Bis benzothiophene Schiff bases: synthesis and in silico-guided biological activity studies

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Abstract: Since benzo [b] thiophene scaffold is one of the privileged structures in drug discovery as this core exhibits-activities for different biological problems, in this study bis (benzo[b]thiophene-2-yl) alkyl methanimine derivatives (1-9) were synthesized by reacting benzo[b]thiophene-2-carbaldehyde with diamines. All newly compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods. Synthesized compounds were investigated using binary QSAR-based models on therapeutic activity prediction of synthesized compounds and they showed high predicted activities in following diseases: bacterial, angina, allergy, depression and obesity. Thus, they were then tested for their antimicrobial and antileishmanial activities as a result of this theoretical study. Compound 1(N, N'- (propane-1,3-diyl) bis (1-(benzo [b] thiophene-2-yl)) methanimine) was found the most active compound in both diseases. Thus, its molecular docking studies were also carried out.

Key words: Benzo [b] thiophene, Schiff base, binary QSAR models, molecular docking, antimicrobial and antileishmanial activities

1. Introduction

Benzo [b] thiophene derivatives display remarkable biological activities such as antiinflammatory, analgesic, antifungal, antidepressant, antiangiogenic, estrogen receptor modulating, antimitotic, anticancer, kinase inhibitors, antituberculosis, anticonvulsant, antimalarial, anthelmintic, antihyperglycemic and pesticide. Benzothiophene derivatives have been used as potential diagnostic agents and amyloid binding in neurodegenerative diseases, treatment of fatty acid amide hydrolase inhibitors (FAAH), BMP-2 upregulators, Alzheimer's disease (AD), human nicotinamide phosphoribosyltransferase inhibitors, BRAF kinase inhibitors, Rho kinase inhibitors, selective linear tachykinin NK2 receptor antagonists, protein tyrosine phosphatase 1B inhibitors, histamine H3 antagonists, antiallergic agents and many other activities [1–7]. By replacing the substituents attached to the benzothiophene ring, some of the FDA-approved anticancer drugs such as arzoxifene and raloxifene were also developed [8,9]. Schiff bases, including azomethine functional group (N=CH), possess biological properties such as analgesic, antifungal, antibacterial, antidepressant, anticancer, anticonvulsant and antiinflammatory [10–15]. Schiff bases are also known to be highly effective in synergistic effects on insecticides and plant growth regulators [16–18].

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Antibiotics are widely used in the treatment of bacterial infections for many years in the early 20th century. Penicillin was the first antibiotic used to treat bacterial infections. Antibiotics, which are effective by killing or stopping the growth of bacteria, cause the emergence of drug-resistant pathogens and the development of resistance if used unconsciously and excessively. In addition, the long-term use of antibiotics damages our microbiota and losing the beneficial microorganisms has negative consequence. Approximately 16 million people die annually due to bacterial infections, while developing new approaches to combating drug-resistant pathogens and infectious diseases. New substances have been synthesized due to technological developments and have been used in various bacterial infections for therapeutic purposes [19,20].

Leishmaniasis is a disease caused by a protozoon called Leishmania which is transmitted by the bite of infected female sand flies. Poor nutrition lack of sanitation weakened immune system and poverty facilitate spread of the disease in underdeveloped and developing countries. The disease occurs in 3 different forms: cutaneous (most common), visceral (also known as kala-azar and the most serious form of the disease), and mucocutaneous. An estimated 700,000 to 1 million new cases are reported annually, and 26,000 to 65,000 disease-related deaths are reported by the World Health Organization.While cutaneous form is endemic in Southeastern Anatolia, visceral form is mostly seen in Mediterranean, Central Anatolian and Aegean Regions andimportant constitutes an important health problem for Turkey [21,22].

Recent advances made in molecular biology and computational chemistry open new avenues in designing novel therapeutic compounds. Molecular biology has provided the crystal structures and cryo-electron microscopy structures of crucial targets for different diseases, which can be used as accurate templates in modeling studies. Computational chemistry offers a range of simulation, multiscale modeling and virtual screening tools for definition and analysis of protein-ligand, protein-protein interactions. Development of new techniques on statistical methods and free energy simulations help to predict novel optimal ligands. Thus, in this study, we performed in silico-guided biological activity studies for a set of newly synthesized compounds. We aimed to synthesize a new series of Schiff bases including benzo [b] thiophene for more efficacious biologic activities. In order to predict the biological activities of synthesized compounds before the experimental studies, therapeutic activities of these novel compounds were predicted. The therapeutic activity value (TAV) of each compound was calculated by binary QSAR models of 25 common disease QSAR models. In silico results represented that synthesized compounds may show potential activity against in following diseases: bacterial, angina, allergy, depression and obesity. Therefore, these compounds were then tested for their antimicrobial and antileishmanial activities. Moreover, molecular docking study for one of the most active compounds as antibacterial agent was performed and its molecular interactions were studied.

2. Experimental

2.1. General method for the synthesis of compounds (1-9)

Benzo [b] thiophene-2-carbaldehyde (2 mmol) and diamines (1 mmol) were mixed and heated in oil bath without solvent for 2–3 h at 150–160 ^{0}C. The reaction content was controlled with TLC examination and cooled to room temperature. The precipitate formed was purified by recrystallization from ethanol-diethylether.

2.1.1. Synthesis of N, N'-(propane-1,3-diyl) bis(1-(benzo[b] thiophene-2-yl) methanimine) (1):

Yield: 87.00%, m.p. 183–184 ^{o}C. IR (KBr, cm^{-1}): 3056 (=CH), 2936 (CH), 1633 (C=N), 1600 (C=N), 1527 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.64 (s, 2H, N=CH), 7.92–7.97 (m, 4H, Arom.H), 7.82 (

bs, 2H,thiop.H), 7.42 (bs, 4H, Arom.H), 3.68 (s, 4H, N-CH$_2$), 1.98 (s, 2H, N-CH$_2$-CH$_2$) ; ^{13}C NMR (100 Hz, DMSO-d$_6$) δ : 156.23, 143.05, 139.99, 139.55, 128.78, 126.64, 125.28, 123.22, 58.28, 32.05.

2.1.2. Synthesis of 1-(benzo[b]thiophene -2-yl)-N-(4-((benzo[b]thiophene-2-ylmethylene) amino) butyl) methanimine(2):

Yield: 88.20%, m.p. 166–167 ^{o}C. IR (KBr, cm^{-1}): 3056 (=CH), 2939 (CH), 1628 (C=N), 1526 (C=N), 1464 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.61 (s, 2H, N=CH), 7.87–7.97 (m, 4H, Arom.H), 7.79 (2H, bs, thiop.H), 7.37–7.44 (m, 4H, Arom.H), 3.64 (s, 4H, N-CH$_2$), 1.68 (s, 4H, N-CH$_2$-CH$_2$) ;^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.87, 143.11, 139.96, 139.65, 128.66, 126.62, 125.22, 123.23, 60.33, 28.64.

2.1.3. Synthesis ofN,N'-(pentane-1,5-diyl)bis(1-(benzo[b] thiophene-2-yl)methanimine) (3):

Yield: 82.65%, m.p. 110–111 ^{o}C. IR (KBr, cm^{-1}): 3053 (=CH), 2935 (CH), 1632 (C=N), 1560 (C=N), 1525 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.58 (s, 2H, N=CH), 7.84–7.93 (m, 4H, Arom.H), 7.75 (2H, bs, thiop.H), 7.37–7.42 (m, 4H, Arom.H), 3.59 (s, 4H, N-CH$_2$), 1.65–1.70 (m, 4H, N-CH$_2$-CH$_2$), 1.35–1.39 (m, 2H, N-CH$_2$-CH$_2$-CH$_2$); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.73, 143.15, 139.64, 128.56, 126.58, 125.19, 123.20, 60.44, 31.44, 24.87.

2.1.4. Synthesis of 1-(benzo[b]thiophene-2-yl)-N-(6-((benzo[b]thiophene-2- ylmethylene) amino) hexyl) methanimine (4):

Yield: 80.28%, m.p. 143–144 ^{o}C. IR (KBr, cm^{-1}): 3057 (=CH), 2934 (CH), 1628 (C=N), 1560 (C=N), 1526 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.58 (s, 2H, N=CH), 7.88–7.95 (m, 4H, Arom.H), 7.76 (2H, bs, thiop.H), 7.41 (bs, 4H, Arom.H), 3.58 (s, 4H, N-CH$_2$), 1.63 (bs, 4H, N-CH$_2$-CH$_2$), 1.37 (bs, 4H, N-CH$_2$-CH$_2$-CH$_2$); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.73, 143.15, 139.64, 128.56, 126.58, 125.19, 123.20, 60.44, 31.44, 24.87.

2.1.5. Synthesis of N,N'-(heptane-1,7-diyl)bis(1-(benzo[b] thiophene-2-yl)methanimine) (5):

Yield: 80.85%, m.p. 117–118 ^{o}C. IR (KBr, cm^{-1}): 3056 (=CH), 2934 (CH), 1673 (C=N), 1629 (C=N), 1517 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.57 (s, 2H, N=CH), 7.87–7.94 (m, 4H, Arom.H), 7.76 (2H, bs, thiop.H), 7.37–7.43 (m, 4H, Arom.H), 3.56 (s, 4H, N-CH$_2$), 1.62 (bs, 4H, N-CH$_2$-CH$_2$), 1.37 (bs, 6H, N-CH$_2$-CH$_2$-CH$_2$-CH$_2$); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.63, 143.16, 139.95, 139.64, 128.54, 126.59, 125.19, 123.22, 60.53, 30.70, 28.93, 27.14.

2.1.6. Synthesis of 1-(benzo[b]thiophene-2-yl)-N-(8-((benzo[b]thiophene -2- ylmethylene) amino) octyl) methanimine (6):

Yield: 84.85%, m.p. 122–123 ^{o}C. IR (KBr, cm^{-1}): 3056 (=CH), 2924 (CH), 1628 (C=N), 1525 (C=N), 1465 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.59 (s, 2H, N=CH), 7.91 (bs, 4H, Arom.H), 7.78 (2H, bs, thiop.H), 7.41 (bs, 4H, Arom.H), 3.39 (s, 4H, N-CH$_2$), 1.62 (bs, 4H, N-CH$_2$-CH$_2$), 1.32 (bs, 8H, N-CH$_2$-CH$_2$-CH$_2$-CH$_2$); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.65, 143.17, 140.91, 128.56, 126.01, 125.20, 123.23, 60.58, 30.78, 29.36, 27.18.

2.1.7. Synthesis of N,N'-(nonane-1,9-diyl)bis(1-(benzo[b] thiophene-2-yl)methanimine) (7):

Yield: 81.20%, m.p. 115–116 ^{o}C. IR (KBr, cm^{-1}): 3057 (=CH), 2923 (CH), 1672 (C=N), 1629 (C=N), 1525(C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.57 (s, 2H, N=CH), 7.89–7.96 (m, 4H, Arom.H), 7.78 (2H, bs, thiop.H), 7.41 (bs, 4H, Arom.H), 3.56 (s, 4H, N-CH$_2$), 1.60 (bs, 4H, N-CH$_2$-CH$_2$), 1.30 (bs, 10H, N-CH$_2$-CH$_2$-CH$_2$- CH$_2$-CH$_2$); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.64, 143.17, 139.95, 139.66, 128.56, 126.60, 125.21, 123.23, 60.57, 30.77, 29.41, 29.18, 27.18.

2.1.8. Synthesis of 1-(benzo[b]thiophene-2-yl)-N-(10-((benzo[b]thiophene-2- ylmethylene) amino) decyl) methanimine (8):

Yield: 83.25%, m.p. 141–142 ^{o}C. IR (KBr, cm^{-1}): 3050 (=CH), 2924 (CH), 1625 (C=N), 1591 (C=N), 1525(C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.58 (s, 2H, N=CH), 7.88–7.94 (m, 4H, Arom.H), 7.77 (2H, bs, thiop.H), 7.40 (bs, 4H, Arom.H), 3.56 (s, 4H, N-CH$_2$), 1.58 (bs, 4H, N-CH$_2$-CH$_2$), 1.28 (bs, 12H, N-CH$_2$-CH$_2$-CH$_2$- CH$_2$-CH$_2$); ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.64, 143.18, 139.70, 139.66, 128.55, 126.59, 125.21, 123.23, 60.57, 30.77, 29.83, 29.41, 27.33.

2.1.9. Synthesis of 1-(benzo[b]thiophene-2-yl)-N-(12-((benzo[b]thiophene-2- ylmethylene) amino) dodecyl) methanimine (9):

Yield: 80.25%, m.p. 122–123 ^{o}C. IR (KBr, cm^{-1}): 3054 (=CH), 2918 (CH), 1632 (C=N), 1560 (C=N), 1526 (C=C); ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 8.58 (s, 2H, N=CH), 7.90–7.95 (m, 4H, Arom.H), 7.79 (2H, bs, thiop.H), 7.41 (bs, 4H, Arom.H), 3.38 (s, 4H, N-CH$_2$), 1.59 (bs, 4H, N-CH$_2$-CH$_2$), 1.24 (bs, 10H, N-CH$_2$-CH$_2$-CH$_2$- CH$_2$-CH$_2$), 1.06 (bs, 4H, N-CH$_2$-CH$_2$-CH$_2$- CH$_2$-CH$_2$-CH$_2$) ; ^{13}C NMR (100 Hz, DMSO-d$_6$) δ :155.65, 139.70, 128.57, 126.60, 125.21, 123.23, 60.57, 30.74, 29.40, 29.18.

2.2. MetaCore/MetaDrug applications

Studied compounds were examined for their therapeutic activity against 25 common diseases using Meta-Core/MetaDrug platform of Clarivate Analytics (Philadelphia, PA, USA). Training set of compounds were collected from FDA approved compounds, candidate drugs in clinical phases and hit compounds from in vivo activities. Results showed that synthesized compounds can be used as antibacterial agents. Following training and test set compound numbers were used in bacterial QSAR model constructions: Training set N = 530, test set N = 97, sensitivity = 0.87, specificity = 0.90, accuracy = 0.89, Matthews correlation coefficient (MCC) = 0.77.

3. Ligand preparation

The synthesized molecules are prepared for further calculations via LigPrep module [23] of Schrodinger's molecular modeling suite with OPLS2005 force field [24]. Epik [25] was used to determine the protonation states of these compounds at neutral pH.

4. Protein preparation

The X-ray diffraction (XRD) structure of the target was used from the Protein Data Bank (PDB) with PDB IDs of 5TW8. Protein preparation module was utilized [26], [27]. Missing side chains are filled via prime module

and PROPKA [28]. was subjected to determine the protonation of side chains at physiological pH. Finally, a restrained minimization in OPLS2005 force field was applied to these optimized structures.

5. Molecular docking

The molecules which possessed desirable pharmacokinetic and toxicity properties were subjected to a grid-based Glide/XP docking protocol from Schrodinger's Maestro molecular modeling package [29]. Binding pocket was determined from cocrystallized ligand and default protocol was employed. Shortly, we used flexible docking approach. Hydroxyl and thiol groups of binding pocket residues were allowed for rotation throughout the docking. Extra precision (XP) protocol of Glide was used. Ten poses were requested at the docking and postdocking minimization was performed.

6. Results and discussion

6.1. Synthesis

Synthesis of bis (benzo[b]thiophene-2-yl) alkyl methanimine derivatives (1-9) was performed according to the reaction outlined in Scheme.

n=3.....1
n=4.....2
n=5.....3
n=6.....4
n=7.....5
n=8.....6
n=9.....7
n=10...8
n=12...9

NH$_2$-(CH$_2$)n-NH$_2$

1-9
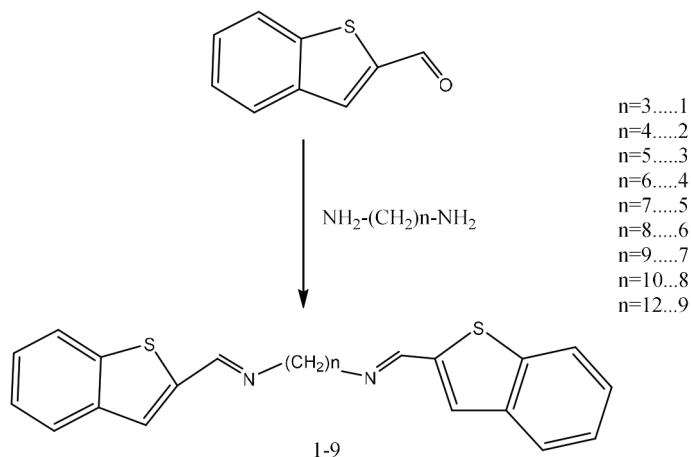
Scheme. Synthesis of Schiff base derivatives with benzo [b] thiophene (1-9).

The peaks of NH$_2$ and C=O belonging to the starting amines and aldehyde respectively disappeared in the IR spectra of the compounds (1-9). Proton signal of imine group (N=CH) obtained by Schiff base reaction was observed as singlet at 8.57–8.64 ppm in the ^{1}H-NMR spectra of the compounds (1-9). Aromatic protons belonging to benzo[b]thiophen ring resonated at 7.37–7.97 ppm and CH$_2$ alkyl protons were seen at 1.28–3.68 ppm in the ^{1}H-NMR spectra of the compounds as expected from alkyl groups. When carbon spectrawere examined, N=CH imine carbon was observed at 155.64–156.23 ppm in the ^{13}C-NMR spectra of compounds **1-9**. Aromatic carbon peaks of benzo [b] thiophene were seen at 123.20–143.19 ppm and alkyl carbon peaks resonated at 24.87–60.58 ppm in the ^{13}C-NMR spectra of the compounds. As a result, spectral data supports structures of the compounds (1-9).

6.2. In silico predictions of therapeutic activities of synthesized compounds

Binary quantitative structure-activity relationships (QSAR) common disease models from Clarivate Analytics MetaCore/MetaDrug platform were used for the therapeutic activity predictions of synthesized compounds. For this aim, 25 different common diseases binary QSAR models were used. All the nine compounds were screened on MetaCore/MetaDrug platform and therapeutic activity values were predicted. Therapeutic activity values in MetaCore/MetaDrug are normalized between 0 and 1 (while 0 represents inactive compound, 1 represents active compounds). Although predicted therapeutic activity value higher than 0.5 indicates compound that may show activity, in the current study we used a higher cutoff value (0.75) for being in the safe zone. Table S1 at the supplementary information show corresponding predicted therapeutic activity values of each studied compound that has activity value equal or more than 0.75. When we check diseases in Table S1 it can be seen that synthesized compounds can be considered mainly for bacterial, angina, allergy, depression and obesity models. Since the investigated compounds are analogs of each other they showed similar TAV values in certain disease models such as antibacterial profile. Thus, from these diseases we considered to perform antibacterial in vitro assays using these compounds. Furthermore, since known antifungal and antileishmanial effect of benzo [*b*] thiophene derivatives, we also performed antifungal and antileishmanial in vitro assays for these compounds.

6.3. In vitro antileishmanial activity studies

Antileishmanial activity results of nine compounds as a result of evaluation Figures 1–2 and minimum inhibitory concentration (MIC) values are given in Table 1. The tests in the positive and negative control wells were found to work as expected.
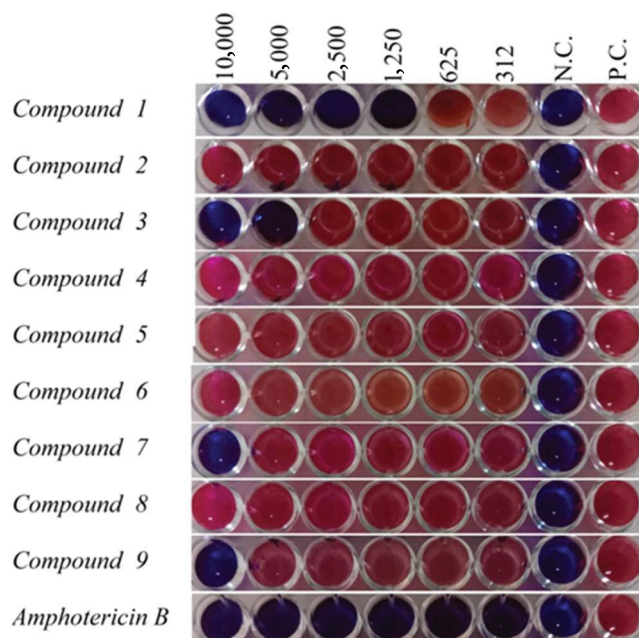
Figure 1. Antileishmanial activity results of the compounds against standard *Leishmania infantum* promastigotes. Control drug: Amphotericin B. Dilution concentrations 10,000 µg/mL to 312 µg/mL. N.C.: Negative control; P.C.: Positive control.
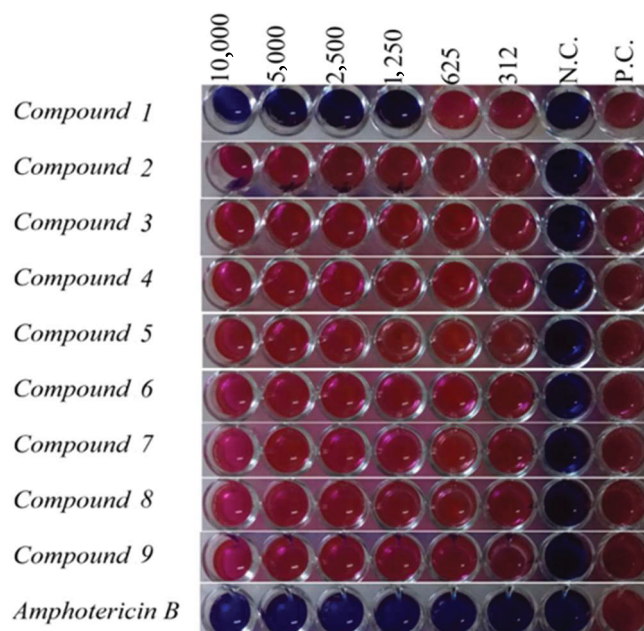
Figure 2. Antileishmanial activity results of compounds against standard *Leishmania tropica* promastigotes. Control drug: Amphotericin B. Dilution concentrations 10,000 µg/mL to 312 µg/mL. N.C.: Negative control; P.C.: Positive control.

Table 1. Minimum inhibitory concentration (MIC) values of the compounds against *Leishmania infantum* and *Leishmania tropica* promastigotes.

Compounds	*L.infantum* MIC values (µg/mL)	*L.tropica* MIC values (µg/mL)
1	1250	1250
2	>10,000	>10,000
3	5000	>10,000
4	>10,000	>10,000
5	>10,000	>10,000
6	>10,000	>10,000
7	10,000	>10,000
8	>10,000	>10,000
9	10,000	>10,000
Amphotericin B	<312	<312

Compound 1 was found to be the most effective compound (MIC: 1250 μg / mL) among the compounds whose antileishmanial activities were evaluated against *Leishmania infantum* promastigotes. In addition, compounds 3, 7 and 9 were found to have antileishmanial activity at different concentrations (MIC: 5000–10,000 μg/mL).

It was found that other compounds did not have antileishmanial activities against *Leishmania infantum* promastigotes at the studied concentrations. Compound 1 was also found to be the most effective compound

against the *Leishmania tropica* promastigotes from compounds whose antileishmanial activities were evaluated (MIC: 1250 μg/mL). It was determined that other compounds did not have antileishmanial activities against *Leishmania tropica* promastigotes at studied concentrations.

The study of Maina et al. revealed the antileishmanial activity of *Clerodendrum myricoides* and *Salvadora persica.* They reported, *Clerodendrum myricoides* water extract demonstrated the best potential antileishmanial activity against *Leishmania major* promastigotes (MIC = 625 μg/mL). Also, the dichloromethane and petroleum ether extract were reported moderate to weak activity against *Leishmania major* promastigotes (MIC = 1250 μg/mL; 2500 μg/mL) and amastigotes respectively [30].

In the study of Ogeto et al., *Aloe secundiflora* water extract was found active against L. major at the lowest concentration of 2000 μg/mL. Methanollic plant extract reported less antileishmanial activity as compared to Pentostam and amphotericn B with MIC of 1000 μg/mL, 250 μg/mL and 125 μg/mL, respectively. They marked higher antileishmanial activities though not of comparative concentrations than most of the reference drugs. The results also showed that plant extracts had lower toxicity against Vero cells as compared to the standard drug amphotericin B [31].

Amphotericin B, which is used as a standard drug, was found to be effective at all concentrations (MIC: <312 µg / mL) for both leishmania species.

6.4. In vitro antibacterial and antifungal activity studies

Different concentrations of some compounds against standard bacterial isolates and yeast isolates were found to have antimicrobial activity, while some compounds were found to be ineffective at the concentrations studied. The test images of compound 1 which have the most effective antibacterial and antifungal effect are given in the Figure 3. The MIC values of the compounds are given in Table 2. It was determined that the compounds showed antibacterial and antifungal activity against 6 of the studied standard bacteria and yeast isolates.
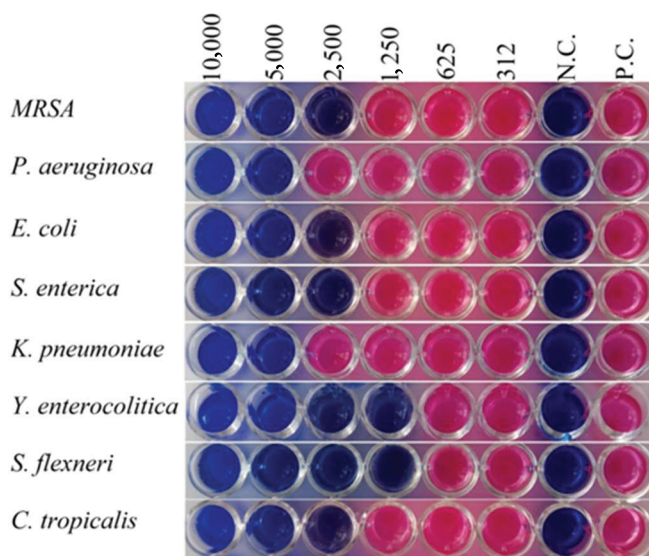
Figure 3. Antibacterial and antifungal activity results of compound 1. Dilution concentrations: 10,000 µg/mL–312 µg/mL. N.C.: Negative control; P.C.: Positive control.

Table 2. Minimum inhibitory concentration (MIC) values of all compounds against bacteria and fungi.

Bacteria/ Fungi Compounds	Staphylococcus aureus (MRSA)	Escherichia coli	Klebsiella pneumoniae	Shigella flexneri	Salmonella enteridis	Yersinia enterocolitica	Pseudomonas aeruginosa	Candida tropicalis
1	2500	2500	5000	1250	2500	1250	5000	2500
2	>10000	>10000	>10000	10000	>10000	>10000	10000	>10000
3	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
4	>10000	>10000	>10000	>10000	>10000	>10000	10000	>10000
5	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
6	>10000	>10000	>10000	>10000	>10000	>10000	10000	>10000
7	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
8	>10000	>10000	>10000	>10000	>10000	>10000	10000	>10000
9	>10000	>10000	>10000	>10000	>10000	>10000	10000	>10000

The compound 1 showed the strongest antibacterial effect against *Shigella flexneri* and *Yersinia entero-colitica* (MIC:1250 μg/mL) (Figure 3). Compound 1 was found to be effective against all bacteria and yeast (Table 2). Antibacterial and antifungal activity were determined in 6 compounds (1, 2, 4, 6, 8 and 9).

When the results of synthesized compounds were evaluated, it was found that compound 1 was the most effective compound for all bacteria and yeast isolates studied. Compound 1 was found to have different levels of antimicrobial activity against *Shigella flexneri*, *Yersinia enterocolitica*, *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Salmonella enteritidis*, *Candida tropicalis*, *Pseudomonas aeruginosa,* and *Klebsiella pneumonia* (respectively MIC values: 1250 μg/mL, 1250 μg/mL, 2500 μg/mL, 2500 μg/mL, 2500 μg/mL, 2500 μg/mL, 5000 μg/mL, and 5000 μg/mL).

It is found that *Klebsiella pneumoniae* is the least affected one by the compounds. All concentrations of the compounds 3, 5 and 7 were found to have no effect on any bacterial and yeast isolates (MIC: >10,000 μg/mL).

Thus, because of its promising results at both disease models, compound 1 can be considered as lead compound. However, further animal in vivo studies must be carried out.

Since the most active compound was found as compound 1 as antibacterial agent, we used crystal structure of wild-type *S. aureus* penicillin binding protein 4 (PBP4), PDB ID: 5TW8, and performed docking simulations. Figure 4 shows 2D and 3D ligand interactions diagram of compound 1 at the binding pocket of the target structure. It can be seen that main nonbonded interactions are constructed with Tyr291 and Tyr268 (π-π stacking interactions) and with Glu297 (ionic interactions). These strong interactions (docking score, -8.33 kcal/mol) show that compound 1 can be considered as promising anti-bacterial agent. Both in silico predictions (ligand-based and target-driven based) as well as performed in vitro assays validate each other for the antibacterial effect of the novel compound (compound 1). Our hybrid molecular modeling approaches (combined ligand-based and structure-based) predicted the biological activity of a set of new compounds and these predictions were investigated by in vitro studies.

7. Conclusion

Nine bis (benzo[*b*]thiophen-2-yl) alkyl methanimine derivatives were synthesized. All newly compounds were characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods.Synthesized compounds were investigated using 25 different binary QSAR disease models. Results showed that synthesized compounds may be considered mainly for bacterial, angina, allergy, depression and obesity models. Our in silico-guided design study lead to a hit compound (Compound 1) for its antibacterial effects. In vitro antileishmanial, antifungal and antibacterial activity studies were performed on all synthesized compounds. According to the test results; Compound 1 showed antileishmanial activity (MIC = 1250 µg/mL) and it was concluded that further studies could be continued for this compound 1. The purpose of the control drug study was made to test whether the experimental study was working properly. The substances studied showed antileishmanial activity even if they had a higher value than the MIC of the standard drug Amphotericin B. In this antileishmanial activity study, while aiming to determine the concentration of the compound that is effective against leishmania, the reliability, toxic effect and side effects of the substances should also be evaluated for new drug candidates. Due to the toxic effects of currently used drugs and the increasing resistance to these drugs, the discovery and development of new therapeutic agents is important.Compound 1 also showed better antibacterial and antifungal activity.
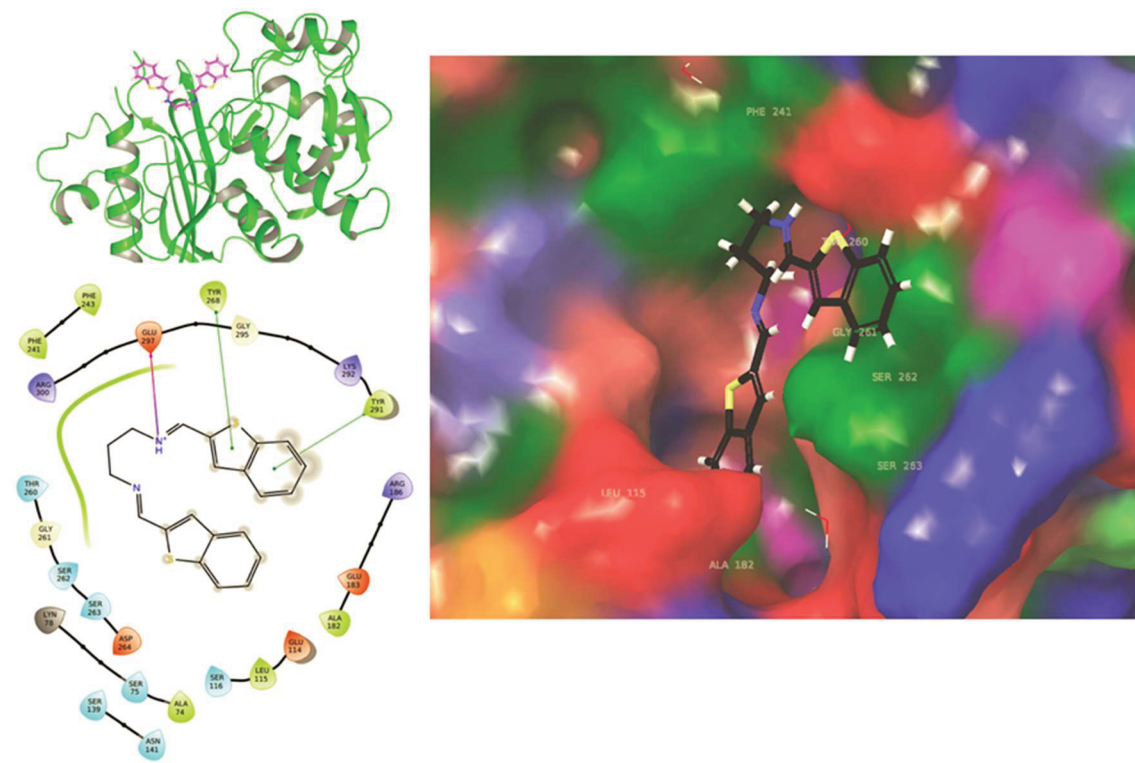
Figure 4. 2D and 3D ligand interactions diagrams of compound 1 at the binding pocket of 5TW8 PDB coded structure.

Acknowledgment

Chemistry and determination of in vitro antileishmanial, antifungal and antibacterial activities were submitted at supplementary information.

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Supplementary information:

S1. Experimental

Chemistry

The ^{1}H-, and ^{13}C-nuclear magnetic resonance spectra were recorded on a Bruker 400 MHz spectrometer, where TMS as an internal standard and DMSO-d$_6$ as solvent was used. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer in KBr pellets. Melting points were measured by an electrothermal apparatus. All chemicals were purchased from commercial suppliers.

S2. Determination of in vitro antileishmanial, antifungal, and antibacterial activities

Antileishmanial activity

In vitro antileishmanial activities of the compounds against *Leishmania infantum* and *Leishmania tropica* promastigotes were determined using liquid microdilution method added alamar blue.

Preparation of *Leishmania infantum* and *Leishmania tropica* promastigotes

In the antileishmanial activity study, axenic standard *Leishmania infantum* and *Leishmania tropica* promastigotes were used. Standard leishmania isolates were produced in RPMI-1640 (R8758 Sigma-Aldrich Corp., St. Louis, MO, USA) medium which containing 10% Fetal Bovine Serum (FBS F4135 Sigma-Aldrich Corp.), 1% Penicillin (P3032 Sigma-Aldrich Corp.) and Streptomycin (S9137 Sigma-Aldrich Corp.) (100,000 units penicillin and 10 mg streptomycin). Promastigotes grown on RPMI-1640 medium were placed in sterile falcon tubes and centrifuged at 1000 g for 10 min. The supernatant was decanted and centrifuged by addition of sterile PBS (phosphate buffered saline). This procedure was repeated 3 times to wash the promastigotes. RPMI-1640 medium was added to the promastigotes and adjusted to approximately 2.5 $\times 10^7$ promastigote/mL by the hemocytometer.

In vitro antileishmanial activity test

Synthesized compounds were dissolved by dimethylsulfoxide (DMSO)/H$_2$O (10%). The stock solutions of the compounds were prepared by adding RPMI-1640 medium to a concentration of 20 mg/mL and sterilized by passing through a 0.45-μm pore diameter sterile membrane filter (EMD Millipore Corp., Bedford, MA, USA). The sterile microplates with 96-well were used for the antileishmanial activity test. Dilution rates in the wells were prepared between 10,000 μg/mL and 312 μg/mL.

The microdilution method with alamar blue was performed as previously described [1–2]. Microplates were incubated for 20 h in a adjusted incubator set at 27 °C and after, 20 µL of alamar blue (Resazurin sodium salt R7017 Sigma-Aldrich Corp.) was added to the

wells and incubated again at 27 ºC for another 4 h. Results were obtained after 24, 48 and 72 h by visual evaluation of the microplates. In addition, 30 µL of fresh samples were prepared from all wells and the viability movements of the promastigotes were observed and confirmed visually. The test was repeated twice for each compound. Amphotericin B was used as control drug. The change of the indicator dye alamar blue to pink color in the wells was interpreted as continuing parasite growth, while the color remaining as blue was evaluated the parasite growth stopped and the compound was interpreted as effective.

Antibacterial activity

In this study, in vitro antimicrobial activities of the compounds against 7 selected standard bacteria and 1 yeast isolate were determined by liquid microdilution method with alamar blue.

Preparation of standard bacterial and fungal isolates

It was aimed to determine the minimal inhibitory concentrations (MIC) of the antimcrobial activities of the compounds against the standard bacterial and yeast isolates by liquid microdilution method with alamar blue (Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing). The standard isolates (*Escherichia coli* ATCC 25922, *Yersinia enterocolitica* ATCC 9610, *Salmonella enteridis* ATCC 13076, *Klebsiella pneumoniae* ATCC 700603, *Shigella flexneri* ATCC 12022, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* (MRSA) ATCC 43300 and *Candida tropicalis* ATCC 750) used in the study were obtained from the American Type Culture Collection (ATCC). Standard bacterial isolates were produced by incubating overnight at 37 °C using blood agar (Merck KGaA, Darmstadt, Germany) and eosin methylene blue agar (EMB, Merck KGaA) media and purity checks were performed. Similarly, standard yeast isolate was produced by incubating overnight at 37 °C using Sabouraud dextrose agar (SDA, Merck KGaA) medium and purity checks were performed.Standard bacterial isolates were prepared by diluting them in sterile saline to approximately 1.5×10^8 cfu / mL according to McFarland 0.5 turbidity chart. For fungi, it was set to be McFarland 1.

In vitro antimicrobial activity test

Antimicrobial activity testing of the compounds was performed using 96-well sterile microplates. The stock solutions of the compounds were prepared by adding sterile distilled water to a concentration of 20 mg/mL and sterilized by filtration through a 0.45-μm pore diameter sterile membrane filter (EMD Millipore Corp.). Firstly, 100 µL MHB medium was added to all wells for antibacterial activity test and Sabouraud dextrose broth medium was used for antifungal activity test. The dilution rates of compounds as previously described [2–3] were performed between 10,000 μg/mL and 312 μg/mL. Standard bacterial or yeast suspension was added to 100 µL wells. Negative and positive control wells were added and incubated in an incubator set at 37 °C. After 20 h of incubation, 20 µL of alamar blue was added onto the microplates and incubated for another 4 h. Microplates were evaluated visually for color change after 24 and 48 h. After the incubation the color unchanging of alamar blue in the wells was interpreted as the absence of proliferation, and the changing to pink from blue was interpreted as proliferation. As a reproductive control, samples were taken from each well with the loop and the presence of reproduction was investigated on the blood agar medium. While the antimicrobial activity test for each compound was repeated twice, amikacin and amphotericin B were used as control drugs.

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Table S1. Therapeutic activity value (TAV) predictions using binary QSAR models.

Compound 1

Disease	TAV
Bacterial	0.78
Angina	0.77
Depression	0.75
Allergy	0.75

Compound 2

Disease	TAV
Bacterial	0.81
Angina	0.79
Allergy	0.77

Compound 3

Disease	TAV
Bacterial	0.81
Angina	0.79
Allergy	0.77

Compound 4

Disease	TAV
Bacterial	0.82
Angina	0.79
Allergy	0.77
Obesity	0.75

Compound 5

Disease	TAV
Allergy	0.77
Angina	0.77
Obesity	0.75

Compound 6

Disease	TAV
Allergy	0.77
Angina	0.77
Depression	0.75
Obesity	0.75

Compound 7

Disease	TAV
Allergy	0.77
Angina	0.77
Obesity	0.75

Compound 8

Disease	TAV
Bacterial	0.82
Allergy	0.77
Alzheimer	0.76
Obesity	0.75

Compound 9

Disease	TAC
Bacterial	0.82
Allergy	0.77
Angina	0.76
Obesity	0.75
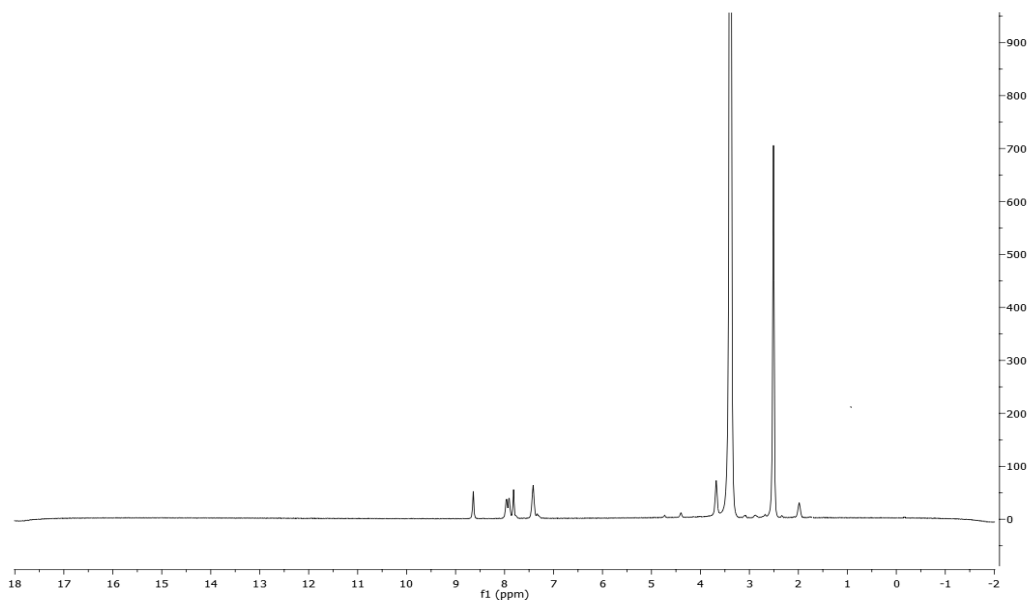
^{1}H-NMR spectrum of compound 1.
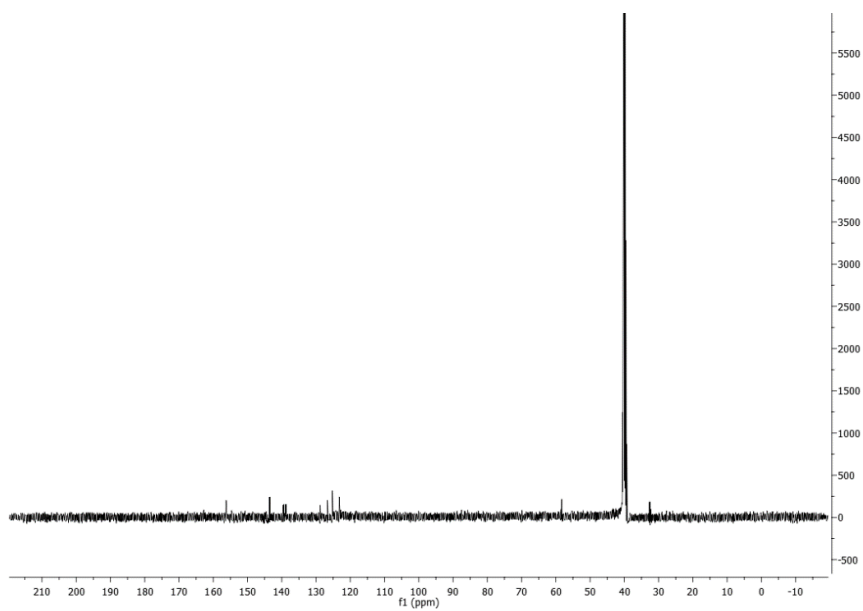
^{13}C-NMR spectrum of compound 1.
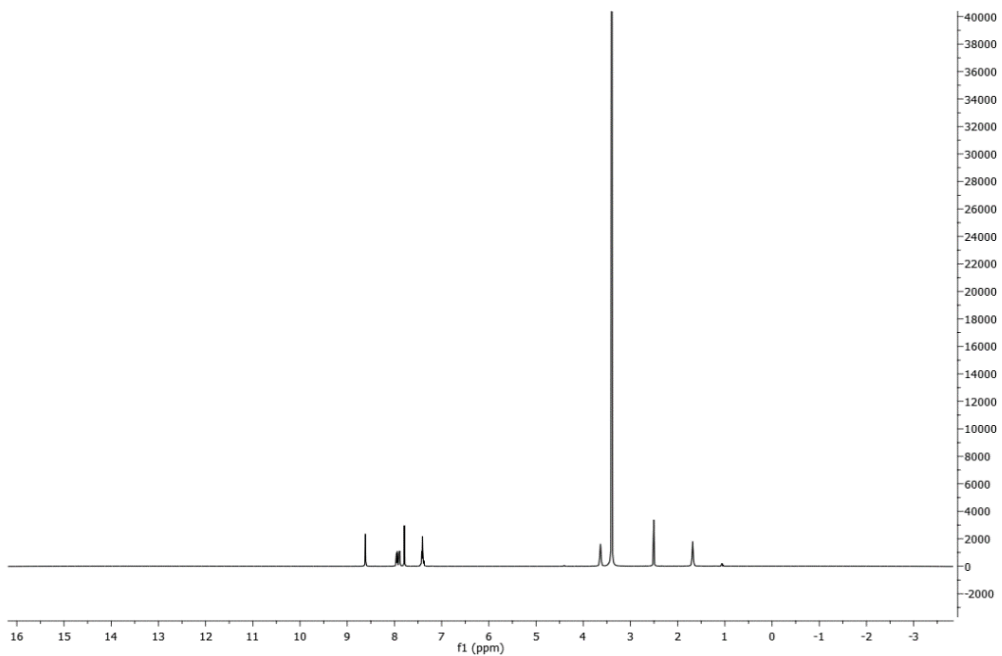
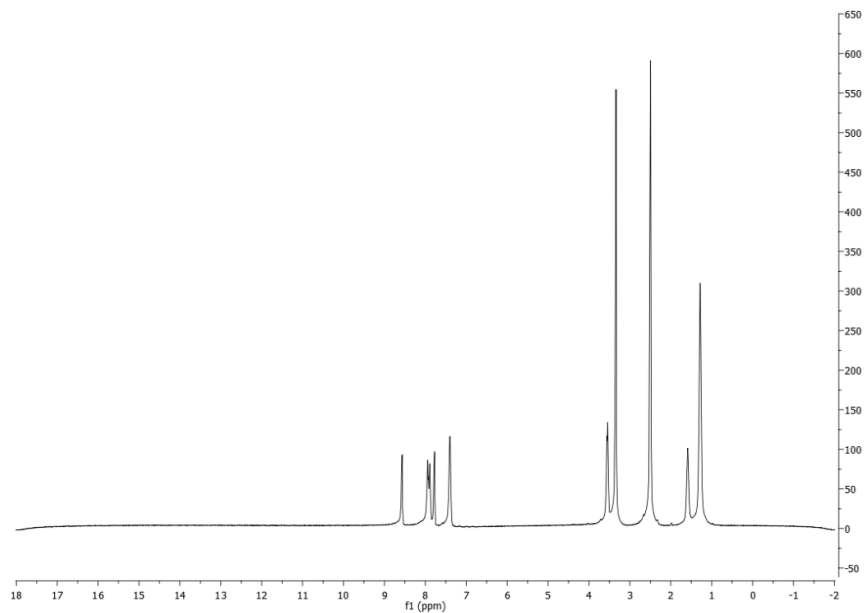^{1}H-NMR spectrum of compound 2.
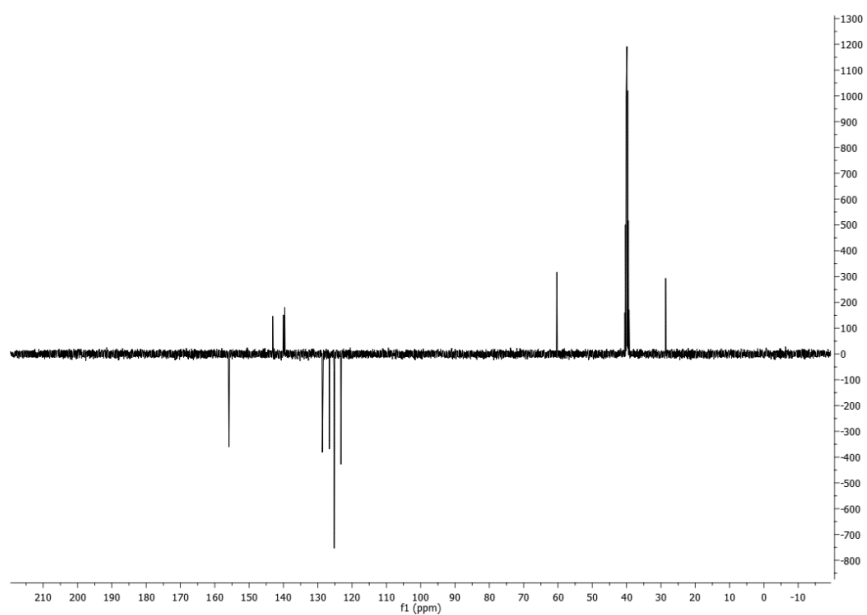
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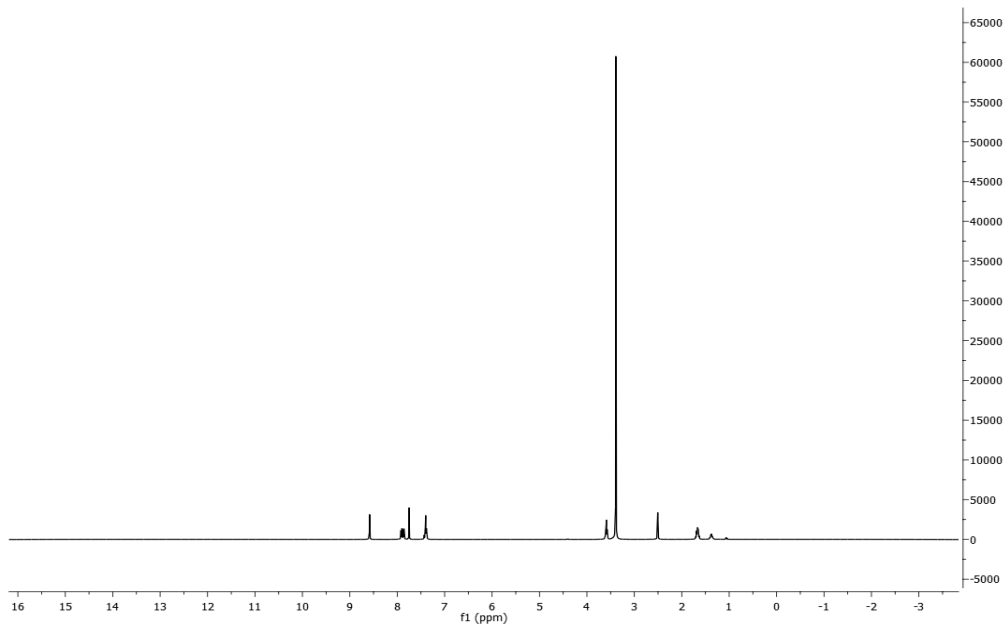
^{1}H-NMR spectrum of compound 3
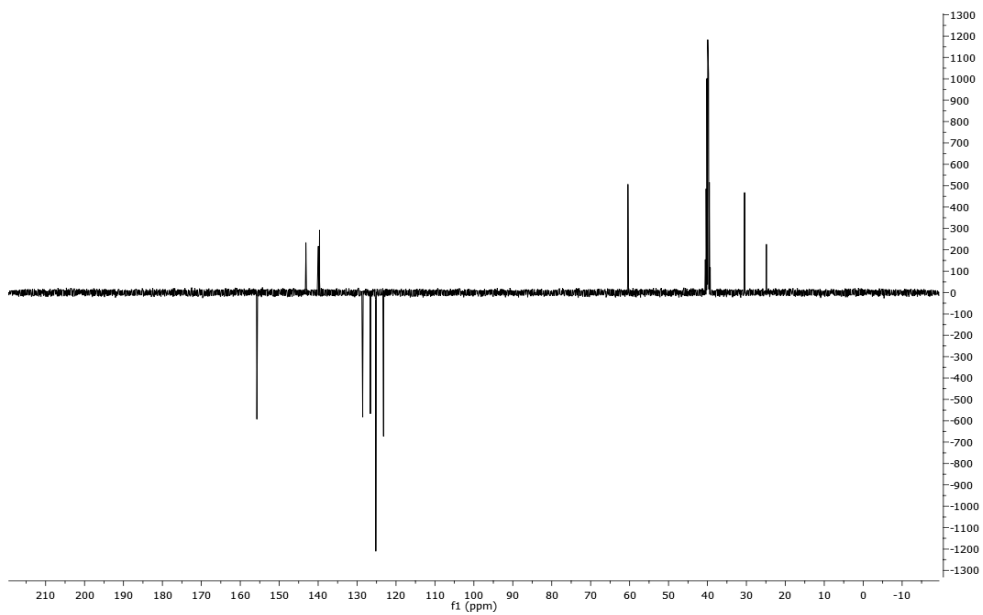
^{13}C-NMR spectrum of compound 3.
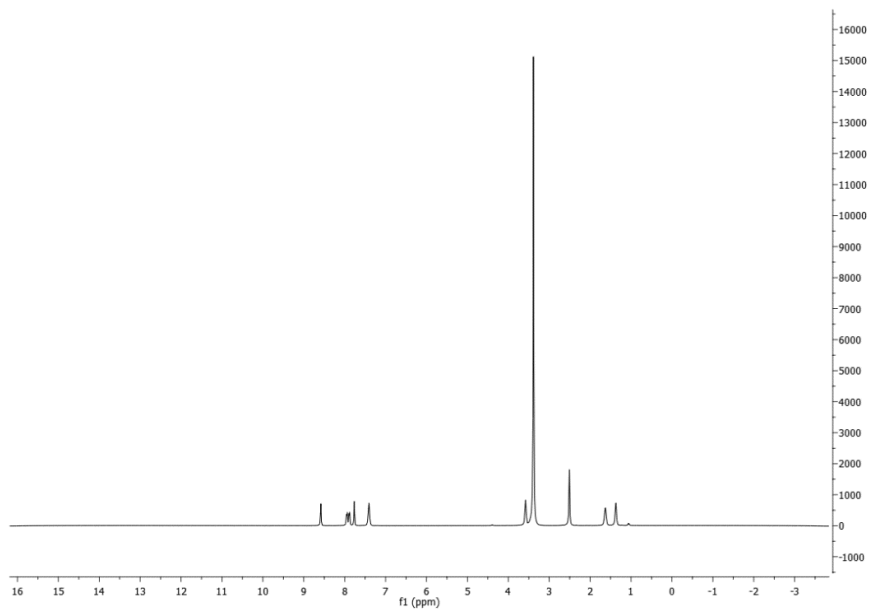
^{1}H-NMR spectrum of compound 4.
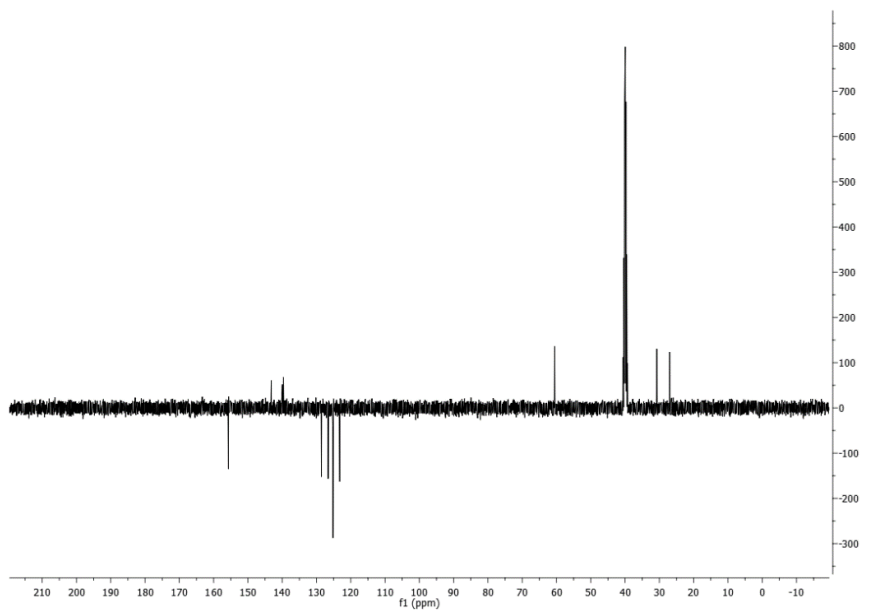
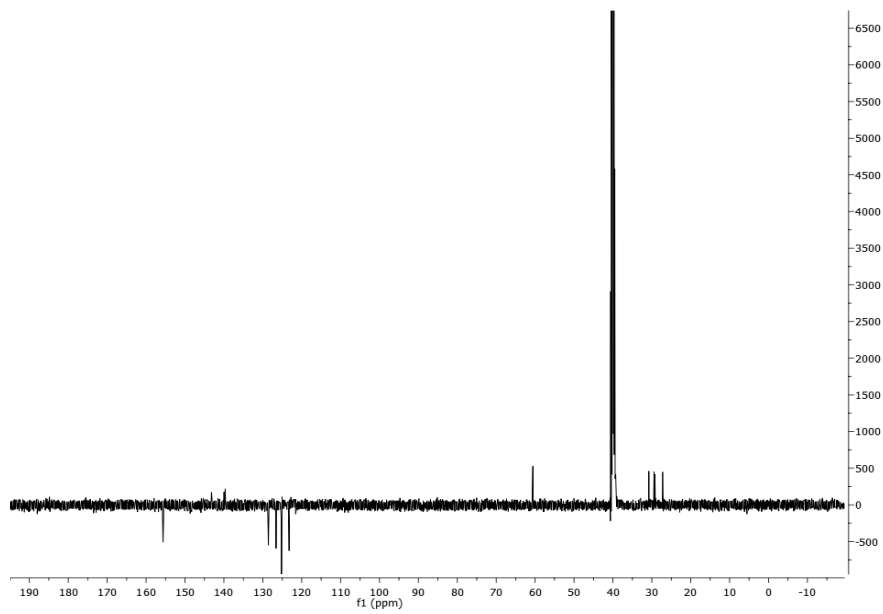^{13}C-NMR spectrum of compound 4.
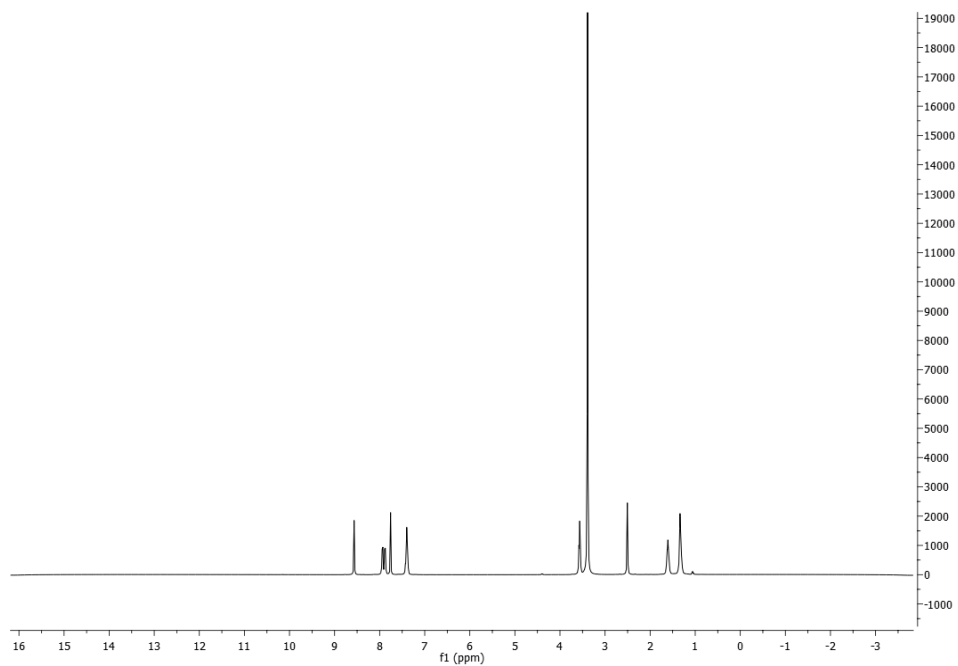
^{1}H-NMR spectrum of compound 5.
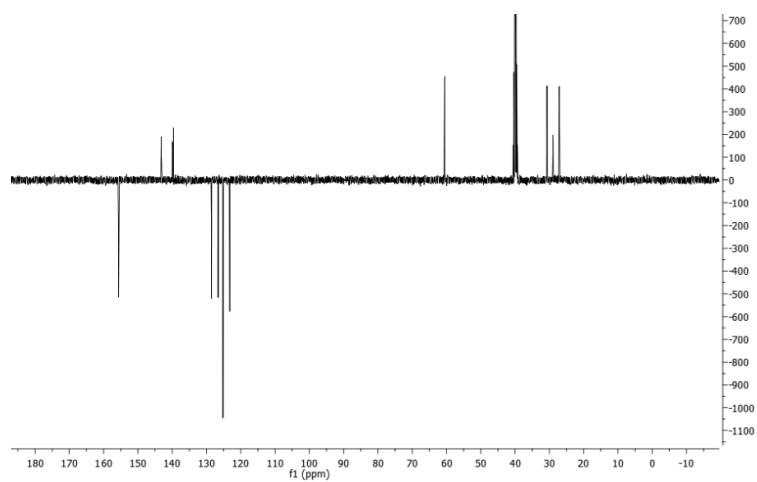
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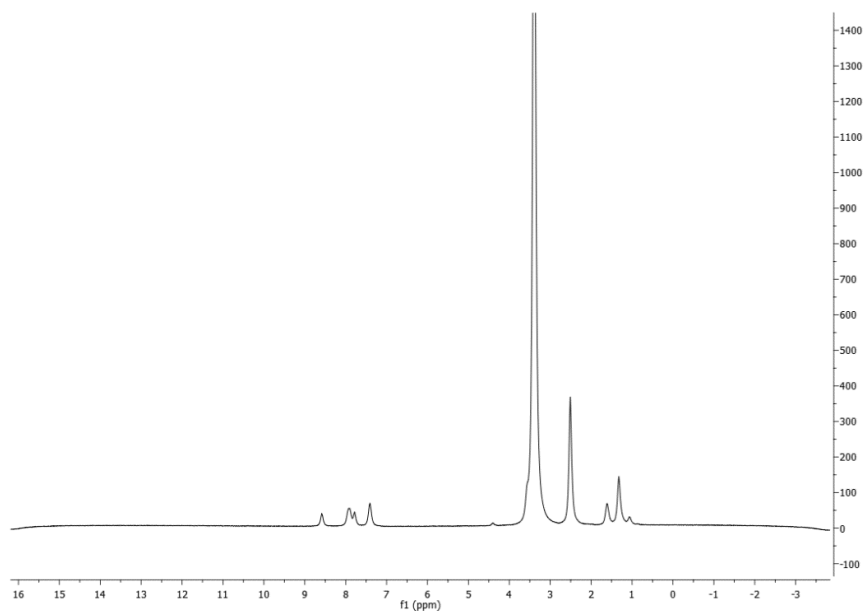
^{1}H-NMR spectrum of compound 6.
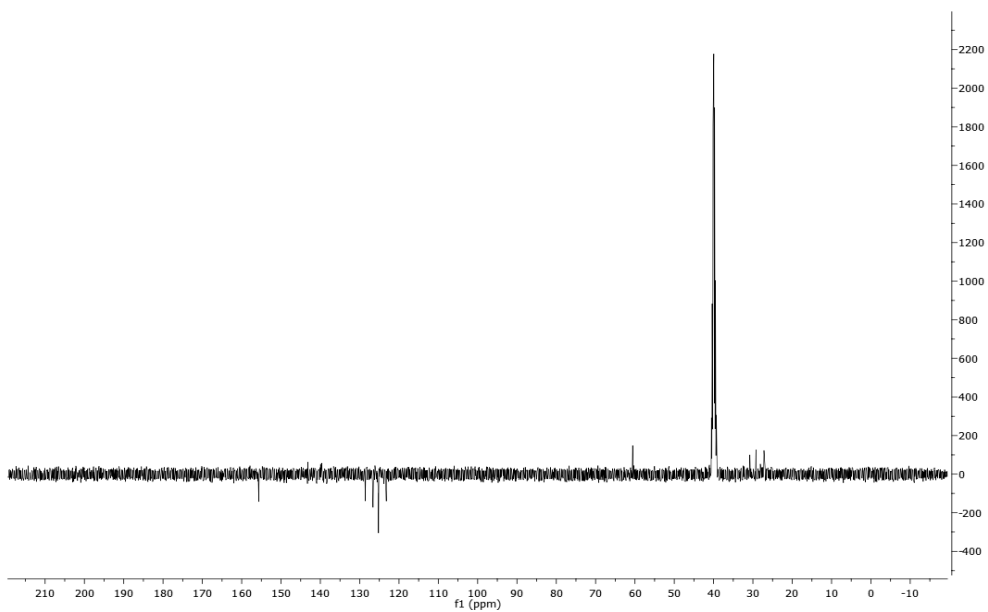
^{13}C-NMR spectrum of compound 6.
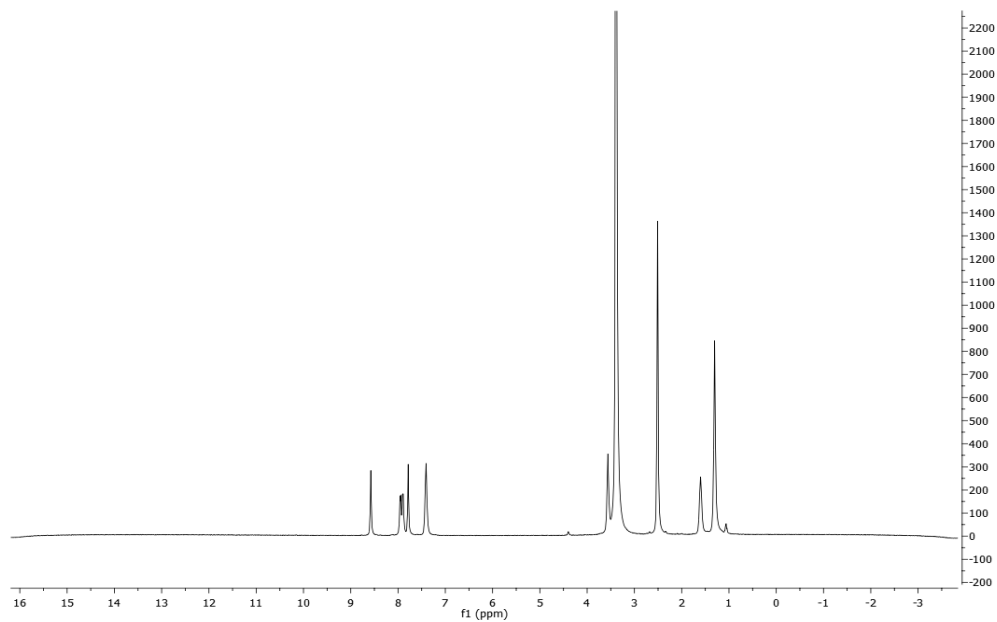
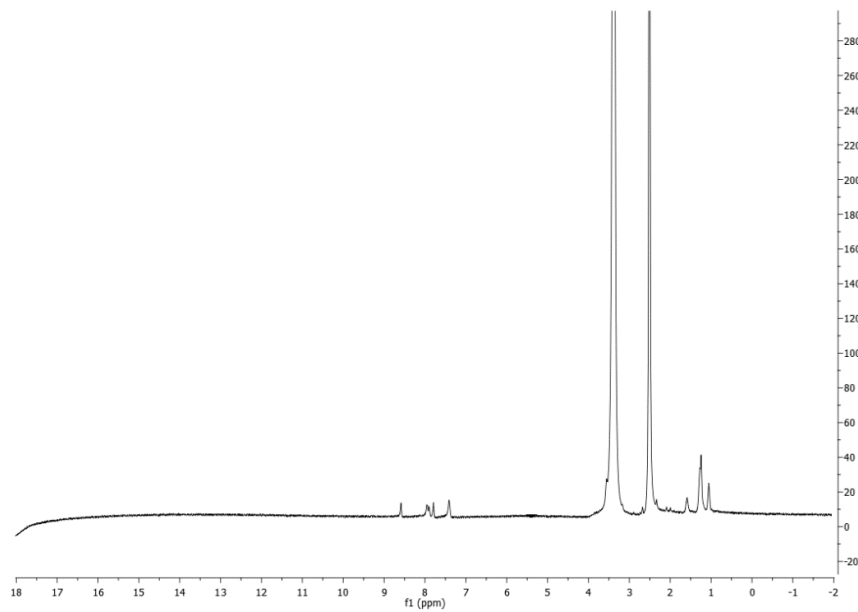^{1}H-NMR spectrum of compound 7.
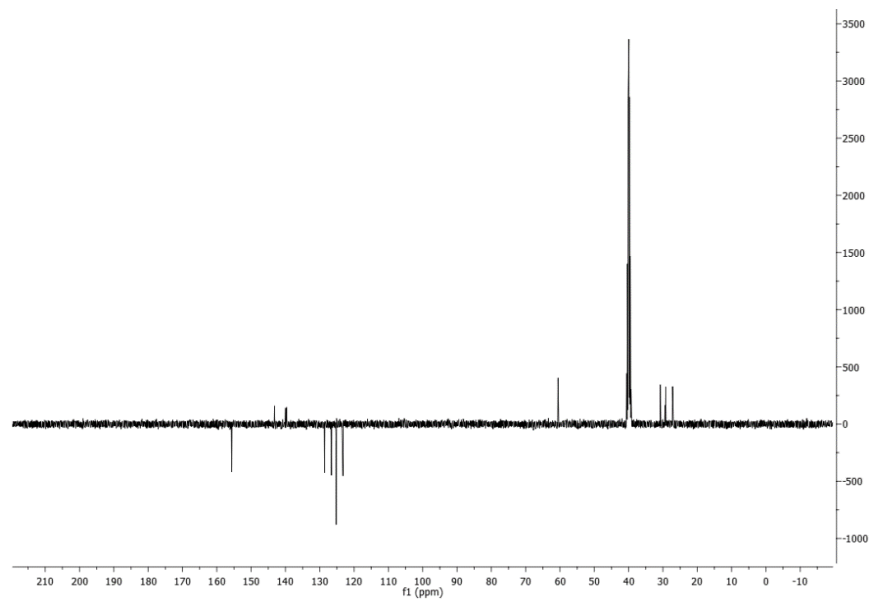
^{13}C-NMR spectrum of compound 7.

^{1}H-NMR spectrum of compound 8.

^{13}C-NMR spectrum of compound 8.

^{1}H-NMR spectrum of compound 9.

^{13}C-NMR spectrum of compound 9.