Synthesis, in vitro antibacterial and antiprotozoan evaluation of 4,7-disubstituted quinolines

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Dissertation accepted in fulfilment of the requirements for the degree Master of Science in Pharmaceutical Chemistry at the North-West University

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Graduation: December 2023

PREFACE

This dissertation is prepared in article format as per the guidelines of the North-West University's General Academic Rules (A.13.7.3).

CHAPTER 1: INTRODUCTION

CHAPTER 2: LITERATURE REVIEW

CHAPTER 3: MANUSCRIPT FOR SUBMISSION

Synthesis, *in vitro* antibacterial and antiprotozoan evaluation of 4,7-disubstituted quinolines

CHAPTER 4: SUMMARY AND CONCLUSION

ACKNOWLEDGEMENTS

- To **Prof. R.M. Beteck**, my supervisor, thank you for granting me the opportunity to conduct research in your esteemed lab. Your guidance and patience throughout the study have been instrumental to my growth and development.
- To **Prof. L.J. Legoabe**, my co-supervisor, thank you for being a constant source of inspiration and motivation.
- To **Miss P. Dube**, my assistant supervisor, thank you for your remarkable laboratory expertise and commitment to teaching me the essential lab skills I possess today. Above all, thank you for being an inspiring role model.
- To the **North-West University**, for their financial support.
- To **Dr J. Jordaan** and **Dr Otto** for HRMS and NMR spectroscopy.
- To all of my **colleagues** at the Centre of Excellence for Pharmaceutical Sciences (Pharmacen – NWU), thank you for your invaluable assistance and warm camaraderie throughout this journey.
- To my dear friend, **Kobie Neethling**, together we have faced and conquered challenges, celebrated triumphs, and made it to the finish line. Your companionship has meant the world to me.
- To my **family** and **friends** for their love and unconditional support in every aspect of my life.
- To my fiancé, **Paul Burger**, your belief in me has been my driving force. Thank you for restoring hope when it seemed lost. Your encouragement and love are deeply cherished.
- Finally, to my **mother**, my greatest inspiration. Your teachings of perseverance and self-belief have shaped my journey. Forever grateful.

ABSTRACT

The rise of antimicrobial resistance has become a critical global health threat, rendering current treatments ineffective against multidrug-resistant pathogens. This issue is further compounded by the limited availability of effective drugs for neglected diseases and their associated toxicity and inadequate drug-like properties. In light of these challenges, the development of novel compounds and therapeutic strategies has become an urgent necessity in the fight against antimicrobial resistance.

Among the diseases with limited treatment options, human African trypanosomiasis (HAT), commonly known as sleeping sickness, stands out as a particularly challenging and lethal protozoal disease. HAT is caused by *Trypanosoma* parasites transmitted through tsetse flies. The currently approved drugs for HAT management suffer from poor drug-like properties and severe side effects. Similarly, African animal trypanosomiasis, a form of trypanosomiasis affecting animals, presents treatment challenges due to limited available drugs and concerns over toxicity.

Leishmaniasis, caused by *Leishmania* parasites transmitted by sandflies, manifests in three main clinical presentations. This disease is prevalent in regions with poor healthcare access, resulting in limited diagnosis, treatment, and prognosis. Although several drugs are used to combat leishmaniasis, the currently most effective treatment options are marred by severe side effects, poor drug-like properties, and emerging drug resistance.

ESKAPE pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., are highly resistant to antimicrobials and contribute significantly to mortality rates. These pathogens pose a major concern, particularly in healthcare settings and among immuno-compromised patients.

Quinolines have demonstrated broad-spectrum biological activities. Aminochalcone, another compound class, has exhibited antimicrobial properties and various other desired pharmacological properties. Nitro-containing carbocycles are increasingly gaining attention from researchers due to their antimicrobial properties. The electron-attracting characteristics of the nitro group contribute to redox reactions in cells, leading to the demise of micro-organisms. Aromatic nitro compounds, including fexinidazole and benznidazole, have demonstrated efficacy against protozoans and several bacterial pathogens.

To address the challenges posed by antimicrobial resistance and the limitations of current drugs, such as toxicity and poor drug-like properties, there is a pressing need to explore novel compounds and therapeutic strategies. This dissertation aims to investigate novel compounds incorporating quinolinyl, aminochalconyl, and aryl nitro moieties as potential drug substrate against pathogens of clinical relevance.

In pursuit of the set aim, we synthesised 18 novel compounds in this study through a multistep synthetic protocol involving *N*-acylation, aldol condensation, and nucleophilic substitution. The compounds were achieved in yields ranging from 8% to 92%. Analytical techniques such as nuclear magnetic resonance, high-resolution mass spectrometry, Fourier-transform infrared spectroscopy, and high-performance liquid chromatography were utilised for compound characterisation and determination of purity. These compounds underwent *in vitro* evaluations against *Trypanosoma brucei rhodesiense*, *Trypanosoma brucei brucei*, *Trypanosoma cruzi*, *Leishmania infantum*, and specific ESKAPE pathogens. Additionally, antifungal screening was performed against *Candida albicans*.

While none of the compounds exhibited activity against the specific ESKAPE pathogens or *C. albicans*, all of them demonstrated significant broad-spectrum antiprotozoal activity, with values ranging from 0.11 µM to 36.76 µM. However, the study revealed that certain potent compounds exhibited considerable levels of toxicity, representing a limitation that necessitates further optimisation in future investigations. Notably, **Compound 4i** exhibited potent broad-spectrum antiprotozoal activity while maintaining low toxicity against normal human cell lines. Additionally, **Compounds 2c**, **2d**, and **3d** showed potent antiprotozoal activity against *T.b. rhodesiense*, *T.b. brucei*, and *L. infantum*, respectively. These compounds showed low toxicity against normal human cell lines.

Keywords: Quinolines, Aminochalcones, Nitro aromatics, Trypanosomiasis, Leishmaniasis, ESKAPE pathogens.

TABLE OF CONTENTS

PREFACE ..... **ii**

ACKNOWLEDGEMENTS ..... **iii**

ABSTRACT ..... **iv**

LIST OF FIGURES ..... **ix**

LIST OF TABLES ..... **xi**

LIST OF SCHEMES ..... **xi**

ABBREVIATIONS AND NOMENCLATURE ..... **xii**

CHAPTER 1: INTRODUCTION **1**

1.1 Introduction 1

1.1.1 Human African trypanosomiasis 3

1.1.2 African animal trypanosomiasis 4

1.1.3 Leishmaniasis 5

1.1.4 ESKAPE pathogens 6

1.2 Problem statement 8

1.3 Aims and objectives 8

CHAPTER 1: REFERENCE LIST **10**

CHAPTER 2: LITERATURE REVIEW **15**

2.1 Introduction 15

2.2 Protozoal diseases 16

2.2.1 Human African trypanosomiasis 16

2.2.1.1 Signs, symptoms and diagnosis 17

2.2.1.2 Current drugs 19

2.2.1.3 Transmission and epidemiology 22

2.2.2 African animal trypanosomiasis 24

2.2.2.1 Signs, symptoms and diagnosis 25

2.2.2.2 Current drugs 27
2.2.2.3 Transmission and epidemiology 28
2.2.3 Leishmaniasis 28
2.2.3.1 Signs, symptoms and diagnosis 30
2.2.3.2 Current drugs 32
2.2.3.3 Transmission and epidemiology 35
2.3 Pathogenic bacteria 37
2.3.1 ESKAPE pathogens....37
2.3.2 Resistance mechanism of ESKAPE pathogens....40
2.4 Quinolines....41
2.4.1 Antiprotozoal activity....42
2.4.1.1 Antileishmanial drugs....43
2.4.1.2 Antitrypanosomal drugs 44
2.4.2 Antibacterial activity....46
2.5 Aminochalcones....47
2.5.1 Antiprotozoal activity....48
2.5.2 Antibacterial activity....49
2.6 Aromatic nitro compounds 50
2.6.1 Antiprotozoal activity....51
2.6.2 Antibacterial activity....52
2.7 Rationale 53
CHAPTER 2: REFERENCE LIST....55
CHAPTER 3: ARTICLE FOR SUBMISSION....67
3.1 Introduction....71
3.2 Results and discussion 73
3.2.1 Chemistry and structure characterisation....73
3.2.2 Antiprotozoal activity....75
3.2.3 Antibacterial and antifungal activities 79

3.3 Materials and methods 79
3.3.1 General methods 79
3.3.2 Synthesis of target compounds 80
3.3.2.1 Synthesis of Compound 1a 80
3.3.2.2 Synthesis of Compounds 2a–2d and 4a–4i 81
3.3.2.3 Synthesis of Compounds 3a–3d 81
3.3.3 *In vitro* antiprotozoal evaluation 85
3.3.4 *In vitro* antibacterial and antifungal evaluation 86
3.3.5 *In vitro* cytotoxicity evaluation 87
3.4 Conclusion 87
CHAPTER 3: REFERENCE LIST 89
CHAPTER 4: SUMMARY AND CONCLUSION 93
CHAPTER 4: REFERENCE LIST 96
ANNEXURE A: SUPPLEMENTARY MATERIAL FOR CHAPTER 3 98

LIST OF FIGURES

Figure 1.1: Structures of 4,7-disubstituted quinolines (chloroquine and amodiaquine) 2

Figure 1.2: The Qiao *et al*. (2012:3554) synthesised aminochalcone 2

Figure 1.3: The Nielsen *et al*. (2005:2671) synthesised aminochalcone.....3

Figure 1.4: Aromatic nitro drugs – fexinidazole and benznidazole 3

Figure 2.1: Summary of the two subspecies of T. brucei responsible for HAT (adapted from Berninger *et al*., 2017:4; Malvy & Chappuis, 2011:987) 17

Figure 2.2: The structure of pentamidine.....20

Figure 2.3: The structure of suramin 20

Figure 2.4: The structure of melarsoprol 21

Figure 2.5: The structure of eflornithine and nifurtimox.....21

Figure 2.6: The structure of fexinidazole 22

Figure 2.7: The distribution of HAT across Africa (Brun *et al*., 2010:149).....23

Figure 2.8: Summary of the three main species responsible for AAT (adapted from Eshetu & Begejo, 2015:117).....25

Figure 2.9: The structures of AAT drugs 28

Figure 2.10: The structures of antileishmanial drugs 33

Figure 2.11: The structure of quinoline.....41

Figure 2.12: The Yousuf *et al*. (2016:4-7) synthesised compounds that had antileishmanial activity 43

Figure 2.13: The Ramírez–Prada *et al*. (2017:2) synthesised compound that exhibited high antileishmanial activity.....44

Figure 2.14: The Nefertiti *et al*. (2018:11-13) synthesised compounds that exhibited high antitrypanosomal activity 44

Figure 2.15: The Lavrado *et al*. (2012:6256-6259) synthesised compounds that exhibited high antitrypanosomal activity 45

Figure 2.16: The Yang *et al*. (2017:4) synthesised compound that exhibited high antibacterial activity 46

Figure 2.17: The structure of chalcone 47

Figure 2.18: The structure of aminochalcones 48

Figure 2.19: Aminochalcones with antitrypanosomal activity (Qiao *et al*., 2012:3554) 49

Figure 2.20: Chalcones exhibiting antiprotozoal activity (Roussaki *et al*., 2013:5) 49

Figure 2.21: Aminochalcones exhibiting antibacterial activity (Nielsen *et al*., 2005:2671) 50

Figure 2.22: The structure of benznidazole, an effective aryl nitro compound (Soriano-Correa *et al*., 2008:1370) 51

Figure 2.23: The Pozas *et al*. (2005:1417) synthesised compounds 52

Figure 2.24: The Andrade *et al*. (2020) synthesised nitro compounds 53

Figure 2.25: The Gupta *et al*. (2019) synthesised nitro compounds 53

Figure 3.1: Drugs used to treat trypanosomatid diseases 72

Figure 4.1: Target compounds exhibiting potent antiprotozoal activity 94

LIST OF TABLES

Table 3.1: Structures, antiprotozoal activities, and cytotoxicity of Compounds 2a–2d, 3d, and 4a–4i.....76

Table 4.1: Target compounds with the most potent MIC values against evaluated protozoal pathogens 94

LIST OF SCHEMES

Scheme 1: Synthesis of target compounds 74

ABBREVIATIONS AND NOMENCLATURE

^{1}H	proton nuclear magnetic resonance
^{13}C	carbon-13 nuclear magnetic resonance
A. baumannii	*Acinetobacter baumannii*
AAT	African animal trypanosomiasis
AmB-D	amphotericin B deoxycholate
AmB-L	lipid formulation of amphotericin
APCI	atmospheric pressure chemical ionisation
CC_{50}	50% cytotoxic concentration
CFU	colony-forming unit
CNS	central nervous system
d	doublet
dd	doublet of doublets
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
E. aerogenes	*Enterococcus aerogenes*
E. cloacae	*Enterococcus cloacae*
E. faecium	*Enterococcus faecium*
E. hormaechei	*Enterococcus hormaechei*
EC_{50}	half maximal effective concentration
ESKAPE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp.
FTIR	Fourier-transform infrared spectroscopy
GI_{50}	50% growth inhibitory concentration
HAT	human African trypanosomiasis
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IC_{50}	half maximal inhibitory concentration
ICU	intensive care unit

J	coupling constant
K. pneumoniae	*Klebsiella pneumoniae*
L. aethiopica	*Leishmania aethiopica*
L. braziliensis	*Leishmania braziliensis*
L. donovani	*Leishmania donovani*
L. infantum	*Leishmania infantum*
L. major	*Leishmania major*
L. panamensis	*Leishmania panamensis*
L. tropica	*Leishmania tropica*
m	multiplet
m.p.	melting point
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
NECT	nifurtimox-eflornithine combination therapy
NMR	nuclear magnetic resonance
P. aeruginosa	*Pseudomonas aeruginosa*
PCR	polymerase chain reaction
ppm	parts per million
s	singlet
S. aureus	*Staphylococcus aureus*
Sb^v	pentavalent antimonial
t	triplet
T. congolense	*Trypanosoma congolense*
T. cruzi	*Trypanosoma cruzi*
T. evansi	*Trypanosoma evansi*
T. vivax	*Trypanosoma vivax*
T.b. brucei	*Trypanosoma brucei brucei*
T.b. gambiense	*Trypanosoma brucei gambiense*
T.b. rhodesiense	*Trypanosoma brucei rhodesiense*
td	doublet of triplets
TLC	thin-layer chromatography

CHAPTER 1: INTRODUCTION

1.1 Introduction

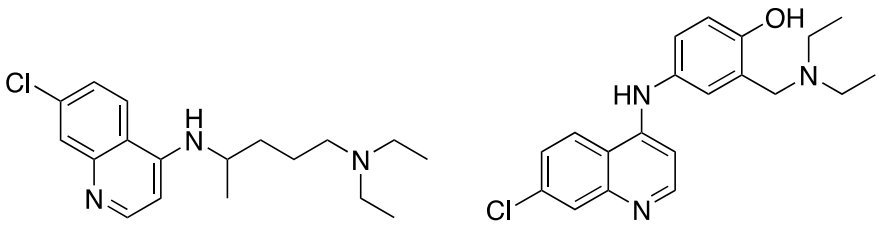
Antimicrobial resistance poses a significant and growing threat to human health, jeopardising the effectiveness of conventional medications (Chinemerem Nwobodo *et al.*, 2022). It refers to the phenomenon where various pathogenic microorganisms, including bacteria, protozoans, viruses, and fungi, develop resistance or reduced sensitivity to previously effective antimicrobial treatments. The emergence of multidrug resistance, wherein microorganisms exhibit resistance to three or more classes of antimicrobial agents, further compounds the challenge by limiting treatment options for diseases caused by these resilient pathogens (Tanwar *et al.*, 2014:1). The development of antimicrobial resistance is influenced by a multitude of factors, including excessive antimicrobial prescribing, inappropriate use of antimicrobials, and the prevalence of substandard pharmaceuticals (Santajit & Indrawattana, 2016:1).

In addition to the formidable threat of antimicrobial resistance, the limited availability of effective drugs, along with concerns regarding drug toxicity and suboptimal pharmacological properties, presents significant obstacles in the field of therapeutics (Di & Kerns, 2015:10). Hence, there is a pressing need for ongoing research and innovation to discover novel compounds and devise innovative therapeutic strategies to address these critical healthcare challenges.

Quinolines have emerged as a promising class of compounds due to their diverse biological activities, making them attractive candidates for combating drug-resistant microorganisms of clinical significance. These compounds have demonstrated antibacterial, antiprotozoal, anti-inflammatory, antiplatelet, insecticidal, and antitumour properties, while exhibiting low toxicity towards mammalian cells (Senerovic *et al.*, 2019:37). The structural diversity inherent in quinoline and its derivatives underpins the multifaceted mechanisms of action associated with this chemical scaffold (Senerovic *et al.*, 2019:37). The specific biological profiles of quinoline compounds depend on the type and position of substitutions on the quinoline ring (Kumar *et al.*, 2022).

This research project aims to investigate the significance of 4,7-disubstituted quinolines, exemplified by well-known compounds such as chloroquine and amodiaquine (see **Figure 1.1** for structures). These compounds have been extensively used as prophylactic and therapeutic

agents against malaria (Chu *et al.*, 2019:15-16). Their mode of action involves intercalation with the deoxyribonucleic acid (DNA) of the pathogen and the presence of an electronegative group, such as chlorine at the seventh position of the quinoline ring, enhances their antimicrobial activity (Bass *et al.*, 1971:275).



Chloroquine **Amodiaquine**

Figure 1.1: *Structures of 4,7-disubstituted quinolines (chloroquine and amodiaquine)*

Another intriguing class of compounds with potential antimicrobial properties is amino-chalcones. These compounds exhibit diverse biological effects, including immuno-suppressive, anti-amoebic, insecticidal, anti-ulcer, antiviral, antiprotozoal, and anticancer activities (Yerragunta *et al.*, 2013:55). Notably, Qiao *et al*. (2012:3553) has recently synthesised an aminochalcone compound (**Figure 1.2**) in their research that demonstrates potency in inhibiting trypanosomal growth, highlighting its potential as a therapeutic agent.

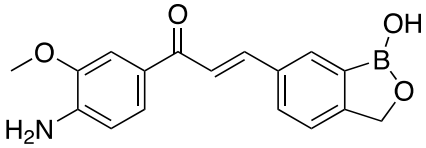


Figure 1.2*: The Qiao et al. (2012:3554) synthesised aminochalcone*

Furthermore, a synthesised compound by Nielsen *et al*. (2005:2671) exhibits significant antibacterial potency, which can be seen in **Figure 1.3**.

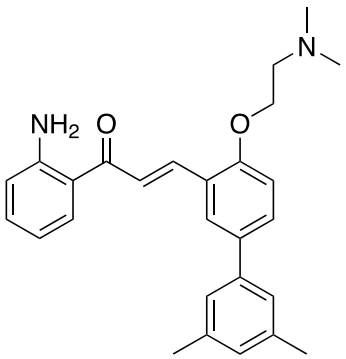
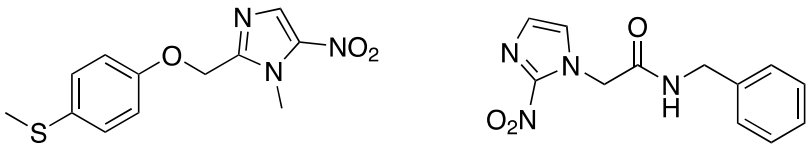


***Figure 1.3**: The Nielsen et al. (2005:2671) synthesised aminochalcone*

The exploration of nitro-containing carbocycles and heterocycles has gained substantial attention in antimicrobial research since the discovery of chloramphenicol in 1947 (Noriega *et al.*, 2022:1). The nitro group's electron-attracting properties, attributed to its strong inductive and resonance effects, have sparked interest in nitro compounds (Jezuita *et al.*, 2021:179). This moiety initiates redox reactions in cells, leading to toxicity and ultimately causing the demise of pathogens such as bacteria and parasites (Noriega *et al.*, 2022:1). Aromatic nitro compounds, such as fexinidazole and benznidazole (see **Figure 1.4**), serve as exemplars of this class and have demonstrated effectiveness against trypanosomiasis (Soriano-Correa *et al.*, 2008:1370; Sundar *et al.*, 2019:5).



Fexinidazole **Benznidazole**

***Figure 1.4**: Aromatic nitro drugs – fexinidazole and benznidazole*

1.1.1 Human African trypanosomiasis

Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is a protozoal disease of significant concern. Despite its impact on public health, there is a critical lack of approved drugs with favourable drug-like properties for the management and treatment of this disease (Welburn *et al.*, 2017:1943-1946).

HAT is caused by the *Trypanosoma* parasite, which is transmitted to mammals through the bite of the tsetse fly. Unlike other vectors, such as mosquitoes and sandflies, both female and

male tsetse flies rely on blood ingestion for survival (Correia *et al.*, 2021:1). There are two species of the *Trypanosoma* parasite responsible for human infection, namely: *Trypanosoma brucei gambiense* (*T.b. gambiense*), which accounts for 98% of HAT cases, primarily affecting West and Central Africa, while *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*) is responsible for the remaining 2% of cases that predominantly affect East and Southern Africa (Tanekou *et al.*, 2022:260). Among the two, *T.b. rhodesiense* infections are the most severe, leading to an acute and rapidly progressive disease course that can result in death within months. *T.b. gambiense* infections, on the other hand, presents as a chronic illness that can persist for several years. Symptoms of HAT vary depending on the disease stage, with the initial stage characterised by intermittent fever, headache, pruritus, endocrine dysfunction, lymphadenopathy, edema, and hepatosplenomegaly. The second stage is marked by neuropsychiatric manifestations, including sleep disorders, tremors, motor weakness, speech disorders, attention deficit, confusion, and emotional instability (Büscher *et al.*, 2017:4-5).

The complex epidemiology and clinical presentation of HAT make it a formidable and life-threatening disease. Currently, there is no available vaccine and existing drug therapies face challenges related to their toxicity profiles (Malvy & Chappuis, 2011:986). While self-cured cases of gambiense HAT have been reported in individuals with robust immune systems, both *T.b. gambiense* and *T.b. rhodesiense* infections can be fatal if left untreated (Büscher *et al.*, 2017:4). The treatment options for HAT are limited, with suramin and pentamidine used for first-stage HAT and nifurtimox, eflornithine, and melarsoprol employed for second-stage HAT (WHO, 2013:150). However, it should be noted that stage 1 drugs are ineffective against the meningoencephalitic stage, underscoring the importance of early detection and treatment initiation before the disease crosses the blood-brain barrier. Furthermore, second-stage treatment approaches, such as nifurtimox-eflornithine combination therapy (NECT) and eflornithine and melarsoprol monotherapy, are more complex and can pose challenges due to the need for effective penetration of the blood-brain barrier and potential toxicity (Büscher *et al.*, 2017:6).

1.1.2 African animal trypanosomiasis

African animal trypanosomiasis (AAT), also known as nagana, is a form of trypanosomiasis that primarily affects animals. It is caused by various species of trypanosome parasites, including *T.b. gambiense* and *T.b. rhodesiense*, which can infect both animals and humans. Another species, *Trypanosoma brucei brucei* (*T.b. brucei*), exclusively causes nagana in animals. Additionally, *Trypanosoma congolense* (*T. congolense*), *Trypanosoma vivax*

(*T. vivax*), and *Trypanosoma evansi* (*T. evansi*) are other trypanosome species that significantly affect livestock (Steverding, 2017:277).

Domestic animals infected with *Trypanosoma* parasites often experience severe and sometimes fatal reactions, whereas wild animals generally exhibit tolerance (Steverding, 2017:277). The prevalence of AAT is particularly high in regions of Africa where tsetse flies are endemic (Connor, 1994:379). Clinical signs of AAT include hair loss, fever, edema, ocular discharge, lethargy, decreased milk production, emaciation, anaemia, and paralysis. As the disease progresses, affected animals weaken progressively (Steverding, 2017:288).

Diagnosis of AAT involves obtaining a blood test as anaemia and the general physical condition of the animal provide initial indications of the disease. Similar to human trypanosomiasis, untreated animals typically face a fatal outcome. However, the available treatment options pose significant challenges, including limited drug choices, high toxicity profiles, and complexities associated with administration (Steverding, 2017:293). Four compounds are commonly used as preventive and therapeutic agents for AAT, namely: isometamidium chloride, quinapyramine, diminazene, and melarsomine (Venturelli *et al.*, 2022:10-11).

1.1.3 Leishmaniasis

Leishmaniasis, caused by *Leishmania* parasites, is a vector-borne disease transmitted to humans through the bite of infected phlebotomine sandflies (Roatt *et al.*, 2020). Belonging to the *Trypanosomatidae* family, *Leishmania* encompasses approximately 20 different species capable of infecting humans. The disease manifests in three primary clinical forms, namely: cutaneous leishmaniasis, visceral leishmaniasis, and mucocutaneous leishmaniasis (Roatt *et al.*, 2020). While cutaneous leishmaniasis can occasionally heal spontaneously, it often results in disfiguring scarring at the site of the bite. Conversely, visceral leishmaniasis can be fatal if left untreated (Alvar *et al.*, 2006:552).

Leishmaniasis exhibits higher prevalence in regions characterised by poverty, malnutrition, and poor hygiene practices (Mann *et al.*, 2021:121). Affected areas span across Africa, Asia, the Americas, and the Mediterranean (Torres-Guerrero *et al.*, 2017:3). It is important to acknowledge that limited access to healthcare services significantly affects the diagnosis, treatment, and prognosis of leishmaniasis, contributing to its overall morbidity and mortality rates (Alvar *et al.*, 2006:552). The disfiguring scars resulting from the disease also give rise to psychosocial suffering due to social isolation and the stigma associated with the condition

(Roatt *et al.*, 2020). Leishmaniasis stands as a neglected disease (Mann *et al.*, 2021:121) and is ranked high among infectious diseases (Alvar *et al.*, 2006). It is estimated to cause approximately 70,000 deaths annually (Torres-Guerrero *et al.*, 2017:1). However, under-reporting of cases is likely, with current estimates ranging from 700,000 to 1.2 million cutaneous leishmaniasis cases per year and around 100,000 visceral leishmaniasis cases per year (Mann *et al.*, 2021:121), of which 59,000 are fatal (Alvar *et al.*, 2006:552).

While various drugs are utilised for the treatment of leishmaniasis, pentavalent antimonials are currently the only ones deemed effective (Torres-Guerrero *et al.*, 2017:11). Other available drugs include amphotericin B deoxycholate and liposomal amphotericin B, miltefosine, paromomycin, pentamidine, and azole antifungal agents. However, the use of these compounds is restricted by severe side effects such as cardiac and hepatic failure, local irritation, anorexia, nausea, vomiting, and the emergence of drug-resistant *Leishmania* strains (Roatt *et al.*, 2020).

1.1.4 ESKAPE pathogens

The ESKAPE pathogens, a group of nosocomial pathogenic bacteria, are notorious for their high resistance to antimicrobial agents, rendering them of significant clinical concern. The pathogens that define the acronym ESKAPE include *Enterococcus faecium* (*E. faecium*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumanni* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter* spp. (Marturano & Lowery, 2019:1). Epidemiological studies indicate that ESKAPE pathogens are responsible for causing 700,000 fatalities annually (Qin *et al.*, 2022:1)

E. faecium is a Gram-positive pathogen commonly associated with healthcare-associated infections, particularly in immunocompromised patients. *Enterococci* pose a significant threat in hospital settings due to their remarkable capacity to colonise the skin and gastrointestinal tract (Pendleton *et al.*, 2013:298-299). They cause diseases such as bacteremia, urinary tract infections, wound infections, endocarditis, and occasionally meningitis (Vu & Carvalho, 2011:359). *Enterococcus* is notably resistant to vancomycin (Denissen *et al.*, 2022:4).

S. aureus is a Gram-positive bacterium commonly found in the environment and as part of normal human flora. Higher-than-normal colonisation rates of *S. aureus* are often observed among healthcare workers, immunocompromised individuals, and hospitalised patients. Transmission occurs person-to-person or through fomites (Taylor & Unakal, 2021). When *S. aureus* forms a biofilm, it becomes a significant pathogen in wound infections. Both chronic

and acute infections and syndromes caused by *S. aureus* include toxic shock syndrome, surgical wound infections, epidural abscesses, furuncles or carbuncles, pyomyositis, and botryomycosis (Archer, 1998:1180).

K. pneumoniae is a clinically significant organism of growing concern in public health due to its extensive antibiotic resistance (Effah *et al.*, 2020:1). Consequently, infections caused by this pathogen are associated with high morbidity and mortality rates (Bengoechea & Sa Pessoa, 2019:123). *K. pneumoniae* affects both hospital and community sectors worldwide, with a higher propensity to infect neonates, the elderly, and individuals with compromised immune systems (Pendleton *et al.*, 2013:300). It is responsible for a broad-spectrum of diseases, including sepsis, pneumonia, and urinary tract infections (Bengoechea & Sa Pessoa, 2019:123). *K. pneumoniae* is recognised for its resistance to third-generation cephalosporins (Denissen *et al.*, 2022:5).

A. baumannii, formerly considered a low-level pathogen, has emerged as a leading cause of community- and hospital-acquired infections (Asif *et al.*, 2018:1250). This Gram-negative coccobacillus and non-fermenter is frequently found in surgical wards and intensive care units (ICUs) (Pendleton *et al.*, 2013:300). However, it has also gained the ability to infect the general population, particularly neonates and immunocompromised individuals (Asif *et al.*, 2018:1250). In a community setting, *A. baumannii* is responsible for tracheobronchitis and bronchiolitis, with a heightened risk of infection among severely ill patients in ICUs. Its mortality rate ranges from 26% to 68% (Eliopoulos *et al*., 2008:1255). *A. baumannii* is known for its resistance to carbapenems (Denissen *et al.*, 2022:5).

P. aeruginosa is a Gram-negative bacterium that can thrive in both aerobic and anaerobic conditions (facultative anaerobe) (Pendleton *et al.*, 2013:301). The formation of biofilms enables *P. aeruginosa* to survive in harsh environments, posing challenges for treatment (Qin *et al.*, 2022:1). This has allowed this pathogen to be prevalent in both hospital and community sectors (Lister *et al.*, 2009:583). *P. aeruginosa* primarily targets immunocompromised individuals with conditions such as cystic fibrosis, chronic obstructive pulmonary disease, burn wounds, cancer, and respiratory infections requiring ventilation, such as those seen in Covid-19 (Qin *et al.*, 2022:1). *P. aeruginosa* is known for its carbapenem resistance (Denissen *et al.*, 2022:6).

Enterobacter species comprise a group of opportunistic Gram-negative bacilli classified as facultative anaerobes. They naturally inhabit the gut microbiota of humans and animals. There are currently 22 identified *Enterobacter* species, namely: *E. aerogenes*, *E. amnigenus*,

E. arachidis, *E. asburiae*, *E. carcinogenus*, *E. cloacae*, *E. cowanii*, *E. dissolvans*, *E. gergoviae*, *E. helveticus*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, *E. nimipressuralis*, *E. oryzae*, *E. pulveris*, *E. pyrinus*, *E. radicincitans*, *E. coli*, *E. taylorae*, and *E. turicensis.* They are grouped together due to their similar genetic and phenotypic characteristics (Davin-Regli *et al.*, 2019:2). The increasing antimicrobial resistance of *Enterobacter* species contributes to their daily rising mortality rate. They are predominantly prevalent in hospital settings, particularly ICUs (Cosgrove *et al.*, 2002:185). Infection with *Enterobacter* species can lead to meningitis, pneumonia, intracranial abscesses, skin infections, sepsis, urinary tract infections, and intestinal/peritoneal infections. *Enterobacter* species are known for their resistance to third-generation cephalosporins (Denissen *et al.*, 2022:6).

1.2 Problem statement

Antimicrobial resistance poses a significant global health threat, particularly in the case of ESKAPE pathogens, which have developed various mechanisms to overcome the action of commonly deployed drugs. Neglected diseases such as HAT, AAT, and leishmaniasis suffer from limited treatment options, poor drug-like properties, and drug resistance, leading to a high number of annual deaths worldwide. It is imperative to develop effective solutions to address these challenges and prevent further loss of lives.

Quinoline derivatives have shown activity against multiple pathogens and have been used extensively in clinical settings, demonstrating their safety and favourable drug-like properties. Therefore, the problem statement focuses on the need to identify and develop novel anti-microbial agents, particularly quinoline derivatives, that can effectively combat antimicrobial resistance, overcome limitations in current treatments for neglected diseases such as HAT, AAT and leishmaniasis, and ultimately reduce the global burden of morbidity and mortality caused by these pathogens.

1.3 Aims and objectives

Aim

The main aim of this project is to synthesise novel 4,7-disubstituted quinolines and establish their antibacterial and antiprotozoal activities *in vitro*.

Objectives

- Synthesis and characterisation of target compounds using spectroscopic and spectrometric techniques such as nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), and high-resolution mass spectrometry (HRMS).
- *In vitro* evaluation of target compounds against ESKAPE pathogens.
- *In vitro* screening of target compounds against *T.b. brucei* and *Leishmania donovani* (*L. donovani*).
- *In vitro* screening of selected compounds against MRC-5 cell line for overt cell toxicity.

CHAPTER 1: REFERENCE LIST

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The issue of drug resistance has become increasingly prevalent with the emergence of novel infectious diseases that pose life-threatening complications. This emphasises the urgent need for greater attention and investment in pharmaceutical research and development by the scientific community. The pandemic character of Covid-19 serves as a prime example of the critical importance of advancing medical innovation (Ajani *et al.*, 2022:18594). In light of such challenges, the global community is now more acutely aware of the crucial role that scientific research and development plays in addressing health crises and ensuring the development of effective treatments and interventions.

Due to the diverse pharmacological responses they elicit, certain compound classes have received significant interest. Among them are quinolines (Ajani *et al.*, 2022:18594), amino-chalcones (Irfan *et al.*, 2020:1), and aromatic nitro compounds (Noriega *et al.*, 2022:1). Remaining current with the latest developments in the field of science, particularly regarding the integration of quinoline, chalcone, and aryl nitro components in the realms of drug discovery and pharmaceutical chemistry, holds paramount significance in safeguarding human health and advancing medical science.

Several neglected diseases have a limited selection of drugs that are available for effective treatment. Protozoan diseases including HAT (Malvy & Chappuis, 2011:986), AAT (Connor, 1994:379), and leishmaniasis (Roatt *et al.*, 2020) are prime examples of such diseases. Unfortunately, no vaccines have been developed to manage or prevent these diseases. Moreover, there is a growing emergence of drug resistance among the pathogens that cause these diseases. Furthermore, some currently available drugs used to treat these diseases exhibit severe toxicity in addition to weak drug-like qualities.

Bacterial pathogens, specifically ESKAPE pathogens, have gained notoriety for their extended drug-resistance profiles. Without the development of novel antibiotics against these bacteria, there is a risk of regressing to the pre-antibiotic era. It is therefore imperative to prioritise the development of novel antimicrobials (Jadimurthy *et al.*, 2022:1).

Chapter 2 delves into the intricacies of these protozoal and bacterial pathogens, highlighting the significance of synthesising novel medications to treat them effectively. Furthermore, this

chapter explores the existing achievements and future prospects of utilising quinolines, aminochalcones, and aromatic nitros as anti-infective agents.

2.2 Protozoal diseases

Protozoa are a diverse and polyphyletic group of organisms that exhibit a wide range of epidemiological traits and clinical manifestations, particularly in subtropical and tropical regions (Monzote & Siddiq, 2011:1). They are multicellular or unicellular organisms with the capacity to infect live cells of plants, animals, and humans. These organisms invade host cells, utilise their nutrients for intracellular proliferation, and upon completion of their replication cycle, induce cell death before entering new cells (De Souza, 2008:822). To effectively address diseases caused by protozoa, the development of novel therapies that adhere to predetermined standards is the only proven method (Senerovic *et al.*, 2020).

2.2.1 Human African trypanosomiasis

HAT, also known as sleeping sickness, is a neglected protozoal disease that poses significant challenges and life-threatening consequences. Its complex epidemiology and clinical manifestations make it particularly difficult to manage. If left untreated, it can result in high mortality rates (Malvy & Chappuis, 2011:986). Unfortunately, there are only a limited number of approved medications available for treating HAT. Moreover, these drugs do not possess optimal pharmacokinetic properties and are associated with potentially fatal side effects (Welburn *et al.*, 2017:1943-1946). These drug-related issues and limitations are thoroughly explained in section 2.2.1.2.

HAT is caused by an infection of the protozoan parasite *T. brucei*, transmitted by tsetse flies of the genus *Glossina* (Malvy & Chappuis, 2011:986). There are two subspecies of *T. brucei* as depicted in **Figure 2.1**, namely *T.b. rhodesiense* that accounts for fewer than 10% of reported cases and *T.b. gambiense* that makes up roughly 90% of HAT cases (Malvy & Chappuis, 2011:987).

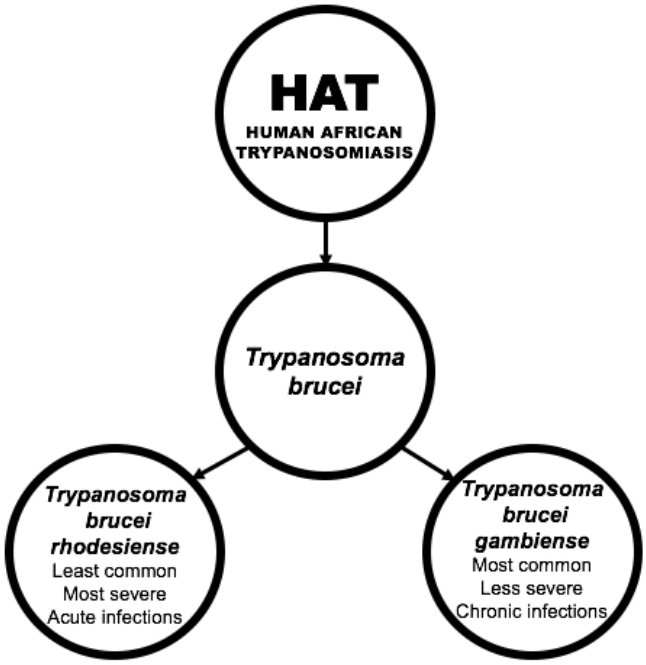


***Figure 2.1**: Summary of the two subspecies of T. brucei responsible for HAT (adapted from Berninger et al., 2017:4; Malvy & Chappuis, 2011:987)*

2.2.1.1 Signs, symptoms and diagnosis

Infection with *T.b. gambiense* typically manifests as a chronic infection, referred to as West African or Gambian African sleeping sickness. It is characterised by a gradual, protracted progression over approximately three years and may be misdiagnosed as a chronic blood disorder (Malvy & Chappuis, 2011:988). On the other hand, *T.b. rhodesiense* causes a more severe form of the illness known as East or Rhodesian African sleeping sickness. This rare and acute infection is highly contagious, and infected individuals often succumb within a few months (Berninger *et al.*, 2017:4). Both *T.b. gambiense* and *T.b. rhodesiense* exhibit similar clinical manifestations. Following a tsetse fly bite, the initial observable consequence is the development of an ulcer, which persists for two to three weeks and appears at the site of parasite presence. This ulceration marks the primary localised damage often accompanied by adjacent redness, warmth, inflammation, and soreness. While *T.b. gambiense* infections rarely exhibit ulcers, they are more commonly observed in individuals infected with *T.b. rhodesiense* and can be numerous (Malvy & Chappuis, 2011:989). Subsequently, the illness progresses through the two typical stages (Berninger *et al.*, 2017:4).

Stage 1, referred to as the haemolymphatic stage, is characterised by recurrent flu-like symptoms that are believed to coincide with repeated infestations by the parasite (Crilly & Mugnier, 2021:1). Early-stage infections commonly present with prominent indications such

as intense pruritis (itching) and excoriation (scratching), cutaneous lesions, headaches, intermittent fever, facial and limb edema, palpable or soft lymphadenopathy, and, to a lesser extent, splenomegaly or hepatomegaly. Eventually, the parasites breach the bloodstream and invade the central nervous system (CNS), marking the onset of the fatal stage of the disease (Walsh & Hill, 2021:936). Initial involvement of the CNS may manifest as mild neurological and endocrine disturbances. Patients frequently report heightened sensory sensitivity as well (Malvy & Chappuis, 2011:989).

Stage 2 represents the active invasion of the CNS by the parasite (Malvy & Chappuis, 2011:989). This neurological stage is characterised by a range of neurological signs attributed to meningoencephalitis and cerebral edema (Crilly & Mugnier, 2021:1). Neuropsychiatric manifestations accompany the symptoms observed in the initial stage, making it challenging to distinguish between the two stages based solely on clinical features (Büscher *et al.*, 2017:4). Neurological symptoms may include tremors, generalised muscle fasciculations, difficulty initiating or executing movements, confusion, abnormal movements, hemiparesis, sensory disturbances with heightened pain sensitivity, and speech impairments (Malvy & Chappuis, 2011:989). The disruption of the hosts circadian rhythm by the parasite leads to sleep disorders characterised by excessive daytime sleepiness and nocturnal insomnia (Büscher *et al.*, 2017:4). Endocrine abnormalities may manifest as either hyper- of hypofunctions of the thyroid and adrenal cortex. Psychiatric symptoms can also be prominent features of the disease (Malvy & Chappuis, 2011:990). Regrettably, patients in this phase of the disease are stigmatised. Neurological and psychiatric consequences are common, with CNS atrophy accompanied by changes in consciousness, resulting in a gradual decline of cognitive function. Ultimately, cachexia or opportunistic infections contribute to the demise of the affected individual (Malvy & Chappuis, 2011:990).

The complexity of diagnostic tests makes their integration into primary healthcare impractical, resulting in most cases of the disease being detected in the later stage, known as stage 2, or meningoencephalitic stage, where the parasites have invaded the CNS. This is in contrast to the earlier, more manageable stage 1, or haemolymphatic stage (Checchi & Barrett, 2008). The accurate diagnosis of HAT involves a three-step process that includes screening, parasitological verification, and staging (Malvy & Chappuis, 2011:990).

Screening of HAT involves the use of rapid diagnostic tests such as the card agglutination test for trypanosomiasis or the SD Bioline™ HAT. These tests have been specifically designed with high specificity and sensitivity to detect various *Trypanosoma* antigens. However, it is important to note that a positive rapid diagnostic test result does not necessarily confirm an

ongoing infection. It can also indicate the presence of an antitrypanosomal immune response resulting from a previous bite by an infected fly. Rapid diagnostic tests cannot distinguish between a current trypanosome infection and a past episode of HAT that has already been treated successfully (Kennedy, 2019).

Ideally, the parasitological diagnosis of HAT involves the direct observation of trypanosomes in lymph nodes or blood samples under a microscope. This method is usually successful in cases of *T.b. rhodesiense* as it is associated with high levels of parasitaemia. However, it is often challenging to detect *T.b. gambiense* infections due to their cyclical parasitaemia, which may be linked to the parasite's better adaptation to the host (Kennedy, 2019).

The staging process includes a cerebrospinal fluid examination following a lumbar puncture. This step is essential to determine the current stage of the disease and is required for all confirmed HAT patients. Treatments can vary significantly between the early and advanced stages, making accurate staging crucial (Malvy & Chappuis, 2011:990).

2.2.1.2 Current drugs

As previously mentioned, HAT is a neglected tropical disease with devastating and often fatal consequences if left untreated. Although there are a few available treatments for HAT, accessing these treatments can be challenging and costly for patients as they often require skilled healthcare professionals (Imran *et al.*, 2022:1). Currently, no vaccines have been developed for HAT, limiting treatment options to existing drugs that have suboptimal drug-like properties and cause life-threatening side effects (Malvy & Chappuis, 2011:986). The choice of treatment depends on the species of *T. brucei* involved and the stage of the disease. Early diagnosis is crucial for successful treatment outcomes (WHO, 2022).

First-stage treatment for *T.b. gambiense* infection typically involves the use of pentamidine (**Figure 2.2**) (WHO, 2022). Pentamidine is either administered intramuscularly once daily for seven days or as an intravenous infusion in saline over two hours (Büscher *et al.*, 2017:6). It is generally well-tolerated by patients, although some may experience undesirable effects (WHO, 2022). The intramuscular injection can result in pain, discomfort, and swelling at the injection site. Other possible side effects include abdominal discomfort, gastrointestinal issues, low blood pressure, and hypoglycaemia. The risk of hypoglycaemia can be reduced by having the patient ingest 10–20 g of sugar. Pentamidine is also used as an alternative treatment for *T.b. rhodesiense* (Büscher *et al.*, 2017:6-7).

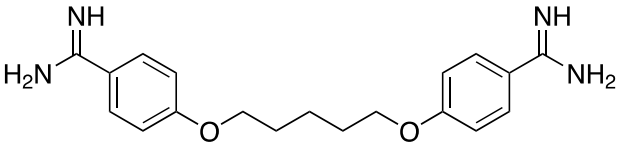


***Figure 2.2**: The structure of pentamidine*

Another treatment option for *T.b. rhodesiense* is suramin (**Figure 2.3**)(WHO, 2022). Suramin is specifically intended for *T.b. rhodesiense* due to the potential co-infection with onchocerciasis in regions where *T.b. gambiense* is endemic (Büscher *et al.*, 2017:7). However, suramin can cause side effects, including nephrotoxicity and hypersensitivity reactions (WHO, 2022). A test dose should be administered before completing the treatment to assess the risk of acute hypersensitivity reactions. Suramin is administered as a slow intravenous infusion and should be injected immediately after dilution as it degrades rapidly when exposed to air. The recommended treatments have a complex schedule and may take up to one month to complete (Büscher *et al.*, 2017:7).

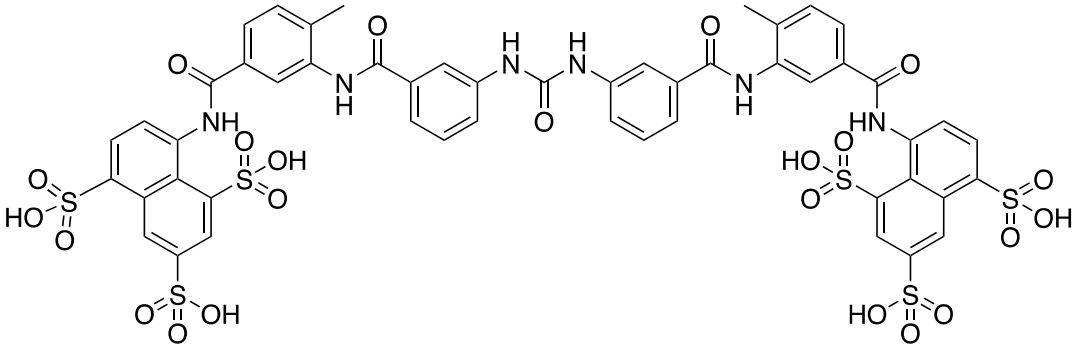


***Figure 2.3**: The structure of suramin*

In the second stage of HAT treatment, several options are available. One approach is the use of NECT, which involves the simultaneous administration of nifurtimox and eflornithine. Alternatively, eflornithine or melarsoprol can be used as monotherapy (Büscher *et al.*, 2017:6). Melarsoprol (**Figure 2.4**), derived from arsenic, is a treatment option primarily recommended for *T.b. rhodesiense* infections but can also be used for *T.b. gambiense* cases, although it is not the preferred choice because of its dangerous side effects. The most severe and potentially fatal side effect includes reactive encephalopathy (encephalopathic syndrome) (WHO, 2022). It is noteworthy that approximately one in 20 HAT patients receiving melarsoprol treatment may die from reactive encephalopathy. Due to its toxicity, melarsoprol is administered cautiously and with close monitoring (Akazue *et al.*, 2019:5).

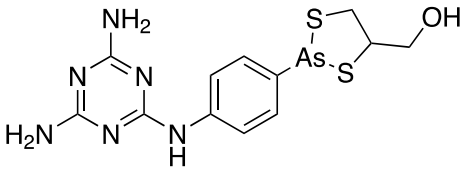
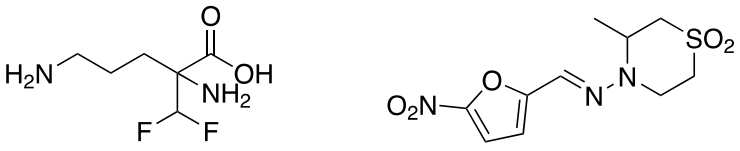


***Figure 2.4**: The structure of melarsoprol*

Eflornithine (**Figure 2.5**) is a less harmful alternative to melarsoprol for the treatment of HAT (WHO, 2022). It is administered as a monotherapy for *T.b. gambiense* infections when nifurtimox (**Figure 2.5**), the accompanying drug, is not available or not recommended. However, it is important to note that eflornithine monotherapy relies on an active immune system. In individuals with impaired immune systems, it may not eliminate all parasites completely. Eflornithine involves a total of 56 infusions administered as a slow-paced intravenous infusion over a period of 14 days. This treatment schedule can be challenging to maintain in resource-limited settings and requires careful attention to prevent complications associated with catheter insertion. Common side effects of eflornithine treatment include fever, headaches, high blood pressure, nausea, vomiting, diarrhoea, abdominal pain, itching, and a decreased number of blood cells (such as anaemia, low white blood cell count, and low platelet count). Infrequent seizures may occur but can be managed and typically occur in isolated instances (WHO, 2022).

The first and recommended treatment option for HAT is NECT unless there are contra-indications or other issues (Büscher *et al.*, 2017:7). NECT is considered more convenient than eflornithine monotherapy as it reduces the treatment duration and the number of intravenous infusions required. However, it is important to note that NECT has not been evaluated for use in second-stage *T.b. rhodesiense* (WHO, 2022). NECT consists of the oral administration of nifurtimox and intravenous administration of eflornithine (Akazue *et al.*, 2019:5). The World Health Organization (WHO) provides both medications free of charge to countries where HAT is prevalent and also supplies the necessary resources for their administration (WHO, 2022).



Eflornithine **Nifurtimox**

***Figure 2.5**: The structure of eflornithine and nifurtimox*

It is important to determine the stage of the disease before initiating treatment because stage 1 medications cannot completely eliminate HAT if it has progressed to the meningoencephalitic stage (Büscher *et al.*, 2017:6). Fexinidazole (**Figure 2.6**) is the only recently approved drug by the European Medicines Agency and the United States Food and Drug Administration for the treatment of both stage 1 (haemolymphatic) and stage 2 (meningoencephalitic) HAT. It is an oral therapy, which makes it more convenient for patients (Imran *et al.*, 2022:3). Fexinidazole is recommended as the first-line treatment for stage 1 *T.b. gambiense* infections and mild to moderate cases in stage 2. Fexinidazole should be taken under the supervision of trained medical personnel for a duration of 10 days, preferably after consuming a solid meal. Clinical trials are currently underway to assess the efficacy of fexinidazole for treating *T.b. rhodesiense* cases (WHO, 2022).

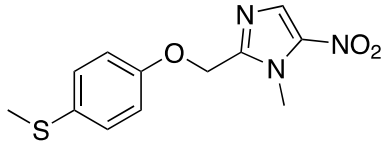


***Figure 2.6**: The structure of fexinidazole*

The existing drugs used to treat HAT are beset with several shortcomings that render them far from ideal. These limitations encompass the expensive manufacturing cost, high toxicity levels, inadequate oral absorption, challenges in drug administration, prolonged treatment duration, and a low rate of effectiveness (Akazue *et al.*, 2019:4-5). Furthermore, the emergence of drug-resistant strains of *T. brucei* poses a significant concern, exacerbated by the limited repertoire of available drugs to combat HAT. Pentamidine, melarsoprol, eflornithine, and nifurtimox have all exhibited numerous cases of resistance. This predicament disproportionally affects economically disadvantaged nations, necessitating the critical development of novel treatment strategies and drugs (Akazue *et al.*, 2019:5).

2.2.1.3 Transmission and epidemiology

The population of *tsetse* flies plays a significant role in the rate and spread of HAT transmission (Alderton *et al.*, 2018:3). Transmission typically occurs when individuals enter tsetse fly inhabited environments for activities such as farming, fishing, and hunting (Steverding, 2017a:294). **Figure 2.7** represents the distribution of HAT across Africa. *T.b. rhodesiense* is the prominent strain in the southern to eastern parts of Africa whereas *T.b. gambiense* is known to affect the western African parts (Brun *et al.*, 2010:149). However,

the concentrated occurrence of HAT remains a mystery as there are regions where tsetse flies exist without any reported cases of sleeping sickness.

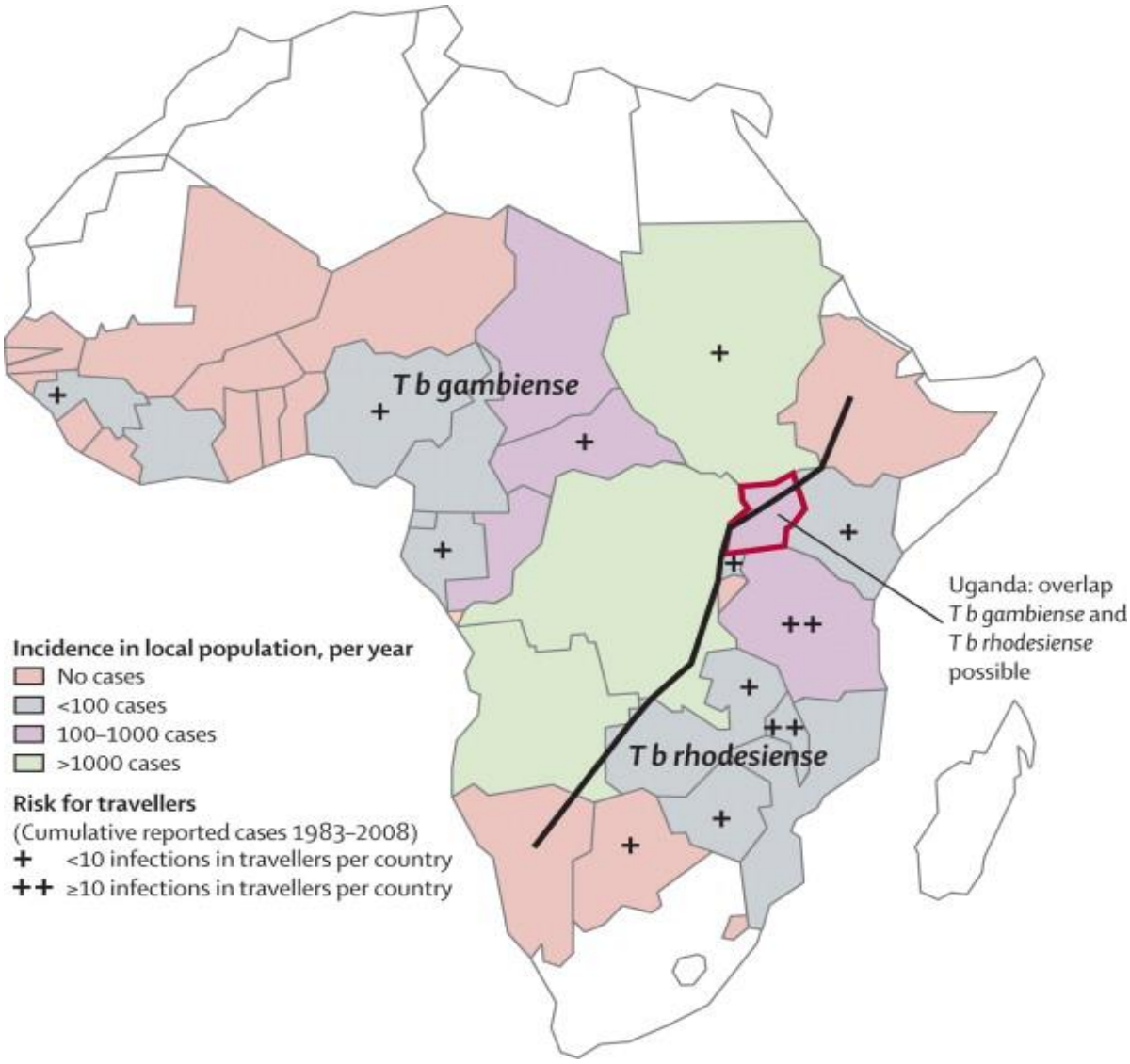


***Figure 2.7**: The distribution of HAT across Africa (Brun et al., 2010:149)*

The palpalis and morsitans tsetse fly groups are key vectors for transmitting protozoan *T. brucei*, with *T.b. gambiense* primarily transmitted by the palpalis group and *T.b. rhodesiense* by the morsitans group (Steverding, 2017a:286). Two factors that could potentially affect tsetse fly populations and the transmission system are the demographic expansion due to abundant food and living space, as well as climate changes influencing tsetse fly behaviour and mortality rates. Enhancing our understanding of population dynamics in tsetse fly populations could yield positive outcomes as such knowledge could facilitate the development of more targeted vector control strategies that have minimal ecological impact

while also providing a deeper comprehension of the HAT transmission system (Alderton *et al.*, 2018:3).

Up to 36 countries in sub-Saharan Africa are affected by HAT, putting 70 million people at risk (Kennedy, 2019). The remote rural locations of these populations pose challenges in terms of accessing healthcare services, which hinders the ability to diagnose, treat, and monitor cases. Additionally, factors such as large-scale displacement of people, armed conflicts, and poverty contribute to the ease of disease transmission (WHO, 2013:161). However, there is some positive news as the number of HAT cases worldwide has decreased significantly since 1998, dropping from 300,000 cases to fewer than 10,000 cases in 2009, fewer than 4,000 in 2014, and fewer than 2,100 in 2016 (Imran *et al.*, 2022:2). This decline has continued in recent years with 992 new cases reported in 2019 and 663 in 2020 (Franco *et al.*, 2022:1). These figures represent the lowest numbers recorded in the past 80 years. While it seems that disease transmission has been interrupted in certain countries, challenges arise in gathering specific data due to unstable social contexts and limited accessibility, which impede monitoring and diagnostics efforts. According to the WHO (2022), approximately 70% of reported cases over the past five years have occurred in the Democratic Republic of the Congo, with an average of fewer than 1,000 cases reported annually.

2.2.2 African animal trypanosomiasis

Trypanosoma protozoans are not only responsible for HAT, but also for AAT – commonly referred to as nagana. The name nagana originates from the Zulu expression *n'gana*, meaning useless/powerless, accurately reflecting the debilitated state of animals affected by *Trypanosoma* protozoans (Steverding, 2017a:288). The impact of this disease on animal well-being and productivity carries significant economic implications (Eshetu & Begejo, 2015:117). Nagana frequently results in fatality, and its consequences extend throughout herds. All aspects of production suffer adverse effects, including reduced fertility, milk yields, and beef production. Similar to HAT, nagana remains a neglected disease with limited and complex treatment options. Therefore, the development of innovative treatments is crucial (Connor, 1994:380).

Livestock are primarily affected by three key species of *Trypanosoma*, namely *T. congolense*, *T. vivax*, and members of the *T. brucei* group (**Figure 2.8**) (Eshetu & Begejo, 2015:117). Among the *T. brucei* group, both *T.b. gambiense* and *T.b. rhodesiense* can infect animals and humans. However, a third species called *T.b. brucei* is exclusively limited to animals infections. This disease primarily affects domesticated animals and can lead to severe and often fatal

symptoms, while wildlife generally exhibits tolerance towards the disease (Steverding, 2017a:287-288).

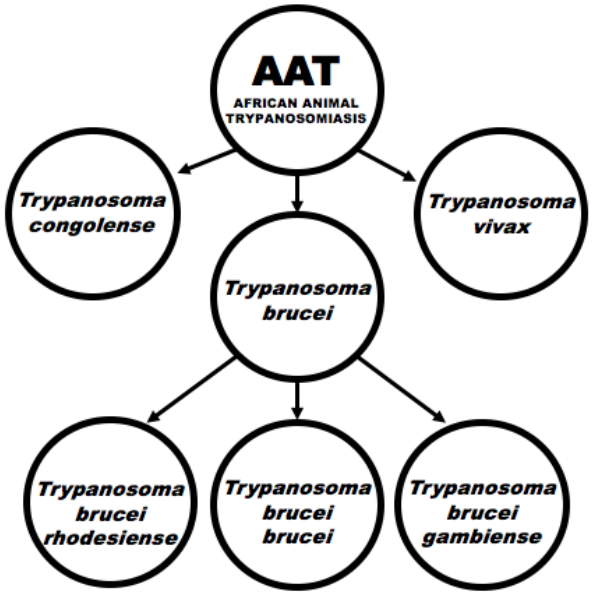


***Figure 2.8**: Summary of the three main species responsible for AAT (adapted from Eshetu & Begejo, 2015:117)*

2.2.2.1 Signs, symptoms and diagnosis

While nagana shares some clinical features with HAT, it also exhibits distinct primary signs and symptoms (Crilly & Mugnier, 2021:1). The disease can manifest as either acute or chronic, and its severity may be exacerbated by concurrent illnesses and inadequate nutrition (Eshetu & Begejo, 2015:119). Cattle and pigs typically display a relatively mild form of the disease, whereas sheep and goats tend to exhibit a moderate presentation. Horses, donkeys, and dogs, on the other hand, may experience a more severe manifestation (Steverding, 2017a:287-288). If left untreated, all three species of trypanosomes can eventually lead to death of their hosts (Eshetu & Begejo, 2015:119).

The primary clinical symptoms of nagana include weight loss, hair shedding, anaemia, periodic fever, swollen lymph nodes, increased tear production, emaciation, miscarriage, and infertility. In cases of chronic infections, the presence of digestive and neurological symptoms often leads to mortality (Eshetu & Begejo, 2015:119). As the infection progresses, affected animals experience increasing debilitation, rendering them unfit for labour. This is the point at which the animals become ‘useless’ and ‘powerless’ (Steverding, 2017a:288).

A likely diagnosis can be established based on clinical evidence, such as the presence of anaemia and the deteriorated condition of the animal. However, before initiating any treatment, it is crucial to perform a direct examination of the animal's blood to confirm the presence of trypanosomes (Steverding, 2017a:290). Alongside clinical diagnosis, there are several other methods for diagnosing trypanosomiasis in animals, each varying in specificity and sensitivity. These methods include direct parasitology, indirect serological testing, animal inoculation, and molecular diagnostics (Eshetu & Begejo, 2015:118).

Parasitological diagnosis methods for trypanosomiasis include wet blood films, fresh lymph preparations, thick blood films, thin blood smears, and thin smears of lymph (Eshetu & Begejo, 2015:119). Wet blood films are simple and provide immediate results, but their drawbacks include limited sensitivity, the need for a field microscope, and inability to identify the trypanosome species (Paris *et al.*, 1982). Fresh lymph preparations can be examined when trypanosomes are not found in blood, but their detection relies on the movement of lymphocytes (Eshetu & Begejo, 2015:119). Thick blood films are stained and easily recognised for their general morphology but lack immediate on-the-spot diagnosis and have limited sensitivity (Uilenberg, 1998). Thin blood smears, fixed and stained, allow for specific identification of trypanosomes but have low sensitivity. Thin smears of lymph, when prepared as fixed and stained, can also aid in specific identification but require very thin smears due to the presence of stained lymphocytes (Eshetu & Begejo, 2015:119).

Serological tests aim to detect specific trypanosomal antibodies or circulating parasitic agents, although a positive result does not necessarily indicate an active infection as antibodies can persist after clearance of the parasites, while the presence of trypanosomal antigens typically indicates the presence of live trypanosomes in the animal (Uilenberg, 1998). Indirect fluorescent antibody tests and enzyme-linked immunosorbent assays are commonly employed for identifying seropositive cattle, but cross-reactions can occur with non-pathogenic trypanosomes (Eshetu & Begejo, 2015:120)

Animal inoculation involves the injection of freshly collected blood from infected animals into laboratory rodents, and subsequent examination of blood samples using the wet film method. This method is highly sensitive but time-consuming, and its effectiveness depends on factors such as the species of strain of trypanosome and the susceptibility of the experimental animals used (Eshetu & Begejo, 2015:120).

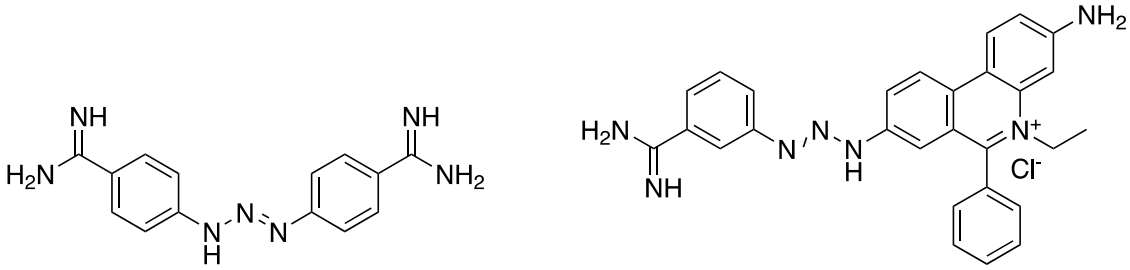
On the other hand, molecular tests, particularly polymerase chain reaction (PCR), have revolutionised trypanosome characterisation, allowing for highly sensitive and specific

detection at the subgenus, species, or strain level by identifying specific nucleotide sequences of the parasite's DNA (Eshetu & Begejo, 2015:120-121). Molecular tests offer exceptional sensitivity, can detect parasites even at low levels, and can be used for both mammalian hosts and insect vectors. PCR, in particular, enables the identification of trypanosomes at levels beyond the detection capabilities of traditional parasitological techniques (Geysen *et al.*, 2003:172).

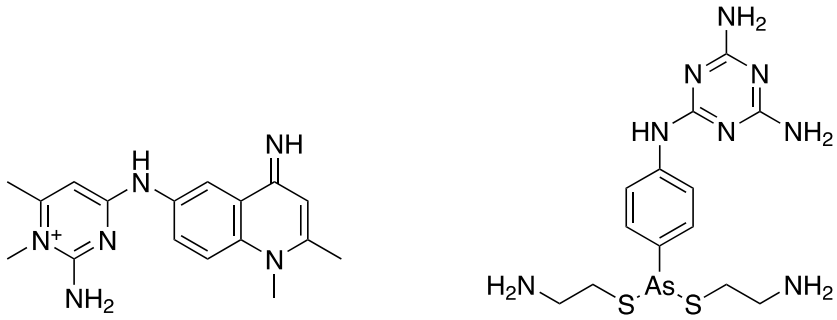
2.2.2.2 Current drugs

Currently, the available treatment options for nagana are limited. These drugs can be seen in **Figure 2.9** and include diminazene aceturate, isometamidium chloride, quinapyramine, and melarsomine dihydrochloride. Pentamidine and suramin, as seen in **Figure 2.2** and **Figure 2.3**, respectively, can also be used to treat AAT (Mabille *et al.*, 2022:22). Isometamidium and quinapyramine can be employed as prophylactic measures during periods of high tsetse fly activity or transhumance, aiming to prevent infection. Diminazene aceturate and quinapyramine are used for both prevention and treatment purposes. Among these, diminazene aceturate emerges as the most commonly employed chemotherapeutic agent due to its efficacy against various *Trypanosoma* species associated with AAT. Following diminazene aceturate, isometamidium chloride, suramin, and pentamidine are also utilised as treatment options (Murray & Gray, 1984:26-27).

However, despite the available treatment options, there are notable limitations associated with these drugs, including toxicity and the alarming rise of drug resistance among the parasites causing AAT (Mabille *et al.*, 2022:21). Quinapyramine serves as a prime example of a drug to which these parasites have developed complete resistance. It is now primarily employed as a prophylactic measure for pigs to prevent *T. brucei* infections (Steverding, 2017a:293). The stagnant state of drug development for nagana over the past several decades underscores the pressing need to explore novel therapeutic approaches, including the development of innovative drugs and potentially vaccines (Mabille *et al.*, 2022:22).



Diminazene aceturate **Isometamidium chloride**



Quinapyramine **Melarsomine dihydrochloride**

Figure 2.9*: The structures of AAT drugs*

2.2.2.3 Transmission and epidemiology

Nagana is prevalent in at least 37 out of the 54 countries in Africa (Yaro *et al.*, 2016:43). The transmission dynamics of the disease are influenced by several factors, including the population of tsetse flies, climatic conditions, host characteristics, types of trypanosome involved, and disease management strategies (Connor, 1994:381). When tsetse fly populations are dense, the morbidity rates tend to be higher (Eshetu & Begejo, 2015:118). Infection usually occurs when livestock are brought by farmers to graze in areas inhabited by infected tsetse flies (Steverding, 2017a:294).

Among the susceptible livestock species, cattle have demonstrated the highest susceptibility for trypanosomal infections, particularly in Nigeria and Ethiopia, followed by pigs and camels. The most frequently detected trypanosome species were *T. congolense* (11.80% to 13.40%) and *T. vivax* (10.50% to 18.80%) (Okello *et al.*, 2022). These findings highlight the significant impact of AAT on livestock, emphasising the need for effective control and management strategies to migrate the disease's burden in affected regions.

2.2.3 Leishmaniasis

Leishmaniasis, an important protozoan disease investigated in this study (Roatt *et al.*, 2020), is recognised by the WHO as one of the seven major neglected tropical diseases. It poses a significant global health threat, characterised by a diverse spectrum of clinical manifestations, some of which can be life-threatening (Torres-Guerrero *et al.*, 2017:1). This complex disease is often associated with inadequate healthcare systems, malnutrition, and a lack of preventative measures (Pinheiro & De Souza, 2022). The available treatment options for

leishmaniasis have several limitations, including low efficiency, adverse effects, high cost, and complex administration. Moreover, the emergence of drug resistance has become a critical concern (Pradhan *et al.*, 2022:516). Despite advances in understanding the pathogenesis of *Leishmania*, a preventative vaccine remains an unmet objective (Pinheiro & De Souza, 2022). Addressing these challenges and developing effective strategies for prevention and treatment is crucial to combat the burden imposed by leishmaniasis on public health.

Leishmaniasis, a disease caused by protozoan parasites of the *Leishmania* genus, is marked by a high degree of complexity, with approximately 20 distinct species known to infect humans. These parasites are classified under the *Trypanosomatidae* family. The mode of transmission of leishmaniasis occurs primarily through the bite of infected sandflies, specifically the *Phlebotomus* or *Lutzomyia* species (Roatt *et al.*, 2020). It's worth noting that, unlike HAT, only female sandflies engage in hematophagy, feeding on the blood of mammals to support their egg development. Once inside their mammalian hosts, *Leishmania* parasites find a unique niche within phagocytic cells, particularly within a specialized structure known as the phagolysosome. This niche provides a protected environment for the parasites to reside and replicate (Steverding, 2017b:1).

However, what makes leishmaniasis particularly intricate is the dynamic interplay between the parasites and the host's immune system. This interaction contributes significantly to the multifaceted pathogenesis of the disease and the wide range of clinical manifestations observed in leishmaniasis (Pinheiro & De Souza, 2022). Understanding the precise mechanisms underlying this intracellular lifestyle of the parasites and the complex host-parasite interplay is paramount. It serves as a fundamental stepping stone in the quest to develop effective therapeutic interventions against this challenging and clinically relevant disease. By unraveling these mechanisms, researchers aim to identify new targets for drug development and innovative treatment strategies to combat leishmaniasis effectively.

Leishmaniasis is divided into three primary forms characterised by the target phagocytic cells, namely visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis (Pradhan *et al.*, 2022:516). Cutaneous leishmaniasis is the most prevalent form, whereas visceral leishmaniasis represents a severe and often fatal manifestation (Pradhan *et al.*, 2022:516). In cutaneous leishmaniasis infections, the parasites infiltrate dermal macrophages, leading to their swelling and subsequent rupture. The released amastigotes invade neighbouring macrophages, continuing the infection (Steverding, 2017b:1). Conversely, in visceral leishmaniasis, the amastigotes are disseminated through the bloodstream, establishing infections in the reticuloendothelial system encompassing the liver, spleen,

marrow, lymph nodes, and gut (Steverding, 2017b:1). Mucocutaneous leishmaniasis occurs when parasites invade macrophages of the mucous membranes, affecting regions such as the mouth, throat, and nose (Steverding, 2017b:1).

2.2.3.1 Signs, symptoms and diagnosis

The clinical presentation of leishmaniasis varies depending on the species of *Leishmania* involved and the immune response of the host (Torres-Guerrero *et al.*, 2017:7). Cutaneous leishmaniasis can lead to disabling if there are multiple lesions, visceral leishmaniasis can be life-threatening if left untreated, and mucocutaneous leishmaniasis is disfiguring in nature (Desjeux, 2004:306).

In the case of cutaneous leishmaniasis, the parasites primarily affect the epidermis of the skin (Steverding, 2017b:1). Cutaneous leishmaniasis lesions commonly occur in areas of the body exposed to insect bites, such as the legs, ears, cheeks, nose, hands and forearms, upper lip, and ankles. Symptoms typically appear one to four weeks after an insect bite, although in rare cases, they can manifest after several years. Initially, an asymptomatic erythematous papule develops at the site of the bite, accompanied by localised swelling and a rise in temperature. The papule, ranging from 1 mm to 10 mm in diameter, may cause itchiness. Within two days, it evolves into a vesicle and eventually forms a pustule. Upon rupture, either spontaneously or due to scratching, the lesion transforms into a rounded ulcer with thick borders and angular, pointed edges. The ulcer persists for a variable duration, ranging from three to five months and up to 15 to 20 years in extreme cases. Granulation tissue is present beneath the ulcer, which can bleed upon contact. In some cases, an adherent crust may develop as a result of excessive drainage (Torres-Guerrero *et al.*, 2017:7). Typically, the ulcer is painless unless it becomes secondarily infected, and it can occur as a solitary lesion or as part of a cluster (Vera-Izaguirre *et al.*, 2006). Autoinoculation may occur, leading to the spread of the infection to other areas of the body that have come into contact with the original ulcer, such as the forearms (Torres-Guerrero *et al.*, 2017:7).

Visceral leishmaniasis primarily affects the reticuloendothelial system, involving organs such as the liver, spleen, marrow, lymph nodes, and gut (Steverding, 2017b:1). It represents the most severe form of leishmaniasis and is often fatal if left untreated. The incubation period for visceral leishmaniasis ranges from three to eight months following a sandfly bite. Certain populations, such as the immunocompromised, preschool children, and malnourished individuals, are particularly vulnerable to the disease. Visceral leishmaniasis has been increasingly observed in large-scale epidemics and is characterised by intermittent fever,

lymphadenopathy, thrombocytopenia, weakness, hepatomegaly, pallor, anaemia, night sweats, leukopenia, anorexia, splenomegaly, asthenia, and cutaneous pigmentation (Torres-Guerrero *et al.*, 2017:9). Following recovery from visceral leishmaniasis, some individuals may experience chronic cutaneous leishmaniasis, requiring a prolonged and costly management plan (Desjeux, 2004:309).

Mucocutaneous leishmaniasis is characterised by disfiguring sores and severe agony, particularly affecting the oral, nasal, and pharyngeal cavities (Desjeux, 2004:309). The invasion of mucosal tissues occurs gradually, resulting in minor ulcerations that progress to necrotic and torn borders. The damage can extend to the tonsils, uvula, and palate roof pillars (Torres-Guerrero *et al.*, 2017:9). Infections usually start in the nasal mucosa and can spread to the larynx, pharyngeal and oral mucosa, as well as the skin of the nose and lips (Desjeux, 2004:309). Nasal cavity involvement may cause atrophy of the nasal turbinate and destruction of the cartilaginous septum, while the oral mucosa lesions vary from discomfort to severe symptoms such as odynophagia or cachexia. Superimposed infections can lead to painful lymph nodes (Reithinger *et al.*, 2007:584). Mucocutaneous leishmaniasis is associated with facial mutilation, unpleasant odour, and, in severe cases, even death (Marsden, 1986).

In the diagnosis of cutaneous and mucocutaneous leishmaniasis, various approaches are employed. While standardised and sensitive tests exists for identifying serum antileishmanial antibodies (Murray *et al.*, 2005:1567), the practical diagnosis is typically performed through microscopic examination of biopsies or smears to detect the presence of amastigotes (Murray *et al.*, 2005:1567). For accurate species identification, DNA analysis using PCR tests is considered the most reliable method. However, it should be noted that the implementation of technically demanding laboratory procedures such as PCR is currently limited in under-developed nations (Murray *et al.*, 2005:1568).

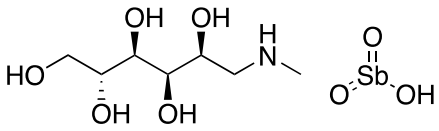
The diagnosis of visceral leishmaniasis involves employing various diagnostic methods depending on the available resources and technical expertise. Traditional serological methods such as the direct agglutination test can be used for serological diagnosis of visceral leishmaniasis (Desjeux, 2004:311). In settings where microscopy is accessible and skilled personnel are available, direct observation of amastigotes in clinical specimens remains the gold standard for diagnosis. In epidemic conditions or locations with limited microscopy capabilities, serological testing for serum antileishmanial immunoglobulin G, in high often direct agglutination tests or other serological procedures, is commonly employed to detect high titres of antibodies indicative of infection. An emerging method involves testing urine samples for leishmanial antigen or antibodies. Additionally, parasite isolation can be

performed by cultivating clinical samples in a central laboratory, and leishmanial DNA can be readily detected through PCR testing, including in peripheral serum and blood samples (Murray *et al.*, 2005:1567-1568).

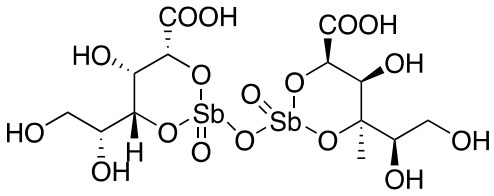
2.2.3.2 Current drugs

The treatment of leishmaniasis is influenced by several factors, including the specific *Leishmania* species involved, the presence of other concurrent diseases, and the geographic location. Leishmaniasis is a treatable and curable condition; however, complete eradication of the parasite from the body is not achieved by medication alone. Therefore, a normal functioning immune system is crucial to minimise the risk of relapse in cases where immuno-suppression occurs (WHO, 2023). It is important to initiate treatment only after a positive diagnosis has been confirmed (WHO, 2010:49).

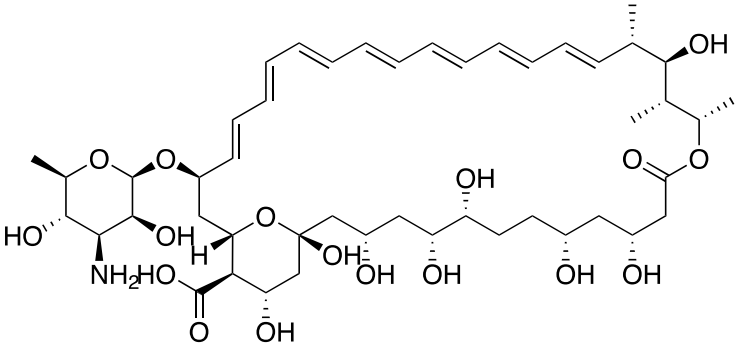
The current drugs used for the treatment of leishmaniasis, as illustrated in **Figure 2.10**, include pentavalent antimonials, amphotericin B (deoxycholate and liposomal formulation), miltefosine, paromomycin, pentamidine, and azole antifungal agents (Roatt *et al.*, 2020). Among these, intravenous pentavalent antimonials are considered the most effective therapy, showing positive clinical and microbiological outcomes for all clinical types of leishmaniasis in most countries. However, it is important to note that drug-resistant strains have been observed in certain countries, such as India, limiting the effectiveness of pentavalent antimonials in that region (Torres-Guerrero *et al.*, 2017:11).



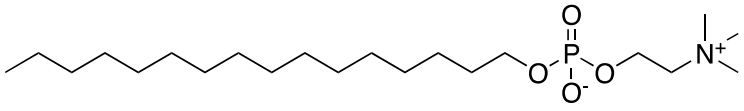
Meglumine antimoniate



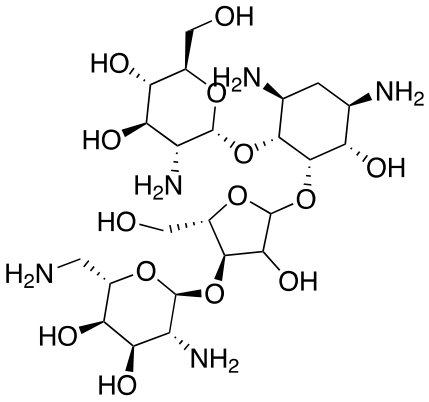
Sodium stibogluconate

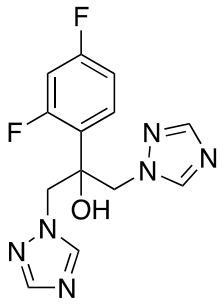


Amphotericin B deoxycholate



Miltefosine





Paromomycin **Fluconazole**

Figure 2.10*: The structures of antileishmanial drugs*

Meglumine antimoniate and sodium stibogluconate are two commonly used pentavalent antimonial (Sb^{v}) agents in the treatment of leishmaniasis. Despite their effectiveness, their use is often limited due to the requirement of regular parenteral administration, high toxicity, and treatment failures (Roatt *et al.*, 2020). In certain regions, such as eastern Africa, a combination of sodium stibogluconate and paromomycin has been proposed as the first-line therapy for visceral leishmaniasis (Musa *et al.*, 2010:2). The Pan American Health Organization previously recommended intralesional meglumine antimoniate for New World cutaneous leishmaniasis, but currently, systemic meglumine antimoniate (20 mg of Sb^{v}/kg/day for 20 days) is the preferred treatment for the majority of New World cutaneous leishmaniasis patients (Roatt *et al.*, 2020).

The use of Sb^{v} is associated with adverse effects such as anorexia, cardiac and hepatic changes, local irritation, nausea, and vomiting (Cota *et al.*, 2013:1). Moreover, in human immunodeficiency virus (HIV)/leishmaniasis co-infected individuals, Sb^{v} therapy has shown treatment-related adverse effects, significant failure and relapse rates, and higher mortality (Cota *et al.*, 2013:2).

In India, amphotericin B deoxycholate (AmB-D) has been established as the primary treatment for visceral leishmaniasis. AmB-D is a broad-spectrum antifungal agent that has demonstrated effectiveness against *Leishmania* species; however, its use is often associated with significant side effects, necessitating hospital care for treated patients. These side effects include hypokalaemia, fever, myocarditis, and most notably, nephrotoxicity (Roatt *et al.*, 2020). To mitigate these adverse effects, several lipid formulations of amphotericin (AmB-L) have been developed in recent years. AmB-L is the preferred choice of therapy for visceral leishmaniasis patients with HIV co-infection, organ transplant recipients, and pregnant women in many countries, although it is not considered cost-effective (Roatt *et al.*, 2020).

For cutaneous and mucocutaneous leishmaniasis, AmB-D therapy has shown comparable efficacy to Sb^{v}. However, due to its severe side effects, AmB-D is not considered an alternative treatment option. As a result, there is limited research exploring the use of AmB-D or its liposomal formulation in cutaneous or mucocutaneous leishmaniasis patients, and no clinical trials have been conducted in this regard (Roatt *et al.*, 2020).

Since its discovery in the 1980s as an effective antileishmanial agent, miltefosine, originally developed as an antineoplastic drug, has been the only oral medication approved for the treatment of leishmaniasis (Roatt *et al.*, 2020). It was widely used as the drug of choice for the elimination of visceral leishmaniasis in India, Nepal, and Bangladesh, thanks to its oral administration advantages, ease of use, and efficacy. However, its effectiveness declined after a decade of use in the Indian subcontinent due to the emergence of drug-resistant strains. As a result, miltefosine has been replaced by single-dose liposomal amphotericin B as the preferred treatment for the visceral leishmaniasis elimination programme in the Indian subcontinent (Chakravarty & Sundar, 2019:1251).

Miltefosine is associated with gastrointestinal changes and, less frequently, hepatotoxicity as side effects. It is important to note that women of childbearing age are advised to use contraception during the treatment period due to the teratogenic effects of miltefosine (Roatt *et al.*, 2020).

Paromomycin, an aminoglycoside antibiotic, serves as an alternative treatment option for leishmaniasis and is administered topically or parenterally (Roatt *et al.*, 2020). In certain regions of India, paromomycin is employed both topically and systemically for cutaneous leishmaniasis, and as a systemic medication for visceral leishmaniasis. However, as a monotherapy, paromomycin demonstrates only moderate efficacy in East Africa (Musa *et al.*, 2010:2). Therefore, in East Africa, paromomycin is commonly used in combination with

sodium stibogluconate for optimal treatment outcomes (Sundar *et al.*, 2019:4). Systemic administration of paromomycin can lead to rare but uncomfortable side effects such as mild hepatotoxicity, ototoxicity, and local discomfort or burning at the application site (Roatt *et al.*, 2020).

Pentamidine, as illustrated in **Figure 2.2**, has also been explored as a potential treatment for leishmaniasis. This compound was initially synthesised in the 1940s and its antileishmanial properties have been studied, particularly in regions where resistance to antimonial drugs, such as the Indian subcontinent, has been reported. However, the use of pentamidine as therapeutic agent for leishmaniasis was discontinued due to its associated toxicity. Adverse effects include hypoglycaemia, hyperkalaemia, hypotension, and pancreatitis, which could potentially lead to the development of diabetes mellitus (Roatt *et al.*, 2020).

Azole antifungal drugs have been investigated as potential treatment options for cutaneous forms of leishmaniasis. In the case of oral fluconazole, increasing the medication concentration has shown a significant improvement in efficacy for Old World cutaneous leishmaniasis, with cure rates reaching approximately 60%. However, it is important to note that oral fluconazole is associated with undesirable side effects, such as hepatotoxicity and cardiotoxicity (Alrajhi *et al.*, 2002:891). A recent comprehensive meta-analysis suggest that none of the azole antifungal drugs are effective as stand-alone treatments for cutaneous forms of leishmaniasis. Therefore, their use should be limited to combination therapy with other drugs (Galvao *et al.*, 2017:2).

2.2.3.3 Transmission and epidemiology

Female sandflies exhibit a feeding behaviour primarily occurring at or after sunset, during which they become infected by ingesting the blood of terrestrial animals, including humans, leading to either zoonotic or anthroponotic transmission patterns of leishmaniasis (Murray *et al.*, 2005:1564). While certain sandfly species display a wide range of hosts such as marsupials, rodents, canines, and hyraxes, others have a greater preference for humans (Steverding, 2017b:1). Notably, the parasites responsible for leishmaniasis are primarily located in the skin rather than in the peripheral circulation or internal organs such as the liver and spleen (Bates, 2007:1099).

Within the gut of the sandfly, *Leishmania* parasites undergo transformation from amastigotes to infectious metacyclic promastigotes. As the sandfly continues to feed on blood, it becomes contagious, enabling the transmission of the parasites. Transmission occurs in groups, with

both the promastigote secretory gel and sandfly saliva playing crucial roles as vector-derived components that aid in parasite survival, establishment in the host, and successful transmission (Serafim *et al.*, 2021:1). Furthermore, the host's immune response following the bite of an infected sandfly also influences the outcome of transmission (Torres-Guerrero *et al.*, 2017:9).

It is important to note that leishmaniasis is a widespread disease affecting 89 different countries (Kashif *et al.*, 2017), with endemic regions in Africa, Asia, the Mediterranean, and the Americas, and approximately 350 million people at risk of contracting the disease (Torres-Guerrero *et al.*, 2017:1). The burden of leishmaniasis falls heavily on some of the world's poorest nations, with factors such as immune system weakness, malnutrition, and substandard housing contributing to its prevalence (WHO, 2023). Annually, there are approximately 70,000 fatalities and 1.5 to 2 million new cases reported, although it is estimated that only 25–45% of visceral leishmaniasis cases and around 200,000 cases of cutaneous leishmaniasis are reported to the WHO (Torres-Guerrero *et al.*, 2017:1).

Visceral leishmaniasis displays regional variations in terms of the *Leishmania* (*L*.) species responsible for the disease. In the Indian subcontinent and Africa, visceral leishmaniasis is caused by *L. donovani*, while in the Mediterranean Basin, Central America, and South America, it is primarily brought on by *L. infantum* (Sundar & Singh, 2016:98). On the other hand, mucocutaneous leishmaniasis is attributed to *L. braziliensis* and *L. panamensis*, with distinct geographical distributions. Cutaneous leishmaniasis is further classified into Old World cutaneous leishmaniasis and New World cutaneous leishmaniasis based on geographic regions (Sundar & Singh, 2016:98). In Old World cutaneous leishmaniasis, *Leishmania* species such as *L. aethiopica*, *L. donovani*, *L. infantum*, *L. major*, and *L. tropica* are prevalent in Southern Europe, the Middle East and parts of Southwest Asia, Central Asia, and Africa. New World cutaneous leishmaniasis predominantly affects Mexico and Latin America, with various *Leishmania* species implicated in the disease (Sundar & Singh, 2016:98).

The distribution of visceral leishmaniasis cases is particularly high in East Africa, Brazil, and India, while cutaneous leishmaniasis cases are concentrated in the Mediterranean Basin, the Americas, the Middle East, and Central Asia, with Ethiopia, Brazil, Peru, and Bolivia accounting for a significant proportion of mucocutaneous leishmaniasis cases (WHO, 2023).

2.3 Pathogenic bacteria

The discovery of antibiotics in the 1930s revolutionised medicine, significantly improving global health outcomes. However, the rise of antimicrobial resistance poses a grave challenge to human health today, resulting in the deaths of over 700,000 individuals annually (Qin *et al.*, 2022:1). Antimicrobial resistance threatens to push us back to the pre-antibiotic era with even minor infections becoming life-threatening (Navidinia, 2016:43).

Antibiotic resistance poses a significant challenge for both developing and developed nations (Wise *et al.*, 1998:609). With each passing year, the arsenal of effective antibiotics against pathogenic bacteria is dwindling, leaving us vulnerable to a future where these pathogens become untreatable. Consequently, there is an urgent demand to develop new treatment strategies to address the issue of drug-resistant infections, especially those caused by ESKAPE pathogens (Mulani *et al.*, 2019:2).

2.3.1 ESKAPE pathogens

The ESKAPE pathogens are primarily responsible for these resistance issues (Rice, 2010:S7). These pathogens have acquired the ability to ‘escape’ the activity and effectiveness of antimicrobials, posing a significant threat to the healthcare system (Jadimurthy *et al.*, 2022:1).

ESKAPE pathogens are responsible for a range of nosocomial infections and have been identified as a significant concern by The Infectious Disease Society of America. These infections contribute to higher mortality rates, prolonged hospital stays, and increased treatment costs (Jadimurthy *et al.*, 2022:2). Currently, these drug-resistant bacteria account for 15.5% of all global healthcare-associated infections (Mulani *et al.*, 2019:1), with a particularly high prevalence in ICUs (Denissen *et al.*, 2022:1). In the ICU setting, over 40% of infections were attributed to ESKAPE pathogens in 2015, highlighting the impact on patient safety (Rice, 2010:S7). These pathogens are associated with life-threatening conditions such as wound infections, urinary tract infections, meningitis, pneumonia, and bacteremia (Denissen *et al.*, 2022:1).

Transmission occurs through various means, including inadequate sterilisation practices, contaminated equipment, inadequate transmission control practices, and infected healthcare workers (Jadimurthy *et al.*, 2022:2). The management of these infections has become

increasingly challenging due to the lack of development and investigation of novel antimicrobials against multidrug-resistant pathogens and the rapid rise of antimicrobial resistance (Rice, 2010:S7). While the development of new antibiotics is crucial, it is equally important to preserve the effectiveness of currently available antibiotics (Arbune *et al.*, 2021:2370). Moreover, ESKAPE pathogens are not limited to healthcare settings and are increasingly being identified in community settings. Environmental reservoirs such as sewage systems, municipal waste, soil, food, water sources, and plants can harbour these pathogens (Denissen *et al.*, 2022:2).

WHO categorises ESKAPE pathogens into three priority levels, namely critical, high, and medium priority (Denissen *et al.*, 2022:1). The categorisation is based on the urgency for developing novel antibiotics to combat these pathogens (Jadimurthy *et al.*, 2022:2). The Gram-positive species *E. faecium* and *S. aureus* are classified as high priority pathogens. On the other hand, the critical priority group comprises Gram-negative pathogens that exhibit multidrug resistance by being resistant to three or more antibiotic classes. This group includes *K. pneumoniae*, *A. baumannii, P. aeruginosa*, and *Enterobacter* spp. (Denissen *et al.*, 2022:1).

E. faecium is a Gram-positive cocci bacterium that exhibits a wide range of habitats. It is commonly found in the digestive microbiota of humans and warm-blooded animals. Additionally, *E. faecium* can be present in various environments such as bodies of water, soil, plants, and a variety of food sources including fermented goods, dairy products, meat, and seafood (Rathnayake *et al.*, 2012:326). In the context of human infections, *E. faecium* is the most frequently encountered species, particularly in hospital settings. These microorganisms possess a remarkable ability to survive and thrive under challenging conditions thanks to their resistance to different environmental stresses such as dehydration, varying temperatures, and diverse pH levels (Cattoir, 2022).

S. aureus is a Gram-positive bacterium that is widely encountered as a human pathogen (Guo *et al.*, 2020:1). Apart from its well-known antibiotic resistance, *S. aureus* exhibits the ability to survive and adapt to diverse extreme conditions, posing challenges in its eradication (Cheung *et al.*, 2021:559). Approximately 60% of the human population are intermittent carriers of *S. aureus*, meaning they intermittently harbour varying populations of resident strains. Meanwhile, about 20% of individuals are persistent carriers, constantly harbouring a stable strain of *S. aureus* (Pendleton *et al.*, 2013:299). The primary colonisation sites for *S. aureus* are the skin and mucosal surfaces. While *S. aureus* typically colonises the human body without causing any infections, it can lead to a wide range of health complications with varying degrees of severity (Cheung *et al.*, 2021:548). Certain populations, including healthcare workers,

immunocompromised individuals, hospital patients, and those who frequently use needles, are often at higher risk of infection (Taylor & Unakal, 2021).

K. pneumoniae is a well-known member of the Gram-negative *Enterobacteriaceae* family and poses a growing health threat both in healthcare settings and the community due to its widespread presence worldwide. It naturally inhabits the intestinal tract and nasal passages of humans and animals. Additionally, it is commonly found in environmental reservoirs such as water, soil, and plants (Denissen *et al.*, 2022:8-9). *Klebsiella* species, including *K. pneumoniae,* are the predominant bacteria responsible for healthcare-associated infections (Santajit & Indrawattana, 2016:1). While individuals with weakened immune systems have traditionally been more susceptible to infections, there has been a rise in the emergence of stronger and more virulent strains of *K. pneumoniae*, increasing the risk of infection even among individuals with robust immune systems (Ali *et al.*, 2021:64).

A. baumannii is a Gram-negative coccobacillus that presents a significant concern as a pathogen. It is commonly found in moist environments, including damp soil, lakes, wetlands, fish farms, wastewater, water treatment facilities, and even marine water (Wong *et al.*, 2017:410). This adaptability allows *A. baumannii* to thrive across a wide range of pH levels, temperatures, and environmental conditions, enabling its presence in both human and environmental reservoirs (Pendleton *et al.*, 2013:300). It is capable of causing infections in various sites, such as the respiratory and urinary systems. Notably, *A. baumannii* exhibits high rates of resistance, presenting a particular challenge in surgical wards and ICUs (Santajit & Indrawattana, 2016:6).

P. aeruginosa is a Gram-negative bacterium that exhibits a unique ability to thrive in both oxygen-rich and oxygen-deprived environments, classifying it as a facultative anaerobe. It is a normal constituent of the gastrointestinal flora in humans (Santajit & Indrawattana, 2016:6). In its biofilm form, *P. aeruginosa* can withstand harsh conditions, including hypoxia, making it particularly resilient. The treatment of *P. aeruginosa* infections is extremely challenging due to its rapid mutation and adaptation, leading to the development of drug resistance. Moreover, *P. aeruginosa* is one of the leading causes of nosocomial infections, commonly found on medical devices such as ventilators (Qin *et al.*, 2022:1).

Enterobacter species are Gram-negative bacilli that form part of the normal flora in the gastrointestinal system of both humans and animals (Davin-Regli *et al.*, 2019:2). These organisms are facultative anaerobes that can be found in various environmental reservoirs, including soil, water, and plants (Denissen *et al.*, 2022:10). The *Enterobacter* genus comprises

22 species and has been associated with causing diseases in plants, animals, and humans. Among the isolated species, *E. aerogenes*, *E. cloacae*, and *E. hormaechei* are of significant concern (Davin-Regli *et al.*, 2019:2). These bacteria are particularly notorious for causing opportunistic infections in immunocompromised individuals, predominantly in hospital settings. These species possess a range of antibiotic resistance mechanisms, posing a significant threat to the healthcare system (Santajit & Indrawattana, 2016:6).

2.3.2 Resistance mechanism of ESKAPE pathogens

ESKAPE pathogens have exhibited resistance to multiple classes of antibiotics, including tetracyclines, fluoroquinolones, macrolides, β-lactams, β-lactam-β-lactamase inhibitor combinations, oxazolidinones, and lipopeptides. In some cases, these pathogens have even developed resistance to last resort antibiotics such as carbapenems, glycopeptides, and polymyxins (Mulani *et al.*, 2019:2). These notorious multidrug-resistant bacteria employ various mechanisms to evade the effects of antibiotics, including upregulating efflux pumps, developing sophisticated efflux systems, altering antibiotic binding sites, enzymatic inactivation, and biofilm formation (Mulani *et al.*, 2019:2).

Drug accumulation mechanisms: One common approach involves upregulating efflux pumps, which actively pump antibiotics out of bacterial cells, thereby impeding the accumulation of drugs to effective levels. Additionally, these pathogens may alter their membrane permeability or decrease the uptake of antibiotics, further hindering drug entry into bacterial cells (Santajit & Indrawattana, 2016:4)

Sophisticated efflux systems: Some pathogens go beyond the basic upregulation of efflux pumps. They develop more complex efflux systems capable of expelling a wide range of antibiotics. This advanced defense mechanism significantly limits the effectiveness of antibiotics (Santajit & Indrawattana, 2016:4)

Alteration of antibiotic binding sites: Another resistance strategy involves genetic mutations or the acquisition of resistance genes by ESKAPE pathogens. These alterations lead to changes in the target sites of antibiotics. As a result, drugs are unable to effectively bind to their intended targets, diminishing their ability to inhibit bacterial growth or kill pathogens (Santajit & Indrawattana, 2016:2-4)

Enzymatic inactivation: ESKAPE pathogens may produce enzymes such as β-lactamases, which enzymatically inactivate antibiotics by degrading or modifying them. For example, β-

lactamases can hydrolyze β-lactam antibiotics like penicillins and cephalosporins, rendering them ineffective in inhibiting bacterial growth (Mulani *et al.*, 2019:2)

Biofilm formation: Many pathogens have the ability to form biofilms, which are protective matrices of extracellular substances. These biofilms serve as physical barriers that prevent antibiotics from reaching bacterial cells, making it challenging for drugs to exert their antibacterial effects (Santajit & Indrawattana, 2016:4-5)

2.4 Quinolines

Quinoline (C_9H_7N) is characterised by its double-ring structure, comprising a pyridine and a benzene ring connected through adjacent carbon atoms, as shown in **Figure 2.11** (Amit *et al.*, 2014). It acts as a weak base and can form salts by reacting with acids. Quinoline is readily soluble in various organic solvents at room temperature and can absorb water molecules from the environment (Jain *et al.*, 2019:4921). It participates in a variety of coupling reactions, nucleophilic reactions, and electrophilic reactions. The reactivity of quinoline is influenced by the type and position of substitutions on its rings, with, for instance, a halogen substitution at position 7 enhancing its propensity for coupling reactions (Kumar *et al.*, 2022).

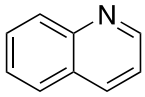


***Figure 2.11**: The structure of quinoline*

Since the discovery of medications, natural remedies have played a vital role in the management and prevention of various diseases (Luo *et al.*, 2021:1123). These organic materials are utilised either directly as crude extracts or as a basis for the synthesis of bio-active compounds (Mushtaq *et al.*, 2018:422). Nitrogen-containing derivatives of these natural compounds have particularly contributed to the development of numerous clinical candidates (Mermer *et al.*, 2021:1). Among them, heterocyclic compounds, including quinoline, have emerged as important molecules due to their various medicinal properties, such as anticancer, antimicrobial, antitumour, and anti-inflammatory activities (Ajani *et al.*, 2022:18595). Quinoline, with its unique structure, provides medicinal chemists with significant versatility in terms of modification and synthesis, making it crucial for diverse drug core structures and biological and pharmacological activities (Kumar *et al.*, 2022).

The isolation of the quinoline core structure can be traced back to the nineteenth century when German scientist Friedlieb Ferdinand Runge first isolated it from coal tar in 1834. Later, French chemist Charles Gerhardt obtained quinoline through the dry distillation of strychnine, cinchonine, and quinine (Kumar *et al.*, 2022). Subsequently, the synthesis of quinoline derivatives became feasible using various starting materials such as anilines, β-ketoanalides, acylaminoacetophenone, and isatin, allowing the creation of structurally diverse quinoline derivatives (Kumar *et al.*, 2022). While the core structure itself lacks biological significance, its derivatives exhibit a wide range of biological activities. Notably, several quinoline derivatives, including chloroquine, primaquine, and amodiaquine, have shown significant antimalarial properties, with their biological profiles influenced by the specific substitutions made on the quinoline ring (Foley & Tilley, 1998:55).

The strategic substitution of the quinoline ring has contributed to the synthesis of a diverse array of antimicrobial agents, encompassing antiprotozoal, antibacterial, antiviral, and antifungal compounds (Da Gama & Soeiro, 2021). Quinoline hybrids have demonstrated efficacy in targeting novel cellular mechanisms, effectively functioning as inhibitors of cell proliferation. These mechanisms include apoptosis induction, cell cycle arrest, interference with cellular migration, angiogenesis disruption, and modulation (Yadav & Shah, 2021). The versatility of quinoline derivatives has made them a pivotal focus of research for scientists worldwide. The continuous development of novel quinoline compounds holds great potential in combating the global challenge of antimicrobial resistance (Kumar *et al.*, 2022).

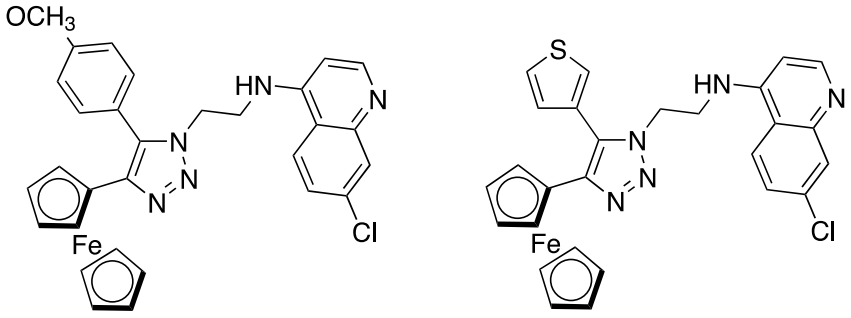
2.4.1 Antiprotozoal activity

Protozoa, as discussed in Section 2.2, exhibit a characteristic intracellular proliferation in host cells by utilising available nutrients. Subsequently, they cause the host cell's demise and seek out new healthy cells for infection (De Souza, 2008:822). Antiprotozoal drugs disrupt the survival and replication of these parasites by either inhibiting nutrient uptake or impeding mitochondrial energy production, leading to starvation and eventual cell death. Key targets involved in nutrient acquisition and growth promotion are regulated by various pumps, channels, and enzymes. Pentamidine, paromomycin, and miltefosine are examples of effective medications that target these pathways. Unfortunately, the emergence of drug resistance, attributable to factors such as reduced drug diffusion, altered drug targets resulting from mutations, increased drug efflux, and enzyme-mediated drug inactivation, has compromised their efficacy (Garcia-Salcedo *et al.*, 2016:2-3). Moreover, these drugs possess unfavourable drug-like properties and associated toxicities. Consequently, there is an urgent need to develop novel antiprotozoal compounds with enhanced affinity and effectiveness

(Kumar *et al.*, 2022). The subsequent section will delve into recent advancements in the development of quinoline-based antiprotozoal drugs.

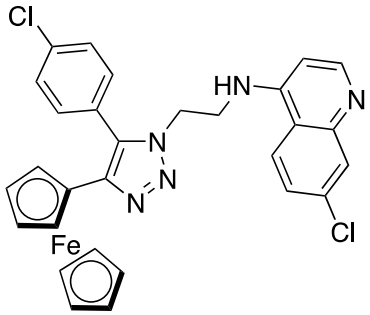
2.4.1.1 Antileishmanial drugs

Yousuf *et al*. (2016) conducted research on the development of novel antileishmanial drugs by synthesising ferrocenylquinoline derivatives containing triazoles, as depicted in **Figure 2.12**. The inhibitory effects of these compounds were assessed against both the promastigote and amastigote stages of *L*. *donovani*. Notably, **Compound 1**, **Compound 2**, and **Compound 3** exhibited significant antipromastigote activity, with half maximal inhibitory concentration (IC_{50}) values of 28.7 μM, 22.1 μM, and 28 μM, respectively. Similarly, these compounds demonstrated remarkable action against the intracellular amastigote stage, with IC_{50} values of 16 μM, 8 μM, and 16 μM, respectively. Importantly, no cytotoxicity towards host phytohemagglutinin-induced murine splenocytes was observed for **Compound 1**, **Compound 2**, or **Compound 3**, which was attributed to the presence of two heterocyclic rings that mechanistically damaged the parasite membranes by inhibiting the enzymatic activity of α-demethylase (CYP51) and altering the sterol composition of the parasitic membrane, ultimately leading to parasite death (Yousuf *et al.*, 2016:4-7).



Compound 1

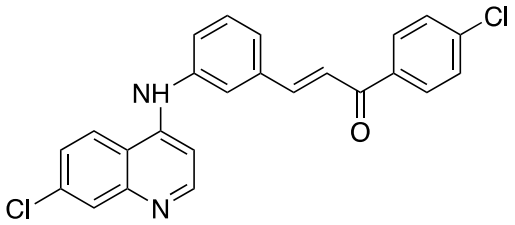
Compound 2



Compound 3

Figure 2.12*: The Yousuf et al. (2016:4-7) synthesised compounds that had antileishmanial activity*

In their study, Ramírez–Prada *et al*. (2017) synthesised a quinoline chalcone derivative, specifically **Compound 4** illustrated in **Figure 2.13**. The researchers evaluated the anti-leishmanial activity of this compound and found it to exhibit remarkable efficacy. **Compound 4** demonstrated the highest antileishmanial activity among the tested compounds, with a half maximal effective concentration (EC_{50}) value of 0.79 µg/mL. This indicates its potency in inhibiting the growth of *Leishmania* parasites, suggesting its potential as a promising antileishmanial agent (Ramírez–Prada *et al.*, 2017:3-4).

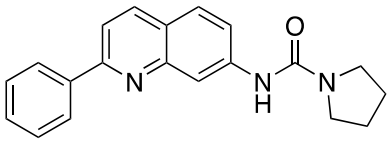


Compound 4

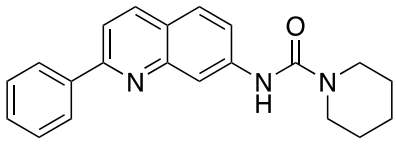
Figure 2.13*: The Ramírez–Prada et al. (2017:2) synthesised compound that exhibited high antileishmanial activity*

2.4.1.2 Antitrypanosomal drugs

In their research, Nefertiti *et al*. (2018) developed and synthesised 7-phenyl-quinoline derivatives, as depicted in **Figure 2.14**, with potent activity against *T. brucei*, the causative agent of African trypanosomiasis. All the investigated quinolines displayed significant *in vitro* anti-African trypanosome activity, with IC_{50} values below 0.25 µM. Among these compounds, **Compound 5** demonstrated exceptional efficacy. When administered three times intra-peritoneally at a dose of 40 mg/kg, **Compound 5** resulted in a remarkable reduction of more than 98% in parasitemia and achieved a 50% cure rate in infected subjects. Furthermore, **Compound 6** exhibited even greater effectiveness, completely eliminating parasitemia and curing 50% of the infected subjects (Nefertiti *et al.*, 2018:11-13).



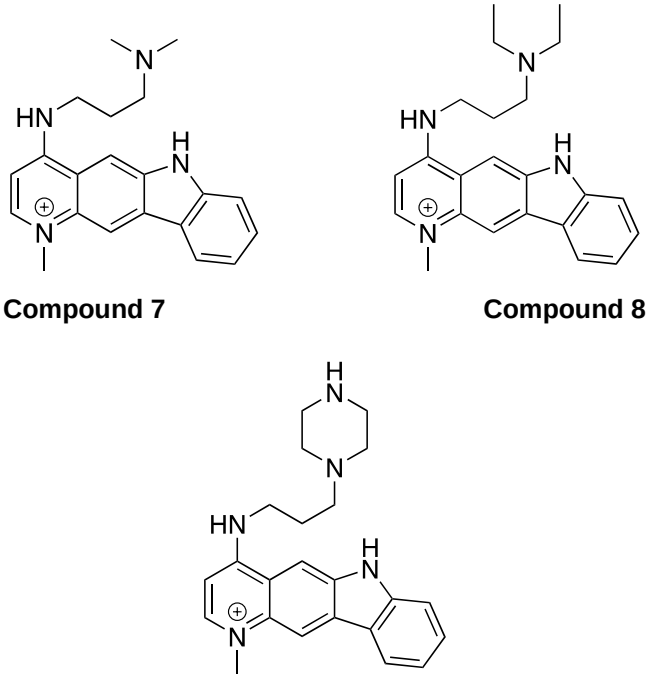
Compound 5



Compound 6

Figure 2.14*: The Nefertiti et al. (2018:11-13) synthesised compounds that exhibited high antitrypanosomal activity*

Lavrado *et al*. (2012) synthesised C11-substituted cryptolepine derivatives with weak basic alkyl diamine side chains, employing the alkaloid cryptolepine and the antimalarial drug chloroquine as models, as illustrated in **Figure 2.15**. Among the collection of cryptolepine analogues, **Compounds 7** and **8** exhibited significant activity against *T.b. brucei* with IC_{50} values below 0.06 µM, along with acceptable cytotoxic activity (CC_{50}) against Vero cells, resulting in high selectivity index values. **Compound 9** emerged as the most selective compound, demonstrating an impressive IC_{50} value of 0.01 µM and a selectivity index value of 6,200 (Lavrado *et al*., 2012:6256-6259)



Compound 7 **Compound 8**

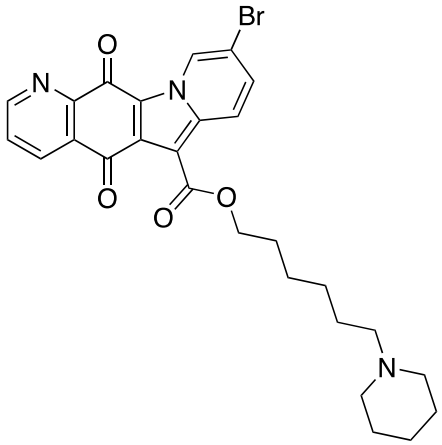
Compound 9

Figure 2.15*: The Lavrado et al. (2012:6256-6259) synthesised compounds that exhibited high antitrypanosomal activity*

These novel compounds represent only a fraction of the vast pool of quinoline derivatives that exhibit activity against protozoal diseases. The extensive research conducted on quinoline derivatives underscores their significant potential in medical investigations. With their diverse chemical structures and versatile pharmacological properties, quinoline derivatives continue to be an attractive area of study for the development of novel therapeutics against protozoal illnesses. The ongoing exploration of quinoline derivatives holds great promise and opens up new avenues for advancing our understanding and treatment of these diseases.

2.4.2 Antibacterial activity

In the context of their research, Yang *et al*. (2017) conducted the synthesis of a series of 9-bromo modified indolizinoquinoline-5,12-dione derivatives and assessed their antibacterial efficacy against various bacterial strains, including the multidrug-resistant methicillin-resistant *S. aureus* (MRSA). Among the synthesised compounds, **Compound 10** (**Figure 2.16**) demonstrated remarkable antibacterial activity and exhibited favourable solubility in water, with a solubility level of 1.98 mg/mL. Specifically, **Compound 10** displayed potent activity against clinical MRSA strains, as indicated by its minimum inhibitory concentration (MIC_{50}) value of 63 ng/mL and MIC_{90} value of 125 ng/mL. Notably, the antimicrobial effectiveness of **Compound 10** surpassed that of vancomycin, a widely utilised antibiotic, by a significant margin of 16-fold (Yang *et al.*, 2017:4).



Compound 10

Figure 2.16*: The Yang et al. (2017:4) synthesised compound that exhibited high antibacterial activity*

2.5 Aminochalcones

Chalcones, a class of open chain flavonoids, possess a unique structure consisting of a three-carbon, α,β-unsaturated carbonyl system that connects two aromatic rings as shown in **Figure 2.17** (Singh *et al.*, 2012:1298). Referred to as 1,3-diphenylprop-2-en-1-one, this structural feature is believed to contribute to their distinctive hydrophobic/hydrophilic characteristics, which has sparked significant research interest, particularly in the field of infectious diseases. Chalcones are commonly synthesised through the Claisen–Schmidt condensation, involving the reaction between benzaldehydes and active methylene ketones (Dhaliwal *et al.*, 2022:3). Chalcones exhibit diverse applications, including their use as food additives, constituents in cosmetic formulations, and treatments for conditions such as stomach cancer, gastritis, discomfort, and various viral and vascular disorders. Additionally, chalcones display a wide range of biological properties, encompassing cytotoxic, immunosuppressive, amoebic, insecticidal, anti-ulcer, antiviral, antiprotozoal, and anticancer effects (Yerragunta *et al.*, 2013:54).

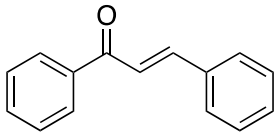
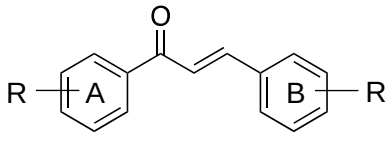


***Figure 2.17**: The structure of chalcone*

Within the class of chalcones, aminochalcones hold particular significance, characterised by the presence of a $-NR_2$ group on either ring A or ring B (**Figure 2.18**). Recent research conducted by several organisations has focused on the synthesis and biological implications of these molecules. Notably, the cytotoxic activity of aminochalcones has garnered attention due to their potential for enhanced electrophilic attack on cellular proteins through hetero-Michael addition, leading to the formation of covalent bonds. This mechanism has stimulated interest in the development of covalent inhibitors, representing a novel area of drug discovery, and aminochalcones have emerged as promising candidates for the creation of inhibitors targeting specific biological proteins (Irfan *et al.*, 2020:2).



R: NH_2, NX_2, NXH

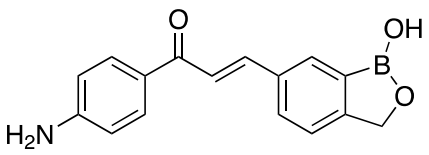
***Figure 2.18**: The structure of aminochalcones*

Chalcones are naturally abundant in various organic foods such as fruits, teas, vegetables, and spices. These compounds exhibit a wide spectrum of bioactivities, including but not limited to anticancer, anti-inflammatory, antimalarial, antioxidant, antibacterial, antifungal, anti-protozoal, anticonvulsant, and anti-ulcer activities (Irfan *et al.*, 2020:2). Notably, chalcones have demonstrated significant antimicrobial potential, particularly aminochalcones, as evidenced by several studies discussed below.

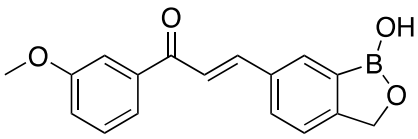
2.5.1 Antiprotozoal activity

A study was conducted demonstrating that chalcones, active against *L. major* and *L. donovani*, inhibit fumarate reductase, an enzyme present in the parasite respiratory chain but absent in mammalian cells. It proposed that fumarate reductase may serve as the specific target for chalcones. Interestingly, although the bloodstream form of *T. brucei* does not rely on fumarate reductase activity, chalcones still exhibit activity against this form of the parasite, suggesting the involvement of alternative mechanisms in their cytotoxicity (Roussaki *et al.*, 2013:8).

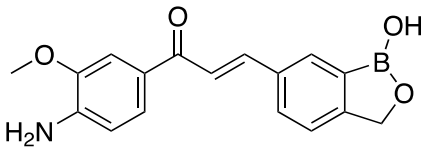
Previous investigations have shown that chalcones inhibit the proliferation of *T. brucei* parasites (Troeberg *et al.*, 2000:660). Building upon this knowledge, Qiao *et al*. (2012) explored the potential synergistic relationship between benzoxaborole and chalcone scaffolds. The authors synthesised chalcone-benzoxaborole hybrid compounds (**Figure 2.19**) and evaluated their ability to inhibit the growth of *T. brucei* in the bloodstream. Among the compounds tested, **Compound 11** (4-NH_2) and **Compound 12** (3-OMe) emerged as lead compounds, exhibiting IC_{50} values of 0.024 µg/mL and 0.022 µg/mL, respectively. Combining the structural features of **Compounds 11** and **12** led to the development of **Compound 13** (4-amino-3-methoxy), which displayed improved activity with an IC_{50} of 0.010 µg/mL (Qiao *et al.*, 2012:3553).



Compound 11



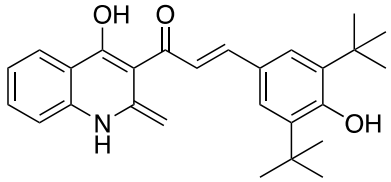
Compound 12



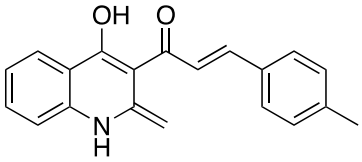
Compound 13

***Figure 2.19**: Aminochalcones with antitrypanosomal activity (Qiao et al., 2012:3554)*

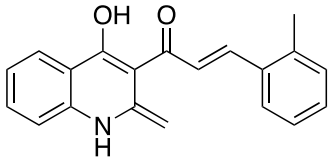
Roussaki *et al*. (2013) synthesised and evaluated a series of quinolinone-chalcone derivatives (**Figure 2.20**) for their biological activity against *T. brucei* and *L. infantum*. Among the compounds tested, they identified **Compound 14** as the most potent anti-*L. infantum* compound, with an IC_{50} value of 1.3 ± 0.1 µM. This compound displayed equipotent anti-parasitic activity against both species. **Compound 15** exhibited efficacy specifically against the amastigote stage of *L. infantum*, with IC_{50} values of 2.1 ± 0.6 µM. **Compound 16** and **Compound 14** also demonstrated promise as trypanocidal agents, with IC_{50} values of 2.6 ± 0.1 µM and 3.3 ± 0.1 µM, respectively (Roussaki *et al.*, 2013:1-2).



Compound 14



Compound 15



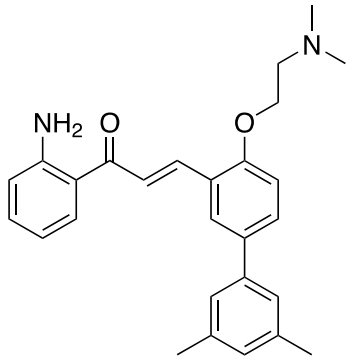
Compound 16

***Figure 2.20**: Chalcones exhibiting antiprotozoal activity (Roussaki et al., 2013:5)*

2.5.2 Antibacterial activity

Nielsen *et al*. (2005) synthesised a series of 2-aminochalcone derivatives (**Figure 2.21**) and assessed their antibacterial activity against MRSA ATCC33591, vancomycin-resistant clinical isolate *E. faecium* 17501, and *E. faecium* ATCC29212 strains. Among the compounds tested, **Compound 17** exhibited significant antibacterial activity, with MIC values ranging from 5 µM to 10 µM against the targeted strains. It was observed that **Compound 17** and related

2-aminochalcones with modifications in ring B showed increased selectivity towards the bacterial membrane. Comparison of the MIC values revealed that **Compound 17** displayed superior antibacterial activity compared with its 3-aminochalcone analogue (MIC = 10 µM) and 4-aminochalcone analogues (MIC = 10 µM and 20 µM), highlighting the influence of the position of the $-NH_2$ group on ring A on antibacterial activity (Nielsen *et al.*, 2005:2671).



Compound 5

***Figure 2.21**: Aminochalcones exhibiting antibacterial activity (Nielsen et al., 2005:2671)*

2.6 Aromatic nitro compounds

Since the discovery of chloramphenicol in 1947, nitro-containing compounds have been the subject of extensive research. These molecules display a diverse array of biological activities, making them appealing to medicinal chemists. Nitro compounds have demonstrated potential as antiparasitic agents, antineoplastic drugs, antibiotics, antihypertensive agents, tranquilisers, herbicides, and more (Noriega *et al.*, 2022:1).

The investigation of nitro compounds has garnered significant attention due to their exceptional electron-attracting properties conferred by the nitro group. This property stems from their strong inductive and resonance effects, endowing them with potent electron-attracting capabilities (Jezuita *et al.*, 2021:179). Consequently, nitro compounds have demonstrated a broad-spectrum of biological activities, functioning as both pharmacophores and structural moieties in various compounds. Among these activities, their antimicrobial properties are particularly noteworthy. By initiating redox processes in cells, nitro compounds induce toxicity, leading to the elimination of microorganisms, including parasites and bacteria. However, it is crucial to recognise that the nitro group can exhibit both pharmacophoric and toxicophoric characteristics, as similar mechanisms of action can also affect human cells (Noriega *et al.*, 2022:1-2).

Researchers often focus on the development of nitrated derivatives of existing pharmaceuticals to enhance their activity or minimise side effects. The strong electron-withdrawing nature of the nitro group profoundly influences the polarity of associated molecules, facilitating interactions with proteins. Additionally, the electron-withdrawing effect observed in aromatic rings plays a crucial role in the design of synthetic routes as it promotes specific positions for electrophilic substitution reactions. Although the nitro group itself is rarely considered a stand-alone pharmacophore, its inclusion is often associated with toxic effects related to the disruption of oxidative stress mechanisms, as observed in antiparasitic agents (Noriega *et al.*, 2022:11).

A widely accepted model proposes that when nitro compounds undergo reduction, they generate harmful intermediates, including nitroso and superoxide species. These reduced nitro species form covalent bonds with DNA, resulting in nuclear damage and cellular demise (Müller *et al.*, 2018:271). Hence, this mechanism underscores the antimicrobial activity exhibited by nitro-containing compounds such as metronidazole, chloramphenicol, and other derivatives. Notably, nitro-based compounds play a crucial role as primary therapeutic agents in treating common infections caused by diverse microorganisms (Noriega *et al.*, 2022:2).

2.6.1 Antiprotozoal activity

NECT (**Figure 2.5**) as mentioned in Section 2.2.1.3, represents the primary treatment choice for second-stage HAT. NECT is the combination of nifurtimox, which is a prime example of an aryl nitro compound, and eflornithine (Büscher *et al.*, 2017:7). Another effective aryl nitro compound for trypanosomiasis treatment is benznidazole as presented in **Figure 2.22**. Both nifurtimox and benznidazole serve as valuable templates for the development of novel nitro compounds with intriguing biological properties (Soriano-Correa *et al.*, 2008:1370).

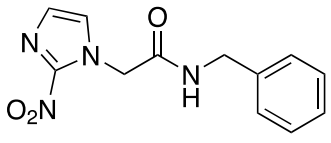


***Figure 2.22**: The structure of benznidazole, an effective aryl nitro compound (Soriano-Correa et al., 2008:1370)*

Pozas *et al*. (2005) synthesised a series of eight novel analogues of nifurtimox incorporating different heterocyclic moieties in the form of alpha-beta unsaturated amides, aiming to explore their structural domains and associated biological activities. Notably, four of these analogues (**Figure 2.23**), containing nitro groups at position 5 of the furan ring, demonstrated significantly enhanced trypanocidal activity compared with nifurtimox itself (Pozas *et al.*, 2005:1417).

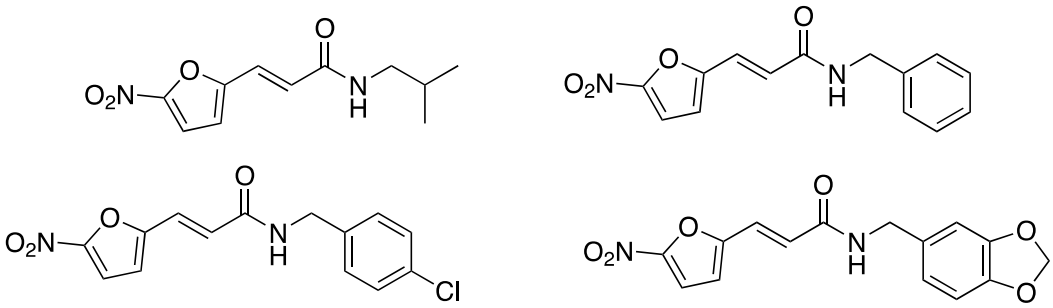
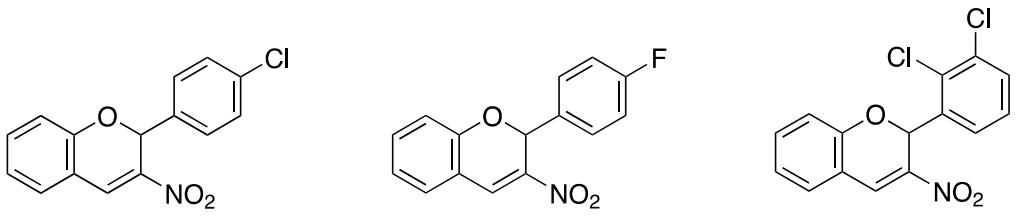


Figure 2.23*: The Pozas et al. (2005:1417) synthesised compounds*

Fexinidazole, depicted in **Figure 2.6**, is an innovative aromatic nitro drug utilised for HAT, as discussed in Section 2.2.1.3. Acting as a prodrug, fexinidazole undergoes enzymatic reduction by nitro-reductases, resulting in the generation of cytotoxic metabolites. These metabolites exhibit damaging effects on DNA, lipids, and proteins. Besides its activity against HAT, recent research has revealed the effectiveness of fexinidazole against leishmaniases as well. *In vitro* studies have demonstrated its potent efficacy against *L. donovani*, and in vivo experiments using a visceral leishmaniasis mouse model have shown promising results. Furthermore, fexinidazole has been reported to be well-tolerated, whether administered as a single dose or over a 14-day period of repeated dosing. Multiple phase I investigations have been conducted to assess the pharmacokinetics and safety profile of fexinidazole (Sundar *et al.*, 2019:5).

2.6.2 Antibacterial activity

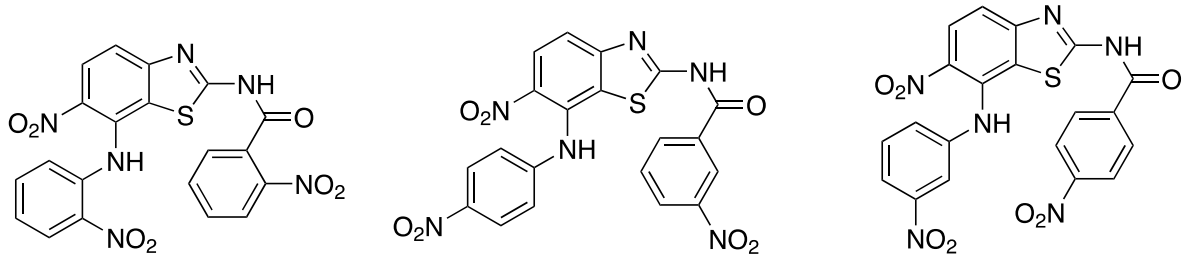
Andrade *et al*. (2020) synthesised various nitro derivatives (**Figure 2.24**) using an oxa-Michael-aldol condensation reaction and assessed their antimicrobial potential against *S. aureus.* Experimental findings revealed that nitro derivatives with halogenation, specifically **Compounds 18**, **19** and **20** exhibited the most promising results, with MIC values ranging from 15.6 µg/mL to 62.5 µg/mL against *S. aureus* (Andrade *et al*., 2020).



Compound 18 **Compound 19** **Compound 20**

***Figure 2.24**: The Andrade et al. (2020) synthesised nitro compounds*

The incorporation of the nitro functional group has been shown to enhance the antibacterial activity of heterocyclic compounds, particularly benzothiazoles. In a recent study, researchers successfully synthesised nitrated benzothiazoles from nitroanilines and potassium thiocyanate using mild reaction conditions. Notably, three specific compounds, namely **Compounds 21, 22,** and **23**, displayed significant antibacterial activity against *P. aeruginosa*, with MIC values of 24 µg/mL, 19 µg/mL, and 19 µg/mL, respectively (**Figure 2.25**). These findings highlight the potential of these three compounds as promising candidates for further investigation as pharmaceutical agents. Intriguingly, the inhibitory effects of these compounds were comparable to those of procaine penicillin, a well-known antibacterial agent (Gupta, 2019).



Compound 21 **Compound 22** **Compound 23**

***Figure 2.25**: The Gupta et al. (2019) synthesised nitro compounds*

2.7 Rationale

The derivatisation of the quinoline scaffold has led to the discovery of a diverse range of anti-microbial agents, encompassing both antiprotozoals and antibacterials. This has propelled quinoline derivatives into the spotlight, attracting widespread attention from researchers (Kumar *et al.*, 2022).

Another class of compounds, chalcones, has demonstrated notable antimicrobial activity owing to their reactive *α,β*-unsaturated system. Aminochalcones, in particular, hold great potential as starting points for designing inhibitors that target specific biological proteins. The development of covalent inhibitors represents a novel avenue in the field of drug discovery (Irfan *et al.*, 2020:2).

Additionally, aryl nitro moiety has been shown to initiate redox reactions in cells, leading to the elimination of bacteria and multicellular organisms such as protozoans (Noriega *et al.*, 2022:2). This property highlights the potential of aromatic nitro compounds as effective agents against numerous pathogens.

Based on these observations, the primary objectives of this project are to synthesise novel 4,7-disubstituted quinolines incorporating aminochalcone and aryl nitro moieties, and assess their antibacterial and antiprotozoal activities *in vitro*. By exploring the potential of these multifunctional compounds, we hope to contribute to the fight against antimicrobial-resistant pathogens and pave the way for the development of novel therapeutic agents.

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CHAPTER 3: ARTICLE FOR SUBMISSION

Chapter 3 is a manuscript to be submitted to *Molecular Diversity* for publication consideration. This manuscript is structured into various sections, including an abstract, graphical abstract, introduction, results and discussion, materials and methods, and a conclusion.

SYNTHESIS, *IN VITRO* ANTIBACTERIAL AND ANTIPROTOZOAN EVALUATION OF 4,7-DISUBSTITUTED QUINOLINES

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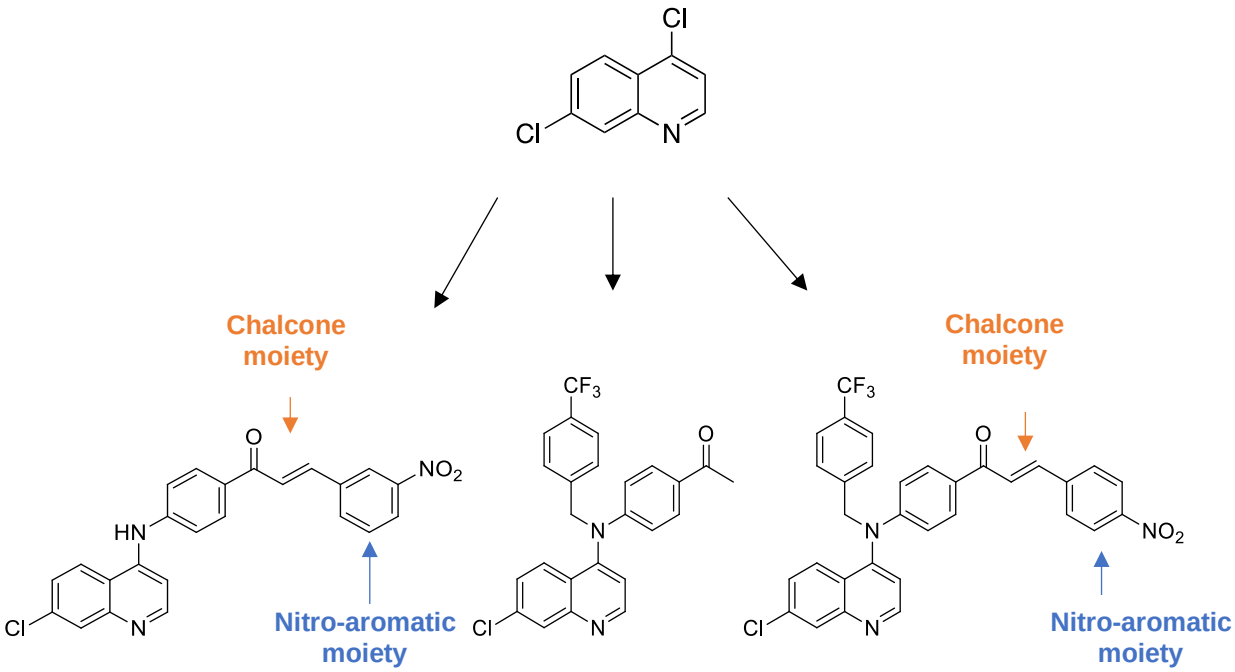
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ABSTRACT

Herein we synthesised several quinoline derivatives incorporating aryl nitro and amino-chalcone moieties. The synthesised compounds were evaluated *in vitro* against a broad panel of trypanosomatid protozoan parasites responsible for sleeping sickness (*Trypanosoma brucei rhodesiense*), nagana (*Trypanosoma brucei brucei*), Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania infantum*). Several of the compounds demonstrated significant antiprotozoal activity. Specifically, **Compounds 2c**, **2d**, and **4i** displayed sub-micromolar activity against *T.b. rhodesiense* with minimum inhibitory concentration (IC_{50}) values of 0.68 µM, 0.46 µM, and 0.14 µM, respectively, and with a high selectivity relative to human lung fibroblasts and mouse primary macrophages (~100-fold). **Compounds 2d** and **4i** also showed considerable activity against *T.b. brucei* with IC_{50} values of 0.29 µM and 0.28 µM, respectively. Some antileishmanial activity was recorded as well with IC_{50} values of 8.83 µM and 6.35 µM for **Compounds 3 d** and **4i**, respectively.

Keywords: Quinolines, Nitro aromatics, Aminochalcones, Protozoans, Trypanosomiasis, Leishmaniasis.

GRAPHICAL ABSTRACT



2d

T.b. brucei IC_{50} 0.29 µM

T.b. rhodesiense IC_{50} 0.46 µM

MRC-5 IC_{50} > 64 µM

PMM IC_{50} > 64 µM

3d

L. infantum IC_{50} 8,83 µM

MRC-5 CC_{50} > 64 µM

PMM CC_{50} > 64 µM

4i

T.b. brucei IC_{50} 0.28 µM

T.b. rhodesiense IC_{50} 0.14 µM

L. infantum IC_{50} 6.35 µM

MRC-5 CC_{50} 25.02 µM

PMM CC_{50} > 64 µM

3.1 Introduction

Trypanosomatids are responsible for a range of neglected parasitic diseases, including human and animal African trypanosomiasis, Chagas disease, and leishmaniasis (Horn, 2022:1). *Trypanosoma brucei gambiense* and *T. brucei rhodesiense* are responsible for human African trypanosomiasis (HAT), commonly referred to as sleeping sickness. HAT occurs in sub-Saharan Africa and its prevalence is largely influenced by the presence of the tsetse fly vector and the implementation of control measures. Unfortunately, control measures tend to be disregarded during political unrest, leading to the re-emergence of the disease (Brun *et al.*, 2010:148).

T.b. gambiense accounts for 98% of HAT cases and is primarily found West and Central Africa, while *T.b. rhodesiense* causes 2% of HAT cases and is mainly prevalent in East and Southern Africa (Tanekou *et al.*, 2022:260). *T.b. rhodesiense* infections are more severe, leading to acute infections and potential death within a few months of initiation. *T.b. gambiense*, on the other hand, causes chronic infections lasting several years (Correia *et al.*, 2021:1). Symptoms for both species vary according to the disease stage, with the first stage exhibiting intermittent fever, headache, pruritus, endocrine dysfunction, lymphadenopathy, edema, and hepatosplenomegaly. During the second stage, when the parasites infiltrate the central nervous system, patients may experience neuropsychiatric disorders such as sleep disturbances, tremors of the fingers and hands, motor weakness, speech disorders, attention deficit, confusion, and emotional lability (Büscher *et al.*, 2017:4-5). If left untreated, this disease can be fatal, particularly if the parasites have already crossed the blood-brain barrier (Berninger *et al.*, 2017:3).

African animal trypanosomiasis, commonly referred to as nagana, is a parasitic disease affecting livestock in sub-Saharan Africa. The causative agents of this disease are *T. congolense*, *T. vivax*, and *T.b. brucei* (Morrison *et al.*, 2016:1). Infection results in debilitating symptoms including anaemia and cachexia that may eventually result in death. Livestock productivity is severely hindered by nagana, which consequently poses a serious risk to food security, and economic growth in sub-Saharan Africa (Holt *et al.*, 2016:1).

T. cruzi, transmitted by kissing bugs and other horizontal and vertical routes (such as blood transfusion, contaminated food and drinks, and congenital transmission), causes Chagas disease, which is mainly prevalent in Latin America. This vector-borne illness affects millions of people and can remain asymptomatic over a prolonged period of time, eventually resulting

in a chronic disease with severe cardiac and gastrointestinal complications (World Health Organization, 2020:86).

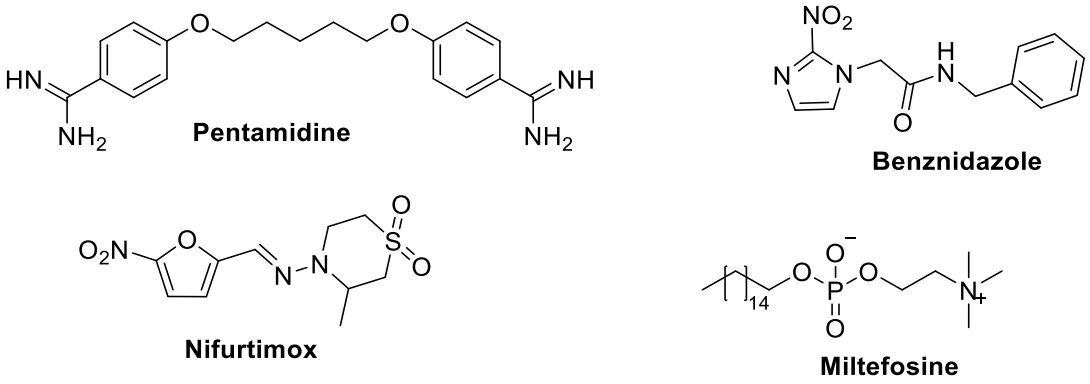


***Figure 3.1**: Drugs used to treat trypanosomatid diseases*

Leishmaniasis is a family of related parasitic diseases caused by hemoflagellate *Leishmania* spp. that are transmitted to humans primarily through the bites of infected female phlebotomine sand flies (Sundar *et al.*, 2019:1). Clinical manifestations vary from a cutaneous, to a destructive and stigmatising mucocutaneous, to a lethal visceral form of the disease (kala-azar) and post-kala-azar dermal leishmaniasis. Leishmaniasis is endemic in at least 88 countries located in different regions of the world and disproportionately affects the poorest populations (Singh & Sivakumar, 2004:320).

Although there have been promising developments for these trypanosomatid diseases (De Rycker *et al.*, 2023:35), several challenges remain and many of the available treatment options (**Figure 3.1**) are plagued by issues such as the need for parenteral administration over prolonged periods of time, toxicity or resistance affecting efficacy, and patient compliance with the treatment regimens. These limitations necessitate the development of newer and more effective therapeutic interventions (Sundar *et al.*, 2019:1).

The ESKAPE pathogens, compromising *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., are notorious for their multidrug resistance and strong virulence (Rice, 2010:1). These pathogens have emerged as major contributors to healthcare-associated infections, posing a significant challenge in clinical settings (Boucher *et al.*, 2009:1-2). The availability of effective drugs against ESKAPE pathogens is declining each year, leading to reliance on last resort antibiotics (Mulani *et al.*, 2019:1). Moreover, these antibiotics are limited in their efficacy and face challenges such as toxicity and the emergence of drug-resistant

strains (Boucher *et al.*, 2009:2). As a result, there is an urgent need to develop novel therapeutic strategies and identify new antibiotics with unique mechanisms of action to combat the ESKAPE bacteria effectively (Mulani *et al.*, 2019:2).

Quinoline is an extensively studied class of heterocyclic compounds. Quinoline derivatives are widely distributed in nature and have garnered great interest in medicinal chemistry due to their ability to serve as versatile scaffolds for the development of compounds with diverse biological activities (Harry *et al.*, 2020:1). Numerous quinoline derivatives have shown efficacy *in vitro* against neglected diseases such as sleeping sickness (Nefertiti *et al.*, 2018:2), leishmaniasis (Yousuf *et al.*, 2016:1), and certain ESKAPE pathogen related diseases (Sun *et al.*, 2017:1). Like quinolines, chalcones exhibit a wide range of biological activities, including immunosuppressive, anti-ulcer, antiviral, antiprotozoal (Angula *et al.*, 2022; Beteck *et al.*, 2019:1), and anticancer activities (Yerragunta *et al.*, 2013). Among the various chalcone derivatives, aminochalcones have potential as antileishmanial (Roussaki *et al.*, 2013:1), anti-trypanosomal (Qiao *et al.*, 2012:3553), and antibacterial agents (Nielsen *et al.*, 2005:2671).

Nitroaromatic chemicals have been found to exhibit inhibitory effects against various pathogenic organisms, including bacteria (Du Preez *et al.*, 2023:718), fungi (Hart *et al.*, 2022), viruses, and protozoans. Typically, they function as pro-drugs, wherein the nitro group is bio-reduced to generate reactive nitrogen species that are responsible for the anti-infective properties of this compound class (Hurdle *et al.*, 2008:1038).

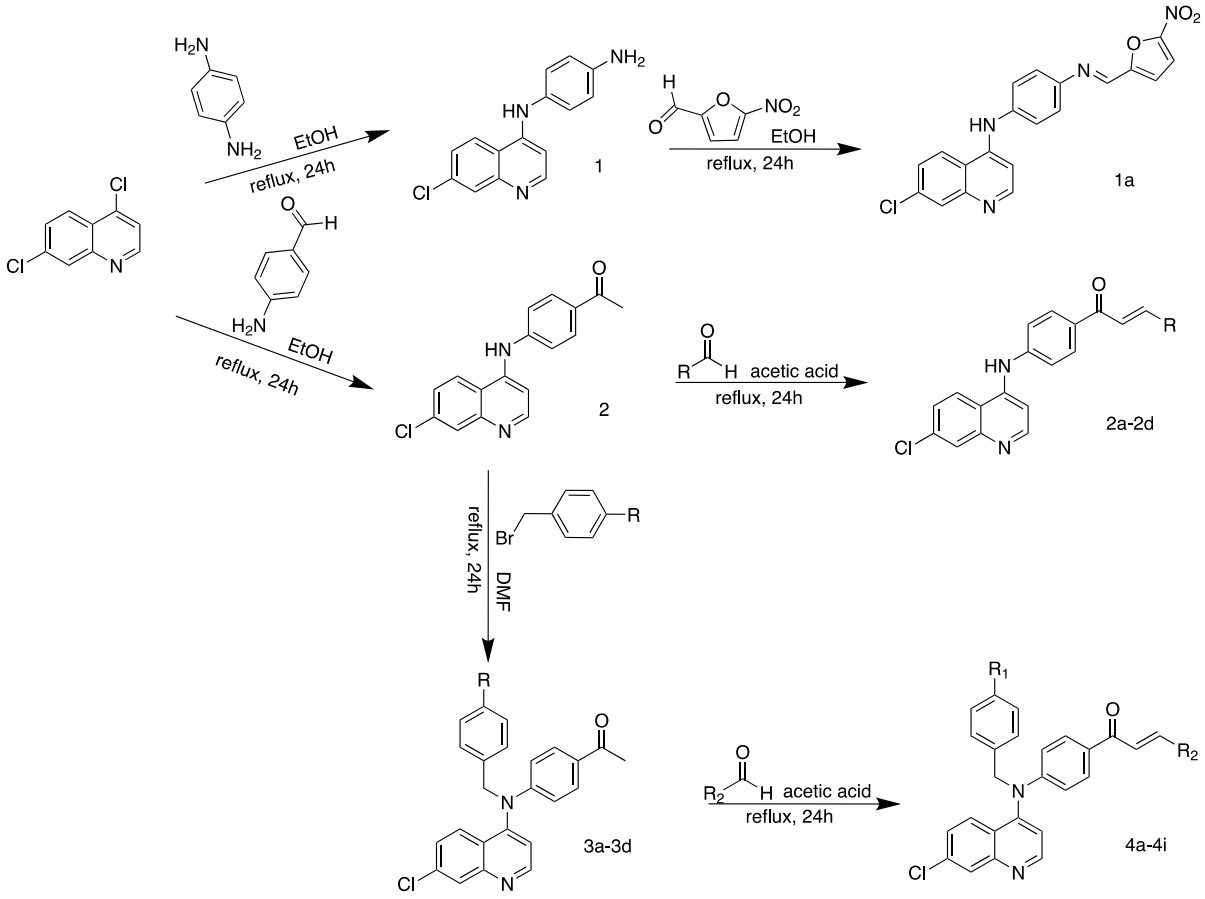
In this study, we conceptualised and synthesised novel compounds incorporating the quinolinyl, aminochalconyl, and the aryl nitro moieties. The compounds were screened against several parasitic antiprotozoans, and they generally showed broad-spectrum antiprotozoal activities.

3.2 Results and discussion

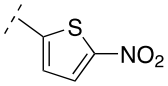
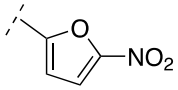
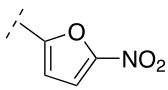
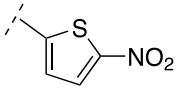
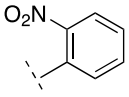
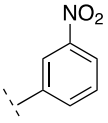
3.2.1 Chemistry and structure characterisation

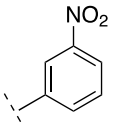
The synthesis of target compounds was achieved using synthetic procedures depicted in **Error! Reference source not found.**. The starting material, 4,7-dichloroquinoline, was subjected to a nucleophilic substitution reaction with appropriate anilines to generate **Intermediates 1** and **2**. Thereafter, **Intermediate 1** was subjected to a Schiff base condensation reaction with 5-nitrofuran-2-carbaldehyde to yield **Compound 1a** with a yield of 59%. **Intermediate 2** was subjected to aldol condensation reaction with various aldehydes,

affording **Compounds 2a–2d** in yields ranging from 30% to 100%. To synthesise **Compounds 3a–3d**, **Intermediate 2** underwent *N*-alkylation with appropriate benzyl halide. This reaction afforded the desired compounds with yields varying from 10% to 80%. Additionally, **Compounds 3a–3d** were subjected to aldol condensation with various aldehydes in acetic acid, resulting in the formation of **Compounds 4a–4i** with yields ranging from 5% to 50%.

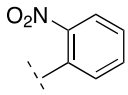


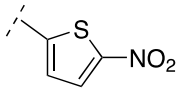
***Scheme 1**: Synthesis of target compounds*

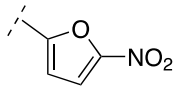
*percentage yield	**R**	
2a: 92%*, 	**3a**: 71%*, CH_3	**4a**: 45%*, R_1: CH_3, R_2: 
2b: 88%*, 	**3b**: 32%*, H	**4b**: 8%*, R_1: CH_3, R_2: 
2c: 32%*, 	**3c**: 18%*, Br	**4c**: 14%*, R_1: CH_3, R_2: 

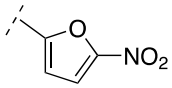
2d: 62%*, 

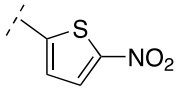
3d: 39%*, CF_3

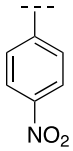
4d: 26%*, R_1: CH_3, R_2: 

4e: 27%*, R_1: H, R_2: 

4f: 22%*, R_1: Br, R_2: 

4g: 35%*, R_1: CF_3, R_2: 

4h: 21%*, R_1: CF_3, R_2: 

4i: 20%*, R_1: CF_3, R_2: 

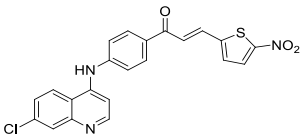
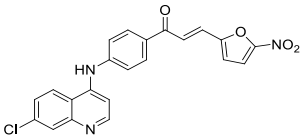
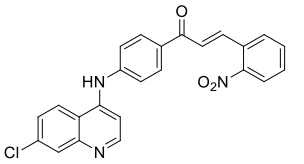
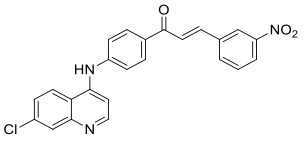
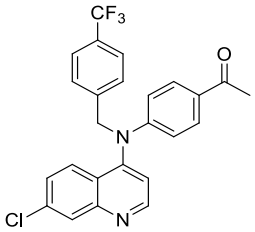
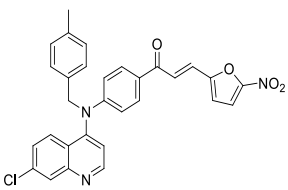
Proton and carbon-13 NMR were used to characterise all target compounds. The appearance or absence/disappearance of certain peaks were used to confirm successful synthetic transformation(s). The absence of a singlet peak at around 5 ppm (attributable to the $-NH_2$ moiety of **Compound 1**) on the proton NMR of **Compound 1a** implies that the Schiff base condensation was successful. The absence of the peaks at around 2.4 ppm and 30 ppm (attributable to the $-CH_3$ moiety of **Compounds 2** and **3a–3d**) in the ^{1}H and ^{13}C of **Compounds 2a–2d** and **4a–4i**, respectively, indicate that the aldol condensation reaction was successful. Due to the low solubility of some compounds in deuterated dimethyl sulfoxide (DMSO)-d_6, all carbon peaks could not be identified. Several target compounds also included small amounts of solvent impurities, such as ethanol, in their NMR spectra. The target compounds were vacuum-dried for 72 hours to get rid of solvent contaminants before biological testing. High-resolution mass spectrometry (HRMS) was used to verify the molecular mass of each compound.

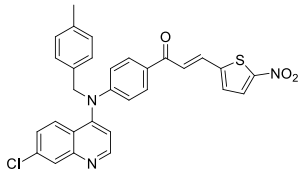
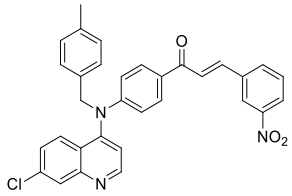
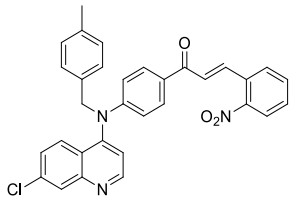
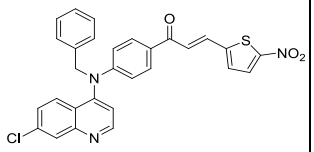
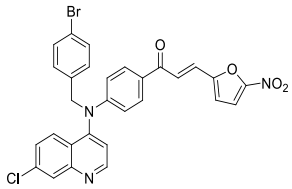
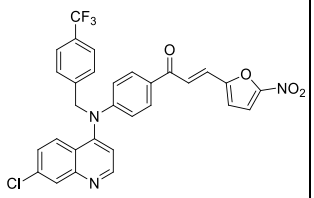
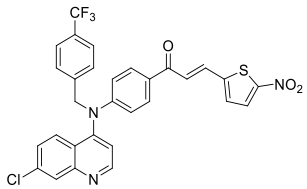
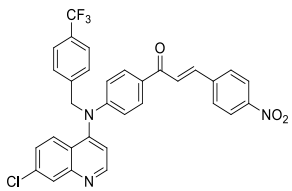
3.2.2 Antiprotozoal activity

The compounds in this study were evaluated for antiprotozoal activities against the causal agents of Chagas disease, leishmaniasis, HAT, and nagana. Benznidazole, miltefosine, and suramin were included as reference drugs. The compounds were also evaluated for overt toxicity using MRC-5 human fibroblasts and primary mouse macrophages. Tamoxifen was included as a reference in the toxicity assays.

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***Table 3.1**: Structures, antiprotozoal activities, and cytotoxicity of Compounds 2a–2d, 3d, and 4a–4i*

ID	Structure	MRC-5 CC_{50}(μM)[a]	*T. cruzi*[b] IC_{50}(μM)[a]	*L. inf*[c] IC_{50}(μM)[a]	*T. bruc*[d] IC_{50}(μM)[a]	*T. rhod*[e] IC_{50}(μM)[a]	PMM[f] CC_{50}(μM)[a]
2a		> 64	8.12	36.76	8.90	7.21	> 64
2b		5.22	1.95	26.49	1.93	1.93	> 64
2c		> 64	32.90	12.70	2.52	0.68	> 64
2d		> 64	31.74	22.63	0.29	0.46	> 64
3d		> 64	> 64	8.83	> 64	> 64	> 64
4a		1.41	0.52	1.59	0.46	0.50	32

ID	Structure	MRC-5 CC_{50}(μM)[a]	*T. cruzi*[b] IC_{50}(μM)[a]	*L. inf*[c] IC_{50}(μM)[a]	*T. bruc*[d] IC_{50}(μM)[a]	*T. rhod*[e] IC_{50}(μM)[a]	PMM[f] CC_{50}(μM)[a]
4b		0.94	0.20	10.08	0.40	0.13	> 64
4c		6.62	1.69	0.50	0.21	0.42	8
4d		6.96	2.00	1.39	0.32	0.47	2
4e		2.36	0.37	2.16	0.47	0.50	> 64
4f		1.15	0.53	1.78	0.50	0.49	8
4g		0.23	0.13	0.63	0.11	0.13	8
4h		1.31	0.46	1.49	0.47	0.50	32
4i		25.02	2.10	6.35	0.28	0.14	> 64

ID	Structure	MRC-5 CC_{50}(µM)[a]	*T. cruzi*[b] IC_{50}(µM)[a]	*L. inf*[c] IC_{50}(µM)[a]	*T. bruc*[d] IC_{50}(µM)[a]	*T. rhod*[e] IC_{50}(µM)[a]	PMM[f] CC_{50}(µM)[a]
TF		10.27					
BZN			1.47				
MF				6.35			
SUR					0.05	0.04	

[a]Inhibitory concentration; [b]*Trypanosoma cruzi*; [c]*Leishmania infantum*; [d]*Trypanosoma brucei*; [e] *Trypanosoma brucei rhodesiense*; [f]primary mouse microphage (PMM). **Reference drugs:** Tamoxifen (TF), benznidazole (BZN), miltefosine (MF), and suramin (SUR).

As indicated in **Table 3.1**, most compounds in this study demonstrated potent and broad-spectrum antiprotozoal activities. However, several of these compounds also exhibited severe cytotoxicity towards both MRC-5 human fibroblasts and primary mouse macrophages. Among the active compounds, **Compounds 2c**, **2d**, and **4i** notably demonstrated potent antiprotozoal activity, while exhibiting little to no cytotoxicity with selectivity indices approaching or surpassing 100 for the African trypanosome species.

Compound 2c and **2d** demonstrated sub-micromolar IC_{50} values against the causal agents of nagana and HAT. **Compound 2c** had an IC_{50} value of 0.68 µM against *T.b. rhodesiense*, while **Compound 2d** showed IC_{50} values of 0.29 µM and 0.46 µM against *T.b. brucei* and *T.b. rhodesiense*, respectively. Both compounds contained a nitro (NO_2) functional group, with the only difference being the position of the nitro moiety. **Compound 2d**, which had the nitro group at the third position, showed higher activity against both *T.b. brucei* and *T.b. rhodesiense* than **Compound 2c**, which had the nitro group at the second position. This suggests that the position of the nitro group on the aromatic ring plays a critical role in determining the potency of the compounds against these protozoan parasites.

Compound 3d exhibited some antileishmanial activity, with an IC_{50} value of 8.83 µM against *L. infantum*. This *in vitro* activity profile is comparable to that exhibited by the antileishmanial drug miltefosine (IC_{50}: 6.35 µM).

Compound 4i had potent IC_{50} values of 0.28 µM against *T.b. brucei*, 0.14 µM against *T.b. rhodesiense*, 2.1 µM against *T. cruzi*, and 6.5 µM against *L. infantum*. The activity profile of this compound against *T. cruzi* and *L. infantum* is comparable to that recorded for the reference drugs. **Compound 4i** does have broad-spectrum antiprotozoal activities and is not

toxic to primary mouse microphages and MRC-5 cells at the concentration required to achieve antiprotozoal activities.

Generally, it could be observed that compounds containing six membered aryl nitro units (**Compounds 2c**–**2d**) possessed better intrinsic antiprotozoal activities than their corresponding analogues bearing five membered aryl nitro units (**2a**–**2b**). Moreover, converting the secondary amine at position 4 of the quinoline ring to a tertiary amine increased antiprotozoal activities and cell toxicity. For example, **Compound 2d** showed higher IC_{50} values against all the targets than its tertiary amine derivative, **Compound 4c**. However, **Compounds 3d** and **4i** were not toxic to MRC-5 and primary mouse macrophages. With regard to compounds containing five membered aryl nitro units, it could be observed that the nitrothiopnenyl-bearing compounds showed lower cell toxicity potential than the nitrofuranyl-bearing compounds. For example, **Compound 2a** was non-toxic for MRC-5 (IC_{50}: > 64 µM), while **Compound 2b** showed low micromolar activity of 5 µM against MRC-5.

3.2.3 Antibacterial and antifungal activities

All compounds were tested for antibacterial activities against *S. aureus* and methicillin-resistant *S. aureus* (MRSA) (ATCC 43300), *K. pneumoniae* (ATCC 700603), *A. baumannii* (ATCC 19606), and *P. aeruginosa* (ATCC 27853). However, none of the compounds had minimum inhibitory concentration $(MIC)_{50}$ values of < 32 µM and were generally not active against these bacteria.

All compounds were tested for antifungal activity against *Candida albicans* (ATCC 90028). However, none of the compounds had MIC_{50} values of < 32 µM.

3.3 Materials and methods

3.3.1 General methods

Reagents and solvents were purchased from chemical vendors such as Sigma-Aldrich, Ace, Rochelle, Ambeed, and were utilised as supplied. Merck $60F_{254}$ silica gel plates supported by 0.20 mm thick aluminium sheets were used to track reactions progression. Samples were dissolved in deuterated DMSO-d_6, and a Bruker Advance III 600 spectrophotometer was used to record their ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra at 600 MHz and 151 MHz, respectively. MestResNova version 10 was used for the analysis of NMR data. Chemical shifts are given in parts per million (ppm) and were compared with solvent peaks (DMSO-d_6: 2.50 and 39.52 ppm for ^{1}H and ^{13}C-NMR, respectively).

Spin multiplicities are denoted by the letters s for singlet, d for doublet, t for triplets, m for multiplet, dt for doublet of triplets, td for triplet of doublets, and dd for doublet of doublets. Coupling constants (*J*) are given in Hz. HRMS results were captured using a Bruker micrOTOF-Q II mass spectrometer in positive ion mode using atmospheric pressure chemical ionisation (APCI). With a capillary voltage of 4,500 V, end plate offset of −500 V, 1.8 bar nebulisers, and collision cell radio frequency voltage of 150 Vpp, a complete scan from 50 m/z to 1,600 m/z was performed.

High-performance liquid chromatography (HPLC) analysis utilising an Agilent 1200 series HPLC system outfitted with a quaternary pump and an Agilent 1200 series diode array detector was used to ascertain the purity of the compound. The mobile phase was initially composed of 30% acetonitrile and 70% Milli-Q water at a flow rate of 1 mL/min, and a Venusil XBP C18 column (4.60 150 mm, 5 m) was employed for separation. The substances were dissolved in acetonitrile of HPLC grade, and 10 µL of the mixture was injected. The samples were examined at 280 nm and 400 nm wavelengths. Each HPLC run lasted 13 minutes with an equilibration time of five minutes in between. A Bruker ALPHA FTIR Routine spectrometer was used to perform Fourier-transform infrared spectroscopy (FTIR).

3.3.2 Synthesis of target compounds

3.3.2.1 Synthesis of Compound 1a

In a round bottom flask equipped with a reflux condenser, 1.0 g (5 mmoles) of 4,7-dichloroquinoline was heated under reflux for 24 hours with (1.2 equivalents, 6.1 mmoles) *p*-phenylenediamine in 20 mL of ethanol as the solvent. The reaction progression was monitored by thin-layer chromatography (TLC). After completion of the reaction, a solid precipitate formed. The reaction mixture was allowed to cool to room temperature and filtered. The resulting solid was dried to yield **Intermediate 1**.

Next, 0.5 g (1.9 mmoles) of **Intermediate 1** was added to a round bottom flask with (1.5 equivalents, 2.8 mmoles) 5-nitro-2-furaldehyde. The reaction was carried out in 15 mL of ethanol with 0.5 mL of acetic acid as the catalyst. The mixture was refluxed for 24 hours and monitored using TLC. Upon completion of the reaction, excess aldehyde was removed by hot filtration. The solid residue was dried to afford **Compound 1a**.

3.3.2.2 Synthesis of Compounds 2a–2d and 4a–4i

A round bottom flask was charged with 0.3 g (1 mmoles) of **Intermediate 2**, 1.5 equivalents of appropriate aldehydes, and 10–15 mL of acetic acid as a solvent, along with catalytic amounts of sulfuric acid. The reaction was refluxed for 24 hours and monitored with TLC. After completion, the reaction was cooled to room temperature and added to ice, whereafter it was stirred for approximately 1 hour. It was then filtered and washed with water to afford target **Compounds 2a–2d**. This same procedure was used to generate **Compounds 4a–4d**, starting with **Intermediate 3a–3d**.

3.3.2.3 Synthesis of Compounds 3a–3d

A round bottom flask was charged with 1.0 g (3.4 mmoles) of **Intermediate 2**, 0.9 g (2 equivalents, 6.7 mmoles) of potassium carbonate (K_2CO_3), 1.5 equivalents of appropriately substituted benzyl bromide, and 20 mL of *N,N*-dimethylformamide as a solvent. The mixture was heated under reflux and monitored by TLC for 24 hours. After completion, the reaction mixture was cooled to room temperature and washed with 20 mL of deionised water. The resulting residue afforded **Compound 3a**.

(E)-7-chloro-N-(4-(((5-nitrofuran-2-yl)methylene)amino)phenyl)quinolin-4-amine (**1a**)

Orange powder; 59% yield; m.p.: 238–240°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.91 (d, J = 9.1 Hz, 1H), 8.73 (s, 1H), 8.56 (d, J = 6.9 Hz, 1H), 8.19 (d, J = 2.1 Hz, 1H), 7.90–7.81 (m, 2H), 7.59–7.40 (m, 4H), 7.49 (d, J = 3.9 Hz, 1H), 6.91 (d, J = 6.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 154.81, 153.15, 152.92, 148.89, 148.78, 144.31, 140.03, 138.71, 136.92, 127.78, 127.01, 126.66, 126.46, 123.45, 120.08, 118.88, 116.70, 114.66, 101.14. HRMS-APCI *m/z* calcd for $C_{20}H_{14}ClN_4O_3$ $[M+H]^+$, 393.0768, found 393.0749.

(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**2a**)

Green powder; 92% yield; m.p.: 189°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (d, J = 5.9 Hz, 1H), 8.55 (d, J = 9.1 Hz, 1H), 8.28 (d, J = 8.5 Hz, 2H), 8.18 (d, J = 4.3 Hz, 1H), 8.05–8.00 (m, 2H), 7.91 (d, J = 15.5 Hz, 1H), 7.80 (dd, J = 15.1, 6.9 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 6.0 Hz, 1H). HRMS-APCI *m/z* calcd for $C_{22}H_{15}ClN_3O_3S$ $[M+H]^+$, 436.0524, found 436.0517.

(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**2b**)

Brown powder; 88% yield; m.p.: 258–260°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 (d, J = 5.9 Hz, 1H), 8.56 (dd, J = 9.3, 9.1 Hz, 1H), 8.22 (d, J = 8.4 Hz, 2H), 8.01 (s, 1H), 7.91 (d, J = 5.5 Hz, 1H), 7.83 (d, J = 3.8 Hz, 1H), 7.81–7.71 (m, 2H), 7.61–7.51 (m, 2H), 7.45 (d, J = 3.9 Hz, 1H), 7.27 (d, J = 6.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.38, 130.64, 128.26, 125.00, 121.22, 117.69, 114.88. HRMS-APCI *m/z* calcd for $C_{22}H_{15}ClN_3O_4$ $[M+H]^+$, 420.0765, found 420.0746.

(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**2c**)

Yellow powder; 32% yield; m.p.: 152–154°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 (d, J = 6.0 Hz, 1H), 8.55 (d, J = 9.0 Hz, 1H), 8.28 (d, J = 8.3 Hz, 2H), 8.10 (d, J = 8.2 Hz, 1H), 8.02–7.93 (m, 3H), 7.85 (t, J = 7.6 Hz, 1H), 7.77 (dd, J = 9.0, 2.2 Hz, 1H), 7.71 (t, J = 7.8 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.25 (s, 1H), 5.75 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 187.32, 148.80, 148.43, 144.26, 138.09, 136.33, 133.75, 132.90, 131.05, 130.69, 129.76, 129.47, 126.65, 126.34, 125.27, 124.70, 121.51, 118.07, 103.64. HRMS-APCI *m/z* calcd for $C_{24}H_{17}ClN_3O_3$ $[M+H]^+$, 430.0947, found 430.0953.

(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**2d**)

Green powder; 62% yield; m.p.: 262°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.81 (t, J = 2.0 Hz, 1H), 8.66 (d, J = 6.2 Hz, 1H), 8.60 (d, J = 9.0 Hz, 1H), 8.38–8.33 (m, 3H), 8.29 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.04 (d, J = 2.2 Hz, 1H), 7.89 (d, J = 15.6 Hz, 1H), 7.83–7.75 (m, 2H), 7.66–7.60 (m, 2H), 7.24 (d, J = 6.2 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 187.91, 148.93, 144.21, 141.72, 137.12, 135.63, 134.03, 131.16, 130.87, 127.34, 125.87, 125.14, 123.43, 122.54, 118.25, 103.66 HRMS-APCI *m/z* calcd for $C_{24}H_{17}ClN_3O_3$ $[M+H]^+$, 430.0954, found 430.0953.

1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)ethan-1-one (**3d**)

Yellow powder; 39% yield; m.p.: 219–220°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.41 (d, J = 8.6 Hz, 1H), 7.97–7.92 (m, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 8.0 Hz, 1H), 7.49–7.43 (m, 3H), 7.33 (dd, J = 8.7, 1.9 Hz, 1H), 6.99–6.94 (m, 2H), 5.93 (d, J = 7.9 Hz, 1H), 5.50 (s, 2H), 2.55 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 196.89, 157.97, 153.70, 142.85, 141.97, 140.32, 136.67, 131.36, 130.47, 128.82, 128.61, 128.14, 127.59, 126.28, 126.25, 125.50,

124.31, 123.85, 123.70, 121.52, 116.12, 100.86, 54.13, 26.86. HRMS-APCI *m/z* calcd for $C_{25}H_{19}ClF_3N_2O$ $[M+H]^+$, 455.1130, found 455.1133.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**4a**)

Brown powder; 45% yield; m.p.: 80–83°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (dd, J = 11.4, 7.2 Hz, 1H), 8.43–8.34 (m, 1H), 8.24 (d, J = 8.3 Hz, 2H), 7.99–7.87 (m, 2H), 7.82 (dd, J = 10.6, 4.3 Hz, 1H), 7.72–7.56 (m, 2H), 7.53–7.40 (m, 3H), 7.19–6.87 (m, 4H), 6.74 (d, J = 51.2 Hz, 1H), 5.67 (s, 2H), 2.27 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.87, 153.35, 151.95, 139.36, 137.45, 130.56, 129.50, 128.29, 126.62, 125.06, 117.66, 114.84, 56.01, 39.94, 39.80, 39.66, 39.52, 39.38, 39.24, 39.10, 20.65 HRMS-APCI *m/z* calcd for $C_{30}H_{23}ClN_3O_4$ $[M+H]^+$, 524.1354, found 524.1372.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**4b**)

Yellow powder; 8% yield; m.p.: 238–240°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (d, J = 8.8 Hz, 1H), 8.37 (d, J = 27.6 Hz, 1H), 8.28 (d, J = 8.5 Hz, 2H), 8.17 (t, J = 5.5 Hz, 1H), 8.03 (d, J = 5.5 Hz, 1H), 7.95–7.88 (m, 2H), 7.81 (d, J = 4.4 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 6.8 Hz, 2H), 7.19 (s, 4H), 6.75–6.55 (m, 1H), 5.65 (s, 2H), 2.27 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.83, 153.84, 151.53, 146.78, 139.37, 137.43, 134.37, 132.48, 130.97, 130.65, 130.58, 129.49, 127.12, 126.61, 125.53, 123.16, 117.33, 101.10, 56.01, 18.54.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**4c**)

Yellow powder; 14% yield; m.p.: 152–154°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (dd, J = 6.7, 4.9 Hz, 1H), 8.62 (dd, J = 2.0, 7.0 Hz, 1H), 8.40–8.18 (m, 5H), 7.96–7.83 (m, 2H), 7.81–7.73 (m, 1H), 7.71–7.60 (m, 1H), 7.39 (s, 2H), 7.26–7.13 (m, 4H), 6.62 (d, J = 9.5 Hz, 1H), 5.63 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 187.51, 153.82, 148.43, 141.02, 139.42, 137.38, 136.68, 135.08, 130.65, 130.36, 129.49, 127.15, 126.59, 124.78, 124.59, 122.94, 101.00, 55.06, 39.10, 20.65 HRMS-APCI *m/z* calcd for $C_{32}H_{25}ClN_3O_3$ $[M+H]^+$, 534.1553, found 534.1579.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**4d**)

Brown powder; 26% yield; m.p.: 141–144°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.70 (d, J = 8.9 Hz, 1H), 8.37–8.31 (m, 3H), 8.24 (dd, J = 7.9, 1.4 Hz, 1H), 8.12 (dd, J = 8.2, 1.3 Hz, 1H), 8.08–7.96 (m, 3H), 7.86 (td, J = 7.6, 1.3 Hz, 1H), 7.77 (s, 1H), 7.76–7.72 (m, 3H), 7.59–7.51 (m, 4H), 7.20 (s, 1H), 5.76 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 188.14, 154.49, 149.31, 139.73, 138.79, 138.05, 134.25, 132.73, 131.57, 131.14, 130.23, 130.01, 129.99, 127.52, 127.18, 126.84, 125.20, 124.19, 101.88, 51.61, 21.14 HRMS-APCI *m/z* calcd for $C_{32}H_{25}CLN_3O_3$ $[M+H]^+$, 534.1570, found 534.1579.

(E)-1-(4-(benzyl(7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**4e**)

Brown powder; 27% yield; m.p.: 295°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 (d, J = 7.4 Hz, 1H), 8.81 (d, J = 9.0 Hz, 1H), 8.64 (dd, J = 22.5, 7.7 Hz, 1H), 8.38 (d, J = 8.3 Hz, 2H), 8.34–8.24 (m, 2H), 8.19 (dd, J = 6.9, 4.3 Hz, 1H), 8.11–7.89 (m, 4H), 7.85 (d, J = 7.9 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.43–7.20 (m, 3H), 5.98 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 146.52, 130.62, 130.57, 129.01, 128.36, 126.72, 125.26, 124.64.

(E)-1-(4-((4-bromobenzyl)(7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**4f**)

Orange powder; 22% yield; m.p.: 248–249°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.51 (d, J = 8.8 Hz, 1H), 8.17– 8.09 (m, 4H), 7.99 (d, J = 6.0 Hz, 1H), 7.92 (dd, J = 5.6, 13.0 Hz, 1H), 7.83 (dd, J = 4.0, 2.3 Hz, 1H), 7.68 (s, 1H), 7.64 (d, J = 2.1 Hz, 1H), 7.54–7.43 (m, 4H), 7.26–7.19 (m, 4H), 5.52 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.95, 186.51, 154.08, 153.98, 152.40, 140.07, 136.06, 132.30, 131.15, 131.07, 129.21, 128.41, 127.94, 126.19, 125.76, 125.64, 125.27, 122.77, 121.48, 119.49, 117.88, 115.35, 101.21, 40.53.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)-3-(5-nitrofuran-2-yl) prop-2-en-1-one (**4g**)

Brown powder; 35% yield; m.p.: 187–190°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.50 (d, J = 8.7 Hz, 1H), 8.18 (d, J = 8.2 Hz, 2H), 7.94 (d, J = 15.6 Hz, 2H), 7.83 (d, J = 3.9 Hz, 1H), 7.76 (d, J = 8.0 Hz, 2H), 7.61 (t, J = 13.7 Hz, 2H), 7.46 (t, J = 5.8 Hz, 4H), 7.18 (d, J = 8.0 Hz, 2H), 6.22 (s, 1H), 5.62 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 186.91, 154.01, 152.39,

141.60, 140.19, 131.07, 128.74, 128.33, 128.03, 127.66, 126.29, 126.26, 125.80, 125.47, 123.67, 122.55, 117.81, 115.32, 101.22, 49.05. HRMS-APCI *m/z* calcd for $C_{30}H_{20}ClF_3N_3O_4$ $[M+H]^+$, 578.1083, found 578.1089.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**4h**)

Brown powder; 21% yield; m.p.: 238–240°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (d, J = 9.0 Hz, 1H), 8.44 (d, J = 9.3 Hz, 1H), 8.32 (d, J = 8.2 Hz, 2H), 8.19 (d, J = 4.3 Hz, 1H), 8.04 (s, 1H), 7.93 (d, J = 15.4 Hz, 1H), 7.83 (d, J = 4.3 Hz, 1H), 7.77– 7.69 (m, 4H), 7.49–7.45 (m, 4H), 7.40 (s, 1H), 5.88 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 187.42, 154.54, 152.08, 147.23, 139.86, 135.01, 131.54, 131.17, 131.09, 128.98, 127.87, 127.68, 126.34, 126.31, 125.98, 40.43. HRMS-APCI *m/z* calcd for $C_{30}H_{20}ClF_3N_3O_3S$ $[M+H]^+$, 594.0832, found 594.0861.

(E)-1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)-3-(4-nitrophenyl) prop-2-en-1-one (**4i**)

Yellow powder; 20% yield; m.p.: 160–162°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.73 (d, J = 8.8 Hz, 1H), 8.37 (d, J = 8.5 Hz, 2H), 8.32 (d, J = 8.5 Hz, 2H), 8.28–8.15 (m, 3H), 8.05 (s, 1H), 7.86–7.76 (m, 5H), 7.59–7.52 (m, 4H), 6.91 (s, 1H), 5.94 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 188.14, 154.69, 148.59, 141.68, 141.55, 139.78, 131.15, 130.38, 129.03, 127.91, 127.60, 126.44, 126.35, 126.33, 124.44, 55.53. HRMS-APCI *m/z* calcd for $C_{32}H_{22}ClF_3N_3O_3$ $[M+H]^+$, 588.1273, found 588.1296.

3.3.3 *In vitro* antiprotozoal evaluation

For the *L. infantum* assay, primary peritoneal mouse macrophages were infected with 4.5×10^5 parasites per well, and 3×10^4 macrophages were used as host cells. After two hours of infection, serial dilutions of samples were added to the cells. Thereafter, the cells were incubated for five days, after which the parasite burden was assessed microscopically by staining with a 10% Giemsa solution. The mean number of amastigotes (parasitic form) per macrophage was determined to evaluate the anti-*Leishmania* activity of the tested compounds.

For the *T. cruzi* assay, the Tulahuen CL_2, β-galactosidase strain (which is sensitive to nifurtimox) was deployed, and MRC-5_{SV2} (human lung fibroblast) was used as host cells. The

cells were infected with 4×10^4 parasites per well. Serial dilutions of test samples were added four hours after infection and incubated at 37°C, whereafter the substrate CPRG (chloro-phenolred ß-d-galactopyranoside) was added. The parasite burden was assessed by measuring the change in colour spectrophotometrically at 540 nm to determine the level of anti-*T. cruzi* activity of the tested compounds.

The drug susceptibility of *T. brucei* Squib 427 and *T.b. rhodesiense* STIB-90050 was assessed using resazurin assay. The parasites were cultured at a density of 1.5×10^4 parasites/well for *T. brucei* Squib 427 and 4×10^3 parasites per well for *T.b. rhodesiense*. Following incubation with varying concentrations of test samples, the parasites were further incubated for 72 hours. Thereafter, resazurin was added, and the cells were incubated for an additional 24 hours for *T. brucei* and six hours for *T.b. rhodesiense*. Fluorescence detection was employed to measure the susceptibility of the parasites to the tested compounds.

In each of the experiments, the growth of the parasites was compared with that of two control groups: the untreated but infected group (which showed 100% growth) and the non-infected group (which showed 0% growth). The results were expressed as the percentage reduction in parasite growth at different drug concentrations, and these values were used to generate dose-response curves and calculate the IC_{50} values (Mabille *et al.*, 2022:22).

3.3.4 *In vitro* antibacterial and antifungal evaluation

This paragraph describes the methodology used to determine the inhibitory activity of compounds against bacteria and *C. albicans*.

To begin, eight-fold serial dilutions of each sample were prepared in 384-well plates with a non-binding surface while ensuring that the final concentration of DMSO did not exceed 0.5%. Bacteria were cultured in cation-adjusted Mueller Hinton broth overnight at 37°C and then diluted 40-fold in fresh broth, followed by incubation at 37°C for 1.5–3 hours. Thereafter, the mid-log phase cultures were diluted, and the concentration was measured by OD_{600} before being added to each well of the compound-containing plates, resulting in a cell density of 5 × 105 CFU/mL and a volume of 50 µL. These plates were incubated at 37°C for 18 hours without shaking.

To determine the inhibition of bacterial growth, the absorbance at 600 nm (OD_{600}) was measured using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well using the negative control (media only) and positive

control (bacteria without inhibitors) on the same plate as references. The MIC, defined as the lowest concentration at which > 80% bacterial growth is inhibited, was determined following Clinical & Laboratory Standards Institute guidelines. Hits were classified as having a MIC ≤ 16 µg/mL in either replicate (n = 2).

For *C. albicans*, the fungus was cultured on yeast extract peptone dextrose agar at 30°C for three days. A suspension of 1–5 × 10^6 CFU/ml was prepared from five colonies, diluted, and added to each well of the compound-containing plates, resulting in a final cell density of 2.5 × 10^3 CFU/m and a volume of 50 µl. Thereafter, the plates were covered and incubated at 35°C for 36 hours without shaking. Growth inhibition was determined by measuring the absorbance at 630 nm (OD_{630}) after adding resazurin (0.001% final concentration) and incubating at 35°C for two hours. The absorbance was measured using a Biotek Multiflo Synergy HTX plate reader (Hart *et al.*, 2022).

3.3.5 *In vitro* cytotoxicity evaluation

To evaluate the cytotoxicity of compounds on MRC-5_{SV2} cells, the same methodology as described in a previous reference was followed. Briefly, 1.5 × 10^5 cells/mL were incubated with different concentrations of the test compounds at 37°C and 5% CO_2. Cell growth was compared with two control groups, namely untreated wells (which showed 100% cell growth) and medium-control wells (which showed 0% cell growth). After incubating for three days, cell viability was measured by adding 50 µL of resazurin per well and then incubating at 37°C for four hours, followed by measuring fluorescence (λ_{ex} 550 nm, λ_{em} 590 nm). The results were expressed as a percentage reduction in cell growth or viability compared with the control wells, and an IC_{50} value was determined accordingly (Mabille *et al.*, 2022:22).

3.4 Conclusion

In this project, we have successfully synthesised novel 4,7-disubstituted quinoline derivatives. The compounds were subjected to *in vitro* evaluation to determine their antiprotozoal activities. Notably, numerous compounds demonstrated significant and selective antiprotozoal activity with IC_{50} values of less than 5 µM. **Compounds 2c**, **2d**, and **4i** displayed notable activity against *T.b. rhodesiense* with IC_{50} values of 0.68 µM, 0.46 µM, and 0.14 µM, respectively. **Compounds 2d** and **4i** also showed potent activity against *T.b. brucei*, with IC_{50} values of 0.29 µM and 0.28 µM, respectively. **Compounds 3d** and **4i** exhibited antileishmanial activity with IC_{50} values of 8.83 µM and 6.35 µM, respectively.

Acknowledgements

This project was funded by MRC-SIR Grant awarded to RMB. The NMR spectra were recorded by Dr Otto Daniel and Dr Johan Jordaan of the SASOL Centre for Chemistry, North-West University. The authors are appreciative of the financial and infrastructural assistance rendered by North-West University, Potchefstroom campus. GC is a co-promotor of the Infla-Med Centre of Excellence (www.uantwerpen.be/infla-med) and participates in COST Action CA21111.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

The supplementary material contains ^{1}H, ^{13}C NMR spectra, and HRMS spectra of compounds reported in this study.

CHAPTER 3: REFERENCE LIST

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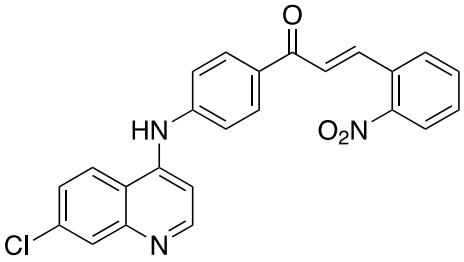
CHAPTER 4: SUMMARY AND CONCLUSION

The efficacies of once-reliable antimicrobial drugs are diminishing due to the emergence and spread of antimicrobial resistance pathogens (Yu *et al.*, 2017:3). Antimicrobial resistance causes increased mortality, morbidity, and associated healthcare cost (Kumar *et al.*, 2022). The alarming number of annual fatalities caused by antimicrobial resistance, already reaching 700 000 in Africa, highlights the urgent need for the development of novel antimicrobials to prevent further escalation (Kariuki *et al.*, 2022).

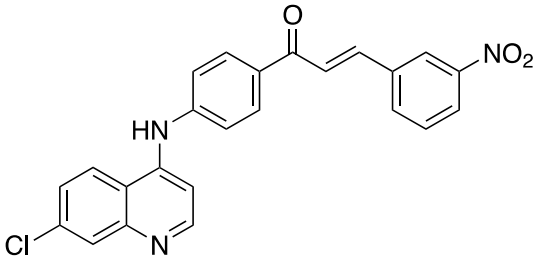
Neglected parasitic diseases, such as sleeping sickness (Malvy & Chappuis, 2011:986), nagana (Connor, 1994:380), and leishmaniasis (Roatt *et al.*, 2020), suffer from limited treatment options. These limited drugs are not ideal with poor drug-like properties, toxicity issues, and drug resistance (Roatt *et al.*, 2020). Therefore, there is a need to identify new molecules with activity against the causal agent(s) of these parasitic diseases

Quinolines have shown promising activity *in vitro* against the causal agents of neglected diseases such as leishmaniasis, sleeping sickness, and certain ESKAPE pathogens (Nefertiti *et al.*, 2018:1; Sun *et al.*, 2017:1; Yousuf *et al.*, 2016:1). Additionally, aminochalcones and aryl nitro compounds have exhibited strong antiprotozoal and antibacterial activities, making them attractive scaffolds for further investigation (Du Preez *et al.*, 2023:718; Irfan *et al.*, 2020:2).

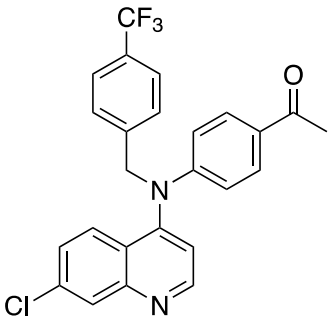
In this study, novel 4,7-disubstituted quinolines containing aminochalcone and aryl nitro moieties were synthesised and characterised successfully using analytical techniques (^{1}H NMR, ^{13}C NMR, and HRMS). These compounds were evaluated *in vitro* for their anti-protozoal, antibacterial, and antifungal activities. Notably, none of the compounds exhibited potent antibacterial activity against neither ESKAPE bacteria nor *C. albicans*. However, most target compounds exhibited potent antiprotozoal activity in the low to sub-micromolar range. Among the synthesised compounds, **Compounds 2c**, **2d**, **3d**, and **4i** (see **Figure 4.1**) emerged as the most notable, displaying potent antiprotozoal activity and low toxicity values.



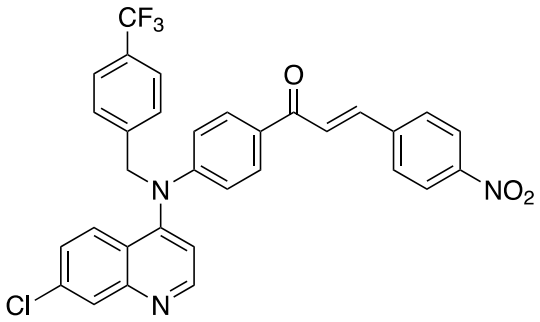
Compound 2c



Compound 2d



Compound 3d



Compound 4i

***Figure 4.1**: Target compounds exhibiting potent antiprotozoal activity*

Compounds 2c, **2d**, and **4i** demonstrated significant activity against *T.b. rhodesiense*, with IC_{50} values of 0.68 µM, 0.46 µM, and 0.14 µM, respectively. Moreover, **Compounds 2d** and **4i** exhibited potent activity against *T. brucei*, with IC_{50} values of 0.29 µM and 0.28 µM, respectively. In addition, **Compounds 3d** and **4i** displayed good antileishmanial activity, with IC_{50} values of 8.83 µM and 6.35 µM, respectively. These findings (**Table 4.1**) highlight the significant potential of these novel quinoline derivatives as promising candidates for the development of effective therapeutics against neglected diseases.

While several other target compounds demonstrated potent activity, their high toxicity levels warrant further investigation. Lowering the toxicity of these compounds is imperative to ensure their safe use as potential therapeutic agents. Therefore, future research should focus on optimising the toxicity profile of these compounds while maintaining their potent antiprotozoal activity. By addressing the issue of high toxicity, these compounds can potentially contribute to the development of safer and more effective treatments for neglected diseases.

***Table 4.1**: Target compounds with the most potent MIC values against evaluated protozoal pathogens*

Pathogen	**Compound**	**MIC**
Trypanosoma brucei rhodesiense	**2c, 2d, 4i**	≤ 0,68 µM
Trypanosoma brucei	**2d, 4i**	≤ 0,29 µM
Leishmania infantum	**3d, 4i**	≤ 8.83 µM

In summary, this study successfully identified novel 4,7-disubstituted quinoline derivatives with potent and broad-spectrum antiprotozoal activities.

CHAPTER 4: REFERENCE LIST

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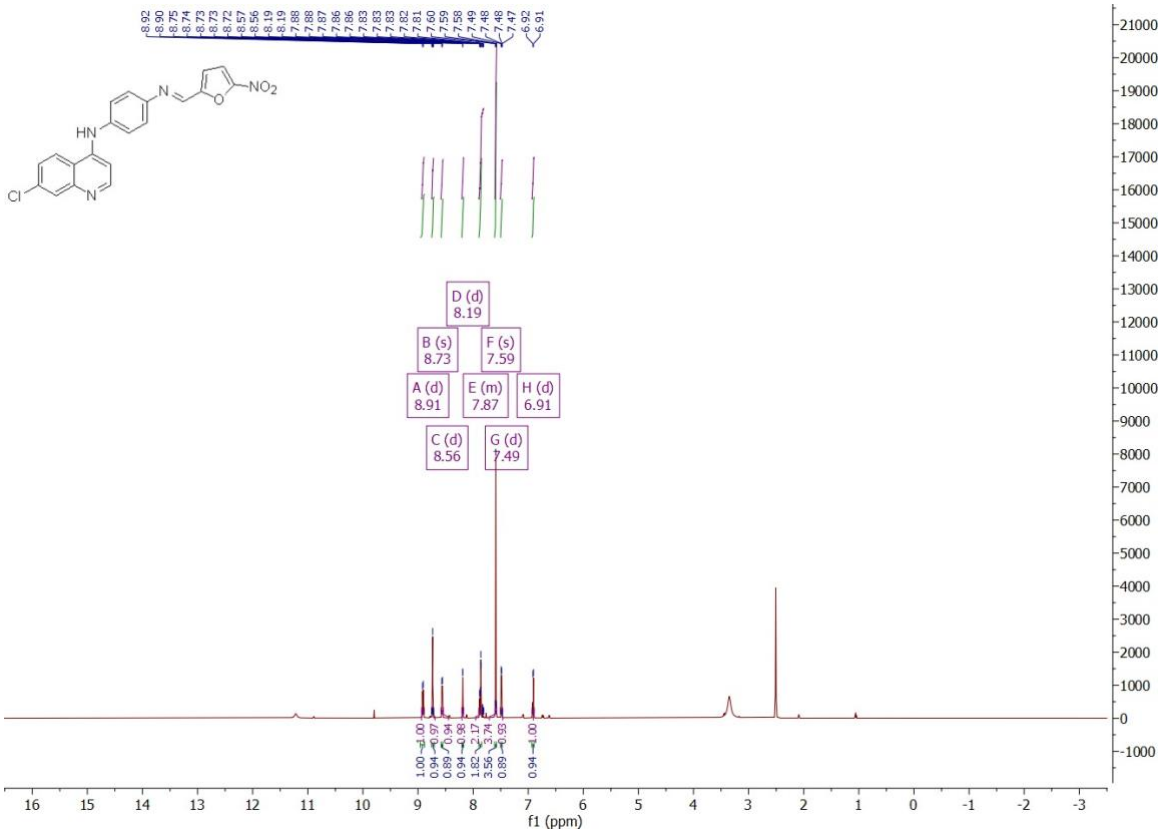
Yousuf, M., Mukherjee, D., Dey, S., Pal, C. & Adhikari, S. 2016. Antileishmanial ferrocenylquinoline derivatives: synthesis and biological evaluation against *Leishmania donovani*. *European Journal of Medicinal Chemistry*, 124:468-479. https://doi.org/10.1016/j.ejmech.2016.08.049

ANNEXURE A: SUPPLEMENTARY MATERIAL FOR CHAPTER 3

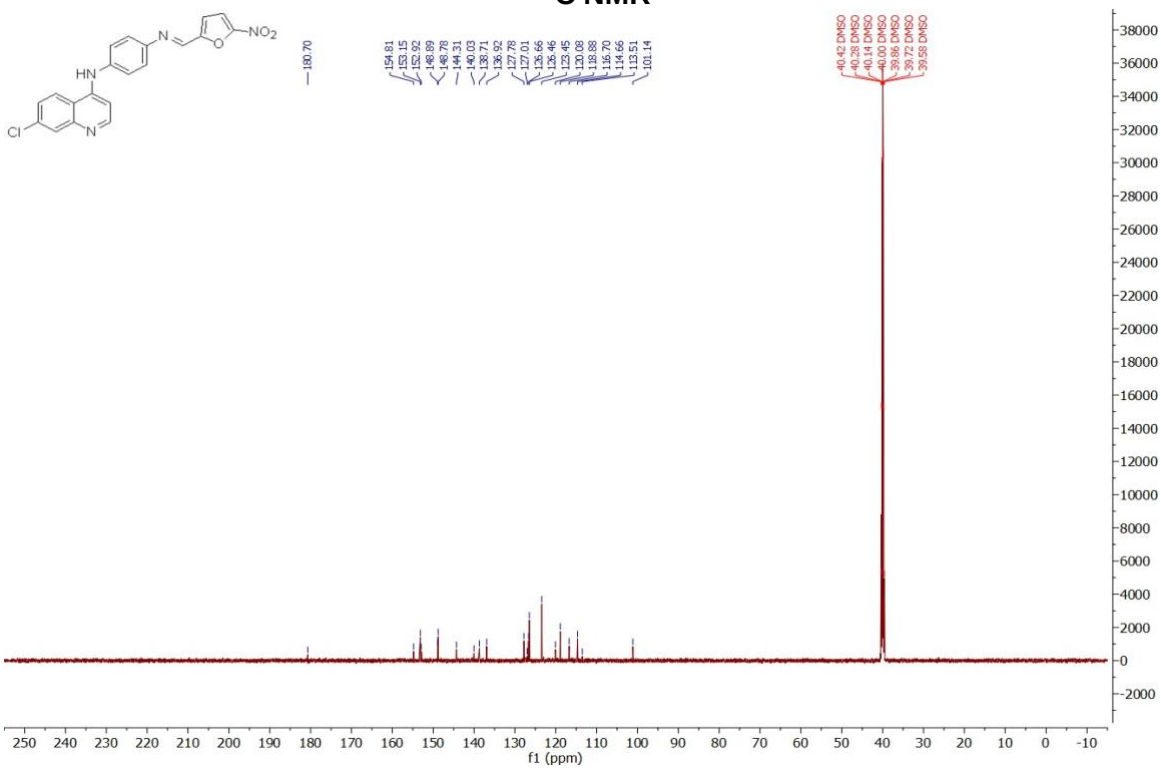
Synthesis, *in vitro* antibacterial and antiprotozoan evaluation of 4,7-disubstituted quinolines

(E)-7-chloro-N-(4-(((5-nitrofuran-2-yl)methylene)amino)phenyl)quinolin-4-amine (**1a**)

^{1}H NMR



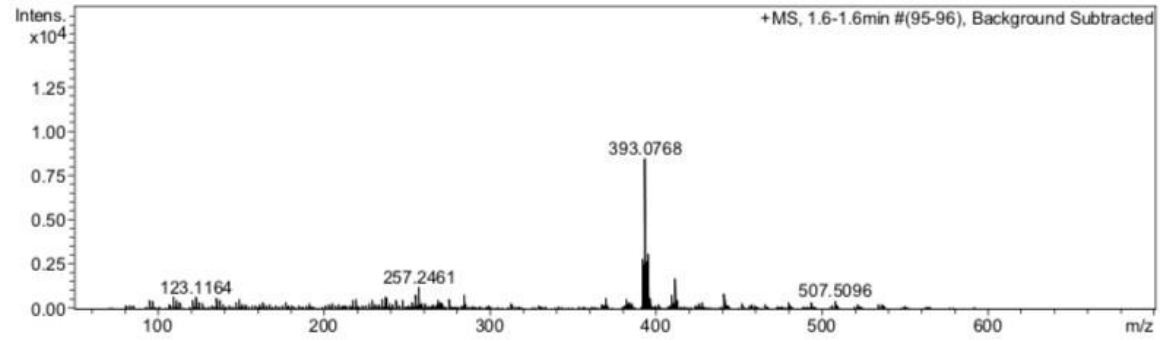
^{13}C NMR



HRMS

Acquisition Parameter

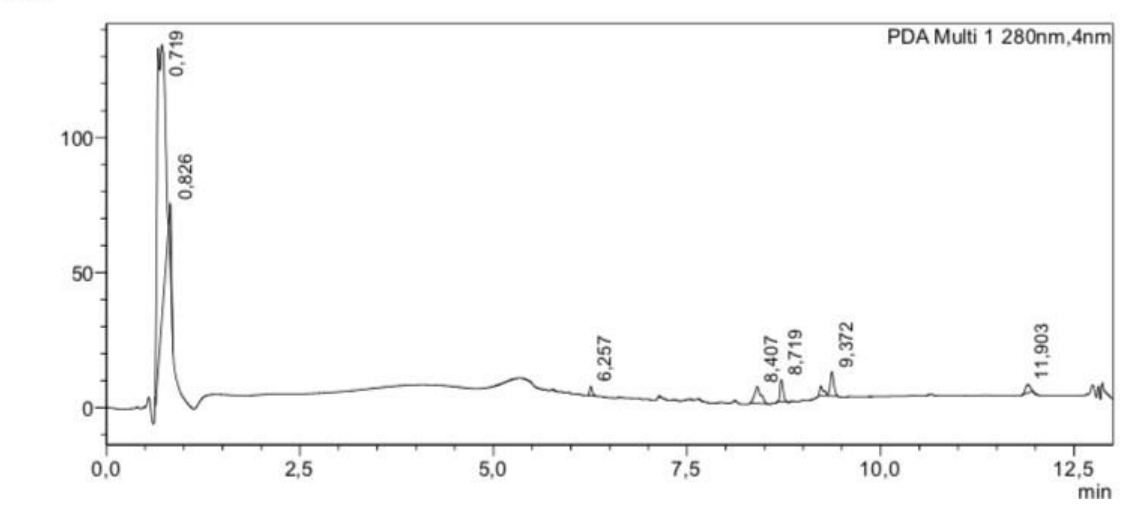
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Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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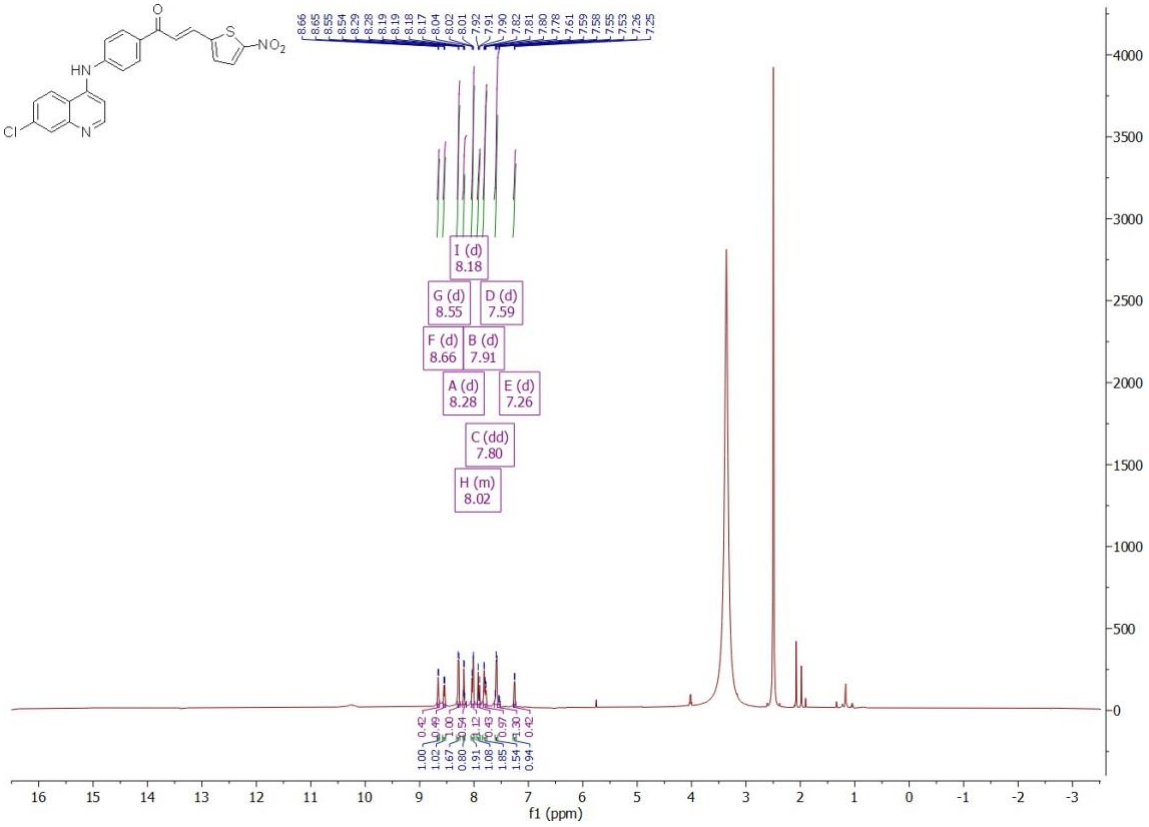
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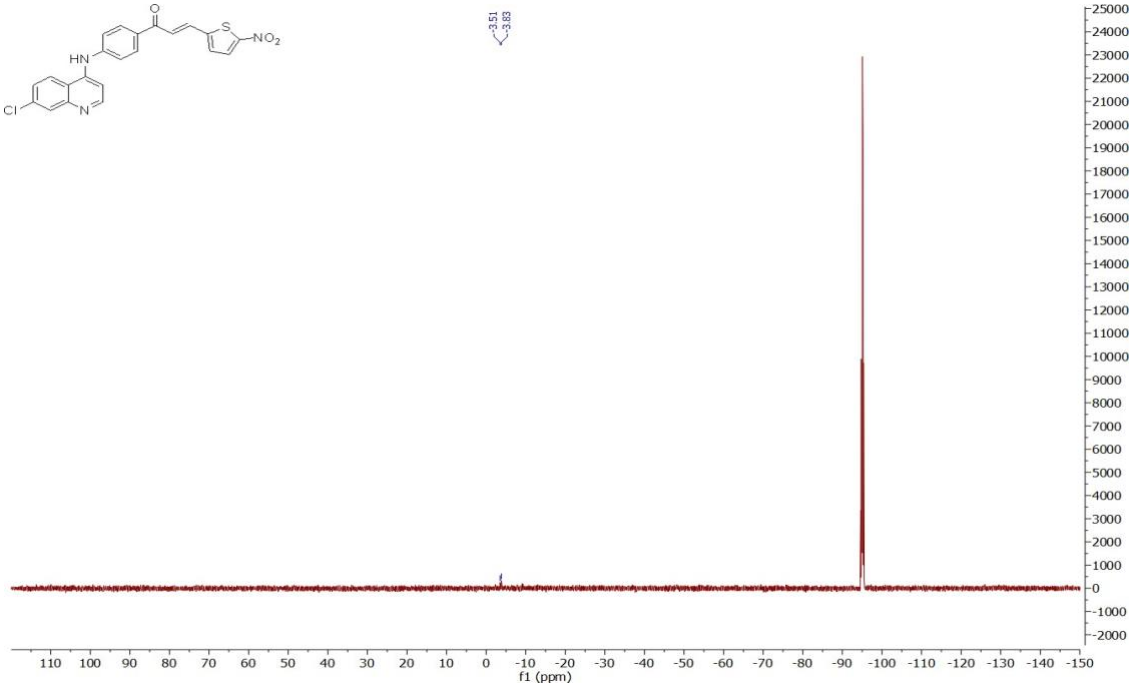


(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**2a**)

^{1}H NMR



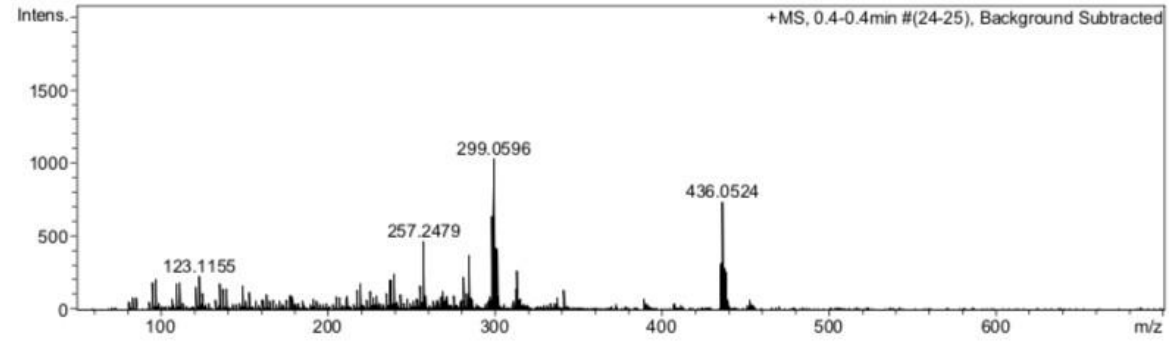
^{13}C NMR



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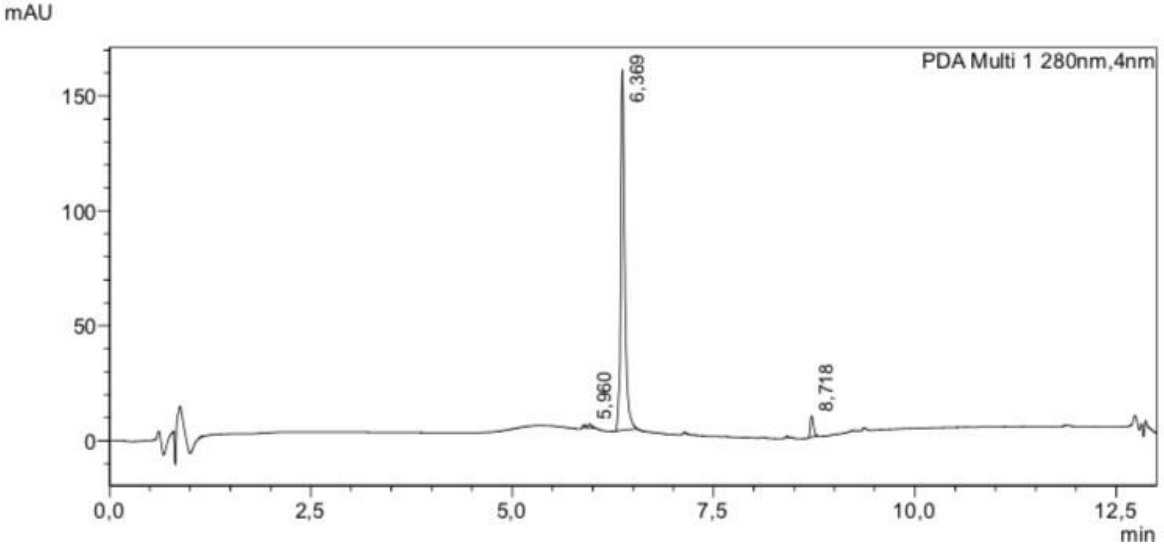
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Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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	2	C 16 H 12 Cl N 2 O 2	100.00	299.0582	-1.4	-4.6	109.9	11.5	even	ok
	3	C 13 H 16 Cl N 2 O 2 S	45.45	299.0616	2.0	6.6	117.0	6.5	even	ok
	4	C 16 H 13 N O 3 S	0.16	299.0611	1.5	5.0	203.6	11.0	odd	ok
	5	C 22 H 7 N 2	0.01	299.0604	0.8	2.7	238.0	20.5	even	ok
	6	C 19 H 9 N O 3	0.00	299.0577	-1.9	-6.3	248.0	16.0	odd	ok
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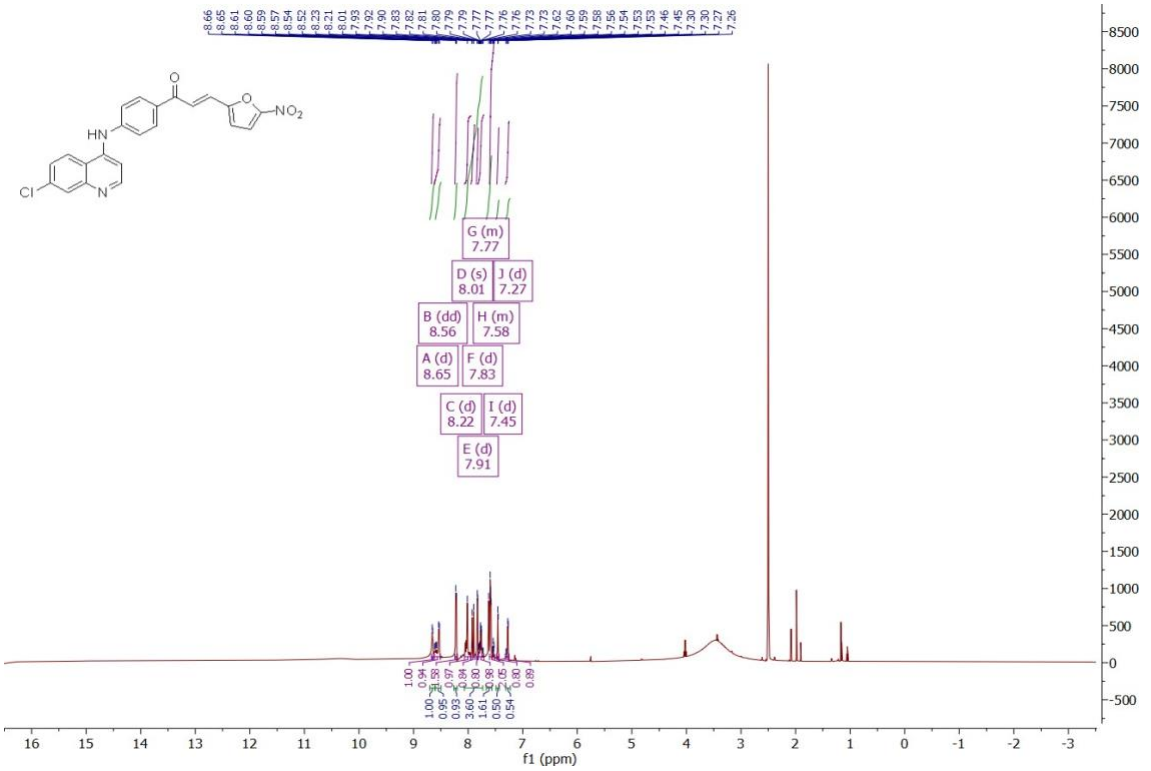
HPLC

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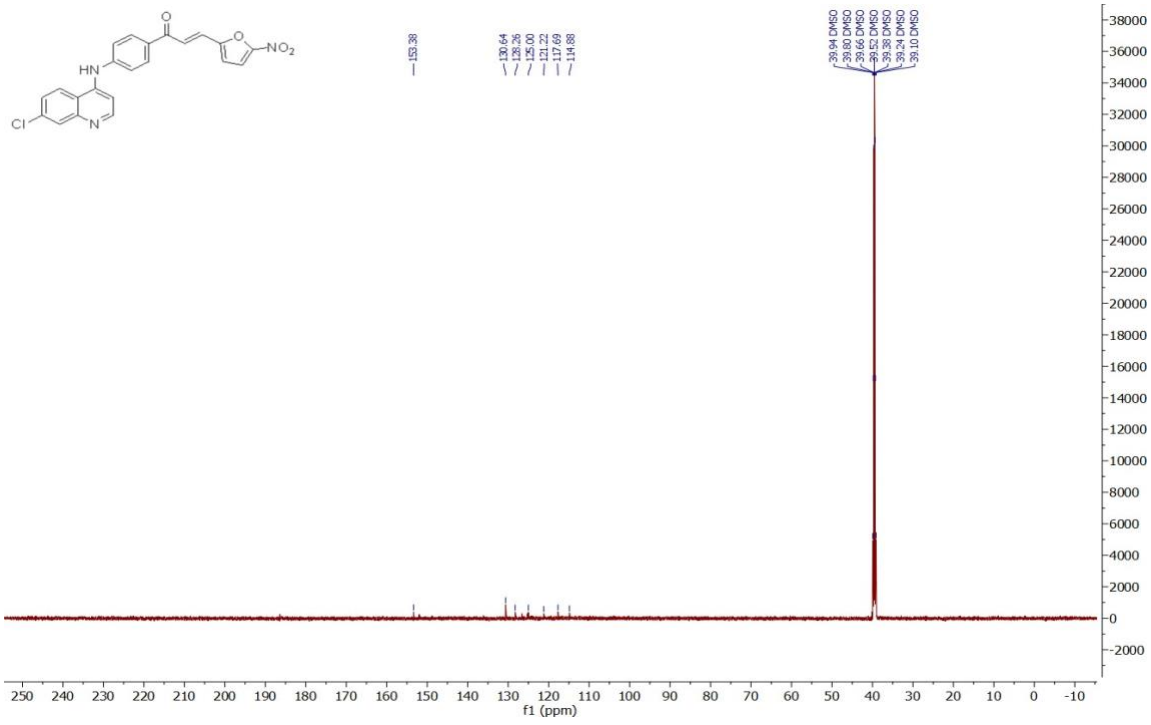


(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**2b**)

^{1}H NMR



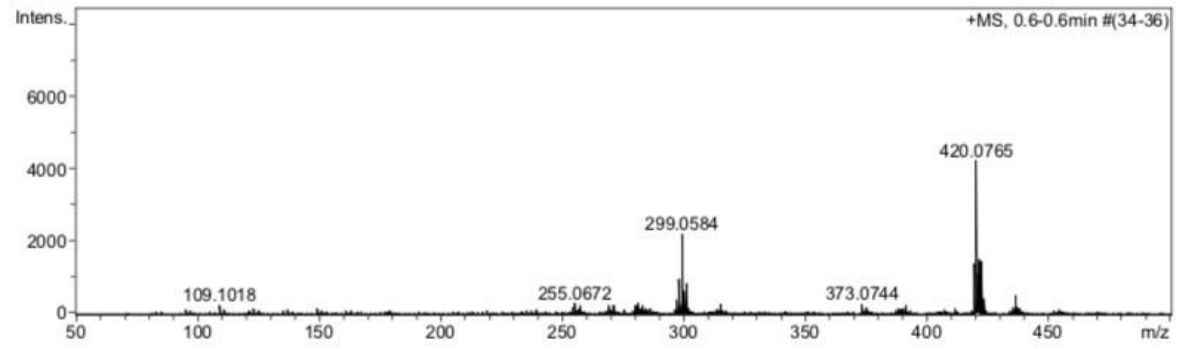
^{13}C NMR



HRMS

Acquisition Parameter

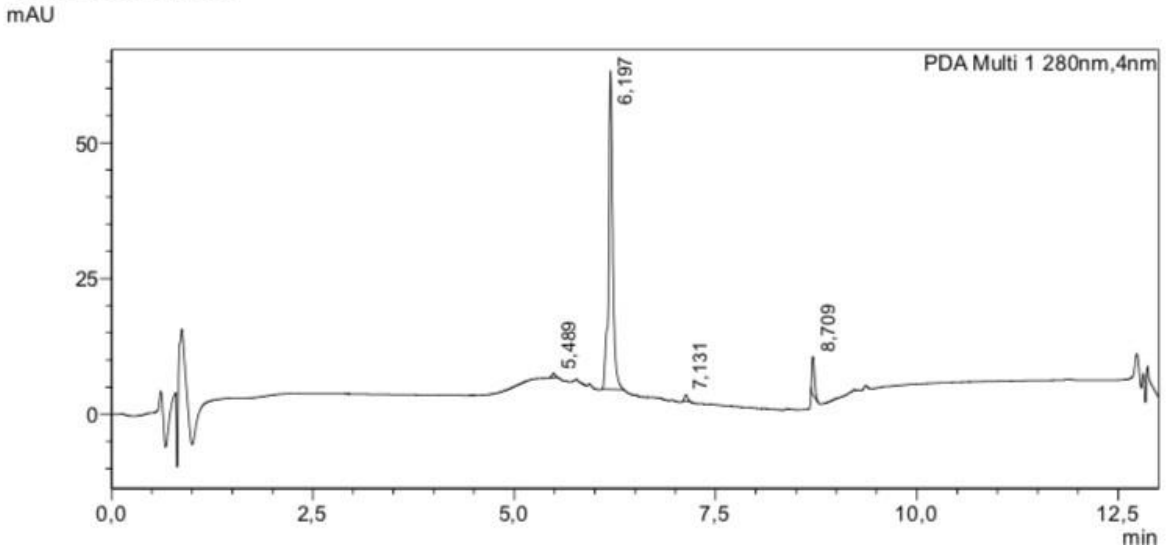
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Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
299.0584	1	C 21 H 12 Cl	10.45	299.0622	3.8	12.8	35.0	15.5	even	ok
	2	C 16 H 12 Cl N 2 O 2	100.00	299.0582	-0.2	-0.6	54.8	11.5	even	ok
	3	C 22 H 7 N 2	0.00	299.0604	2.0	6.7	207.1	20.5	even	ok
	4	C 19 H 9 N O 3	0.01	299.0577	-0.7	-2.2	212.0	16.0	odd	ok
420.0765	1	C 22 H 15 Cl N 3 O 4	100.00	420.0746	-2.0	-4.7	49.2	16.5	even	ok

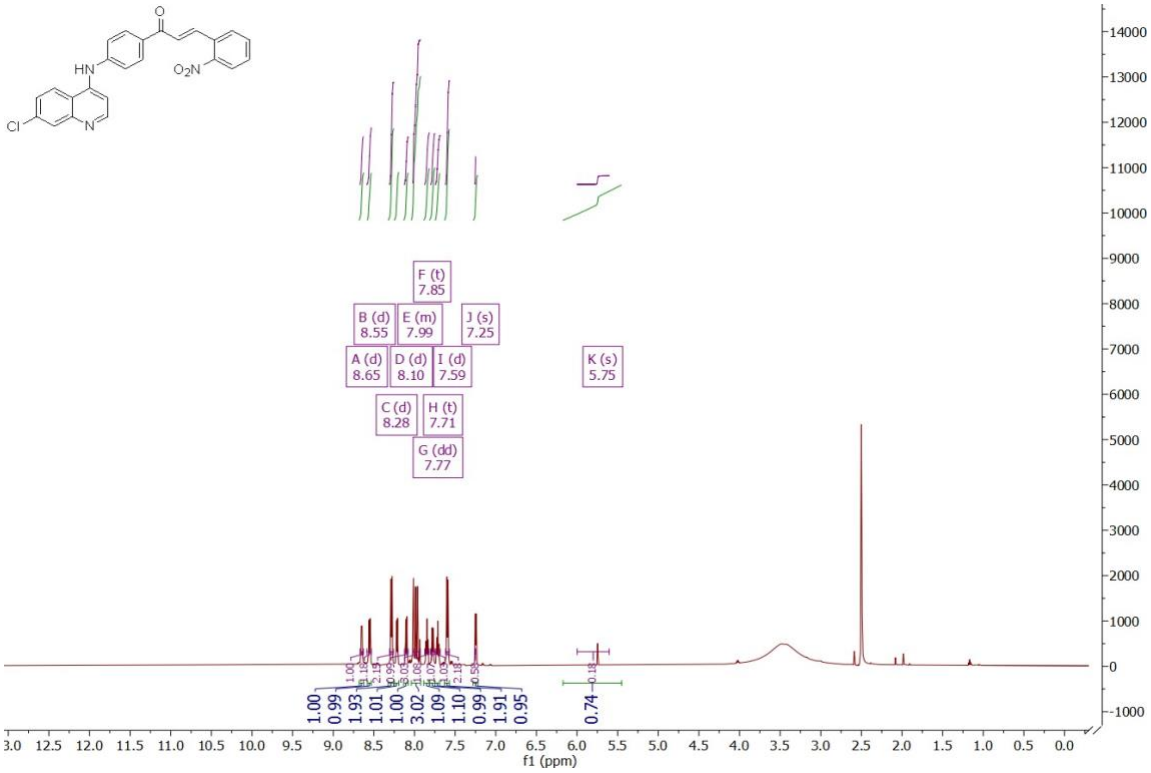
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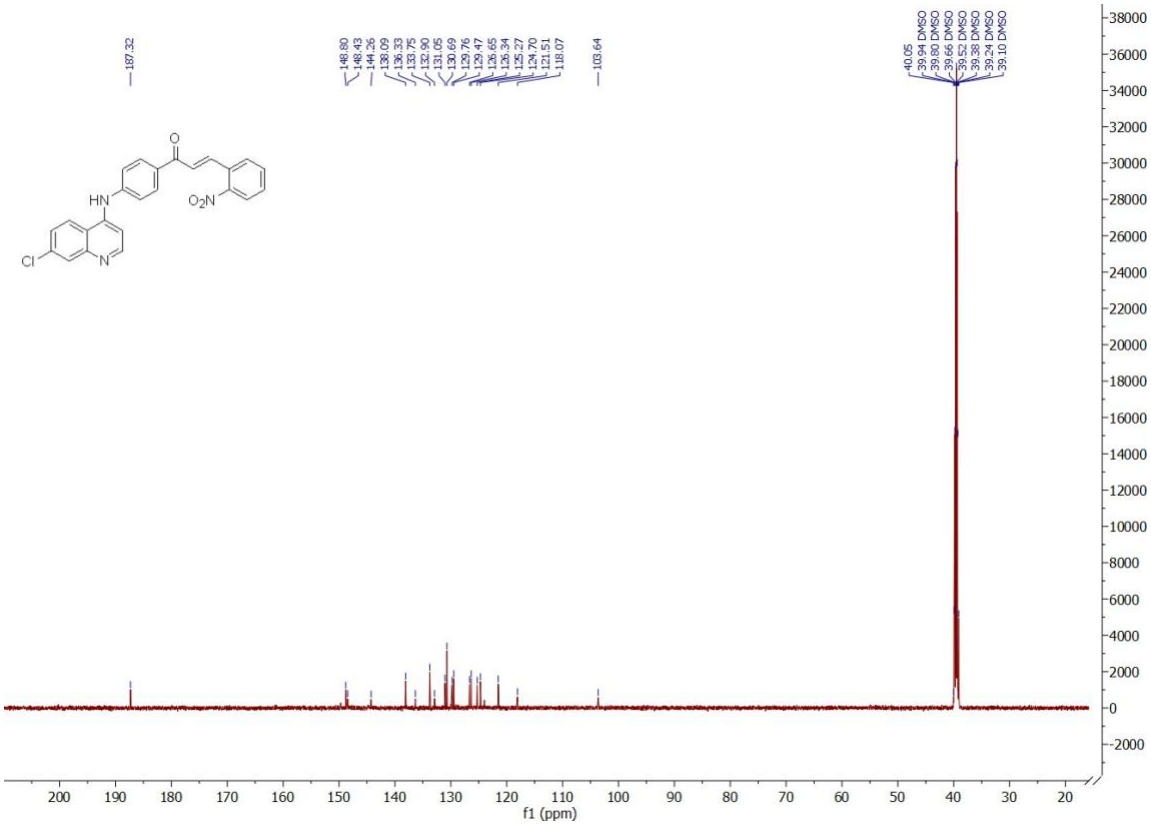


(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**2c**)

^{1}H NMR



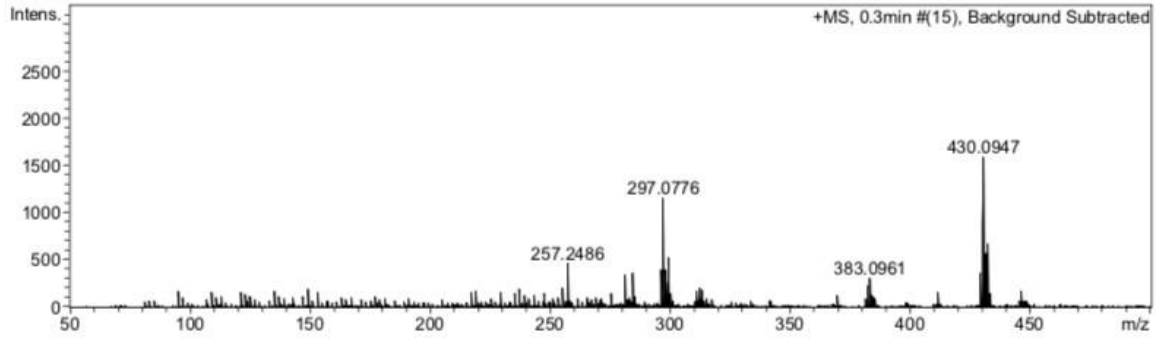
^{13}C NMR



HRMS

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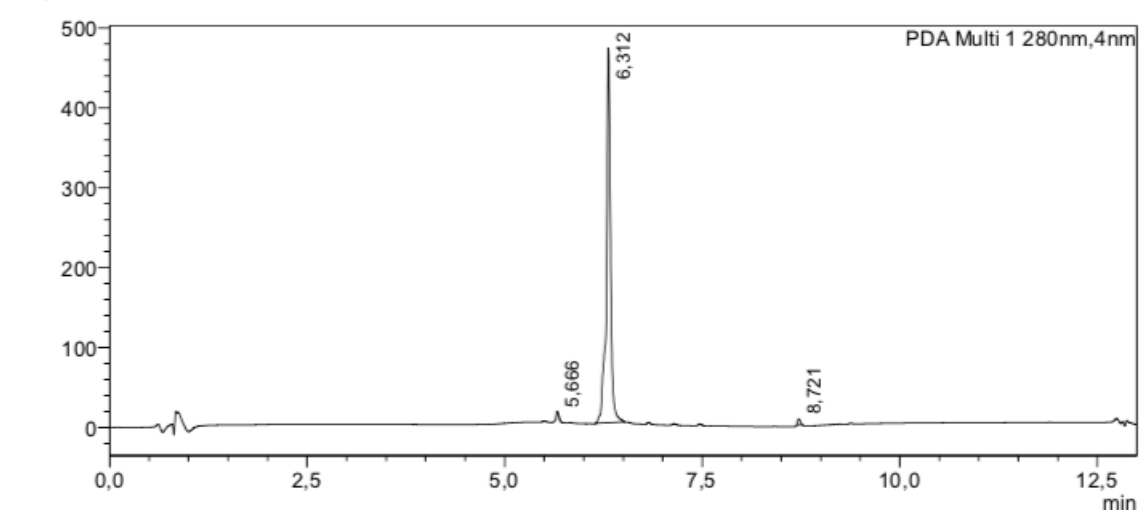
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Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



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	2	C 20 H 11 N O 2	0.00	297.0784	0.8	2.6	259.6	16.0	odd	ok
430.0947	1	C 24 H 17 Cl N 3 O 3	100.00	430.0953	0.6	1.5	64.1	17.5	even	ok

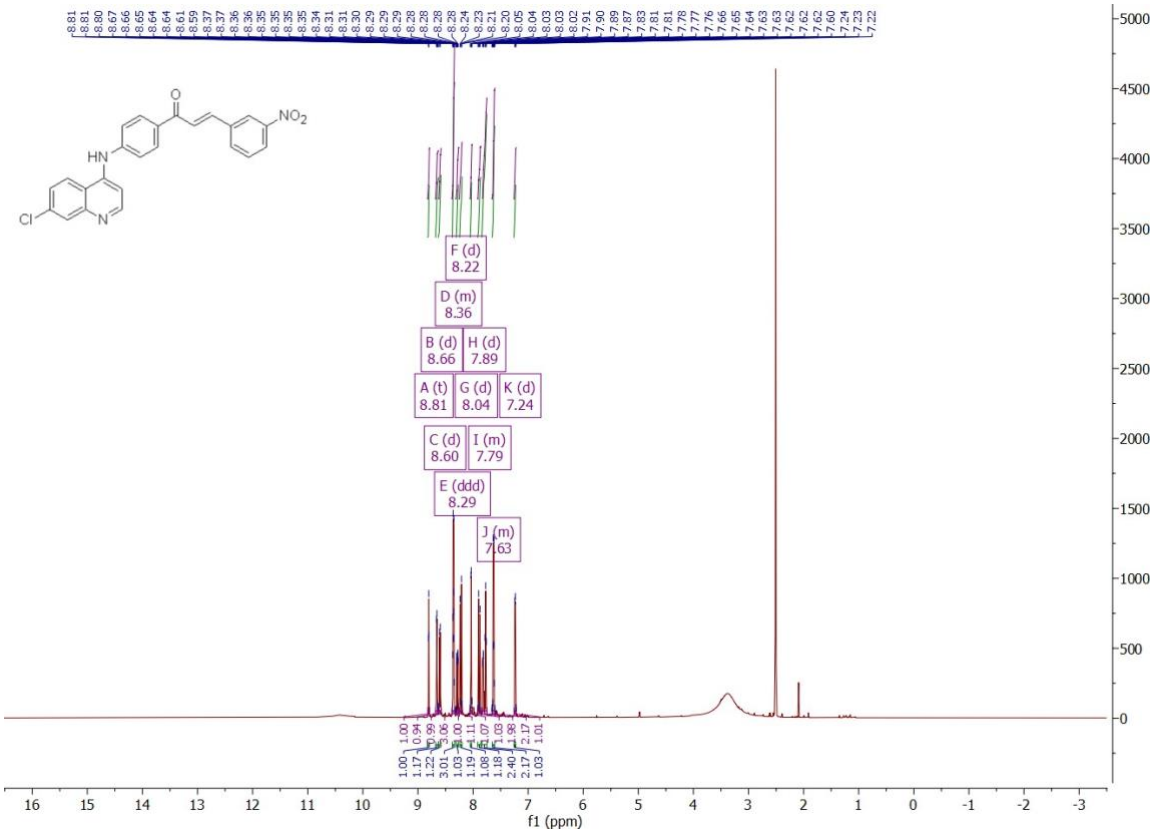
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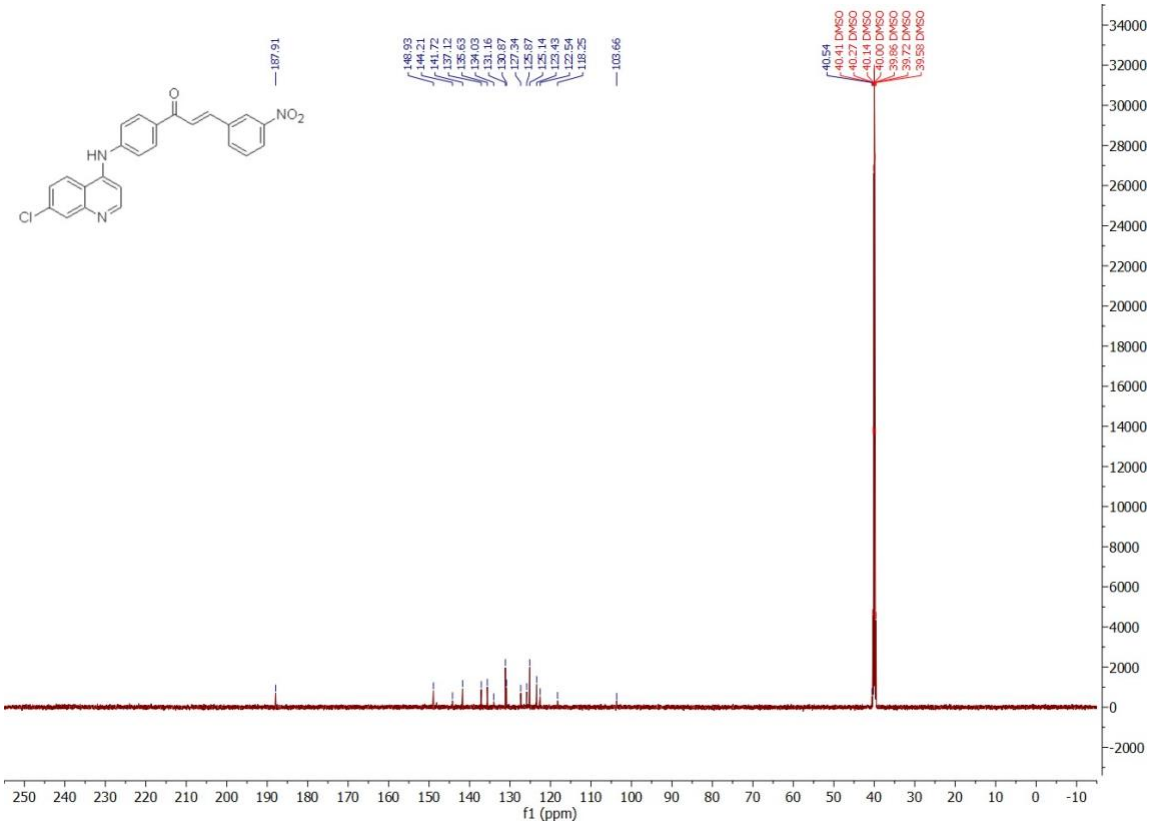


(E)-1-(4-((7-chloroquinolin-4-yl)amino)phenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**2d**)

^{1}H NMR



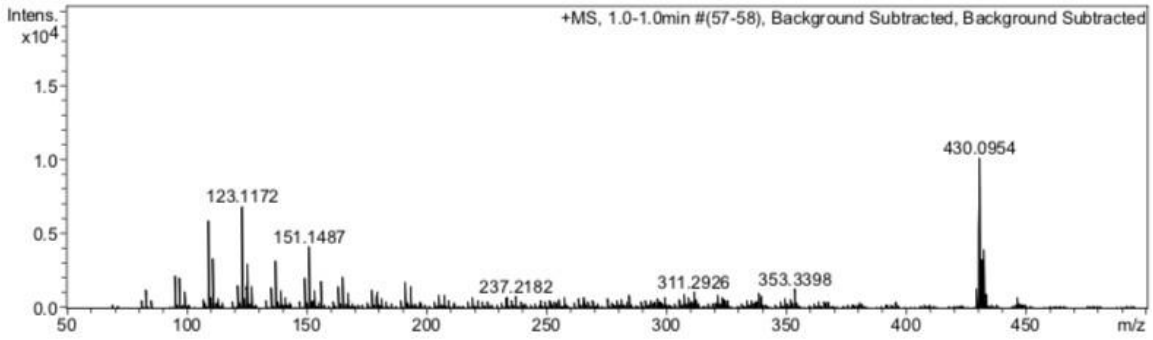
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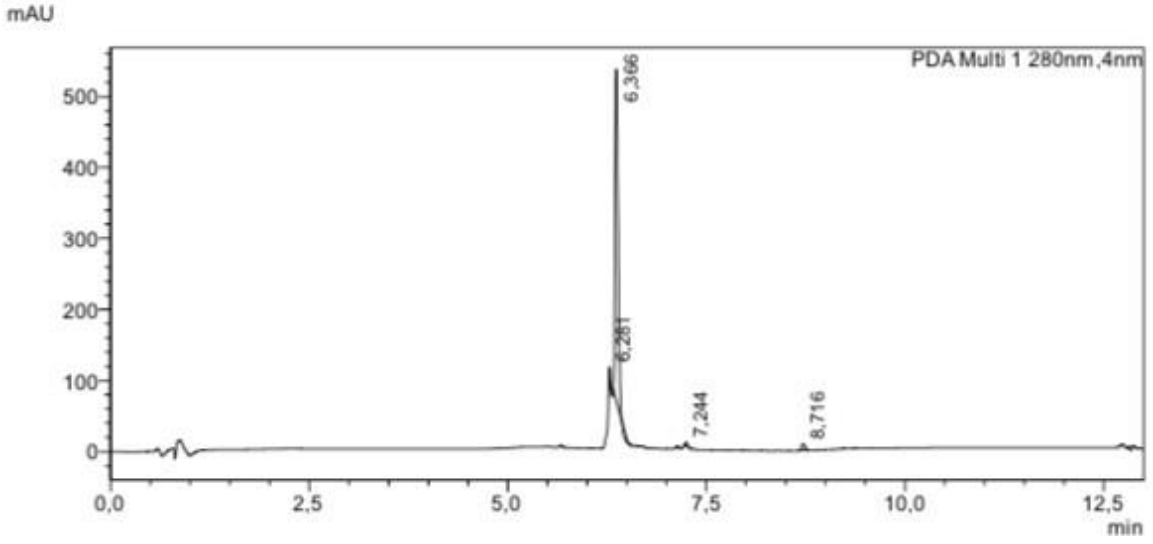
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Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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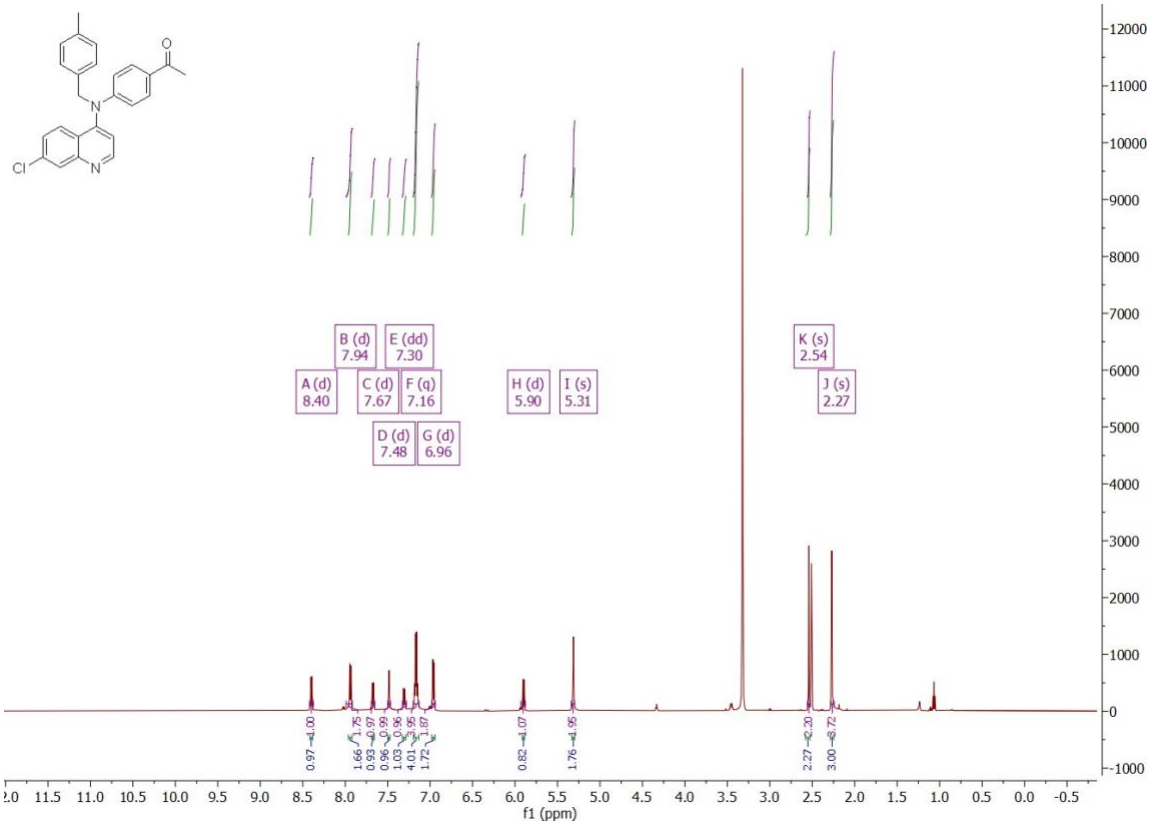
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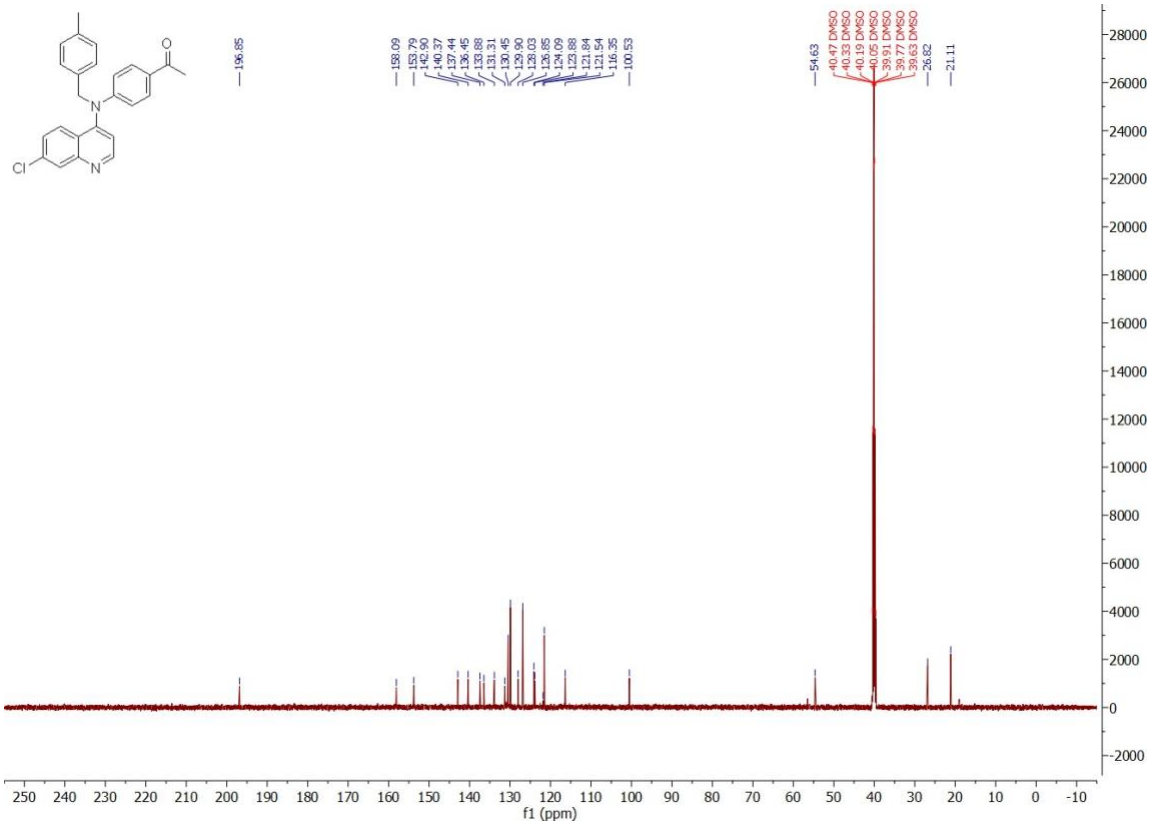


1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)ethan-1-one (**3a**)

^{1}H NMR



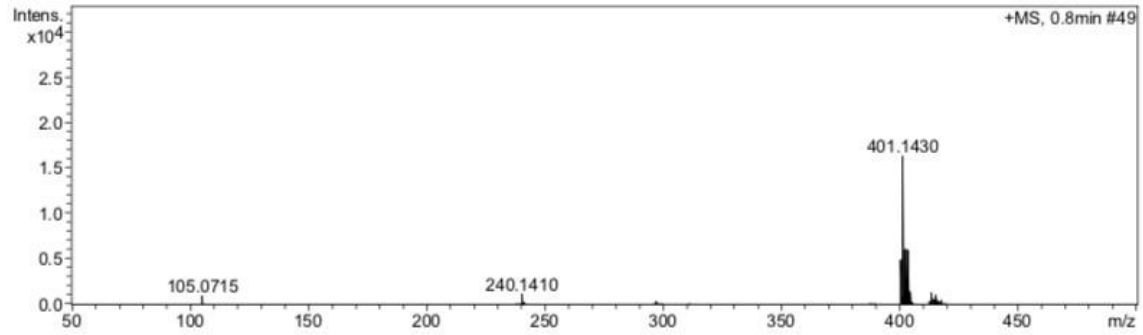
^{13}C NMR



HRMS

Acquisition Parameter

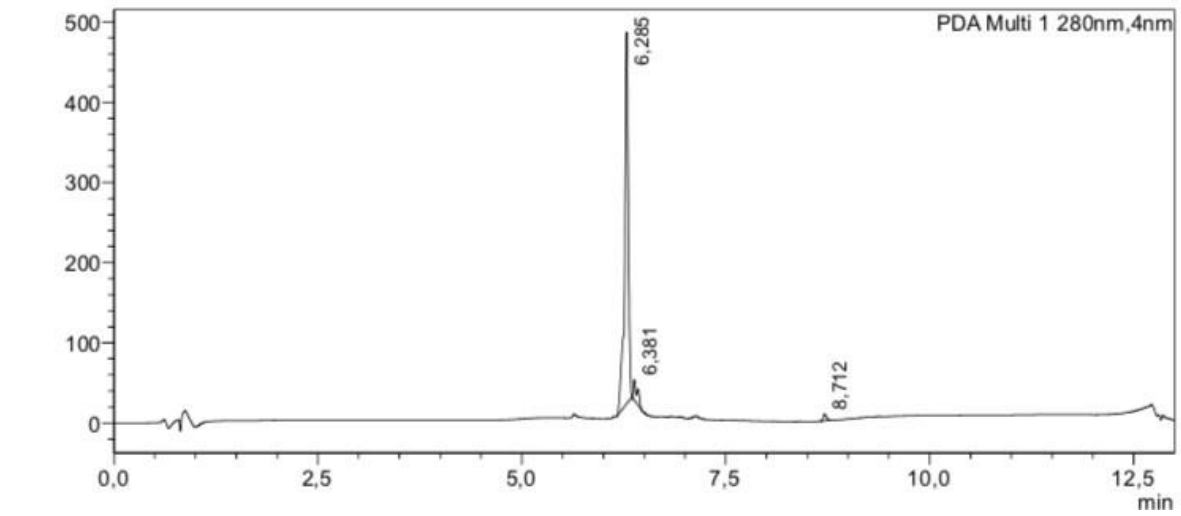
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Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
401.1430	1	C 25 H 22 Cl N 2 O	100.00	401.1415	-1.5	-3.7	43.4	15.5	even	ok

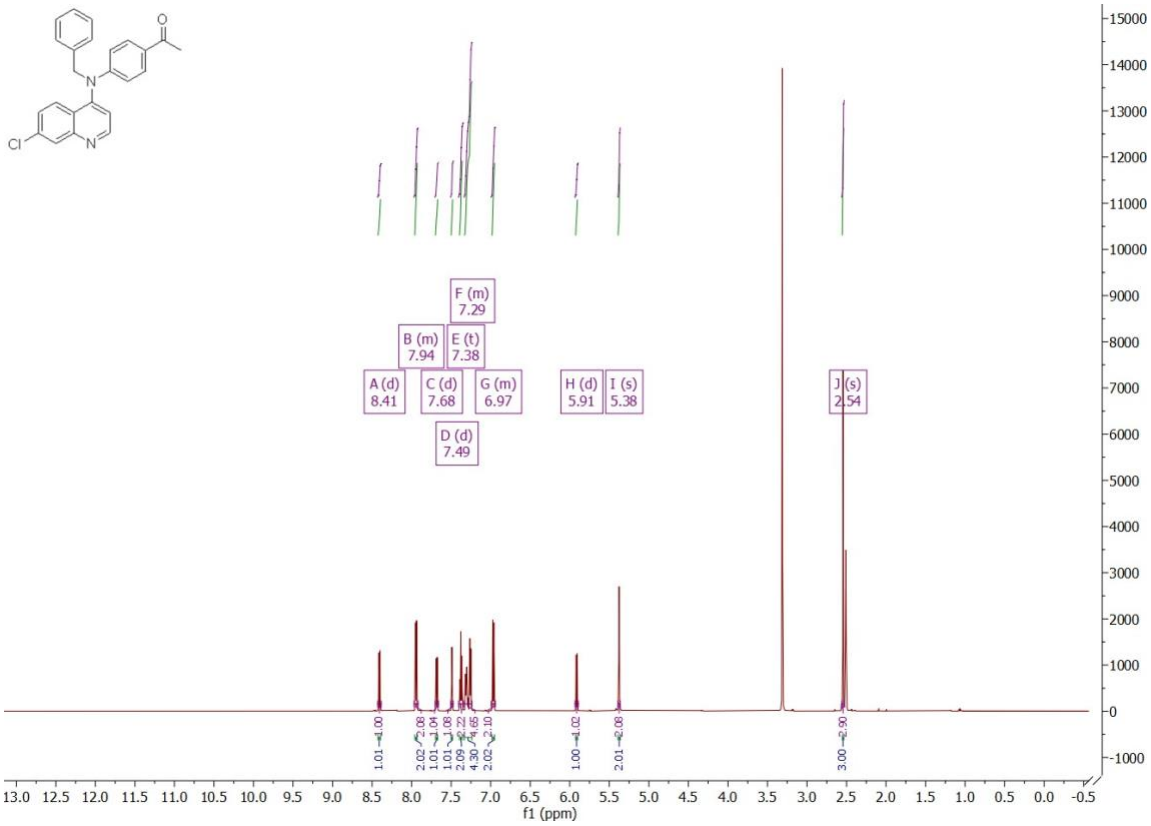
HPLC

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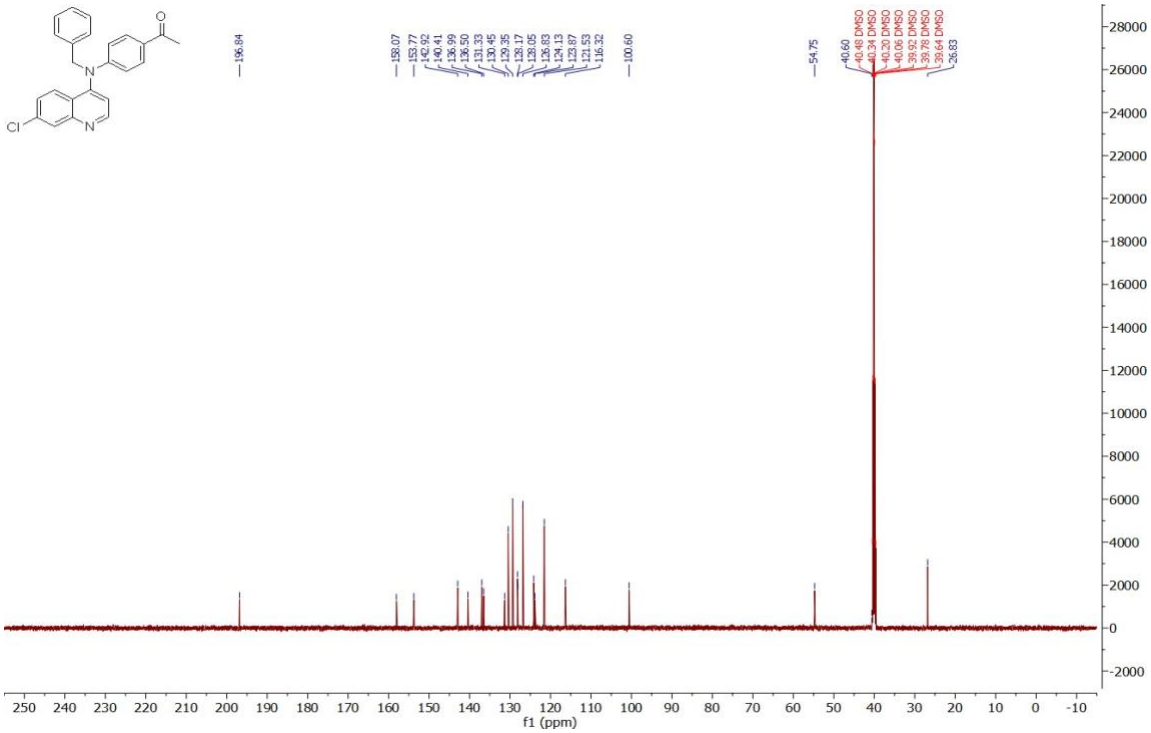


1-(4-(benzyl(7-chloroquinolin-4-yl)amino)phenyl)ethan-1-one (**3b**)

^{1}H NMR



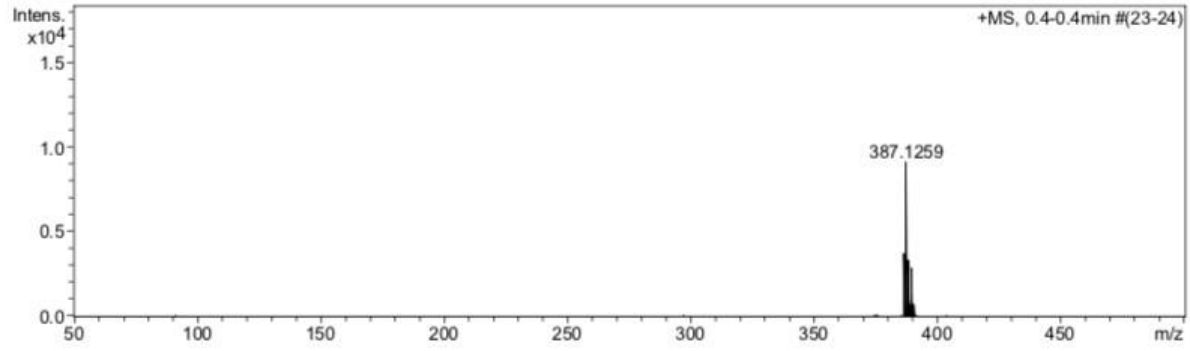
^{13}C NMR



HRMS

Acquisition Parameter

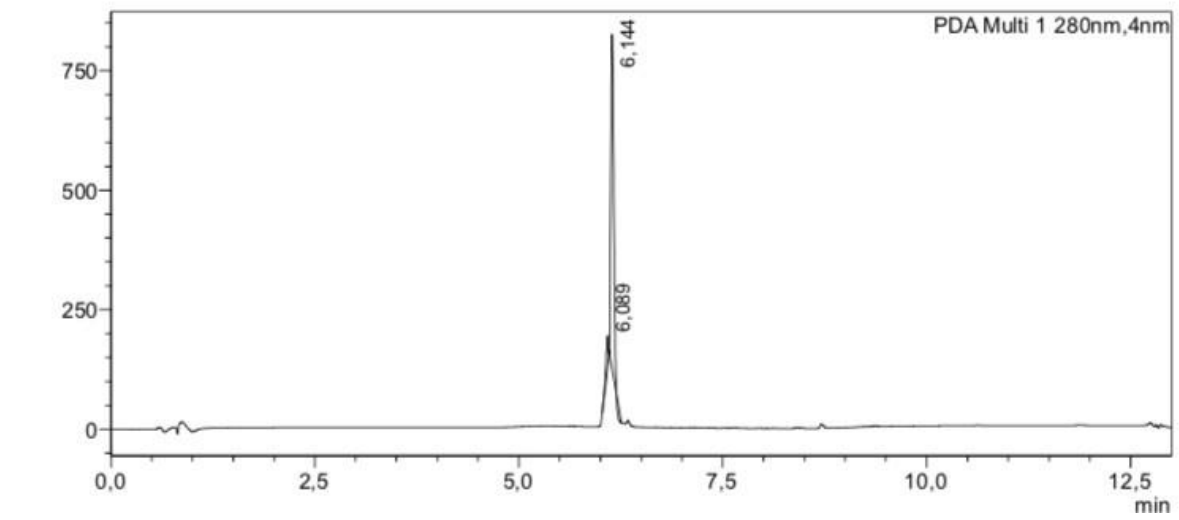
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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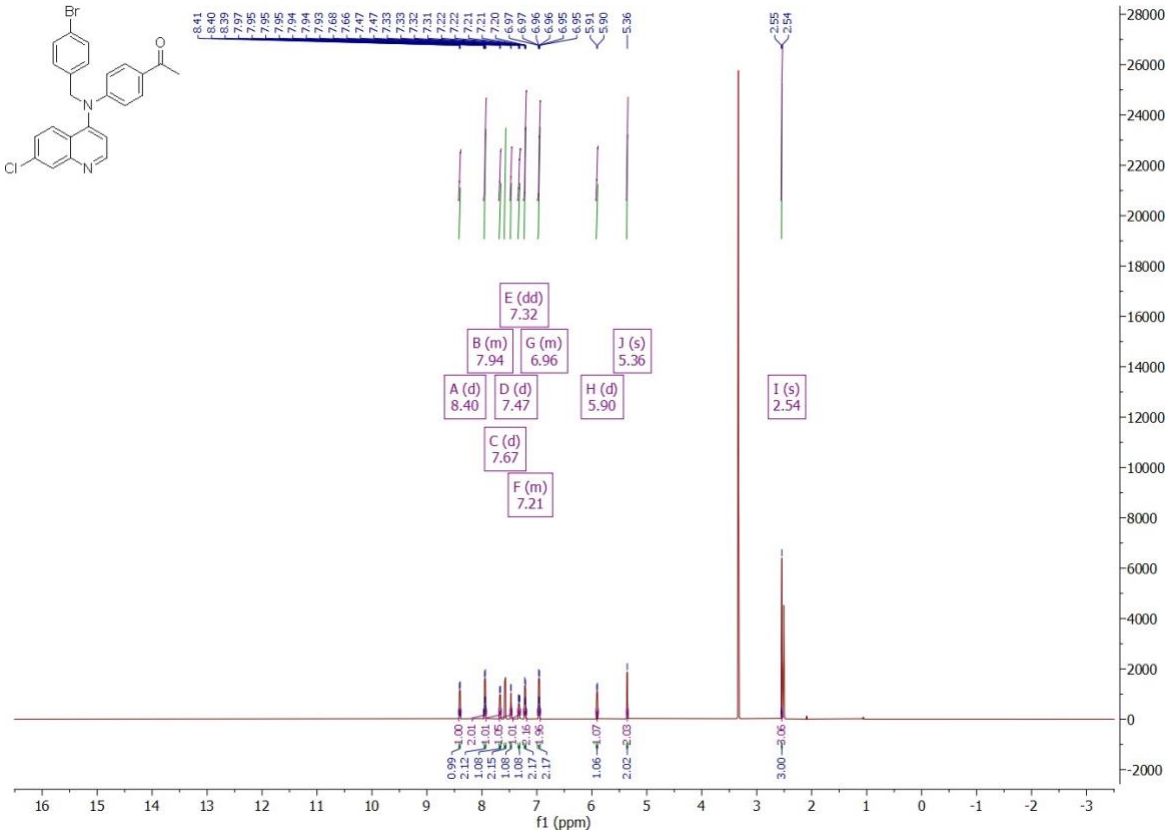
HPLC

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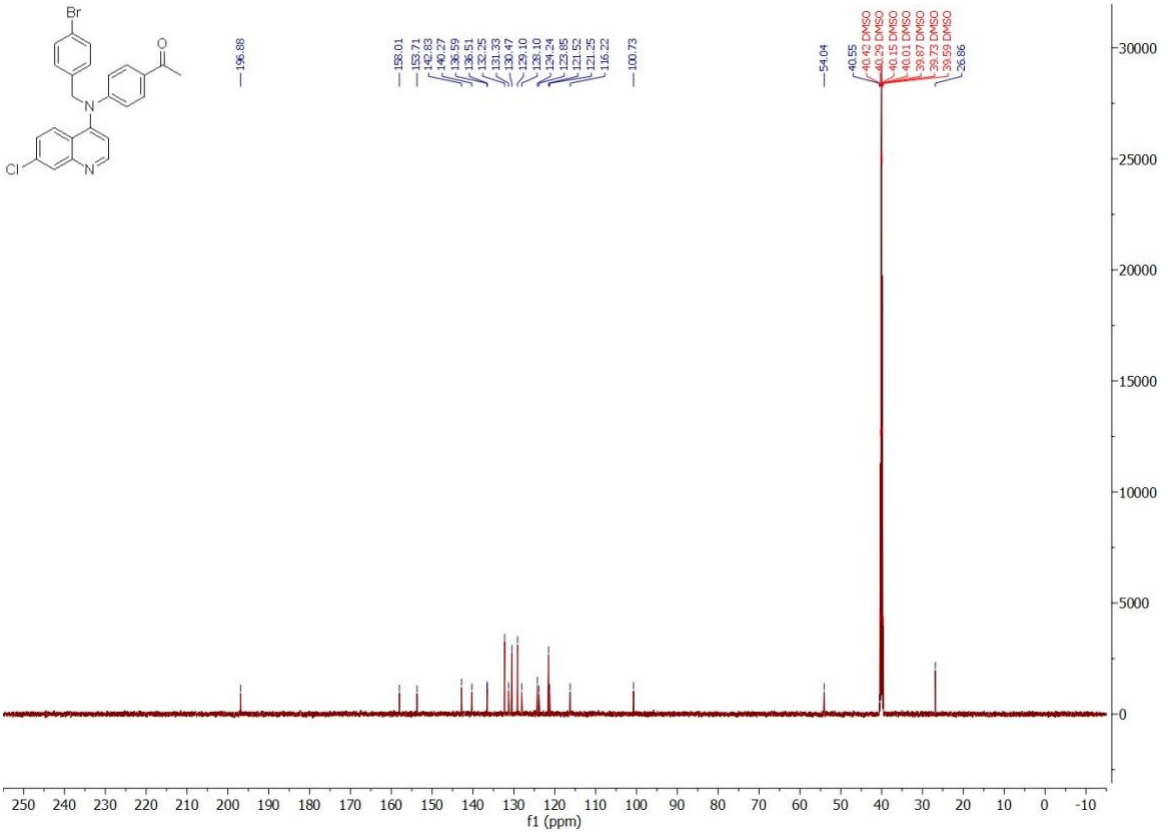


1-(4-((4-bromobenzyl)(7-chloroquinolin-4-yl)amino)phenyl)ethan-1-one (**3c**)

^{1}H NMR



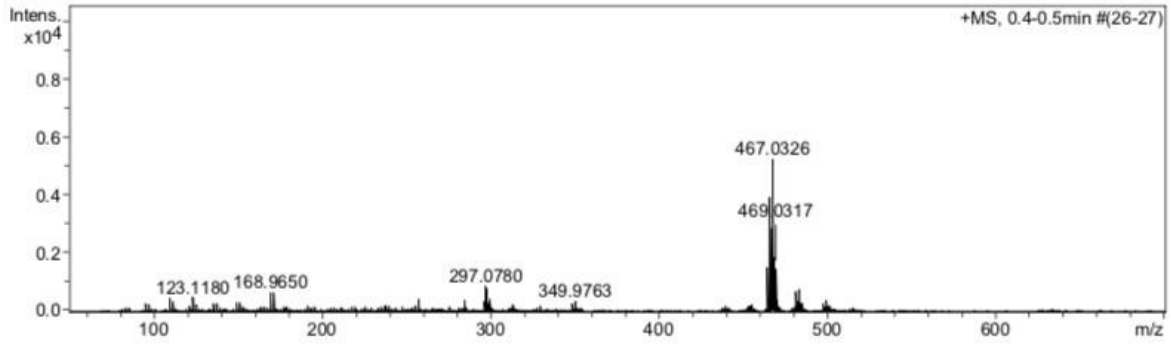
^{13}C NMR



HRMS

Acquisition Parameter

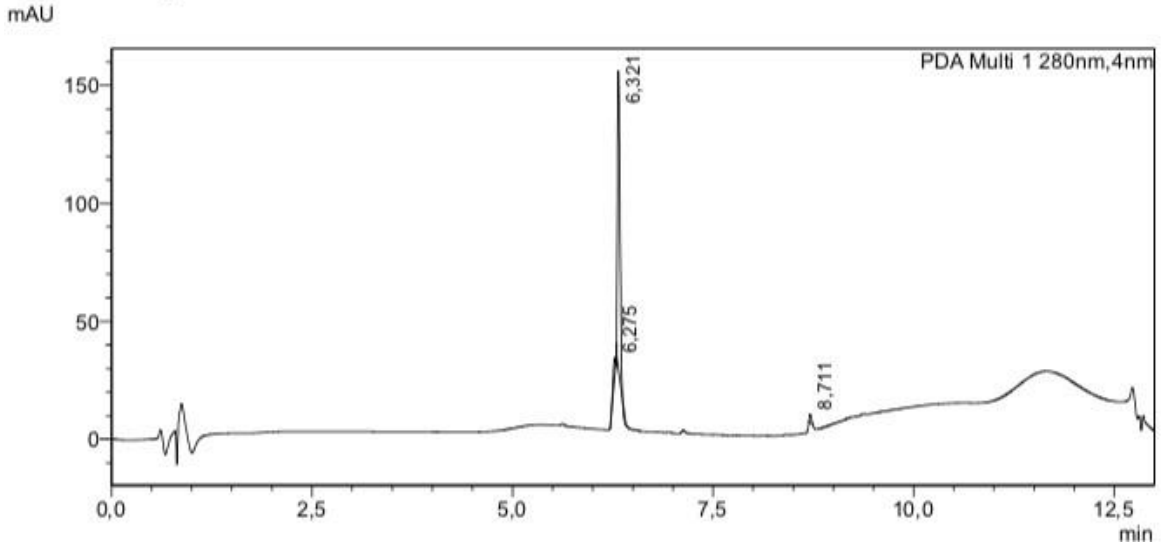
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Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
464.0280	1	C 24 H 18 Br Cl N 2 O	100.00	464.0286	0.5	1.2	436.2	16.0	odd	ok
465.0353	1	C 24 H 19 Br Cl N 2 O	100.00	465.0364	1.1	2.3	133.9	15.5	even	ok
466.0298	1	C 25 H 20 Br Cl O 2	43.24	466.0330	3.1	6.7	404.4	15.0	odd	ok
	2	C 23 H 18 Br Cl N 3 O	100.00	466.0316	1.8	3.8	406.3	15.5	even	ok
	3	C 26 H 15 Br N 2 O 2	0.00	466.0311	1.3	2.8	475.8	20.0	odd	ok

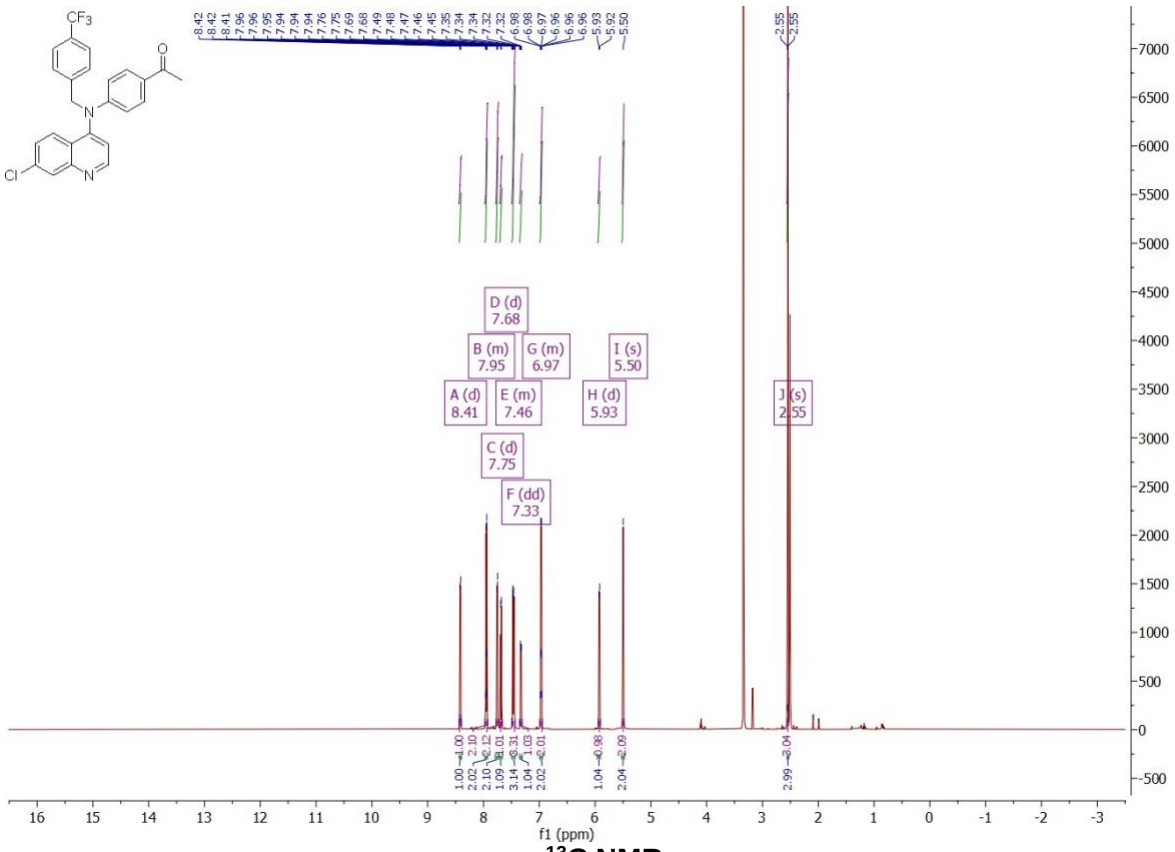
HPLC

<Chromatogram>

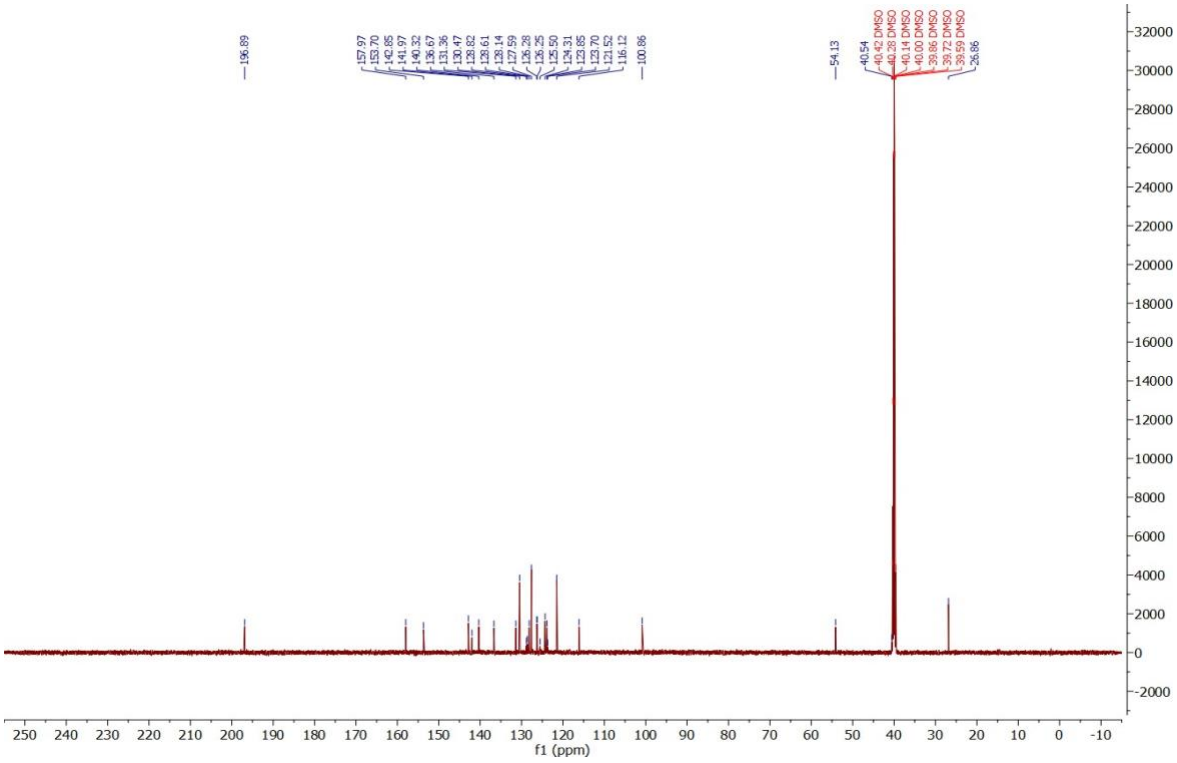


1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)ethan-1-one (**3d**)

^{1}H NMR



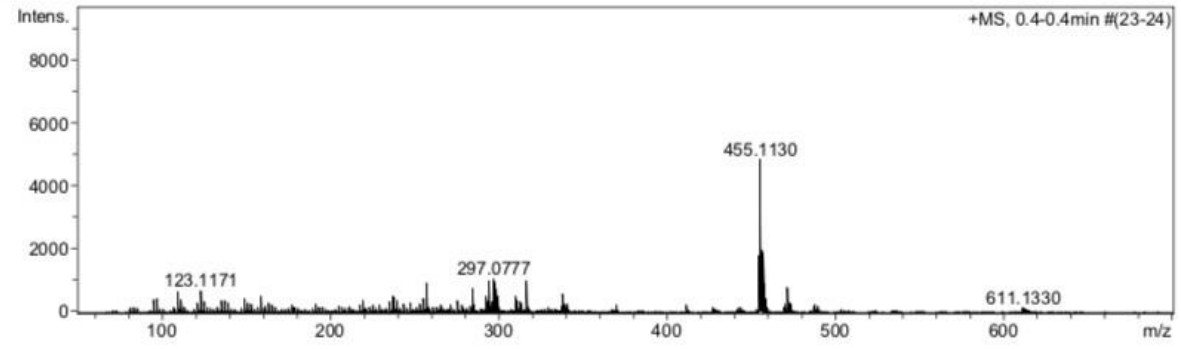
^{13}C NMR



HRMS

Acquisition Parameter

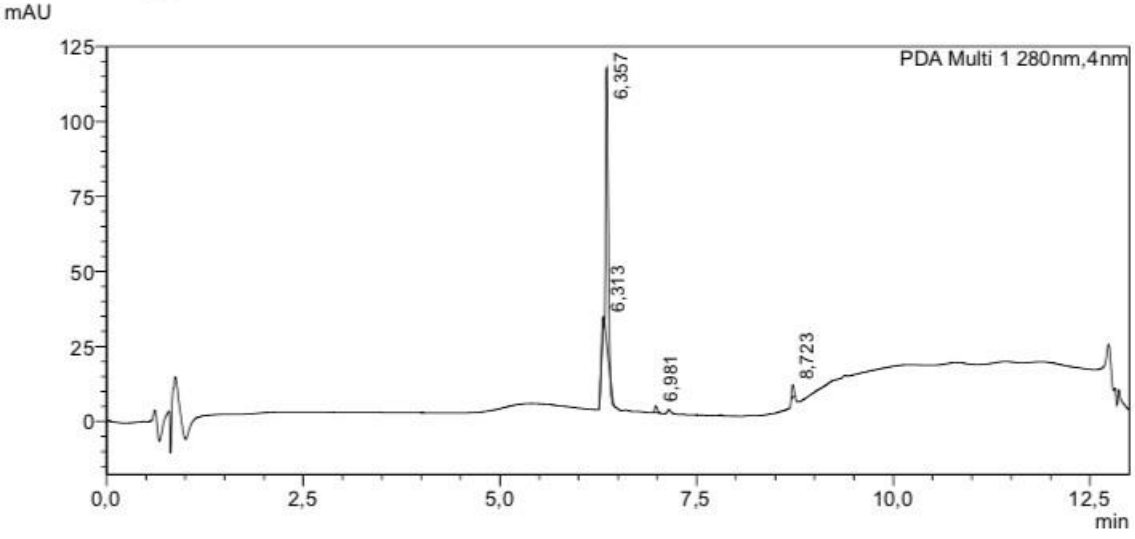
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Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
455.1130	1	C 25 H 19 Cl F 3 N 2 O	100.00	455.1133	0.3	0.6	59.8	15.5	even	ok

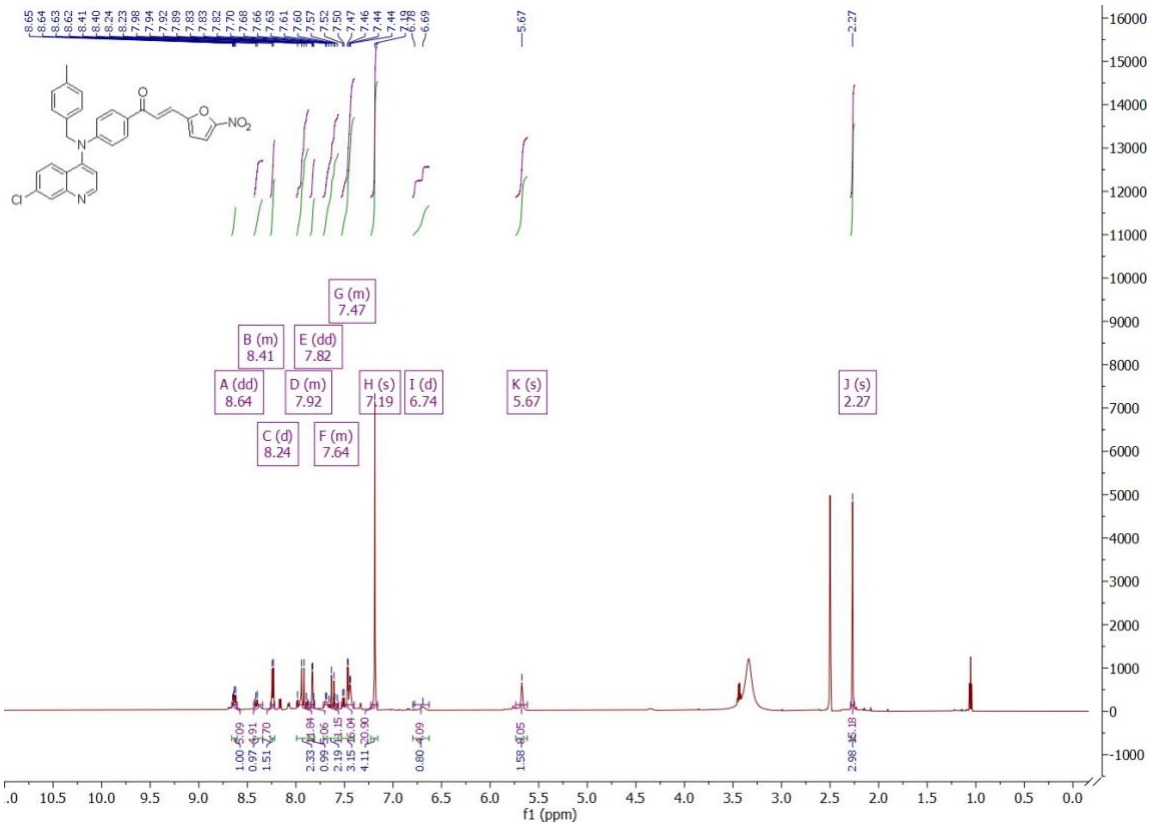
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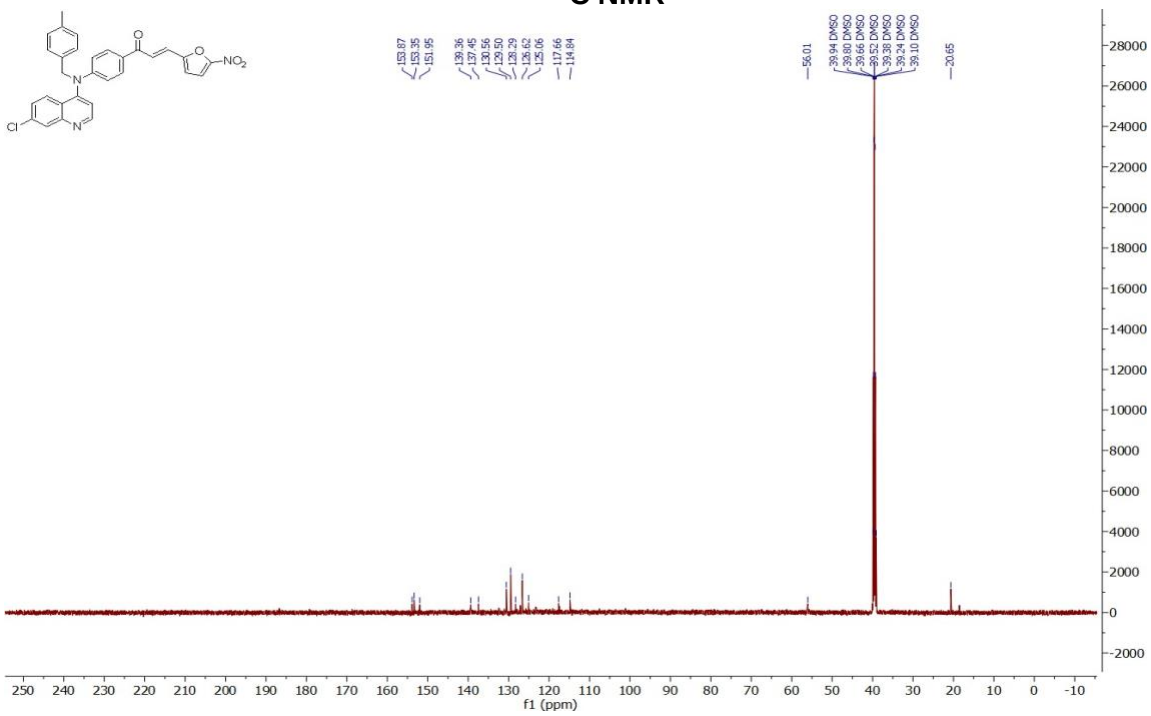


(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**4a**)

^{1}H NMR



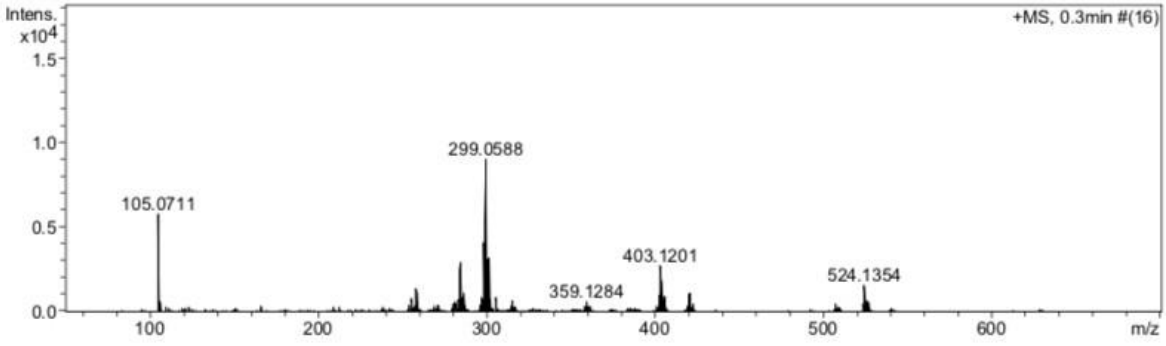
^{13}C NMR



HRMS

Acquisition Parameter

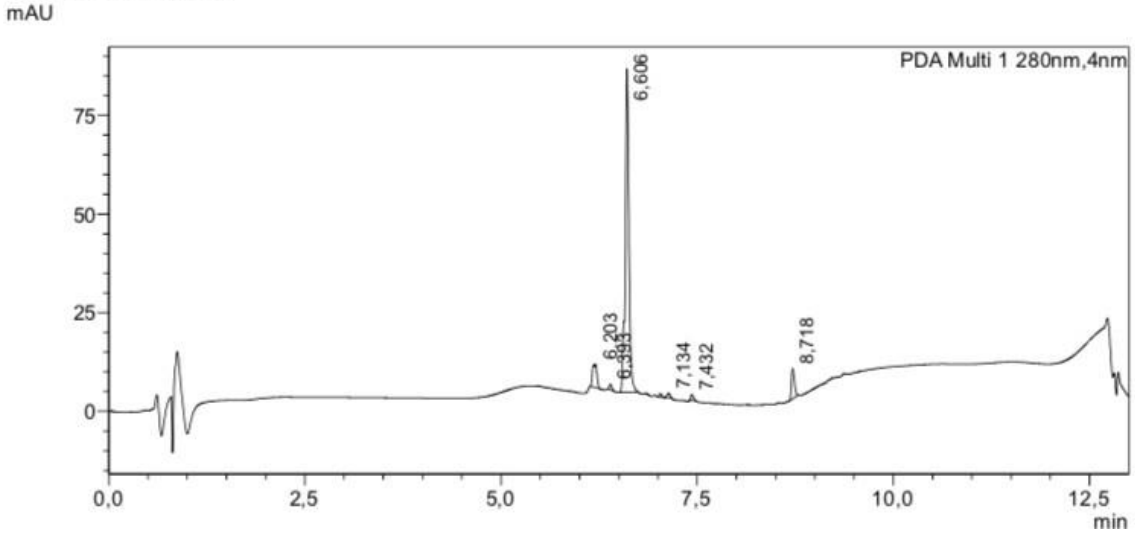
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Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
523.1310	1	C 30 H 22 Cl N 3 O 4	100.00	523.1293	-1.6	-3.2	447.2	21.0	odd	ok
524.1354	1	C 30 H 23 Cl N 3 O 4	100.00	524.1372	1.8	3.4	66.2	20.5	even	ok

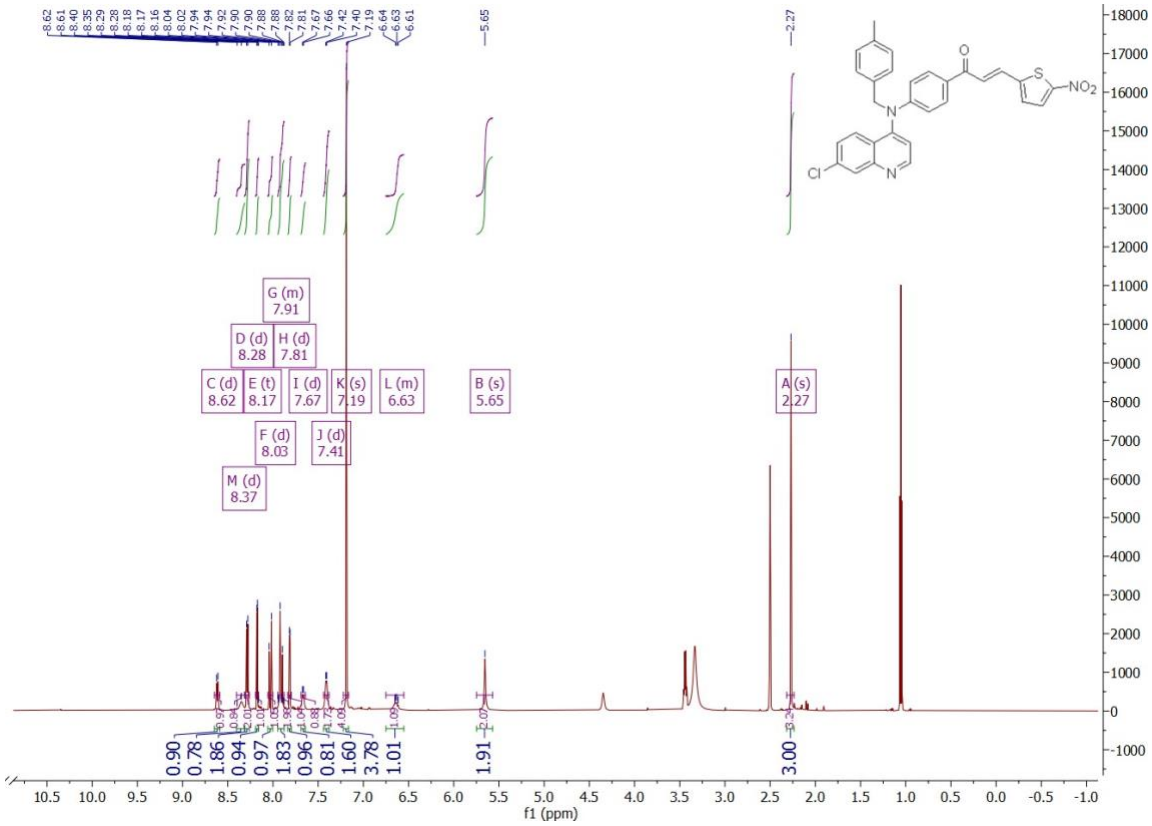
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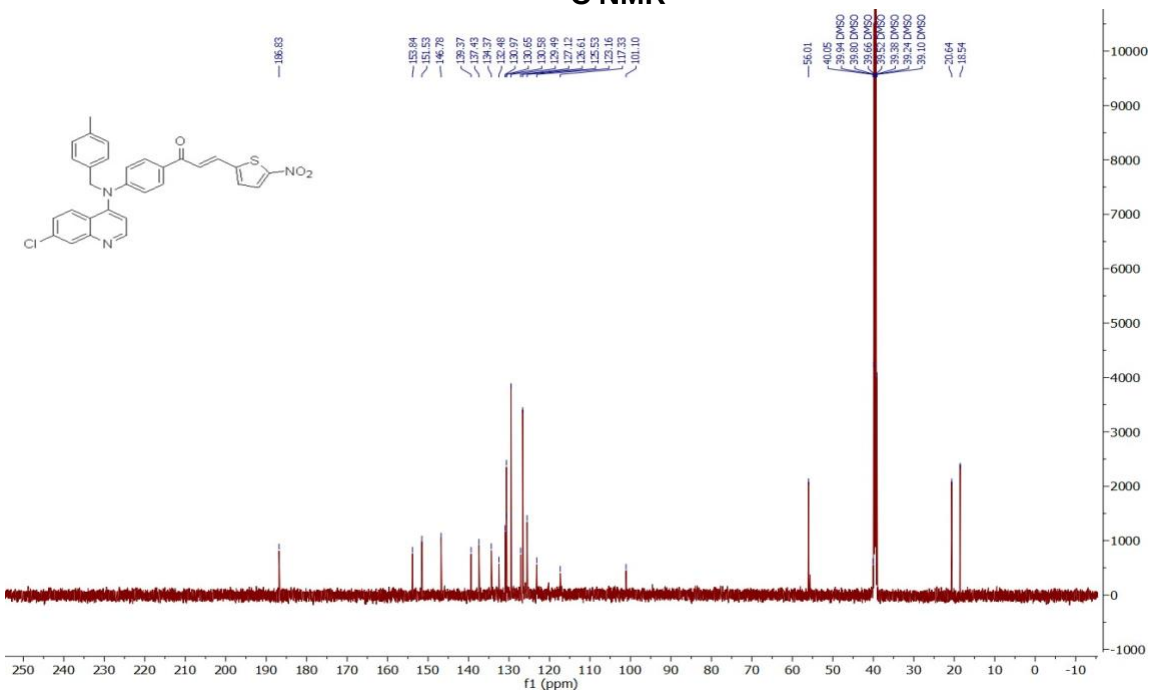


(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**4b**)

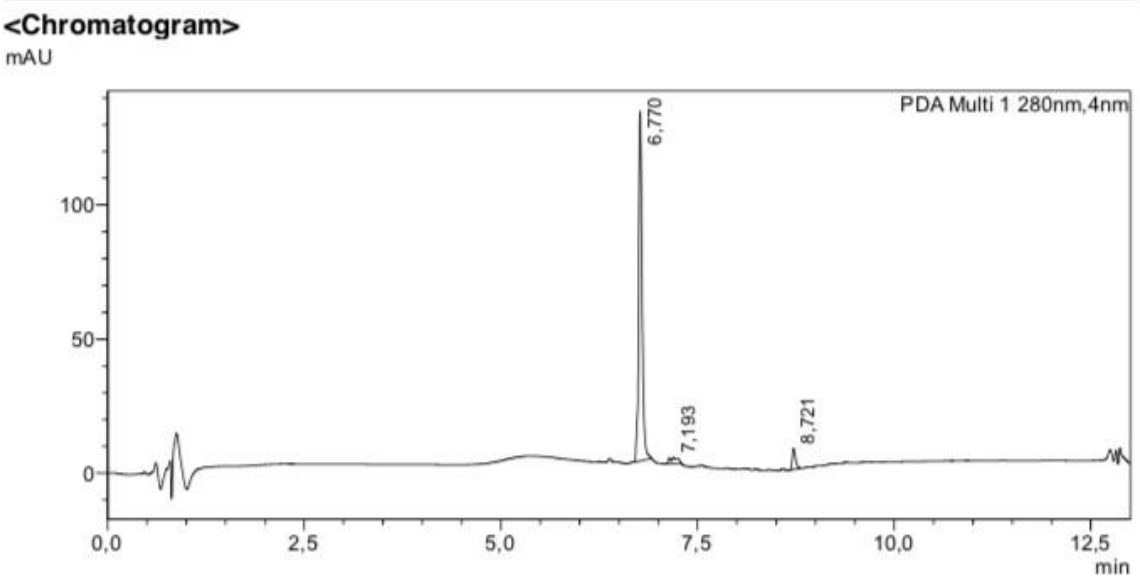
^{1}H NMR



^{13}C NMR

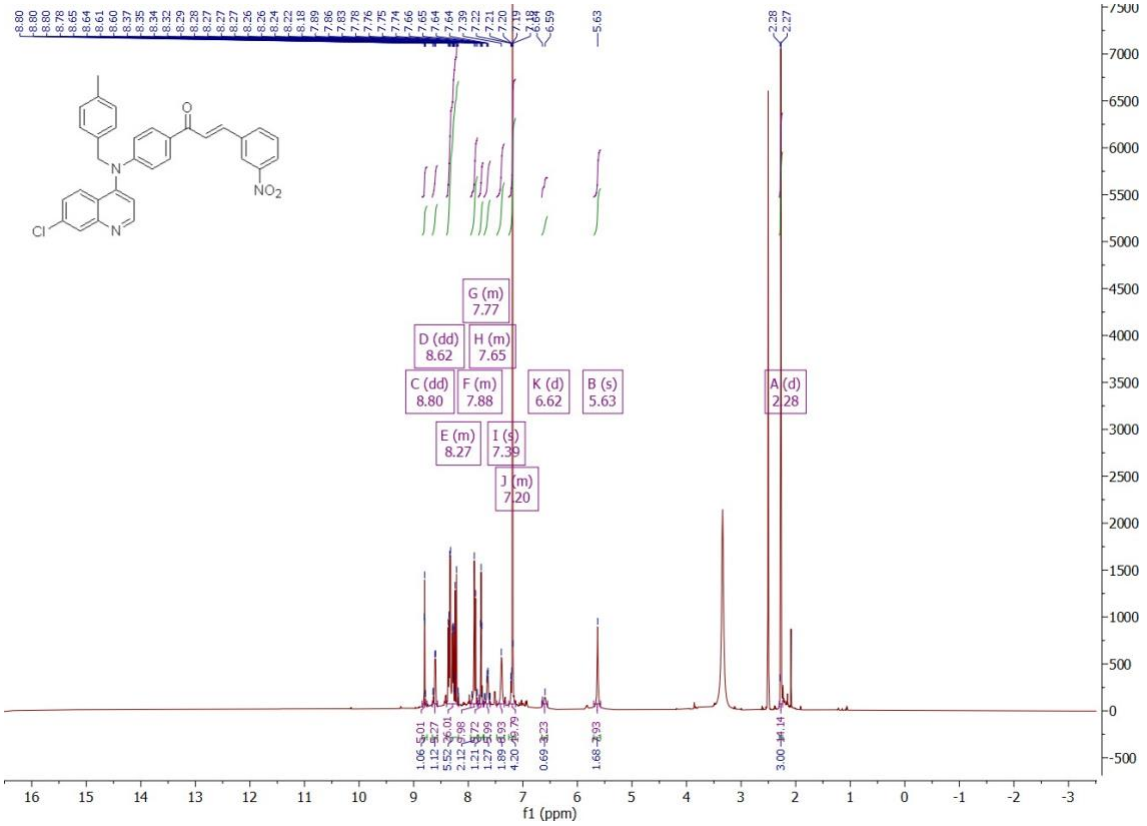


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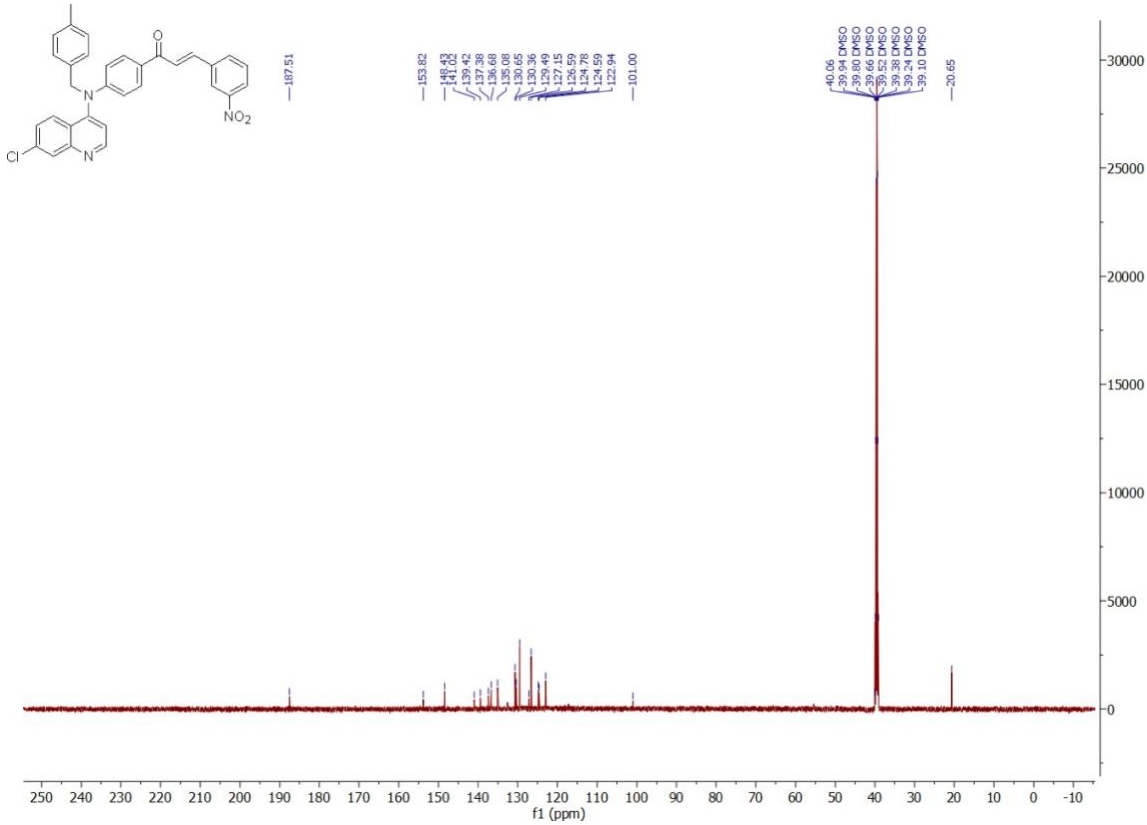

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min

(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**4c**)

^{1}H NMR



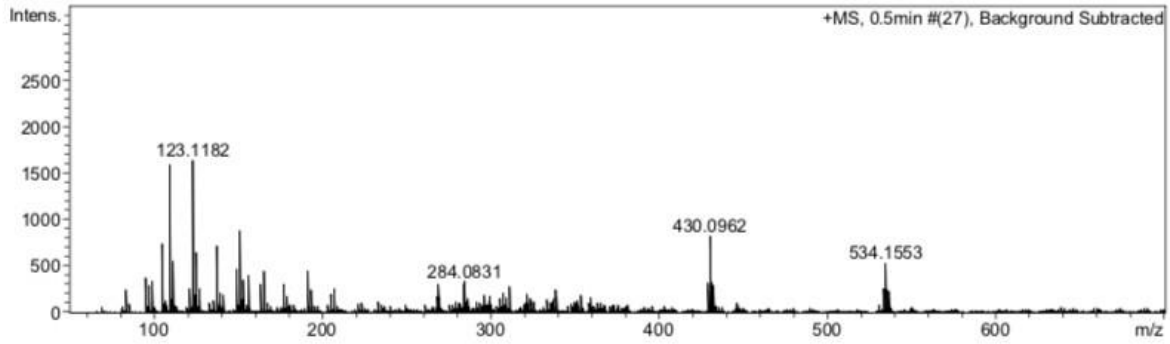
^{13}C NMR



HRMS

Acquisition Parameter

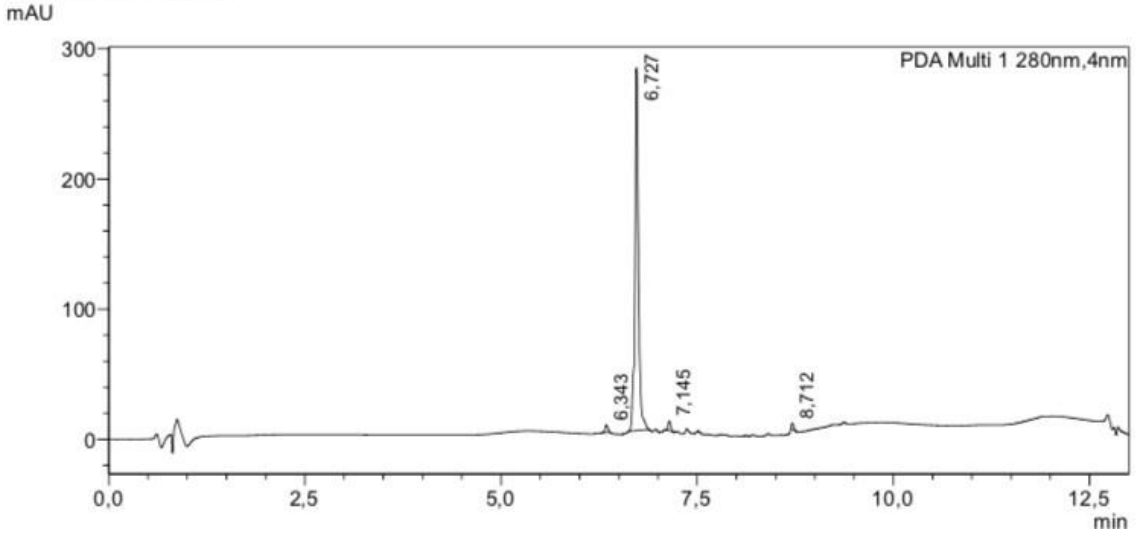
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
430.0962	1	C 32 H 14 O 2	37.81	430.0988	2.6	6.1	45.0	26.0	odd	ok
	2	C 30 H 12 N 3 O	100.00	430.0975	1.3	3.0	46.5	26.5	even	ok
	3	C 27 H 14 N 2 O 4	58.99	430.0948	-1.4	-3.2	61.4	22.0	odd	ok
	4	C 29 H 17 Cl N O	0.03	430.0993	3.1	7.3	176.6	21.5	even	ok
	5	C 24 H 17 Cl N 3 O 3	0.14	430.0953	-0.9	-2.1	177.7	17.5	even	ok
	6	C 26 H 19 Cl O 4	0.16	430.0966	0.4	1.0	178.5	17.0	odd	ok
534.1553	1	C 32 H 25 Cl N 3 O 3	100.00	534.1579	2.6	4.8	242.3	21.5	even	ok

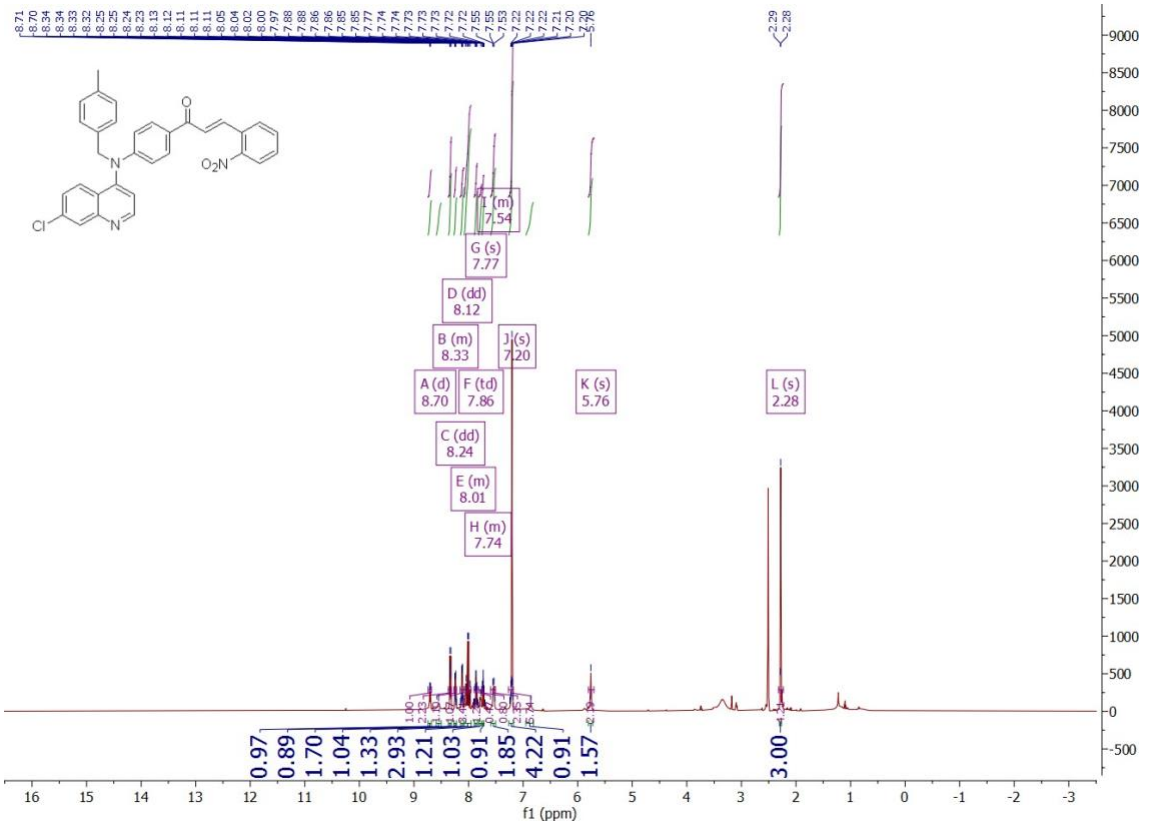
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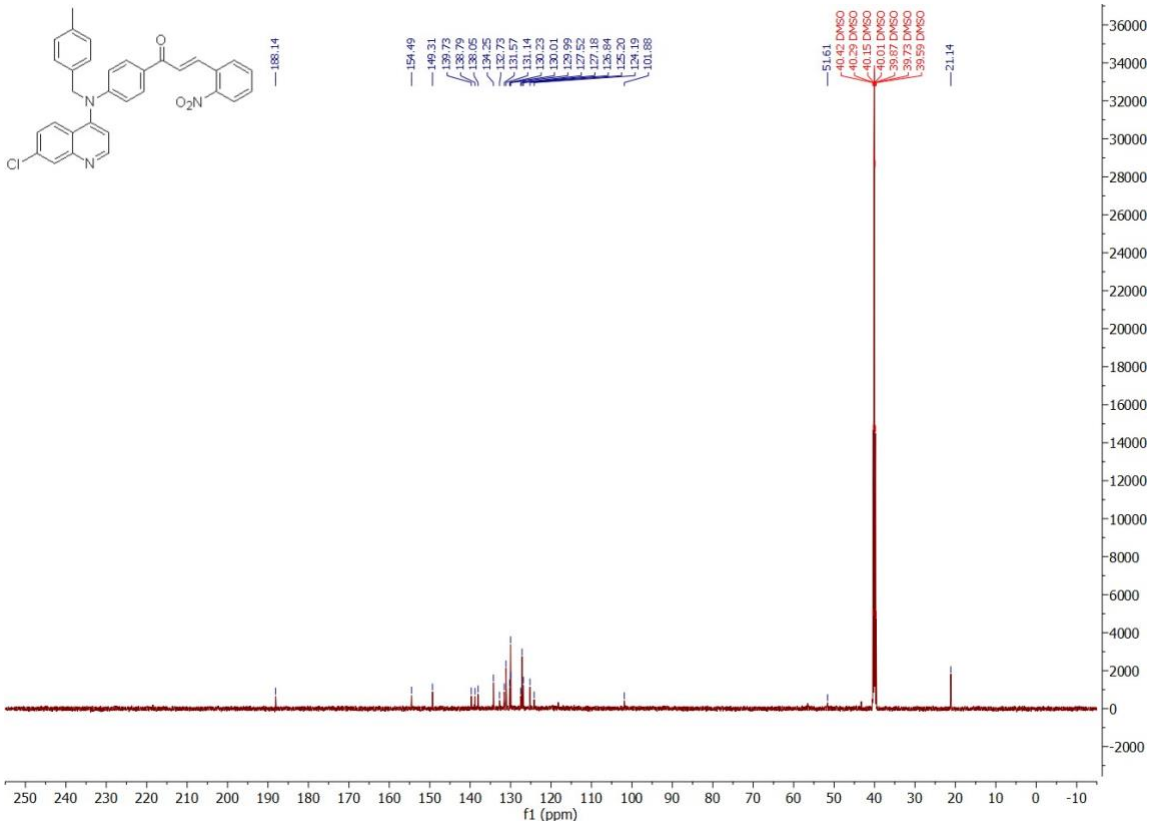


(E)-1-(4-((7-chloroquinolin-4-yl)(4-methylbenzyl)amino)phenyl)-3-(2-nitrophenyl)prop-2-en-1-one (**4d**)

^{1}H NMR



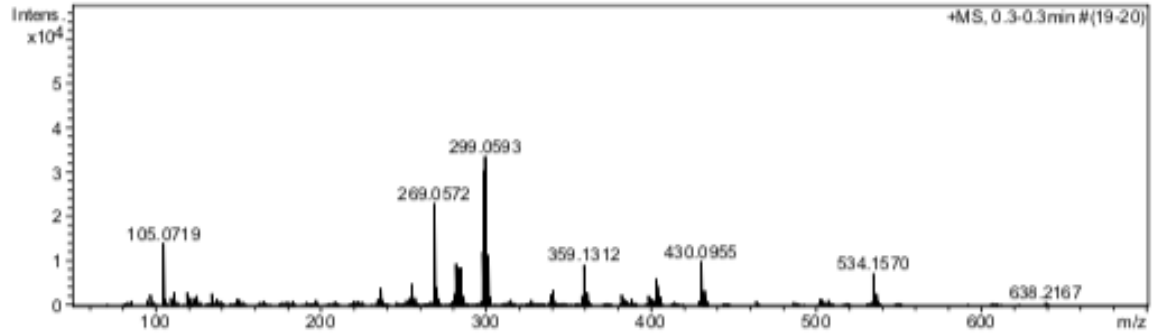
^{13}C NMR



HRMS

Acquisition Parameter

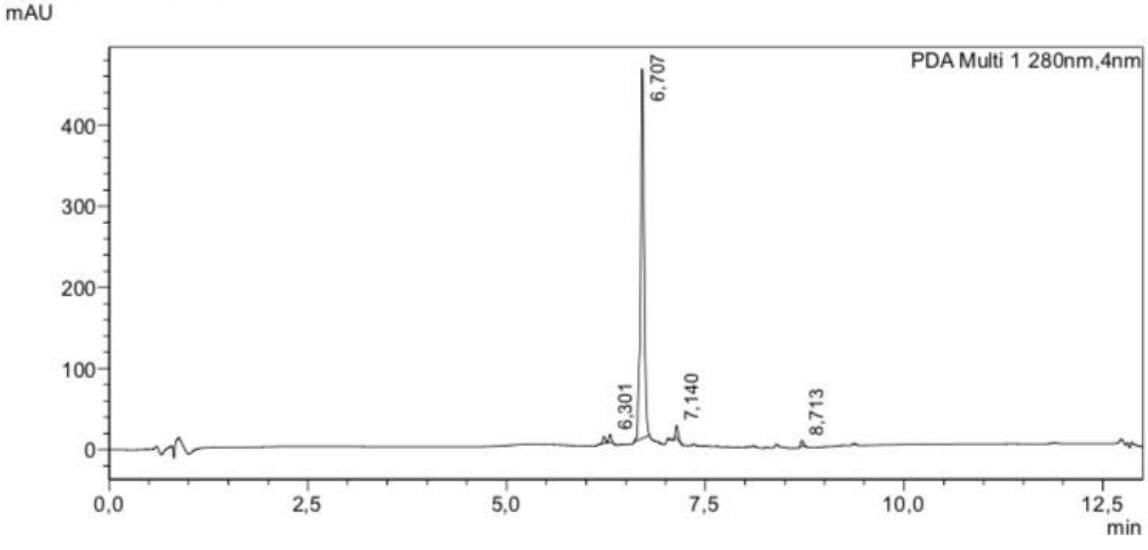
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Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
105.0719	1	C 8 H 9	100.00	105.0699	-2.1	-19.6	1.4	4.5	even	ok
269.0572	1	C 19 H 9 O 2	38.42	269.0597	2.5	9.1	24.7	15.5	even	ok
	2	C 17 H 7 N 3 O	100.00	269.0584	1.1	4.2	25.2	16.0	odd	ok
	3	C 14 H 9 N 2 O 4	73.46	269.0557	-1.6	-5.8	26.3	11.5	even	ok
	4	C 16 H 12 Cl N O	0.51	269.0602	2.9	11.0	124.3	11.0	odd	ok
	5	C 11 H 12 Cl N 3 O 3	0.85	269.0562	-1.1	-4.0	140.1	7.0	odd	ok
	6	C 13 H 14 Cl O 4	1.21	269.0575	0.3	1.0	141.1	6.5	even	ok
299.0593	1	C 21 H 12 Cl	43.93	299.0622	2.9	9.7	27.7	15.5	even	ok
	2	C 16 H 12 Cl N 2 O 2	100.00	299.0582	-1.1	-3.8	47.8	11.5	even	ok
	3	C 22 H 7 N 2	0.11	299.0604	1.1	3.6	178.7	20.5	even	ok
	4	C 19 H 9 N O 3	0.05	299.0577	-1.6	-5.4	183.8	16.0	odd	ok
359.1312	1	C 23 H 20 Cl N 2	100.00	359.1310	-0.2	-0.6	23.0	14.5	even	ok
	2	C 20 H 22 Cl N O 3	11.23	359.1283	-2.9	-8.1	37.6	10.0	odd	ok
	3	C 26 H 17 N O	0.12	359.1305	-0.7	-2.0	165.1	19.0	odd	ok
	4	C 23 H 19 O 4	0.01	359.1278	-3.4	-9.5	168.2	14.5	even	ok
403.1213	1	C 29 H 20 Cl	10.57	403.1248	3.5	8.8	15.6	19.5	even	ok
	2	C 24 H 20 Cl N 2 O 2	100.00	403.1208	-0.5	-1.2	20.9	15.5	even	ok
	3	C 30 H 15 N 2	0.19	403.1230	1.7	4.2	150.6	24.5	even	ok
	4	C 27 H 17 N O 3	0.27	403.1203	-1.0	-2.4	152.3	20.0	odd	ok
430.0955	1	C 26 H 19 Cl O 4	68.55	430.0966	1.1	2.6	11.1	17.0	odd	ok
	2	C 29 H 17 Cl N O	6.72	430.0993	3.8	8.8	12.3	21.5	even	ok
	3	C 24 H 17 Cl N 3 O 3	100.00	430.0953	-0.2	-0.5	14.9	17.5	even	ok
	4	C 32 H 14 O 2	0.03	430.0988	3.3	7.7	154.9	26.0	odd	ok
	5	C 30 H 12 N 3 O	0.07	430.0975	2.0	4.6	158.1	26.5	even	ok
	6	C 27 H 14 N 2 O 4	0.16	430.0948	-0.7	-1.7	158.3	22.0	odd	ok
534.1570	1	C 32 H 25 Cl N 3 O 3	100.00	534.1579	0.9	1.6	16.6	21.5	even	ok

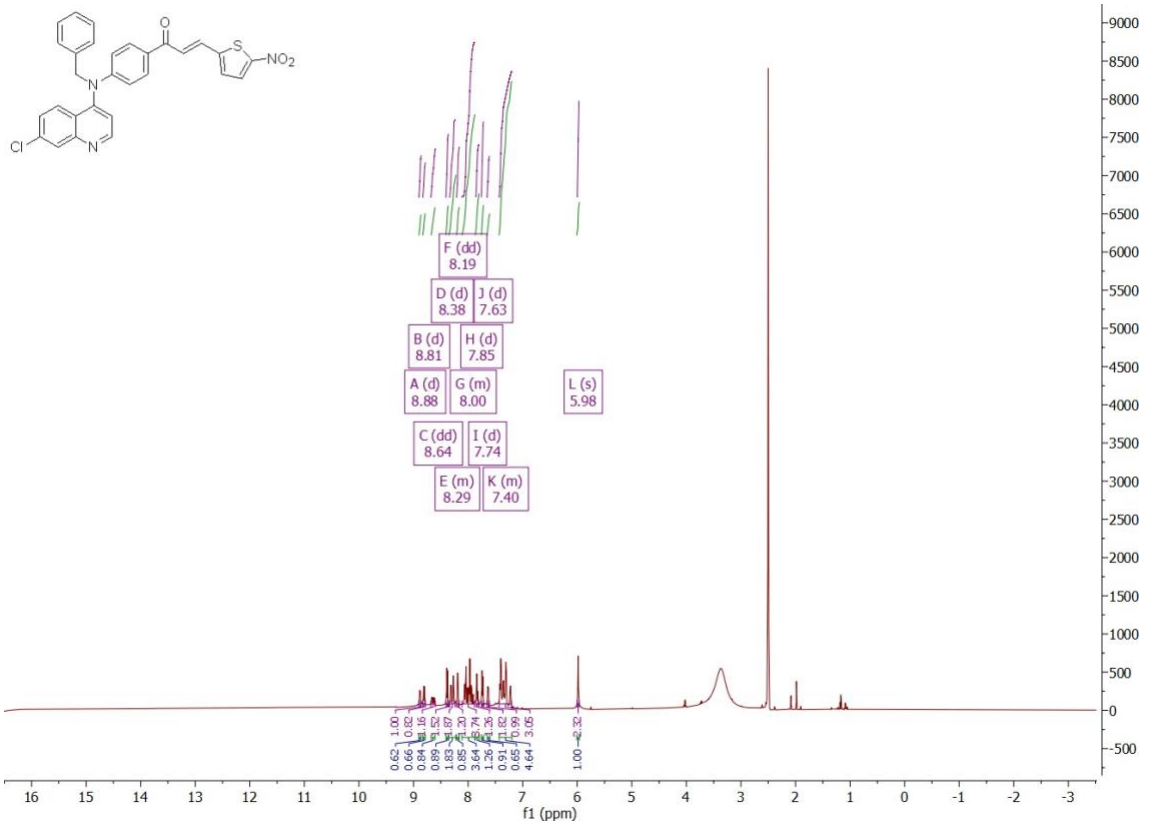
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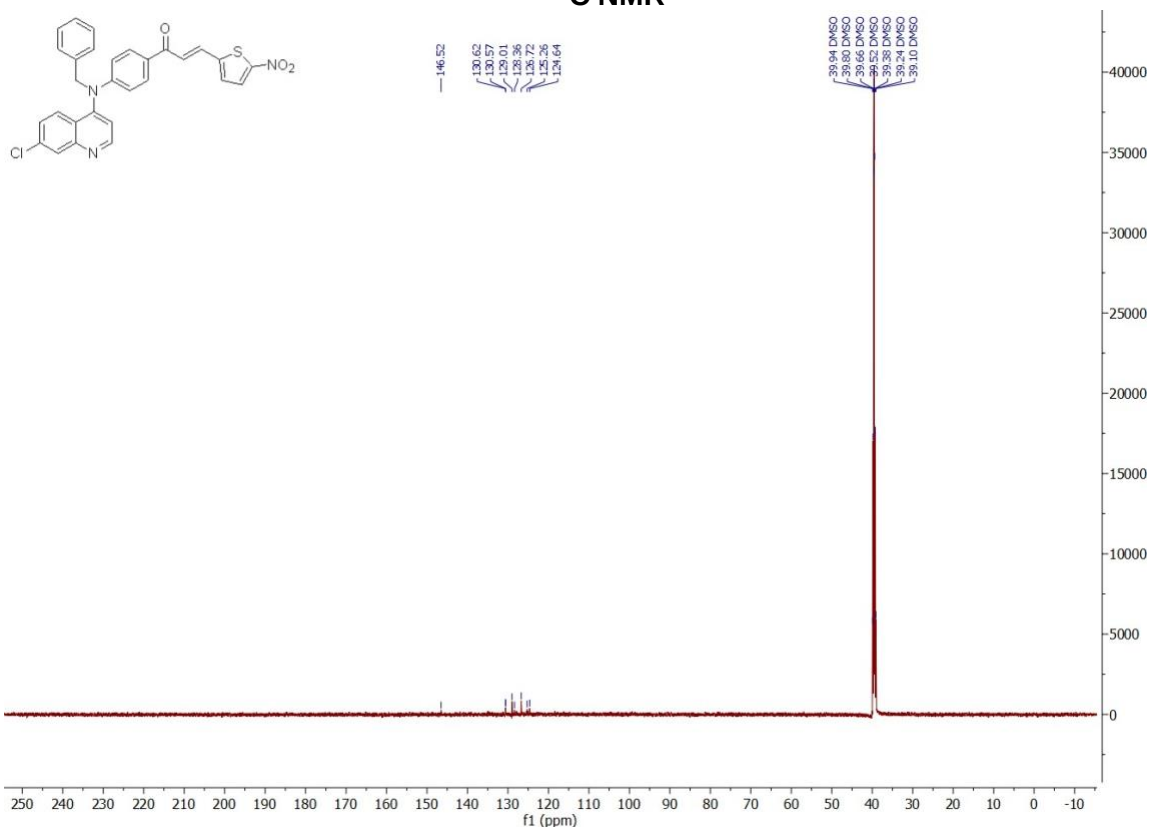


(E)-1-(4-(benzyl(7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**4e**)

^{1}H NMR

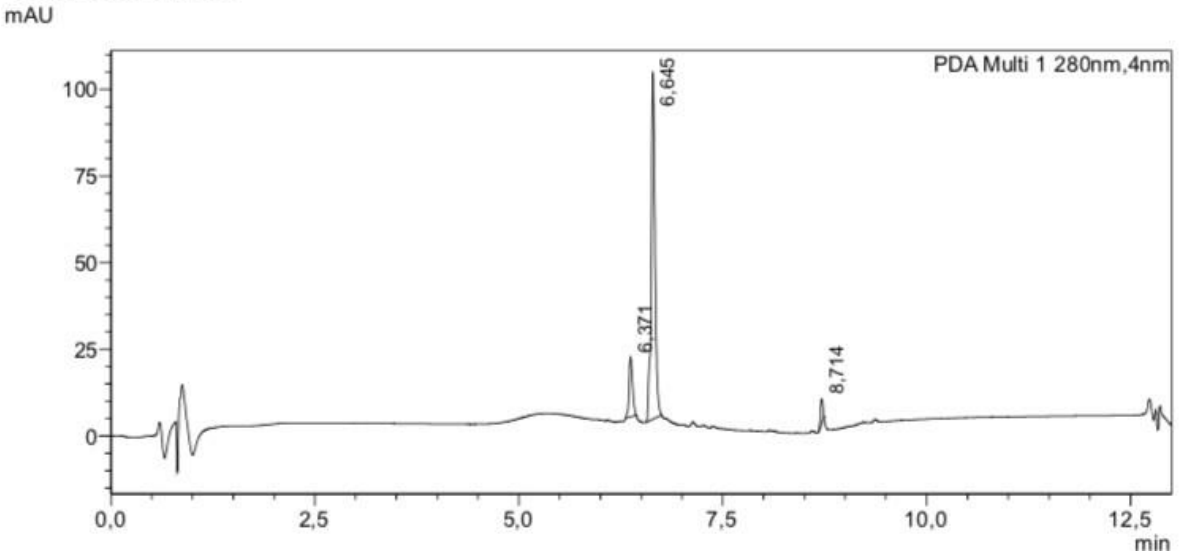


^{13}C NMR



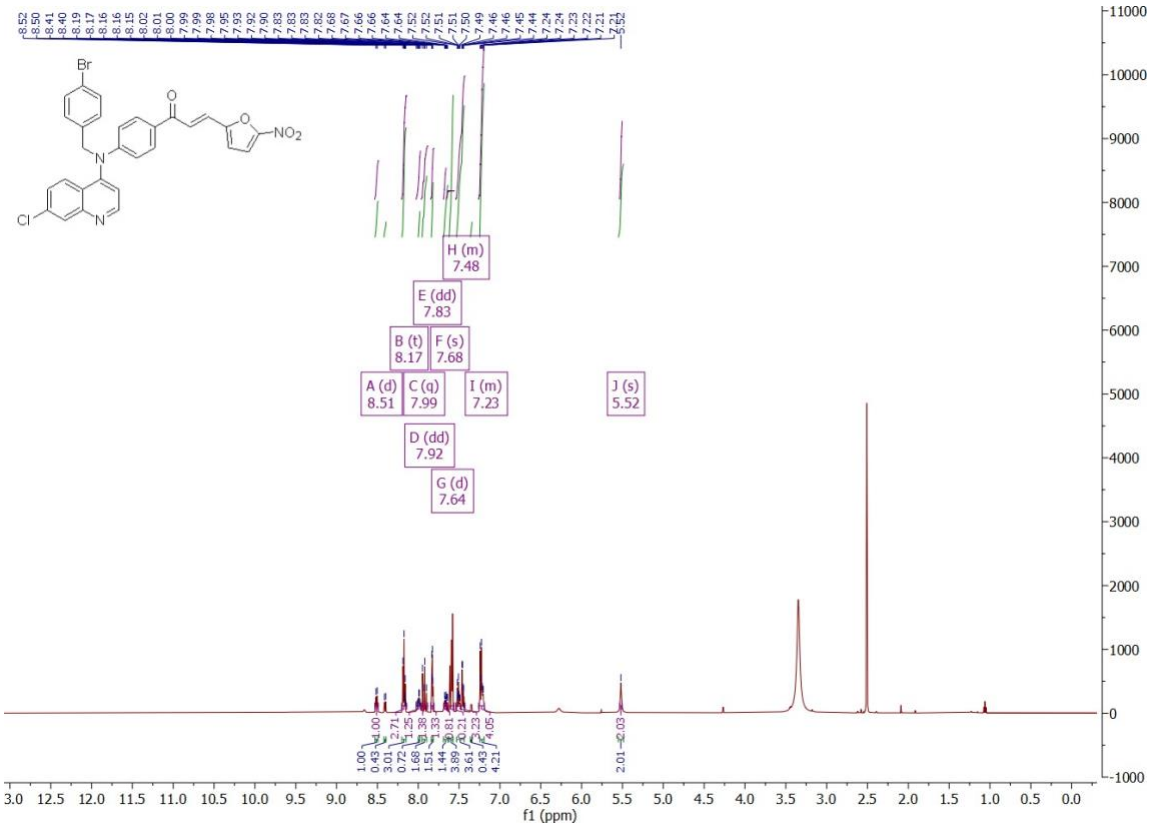
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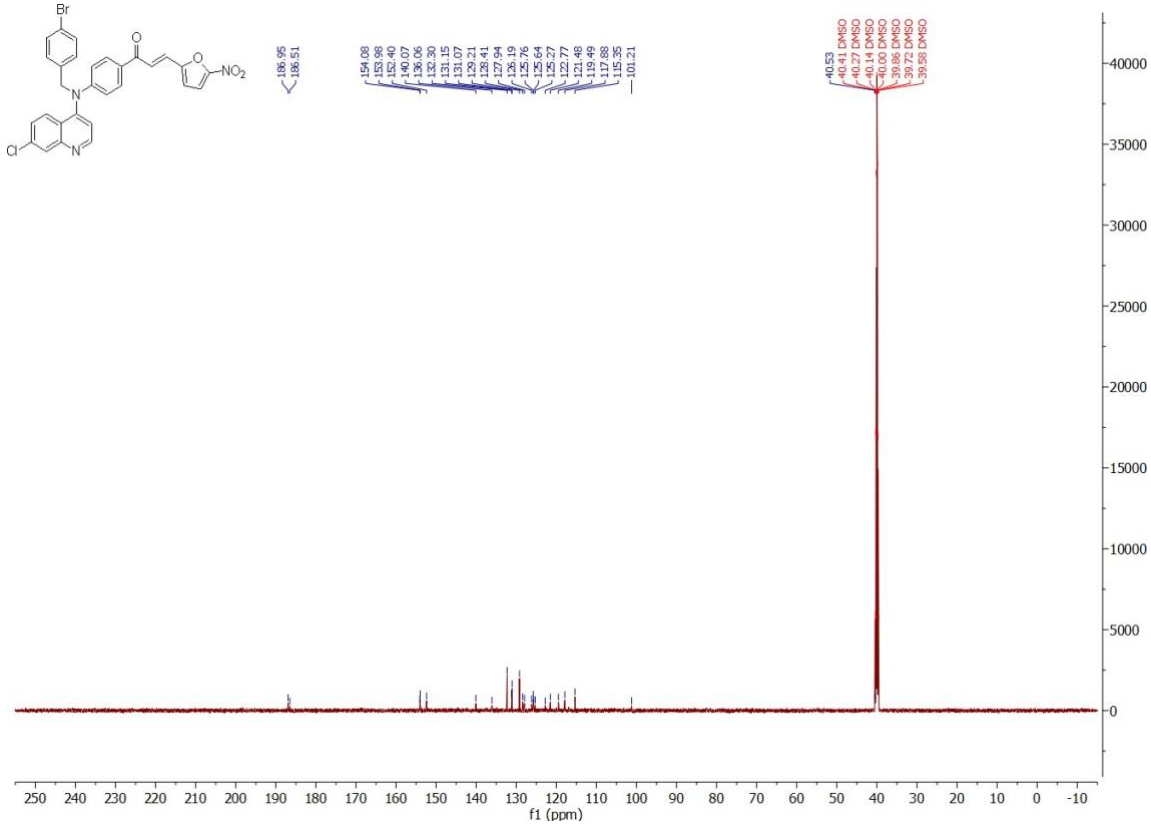


(E)-1-(4-((4-bromobenzyl)(7-chloroquinolin-4-yl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**4f**)

^{1}H NMR

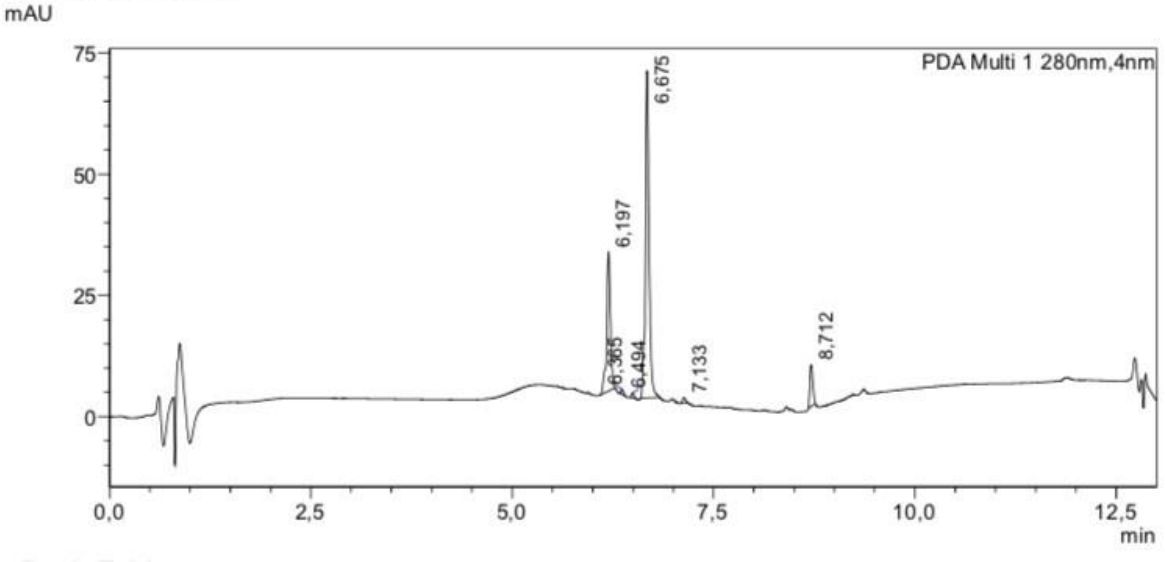


^{13}C NMR



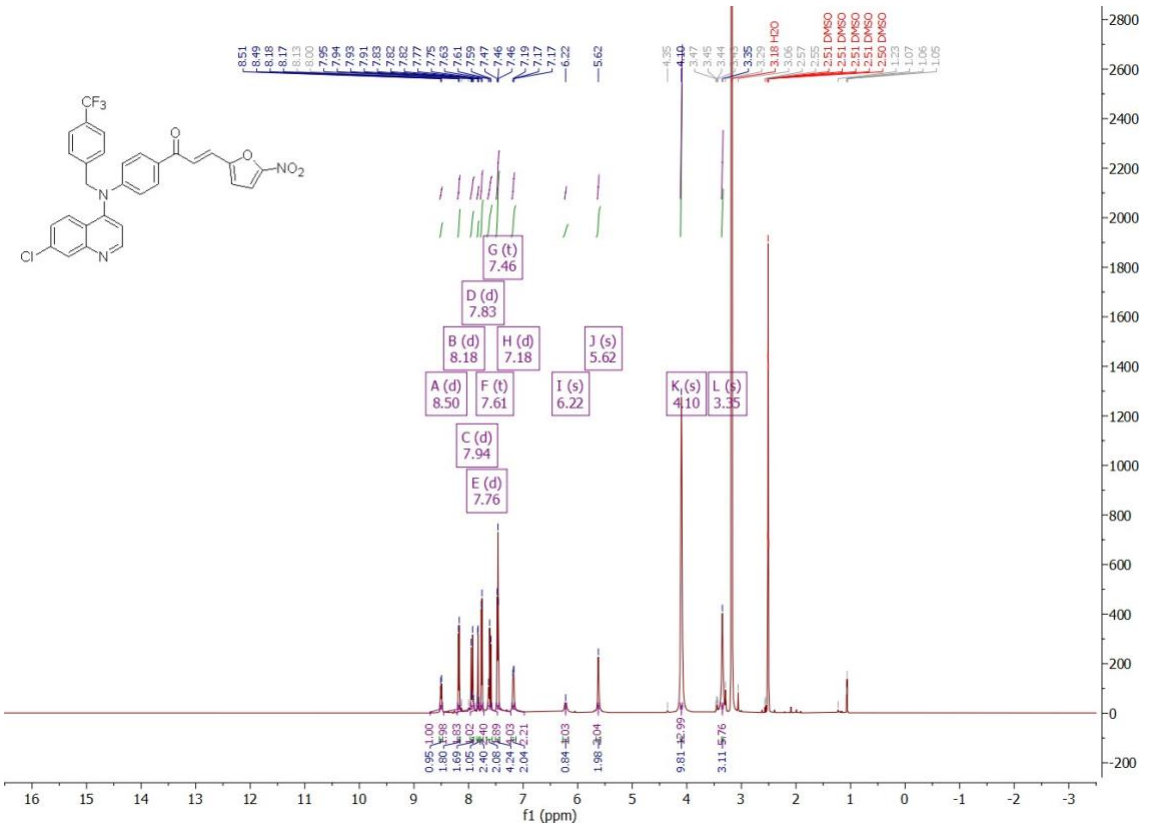
HPLC

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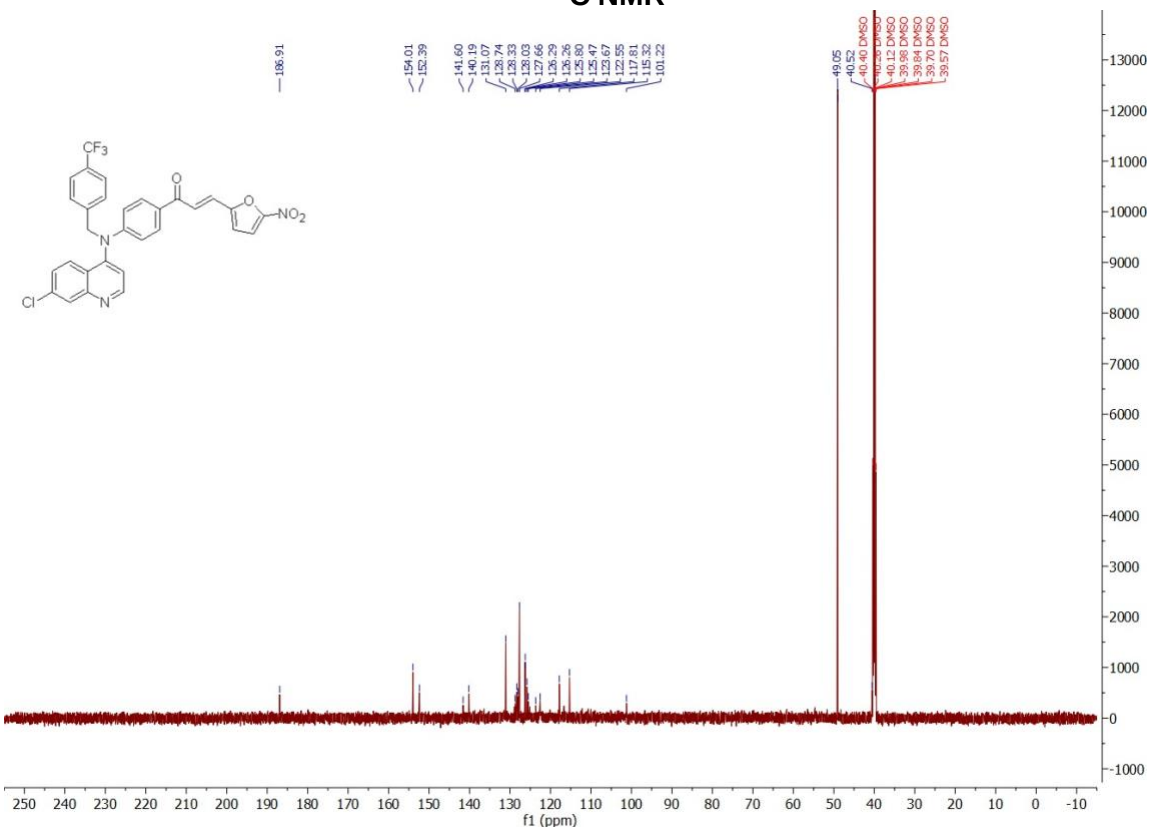


(E)-1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)-3-(5-nitrofuran-2-yl)prop-2-en-1-one (**4g**)

^{1}H NMR



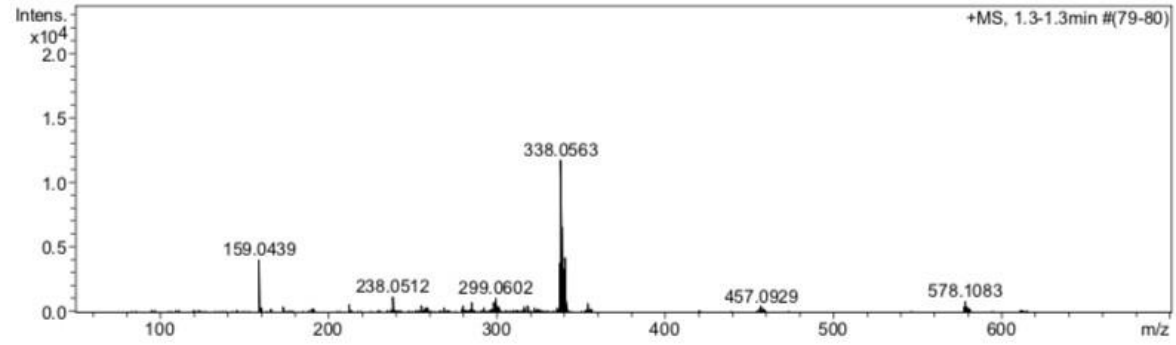
^{13}C NMR



HRMS

Acquisition Parameter

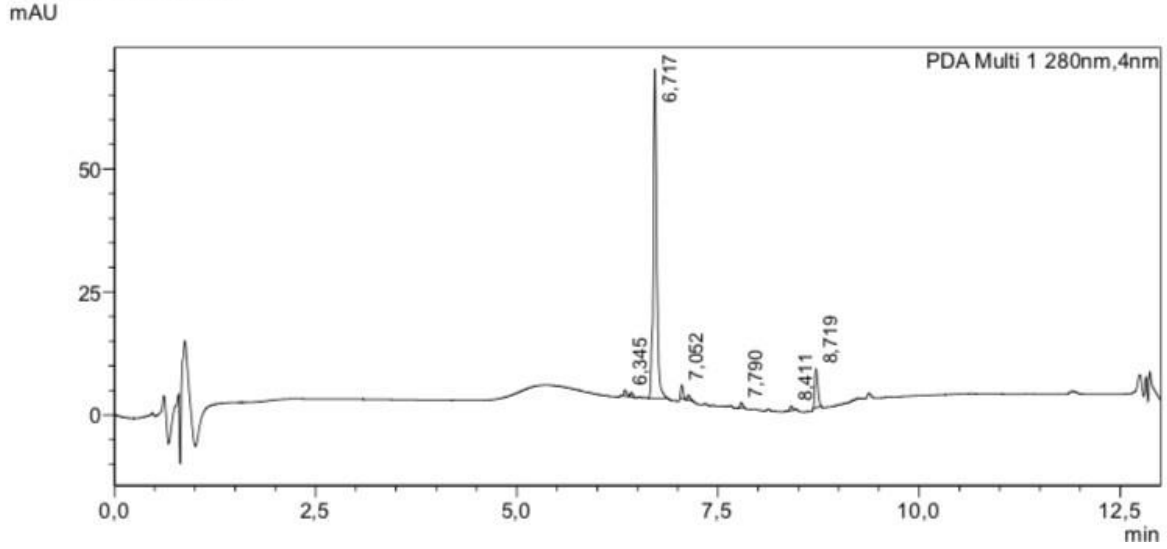
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
338.0563	1	C 20 H 11 Cl F 2 N	75.76	338.0543	-2.1	-6.1	30.8	14.5	even	ok
	2	C 19 H 13 Cl N O 3	100.00	338.0578	1.5	4.5	35.1	13.5	even	ok
	3	C 26 H 7 F	0.01	338.0526	-3.7	-10.9	187.8	23.0	odd	ok
	4	C 25 H 8 N O	0.00	338.0600	3.7	11.0	189.0	22.5	even	ok
	5	C 22 H 10 O 4	0.04	338.0574	1.0	3.1	191.3	18.0	odd	ok
	6	C 23 H 8 F 2 O	0.01	338.0538	-2.5	-7.5	193.8	19.0	odd	ok
	7	C 20 H 8 N 3 O 3	0.04	338.0560	-0.3	-0.9	194.8	18.5	even	ok
	8	C 21 H 6 F 2 N 3	0.00	338.0524	-3.9	-11.5	195.9	19.5	even	ok
	9	C 20 H 9 F 3 O 2	0.02	338.0549	-1.4	-4.2	198.5	15.0	odd	ok
578.1083	1	C 30 H 20 Cl F 3 N 3 O 4	100.00	578.1089	0.6	1.0	80.0	20.5	even	ok

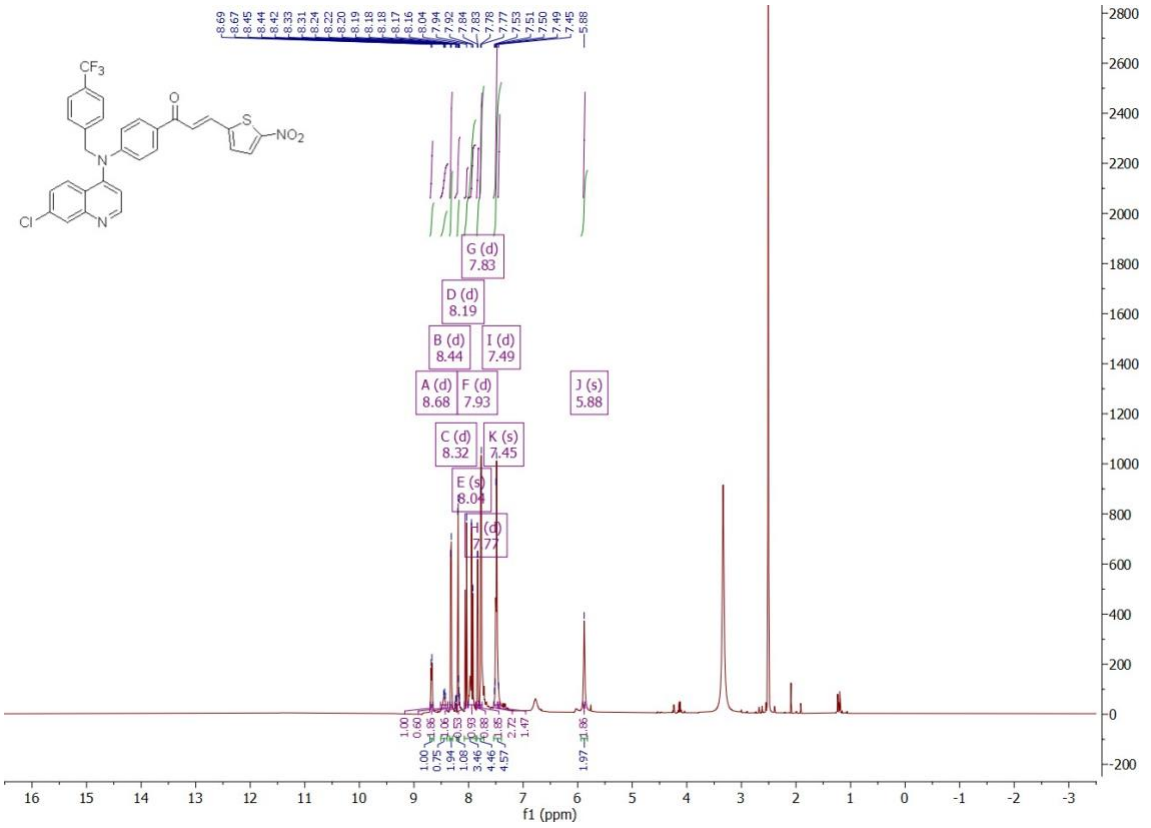
HPLC

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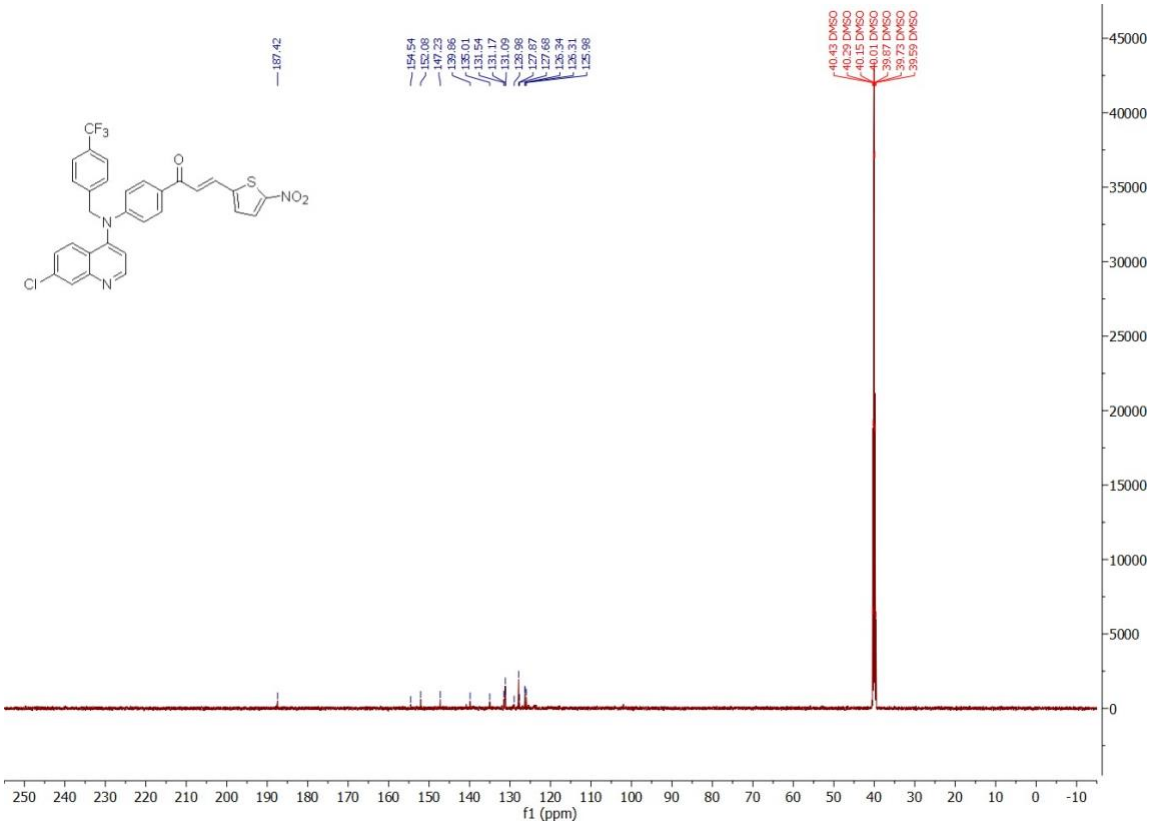


(E)-1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)-3-(5-nitrothiophen-2-yl)prop-2-en-1-one (**4h**)

^{1}H NMR



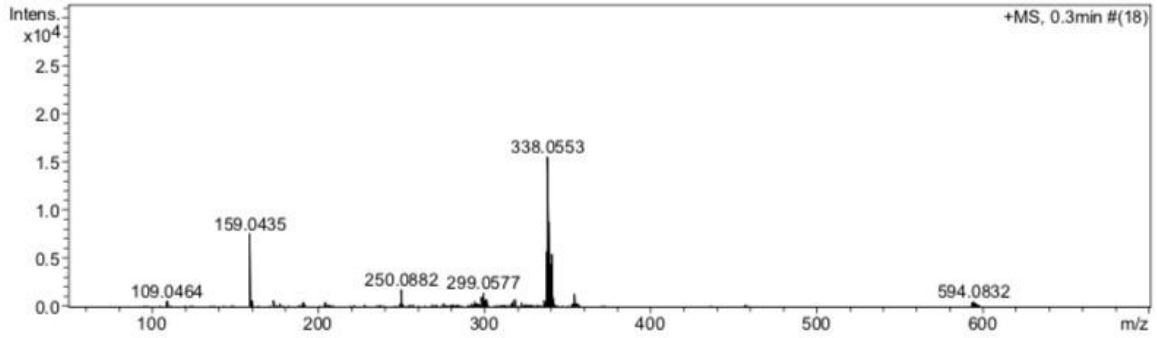
^{13}C NMR



HRMS

Acquisition Parameter

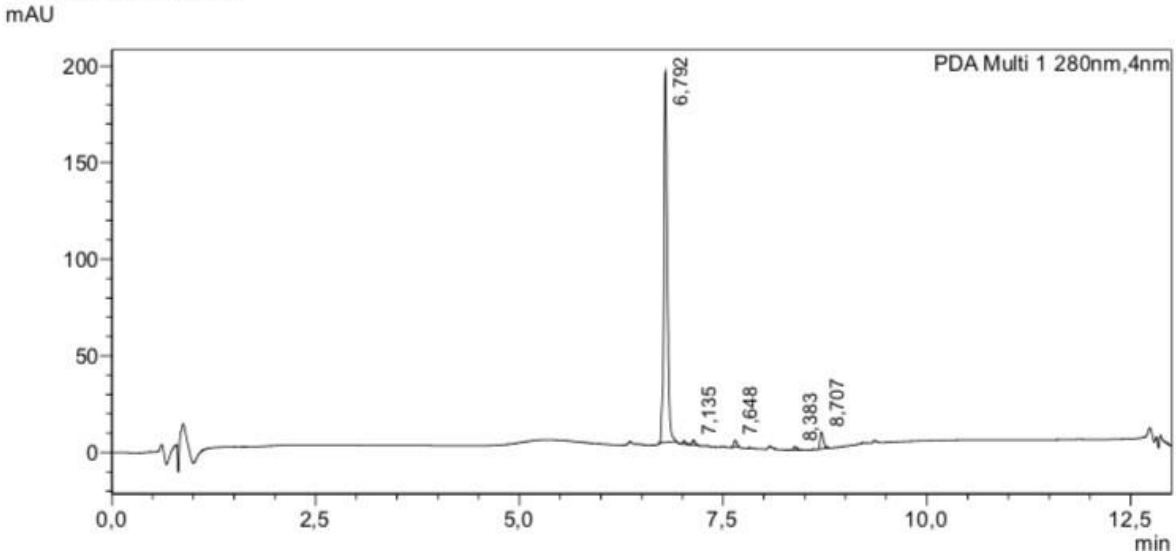
Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
593.0757	1	C 30 H 19 Cl F 3 N 3 O 3 S	100.00	593.0782	2.5	4.3	321.2	21.0	odd	ok
594.0832	1	C 30 H 20 Cl F 3 N 3 O 3 S	100.00	594.0861	2.9	4.8	114.2	20.5	even	ok

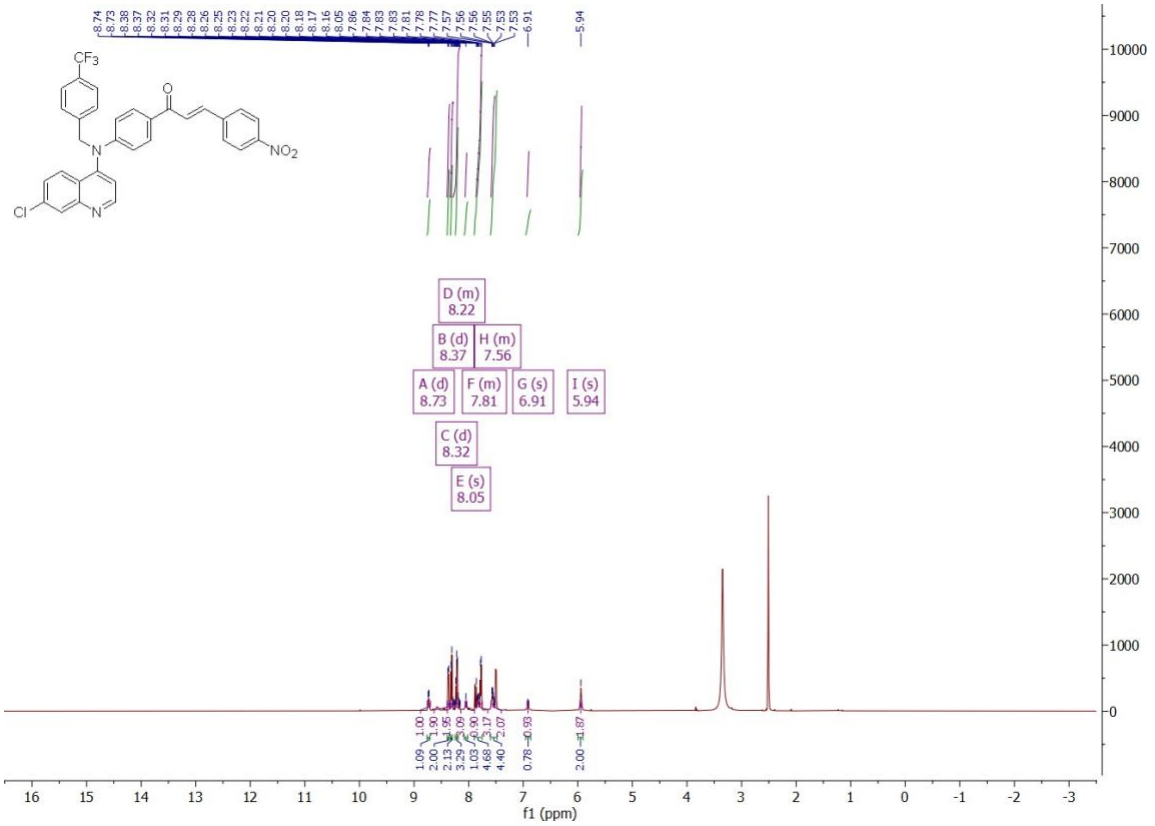
HPLC

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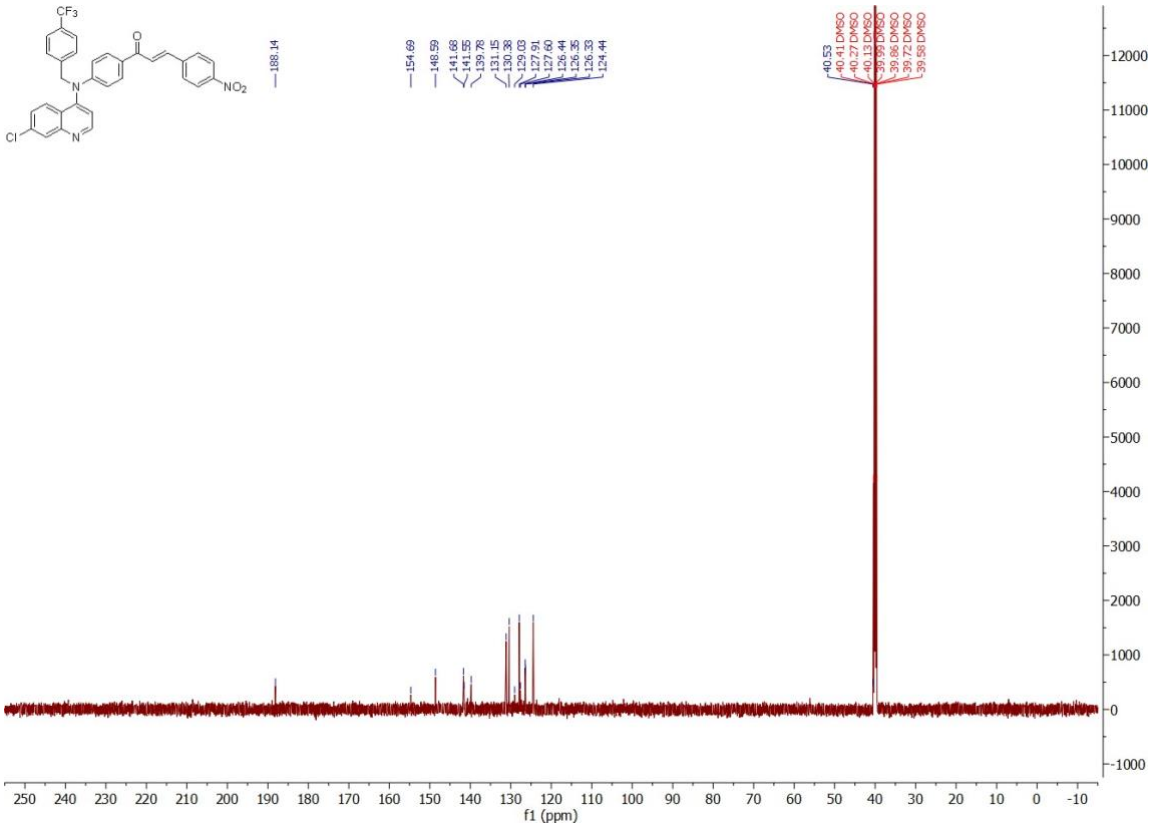


(E)-1-(4-((7-chloroquinolin-4-yl)(4-(trifluoromethyl)benzyl)amino)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (**4i**)

^{1}H NMR



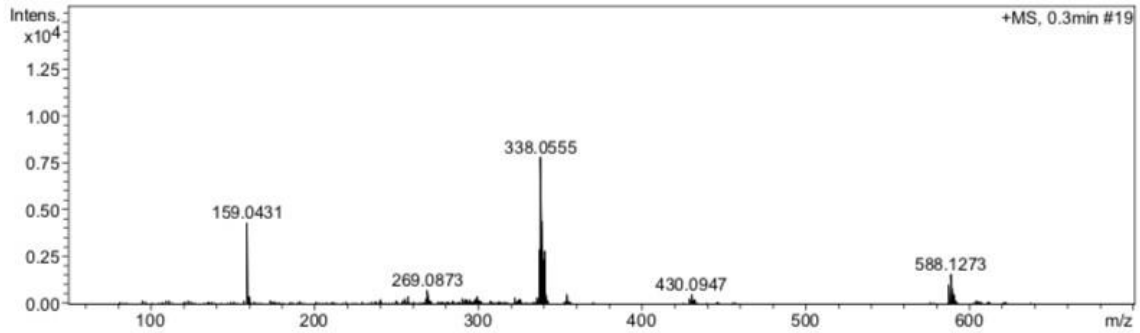
^{13}C NMR



HRMS

Acquisition Parameter

Source Type	APCI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	100.0 Vpp	Set Divert Valve	Waste



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
159.0431	1	C 7 H 10 Cl N O	100.00	159.0445	1.4	8.8	751.7	3.0	odd	ok
	2	C 10 H 7 O 2	0.56	159.0441	0.9	5.7	769.6	7.5	even	ok
	3	C 8 H 5 N 3 O	0.06	159.0427	-0.4	-2.7	777.7	8.0	odd	ok
	4	C 8 H 6 F 3	0.01	159.0416	-1.5	-9.7	782.0	4.5	even	ok
	5	C 7 H 8 F O 3	0.00	159.0452	2.1	12.9	783.6	3.5	even	ok
	6	C 5 H 7 N 2 O 4	0.00	159.0400	-3.1	-19.6	788.2	3.5	even	ok
	7	C 5 H 6 F N 3 O 2	0.00	159.0439	0.7	4.5	788.4	4.0	odd	ok
	8	C 4 H 9 F 2 O 4	0.00	159.0463	3.2	20.1	795.6	-0.5	even	ok
338.0555	1	C 20 H 11 Cl F 2 N	100.00	338.0543	-1.3	-3.7	34.4	14.5	even	ok
	2	C 26 H 7 F	0.01	338.0526	-2.9	-8.5	189.1	23.0	odd	ok
	3	C 22 H 10 O 4	0.02	338.0574	1.8	5.4	193.3	18.0	odd	ok
	4	C 23 H 8 F 2 O	0.01	338.0538	-1.7	-5.2	195.6	19.0	odd	ok
	5	C 20 H 8 N 3 O 3	0.03	338.0560	0.5	1.5	196.9	18.5	even	ok
	6	C 21 H 6 F 2 N 3	0.00	338.0524	-3.1	-9.1	197.9	19.5	even	ok
	7	C 20 H 9 F 3 O 2	0.02	338.0549	-0.6	-1.8	200.7	15.0	odd	ok
588.1273	1	C 32 H 22 Cl F 3 N 3 O 3	100.00	588.1296	2.3	3.9	107.4	21.5	even	ok

HPLC

<Chromatogram>

