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**DESENHO RACIONAL DE FÁRMACOS AUXILIADO POR COMPUTADOR DE NOVOS
DERIVADOS 2-AMINO-TIOFENO COMO AGENTE ANTI-*LEISHMANIA* E ANTIFÚNGICO**

JOÃO PESSOA - PB

2023

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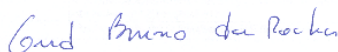
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“Pois dele, por ele e para ele são todas as
coisas. A ele seja a glória para sempre!
Amém.”

Romanos 11.36

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RESUMO

O desenvolvimento de fármacos é um processo desafiador, caro e demorado, embora esse processo tenha sido acelerado devido ao desenvolvimento de ferramentas e metodologias computacionais. A química medicinal possui papel central em vários processos que visam à identificação de substâncias bioativas e ao desenvolvimento de compostos-líderes com propriedades farmacodinâmicas e farmacocinéticas otimizadas. Portanto, este estudo tem como objetivo realizar estudos planejamento de fármacos auxiliados por computador (CADD) e sintetizar moléculas promissoras com atividade biológica para as leishmanioses e micoses. No primeiro capítulo, uma série de derivados de 2-amino-tiofeno (2-AT) foi triada virtualmente usando ferramentas QSAR, filtros ADMET e modelos de predição, permitindo a síntese direta de compostos, que foram avaliados *in vitro* contra as formas promastigotas e amastigotas axênicas de *Leishmania amazonensis*. Todos os compostos foram devidamente sintetizados e 8 deles demonstraram ser ativos frente a pelo menos uma das formas evolutivas do parasita apresentando valores de IC₅₀ inferiores a 10 µM. Compostos **8CN** e **DCN-83**, respectivamente foram os mais ativos frente as formas promastigotas e amastigotas, com valores de IC₅₀ iguais a 1,20 e 0,71 µM. Como resultado, esses achados demonstram que a técnica de triagem virtual baseada em ligantes (LBDD) provou ser bastante eficaz e economizou tempo, esforço e dinheiro na seleção de potenciais agentes anti-*Leishmania*. No segundo capítulo, 17 novos derivados de 2-AT foram projetadas e sintetizadas usando simplificação molecular através de procedimentos baseados nos princípios da química verde para melhorar a lipofilicidade dessas moléculas frente a doenças causadas por fungos dermatófitos. Através do uso de síntese mediada por microondas, os compostos foram obtidos em rendimentos variando de 35-85%. Os compostos apresentaram boa druglikeness e perfil farmacocinético, além de não apresentarem citotoxicidade em concentrações inferiores a 100µM. Os compostos com melhor perfil antifúngico foram **4e**, **4f**, **4g**, **4k**, **4l**, **4m**, **4o** e **4p** as quais apresentaram valores de MIC nas concentrações de 16 - 64 µg.mL⁻¹, para mais de uma cepa fúngica, sendo essa atividade superior à droga padrão fluconazole, em alguns casos. Com resultado, a estratégia de planejamento baseado na estrutura do ligante, as relações estrutura-atividade (SAR) e avaliação citotóxica dos compostos mais ativos foram um sucesso, melhorando o perfil farmacocinético e solubilidade em água, desenvolvendo a partir dos derivados 2-AT novas drogas promissoras antifúngicas.

Palavras-chave: Derivados 2-amino-tiofeno; Planejamento de Fármacos Auxiliado por Computador; Triagem Virtual; Leishmanioses; Infecções fúngicas.

SUMMARY

Drug development is a challenging, expensive, and time-consuming process, although this process has been accelerated due to the development of computational tools and methodologies. Medicinal chemistry plays a central role in several processes aimed at identifying bioactive substances and developing lead compounds with optimized pharmacodynamic and pharmacokinetic properties. Therefore, this study aims to perform computer-aided drug design (CADD) studies and synthesize promising molecules with biological activity for leishmaniasis and fungal diseases. In the first chapter, a series of 2-amino-thiophene (2-AT) derivatives were virtually screened using QSAR tools, ADMET filters and prediction models, allowing the direct synthesis of compounds, which were evaluated in vitro against promastigotes and axenic amastigotes of *Leishmania amazonensis*. All compounds were properly synthesized and 8 of them proved to be active against at least one of the evolutionary forms of the parasite with IC_{50} values lower than 10 μ M. Compounds **8CN** and **DCN-83**, respectively, are the most active against promastigote and amastigote forms, with IC_{50} values of 1.20 and 0.71 μ M. As a result, these findings demonstrate that the ligand-based virtual screening (LBDD) technique proved to be quite effective and saved time, effort, and money in the selection of potential anti-*Leishmania* agents. In the second chapter, 17 new 2-AT derivatives were designed and synthesized using molecular simplification through green chemistry procedures to improve the lipophilicity of these molecules for use against dermatophyte fungi diseases. Using microwave assisted synthesis compounds were obtained in good yields (35-85 %). Compounds 4a-q presented good druglikeness and pharmacokinetic profiles and no cytotoxicity up to 100 μ M. The compounds presenting the best antifungal profiles were **4e**, **4f**, **4g**, **4k**, **4l**, **4m**, **4o** and **4p** with MIC values between 16 - 64 μ g.mL⁻¹, against more than one fungi specie, being in some cases better than the reference drug fluconazole. As a result, the planning strategy based on the structure of the ligand, the structure-activity relationships (SAR) and cytotoxic evaluation of the most active compounds was a success, improving the pharmacokinetic profile and solubility in water, developing from the 2-AT derivatives new promising antifungal drugs.

Keywords: 2-amino-thiophene derivatives; Computer-Aided Drug Design; Virtual Screening; Leishmaniasis; Mycosis.

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INTRODUÇÃO



O planejamento e desenvolvimento de um novo medicamento é um processo longo, caro e depende de muitas etapas para sua regulamentação. Cada fármaco em desenvolvimento deve ter sua eficácia comprovada em todos os grupos raciais, em diferentes faixas etárias e situações fisiopatológicas, além de ter sua segurança comprovada em cada etapa do processo.^{1,2}

Os primeiros passos para a criação do fármaco ocorrem na etapa de pesquisa, na qual grandes empresas farmacêuticas trabalham muitas vezes em conjunto com universidades e centros de pesquisa. O trabalho consiste inicialmente em achar uma molécula promissora (*lead*), que seja candidata a se tornar uma nova droga, e realizar os primeiros testes e otimização química visando a sua eficácia e segurança.

Nas próximas etapas, o fármaco passa por testes não clínicos laboratoriais (*in vitro*) e em animais (*in vivo*) com objetivo de determinar sua segurança para ser testado em humanos. A última etapa consiste nos testes clínicos, na qual o fármaco é testado em diferentes grupos de pessoas. O resultado da pesquisa é apresentado aos órgãos regulatórios e a comitês de ética em pesquisa, que aprovam ser comercializado.

O tempo para o desenvolvimento de um medicamento é variado, se o objetivo tiver sido bem traçado, em aproximadamente 1 ano são selecionados os compostos *lead* com atividade biológica e leva mais 1 a 3 anos para otimizá-los. O uso dos modelos animais, as avaliações de segurança e eficácia em ensaios clínicos podem levar em média mais 5 anos. Após essas etapas, são necessários entre 1 e 2 anos para obter aprovação regulatória e efetuar o lançamento no mercado, totalizando entre 11 e 16 anos no desenvolvimento total até lançamento do fármaco no mercado.³

Portanto, o lançamento de um medicamento inédito é um processo longo e possui muitas etapas complexas. Atualmente o investimento estimado para o desenvolvimento de um novo medicamento encontra-se entre 2 e 3 bilhões de dólares distribuídos num período de quase 15 anos.⁴

Diante desse quadro, a Química Medicinal tem utilizado do planejamento de fármacos auxiliados por computador (*Computer-Aided Drug Design*, CADD) fazendo uso de métodos computacionais para obter informações úteis de banco de dados químicos e biológicos para a descoberta de novos compostos com atividade ou propriedades desejadas. Este método permite diminuir consideravelmente o número de compostos sintetizados e avaliados biologicamente, consequentemente, reduzir o tempo e o custo de todo o processo de desenvolvimento de novos candidatos a fármacos.^{1, 5, 6}

Entre as áreas de estudo diretamente relacionadas ao planejamento de fármacos, o estudo das relações quantitativas entre estrutura química e atividade biológica

(*Quantitative Structure-Activity Relationships*, QSAR) busca a obtenção de modelos que permitam a previsão de propriedades relacionadas aos fármacos, com uso de descritores frequentemente calculados por métodos de modelagem molecular. O QSAR busca determinar a relação entre estrutura e atividade biológica, geralmente *in vitro*, mesmo que não se conheça o alvo; é representada por variáveis que descrevem alterações estruturais (descritores moleculares), com sua respectiva influência sobre a atividade biológica e por modelos matemáticos relacionando à características estruturais mais importantes de um composto.

Pensando em fármacos que têm atraído grande atenção pela comunidade científica nos últimos anos, os compostos 2-aminotiofenos (2-AT) possuem uma ampla gama de atividades biológicas descritas, como agente antibacterianos, antifúngicos, antiamebas, antitumorais, antioxidantes, anticoagulantes, antitrombóticos e anti-Leishmania.⁷⁻¹⁰

Portanto, usaremos metodologias *in silico* do planejamento de fármacos baseados na estrutura de ligantes conhecidos (*Ligand-Based Drug Design*, LBDD), na qual pequenas moléculas, como os derivados 2-AT, podem ser definidos com o auxílio de técnicas envolvendo, como por exemplo, a mecânica molecular, dinâmica molecular, mecânica quântica e análise conformacional; enquanto propriedades estruturais e físico-químicas de compostos ativos e inativos, além de relações estrutura-atividade, podem ser utilizadas para caracterizar topograficamente as enzimas, em um procedimento conhecido como mapeamento de biorreceptores ou identificação de farmacóforos.¹¹⁻¹⁴

Neste contexto, o presente trabalho apresenta estudos computacionais baseados na estrutura do ligante, e aprendizado de máquina, com o objetivo de selecionar moléculas derivadas de 2-aminotiofenos com possível potencial atividade leishmanicida e antifúngica.

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CAPÍTULO 1



A leishmaniose é uma doença negligenciada causada por um grupo de protozoários parasitas do gênero *Leishmania* cujo tratamento é limitado, obsoleto, tóxico e ineficaz em certos casos. Essas características motivam pesquisadores em todo o mundo a planejar novas alternativas terapêuticas para o tratamento da leishmaniose, o uso de ferramentas quimioinformáticas aplicadas ao design de fármacos permitiu que a pesquisa fizesse grandes avanços na busca de novos candidatos a fármacos.

Neste estudo, uma série de derivados de 2-amino-tiofeno (2-AT) foi triada virtualmente usando ferramentas QSAR, filtros ADMET e modelos de predição, permitindo a síntese direta de compostos, que foram avaliados *in vitro* contra as formas promastigotas e amastigotas axênicas de *Leishmania amazonensis*.

A combinação de diferentes descritores e métodos de aprendizado de máquina levou à obtenção de modelos QSAR robustos e preditivos, com taxas de classificação corretas variando de 0,53 (para amastigotas) a 0,91 (para promastigotas), permitindo selecionar onze derivados 2-AT, com boas probabilidades de $\leq 70\%$ de potencial atividade contra as duas formas evolutivas do parasita, que não violam a regra de Lipinski e exibiram um bom perfil *druglikeness*

Todos os compostos foram devidamente sintetizados e 8 deles demonstraram ser ativos contra pelo menos uma das formas evolutivas do parasita com valores de IC_{50} menores que 10 μM .

Os compostos **8CN** e **DCN-83**, são os mais ativos contra as formas promastigota e amastigota, com valores de IC_{50} de 1,20 e 0,71 μM , respectivamente. Em conjunto, esses achados demonstram que o uso de triagem virtual baseada em ligantes provou ser bastante eficaz e economizou tempo, esforço e dinheiro na seleção de potenciais agentes anti-*Leishmania*, e confirmam, mais uma vez, que os derivados de 2-AT são promissores compostos para o desenvolvimento de novos agentes anti-*Leishmania*.

COMPUTER-AIDED DRUG DESIGN OF NEW 2-AMINO-THIOPHENE DERIVATIVES AS ANTI-LEISHMANIAL AGENTS

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Abstract: The leishmaniasis is a neglected disease caused by a group of protozoan parasites from the genus *Leishmania* whose treatment is limited, obsolete, toxic, and ineffective in certain cases. These characteristics motivate researchers worldwide to plan new therapeutic alternatives for the treatment of leishmaniasis, where the use of cheminformatics tools applied to computer-assisted drug design has allowed research to make great advances in the search for new drugs candidates. In this study, a series of 2-amino-thiophene (2-AT) derivatives was screened virtually using QSAR tools, ADMET filters and prediction models, allowing direct the synthesis of compounds, which were evaluated *in vitro* against promastigotes and axenic amastigotes of *Leishmania amazonensis*. The combination of different descriptors and machine learning methods led to obtaining robust and predictive QSAR models, with correct classification rates ranging from 0.53 (for amastigotes) to 0.91 (for promastigotes), allowing to select eleven 2-AT derivatives, which do not violate Lipinski's rules, exhibit good druglikeness, and with probability $\leq 70\%$ of potential activity against the two evolutionary forms of the parasite. All compounds were properly synthesized and 8 of them were shown to be active at least against one of the evolutionary forms of the parasite with IC₅₀ values lower than 10 μM . Compounds **8CN** and **DCN-83**, respectively, are the most active against promastigote and amastigote forms, with IC₅₀ values of 1.20 and 0.71 μM . Taken together, these findings demonstrate that the use of ligand-based virtual screening proved to be quite effective and saved time, effort, and money in the selection of potential anti-leishmanial agents, and confirm, once again that 2-AT derivatives are promising hit compounds for the development of new anti-leishmanial agents.

Keywords: 2-amino-thiophene, Computer-Aided Drug Design Virtual Screening, Anti-leishmanial, Neglected Diseases.

1. Introduction

Leishmaniasis is a neglected disease that affects more than 12 million people in 98 different countries. It is caused by a group of protozoan parasites from the genus *Leishmania*, which are known over 20 infectious species that transmit the disease by the bite of female sandflies belonging to the genus *Lutzomyia* and *Phlebotomus*.^[1-3]

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis, and is typically characterized by localized, diffuse, or disseminated skin lesion.^[4] In the old world (southern Europe, the Middle East, southwest Asia, and Africa), approximately 20 different *Leishmania* species are responsible for the transmission of CL, including *Leishmania tropica*, *L. major*, *L. aethiopica*, *L. infantum*, and *L. donovani*, being the *L. major* the most common.^[5] In the new world (from the southern United States through Latin America to South America), *L. mexicana*, *L. venezuelensis*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. guyanensis*, and *L. peruviana* are the main species that cause CL.^[6]

The present study is focused on the species of *Leishmania amazonensis* described for the first time in 1972 by Ralph Lainson and Jeffrey Shawn, belonging to the *L. mexicana* complex.^[7] This species has been described as an important etiologic agent of cutaneous leishmaniasis in humans, presenting a wide range of clinical manifestations in infections.^[8]

Currently, chemotherapy treatments for CL are limited, there is no human vaccine available and therapeutic options are restricted to 3-4 drugs, which presents toxic side effects, which require long periods of treatment and with questioned effectiveness due to the emergence of some cases of resistance.^[9]

In this context, the need to research new therapeutic alternatives that can be used as better options for the treatment of leishmaniasis is evident. Our research group has been searching for drug candidates for leishmaniasis for more than a decade, and the 2-amino-thiophene (2-AT) scaffold has been shown to be one of the most promising.

In the studies by Rodrigues et al,^[10] and Felix et al.^[11] several 2-AT were identified with low cytotoxicity for mammalian cells, and with *in vitro* antileishmania activity, against the two evolutionary forms of *Leishmania amazonensis* (amastigotes and promastigotes) with IC₅₀ values lower than 5 μ M. Years later, Rodrigues et al,^[10] showed the orally *in vivo* activity of one of these derivatives (SB83), with activity

superior to the reference drugs (Figure 1).

More recently, and using Computer-Aided Drug Design (CADD) techniques, Félix et al.^[12] through the construction of prediction models, predicted the anti-leishmanial activity of six 2-AT, of which one-third showed very promising anti-leishmanial activities showing IC₅₀ values in the range of 2 μ M (against promastigotes) and 1 μ M (against amastigotes).

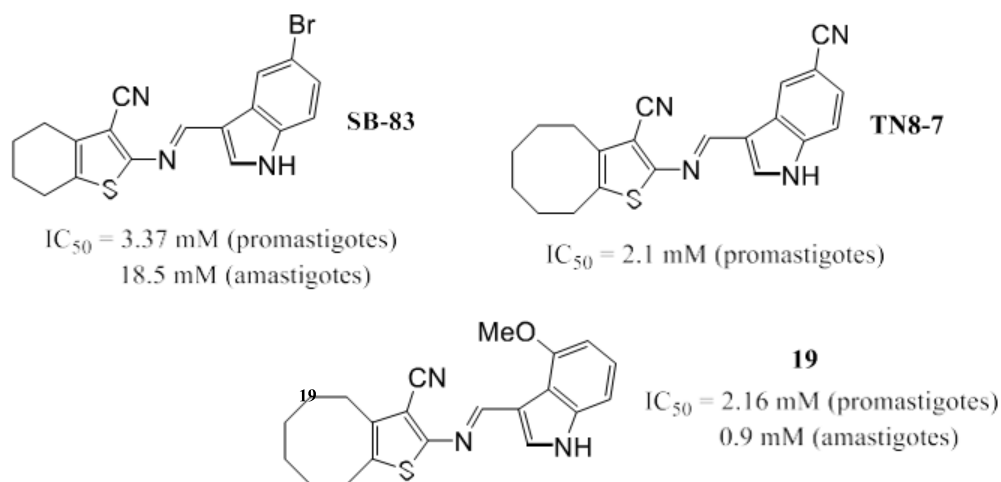


Figure 1. 2-Amino-thiophenes as potential anti-leishmanial agents.

In view of these considerations, and considering our experience, associated with the advantages offered by computational tools for the rational drug design process.^[13-15] In the present study, we decided to carry out a virtual screening to select 2-AT derivatives with potential anti-*Leishmania amazonensis* activity, against the two evolutionary forms of the parasite, thus allowing to direct the synthesis steps and increase the chances of success in the search for new 2-AT with anti-leishmanial activity.

2. Results and Discussion

2.1 Virtual Screening

The dataset of 1,862 compounds was analyzed to find a chemical pattern between the chemical compound and anti-leishmanial activity, including forms activities: amastigote and promastigote. Among them, 1,506 had some of the analyzed activities with median inhibitory concentrations (IC₅₀) in *in vitro* cultures. The compounds remaining (176) had no anti-leishmania activity or activity had not been reported.

The MuDRA managed to find a chemical pattern between the chemical composition of 2-AT and anti-leishmanial activity. In parallel, we chose to check if this

chemical pattern is also found by using a supervised algorithm, known as Random Forest (RF). The RF model was generated using the 5-fold external cross-validation technique;^[16-18] this means that the entire data set is partitioned five times into a modeling set (training set) including 80% of the compounds the set, and the external cross validation data set, comprising the remaining 20% of the compounds the data set. After this, only the modeling set is used to build the models and then the models are validated with the external cross validation technique. Its performance was evaluated through statistics such as specificity, sensitivity, which obtained satisfactory values that corroborate the accuracy of the superior model, at 70%. The performances can be observed in Table 1, these parameters are an average between the five models. During the creation of the model, we also observed the domain of applicability to ensure that the samples tested were within the chemical space of each model.

Table 1 Statistical characteristics of binary QSAR models for anti-leishmania by 5-fold cross-validation generated with MuDRA algorithm.

Evolutionary Leishmania form	Sensitivity	Specificity	CCR	PPV	NPV	Coverage
Amastigote	0.47	0.60	0.53	0.90	0.89	1
Promastigote	0.96	0.85	0.91	0.93	0.92	1

CCR: correct classification rate; PPV: positive predictive value; NPV: negative predictive value.

The combination of different descriptors and machine learning methods have led to obtain robust and predictive QSAR (Quantitative Structure-Activity Relationship) models (Figure 2), with correct classification rate (CCR) ranging from 0.53 to amastigote forms and 0.91 to promastigote forms, showing statistically significant values for classification of 2-AT compounds with high accuracy.

We see that the hit rate for true positive rate (PPV) and true negative rate (NPV) both in training sets and in test sets were higher than 0.7, showing that the SOM model is robust. Model accuracy assessment gives information about the overall performance of the model, indicating the overall hit rate. The hit rate is the rate that evaluates how well the model correctly classified the PPV and NPV. Accuracy values vary between 0 and 1. Models with accuracy rate closer to 1 represent the higher model's hit rate; while an accuracy rate equal to or greater than 0.7 is considered models of optimal performance.^[19]

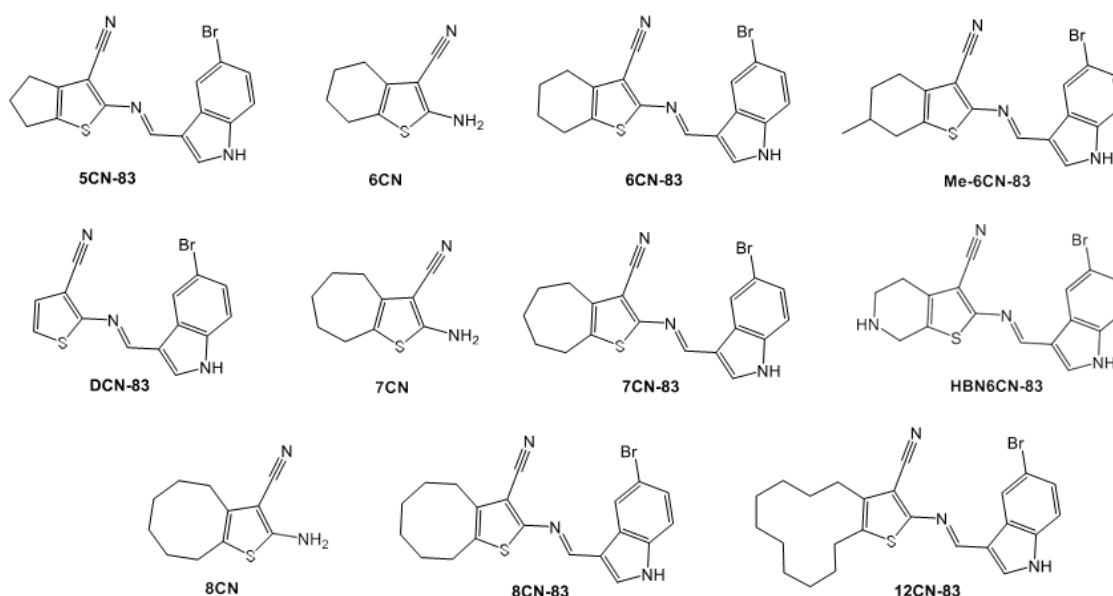


Figure 2. Chemical structures of the molecules selected for MuDRA model.

Obtaining high PPV values is crucial for indicating the ability of models to accurately predict compounds. For anti-leishmania, we can see that PPV values ranged from 0.90 for amastigote and 0.93 promastigote forms. Overall, PPV values were above acceptable thresholds and reached high values (0.9), meaning that a prediction made by the two best models generated in this study for anti-leishmania activity would be correct with more than 91% certainty. Likewise, high NPV values are equally important as they provide the certainty of the prediction made by the model regarding the non-active ones for the two forms of *Leishmania*. NPV values for the models built in our study ranged from 0.89 for amastigote and 0.92 promastigote forms. This shows that negative predictions made using our best models have at least 90% certainty.

After verification of the MuDRA model, virtual screening selected 11 of the 27 proposed 2-AT molecules with probability greater than or equal to 70% of potential activity against both the amastigote and promastigote forms. These compounds were then screened through ADMET (absorption, distribution, metabolism, excretion, and toxicity) and pharmacokinetics studies.

2.2 ADMET and pharmacokinetics studies

The results of ADMET and pharmacokinetics of the 2-aminothiophenes derivatives are depicted in Tables 2 and 3.

None of the compounds designed violate greater than two of the acceptable thresholds established by Lipinski's rule of five filters for small molecules.

Accordingly, they were expected to be permeable across the cell membrane, easily absorbed, transported, and diffused.^[20]

The organic synthesis of new chemotherapeutic compounds against neglected diseases, including leishmaniasis, may be hindered when the methods used are complex, time-consuming, expensive and have low possibility of large-scale production. In a previous work we used click chemistry methodology to efficiently synthesize 2-aminothiophene (2-AT) derivative, was selected for the analysis of its anti-leishmanial potential on the etiological agents.

Table 2. Predicted drug-likeness properties of the designed compounds in the pkCSM software.

ID	MW	TPSA	WLogP	HBA	HBD	RO5
5CN-83	304.46	256.48	4.56	3	1	0
6CN	206.27	160.16	2.07	3	1	0
6CN-83	164.23	127.21	1.69	2	1	0
7CN	178.26	140.97	2.03	2	1	0
7CN-83	267.35	205.98	2.90	4	1	0
8CN	192.29	154.73	2.37	2	1	0
8CN-83	281.38	219.74	3.24	4	1	0
12CN-83	248.35	201.44	3.61	3	1	0
DCN-83	166.20	132.38	1.17	3	1	0
HBN6CN83	221.28	171.62	0.72	4	2	0
Me-6CN-83	398.32	152.72	2.68	3	1	0

MW: molecular weight; TPSA: topological polar surface area; WLogP: partition coefficient value; HBA: hydrogen bond acceptors, HBD: hydrogen bond donors, RO5: rule of five.

The results of ADMET studies of the designed compounds illustrates that they have the potential of crossing blood brain barrier and central nervous system since for a molecule, blood–brain barrier (LogBB), and the penetrability of central nervous system (LogPS) approved values are > 0.23 to < 0.005 log BB and > -0.75 to < -2.89 logPS respectively. The class of super enzymes Cytochrome P450 (CYP450) have a vigorous role in drug metabolism since it is the key liver protein system responsible for phase-1 metabolism (oxidation). Up to today, only seventeen CYP families were recognized in humans, among which only (CYP1, CYP2, CYP3, and CYP4, respectively) are involved in the drug metabolism, with a CYP (1A2, 2C9, 2C19, 2D6 and 3A4, respectively) were known to be accountable for the biotransformation of at least 90% of the drugs that undertake phase-I metabolism^[21] and have been predicted and presented in Table 3. Clearance designates the correlation between the body’s drug levels to its elimination per unit time. A minimal value of the total clearance suggests good perseverance of the drugs in the human body,^[22] and all the designed compounds displayed a promising persistence in the body for the drug. Additionally, it is advisable to explore the toxicity level of the designed compounds as it plays an important part in screening of drugs candidates.

Table 3. Predicted ADMET properties of the designed compounds in the pkCSM software.

ID	Absorption Intestinal absorption	Distribution		Metabolism							Excretion Total Clearance	Toxicity	
		LogBB	LogPS	Substrate		Inhibitors						AMES toxicity	Hepato toxicity
				CYP 2D6	CYP 3A4	CYP 1A2	CYP 2C19	CYP 2C9	CYP 2D6	CYP 3A4			
5CN-83	91.53	0.11	-1.38	No	Yes	Yes	Yes	Yes	No	Yes	0.07	No	No
6CN	93.18	0.05	-1.95	No	No	Yes	No	No	No	No	-0.07	Yes	No
6CN-83	91.55	0.10	-1.30	No	Yes	Yes	Yes	Yes	No	Yes	0.02	No	No
7CN	93.956	0.11	-2.76	No	No	Yes	No	No	No	No	-0.06	No	No
7CN-83	92.312	0.21	-1.19	No	Yes	Yes	Yes	No	No	Yes	0.04	No	No
8CN	93.567	0.13	-2.74	No	No	Yes	No	No	No	No	-0.05	No	No
8CN-83	91.924	0.23	-1.10	No	Yes	Yes	Yes	Yes	No	Yes	0.05	No	No
12CN-83	88.908	0.20	-0.75	No	Yes	No	Yes	Yes	No	No	0.10	No	No
DCN-83	89.205	0.16	-1.50	No	Yes	Yes	Yes	Yes	No	No	0.23	No	No
HBN6CN-83	88.270	0.08	-1.60	No	Yes	Yes	No	No	No	No	0.92	Yes	No
Me-6CN-83	90.751	0.03	-1.22	No	Yes	Yes	Yes	Yes	No	No	0.01	Yes	No

Finally, all the designed compounds are found to be non-toxic studies. We analyzed the toxicity profile of these compounds through the DataWarrior program, a program that can perform chemical analyzes in silico through a database to generate toxicity predictions from chemical compounds that is calculated using algorithms and a bank of fragments with known toxicity.

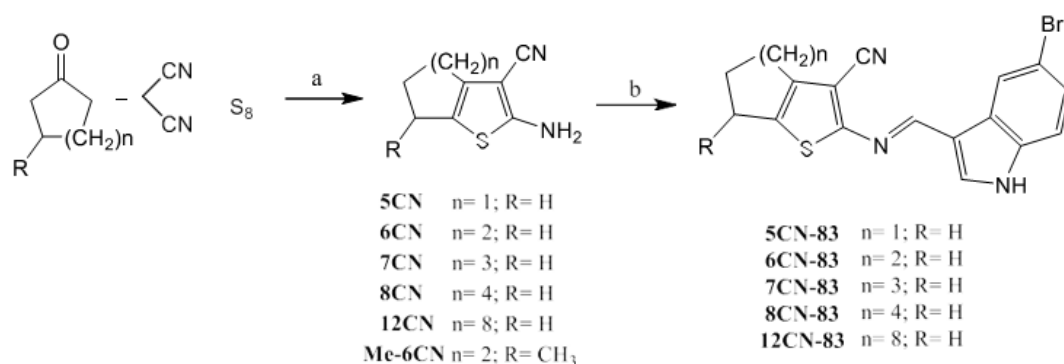
By comparing the molecules that we want to predict toxicity with the bank of fragments with known toxicity, DataWarrior will predict their respective toxicity profiles. We thus evaluated the toxicity profile for mutagenicity, tumorigenicity, reproductive toxicity and skin irritation. The predictions are classified into no risk, low risk, medium risk, and high risk. The result of the analysis showed that all tested molecules were considered without risk, that is, non-toxic by the evaluated parameters.

2.3 Synthesis

For the synthesis of the selected compounds, several synthetic methodologies were used, starting with one of the versions of the Gewald reaction following previous procedures described by our group, and depicted in the schemes below.

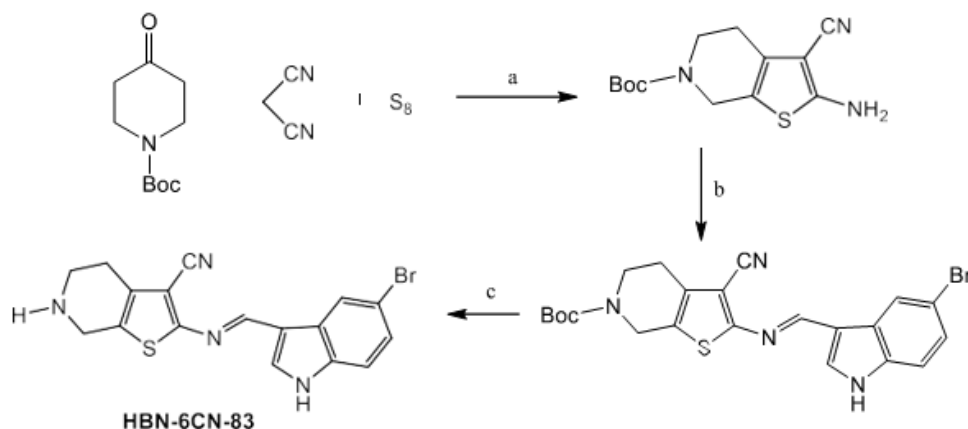
Compounds **5CN-83**, **6CN-83**, **Me-6CN-83**, **7CN-83**, **8CN-83**

and **12CN-83** were synthesized in two step synthesis. The first step was the first version of the Gewald reaction^[23-25] where the Gewaldadducts were obtained (**5CN**, **6CN**, **Me-6CN**, **7CN**, **8CN** and **12CN**). The target compounds were synthesized reacting these adducts with 5-bromo-indol-3-carboxaldehyde according to procedure described in Rodrigues et al.^[10, 26]



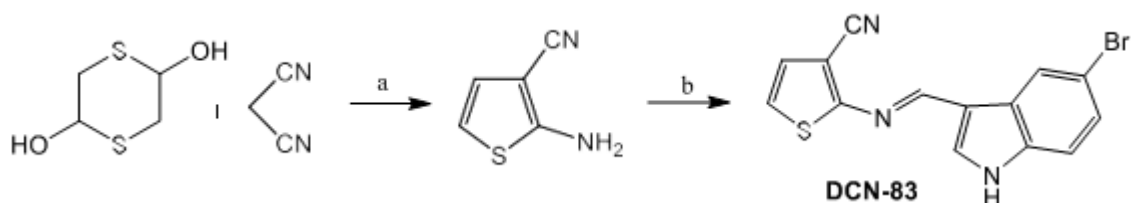
Scheme 1. Synthetic route for obtaining 2-aminothiophene derivatives (**5CN**, **6CN**, **Me-6CN**, **7CN**, **8CN**, **12CN**, **5CN-83**, **6CN-83**, **Me-6CN-83**, **7CN-83**, **8CN-83** and **12CN-83**): (a) morpholine, ethanol, reflux, 2-3 h; (b) 5-bromo-indol-3-carboxaldehyde, ethanol, acetic acid, reflux, 4 h.

HBN-6CN-83 was synthesized in three steps, starting from the first version of the Gewald reaction using as cyclic ketone, the 4- Boc-piperidone. Gewald adduct was condensed with 5-bromo-indol-3-carboxaldehyde as describe in Rodrigues et al.^[10, 26] and Boc deprotection was performed as previously described in da Cruz et al.^[27] using a mixture of dichloromethane and trifluoroacetic acid (8:2), at 0-5°C for 1h.



Scheme 2. Synthetic route for obtaining **HBN-6CN-83**: (a) morpholine, ethanol, reflux, 3 h; (b) 5-bromo-indol-3-carboxaldehyde, ethanol, acetic acid, reflux, 4 h; (c) CH_2Cl_2/TFA (8:2), 0-5°C, 1h.

DCN-83 was synthesized through the fourth version of the Gewald reaction. The Gewald adduct was obtained reacting malononitrile with 1,4-dithiane-2,5-diol in ethanol, using morpholine as base.^[23,24] The target compound (**DCN-83**), was synthesized by condensation of 5-bromo-indol-3-carboxaldehyde with the Gewald adduct, as described in Luna et al.^[28]



Scheme 3. Synthetic route for obtaining **DCN-83**: (a) morpholine, ethanol, reflux, 2-3 h; (b) 5-bromo-indol-3-carboxaldehyde, ethanol, acetic acid, reflux, 4 h.

All compounds were properly obtained. Compounds **6CN**, **7CN**, **6CN-83**, **7CN-83** and **8CN** were resynthesized according previously described in literature.^[10, 26]

All new compounds (**5CN-83**, **8CN-83**, **12CN-83**, **Me-6CN-83**, **HBN-6CN-83** and **DCN-83**) had their structures confirmed by Nuclear Magnetic Resonance of Carbon and Hydrogen (¹³C NMR and ¹H NMR), and by High Resolution Mass Spectra (HRMS). (spectra available in supplementary material)

As observed by Félix et al.^[12] in HRMS all compounds containing bromine atom exhibited a molecular peak $[M+2]^+$ with equivalent intensity to the molecular ion peak $[M+H]^+$ confirming the presence of the heaviest isotope of bromide atom (Br^{81}).

2.4 *In vitro* anti-leishmanial activity

The results of the anti-Leishmanial activity of the selected and synthesized 2-AT derivatives, compared with the reference drugs amphotericin B and meglumine antimoniate, against promastigote and axenic amastigote of *L. amazonensis* are presented in Table 4.

As shown in Table 4, among the eleven compounds selected and evaluated up to a maximum concentration of 10 μ M (highest concentration evaluated), eight of them presented some degree of anti-leishmanial activity, at least against one of the evolutionary forms of the parasite. All active compounds were more active than meglumine antimoniate and less active than amphotericin B against both evolutionary forms of the parasite.

Table 4. Anti-leishmanial activity of meglumine antimoniate, amphotericin B and selected 2-aminothiophene derivatives against promastigotes and axenic amastigotes of *L. amazonensis*. (CI₅₀ values in μ M).

Compounds	Anti-leishmanial Activity (CI ₅₀)	
	Amastigotes	Promastigotes
5CN-83	> 10.00	> 10.00
6CN	> 10.00	4.50 [#]
6CN-83	18.50 [*]	3.37 [*]
7CN	2.79	3.20 [#]
7CN-83	20.09 [*]	3.65 [*]
8CN	9.80	1.20
8CN-83	3.80	4.75
12CN-83	> 10.00	> 10.00
DCN-83	0.71	9.70
HBN-6CN-83	> 10.00	> 10.00
Me-6CN-83	8.72	9.90
Meglumine antimoniate	1,017.4 ^{a, *}	25,740 ^{a, *}
Amphotericin B	0.23 [*]	0.17 [*]

^aValues in mg/L.

[#] Values from Félix et al.^[11]

^{*} Values from Rodrigues et al.^[10,26]

For the Gewald adducts, compounds **6CN**, **7CN** and **8CN**, increasing the size of the cycloalkane gradually promotes an increase in anti-promastigote activity (respectively IC₅₀ = 4.50, 3.20 and 1.20 μ M). Being **8CN** the compound showing the best anti-promastigote activity in the series. The same pattern is not observed for anti-amastigote activity, in which the adduct with the best activity was the cyclohepta[*b*]thiophene (**7CN**), with IC₅₀ = 2.79 μ M.

For the cycloalka[*b*]thiophene derivatives associated with a 5- bromo-indole moiety it was observed that the size of the cycloalka ring exerts a great influence on the anti-leishmania activity against the two evolutionary forms of the parasite.

Rings smaller than 6 (like **5CN-83**) and larger than 8 carbons (like **12CN-83**) do not show activity at the evaluated concentrations. Cyclohexa[*b*]thiophene (**6CN-83** and **Me-6CN-83**), cyclohepta[*b*]thiophene (**7CN-83**) and cycloocta[*b*]thiophene (**8CN-83**) rings are active, and for anti-amastigote activity follow the same pattern observed for the Gewald adducts, with the 8- membered ring being the most active (**8CN-83**, IC₅₀ = 3.80 μ M). And they follow inverse order of activity against the promastigote form, presenting IC₅₀ values of 3.37, 3.65 and 4.75, respectively for **6CN-83**, **7CN-83** and **8CN-83**.

The bioisosteric replacement of a -CH₂- by a -NH- seems to promote a loss and/or reduction in the anti-leishmanial activity, as observed for the anti-promastigote activity of **6CN-83** and **HBN6CN-83** (decreasing IC₅₀ = 3.37 μ M to inactive > 10. μ M).

Finally, and despite the importance and influence of the presence of a cycloalka ring for anti-leishmania activity, this presence does not seem to be essential for activity, as can be observed for the **DCN-83** derivative, which presented IC_{50} less than 10.0 μM against the two forms of the parasite, still being the compound with the most pronounced anti-amastigote activity, among the evaluated compounds, presenting an IC_{50} value = 0.71 μM .

3. Conclusions

In summary, the virtual screening performed in this study using combined *in silico* approaches was able to identify eleven 2- amino-thiophene derivatives with potential activity against amastigote and promastigote forms of *L. amazonensis*. After being properly synthesized and evaluated *in vitro* in anti-leishmania activity assays, eight of them were able to inhibit the growth of at least one of the evolutionary forms of *L. amazonensis* at concentrations lower than 10 μM .

The use of ligand-based virtual screening (LBVS) proved to be quite effective and saved time, effort, and money in the selection of potential anti-leishmanial agents. Our results prove, once again, that 2-AT derivatives are promising hit compounds for the development of new anti-leishmanial agents.

4. Experimental Section

4.1 Leishmania database

The publicly available data from the ChEMBL database (<https://www.ebi.ac.uk/chembl/>)^[29] were used for created Leishmania dataset, initially composed of 1,862 records that were classified according to their predicted activity against *Leishmania amazonensis*, 356 records were removed from the database leaving 1,506 records. These records were classified according to pIC_{50} values [$-\log IC_{50}$ (mol/L)]; therefore, we stratified them into active ($pIC_{50} \geq 5.0$) and inactive ($pIC_{50} < 5.0$) structures was selected 371 compounds for amastigote and 896 compounds promastigotes forms. Data curation of the datasets was performed according to the suggested procedures in the literature.

4.2 Data curation

For all structures of the ChEMBL database the SMILES codes were generated and used as the input data for Marvin 19.27.0, 2019, ChemAxon (<http://www.chemaxon.com>)^[30] and Standardizer software [JChem 19.27.0, 2019; ChemAxon]^[30] to convert the chemical structures into curated and canonical representations. This standardization is of paramount importance to create consistent compound libraries and is done through the following steps: addition of hydrogens, aromatization, generation of 3D structure and exporting the compounds in SDF format. For a more detailed description on how the dataset was curated, please refer to the workflows described by Fourches et al.^[31-33]

4.3 MuDRA model

We used the Knime software to generate the MuDRA model. The prediction model was constructed from the bank of molecules with known anti-leishmania activity, to predict the activity of the Leishmania bank. The model was constructed according to the methodology of Alves et al.^[34] The model is called MuDRA and uses instance-based machine learning, meaning, it compares new instances with seen instances in the training set, rather than performing explicit generalizations. This method provides an alternative to the set-modeling approach, facilitating the implementation and lower computational cost.^[35,36]

We followed a 5-fold external cross-validation procedure^[35,36] using the complete datasets in the original publications, and randomly selected 20 % of the dataset as an external set using stratified sampling to standardize the analysis within this study. For MuDRA modeling, four different types of molecular descriptors are used: Morgan, Avalon, MACCS and RDkit. The MuDRA plating depends on the chemical similarity and comparisons of biological responses, where structural similarity is identified within a chemical space defined by the different types of molecular descriptors. In each chemical space, several nearest neighbors are selected based on their similarity to the compounds that have known activity, and the predicted activity is calculated based on the Tanimoto coefficient as a function of the Jaccard distance.^[37-41]

Using Knime nodes, the most important descriptors in the generation of each prediction model were evaluated. The external performances of the selected models were analyzed for sensitivity (true positive rate, i. e., active rate), specificity (true negative rate, i. e., inactive rate) and accuracy (overall predictability). The positive (PPV) and

negative (NPV) predictive values inform us about the probability of predicted positives (PPV) and negatives (NPV) being the true positives and negatives, respectively. In addition, the sensitivity and specificity of the Receiver Operating Characteristic (ROC) curves were found to describe true performance with more clarity than accuracy.

The models were also analyzed by the Matthews coefficient, a way to evaluate the model globally from the results obtained from the confusion matrix. The MCC is a correlation coefficient between observed and predictive binary classifications. It results in a value between -1 and +1, where a coefficient of +1 represents a perfect forecast, 0 is nothing more than a random forecast, and -1 indicates total disagreement between forecast and observation.^[42]

The Matthews correlation coefficient can be calculated from the following formula:

$$MCC = \frac{VP \times VN - FP \times FN}{\sqrt{(VP + FP)(VP + FN)(VN + FP)(VN + FN)}}$$

Where VP is the value of true positive, VN is the value of true negative, FP is the value of false positives and FN of false negatives.

4.4 Virtual screening

Virtual screening was performed using the predictive models created for MuDRA. The Leishmania dataset was screened in the two models, amastigote and promastigote forms, the results were analyzed through a consensual analysis between the forms.^[43,44]

MuDRA models were developed in KNIME analytics platforms version 3.1.0 according to the method described by Alves et al.^[34] using Morgan fingerprints (radius = 3 bonds and 2048 bits), RDKit whole-molecule descriptors, MACCS keys, and Avalon fingerprints. This method is a simple and reliable instance-based technique related to the popular kNN approach, albeit it uses different types of chemical descriptors during similarity assessment. MuDRA model generation included the following steps: (i) calculation of various chemical descriptors for the training set, (ii) calculation of Tanimoto similarity for the query compound against all chemicals in the training set in all descriptor spaces, and (iii) activity prediction for the query compound based on the weighted similarity to the nearest neighbors of the query in each chemical space.^[45-47]

4.5 Druglikeness and in silico pharmacokinetics ADMET prediction

The prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET)^[48] parameters of 2-AT and the reference drugs for leishmaniasis was

performed using the online pkCSM server (<http://biosig.unimelb.edu.au/pkcsml/>)^[49-51]. The physical–chemical parameters, the potential for oral bioavailability and the similarity with other compounds already described were also predicted using OSIRIS Data Explorer and DataWarrior version 5.5 software (<http://www.openmolecules.org/datawarrior/>) and compared with the descriptors obtained for the reference drugs.

4.6 Chemistry

4.6.1 General Methods

All chemicals, solvents, and reagents (reagent grade) used were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The reaction was monitored by TLC, run on silica gel-coated aluminum sheets (silica gel 60 GF254, E. Merck, Germany) and revealed by UV light (254 or 365 nm). ¹H, ¹³C Nuclear Magnetic Resonance (NMR) spectra were registered in a Bruker Avance 400 MHz NMR spectrometer in deuterated DMSO solution. The chemical shifts (δ) were reported in parts per million (ppm) downfield to tetramethylsilane (δ = 0). The coupling constant (*J*) was expressed in Hertz (Hz). The following abbreviations were reported in the following order: chemical shift, multiplicity, number of protons and coupling constant. The multiplicity of proton signals was reported as s: singlet, bs: broad singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublets, dt: doublet of triplets and m: multiplet. High Resolution Mass Spectra (HRMS) were performed using direct infusion on a micrOTOF II mass spectrometer (Bruker Daltonics, Billerica, MA, USA) containing an electrospray ion (ESI) source was used to obtain high resolution mass spectra. The parameters applied were as follows: capillary 4.5 kV, ESI in negative mode, final plate offset 500 V, 40 psi nebulizer, dry gas (N₂) with a flow rate of 8 mL/min and a temperature of 200 °C. The mass spectra (*m/z* 50–1000) were recorded every 2 s.

4.6.2 Synthesis

Compounds **6CN**, **7CN**, **8CN**, **6CN-83**, and **7CN-83** were synthesized, and their structures have been confirmed by comparison with ¹H NMR spectra data from the literature [Rodrigues et al., 2015; Félix et al., 2016].^[10,11]

Compounds **5CN-83**, **8CN-83**, **12CN-83** and **Me-6CN-83** were synthesized as previously described in [Rodrigues et al., 2015; Félix et al., 2016].^[10,11]

2-(((5-bromo-1H-indol-2-yl)methylene)amino)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (**5CN-83**) Yield 79.1%; ¹H NMR (DMSOd6, 500MHz): δ 2.31-2.37 (m, 2H, CH₂), 2.75-2.78 (m, 2H, CH₂), 2.85-2.88 (m, 2H, CH₂), 7.39 (dd, 1H, *J* = 8.5, 2.0 Hz, CH), 7.47 (d, 1H, *J* = 9.0 Hz, CH), 8.21 (d, 1H, *J* = 2.5 Hz, CH), 8.56 (d, 1H, *J* = 1.5 Hz, CH), 8.72 (s, 1H, HC=N), 12.20 (sl, 1H, NH); ¹³C NMR (DMSOd6, 125MHz): δ 27.3, 28.1, 30.2, 99.2, 114.2, 114.9, 114.9, 115.6, 124.8, 126.5, 126.7, 135.3, 136.6, 137.8, 143.3, 154.5, 168.3; HRMS (ESI- TOF+) *m/z* calculated for C₁₇H₁₂BrN₃S [M+H]⁺ = 369.9935; found 369.9991.

2-(((5-bromo-1H-indol-2-yl)methylene)amino)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carbonitrile (**8CN-83**) Yield 87.5%; ¹H NMR (DMSOd6, 500MHz): δ 1.36-1.37 (m, 4H), 1.61 (m, 4H), 2.68-2.71 (m, 2H), 2.79-2.81 (m, 2H), 7.38 (dd, 1H, *J* = 8.5, 2.0 Hz), 7.47 (d, 1H, *J* = 9.0 Hz), 8.19 (d, 1H, *J* = 2.5 Hz), 8.56 (d, 1H, *J* = 2.0 Hz), 8.70 (s, 1H), 12.20 (sl, 1H) ¹³C NMR (DMSOd6, 125MHz): δ 25.46, 25.72, 25.75, 26.96, 30.10, 31.77, 104.63, 114.21, 114.87, 114.91, 115.54, 124.80, 126.46, 126.75, 132.76, 136.60, 137.06, 137.66, 155.57, 162.30; HRMS (ESI-TOF+) *m/z* calculated for C₂₀H₁₈BrN₃S [M+H]⁺ = 412.0405; found 412.0482.

2-(((5-bromo-1H-indol-2-yl)methylene)amino)-4,5,6,7,8,9,10,11,12,13-decahydrocyclododeca[b]thiophene-3-carbonitrile (**12CN-83**) Yield 6.6%; ¹H NMR (DMSOd6, 500MHz): δ 1.26-1.30 (m, 2H, CH₂), 1.34 (m, 6H, CH₂), 1.45-1.47 (m, 4H, CH₂), 2.43-2.67 (m, 2H, CH₂), 2.76-2.79 (m, 2H, CH₂), 7.41 (dd, 1H, *J* = 8.0, 4.0 Hz, CH), 7.50 (d, 1H, *J* = 12.0 Hz, CH), 8.23 (s, 1H, CH), 8.58 (d, 1H, *J* = 4.0 Hz, CH), 8.73 (s, 1H, HC=N), 12.25 (sl, 1H, NH). ¹³C NMR (DMSOd6, 125MHz): 22.2, 22.7, 24.0, 24.4, 24.8, 25.0, 25.4, 27.4, 29.8, 104.5, 114.2, 115.0, 115.0, 115.7, 124.8, 126.5, 126.8, 134.8, 136.3, 136.6, 138.0, 156.0, 163.3;

2-(((5-bromo-1H-indol-2-yl)methylene)amino)-6-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (**Me-6CN-83**) Yield 78.2% ¹H NMR (DMSOd6, 500MHz): δ 1.01 (d, 3H, *J* = 5.4 Hz), 1.36-1.43 (m, 1H), 1.84-1.87 (m, 2H), 2.23-2.28 (m, 1H), 2.54-2.64 (m, 2H), 2.73 (dd, 1H, *J* = 13.2, 3.8 Hz), 7.39 (dd, 1H, *J* = 6.8, 1.5 Hz), 7.47 (d, 1H, *J* = 6.8 Hz), 8.20 (d, 1H, *J* = 2.4 Hz), 8.55 (d, 1H, *J* = 1.4

Hz), 8.68 (s, 1H), 12.20 (sl, 1H)) ^{13}C NMR (DMSO d_6 , 125MHz): δ 21.51, 24.05, 29.50, 30.17, 32.97, 103.48, 114.20, 114.87, 114.93, 115.26, 124.82, 126.48, 126.74, 129.87, 133.63, 136.60, 137.75, 155.46, 162.99; HRMS (ESI-TOF+) $\text{C}_{19}\text{H}_{16}\text{BrN}_3\text{S}$ $[\text{M}+\text{H}]^+$: calcd m/z = 398.0248; found 398.0314.

Compound **HBN-6CN-83** was synthesized according previous described in [Rodrigues et al., 2015; and da Cruz et al., 2020].^[10,27]

2- [(5- bromo- 1H- indol- 3- yl)methylidene]amino]- 4,5,6,7-tetrahydro-tieno[2,3- c]pyridine- 3- carbonitrile (**HBN-6CN-83**) Yield 2.1%; ^1H NMR (DMSO d_6 , 500MHz): δ 2.56(t, 2H, J = 4.0 Hz, CH), 3.00 (t, 2H, J = 8.0 Hz, CH), 3.81 (s, 2H, CH $_2$), 7.41 (dd, 1H, J = 8.0, 4.0 Hz, CH), 7.50 (d, 1H, J = 12.0 Hz, CH), 8.24 (s, 1H, CH), 8.57 (d, 1H, J = 4.0 Hz, CH), 8.72 (s, 1H, HC=N), 12.26 (sl, 1H, NH) ^{13}C NMR (DMSO d_6 , 125MHz): δ 25.0, 42.4, 44.4, 100.0, 103.6, 114.2, 114.9, 115.0, 115.1, 124.8, 126.6, 126.7, 129.9, 133.0, 136.6, 138.0, 155.7, 163.1.

Compound **DCN-83** was synthesized as previously described in [Luna et al., 2021].^[28]

2-(((5-bromo-1H-indol-3-yl)methylene)amino)thiophene- 3-carbonitrile (**DCN-83**) Yield 67.6%; ^1H NMR (DMSO d_6 , 500MHz): ^1H NMR (400 MHz, DMSO d_6) δ 7.41 (d, 1H, J = 5.6 Hz, H-4), 7.60 (d, 1H, J = 6.0 Hz, H-5), 7.77 (d, 2H, J = 8.8 Hz, ArH), 7.94 (d, 2H, J = 8.8 Hz, ArH), 8.78 (s, 1H, CH=N); ^{13}C NMR (125 MHz, DMSO d_6) δ 105.7, 114.5, 124.0, 126.5, 127.7, 131.0, 132.1, 133.8, 161.3, 162.7.

4.7 Maintenance of parasites

Leishmania (Leishmania) amazonensis (IFLA/BR/67/PH8) was used to determine the anti-leishmanial activity. The parasites were cultured in Schneider medium, pH 7, supplemented (20% heat-inactivated fetal bovine serum and 1% penicillin 100 U/mL and streptomycin 100 $\mu\text{g/mL}$) and incubated at 26°C in a Biochemical Demand oven. Oxygen (BOD).^[10] Axenic amastigote forms were obtained from the differentiation of promastigote forms of *L. amazonensis* (IFLA/BR/67/PH8) according to Rodrigues et al.^[26], applying an increase in temperature to 32°C and a decrease in the pH of the medium to 5.5.

4.8 Anti-leishmanial activity on promastigotes and axenic amastigotes

Anti-leishmanial activity on promastigotes and axenic amastigotes was evaluated using the MTT colorimetric assay. Promastigotes or axenic amastigotes in log phase of growth were cultured in 96-well culture plates at 1×10^6 parasites per well in 100 mL of Schneider medium supplemented at different concentrations previously diluted in DMSO, starting at 10 μ M. The plates were incubated for 72 h in BOD at 26°C. Then 10 μ L of MTT was added to the plates (final concentration 5 mg/mL) and the cells were incubated for a further 4 h. At the end of the process, 50 μ L of 10% SDS were added to solubilize the formazan crystals. Absorbances were measured using an ELISA plate reader (BioSystems model ELx800, Curitiba, PR, Brazil) at 540 nm.^[26]

4.9 Statistical analysis

The different mean concentrations on promastigote forms (IC₅₀) were calculated by non-linear regression. Variance was calculated using ANOVA, followed by Tukey's test through a statistical program, taking the value of $p < 0.05$ as the maximum level of statistical significance.

Conflicts of Interest

The authors declare no conflict of interests, financial or otherwise, in this paper.

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Supplementary data

¹H NMR and ¹³C NMR spectra; HRMS spectra.

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Supporting Information

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2.1 General information

2.2 Synthetic procedures

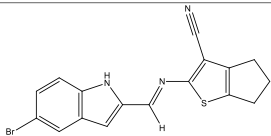
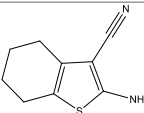
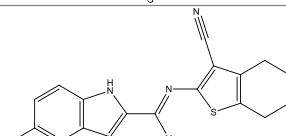
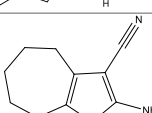
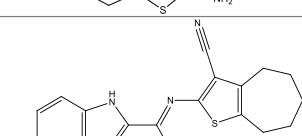
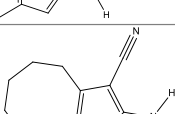
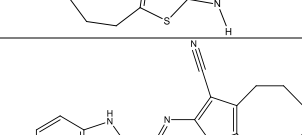
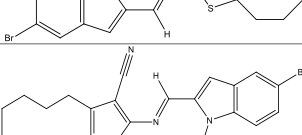
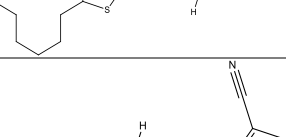
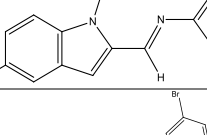
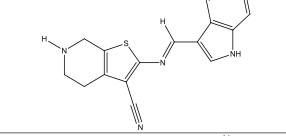
2.2.1 2-Aminothiophenes

3 References

4 Copies of NMR spectra

1 List of the studied compounds

Table S1. List of the studied 2-aminothiophene derivatives and references for their synthesis.

Compound	Structure	Synthetic procedure
5CN-83		see section 2.2
6CN		Puterová et al. 2010 ¹
6CN-83		Rayssa et al. 2020 ²
7CN		Puterová et al. 2010 ¹
7CN-83		Rayssa et al. 2020 ²
8CN		Puterová et al. 2010 ¹
8CN-83		see section 2.2
12CN-83		see section 2.2
DCN-83		Luna et al. 2021 ³
HBN6CN-83		see section 2.2
Me-6CN-83		see section 2.2

2 Synthesis

2.1 General information

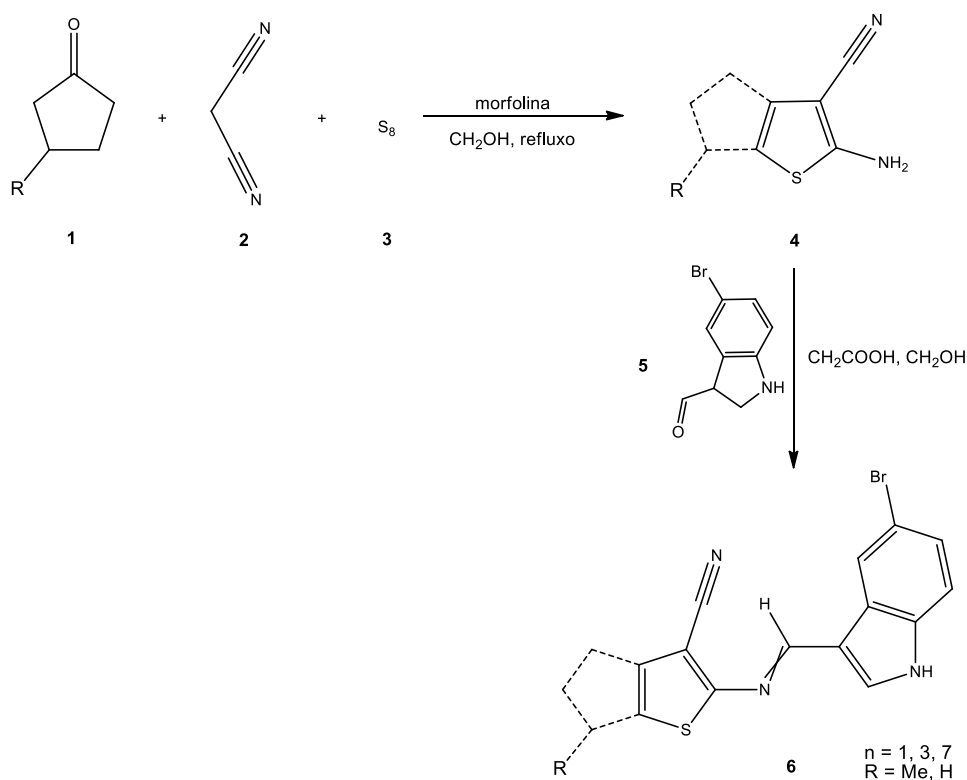
The identity of the synthesized compounds was confirmed with ^1H NMR, ^{13}C NMR and mass spectrometry. NMR analyses were carried out using 400 and 125 MHz spectrometers, respectively, and chemical shifts are reported in parts per million (ppm) relative to the residual solvent signals.

2.2 Synthetic procedures

2.2.1 2-Aminothiophenes

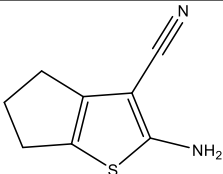
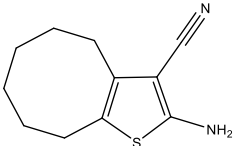
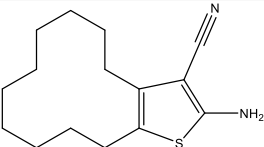
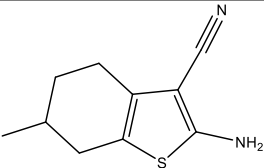
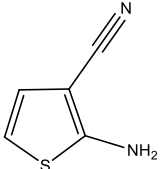
In the synthesis of 2-aminothiophene derivatives, the synthetic methodology based on the Gewald reaction^[1,2] was used, followed by a condensation reaction (Scheme S1). In general, the experimental procedure used consisted of an equimolar mixture of ketone (1), malononitrile (2), sulfur (3) in methanolic medium, then added to morpholine drop by drop in an ice bath, after adding the base it was The reaction was refluxed at $\sim 60^\circ\text{C}$. Since the product, the intermediate 2-aminothiophene-3-carbonitrile (4), presents a highly conjugated system, its formation is perceptible, since, in most cases, we observe a change in color.

Scheme S1. General scheme for obtaining 2-aminothiophene derivatives.



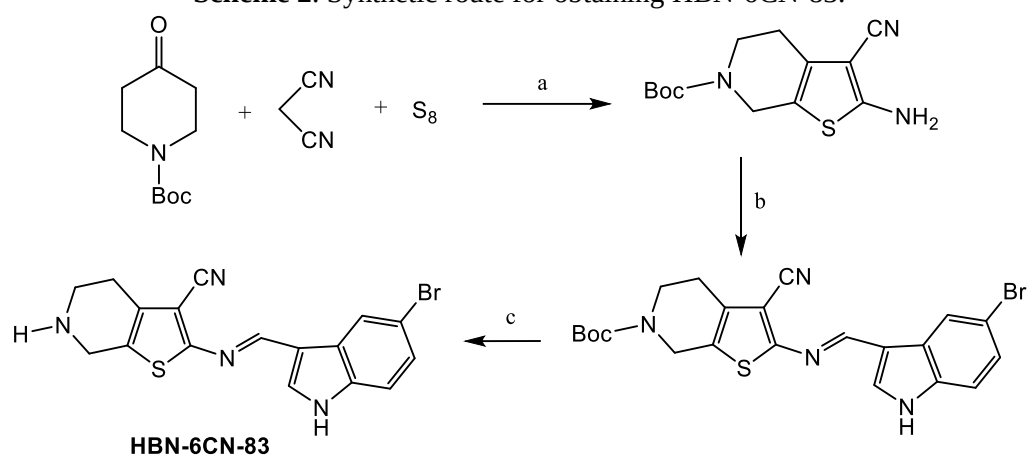
The second step of the reaction occurred equimolar between the starting reagent (4) (described in Table 2) and 5-bromoindole-3-carboxaldehyde (5), methanol and acetic acid at ~50°C. Both reaction steps were followed by Thin Layer Chromatography (TLC) and the isolation process of the product (6) was carried out by filtration, when purification was necessary, recrystallization and extraction generally became efficient.

Table S2. Reaction conditions involved in obtaining 2-aminothiophene derivatives.

Compounds	Starting reagent	Number of moles	Heavy mass	Reaction time
5CN-83		0,00206mol	0,339g	21h
8CN-83		0,00192mol	0,347g	23h
12CN-83		0,00068mol	0,180g	24h
Me-6CN-83		0,00078 mol	0,150g	24h
DCN		0,0016 mmol	0,250g	4h

HBN-6CN-83 was synthesized in three steps (Scheme S1), starting from the first version of the Gewald reaction using as cyclic ketone, the 4-Boc-piperidone. Gewald adduct was condensed with 5-bromo-indol-3-carboxaldehyde as describe in Rodrigues et al.^[4,5] and Boc deprotection was performed as previously described in da Cruz et al.^[6] using a mixture of dichloromethane and trifluoroacetic acid (8:2), at 0-5°C for 1h.

Scheme 2. Synthetic route for obtaining HBN-6CN-83.

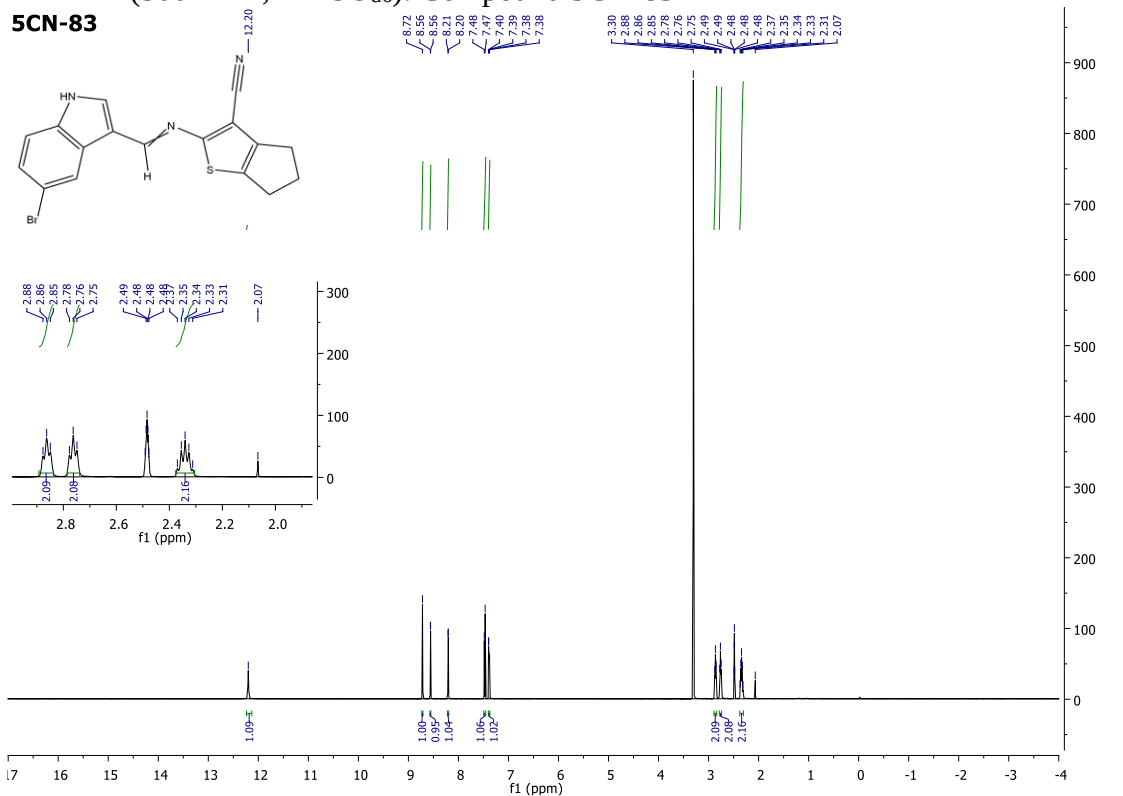


3 References

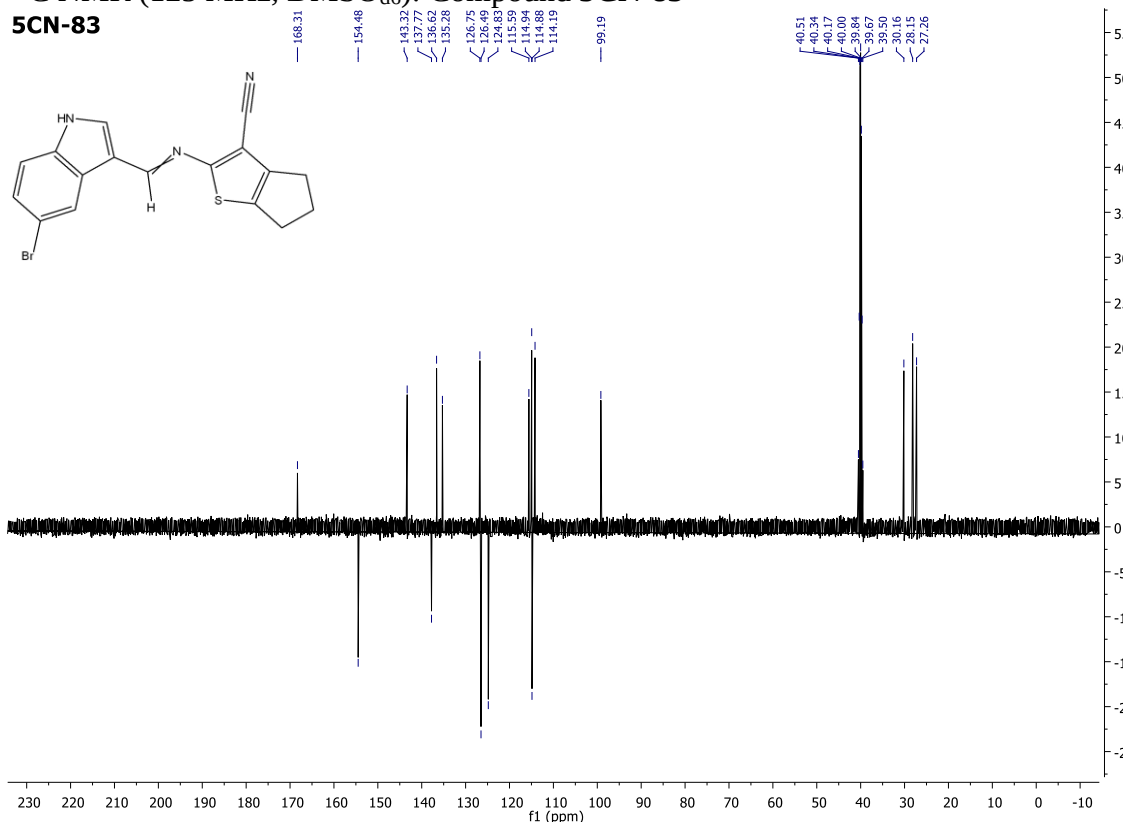
- [1] Puterová, Z., Krutošíková, A. Véghc, D. Gewald Reaction Synthesis, Properties and Applications of Substituted 2-Aminothiophenes. ARKIVOC,(2010) 209-246. <http://dx.doi.org/10.3998/ark.5550190.0011.105>.
- [2] Rayssa M. D. da Cruz, Renaud Zelli, Sarah Benshain, Ryldene M. D. da Cruz, José P. Siqueira-Júnior, Jean-Luc Décout, Marie-Paule Mingeot-Leclercq, Francisco J. B. Mendonça-Junior. Synthesis and Evaluation of 2-Aminothiophene Derivatives as *Staphylococcus aureus* Efflux Pump Inhibitors. ChemMedChem, 15 (2020) 716-725, 2020. <https://doi.org/10.1002/cmdc.201900688>.
- [3] Luna, I.S. Neves, W.W. Lima-Neto, R.G. Albuquerque, A.P.B. Pitta, M.G.R. Rêgo, M.J.B.M. Neves, R.P. Scotti, M.T. Mendonça-Junior, F.J.B. Design, Synthesis and Antifungal Activity of New Schiff Bases Bearing 2-Aminothiophene Derivatives Obtained by Molecular Simplification. J. Braz. Chem. Soc. 32 (2021) 1017-1029. <https://doi.org/10.21577/0103-5053.20210004>.
- [4]Rodrigues, K.A.F. Dias, C.N.S. Nêris, P.L. do N. Rocha, J.C. Scotti, M.T. Scotti, L., Mascarenhas, S.R. Veras, R.C. Medeiros, I.A. Keesen, T.S.L. de Oliveira, T.B., de Lima, M.C., Balliano, T.L., de Aquino, T.M. de Moura, R.O., Mendonça Junior, F.J.B., de Oliveira, M.R. 2-amino-thiophene derivatives present antileishmanial activity mediated by apoptosis and immunomodulation in vitro, Eur. J. Med. Chem. 106 (2015) 1–14, <https://doi.org/10.1016/j.ejmech.2015.10>.
- [5] Rodrigues, K. A. F. Amorim, L.V. Dias, C.N. Moraes, D.F.C. Carneiro, S.M.P. Carvalho, F.A.A. *Syzygium cumini* (L.) Skeels essential oil and its major constituent α -pinene exhibit anti-*Leishmania* activity through immunomodulation in vitro. J. Ethnopharmacol. 160 (2015) 32-40. <https://doi.org/10.1016/j.jep.2014.11.024>.
- [6] da Cruz, R.M.D. Zelli, R. Benshain, S. da Cruz, R.M.D. Siqueira-Júnior, J.P. Décout, J.-L. Mingeot-Leclercq, M.-P. Mendonça-Junior, F.J.B. Synthesis and Evaluation of 2-Aminothiophene Derivatives as *Staphylococcus aureus* Efflux Pump Inhibitors. ChemMedChem 15 (2020) 716-725. <https://doi.org/10.1002/cmdc.201900688>.

4 Copies of NMR spectra

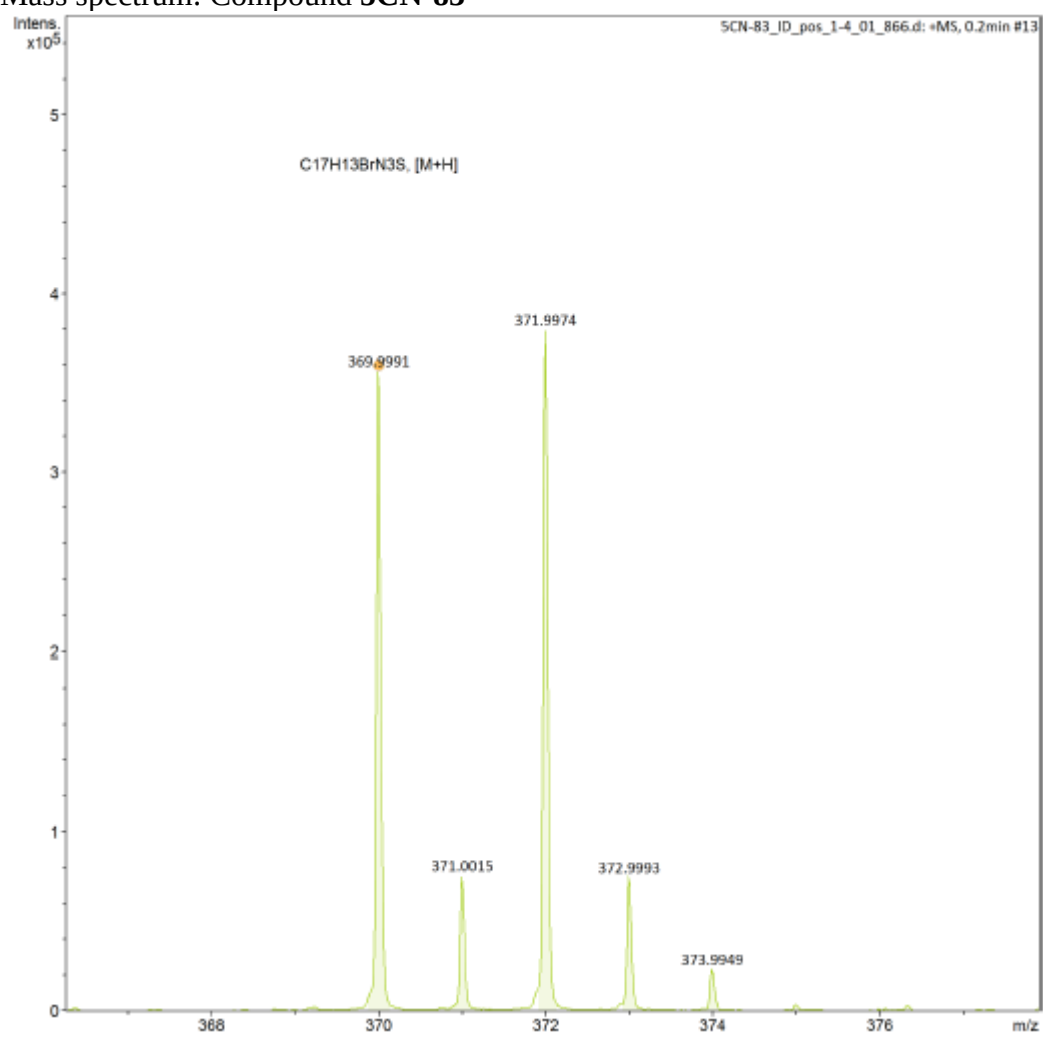
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¹³C NMR (125 MHz, DMSO_d₆): Compound 5CN-83



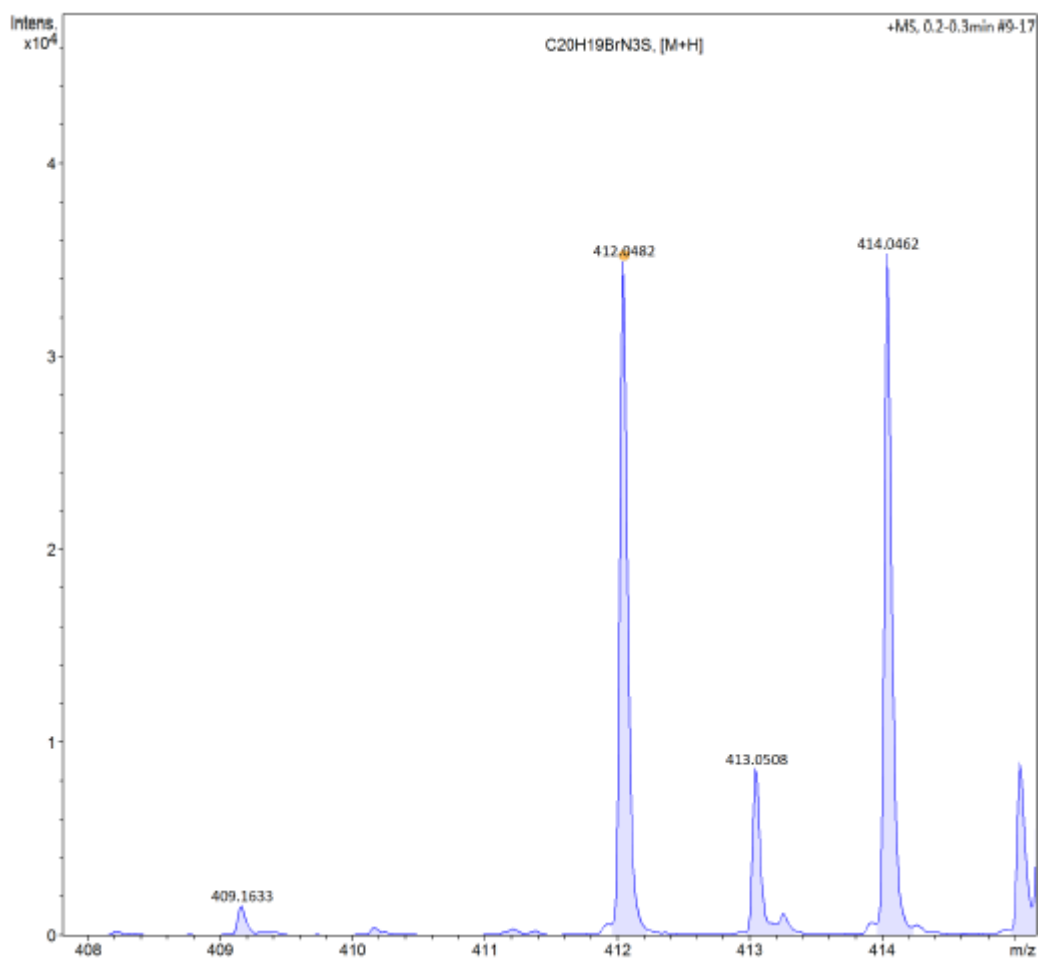
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8CN-83

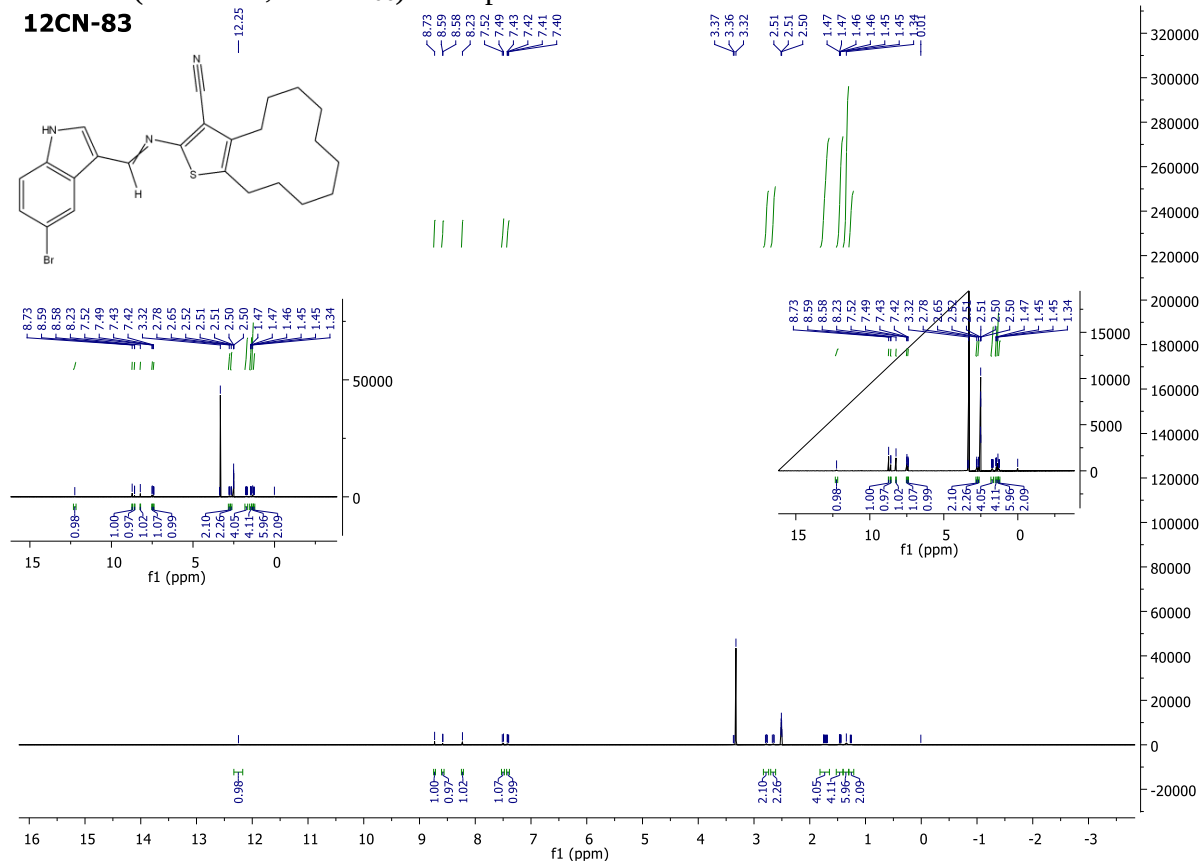
8CN-83

Mass spectrum: Compound **8CN-83**



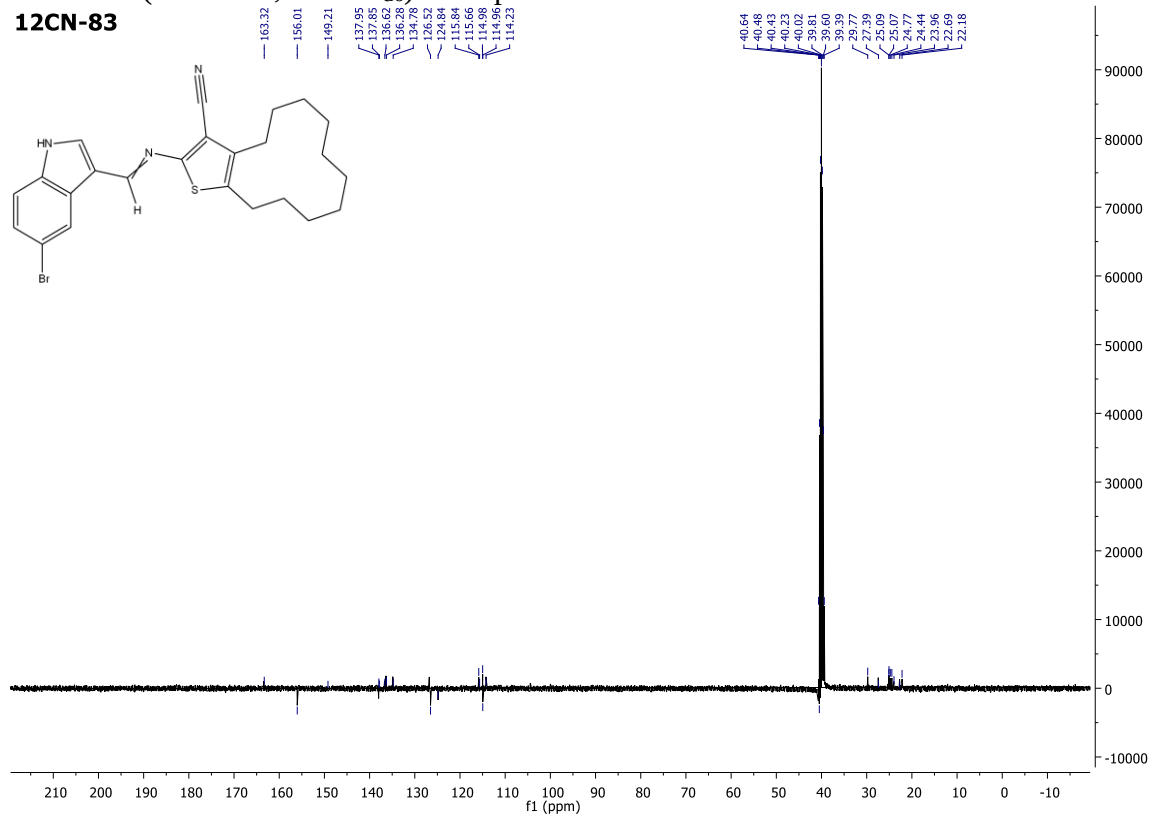
¹H NMR (500 MHz, DMSO_d₆): Compound **12CN-83**

12CN-83

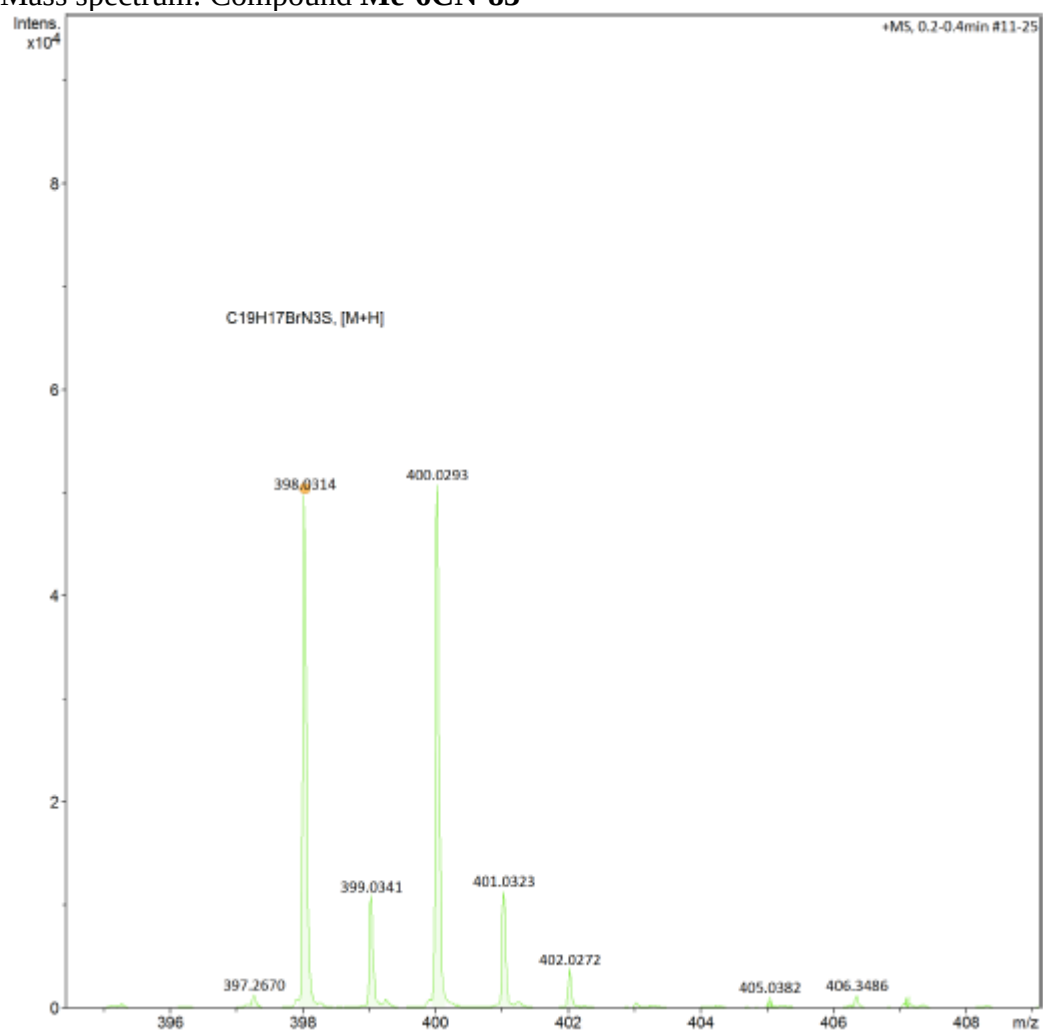


¹³C NMR (125 MHz, DMSO_d₆): Compound **12CN-83**

12CN-83

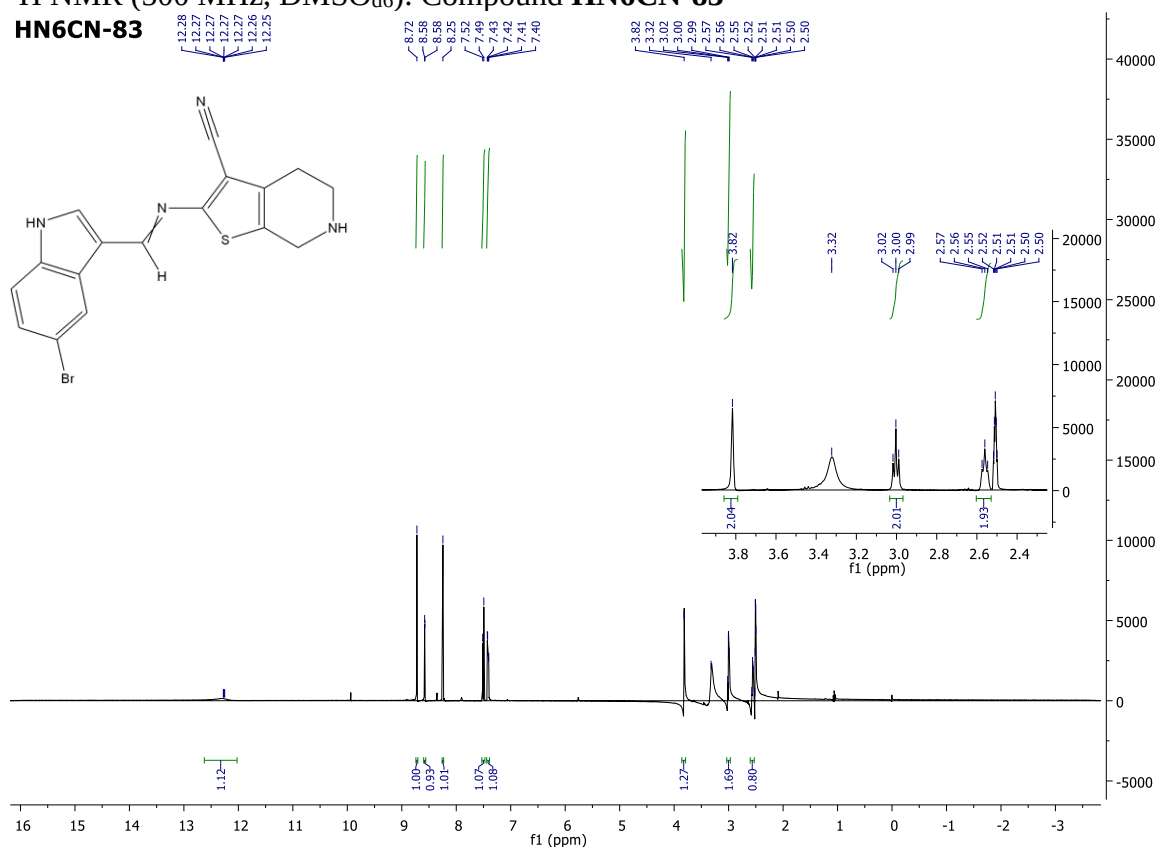


Mass spectrum: Compound **Me-6CN-83**



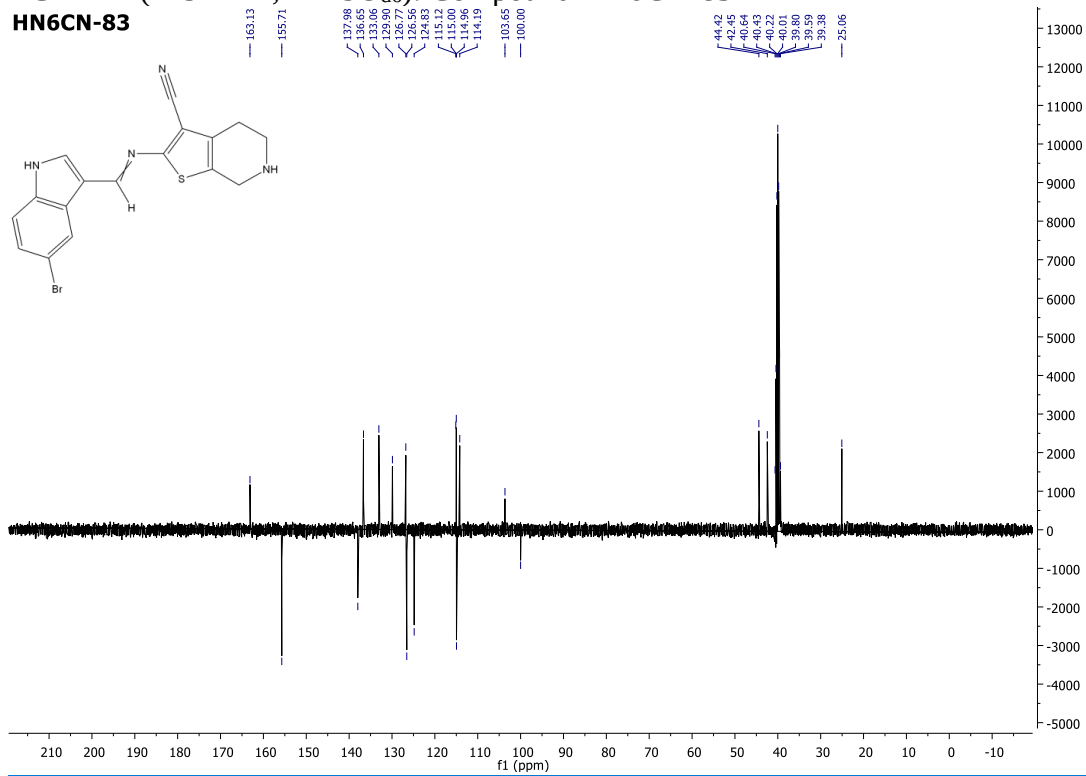
¹H NMR (500 MHz, DMSO_d₆): Compound **HN6CN-83**

HN6CN-83



¹³C NMR (125 MHz, DMSO_d₆): Compound **HN6CN-83**

HN6CN-83



CAPÍTULO 2



Neste capítulo dezessete (17) bases de Schiff contendo derivados de 2-aminotiofeno foram projetadas e sintetizadas usando simplificação molecular para melhorar a lipofilicidade dessas moléculas. Os compostos resultantes (4a-q) foram avaliados quanto à sua atividade antifúngica *in vitro* contra dermatófitos. Foi realizada a predição de sua semelhança com drogas e propriedades farmacocinéticas, estabelecimento de suas relações estrutura-atividade (SAR) e avaliação citotóxica dos compostos mais ativos.

Usando um procedimento ecologicamente correto, a síntese assistida por micro-ondas resultou em compostos com bons rendimentos (35-85%). Os compostos 4a-q apresentaram bons perfis farmacocinéticos e nenhuma citotoxicidade para nenhuma linha celular testada até 100 μ M.

Os compostos que apresentaram os melhores perfis antifúngicos foram 4e, 4f, 4g, 4k, 4l, 4m, 4o e 4p com mais de um valor de MIC ocorrendo entre 16 - 64 μ g.mL⁻¹, portanto, em alguns casos melhor que o medicamento de referência (fluconazol).

O teste de SAR indicou que a presença de halogênios e substituintes nitro aumenta a atividade antifúngica. Tomados em conjunto, os resultados demonstram que os derivados de 2-aminotiofeno são compostos líderes promissores para o desenvolvimento de drogas antifúngicas.

DESIGN, SYNTHESIS AND ANTIFUNGAL ACTIVITY OF NEW SCHIFF BASES BEARING 2-AMINOTHIOPHENE DERIVATIVES OBTAINED BY MOLECULAR SIMPLIFICATION

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Artigo encontra-se publicado.

Abstract: Seventeen (17) Schiff bases bearing 2-aminothiophene derivatives were designed and synthesized using molecular simplification. The resulting compounds (**4a-q**) were evaluated for their *in vitro* antifungal activity against dermatophytes. Prediction of their *druglikeness* and pharmacokinetic properties, establishment of their structure-activity relationships (SAR), and cytotoxic evaluation of the most active compounds was performed. Using an eco-friendly procedure, microwave assisted synthesis resulted in compounds in good yields (35-85 %). Compounds **4a-q** presented good *druglikeness* and pharmacokinetic profiles and no cytotoxicity for any cell line tested up to 100 μM . The compounds presenting the best antifungal profiles were **4e**, **4f**, **4g**, **4k**, **4l**, **4m**, **4o** and **4p** with more than one MIC value occurring between 16 - 64 $\mu\text{g.mL}^{-1}$, thus, in some cases better than the reference drug (fluconazole). SAR testing indicated that the presence of halogens and nitro substituents increases antifungal activity. Taken together, the results demonstrate that 2-aminothiophene derivatives are promising lead compounds for the development of antifungal drugs.

Keywords: 2-aminothiophene, ADMET properties, antifungal agents, drug design, Schiff base.

1. Introduction

During the past few decades, the incidence of fungal infection has substantially increased worldwide, and dermatophytoses, being one of the most common mycoses in humans, represent approximately 25% of this increase.^{1,2} Dermatophytes are filamentous fungi that cause dermatophytosis or tinea. They belong to the oldest groups of microorganisms and are recognized as agents of human disease, infecting keratinized tissues such as skin, hair and nails.^{3,4} The emergence of resistant dermatophytes in recent years has become a matter of worldwide concern, provoking interest in the development of new drugs with antifungal potential.^{5,6}

Despite the existence of various classes of antifungal agents such as azoles, polyenes, allylamines, antimetabolites, and echinocandins, the search for new agents against pathogenic fungi is of great importance and urgency, since the currently available antifungal drugs present severe side effects and toxicity. The most common side effects associated with the use of antifungals are serious: nephrotoxicity is observed with amphotericin B, the most common polyene,⁷ hepatotoxicity is associated with all azoles,⁸ hepatotoxicity and bone marrow depression are caused flucytosine,⁹ heart disease is associated with echinocandin,¹⁰ and gastrointestinal and hepatobiliary disorders are associated with the use of terbinafine.¹¹

As if this was not enough, antifungal resistance to all of these drugs is common and often reported. This is directly reflected in increased morbidity and mortality.^{12,13} There is a real need for new antifungal drugs.

Drug design and development is a complex and multidisciplinary process that aims to obtain new chemical entities with both the desired pharmacological effect and clinical safety (toxicity thus being absent or negligible). To achieve such characteristics, a crucial step is the choice of a pharmacophore group (or groups) that will lend to the drug candidate an appropriate pharmacodynamic and pharmacokinetic (absorption, distribution, metabolism, excretion and toxicity (ADMET)) profile.

Due to their great chemical versatility and valuable pharmacological properties, Schiff bases and thiophene derivatives are widely known.¹⁴⁻¹⁸ Their roles in the field of antifungal therapy stand out. Commercial antifungals with applications in dermatology, such as sertaconazole, oxiconazole,¹⁹ and imibenconazole²⁰ (Figure 1) present these pharmacophoric groups. Other examples of antifungal candidates presenting Schiff bases or thiophene scaffolds in their chemical structures are presented in figure 1.

These include: (a) Schiff bases containing a 2,4-dichloro-5-fluorophenyl group (**I**), being equipotent to fluconazole against *Aspergillus flavus*, *A. fumigatus*, *Penicillium marneffe* and *Trichophyton mentagrophytes* strains;²¹ (b) Schiff bases bearing salicylaldehyde (**II**) with anti-*Candida albicans* activity comparable to fluconazole;²² (c) imine containing a kakuol moiety (**III**) against phytopathogenic fungi, especially against *Alternaria Alternata*;²³ (d) Schiff bases containing piperonyl (**IV**) capable of inhibiting the growth of *Epidermophyton floccosum* and *Trichophyton rubrum*;²⁴ and (e) Schiff bases of 4-(4-aminophenyl)-morpholine (**V**) with antifungal properties against *Aspergillus niger* and *Candida albicans*.²⁵

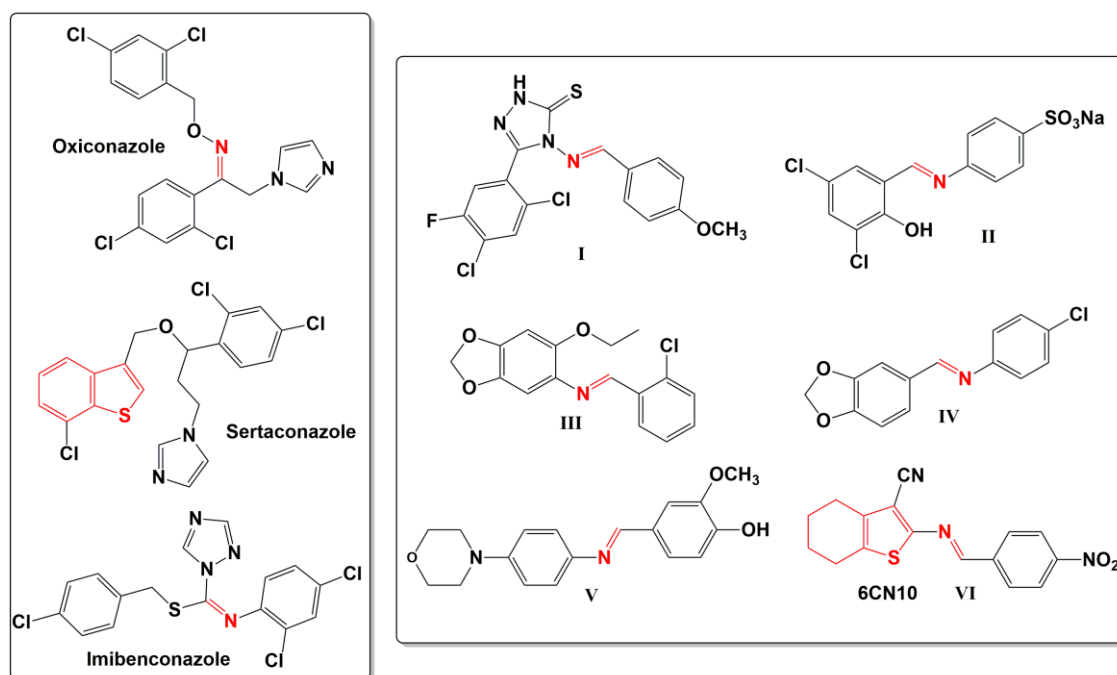


Figure 1. Antifungal agents under development or marketed containing Schiff bases and thiophene derivatives.

In recent years, our research group has investigated the potential of Schiff bases bearing cycloalka[*b*]thiophene derivatives for inhibiting the growth of various fungal species, especially *Cryptococcus neoformans*.²⁶⁻²⁸ Various compounds, such as 2-(4-nitrobenzylidene)-amino-4,5,6,7-tetrahydro-4*H*-benzo[*b*]thiophene-3-carbonitrile (named 6CN10) (**VI**) (Figure 1) are promising. However, the low water solubility of these compounds is a limiting factor, especially when conducting *in vivo* studies.²⁷ To overcome these limits, since 2014, efforts have been made to prepare adequate formulations that contain the most promising Schiff bases; those bearing cycloalka[*b*]thiophene derivatives. Inclusion complexes with 2-hydroxypropyl- β -cyclodextrin (HP- β -CD),²⁹ microemulsions,³⁰ and nanoparticles

(nanospheres/nanocapsules)³¹ have been prepared with a view to increase water solubility and antifungal activity. In view of these considerations and continuing our program to develop compounds with potent antifungal activity, we have (through a strategy of molecular simplification³²⁻³⁴) designed a new series of Schiff bases bearing 2-aminothiophene derivatives obtained from 1,4-dithiane-2,5-diol. This makes it possible to obtain unsubstituted Gewald adducts C-4 and C-5 (Figure 2).^{35,36} The new compounds present lower LogP values when compared with their cycloalka[*b*]thiophene analogues,^{26, 28} improving both water solubility and their pharmacokinetic profiles; and resulting in a potential increase in antifungal activity.

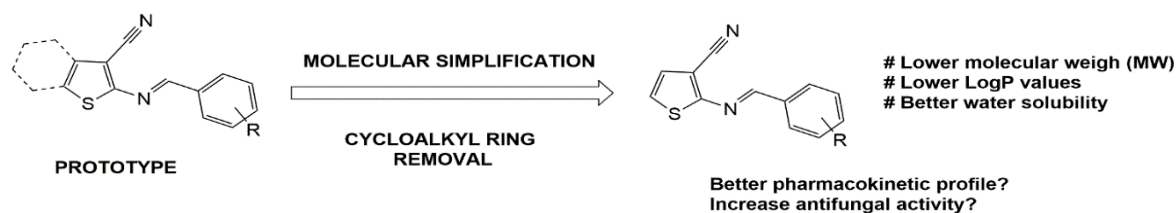


Figure 2. Rationale for the synthesis of new Schiff bases bearing 2-aminothiophene derivatives with improved antifungal activity.

The aim of this study was to evaluate the *in vitro* antifungal activity against dermatophytes of new Schiff bases bearing 2-aminothiophene derivatives, synthesized using a molecular simplification strategy. Prediction of their *druglikeness* and pharmacokinetic (ADMET) properties, establishment of their structure-activity relationships (SAR), and cytotoxic evaluation of the most active compounds was also performed.

2. Experimental

2.1 Chemistry

All reagents and solvents were purchased from Sigma-Aldrich, Fluka, Neon, Vetec and used without further purification. Melting points were measured using a model MA-381 melting point (Marconi, Brazil), apparatus and are uncorrected. Synthesis under microwave irradiation was carried out on a Discover-System (CEM). ¹H-NMR, ¹³C-NMR, two-dimensional NMR (HSQC and HMQC) spectra were recorded in DMSO-d₆ on a Varian Unity Plus 400 MHz spectrometer. Chemical shifts were reported in ppm (δ) and coupling constants (*J*) were reported in Hertz. Chemical shifts (δ) are given in parts per million (ppm). Multiplicities are reported as singlet (s), doublet

(d), doublet of doublets (dd), triplet (t), quartet (q) and multiplet (m), and the observed coupling constants (J) provided in Hertz (Hz). Peaks are assigned where possible. Infrared spectra were acquired on a VERTEX 70 series FT-IR spectrometer using KBr discs, and the wavenumbers related to the transmittance minima (ν_{max}) in cm^{-1} are reported. Exact mass measurements of the molecular ions were obtained on a Bruker Daltonics the micrOTOF-Q IITM ESI-Qq-TOF mass spectrometer. The chromatographic analysis had reaction progress was monitored by thin layer chromatography using Merck TLC Silica gel 60 F₂₅₄ aluminium sheets and detection by short and long wavelength ultraviolet light (254 and 365 nm).

2.1.1 General

2.1.2 General procedure for the synthesis of compounds 4a-q

Method A: In a round bottom flask were added 1,4-dithiane-2,5-diol (**1**) (1 eq) and malononitrile (**2**) (2 eq.). Methanol (MeOH) was added to dissolve the mixture, and then triethylamine (0.6 eq.) was added dropwise. The reaction proceeded at 60 °C with stirring for 4 h. After 4 h, substituted aromatic aldehydes (2 eq.) and acetic acid (0.7 eq.) were added in the reaction medium, and the reaction proceeded at room temperature overnight. After completion of the reaction, the final products (**4a-q**) were filtered and washed successively with cold water and MeOH to obtain chromatographically pure solid residues.

Method B: In a microwave tube were added 1,4-dithiane-2,5-diol (**1**) (1 eq), malononitrile (**2**) (2 eq.), sodium bicarbonate (1.3 eq.) and 2mL of ethylene glycol. The reaction mixture was irradiated (150 W) at 80 °C for 8 min (ramp time: 5 min) in the microwave apparatus. After cooling, the microwave tube was opened, and were added substituted aromatic aldehydes (2 eq.), acetic acid (1.5 eq.) and 0.5 mL of ethylene glycol. Then, the reactional mixture was subjected under microwave irradiation (150 W) at 50 °C for 2 min (ramp time: 2 min 30 s). After completion of the reaction, the final products (**4a-q**) were filtered and washed successively with cold MeOH/water (1:1) to obtain chromatographically pure solid residues.

2-(4-methoxybenzylidene)aminothiophene-3-carbonitrile (4a): Green crystals; yield 86 % (method A), 85 % (method B); m.p. 133.4-135.2 °C; ¹H NMR (400 MHz, DMSO-d₆) δ

3.61 (s, 3H, OCH₃), 6.87 (d, 2H, *J* 8.8 Hz, ArH), 7.11 (d, 1H, *J* 5.6 Hz, H-4), 7.25 (d, 1H, *J* 6.0 Hz, H-5), 7.71 (d, 2H, *J* 8.8 Hz, ArH), 8.43 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO-d₆) δ 56.0, 106.1, 115.1, 115.3, 123.1, 124.5, 127.9, 129.4, 132.0, 162.2; IR (KBr) ν_{max}, cm⁻¹: CH (3101), CN (2227), C=N (1593); HRMS (ESI) *m/z*, observed: 243.0593; C₁₃H₁₀N₂OS [H]⁺ requires: 243.0595.

2-(4-Chlorobenzylidene)aminothiophene-3-carbonitrile (4b): Green crystals; yield 66 % (method A), 52 % (method B); m.p. 104.3-105.9 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 7.39 (d, 1H, *J* 5.6 Hz, H-4), 7.58 (d, 1H, *J* 5.6 Hz, H-5), 7.61 (d, 2H, *J* 1.4 Hz, ArH), 7.99 (d, 2H, *J* 1.0 Hz, ArH), 8.77 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO-d₆) δ 99.4, 105.6, 114.4, 123.8, 127.5, 129.1, 130.8, 133.4, 137.3, 161.0; IR (KBr) ν_{max}, cm⁻¹: CH (3111), CN (2229), C=N (1590); HRMS (ESI) *m/z*, observed: 247.0097; C₁₂H₇ClN₂S [H]⁺ requires: 247.0095.

2-(4-Fluorobenzylidene)aminothiophene-3-carbonitrile (4c): Green crystals; yield 55 % (method A), 66 % (method B); m.p. 109.3-110.1 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 7.43 (d, 1H, *J* 6 Hz, H-4), 7.65 (d, 1H, *J* 5.6 Hz, H-5), 7.85 (t, 1H, *J* 8.0 Hz, ArH), 8.39-8.42 (m, 2H, ArH), 8.77-8.78 (m, 1H, ArH), 8.95 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO-d₆) δ 107.0, 114.9, 124.0, 125.2, 127.1, 128.3, 131.2, 135.3, 136.7, 161.0; IR (KBr) ν_{max}, cm⁻¹: CH (3128), CN (2228), C=N (1580); HRMS (ESI) *m/z*, observed: 231.0393; C₁₂H₇FN₂S [H]⁺ requires: 231.0370.

2-(2,4-Dichlorobenzylidene)aminothiophene-3-carbonitrile (4d): Yellow crystals; yield 49 % (method A), 35 % (method B); m.p. 152.6-153 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 7.43 (d, 1H, *J* 5.6 Hz, H-4), 7.61 (dd, 1H, *J* 8.4, 2.0 Hz, ArH), 7.65 (d, 1H, *J* 5.6 Hz, H-5), 7.80 (d, 1H, *J* 2.0 Hz, ArH), 8.15 (d, 1H, *J* 8.8 Hz, ArH), 8.83 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO-d₆) δ 106.9, 114.8, 125.2, 126.8, 128.4, 128.8, 130.3, 130.4, 132.8, 136.8, 138.4, 156.8; IR (KBr) ν_{max}, cm⁻¹: CH (3121), CN (2232), C=N (1582); HRMS (ESI) *m/z*, observed: 280.9708; C₁₂H₆Cl₂N₂S [H]⁺ requires: 280.9700.

2-(2,6-Dichlorobenzylidene)aminothiophene-3-carbonitrile (4e): Yellow crystals; yield 56 % (method A), 83 % (method B); m.p. 150.1-151.5 °C; ¹H NMR (400 MHz, DMSO_{d6}): δ 7.46 (d, 1H, *J* 5.6 Hz, H-4), 7.55 (dd, 1H, *J* 8.8, 7.2 Hz, ArH), 7.63 (d, 2H, *J* 8.0 Hz, ArH), 7.69 (d, 1H, *J* 5.6 Hz, H-5), 8.90 (s, 1H, CH=N); ¹³C NMR (100MHz, DMSO_{d6}): δ 106.6, 114.2, 125.1, 128.1, 129.6, 130.2, 132.8, 134.7, 157.9, 161.5; IR (KBr) $\nu_{\max}$, cm⁻¹: CH (3107), CN (2225), C=N (1578); HRMS (ESI) *m/z*, observed: 280.9708; C₁₂H₆Cl₂N₂S [H]⁺ requires: 280.9707.

2-(2-Nitrobenzylidene)aminothiophene-3-carbonitrile (4f): Green crystals; yield 44 % (method A), 52 (method B); m.p. 179.8–80.3 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 7.46 (d, 1H, *J* 5.6 Hz, H-4), 7.66 (d, 1H, *J* 5.6 Hz, H-5), 7.83 (t, 1H, *J* 7.2 Hz, ArH), 7.92 (t, 1H, *J* = 7.6 Hz, ArH), 8.16 (d, 1H, *J* 8.0 Hz, ArH), 8.21 (d, 1H, *J* 7.6 Hz, ArH), 9.04 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO_{d6}) δ 108.8, 114.7, 117.2, 125.3, 125.4, 128.5, 130.0, 131.5, 133.4, 134.5, 159.1, 164.5; IR (KBr) $\nu_{\max}$, cm⁻¹: CH (3100), CN (2233), C=N (1509); HRMS (ESI) *m/z*, observed: 258.0338; C₁₂H₇N₃O₂S [H]⁺ requires: 258.0329.

2-(3-Nitrobenzylidene)aminothiophene-3-carbonitrile (4g): Green crystals; yield 64 % (method A), 78% (method B); m.p. 196.3-197.6 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 7.19 (d, 1H, *J* 5.6 Hz, H-4), 7.41 (d, 1H, *J* 5.6 Hz, H-5), 7.60 (t, 1H, *J* 8.0 Hz, ArH), 8.14-8.18 (m, 2H, ArH), 8.53 (t, 1H, *J* 2.0 Hz, ArH), 8.71 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO_{d6}) δ 107.0, 114.9, 124.0, 125.2, 127.1, 127.2, 128.3, 131.2, 135.3, 136.7, 160.9, 161.0; IR (KBr) $\nu_{\max}$, cm⁻¹: CH (3096), CN (2232), C=N (1522); HRMS (ESI) *m/z*, observed: 258.0338; C₁₂H₇N₃O₂S [H]⁺ requires: 258.0333.

2-(2-Hydroxybenzylidene)aminothiophene-3-carbonitrile (4h): Green crystals; yield 45 % (method A), 79 % (method B); m.p. 131.5-133.4 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 6.98-7.02 (m, 2H, ArH), 7.40 (d, 1H, *J* 5.6 Hz, H-4), 7.47 (dt, 1H, *J* 7.2, 1.6 Hz, ArH), 7.58 (d, 1H, *J* 5.6 Hz, H-5), 7.82 (dd, 1H, *J* 8.0, 1.6 Hz, ArH), 8.97 (s, 1H, CH=N), 11.49 (s, 1H, OH); ¹³C NMR (100 MHz, DMSO_{d6}) δ 104.7, 114.4, 116.8, 119.4, 119.7, 123.5, 127.3, 131.0, 134.8, 159.9, 161.7, 162.2; IR (KBr) $\nu_{\max}$, cm⁻¹: CH (3115), CN (2228), C=N (1604); HRMS (ESI) *m/z*, observed: 229.0454; C₁₂H₈N₂OS [H]⁺ requires: 229.0427.

2-(4-Methylbenzylidene)aminothiophene-3-carbonitrile (4i): Green crystals; yield 59 % (method A), 27 % (method B); m.p. 116.5-117.1 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 2.39 (s, 3H, CH₃), 7.37 (d, 2H, *J* 8.0 Hz, ArH), 7.38 (d, 1H, *J* 5.6 Hz, H-4), 7.54 (d, 1H, *J* 5.6 Hz, H-5), 7.90 (d, 2H, *J* 8.0 Hz, ArH), 8.72 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO_{d6}) δ 21.3, 104.8, 114.7, 123.2, 127.5, 129.4, 129.7, 132.0, 143.3, 162.3, 163.4; IR (KBr) ν_{max}, cm⁻¹: CH (3109), CN (2222), C=N (1599); HRMS (ESI) *m/z*, observed: 227.0644; C₁₃H₁₀N₂OS [H]⁺ requires: 227.0626.

2-((4-(Dimethylamino)benzylideneamino)thiophene-3-carbonitrile (4j): Orange crystals; yield 31 % (method A), 55 % (method B); mp 143.5-144.3 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 3.05 (s, 6H, CH₃), 6.81 (d, 2H, *J* 8.8 Hz, ArH), 7.28 (d, 1H, *J* 5.6 Hz, H-4), 7.37 (d, 1H, *J* 6.0 Hz, H-5), 7.81 (d, 2H, *J* 8.8 Hz, ArH), 8.49 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO_{d6}) δ 39.6, 102.2, 111.5, 115.2, 120.9, 121.7, 127.1, 131.5, 153.3, 161.5, 165.3; IR (KBr) ν_{max}, cm⁻¹: CH (3094), CN (2225), C=N (1579); HRMS (ESI) *m/z*, observed: 256.0909; C₁₄H₁₃N₃S [H]⁺ requires: 256.0914.

2-(4-Nitrobenzylidene)aminothiophene-3-carbonitrile (4k): Brown crystals; yield 60 % (method A), 90 % (method B); m.p. 153.8-155.4 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 7.47 (d, 1H, *J* 5.6 Hz, H-4), 7.70 (d, 1H, *J* 5.6 Hz, H-5), 8.25 (d, 2H, *J* 8.8 Hz, ArH), 8.41 (d, 2H, *J* 8.4 Hz, ArH), 8.95 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO_{d6}) δ 107.9, 115.3, 124.7, 125.1, 126.1, 128.9, 131.2, 131.5, 141.1, 150.3, 161.3, 162.8; IR (KBr) ν_{max}, cm⁻¹: CH (3093), CN (2230), C=N (1720); HRMS (ESI) *m/z*, observed: 258.0338; C₁₂H₇N₃O₂S [H]⁺ requires: 258.0347.

2-(3,4,5-Trimethoxybenzylidene)aminothiophene-3-carbonitrile (4l): Yellow crystals; yield 39 % (method A), 57% (method B); m.p. 160.6-161.7 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 3.77 (s, 3H, OCH₃), 3.86 (s, 6H, OCH₃), 7.36 (s, 2H, ArH), 7.39 (d, 1H, *J* 5.6 Hz, H-4), 7.55 (d, 1H, *J* 5.6 Hz, H-5), 8.69 (s, 1H, CH=N); ¹³C NMR (100 MHz, DMSO_{d6}) δ 55.9, 60.2, 104.6, 106.7, 114.7, 123.2, 127.6, 129.9, 141.6, 153.1, 162.2, 163.3; IR (KBr) ν_{max}, cm⁻¹: CH (3091), CN (2222), C=N (1570); HRMS (ESI) *m/z*, observed: 303.0804; C₁₅H₁₄N₂O₃S [H]⁺ requires: 303.0795.

2-(Benzylidene)aminothiophene-3-carbonitrile (4m): Green crystals; yield 49 % (method A), 63% (method B); m.p. 113.3-114.1 °C; ¹H NMR (400 MHz, DMSO_{d6}) δ 7.40 (d, 1H, *J* 6.0 Hz, H-4), 7.55-7.63 (m, 4H, ArH + H-5), 8.01 (d, 2H, *J* 6.8 Hz, ArH),

8.78 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ 105.7, 115.1, 124.1, 128.1, 129.5, 129.9, 133.3, 135.1, 163.0, 163.6; IR (KBr) ν_{max} , cm^{-1} : CH (3096), CN (2224), C=N (1571); HRMS (ESI) m/z , observed: 213.0487; $\text{C}_{14}\text{H}_{14}\text{N}_2\text{S}$ $[\text{H}]^+$ requires: 213.0472.

2-(2,3-Dichlorobenzylidene)aminothiophene-3-carbonitrile (4n): Yellow crystals; yield 56 % (method A), 46 % (method B); m.p. 175.5-176.4 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.46 (d, 1H, J 6.0 Hz, H-4), 7.57 (t, 1H, J 8.0 Hz, ArH), 7.68 (d, 1H, J 5.6 Hz, H-5), 7.88 (d, 1H, J 8.0 Hz, ArH), 8.15 (d, 1H, J 8.0 Hz, ArH), 8.94 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ 107.2, 114.8, 125.5, 127.8, 128.5, 129.2, 132.2, 133.8, 134.2, 134.5, 157.8, 162.3; IR (KBr) ν_{max} , cm^{-1} : CH (3092), CN (2236), C=N (1552); HRMS (ESI) m/z , observed: 280.9708; $\text{C}_{12}\text{H}_6\text{Cl}_2\text{N}_2\text{S}$ $[\text{H}]^+$ requires: 280.9696.

2-(4-Bromobenzylidene)aminothiophene-3-carbonitrile (4o): Green crystals; yield 46 % (method A), 63% (method B); m.p. 95,8-98,4 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.41 (d, 1H, J 5.6 Hz, H-4), 7.60 (d, 1H, J 6.0 Hz, H-5), 7.77 (d, 2H, J 8.8 Hz, ArH), 7.94 (d, 2H, J 8.8 Hz, ArH), 8.78 (s, 1H, CH=N); ^{13}C NMR (100 MHz, DMSO-d_6) δ 105.7, 114.5, 124.0, 126.5, 127.7, 131.0, 132.1, 133.8, 161.3, 162.7; IR (KBr) ν_{max} , cm^{-1} : CH (3094), CN (2230), C=N (1584); HRMS (ESI) m/z , observed: 290.9592; $\text{C}_{12}\text{H}_7\text{BrN}_2\text{S}$ $[\text{H}]^+$ requires: 290.9590.

2-((4-Nitro-1H-indol-3-yl)methyleneamino)thiophene-3-carbonitrile (4p): Yellow crystals; yield 64 % (method A), 50 % (method B); m.p. 172-174.1 °C, ^1H NMR (400 MHz, DMSO-d_6) δ 7.31 (d, 1H, J 5.6 Hz, H-4), 7.40 (d, 1H, J 6.4 Hz, H-5), 7.44 (d, 1H, J 8.0 Hz, ArH), 7.96 (d, 1H, J 8.4 Hz, ArH), 8.00 (d, 1H, J 8.0 Hz, ArH), 8.58 (s, 1H, ArH), 9.10 (s, 1H, CH=N), 12.93 (sl, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 102.2, 112.3, 115.0, 117.1, 119.2, 119.6, 121.1, 122.1, 127.4, 135.5, 139.5, 142.1, 158.3, 165.3; IR (KBr) ν_{max} , cm^{-1} : CH (3225), CN (2231), C=N (1512); HRMS (ESI) m/z , observed: 297.0447; $\text{C}_{14}\text{H}_8\text{N}_4\text{OS}$ $[\text{H}]^+$ requires: 297.0427.

2-((5-Methoxy-1H-indol-3-yl)methyleneamino)thiophene-3-carbonitrile (4q): Blue crystals; yield 23 % (method A), 71% (method B); m.p. 278.4-281.3 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 3H, OCH_3), 6.91 (dd, 1H, J 8.8, 2.4 Hz, ArH), 7.30 (d, 1H, J 6.0 Hz, H-4), 7.35 (d, 1H, J 6.0 Hz, H-5), 7.42 (d, 1H, J 8.8 Hz, ArH), 8.06 (d, 1H, J 2.8 Hz, ArH), 8.15 (d, 1H, J 3.2 Hz, ArH), 8.82 (s, 1H, CH=N), 12.02 (sl, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.1, 102.3, 103.9, 113.1, 113.4, 114.1, 115.5, 120.2,

125.4, 126.6, 132.0, 136.7, 155.4, 156.4, 166.2; IR (KBr) $\nu_{\max}$, cm^{-1} : CH (3305), CN (2218), C=N (1578); HRMS (ESI) m/z , observed: 282.0702; $\text{C}_{15}\text{H}_{11}\text{N}_3\text{OS}$ $[\text{H}]^+$ requires: 282.0714.

2.2 Prediction of biological activity

2.2.1 Prediction of druglikeness and pharmacokinetics (ADMET) properties

The *druglikeness*, pharmacokinetics properties, and toxicity profile of compounds **4a-q** were determined using an ADMET descriptors algorithm protocol of pkCSM.⁶⁸ This software has been used as evidence to train accurate molecular predictors of important physicochemical parameters such as molecular weight (MW), topological polar surface area (TPSA), partition coefficient (octanol-water) -LogP, number of hydrogen bond acceptor (*n*HBA), number of hydrogen bond donors (*n*HBD) and rotatable bonds (ROTB). Analysis of these aspects were used to verify the *druglikeness* properties of 2-aminothiophenes based on the guidelines of the Lipinski's and Weber's rules.⁶⁹ Absorption properties were analyzed based on membrane permeability (indicated by colon cancer cell line (Caco-2) permeability), skin permeability and the categorical classification of 2-aminothiophene derivatives. Drug distribution was predicted according the blood-brain barrier permeability (logBB) and central nervous system permeability (logPS). Metabolism property was evaluated based on the CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4). Excretion aspect was predicted by the total clearance model and the categorical classification of 2-aminothiophene derivatives as a renal OCT2 substrate. Toxicity of compounds was obtained based on AMES toxicity, hERG I and II inhibition, Oral rat acute and chronic toxicity, hepatotoxicity, skin sensitization, *T. pyriformis* toxicity and Minnow toxicity. After the results, all the PK parameters were calculated and checked for compliance with their standard ranges.⁷⁰

2.3 Pharmacological/biological assays

2.3.1 Antifungal activity

2.3.1.1 Preparation of samples and standards

All compounds (**4a-q**) were dissolved in dimethylsulfoxide (DMSO). Fluconazole (used as reference drug) was solubilized in sterile distilled water. Then all solutions were filtered through a 22 mm diameter filters. Subsequently all drugs solutions were diluted in RPMI 1640 medium buffered with 4-morpholinepropanesulfonic acid (MOPS), to obtain 1.024 $\mu\text{g}/\text{mL}$ solutions. Compounds **4a-q** were tested in concentrations ranged from 1 to 1.024 $\mu\text{g}\cdot\text{mL}^{-1}$. Fluconazol in concentrations ranging from 0.125 to 64 $\mu\text{g}\cdot\text{mL}^{-1}$.

2.3.1.2 Antifungal sensitivity

E. floccosum, *T. tonsurans*, *T. mentagrophytes*, *T. rubrum* and *C. parapsilosis* ATCC 22019 (reference strain) used in the test were deposited in Micoteca URM of the Mycology Department of the Federal University of Pernambuco and are showed in table 4. The tests were performed according to CLSI (CLSI, 2008a), standard M38-A2. Dermatophytes species were grown on Sabouraud Dextrose Agar (Merck KGaA, Darmstadt, Germany) at 24 °C four to seven days. For the test, was removed an inoculum culture and suspended in 5.0 mL of sterile saline 0.085 % and placed in vortex for 15 s. The cell density was adjusted by a spectrophotometer Genesys UV-VIS 10S (Thermo Scientific, Waltham, USA) to obtain the transmittance equivalent to a standard solution of 0.5 McFarland wavelength of 530 nm. The suspension for testing was obtained by making a 1/100 dilution followed by a 1/50 dilution in RPMI 1640 medium with MOPS for dermatophytes.

2.3.2 Cytotoxicity

2.3.2.1 Cell Culture

Immortalized non-tumor cell lines from human fibroblasts (MRC-05), monkey kidney epithelial cells (VERO) and murine fibroblasts (3T3) from the Bank of Cells of Rio de Janeiro and stocked in the Tumor Cell Bank of LINAT / UFPE. VERO and MRC-05 cells were cultured in Low Glucose DMEM medium and 3T3 cells in RPMI 1640 medium both supplemented with 10% Bovine Fetal Serum, 10 mM HEPES and 1% U/ml penicillin/streptomycin. The medium of the MRC-05 cells was supplemented with 20% Bovine Fetal Serum, 10% Insulin and 10% Fibroblast Growth Factor. The

lineages were kept in an oven containing 5% CO₂ at 37 °C and routinely evaluated for mycoplasma contamination.

2.3.2.2 Cytotoxicity in Non-transformed Cells

Cells were plated (1×10^4 cells/well) and incubated in a humid atmosphere containing 5 % CO₂ at 37 °C for 24 h. Then the **4e**, **4f**, **4g**, **4k**, **4l**, **4m** and **4o** derivatives were added at the concentrations of 1, 10, 50 and 100 μ M and the plates were incubated for 72 h. Vehicle control (DMSO) was added at the same concentration present in the final solutions. Then, 20 μ L of the MTT solution was added to each well and after 3 h of incubation and 130 μ L of 20 % SDS were added for the dissolution of the formazan crystals, keeping the reaction sheltered from light for 24 h. After this period, the optical density was read in a spectrophotometer in the microplate reader (EL808 - Biotek®) at 570 nm absorbance. The mean optical density of the test conditions was compared to the vehicle mean 0.5% for the determination of cell viability.

2.3.3 Statistical analysis

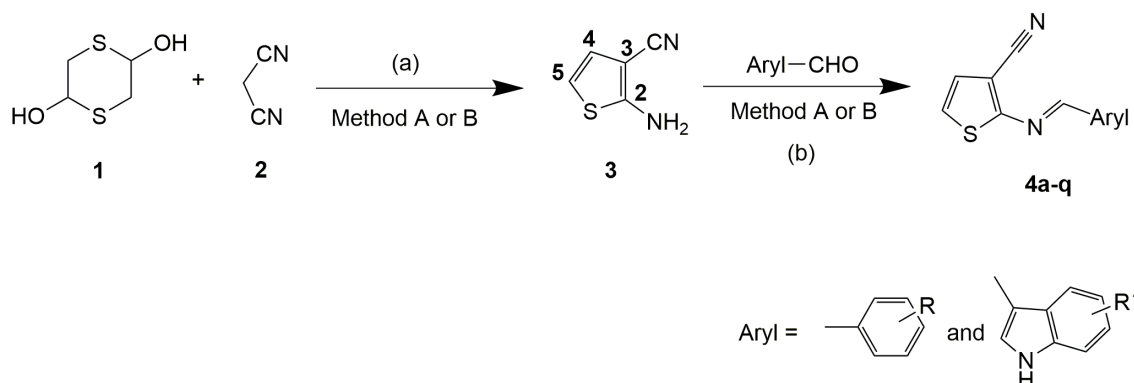
The analysis of mean, standard deviation and viability was performed in Excel® software. In all the tests three independent experiments were performed and each condition was evaluated in technical replicates.

3. Results and Discussion

3.1 Chemistry

The Schiff bases bearing 2-aminothiophene derivatives (**4a-q**) were obtained through a molecular simplification strategy being synthesized in a two-step reaction in two procedures, as shown in scheme 1. 2-Aminothiophene-3-carbonitrile (**3**) was synthesized using the fourth version of Gewald-type reaction.³⁵ Reacting commercial 1,4-dithiane-2,5-diol (**1**), with malononitrile (**2**) in methanol and triethylamine and magnetic stirring (Method A), or in ethylene glycol and sodium bicarbonate under microwave irradiation (Method B), we obtained compounds **4a-q**. Various aromatic aldehydes were added to each reaction media, along with a few drops of acetic acid, to yield the target compounds in yields ranging from 19-86 % (Method A) and 35-85 %

(Method B) (Table 1). The compound structures were confirmed using nuclear magnetic resonance (NMR) (^1H NMR, ^{13}C NMR, Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC)), infrared (IR), and High Resolution Mass Spectrum (HRMS) analyses (available in the supplementary material).



Scheme 1 Synthesis of Schiff bases bearing 2-aminothiophene derivatives (**4a-q**). Reagents and conditions: Method A: (a) triethylamine, methanol, 60°C, 4 h; (b) acetic acid, r.t., overnight. Method B: (a) sodium bicarbonate, ethylene glycol, MW, 80°C for 8 min; (b) acetic acid, MW, 50°C, 2 min.

The synthetic Methodologies used to obtain the target compounds; conventional (Method A) and microwave irradiation (Method B) proved to be both viable and efficient. Hesse *et al.*³⁷ obtained 2-aminothiophene-3-carbonitrile (compound **3**), using both microwave and conventional Methods in respective yields of 60% and 58%. In our procedures, compound **3** was obtained without isolation, and after reacting with aromatic aldehydes it provided several final compounds in final global yields of greater than 60% (after two-step reaction), demonstrating that our procedures were better than previously described.

The reactions conducted using Method B were more efficient (providing better yields) in 60% of the procedures (11 of 18 reactions). The microwave assisted syntheses of Schiff bases bearing 2-aminothiophene derivatives presented specific advantages such as: shorter reaction time (10 minutes), energy savings, and the use of an inorganic base (sodium bicarbonate) instead of the traditional organic bases used in Gewald-type reactions; an eco-friendly procedure.³⁸

Table 1. Chemical structures and yields using Schiff bases bearing 2-aminothiophene derivatives obtained by conventional (Method A) and microwave irradiation (Method B) Methods.

Compound	R	R'	Yield (%)	
			Method A	Method A
4a	4-OCH ₃	-	86	85
4b	4-Cl	-	65.6	52
4c	4-F	-	54.9	66
4d	2,4-Cl	-	49	35
4e	2,6-Cl	-	56	83
4f	2-NO ₂	-	44	52
4g	3-NO ₂	-	65	78
4h	4-OH	-	45	79
4i	4-CH ₃	-	59	66
4j	4-N(CH ₃) ₂	-	31	55
4k	4-NO ₂	-	60	49
4l	3,4,5-OCH ₃	-	39	56
4m	H	-	49	44
4n	2,3-Cl	-	56	64
4o	4-Br	-	47	45
4p	-	4-NO ₂	64	50
4q	-	5-OCH ₃	23	71

3.2 Druglikeness prediction

The *druglikeness* of compounds (**4a-q**) was determined based on Lipinski's and Veber's rules. As can be seen in Table 2, none of the compounds violated Lipinski's rule of five: (presenting no more than 5 hydrogen bonding donors (HBD); presenting no more than 10 hydrogen bonding acceptors (HBA); having a molecular weight (MW) of less than 500 Da; and presenting partition coefficient (Log*P*) of less than 5).^{39,40} Likewise, none of the Weber parameters were breached: (presenting a polar surface area (PSA) of less than 140 Å; and presenting less than 10 rotatable bonds (*n*Rotb)).^{41,42} Based on these data, the compounds possess a drug candidate profile with good oral bioavailability.

Table 2. *Druglikeness* of compounds **4a-q** based on Lipinski and Weber rules.

Compound	Lipinski's				Weber's	
	log <i>P</i> ^a	HBA ^b	HBD ^c	MW ^d	<i>n</i> Rotb ^e	PSA ^f
4a	3.3	4	0	242.303	3	103.625
4b	4.0	3	0	246.722	2	102.450
4c	3.5	3	0	230.267	2	96.312
4d	4.6	3	0	281.167	2	112.753
4e	4.6	3	0	281.167	2	112.753
4f	3.2	5	0	257.274	3	106.800
4g	3.2	5	0	257.274	3	106.800
4h	3.0	4	1	228.276	2	96.941
4i	3.6	3	0	226.304	2	98.512
4j	3.4	4	0	255.346	3	110.636
4k	3.2	5	0	257.274	3	106.800
4l	3.3	3	0	212.277	2	92.147
4m	4.1	3	0	291.173	2	106.014
4n	4.0	3	0	246.722	2	102.450
4o	5.0	3	0	288.375	3	127.204

4p	3.7	5	1	296.311	3	122.690
4q	3.8	4	1	281.340	3	119.516

^apartition coefficient; ^bhydrogen bond acceptor; ^chydrogen bond donor; ^dmolecular weight; ^erotatable bond number; ^fpolar surface area.

3.3 Prediction of pharmacokinetic (ADMET) properties

In the process of drug discovery and development, prediction of pharmacokinetic properties is a common procedure which during the initial planning phase allows identification of compounds with poor ADMET properties. This reduces the number of compounds to be tested in the early stages of research, and also the number of compounds which might fail in clinical trials.⁴³⁻⁴⁵ pKCSM software was used to predict certain ADMET parameters for compounds **4a-q** (table with all results is available in the supplementary material).

The absorption of orally administered drugs depends on their ability to cross the gastrointestinal tract (GIT) walls.⁴⁶ Caco-2 cells extracted from human epithelial colorectal adenocarcinoma are widely used since these cells mimic the gastrointestinal epithelium. They present a validated assay system for oral absorption studies.⁴⁷⁻⁴⁹ Compounds with a Papp value of > 0.90 present high Caco-2 permeability, and so, the potential for GIT absorption.⁵⁰ The data presented in Table 3 show that compounds **4a-q**, (except those presenting nitro substituents: **4f**, **4g**, **4k** and **4p**), present high Caco-2 permeability, indicating that the compounds are highly likely to be absorbed by the GIT.

Skin permeation was also predicted, and most of compounds (except indolic derivatives **4p** and **4q**) demonstrated relatively low skin permeability, $\log K_p > -2.5$.⁵⁰ This value indicates that if administrated topically, the compounds will not be well absorbed by the skin.

Distribution analysis (concerning the ability to leave circulation and enter extravascular tissues) of compounds **4a-q** was based on blood brain barrier (BBB) and central nervous system (CNS) permeability (Table 3).

Blood brain barrier (BBB) permeability is an important parameter since it is composed of endothelial cells which regulate (as a protective function) the passage of compounds from the blood to the CNS.⁴⁶

According to the pkCSM model, compounds with a logBB of > 0.3 can readily cross the BBB while compounds with a logBB of < -1 present difficulty in distribution to the brain. Compounds with a logPS of > -2 can penetrate to the CNS, while those with a logPS of < -3 are considered unable to penetrate to the CNS.⁵⁰

With regard to distribution, compounds **4a-q** presented intermediate BBB permeability values, suggesting somewhat limited brain distribution, yet they were able to penetrate and likely present effects at the CNS level.

First pass metabolic characteristics depend on interaction with several microsomal enzymes (known as the cytochrome P450 (CYP450) superfamily.⁴⁶ These enzymes are mostly located in the liver being responsible for the majority of first-pass drug metabolism. CYP3A4 performs almost 50% of xenobiotic metabolism in humans and is highlighted.^{51, 52}

ADMET predictions revealed that compounds **4a-q** are substrate to CYP3A4 isoforms, and non-substrate to CYP2D6 isoforms, excepting compound **4q** for CYP3A4, and **4o** for CYP2D6. The compounds were also inhibitors of CYP1A2, CYP2C19, and CYP2C9 isoforms, and non-inhibitors of CYP2D6, and CYP3A4 isoforms, (except compound **4o** for CYP2C9, and **4p** for CYP3A4) (Table 3).

Excretion is another important pharmacokinetic parameter related to removal of an intact drug or its metabolites from the body, thus determining the amount of time the drug will remain in the organism as well as its volume of distribution.^{44, 51, 52}

This parameter was analyzed based on total clearance, (values were estimated, and from this, the rate of excretion can be predicted), and evaluation of the behavior of the compounds as a renal OCT2 substrate (Table 3). There is no delimited range for total clearance, the higher the value, the faster the excretion process will be.⁵⁰

OCT2 is a renal uptake transporter responsible for renal drug clearance.⁵³ The ability of a compound to bind to OCT2 is an indication of its clearance which (to achieve a steady-state in the blood plasma) is important for determining posology.⁵⁴ In accordance with Table 3, compounds **4a-q** were unable to interact with or bind OCT2, which limits excretion using this pathway.

Drug toxicity is the most common impairment of drug discovery and development.⁵⁵ Using pkCSM, induced toxicity was evaluated for the Schiff bases bearing 2-aminothiophenes (Table 3).

Genetic toxicity was assessed by the AMES test; screening compounds **4a-q** for mutagenic or non-mutagenic potential. Compounds that present a positive AMES test

result may be mutagenic.^{50, 56} The results revealed seven compounds with a negative result (probably not mutagenic), while ten compounds were positive, presenting risks of mutagenicity.

Cardiotoxicity was evaluated by testing whether the compounds might be hERG I and/or hERG II inhibitors. hERG channels play an important role in cardiac repolarization.⁵⁷⁻⁵⁹ hERG current inhibition is the most likely mechanism involved in drug-induced QT interval prolongation, and severe cardiac arrhythmias are an important reason for drug failures during preclinical study.^{50, 52} Excepting compounds **4o** and **4q** (which tested positive for hERG II inhibition), compounds **4a-q** did not test positive for either hERG I or hERG II inhibition,.

A principal motive for post-market removal of a medication is hepatotoxicity.⁶⁰ Drug-induced liver injury can lead to acute liver failure and even death.^{61, 62} In accordance with Table 3, compounds **4a-q** were unable to disrupt normal liver function and are therefore not considered hepatotoxic.

Skin sensitization is a potential adverse effect of drugs that are applied topically.⁵⁰ Table 3 reveals that the compounds **4a-q**, except **4m**, presented no skin sensitization potential.

Tetrahymena pyriformis is a protozoan whose toxicity is often used as a toxic endpoint. Compounds with logIGC values of > -0.5 are considered toxic.^{44, 50} All of the logIGC values were greater than -0.5 , and no compounds presented toxicity to *T. pyriformis*.

3.4 Antifungal activity

The *in vitro* antifungal activity of compounds **4a-q** together with fluconazole (reference drug) against 8 dermatophyte fungi strains including *Epidermophyton floccosum*, *Trichophyton tonsurans*, *T. mentagrophytes*, *T. rubrum*, and *Candida parapsilosis* ATCC 22019 (reference strain) was determined using the microdilution broth Method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) standard M38-A2.⁶³ The MIC (minimal inhibitory concentration) values are summarized in Table 3. For each antifungal susceptibility experiment, the inoculum controls presented clear detectable growth after the incubation period, indicating that all of the isolates were viable and that the conditions were suitable for

fungal growth. The MIC of fluconazole against the reference strain was 0.5 $\mu\text{g.mL}^{-1}$, confirming the assay's reproducibility.

The results revealed that all of the compounds exhibit antifungal activity in different degrees for at least one of the analyzed dermatophytes. The compounds with the better antifungal profiles were **4e**, **4f**, **4g**, **4k**, **4l**, **4m**, **4o** and **4p**, each presenting at least one MIC value ranging between 16 - 64 $\mu\text{g.mL}^{-1}$. This in some cases was better than or equivalent to the reference drug fluconazole. In the case of the reference drug fluconazole, it can be seen that (with the exception of the reference strain ATCC 22019 and *E. floccosum* 6754), all isolates were either resistant ($\text{MIC} \geq 64 \mu\text{g.mL}^{-1}$) or presented inhibited growth in a dose-dependent pattern ($\text{MIC} = 16$ or $32 \mu\text{g.mL}^{-1}$).⁶⁴

E. floccosum 6754 proved to be more resistant to the 2-aminothiophene derivatives than *E. floccosum* 6999, which in high concentrations ($\text{MICs} = 512$ and $1,024 \mu\text{g.mL}^{-1}$) was sensitive to only 6 compounds (**4a**, **4h**, **4i**, **4k**, **4m** and **4o**). *E. floccosum* 6999 was sensitive to 14 compounds, presenting MIC values of up to 16 $\mu\text{g.mL}^{-1}$ (for **4m**), being equipotent to fluconazole.

For all of the *Trichophyton* isolates tested, it was observed that about half of the compounds inhibited the growth of the fungi. Against *T. tonsurans*, compound **4m** presented MIC values of 32 $\mu\text{g.mL}^{-1}$, for both isolates, a better profile than that of the reference drug. Compounds **4e** and **4o** presented MIC values of 64 $\mu\text{g.mL}^{-1}$ against *T. tonsurans* 700, also superior to fluconazole. *T. rubrum* 6753 was the most sensitive isolate, with 5 compounds testing as equipotent to fluconazole ($\text{MIC} = 64 \mu\text{g.mL}^{-1}$ - **4k**, **4l** and **4p**) or better ($\text{MIC} = 32 \mu\text{g.mL}^{-1}$ - **4e** and **4o**).

Table 3. Antifungal activity of compounds 4a-q and fluconazole against dermatophyte isolates. Values expressed in $\mu\text{g.mL}^{-1}$. ^aFluconazole; ^bCollection of URM cultures; ^cAmerican Type Culture Collection; ^dGrowth at the highest concentration tested.

Compound	N° URM ^b								
		<i>Candida parapsilosis</i> ATCC ^c 22019	<i>Epidermophyton floccosum</i> 6999	<i>Epidermophyton floccosum</i> 6754	<i>Trichophyton tonsurans</i> 700	<i>Trichophyton tonsurans</i> 2822	<i>Trichophyton mentagrophytes</i> 5431	<i>Trichophyton mentagrophytes</i> 5432	<i>Trichophyton rubrum</i> 6753
4a		1.024	256	1.024	1.024	1.024	1.024	1.024	1.024
4b		1.024	128	G ^d	G	1.024	1.024	1.024	1.024
4c		512	256	G	256	G	256	512	G
4d		G	128	G	G	G	G	G	G
4e		G	G	G	64	256	G	G	32
4f		128	32	G	G	G	G	G	G
4g		G	64	G	G	G	G	G	128
4h		1.024	G	1.024	1.024	1.024	G	1.024	G
4i		G	G	51	G	G	128	512	G
4j		1.024	128	G	256	1.024	1.024	512	1.024
4k		1.024	1.024	512	512	G	G	G	64
4l		G	256	G	128	G	256	128	64
4m		G	16	512	32	32	G	256	G
4n		512	256	G	G	G	G	G	G
4o		128	512	512	64	1.024	128	1.024	32
4p		G	1.024	G	128	512	G	512	64
4q		128	G	G	G	G	G	G	G
Flu ^a		2	16	2	>64	32	32	64	64

^aFluconazole; ^bCollection of URM cultures; ^cAmerican Type Culture Collection;

^dGrowth at the highest concentration tested.

2.5 Cytotoxicity of selected compounds

The most active compounds (**4e**, **4f**, **4g**, **4k**, **4l**, **4m** and **4o**) were evaluated for cytotoxicity at concentrations of 1-100 μM , using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay⁶⁵ with three non-tumor cell lines (VERO (monkey kidney-epithelial cells), MRC-05 (immortalized non-tumor cell lines from human fibroblasts), and 3T3 (murine fibroblasts)).

No difference in cell viability was observed for any of the compounds, at any of the concentrations evaluated, after 72 h of incubation. All of the compounds presented similar profiles. None of the compounds showed toxicity for any of the cell lines to the highest concentration evaluated (100 μM), (graphs and table of cell viability for each cell line are available in the supplementary material).

3.6 Structure-activity relationships (SAR)

Based on the results of the antifungal activity in Table 4, the presence of nitro substituents (NO_2) attached to the arylidene moiety (as observed in compounds **4f**, **4g**, **4k** and **4p**) is important for the antifungal activity. The results demonstrate that all of the tested compounds containing nitro substituents presented better activity profiles, (with MIC values of less than or equal to the reference drug ($\text{MIC} \geq 64 \mu\text{g.mL}^{-1}$)). For the benzylidene derivatives presenting nitro substituents in positions 2, 3, and 4 (respectively compounds **4f**, **4g**, **4k**), it was not possible to associate substituent position with an increase in activity. For the indole methylidene derivatives (**4p** and **4q**), the presence of NO_2 in **4p** was essential for antifungal activity. Replacing NO_2 with an electron donor group (OCH_3 in **4q**) resulted in a compound without activity.

The importance of the NO_2 substituent for antifungal activity in Schiff bases bearing cycloalkyl[b]thiophene derivatives has been discussed by our group. In work reported by Mendonça Junior *et al.*²⁶, the compound 2-(4-nitrobenzylidene)-amino-4,5,6,7-tetrahydro-4*H*-benzo[b]thiophene-3-carbonitrile (6CN10) was found to be the most active against *Candida* and *Cryptococcus neoformans* strains.

In general, the presence of electron donor groups, observed in **4a** (4- OCH_3), **4h** (4- OH), **4i** (4- CH_3), **4j** (4- $\text{N}(\text{CH}_3)_2$) and **4q** (5- OCH_3), leads to poor antifungal activity. The only exception observed was compound **4l**, which presents three methoxyl

substituents (OCH₃) at positions 3, 4 and 5, being equipotent to fluconazole against *T. rubrum* 6753 (MIC = 64 µg.mL⁻¹), and against *T. tonsurans* 700 (MIC = 128 µg.mL⁻¹), where resistance to fluconazole is defined at an MIC ≥ 64 µg.mL⁻¹.

Similar results were observed in the study conducted by Wang *et al.*⁶⁶, where it was found that the presence of an electron-donating group such as 4-OCH₃, or 4-CH₃ was harmful to the antifungal activity of fenfuram-diarylamine hybrids against phytopathogenic fungi.

Both type and position of halogens at the benzylidene moiety present significant effects on antifungal activity. For the mono-substituted derivatives **4c** (4-F), **4b** (4-Cl), and **4o** (4-Br), the size of the halogen was a great influence on both activity and spectrum of action.

Compound **4o**, containing the bromine atom (with the highest atomic volume), was the only derivative causing sensitivity in all tested fungal isolates, and presenting better activity than fluconazole, when compared against the *T. tonsurans* 700 (MIC = 64 µg.mL⁻¹) and *T. rubrum* 6753 (MIC = 32 µg.mL⁻¹) isolates.

For the di-substituted halogenated derivatives **4d** (2,4-Cl), **4e** (2,6-Cl), and **4n** (2,3-Cl), the presence of the two chlorine atoms in the *ortho* position (2,6-Cl) increased the spectrum of activity for compound **4e**. Compounds **4d** and **4n** were only active against *E. floccosum* 6999, with respective MICs = 128 and 256 µg.mL⁻¹. Compound **4e** was active against *T. tonsurans* 2822 (MIC = 256 µg.mL⁻¹), *T. tonsurans* 700 (MIC = 64 µg.mL⁻¹), and *T. rubrum* 6753 (MIC = 32 µg.mL⁻¹). Its antifungal activity against these last two isolates was superior to fluconazole.

The importance of the presence of halogens for the antifungal activity of new drug candidates is widely described. In a study conducted by Sui *et al.*²³ the most active imine derivatives bearing a kakuol moiety against four phytopathogenic fungi were those presenting halogens, especially chlorine in position 2 (*ortho*). The same authors also demonstrated that the presence of two chlorine atoms is beneficial for both antifungal activity, and antibacterial activity against Gram-positive bacteria. Karthikeyan *et al.*²¹ also reports that Schiff bases containing 2,4-dichloro-5-fluorophenyl substituents present antifungal activity equal to fluconazole against strains of *Aspergillus flavus*, *A. fumigatus*, *Penicillium marneffeii*, and *T. mentagrophytes*. Echevarria reported that Schiff bases bearing a piperonyl moiety containing a halogen (Cl, Br, or I) in position 4 (*para*) were the most active compounds against *E. floccosum* and *T. rubrum*.²⁴

According to Mendonça Júnior *et al.*²⁶, the presence of halogens (chlorine and fluorine) is closely related to the chemical structures found in most azole derivatives, such as miconazole, clotrimazole, econazole, and ketoconazole. Halogens contribute to increase lipophilicity for the compounds, facilitating their membrane permeability, and allowing more intense hydrophobic interactions with specific enzymes and receptors.²¹

5. Conclusions

A series of novel Schiff bases bearing 2-aminothiophene derivatives was designed, synthesized and spectroscopically characterized. The use of microwave-assisted synthesis produced the compounds in shorter reaction times, with good yields, and using an inorganic base; thus being an eco-friendly procedure based on principles of green chemistry. The importance of the cycloalkyl ring attached to the 2-aminothiophene core for antifungal activity was investigated using molecular simplification. The cycloalkyl ring of cycloalka[*b*]thiophenes was therefore excluded, giving rise to C-4 and C-5 unsubstituted 2-aminothiophenes. The reduction of “molecular obesity”⁶⁷ (lower molecular weight (MW), and lipophilicity) promoted by the removal of the cycloalkyl ring resulted in compounds with better *druglikeness*, adequate pharmacokinetic (ADMET) profiles, low toxicity, and good antifungal profiles, in some cases better than the reference drug.

The structure-activity relationships revealed that the halogen (F, Cl, and Br) and nitro (NO₂) substituents in the benzylidene moiety led to the most active compounds.

Taken together, these results demonstrate that the molecular simplification strategy was a success, improving *druglikeness*, pharmacokinetic profiles, and water solubility; while increasing antifungal activity for Schiff bases bearing 2-aminothiophene derivatives, thus making them promising lead compounds for the development of new antifungal drugs.

Supplementary Information

When applicable, use this section to inform the reader that “Supplementary Information (detail here the kind of information) is available free of charge at <http://jbcs.sbq.org.br>”.

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Author Contributions

Isadora S. Luna was responsible for data curation, formal analysis, investigation, writing original draft; Wendell W. Neves for data curation, formal analysis, investigation, writing original draft; Reginaldo G. de Lima- Neto for data curation, resources, writing review; Amanda P. B. Albuquerque for formal analysis, investigation, writing original draft; Maira G. R. Pitta, for funding acquisition, resources, validation, review; Moacyr J. B. de Melo Rego for funding acquisition, resources, validation, review; Rejane P. Neves for funding acquisition, resources, validation, review; Marcus T. Scotti for conceptualization, resources, software, validation, review and editing; Francisco J. B. Mendonca-Junior, for conceptualization, funding acquisition, resources, project administration, validation, review and editing.

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Supplementary Information

Design, synthesis and antifungal activity of new Schiff bases bearing 2-aminothiophene derivatives obtained by molecular simplification

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Table S1. Pharmacokinetics (ADMET) parameters of compounds **4a-q**.

Cpd	ABSORPTION			DISTRIBUTION			METABOLISM									
	Water solubility	Caco-2 permeability	Skin Permeability	Fraction unbound (human)	Bloond brain barrier permeability	Central Nervous System permeability	2D6	3A4	1A2	2C19	2C9	2D6	3A4			
							Substrate			Inhibitor						
							Categorical (Yes/No)									
(log mol.L ⁻¹)	(log Papp in 10 ⁻⁶ cm.s ⁻¹)	Numeric (log Kp)	Numeric (Fu)	(logBB)	Numeric (LogPS)											
4a	-4.146	1.376	-2.076	0.074	0.137	-1.472	No	Yes	Yes	Yes	Yes	No	No			
4b	-4.814	1.46	-1.727	0.059	0.265	-1.37	No	Yes	Yes	Yes	Yes	No	No			
4c	-4.045	1.588	-1.788	0.092	0.258	-1.396	No	Yes	Yes	Yes	Yes	No	No			
4d	-5.679	1.423	-1.814	0.035	0.232	-1.358	No	Yes	Yes	Yes	Yes	No	No			
4e	-5.588	1.598	-1.845	0.021	0.203	-1.369	No	Yes	Yes	Yes	Yes	No	No			
4f	-4.494	0.815	-2.456	0.034	-0.444	-1.907	No	Yes	Yes	Yes	Yes	No	No			
4g	-4.468	0.779	-2.45	0.021	-0.422	-1.889	No	Yes	Yes	Yes	Yes	No	No			
4h	-3.236	1.342	-2.02	0.114	0.014	-1.864	No	Yes	Yes	Yes	Yes	No	No			
4i	-4.392	1.453	-1.688	0.075	0.278	-1.37	No	Yes	Yes	Yes	Yes	No	No			
4j	-4.51	1.406	-1.894	0.088	0.173	-1.402	No	Yes	Yes	Yes	Yes	No	No			
4k	-4.501	0.876	-2.452	0.031	-0.435	-1.891	No	Yes	Yes	Yes	Yes	No	No			
4l	-3.996	1.559	-1.731	0.076	0.259	-1.393	No	Yes	Yes	Yes	Yes	No	No			
4m	-4.957	1.459	-1.735	0.052	0.263	-1.37	No	Yes	Yes	Yes	Yes	No	No			
4n	-4.886	1.478	-1.737	0.064	0.221	-1.388	No	Yes	Yes	Yes	Yes	No	No			
4o	-6.231	1.601	0.19	0.065	0.19	-1.11	Yes	Yes	Yes	Yes	No	No	No			
4p	-4.472	0.089	-2.782	0.05	-0.439	-1.811	No	Yes	Yes	Yes	Yes	No	Yes			
4q	-4.34	1.336	-2.683	0.072	0.04	-1.815	No	No	Yes	Yes	Yes	No	No			

Table S1. Continued.

Cpd	EXCRETION		TOXICITY						
	Total clearance	Renal OCT2 substrate	AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Hepatotoxicity	Skin Sensitisation	<i>T. pyriformis</i> toxicity
	Numeric (log ml/min/kg)	(Yes/No)	(Yes/No)	Numeric (log mg/Kg/day)	(Yes/No)	(Yes/No)	(Yes/No)	(Yes/No)	Numeric (log ug.L ⁻¹)
4a	0.13	No	No	0.675	No	No	No	No	2.075
4b	0.085	No	Yes	0.61	No	No	No	No	2.869
4c	0.066	No	No	0.607	No	No	No	No	2.613
4d	0.201	No	No	0.608	No	No	No	No	3.269
4e	0.27	No	No	0.55	No	No	No	No	3.169
4f	0.321	No	Yes	0.397	No	No	No	No	2.217
4g	0.241	No	Yes	0.389	No	No	No	No	2.232
4h	0.105	No	Yes	0.548	No	No	No	No	2.285
4i	0.159	No	Yes	0.616	No	No	No	No	2.594
4j	0.188	No	Yes	0.564	No	No	No	No	2.982
4k	0.117	No	Yes	0.388	No	No	No	No	2.217
4l	0.219	No	Yes	0.568	No	No	No	No	2.204
4m	0.064	No	Yes	0.605	No	No	No	Yes	2.925
4n	0.207	No	Yes	0.606	No	No	No	No	2.857
4o	0.150	No	No	0.485	No	Yes	No	No	0.875
4p	0.466	No	No	0.099	No	No	No	No	1.31
4q	0.323	No	No	0.246	No	Yes	No	No	1.61

Figure S1. Cytotoxicity of the most active compounds (**4e**, **4f**, **4g**, **4k**, **4l**, **4m** and **4o**) against VERO MRC-05 and 3T3 cell lines using MTT assay. The data are expressed in percentages of cell viability.

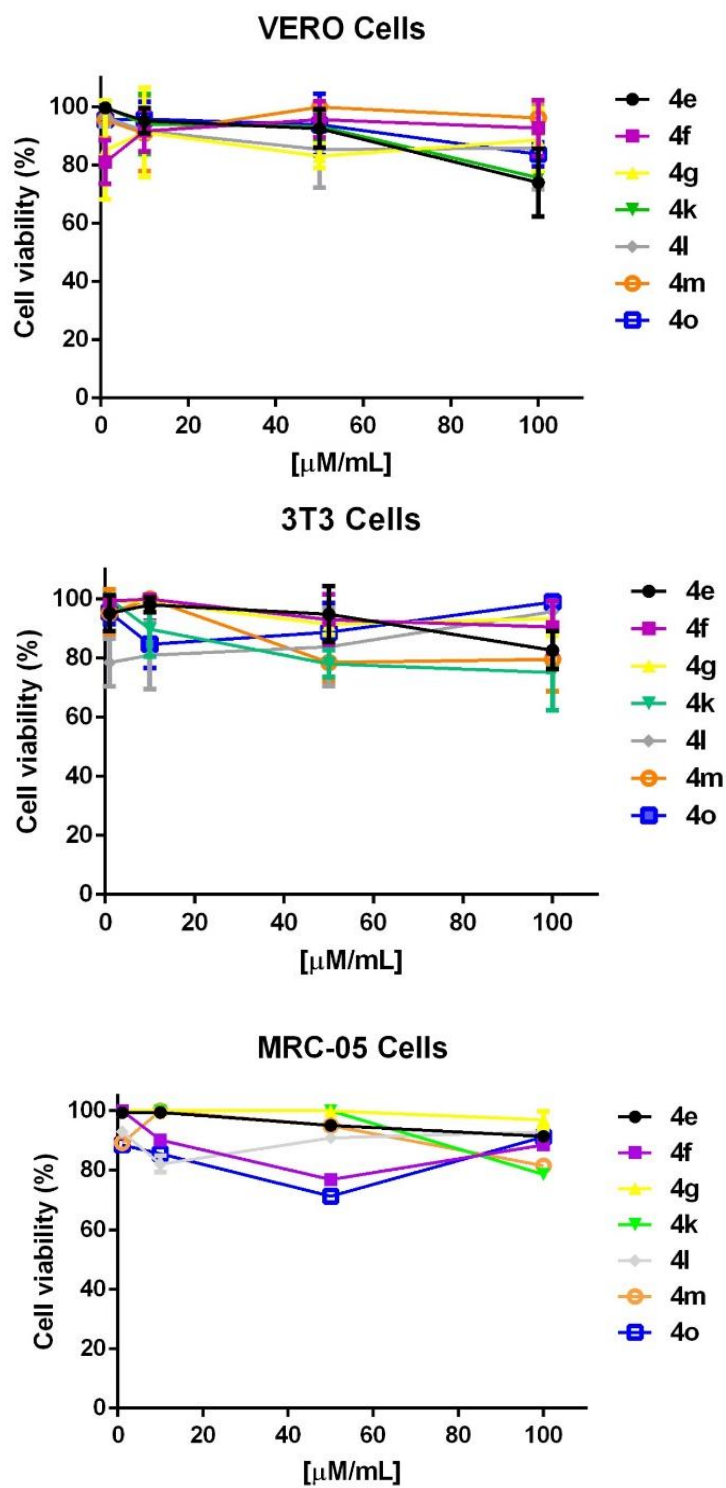


Table S2. Cellular cytotoxicity demonstrated by IC₅₀ concentration against the non-tumor VERO, MRC-05 and 3T3 cell lines.

Compounds	Cell lines		
	VERO	MRC-05	3T3
4e	>100±0	>100±0	>100±0
4f	>100±0	>100±0	>100±0
4g	>100±0	>100±0	>100±0
4k	>100±0	>100±0	>100±0
4l	>100±0	>100±0	>100±0
4m	>100±0	>100±0	>100±0
4o	>100±0	>100±0	>100±0

Figure S3. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **4a**

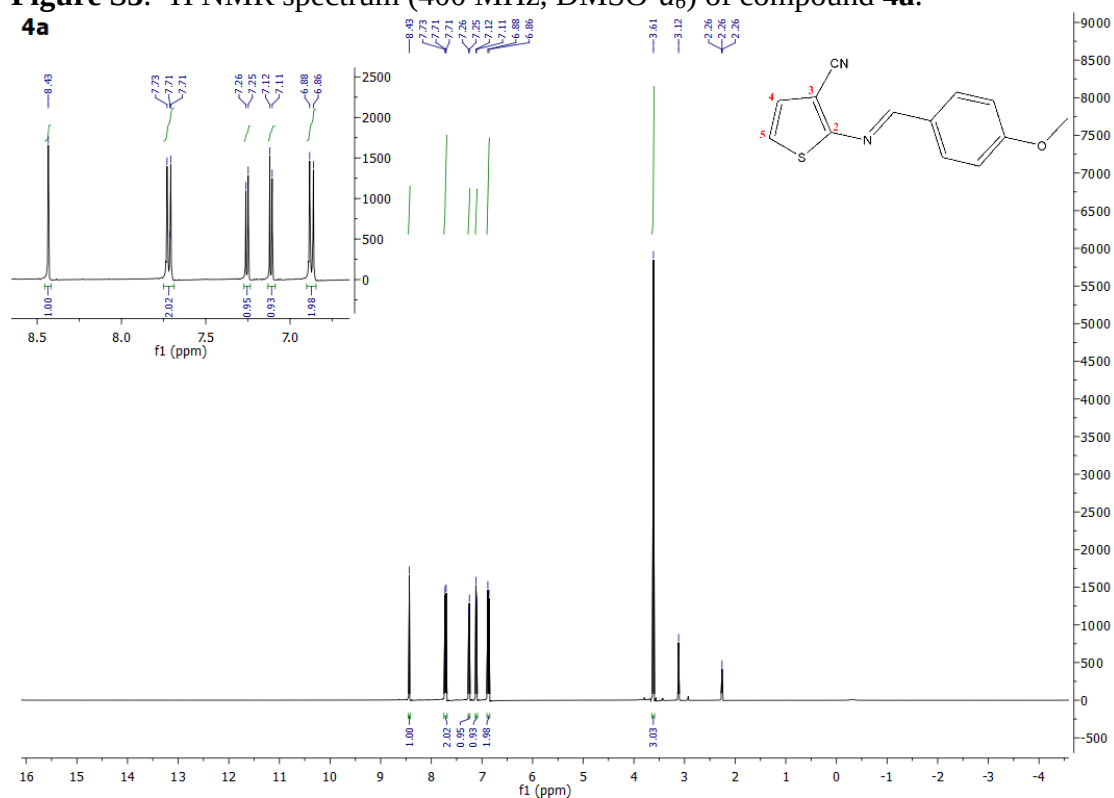


Figure S4. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4a**

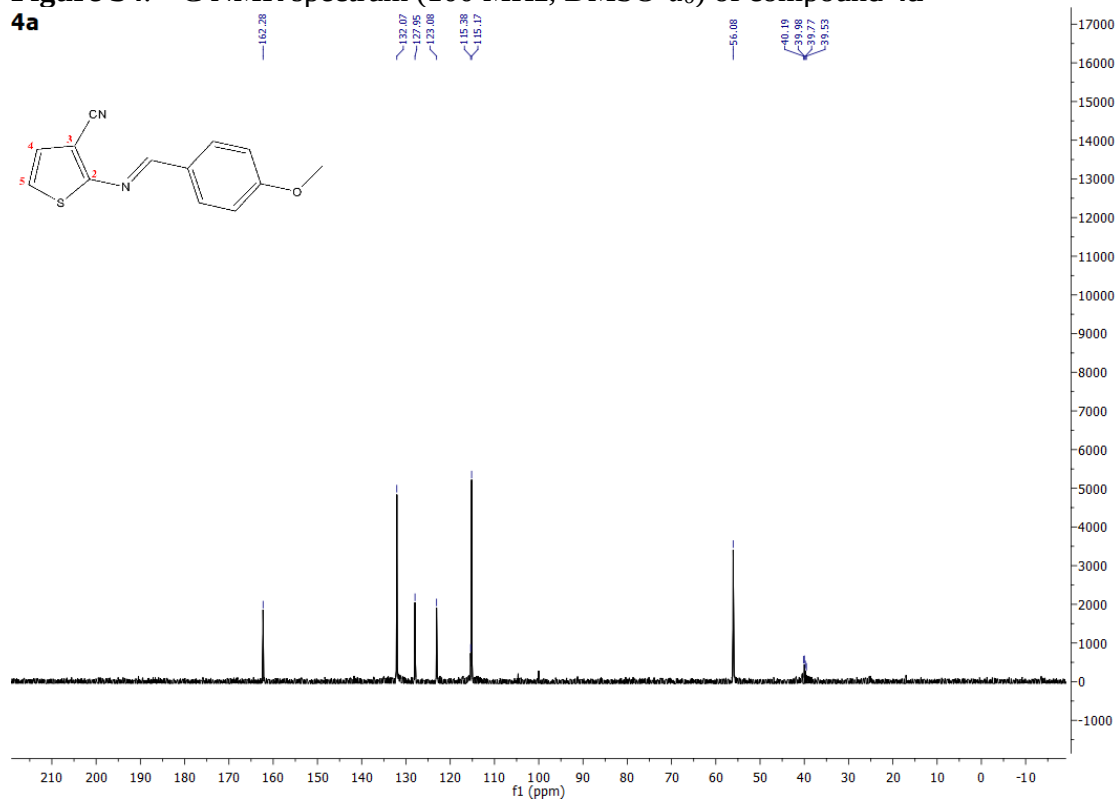


Figure S5. HSQC spectrum of compound **4a**

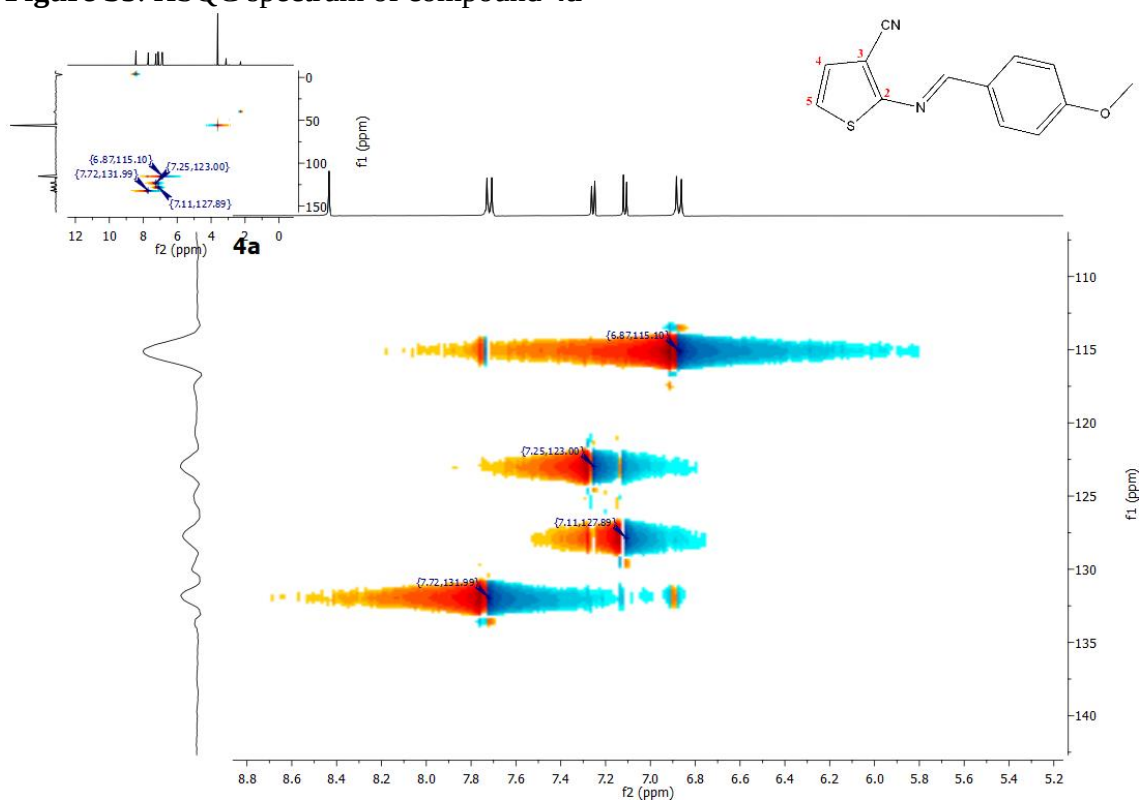
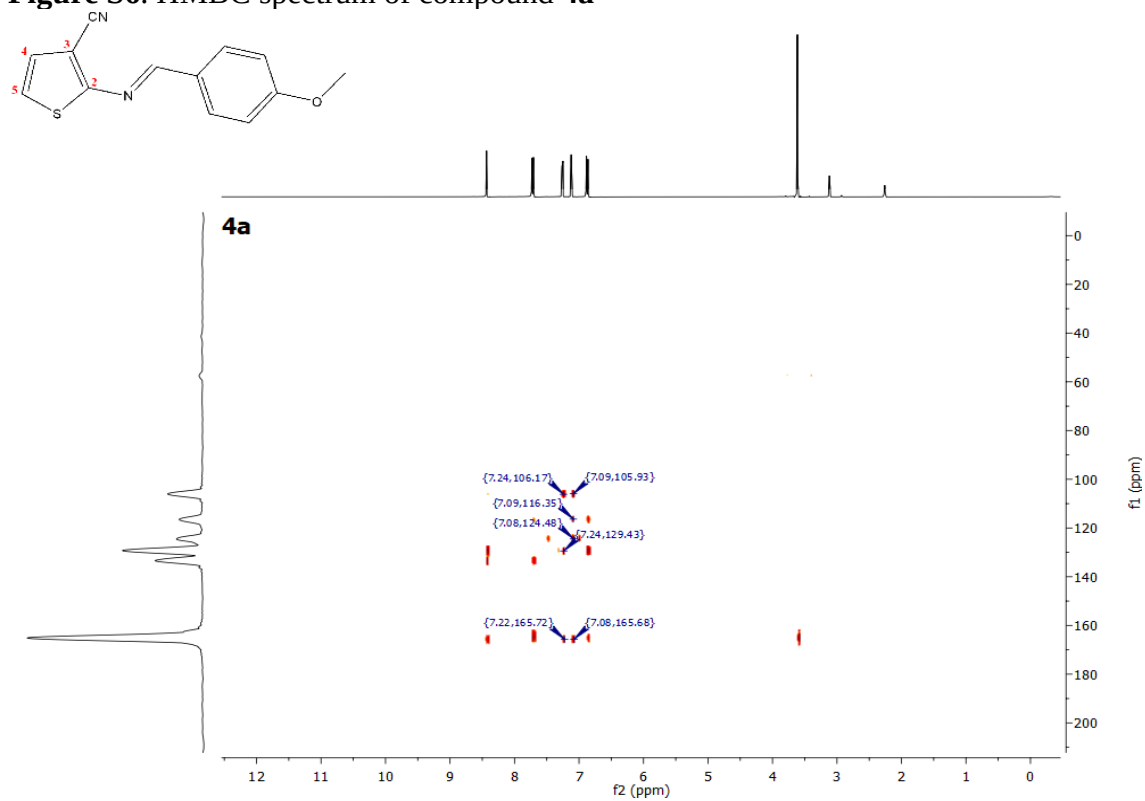


Figure S6. HMBC spectrum of compound **4a**



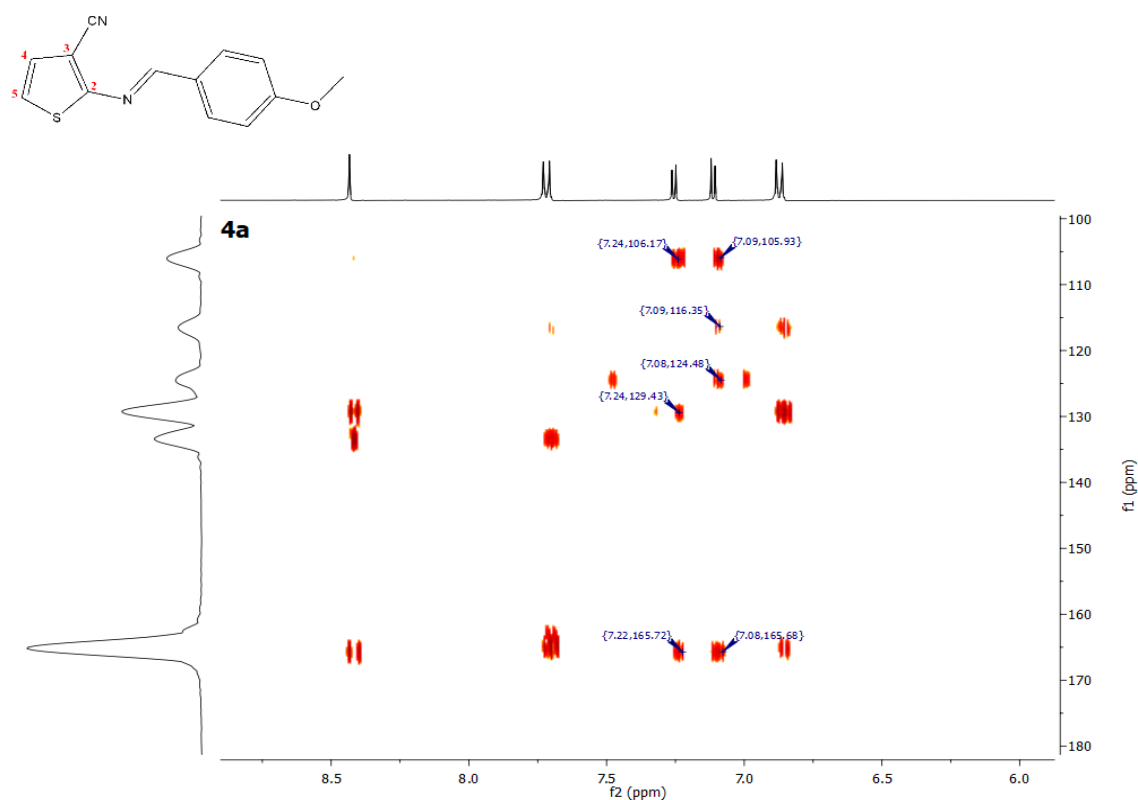


Figure S7. FTIR (KBr) spectrum of compound **4a**

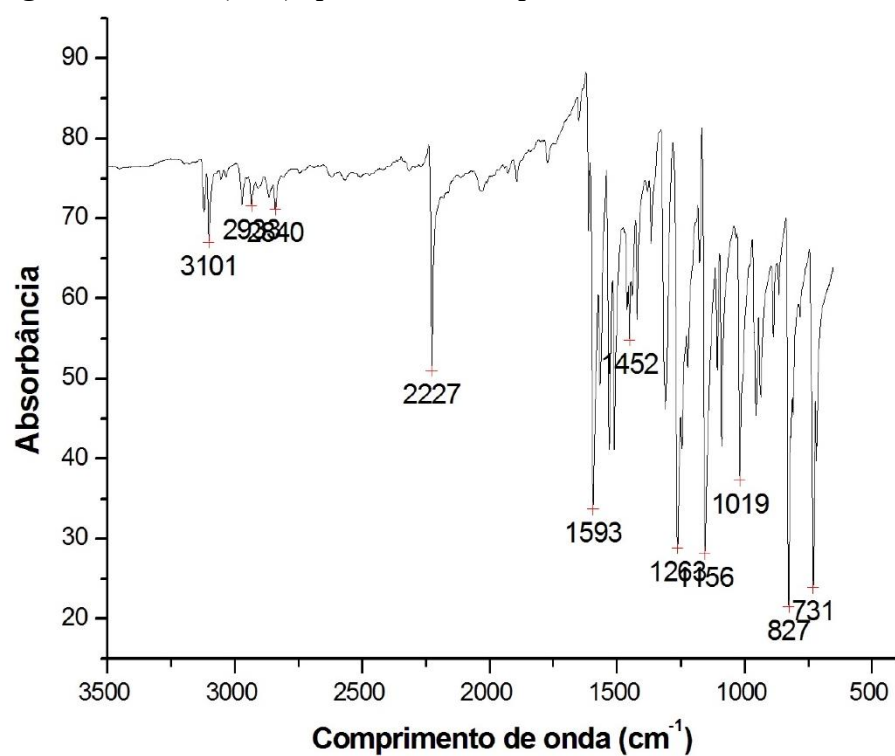


Figure S8. Mass spectrum of compound **4a**

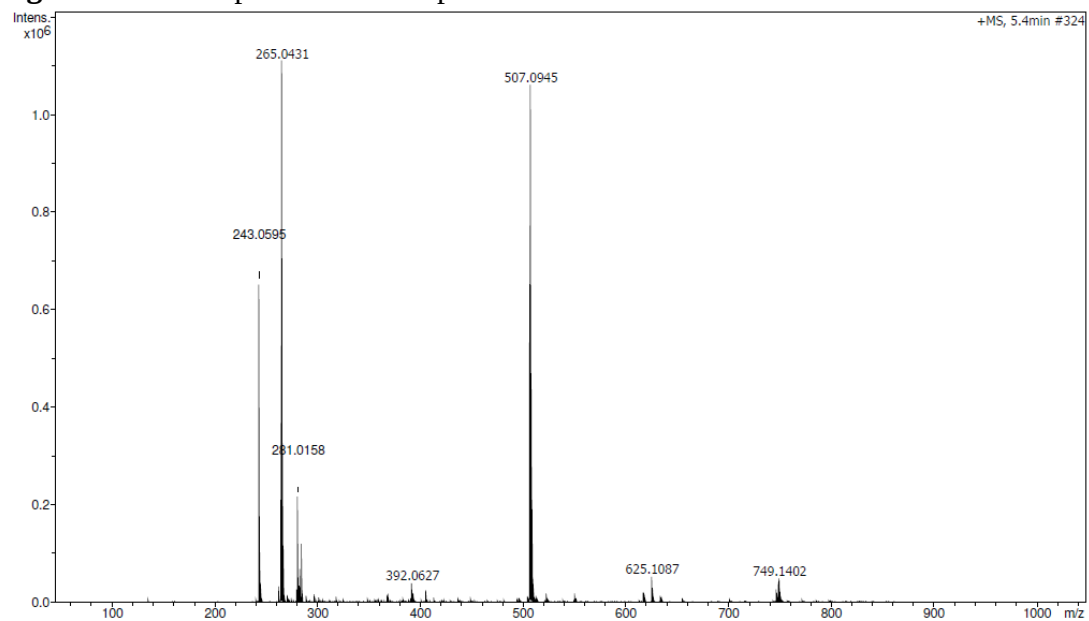


Figure S9. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4b**

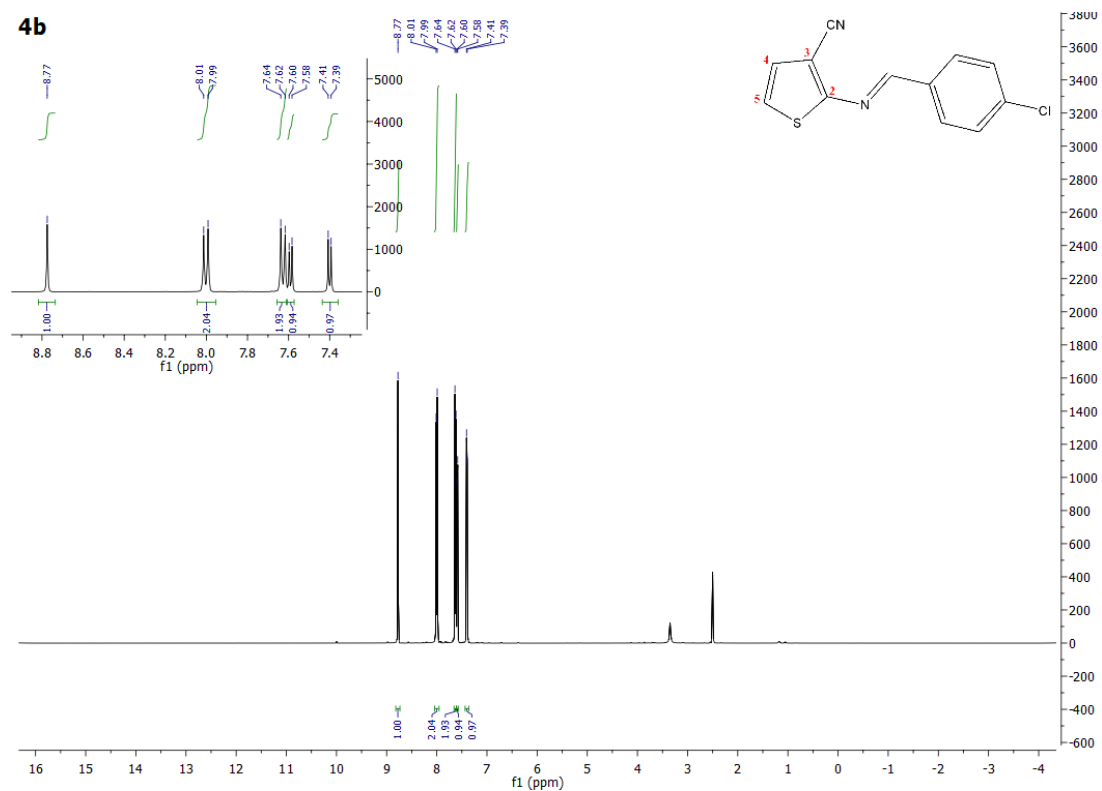


Figure S10. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4b**

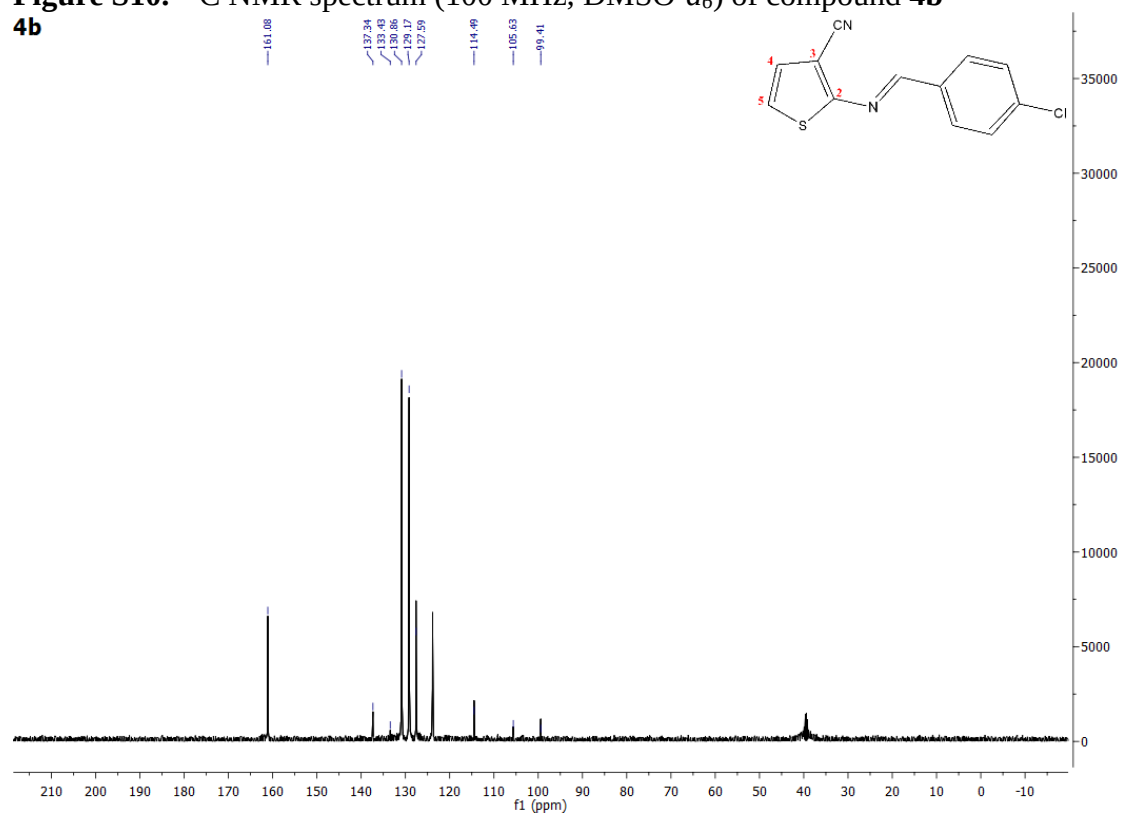


Figure S11. HSQC spectrum of compound **4b**

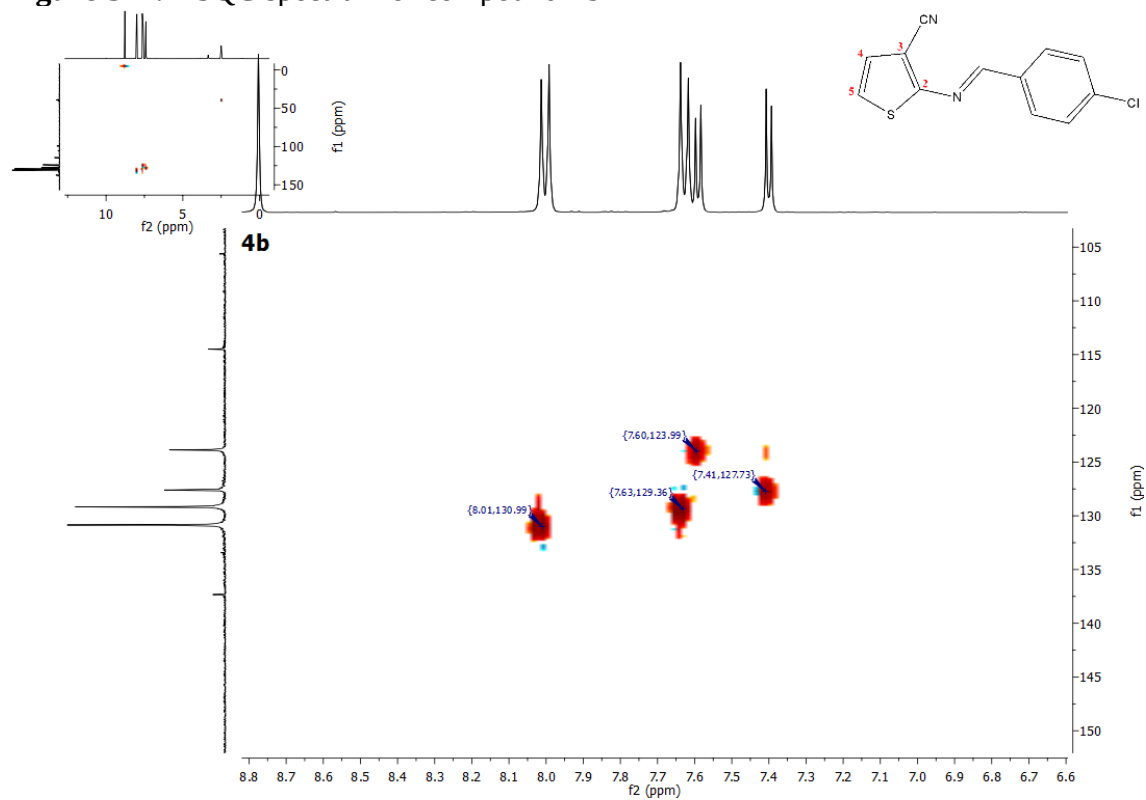


Figure S12. HMBC spectrum of compound **4b**

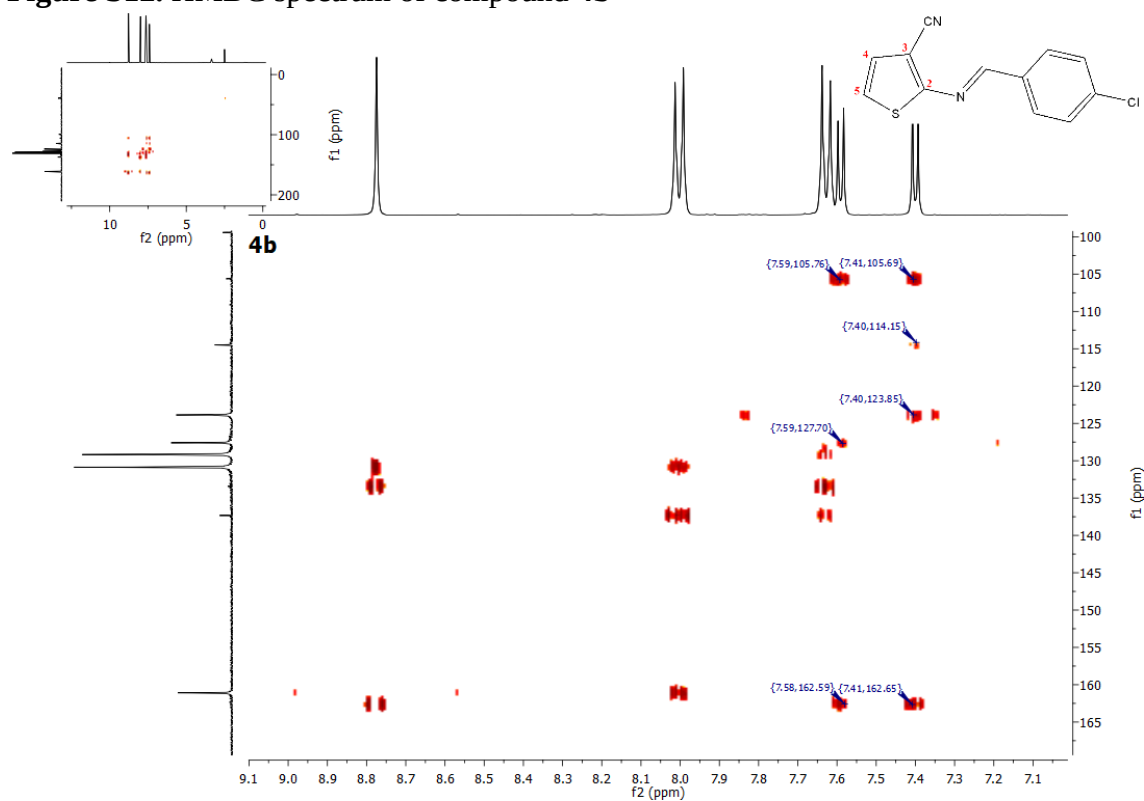


Figure S13. FTIR (KBr) spectrum of compound **4b**

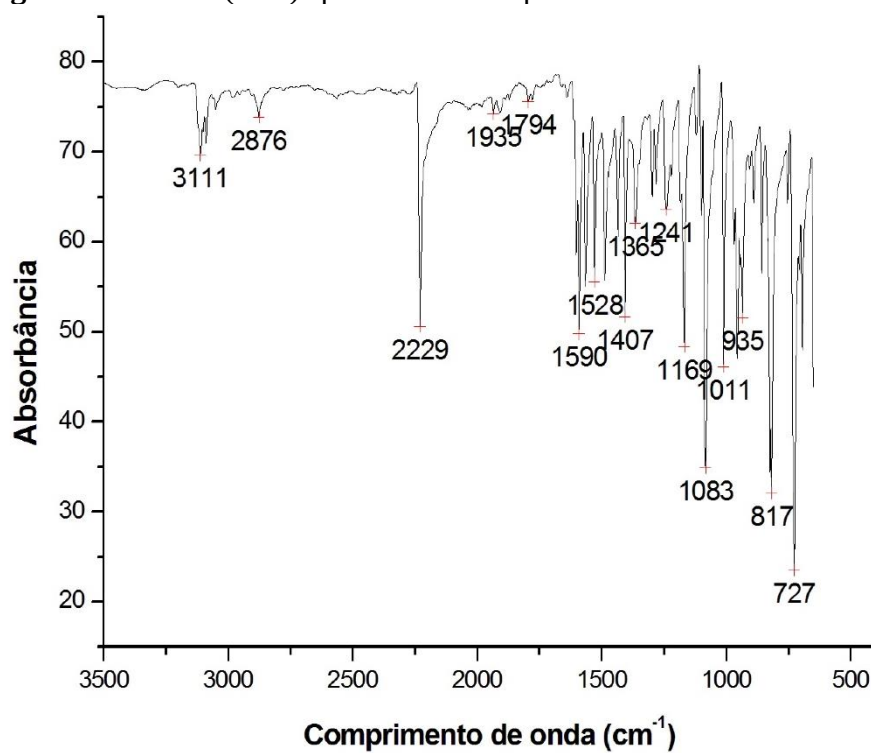


Figure S14. Mass spectrum of compound **4b**

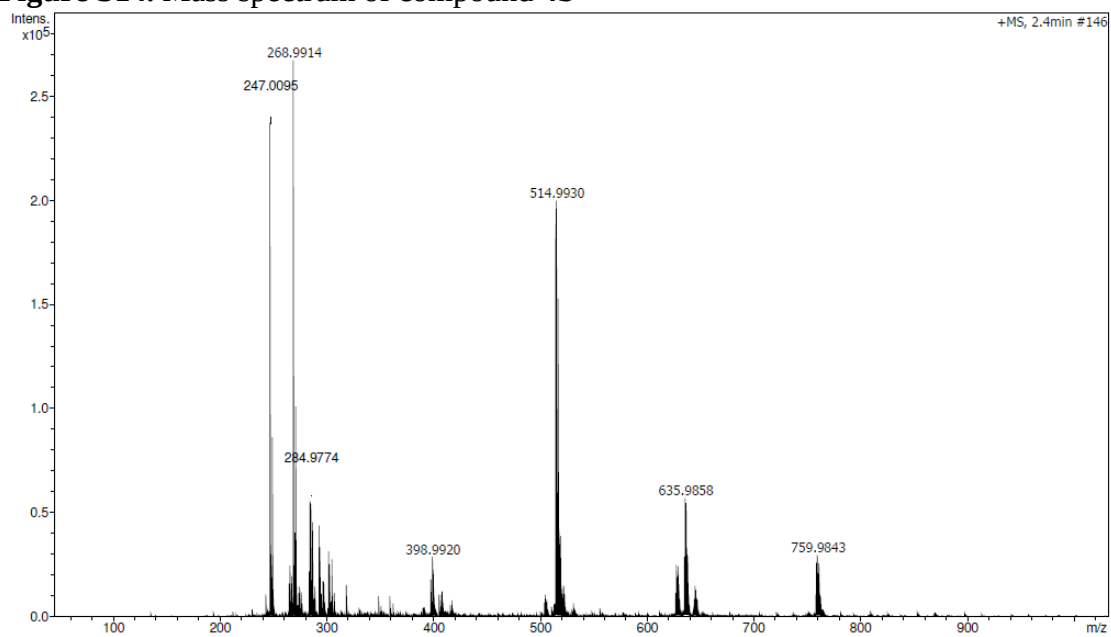


Figure S15. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4c**

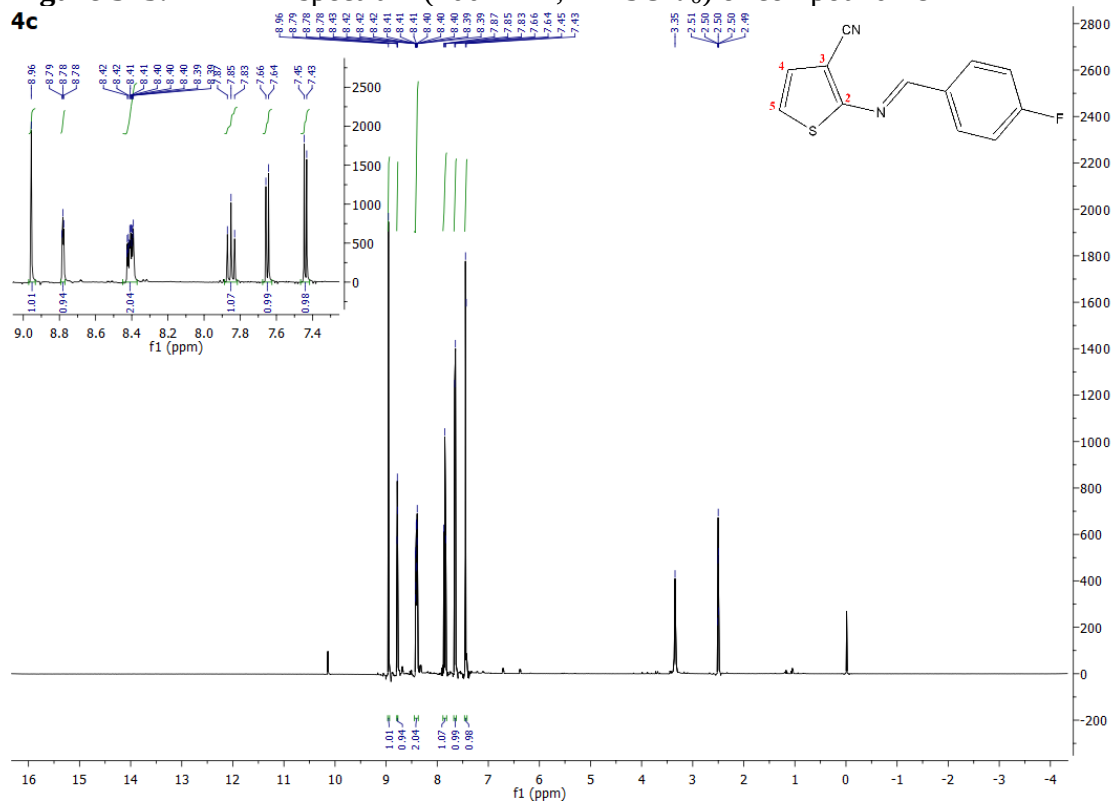


Figure S16. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **4c**

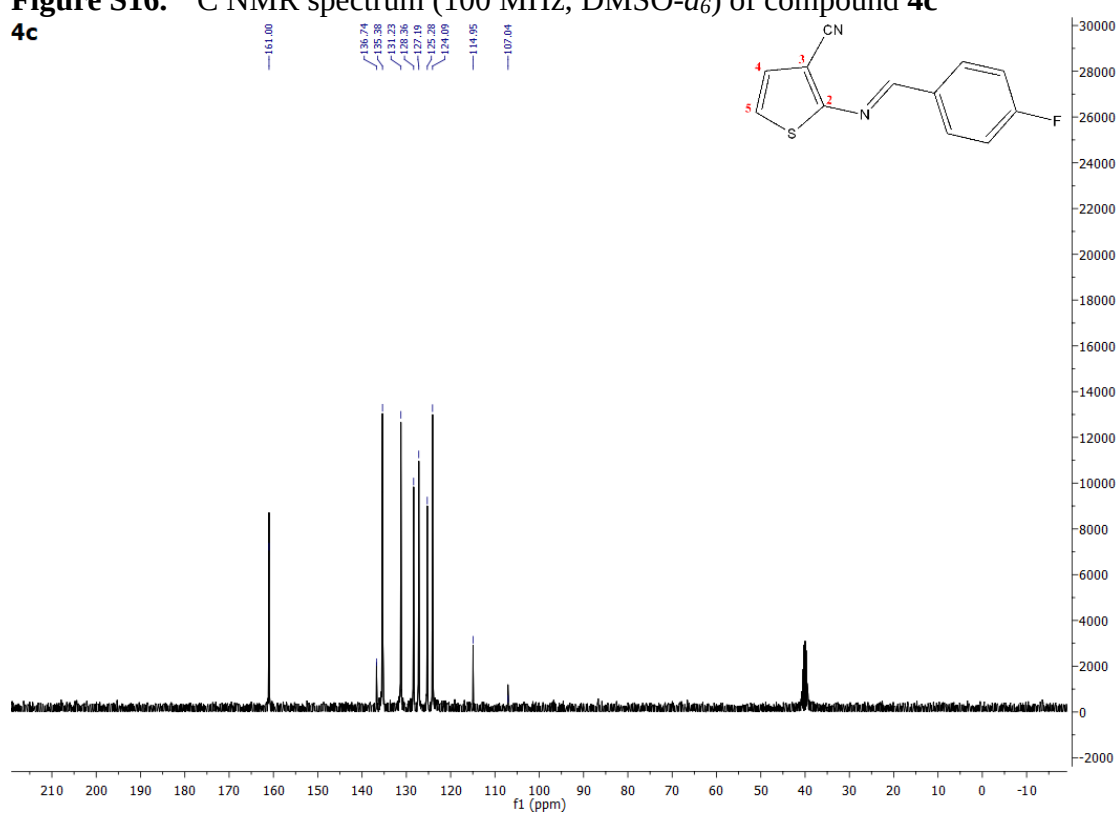


Figure S17. HSQC spectrum of compound **4c**

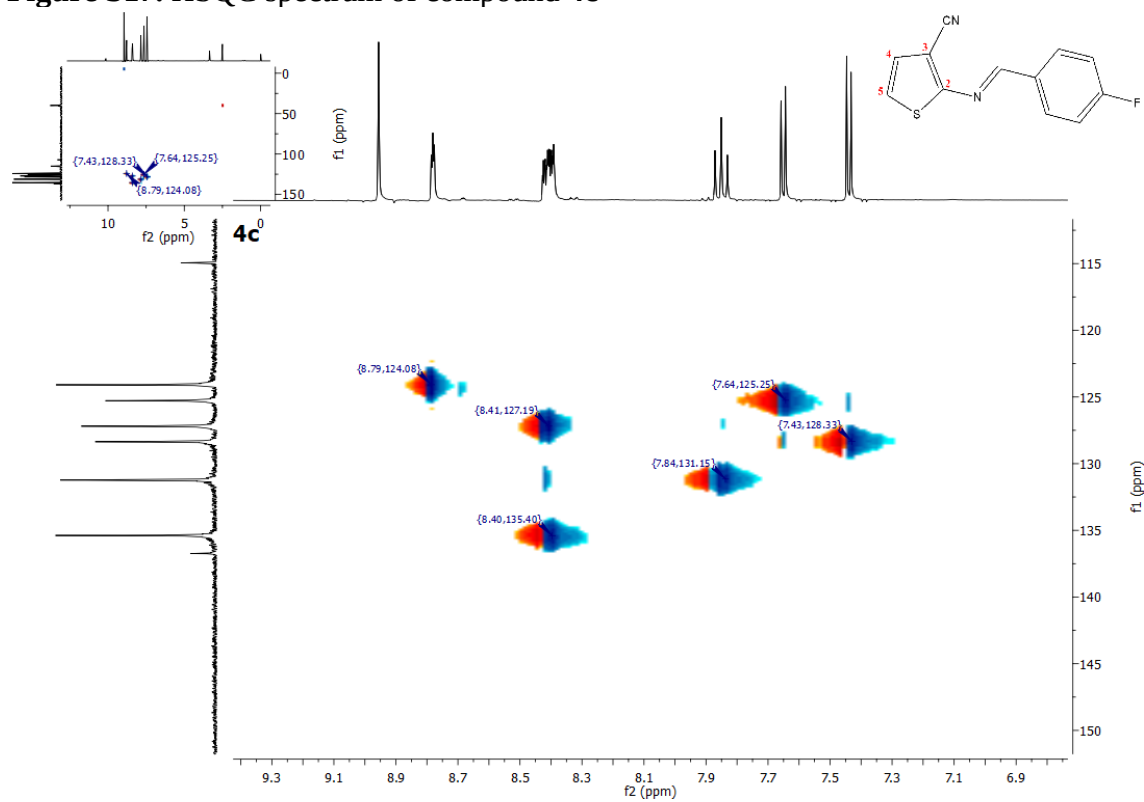


Figure S18. HMBC spectrum of compound **4c**

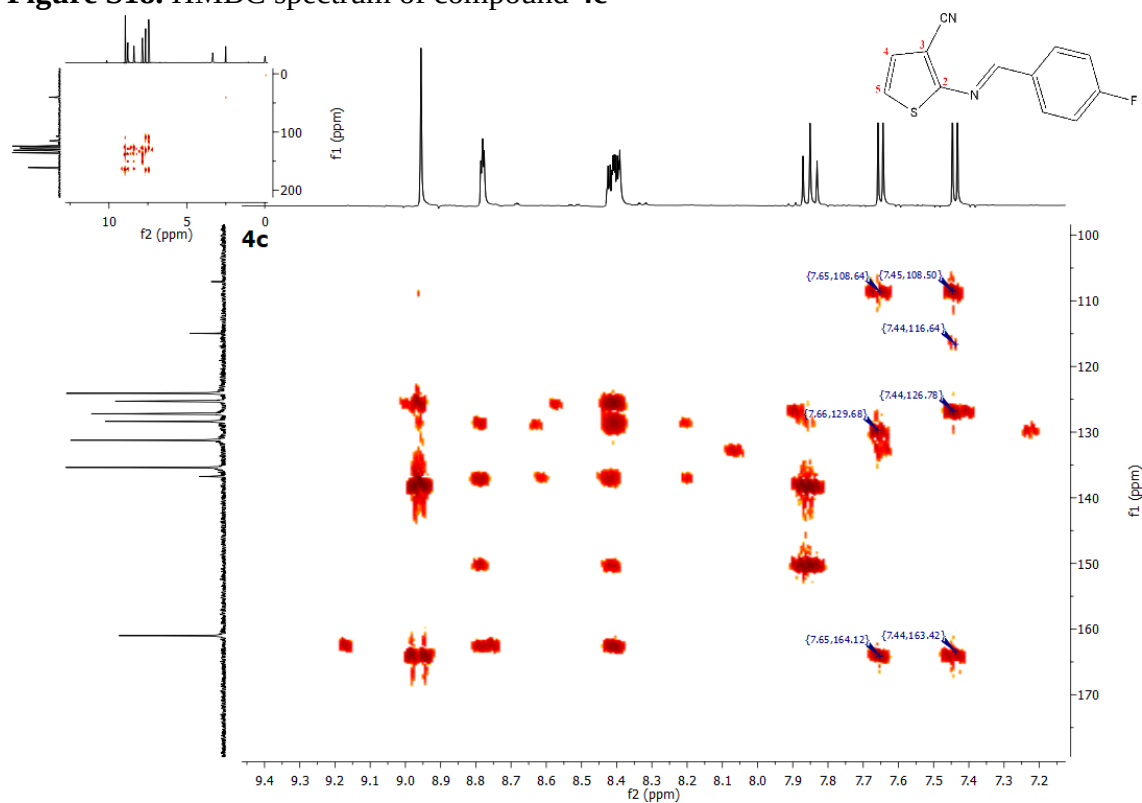


Figure S19. FTIR (KBr) spectrum of compound **4c**

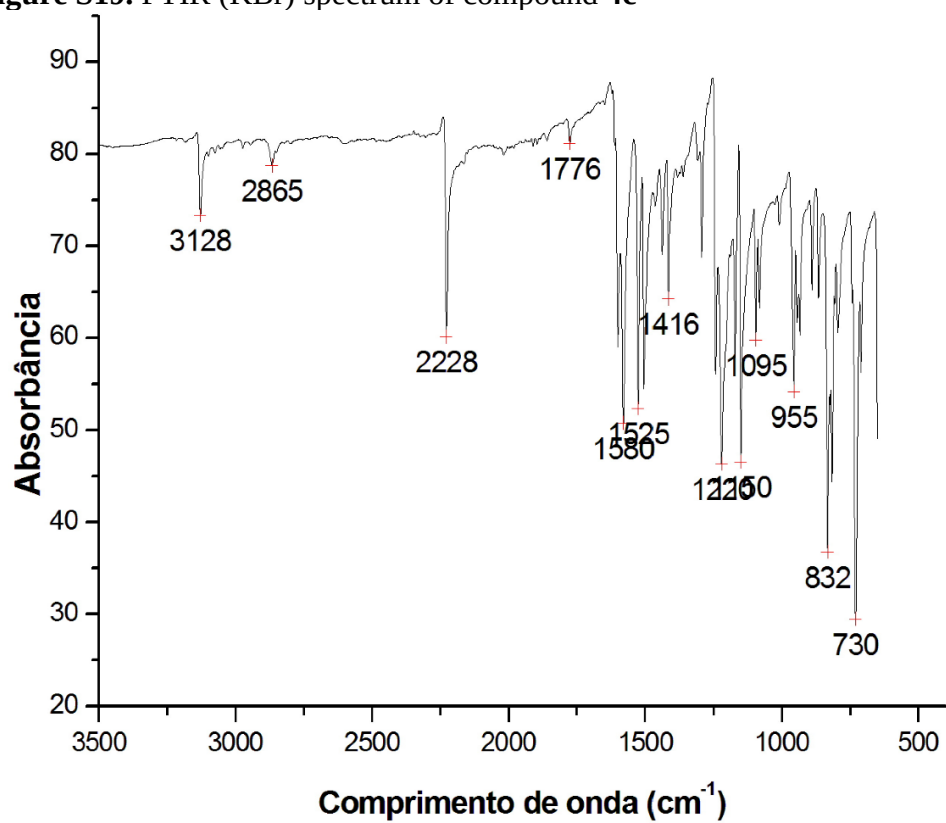


Figure S20. Mass spectrum of compound **4c**

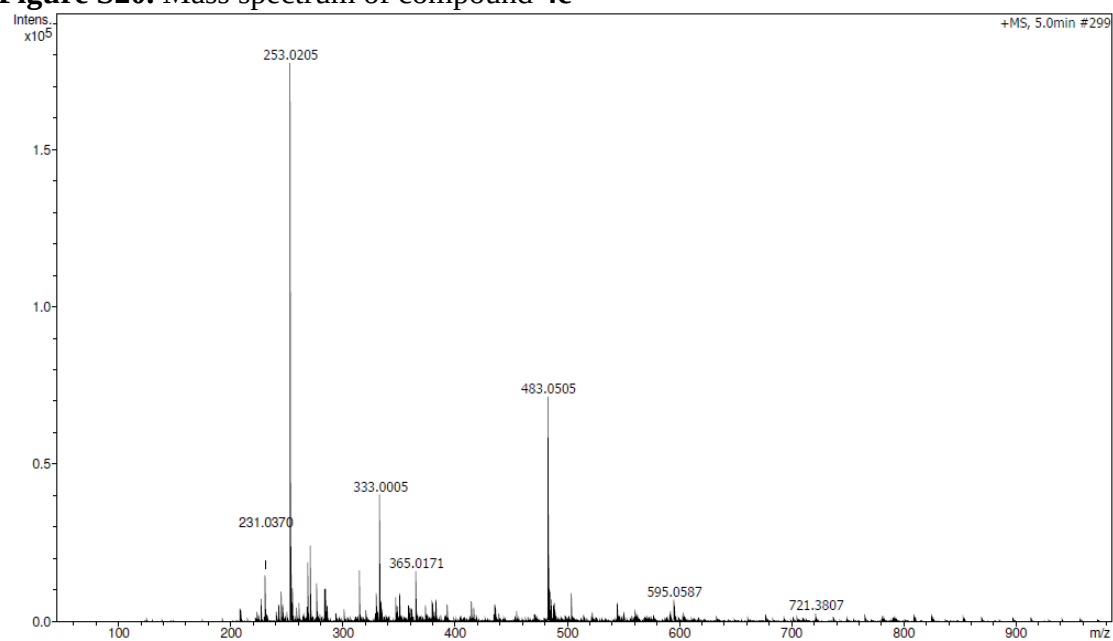


Figure S21. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4d**

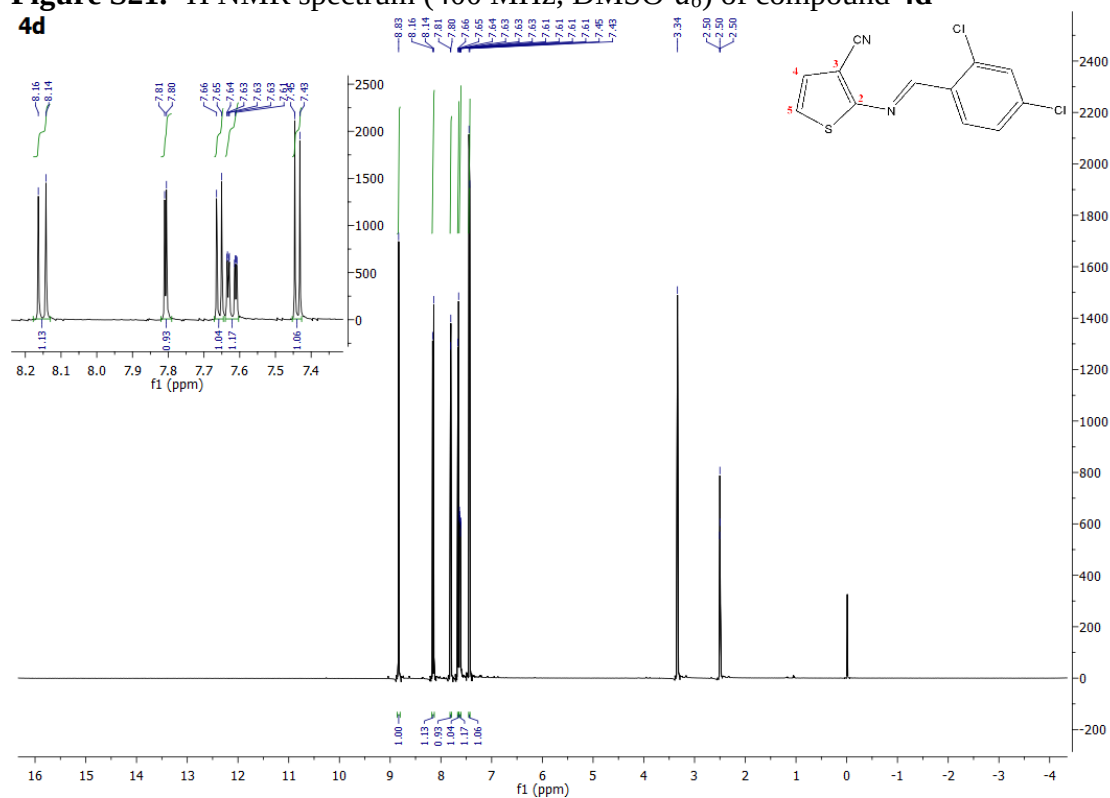


Figure S22. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **4d**

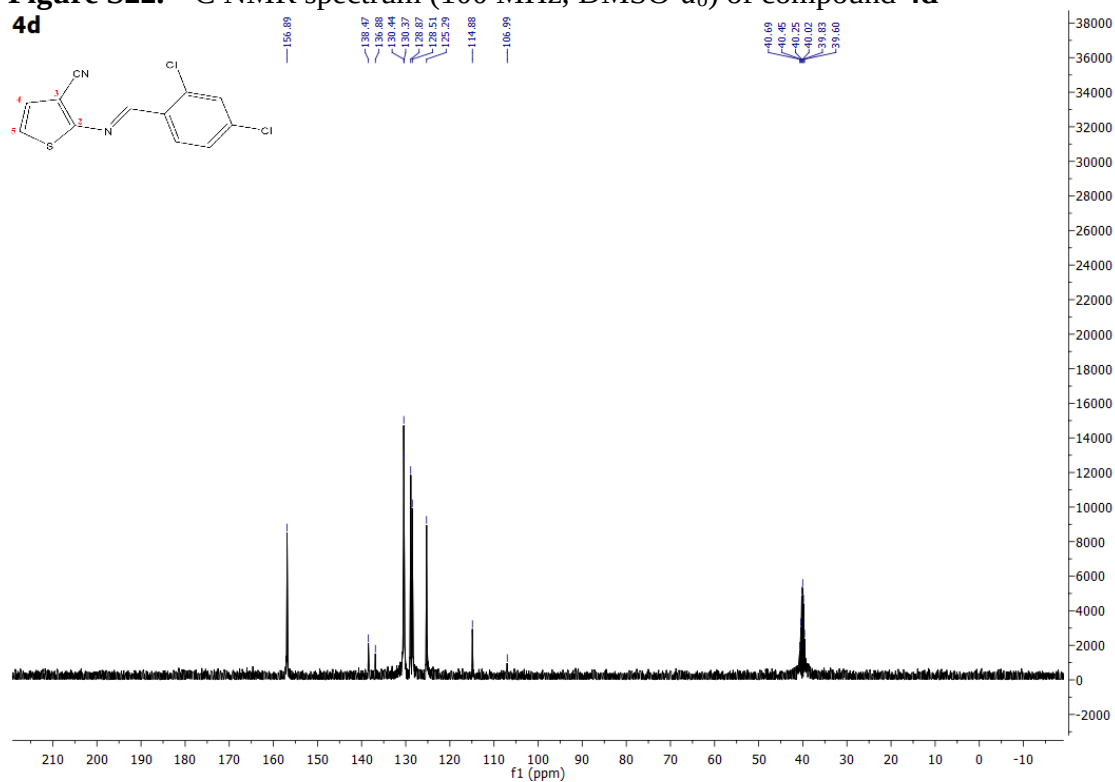


Figure S23. HSQC spectrum of compound **4d**

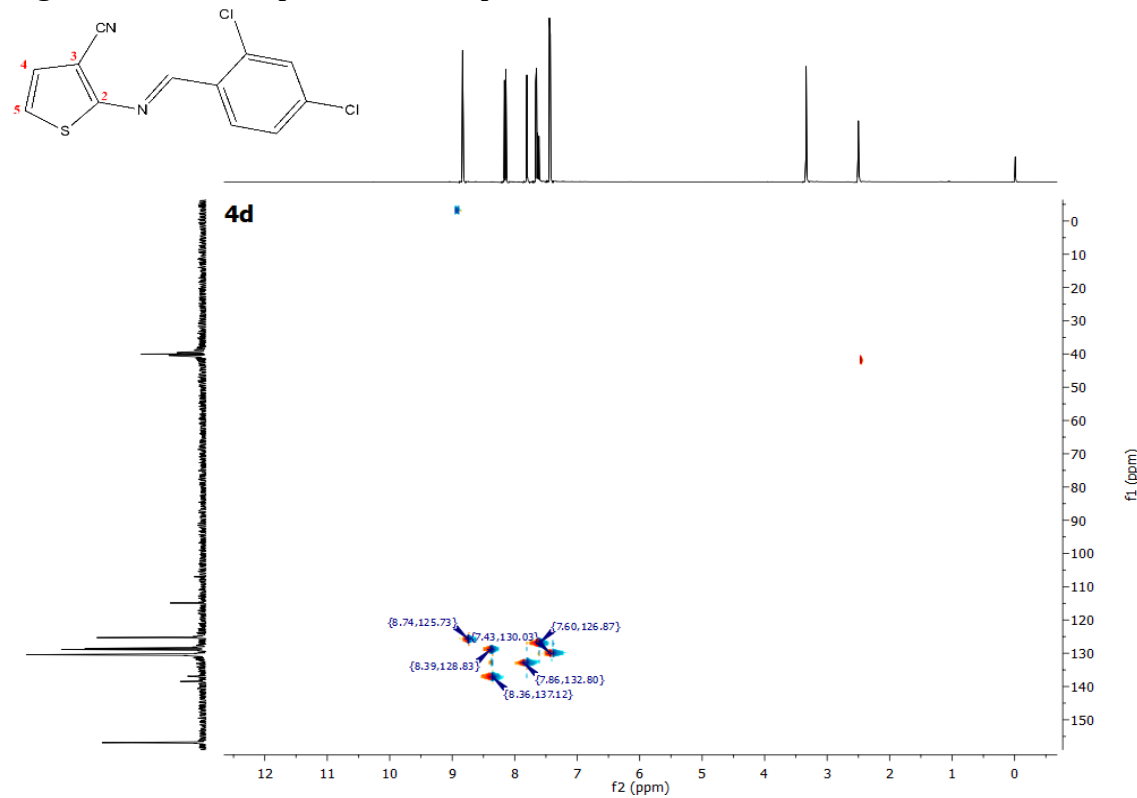


Figure S24. HMBC spectrum of compound **4d**

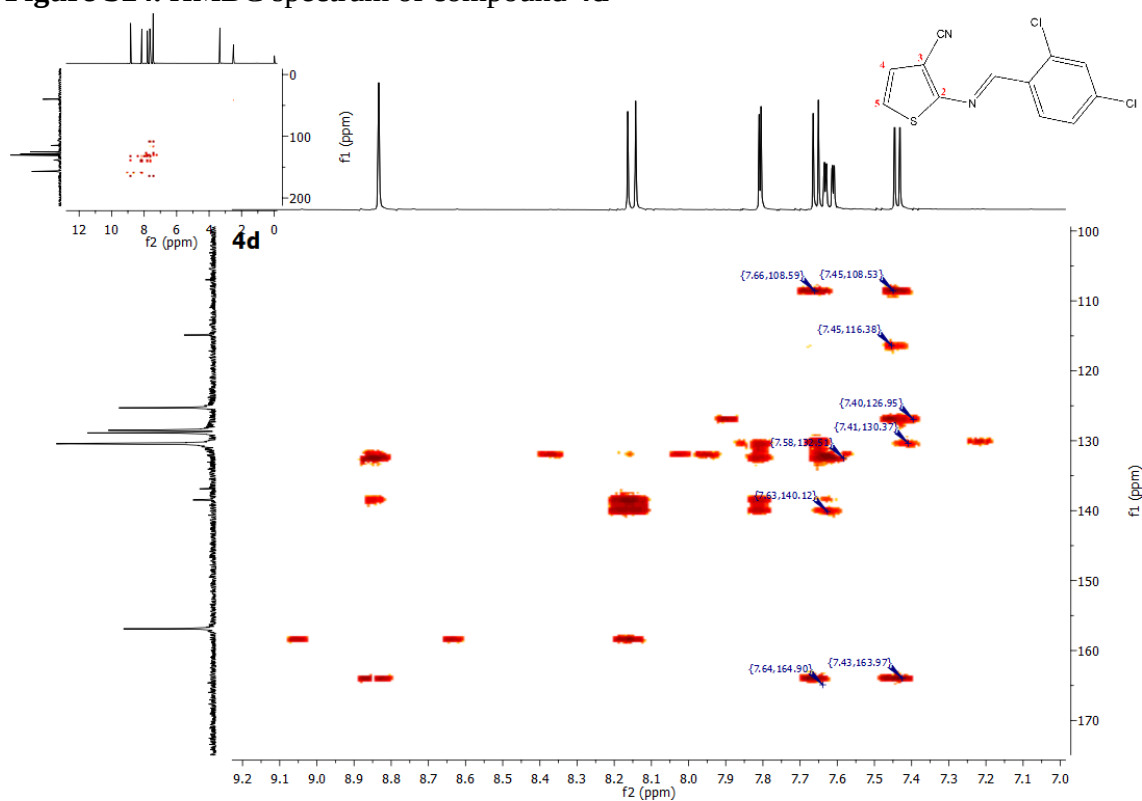


Figure S25. FTIR (KBr) spectrum of compound **4d**

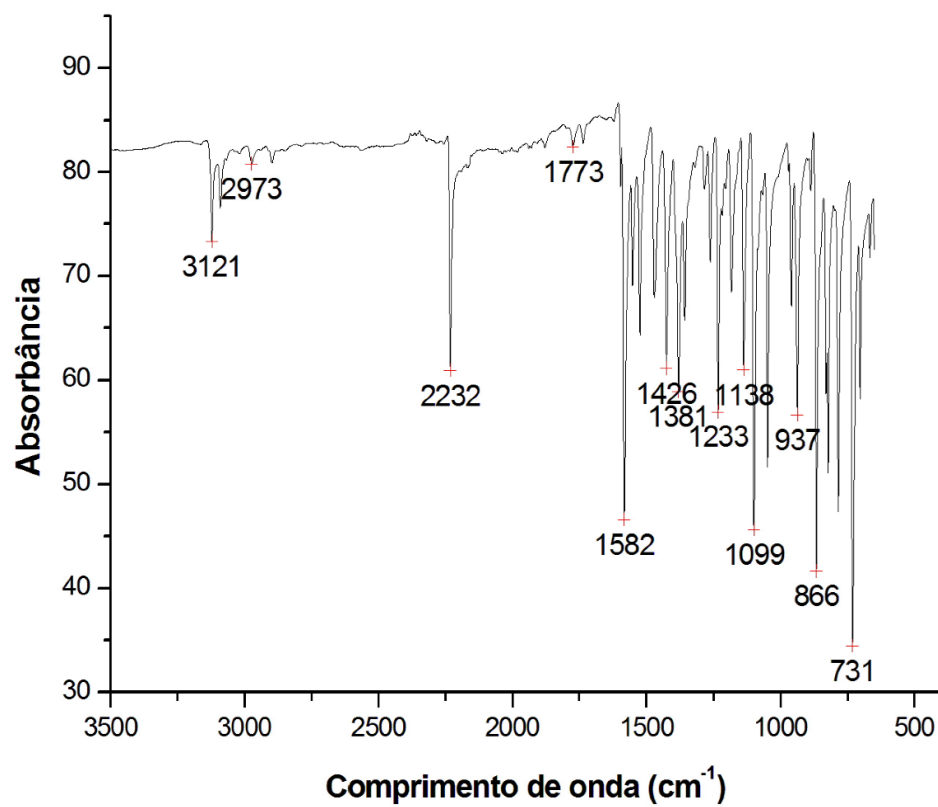


Figure S26. Mass spectrum of compound **4d**

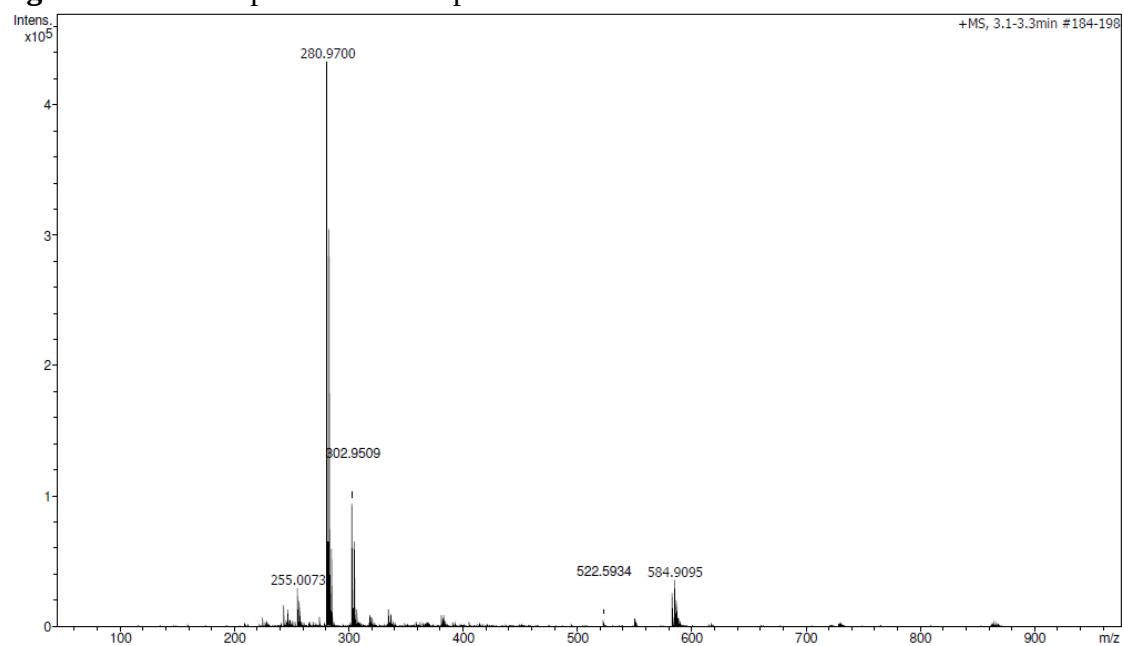


Figure S27. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4e**

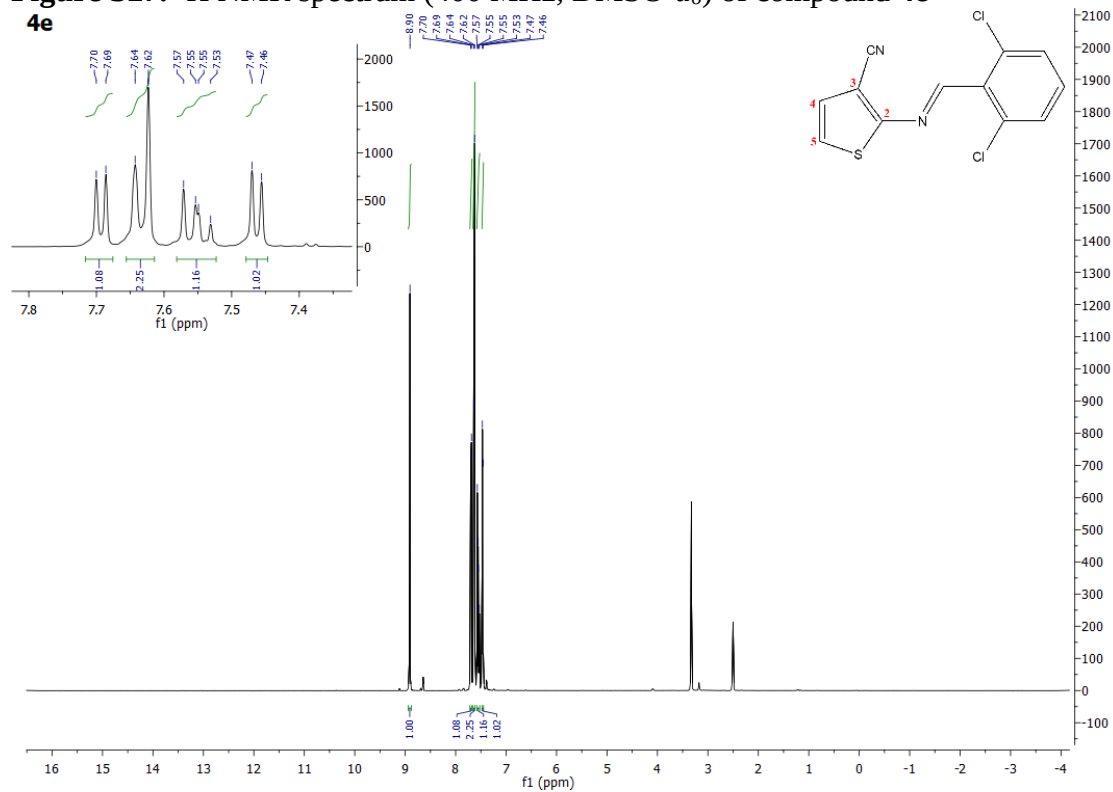


Figure S28. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4e**

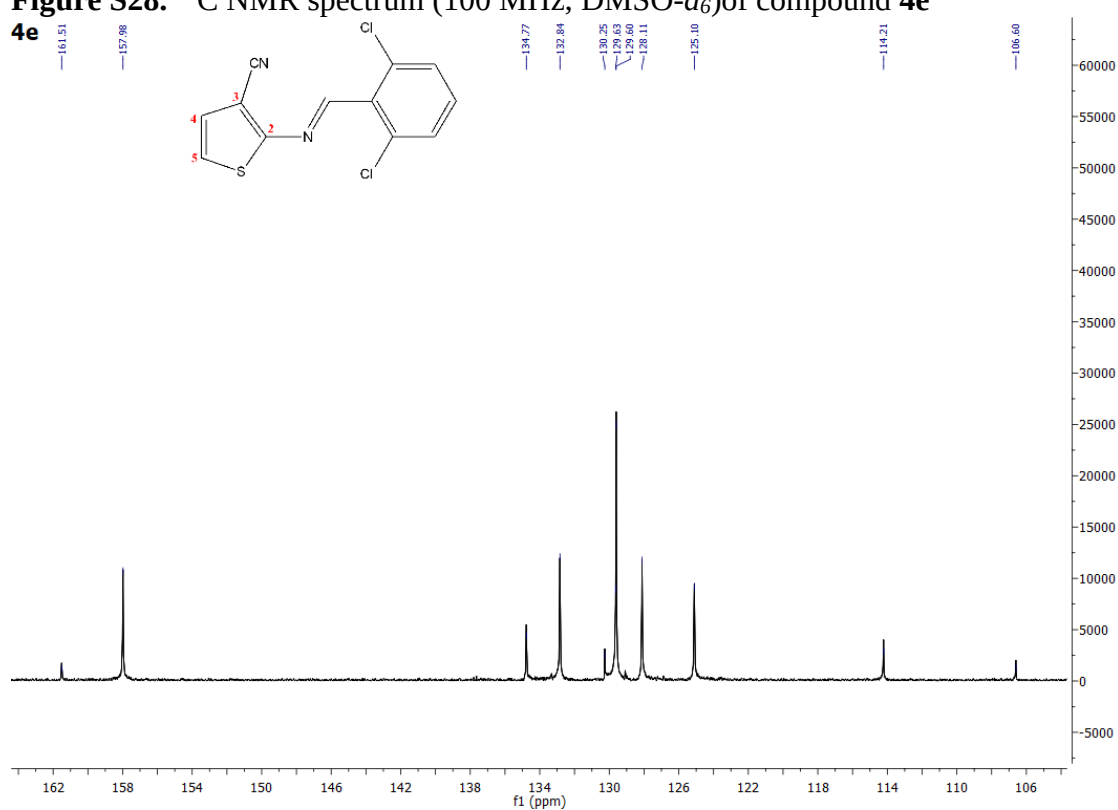


Figure S29. HSQC spectrum of compound **4e**

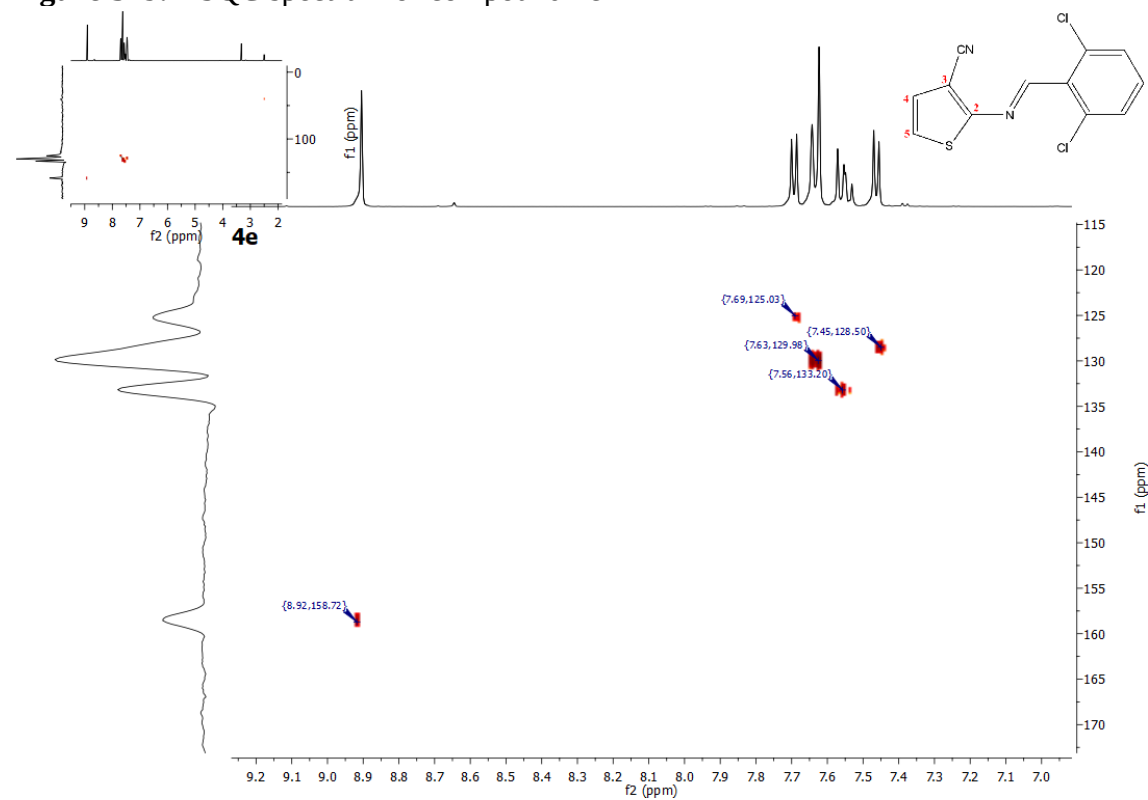


Figure S30. HMBC spectrum of compound **4e**

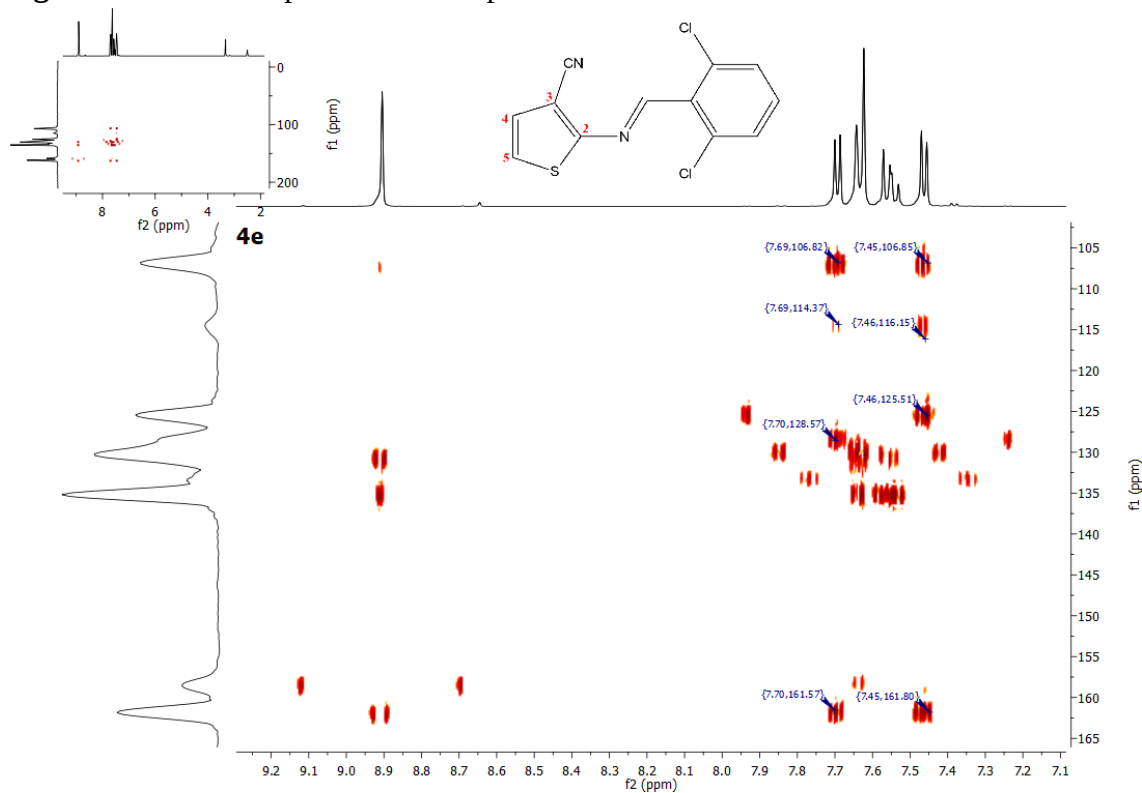


Figure S31. FTIR (KBr) spectrum of compound **4e**

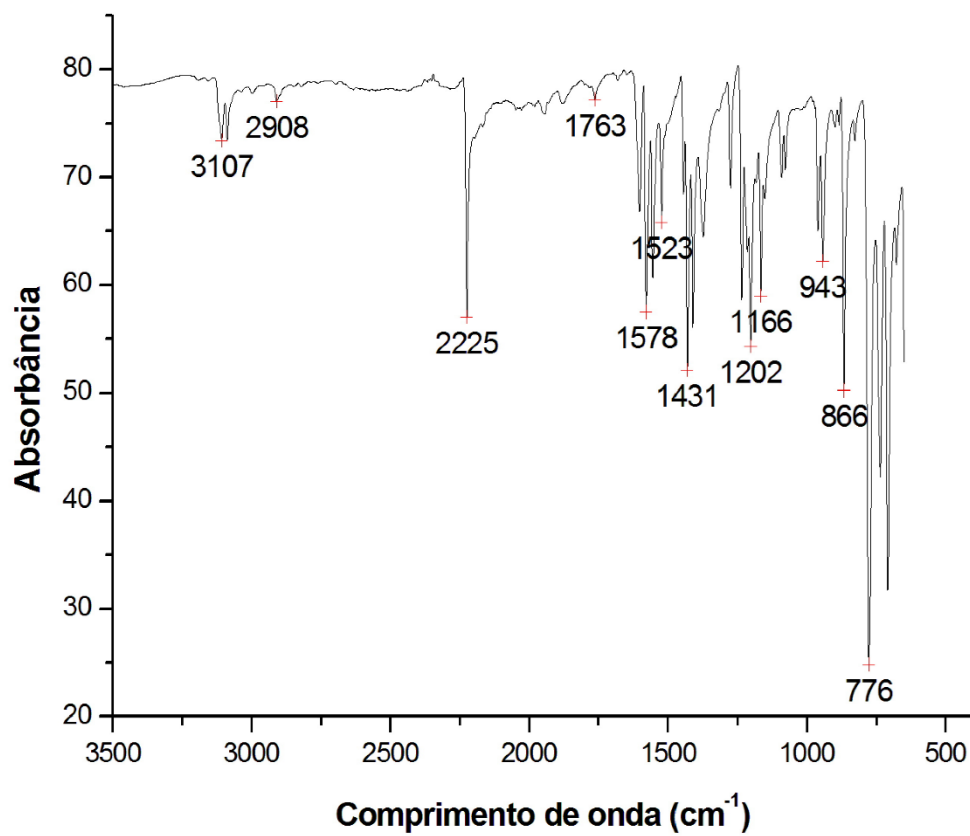


Figure S32. Mass spectrum of compound **4e**

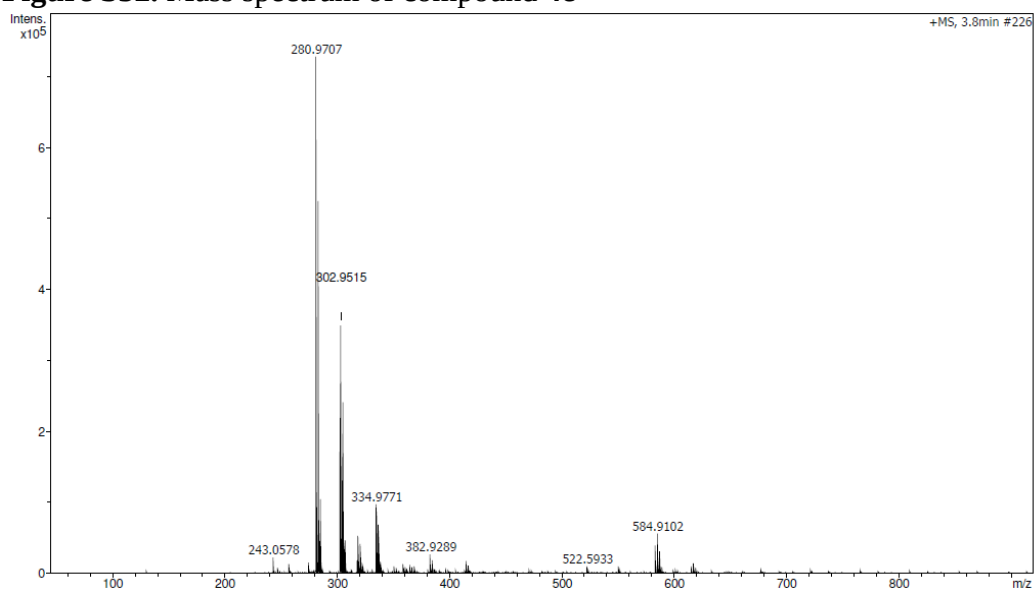


Figure S33. ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **4f**

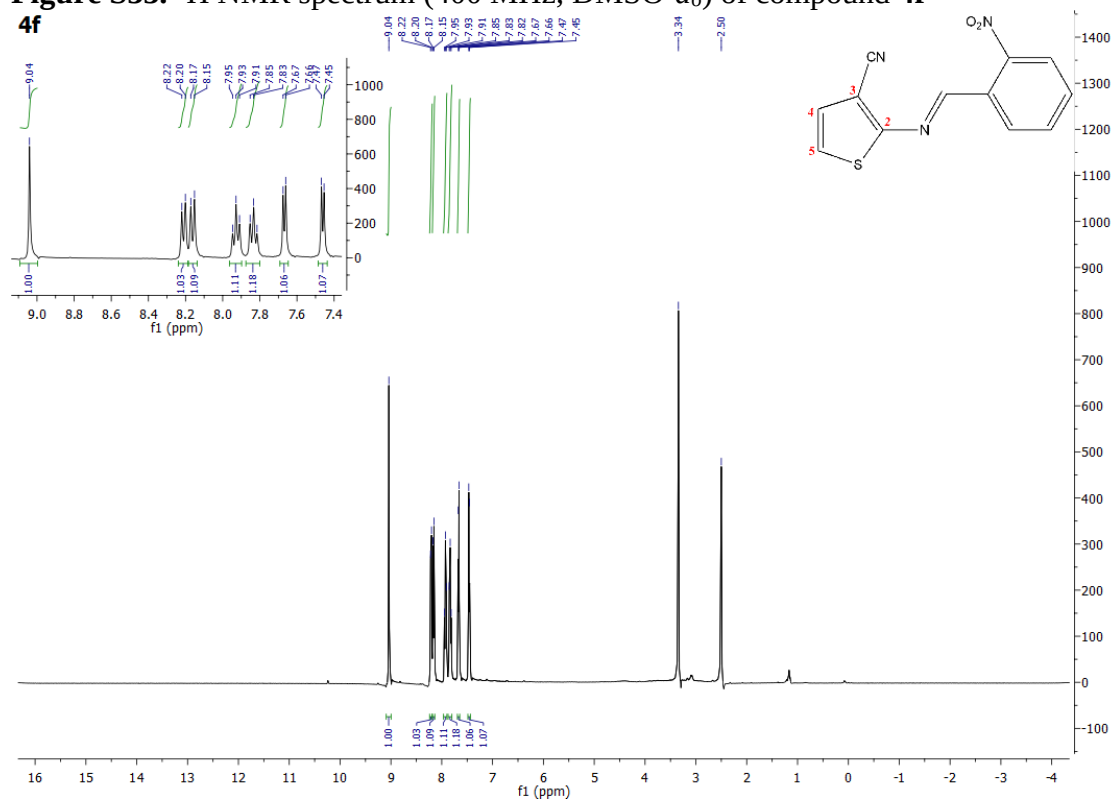


Figure S34. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4f**

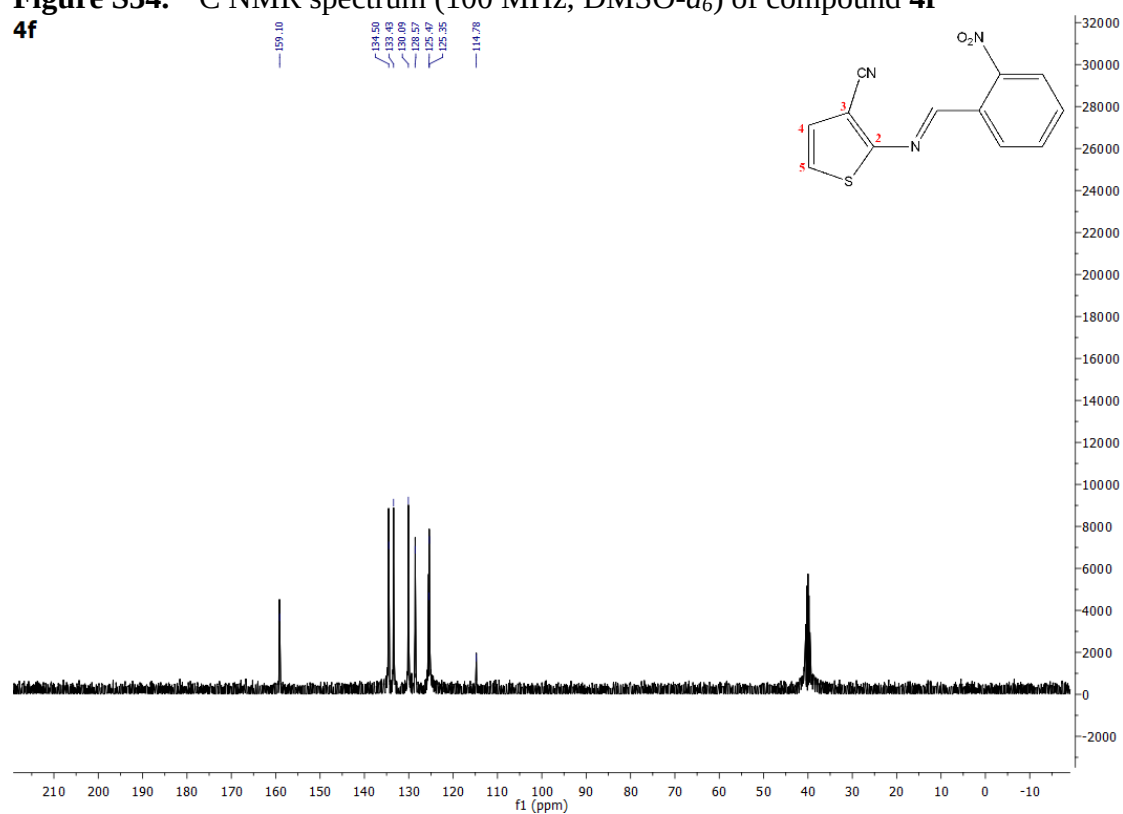


Figure S35. HSQC spectrum of compound **4f**

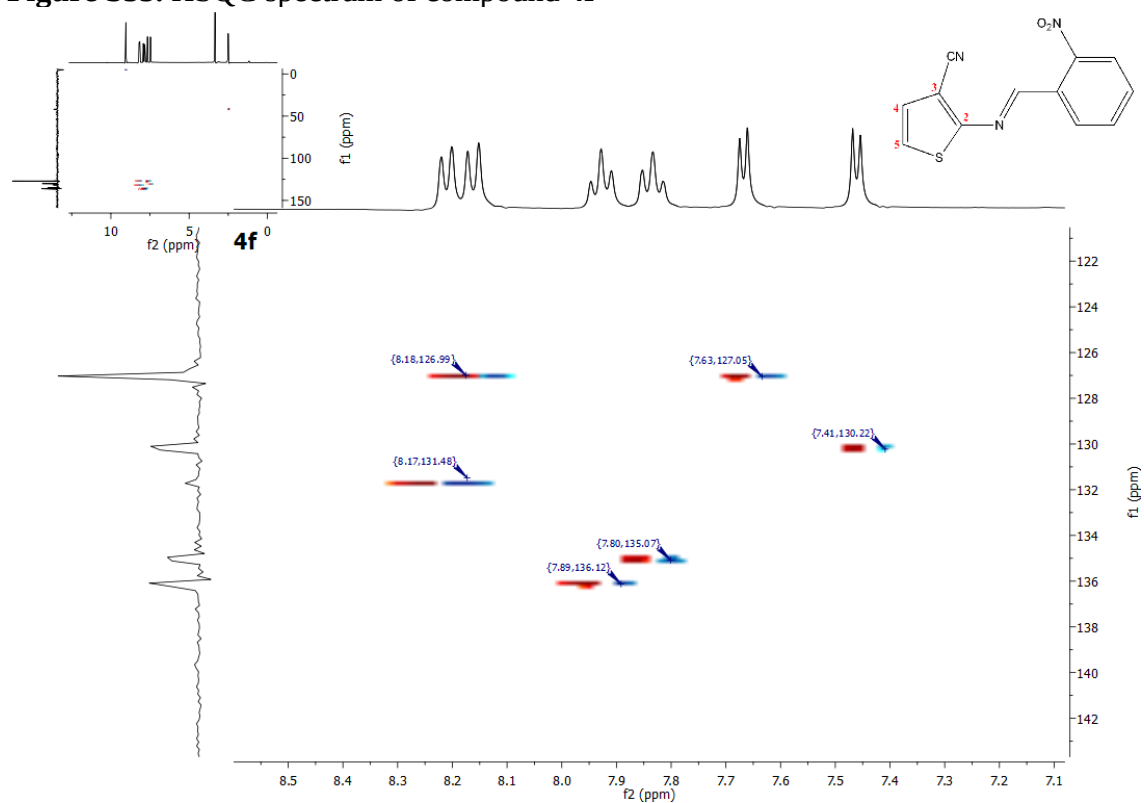


Figure S36. HMBC spectrum of compound **4f**

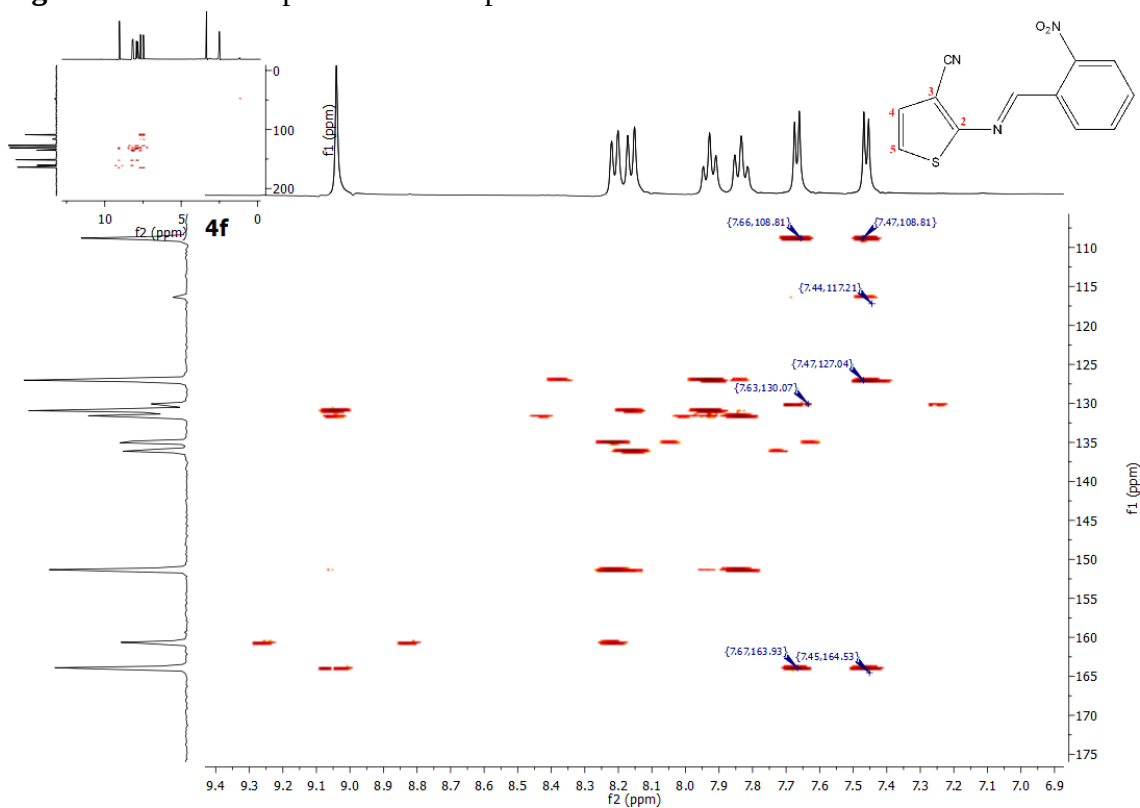


Figure S37. FTIR (KBr) spectrum of compound **4f**

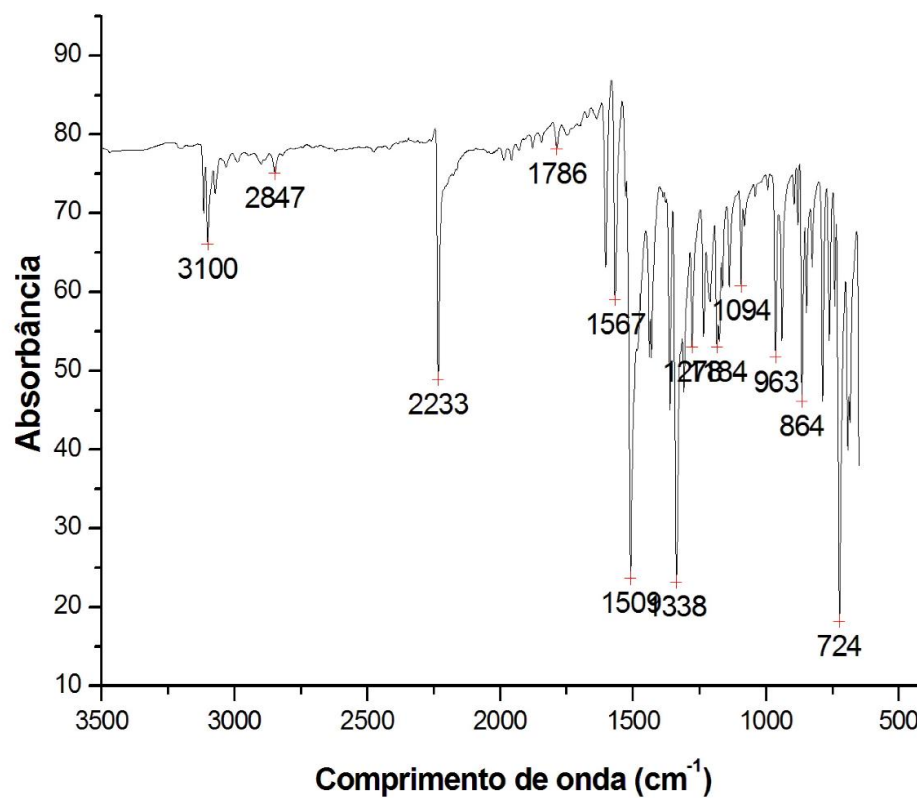


Figure S38. Mass spectrum of compound **4f**

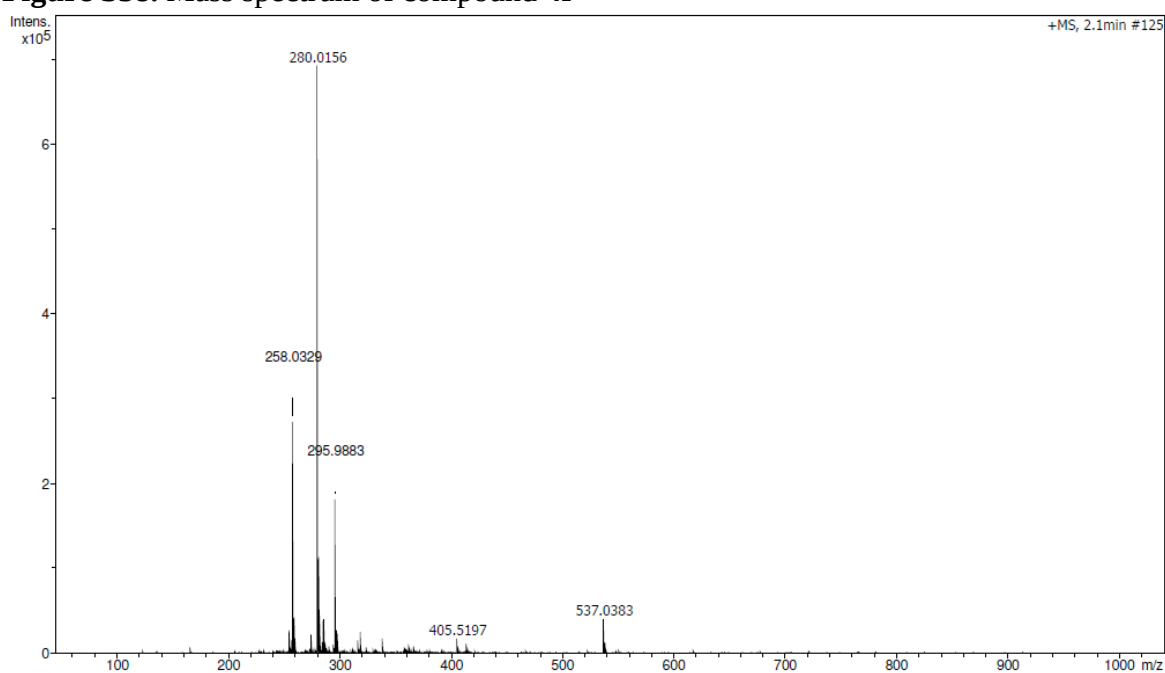


Figure S39. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4g**

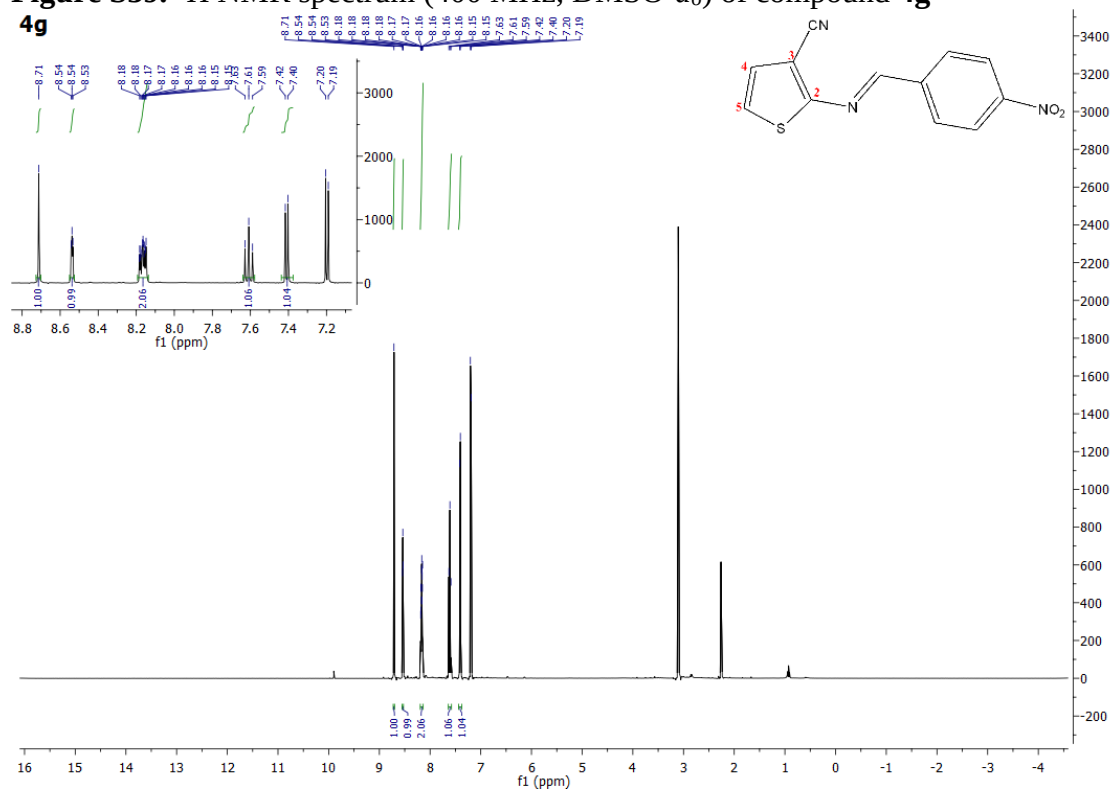


Figure S40. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4g**

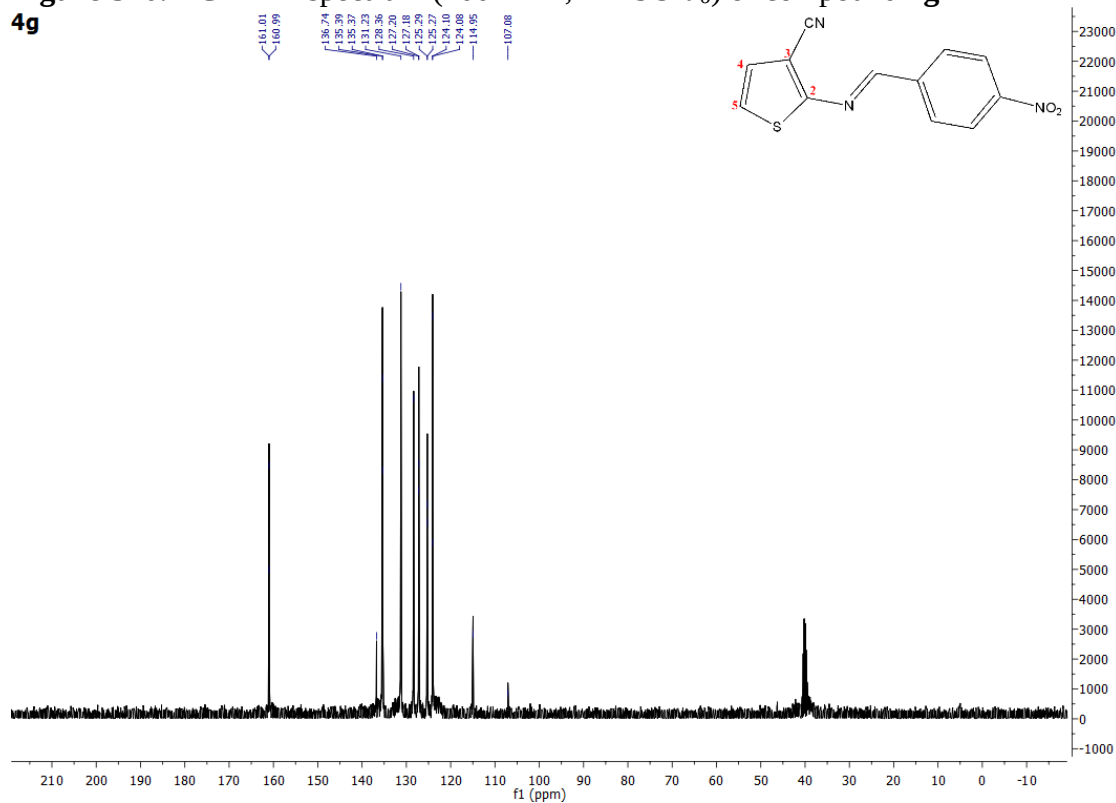


Figure S41. HSQC spectrum of compound **4g**

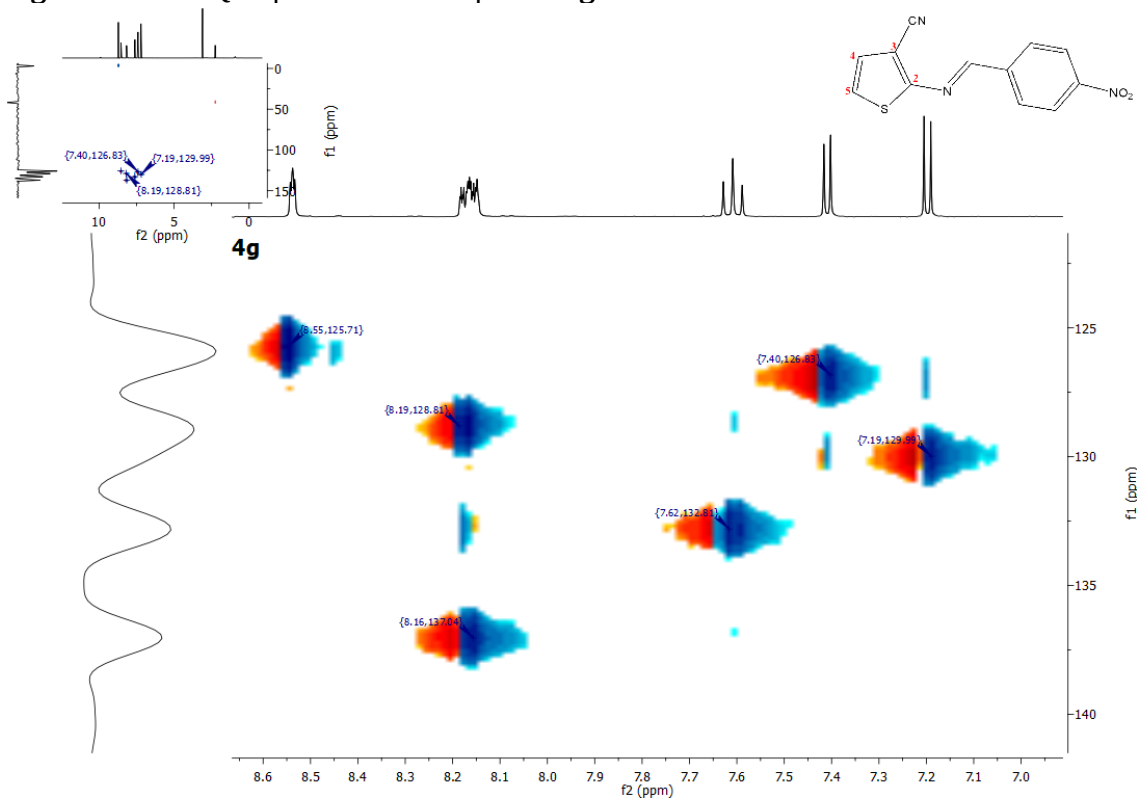


Figure S42. HMBC spectrum of compound **4g**

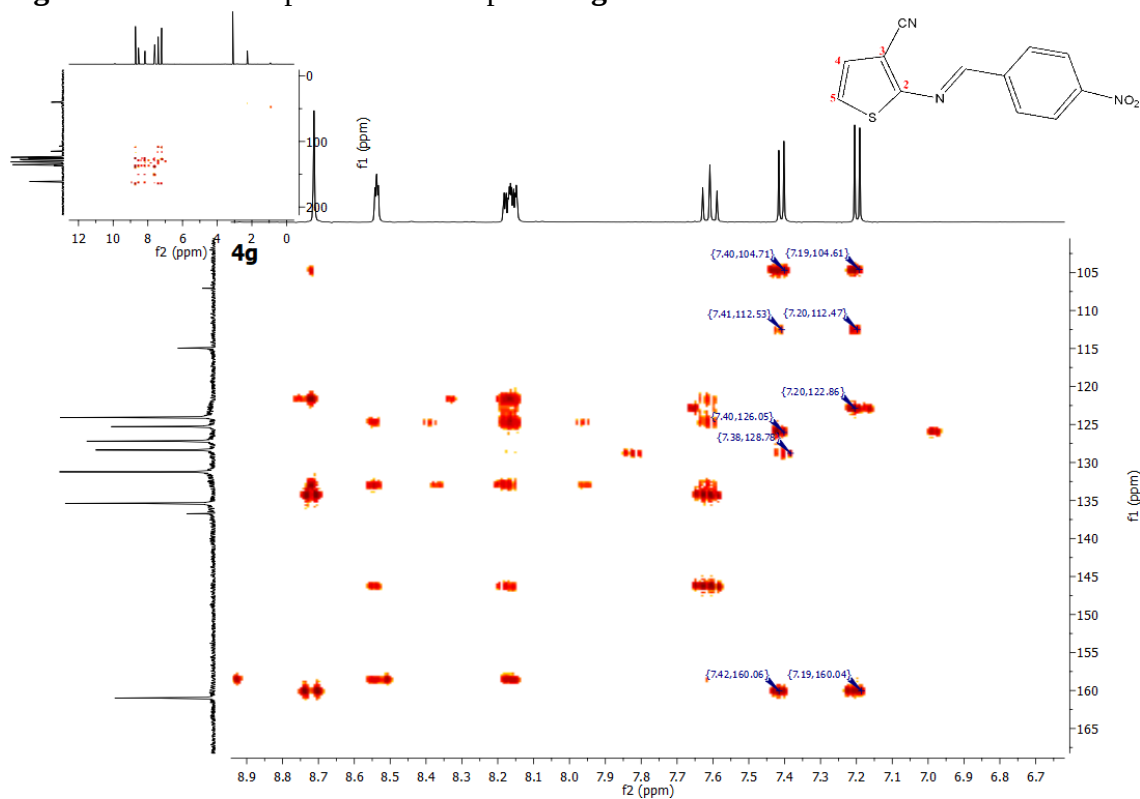
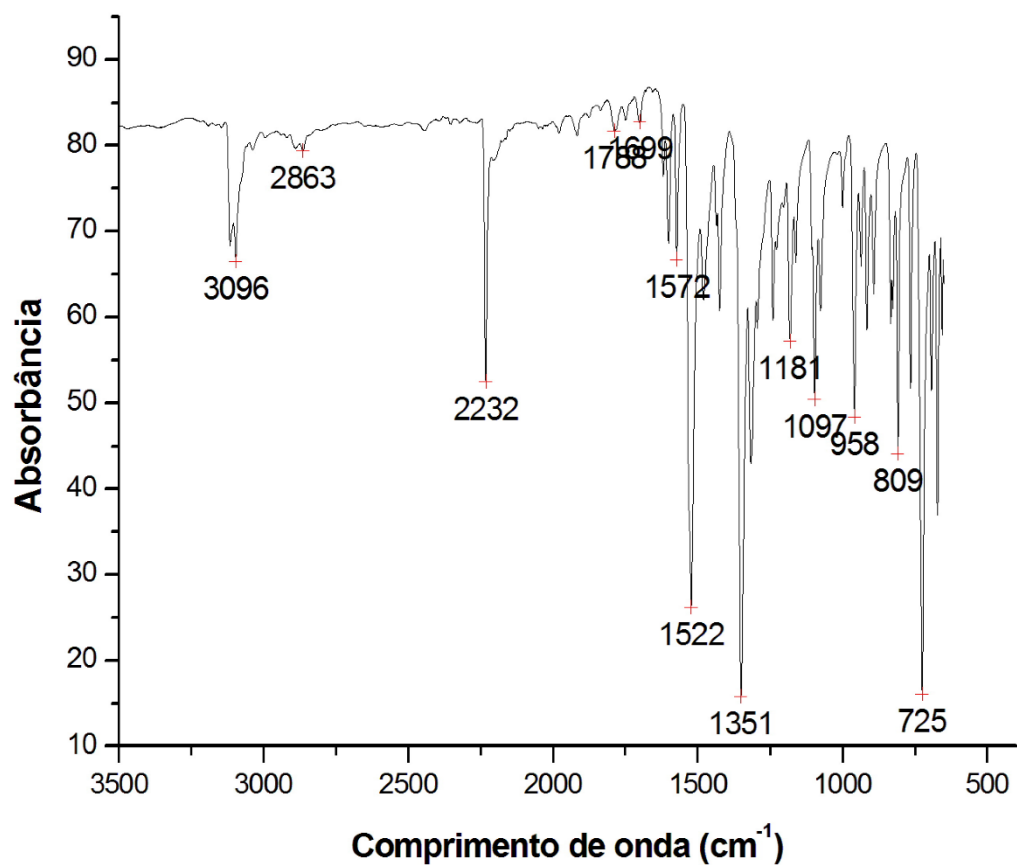


Figure S43. FTIR (KBr) spectrum of compound **4g**



Mass spectrum plot showing intensity (x10⁵) versus m/z. The x-axis ranges from 0 to 1000 m/z, and the y-axis ranges from 0 to 6 x 10⁵. The base peak is at m/z 258.0333. Other significant peaks are labeled at m/z 280.0144 and 537.0373. The plot is titled '+MS, 3.2-3.6min #193-212'.

Figure S-13. ¹H NMR spectrum (400 MHz, DMSO-d₆) of compound **4h**.

Chemical structure of compound **4h** is shown in the top right corner. The structure is a thienothiopyran derivative with a thienothiopyran core, a thienothiopyran methine group, a thienothiopyran methyl group, and a thienothiopyran methoxy group.

The ¹H NMR spectrum (400 MHz, DMSO-d₆) of compound **4h** is displayed. The x-axis represents the chemical shift in ppm (f1), ranging from 0 to 10. The y-axis represents the intensity in arbitrary units (a.u.), ranging from 0 to 3800. The spectrum shows several peaks, with the following chemical shifts (ppm) and integration values (a.u.) labeled:

- 9.51 (2.51)
- 8.98 (1.03)
- 7.94 (1.03)
- 7.84 (1.03)
- 7.82 (1.03)
- 7.59 (1.03)
- 7.48 (1.03)
- 7.47 (1.03)
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- 7.45 (1.03)
- 7.41 (1.03)
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Figure S46. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **4h**

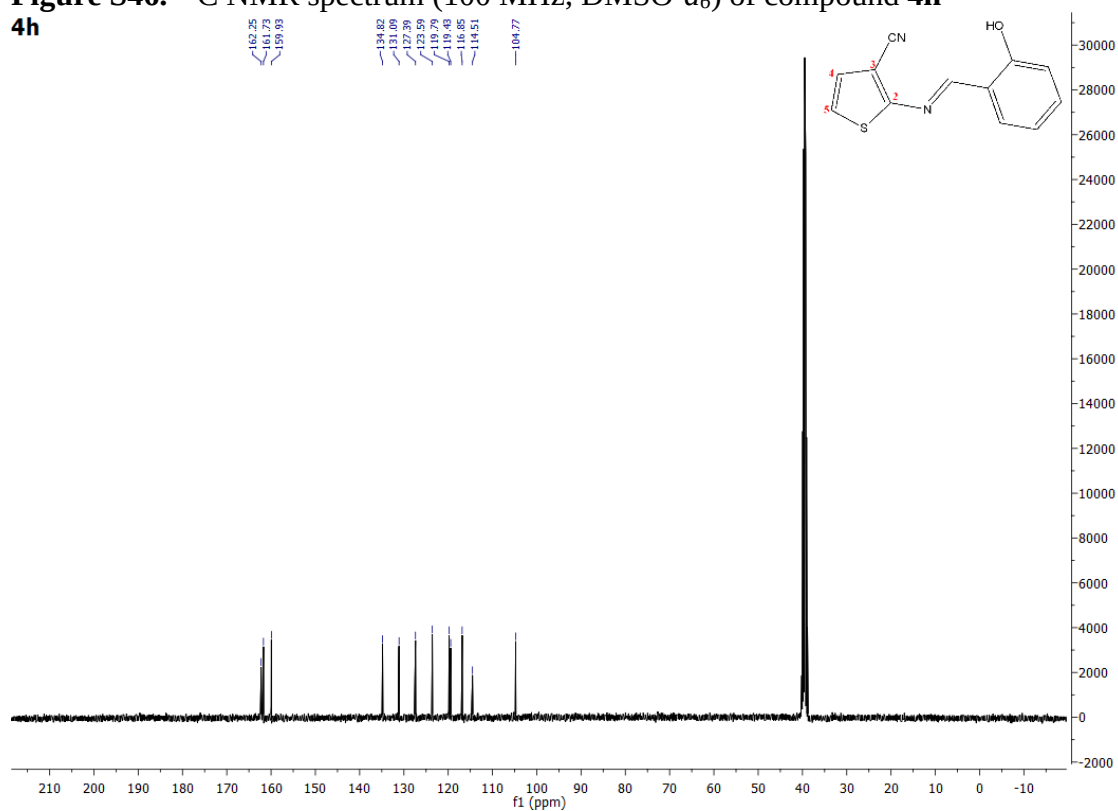


Figure S47. HSQC spectrum of compound **4h**

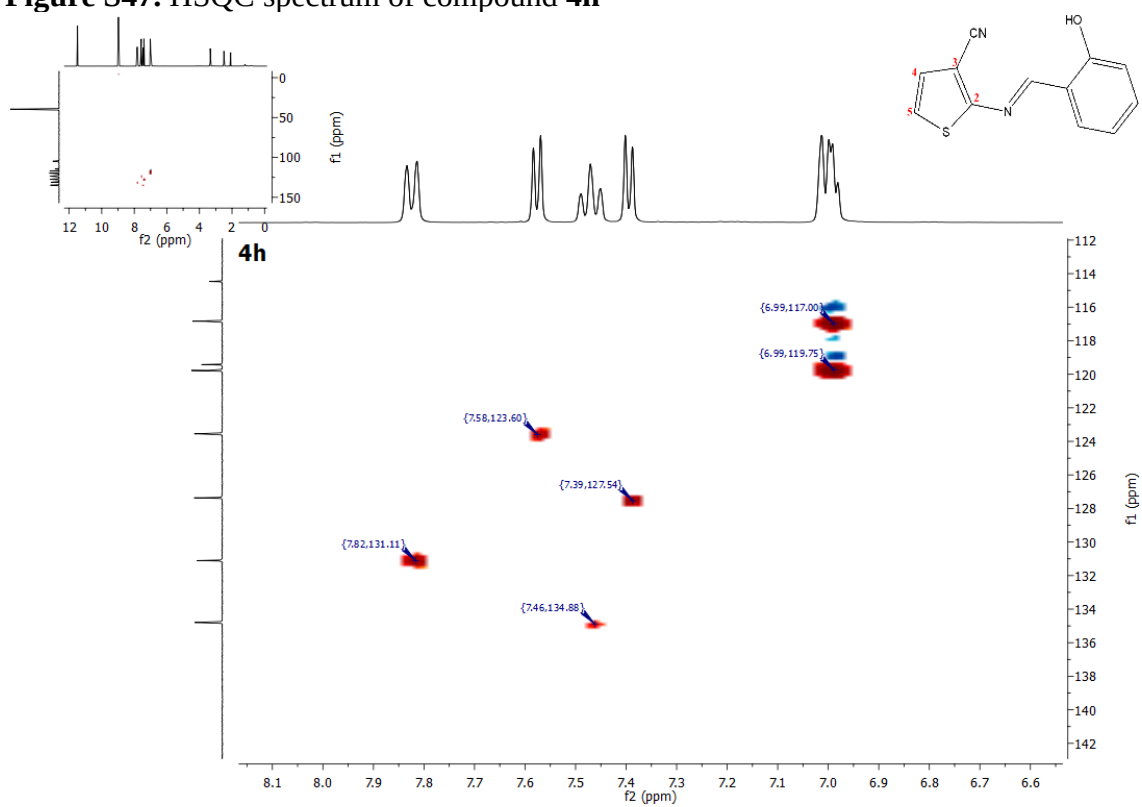


Figure S48. HMBC spectrum of compound **4h**

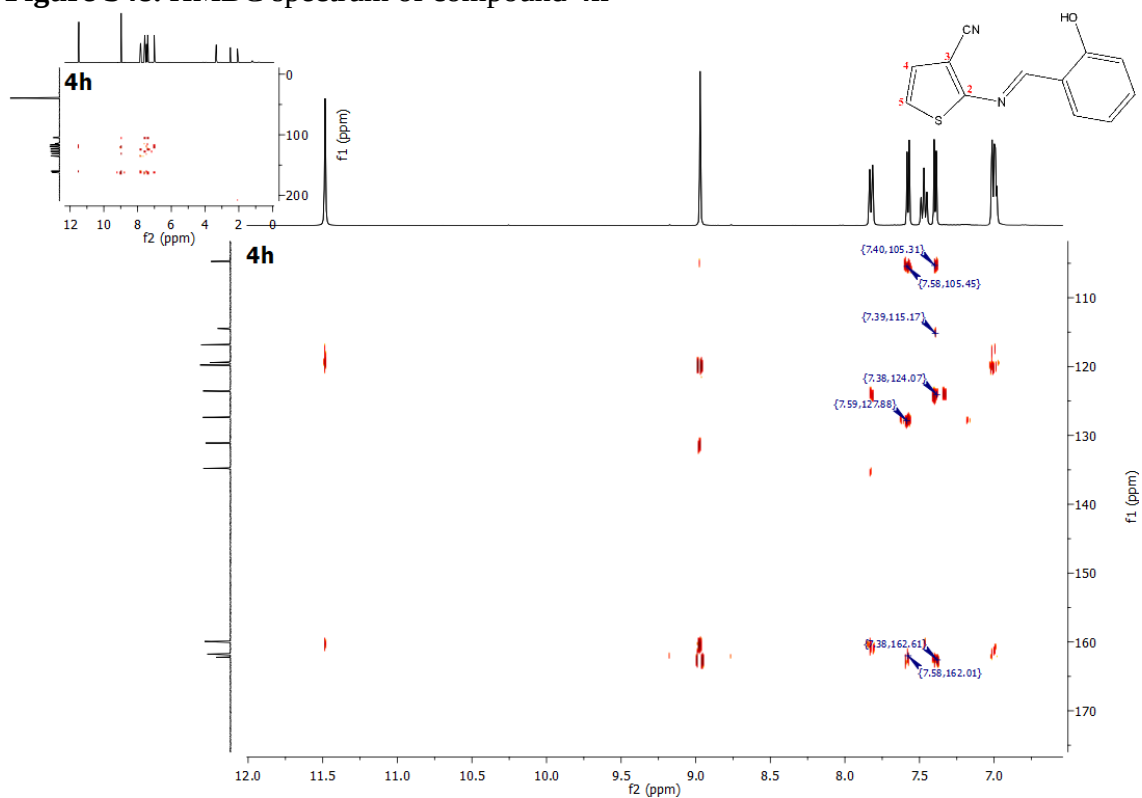
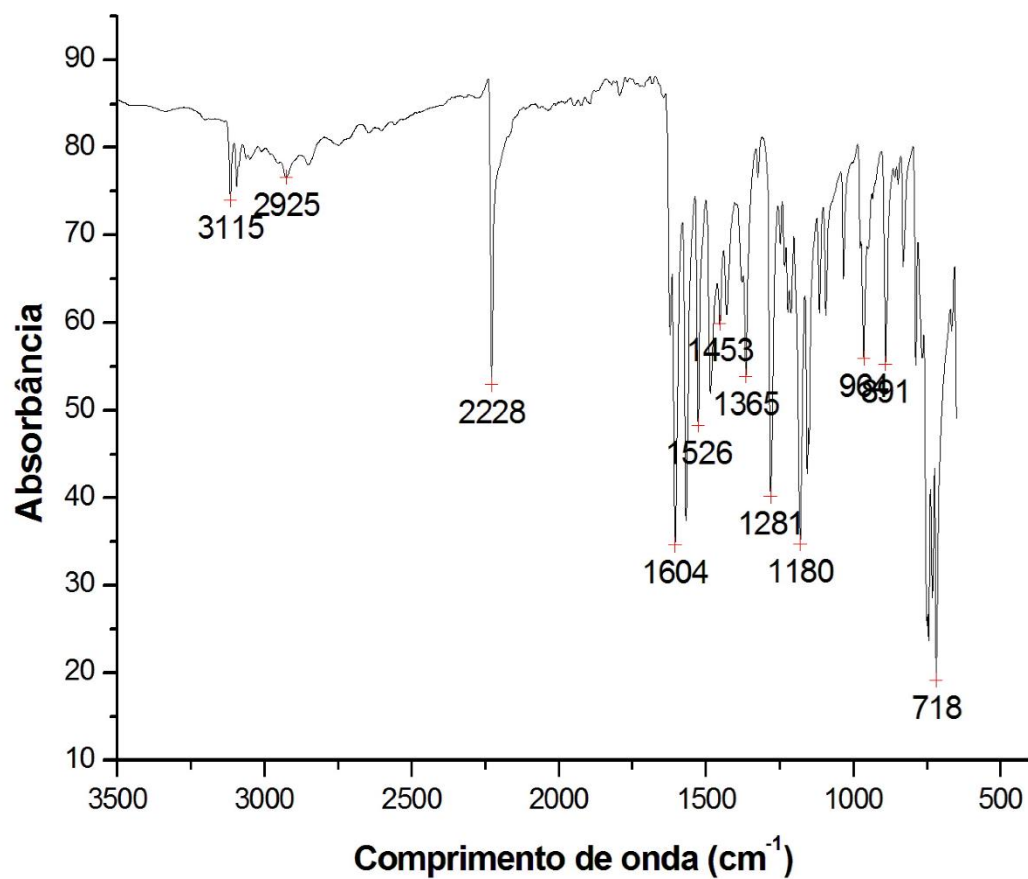


Figure S49. FTIR (KBr) spectrum of compound **4h**



Mass spectrum plot showing intensity (x10⁵) versus m/z. The base peak is at m/z 251.0255. Other labeled peaks include 229.0427, 266.9976, 359.2298, 391.3147, 405.2956, 479.0589, 550.6244, and 765.4132. The plot is titled '+MS, 2.2min #133'.

Figure S31. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4i**.

The ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4i** displays the following chemical shifts (ppm) and integration values:

- 8.72 (1.00)
- 7.91 (2.05), 7.89 (0.96)
- 7.56 (0.96), 7.54 (3.06)
- 7.39 (0.96), 7.38 (3.06), 7.37 (2.99)
- 2.40 (2.99)

The chemical structure of compound **4i** is shown as an inset, featuring a thiazole ring substituted with a cyano group (CN) and a 4-methylphenyl group (Me).

Figure S52. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **4i**

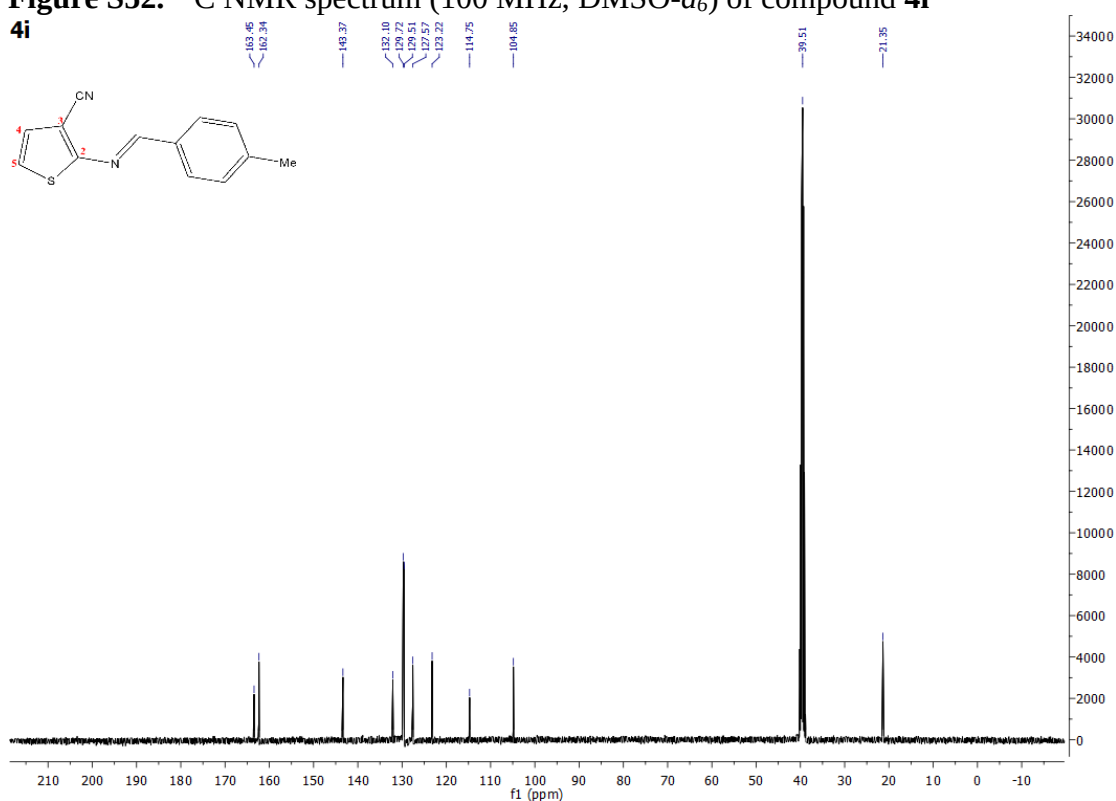


Figure S53. FTIR (KBr) spectrum of compound **4i**

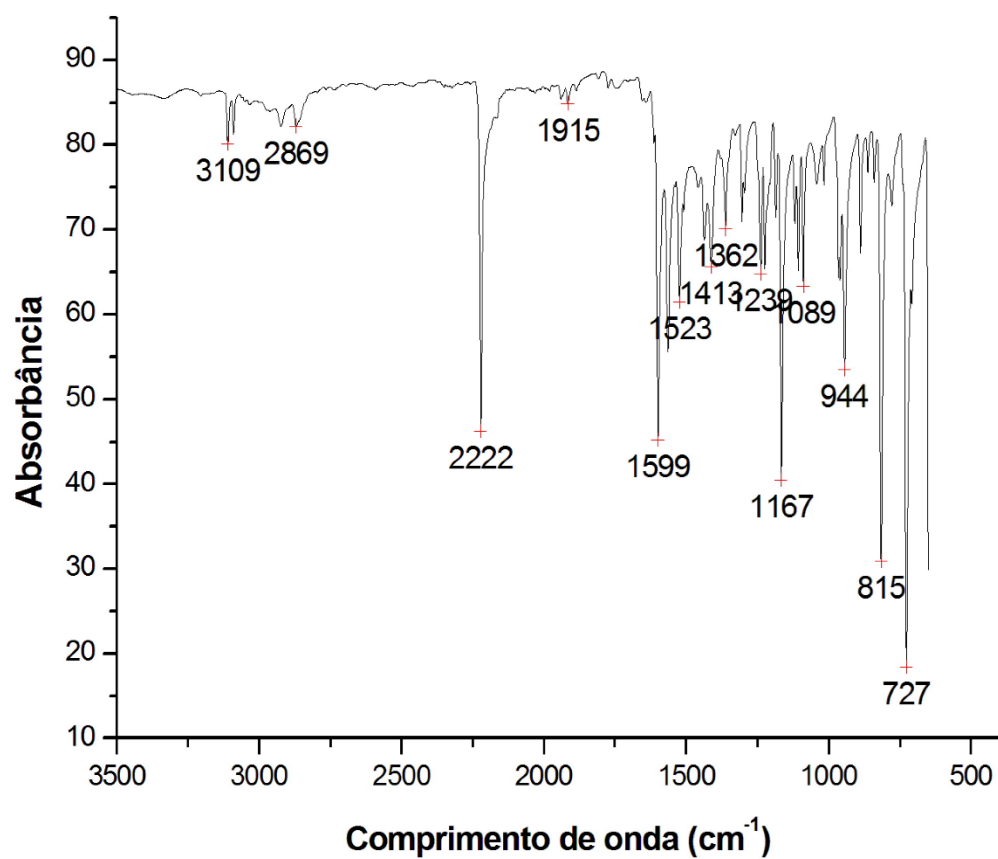


Figure S54. Mass spectrum of compound **4i**

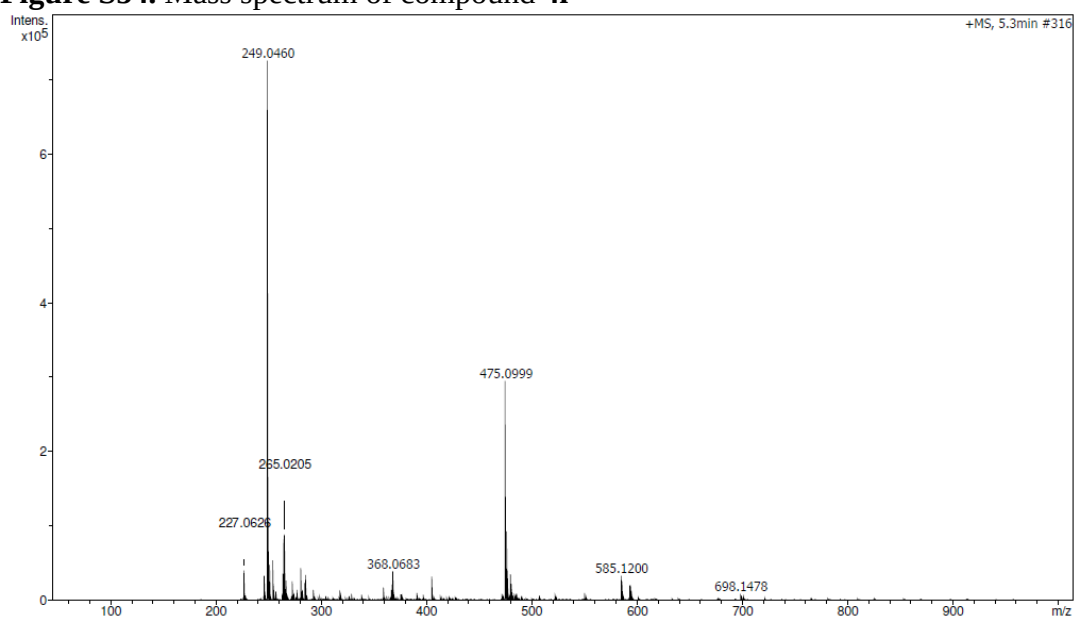


Figure S55. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4j**

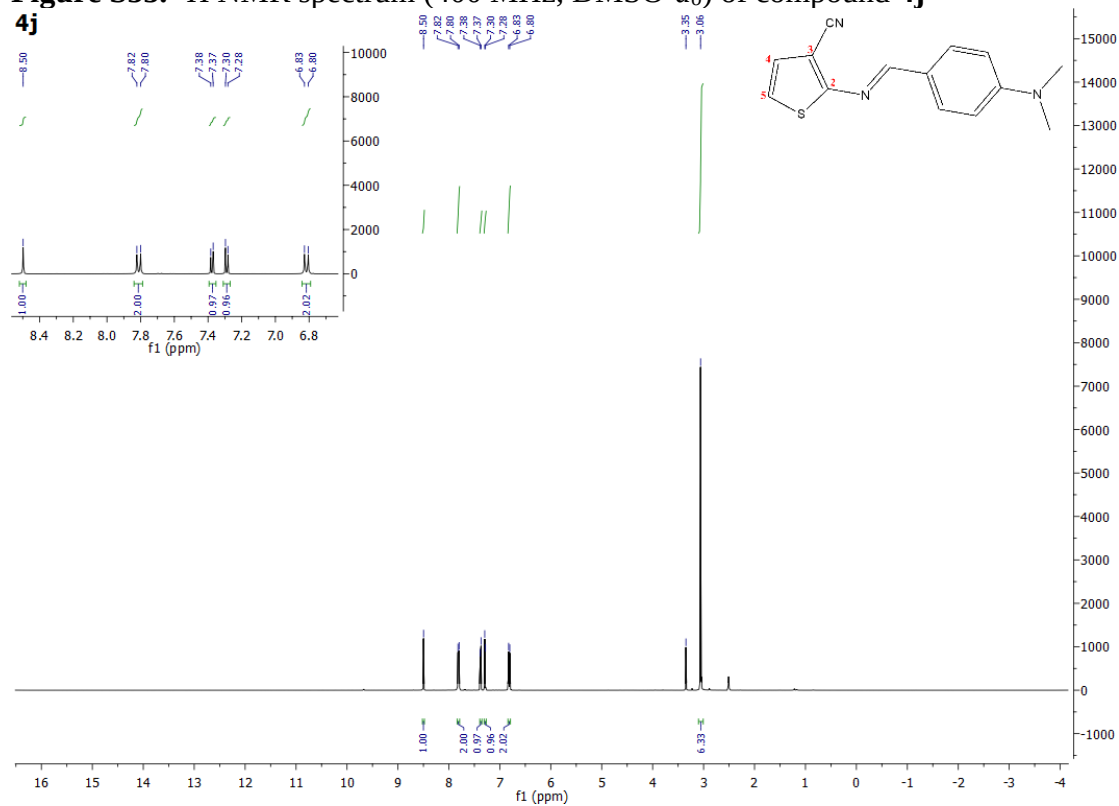


Figure S56. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **4j**

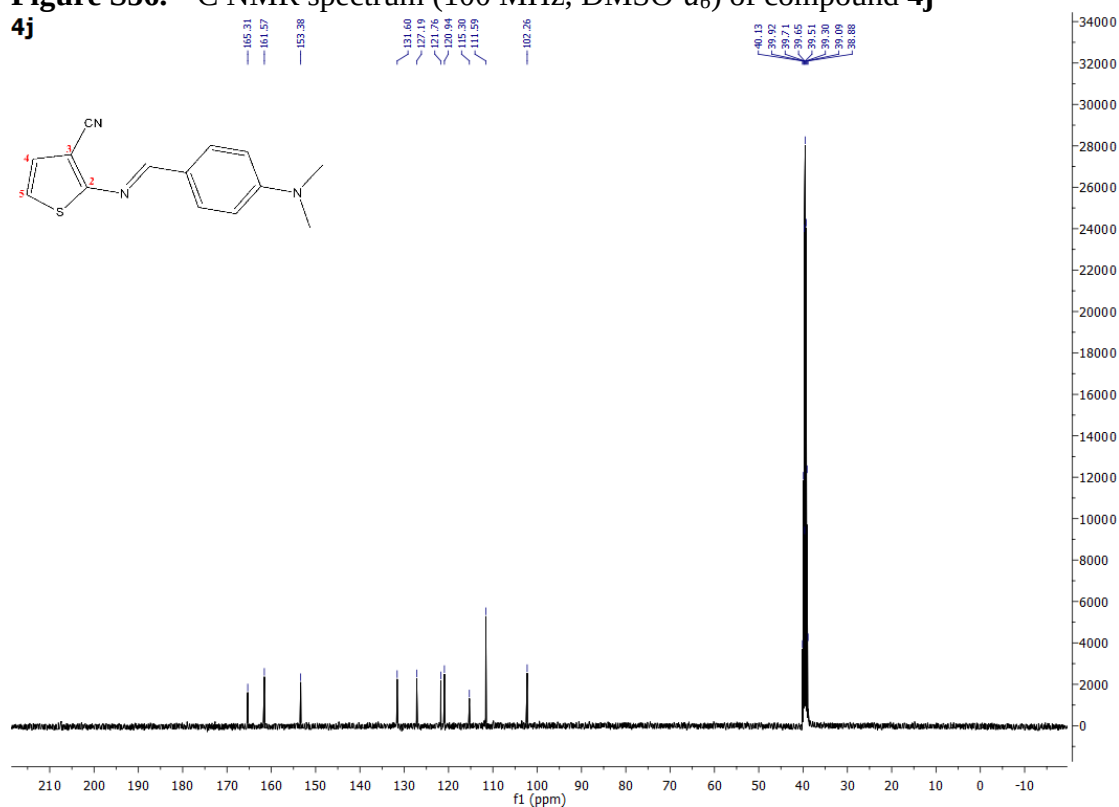


Figure S57. FTIR (KBr) spectrum of compound **4j**

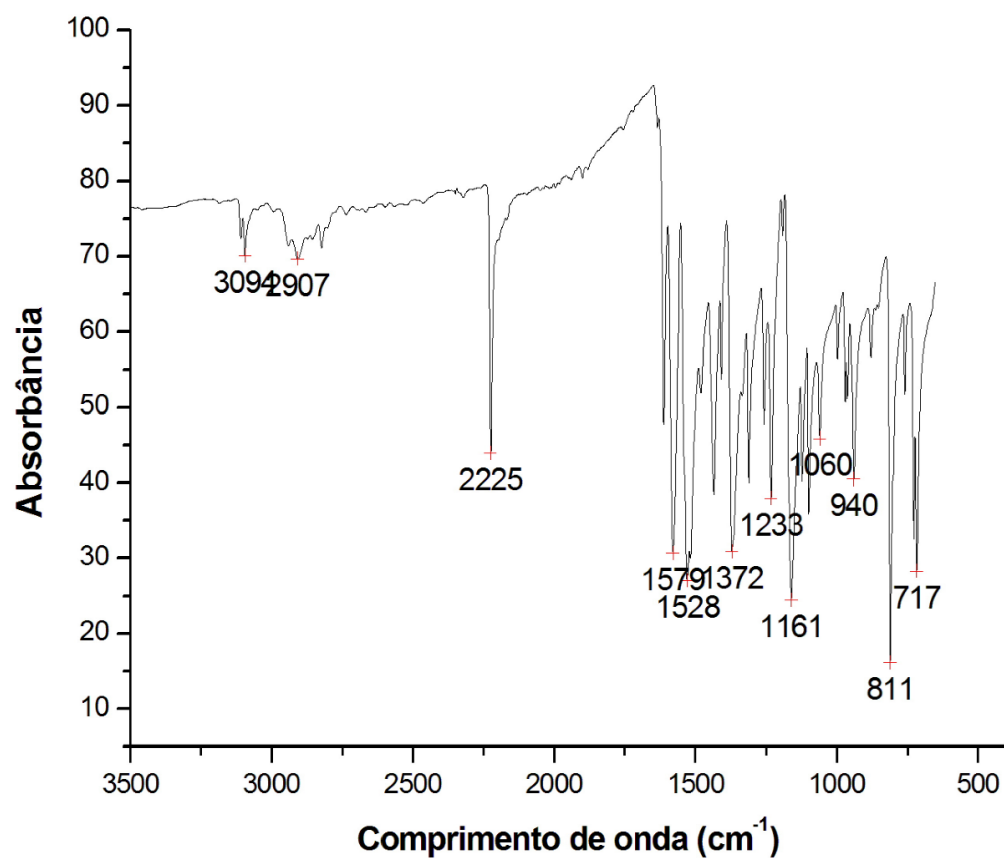


Figure S58. Mass spectrum of compound **4j**

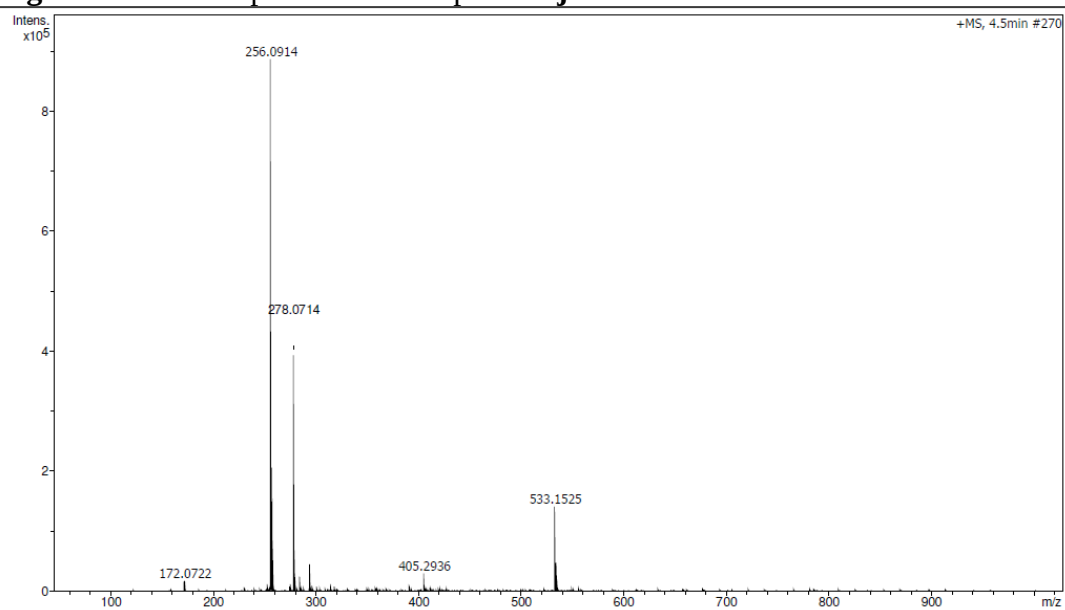


Figure S59. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4k**

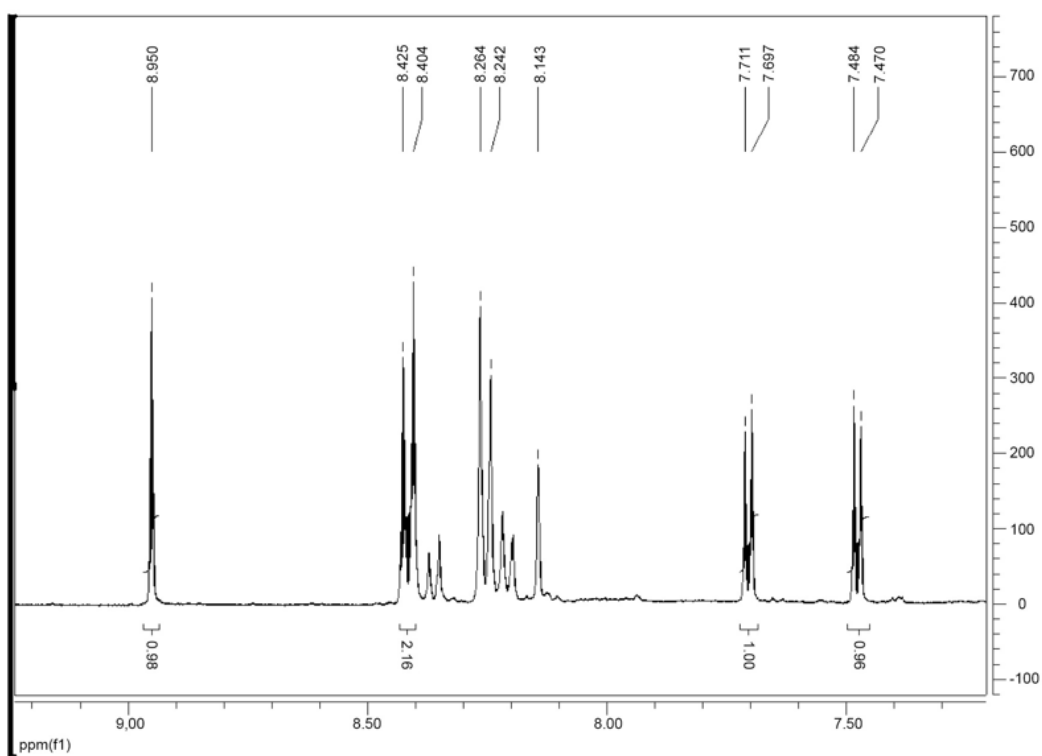


Figure S60. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4k**

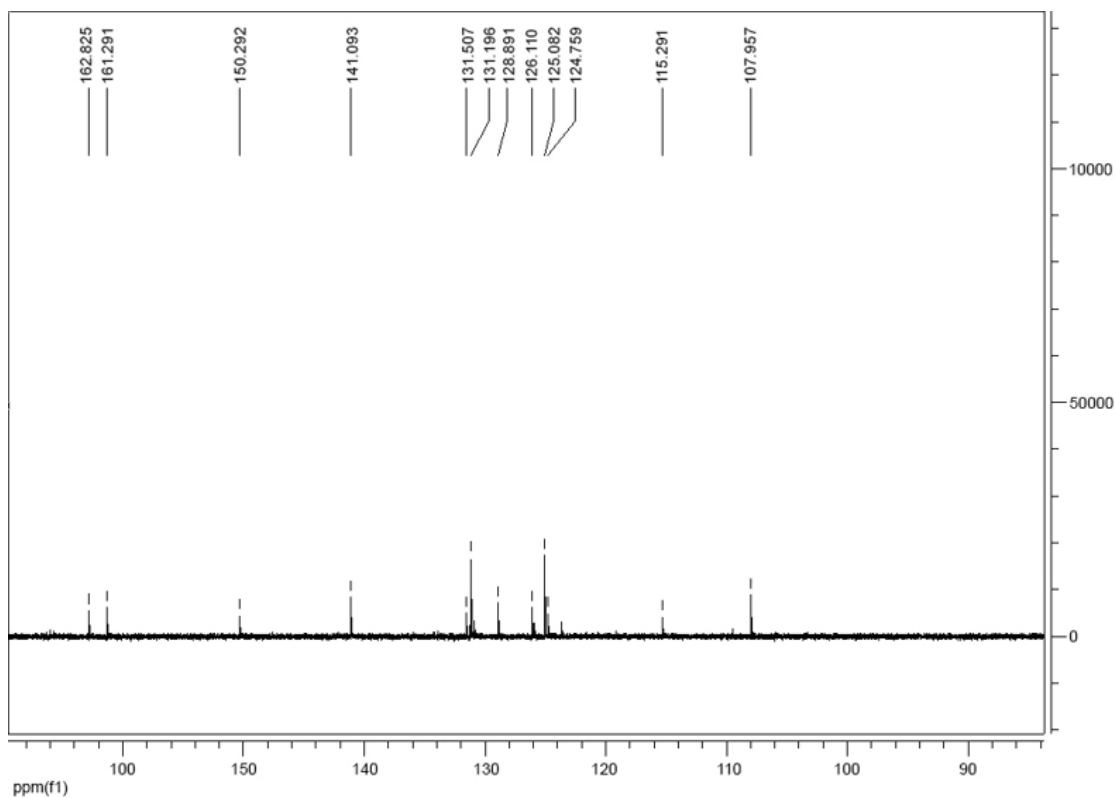


Figure S61. FTIR (KBr) spectrum of compound **4k**

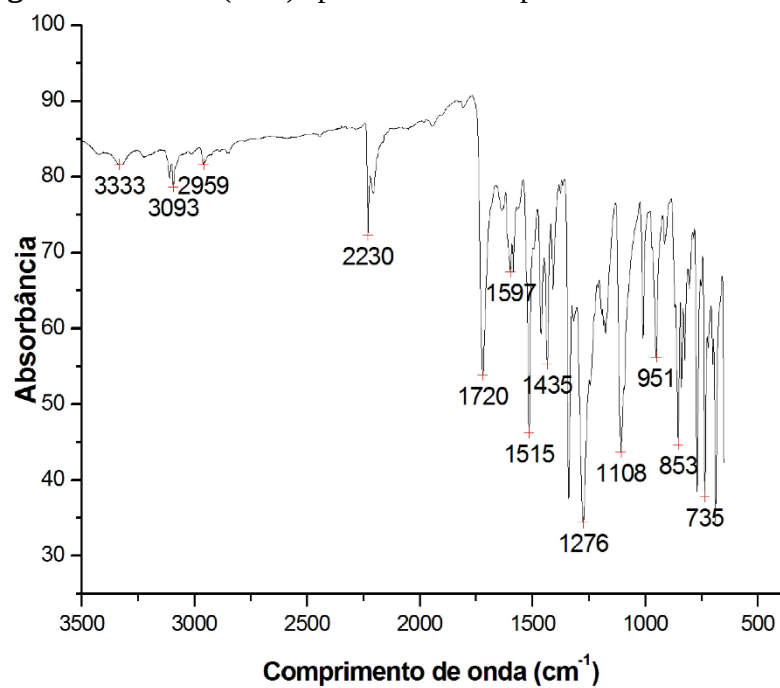


Figure S62. Mass spectrum of compound **4k**

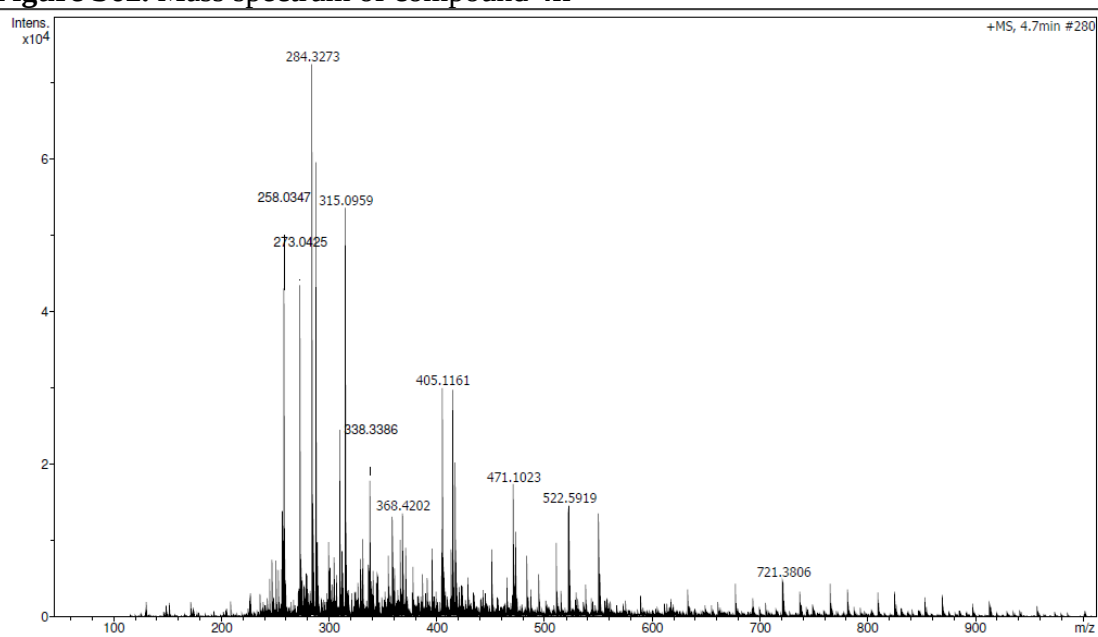


Figure S63. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of compound **4l**

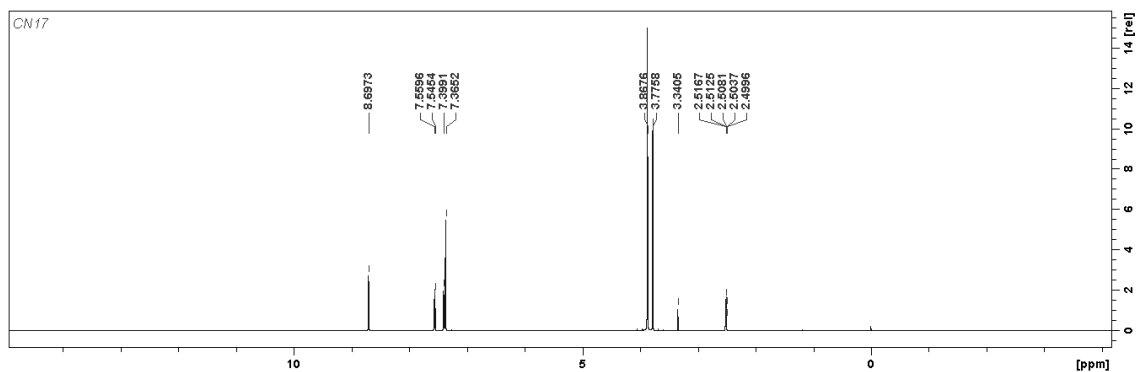


Figure S64. ¹³C NMR spectrum (100 MHz, DMSO-*d*₆) of compound **4l**

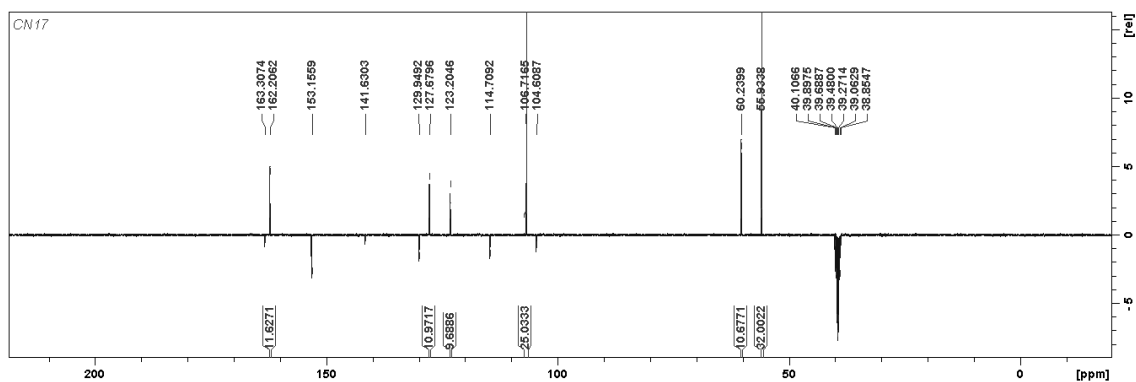


Figure S65. FTIR (KBr) spectrum of compound **4l**

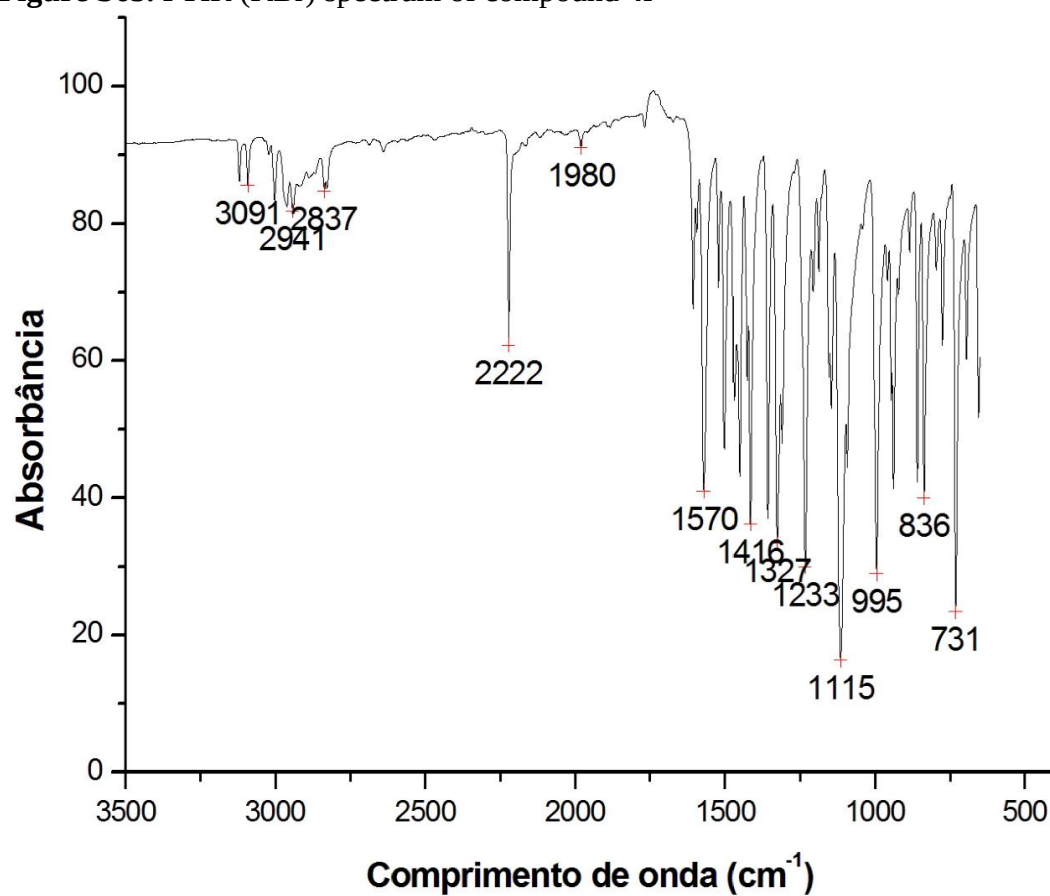


Figure S66. Mass spectrum of compound **4l**

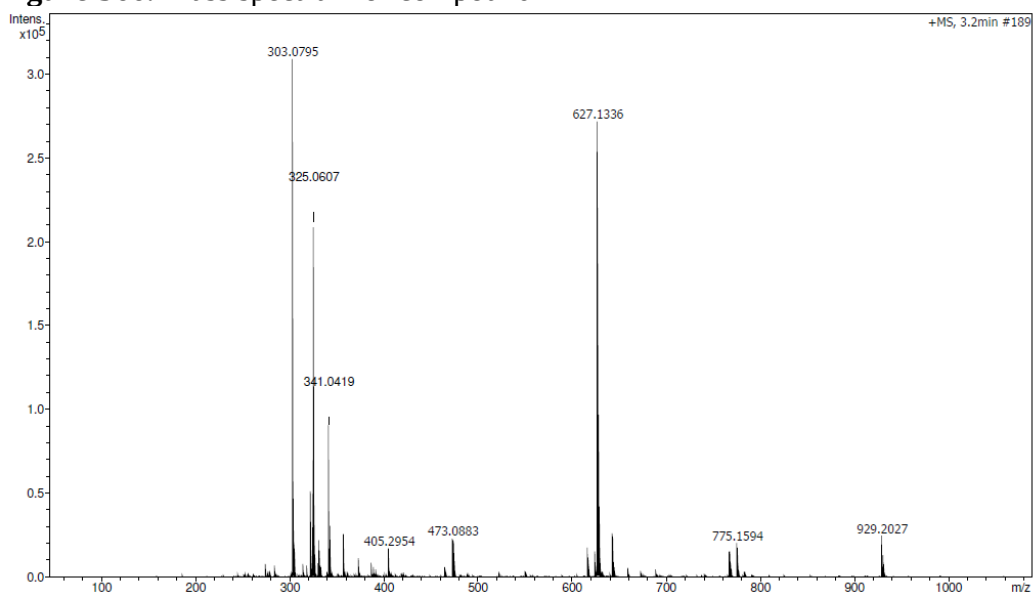


Figure S67. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **4m**

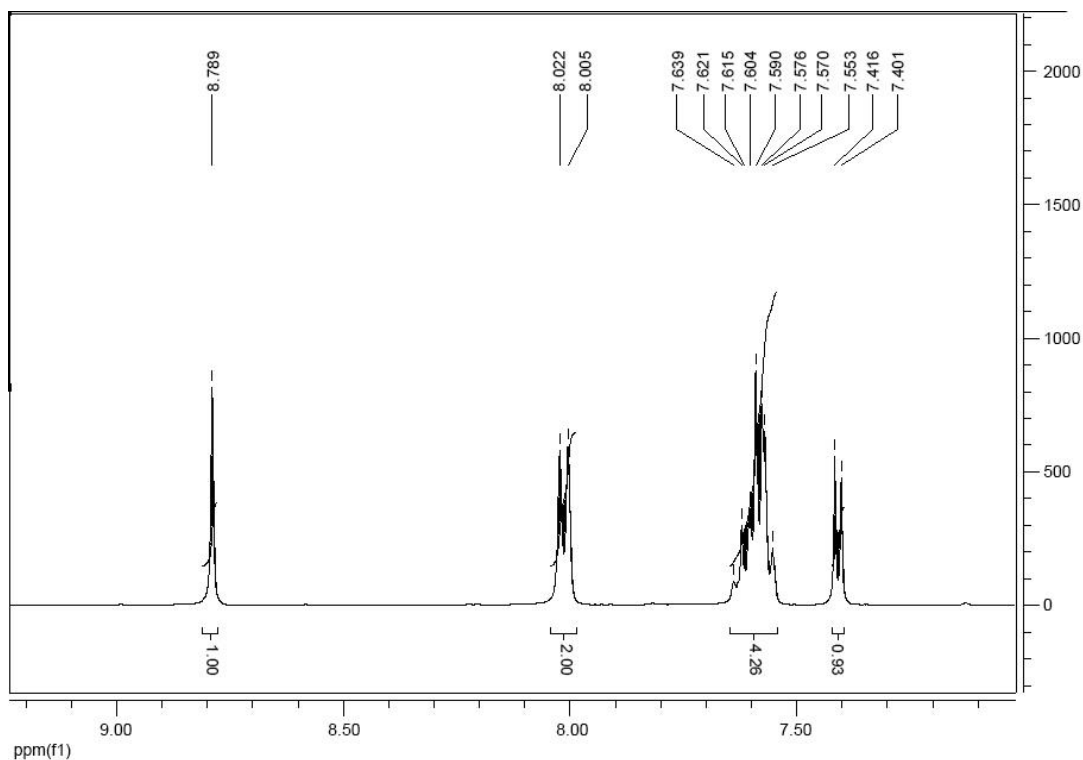


Figure S68. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4m**

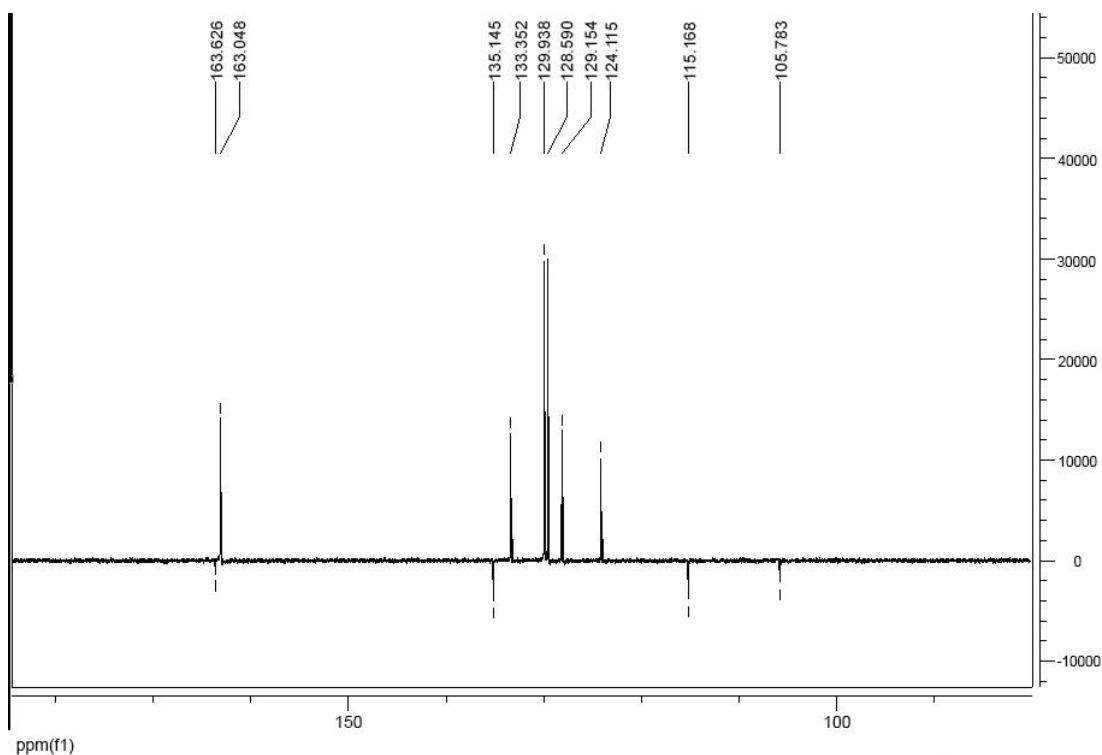


Figure S69. FTIR (KBr) spectrum of compound **4m**

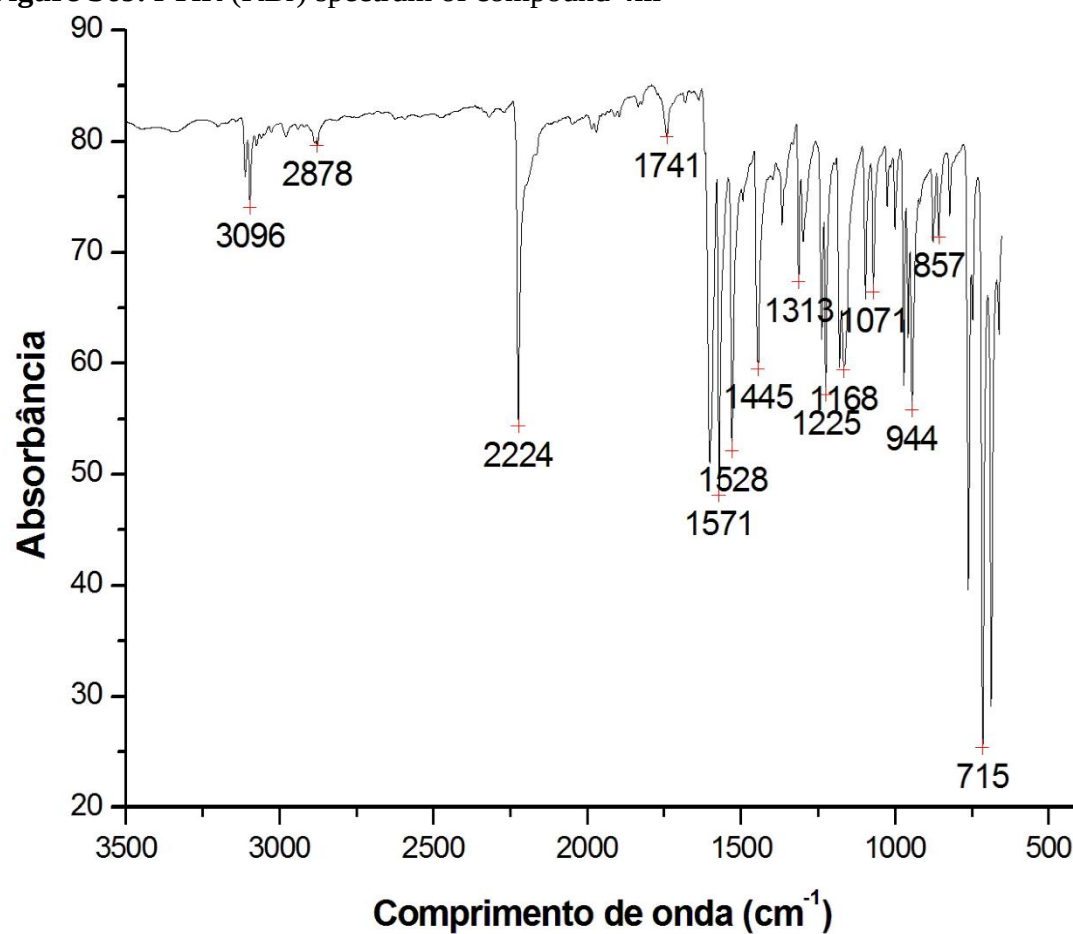


Figure S70. Mass spectrum of compound **4m**

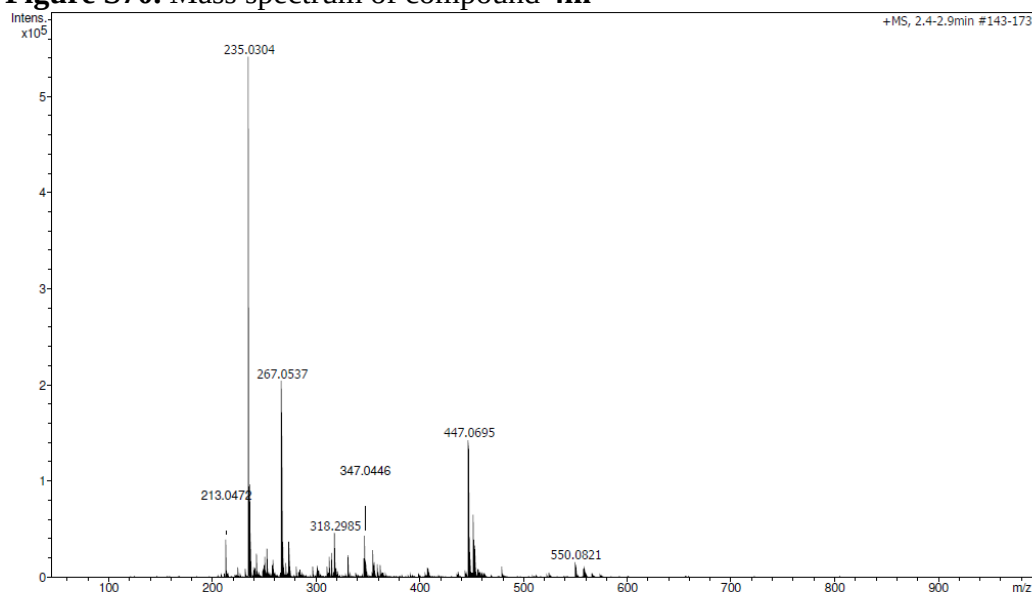


Figure S71. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **4n**

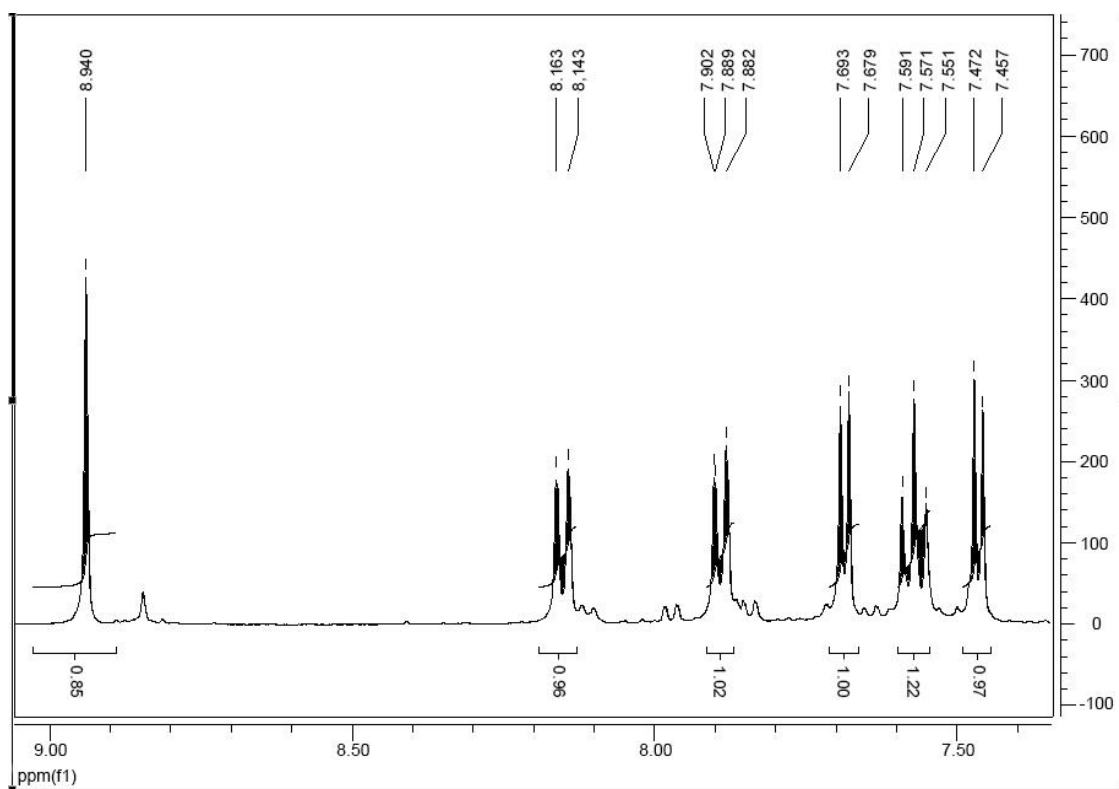


Figure S72. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4n**

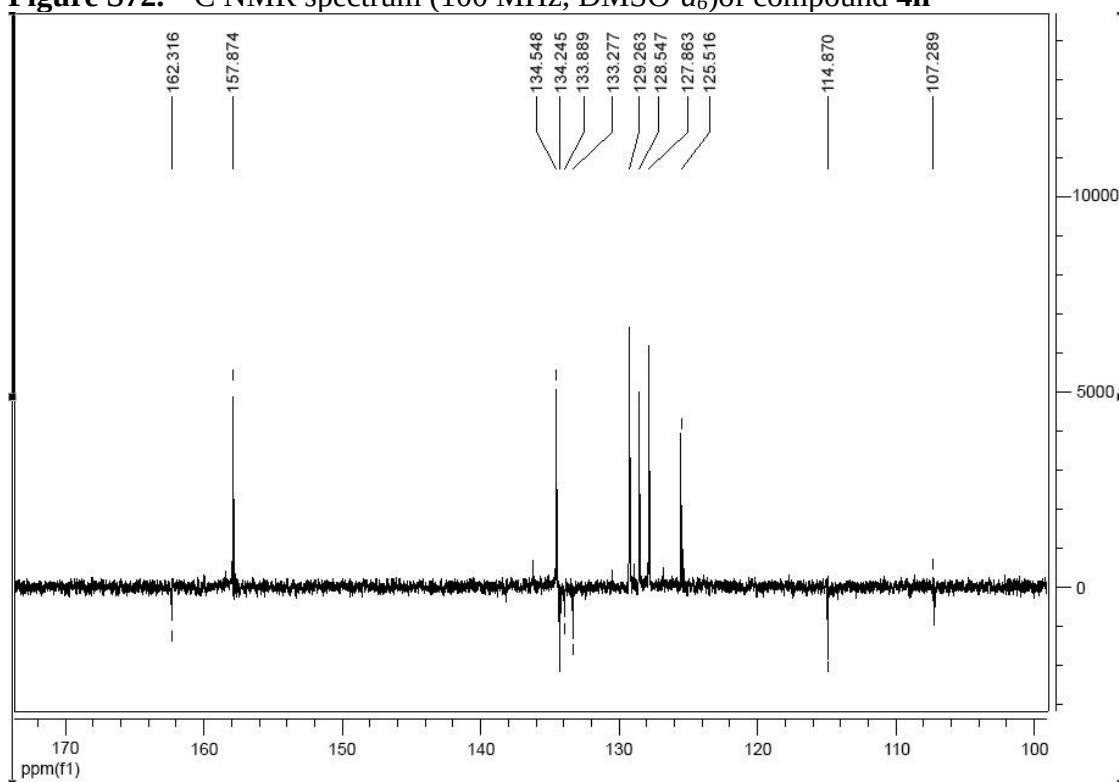


Figure S73. FTIR (KBr) spectrum of compound **4n**

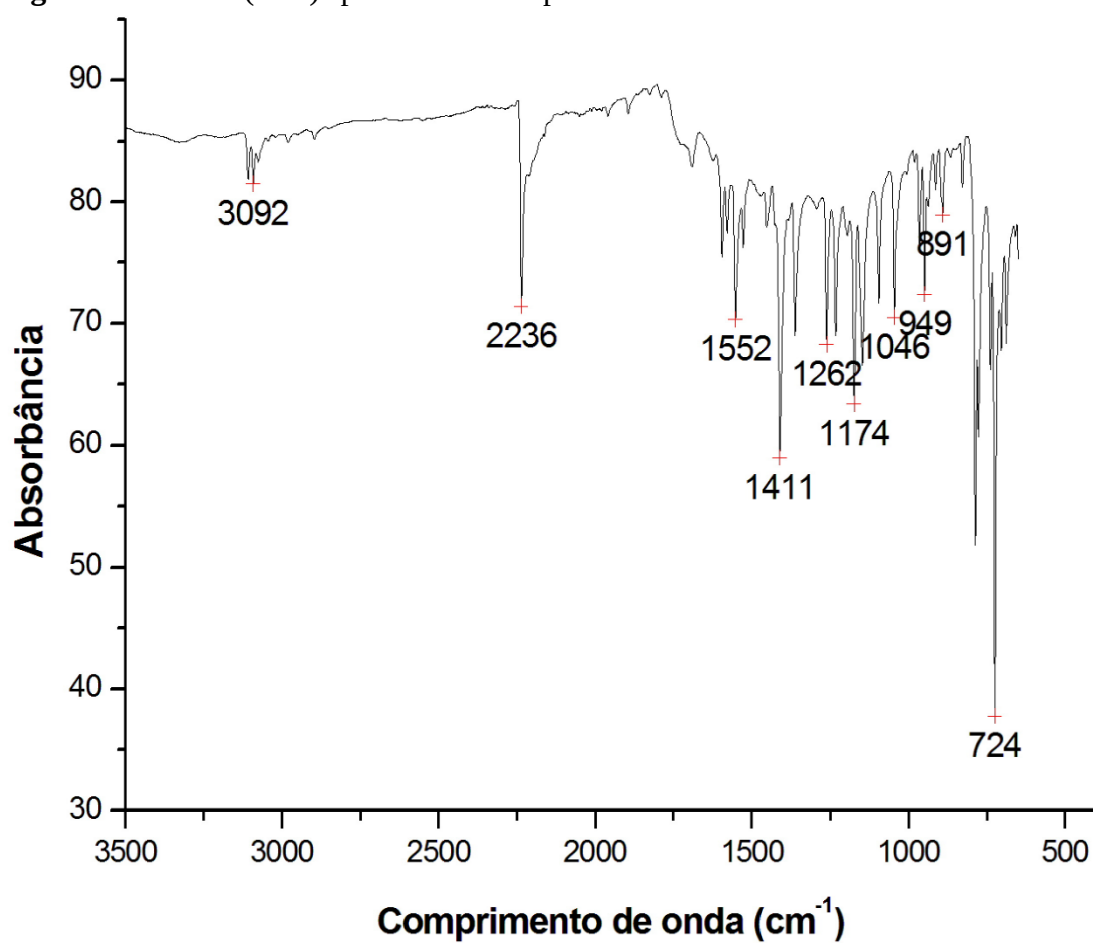


Figure S74. Mass spectrum of compound **4n**

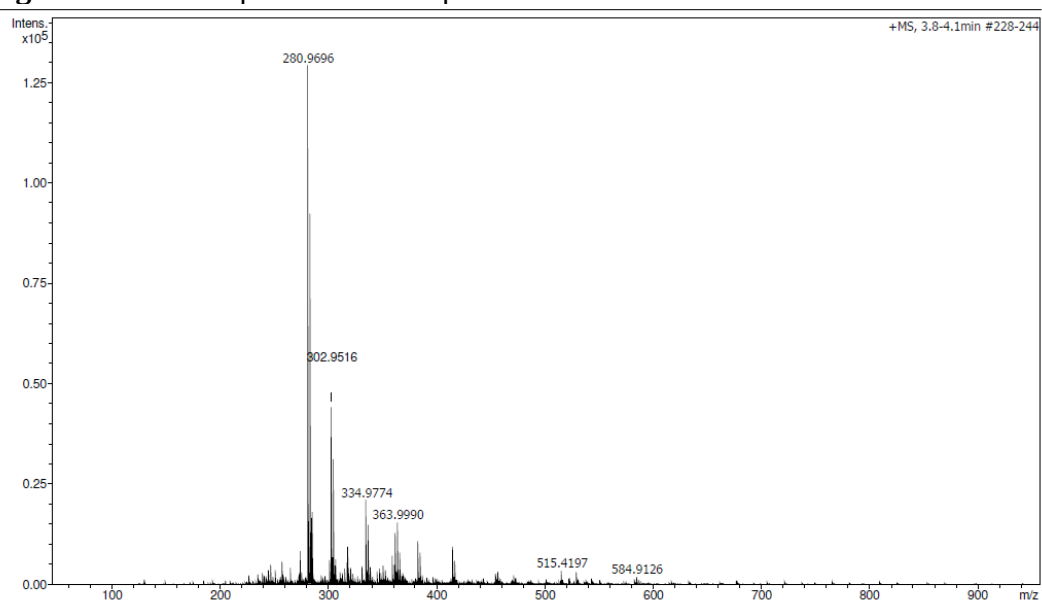


Figure S75. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **4o**

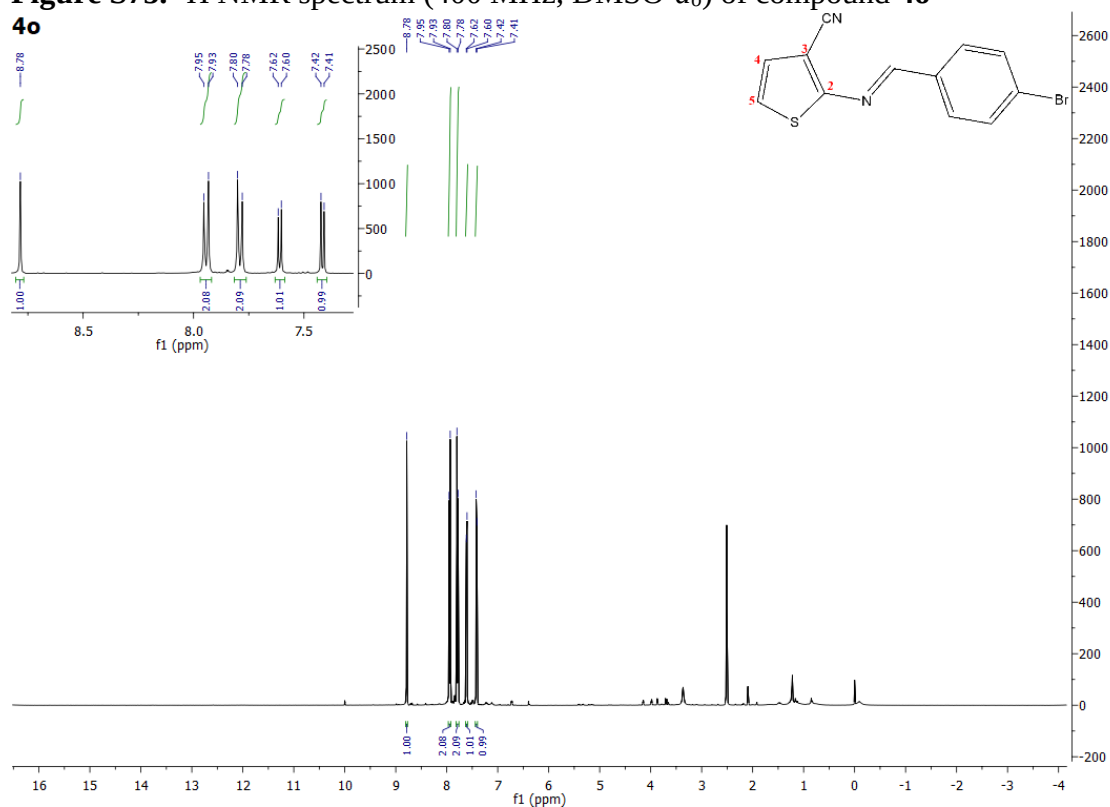


Figure S76. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4o**

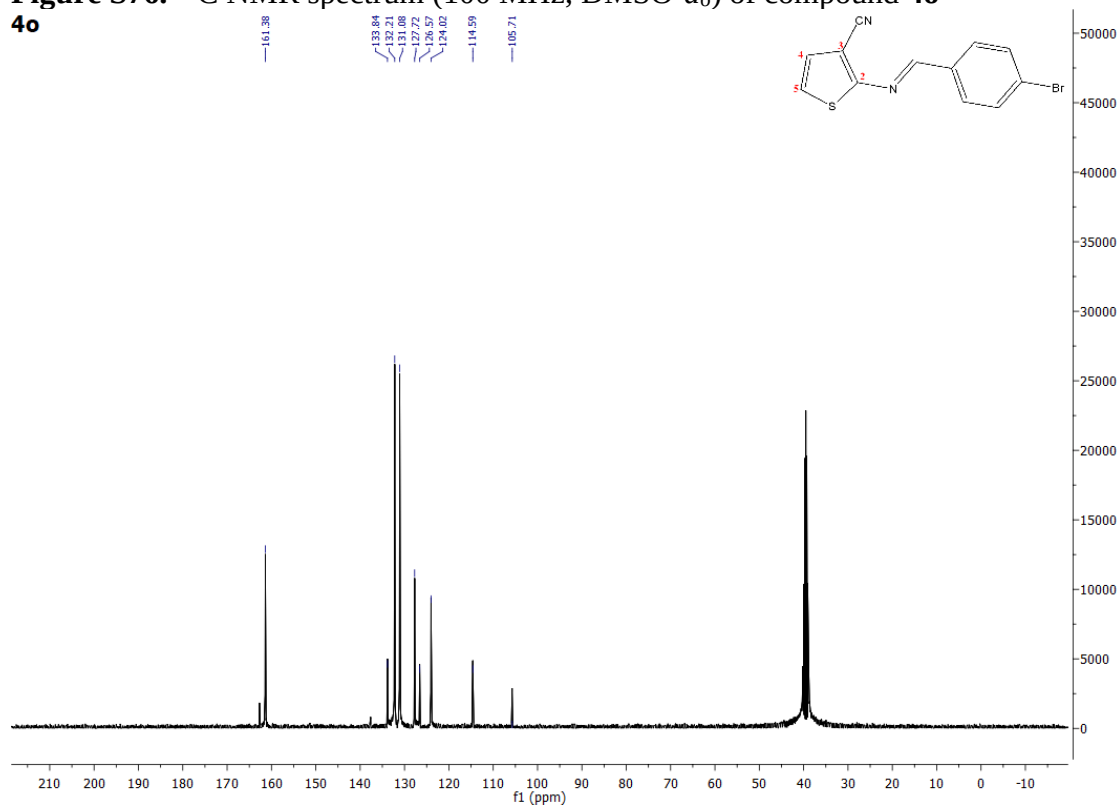


Figure S77. HSQC spectrum of compound **4o**

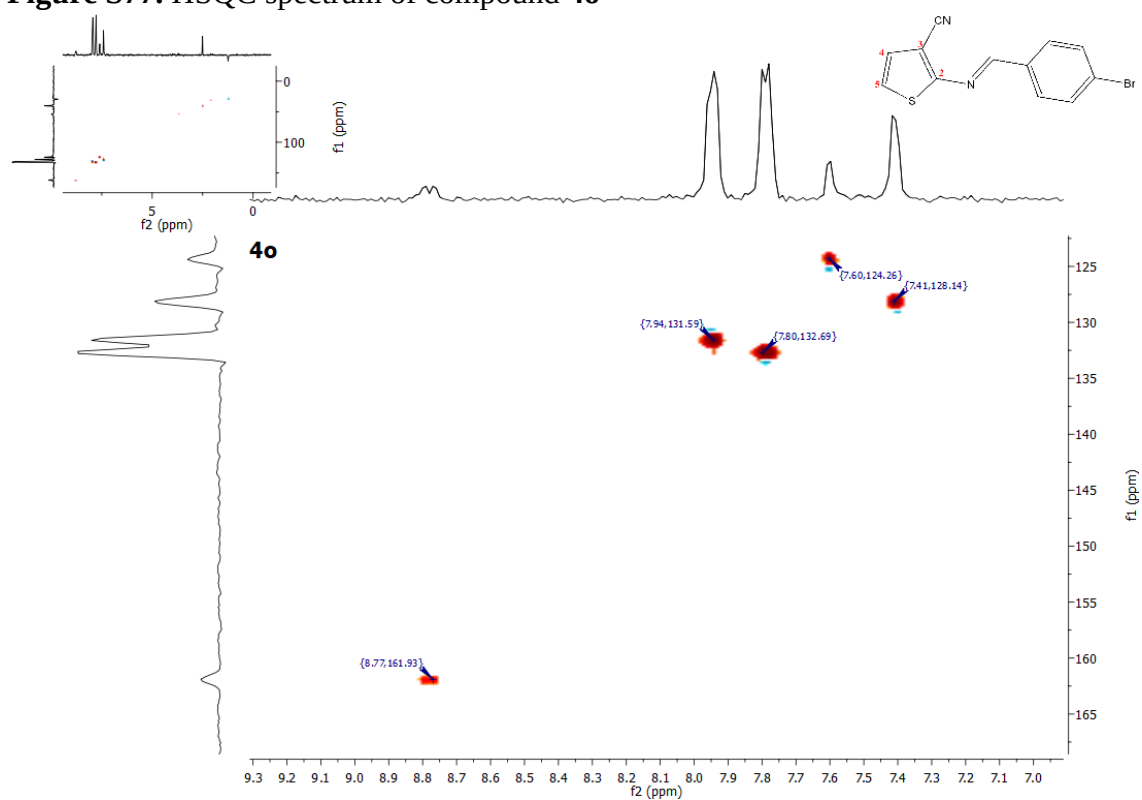


Figure S78. HMBC spectrum of compound **4o**

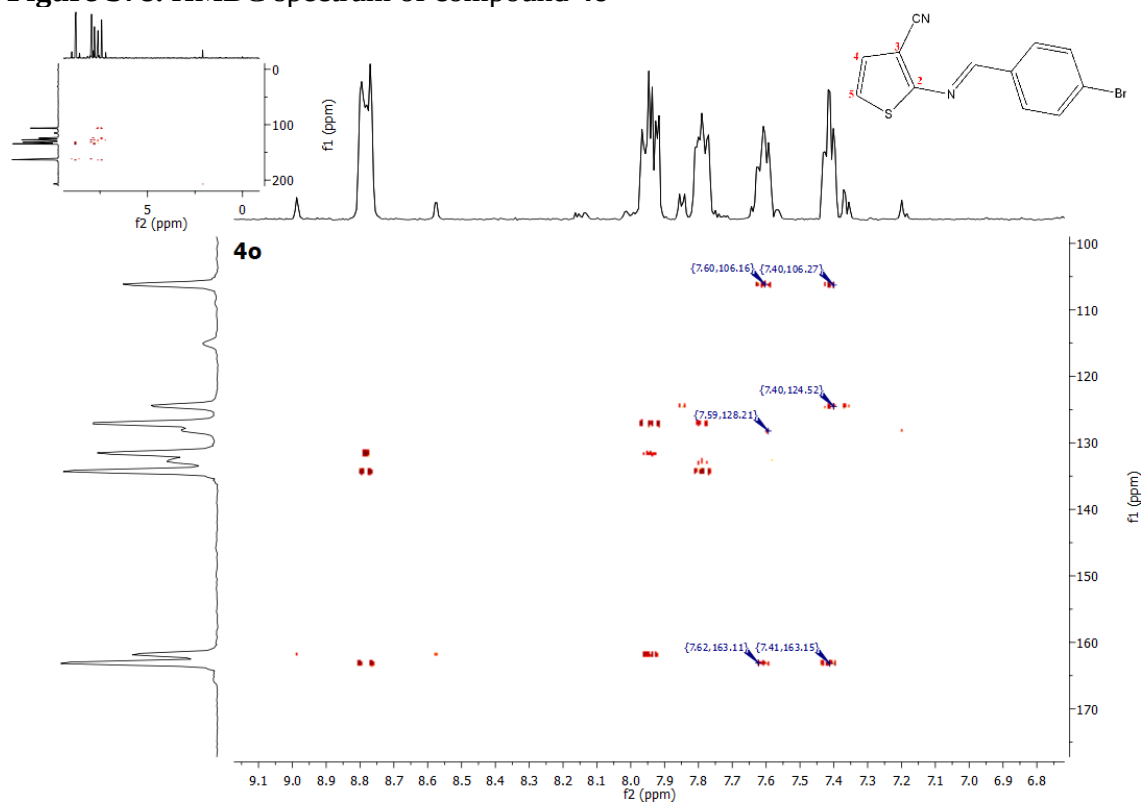


Figure S79. FTIR (KBr) spectrum of compound **4o**

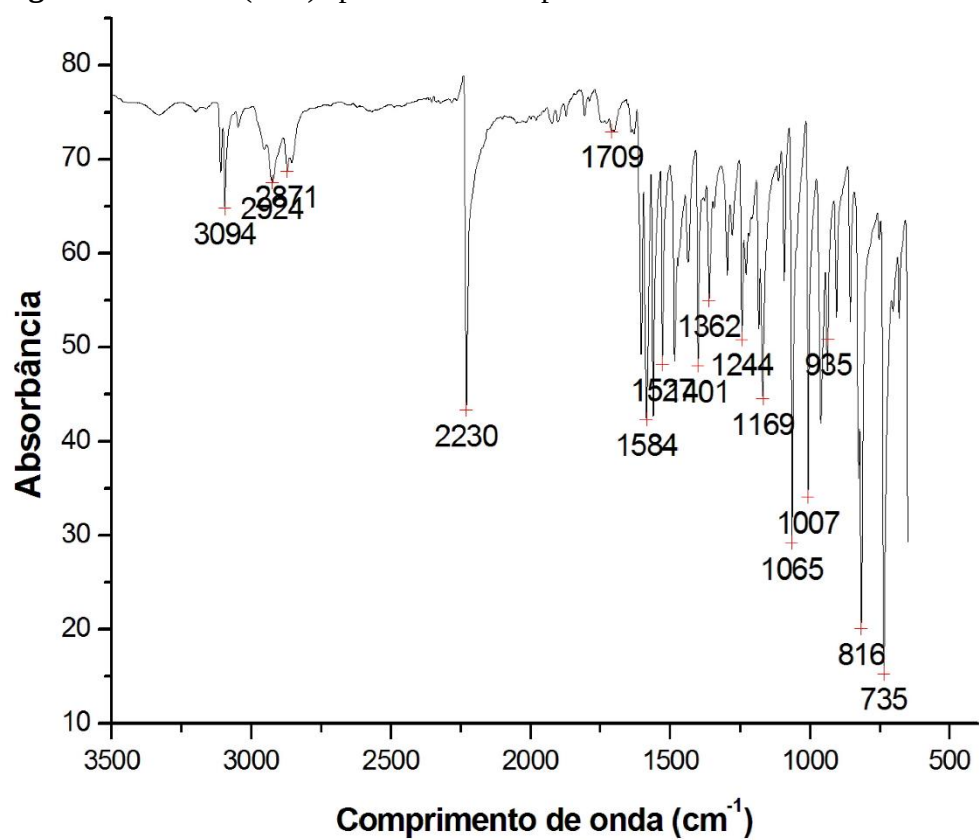
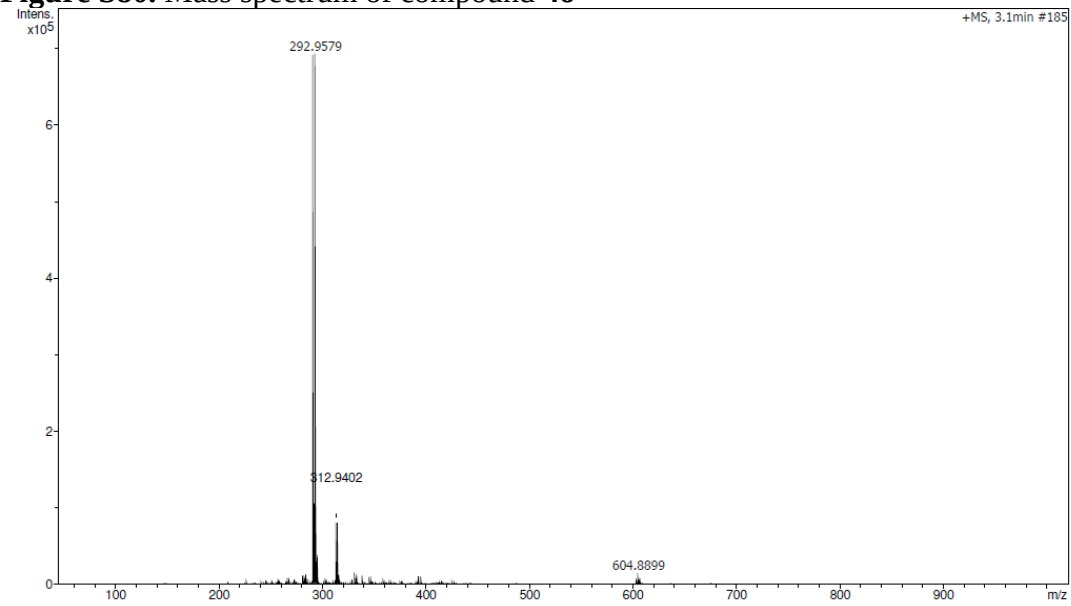


Figure S80. Mass spectrum of compound **4o**



NO₂

of

Figure S81. ^1H NMR spectrum (400 MHz, $\text{DMSO-}d_6$) of compound **4p**
5a

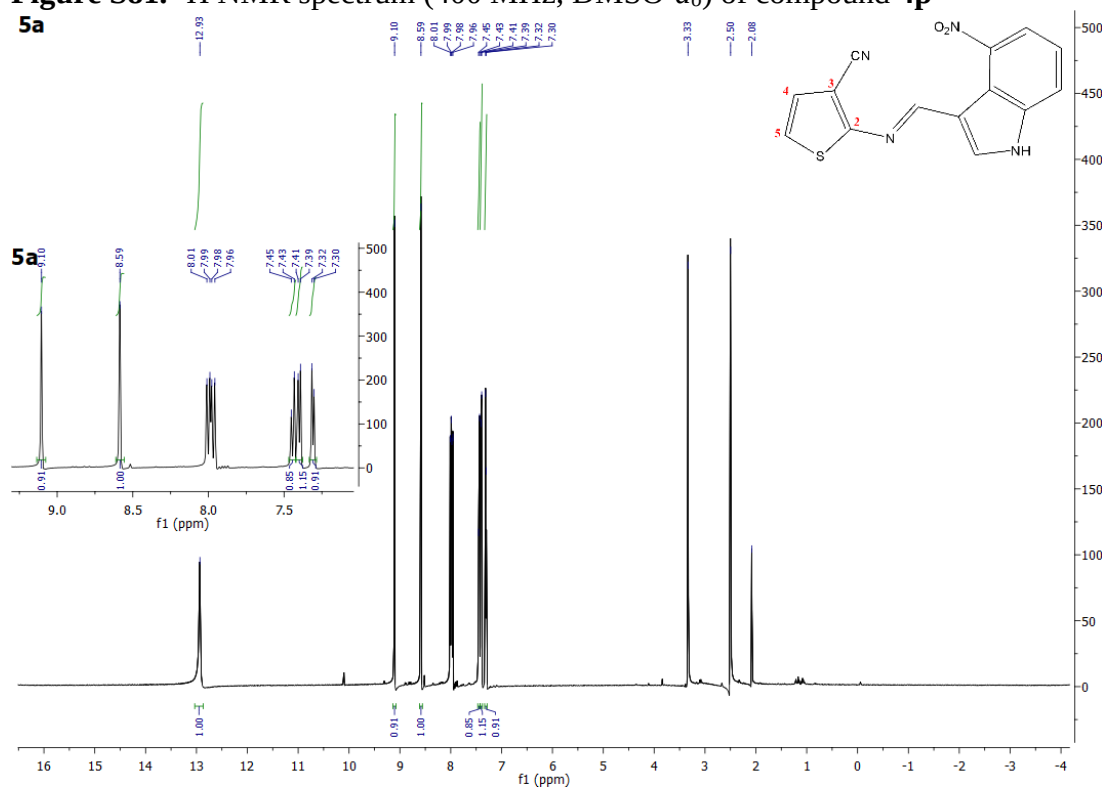


Figure S82. ^{13}C NMR spectrum (100 MHz, $\text{DMSO-}d_6$) of compound **4p**
5b

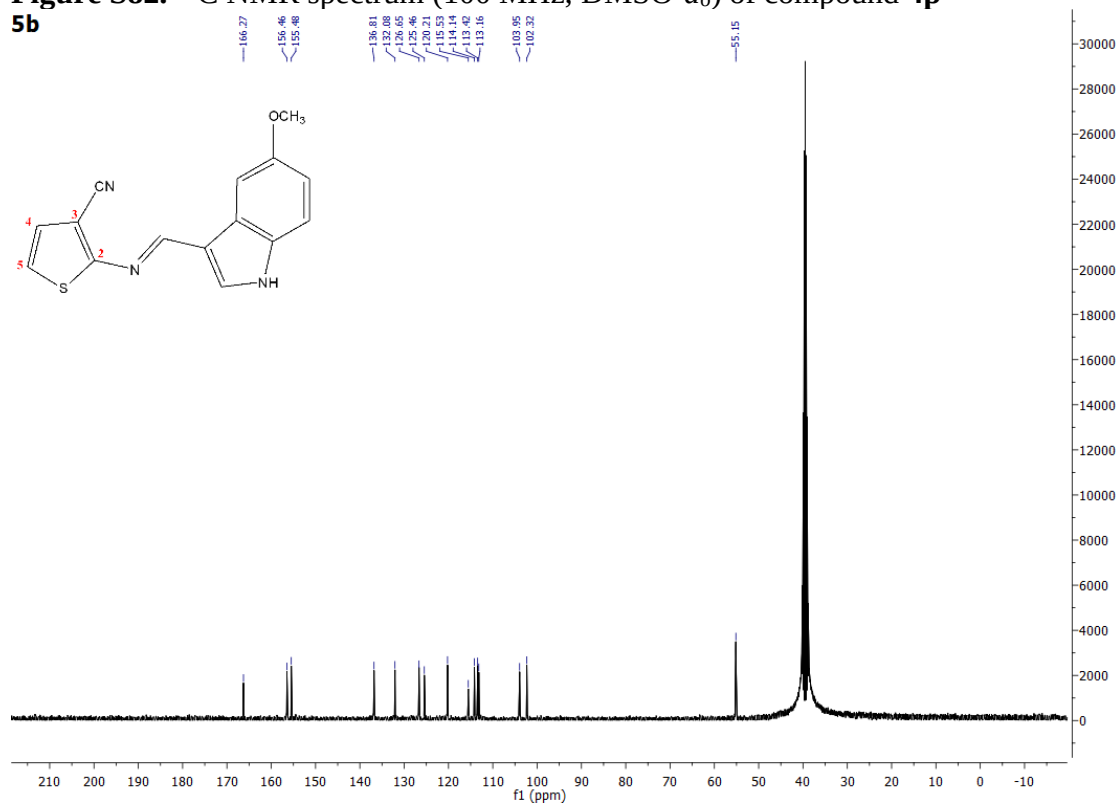


Figure S83. FTIR (KBr) spectrum of compound **4p**

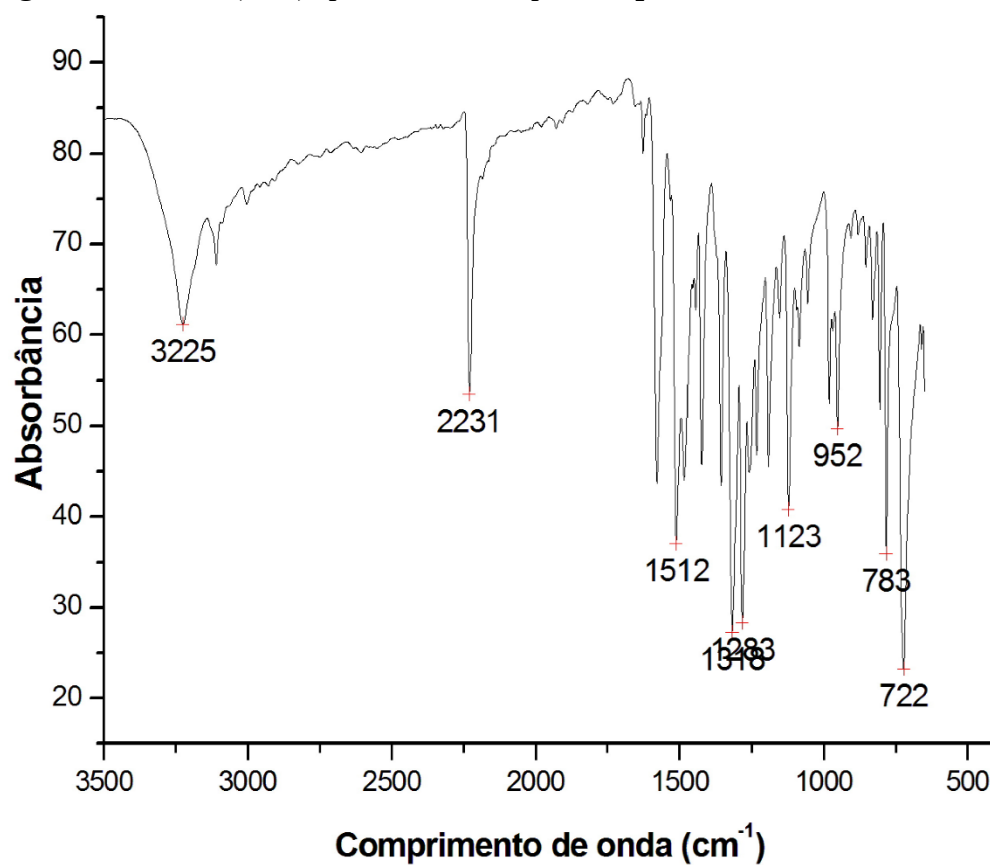


Figure S84. Mass spectrum of compound **4p**

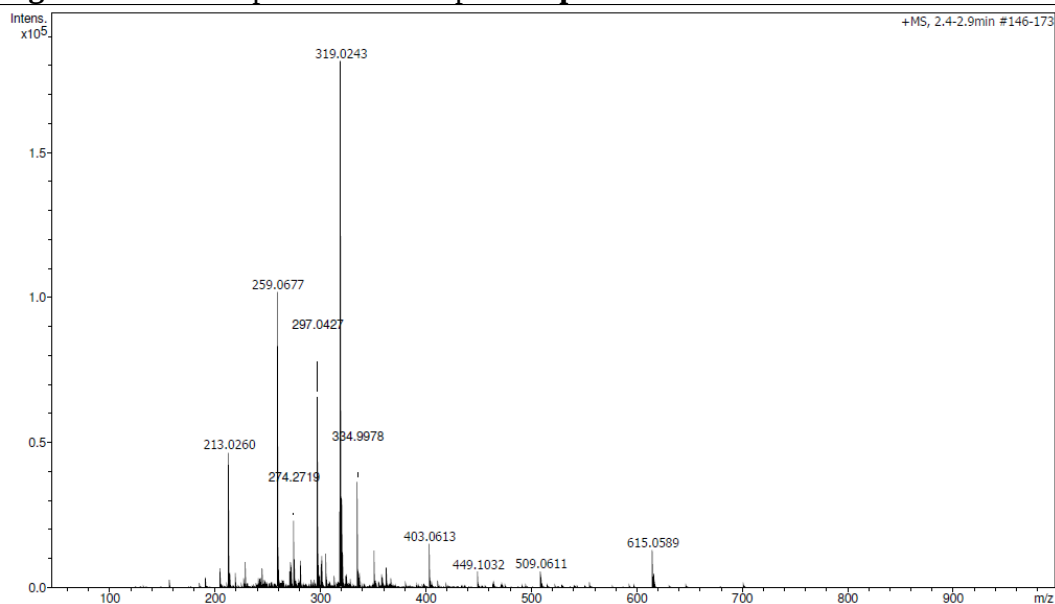


Figure S85. ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound **4q**
5b

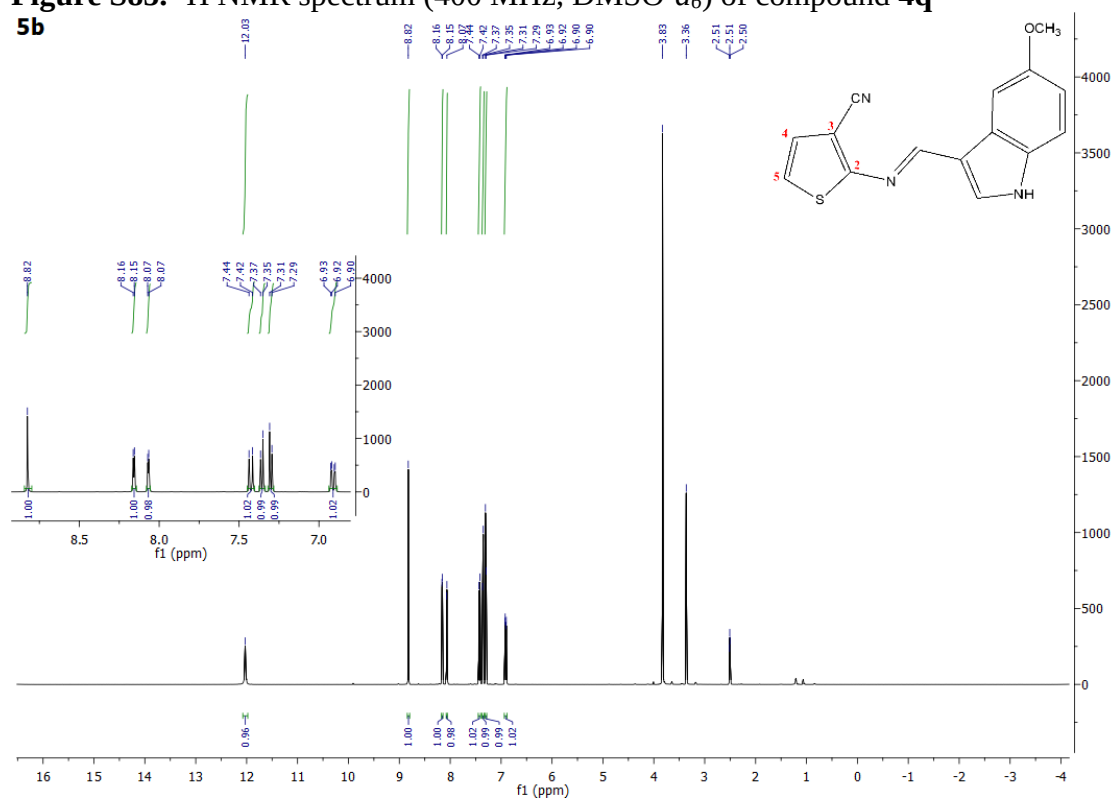


Figure S86. ^{13}C NMR spectrum (100 MHz, $\text{DMSO}-d_6$) of compound **4q**
5b

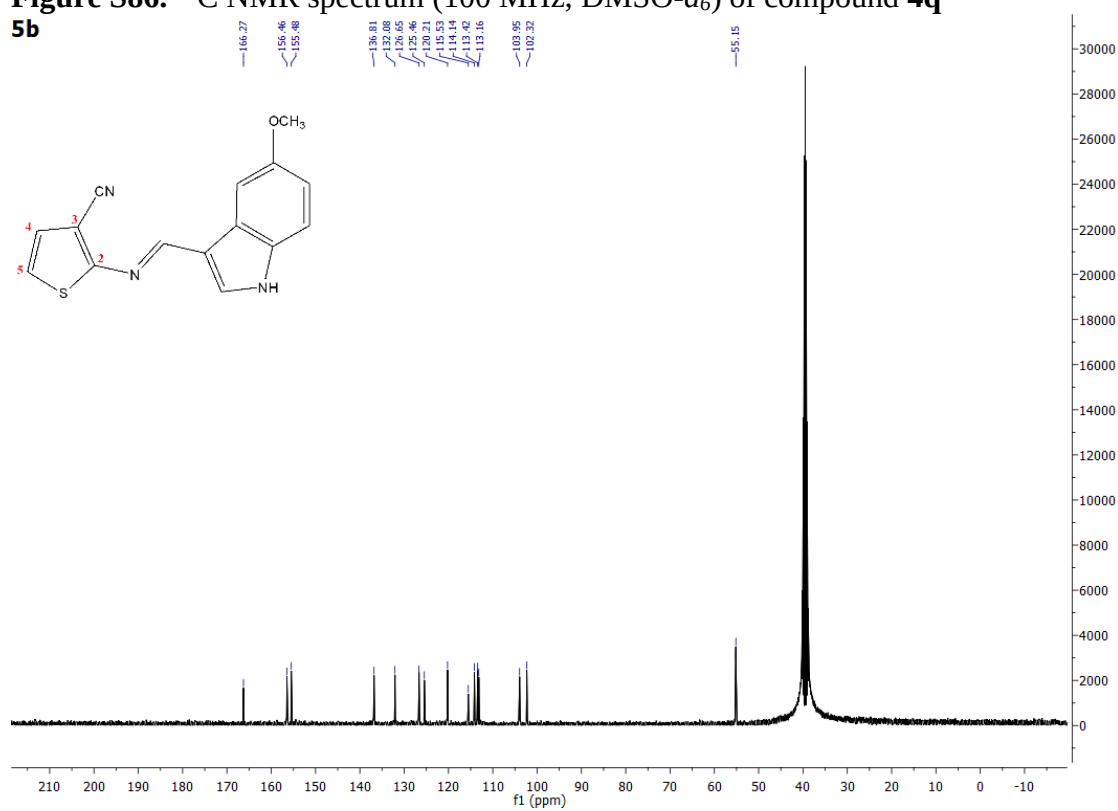


Figure S87. HSQC spectrum of compound **4q**

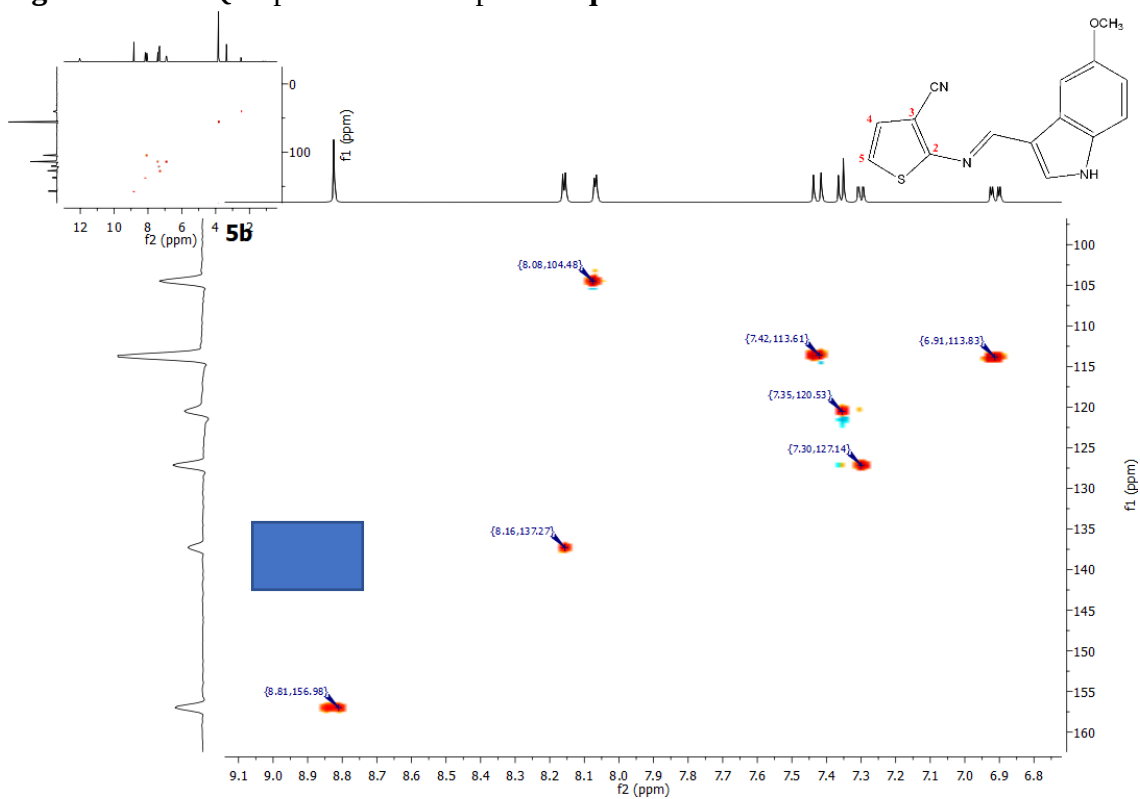


Figure S88. HMBC spectrum of compound **4q**

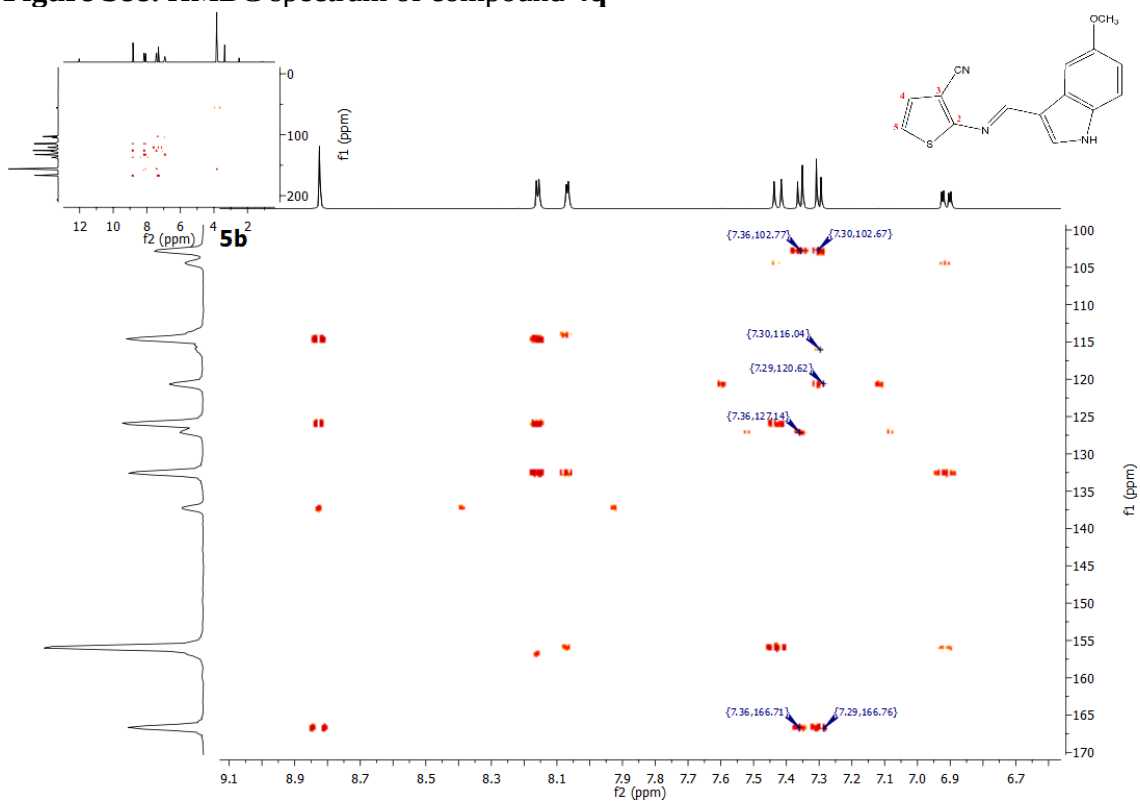


Figure S89. FTIR (KBr) spectrum of compound **4q**

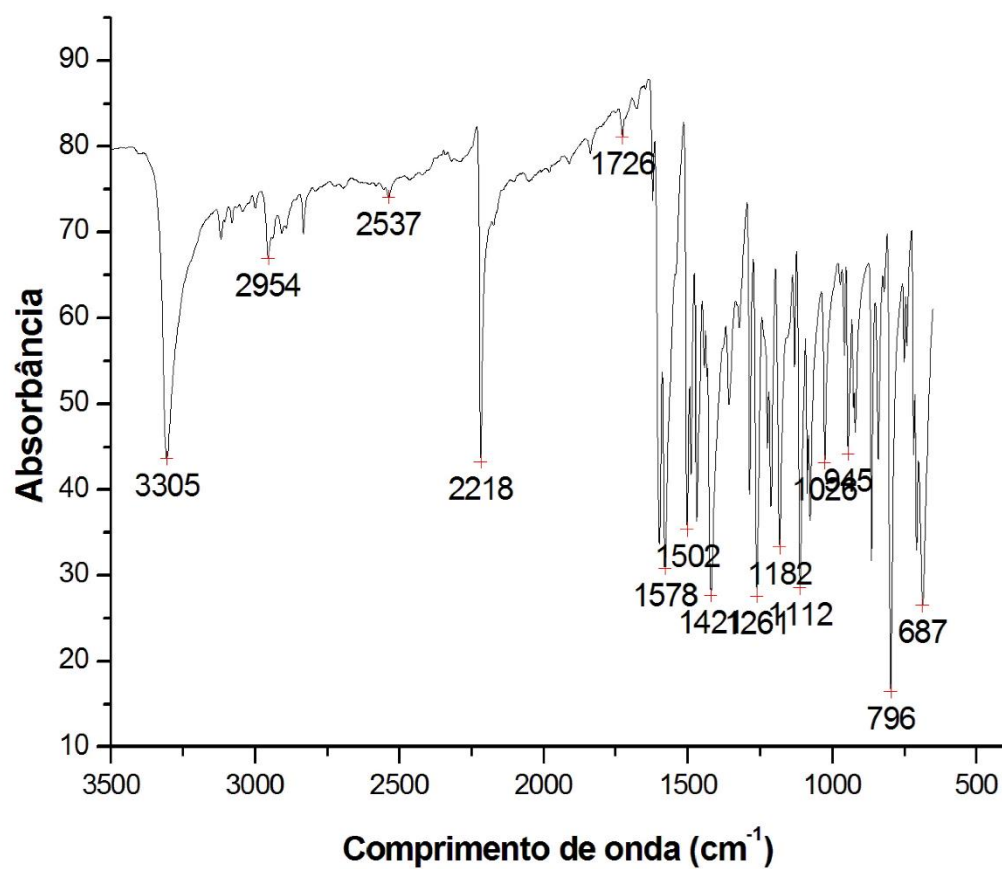
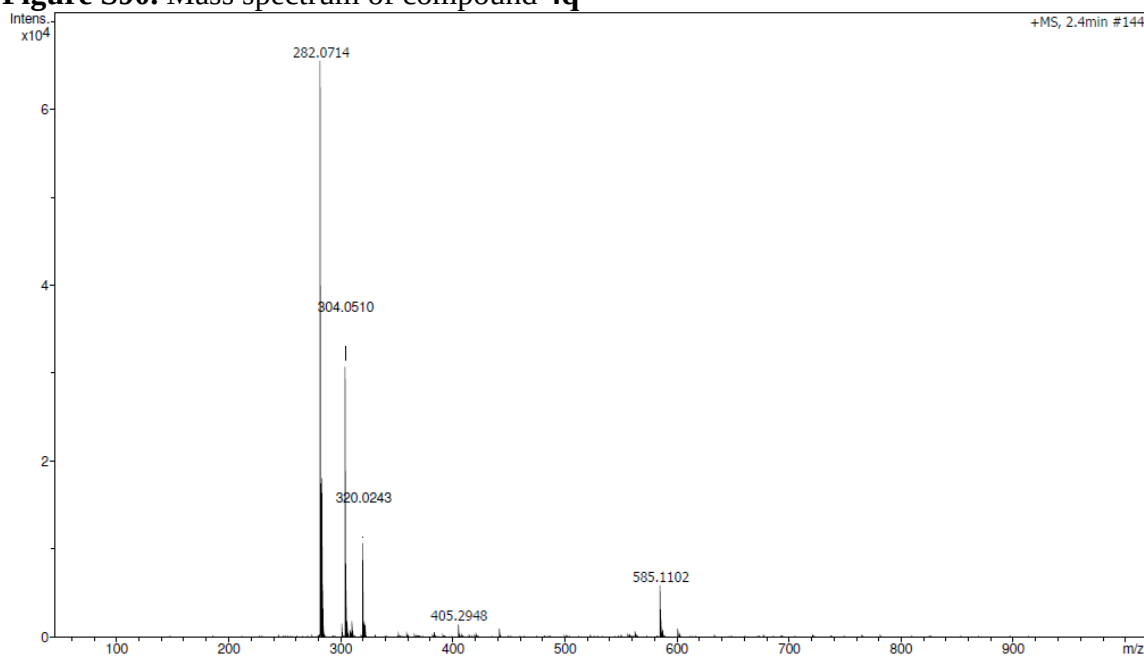


Figure S90. Mass spectrum of compound **4q**



CONCLUSÃO

CONCLUSÃO

Duas pesquisas com diferentes atividades biológicas foram realizadas, seguindo estratégias baseados em planejamento de fármacos auxiliados por computador (CADD). No primeiro estudo, as descobertas demonstram que o uso de triagem virtual baseada em ligantes (LBDD) provou ser bastante eficaz e economizou tempo, esforço e dinheiro na seleção de potenciais agentes anti-*leishmania* e confirma que os derivados de 2-AT são compostos de sucesso promissores para o desenvolvimento de novos agentes anti-*leishmania*.

No segundo estudo, uma nova série contendo 2-aminotiofeno derivados foram projetados, sintetizado e espectroscopicamente caracterizado. O uso da síntese assistida por micro-ondas produziu os compostos em tempos de reação mais curtos, com bons rendimentos. Tomados em conjunto, esses resultados demonstram que a estratégia planejamento baseado na estrutura do ligante foi um sucesso, melhorando semelhança com drogas, perfis farmacocinéticos e solubilidade em água; mostrando que os 2-aminotiofenos são compostos promissores para o desenvolvimento de novas drogas antifúngicas.



ANEXOS



ANEXOS

Artigos e capítulos de livros publicados como primeiro autor

Computer-Aided Drug Design of new 2-amino-thiophene derivatives as antileishmanial agents

Journal: European Journal of Medicinal Chemistry

Impact Factor: 7.088



European Journal of Medicinal Chemistry
journal homepage: www.elsevier.com

Computer-Aided Drug Design of new 2-amino-thiophene derivatives as anti-leishmanial agents

Isadora Silva Luna,^{a,b} Thalisson Amorim de Souza,^c Marcelo Sobral da Silva,^c Klinger Antonio da Franca Rodrigues,^d Luciana Scotti,^b Marcus Tullius Scotti,^b and Francisco Jaime Bezerra Mendonça-Junior,^{a,b,*}

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ABSTRACT

The leishmaniasis is a neglected disease caused by a group of protozoan parasites from the genus *Leishmania* whose treatment is limited, obsolete, toxic, and ineffective in certain cases. These characteristics motivate researchers worldwide to plan new therapeutic alternatives for the treatment of leishmaniasis, where the use of cheminformatics tools applied to computer-assisted drug design has allowed research to make great advances in the search for new drugs candidates. In this study, a series of 2-amino-thiophene (2-AT) derivatives was screened virtually using QSAR tools, ADMET filters and prediction models, allowing direct the synthesis of compounds, which were evaluated *in vitro* against promastigotes and axenic amastigotes of *Leishmania amazonensis*. The combination of different descriptors and machine learning methods led to obtaining robust and predictive QSAR models, with correct classification rates ranging from 0.53 (for amastigotes) to 0.91 (for promastigotes), allowing to select eleven 2-AT derivatives, which do not violate Lipinski's rules, exhibit good druglikeness, and with probability $\leq 70\%$ of potential activity against the two evolutionary forms of the parasite. All compounds were properly synthesized and 8 of them were shown to be active at least against one of the evolutionary forms of the parasite with IC_{50} values lower than 10 μ M. Compounds **8CN** and **DCN-83**, respectively, are the most active against promastigote and amastigote forms, with IC_{50} values of 1.20 and 0.71 μ M. Taken together, these findings demonstrate that the use of ligand-based virtual screening proved to be quite effective and saved time, effort, and money in the selection of potential anti-leishmanial agents, and confirm, once again that 2-AT derivatives are promising hit compounds for the development of new anti-leishmanial agents.

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Design, Synthesis and Antifungal Activity of New Schiff Bases Bearing 2-Aminothiophene Derivatives Obtained by Molecular Simplification

Journal: Journal of the Brazilian Chemical Society

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Article

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Design, Synthesis and Antifungal Activity of New Schiff Bases Bearing 2-Aminothiophene Derivatives Obtained by Molecular Simplification

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Seventeen Schiff bases bearing 2-aminothiophene derivatives were designed and synthesized using molecular simplification. The resulting compounds (**4a-4q**) were evaluated for their *in vitro* antifungal activity against dermatophytes. Prediction of their druglikeness and pharmacokinetic properties, establishment of their structure-activity relationships (SAR), and cytotoxic evaluation of the most active compounds were performed. Using an eco-friendly procedure, microwave assisted synthesis resulted in compounds in good yields (35-85%). Compounds **4a-4q** presented good druglikeness and pharmacokinetic profiles and no cytotoxicity for any cell line tested up to 100 μ M. The compounds presenting the best antifungal profiles were **4e**, **4f**, **4g**, **4k**, **4l**, **4m**, **4o** and **4p** with more than one minimum inhibitory concentration (MIC) value occurring between 16-64 μ g mL⁻¹, thus, in some cases better than the reference drug (fluconazole). SAR testing indicated that the presence of halogens and nitro substituents increases antifungal activity. Taken together, the results demonstrate that 2-aminothiophene derivatives are promising lead compounds for the development of antifungal drugs.

Keywords: 2-aminothiophene, ADMET properties, antifungal agents, drug design, Schiff base

Synthesis, *in silico* characterization and *ex vivo* evaluation of the novel organic nitrate NDIBP as a potential vasorelaxant agent

Journal: Asian Journal of Pharmaceutical Sciences

Impact Factor: 9.289

ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH



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Research Article

SYNTHESIS, *IN SILICO* CHARACTERIZATION AND *EX VIVO* EVALUATION OF THE NOVEL ORGANIC NITRATE NDIBP AS A POTENTIAL VASORELAXANT AGENT

AIRLLA LAANA DE MEDEIROS CAVALCANTI^{1†}, PATRÍCIA KEYTTH LINS ROCHA^{1†}, ISADORA SILVA LUNA¹, MARIA CLÁUDIA RODRIGUES BRANDÃO², EMMELY OLIVEIRA DA TRINDADE², GEOVANI PEREIRA GUIMARÃES³, PETRÔNIO FILGUEIRAS ATHAYDE-FILHO², EUGENE MURATOV⁴, BARKAT KHAN⁵, MARCUS TULLIUS SCOTTI¹, VALDIR DE ANDRADE BRAGA^{1,*}

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ABSTRACT

Objective: This study aimed to describe the synthesis and biological/pharmacokinetic potential of the 1,3-diisobutoxypropan-2-yl nitrate (NDIBP) using *in silico* and *ex vivo* approaches.

Methods: The compound was characterized by Fourier-transform infrared spectroscopy and ¹H and ¹³C- nuclear magnetic resonance spectra. NDIBP biological activity spectrum was obtained by Prediction of Activity Spectra for Substances (PASS). The pharmacological effect was validated in *ex vivo* studies using mesenteric artery. Drug-like properties and Absorption Distribution Metabolism Excretion and Toxicity (ADMET) studies were carried out by pkCSM (Predicting Small-Molecule Pharmacokinetic Properties Using Graph-Based Signatures) software.

Results: PASS prediction indicated NDIBP as nitric oxide (NO) donor with vasodilator effect. *Ex vivo* studies validated PASS analysis and showed the NDIBP vasorelaxant activity in mesenteric arteries. Physicochemical parameters and ADMET prediction suggested that NDIBP is a drug-like molecule with a good theoretical oral bioavailability, good absorption in the gastrointestinal tract, and a low distribution in the tissues.

Conclusion: All the data indicated that NDIBP possesses biological activities and drug-like properties to be considered as a vasorelaxant agent and a good candidate for further investigation in the treatment of arterial hypertension and drug development studies.

RMD86, a thiophene derivative, promotes antinociceptive and antipyretic activities in mice

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Research article

RMD86, a thiophene derivative, promotes antinociceptive and antipyretic activities in mice



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ABSTRACT

Treatment of pain and fever remains an important challenge for modern medicine. Non-steroidal anti-inflammatory drugs (NSAIDs) are the pharmacological options most often used, but their frequent use exposes the patient to serious side effects and dangerous drug interactions. In this context, thiophene derivatives are promising therapeutic alternatives. In this study, we evaluated the *in vivo* and *in silico* antinociceptive and antipyretic properties of RMD86, a thiophene derivative. At 100 mg/kg, RMD86 induced no significant changes in the motor coordination of mice in the Rotarod test. At 25, 50, and 100 mg/kg RMD86 significantly reduced the number of abdominal contortions induced by acetic acid (antinociceptive activity) in mice when compared to the control. In the formalin test, for the first phase, there was a reduction in licking times at doses of 50 and 100 mg/kg. In the second phase, reduction occurred at all doses. In the hot plate test, RMD86 (at 100 mg/kg) increased latency time in the first 30 min. For antipyretic activity, RMD86, when compared to the reference drug acetaminophen (250 mg/kg), significantly reduced pyrexia at 30, 60, and 120 min, at dosages of 25, 50 and 100 mg/kg. Molecular docking studies revealed that RMD86 presents a greater number of interactions and lower energy values than both the co-crystallized ligand and the reference drug (meloxicam) against COX-1 and COX-2 isoenzymes. The results give evidence of the analgesic and antipyretic properties like NSAIDs suggesting its potential for pain therapy.

Review Article

Active Essential Oils and Their Components in Use against Neglected Diseases and Arboviruses

Emanuela Coutinho Luna,¹ Isadora Silva Luna,¹ Luciana Scotti^{2,3}, Alex France M. Monteiro,³ Marcus Tullius Scotti³, Ricardo Olimpio de Moura,¹ Rodrigo Santos Aquino de Araújo^{3,1}, Kadja Luana Chagas Monteiro,⁴ Thiago Mendonça de Aquino,⁵ Frederico Fávoro Ribeiro,¹ and Francisco Jaime Bezerra Mendonça Junior¹

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The term *neglected diseases* refers to a group of infections caused by various classes of pathogens, including protozoa, viruses, bacteria, and helminths, most often affecting impoverished populations without adequate sanitation living in close contact with infectious vectors and domestic animals. The fact that these diseases were historically not considered priorities for pharmaceutical companies made the available treatments options obsolete, precarious, outdated, and in some cases nonexistent. The use of plants for medicinal, religious, and cosmetic purposes has a history dating back to the emergence of humanity. One of the principal fractions of chemical substances found in plants are essential oils (EOs). EOs consist of a mixture of volatile and hydrophobic secondary metabolites with marked odors, composed primarily of terpenes and phenylpropanoids. They have great commercial value and were widely used in traditional medicine, by phytotherapy practitioners, and in public health services for the treatment of several conditions, including neglected diseases. In addition to the recognized cytoprotective and antioxidative activities of many of these compounds, larvicidal, insecticidal, and antiparasitic activities have been associated with the induction of oxidative stress in parasites, increasing levels of nitric oxide in the infected host, reducing parasite resistance to reactive oxygen species, and increasing lipid peroxidation, ultimately leading to serious damage to cell membranes. The hydrophobicity of these compounds also allows them to cross the membranes of parasites as well as the blood-brain barrier, collaborating in combat at the second stage of several of these infections. Based on these considerations, the aim of this review was to present an update of the potential of EOs, their fractions, and their chemical constituents, against some neglected diseases, including American and African trypanosomiasis, leishmaniasis, and arboviruses, specially dengue.

1,4-Dithiane-2,5-diol: A Versatile Synthon for the Synthesis of Sulfur-containing Heterocycles

Journal: Current Organic Synthesis

Impact Factor: 2.276

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1

REVIEW ARTICLE

1,4-Dithiane-2,5-diol: A Versatile Synthon for the Synthesis of Sulfur-containing Heterocycles

Isadora Silva Luna^{a,b}, Rayssa Marques Duarte da Cruz^{a,b}, Ryldene Marques Duarte da Cruz^{a,b}, Rodrigo Santos Aquino de Araújo^a and Francisco Jaime Bezerra Mendonça-Junior^{a,b}

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Abstract: Background: 1,4-Dithiane-2,5-diol (1,4-DTD) is the stable dimer of α -mercapto acetaldehyde. This commercially available ambidentate compound is characterized as having in its chemical structure one group that acts as an electrophile and another that acts as a nucleophile, this permits its use as versatile and efficient synthon in synthetic heterocycle procedures.

Objective: The aim of this review is to present synthetic applications of 1,4-DTD in heterocyclic chemistry and their applicability to the synthesis of bioactive compounds.

Conclusion: Gewald reactions to obtain C-4 and C-5 unsubstituted 2-amino-thiophene derivatives; sulfa-Michael/Henry and sulfa-Michael/aldol sequences to obtain polysubstituted tetrahydrothiophenes, and other heterocyclic reactions that allow synthesizing several functionalized sulfur-containing heterocycles such as thiazolidines, oxathiazinones and thiazoles are presented and discussed. The use of such heterocyclics in subsequent reactions allows obtaining various bioactive compounds including the antiretroviral lamivudine which is one of the examples presented in this review.

Keywords: 1,4-dithiane-2,5-diol, 2-amino-thiophene, Gewald reaction, sulfur heterocycles, sulfanyl carbonyl, tetrahydrothiophene.

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Título da Invenção ou Modelo de Utilidade: derivados 2-amino-tiofênicos como agentes antifúngicos

Depositante: Universidade Federal de Pernambuco

Inventores: Wendell Wons Neves, Isadora Silva Luna, Reginaldo Gonçalves De Lima Neto, Francisco Jaime Bezerra Mendonça Junior, Rejane Pereira Neves

Número de Processo: BR 10 2019 019689 0



Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

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Depositante 1 de 1

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CPF/CNPJ: 24134488000108

Nacionalidade: Brasileira

Qualificação Jurídica: Instituição de Ensino e Pesquisa

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***In silico* analysis of cytotoxicity, rate of absorption and molecular docking of natural products against protease, integrase and HIV-1 reverse transcriptase**

Authors: Alex France Messias Monteiro, **Isadora Silva Luna**, Marcus Tullius Scotti, Luciana Scotti

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***In silico* analysis of cytotoxicity, rate of absorption and molecular docking of natural products against protease, integrase and HIV-1 reverse transcriptase**

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Received: / Accepted: / Published:

Abstract: AIDS is an infectious disease characterized by compromised defense cells, is caused by Human Immunodeficiency Virus - HIV, which affects about 36.7 million people. In its viral multiplication process, HIV requires protease, integrase and reverse transcriptase which are important enzymes in the process. Many therapeutic alternatives in the anti-HIV treatment are in the inhibition of these enzymes, many researches are being directed towards the search of new inhibitors that present better pharmacological profiles. The use of natural products in anti-HIV research has been growing substantially, research groups are betting on these substances in an attempt to offer potent drugs with reduced side effects. The objective of this study was to carry out chemoinformatic studies using cytotoxicity risk prediction tools; prediction of absorption and molecular docking of natural products found in the database of chemical structures (ChEMBL) and literature. The interactions with the targets, the % ABS and cytotoxicological analysis were evaluated. 243 natural products and 18 anti-HIV drugs were analyzed. All molecules had their 3D structures optimized by the methods of mechanical molecules (MM+) and Semi-empirical methods (AM1) (RMS 0.1 kcal /Å.mol in 600 cycles) through HyperChemTM 8.0 software. Structures were imported into the software OSIRIS DataWarrior 4.3.7 for prediction of cytotoxicity risks and absorption rate was calculated based on TPSA. Finally, molecular docking was performed using the software Molegro Virtual Docker 6.0 to calculate the energies of interaction with the protease receptors (PDB ID: 1OHR; reverse transcriptase PDB ID: 1REV and integrase (PDB ID: 3WNH). Of the 243 molecules of natural products with anti-HIV activity, 7 were promising because they did not present cytotoxicity risks (mutagenicity, carcinogenicity, skin irritability and reproductive system effect), better MolDockScore energies for all targets studied simultaneously by varying the interaction energies binder-receptor of -209.47kJ.mol⁻¹ at -60.20kJ.mol⁻¹ and absorption rate (% ABS) between 34.26% and 90.24%.

Keywords: molecular docking; natural products; HIV; cytotoxicity; rate of absorption.

IN SILICO STUDY, SYNTHESIS AND EVALUATION OF THE ANTIFUNGAL POTENTIAL OF NEW 2-AMINO-THIOPHENE DERIVATIVES

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ABSTRACT

INTRODUCTION: 2-Aminothiophene derivatives are important synthetic intermediates for medicinal chemistry, allowing to obtaining several bioactive compounds, with emphasis on antifungal activities^{1,2}. Previous studies carried out by the Laboratory of Synthesis and Vectorization of Molecules have identified promising antimicrobial activity of cycloalquil[b]thiophene derivatives, yet theoretical studies have demonstrated that their high LogP values are one of the main factors limiting their biological potential^{3,4}. **AIMS:** Carry out a rational design of new 2-amino thiophenes obtained from 1,4-dithiane-2,5-diol in order to obtain new molecules with more suitable pharmacokinetic characteristics, and determine the *in vitro* antifungal activity of these new drug candidates. **METHODS:** To perform the *in silico* studies and analyse the pharmacokinetic and toxicological properties was used the method "Predicting Small- Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures (pkCSM)". The synthesis was conducted through the fourth version of Gewald reaction⁵. After structural confirmation, all compounds had their antifungal activities determined against dermatophyte fungi by determination of the minimum inhibitory concentrations (MICs) which were conducted by the broth microdilution method according to

Certificado



I Simpósio Paraibano de Estudos Químicos de Produtos Naturais

Certificamos que **ISADORA SILVA LUNA** participou no I Simpósio Paraibano de Estudos Químicos de Produtos Naturais, realizado pelo PPgPNSB/CCS/UFPB, no período de 9 a 11 de outubro de 2018, com carga horária de 24 horas, como membro da COMISSÃO ORGANIZADORA.

9 a 11 de outubro de 2018.

Marcus Tullius Scotti
Coordenador Geral

Vicente Carlos de Oliveira Costa
Vice-coordenador Geral

Realização:



CERTIFICATE OF ONLINE ATTENDANCE

Isadora Luna

UFPB

HAS ATTENDED THE

XXVIII Symposium on

Bioinformatics and Computer-Aided Drug Discovery

2022 / May 24 - 26

Symposium Co-Chairs



Corr. Member of Rus. Acad. Sci.
Vladimir V. Poroikov



Professor
Roman G. Efremov

XXIII Encontro de Genética do Nordeste

EN GENE



Certificado

Certificamos que o trabalho intitulado **Bioativação do nitrato orgânico benzoato de 4-nitrooxibutil: abordagens ex vivo e in silico**, de autoria de *Patrícia Keytth Lins Rocha; Isadora Silva Luna; Helivaldo Diógenes da Silva Souza; Priscila Santos Vieira de Lima; Petrónio Filgueiras de Athayde-Filho; Juliana Franco de Almeida; Marcus Tullius Scotti; Valdir de Andrade Braga* foi apresentado em formato de Comunicação Oral durante as Atividades do **XXIII ENCONTRO DE GENÉTICA DO NORDESTE**, evento científico realizado de **13 a 16 de Abril de 2021**, em formato online.

Profa. Dra. Gisele Veneroni Gouveia
Presidente do Evento

Promoção



Realização



Certificate

Isadora Silva Luna

has participated at the 1st Brazil-France Symposium on Medicinal Chemistry – *Drugs for Infectious Diseases and Cancer* held from 2-4 December 2019 at the Universidade Federal de Pernambuco, Recife, Brazil.

- PÔSTER: *IN SILICO* STUDY, SYNTHESIS AND EVALUATION OF THE ANTIFUNGAL POTENTIAL OF NEW 2-AMINO-THIOPHENE DERIVATIVES
Isadora Silva Luna; Wendell Wons Neves; Marcus Tullius Scotti; Francisco Jaime Bezerra Mendonça Junior.

Teressinha G. Silva

Profa. Teressinha Gonçalves da Silva
President of the BFSMC



Certificado



Encontro Comemorativo dos 40 Anos do Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos

Certificamos que **Isadora Silva Luna** participou do Encontro Comemorativo dos 40 Anos do Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos, realizado pelo PGPNSB/CCS/UFPB, nos dias 22 e 23 de novembro de 2018, com carga horária de **20** horas.

22 a 23 de novembro de 2018.


Marcelo Sobral da Silva
Coordenador Geral

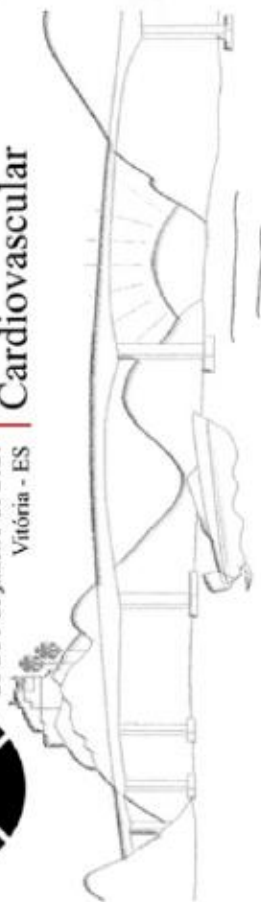

Fabiana de Andrade Cavalcante
Vice-coordenadora Geral



Realização:



XXV Simpósio Brasileiro
de Fisiologia
Cardiovascular
28 a 30 de janeiro de 2021
Vitória - ES



CERTIFICADO

Certificamos que **ROCHA, P.K.L.** submeteu o trabalho: **Avaliação in silico, ex vivo e in vivo do efeito farmacológico do doador de óxido nítrico benzoato de 4-nitrooxibutyl sobre o sistema cardiovascular na modalidade Farmacologia de produtos naturais: ROCHA, P.K.L., GALVÃO, A.C., LUNA, I.S., ALMEIDA, J.F., SCOTTI, M.T., BRAGA, V.A.** trabalho apresentado na XXV Simpósio Brasileiro de Fisiologia Cardiovascular, realizado no período de 28/01/2021 a 30/01/2021.

Prof. Dr. Roger Lyrio dos Santos
Presidente da Comissão Organizadora
XXV SBFCV

Profa. Dra. Carmem Luiza Sartório
Vice-presidente da Comissão Organizadora
XXV SBFCV



WebConai

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Certificamos que

ISADORA SILVA LUNA

Participou na qualidade de congressista da Web Conferência de Análises
Instrumentais - WEBCONAI ocorrido de 03 a 05 de Agosto de 2020 com carga
horária de 40 horas.

Ingrid Ferreira Costa,
Organizadora do WebConai



Realização
CNPJ 28.289.385/0001-78





Universidade
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I Congresso de

**Ciências Aplicadas à
Farmácia**
Online



CERTIFICADO DE PARTICIPAÇÃO

Certificamos que **ISADORA SILVA LUNA** participou do I Congresso de
Ciências Aplicadas à Farmácia - CONCAF, online, realizado durante os dias 15
a 18 de setembro de 2020, com carga horária de 25 horas.



Prof. Dr. João Augusto Oshiro Junior
Coordenador do CONCAF



Prof.ª Dr.ª Léila Aparecida Chivacchi
Coordenadora do CONCAF