Supplementary Material

Synthesis and Trypanocidal Activity of Substituted 2,4-Diarylquinoline Derivatives

Kola A. Oluwafemi[a†], Siyolise Phunguphungu[a], Sinalo Gqunu[a], Michelle Isaacs,[c] Heinrich C. Hoppe [b,c], Rosalyn Klein[a,b*] and Perry T. Kaye[a,b*]

[a] Department of Chemistry, Rhodes University, Makhanda/Grahamstown, 6140, South Africa.
[b] Centre for Chemico-and Biomedical Research, Rhodes University, Makhanda/Grahamstown, 6140, South Africa
[c] Department of Biochemistry and Microbiology, Rhodes University, Makhanda/Grahamstown, 6140, South Africa
Email: r.klein@ru.ac.za; p.kaye@ru.ac.za

Table of Contents

1. ^{1}H- and ^{13}C-NMR spectra S2
2. Bioassay protocols and data S12

I. ^{1}H and ^{13}C NMR Spectra of compounds **8**, **4a-g** and **5h**,**i.**

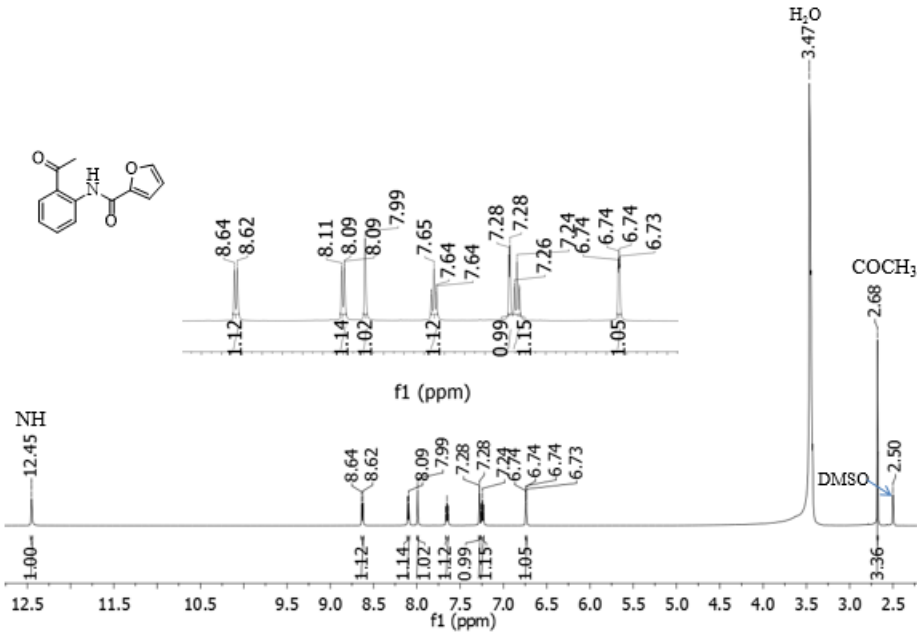


Figure S1. 600 MHz ^{1}H NMR spectrum of **8** in DMSO-d_6

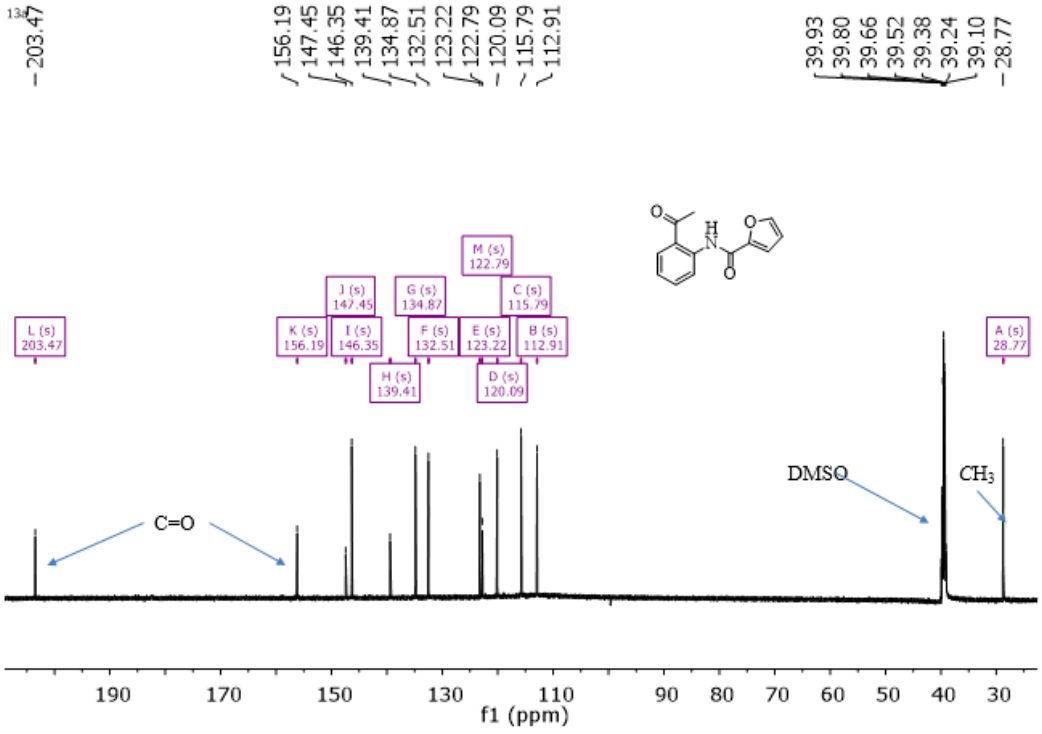


Figure S2. 150 MHz ^{13}C NMR spectrum of **8** in DMSO-d_6

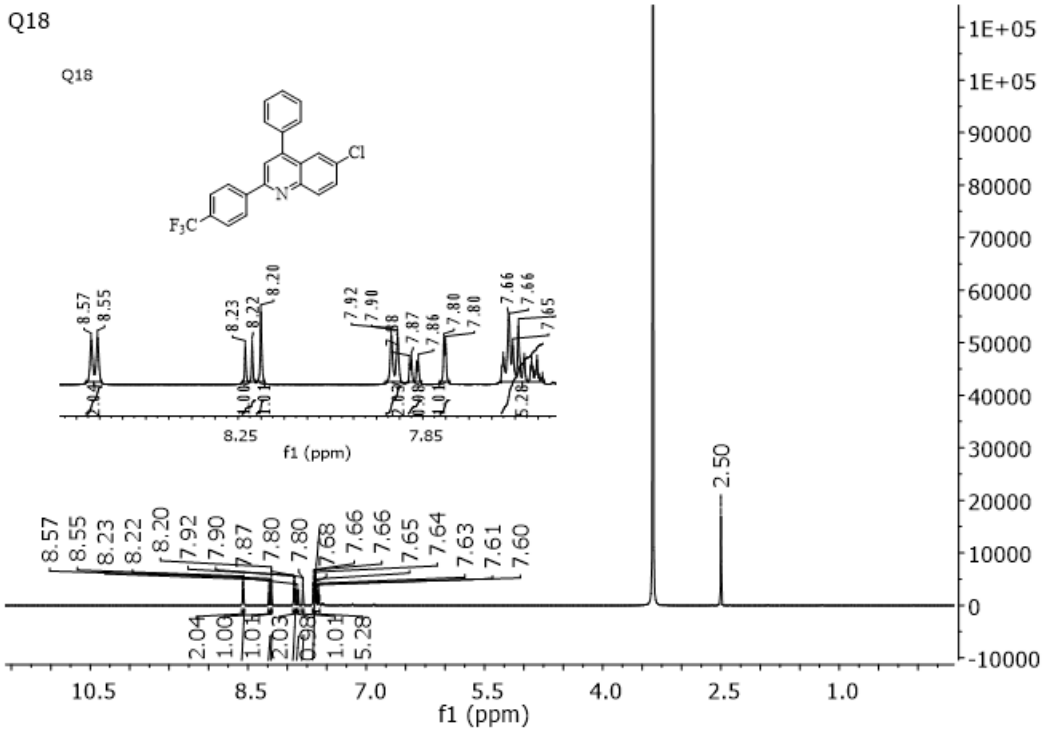


Figure S3. 600 MHz ^{1}H NMR spectrum of **4a** in DMSO-d_6.

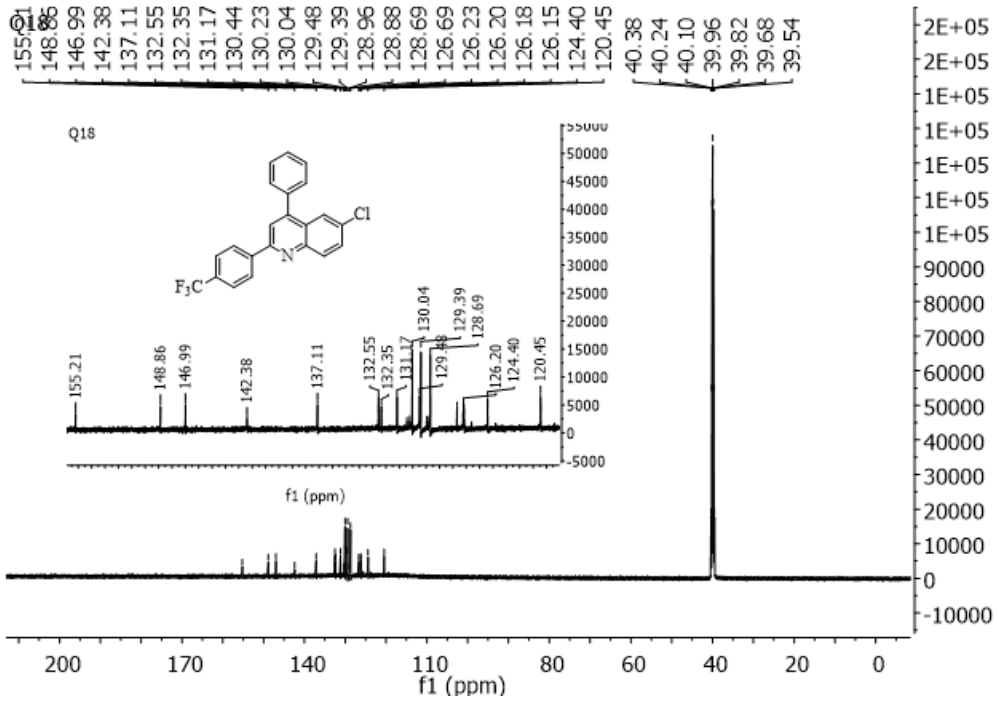


Figure S4. 150 MHz ^{13}C NMR spectrum of **4a** in DMSO-d_6.

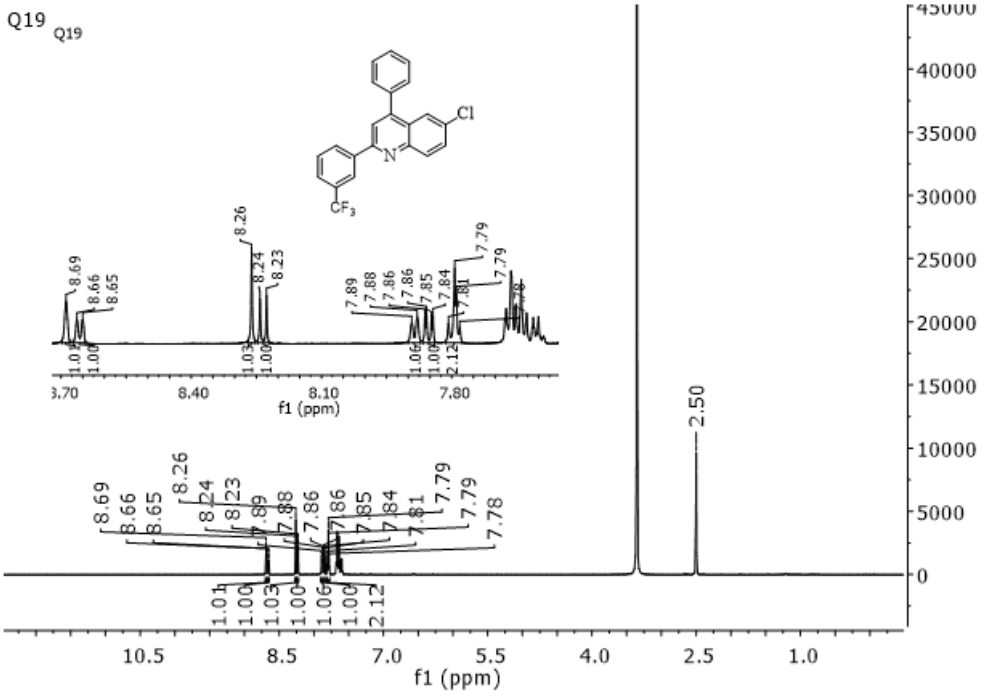


Figure S3. 600 MHz ^{1}H NMR spectrum of **4b** in DMSO-d_6

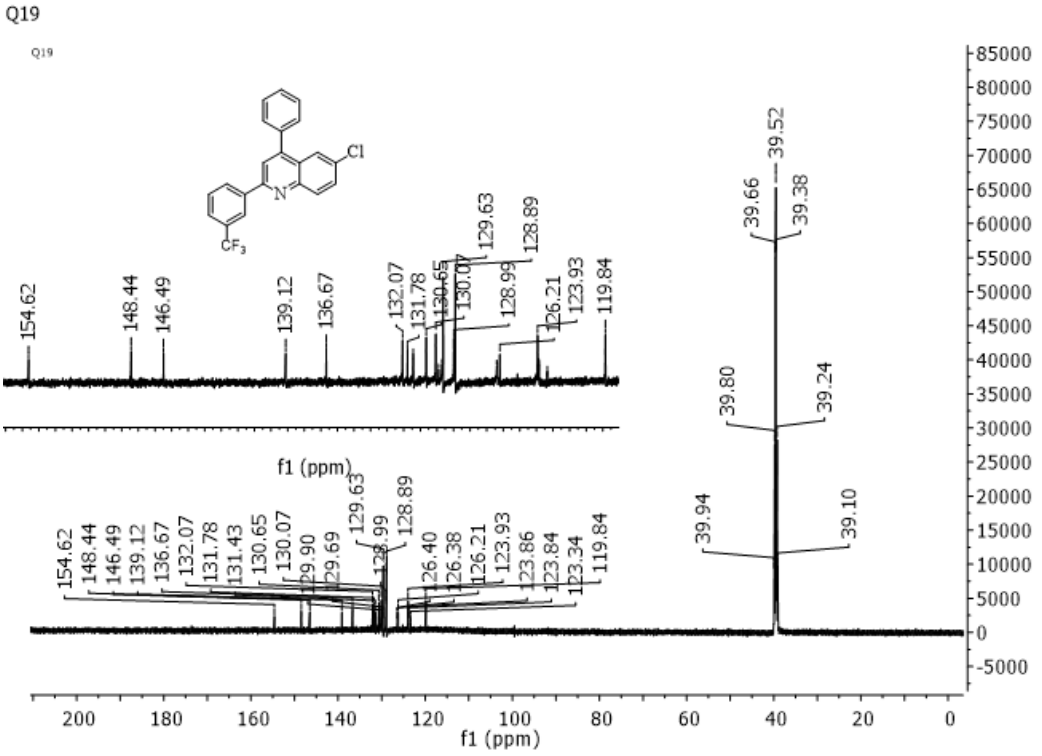


Figure S4. 150 MHz ^{13}C NMR spectrum of **4b** in DMSO-d_6.

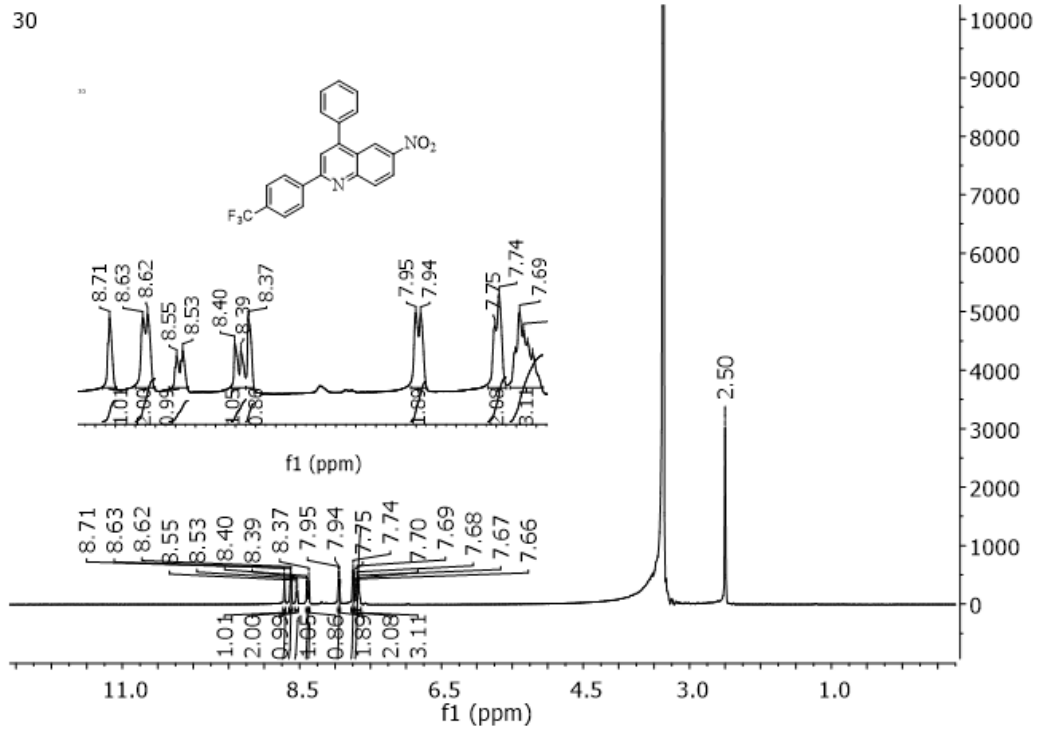


Figure S6. 600 MHz ^{1}H NMR spectrum of **4c** in DMSO-d_6.

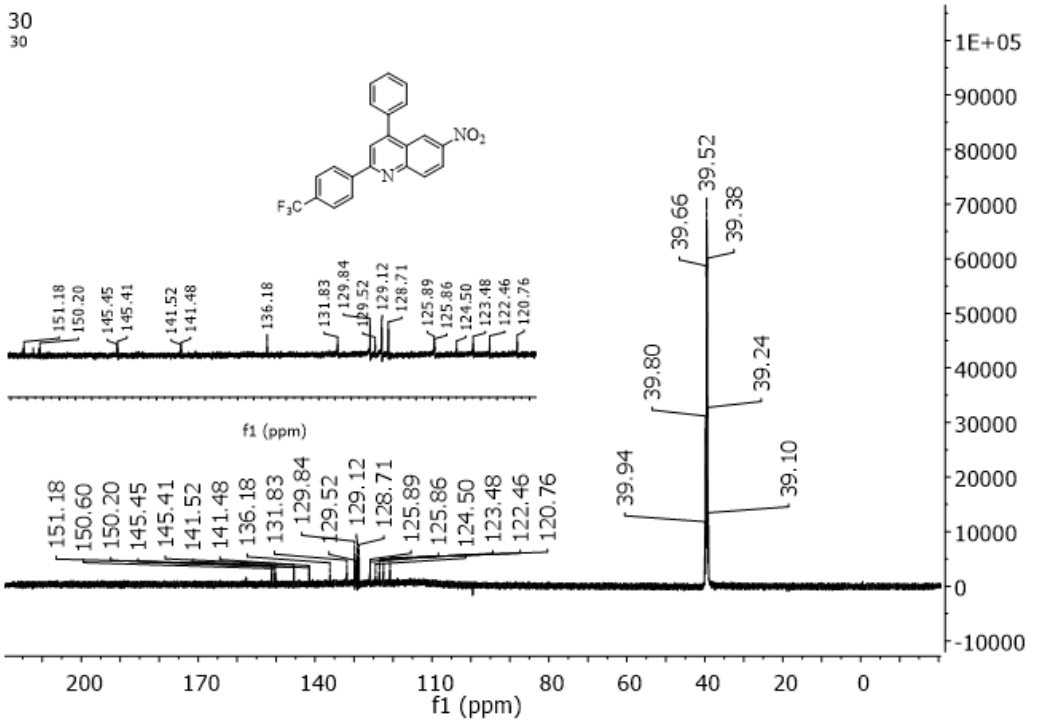


Figure S7.150 MHz ^{13}C NMR spectrum of **4c** in DMSO-d_6.

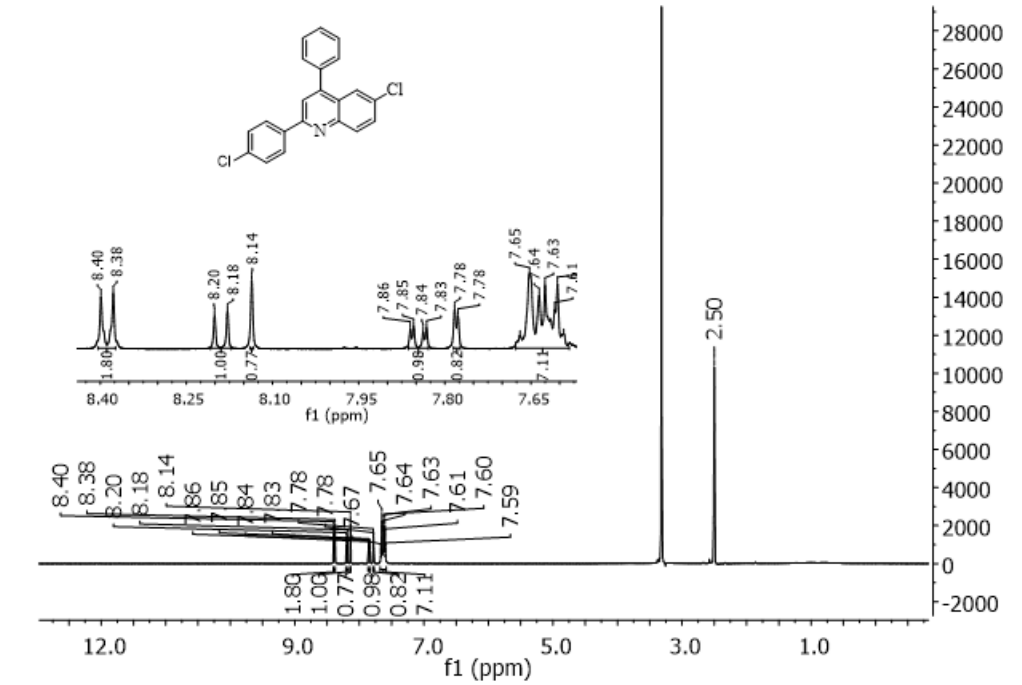


Figure S8. 300 MHz ^{1}H NMR spectrum of **4d** in DMSO-d_6.

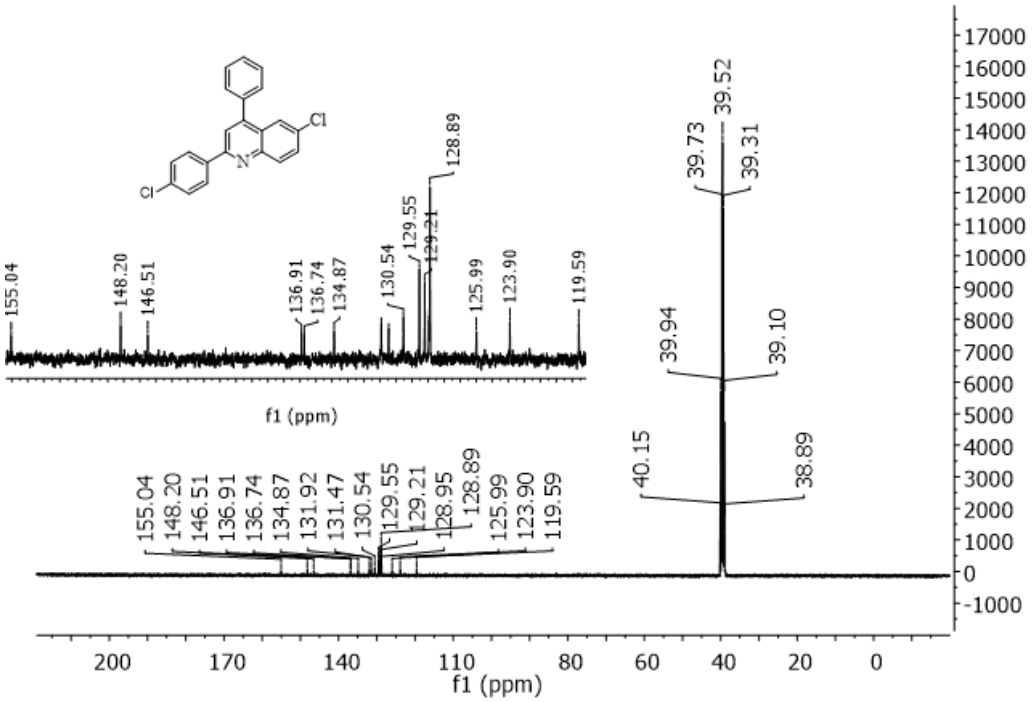


Figure S9. 100 MHz ^{13}C NMR spectrum of **4d** in DMSO-d_6.

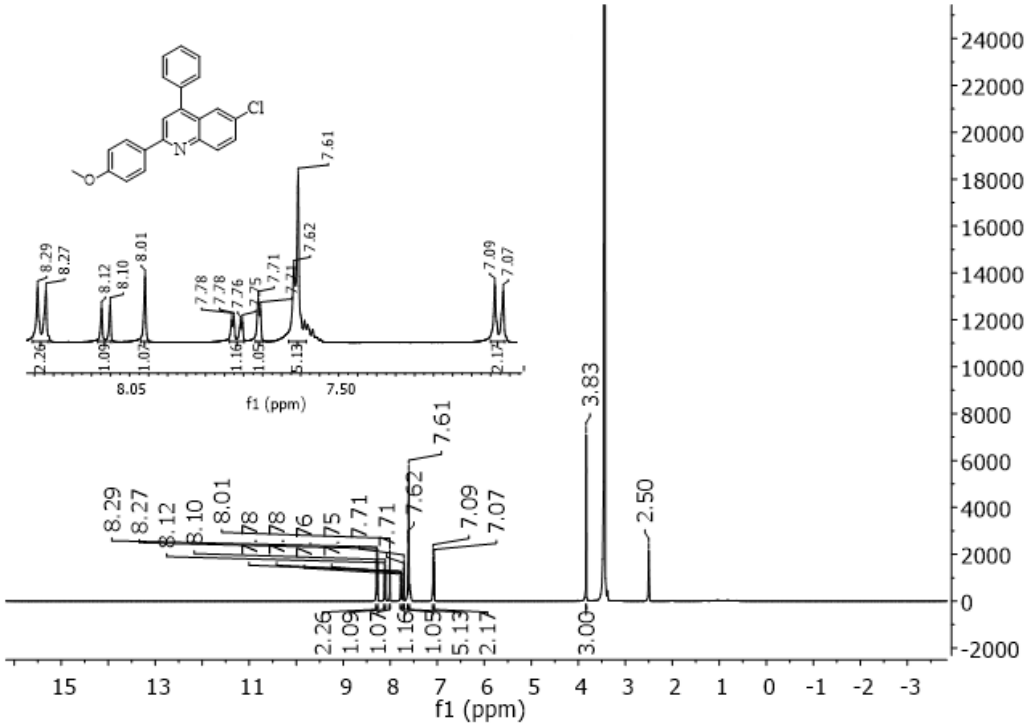


Figure S10. 400 MHz ^{1}H NMR spectrum of **4e** in DMSO-d_6.

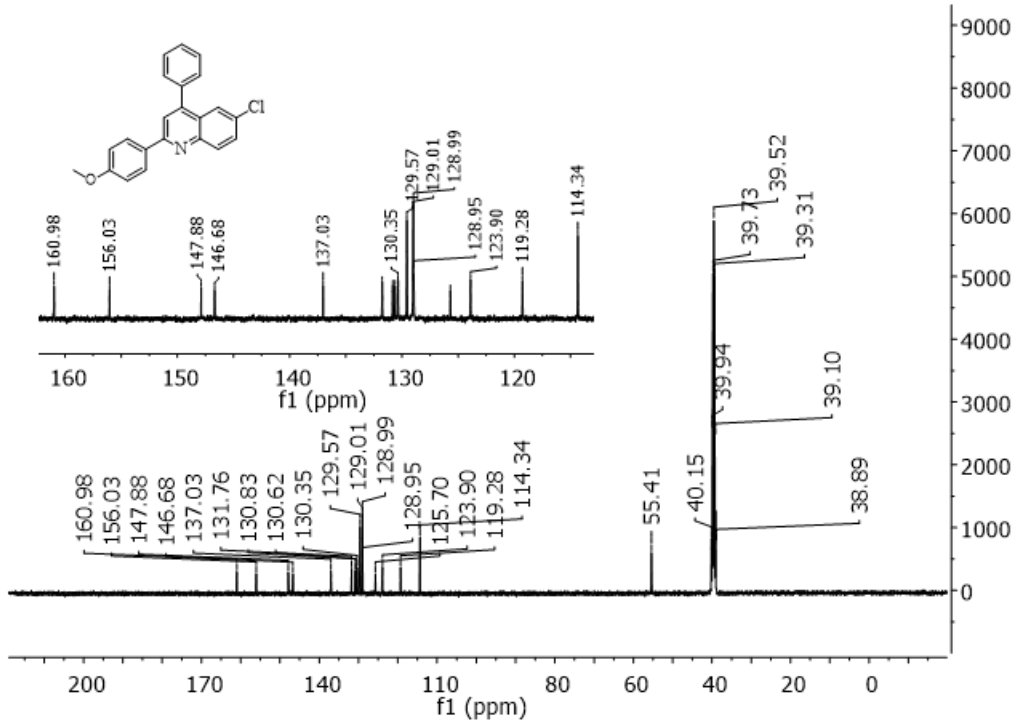


Figure S11. 150 MHz ^{13}C NMR spectrum of **4e** in DMSO-d_6.

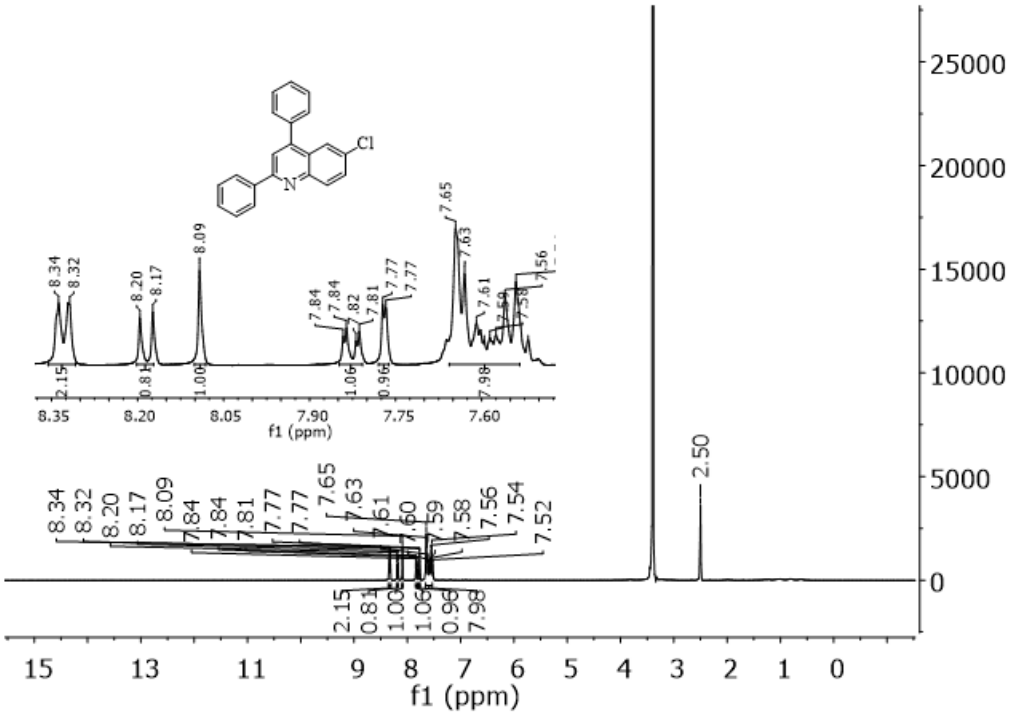


Figure S12. 400 MHz ^{1}H NMR spectrum of **4f** in DMSO-d_6

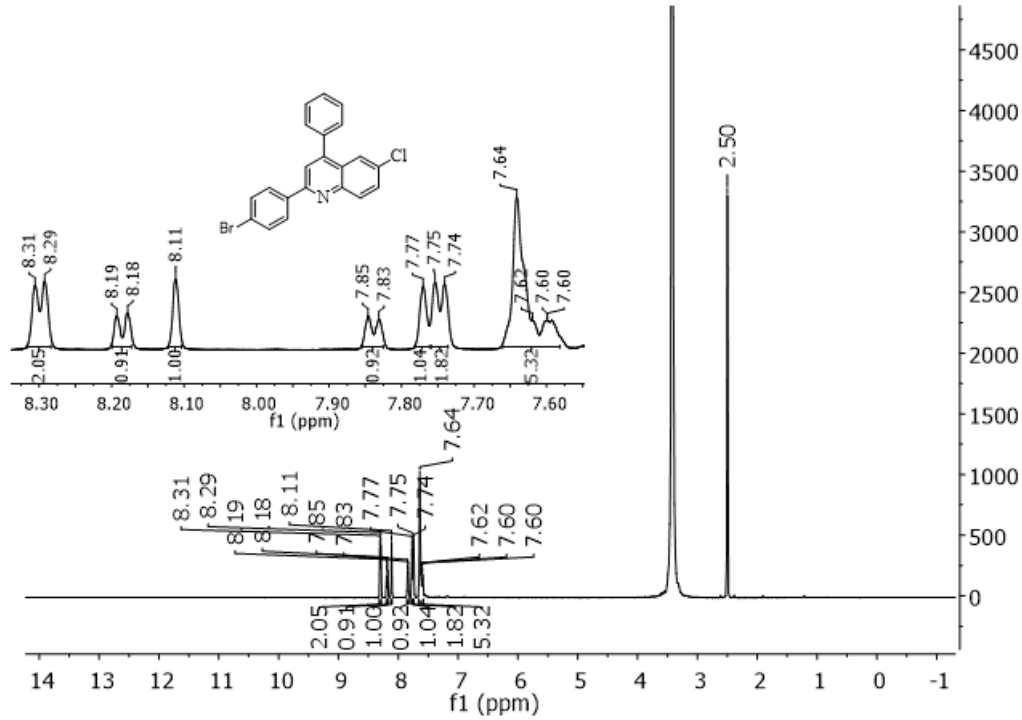


Figure S13. 400 MHz ^{1}H NMR spectrum of **4g** in DMSO-d_6.

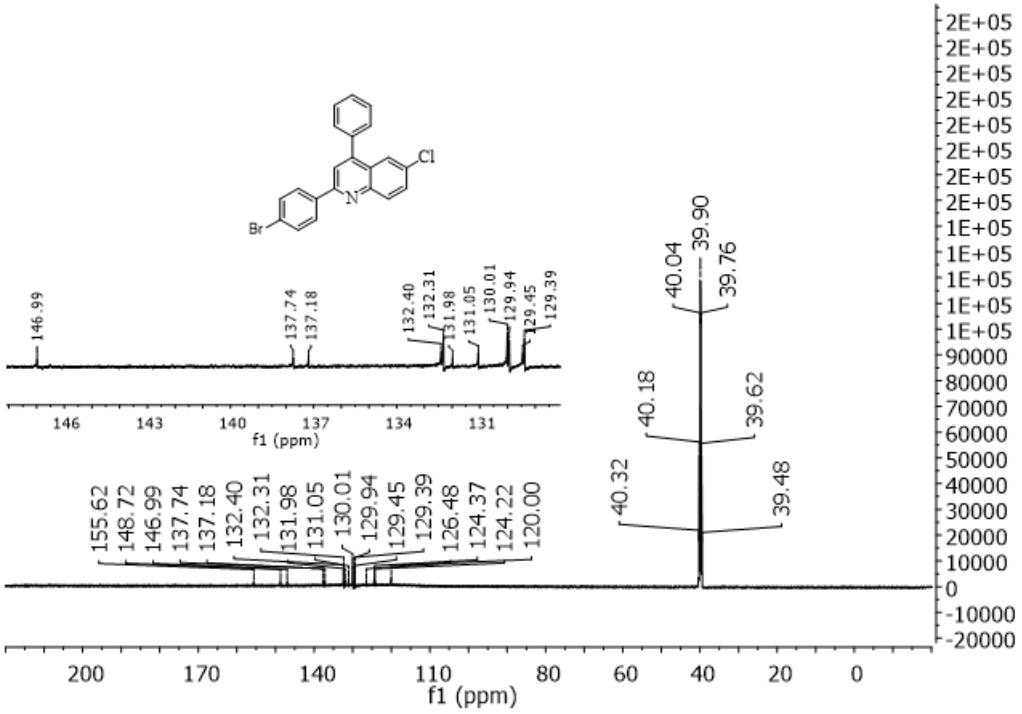


Figure S14. 100 MHz ^{13}C NMR spectrum of **4g** in DMSO-d_6.

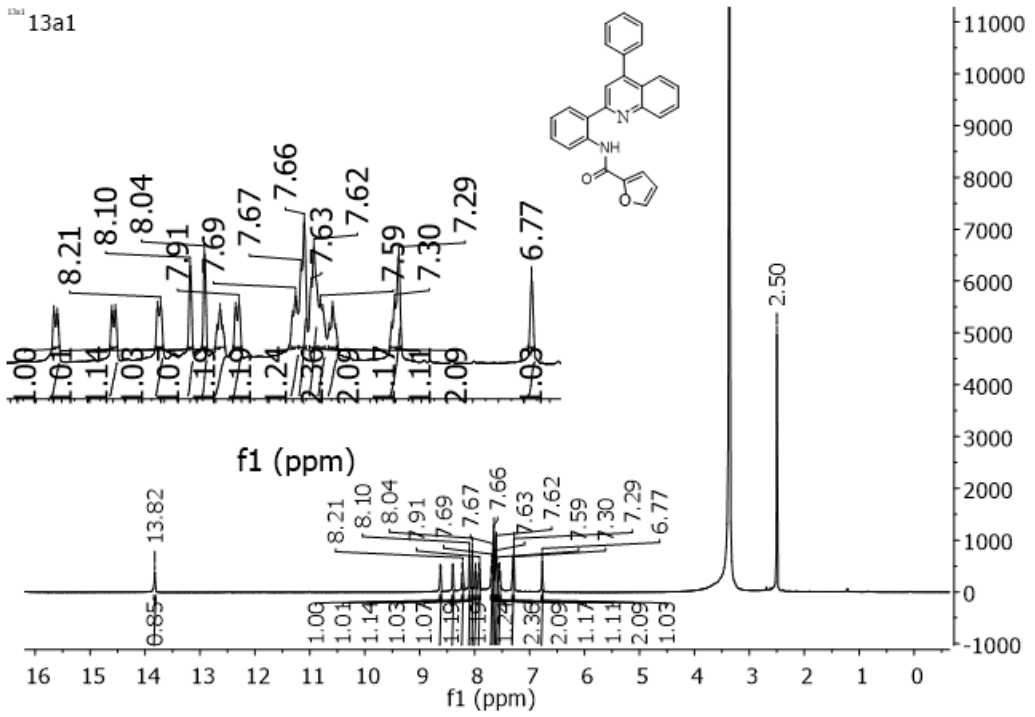


Figure S16. 600 MHz ^{1}H NMR spectrum of **5h** in DMSO-d_6.

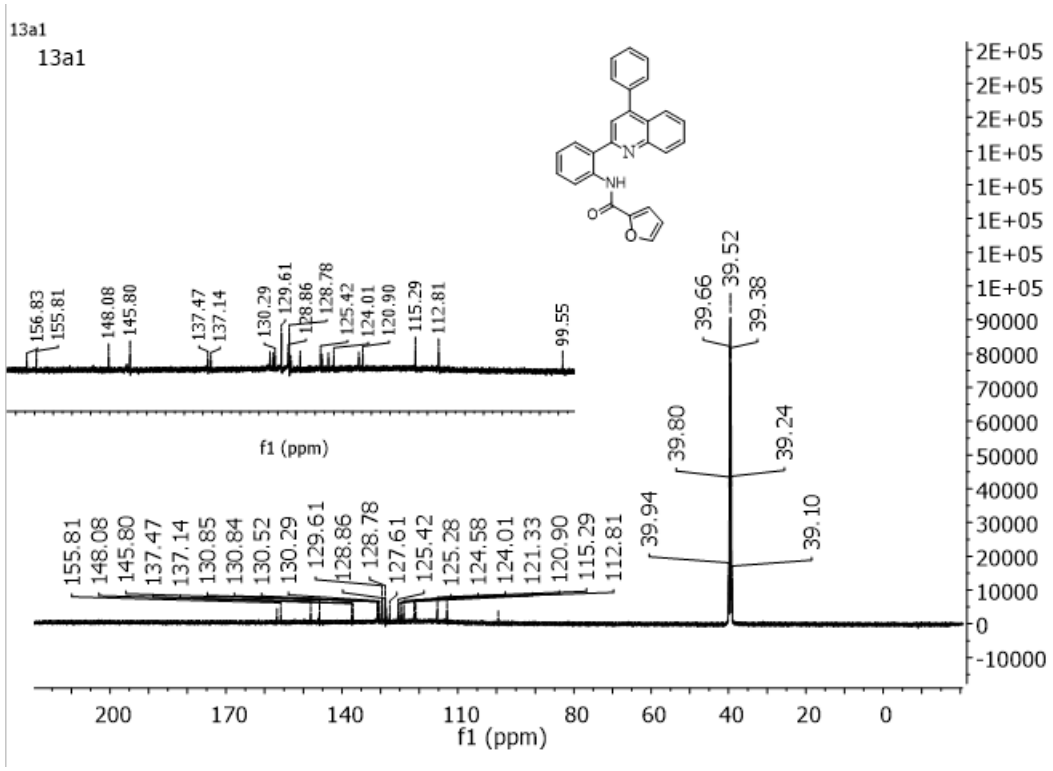


Figure S17. 150 MHz ^{13}C NMR spectrum of **5h** in DMSO-d_6.

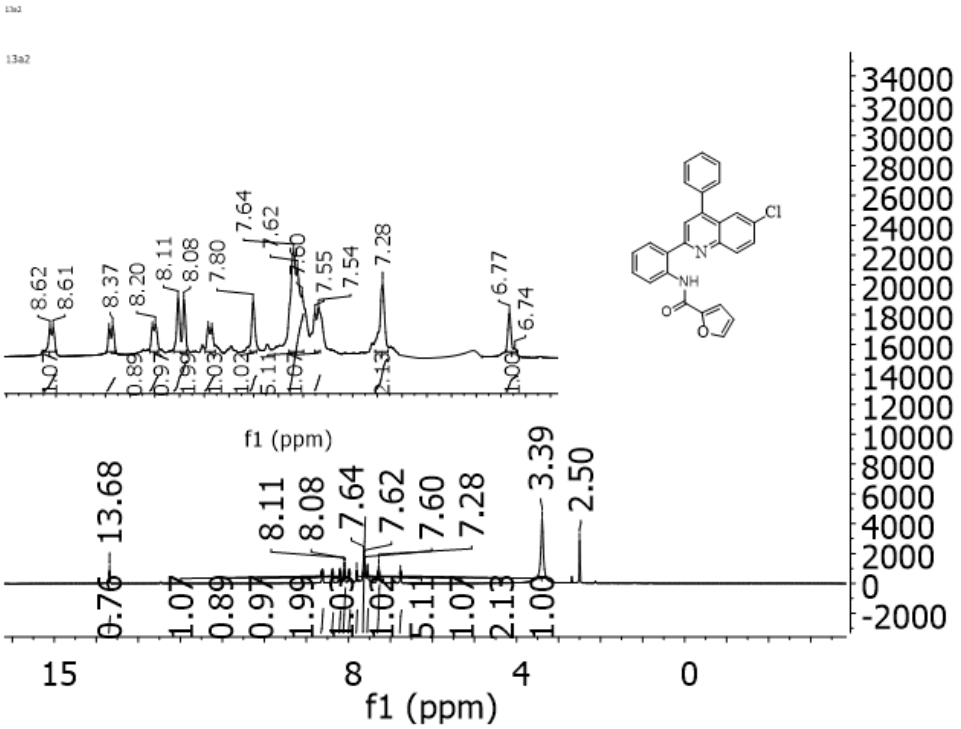


Figure S18. 600 MHz ^{1}H NMR spectrum of **5i** in DMSO-d_6.

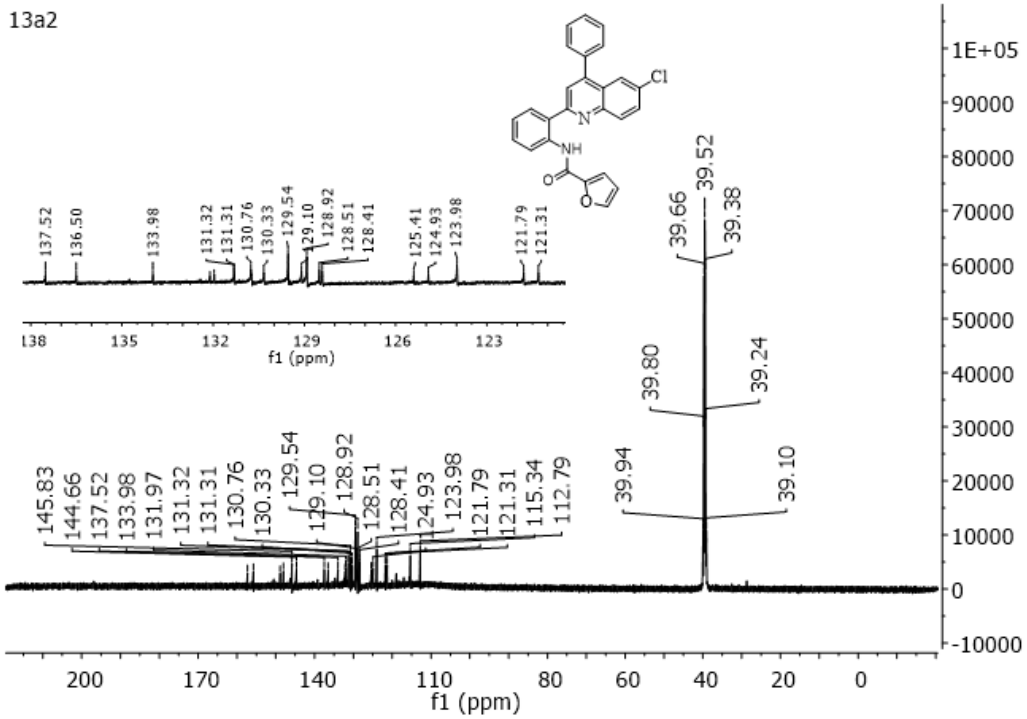


Figure S19. 150 MHz ^{13}C NMR spectrum of **5i** in DMSO-d_6.

II. Bioassay protocols and data

Cytotoxicity determination

To assess the overt cytotoxicity of the compounds, they were incubated at a fixed concentration of 20 μM in 96-well plates containing HeLa (human cervix adenocarcinoma) cells for 48 hours. After the incubation, residual cell viability was determined using a resazurin assay, as previously described (Veale and Hoppe, 2018). Results were expressed as % cell viability – the resorufin fluorescence in compound-treated wells relative to untreated controls, after subtracting background readings obtained from wells without cells. Compounds were tested in duplicate wells. Emetine was used as a control drug standard.

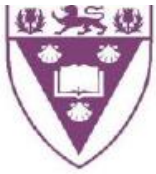
Anti-trypanosomal Assay

To assess trypanocidal activity, compounds were incubated with *in vitro* cultures of *T.b. brucei* (strain 427) in 96-well plates at a fixed concentration of 20 µM or as 3-fold serial dilutions, and parasite viability assessed by a resazurin assay as described previously (Veale and Hoppe). Pentamidine was used a control drug standard

Veale C.G.L., Hoppe H.C. *Med. Chem. Commun*. **2018**, 9, 2037.

The following key correlates compound numbers in the paper with the sample codes in the following Bioassay reports

4a - AKO-Q-18
4b - AKO-Q-19
4c - sinalo 3
4d - AKO-Q-1
4e - AKO-Q-2
4f - AKO-Q-10
4g - AKO-Q-5
5h - AKO-Q-13a1
5i - AKO-Q-13a2



Rhodes University
Centre for Chemico- and Biomedicinal Research
MRC SA University Flagship Programme

Cytotoxicity assay – single concentration screen

Background

To assess the overt cytotoxicity of the compounds, they are incubated at a fixed concentration of 20uM for Pure compounds and 50ug/ml for extracts (unless otherwise stated) in 96-well plates containing HeLa (human cervix adenocarcinoma) cells/ cells for 48 hours. The numbers of cells surviving drug exposure are also determined by using the resazurin based reagent and reading resorufin fluorescence in a multiwell plate reader.

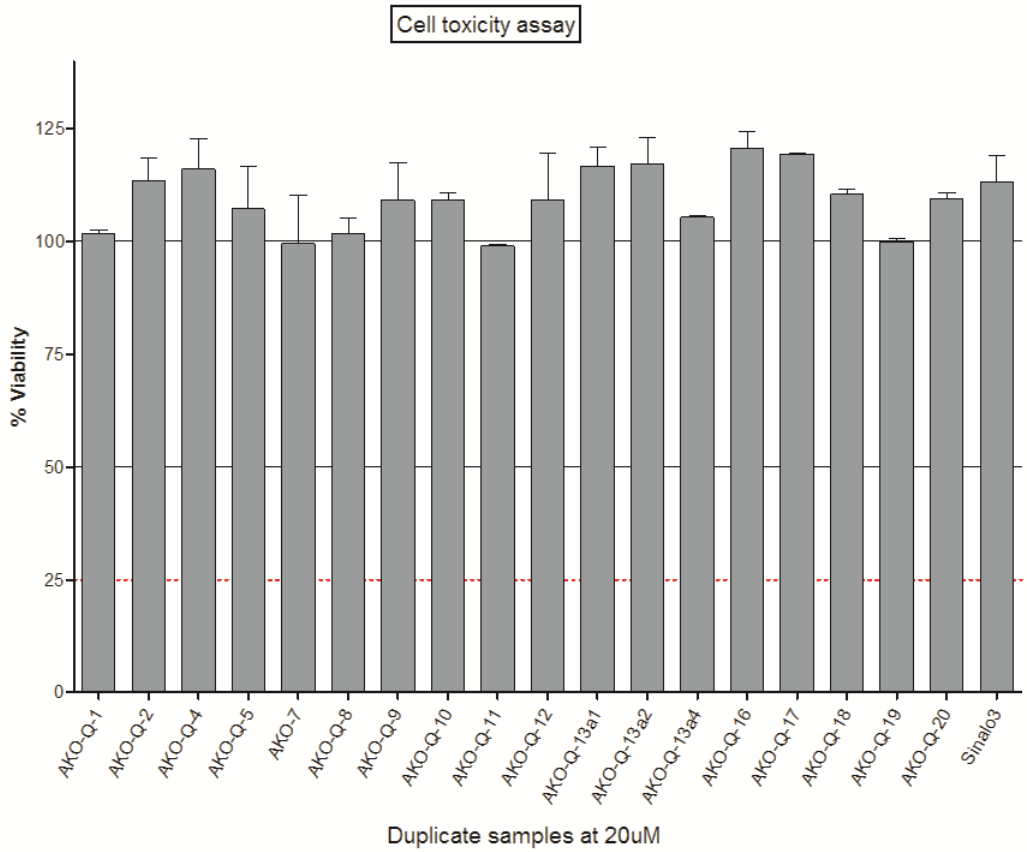
Results are expressed as **% viability** – the resorufin fluorescence in compound-treated wells relative to untreated controls. Compounds are usually tested in duplicate wells, and a standard deviation (SD) is also included. Emetine (which induces cell apoptosis) is used as a positive control drug standard.
(*Note: for publication purposes, a detailed description of the method is available on request*)

Assay details

Date: 16 June 2017
Concentration used: 20uM

Results

The bar graph and table below show the % HeLa cell viability ±SD obtained for the individual compounds.

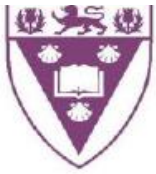


Compound at 20uM unless otherwise specified	Viability %	SD
AKO-Q-1	101.7	0.8
AKO-Q-2	113.4	5.2
AKO-Q-4	116.1	6.6
AKO-Q-5	107.2	9.4
AKO-7	99.5	10.8
AKO-Q-8	101.8	3.4
AKO-Q-9	109.2	8.3
AKO-Q-10	109.3	1.4
AKO-Q-11	99.0	0.4
AKO-Q-12	109.2	10.5
AKO-Q-13a1	116.7	4.3
AKO-Q-13a2	117.1	6.0
AKO-Q-13a4	105.4	0.5
AKO-Q-16	120.6	3.9
AKO-Q-17	119.4	0.03
AKO-Q-18	110.4	1.1
AKO-Q-19	99.9	0.7
AKO-Q-20	109.4	1.4
Sinalo3	113.2	5.9

		Emetine
Conc (µM)	Log(Conc)	Percentage Viability
1	0	28.2
0.333333	-0.47712	38.5
0.111111	-0.95424	50.1
0.037037	-1.43136	77.4
0.012346	-1.90849	93.1
0.004115	-2.38561	115.8
0.001372	-2.86273	118.1
0.000457	-3.33985	104.3
	IC50	0.047

Conclusion

None of the samples caused significant cytotoxic effects at a concentration of 20 µM (none reduced the viability of HeLa cells to below 50%).



Rhodes University
Centre for Chemico- and Biomedicinal Research
MRC SA University Flagship Programme

Trypanosoma brucei assay – single concentration screen

Background

Trypanosoma brucei (T.b.) parasites are the causative agents of African sleeping sickness (human African trypanosomiasis) in humans and Nagana (animal African trypanosomiasis) in cattle. The subspecies responsible for Nagana (*T.b. brucei*) is not infective to humans and is commonly used for drug screening. To assess anti-trypanocidal activity, compounds are added to *in vitro* cultures of *T.b. brucei* in 96-well plates at a fixed concentration of 20µM for pure compounds or 25µg/mL for natural extracts (unless otherwise stated). After an incubation period of 48 hours, the numbers of parasites surviving drug exposure are determined by adding a resazurin based reagent. The reagent contains resazurin which is reduced to resorufin by living cells. Resorufin is a fluorophore (Exc_{560}/Em_{590}) and can thus be quantified in a multiwell fluorescence plate reader.

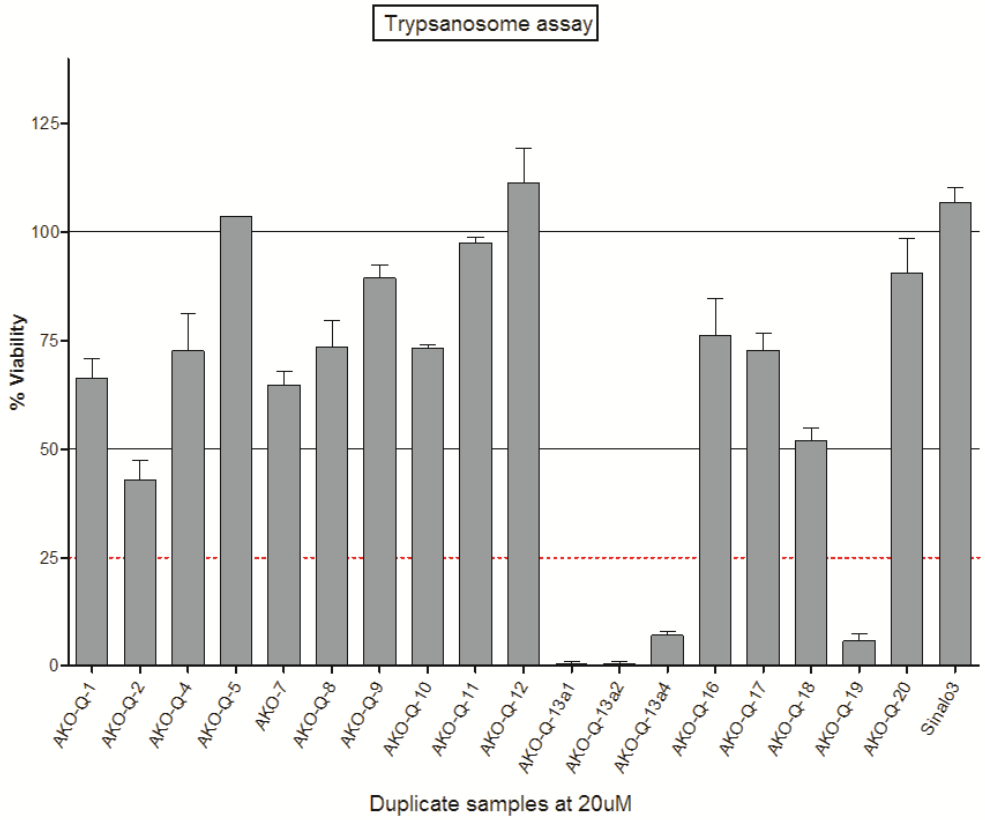
Results are expressed as **% parasite viability** – the resorufin fluorescence in compound-treated wells relative to untreated controls. Compounds are usually tested in duplicate wells, and a standard deviation (SD) is also included. Generally, compounds/extracts that reduce parasite viability to < 10-20% may be considered for further testing (e.g. dose-response and cytotoxicity assays). Pentamidine (an existing drug treatment for trypanosomiasis) is used as a positive control drug standard.

Assay details

Date: 15 June 2017
Concentration used: 20 uM

Results

The bar graph and table below show the residual % parasite viability ±SD obtained for the individual compounds.



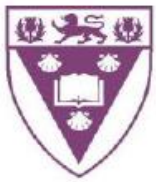
Compound at 20uM	Viability %	SD
AKO-Q-1	66.4	4.4
AKO-Q-2	42.9	4.5
AKO-Q-4	72.6	8.5
AKO-Q-5	103.5	0.06
AKO-7	64.8	3.2
AKO-Q-8	73.4	6.3
AKO-Q-9	89.4	3.0
AKO-Q-10	73.2	0.8
AKO-Q-11	97.6	1.2
AKO-Q-12	111.4	7.9
AKO-Q-13a1	0.49	0.7
AKO-Q-13a2	0.53	0.5
AKO-Q-13a4	7.07	1.0
AKO-Q-16	76.1	8.5
AKO-Q-17	72.8	4.1
AKO-Q-18	51.9	2.8
AKO-Q-19	5.8	1.7
AKO-Q-20	90.5	7.9
Sinalo 3	106.8	3.6

Pentamidine standard:-

Concentration (uM)	Log	Percentage activity	Std dev
1	0	-0.3	0.08
0.333333	-0.47712	2.0	0.16
0.111111	-0.95424	2.1	0.16
0.037037	-1.43136	2.7	0.39
0.012346	-1.90849	4.0	1.2
0.004115	-2.38561	14.8	0.93
0.001372	-2.86273	88.9	2.2
0.000457	-3.33985	84.4	2.6
	IC50	0.0037	

Conclusion

The samples highlighted in red significantly affected the viability of the Trypanosomes at 20 µM. These samples will be subjected to IC_{50} evaluations if they are not cytotoxic (the results need to be looked at in conjunction with the Cell toxicity assay results).



Rhodes University

Centre for Chemico- and Biomedicinal Research

MRC SA University Flagship Programme

Trypanosoma brucei assay – dose response

Background

Trypanosoma brucei (T.b.) parasites are the causative agents of African sleeping sickness (human African trypanosomiasis) in humans and Nagana (animal African trypanosomiasis) in cattle. The subspecies responsible for Nagana (*T.b. brucei*) is not infective to humans and is commonly used for drug screening. To determine the anti-trypanocidal potency of test compounds, serial dilutions of the compounds are added to *in vitro* cultures of *T.b. brucei* in 96-well plates and incubated for 48 hours. The numbers of parasites surviving exposure to the individual compound concentrations are determined by adding a resazurin based reagent. The reagent contains resazurin which is reduced to resorufin by living cells. Resorufin is a fluorophore (Exc_{560}/Em_{590}) and can thus be quantified in a multiwell fluorescence plate reader.

For each compound concentration, **% parasite viability** – the resorufin fluorescence in compound-treated wells relative to untreated controls – is calculated. Compounds are usually tested in duplicate wells, and a standard deviation (SD) is derived. For each compound, percentage viability is then plotted against Log(compound concentration) and the **IC_{50}** (50% inhibitory concentration) obtained from the resulting dose-response curve by non-linear regression. For comparative purposes, pentamidine (an existing drug treatment for trypanosomiasis) is used as a drug standard and yields IC_{50} values in the range 0.001-0.005 µM.

Assay details

Date: 22 June 2017

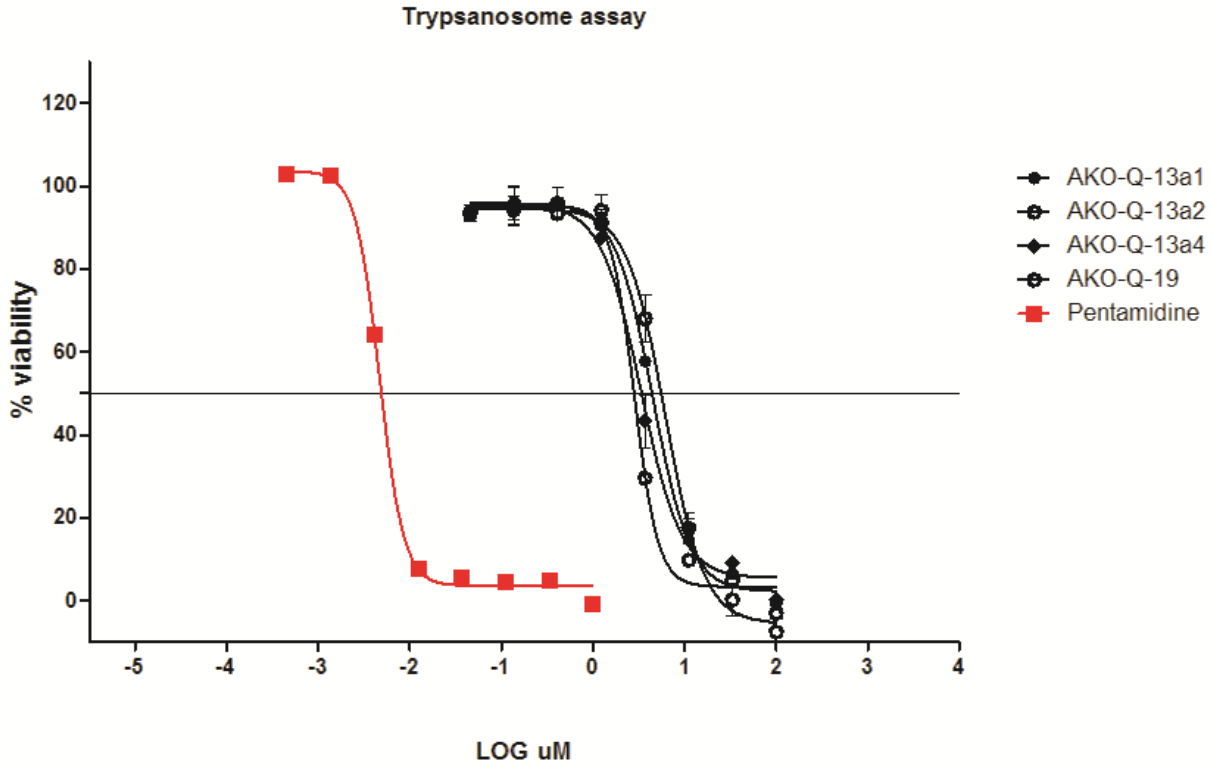
Concentration used: 3-fold serial dilutions with a starting concentration of 100 µM

Results

The table below shows the IC_{50} values obtained for individual compounds, followed by the dose-response plots and % viability ±SD data used to prepare the graphs.

Compound	IC50 (uM)
AKO-Q-13a1 (5i)	4.5
AKO-Q-13a2 (5j)	2.8
AKO-Q-13a4 (5k)	3.4
AKO-Q-19 (4b)	6.2
Pentamidine	0.005

* Approximate



		AKO-Q-13a1		AKO-Q-13a2		AKO-Q-13a4	
uM	log	%Viab	SD	%Viab	SD	%Viab	SD
100	2	-0.6594	0.024711	-3.12057	0.477104	0.212371	0.244101
33.33333	1.522879	6.479433	0.493322	4.828215	0.757764	9.155692	0.44266
11.11111	1.045757	14.71262	0.814962	9.761726	0.488219	17.31352	2.330072
3.703704	0.568636	57.74025	0.523188	29.56382	1.264962	43.34812	6.399081
1.234568	0.091515	91.97546	1.528426	90.82199	3.35642	87.65957	1.139119
0.411523	-0.38561	95.96166	3.627241	95.76089	0.808916	95.95919	3.523715
0.137174	-0.86273	95.98511	3.989653	95.32986	4.47013	94.10931	3.579952
0.045725	-1.33985	93.40788	0.56873	93.5491	0.863685	93.4525	1.997981

		AKO-Q-19	
uM	log	%Viab	SD
100	2	-7.4997	2.972549
33.33333	1.522879	0.222726	3.86748
11.11111	1.045757	17.4995	3.650694
3.703704	0.568636	68.00697	5.707235
1.234568	0.091515	94.13371	3.88022
0.411523	-0.38561	93.31949	0.911201
0.137174	-0.86273	94.13203	0.116965
0.045725	-1.33985	93.18314	1.021578

Pentamidine		
Conc (µM)	Log(Conc)	%Viab
1	0	-0.89291
0.333333	-0.47712	5.04438
0.111111	-0.95424	4.610519
0.037037	-1.43136	5.654776
0.012346	-1.90849	7.847016
0.004115	-2.38561	64.11454
0.001372	-2.86273	102.5856
0.000457	-3.33985	102.797

Conclusion

The IC_{50} values of the samples are listed in the first table.