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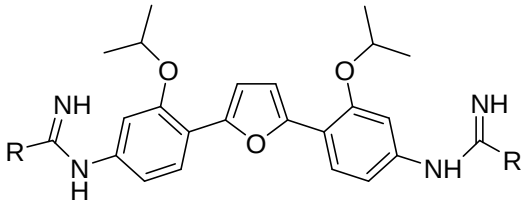

R= various 5 and 6 membered ring heterocyclces

10 of 15 compounds are very active vs. *Plasmodium falciparum* (IC_{50} = 9-87 nM)

Synthesis and antiparasitic activity of new bis-arylimidamides: DB766 analogs modified in the terminal groups

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Abstract: Fifteen novel bis-arylimidamide derivatives with various 6-membered (**7a-c)** and 5-membered (**7d-o)** heterocyclic rings replacing the terminal pyridyl rings of the lead compound DB766{(2,5-bis[2-*i*-propoxy-4-(2-pyridylimino)aminophenylfuran]}, were prepared and evaluated versus *Trypanosoma cruzi, Leishmania amazonensis, Trypanosoma brucei rhodesiense* and *Plasmodium falciparum*. Compound **7a** with pyrimidine replacing the pyridine rings showed good activity versus *T. cruzi*, *T. brucei rhodesiense* and *P. falciparum* (IC_{50} = 200 nM, 32 nM and 8.5 nM, respectively). Three compounds (**7g, 7i, 7j**) with thiazole replacing the pyridine rings gave low micromolar (0.17- 0.3 μM) IC_{50} values versus *L. amazonensis*, however only **7g** exhibited an acceptable selectivity index (SI = 27). Compounds **7a**, **7j**, and **7m** exhibited potent activity against *T. brucei rhodesiense* (IC_{50} = 12-60 nM). Ten of the 15 compounds with pyrimidine, pyrrole, thiazole and imidazole terminal units were highly active against *P. falciparum* (IC_{50} = 9-87 nM). Both pyrimidine and pyridine terminal groups are advantageous for anti-*T. cruzi* activity and several different heterocyclic terminal units are effective versus *P. falciparum*, both findings merit further investigation.

Keywords: bis-arylimidamides, DB766, antiparasitic agents

1. Introduction

Parasitic diseases including African sleeping sickness, Chagas' disease, leishmaniasis, malaria and others have been a major threat for mankind for centuries and continue to cause significant public health problems [1, 2]. Protozoan parasitic diseases are major causes of mortality and morbidity in tropical and subtropical countries, and are also responsible for important economic losses [1-5]. Chagas' disease is a neglected tropical illness caused by *Trypanosoma cruzi* that affects approximately 9 million people in Latin America, with an expected mortality of between 23 and 45 thousand individuals [5, 6]. Currently there are an estimated 300,000 cases in the United States [7]. African sleeping sickness is caused by the protozoan parasite *Trypanosoma brucei* exclusively in sub-Saharan Africa. The number of reported cases is decreasing and currently at less than 8000 per year but the estimated number is likely to be around 25,000 [8]. Leishmaniasis, a neglected tropical disease caused by parasitic protozoa of the genus *Leishmania*, is responsible for 1- 2 million new clinical cases each year in 88 countries where up to 350 million people are at risk of infection [9]. Malaria is a major public health problem today in approximately 100 countries where it is estimated that 219 million clinical cases occur annually, with mortality estimated at 660 thousand persons per year [10]. Approximately half the world population is at risk of contract malaria [10]. However, up to now, for all of these parasitic diseases, effective vaccines are lacking and the approved chemotherapeutic compounds are costly, toxic, require long periods of treatment and generally are losing their effectiveness due to the development of resistant parasites. Therefore,

new effective, more selective and safer antiparasitic agents are urgently needed.
Diamidines such as pentamidine (Figure 1) are DNA minor groove binders and represent a potent class of antiparasitic agents with a broad spectrum of activities against human and veterinary pathogens [11]. However, these compounds often have poor bioavailability and cause considerable side effects. Many analogues and derivatives, including prodrugs, have been synthesized and screened *in vitro* and *in vivo* in order to overcome these limitations [2, 11]. In our efforts to identify promising antiprotozoal agents from a library of aromatic diamidines and their analogues, we have identified arylimidamides (AIAs), which have demonstrated high potency against several parasites including *T. cruzi* [2, 12-14], *Leishmania* spp. [2,13-16], *Neospora caninum* [17,18], *Besnoitia besnoiti* [19] and *Echinococcus multilocularis* [20]. The initial AIAs were modified versions of furamidine (Figure 1) which were designed as DNA minor groove binders and which have pKa values of approximately 7 in comparison to furamidine and other classical amidines which exhibit pKa values of 10-11 [21]. The lower pKa values were expected to improve the oral bioavailability of these modified analogues. Furamidine and analogues showed only moderate anti-*T.cruzi* and anti-*leishmanial* effects [22, 23] and the AIAs with altered physiochemical properties displayed improved activity. DB766 (Figure 1), one of the most potent antileishmanial AIAs, exhibited *in vitro* activity with IC_{50} values of 0.036 and 0.087 μM against *L.dono vani* and *L. amazonensis*, respectively, and notably reduced liver parasitemia in both mice and hamsters [21]. DB766 also exhibited strong anti-*T. cruzi* activity and excellent selectivity for bloodstream trypomastigotes and intracellular amastigotes (Y strain) [12]. Studies *in vivo* with DB766 demonstrated a reduction in the parasite load levels in the blood and cardiac tissue with similar trypanocidal activity as that of benznidazole in a mouse model of acute *T. cruzi* infection [12,13]. The excellent activity of DB766 motivated the design and synthesis of novel structurally related compounds [16,24]. The mode of antiparasitic action of classical diamidines has been suggested to involve, at least in part, kDNA binding leading to mitochondria disruption [25], however the mode of action of AIAs, which has not been extensively studied, seems likely to be different and more complex than that of the parent diamidines. For example, the anti-*T. cruzi* activity of AIAs is not correlated with kDNA binding affinities [26]; despite the fact that AIAs do bind to the minor groove of DNA in a highly structure dependent manner which has been studied in detail [27]. However, intracellular *T. cruzi* amastigotes exposed to AIAs showed kDNA disruption, mitochondrion swelling and other manifestations including loss of subpellicular microtubules and induced an atypical organization of multiple flagella in the trypomastigote stage [28]. AIA mechanistic studies appear to be lacking against *L. sp., T. brucei rhodesience* and *P. falciparum.*

Figure 1 here

We have described the antileishmanial SAR for arylimidamides with multiple variations on the diphenylfuran core of furamidine with the terminal groups held constant with 2-pyridyl groups [14-16]. We have recently shown that relatively low molecular weight AIAs derived from DB766 can retain high activity against *Leishmania* spp., however these smaller compounds are toxic [29]. We have also shown that DB766 analogues that possess unsymmetrical substitutions on the diphenylfuran linker displayed nanomolar *in vitro* potency against intracellular *Leishmania* with selectivity indexes >100 compared to J774 macrophages [16]. However, relatively little variation in the terminal groups have been explored [15]. In order to accomplish our drug development and molecular recognition goals, a variety of analogs of DB766 were prepared and are reported herein, in which the terminal pyridyl groups have been replaced with various heterocyclic rings. The synthetic compounds were evaluated for their activity *in vitro* against *T. cruzi*, *L. amazonensis*, *T. brucei rhodesiense* and *P. falciparum*.

2. Results and discussion

2.1. Chemistry

The syntheses for the target bis-arylimidamides **7a-7o** require the corresponding diamino compound **4** as the key intermediate (Scheme 1). The preparation of the diamino compound **4** was conveniently achieved in two steps starting with a Stille reaction between 1-bromo-2-isopropoxy-4-nitrobenzene (**1**) and 2,5-bis(tri-*n*-butylstannyl)furan (**2**) to form the corresponding 2,5-bis [4-nitro-2-(2-propoxy)phenyl] furan **3** in good yield (70%). Reduction of the 2,5-bis[4-nitro-2-(2-propoxy)phenyl]furan **3** by catalytic hydrogenation produced the desired diamino compound **4** in excellent yield (96%). 1-Bromo-2-isopropoxy-4-nitrobenzene (**1**) was obtained

starting with the bromination of commercially available 2-amino-5-nitrophenol using sulfamic acid and sodium nitrite in aqueous HBr to form 2-bromo-5-nitrophenol in 78% yield [16, 30, 31]. The phenolic group was then alkylated with 2-iodopropane in the presence of potassium *t*-butoxide to give **1** in 68% yield [13, 15]. 2,5-Bis-(tri-*n*-butylstannyl)furan **2** was prepared from furan via lithiation with *sec*-butyllithium and subsequent treatment with tri-*n*-butyltin chloride as previously described [16, 32].

Scheme 1 here

The bis-arylimidamides **7a**-**7o** (30-82% yields) were prepared in a one-step process in which **4** was reacted with two equivalents of the various *S*-(2-naphthylmethyl)-2-thioimidatehydrobromides **6** in ethanol/acetonitrile (Scheme 2) [33]. The *S*-(2-naphthylmethyl)-2-thioimidatehydrobromides **6** were obtained by reacting the heterocyclicthioamides **5** with 2-(bromomethyl)naphthalene in refluxing $CHCl_3$ according to the literature procedure [33].

Scheme 2 here

2.2. Biological Evaluations：In vitro antiparasitic activities

The results for the evaluation of the 15 bis-arylimidamides against *T. cruzi*, *L. amazonensis*, *T. brucei rhodesiense* and *P. falciparum* along with their cytotoxicity to L6 rat myoblast cells are shown in Table 1. For comparison purposes, Table 1 includes data for DB766 and several standard antiparasitic drugs. Four (**7a**, **7c**, **7j**, **7m**) of the 15 new compounds showed anti-*T. cruzi* IC_{50} values between 0.2 and 1.6 μM, which are lower than or comparable to that of benznidazole (IC_{50} = 1.87 μM); however only **7a** gave a reasonable selectivity index (SI = 29). Compound **7a** gave a quite low IC_{50} value of 0.2 μM against *T. cruzi*, which approached that of DB766 and is about 9-fold more potent than that of benznidazole. Eight (**7a**, **7g**, **7i**, **7j**, **7k**, **7l**, **7m**, **7o**) of the 15 compounds gave IC_{50} values of 0.9 μM or less against *L. amazonensis*, comparable to that of pentamidine (IC_{50} = 0.83 μM). The most active compound (which has an acceptable SI value of 27) of this set **7g** (IC_{50} = 0.17 μM) has an IC_{50} value comparable to amphotericin B but is two-fold less active than DB766. Three compounds (**7a**, **7j**, **7m**) gave potent activity against *T. brucei rhodesiense* with IC_{50} values between 12 and 60 nM and acceptable selectivity indices (26-181) however; they were not as active as pentamidine (IC_{50} = 2.3 nM). Ten (**7a**, **7g**-**7o**) of the 15 compounds showed good activity against *P. falciparum* with IC_{50} values ranging from 9 to 87 nM all of which are more active than chloroquine (IC_{50} = 125 nM); all ten compounds have acceptable selectivity indices (from 20 to 682). Compounds **7a** and **7j** are the most potent ones against *P. falciparum* with IC_{50} values of 9 nM, 5-fold more potent than pentamidine.

Three new compounds (**7a-7c**), with nitrogen containing 6-membered rings similar to the lead compound DB766, were prepared. When the pyridine rings of DB766 are replaced by pyrimidine rings (**7a**), with both nitrogen atoms *ortho* to the amidine, a 2-fold loss of activity versus *T. cruzi* and an 8-fold decrease against *L. amazonensis* is observed. The selectivity index for the *T. cruzi* activity is acceptable however that is not the case for *L. amazonensis*. Interestingly, the activity of **7a** against *T. brucei rhodesiense* and *P. falciparum* shows an approximately 4-fold and 18-fold increase, respectively, compared to that of DB766. Both compounds **7b** (terminal pyrazines) and **7c** (terminal pyridizines), which are isomeric to **7a**, exhibited a significant loss in potency against all four parasites tested compared to that of compound **7a**.

Twelve analogues of DB766 were prepared in which the terminal pyridine rings were replaced with various 5-membered ring heterocycles. When the pyridine rings were replaced with bromothiophenes (**7d** and **7e**), essentially no antiparasitic activity was retained. On replacement of the pyridine rings with thiazoles (**7f**-**7k**) significant variation in activity with structure and parasite was observed. None of the six thiazoles gave anti-*T. cruzi* activity below 1 μM and their selectivity indices were generally one or less. Three of the thiazoles (**7g, 7i, 7j**) gave low micromolar (0.17- 0.3 μM) IC_{50} values versus *L. amazonensis*, however only **7g** exhibited an acceptable selectivity index (SI = 27). Against *T. brucei rhodesiense* , **7j** was the only thiazole to show promising activity (IC_{50} = 12 nM; SI = 50). In contrast to the previous results, five of six thiazoles (**7g**-**7k**) show promising activity (IC_{50} values between 9 and 86 nM) against *P. falciparum* and reasonable selectivity

(SI = 22-94). The results for the thiazoles against *P. falciparum* is an interesting finding and merits follow-up. The antiparasitic results for the pyrrole analogue **7l** are poor, similar to the thiophenes (**7d**-**e**), except against *P. falciparum*, which yielded an IC_{50} value of 25 nM and acceptable selectivity (SI = 40). The two imidazole analogues studied (**7m** and **7n**) both show only modest activity against *T. cruzi* and *L. amazonensis* with poor selectivity. The imidazole **7m** shows reasonable activity and selectivity versus *T .brucei rhodesience* (IC_{50} = 60 nM; SI = 26) and both **7m** (IC_{50} = 15 nM; SI = 106) and **7n** (IC_{50} = 54 nM; SI = 148) provide good activity and selectivity versus *P. falciparum*. The pyrazole **7o** shows only modest activity against *T. cruzi, L. amazonensis* and *T. brucei rhodesiense*, however it is reasonably active and selective for *P. falciparum* (IC_{50} = 70 nM; SI = 20).

Table 1 here

3. Conclusion

After evaluation of 15 new compounds in which the terminal pyridine groups of DB766 have been replaced with various 5- and 6-membered heterocyclic rings, the pyridine terminal groups remain the best choice for activity and selectivity against *T. cruzi* and *L. amazonensis.* Terminal pyrimidine units (**7a**) are tolerated for anti-*T. cruzi* activity, albeit not as effect as pyridine, and the 2-methylthiazole system (**7g**) performs similarly against *L. amazonensis.* Against *T. brucei rhodesiense* the pyridine unit (**7a**), the imidazole system (**7m**) and the thiazole analogue (**7j**) show encouraging activity and selectivity. The major new finding in this report is that this class of compounds seems generally active and selective against malaria with 10 of the 15 compounds giving IC_{50} values of 87 nM or less. These results suggest that lead optimization of this series against malaria would likely be a fruitful endeavor. Our previous studies have shown that the number and location of nitrogen atoms have a significant impact on the antitrypanosomal and antileshmanial activity of related diamidines [37, 38]. The results from the current study of these bis-arylimidamides against *T. cruzi*, *L. amazonensis*, *T. brucei rhodesiense* and *P. falciparum* are generally consistent with previous observations. For compounds **DB766, 7a, 7g, 7m, 7j,** which show potent antiparasitic activity, one or two nitrogen atoms are *ortho* to the amidine groups in the terminal rings, which suggests that nitrogen atoms *ortho* to the amidine group is important for the antiparasitic activity of bis-arylimidamides.

4. Experimental section

4.1. Chemistry

All commercial solvents and reagents were used without purification. Melting points were recorded using a Mel-Temp 3.0 capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded employing a Bruker 400 Ultrashield ^{TM} instrument, chemical shifts (δ) are in ppm relative to internal TMS. Mass spectra were recorded on a VG analytical 70-SE spectrometer. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or other solvents; in each case ^{1}H NMR spectra were consistent with the presence of water or organic solvent(s). Elemental analyses were obtained from Atlantic Microlab, Inc. (Norcross, GA) and are within ± 0.4 of the theoretical values.

*4.1.1 2,5-Bis[4-nitro-2-(2-propoxy)phenyl]furan (**3**)*

A mixture of 1-bromo-2-isopropoxy-4-nitrobenzene [16] (4.16 g, 16 mmol), 2,5-bis(tri- methylstannyl)furan [30, 31] (3.16 g, 8 mmol) and tetrakis(triphenyl-phosphine)palladium(0) (0.37 g) in dry 1,4-dioxane (50 ml)

was heated under nitrogen at 80–90 □ for overnight, cooled to room temperature and filtered to give, after rinsing with hexanes, an orange solid **3** (2.4 g, 70%). Mp 253-254 □; ^{1}H NMR ($CDCl_3$): δ 1.55 (d, *J* = 6.0 Hz, 12H), 4.89 (m, 2H), 7.39 (s, 2H), 7.85 (d, *J* = 2.0 Hz, 2H), 7.95 (dd, *J* = 2.0 Hz, 8.8 Hz, 2H), 8.13 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 21.9, 72.1, 108.5, 115.9, 126.0, 126.1, 147.6, 149.2, 154.1; HRMS (ESI) calcd. for $C_{22}H_{23}N_2O_7$ (M^++H): 427.1505, found: 427.1511.

*4.1.2 2,5-Bis[4-amino-2-(2-propoxy)phenyl]furan (**4**)*
To a suspension of **3** (3g, 7.0 mmol) in EtOAc (50 ml) and absolute EtOH (10 ml) was added Pd/C (10%) (0.3 g) and the mixture was hydrogenated on a Parr apparatus at an initial pressure of about 50 psi for 3 h, the resulting solution was filtered over celite and the filtrate was concentrated in vacuo near dryness to give, after dilution with hexanes, the pure diamino as a yellow/tan solid (2.47 g, 96%). Mp 77-79 □; ^{1}H NMR ($CDCl_3$): δ 1.44 (d, *J* = 6.0 Hz, 12H), 3.71 (s, 4H), 4.64 (heptet, *J* = 6.0 Hz, 2H), 6.32 (s, 2H), 6.37 (d, *J* = 8.2 Hz, 2H), 6.86 (s, 2H), 7.77 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 22.3, 70.2, 100.6, 107.5, 109.5, 112.7, 127.0, 146.2, 148.5, 154.8; Anal. Calcd. for $C_{22}H_{26}N_2O_3$. 0.25 H_2O: C, 71.23; H, 7.20; N, 7.55. Found: C, 71.31; H, 7.13; N, 7.46.

*4.1.3 General procedure for synthesis of S-2-naphthylmethyl thioimidatehydrobromides **6***
To a stirred solution of thioacetamide (5 mmol) in $CHCl_3$ (10 ml) was added 2-(bromomethyl)naphthalene (6 mmol). The mixture was heated to reflux for 1.5~10 h, cooled to room temperature and placed in an ice bath. The resulting solid was collected and dried in vacuo to afford title compounds (68 ~ 96% in yield) as a solid and used directly in the next step.

4.1.4 General procedure for compounds **7a**-**7o**
To a solution of 2,5-bis(4-amino-2-(2-propoxy**)**phenyl)furan (1.5 mmol) in dry MeCN (8 mL) was added dry EtOH (24 mL), and the solution was chilled briefly in an icewater bath. The appropriate *S*-(2-naphthylmethyl) thioacetimidates (3.2 mmol) was then added, and the mixture was stirred overnight at room temperature. The resulting solution was concentrated to near dryness, which was triturated with ether to give a yellow solid. The solid was collected, dissolved in EtOH, and basified with NaOH (1 N), and the free base was extracted into $CHCl_2$. After drying (K_2CO_3) and partially concentrating, the resulting suspension was diluted with hexane to give a fluffy yellow solid that was collected by filtration. The free base (1 mmol) was used directly by suspending in EtOH (3 ml) and treated with HCl saturated ethanol (5 ml) for 1 h at room temperature. The resulting solution was then concentrated under reduced pressure to near dryness to give a suspension that was diluted with ether and filtered to yield an orange/ red powder.

*4.1.4.1. 2,5-Bis[2-(2-propoxy)-4-(2-pyrimidylimino)aminophenyl]furan hydrochloride salt (**7a**)*
Yield: 70%; mp 162-194 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.44 (d, *J* = 6.0 Hz, 12H), 4.83 (m, 2H), 7.16 (dd, *J* = 1.6 Hz, 8.4 Hz, 2H), 7.20 (s, 2H), 7.32 (d, *J* = 1.6 Hz, 2H), 8.00 (t, *J* = 5.0 Hz, 2H), 8.14 (d, *J* = 8.4 Hz, 2H), 9.23 (d, *J* = 5.0 Hz, 4H), 9.70 (s, 2H), 10.18 (s, 2H), 11.99 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.1, 111.7, 113.6, 118.2, 119.7, 125.5, 126.9, 134.6, 148.6, 153.6, 154.4, 157.3, 158.9; Anal. Calcd. for $C_{32}H_{32}N_8O_3$. 2HCl. 2.7H_2O: C, 55.05; H, 5.69; N, 16.05. Found: C, 55.37; H, 5.54; N, 15.68.

*4.1.4.2 2,5-Bis[2-(2-propoxy)-4-(2-pyrazinylimino)aminophenyl]furan hydrochloride salt (**7b**)*
Yield: 30%; mp 191-193 □ (dec.); ^{1}H NMR (DMSO-*d*6):δ 1.45 (d, *J* = 6.0 Hz, 12H), 4.84 (m, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.21 (s, 2H), 7.33 (s, 2H), 8.17 (d, *J* = 8.4 Hz, 2H), 9.01 (t, *J* = 2.2 Hz, 2H), 9.11 (d, *J* = 2.2 Hz, 2H), 9.52 (s, 2H), 9.59 (s, 2H), 10.28 (s, 2H), 12.05 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.1, 111.4, 113.6, 118.0, 119.7, 127.0, 134.7, 141.5, 144.9, 145.7, 148.6, 149.6, 154.5, 158.8; Anal. Calcd. for $C_{32}H_{32}N_8O_3$. 2HCl. 2.65H_2O: C, 55.17; H, 5.69; N, 16.09. Found: C, 55.57; H, 5.63; N, 15.49.

*4.1.4.3. 2,5-Bis[2-(2-propoxy)-4-(2-pyridazinylimino)aminophenyl]furan hydrochloride salt (**7c**)*
Yield: 32%; mp 213-215 ^{o}C (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.45 (d, *J* = 5.8 Hz, 12H), 4.87 (m, 2H), 7.19 (s, 2H), 7.21 (s, 2H), 7.36 (s, 2H), 8.15 (s, 2H), 8.17 (d, *J* = 8.4 Hz, 2H), 8.70 (d, *J* = 8.4 Hz, 2H), 9.63 (s, 2H), 9.64 (s, 2H), 10.48 (s, 2H), 12.32 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.0, 70.7, 111.0, 113.2, 117.6, 119.3, 126.6, 128.1, 134.2, 148.2, 149.9, 154.1, 154.5, 158.7; Anal. Calcd. for $C_{32}H_{32}N_8O_3$. 2HCl. 4.05H_2O: C, 53.19;

H, 5.87; N, 15.50. Found: C, 53.58; H, 5.49; N, 14.96.

*4.1.4.4. 2,5-Bis[2-(2-propoxy)-4-(5-bromo-2-thiophenylimino)aminophenyl]furan hydrochloride salt (**7d**)*
Yield: 82%; mp 203-205 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.42 (d, *J* = 6.0 Hz, 12H), 4.81 (m, 2H), 7.09 (d, *J* = 8.6 Hz, 2H), 7.17 (s, 2H), 7.27 (s, 2H), 7.57 (d, *J* = 4.0 Hz, 2H), 7.98 (d, *J* = 4.0 Hz, 2H), 8.10 (d, *J* = 8.6 Hz, 2H), 9.06 (s, 2H), 9.87 (s, 2H), 11.7 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.1, 111.3, 113.5, 117.9, 119.4, 121.2, 127.0, 131.1, 132.4, 135.1, 136.0, 148.6, 154.5, 155.8; Anal. Calcd. for $C_{32}H_{30}Br_2N_4O_3S_2$. 2HCl. $2H_2O$: C, 45.14; H, 4.26; N, 6.58. Found: C, 45.00; H, 4.06; N, 6.44.

*4.1.4.5. 2,5-Bis[2-(2-propoxy)-4-(4-bromo-2-thiophenylimino)aminophenyl]furan hydrochloride salt (**7e**)*
Yield: 80%; mp 187-189 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 6.0 Hz, 12H), 4.82 (m, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.18 (s, 2H), 7.31 (s, 2H), 8.12 (d, *J* = 8.4 Hz, 2H), 8.24 (s, 2H), 8.30 (s, 2H), 9.18 (s, 2H), 10.05 (s, 2H), 11.90 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.0, 110.3, 111.3, 113.5, 117.8, 119.4, 126.9, 131.3, 132.3, 135.2, 136.7, 148.6, 154.4, 155.8; Anal. Calcd. for $C_{32}H_{30}Br_2N_4O_3S_2$. 2HCl. $1H_2O$: C, 46.11; H, 4.11; N, 6.72. Found: C, 46.20; H, 3.91; N, 6.65.

*4.1.4.6. 2,5-Bis[2-(2-propoxy)-4-(4-methyl-2-thiazolylimino)aminophenyl]furan hydrochloride salt (**7f**)*
Yield: 67%; mp 192-194 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 6.0 Hz, 12H), 2.56 (s, 6H), 4.83 (m, 2H), 7.12 (d, *J* = 8.2 Hz, 2H), 7.18 (s, 2H), 7.29 (s, 2H), 7.99 (s, 2H), 8.11 (d, *J* = 8.2 Hz, 2H), 9.33 (s, 2H), 10.08 (s, 2H), 11.97 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 17.0, 22.4, 71.0, 111.4, 113.5, 117.9, 119.5, 123.8, 126.9, 135.1, 148.6, 154.1, 154.5, 154.8; Anal. Calcd. for $C_{32}H_{34}N_6O_3S_2$. 2HCl. $2.8H_2O$: C, 52.13; H, 5.69; N, 11.41. Found: C, 52.24; H, 5.52; N, 11.36.

*4.1.4.7. 2,5-Bis[2-(2-propoxy)-4-(2-methyl-4-thiazolylimino)aminophenyl]furan hydrochloride salt (**7g**)*
Yield: 72%; mp 231-233 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 5.8 Hz, 12H), 2.82 (s, 6H), 4.84 (m, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 7.19 (s, 2H), 7.28 (s, 2H), 8.12 (d, *J* = 8.6 Hz, 2H), 9.01 (s, 2H), 9.12 (s, 2H), 9.93 (s, 2H), 11.69 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 19.3, 22.4, 71.0, 111.7, 113.5, 118.2, 119.5, 126.9, 129.7, 134.6, 142.2, 148.6, 154.4, 154.9, 168.2; Anal. Calcd. for $C_{32}H_{34}N_6O_3S_2$. 2HCl. 1.3 H_2O: C, 54.12; H, 5.48; N, 11.84. Found: C, 54.34; H, 5.49; N, 11.64.

*4.1.4.8. 2,5-Bis[2-(2-propoxy)-4-(2-amino-4-thiazolylimino)aminophenyl]furan hydrochloride salt(**7h**)*
Yield: 60 %; mp 264-266 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 6.0 Hz, 12H), 4.83 (m, 2H), 7.08 (d, *J* = 8.2 Hz, 2H), 7.17 (s, 2H), 7.24 (s, 2H), 7.48 (s, 4H), 8.09 (d, *J* = 8.2 Hz, 2H), 8.92 (s, 2H), 9.51 (s, 2H), 11.25 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.0, 111.5, 113.4, 117.7, 118.0, 119.4, 126.8, 134.7, 138.6, 148.6, 154.4, 155.2, 169.1; Anal. Calcd. for $C_{30}H_{32}N_8O_5S_2$. 2.6HCl. $2.2H_2O$: C, 47.97; H, 5.23; N, 14.92. Found: C, 47.98; H, 5.10; N, 14.56.

*4.1.4.9. 2,5-Bis[2-(2-propoxy)-4-(2-methanamine-4-thiazolylimino)aminophenyl]furan hydrochloride salt (**7i**)*
Yield: 73%; mp 236-238 □(dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 6.0 Hz, 12H), 2.97 (s, 6H), 4.85 (m, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.18 (s, 2H), 7.27 (s, 2H), 8.12 (d, *J* = 8.4 Hz, 2H), 8.19 (s, 2H), 8.94 (s, 2H), 9.54 (s, 2H), 11.30 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 31.3, 71.0, 111.7, 113.5, 117.1, 118.2, 119.5, 126.9, 134.6, 138.5, 148.6, 154.4, 155.3, 169.4; Anal. Calcd. for $C_{32}H_{36}N_8O_3S_2$. 2.5HCl. $3.6H_2O$: C, 47.99; H, 5.75; N, 13.99. Found: C, 47.99; H, 5.39; N, 13.80.

*4.1.4.10. 2,5-Bis[2-(2-propoxy)-4-(2-acetamide-4-thiazolylimino)aminophenyl]furan hydrochloride salt (**7j**)*
Yield: 71%; mp 271-273 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 5.2 Hz, 12H), 2.26 (s, 6H), 4.84 (m, 2H), 7.13 (d, *J* = 8.2 Hz, 2H), 7.19 (s, 2H), 7.30 (s, 2H), 8.12 (d, *J* = 8.2 Hz, 2H), 8.65 (s, 2H), 9.17 (s, 2H), 9.72 (s, 2H), 11.54 (s, 2H), 12.53 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.0, 22.6, 70.6, 111.1, 113.1, 117.6, 119.1, 122.7, 126.5, 134.2, 137.2, 148.2, 154.1, 155.2, 158.8, 169.8; Anal. Calcd. for $C_{34}H_{36}N_8O_5S_2$. 2HCl. $3.9H_2O$: C, 48.38; H, 5.47; N, 13.28. Found: C, 48.37; H, 5.48; N, 13.05.

*4.1.4.11. 2,5-Bis[2-(2-propoxy)-4-(2-methylacetamide-4-thiazolylimino)aminophenyl]furan hydrochloride salt (**7k**)*

Yield: 68 %; mp >300 □; ^{1}H NMR (DMSO-*d*6): δ 1.44 (d, *J* = 5.4 Hz, 12H), 2.46 (s, 6H), 3.82 (s, 6H), 4.87 (m, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.21 (s, 2H), 7.34 (s, 2H), 8.17 (d, *J* = 8.0 Hz, 2H), 8.57 (s, 2H), 9.05 (s, 2H), 9.77 (s, 2H), 11.37 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 23.3, 35.9, 71.1, 112.1, 113.7, 118.6, 119.8, 124.1, 127.1, 134.3, 136.6, 148.6, 154.6, 155.6, 160.5, 172.2; Anal. Calcd. for $C_{36}H_{40}N_8O_5S_2$. 2HBr: C, 48.54; H, 4.75; N, 12.58. Found: C, 48.63; H, 4.75; N, 12.22.

*4.1.4.12. 2,5-Bis[2-(2-propoxy)-4-(1H-2-pyrrolylimino)aminophenyl]furan hydrochloride salt (**7l**)*
Yield: 76%; mp 231-233 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 6.0 Hz, 12H), 4.84 (m, 2H), 6.41 (m, 2H), 7.09 (dd, *J* = 1.6 Hz, 8.4 Hz, 2H), 7.17(s, 2H), 7.24 (d, *J* = 1.6 Hz, 2H), 7.42 (s, 2H), 7.44 (s, 2H), 8.11 (d, *J* = 8.4 Hz, 2H), 8.63 (s, 2H), 9.45 (s, 2H), 11.25 (s, 2H), 12.65 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.0, 111.4, 113.3, 117.8, 117.9, 119.2, 119.5, 126.9, 127.2, 134.8, 148.6, 153.5, 154.5; Anal. Calcd. for $C_{32}H_{34}N_6O_3$. 2HCl. 1.5H_2O: C, 59.15; H, 6.05; N, 12.94. Found: C, 59.13; H, 5.93; N, 12.81.

*4.1.4.13. 2,5-Bis[2-(2-propoxy)-4-(1H-2-imidazolylimino)aminophenyl]furan hydrochloride salt (**7m**)*
Yield: 69%; mp 254-256 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 3.6 Hz, 12H), 4.84 (m, 2H), 7.12 (d, *J* = 7.6 Hz, 2H), 7.18 (s, 2H), 7.28 (s, 2H), 7.61 (s, 4H), 8.11 (d, *J* = 7.6 Hz, 2H), 9.26 (s, 2H), 9.98 (s, 2H), 11.80 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 71.0, 111.4, 113.5, 118.0, 119.4, 121.9, 126.9, 134.3, 135.3, 148.6, 151.4, 154.4; Anal. Calcd. for $C_{30}H_{32}N_8O_3$. 2HCl. 2.2H_2O: C, 54.17; H, 5.82; N, 16.85. Found: C, 54.20; H, 5.60; N, 16.64.

*4.1.4.14. 2,5-Bis[2-(2-propoxy)-4-(1-methyl-1H-4-imidazylimino)aminophenyl]furan hydrochloride salt (**7n**)*
Yield: 75%; mp 191-193 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 6.0 Hz, 12H), 3.84 (s, 6H), 4.84 (m, 2H), 7.09 (dd, *J* = 1.4 Hz, 8.2 Hz, 2H), 7.17 (s, 2H), 7.22 (d, *J* = 1.4 Hz, 2H), 8.05 (s, 2H), 8.09 (d, *J* = 8.2 Hz, 2H), 8.42 (s, 2H), 8.77 (s, 2H), 9.57 (s, 2H), 11.34 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 34.5, 71.0, 111.5, 113.3, 118.0, 119.2, 126.8, 127.9, 129.4, 134.8, 140.7, 148.6, 154.4, 155.7; Anal. Calcd. for $C_{32}H_{36}N_8O_3$. 2HCl. 2.3H_2O: C, 55.30; H, 6.18; N, 16.12. Found: C, 55.62; H, 6.01; N, 15.77.

*4.1.4.15. 2,5-Bis[2-(2-propoxy)-4-(1-methyl-1H-3-pyrazolylimino)aminophenyl]furan hydrochloride salt (**7o**)*
Yield: 75%; mp 196-198 □ (dec.); ^{1}H NMR (DMSO-*d*6): δ 1.43 (d, *J* = 5.8 Hz, 12H), 4.06 (s, 6H), 4.85 (m, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 7.18 (s, 2H), 7.26 (s, 2H), 7.42 (s, 2H), 8.10 (d, *J* = 8.2 Hz, 2H), 8.11 (s, 2H), 9.00 (s, 2H), 9.83 (s, 2H), 11.63 (s, 2H); ^{13}C NMR (DMSO-*d*6): δ 22.4, 39.6, 71.0, 109.1, 111.7, 113.4, 118.2, 119.4, 126.9, 134.5, 134.6, 139.9, 148.6, 154.4, 155.4; Anal. Calcd. for $C_{32}H_{36}N_8O_3$. 2HCl. 3.6H_2O: C, 53.55; H, 6.35; N, 15.62. Found: C, 53.46; H, 6.13; N, 15.26.

4.2. Biology

4.2.1. In vitro antiparasitic activity:

In vitro antiprotozoal activities against intracellular amastigote *T. cruzi* strain Tulahuen LacZ/C4, the trypomastigote bloodstream form of *T. b. rhodesiense* strain STIB900 and the erythrocytic stages of the chloroquine resistant *P. falciparum* strain K1, were measured following established protocols **[**35]. Serial drug dilutions were tested against the relevant parasite forms and plates incubated at 37°C. The drug exposure time of the intracellular *T. cruzi* assay was 4 days. The endpoint of the reporter gene assay was measured by addition of the substrate (CPRG) for β-galactosidase resulting in a color change. The drug exposure time was 3 days in the axenic *T. b. rhodesiense* Alamar blue viability assay. Viability of *P. falciparum* was assessed after 3 days drug exposure using the tritium labeled *hypoxanthine* incorporation *assay* [35]. The in vitro activities against intracellular *L. amazonensis* were determined following established methodology [36]. The IC_{50} values are the mean of two independent assays. Coefficients of variation were less than 50%.

4.2.2. In vitro cytotoxicity assay:

The *in vitro* cytotoxicity of the compounds was determined in a 3 day drug exposure Alamar blue viability assay as previously described [34]. The IC_{50} values are the mean of two independent assays. Coefficients of variation were less than 50%.

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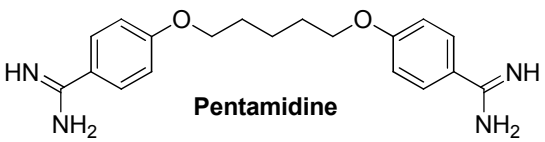
Table 1. Antiparasitic activities and cytotoxicity data for bis-arylimidamides DB766 analogs.

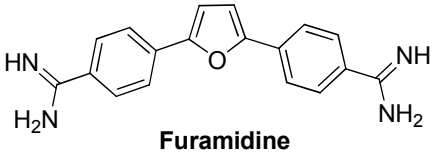
Code	Cyto toxicity	*T. c.*		*L. a.*		*T. b. r*		*P. f.*	
	IC_{50} [a] (μM)	IC_{50} [b] (μM)	SI [d]	IC_{50} [c] (μM)	SI [e]	IC_{50} [b] (μM)	SI [f]	IC_{50} [b] (μM)	SI [g]
DB766	3.0	0.1	30	0.087	34.5	0.12	25	0.149	20.13
7a	5.8	0.2	29	0.7	8.29	0.032	181.3	0.0085	682.3
7b	>129	16	8.1	3.7	34.8	8.077	15.97	0.552	233.7
7c	1.8	1.5	1.2	1.1	1.64	1.70	1.06	0.582	3.09
7d	>110	>110	1	ND [h]	ND	17.0	6.47	0.914	120
7e	>108	>108	1	ND	ND	43.9	2.46	0.992	108.9
7f	>122	>122	1	>10	>12.2	78.6	1.55	0.999	122.1
7g	4.7	2.3	2.04	0.17	27.6	0.74	6.36	0.087	54.0
7h	3.7	5.6	0.66	1.7	2.18	0.357	10.4	0.039	94.9
7i	0.8	2.3	0.35	0.30	2.67	0.092	8.69	0.031	25.8
7j	0.6	1.6	0.38	0.22	2.73	0.012	50	0.009	66.7
7k	0.8	3.1	0.26	0.81	0.99	0.078	10.3	0.035	22.6
7l	1.0	4.1	0.24	0.90	1.11	0.115	8.69	0.025	40
7m	1.6	0.6	2.67	0.37	4.32	0.060	26. 7	0.015	106.6
7n	8.0	18	0.44	5.0	1.6	0.108	74.1	0.054	148.1
7o	1.4	6.1	0.23	0.74	1.89	0.436	3.21	0.070	20
pentamidine	46.6	7.1	6.53	0.83	55.9	0.0023	20173	0.0464	1004
amphotericin B		ND		0.14		ND		ND	
benznidazole	>150	1.87	>80	ND		ND		ND	
chloroquine	76.5	ND		ND		ND		0.125	612
melarsoprol	5.1					0.004	1275		
podophyllotoxin	0.017								

[a] Cytotoxicity was evaluated using cultured L6 rat myoblast cells [34].
[b] The in vitro activities were obtained using intracellular amastigote *T. cruzi* strain Tulahen LacZ/C4, the trypomastigote bloodstream form of *T. brucei rhodesiense* strain STIB900 and the erythrocytic stages of the chloroquine resistant *P. falciparum* strain K1. The IC_{50} values are the mean of two independent assays. Coefficients of variation were less than 50% [35].
[c] Intracellular *L. amazonensis* were used and some values are reported in ref [15]; all were determined as described in ref [36].
[d] Selectivity index for *T. cruzi* expressed as the ratio: IC_{50} (L6)/IC_{50} (*T. cruzi*).
[e] Selectivity index for *L. amazonensis* expressed as the ratio: IC_{50} (L6)/IC_{50} (*L. . amazonensis*).
[f] Selectivity index for *T. brucei rhodesiense* expressed as the ratio: IC_{50} (L6)/IC_{50} (*T. brucei rhodesiense*.).
[g] Selectivity index for *P. falciparum* expressed as the ratio: IC_{50} (L6)/IC50 (*P. falciparum*).
[h] ND, not determined.

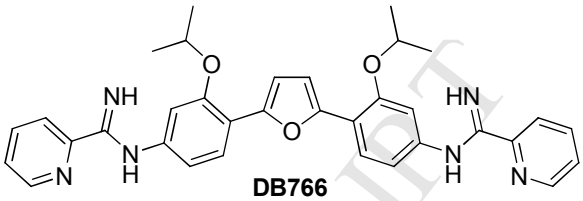
Figure 1. Antiparasitic Leads



Pentamidine



Furamidine



DB766

Highlights

. 15 new DB766 analogues with 5- or 6-heterocyclic ring terminal groups were made

. compounds evaluated vs. *T. cruzi*, *T. b. rhodesiense*, *P. falciparum* and *L. amazonensis*

. generally the pyridine terminal unit remains the most active

. major finding: 10 of 15 compounds are very active vs. *P. falciparum* (IC_{50} = 9-87 nM)

Synthesis and antiparasitic activity of new bis-arylimidamides: DB766 analogs modified in the terminal groups

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[b]*Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, People's Republic of China,*[c]*Swiss Tropical and Public Health Institute, Department of Medical Parasitology and Infection Biology 4002 Basel, Switzerland,* [d]*University of Basel, 4003 Basel, Switzerland,*[e]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA.*

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^{1}H NMR and 13C NMR scans for 7a-7o follow.

Figure 1: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7a.

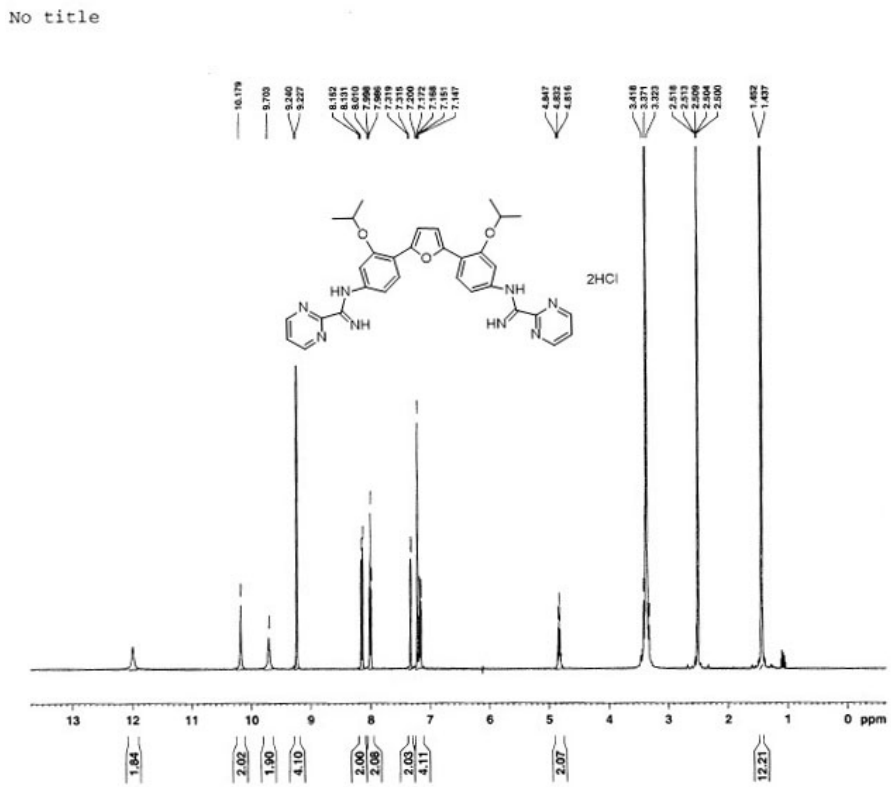


Figure 2: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7a.

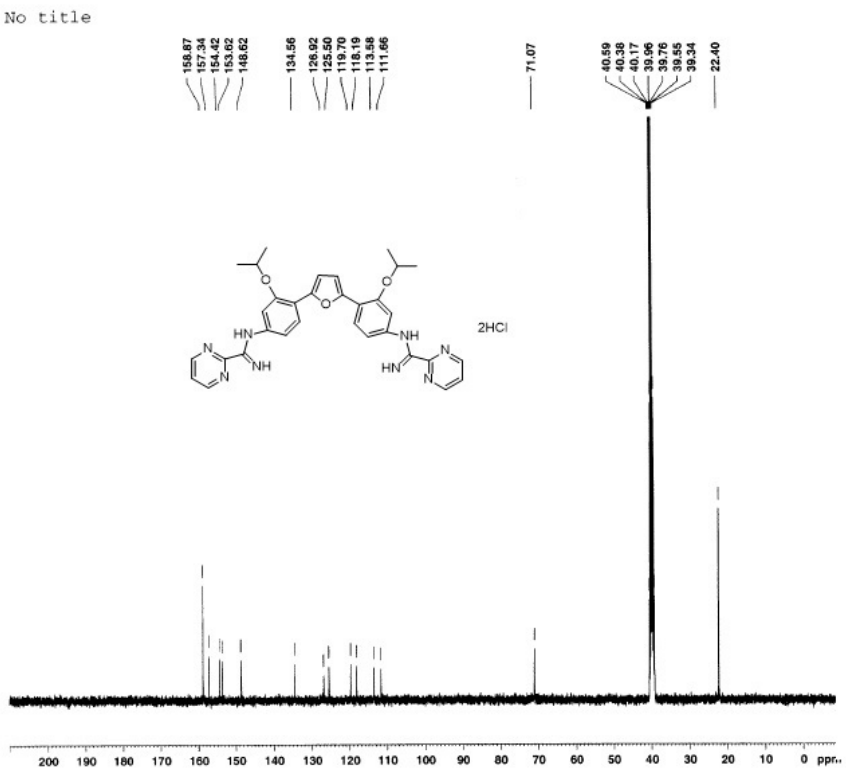


Figure 3: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7b.

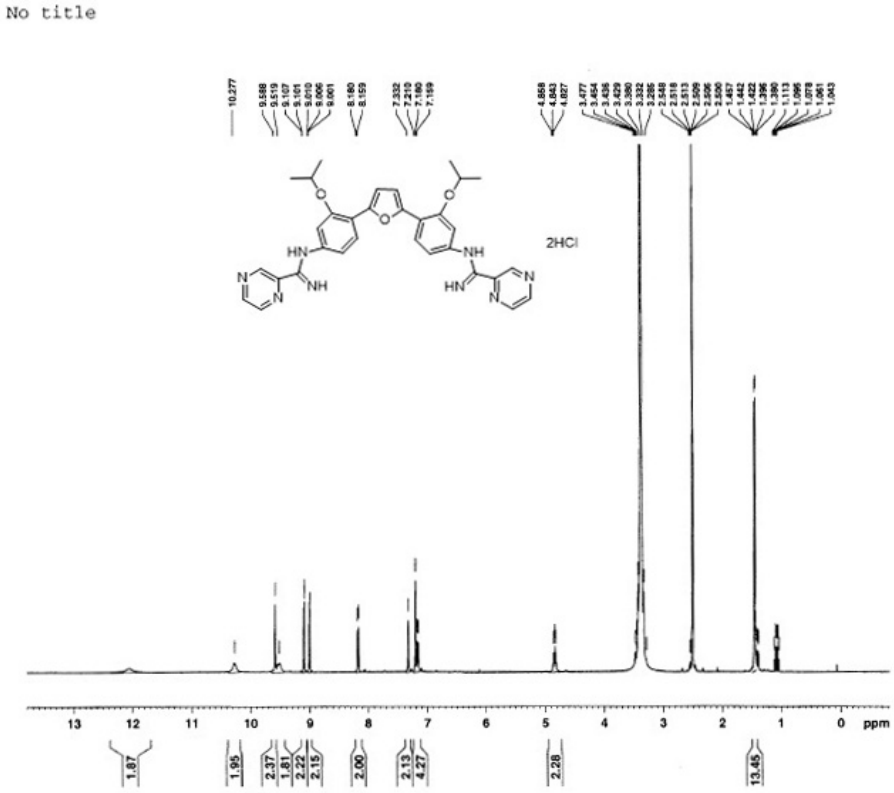


Figure 4: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7b.

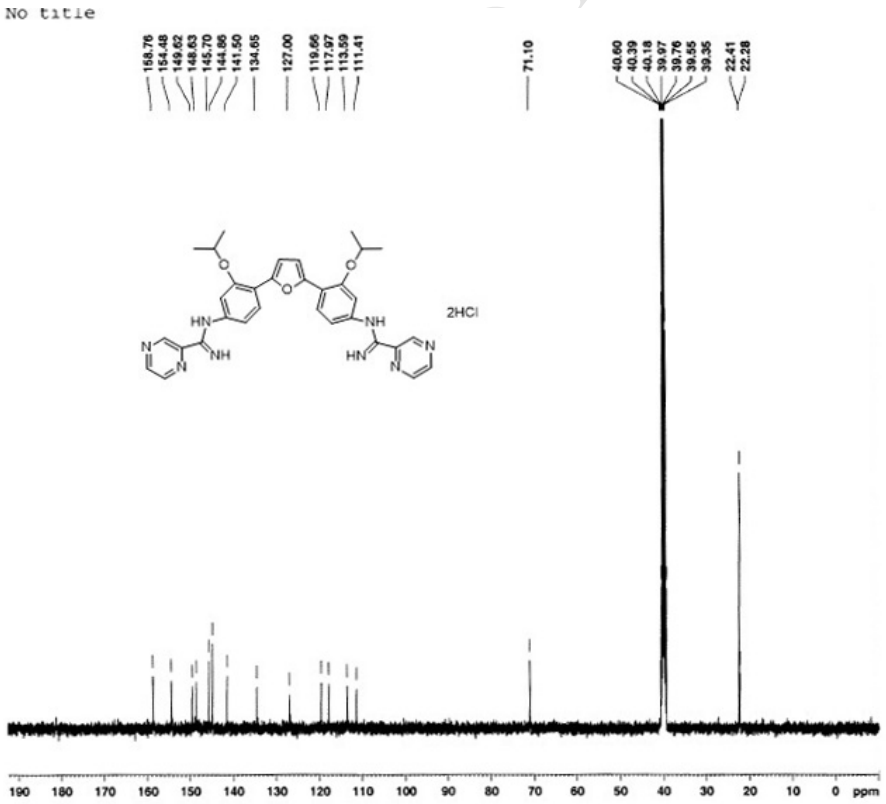


Figure 5: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7c.

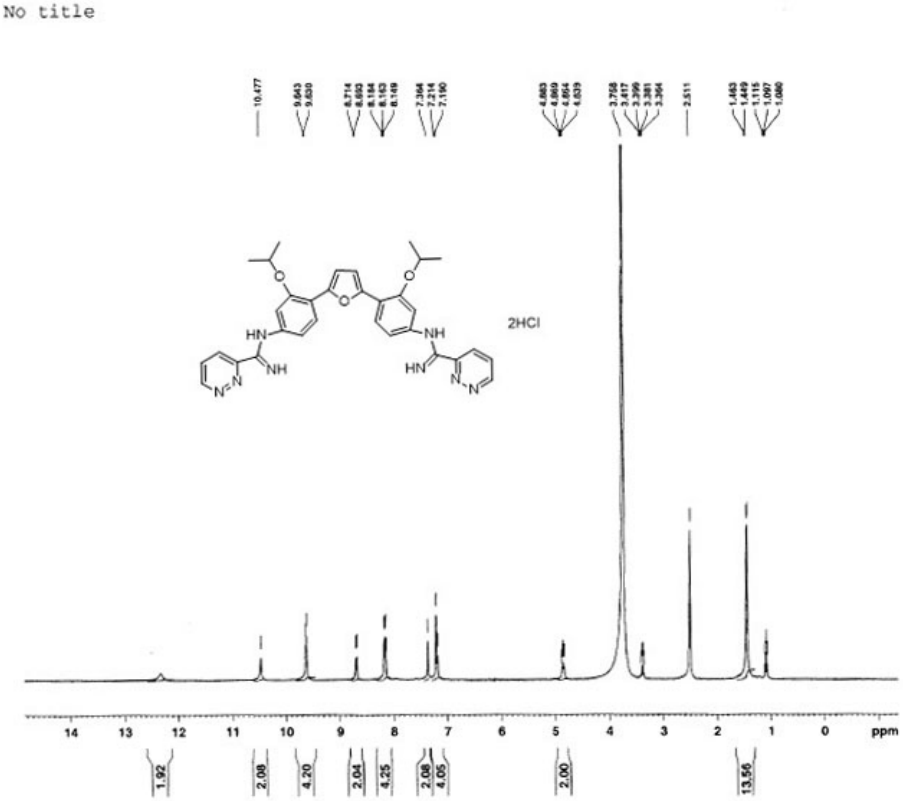


Figure 6: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7c.

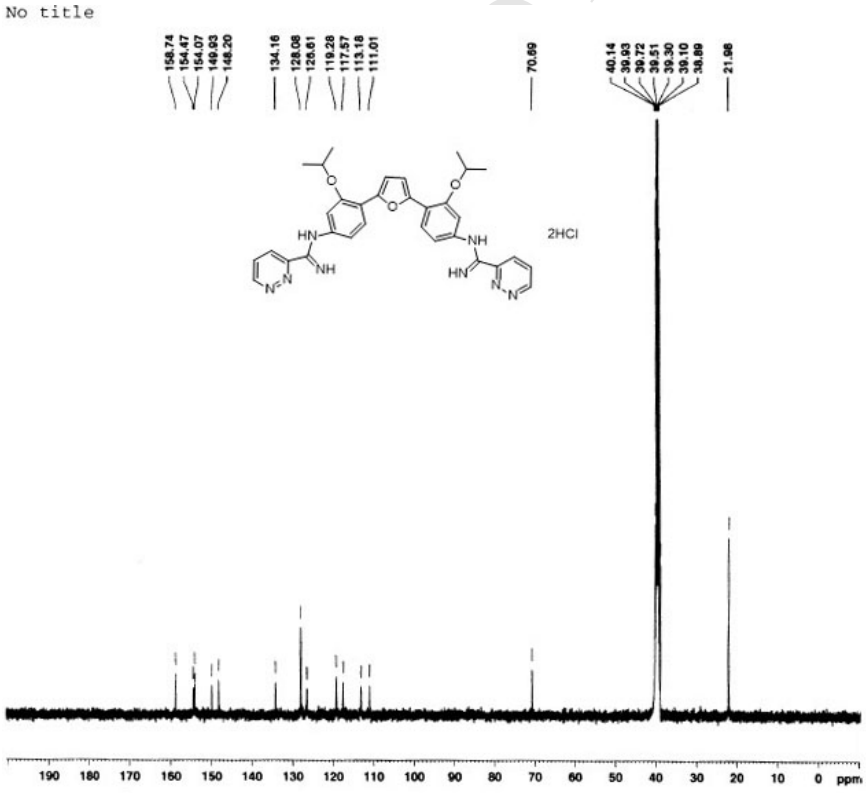


Figure 7: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7d.

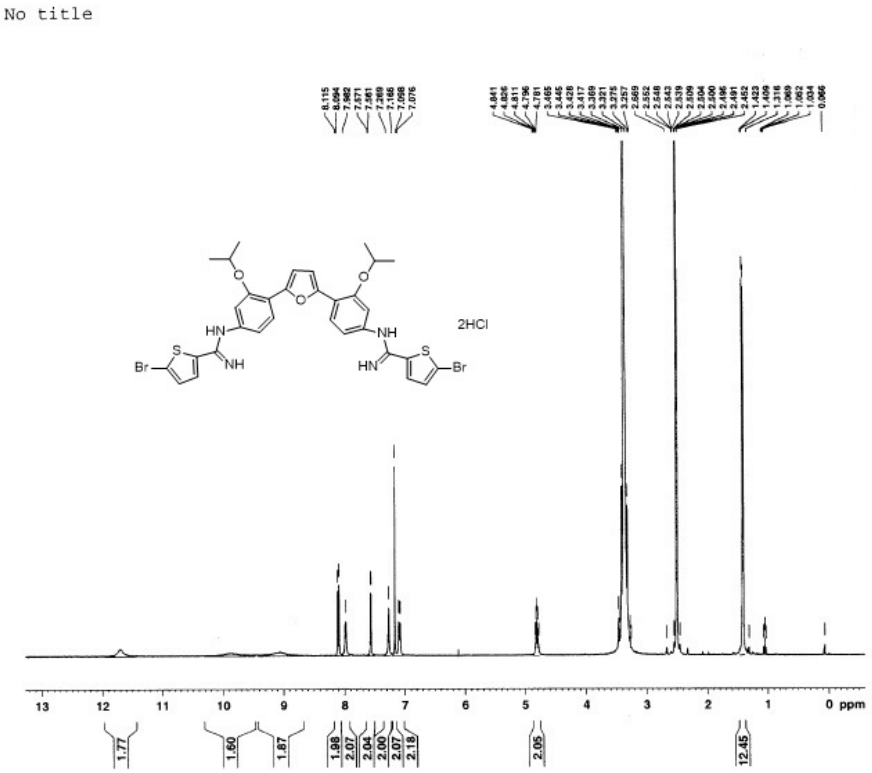


Figure 8: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7d.

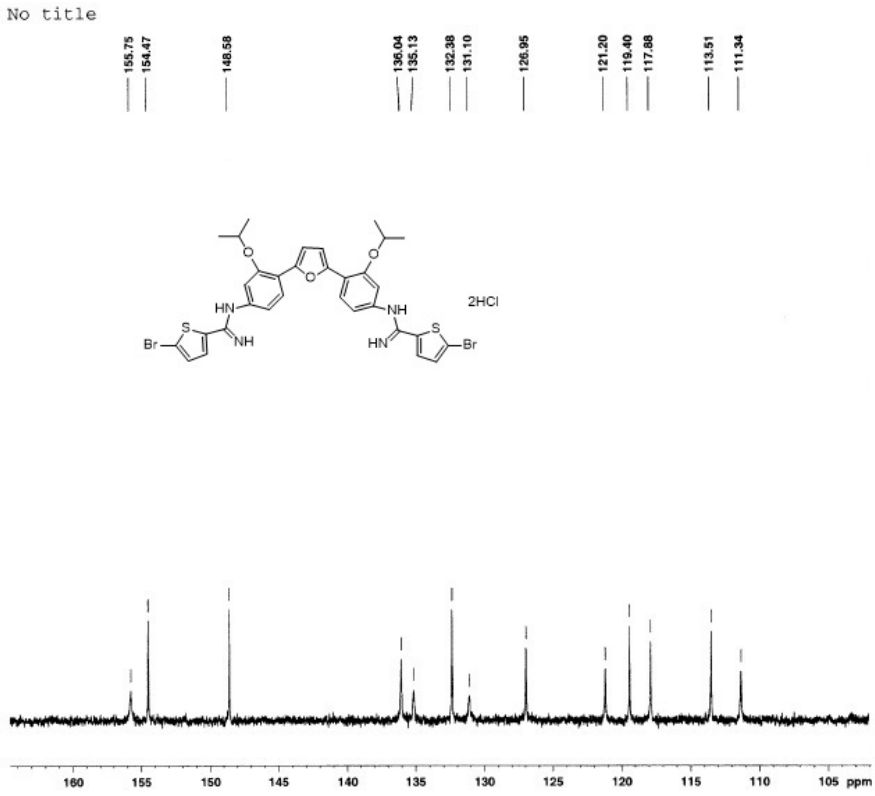


Figure 9: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7e.

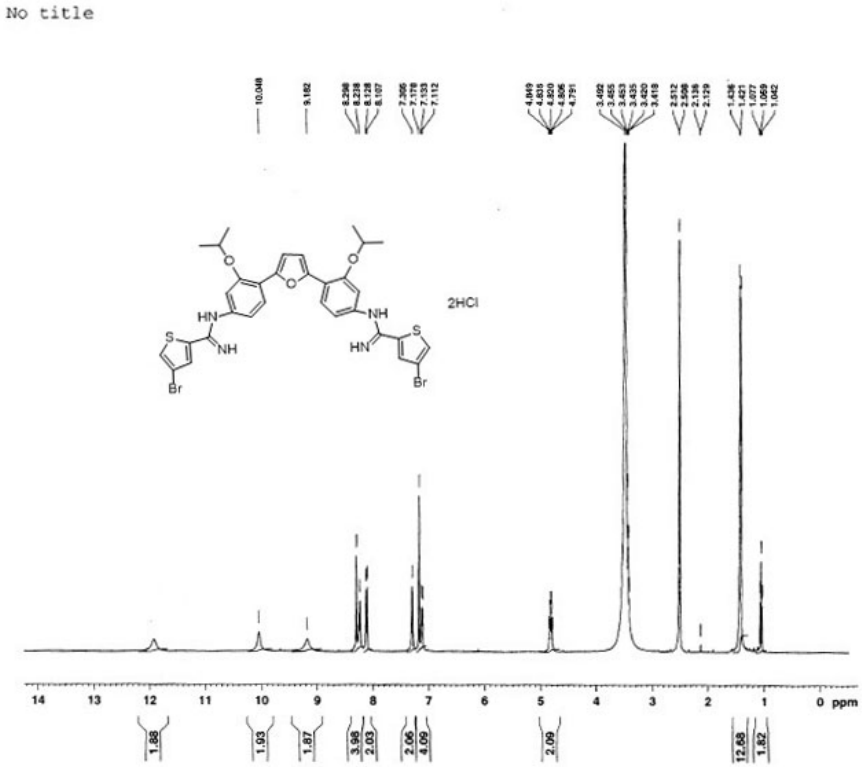


Figure 10: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7e.

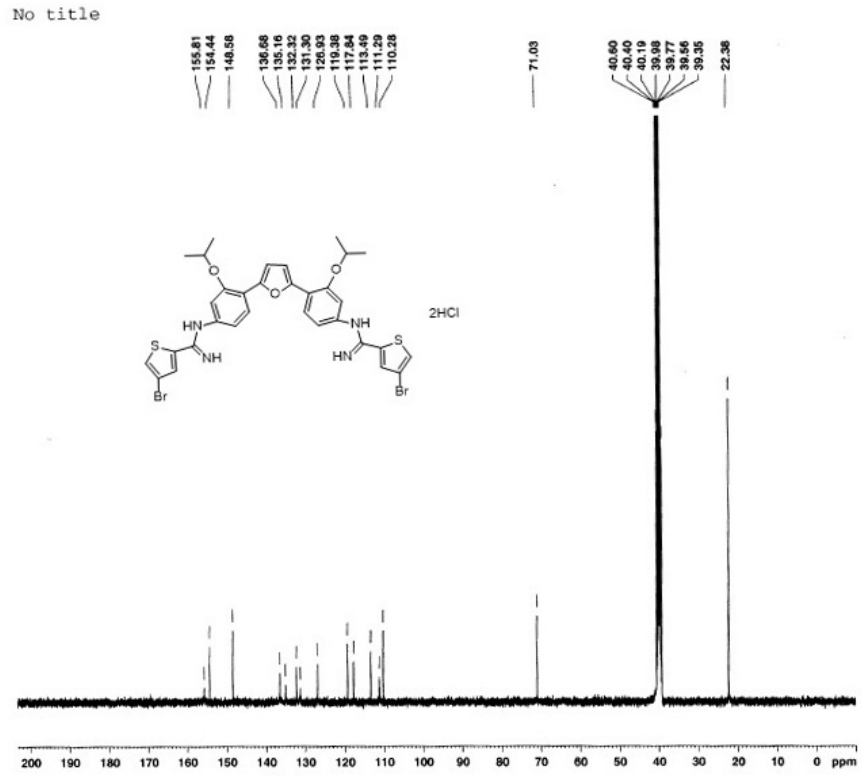


Figure 11: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7f.

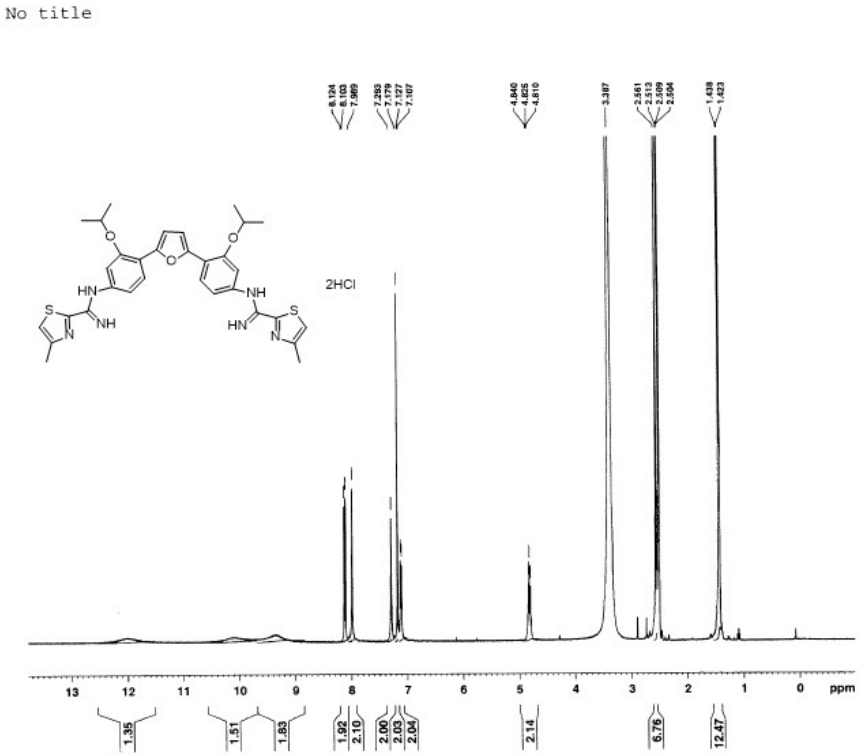


Figure 12: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7f.

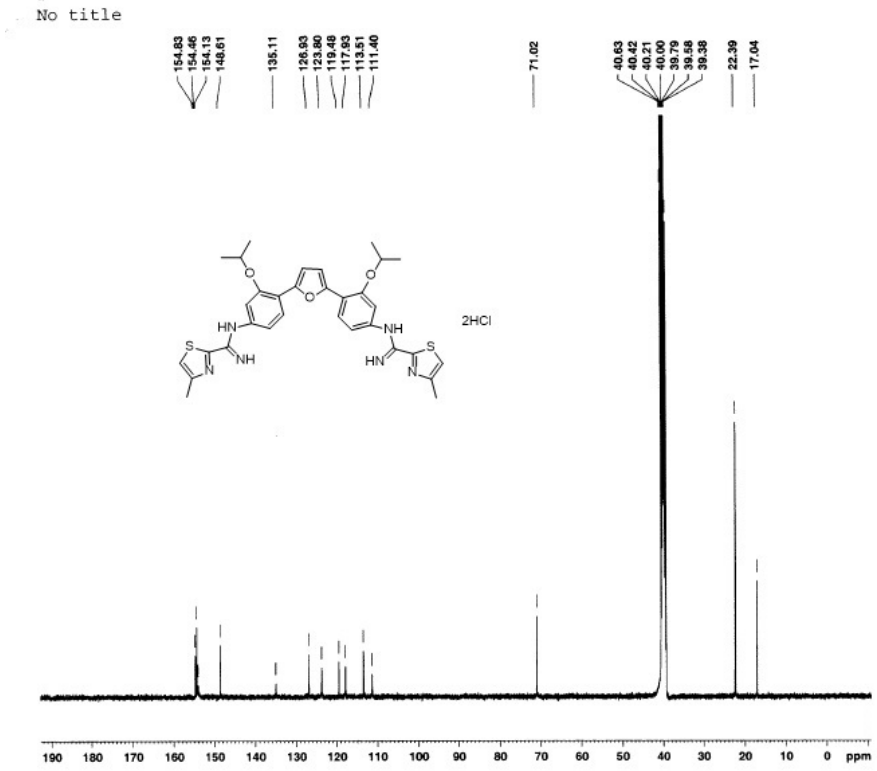


Figure 13: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7g.

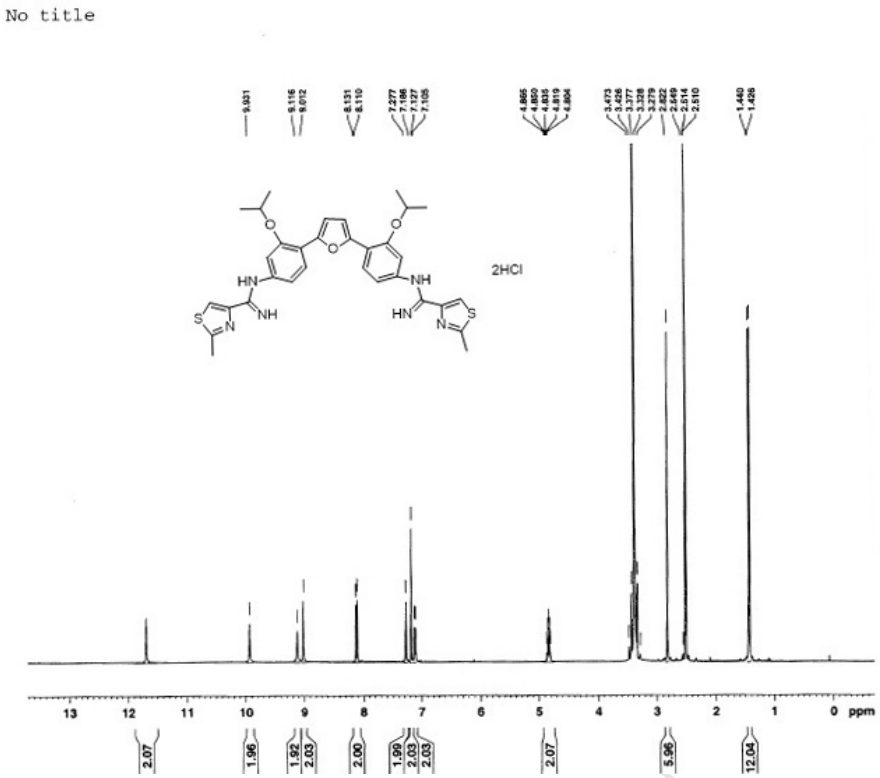


Figure 14: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7g.

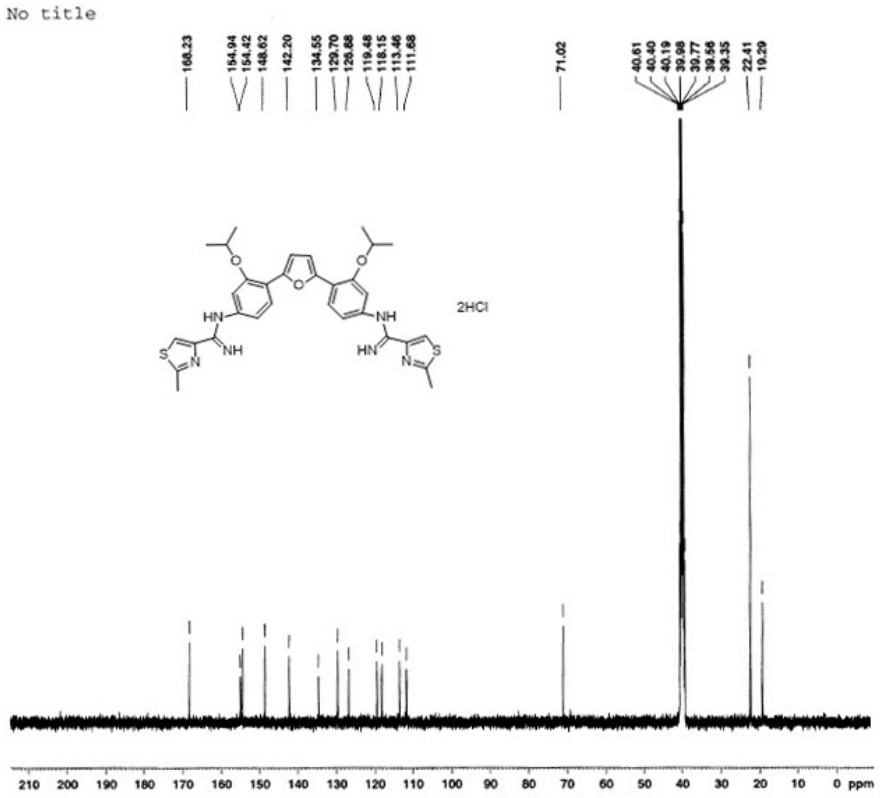


Figure 15: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7h.

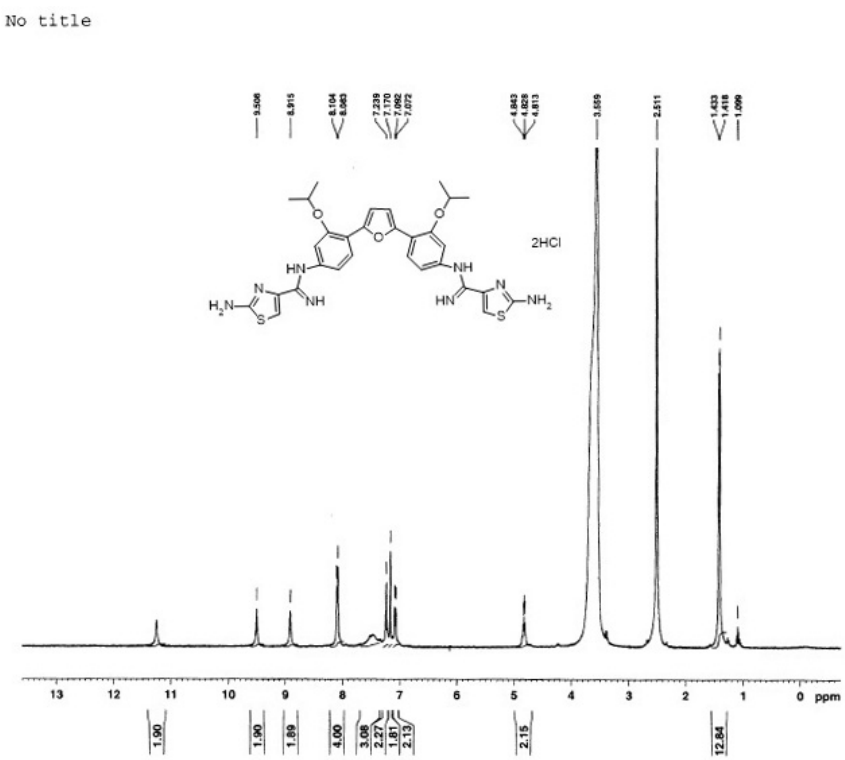


Figure 16: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7h.

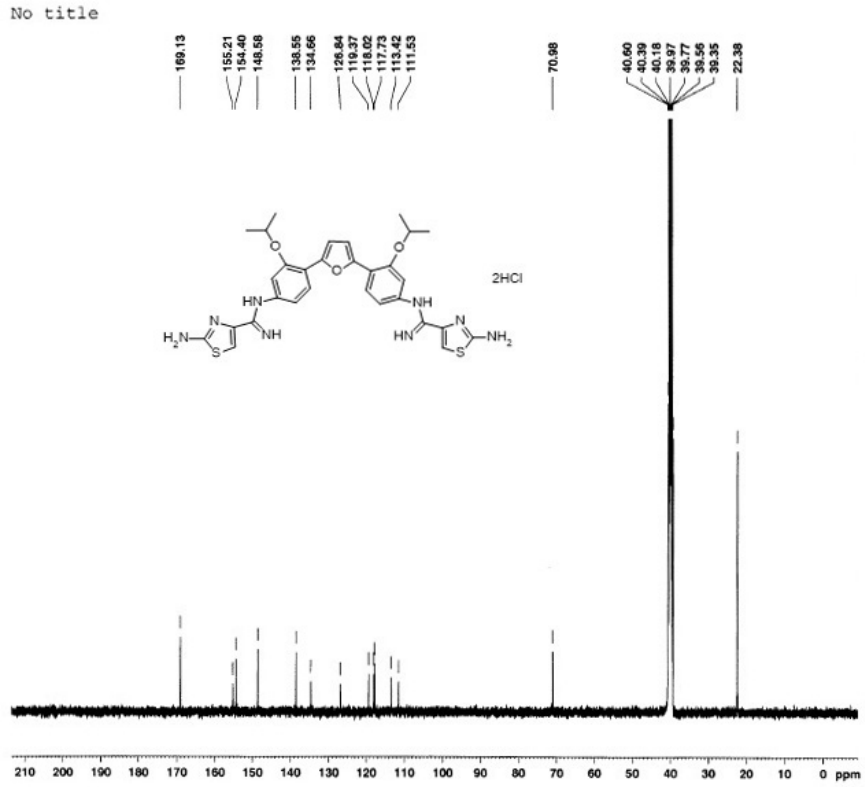


Figure 17: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7i.

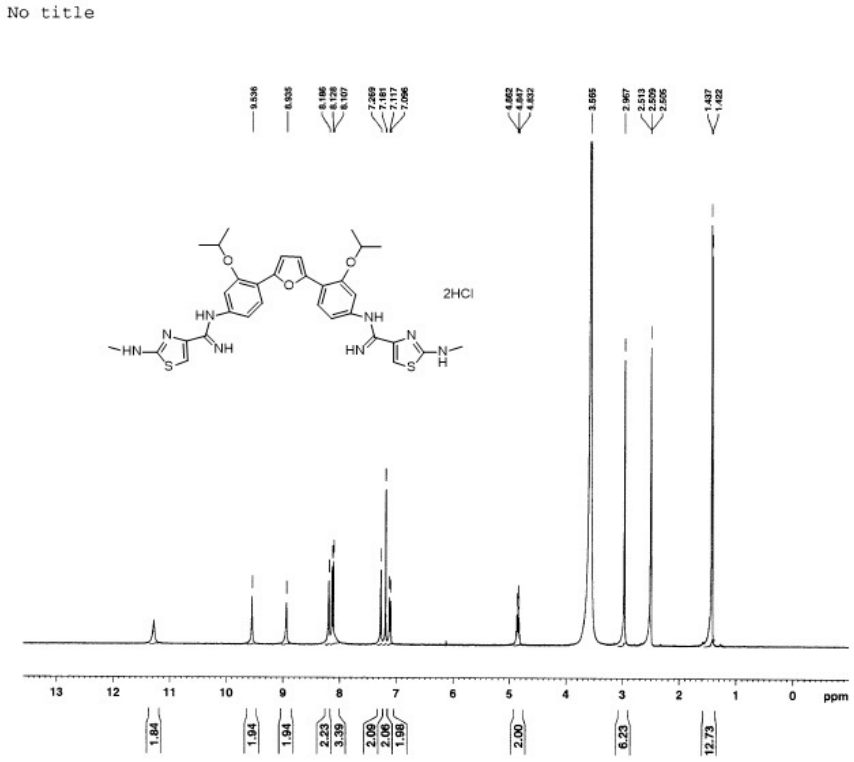


Figure 18: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7i.

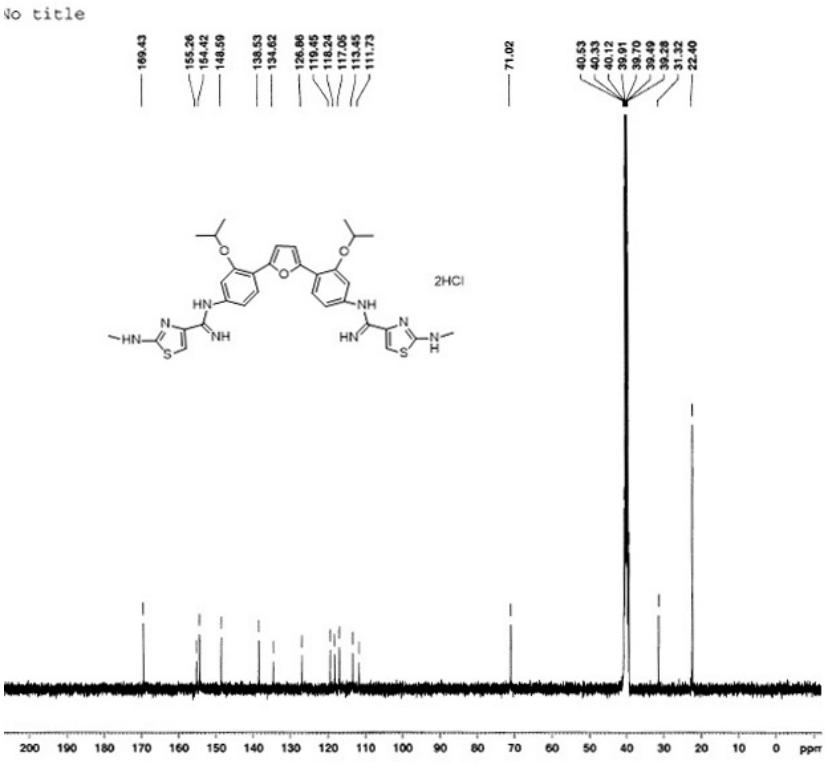


Figure 19: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7j.

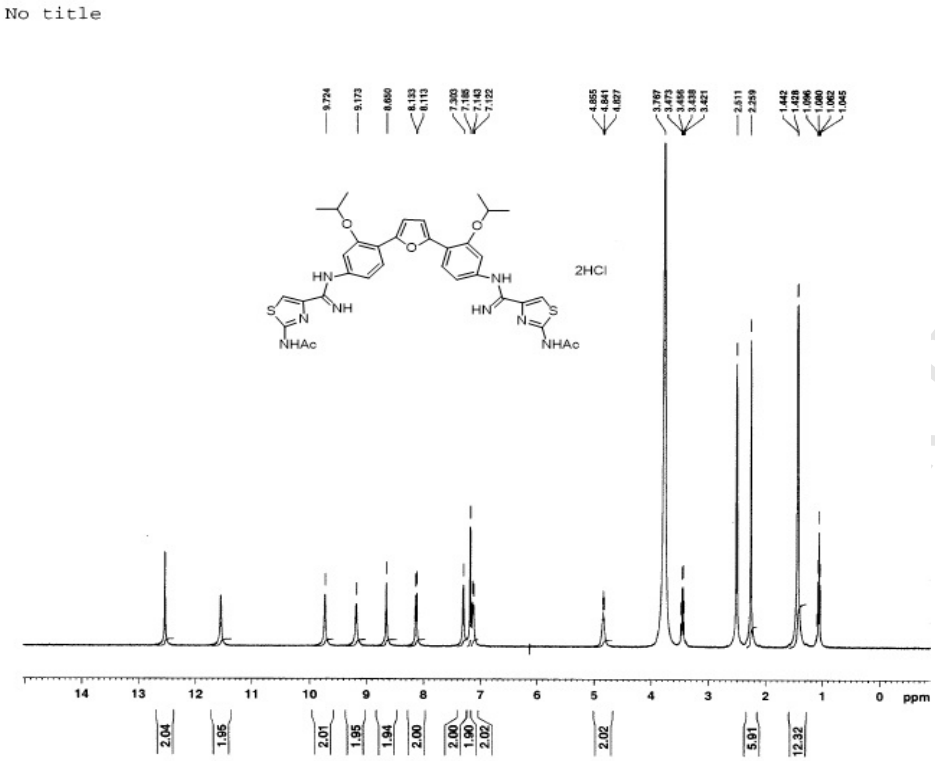


Figure 20: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7j.

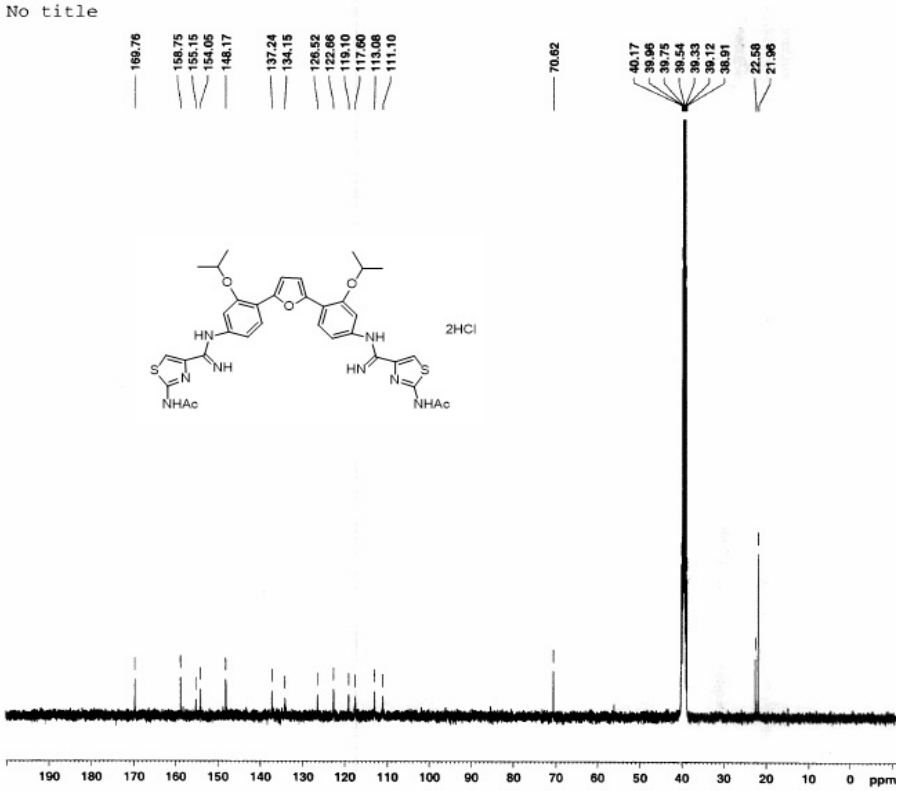


Figure 21: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7k.

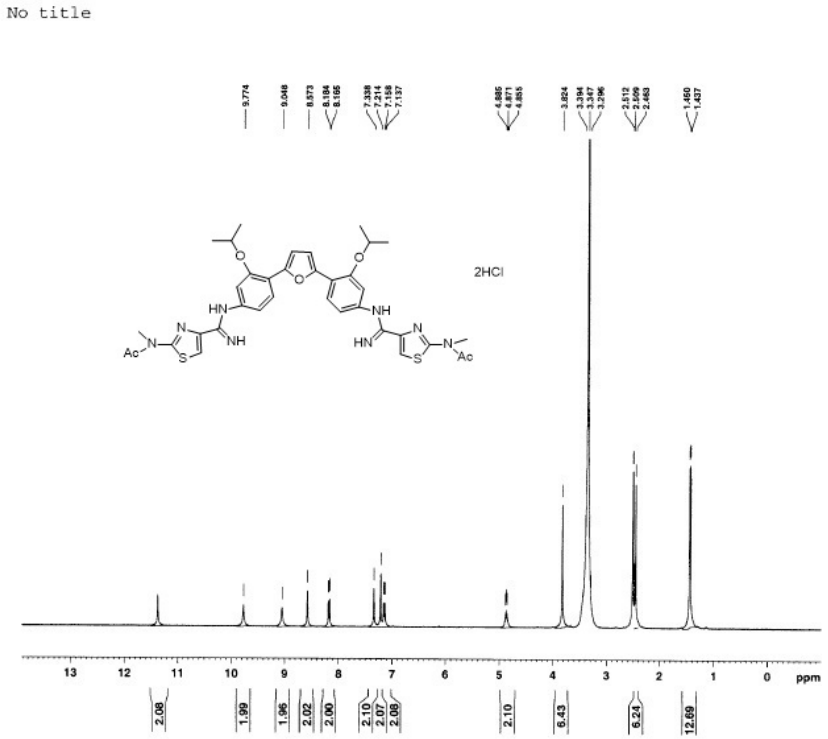


Figure 22: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7k.

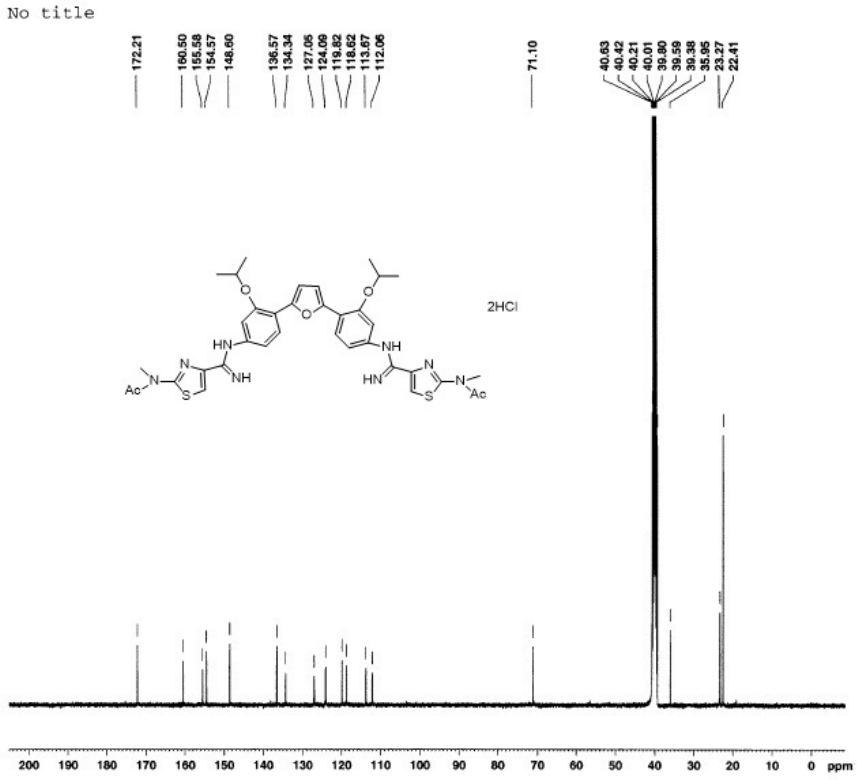


Figure 23: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7l.

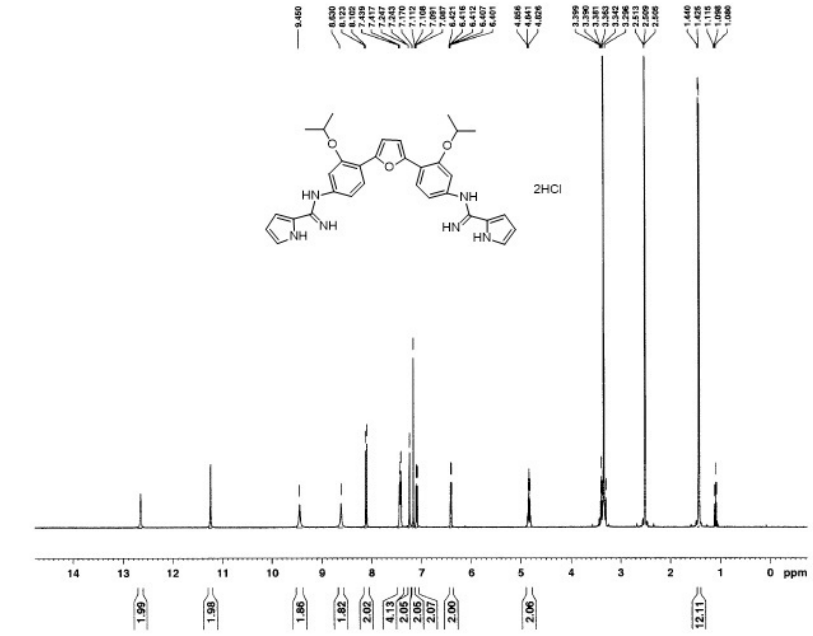


Figure 24: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7l.

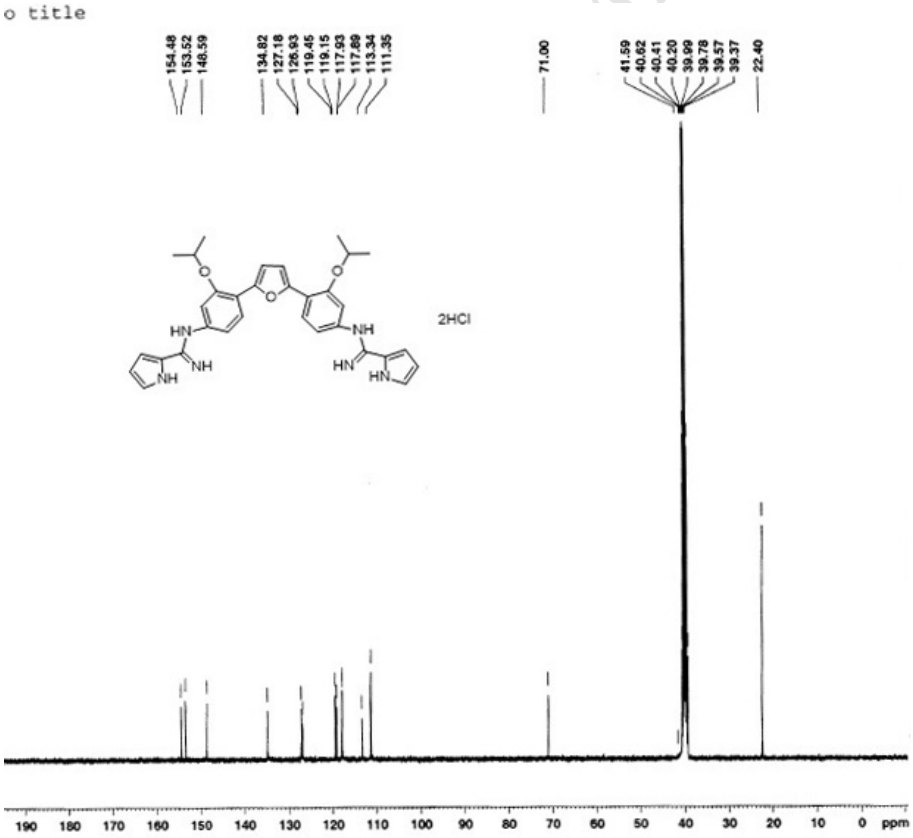


Figure 25: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7m.

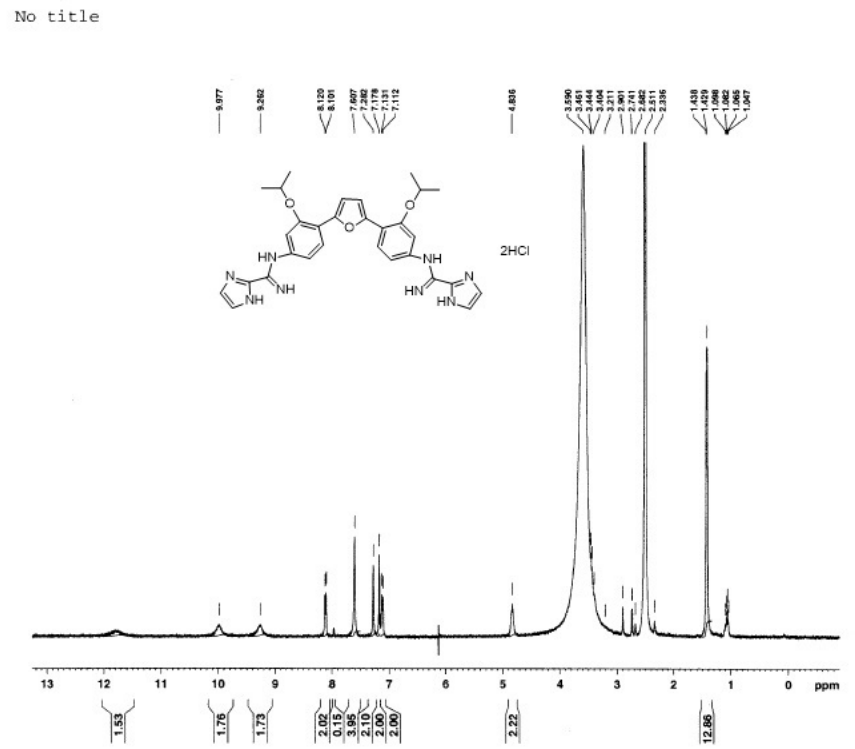


Figure 26: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7m.

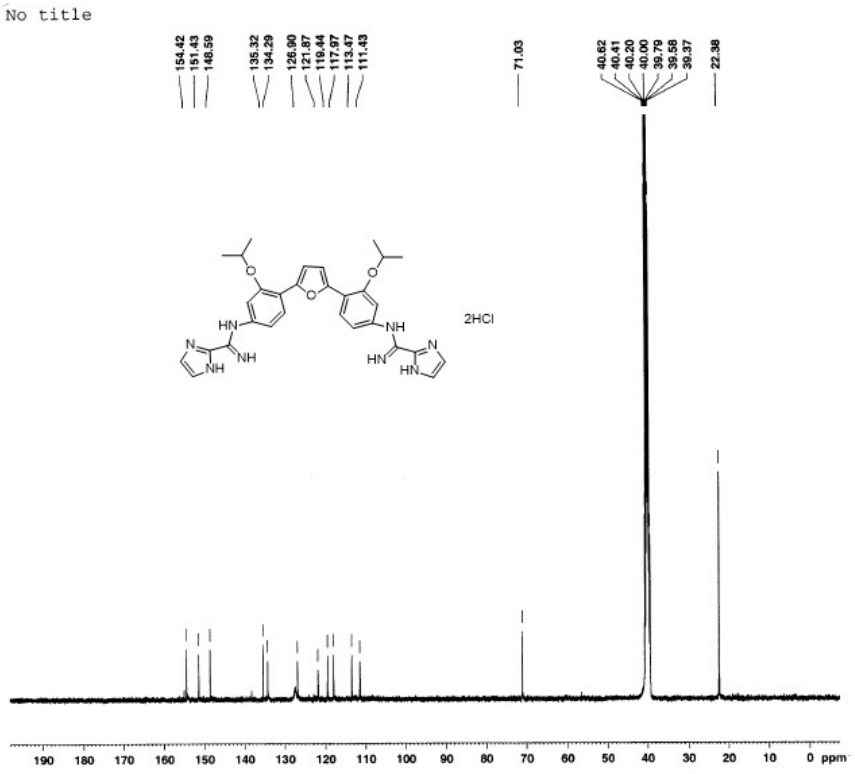


Figure 27: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7n.

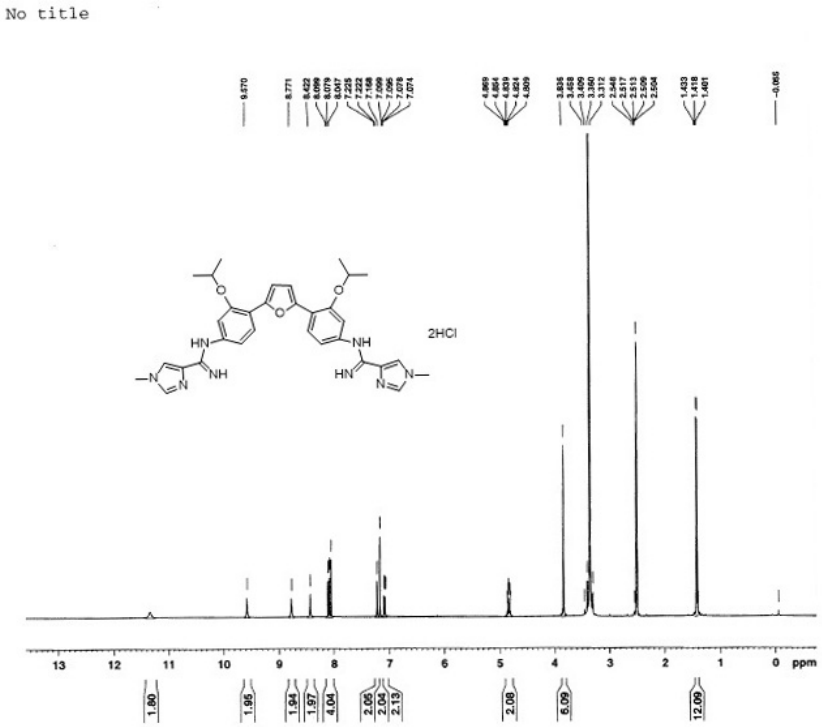


Figure 28: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7n.

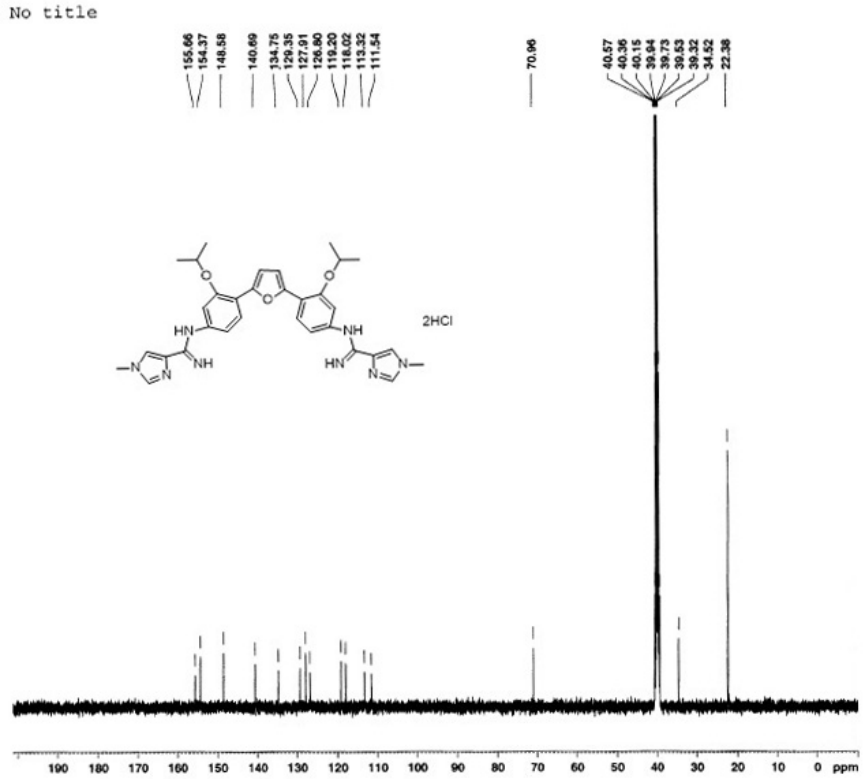


Figure 29: ^{1}H NMR (400 MHz, DMSO-*d6*) of 7o.

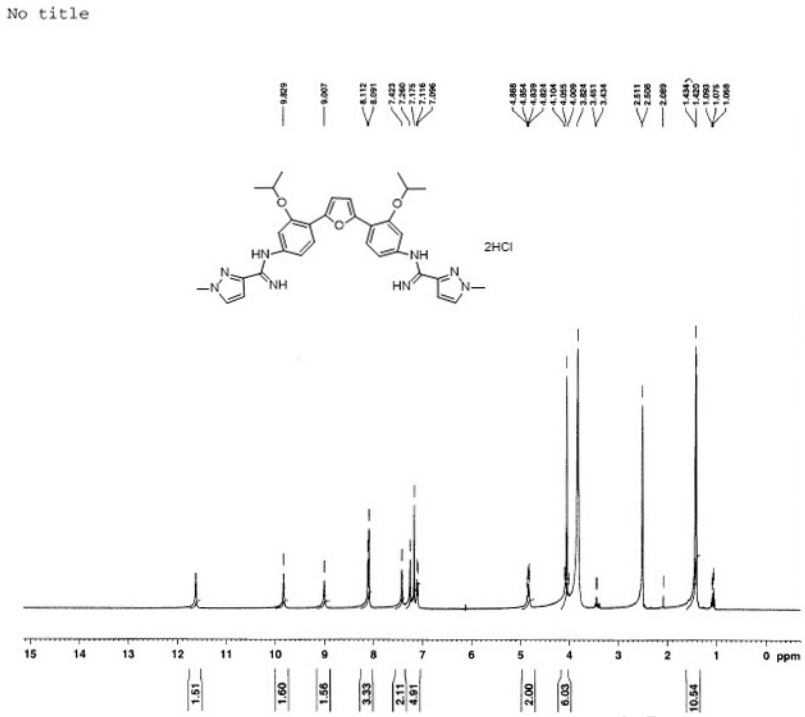
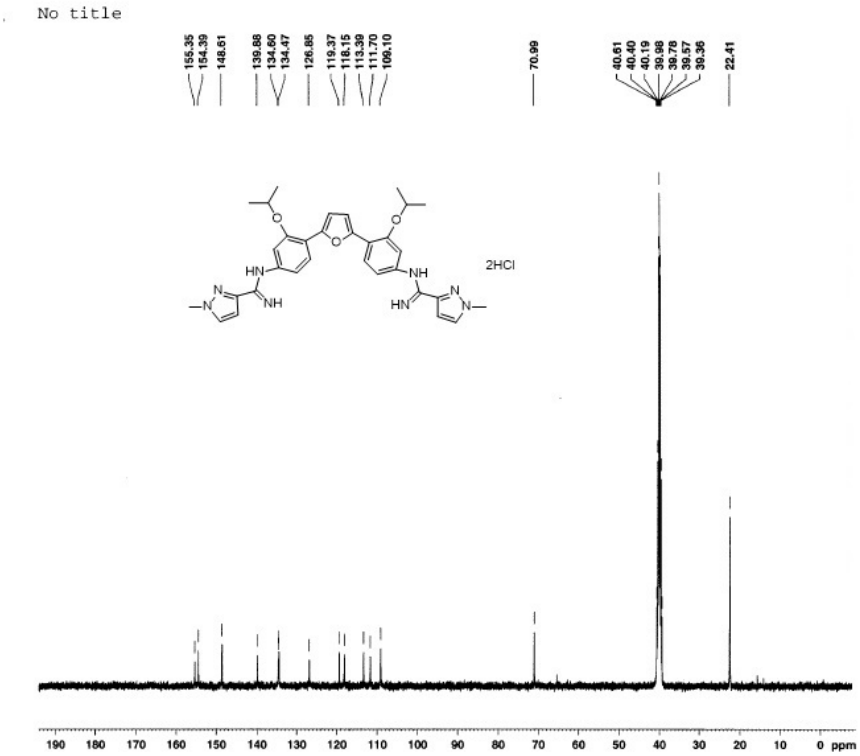
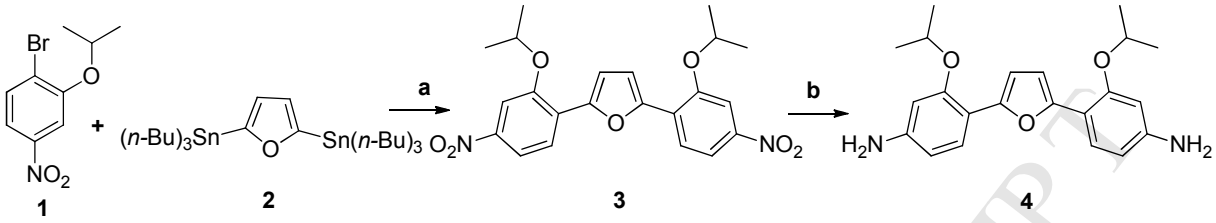


Figure 30: ^{13}C NMR (400 MHz, DMSO-*d6*) of 7o.

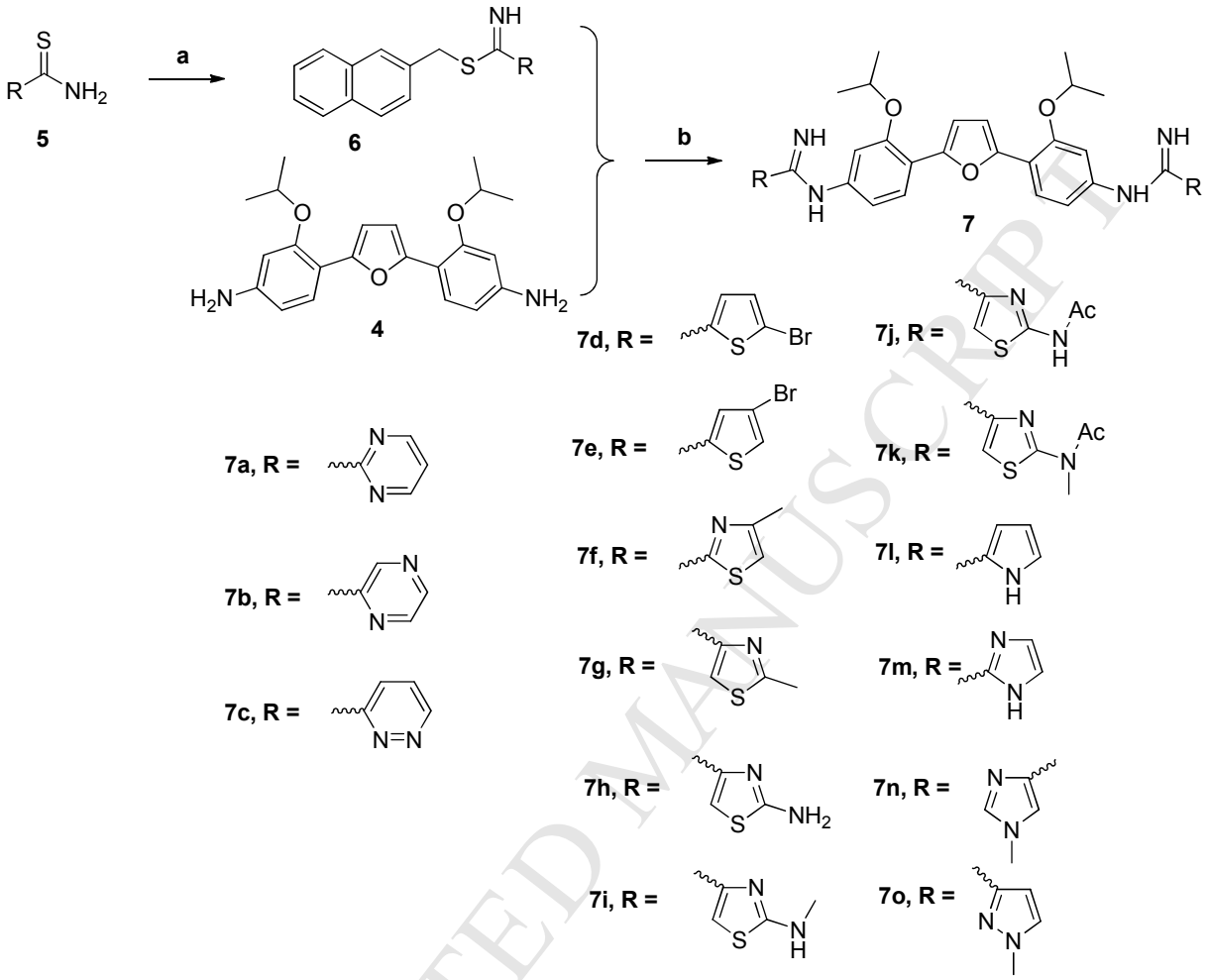


Scheme 1.



Reagents and conditions: a. $Pd(PPh_3)_4$, 1,4-dioxane, reflux b. H_2, EtOAc, EtOH, rt

Scheme 2.



Reagents and conditions: a. 2-(bromomethyl)naphthalene, $CHCl_3$, reflux b. MeCN, EtOH, rt