SYNTHESIS OF NOVEL RILUZOLE ANALOGUES

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease that is universally fatal. The only drug that is currently FDA approved for the treatment of ALS is Riluzole (Figure 1), which improves life expectancy by a few months *via* an unknown mechanism.

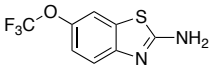
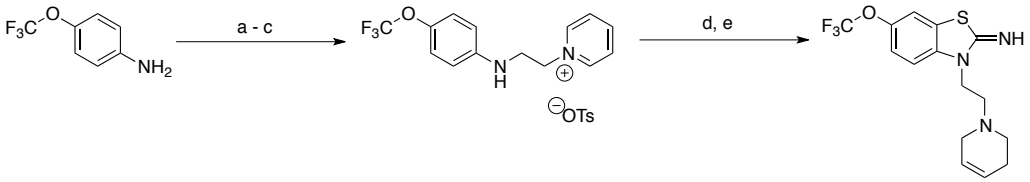


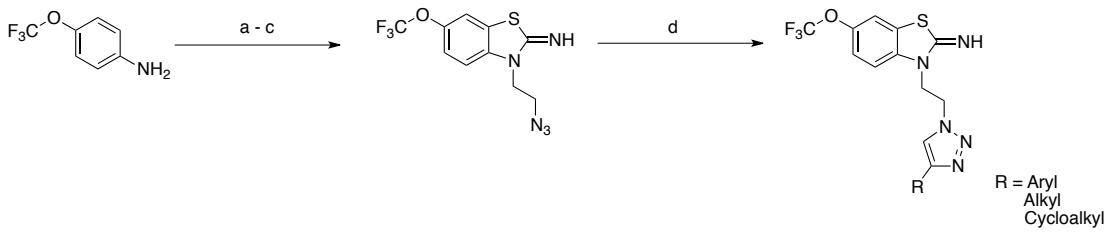
***Figure 1**: Riluzole*

This thesis describes the preparation of novel Riluzole analogues with the overall aim of improving neuroprotective activity against ALS. Two libraries are reported based respectively on the incorporation of tetrahydropyridine and 1,4-substituted-1,2,3-triazole functionality to the benzothiazole ring. Both of these functional groups have been reported in pharmaceutically active drugs either already on the market or in late clinical trials related to the treatment towards motor neuron diseases. Tetrahydropyridine analogues were synthesised in a five step process by the generation of a diamine intermediate, followed by reaction with Zincke salt, cyclisation and reduction (Scheme 1).



***Scheme 1**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)_2NH_2.HBr$, toluene, reflux, 24 h, 65 %; (b) 1.0 equiv. N-(2,4-dinitrophenyl)pyridinium chloride, MeOH, RT - reflux, 20 h; (c) 1.0 equiv. Sodium p-toluenesulfonate, EtOAc, reflux, 12 h, 78 % (over two steps); (d) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 16 h; (e) 2.8 equiv. $NaBH_4$, MeOH, 0 °C - RT, 16 h, 69 % (over two steps)*

1,4-Substituted-1,2,3-triazole analogues were synthesised by reaction of a terminal alkyne with an azide Riluzole analogue with substoichiometric amounts of Cu^I (Scheme 2). The azide analogue was generated by carrying out a diazotransfer reaction on the diamine intermediate followed by cyclisation with KSCN and Br_2.



***Scheme 2**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)NH_2.HBr$, toluene, reflux, 24 h, 65 %; (b) 1.2 equiv. Imidazole-1-sulfonyl azide hydrochloride, 2.3 equiv. K_2CO_3, 0.001 equiv. $CuSO_4.5H_2O$, MeOH, RT, 2 h, 63 %; (c) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 2 h, 63 %; (d) 1.5 equiv. Terminal Alkyne, THF/H_2O, 1M $CuSO_4$, 1M NaAsc, 20 °C, 2 h, 35 - 98 %*

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Furthermore, I would like to extend my thanks to the members of our research group for their constant help and support throughout this PhD. A special thanks to Sam Moss and Anthony Walsh for their friendship and support throughout. You have been fantastic throughout and I will miss all the tea and coffee breaks we have had over the years, chatting about anything and everything. I would also like to include in this little thank you all researchers I have met at both Reading and Huddersfield University, who have made this PhD an enjoyable experience.

The only people left to thank are all my fantastic friends, family and boyfriend, you really have been my rock on this rollercoaster experience and I can't thank you enough. Mum, Dad thank you for being there every step of the way through every decision I have made and pushing me to achieve my goals when I have had doubt. Thank you. One thing left to say is unfortunately Mum, 42 has not been the answer to any exam I have taken, yet!

ABBREVIATIONS

Ac	Acetyl
AD	Alzheimer's Disease
ADME	Adsorption, Distribution, Metabolism and Excretion
ALS	Amyotrophic Lateral Sclerosis
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AOP	Aryloxanyl pyrazolone
app	Apparent
Ar	Aromatic
ASP	Arylsulfanyl Pyrazolones
ATF3	Activating Transcription Factor 3
ATP	Adenosine Triphosphate
b	Broad
BBB	Blood Brain Barrier
BINAP	2,2'-bis(Diphenylphosphino)-1,1'-binaphthyl
CAI	Carboxyamidotriazole
CDDO-EA	2-Cyano-3,12-dioxoolean-1,9-diene-28-oic acid-ethylamide
CDDO-TFEA	2-Cyano-3,12-dioxoolean-1,9-diene-28-oic acid-trifluoroethylamide
CHD	Cyclohexane-1,3-diones
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CuAAC	Copper^{I} catalysed azide-alkyne cycloaddition
Cu^{II}(btsc)	Bis(thiosemicarbazonato)copper^{II}
CY	Cyclohexane
CYP	Cytochrome P450
CPN-9	*N*-(5-(2-Pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethyl-phenoxy)acetamide
d	Doublet
DAO	D-Amino acid oxidase
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIEA	N,N-Diisopropylethylamine
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
DNP	2,4-Dinitrophenyl
DPPF	1,1′-Bis(diphenylphosphino)ferrocene
EAAT-2	Excitatory Amino Acid Transporter-2
EC_{50}	Half Maximal Effective Concentration

ED_{50}	Median Effective Dosage
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ELISA	Enzyme-linked Immunosorbent Assay
ER	Endoplasmic Reticulum
fALS	Familial Amyotrophic Lateral Sclerosis
FDA	Food and Drug Administration
FU	Fluorescent Unit
GABA	γ-Aminobutyric acid
GFP	Green Fluorescent Protein
GLT	Glial Glutamate Transporter
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
Hex	Hexane
HO-1	Heme Oxygenase 1
HSP	Heat-Shock Protein
HTS	High-throughput Screen
HD	Huntington's Disease
i.p.	Intraperitoneal injection
IgG	Immunoglobulin G
IR	Infrared
JNK	c-Jun N-terminal Kinase
log P	Partition Coefficient
m	Multiplet
MAP2	Microtubule-associated Protein 2
m-CPBA	*meta*-Chloroperoxybenzoic acid
MEK	Butanone
MND	Motor Neuron Disease
m.p.	Melting Point
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Ms	Mesyl
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NAIP	Neuronal Apoptosis Inhibitory Protein
NaAsc	Sodium Ascorbate
NMDA	*N*-Methyl-D-aspartate
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
non	Nonet
Nrf2/ARE	NF-E2 related factor 2/antioxidant response element
NSAIDs	Non-steroidal anti-inflammatory drugs

NSC	Neuroblastoma-Spinal Cord
Nu	Nucleophile
OATBs	Organic Ammonium Tribromides
PBP	Progressive Bulbar Palsy
PD	Parkinson Disease
PE	Petroleum Ether
Ph	Phenyl
PLS	Primary Lateral Sclerosis
PMA	Progressive Muscular Atrophy
ppm	parts per million
PK	Pharmacokinetics
Py	Pyridine
q	Quartet
quin	Quintet
PYT	Pyrimidine-2,4,6-triones
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RT	Room Temperature
s	Singlet
sALS	sporadic Amyotrophic Lateral Sclerosis
SAR	Structure Activity Relationship
sept	Septet
sext	Sextet
SG	Stress Granules
SOD1	Superoxide Dismutase Type-1
SR	Serine Racemase
t	Triplet
TARDBP	TAR DNA Binding Protein
TDP-43	Transactive Response DNA Binding Protein 43
TfN_3	Trifluoromethanesulfonyl azide
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
THPYy	Tetrahydropyridine
Ts	Tosyl
TSAO	*tert*-Butyldimethylsilylspiroaminooxathioledioxide
VDCC	Voltage Dependant Calcium Channels

CHAPTER 1: INTRODUCTION

1.1 Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS) was first described in 1869.[1] It is also referred to as 'Lou Gehrig's Disease' in memory of the baseball player who died from ALS in 1941.[2] ALS along with other neurodegenerative diseases, including Alzheimer's, Parkinson's and Huntington's Disease are caused by a combination of events impairing normal neuronal functions (Figure 2).[3,4]

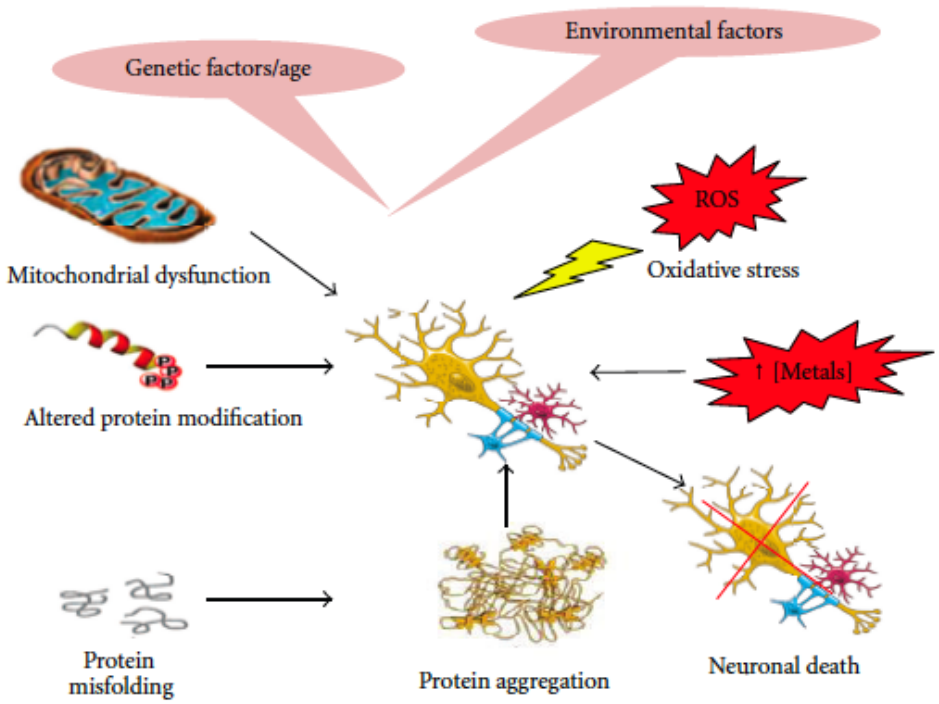


***Figure 2**: Different factors associated with neurodegenerative disease (Reproduced with permission, from Sheikh (2013))*[4]

ALS is the degeneration of motor neurons, which leads to respiratory failure after three to five years through a combination of muscle weakness and wasting of upper and lower voluntary muscles.[5,6] The exact mechanism by which disease progresses manifests for ALS is not known: a number of theories, which have been put forth will be outlined below.

(1) Autoimmune Considerations in ALS Patients

Over the last two decades a number of autoimmune considerations have been proposed as the underlying cause of ALS.[7] There is growing evidence that unusual antibodies may be present in a significant number of patients with motor neuron disease (MND) and ALS, which are indicative of a pathogenic humoral effect.[7,8] MND is a class of neurodegenerative disease which selectively affects upper and lower motor neurons. The following five disorders are grouped under this classification: ALS, Primary Lateral Sclerosis (PLS), Progressive Muscular

Atrophy (PMA), Progressive Bulbar Palsy (PBP) and Pseudobulbar Palsy. Conventional autoimmune symptoms/disorders, inflammation and/or antibodies are observed within affected cells, whereas in regards to ALS patients increased serum levels of antibodies are not recorded. Instead an increase of antibodies is reported within the nerves. It has been hypothesised that antibodies interact with the ganglioside-rich motor nerve terminals, which act as antigens, allowing antibodies to enter the motor neurons where they can then inflict damage.[7] Additionally, the presence of IgG, a type of antibody in ALS motor neurons provides further evidence that these are autoimmune considerations to the pathenogenesis of the disease. In ALS model systems IgG is found to enhance calcium currents, either by antibodies directly interacting with voltage-dependent calcium channels (VDCC) or by modifying calcium currents, which will both result in cell death due to increased cellular calcium levels.[8] The higher concentrations of these antibodies leads to an increased number of T-cells, which leads to ALS patients generating T-cell-independent B-cell responses. These are generally harder to suppress than T-dependant responses, and could result in cellular degeneration without signs of inflammation.

Identification of the auto-antigens involved will help elucidate the mechanism further and also help in the development of designing rational therapies for molecular targets.[9]

(2) Excessive activation of Glutamate in ALS Patients

ALS patients have been reported to have a three-fold higher concentration of cerebrospinal fluid (CSF), which can lead to cell death through excitotoxicity (Figure 3), which is an over activation of glutamate, aspartate, *N*-acetyl-aspartyl glutamate and *N*-acetylaspartate.[1]

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and is responsible for cell communication. Glutamate is synthesised from the reductive deamination of α-ketoglutarate by glutamate dehydrogenase and from the activation of amino-transferase on amino acids. Approximately 20 % of the glutamate pool is found in the presynaptic nerve terminal.[1]

Neurotransmission is initiated by the presynaptic neuron being depolarised by the influx of Ca^{2+}, which then causes glutamate to diffuse across the synaptic cleft and activate the post-synaptic glutamatergic receptors. Excitatory signals are terminated by the removal of glutamate from the synaptic cleft with the help of Na^{+}-dependant glutamate transporters such as the excitatory amino acid transporter-2 (EAAT-2), which are present in astrocytic processes that envelop the synapse.[10,11,12] These transporters are responsible for removing up to 94 % of glutamate from the synaptic cleft.[1] Within the astrocytes, glutamate is converted to glutamine by glutamine synthetase and is subsequently returned to the neuron for glutamate re-synthesis.

Therefore an excessive activation of glutamatergic receptors located on the post-synaptic neuron due to an increase in glutamate increases the concentration of Ca^{2+} in the post-synaptic neuron. This in turn causes the activation of degenerative enzymes, for example phospholipase A, proteases and nitric oxide synthase.[12] An increase in Ca^{2+} also causes mitochondrial function to be altered, resulting in the production of free radicals and impairing the production of adenosine triphosphate (ATP). Depletion of ATP combined with the production of nitric oxide and other free radical species results in the deactivation of the Na^{+}/K^{+} pump, raising intracellular Na^{+} concentrations, and subsequently results in neuronal depolarisation. The reverse operation of Na^{+}/Ca^{2+} exchange required to normalise intracellular Na^{+} concentrations further increases Ca^{2+} uptake, elevating already high levels of Ca^{2+} and increasing the likelihood of cell death.

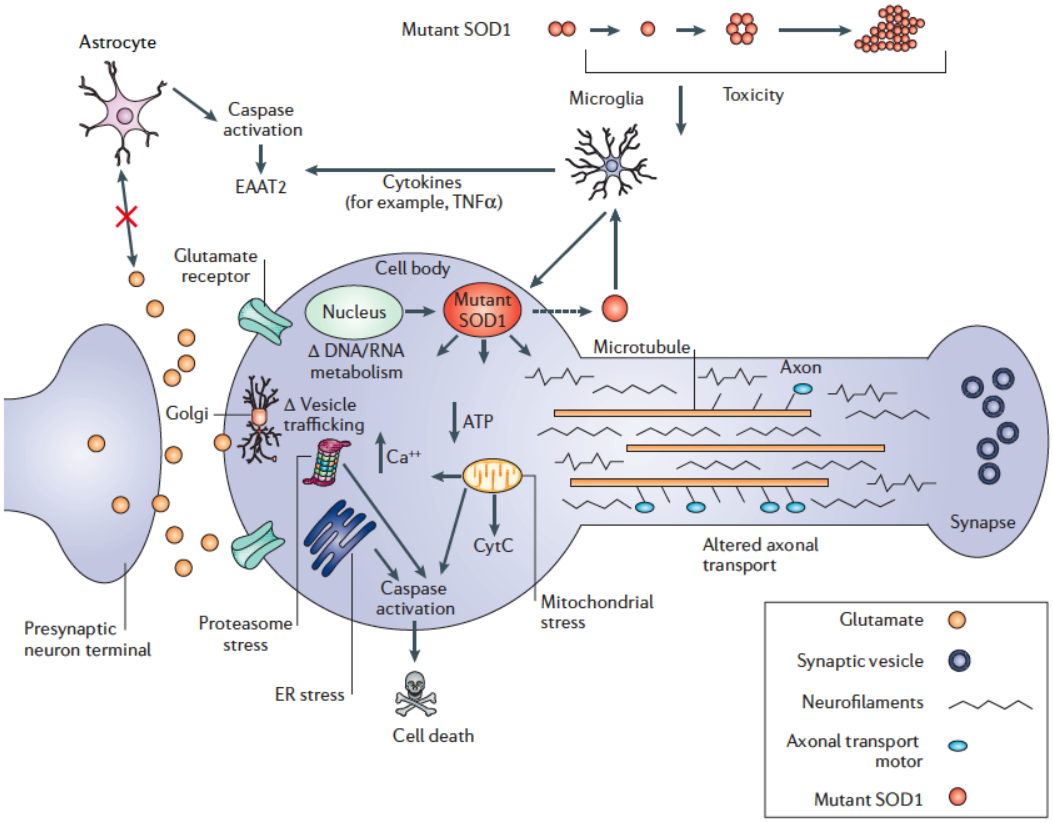


***Figure 3**: The pathogenic process that triggers motor neuron degeneration in ALS patients (Reproduced with permission, from Pasinelli (2006))* [13]

(3) Misfolding of Superoxide Dismutase Type-1 (SOD1) in ALS Patients

Superoxide dismutase type-1 (SOD1), also known as Cu/Zn superoxide dismutase is a free radical scavenger. Its function is the detoxification or dismutation of the superoxide anion to form hydrogen peroxide, which then in turn is converted to water through the action of catalase and glutathione peroxidase (Scheme 3).[14] The activity of SOD1 plays a crucial role in the regulation of oxidative stress and in the protection against oxygen radical-induced cellular damage.

$$O_2^{\bullet-} \xrightarrow{\text{SOD1}} H_2O_2 \xrightarrow[\text{glutathione peroxidase}]{\text{catalase}} H_2O$$

***Scheme 3**: Conversion of superoxide anion to water* [14]

Misfolding of SOD1 can arise from mutations in the gene. The first mutations of SOD1 linked to ALS were reported in 1993.[5] To date, 20 % of familial ALS (fALS) and 1 - 4 % of sporadic ALS (sALS) cases are likely to be caused by mutations in the SOD1 gene.[5] The exact mechanism of how mutated SOD1 is linked to motor neuron death and ALS is unknown, but over the years a number of hypotheses have been proposed and investigated (Figure 4).

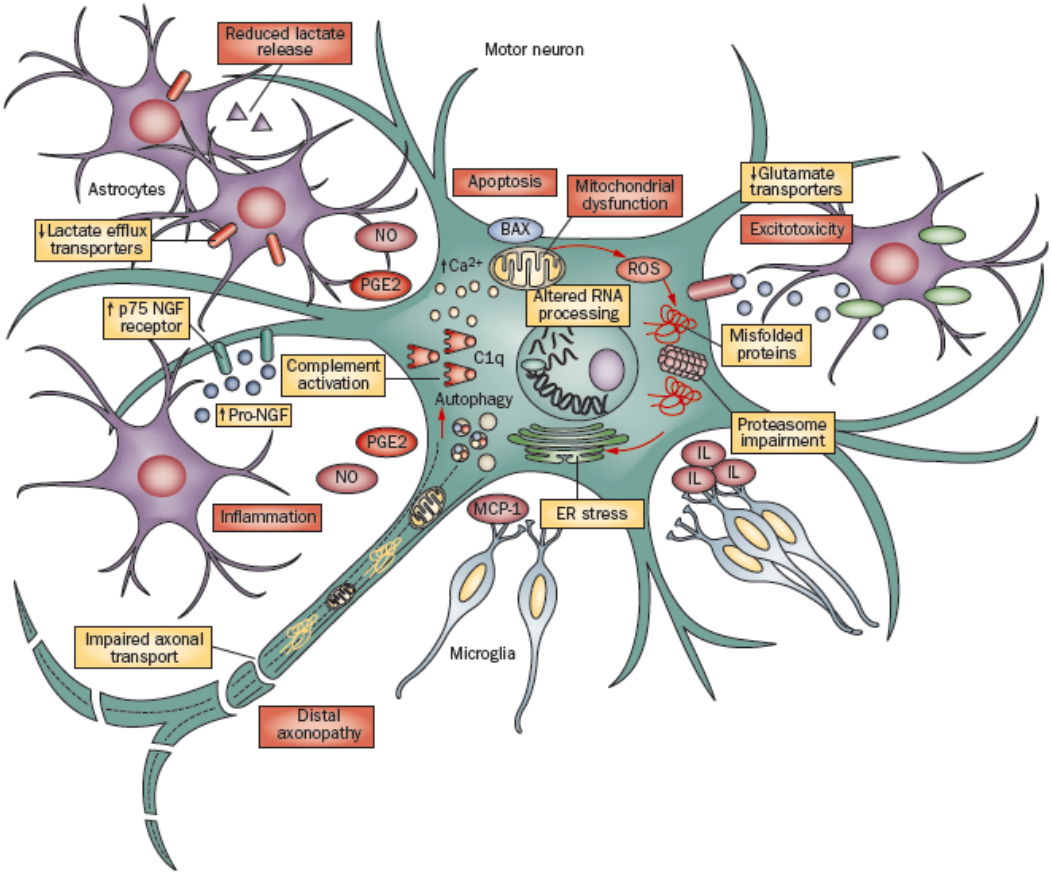


***Figure 4**: Molecular mechanisms of motor neuron injury in ALS caused by mutated SOD1 (Reproduced with permission, from Ferraiuolo (2011))* [15]

Protein misfolding of the SOD1 gene has resulted in the formation of mutated SOD1 genes. Mutations in the SOD1 gene has resulted in the reduction of cellular activity due to diminished stability, reducing the proteins half-life by 30 - 75 %.[14] Misfolded proteins cause an endoplasmic reticulum (ER) stress response (Figure 4) that attempts to correct the protein folding. If this ER correction process is not successful it can result in cell death. Mutations in the SOD1 gene can also lead to oxidative stress from an imbalance between the generation and removal of reactive oxygen species (ROS) as a result of the SOD1 gene being structurally damaged protein misfolding. It has been reported that the CSF of people with

ALS have higher levels of free radicals, which can impair cellular ability to cope with a toxic insult in cellular injury and neuronal death in non-replicating neurons during aging.[15,16] Aggregates of mutant SOD1 found in the neuronal cell lines or in cultured primary motor neurons depolarise mitochondria, impairing calcium homeostasis and reducing ATP production, which as a result will result in apoptosis.[13] Raised levels of intracellular Ca^{2+}, more ROS, perturbation of mitochondria function and ATP production caused by mutant SOD1 can cause excitotoxicity, which will result in neuronal injury from excessive activation of glutamate receptors. Excess glutamate release can be counteracted by increased uptake by astrocytes, but when in the presence of mutated SOD1 there function is reduced and they secrete inflammatory mediators due to neuroinflammation.

(4) Accumulation of Transactive Response DNA Binding Protein 43 (TDP-43) in ALS Patients

TDP-43 is a cellular protein, encoded by the TARDBP gene, which is a modular DNA/RNA binding protein. TDP-43 is localised to the cytosol and the nucleus, and is involved in RNA splicing, gene transcription, microRNA processing, stabilisation and the transport of RNA.[17] In 2008 mutations were identified in the TARDBP gene encoding TDP-43, the result of which is believed to be the accumulation of the protein in cyctoplasmic inclusions, where it can take refuge in cyctoplasmic RNAs in stress granules (SG).[6,18] Therefore, neurons of ALS sufferers have decreased levels of TDP-43. The dysfunction of TDP-43 has been established as contributing towards up to 6.5 % of fALS cases, but the exact mechanism of how TDP-43 causes neurotoxicity in neurons is currently not fully understood.[17]

1.2 Therapeutic Targets for the Treatment of ALS

As described previously, there are several proposed mechanisms towards the understanding of ALS. To date, there is no single definitive mechanism for understanding how ALS manifests, which therefore widens the scope in finding a suitable drug target. There have been several drugs tested at phase III clinical trials in regards to their potent properties against ALS.[19] Phase III clinical trials are performed on a large number of patients and all new treatments are compared against the best treatment currently available, which for ALS this is Riluzole (**1**, Figure 5). The following drugs shown in Figure 5 have all been subjected to phase III clinical trials;[19]

(1) **Dexpramipexole** (**2**) is highly related to Pramipexole, which is a treatment for Parkinson's Disease (PD). Dexpramipexole has been shown to slow the progression of ALS by maintaining mitochondrial function in deteriorating motor neurons. However, in 2012 phase III trials completed on Dexpramipexole showed no statistically significant evidence with regards to Dexpramipexole slowing ALS disease progression.[20]

(2) **Arimoclomol** (**3**) is hypothesised to reduce the levels of protein aggregates identified as a possible cause of ALS in motor neurons. Arimoclomol is understood to boost the expression

of chaperones Hsp70 and Hsp90, which help newly synthesised proteins to fold properly. Phase II/III clinical trials are still ongoing.[21]

(3) **Olesoxime** (**4**) is understood to protect mitochondria in the motor neurons of ALS patients *via* putative mitochondrial permeability transition pore modulators, which will result in the slowing of disease progression.[22] Phase III clinical trails for Olesoxime were dropped due to a statistically insignificant increase in survival *vs*. placebo.[23]

(4) **Ceftriaxone** (**5**) is an antibiotic that protects cultured neurons from glutamate-induced excitotoxicity, brought about by reducing glutamate levels and increasing the expression of glutamate transporter EAAT-2. Phase III trials reported that this particular antibiotic had no effect on disease progression.[24]

(5) **Edaravone** (**6**) is a neuroprotective agent, which acts as a potent antioxidant and is also a strong scavenger of free radicals. These activities protect against oxidative stress and neuronal apoptosis.[25] Treatment of patients with Edaravone has shown a reduction in CSF levels of 3-nitrotyrosine, a marker of oxidative stress. Edaravone is showing promising results in the slowing down of disease progression. Phase III trials are ongoing.

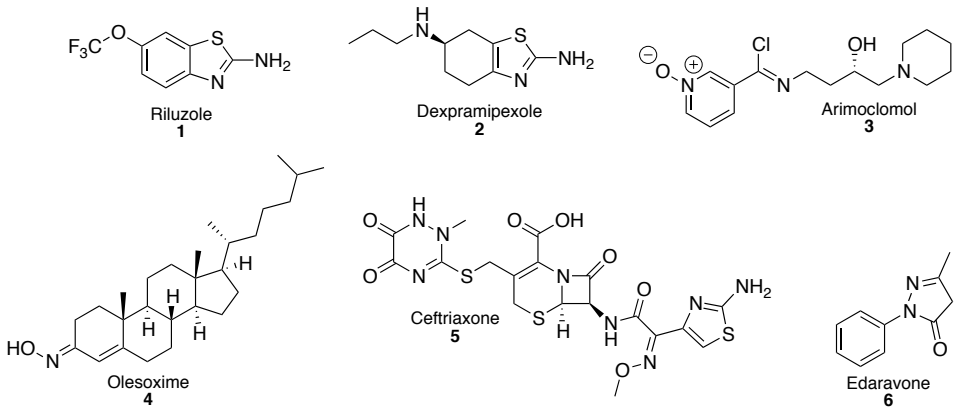


***Figure 5**: A number of small molecules tested for their properties against ALS at phase III*[19]

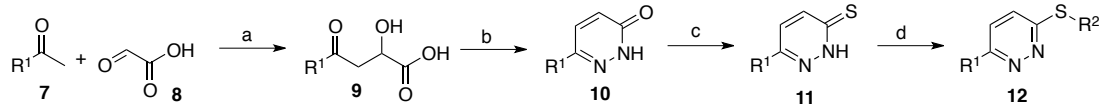
(1) Targeting Reduction in Glutamate Toxicity

Riluzole (**1**) is an FDA approved drug, which is currently the only approved treatment for attenuating disease progression in ALS patients. Riluzole is hypothesised to inhibit the release of glutamate and to non-competitively inhibit postsynaptic *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors.[26] The modest success of Riluzole against ALS has highlighted the role of glutamate excitotoxicity in numerous disease states, which has put focus on further developing drugs which will target the modulation of glutamate signalling.

A potential approach in preventing excitotoxicity is to enhance glutamate reuptake, *via* EAAT-2. EAAT-2 is a major glutamate transporter that removes glutamate from the synapse. An approach in increasing EAAT-2 activity is the development of a library of small molecules, which could enhance EAAT-2 expression. Colton *et al*. developed and used a cell based

ELISA for EAAT-2 protein expression to screen a library of 140,000 small molecules: including compounds approved by the FDA, a purified natural products library, and compounds purchased from Peakdale, Maybridge Plc, Cerep, Bionet Research Ltd, Prestwick, Specs and Biospecs, ENAMINE, Life Chemicals, Inc, MicroSource Diversity System's NINDS customs collection, Chemical Diversity Labs, Chembridge, and small molecules obtained from various academic institutions.[26] This screen found 293 hits with 61 compounds showing a dose dependent increase in EAAT-2 expression. Three out of the 61 compounds tested were selected for further optimisation based on their low potency ($EC_{50} < 1$ μM), lack of toxicity and chemical traceability. It should be noted that the structures of these compounds has not been disclosed.[19,26]

Xing *et al.* performed chemical optimisation on the identified three lead compounds to develop additional analogues for potential use as therapeutic agents.[27] It was found through structure-activity relationship (SAR) studies that the thioether and pyridazine functionality are essential molecular components for increasing EAAT-2 protein levels. Thiopyridazine analogues can be synthesised *via* a four-step process (Scheme 4). Firstly, a known ketone in the presence of K_2CO_3 is reacted with glyoxylic acid to generate an aldol addition product. This is then directly reacted with hydrazine to yield pyridazinone **10**. Reacting pyridazinone **10** with P_2S_5 and pyridine at 120 °C yields pyridazinethione **11**, which is further converted to thiopyridazine **12** *via* alkylation.[27]



R^1 = 2-Py. and R^2 = 2,6-Di-Me-Bn, 2,4-Di-Me-Bn, 2,6-Di-Cl-Bn or 1-(2-Cl-6-F-phenyl)ethyl

***Scheme 4**: Reagents and conditions: (a) 2.0 equiv. K_2CO_3, H_2O, RT, 20 h; (b) 1.2 equiv. NH_2NH_2, AcOH, 100 °C, 2.5 h, 20 % (over two steps); (c) 1.9 equiv. P_2S_5, Py. 120 °C, 3 h, 80 %; (d) 2.0 equiv. R^2Br, 2.0 equiv. K_2CO_3, DMF, RT, 1 h, ~ 90 %* [27]

Of the analogues synthesised in this study, several thiopyridazine derivatives (Figure 6) were found to exhibit an increase in EAAT-2 levels. The thiopyridazine derivatives were found to increase EAAT-2 levels greater than six-fold over endogenous levels in primary astrocytes at concentrations of less than 5 μM.[19] Additionally, thiopyridazine derivative **15** was found to increase EAAT-2 levels three- to four-fold at a low EC_{50} value, 0.5 μM. These compounds will prove useful for determining the biological mechanism for regulating EAAT-2 levels and also for further assessing the role of glutamate excitotoxicity in cellular systems, potentially in animal models of acute and chronic neurodegeneration.[27]

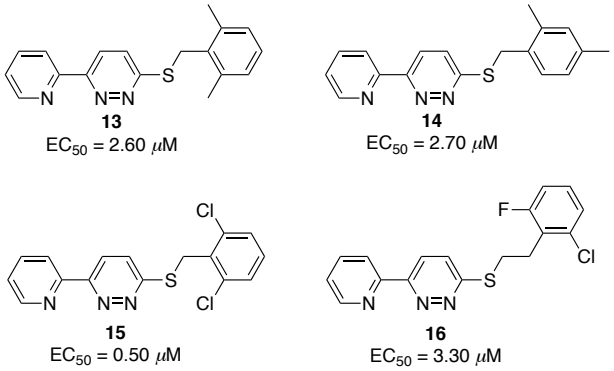


***Figure 6**: Thiopyridazines developed to increase EAAT-2 protein levels* [27]

In addition to finding small molecules which will enhance glutamate uptake through the increase of EAAT-2 levels, other studies have also reported that elevated levels of D-serine can also contribute to glutamate excitotoxicity.[19] D-Serine serves as a co-agonist at the glycine site of the NMDA glutamate receptor. An increase in D-serine has been reported in the spinal cord of ALS patients.[28] Usually excess D-serine is removed by D-amine acid oxidase (DAO) *via* metabolism, but ALS patients have been reported to have a reduction in DAO, which could account for these high levels of D-serine.[19] Recently, a new mutation in DAO has been reported to contribute to fALS. This new mutation - R199W DAO is understood to inhibit DAO's original function by increasing small protein-containing aggregates and reducing cell viability when expressed in neuroblastoma-spinal cord (NSC)-34 cells, a motor neuron cell line.[29,30] Therefore, finding small molecules which will contribute towards the reduction of D-serine either by enhancing the activation of DAO or the reduction of serine racemase (SR), which is responsible for D-serine synthesis, may be therapeutically beneficial.[31]

(2) Targeting SOD1 Mutation

Developing a library of drugs which reduces SOD1 protein levels in ALS patients will reduce the levels of the mutated SOD1 gene, therefore resulting in cells building up a resistance to ALS-induced cellular death. Murakami *et al.* developed a high-throughput screen (HTS), which screened a total of 9600 small molecules.[32] From this screen, 325 compounds were identified as hits, with only two compounds demonstrating selectivity in downregulating SOD1 protein levels without apparent cellular toxicity following secondary assays. One of the two compounds, 3-(1*H*-benzo[*d*]imidazol-2-yl)-6-chloro-4*H*-chromen-4-one, 052C9 (**17**, Figure 7), was put forward for further analysis due to its considerably lower EC_{50} and was also found to reduce phosphorylation of the transcription factor Nrf2, a known activator of cellular stress gene as well as an upregulator of SOD1 transcription.[19,32]

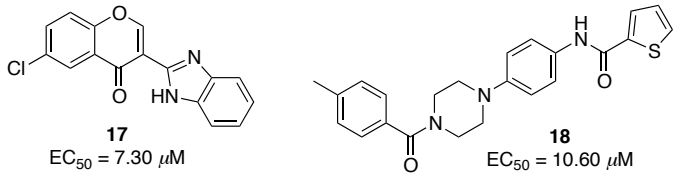
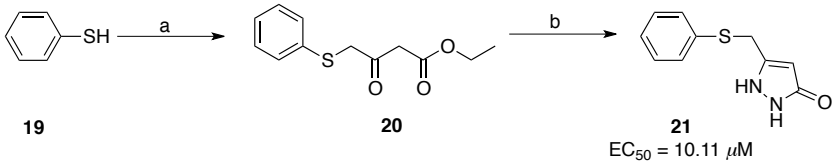


***Figure 7**: Compounds shown to reduce SOD1 expression* [19]

Similarly, Wright *et al.* performed a HTS on 30,000 small molecules focusing on repressing SOD1 transcription. From this HTS 20 compounds were identified as hits. Compound 7687685 (**18**, Figure 7) from this HTS demonstrated both reduction in endogenous SOD1 protein levels in human cells and also repressed several other genes implicated in ALS. However, when *in vivo* studies of compound **18** were performed in SOD1 G93A transgenic mice, only a small reduction of SOD1 protein levels in spinal-cord extracts were recorded, suggesting that this would not be a useful treatment for ALS patients.[19]

As previously stated, mutation in SOD1 has also been reported to lead to cellular toxicity through loss of function of the SOD1 protein whereby mutant proteins in intercellular inclusion lead to cellular dysfunction. Therefore compounds, which reduce the aggregation of SOD1 protein, could be found beneficial in protecting cells from neuronal damage. Benmohamed *et al.* performed a HTS on 50,000 small molecule compounds focusing on their ability to reduce mutant SOD1 aggregates in a cell-culture model.[33] Hits from this HTS were then subjected to further screens and from this, three distinct chemical series were identified for further optimisation based on their ability to reduce both cellular toxicity and mutant SOD1 protein aggregation: arylsulfanyl pyrazolones (ASP) **21**, cyclohexane-1,3-diones (CHD) **22** and pyrimidine-2,4,6-triones (PYT) **23**.[19]

The general method to generate ASP **21** is achieved in two-steps (Scheme 5). Thiophenol **19** and ethyl 4-chloroacetate **20** react *via* an S_N2 nucleophilic displacement to give β-ketoester, which is further treated with hydrazine to generate ASP **21**.[33]



***Scheme 5**: Reagents and conditions for the general synthesis of ASP compounds: (a) 1.0 equiv. ethyl 4-chloroacetoacetate, 1.1 equiv. Et_3N, DCM, 0 °C, 30 mins; (b) 1.0 equiv. NH_2NH_2, EtOH, RT, overnight, 67 % (over two steps)* [33]

A number of ASP analogues were synthesised as shown in Scheme 5 with different functionalised aromatic rings within the ASP scaffold. The first set of SAR studies carried out looked at *para* substitution on the aryl ring with electron-withdrawing and electron-donating

substituents of varying size. From this first study the only ASP compound that showed promising reduction in mutant SOD1 was an ASP analogue containing a chloro group at the *para* position of the aromatic ring with an EC_{50} of 1.93 μM. Further studies focused on multiple substitutions on the aromatic ring, which included chloride as one of the substituents. These studies identified two promising compounds **24** and **25**, which showed potent activity towards the reduction of mutant SOD1 (Figure 8).[33]

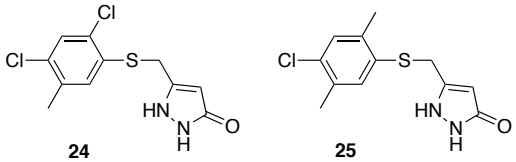


***Figure 8**: Two ASP hit compounds **24** and **25** [33]*

Both of these ASP compounds **24** and **25** have shown promising properties with good potency and the capability of the ASP scaffold to permeate into the brain. Penetration across the BBB is achievable with a minimum number of polar groups on a pharmaceutical compound, or the polar groups are temporarily masked. Even though compounds **24** and **25** exhibit good potency and the capability to cross the blood brain barrier (BBB), which is required for a drug targeting neurodegenerative diseases they have been report to have poor metabolic stability. It has been reported that the sulfur linker when in the presence of either microsomal or plasma enzymes is oxidised to the sulfoxide **29** hindering its ability to reduce SOD1 aggregation.[34] The synthesis of compound **29** was confirmed by analysis of mass spectroscopy between compound **27** and nicotinamide adenine dinucleotide phosphate (NADPH), which reported the rate of formation of **29** in relation to the loss of **27**. As metabolism of the sulfide linker is fast ASP compounds require microsomal and plasma enzyme protection. This was achieved by replacing the sulfur linker with either a sulfoxide, sulfone or ether linker. Potency of these different linkers increased (EC_{50} decreases) in the order of ether > sulfide > sulfone >> sulfoxide (Figure 9).[34]

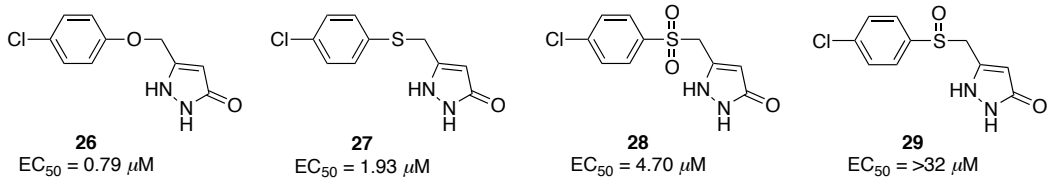
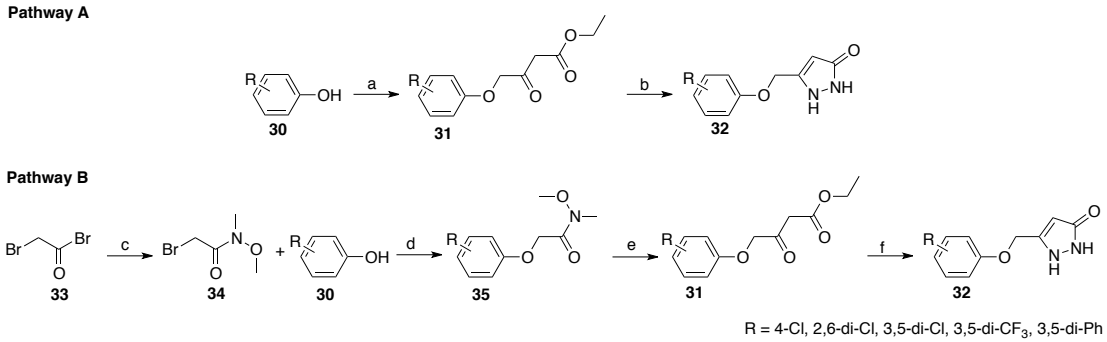


***Figure 9**: Structures for the Ether, Sulfide Sulfone and Sulfoxide Linkers*

Replacement of the sulfur linker with an ether linker enhanced the reduction of SOD1 protein aggregates. Chen and co-workers investigated and reported the synthesis and activity of a number of aryloxanyl pyrazolones (AOP).[34] Initial preparation of AOP **32** was achieved in a two-step process (Scheme 6, Pathway A). Phenol was reacted with ethyl 4-chloroacetoacetate to give intermediate β-hydroxyester **31** *via* an S_N2 nucleophilic

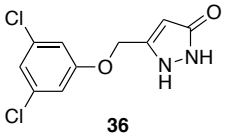
displacement. Intermediate **31** was then treated with hydrazine to give AOP **32**. This two-step synthetic pathway was simple and direct, but was not efficient due to the instability of the enolate intermediates obtained. These issues were overcome by generating AOP **32** in a four-step process (Scheme 6, Pathway B). Phenol and 2-bromo-*N*-methoxy-*N*-methylacetamide were reacted together to generate hydroxamide **35**. The hydroxamide intermediate was then reacted with ethyl acetate to generate intermediate β-hydroxyester **31**, which was then further reacted with hydrazine to give AOP **32**.



***Scheme 6**: Reagents and conditions for general synthesis of AOP compounds: **Pathway A** (a) 1.5 equiv. ethyl 4-chloroacetoacetate, 2.5 equiv. NaH, THF, DMF, -20 °C - 70 °C; (b) 1.0 equiv. NH_2NH_2, EtOH, RT, overnight, 2 - 30 % (over two steps);*

***Pathway B** (c) 1.0 equiv. N,O-dimethylhydroxylamine hydrochloride, 2.2 equiv. K_2CO_3, Et_2O, H_2O, RT, 30 mins, 74 %; (d) 1.0 equiv. NaOEt, EtOH, 70 °C, overnight; (e) 1.0 equiv. EtOAc, 2.3 equiv. LiHMDS, THF, -78 °C, overnight; (f) 1.0 equiv. NH_2NH_2, EtOH, RT, overnight, 2 - 27 % (over three steps)*

After a number of AOP analogues containing mono or disubstituted functionality, such as aryl, alkyl or halide were successfully synthesised using Pathway B of Scheme 6 they were subjected to adsorption, distribution, metabolism and excretion (ADME) testing. The following analogue **36** was found to be the most potent within this library of AOPs generated by Chen and co-workers (Figure 10). Compound **36** showed promising results with good aqueous solubility, good BBB penetration, good metabolic stability and a life extension of G93A ALS mice of 13.3 % at 20 mg/kg.[34] These initial findings show that AOP analogue **36** could be a novel drug candidate for the treatment of ALS.[34]

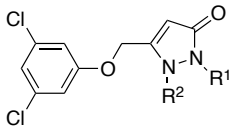


36
EC_{50} = 0.067 μM

***Figure 10**: AOP analogue **36** [34]*

Tripper and co-workers reported that if R^2 on the pyrazolone ring **37** (Figure 11) is altered from a simple hydrogen bond AOP analogues become inactive, suggesting this position is essential for cellular activity. Varying the functionality of R^1 on the pyrazolone ring gave a number of active AOP analogues. Addition of sterically demanding substituents at R^1 had

little effect on the efficacy, suggesting the possibility of a large open pocket or corridor within the target structure. Overall, this study identified that when R^1 is a benzyl group, enhanced potency towards the reduction of mutant SOD1 is reported.[35]

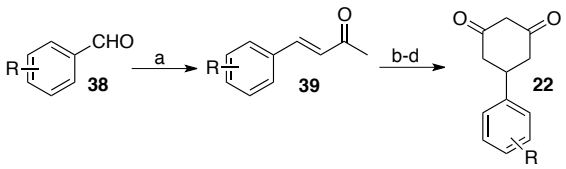


37

R^1 = Me,CH_2CH_2OH, Bn, CHO and R^2 = Me or H

***Figure 11**: Varying R^1 and R^2 substituents on the pyrazolone ring*

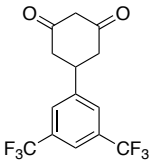
CHD analogues are generated over two-steps using the general method shown below (Scheme 7).[36] Commercially available or previously synthesised aldehydes were used as starting materials, which were converted to intermediate **39** *via* the Wittig reaction. Intermediate **39** was then converted to CHD analogues **22** after Michael addition, cyclisation, hydrolysis and decarboxylation.[37]



R = 3,5-di-F, 3,5-di-CF_3, 3,5-di-Me, 3,5-di-Cl

***Scheme 7**: Reagents and conditions for the general synthesis of CHD compounds: (a) 1.0 equiv. 1-(triphenylphosphoranylidene)-2-propanone, THF, RT, 12 h; (b) 1.0 equiv. diethyl malonate, 2.0 equiv. EtONa, EtOH, RT, 12 h; (b) 3N aq. NaOH, H_2O, RT, 12 h; (c) 3N aq. HCl, Et_2O, RT, 4 h, 80 % (over four steps)* [36]

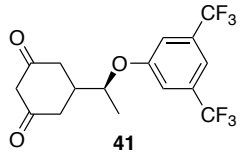
A number of CHD analogues synthesised by Zhang and co-workers containing functionality on the aromatic ring were found to be active candidates (Scheme 7, general procedure).[36] All active CHD analogues were tested for their cell survival against the formation of mutant SOD1 aggregates. These mutant SOD1 aggregates are observed to be generated from treatment with proteasome inhibitor MG132. SAR studies lead to several conclusions: (1) electronic properties of the substituted groups do not affect the activity of analogues; (2) the *meta* position is much more important than the other positions; (3) the size of the *meta*-substituent is crucial; and (4) trifluoromethyl is a favoured substituent to increase potency. Over 120 analogues were obtained using Scheme 7, which lead to the discovery of CHD analogue **40**, which has an EC_{50} of 0.70 μM (Figure 12).[36]



40
EC_{50} = 0.70 μM

Figure 12**: CHD analogue **40

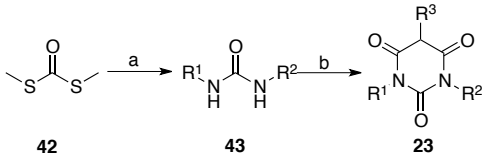
Even though CHD analogue **40** showed favourable pharmacokinetics (PK), demonstrating high plasma stability, oral bioavailability and brain accumulation, it did not show any beneficial effects when tested in SOD1 G93A transgenic mice. Additional studies demonstrated that CHD analogue **40** exhibited minimal activity in primary cortical neurons due to low penetration of neuronal cells.[19] Due to the poor therapeutic benefits demonstrated by compound **40** further SAR studies around this series were investigated, which lead to new chiral CHD analogues, such as **41**. CHD analogue **41** exhibited enhanced activity of cortical neurons with good PK properties, whilst also retaining activity in the PC12 assay (Figure 13). Additionally this analogue was found to extend the life expectancy of an ALS mouse by 13 %, which is slightly longer than that previously reported for Riluzole using the same mouse model.[37]



41
EC_{50} = 0.30 μM

Figure 13**: CHD analogue **41

PYT analogues, such as **23** are synthesised in two-steps (Scheme 8, general procedure). Firstly commercially available *S,S*-dimethyl carbonodithioate and amines are reacted together to generate a urea intermediate **43**. This urea intermediate is then further treated with malonic acid in an acetic acid/acetic anhydride medium generating PYT analogues **23**.[38]

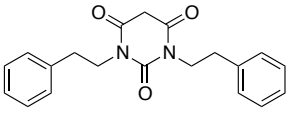


42 **43** **23**

R^1/R^2 = aryl, alkyl, or arylalkyl groups and R^3 = unsubstituted alkene or alkyl

***Scheme 8**: Reagents and conditions for the general synthesis of PYT compounds: (a) 2.1 equiv. RNH_2, MeOH, 60 °C, 24 h; (b) 1.0 equiv. (substituted) malonic acid, AcOH/Ac_2O (3:2), 60 °C - 90 °C, 4 h* [38]

PYT analogues were identified as novel potential drug candidates with good potency and ADME properties towards the reduction of SOD1 protein aggregation. PYT analogue **44** (Figure 14), was found to have a desirable combination of potency, ADME properties, low toxicity, brain penetration and oral bioavailability.[38]



44
EC_{50} = 1.68 μM

Figure 14**: PYT analogue **44

Conclusions drawn from the above three distinct chemical series, ASP **21**, CHD **22** and PYT **23** identified initially from HTS preformed by Benmohamed *et al.* have all shown positive results towards the reduction of mutant SOD1 aggregation. In particular compound **36**, which is a modification of the ASP library and has been reported to display an increased lifespan compared to controls.[34] Reduction in mutant SOD1 aggregation will result in normal cell function, therefore reducing the chances of excitotoxicity, which is a proposed mechanism in contributing to ALS. Therefore, investigating these libraries further could help towards finding alternative methods in reducing disease progression for patients with ALS.

An alternative method to prevent the aggregation of SOD1 was to design a compound that acts as a pharmaceutical chaperone stabilising the SOD1 native dimer. Ray *et al.* tested a number of compounds from 15 commercially available libraries to find out their potential towards binding at the SOD1 dimer interface and stabilise the dimer. From this initial *in silico* screening 100 compounds were found to be hits, of which were further screened in SOD1 A4V aggregation assay. From this screening 15 compounds were identified to inhibit the aggregation of SOD1 A4V (most common ALS-causing mutation) proteins and other SOD1 mutants.[19,39]

When the 15 hit compounds were tested for SOD1 protein-binding in the presence of human plasma, they showed poor binding affinity relative to other protein components in the plasma. Nowak *et al.* carried out a number of docking calculations on a database of small molecules to model the aggregation inhibitors at the dimer interface binding pocket.[40] From these docking calculations 20 compounds were identified to have satisfactory docking constraints (hydrogen bonds). These hits were further tested for their ability to block aggregation of SOD1 and specifically bind SOD1 over blood plasma components. This screening resulted in at least six compounds (Figure 15) having high selectivity towards the blocking of SOD1 aggregation and were found to perform significantly better than original azauracil-based molecules tested in blood plasma. These six compounds indicate an excellent starting point towards the therapeutic development of ALS.[19,40]

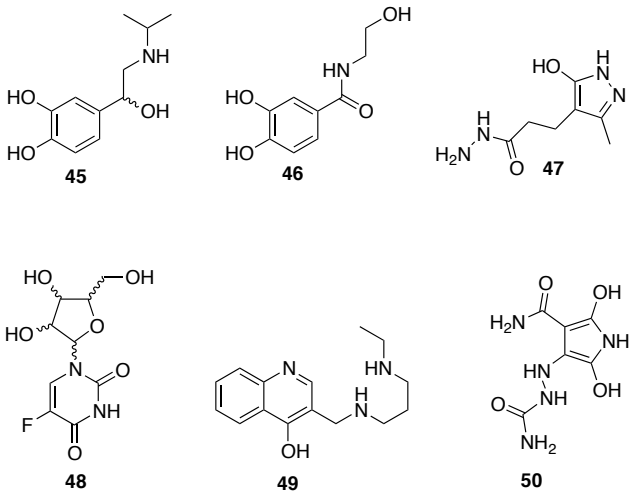


***Figure 15**: Compounds that selectively bind SOD1 over human plasma and inhibit A4V-SOD1 aggregation*[40]

(3) Targeting TDP-43

Mutation in the TARDBP gene encoding TDP-43 is estimated to be responsible for up to 6.5 % of fALS cases.[17] TDP-43 mutations are highly toxic to the cell.[19,41] One approach to reduce the pathology caused by mutant TDP-43 is to identify small molecules that inhibit the binding of TDP-43 to nucleotides. Cassel *et al.* developed a HTS assay to measure oligonucleotide binding to TDP-43. A total of 7360 small molecules which are known to disrupt oligonucleotide binding to TDP-43 protein were screened.[42] From the HTS a series of 4-aminoquinoline derivatives (Figure 16) were tested for their ability to regulate TDP-43. Cassel *et al.* hypothesised that this series would stimulate capase-7 mediated cleavage of TDP-43, affecting its cellular accumulation.[43] Caspase-7 can mediate the reduction of TDP-43 protein levels *via* cleavage of the TDP-43 and subsequent clearance of the cleaved products by the proteasome. The 4-aminoquinoline derivatives shown in Figure 16 were found to bind to TDP-43 decreasing its association with oligonucleotide and increasing caspase-mediated cleavage of the protein. When these compounds were further tested in H4 cells they were found to modestly reduce intracellular levels of TDP-43 and proteins known to be regulated by TDP-43.[41] Reduction of TDP-43 levels within motor neurons will prove to be beneficial towards ALS treatment as this will restore normal TDP-43 functions, including RNA transport. Therefore further development and validation of these small molecules could prove valuable for future therapeutic development.

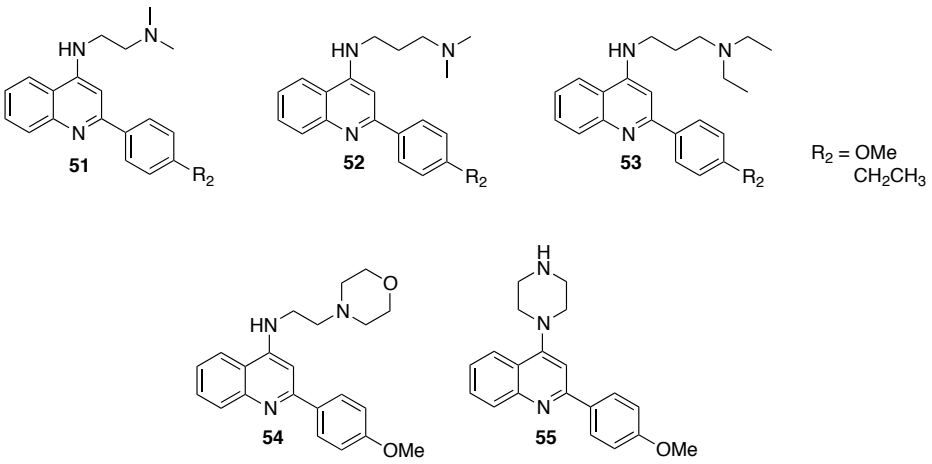


***Figure 16**: 4-aminoquinolines developed for disruption of oligonucleotide/ TDP-43 binding* [43]

Another mechanism to diminish TDP-43 toxicity in cells is the reduction of intracellular inclusions. Bis(thiosemicarbazonato)copper^{II} complexes (Cu^{II} (btsc)s **56**, Figure 17) were tested on treated SH-SY5Y cells with paraquat, which induces cellular stress through mitochondrial inhibition, leading to the formation of TDP-43 aggregates in the cytoplasm.[18] The TDP-43-containing cellular inclusions are dependent on the activation of stress-induced kinases such as c-Jun N-terminal kinase (JNK). Results obtained for compound **56** and other copper complexes showed reduced stress-induced kinase activity and prevented TDP-43 aggregation. These copper complexes have previously demonstrated neuroprotective effects in mouse models of neurodegeneration, therefore making them beneficial in the treatment of ALS as they modulate kinase activity and reduce protein aggregation.[19,44]

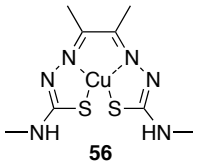


***Figure 17**: Cu^{II}(atsm), an example of a Cu^{II}(btsc) copper complex*

Alternatively a way to reduce TDP-43-containing cytoplasmic inclusions (foreign substances contained within the cell membrane) is to induce autophagy, which can be achieved by using known pharmacological activators such as Tamoxifen (**57**), Carbamazepine (**58**), Spermidine (**59**), or Rapamycin (**60**, Figure 18). Autophagy is a process in which dysfunctional proteins and organelles are removed from the cell and then degraded by lysosomes. These compounds were trialled to see if they could enhance autophagy in disease models with TDP-43 proteinopathies. It was found that clearance of cytoplasmic TDP-43, along with a reduction in caspase activation and cellular death corresponded to an upregulation of these autophagic markers.[45] The use of enhancing autophagy may reduce cellular death and behavioural dysfunction associated with TDP-43 mutations.[19]

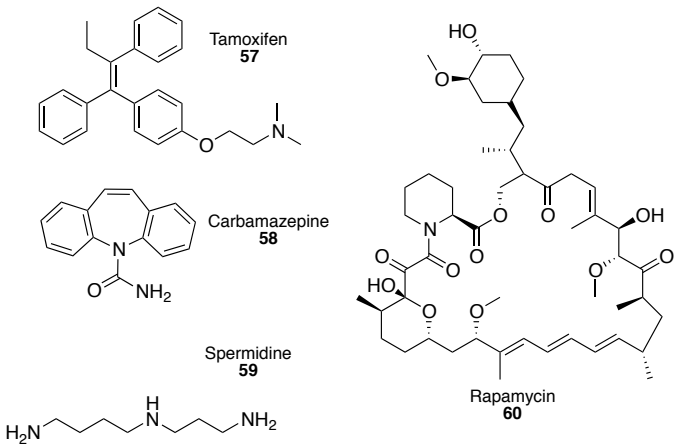


***Figure 18**: Pharmaceutical inducers of autophagy*

(4) TARGETING REDUCTION IN OXIDATIVE STRESS AND INFLAMMATION

Oxidative stress is the result of an imbalance between the production of ROS and cellular antioxidant defence systems, which play a crucial role towards neurodegenerative conditions. Several treatment strategies have focused on minimising the imbalance between the production of ROS and cellular antioxidant defence systems within cellular pathways. One method of reducing oxidative stress in the neurons is the upregulation of signalling through the NF-E2 related factor 2/ antioxidant response element (Nrf2/ARE) pathway, which is responsible for the upregulation of antioxidants and prosurvival genes and reduction of cell apoptosis under cytotoxic conditions. Neymotin *et al*. tested two triterpenoids, 2-cyano-3,12-dioxoolean-1,9-diene-28-oic acid-ethylamide (CDDO-EA **61**) and CDDO-trifluoroethylamide (CDDO-TFEA **62**) for their ability to activate Nrf2/ARE signalling in cell culture and mouse models of ALS (Figure 19). Treatment of NSC-34 cells expressing SOD1 G93A with CDDO-TFEA (**62**) gave elevated levels of Nrf2 and Nrf2 regulated genes. When (**61**) and (**62**) were orally administered to transgenic SOD1 G93A mice, results obtained showed an increase in Nrf2 expression and nuclear localisation. Both of these triterpenoid compounds activated the Nrf2/ARE system, which results in the reduction of oxidative stress in cell cultures and mice models of ALS therefore resulting in a neuroprotective response.[19,46]

Kanno *et al.* used a virtual screening system to discover oxidative-stress reducing agents, which identified *N*-(5-(2-pyridyl)(1,3-thiazol-2-yl))-2-(2,4,6-trimethyl-phenoxy)acetamide (CPN-9 **63**, Figure 19). Initial testing of CPN-9 (**63**) gave highly cytoprotective properties against pharmacologically induced oxidative stress. Further testing of CPN-9 (**63**) in a variety of cell-stress inducers all showed protection against cellular death when induced by oxidative-stress pathways only. Overall, CPN-9 (**63**) showed resistance to oxidative stress through upregulation of the Nrf2/ARE transcriptional pathway and inhibition of cellular death. Treatment of transgenic mice expressing the hDOD1 H46R mutant gene with CPN-9 (**63**)

showed an increase in motor neuron function within the spinal cord and extended survival following disease onset.[19,47]

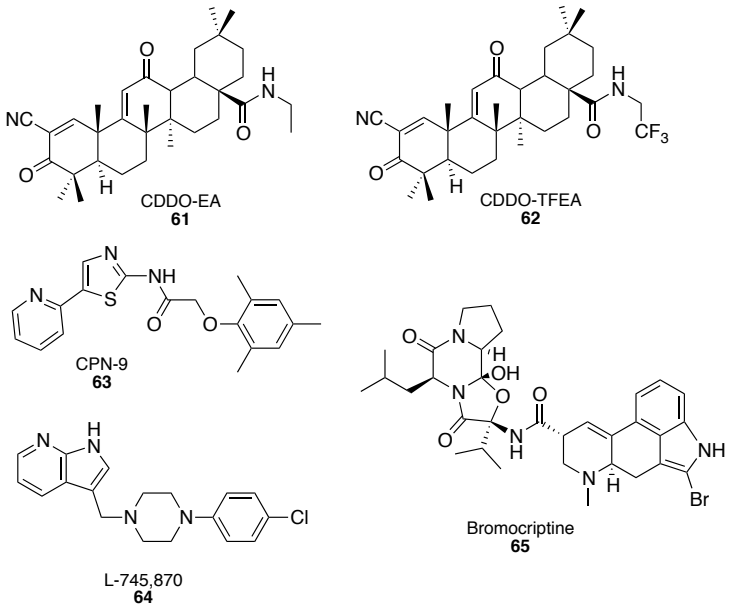


***Figure 19**: Compounds developed to reduce oxidative stress and inflammation*

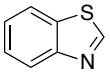
Tanaka *et al.* performed further studies into the reduction of oxidative stress in ALS utilising dopamine D4 receptor antagonist, L-745,870 (**64**, Figure 19) to selectively inhibit oxidative stress induced cell death. This compound was previously determined to upregulate neuronal apoptosis inhibitory protein (NAIP), BIRC1, a cytoprotective protein that improves oxidative stress induced cellular death.[48] Treatment of (**64**) administered within the stomach of SOD1 H46R mice before symptom onset was discovered to delay symptom onset as determined by limb movement, rearing activity and foot clasping behaviours. It was also found that treatment with (**64**) delayed weight loss and motor dysfunction as examined by a balance-beam test.[19,49]

Further work within this group identified that dopamine D2 receptor agonist bromocriptine (**65**), an NAIP upregulating compound, reduced oxidative stress through the upregulation of antioxidant proteins, such as activating transcription factor 3 (ATF3) and heme oxygenase 1(HO-1). The delay in disease progression of compound (**65**) was identified by *in vivo* studies of compound (**65**) administered to SOD1 H46R mice following symptom presentation. These studies indicate that the depletion of oxidative stress pathways through the upregulation of antioxidant genes can reduce disease progression in ALS models.[19,50]

1.3 Riluzole

2-Amino-6-trifluoromethyoxybenzothiazole also known as Riluzole and Rilutek (**1**), is a derivative of benzothiazole **66** (Figure 20). Riluzole is bicyclic and comprises of a benzene ring, fused to a five-membered ring containing a nitrogen and sulfur atom.[1] Benzothiazole

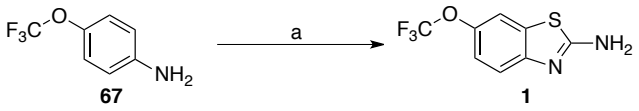
derivatives were first identified in the 1950s as potential muscle relaxants, but were later investigated as anticonvulsant and neuroprotective agents. In 1995 Riluzole was approved by the FDA for the treatment of ALS and, to date, remains to be the only approved drug in suppressing ALS symptoms.[51] Riluzole is taken twice a day with a fixed regimen of 50 mg dosages, prolonging life expectancy by approximately 3 - 6 months.[52,53]



66

Figure 20**: Benzothiazole **66

In the 1960s Yagupol'skii and Gandel'sman reported the first synthetic method of generating Riluzole, which was then patented by Rhône-Poulenc Rorer.[1] The original method and commercial production of Riluzole is done in one-pot by reacting 4-trifluoromethoxyaniline, ammonium thiocyanate and a source of bromine together (Scheme 9).



67 **1**

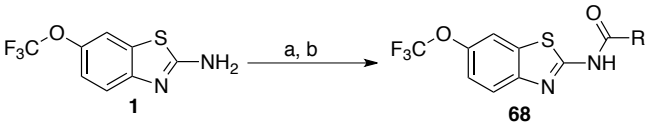
***Scheme 9**: Synthesis of Riluzole via the Hugerschoff Reaction:*
Reagents and conditions (a) 1.0 equiv. NH_4SCN, 1.0 equiv. $PhCH_2NMe_3Br_3$, MeCN, 24 h, 80 % [54]

Most pharmaceutical drugs are designed to have a single molecular targets, however the neuroprotective effects shown by Riluzole are thought to exert their effects through a number of interdependent pathways. The exact mechanism of action in which Riluzole slows disease progression is still unknown but it is understood that Riluzole both inhibits the release of glutamate and also non-competitively inhibits postsynaptic NMDA and AMPA receptors. Potential modes of action include (1) anti-glutamatergic actions (2) Ca^{2+} channel blockade (3) Na^{+} channel blockade and (4) GABAergic mechanisms.[1]

PK studies of Riluzole have demonstrated variable drug exposure in addition to serum concentrations varying greatly for patients with ALS, following oral administration.[19,53] This variation correlates to the concentrations of CYP isoform CYP1A2 found in ALS patients. The enzyme CYP1A2 converts Riluzole to *N*-hydroxyriluzole, *via* oxidative metabolism making the drug inactive.[52] Individual dosing of Riluzole may lead to an increased efficacy in patients who are fast metabolisers and a decrease of side-effects in others who are slow metabolisers. This is not a feasible approach because, if a large variability exists within a group of individuals, it may be due to individual characteristics and variability.[53] Studies have focused around creating Riluzole prodrugs that would exhibit higher stability *in vivo*. Prodrugs are inactive pharmaceutical medications, which when administered are converted to active pharmaceutical medications through a normal metabolic process, such as hydrolysis of an

ester. Prodrugs might be used over drug administration to improve ADME, bioavailability, selectivity and reduce undesirable side effects.

McDonnell *et al.* identified and screened 23 Riluzole prodrugs against glutamate toxicity in ALS and other disorder models.[19] Preparation of Riluzole prodrugs candidates are achieved by converting the endocyclic amine to single α-amino amide, carbamate, succinamide, and amide linkage from γ-aminobutyric acids (Scheme 10).[55]

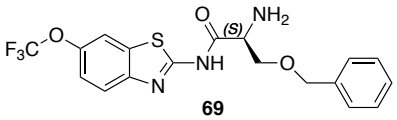


1 **68**

R = $C(NH_2)$((S)-Bu), $C(NH_2)$((R)-Bn), OMe, OEt

Scheme 10*: Preparation of Riluzole prodrugs. Reagents and conditions: (a) 1.0 equiv. RCO_2H, 1.5 equiv. EDCI, DCM, RT, 4 days, 34 - 83 %; or 1.8 equiv. ROCOCl, 1.5 equiv. Et_3N, DCM, RT, 24 h, 13 - 77 %; or 1.0 equiv. RCO_2COR^1, DMF, RT, 24 h then 1.0 equiv. R^1R^2NH, 1.0 equiv. HATU, 1.0 equiv. Et_3N, DMF, RT, 24 h, 9 - 16 %; (b) 5.0 equiv. TFA, DCM, 2h if deprotection is required* [55]

These Riluzole prodrugs would be cleaved in the plasma by esterase and amidase enzyme to regenerate Riluzole. The stability of these analogues were tested in simulated gastric fluid, intestinal fluid and liver microsomes to determine whether the prodrugs would enter the plasma intact. Further, the cleavage of the Riluzole prodrug to Riluzole was analysed in the plasma. From this rigorous testing one compound was shown to be a Riluzole prodrug candidate for *in vivo* testing, the *O*-bezylserine derivative **69** (Figure 21). This prodrug showed good stability in *in vitro* intestinal and microsomal assays and was also able to withstand metabolism by CYP1A2. Development of this prodrug could help Riluzole to be a more effective treatment against ALS.[19,55]



69

Figure 21*: O-benzylserine Riluzole prodrug* ***69***

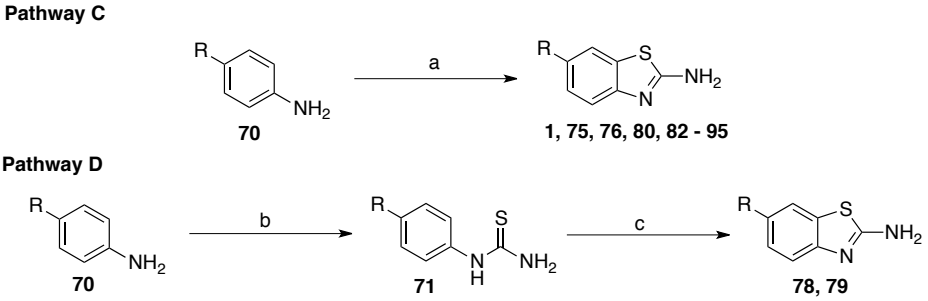
Both the 6- and 3-position of the core Riluzole structure offer attachment points for the incorporation of diversity generating substructures. Jimonet *et al.* reported the synthesis of several analogues of Riluzole (**1**) showing the crucial importance of the 6-substitutent on benzothiazoles and the preparation of 3-substituted derivatives of Riluzole and their *in vivo* 'antiglutamate' activity. For all compounds synthesised *in vivo* antiglutamate activity was recorded as the median effective dosage (ED_{50}), which was calculated by injecting 6- and 3-substituted Riluzole derivatives (Table 1 and 2) into male rats that had been injected with 12.5 μM mol/kg solution of L-glutamic acid in saline. The ED_{50} values obtained for each compound

was evaluated together with reference drugs for their ability to protect against seizures induced by intracerebroventricular administration of glutamic acid in rats.[56]

(1) 6-Substituted Riluzole Derivatives

A number of 2-benzothiazolamines bearing various substituents in the 6-position were synthesised *via* a number of routes (Scheme 11), as one general method was not feasible. All 6-substituted Riluzole analogues generated are listed in Table 1.

Pathway C (Scheme 11) is one of two versatile routes to prepare 6-substituted Riluzoles analogues. This route generated analogues **1, 75, 76, 80, 82 - 95** (Table 1) *via* a one-pot reaction between an appropriate aniline and thiocyanogen, which is generated from bromine and an alkaline thiocyanate in an acetic acid medium. Pathway D (Scheme 11) generated analogues **78** and **79** (Table 1), which were achieved *via* an alternative route. This route went *via* intermediate phenythiourea. Phenythiourea was generated by reacting an appropriate aniline and thiocyanate together and on addition of bromine resulted in ring cyclisation, which generated 6-substituted Riluzole analogues. Pathways outlined in Scheme 11 did not generate Riluzole analogues **74** and **81** reported in Table 1. These two analogues were generated *via* further reactions to previously generated Riluzole analogues. Analogue **74** was generated by the reduction of analogue **77**, which is commercially available and analogue **81** was generated by reacting commercially available 6-bromo Riluzole analogue with *n*-butyllithium and chlorotrimethylsilane, which was then followed by hydrolysis.



***Scheme 11**: Reagents and conditions: **Pathway C** (a) 4.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 21 h, 11 - 71 %;*

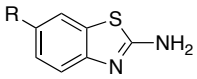
***Pathway D** (b) 1.1 equiv. NH_4SCN, 0.006 equiv. $NaHSO_3$, 2.0 equiv. 20 % aq HCl, 90 °C, 14 h; (c) 2.0 equiv. Br_2, DCM, reflux, 2.5 h, RT, 18 h, 22 - 41 % (over two steps)*

All 6-substituted-2-benzothiazolamines analogues **73** - **82** (Table 1) displayed very weak activity compared to that of Riluzole.[56] These results suggest that electronic factors are relatively unimportant, as substitutions with electron-donating and electron-withdrawing groups at the 6-position did not increase antiglutamate activity when compared to analogue **72**. Both chloro and trifluoromethoxy groups are known to deactivate the aromatic ring by an inductive electron withdrawal and also donate electrons by resonance. When the

trifluoromethoxy substituent of analogue **1** is replaced with a chloro substituent generating analogue **73** the activity is drastically reduced.[57] This difference in activity has been seen in other medicinal drugs where a chloro substituent is replaced by such a 'pseudohalogen' resulting in an active compound; for example when the 7-chloro substituent in Diazepam is replaced with a trifluoromethoxy group.[58] Therefore, Riluzoles *in vivo* activity depends essentially on non-electronic factors such as lipophilicity, which is the ability for a compound to dissolve in fats, oils, lipids and non-polar solvents.

All active 6-substituted Riluzole analogues fell into two sets: (a) analogues bearing large alkyl substituents or (b) analogues bearing a polyfluoroalkyl or a polyfluoroalkoxy substituents similar to that of Riluzole. A variety of alkyl chains varying in length and size were investigated to determine an optimum chain length related to antiglutamate activity. For linear substituents it was found that the most potent analogue was **86** and shortening or lengthening of this chain decreased the potency of Riluzole analogues. Altering the butyl chain with a branched chain such as **81** and **87** did not affect activity, but when the branched chain was shortened or lengthened this led to compounds with weaker activity **85** and **89**. Analogues **93 - 95** contained a polyfluoroalkyl or a polyfluoroalkoxy substituents and showed strong antiglutamate activity, but when one or more of the fluorine groups were replaced with hydrogen, the activity decreased. These results are consistent with lipophilicity of the substituent at the 6-position being a major contributing factor for antiglutamate activity.

Table 1: Antiglutamate activity of 6-substituted-2-benzothiazolamines



Compound	R	Reaction Pathway	ED_{50}, mg/kg i.p.
1	OCF_3	C	3.2
72[a]	H		>10
73[a]	Cl		> 10
74[b]	NH_2		> 10
75	CN	C	> 10
76	CO_2Et	C	> 10
77[a]	NO_2		> 10
78	SO_2Me	D	> 10
79	OMe	D	> 10
80	O-*t*-Bu	C	> 10
81[b]	$SiMe_3$		5
82	Me	C	> 10
83	Et	C	7

84	*n*-Pr	C	6
85	*i*-Pr	C	> 10
86	*n*-Bu	C	4
87	*t*-Bu	C	4
88	*n*-Pen	C	7.5
89	*t*-Pen	C	> 10
90	*n*-Hex	C	7
91	*n*-Hep	C	> 10
92	$COCF_3$	C	> 10
93	OC_2F_5	C	2.5
94	C_2F_5	C	2.5
95	CF_3	C	2.5

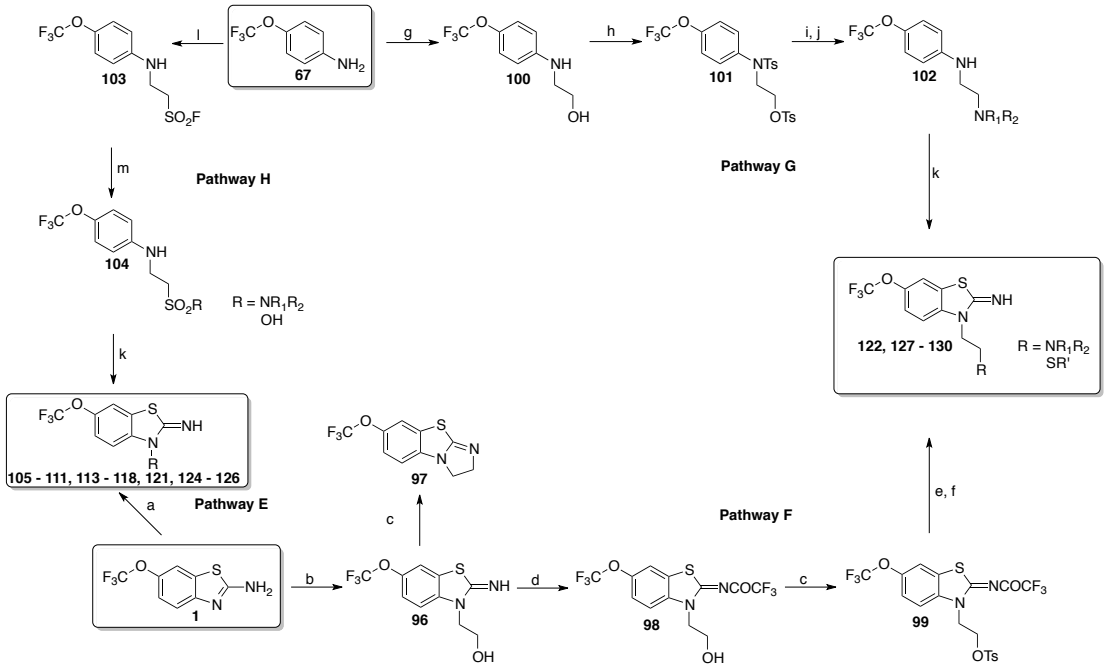
[a] commercially available product from Aldrich; [b] obtained *via* further reactions to existing Riluzole analogues

(2) 3-Substituted Riluzole Derivatives

Alkylation of 6-trifluoromethoxy-2-benzothiazolamine **1** occurred exclusively at the endocyclic nitrogen.[59] Jimonet *et al.* published four different reaction pathways in which functionalisation was introduced to the endocyclic nitrogen of Riluzole (Scheme 12) generating a library of 3-substituted Riluzole analogues (Table 2).[56,60]

Pathway E (Scheme 12) generates 3-substituted Riluzole derivatives *via* simple alkylation of Riluzole with commercially available alkylating reagents, in either an alcohol, methylethyl ketone, or dimethylformamide solution under reflux for several hours. The simplicity of this reaction and the availability of many alkylating agents allowed rapid synthesis of a large number of analogues **105** - **111**, **113**, **116** - **118**, **121** and **124** - **126** (Table 2). Pathway F (Scheme 12) generates thiolate and amine functionalised 3-substituted Riluzole derivatives, such as **122** and **130** (Table 2), which cannot be generated *via* simple alkylation reaction of Riluzole. Riluzole is firstly alkylated with hydroxylethyl bromide. This is then followed by protection of the imine with a suitable electron-withdrawing group such as trifluoroacetyl. The hydroxyethyl chain is then activated with *p*-toluenesulfonyl chloride and reacted with either a thiolate or amine, generating either a thiolate or amine functionalised 3-substituted Riluzole analogue via an S_N2 displacement reaction. Protection of the imine is essential otherwise intramolecular nucleophilic attack occurs between the nucleophilic imino and tosylate group forming an unwanted tricyclic derivative **97**. Pathway G (Scheme 12) introduces functionality at the *N*-3 position before cyclisation, therefore reducing the generation of unwanted cyclised side-products experienced in other pathways. 4-Trifluoromethoxyaniline and 2-bromoethanol are reacted together generating an amino alcohol compound. This amino alcohol compound

is then reacted with two equivalents of *p*-toluenesulfonyl chloride giving the ditosylate salt **101**. Nucleophilic displacement of **101** with an appropriate amine, followed by hydrolysis and cyclisation gives analogues **127** - **130** (Table 2). Pathway H (Scheme 12) also adds functionality before cyclisation. Firstly ethenesulfonyl fluoride is reacted with 4-trifluoromethoxyaniline *via* a Michael addition to give **103**. This is then converted to either a sulfonamide or sulfonic acid compound, such as **104**, which is achieved *via* an S_N2 displacement between the sulfonyl fluoride compound and an appropriate amine or sulfonic acid. The final step in the pathway is ring-cyclisation yielding either a sulfonamide or sulfonic acid functionalised 3-substituted Riluzole analogue, such as analogues **114** and **115**.

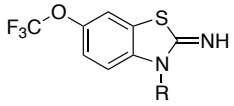


***Scheme 12**: Reagents and conditions: (a) 1.2 equiv. RX, EtOH, MEK or DMF, reflux, 24 h; (b) 1.0 eq. $Br(CH_2)_2OH$, EtOH, reflux, 1.5 h; (c) 1.1 equiv. TsCl, excess Py. 0 °C - RT, 90 % (over two steps); (d) 1.2 equiv. CF_3CO_2Et, 1.1 equiv. Et_3N, EtOH, RT, 18 h, 85 %; (e) 1.1 equiv. R^1R^2NH, 1.0 equiv. $NaHCO_3$, DMF, 80 °C, 18 h or 1.1 equiv, R^1SNa, DMF, 80 °C, 18 h; (f) aq K_2CO_3, MeOH, RT, 5 h; (g) 1.0 equiv. $Br(CH_2)_2OH$, 160 °C, 1.5 h, 49 %; (h) 2.0 equiv. TsCl, 2.0 equiv. Et_3N, DCM, 0 °C - RT, 1.5 h, 55 %; (i) 2.1 equiv. R^1R^2NH, 2.1 equiv. $NaHCO_3$, DMF, 80 °C, 18 h (j) HCl, aq AcOH, reflux; (k) 1.0 equiv. Br_2, 4.0 equiv. KSCN, AcOH, RT, 18 h; (l) 1.0 equiv. CH_2CHSO_2F, DMF, RT, 2 h, 75 %; (m) 8.0 equiv. R^1R^2NH, acetone, reflux, 1 h or 8.0 equiv. AcOH, reflux, 1 h.*

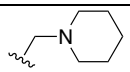
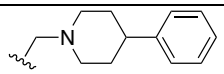
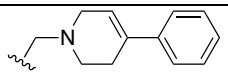
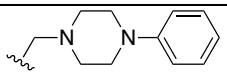
Riluzole analogues generated with *N*-3 functionality being small aliphatic chains, such as methyl **105**, ethyl **106** and propyl **107** showed good antiglutamate activity, but the small variation in chain length did not drastically affect antiglutamate activity overall. When bulky alkyl and aromatic substituents were introduced, for example derivatives **108** and **109**, potency decreased dramatically. *N*-3 Riluzole derivatives containing esters **111**, amides **113**, sulfonamides **114**, carboxylic acid **112**, sulfonic acid **115**, and alcohol **116**, functionality did not show improved potency compared to Riluzole.

Antiglutamate activity was shown to significantly increase when nitrogen or sulfur functionality was introduced to the *N*-3 position of Riluzole. For the sulfur substituents the most potent derivative was methylthioethyl **118**, which gave an ED_{50} of 1 mg/kg i.p. Two other sulfur substituents similar to **118** were sulfoxide **119** and sulfone **120** all of which suggest that the active molecule *in vivo* is in fact a common metabolite.[56] When the chain length to the thio group was increased or decreased or altered from the methythio group to thiol or something bulkier, such as a phenyl ring (**117**, **121** and **122**) this resulted in a loss of anticonvulsant activity. From the *N*-3 amino Riluzole substituents generated it was found that dimethylaminoethyl **124** and cyclic **125** substituted benzothiazoline analogues demonstrated good anticonvulsant potency over monoethylated substituents **123**. Increasing the chain length from a two-carbon chain to a three-carbon chain for dimethylaminoethyl substituted benzothiazoline **126** or changing one of the methyl groups for an aromatic substituent **127** reduced potency. The last three entries of Table 2, 4-phenylpiperidinyl **128**, 4-phenyl-1,2,3,6-tetrahydropyridyl **129**, and 4-phenyl-piperazinyl **130** all show unexpectedly high levels of antiglutamate activity.

Table 2: Antiglutamate activity of 3-substituted-2-imino-benzothiazolamines



Compound	R	Reaction pathway	ED_{50}, mg/kg i.p.
1[a]	H		3.2
105	Me	E	5.0
106	Et	E	5.0
107	n-Pr	E	4.5
108	n-Bu	E	10.0
109	CH_2Ph	E	> 10
110	$(CH_2)_2Ph$	E	> 10
111	CH_2CO_2Me	E	> 10
112[b]	CH_2CO_2H		> 10
113	CH_2CONH_2	E	7.0
114	$(CH_2)_2SO_2NH_2$	H	6.0
115	$(CH_2)_2SO_3H$	H	> 10
116	$(CH_2)_2OH$	E	8.0
117	CH_2SMe	E	3.0
118	$(CH_2)_2SMe$	E	1.0

119[c]	$(CH_2)_2SOMe$		1.1
120[c]	$(CH_2)_2SO_2Me$		1.8
121	$(CH_2)_2SPh$	E	4.5
122	$(CH_2)_3SMe$	F	4.2
123	$(CH_2)_2NHMe$		> 10
124	$(CH_2)_2NMe_2$	E	2.3
125		E	2.0
126	$(CH_2)_3NMe_2$	E	9.0
127	$(CH_2)_2N(Me)CH_2Ph$	G	7.0
128		G	3.0
129		G	2.2
130		F	3.5

[a] commercially available product from Aldrich; [b] could not be obtained directly from Riluzole thus compound **112** was prepared in two-steps after acidic hydrolysis of the benzothiazoline precursor **111**; [c] sulfoxide **119** and sulfone **120** were prepared from their respective thioethers *via* oxidation with *m*CPBA.

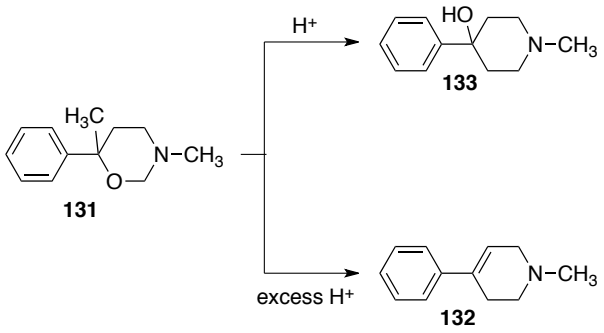
In conclusion a library of 6-substituted-2-benzothiazolamines and 3-substituted-2-imino-benzothiazolines were successfully evaluated for their antiglutamate properties against seizures induced by intracerebroventricular administration of glutamic acid in rats. For the 6-substituted-2-benzothiazolamine library two sets of products were found to be active, compounds bearing a large but not too large alkyl substituent **86** and compounds bearing a polyfluoroalkyl or a polyfluoroalkoxy substituent **93 - 95**. Active analogues from this library suggest that 6-substituted-2-benzothiazolamine possess excitatory amino acid antagonist activity, which could result in some of the analogues synthesised from this library exhibiting interesting anticonvulsive and neuroprotective properties similar to Riluzole. For the 3-substituted-2-imino-benzothiazolines it was shown that a variety of analogues could be synthesised *via* a number of pathways (Scheme 12) and a number of these analogues showed similar or elevated activity compared to Riluzole. Benzothiazoline analogues exhibiting strong antiglutamate activity were found to bear heteroatoms in the β-position of the alkyl substituent, which included the cycloalkylamino- **125**, ethylamino- **124**, and uncharged or oxidised alkylthioethyl-benzothiazoline analogues **118**.

Other methods investigated towards enhancing Riluzoles antiglutamate activity included a combination therapy with Neramexane in which both active ingredients are administered as a single pharmaceutical composition. Neramexane is categorised as either an NMDA

antagonist or an NMDA receptor antagonist, therefore containing neuroprotective properties which inhibit the effects of excessive glutamate at the NMDA receptors to nerve cells. Initial studies were carried out on mice with an overexpression of the SOD1 gene mutation present in ALS patients. Initial results from this study showed that a combination therapy containing both Riluzole and Neramexane retarded disease progression. This study was carried forward and tested in 150 patients suffering from ALS. Results obtained from this study concluded that a combination therapy containing both Riluzole and Neramexane reduced disease progression to a greater extent then Riluzole.[61]

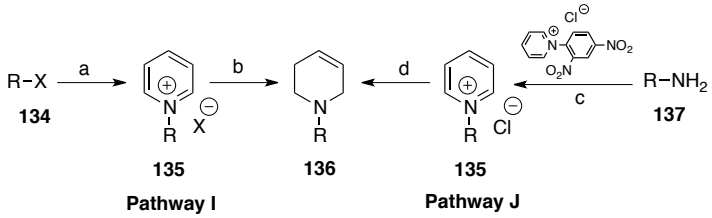
1.4 1,2,3,6-Tetrahydropyridine

One of the first reported synthesis of a tetrahydropyridine (THPy) was of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP **132**), which was discovered in the late 1970s as a trace impurity in a synthetic fentanyl derivative known as meperidine.[62] It is a potent neurotoxin in the domaminergic system, which produced Parkinson-like symptoms amongst other side effects.[63] MPTP is generated through the rearrangement of 3,6-dimethyl-6-phenyl-tetrahydro-1,3-oxazine (**131**) using either sulphuric or hydrochloric acid (Scheme 13). Under relatively mild conditions 1-methyl-4-phenyl-4-piperidinol **133** was also obtained as a minor product.[64,65]



***Scheme 13**: Synthesis of MPTP*

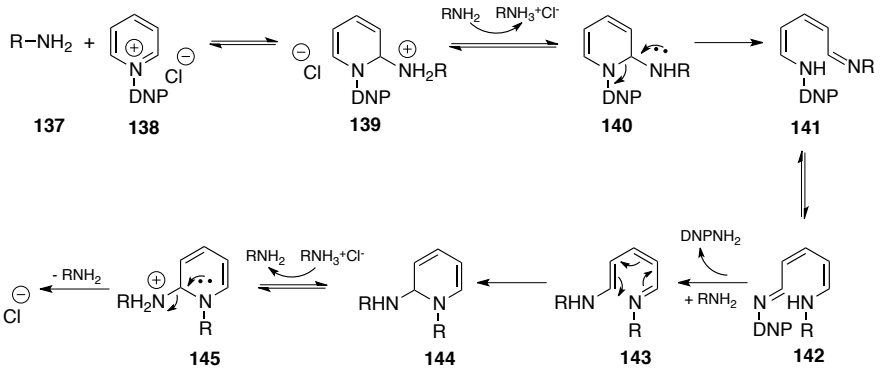
The reduction of *N*-alkylpyridiniums with sodium borohydride is a widely used method for the synthesis of pharmacologically interesting THPys **136**.[64,66] Unfunctionalised *N*-alkylpyridiniums **135** can be synthesised either *via* S_N2 nucleophilic displacement between pyridine and an alkyl or aryl halide or *via* the Zincke reaction (Scheme 14).[67,68]



***Scheme 14**: Synthesis of THPy Conditions and reagents: **Pathway I** (a) excess Py. reflux, 24 h; (b) 2.8 equiv $NaBH_4$, MeOH, RT, 18 h;*

***Pathway J** (c) MeOH, RT - reflux, 24 h; (d) 2.8 equiv. $NaBH_4$, MeOH, RT, 18 h*

The Zincke reaction is a versatile method that allows the synthesis of pyridinium salts that cannot be made by direct *N*-functionalisation of pyridines.[69] Zincke reported the preparation of pyridinium salts and their reactions with primary amines in a series of papers between 1903 and 1905.[68,69] The mechanism by which the Zincke reaction occurs is, that a primary amine reacts with a highly electrophilic *N*-2,4-dinitrophenylpyridinium salt **138**, resulting in the ring-opening of **138** to afford dianil salts **141** and **142**. Reacting dianil salt **142** with another equivalent of primary amine generates **143** alongside the release of 2,4-dinitroaniline. Cyclisation of **139** results in the generation of pyridinium salt **135** (Scheme 15).[70]



***Scheme 15**: Mechanism for the Zincke reaction*

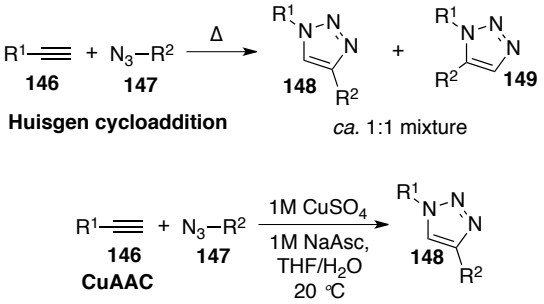
The THPy moiety is present in a variety of pharmaceutical compounds, including dopamine autoreceptor agonists, GABA uptake inhibitors as anticonvulsant and sedatives, muscarinic agonists in the treatment of AD and non-steroidal anti-inflammatory drugs (NSAIDs).[64] Dopamine autoreceptor agonists, GABA uptake inhibitors and Muscarinic agonists which contain THPy moiety were reported to maintain healthy neurotransmission, which had previously been damaged by a dysfunction within the nervous system through excessive neurotransmission, which can lead to a variety of neurodegenerative diseases such as, AD, PD and ALS. NSAIDs containing THPy functionality report the reduction of inflammation from a biological response by protecting the body from infection and reducing tissue damage through injury. As stated early the exact mechanism in which ALS progresses in unknown, but through a number of proposed theories it is thought to be a combination of pathways including autoimmune, excitotoxicity, misfolding of the SOD1 gene and accumulation of TDP-43. Therefore tetrahydropyridine moiety incorporated with Riluzole could potentially enhance antiglutamate activity as both have been reported to reduce inflammation and regulate neurotransmission to a healthy level.

1.5 1,4-Substituted-1,2,3-Triazole Moiety in a Variety of Drug Candidates

The term 'click chemistry' was first conceived by Sharpless and co-workers in 2001, who laid out a set of high benchmarks which needed to be met if a reaction was to be termed a 'click' reaction.[71] These benchmarks were; 'the reaction must be modular, wide in scope, give very high yields, and be stereospecific (but not necessarily enantioselective). The required

process characteristics include simple reaction conditions, readily available starting materials and reagents, the use of no solvents or a solvent that is benign (such as water) or easily removed, and simple product isolation.[72]

The Huisgen cycloaddition, also known, as the 1,3-dipolar cycloaddition, is a reaction between an azide and a terminal alkyne, which generates 1,4- and 1,5-substituted-1,2,3-triazoles (Scheme 16). This reaction was first described in the 1960s.[73] The Huisgen cycloaddition reaction does not quite fit the 'click' reaction criteria as for the past 40 years this reaction has suffered from a lack of selectively yielding a mixture of 1,4- and 1,5-regioisomers due to activation energies of the two triazoles being very similar in energy 25.7 kcal/mol and 26.0 kcal/mol.[74] Furthermore, this transformation required heating and long reaction times to achieve completion, and the resulting regioisomers obtained require the use of potentially laborious separation techniques.[73] In 2002 Medal, then Sharpless, discovered that Cu^{I} salts could be used to catalyse the Huisgen cycloaddition, which allowed the reaction to be regioselective and also eliminated the harsh reaction conditions previously reported.[75] The reaction could also now be performed at room temperature (RT) with very short reaction times (Scheme 16). The copper^{I} catalysed azide-alkyne cycloaddition (CuAAC) is a close fit to the definition of 'click chemistry'.[75]

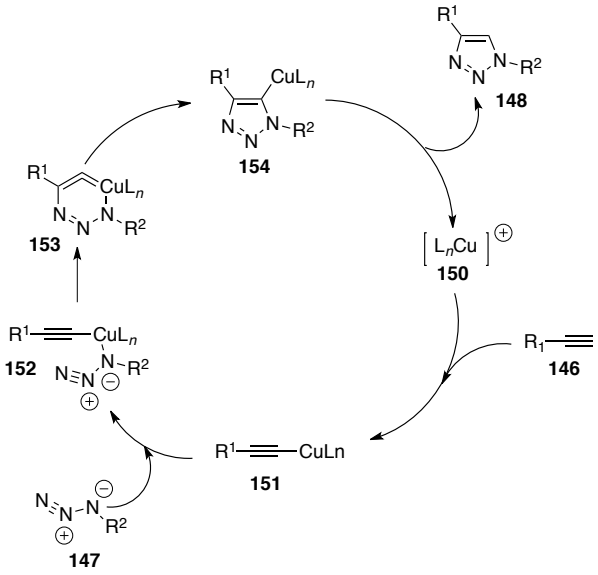


***Scheme 16**: The 1,3-dipolar cycloaddition between azides and alkynes*

The CuAAC reaction to generate 1,4-substituted-1,2,3-triazoles tolerates most organic functional groups and shows a wide scope with respect to alkyne and azide reactants. The reaction proceeds in a variety of solvents, tolerates a wide range of pH values, and performs well over a broad temperature range.[76] The use of Cu^{I} in the 1,3-dipolar cycloaddition reaction lowers the activation barrier of the uncatalysed process by as much as 11 kcal/mol, which is sufficient to explain the incredible rate enhancement observed under Cu^{I} catalysis.[76]

The CuAAC reaction proceeds *via* a stepwise sequence on the basis of calculation and kinetic studies.[76] Although thermal cycloaddition of azides and alkynes occurs through a concerted [2+3] cycloaddition mechanism, DFT calculations on monomeric copper acetylide complexes indicate that the concerted mechanism is strongly disfavoured. Overall a concerted mechanism is disfavoured as the calculated activation barrier for the cycloaddition

between an appropriate azide and copper-acetylene π complex is similar to the uncatalysed process, 25.7 kcal/mol.[74,76] The stepwise catalytic cycle compared to the uncatalysed process lowers the activation energy by as much as 11 kcal/mol.[76] The catalytic sequence begins with the coordination of the alkyne to the Cu^{I} species, forming a copper acetylide intermediate **151**. Cu^{I} is generated from an *in situ* reduction of Cu^{II}, such as $CuSO_4.5H_2O$ with sodium ascorbate or ascorbic acid. Introduction of the azide to this copper acetylide intermediate **151** generates an acetylide-azide complex **152**. The terminal nitrogen on the azide of the acetylide-azide complex **152** attacks the *C*-2 carbon of acetylide forming a six-membered Cu^{III} metallocycle **153**. This is followed by ring contraction, which results in the generation of a copper triazolide intermediate **154** which on elimination yields the 1,4-substituted-1,2,3-triazole **148** upon protonation (Scheme 17).[76] Formation of 1,4-substtuted-1,2,3-triazoles is confirmed by nuclear overhauser effect (NOE) data as only the 1,4-substituted-1,2,3-triazole gives NOE interactions to both substituents for the triazole proton and no NOE between the substituents. NOE is the transfer of nuclear spin polarization from one nuclear spin population to another *via* cross-relaxation



***Scheme 17**: Proposed catalytic cycle for the Cu^{I}-catalysed ligation*

There are a few 1,2,3-triazole containing molecules on the market or in the last stages of clinical trials, which include the anti-cancer compound carboxyamidotriazole (CAI **155**), the nucleoside derivative non-nucleoside reverse transcriptase inhibitor *tert*-butyldimethylsilylspiroaminooxathioledioxide (TSAO **156**), β-lactam antibiotic tazobactum (**157**) and the cephalosporine cefatrizine (**158**, Figure 22).[77] Triazole moiety has been incorporated into a wide variety of therapeutically interesting drug candidates to date, which includes anti-inflammatory agents, CNS stimulants, sedatives, anti-anxiety and antimicrobial agents as well as showing anti-fungal activity.[78] Compounds containing 1,2,3-triazole moiety have also shown a wide range of pharmacological uses such as anti-malarial, analgesic, anti-

inflammatory, anti-convulsant, anti-neoplastic, anti-malarial, anti-viral, anti-proliferative and anti-cancer activities.[78]

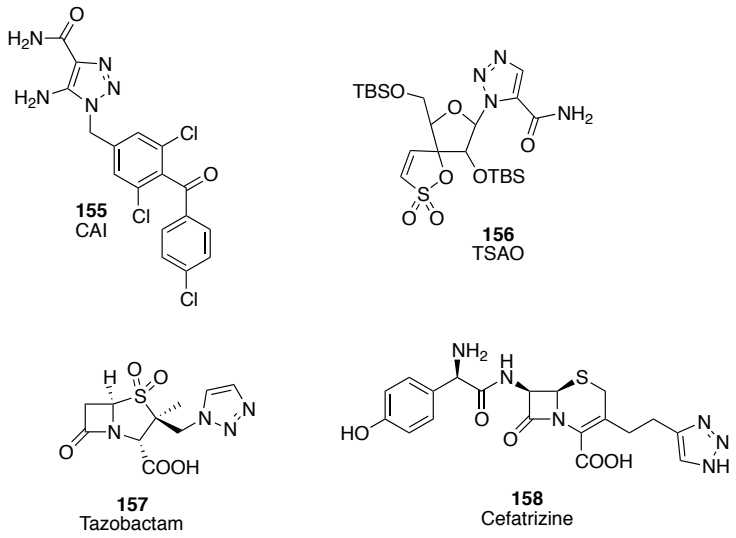
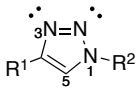


***Figure 22**: Potential pharmaceuticals based on 1,2,3-triazoles*

1,2,3-Triazole functionality is an attractive connectivity unit in drug discovery because of its stability against metabolic degradation and capable hydrogen bond acceptors from the N(2) and N(3) of the triazole ring (Figure 23).[79] Hydrogen bonding is a favourable property in relation to the binding of biomolecular targets and can also improve the overall solubility of the pharmaceutically active compound.[77]



148

***Figure 23**: 1,4-substituted-1,2,3-triazole*

In conclusion, click chemistry has had a dramatic and diverse impact on drug discovery and development in generating novel 1,4-substituted-1,2,3-triazole, which can be achieved *via* combinational chemistry. Triazole moiety can be found in a range of pharmaceutically active compounds including CNS treatments.

CHAPTER 2: PROJECT

To date Riluzole (**1**) is the only approved neuroprotective drug on the market, which prolongs the life expectancy of ALS patients *via* an unknown mechanism. While Riluzole is the only neuroprotective compound against ALS it has relatively low efficacy. This low efficacy means better therapies need to be investigated, which this thesis will do by modifying the chemical structure of Riluzole in the aim of producing a compound that has beneficial properties of Riluzole, but is predicted to be more effective in people living with ALS. This will be achieved by looking into designing two novel synthetic routes incorporating tetrahydropyridine **159** and 1,4-substituted-1,2,3-triazole **160** moiety at the *N*-3 position of Riluzole (Figure 24). Once simple synthetic methods have been developed for these two *N*-3 Riluzole libraries all pure *N*-3 Riluzole derivatives generated will be tested at Bradford University for their antiglutamate activity and compared to Riluzole.

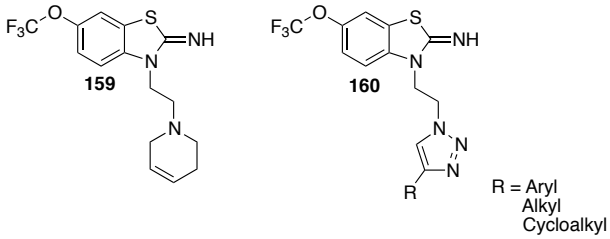


***Figure 24**: N-3 Riluzole Derivatives containing tetrahydropyridine and 1,4-substituted-1,2,3-triazole moiety*

CHAPTER 3: RESULTS AND DISCUSSION

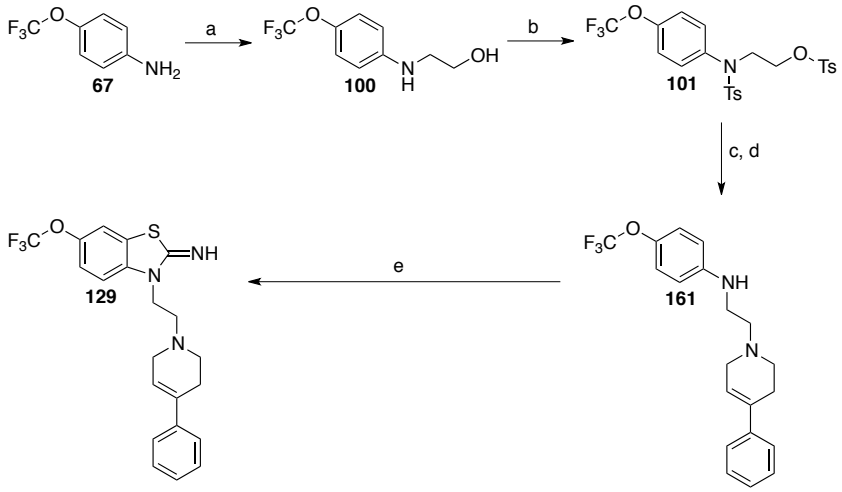
All *N*-3 Riluzole compounds synthesised were synthesised in accordance with Lipinski's rule of five, which are as follows; (1) the molecule being synthesised does not exceed a molecular weight of 500 g/mol^{-1}, (2) there are no more than five hydrogen bond donor groups, (3) there are no more than ten hydrogen bond acceptors, and (4) there is a calculated log P value of less than +5 (log P is a measure of drug's lipophilicity).[80] Pure *N*-3 Riluzole compounds obtained were then biological tested for their antiglutamate activity compared to Riluzole by Victoria Pugh who is carrying out a PhD at the University of Bradford, 'Evaluation and Mechanism of Novel Neuroprotective Compounds for the Treatment of Motor Neurone Disease'.

3.1 Tetrahydropyridine Synthesis

The addition of tetrahydropyridine functionality to the *N*-3 position of Riluzole as reported by Jimonet and co-workers compound **129** has shown an increase of antiglutamate activity compared to Riluzole by approximately an EC_{50} of 1.0 mg/kg. Therefore synthesising a library of *N*-3 Riluzole analogues containing tetrahydropyridine moiety could result in an increase in antiglutamate activity reducing motor neuron damage, which could potentially lead to an increase in life expectancy for ALS patients.

3.1.1 Synthesis of Tetrahydropyridines without Functionality on the Tetrahydropyridine Ring

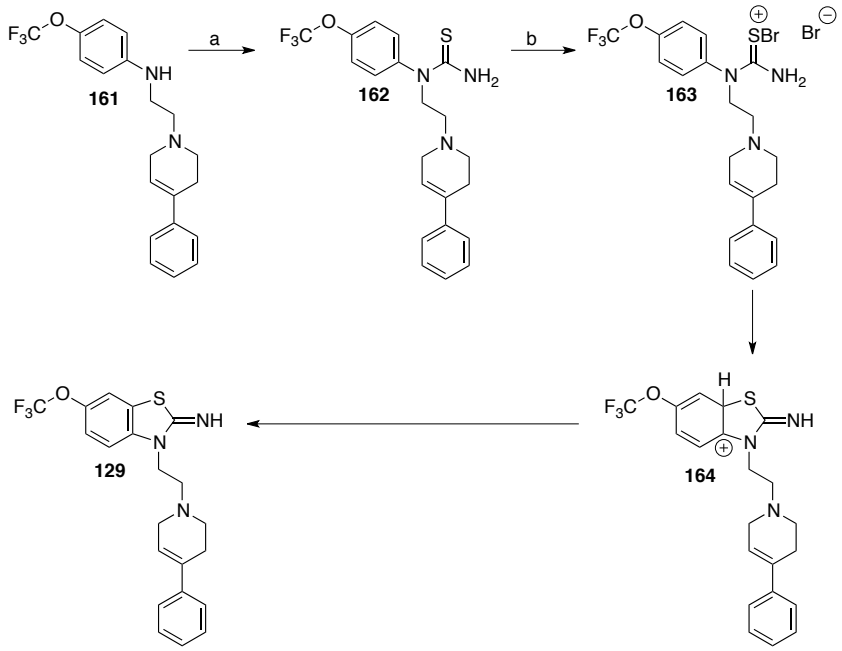
In 1999 Jimonet and co-workers reported the synthesis of a number of *N*-3 substituted Riluzole compounds, including 2-imino-3-[2-(4-phenyl-1,2,3,6-tetrahydro-1-pyridyl)-ethyl]-6-trifluoromethoxy-benzothiazoline **129** with higher antiglutamate activity than Riluzole (Scheme 18).[56] Scheme 18 shows a direct route to generating *N*-3 Riluzole analogues with tetrahydropyridine moiety, which can be further adopted to generating 3-(2-(4,5-dihydropyridin-1-yl)ethyl)-6-trifluoromethoxy)benzothiazole-2-imine **159**, which is the starting point towards generating a library of *N*-3 Riluzole analogues with the aim of enhancing Riluzoles antiglutamate activity.



***Scheme 18**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)_2OH$, 160 °C, 1.5 h, 49 %; (b) 2.0 equiv. TsCl, 2.0 equiv. Et_3N, DCM, 0 °C, 1.5 h, 55 %; (c) 2.1 equiv. 4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride, 2.1 equiv. $NaHCO_3$, DMF, 80 °C, 18 h; (d) HCl, aq. AcOH, reflux, 3 h; (e) 1.0 equiv. Br_2, 4.0 equiv. KSCN, AcOH, RT, 18 h*[56]

Compound **129** was synthesised in four-steps starting from 4-trifluoromethoxyanilne **67**. Reacting 4-trifluoromethoxyaniline with 2-bromoethanol generated amino alcohol **100** *via* an S_N2 reaction. Compound **100** was further reacted with two equivalents of *p*-toluenesulfonyl chloride to give the ditosylated amino alcohol **101**. Reacting compound **101** with 4-phenyl-1,2,3,6-tetrahydropyridine resulted in the displacement of $^-$OTs, which followed by acidic desulfonylation gave compound **161**. The final step in the generation of compound **129** was achieved by reacting compound **161** with KSCN and Br_2.

Cyclisation of compound **161** was achieved by firstly reacting a secondary amine with KSCN to form the thiourea intermediate **162**, which then in the presence of Br_2 underwent an electrophilic addition at sulfur to afford **163** as an intermediate. This intermediate is then attacked by the π system of the aromatic ring to give **164**, which is followed by rapid re-aromatisation to form the benzothiazole ring (Scheme 19).[81]



***Scheme 19**: Proposed bromine mediated cyclisation to afford the benzothiazole core. Reagents and conditions: (a) 4.0 equiv. KSCN, AcOH, RT, 14 h; (b) 1.0 equiv. Br_2, AcOH, RT, 18 h*

In generating compound **159** the first two-steps of Jimonet and co-workers pathway (Scheme 18) were repeatable, but nucleophilic displacement between ⁻OTs and pyridine to generate the pyridinium intermediate was unsuccessful with only starting material being recovered from the reaction between compound **101** and pyridine, the nucleophile. Altering the ⁻OTs for an iodide leaving group *via* the Finklestein reaction generated compound **165** in high yield (Scheme 21).

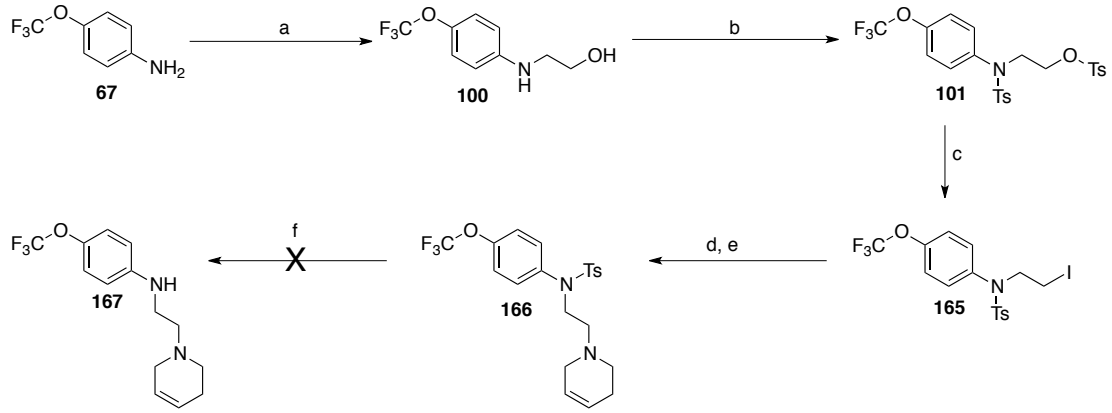
The first-halogen exchange was reported in the mid 1800s by Perkin.[82] However, the process became known as the Finklestein reaction after a number of systematic studies on the reaction were conducted by Finklestein several decades later, in 1910.[82] The reaction proceeds by S_N2 substitution of the leaving group, typically bromide or chloride, with an iodide (Scheme 20). The reaction exploits the differing solubilities of sodium halide salts in acetone. Sodium chloride and sodium bromide are not soluble in acetone, so therefore precipitate out of the reaction mixture, thus driving the reaction to completion.

X = I, R = 1º and 2º alkyl, allyl, benzyl and X' = Cl, Br, OMs, OTs

***Scheme 20**: The mechanism of the Finklestein reaction*

The pyridinium intermediate was then reduced with sodium borohydride to generate tetrahydropyridine intermediate **166** *via* a double iminium reduction. Desulfonylation of the secondary amine with strong acid to yield **167** was unsuccessful with only starting material

being recovered (Scheme 21). Despite repeating this reaction, the desulfonylation was unsuccessful, which precluded the cyclisation pathway required to prepare an *N*-3 unsubstituted tetrahydropyridine derivative of Riluzole.



***Scheme 21**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)_2OH$, 160 °C, 1.5 h, 36 %; (b) 2.0 equiv. TsCl, 2.0 equiv. Et_3N, DCM, 0 °C, 1.5 h, 48 %; (c) 2.0 equiv. NaI, acetone, reflux, 60 h, 97 %; (d) excess Py. reflux, 20 h, (e) 2.8 equiv. $NaBH_4$, MeOH, 0 °C - RT, 42 h, 71 % (over two steps); (f) HCl, aq. AcOH, reflux, 3 h, 0 %*

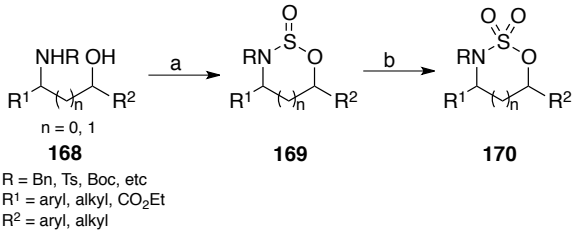
Repetition of Jimonet and co-workers methodology to generate *N*-3 Riluzole analogues with tetrahydropyridine moiety was unsuccessful as desulfonylation of compound **167** was found to retard this synthetic pathway, even though Jimonet and co-workers have reported the generation of a number Riluzole analogues using this route. Therefore alternative routes, which don't require protecting groups within the reaction pathway, should be investigated, such as the generation of sulfamidate and aziridine intermediates.

3.1.1.1 Synthesis of Unfunctionalised Tetrahydropyridine Riluzole Derivatives *via* Sulfamidate Chemistry

Cyclic sulfamidates are synthetically versatile electrophiles, which can be directly synthesised from readily available 1,2- and 1,3-amino alcohols. Generating these cyclic sulfamidate intermediates in the generation of *N*-3 Riluzole tetrahydropyridines will remove previous problems highlighted by Jimonet and co-workers experimental procedure. Pyridine then reacts at the C-O bond of the sulfamidate, which followed by desulfonylation and re-aromatisation yields an *N*-3 Riluzole tetrahydropyridine.

Constrained 1,2-amino alcohols, such as prolinol, are directly reacted with sulfuryl chloride to yield cyclic sulfamidates.[83] Using this as a generalised method for the synthesis of cyclic sulfamidates from 1,2- and 1,3-amino alcohols is not feasible as this process can result in competitive chlorination and aziridination.[83] A two-step approach is preferred instead. Firstly 1,2- and 1,3-amino alcohols are treated with thionyl chloride in the presence of imidazole generating 1,2- and 1,3-cyclic sulfamidites **169** in a highly efficient manner. Cyclic sulfamidite

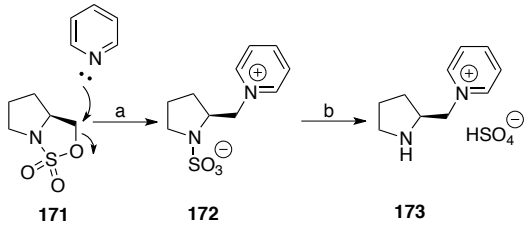
169 then undergoes oxidation with RuO_4 or $RuCl_3$ and $NaIO_4$ in aqueous solvent to yield the cyclic sulfamidate **170**. A number of alternative oxidising agents reported in the literature, such as *m*-CPBA and $KMnO_4$ have been tested previously, but reagents RuO_4 or $RuCl_3$ and $NaIO_4$ have been reported as the most effective systems giving yields greater than 80 % (Scheme 22).[83,84]



***Scheme 22**: Reagents and conditions: (a) 1.1 equiv. $SOCl_2$, 2.2 equiv. Et_3N, 4.0 equiv. imidazole, DCM, RT, 12 h; (b) 0.1 mol % $RuCl_3$, 1.1 equiv. $NaIO_4$, MeCN/H_2O, RT, 12 h*

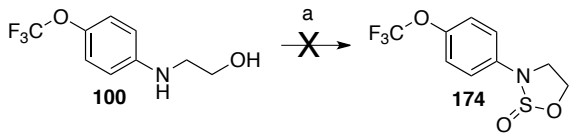
Nucleophilic attack directed towards cyclic sulfamidates occurs exclusively at the oxygen-bearing carbon *via* an S_N2 nucleophilic substitution (Scheme 23).[84] The reactivity of cyclic sulfamidates is similar to activated aziridines and azetidines, but not through ring strain. Cyclic sulfamidates can undergo ring-opening with a variety of nucleophiles, such as sulfur, oxygen, nitrogen, carbon and halogen nucleophiles.[83] From here on in, this thesis will focus solely on the ring-opening of cyclic sulfamidates with nitrogen nucleophiles.

Zhang and co-workers reported the facile synthesis of functionalised chiral ionic liquids *via* ring-opening of sulfamidates with pyridine (Scheme 23).[85] This reaction is of relevance to generating 3-(2-(4,5-dihydropyridinin-1-yl)ethyl)-6-trifluoromethoxy)benzothiazole-2-imine **159** as an uncyclised pyridinium ionic liquid will be generated as one of the intermediate compounds in the synthesis towards obtaining compound **159**. The generation of an uncyclised pyridinium compound can be obtained by generating a sulfamidate compound from compound **100**, which is then ring-opened with pyridine. This uncyclised pyridinium intermediate will then undergo re-aromatisation and reduction to yield compound **159**. Pyridine behaves like a tertiary aliphatic or aromatic amine in reactions that involve bond formation through the lone pair of electrons on the nitrogen ring. Pyridine attacks the C-O bond of the sulfamidate generating the desired pyridinium cation.[86]



***Scheme 23**: Reagents and conditions (a) toluene, 80 °C, 10 h, 72 %; (b) excess HCl, EtOH, 80 °C, 6 h, 88 %*

The synthesis of cyclic sulfamidates with 1,2-amino alcohols followed by ring-opening with pyridine was adopted as a route for preparing Riluzole derivatives with tetrahydropyridine substituents at the *N*-3 position. Compound **100** was treated with thionyl chloride in the presence of pyridine to generate cyclic sulfamidite intermediate **174** (Scheme 24).[87] This reaction was attempted numerous times but only starting material was recovered from the reaction.



***Scheme 24**: Reagents and conditions: (a) 3.3 equiv. $SOCl_2$, excess Py. DCM, 0 °C - RT, 1.5 h, 0 %*

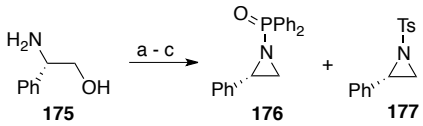
Reasoning behind why this reaction was unsuccessful could be due to the trifluoromethoxy group having electron deficient characteristics, which will in turn result in the lone pair of the nitrogen delocalising within the electron deficient aromatic ring. Delocalisation of the nitrogen lone pair within the electron deficient ring will in turn retard cyclisation and sulfamidite generation, as nucleophilic attack between the nitrogen and the sulfurochloridite is less likely to occur.

3.1.1.2 Synthesis of *N*-3 Riluzole Derivatives with Tetrahydropyridine Functionality *via* Aziridine Chemistry

Aziridine functionality represents one of the most valuable three-membered ring systems in modern synthetic chemistry due to its widely recognised versatility as a significant building block for chemical bond elaborations and functional group transformations.[88] Synthesising aziridine intermediates in the generation of *N*-3 Riluzole tetrahydropyridine will remove the use of protecting groups and also the number of steps required to get to the desired tetrahydropyridine product. Also, aziridines are very constrained three-membered rings, which readily ring-open with a variety of nucleophiles, which include carbon, oxygen, sulfur, and nitrogen heteroatoms. Over the years a number of different synthetic methods have been published regarding the synthesis of aziridines and this thesis focuses on three.

3.1.1.2.1 Ring-opening of Aziridines *via O*-Diphenylphosphinyl Protection to Generate Unfunctionalised Tetrahydropyridine Riluzole Derivatives

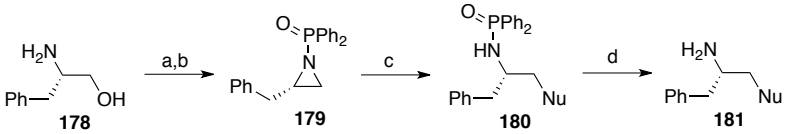
In 1993 Sweeney and co-workers first reported the preparation of *N*-phosphinylated aziridine **176**, which was efficiently prepared from 1,2-hydroxyamines *via* a three-step process (Scheme 25).[89]



***Scheme 25**: Reagents and conditions: (a) 1.0 equiv. Ph_2POCl, 1.0 equiv Et_3N, DCM, 0 °C - RT, 4 h; (b) 1.1 equiv. TsCl, 3.0 equiv Et_3N, 0 °C - RT, 18 h; (c) 2.0 equiv. NaH, THF, 0 °C - RT, 24 h, 52 % (over three steps)*

N-phosphinylated aziridines **176** are generated from 1,2-amino alcohols **175**. 1,2-Amino alcohols **175** was reacted with one equivalent of *p*-toluenesulfonyl chloride, and then one equivalent of diphenylphosphonic chloride to give 2-((diphenylphosphoryl)amino)ethyl-4-methylbenzenesulfonate. This was further treated with NaH resulting in the cyclisation of 2-((diphenylphosphoryl)amino)ethyl-4-methylbenzenesulfonate to yield *N*-phosphinylated aziridine **176** in good yield. Small quantities of *N*-tosylaziridine **177** are also generated as a byproduct of this reaction. This byproduct **177** is speculated to have been generated by incomplete phosphinylation generating *N*,*O*-ditosylated compounds which upon treatment with base yields the undesired aziridine **177**. Alternatively the phosphinyl group migrates from the nitrogen to the oxygen during tosylation, which then upon cyclisation with base will yield the undesired aziridine **177**.[89]

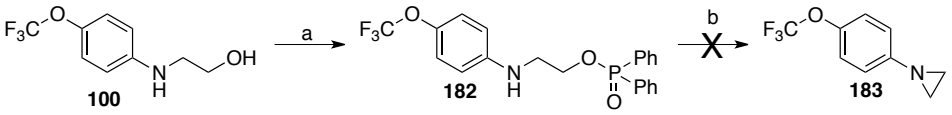
Sulfonyl groups are commonly used as activating groups for aziridines due to their excellent activation properties for ring-opening reactions with a variety of nucleophiles. When these *N*-tosylaziridnes are ring-opened they yield sulfonamides, which then require harsh conditions to achieve desulfonylation. However as discussed earlier desulfonylation of the nitrogen on compound **177** has proven to be unsuccessful, which is what this chemistry will be applied to. Diphenylphosphinyl groups have similar activating effects to toluenesulfonyl group as the P=O bond is highly polar and compared to sulfonyl groups diphenylphosphonic groups can be easily removed because there is a smaller interaction between the phosphorus and lone pair on the nitrogen. Therefore, N-P bonds can be cleaved under much milder conditions (Scheme 26).[90]



***Scheme 26**: Reagents and conditions: (a) 2.0 equiv. Ph_2POCl, 3.0 equiv. Et_3N, THF, 0 °C - RT, 5 h; (b) excess NaH, THF, 0 °C - RT, 24 h, 99 % (over three steps) (c) Nu; (d) excess BF_3OEt_2, MeOH, DCM, RT, 18 h, 68 - 92 % (over two steps)* [91]

Scheme 25 shows a plausible experimental procedure to generate *O*-diphenylphosphinoylated compounds with 1,2-amino alcohols, which can then be cyclised *via* nucleophilic attack with a nucleophilic amine generated by using a strong base, such as NaH

to generate aziridines. Scheme 26 reports a repeatable deprotection procedure to regenerate free amines. These experimental procedures were applied to amino alcohol **100** in the aim of generating Riluzole derivatives with tetrahydropyridine substituents at the *N*-3 position from a secondary amine instead of a primary amine, which has been reported in the above schemes 25 and 26. Reacting compound **100** with one equivalent of diphenylphosphonic chloride successfully gave compound **182** in moderate yield (Scheme 27). Further reacting compound **182** with two equivalents of NaH did not generate the desired *N*-aryl aziridine **183** only starting material was recovered (Scheme 27). After numerous attempts this method was sidelined.



***Scheme 27**: Reagents and conditions: (a) 1.0 eq Ph_2POCl, Et_3N, DCM, 0 °C - RT, 94 %; (b) 2.0 equiv. NaH, THF, 0 °C - RT, 24 h, 0 %*

As discussed in section 3.1.1.1 the generation of a sulfamidate intermediate **174** in the preparation of generating *N*-3 Riluzole analogues with tetrahydropyridine moiety was unsuccessful due to the nitrogen lone pair not being available for nucleophilic attack, as a result of the nitrogen being delocalised in the electron deficient ring. The reasoning provide for compound **174** is also true for aziridine **183** not being generated.

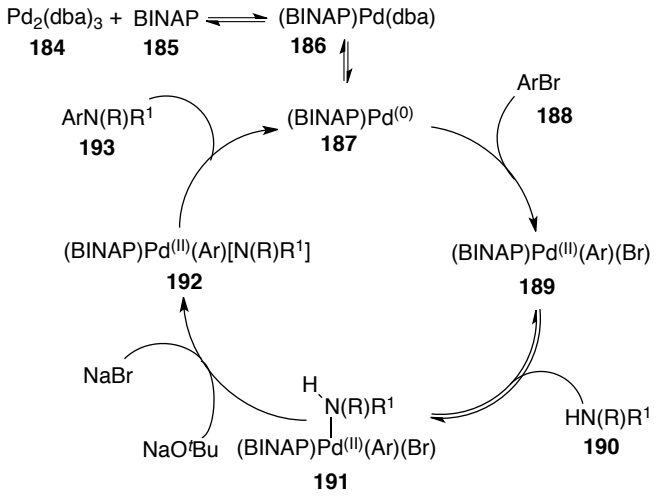
3.1.1.2.2 Ring-opening of Aziridines *via* Buchwald-Hartwig Cross-Coupling to Generate Unfunctionalised Tetrahydropyridine Riluzole Derivatives

Palladium-catalysed carbon-nitrogen bond-forming reactions have received considerable attention in recent years, with the first reported synthesis of palladium-catalysed amination of aryl halides being published by Kosugi *et al.* in 1983.[92] Using this methodology to generate a Riluzole aziridine intermediate **183** between an aryl halide and aziridine will remove previous issues experienced in which cyclisation has not occurred due to the nitrogen lone pair being delocalised within the electron deficient ring preventing nucleophilic attack.

Buchwald and Hartwig concurrently investigated both the mechanistic and synthetic process of the palladium-catalysed amination of aryl halides 10 years after it was first reported. In 1995 both Hartwig and Buchwald separately reported the replacement of tin reagents for either alkoxide or silylamide bases as the generation of tin amides, are known to be toxic, thermally unstable, air-sensitive and can only be applied to electron-neutral aryl halides.[92] Exploration of alkoxide bases, such as NaO^tBu, K_2CO_3 and Cs_2CO_3 by Buchwald and co-workers reported that they could be applied to the palladium-catalysed amination between a variety of electron-withdrawing and electron-donating aryl bromides with either primary or secondary amines.[93] In 1996 Hartwig and Buchwald published a number of back-to-back

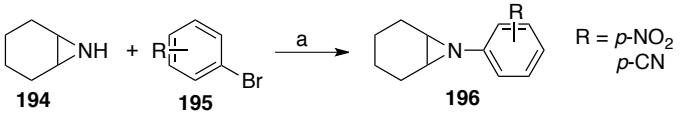
papers in which the palladium catalyst is complexed with chelating phosphine type ligands such as BINAP and DPPF.[92] It was reported that these palladium complexes catalysed the amination of aryl bromides and iodides with primary alkyl amines, cyclic secondary amines, and anilines. Overall, BINAP is the preferred ligand of choice as amination between either alkyl amines or alkyl halides can be applied to a number of electron-rich, electron-poor, hindered, unhindered or neutral aryl bromides, iodides or amines and will give high yields.

Buchwald proposed the catalytic cycle by which palladium-catalysed cross-coupling amination generates new C-N bonds between suitable amines and aryl halides.[94] Firstly, $Pd^{(0)}$ undergoes oxidative addition with a suitable aryl halide to generate **189**, which is followed by amine addition to give **191**. The introduction of an alkoxide base generates **192** a $Pd^{(II)}$-aryl amide, which is then reductively eliminated to generate a new C-N bond and also the regeneration of the $Pd^{(0)}$ catalyst (Scheme 28).



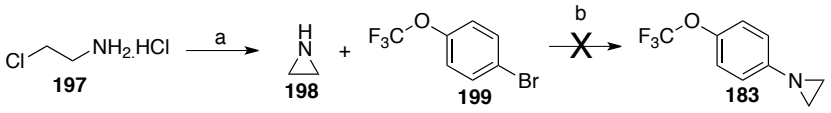
***Scheme 28**: The catalytic cyclic for the formation of C-N bonds*

Palladium-catalysed amination reactions between aryl halides and aziridines were not reported until 2003 where Yudin and co-workers described the reaction between cyclohexeneimine **194** and a variety of electron-withdrawing and electron-donating groups at the *para* and *ortho* potion of the aryl halide (Scheme 29).[95,96] Palladium cross-coupling conditions reported by Yudin and co-workers are of interest towards the synthesis of compound **183** as electron-withdrawing aryl halides can be reacted with aziridines.



***Scheme 29**: Reagents and conditions: (a) 2 mol % $Pd_2(dba)_3$, 2 mol % BINAP, 1.4 equiv. ^tBuONa, toluene, 50 °C - 80 °C, 2 - 24 h, 76 - 96 %*

The experimental procedure reported by Yudin and co-workers was adapted to synthesising *N*-3 tetrahydropyridine Riluzole analogue. As previously discussed the generation of a Riluzole aziridine intermediate *via* intramolecular cyclisation has not been successful due to the nitrogen lone being delocalised within the electron deficient aromatic ring. The generation of a Riluzole aziridine intermediate using Yudin and co-workers methodology removes intramolecular cyclisation and instead palladium catalysed cross-coupling between 4-trifluoromethoxy bromobenzene and aziridine will be attempted. Palladium cross-coupling between 4-trifluoromethoxy bromobenzene and aziridine, which was generated *in situ* by reacting 2-chloroethylammonium hydrochloride with 0.02 M sodium hydroxide solution did not generate compound **183** (Scheme 30).[97] 4-Trifluoromethoxy bromobenzene was seen to be fully consumed, but product peaks were not observed in the crude ^{1}H NMR. Overall the ^{1}H NMR obtained was inconclusive.



197 **198** **199** **183**

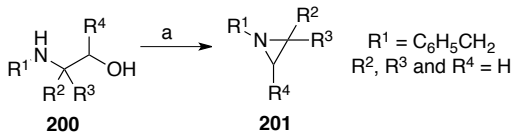
***Scheme 30**: Reagents and conditions: (a) 2.0 equiv. 0.02 M aq NaOH, H_2O, 50 °C, 45 mins[97]; (b) 2 mol %, $Pd_2(dba)_3$, 2 mol % rac-BINAP, 1.4 equiv. Na^{t}OBu, toluene, 50 °C, 12 h, 0 %*

Reasoning for the lack of success with this reaction could be related to the conditions used to cross-couple aziridine with 4-trifluoromethoxy bromobenzene, as Yudin and co-workers only reported palladium cross-coupling with substituted or kinetically stable aziridines, such as cyclohexeneimine **194**. Cross-coupling with aziridine has proven to be problematic firstly due to its low basicity (the pK_a of the aziridinium ion is 8.0, whereas that of a secondary amine is *ca.* 11) and secondly its inherent ring strain of approximately 111 kJ mol^{-1}.[98] Low basicity of the aziridine results in weak bond formation of the amine within the aziridine and activated palladium catalyst, which means the generation of palladium mediated *N*-arylated compounds are less likely to be generated. Palladium mediated *N*-arylated products are less likely to be generated from aziridines having low basicity instead of aziridines being ring-opened because of the ring strain associated which results in the generation of unwanted ring-opened products. Ring-opening of aziridines can be induced by either nucleophilic attack at the carbon or Lewis acid coordination at the nitrogen. In general, ring-opening reactions of aziridines and *N*-arylaziridines can be achieved under relatively mild conditions.[99]

3.1.1.2.3 Generation of Aziridines *via* the Mitsunobu Reaction Followed by Ring-Opening to Generate Unfunctionalised Tetrahydropyridine Riluzole Derivatives

Mitsunobu conditions have been widely reported for the synthesis of aziridines from 1,2-amino alcohols.[100] In 1984 Pfister reported the synthesis of aziridines *via* intramolecular dehydrogenation of 2-aminoethanol using a combination of DEAD and triphenylphosphine

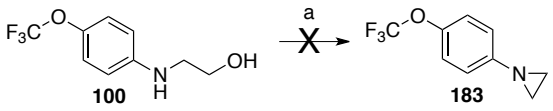
(Scheme 31).[101] Since then a number of aziridines have been generated from 1,2-amino alcohols using this methodology.[102]



200 **201**

***Scheme 31**: Reagents and conditions: (a) 1.5 equiv. DEAD, 1.5 equiv. PPh_3, THF/Et_2O, 0 °C - RT, 2 h, 18 %*

Using Mitsunobu reaction conditions to generate a Riluzole aziridine intermediate will remove issues previously experienced with protecting groups reported by Jimonet and co-workers methodology, which did not successfully generate an *N*-3 Riluzole analogue with tetrahydropyridine moiety as desulfonylation was not repeatable. Applying the following methodology to compound **100** did not yield a Riluzole aziridine intermediate **183** only starting material was recovered (Scheme 32).



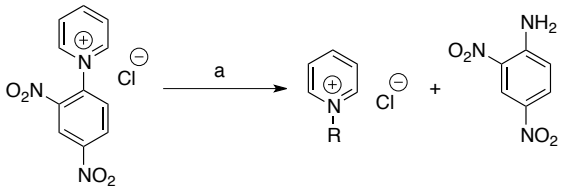
100 **183**

***Scheme 32**: Reagents and conditions: (a) 1.4 equiv. DEAD, 2.0 equiv. PPh_3, THF/Et_2O, 0 °C - RT, 24 h, 0 %*

The three-step intramolecular Mitsunobu reaction in which the amino alcohol **100** is converted to a Riluzole aziridine intermediate **183** was as stated above unsuccessful. The recovery of starting material could be the result of the trifluoromethoxy group attached at the *para* position of aniline being electron withdrawing with a hammett value of $\sigma_p = 0.35$.[103] Having an electron deficient aromatic ring attached to the nitrogen will reduce intramolecular S_N2 nucleophilic displacement between the nitrogen and the activated alcohol reducing the chances of generating a Riluzole aziridine intermediate.

3.1.1.3 Zincke Reaction

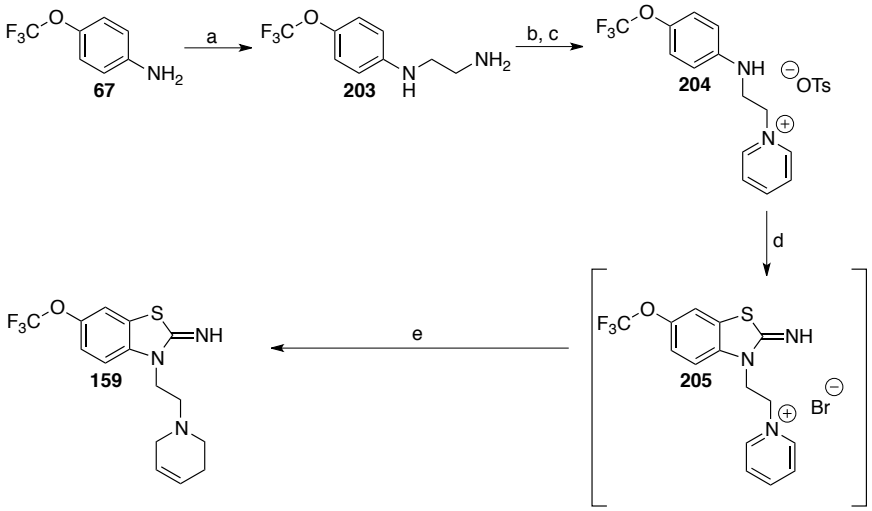
The Zincke reaction is an amine exchange process in which primary amines are converted to pyridinium salts using *N*-(2,4-dinitrophenyl) pyridinium chloride **138** (Scheme 33).[104]



138 **136** **202**

***Scheme 33**: Zincke Reaction Reagents and Conditions: (a) 2.0 equiv R-NH_2, EtOH, reflux, 12 h*

Incorporating the Zincke reaction into the synthesis of *N*-3 Riluzole with tetrahydropyridine moiety will remove focus from previous chemistry, which so far has focused on the secondary amine attached to an electron deficient aromatic ring. All reaction pathways so far focusing on the secondary amine attached to an electron deficient ring have ended in failure therefore taking a new approach could result in success. The aim is to generate a diamine compound which will react with *N*-(2,4-dintrophenyl) pyridinium chloride *via* Zincke reaction conditions generating an uncyclised pyridinium compound **204**. Once this intermediate has been generated it will then undergo cyclisation and reduction to generate an *N*-3 Riluzole tetrahydropyridine analogue (Scheme 34).



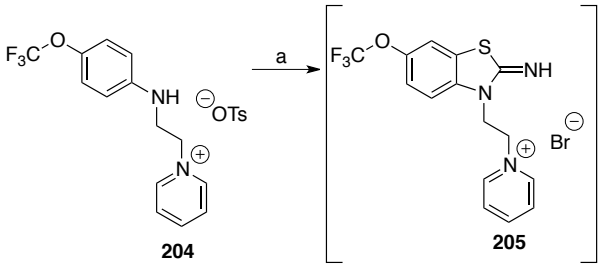
***Scheme 34**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)_2NH_2.HBr$, toluene, reflux, 24 h, 65 %; (b) 1.0 equiv. N-(2,4-dintirophenyl) pyridinium chloride, MeOH, RT - reflux, 20 h; (c) 1.0 equiv. sodium p-toluenesulfonate, EtOAc, reflux 12 h, 78 % (over two steps); (d) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 16 h; (e) 2.8 equiv. $NaBH_4$, MeOH, 0 °C - RT, 16 h, 69 % (over two steps)*

Firstly 4-trifluoromethoxyaniline underwent an S_N2 nucleophilic displacement with 2-bromoethylamine hydrobromide to generate diamine **203**.[105] The diamine **203** is then reacted with *N*-(2,4-dinitrophenyl) pyridinium chloride **138** under Zincke reaction conditions to yield an uncyclised pyridinium salt **204**, which proceeds *via* Scheme 15, found on pg. 36. Reacting compounds **203** and **67** together generates a ring-opened dianil salt. Reacting ring-opened dianil salt with another equivalent of compound **203** results in the generation of a diamine chain and the release of 2,4-dintiroanilne. The diamine chain then undergoes cyclisation to yield compound **204** after counter-ion exchange. Compound **204** then undergoes cyclisation with the following reagents KSCN and Br_2 under acidic conditions to generate intermediate **205**, which is then reduced *in situ* with $NaBH_4$ to yield compound **159** (Scheme 34).

Uncyclised pyridinium **204** is generated over uncyclised tetrahydropyridine as cyclisation of the latter has been reported to yield a dibromopiperidine ring. Optimisation studies of cyclising uncyclised pyridinium **204** to the crude intermediate **205** were investigated as

following the exact experimental procedure reported by Jimonet and co-workers gave a 1:1 ratio of **204** and **205**. This optimisation study focused on varying the equivalents of KSCN and Br_2, and also looked at using alternative bromine source (Table 3). All other variables time, temperature and solvent were kept constant throughout this study.

***Table 3**: Varying the equivalents of KSCN, Br_2 or $PhCH_2NMe_3Br_3$ source*



204 **205**

Reagents and conditions: (a) 4.0 - 12.0 equiv. KSCN, 1.0 - 2.0 equiv. Br_2 or $PhCH_2NMe_3Br_3$, AcOH, RT, 24 h

Entry	KSCN	Br_2	$PhCH_2NMe_3Br_3$	204:205 [a]
1	4.0 eq	1.0 eq	-	1:1
2	8.0 eq	1.0 eq	-	1:4
3	12.0 eq	1.0 eq	-	1:8
4	4.0 eq	2.0 eq	-	1:1
5	8.0 eq	2.0 eq	-	1:1
6	12.0 eq	2.0 eq	-	1:4
7	4.0 eq	-	1.0 eq	1:2
8	8.0 eq	-	1.0 eq	1:3
9	12.0 eq	-	1.0 eq	1:5
10	4.0 eq	-	2.0 eq	1:1
11	8.0 eq	-	2.0 eq	1:1
12	12.0 eq	-	2.0 eq	1:4

[a] ^1H NMR were run in $CDCl_3$ after working up the reaction

Entries 1-6 in Table 3 vary the equivalents of KSCN and Br_2. Ratios of starting material **204** to crude intermediate **205** were determined by comparing proton integration corresponding to the two compounds after work-up with all ^1H NMRs run in $CDCl_3$. The highest conversion of **204** to **205** used twelve equivalents of KSCN and one equivalent of Br_2, entry 3. Entries 7-12 of Table 3 vary the equivalents of KSCN and an alternative bromine source, trimethylphenylammonium tribromide ($PhCH_2NMe_3Br_3$), which is an organic ammonium tribromide (OATB). OATBs compared to liquid bromine have higher molecular weights so are found as crystalline solids. Advantages of using OATBs over liquid bromine include higher stability and accurate masses as OATBs are less volatile and take solid forms.[81] Overall for

all entries in Table 3 a trend can be observed, increasing the equivalents of KSCN from four to twelve equivalents increases the conversion of **204** to **205**. Altering the equivalents of bromine from one to two equivalents shows no enhanced improvement in conversion of compound **204** to crude intermediate **205**. Also altering the bromine source from Br_2 liquid to an OATB showed no improvement in conversion from **204** to **205**. To conclude this optimisation study has reported that the reaction conditions required for a high conversion of compound **204** to intermediate **205** requires twelve equivalents of KSCN and one equivalent of Br_2, entry 3.

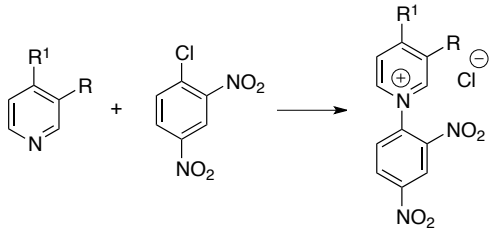
Riluzole derivative **159** was obtained in a four-stage synthesis from 4-trifluoromethoxyaniline with an overall yield of 13 %. Biological testing on compound **159** was not conducted as standard column chromatography failed to give sufficiently pure compound.

3.1.2 Synthesis of Functionalised Tetrahydropyridines

3.1.2.1 Using the Zincke Reaction

Generating an *N*-3 Riluzole tetrahydropyridine analogue was successful using Zincke reaction chemistry. The synthesis of *N*-3 Riluzole tetrahydropyridine analogues with substitution on the tetrahydropyridine ring can be generated using the same method describe for compound **159** but instead substituted Zincke salts are used. Synthesising a variety of substituted *N*-3 Riluzole tetrahydropyridine analogues will produce a variety of *N*-3 Riluzole substituted tetrahydropyridine analogues, which can be tested for their antiglutamate activity against Riluzole in the aim of generating an analogue with greater antiglutamate activity.

Substituted Zincke salts are generated by reacting a substituted pyridine with 1-chloro-2,4-dinitrobenzene (Scheme 35). The substituted Zincke salt **208** is then reacted with a primary amine to yield a substituted pyridinium compound, which when reduced yields a substituted tetrahydropyridine. When generating a substituted Zincke salts there are a few important aspects to consider: (1) pyridines with functionality at either the 2- or 6-position do not generate Zincke salts as this retards primary amine attack due to unfavourable steric effects, (2) generation of Zincke salts between 1-chloro-2,4-dinitrobenzene and pyridines with strongly electron deficient functionality, with R being CN, or NO_2 or where R^1 is also CN or NO_2 are not obtainable due to the pyridines lone pair being pulled towards the electron deficient functional group so therefore stopping S_NAr substitution, and (3) Zincke salts with the following substituted pyridines having R as Br or COOEt or R^1 as COOEt can be obtained but require elevated temperatures.[68]



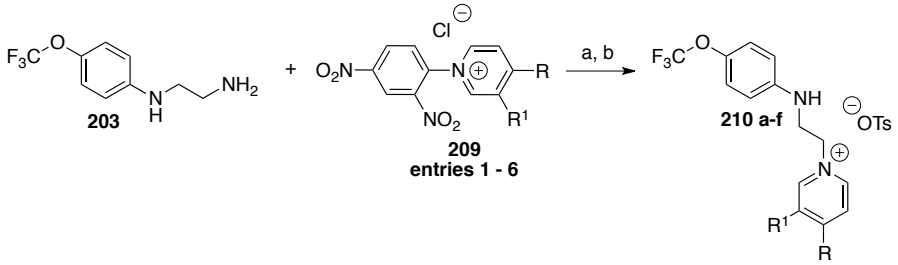
206 **207** **208**

***Scheme 35**: Generation of substituted N-(2,4-dinitrophenyl)pyridinium chloride salts; Reagents and conditions: (a) Acetone or MeOH, reflux, 48 h, 63 - 85 %*[70]

The rate in which pyridinium salts are generated *via* the nucleophilic substitution between a substituted Zincke salt and a primary amine are determined by the functionality on the Zincke salt **208**. It has been reported that the reaction between primary amines and mildly electron-deficient Zincke salts generates pyridinium salts quickly as fast ring-opening of the Zincke salt has been recorded. Whereas, reacting primary amines with electron-rich Zincke salts generates pyridinium slats slowly as slow ring-opening of the Zincke salt has been recorded. This sluggish reaction with electron-rich Zincke salts can be overcome by raising the reaction temperature, changing the solvent the reaction is run in, or changing the Zincke salts counteranion from chloride to dodecyl sulfate. Altering the Zincke salts counter-ion will lower nucleophilic anion properties, which will then allow Zincke salts to dissolve in a broader range of organic solvents.[68,106]

Zincke salts **209** entries 1-6 of Table 4 were generated *via* an S_NAr substitution between 1-chloro-2,4-dinitrobenzene and substituted pyridines giving moderate to high yield 49 - 92 %. These Zincke salts generated were then reacted with diamine **203** to give crude uncyclised substituted pyridinium salts, which then underwent counter-ion exchange with sodium *p*-toluenesulfonate to yield pure uncyclised substituted pyridinium tosylate salts **210 a - f** in low to good yields over two-steps. Having substitution at the 3-position of the pyridinium tosylate salts decreased in yield as you increased the alkyl chain. Whereas, substitution at the 4-position of the pyridinium tosylate salt decreased in yield as the alkyl chain was increased and using bulkier groups increased the yield. Overall compound **210 f** gave the best yield of pyridinium tosylate salt 89 %.

***Table 4**: Results obtained from the reaction of compound **203** with substituted zincke salts*



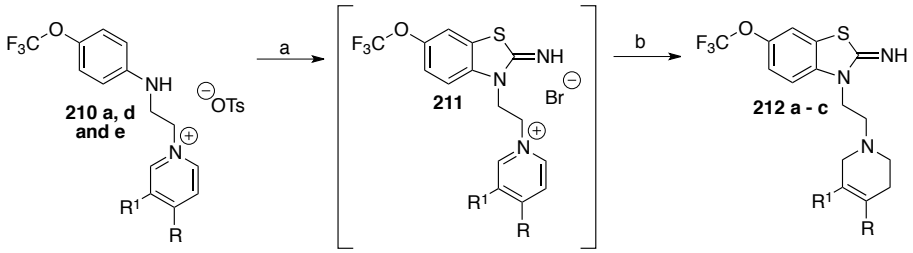
Reagents and conditions: (a) 1.0 equiv. Zincke salt, solvent, RT - reflux, 18 h; (b) 1.0 equiv. sodium p-toluenesulfonate, EtOAc, reflux, 12 h, 18 - 89 % (over two steps)

Entry	Zincke Salt 209		Solvent	Time (hrs)	Compound	% Yield
	R	R^1				
1 [107]	Me	H	*n*-butanol	18[a]	210a	41
2 [108]	H	Me	*n*-butanol	18[a]	210b	73
3 [107]	Et	H	MeOH	18[b]	210c	18
4 [109]	H	Et	MeOH	18[b]	210d	63
5 [110]	Ph	H	EtOH	18[a]	210e	44
6 [111]	*t*-Bu	H	n-butanol	18[a]	210f	89

[a] = Refluxed; [b] = 2 hrs RT-16 hrs reflux

All uncyclised pyridinium tosylate salts were subjected to ring cyclisation with KSCN and Br_2 under an acidic medium. The following uncyclised pyridinium tosylate salts **210 a, d** and **e** successfully yielded crude ring cyclised pyridinium salt **211 a - c**. These crude ring cyclised pyridinium salts were then subjected to *in situ* reduction with $NaBH_4$ to yield crude *N*-3 functionalised tetrahydropyridine Riluzole derivative **212 a** - **c** as oils (Table 5). Yields for compounds **212 a - c** were calculated from crude mixtures after work-up. Following on from observation observed for the uncyclised pyridinium tosylate salts similar trends were seen. For tetrahydropyridine Riluzole analogues containing substitution at the 4-position it was observed that compound **212 c** gave a higher crude yield than **212 a** with a crude yield of 68 % compared 30 %. For the tetrahydropyridine Riluzole analogue containing substitution at the 3-position a crude yield of 69 % was obtained.

***Table 5**: Synthesis of N-3 substituted tetrahydropyridine derivatives of Riluzole*



Reagents and conditions: (a) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 16 h; (b) 2.8 equiv. $NaBH_4$, MeOH, 0 °C - RT, 16 h, 30 - 69 % crude (over two steps)

Entry	Uncyclised pyridinium tosylate 210		Compound	Yield (%)[a]
	R	R^1		
1	Me	H	212a	30
2	H	Et	212b	69
3	Ph	H	212c	68

[a] *isolated crude yield after reaction work-up*

Crude proton NMRs of compounds **212 a - c** were obtained by running the sample in $CDCl_3$ after worked-up. The crude proton NMR results obtained for compounds **212 a - c** show that the ring cyclised pyridinium previously synthesised have successfully undergone reduction as a broad singlet is found in the region between 6.5 and 4.5 ppm, which corresponds to the alkene region (Figure 25). Compound **212 c**, which contains the phenyl substituent at the *para* position was observed further downfield compared to the other two analogues due to its aromatic functionality. The other distinct region within these crude ^{1}H NMRs is between 7.5 and 6.5 ppm, which corresponds to the benzothiazole ring formation as prior to cyclisation this splitting pattern is not observed. The ^{1}H NMRs are going from two doublets in the aromatic region, which corresponds to the four aromatic protons attached to the trifluoromethoxy group of the uncyclised compound **210 a, d and e** to two doublets and a singlet, which corresponds to the three protons of the benzothiazole ring.

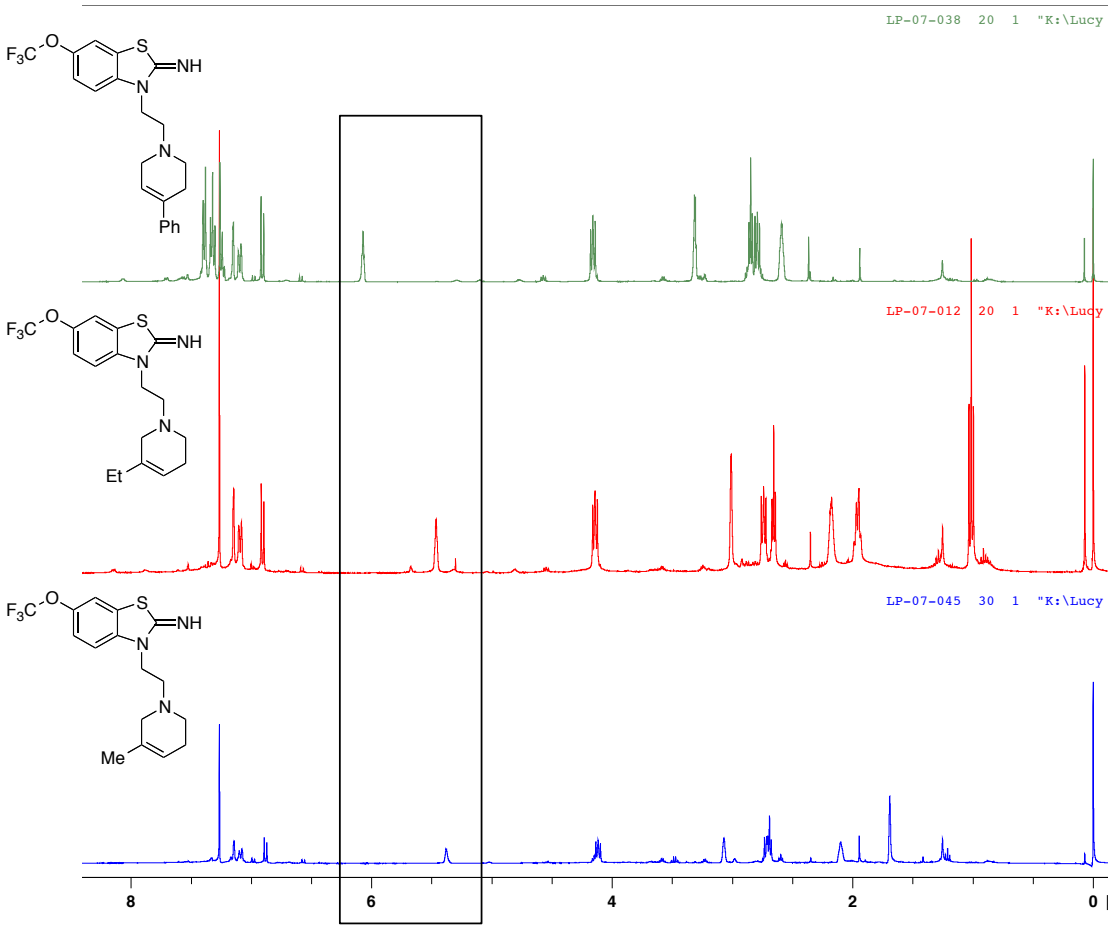


Figure 25**: Crude ^{1}H NMR of 3- and 4-substituted tetrahydropyridines; **212 a**, **212 b**, **212 c

N-3 functionalised tetrahydropyridine Riluzole derivatives were obtained *via* the Zincke reaction from 4-trifluoromethoxyaniline in moderate yields. Biological testing of compounds **212 a - c** has not been reported because the following tetrahydropyridine Riluzole derivatives could not be purified *via* simple column chromatography. Column chromatography was not a viable method of purification because of their polarity. The following solvent, MeOH moved these compounds of the baseline, but in doing so this did not remove any possible impurities or improve the physical appearance of the compounds.

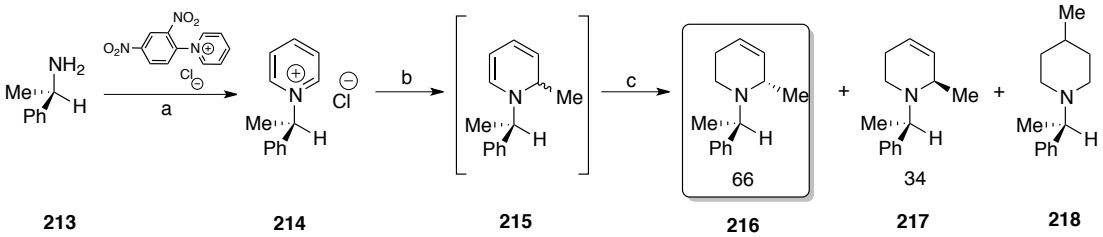
3.1.2.2 Using Grignard Reagents

The Zincke reaction method described in section 2.1.2.1 has provided a viable method to generating an *N*-3 Riluzole containing tetrahydropyridine functionality. This synthetic pathway described, Scheme 34 can be modified to incorporate a Grignard reaction, which can then go on to generate a library of *N*-3 Riluzole tetrahydropyridine analogues with substitution at the 2-position, which is not possible *via* substituted Zincke salts. Having substitution at the 2-position as well as the 3- and 4-position *via* substituted Zincke salt reactions will provide a variety of analogues to be tested for their antiglutamate activity compared to Riluzole and

having substitution at the *ortho*, *para* and *meta* position will highlight any possible trends observed when biologically tested.

In 1909 Freund and Bode reported the formation of 1,2- and 1,4- dihydropyridines by reacting pyridine with a range of suitable Grignard reagents.[112] Since this first report, a number of publications have described the addition of carbon nucleophiles to pyridinium salts at the 2-, 4- and 6-position generating 2-, 4- and 6-functionalised dihydropyridine intermediates. *N*-alkyl dihydropyridine intermediates are further reduced to tetrahydropyridines or piperidines due to the intermediate dihydropiperidines being unstable.[113] Nucleophiles that have been reported to have undergone nucleophilic addition with pyridine and *N*-alkyl pyridinium salts, include alkyllithium reagents such as phenyllithium and *tert*-butyllithium which react exclusively at the 2-position; Grignard reagents which, depending on the bulkiness of the Grignard reagent, react at either the 2- or 4-position; and lithium organocuperates which, react solely at the 4-position.[114] The remainder of this subchapter will focus on the reaction between *N*-alkyl pyridinium salts and Grignard reagents.

Guillotea-Bertin and co-workers reported the synthesis of 2-functionalised tetrahydropyridines by reacting pyridinium salt **214** with suitable Grignard reagents (Scheme 36).[115] This is achieved by firstly alkylating the pyridinium salt with a suitable Grignard reagent, which yields an unstable 1,2-dihydropyridine **215**. The unstable 1,2-dihydropyridine is then reduced with $NaBH_4$ affording tetrahydropyridine **216** as the major product and **217** and **218** as the minor products. Guillotea-Bertin and co-workers observed that regioselective attack at the 2-position of the pyridinium ring decreases with relatively hindered Grignard reagents, such as benzyl and isopropylmagnesium bromide which attack predominately at the 4-position of the pyridinium ring, resulting in the formation of piperidines **218** as the major product, after reduction.[115]

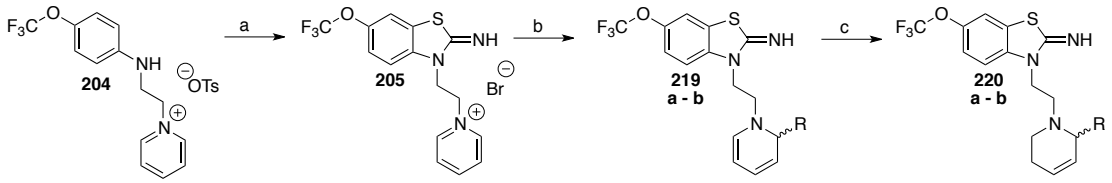


***Scheme 36**: Reagents and conditions: (a) DCM, 20 °C - Reflux, 36 h[116]; (b) 3.0 equiv. MeMgCl, THF/toluene, -20 °C - 0 °C, 3 h; (c) 6.0 equiv. $NaBH_4$, aq. MeOH, reflux, 1 h, 46 % (over three steps)*

Guillotea-Bertin and co-workers methodology was applied to the synthesis of *N*-3 Riluzole analogues with 2-substituted tetrahydropyridines. Firstly compound **204**, which is generated by reacting diamine **203** with Zincke salt, 2,4-dinitrophenyl pyridinium chloride followed by a counter-ion exchange is ring cyclised generating *N*-3 Riluzole pyridinium salt **205**. Pyridinium

salt **205** is then alkylated with one of the following Grignard reagents, methylmagnesium bromide, ethylmagnesium chloride or phenylmagnesium chloride (Table 6). This is followed by *in-situ* reduction to generate *N*-3 Riluzole 2-substituted tetrahydropyridine analogues.

***Table 6**: Synthesis of 2-substituted tetrahydropyridines*



Reagents and conditions: (a) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 16 h; (b) 3.0 equiv. RMgX, THF, -20 °C - 0 °C, 3 h; (c) 6.0 equiv. $NaBH_4$, 90 % aq MeOH, reflux, 1 h, 0 - 62 % (over three steps)

Entry	Grignard Reagent (R)	Compound	Yield (%)[a]
1	MeMgBr	220a	42
2	EtMgCl	220b	62
3	PhMgCl	220c	0

[a] % yields are calculated as a whole from the crude reaction mixture after work and ^{1}H NMR were run in $CDCl_3$

Running the reaction in THF/Toluene as reported by Guillotea-Bertin solely yielded compound **205**. It was observed that compound **205** did not dissolve in this solvent system, which gives indication into why only compound **205** was recovered. Running the reaction solely in THF rectified solubility issues previously experienced and successfully yielded crude *N*-3 Riluzole 2-substituted tetrahydropyridines. Alkylating compound **205** with methylmagnesium bromide and ethylmagnesium chloride successfully generated *N*-3 Riluzole analogues with 2-substituted tetrahydropyridines. Crude yields obtained indicate that increasing the alkyl chain of the Grignard reagent increases the percentage yield of 2-substituted tetrahydropyridines. Alkylating compound **205** with phenylmagnesium chloride does not yield *N*-3 Riluzole 2-phenyl tetrahydropyridine and starting material is not recovered (Figure 26). The crude ^{1}H NMR for the alkylation of compound **205** with phenylmagnesium chloride followed by *in-situ* reduction does not show the two protons corresponding to the alkene region of the tetrahydropyridine ring. This distinct alkene region of the tetrahydropyridine ring is observed for compounds **220 a and b**, highlighted in the black box, Figure 26. Guillotea-Bertin and co-workers have previously discussed that the alkylation of pyridinium salts with hindered Grignard reagents, such a phenylmagnesium chloride do not yield 2-substituted tetrahydropyridines, but instead generate 4-substituted piperidine rings, which would highlight why an alkene region is not seen for compound **220 c**. Due to compound **220 c** crude ^{1}H NMR being unclear mass spectrometry was performed to determine whether one of these

products had been generated, however mass spectrometry analysis did not detect the mass of the *N*-3 4-substituted piperidine Riluzole or the masses for compound **205**.

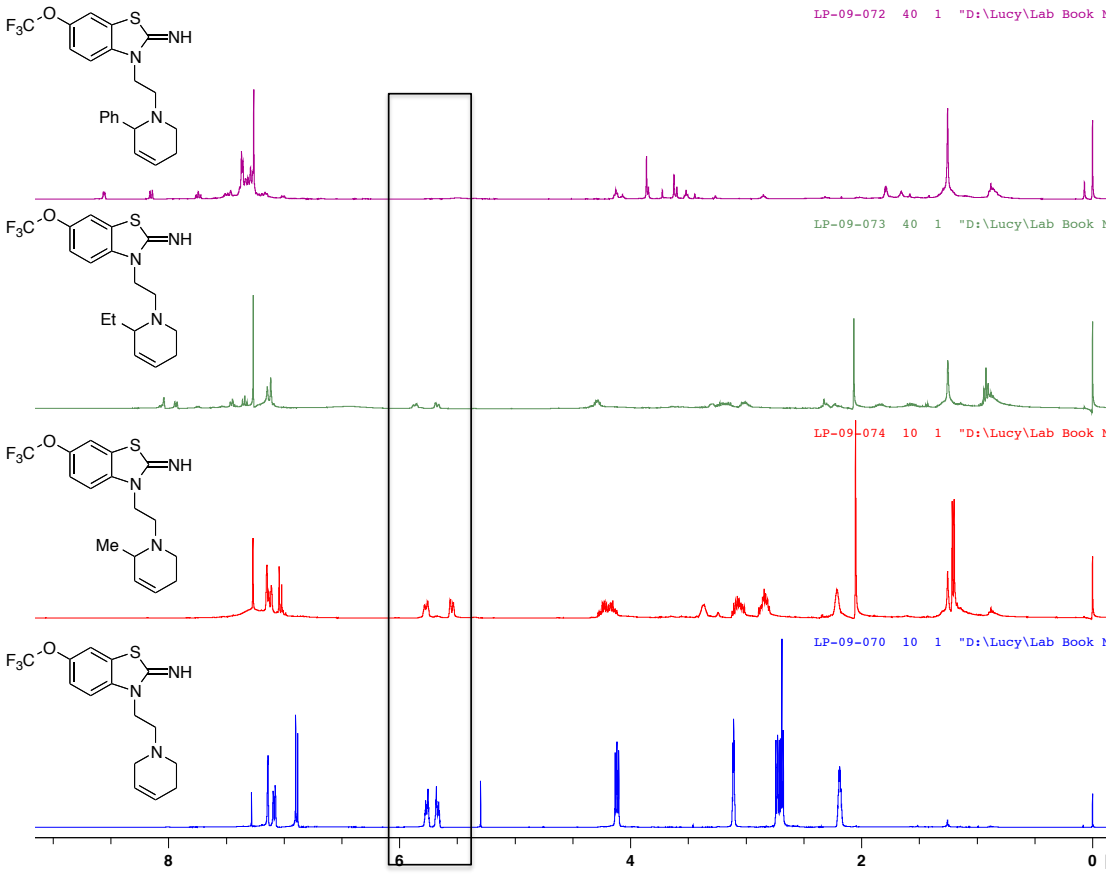


Figure 28**: Crude ^{1}H NMR for compounds **159**, **220 a**, **220 b** and **220 c

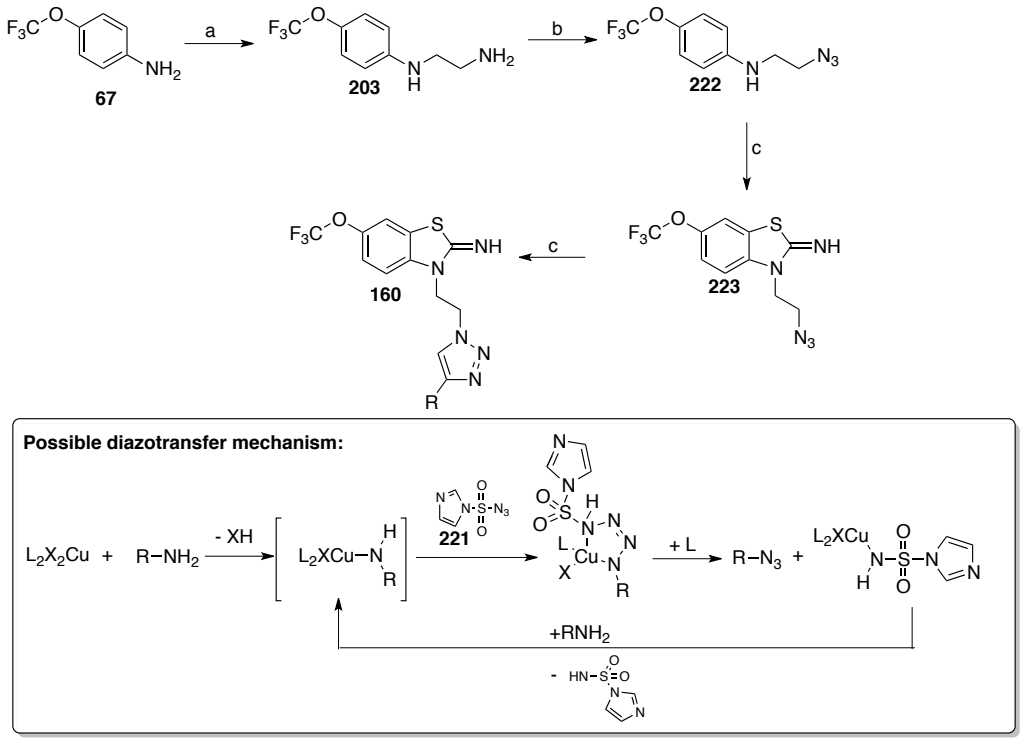
In summary, crude 2-substituted tetrahydropyridine Riluzole derivatives can be generated in four-steps from 4-trifluoromethoxyaniline, with methyl and ethyl Grignard reagents. Purification of these crude 2-substituted tetrahydropyridine compounds *via* simple column chromatography has proven difficult, as pure samples for biological testing was not achievable. Purifying compounds **220 a** and **b** using HPLC successfully generated a pure sample of compound **220 a**, but when compound **220 b** was purified using the same HPLC solvent system the compound was found to break down on the column. Overall this synthetic method to generate a library of novel *N*-3 substituted tetrahydropyridine Riluzole derivatives is not versatile as bulky Grignard reagents do not yield the desired tetrahydropyridine analogues and also a versatile purification method has not be achieved.

3.2 Synthesis of *N*-3 1,4-Substituted-1,2,3-Triazole Derivatives of Riluzole Using Click Chemistry

1,2,3-Triazoles generated to date have a lot of interest in drug discovery including combinational chemistry. Therefore click chemistry will be investigated to generate a library

of *N*-3 Riluzole 1,4-substituted-1,2,3-triazole analogues.[79] The 1,2,3-triazole nucleus has diverse biological activities including anticancer, antifungal, antibacterial, antiturberculosis and antivirus. Triazoles exhibit both basic and acidic properties and are also found to be very stable to metabolic and chemical degradation making them rather inert to severe hydrolytic, oxidising and reducing conditions, even at high temperatures.[79] All these properties described above for triazole moiety are favourable characteristics in designing novel pharmaceutically active compounds. Triazole analogues synthesised within this thesis will be tested for their antiglutamate activity against Riluzole.

1-4-Substituted-1,2,3-triazole derivatives of Riluzole **160** will be generated as follows firstly 4-trifluoromethoxyaniline **67** will undergo S_N2 nucleophilic displacement with 2-bromoethylamine hydrobromide to generate diamine **203**. Diamine **203** then undergoes diazotransfer with imidazole-1-sulfonyl azide hydrochloride to yield azide **222**. Imidazole-1-sulfonyl azide hydrochloride **221** is synthesised in two-steps. Firstly equimolar amounts of sulfuryl chloride and sodium azide are reacted together to yield chlorosulfonyl azide, which is then reacted an excess of imidazole generating the diazotransfer reagent in moderate yield, 48 %.[117] Azide **222** is then ring cyclised in the presence of KSCN and Br_2 to yield azide **223**. Azide **223** will then be reacted with a terminal alkyne in the presence of substoichiometric amounts of Cu^I, which is generated *in situ* to yield a 1,4-substituted-1,2,3-triazole Riluzole analogue **160** (Scheme 37).[118] The catalytic cycle by which 1,4-substituted-1,2,3-triazoles are synthesised is shown on pg. 38 Scheme 17.

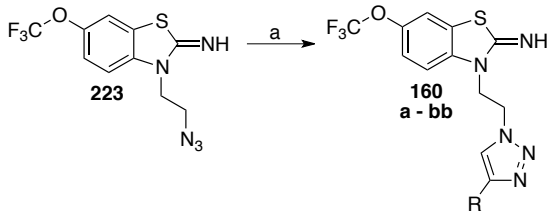


***Scheme 37**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)_2NH_2.HBr$, toluene, reflux, 24 h, 65 %; (b) 1.2 equiv. Imidazole-1-sulfonyl azide hydrochloride, 2.3 equiv. K_2CO_3, 0.001 equiv. $CuSO_4.5H_2O$, MeOH, RT, 2 h, 25 %; (c) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 2 h, 63 %; (d) 1.5 equiv. Terminal Alkyne, THF/H_2O, 1.0 equiv. 1M $CuSO_4$, 2.0 equiv. 1M NaAsc, 20 °C, 2 h, 27 - 98 %* [105,117,118,119]

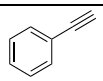
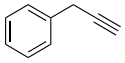
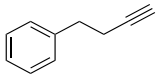
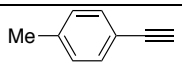
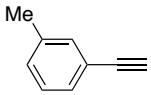
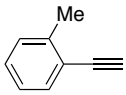
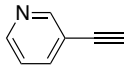
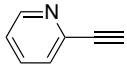
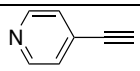
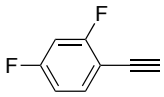
Conversion of diamine **203** to azide **222** is obtained *via* a diazotransfer instead of nucleophilic displacement with an azide anion because an azide anion could result in the formation of elimination products or products with the incorrect stereochemical configuration.[117] Ruff[120] first described the conversion of primary amines to azides using the following diazotransfer reagent, trifluoromethanesulfonyl azide (TfN_3) in the presence of catalytic amounts of Cu^{II}, which generates organic azides in high yields and preserves any existing stereochemistry.[121,122] However, using TfN_3 as a diazotransfer reagent in the conversion of primary amines to azides has some major drawbacks which include its explosive nature, relatively poor shelf life, difficulty in extraction from polar compounds and the expense of the starting material trifluoromethanesulfonic anhydride in generating TfN_3.[117] Hanessian and Vatèle reported diazotransfer reactions with the diazotransfer reagent imidazole-1-sulfonyl azide hydrochloride, which resulted in the conversion of primary amines to azides.[117,123] Imidazole-1-sulfonyl azide hydrochloride is reported to have similar reactivity to that of trifluorosulfonates, but compared to TfN_3, imiadazole-1-sulfonyl azide hydrochloride has a longer shelf live and is less expensive to prepare.

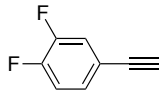
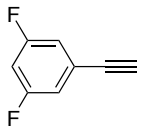
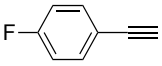
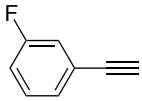
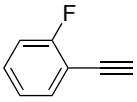
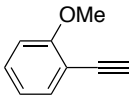
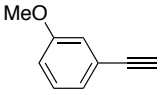
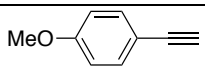
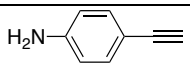
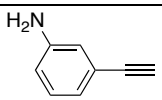
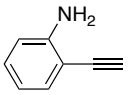
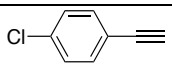
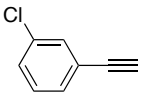
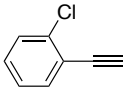
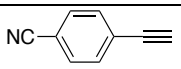
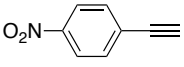
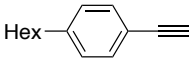
Azide **223** was successfully generated in three-steps starting from 4-trifluoromethoxyaniline **67** (Scheme 37). Reacting azide **223** with a number of terminal alkynes including alkyl chains, cyclic alkyls, alkyl esters, alkyl benzenes, heterocycles and electron-donating and electron-withdrawing aromatic rings in the presence of substoichiometric amounts of Cu^{I} generated *in situ* between copper sulfate and sodium ascorbate successfully generated 1,4-substituted-1,2,3-triazole *N*-3 Riluzole analogues **160** (Table 7). All reactions were run at 20 °C and reactions were monitored by TLC.

***Table 7**: Synthesis of N-3 1,4-substituted-1,2,3-triazole Riluzole compounds*



Reagents and conditions: (a) 1.5 equiv. Terminal Alkyne, THF/H_2O, 1.0 equiv. 1M $CuSO_4$, 2.0 equiv. 1M NaAsc, 20 °C, 2 h, 27 - 98 %

Entry	Alkyne (R)	Compound	Time (hrs)[a]	Yield (%)[b]
7		160a	3	39
8		160b	3	50
9		160c	2	47
10		160d	2	56
11		160e	2	59
12		160f	2	37
13		160g	2	64
14		160h	2	53
15		160i	2	46
16		160j	2	50

17		160k	2	44
18		160l	2	41
19		160m	2	56
20		160n	2	44
21		160o	2	52
22		160p	2	61
23		160q	2	67
24		160r	2	47
25		160s	2	27
26		160t	2	45
27		160u	2	49
28		160v	2	55
29		160w	2	58
30		160x	2	53
31		160y	2	59
32		160z	2	63
33		160aa	2	72
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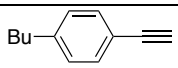
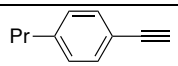
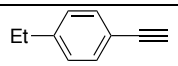
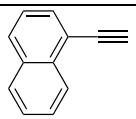
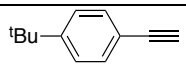
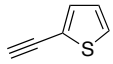
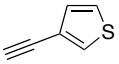
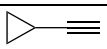
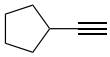
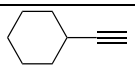
35		160ac	2	72
36		160ad	2	75
37		160ae	2	67
38		160af	2	61
39		160ag	2	73
40		160ah	2	53
41		160ai	2	55
42		160aj	2	67
43		160ak	2	59
44		160al	2	55
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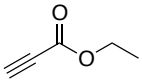
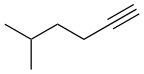
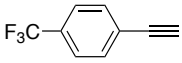
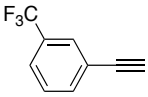
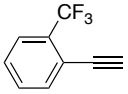
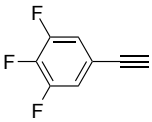
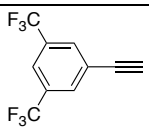
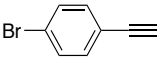
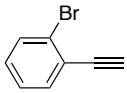
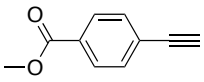
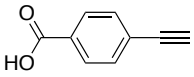
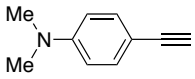
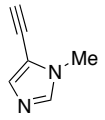
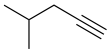
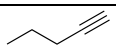
46		160an	2	61
47		160ao	2	63
48		160ap	2	35
49		160aq	2	66
50		160ar	2	59
51		160as	2	74
52		160at	2	87
53		160au	2	69

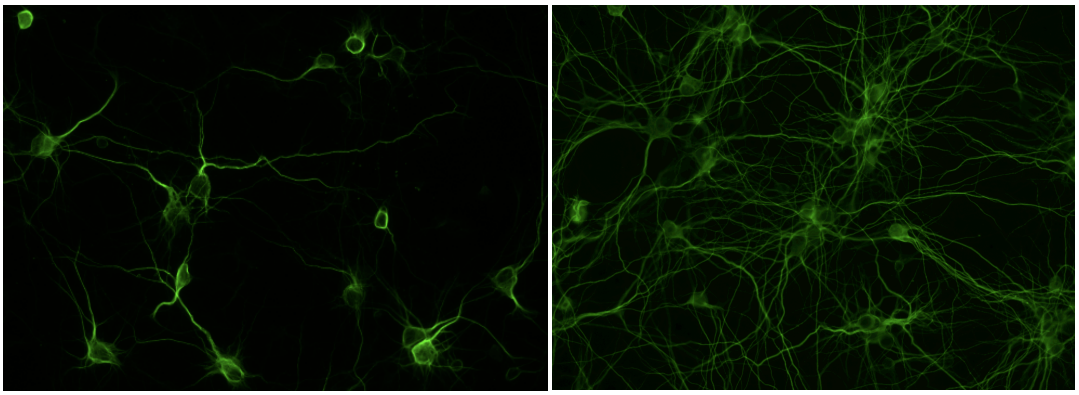
54		160av	2	50
55		160aw	2	59
56		160ax	2	59
57		160ay	2	58
58		160az	2	67
59		160ba	2	98
60		160bb	2	44

[a] completion of reaction was monitored *via* TLC; [b] isolated yields after column chromatography

All 1,4-substituted-1,2,3-triazole *N*-3 Riluzole analogues were generated in moderate to high yields. Altering the alkyne chain length for the benzyl group analogues **160 a - c** showed that the highest yielding analogue had a chain length of two with 50 %. Percentage yields obtained between azide **223** and alkynes containing electron-withdrawing aromatic rings varied from 35 - 74 % with analogue **160 ap** being the lowest and analogue **160 as** being the highest yielding. Compound **160 at** was not included due to its molecular mass being greater than 500 g/mol^{-1}. Percentage yields obtained between azide **223** and alkynes containing electron-donating aromatic rings varied from 27 - 75 % with analogue **160 s** being the lowest and analogue **160 ad** being the highest yielding. No major trends are observed between electron-withdrawing and electron-donating groups. Analogues with the same functionality positioned at the *para*, *meta* or *ortho* position have shown higher yields at the *meta* position. Increasing the ring size of the cycloalkyl alkyne decreases percentage yield and increasing branched alkyne alkyl chains also decreases the percentage yield of 1,4-substituted-1,2,3-trialozle *N*-3 Riluzole analogues obtained.

All triazole compounds **160 a - bb** were subjected to biological testing in primary cortical neurons cultured from E15 Swiss mouse embryos. All compounds were made up to 1 μM concentrations in DMSO and after 30 mins alone on the cultivated cortical neuron cells a 100 μM concentration of kainate in water was added. This was left for 18 hrs at 37 °C in an incubator with 5 % CO_2 levels. Each compound was repeated four times generating a total of 24 MAP2 images *per* experimental condition. Measurement of the neuroprotective activity for each triazole compound was measured by all cells being incubated with a primary antibody, which selectively binds to MAP2 proteins in the neuron. The cells were then further incubated

with a fluorescent secondary antibody, which will selectively bind to primary antibodies. Therefore when fluorescent light is shone on the cell the fluorescent secondary antibodies will be visible in regards to parts of the cell where MAP2 is present. The more fluorescence observed corresponds to less of the cell being damaged by kainate (Figure 27).



A B

***Figure 27**: (**A**) less fluorescents means more cell damage; (**B**) more fluorescents means less cell damage*

Figures 28 - 31 which present preliminary data obtained for subjecting compounds **160 a** - **bb** to primary cortical neurons cultured from E15 Swiss mouse embryos, method described above were obtained from Bradford University. The data is shown in two ways; (1) Figure 28 and 29 focus on the raw averages of MAP2 fluorescence normalised to kainate, and (2) Figure 30 and 31 look at each drug treatment normalised to a cortical neuron cell treated with kainate. All 1,4-substituted-1,2,3-triazole compounds **160** are compared to that of kainate because kainate has similar properties to glutamate in which they both act on the same post-synaptic receptors and if either kainate or glutamate is generated in excess they will both cause a negative response known as excitotoxicity.

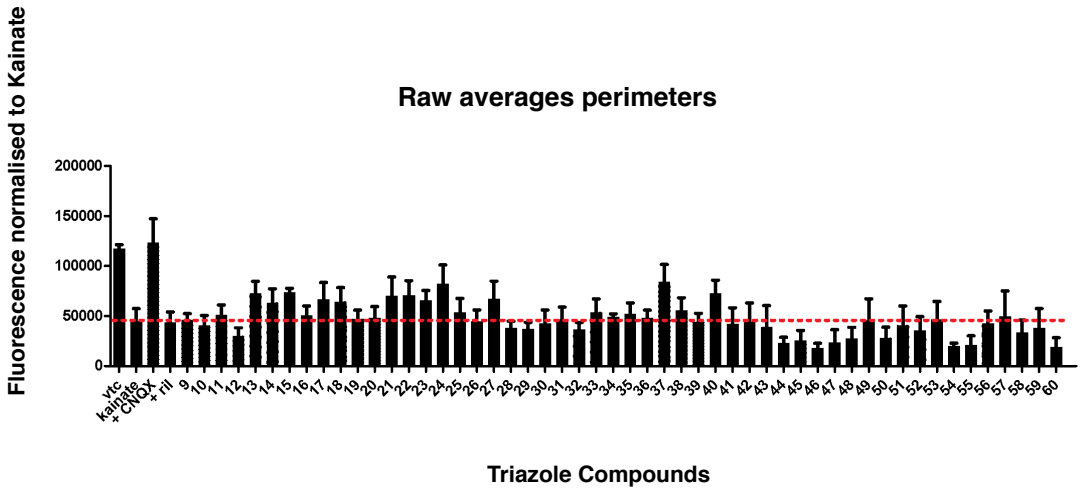


Figure 28**: Raw Averages Perimeters for Compounds **160 a - bb

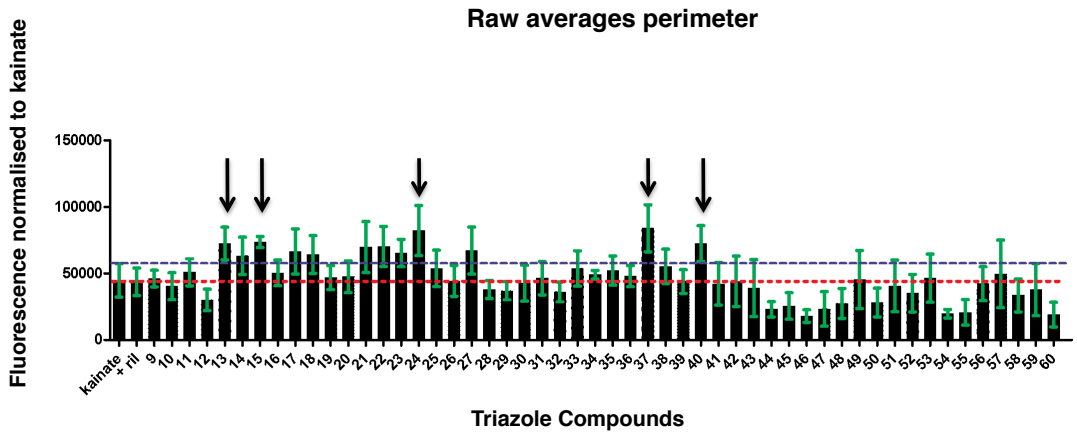


Figure 29**: Raw Averages Perimeters with Error Bars for Compounds **160 a - bb

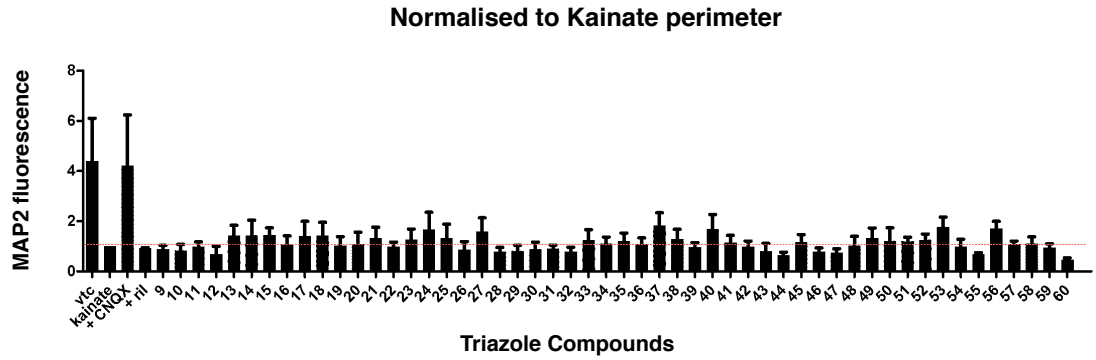


Figure 30**: Normalised to Kainate Perimeter for Compounds **160 a - bb

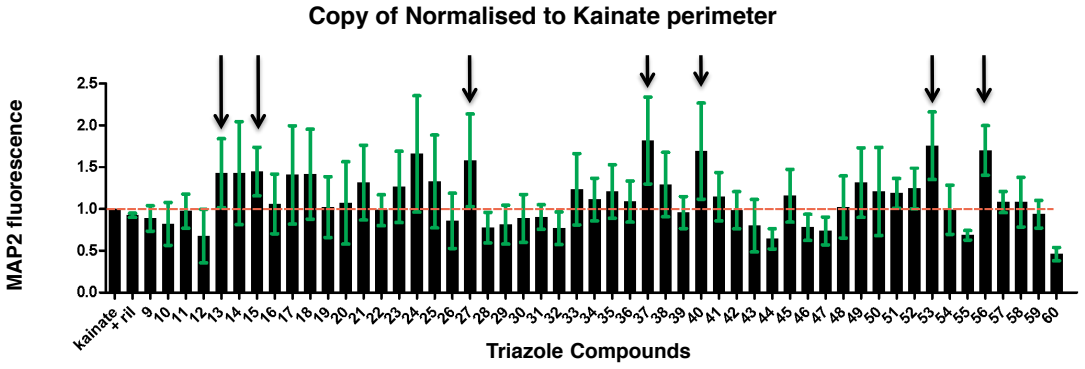


Figure 31**: Normalised to Kainate Perimeter with Error Bars for Compounds **160 a - bb

Figures 28 and 29 record the fluorescence emitted for each triazole compound treated with kainate on cortical neuron cells and are compared to a cortical neuron cell treated with only kainate. Compounds emitting more fluorescence than kainate (Figure 28) are of potential interest as higher emissions of fluorescence recorded on cortical neuron cells means less of the cell has been damaged by an excess of kainate. Figure 29 develops on Figure 30 by

stating that any compounds with fluorescent error bars overlaying the error bar of kainate, shown by the blue line are not of interest. Overall these two Figures report the success of five compounds **160 g**, **i**, **r**, **ae** and **ah** which have shown high fluorescence and cell protection in cortical neurons, so will therefore be considered for future biological testing. Figures 30 and 33 standardise kainate to one fluorescent unit (FU). All compounds fluorescence is compared to the fluorescence emitted from a cortical neuron treated with only kainate standardised to 1 FU. Compounds emitting fluorescence below the red line of kainate (Figure 30) have low cell protection levels compared to kainate so are therefore not of interest for future biological testing. Figure 31 includes fluorescent error bars for each compound treated with kainate and all results that have error bars that either overlay or are below the red line of kainate which has been standardised to 1 FU show cell protection similar to or worse than cortical neuron cells treated with kainate. From theses two Figures the following compounds **160 g**, **i**, **u**, **ae**, **ah**, **au** and **ax** have shown high fluorescence against kainate and will be put forward for future biological testing.

In conclusion, a library of 1,4-substituted-1,2,3-triazole *N*-3 Riluzole compounds were successfully synthesised in four-steps using diazotransfer and click chemistry in moderate to high yields. All analogues synthesised were biologically tested for their antiglutamate activity against Riluzole with eight analogues expressing higher antiglutamate activity over Riluzole (Figure 32). These analogues range from heterocyclic compounds 3- and 4-pyridine and 2-thiophene to aromatic rings with electron-donating groups 2-aniline, 4-anisole and 4-ethyl benzene and electron-withdrawing groups 4-bromo benzene and 4-benzoic acid. There are no obvious trends, such as substitution observed at one position on the ring. These hits show substitution at all positions and also a range of donating and withdrawing substitutions as well as heterocycles exhibiting antiglutamate activity greater than Riluzole.

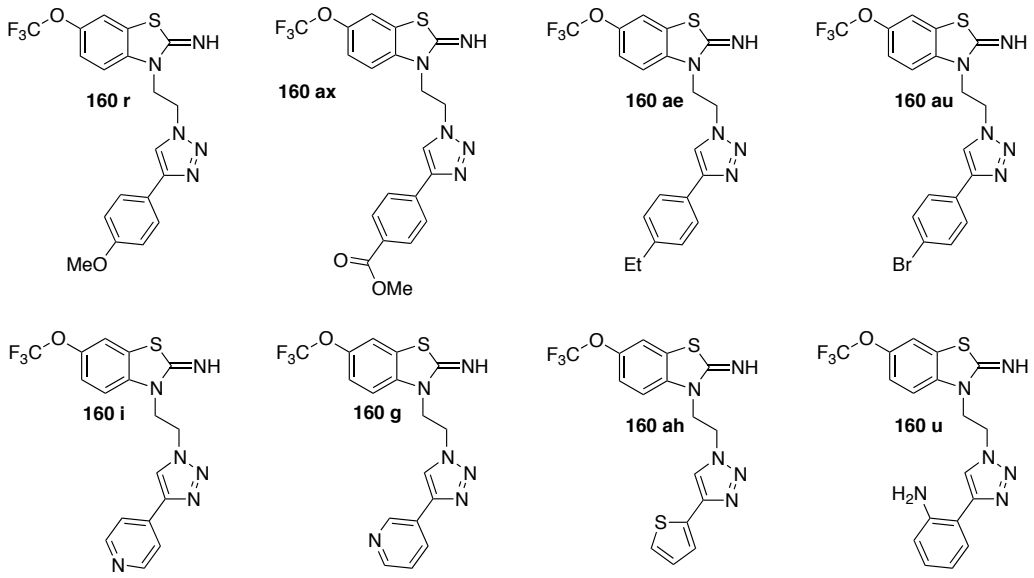
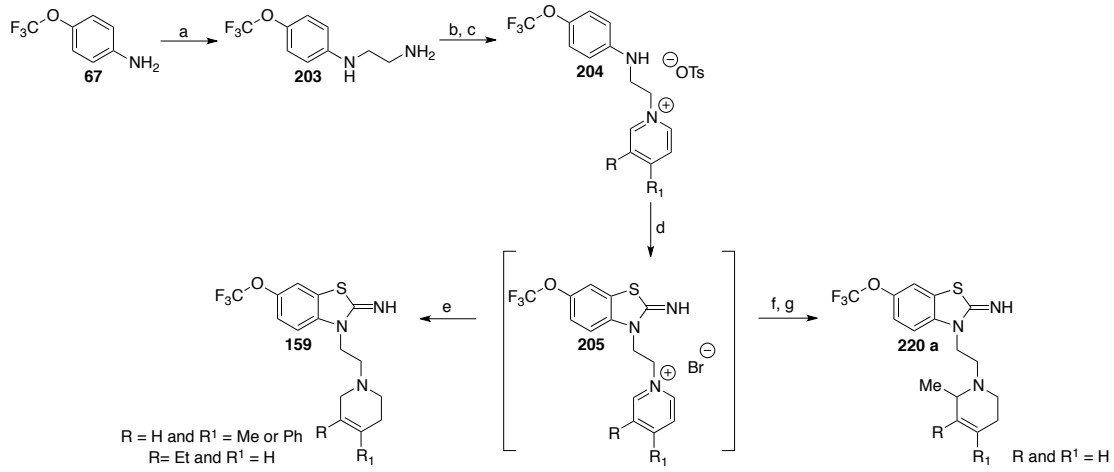


***Figure 32**: Hit 1,4-substituted-1,2,3-triazole N-3 Riluzole Analogues*

CHAPTER 4: CONCLUSIONS AND FUTURE WORK

The aim of this thesis was to generate two novel *N*-3 Riluzole libraries, one with tetrahydropyridine moiety and the other containing 1,4-substituted-1,2,3-triazole moiety. Once this had been achieved all pure *N*-3 analogues from the two novel libraries would be tested for their antiglutamate activity and compared to Riluzole.

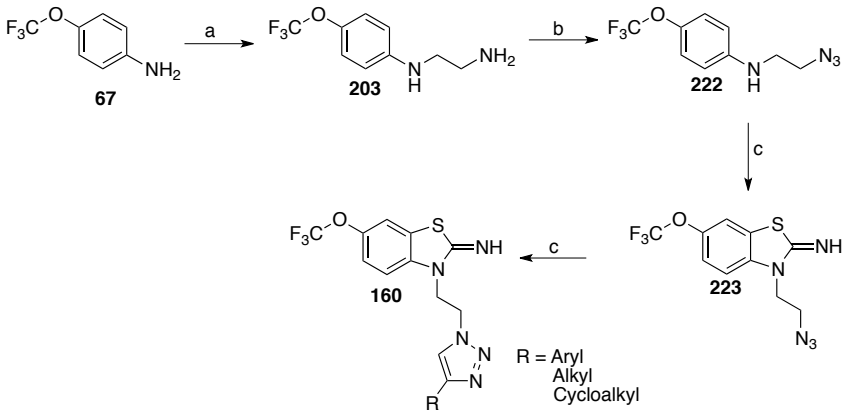
A number of routes were investigated in generating *N*-3 Riluzole analogues with tetrahydropyridine moiety. Routes, which looked at nucleophilic displacement or attack with pyridine between a tosyl, sulfamidite or aziridine, were all unsuccessful (Schemes 21, 24, 27, 30 and 32). Attempts at generating the following azide **183** from 4-trifluoromethoxyaniline **67** using Mitsunobu (Scheme 32), Buchwald-Hartwig cross-coupling (Scheme 30) or protection chemistry (Scheme 27) were unsuccessful therefore retarding nucleophilic attack to yield the uncyclised pyridinium compound **205**. The synthesis of a sulfamidite compound from the sulfamidate **174**, which is generated from 4-trifluoromethoxyaniline **67** to yield the uncyclised pyridinium compound **205** after nucleophilic attack with pyridine was also unsuccessful (Scheme 24). Reasoning's into why these synthetic routes were unsuccessful are due to the aromatic ring being electron-deficient which was hindering the nitrogen's lone pair from reacting with the necessary group to allow ring cyclisation to yield either the aziridine or sulfamidite intermediate after oxidation of the sulfamidate. However, generating a diamine compound **203** from 4-trifluoromethoxyaniline **67** and then reacting it with a suitable Zincke salt overcame previous issues generating an *N*-3 Riluzole analogues with tetrahydropyridine moiety (Scheme 38). This synthetic route generated tetrahydropyridine rings with functionality at the 3- and 4-position. Limited functionality at the 2-position was achieved by reacting the ring-cyclised pyridinium salt generated by reacting diamine **203** with 2,4-dinitrophenyl pyridinium chloride **138** with a suitable Grignard reagent followed by *in-situ* sodium borohydride reduction (Scheme 38).



***Scheme 38**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)NH_2.HBr$, toluene, reflux, 24 h, 65 %; (b) MeOH, RT - reflux, 20 h; (c) 1.0 equiv. Sodium p-toluenesulfonate, EtOAc, reflux, 12 h, 18 - 89 % (over two steps); (d) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 16 h; (e) 2.8 equiv. $NaBH_4$, MeOH, 0 °C - RT, 16 h, 30 - 71 % (over two steps); (f) 3.0 equiv. MeMgBr, THF, -20 °C - 0 °C, 3 h; (g) 6.0 equiv. $NaBH_4$, 90 % MeOH (aq), reflux, 1 h, 42 %*

Even though these synthetic routes were successful and could generate a range a tetrahydropyridine analogues with functionality at the 2,- 3- and 4-position these compounds proved difficult to purify to the standards required for biological testing. Future purification into rectifying this problem could look at either improving the ring-cyclised pyridinium intermediate **205**, which could be achieved by improving its crystallinity. This can be achieved by altering the bromine counter-ion for either a tosylate or tetraphenylborate counter-ion. Or, alternatively looking at generating *N*-3 Riluzole tetrahydropyridine analogues as salts, which has been reported in the literature, such as Jimonet and co-workers synthesising 2-imino-3-[2-(4-phenyl-1,2,3,6-tetrahydro-1-pyridyl)-ethyl]-6-trifluoromethoxy-benzothiazoline **129** as the dihydrochloride salt.[56]

A number of *N*-3 Riluzole triazole analogues were generated in a four-step process from 4-trifluoromethoxyaniline **67** in moderate to high yields (Scheme 39). All Riluzole analogues generated were biologically tested in cortical neuron cells at Bradford University and their antiglutamate activity was compared to Riluzole. Compounds which expressed increased antiglutamate activity over Riluzole were triazole analogues **160 g**, **i**, **r, u**, **ae**, **ah**, **au** and **ax** (Figure 32).



***Scheme 39**: Reagents and conditions: (a) 1.0 equiv. $Br(CH_2)_2NH_2.HBr$, toluene, reflux, 24 h, 65 %; (b) 1.2 equiv. Imidazole-1-sulfonyl azide hydrochloride, 2.3 equiv. K_2CO_3, 0.001 equiv. $CuSO_4.5H_2O$, MeOH, RT, 2 h, 25 %; (c) 12.0 equiv. KSCN, 1.0 equiv. Br_2, AcOH, RT, 2 h, 63 %; (d) 1.5 equiv. Terminal Alkyne, THF/H_2O, 1.0 equiv. 1M $CuSO_4$, 2.0 equiv. 1M NaAsc, 20 °C, 2 h, 27 - 98 %*

Future work on these hit eight compounds will include optimisation studies looking at finding an optimum potency required for maximum neuron protection. Concentrations that will be tested are 100 nM, 500 nM, 2 μM and 5 μM. Once this has been achieved the hit compounds will be subjected to testing in primary spinal cord motor neurons cultured from E15 Swiss mouse embryos. Alongside finding the optimum potency for *N*-3 Riluzole triazole analogues optimisation studies on the CuAAC reaction need to be investigated as well as generating more 1,4-substituted-1,2,3-traizole analogues derived from the existing hit compounds (Figure 33).

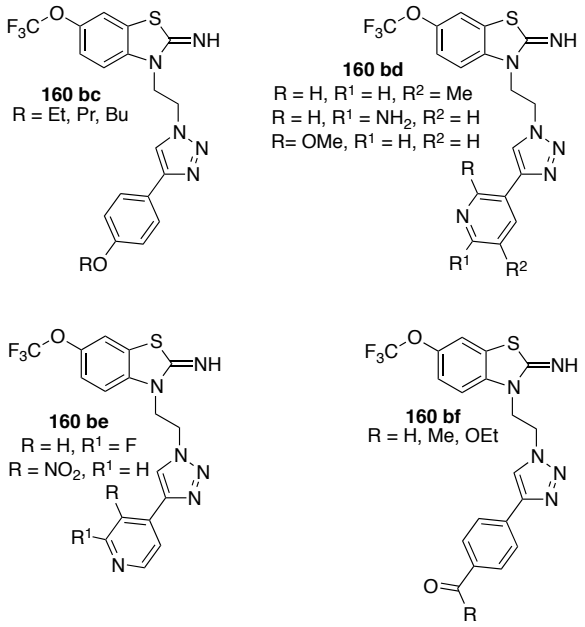


***Figure 33**: Potential 1,4-substituted-1,2,3-triazole derivatives of Riluzole, which could be of future interest*

For hit compound **160 r** a focus will be put into looking at whether varying the alkyl chain of the ether linkage at the *para* position of the aromatic ring will improve neuronal protection **160 bc**. For the two pyridine analogues **160 g** and **160 i** a range of functional groups ranging from electron-withdrawing to electron-donating groups at the *ortho*, *meta* and *para* position attached to 3- and 4-pyridine will be investigated **160 bd** and **160 be**. For hit compound **160 ax** carbonyl functionality of the benzoic acid will be altered to see if this improves neuron protection. Changing benzoic acid to benzoate **160 aw** has already been generated (Table 7) and was found to drastically reduced neuronal protection. Alternative benzyl carbonyl functionality **160 bf** generated will include a variety of benzyl alkyl ketones and benzaldehyde.

Optimisation of the CuAAC reaction will look at removing column chromatography purification in the case of this reaction being scaled up. This could either be achieved by finding a versatile crystallisation method that can be applied to all 1,4-substituted-1,2,3-triazole Riluzole analogues or varying the equivalents of terminal alkyne added to the reaction, until there isn't an excess observed in the crude ^{1}H NMR.

CHAPTER 5: EXPERIMENTAL

Spectroscopy

NMR spectra were recorded on either a Bruker 400 or 500 MHz Ultrashield Plus spectrometer with the stated deuterated solvent. Chemical shifts (δ) in ^{1}H NMR are reported in ppm, downfield from TMS, and as in ^{13}C NMR, are referenced to the residual solvent peak. Multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and combinations thereof, or multiplet (m). Coupling constants (J) are quoted in Hertz and rounded to the nearest 0.5 Hz. All ^{13}C resonances were assigned *via* distortionless enhancement by polarisation transfer (DEPT) experiments. Melting points were recorded on a Stuart melting point apparatus, model smp10 and are uncorrected. Infrared spectra were recorded using Nicolet 380 FTIR spectrometer. Mass spectroscopy was performed using an Agilent 6210 100SL-TOF LC/MS.

Reagents

Reagents were purchased from Sigma Aldrich, Fischer Scientific and TCI. They were used as supplied or, purified in accordance with the procedures of Perrin and Armarego.[124] Acetonitrile and dichloromethane were distilled from CaH_2 under an atmosphere of nitrogen. Tetrahydrofuran was distilled from sodium wire and benzophenone under an atmosphere of nitrogen.

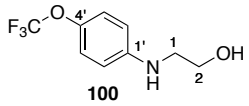
Chromatography

Thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} aluminium sheets. Visualisations of the developed plates were carried out by UV quenching at 254 nM. Column chromatography was carried out using silica gel 60Å, particle size 63-200 μM obtained from Fischer Scientific.

Caution: When handling sodium azide or any organic azide use a blast screen, as these compounds are potentially explosive. Also seek appropriate safety guidelines outlined from Sigma Aldrich or wherever purchased whenever handling.

5.1 Synthesis of *N*-3 Unfunctionalised and Functionalised Tetrahydropyridine Derivatives of Riluzole

2-((4-(Trifluoromethoxy)-phenyl)amino) ethanol 100[56]

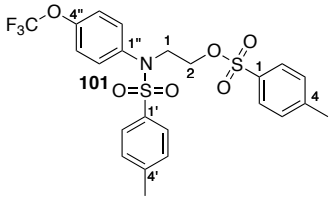


100

A solution of 4-trifluoromethoxyaniline (0.8 mL, 5.65 mmol, 2.0 equiv.) and 2-bromoethanol (0.2 mL, 2.82 mmol, 1.0 equiv.) was stirred at 160 °C for 1.5 h. Once cooled 20.0 mL DCM was added to the reaction mixture and the resulting precipitate was filtered off. The filtrate was collected and concentrated under reduced pressure to yield a crude orange/brown oil. The crude oil was purified *via* column chromatography using 4:6 EtOAc:CY to yield 2-((4-(trifluoromethoxy)-phenyl)amino) ethanol (**100**, 0.23 g, 1.02 mmol, 36 %) as a dark orange oil; $\mathbf{R_f}$ 0.22 (4:6 EtOAc:CY).

IR ν_{max}/cm^{-1} 3372, 2947, 2883, 1617, 1516, 1250; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.04 (2H, d, J = 8.0 Hz, H-3' and H-5'), 6.61 (2H, d, J = 8.0 Hz, H-2' and H-6'), 4.09 (1H, bs, N**H**), 3.85 (2H, t, J = 4.0 Hz, H-2), 3.30 (2H, t, J = 4.0 Hz, H-1), 1.65 (1H, bs, O**H**); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 46.2 (C-1), 61.2 (C-2), 113.4 (C-2' and C-6'), 122.5 (C-3' and C-5'), 140.7 (ArC), 140.9 (ArC), 147.1 (Ar(O**C**F_3)); **MS** m/z $[M+H]^+$ $C_9H_{11}F_3NO_2$ requires 222.07, found 220.07.

2-(4-Methyl-*N*-(4-(trifluoromethoxy)phenyl)phenylsulfonamido)ethyl-4-methylbenzenesulfonate 101[56]

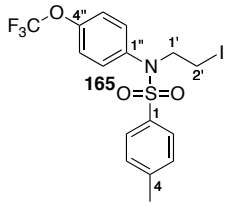


To a cooled solution amino alcohol **100** (2.00 g, 9.05 mmol, 1.0 equiv.) and Et_3N (2.5 mL, 18.09 mmol, 2.0 equiv.) in 40.0 mL DCM was added *p*-toluenesulfonyl chloride (3.45 g, 18.09 mmol, 2.0 equiv.) portion-wise. After 5 h of stirring at 0 °C the reaction mixture was warmed to RT, diluted with 40.0 mL DCM and washed with 40.0 mL H_2O. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a crude orange oil. The crude oil was recrystallised with 100 % EtOH to yield 2-(4-methyl-*N*-(trifluoromethoxy) phenyl) phenylsulfonamido)ethyl-4-methylbenzenesulfonate (**101**, 2.28 g, 4.30 mmol, 48 %) as a colourless solid; **m.p.** 92 - 95 °C (Lit.[56] **m.p.** 88 °C)

IR ν_{max}/cm^{-1} 3026, 2952, 1502, 1350, 1251, 1160; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.69 (2H, d, J = 8.0 Hz, TsH-2 and TsH-6), 7.44 (2H, d, J = 8.0 Hz, TsH-2' and TsH-6'), 7.33 (2H, d, J = 8.0 Hz, TsH-3 and TsH-5), 7.26 (2H, appd, J = 8.0 Hz, TsH-3' and TsH-5'), 7.09 (2H, d, J = 8.0

Hz, H-3'' and H-5''), 6.96 (2H, d, J = 8.0 Hz, H-2'' and H-6''), 4.13 (2H, t, J = 4.0 Hz, H-2), 3.80 (2H, t, J = 4.0 Hz, H-1), 2.45 (6H, d, J = 12.0 Hz, Ts(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 21.5 (Ts(**C**H_3), 21.6 (Ts(**C**H_3)), 49.9 (C-1), 67.4 (C-2), 119.0 (ArC), 121.4 (C-3'' and C-5''), 127.7 (TsC-2' and TsC-6'), 127.9 (TsC-2 and TsC-6), 129.7 (TsC-3' and TsC-5'), 129.9 (TsC-3 and TsC-5), 130.4 (C-2'' and C-6''), 132.5 (ArC), 134.5 (ArC), 137.6 (ArC), 144.2 (ArC), 145.2 (ArC), 148.6 (Ar(O**C**F_3)); **MS** m/z $[M+H]^+$ $C_{23}H_{23}F_3NO_6S_2$ requires 530.08, found 530.09.

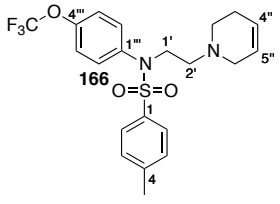
***N*-(2-Iodoethyl)-4-methyl-*N*-(4-trifluoromethoxy)phenyl)benzenesulfonamide 165**



A solution of **101** (0.40 g, 0.76 mmol, 1.0 equiv.) and NaI (0.23 g, 1.51 mmol, 2.0 equiv.) in 20.0 mL acetone stirred at reflux for 60 h. This was then cooled and concentrated under reduced pressure to yield a crude pale yellow solid. The crude solid was purified *via* column chromatography using 5:5 PE 40-60 °C:EtOAc to yield *N*-(2-iodoethyl)-4-methyl-*N*-(4-(trifluoromethoxy)phenyl)benzenesulfonamide (**165**, 0.26 g, 0.54 mmol, 71 %) as a pale yellow solid; **R$_f$** 0.91 (5:5 PE 40-60 °C:EtOAc), **m.p**. 97 - 99 °C

IR ν_{max}/cm^{-1} 3056, 2806, 1504, 1352, 1272, 1154; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.40 (2H, d, J = 8.0 Hz, TsH-2 and TsH-6), 7.21 (2H, d, J = 8.0 Hz, TsH-3 and TsH-5), 7.11 (2H, d, J = 8.0 Hz, H-3'' and H-5''), 7.03 (2H, d, J = 8.0 Hz, H-2'' and H-6''), 3.77 (2H, t, J = 8.0 Hz, H-1'), 3.11 (2H, t, J = 8.0 Hz, H-2'), 2.37 (3H, s, Ts(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 1.2 (C-2'), 21.6 (Ts(**C**H_3)), 53.4 (C-1'), 120.4 (C-3'' and C-5''), 126.5 (TsC-2 and TsC-6), 128.6 (TsC-3 and TsC-5), 129.3 (C-2'' and C-6''), 134.8 (ArC), 137.2 (ArC), 144.1 (ArC), 146.0 (ArC), 148.8 (Ar(O**C**F_3)); **MS** m/z $[M+Na]^+$ $C_{16}H_{15}F_3INO_3SNa$ requires 507.97, found 507.97.

***N*-(2-(4,5-Dihydropyridinyl)ethyl)-4-methyl-*N*-(4-(trifluoromethoxy)phenyl)benzenesulfonamide 166**

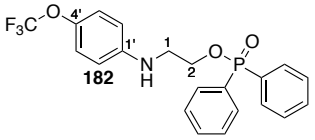


A solution of **165** (0.40 g, 0.82 mmol, 1.0 equiv.) in 5.0 mL pyridine was stirred at reflux for 20 h. This was then cooled and concentrated under reduced pressure to yield the crude pyridinium salt as a deep brown solid. To a cooled solution of the crude pyridinium salt (0.48 g, 0.93 mmol, 1.0 equiv.) in 12.5 mL MeOH at 0 °C was added $NaBH_4$ (0.10 g, 2.60 mmol, 2.8

equiv.) portion-wise. This was brought to RT and left to stir for a further 42 h. The reaction mixture was then diluted with H_2O and washed five times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield *N*-(2-(4,5-dihydropyridinyl)ethyl)-4-methyl-*N*-(4-(trifluoromethoxy)phenyl)benzenesulfonamide (**166**, 0.29 g, 0.66 mmol, 71 %) as an orange oil.

IR ν_{max}/cm^{-1} 3038, 2920, 1660, 1505, 1349, 1258, 1155; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.50 (2H, d, J = 8.0 Hz, TsH-2 and TsH-6), 7.37 (2H, d, J = 8.0 Hz, TsH-3 and TsH-5), 7.26 (2H, d, J = 8.0 Hz, H-3''' and H-5'''), 7.21 (2H, d, J = 8.0 Hz, H-2''' and H-6'''), 5.64 (1H, dt, J = 4.0 Hz and 8.0 Hz, H-5''), 5.54 (1H, dt, J = 4.0 Hz and 8.0 Hz, H-4''), 3.62 (2H, t, J = 4.0 Hz, H-1') 2.87 (2H, appq, J = 4.0 Hz, H-6''), 2.58 - 2.52 (4H, m, H-2' and H-2''), 2.35 (3H, s, Ts(C**H**$_3$)), 2.03 - 2.02 (2H, m, H-3''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 21.5 (Ts(**C**H_3)), 26.0 (C-2''), 48.5 (C-1'), 50.1 (C-2'), 52.7 (C-3''), 56.4 (C-6''), 121.3 (C-3''' and C-5'''), 124.9 (C-5''), 125.1 (C-4''), 127.7 (TsC-2 and TsC-6), 129.5 (TsC-3 and TsC-5), 130.3 (C-2''' and C-6'''), 135.0 (ArC), 137.9 (ArC), 143.7 (ArC), 145.5 (ArC), 148.4 (Ar(O**C**F_3)); **MS** m/z $[M+H]^+$ $C_{21}H_{24}F_3N_2O_3S$ requires 441.14, found 441.15.

2-((4-(Trifluoromethoxy)phenyl)amino)ethyl Diphenylphosphinate 182

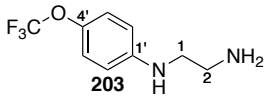


182

To a cooled solution of **100** (0.25 g, 1.13 mmol, 1.0 equiv.) in 10.0 mL DCM was added diphenylphosphonic chloride (0.2 mL, 1.13 mmol, 1.0 equiv.) and Et_3N (0.2 mL, 1.13 mmol, 1.0 equiv.) dropwise. After stirring at 0 °C for an hour the reaction mixture was warmed to RT and stirred for a further 4 h. This was then diluted with H_2O and washed twice with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield a crude orange oil. The crude oil was purified *via* column chromatography using 1:1 EtOAc:Hex to yield 2-((4-trifluoromethoxy)phenylamino)ethyl diphenylphosphinate (**182**, 0.45 g, 1.07 mmol, 94 %) as an off-white solid; **R_f** 0.13 (1:1 EtOAc:Hex), **m.p.** 85 - 89 °C

IR ν_{max}/cm^{-1} 3280, 3051, 2974, 1614, 1532, 1506, 1273, 1024; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.82 - 7.76 (4H, m, ArH), 7.55 - 7.51 (2H, m, ArH), 7.47 - 7.41 (4H, m, ArH), 7.01 (2H, d, J = 7.5 Hz, H-3' and H-5'), 6.56 (2H, d, J = 7.5 Hz, H-2' and H-6'), 4.23 (2H, quin, J = 5.0 Hz, H-2), 3.44 (2H, t, J = 5.0 Hz, H-1); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 44.4 (C-1), 64.0 (C-2), 113.1 (C-2' and C-6'), 119.5 (ArC), 122.4 (C-3' and C-5'), 128.6 (ArCH), 130.1 (ArC), 131.6 (ArCH), 132.5 (ArCH), 140.5 (Ar(O**C**F_3)), 146.6 (ArC); **MS** m/z $[M+H]^+$ $C_{21}H_{20}F_3NO_3P$ requires 422.11, found 422.11.

***N*-(4-Trifluoromethoxy)phenyl)ethane-1,2-diamine 203**[105]



203

A solution of 4-trifluoromethoxyaniline (2.7 mL, 20.00 mmol, 2.0 equiv.) and 2-bromoethylamine hydrobromide (2.05 g, 10.00 mmol, 1.0 equiv.) in 10.0 mL toluene was stirred at reflux overnight. Once cooled the reaction mixture was diluted with 13.0 mL 30 % NaOH solution and washed twice with toluene. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield a crude deep orange oil. The crude oil was purified *via* column chromatography using 9:1 DCM:MeOH with 1.25 % NH_4OH to yield *N*-(4-trifluoromethoxy)phenyl)ethane-1,2-diamine (**203**, 1.43 g, 6.50 mmol, 65 %) as a pale orange oil; $\mathbf{R_f}$ 0.42 (9:1 DCM:MeOH with 1.25 % NH_4OH).

IR ν_{max}/cm^{-1} 3317, 3040, 2938, 1614, 1514, 1252; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.03 (2H, d, J = 9.0 Hz, H-3' and H-5'), 6.58 (2H, d, J = 9.0 Hz, H-2' and H-6'), 4.23 (1H, bs, N**H**), 3.16 (2H, t, J = 6.0 Hz, H-1), 2.97 (2H, t, J = 6.0 Hz, H-2), 1.70 (2H, bs, N$\mathbf{H_2}$); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 40.8 (C-2), 46.3 (C-1), 113.0 (C-2' and C-6'), 122.4 (C-3' and C-5'), 140.4 (Ar(O**C**F_3)), 147.2 (ArC), 147.5 (ArC); **MS** m/z $[M+H]^+$ $C_9H_{12}F_3N_2O$ requires 221.08, found 221.10.

Formation of Zincke Salts

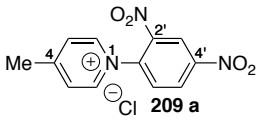
***N*-(2,4-Dinitrophenyl)pyridinium Chloride 138**[104]

138

1-Chloro-2,4-dintirobenzene (10.01 g, 49.42 mmol, 1.0 equiv.) was added portion-wise to pyridine (4.0 mL, 49.42 mmol, 1.0 equiv.). This was stirred at 95 °C for an hour. Once cooled the precipitate was triturated with acetone and filtered to yield *N*-(2,4-dinitrophenyl)pyridinium chloride (**138**, 11.83 g, 42.09 mmol, 85 %) as an off-white powder; **m.p.** 212 - 216 °C (Lit.[104] **m.p.** 193 - 194 °C).

IR ν_{max}/cm^{-1} 3040, 1617, 1537, 1477, 1343; **^{1}H NMR** (400MHz, D_2O) δ; 9.34 (2H, dd, J = 1.0 Hz and 6.0 Hz, H-2 and H-6), 9.31 (1H, d, J= 5.0 Hz, H-3'), 8.99 (1H, tt, J = 5.0 Hz and 10.0 Hz, H-4), 8.95 (1H, dd, J = 5.0 Hz and 10.0 Hz, H-5'), 8.44 (2H, dd, J = 7.0 Hz and 8.0 Hz, H-3 and H-5), 8.34 (1H, d, J = 10 Hz, H-6'); **^{13}C NMR** (100MHz, D_2O) δ; 122.6 (C-3'), 128.4 (C-3 and C-5), 130.5 (C-5'), 131.1 (C-6'), 138.6 (ArC), 142.8 (ArC), 145.4 (C-2 and C-6), 149.1 (C-4), 149.6 (ArC); **MS** m/z $[M]^+$ $C_{11}H_8N_3O_4^+$ requires 246.05, found 246.05.

***N*-(2,4-Dinitrophenyl)-4-methylpyridinium Chloride 209a**[107]

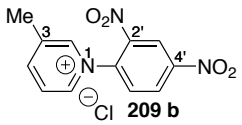


209 a

To a solution of 4-picoline (1.0 mL, 9.87 mmol, 1.0 equiv.) in 20.0 mL MeOH was added 1-chloro-2,4-dinitrobenzene (2.00 g, 9.87 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 96 h. Once cooled the reaction mixture was concentrated under reduced pressure to yield a crude black tar. The crude tar was purified *via* column chromatography *via* a gradient moving from 9:1 DCM:MeOH to 100% MeOH to yield *N*-(2,4-dinitrophenyl)-4-methylpyridinium chloride (**209a**, 1.63 g, 5.51 mmol, 56 %) as a black solid; $\mathbf{R_f}$ 0.22 (9:1 DCM:MeOH to 100% MeOH), **m.p** 82 - 84 °C (Lit.[107] **m.p.** 142 - 145 °C).

IR ν_{max}/cm^{-1} 3002, 2942, 1609, 1537, 1467, 1339; **^{1}H NMR** (400MHz, MeOD) δ; 9.28 (1H, d, J = 2.5 Hz, H-3'), 9.13 (2H, d, J = 7.0 Hz, H-2 and H-6), 8.93 (1H, dd, J = 3.0 Hz and 8.5 Hz, H-5'), 8.31 (1H, d, J = 8.5 Hz, H-6'), 8.24 (2H, d, J = 6.5 Hz, H-3 and H-5), 2.89 (3H, s, C**H**$_3$(Py.)); **^{13}C NMR** (100MHz, MeOD) δ; 21.4 (**C**H$_3$(Py.)), 121.8 (C-3'), 128.5 (C-3 and C-5), 129.7 (C-5'), 131.3 (C-6'), 138.6 (ArC), 143.3 (ArC), 144.5 (C-2 and C-6), 149.7 (ArC), 164.1 (ArC); **MS** m/z $[M]^+$ $C_{12}H_{10}N_3O_4^+$ requires 260.07, found 260.07.

***N*-(2,4-Dinitrophenyl)-3-methylpyridinium Chloride 209b**[108]

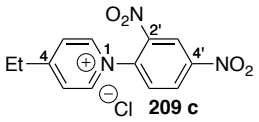


209 b

To a solution of 3-picoline (0.5 mL, 4.94 mmol, 1.0 equiv.) in 10.0 mL acetone was added 1-chloro-2,4-dintirobenzene (1.00 g, 4.94 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h. Once cooled the reaction mixture was filter to yield *N*-(2,4-dinitrophenyl)-3-methylpyridinium chloride (**209b**, 1.01 g, 3.90 mmol, 79 %) as an off-white solid; **m.p.** 221 - 223 °C (Lit.[108] **m.p.** 206 - 207 °C).

IR ν_{max}/cm^{-1} 3056, 2914, 1531, 1475, 1342; **^{1}H NMR** (400MHz, MeOD) δ; 9.30 (1H, bs, H-3'), 9.22 (1H, bs, H-2), 9.13 (1H, d, J = 6.0 Hz, H-6), 8.94 (1H, d, J = 8.5 Hz, H-5'), 8.81 (1H, d, J = 8.0 Hz, H-4), 8.32 - 8.28 (2H, m, H-5 and H-6'), 2.71 (3H, s, CH_3(Py.)); **^{13}C NMR** (100MHz, MeOD) δ; 17.1 (CH_3(Py.)), 121.8 (C-3'), 127.4 (C-5), 129.7 (C-5'), 131.2 (C-6'), 138.8 (ArC), 140.2 (ArC), 143.0 (C-6), 143.2 (ArC), 145.2 (C-2), 149.2 (C-4), 149.8 (ArC); **MS** m/z $[M]^+$ $C_{12}H_{10}N_3O_4^+$ requires 260.07, found 260.07.

***N*-(2,4-Dinitrophenyl)-4-ethylpyridinium Chloride 209c**[107]

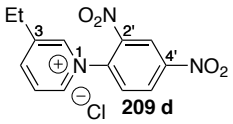


209 c

To a solution of 4-ethylpyridine (0.6 mL, 5.43 mmol, 1.1 equiv.) in 5.0 mL PE 40-60 °C was added 1-chloro-2,4-dintirobenzene (1.00 g, 4.94 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 96 h. Once cooled the reaction mixture was filter to yield *N*-(2,4-dinitrophenyl)-4-ethylpyridinium chloride (**209c**, 1.06 g, 3.43 mmol, 75 %) as a black solid; **m.p.** 154 - 157 °C (Lit.[107] **m.p.** 133 - 135 °C)

IR ν_{max}/cm^{-1} 3019, 2971, 1610, 1540, 1463, 1345; **^{1}H NMR** (400MHz, MeOD) δ; 9.29 (1H, d, J = 3.5 Hz, H-3'), 9.16 (2H, d, J = 6.0 Hz, H-2 and H-6), 8.93 (1H, dd, J = 3.0 Hz and 8.5 Hz, H-5'), 8.31 (1H, d, J = 8.5 Hz, H-6'), 8.28 (2H, d, J = 6.0 Hz, H-3 and H-5), 3.20 (2H, q, J = 8.5 Hz, C**H**$_2$CH$_3$(Py.)), 1.50 (3H, t, J = 7.5 Hz, CH$_2$C**H**$_3$(Py.)); **^{13}C NMR** (100MHz, MeOD) δ; 12.5 (**C**H$_2$CH$_3$(Py.)), 29.1 (CH$_2$**C**H$_3$(Py.)), 121.9 (C-3'), 127.5 (C-3 and C-5), 129.8 (C-5'), 131.3 (C-6'), 138.7 (ArC), 143.4 (ArC), 145.0 (C-2 and C-6), 149.9 (ArC), 169.1 (ArC); **MS** m/z $[M]^+$ $C_{13}H_{12}N_3O_4^+$ requires 274.08, found 274.08.

***N*-(2,4-Dinitrophenyl)-3-ethylpyridinium Chloride 209d**[109]

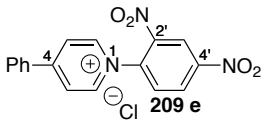


209 d

To a solution of 3-ethylpyridine (0.6 mL, 5.43 mmol, 1.1 equiv.) in 5.0 mL acetone was added 1-chloro-2,4-dintirobenzene (1.00 g, 4.94 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h. Once cooled the reaction mixture was filter to yield *N*-(2,4-dinitrophenyl)-3-ethylpyridinium chloride (**209d**, 0.92 g, 2.98 mmol, 61 %) as an off white powder; **m.p.** 197 - 199 °C (Lit.[109] **m.p.** 194 - 196 °C)

IR ν_{max}/cm^{-1} 3000, 2925, 1607, 1535, 1449, 1346; **^{1}H NMR** (400MHz, MeOD) δ; 9.31 (1H, d, J = 4.5 Hz, H-3'), 9.25 (1H, bs, H-2), 9.15 (1H, bd, J = 6.0 Hz, H-6), 8.95 (1H, dd, J = 3.5 Hz and 9.0 Hz, H-5'), 8.69 (1H, bd, J = 7.5 Hz, H-4), 8.24 (1H, t, J = 8.5 Hz, H-5), 8.31 (1H, d, J = 9.0 Hz, H-6'), 3.05 (2H, q, J = 7.5 Hz, C**H**$_2$CH$_3$(Py.)), 1.44 (3H, t, J = 7.5 Hz, CH$_2$C**H**$_3$(Py.)); **^{13}C NMR** (100MHz, MeOD) δ; 13.4 (CH$_2$**C**H$_3$(Py.)), 25.6 (**C**H$_2$CH$_3$(Py.)), 121.8 (C-3'), 127.7 (C-6'), 129.8 (C-5'), 131.1 (C-5), 138.8 (ArC), 143.2 (ArC), 143.3 (C-6), 144.8 (C-2), 145.7 (ArC), 148.2 (C-4), 149.7 (ArC); **MS** m/z $[M]^+$ $C_{13}H_{12}N_3O_4^+$ requires 274.08, found 274.08.

***N*-(2,4-Dinitrophenyl)-4-phenylpyridinium Chloride 209e**[110]

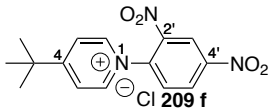


209 e

To a solution of 4-phenylpyridine (0.77 g, 4.94 mmol, 1.0 equiv.) in 10.0 mL acetone was added 1-chloro-2,4-dintirobenzene (1.00 g, 4.94 mmol, 1.0 equiv.) portion-wise. This was left to stir at reflux for 12 h. Once cooled the reaction mixture was filter to yield *N*-(2,4-dinitrophenyl)-4-phenylpyridinium chloride (**209e**, 0.86 g, 2.41 mmol, 49 %) as an off-white solid; **m.p.** 195 - 199 °C (Lit.[110] **m.p.** 186 - 187 °C)

IR ν_{max}/cm^{-1} 3002, 1608, 1533, 1337; **^{1}H NMR** (400MHz, MeOD) δ; 9.31 (1H, bs, C-3'), 9.26 (2H, d, J =6.5 Hz, H-2 and H-6), 8.95 (1H, d, J = 8.5 Hz, H-5'), 8.73 (2H, d, J = 6.5 Hz, H-3 and H-5), 8.34 (1H, d, J = 8.5 Hz, H-6'), 8.20 (2H, d, J = 7.5 Hz, ArH(Py.)), 7.80 - 7.72 (3H, m, ArH(Py.)); **^{13}C NMR** (100MHz, MeOD) δ; 121.8 (C-3'), 124.4 (C-3 and C-5), 128.4 (ArCH(Py.)), 129.7 (C-5'), 129.8 (ArCH(Py.)), 131.4 (C-6'), 133.1 (ArCH(Py.)), 133.4 (ArC), 138.6 (ArC), 143.4 (ArC), 145.5 (C-2 and C-6), 149.7 (ArC), 159.4 (ArC); **MS** m/z $[M]^+$ $C_{17}H_{12}N_3O_4^+$ requires 322.08, found 322.09.

***N*-(2,4-Dinitrophenyl)-4-*tert*-butylpyridinium Chloride 209f**[111]



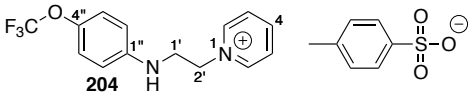
209 f

To a solution of 4-*tert*-butylpyridine (0.7 mL, 4.94 mmol, 1.0 equiv.) in 20.0 mL MeOH was added 1-chloro-2,4-dinitrobenzene (1.00 g, 4.94 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 65 h. Once cooled the reaction mixture was concentrated under reduced pressure to yield *N*-(2,4-dinitrophenyl)-4-*tert*-butylpyridinium chloride (**209f**, 1.37 g, 4.55 mmol, 92 %) as a yellow solid; **m.p.** 137 - 150 °C (Lit.[111] **m.p.** 154 -156 °C)

IR ν_{max}/cm^{-1} 3042, 2971, 1608, 1533, 1458, 1339; **^{1}H NMR** (400MHz, MeOD) δ; 9.29 (1H, d, J = 2.5 Hz, H-3'), 9.16 (2H, d, J = 7.0 Hz, H-2 and H-6), 8.93 (1H, dd, J = 2.5 Hz and 8.5 Hz, H-5'), 8.44 (2H, d, J = 7.0 Hz, H-3 and H-5), 8.29 (1H, d, J = 8.5 Hz, H-6'), 1.57 (9H, s, C(C**H**$_3$)$_3$(Py.)); **^{13}C NMR** (100MHz, MeOD) δ; 29.1 (C(**C**H$_3$)$_3$(Py.)), 122.6 (C-3'), 124.5 (ArC), 125.6 (C-3 and C-5), 130.5 (C-5'), 131.3 (C-6'), 138.5 (ArC), 142.8 (ArC), 144.3 (C-2 and C-6), 149.4 (ArC), 175.7 (ArC); **MS** m/z $[M]^+$ $C_{15}H_{16}N_3O_4$ requires 302.11, found 302.12.

Formation of Pyridinium Tosylates

***N*-(2-((4-Trifluoromethoxy)phenyl)amino)ethyl)pyridinium Tosylate 204**

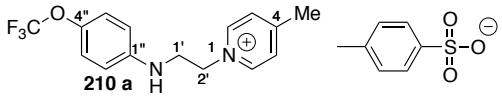


204

To a solution of **203** (1.99 g, 9.06 mmol, 3.0 equiv.) in 30.0 mL MeOH was added Zincke salt **138** (0.85 g, 3.02 mmol, 1.0 equiv.) portion-wise. This was stirred at RT for 2 h and then refluxed for a further 18 h. Once cooled the reaction mixture was, diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised pyridinium chloride as a crude yellow oil. To a solution of the crude uncyclised pyridinium chloride (0.96 g, 3.02 mmol, 1.0 equiv.) in 30.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.59 g, 3.02 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate was concentrated under reduced pressure to yield a crude yellow slurry, which was purified *via* crystallisation with 100 % EtOAc to yield *N*-(2-((4-trifluoromethoxy)phenyl)amino)ethyl)pyridinium tosylate (**204**, 1.07 g, 2.35 mmol, 78 %) as a fine pale yellow powder; **m.p.** 111 - 118 °C

IR ν_{max}/cm^{-1} 3291, 3060, 2855, 1609, 1506, 1251, 1160; **^{1}H NMR** (400MHz, MeOD) δ; 8.91 (2H, d, J = 6.0 Hz, H-2 and H-6), 8.58 (1H, t, J = 8.0 Hz, H-4), 8.09 (2H, t, J = 7.0 Hz, H-3 and H-5), 7.72 (2H, d, J = 8.0 Hz, TsH), 7.25 (2H, d, J = 8.0 Hz, TsH), 7.03 (2H, d, J = 9.0 Hz, H-3'' and H-5''), 6.65 (2H, d, J = 9.0 Hz, H-2'' and H-6''), 4.81 (2H, t, J = 5.0 Hz, H-2'), 3.79 (2H, t, J = 5.0 Hz, H-1'), 2.39 (3H, s, Ts(C**H**$_3$)); **^{13}C NMR** (100MHz, MeOD) δ; 21.2 (Ts(**C**H$_3$)), 44.4 (C-1'), 60.7 (C-2'), 113.1 (C-2'' and C-6''), 119.3 (ArC), 122.2 (C-3'' and C-5''), 125.8 (TsCH), 127.6 (C-3 and C-5), 129.0 (TsCH), 140.0 (ArC), 140.3 (Ar(O**C**F$_3$)), 143.4 (ArC), 144.4 (ArC), 145.7 (C-2 and C-6), 146.5 (C-4); **MS** m/z $[M]^+$ $C_{14}H_{14}F_3N_2O$ requires 283.11, found 283.10.

4-Methyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium Tosylate 210a



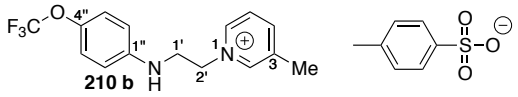
210 a

To a solution of **203** (0.30 g, 1.35 mmol, 1.1 equiv.) in 20.0 mL *n*-butanol was added Zincke salt **209a** (0.36 g, 1.23 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 18 h. Once cooled the reaction mixture was diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised 4-methyl pyridinium chloride as a crude mustard yellow oil. To a solution of the crude uncyclised 4-methyl pyridinium chloride (0.41 g, 1.23 mmol, 1.0 equiv.) in 20.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.24 g, 1.23 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate was concentrated under reduced pressure to yield a crude brown slurry,

which was purified *via* crystallisation with 100 % EtOAc to yield 4-methyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl pyridinium tosylate (**210a**, 0.24 g, 0.50 mmol, 41 %) as a fine pale yellow powder; **m.p.** 120 - 124 °C

IR ν_{max}/cm^{-1} 3302, 3053, 2913, 1610, 1476, 1518, 1255, 1141; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.99 (2H, d, J = 6.5 Hz, H-2 and H-6), 7.78 (2H, d, J = 8.0 Hz, TsH), 7.48 (2H, d, J = 6.5 Hz, H-3 and H-5), 7.17 (2H, d, J = 8.0 Hz, TsH), 6.83 (2H, d, J = 8.5 Hz, H-3'' and H-5''), 6.49 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 4.98 (2H, t, J = 5.0 Hz, H-2'), 3.68 (2H, t, J = 5.0 Hz, H-1'), 2.45 (3H, s, Ts(C**H**$_3$)), 2.37 (3H, s, C**H**$_3$(Py.)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 21.2 (**C**H_3(Py.)), 21.9 (Ts(**C**H_3)), 44.4 (C-1'), 59.9 (C-2'), 113.1 (C-2'' and C-6''), 119.3 (ArC), 122.3 (C-3'' and C-5''), 125.8 (TsCH), 128.2 (C-3 and C-5), 128.9 (TsCH), 140.0 (ArC), 140.3 (Ar(O**C**F_3)), 143.2 (ArC), 144.7 (C-2 and C-6), 146.5 (ArC), 158.5 (ArC); **MS** m/z $[M]^+$ $C_{15}H_{16}N_2OF_3$ requires 297.12, found 297.12.

3-Methyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium Tosylate 210b



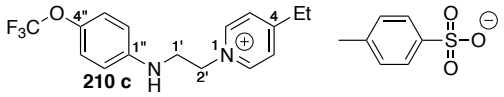
210 b

To a solution of **203** (0.48 g, 2.19 mmol, 1.1 equiv.) in 10.0 mL *n*-butanol was added Zincke salt **209b** (0.59 g, 2.19 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 18 h. Once cooled the reaction mixture was diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised 3-methyl pyridinium chloride as a crude yellow oil. To a solution of the crude uncyclised 3-methyl pyridinium chloride (0.66 g, 1.99 mmol, 1.0 equiv.) in 15.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.38 g, 1.99 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate was concentrated under reduced pressure to yield a crude brown slurry, which was purified *via* crystallisation with 100 % EtOAc to yield 3-methyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium tosylate (**210b**, 0.68 g, 1.45 mmol, 73 %) as a fine pale yellow powder; **m.p.** 221 - 223 °C

IR ν_{max}/cm^{-1} 3418, 3054, 2837, 1639, 1508, 1477, 1252, 1188; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 9.10 (1H, s, H-2), 9.04 (1H, d, J = 6.0 Hz, H-6), 7.88 (1H, d, J = 8.0 Hz, H-4), 7.80 (2H, d, J = 7.5 Hz, TsH), 7.59 (1H, t, J = 6.5 Hz, H-5), 7.18 (2H, d, J = 7.5 Hz, TsH), 6.80 (2H, d, J = 8.5 Hz, H-3'' and H-5''), 6.47 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 5.93 (1H, t, J = 6.0 Hz, N**H**), 5.04 (2H, t, J = 4.5 Hz, H-2'), 3.72 (2H, q, J = 5.0 Hz, H-1'), 2.38 (3H, s, C**H**$_3$(Py.)), 2.36 (3H, s, TsC(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 17.2 (**C**H_3(Py.)), 20.2 (Ts(**C**H_3)), 43.3 (C-1'), 59.6 (C-2'), 112.9 (C-2'' and C-6''), 119.2 (ArC), 122.1 (C-3'' and C-5''), 125.7 (TsCH), 126.9 (C-5),

128.9 (TsCH), 139.0 (ArC), 140.0 (ArC), 140.1 (Ar(O**C**F_3)), 142.8 (C-6), 143.3 (ArC), 145.0 (C-4), 145.3 (C-2), 146.6 (ArC); **MS** m/z $[M]^+$ $C_{15}H_{16}N_2OF_3$ requires 297.12, found 297.12.

4-Ethyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridin-1-ium Tosylate 210c

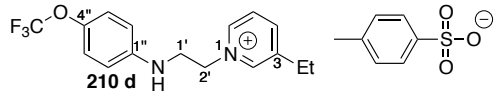


210 c

To a solution of **203** (1.02 g, 4.62 mmol, 3.0 equiv.) in 20.0 mL MeOH was added Zincke salt **209c** (0.48 g, 1.54 mmol, 1.0 equiv.) portion-wise. This was stirred for 2 h at RT and then refluxed for a further 16 h. Once cooled the reaction mixture was diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised 4-ethyl pyridinium chloride as a crude orange slurry. To a solution of the crude uncyclised 4-ethyl pyridinium chloride (0.53 g, 1.54 mmol, 1.0 equiv.) in 20.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.30 g, 1.54 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate was concentrated under reduced pressure to yield a crude orange slurry, which was purified *via* crystallisation with 100 % EtOAc to yield 4-ethyl-1-(2-((4-(trifluoromethoxy)phenyl)ethyl amino)ethyl pyridinium tosylate (**210c**, 0.13 g, 0.28 mmol, 18 %) as a fine pale orange powder; **m.p.** 142 - 145 °C

IR ν_{max}/cm^{-1} 3301, 3055, 2957, 1609, 1518, 1476, 1255, 1185; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 9.06 (2H, d, J = 6.0 Hz, H-2 and H-6), 7.80 (2H, d, J = 8.0 Hz, TsH), 7.55 (2H, d, J = 6.0 Hz, H-3 and H-5), 7.18 (2H, d, J = 8.0 Hz, TsH), 6.85 (2H, d, J = 8.5 Hz, H-3''and H-5''), 6.51 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 5.95 (1H, bs, N**H**), 5.04 (2H, t, J = 5.0 Hz, H-2'), 3.73 (2H, bs, H-1'), 2.78 (2H, q, J = 7.5 Hz, C**H**$_2$CH$_3$(Py.)), 2.36 (3H, s, Ts(C**H**$_3$)), 1.24 (3H, t, J = 7.5 Hz, CH$_2$C**H**$_3$(Py.)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 13.0 (CH$_2$**C**H$_3$(Py.)), 21.3 (Ts(C**H**$_3$)), 28.7 (**C**H$_2$CH$_3$(Py.)), 44.4 (C-1'), 60.2 (C-2'), 113.0 (C-2'' and C-6''), 119.3 (ArC), 122.2 (C-3'' and C-5''), 125.8 (TsCH), 126.8 (C-3 and C-5), 129.0 (TsCH), 139.9 (ArC), 140.0 (ArC), 140.3 (Ar(O**C**F_3)), 144.8 (C-2 and C-6), 144.9 (ArC), 146.5 (ArC); **MS** m/z $[M]^+$ $C_{16}H_{18}N_2OF_3$ requires 311.14, found 311.14.

3-Ethyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium Tosylate 210d



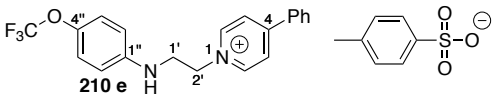
210 d

To a solution of **203** (1.01 g, 4.59 mmol, 3.0 equiv.) in 50.0 mL MeOH was added Zincke salt **209d** (0.47 g, 1.53 mmol, 1.0 equiv.) portion-wise. This was stirred at RT for 2 h and then refluxed for a further 16 h. Once cooled the reaction mixture was diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised 3-ethyl

pyridinium chloride as a crude yellow oil. To a solution of the crude uncyclised 3-ethyl pyridinium chloride (0.53 g, 1.53 mmol, 1.0 equiv.) in 25.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.30 g, 1.53 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate collected was concentrated under reduced pressure to yield a crude yellow powder, which was purified *via* crystallisation with 100 % EtOAc to yield 3-ethyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium tosylate (**210d**, 0.45 g, 0.94 mmol, 63 %) as a fine pale yellow powder; **m.p.** 104 - 106 °C

IR ν_{max}/cm^{-1} 3287, 3054, 2974, 1613, 1509, 1476, 1248, 1154; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 9.15 (1H, d, J = 6.0 Hz, H-6), 9.13 (1H, s, H-2), 7.91 (1H, d, J = 8.0 Hz, H-4), 7.80 (2H, d, J = 8.0 Hz, TsH), 7.62 (1H, t, J = 6.5 Hz, H-5), 7.17 (2H, d, J = 8.0 Hz, TsH), 6.77 (2H, d, 8.5 Hz, H-3'' and H-5''), 6.45 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 6.02 (1H, bt, J = 6.0 Hz, N**H**), 5.07 (2H, bt, J = 4.5 Hz, H-2'), 3.73 (2H, q, J = 5.0 Hz, H-1'), 2.68 (2H, q, J = 7.5 Hz, C**H**$_2$CH$_3$(Py.)), 2.35 (3H, s, Ts(C**H**$_3$)), 1.16 (3H, t, J = 7.5 Hz, CH$_2$C**H**$_3$(Py.)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 14.1 (CH$_2$**C**H$_3$(Py.)), 21.2 (Ts(**C**H$_3$)), 25.6 (**C**H$_2$CH$_3$(Py.)), 44.2 (C-1'), 60.7 (C-2'), 112.8 (C-2'' and C-6''), 119.3 (ArC), 121.8 (ArC), 122.1 (C-3'' and C-5''), 125.8 (TsCH), 127.1 (C-5), 128.9 (TsCH), 139.8 (ArC), 140.1 (ArC), 143.1 (C-6), 143.6 (Ar(O**C**F$_3$)), 144.8 (C-4), 145.0 (C-2), 146.7 (ArC); **MS** m/z $[M]^+$ $C_{16}H_{18}N_2OF_3$ requires 311.14, found 311.14.

4-Phenyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium Tosylate 210e



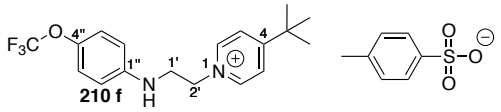
210 e

To a solution of **203** (0.19 g, 0.86 mmol, 2.0 equiv.) in 10.0 mL EtOH was added Zincke salt **209e** (0.15 g, 0.43 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 18 h. Once cooled the reaction mixture was diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised 4-phenyl pyridinium chloride as a crude orange oil. To a solution of the crude uncyclised 4-phenyl pyridinium chloride (0.17 g, 0.43 mmol, 1.0 equiv.) in 15.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.08 g, 0.43 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate collected was concentrated under reduced pressure to yield a crude yellow powder, which was purified *via* crystallisation with 100 % EtOAc to yield 4-phenyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium tosylate (**210e**, 0.10 g, 0.19 mmol, 44 %) as a fine pale yellow powder; **m.p.** 161 - 164 °C

IR ν_{max}/cm^{-1} 3304, 3043, 1609, 1504, 1254, 1186; **^{1}H NMR** (400MHz, MeOD) δ; 8.84 (2H, d, J = 7.0 Hz, H-2 and H-6), 8.36 (2H, d, J = 7.0 Hz, H-3 and H-5), 7.98 (2H, dd, J = 2.0 Hz and

5.0 Hz, TsH), 7.72 (2H, d, J = 8.0 Hz, ArH(Py.)), 7.68 - 7.62 (3H, m, ArH(Py.)), 7.24 (2H, d, J = 8.0 Hz, TsH), 7.04 (2H, d, J = 8.0 Hz, H-3'' and H-5''), 6.67 (2H, d, J = 8.0 Hz, H-2'' and H-6''), 4.79 (2H, t, J = 5.5 Hz H-2'), 3.80 (2H, t, J = 5.5 Hz, H-1'), 2.38 (3H, s, Ts(C**H**$_3$)); **^{13}C NMR** (100MHz, MeOD) δ; 19.9 (Ts(**C**H$_3$)), 43.6 (C-1'), 59.7 (C-2'), 112.6 (C-2'' and C-6''), 122.0 (ArC), 122.2 (C-3'' and C-5''), 124.4 (C-3 and C-5), 125.5 (ArCH(Py.)), 127.7 (TsCH), 128.4 (TsCH), 129.6 (ArCH(Py.)), 132.1 (ArCH(Py.)), 133.9 (ArC), 140.3 (ArC), 140.4 (Ar(O**C**F$_3$))), 142.1 (ArC), 144.9 (C-2 and C-6), 146.4 (ArC), 156.7 (ArC); **MS** m/z [M]$^+$ $C_{20}H_{18}N_2OF_3$ requires 359.14, found 359.14.

4-*tert*-Butyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium Tosylate 210f



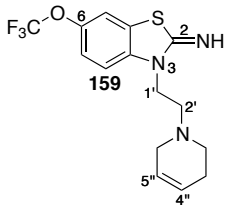
210 f

To a solution of **203** (0.16 g, 0.73 mmol, 1.1 equiv.) in 10.0 mL *n*-butanol was added Zincke salt **209f** (0.22 g, 0.66 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 18 h. Once cooled the reaction mixture was diluted with H_2O and washed several times with DCM until no further colour was observed in the organic layer. The aqueous layer was concentrated under reduced pressure to yield uncyclised 4-*tert*-butyl pyridinium chloride as a crude orange oil. To a solution of the crude uncyclised 4-*tert*-butyl pyridinium chloride (0.25 g, 0.66 mmol, 1.0 equiv.) in 20.0 mL EtOAc was added sodium *p*-toluenesulfonate (0.13 g, 0.66 mmol, 1.0 equiv.) portion-wise. This was stirred at reflux for 12 h then cooled and filtered. The filtrate collected was concentrated under reduced pressure to yield a crude yellow powder, which was purified *via* crystallisation with 100 % EtOAc to yield 4-*tert*-butyl-*N*-(2-((4-(trifluoromethoxy)phenyl)amino)ethyl)pyridinium tosylate (**210f**, 0.30 g, 0.58 mmol, 89 %) as a fine pale yellow powder; **m.p.** 81 - 86 °C

IR ν_{max}/cm^{-1} 3304, 3053, 2972, 1644, 1516, 1476, 1248, 1186; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 9.19 (2H, d, J = 6.5 Hz, H-2 and H-6), 7.82 (2H, d, J = 8.0 Hz, TsH), 7.62 (2H, d, J = 6.5 Hz, H-3 and H-5) 7.18 (2H, d, J = 8.0 Hz, TsH), 6.78 (2H, d, J = 8.5 Hz, H-3'' and H-5''), 6.48 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 5.98 (1H, t, J = 6.0 Hz, N**H**), 5.05 (2H, t, J = 5.0 Hz, H-2'), 3.70 (2H, q, J = 4.5 Hz, H-1'), 2.35 (9H, s, C(C**H**$_3$)$_3$(Py.)), 1.25 (3H, s, Ts(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 21.3 (C(**C**H$_3$)$_3$(Py.)), 29.9 Ts(**C**H$_3$)), 36.3 (ArC), 44.4 (C-1'), 59.9 (C-2'), 113.0 (C-2'' and C-6''), 122.2 (C-3'' and C-5''), 124.6 (C-3 and C-5), 125.9 (TsCH), 128.9 (TsCH), 132.2 (ArC) 140.0 (ArC), 140.3 (Ar(O**C**F$_3$)), 143.2 (ArC), 145.1 (C-2 and C-6), 146.5 (ArC), 170.8 (ArC); **MS** m/z [M]$^+$$C_{18}H_{22}N_2OF_3$ requires 339.17, found 339.17.

Formation of *N*-3 Riluzole Tetrahydropyridines *via* the Zincke Pathway

3-(2-(4,5-Dihydropyridin-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 159

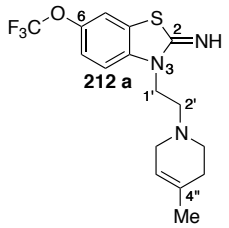


159

To a solution of **204** (0.32 g, 0.70 mmol, 1.0 equiv.) and KSCN (0.82 g, 8.40 mmol, 12.0 equiv.) in 6.0 mL AcOH was added a solution of Br_2 (0.1 mL, 0.70 mmol, 1.0 equiv.) in 6.0 mL AcOH dropwise. This was stirred at RT for 16 h then diluted with 36 mL H_2O, neutralised with 30 % NaOH solution and washed twice with 100 % EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield ring-cyclised pyridinium bromide as a crude oil. To a cooled solution of the crude ring-cyclised pyridinium bromide (0.29 g, 0.70 mmol, 1.0 equiv.) in 30.0 mL MeOH at 0 °C was added $NaBH_4$ (0.07 g, 0.96 mmol, 2.8 equiv.) portion-wise. This was warmed to RT and stirred for a further 18 h. This was then diluted with H_2O and washed five times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield 3-(2-(4,5-dihydropyridin-1-yl)-6-trifluoromethoxy)benzothiazol-2-imine (**159**, 0.16 g, 0.48 mmol, 69 %) as a pale yellow oil.

IR ν_{max}/cm^{-1} 3054, 2917, 1645, 1503, 1485, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.15 (1H, d, J = 1.5 Hz, H-7), 7.09 (1H, dd, J = 2.0 Hz and 8.5 Hz, H-5), 6.89 (1H, d, J = 8.5 Hz, H-4), 5.80 - 5.74 (1H, m, H-5''), 5.70 - 5.65 (1H, m, H-4''), 4.12 (2H, t, J = 7.5 Hz, H-2'), 3.11 (2H, quin, J = 3.0 Hz, H-6''), 2.73 (2H, appt, J = 6.5 Hz, H-2''), 2.70 (2H, appt, J = 6.0 Hz, H-1'), 2.21 - 2.17 (2H, m, J = 3.0 Hz, H-3''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 26.0 (C-3''), 41.1 (C-2'), 50.3 (C-1'), 52.9 (C-6''), 54.1 (C-2''), 109.4 (C-4), 115.2 (C-7), 119.6 (C-5), 121.8 (ArC), 123.8 (ArC), 124.9 (C-4''), 125.3 (C-5''), 139.4 (ArC), 143.4 (Ar(O**C**F_3)), 161.2 (ArC); **MS** m/z $[M]^+$ $C_{15}H_{17}F_3N_3OS$ requires 344.10, found 344.10.

3-(2-(4-Methyl-5,6-dihydropyridin-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 212a



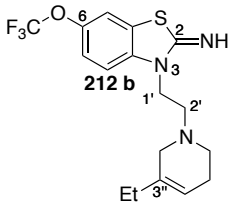
212 a

To a solution of **210a** (0.17 g, 0.39 mmol, 1.0 equiv.) and KSCN (0.45 g, 4.68 mmol, 12.0 equiv.) in 2.0 mL AcOH was added a solution of Br_2 (0.02 mL, 0.39 mmol, 1.0 equiv.) in 2.0 mL AcOH dropwise at RT and stirred for 16 h. This was then diluted with 12.0 mL H_2O,

neutralised with 30 % NaOH solution and washed twice with 100 % EtOAc. The organic layers were combined, dried over Na_2SO_4, filter and concentrated under reduced pressure to yield ring-cyclised 4-methylpyridinium bromide as a crude oil. To a cooled solution of the crude ring-cyclised 4-methylpyridinium bromide (0.17 g, 0.39 mmol, 1.0 equiv.) in 10.0 mL MeOH at 0 °C was added $NaBH_4$ (0.04 g, 1.10 mmol, 2.8 equiv.) portion-wise. This was warmed to RT and stirred for a further 18 h. This was then diluted with H_2O and washed five times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield 3-(2-(4-methyl-5,6-dihydropyridin-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**212a**, 0.04 g) as a crude red/brown oil.

IR ν_{max}/cm^{-1} 2911, 1650, 1585, 1485, 1255, 1160; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.14 (1H, s, H-7), 7.09 (1H, d, J = 9.0 Hz, H-5), 6.88 (1H, d, J = 9.0 Hz, H-4), 5.37 (1H, appquin, J = 1.5 Hz, H-3''), 4.12 (2H, t, J = 7.5 Hz, H-2'), 3.07 (2H, bs, H-2''), 2.71 - 2.68 (4H, m, H-1' and H-6''), 2.01 (2H, bs, H-5''), 1.69 (3H, s, C**H**$_3$(THPy.)); **MS** m/z $[M+H]^+$ $C_{16}H_{19}F_3N_3OS$ requires 358.11, found 358.12.

3-(2-(3-Ethyl-5,6-dihydropyridin-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 212b



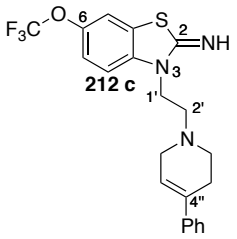
212 b

To a solution of **210d** (0.20 g, 0.40 mmol, 1.0 equiv.) and KSCN (0.47 g, 4.80 mmol, 12.0 equiv.) in 2.0 mL AcOH was added a solution of Br_2 (0.02 mL, 0.40 mmol, 1.0 equiv.) in 2.0 mL AcOH dropwise at RT and stirred for 16 h. This was then diluted with 12.0 mL H_2O, neutralised with 30 % NaOH solution and washed twice with 100 % EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield ring-cyclised 3-ethylpyridinium bromide as a crude oil. To a cooled solution of the crude ring-cyclised 3-ethyl pyridinium bromide (0.18 g, 0.40 mmol, 1.0 equiv.) in 10.0 mL MeOH at 0 °C was added $NaBH_4$ (0.04 g, 1.12 mmol, 2.8 equiv.) portion-wise. This was warmed to RT and stirred for a further 18 h. This was then diluted with H_2O and washed five times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield 3-(2-(3-ethyl-5,6-dihydropyridin-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**212b**, 0.08 g) as a crude orange oil.

IR ν_{max}/cm^{-1} 2934, 1648, 1486, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.15 (1H, bs, H-7), 7.09 (1H, bd, J = 8.5 Hz, H-5), 6.91 (1H, d, J = 8.5 Hz, H-4), 5.47 (1H, appsept, J = 1.5 Hz, H-4''), 4.12 (2H, t, J = 7.5 Hz, H-2'), 3.01 (2H, bs, H-2''), 2.74 (2H, t, J = 7.5 Hz, H-1'), 2.66 (2H, t, J

= 6.0 Hz, H-6''), 2.19 - 2.17 (2H, m, H-5''), 1.96 (2H, appq, J = 7.5 Hz, C**H**$_2$CH$_3$(THPy.)), 1.02 (3H, t, J = 7.5 Hz, CH$_2$C**H**$_3$(THPy.)); **MS** m/z $[M+H]^+$ $C_{17}H_{21}F_3N_3OS$ requires 372.13, found 372.14.

3-(2-(4-Phenyl-5,6-dihydropyridin-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 212c

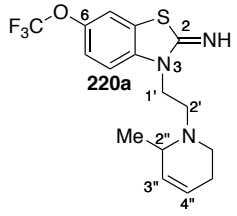


To a solution of **210e** (0.10 g, 0.20 mmol, 1.0 equiv.) and KSCN (0.22 g, 2.30 mmol, 12.0 equiv.) in 2.0 mL AcOH was added a solution of Br_2 (0.01 mL, 0.20 mmol, 1.0 equiv.) in 2.0 mL AcOH dropwise at RT and stirred for 16 h. This was then diluted with 12.0 mL H_2O, neutralised with 30 % NaOH solution and washed twice with 100 % EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield ring-cyclised 4-phenyl pyridinium bromide as a crude oil. To a cooled solution of the crude ring-cyclised 4-phenyl pyridinium bromide (0.10 g, 0.20 mmol, 1.0 equiv.) in 10.0 mL MeOH at 0 °C was added $NaBH_4$ (0.02 g, 0.56 mmol, 2.8 equiv.) portion-wise. This was warmed to RT and stirred for a further 18 h. This was then diluted with H_2O and washed five times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield 3-(2-(4-phenyl-5,6-dihydropyridin-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**212c**, 0.05 g) as a crude yellow oil.

IR ν_{max}/cm^{-1} 3058, 2923, 1654, 1485, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.40 - 7.24 (5H, m, ArH(THPy.)), 7.15 (1H, d, J = 1.5 Hz, H-7), 7.12 (1H, dd, J = 1.0 Hz and 8.5 Hz, H-5), 6.91 (1H, d, J = 8.5 Hz, H-4), 6.07 (1H, quin, J = 2.0 Hz, H-3''), 4.16 (2H, t, J = 7.5 Hz, H-2') 3.31 (2H, appq, J = 2.5 Hz, H-2''), 2.85 (2H, t, J = 6.0 Hz, H-6''), 2.79 (2H, t, J = 7.5 Hz, H-1'), 2.59 - 2.58 (2H, m, H-5''); **MS** m/z $[M+H]^+$ $C_{21}H_{21}F_3N_3OS$ requires 420.13, found 420.14.

Formation of *N*-3 Riluzole Tetrahydropyridines via the Grignard Pathway

3-(2-(2-Methyl-3,4-dihydropyridin-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 220a

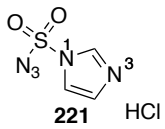


To a solution of **204** (0.35 g, 1.12 mmol, 1.0 equiv.) and KSCN (1.30 g, 13.44 mmol, 12.0 equiv.) in 3.0 mL AcOH was added a solution of Br_2 (0.06 mL, 1.12 mmol, 1.0 equiv.) in 3.0 mL AcOH dropwise. This was stirred at RT for 16 h. The reaction mixture was then diluted with 18.0 mL H_2O, neutralised with 30 % NaOH solution and washed twice with 100 % EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield ring-cyclised pyridinium bromide as a crude oil. To a solution of the crude ring-cyclised pyridinium bromide (0.51 g, 1.22 mmol, 1.0 equiv.) in 5.0 mL THF cooled to -20 °C under an atmosphere of N_2 was added methylmagnesium bromide (1.2 mL) dropwise. After 1 h at -20 °C the reaction mixture was warmed to 0 °C and stirred for a further 2 h. This was then diluted with 5.0 mL 90 % aq. MeOH and $NaBH_4$ (0.28 g, 7.29 mmol, 6.0 equiv.) was added portion-wise. This was stirred at reflux for 1 h and then concentrated under reduced pressure, diluted with 10.0 mL H_2O, and washed twice with 10.0 mL Et_2O. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield 3-(2-(2-methyl-3,4-dihydropyridin-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**220a**, 0.18 g) as a crude orange oil.

IR ν_{max}/cm^{-1} 2926, 1660, 1585, 1485, 1254, 1164; **^{1}H NMR** (400 MHz, $CDCl_3$) δ; 7.14 (1H, s, H-7), 7.08 (1H, d, J = 9.0 Hz, H-5), 6.89 (1H, d, 9.0 Hz, H-4), 5.73 - 5.69 (1H, m, H-3''), 5.00 (1H, ddd, J = 2.0 Hz, 4.0 Hz and 8.0 Hz, H-4''), 4.15 - 3.98 (2H, m, H-2'), 3.12 - 3.09 (1H, m, H-2''), 3.01 - 2.92 (2H, m, H-1'), 2.68 - 2.56 (2H, m, H-6''), 2.14 - 2.08 (2H, m, H-5''), 1.09 (3H, d, J = 6.5 Hz, CH_3(THPy.)); **MS** m/z $[M+H]^+$ $C_{16}H_{19}F_3N_3OS$ requires 358.11, found 358.11.

5.2 Triazole

Imidazole-1-Sulfonyl Azide Hydrochloride 221[117]



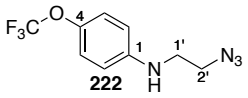
221 HCl

To a solution of NaN_3 (1.30 g, 20.00 mmol, 1.0 equiv.) in 20.0 mL MeCN under an atmosphere of N_2 cooled to 0 °C was added sulfuryl chloride (1.6 mL, 20.00 mmol, 1.0 equiv.) dropwise. The reaction mixture was warmed to RT and left to stir overnight. This was then cooled to 0 °C and imidazole (2.59 g, 38.00 mmol, 1.9 equiv.) was added portion-wise, and stirred for 3 h at RT. The reaction mixture was then diluted with 40.0 mL EtOAC, washed twice with 40.0 mL H_2O and then with 40.0 mL saturated $NaHCO_3$. The organic layer collected was dried over $MgSO_4$ and filtered. A solution of HCl in EtOH [obtained by the dropwise addition of AcCl (2.1 mL, 30.00 mmol, 1.5 equiv.) to 7.5 mL dry ethanol cooled to 0 °C] was added dropwise to the cooled filtrate to give a colourless precipitate which when filtered and washed three times with 10.0 mL EtOAc yielded imidazole-1-sulfonyl azide

hydrochloride (**221**, 1.64 g, 7.85 mmol, 48 %) as a colourless solid; **m.p.** 118 - 120 °C (Lit.[117] **m.p.** 100 - 102 °C)

IR ν_{max}/cm^{-1} 3054, 2169, 1322, 1159; **^{1}H NMR** (400MHz, D_2O) δ; 9.05 (1H, bs, H-5), 7.84 (1H, bs, H-4), 7.42 (1H, bs, H-2); **^{13}C NMR** (100MHz, D_2O) δ; 119.8 (C-4), 124.4 (C-2), 137.7 (C-5); **MS** m/z $[M+H]^+$ $C_3H_4N_5O_2S$ requires 174.01, found 174.01.

***N*-(2-Azidoethyl)-4-(trifluoromethoxy)aniline 222**

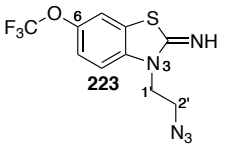


222

To a solution of **203** (0.83 g, 3.75 mmol, 1.0 equiv.), K_2CO_3 (1.21 g, 8.75 mmol, 2.3 equiv.) and $CuSO_4.5H_2O$ (9 mg, 0.04 mmol, 0.001 equiv.) in 20.0 mL MeOH under an atmosphere of N_2 was added imidazole-1-sulfonyl azide hydrochloride **221** (0.94 g, 4.50 mmol, 1.2 equiv.) portion-wise. The reaction mixture was stirred at RT for 2 h and then diluted with 60.0 mL H_2O, acidified with conc. HCl and washed three times with 40.0 mL EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a crude orange oil. The crude oil was purified *via* column chromatography using 9:1 PE 40-60 °C:EtOAC to yield *N*-(2-azidoethyl)-4-(trifluoromethoxy)aniline (**222**, 0.23 g, 0.93 mmol, 25 %) as a pale yellow oil; **R_f** 0.38 (9:1 PE 40-60 °C:EtOAc)

IR ν_{max}/cm^{-1} 3411, 3029, 2926, 2102, 1613, 1448, 1515, 1250; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.06 (2H, d, J = 8.0 Hz, H-3 and H-5), 6.61 (2H, d, J = 8.0 Hz, H-2 and H-6), 3.55 (2H, t, J = 6.0 Hz, H-1'), 3.34 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.3 (C-2'), 50.4 (C-1'), 113.5 (C-2 and C-6), 119.4 (ArC), 122.6 (C-3 and C-5), 140.9 (Ar(O**C**F_3)), 145.9 (ArC); **MS** m/z $[M+H]^+$ $C_9H_{10}N_4F_3O$ requires 247.08, found 247.08.

3-(2-Azidioethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 223



223

To a solution of uncyclised azide **222** (0.36 g, 1.46 mmol, 1.0 equiv.) and KSCN (1.70 g, 17.52 mmol, 12.0 equiv.) in 4.0 mL AcOH was added a solution of Br_2 (0.1 mL, 1.46 mmol, 1.0 equiv.) in 4.0 mL AcOH dropwise. This was stirred at RT for 2 h. The reaction mixture was then diluted with 24.0 mL H_2O, neutralised with 30 % NaOH solution and washed twice with EtOAc. The organic layers were combined dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield a crude yellow oil. The crude oil was purified *via* column chromatography using 100 % EtOAc to yield 3-(2-azidioethyl)-6-

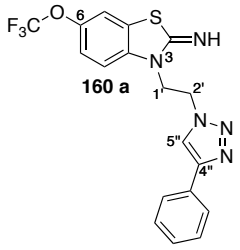
(trifluoromethoxy)benzothiazol-2-imine (**223**, 0.18 g, 0.59 mmol, 63 %) as a yellow oil; **R_f** 0.43 (100 % EtOAc)

IR ν_{max}/cm^{-1} 3044, 2929, 2110, 1610, 1584, 1485, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.20 (1H, bs, H-7), 7.14 (1H, bd, J = 9.0 Hz, H-5), 7.00 (1H, d, J = 9.0 Hz, H-4), 4.14 (2H, t, J = 6.0 Hz, H-1'), 3.73 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.7 (C-1'), 48.5 (C-2'), 109.7 (C-4), 115.3 (C-7), 119.7 (C-5), 121.8 (ArC), 123.8 (ArC), 139.2 (ArC), 143.8 (Ar(O**C**F_3)), 161.2 (ArC); **MS** m/z $[M+H]^+$ $C_{10}H_9N_5F_3OS$ requires 304.05, found 304.05.

General Procedure for the [3 + 2] Cycloaddition of Azides and Terminal Alkynes

To a solution of ring-cyclised azide **223** (1.0 equiv.) and alkyne (1.5 equiv.) in a 1:1 mixture of THF and H_2O heated to 20 °C was added 1M $CuSO_4$ (aq) (1.0 equiv.) and freshly prepared 1M sodium ascorbate (aq) (2.0 equiv.). The reaction was monitored by TLC. After total consumption of azide **223** the reaction mixture was concentrated under reduced pressure, diluted with 2:1 DCM: conc. NH_4OH and then left to stir for 30 mins at RT. This was then washed twice with H_2O and once with brine. The organic layer collected was dried over $MgSO_4$, filtered and concentrated under reduced pressure to yield the crude *N*-3 Riluzole 1,4-substituted-1,2,3-triazole, which was further purified *via* flash column chromatography in a suitable solvent system.

3-(2-(4-Phenyl-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160a

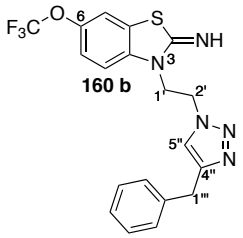


Using the general procedure; to a solution of azide **223** (0.27 g, 0.89 mmol, 1.0 equiv.) and phenylacetylene (0.2 mL, 1.32 mmol, 1.5 equiv.) in 12.0 mL H_2O and 12.0 mL THF heated to 20 °C was added 0.9 mL 1M $CuSO_4$ (aq) and 1.8 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 3 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-phenyl-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160a**, 0.14 g, 0.35 mmol, 39 %) as an off-white solid; **R_f** 0.21 (100 % EtOAc), **m.p.** 195 - 201 °C

IR ν_{max}/cm^{-1} 3250, 3085, 2954, 1615, 1584, 1483, 1259; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.65 (2H, d, J =18.5 Hz, ArH), 7.61 (1H, s, H-5''), 7.38 (2H, t, J = 7.0 Hz, ArH), 7.33 - 7.29 (1H, m, ArH), 7.09 (1H, bs, H-7), 6.94 (1H, bd, J = 8.5 Hz, H-5), 6.54 (1H, d, J = 9.0 Hz, H-4), 4.82

(2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.6 (C-2'), 46.9 (C-1'), 109.0 (C-4), 115.3 (C-7), 119.8 (C-5), 120.8 (C-5''), 121.7 (ArC), 123.2 (ArC), 125.8 (ArCH), 128.3 (ArCH), 128.8 (ArCH), 130.2 (ArC), 138.6 (ArC), 143.9 (Ar(O**C**F_3)), 148.2 (ArC), 161.0 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{15}F_3N_5OS$ requires 406.10, found 406.09.

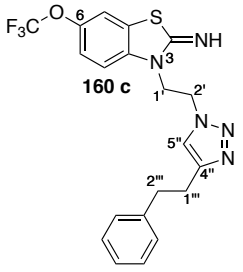
3-(2-(4-Benzyl-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160b



Using the general procedure; to a solution of azide **223** (0.22 g, 0.72 mmol, 1.0 equiv.) and 3-phenyl-1-propyne (0.1 mL, 1.07 mmol, 1.5 equiv.) in 8.6 mL H_2O and 8.6 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 3 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-benzyl-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160b**, 0.15 g, 0.34 mmol, 50 %) as a pale yellow solid; **R_f** 0.15 (100 % EtOAc), **m.p.** 103 - 106 °C

IR ν_{max}/cm^{-1} 3232, 3064, 2970, 1602, 1580, 1484, 1256; **^{1}H NMR** (400MHz, $CDCl_3$); 7.24 - 7.20 (3H, m, ArH), 7.10 (1H, bs, H-5''), 7.00 - 6.99 (3H, m, ArH and N**H**), 6.96 (1H, s, H-7), 6.91 (1H, bd, J = 9.0 Hz, H-5), 6.37 (1H, d, J = 9.0 Hz, H-4), 4.71 (2H, t, J = 6.0 Hz, H-1'), 4.37 (2H, t, J = 6.0 Hz, H-2'), 3.92 (2H, s, H-1'''); **^{13}C NMR** (100MHz, $CDCl_3$); 31.0 (C-1'''), 42.8 (C-2'), 45.7 (C-1'), 107.8 (C-4), 114.0 (C-5''), 118.1 (ArC) 118.7 (C-5), 120.7 (ArC), 121.7 (ArC), 122.1 (C-7), 125.5 (ArCH), 127.5 (ArCH), 127.5 (ArCH), 137.6 (ArC), 142.7 (Ar(O**C**F_3)), 147.1 (ArC), 159.6 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5OS$ requires 420.11, found 420.11.

3-(2-(4-Phenylethyl-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160c

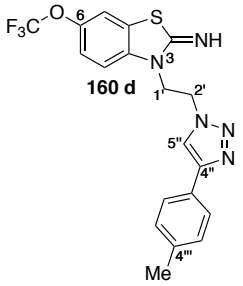


Using the general procedure; to a solution of azide **223** (0.18 g, 0.59 mmol, 1.0 equiv.) and 4-phenyl-1-butyne (0.1 mL, 0.89 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified

using 100 % EtOAc to yield 3-(2-(4-phenylethyl-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy) benzothiazol-2-imine (**160c**, 0.12 g, 0.28 mmol, 47 %) as an off-white solid; $\mathbf{R_f}$ 0.13 (100 % EtOAc), **m.p.** 153 - 157 °C

IR ν_{max}/cm^{-1} 3263, 3030, 2971, 1614, 1585, 1484, 1260; **^{1}H NMR** (500MHz, $CDCl_3$) δ; 7.25 (2H, appt, J = 7.5 Hz, ArH), 7.19 (1H, t, J = 6.0 Hz, ArH), 7.11 (1H, s, H-7), 7.07 (2H, d, J = 7.0 Hz, ArH), 7.03 (1H, bs, N**H**), 6.96 - 6.94 (2H, m, H-5 and H-5''), 6.39 (1H, d, J = 9.0 Hz, H-4), 4.69 (2H, t, J = 5.5 Hz, H-1'), 4.39 (2H, t, J = 6.0 Hz, H-2'), 2.89 (2H, t, J = 7.5 Hz, H-2'''), 2.77 (2H, t, J = 8.5 Hz, H-1'''); **^{13}C NMR** (125MHz, $CDCl_3$) δ; 26.1 (C-2'''), 34.4 (C-1'''), 42.8 (C-2'), 45.8 (C-1'), 107.9 (C-4), 114.1 (C-7), 118.1 (ArC), 118.8 (C-5), 122.1 (C-5''), 123.2 (ArC), 125.1 (ArCH), 127.3 (ArCH), 127.4 (ArCH), 137.7 (ArC), 140.0 (ArC), 142.7 (Ar(O**C**F_3)) 146.6 (ArC), 159.67 (ArC); **MS** m/z $[M+H]^+$ $C_{20}H_{19}F_3N_5OS$ requires 434.13 found 434.13.

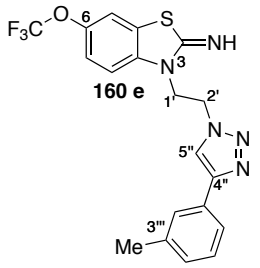
3-(2-(4-(*p*-Toly)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160d



Using the general procedure; to a solution of azide **223** (0.31 g, 1.02 mmol, 1.0 equiv.) and 4-ethynyltoluene (0.1 mL, 1.07 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(*p*-toly)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160d**, 0.17 g, 0.40 mmol, 56 %) as a pale yellow solid; $\mathbf{R_f}$ 0.27 (100 % EtOAc), **m.p.** 199 - 203 °C

IR ν_{max}/cm^{-1} 3273, 3014, 2943, 1626, 1586, 1479, 1386, 1252; **^{1}H NMR** (400MHz, $CDCl_3$); 7.57 (1H, s, H-5''), 7.54 (2H, d, J = 8.0 Hz, ArH), 7.19 (2H, d, J = 8.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.8 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 2.36 (3H, s, Ar(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$); 20.2 (Ar(**C**H_3)), 42.6 (C-2'), 45.8 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.1 (ArC), 118.8 (ArC), 119.4 (C-5), 120.6 (C-5''), 122.2 (ArC), 124.6 (ArCH), 126.3 (ArC), 128.4 (ArCH), 137.1 (ArC), 142.8 (Ar(O**C**F_3)), 147.2 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5OS$ requires 420.11, found 420.11.

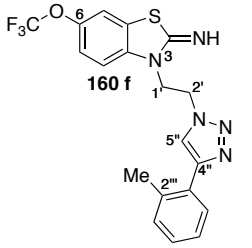
3-(2-(4-(*m*-Toly)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160e



Using the general procedure; to a solution of azide **223** (0.18 g, 0.60 mmol, 1.0 equiv.) and 3-ethynyltoluene (0.1 mL, 0.90 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(*m*-toly)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160e**, 0.15 g, 0.36 mmol, 59 %) as a pale yellow solid; $\mathbf{R_f}$ (100 % EtOAc), **m.p.** 178 - 182 °C

IR ν_{max}/cm^{-1} 3280, 3029, 2954, 1625, 1586, 1480, 1384, 1267; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.60 (1H, s, ArH), 7.52 (1H, s, H-5''), 7.41 (1H, d, J = 7.5 Hz, ArH), 7.26 (1H, appt, J = 6.5 Hz, ArH), 7.13 (1H, d, J = 7.5 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'), 2.37 (3H, s, Ar(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 20.3 (Ar(**C**H_3)), 42.6 (C-2'), 46.9 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.1 (ArC), 118.8 (ArC), 119.7 (C-5), 120.6 (C-5''), 122.2 (ArCH), 123.2 (ArC) 125.4 (ArCH), 127.6 (ArCH), 128.0 (ArCH), 129.0 (ArC), 137.5 (ArC), 142.8 (Ar(O**C**F_3)), 147.2 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5OS$ requires 420.11, found 420.11.

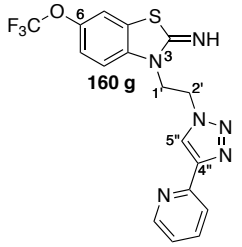
3-(2-(4-(*o*-Toly)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160f



Using the general procedure; to a solution of ring-cyclised azide **223** (0.22 g, 0.73 mmol, 1.0 equiv.) and 2-ethynyltoluene (0.1 mL, 1.09 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ and 1.5 mL freshly prepared 1M sodium ascorbate dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(*o*-toly)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160f**, 0.11 g, 0.27 mmol, 37 %) as an off white solid; $\mathbf{R_f}$ 0.15 (100 % EtOAc), **m.p.** 165 - 170 °C

IR ν_{max}/cm^{-1} 3227, 3077, 2960, 1601, 1581, 1484, 1382, 1260; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.45 - 7.42 (2H, m, ArH and H-5''), 7.24 - 7.19 (3H, m, ArH), 7.09 (2H, bs, H -7 and N**H**), 6.92 (1H, d, J = 8.5 Hz, H-5), 6.44 (1H, d, J = 9.0 Hz, H-4), 4.86 (2H, t, J = 5.5 Hz, H-1'), 4.47 (2H, t, H-2', J = 5.5 Hz), 2.16 (3H, s, Ar(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 19.6 (Ar(**C**H_3)), 42.9 (C-2'), 45.9 (C-1'), 107.8 (C-4), 114.2 (C-7), 118.07 (ArC), 118.9 (C-5), 120.6 (ArC), 121.9 (C-5''), 122.1 (ArC), 125.0 (ArCH), 127.3 (ArCH), 128.0 (ArCH), 128.5 (ArC), 129.6 (ArCH), 134.6 (ArC), 137.66 (ArC), 142.8 (Ar(O**C**F_3)), 146.4 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5OS$ requires 420.11, found 420.11.

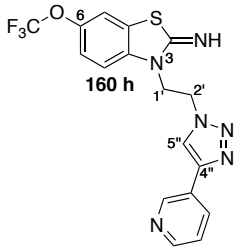
3-(2-(4-(Pyridin-2-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160g



Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.67 mmol, 1.0 equiv.) and 2-ethynylpyridine (0.1 mL, 1.01 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using a gradient solvent system from 100 % EtOAc to 100 % MeOH to yield 3-(2-(4-(pyridin-2-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160g**, 0.15 g, 0.36 mmol, 53 %) a pale yellow solid; **R_f** 0.07 (100 % EtOAc), **m.p.** 156 - 160 °C

IR ν_{max}/cm^{-1} 3206, 3056, 2950, 1602, 1581, 1484, 1260; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.52 (1H, d, J = 4.5 Hz, CH(Py.)), 8.08 (1H, d, J = 8.0 Hz, CH(Py.)), 8.02 (1H, s, H-5''), 7.75 (1H, t, J = 7.5 Hz, CH(Py.)), 7.21 (1H, t, J = 5.0 Hz, CH(Py.)), 7.11 (1H, bs, N**H**), 7.07 (1H, s, H-7), 6.91 (1H, d, J = 9.0 Hz, H-5), 6.54 (1H, d, J = 9.0 Hz, H-4), 4.83 (2H, d, J = 6.0 Hz, H-1'), 4.49 (2H, d, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.5 (C-2'), 46.0 (C-1'), 107.8 (C-4), 114.3 (C-7), 119.1 (C-5), 121.9 (CH(Py.)), 122.2 (CH(Py.)), 122.4 (C-5''), 135.8 (CH(Py.)), 137.6 (ArC), 142.7 (Ar(O**C**F_3)), 144.4 (ArC), 147.6 (ArC), 148.3 (CH(Py.)), 148.8 (ArC), 149.8 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{17}H_{14}F_3N_6OS$ requires 407.09, found 407.09.

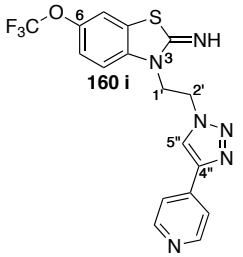
3-(2-(4-(Pyridin-3-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160h



Using the general procedure; to a solution of ring-cyclised azide **223** (0.16 g, 0.51 mmol, 1.0 equiv.) and 3-ethynylpyridine (0.08 g, 0.77 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2.5 h. After work-up the crude was column purified using a gradient solvent system from 100 % EtOAc to 100 % MeOH to yield 3-(2-(4-(pyridin-3-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160h**, 0.13 g, 0.32 mmol, 64 %) as a pale yellow solid; $\mathbf{R_f}$ 0.08 (100 % EtOAc), **m.p.** 195 - 197 °C

IR ν_{max}/cm^{-1} 3217, 3035, 2971, 1618, 1585, 1482, 1262; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.82 (1H, s, CH(Py.)), 8.56 (1H, d, J = 4.0 Hz, CH(Py.)), 8.04 (1H, d, J = 8.0 Hz, CH(Py.)), 7.69 (1H, s, H-5''), 7.33 (1H, q, J = 5.0 Hz, CH(Py.)), 7.09 (1H, s, H-7), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.84 (2H, t, J = 6.0 Hz, H-1'), 4.48 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 45.9 (C-1'), 107.8 (C-4), 114.3 (C-7), 118.8 (C-5), 120.1 (C-5''), 120.6 (ArC), 122.2 (ArC), 122.7 (ArC), 125.3 (CH(Py.)), 132.0 (CH(Py.)), 137.4 (ArC), 142.8 (Ar(O**C**F_3)), 144.0 (ArC), 145.9 (CH(Py.)), 148.3 (CH(Py.)), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{17}H_{14}F_3N_6OS$ requires 407.09, found 407.09.

3-(2-(4-(Pyridin-4-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160i

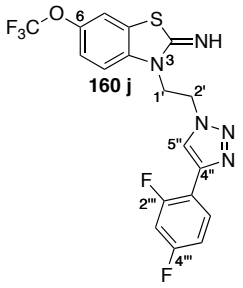


Using the general procedure; to a solution of ring-cyclised azide **223** (0.25 g, 0.82 mmol, 1.0 equiv.) and 4-ethynylpyridine (0.17 g, 1.23 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.8 mL 1M $CuSO_4$ (aq) and 1.6 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 9:1 DCM:MeOH to yield 3-(2-(4-(pyridin-4-yl)-1,2,3-triazol-1-

yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160i**, 0.15 g, 0.38 mmol, 46 %) as an off white solid; **R_f** 0.21 (9:1 DCM:MeOH), **m.p.** 209 - 211 °C

IR ν_{max}/cm^{-1} 3221, 3028, 2971, 1604, 1583, 1482, 1266; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.63 (2H, bs, CH(Py.)), 7.77 (1H, s, H-5''), 7.57 (2H, d, J = 5.0 Hz, CH(Py.)), 7.10 (1H, s, H-7), 6.91 (1H, d, J = 9.0 Hz, H-5), 6.54 (1H, d, J = 9.0 Hz, H-4), 4.85 (2H, t, J = 6.0 Hz, H-1'), 4.48 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 46.0 (C-1'), 107.8 (C-4), 114.3 (C-7), 118.0 (ArC), 118.8 (C-5), 120.6 (ArC), 121.2 (CH(Py.)), 122.2 (C-5''), 136.4 (ArC), 137.4 (ArC), 142.8 (Ar(O**C**F_3)), 144.6 (ArC), 149.4 (CH(Py.)), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{17}H_{14}F_3N_6OS$ requires 407.09, found 407.09.

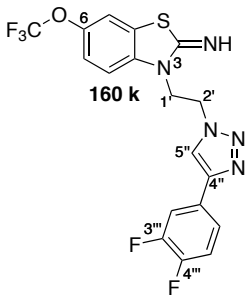
3-(2-(4-(2,4-Difluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160j



Using the general procedure; to a solution of ring-cyclised azide **223** (0.31 g, 1.02 mmol, 1.0 equiv.) and 1-ethynyl-2,4-difluorobenzene (0.2 mL, 1.53 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 1.0 mL 1M $CuSO_4$ (aq) and 2.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. The crude was column purified using 100 % EtOAc to yield 3-(2-(4-(2,4-difluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160j**, 0.22 g, 0.51 mmol, 50 %) as an off-white solid; **R_f** 0.33 (100 % EtOAc), **m.p.** 194 - 200 °C

IR ν_{max}/cm^{-1} 3234, 3028, 2957, 1601, 1578, 1483, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.16 (1H, td, J = 5.0 Hz and 9.0 Hz, ArH), 7.71 (1H, d, J = 3.5 Hz, H-5''), 7.09 (2H, bs, H-7 and N**H**), 6.97 (1H, td, J = 3.0 Hz and 9.0 Hz, ArH), 6.91 (1H, d, J = 7.0 Hz, H-5), 6.83 (1H, ddd, J = 2.5 Hz, 9.0 Hz and 11.0 Hz, ArH), 6.49 (1H, d, J = 9.0 Hz, H-4), 4.83 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2'), 46.9 (C-1'), 103.8 (ArC), 104.0 (ArCH), 104.3 (ArC), 108.8 (C-4), 111.9 (ArCH), 112.1 (ArC), 114.6 (ArC), 115.3 (C-7), 119.1 (ArC), 119.8 (C-5), 123.3 (C-5''), 123.4 (ArC), 128.8 (ArCH), 138.5 (ArC), 140.8 (ArC), 143.8 (Ar(O**C**F_3)); **MS** m/z $[M+H]^+$ $C_{18}H_{13}F_5N_5OS$ requires 442.08, found 442.08.

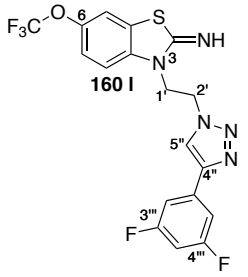
3-(2-(4-(3,4-Difluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160k



Using the general procedure; to a solution of ring-cyclised azide **223** (0.15 g, 0.51 mmol, 1.0 equiv.) and 3,4-difluorophenylacetylene (0.1 mL, 0.76 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(3,4-difluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160k**, 0.10 g, 0.23 mmol, 44 %) as an off-white solid; $\mathbf{R_f}$ 0.17 (100 % EtOAc), **m.p.** 179 - 184 °C

IR ν_{max}/cm^{-1} 3227, 3043, 1580, 1483, 1266; ^{1}**H NMR** (400MHz, $CDCl_3$) δ; 7.57 (1H, s, H-5''), 7.50 (1H, dddd, J = 2.0 Hz, 7.5 Hz and 10.0 Hz, ArH), 7.36 - 7.33 (1H, m, ArH), 7.16 (1H, q, J = 8.5 Hz, ArH), 7.10 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.51 (1H, d, J = 9.0 Hz, H-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); ^{13}**C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 45.9 (C-1'), 107.8 (C-4), 113.9 (ArC), 114.3 (ArCH), 116.6 (C-7), 116.7 (ArCH), 118.8 (C-5), 119.9 (C-5''), 120.7 (ArC), 122.2 (ArCH), 126.2 (ArC), 137.5 (ArC), 142.8 (Ar(O**C**F_3)), 145.3 (ArC), 148.2 (ArC), 150.6 (ArC), 159.7 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{13}F_5N_5OS$ requires 442.08, found 442.07.

3-(2-(4-(2,4-Difluorophenyl)-1,2,3-triazol-1-ly)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160l

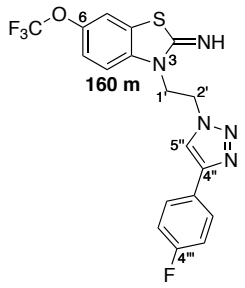


Using the general procedure; to a solution of ring-cyclised azide **223** (0.19 g, 0.61 mmol, 1.0 equiv.) and 1-ethynyl-3,5-difluorobenzene (0.1 mL, 0.92 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(2,4-difluorophenyl)-

1,2,3-triazol-1-ly)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160l**, 0.11 g, 0.25 mmol, 41 %) as an off-white solid; **R_f** 0.23 (100 % EtOAc), **m.p.** 183 - 185 °C

IR ν_{max}/cm^{-1} 3259, 3087, 2954, 1617, 1588, 1484, 1262; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.62 (1H, s, H-5''), 7.19 (2H, d, J = 6.5 Hz, ArH), 7.10 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.75 (1H, tt, J = 2.0 Hz and 9.0 Hz, ArH), 6.51 (1H, d, J = 9.0 Hz, H-4), 4.83 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 46.0 (C-1'), 102.5 (ArCH), 107.4 (ArCH), 107.6 (ArC), 107.8 (C-4), 114.3 (C-7), 118.8 (C-5), 120.4 (C-5''), 122.2 (ArC), 132.1 (ArC), 137.4 (ArC), 142.8 (Ar(O**C**F_3)), 145.1 (ArC), 161.1 (ArC), 163.5 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{13}F_5N_5OS$ requires 442.08, found 442.07.

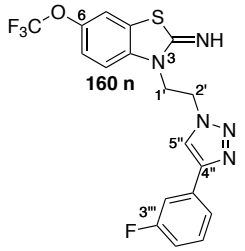
3-(2-(4-(4-Fluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160m



Using the general procedure; to a solution of ring-cyclised azide **223** (0.21 g, 0.70 mmol, 1.0 equiv.) and 1-ethynyl-4-fluorobenzene (0.1 mL, 1.05 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-fluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160m**, 0.16 g, 0.39 mmol, 56 %) as an off-white solid; **R_f** 0.15 (100 % EtOAc), **m.p.** 193 - 197 °C

IR ν_{max}/cm^{-1} 3230, 3014, 1601, 1582, 1484, 1261; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.61 (2H, dd, J = 5.5 Hz and 8.5 Hz, ArH), 7.56 (1H, s, H-5''), 7.09 (2H, bs, H-7 and N**H**), 7.06 (2H, appt, J = 8.5 Hz, ArH), 6.93 (1H, d, J = 8.5 Hz, H-5), 6.52 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.5 (C-2'), 45.9 (C-1'), 107.9 (C-4), 114.2 (C-7), 114.7 (ArCH), 114.9 (ArCH), 118.8 (C-5), 119.5 (C-5''), 122.2 (ArC), 126.4 (ArC), 126.5 (ArC), 137.5 (ArC), 142.8 (Ar(O**C**F_3)), 146.3 (ArC), 159.8 (ArC), 162.9 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}F_4N_5OS$ requires 424.09, found 424.08.

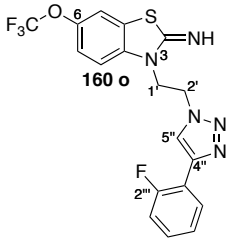
3-(2-(4-(3-Fluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160n



Using the general procedure; to a solution of ring-cyclised azide **223** (0.26 g, 0.84 mmol, 1.0 equiv.) and 1-ethynyl-3-fluorobenzene (0.2 mL, 1.27 mmol, 1.5 equiv.) in 15.0 mL H_2O and 15.0 mL THF heated to 20 °C was added 0.8 mL 1M $CuSO_4$ (aq) and 1.7 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(3-fluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160n**, 0.16 g, 0.37 mmol, 44 %) as an off-white solid; $\mathbf{R_f}$ 0.19 (100 % EtOAc), **m.p.** 185 - 190 °C

IR ν_{max}/cm^{-1} 3242, 3079, 2953, 1614, 1583, 1482, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.61 (1H, s, H-5''), 7.40 (2H, appt, J = 7.5 Hz, ArH), 7.34 (1H, appq, J = 8.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 7.01 (1H, t, J = 8.5 Hz, ArH), 6.94 (1H, d, J = 9.5 Hz, H-5), 6.52 (1H, d, J = 9.0 Hz, H-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2'), 47.0 (C-1'), 108.9 (C-4), 112.6 (ArC), 112.8 (ArCH), 115.0 (ArCH), 115.2 (ArC), 115.3 (C-7), 119.9 (C-5), 121.1 (C-5''), 121.3 (ArCH), 123.2 (ArC), 130.4 (ArCH), 132.2 (ArC), 138.5 (ArC), 143.9 (Ar(O**C**F_3)), 147.1 (ArC), 160.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}F_4N_5OS$ requires 424.09, found 424.08.

3-(2-(4-(2-Fluorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160o

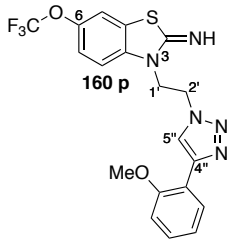


Using the general procedure; to a solution of ring-cyclised azide **223** (0.28 g, 0.93 mmol, 1.0 equiv.) and 1-ethynyl-2-fluorobenzene (0.2 mL, 1.39 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.9 mL 1M $CuSO_4$ (aq) and 1.9 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(2-fluorophenyl)-1,2,3-triazol-1-

yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160o**, 0.21 g, 0.49 mmol, 52 %) as an off-white solid; **R_f** 0.33 (100 % EtOAc), **m.p.** 172 - 175 °C

IR ν_{max}/cm^{-1} 3243, 3068, 2958, 1576, 1483, 1254; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.17 (1H, td, J = 1.5 Hz and 7.5 Hz, ArH), 7.77 (1H, d, J = 3.5 Hz, H-5''), 7.31 - 7.28 (1H, m, ArH), 7.22 (1H, t, J = 7.5 Hz, ArH), 7.09 - 7.00 (3H, m, ArH, N**H**, and H-7), 6.91 (1H, d, J = 8.5 Hz, H-5), 6.50 (1H, d, J = 9.0 Hz, H-4), 4.84 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.5 (C-2'), 45.8 (C-1'), 107.8 (C-4), 114.2 (C-7), 114.5 (ArCH), 117.1 (ArC), 118.7 (C-5), 122.2 (ArC), 122.8 (C-5''), 123.5 (ArCH), 126.7 (ArCH), 128.4 (ArCH), 137.5 (ArC), 140.4 (ArC), 142.7 (Ar(O**C**F_3)), 156.8 (ArC), 159.8 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}F_4N_5OS$ requires 424.09, found 424.08.

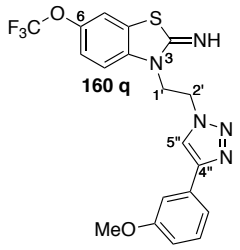
3-(2-(4-(2-Methoxyphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160p



Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.67 mmol, 1.0 equiv.) and 2-ethynylanisole (0.1 mL, 1.01 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(2-methoxyphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160p**, 0.22 g, 0.52 mmol, 77 %) as a pale yellow solid; **R_f** 0.20 (100 % EtOAc), **m.p.** 155 - 160 °C

IR ν_{max}/cm^{-1} 3287, 3011, 2835, 1632, 1585, 1481, 1245; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.21 (1H, d, J = 7.5 Hz, ArH), 7.86 (1H, s, H-5''), 7.29 (1H, appt, J = 8.0 Hz, ArH), 7.08 (2H, bs, C-7 and N**H**), 7.05 (1H, appt, J = 7.5 Hz, ArH), 6.92 (2H, d, J = 8.5 Hz, ArH and C-5), 6.51 (1H, d, J = 9.0 Hz, C-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 3.82 (3H, s, Ar(OC**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.6 (C-2'), 45.7 (C-1'), 54.2 (Ar(O**C**H_3)), 108.0 (C-4), 109.7 (ArCH), 114.0 (C-7), 118.0 (ArC), 118.8 (C-5), 120.0 (ArCH), 120.6 (ArC), 122.1 (ArC), 123.1 (C-5''), 126.6 (ArCH), 128.0 (ArCH), 137.7 (ArC), 142.4 (ArC), 142.7 (Ar(O**C**F_3)), 154.5 (ArC), 159.9 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5O_2S$ requires 436.11, found 436.10.

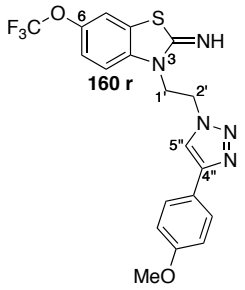
3-(2-(4-(3-Methoxyphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160q



Using the general procedure; to a solution of ring-cyclised azide **223** (0.18 g, 0.58 mmol, 1.0 equiv.) and 3-ethynylanisole (0.1 mL, 0.87 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(3-methoxyphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160q**, 0.17 g, 0.39 mmol, 67 %) as an off white solid; $\mathbf{R_f}$ 0.18 (100 % EtOAc), **m.p.** 170 - 174 °C

IR ν_{max}/cm^{-1} 3253, 3097, 2835, 1606, 1584, 1485, 1364, 1258; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.61 (1H, s, H-5''), 7.28 (2H, d, J = 5.0 Hz, ArH), 7.17 (1H, d, J = 7.5 Hz, ArH), 7.09 (2H, bs, C-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, C-5), 6.86 (1H, d, J = 7.0 Hz, ArH), 6.54 (1H, d, J = 9.0 Hz, C-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'), 3.85 (3H, s, Ar(OC**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.5 (C-2'), 45.9 (C-1'), 54.3 (Ar(O**C**H$_3$)), 108.0 (C-4), 109.8 (ArCH), 113.3 (ArCH), 114.2 (C-7), 117.1 (ArCH), 118.1 (ArC), 118.8 (C-5), 120.0 (C-5''), 120.6 (ArC), 122.2 (ArC), 128.8 (ArCH), 130.4 (ArC), 137.5 (ArC), 142.8 (Ar(O**C**F$_3$)) 147.0 (ArC), 158.9 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5O_2S$ requires 436.11, found 436.11.

3-(2-(4-(4-Methoxyphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160r

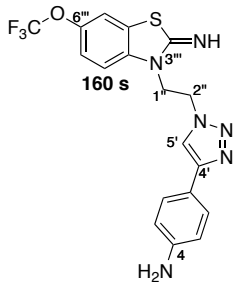


Using the general procedure; to a solution of ring-cyclised azide **223** (0.14 g, 0.46 mmol, 1.0 equiv.) and 4-ethynylanisole (0.1 mL, 0.69 mmol, 1.5 equiv.) in 5.0 mL H_2O and 5.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 0.9 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-methoxyphenyl)-1,2,3-triazolyl)ethyl)-6-

(trifluoromethoxy)benzothiazol-2-imine (**160r**, 0.09 g, 0.21 mmol, 47 %) an off white solid; $\mathbf{R_f}$ 0.21 (100 % EtOAc), **m.p.** 187 - 197 °C

IR ν_{max}/cm^{-1} 3262, 3029, 2835, 1619, 1584, 1485, 1362, 1265; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.57 (2H, d, J = 9.0 Hz, ArH), 7.52 (1H, s, H-5''), 7.09 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.0 Hz, H-5), 6.91 (2H, d, J = 9.0 Hz, ArH), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.80 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 3.83 (3H, s, Ar(OC**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.6 (C-2'), 45.8 (C-1'), 54.3 (Ar(O**C**H_3)), 108.0 (C-4), 113.2 (ArCH), 114.2 (C-7), 118.1 (ArC), 118.8 (C-5), 119.0 (C-5''), 121.8 (ArC), 122.2 (ArC), 126.0 (ArCH), 137.6 (ArC), 142.8 (Ar(O**C**F_3)), 147.0 (ArC), 158.6 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{17}F_3N_5O_2S$ requires 436.11, found 436.10.

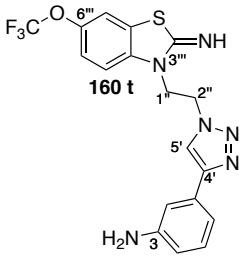
4-(1-(2-(2-Imino-6-trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)aniline 160s



Using the general procedure; to a solution of ring-cyclised azide **223** (0.24 g, 0.78 mmol, 1.0 equiv.) and 4-ethynylaniline (0.14 g, 1.17 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.8 mL 1M $CuSO_4$ (aq) and 1.6 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 4-(1-(2-(2-imino-6-trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)aniline (**160s**, 0.09 g, 0.21 mmol, 27 %) as a pale yellow solid; $\mathbf{R_f}$ 0.10 (100 % EtOAc), **m.p.** 196 - 200 °C

IR ν_{max}/cm^{-1} 3373, 3318, 3028, 2962, 1609, 1585, 1484, 1252; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.47 (1H, s, H-5'), 7.44 (2H, d, J = 8.5 Hz, ArH), 7.09 (2H, bs, H-7''' and N**H**), 6.94 (1H, d, J = 9.0 Hz, H-5''') 6.68 (2H, d, J = 8.5 Hz, ArH), 6.53 (1H, d, J = 9.0 Hz, H-4'''), 4.78 (2H, t, J = 6.0 Hz, H-1''), 4.45 (2H, t, J = 6.0 Hz, H-2''), 3.74 (2H, bs, Ar(N**H**$_2$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.6 (C-2''), 46.8 (C-1''), 109.1 (C-4'''), 115.2 (ArCH and C-7'''), 119.6 (C-5'), 119.9 (C-5'''), 120.6 (ArC), 121.7 (ArC), 123.2 (ArC), 127.0 (ArCH), 138.6 (ArC), 143.8 (Ar(O**C**F_3)), 146.6 (ArC), 148.5 (ArC), 160.9 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{16}F_3N_6OS$ requires 421.11, found 421.11.

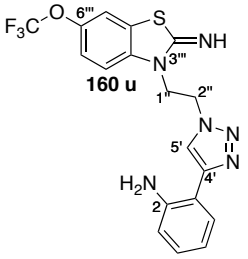
3-(1-(2-(2-Imino-6-trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)aniline 160t



Using the general procedure; to a solution of ring-cyclised azide **223** (0.25 g, 0.82 mmol, 1.0 equiv.) and 3-ethynylaniline (0.1 mL, 1.23 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.8 mL 1M $CuSO_4$ (aq) and 1.6 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(1-(2-(2-imino-6-trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)aniline (**160t**, 0.15 g, 0.37 mmol, 45 %) as a pale yellow solid; $\mathbf{R_f}$ 0.10 (100 % EtOAc), **m.p.** 196 - 199 °C

IR ν_{max}/cm^{-1} 3461, 3295, 3036, 2919, 1620, 1586, 1481, 1262; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.60 (1H, s, H-5'), 7.14 (1H, t, J = 7.5 Hz, ArH), 7.10 (2H, s, H-7''' and ArH), 6.95 (2H, d, J = 8.0 Hz, H-5''' and ArH), 6.64 (1H, dd, J = 1.5 Hz and 8.0 Hz, ArH), 6.56 (1H, d, J = 9.0 Hz, H-4'''), 5.30 (2H, s. Ar(N**H**$_2$)), 4.80 (2H, t, J = 6.0 Hz, H-1''), 4.47 (2H, t, J = 6.0 Hz, H-2''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.5 (C-2''), 45.8 (C-1''), 108.0 (C-4'''), 111.2 (ArCH), 114.0 (ArCH), 114.2 (C-7'''), 115.0 (ArCH), 118.8 (C-5'''), 119.8 (C-5'), 120.6 (ArC), 122.2 (ArC), 128.7 (ArCH), 130.1 (ArC), 137.5 (ArC), 142.8 (Ar(O**C**F_3)), 145.8 (ArC), 147.2 (ArC), 160.0 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{16}F_3N_6OS$ requires 421.11, found 421.11.

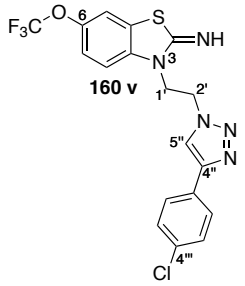
2-(1-(2-(2-Imino-6-trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)aniline 160u



Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.65 mmol, 1.0 equiv.) and 2-ethynylaniline (0.1 mL, 0.97 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 2-(1-(2-(2-imino-6-trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)aniline (**160u**, 0.13 g, 0.32 mmol, 49 %) as a pale yellow solid; $\mathbf{R_f}$ 0.20 (100 % EtOAc), **m.p.** 193 - 195 °C

IR ν_{max}/cm^{-1} 3345, 3278, 3029, 2971, 1610, 1585, 1484, 1255; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.63 (1H, s, H-5'), 7.12 - 7.08 (4H, m, H-7''', ArH and N**H**), 6.96 (1H, d, J = 8.5 Hz, H-5'''), 6.73 (1H, d, J = 8.0 Hz, ArH), 6.67 (1H, t, J = 7.5 Hz, ArH), 6.53 (1H, d, J = 9.0 Hz, H-4'''), 5.24 (2H, bs, Ar(N**H**$_2$)), 4.83 (2H, t, J = 6.0 Hz, H-1''), 4.47 (2H, t, J = 6.0 Hz, H-2''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2''), 46.9 (C-1''), 108.9 (C-4'''), 113.4 (ArC), 115.3 (C-7'''), 116.6 (ArCH), 117.4 (ArCH), 119.9 (C-5'''), 121.1 (C-5'), 123.3 (ArC), 127.8 (ArCH), 129.2 (ArCH), 138.6 (ArC), 143.9 (Ar(O**C**F$_3$)), 148.7 (ArC), 154.1 (ArC), 157.9 (ArC), 160.1 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{16}F_3N_6OS$ requires 421.11, found 421.11.

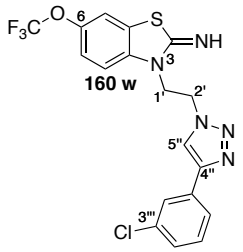
3-(2-(4-(4-Chlorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160v



Using the general procedure; to a solution of ring-cyclised azide **223** (0.25 g, 0.81 mmol, 1.0 equiv.) and 1-chloro-4-ethynylbenzene (0.17 g, 1.21 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.8 mL 1M $CuSO_4$ (aq) and 1.6 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-chlorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160v**, 0.19 g, 0.44 mmol, 55 %) as an off-white solid; **R$_f$** 0.18 (100 % EtOAc), **m.p.** 206 -209 °C

IR ν_{max}/cm^{-1} 3255, 3086, 2958, 1617, 1586, 1484, 1237, 827; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.60 (1H, s, H-5''), 7.58 (2H, d, J = 5.0 Hz, ArH), 7.35 (2H, d, J = 8.5 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2'), 46.9 (C-1'), 108.9 (C-4), 115.3 (C-7), 119.9 (C-5), 120.8 (C-5''), 123.2 (ArC), 127.0 (ArCH), 129.0 (ArCH), 132.9 (ArC), 134.0 (ArC), 138.5 (ArC), 143.9 (Ar(O**C**F$_3$)), 147.1 (ArC), 154.7 (ArC), 160.9 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}ClF_3N_5OS$ requires 440.06, found 440.05.

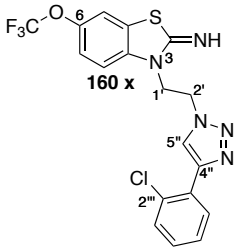
3-(2-(4-(3-Chlorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160w



Using the general procedure; to a solution of ring-cyclised azide **223** (0.30 g, 0.98 mmol, 1.0 equiv.) and 3-chloro-1-ethynylbenzene (0.2 mL, 1.47 mmol, 1.5 equiv.) in 15.0 mL H_2O and 15.0 mL THF heated to 20 °C was added 1.0 mL 1M $CuSO_4$ (aq) and 2.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(3-chlorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160w**, 0.25 g, 0.57 mmol, 58 %) as an off-white solid; $\mathbf{R_f}$ 0.23 (100 % EtOAc), **m.p.** 183 - 187 °C

IR ν_{max}/cm^{-1} 3242, 3043, 2953, 1612, 1581, 1481, 1254, 794; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.67 (1H, s, ArH), 7.63 (1H, s, H-5''), 7.53 (1H, d, J = 7.0 Hz, ArH), 7.33 - 7.26 (2H, m, ArH), 7.10 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2'), 47.0 (C-1'), 108.9 (C-4), 115.3 (C-7), 119.9 (C-4), 121.1 (C-5''), 123.2 (ArC), 123.8 (ArCH), 125.8 (ArCH), 128.3 (ArCH), 130.1 (ArCH), 131.9 (ArC), 134.8 (ArC), 138.5 (ArC), 143.8 (Ar(O**C**F_3)), 146.9 (ArC), 156.6 (ArC), 160.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}ClF_3N_5OS$ requires 440.06, found 440.05.

3-(2-(4-(2-Chlorophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160x

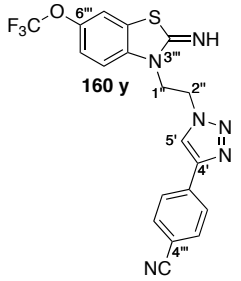


Using the general procedure; to a solution of ring-cyclised azide **223** (0.17 g, 0.56 mmol, 1.0 equiv.) and 1-chloro-2-ethynylbenzene (0.1 mL, 0.84 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(2-chlorophenyl)-1,2,3-triazol-

1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160x**, 0.13 g, 0.30 mmol, 53 %) as a pale yellow solid; $\mathbf{R_f}$ 0.30 (100 % EtOAc), **m.p.** 154 - 157 °C

IR ν_{max}/cm^{-1} 3245, 3093, 2955, 1614, 1584, 1484, 1253, 757; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.03 (1H, d, J = 8.0 Hz, ArH), 7.94 (1H, s, H-5''), 7.37 (1H, d, J = 8.0 Hz, ArH), 7.33 (1H, t, J = 7.0 Hz, ArH), 7.26 -7.23 (1H, m, ArH), 7.08 (2H, bs H-7 and N**H**), 6.90 (1H, d, J = 9.0 Hz, H-5), 6.42 (1H, d, J = 9.0 Hz), 4.86 (2H, d, J = 5.5 Hz, H-1'), 4.46 (2H, d, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.6 (C-2'), 45.9 (C-1'), 107.7 (C-4), 114.2 (C-7), 118.8 (C-4), 120.6 (ArC), 122.2 (ArC), 123.3 (C-5''), 126.1 (ArCH), 127.8 (ArC), 128.1 (ArCH), 128.8 (ArCH), 129.0 (ArCH), 130.2 (ArC), 137.6 (ArC), 142.8 (Ar(O**C**F_3)), 143.3 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}ClF_3N_5OS$ requires 440.06, found 440.05.

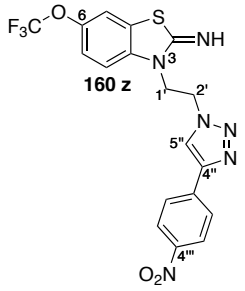
4-(1-(2-(2-Imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl) benzonitrile 160y



Using the general procedure; to a solution of ring-cyclised azide **223** (0.21 g, 0.70 mmol, 1.0 equiv.) and 4-ethynylbenzonitrile (0.13 g, 1.05 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 4-(1-(2-(2-imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl) benzonitrile4 (**160y**, 0.18 g, 0.32 mmol, 59 %) as an off-white solid; $\mathbf{R_f}$ 0.12 (100 % EtOAc), **m.p.** 193 - 198 °C

IR ν_{max}/cm^{-1} 3320, 3048, 2938, 2222, 1612, 1584, 1483, 1252; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.77 (2H, d, J = 8.0 Hz, ArH), 7.71 (1H, s, H-5'), 7.67 (2H, d, J = 8.0 Hz, ArH), 7.10 (2H, bs, H-7''' and N**H**), 6.93 (1H, d, J = 8.5 Hz, H-5'''), 6.53 (1H, d, J = 9.0 Hz, H-4'''), 4.85 (2H, t, J = 6.0 Hz, H-1''), 4.48 (2H, t, J = 6.0 Hz, H-2''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.4 (C-2''), 47.0 (C-1''), 108.8 (C-4'''), 111.7 (ArC), 115.4 (C-7'''), 118.7 (Ar(**C**N)), 119.1 (ArC), 119.9 (C-5'''), 121.9 (C-5'), 123.3 (ArC), 126.1 (ArCH), 132.7 (ArCH), 134.5 (ArC), 138.5 (ArC), 143.8 (Ar(O**C**F_3)), 146.3 (ArC), 160.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{14}F_3N_6OS$ requires 431.09, found 431.09.

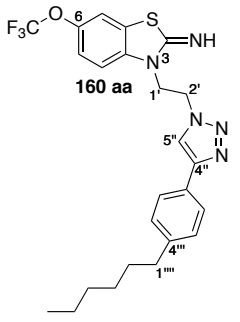
3-(2-(4-(4-Nitrophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160z



Using the general procedure; to a solution of ring-cyclised azide **223** (0.15 g, 0.48 mmol, 1.0 equiv.) and 1-ethynyl-4-nitrobenzene (0.11 g, 0.72 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-nitrophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160z**, 0.14 g, 0.30 mmol, 63 %) as a pale yellow solid; $\mathbf{R_f}$ 0.15 (100 % EtOAc), **m.p.** 181 - 186 °C

IR ν_{max}/cm^{-1} 3322, 3077, 2950, 1605, 1581, 1514, 1484, 1331, 1257; ^{1}**H NMR** (400MHz, $CDCl_3$) δ; 8.25 (2H, d, J = 8.5 Hz, ArH), 7.84 (2H, d, J = 8.5 Hz, ArH), 7.77 (1H, s, H-5''), 7.10 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 9.0 Hz, H-5), 6.55 (1H, d, J = 9.0 Hz, H-4), 4.86 (2H, t, J = 6.0 Hz, H-1'), 4.49 (2H, t, J = 6.0 Hz, H-2'); ^{13}**C NMR** (100MHz, $CDCl_3$) δ; 42.3 (C-2'), 46.0 (C-1'), 107.8 (C-4), 114.5 (C-7), 118.8 (C-5), 120.6 (ArC), 121.3 (C-5''), 122.3 (ArC), 123.2 (ArCH), 125.1 (ArC), 135.4 (ArC), 137.4 (ArC), 142.8 (Ar(O**C**F_3)), 144.8 (ArC), 146.3 (ArC), 159.7 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}F_3N_6O_3S$ requires 451.08, found 451.08.

3-(2-(4-(4-Hexylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160aa

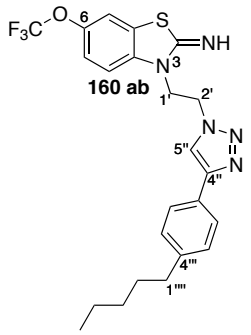


Using the general procedure; to a solution of ring-cyclised azide **223** (0.18 g, 0.61 mmol, 1.0 equiv.) and 1-ethynyl-4-hexylbenzene (0.2 mL, 0.91 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-hexylphenyl)-1,2,3-

triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160aa**, 0.21 g, 0.44 mmol, 72 %) as a pale yellow solid; **R_f** 0.24 (100 % EtOAc), **m.p.** 175 - 180 °C

IR ν_{max}/cm^{-1} 3276, 3044, 2926, 1626, 1585, 1481, 1381, 1255; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.57 (1H, s, H-5''), 7.55 (2H, d, J = 8.0 Hz, ArH), 7.19 (2H, d, J = 8.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 9.0 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, d, J = 6.0 Hz, H-1'), 4.45 (2H, t, J = 6.0 Hz, H-2'), 2.60 (2H, t, J = 7.5 Hz, H-1''''), 1.59 (2H, quin, H-2''''), 1.34 - 1.30 (6H, m, H-3'''', H-4'''' and H-5''''), 0.88 (3H, t, J = 6.5 Hz, H-6''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 13.1 (C-6''''), 21.6 (C-5''''), 27.9 (C-3''''), 30.3 (C-2''''), 30.7 (C-4''''), 34.7 (C-1''''), 42.6 (C-2'), 45.8 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.8 (C-5), 119.4 (C-5''), 120.6 (ArC), 122.2 (ArC), 124.6 (ArCH), 126.5 (ArC), 127.8 (ArCH), 137.6 (ArC), 142.2 (ArC), 142.8 (Ar(O**C**F_3)), 147.2 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{24}H_{27}F_3N_5OS$ requires 490.19, found 490.19.

3-(2-(4-(4-Pentylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ab

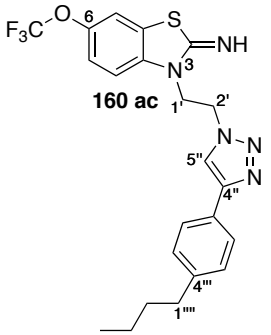


Using the general procedure; to a solution of ring-cyclised azide **223** (0.17 g, 0.55 mmol, 1.0 equiv.) and 1-ethynyl-4-pentylbenzene (0.2 mL, 0.82 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-pentylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ab**, 0.18 g, 0.38 mmol, 68 %) as a pale yellow solid; **R_f** 0.21 (100 % EtOAc), **m.p.** 184 - 187 °C

IR ν_{max}/cm^{-1} 3276, 3044, 2926, 1626, 1585, 1481, 1382, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.59 (1H, s, H-5''), 7.56 (2H, d, J = 8.0 Hz, ArH), 7.19 (2H, d, J = 8.0 Hz, ArH), 7.10 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.55 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 2.60 (2H, t, J = 7.5 Hz, H-1''''), 1.65 - 1.58 (2H, m, H-2''''), 1.33 - 1.32 (4H, m, H-3'''' and H-4'''') 0.89 (3H, t, J = 6.5 Hz, H-5''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 13.2 (C-5''''), 21.5 (C-4''''), 30.0 (C-2''''), 30.4 (C-3''''), 34.7 (C-1''''), 42.6 (C-2'), 45.8 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.8 (C-5), 119.4 (C-5''), 120.6 (ArC), 123.2 (ArC), 124.6

(ArCH), 126.5 (ArC), 127.8 (ArCH), 137.6 (ArC), 142.2 (ArC), 142.8 (Ar(O**C**F_3)), 147.2 (ArC), 159.9 (ArC); **MS** m/z $[M+H]^+$ $C_{23}H_{25}F_3N_5OS$ requires 476.18, found 476.17.

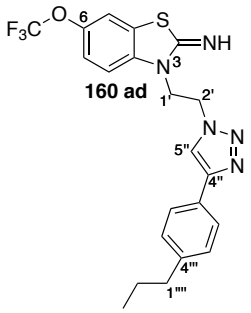
3-(2-(4-(4-Butylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ac



Using the general procedure; to a solution of ring-cyclised azide **223** (0.15 g, 0.49 mmol, 1.0 equiv.) and 1-butyl-4-ethynylbenzene (0.1 mL, 0.74 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-butylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ac**, 0.16 g, 0.35 mmol, 72 %) as a pale yellow solid; $\mathbf{R_f}$ 0.21 (100 % EtOAc), **m.p.** 183 - 186 °C

IR ν_{max}/cm^{-1} 3230, 3048, 2967, 1617, 1581, 1484, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.58 (1H, s, H-5"), 7.56 (2H, d, J = 7.5 Hz, ArH), 7.19 (2H, d, J = 7.5 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 7.5 Hz, H-5), 6.54 (1H, d, J = 8.5 Hz, H-4), 4.81 (2H, bs, H-1'), 4.48 (2H, bs, H-2'), 2.61 (2H, t, J = 7.5 Hz, H-1''''), 1.60 (2H, quin, J = 7.5 Hz, H-2''''), 1.36 (2H, sext, J = 7.5 Hz, H-3''''), 0.92 (3H, t, J = 7.5 Hz, H-4''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 12.9 (C-4''''), 21.3 (C-3''''), 32.5 (C-2''''), 34.4 (C-1''''), 42.6 (C-2'), 45.8 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.8 (C-5), 119.5 (C-5"), 120.6 (ArC), 122.2 (ArC), 124.6 (ArCH), 126.5 (ArC), 127.8 (ArCH), 137.6 (ArC), 142.2 (ArC), 142.8 (Ar(O**C**F_3)), 147.3 (ArC), 160.3 (ArC); **MS** m/z $[M+H]^+$ $C_{22}H_{23}F_3N_5OS$ requires 462.16, found 462.16.

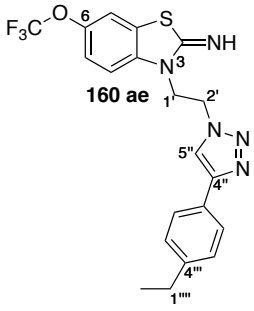
3-(2-(4-(4-Propylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ad



Using the general procedure; to a solution of ring-cyclised azide **223** (0.18 g, 0.56 mmol, 1.0 equiv.) and 1-ethynyl-4-propylbenzene (0.1 mL, 0.87 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-propylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ad**, 0.19 g, 0.42 mmol, 75 %) as an off-white solid; $\mathbf{R_f}$ 0.23 (100 % EtOAc), **m.p.** 190 - 196 °C

IR ν_{max}/cm^{-1} 3274, 3043, 2931, 1626, 1585, 1480, 1380, 1252; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.59 (3H, d, J = 8.0 Hz, H-5'' and ArH), 7.19 (2H, d, J = 8.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.93 (1H, d, J = 9.0 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 2.59 (2H, t, J = 7.5 Hz, H-1''''), 1.63 (2H, sext, J = 7.5 Hz, H-2''''), 0.94 (3H, t, J = 7.5 Hz, H-3''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 12.7 (C-3''''), 23.4 (C-2''''), 36.8 (C-1''''), 42.6 (C-2'), 45.8 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.8 (C-5), 119.5 (C-5''), 120.6 (ArC), 122.2 (ArC), 124.6 (ArCH), 126.6 (ArC), 127.8 (ArCH), 137.6 (ArC), 141.9 (ArC), 142.8 (Ar(O**C**F_3)), 147.2 (ArC), 159.9 (ArC); **MS** m/z $[M+H]^+$ $C_{21}H_{21}F_3N_5OS$ requires 448,14, found 448.14.

3-(2-(4-(4-Ethylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ae

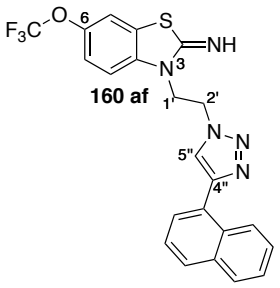


Using the general procedure; to a solution of ring-cyclised azide **223** (0.19 g, 0.62 mmol, 1.0 equiv.) and 1-ethyl-4-ethynylbenzene (0.1 mL, 0.93 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared

1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-ethylphenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ae**, 0.17 g, 0.40 mmol, 67 %) as an off-white solid; **R_f** 0.20 (100 % EtOAc), **m.p.** 189 - 193 °C

IR ν_{max}/cm^{-1} 3278, 3044, 2930, 1626, 1585, 1481, 1382, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.57 (3H, d, J = 7.0 Hz, H-5'' and ArH), 7.21 (2H, d, J = 8.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.93 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 2.66 (2H, q, J = 7.5 Hz, H-1''''), 1.24 (3H, t, J = 7.5 Hz, H-2''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 14.5 (C-2''''), 27.6 (C-1''''), 42.6 (C-2'), 45.8 (C-1'), 108.0 (C-7), 114.2 (C-4), 118.8 (C-5), 119.5 (C-5''), 120.6 (ArC), 122.2 (ArC), 124.7 (ArCH), 126.5 (ArC), 127.3 (ArCH), 137.6 (ArC), 142.8 (Ar(O**C**F_3)), 143.5 (ArC), 147.2 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{20}H_{19}F_3N_5OS$ requires 434.13, found 434.12.

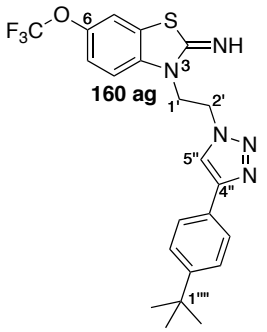
3-(2-(4-(Napthalen-1-yl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160af



Using the general procedure; to a solution of ring-cyclised azide **223** (0.17 g, 0.55 mmol, 1.0 equiv.) and 1-ethynylnapthalene (0.1 mL, 0.83 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(napthalen-1-yl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160af**, 0.15 g, 0.34 mmol, 61 %) as a red solid; **R_f** 0.20 (100 % EtOAc), **m.p.** 161 - 163 °C

IR ν_{max}/cm^{-1} 3273, 3014, 2939, 1632, 1582, 1481, 1254; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.87 - 7.84 (3H, m, ArH), 7.60 (1H, s, H-5''), 7.52 - 7.39 (4H, m, ArH), 7.12 (2H, bs, H-7 and N**H**), 6.95 (1H, d, J = 8.5 Hz, H-5), 6.49 (1H, d, J = 9.0 Hz, H-4), 4.93 (2H, t, J = 5.5 Hz, H-1'), 4.52 (2H, t, J = 5.5 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.9 (C-2'), 46.0 (C-1'), 107.8 (C-4), 114.2 (C-7), 118.8 (C-4), 120.6 (ArC), 122.2 (ArC), 122.8 (C-5''), 123.9 (ArCH), 124.2 (ArCH), 125.0 (ArCH), 125.5 (ArCH), 126.2 (ArCH), 126.6 (ArC), 127.3 (ArCH), 127.9 (ArCH), 130.0 (ArC), 132.7 (ArC), 138.6 (ArC), 142.8 (Ar(O**C**F_3)), 146.0 (ArC), 159.7 (ArC); **MS** m/z $[M+H]^+$ $C_{22}H_{17}F_3N_5OS$ requires 456.11, found 456.11.

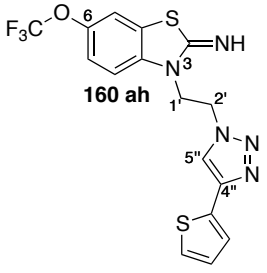
3-(2-(4-(4-(*tert*-Butyl)phenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ag



Using the general procedure; to a solution of ring-cyclised azide **223** (0.21 g, 0.68 mmol, 1.0 equiv.) and 4-*tert*-butylphenylacetylene (0.2 mL, 1.01 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-(*tert*-butyl)phenyl)-1,2,3-triazolyl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ag**, 0.23 g, 0.50 mmol, 73 %) as a pale yellow solid; $\mathbf{R_f}$ 0.24 (100 % EtOAc), **m.p.** 176 - 180 °C

IR ν_{max}/cm^{-1} 3321, 3007, 2953, 1604, 1583, 1483, 1361, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.58 (3H, d, J = 7.0 Hz, ArH and H-5''), 7.40 (2H, d, J = 8.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.93 (1H, d, J = 9.0 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.81 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'), 1.32 (9H, s, ArC(C**H**$_3$)$_3$); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 30.2 (ArC(**C**H$_3$)$_3$), 33.6 (ArC), 42.6 (C-2'), 45.9 (C-1'), 107.8 (C-4), 114.2 (C-7), 118.8 (C-5), 119.5 (C-5''), 120.6 (ArC), 122.2 (ArC), 124.5 (ArCH), 124.7 (ArCH), 126.3 (ArC), 137.6 (ArC), 142.8 (Ar(O**C**F$_3$)), 147.1 (ArC), 150.4 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{22}H_{23}F_3N_5OS$ requires 462.16, found 462.16.

3-(2-(4-(Thiophen-2-yl)-1,2,3-triazol-1-ly)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ah

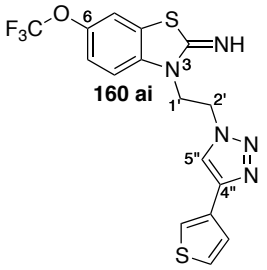


Using the general procedure; to a solution of ring-cyclised azide **223** (0.21 g, 0.70 mmol, 1.0 equiv.) and 2-ethynylthiophene (0.1 mL, 1.05 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude

was column purified using 100 % EtOAc to yield 3-(2-(4-(thiophen-2-yl)-1,2,3-triazol-1-ly)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ah**, 0.15 g, 0.37 mmol, 53 %) as a pale yellow solid; **R_f** 0.19 (100 % EtOAc), **m.p.** 172 - 176 °C

IR ν_{max}/cm^{-1} 3245, 3068, 2954, 1605, 1583, 1483, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.54 (1H, s, H-5''), 7.28 - 7.26 (1H, m, ArH), 7.23 (1H, d, J = 3.0 Hz, ArH), 7.10 (2H, bs, H-7 and N**H**), 7.03 (1H, t, J = 4.0 Hz, ArH), 6.96 (1H, d, J = 8.5 Hz, H-5), 6.54 (1H, d, J = 9.0 Hz, H-4), 4.80 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 45.9 (C-1'), 107.9 (C-4), 114.2 (C-7), 118.8 (C-5), 119.3 (C-5''), 120.6 (ArC), 122.2 (ArC), 123.3 (ArCH), 124.2 (ArCH), 126.5 (ArCH), 131.3 (ArC), 137.5 (ArC), 142.0 (ArC), 142.8 (Ar(O**C**F_3)), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{16}H_{13}F_3N_5OS_2$ requires 412.05, found 412.05.

3-(2-(4-(Thiophen-3-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ai

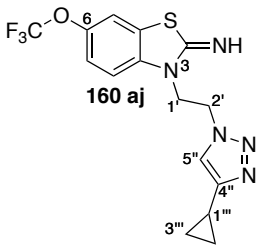


160 ai

Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.67 mmol, 1.0 equiv.) and 3-ethynylthiophene (0.1 mL, 1.00 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(thiophen-3-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ai**, 0.15 g, 0.37 mmol, 55 %) as an off white solid; **R_f** 0.17 (100 % EtOAc), **m.p.** 185 - 188 °C

IR ν_{max}/cm^{-1} 3246, 3081, 2954, 1615, 1585, 1483, 1258; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.55 (1H, d, J = 2.0 Hz, ArH), 7.53 (1H, s, H-5''), 7.35 - 7.33 (1H, m, ArH), 7.30 (1H, d, J = 5.0 Hz, ArH), 7.09 (2H, bs, H-7 and N**H**), 6.95 (1H, d, J = 8.5 Hz, H-5), 6.55 (1H, d, J = 9.0 Hz, H-4), 4.80 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.5 (C-2'), 45.8 (C-1'), 108.0 (C-4), 114.2 (C-7), 118.8 (C-5), 119.6 (C-5''), 120.2 (ArCH), 120.6 (ArC), 122.2 (ArC), 124.7 (ArCH), 125.3 (ArCH), 130.3 (ArC), 137.5 (ArC), 142.8 (Ar(O**C**F_3)), 143.2 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{16}H_{13}F_3N_5OS_2$ requires 412.05, found 412.05.

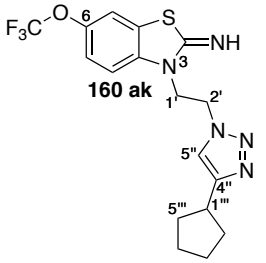
3-(2-(4-Cyclopropyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160aj



Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.66 mmol, 1.0 equiv.) and cyclopropylacetylene (0.1 mL, 0.99 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-cyclopropyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160aj**, 0.16 g, 0.44 mmol, 67 %) as a pale yellow solid; $\mathbf{R_f}$ 0.10 (100 % EtOAc), **m.p.** 149 - 152 °C

IR ν_{max}/cm^{-1} 3220, 3089, 2950, 1601, 1580, 1484, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.10 (1H, s, H-7), 7.01 (1H, s, H-5''), 6.93 (1H, d, J = 9.0 Hz, H-5), 6.33 (1H, d, J = 9.0 Hz, H-4), 4.70 (2H, t, J = 5.5 Hz, H-1'), 4.37 (2H, t, J = 6.0 Hz, H-2'), 1.78 (1H, tt, J = 5.0 Hz and 13.5 Hz, H-1'''), 0.83 (2H, dt, J = 4.5 Hz and 15.0 Hz, H-2''' and H-3'''), 0.57 (2H, dt, J = 4.5 Hz and 11.0 Hz, H-2''' and H-3'''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 5.3 (C-1'''), 6.5 (C-2''' and C-3''''), 42.7 (C-2'), 45.7 (C-1'), 107.8 (C-4), 114.1 (C-7), 118.5 (C-5), 119.8 (C-5''), 120.7 (ArC), 122.0 (ArC), 137.6 (ArC), 142.7 (Ar(O**C**F_3)), 149.7 (ArC), 159.6 (ArC); **MS** m/z $[M+H]^+$ $C_{15}H_{15}F_3N_5OS$ requires 370.10, found 370.10.

3-(2-(4-Cyclopentyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ak

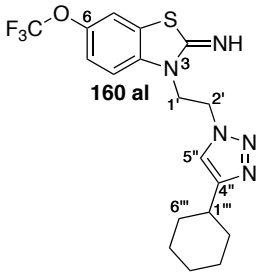


Using the general procedure; to a solution of ring-cyclised azide **223** (0.21 g, 0.68 mmol, 1.0 equiv.) and cyclopentylacetylene (0.1 mL, 1.02 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-cyclopentyl-1,2,3-triazol-1-yl)ethyl)-6-

(trifluoromethoxy)benzothiazol-2-imine (**160ak**, 0.16 g, 0.40 mmol, 59 %) as an off white solid; **R_f** 0.13 (100 % EtOAc), **m.p.** 174 - 179 °C

IR ν_{max}/cm^{-1} 3248, 3078, 2948, 1600, 1579, 1484, 1261; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.09 (1H, s, H-7), 7.06 (1H, bs, NH), 7.01 (1H, s, H-5''), 6.91 (1H, dd, J = 1.5 Hz and 9.0 Hz, H-5). 6.29 (1H, d, J = 9.0 Hz, H-4), 4.73 (2H, t, J 5.5 Hz, H-1'), 4.37 (2H, t, J = 6.0 Hz, H-2'), 3.01 (1H, quin, J = 8.0 Hz, H-1'''), 1.94 - 1.86 (2H, m, H-2''' and H-5'''), 1.63 - 1.53 (4H, m, H-3''' and H-4'''), 1.44 - 1.32 (2H, m, H-2''' and H-5'''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 23.9 (C-3''' and C-4'''), 32.1 (C-2''' and C-5'''), 35.4 (C-1'''), 42.9 (C-2'), 45.7 (C-1'), 107.8 (C-4), 114.0 (C-7), 118.8 (C-5), 119.8 (C-5''), 120.7 (ArC), 121.9 (ArC), 137.7 (ArC), 142.7 (Ar(OCF_3)), 152.2 (ArC), 159.6 (ArC); **MS** m/z $[M+H]^+$ $C_{17}H_{19}F_3N_5Os$ requires 398.13, found 198.13.

3-(2-(4-Cyclohexyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160al

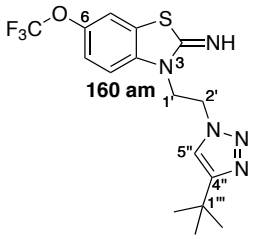


160 al

Using the general procedure; to a solution of ring-cyclised azide **223** (0.23 g, 0.75 mmol, 1.0 equiv.) and cyclohexylacetylene (0.2 mL, 1.12 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.8 mL 1M $CuSO_4$ (aq) and 1.5 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-cyclohexyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160al**, 0.17 g, 0.41 mmol, 55 %) as a pale yellow solid; **R_f** 0.15 (100 % EtOAc), **m.p.** 178 - 182 °C

IR ν_{max}/cm^{-1} 3220, 3028, 2925, 1581, 1483, 1254; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.09 (2H, bs, H-7 and NH), 6.99 (1H, s, H-5''), 6.90 (1H, d, J = 9.0 Hz, H-5), 6.27 (1H, d, J = 9.0 Hz, H-4), 4.74 (2H, t, J = 5.5 Hz, H-1'), 4.37 (2H, t, J = 5.5 Hz, H-2'), 2.58 (1H, ttt, J = 3.5 Hz and 11.5 Hz, H-1'''), 1.78 - 1.75 (2H, m, H-2''' and H-6'''), 1.70 -1.64 (3H, m, H-3''', H-4''' and H-5'''), 1.35 - 1.25 (2H, m, H-2''' and H-6'''), 1.20 - 1.06 (3H, m, H-3''', H-4''' and H-5'''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 25.1 (C-3''', C-4''' and C-5'''), 31.9 (C-2''' and C-6'''), 34.0 (C-1'''), 42.9 (C-2'), 45.7 (C-1'), 107.8 (C-4), 114.0 (C-7), 118.1 (ArC), 118.8 (C-5), 119.6 (C-5''), 120.6 (ArC), 121.9 (ArC), 137.7 (ArC), 142.6 (Ar(O**C**F_3)), 153.1 (ArC), 159.6 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{21}F_3N_5OS$ requires 412.14, found 412.14.

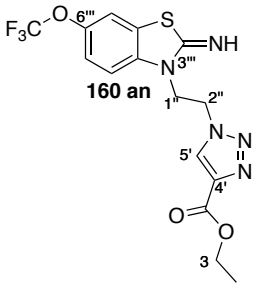
3-(2-(4-(*tert*-Butyl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160am



Using the general procedure; to a solution of ring-cyclised azide **223** (0.16 g, 0.53 mmol, 1.0 equiv.) and 3,3-dimethyl-1-butyne (0.1 mL, 0.80 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(*tert*-butyl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160am**, 0.13 g, 0.34 mmol, 64 %) as a pale yellow solid; $\mathbf{R_f}$ 0.13 (100 % EtOAc), **m.p.** 150 - 154 °C

IR ν_{max}/cm^{-1} 3256, 3068, 2979, 1605, 1580, 1484, 1384, 1258; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.09 (1H, s, H-7), 7.07 (1H, bs, NH), 6.98 (1H, s, H-5''), 6.89 (1H, d, J = 9.0 Hz, H-5), 6.25 (1H, d, J = 9.0 Hz, H-4), 4.74 (2H, t, J = 5.5 Hz, H-1'), 4.36 (2H, t, J = 5.5 Hz, H-2'), 1.12 (9H, s, ArC(C**H**$_3$)$_3$); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 29.1 (ArC(**C**H$_3$)$_3$), 42.9 (C-2'), 45.6 (C-1'), 107.7 (C-4), 114.0 (C-7), 118.1 (ArC), 118.7 (C-5), 118.8 (C-5''), 120.7 (ArC), 123.2 (ArC), 137.7 (ArC), 142.6 (Ar(O**C**F$_3$)), 157.1 (ArC), 159.6 (ArC); **MS** m/z $[M+H]^+$ $C_{16}H_{19}F_3N_5OS$ requires 386.13, found 386.13.

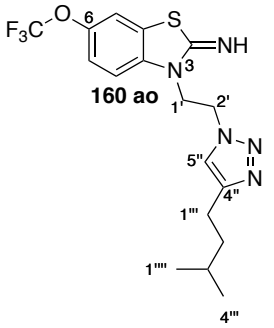
Ethyl 1-(2-(2-Imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazole-4-carboxylate 160an



Using the general procedure; to a solution of ring-cyclised azide **223** (0.15 g, 0.48 mmol, 1.0 equiv.) and ethyl propiolate (0.1 mL, 0.72 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield ethyl 1-(2-(2-imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazole-4-carboxylate (**160an**, 0.12 g, 0.29 mmol, 61 %) as a pale yellow solid; $\mathbf{R_f}$ 0.15 (100 % EtOAc), **m.p.** 159 - 163 °C

IR ν_{max}/cm^{-1} 3276, 3043, 2979, 1721, 1631, 1583, 1485, 1377, 1261; **^{1}H NMR** (400MHz, $CDCl_3$); 7.95 (1H, s, H-5'), 7.11 (1H, s, H-7'''), 7.09 (1H, bs, N**H**), 6.97 (1H, d, J = 8.5 Hz, H-5'''), 6.49 (1H, d, J = 9.0 Hz, H-4'''), 4.84 (2H, t, J = 6.0 Hz, H-1''), 4.46 (2H, t, J = 6.0 Hz, H-2''), 4.37 (2H, q, J = 7.0 Hz, H-3), 1.37 (3H, t, J = 7.0 Hz, H-4); **^{13}C NMR** (100MHz, $CDCl_3$); 14.2 (C-4), 43.3 (C-2''), 47.2 (C-1''), 61.4 (C-3), 108.7 (C-4'''), 115.4 (C-7'''), 119.9 (C-5'''), 121.7 (ArC), 123.4 (ArC), 128.6 (C-5'), 138.4 (ArC), 140.4 (ArC), 143.9 (Ar(O**C**F_3)), 160.4 (ArC), 160.8 (ArC); **MS** m/z $[M+H]^+$ $C_{15}H_{15}F_3N_5O_3S$ requires 402.09, found 402.08.

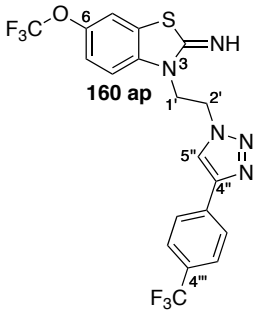
3-(2-(4-Isopentyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ao



Using the general procedure; to a solution of ring-cyclised azide **223** (0.16 g, 0.54 mmol, 1.0 equiv.) and 5-methyl-1-hexyne (0.1 mL, 0.81 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-isopentyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ao**, 0.14 g, 0.34 mmol, 63 %) as a pale yellow solid; **R_f** 0.11 (100 % EtOAc), **m.p.** 126 - 129 °C

IR ν_{max}/cm^{-1} 3232, 3072, 2958, 1602, 1581, 1484, 1384, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.09 (2H, bs, H-7 and N**H**), 7.07 (1H, s, H-5''), 6.92 (1H, d, J = 9.0 Hz, H-5), 6.36 (1H, d, J = 9.0 Hz, H-4), 4.73 (2H, t, J = 5.5 Hz, H-1'), 4.39 (2H, t, J = 6.0 Hz, H-2'), 2.54 (2H, t, J = 8.0 Hz, H-1'''), 1.46 - 1.36 (1H, m, H-3'''), 1.34 - 1.28 (2H, m, H-2'''), 0.85 (6H, d, J = 6.5 Hz, H-4''' and H-1''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 22.2 (C-4''' and C-1''''), 26.3 (C-1'''), 37.4 (C-2''' and C-3'''), 42.8 (C-2'), 45.7 (C-1'), 107.9 (C-4), 114.0 (C-7), 118.1 (ArC), 118.7 (C-5), 120.7 (C-5''), 122.1 (ArC), 137.6 (ArC), 142.7 (Ar(O**C**F_3)), 147.9 (ArC), 159.7 (ArC); **MS** m/z $[M+H]^+$ $C_{17}H_{21}F_3N_5OS$ requires 400.14, found 400.14.

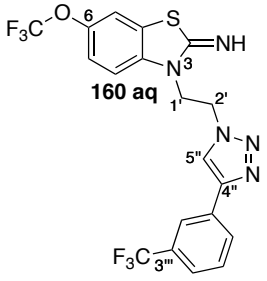
6-(Trifluoromethoxy)-3-(2-(4-(4-(trifluoromethyl)phenyl)-1,2,3-triazol-1-yl)ethyl)benzothiazol-2-imine 160ap



Using the general procedure; to a solution of ring-cyclised azide **223** (0.22 g, 0.72 mmol, 1.0 equiv.) and 4-ethynyl-α,α,α-trifluorotoluene (0.2 mL, 1.09 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.4 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 6-(trifluoromethoxy)-3-(2-(4-(4-(trifluoromethyl)phenyl)-1,2,3-triazol-1-yl)ethyl)benzothiazol-2-imine (**160ap**, 0.12 g, 0.25 mmol, 35 %) as a pale yellow solid; $\mathbf{R_f}$ 0.17 (100 % EtOAc), **m.p.** 218 - 221 °C

IR ν_{max}/cm^{-1} 3253, 3021, 2953, 1613, 1584, 1484, 1326, 1234; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.78 (2H, d, J = 8.0 Hz, ArH), 7.69 (1H, s, H-5''), 7.63 (2H, d, J = 8.0 Hz, ArH), 7.10 (2H, d, J = 1.5 Hz, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.84 (2H, t, J = 5.5 Hz, H-1'), 4.48 (2H, t, J = 5.5 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2'), 47.0 (C-1'), 108.9 (C-4), 115.5 (C-7), 119.9 (C-5), 121.6 (C-5''), 122.7 (ArC), 123.3 (Ar(**C**F_3)), 123.7 (ArC), 125.8 (ArCH), 129.7 (ArC), 130.0 (ArC), 130.3 (ArC), 133.6 (ArC), 138.6 (Ar(O**C**F_3)), 143.9 (ArC), 146.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{14}F_6N_5OS$ requires 474.08, found 474.08.

6-(Trifluoromethoxy)-3-(2-(4-(3-(trifluoromethyl)phenyl)-1,2,3-triazol-1-yl)ethyl)benzothiazol-2-imine 160aq

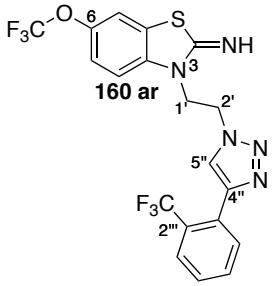


Using the general procedure; to a solution of ring-cyclised azide **223** (0.18 g, 0.59 mmol, 1.0 equiv.) and 3-ethynyl-α,α,α-trifluorotoluene (0.1 mL, 0.88 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.2 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-

up the crude was column purified using 100 % EtOAc to yield 6-(trifluoromethoxy)-3-(2-(4-(3-(trifluoromethyl)phenyl)-1,2,3-triazol-1-yl)ethyl)benzothiazol-2-imine (**160aq**, 0.18 g, 0.39 mmol, 66 %) as a yellow/green solid; $\mathbf{R_f}$ 0.21 (100 % EtOAc), **m.p.** 145 - 149 °C

IR ν_{max}/cm^{-1} 3246, 3071, 2958, 1578, 1483, 1322, 1253; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.91 (1H, s, ArH), 7.85 (1H, d, J = 7.5 Hz, ArH), 7.70 (1H, s, H-5''), 7.57 (1H, d, J = 8.0 Hz, ArH), 7.50 (1H, t, J = 7.5 Hz, ArH), 7.10 (2H, bs, H-7 and N**H**), 6.94 (1H, d, J = 8.0 Hz, H-5), 6.54 (1H, d, J = 9.0 Hz, H-4), 4.84 (2H, bs, H-1'), 4.48 (2H, bs, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 45.9 (C-1'), 107.8 (C-4), 114.3 (C-7), 118.8 (C-5), 120.3 (C-5''), 120.6 (ArC), 121.4 (ArCH), 121.6 (ArC), 122.2 (Ar(**C**F_3), 123.8 (ArCH), 124.3 (ArC), 127.8 (ArCH), 128.3 (ArCH), 130.1 (ArC), 130.4 (ArC), 137.5 (Ar(O**C**F_3)), 142.8 (ArC), 145.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{14}F_6N_5OS$ requires 474.08, found 474.08.

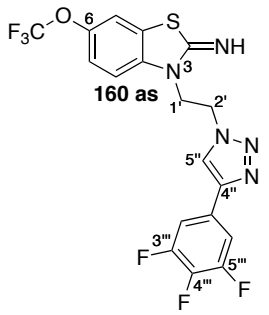
6-(Trifluoromethoxy)-3-(2-(4-(2-(trifluoromethyl)phenyl)-1,2,3-triazol-1-yl)ethyl)benzothiazol-2-imine 160ar



Using the general procedure; to a solution of ring-cyclised azide **223** (0.22 g, 0.74 mmol, 1.0 equiv.) and 2-ethynyl-α,α,α-trifluorotoluene (0.2 mL, 1.11 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.5 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 6-(trifluoromethoxy)-3-(2-(4-(2-(trifluoromethyl)phenyl)-1,2,3-triazol-1-yl)ethyl)benzothiazol-2-imine (**160ar**, 0.21 g, 0.43 mmol, 59 %) as an off white solid; $\mathbf{R_f}$ 0.32 (100 % EtOAc), **m.p.** 186 - 190 °C

IR ν_{max}/cm^{-1} 3245, 3081, 2954, 1616, 1585, 1485, 1318, 1256; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.69 (2H, t, J = 7.5 Hz, ArH), 7.57 (2H, t, J = 9.5 Hz, ArH and H-5''), 7.45 (1H, t, J = 7.5 Hz, ArH), 7.08 (2H, bs, H-7 and NH), 6.93 (1H, d, J = 9.0 Hz, H-5), 6.45 (1H, d, J = 9.0 Hz, H-4), 4.87 (2H, t, J = 5.5 Hz, H-1'), 4.47 (2H, t, J = 5.5 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.8 (C-2'), 46.0 (C-1'), 107.7 (C-4), 114.2 (C-7), 118.7 (C-4), 122.2 (ArC), 123.0 (Ar(CF_3)), 124.2 (C-5''), 125.0 (ArC), 126.2 (ArCH), 126.5 (ArC), 127.3 (ArC), 128.0 (ArCH), 130.6 (ArCH), 130.9 (ArCH), 137.6 (Ar(OCF_3)), 142.7 (ArC), 143.6 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{14}F_6N_5OS$ requires 474.08, found 474.08.

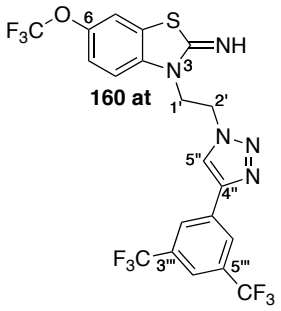
6-(Trifluoromethoxy)-3-(2-(4-(3,4,5-trifluorophenyl)-1,2,3-triazol-1yl)ethyl)benzothiazol-2-imine 160as



Using the general procedure; to a solution of ring-cyclised azide **223** (0.14 g, 0.46 mmol, 1.0 equiv.) and 3,4,5-trifluorophenylacetylene (0.11 g, 0.69 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 0.9 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 6-(trifluoromethoxy)-3-(2-(4-(3,4,5-trifluorophenyl)-1,2,3-triazol-1yl)ethyl)benzothiazol-2-imine (**160as**, 0.16 g, 0.34 mmol, 74 %) as a pale yellow solid; $\mathbf{R_f}$ 0.21 (100 % EtOAc), **m.p.** 166 - 170 °C

IR ν_{max}/cm^{-1} 3229, 3077, 2958, 1607, 1518, 1483, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.59 (1H, s, H-5''), 7.32 - 7.24 (2H, m, ArH), 7.11 (2H, d, J = 1.5 Hz, H-7 and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5), 6.51 (1H, d, J = 9.0 Hz, H-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 46.0 (C-1'), 107.8 (C-4), 108.6 (ArCH), 108.9 (ArCH), 114.4 (C-7), 118.8 (C-5), 120.2 (C-5''), 122.2 (ArC), 137.4 (ArC), 139.8 (Ar(**C**F)), 142.8 (Ar(O**C**F_3)), 144.4 (ArC), 149.3 (ArC), 151.8 (Ar(**C**F)), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{12}F_6N_5OS$ requires 460.07, found 460.07.

6-(Trifluoromethyoxy)-3-(2-(4-(3,5-bis(trifluoromethyl)phenyl)-1,2,3-triazol-1-ly)ethyl)benzothiazol-2-imine 160at

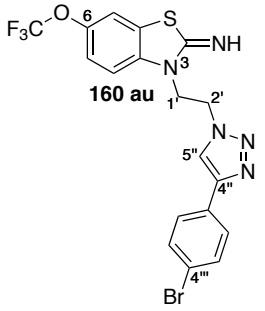


Using the general procedure; to a solution of ring-cyclised azide **223** (0.14 g, 0.47 mmol, 1.0 equiv.) and 1-ethynyl-3,5-bis(trifluoromethyl) benzene (0.1 mL, 0.71 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 0.9 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 6-(trifluoromethyoxy)-3-(2-

(4-(3,5-bis(trifluoromethyl)phenyl)-1,2,3-triazol-1-ly)ethyl)benzothiazol-2-imine (**160at**, 0.22 g, 0.41 mmol, 87 %) as a pale yellow solid; **R_f** 0.31 (100 % EtOAc), **m.p.** 184 - 188 °C

IR ν_{max}/cm^{-1} 3217, 3043, 2954, 1624, 1587, 1484, 1324, 1275; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.12 (2H, s, ArH), 7.81 (1H, s, ArH), 7.79 (1H, s, H-5''), 7.10 (2H, d, J = 1.5 Hz, H-7 and N**H**), 6.95 (1H, d, J = 8.5 Hz, H-5), 6.55 (1H, d, J = 9.0 Hz, H-4), 4.87 (2H, t, J = 6.0 Hz, H-1'), 4.49 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.4 (C-2'), 46.1 (C-1'), 107.8 (C-4), 114.4 (C-7), 118.9 (C-5), 121.8 (C-5''), 120.7 (Ar(**C**F_3)), 120.9 (ArCH), 123.5 (ArC), 124.5 (ArCH), 131.1 (ArC), 131.2 (ArC), 131.4 (ArC), 137.4 (Ar(O**C**F_3)), 143.9 (ArC), 144.4 (ArC), 155.6 (ArC); **MS** m/z $[M+H]^+$ $C_{20}H_{13}F_9N_5OS$ requires 542.07, found 542.07.

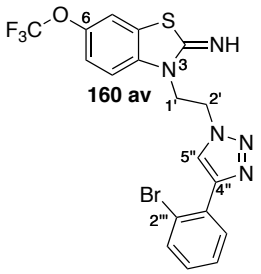
3-(2-(4-(4-Bromophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160au



Using the general procedure; to a solution of ring-cyclised azide **223** (0.17 g, 0.55 mmol, 1.0 equiv.) and 1-bromo-4-ethynylbenzene (0.15 g, 0.82 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.6 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(4-bromophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160au**, 0.18 g, 0.38 mmol, 69 %) as an off-white solid; **R_f** 0.17 (100 % EtOAc), **m.p.** 213 - 215 °C

IR ν_{max}/cm^{-1} 3240, 3087, 2950, 1614, 1584, 1484, 1254, 757; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.61 (1H, s, H-5''), 7.54 - 7.49 (4H, m, ArH), 7.10 (2H, d, J = 1.5 Hz, H-7 and N**H**), 6.94 (1H, dd, J = 1.5 Hz and 9.0 Hz, H-5), 6.53 (1H, d, J = 9.0 Hz, H-4), 4.82 (2H, t, J = 6.0 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-1'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-1'), 46.9 (C-2'), 108.9 (C-4), 115.3 (C-7), 119.8 (C-5), 120.9 (C-5''), 121.7 (ArC), 122.2 (ArC), 123.2 (ArC), 127.2 (ArCH), 129.1 (ArC), 132.0 (ArCH), 138.5 (ArC), 143.9 (Ar(O**C**F_3)), 147.1 (ArC), 160.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}BrF_3N_5OS$ requires 484.01, found 484.00.

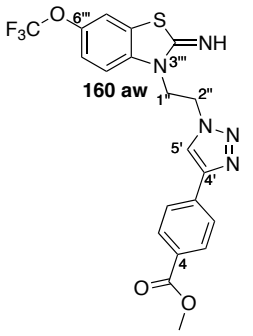
3-(2-(4-(2-Bromophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine 160av



Using the general procedure; to a solution of ring-cyclised azide **223** (0.16 g, 0.53 mmol, 1.0 equiv.) and 1-bromo-2-ethynylbenzene (0.1 mL, 0.80 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was left to stir at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-(2-bromophenyl)-1,2,3-triazol-1-yl)ethyl)-6-trifluoromethoxy)benzothiazol-2-imine (**160av**, 0.13 g, 0.26 mmol, 50 %) as a pale yellow solid; $\mathbf{R_f}$ 0.25 (100 % EtOAc), **m.p.** 169 - 174 °C

IR ν_{max}/cm^{-1} 3254, 3083, 2961, 1616, 1585, 1484, 1234; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.97 (1H, s, H-5''), 7.87 (1H, dd, J = 1.5 Hz and 8.0 Hz, ArH), 7.57 (1H, dd, J = 1.0 Hz and 8.0 Hz, ArH), 7.36 (1H, td, J = 1.0 Hz and 7.5 Hz, ArH), 7.17 (1H, td, J = 1.5 Hz and 8.0 Hz, ArH), 7.08 (2H, d, J = 1.5 Hz, H-7 and N**H**), 6.90 (1H, dd, J = 1.5 Hz and 9.0 Hz, H-5), 6.41 (1H, d, J = 9.0 Hz, H-4), 4.87 (2H, t, J = 5.5 Hz, H-1'), 4.46 (2H, t, J = 6.0 Hz, H-2'); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 42.8 (C-2'), 46.0 (C-1'), 107.8 (C-4), 114.2 (C-7), 118.8 (C-5), 120.2 (ArC), 120.6 (ArC), 122.2 (ArC), 123.2 (C-5''), 126.6 (ArCH), 128.4 (ArCH), 129.5 (ArCH), 129.8 (ArC), 132.3 (ArCH), 137.6 (ArC), 142.8 (Ar(O**C**F_3)), 144.7 (ArC), 159.8 (ArC); **MS** m/z $[M+H]^+$ $C_{18}H_{14}BrF_3N_5OS$ requires 484.01, found 484.00.

Methyl 4-(1-(2-(2-imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)benzoate 160aw

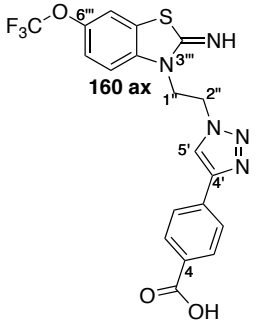


Using the general procedure; to a solution of ring-cyclised azide **223** (0.14 g, 0.45 mmol, 1.0 equiv.) and methyl-4-ethynylbenzoate (0.11 g, 0.67 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 0.9 mL freshly prepared

1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield methyl 4-(1-(2-(2-imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)benzoate (**160aw**, 0.12 g, 0.26 mmol, 59 %) as an off white solid; $\mathbf{R_f}$ 0.19 (100 % EtOAc), **m.p.** 196 - 199 °C

IR ν_{max}/cm^{-1} 3276, 3099, 2955, 1721, 1604, 1582, 1485, 1366, 1261; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 8.05 (2H, d, J = 8.5 Hz, ArH), 7.74 (2H, d, J = 8.5 Hz, ArH), 7.71 (1H, s, H-5'), 7.10 (2H, d, J = 1.5 Hz, H-7''' and N**H**), 6.94 (1H, d, J = 8.5 Hz, H-5'''), 6.55 (1H, d, J = 9.0 Hz, H-4'''), 4.84 (2H, t, J = 6.0 Hz, H-1''), 4.48 (2H, t, J = 6.0 Hz, H-2''), 3.93 (3H, s, H-3''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 43.5 (C-2''), 47.0 (C-1''), 52.2 (C-3''''), 109.0 (C-4'''), 115.3 (C-7'''), 119.1 (ArC), 120.0 (C-5'''), 121.7 (C-5'), 123.3 (ArC), 125.5 (ArCH), 129.7 (ArC), 130.2 (ArCH), 134.4 (ArC), 138.5 (ArC), 143.9 (Ar(O**C**F_3)), 147.1 (ArC), 161.0 (ArC), 166.7 (ArC); **MS** m/z $[M+H]^+$ $C_{20}H_{17}F_3N_5O_3S$ requires 464.10, found 464.10.

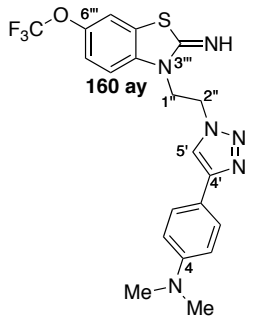
4-(1-(2-(2-Imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)benzoic acid 160ax



Using the general procedure; to a solution of ring-cyclised azide **223** (0.16 g, 0.53 mmol, 1.0 equiv.) and 4-ethynylbenzoic acid (0.12 g, 0.79 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. Once washed with brine a precipitate formed. This was filtered to yield 4-(1-(2-(2-imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)benzoic acid (**160ax**, 0.14 g, 0.31 mmol, 59 %) as an off-white solid; **m.p.** 380 - 384 °C

IR ν_{max}/cm^{-1} 3238, 2974, 1585, 1484, 1262; **^{1}H NMR** (500MHz, MeOD) δ; 8.32 (1H, s, H-5'), 8.00 (2H, d, J = 10.0 Hz, ArH), 7.71 (2H, d, J = 5.0 Hz, ArH), 7.33 (1H, s, H-7'''), 6.99 (1H, d, J = 10.0 Hz, H-5'''), 6.77 (1H, d, J = 10.0 Hz, H-4'''), 4.88 (2H, t, J = 5.0 Hz, H-1''), 4.52 (2H, t, J = 5.0 Hz, H-2''); **^{13}C NMR** (125MHz, MeOD) δ; 42.7 (C-2''), 46.7 (C-1''), 109.2 (C-4'''), 115.1 (C-7'''), 119.1 (C-5'''), 119.6 (ArC), 122.3 (C-5'), 123.9 (ArC), 124.6 (ArCH), 129.6 (ArCH), 132.0 (ArC), 138.7 (Ar(O**C**F_3)), 143.7 (ArC), 147.4 (ArC), 157.9 (ArC), 162.3 (ArC), 173.0 (ArC); **MS** m/z $[M+H]^+$ $C_{19}H_{15}F_3N_5O_3S$ requires 450.09, found 450.08.

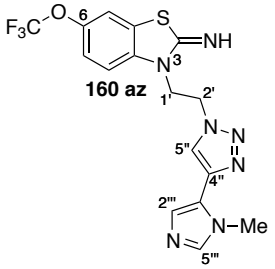
4-(1-(2-(2-Imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)-*N*,*N*-dimethylaniline 160ay



Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.67 mmol, 1.0 equiv.) and 4-ethynyl-N,N-dimethylaniline (0.15 g, 1.01 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 4-(1-(2-(2-imino-6-(trifluoromethoxy)benzothiazol-3-yl)ethyl)-1,2,3-triazol-4-yl)-*N*,*N*-dimethylaniline (**160ay**, 0.17 g, 0.39 mmol, 58 %) as a pale yellow solid; $\mathbf{R_f}$ 0.17 (100 % EtOAc), **m.p.** 214 - 217 °C

IR ν_{max}/cm^{-1} 3246, 3014, 2945, 1614, 1584, 1483, 1352, 1251; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.53 (2H, d, J = 9.0 Hz, ArH), 7.49 (1H, s, H-5'), 7.09 (2H, d, J = 1.5 Hz, H-7''' and N**H**), 6.94 (1H, d, J = 9.0 Hz, H-5'''), 6.72 (2H, d, J = 9.0 Hz, ArH), 6.55 (1H, d, J = 9.0 Hz, H-4'''), 4.80 (2H, t, J = 6.0 Hz, H-1''), 4.45 (2H, t, J = 6.0 Hz, H-2''), 2.99 (6H, s, ArN(C**H**$_3$)$_2$); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 40.4 (ArN(**C**H_3)$_2$), 43.6 (C-2''), 46.8 (C-1''), 109.1 (C-4'''), 112.4 (ArCH), 115.2 (C-7'''), 118.4 (ArC), 119.3 (C-5'), 119.8 (C-5'''), 121.7 (ArC), 123.2 (ArC), 126.7 (ArCH), 138.6 (ArC), 143.8 (Ar(O**C**F_3)), 148.7 (ArC), 150.5 (ArC), 160.9 (ArC); **MS** m/z $[M+H]^+$ $C_{20}H_{20}F_3N_6OS$ requires 449.14, found 449.14.

3-(2-(4-(1-Methyl-1*H*-imidazol-5-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160az

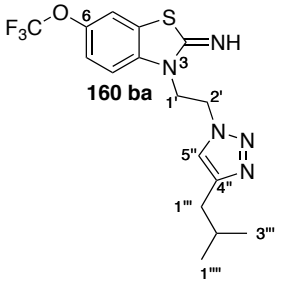


Using the general procedure; to a solution of ring-cyclised azide **223** (0.20 g, 0.66 mmol, 1.0 equiv.) and 5-ethynyl-1-methyl-1H-imidazole (0.1 mL, 0.99 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.7 mL 1M $CuSO_4$ (aq) and 1.3 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 9:1 DCM:MeOH to yield 3-(2-(4-(1-methyl-1*H*-

imidazol-5-yl)-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160az**, 0.18 g, 0.44 mmol, 67 %) as an off white solid; $\mathbf{R_f}$ 0.52 (9:1 DCM:MeOH), **m.p.** 220 - 225 °C

IR ν_{max}/cm^{-1} 3161, 2958, 1626, 1585, 1484, 1386, 1267; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.52 (1H, s, H-5''), 7.48 (1H, bs, H-2'''), 7.11 (1H, d, J =1.5 Hz, H-7), 7.06 (1H, bs, H-5'''), 6.94 (1H, dd, J = 1.5 Hz and 9.0 Hz, H-5), 6.51 (1H, d, J = 9.0 Hz, H-4), 4.84 (2H, t, J = 5.5 Hz, H-1'), 4.47 (2H, t, J = 6.0 Hz, H-2'), 3.73 (3H, s, N(C**H**$_3$)); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 32.2 (N(**C**H_3)), 42.5 (C-2'), 45.8 (C-1'), 107.7 (C-4), 114.3 (C-7), 118.1 (ArC), 118.6 (C-5), 120.7 (ArC), 121.4 (C-5''), 122.4 (ArC), 123.2 (ArC), 129.1 (C-5'''), 137.5 (C-2'''), 137.6 (ArC), 142.8 (Ar(O**C**F_3)), 159.6 (ArC); **MS** m/z $[M+H]^+$ $C_{16}H_{15}F_3N_7OS$ requires 410.10, found 410.10.

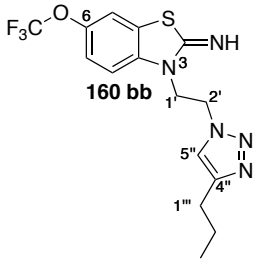
3-(2-(4-Isobutyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160ba



Using the general procedure; to a solution of ring-cyclised azide **223** (0.14 g, 0.48 mmol, 1.0 equiv.) and 4-methyl-1-pentyne (0.1 mL, 0.72 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.0 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-isobutyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160ba**, 0.18 g, 0.47 mmol, 98 %) as an off-white solid; $\mathbf{R_f}$ 0.19 (100 % EtOAc), **m.p.** 165 -170 °C

IR ν_{max}/cm^{-1} 3237, 3065, 2955, 1580, 1485, 1383, 1263; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.07 (3H, bs, H-7, H-5'' and N**H**), 6.91 (1H, dd, J = 1.5 Hz and 9.0 Hz, H-5), 6.38 (1H, d, J = 9.0 Hz, H-4), 4.76 (2H, t, J = 5.5 Hz, H-1'), 4.40 (2H, t, J = 6.0 Hz, H-2'), 2.42 (2H, d, J = 6.0 Hz, H-1'''), 1.70 (1H, sept, J = 6.5 Hz, H-2'''), 0.72 (6H, d, J = 6.5 Hz, H-3''' and H-1''''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 20.9 (C-1'''' and C-3'''), 27.5 (C-2'''), 33.4 (C-1'''), 42.9 (C-2'), 45.7 (C-1'), 107.9 (C-4), 114.1 (C-7), 118.8 (C-5), 120.7 (ArC), 121.4 (C-5''), 122.1 (ArC), 137.7 (ArC), 142.7 (Ar(O**C**F_3)), 146.4 (ArC), 159.7 (ArC); **MS** m/z $[M+H]^+$ $C_{16}H_{18}F_3N_5OS$ requires 386.13, found 386.13.

3-(2-(4-Propyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine 160bb



Using the general procedure; to a solution of ring-cyclised azide **223** (0.16 g, 0.53 mmol, 1.0 equiv.) and 1-pentyne (0.1 mL, 0.79 mmol, 1.5 equiv.) in 10.0 mL H_2O and 10.0 mL THF heated to 20 °C was added 0.5 mL 1M $CuSO_4$ (aq) and 1.1 mL freshly prepared 1M sodium ascorbate (aq) dropwise. This was stirred at 20 °C for 2 h. After work-up the crude was column purified using 100 % EtOAc to yield 3-(2-(4-propyl-1,2,3-triazol-1-yl)ethyl)-6-(trifluoromethoxy)benzothiazol-2-imine (**160bb**, 0.09 g, 0.23 mmol, 44 %) as a pale yellow solid; $\mathbf{R_f}$ 0.14 (100 % EtOAc), **m.p.** 134 - 138 °C

IR ν_{max}/cm^{-1} 3334, 3072, 2961, 1602, 1580, 1484, 1383, 1257; **^{1}H NMR** (400MHz, $CDCl_3$) δ; 7.09 (2H, d, J = 1.5 Hz, H-7 and N**H**), 7.06 (1H, s, H-5''), 6.92 (1H, dd, J = 1.5 Hz and 9.0 Hz, H-5), 6.35 (1H, d, J = 9.0 Hz, H-4), 4.74 (2H, t, J = 5.5 Hz, H-1'), 4.39 (2H, t, J = 6.0 Hz, H-2'), 2.52 (2H, t, J = 7.5 Hz, H-1'''), 1.44 (2H, sext, J = 7.5 Hz, H-2'''), 0.78 (3H, t, J = 7.5 Hz, H-3'''); **^{13}C NMR** (100MHz, $CDCl_3$) δ; 12.4 (C-3'''), 21.6 (C-2'''), 26.3 (C-1'''), 42.9 (C-2'), 45.7 (C-1'), 107.8 (C-4), 114.1 (C-7), 118.8 (C-5), 120.7 (ArC), 120.9 (C-5''), 122.0 (ArC), 137.7 (ArC), 142.7 (Ar(O**C**F_3)), 147.5 (ArC), 159.7 (ArC); **MS** m/z $[M+H]^+$ $C_{15}H_{16}F_3N_5OS$ requires 372.11, found 372.11.

CHAPTER 6: REFERENCES

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