SYNTHESIS AND BIOLOGICAL ACTIVITIES
OF 2-HYDROXYPHENYLACYL AZOLES AND
THEIR OXIME DERIVATIVES

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SYNTHESIS AND BIOLOGICAL ACTIVITIES
OF 2-HYDROXYPHENYLACYL AZOLES AND
THEIR OXIME DERIVATIVES

A THESIS

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Collage of Science and Agriculture,

by
ADELE CHEDDIE

2012

Supervisor: Dr N.A. Koorbanally
Preface

This study represents original work by the author and has not been submitted in any other form to another university. Where use was made of work of others it has been duly acknowledged in the text.

Candidate:

Signed:___________________ Name:___________________ Date:____________________

As the Candidate’s supervisor, I have approved this thesis for submission.

Supervisor:

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ABSTRACT

Fourteen phenylacyl and 2-hydroxyphenylacyl azoles and their oxime derivatives of which three were new, 2-(2-methyl-1H-imidazol-1-yl)-1-phenylethanone oxime 7; 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl)ethanone oxime 13 and 1-(2-hydroxyphenyl)-2-(1H-1,2,4 triazol-1-yl)ethanone oxime 14. The yields obtained for the ketone derivatives were between 50 and 60%, and between 80 and 95% for the oxime derivatives. All compounds were characterized using NMR, IR, UV and GCMS, and were tested for their antifungal and antibacterial activity. These compounds possessed moderate antifungal and weak antibacterial activity, with compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl)ethanone oxime showing the highest antifungal activity with a MIC value of 1 µg mL⁻¹. In general, the 2'-hydroxy substituted compounds were shown to have better antifungal and antibacterial activity than the unsubstituted compounds.
STRUCTURES OF COMPOUNDS

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ABBREVIATIONS

$^1$H NMR  proton nuclear magnetic resonance spectroscopy
$^{13}$C NMR  C-13 nuclear magnetic resonance spectroscopy
CDCl$_3$  deuterated chloroform
COSY  correlated spectroscopy
DMSO-d$_6$  deuterated dimethyl sulfoxide
DCM  Dichloromethane
EIMS  electron impact mass spectroscopy
EtOH  ethanol
HMBC  heteronuclear multiple bond coherence
HREIMS  high resolution electron impact mass spectroscopy
HSQC  heteronuclear single quantum coherence
MeOH  methanol
MIC  minimum inhibitory concentration
NOESY  nuclear overhauser effect spectroscopy
TLC  thin layer chromatography
UV  ultraviolet
d  doublet
dd  double doublet
Hz  hertz
IR  infrared
Mp  melting point
m  multiplet
Rf  Retention factor
s  singlet
t  triplet
td  triplet of doublets
DECLARATION

DECLARATION 1 – PLAGIARISM

I, ADELE CHEDDIE declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.

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.

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   a. Their words have been re-written but the general information attributed to them have been referenced
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Signed ………………………………………………………………..
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Chapter 1. Introduction

The search for new compounds that could have useful practical applications is an ongoing process. Compounds containing an azole ring possess properties which make them valuable in medicinal applications. Azoles are five-membered heterocyclic rings which contain nitrogen and at least one other heteroatom (Figure 1-1). They are known to have a wide range of applications, such as antimicrobial, antitumor, anti-tubercular, and most commonly, antifungal activities. We intend to evaluate the antifungal activity of compounds containing a phenyl ethanone backbone bonded to five membered heterocycles such as those below (Figure 1-1).

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{N} & \quad \text{N} \\
\text{1H-imidazole} & \quad \text{1H-1,2,4-triazole} & \quad \text{2-methyl-1H-imidazole}
\end{align*}
\]

Figure 1-1  Structure of azole rings, imidazole, triazole and 2-methylimidazole

Most antifungal drugs are highly toxic, and ineffective against new or re-emerging fungi due to the appearance of resistant strains. In spite of ongoing research on antifungal agents, azoles are still the drug of choice against invasive fungal infections. This is due to their low toxicity, fungistatic, and broad-spectrum properties (Aher et al., 2009). The discovery of new and potent antifungal azoles is the best way forward in overcoming resistance and developing effective therapies.
There is a vast amount of literature reported on the synthesis of structurally modified analogues of azoles. In this study we have chosen to focus our attention on phenylacyl and hydroxyphenylacyl azoles and their oxime compounds (Figure 1-2). Emami and co-workers (2008) developed an efficient protocol for the synthesis of 2-hydroxyphenylacyl azoles and its oxime derivative, where the azole and azolium compounds synthesized showed significant inhibition of fungal growth \textit{in vitro} when compared to the reference drug fluconazole. The study also indicated that the hydroxyl group and 4'- substitution on the phenyl ring increased the antifungal activity of the compound. This was also shown by Chai et al. (2011), where they used molecular docking to show that substitution at the 2' and 4' positions on phenylacyl azoles form hydrophobic and van der Waals interactions with hydrophobic regions on lanesterol. These substitutions increase the binding of these phenylacyl azole compounds to the active site; as shown by their antifungal activity at concentrations less than 1µg mL$^{-1}$ (Chai et al., 2011). Therefore, 2-hydroxyphenylacyl azoles may be considered as promising new antifungal agents.

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{figure1.png}
\caption{Structure of 2-hydroxyphenylacyl azole and its oxime derivative}
\end{figure}
1.1 Azole antifungals

Fungi are eukaryotic microorganisms such as yeast and molds. Many fungi have pathogenic potential and pose a continuous threat to human health and life. Healthy individuals are susceptible to a host of superficial mycoses (mycoses of skin, nails and hair), as well as severe life-threatening diseases such as histoplasmosis (Pfaller and Diekema, 2010; Kathiravan et al., 2012). Invasive fungal infections are a significant cause of morbidity and mortality in individuals with increased vulnerability. These are patients receiving immunosuppressive therapy, organ transplant patients and those infected with the human immunodeficiency virus (HIV) (Ranganathan, 2012).

The development of polyene antifungals was the first major milestone in medical mycology and Amphotericin B quickly became the ‘Gold standard’ treatment for serious infections. Due to its numerous side effects and high toxicity a continued search for new drugs led to the discovery of azoles as potent antifungals (Maertens, 2004).

These azoles were first reported to show antifungal activity by Woolley in 1944. Interest in the antifungal activity ofazole compounds only began to increase in the late 1960’s with the discovery of miconazole, econazole and ketoconazole, by Janssen pharmaceutica (Beerse, Belgium) (Maertens, 2004). It wasn’t until the late 1980’s that the most useful alternative emerged through the development of triazole derivatives, fluconazole and itraconazole (Figure 1-3).
The most active antifungals contain an imidazole or triazole ring, which share the same mechanism of action. The imidazoles are used to treat superficial mycoses, whereas the triazoles are more active in treating both superficial and systemic invasive fungal infections (Sheehan et al., 1999).

![Structures of some antifungal agents](image)

**Figure 1-3 Structures of some antifungal agents**

### 1.1.1 Mechanism of Action

The mode of action of all azole antifungals is to inhibit the synthesis of ergosterol from lanosterol in fungal cell membranes. Ergosterol is an essential component of fungal cell membranes, regulating its fluidity, asymmetry and integrity. Azoles target the 14α-demethylase enzyme, which catalyze the synthesis of lanosterol to ergosterol, which results in
a decrease in ergosterol and an accumulation of methyl steroids leading to the inhibition of fungal growth and replication (Maertens, 2004; Lass-Flörl, 2011; Kathiravan et al., 2012). Different targets for antifungal drugs are shown in Figure 1-4 (Kathiravan et al. 2012).

![Figure 1-4 Target sites for different antifungal drugs (Kathiravan et al., 2012)](image)

**1.1.2 Prevalence of fungal infections**

The frequency of invasive fungal infections has clearly increased in recent years. This was reported in a study of the epidemiology of sepsis in the United States, where the results showed that the annual number of cases of sepsis caused by fungal organisms increased by more than two-fold between 1979 and 2000 (Martin et al., 2003). Data collected from 49 hospitals over a 7-year period (1995-2002) revealed that *Candida* species had become the fourth most common cause of nosocomial blood stream infections (Wisplinghoff, 2004).
*Candida* and *Aspergillus* remain the most well-known cause of opportunistic pathogens, with an annual incidence of 72 – 290 infections per million people for *Candida* and 12-34 infections per million people for *Aspergillus* (Rees *et al*., 1998). Although there have been significant advances in antifungal drug development, the available drugs do not meet all the requirements of managing infections in the complex patient populations. The development of more antifungal drugs is urgently needed.

1.1.3 **Antifungal resistance to azoles**

The development of resistance of fungal species to therapeutic drugs has become increasingly apparent, and is a dominant factor for the concomitant rise in invasive fungal infections. Resistance is defined as the inability of an antifungal agent to inhibit the growth of the organism at concentrations that are achieved with normal dosing (Pfaller, 2012). Clinical resistance is defined as the inability to eradicate a fungal infection despite the use of antifungal agents at normal dosage (Pfaller, 2012).

The four mechanisms for azole resistance was described by Kanafani (2008); the first is the regulation of efflux pumps, which decreases the concentration of the drug at the target site, the second is point mutations which alter the target site, the third is over expression of the altered site inhibiting binding, and fourth is the development of pathways that bypass the disruptive effects of the drugs (Casalinuovo *et al*., 2004; Kanafani and Perfect, 2008).

The epidemiology of invasive fungal infections has been reported extensively. The increase in resistance by *Candida* species to fluconazole, as well as cross resistance to other azoles have been reported in a study by Pfaller *et al.* (2009). The results showed a rise in
fluconazole resistance from 9% to 14% during the period 2001 to 2007. A recent study conducted by the SENTRY antimicrobial surveillance program in 2008 to 2009, reported on the frequency of fungal infections and its associated resistance toazole antifungal agents (Pfaller et al., 2010). These results showed a total of 1239 infections of Candida species with the highest resistance rates to fluconazole (16.7%), voriconazole (11%) and posaconazole (5%) (Pfellar, 2010).

The emergence of multidrug resistant species of fungi is a real concern since neither theazole nor polyene antifungals are effective against them. The discovery of new and potent antifungal azoles is the best way forward in overcoming resistance and developing effective therapies.

1.2 Other Uses of Azoles

1.2.1 Anti-malarial

Rodrigues et al. (2011) and Tien Huy et al. (2002) reported known azole mycotics, such as ketoconazole and fluconazole, to be active against malaria causing Plasmodium spp. Havaldar and Patil (2008), synthesized and screened a series of triazole derivatives for anti-malarial activity. Among these derivatives, 3-{4-[4-(4-fluorophenyl)-4H-[1,2,4]-triazol-3-ylmethoxy]-phenyl}-2-phenyl-3H-quinazolin-4-one (Figure 1-5) was found to be most active against Plasmodium falciparum strains.
1.2.2 Anti-tubercular

Banfi et al. (2006) reported the imidazole derivative (Figure 1-6) as well as miconazole to have good anti-tubercular activity when tested against different strains of *Mycobacterium tuberculosis* (MTB) with MICs in the range of 4 to 64 µg mL⁻¹. Guardiola-Diaz et al. (2001) also showed imidazole containing antimycotics, ketoconazole and econazole to inhibit growth in mycobacterial strains at 100 µM and 1 µM respectively.
1.2.3 Anticancer

Compounds having triazole moieties such as vorozole, letrozole and anastrozole are reported to be good inhibitors for preventing breast cancer (Clemons et al., 2004). Demirbas et al. (2004) showed the therapeutic activity of the triazole derivative 4-amino-3-phenyl-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl acetic acid 2,4-dichlorobenzylidene hydrazide (Figure 1-7) for the treatment of breast cancer, with an activity range of 24 to 50 µM against breast cancer cells.

![Figure 1-7 Structure of 4-amino-3-phenyl-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl acetic acid 2,4-dichlorobenzylidene hydrazide (Demirbas et al., 2004)](image)
1.3  Synthesis of Azoles and its derivatives:

The general phenylacyl azole moiety (Figure 1-8), seen in many well know azole derivatives, is thought to be largely responsible for imparting antifungal activity.

![Az: Azole ring](image)

**Figure 1-8  General structure of the phenylacyl azole moiety**

Azole derivatives have been synthesized using a variety of synthetic methodologies. Conventional methods using polar aprotic solvents, methods using transition metal salts as catalysts and microwave assisted methods have been reported.

1.3.1  Conventional method

The conventional synthetic approach reported for phenylacyl azoles is a two-step reaction which involves a nucleophilic substitution reaction of the azole with 2-bromoacetophenone in the presence of a polar aprotic solvent (Scheme 1-1) since protic solvents would protonate the nitrogen of the azoles.

Güven *et al.* (2007) reported yields of 60\% with the use of dioxane-ether as a solvent whilst stirring for 18 hours under ambient conditions. Mamolo *et al.* (2004) and Clader *et al.* (1995) reported very good yields of 83 and 70\% respectively using tetrahydrofuran (THF) as a
solvent. Although the reaction time used by Mamolo et al. (2004) was considerably longer (12 hours) as opposed to the short 1.5 hour reaction time used by Clader et al. (2005). The method proposed by Emami et al. (2008) had proved to be most efficient obtaining yields of 60%. This method employed the use of dimethylformamide (DMF), while refluxing at 40-45 °C for 4 to 8 hours.

Scheme 1-1 Mechanism for N-alkylation of an azole with bromoacetophenone
1.3.1.1 Bromoacetophenones as intermediates for azole substitution

Various bromination methods of carbonyl compounds have previously been reported, the most common being the use of Br₂ (Siddiqui and Ahsan, 2010). Copper-bromide (CuBr₂) (King and Ostrum, 1964) and N-bromosuccinimide (NBS) (Heasley et al., 1988; Tanemura, et al., 2004) have also been used as milder reagents for bromination (Scheme 1-2). The advantage of using CuBr₂ and NBS is that they are less toxic and easier to handle. The use of these milder agents did not affect the overall result of the reaction; in fact they were reported to have excellent yields of 100 % by King and Ostrum (1964) and 80 % by Tanemura et al. (2004).

![Scheme 1-2 Bromination of carbonyl compounds](image)

The reactivity of bromoacetophenone is due to the inductive effect of the carbonyl group which withdraws electrons toward the oxygen of the carbonyl group resulting in a more electrophilic α carbon atom and a weaker C-Br bond (Erian et al., 2003). The interaction between the carbonyl group and the heteroatom is mainly electrostatic and the reaction is thought to proceed through an alkoxide intermediate resulting from the nucleophilic azole attacking the carbonyl group first due to the polarization interaction caused by the smaller
steric requirement of the ketone group as compared to a carbon of the alkyl bromide moiety (Erian et al., 2003) (Scheme 1-3). This is followed by an intramolecular S\textsubscript{N}2 reaction where the azole group is substituted for the weaker bromide base. The absence of protons to form a hydroxy group further promotes this and therefore non-protic solvents are ideal for this reaction.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {Bromoacetophenone};
\node (b) at (2,0) {Imidazole};
\node (c) at (4,0) {Slow};
\node (d) at (6,0) {$\text{Br}^\text{-}$};
\node (e) at (8,0) {2-(1\textit{H}-imidazol-1-yl)-1-phenylethanone};
\node (f) at (0,-2) {Bromoacetophenone};
\node (g) at (2,-2) {Imidazole};
\node (h) at (4,-2) {$\text{Br}^\text{-}$};
\node (i) at (8,-2) {2-(1\textit{H}-imidazol-1-yl)-1-phenylethanone};
\draw[->] (a) -- (b);
\draw[->] (b) -- (c);
\draw[->] (c) -- (d);
\draw[->] (d) -- (e);
\draw[->] (e) -- (f);
\draw[->] (f) -- (g);
\draw[->] (g) -- (h);
\draw[->] (h) -- (i);
\end{tikzpicture}
\end{center}

Scheme 1-3 Mechanism for the S\textsubscript{N}2 reaction of an azole with bromoacetophenone (Erian et al., 2003)
1.3.1.2 Formation of oxime derivatives

The oxime functional group is incorporated into many medicinal agents, including the antifungal agent, Oxiconazole. Oximes are formed by nucleophilic attack of hydroxylamine with the carbonyl carbon of a ketone to form an unstable carbinolamine intermediate (Emami et al., 2002). The carbinolamine intermediate is then broken down to an oxime by an acid catalyzed reaction (Emami et al., 2002) (Scheme 1-4).
Scheme 1-4 Mechanism for formation of the oxime derivatives
1.3.2 Microwave assisted methods

There are many heterocyclic reactions that can be used for automated medicinal chemistry in drug synthesis. These reactions have traditionally been carried out using solvents and with long reaction times. Microwave synthesis is a technique that has been shown to enhance the speed and reproducibility of the reaction (Bougrin et al., 2005). This was demonstrated by Kidwai et al. (2000), who synthesized oxazoles using microwave irradiation by reacting reagents on basic alumina in a microwave. Results showed a dramatic increase in yield and reduction of the reaction time as compared to the conventional method.

Scheme 1-5 The condensation reaction of urea with 1-(4-chlorophenyl)-2-bromoethanone producing an amino-oxazole, via microwave irradiation (MWI) (Kidwai et al., 2000)
1.3.3 Methods using transition metals

Limitations such as low yields, long reaction times, difficult work-up and occurrence of side reactions warranted the need for better catalysts for the synthesis of azole derivatives. Palladium, copper and platinum are the preferred transition metal catalysts used in these reactions (Aromi et al., 2011). Huang et al. (2008), showed the amination of aryl halides using imidazole to give higher yields in a shorter reaction time under milder reaction conditions, when using copper (II) acetate (Cu(OAc)$_2$) as a catalyst (Scheme 1-6).

\[
\text{Bromophenyl} + \text{imidazole} \xrightarrow{\text{Cu(OAc)$_2$, DBU, DMSO, MWI}} \text{1-phenyl-1H-imidazole}
\]

**Scheme 1-6 Mechanism for the Cross-Coupling of Aryl Halides with imidazole (Huang et al., 2008)**

The copper (II) acetate forms a four-coordination complex with the imidazole and 1,8-diazabicycloundec-7-ene (DBU), which then undergoes oxidative addition with the aryl halides to form a five-coordination copper complex, which is then reduced to produce the phenyl-1H-imidazole (Huang et al., 2008) (Scheme 1-7).
Scheme 1-7  Possible mechanism of N-arylation promoted by copper (II) acetate
(Huang et al., 2008)
1.4 Aims and objectives:

Since the azole functional group is present in many antibacterials and antifungals such as miconazole, econazole and ketoconazole, it was hypothesised that this functional group present in other carbon backbones could also possess antifungal and antibacterial activity. Hence we decided to explore the phenylacyl and 2-hydroxyphenylacyl azoles and their oxime derivatives.

The specific aim of the project was to synthesise a small library of phenylacyl and 2-hydroxyphenylacyl azoles and their oxime derivatives and to assess the synthesized compounds for their antimicrobial activity against different strains of fungi and bacteria.

Objectives of the study

1. To synthesize substituted phenylacyl and 2-hydroxyphenylacyl azoles and their oxime derivatives in a three step reaction from acetophenone and 2-hydroxyacetophenone.

2. To investigate and compare the effect that the oxime group and hydroxy substitution at the 2'st position has on antifungal activity.

3. To conduct primary screening for antifungal and antibacterial activity of the synthesized compounds.
Chapter 2. Experimental

2.1 General

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AVANCE III 400 MHz spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded at 400 MHz and 100 MHz respectively, at 30 °C with chemical shifts (δ) recorded against the internal standard, tetramethysilane (TMS). Melting points were recorded on an Ernst LeitzWetziar micro-hot stage melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F$_{254}$ plates. Column chromatography was carried out using silica gel 60. Infrared (IR) spectra were recorded neat from the Perkin Elmer spectrum100 instrument with a universal ATR attachment. Ultra Violet (UV) spectra were obtained on a Varian Cary UV-VIS Spectrophotometer in methanol. For Gas chromatography Mass spectrometry (GCMS) analyses, the samples were analysed on an Agilent GC–MSD apparatus equipped with DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column. Helium (at 2 mL min$^{-1}$) was used as a carrier gas. The MS was operated in the EI mode at 70 eV.
2.2 Synthetic procedures

2.2.1 Synthesis of 2-bromo-1(2-hydroxyphenyl) ethanone intermediate.

Bromine (0.044 mol, 10 mL) was dissolved in chloroform (50.0 mL) and added drop wise to a cold solution of substituted acetophenone (0.040 mol, 5 g) in chloroform (50.0 mL). The reaction mixture was stirred for an additional two hours at room temperature. The solvent was removed in vacuo to yield brominated acetophenones 1 (85% yield) and 2 (70% yield).

2-Bromo-1-phenyl ethanone (1);

\[
\begin{align*}
 & \text{C}_8\text{H}_7\text{BrO (199.04 g mol}^{-1}) \; ; \\
 & 85 \% \text{ yield; } \\
 & \text{White crystals; } \\
 & \text{Mp: 47 - 49 °C; } \\
 & \text{R}_f = 0.7 \text{ (Ethyl acetate/Hexane 10:90); } \\
 & \text{UV } \lambda_{\text{max}} \text{ nm (log } \varepsilon) : \text{250 (6.40), 210 (6.32); } \\
 & \text{IR } \nu_{\text{max}} \text{ (cm}^{-1}) : \text{3058, 3030, 2942, 1679 (C=O), 1595, 1447, 1277, 1193; } \\
 & \text{1H NMR (400 MHz, CDCl}_3\text{) } \delta : \text{7.96 (d, 2H, } J = 7.36 \text{ Hz, H-4/8), 7.59 (t, 1H, } J = 7.44 \text{ Hz, H-6), 7.47 (t, 2H, } J = 8.00 \text{ Hz, H-5/7), 4.43 (s, 2H, CH}_2\text{); } \\
 & \text{13C NMR (100 MHz, CDCl}_3\text{) } \delta : \text{191.49 (C-1), 134.16 (C-3), 129.13 and 129.06 (C4 –C-8), 31.11 (C-2); } \\
 & \text{EIMS } m/z \text{ (rel.int.): 202 [M}^+\text{, Br}^{81} \text{] (7), 199 [M}^+\text{, Br}^{79} \text{] (8), 91 (18), 44 (25), 32 (100).}
\end{align*}
\]
2-Bromo-1-(2-hydroxy-phenyl) ethanone (2);

C₈H₇BrO₂ (215.04 g mol⁻¹);
70% yield;
White crystals;
Mp: 50 - 52 °C;

Rᶠ = 0.7 (Ethyl acetate/Hexane 10:90); UV λmax nm (log ε): 330 (6.56), 257 (6.46), 210 (6.38); IR νmax (cm⁻¹): 3054, 3008, 2950, 1640 (C=O), 1458, 1448, 1221, 1156, 690 (C-Br);

¹H NMR (400 MHz, CDCl₃) 8: 11.70 (s, 1H, OH), 7.72 (dd, 1H, J = 8.08, 1.48 Hz, H-8), 7.50 (td, 1H, J = 8.60, 1.48 Hz, H-7), 6.99 (dd, 1H, J = 8.50, 0.68 Hz, H-5), 6.91 (td, 1H, J = 7.16, 0.84 Hz, H-7), 4.42 (S, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃) ⁸: 197.19 (C-1), 163.37 (C-4), 137.62 and 130.52 (C-6/8), 119.47 (C-5), 119.11 (C-7), 117.22 (C-3), 30.14 (C-2); EI MS m/z (rel.int.): 214 [M⁺, Br⁷⁹] (30), 201 (26), 134 (21), 121 (43), 105 (24), 32 (100).

2.2.2 Synthesis of 2-hydroxyphenylacyl azoles

The desired azole (0.015 mol, 1.0 g) was added to a solution of β-bromoketone (1 – 2, 0.005 mol, 1.0 g) in DMF (5.0 mL). The reaction mixture was refluxed with stirring at 65 °C for 4-8 hours; thereafter it was poured into water and extracted with ethyl acetate. The organic phase was concentrated in vacuo and further purified by column chromatography using dichloromethane (DCM): methanol (MeOH) (98:2). The appropriate fractions were evaporated to yield crystalline products (3 – 5, 9 – 11). The reaction is given in Scheme 2-1.
2.2.3 Synthesis of oxime derivatives of 2-hydroxyphenylacetyl azoles

A 6.0 mL solution of ethanol and water (1:1) containing the desired phenylacyl azole (0.005 mol, 1.0 g) (3 – 5, 9 – 11), sodium hydroxide (0.015 mol, 0.6 g) and hydroxylammonium chloride (0.015 mol, 1.1 g) was refluxed with stirring for two hours at 100 °C. The reaction mixture was cooled and the resulting precipitate was vacuum filtered, washed with cold water (20.0 mL) and dried to give the oxime derivatives (6 – 8, 12 – 14). The reaction is given in Scheme 2-1.
2.3 Physical characteristics, UV, IR and NMR data for phenylacyl azoles and their oxime derivatives

2-Imidazol-1-yl-1-phenyl ethanone (3)

- $C_{11}H_{10}N_2O$ (186.21 g mol$^{-1}$);
- 60% yield;
- Yellow crystals;
- Mp: 115 - 117 °C;

$R_f = 0.5$ (DCM/MeOH 95:5); UV $\lambda_{\text{max}}$ nm (log $\varepsilon$): 244 (6.26), 208 (6.19); IR $\nu_{\text{max}}$ (cm$^{-1}$):

- 3380, 3133, 2935, 1691 (C=O), 1560, 1595 (C=N), 1516, 1226 (CH$_2$ -N), 1058;

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.93 (d, 2H, $J = 7.80$ Hz, H-7/11), 7.63 (t, 1H, $J = 7.48$ Hz, H-9), 7.50 (t, 2H, $J = 7.92$ Hz, H-8/10), 7.48 (s, 1H, H-5), 7.10 (s, 1H, H-4), 6.92 (s, 1H, H-3) 5.37 (s, 2H, CH$_2$);

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 191.60 (C-1), 138.15 (C-5), 134.41 (C-9), 134.18 (C-6), 129.57 (C-4), 129.13 (C-8/10), 127.97 (C-7/11), 120.30 (C-3), 52.47 (C-2);

EIMS m/z (rel. int.): 186 [M$^+$ ] (15), 105 (100), 77 (61), 51 (18).

2-(5-Methyl-1H-imidazol-1-yl)-1-phenylethanone (4)

- $C_{12}H_{12}N_2O_2$ (200.23 g mol$^{-1}$);
- 59% yield;
- Yellow crystals;
- Mp: 134 - 136 °C;
$R_f = 0.5$ (DCM/MeOH 95:5); **UV** $\lambda_{max}$ nm (log $\varepsilon$): 244 (6.39), 205 (6.31); **IR** $\nu_{max}$ cm$^{-1}$: 3362, 3135, 2939, 1686 (C=O), 1597 (C=N), 1432, 1228 (CH$_2$-N); **$^1$H NMR (400 MHz, CDCl$_3$)** $\delta$: 7.95 (d, 2H, $J = 7.28$ Hz, H-8/12), 7.63 (t, 1H, $J = 7.44$ Hz, H-10), 7.51 (t, 2H, $J = 7.65$ Hz, H-9/11), 6.97 (d, 1H, $J = 1.28$ Hz, H-4), 6.79 (d, 1H, $J = 1.20$ Hz, H-3), 5.27 (s, 2H, CH$_2$), 2.29 (s, 3H, CH$_3$); **$^{13}$C NMR (100 MHz, CDCl$_3$)** $\delta$: 191.75 (C1), 145.72 (C-5), 134.58 (C-10), 134.51 (C-7), 129.35 (C-9/11), 128.19 (C-8/12), 127.68 (C-4), 120.57 (C-3), 52.21 (C-2), 13.07 (C-6); **EIMS m/z (rel. int.):** 200 [M$^+$] (23), 105 (100), 95 (7), 77 (42), 51 (12).

**1-Phenyl-2-[1,2,4]triazol-1-yl ethanone (5)**

![Chemical Structure](attachment:image.png)

C$_{10}$H$_9$N$_3$O (187.20 g mol$^{-1}$);

65% yield;

Pale yellow crystals;

Mp: 114 - 116 °C;

$R_f = 0.6$ (DCM/MeOH 95:5); **UV** $\lambda_{max}$ nm (log $\varepsilon$): 246 (6.36), 206 (6.28); **IR** $\nu_{max}$ cm$^{-1}$: 3114, 2993, 2955, 1697 (C=O), 1597 (C=N), 1505, 1346 (C-N, aromatic), 1226 (CH$_2$-N); **$^1$H NMR (400 MHz, CDCl$_3$)** $\delta$: 8.22 (s, 1H, H-3), 7.98 (s, 1H, H-4), 7.96 (d, 1H, $J = 7.32$ Hz H-6/10), 7.64 (t, 1H, $J = 7.44$ Hz, H-8), 7.51 (t, 2H, $J = 7.66$ Hz, H-7/9), 5.65 (s, 2H, CH$_2$); **$^{13}$C NMR (100 MHz, CDCl$_3$)** $\delta$: 190.74 (C-1), 152.10 (C-4), 145.06 (C-3), 134.76 (C-8), 134.20 (C-5), 129.36 (C-6/10), 128.30 (C-7/9), 55.20 (C-2); **EIMS m/z (rel. int.):** M$^+$ not detected, 105 (100), 77.1 (50), 51 (14).
2-(1H-Imidazol-1-yl)-1-phenylethaone oxime (6)

\[ \text{C}_{11}\text{H}_{11}\text{N}_{3}\text{O} \ (201.22 \text{ g mol}^{-1}) \].

98% yield;

Pale yellow crystals;

MP: 154 - 164 °C;

\[ R_f = 0.5 \ (\text{DCM}/\text{MeOH} \ 95:5) \];

UV \( \lambda_{\text{max}} \text{ nm} (\log \varepsilon) \): 246 (6.39), 206 (6.31);

IR \( \nu_{\text{max}} \text{ cm}^{-1} \):

3121, 3003, 2817, 2681 (N-OH), 1238 (CH\(_2\)-N), 1515, 1499, 1471, 1439, 1287, 957, 938, 924, 913;

\(^1\)H NMR (400 MHz, DMSO-\(\text{d}_6\)) \( \delta \):

7.66-7.68 (m, 2H, H-7 /11), 7.65 (s, 1H, H-5), 7.36-7.38 (m, 3H, H-8/9/10), 7.04 (s, 1H, H-3), 6.81 (s, 1H, H-4), 5.32 (s, 2H, CH\(_2\));

\(^{13}\)C NMR (100 MHz, DMSO-\(\text{d}_6\)) \( \delta \):

128.33 (C-4), 128.52 (C-8/10), 129.15 (C-9), 126.00 (C-7 & C-11), 119.53 (C-3), 38.94 (C-2);

EIMS \( m/\text{z} \) (rel. int.): 201 [M\(^+\)] (77), 103 (65), 91 (23), 81 (100), 77 (57), 69 (45).

2-(2-Methyl-1H-imidazol-1-yl)-1-phenylethanone oxime (7)

\[ \text{C}_{12}\text{H}_{13}\text{N}_{3}\text{O} \ (215.25 \text{ g mol}^{-1}) \]

98% yield;

White crystals;

MP: 170 - 185 °C;

\[ R_f = 0.5 \ (\text{DCM}/\text{MeOH} \ 95:5) \];

UV \( \lambda_{\text{max}} \text{ nm} (\log \varepsilon) \): 240 (6.38), 204 (6.30);

IR \( \nu_{\text{max}} \text{ cm}^{-1} \):

2995, 2586 (N-OH), 1497, 1422, 1277 (CH\(_2\)-N), 974, 731, 692;

\(^1\)H NMR (400 MHz, DMSO-\(\text{d}_6\)) \( \delta \):

7.50-7.52 (m, 2H, H-8/12), 7.32-7.34 (m, 3H, H-9/10), 6.89
(s, 1H, H-3), 6.60 (s, 1H, H-4), 5.22 (s, 2H, CH₂), 2.19 (s, 3H, CH₃); \(^{13}\)C NMR (100 MHz, DMSO-d₆) δ: 152.82 (C-1), 144.67 (C-5), 134.38 (C-7), 129.62 (C-10), 128.91 (C-9/11), 126.88 (C-8/12), 126.27 (C-4), 120.68 (C-3), 39.60 (C-2), 12.71 (C-3); EIMS m/z (rel. int.): 215 [M⁺] (92), 198 (26), 103 (70), 95 (100), 77 (53); HRMS [M⁺ + H] 216.1136, calculated for C₁₂H₁₄N₃O [M⁺ + H] 216.1137.

1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime (8)

\[ \text{C}_{10}\text{H}_{10}\text{N}_{4}\text{O} \]

98% yield;

White crystals;

Mp: 145 - 155 °C;

Rᵢ = 0.6 (DCM/MeOH 95:5); UV \( \lambda_{\text{max}} \text{ nm} \) (log ε): 247 (6.39), 205 (6.31); IR \( \nu_{\text{max}} \text{ cm}^{-1} \): 3415, 3103, 3010, 2824, 2764, 1515, 1473, 1444, 1279, 1136, 927; \(^{1}\)H NMR (400 MHz, DMSO-d₆) δ: 7.68 (s, 1H, H-3), 7.01 (s, 1H, H-4), 6.81-6.84 (m, 2H, H-6 /10), 6.50-6.52 (m, 3H, H-7/8/9), 4.65 (s, 2H, CH₂); \(^{13}\)C NMR (100 MHz, DMSO-d₆) δ: 151.12 (C-1), 150.36 (C-4), 145.02 (C-3), 134.35 (C-5), 129.02 (C-8), 128.40 (C-7/9), 126.25 (C-6 /10), 42.43 (C-2); EIMS m/z (rel. int.): 202 [M⁺] (19), 103 (100), 77 (30), 70 (45).
2.4 Physical characteristics, UV, IR and NMR data for 2-hydroxyphenylacyl azoles and their oxime derivatives

1-(2-Hydroxy-phenyl)-2-imidazol-1-yl ethanone (9)

C_{11}H_{10}N_{2}O_{2} (202.21 g mol^{-1});
58% yield;
Pale yellow crystals;
Mp: 175 - 177 °C;

R_{f} = 0.4 (DCM/MeOH 95:5); UV \lambda_{max} \text{ nm (log} \varepsilon) : 325 (6.42), 251(6.30), 215(6.24); IR \nu_{max} (cm^{-1}): 3123, 2934, 2427 (Ph-OH), 1667 (C=O), 1594 (C=N), 1452, 1231 (CH_{2}- N); \ H \text{ NMR (400 MHz, CDCl}_{3} \delta): 7.70 (d, 1H, J = 8.00 Hz, H-11), 7.54 – 7.61 (m, 2H, H-5/9), 7.16 (s, 1H, H-4), 7.04 (d, 1H, J = 8.40 Hz, H-8), 6.94-6.96 (m, 2H, H-3/10), 5.44 (s, 1H, CH_{2}); \ C \text{ NMR (100 MHz, CDCl}_{3} \delta): 196.88 (C-1), 162.82 (C-7), 140.35 (C-5), 137.82 (C-9), 131.07 (C-4), 128.64 (C-11), 121.31 (C-6), 119.79 (C-10), 119.38 (C-8), 117.49 (C-4), 52.12 (C-2); EIMS m/z (rel. int.): 202 [M^+](23), 121 (100), 93 (23), 82 (80).

1-(2-Hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone (10)

C_{12}H_{12}N_{2}O_{2}, (216.23 g mol^{-1});
62% yield;
Yellow crystals;
Mp: 164 - 166 °C;
$R_f = 0.4$ (DCM/MeOH 95:5);  
**UV $\lambda_{\text{max}}$ nm (log $\varepsilon$):** 328 (6.52), 250 (6.40), 216 (6.33);  
**IR $\nu_{\text{max}}$ (cm$^{-1}$):** 1651 (C=O), 1577 (C=N), 1194 (CH$_2$-N);  
**$^1$H NMR (400 MHz, CDCl$_3$) $\delta$:** 7.72 (dd, 1H, $J = 8.08$, 1.4 Hz, H-12), 7.55 (td, 1H, $J = 7.41$, 1.56 Hz, H-10), 7.04 (d, 1H, $J = 8.52$ Hz, H-9), 6.97 (td, 1H, $J = 8.04$, 0.84 Hz, H-11), 6.99 (d, 1H, $J = 1.2$ Hz, H-4), 6.80 (d, 1H, $J = 1.32$ Hz, H-3), 5.32 (s, 2H, CH$_2$), 2.30 (s, 3H, CH$_3$);  
**$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$:** 197.07 (C-1), 162.82 (C-8), 145.88 (C-5), 137.78 (C-10), 128.62 (C-12), 127.88 (C-4), 120.60 (C-3), 119.74 (C-11), 119.40 (C-9), 117.57 (C-7), 51.51 (C-2), 13.07 (C-6);  
**EIMS $m/z$ (rel. int.):** 216 [M$^+$] (30), 121 (100), 96 (84), 65 (30).  

**1-(2-Hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone (11)**

C$_{10}$H$_9$N$_3$O$_2$ (203.19 g mol$^{-1}$);  
62% yield;  
Pale yellow crystals;  
Mp: 135 - 137 °C;  

$R_f = 0.5$ (DCM/MeOH 95:5);  
**UV $\lambda_{\text{max}}$ nm (log $\varepsilon$) 322 (6.50), 251 (6.40), 212 (6.33);  
**IR $\nu_{\text{max}}$ (cm$^{-1}$) 3126, 2924, 1663 (C=O), 1597 (C=N), 1453, 1229 (CH$_2$-N);  
**$^1$H NMR (400 MHz, CDCl$_3$) $\delta$:** 11.41 (s, 1H, OH), 8.21 (s, 1H, H-3), 8.00 (s, 1H, H-4), 7.70 (d, 1H, $J = 7.88$ Hz, H-10), 7.54 (t, 1H, $J = 8.12$ Hz, H-8), 7.02 (d, 1H, $J = 8.48$ Hz, H-7), 6.96 (t, 1H, $J = 7.40$ Hz, H-9), 5.68 (s, 2H, CH$_2$);  
**$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$:** 195.77 (C-1), 162.79 (C-6), 152.29 (C-4), 145.25 (C-3), 137.96 (C-8), 128.80 (C-10), 119.86 (C-9), 119.29 (C-7), 117.36 (C-5), 54.44 (C2);  
**EIMS $m/z$ (rel. int.):** 203 [M$^+$] (7), 121 (100), 93 (15), 65 (22).
1-(2-Hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime (12)

![Chemical structure of compound 12](image)

C₁₁H₁₁N₃O₂ (217.22 g mol⁻¹)

97% yield;

Pale yellow crystals;

Mp: 165 - 177 °C;

Rₛ = 0.4 (DCM/MeOH 95:5); UV λₚₐₓ nm (log ε): 318 (6.34), 258 (6.23), 221 (6.32);

IR νₚₐₓ (cm⁻¹): 3124, 2577 (N-OH), 1579 (C=N), 1510, 1478, 1281, 1242 (CH₂-N), 1068;

¹H NMR (400 MHz, DMSO-d₆) δ: 12.06 (s, 1H, OH), 10.58 (s, 1H, OH), 7.60 (s, 1H, H-5), 7.35 (dd, 1H, J = 7.52, 1.56 Hz, H-11), 7.21 (td, 1H, J = 8.48, 1.48 Hz, H-9), 7.02 (s, 1H, H-3), 6.85 (d, 1H, J = 8.28 Hz, H-8), 6.80 (t, 2H, J = 7.96 Hz, H-4/10), 5.30 (s, 2H, CH₂);

¹³C NMR (100 MHz, DMSO-d₆) δ: 156.27 (C-7), 154.29 (C-1), 137.70 (C-5), 130.44 (C-9), 128.95 (C-11), 128.07 (C-3), 119.64 (C-4), 119.29 (C-6), 118.98 (C-10), 116.19 (C-8), 40.69 (C-2); EIMS m/z (rel. int.): 199 [M⁺-H₂O], 172 (11), 132 (23), 81(90), 32 (100).

1-(2-Hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime (13)

![Chemical structure of compound 13](image)

C₁₂H₁₃N₃O₂ (231.25 g mol⁻¹)

95% yield;

Cream crystals;

Mp: 176 - 188 °C;

Rₛ = 0.4 (DCM/MeOH 95:5); UV λₚₐₓ nm (log ε): 312 (6.49), 259 (6.41), 225 (6.35);

IR νₚₐₓ (cm⁻¹): 2463 (N-OH), 1820, 1586 (C=N), 1447, 1424, 1298, 1272, 1254 (CH₂-N),
1064, 989, 749, 728; $^1$H NMR (400 MHz, DMSO) $\delta$: 11.72 (s, 1H, OH), 10.15 (s, 1H, OH), 7.02 -7.07 (m, 2H, H-10/12), 6.71 (d, 1H, $J = 8.12$ Hz, H-9), 6.69 (d, 1H, $J = 0.64$ Hz, H-3), 6.63 (t, 1H, $J = 7.24$ Hz, H-11), 6.46 (d, 1H, $J = 0.60$ Hz H-4), 5.08 (s, 2H, CH$_2$), 2.06 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO) $\delta$: 155.75 (C-8), 153.79 (C-1), 143.96 (C-5), 130.26 (C-10), 129.40 (C-12), 125.99 (C-4), 120.33 (C-7), 119.87 (C-3), 119.90 (C-11), 115.79 (C-9), 39.48 (C-2), 12.39 (C-6); EIMS $m/z$ (rel.int.): 213 [M$^+$] (100), 133 (80), 132 (78), 105 (26), 95 (88), 77 (38); HRMS [M$^+$ + H] 232.1081, calculated for C$_{12}$H$_{14}$N$_3$O$_2$ (M$^+$ + H) 232.1086.

1-(2-Hydroxyphenyl)-2-(1 H- 1,2,4 triazol-1-yl) ethanone oxime (14)

$\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}_2$ (218.21 g mol$^{-1}$).

97% yield;

White crystals;

Mp: 230 - 241 °C;

$R_f$ = 0.5 (DCM/MeOH 95:5); UV $\lambda_{\text{max}}$ nm (log $\varepsilon$): 308 (6.49), 255(6.40), 216 (6.33);

IR $\nu_{\text{max}}$ (cm$^{-1}$): 2650.56 (N-OH), 1577.55 (C=N), 1277.60 CH$_2$-N; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$: 8.44 (s, 2 × H-3), 7.84 (s, H-4), 7.82 (s, H-4), 7.38 (d, $J = 2.28$ Hz, H-10), 7.32 (t, $J = 7.68$ Hz, H-8), 7.30 (d, $J = 2.32$ Hz, H-10), 7.18 (td, $J = 7.12$, 0.92 Hz, H-8), 6.80 (t, $J = 8.64$ Hz, 2 × H-9), 6.76 (d, $J = 8.72$ Hz, 2 × H-7), 5.50 (s, H-2) and 5.48 (s, H-2); $^{13}$C NMR (100 MHz, DMSO-d$_6$) $\delta$: 155.93 (C-6), 155.15 (C-6), 153.13 (C-1), 151.88 (C-1), 151.21 (C-4), 151.10 (C-4), 145.01 (C-3), 144.88 (C-3), 132.69 (C-10), 131.55 (C-10), 130.30 (C-8),
129.30 (C-8), 122.62 (C-5), 119.76 (C-5), 118.89 (C-7), 118.01 (C-7), 115.94 (C-9), 43.58 (C-2), 43.20 (C-2). **EIMS m/z (rel. int.):** 200 [M$^+$ -H$_2$O] (64), 82 (68), 55 (100); **HRMS [M$^+$-H]** 217.0732, calculated for C$_{10}$H$_9$N$_4$O$_2$ (M$^+$-H) 217.0726.
Chapter 3. Results and discussion

This chapter contains the chemistry involved in synthesising six phenylacyl azoles and their oxime derivatives. A full structural elucidation is given for some of the compounds and the $^1$H and $^{13}$C NMR data compared to these compounds for complete assignment of the resonances.

3.1 Chemistry

The synthesis of 2-azolyl-2-hydroxyphenyl ethanone derivatives involves two steps (Scheme 3-1). The first step involves the bromination of acetophenone using Br$_2$ and chloroform to form the $\alpha$-bromoketones 1 – 2 and the second step entails reacting the $\alpha$-bromoketone with an excess of azole in dimethylformamide (DMF) at 65 °C under reflux to produce the corresponding azole derivatives (3 – 5 and 9 – 11). The ketones were converted into their oxime derivatives by reacting them with an excess of HONH$_2$.HCl in ethanol and water at 100 °C.

The first step of this synthesis involves the use of one equivalent of bromine to displace one hydrogen atom. It is important that only one equivalent of bromine is used as excess bromine results in the removal of both hydrogen atoms from the methyl group of acetophenone, and a mixture of di-bromoketone and mono-bromoketone is formed. The use of copper (II) bromide (CuBr$_2$) and NBS was also investigated since these are milder and less toxic reagents.
than elemental bromine. The yields obtained with these reagents were however much lower (45 - 60%) (Table 3-1).

![Scheme 3-1  Synthesis of 2-azolyl-2-hydroxyphenyl ethanone and its oxime derivatives](image)

**Table 3-1  Yields obtained for α-bromoketones using different brominating agents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Reaction time (Hours)</th>
<th>Yield (%)</th>
<th>Literature yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br₂</td>
<td>2</td>
<td>80-90</td>
<td>88-96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CuBr₂</td>
<td>6-8</td>
<td>58</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NBS</td>
<td>12</td>
<td>47</td>
<td>71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>- (Cowper and Davidson, 1943),  <sup>b</sup>- (King and Ostrum, 1964),  <sup>c</sup>- (Tanemura et al., 2004)
The synthetic approach reported for phenylacyl azoles involves nucleophilic substitution of the desired azole with an α-bromoketone in the presence of an aprotic solvent. The use of different solvents and reaction times were investigated to determine optimum yields for phenylacyl azoles. Emmai et al., (2008) used DMF in a 4 – 8 hour reaction which produced 58% yields. Güven et al. (2007) used dioxane-ether as the solvent for the reaction, which took 18 hours at ambient conditions and produced yields of 60%. Clader et al. (1995) reported yields of 70% with THF for the same reaction. The use of both THF and dioxane was also investigated in this work, where the reactions took between 12 – 20 hours at 65 °C and produced between 25 – 40 % of the desired product. The use of DMF had been the most efficient, obtaining yields of 60% with reaction times of 4 – 8 hours (Table 3-2). The azole derivatives (3 – 5 and 9 – 11) were obtained as white or yellow crystals, after further purification by column chromatography with DCM: MeOH (95:5).

**Table 3-2  Yields obtained for phenylacyl azoles using different solvents**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Reaction time (Hours)</th>
<th>Yield (%)</th>
<th>Literature yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>4-8</td>
<td>60</td>
<td>58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>THF</td>
<td>12-20</td>
<td>25</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dioxane-ether</td>
<td>12-20</td>
<td>30-40</td>
<td>60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>- (Emmai et al., 2008), <sup>b</sup>- (Clader et al., 1995), <sup>c</sup>- (Güven et al., 2007)
The ketone group in compounds (3 – 5 and 9 – 11) was converted into their oxime derivatives by the addition of excess sodium hydroxide and hydroxyaluminium chloride. The reaction was refluxed at 100 °C for two hours, and yields of greater than 95% were obtained. The high temperature was needed since a mixture of water and ethanol was used as the solvent. All the oxime derivatives (6 – 8 and 12 – 14) had precipitated out of solution once the reaction was complete. They were then washed with ethyl acetate and then cold water to remove unconverted ketone derivatives.

The yields and melting points of all 14 synthesized compounds are shown in Table 3-3. The melting points of the α-bromoketones was 47 - 49 °C for 1 and 50 - 52 °C for 2. Upon substitution with an azole moiety the melting points had increased and were greater than 100°C. The 2-hydroxy substituted compounds had much greater melting points than the unsubstituted compounds. The melting points of the 2-azolyl-2-hydroxyphenyl ethanone oxime derivatives showed an increase compared to their corresponding ketone derivatives. The oxime derivatives showed a broad melting point range with a difference of up to 15°C, this could be due to the racemic mixtures of the $E$ and $Z$ isomers.
Table 3-3  Melting points and yields for the 14 synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R’</th>
<th>Az</th>
<th>Physical Characteristic</th>
<th>Yield (%)</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>C</td>
<td>H</td>
<td>White</td>
<td>85</td>
<td>47 - 49</td>
</tr>
<tr>
<td>2</td>
<td>OH</td>
<td>C</td>
<td>H</td>
<td>White</td>
<td>70</td>
<td>50 - 52</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>C</td>
<td>Imidazole</td>
<td>Yellow</td>
<td>60</td>
<td>115 - 117</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>C</td>
<td>2-methylimidazole</td>
<td>Yellow</td>
<td>59</td>
<td>134 - 136</td>
</tr>
<tr>
<td>5</td>
<td>H</td>
<td>C</td>
<td>1,2,4-triazole</td>
<td>Yellow</td>
<td>65</td>
<td>114 - 116</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>N-OH</td>
<td>Imidazole</td>
<td>Yellow</td>
<td>98</td>
<td>154 - 164</td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>N-OH</td>
<td>2-methylimidazole</td>
<td>White</td>
<td>98</td>
<td>170 - 185</td>
</tr>
<tr>
<td>8</td>
<td>H</td>
<td>N-OH</td>
<td>1,2,4-triazole</td>
<td>White</td>
<td>98</td>
<td>145 - 155</td>
</tr>
<tr>
<td>9</td>
<td>OH</td>
<td>C</td>
<td>Imidazole</td>
<td>Yellow</td>
<td>58</td>
<td>175 - 177</td>
</tr>
<tr>
<td>10</td>
<td>OH</td>
<td>C</td>
<td>2-methylimidazole</td>
<td>Yellow</td>
<td>62</td>
<td>164 - 166</td>
</tr>
<tr>
<td>11</td>
<td>OH</td>
<td>C</td>
<td>1,2,4-triazole</td>
<td>Yellow</td>
<td>62</td>
<td>135 - 137</td>
</tr>
<tr>
<td>12</td>
<td>OH</td>
<td>N-OH</td>
<td>Imidazole</td>
<td>Yellow</td>
<td>97</td>
<td>165 - 177</td>
</tr>
<tr>
<td>13</td>
<td>OH</td>
<td>N-OH</td>
<td>2-methylimidazole</td>
<td>White</td>
<td>95</td>
<td>176 - 188</td>
</tr>
<tr>
<td>14</td>
<td>OH</td>
<td>N-OH</td>
<td>1,2,4-triazole</td>
<td>White</td>
<td>91</td>
<td>230 - 241</td>
</tr>
</tbody>
</table>
3.2 Characterization

All the synthesised compounds were characterized using $^1$H and $^{13}$C NMR, infrared (IR) and ultra violet (UV) spectroscopy and gas-chromatography mass spectrometry (GC-MS). The three novel compounds (7, 13 and 14) were also elucidated using 2D – NMR and high resolution mass spectroscopy (HRMS).

3.2.1 Structural elucidation of phenylacyl azoles and their oxime derivatives

The IR spectrum of 1 showed a carbonyl absorption at 1679 cm$^{-1}$ indicating the presence of the carbonyl group. The mass spectrum showed molecular ion peaks typical for that of the brominated compound with the Br$^{81}$ and Br$^{79}$ molecular ion isotopes at $m/z$ 201 and 199 respectively. The peak at $m/z$ 91 is typical of the tropylium cation. The $^1$H NMR spectrum showed the aromatic proton resonances at $\delta_H$ 7.96, a doublet assigned to H-4/8, $\delta_H$ 7.47, a triplet ascribed to H-5/7 and another triplet at $\delta_H$ 7.59 which is that of H-6. The coupling constants of the three resonances were between 7.36 and 7.56 Hz, characteristic of ortho coupling. The methylene resonance, H-2 appears as a singlet resonance at $\delta_H$ 4.43 and is deshielded because of the electronegative bromine atom. The $^{13}$C NMR spectrum shows the presence of the carbonyl group resonance at $\delta_C$ 191.49, together with the methylene resonance at $\delta_C$ 31.11 and the aromatic resonances at $\delta_C$ 129.13 and 129.06 (C-4 – C-8), and $\delta_C$ 134.16 (C-3). The $^1$H and $^{13}$C NMR resonances matched those from the literature (Steiner, et al., 2005).
Upon replacing the bromine in 1 with an imidazole ring in 3, the carbonyl peak in the IR spectrum showed a slight shift in wavelength from 1979 to 1691 cm\(^{-1}\). The imidazole aromatic bands were observed at 642-682 cm\(^{-1}\) in the IR spectrum. The structure was also verified by the molecular ion peak at \(m/z\) 186 in the mass spectrum. There was no change to the aromatic resonances in the \(^1\)H NMR spectrum as compared to 1, however as expected there was a downfield shift in the methylene resonance H-2 from \(\delta_H 4.43\) in 1 to \(\delta_H 5.37\) in 3 due to the electronegative nitrogen atom of the imidazole group. The H-3, H-4 and H-5 proton resonances of the imidazole moiety were present at \(\delta_H 6.92\), \(\delta_H 7.10\) and \(\delta_H 7.48\) respectively, the H-5 resonance overlapping with the H-8/10 resonance. The \(^{13}\)C NMR spectrum showed changes in the chemical shifts with respect to the aromatic carbon resonances and although these are in the same chemical shift region, the C-9, C-8/10, C-7/11 can be clearly distinguished at \(\delta_C 134.41\), 129.13 and 127.97 respectively. The remaining aromatic resonance C-6 appears at \(\delta_C 134.18\) and the imidazole carbon resonances appear at \(\delta_C 120.30\), \(\delta_C 129.57\) and \(\delta_C 138.15\). The carbonyl resonance is present at \(\delta_C 191.60\) and C-2 at \(\delta_C 52.47\), much more deshielded than its corresponding resonance in 1 due to being bonded to the imidazole ring. The proton and carbon resonance assignments were made in comparison to 7 below, which were elucidated using HSQC and HMBC data.

The IR spectrum of 4 showed a carbonyl stretching band at 1686 cm\(^{-1}\), which was similar to compound 3 at 1691 cm\(^{-1}\). The aromatic stretching bands of the 2-methylimidazole ring was observed in the region of 624-688 cm\(^{-1}\). The mass spectrum of 4 showed the molecular ion peak at \(m/z\) 200, and the base peak at \(m/z\) 105, which was indicative of the phenacetyl
fragment. In 4 all the aromatic proton and carbon resonances as well as that for methylene resonance and the carbonyl resonance had similar chemical shifts and splitting patterns to that of 1 and 3. However in 4, there were only two imidazole proton resonances as opposed to three in 3. The H-5 resonance that overlapped with H-8/10 at $\delta_H$ 7.48 was absent as indicated by the resonance only integrating to two protons in 4 as opposed to three in 3. This occurred with a concurrent appearance of an olefinic methyl resonance at $\delta_H$ 2.29 and a corresponding methyl carbon resonance at $\delta_C$ 13.07. This was assigned to the methyl group at C-5 on the imidazole ring.

The H-2 methylene proton resonance showed HMBC correlations to the carbon resonances at $\delta_C$ 120.57, 145.72 and the carbonyl resonance at $\delta_C$ 191.75. Since the resonance at $\delta_C$ 120.57 was protonated, this was attributed to C-3 with a corresponding doublet proton resonance at $\delta_H$ 6.79 ($J = 1.28$ Hz). It was now able to assign the remaining olefinic resonance at $\delta_H$ 6.97 to H-4. The other resonance showing a HMBC correlation to H-2 at $\delta_C$ 145.72 was accordingly ascribed to C-5 as this was a non-protonated carbon resonance, which was distinguished from the other non-protonated resonance at $\delta_C$ 134.51 because of HMBC correlations to H-3 and H-4. This resonance ($\delta_C$ 134.51) was thus assigned to C-7. There was also a weak HMBC correlation to H-2 which supported this assignment.

The triazole substitution in 5, afforded a slight shift in the carbonyl stretching band in the IR spectrum which was observed at 1697 cm$^{-1}$. The azole aromatic bands were observed in the same region as 4 at 635 - 688 cm$^{-1}$. The molecular ion peak in the mass spectrum for 5 was
observed at \( m/z \) 187. The fragmentation pattern was the same as that in compounds 3 and 4, showing the phenacyl fragment as the base peak at \( m/z \) 105, and the phenyl ring fragment at \( m/z \) 77.

In the \(^1\text{H}\) NMR spectrum of 5, most of the resonances were similar to that of 3 with the exception of the azole proton resonances and the methylene proton resonance H-2. The azole proton resonances now occurred at \( \delta_H \) 8.22 for H-3 and \( \delta_H \) 7.98 for H-4. These assignments were made in accordance with the assignments made for 12 using HMBC correlations (discussed below). The methylene resonance was more deshielded being present at \( \delta_H \) 5.65 as opposed to 5.37 in 3. This could probably be due to hydrogen bonding with the additional nitrogen atom in the azole ring. As expected there were now only two imidazole carbon resonances which were much more deshielded at \( \delta_C \) 145.06 (C-3) and \( \delta_C \) 152.10 (C-4) than their corresponding resonances in 3.

The formation of the imidazole oxime derivative 6 was verified by IR and mass spectroscopy. The disappearance of the carbonyl stretching band at 1691 cm\(^{-1}\) was observed in the IR spectrum of 3 and a broad N-OH band at 2681 cm\(^{-1}\) appeared in 6. This is indicative that formation of the oxime had occurred. The mass spectrum for compound 6 showed the molecular ion peak at \( m/z \) 201. The base peak was observed at \( m/z \) 81 which correlates to the imidazole moiety; the other major peak was the benzonitrile cation at \( m/z \) 103 (Scheme 3-2).
The H-7/11 resonance in 6 appeared as a multiplet at $\delta_H 7.66$, slightly more shielded than H-7/11 in 3. The H-8, H-9 and H-10 resonances overlap as a multiplet at $\delta_H 7.36$, also slightly more shielded than their corresponding resonances in 3. The H-5 resonance however is more deshielded than that in 3, appearing at $\delta_H 7.65$ in 6. The methylene resonance H-2 and the H-3 and H-4 resonances of the imidazole ring did not differ much from 3. There was also the presence of the extra oxime hydroxy resonance at $\delta_H 12.06$ in the $^1$H NMR spectrum further indicating that the oxime had formed. It must be noted however that the $^1$H NMR data was acquired in DMSO whereas that for 3 was acquired in CDCl$_3$ and therefore solvent effects could also account for the differences in the $^1$H NMR chemical shifts. In the $^{13}$C NMR spectrum, there was only a difference in the oxime carbon, which occurred at $\delta_C 151.90$ as opposed to the carbonyl resonance at $\delta_C 191.60$ in 3. This was accompanied by an upfield shift of the C-2 carbon, which occurred together with the solvent peak at $\delta_C 38.94$, determined by HSQC correlations. The $^1$H and $^{13}$C NMR resonances of 6 matched those in the literature (Rad, et al., 2009).
Scheme 3-2  Fragmentation pattern of the oxime derivative 6

In the other two oxime compounds with an unsubstituted phenyl ring (7 and 8), a similar splitting pattern was observed for the phenyl ring having two multiplets with similar chemical shifts. These occurred at $\delta_H$ 7.33 and $\delta_H$ 7.50 in 7 and $\delta_H$ 6.50 and $\delta_H$ 6.81 in 8. Similar to 6, the H-2 resonance did not shift much for 7; however there was a noticeable difference in chemical shift for H-2 in 8 at $\delta_H$ 4.65 as opposed to 5.66 in 5, the carbonyl derivative. For the triazole oxime derivative 8, the H-3 and H-4 resonances were seen to be shifted to $\delta_H$ 7.68 and 7.01 in comparison to $\delta_H$ 8.22 and 7.97 in 5. In the 2-methylimidazole oxime derivative 7, the H-3 and H-4 resonances appeared at $\delta_H$ 6.89 and $\delta_H$ 6.60 compared to $\delta_H$ 6.97 and $\delta_H$ 6.79 in 4. We speculate that this could be either due to a lower local magnetic field produced
by the pi electrons or this could be due to interaction with the solvent since both these compounds were also acquired in DMSO. The 2-methyl resonance did not change much from 4 to 7.

In the $^{13}$C NMR spectrum of 8, there was the expected shift of the carbonyl resonance from $\delta_C$ 190.74 in 5 to $\delta_C$ 151.12 in 8 and a shift of C-2 from $\delta_C$ 55.21 in 5 to $\delta_C$ 42.43 in 8, similar to that observed in compound 6. There was also an additional shift of the C-8 resonance from $\delta_C$ 134.76 in 5 to 129.02 in 8. The same trend was observed in 7 with the oxime carbon resonance being present at $\delta_C$ 152.82, C-2 at $\delta_C$ 39.60 and C-10 at $\delta_C$ 129.62.

The IR spectrum for the oxime derivatives 7 and 8 were similar to 6, showing respective broad N-OH stretching bands at 2586 and 2764 cm$^{-1}$. The mass spectrum for 7 and 8 showed the same trend as 6 by the presence of the benzonitrile ion at $m/z$ 103 for 7 and 8, and the 2-methylimidazole moiety at $m/z$ 95 for 7. The molecular ion peaks were observed at $m/z$ 215 for 7 and $m/z$ 202 for 8.

### 3.2.2 Structural elucidation of 2-hydroxyphenylacyl azoles and their oxime derivatives

The IR spectrum of 2 showed a carbonyl absorption at 1640 cm$^{-1}$ which had more single bond character compared to 1679 cm$^{-1}$ in 1. The mass spectrum showed a molecular ion peak for Br$^{79}$ at $m/z$ 214. The base peak at $m/z$ 121 indicated a 2-hydroxyphenacyl cation, due to $\alpha$-cleavage on the side of the azole ring.
With the 2-hydroxy substituted bromo compound, 2, the splitting patterns of H-5 and H-8 were each double doublets and occurred at $\delta_H$ 6.99 ($J = 8.50; 0.68$ Hz) and $\delta_H$ 7.72 ($J = 8.08; 1.48$ Hz). The H-6 and H-7 resonances appeared as two triplets of doublets at $\delta_H$ 7.50 ($J = 8.60$ and 1.48 Hz) and $\delta_H$ 6.91 ($J = 7.16$ and 0.84 Hz). The H-8 and H-5 resonances as well as the H-6 and H-7 resonances were distinguished based on their electronic effects due to the hydroxy group at C-4 where the ortho and para proton resonances are more shielded than the meta resonances. The shielding is due to electron donation by resonance to the oxygen of the hydroxy group. The phenolic hydroxyl resonance was also observed at $\delta_H$ 11.70 due to hydrogen bonding with the carbonyl group. The H-2 proton resonance did not change much as compared to 1, appearing at $\delta_H$ 4.42 as a singlet resonance. In the $^{13}$C NMR spectrum of 2, the carbonyl resonance was present at $\delta_C$ 197.19 and the C-2 resonance at $\delta_C$ 30.14. The six aromatic resonances were present at $\delta_C$ 163.37 for (C-4), $\delta_C$ 117.22 (C-3), 119.48 and 119.11 (C-5 and C-7 and could be interchangeable), $\delta_C$ 130.52 (C-8) and $\delta_C$ 137.62 (C-6).

On formation of the imidazole derivative 9, there were no differences in terms of chemical shifts with regard to the aromatic protons, H-8 to H-11, however due to overlap with the imidazole protons as indicated by the integration of the spectrum, H-3 with H-10 and H-5 with H-9 the splitting pattern of the H-9 and H-10 proton resonances could not clearly be seen and therefore were recorded as multiplets. The $^1$H NMR resonances of 9 occurred at $\delta_H$ 6.95 (m, H-3/10), $\delta_H$ 7.04 (d, $J = 8.40$, H-8), $\delta_H$ 7.16 (s, H-4), $\delta_H$ 7.54-7.61 (m, H-5/9), $\delta_H$ 7.70 (d, $J = 8.00$ Hz, H-11) and $\delta_H$ 5.44 (s, H-2). The change in chemical shift for H-2 as
well as the appearance of the imidazole proton resonances in 9 was evidence that the imidazole ring had successfully substituted the bromide. With reference to the $^{13}$C NMR spectrum of 9, the only resonance that shifted was understandably the C-2 resonance which appeared at $\delta_C$ 52.12 because of the imidazole being substituted for the bromide. All the other resonances of the phenyl ring were similar to that of 2.

The IR spectrum of 9 shows the carbonyl stretching band shift from 1640 cm$^{-1}$ in 2 to 1667 cm$^{-1}$ in 9. The substitution of the imidazole moiety was also validated by the presence of imidazole aromatic stretching bands which occurred at 619-657 cm$^{-1}$. The molecular ion peak of 9 was shown at $m/z$ 202. The two other major peaks in the mass spectrum of 9 were the 2-hydroxyphenyacetyl cation at $m/z$ 121, a product of $\alpha$-cleavage, and the imidazole cation at $m/z$ 82.

The 2-methylimidazole 10 and the triazole 11 derivatives of the substituted 2-hydroxy compounds were subsequently compared to 2 to determine if there were any changes to the phenyl ring. The azole rings were also compared to the corresponding un-substituted compounds 4 and 5 to determine if there were changes to the azoles protons in formation of the 2-hydroxy derivatives. The resonances of these two moieties were similar to those they were being compared to and therefore a discussion of these compounds is not included here.
The mass spectra of 10 and 11 showed the same fragmentation pattern as 9 with the base peak being present at $m/z$ 121 (2-hydroxyphenacyl cation) for both 10 and 11. The molecular ion peak was detected at $m/z$ 216 for 10 and $m/z$ 203 for 11. The IR spectra were also similar to 9. The carbonyl stretching bands were visible at 1651 cm$^{-1}$ and 1663 cm$^{-1}$ for 10 and 11 respectively.

The oxime derivatives of the 2-hydroxy substituted ketones (12 – 14) were acquired in DMSO and the chemical shifts in the $^1$H and $^{13}$C NMR spectra were different to that of the other compounds therefore a discussion on these three compounds are given below.

The $^1$H NMR spectrum of 12 showed the characteristic splitting pattern for the 2-hydroxy substituted phenyl ring having a double doublet at $\delta_H$ 7.35 ($J = 7.52, 1.56$ Hz) for H-11, a triplet of doublets at $\delta_H$ 7.20 ($J = 8.48, 1.48$ Hz) for H-9, a doublet at $\delta_H$ 6.84 ($J = 8.28$ Hz) for H-8 and a triplet at $\delta_H$ 6.80 ($J = 7.96$ Hz) attributed to H-10. The H-4 resonance overlapped with the triplet at $\delta_H$ 6.80, the H-3 resonance occurred at $\delta_H$ 7.02 and the H-5 resonance occurred at $\delta_H$ 7.60 as broad singlets. The C-5 carbon resonance was indicated by a HMBC correlation between C-5 and H-3. The two hydroxy group proton resonances were present at $\delta_H$ 12.07 and 10.58. The H-2 resonance was present at $\delta_H$ 5.31 as a singlet. The corresponding carbon resonances were assigned using the HSQC spectrum and the assignments confirmed by HMBC correlations; 2H-2 with C-3, C-5 and C-1; C-7 with H-11, H-9 and H-8. The oxime carbon resonance was present at $\delta_C$ 154.29 and the C-7 oxygenated.
carbon resonance present at $\delta_C$ 156.27. The $^1$H and $^{13}$C NMR resonances of 12 matched those from the literature (Emami, et al., 2008).

For the 2-methylimidazole oxime derivative 13, proton resonances ortho and para to the hydroxy group (H-9 and H-11) retained the splitting pattern but were more shielded at $\delta_H$ 6.71 (d, $J = 8.12$ Hz) and $\delta_H$ 6.63 (t, $J = 7.24$ Hz). The meta protons H-10 and H-12 both overlapped at $\delta_H$ 7.02 – $\delta_H$ 7.07 and was recorded as a multiplet. The C-3 and C-5 carbon resonance of the 2-methylimidazole ring was determined by HMBC correlations to H-2, which allowed H-3 to be identified by the HSQC spectrum and the remaining H-4 resonance was then determined by elimination. Thus, H-3 occurred at $\delta_H$ 6.69 (d, $J = 0.64$ Hz) and H-4 occurred at $\delta_H$ 6.46 (d, $J = 0.60$ Hz). The carbon resonances of C-3, C-4 and C-5 occurred at $\delta_C$ 119.87, 125.99 and 143.97 respectively.

Compound 14, the oxime derivative of the 2-hydroxyphenyl triazole ketone was synthesised as a mixture of E and Z isomers (Figure 3-1). This was evident by the twin resonances for H-2, H-4, H-8 and H-10. The two H-8 and two H-10 resonances were most notably separated from each other and upon adding the integration of the resonances, it is evident that the two isomers are present in a racemic mixture. Various attempts at separating the mixture failed and therefore, these are reported as a mixture of isomers.
The two H-2 resonances appeared at $\delta_H$ 5.50 and 5.48. This resonance was used in the HMBC spectrum to identify the two C-5 resonances at $\delta_C$ 122.62 and 119.76, the two C-3 resonances at $\delta_C$ 145.01 and 144.88 and the two C-1 resonances at $\delta_C$ 153.13 and 151.88. Using the HSQC spectrum, H-3 was identified at $\delta_H$ 8.44. The H-3 resonances for both isomers overlapped at this resonance. This H-3 resonance was then used to identify the C-4 resonances due to a HMBC correlation between H-3 and the two C-4 resonances at $\delta_C$ 151.21 and 151.10.

The two C-6 resonances were then identified at $\delta_C$ 155.93 and 155.15 since these resonances are in the aromatic C-O region of the spectrum. Due to electronic effects by the 2-hydroxy group, the ortho (2 x H-7) and para (2 x H-9) resonances were identified more upfield at $\delta_H$ 6.76 (d, $J = 8.72$ Hz) and $\delta_H$ 6.80 (t, $J = 8.64$ Hz) respectively to the two meta protons of H-8 at $\delta_H$ 7.32 (t, $J = 7.68$ Hz) and $\delta_H$ 7.18 (td, $J = 7.12$, 0.92 Hz) and H-10 at $\delta_H$ 7.38 (d, $J = 2.28$ Hz) and $\delta_H$ 7.30 (d, $J = 2.32$ Hz). We recognize that the coupling constant for the H-10 resonances are rather small (approximately 2.30 Hz) and should be approximately 8.0 Hz, but are unable to explain why this occurs. The only postulated explanation could be that rapid
conversion between the $E$ and $Z$ isomers affect the coupling constant of H-10 as this proton is in the vicinity of the oxime but could not find any literature on this phenomenon. There are two corresponding carbon resonances for each of C-7 ($\delta_C$ 118.89 and 118.01), C-8 ($\delta_C$ 130.30 and 129.30) and C-10 ($\delta_C$ 132.69 and 131.55), but only one for C-9 at $\delta_C$ 115.94. The two C-9 resonances for each of the isomers may overlap.

The molecular ion peaks for the 2-hydroxy substituted oxime derivatives (12 – 14) could not be detected in the mass spectra, but a $M^+ - H_2O$ peak was observed in each of the mass spectra ($m/z$ 199 for 12; $m/z$ 213 for 13 and $m/z$ 200 for 14). The fragmentation patterns were similar to the corresponding ketones. The base peak for 12 at $m/z$ 81, was shown to be the imidazole moiety which was cleaved at C-1. The 1,2-dimethyl-1H-imidazole fragment was detected at $m/z$ 95 for 13. The IR spectrum for compounds (12 – 14) all showed the absence of the carbonyl peak, indicating the formation of the oxime group. All oxime derivatives showed broad stretching at 2577 cm$^{-1}$ for 12, 2463 cm$^{-1}$ for 13 and 2650 cm$^{-1}$ for 14, indicative of the N-OH group.

The UV spectra for all 14 compounds are in the range of 200 – 260 nm. The $\pi \to \pi^*$ transitions of the phenyl ring was detected at 200 – 220 nm in all 14 compounds. The 2-hydroxy substituted compounds showed three absorption peaks, as compared to two in the unsubstituted compounds.
Chapter 4. Antifungal and antibacterial activity

4.1 Introduction

Antimicrobial susceptibility testing is an essential technique in many different disciplines, from pathology through to drug development (Powers, 2004). These tests are used to determine the efficacy of novel antimicrobials against a number of different microbial species (Powers, 2004).

*In vitro* susceptibility tests for measuring antifungal activity have been available since the 1950s. The methods used currently include broth dilution, agar dilution and disc diffusion. Each method has its own advantages and disadvantages, including the type of organism that may be accurately tested by the method. Susceptibility testing with antifungal agents (especially with azoles) against yeasts is markedly influenced by medium composition, length and temperature of incubation, as well as inoculum size (Pfaller, *et al*., 1990).

The broth dilution method determines whether a compound has microbicidal or microbiostatic action at a particular concentration. This procedure involves preparing two-fold dilutions of drugs in a liquid growth medium dispensed in 96 well plates. The plates are then inoculated with a standardized bacterial suspension. The lowest concentration of the drug that prevents microbial growth represents the minimal inhibitory concentration (MIC). Currently, the broth dilution method is recommended for testing commercial antifungal drugs targeting yeasts using either the National Committee for Clinical Laboratory Standards (NCCLS) reference procedure M27-A2 or EUCAST (European Committee on Antibiotic
The advantages of the broth dilution procedure include the generation of reproducible MICs, and assistance in generating computerized reports if an automated 96 well plate reader is used (Jorgensen and Ferraro, 2009).

The disk diffusion susceptibility method is widely used since it is cost effective and simple to use. The test is performed by applying absorbent discs containing various concentrations of the drug on agar growth medium inoculated with bacteria. The diameter of the clear zone around the disc is measured at the end of the incubation period and compared with standard drugs (Scorzoni, et al., 2007). One other advantage of the disk diffusion method is that it does not require any specialized equipment and the results can be easily interpreted (Jorgensen and Ferraro, 2009). The disadvantage of using this test method is the lack of automation of the test; it is also limiting in that not all bacteria and fungi can be accurately tested (Jorgensen and Ferraro, 2009).

The current study was conducted to investigate the antifungal activity of the 12 synthesized compounds since azole derivatives are known as potent antifungal agents. The susceptibility testing was evaluated by the broth micro-dilution method in accordance with the standardized protocol developed by the NCCLS.
4.2 Experimental

4.2.1 Microbial strains

The antimicrobial activities of the synthesized compounds 3 – 14 were investigated against several representative pathogenic organisms, yeast (*Candida albicans* ATCC 90028, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, and *Candida parapsilosis* ATCC 22019), two gram positive bacteria (*Staphylococcus aureus* ATCC 43300 and *Enterococcus faecalis* ATCC 5129) and three gram negative bacteria (*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853).

Fungal test organisms were grown on sabouraud dextrose agar (30 g L⁻¹), and bacterial test organisms on Muller-Hinton agar (21 g L⁻¹) at 35-37 °C in a CO₂ incubator for 24 hours. Organisms were suspended in saline and the turbidity was adjusted to 0.5 McFarland standard. This suspension was further diluted with saline to give a final suspension of 1-5 x 10⁴ colony forming units (CFU) mL⁻¹.

4.2.2 Reagents

For susceptibility testing procedures a synthetic medium Roswell Park Memorial Institute (RPMI) 1640 medium was used. This was supplemented with glucose and buffered with 0.165 M morpholine propane sulfonic acid (MOPS, Sigma-Aldrich) to pH 7.0. Sabouraud dextrose agar (Merck) and Muller-Hinton agar were reconstituted according to the manufacturer’s instructions and 25 mL aliquots were poured into petri dishes. The 3-(4, 5-
dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS, Promega Corporation, Madison, USA) was prepared by dissolving the MTS salt in phosphate buffered saline. Iodonitrotetrazolium chloride (INT) solution was freshly prepared in phosphate buffered saline to a final concentration of 200 µg mL\(^{-1}\). Each microplate well was measured after 24 hours of incubation with a 96-well microplate spectrophotometer (Mindray MR-96A) set at a wavelength of 490 nm.

### 4.2.3 Preparation of antimicrobial agents

Stock solutions (10 µg mL\(^{-1}\)) of the synthesized compounds 3-14 and reference drugs, Amphotericin B and Neomycin (Sigma Aldrich) were freshly prepared in dimethyl sulfoxide (DMSO). The final concentration of test compounds ranged from 0.0012 to 200 µg mL\(^{-1}\) and Amphotericin-B ranged from 0.0015 to 100 µg mL\(^{-1}\) and Neomycin from 0.0076 to 500 µg mL\(^{-1}\). All drug dilutions were carried out in 96-well flat bottom microtitre plates; each well contained 100 µL of two fold serial dilutions of the drugs tested and were stored at -70 °C until the day of testing.

### 4.2.4 Antifungal susceptibility testing

Evaluation of the susceptibility of Candida albicans and non- Candida albicans species was performed using the broth microdilution method according to the NCCLS reference procedure M27-A2. Yeast strains were grown aerobically overnight at 35 °C on Sabouraud dextrose agar plates. Yeasts were harvested and suspended in 1% sterile saline and the
turbidity of the supernatants measured spectrophotometrically at 625 nm with an absorbance of 0.08-0.1 equivalents to the No. 0.5 Mc Farland standard according to the NCCLS M27-A2 guidelines. The working suspension was diluted to 1:20 in RPMI 1640. The working suspension was further diluted with the medium (1:50) to obtain the final test inocula (1.0 - 1.5 x10^3 CFU mL^{-1}). The microtitre plates containing different concentrations of test compounds were allowed to thaw and equilibrate to room temperature under aseptic conditions. Aliquots of 100 µL of working inoculum suspensions were dispensed into each well and the plates incubated in an aerobic environment at 35 °C for 24 hours. After incubation, 20 µL of MTS salt was added directly to each well and incubated at 37 °C for 4 hours. The formation of formazan crystals was quantified by measuring the absorbance at 490 nm on a 96-well plate reader. All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

4.2.5 Antibacterial susceptibility test:

Bacterial susceptibility testing was carried out using the micro broth dilution method (Eloff, 1998; NCCLS M7-A5, 2000; Schwalbe et al., 2007). The 16 – 18 hour overnight cultures, which were incubated at 37 °C, were adjusted to turbidity comparable to a No. 0.5 McFarland standard. The inoculum was adjusted to an absorbance between 0.08-0.1 at 625 nm to yield a stock suspension of 0.4 - 0.5 x 10^8 CFU mL^{-1} which was diluted one hundred fold to obtain a working suspension of 1.0 x 10^6 CFU mL^{-1}. Microtitre plates were placed in the laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots (100 µL) of
bacterial inoculum were added to the microtiter plates containing appropriately diluted test compounds. Plates were incubated aerobically for 16 - 18 hours at 37 °C. Following incubation, 40 µL of freshly prepared iodonitrotetrazolium chloride solution was added to each well and the plate was further incubated for 45 minutes at 37 °C in the dark. The reduced iodonitrotetrazolium formazan (INTF) was measured after extraction with N,N-dimethyl formamide (DMF). Persistent growth of bacteria is indicated by a red colour change, while no colour change denotes the lack of bacterial growth. Neomycin was used as the control drug in this study. All analyses were performed in triplicate, and the data is reported as the mean ± standard error of the mean of ≤ 5.
4.3 Results and Discussion

4.3.1 Antifungal activity:

The in vitro antifungal activities of the synthesized compounds 3 - 14 were investigated against four pathogenic strains of Candida species (Table 4-1) using the broth-dilution method. The Minimum Inhibitory Concentration (MIC) values were determined by comparison to Amphotericin B (Amp-B) as the reference drug. The results indicate that compounds (9 - 14) with a 2'-hydroxy substitution on the phenyl ring showed better antifungal activity than the other test compounds, especially against Candida albicans (ATCC 90228) and Candida parapsilosis.

The 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime derivative 12 was the most potent against C. albicans (ATCC 10231), with a MIC value of 1 µg mL\(^{-1}\), and was comparable to that of the reference drug Amphotericin B (MIC 1.2 µg mL\(^{-1}\)). Among the compounds tested, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone 4 and 1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone 10 showed much higher activity than the rest of the test compounds against C. albicans (ATCC 90228) with MIC values of 12.5 and 25 µg mL\(^{-1}\) respectively.

In general, the compounds containing a triazole or 2-methylimidazole moiety showed better activity than its imidazole counterparts. The oxime derivatives of compounds containing imidazole 6 and triazole 8 and 14, showed better antifungal activity against C. albicans (ATCC 90028) than their ketone derivatives, whereas the oxime compounds containing 2-
methylimidazole (7 and 12) had no activity. *C krusei* ATCC-5129 was resistant to all the test compounds (3 – 14).

**Table 4-1 Minimum Inhibitory Concentration (MIC µg mL⁻¹) of test compounds on* Candida *species**

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>C. albicans</em> ATCC 90028</th>
<th><em>C. albicans</em> ATCC 10231</th>
<th><em>C. krusei</em> ATCC 6258</th>
<th><em>C. parapsilosis</em> ATCC 22019</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
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<td>5</td>
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<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>12.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>12.5</td>
</tr>
<tr>
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</tr>
<tr>
<td>13</td>
<td>-</td>
<td>25</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>14</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Amp-B</strong></td>
<td>1.2</td>
<td>1.2</td>
<td>5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

-: indicates no activity at 200 µg mL⁻¹. Data reported as the mean ± standard error of the mean ≤ 5.

4.3.2 Antibacterial activity

The antibacterial activity of compounds 3 – 14 were evaluated by the *in vitro* broth dilution assay and the results are presented in Table 4-2. Two Gram-positive (*S. aureus* and *E. faecalis*) and three Gram negative (*E. coli, K. pneumoniae* and *P. aeruginosa*) bacterial
strains were employed in the present study and Neomycin (Sigma Aldrich) was used as the reference drug. In general the antibacterial activity was not as good as the antifungal activity with moderate activity being exhibited by 6 against S. aureus (MIC of 200 µg mL\(^{-1}\)) and K. pneumonia (100 µg mL\(^{-1}\)), 9 against E. coli (MIC of 100 µg mL\(^{-1}\)) and 5 against P. aeruginosa (200 µg mL\(^{-1}\)). Both compounds 6 and 9 contain an imidazole moiety. All compounds were inactive against E. faecalis.

Compounds containing an oxime group (6 – 8, 12 – 14) as well as 10 containing a 2-methylimidazole group showed inhibitory activity against K. pneumonia, although all showed moderate in vitro antibacterial activity compared to the reference drug Neomycin.

Table 4-2 Minimum inhibitory concentration of the test compounds (MIC µg mL\(^{-1}\)) on gram positive and gram negative bacteria

<table>
<thead>
<tr>
<th>Compound</th>
<th>S.aureus ATCC-43300</th>
<th>E.faecalis ATCC-5129</th>
<th>E.coli ATCC-35218</th>
<th>K. pneumonia ATCC-700603</th>
<th>P. aeruginosa ATCC-27853</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
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<td>200</td>
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<td>100</td>
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<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>Neomycin</td>
<td>10</td>
<td>20</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

*: indicates no activity at 200 µg mL\(^{-1}\). Data reported as the mean sd ±5.

* compounds 3, 4, 7, 11 and 14 showed no antibacterial activity to the strains tested.
4.4 Conclusion

The antifungal and antibacterial results indicate that the synthesized compounds (3 - 14) possess moderate antifungal activity and weak antibacterial activity. These results are in agreement with the literature on antimicrobial activity for azole compounds, which show them to have antifungal properties.

In this study, we found the most active compound to be 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl)ethanone oxime 12, at 1 µg mL\(^{-1}\) against *C. albicans* (ATCC 10231). In general, the 2-hydroxy substituted compounds are shown to be more active against the different *Candida* species, as compared to their unsubstituted counterparts. The compounds containing a triazole or 2-methylimidazole moiety showed higher activity as compared to compounds containing an imidazole moiety.

The antibacterial results also indicated that in general, the 2-hydroxy substituted compounds showed better activity.

These results are only a preliminary screening for the antifungal activity of these compounds. Further antifungal testing is needed on non-candida species of yeasts, as well as molds and dermatophytes. From this study it is concluded that 2-hydroxyphenylacyl azole compounds have the potential to be antifungal agents and further studies on their structure-activity relationships and mode of action may provide us with a better understanding of their antifungal activities.
Chapter 5. Conclusion

The synthesis of the target compounds 1 – 14, were synthesised fairly easily in three steps with the only problem being that 14 was not separated into the E and Z isomers. The yields of the reactions were all between 58 and 98%, with almost half of the compounds having yields of greater than 90%.

The use of DMF proved to be the best solvent for the reaction with yields of 60%. The conversion of the ketones to their oxime derivatives was successfully accomplished with 95% of the ketone derivatives being converted. The oxime derivatives that formed were either E or Z stereoisomers and could not be separated.

Further work is needed in order to prepare pure isomers of this compound, or to further purify the racemic mixtures. This could lead to further testing of the purified isomers to investigate if the E or Z configuration of the oxime group has an effect on the antifungal activity of the compound.

Three novel compounds, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime 7; 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime 13; and 1-(2-hydroxyphenyl)-2-(1H-1,2,4 triazol-1-yl) ethanone oxime 14, were synthesized and their structures determined by NMR and confirmed by high resolution mass spectroscopy (HRMS).

Target compounds possessed moderate antifungal activity and weak antibacterial activity against several pathogenic fungi and bacteria. The most active antifungal compound was 10.
with a MIC value of 1µg mL\(^{-1}\) against \textit{C. albicans}. The triazole and 2-methylimidazole derivatives showed better antifungal activity with MIC values in the range of 12.5 to 50 µg mL\(^{-1}\). In general, the 2-hydroxy substituted derivatives showed better antifungal and antibacterial activity when compared to their corresponding unsubstituted derivatives.

The present study shows that 2'-methyl substitution on the azole ring and 2'-hydroxyl substitution on the aryl ring lead to enhanced inhibitory activity of the target compounds. Further optimization and antifungal screening against a wider range of pathogenic fungi is needed to assess the extent of the antifungal activity. Cytotoxicity screening is also needed to validate the results.
References


Demirbas, N., Karaoglu, S.A., Demirbas, A. and Sancak, K. 2004. Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]-thiadiazol-2-yl)-methyl-5-oxo-[1,2,4]-triazole and 1-(4-phenyl-5-thioxo-[1,2,4]-triazol-3-yl)-methyl-5-oxo-[1,2,4]-triazole derivatives. *European Journal of Medicinal Chemistry*. **39**: 793–804.


APPENDIX A
$^1$H NMR spectrum of compound 1,2-bromo-1-phenylethanone
$^1$H NMR spectrum of compound 1,2-bromo-1-phenylethanone
$^{13}$C NMR of compound 1, 2-bromo-1-phenylethanone
Ultra Violet spectrum of compound 1, 2-bromo-1-phenylethanone

Chemical Formula: C₇H₇BrO
Exact Mass: 197.9680
Mass spectrum of compound 1, 2-bromo-1-phenylethanone

Chemical Formula: C₆H₅BrO
Exact Mass: 197.9680
$^1$H NMR spectrum of compound 2, 2-bromo-1-(2-hydroxy-phenyl) ethanone
$^1$H NMR spectrum of compound 2, 2-bromo-1-(2-hydroxy-phenyl) ethanone
$^{13}$C NMR of compound 2, 2-bromo-1-(2-hydroxy-phenyl) ethanone.
Ultra Violet spectrum of compound 2, 2-bromo-1-(2-hydroxyphenyl)ethanone
Infrared spectrum of compound 2, 2-bromo-1-(2-hydroxy-phenyl) ethanone
Mass spectrum of compound 2, 2-bromo-1-(2-hydroxy-phenyl) ethanone
$^1$H NMR spectrum of compound 3, 2-Imidazol-1-yl-1-phenyl ethanone
$^1$H NMR spectrum of compound 3, 2-Imidazol-1-yl-1-phenyl ethanone
$^{13}$C NMR of compound 3, 2-Imidazol-1-yl-1-phenyl ethanone
$^13$C NMR of compound 3, 2-Imidazol-1-yl-1-phenyl ethanone
Ultra Violet spectrum of compound 3, 2-(1H-imidazol-1-yl)-1-phenylethanone
Infrared spectrum of compound 3, 2-Imidazol-1-yl-1-phenyl ethanone
Mass spectrum of compound 3, 2-Imidazol-1-yl-1-phenyl ethanone
$^1$H NMR spectrum of compound 4$_2$2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
$^1$H NMR spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
$^{13}$C NMR of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
COSY spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
HSQC spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
HSQC spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
HMBC spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
HMBC spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
NOESY spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
NOESY spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
Ultra Violet spectrum of compound 4, 2-(2-methyl-1H-imidazol-1-yl)-1-phenylethanone
Infrared spectrum of compound 4', 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
Mass spectrum of compound 4, 2-(5-methyl-1H-imidazol-1-yl)-1-phenylethanone
'H NMR spectrum of compound 5, 1-Phenyl-2-[1,2,4]triazol-1-yl ethanone
$^1$H NMR spectrum of compound 5, 1-phenyl-2-[1,2,4]triazol-1-yl ethanone
$^{13}$C NMR of compound 5, 1-Phenyl-2-[1,2,4]triazol-1-yl ethanone
Ultra Violet spectrum of compound 5, 1-phenyl-2-(1H-1,2,4-triazol-1-yl)ethanone
Infrared spectrum of compound 5, 1-Phenyl-2-[1,2,4]triazol-1-yl ethanone
Mass spectrum of compound 5, 1-Phenyl-2-[1,2,4]triazol-1-yl ethanone
1H: 2-imidazole-phenyl ethanone oxime in DMSO

Chemical Formula: C_{11}H_{11}N_{2}O
Exact Mass: 201.0902

1H NMR spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime
$^1$H NMR spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethanoone oxime
$^{13}$C NMR of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethane oxime
$^{13}\text{C} \text{NMR of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime}$
COSY spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime
COSY spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime
HSQC spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime
HSQC spectrum of compound 6H, 2-(1H-imidazol-1-yl)-1-phenylethane oxime
HMBC spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethanoic oxime
HMBC spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime
NOESY spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethane oxime
NOESY spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethanoate oxime
Ultra Violet spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethanone oxime
Infrared spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethethaone oxime
Mass spectrum of compound 6, 2-(1H-imidazol-1-yl)-1-phenylethaone oxime
$^{1}H$ NMR spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
IH: 2-(2-methylimidazole)-1-phenyl ethanone oxime in DMSO

Chemical Formula: C_{17}H_{13}N_{5}O
Exact Mass: 215.1059

1H NMR spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
$^{13}$C NMR of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime.
COSY spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
COSY spectrum of compound 7\(^{15}\), 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
HSQC spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
HSQC spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
HMBC spectrum of compound \(7\), 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
HMBC spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
NOESY spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
Ultra Violet spectrum of compound 7\% 2-(2-methyl-1H-imidazol-1-yl)-1-phenylethanone oxime
Infrared spectrum of compound 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
Mass spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
Adive 3 2 (0.017) Cm (1:31)
TOF MS ESI+

HRMS spectrum of compound 7, 2-(2-methyl-1H-imidazol-1-yl)-1-phenyl ethanone oxime
**1H NMR spectrum of compound 8, 1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime**
$^{1}$H NMR spectrum of compound 8, 1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime
$^{13}$C NMR of compound $8$, 1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime
$^{13}$C NMR of compound 8, 1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime
Ultra Violet spectrum of compound 8l, 1-phenyl-2-(1H-1,2,4-triazol-1-yl)ethanone oxime
Infrared spectrum of compound 8', 1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime
Mass spectrum of compound 8\textsuperscript{1}, 1-phenyl-2-[1,2,4]triazol-1-yl-ethanone oxime
$^1$H NMR spectrum of compound 9,1-(2-hydroxy-phenyl)-2-imidazol-1-yl ethanone
$^1$H NMR spectrum of compound 9, 1-(2-hydroxy-phenyl)-2-imidazol-1-yl ethanone
\(^{13}\text{C}\) NMR of compound 9, 1-(2-hydroxy-phenyl)-2-imidazol-1-yl ethanone
Ultra Violet spectrum of compound 9, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl)ethanone
Infrared spectrum of compound 9, 1-(2-hydroxy-phenyl)-2-imidazol-1-yl ethanone
Mass spectrum of compound 9, 1-(2-hydroxy-phenyl)-2-imidazol-1-yl ethanone
$^{1}H$ NMR spectrum of compound 1H-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
1H NMR spectrum of compound 10(1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
$^{13}$C NMR of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
COSY spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
COSY spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
HSQC spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
HSQC spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
HMBC spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
HMBC spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
NOESY spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone.
NOESY spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone.
Ultra Violet spectrum of compound 10,1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl)ethanone
Infrared spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
Mass spectrum of compound 10,1-(2-hydroxyphenyl)-2-(5-methyl-1H-imidazol-1-yl)ethanone
1H NMR spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
\(^1\)H NMR spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
$^{13}$C NMR of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
$^{13}$C NMR of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
COSY spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
COSY spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
HSQC spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
HSQC spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
HMBC spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
HMBC spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
NOESY spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
NOESY spectrum of compound 2, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
Ultra Violet spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl)ethanone oxime

Chemical Formula: C_{11}H_{13}N_{3}O_{2}
Exact Mass: 217.0851
Infrared spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
Mass spectrum of compound 12, 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl) ethanone oxime
$^1$H NMR spectrum of compound 11,1-(2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone
\[ ^1H \text{NMR spectrum of compound 1-(2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone} \]
$^{13}$C NMR of compound 1H,1-(2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone
Ultra Violet spectrum of compound 11,1-((2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone

Chemical Formula: C_{10}H_{12}N_{2}O_{2}
Exact Mass: 203.0695
Infrared spectrum of compound 11,1-(2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone
Mass spectrum of compound 11,1-(2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone
$^{1}$H NMR spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
"\textsuperscript{1}H NMR spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime"
$^{13}$C NMR of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
COSY spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
Cosy: 2-hydroxyphenyl-2-methylimidazole ethanone oxime in DMSO

COSY spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
HSQC spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
HSQC spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime.
HMBC spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
HMBC spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime.
NOESY spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
NOESY spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
Ultra Violet spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl)ethanone oxime
Infrared spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
Mass spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
16 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
Adele 2.8 (0.119) Cm (1.31)
TOF MS ES+

HRMS spectrum of compound 13, 1-(2-hydroxyphenyl)-2-(2-methyl-1H-imidazol-1-yl) ethanone oxime
$^1$H NMR spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1H-1,2,4 triazol-1-yl) ethanone oxime
1H NMR spectrum of compound 14. 1-(2-hydroxyphenyl)-2-(1 H- 1,2,4 triazol-1-yl) ethanone oxime
$^{13}$C NMR of compound 14, 1-(2-hydroxyphenyl)-2-(1H-1,2,4 triazol-1-yl) ethanone oxime
$^{13}$C NMR of compound 14, 1-(2-hydroxyphenyl)-2-(1H-1,2,4 triazol-1-yl) ethanone oxime
COSY spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H-1,2,4 triazol-1-yl) ethanone oxime.
HSQC spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H- 1,2,4 triazol-1-yl) ethanone oxime
HSQC spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H- 1,2,4 triazol-1-yl) ethanone oxime
HMBC spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1H-1,2,4 triazol-1-yl) ethanone oxime.
HMBC spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H- 1,2,4 triazol-1-yl) ethanone oxime
NOESY spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H-1,2,4 triazol-1-yl) ethanone oxime
Ultra Violet spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone oxime
Infrared spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H-1,2,4 triazol-1-yl) ethanone oxime
Mass spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H-1,2,4 triazol-1-yl) ethanone oxime
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
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 50 best isotopic matches for each mass)
Elements Used:
C: 10-15 H: 5-10 N: 0-5 O: 0-5
Adele 1.15 (0.239) Cm (1.31)
TOF MS ES-

HRMS spectrum of compound 14, 1-(2-hydroxyphenyl)-2-(1 H-1,2,4 triazol-1-yl) ethane oxime