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MICROWAVE SYNTHESIS AND CHARACTERIZATION OF CERTAIN AMINE SUBSTITUTED 5-CHLORO-2(3H)-BENZOXAZOLONES

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Advisor

Assist. Prof. Dr. Banu KEŞANLI

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To my parents

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This thesis would not have been possible without the help, support and patience of my supervisor, my deepest appreciation goes to Assist. Prof. Dr. Banu KEŞANLI for her relentless encouragement and guidance. She has walked me through all the stages of writing of my thesis. Without her consistent and illuminating instruction, this thesis could not have reached its present form.

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ABSTRACT

2(3H)-Benzoxazolone derivatives are known to exhibit many different biological activities according to the literature. Three different Mannich bases of 5-chloro-2(3H)-benzoxazolone derivatives having a piperazine or piperidine group at the third position of the ring were synthesized in this study using a classic Mannich reaction. In this present work, we have developed a facile and efficient approach for the synthesis of these compounds under microwave condition. The reactions were also carried out by reflux so as to draw a comparison between these two different methods. Short reaction time, improved yield were observed under microwave condition thus less energy was required compared to that of reflux method. The reactions were monitored by TLC and melting point determination, whereas chemical structures of the compounds were elucidated using FT-IR and ^{1}H-NMR analysis.

Keywords: 5-chloro- 2(3H)-Benzoxazolone, Mannich reaction, Microwave Synthesis

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LIST OF ABBREVIATIONS

NSAIDS	Non-steroidal Anti-inflammatory Drugs
PPA	Polyphosphoric acid
DCM	Dichloromethane
DMF	Dimethylformamide
THF	Tetrahydrofuran
COX	Cyclooxygenase
FT-IR	Fourier Transform-Infrared
NMR	Nuclear Magnetic Resonance
UV-Vis	Ultraviolet-visible
TLC	Thin Layer Chromatography
MW	Microwave
MAOS	Microwave-Assisted Organic Synthesis

1. INTRODUCTION

Opioids (such as codeine and morphine) and non-steroidal anti-inflammatory drugs (NSAIDs) (such as aspirin, ibuprofen and paracetamol) are the most dominant prescribed analgesics widely used.[1] The opioids exert an action mainly on the central nervous system and not only hinder or block the incoming sensory nociceptive signals to the brain but also control the effective components of the pain at higher brain center. Consequently, this results in addiction, tolerance and dependence, hence restricting their clinical use.[1, 2] Meanwhile, NSAIDs act peripherally by inhibiting the formation of the enzyme cyclooxygenase (COX) in the initial pathway of the synthesis of prostaglandin at the site of injury. This could lead to gastrointestinal damage with gastric upset and irritation which is a major drawback.[1, 3-5] Long term administering of these drugs is not widely advisable. Therefore, there has been an interest in searching for novel, potent and selective analgesics and anti-inflammatory agents destitute of or with minimal side effects.

With emergence of soporific activities of 2(3H)-Benzoxazolone, numerous derivatives of this compound such as chlorzoxazone, have been tested for various activities such as analgesics, antifungal, antibacterial, cardiotonic, antimicrobial and anti-inflammatory activities.[6, 7]

Benzoxazolone nucleus is regarded as important scaffolds in the designed synthesis of various pharmacological probes. Heteroatomic nitrogen in position 3 is of interest because it allows various important chemical transformations to take place. One of the possible reactions is attachment of amine group to the N-atom of the benzoxazolone ring.

The aim of this work is to attach different piperazine derivatives (i.e., phenylpiperazine and trifluoromethylphenylpiperazine) and 4-methylpiperadine on heteroatomic nitrogen position 3 through Mannich reaction under two different conditions (reflux and microwave heating methods). Although these compounds were previously synthesized under reflux condition,[8, 9] in this present work they were synthesized with microwave and also with reflux at the same time so as to compare the yields and purity.

The objective is to compare both results and find suitable conditions for these reactions and also to make proper implementation of the principle of green chemistry.

These three compounds were thoroughly characterized by Fourier Transform Infrared (FT-IR) and Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy. The purity was determined by melting point and thin layer chromatography (TLC).

2. LITERATURE REVIEW

2.1. Analgesics and Analgesic Effects

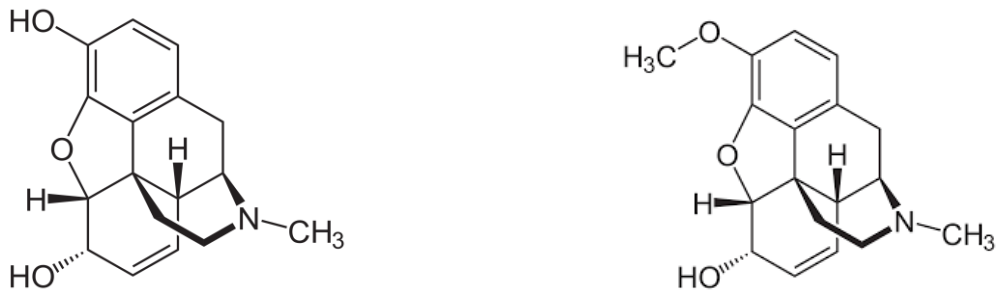
Analgesics can be referred to as any drug that has the capability of relieving pain in selective manner without hindering or blocking the conduction of sensory nerve impulses, markedly changing sensory perception or affecting consciousness.[10] Analgesics are primarily classified into two major classes: opioid (narcotic) and non-opioid (non-narcotic) analgesics or non-steroidal anti-inflammatory drugs (NSAIDs).

2.1.1. Opioid Analgesics

Opioid analgesics are used to alleviate moderate to severe pain. They are either natural alkaloids 'opiates' (derived from the opium poppy), Fig. 2.1 or synthetic agent (opiate-like) which are together called opioids.

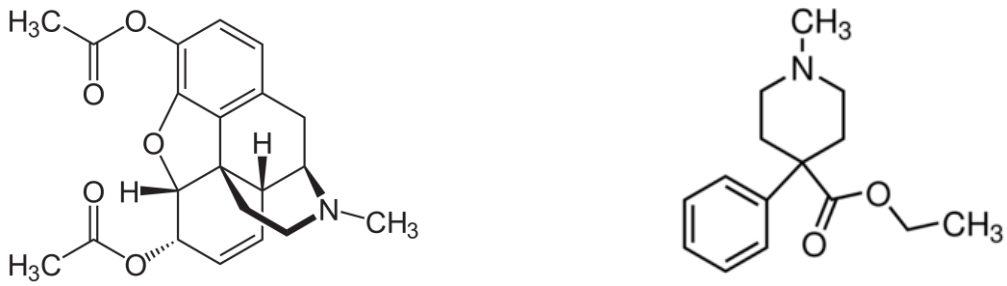
Figure 2.2: Images of opium poppy flower and fruit

Morphine and codeine are opiates while heroin and meperidine are examples of semi synthetic and synthetic agents, respectively.[11]



(a) (b)

Figure 2.3: Structures of opiates (a) morphine and (b) codeine



(a) (b)

Figure 2.4: Structures of (a) heroin and (b) meperidine

Opioid analgesic agent are classified as agonists (e.g., morphine), antagonists (e.g., naloxone) and mixed agonist-antagonists (e.g., nalbuphine). The chemical structures of these agents are shown in Fig. 2.4.

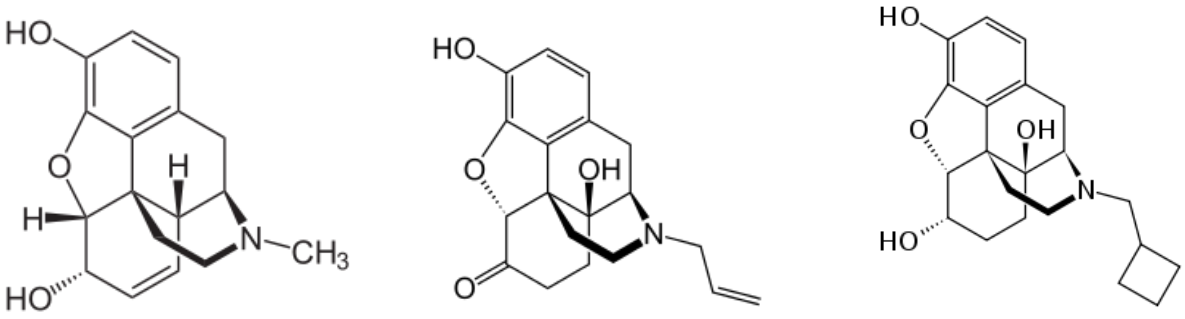


Figure 2.5: Structures of (a) morphine (b) naloxone and (c) nalbuphine

Morphine opiate served as a 'lead' to several synthetic opioids.[11,] Some examples of morphine opiate analogs which differ in either position (3, 6, N or 14) include: heroin, codeine, levorphanol, dihydrocodeine, nalorphine, nalbuphine, naloxone and so forth.

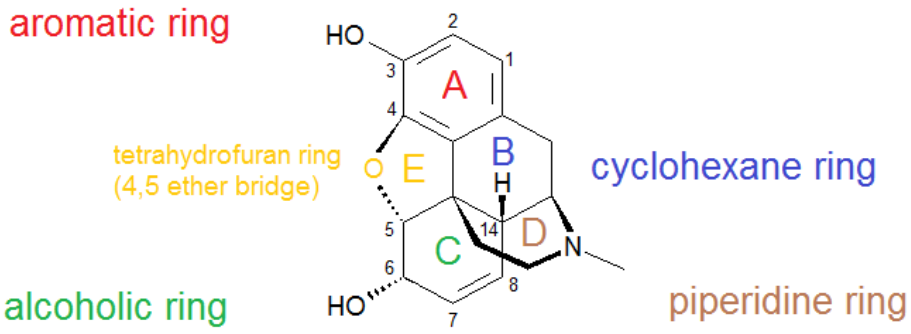


Figure 2.6: Structure and numbering of morphine

2.1.2. Non-Opioid Analgesics

Non-opioid analgesics are weak analgesics (non-narcotic analgesics or non-steroidal anti-inflammatory drugs). Aspirin was first synthesized by Gerhardt C. (1853) and is still one of the most widely used mild analgesic and NSAIDs. It had its medicinal origin in the salicylates and glucosides of willow bark, long used to treat rheumatic diseases and gout mild pains.[12]

2.1.2.1. General Structure and Properties of NSAIDs

Generally, NSAIDs are structurally made up of a carboxylic acid and enols (acidic moiety) attached to a planar aromatic functionality. Some analgesics derivatives also have a polar linking group that attached the planar moiety to an additional lipophilic group.[13] The general structure is shown in Fig. 2.6.

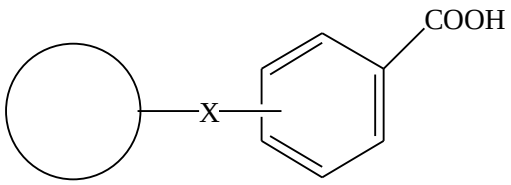


Figure 2.7: General structure of NSAIDs

NSAIDs are characterized by the following pharmacological and chemical properties; they are relatively week organic acid with 3-5 pK_a range. Though not all are carboxylic but most, and the acidic group is vital for COX inhibitory activity. During drug interaction, NSAIDs are highly bonded by plasma protein through major ionic binding with the carboxylic acid group. The acidic group also serves as a major site of metabolism by conjugation.[13]

Based on chemical structure, NSAIDs are reported to be classified as follows; Salicylates (aspirin), Propionic acids (profens), Anthranilates (fenamates), Aryl and Heterocyclic acid, Oxicams (enol acids), Phenylpyrazolones and Anilides.[13] General structures and some examples of NSAIDs are presented in the figures below.

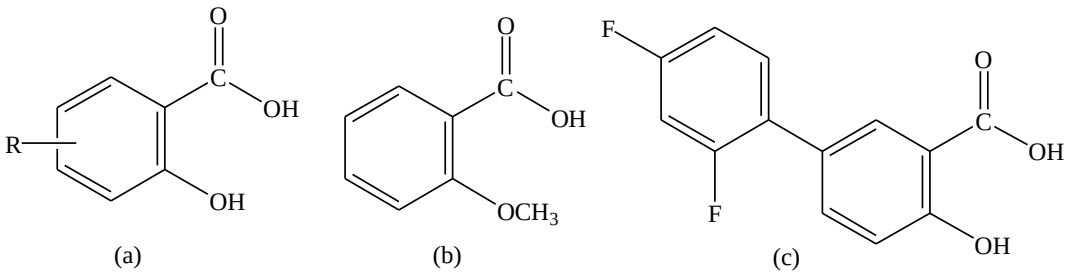


Figure 2.8: (a) General structure of salicylates, (b) aspirin and (c) diflunisal

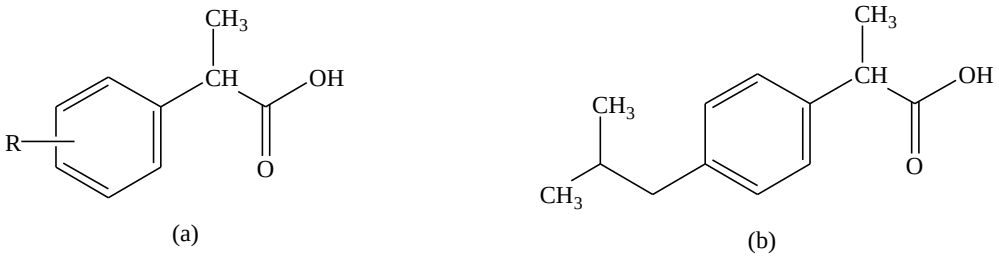


Figure 2.9: (a) General structure of propanoic acid NSAIDs and (b) ibuprofen

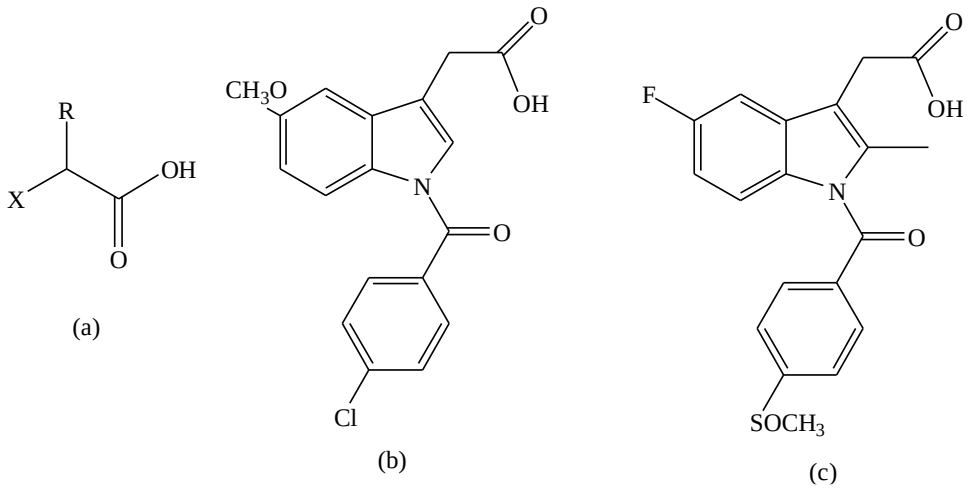
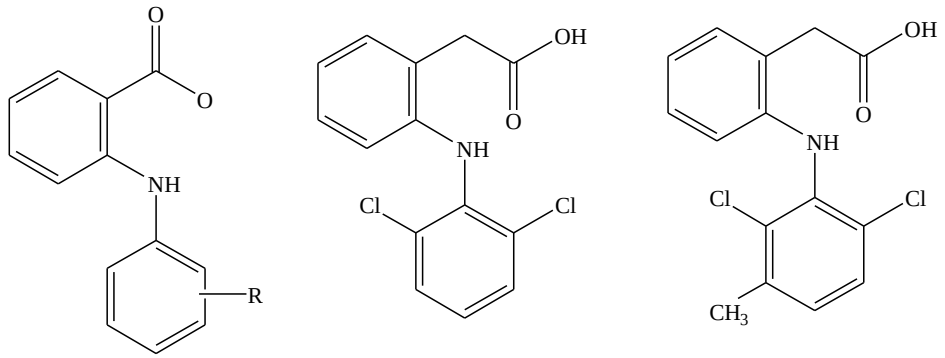
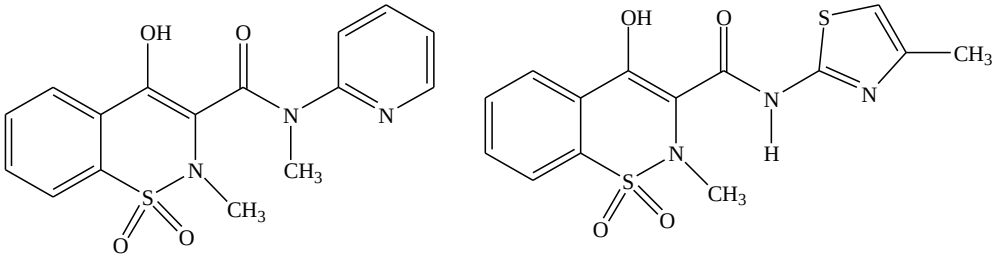


Figure 2.10: (a) General structure for heterocyclic acetic acid where X is a heterocycle, (b) indomethacin and (c) clinoril



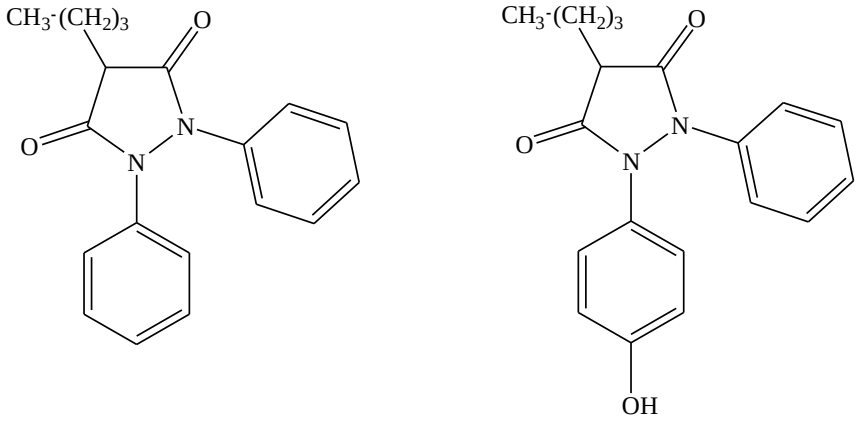
(a) (b) (c)

Figure 2.11: (a) General structure of anthranilates, (b) diclofenac and (c) meclofenamate



(a) (b)

Figure 2.12: The structure of oxicams: (a) piroxicam and (b) meloxicam



(a) (b)

Figure 2.13: The structure of phenylpyrazolones: (a) phenylbutazone and (b) oxyphenbutazone

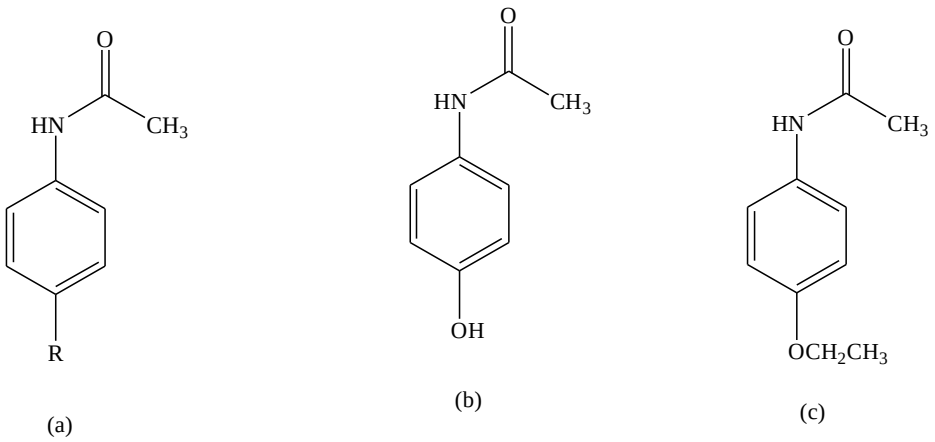
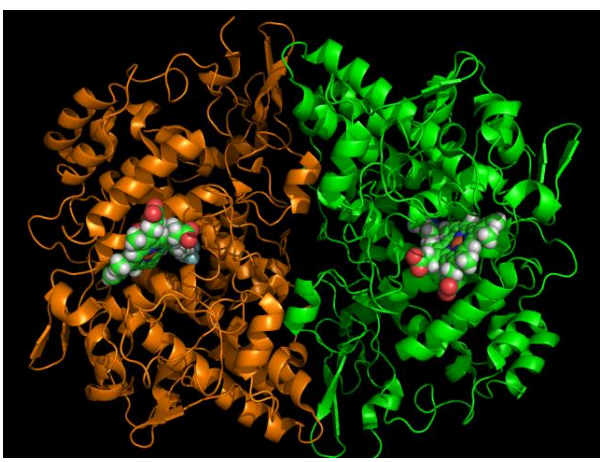


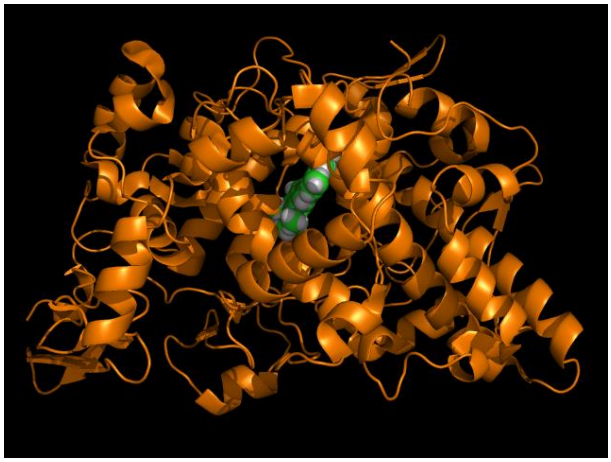
Figure 2.14: (a) General structure for anilides (b) acetaminophen and (c) phenacetin

2.1.2.2. Mechanism of Action of NSAIDs

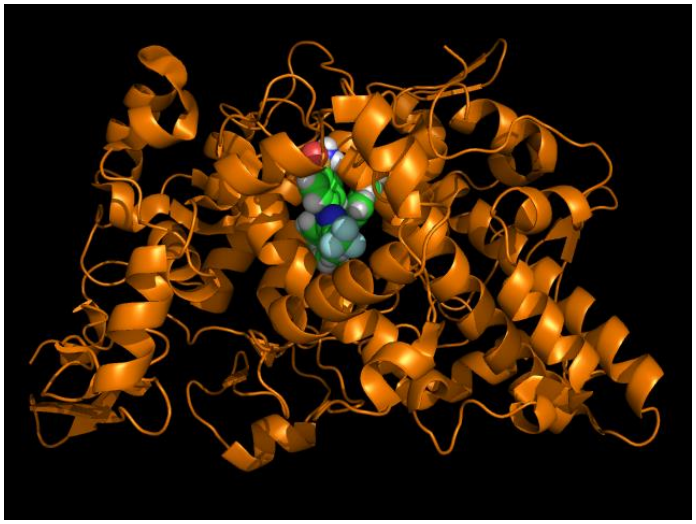
NSAIDs induce their therapeutic effects (antipyretic, analgesic and anti-inflammatory activities) by inhibition of prostaglandin (PG) synthesis. NSAIDs specifically (for the most part) inhibit cyclooxygenases (COX-1 and COX-2, Fig. 2.14), the enzymes that catalyze the synthesis of cyclic endoperoxides from arachidonic acid to form prostaglandins. COX-1, expressed constitutively, is synthesized continuously and is present in all tissues and cell types, most notably in platelets, endothelial cells and the gastrointestinal (GI) tract. Thus COX-1 is important for the production of prostaglandins of homeostatic maintenance, such as the regulation of blood flow in the kidney and stomach, platelet aggregation and the regulation of gastric acid secretion. The major contributor to NSAID GI toxicity is considered to be caused by inhibition of COX-1 activity.[13, 14] Though there is some constitutive expression in the kidney, brain, bone, female reproductive system and GI tract, COX-2 is regarded as an inducible isoenzyme. The COX-2 isoenzyme plays an important role in pain and inflammatory processes.



(a)



(b)



(c)

Figure 2.15: (a) COX-1 and COX-2, (b) and (c) shows how NSAIDs targeted COX enzymes

NSAIDs in general, inhibit both COX-1 and COX-2. The majority of NSAIDs are mainly COX-1 selective (e.g., aspirin, indomethacin and piroxicam), some are slightly selective for COX-1 (e.g. ibuprofen, naproxen and diclofenac) and others may be considered slightly selective for COX-2 (e.g, meloxicam and etodolac), although it has been reported in the analysis of the therapeutic level of meloxicam that meloxicam is not a specific COX-2 inhibitor.[15]

2.1.2.3. Side Effects of NSAIDs

NSAIDs may cause damage distil to the duodenum, bleeding, perforation, ulcer mainly associated with large and small intestine occasionally due to taking this drug.[16] Relapse of classical inflammatory bowel disease may also be associated with NSAIDS[17] Patients who are administered to NSAIDs have an increased risk of mucosal damage in the upper gastrointestinal tract.[3] Prospective study has confirmed that the dose of NSAIDs is associated with increased risk of gastric, duodenal and peptic ulcer.[18-20] NSAIDs as reported by the Food and Drug Administration FDA (2015), may increase the risk of stroke and heart attack, both of which can lead to death in rare cases.[5, 21]

Due to these side effects, there has been an interest to develop COX-2 selective analgesics and many studies have been reported.[1, 7]

2.2. Chemistry of 2(3H)-Benzoxazolone

2(3H)-Benzoxazolone is a heterocycle bicyclic aromatic ring system composed of a benzene ring fused to a carbamate. It is a light brown powder with the molecular formula $C_7H_5NO_2$, molecular weight (135.12 g mol^{-1}) with one H-bonding donating site and two H-bonding accepting site. Lipophilic character in one side and hydrophilic character in the other face with high dipole moment of (4.47 Debye), a discrete partition coefficient (log P = 0.97) and pK_a value of 8.7, hence it is a weak acid in aqueous solution. Fig. 2.15, shows the structure of 2(3H)-Benzoxazolone

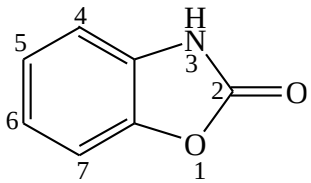


Figure 2.16: The structure of 2(3H)-Benzoxazolone

2(3H)-Benzoxazolone nucleus is one of the most important and versatile heterocyclic rings and serves as a lead to various compounds with a wide range of biological activities such as antibacterial, antifungal, analgesics, anti-inflammatory, antimalarial, anticancer anti nociceptive.[6, 7, 22]

2.2.1 Synthesis of 2(3H)-Benzoxazolone

Srikanth,[23] synthesized 2(3H)-Benzoxazolone by first synthesis of *o*-hydrophenylurea, where finely ground urea was thoroughly mixed with 2-aminophenol and heated at 160 ^{o}C under reflux for 25 min. Dried *o*-hydrophenylurea formed were then heated at 160 ^{o}C for 15 min and recrystallized with methanol to obtain pure 2(3H)-Benzoxazolone crystals, as shown in Fig. 2.16.

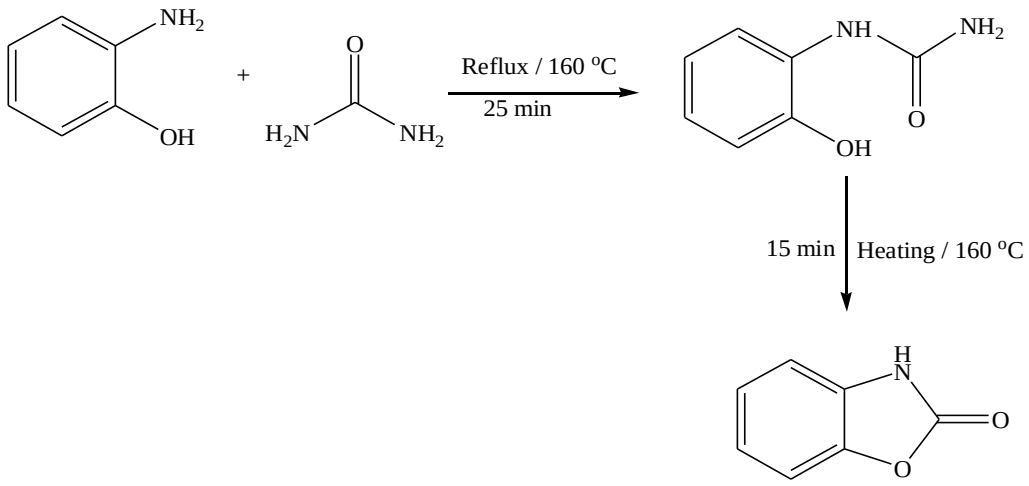


Figure 2.17: Synthesis of 2(3H)-Benzoxazolone under reflux condition

Eren,[24] also synthesized 2(3H)-Benzoxazolone by reacting 2-aminophenol with urea under microwave irradiation at 140 ^{o}C for 10 min, as shown in Fig. 2.17.

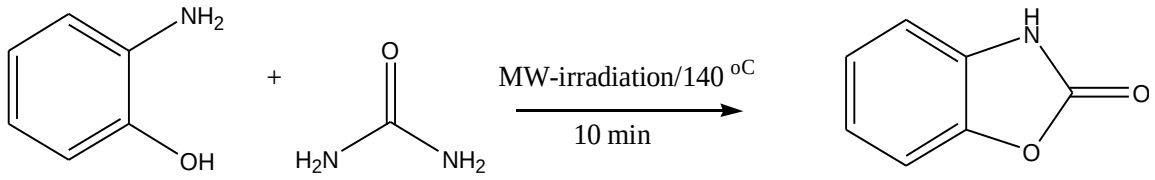


Figure 2.18: Microwave synthesis of 2(3H)-Benzoxazolone

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

2(3H)-Benzoxazolone could undergo three different kinds of reactions; ring opening/ring expansion, Friedel-Crafts alkylation/acylation (N-substitution) and electrophilic substitution reaction on aromatic ring such as chlorination reaction to yield 5-chloro-2(3H)-Benzoxazolone (chlorzoxazone).[7]

2.3. 5-Chloro-2(3H)-Benzoxazolone

Chlorzoxazone is a 2(3H)-Benzoxazolone derivative. It is a bicyclic ring system made up of chlorophenyl fused to a carbamate. It is a white to off-white powder with the molecular formula $C_7H_4ClNO_2$, molecular weight (169.565 g mol^{-1}), octanol/H_2O partition coefficient (log P = 1.6) and also have one H-bond donor and two H-bond acceptor sites having satisfied all the Lipinski's rule of five.[25] 5-chloro-2(3H)-Benzoxazolone structure is shown in Fig. 2.18.

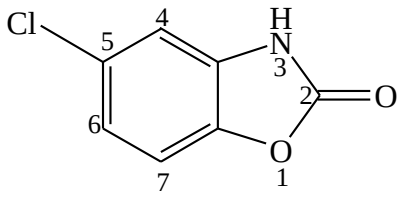


Figure 2.19: Structure and numbering of 5-chloro-2(3H)-Benzoxazolone nucleus

2.3.1. Bioisosterism of 5-Chloro-2(3H)-Benzoxazolone

5-chloro-2(3H)-Benzoxazolone (Fig. 2.19, a) in numerous plans serves as phenol substitute. To some degree, the sulphur bioisoster, i.e., 5-chloro-2(3H)-Benzthiazolone (b), methylene bioisoster i.e., 2-oxindole (c), 5-chloro-benzimidazol-2-one (d), as nitrogen bioisoster. chlorobenzoxazinone (e) also prepared by the same methodology of bioisosterism of ring expansion of derivative (a) as display in Fig. 2.19.

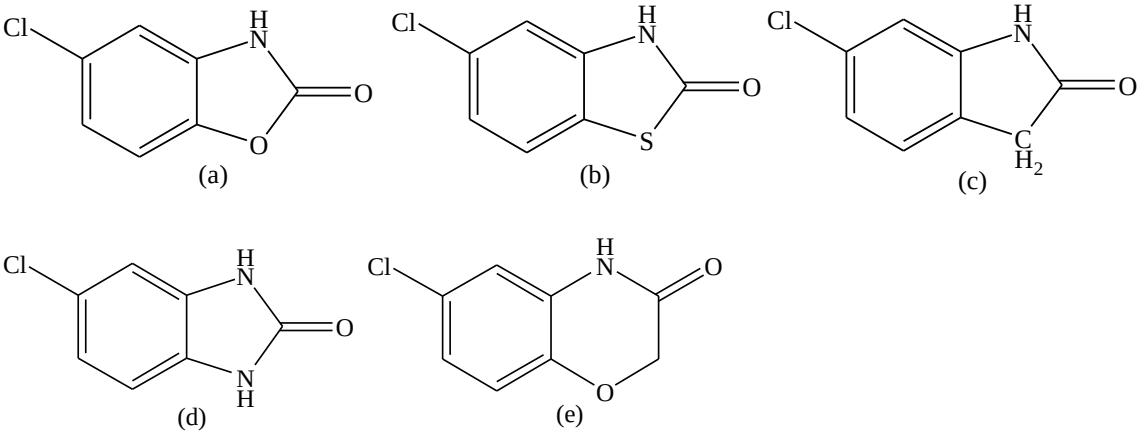


Figure 2.20: Some example of bioisosters of 5-chloro-2(3H)-Benzoxazolone

This concept of bioisosterism presents local steric and electronic adjustment or modifications to a lead compound. This may bring about a change in receptor affinity and selectivity and/or agonist-antagonist character.

2.3.2. Chemical Reactivity of 5-Chloro-2(3H)-Benzoxazolone

As with 2(3H)-Benzoxazolone, 5-chloro-2(3H)-Benzoxazolone also undergoes three different types of reactions; ring opening and ring expansion, N-substitution (Friedel-Crafts acylation or alkylation) and electrophilic substitution reactions such nitration, halogenation and sulfonation etc. Many important transformations occur on the heterocyclic N-atom at the 3rd position due to enolizable character of amide functionality. N-acylation of 5-chloro-2(3H)-Benzoxazolone under

acid-base catalysis generate derivative (b), meanwhile base-catalyzed reaction gives derivative (c),[25] as seen in Fig. 2.20.

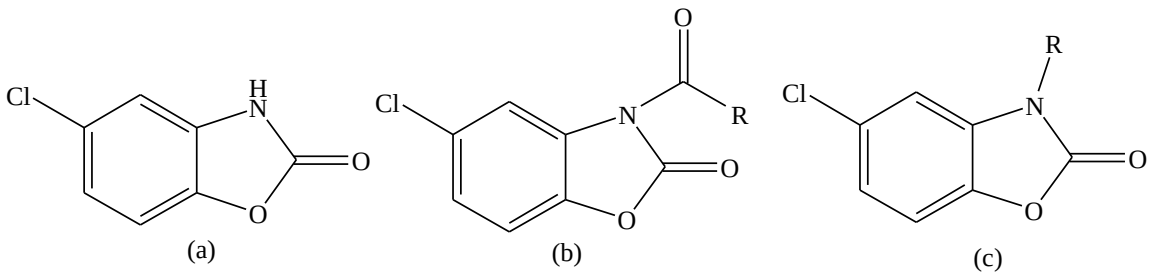


Figure 2.21: The structure of (a) 5-chloro-2(3H)-Benzoxazolone nucleus (b) product of N-acylation (c) product of N-alkylation, at position 3

Michael addition of acrylonitrile (C_3H_3N) to 5-chloro-2(3H)-Benzoxazolone under base-catalyzed condition give N-cyanoethyl derivative (Fig 2.21, a). N-substitution reaction of 5-chloro-2(3H)-Benzoxazolone with hydroxaminosulfuric acid leads to compound (Fig. 2.21, b), a cyclic hydrazide. Mannich reaction (condensation) gives prepared access to N-aminomethyl subordinate (Fig. 2.21, c), [23] as displayed in Fig. 2.21.

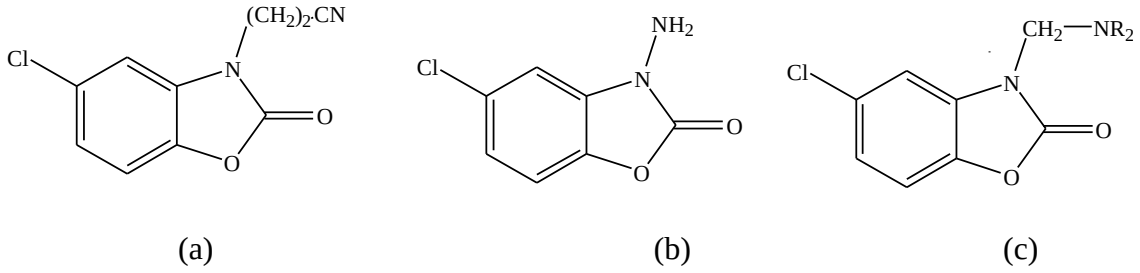


Figure 2.22: Product of (a) Michael addition (b) cyclic hydrazide (c) Mannich reaction, formed by transformation of α-hydrogen (active) on heteroatom (N) at position 3.

Electrophilic substitution on aromatic ring is controlled by the enormous desire for the 6th position, which is observed not only for simple nitration, halogenation and sulfonation reactions but also for Friedel-Crafts acylation.[25, 26] However, in the specific instance of Friedel-Crafts reaction, due to high electron-rich character of 5-chloro-2(3H)-Benzoxazolone, the heterocyclic atom is broadly complexed or get protonated by the Lewis acid present in the medium which serves as required catalyst. While 5-chloro-2(3H)-Benzoxazolone as a strongly activated substrate in normal electrophilic substitution reaction, it behaves as a strongly deactivating substrate towards electrophilic attack of acylium ion due to broad complexation experienced in the Friedel-Crafts reaction. Ideally, this reaction is carried out either by using $AlCl_3$.DMF complex to yield compound (a) or by using less reactive electrophilic species such as polyphosphoric acid (PPA) as a solvent and acylating agent to yield 6-acyl derivative (b)[27] as demonstrated in Fig. 2.22.

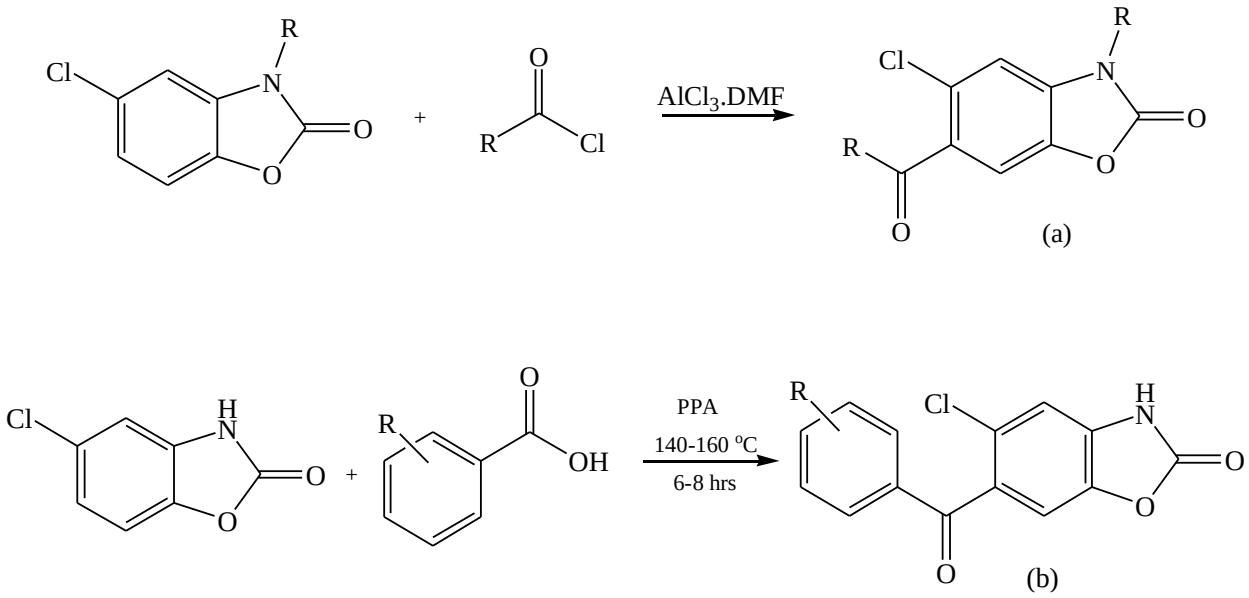


Figure 2.23: Synthetic pathway of 6-acyl-5-chloro-2(3H)-2-Benzoxazolone using (a) $AlCl_3$.DMF complex and (b) the less reactive electrophilic species PPA as a solvent and an acylating agent

5-chloro-2(3H)-Benzoxazolonene also exists as a tautomer (keto-enol) form due to enolizable character of amide moiety which allows useful modifications to occur on the heteroatomic nitrogen at position 3. The keto-enol forms of this compound are shown in Fig. 2.23.

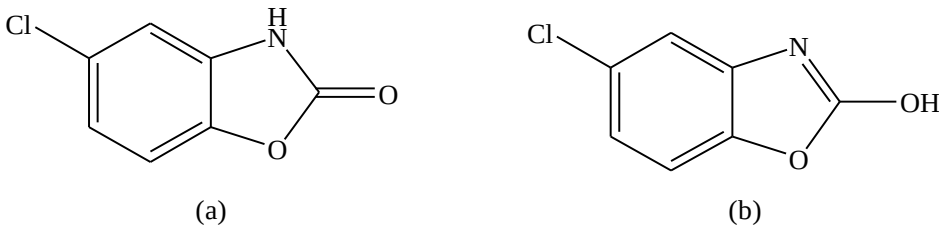


Figure 2.24: (a) Keto form and (b) Enol form

2.4. Biological Activity of 2(3H)-Benzoxazolone Derivatives

A numerous derivatives of 2(3H)-Benzoxazolone such as 5-chloro-2(3H)-Benzoxazolone, have been tested for various biological activities including analgesics, antifungal, antibacterial, cardiotonic, antimicrobial and anti-inflammatory activities.[6, 7, 28]

Köksal et al.[29] discovered a new series of Mannich bases 5-nitro-3-substituted piperazinomethyl-2(3H)-Benzoxazolones and anti-inflammatory and analgesics activities of the compounds were examined in two different bioassays, namely, *p*-benzoquinone-induced abdominal constriction test and carrageenan-induced hind paw edema test in mice. Ulcerogenic effects of these compounds were also examined. Among the tested derivatives are compounds with electron deactivating or withdrawing groups (e.g., fluorine, chlorine and acetyl) substituted in ortho-para position of phenyl nucleus on the piperazine ring at the 3rd position of 2(3H)-Benzoxazolone moiety (a, b, c, d, e), which were synthesized via Mannich reactions as shown in Fig. 2.24.

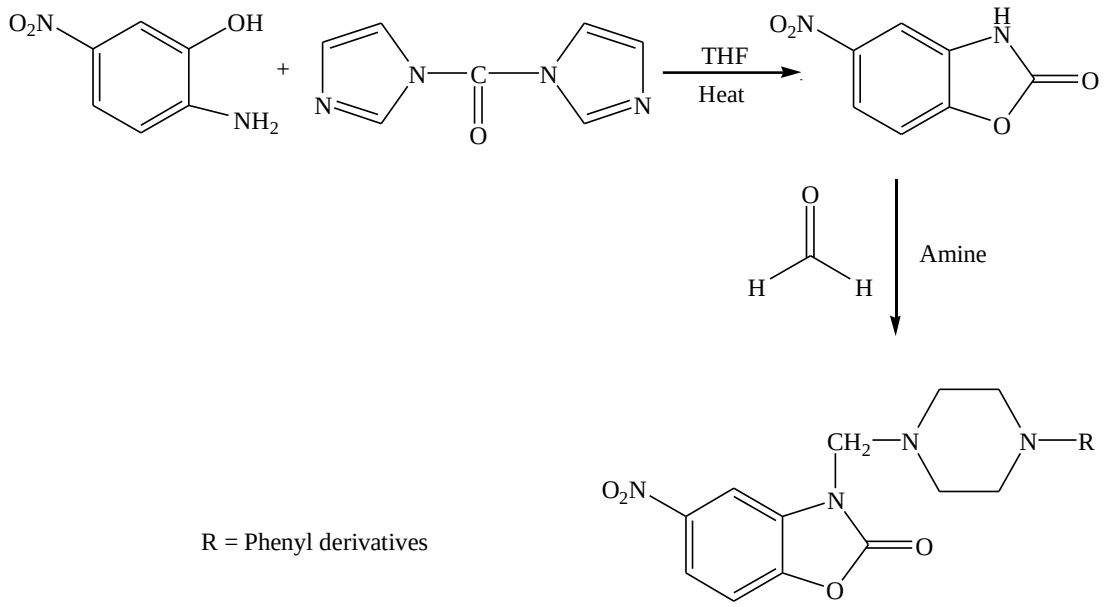


Figure 2.25: Synthesis of 5-nitro-3-substituted piperazinomethyl-2(3H)-Benzoxazolones derivatives

All compounds show analgesics activities higher than their anti-inflammatory activities even though inhibitory ratios towards anti-inflammatory for all the compounds were above 30%. In light of this, these compounds were worthy more attention for further assessment.

In a similar research, Gökhan et al.[30] synthesized some Mannich bases of 5-methyl-3-substituted piperazinomethyl-2(3H)-Benzoxazolone through Mannich reaction. Analgesic and anti-inflammatory activities of the compounds were examined *in vivo* in two different bioassays similar to reference [29] above. The most promising results obtained from the tested compounds are the once bearing electron withdrawing substituents in the para position of phenyl nucleus on piperazine ring at position 3 of 2(3H)-Benzoxazolone moiety shown in Fig. 2.25. Analgesics activity of all the compounds are greater than their anti-inflammatory activities hence, the compounds could shows central effect due to the high analgesic activity they exhibited.

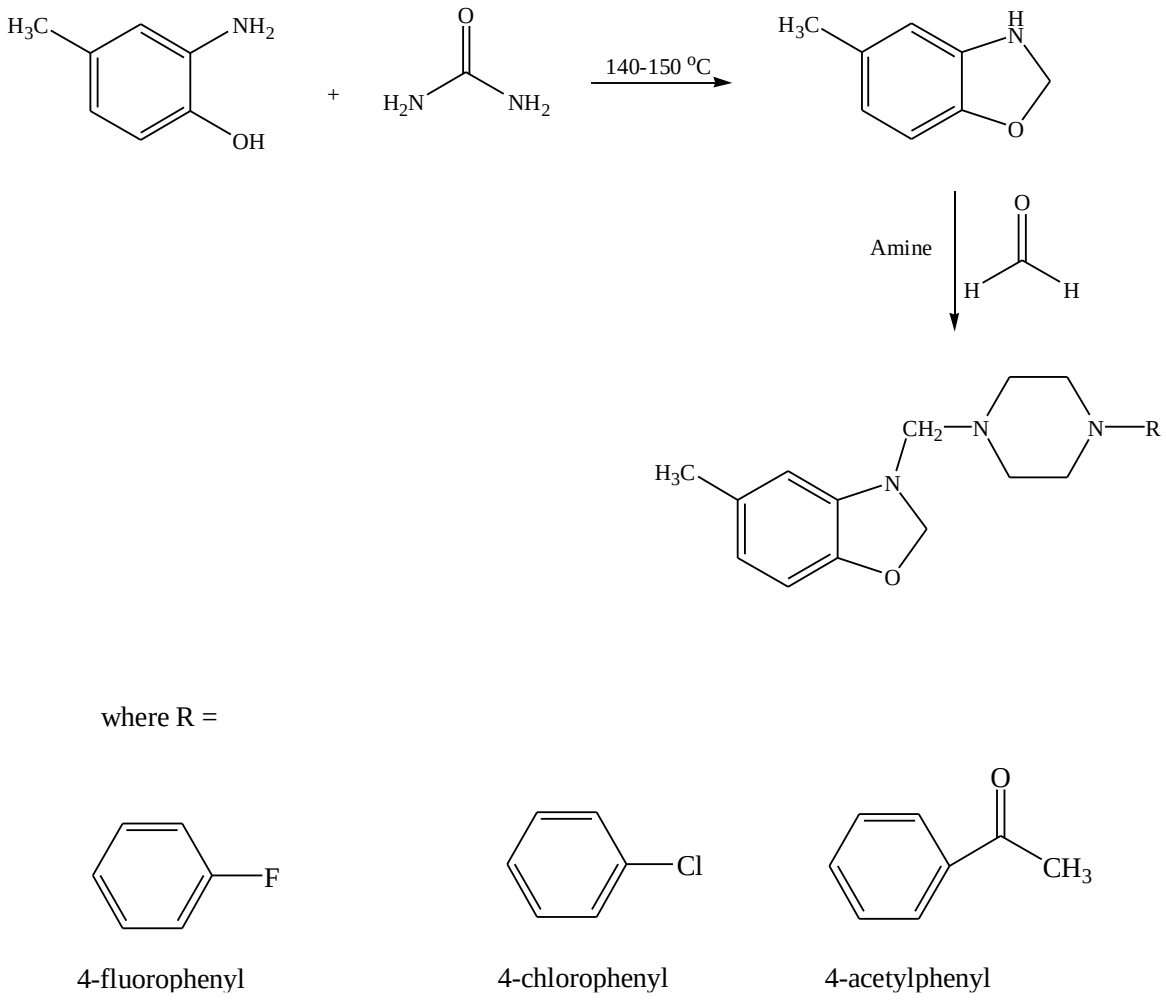
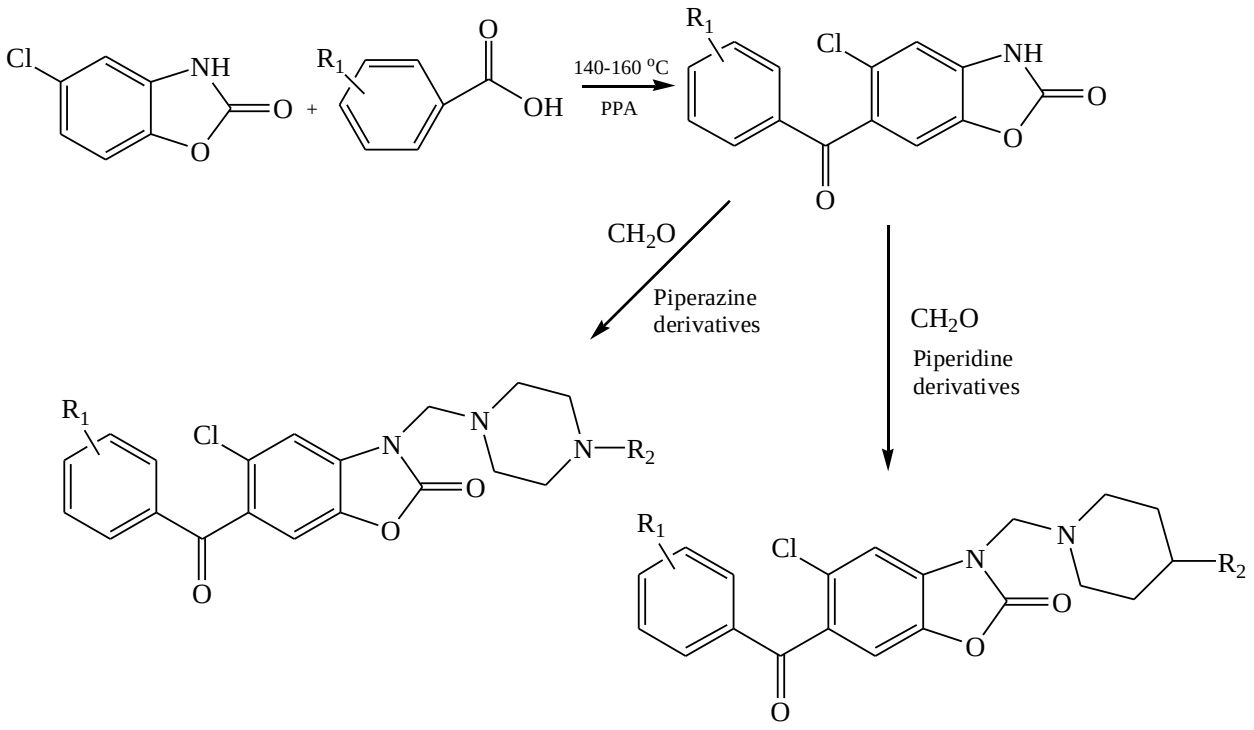


Figure 2.26: Synthesis of Mannich bases of 5-methyl-3-substituted piperazinomethyl-2(3H)-Benzoxazolone with electron withdrawing substituents on para position of phenyl nucleus on piperazine

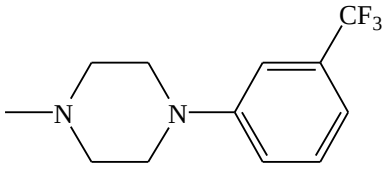
Özkanli [27] conducted experiment to synthesize some new Mannich bases of 6-acyl-5chloro-3-substituted piperazine/piperadinomethyl-2(3H)-Benzoxazolone via Mannich reaction. Seven derivatives of the compounds were synthesized with yield between 40 – 60%. Some of the derivatives are demonstrated in Fig. 2.26.



where,

R_1 = and R_2

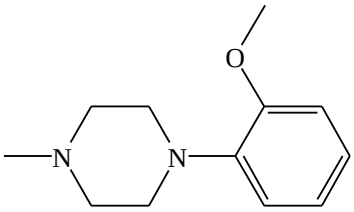
2,6-Difluoro,



4-(3-trifluoromethylphenyl)methylpiperazine

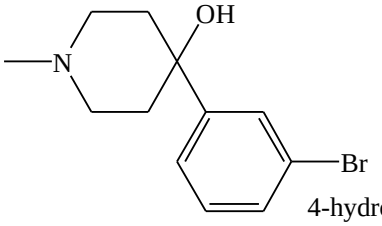
R_1 = and R_2

2,6-Difluoro,



4-(2-methoxyphenyl)methylpiperazine

2,6-Difluoro,



4-hydroxy-4-(3-bromophenyl)methylpiperidine

Figure 2.27: Synthesis of new Mannich bases derivatives of 6-acyl-5-chloro-3-substituted piperazine/piperidino methyl-2-(3H)-Benzoxazolone via Mannich reaction

Gökhan et al.[31] synthesized and screened analgesics and anti-inflammatory activities of 4-(5-chloro-2-oxa-3H-benzoxazol-3-yl) butanamide derivatives and also its gastric ulceration potential in tested organisms. They reported that, 2-oxa-3H-benzoxazole derivatives possess broad range of analgesic and anti-inflammatory activities especially 3-substituted-2-oxa-3H-benzoxazoles derivatives. It was also suggested that, 6-acyl function attached to benzene ring in the 6th position was the reasons for potent analgesic activity in these derivatives. Further studies show that some Mannich bases of 3-substituted-2-oxo-3H-benzoxazole having pyridine with methyl substituent in particular, exhibited more potent anti-inflammatory and analgesic activities. Among the compounds synthesized are, (a, b, c) shown in Fig. 2.27. It was reported that none of the compounds caused gastric problems or bleeding except compound (c) towards the tested animals.

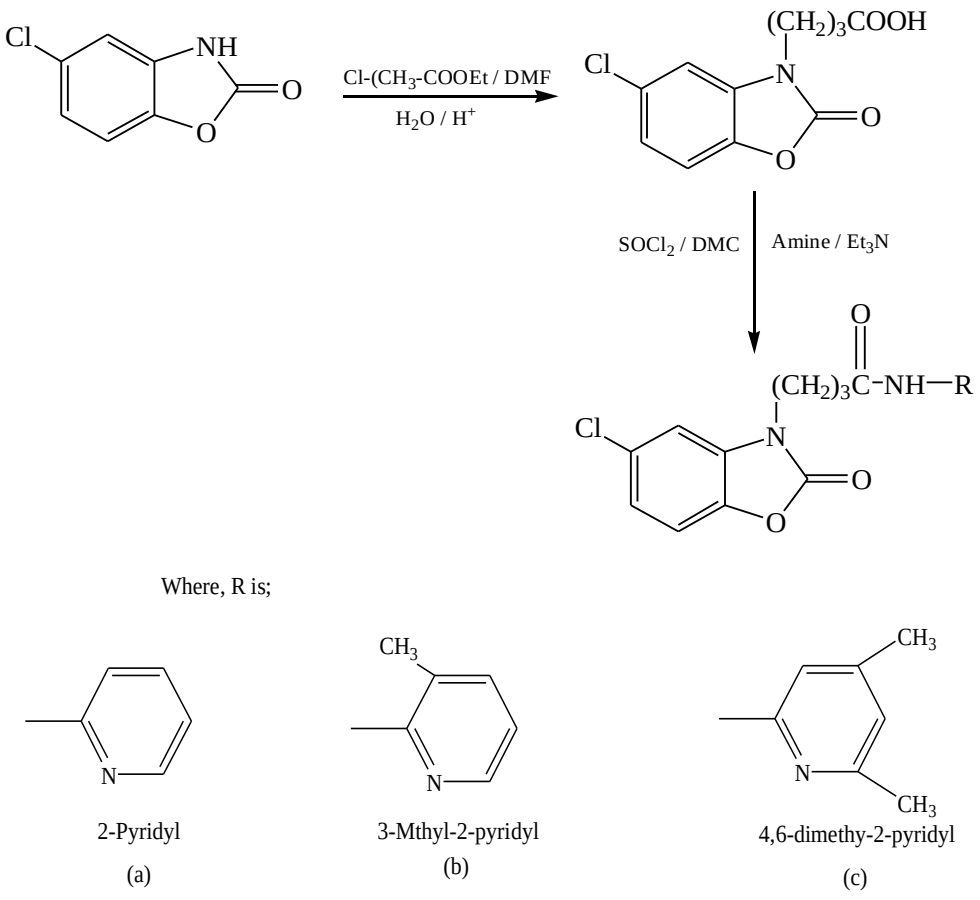


Figure 2.28: Synthesis of 4-(5-chloro-2-oxo-3H-benzoxazol-3-yl)butanamide derivatives

Soyer et al.[32] also synthesized N-substituted-5-chloro-2(3H)-benzoxazolone derivatives through Mannich reaction, but this time, acetylcholinesterase inhibitory activities were investigated instead. Some of the derivatives of this compound are (a, b, c) as displayed in Fig. 2.28.

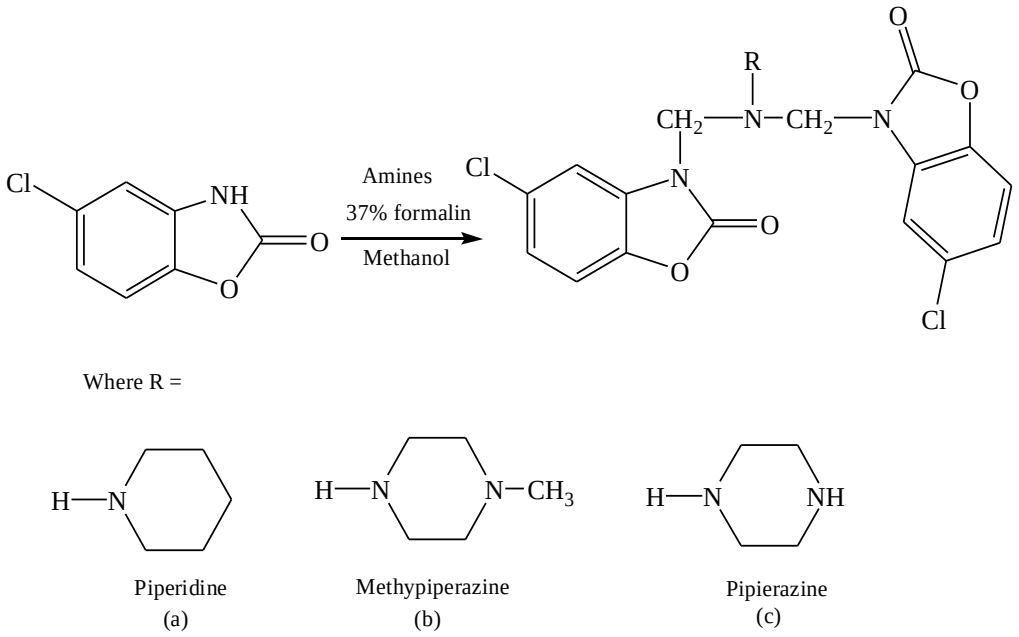
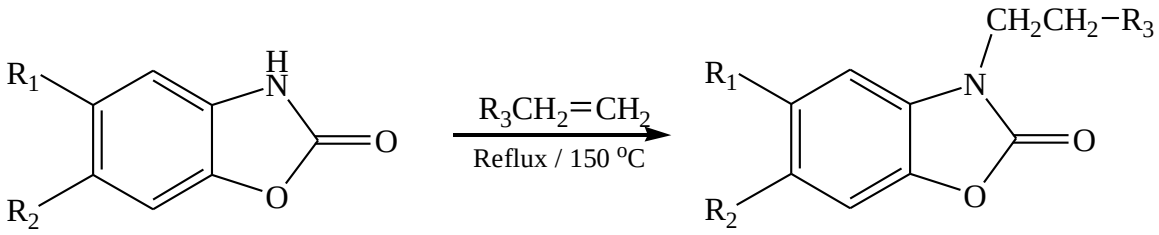


Figure 2.29: Synthesis of Mannich bases of N-substituted-5-chloro-2-(3H)-Benzoxazolone derivatives

Şafak et al.[33] also synthesized and screened for analgesic and anti-inflammatory activities of 3-(2-pryidylethyl)benzoxazolone derivatives and almost all the derivatives were reported to show analgesic activity higher than that of the reference drug, aspirin, and also high anti-inflammatory activity compared to indomethacin and those without a substituent at the 6th position of the ring showed more activity than the rest of the group. Compounds (a and b) are among those that showed higher activity towards the tested organisms as shown in Fig. 2.29.



3-[2-(2- and 4-pyridyl)ethyl]benzoxazolone

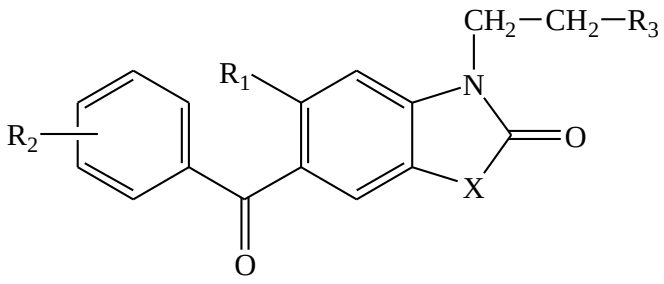
where,

R_1 = Cl R_2 = H R_3 = 2-Pyridine (a)

R_1 = Cl R_2 = H R_3 = 4-Pyridine (b)

Figure 2.30: Synthesis of 2-[2-(2- and 4-pyridyl)ethyl]Benzoxazolones derivatives

Gökhan et al.[34] synthesized similar compounds of new pyridylethylated benza(thia)zolinones and screened their analgesic activities in redesigned koster and hot-plate tests. Most of the compounds tested at 100 kg dose level showed analgesic activities higher than the reference drug (aspirin). Additional studies showed that flouro substituent at the 6th position of the phenyl ring seemed to show higher activity than those with bromine substituent. Some compounds such as (a) showed high activity in hot-plate test but inactive in koster test while others like compound (b) showed significant activity in koster but inactive in hot-plate test. The structure and some derivatives of the compound are shown in Fig. 2.30.



X = O or S

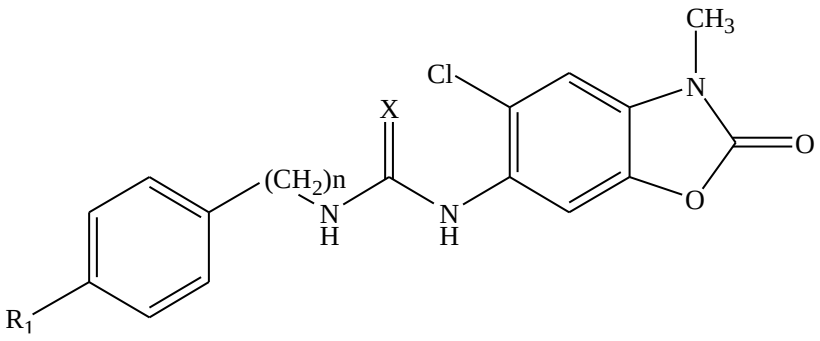
Where,

R_1 = Cl R_2 = 3-fluoro R_3 = 2-pyridyl X = O (a)

R_1 = Cl R_2 = 2-bromo R_3 = 4-pyridyl X = O (b)

Figure 2.31: Synthesis of a new pyridylethylated benza(thia)zolinones

Gülkok et al.[35] synthesized some 5-chloro-2(3H)-benzoxazolone derivatives and screened them for their antibacterial and antifungal activities against some pathogenic strains. Compounds (a and b) as in Fig. 2.31 urea derivatives and compounds (c and d) as Fig. 2.31 thiourea derivatives showed significant inhibitory activity against E. coli specie.

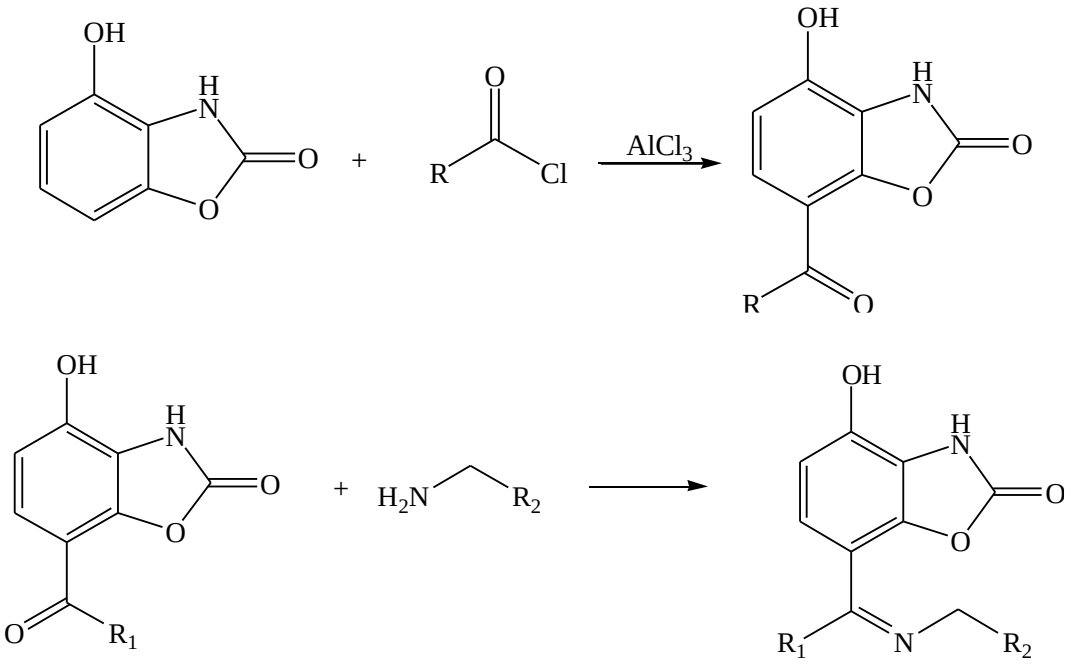


where

R_1 = H	X = O	n = 0	(a)
R_1 = Cl	X = O	n = 0	(b)
R_1 = H	X = S	n = 0	(c)
R_1 = H	X = S	n = 1	(d)

Figure 2.32: Synthesis of some 5-chloro-2(3H)-Benzoxazolone compounds of urea and thiourea derivatives

Guangjin et al.[36] also synthesized chlorzoxazone bioisoster (4-hydroxy-2-benzoxazolone) and screened for anti-inflammatory and analgesic activities of its various derivatives using carrageenan rat paw edema and hot-plate test, respectively. Some of the synthesized and tested compounds are demonstrated in Fig. 2.32.



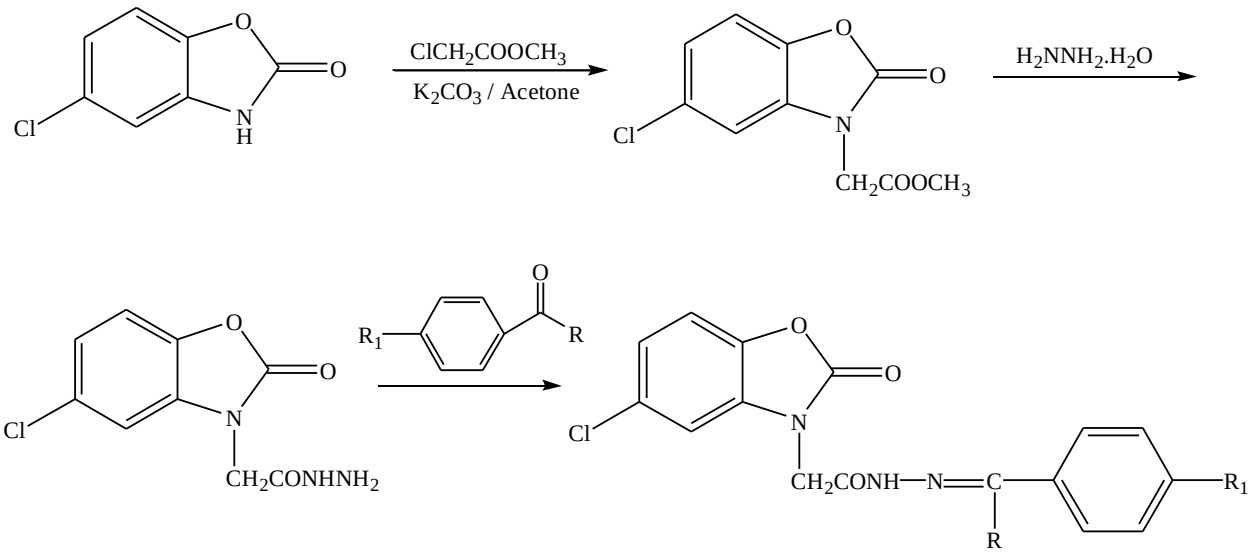
where,

$R_1 = -CH_3$ $R_2 = -CH_2OH$

$R_1 = -CH_2CH_2CH_3$ $R_2 = -CH_2COOH$

Figure 2.33: Synthesis of derivatives of 4-hydroxy-2-benzoxazolone

Onkol et al.[37] synthesized 5-chloro-2(3H)-Benzoxazolinone-3-acetyl-2-(*p*-substituted benzal)hydrazine and 5-chloro-2(3H)-Benzoxazolinone-3-acetyl-2-(*p*-substituted acetophenone)hydrazone derivatives under microwave condition and screened for antimicrobial activities. Some derivatives are effective against Staphylococcus aureus as standard reference drug (ampicillin), some showed moderate activity towards Gram-positive and Gram-negative bacteria compared to ampicillin but the entire compounds showed pronounced antifungal activity higher than the reference drug (fluconazole) against Canadian albicans. Synthesis and derivatives of this compound are shown in Fig. 2.33.



Where,

R = H, CH_3 R_1 = Halogens

Figure 2.34: Synthesis of 5-chloro-2(3H)-Benzoxazolinone-3-acetyl-2-(*p*-substituted benzal)hydrazone and 5-chloro-2(3H)-Benzoxazolinone-3-acetyl-2-(*p*-substituted acetophenone)hydrazone derivatives under microwave condition.

5-Chloro-2(3H)-Benzoxazolone was also reported to have skeletal muscle relaxant activity and was also used to decrease muscle tone and tension, relieve spasm and pain associated with musculoskeletal disorders.[38]

2.5. Mannich Reaction

Mannich reaction is an organic reaction used to convert a primary or secondary amine and two carbonyl compounds (enolizable and non-enolizable) to a β-amino carbonyl compound (Mannich base) using acid or base catalyst. The solvents that are usually used are protic solvents such as methanol, ethanol and acetic acid, etc. This is to ensure a sufficient high concentration of

electrophilic iminium ion. The schematic representation of Mannich reaction is shown in Fig. 2.34.

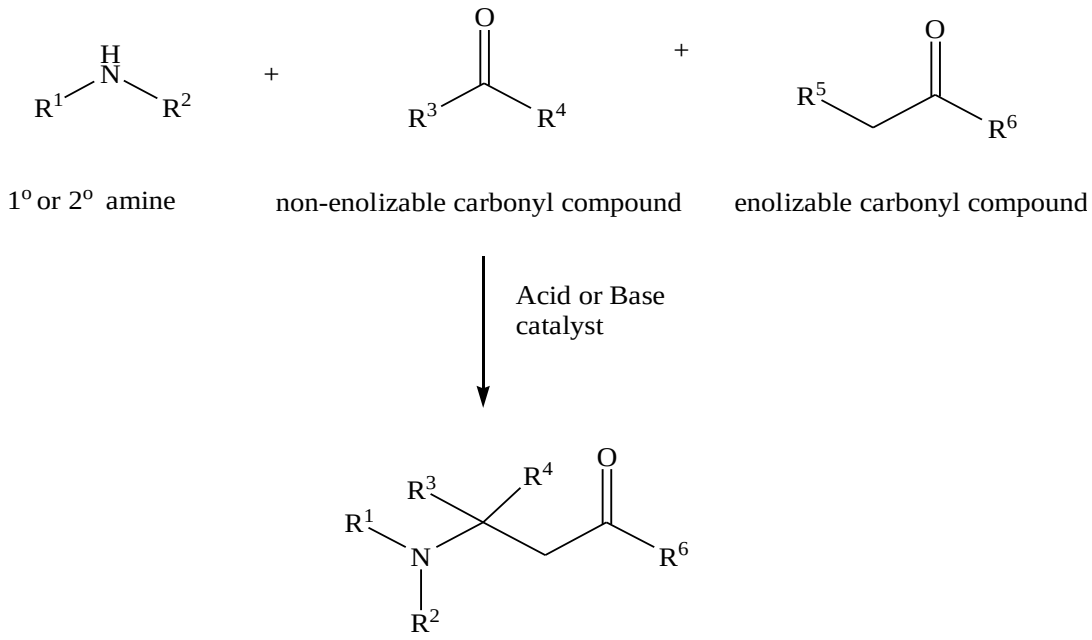


Figure 2.35: Schematic representation of general Mannich reaction

2.5.1. Mannich Base

Mannich base is a β-amino ketone carrying compound and or the end product of a Mannich reaction. Mannich base serves as a very important pharmacophores or bioactive "lead" which serves as a starting material and an active agent for the synthesis of various natural products especially alkaloids with higher medicinal value such as cocaine (a) procyclidine (b) and fluoxetine (c) in Fig. 2.35 which both possess aminoalkyl chain.[39]

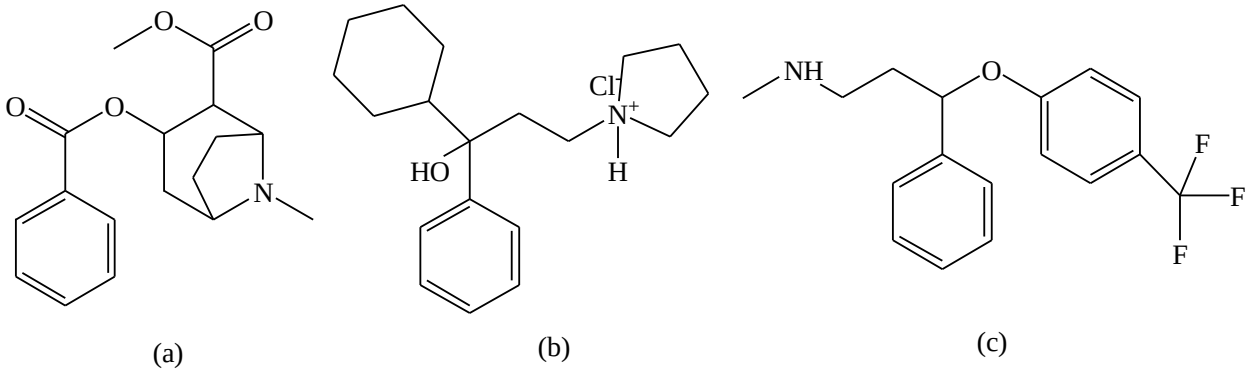


Figure 2.36: Some active natural products (a) cocaine (b) procyclidine and (c) fluoxetine which possess aminoalkyl chain

2.5.2. Synthetic Application of Mannich Base

A Mannich base plays a vital role in developing synthetic route in medicinal and pharmaceutical chemistry, being very reactive and can easily be transform and converted to other compounds, for example, reduced to form amino alcohol.[40] A Mannich base is also known to possess potent activities such as anti-inflammatory, antibacterial, antifungal, antiviral and analgesic.[9] Some synthetic applications of Mannich bases are giving below;

I. Treatment of Mannich base (benzimidazol-2-thiol) with excess formalin (HCHO) solution in water or methanol at refluxing temperature to yield 1,3-dihydromethylbenzimidazol-2-thione.[41]

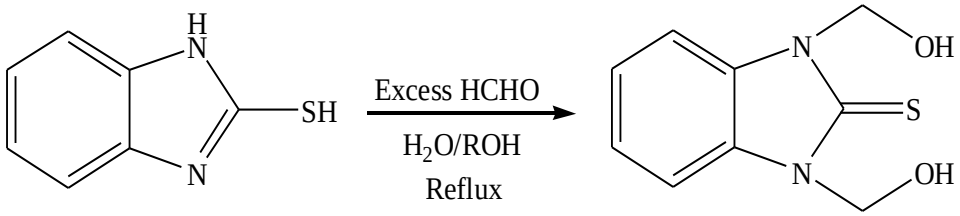


Figure 2.37: Synthesis of a Mannich base, 1,3-dihydromethylbenzimidazol-2-thione

II. Synthesis of 3,3-[piperazine-1,4-diylbis(methylene))bis(5-chlorobenzo[d]oxazol-2(3H)-one), by reaction of Mannich base (chlorzoxazone), formalin and appropriate secondary amine(piperazine) and methanol as a solvent.[32]

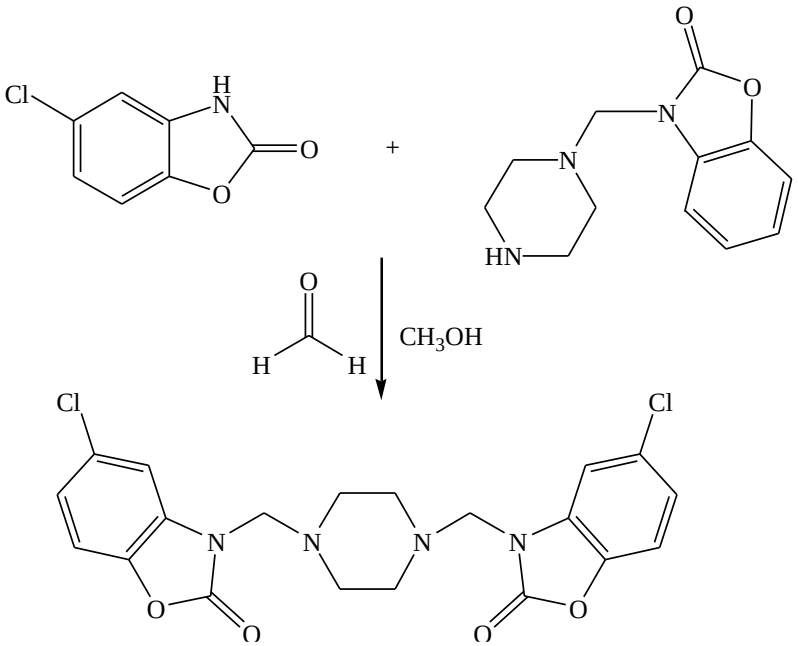


Figure 2.38: Synthesis of a Mannich base, 3,3-[piperazine-1,4-diylbis(methylene))bis(5-chlorobenzo[d]oxazol-2(3H)-one)

III. 6-Acyl-5-chloro-3-(4-phenylpiperazin-1-yl)methyl-5-chloro-2(3H)-Benzoxazolone derivative can also be prepared by the reaction of the Mannich base (6-Acyl-5-chloro-2(3H)-Benzoxazolone) with a secondary amine (piperazine derivative), formalin solution and methanol as a solvent under reflux condition.[27]

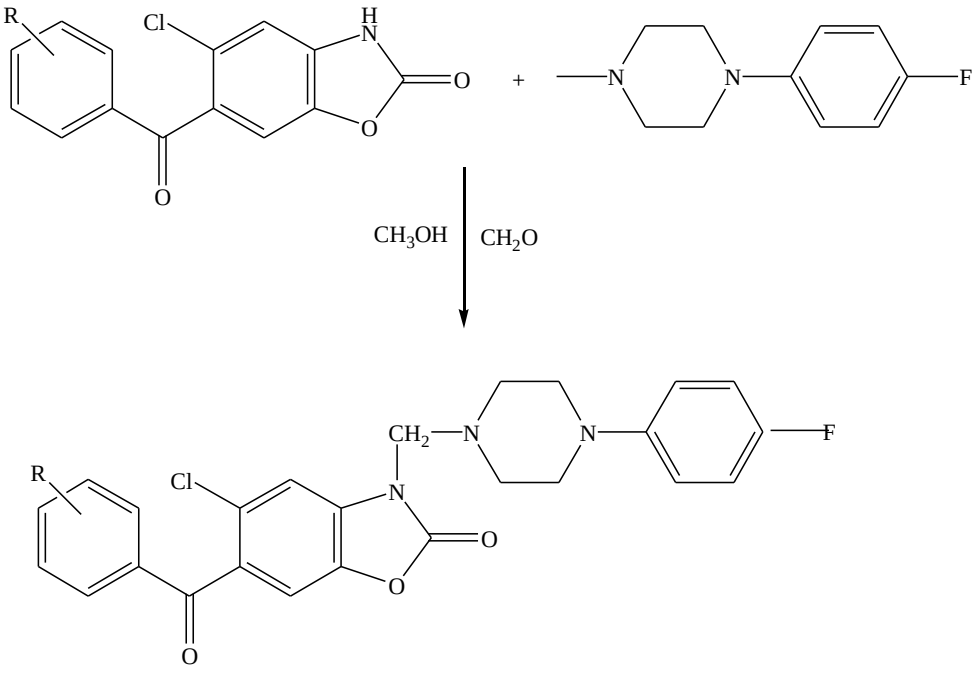


Figure 2.39: Synthesis N-substituted Mannich base with acyl derivatives

2.6. High Temperature Synthesis Method

2.6.1. Reflux Heating Method

The rates of chemical reactions vary greatly. Some reactions are instantaneous, some are slow while others may reach their equilibrium at a very long time. The measure of change in concentration of the reactants or the products per unit time is referred to as reaction rate. The rate of reaction usually increases with increased temperature and vice-versa. Some organic reactions are extremely slow and take a long time to achieve any noticeable yield. To increase the rate of such reactions, heating is usually explored. However, some organic compounds have low boiling point and may vaporize upon exposure to such heat, thereby inhibiting the reaction to proceed at a satisfactory rate. Therefore heating the mixture under reflux is the solution to overcome this problem.

Figure 2.40: Reflux heating set up

Reflux heating is a safe means of heating a mixture of a solution using a reflux condenser to make sure that volatile substances vaporize and recondense and safely drip back into the reaction mixture without loss of solvent or reagent due to evaporation. This means that with reflux, liquid, can might be boiled without losing its volume.[42]

2.6.2. Microwave-Assisted Organic Synthesis

Organic synthesis is one of the major areas of research in chemistry. Synthesis of new chemical entities is major stumbling block in research and drug discovery. Conventional strategies of various chemical synthesis are generally used. In 1855, Robert Bunsen invented the burner which served as an energy source for heating a reaction vessel. It was superseded by isothermal, oil bath or hot plate methods (reflux heating), but the danger of heating though remain the same. Microwave-assisted organic synthesis (MAOS), has emerged as a new "lead" in organic synthesis and has been regarded as superior to reflux heating method.[43] Microwave techniques offer simple, clean, fast, efficient, improved purity and economical.[44, 45] It is also regarded as an

important “green chemistry” approach due to its environmentally sound, clean procedures and eco-friendly nature.[46, 47]

Figure 2.41: Microwave heating set up

This techniques has gained popularity in the past decade as a powerful tool for rapid and efficient synthesis of a variety of compounds and become the cutting edge technology across medicinal, pharmaceutical, biotechnological and fine chemical industries.[1, 45, 47, 48]

2.6.2.1. Brief History of Microwave

J. Maxwell (1864), theorized that microwave energy could be able to travel through space in a wave-like nature. “Magnetron” was the first generator of microwave power for radar, which was invented at Birmingham University in 1940’s. Percy Spencer, was an engineer who observed melting of peanut chocolate bar in his pocket while working with a new microwave vacuum tube

magnetron at Raytheon Corp, US in 1986, and first food deliberately cooked with Spencer microwave was popcorn, second was an egg, which accidently explode in the face of one of the experimenters.[45] Microwave irradiation got its application in organic synthesis as reported by Gedye et. al (1986),[45] where four 4 different types of reactions were carried out, namely; acid hydrolysis of benzamide, permanganate oxidation of toluene, esterification of benzoic acid with alcohol and S_N2 reaction of sodium-4-cyanophenoxide with benzylchloride in ethanol.[49, 50] Acid hydrolysis of benzamide to benzoic acid is shown in Table 1.

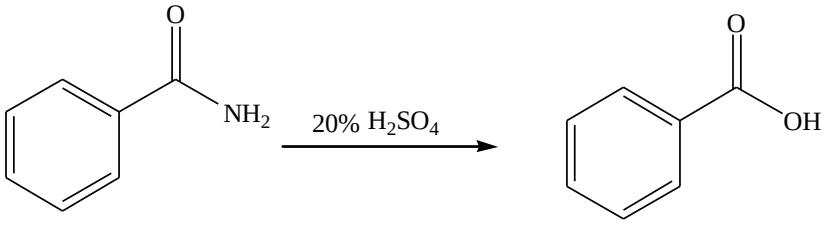


Figure 2.42: Acid hydrolysis of benzamide to benzoic acid

Table 1: Comparison of reflux and microwave heating methods

Method	Reaction time (min)	Yield (%)
Reflux	60	90
Microwave	10	99

These synthetic methods were applied to various organic transformations. Since mid-90's, a significant number of publications has been produced. At the present, application of microwave irradiation has become increasingly acceptable in almost all specialized areas of chemistry and researches especially microwave-assisted organic synthesis. Literally, "all new compounds have their first synthesis in a microwave."[51]

2.6.2.2. What Are Microwaves?

Microwaves are electromagnetic waves having a wavelength of 1m-1mm correspond to frequencies in the range of 0.3-300 GHz. Microwaves lie between infrared (IR) and radio wave region in the electromagnetic spectrum Fig. 2.42

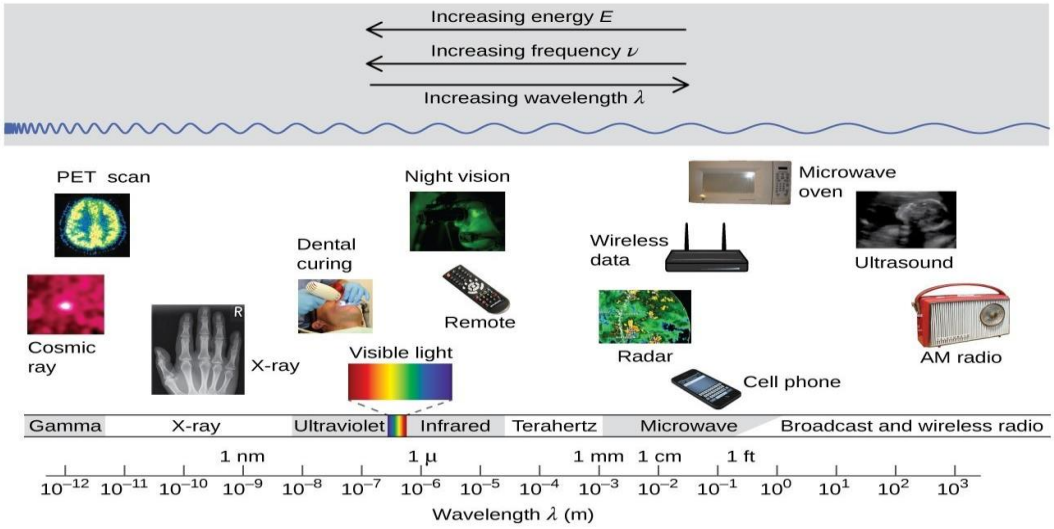


Figure 2.43: Electromagnetic spectrum

Microwaves can be ionizing or non-ionizing. The non-ionizing microwaves are the ones used in radios, cell phones, telecommunications, televisions, wireless network and radars. Microwave irradiation usually operate at wavelength of, 12.2cm, frequency of 2.45 GHz and the energy (E) of microwave photon of this frequency, 0.037 KCal is too low to cleave the molecular bond (unlike UV radiations) but do cause molecular vibration and rotation.[52, 53]

2.6.2.3. Principles of Microwave Activation

Organic transformations can be caused to occur via two ways, by conventional (reflux) or through microwave heating.

I. Reflux heating is a conduction heating method in which the heat is transferred into the substance first from the wall, surface of the vessel, to the mixture and eventually to the reacting species. This leads to slow activation of the materials. Hence, the method could be slow.

II. Unlike reflux heating, microwave heating is an electromagnetic heating method in which the core mixture is heated directly regardless of the transfer of heat from the vessel to the reacting mixture which results into a rapid rise in the temperature (increase in rate) and instantaneous local super heating can also acieved.[53, 54]

2.6.2.4. Differences between Reflux and Microwave Heating

A summary of the differences among the two methods is given in Table 2.[52, 54, 55]

Table 2: Difference between reflux and microwave heating methods

	Reflux heating	**Microwave heating**
Heating	by thermal or electric source	by electromagnetic source
Mechanism of heating	by conduction	by energetic coupling, it involves dielectric polarization and conduction
Transfer of energy	occurs from the wall to the surface of the vessel to the reacting mixture and eventually to the reacting species	the core of the reacting mixture is heated directly
Super heating	absence of superheating	super heating occurs (i.e., the temperature of the mixture can rise above its boiling point
Heating selectivity	non-selective heating (i.e., all components of the mixture are heated equally)	Selective heating (i.e., specific components can be heated specifically
Safety	High probability risk	safe, simple and clean procedure

2.6.2.5. Microwave Solvents

Every solvent and reagent absorbs microwave irradiation in different ways as they have different degree of polarities within the molecule. Thus, they will be affected either less or more by changing the microwave energy. Polar solvents with high dielectric constant couple with microwaves and reach high temperature in a short time (i.e., are microwave-active), while non-polar solvents are transparent to microwave energy and hence, microwave inactive.[56] However, polarity of the solvent is not the only determining factor of the true absorbance of microwave energy nevertheless, may give insight and provide good frame of reference. Other factors such as, loss tangent (δ) should also be considered when choosing a suitable solvent for microwave synthesis. The ability of a substance to absorb microwave energy and convert it into heat is referred to as loss of tangent and is expressed in term of tangent value:

$$\tan\delta = \acute{\varepsilon}/\check{\varepsilon} \qquad \text{equation (1)}$$

where έ is the dielectric constant (polarity) and ε̌ is the dielectric loss factor.

The higher the tangent value ($tan\delta$), the better is the solvent at absorbing microwave energy and thus better heat is generated. Table 3 shows the loss tangent values of some common pure solvents at room temperature.[57]

Table 3: Dielectric constant and loss of tangent values of some common pure solvents

Solvent	**Dielectric constant (έ)**	**Loss of tangent ($tan\delta$) / 2.45 GHz**
Ethylene glycol	38	1.17
Ethanol	24	0.94
Dimethyl sulfoxide	47	0.82
Methanol	33	0.66
Acetic acid	6.1	0.17
Dimethylformamide	37	0.16
Water	80	0.12
Chloroform	4.8	0.091
Acetonitrile	38	0.062
Ethyl acetate	6.0	0.059
Acetone	21	0.054
Tetrahydrofuran	7.6	0.047
Dichloromethane	9.1	0.047

From Table 3, it is evident that the like of water has high dielectric constant (more polar) than ethanol but ethanol is more active solvent under microwave condition than water why because the former has higher loss tangent value than the later.

Another important point to be considered is that most organic solvents are non-polar and as mentioned earlier, non-polar solvents are microwave inactive. So, with this regard, two conditions are possible for that reaction mixture to be heated under microwave.[58]

I. Non-polar solvent but polar reagents or at least one polar reagent,
II. When both the solvent and the reagents are non-polar, weflon can be added in order to heat the reaction mixture.

2.6.2.7. Advantages of Using Microwave in Organic Synthesis

The followings are some of the advantages of using microwave energy particularly in the synthesis of organic molecules[59, 60]

- Rapid reaction
- High degree of purity
- Less by products
- Better and improved yield
- Experimentally simple and feasible
- Wider temperature range
- High energy efficiency
- Selectively directive
- Modular system enable changing from mg to kg
-

2.6.2.8. Disadvantage of Using Microwave

- The reaction cannot be monitored directly or visually inspected as it progresses
- Addition of a catalyst or a reagent while running the reaction may disrupt the entire process
- The equipment is expensive compared to the conventional once
- Closed reacting vessel can be burst
- Solvent at high pressure may result in pressure build up, explosion potential may increase

3. MATERIALS AND METHODS

3.1 Materials

5-chloro-2(3H)-Benzoxazolone, 1-phenylpiperazine, trifluoromethylphenylpiperazine, 4-methylpiperadine, 37% (w/v) formalin solution and methanol used as starting materials were purchased from Sigma-Aldrich (Germany) and were used without further drying.

3.2. Thin Layer Chromatographic Method

3.2.1. Material

Thin-layer chromatography (TLC) was carried out on Silica gel/TLC-plates (DC-Alufplien-Kieselgel, Germany) and solvents used were benzene, ethyl acetate, hexane and methanol. Silica gel plate was detected under UV-light (254 nm).

Three different mobile phases were prepared with different solvents at different ratios as follows;

J_1: Benzene – Methanol (5: 1, v/v)

J_2: Ethyl acetate – Hexane (3: 6, v/v)

J_3: Benzene – Methanol (9: 1, v/v)

3.2.2. Method

The mobile phase (solvents) was poured into the TLC chamber to a depth of about 0.5 cm. The chambers were covered with watch glass, gently swirled and allowed to stand while assembling the plates.

6×3 cm plates were prepared for three different spots while 0.5 cm line of origin was gently drawn away from the bottom with a pencil.

5-chloro-2(3H)-Benzoxazolone, piperazine, piperidine derivatives (starting materials) were dissolved in methanol and the products were dissolved in an appropriate solvent. Spots were made on the plate with the aid of a microcapillary and gently placed to the TLC chamber, covered with watch glass and left undisturbed. It was allowed to develop until it moved to the solvent front. The plate was removed, the solvent front was marked with a pencil and the plate was allowed to dry. UV-light at wavelength (254 nm) was used to visualize the spots and the retention factor values were calculated.

3.3. Melting Point

Melting point of the compounds was recorded on the Mettler Toledo FP900 Thermosystem digital melting point apparatus and the values are uncorrected.

3.4. Microwave

Microwave irradiation was carried out in a microwave oven (MicroSYNTH, Milestone, Italy).

3.5. Spectroscopy

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, Department of Chemistry, Science and Arts Faculty (Istanbul, Turkey).

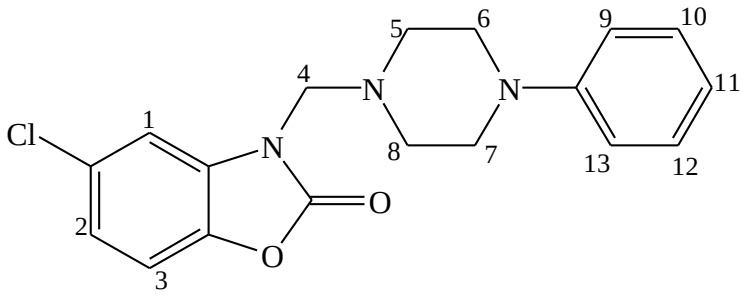
^{1}H-NMR Spectra: The ^{1}H-NMR spectra of the compounds were recorded on a Mercury Varian 400 MHz NMR Spectrometer using deuterated chloroform ($CDCl_3$) as a solvent at Boğaziçi University, Research and Development Laboratories (Istanbul, Turkey). Chemical shifts (δ) values were reported in parts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

3.6. Experimental

Experimental procedures were taken from the literature.[8, 29]

3.6.1. Synthesis of Compound 1

3-(4-phenylpiperazin-1-yl)methyl-5-chloro-2(3H)-Benzoxazolone



Reflux

200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone and 0.18 ml (0.001 mol) of 1-phenylpiperazine were dissolved in 8 mL of methanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of methanol and then poured into the reaction mixture. The solution was refluxed in a water bath for 60 min. After completion, the mixture was poured into crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method to yield a crude product which was subsequently washed with ethanol and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with ethanol.

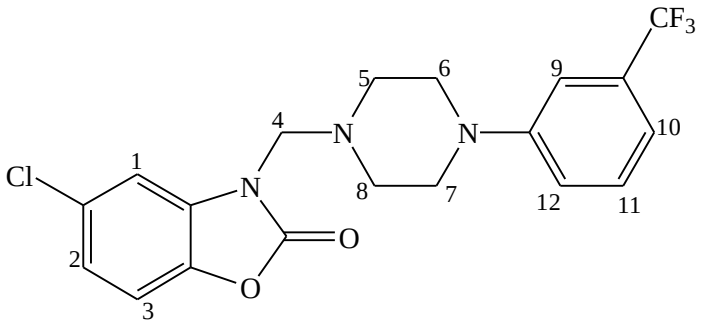
Microwave

200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone and 0.18 ml (0.001 mol) of 1-phenylpiperazine were dissolved in 8 mL of methanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of methanol and then poured into the reaction mixture. The solution were placed in a microwave oven and irradiated at 3 min, 150 w, 65 ^{o}C / 5 min, 100 w, 65 ^{o}C the mixture was poured into crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method to yield a crude product which

was subsequently washed with ethanol and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with ethanol.

3.6.2. Synthesis of compound 2

3-[4-(trifluoromethylphenyl)piperazin-1-yl]methyl-5-chloro-2(3H)-Benzoxazolone



Reflux

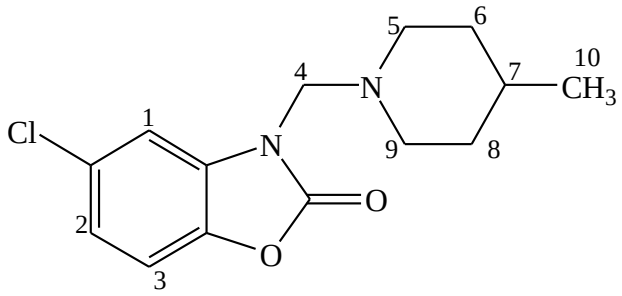
200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone and 271.56 mg (0.001 mol) of 1-[3-(trifluoromethyl)phenyl]piperazine were dissolved in 8 mL of methanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of methanol and then poured into the reaction mixture. The solution was refluxed in a water bath for 60 min. After completion, the mixture was poured into crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method to yield a crude product which was subsequently washed with methanol and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with methanol.

Microwave

200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone and 271.56 mg (0.001 mol) of 1-[3-(trifluoromethyl)phenyl]piperazine were dissolved in 8 mL of methanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of methanol and then poured into the reaction mixture. The reaction mixture were placed in a microwave oven and irradiated at 3 min, 150 w, 65^{o}C / 5 min, 100 w, 65^{o}C and the resulting solid was filtered using vacuum filtration method to yield a crude product which was subsequently washed with methanol and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with methanol.

3.6.3. Synthesis of compound 3

3-(4-phenylpiperidin-1-yl)methyl-5-chloro-2(3H)-Benzoxazolone



Reflux

200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone, and 0.14 ml (0.001 mol) of 4-methylpiperidine were dissolved in 8 mL of methanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of methanol and then poured into the reaction mixture and refluxed in a water bath for 60 min. After completion, the mixture was poured into

crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method to yield a crude product which was subsequently washed with cyclohexane and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with cyclohexane.

Microwave 1

200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone, and 0.14 ml (0.001 mol) of 4-methylpiperidine were dissolved in 8 mL of methanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of methanol and then poured into the reaction mixture. The mixture is placed in a microwave oven and irradiated at 3 min, 150 w, 65 ^{o}C / 5 min, 100 w, 65 ^{o}C. After completion, the mixture was poured into crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method to yield a crude product which was subsequently washed with cyclohexane and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with cyclohexane.

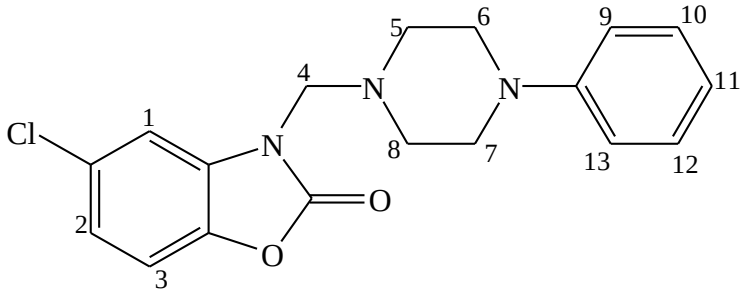
Microwave 2

200 mg (0.001 mol) of 5-Chloro-2(3H)-Benzoxazolone, and 0.14 ml (0.001 mol) of 4-methylpiperidine were dissolved in 8 mL of ethanol in 50 ml round bottom flask. 0.2 ml of 37% (w/v) formalin solution were mixed with 2 ml of ethanol and then poured into the reaction mixture. The mixture is placed in a microwave oven and irradiated at 3 min, 150 w, 65 ^{o}C / 5 min, 100 w, 65 ^{o}C. After completion, the mixture was poured into crushed ice upon which a precipitate was formed. The resulting solid was filtered using vacuum filtration method to yield a crude product which was subsequently washed with cyclohexane and allowed to dry at room temperature. The reactions were monitored by TLC and resulting precipitate was purified by recrystallization with cyclohexane.

4. RESULTS AND DISCUSSION

4.1. Results

Compound 1



Reflux

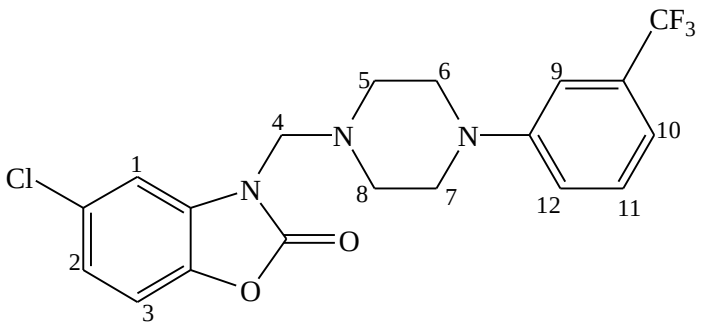
- White crystalline solid was obtained with a yield of 51.3 % (176.23 mg) and a melting point of 165.6 ºC.
- TLC in the J_1, J_2, and J_3 mobile phases gave R_f values of 0.55, 0.54, 0.41 respectively.
- Fourier Transform Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed stretches at 2828.5 cm^{-1} (C-H stretch) and 1782 cm^{-1} carbonyl group (C=O stretch).

Microwave

- White crystalline solid was obtained with yield of 67.7 % (243.74 mg) of melting point of 165 ºC.
- TLC in the J_1, J_2, and J_3 mobile phases gave R_f values of 0.56, 0.54, 0.48 respectively

- Fourier Transform Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed stretches at 2828.3 cm^{-1} (C-H stretch) and 1782 cm^{-1} carbonyl group (C=O stretch).
- Proton Nuclear Magnetic Resonance Spectroscopy (^1H NMR, $CDCl_3$; ppm) δ: 6.8-7.4 (m; 8H; Aromatic-H); 4.6 (s; 2H; H^4); 3.2 (t; 4H; pip H^6-H^7); 2.8 (t; 4H; pip H^5-H^8) ppm.

Compound 2



Reflux

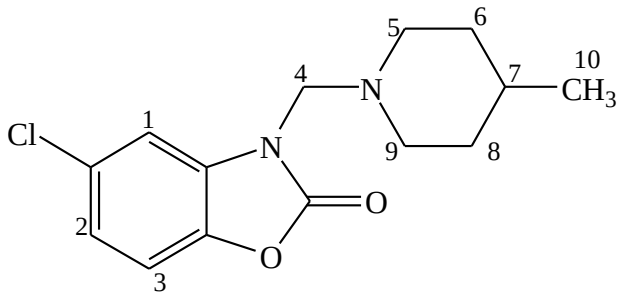
- White crystalline solid was obtained with yield of 38.7 % (154.6 mg) of melting point of 145 ^oC.
- TLC in the J_1, J_2 and J_3 mobile phases gave R_f values of 0.65, 0.62, 0.54 respectively.
- Fourier Transforms Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed stretches at 2937.3 – 2855.2cm^{-1} (C-H stretch) and 1770 cm^{-1} carbonyl group (C=O stretch).

Microwave

- White crystalline solid was obtained with yield of 50.1 % (198.26 mg) of melting point of 145 ^oC.
- TLC in the J_1, J_2 and J_3 mobile phases gave R_f values of 0.66, 0.63, 0.53 respectively.

- Fourier Transforms Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed broad peak at 3361.7cm^{-1} (O-H stretch) and 1768.8 cm^{-1} carbonyl group (C=O stretch).
- Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR, $CDCl_3$; ppm) δ: 6.9-7.4 (m; 7H; Aromatic-H); 4.6 (s; 2H; H^4); 3.2 (t; 4H; pip H^6-H^7); 2.8 (t; 4H; pip H^5-H^8) ppm.

Compound 3



Reflux

- White crystalline solid was obtained with yield of 52.2 % (146.3 mg) of melting point of 91.74 ºC.
- TLC in the J_1, J_2 and J_3 mobile phases gave the R_f values of 0.64, 0.48, 0.51 respectively.
- Fourier Transforms Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed a peak at 2923.5 cm^{-1} (C-H stretch) and 1780.8 cm^{-1} carbonyl group (C=O stretch).
- Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR, $CDCl_3$; ppm) δ: 7.1-7.3 (m; 3H; Ph-H); 4.6 (s; 2H; H^4); 3 and 1.6 (dd; 4H; pipd H^6-H^8); 2.3 (t; 4H; pipd H^5-H^9); 1.2-1.4 (m; 1H; pipd H^7); 0.8 (d; 3H; H^{10}) ppm.

Microwave 1

- White crystalline solid was obtained with yield of 28.8 % (80.6 mg) melting point of 92 ^{o}C.

- TLC in the J_1, J_2 and J_3 mobile phases gave R_f values of 0.55, 0.40, 0.52 respectively.

- Fourier Transform Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed a peak at 2923.7 cm^{-1} (C-H stretch) and 1779.6 cm^{-1} carbonyl group (C=O stretch).
- Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR, $CDCl_3$; ppm) δ: 7.1-7.3 (m; 3H; Aromatic-H); 4.6 (s; 2H; H^4); 3 and 1.6 (dd; 4H; pip H^6-H^8); 2.3 (t; 4H; pip H^5-H^9); 1.2-1.4 (m; 1H; pip H^7); 0.8 (d; 3H; H^{10}) ppm.

Microwave 2

- White crystalline solid was obtained with yield of 50.1 % (140.5 mg) of melting point of 90.7 ^{o}C.

- TLC in the J_1, J_2 and J_3 mobile phases gave R_f values of 0.56, 0.48, 0.52 respectively

- Fourier Transform Infrared (FT-IR) spectroscopy (ν_{max})**:** FT-IR showed a peak at 2723.7 cm^{-1} (C-H stretch) and 1779.9 cm^{-1} carbonyl group (C=O stretch).

4.2. Discussion

In this research, three compounds have been synthesized by following literature procedures based on 5-chloro-2(3H)-Benzoxazolone structure. These compounds have been previously synthesized under reflux condition.[8, 9] In this present work, however, a facile and efficient approach for the synthesis of these compounds under microwave condition was studied. Reactions were also conducted under reflux condition to maintain the reaction conditions so as to draw a comparison between these two different methods.

Two piperazine and one piperidine derivative were attached to heteroatomic N-atom at position 3 of 5-chloro-2(3H)-Benzoxazolone via Mannich reaction to give the target compounds. The structures of the synthesized Mannich bases are shown in the figure below;

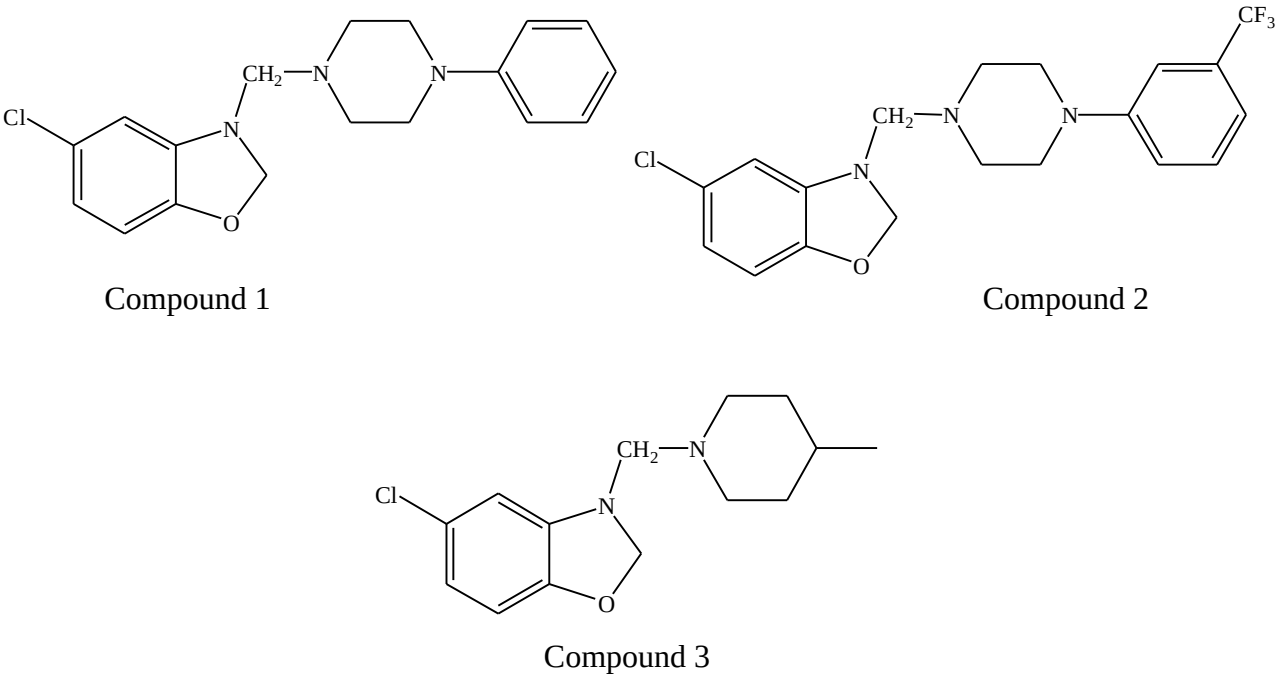
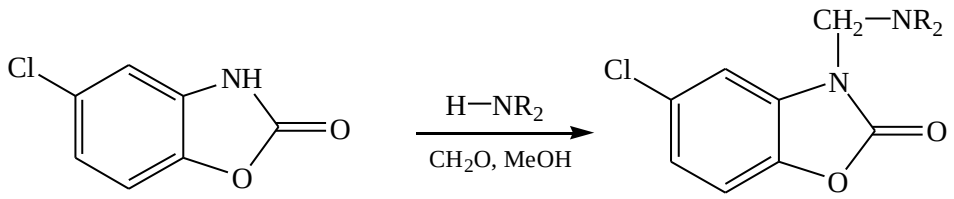


Figure 4.1: Structures of compounds 1, 2 and 3

The core structures of these three compounds are the same. They only differ in the amine moiety on position 3 of the 5-chloro-2(3H)-Benzoxazolone structure.

Compound 1 has 1-phenylpiperazine, compound 2 has 1-[3-(trifluoromethyl)phenyl]piperazine while compound 3 has 4-methylpiperidine on position 3 respectively. Fig. 4.2, and 4.3, give the general synthesis and Mannich reaction mechanism of the compounds synthesized in this study.



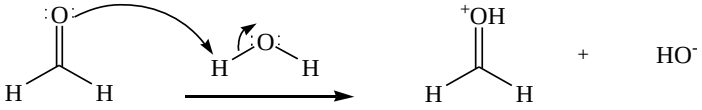
Where,

R_2 = 1-phenylpiperazine	Compound 1
R_2 = 1-[3-(trifluoromethyl)phenyl]piperazine	Compound 2
R_2 = 4-methypiperidine	Compound 3

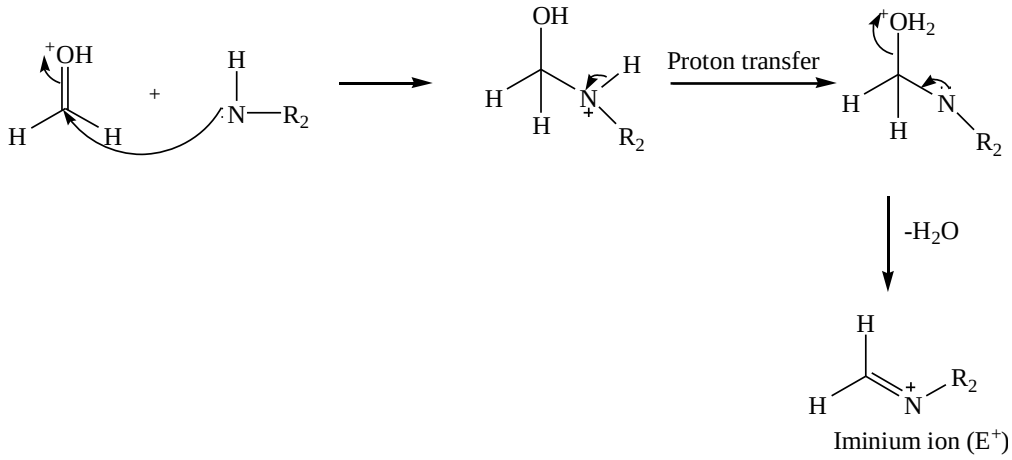
Figure 4.2: General synthesis of 5-chloro-3-substituted benzoxazolone molecules

The general reaction mechanism for this reaction follows two major steps; formation of iminium ion and attacking of iminium ion by the substrate (5-chloro-2-2(3H)-Benzoxazolone nucleus) as a nucleophile.

Formalin in solution, 37% w/v in H_2O



First step: formation of iminium ion



Second step: involve attacking iminium ion by the substrate and deprotonation of active hydrogen by nucleophile to give the target product.

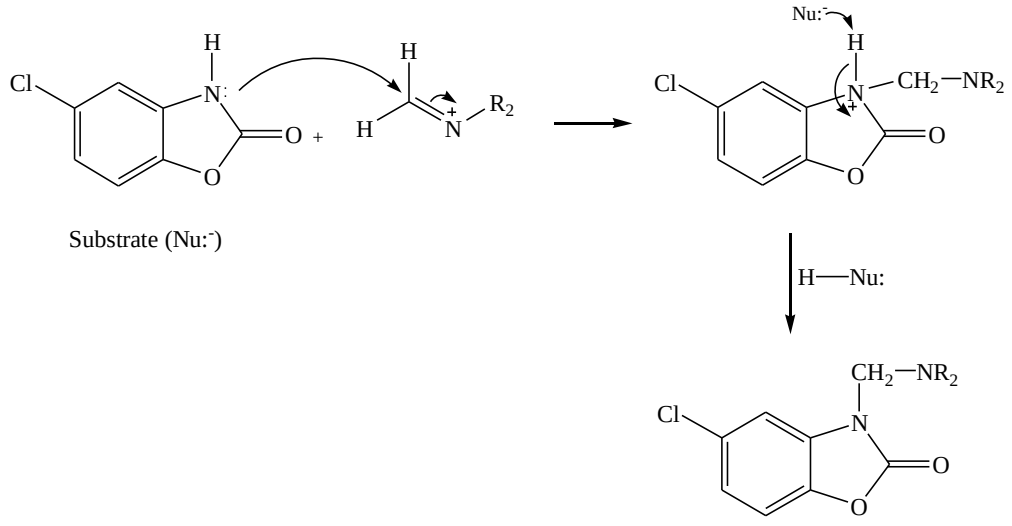
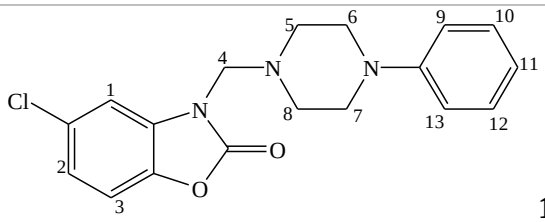
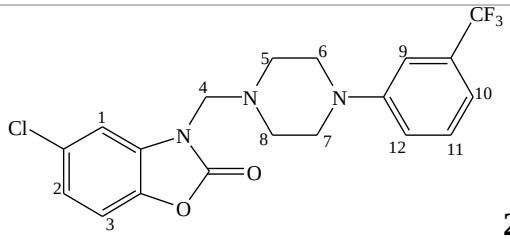
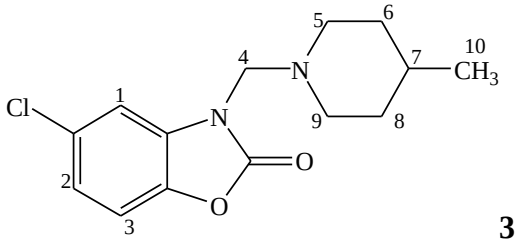


Figure 4.3: Mannich reaction mechanism of 5-chloro-2(3H)-Benzoxazolone derivative of phenyl piperazine/piperidine

In the microwave synthesis method, the reaction times for all the compounds were greatly reduced from 60 min to 8 min as compared to reflux as expected. Under microwave reaction condition, the yields were slightly improved as well for compounds 1 and 2 but surprisingly for piperidine derivative (compound 3) synthesized in methanol, the yield was less compared to the yield obtained from reflux condition. Comparison of the yields and time of microwave synthesis method versus the conventional reflux method is shown detail in Table 4. Further optimization for microwave synthesis is needed to be able to make a better comparison.

Table 4: Comparison of % yields and time between reflux and microwave synthesis method

Compound	Method	Melting point (ºC)	Yield (%)	Time (min)
 **1**	**Reflux**	165.6	51.3	60
	Microwave	165	67.7	8
 **2**	**Reflux**	145	38.7	60
	Microwave	145	50.1	8
 **3**	**Reflux**	91.7	52.2	60
	Microwave 1	92	28.8	8
	Microwave 2	90.7	50.1	8

Compound 3 was also synthesized via microwave in ethanol and the yield was observed to have increased from 28.8 % to 50.11 %. This may be due to relatively higher boiling point and higher loss of tangent value of ethanol which required more heat compared to methanol. However, it is necessary to repeat this reaction under reflux condition by using ethanol as a solvent to have a reliable comparison.

The synthesized compounds were characterized by Fourier Transform Infra-Red (FT-IR) 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 all the three synthesized compounds show the absence of N-H stretch which is reported to come around 3146 cm^{-1}, which indicates that the reaction has actually taken place at position 3 of 5-chloro-2(3H)-benzoxazolone.

As expected at 1768 – 1782 $cm,^{-1}$ carbonyl (C=O) stretch was observed in FT-IR spectra of all three compounds similar to reported values in the literature. Also C-H stretches are observed around 3796-3065 cm^{-1} as expected. Broad peak at 3361.7 cm^{-1} of compound 2 shows the presence of O-H stretches which should not be present but maybe due to the presence of moisture in the compound. The FT-IR spectra of the compounds synthesized via microwave are shown in Fig. 4.4, Fig. 4.5 and Fig. 4.6 below;

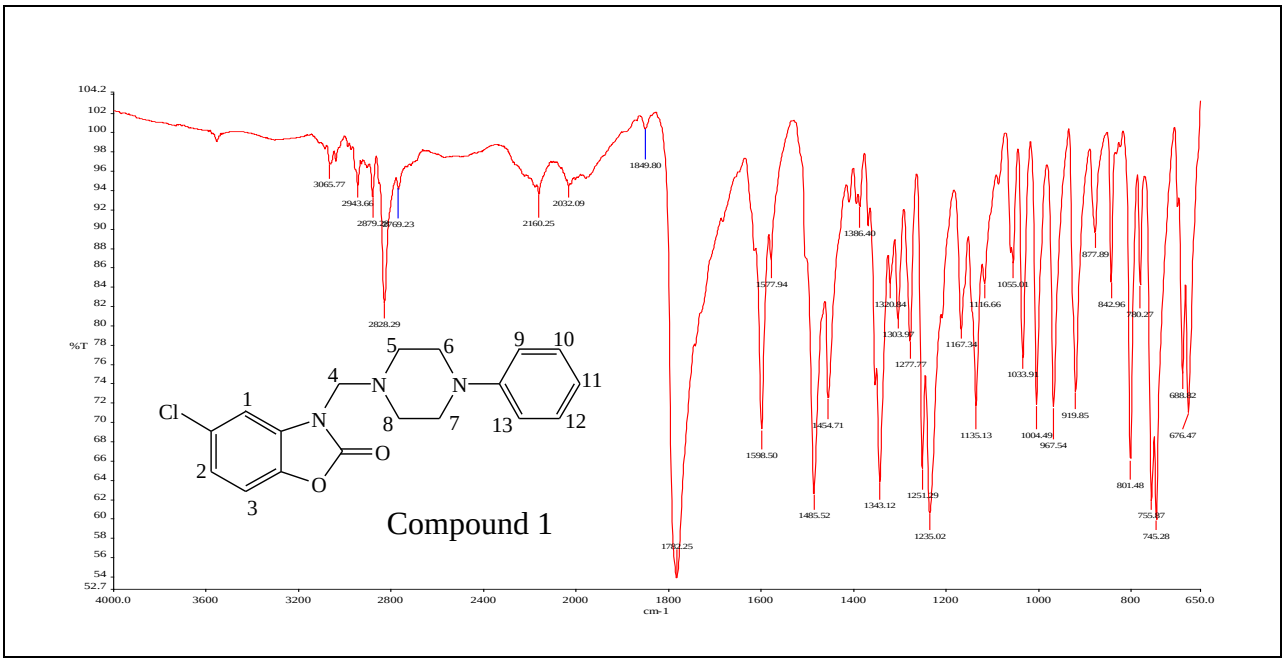


Figure 4.4: FT-IR Spectrum of 3-(4-phenylpiperazin-1-yl)methyl-5-chloro-2(3H)-Benzoxazolone

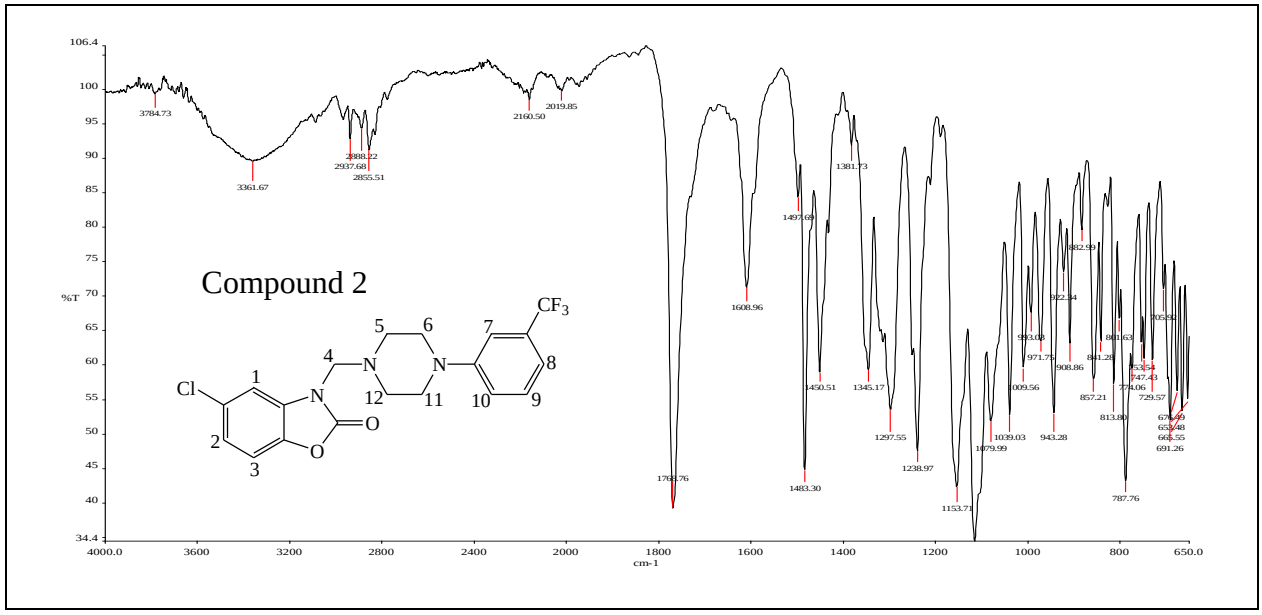


Figure 4.5: FT-IR Spectrum of 3-[4-(trifluoromethylphenyl)piperazin-1-yl]methyl-5-chloro-2(3H)-Benzoxazolone

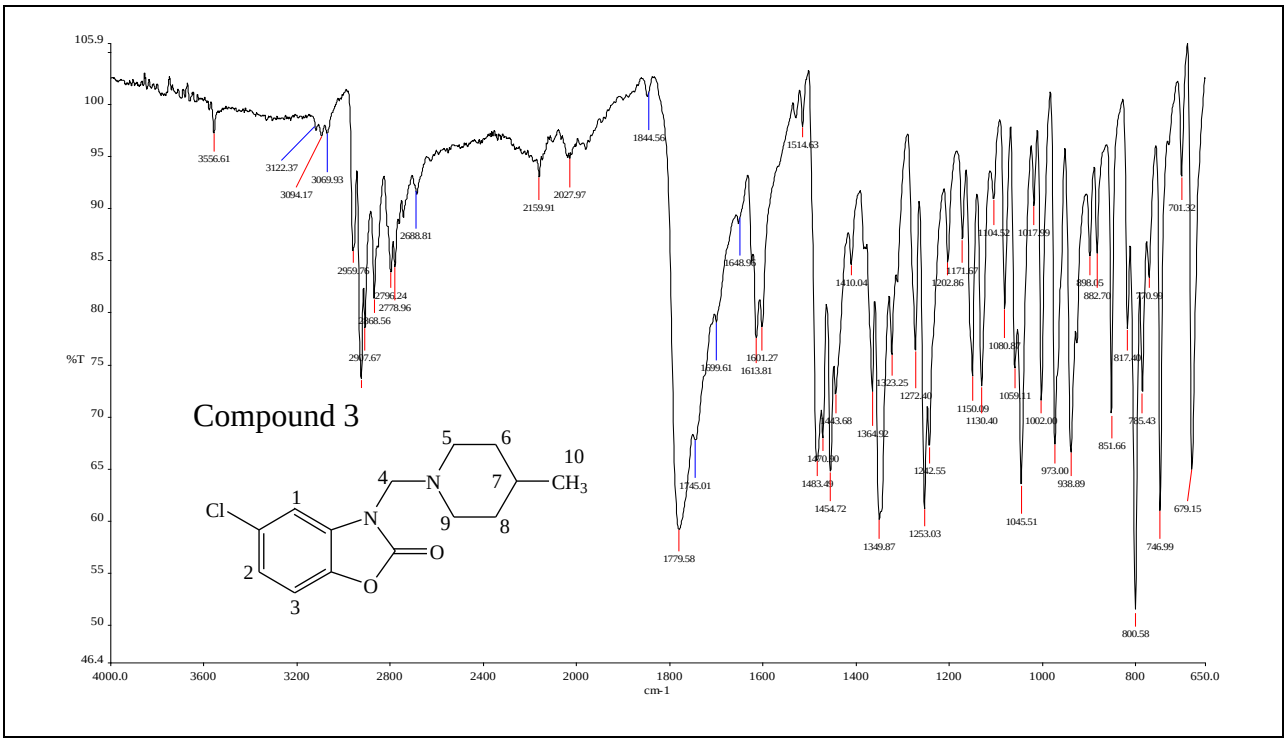


Figure 4.6: FT-IR Spectrum of 3-(4-phenylpiperidin-1-yl)methyl-5-chloro-2(3H)-Benzoxazolone

^{1}H-NMR spectra of compounds 1, 2 and 3 in $CDCl_3$ show peaks at expected chemical shifts values. In all spectra, relative to the starting material (5-chloro2-3(H)-Benzoxazolone), there is an additional CH_2 (methylene) peak as a singlet observed at 4.6 ppm for both compounds. This also proves that the reaction has taken place at the N-atom in position 3 and piperazine or piperidine is bonded to 5-chloro-2(3H)-Benzoxazolone via a CH_2 bridge.

Further investigations of ^{1}H-NMR spectra reveal the presence of aromatic peaks as multiplets between 6.8 to 7.3 ppm which is expected. The piperazine protons (H^6 and H^7) and (H^5 and H^8) were seen as triplets at 2.8 and 3.2 ppm respectively for both compounds 1 and 2. This indicated that less shielded protons (H^6 and H^7) are closer to the piperazine nitrogen next to the electron withdrawing group, benzene, while more shielded protons (H^5 and H^9) are closer to the piperazine nitrogen next to the electron releasing group methylene.

Additional peaks are observed in compound 3 due to asymmetric hydrogen of piperidine.

^{1}H-NMR spectra of compounds 1, 2 and 3 in $CDCl_3$ are shown in Fig. 4.7, Fig. 4.8 and Fig. 4.9.

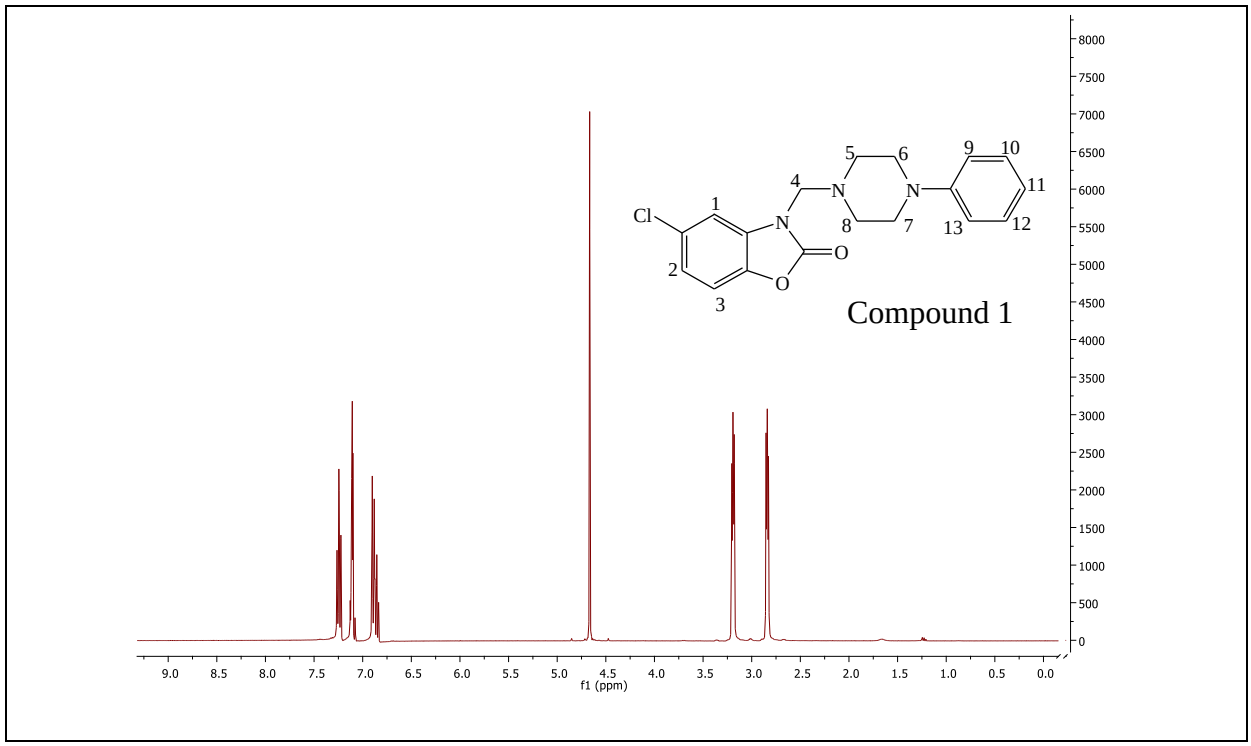


Figure 4.7: ^{1}H NMR Spectrum of 3-(4-phenylpiperazin-1-yl)methyl-5-chloro-2(3H)-benzoxazolone

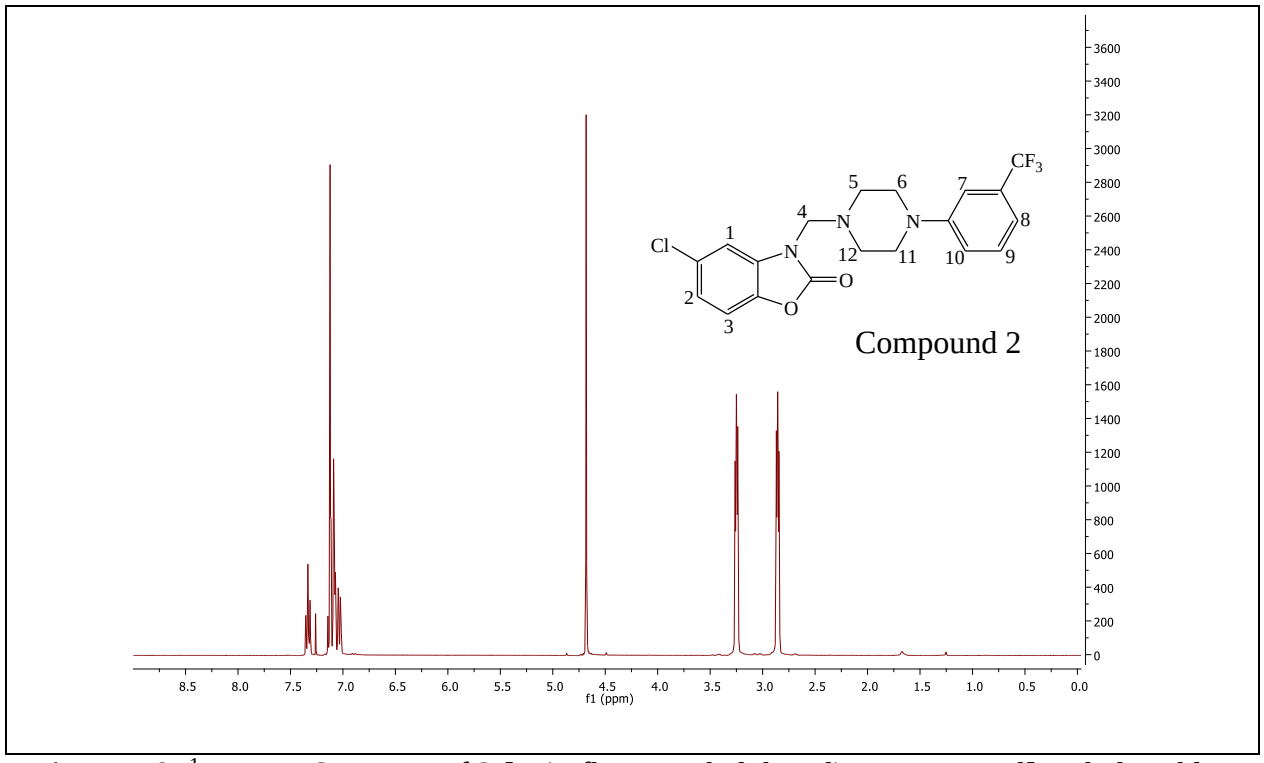


Figure 4.8: ^{1}H NMR Spectrum of 3-[4-(trifluoromethylphenyl)piperazin-1-yl]methyl-5-chloro-2(3H)-Benzoxazolone

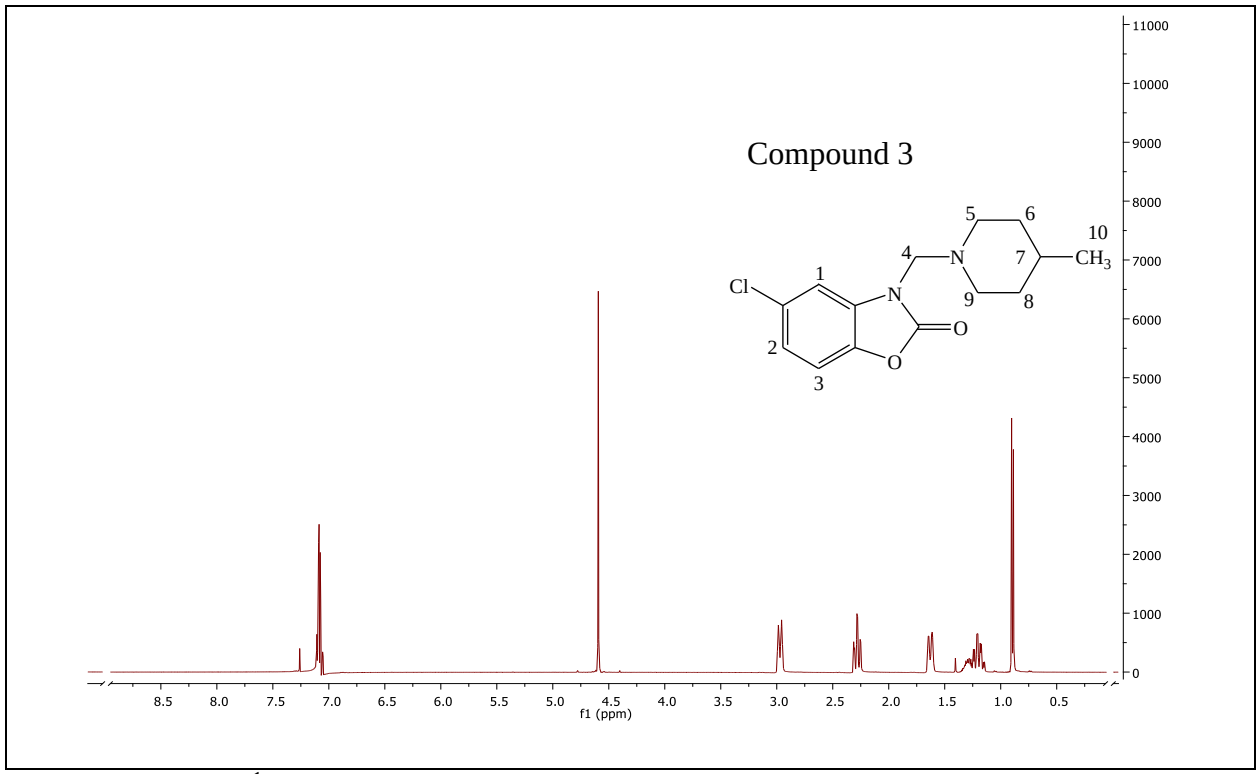


Figure 44: ^{1}H NMR Spectrum of 3-(4-phenylpiperidin-1-yl)methyl-5-chloro-2(3H)-Benzoxazolone

5. CONCLUSION

Three different Mannich bases of 5-chloro-3-substituted benzoxazolone derivatives were synthesized in this study using the classical Mannich reaction via two different methods; reflux and microwave synthesis. Short reaction time, improved yield, simple, clean and less energy required are the advantage of microwave over the conventional synthesis method. So, in the future studies, the use of microwave synthesis with different solvents and different heating rates need to be developed to improve yield and efficiency.

Biological activity of all the synthesized compounds were not conducted due to time constrained. However, based on the literature studies, all of the three compounds synthesized in this study might have some biological properties. Activity studies apart from analgesic and anti-inflammatory activities are intended to be made in the future since it is possible to do different substitutions at different sites of 5-chloro-2(3H)-Benzoxazolone structure and different amine groups can also be substituted at heteroatomic nitrogen in position 3, that could potentially change the biological activities of these types of compounds.

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