SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SUBSTITUTED 2-QUINOLINE THIOSEMICARBAZONES

2017

SIBONISO SHEZI
SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SUBSTITUTED 2-QUINOLINE THIOSEMICARBAZONES

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2017

A thesis submitted to the School of Chemistry and Physics in the College of Agriculture, Engineering and Science for the fulfilment of the degree of Master of Science.
Preface

I hereby declare that the thesis entitled “Synthesis, characterisation and antibacterial activity of substituted 2-quinoline thiosemicarbazones” submitted to the University of KwaZulu-Natal for the award of the degree of Master of Science in Chemistry under the supervision of Professor Neil A. Koorbanally represents original work by the author and has not been submitted in full or part for any degree or diploma at this or any other University.

Where use was made of the work of others it has been duly acknowledged in the text. This work was carried out in the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus, Durban, South Africa.

Signed: ____________________   Date: ___________________

Siboniso Shezi, BSc(HONS)

As the candidate’s supervisor, I have approved this dissertation for submission

Signed: ____________________   Date: ___________________

Professor Neil A. Koorbanally, PhD (Natal)
DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, Siboniso Shezi declare that

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2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed …………………………………………………………………………
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- To the National Research Foundation (NRF) for generous financial support through a Scarce Skills Scholarship.

- All glory to the almighty God who has been everything to me.
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
<td>bd</td>
<td>broad doublet</td>
</tr>
<tr>
<td>bs</td>
<td>broad singlet</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>proton nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>carbon-13 nuclear magnetic resonance spectroscopy</td>
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<tr>
<td>COSY</td>
<td>correlation spectroscopy</td>
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<td>doublet</td>
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<td>doublet of doublets</td>
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<td>ddd</td>
<td>doublet of doublet of doublets</td>
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<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DMSO-$d_6$</td>
<td>deuterated dimethyl sulfoxide</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single quantum coherence</td>
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<td>Hz</td>
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</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography–mass spectrometry</td>
</tr>
<tr>
<td>MBC</td>
<td>minimum bactericidal concentration</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>MHA</td>
<td>Mueller-Hinton agar</td>
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<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>NHP</td>
<td>N-hydroxyphthalimide</td>
</tr>
<tr>
<td>NOESY</td>
<td>nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>PIDA</td>
<td>phenylidione(III) diacetate</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
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<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
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<tr>
<td>TMS</td>
<td>tetramethylsilane</td>
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ABSTRACT

Fifteen quinoline-2-thiosemicarbazone hybrid derivatives were synthesised in a three step reaction involving formation of the quinoline, oxidation of the 2-methyl group and condensation to the thiosemicarbazones. The phenylhydrazines were prepared in an additional step prior to the condensation. Twelve of the fifteen hybrid molecules were novel. A full structural elucidation of all synthesised compounds were carried out using amongst others 1D and 2D NMR spectroscopy and mass spectrometry. The data presented here will provide a basis for the identification of further molecules of this type. The hybrid molecules were then subjected to antibacterial bioassays against Gram -ve and Gram +ve bacterial strains. Unfortunately, only one compound, the 6-bromo-4'-fluoro derivative on the (E)-2-quinoline-2-yl)methylene-N-phenylhydrazinecarbothiamide framework showed any antibacterial activity. They were active against both Staphylococcus aureus and methicillin resistant Staphylococcus aureus (MRSA) at 387 μM.

Keywords: Quinoline-2-thiosemicarbazone, antibacterial, Doebner-von Miller, structural elucidation
STRUCTURES OF THE SYNTHESISED COMPOUNDS REPORTED IN THIS THESIS

![Chemical Structure](image)

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Chapter 1. Introduction

Since the discovery of penicillin in 1928 by Sir Alexander Fleming, antibiotics have been used to treat bacterial infections, preventing secondary infections and even death. Even prior to this, there was evidence of antibiotics being used, with traces of tetracycline being found in human skeletal remains of Egyptian and Sudanese Nubia populations dating back to 350-550 CE (Aminov, 2010). These antibiotics have also played an important role in medicine and surgery preventing bacterial infections from setting in after surgery (Ventola, 2015). However, over the years, overuse, inappropriate and incorrect prescribing, self-medicating and extensive agricultural use of antibiotics has led to antibiotic resistance (Ventola, 2015; Rather et al., 2017).

Antibiotic resistance occurs when bacteria modifies its structure and form in some way that reduces or eliminates antibiotic binding reducing or eliminating their effectiveness (Bisht et al., 2009). As a result, resistant bacteria are able to survive and mutate, leading to bacteria that are not susceptible to known antibiotics. The consequences are prolonged illnesses, greater risk of death, longer periods of hospitalization and an increase in infections. A huge percentage of bacteria (at least 70%) has become resistant to at least one of the most commonly used antibiotics used for treatment (Bisht et al., 2009).

To combat this problem, scientists and pharmaceutical companies have developed and introduced many new antibiotics. This was rampant from 1940-1980, but significantly declined thereafter, largely contributing to the antibiotic resistance crisis we find ourselves in today (Martens and Demain, 2017). This has been attributed to the merging of pharmaceutical companies, leading to less productive companies, a decline in Natural Products research, which inspired or contributed to the development of antibiotics and the cost involved in producing
these drugs (12-15 years and costing in the region of 1.2 billion US dollars) (Martens and Demain, 2017).

However, antibiotic resistance has now become a crisis, since the rate at which these antibiotics are being rendered inactive due to antibiotic resistance is alarming with more and more bacteria becoming resistant to commonly used drugs. For Gram positive bacteria, this has been described as a crisis that is under control and for Gram negative bacteria, a crisis going out of control (Rossolini et al., 2014). The inactivity of current antibiotics through antibiotic resistance will lead to loss of human lives if alternative antibiotics are not discovered soon. There is thus an urgent need for the development of new antimicrobials to treat bacteria that have become resistant to first line antibacterial agents used in treatment.

The current project aims to address this by synthesising new molecules, which have a good chance of having antibacterial activity and hence could be developed into alternative antibiotics to currently marketed drugs. We aim to use an approach of creating hybrid molecules. Hybrid molecules are those incorporating the frameworks of two molecular scaffolds, each with bioactivity of their own. These compounds contain the basic framework of each of the individual structures. The desired effect of the hybrid molecule is to have a synergistic effect between the different pharmacophores, increasing the activity of either of them alone. The ultimate prize is one drug which can treat more than one disease or medical condition or enhance the therapeutic potential of an existing drug. These molecules are usually formed by an organic reaction with two functional groups, one on each molecular framework. Quinoline hybrids have produced compounds with increased bioactivity (Vandekerckhove and D’Hooghe, 2015), for example anti-malarial (Burgess et al., 2006), antitubercular (Jain et al., 2016),
anticancer (Solomon et al., 2010; Alegaon et al., 2017), antileishmanial (Coa et al., 2017) and antimicrobial activities (Desai et al., 2013) amongst others. Likewise, thiosemicarbazide hybrids have also shown good bioactivity such as antidiabetic (Taha et al., 2016), antibacterial, antitubercular (Rane et al., 2014) and anticancer agents (Rane et al., 2015).

When a quinoline with an aldehyde or ketone substituent reacts with a thiosemicarbazide moiety, the hybrid molecule that forms is termed a quinoline-thiosemicarbazone. These quinoline-thiosemicarbazone hybrids have shown anticancer (Huang et al., 2010), antimicrobial (Abdel-Moty et al., 2005; Kulkarni et al., 2010), anti-oxidant (Ramachandran et al., 2012), anti-HIV, antitubercular (Banerjee et al., 2010), anticonvulsant and analgesic activities (Aly et al., 2010).

1.1 Quinolines

Quinoline is an aromatic heterocyclic compound consisting of two fused rings, a benzene ring fused with a pyridine ring (Figure 1.1), and has the synonyms benzopyridine, benzo[b]pyridine, and benzazine. The quinoline nucleus occurs in several naturally occurring compounds (O'Donnell et al., 2006; Michael, 2008; Zhang et al., 2012) and pharmacologically active substances displaying a broad range of biological activity (Gopaul et al., 2015). These include antimalarial (Vandekerckhove and D'Hooghe, 2015), anti-cancer (Afzal et al., 2015; Gopaul et al., 2015), antioxidant (Maddela et al., 2015), anti-HIV (Luo et al., 2009), antimicrobial (Musiol et al., 2011), antileishmanial (Gopinath et al., 2013), antimycobacterial (Eswaran et al., 2010), and anticonvulsant activities (Siddiqui, 2010).
Several natural occurring compounds that contain the quinoline moiety are either pharmaceuticals or employed as lead compounds for the development of new and more potent drugs (Kumar et al., 2009). One such example is quinine (Figure 1.2) isolated from the bark of the cinchona tree, used in the treatment of malaria (Kumar et al., 2009). This initial discovery was followed by the development of more potent drugs such as chloroquine, primaquine and mefloquine (Figure 1.2) (Kumar et al., 2009).

Synthesis

Many methods are used to synthesise quinolines (Yamashkin and Oreshkina, 2006; Madapa et al., 2008). Most start with simple anilines and make use of electrophiles, which form a heterocyclic ring from their reaction. Reactions used to synthesise quinolines include the Skraup, Conrad-Limpach, Combes, Pavarov, Doebner and Doebner-von Miller reaction (Li and Corey, 2004; Ramann and Cowen, 2016).
The Skraup synthesis involves anilines, such as \( m \)-toluidine reacting with glycerol in the presence of a sulphuric acid catalyst and nitrobenzene (an oxidising agent in the reaction) forming a mixture of 7-methylquinoline and 5-methylquinoline (Scheme 1-1) (Zibaseresht et al., 2013).

![Scheme 1-1 Skraup quinoline synthesis](image)

In the Combes synthesis, condensation of aniline with \( \beta \)-diketones take place to form 2,4-substituted quinolines in the presence of an acid catalyst (Scheme 1-2) (Aribi et al., 2016).

![Scheme 1-2 Combes quinoline syntheses](image)

The Conrad-Limpach synthesis conditions are like those of the Combes synthesis but uses \( \beta \)-ketoesters, which results in quinolones being formed (Scheme 1-3). A modification of this synthesis makes use of a vinyl ether instead of ethyl acetoacetate. With a deactivating \( p \)-nitro substituent on the aniline, the 4-hydroxyquinoline predominates (Scheme 1-4) (Brouet et al., 2009).
Anilines reacted with substituted aldehydes and pyruvic acid forming quinoline-4-carboxylic acids in a reaction called the Doebner reaction (Scheme 1-5) (Wang et al., 2016). The resultant quinoline products have a carboxyl group at C-4 and substituent at C-2 depending on the aldehyde used in the synthesis.

The Doebner-von Miller synthesis makes use of $\alpha,\beta$-unsaturated ketones and aldehydes in the presence of an acid catalyst (Scheme 1-6) (Gopaul and Koorbanally, 2016). When aldehydes are used, 2-substituted quinolines are formed and when ketones are used, 2,4-disubstituted quinolines result (Chaskar et al., 2010).
In the Pavarov synthesis, condensation between an aniline and aromatic aldehyde first takes place forming an imine, which then undergoes a cycloaddition reaction with an alkene having an electron donating substituent such as ethoxyethene to form 2-phenylquinolines. This reaction uses boron trifluoride as a Lewis acid catalyst (Scheme 1-7) (Kouznetsov, 2009).

This reaction proceeds by imine formation from a condensation reaction between aniline and benzaldehyde followed by a 2+2 cycloaddition reaction between the imine and ethoxyethene forming an unstable four membered ring intermediate. Ring opening and cyclisation to a six-membered intermediate then occurs, after which aromaticity is restored forming the product (Scheme 1-8).
In the Friedlander synthesis, o-aminobenzaldehyde reacts with ketones, aldehydes or other carbonyl compounds containing reactive α-methylene groups to form 2,3-disubstituted quinoline derivatives (Scheme 1-9) (Shiri et al., 2011).

The Niementowski quinoline synthesis is similar to the Friedlander synthesis, however instead of the aldehyde, a carboxylic acid group is present in the aniline derivative. When this reagent is reacted with a β-diketo ester, a pseudo coumarin skeleton occurs in an angular fashion on the quinoline framework (Scheme 1-10) (Poronik et al., 2017).
The Pfitzinger quinoline synthesis uses isatin instead of aniline derivatives, which reacts with carbonyl compounds in the presence of strong bases such as potassium hydroxide. This reaction produces quinolines with a carboxyl group at C-4 (Scheme 1-11) (Sangshetti et al., 2014).

In the present work, the Doebner-Miller reaction was used to synthesise quinolines since the design of the target molecules used crotonaldehyde as a reagent along with aniline to form 2-methylquinolines. This was an essential intermediate for subsequent oxidation to quinoline-2-carbaldehyde from which thiosemicarbazones were synthesised.

More about the Doebner-von Miller reaction

This reaction is normally conducted in a biphasic solvent system consisting of toluene and aqueous hydrochloric acid, in order to keep nucleophiles away from the aldehyde moiety, preventing polymerisation of the aldehyde. The biphasic system also helps in the isolation of the quinoline derivative in that the quinoline remains in the organic phase and other polar
compounds in the aqueous phase (Matsugi et al., 2000). This reaction has some limitations, such as being limited to sterically accessible aldehydes such as crotonaldehyde. Sterically bulky aldehydes such as cinnamaldehyde does not produce corresponding phenylquinolines under similar reaction conditions (Reynolds et al., 2010).

The Doebner-von Miller reaction involves cyclization of the amino group from aromatic amines with $\alpha,\beta$ unsaturated compounds (Reynolds et al., 2010). The four carbon crotonaldehyde (an $\alpha,\beta$-unsaturated compound) can be formed by the reaction of two molecules of acetaldehyde (Snell et al., 2010). Aniline then condenses with crotonaldehyde forming an enol. Subsequent departure of the hydroxy group after protonation results in a conjugated imine. A 2+2 cycloaddition reaction of two molecules of the imine then forms a four-membered intermediate, which opens and cyclises to a six-membered intermediate. After aromatisation is restored, one of the nitrogen atoms (that which is not adjacent to the aromatic ring) is then protonated, catalysing the opening of the six-membered heterocyclic ring. Cyclisation then occurs with the adjacent alkene moiety and the aromatic amine is then released. The final step involves a dehydrogenation step to yield the quinoline (Scheme 1-12) (Eisch and Dluzniewski, 1989).

This reaction takes place at temperatures between 80 – 111 °C (Reynolds et al., 2010; Ramann and Cowen, 2015) and is usually catalysed by Brønsted acids such as hydrochloric acid (Gopaul and Koorbanally, 2016), sulfuric acid (Yadav et al., 2016) and heteropoly acids such as phosphomolybdic acid (Chaskar et al., 2010) and phosphotungstic acid (Sivaprasad et al., 2006). Ranu et al. (2003) also demonstrated that quinolines and dihydroquinolines could be synthesised using solvent free microwave reactions on the surface of silica gel impregnated with In (III).
Oxidation of 2-methylquinoline to quinoline-2-carboxaldehyde

The oxidation of 2-methylquinoline (quinaldine) to either quinoline-2-carbaldehyde or quinoline-2-carboxylic acid (quinaldinic acid) creates functionality at C-2 on the quinoline nucleus, which can be used for further nucleophilic and coupling reactions. Selenium dioxide (SeO₂) is a commonly used oxidant for the oxidation of quinaldine to quinoline-2-carboxaldehyde (Scheme 1-13) (Achremowicz, 1996; Pérez-Melero et al., 2004; Gopinath et al., 2013; Wang et al., 2015; Gopaul and Koorbanally, 2016).

Scheme 1-12 Proposed mechanistic pathway for the Doebner-von Miller synthesis (Eisch and Dluzniewski, 1989)
Precautions are to be taken when working with SeO₂ as it is known to be toxic and to form selenous acid, a severe skin irritant when in contact with the body fluids such as perspiration and tears (Mlochowski and Wojtowicz-Mlochowska, 2015). The oxidation was carried out in boiling aprotic solvents such as dioxane with yields as high as 90% (Wang et al., 2015) or xylene (Pérez-Melero et al., 2004) with yields of between 50 – 80%. Using SeO₂ in the absence of reflux conditions produces yields of 70%, but only after two weeks (Achremowicz, 1996).

Scheme 1-13 Oxidation of 2-methylquinoline to quinoline-2-carbaldehyde

A much greener photo-oxidation of 2-methylquinoline (quinaldine) to quinoline-2-carboxaldehyde in good yield (65%) was reported where the methyl group was oxidised with TFA and I₂ by irradiation under an oxygen atmosphere for 20 hours (Nagasawa et al., 2016). Aerobic oxidation of 2-methylquinolines using copper catalysts in DMF at 130 °C in 12 hours was also carried out (Zheng et al., 2016). Microwave assisted oxidation of methylquinolines to quinoline aldehydes was demonstrated using phenyliodine(III) diacetate (PIDA) as a catalyst in DMSO in 18 to 42 minutes (Jiang et al., 2016).

Quinaldine (2-methylquinoline) can also be oxidized to quinoline-2-carboxylic acid (quinaldinic acid) when using selenium dioxide in dioxane refluxing for 2-8 hours (Musiol et al., 2009; Chandrasekhar et al., 2011) (Scheme 1-14). An aerobic oxidation of quinaldine to quinaldinic acid was also carried out using N-hydroxyphthalimide (NHP)/Co(OAc)₂/Mn(OAc)₂ catalysts with a NO₂ initiator (Sakaguchi et al., 2002).
Quinoline-2-carbaldehyde was easily oxidized to quinaldinic acid using hydrogen peroxide in formic acid (Dodd and Le Hyaric, 1993) or hydrogen peroxide in water catalysed by a sulfonylalkyl-substituted N-heterocyclic carbene catalyst (Scheme 1-15) (Yoshida et al., 2009).

\[ \text{SeO}_2 \xrightarrow{\text{Dioxane}} \text{quinoline-2-carbaldehyde} \xrightarrow{\text{H}_2\text{O}_2, \text{formic acid or catalyst, water}} \text{quinoline-2-carboxylic acid} + \text{Se} + \text{H}_2\text{O} \]

**Scheme 1-15** Oxidation of quinoline-2-carbaldehyde to quinaldinic acid

**Mechanism of oxidation**

In the proposed mechanism by Trump and Zhou (1993) (Scheme 1-16), nucleophilic attack by methylquinoline to selenium dioxide followed by proton transfer, leads to an unstable four-membered cyclic intermediate, resulting in the oxygen adding to the 2-methyl group to open up the four-membered ring by breaking the C-Se bond. In the final step, the oxygen bound to Se abstracts the proton from the 2-methylene group, forming quinoline-2-carbaldehyde and releasing selenium and water.

In SeO\(_2\) oxidation of methylquinoline, Se\(^{4+}\) is reduced to Se, which is subsequently removed from the reaction mixture by filtration. The aldehyde formed is then separated by biphasic extraction and purified by column chromatography.
Thiosemicarbazides and thiosemicarbazones

Thiosemicarbazides are an important class of compounds, which together with their derivatives have good biological activity (Singhlal et al., 2013), including anticancer (Arora et al., 2014), antitubercular (Patel et al., 2014), antibacterial (Nevagi and Dhake, 2013), antiproliferative (Pitucha et al., 2016), and antifungal (Er et al., 2017) activity. When thiosemicarbazides form imines with aldehydes or ketones in an additional step, the resultant molecules are termed thiosemicarbazones (Figure 1.3) (Singh et al., 1978). Thus, thiosemicarbazones have an unsaturated N-1 whereas thiosemicarbazides have a fully saturated N-1. Thiosemicarbazones have shown antibacterial (Souza et al., 2013), anticancer (Thanigaimalai et al., 2012), antiparasitic (Glinma et al., 2014) and antioxidant activities (Nguyen et al., 2013).
Thiosemicarbazones have also demonstrated their ability to complex with metal ions, such as Fe(II), Cu(II), Pd (II) and Zn(II) resulting in pharmaceutically active compounds (Zeglis et al., 2011; Yu et al., 2009; Hernandez et al., 2013). The metallation has been shown to play a role in bioactivity such as topoiosomerase IIα inhibition and antiproliferative activity (Zeglis et al., 2011). Examples of commercially available thiosemicarbazone based drugs are Marboran® (methisazone), an antiviral used to treat smallpox, and Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) which is an anti-cancer drug (Figure 1.4).

Synthesis

Thiosemicarbazide synthesis involves the preparation of isothiocyanates in situ by nucleophilic addition of amines to carbon disulfide, resulting in potassium arylcarbamodithioates. These intermediates then react with methyl iodide, which replaces the potassium to afford N-aryl methyldithiocarbamates, which undergo hydrazinolysis to give 4-arylthiosemicarbazides (Scheme 1-17). They can also be formed by starting directly from isothiocyanates or ammonium thiocyanates which undergo a condensation reaction with carbohydrazines (Scheme 1-18) (Metwally et al., 2011).

![Marboran and Triapine](image)

**Figure 1.4** Examples of thiosemicarbazone based drugs available on the market
Substituted phenylthiosemicarbazides were synthesized by nucleophilic addition between aromatic isothiocyanates and hydrazine (Bečka et al., 2017). A proposed mechanism for the reaction involves nucleophilic attack by hydrazine on the carbon of the isothiocyanate followed by proton transfer, resulting in the formation of phenylthiosemicarbazides (Scheme 1-19).

This reaction was carried out in protic solvents such as ethanol under both under reflux (for 7 hours) (Foroughifar et al., 2014), and at room temperature (for 30 mins) (Benmohammed et al., 2014). Water can also be used with hydrochloric acid as a catalyst for selected thiosemicarbazides such as 1-(2-hydroxybenzoyl) thiosemicarbazide at 95 °C for 4 hours (Nurkenov et al., 2016).

This moiety also reacts with compounds containing C=O and C=N groups to form a variety of five or six membered heterocyclic molecules, including thiazoles, pyrazoles, thiadiazoles,
triazoles, oxadiazoles and thiazines, which have shown potential biological activities (Metwally et al., 2011; Gazieva and Kravchenko, 2012; Singhal et al., 2013).

1.3 Quinoline thiosemicarbazone hybrids

Quinoline thiosemicarbazide hybrid molecules contain both quinoline and thiosemicarbazone moieties (Figure 1.5). The thiosemicarbazone moiety can be linked to the quinoline framework at a variety of positions. These molecules have shown anti-tubercular (Patel et al., 2014), anticancer (Huang et al., 2010), antimalarial (Pingaew et al., 2010), antifungal (Abdel-Moty et al., 2005; Degola et al., 2015) and anticonvulsant (Singh et al., 2011) activity. The Cu (II) and Ni (II) complexes of quinoline-2-carboxaldehyde thiosemicarbazone derivatives were also shown to have anticancer and antifungal activity (Kulkarni et al., 2010; Bisceglie et al., 2015). 5-Acetyl (or 5 benzoyl)-8-hydroxyquinoline-4-phenyl thiosemicarbazones and 2-hydroxyquinoline-3-thiosemicarbazones were tested for the antibacterial activity, but proved to be mildly active or inactive against the bacterial strains tested against (Abdel-Moty et al., 2005; Kulkarni et al., 2010). There have been no reports on the antibacterial activity of quinoline-2-thiosemicarbazones in the literature.

![Figure 1.5](image.png) The structure of a quinoline-thiosemicarbazone hybrid

These molecules have been prepared by a condensation reaction between quinoline-2-carboxaldehyde and thiosemicarbazides (Scheme 1-20) (Biscegli et al., 2015; Degola et al., 2015; Huang et al., 2010). The reaction is quick and simple and occurs by adding equimolar
quantities of the thiosemicarbazide and quinoline-2-carbaldehyde in methanol or ethanol at room temperature with a few drops of glacial acetic acid to catalyse the reaction and left to stir in an ice bath for 20 hours (Bisceglie et al., 2015; Degola et al., 2015). Variations of the synthesis involve changing the solvent to isopropanol and duration of the reaction (2-8 hours) (Huang et al., 2010).

Scheme 1-20 Condensation reaction of quinoline-2-carbaldehyde with thiosemicarbazides

A similar acid catalysed (HCl) condensation reaction between quinoline with ketone substituent at C-5 and thiosemicarbazide has also been reported (Scheme 1-21) (Abdel-Moty et al., 2005). In this reaction, alkanoyl chlorides were first reacted with 8-hydroxyquinoline to functionalise C-5 with a ketone from which quinoline-thiosemicarbazones were formed.

Scheme 1-21 Synthesis of 8-hydroxyquinoline bearing a thiosemicarbazone moiety

Serda et al. (2010) used a microwave assisted reaction between quinolones and thiosemicarbazide in ethanol using polyphosphoric acid as a catalyst to synthesise quinoline-thiosemicarbazones where the thiosemicarbazone moiety was directly attached to the quinoline skeleton rather than a functional group on the ring. The quinoline substrate was first nitrated
in order to deactivate the aromatic quinoline system, making the amide carbonyl more reactive toward nucleophilic addition (Scheme 1-22).

**Scheme 1-22** Synthesis of quinoline-diylidene bearing a thiosemicarbazone moiety

The proposed mechanism of thiosemicarbazone formation proceeds by nucleophilic attack of the thiosemicarbazide on quinoline-2-carboxyaldehyde. This is followed by proton transfer and dehydration to form an imine (Scheme 1-23).

**Scheme 1-23** Proposed mechanistic pathway for the synthesis of substituted quinoline-2-thiosemicarbazones
1.4 Hypothesis and aims

Hypothesis

Since both the quinoline and thiosemicarbazide moieties each have good biological activity on their own, combining these two structural motifs into one molecule may enhance the activity that each of them have on their own. Furthermore, synthesising novel molecules with each of these moieties may lead to a new drug with possible anticancer, anti-HIV, antimalarial, antitubercular and antibacterial activity.

Aims

1. To synthesise a small library of quinoline thiosemicarbazone derivatives, with varying functional groups on both the quinoline and thiosemicarbazide moieties.
2. To characterise the synthesised compounds using NMR spectroscopy and mass spectrometry.
3. To determine the antibacterial activity of the synthesised compounds.
4. To conduct a structure activity relationship analysis of the synthesised compounds with regard to antibacterial activity.
Chapter 2. Results and Discussion

2.1 Chemistry

Fifteen 2-quinoline thiosemicarbazones (6a-o), of which 12 were novel (6b-e, g-j and l-o) were synthesised in a four-step reaction (Scheme 2-1). The 2-methylquinolines were first synthesised from fluoro, chloro and bromo substituted anilines (1a-c) and crotonaldehyde under acidic conditions using the Doebner-von Miller reaction. The Doebner-von Miller reaction was used since this is the most popular method to synthesise 2-methylquinolines (Gopaul et al., 2015). The resultant 2-methylquinolines were oxidised immediately without further purification to the quinoline-2-carboxaldehydes (3a-c), which were then purified. The thiosemicarbazide intermediates 5a-e were formed by a nucleophilic addition reaction between hydrazine hydrate and para substituted isothiocynates and thereafter reacted with the carboxaldehyde intermediates (3a-c) to form quinoline-2-thiosemicarbazone derivatives 6a-o by a condensation reaction in yields of between 55 to 65%.

Scheme 2-1 Reaction scheme for the synthesis of quinoline-2-carboxaldehyde thiosemicarbazone derivatives 6a-o
Characterisation of the 6-chloroquinoline-2-carbaldehyde intermediates (3a-c)

The intermediates were characterised by $^1$H and $^{13}$C NMR spectroscopy as well as 2D NMR spectroscopy. For example, the 6-chloroquinoline-2-carbaldehyde (3b) showed six proton resonances in the $^1$H NMR spectrum, a sharp singlet at $\delta$ 10.17 assigned to the aldehyde group, and two olefinic doublets at $\delta$ 8.19 and $\delta$ 8.01 with $J$ values of 8.5 Hz. The H-5, H-7 and H-8 resonances appeared at $\delta$ 7.86 ($J$ = 2.2 Hz), 7.73 ($J$ = 9.0, 2.2 Hz) and 8.16 ($J$ = 9.0 Hz) as a doublet, double doublet and doublet respectively (Figure 2.1).

![Figure 2.1](6-chloro aldehyde in cdcl3)

$^1$H NMR spectrum for 6-chloroquinoline-2-carbaldehyde 3b

The $^{13}$C NMR spectrum showed the presence of ten carbon resonances, which includes the aldehyde carbon at $\delta$ 193.4 and five aromatic methine carbon resonances at $\delta$ 118.3 (C-3), 136.4 (C-4), 126.6 (C-5), 131.6 (C-7) and 132.0 (C-8). The remaining four carbon resonances were quaternary, the most deshielded being C-2 at $\delta$ 152.7, adjacent to the aldehyde and the
nitrogen atom, followed by C-8a at δ 146.3, adjacent to only the nitrogen and then followed by C-4a and C-6 at δ 136.4 and δ 130.6 respectively (Figure 2.2).

Figure 2.2 $^{13}$C NMR spectrum of 6-chloroquinoline-2-carbaldehyde (3b)

H-5, H-7 and H-8 could be assigned by their splitting patterns and coupling constants. H-5 is a doublet with a 2.2 Hz coupling to H-7, which appears as a double doublet with an additional 9.0 Hz coupling to H-8 appearing as a doublet. However, H-4 was assigned due to a HMBC correlation to C-8a. Its coupled resonance was assigned to H-3. The quaternary C-4a was assigned to δ 135.2 due to a HMBC with H-3 (Figure 2.3). The remaining quaternary resonance at δ 130.6 was assigned to C-6. The other halogenated quinoline-2-carbaldehydes were elucidated in a similar manner.

Figure 2.3 Selected HMBC correlations for 3b used for the structural assignment
In 3a, the splitting pattern of H-5, H-7 and H-8 becomes rather complex, due to the presence of the 6-fluoro group. H-5 is now a double doublet with $J_{H5,F} = 8.6$ Hz and $J_{H5,H7} = 2.8$ Hz, H-7 is a doublet of doublet of doublets (ddd) with $J_{H7,F} = 8.2$ Hz, $J_{H7,H8} = 9.2$ Hz and $J_{H5,H7} = 2.8$ Hz and H-8 appeared as a double doublet ($J_{H7,H8} = 9.2$ Hz and $J_{H8,F} = 5.0$ Hz) (Figure 2.4). Changes in the $^{13}$C NMR spectrum were also evident due to the presence of fluorine. The C-6 resonance was seen as a doublet with a large $J$ value of 253.0 Hz at $\delta$ 162.0, the ortho carbon resonances were doublets with smaller coupling constants of 26.1 Hz (C-7 at $\delta$ 121.1) and 22.0 Hz (C-5 at $\delta$ 111.0) and the meta carbon resonances appeared at $\delta$ 133.2 and 131.0 as doublets with $J$ values of 9.6 and 10.5 Hz respectively (Figure 2.5).

![Figure 2.4](image)

**Figure 2.4** Aromatic region of the $^1$H-NMR spectrum of compound 3a
Since the quinolines were reaction intermediates, it was found that the yields could be maximised by using the methylquinoline reaction mixture directly (without isolating it) in the oxidation reaction with selenium dioxide as an oxidant to form the desired quinoline-2-carboxyaldehydes 3a-c. The reaction was monitored to completion using thin layer chromatography. The yields of the substituted quinoline-2-carboxyaldehydes ranged from 55-65%. It was found that chloro and bromo substituents at C-6 on the quinoline moiety have higher oxidation yields (63-65%) than when a fluoro group is present at the same position (55%), probably due to the high electronegativity of fluorine, making the nitrogen on the quinoline ring less nucleophilic. This affects the nucleophilic addition to the selenium in the first instance. The mechanism for this reaction is shown in Scheme 1-16 on page 12.
Synthesis and characterisation of the thiosemicarbazides 5a-e

The thiosemicarbazides 5a-e were obtained in good yields (80 to 90%) by a straightforward nucleophilic addition reaction between hydrazine hydrate and various para substituted isothiocyanates in isopropanol at ambient temperature. The mechanism for this reaction is shown in Scheme 1-19 on page 14. These compounds crystallised out of solution upon completion of the reaction. Their $^1$H NMR spectra were recorded and their structures confirmed by mass spectroscopy.

The NMR spectrum of a typical phenyl thiosemicarbazide, for example 5a contained the aromatic resonances H-2/6 at $\delta$ 7.64 (d, $J = 7.5$ Hz), H-3/5 at $\delta$ 7.29 (t, $J = 7.5$ Hz) and H-4 at $\delta$ 7.09 (t, $J = 7.5$ Hz). The NH$_2$ proton resonance integrating to two protons appeared at $\delta$ 4.79 and H-1' and H-3', the two NH protons, appeared at $\delta$ 9.11 as a sharp singlet and 9.64 as a broad singlet. These two resonances are interchangeable. The HMBC spectrum showed no correlation between NH-1 and H-2/6, which would have allowed these two resonances to be distinguished (Figure 2.6).

![Figure 2.6 $^1$H NMR spectrum for phenyl thiosemicarbazide 5a](image)
The mass spectra of all the compounds 5a-e contained the molecular ion peak, which indicated that the hydrazide had formed. For example, in 5a, a mass at m/z 168 was obtained.

The desired substituted quinoline-2-thiosemicarbazone derivatives 6a-o were obtained via a simple condensation reaction between the quinoline aldehydes 3a-c and the para substituted thiosemicarbazides 5a-e (Table 2-1). The resulting quinoline-2-thiosemicarbazone derivatives were obtained in good yields (59 to 80%). Similar compounds were synthesised previously using the same condensation reaction (Biscegli et al., 2015; Huang et al., 2010), however the compounds in this work, 6a-o differ from those synthesised previously in that they have substitution on the quinoline scaffold as well as para substituted phenylthiosemicarbazides used for the first time to form novel derivatives. The mechanism for this reaction is shown in Scheme 1-23 on page 17.

Table 2-1 The yields of the synthesised quinoline-2-thiosemicarbazone derivatives

<table>
<thead>
<tr>
<th>Compound 6</th>
<th>R₁</th>
<th>R₂</th>
<th>Melting point °C</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>F</td>
<td>H</td>
<td>193-210</td>
<td>75</td>
</tr>
<tr>
<td>b</td>
<td>F</td>
<td>F</td>
<td>200-210</td>
<td>61</td>
</tr>
<tr>
<td>c</td>
<td>F</td>
<td>Cl</td>
<td>188-193</td>
<td>67</td>
</tr>
<tr>
<td>d</td>
<td>F</td>
<td>CH₃</td>
<td>185-195</td>
<td>82</td>
</tr>
<tr>
<td>e</td>
<td>F</td>
<td>NO₂</td>
<td>195-205</td>
<td>72</td>
</tr>
<tr>
<td>f</td>
<td>Cl</td>
<td>H</td>
<td>205-210</td>
<td>68</td>
</tr>
<tr>
<td>g</td>
<td>Cl</td>
<td>F</td>
<td>215-225</td>
<td>62</td>
</tr>
<tr>
<td>h</td>
<td>Cl</td>
<td>Cl</td>
<td>185-205</td>
<td>65</td>
</tr>
<tr>
<td>i</td>
<td>Cl</td>
<td>CH₃</td>
<td>198-205</td>
<td>80</td>
</tr>
<tr>
<td>j</td>
<td>Cl</td>
<td>NO₂</td>
<td>205-214</td>
<td>64</td>
</tr>
<tr>
<td>k</td>
<td>Br</td>
<td>H</td>
<td>190-205</td>
<td>70</td>
</tr>
<tr>
<td>l</td>
<td>Br</td>
<td>F</td>
<td>215-220</td>
<td>60</td>
</tr>
<tr>
<td>m</td>
<td>Br</td>
<td>Cl</td>
<td>205-220</td>
<td>66</td>
</tr>
<tr>
<td>n</td>
<td>Br</td>
<td>CH₃</td>
<td>212-220</td>
<td>76</td>
</tr>
<tr>
<td>o</td>
<td>Br</td>
<td>NO₂</td>
<td>208-219</td>
<td>59</td>
</tr>
</tbody>
</table>
In general, the yield for the thiosemicarbazides with electron withdrawing groups were lower than those with electron donating groups. The synthesised compounds were then characterised by NMR and mass spectroscopy.

The $^1$H NMR spectrum of 6f containing a chloro group at position 6, had resonances similar to both of the starting materials, 3b and 5a. Two of the resonances are however worth noting, H-9 and H-3. H-9 is the imine singlet proton and occurs as the result of the condensation between the aldehyde and thiosemicarbazone. This is an indication that the reaction was successful. H-3, which occurred at $\delta$ 8.01 in the carbaldehyde, now occurs more downfield at $\delta$ 8.66. This indicates that the thiosemicarbazide moiety has decreased the electron density at C-3, probably by induction after introducing all the electronegative groups present in the thiosemicarbazide moiety. The other resonance which had a slight shift was H-5, which shifted from $\delta$ 7.86 in 3b to $\delta$ 8.12 in 6f (Figure 2.7). The reason for this cannot be explained. Resonance and inductive effects were discussed, but a reasonable explanation could not be provided.

The $^{13}$C NMR spectrum of 6f contained similar resonances to that of its precursors, 3b and 5a. There were slight differences in the chemical shifts, however none were worth noting with the exception of the aldehyde carbon in 3b at $\delta$ 193.3 now being absent and appearing as an imine carbon in 6f at $\delta$ 142.6, also an indication that the reaction had occurred (Figure 2.8).
Figure 2.7  Aromatic region of the $^1$H NMR spectrum for compound 6f

Figure 2.8  $^{13}$C NMR spectrum for compound 6f
2.2 Antibacterial activity of compounds 6a-o

The synthesised compounds were tested for their antibacterial activity against six bacterial strains, two Gram +ve strains, *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) and four Gram –ve strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Klebsiella pneumonia*. These gram negative bacteria are on the WHO’s priority 1 (critical) list and the *Staphylococcus* species on the priority 2 (high) list for bacterial strains where new drugs are needed (www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/).

*Pseudomonas* is no. 2 on the critical list with the *Enterobacteriaceae*, such as *Escherichia*, *Salmonella* and *Klebsiella* being no. 3. *Staphylococcus aureus* and MRSA is no. 2 on the high priority list.

A disc diffusion assay was first used as a preliminary screening method to select compounds for further minimum bactericidal concentration (MBC) tests. Compounds 6c, 6g, 6h, 6l and 6o showed activity against four or more strains (Table 2-2). This set contained all compounds with a para substituted fluoro group on the thiosemicarbazide portion of the molecule 6c, 6g and 6l. These three compounds were also active against both *S. aureus* and MRSA.

Compound 6h (with a 6-Cl, 4'-Cl substitution pattern) was active in all four of the Gram –ve bacterial strains. The activity was lost when a fluoro group replaced the chloro group at C-4' (6g), resulting in loss of activity with *E. coli*, however this resulted in added activity with MRSA. The compound with a bromo group at C-6 and methyl group at C-4' (6o) had the least activity among these five compounds, showing activity in four of the bacterial strains when the other four compounds showed activity in five of the bacterial strains. Thus, the best lead for
antibacterial activity was fluoro substitution at the *para* position of the thiosemicarbazide moiety.

**Table 2-2** Antibacterial activity of 6a-o using the disc diffusion assay

<table>
<thead>
<tr>
<th>Substituents</th>
<th>Gram-Positive</th>
<th>Gram-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
<td><strong>R₁</strong></td>
<td><strong>R₂</strong></td>
</tr>
<tr>
<td>6a</td>
<td>F</td>
<td>H</td>
</tr>
<tr>
<td>6b</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>6c</td>
<td>F</td>
<td>Cl</td>
</tr>
<tr>
<td>6d</td>
<td>F</td>
<td>NO₂</td>
</tr>
<tr>
<td>6e</td>
<td>F</td>
<td>CH₃</td>
</tr>
<tr>
<td>6f</td>
<td>Cl</td>
<td>H</td>
</tr>
<tr>
<td>6g</td>
<td>Cl</td>
<td>F</td>
</tr>
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<td>6h</td>
<td>Cl</td>
<td>Cl</td>
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<td>6i</td>
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<tr>
<td>6j</td>
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<td>6k</td>
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<td>Br</td>
<td>NO₂</td>
</tr>
<tr>
<td>6o</td>
<td>Br</td>
<td>CH₃</td>
</tr>
</tbody>
</table>

The five compounds (6c, 6l, 6g, 6h and 6o) showing activity in four or more bacterial strains were then diluted to different concentrations and spotted directly on agar plates containing the
different microorganisms to determine their MBC values. This method was used since the compounds were insoluble in aqueous media and precipitated out on trying the broth dilution method. Unfortunately, most of the compounds had MBC values of > 625 µM, with the exception of 6l, containing a 6-Br substitutent and fluoro group at the para position of the thiosemicarbazide moiety (Table 2-3). Thus, the larger and more polarisable bromo group on the quinoline scaffold was essential for this antimicrobial activity as when the smaller chloro and fluoro groups occupy the same position, activity is decreased.

**Table 2-3** Minimum bactericidal concentration (MBC in µM) of selected quinoline-2-thiosemicarbazone derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>Sa</th>
<th>MRSA</th>
<th>Ec</th>
<th>Pa</th>
<th>St</th>
<th>Kp</th>
</tr>
</thead>
<tbody>
<tr>
<td>6c</td>
<td>F</td>
<td>Cl</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
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<td>&gt; 625</td>
</tr>
<tr>
<td>6l</td>
<td>Br</td>
<td>F</td>
<td>387</td>
<td>387</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
</tr>
<tr>
<td>6g</td>
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<td>Cl</td>
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<td>&gt; 625</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
<td>&gt; 625</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td>94.3</td>
<td>188.6</td>
<td>2.95</td>
<td>188.6</td>
<td>2.95</td>
<td>11.8</td>
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<td>86.5</td>
<td>0.34</td>
<td>345</td>
<td>21.6</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Sa = *Staphylococcus aureus*; MRSA = Methicillin resistant *Staphylococcus aureus*; Ec = *Escherichia coli*; Ps = *Pseudomonas aeruginosa*; St = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*.

It was already established from the disc diffusion assays that fluoro substitution on the thiosemicarbazide moiety resulted in antimicrobial activity. Thus, the 6-Br, 4'-F substitution pattern on these quinoline-2-thiosemicarbazides was the best substitution pattern on this particular quinoline-thiosemicarbazide scaffold for antibacterial activity against *Staphylococcus aureus* and MRSA to occur. However, the results were not comparable to the
standard antibiotics, ciprofloxacin and levofloxacin (Table 2-3), hence cell viability and cytotoxicity studies on 6l were not carried out.

Our results corroborated findings by Kulkarni et al. (2010) and Abdel-Moty et al. (2005) who also found that 5-Acetyl (or 5 benzoyl)-8-hydroxyquinoline-4-phenyl thiosemicarbazones and 2-hydroxyquinoline-3-thiosemicarbazones showed weak to no activity in the bacterial strains tested against. However, 6l (6-Br, 4'-F derivative) did show interesting antibacterial activity and further research on this particular molecule, perhaps by adding other groups to the molecule using this as a basic scaffold can be determined in order to identify a compound that can be developed into a potential antibiotic.
Chapter 3. Experimental

3.1 General Experimental Procedures

Chemicals and reagents used in this study were purchased from Sigma-Aldrich through Capital Laboratories, South Africa. Organic solvents were redistilled according to standard procedures. TLC analysis was carried out on silica gel 60 F$_{254}$ plates purchased from Merck South Africa. Purifications were carried out by column chromatography using silica gel (60-120 mesh) as the stationery phase and varying ratios of ethyl acetate and hexane as the mobile phase. IR spectra were recorded using a Perkin Elmer 100 FT-IR spectrometer. $^1$H, $^{13}$C and 2D NMR experiments were carried out in DMSO-$d_6$ using a Bruker Avance 400 MHz instrument. Tetramethylsilane (TMS) was used as the internal standard with chemical shifts reported in (ppm) and coupling constants ($J$) in Hz, referenced to the DMSO-$d_6$ solvent line (2.50 ppm for $^1$H and 39.52 ppm for $^{13}$C). Spectra were analysed using Topspin 3.1 software (2013). High-Resolution mass spectral data (HRMS) were obtained at ambient temperature using a Bruker micro TOF-Q II ESI instrument. Melting points were carried out on a Stuart Scientific melting point apparatus SMP3 and UV data were obtained on a Shimadzu UV-3600 UV-Vis-IR spectrometer.

3.2 Synthesis

Synthesis of 6-substituted quinoline-2-aldehyde derivatives 3a-c

The respective para substituted anilines 1a-c (40.0 mmol) were reacted with crotonaldehyde (60.0 mmol, 5.00 mL) in 40 mL of HCl:H$_2$O (1:1) under reflux at 100 °C for 12 h. The reaction was monitored by TLC. Upon completion, the mixture was basified with NaHCO$_3$ to pH 8 and NaCl added to the mixture, which was subsequently extracted with EtOAc (3 × 60 mL). The
mixture was then dried over anhydrous Na$_2$SO$_4$ and concentrated using a rotary evaporator to yield the crude 2-methylquinoline intermediates 2a-c as brown gummy residues. These intermediates were not purified, but were oxidized directly using SeO$_2$ (5.00 g, 40.0 mmol) in 15 mL of 1,4-dioxane. Selenium dioxide was added gradually over a period of 5 min and the reaction left to stir under reflux at 110 °C for 1.5 h to produce the 6-substituted quinoline-2-aldehydes 3a-c (Scheme 3-1). The reaction was monitored by TLC until completion. Once cooled, the reaction mixture was filtered through celite, diluted with water and extracted with ethyl acetate. The organic extract was dried over anhydrous MgSO$_4$, concentrated and subjected to column chromatography on silica gel using a mobile phase of hexane:ethyl acetate (95:5) in yields of between 55 to 65%.

Scheme 3-1 Reactions for the synthesis of 6-substituted quinoline-2-aldehydes 3a-c

6-fluoroquinoline-2-carbaldehyde (3a); Orange solid residue; 55% yield; $^1$H NMR (DMSO-d$_6$, 400 MHz) δ 10.18 (s, H-9), 8.25 (1H, d, $J$ = 8.5 Hz, H-4), 8.24 (1H, dd, $J$ = 9.2, 5.0 Hz, H-8), 8.03 (1H, d, $J$ = 8.5 Hz, H-3), 7.58 (1H, ddd, $J$ = 9.2, 8.2, 2.8 Hz, H-7), 7.50 (1H, dd, $J$ = 8.6, 2.8 Hz, H-5); $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ 193.4 (C-9), 162.0 (d, $J_{CF}$ = 253.0 Hz, C-6), 152.2 (d, $J_{CF}$ = 2.9 Hz, C-2), 145.0 (C-8a), 136.7 (d, $J_{CF}$ = 5.6 Hz, C-4), 133.2 (d, $J_{CF}$ = 9.6 Hz, C-8), 131.0 (d, $J_{CF}$ = 10.5 Hz, C-4a), 121.1 (d, $J_{CF}$ = 26.1 Hz, C-7), 118.1 (C-3), 111.0 (d, $J_{CF}$ = 22.0 Hz, C-5).
6-chloroquinoline-2-carbaldehyde (3b); Cream white; 65% yield; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 10.17 (s, H-9), 8.20 (1H, d, $J = 8.5$ Hz, H-4), 8.16 (1H, d, $J = 9.0$ Hz, H-8), 8.01 (1H, d, $J = 8.5$ Hz, H-3), 7.86 (1H, d, $J = 2.2$ Hz, H-5), 7.73 (1H, dd, $J = 9.0$, 2.2 Hz, H-7); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 193.3 (C-9), 152.7 (C-2), 146.3 (C-8a), 136.4 (C-4), 135.2 (C-4a), 132.0 (C-8), 131.6 (C-7), 130.6 (C-6), 126.6 (C-5), 118.3 (C-3).

6-Bromoquinoline-2-carbaldehyde (3c); light brown solid residue; 63% yield; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 10.18 (s, H-9), 8.20 (1H,d, $J = 8.5$ Hz, H-4), 8.10 (1H, d, $J = 9.0$ Hz, H-8), 8.04 (1H, d, $J = 2.1$ Hz, H-5 ), 8.02 (1H, d, $J = 8.5$ Hz, H-3), 7.86 (1H, dd, $J = 9.0$, 2.3 Hz, H-7); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ 193.3 (C-9), 152.8 (C-2), 146.5 (C-8a), 136.4 (C-4), 134.1 (C-8), 132.0 (C-7), 131.0 (C-4a), 130.0 (C-5), 123.6 (C-6), 118.3 (C-3).

Synthesis of para substituted phenylisothiosemicarbazides 5a-e

Para substituted phenylisocyanates 4a-e (6.70 mmol) were dissolved in isopropanol (10 mL), to which hydrazine hydrate (0.66 mL, 13.40 mmol) was added and the mixture stirred for 15 min at room temperature and then placed in an ice-bath for 30 min. The crystals that formed were filtered and washed with isopropanol to yield the phenylisothiosemicarbazides 5a-e in yields of between 82 and 90% (Scheme 3-2).

**Scheme 3-2** The reaction of phenylisocyanates with hydrazine hydrate to produce 4-substituted thiosemicarbazides 5a-e

![Scheme 3-2](image)
N-phenylhydrazinecarbothioamide (5a); white solid residue: 90% yield: $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.64 (s, 3'-NH), 9.11 (s, 1'-NH) 7.64 (2H, bd, $J = 8.1$ Hz, H-2/6), 7.29 (2H, dd, $J = 8.1, 7.3$ Hz, H-3/5), 7.09 (1H, t, $J = 7.3$ Hz, H-4), 4.79 (s, 4'-NH).

N-(4-fluorophenyl) hydrazinecarbothioamide (5b); white solid residue: 86% yield: $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.60 (s, 3'-NH), 9.11 (s, 1'-NH), 7.60 (bs, H-2/6), 7.12 (2H, t, $J = 8.9$ Hz, H-3/5), 4.76 (s, 4'-NH).

N-(4-chlorophenyl) hydrazinecarbothioamide (5c); white solid residue: 84% yield: $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.19 (s, 1'-NH), 7.68 (2H, bd, $J = 8.7$ Hz, H-2/6), 7.33 (2H, d, $J = 8.7$ Hz, H-3/5), 4.81 (s, 3'-NH).

N-(4-nitrophenyl) hydrazinecarbothioamide (5d); yellow solid residue: 80% yield: $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.35 (s, 3'-NH), 8.13 (2H, d, $J = 9.2$ Hz, H-3/5), 7.80 (3H, bs, H-2/6, 1'-N-H), 4.45 (s, 4'-NH).

N-p-tolylhydrazinecarbothioamide (5e); yellow solid residue: 88% yield: $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.75 (s, 3'-NH), 7.41 (2H, d, $J = 8.2$ Hz, H-2/6), 7.10 (2H, d, $J = 8.2$ Hz, H-7/9), 2.26 (s, CH$_3$).

Synthesis of quinoline-2-carboxyaldehyde thiosemicarbazone derivatives 6a-o

Phenylthiosemicarbazides 5a-e (0.482 mmol) were dissolved in ethanol by heating slightly while stirring in an oil bath. The substituted quinoline aldehydes 3a-c (0.583 mmol) were then added separately to each of the dissolved phenylthiosemicarbazides (5a-e) and the resulting
mixture refluxed for 5 h at 110 °C. The reaction mixture was then placed in an ice bath for 30 min, where the product crystallised out and was filtered to yield the products 6a-o in yields of between 59 and 80% (Scheme 3-3).

**Scheme 3-3** The reaction of quinoline-2-carbaldehydes and thiosemicarbazides to form quinoline-2-carboxaldehyde thiosemicarbazone derivatives 6a-o

(E)-2-((6-fluoroquinolin-2-yl)methylene)-N-phenylhydrazine carbothioamide (6a); light yellow solid: 75% yield: mp 193-210 °C: UV $\lambda_{\text{max}}$ (EtOAc) nm (log $\varepsilon$) 250 (4.24), 287 (4.15), 326 (4.13), 341 (4.16), 355 (4.20); IR (KBr) $\nu_{\text{max}}$: 3317 (N-H), 3105 (C-H), 2939 (C-H), 1542 (C=C), 1498 (C=C), 1181 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.20 (s, 11-NH), 10.38 (s, 13-NH), 8.65 (1H, d, $J = 8.8$ Hz, H-3), 8.38 (1H, d, $J = 8.8$ Hz, H-4), 8.33 (s, H-9), 8.10 (1H, dd, $J = 9.2$, 5.5, Hz, H-8), 7.80 (1H, dd, $J = 9.3$, 2.8 Hz, H-5), 7.70 (1H, ddd, $J = 9.2$, 8.9, 2.8 Hz, H-7), 7.56 (2H, d, $J = 7.8$ Hz, H-2'/6'), 7.41 (2H, t, $J = 7.8$ Hz, H-3'/5'), 7.25 (1H, t, $J = 7.8$ Hz, H-4'); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.6 (C-12), 160.0 (d, $J_{\text{CF}} = 245.2$ Hz, C-6), 153.4 (d, $J_{\text{CF}} = 2.5$ Hz, C-2), 144.5 (C-8a), 142.7 (C-9), 138.9 (C-1'), 135.8 (d, $J_{\text{CF}} = 5.0$ Hz, C-4), 131.6 (d, $J_{\text{CF}} = 9.4$ Hz, C-8), 128.7 (d, $J_{\text{CF}} = 10.3$ Hz, C-4a), 128.1 (C-2'/6'), 126.3 (C-3'/5'), 125.7 (C-4'), 119.9 (d, $J = 25.8$ Hz, C-7), 119.2 (C-3), 111.2 (d, $J = 21.9$ Hz, C-5).
(E)-N-(4-fluorophenyl)-2-((6-fluoroquinolin-2-yl)methylene)hydrazine carbothioamide (6b);
yellow solid residue: 78% yield: mp 200-210 °C: UV $\lambda_{\text{max}}$ (Ethylacetate)nm (log $\varepsilon$) 250 (4.24), 326 (4.31), 340 (4.35), 354 (4.37); IR (KBr) $\nu_{\text{max}}$: 3302 (N-H), 3117 (C-H), 2977 (C-H), 1504 (C=C), 1176 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.22 (s, 11-NH), 10.37 (s, 13-NH), 8.64 (1H, d, $J = 8.8$ Hz, H-3), 8.39 (1H, d, $J = 8.8$ Hz, H-4), 8.32 (s, H-9), 8.10 (1H, dd, $J = 8.8, 8.8, 2.9$ Hz, H-7), 7.55 (2H, dd, $J = 8.9, 5.1$ Hz, H-2'/6'), 7.24 (2H, dd, $J = 8.8, 8.8$ Hz, H-3'/5'); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.9 (C-12), 160.1 (d, $J_{CF} = 245.1$ Hz, C-6), 159.9 (d, $J_{CF} = 241.3$ Hz, C-4'), 153.4 (d, $J_{CF} = 2.7$ Hz, C-2), 144.5 (C-8a), 142.9 (C-9), 135.8 (d, $J_{CF} = 5.1$ Hz, C-4), 135.2 (C-1'), 131.6 (d, $J_{CF} = 9.3$ Hz, C-8), 128.7 (d, $J_{CF} = 10.6$ Hz, C-4a), 128.5 (d, $J = 8.4$ Hz, C-2'/6'), 119.9 (d, $J_{CF} = 25.5$ Hz, C-7), 119.1 (C-3), 114.8 (d, $J = 22.3$ Hz, C-3'/5'), 111.2 (d, $J = 21.7$ Hz, C-5); HRMS (pos) (m/z): calculated for C$_{17}$H$_{12}$F$_2$N$_4$S (M + H)$^+$: 341.0672, found: 341.0680.

(E)-N-(4-chlorophenyl)-2-((6-fluoroquinolin-2-yl)methylene)hydrazine carbothioamide (6c);
yellow solid residue: 67% yield: mp 188-193 °C: UV $\lambda_{\text{max}}$ (Ethylacetate)nm (log $\varepsilon$) 249 (4.29), 326 (4.39), 340 (4.33), 354 (4.44); IR (KBr) $\nu_{\text{max}}$: 3303 (N-H), 3118 (C-H), 2972 (C-H), 1503 (C=C), 1177 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.27 (s, 11-NH), 10.40 (s, 13-NH), 8.63 (1H, d, $J = 8.8$ Hz, H-3), 8.39 (1H, d, $J = 8.8$ Hz, H-4), 8.33 (s, H-9), 8.10 (1H, dd, $J = 8.8, 5.5$ Hz, H-8), 7.80 (1H, dd, $J = 9.3, 2.8$ Hz, H-5), 7.70 (1H, ddd, $J = 8.8, 8.8, 2.9$ Hz, H-7), 7.61 (1H, dd, $J = 8.9, 5.1$ Hz, H-2'/6'), 7.46 (1H, dd, $J = 8.8, 8.8$ Hz, H-3'/5'); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.6 (C-12), 160.1 (d, $J_{CF} = 245.1$ Hz, C-6), 153.3 (C-2), 144.5 (C-8a), 143.1 (C-9), 137.9 (C-1'), 135.8 (d, $J_{CF} = 4.9$ Hz, C-4), 131.7 (d, $J_{CF} = 9.3$ Hz, C-8), 129.7 (C-4'), 128.7 (d, $J_{CF} =$
10.6 Hz, C-4a), 128.0 (C-3'/5'), 127.9 (C-2'/6'), 119.9 (d, J_{CF} = 25.6 Hz, C-7), 119.2 (C-3), 111.2 (d, J_{CF} = 22.0 Hz, C-5); HRMS(pos) (m/z): calculated for: C_{17}H_{12}ClFN_{4}S (M-H)^{+}: 358.05, found: 357.081.

(E)-2-((6-fluoroquinolin-2-yl)methylene)-N-(4-nitrophenyl)hydrazine carbothioamide (6d); yellow solid residue: 82% yield: mp 200-210 °C: UV \( \lambda_{max} \) (Ethylacetate) nm (log \( \varepsilon \)) 249 (4.30), 330 (4.67), 344 (4.62); IR \( \nu_{max} \) 3359 (N-H), 3083 (C-H), 2927 (C-H), 1702*, 1596 (C=C), 1529, 1499, 1485, 1322, 1299, 1222, 1153, 1106 (C-F) cm\(^{-1}\); \(^{1}\)H-NMR(DMSO-\(d_6\), 400 MHz) \( \delta \) 11.49 (s, 11-NH), 9.71 (s, 13-NH), 8.56 (1H, d, \( J = 8.8 \) Hz, H-3), 8.42 (1H, d, \( J = 8.8 \) Hz, H-4), 8.24 (d, \( J = 9.3 \) Hz, H-3'/5'), 8.17 (s, H-9), 8.08 (1H, dd, \( J = 9.1, 5.4 \) Hz, H-8), 8.01 (d, \( J = 9.3 \) Hz, H-2'/6'), 7.82 (1H, dd, \( J = 9.4, 2.9 \) Hz, H-5), 7.69 (1H, ddd, \( J = 9.1, 8.9, 2.9 \) Hz, H-7); \(^{13}\)C-NMR (DMSO-\(d_6\), 100 MHz) \( \delta \) 160.0 (d, \( J_{CF} = 244.7 \) Hz, C-6), 153.2 (d, \( J_{CF} = 2.8 \) Hz, C-2), 152.4 (C-12), 145.6 (C-4'), 144.5 (C-8a), 142.3 (C-9), 141.7 (C-1'), 135.8 (d, \( J_{CF} = 5.0 \) Hz, C-4), 131.6 (d, \( J_{CF} = 9.4 \) Hz, C-8), 128.6 (d, \( J_{CF} = 10.4 \) Hz, C-4a), 124.7 (C-2'/6'), 119.9 (d, \( J_{CF} = 25.6 \) Hz, C-7), 119.2 (C-3'/5'), 118.9 (C-3), 111.2 (d, \( J_{CF} = 22.0 \) Hz, C-5). *These absorption bands only appear for the nitro substituted compounds, however the frequency is higher than usual for a nitro stretch, typically occurring at 1535-1600 cm\(^{-1}\).

(E)-2-((6-fluoroquinolin-2-yl)methylene)-N-p-tolylhydrazine carbothioamide(6e); yellow solid residue: 72% yield: mp 186-195 °C: UV \( \lambda_{max} \) (Ethylacetate)nm (log \( \varepsilon \)) 250 (4.27), 326 (4.38), 340 (4.42), 354 (4.44); IR \( \nu_{max} \) 3300 (N-H), 3113 (C-H), 2962 (C-H), 1509 (C=C), 1178 (C-F) cm\(^{-1}\); \(^{1}\)H-NMR (DMSO-\(d_6\), 400 MHz) \( \delta \) 12.14 (s, 11-NH), 10.31 (s, 13-NH), 8.65 (1H, d, \( J = 8.8 \) Hz, H-3), 8.38 (1H, d, \( J = 8.8 \) Hz, H-4), 8.31 (s, H-9), 8.10 (1H,dd, \( J = 9.1, 4.9 \) Hz,
(E)-2-((6-chloroquinolin-2-yl)methylene)-N-(4-fluorophenyl)hydrazine carbothioamide (6f); light yellow solid residue: 62% yield: mp 200-210 °C: UV \( \lambda_{\text{max}} \) (Ethylacetate) nm (log \( \varepsilon \)) 250 (4.19), 270 (4.10), 330 (4.16), 344 (4.22), 359 (4.25); IR \( \nu_{\text{max}} \) 3313 (N-H), 3110 (C-H), 2962 (C-H), 1532 (C=C), 1505, 1487, 1250, 1186 (C-F) cm\(^{-1}\); \(^1\)H-NMR (DMSO-\(d_6\), 400 MHz) \( \delta \) 12.22 (s, 11-NH), 10.39 (s, 13-NH), 8.66 (1H, d, \( J = 8.8 \) Hz, H-3), 8.37 (1H, d, \( J = 8.8 \) Hz, H-4), 8.32 (s, H-9), 8.12 (1H, d, \( J = 2.3 \), H-5), 8.04 (1H, d, \( J = 9.0 \) Hz, H-8), 7.79 (1H, dd, \( J = 9.0, 2.3 \) Hz, H-7), 7.56 (2H, d, \( J = 7.7 \) Hz, H-2'/6'), 7.41 (2H, t, \( J = 7.8 \) Hz, H-3'/5'), 7.25 (1H, t, \( J = 7.4 \) Hz, H-4'); \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \( \delta \) 176.6 (C-12), 154.3 (C-2), 145.8 (C-8a), 142.6 (C-9), 138.9 (C-1'), 135.5 (C-4), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.1 (C-2'/6'), 126.7 (C-5), 126.3 (C-3'/5'), 125.7 (C-4'), 119.4 (C-3).

(E)-2-((6-chloroquinolin-2-yl)methylene)-N-phenylhydrazine carbothioamide (6f); light yellow solid residue: 62% yield: mp 200-210 °C: UV \( \lambda_{\text{max}} \) (Ethylacetate) nm (log \( \varepsilon \)) 250 (4.19), 270 (4.10), 330 (4.16), 344 (4.22), 359 (4.25); IR \( \nu_{\text{max}} \) 3313 (N-H), 3110 (C-H), 2962 (C-H), 1532 (C=C), 1505, 1487, 1250, 1186 (C-F) cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \( \delta \) 12.22 (s, 11-NH), 10.39 (s, 13-NH), 8.66 (1H, d, \( J = 8.8 \) Hz, H-3), 8.37 (1H, d, \( J = 8.8 \) Hz, H-4), 8.32 (s, H-9), 8.12 (1H, d, \( J = 2.3 \), H-5), 8.04 (1H, d, \( J = 9.0 \) Hz, H-8), 7.79 (1H, dd, \( J = 9.0, 2.3 \) Hz, H-7), 7.56 (2H, d, \( J = 7.7 \) Hz, H-2'/6'), 7.41 (2H, t, \( J = 7.8 \) Hz, H-3'/5'), 7.25 (1H, t, \( J = 7.4 \) Hz, H-4'); \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \( \delta \) 176.6 (C-12), 154.3 (C-2), 145.8 (C-8a), 142.6 (C-9), 138.9 (C-1'), 135.5 (C-4), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.1 (C-2'/6'), 126.7 (C-5), 126.3 (C-3'/5'), 125.7 (C-4'), 119.4 (C-3).
Hz, H-3), 8.38 (1H, d, J = 8.8 Hz, H-4), 8.32 (s, H-9), 8.12 (1H, d, J = 2.4 Hz, H-5), 8.04 (1H, d, J = 9.0 Hz, H-8), 7.79 (1H, dd, J = 9.0, 2.4 Hz, H-7), 7.55 (1H, dd, J = 8.9, 5.1 Hz, H-2'/6'), 7.24 (1H, t, J = 8.8 Hz, H-3'/5'); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.9 (C-12), 159.9 (d, $J_{CF}$ = 242.5 Hz, C-4'), 154.2 (C-2), 145.8 (C-8a), 142.7 (C-9), 135.5 (C-4), 135.2 (d, $J_{CF}$ = 2.6 Hz, C-1'), 131.5 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.5 (d, $J_{CF}$ = 8.3 Hz, C-2'/6'), 126.7 (C-5), 119.4 (C-3), 114.8 (d, $J_{CF}$ = 22.4 Hz, C-3'/5'); HRMS (pos) (m/z): calculated for C$_{17}$H$_{12}$ClFN$_4$S (M + H)$^+$: 358.05, found: 358.0416.

(E)-N-(4-chlorophenyl)-2-((6-chloroquinolin-2-yl)methylene)hydrazine carbothioamide (6h);

yellow solid residue: 80% yield: mp 185-205°C:

UV $\lambda_{max}$ (Ethylacetate) nm (log $\varepsilon$) 250 (4.32), 330 (4.44), 343 (4.50), 359 (4.53); IR $\nu_{max}$ 3316 (N-H), 3133 (C-H), 2989 (C-H), 1588 (C=C), 1536, 1509, 1488, 1190 (C-F), 1086 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.29 (s, 11-NH), 10.41 (s, 13-NH), 8.64 (1H, d, J = 8.8 Hz, H-3), 8.38 (1H, d, J = 8.8 Hz, H-4), 8.32 (s, H-9), 8.12 (1H, d, J = 2.4 Hz, H-5), 8.04 (1H, d, J = 9.0 Hz, H-8), 7.79 (1H, dd, J = 8.5, 2.3 Hz, H-7), 7.60 (1H, d, J = 8.7 Hz, H-2'/6'), 7.46 (1H, d, J = 8.7 Hz, H-3'/5'); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.6 (C-12), 154.2 (C-2), 145.8 (C-8a), 142.9 (C-9), 137.9 (C-1'), 135.6 (C-4), 131.5 (C-4a), 130.8 (C-7), 130.4 (C-8), 129.7 (C-4'), 128.6 (C-6), 128.0 (C-2'/6'), 127.9 (C-3'/5'), 126.7 (C-5), 119.4 (C-3); HRMS (pos) (m/z): calculated for :C$_{17}$H$_{12}$ClN$_4$S (M + H)$^+$: 374.02, found: 374.0122.

(E)-2-((6-chloroquinolin-2-yl)methylene)-N-(4-nitrophenyl)hydrazine carbothioamide (6i);

yellow solid residue: 64% yield: mp 198-215 °C: UV $\lambda_{max}$ (Ethylacetate) nm (log $\varepsilon$) 249 (4.47), 321 (4.58), 334 (4.61), 349 (4.55); IR $\nu_{max}$ 3364 (N-H), 3072 (C-H), 2921 (C-H), 1711*,
1598 (C=C), 1539, 1491, 1332, 1302, 1223, 1144, 1110 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_{6}$, 400 MHz) $\delta$ 11.52 (s, 11-NH), 9.71 (s, 13-NH), 8.57 (1H, d, $J = 8.8$ Hz, H-3), 8.42 (1H, d, $J = 8.8$ Hz, H-4), 8.24 (1H, d, $J = 9.3$ Hz, H-3'/5'), 8.17 (s, H-9), 8.14 (1H, d, $J = 2.4$ Hz, H-5), 8.03 (1H, d, $J = 8.8$ Hz, H-8), 8.02 (1H, d, $J = 9.3$ Hz, H-2'/6'), 7.79 (1H, dd, $J = 9.0, 2.4$ Hz, H-7); $^{13}$C-NMR (DMSO-$d_{6}$, 100 MHz) $\delta$ 154.1 (C-12), 152.2 (C-4'), 145.7 (C-2), 145.5 (C-8a), 142.1 (C-9), 141.7 (C-1'), 135.6 (C-4), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.5 (C-6), 126.7 (C-5), 124.7 (C-3'/5'), 119.2 (C-2'/6'), 119.1 (C-3). *These absorption bands only appear for the nitro substituted compounds, however the frequency is higher than usual for a nitro stretch, typically occurring at 1535-1600 cm$^{-1}$.

$(E)$-2-((6-chloroquinolin-2-yl)methylene)-N-p-tolylhydrazine carbothioamide (6j); yellow solid residue: 80% yield: mp 198-205 °C; UV $\lambda_{\text{max}}$ (Ethylacetate) nm (log $\varepsilon$) 251 (4.40), 330 (4.26), 360 (4.37); IR $\nu_{\text{max}}$ 3348 (N-H), 3308, 3114 (C-H), 2964 (C-H), 1513 (C=C), 1250, 1177 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_{6}$, 400 MHz) $\delta$ 12.17 (s, 11-NH), 10.32 (s, 13-NH), 8.66 (1H, d, $J = 8.8$ Hz, H-3), 8.37 (1H, d, $J = 8.8$ Hz, H-4 ), 8.31 (s, H-9), 8.12 (1H, d, $J = 2.4$ Hz, H-5), 8.04 (1H, d, $J = 9.0$ Hz, H-8), 7.79 (1H, dd, $J = 9.0, 2.4$ Hz, H-7), 7.41 (1H, d, $J = 8.3$ Hz, H-2'/6'), 7.20 (1H, d, $J = 8.3$ Hz, H-3'/5'); $^{13}$C-NMR (DMSO-$d_{6}$, 100 MHz) $\delta$ 176.6 (C-12), 154.3 (C-2), 145.8 (C-8a), 142.4 (C-9), 136.3 (C-1'), 135.5 (C-4), 134.9 (C-4'), 131.4 (C-4a), 130.8 (C-7), 130.4 (C-8), 128.6 (C-6), 128.0 (C-3'/5'), 126.6 (C-5), 126.2 (C-2'/6'), 119.4 (C-3), 20.6 (C-7'); HRMS (pos) ($m/z$): calculated for C$_{17}$H$_{12}$F$_2$N$_4$S (M + Na)$^+$: 354.07, found: 377.0609.
(E)-2-(((6-bromoquinolin-2-yl)methylene)-N-phenylhydrazine carbothioamide (6k); light yellow solid residue: 70% yield: mp 190-205 °C; UV $\lambda_{\text{max}}$ (Ethylacetate) nm (log $\varepsilon$) 249 (4.37), 331 (4.30), 347 (4.35), 360 (4.40); IR $\nu_{\text{max}}$ 3327 (N-H), 3139 (C-H), 2993 (C-H), 1533 (C=C), 1511, 1245, 1188 (C-F), cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.22 (s, 11-NH), 10.39 (s, 13-NH), 8.66 (1H, d, $J$ = 8.8 Hz, H-3), 8.37 (1H, d, $J$ = 8.8 Hz, H-4), 8.32 (s, H-9), 8.28 (1H, d, $J$ = 2.1 Hz, H-5), 7.97 (1H, d, $J$ = 9.0 Hz, H-8), 7.89 (1H, dd, $J$ = 9.0, 2.3 Hz, H-7), 7.56 (2H, d, $J$ = 7.6 Hz, H-2'/6'), 7.41 (2H, t, $J$ = 7.8 Hz, H-3'/5'), 7.25 (1H, t, $J$ = 7.4 Hz, H-4'); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.6 (C-12), 154.3 (C-2), 146.0 (C-8a), 142.6 (C-9), 138.9 (C-1'), 135.4 (C-4), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.1 (C-4a), 128.1 (C-2'/6'), 126.3 (C-3'/5'), 125.7 (C-4'), 120.1 (C-6), 119.4 (C-3).

(E)-2-(((6-bromoquinolin-2-yl)methylene)-N-(4-fluorophenyl)hydrazine carbothioamide (6l); yellow solid residue: 60% yield: mp 215-220 °C: UV $\lambda_{\text{max}}$ (Ethylacetate) nm (log $\varepsilon$) 250 (4.42), 285 (4.17), 310 (4.23), 361 (4.37); IR $\nu_{\text{max}}$ 3303 (N-H), 3107 (C-H), 2973 (C-H), 1505 (C=C), 1176 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.24 (s, 11-NH), 10.38 (s, 13-NH), 8.65 (1H, d, $J$ = 8.8 Hz, H-3), 8.38 (1H, d, $J$ = 8.8 Hz, H-4), 8.31 (s, H-9), 8.28 (1H, d, $J$ = 2.1 Hz, H-5), 7.97 (1H, d, $J$ = 9.0 Hz, H-8), 7.90 (1H, dd, $J$ = 9.0, 2.1 Hz, H-7), 7.54 (1H, dd, $J$ = 9.0, 5.1 Hz, H-2'/6'), 7.24 (1H, t, $J$ = 8.7 Hz, H-3'/5'); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.9 (C-12), 159.9 (d, $J_{CF}$ = 240.2 Hz, C-4'), 154.3 (C-2), 146.0 (C-8a), 142.7 (C-9), 135.4 (C-4), 134.2 (C-1'), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.1 (C-4a), 128.5 (d, $J_{CF}$ = 8.3 Hz, C-2'/6'), 120.1 (C-6), 119.3 (C-3), 114.8 (d, $J_{CF}$ = 22.3 Hz, C-3'/5'); HRMS (pos) (m/z): calculated for C$_{17}$H$_{12}$BrFN$_4$S (M + H)$^+$: 402.00, found: 402.9850.
(E)-2-((6-bromoquinolin-2-yl)methylene)-N-(4-chlorophenyl)hydrazine carbothioamide (6m); yellow solid residue: 66% yield: mp 205-220 °C; UV \( \lambda_{\text{max}} \) (Ethylacetate) nm (log \( \varepsilon \)) 228 (4.70), 275 (4.58), 333 (4.48); IR \( \nu_{\text{max}} \) 3313 (N-H), 3284, 3129 (C-H), 2990 (C-H), 1510 (C=C), 1176 (C-F) cm\(^{-1}\); \(^1\)H-NMR (DMSO-\(d_6\), 400 MHz) \( \delta \) 12.29 (s, 11-NH), 10.41 (s, 13-NH), 8.64 (1H, d, \( J = 8.8 \) Hz, H-3), 8.38 (1H, d, \( J = 8.8 \) Hz, H-4), 8.32 (s, H-9), 8.29 (1H, d, \( J = 2.1 \) Hz, H-5), 7.97 (1H, d, \( J = 9.0 \) Hz, H-8), 7.90 (1H, dd, \( J = 9.0, 2.3 \) Hz, H-7), 7.61 (1H, d, \( J = 9.0 \) Hz, H-2'/6'), 7.46 (1H, d, \( J = 8.7 \) Hz, H-3'/5'); \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \( \delta \) 176.6 (C-12), 154.2 (C-2), 146.0 (C-8a), 142.9 (C-9), 137.9 (C-1'), 135.5 (C-4), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.7 (C-4'), 129.1 (C-4a), 128.0 (C-3'/5'), 127.9 (C2'/6'), 120.1 (C-6), 119.3 (C-3); HRMS (pos) (\( m/z \)): calculated for C\(_{17}\)H\(_{12}\)BrClN\(_4\)S (M + H): 417.97, found: 418.9957.

(E)-2-((6-bromoquinolin-2-yl)methylene)-N-(4-nitrophenyl)hydrazine carbothioamide (6n); yellow solid residue: 76% yield: mp 195-205 °C; UV \( \lambda_{\text{max}} \) (Ethylacetate) nm (log \( \varepsilon \)) 249 (4.32), 323 (4.63), 334 (4.67), 349 (4.61); IR \( \nu_{\text{max}} \) 3366 (N-H), 3071 (C-H), 2914 (C-H), 1721* (C=C), 1539, 1504, 1333, 1145 (C-F) cm\(^{-1}\); \(^1\)H-NMR (DMSO-\(d_6\), 400 MHz) \( \delta \) 11.52 (s, 11-NH), 9.71 (s, 13-NH), 8.57 (1H, d, \( J = 8.8 \) Hz, H-3), 8.41 (1H, d, \( J = 8.8 \) Hz, H-4), 8.30 (1H, d, \( J = 2.1 \) Hz, H-5), 8.24 (1H, d, \( J = 9.4 \) Hz, H-3'/5'); 8.16 (s, H-9), 8.01 (1H, d, \( J = 9.2 \) Hz, H-2'/6'), 7.96 (1H, d, \( J = 9.0 \) Hz, H-8), 7.89 (1H, dd, \( J = 9.0, 2.2 \) Hz, H-7), \(^{13}\)C-NMR (DMSO-\(d_6\), 100 MHz) \( \delta \) 154.1 (C-12), 152.4 (C-4'), 145.9 (C-2), 145.6 (C-8a), 142.1 (C-9), 141.7 (C-1'), 135.5 (C-4), 133.0 (C-7), 130.9 (C-8), 130.0 (C-5), 129.0 (C-4a), 124.7 (C-3'/5'), 120.0 (C-6), 119.2 (C-2'/6'), 119.1 (C-3). *These absorption bands only appear for the nitro substituted compounds, however the frequency is higher than usual for a nitro stretch, typically occurring at 1535-1600 cm\(^{-1}\).
(E)-2-((6-bromoquinolin-2-yl)methylene)-N-p-tolylhydrazine carbothioamide (6o); yellow solid residue: 59% yield: mp 212-220 °C: UV $\lambda_{\text{max}}$ (Ethylacetate) nm (log $\varepsilon$) 229 (4.71), 280 (4.64), 330 (4.57); IR $\nu_{\text{max}}$ 3309 (N-H), 3123 (C-H), 2963 (C-H), 1592 (C=C), 1537, 1514, 1485, 1249, 1177 (C-F) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.17 (s, 11-NH), 10.32 (s, 13-NH), 8.66 (1H, d, $J$ = 8.8 Hz, H-3), 8.37 (1H, d, $J$ = 8.8 Hz, H-4), 8.31 (s, H-9), 8.28 (1H, d, $J$ = 2.2 Hz, H-5), 7.97 (1H, d, $J$ = 9.0 Hz, H-8), 7.90 (1H, dd, $J$ = 9.0, 2.3 Hz, H-7), 7.41 (1H, d, $J$ = 8.3 Hz, H-2'/6'), 7.20 (1H, d, $J$ = 8.2 Hz, H-3'/5'); $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) $\delta$ 176.6 (C-12), 154.4 (C-2), 146.0 (C-8a), 142.4 (C-9), 136.3 (C-1'), 135.4 (C-4), 134.9 (C-4'), 133.0 (C-7), 130.9 (C-8), 129.9 (C-5), 129.1 (C-4a), 128.6 (C-3'/5'), 126.2 (C2'/6'), 120.1 (C-6), 119.4 (C-3), 20.6 (C-7'); HRMS (pos) (m/z): calculated for C$_{17}$H$_{12}$F$_2$N$_4$S (M + Na)$^+$: 398.02, found: 421.0108.

3.3 In-vitro antimicrobial studies

The antimicrobial activities of the synthesised compounds 6a-o were tested against two Gram +ve strains (Staphylococcus aureus ATCC 25923 and Methicillin resistant Staphylococcus aureus ATCC BAA-1683) and four Gram –ve strains (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium ATCC 14026 and Klebsiella pneumonia ATCC 314588) using levofloxacin and ciprofloxacin as standards for comparison. The disc diffusion method was used for initial screening. Those compounds that showed a broad spectrum of activity across all the bacterial strains, showing activity in at least four of the six strains were chosen to determine their minimum bactericidal activity (MBC).
For the disc diffusion assay, the bacterial micro-organisms were grown overnight at 37 °C in nutrient broth (Biolab, South Africa) and adjusted to a 0.5 McFarland standard using distilled water. Mueller-Hinton agar (MHA) (Biolab, South Africa) plates were prepared by dissolving 38 g of agar in 1 L of water and pouring these into sterile petri dishes, which were then allowed to set and dry at room temperature. They were then inoculated with the respective strains of bacteria by streaking a swab (dipped into the micro-organism solution) evenly over the entire sterile agar surface. A volume of 5 μL of a 10 mg mL^{-1} solution of each test compound was then impregnated onto antibiotic test discs (12 mm) and placed onto the Mueller-Hinton plates. They were then left to incubate for 24 hours at 37 °C, and the zones of inhibition measured in millimeters.

For the determination of MBCs, a swab of the microbial cultures (adjusted to 0.5 McFarland) prepared as described previously were again evenly streaked over sterile agar plates. The test compounds were dissolved in DMSO and serially diluted in 1 mL Eppendorf tubes. A volume of 5 μL of concentrations of 19.5 to 625 µg mL^{-1} were directly spotted on MHA plates containing the respective bacterial strains and incubated at 37 °C for 20 h to determine the MBCs. The MBC was the lowest concentration showing a zone of inhibition around the spotted compound. DMSO was used as a control and showed no zones of inhibition to any of the bacterial strains tested against. Levofloxacin and ciprofloxacin served as the standard drugs for all antimicrobial studies. All experiments were conducted in triplicate.
Chapter 4. Conclusion

Fifteen 6-substituted quinoline-2-thiosemicarbazones were synthesised in good yields from a Doebner-von Miller reaction between para-substituted anilines and crotonaldehyde to form quinoline intermediates. Oxidation with SeO$_2$ to form quinoline-2-carbaldehydes proved to be a useful method to functionalise the 2-position for further reactions and to hybridise the quinoline framework. Formation of thiosemicarbazones was an easy derivatisation and way in which the quinolines could form quinoline thiosemicarbazone hybrid molecules. Thus, the reaction sequence carried out was a good way of forming quinoline thiosemicarbazone hybrids which have the attributes of both quinolines and thiosemicarbazones.

A full structural elucidation of all the compounds synthesised was carried out and will provide a basis for the identification of similar molecules in future research. The different substitution patterns were found to have an effect on the antibacterial activity of the compounds with compound 6l, containing a 6-Br substituent on the quinoline moiety and fluoro group at the para position of the thiosemicarbazide moiety showing the best antibacterial activity at 387 µM. Unfortunately, the antibacterial activity of the synthesised compounds were inactive as antibacterial agents even though the individual constituents, quinolines and thiosemicarbazones have previously demonstrated good antibacterial activity. This could partly be due to the insolubility of the compounds in aqueous media.

Future work will involve modifying the structure of 6l (6-Br, 4'-F derivative), by adding other groups to the molecule using this as a basic scaffold. Compound 6l was the only compound that showed good antibacterial activity. It is hoped that modifications of the structure will identify a compound that can be developed into a potential antibiotic. The modifications can include groups that will increase the solubility of the product.
Chapter 5. References


Li, J. J., Corey, E. J. (Eds) Name reactions in heterocyclic chemistry. *John Wiley & Sons*, Hoboken, New Jersey, **2005**.


Supporting Information

SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SUBSTITUTED 2-QUINOLINE THIOSEMICARBAZONES

Siboniso Shezi
Supervisor: Prof. N. Koobarnally
6-fluoro-2-methyl quinolinaldehyde

$^1$H-NMR spectrum of compound 3a
6-fluoro-2-methyl quinolinealdehyde

Aromatic region $^1$H-NMR spectrum of compound 3a
6-fluoro-2-methyl quinolinaldehyde

$^{13}$C-NMR spectrum of compound 3a
6-chloro aldehyde in CDCl3

$^1$H-NMR spectrum of compound 3b
Aromatic region $^1$H-NMR spectrum of compound 3b
6-chloro aldehyde in cdcl3

\[ ^{13}C\text{-NMR spectrum of compound 3b} \]
COSY spectrum of compound 3b
HSQC spectrum of compound 3b
HMBC spectrum of compound 3b

6-chloro aldehyde in CDCl3
6-bromo-2-methyl aldehyde in CDCl3

1H-NMR spectrum of compound 3c
6-bromo-2-methyl aldehyde in CDCl3

$^1$H-NMR spectrum of compound 3c
6-bromo-2-methyl aldehyde in CDCl3

13C-NMR spectrum of compound 3c
phenylthiosemicarbazide 1

1H-NMR spectrum of compound 5a
$^1$H-NMR spectrum of compound 5b
4-Chloro phenyl thiosem

$^1$H-NMR spectrum of compound 5c
NO2-TSCarbazide

$^1$H-NMR spectrum of compound 5d
4-methyl phenyl thiosemicarbazide

\[ \text{1H-NMR spectrum of compound 5e} \]
QT2 (F, phenyl) in dms

$^1$H-NMR spectrum of compound 6a
Aromatic region $^1$H-NMR spectrum of compound 6a
13C-NMR spectrum of compound 6a
UV spectrum of compound 6a

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IR spectrum of compound 6a
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Aromatic region $^1$H-NMR spectrum of compound 6b
$^{13}$C-NMR spectrum of compound 6b
COSY spectrum of compound 6b
Aromatic region COSY spectrum of compound 6b
HSQC spectrum of compound 6b
Aromatic region HSQC spectrum of compound 6b
Aromatic region HMBC spectrum of compound 6b
**Elemental Composition Report**

**Single Mass Analysis**
- Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
- Element prediction: Off
- Number of isotope peaks used for i-FIT = 3

**Monoisotopic Mass, Even Electron Ions**
- 14 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)

**Elements Used**
- C: 15-20  H: 10-15  N: 0-5  F: 0-2  S: 0-1
- QTSC: 2a 54 (1.788) Cm (1.61)
- TOF MS ES-

**HRMS of compound 6b**
$\lambda_{\text{max}}$ & $\text{Absorbance}$ & $\text{Log } \varepsilon$ \\
250 & 0.46 & 4.24 \\
326 & 0.54 & 4.31 \\
340 & 0.59 & 4.35 \\
354 & 0.62 & 4.37 \\

UV spectrum of compound 6b
IR spectrum of compound 6b
F-Cl QuinThiasem

$^{1}$H-NMR spectrum of compound 6c
Aromatic region $^1$H-NMR spectrum of compound 6c

- F-Cl QuinThiosem
FCl QuinThioser

13C-NMR spectrum of compound 6c
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
QTSC 2b 2 (0.068) Cm (1:60)
TOF MS ES-

HRMS of compound 6c
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UV spectrum of compound 6c
IR spectrum of compound 6c
Aromatic region $^{1}$H-NMR spectrum of compound 6d
F-No2 QTSC

\[ \text{13C-NMR spectrum of compound 6d} \]
UV spectrum of compound 6d

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IR spectrum of compound 6d
1H-NMR spectrum of compound 6e
Aromatic region ¹H-NMR spectrum of compound 6e
13C-NMR spectrum of compound 6e
**Single Mass Analysis**

**Tolerance** = 5.0 PPM  /  DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)

Elements Used:
C: 15-20  H: 15-20  N: 0-5  F: 0-1  Na: 0-1  S: 0-1
QTSC 4a 61 (2.024) Cm (1:61)
TOF MS ES+

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UV spectrum of compound 6e
IR spectrum of compound 6e
quin thio 2 (r=cl, r1=-phenyl) in DMSO
quin thio 2 (r=Cl, r1=phenyl) in DMSO

Aromatic region $^1$H-NMR spectrum of compound 6f
Chloro QTSC

$^{13}$C-NMR spectrum of compound 6f
UV spectrum of compound 6f

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IR spectrum of compound 6f
$^1$H-NMR spectrum of compound 6g
Aromatic region $^1$H-NMR spectrum of compound 6g
$^{13}$C-NMR spectrum of compound 6g
**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:

QTSC 1b 61 (2.025) Cm (1:61)
TOF MS ES-

Minimum: 357.0385
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**HRMS of compound 6g**
UV spectrum of compound 6g

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<td>4.42</td>
</tr>
<tr>
<td>295</td>
<td>0.44</td>
<td>4.33</td>
</tr>
<tr>
<td>384</td>
<td>0.53</td>
<td>4.31</td>
</tr>
</tbody>
</table>

Cl

[Chemical structure of compound 6g]
IR spectrum of compound 6g
$^1$H-NMR spectrum of compound 6h

- H$_2$O
- DMSO

Cl-Cl QTSC
Aromatic region $^1$H-NMR spectrum of compound 6h
13C-NMR spectrum of compound 6h
Elemental Composition Report

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20  H: 10-15  N: 0-5  S: 0-1  Cl: 0-2
QTSC 1a 2 (0.034) Cm (1:61)
TOF MS ES-

Minimum:
Maximum:
Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  i-FIT (Norm)  Formula
373.0090  373.0081  0.9  2.4  13.5  645.4  0.0  C17 H11 N4 S Cl2

HRMS of compound 6h
UV spectrum of compound 6h
IR spectrum of compound 6h
$^1$H-NMR spectrum of compound 6i
Aromatic region $^1$H-NMR spectrum of compound 6i
$^{13}$C-NMR spectrum of compound 6i
UV spectrum of compound 6i

<table>
<thead>
<tr>
<th>$\lambda_{\text{max}}$</th>
<th>Absorbance</th>
<th>Log $\varepsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td>249</td>
<td>0.69</td>
<td>4.47</td>
</tr>
<tr>
<td>321</td>
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<td>4.61</td>
</tr>
<tr>
<td>349</td>
<td>0.84</td>
<td>4.55</td>
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</tbody>
</table>

$\lambda_{\text{max}}$, Absorbance, Log $\varepsilon$
IR spectrum of compound 6i
1H-NMR spectrum of compound 6j
Aromatic region $^1$H-NMR spectrum of compound 6j
13C-NMR spectrum of compound 6j
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20  H: 15-20  N: 0-5  Na: 0-1  S: 0-1  Cl: 0-1
QTSC 4b 21 (0.675) Cm (1:61)
TOF MS ES+

1.30e+005

HRMS of compound 6j

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>i-FIT (Norm)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>377.0609</td>
<td>377.0604</td>
<td>0.5</td>
<td>1.3</td>
<td>12.5</td>
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<td>C18 H15 N4 Na S Cl</td>
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</table>
UV spectrum of compound 6j

<table>
<thead>
<tr>
<th>λmax</th>
<th>Absorbance</th>
<th>Log ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>251</td>
<td>0.70</td>
<td>4.40</td>
</tr>
<tr>
<td>330</td>
<td>0.51</td>
<td>4.26</td>
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<tr>
<td>360</td>
<td>0.66</td>
<td>4.37</td>
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</tbody>
</table>
IR spectrum of compound 6j
QT2(Br, phenyl, in DMSO)

1H-NMR spectrum of compound 6k
Aromatic region $^1$H-NMR spectrum of compound 6k
Bromo QTSC

13C-NMR spectrum of compound 6k
UV spectrum of compound 6k

<table>
<thead>
<tr>
<th>λmax</th>
<th>Absorbance</th>
<th>Log ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>249</td>
<td>0.60</td>
<td>4.37</td>
</tr>
<tr>
<td>331</td>
<td>0.51</td>
<td>4.30</td>
</tr>
<tr>
<td>347</td>
<td>0.57</td>
<td>4.35</td>
</tr>
<tr>
<td>360</td>
<td>0.64</td>
<td>4.40</td>
</tr>
</tbody>
</table>
IR spectrum of compound 6k
Br-F Product

1H-NMR spectrum of compound 6l
Aromatic region $^1$H-NMR spectrum of compound 6l
$^{13}$C-NMR spectrum of compound 6l

Br-F Product

![C-NMR spectrum diagram with molecular structure and chemical shifts]
HRMS of compound 6l
UV spectrum of compound 6l
IR spectrum for compound 6l
Br-CI Product

1H-NMR spectrum of compound 6m
Br-Cl Product

Aromatic region $^1$H-NMR spectrum of compound 6m
Br-Cl Product

13C-NMR spectrum of compound 6m
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20  H: 10-15  N: 0-5  S: 0-1  Cl: 0-1  Br: 0-1

QTSC 3a 37 (1.214) Cm (1.61)
TOF MS ES-

HRMS of compound 6m
UV spectrum of compound 6m

<table>
<thead>
<tr>
<th>$\lambda_{\text{max}}$</th>
<th>Absorbance</th>
<th>Log $\varepsilon$</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>333</td>
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<td>4.48</td>
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</table>
IR spectrum of compound 6m
$^1$H-NMR spectrum of compound 6n
Aromatic region $^1$H-NMR spectrum of compound 6n
QTSC NO2

\[\text{13C-NMR spectrum of compound 6n}\]
UV spectrum of compound 6n

<table>
<thead>
<tr>
<th>λmax</th>
<th>Absorbance</th>
<th>Log ε</th>
</tr>
</thead>
<tbody>
<tr>
<td>249</td>
<td>0.46</td>
<td>4.32</td>
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<tr>
<td>323</td>
<td>0.93</td>
<td>4.63</td>
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<tr>
<td>349</td>
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<td>4.61</td>
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</tbody>
</table>
IR spectrum of compound 6n
Br-CH$_3$ QuinThiosem

$^1$H-NMR spectrum of compound 6o
Br-CH3 QuinThiosem

$^{13}$C-NMR spectrum of compound 60
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
32 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20  H: 15-20  N: 0-5  Na: 0-1  S: 0-1  Br: 0-1

QTSC 4c 31 (1.013) Cm (1:61)
TOF MS ES+

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>i-FIT (Norm)</th>
<th>Formula</th>
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</thead>
<tbody>
<tr>
<td>421.0108</td>
<td>421.0098</td>
<td>1.0</td>
<td>2.4</td>
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<td>C18 H15 N4 Na S Br</td>
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</table>

HRMS of compound 6o
UV spectrum of compound 6o
IR spectrum of compound 6o