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HEALTH SCIENCES INSTITUTE

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PHARMACEUTICAL CHEMISTRY

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This study has been accepted by the thesis committee for the degree of Master of Science in Pharmaceutical Chemistry.

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According to the relevant articles of the Near East University Postgraduate Study – Education and Examination Regulations, this thesis has been approved by the member of the thesis committee and the decision of the Board of Directors of the Institute.

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I am grateful to God Almighty, who has supported, cared and in all ways expresses Himself as the Lord over my life. I am also grateful to my dear mother for her moral teachings, spiritual guidiance and her financial support. May the lord reward you greatly ma.

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DEDICATION

I dedicate this thesis work to my dearest mother for her ever supportive personality and for always believing in me.

ABSTRACT

Non Steroidal Anti-Inflammatory Drugs functions by hindering cyclooxygenase (COX) enzymes. This results in complications such as gastrointestinal damage, ulcer amongst others.

The disclosure of COX-2 specific inhibitors has made it possible for the production of drugs that reduces inflammation without evacuating the defensive prostaglandins created by COX-1 enzymes in the stomach and kidney.

2(3H)- benzoxazolone is one of the flexible heterocyclic compound that shows an extensive variety of pharmacological properties which includes analgesic and anti-inflammatory activities. In this study, 2-fluorophenyl piperazine was made to react with 2(3H)-benzoxazolone at postion 3 using Mannich reaction (**Compound 1**). Also, phenyl piperazine was made to react with 6-bromoacetyl benzoxazolone at the 6^{th} position at room temperature. (**Compound 2**)

The reactions were monitored by TLC and melting point. FT-IR and ^{1}H-NMR analysis were used to determine the chemical structures of the synthesized compounds.

Keywords: 2(3H)-benzoxazolone, 6-bromoacetylbenzoxazolone, Mannich reaction, phenylpiperazine, analgesics, anti-inflammatory.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

NSAIDS	Non-steroidal Anti-inflammatory Drugs
PPA	Polyphosphoric acid
DMF	Dimethylformamide
TEA	Triethylamine
THF	Tetrahydrofuran
COX	Cyclooxygenase
FT-IR	Fourier Transform-Infrared
NMR	Nuclear Magnetic Resonance
UV-Vis	Ultraviolet-Visible
TLC	Thin Layer Chromatography
DMSO-d_6	Dimethyl sulfoxide

LIST OF TABLE

INTRODUCTION

Pain is an upsetting emotional ordeal ordinarily started by noxious stimulus and communicated over a particular neural system to the brain where it is translated thusly.[1]It is also referred to as anemotional experience sprouting from real or potential damaged tissue. Medically, it is regarded as a symptom of an underlying condition. It involves physical, psychologic, and even cultural factors.

There are three types of pains: the nociceptive pain caused by damage to the body tissue.[2].Another type is the inflammatory pain which can be caused by or from initiation of the immune system by tissue damage or contamination.Lastly the neophatic pain which can occur when there is a nerve damage.[3] These types of pains can either be in acute state or chronic state.

Non Steroidal Anti-inflammatory Drugs(NSAIDS) and opioids are types of drugs that are used in the treatment of pain. The most predominant symptoms of the utilization of these analgesics is event of gastrointestinal harm with gastric upset and aggravation being the real issues. Opioid leads to tolerance, physical dependency and addiction. It was reported that 15–35 % of all peptic ulcer complications are caused by NSAIDs.[4]In view of these side effects, investigation of new anti inflammatory agents with low side effects is being embarked on.

Some heterocyclic molecules have been used as functional scaffolds and these are known pharmacophores of a good number of medically useful molecules with physiological effects. Benzoxazolone and its bioisteres such as benzothiazolone and benzoxazinone are found to be amongst these useful heterocycles.In endeavor to orchestrate new anti-inflammatory drugs with minimal gastrointestinal symptoms, benzoxazolinones have showed up as a promising group.[5]

The firstsleep inducing properties of 2-benzoxazolinone discovered made a stage for the blend of various derivatives of this compound. These were tested for various pharmacological activities such asanticonvulsant,analgesics, anti-inflammatory, antiulcer, antibacterial, antimicrobial effects[6-9].

The subsidiaries of 2(3H)- Benzoxazolone are viewed as reasonable platform for union of medication candidates. They have been of enthusiasm for medicinal science since they are

effortlessly accessible, reasonable, open to changes and in particular demonstrate an extraordinary assortment of biological activities.[10]

The aim of this thesis is to synthesize some 2-benzoxazolinone derivatives by reacting with piperazine substitutes in the search for new COX-2 selective inhibitors. Two compounds were synthesized in this study (**compounds 1 and 2**). The experiments for the synthesis of **compounds 1 and 2** are Mannich reaction and reaction at room temperature respectively.Purification of compounds was determined using Thin Layer Chromatography (TLC) and melting point. Characterization of these compounds was determined by Proton Nuclear Resonance (^{1}H-NMR), and Fourier Transform Infra-red Spectroscopy(FT-IR).

2.LITERATURE REVIEW

2.1. Analgesics

Analgesics are drugs designed to specifically to relieve pain.They are drugs that ease pain by acting in central or on peripheral sensory system without particularly evolving cognizance.They are medically made to reduce symptoms of pain.[11]Types of analgesics are:

- Opioids
- Non steroidal anti inflammatory drugs (NSAIDS)

2.1.1. Opioids

Opioids are a class of pain controlling drugs that contain natural or synthetic chemicals based on morphine. Morphine is the main active constituent of opioids.[12] They are referred to as narcotics. Opioids acts mostly on the body's receptorswhich are majorly found in the central nervous system and the gastrointestinal tract. The receptors brings about both the beneficial effects, and the unwanted side effects.[13]

Opioids are classified into three types which include: natural opium alkaloids such asmorphine, codeine and thebaine, these are extracted naturally from opium. The second class is the semi synthetic opiates for example, diacetylmorphine (heroin), hydromorphone, oxycodone amongst others. Lastly are the synthetic opiates such as fentanyl and methadone.There are opioids produced by the body, they are referred to as endogenous opioids or opioid peptides examples include endorphins, enkephalins, dynorphins and endomorphins. They are released when the body encounters any sort of stress or pain.[14]

Fig 2.1 Opium poppy plant

2.1.1.1. Morphine

Morphine is a drug for pain which is discovered normally in various plants and anmals. It was the first medicinal plant alkaloid ever isolated. The primary use of this medication is to treat pain associated with surgical conditions, trauma, burns, pain from kidney stones, severe chronic pains like cancer.[15] Morphine is commonly given through an IV to patients who have undergone surgery.

2.1.1.2. Codeine

Codeine (3-methylmorphine) is a characteristic alkaloid and a component of opium, alongside morphine, papaverine and thebaine.These all belonging to the class of opiates. It is a prodrug of morphine.Codeine was first confined from opium in 1832, by the French scientist P.- J. Robiquet. It is mostly utilized as a pain relieving opiate, a cough depressant and an antidiarrheal drug. Codeine can be synthesized by simple methylation of morphine using dimethyl sulfate as shown in fig 2.2 below.

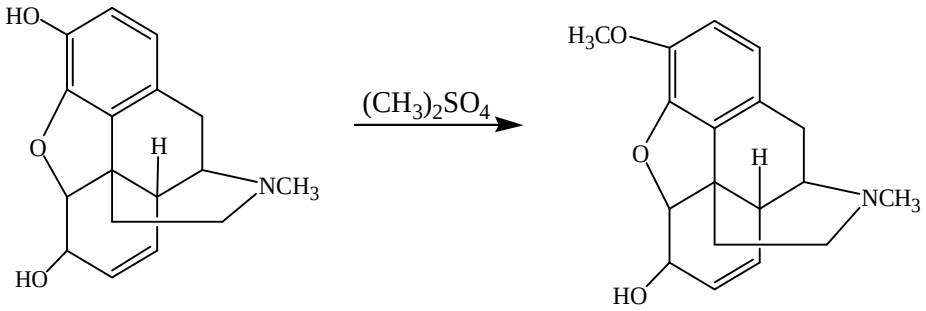


Fig 2.2 Synthesis of codeine from morphine

2.1.1.3.Opioid antagonist

Opioid antagonists, also referred to as narcotic antagonists are referred to as agents that have high affinity for opiate receptors but do not activate these receptors. They attenuate and/or reverse opioids' agonist effect. These drugs bind strongly or with a high attraction to the opioids receptors in the body than most agonists thereby resulting in the blockage of the opiate receptors. They also prevent the body from reacting to drugs such as heroin or other opiates.

In 1915, there was a first report of agents that exhibitedantagonist-like properties. N-allylnorcodeine was seen to hinder the respiratory-depressant impacts of morphine and heroin. Another opioid opponent, nalorphine was synthesized in the 1940s; be that as it may, it was found that it have an incomplete agonist action and its agitating impacts on mind-set (that is, it causes dysphoria) disheartened broad use for treating opioid inebriation or overdose. Naloxone was produced in 1960 as a more powerful and less harmful adversary operator than nalorphine.[16]

Naxolone and Naltrexone are the two opioid antagoists that have been most concentrated to a vast degree and are monetarily accessible today. For a long time, opioids enemy have been outstanding for their applications in treating addictions (naltrexone) and overdose. [17]

(a) (b)

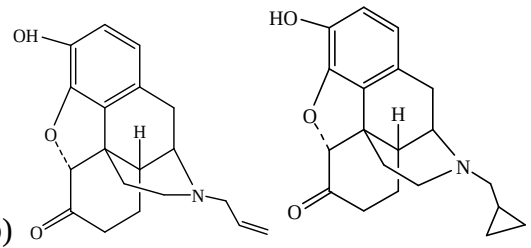


Fig 2.3 Structures of (a) naloxone, (b) naltrexone

2.1.2. Non steroidal anti inflammatory drugs (NSAIDs)

Non-steroidal calming drugs are a class of pain relieving pharmaceutical that lessens pain, fever and irritation. The improvement of the first of the classification of what are presently known as the Non Steroidal Anti-Inflammatory Drugs (NSAIDs) of which aspirin has now turned out to be perceived as the forebear, was phenylbutazone in 1946 (by JR Geigy, Basel, Switzerland) and later indomethacin in the 1960's (by Merck and Co, Rahway, NJ, USA).NSAIDs are more than just pain relievers. They also aid a relieve ininflammation, lower fevers andstops blood from clotting. [18]

NSAIDs works by blocking an enzyme called cyclooxygenase or COX (a protein that triggers changes in the body) from carrying out its functions. They produce their main pharmacological effects by inhibition of this enzyme. COX-1 and COX-2 are two forms of cyclooxygenase.COX-1 shields the stomach lining from cruel acids and stomach related chemicals. It likewise helps proper working of the kidney. COX-2 is generally created when joints are disabled or aggravated.[19]

The most prevalent risk of standard NSAIDs such as aspirin, ibuprofen, naproxen, amongst others is that they can lead to ulcers and other problems in the oesophagus, stomach or small intestine. This is because these drugs represses both COX-1 and COX-2 therebypreventing the creation of prostaglandins (a group of lipids made at sites of tissue damage or infection produced by COX-1 enzymes) whichare involved in dealing with injury and illness. They are made by chemical reactions at the sites where they are required and they additionally help to ensure the covering of the stomach and gastrointestinal (GI) tract. With prostagladins' defenses alterated, GI tract becomes inflammed and damaged by normal gastric acids and for this reason,substances that specifically restrain COX-2 without meddling with the defensive parts of COX-1 are

preferable.NSAIDs are classified into salicylates such as aspirin, propionic alkanone derivatives such as nabumetone aryl acetic acid derivatives such as diclofenac, indole derivatives such as indomethacin, etodolac, arylacetic acids(oxazole acetic acids) such as oxaprozin, para amino phenol derivatives such as paracetamol, alkanone derivatives such as nabumetone and oxicams such as piroxicam and meloxicam[20] as shown in fig 2.4.

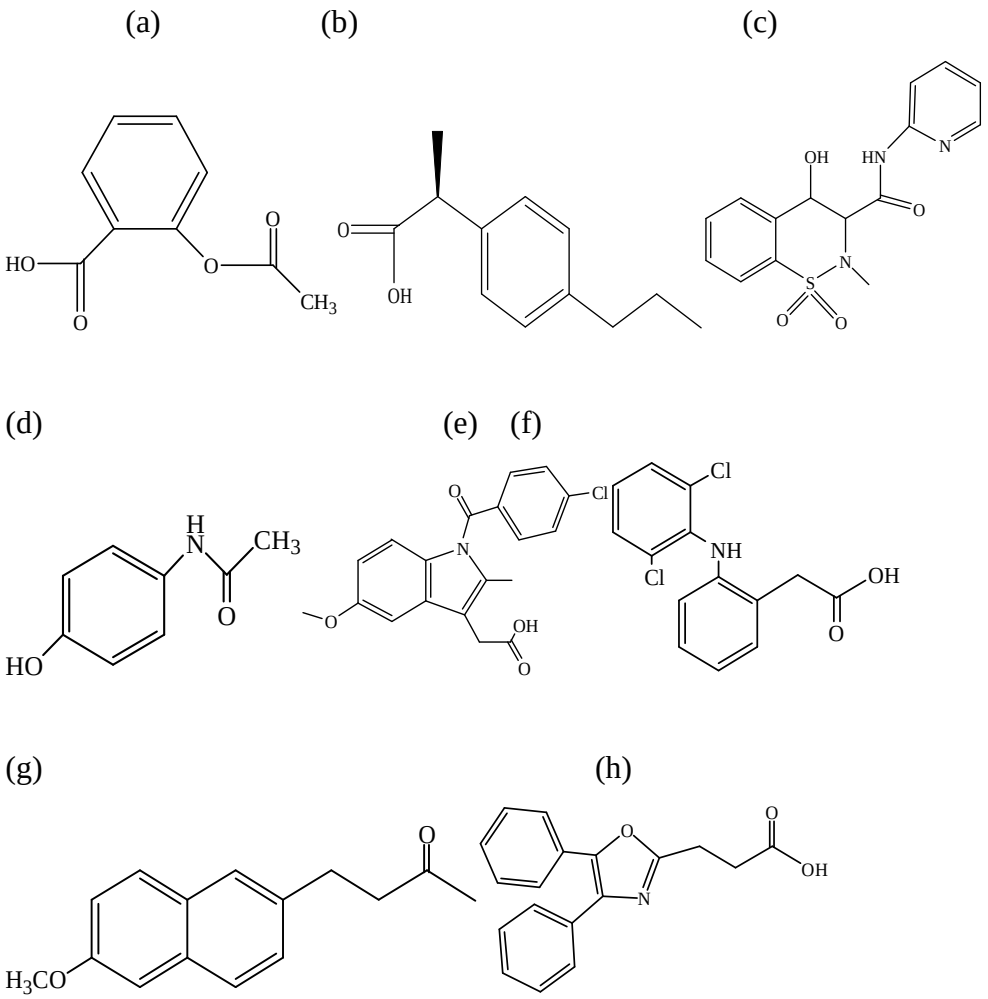


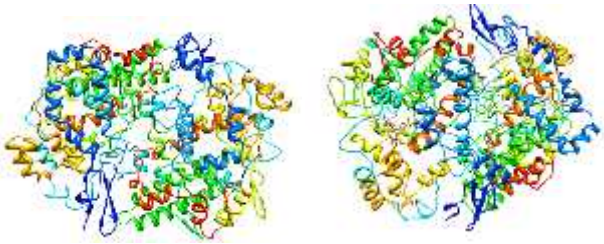
Fig 2.4 Structures of some NSAIDs(a) aspirin, (b)ibuprofen (c)piroxicam (d) paracetamol (e) indomethacin (f) diclofenac (g)nabumetone (h) oxaprozin

2.2COX-1 and COX-2 enzymes

Cyclooxygenase is used by the body to produce different prostanoids such as prostaglandins, prostacylin and thromboxane. These are a huge arbiter that assumes an imperative part in the development of torment and aggravation in the body. COX-1 and COX-2 are the two related isoforms of cyclooxygenase.These two enzymes are similar in structures but they are expressed in different parts of the body. They convert arachidionic acid, a fatty acid in cell membranes into prostaglandins[21]. Prostaglandins are produced by both cox-1 and cox-2 enzymes but each get converted into different paracrine hormones.(paracrine are hormones that only work in the immediate area where are produced).The cyclooxygenase inhibitorsbinds into the cyclooxygenase site of the enzymes and because of the similarity of both COX-1 and COX-2, most NSAIDs inhibits both enzymes.[22]

COX-1 is obvious in many tissues, however it fluctuates. It is delineated as a "housekeeping" protein. It controls typical cell forms, (for example, gastric cytoprotection, vascular homeostasis, platelet collection, and kidney capacity), and it is fortified by hormones or development factors. It is found in the kidney, stomach and platelets and for the most part in the gastrointestinal coating as it keeps up the ordinary covering of the stomach.[23]

COX-2 is an influenceble enzyme as it is produced under certain specific conditions like inflammation. It is found in macrophages, leukocytes and fibroblasts. It is in control of the production of prostanoids in response to diverse of stimuli (which includes cytokines, growth factors, mitogens and tumor promoters) in various tissues and for the mediation of inflammation and pain in some diseases.[24][25]



COX-1 COX-2

Fig 2.5. Crystallographic structure of COX-1 and COX-2

2.2.1COX-2 selective inhibitors

Cyclooxygenase-2 (COX-2) inhibitors are a sort of nonsteroidal anti-inflammatory drug (NSAID) that particularly hinders COX-2 enzymes. COX-2 inhibitors relieve inflammation and pain with less harmful gastrointestinal effects than NSAIDs that inhibit both COX-1 and COX-2 enzymes. The finding of COX-2 has made it conceivable to configuration medicates that lessen aggravation without expelling the defensive prostaglandins in the stomach and kidney made by COX-1.However, they are not totally void of gastrointestinal effects, and their uses corresponds to that ofNSAIDs which makes them to also exhibit exhibit a higher risk of stroke and heart attack. Rofecoxib, valdecoxib, parecoxib and celecoxib are examples of COX-2 inhibitors.[26]

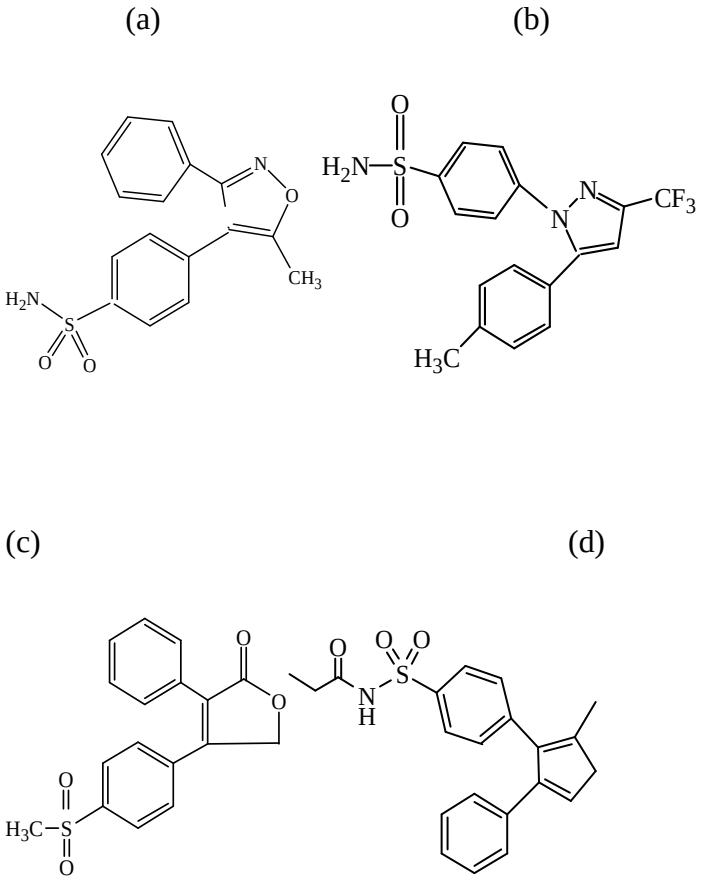


Fig 2.6. Structures of some COX-2 selective inhibitors:(a) Valdecoxib (b) Celecoxib (c)Rofecoxib(d) Palecoxib

2.3.2-Benzoxazolinone

2-Benzoxazolinone or Benzoxazolone is a characteristic substance produced by rye (Secale cereale)[27] it has solid phytotoxic properties. Benzoxazolones are broadly contained in plants and are of developing enthusiasm for an assortment of pharmacological properties, for example, detoxification, antibacterial, hostile to HIV, anti-infllamatory, and tranquilizers. It is a heterocyclic exacerbate that involves a benzene ring intertwined to a five-membered ring containing oxygen and nitrogen as the hetero particles (fig 2.7). 2-benzoxazolinone has a molecular formular of $C_7H_5NO_2$ and a molecular weight of 135.2g/mol.

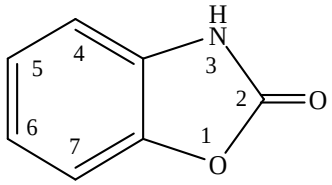


Fig 2.7. Structure of benzoxazolinone

In 1955, Virtanon and Hietala discovered 2(3H) benzoxazolones as an anti-fusarim factor in rye seedlings. In 1984, Dr Murty and his fellow workers obtained it from the leaves of the Indian *Acanthus ilicifolius* which makes it the first time that 2(3H) benzoxazolone was reported from the genus Agantus. D'Souza and his coworkers gave a report on a type of dimeric oxazolinone, (5,5'-bis-benzoxazoline-2,2'-dione), which they obtained from the leaves of the Indian *A. ilicifolius*.[28] The structure of this compound is given in fig 2.8 below

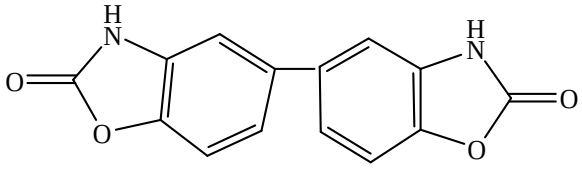


Fig 2.8. Structure of 5,5'-bis-benzoxazoline-2,2'-dione.

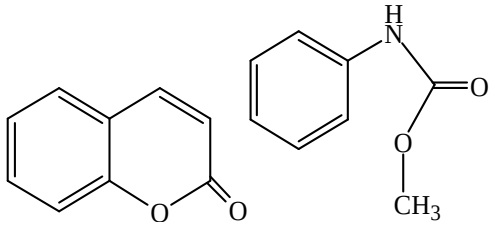
Since the firsthypnotic properties of 2(3H)-benzoxazolinone was discovered, the ring has turned out to be vital in therapeutic science. It has additionally realized the revelation of different derivatives of the compound.These derivatives produces analgesicand anti-inflammatory effects. The structure of 2-benzoxazolinone characterizes many important sequels for medicinal

purposes. This is because it constitute a scaffold of a good advantage in organic synthesis, thereby giving chances for wide varieties of chemical modifications. This suggests a decent directionality in the uses of the side chains on a rigid scaffold.[29]

In aqueous solution, benzoxazolone is a weak acid with pKa of 8.7. This is somewhat comparable to pyrocatechol (pKa = 9.2), and for this reasonit is frequently alluded to as a pyrocatechol bioisoster.It was reported that its structure resembles that of phenylurethane and coumarin, hence it is possesses the hyptonic, analgesic and antipyretic properties of phenylurethanes and the anti-inflammatory, anti-tumor, and antibacterial properties of coumarin.[30,31]

W.J. Close and his colleagues additionally made a provided details regarding how Benzoxazolone is basically identified with 2,4-oxazolidinedione, which is utilized as the premise of various mixes with pain relieving and anticonvulsant activities.[32]

(a) (b)



(c) (d)

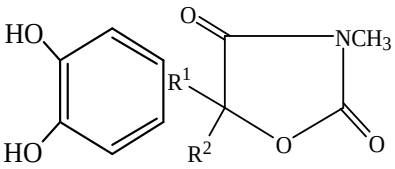


Fig 2.9. Structure (a) Coumarin (b) Phenylurethane (c) pyrocatechol(d) 2,4-oxazolidinedione

Scientists have uncovered that different pharmacodynamic parts of benzoxazolinones have solid organic activities, for example, dopamine receptor agonist, cardiotonic, antihypertensive, antiulcer activities.[33,36]

2(3H)- Benzoxazolone and its bioisosters are viewed as 'favored platforms' in the outline of pharmacological tests. They have gotten critical acknowledgment from the restorative scientific experts because of their ability tobehave like a phenol or catechol moiety in a metabolically stable format.[37] Various derivatives of 2(3H)-benzoxazolone have been marketed as drugs. Examples of thesesdrugs are like chlorzoxazone (paraflex) and vinyzene (topical antiseptic) as shown in fig 2.10

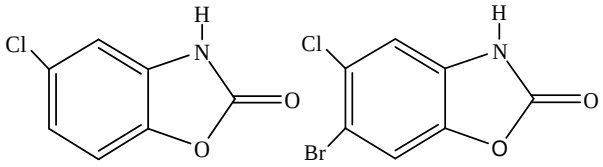
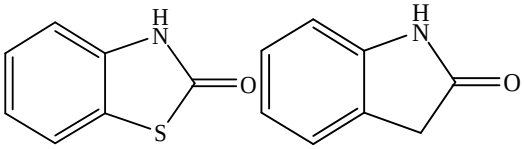


Fig 2.10. Structures of chorzoxazolone and vinylene

In many designs, benzoxazolone have been used as a substitute for phenol. To a specific level, 2(3H)- benzothiazolone, which is the sulfur bioisoster of benzoxazolone, the methylene bioisoster, i.e. 2-oxindole, and furthermore the nitrogen bioisoster, i.e. benzimidazol-2-one have been utilized as a part of circumstances where there is a requirement for either a phenol or catechol to be supplanted by a more satisfactory substitute.The results were great success.[37]

(a) (b)



(c)

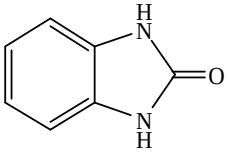
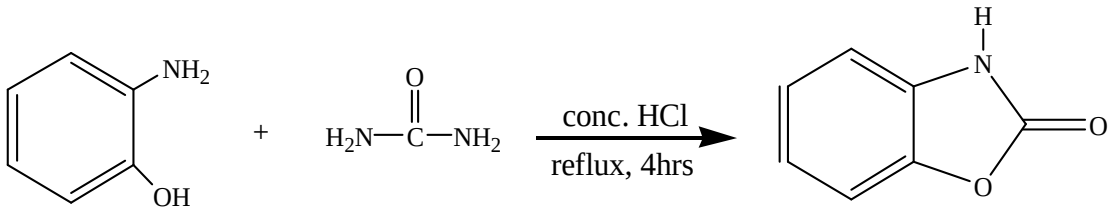


Fig 2.11. Structure of (a) benzothiazole (b) oxindole (c) benzimidazol- 2-one

2.3.1 Synthesis of benzoxazolinone

Safakish et al synthesized benzoxazolinone by heating the mixture of urea with o-aminophenol at 140-170°C under reflux for 4 hours.[38]



R^1= H, Cl;

Fig 2.12. Synthesis of 2-Benzoxazolone from aminophenol and urea.

Nachman et al additionally synthesized 2(3H)- benzoxazolones by refluxing 1,1'-carbonyldiimidazole and 2-aminophenol in dry tetrahydrofuran (THF).The resulting 2(3H)-benzoxazolones was otbtained in excellent yield.[39] The reaction is shown in Fig 2.13 below.

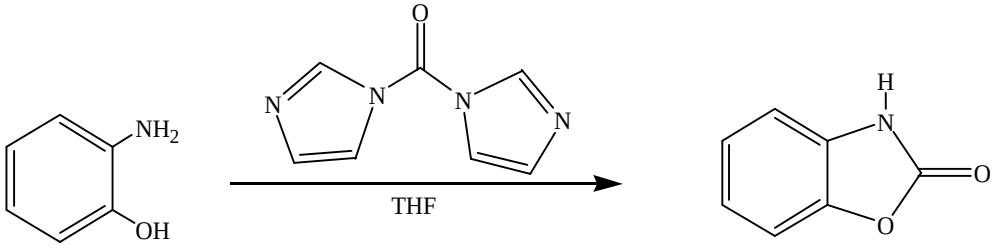


Fig 2.13 Synthesis of benzoxazolone from aminophenol and 1,1'- carbonyldiimidazole

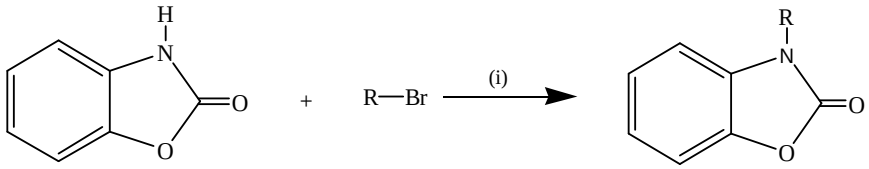
2.3.2. Reactivity of benzoxazolone

Reactivity of 2-benzoxazolinone can be found in its capacity to experience the N-substitution (either alkylation or acylation)[40], aromatic ring electrophillic substitution and ring opening or extension response at 6-position.

2.3.2.1 N-substitution reaction

The enolizable character enables useful transformations can take place at the 3rd-position of the heterocycle as seen in fig 2.14[41]. Suchtransformations alsoinclude the N-aminomethyl derivatives which is readily accessed by Mannich condensation reaction (fig 2.15),[42]also base

catalyzed expansion of acrylonitrile prompts N-cyanoethyl subsidiaries. Another case of N-substitution which results in the cyclic hydrazide structure is the reaction of benzoxazolone with hydroxaminosulfuric corrosive.[43]



where R=3-chloropropyl, allyl, benzyl, 3-butenyl, 3-methyl-2-butenyl

Fig 2.14 N-alkylation of benzoxazolone (i) K_2CO_3, Acetonitrile, rt; or : CsF-Celite, THF, rt;

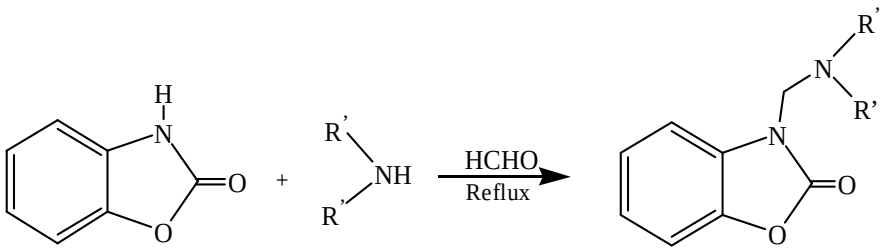


Fig 2.15 synthesis of N- aminomethylation derivative of 2-benzoxazolone through Mannich reaction where R'= CH_3

2.3.2.2 Aromatic Electrophilic Reaction

Aromatic electrophilic substitution is controlled by its strongchoicefor the 6th position in reactions like sulfonation, chlorosulfonation reaction, halogenation and alsofor the Friedel Craft acylation.[44]Particularly to Friedel Craft response, because of the electron-rich nature of 2(3H)-benzoxazolone. The benzoxazolone ring is vastly protonated by the nearness of Lewis acid in the reaction medium, which goes about as a totally obligatory catalyst.

2(3H)- benzoxazolone can act as a solidly actuated substrate in an electrophilic substitution conditions, (for example, bromination), but the tremendous complexation faced in the Friedel-Crafts response emphatically deactivates this kind of substrate towards the electrophilic attack of acylium ions. Keeping in mind that the end goal to overcome this type of issue, acylation of

benzoxazolinone was proficient before by two techniques which include the utilization of less reactive electrophillic species, in which either DMF– $AlCl3$[45] and acid chloride or polyphosphoric acid (PPA) and carboxylic acids.[46] Reports have it that the two strategies gave 6-acylbenzoxazolinone derivatives at close yields

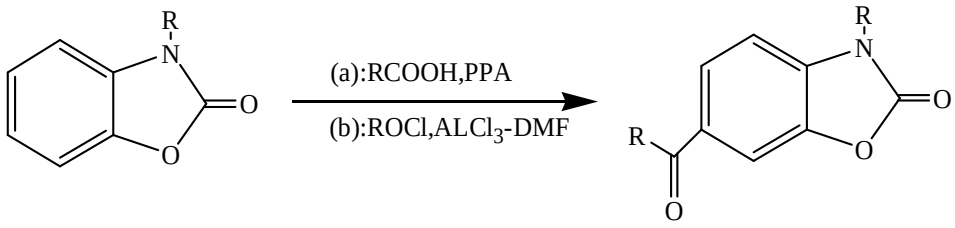
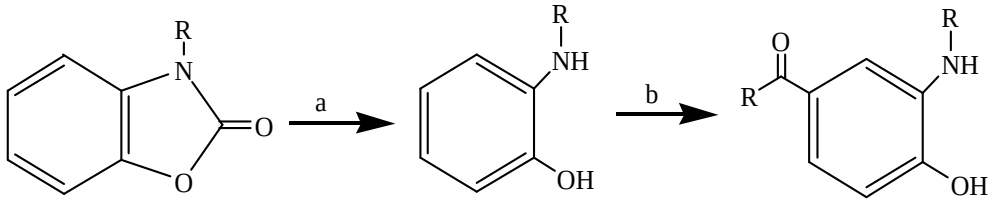


Fig 2.16 Acylation of 2-benzoxazolone derivatives at 6-position

2.3.2.3Ring Opening and Expansion Reaction

The derivatives of 2-benzoxazolinone are comparatively soluble in acidic medium. They have ability to hydrolyze very fast in basic medium.This prompts ring opening products, for example, 2-aminophenols which can be acylated at fourth position.[47]



(a)= aq. NaOH, Δ; (b)= RCOCl, $AlCl_3$.DMF;

Fig 2.17 Ring opening reaction of 2- benzoxazolone and the acylation of aminophenol at 4th position.

2.3.3. Biological activities of 2-Benzoxazolone derivatives

2(3H)- Benzoxazolone, stands out amongst the most multi-useful heterocyclic ring, it has created diverse derivatives with numerous assortments of organic activities, for example, anti- HIV, anticancer, analgeics, antiinflammatory, and antinociceptive. [48]

5-chloro-2(3H)benzoxazolinone (Chlorzoxazone), a derivative of 2- benzoxazolinone, is anactive muscle relaxant used in the treatment of muscle spasm including the pain and discomfort that arises from the spasm. Chlorzoxazone may act by hindering the influx of calcium and potassium and leads to neuronal inhibition and muscle relaxation.[49]Chlorzoxazone acts significantly at the level of the spinal cordand subcortical areas of the brain where it hinders multisynaptic reflex arcs that takes part in growing and maintaining skeletal muscle spasm.[50]

Chlorzoxazone is synthesized by a hetercyclization reaction of 2-amino-4-chlorophenol with carbonyl dichloride (phosgene).[51]

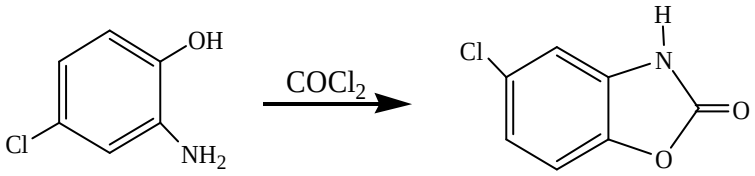


Fig 2.18 Synthesis of chlorzoxazolone

4-hydroxy-2-benzoxazolone is one of the derivatives of 2-benzoxazolone. Itis a compound that has shown analgesic and anti-inflammatory effects. It is a bioisosterism of Chlorzoxazone and has the possibility to be a kind of nonsteroidal anti-inflammatory drugs. 4-hydroxy-2-benzoxazolone is one of the primary pharmacologically dynamic compounds in customary Chinese ˇ herb sedate Acanthus ilicifolius which plainly has calming and pain relieving activities.

Li Tang et al[52] snthesized 4-hydroxy-2-benzoxazolone derivatives and screened them for human soluble epoxide hydrolase (sEH) inhibitors and anti-inflammatory activities. Three compounds displayed solid anti-inflammatory activities in vivo. 2-Aminoresorcinol responded with bis(trichloromethyl) carbonate ($Cl_3COCOOCCl_3$) under a nitrogen atmosphere with reflux toproduce 4-hydroxy-benzoxazolone which is the key intermediate to synthesizing other 4-substituted benzoxazolone derivatives.

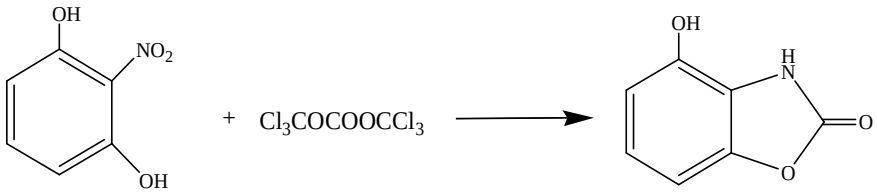


Fig 2.19 Synthesis of 4-hydroxy-2-benzoxazolone from aminoresorcinol

Guangjin Zheng et al[53]prepared 7-chloro-4-hydroxy-2-benzoxazolone via chlorination reaction using Friedel-Crafts acylation on 4-hydroxy-2-benzoxazolone . It reacted with acetic acid, HCl and hydrogen peroxide (in drops) at a temperature of 80^{o}C.

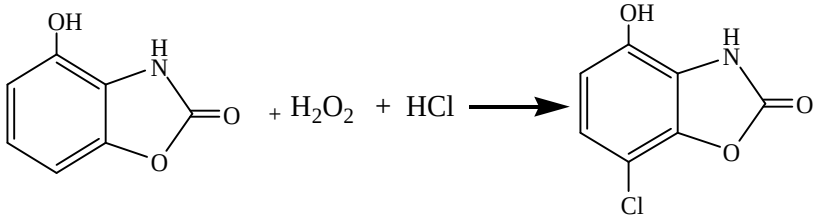
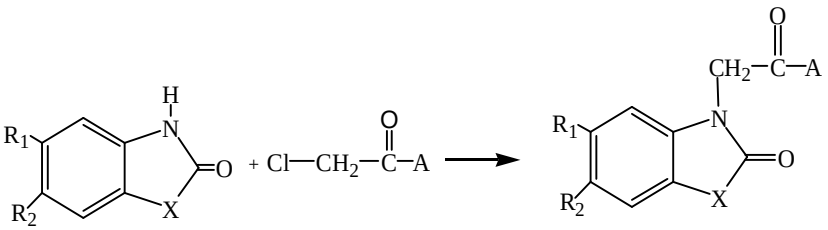


Fig 2.20. Synthesis of 7-chloro-4-hydroxyl-2-benzoxazolone from 4-hydroxy-2-benzoxazolone

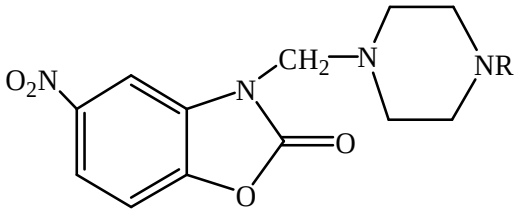
Dogruer et al[54] tested a progression of (2-benzothiazolone-3-yl and 2-benzoxazolone-3-yl) acidic corrosive subsidiaries for anti nociceptive and anti-inflammatorys. 4-[2-(Benzoyl-2-benzoxazolone-3-yl) acetyl] morpholine, 4-(2-[6-(2-chloro-benzoyl) - 2-benzoxazolone-3-yl] acetyl] morpholine, N,N-diethyl-2-(2-benzothiazolone-3-yl) acetamide were compounds that showed anti-infammatory properties.



X=O, S ; A= OH, OCH_3, OC_2H_5, R_1=H, Cl, R_2=H, CH_3

Fig 2.21 synthesis of series of (2-benzothiazolone-3-yl and 2-benzoxazolone-3-yl) acetic acid derivatives

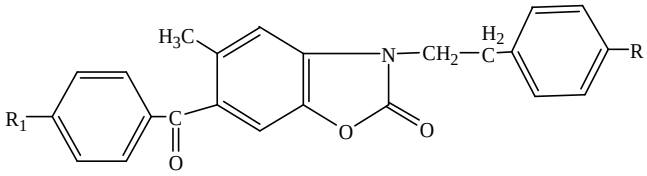
Koksal et al[55] integrated novel series of Mannich bases of 5-nitro-3-substituted piperazinomethyl-2-benzoxazolinones and screened them for analgesics and anti-inflammatory activiyies. Among the tried derivatives, most encouraging outcomes were acquired from the compound which bears electron-withdrawing substituents (F, CI, COCH3) in the ortho/para positions of the phenyl core on the piperazine ring at 3 position of benzoxazolinone moiety.



R=F, Cl, $COCH_3$

Fig 2.225-nitro-3-substituted piperazinomethyl-2-benzoxazolinone derivatives

Gokhan et al[56] synthesized, characterized and screened for the pharmacological properties of new series of (6-difluorobenzoyl)-5-methyl-3-benzoylmethyl-2-(3H)-benzoxazolone and 5-methyl-3-(2-hydroxyl-2-phenylethyl)-2-(3H)-benzoxazolone which are derivatives of 5-methyl-2(3H)-benzoxazolone. .amongst all, 6-(2,5difluyorobenzoyl)-3-(4-bromobenzoylmethyl)-2(3H)-benzoxazolone emerged to be the most encouraging compound for analgesic activity.



R=Br; R_1=2,5-diF

Fig 2.235-methyl-3-substituted piperazinomethyl-2-benzoxazolinone derivatives

The derivatives of 6-acyl-2(3H)- benzoxazolone has remarkably fascinating anti-inflammatory, antiepileptic, pain relieving and antiviral properties. They can be utilized for the management of chronic to moderate pain in the body. In perspective of the discernible clinical significance of these compoundss, an enhanced study of their synthesis was performed.

Huseyin et al[57] revealed a unique technique for acylation on the 6th-position of 2(3H)benzoxazolone and 2(3H) benzothiazolones. This strategy was done in two stages which includes the rearrangement of acyl group from the N-position to the 6-position of the heterocycle and catalyzed by $AlCl_3$. They discovered that this technique is more productive with respect to the utilization of AlCl3 and a yield of around 76-90%.

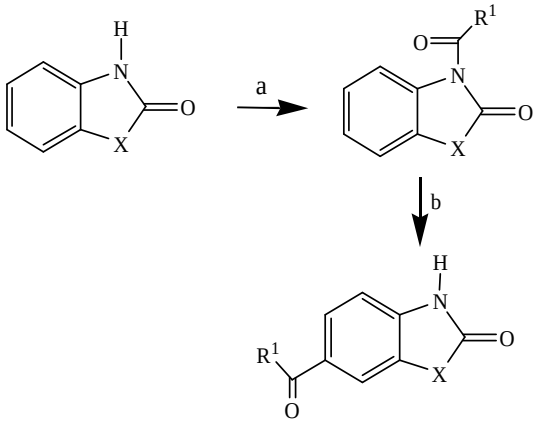


Fig 2.24. Synthesis of 6-acylation of 2(3H)-benzoxazolone and 2(3H) benzothiazolone. Conditions: a.R^1COCl or $R^1(CO)_2O$, TEA, THF, reflux, 2h; b. $AlCl_3$, 165^0C, 3h.

Likewise in efforts to produce new non-steroidal anti-inflammatory agents, Fügen Özkanli [58] prepared and evaluated a series of 6-acyl-3-aminomethyl-2-benzoxazolinones by reacting formaldehyde and arylpiperazine with 6-acyl-2-benzoxazolinone via mannich condensation. The intermediate compound synthesized by allowing 2-benzoxazolone to react with difluorobenzoic acids in the presence of polyphosphoric acid at 140-160^{o}C

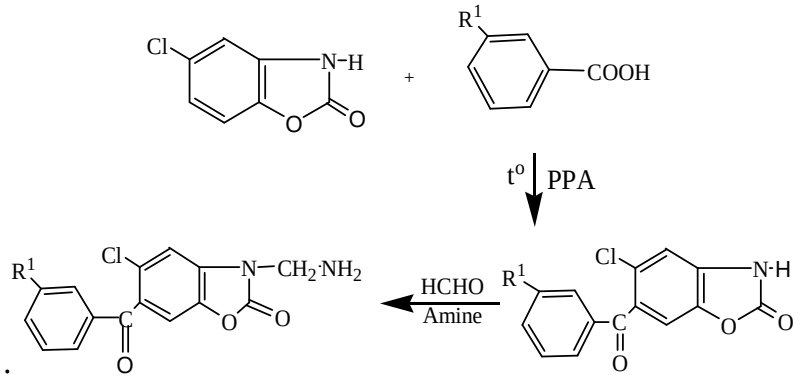
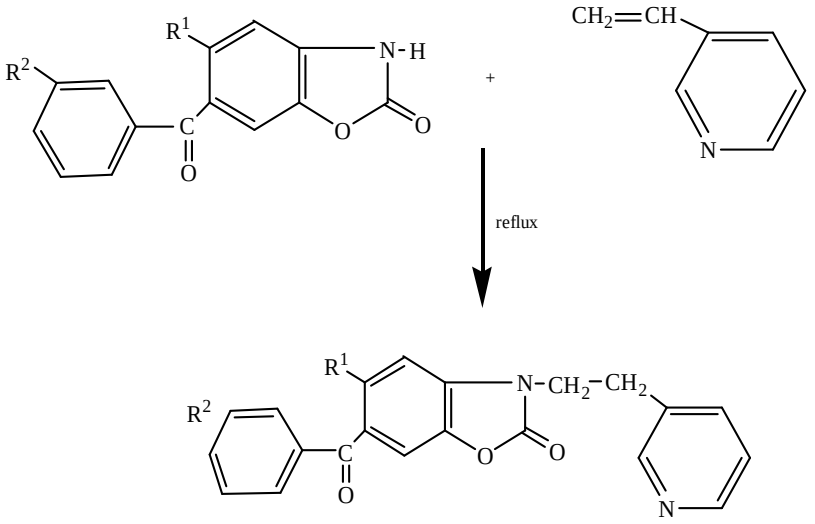


Fig 2.25. Synthesis of 6-acyl-3-aminomethyl-2-benzoxazolinones

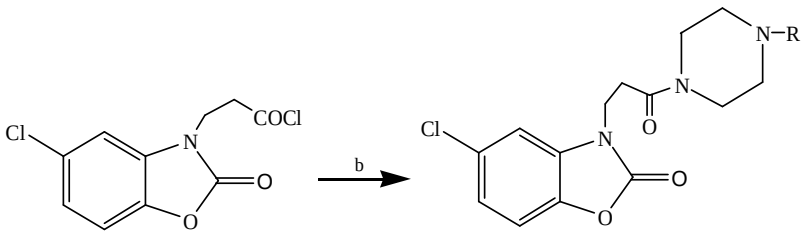
The analgesic properties of pyridylethylatedbenzoxa(Thia)zolinones were tested by Gokhan et al.[35]These compounds were synthesized in one step by reacting 6-Acyl-(5-chloro)-2-benzoxazolinone (2-benzothiazolinone) with 2-and 4-vinylpyridine with reflux in an oil bath.It was observed that compound with bromo substituents on the phenyl ring in the 6-position of the main ring appeared to indicate less activities than those with fluorine.



R^1=H, Cl; X=O, S; R^2=Br, F

Fig 2.26 Synthesis of 6-acyl-3- pyridylethylated -2-benzoxazolinone

5- position 2(3H)-benzoxazolone derivatives has also been tested for anti-nociceptive activities. Onkol et al[59] synthesised (5-chloro-2(3H)-benzoxazolon-3-yl)propanamide derivatives by reacting (5-chloro-2(3H)-benzoxazolon-3-yl)propanoyl chloride derivatives with sodium carbonate, secondary amine derivatives, and tetrahydrofuran, under reflux for about 3-20 h, and stirred at room temperature for 18 hours According to their report, piperazine substitute such as –(4-chlorophenyl)piperazine, -(2-trifluoromethylphenyl) piperazine and –(2-pyridyl) piperazine have been found to be significantly more active than the others and were standards in all the tests.



'Fig 2.27 Synthesis of (5-chloro-2(3H)-benzoxazolon-3-yl)propanamide; b=piperazine derivatives, $NaHCO_3$, THF, reflux

The wide range biological activity of 2(3H)-benzoxazolone derivatives is also seen in its potency to inhibit acetylcholinesterase in the treatment of Alheimer's disease. This is one of the recommended mechanisms for treating of Alzheimer's diseases.

Soyer et al[60] synthesized a good number of N-substituted-5-chloro-2(3H)- benzoxazolone subsidiaries and assessed their acetylcholinesterase inhibitory activity. Depending on structural contrasts, the screening made it apparent that 5-chloro-2-(3H)- benzoxazolone platform had different inhibition range against acetylcholinesterase chemical. In the study, it was found that bis 5-chloro-2(3H)- benzoxazolone demonstrated higher action than others like pyrollidine and piperidine derivatives and it was observed to be more active than other compounds in the series.

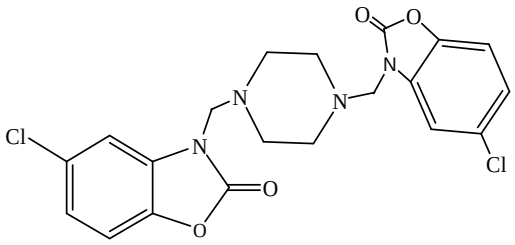
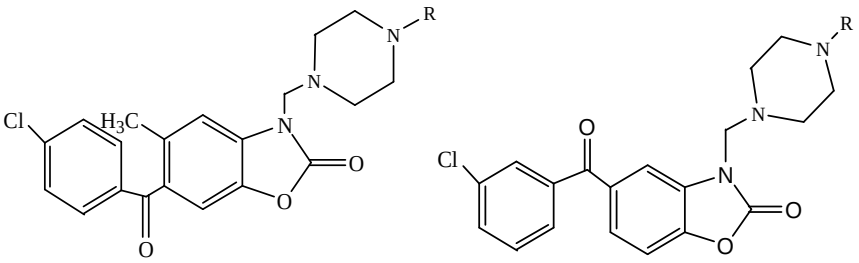


Fig. 2.28 Synthesis of N-substituted-5-chloro-2(3H)-benzoxazolone; 37% formalin, MeOH

Köksal et al.[61] synthesized and screened derivatives of 5-acyl-3(4-substututed-1-piperazinylmethyl)-2-benzoxazolinones and 6-acyl-3-(4-substituted-1-piperazinylmethyl)-2-benzoxazolinones for analgesic and anti-inflammatory activities. In terms of analgesic and anti-inflammatory activities. it was observed that the derivatives of 6-acyl were more active than 5-acyl derivative.The structures of 5-acyl and 6-acyl derivatives are given in fig 2.29

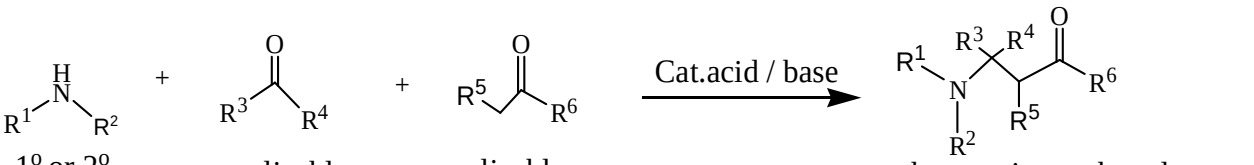


R=C_6H_5, 2-$CH_3OC_6H_5$, 3-$CF_3C_6H_5$, 4-$CH_3COC_6H_5$, 4-$CH_3COC_6H_5$, 2-$CH_3OC_6H_5$

Fig 2.29 6-acyl and 5-acyl-3-(4-substituted-1-piperazinylmethyl)-2-benzoxazolinones compounds that have shown analgesic andanti-inflammatory activities

2.4MANNICH REACTION

Mannich reaction is an important organic reaction that is utilized to change a primary or secondary amine and two carbonyl compound (one non-enolizable and one enolizable) to a β-amino carbonyl compound using an acid or base catalyst. The resulting compound is called Mannich base. In the reaction, the enolizable carbonyl compound, which has a α-hydrogen, gets deprotonated to form an enol intermediate. The non-enolizable carbonyl compound reacts with the amine to form an iminium ion. The enol intermediate attacks the iminium particle which after it is deprotonated finally yields the Mannich base product.[62]



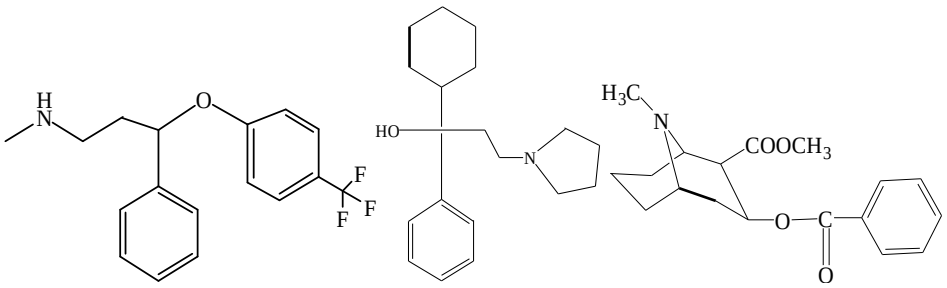
1° or 2° amine | non enolizable carbonyl compound | enolizable carbonyl compound | beta-amino carbonyl group (mannich base)

2.4.1 Mannich Bases

Mannich bases are referred to as beta-amino ketones carrying compound. They are the end products of mannich reaction.[63]Theyfunction as bioactive leads which makes them to be further useful for the synthesis of various potential agents of high medicinal value which contains aminoalkyl (aminoalkylation may increase the hydrophilic properties of drugs through the introduction of a polar function in their structure, the long-known rolicycline is a common example)[64]chain.The examples of clinically useful mannich bases which consist of aminoalkyl chain are cocaine, fluoxetine, atropine, trihexyphenidyl, procyclidine, ranitidine, biperiden[65] as shown in fig 2.29.

Mannich bases are referred to assume an essential role as pharmaceutical science continues developing now and again. Various contemplates have revealed that Mannich bases are extremely reactive and can be effectively changed over to different compounds, for instance, they can be reduced to form pharmacologically active amino alcohol. They show strong activities like anti-inflammatory, anticancer, antibacterial, antifungal, anticonvulsant, analgesics[66-71] and numerous others.

(a) (b) (c)



(d)

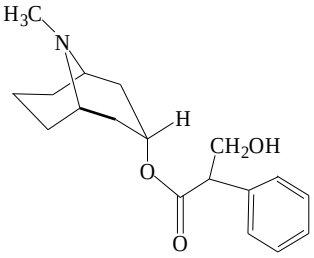


Fig 2.30 Structures of (a) fluoxetine, (b) prociclydine (c) cocaine (d) atropine

3. MATERIALS AND METHOD

3.1 MATERIALS

All reactions were carried out using standard laboratory equipment and glasswares. The chemicals used in this research work such as 2-benzoxazolinone, 6-bromoacetyl-2-benzoxazolone, 2-fluorophenyl piperazine, formaldehyde and phenyl piperazine, were purchased from Sigma Aldrich chemical company and these chemicals were used as received.

3.2METHOD

3.2.1 COMPOUND 1 (MANNICH REACTION)

3-(2-fluorophenylpiperazin-1-yl)methyl-2(3H)-Benzoxazolone

This reaction was proceeded by dissolving solution of 0.2g(0.001mol) 2-benzoxazolinone and 0.23ml (0.001mol)of 2-fluorophenylpiperazine in 2ml of methanol in a round bottom flask. 0.2ml of 37%(w/v) formalin solution was mixed with 2ml of methanol and was added to the mixture in the flask. The mixture was refluxed for 60min. After reflux, solution was poured into crushed ice and filtered using the vacuum filtration method. The resulting crude precipitate was subsequently washed with appropriate alcohol. The resulting precipitate was purified by recrystallization using an appropriate solvent.

3.2.2 COMPOUND 2 (AT ROOM TEMPERATURE)

6-2-(4-phenylpiperazine-1-yl)acetyl-2-benzoxazolone

0.35g (0.001mol) of 6-bromoacetyl-2-benzoxazolone was dissolved in 7ml DMF. The mixture was added dropwisely to a solution containing0.18ml (0.001mol)of phenyl piperazine and 0.4ml (0.002 mol)of triethylamine (TEA) in 3ml DMF. The mixture was stirred at room temperature for 30hours. After which it was poured into crushed ice and filtered using vacuum filtration. The resulting precipitate was washed and dried. [72]

3.3Melting Point Determination

The melting points of synthesized products were determined with on the Mettler Toledo FP900 thermosystem digital melting point apparatus and the values were recorded.

3.4 Thin Layer Chromatography

3.4.1 Materials

The solvents used for the mobile phase are methanol and benzene. The stationary phase plate is made of silica gel.

A_1:Benzene-Methanol (9:1)

A_2:Benzene-Methanol (5:1)

3.4.2Method

The mobile phases were poured in to the TLC chambers to a depth of about 0.5cm. The chambers were covered, swirled gently and allowed to stand while the plates are being assembled.

The plates were prepared for three different spots while o.cm line 0.5cm of origin were gently drawn away from the bottom and top with pencil.

2(3H)-benzoxazolone, 6-bromoacetyl-2-benzoxazolone and piperazinederivatives (starting materials) were dissolved in chloroform and the products were dissolved in appropriate solvents. With the aid of micocapillary spots were made on the origin line at the bottom of the plats. The plates were gently placed in the TLC chambers, covered and left undisturbed to allow the solvent to move to the line at the top of the plate. The plate was removed and allowed to dry. UV-light at wavelength (254nm) was used for visualizing the spots and to obtain the retention factor values (R_f).

3.5 Spectroscopy

3.5.1 Fourier Transform Infra Red (FT-IR): The FT-IR spectra of the product was recorded onAgilent Carry 630 Spectrometer at Ankara University, central Instrumental Analysis Laboratory, Faculty of Pharmacy.

3.5.2 Proton Nuclear Magnetic Resonance (^{1}H-NMR):The ^{1}H-NMR was recorded on a mercury Varian 400MHz spectrometer wheredeuterated solvent of dimethyl sulphoxide (DMSO-d_6) was used. The test was conducted at Ankara University, central Instrumental Analysis Laboratory, Faculty of Pharmacy. Chemical shifts (δ) values were reported in parts per million (ppm).

4. Result and Discussion

4.1.Results

Compound1: 3[4-(2-fluorophenyl)piperazine]methyl-2-benzoxazolone

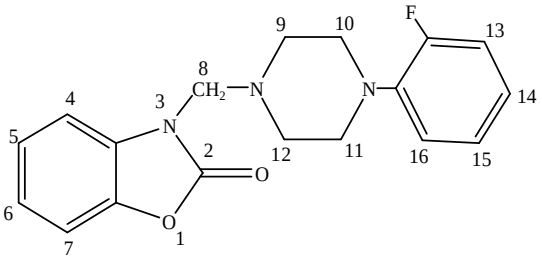


Fig 4.1 3[4-(2-fluorophenyl)piperazine]methyl-2-benzoxazolone

The above **compound 1** was synthesized using reflux as described in the experimentalsection

A brown crystalline compound was obtained; Melting point:174°C

TLC in A_1 and A_2 solvent gave R_f values of 0.49 and 0.57 respectively.

Fourier Transform Infra-Red(FT-IR) spectroscopy (IR υ_{max}): The FT-IR shows aromatic C-H stretches at 2819.7cm^{-1}, carbonyl group (C=O) at 1757cm^{-1}.

Proton Nuclear Magnetic Resonance Spectroscopy(^{1}H-NMR in DMSO-d_6):^{1}H NMR showed peaks at 7.3-6.8 (8H; m; Arom-H); 4.8 (2H; s; CH_2), 2.8 (t; 4H; pip H^{10}, H^{11}); 3.1 (t; 4H; pipH^9, H^{12}). ppm.

Compound 2: 6-2-(4-phenylpiperazine-1-yl)acetyl-2-benzoxazolone

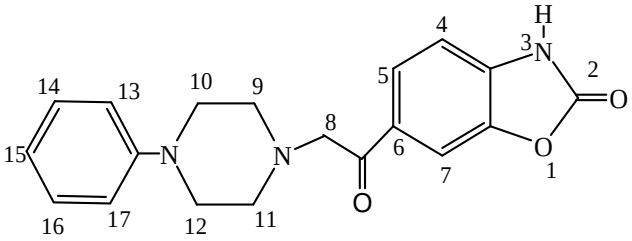


Fig 4.2 6-2-(4-phenylpiperazine-1-yl)acetyl-2-benzoxazolone

Compound 2was synthesized at room temperature as described in the experimental section

Anoff-white compound was observed; Melting point: 163.5°C

TLC in A_1 and A_2 solvents gave R_f values of 0.2 and 0.48 respectively.

Frourier Transform Infra-Red (FT-IR) Spectroscopy(IR υ_{max}): FT-IR showed a broad N-H peak at 3483.2cm^{-1}, C-H stretch at 2816.0cm^{-1}, a carbonyl (C=O) group at1748.1cm^{-1}.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR in DMSO-d_6): ^{1}H-NMR showed resonances at 8-6.8ppm(8H. m, Ar-H), 3.8ppm(2H, s, CH_2), 3.2(t; 4H; pip H^9, H^{11}); 2.49 (t;4H; pipH^{10}, H^{12}).

4.2 Discussion

In this research, two compounds were synthesized by following literature procedures which are based on 2-benzoxazolone structure.[72] **Compound 1** was synthesized via Mannich and was carried out underreflux so as to maintain the reaction conditions. **Compound 2** was synthesized at room temperature.

For **compound 1**, piperazine derivative (2-fluorophenyl piperazine) was attached on position 3 of 2(3H)-benzoxazolone to give Mannich base. For compound 2, piperazine derivative (phenylpiperazine) was attached on position of 6-bromoacetyl-2(3H)-benzoxazolone. The reactions for both **compound 1 and 2** were done under reflux and at room temperature respectively. The general Mannich reaction for compound 1 and reaction for the 6-position reaction of compound 2 at room temperature are given below.

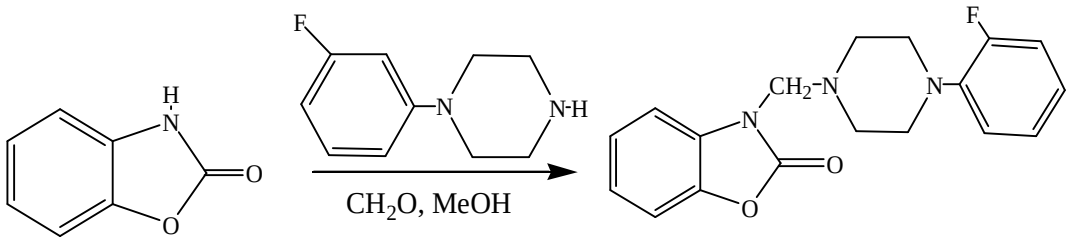


Fig 4.3 Synthesis of Compound 1

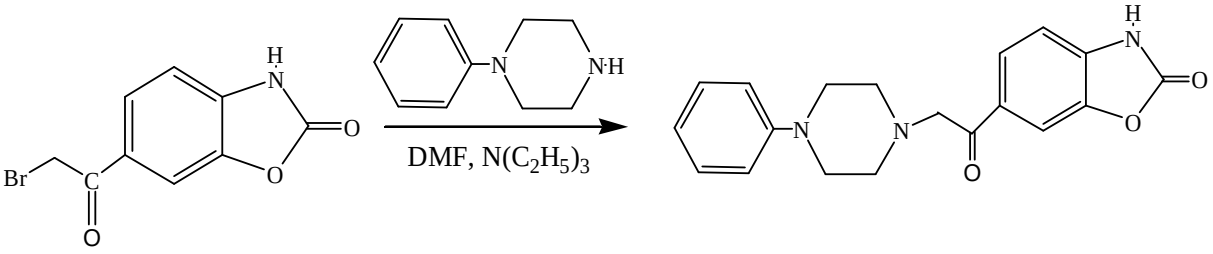
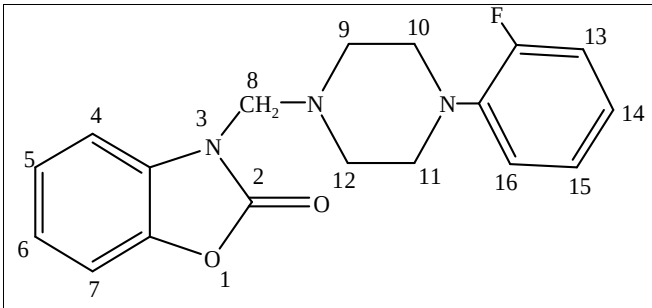
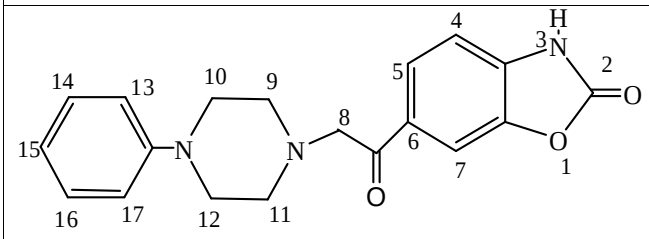


Fig 4.4 Synthesis of Compound 2

Table 4.1summary of the results of compound **1** and **2**.

Structures of compound 1 and 2	Condition/time	Melting point(°C)	R_f Values

 **Compound 1**	Reflux/1hr	174°C	0.49,0.57
 **Compound 2**	room temperature/30hr	163.5°C	0.2,0.48

The structures of the compound that wassynthesized were verified by Fourier Transform Infra-Red (FT-IR) and Proton Nuclear Magnetic Resonance (1H-NMR) Spectroscopy.

The FT-IR spectrum of **compound 1** synthesized compounds showed absence of (N-H) stretch which is expected to appear at (3100-3550) cm^{-1}, this indicates that the reaction had taken place at 3-position as expected.A C=O stretch band of 2(3H)-benzoxazolone was seen at 1757.4cm^{-1}, the (C-H) stretch for the compound also appear at 2819.7 cm^{-1}.

For **compound 2**, a peak at 3483.2cm^{-1} indicates the presence of N-H stretch, a strong C=O stretch band of 2(3H)-benzoxazolone was seen at 1748.1$cm^{-1}$and the (C-H) stretch for the compound also appear at 2816.0 cm^{-1}.

FT-IR spectra of compound 1 and 2 made under different reaction conditions are given in Figure 4.5 and 4.6 respectively.

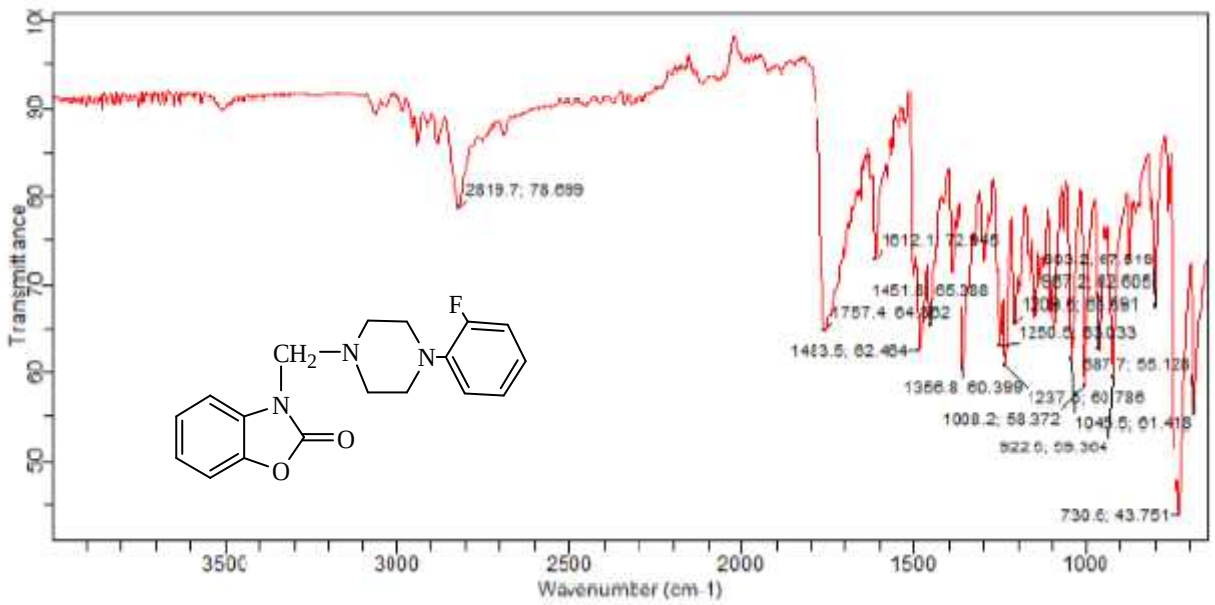


Fig 4.5:FT-IR Spectrum of 3[4-(2-fluorophenyl)piperazine]methyl-2-benzoxazolone

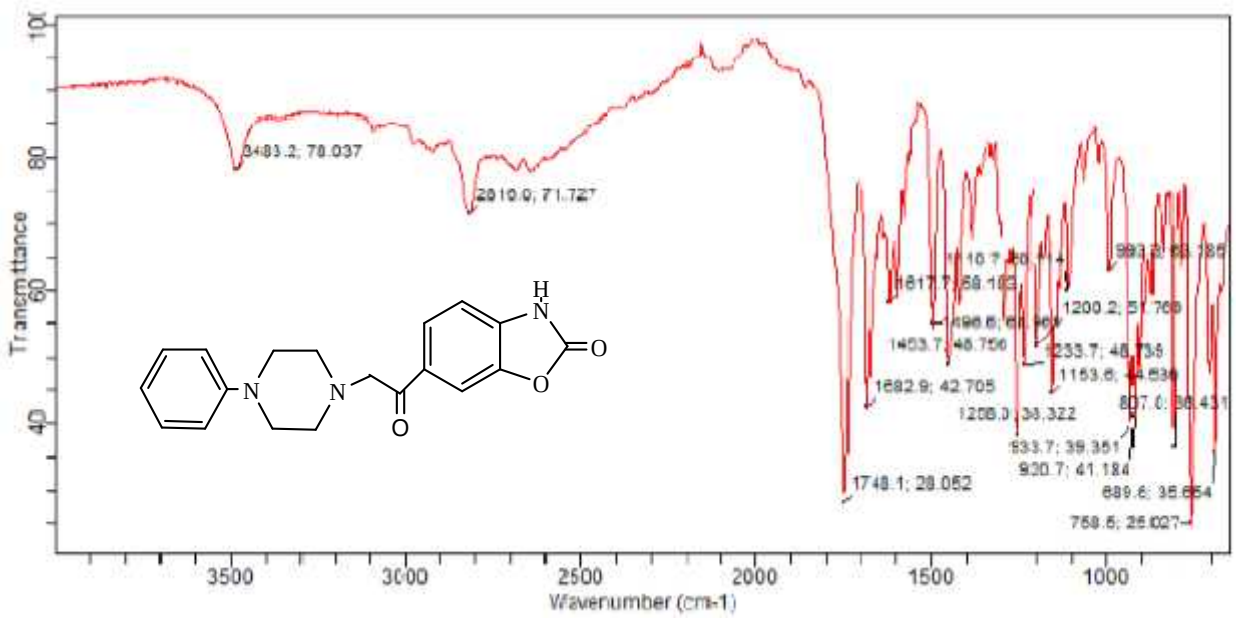


Fig 4.6:FT-IR Spectrum of 6-2-(4-phenylpiperazine-1-yl)acetyl-2-benzoxazolone

^{1}H-NMR spectrum of **compound 1** in DMSO-d_6 shows peaks at expected chemical shifts. In **compound 1** spectrum, relative to the starting material 2(3H)-benzoxazolone, there is a bridging CH_2 (methylene) peak as singlet observed 4.8ppm for this compound. The absence of the N-H

peak in the spectrum of this compound proves that the reaction have taken place in position 3 and piperazine is bonded to 2(3H)-benzoxazolone via CH_2.

^{1}HNMR spectrum of **compound 2** in DMSO-d_6, the presence of aromatic peaks as multiplets between 6.8 to 8ppm The piperazine protons (H^{10} and H^{12}) and (H^9 and H^{11}) were seen as triplets at 2.8 and 3.2 ppm which indicated that, the less shielded protons in ($H^{10}$and H^{12}) are closer to the piperazine nitrogen attached to an electron withdrawing group which is benzene, while more shielded protons (H^9 and H^{11}) are more closer to the piperazine nitrogen next to electron releasing group (CH_2-).

The ^{1}H NMR Spectrum of compounds 1 and 2 are given in fig 4.7 and 4.8 respectively.

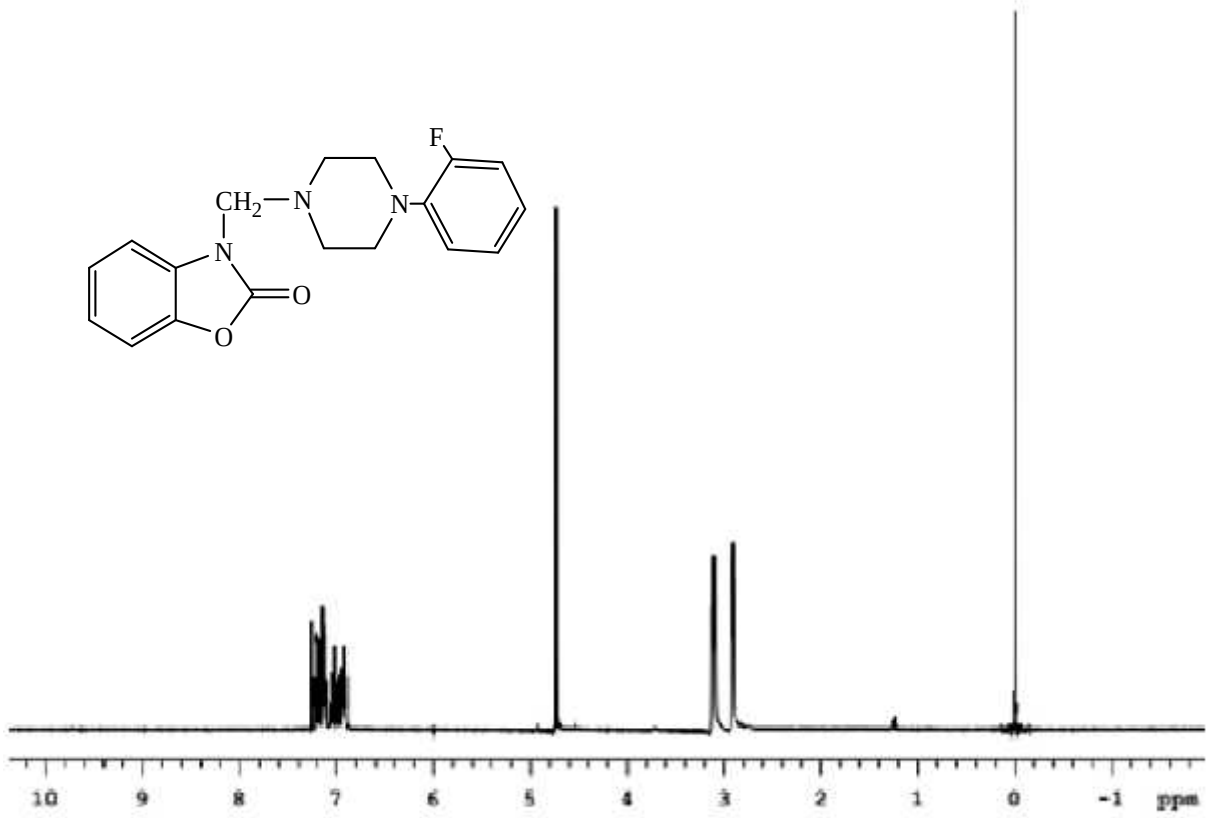


Fig 4.7: ^{1}H NMR Spectrum of 3[4-(2-fluorophenyl)piperazine]methyl-2-benzoxazolone

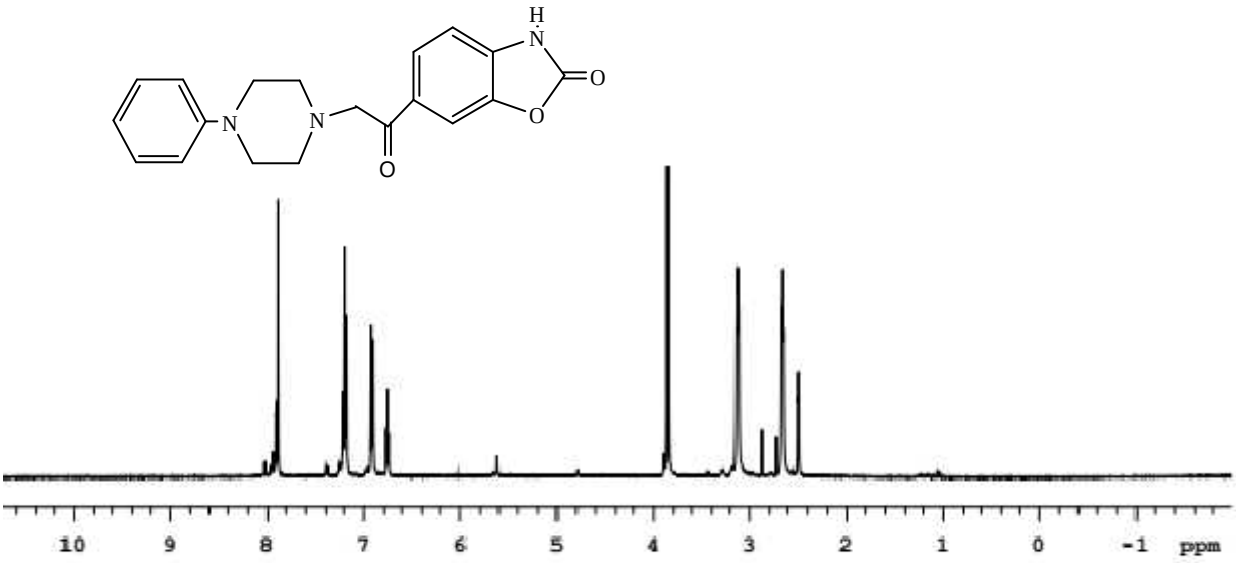


Fig 4.8: ^{1}H NMR Spectrum of 6-2-(4-phenylpiperazine-1-yl)acyl-2-benzoxazolone

5. CONCLUSION

In this study, two different substitutions on 2-benzoxazolone was carried out at 3 and 6 position. The experiments were carried out using Mannich reaction and the reaction at room temperature at the position 3 and 6 respectively.

This has shown according to literature, that it is possible to do substitutions at different sites of benzoxazolone structure. As a result, by changing the position of substituent on 2-benzzoxazolone, it might be possible to introduce other biological activities than analgesics and anti-inflammatory activities.

Based on literature, the synthesized compoundsare expected introduce analgesic and anti-inflammatory activities.

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