Cytotoxic Hydrogen Bridged Ruthenium Quinaldamide Complexes Showing Induced Cancer Cell Death by Apoptosis

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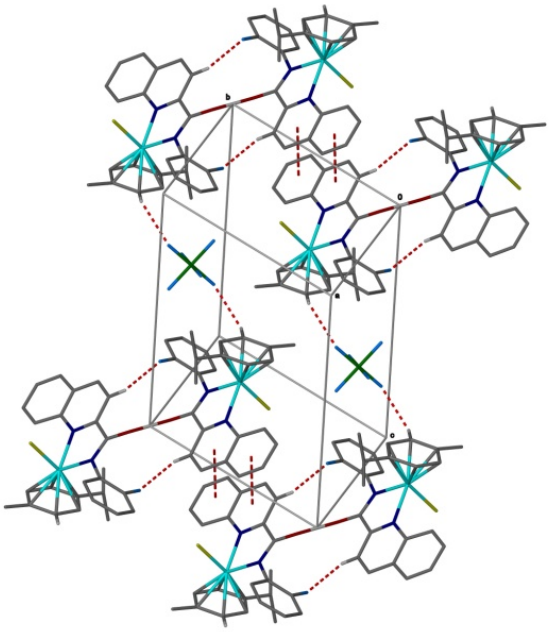
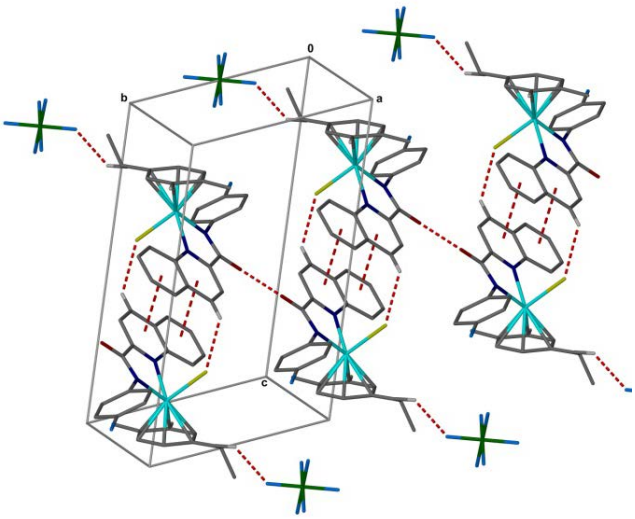
Packing Diagrams and Interactions

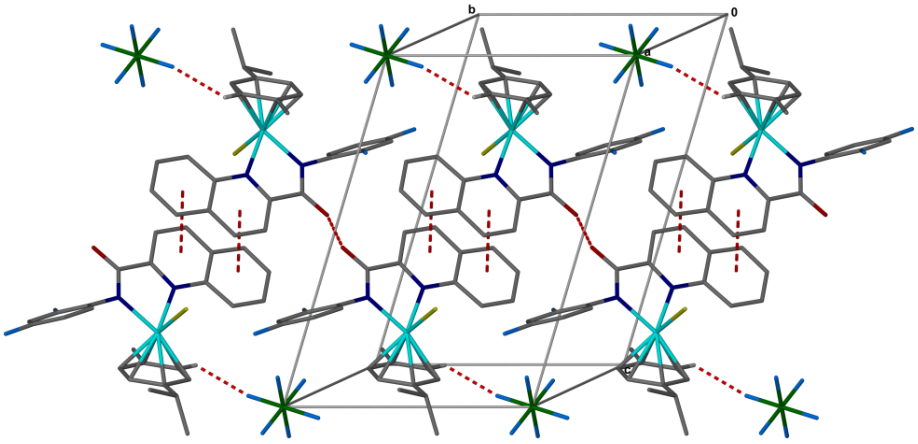
Complexes **1**-**6** all show interesting bonding and crystal packing interactions. **Figure S1** shows the packing diagrams for the ruthenium chloride complexes **1**-**3** and **Figure S2** shows the packing diagram for the ruthenium bromide complexes **4**-**6**. **Tables S1 a-f** summarise the intramolecular and intermolecular interactions for complexes **1**-**6**, which could be contributing to the stability of these hydrogen-bridged dimers. The X-ray crystallographic data is presented in **Table S2**.



Compound **1** viewed along the *c* axis

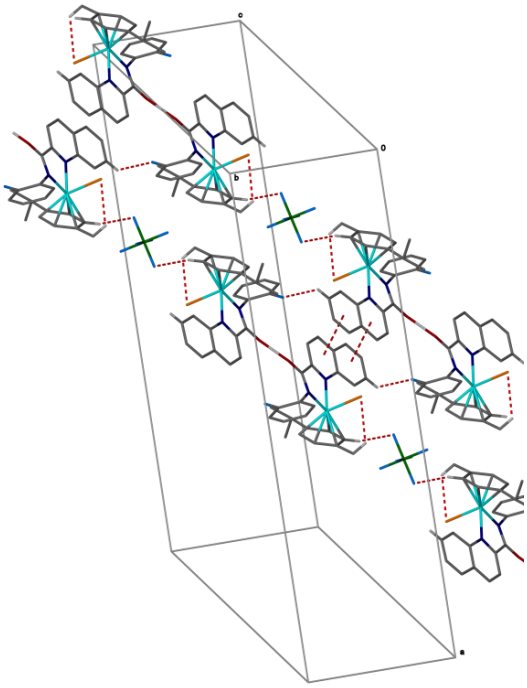


Compound **2** viewed along the *b* axis

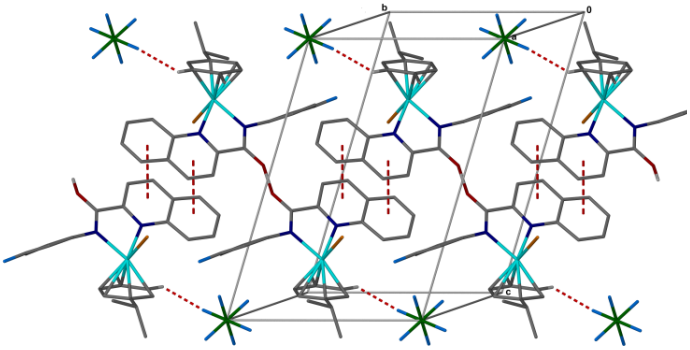


Compound **3** viewed along the *a* axis

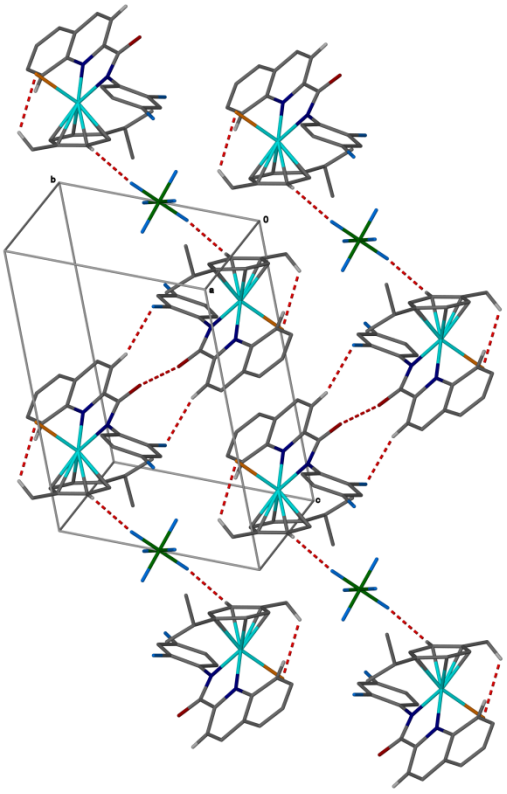
Figure S1 *Packing diagrams of the ruthenium chloride complexes* ***1***-***3***



Compound **4** viewed along the *b* axis



Compound **5** viewed along the *a* axis



Compound **6** viewed along the *b* axis

Figure S2 *Packing diagrams of the ruthenium bromide complexes* ***4****-****6***

Table S1a *Interactions for complex **1**, s.u.s in parenthesis*

	Complex **1**	D…A (Å)
Intramolecular	C(8)-H(8)…F(1)	3.362(3)
	C(19)-H(19)…F(3A)	3.082(17)
Intermolecular	C(17)-H(17B)…Cg(4)	3.571(3)
	C(26)-H(26C)…Cg(1)	3.890(4)
	Cg(1)…Cg(1)	3.7759(16)
	Cg(1)…Cg(2)/Cg(2)…Cg(1)	3.9360(15)/3.9359(15)
	Cg(3)…Cg(3)	3.8240(15)

Table S1b *Interactions for complex **2**, s.u.s in parenthesis*

	Complex **2**	D…A (Å)
Intermolecular	C(4)-H(4)…F(1)	3.335(4)
	C(7)-H(7)…Cl(1)	3.585(3)
	C(8)-H(8)…O(1)	3.141(4)
	C(19)-H(19)...F(3)	3.062(4)
	C(26)-H(26B)…F(3)	3.355(5)
	C(13)-H(13)…Cg(1)	3.743(4)
	Ru(1)-Cl(1)…Cg(4)	3.6904(15)
	Cg(1)…Cg(1)	3.6800(17)
	Cg(1)…Cg(2)/Cg(2)…Cg(1)	3.7526(17)/3.7527(17)

Table S1c *Interactions for complex **3**, s.u.s in parenthesis*

	Complex **3**	D…A (Å)
Intermolecular	C(4)-H(4)…F(2)	3.319(3)
	C(7)-H(7)…Cl(1)	3.562(2)
	C(8)-H(8)…O(1)	3.148(3)
	C(22)-H(22)…F(5)	3.057(3)
	C(15)-H(15)…Cg(1)	3.701(3)
	Ru(1)-Cl(1)…Cg(4)	1.6814(9)
	Cg(1)…Cg(1)	3.6989(11)
	Cg(1)…Cg(2)/Cg(2)…Cg(1)	3.7737(12)/3.7738(12)

Table S1d *Interactions for complex* ***4****, s.u.s in parenthesis*

Complex **4**		D…A (Å)
Intramolecular	C(17)-H(17B)…Br(1)	3.518(4)
Intermolecular	C(3)-H(3)…F(1)	2.948(3)
	C(8)-H(8)…O(1)	3.232(3)
	C(13)-H(13)…O(1)	3.432(3)
	C(23)-H(23)…F(4)	3.402(4)
	C(17)-H(17C)…Cg(4)	3.539(4)
	Cg(1)…Cg(1)	3.9482(16)
	Cg(1)…Cg(2)/Cg(2)…Cg(1)	3.9198(17)/3.9199(17)

Table S1e *Interactions for complex* ***5****, s.u.s in parenthesis*

Complex **5**		D…A (Å)
Intermolecular	C(8)-H(8)…O(1)	3.133(5)
	C(7)-H(7)…Br(1)	3.730(4)
	C(23)-H(23)…F(2)	3.128(6)
	C(26)-H(26B)…F(2)	3.352(7)
	C(15)-H(15)….Cg(1)	3.815(5)
	C(17)-H(17B)…Cg(4)	3.709(6)
	Cg(1)…Cg(1)	3.775(2)
	Cg(1)…Cg(2)/Cg(2)…Cg(1)	3.787(2)

Table S1f *Interactions for complex* ***6****, s.u.s in parenthesis*

Complex **6**		D…A
Intermolecular	C(4)-H(4)…F(1)	3.191(6)
	C(8)-H(8)…F(2)	3.412(5)
	C(8)-H(8)…O(1)	3.209(5)
	C(19)-H(19)…F(5)	3.212(8)
	C(17)-H(17B)…Cg(4)	3.557(6)
	C(26)-H(26F)…Cg(1)	3.894(18)
	P(1)-F(5)…Cg(3)	3.958(7)
	Cg(1)…Cg(1)	3.873(2)
	Cg(3)…Cg(3)	3.950(3)
	Cg(1)…Cg(2)/Cg(2)…Cg(1)	3.883(2)

X-ray Crystallographic Data

Table S2 *X-ray crystallography data for complexes* ***1-6****, s.u.s in parenthesis*

Complex	**1**	**2**	**3**	**4**	**5**	**6**
formula	$C_{52}H_{49}Cl_2F_2N_4O_2$ $Ru_2{\bullet}PF_6$	$C_{52}H_{49}Cl_2F_2N_4O_2$ $Ru_2{\bullet}PF_6$	$C_{52}H_{47}Cl_2F_4N_4O_2$ $Ru_2{\bullet}PF_6$	$C_{52}H_{49}Br_2F_2N_4O_2$ $Ru_2{\bullet}PF_6$	$C_{52}H_{49}Br_2F_2N_4O_2$ $Ru_2{\bullet}PF_6$	$C_{52}H_{47}Br_2F_4N_4O_2$ $Ru_2{\bullet}PF_6$
formula wt	1217.96	1217.96	1253.95	1306.88	1306.88	1342.86
cryst syst	Triclinic	Triclinic	Triclinic	Monoclinic	Triclinic	Triclinic
space group	P-1	P-1	P-1	C2/c	P-1	P-1
a (Å)	9.2730(2)	9.4160(19)	9.4250(10)	31.3090(3)	9.4885(4)	9.6940(3)
b (Å)	9.9670(2)	9.7140(19)	9.8120(2)	10.0915(9)	9.8280(3)	10.1017(3)
c (Å)	13.7840(4)	14.631(3)	14.6230(2)	18.4285(16)	14.7862(6)	13.7167(5)
α (°)	101.1000(10)	73.68(3)	72.4320(10)	90	72.595(3)	99.953(3)
β (°)	93.4141(10)	85.08(3)	85.7400(10)	118.3250(4)	83.935(4)	94.128(3)
γ (°)	100.4010(10)	74.22(3)	74.8270(10)	90	73.979(3)	102.191(2)
V (Å^3)	1223.71(5)	1235.8(5)	1244.28(3)	5125.5(8)	1264.22(9)	12847.93(7)
Z	1	1	1	4	1	1
density (mg/m^3)	1.653	1.637	1.673	1.694	1.717	1.707
absorp coeff (mm^{-1})	0.836	0.828	0.830	2.253	2.284	2.255
λ[Mo−Kα] (Å)	0.71073	0.71073	0.71073	0.71073	0.71073	0.71073
T (K)	148(2)	150(2)	148(2)	148(2)	148(2)	148(2)
reflns collected	24563	22510	25291	33840	15986	32571
independent reflns	5583	5590	5690	6751	15986	17044
R_1	0.0351	0.0394	0.0378	0.0326	0.0479	0.0590
wR_2	0.0953	0.1061	0.0800	0.0694	0.1323	0.1259
GOOF	1.042	1.033	1.045	1.011	1.098	1.011

Chemosensitivity Studies

Activity towards Cisplatin-resistance Cancer Cells

Table S3 shows the resistance factor defined as the IC_{50} in A2780cis divided by IC_{50} in A2780 cells. An RF of 1 indicates equal potency against both cell lines. An RF > 1 indicates that the A2780cis is more resistant than A2780. An RF < 1 indicates that the A2780cis is more sensitive than the A2780 cells.

Table S3 *Resistance Factors of compounds* ***1-6***

Compound	Resistance Factor
1	2.45
2	1.24
3	1.85
4	1.7
5	1.56
6	2.18
Cis	10.61

Cancer Cell Selectivity

Table S4 show the selectivity index defined as the IC_{50} in ARPE-19 divided by IC_{50} relevant cancerous cells. An SR = 1 indicates equitoxic potency against tumour and normal cells. An SR > 1 indicates preferential selectivity for tumour cells compared to normal cells. An RF < 1 indicates poor selectivity (greater cytotoxicity towards ARPE cells compared to normal cells).

Table S4 *Selectivity Index for compounds* ***1-6*** *and cisplatin against HCT116, A2780 and A2780cis*

Compound	HCT116	A2780	A2780cis
1	0.63	0.89	0.37
2	>1.27	>2.85	>2.29
3	0.62	0.91	0.49
4	0.76	0.95	0.56
5	1.19	1.16	0.74
6	0.74	0.96	0.44

Induction of Cancer Cell Death by Apoptosis

The cell viable was studied using the Annexin-V/propidium iodide assay and **Table S5** shows the percentage of apoptotic HCT116 cells after 48 hours incubation with compounds **1-6.**

Table S5 *Show the percentage of apoptotic HCT116 cells for compound **1-6** at concentrations ranging for 0-60 μM*

	Concentration of compound (μM)					
	0	**5**	**10**	**20**	**40**	**60**
Control	12.9%					
1	-	15.05%	16.35%	47.4%	-	-
2	-	-	-	-	18.8%	33%
3	-	13.55%	20.75%	76%	-	-
4	-	27.9%	53.55%	66.65%	-	-
5	-	11.6%	12.95%	35.5%	-	-
6	-	36.25%	66.2%	84.1%	-	-

pKa Studies

As the pK_a values of coordinated water can have an important influence on the reactivity of Ru(II) arene complexes. Solution studies measuring the pK_a of the bound water molecule indicates which species is present in solution, and thus whether the complex will be reactive towards potential target molecules. **Table S6** shows the pKa for the hydrolysis of compounds **1**, **4** and **6**. The pK_a values were determined by a ^1H NMR titration of in 95% D_2O/5% MeOH pH titration experiment. The pH values of solutions were measured at ambient temperature directly in the NMR tube, before and after recording NMR spectra using a 0.1 M $NaClO_4$ solution as an electrolyte. The pKa values were determined by fitting a pH titration curves to the extended Henderson–Hasselbalch equation. The proton resonances that were followed were chosen due to their chemical shifts as they were in a region that was not obstructed by other peaks.

Table S6 *pKa values for the hydrolysis of compounds **1, 4** and **6***

Compound	pKa
1	2.48 ± 0.2
4	4.54 ± 0.01
6	4.00 ± 0.03

Compounds **4** and **6** have higher pK_a values of 4.54 and 4.00 respectively and indicate that the compounds should exist as the deprotonated form at pH 7.4, however the bridged proton should also be considered. It is predicted that the bromide complexes would not form the deprotonated hydroxy [Ru-OH][HBr] in solution as the strong HBr acid would dissociate and protonated the hydroxyl group. Therefore, compounds **4** and **6** are thought to remain as the aqua adducts in solution, [Ru-OH_2][Br]. Compound **1** has a pK_a value of 2.48, which is lower than the corresponding bromo analogue (**4**), which suggests the chloride complexes may exist as the hydroxyl adducts in

solution, [Ru-OH][HCl]. It is known that the aqua adducts are more active *in vitro* than the hydroxyl analogues and the cytotoxicity results show that in all cases the bromides (aqua) are more cytotoxic than the chlorides (hydroxy). A direct correlation is observed between pKa value and IC_{50} value, in which the bromide complexes have the highest pKa values, form aqua adducts and are the most active against all cell lines.

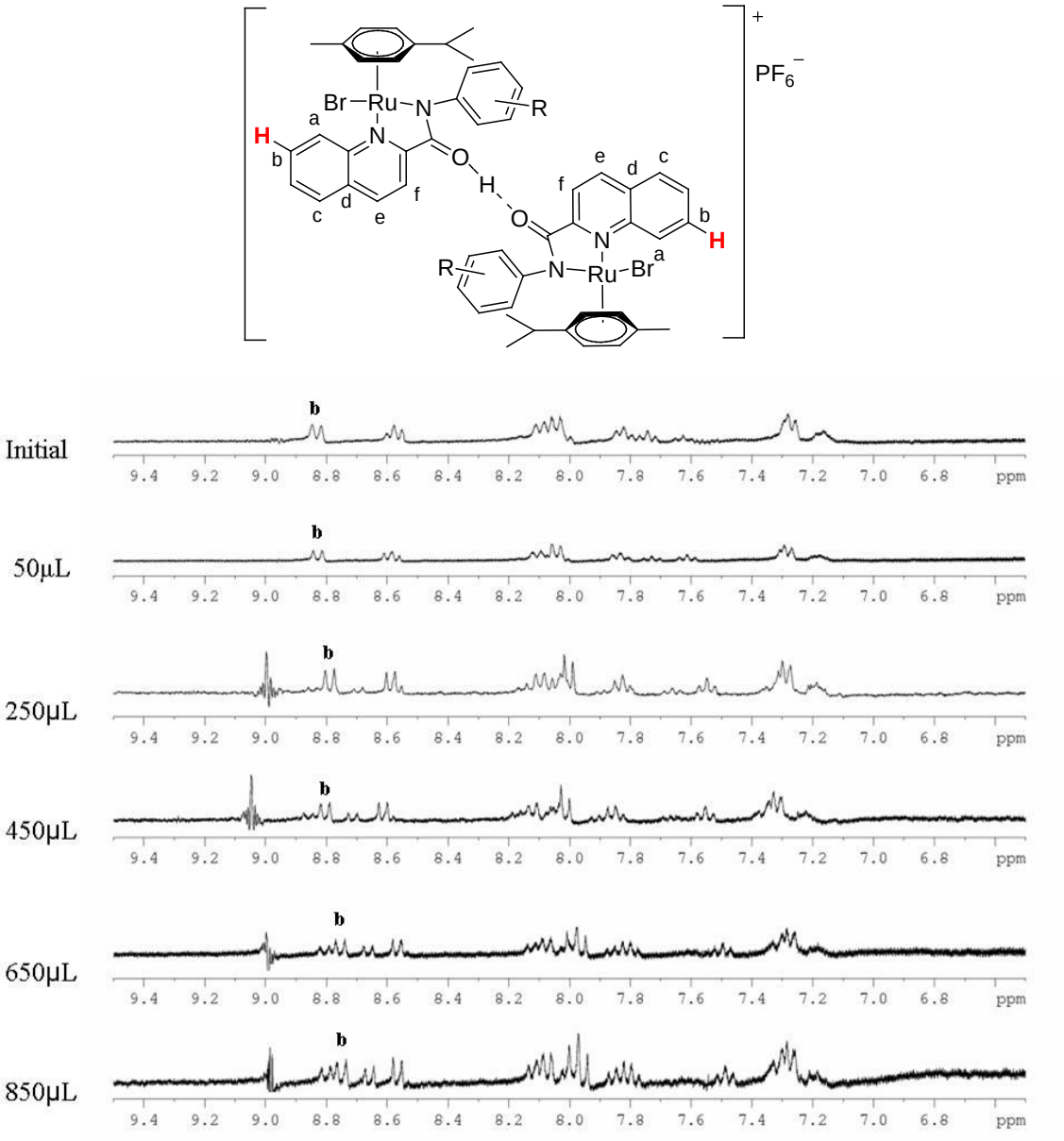


Figure S3 *^{1}H NMR spectra of compound **6** at different volumes of $NaClO_4$ showing the change in chemical shift of proton **Hb**.*

Figure S3 shows the ^{1}H NMR spectra for compound 6 with different volumes of $NaClO_4$. The appearance of a second doublet next to the doublet of **Hb** indicates the formation of another species in solution, however its structure is unknown as the species could not be isolated. **Figure S4** shows the ^{1}H NMR chemical shift of **Hb** versus pH for compound **6**, the curve shows a typical pH titration curve.

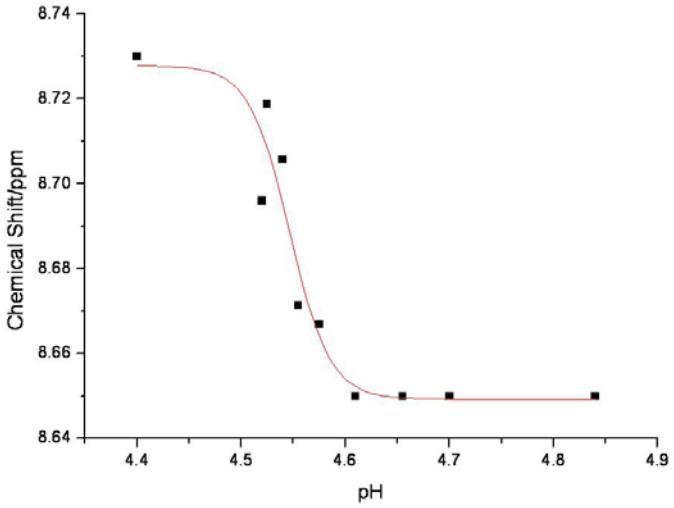


Figure S4 *Dependence of ^{1}H NMR chemical shift on pH of compound* ***6*** *(95% D_2O/5% MeOH, 0.1 M $NaClO_4$, 298 K)*