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

SYNTHESIS AND CHARACTERIZATION OF SOME 3-SUBSTITUTED BENZOXAZOLONES

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DEDICATION

To the four pillars of my life: God, my husband, my son, and my mother. Without you, my life would fall apart.

I might not know where the life's road will take me, but walking with You, God, through this journey has given me strength and all I need.

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I heartily recognize the sacrifices of all my family members and will not hesitate to single out Marcel Suiven and Justice Kernyuy for their sacrifices to me. Am also thinking of Mr/Mrs Ngoran Pius, Mr/Mrs Ngoran Charles, Ms Ngoran Bernice, and Yvonne for their support.

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ABSTRACT

According to literature, 2(3H)-benzoxazolone constitutes a scaffold of high versatility in organic synthesis, allowing for a wide variety of chemical modifications to different positions of the molecule. Also many studies in literature have shown that derivatives of 2(3H)-benzoxazolone show diverse biological activities, hence the need to synthesize new 2(3H)-benzoxazolone derivatives. Herein synthesis and characterization of two 5-chloro-3-substituted-2-benzoxazolone molecules are presented. The molecules were synthesized via classical Mannich reaction starting from 5-chloro-2(3H)-benzoxazolone in the presence of different substituted piperazine molecules and 35% formaldehyde. All two 5-chloro-3-substituted-2-benzoxazolone derivatives were characterized by melting point, TLC, FT-IR and ^{1}H NMR techniques.

Key Words: 5-chloro-2(3H)-benzoxazolone, Mannich Reaction, Analgesics.

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List of Abbreviations

PPA	Polyphosphoric Acid
DMF	N, N -dimethylformamide
THF	Tetrahydrofuran
COX	Cyclooxygenase
FT-IR	Fourier Transform Infrared
UV-VIS	Ultra Violet and Visible Spectroscopy
NMR	Nuclear Magnetic Resonance
ATR	Attenuated Total Reflection
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
TLC	Thin Layer Chromatography
TEA	Triethylamine
ppm	Parts Per Million
nm	Nanometers
cm	Centimeters
s	Singlet
m	Multiplet
t	Triplet
pip	Piperazine
Arom	Aromatic
λ	Lambda
TMS	Trimethylsilane
^{1}H	Proton
Et	Ethyl

1. INTRODUCTION

2(3H)-benzoxazolones and their derivatives are an important class of biologically active molecules. Their importance is due to their potential application in the field of pharmaceuticals and in chemical systems. Many 2(3H)-benzoxazolone derivatives have been described in therapeutics as possessing a wide variety of pharmacological activities. Scientist have investigated the 2(3H)-benzoxazolones in the past years and their findings showed that compounds with this structure have analgesic, hypnotic, anti-inflammatory, antineoplastic, anticonvulsant and antimicrobial activities [1-3].

It has been reported that chlorinated 2(3H)-benzoxazolone compounds have valuable analgesic and fungicidal properties and are therefore suitable for the protection of organic material from attack by fungi and from damage due to rot [4]. The literature also reveals that 6-acyl-2-benzoxazolone derivatives show significant analgesic activity [5].

2(3H)-benzoxazolone constitutes a scaffold of high versatility in organic synthesis, allowing for a wide variety of chemical modifications to different positions of the molecule.

In this research study, two 3-substituted-5-chlorobenzoxazolones were synthesized via Mannich reaction with substitutions of piperazine derivatives in position 3, these two molecules were characterized by Fourier Transform Infra-Red (FT-IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR) and ultraviolet-visible (UV-Vis) spectroscopy. The purity was determined by melting point and thin layer chromatography (TLC).

2. LITERATURE REVIEW

2.1. Analgesics

Analgesic is a drug that reduces or eliminates pain. Two main groups of analgesics in the market are opioids such as morphine and codeine and non-steroidal anti-inflammatory agents (NSAIDs) such as aspirin, paracetamol and ibuprofen.

2.1.1. Narcotic (Opioid) Analgesics

Narcotic agents are potent analgesics which are effective for the relief of severe pain. They act on the central nervous system which can lead to dependence, hence limiting their clinical use [6].

Narcotic agents may be classified into four categories:

- Morphine and codeine - natural alkaloids of opium.
- Synthetic derivatives of morphine such as heroin
- Synthetic agents which resemble the morphine structure such as methadone and meperidine.
- Narcotic antagonists which are used as antidotes for overdoses of narcotic analgesic

2.1.1.1. Natural Alkaloids of Opium

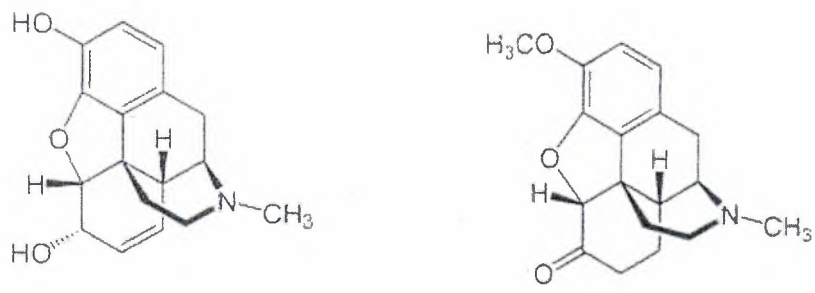
Morphine was first isolated in pure form from dried poppy resin by German apothecary Friedrich W. Sertürner (1783-1841) in the early 1800s. He named the compound "morphium" after Morpheus--the Greek god of dreams [7].

(a) (b)

Figure 2.1. Picture of the (a) poppy flower and (b) dry poppy seed.

Morphine is extracted from opium resin or poppy straw in a series of extraction and purification steps involving water, organic solvents, and pH adjustment. Morphine exerts a narcotic action manifested by analgesia, drowsiness, changes in mood, and mental clouding. The major medical action of morphine sought in the CNS is analgesia. Opiates (The term opiate is used to describe alkaloid molecules derived from opium, which in turn is derived from the juice of the opium poppy Papaver somniferum) suppress the "cough center" which is also located in the brain stem, the medulla. Such an action is thought to underlie the use of opiate narcotics as cough suppressants.

Codeine and thebaine (paramorphine) are only present as trace amounts in poppy cultivated for the purpose of morphine extraction. Approximately 95% of the morphine extracted from licit opium is chemically converted to codeine, a more versatile pharmaceutical. Codeine appears to be particularly effective in this action and is widely used for this purpose [8, 9]. The structures of morphine and codeine are shown in figure 2.2.



(a) (b)

Figure 2.2. Structure of (a) morphine and (b) codeine

2.1.1.2. Synthetic Derivatives of Morphine

Heroin is synthesized from morphine by a relatively simple esterification reaction of two alcohol (phenol) groups with acetic anhydride (equivalent to acetic acid) (figure 2.3). Heroin is much more potent than morphine but without the respiratory depression effect. A possible reason may be that heroin passes the blood-brain barrier much more rapidly than morphine. Once in the brain, the heroin is hydrolyzed to morphine which is responsible for its activity [10].

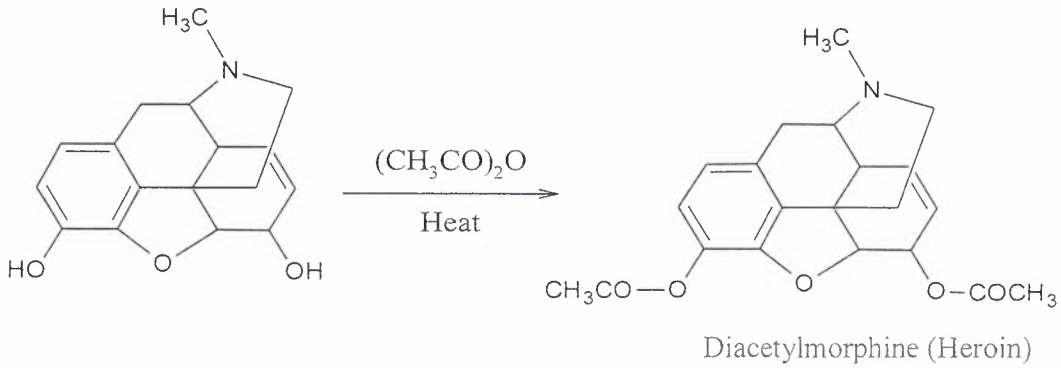


Figure 2.3. Acetylation of morphine to heroin

2.1.1.3. Synthetic Agents which Resemble Morphine Structure

There are mainly two agents which are considered as synthetic agents which resemble morphine. Meperidine (1-methyl-4-phenyl-, ethyl ester, shown in figure 2.4), is the most common substitute for morphine. It was the first synthetic opioid synthesized in 1932 by the chemist Otto Eislib. It exerts several pharmacological effects: analgesic, local anesthetic, and mild antihistamine. This multiple activity may be explained by its structural resemblance to morphine, atropine, and histamine [11].

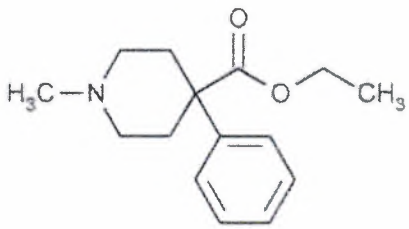


Figure 2.4. Structure of meperidine

Methadone (6-(dimethylamino)-4,4-diphenyl-3-heptanone) on the other hand is a synthetic opioid and it is more active and more toxic than morphine. It can be used for the relief of many types of pain. In addition it is used as a narcotic substitute in addiction treatment because it prevents morphine abstinence syndrome [12]. Structure of methadone is given in figure 2.5.

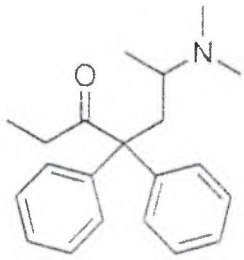
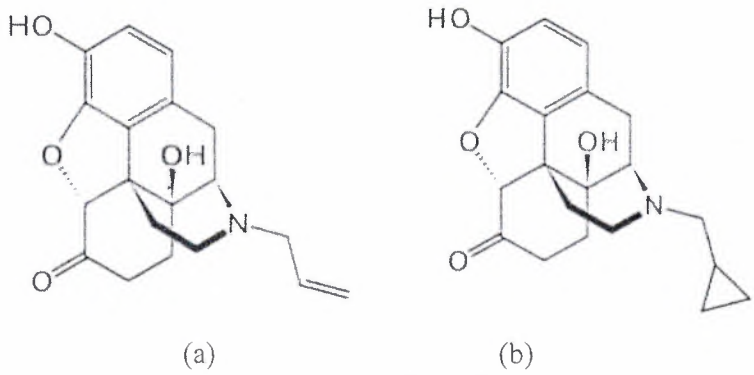


Figure 2.5. Structure of Methadone

2.1.1.4. Narcotic Antagonists

Narcotic antagonists prevent or abolish excessive respiratory depression caused by the administration of morphine or related compounds. They act by competing for the same analgesic receptor sites. They are structurally related to morphine with the exception of the group attached to nitrogen.

Naloxone is an opioid antagonist drug in the 1960s and is used to counter the effects of opiate overdose, for example heroin or morphine overdose while naltrexone is an opioid receptor antagonist used primarily in the management of alcohol dependence and opioid dependence [13]. Figure 2.6 shows the structures of naloxone and naltrexone.



(a) (b)

Figure 2.6. Structures of (a) naloxone and (b) naltrexone

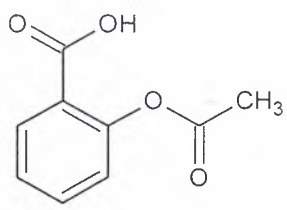
2.1.2. Non-Steroidal Anti-Inflammatory Agents (NSAIDs)

NSAIDs are a class of drugs that relieve a wide range of conditions: Headaches, painful periods, toothache, sprains and strains, infections, such as the common cold or the flu, inflammation of the joints (arthritis) and other tissues.

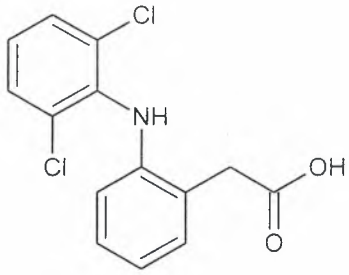
Given the reluctance to use opiates because of their liability towards physical dependence, tolerance, respiratory depression, and constipation and limitation of efficacy of the peripheral analgesics associated to classical drawbacks i.e. gastrointestinal lesions [14]. Analgesic action of NSAIDs is by inhibition of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), and thereby, the synthesis of prostaglandins and thromboxanes. This quest led to the development of new potent analgesic agents with the efficacy of morphine without the undesired use limiting side-effects.

There are many different types of NSAIDs, which may be classified based on their chemical structure or mechanism of action. Older NSAIDs were known long before their mechanism of action was elucidated and were for this reason classified by chemical structure or origin. Newer substances are more often classified by mechanism of action. Figure 2.7 presents medications grouped by types:

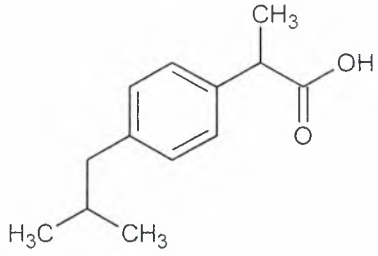
Salicilates
e.g. Aspirin



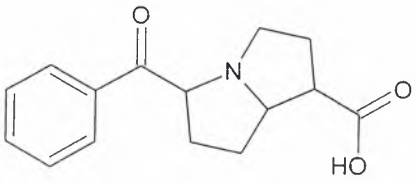
Arylalkanoic Acid
e.g. Diclofenac



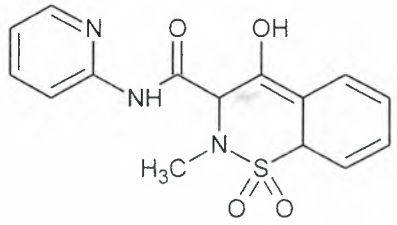
Arylpropanoic Acids
e.g. Ibuprofen



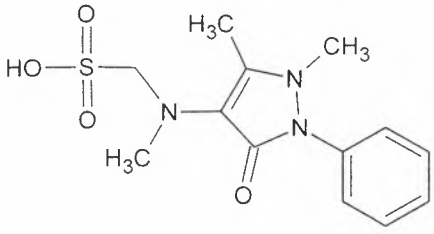
Pyrroles
e.g. Kentorolac



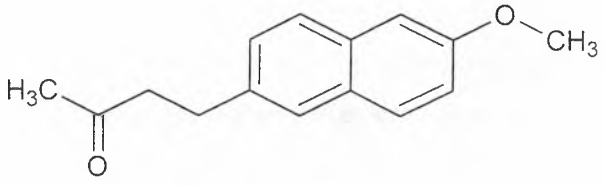
Enolic Acids
e.g. Piroxicam



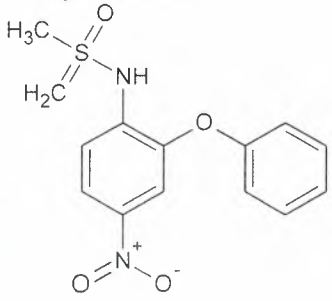
Pyrazolones
e.g. Metamizole



Napthylalkanones
e.g. Nabumetone



Sulphonanilides
e.g. Nimsulide



Fenamates
e.g. Mefenamic acid

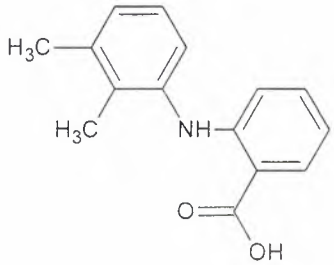


Figure 2.7. Some examples of NSAIDs.

2.1.2.1. COX-1 AND COX-2 Enzymes

In the 1990s, researchers discovered that two different COX enzymes existed, now known as COX-1 and COX-2. Cyclooxygenase-1 (COX-1) is known to be present in most tissues.

In the gastrointestinal tract, COX-1 maintains the normal lining of the stomach. The enzyme is also involved in kidney and platelet function. Cyclooxygenase-2 (COX-2) is primarily present at sites of inflammation.

While both COX-1 and COX-2 convert arachidonic acid to prostaglandin, resulting in pain and inflammation, their other functions make inhibition of COX-1 undesirable while inhibition of COX-2 is considered desirable.

COX-2 closely resembles COX-1 with the exception that the COX-2 active site accommodates larger chemical structures owing to substitution of the isoleucine at position 523 in COX-1 with a valine in COX-2. The loss of a single methyl group arising from the isoleucine to valine substitution is sufficient to open up a secondary internal hydrophobic side pocket in COX-2 that increases the volume of the active site by approximately 25%. Larger pocket size in COX-2 allows drugs with large substituents to enter to active site [15-16]. Figure 2.8 illustrates the structural differences between COX-1 and COX-2 enzymes.

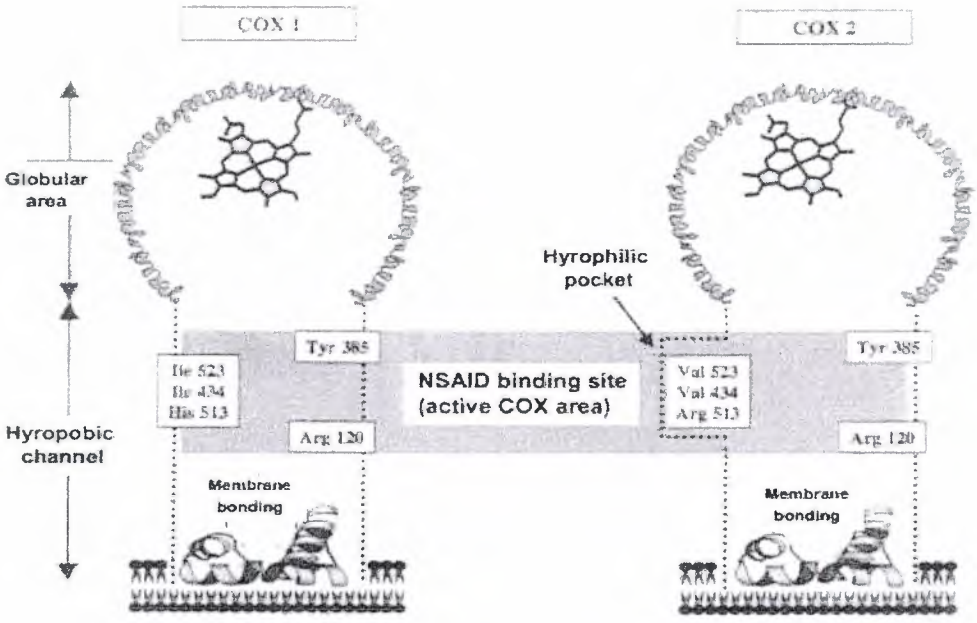


Figure 2.8. Structures of COX-1 and COX-2 Enzymes

2.1.2.2. COX-2 Selective Inhibitors

COX-2 selective inhibitors are NSAIDs that selectively block the COX-2 enzyme and not the COX-1 enzyme. Blocking this enzyme prevents the production of prostaglandins by the COX-2 enzyme that often causes the pain and swelling of inflammation and other painful conditions.

Selective blocking of the COX-2 enzyme and not the COX-1 enzyme makes COX-2 drugs uniquely different from traditional NSAIDs which usually block both COX-1 and COX-2 enzymes.

COX-2 Selective inhibitors are advantageous in that, they do not present the risk of injuring the stomach that medications also blocking COX-1 can. Examples of COX-2 inhibitors

include celecoxib, enterocoxib, and Valdecoxib [17]. Examples of COX-2 inhibitors are shown in figure 2.9.

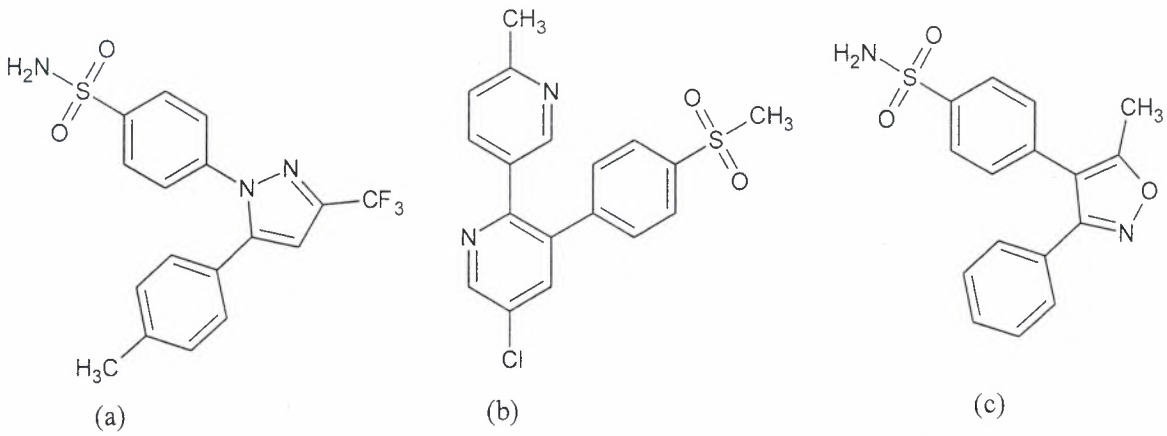


Figure 2.9. Structures of some COX-2 selective inhibitors: (a) Celecoxib, (b) Enterocoxib and (c) Valdecoxib

2.2. 2(3H)-Benzoxazolone

The 2(3H)-benzoxazolone is a heterocycle with bicyclic ring system made of a phenyl ring fused to a carbamoate. This particular structural feature has several important consequences for the medicinal chemist: (i) one edge is lipophilic, while the other one is hydrophilic with two hydrogen bonding accepting sites and a single hydrogen bonding donating site; (ii) this dichotomy is reflected by a rather high dipole moment (4.47Debye) and a discrete partition coefficient (log P = 0.97); (iii) 2(3H)-benzoxazolone is a weak acid in aqueous solution (pKa = 8.7). The structure of 2(3H)-benzoxazolone is shown in figure 2.10.

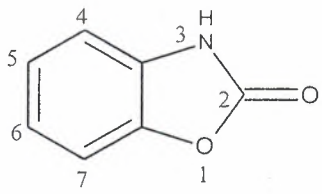


Figure 2.10. Structure and numbering of 2(3H)-benzoxazolone

The 2(3H)-Benzoxazolone, as one of the most versatile heterocyclic rings, is shown to produce diverse compounds with a wide range of biological activities such as antibacterial-antifungal, analgesic-anti-inflammatory, anticonvulsant, antitubercular, antimalarial, anticancer, antinociceptive, and antioxidant dopaminergic, and human immunodeficiency virus (HIV)-1 reverse transcriptase activities [18,19].

2.2.1. 2(3H)-Benzoxazolones in Nature

2(3H)-Benzoxazolone was first discovered in nature by Virtanen and Hietala as an anti-fusarium factor in rye seedlings in 1955. Secondly it was obtained by Murty and his coworkers from the leaves of the Indian A. ilicifolius in 1984. This is the first time that 2(3H)-benzoxazolone was reported from the genus Acanthus. Later, it was obtained again from the seeds of A. mollis and from the whole plant of the Thailand, Indian, and Chinese A. ilicifolius. A dimeric oxazolinone, 5, 5'-bis-benzoxazoline-2, 2'- dione was reported by D'Souza and his coworkers from the leaves of the Indian A.ilicifolius. A benzoxazolinone glucoside has been recently reported from the Chinese A. ilicifolius [20-26]. Figure 2.11 presents the above structures.

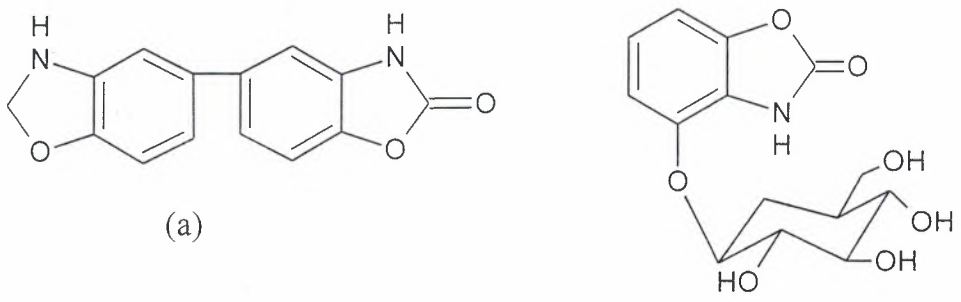
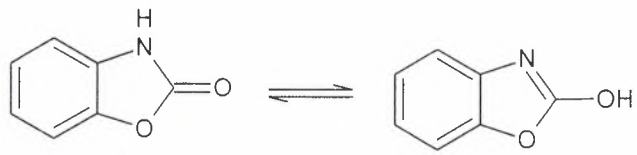


Figure 2.11. Structure of (a) 5, 5'-bis-benzoxazoline-2, 2'- dione and (b) benzoxazolinone glucoside

2.2.2. Chemical Reactivity of 2(3H)-Benzoxazolone

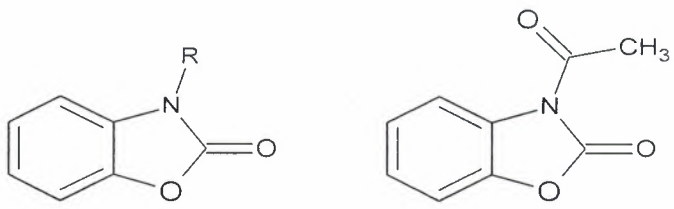
The reactivity of 2(3H)-benzoxazolone permits to define three major types of reactions: N-substitution (either alkylation or acylation), aromatic ring electrophilic substitution and ring opening or expansion reactions [27]. The enolizable (figure 2.12) character of the amide moiety allows for several useful transformations at the third position (N-atom) of the heterocycle.



Keto form Enol form

Figure 2.12. Tautomers of 2(3H)-benzoxazolone

N-alkylation of 2(3H)-benzoxazolone proceeds under base-catalyzed conditions to give N-alkyl derivatives, while N-acylation is submitted to generalized acid-base catalysis to give N-acyl derivatives [28] as shown in figure 2.13.



(a) (b)

Figure 2.13. (a) N-alkyl and (b) N-acyl derivatives of 2(3H)-benzoxazolone

Mannich reaction with 2(3H)-benzoxazolone provides ready access to N-aminomethyl derivatives [29] as shown in figure 2.14.

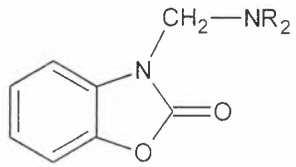


Figure 2.14. N-aminomethyl derivative of 2(3H)-benzoxazolone

Base catalyzed Michael addition of acrylonitrile leads to N-cyanoethyl derivative [30] as shown in figure 2.15.

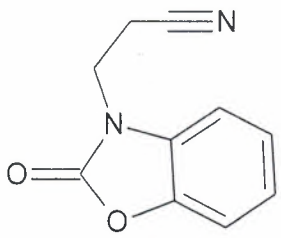


Figure 2.15. N-cyanoethyl derivative of 2(3H)-benzoxazolone

Reaction of 2(3H)-benzoxazolone with hydroxaminosulfuric acid is another example of N-substitution which gives the cyclic hydrazide structure [31] as shown in figure 2.16.

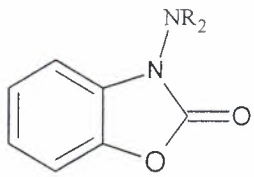


Figure 2.16. Cyclic hydrazide derivative of 2(3H)-benzoxazolone

Aromatic electrophilic substitution is governed by the overwhelming preference for the 6-position which is observed not only for the straightforward halogenation, nitration, sulfonation, and chlorosulfonation reactions, but also for the more troublesome Friedel-Crafts acylation [32] shown in figure 2.17.

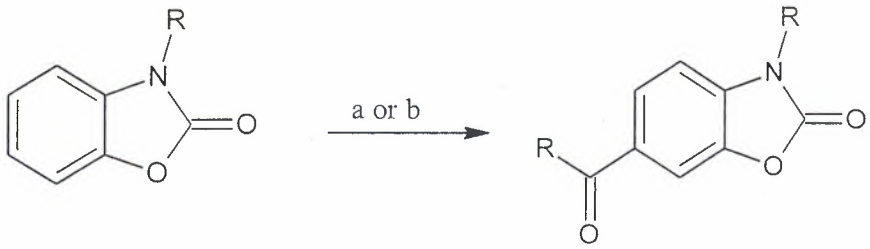


Figure 2.17. General synthesis of 6-acyl-2(3H)-benzoxazolone derivatives.
Methods: (a) RCOOH, PPA, (b) RCOCl, $AlCl_3$, DMF

Indeed, in the particular case of the Friedel-Crafts reaction, due to the electron-rich character of 2(3H)-benzoxazolone, the heterocycle is extensively protonated by the Lewis acid present in the reaction medium, which acts as an absolutely mandatory catalyst. As a paradoxical consequence, while 2(3H)-benzoxazolone behaves as a strongly activated substrate in normal electrophilic substitution conditions (such as bromination, for example), the extensive protonation encountered in the Friedel-Crafts reaction strongly deactivates this type of substrate towards the electrophilic attack of acylium ions. To overcome this problem, the reaction can be run using either a less reactive electrophilic species (polyphosphoric acid, PPA, for example) or preferably the $AlCl_3$, DMF complex, to give 6-acyl derivatives [33].

As a most fruitful alternative, N-acyl derivatives can be rearranged at high temperature (160°C) in a Fries-like reaction promoted by $AlCl_3$, to 6-acyl derivatives as in figure 2.18.

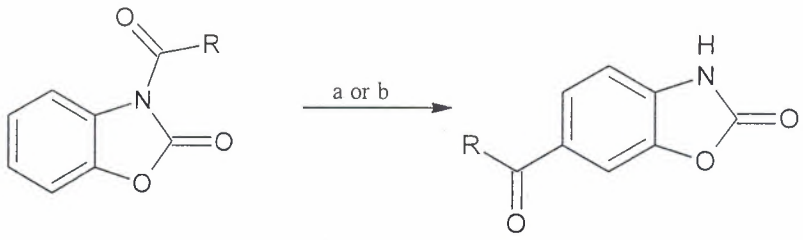


Figure 2.18. General synthesis of 6-acyl-2(3H)-benzoxazolone derivatives. Methods: (a) RCOOH, PPA, (b) RCOCl, $AlCl_3$, DMF

While 2(3H)-benzoxazolone derivatives are fairly stable in acid medium, they are quickly hydrolyzed in basic medium, leading to ring opening products such as 2-aminophenols (route a). These 2-aminophenols can be acylated at 4th position (route b), subsequent ring closure leads to the otherwise inaccessible 5-acyl-2(3H)-benzoxazolone derivatives (route c). Ring expansion of 2(3H)-benzoxazolone derivatives to benzoxazinones can be effected via the same 2-aminophenols (d) [33, 34]. General synthesis of (3H)-benzoxazolone derivatives is given in Figure 2.19.

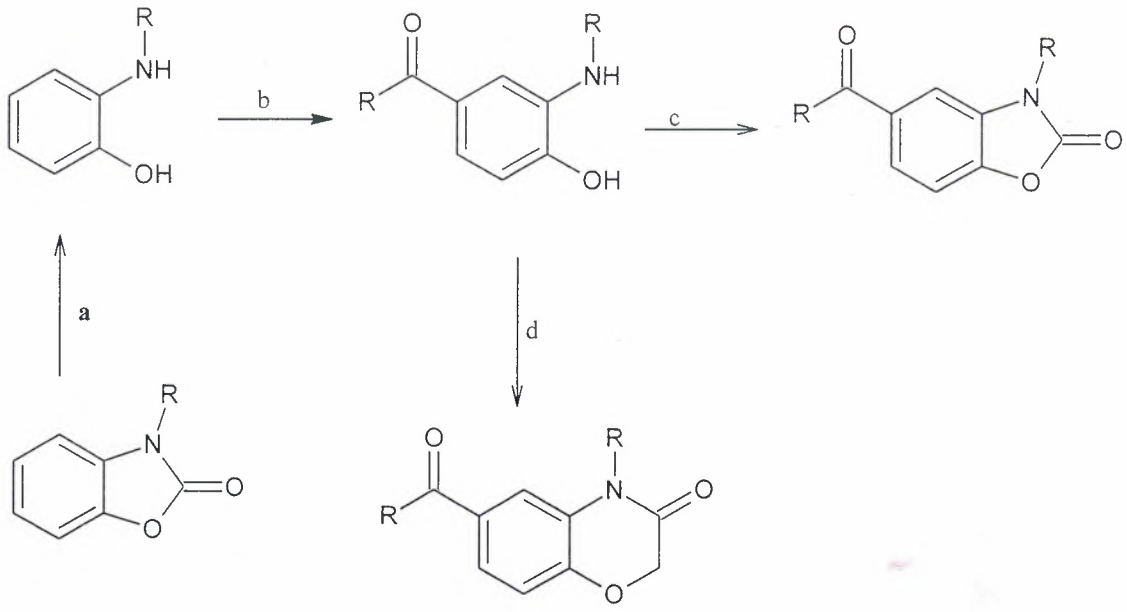


Figure 2.19. General Synthesis of 5-acyl-2(3H)-benzoxazolone and benzoxazinone derivatives. Methods: (a) aq. NaOH, (b) RCOCl, $AlCl_3$.DMF; (c) ClCOOEt, TEA; (d) $BrCH_2COOEt$, TEA

2.2.1. 2(3H)-Benzoxazolone Drugs

2(3H)-Benzoxazolone and its bioisosters are considered 'privileged scaffolds'"(meaning: a substructure or template that when incorporated in a pharmacophore has a high degree of drug likeliness due to the presence of atom or functional group properties that are relevant in ligand binding) in the design of pharmacological probes. Simple derivatives of 2(3H)-benzoxazolone have been marketed for example: Chlorzoxazone (5-chloro-2(3H)-benzoxazolone: sedative analgesic). Studies have shown that the presence of an electronegative atom, Cl, in position 5 of 2-benzoxazolone compounds provided better

analgesic activity than un-substituted benzoxazolone derivatives [4, 35]. Structure of 5-chloro-2(3H)-benzoxazolone is shown in figure 2.20.

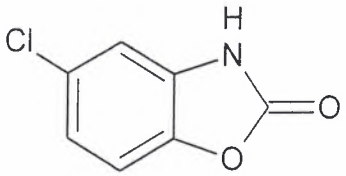
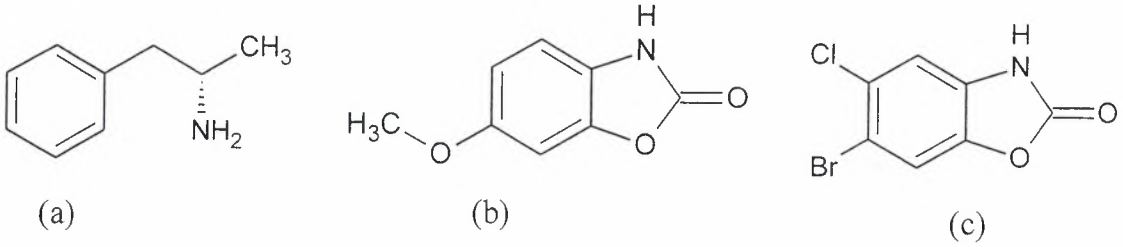


Figure 2.20. Structure of 5-chloro-2(3H)-benzoxazolone (chlorzoxazone)

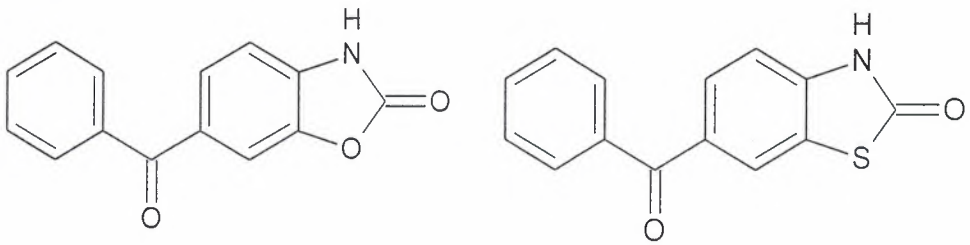
Benzolone (open derivative of 2(3H)-benzoxazolone; myorelaxant) [36], 6-Methoxy-2(3H)-benzoxazolone and 6-bromo-5-chloro-2(3H)-benzoxazolone (vinizene; topical antiseptic) derivatives (given in figure 2.21.) have also been reported with various biological activities such as anti-inflammatory and analgesic [37- 38].



(a) (b) (c)

Figure 2.21. Structure of (a) benzolone, (b) 6-methoxy-2(3H)-benzoxazolone and (c) 6-bromo-5-chloro-2(3H)-benzoxazolone

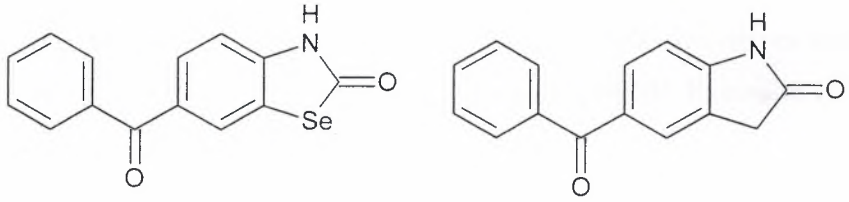
6-Benzoyl-2(3H)-benzoxazolone and its sulfur bioisoster [39] underwent clinical trials until phase II as analgesic as seen in figure 2.22.



(a) (b)

Figure 2.22. Structure of (a) 6-benzoyl-2(3H)-benzoxazolone and its (b) sulfur bioisoster

The 6-benzoylbenzoselenozolinone initially designed as glutathione peroxidase mimetic was found to be a potent inhibitor of the cyclooxygenase and lipoxygenase pathways in vitro and it has shown interesting anti-inflammatory properties in vivo. The 5-benzoylindolin-2-one, also showed anti-inflammatory properties [40] as shown in figure 2.23.



(a) (b)

Figure 2.23. Structure of (a) 6-benzoylbenzoselenozolinone and (b) 5-benzoylindolin-2-one

2.2.2. Biological Importance of the 2(3H)-benzoxazolones

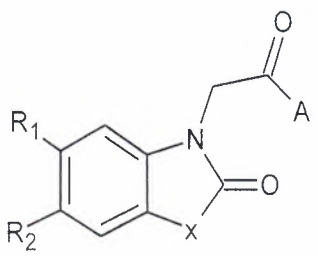
2(3H)-Benzoxazolone derivatives have been associated with various types of biological properties. Various 2(3H)-benzoxazolone derivatives had shown anticonvulsant,

antiinflamatory, analgesic, antipyretic, antimicrobial and anticholinergic activities [41-44]. The pronounced biological activity of many 2(3H)-benzoxazolone derivatives and the medicinal value of 5-chloro-2(3H)-benzoxazolone prompted the investigation of 3-substituted-2(3H)-benzoxazolones.

Lespagnol and coworkers prepared and tested a number of derivatives of 2(3H)-benzoxazolones for their anticonvulsive, hypnotic, antipyretic and analgesic properties [45].

2(3H)-Benzoxazolone derivatives have emerged during the last two decades as potential analgesic and anti-inflammatory agents. Close and co-workers studied the analgesic activities of 2(3H)-benzoxazolones, they have been structurally modified at the positions 3, 5 and 6 in order to screen for their antinociceptive properties [46]. (6-Acyl-2(3H)-benzoxazolone-3-yl) alkanoic acid and ethyl ester derivatives also exhibited analgesic activity [47, 48].

Various (2(3H)-benzoxazolon-3-yl) and (2(3H)-benzothiazolon-3-yl) acetic acid derivatives and (2(3H)-benzoxazolon-3-yl) procainamide and propanoic acid derivatives were reported as potential antinociceptive and anti-inflammatory agents [49-50]. Examples are shown in figure 2.24, 2.25 and 2.26 respectively.

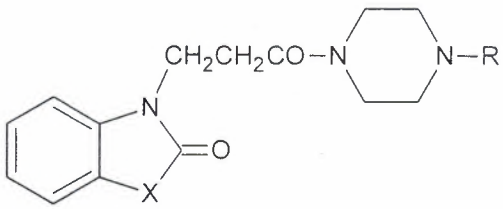


X= O, S; R_1=H, Cl

R_2 = H, CH_3, C_6H_5-CO, 2-ClC_6H_4-CO,

A= 1-Morpholinyl, 1-pyrrolidinyl, diethylamino, OCH_3 , OC_2H_5OH

Figure 2.24. Examples of (2(3H)-benzoxazolon-3-yl) procainamide derivatives



X= O, S;

R=H, CH_3, C_6H_5, 2-OCH_3-C_6H_4, 2Cl-C_6H_4,

4F-C_6H_4, CH_2, C_6H_4, 2-Pyridinyl.

Figure 2.25. Examples of (2(3H)-benzothiazolon-3-yl) acetic acid derivatives

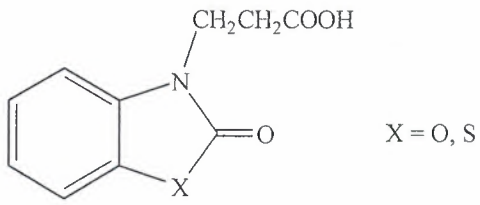
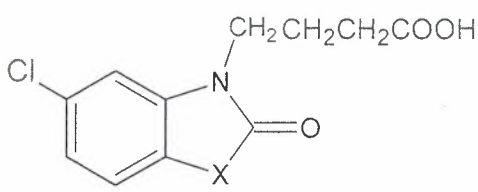
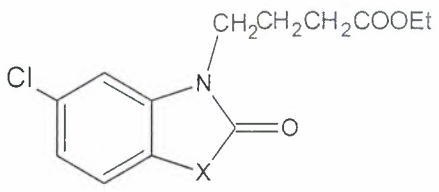


Figure 2.26. Examples of (2(3H)-benzoxazolon-3-yl)propanoic acid derivatives

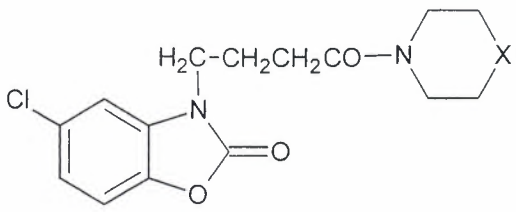
Gülcan and co-workers synthesized 4-(5-chloro-2(3H)-benzoxazolone-3-yl) butanoic acid, its ethyl ester and amide derivatives and found carboxylic acid derivative as most potent antinociceptive and anti-inflammatory agent [51]. Figure 2.27 gives examples of compounds synthesized in this study.



(a)



(b)



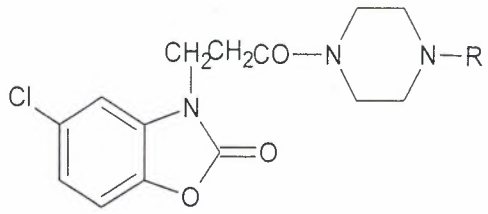
(c)

X= O, CH_2, N-C_6H_5, N-2-OCH_3-C_6H_4 , N-4-F-C_6H_4,N-$_2$-F-C_6H_4, N-CH_2-C_6H_5 , $CHCH_2$-C_6H_5, N-2-Pyridyl

Figure 2.27. Examples of (a) 4-(5-chloro-2(3H)-benzoxazolone-3-yl) butanoic acid, (b) ethyl

ester and (c) amide derivatives

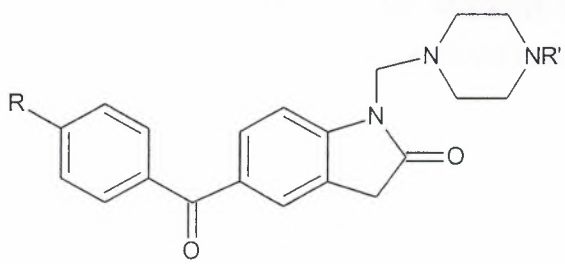
Later Onkol and co-workers synthesized (5-chloro-2(3H)-benzoxazolon-3-yl) propanamide derivatives as potent antinociceptive agents [52]. Examples of synthesized compounds are shown in figure 2.28.



R= C_6H_5, 2-OCH_3-C_6H_4, 3-Cl-C_6H_4, 4-Cl-C_6H_4,
3-CF_3-C_6H_4, 2-F-C_6H_4, 4-F-$C_6H_4CH_2$-C_6H_5, 2-Pyridyl

Figure 2.28. Examples of (5-chloro-2(3H)-benzoxazolon-3-yl) propanamide derivatives

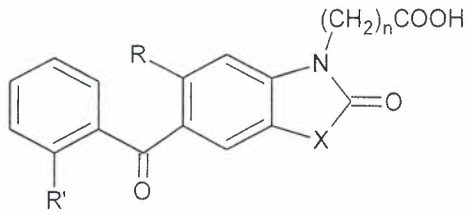
Gökhan et al. synthesized various 6-acyl-3-piperazinomethyl-2-benzoxazolinone derivatives and screened for analgesic and anti-inflammatory activity [53]. Some compounds shown higher analgesic activity than that of aspirin and some compounds have shown equal or higher anti-inflammatory activity than that of indomethacin. The structure of 6-acyl-3-piperazinomethyl-2-benzoxazolinone derivatives is shown in figure 2.29.



R=Br, Cl, OCH_3; R'= 2-NO_2-C_6H_4,
4-CH_3CO-C_6H_4, 2-Pyridyl

Figure 2.29. Examples of 6-acyl-3-piperazinomethyl-2-benzoxazolinone derivatives

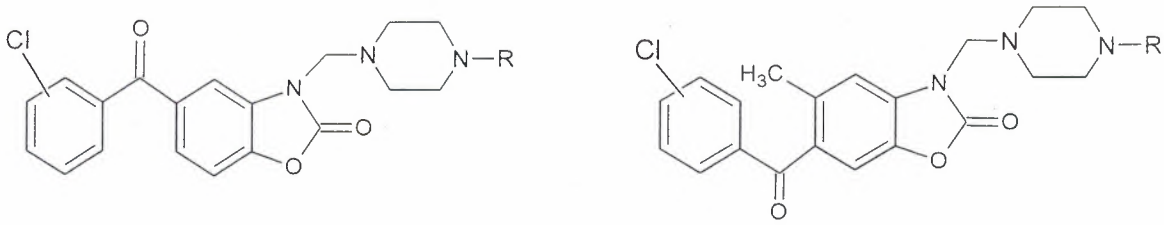
Ünlü *et al.* synthesized 6-acyl-2-benzoxazolinone and 6-acyl-2-benzothiazolinone derivatives with acetic acid and propanoic acid side chain, and screened for analgesic and anti-inflammatory activities [54] synthesized compounds are seen in figure 2.30.



X=O, S; R=H, Cl; R'=H, Cl, F; n=1, 2

Figure 2.30. 6-Acyl-2-benzoxazolinone and 6-acyl-2-benzothiazolinone derivatives

Köksal *et al.* synthesized 5-acyl-3-(4-substituted-1-piperazinylmethyl)-2-benzoxazolinone and 6-acyl-3-(4-substituted-1-piperazinylmethyl)-2-benzoxazolinone derivatives and screened for analgesic and anti-inflammatory activities [55]. 6-Acyl derivatives were found more active than 5-acyl derivatives in terms of the analgesic and anti-inflammatory activities. Structures of 6-acyl and 5-acyl derivatives are shown in figure 2.31.



2-Cl or 4-Cl;
R= C_6H_5, 2-OCH_3-C_6H_4, 3-Cl-C_6H_4,
4-Cl-C_6H_4,3-CF_3-C_6H_4, 2-F-C_6H_4,
4-F-C_6H_4, 4-$CH_3COC_6H_4$, 2-Pyridyl

(a)

2-Cl or 4-Cl;
R= C_6H_5, 2-OCH_3-C_6H_4, 3-Cl-C_6H_4,
4-Cl-C_6H_4,3-CF_3-C_6H_4, 2-F-C_6H_4,
4-F-C_6H_4, 4-$CH_3COC_6H_4$, 2-Pyridyl

(b)

Figure 2.31. 5-acyl and 6-acyl-3-(4-substituted-1-piperazinylmethyl)-2-benzoxazolinone compounds shown to have analgesic and antiinflamatory properties

Recently various 5-methyl-3-benzoylmethyl-2(3H)-benzoxazolone, 5-methyl-3-(2-hydroxyl-2-phenylethyl)-2(3H)-benzoxazolone and (6-difluorobenzoyl)-3-benzoylmethyl-2(3H)-benzoxazolone were prepared and tested for analgesic and anti-inflammatory activities [56]. Among the synthesized compounds, 6-acyl series compounds found most promising for analgesic activity while reduced compounds i.e. displayed considerable anti-inflammatory activity compared to the other derivatives. Synthesized compounds are shown in figure 2.32.

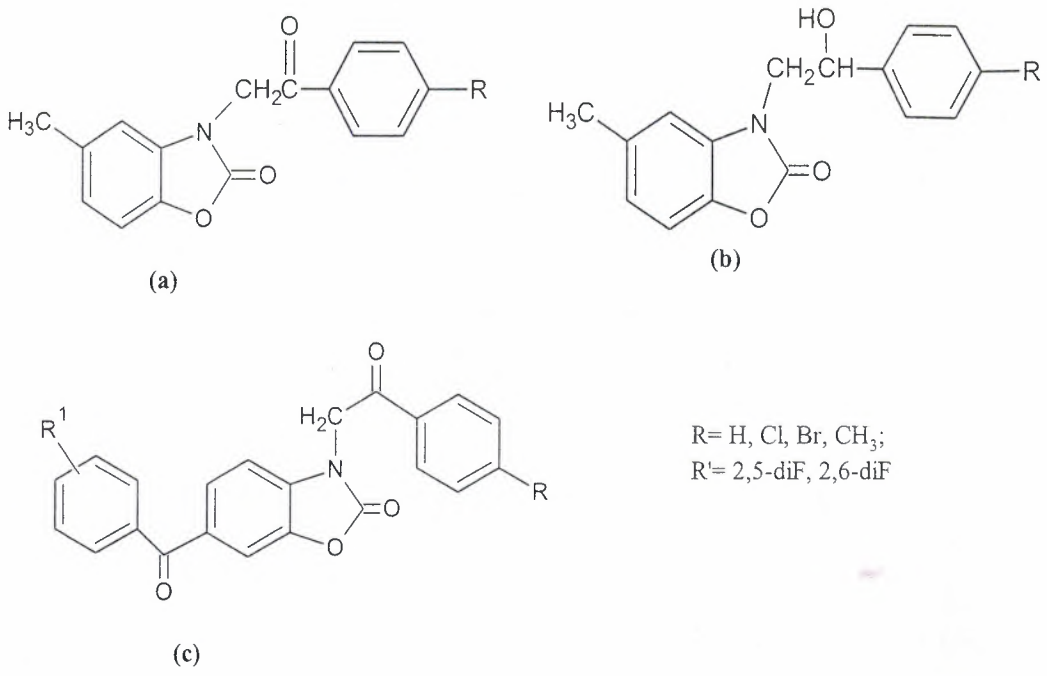
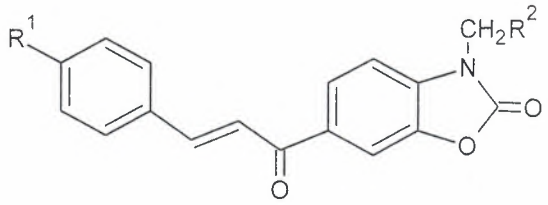


Figure 2.32. 5-methyl-3-benzoylmethyl (a), 5-methyl-3-(2-hydroxyl-2-phenylethyl) (b), and (6-difluorobenzoyl)-3-benzoylmethyl-2(3H)-benzoxazolone (c) derivatives shown to have analgesic and antiinflamatory activities

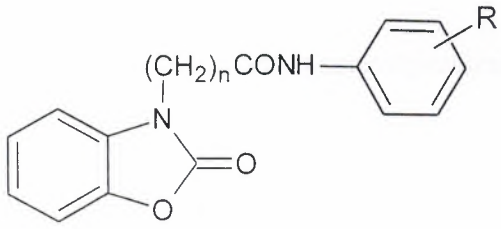
Ivanova *et al.* had synthesized a series of new Mannich bases with chalcone core structure as potential antineoplastic agents [57] examples of synthesized compounds are shown in figure 2.33.



R^1= H, OCH_3
R^2=piperidine,morpholine,thiomorpholine,
$N(CH_3)_2$, N-benzylpiperizine,
N-2-methoxyphenyl

Figure 2.33. Mannich base with chalcone core structure.

Soyer and co-workers had synthesized various ω-[2-oxo-3H-benzoxazol-3-yl]-N-phenylacetamide and propionamide derivatives (shown in figure 2.34) with potential in vitro leukocyte myeloperoxidase (MPO) chlorinating activity [58].



n= 1, 2; R= H, 2-CH_3, 3-CH_3, 4-CH_3,
2-Cl, 3-Cl, 4-Cl, 2-NO_2, 3-NO_2, 4-NO_2

Figure 2.34. ω-[2-oxo-3H-benzoxazol-3-yl]-N-phenylacetamide and propionamide derivatives

Recently Yekini and co-workers had studied antioxidant activity of benzoxazolinonic (a) and benzothiazolinonic (b) derivatives in the LDL (Lithium Di-isopropyl Amide) oxidation model [59]. Benzothiazolones had shown higher antioxidant activity than benzoxazolones. Figure 2.35 gives examples of benzoxazolonic and benzithiaxolonic derivatives with antioxidant properties.

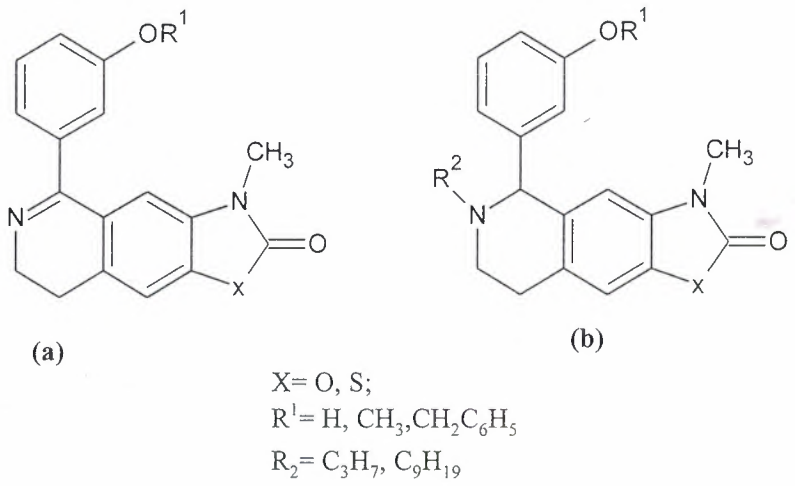


Figure 2.35. (a) Benzoxazolinonic and (b) benzothiazolinonic derivatives

Deng *et al.* synthesized a series of novel alkenyldiarylmethanes (ADAMs) containing benzoxazolone ring and tested for anti- HIV activity [60]. The incorporation of benzoxazolone rings into the alkenyldiarylmethane (ADAMs) system generated several active compounds, of which most potent compound (R^1= CH_3; R^2= OCH_3; R^3=$COOCH_3$) exhibited potencies near those of the standard nevirapine and efavirenz which are commonly used as anti HIV agents. Structures of synthesized alkendiarylmethanes are seen in figure 2.36.

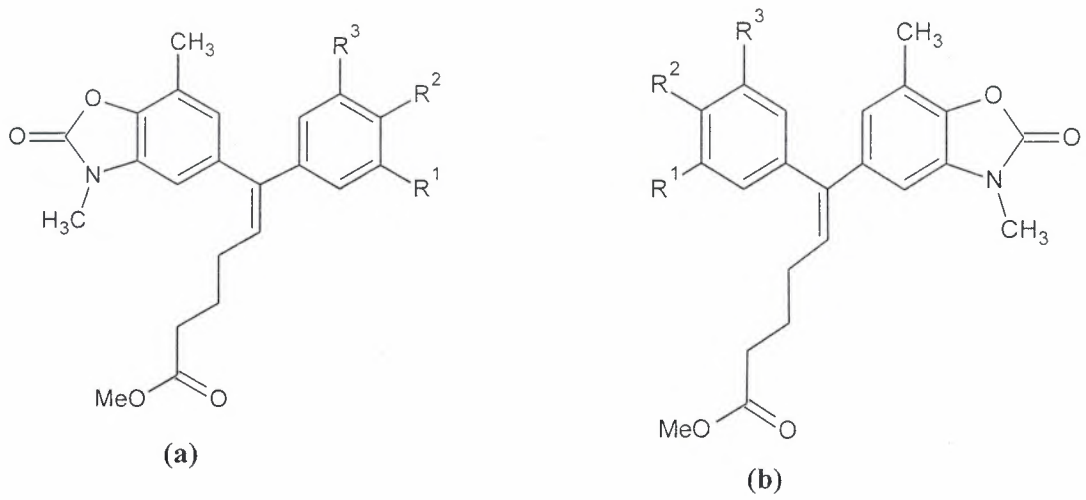
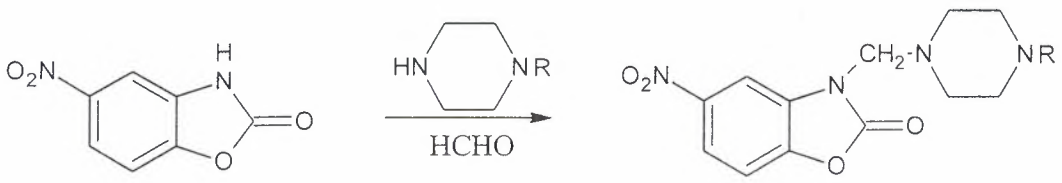


Figure 2.36. Alkenyldiaryl methanes (ADAMs) containing benzoxazolone ring

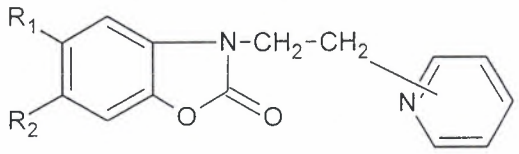
Köksal *et al* in 2005 synthesized a novel series of mannich bases of 5-nitro-N-substituted piperazinomethyl-2-benzoxazolones and tested them for analgesic and antiinflamatory activities [61]. In their studies, most promising results were obtained from compounds bearing electron withdrawing substituents in the ortho/para position of the phenyl nucleus in the piperazine ring at 3 position of the benzoxaxolone moiety. Among the synthesized compounds, 4-flourophenyl, 2-flourophenyl, 4-chlorophenyl, 2-chlorophenyl and 3-methoxyphenyl substituents possessed the most prominent and consistent activity (Figure 2.37).



R= 4-Flourophenyl, 2-Flourophenyl, 4-Chlorophenyl, 2-Fhlorophenyl and 3-Methoxyphenyl

Figure 2.37. Synthesis of 5-nitro-3-piperazinomethyl-2-benzoxazolones

Şafak *et al.* synthesized 3-(2-pyridylethyl)benzoxazolone (figure 2.38) derivatives as potent analgesic and antiinflamatory compounds inhibiting prostaglandins E_2. All the synthesized compounds showed higher antiinflamatory activities compared to indomethacin. Those without substituents on the 6th position of the ring were significantly more active than the rest of the members [62].

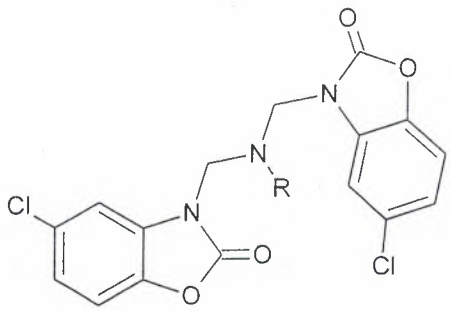


R_1 = -H, -Cl,

R_2 = -C_6H_4Cl (o), -$C_6H_4OCH_3$ (p)

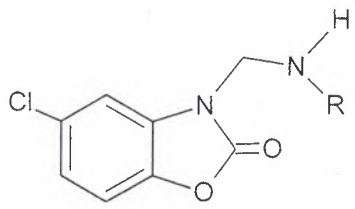
Figure 2.38. Structure of 3-[2-(2and 4-pyridylethyl)] benzoxazolones

Soyer et al synthesized N-substituted-5-chloro-2(3H)-benzoxaxolone derivatives [63] and tested their acetyl cholinesterase inhibitory activity as seen in figure 2.39.

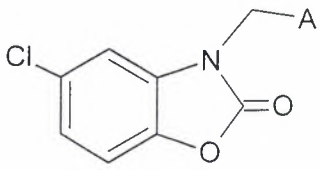


R= Ethyl, Phenethyl, P-chlorophenethyl,

3, 4-dimethoxyphenethyl



R= Phenyl, 1-Napthy, Quinoline-8-yl



A= Pyrolidine, Piperidine, Methylpiperazine

Figure 2.39. Structure of N-substituted-5-chloro-2(3H)-benzoxaxolone derivatives

In the above study, bis-5-chloro-2(3H)-benzoxaxolone (figure 2.40) exhibited higher activity and was the most active compound [63].

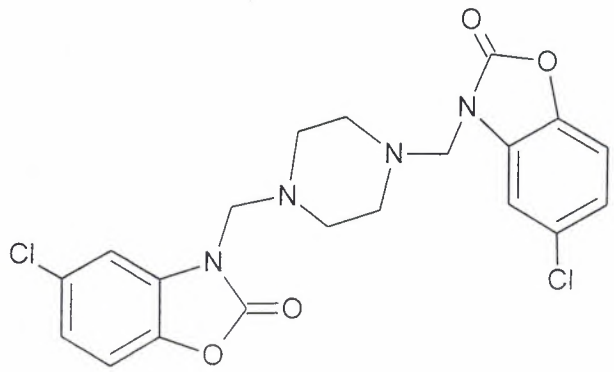


Figure 2.40. Structure of bis-5-chloro-2(3H)-benzoxaxolone

2.3. Mannich Reaction

Mannich reaction is one of the most fundamental and important, C–C bond forming reactions in organic synthesis [64]. Mannich reaction is a nucleophilic addition reaction which involves the condensation of a compound with active hydrogen(s) with an amine (primary or secondary) and formaldehyde (any aldehyde) [65, 66].

Mannich reaction withstands a large diversity of functional groups and hence it has been witnessing a continuous growth in the field of organic chemistry. The surge of literature on Mannich reaction provides an outstanding evidence for the diversity and applications of the reaction [67, 68].

The schematic representation of general Mannich reaction is given in figure 2.41.

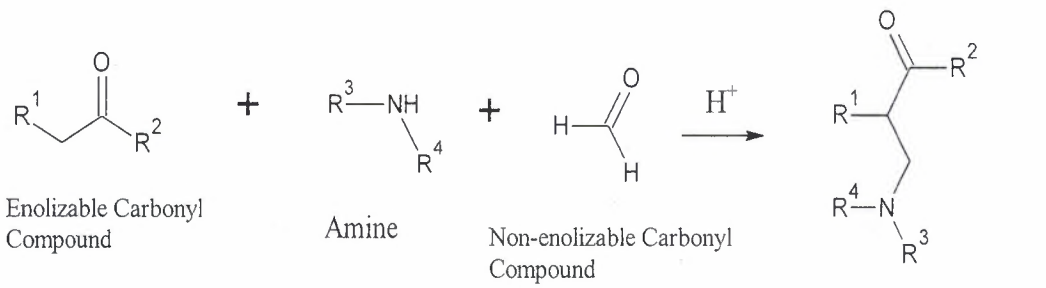
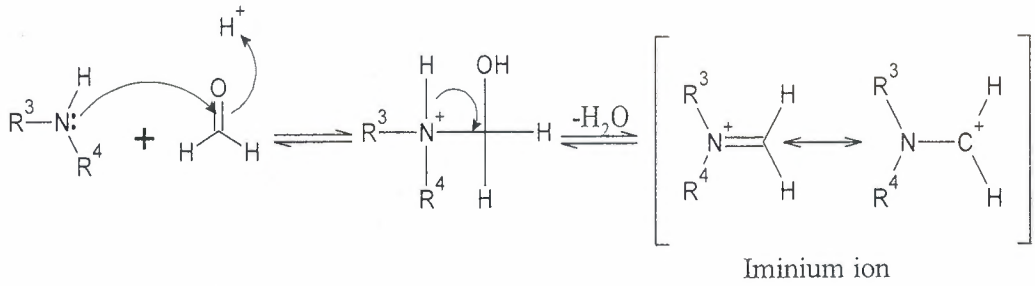


Figure 2.41. General scheme of Mannich reaction

2.3.1. Mechanism of Mannich Reaction



Since the reaction is carried out in acidic conditions, the enolizable carbonyl compound is converted to enol form, which attacks the iminium ion at positively charged carbon adjacent to nitrogen to give finally a ß-aminocarbonyl compound.

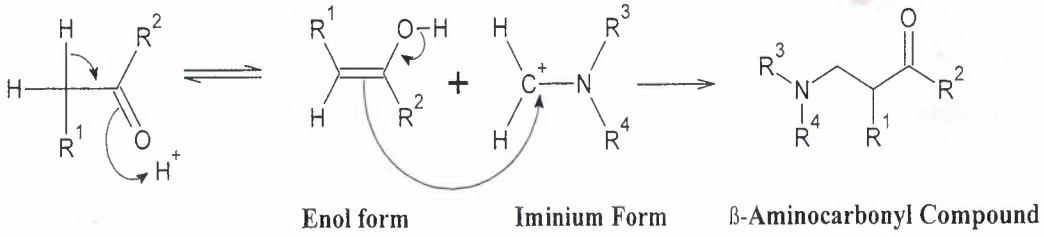


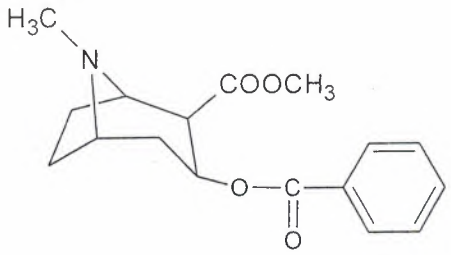
Figure 2.42. Mechanism of Mannich reaction

2.3.2. Mannich Base

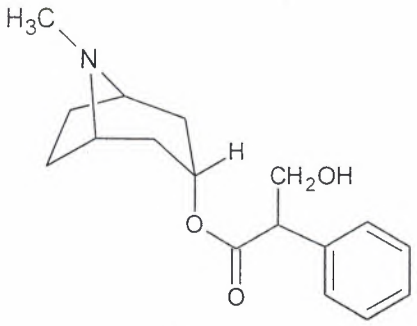
Mannich bases (beta-amino ketones carrying compounds) are the end products of mannich reaction. Mannich bases also act as important pharmacophores or bioactive leads which are further used for synthesis of various potential agents of high medicinal value which possess aminoalkyl chain. The examples of clinically useful Mannich bases which consist of aminoalkyl chain are cocaine (stimulant), fluoxetine (antidepressant), atropine (scompetitive antagonist of the muscarinic receptor), ethacrynic acid (loop diuretic), procyclidine

(anticholinergic drug), Ranitidine (histamine H_2- receptor antagonist), biperiden (antiparkinsonian agent) [69, 70].

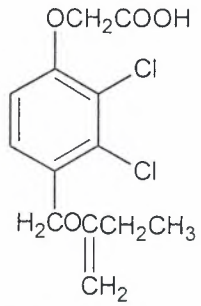
Mannich bases are known to play a vital role in the development of synthetic pharmaceutical chemistry. The literature studies revealed that Mannich bases are very reactive and can be easily converted to other compounds, for example, reduced to form physiologically active amino alcohols [71]. Along with biological activities Mannich bases are also known for their uses in detergent additives, resins, polymers, surface active agents [71]. Mannich bases (optically pure chiral) of 2-naphthol are employed for catalysis (ligand accelerated and metal mediated) of the enantioselective carbon-carbon bond formation. Mannich bases and their derivatives are intermediates for the synthesis of bioactive molecules. Mannich reaction is widely used for the construction of nitrogen containing compounds. Mannich bases have gained importance due to their application in antibacterial activity and other applications are in agrochemicals such as plant growth regulators [72]. Figure 2.43 gives examples of clinicaly useful mannich bases.



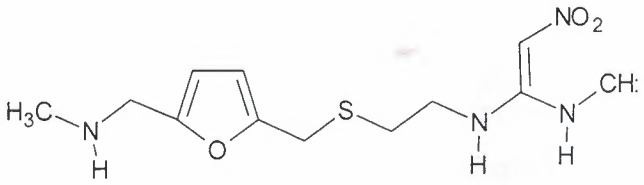
Cocaine (benzoylmethylecgonine)



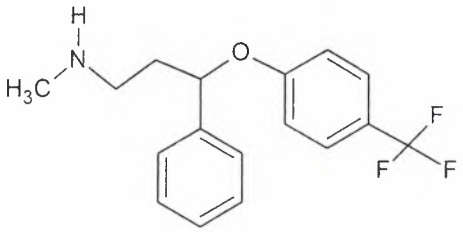
Atropine



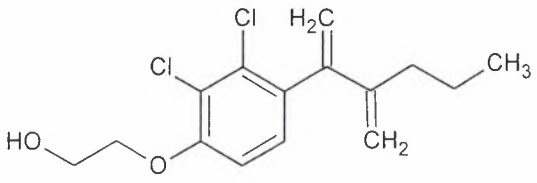
Ethacrynic acid



Ranitidine



Fluoxetine



Ethacrynic acid

Figure 2.43. Examples of clinically useful Mannich bases

3. MATERIALS AND METHOD

3.1. Materials

The starting materials, 5-chloro-2 (3H)-benzoxazolone, 1- (4-fluorophenyl) piperazine, phenyl piperazine, methanol and 35% formaldehyde, used in this study were purchased from Sigma Aldrich Chemical Company and were used without further purification.

3.2. Method

The compounds were prepared by stirring a solution of calculated amounts of 0.002 mol of 5-chloro-2-benzoxazolinone and 0.002 mol of phenyl piperazine in 10ml of methanol followed by addition of 0.002 mol formaldehyde (35%w/v) to the mixture then refluxed in a water bath for 1 hour. The reaction mixture was then poured onto crushed ice and the resulting precipitate was filtered off, washed with methanol, dried and purified by crystallization using ethanol as solvent.

3.3. Melting Point Determination

The melting point was detected with a Mettler Toledo (FP8 1HT MBC cell) melting point apparatus.

3.4. Thin Layer Chromatography

3.4.1. Materials

The plate was made of silica gel and the solvents used were, benzene, ethyl acetate, hexane and methanol with ratios as follows:

C-1: Benzene – Methanol (5:1)
C-2: Benzene – Methanol (9:1)
C-3: Ethylacetate – Hexane (1:5)

3.4.2. Method

The solvent was poured into the chamber to a depth of just less than 0.5 cm, swirled gently, and allowed to stand while the TLC plate prepared.

TLC plates were cut horizontally into plates of about 5 cm tall by various widths and a line drawn across the plat at 0.5 cm from the bottom of the plate using a pencil.

The starting materials and product were dissolved in methanol and chloroform respectively and with the aid of a micro capillary spots were made on the TLC plate and the prepared plate gently placed in the chamber. The plate was allowed to develop until the solvent front was about half a centimeter below the top of the plate and the plate was removed, and solvent front marked with a pencil and allowed to dry. The spots were viewed under UV light at 254 nm and R_f values calculated.

3.5. Spectroscopy

UV-VIS Spectra: The UV-VIS spectra of the compounds with 0.5 x 10^{-4} M concentrations were recorded in a 1 cm quartz cuvette, using a PG Instrument T70 UV / VIS spectrophotometer.

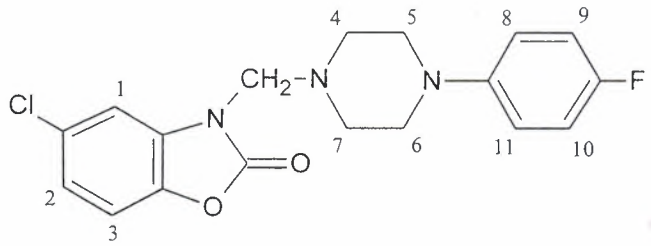
FT-IR Spectra: The FT-IR spectra of the compounds were recorded on a Perkin Elmer Spectrum 100 spectrophotometer with attenuated total reflection (ATR) (in wave numbers) in cm^{-1} at Marmara University Science and Arts Faculty, Department of Chemistry.

^{1}H-NMR Spectra: The ^{1}H-NMR spectra of the compounds were recorded on a Mercury Varian 400MHz NMR Spectrometer using deuterated chloroform ($CDCl_3$) as solvent at Boğaziçi University, Research and Development Laboratories. Chemical shifts were reported in parts per million (ppm).

4. RESULTS AND DISCUSSION

4.1. Results

5-chloro-3[4-(4-florophenyl)piperazino]methyl-2-benzoxazolone (Compound 1)



The above compound was synthesized using the method described in experimental section. 340.30 mg (0.002 mol) of 5-Chloro-2(3H)-benzoxazolinone, and 380.00 mg (0.002 mol) of 1,4-florophenyl piperazine were dissolved in 10 mL of methanol and 0.2 mL (0.002 mol) of 35% formaldehyde was added to the mixture then refluxed in a water bath for one hour.

A white crystalline compound was obtained: Yield: 53.3.7% (385.70 mg); mp 167.2°C.

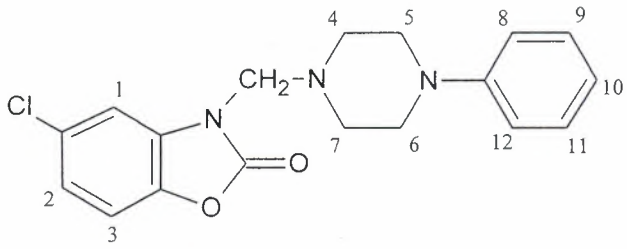
TLC in the C-1 and C-2 solvent system gave R_f values of 0.56 and 0.81 respectively.

Ultraviolet-Visible Absorption Spectroscopy: λ_{max} (acetonitrile) =UV revealed absorptions at 282 nm (log ε = 2.18), 234 nm (log ε = 2.86), and 210 nm (log ε = 1.48) nm.

Fourier Transforms Infra-Red (FT-IR) Spectroscopy ($IR\nu_{max}$)**:** FT-IR showed stretches at 2809-3020 cm^{-1} (C-H) and 1765 cm^{-1}(C=O of carbonyl).

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR in $CDCl_3$): ^{1}H NMR showed peaks at 7.3-6.9 (7H; m; Arom-H); 4.7 (2H; s; CH_2); 3.1 (4H; t; pip H^4-H^7); 2.9-2.8 (4H; t; pip H^5-H^6) ppm.

5-chloro-3[4-(phenyl)piperazino]methyl-2- benzoxazolone (Compound 2)



The above compound was synthesized using the method described in experimental section, 340 mg (0.002 mol) of 5-Chloro-2(3H)-benzoxazolinone and 0.32 mL (0.002 mol) phenylpiperazine were dissolved in 10mL of methanol then 0.2 mL (0.002 mol) of 35% formaldehyde was added and refluxed in a water bath for an hour.

A white crystalline compound was obtained: Yield: 52.7% (362.36 mg); mp 164.7°C.

TLC in the C-2 and C-3 solvent system gave R_f values of 0.73 and 0.54 respectively.

Ultraviolet-Visible Absorption Spectroscopy: λ_{max} (acetonitrile) =UV revealed absorptions at 282 nm (log ε = 3.66), 236 nm (log ε = 4.44) and 214 nm (log ε = 2.64).

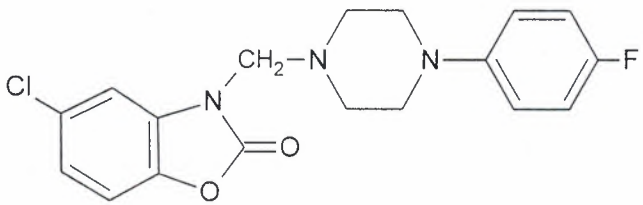
Fourier Transforms Infra-Red (FT-IR) Spectroscopy (IR ν_{max})**:** FT-IR showed stretches at 2826cm^{-1} (C-H Stretch), 1785cm^{-1}(C=O of carbonyl).

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR in $CDCl_3$); ^{1}H NMR showed resonances at 7.3-6.8 (7H; m; Arom-H); 4.7 (2H; s; CH2); 3.2(4H; t; pip H^4-H^7); 2.9 (4H; t; pip H^5-$H^{\backslash 6}$) ppm.

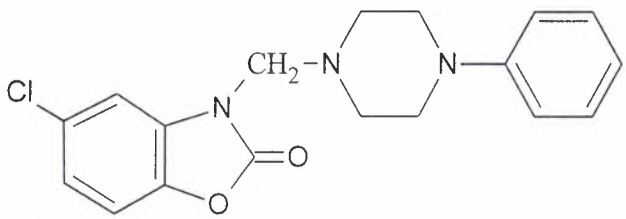
4.2. DISCUSSION

In this research, two compounds have been synthesized by following literature procedures based on 5-chloro-2(3H)-benzoxazolone structure. These compounds have been previously synthesized [39].

Piperazine derivatives were attached on position 3 of 5-chloro-2(3H)-benzoxazolone to give mannich bases. The structure of the synthesized mannich bases are shown in the figure below.



Compound 1.



Compound 2.

Figure 4.1. Structures of compounds 1 and 2

The core structures of these two compounds are the same. They only differ in the piperazine derivative on position 3 of the 5-chloro-2(3H)-benzoxazolone structure.

Compound 1 has 4-florophenyl piperazine on position 3 while compound 2 has phenylpiperazine on position 3. The figure below gives the general mannich reaction of the compounds synthesized in this study.

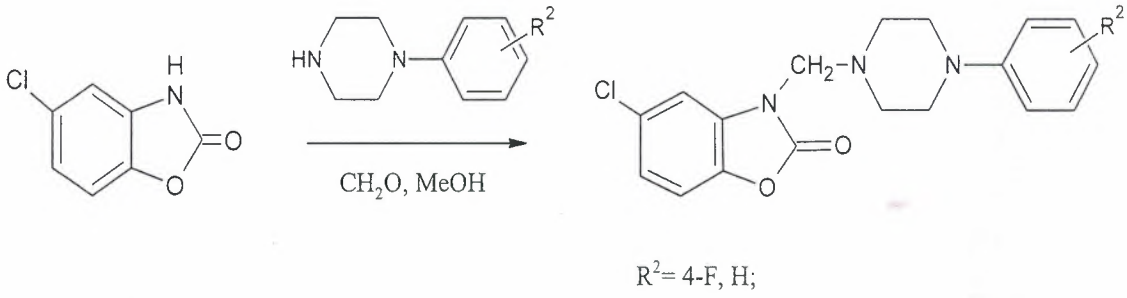


Figure.4.2. General synthesis of 5-Chloro-3-substituted benzoxazolone molecules

The synthesized compounds were characterized by: Fourier Transform Infra-Red (FT-IR), UV-visible spectrometer and Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR).

Thin layer chromatography and melting point were used to check the purity and also to cross reference to previously synthesized compounds.

The FT-IR- spectra of the compounds shows the absence of N-H stretch which is reported to come around 3146cm^{-1}, this indicates that the reaction have actually taken place at position 3 of 5-chloro-2(3H)-benzoxazolone.

As expected at 1765 and 1785cm^{-1} C=O stretch observed similar to reported values at literature. Also C-H stretches are observed around 3809-3020cm^{-1} as expected. The FT-IR spectra of the compounds are shown in figures 4.3 and 4.4 below.

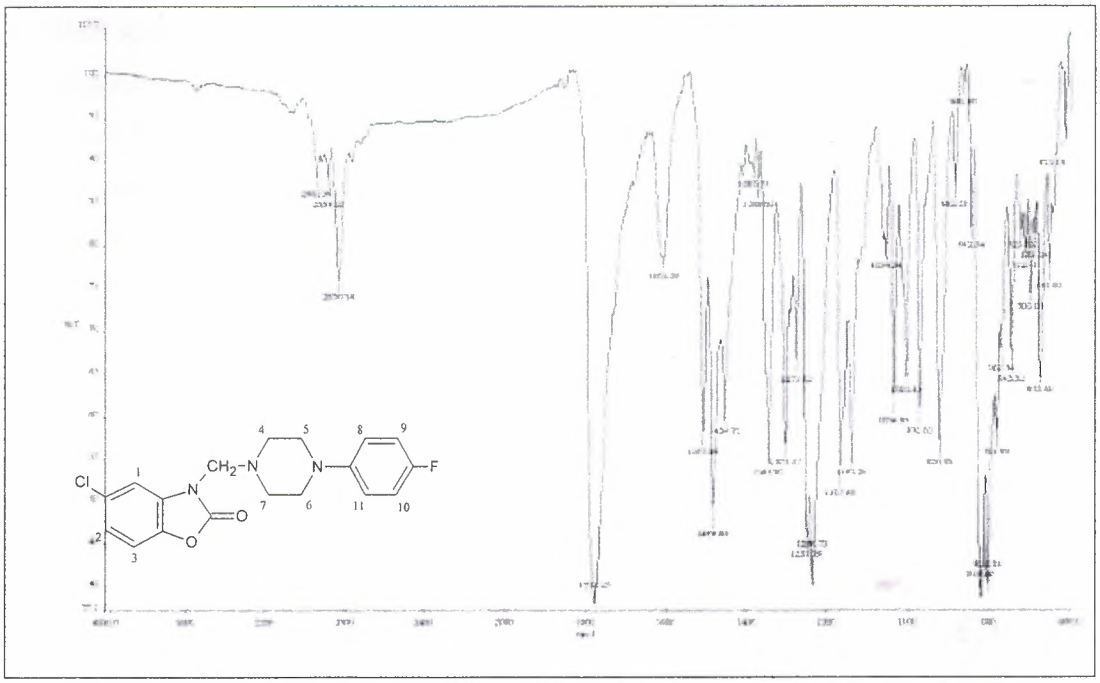


Figure 4.3. IR Spectrum of 5-chloro-3[4-(4-florophenyl)piperazino]methyl-2-benzoxazolone

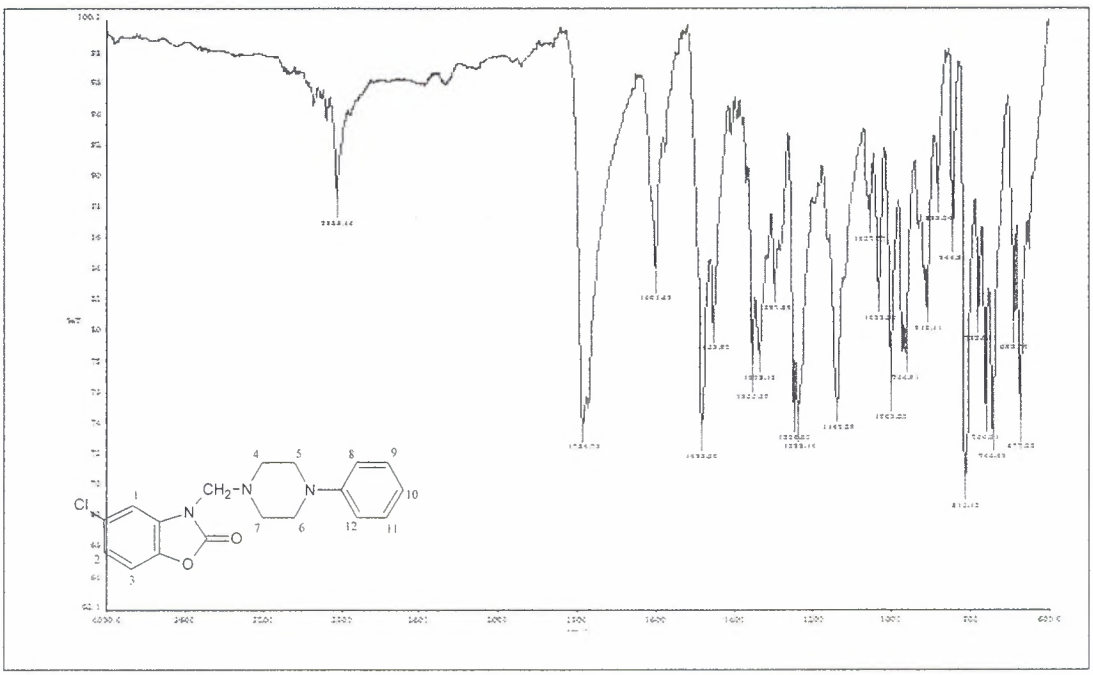


Figure 4.4. IR Spectrum of 5-chloro-3[4-(phenyl)piperazino]methyl-2-benzoxazolone

^{1}H-NMR spectra of compounds 1 and 2 in $CDCl_3$ shows peaks at expected chemical shifts. In both spectra, relative to the starting material (5-chloro2-3(H)-benzoxazolone), there is an additional CH_2 (methylene) peak as singlet observed 4.7 ppm for both compounds. This again proves that the reaction have taken place in position 3 and piperazine is bonded to 5-chlorobenzoxazolone via CH_2.

Further investigations reveal aromatic peaks as multiplets between 6.8 and 7.3 ppm which is similar to literature. The piperazine protons (H^4 and H^7) and (H^5 and H^6) were seen as triplets at 2.8 and 3.2 ppm respectively for both compounds.

The integral values of the peaks on ^{1}H-NMR spectra also matches to the number of protons proposed in structures of the compounds 1 and 2.

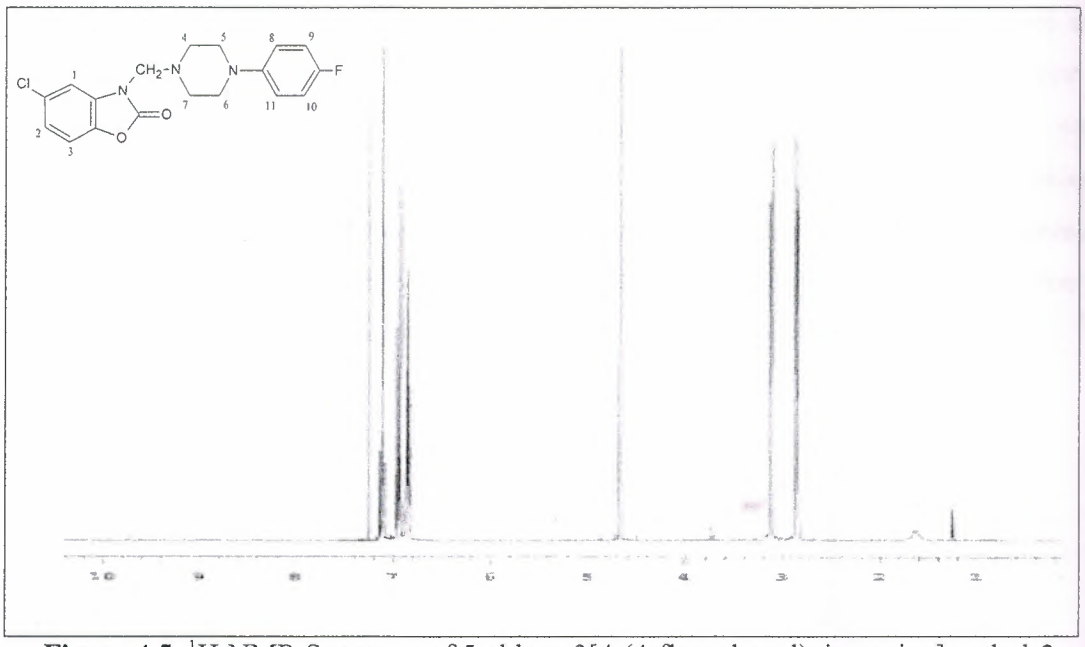


Figure 4.5. ^{1}H-NMR Spectrum of 5-chloro-3[4-(4-florophenyl)piperazino]methyl-2-benzoxazolone

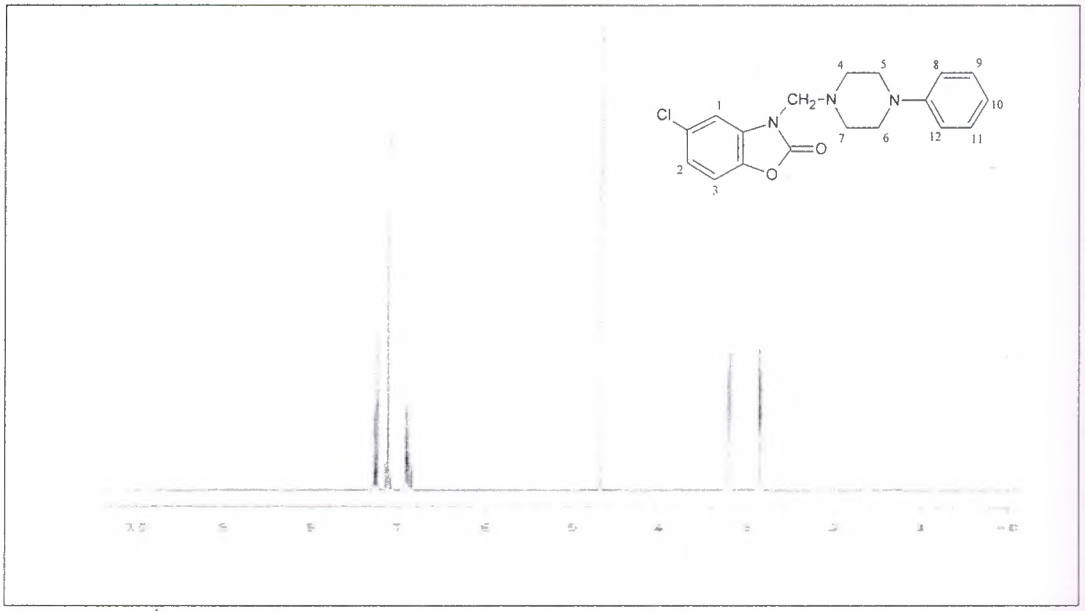


Figure 4.6. ^{1}H-NMR Spectrum of 5-chloro-3[(phenyl)piperazino]methyl-2-benzoxazolone

5. CONCLUSION

Since it is possible to do substitutions at different sites of benzoxazolone structure and furthermore obtain potentially biologically active molecules, it has long attracted interest in medicinal chemistry. Two 5-chloro-3-substituted benzoxazolone derivatives were synthesized in this study by using classical mannich reaction. Simple synthetic conditions and short reaction time required for this synthesis allows it to be favorable for synthesis of biologically active new molecules. Different amines can be substituted to 3-position in efforts to make drug candidates with less side effects and higher activities.

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