

T.R.N.C
NEAR EAST UNIVERSITY
HEALTH SCIENCES INSTITUTE

**STUDY ON SYNTHESIS AND CHARACTERIZATION OF SOME 2-
BENZOXAZOLINONE DERIVATIVES**

ABDULLAHI GARBA USMAN

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Advisor

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DEDICATION

It is a pride to dedicate this laudable effort to my late father Engr. Garba Usman, who died on the 16th of June 2010 may his soul continue to rest in perfect peace. This work is also dedicated to my hardworking, tireless, dedicated and lovely mother who always pray day and night for the actualization of my success. May Allah reward her abundantly and may she live long to see other victories of mine.

To my family and my society (Mubhammasar)

ABSTRACT

According to the literature, 2-(3H)-benzoxazolone and its derivatives are compounds having different pharmacological activities more especially analgesic and anti-inflammatory. Its ability for modification at different positions makes it be of great interest in medicinal and pharmaceutical chemistry. Consequently they can be used in the development of new drug candidates that are COX-2 selective with less side effects.

In this research we synthesized two compounds, **Compound 1** via Mannich reaction, under reflux condition which involves modification of the 3rd position of 2-(3H)-benzoxazolone. **Compound 2** through a reaction at room temperature which involves the modification of the 6th position of 6-(2-bromo-acetyl)-2(3H)-benzoxazolone.

The synthesized compounds were characterized using FT-IR and ¹H-NMR spectroscopy. Their purity was checked using melting point determination and thin layer chromatography.

Keywords: 2-(3H)-benzoxazolone, 6-(2-bromo-acetyl)-2(3H)-benzoxazolone, Analgesics, Anti-inflammatory, Mannich reaction, Reflux

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List of Abbreviations

PPA:	Polyphosphoric acid
DMF:	N,N-dimethyl formamide
COX:	Cyclo-oxygenase
FT-IR:	Fourier Transform Infrared
NMR:	Nuclear Magnetic Resonance
NSAIDs:	Non-steroidal Anti-inflammatory drugs
TLC:	Thin Layer Chromatography
Ppm:	Part per million
nm	Nanometer
s:	singlet
m:	multiplets
t:	triplet
d:	duplet
pip:	piperazine
TMS:	Trimethyl silane
^1H :	Proton
Arom:	Aromatic
DMSO:	Dimethyl sulfoxide

1. INTRODUCTION

The basic and fundamental principle for the production of analgesics is to reduce, cure or minimize pain. According to Farlex Medical dictionary, pain is defined as, “An unpleasant sensation associated with actual or potential tissue damage, and mediated by specific nerve fibers to the brain, where its conscious appreciation may be modified by various factors” [1]. Pain is a result of many cases. The usual cause is an injury, even though pain may also be as a result of illness. Pain can further be classified into chronic and acute pain [2].

There are two types of analgesics, namely narcotics and non-steroidal anti-inflammatory drugs but the most used analgesics used globally are Nonsteroidal anti-inflammatory drugs (NSAIDS), and these NSAIDS have great side effects which can result into gastrointestinal bleeding and gastroduodenal ulcers. This brings about the idea for the production of new pain relievers with little or no side effects [3].

2-Benzoxazolone derivatives are used for synthesis of new drug candidates [4]. These benzoxazolone derivatives are of great interests due to the fact that they are readily accessible, cheap, and susceptible to structural and chemical modifications and most importantly they have varieties of biological properties. Their pharmacological effects constitute of antifungal, antibacterial, analgesics-anti-inflammatory, anti-cancer, anti-HIV and also used as COX-2 selective inhibitors [5].

The aim of this research is to synthesize some 2-benzoxazolinone derivatives through modification on the 6th position of the compound as well as through modification of the 3rd position using Mannich reaction. The compounds were characterized by Fourier Transform Infrared (FT-IR) and proton Nuclear Magnetic Resonance (^1H -NMR) spectroscopy. The purity was also determined using both melting point and thin layer chromatography (TLC).

2. LITERATURE REVIEW

2.1 Analgesics

Analgesics are medicines that are also known as anodynes which is derived from a Greek word and which means “without” and algia which means “pain”. This class constitute of all herbs, active compounds and drug molecules that have the ability to reduce, minimize, eliminate and ameliorate pain. The mechanism of action of analgesics generally act on either the central or peripheral nervous systems through multifarious pathways [6]. Furthermore, analgesics administration involves the use of pharmacological materials or agents to eliminate, reduce or relieve pain in the human body through different routes [7]. Analgesics are generally classified into narcotic (opioid) and non-narcotic (non-opioid) analgesics or non-steroidal anti-inflammatory drugs.

2.1.1 Opioid Analgesics

Opioids are regarded as the prototypical analgesics through which other analgesics are compared. These kinds of alkaloids are usually isolated from poppy seeds [8]. These analgesics are very active for the relief, minimizing and reduction of severe pain. They exert their action on the central nervous system [9]. They are either natural which are derivatives of opium e.g Morphine and Codeine or synthetic derivative such as heroin and meperidine.



Figure 2.1 Image of poppy flower and seed

2.1.1.1 Morphine

Morphine is among the key ingredients of opium, morphine is the most popular, influential and abused substance used so far in human history. It is also used for deadening, suppressing and lessening of pain in the human body. Both morphine and opium have long history of application for both recreational and medicinal uses. This has led to a great need to find alternatives to morphine and opium.

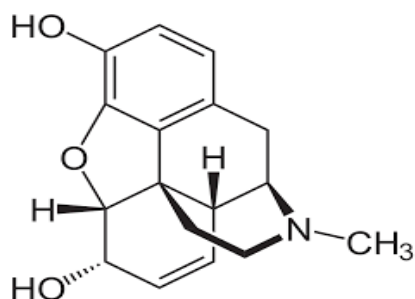


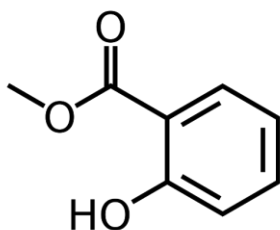
Figure 2.2 Morphine chemical structure

2.1.1.2 Codeine

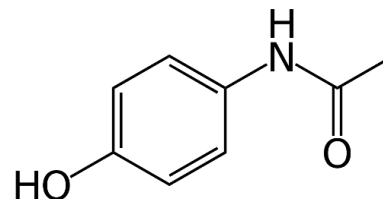
This is a slightly weak narcotic agent, known as a narcotic prodrug and its one of the most used and prescribed opiate drug used orally. It is also used frequently in comparative analgesic effect studies [10]. Sometimes when other common analgesics such as paracetamol e.g Aspirin were unable to provide pain relief to patients Codeine may be added to such analgesics to provide the desired effects and action. Codeine exert its action at different places in the central nervous system [11]. Codeine can also be used in effective controlling of cough and that is why it is considered as “gold standard” cough suppressant [12].

when phenylbutazene was first introduced three years after. These NSAIDs makes life comfortable through reducing and minimizing pain and through minimizing swelling in rheumatoid arthritis, osteoarthritis and much more types of arthritis and they are the best drugs used for acute gout.

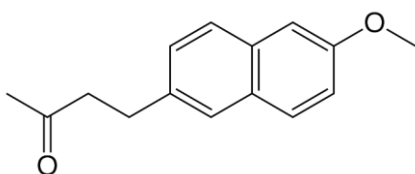
Their modes of action is through inhibition of prostaglandin synthetase (cyclo-oxygenase) even though other mechanisms are involved [13]. NSAIDs can be classified into; salicylic acid derivatives (2-hydroxybenzoic acid) drugs such as Aspirin, para-amino phenol derivatives drugs such as paracetamol, alkanones drugs such as Nabumetone and Indole derivatives drugs such as indomethacin.



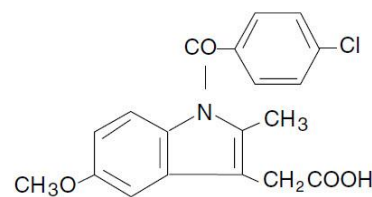
Aspirin



paracetamol



Nabumetone



Indomethacin

Figure 2.5: Chemical structure of some non-steroidal anti-inflammatory drugs (NSAIDs)

2.1.2.5 Mechanisms of action of NSAIDs

Previously, the analgesic effects and properties of non-steroidal anti-inflammatory drugs (NSAIDs) has been described based on the inhibition of certain enzymes used during the synthesis of prostaglandins [14]. Although it was clearly known that NSAIDs produced their analgesic action not only through the inhibition of prostaglandin synthesis in the peripheral nervous system but also through many other central and peripheral mechanisms [15].

It is currently known that there are two structural forms of cyclo-oxygenase enzymes (COX-1 and COX-2). Whereas, COX-1 is one of the members of normal cells but COX-2 is the one induced in inflammatory cells [16]. The analgesic mechanism of action of non-steroidal anti-inflammatory drugs is mostly due to inhibition of COX-2 activity. The ratio of inhibition between COX-1 and COX-2 by NSAIDs is used to determine the likelihood of adverse effects of the analgesics non-steroidal anti-inflammatory drug, though few NSAIDs may inhibit the lipoxygenase pathway, which can lead to the production of some algogenic metabolites, also interference of the NSAIDs with G-protein mediated signal may result in the formation of an analgesic mechanism which is not even related to the inhibition of prostaglandin synthesis.

2.1.2.6 Side effects of NSAIDs

It was not highly accepted that NSAIDs can cause damage and may have side effects and can cause damage distal to the duodenum. Certain reviews on the adverse effects of NSAIDs on “the intestines, the clinical implications and pathogens” which shows that ingested NSAIDs can cause a non-specific colitis and also research has shown that many people suffering from collagenons colitis are taking NSAIDs [17]. Also, other diseases such as large intestinal ulcers, perforations and bleeding are caused by NSAIDs. They also cause relapse of classic inflammatory bowel disease and can also cause fistula. It may occasionally cause strictures that requires surgery, small intestinal perforation and small intestinal inflammation. In conclusion, the treatments of these anomalies caused by these NSAIDs are been undergone trials, so the adverse effects of most of the NSAIDs are asymptomatic [18].

2.2 Cyclo-oxygenase (COX) Enzymes

Arachidonic acid is the acid used in the formation of prostaglandins (PG) in which cyclo-oxygenase is the first enzyme involved in this formation. The metabolites of cyclo-oxygenase have a very large and variety of pathophysiological and physiological effects and are used in some homeostatic processes. It is due to the actions of these metabolites in inflammatory edema and cardiovascular homeostasis and mostly pain lead to the therapeutic advantage of cyclo-oxygenase which affects some of the people at a particular stage in their life time.

The NSAIDs such as aspirin have some therapeutic advantages as well some negative side effects in the inhibition of cyclo-oxygenase. The beneficial therapeutic action and negative menace effects of NSAIDs are due to inhibition of one cyclo-oxygenase enzyme. Whereas cyclo-oxygenase inhibition at gastric mucosa explains their gastrototoxic effects but cyclo-oxygenase inhibition at inflammatory sites shows their therapeutic effects. Therefore, these reveals that there are two types of cyclo-oxygenase. A constitutive form also known as cyclo-oxygenase-1 and an inducible form also known as cyclo-oxygenase 2 enzymes. The inhibition of cyclo-oxygenase-1 (constitutive form) explains the side effects whereas inhibition of cyclo-oxygenase -2 explains the therapeutic advantages of nonsteroidal anti-inflammatory drugs [19].

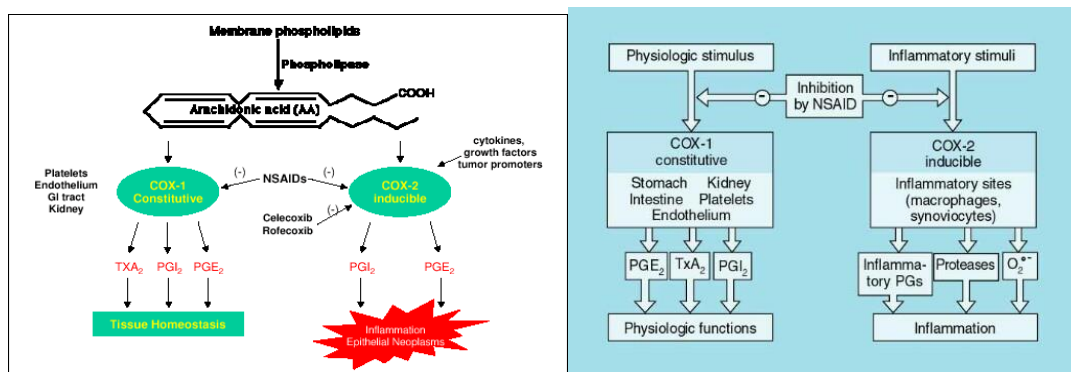
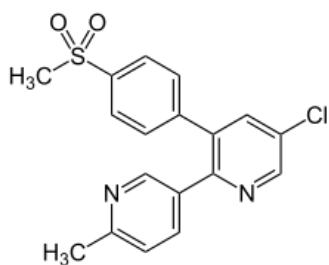


Figure: 2.6: Cyclo-oxygenase (COX-1 and COX-2) interaction with NSAIDs

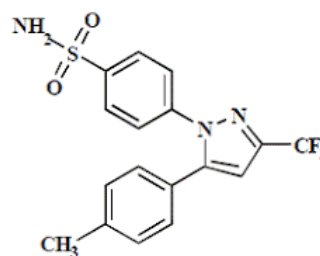
2.2.1 Selective COX-2 inhibitors

These are NSAIDs that selectively inhibits the COX-2 enzyme but not the COX-1 enzyme. COX-2 enzyme produces prostaglandins that causes inflammation, fever and other painful conditions.

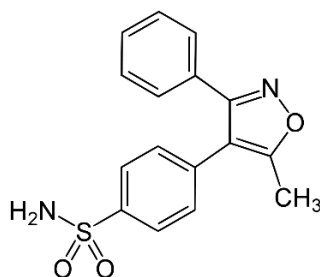
The selective inhibition of COX-2 enzyme by some NSAIDs makes these drugs unique and different from other traditional NSAIDs that mainly blocks both the COX-1 and COX-2 enzymes. COX-2 selective blocking NSAIDs are so important that they do not cause risk of injuring the gastro intestinal mucosa lining of the stomach which can subsequently leads to bleeding due to the inhibition of the COX-1 enzyme. Example of COX-2 inhibitors include; Etoricoxib, celecoxib and valdecoxib.



Etoricoxib



Celecoxib



Valdecoxib

Figure 2.7: Examples of some COX-2 selective inhibitors

2.3 2(3H)-Benzoxazolone

It is a heterocyclic bicyclic compound which composed of a benzene ring which was fused to a carbamate. The benzoxazolone structure composed of two parts which draw the attentions of pharmaceutical and medicinal chemists [20]. It composed of hydrophilic part and lipophilic part. The carbamate part of the benzoxazolone consists

of oxygen and nitrogen which attributes to the hydrophilic property of the compound, this oxygen and nitrogen contribute in the hydrogen bonding and hence increase the dipole moment of the compound. The lipophilicity of the compound is attributed due to its bulkiness. These bi-philic properties of benzoxazolone plays a vital role in the human body especially in absorption, distribution, metabolism and excretion (ADME) [21]. The 2-Benzoxazoline, being among the versatile bicyclic heterocyclic compounds, have been shown to have produced many compounds with large range of biological activities such as anti-cancer, anti-malaria, analgesics-anti-inflammatory, anti-convulsant, anti-tubercular, anti-bacterial, anti-HIV and anti-fungal [22].

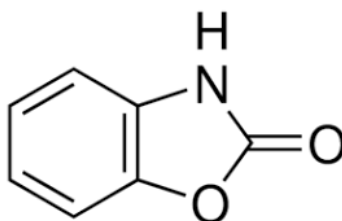


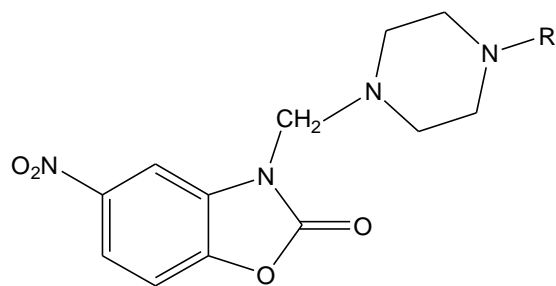
Figure 2.8: Chemical structure of 2(3H)-Benzoxazolone

2.3.1 2-(3H)-Benzoxazone in Nature

These 2-(3H)-benzoxazolinone derivatives were known to be derived from the class of phytoalexins which was reported to be present in some plant kingdom such as Pacea family (wheat, maize and rice) [23]. 1940 these phytoalexins were discovered and since then they were studied extensively in the field of medicinal and pharmaceutical chemistry considering its potentials against pathogens, bacterias, virus and other micro-organisms. [24-25].

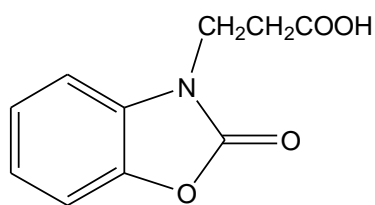
2.3.2 Some 2 (3H)-Benzoxazolone derivatives and their Biological Activities

Numerous derivatives of 2-(3H)-benzoxazolone have been tested for various biological activities including anti-fungal, antimicrobial, analgesics and anti-inflammatory activities [26]. Koksai et al [27] discovered a new series of Mannich bases 5-nitro-3-substituted piperazinomethyl 2(3H)-benzoxazolone and their analgesics and anti-inflammatory were tested.

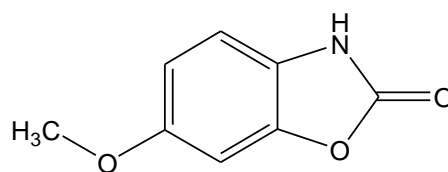


5-nitro-3-substituted piperazinomethyl-2(3H)-benzoxazolone
(Analgesics and anti-inflammatory activity)
(1)

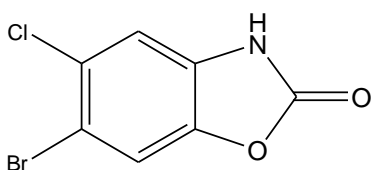
Gulcan and Co-workers synthesized some benzoxazolinone derivatives, butanoic acid acid derivative is found to be the most potent antinociceptive and anti-inflammatory.



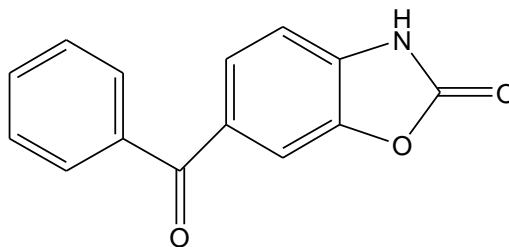
(2(3H)-benzoxazolone-3-yl) butanoic acid
(Antinociceptive and anti-inflammatory activity)
(2)



6-methoxy-2(3H)-benzoxazolone
(Antiseptic activity)
(3)

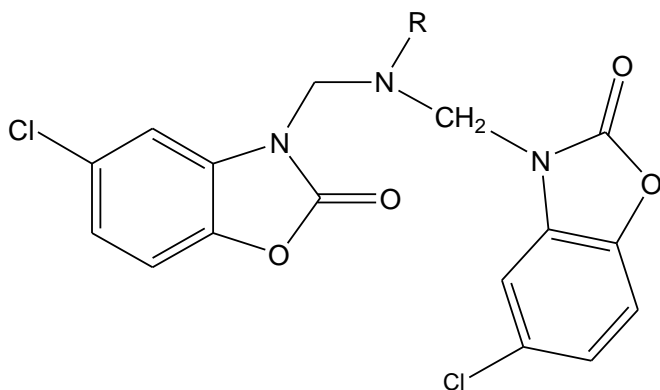


6-bromo-5-chloro-2(3H)-benzoxazolone
(Anti-inflammatory activity)
(4)



6-benzoyl-2(3H)-benzoxazolone
(Analgesic activity)
(5)

Soyer et al [28] also synthesized N-substituted-5-chloro-2(3H)-benzoxazolone derivatives via Mannich reaction but this time acetyl cholinesterase inhibitory activities were tested and examined.

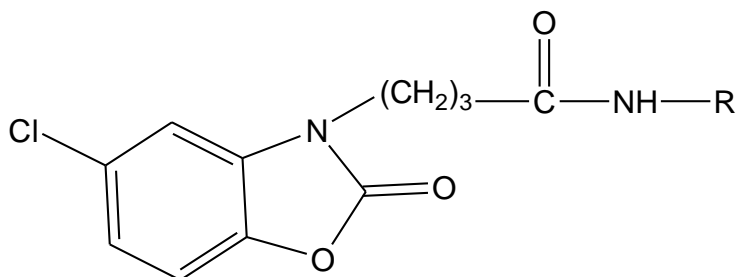


N-substituted 5-chloro-2-(3H)-benzoxazolone
(Acetylcholinesterase inhibitory activity)

(6)

Where R= Piperidine, piperazine, Methylpiperazine

Gokhan et al [29] synthesized and screened analgesic and anti-inflammatory activities of 4-(5-chloro-2-oxa-3H-benzaxazol-3-yl) butanamide derivatives.

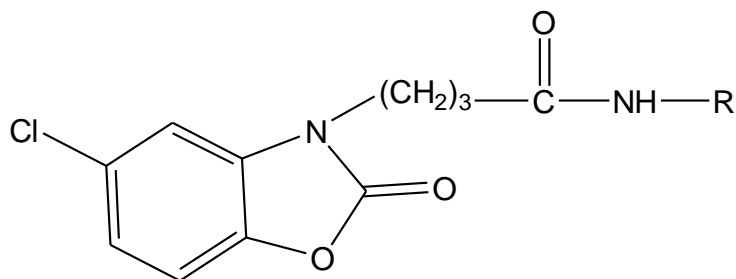


4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanamide
derivative

(Anti-inflammatory activity)

(7)

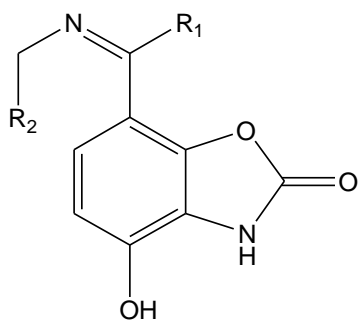
Where R= 4-Fluoro phenyl, 4-chloro phenyl, 4-acetyl phenyl



4-(5-Chloro-2-oxo-3H-benzoxazol-3-yl)butanamide
derivative
(Anti-inflammatory activity)
(8)

Where R= 2,4-Dimethyl-pyridine, 4-methyl-pyridine

Guangjian et al [30] also synthesized and screened chlorozoxazone bioisoter (4-hydroxy-2-benzoxazolone) for anti-inflammatory and analgesic activities of its various derivatives using carrageenan rat pat edema and hot-plate test, respectively.



4-hydroxy-2-benzoxazolone
(9)

(Anti-inflammatory and analgesic activity)

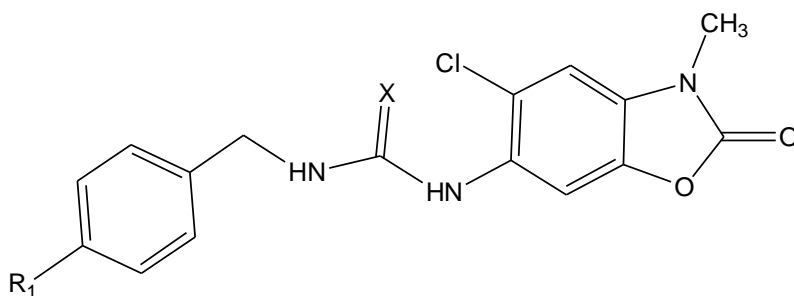
Where

R₁= CH₃

R₂=CH₂OH

R₁= CH₂CH₂CH₃ R₂=CH₂COOH

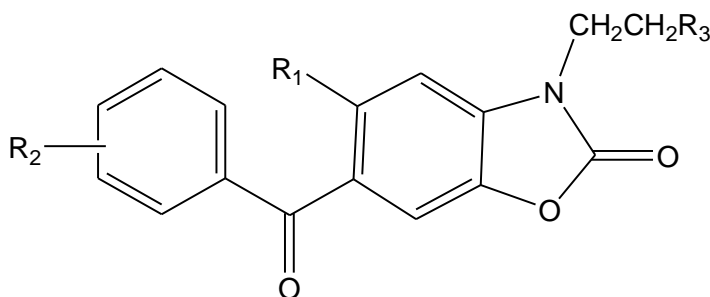
Sieman et al [31] test for urea and thio urea derivatives of 5-chloro-2(3H)



5-Chloro-2(3H)-benzoxazolone compounds of urea and thiourea derivatives
(Anti-fungal and antibacterial activities)
(10)

Where

R1= H	X=O
R1=Cl	X=O
R1=H	X=S
R1=H	X=S



Pyridalethylated benza (thia) zolones
(Anti-inflammatory and analgesic activity)
(11)

Where X=O,S

R1=Cl,	R2= 3-Fluoro,	R3= 2-Pyrimydy
R1=Cl,	R2= 2-bromo,	R3= 4-Pyridyl

Figure 2.9: Some 2 (3H)-Benzoxazolone derivatives and their Biological Activities

2.3.3 Reactions and Reactivity of 2(3H)-Benzoxazolone

2 (3H)-Benzoxazolone have the ability to undergo three different types of chemical reactions which are; (a) Aromatic ring electrophilic substitution (b) Ring opening or expansion reaction (c) N-substitution (Either alkylation or acylation)

2.3.3.1 Aromatic Ring Electrophilic Substitution

This kind of substitution reaction is governed and has preference on the 6-position of the Benzoxazolone molecule. This is achieved not only for the direct halogenation, sulfonation, nitration and chlorosulfonation reactions but even for Friedel-Craft acylation. Since Benzoxazolone has an electron rich character. The reaction encounter problems by the lewis acid which is in the reaction medium. To overcome this hinderance, the reaction should be perform with less reactive electrophilic species such as polyphosphoric acid or more preferably the dimethyl formamide, aluminum chloride complex so that it produces 6-acyl derivatives.

The actual and accurate position of acylation reaction can be detected in the 6-benzoyl-2(3H)-benzoxazolone through $^1\text{H-NMR}$ and by X-ray single-crystal diffraction studies. The 6-acyl (acylation at 6th position) is the only product that can be isolated from the reaction medium but for the case of 5-acyl (acylation at 5th-position), the derivatives at 5th-position there is no evidence be it from HPLC and H-NMR study that shows the concomitant evidence of the formation of derivatives of 5-acyl. Rather 5-acyl derivatives can be synthesized through another alternative route [32].

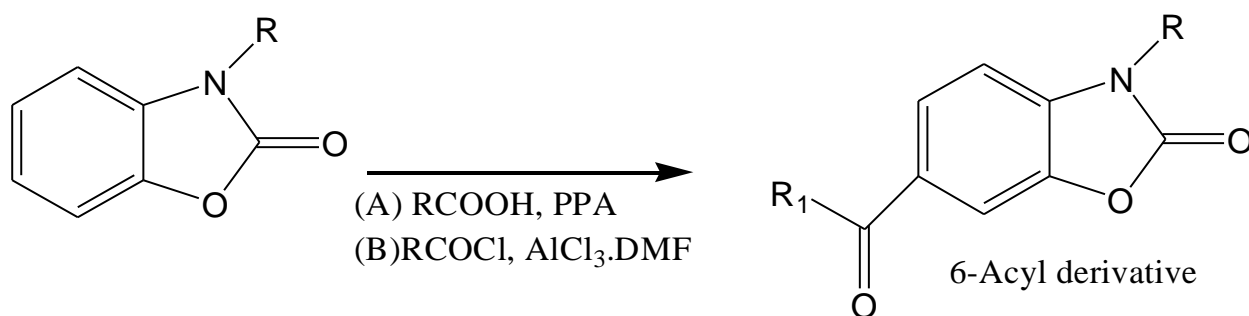


Figure 2.10: Aromatic substitution reaction of benzoxazolone at 6th position

2.3.3.2 Ring Opening or Expansion Reaction

2 (3H)-Benzoxazolone and its derivatives are slightly stable in acidic medium, but they rapidly hydrolysed in basic medium, which results in ring opening products as in 2-aminophenols. Where by the 2-aminophenols can easily be acylated in the 4th-position

and subsequently it leads to the closure of the ring and hence produces in-accessible 5-acyl-benzoxazolone derivative. Moreover the ring expansion of benzoxazolone derivatives to give benzoxazolinones can be affected through same 2-aminophenols [33].

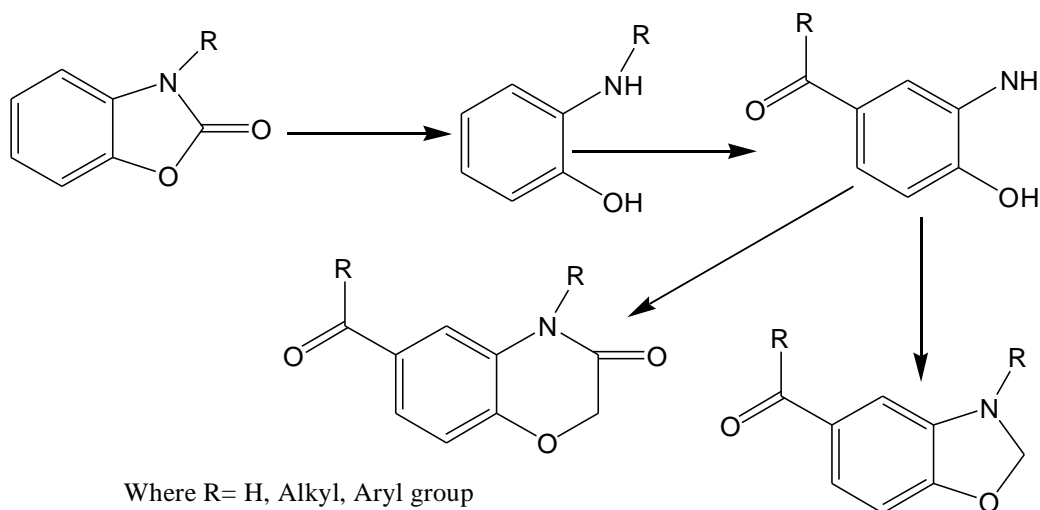
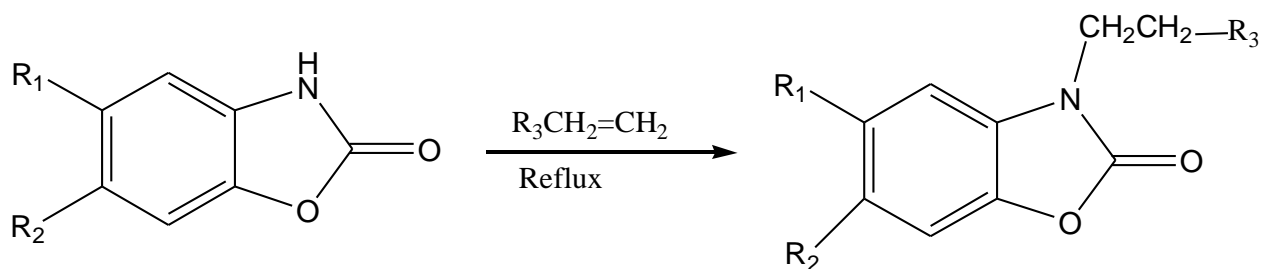


Figure 2.11: Ring opening reaction of 2-Benzoxazolone

2.3.3.3 N-Substitution

This kind of substitution reaction is divided into two; N-alkylation which occurs base-catalyzed conditions and N-acylation that occurs under acid-base catalyzed condition [34]. N-substitution reaction leads to the formation of derivatives such as;



Where

$\text{R}_1 = \text{Cl}$ $\text{R}_2 = \text{H}$ $\text{R}_3 = 2\text{-Pyridine (a)}$

$\text{R}_1 = \text{Cl}$ $\text{R}_2 = \text{H}$ $\text{R}_3 = 4\text{-Pyridine (b)}$

Figure 2.12: Modification of the 3rd position of 2-benzoxazolone

2.3.3.4 Other reactions

Reaction with hydrochloric Acid: - 2-(3H)-Benzoxalone react with hydrochloric acid to produce 2-Amino-phenols.

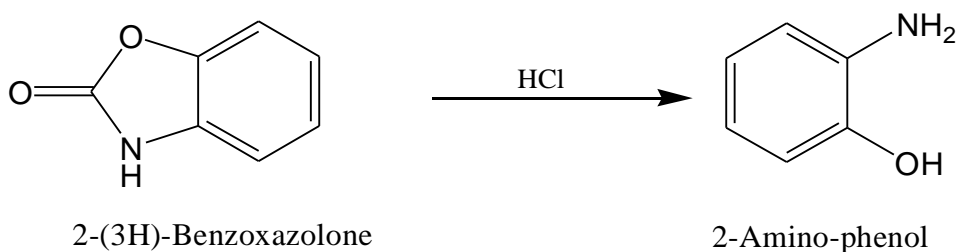


Figure 2.13: Synthesis of 2-Amino-phenol from 2-(3H)-benzoxazolone

2.3.4 Synthesis of 2 (3H)-Benzoxazolone

This compound and its derivatives can be synthesized using different methods and different reaction conditions such as at room temperature, using microwave-assisted technique and using reflux method [31-38].

2.3.4.1 Synthesis of 2-Benzoxazolone and derivatives under room temperature.

2-Benzoxazolone with substitution at 5th position can be synthesized from corresponding 4-substituted 2-aminophenol through its condensation or fusion with phosgene (COCl₂) at room temperature.

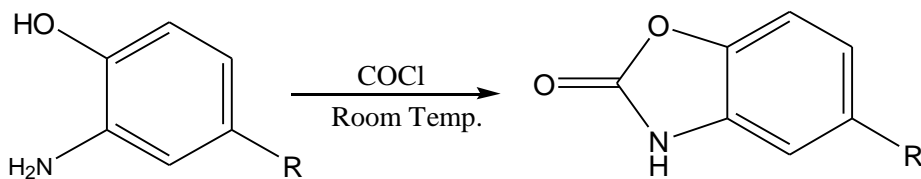
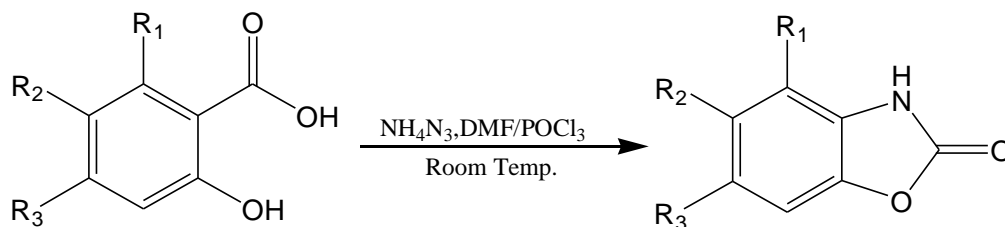


Figure 2.14: Synthesis of 5th position modified benzoxazolone

2-(3H)-Benzoxazolone derivatives can also be synthesized through Perumal *et al* procedure, which involves the reaction between salicylic acid, ammonium azide and vilsmier complex.



Where R₁, R₂, R₃= H

Figure 2.15: Synthesis of benzoxazolone derivatives from salicylic acid

2.3.4.2 Synthesis of 2-Benzoxazolone using microwave assisted method

2 (3H)-Benzoxazolone can be synthesized through reacting urea with 2-aminophenol under microwave irradiation for almost 15 minutes and at a temperature of 140 °C.

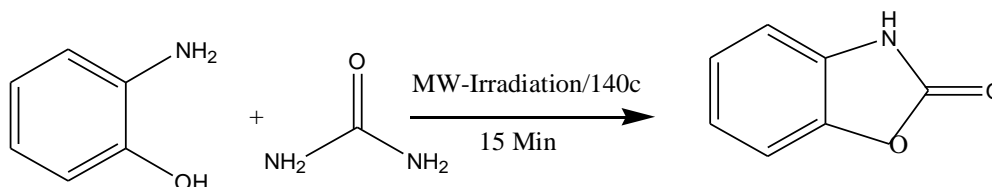


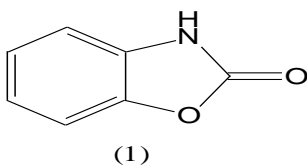
Figure 2.16: Synthesis of benzoxazolone using Microwave-assisted technique

It can also be synthesized using reflux method by reacting finely ground urea with 2-aminophenol and heated at a temperature of about 25 min. o-hydrophenylurea is then formed as an intermediate, which is then heated also at about 160 °C. for 20 min and it is further recrystallized using methanol to get pure 2(3H)-Benzoxazolone.

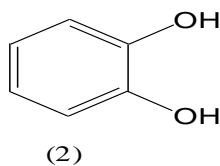
2.4 Bioisosterism of Benzoxazolone

2-benzoxazolone is a cyclic isoster of other compounds such as coumarin, where by its antibacterial properties has been characterized [39-44].

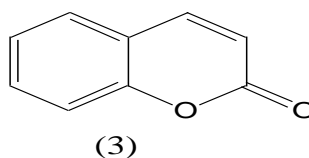
2-Benzoxazolone have resemblance based on its structural arrangements with coumarin and phenylurethane and hence endowed with bactericide properties of the former and analgesics, antipyretic and hypnotic properties of the later [45].



Benzoxazolone



Catechol



Coumarin

Figure 2.17: Some bioisosters of benzoxazolone

2-Benzoxazolone (1) in most cases can act as phenol substitute. At some point even the sulphur bioisoster, which is 2(3H)-benzothiazolone (4), the nitrogen bioisoster which is benzimidazol-2-one (5) and the methylene bioisoster, which is oxindole (6) have been used in places where either a catechol or phenol need to be substituted by a compound having adequate and abundant residue. Also the expansion of the methylene

ring of 2-(3H)-Benzoxazolone, which is benzoxazolinone (7) obeys the same bioisosterism strategy [46-48].

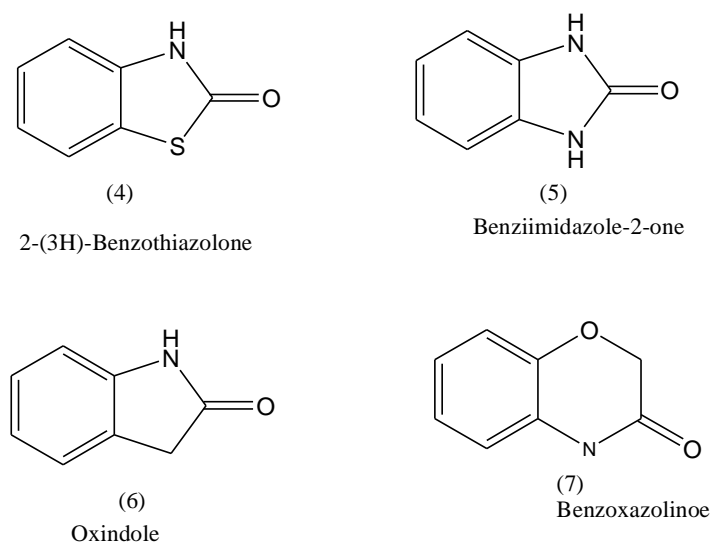


Figure 2.18: Bioisosterism of 2-Benzoxazolone with other compounds

2.5 Mechanism of Mannich Reaction

This kind of reaction is commonly used in synthesis of organic compounds. It normally undergoes reaction of methylene and methynyl compounds in both basic and acidic media [49]. In this reaction there is combination of an ammonia or a primary or secondary amine with an aldehyde mostly a formaldehyde with another compound containing an activated hydrogen. This reaction can be written using this equation.

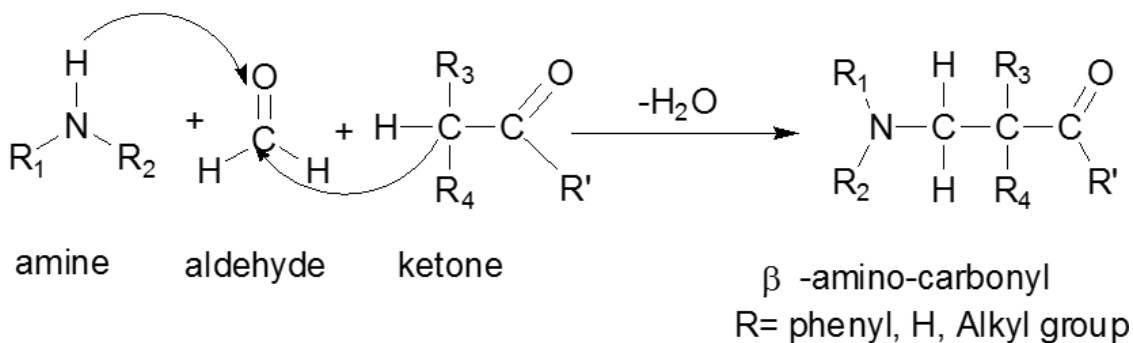


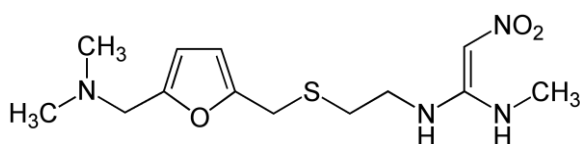
Figure 2.19: Mechanism of Mannich Reaction

Usually the active hydrogen is normally a ketone even though recent scientific research has shown that nitoalkanes can be used. [50]. In conclusion, all this researches shows

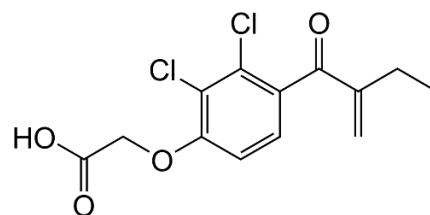
that the rate-limiting step should show a primary salt effect and mostly the mechanism was mainly postulated in acidic media [51].

2.5.1 Mannich Base

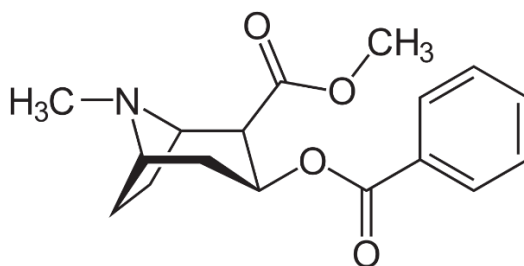
This are also known as beta-amino-ketones carrying compounds, and are also the end products of the mannich chemical reaction [52]. Mannich bases are known to be very good precursors in the development of bioactive molecules [53]. Mannich reaction is used mainly in the production of nitrogenous compounds. Mannich bases have been found to have active biological activities such as antifungal, antiviral, antibacterial and antimalarial [54]. Examples of chemically important Mannich base are; Rantidine, Cocaine and Ethacrynic acid.



Rantidine



Ethacrynic Acid



Cocaine

Figure 2.20: Chemical structures of some important Mannich bases

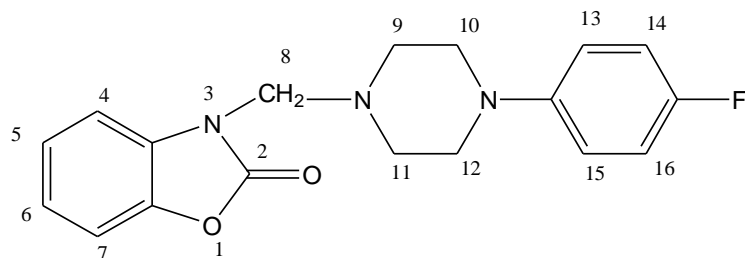
3. Materials and methods

3.1 Materials

All the chemicals used in this research work 2 (3H)-benzoxazolone, (4-fluorophenyl) piperazine, (2-methoxyphenyl) piperazine, methanol, 37% formalin solution, n-hexane, ethylacetate, chloroform, triethyl amine, dioxane and ethanol were all purchased from Sigma Aldrich chemical company and used without any further purification.

3.2 Synthesis of compound 1

1-(4-fluoro phenyl piperazin-1-yl) methyl-2 (3H)-benzoxazolone

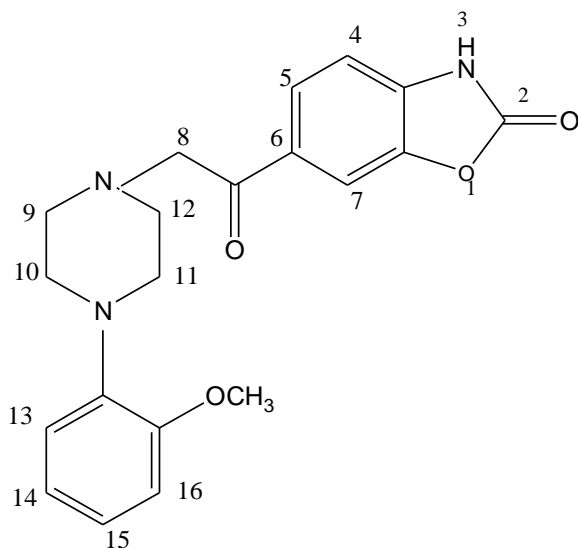


Reflux

200 mg (0.001 mol) of 2(3H)-benzoxazolone and 267 mg (0.001 mol) of 1-(4-fluorophenyl piperazine) were dissolved in 8 mL of methanol in a 50 ml round bottom flask. 0.2 mL (0.005 mol) of formalin solution 37% (w/v) was mixed with 2 mL methanol and then transferred into the reaction mixture. The mixture was then refluxed for 60 minutes in water bath. After the completion of the reaction, the reaction mixture was poured into crushed ice upon which a precipitate was formed. Later on the product was filtered by 'vacuum filtration' to yield a pure product which was later washed with ethanol and allowed to dry at room temperature. The compound was then recrystallized by using ethanol.

3.3 Synthesis of compound 2

6-{2-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-acetyl}-2-(3H)-benzoxazolone



Reaction at Room Temperature (Modification of 6th position)

350 mg (0.001 moles) of 6-(2-bromoacetyl)-2-(3H)-benzoxazolone was dissolved in 7 ml of dimethylformamide (DMF) solution. 0.25ml (0.001 moles) of 2-methoxyphenylpiperazine was mixed with 0.4ml (0.002 moles) of triethyl amine solution in 3 ml dimethyl formamide. And then the mixture containing 6-(2-bromoacetyl)-2(3H)-benzoxazolone in DMF was added dropwise into the reaction mixture. The mixture was stirred at room temperature for 30 hours and then poured into crushed ice and then filtered using vacuum filtration method. The synthesized compound was then washed with water and dried.

3.4 Thin Layer Chromatography

This process was carried out on silica gel-plates having a fluorescent indicator at 254 nm to check the progress of the reaction using chloroform as the stationary phase. Three different mobile phases were prepared and used. Which are:

A1- Benzene/ methanol: (9:1)

A2- Benzene/ methanol: (5:1)

Both the starting material and product were dissolved in chloroform as the stationary phase. The mobile phase was transferred into the TLC chamber and gently swirled. The silica gel plate containing spots made with the aid of micro capillary of both the starting

material solution and the solution of the product was carefully transferred into the mobile phase chamber. It is then allowed to move undisturbed up to the desired height and then gently removed and allowed to dry. It was then visualized under a UV-light having a wavelength of 254nm and the spots were marked with a pencil. The retention factor values (R_F values) were then calculated.

3.5 Melting point determination

This process was conducted using Mettler Toledo (FP90 central processor) melting point apparatus to determine the melting points of the compounds synthesized.

3.6 Spectroscopy

3.6.1 Fourier Transform infra-Red (FT-IR) (ν_{\max})

The FT-IR spectra of the product was recorded on Agilent carry 630 spectrometer at Ankara University, Central Instrumental Analysis Laboratory, Faculty of Pharmacy

3.6.2 Proton Nuclear Magnetic Resonance (^1H -NMR)

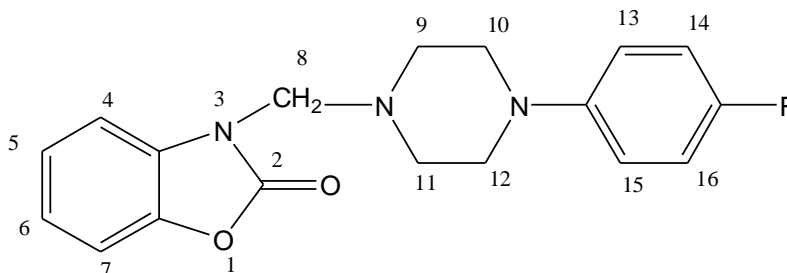
The ^1H -NMR spectra of the product was recorded on a Mercury Varian 400 MHz Spectrometer where deuterated solvent of dimethyl sulfoxide (DMSO) was used. The test was conducted at Ankara University, Central Instrumental Analysis Laboratory, faculty of Pharmacy. Chemical shift (δ) values were reported in parts per million (ppm).

4. RESULTS AND DISCUSSION

4.1 Results

Compound 1

1-(4-fluorophenylpiperazin-1-yl) methyl-2 (3H)-benzoxazolone



The above compound was synthesized by reflux method, mentioned in the experimental section using the procedure from the literature [5].

Reflux

- Brown crystalline solid was obtained
- Melting point: 147 °C.

Thin layer chromatography:

The TLC in A1 and A2 mobile phases gave a retention factor values (R_f values) of 0.48 and 0.55 respectively.

Fourier Transforms Infrared (FT-IR) spectroscopy (IR ν max)

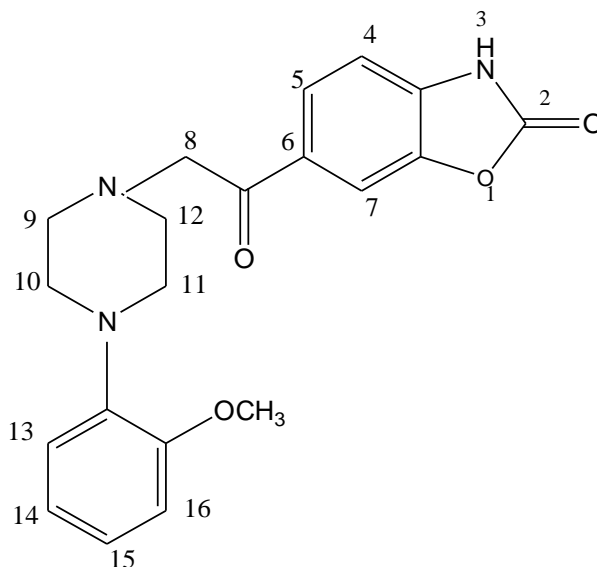
FT-IR showed absorption band at 2823-2956 cm^{-1} for aromatic and aliphatic (C-H) stretches and 1753 cm^{-1} carbonyl group (C=O stretch).

Proton Nuclear Magnetic Resonance spectroscopy (^1H -NMR in DMSO- d_6)

^1H -NMR showed chemical shift at 7.2-6.8 (8H, m; Ar-H), 4.7 (2H, s; CH_2), 2.8-3.2 (8H, t; pip H^9 - H^{12}) ppm.

Compound 2

6-{2-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-acetyl}-2-(3H)-benzoxazolone



Reaction at Room Temperature (Modification of 6th position)

- Pale yellow solid was formed
- Melting point: 200.4 °C.

Thin layer chromatography:

The TLC in A1 and A2 mobile phases gave a retention factor values (R_f values) of 0.22 and 0.29 respectively.

Fourier Transforms Infrared (FT-IR) Spectroscopy (IR ν max)

FT-IR showed a single stretch at 3352 indicating the presence of an amine (N-H), an absorption band at 2827-2988 for aromatic and aliphatic (C-H) stretch and 1777 cm^{-1} carbonyl group (C=O stretch).

Proton Nuclear Magnetic Resonance spectroscopy (^1H -NMR in DMSO- d_6)

^1H -NMR showed chemical shift at 12 (1H, s; N-H), 6.8-8.0 (7H, m; Ar-H), 4.8 (2H, s; CH_2), 3.8 (3H, s; OCH_3), 2.5-3.2 (8H, t; pip H^9 - H^{12}) ppm.

4.2 DISCUSSION

In this research two compounds were synthesized following procedures from literature [5] based on 2-(3H)-benzoxazolone and 2-bromoacyl-2-(3H)-benzoxazolone. The **compound 1** was synthesized involving the modification of the 3rd position through employing Mannich reaction method. On the other hand, **Compound 2** was made by a reaction at room temperature which involves the modification of the 6th-position of 2-bromoacyl-2-(3H)-benzoxazolone. These reactions were conducted to check the reactivity of 2-(3H)-benzoxazolone at different positions (3rd and 6th-positions) as stated in the literature. 4-Fluorophenylpiperazine was studied for molecule with substitution at 3rd position of 2-(3H)-benzoxazolone via Mannich reaction to produce the target compound. The structure of the synthesized compound is shown below.

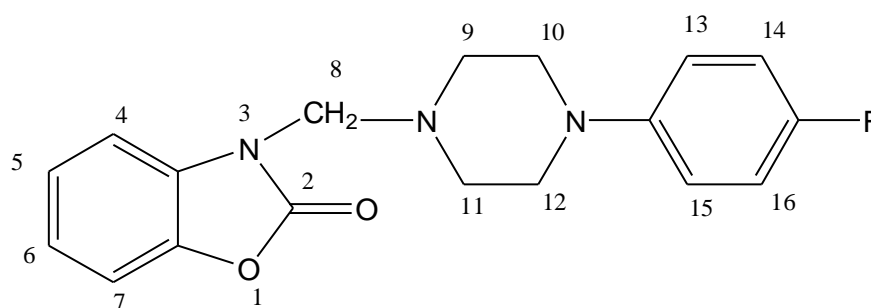


Figure 4.1: Structure of compound 1

The synthesized compounds were characterized using Fourier Transform Infra-Red (FT-IR) and Proton Nuclear Magnetic Resonance Spectroscopy (¹H-NMR).

In compound 2, 2-methoxyphenylpiperazine was attached to the 6th position of 2-bromoacyl-2-(3H)-benzoxazolone at room temperature to give the target compound. The structure of the synthesized compound is shown below.

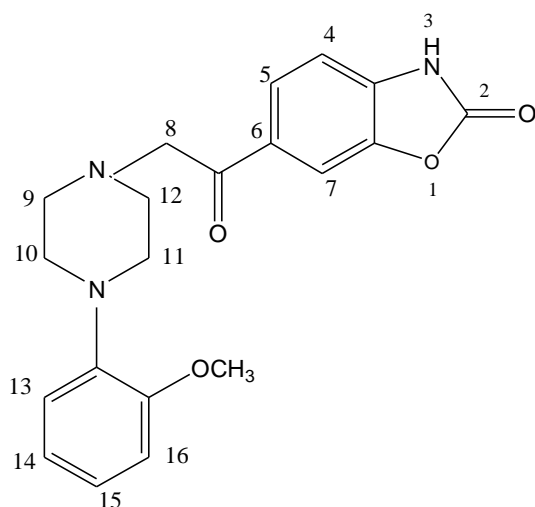


Figure 4.2: Structure of compound 2

The core structure of the two compounds are the same. They only differs in the amine moiety attached on the 3rd and 6th positions respectively.

Compound 1 has 4-fluorophenylpiperazine attached to the 3rd position of 2-(3H)-benzoxazolone while **compound 2** has 2-methoxyphenylpiperazine attached on the 6th position of 2-bromoacyl-2-(3H)-benzoxazolone. Fig. 4.3 and 4.4, give the general synthesis in this research.

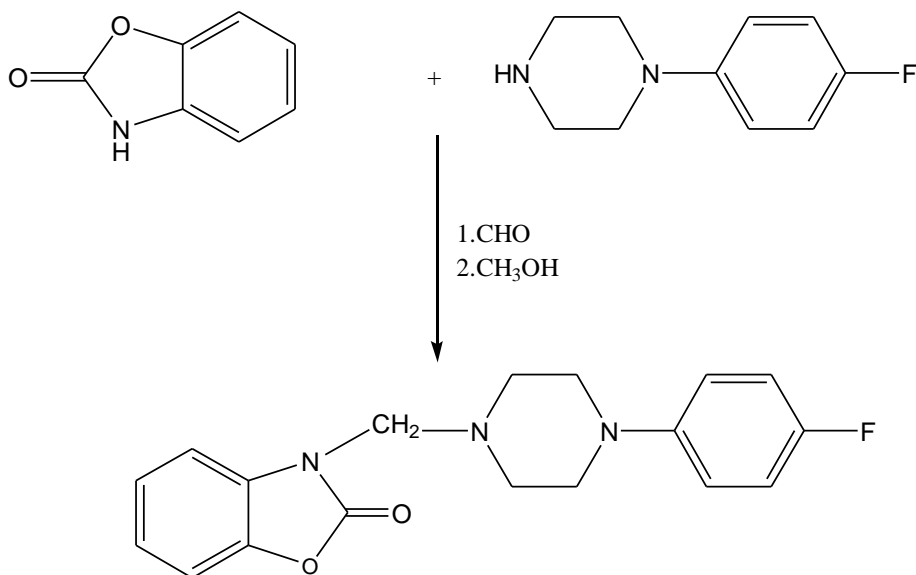


Figure 4.3: Synthesis of compound 1 via Mannich reaction

The general mechanism of this reaction involves two major steps; formation of iminium ion and attacking of iminium ion by the substrate (2(3H)-Benzoxazolone nucleus) as a nucleophile.

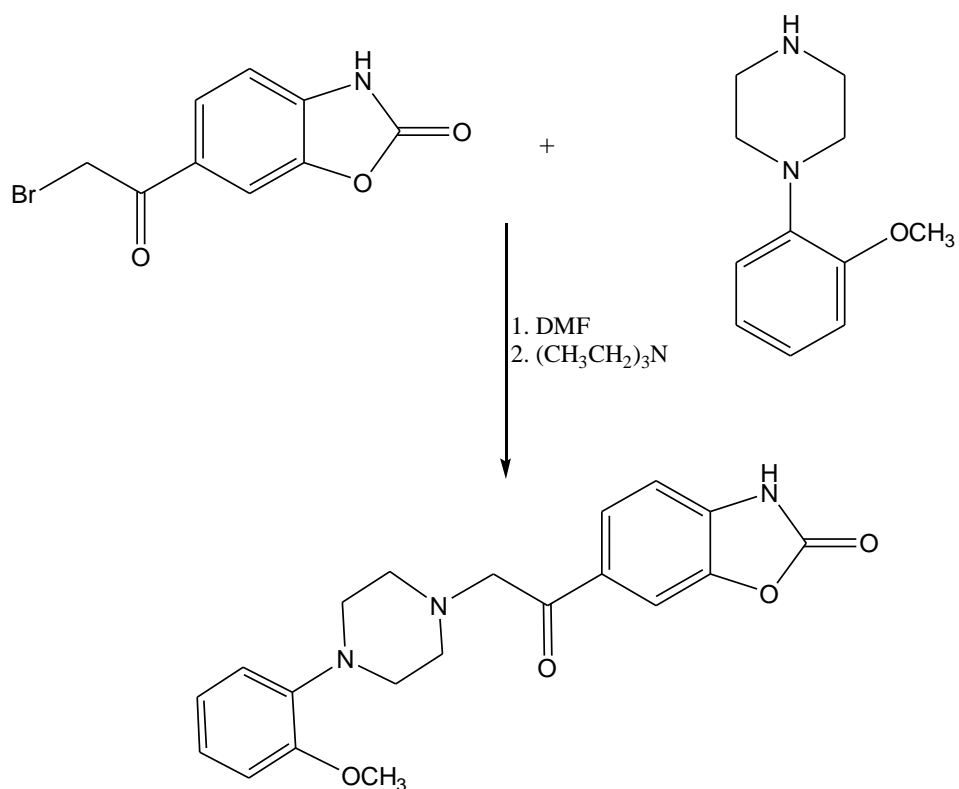
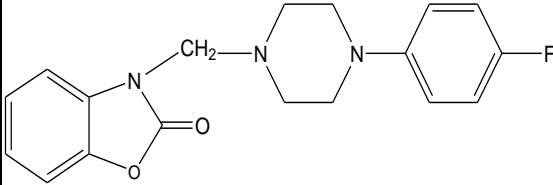
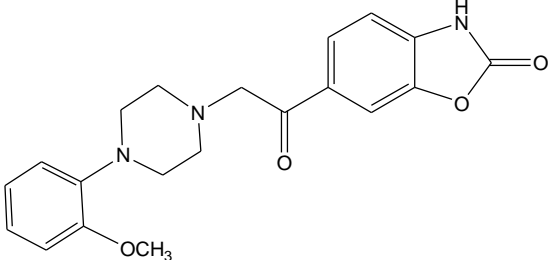


Figure 4.4: Synthesis of compound 2 through modification of 6th position

The chemical structure of the compounds synthesized and their starting materials, methods of preparation, R_f values and melting points are shown in the table 4.1 below.

Table 4.1: Comparison of the R_f values and melting points of the starting materials and the compounds synthesized.

Method	Chemical structure	Melting point ($^{\circ}\text{C}$)	R_f values
Reflux (Modification at 3 rd -position)	 <p>Compound 1</p>	147	A1=0.48 A2=0.55
Reaction at room temp. (Modification at 6 th -position)	 <p>Compound 2</p>	200.4	A1=0.22 A2=0.29

Compound 1 and 2 were characterized using Fourier Transform Infra-Red (FT-IR) and Proton Nuclear Magnetic Resonance Spectroscopy (^1H -NMR).

The FT-IR of **compound 1** shows absence of N-H stretch which has been reported to be around 3146 cm^{-1} this shows the reaction has taken place at the 3rd position of 2(3H)-benzoxazolone as expected. The C=O stretch appears at 1754 and C-H stretches are seen at around $2958\text{--}2824\text{ cm}^{-1}$ as expected. The FT-IR spectrum of compound 1 synthesized is shown in fig. 4.5 below.

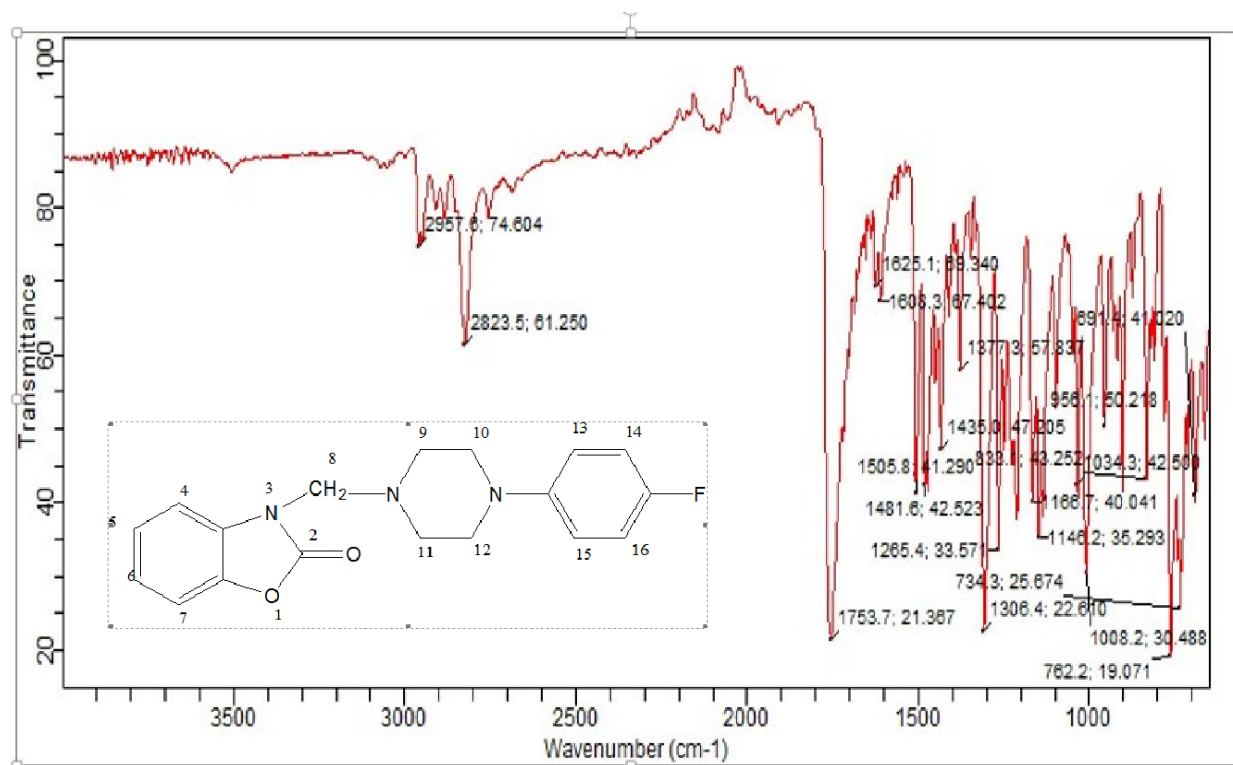


Figure 4.5: FT-IR spectrum of 1-(4-fluorophenylpiperazin-yl) methyl-2(3H)-benzoxazolone

^1H -NMR spectra of **compound 1** in $\text{DMSO-}d_6$ shows peaks at the expected chemical shifts values, which is relative to the starting material (2-(3H)-benzoxazolone), there is additional CH_2 (methylene) peak as a singlet observed at 4.7 ppm of the compound. This shows that the reaction has taken place at the N-atom in the 3rd position and the piperazine derivative is bounded to 2 (3H)-benzoxazolone through the CH_2 bridge.

Further analysis of the ^1H -NMR spectra reveals the presence of aromatic peaks as multiplets between 6.8-7.3ppm as expected. The piperazine protons ($\text{H}^9\text{-H}^{12}$) were seen as triplets at 2.8-3.2 ppm for **compound 1**.

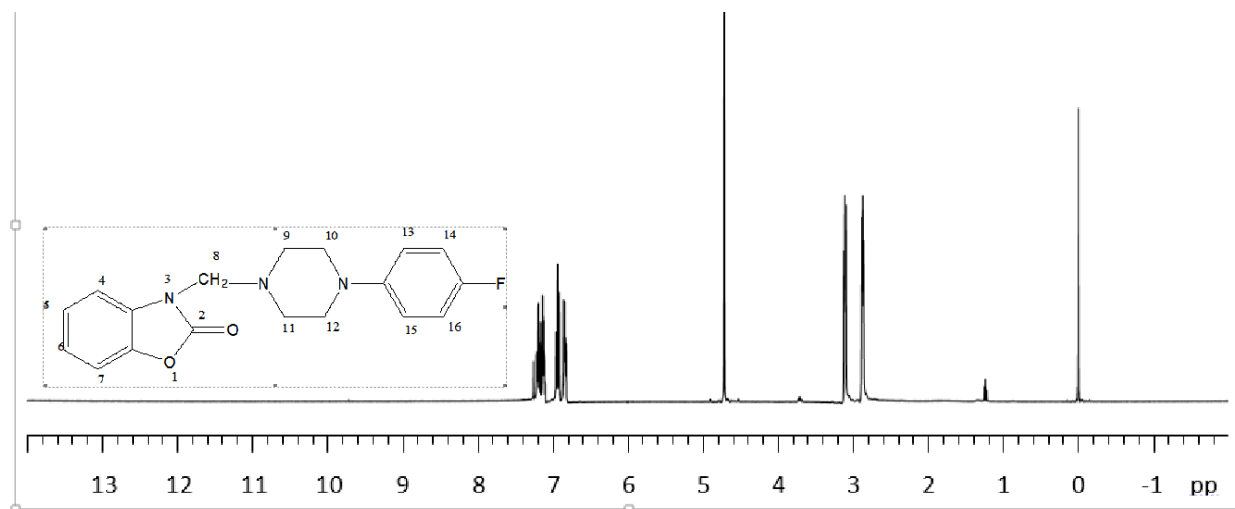


Figure 4.6: ¹H-NMR spectrum of 1-(4-fluorophenylpiperazin-yl) methyl-2(3H)-benzoxazolone

The FT-IR of **compound 2** shows presence of N-H stretch at 3351.9 cm⁻¹ as expected. The C=O stretch also appears at 1777.67 cm⁻¹ and C-H stretch are seen at around 2988-2827 as expected. Presence of N-H indicates the reaction did not take place at the 3rd position. The FT-IR spectra of **compound 2** synthesized is shown in fig. 4.7 below

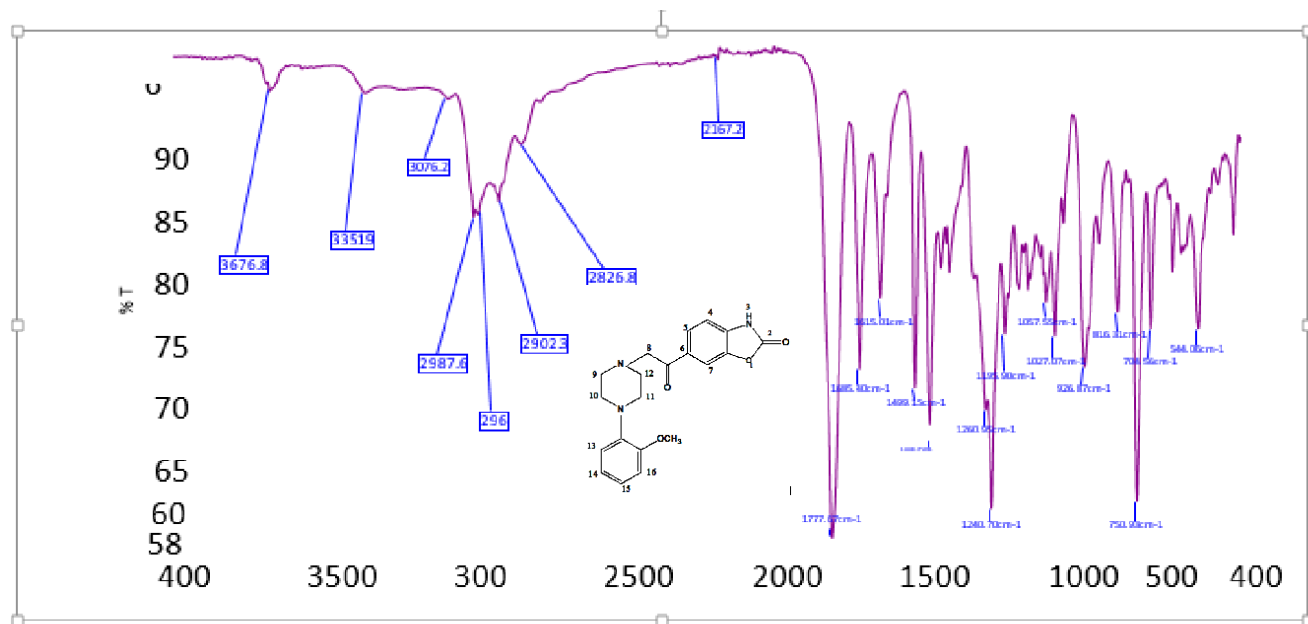


Figure 4.7: FT-IR spectrum of 6-{2-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-acetyl}-2-(3H)-benzoxazolone

^1H -NMR spectra of **compound 2** in $\text{DMSO-}d_6$ shows peaks at the expected chemical shifts values, which is relative to the starting material 6-(2-bromo-acetyl)-2-(3H)-benzoxazolone, there is additional piperazine protons (H^8 - H^{11}) peaks as triplets observed at 2.5-3.2. Further analysis of the ^1H -NMR spectra reveals the presence of CH_2 (methylene) peak as a singlet at 3.8 ppm, aromatic peaks as multiplets between 6.8-8.0 ppm as expected and O-CH_3 (methoxy) peak as singlet at around 3.7 ppm also as expected. Presence of N-H peak as singlet at 12ppm This shows that the reaction has taken place at the 6th position of and the piperazine derivative is bounded to 6-(2-bromo-acetyl) 2-(3H)-benzoxazolone.

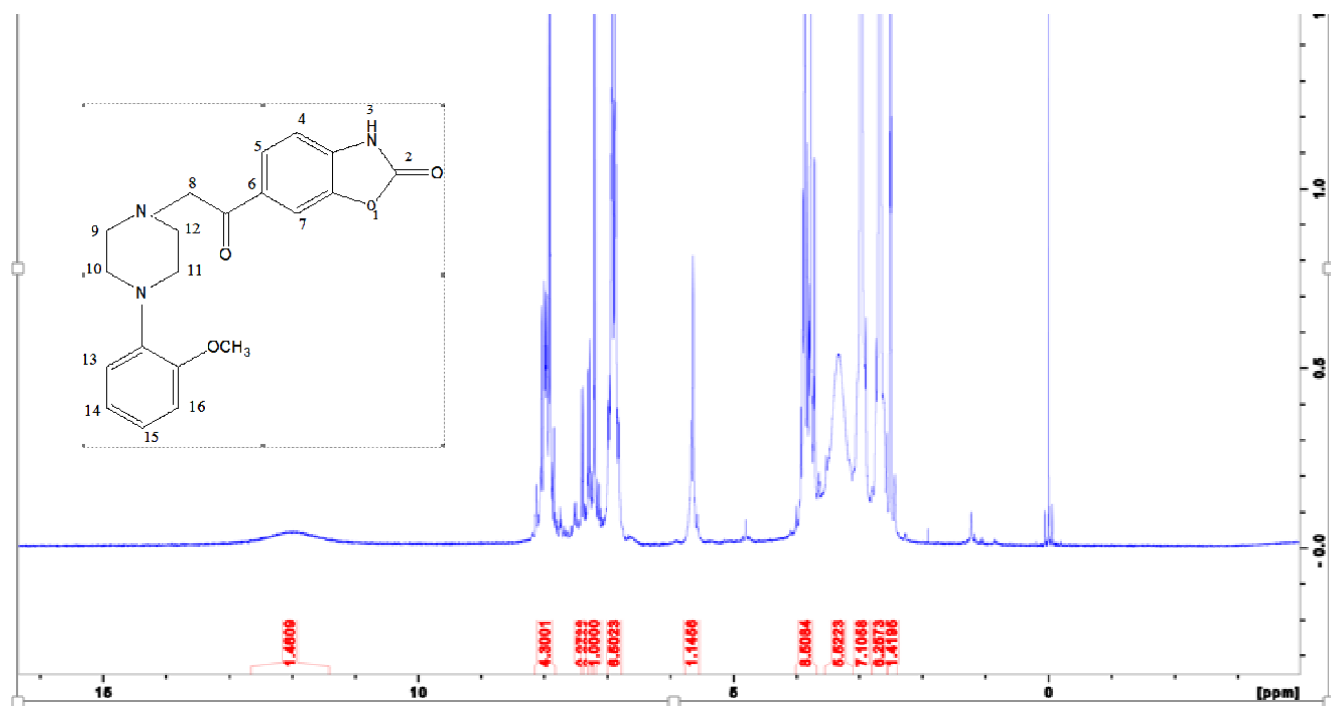


Figure 4.8: ^1H -NMR spectrum of 6-{2-[4-(2-Methoxy-phenyl)-piperazin-1-yl]-acetyl}-2-(3H)-benzoxazolone

5. Conclusion

This research studies the modification of 2-(3H)-benzoxazolone since it's possible to do substitution at different positions. It involves the modification of the 3rd position 2-(3H)-benzoxazolone via Mannich reaction and also modification of the 6th position of 6-(2-bromoacetyl) 2-(3H) benzoxazolone using reaction at room temperature with different piperazine substituents.

Biological activity of the synthesized compounds were not conducted due to time constrains, though based on the literature the two compounds might have some biological activities. Since, it's possible to do substitution at different positions of the starting material, by using different amine groups to do substitution at the 3rd and 6th positions which can change the biological activities of these types of compounds. Moreover, **compound 1 and 2** could be studied for COX-2 selectivity inhibition.

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