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HEALTH SCIENCES INSTITUTE

SYNTHESIS AND CHARACTERIZATION OF 3-METHYLPIPERIDINE SUBSTITUTED BENZOXAZOLINONE

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Advisor

Assist. Prof. Dr. Yusuf MÜLAZİM

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THE DIRECTORATE OF HEALTH SCIENCE INSTITUTE

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

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

Prof. Dr. K. Hüsnü Can Başer

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Firstly, I am grateful to my wife and mum (Hajia Sa'adatu) for their enormous support towards house keeping in my absent and numbers friends who endured this long process with me, always offering support and prayers toward my programme. I always remember you in mind with prayer late Father, Alhaji Mahmoud (Bako) may almighty Allah made pradise be your final distination.

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DEDICATION

I dedicate this thesis work to my parent for their support towards my education and good upbringing which are the driving keys for growth and development that lead to so many opportunities for entire life and success.

ABSTRACT

2(3)-Benzoxazolinone and its derivatives are important compounds reported to have diverse biological activities, particularly analgesic and anti-inflammatory. Amines such as piperidine as a substituent to benzoxazolinone structure reported to increase the analgesic and anti-inflammatory activities of such compounds.

This reseach work, focus on the synthesis of 3-methylpiperidine on 3rd position of benzoxazolinone structure using classic Mannich reaction by reflux and microwave (mw) heating techniques in effort to prepare bioactive compounds. The reactions was monitored by TLC and melting point determination, while the chemical structure of the compound was determined by FT-IR and ^{1}H-NMR analysis.

Keywords: 2(3H)-benzoxazolinone, Microwave, Mannich reaction, 3-methylpiperidine, Analgesics.

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ABBREVIATION

PPA - Polyphosphoric acid

DMF - *N*,*N*-dimethylformamide

THF - Tetrahydrofuran

COX - Cyclooxygenase

FT-IR - Fourier Transform - Infrared

UV-VIS - Ultra Violet - Visible Spectroscopy

NMR - Nuclear Magnetic Resonance

ATR - Attenuated Total Reflection

NSAID - Non-Steroidal Anti-inflammatory Drugs

TLC - Thin Layer Chromatography

TEA - Triethylamine

PPM - Parts per million

1. INTRODUCTION

Heterocyclic compounds such as indole, piperazine, furan, pyrrole and thiazole etc reported to have great diversity of biological activity and found interest in drugs desingn. [1-2] Benzoxazolinones also reported to be a promising group that provide an interesting building blocks for the synthesis of various biological active molecule for clinical used for example as a skeletal muscles relaxant. [3] Furthermore, Benzoxazolinone derivatives are biologically significant compounds which known to exhibit various biological activities such as anti-cancer, anti-microbial and anti-convulsant cardiotonic, antiulcer. [4]

Many studies reported in literature reveal that, Benzoxazolinone derivatives shows promoting analgesic and antiinflammatory activities. Especially, when there is an amine group at 3-position of the heterocycle, the analgesic activity seems to improve. Moreover, the side effects observed in some NSAIDs available on market have been reported to be less in these compounds. Therefore, there has been a great interest to utilizes benzoxazolinones and it derivatives to prepare COX-2 selective NSAIDs.

This research work, focus on synthesis of 3-[(3-methylpiperidine-1-yl)methyl]-2-benzoxazolinone which was reported to have exhibit anti-inflammatory activities. [5-11] Two different methods (microwave and reflux) were used in this studies to improve yield and reaction time. The synthesized compound was characterized by Fourier Transform Infra-Red (FT-IR) and Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopic methods. The purity of the compound was determined by melting point determination and thin layer chromatography (TLC).

2. LITERATURE REVIEW

2.1. Analgesics

Analgesics are types of drugs that normally use to eliminate or relieve pain from human body and it's also known as a pain killer. Therefore, these class of drugs can only relieve pain not used for treatment of diseases. Analgesics have been identified as one of the most commonly drugs prescribed among physicians nowadays. [12]

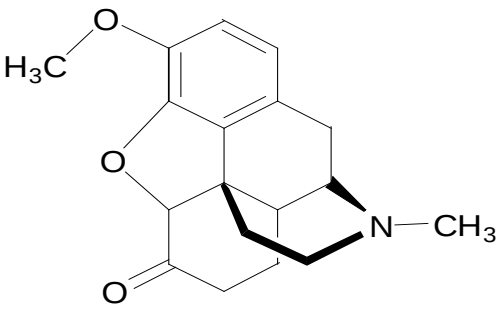
There are two major classification of analgesics drugs;

(I) Opioids (Narcotic) which include Morphine and Codeine

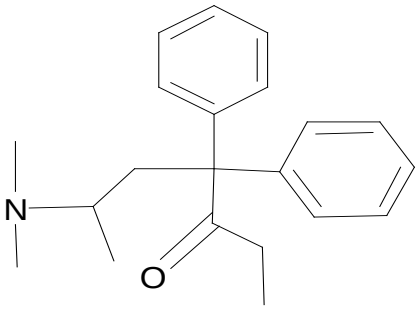
(II) Non-Opioids (non-narcotic) or Non-Steroidal Anti-inflammatory Drugs (NSAIDs) including aspirin and ibuprofen among others. [13]

2.1.1. Narcotic Analgesics

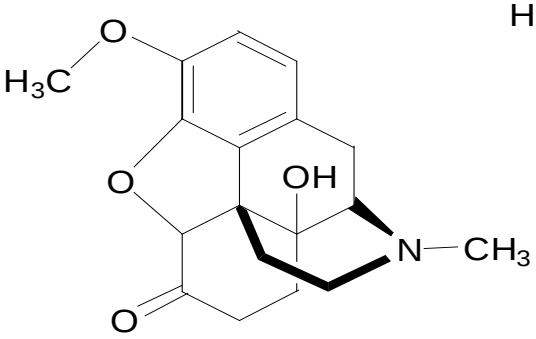
Opium was found to be among class of naturally occurring alkaloids known as opiates. Narcotics (opioids) are plant derivatives earliest known to be the most powerful native medications available that are usually apply for the treatment of pain such as cancer pain and relatives cases of serious pain. [14] It was reported that, narcotic analgesics affect central nervous system, narcotic analgesics drugs include hydrocodone, methadone, oxycodone and stadol among others are also found in some drugs for cough control. [15] Examples of these narcotic analgesic drugs are given in Figure 2.1.



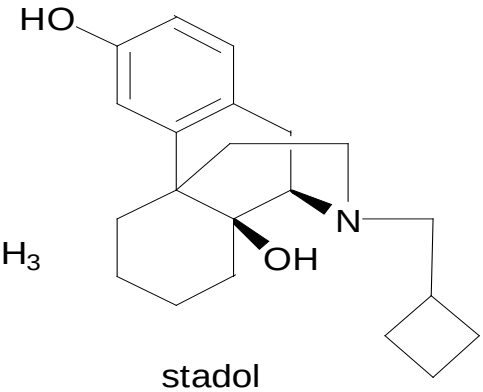
Hydrocodone



Methadone



Oxycodone



stadol

Figure 2.1: Examples of some narcotic analgesics drugs

2.1.1.1. History of Opium Plant

Opium is a natural plant substance extracted from unripe pods of the poppy flower and it belongs to species known as *papaver somni ferum*. Opium was first discovered in 15th century. It serves as a raw material for production of numerous drugs both legal (morphine, codeine, hydromorphone, oxycodone, hydrocodone, etc.) and illegal (heroin). Morphine and codeine are the most constituent opiates responsible for the pharmacological effects of opium which act by binding to opioid receptors throughout the body. [16] These analgesic compounds found to have used for over thousands of years for both recreational and medicinal purposes. Opium and it derivatives such as morphine, codeine and thebaine are highly addictive narcotic drugs. [17] Opium poppy flower and fruit is given in Figure 2.2 below.

Figure 2.2: Images of opium poppy flower and fruit

2.1.1.2. Morphine

Morphine is an alkaloid usually obtained from opium poppy plant known as *Papaver somniferum*, it's a natural product and it's derivatives are referred to as opiate. [18] Morphine is a non-synthetic narcotic agonist with high potential for abuse and its derivatives are clinically known to be the most powerful analgesic drugs for the treatment of acute and chronic pain. [19]

However, morphine tolerance and dependence are of two major health challenges over their effectiveness and usage. [20] The structural of morphine give in Figure 2.3.

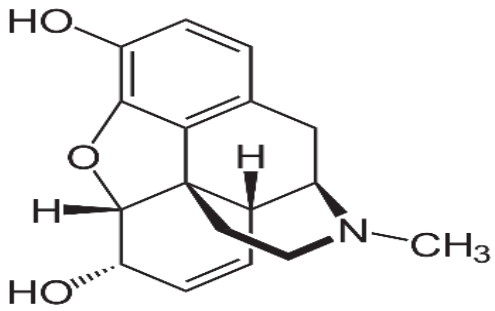
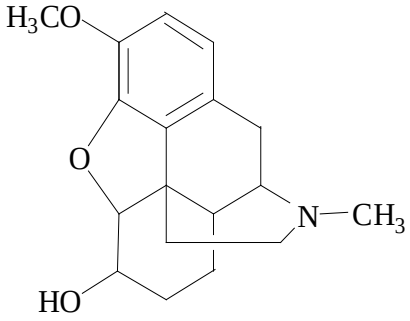


Figure 2.3: Structure of morphine

2.1.1.3. Codeine (Methylmorphine)

Codeine known as methylmorphine is an alkaloid extracted naturally from poppy plants which belongs to the family of opiates and it could be synthesized from morphine via methylation process. [21] Codeine is the most commonly used opioid analgesic and anti-tussive agent with minimal effect. [22]

However, onother formulation of opioid abuses are major serious concerned over the chronic opioid used may lead to addiction which can result to development of physical dependence and tolerance as a result of continued opioid over doses that occur outside medical supervision. [23] The structural given in Figure 2.4.

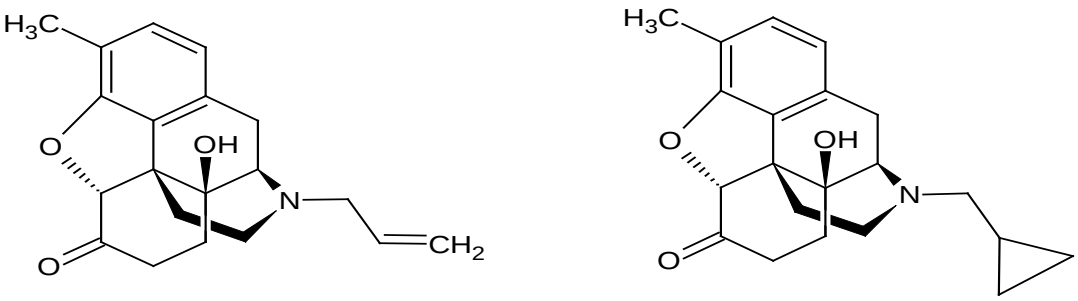


Codeine

Figure 2.4: Structure of codeine

2.1.1.4. Narcotic Antagonists

Narcotic antagonist includes naloxone and naltrexone. Naloxone is used in emergency conditions to counter the effect of opioid (morphine and codeine) overdose while naltrexone on the other hand is used primarily in the management of alcohol dependence and opioid dependence. [24]



(a) (b)

Figure 2.5: Structures of (a) naloxone and (b) naltrexone

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

Non-steroidal anti-inflammatory drugs are among major group of compounds used in a clinical analgesia which act on peripheral nervous system. These drugs agent, that provide analgesic, anti-inflammatory, and anti-pyretic effects are used for the treatment of a variety of disorders. [25]

The NSAIDs inhibit the rate-limiting enzyme cyclooxygenase (COX) in prostaglandin synthesis. Therefore, NSAIDs can be classified in two classes.

1) Non-selective inhibitors and

2) Selective inhibitors.

Non-steroidal anti-inflammatory drugs agents that inhibit both cyclooxygenase COX-1 and COX-2 are refers to **Non-selective inhibitors** while NSAIDs agent that inhibit specifically to COX-2 enzymes are called **Selective inhibitors**.

The inhibition of cyclooxygenase COX-1 by non-steroidal anti-inflammatory (NSAIDs) in most cases lead to uncontrolled bleeding or gastrointestinal ulcer formation, while those drugs that are selectively inhibition of cyclooxygenase COX-2 may have less gastrointestinal toxicity. [26]

2.1.2.1. Non-Steroidal Anti-inflammatory Drugs Non-Selective Inhibitors

Non-selective NSAIDs are drugs that inhibit both types of the COX enzymes are associated with an increased risk of gastric ulceration, presumed to be both through the reduction in gastric protection that is provided by prostaglandins and direct irritation of the gastric lining. [26] Examples of non-selective NSAIDS include: a) para-amino phenol derivatives. e.g. paracetamol b) Salicylic acid derivatives. e.g. aspirin c) Indole and indene acetic acids e.g. Indomethacin d) Propionic acid derivatives e.g. ibuprofen.

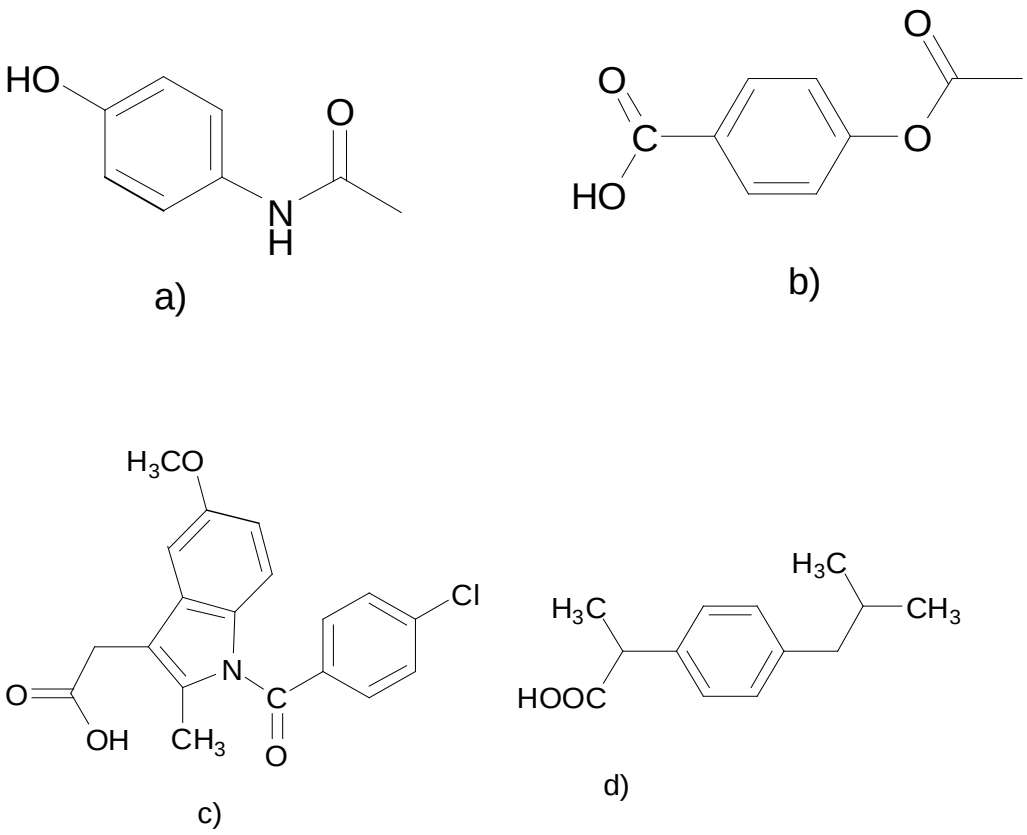


Figure 2.6: Structure of non-selective drugs (NSAIDs) inhibitors

2.1.2.2. COX-2 Selective inhibitors

Selective COX-2 inhibitors, which are specifically targeting COX-2 enzymes prevents the production of prostaglandins that often cause the pain and swelling of inflammation and other painful conditions. These drugs agents usually work as selective COX-2 inhibitors which, the type of drugs includes, a) Celecoxib, b) valdecoxib c) Rofecoxib and d) Etoricoxib are commonly used for the treatment of pain, rheumatoid arthritis. [27-28]

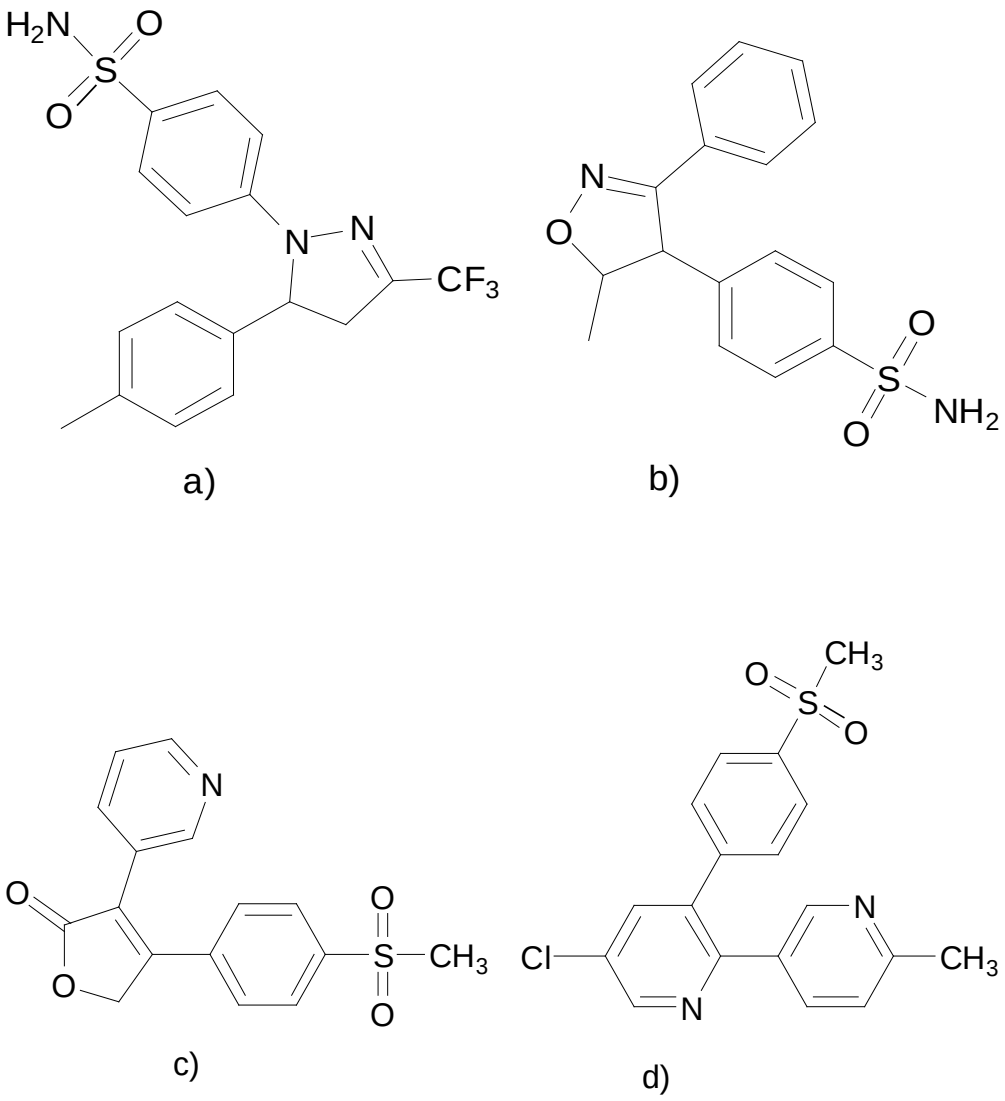


Figure 2.7: Structure of selective drugs (NSAIDs) inhibitors: a) Celecoxib, b) Valdecoxib c) Rofecoxib and d) Etoricoxib

2.1.2.3. Cyclooxygenase Enzyme System

Cyclooxegenase (COX-Enzymes) are membrane-bound enzymes that are responsible for biotransformation (oxidation) of arachidonic acid (AA) to prostagladins endoperoxide and these are called *prostagladins synthase(G/H).* [29] The catalytic rate limiting steps of prostanoid biosynthesis and significant roles of these cyclooxygenase (COX-1and-2) isoenzymes were discovered by Neeleman and Bailey 37 years ago. [30]

Prostaglandins are powerful signaling agents in human and animal body which play a vital role in restoring and maintaining inflammation processes by increasing vascular permeability and amplifying effects of other inflammatory mediators such as kinins, serotonin and histamine but excess production harm the tissues. [31]

Cyclooxygenase isoenzymes exist into two different isoform; The constitutive cyclooxygenase (COX-1) and the inducible cyclooxygenase (COX-2). [29]

Cyclooxygenase-1 (COX-1) enzymes are present in low abundance in tissues and act by regulating normal physiological functions and maintenance of kidney function, platelet aggregation etc and gastro protection. [32] Cyclooxygenase-2 (COX-2) enzymes, are responsible of metabolism of arachidonic acid to prostanoids which is more stable (PG) and induced response to cellular activation by hormones, proinflammatory cytokines, growth factors, and tumor promoters. [33]

Cyclooxygenase isoenzymes work in line with potential active enzymes called peroxidase (POX) which helps to oxidize varieties of co-substrates and are needed for the optimal enzymatic synthesis of prostanoids (PG). Non-steroidal anti-inflammatory drugs do not inhibit the activity of peroxidase only cyclooxygenase isoenzymes activity is been blocked by NSAID agent. Peroxidase enzymes acts towards COX isoenzymes by activation of cellular redox-dependent signalling events and stimulates nuclear factor (NFkB) activity. [34]

2.1.2.4. Structural Differences of COX-1 and COX-2 Enzymes

The crystallographic structures show the selective and nonselective inhibitors that bind in two different ways. In COX-1, the space of the selectivity pocket is reduced due to the presence of isoleucine (Ile) 523, while in COX-2 the presence of valine (Val) 523 extended the available space providing a more stable binding possibility for selective inhibitors allow drugs with large substituents to enter to active site. [35]

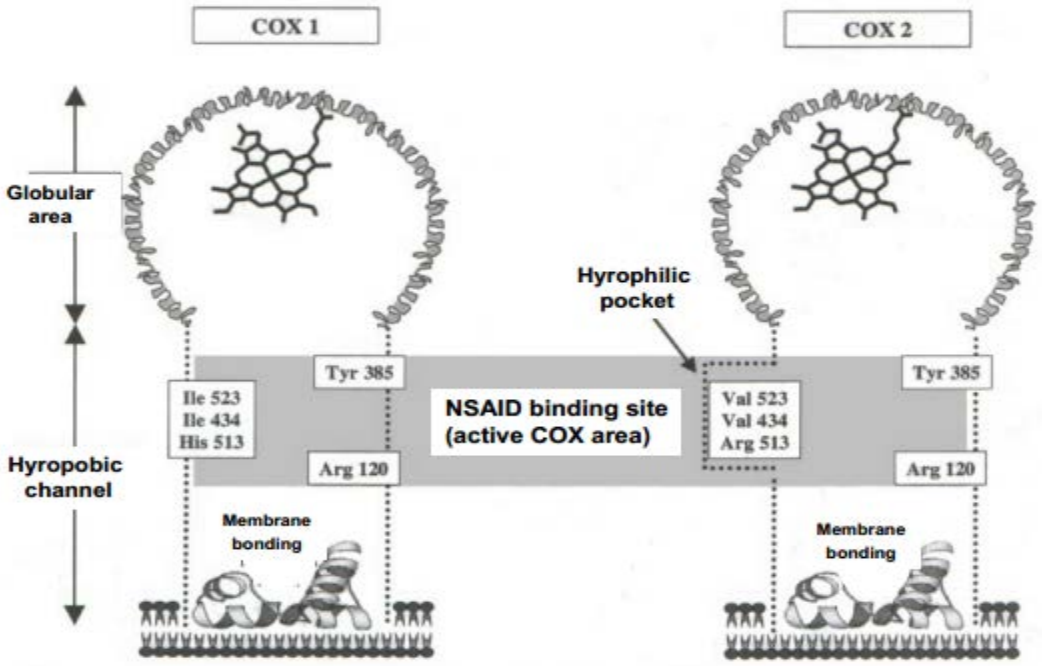


Figure 2.8: Crystallographic structure of cyclooxygenase 1 and 2.

2.2. 2(3H)-Benzoxazolinone

2*(3H)*-Benzoxazolone is a light brown, powdered material, having 138 ºC melting point. It is heterocyclic and bicyclic ring system, composed of a phenyl ring fused to a carbamate. The structure of the benzoxazolinone possesses two important properties, which attracted the attention of medicinal and pharmaceutical chemists: the hydrophilicity and lipophilicity. Hydrophilicity is attributed to the presence of nitrogen and oxygen which participate in hydrogen bonding and contributed to high dipole moment of the compound. The bulkiness of the compound is also good factor in lipophilicity of benzoxazolinone. Its lipophilicity helps the structure for proper binding to hydrophobic protein receptors. These properties of benzoxazolinone play a vital role in its absorption, distribution, metabolism, and excretion (ADME) in the body. Many researchers had subjected benzoxazolinone to derivatization at 3rd position, and have found it shows different biological activities which includes; analgesic and anti-inflammatory effects. [37]

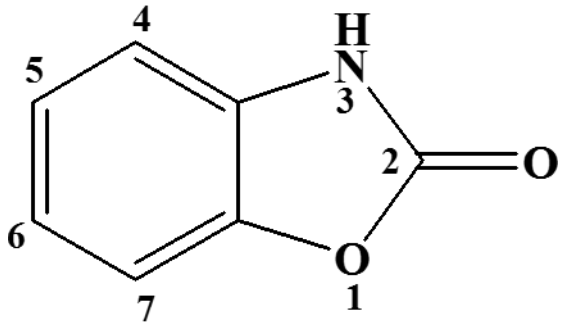


Figure 2.9: Structure and numbering of 2*(3H)*-benzoxazolinone

2.2.1. Benzoxazolinone In Nature

The benzoxazolinone derived from the class of phytoalexins which was reported to be present in some plant kingdom such as Paceae family (wheat, maize and rye). [38] This phytoalexin component was discovered in the year 1940, since then, the field of medicinal chemistry have been extensively in research towards the potential of it against pathogens and their health-promoting effects. [39-40]

2.2.2. Synthesis of Benzoxazolinones

Literature review shows different method indicating the synthesis of benzoxazolinone.

Nachman et al. reported the synthesis of benzoxazolinone in an excellent yield from 2-aminophenol by reflux method in present of carbonyl diimidazole and in dry tetrahydrofuran (THF) in 4-hours. [43]

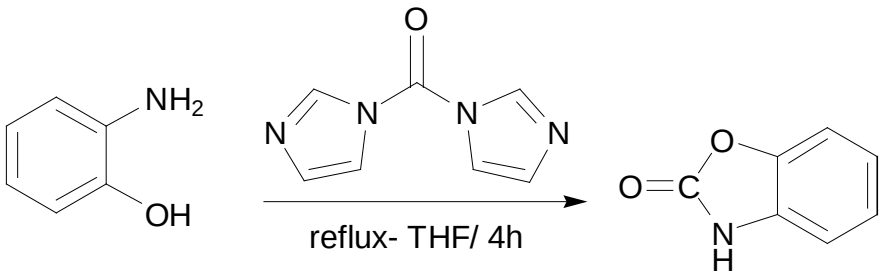


Figure 2.10: Synthesis of benzoxazol-2(3H)-ones from 2-aminophenol

Suman, Bala et al., reported to have synthesized benzoxazolinone from 1-(2-hydroxyphenyl) urea in the present of CDI and THF using reflux method at room for six hours. [43]

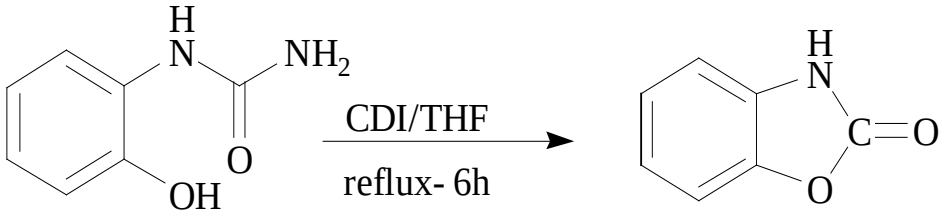


Figure 2.11: Benzoxazolinone from 1-(2-hydroxyphenyl) urea

2.2.3. Chemical Reactivity of Benzoxazolinone Structure

Many studies reported in literature reveal the reactivity of benzoxazolinones as two major types of reactions: *N*-Substitution either alkylation / acylation or Aromatic substitution.

Alkylation of benzoxazolinone at N atom at position three proceeds using mannich reaction procedure yield (61- 90 %) to give mannich base 5-methyl-6acyl-(3-substituted piperazinomethyl)-2-benzoxazolinones. [44]

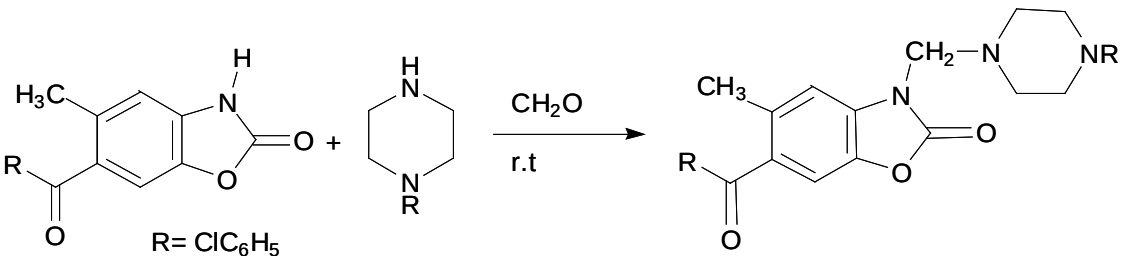


Figure 2.12: N-substitution reaction of benzoxazolinone

Aromatic electrophilic substitution reaction of benzoxazolinone usually occur at position 4, 5, 6 or 7 with different analgesic activity. This reaction occur under Friedel craft acylation to give 5-Methyl-6-acyl-2-benzoxazolinones compound. [44]

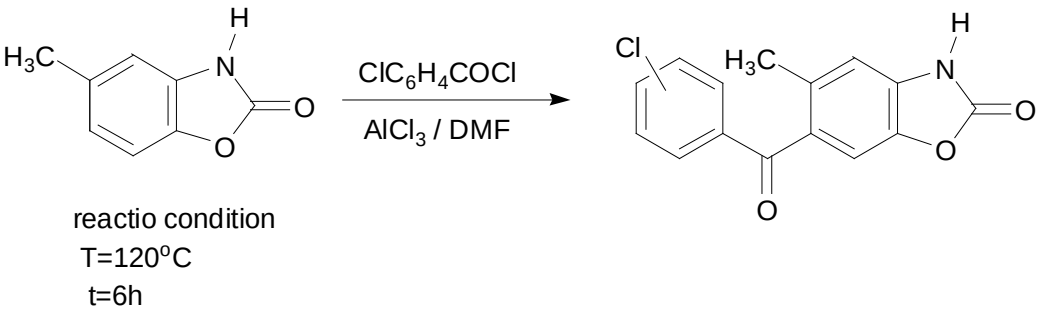
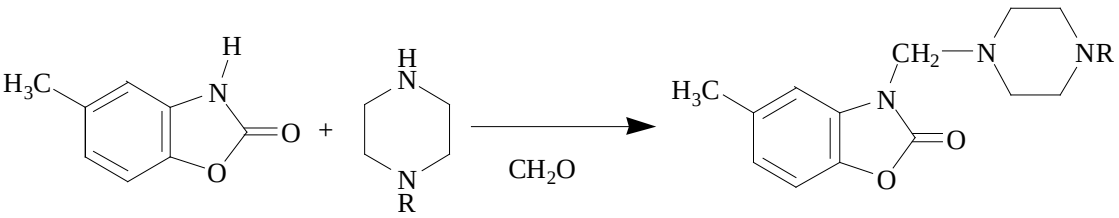


Figure 2.13: Aromatic ring substitution reaction of benzoxazolinone derivative

2.2.4. Bioactivity of Benzoxazolinone Derivatives

Benzoxazolinone derivatives have been tested for various biological activities such as, analgesics, anti-fungal, anti-bacterial, cardiotonic, anti-microbial and anti-inflammatory activities. [45]

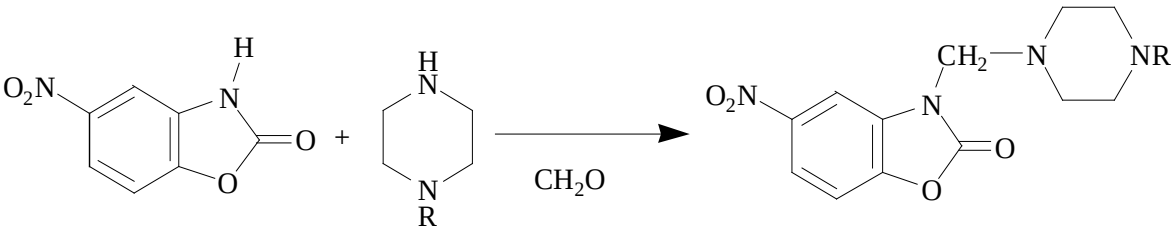
Gokhan, N et. al., synthesized compound of 5-methyl-2-benzoxazolinone phenylpiperazine derivatives at 3-position through Mannich reaction. The studies compound reported that if the substituent is at the meta position of the phenyl ring, the molecule shows antinociceptive activity while at para position it will gives different analgesic activity. [46]



R = H, phenyl or Cl

Figure 2.14: 5-methyl-3-substituted piperazinomethyl-2-benzoxazolinone

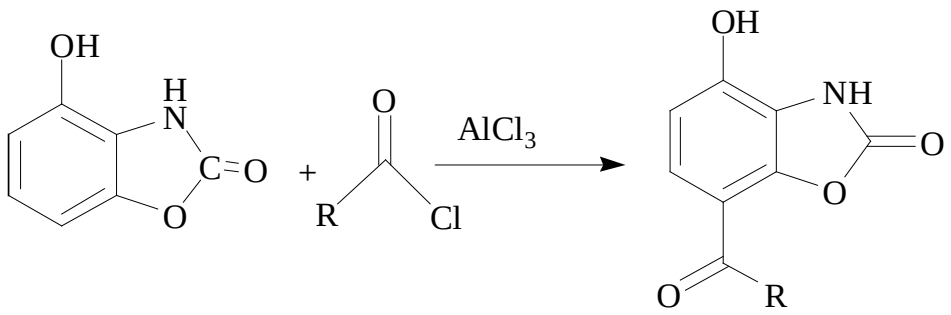
Köksal M et. al., synthesized a novel series of mannich bases of 5-nitro-3-substituted piperazinomethyl-2-benzoxazolinones and the group was screened for anti-inflammatory activities. The tested compounds suggest that, those attached with electron withdrawing groups such as (F, Cl, $COCH_3$) on ortho/para position of the phenyl substituent show higher analgesic and anti-inflammatory activity. [47]



R = H, phenyl or Cl

Figure 2.15: 5-nitro-3-substituted piperazinomethyl-2-benzoxazolinone

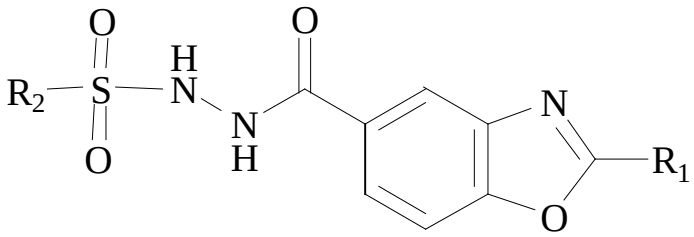
Guangjin, Zheng et. al., focused on the sythesis of 4 and 6-substituted hydroxy-2-benzoxazolone (HBOA) which reported to be used in recent time by Chinese community as a traditional medicine to treat analgesic related issues. The substituted compounds were tested in mice and found to have exhibited more potent analgesic and anti-inflammatory activities compared to aspirin as a reference drug and it can be promising bioactive agents. [48]



R = -CH_3, -CH_2OH or -$CH_2(CH_2)nCO_2H$

Figure 2.16: 4-Hydroxyl-7-Acetyl of benzoxazolinone derivatives

A series of mannich bases, 5-substituted benzoxazoles were synthesized and reported to have bioactive component, but the substituted at its 5th position shows more potent due to its lipophilic part which proved to be a promising moiety for analgesic and the tested compounds showed significant anti-inflammatory activity compared with the standard drug Ibuprofen. [49]



R_1 = H, CH_3
R_2 = para-Cl, p-aceto amido

Figure 2.17: N-[substituted sulfonyl]-1,3-benzoxazole-5-carbohydrazide

The synthesized compounds of 5-Methyl-3-(4-substituted benzoylmethyl)-2(3H)-benzoxazolonone (I) reported that, the substituent bearing 4-substituted benzoylmethyl in the third position of 2(3H)-benzoxazolone decreases the activity of the derivatives when compared to ketone and alcohol groups. It was seen that, reducing ketonic group derivatives to alcoholic group shows more effective than those of ketone. While 5-methyl-3-[2-hydroxyl-2-(4-substitutedmophenyl)ethyl]-2-(3H)-benzoxazolone (II) which shows that, (H) bearing at phenyl ring indicated no substituent thus, exhibited much significant activity when compared to indomethacin. but it was shown that compound having electro-donating groups such as Br, Cl. substituent have more significant activity among the reduced derivatives of ketonic group. [50]

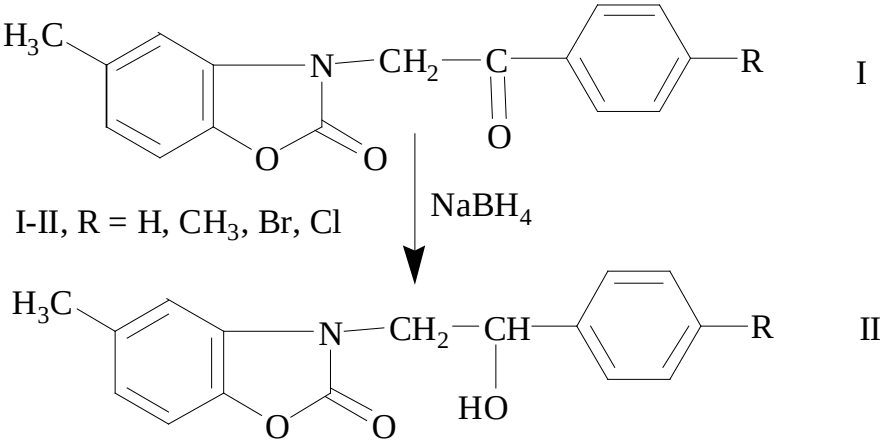
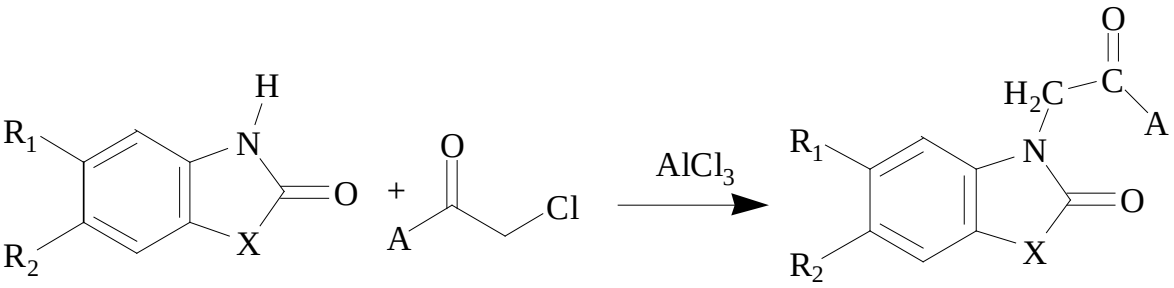


Figure 2.18: Synthesis the derivatives of 5-Methyl-3-(4-substituted benzoylmethyl)-2(3H)-benzoxazolonone

Doğruer, D.S. et. al., Synthesized and analyzed 4-(2-[6-(2-chloro-benzoyl)-2-benzoxazolone-3-yl] acetyl} morpholine, where the compound reported to have shown potent anti-nociceptive activity and the o-chloro-substituted molecule reported to reduce the toxicological effect. [51]



X = O, S / R_1, R_2 = H, Cl,C_6H_5CO
A = OH, morpholinyl

Figure 2.19: Structure of 4-(2-[6-(2-chloro-benzoyl)-2-benzoxazolon-3-yl] acetyl} morpholines

Onkol et al., showed synthesis pathway of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzyl) hydrazine derivatives under microwave synthesis (400W, 76-78 °C) at 15 min with maximum yield of 81%. The compounds was tested and shows effective anti-fungal activity when compared to *Staphylococcus aureus* used as a reference standard. [52] Therefore, presence of an electronegative substituent on phenyl ring give different activity of the compound to anti-bacterial activity. [53]

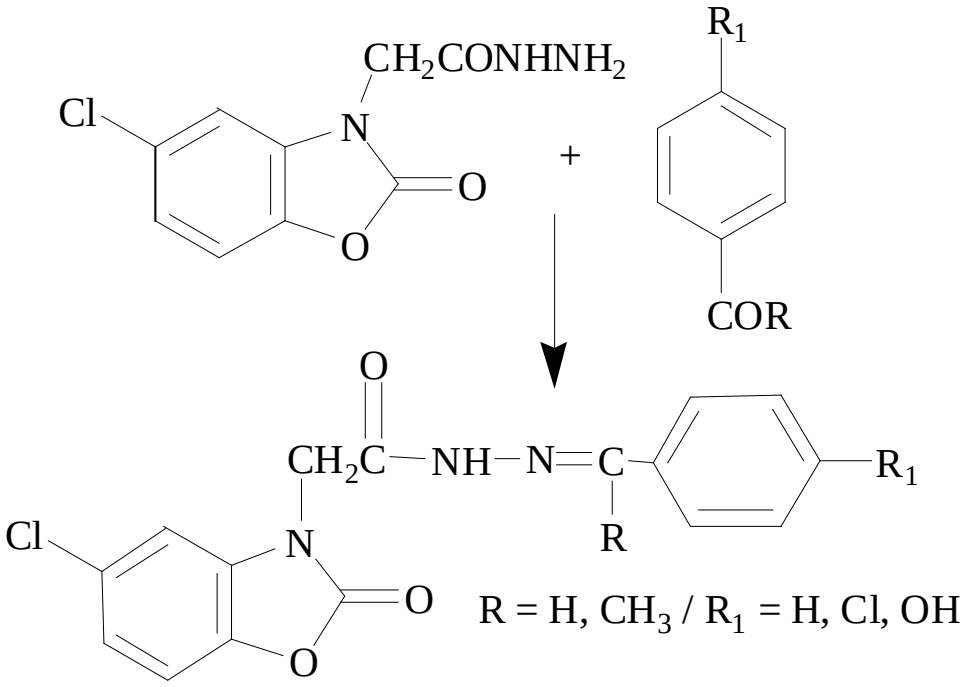


Figure 2.20: Mannich base of 5-chloro-2(3H)-benzoxazolinone-3-acetyl-2-(p-substituted benzal) hydrazone

Synthesis of benzoxazolinone derivatives containing *bis*-heterocyclic base of 1,2,3-triazole was reported by Saqlain Haider et. al., that, the compounds were found to stronger analgesic effect due to the present of π-π bonds of the triazole group in addition to benzoxazolinone. The anti-inflammatory activities of the compounds are reported to be higher when compared to indomethacin standard with minimal side effect of gastric ulceration. [54]

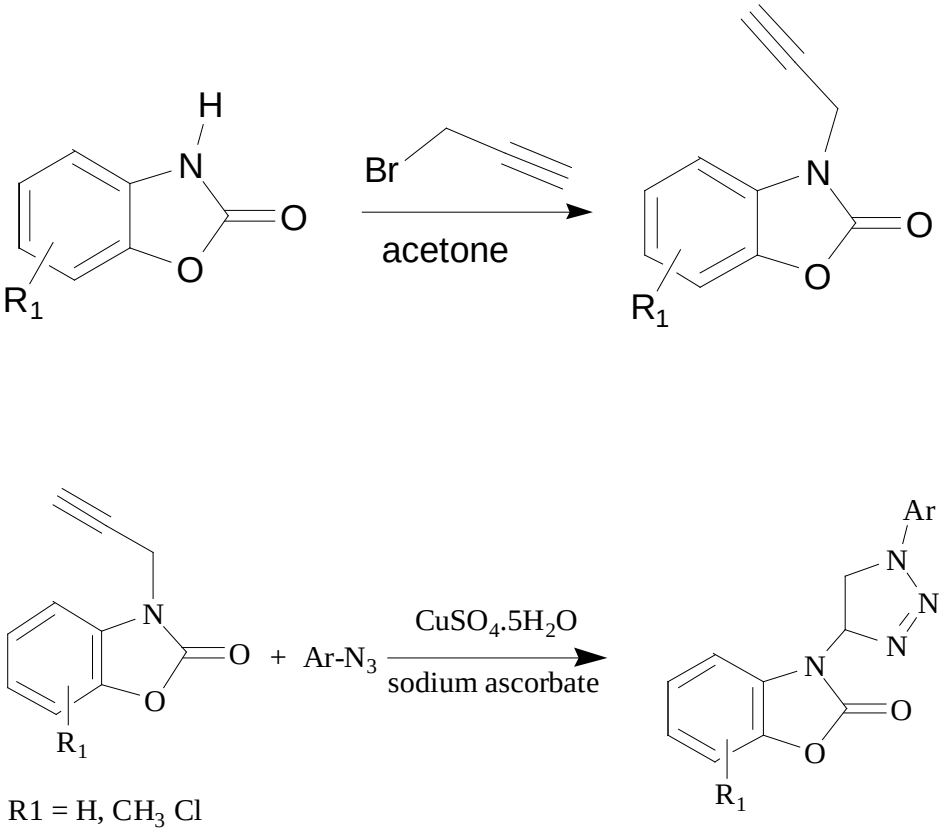
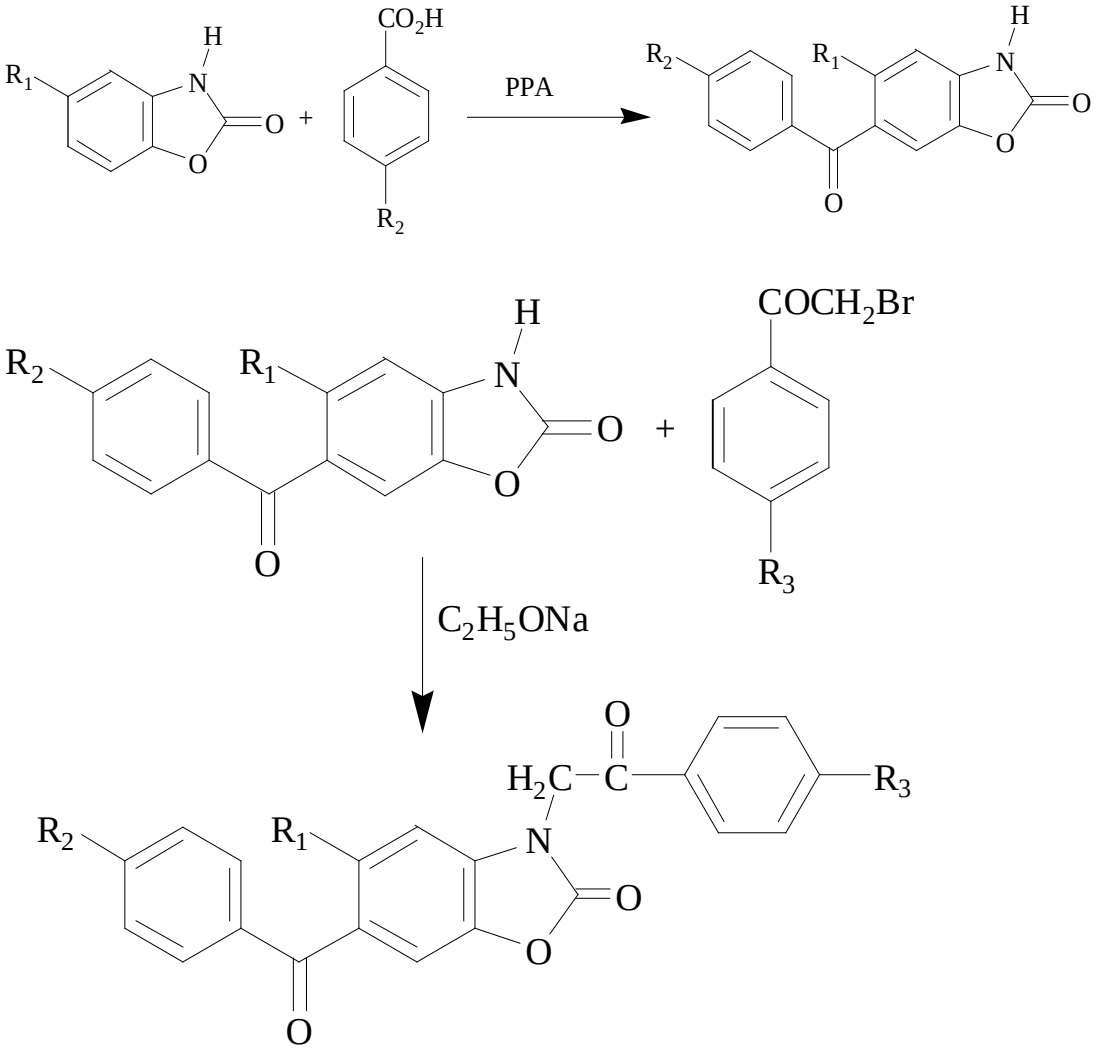


Figure 2.21: Synthesis of benzoxazolinone derivatives containing bis-heterocyclic bases of 1, 2, 3-triazole

A series of 3-(4-substituted benzoylmethyl)-2-benzoxazolinones was screened for anti-microbial activity and evaluated against two gram-positive, one gram-negative bacteria and compared with three different fungi standards e.g Candida albicans, Candida krusei, Candida parapsilosis. It was shown that when present of chlorine substituent of the increases the anti-fungal activities. [55]



R_1=H, Cl / R_2= 4-floro / R_3=Cl, Br, CH_3

Figure 2.22: Synthesis of 3-(4-substituted benzoylmethyl)-2-benzoxazolinone

2.3. Mannich Reaction

Mannich reaction is an organic synthesis which involves reaction of two carbonyl compounds with amines groups to give a *β*-amino-carbonyl compound through C-C single bond-formation in the presence of a proper catalyst. Hence *β*-amino-carbonyl compounds are important intermediates that are used as an agent for the synthesis in pharmaceutical industries or natural products. [56]

Mannich reaction is the condensation of CH-activated (α-position) compound, usually a ketone or aldehyde, with a 1° or 2° amine (or ammonia) and a non-enolizable aldehyde or ketone to produce an amino-alkylated derivative where the reaction condition occurs in protic solvents such as ethanol, methanol, water or acetic acid. This is to ensure formation of a high amount of the electrophilic iminium ion.

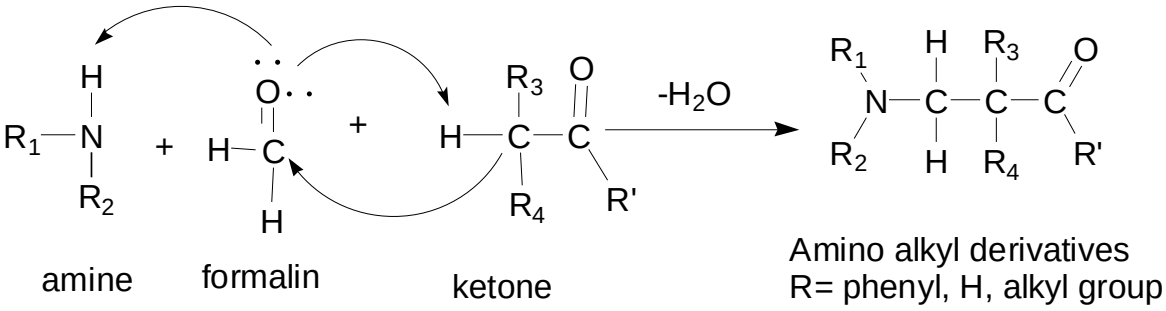


Figure 2.23: Mannich Reaction of Amino-Alkylated derivatives

Some examples of some bioactive drugs molecule which was synthesized under one-pot Mannich reaction. [57-58] The clinically used drugs include zoniporide which is anti-inflammatory NSAIDs and is selective COX isoform enzymes, Rimonabant also anti-inflammatory drug which act on anti-obesity, Celecoxib is non selective cox-2 inhibitors drugs while Lonazolac it's for both analgesic and antipyretic it can be seen in Figure 2.24.

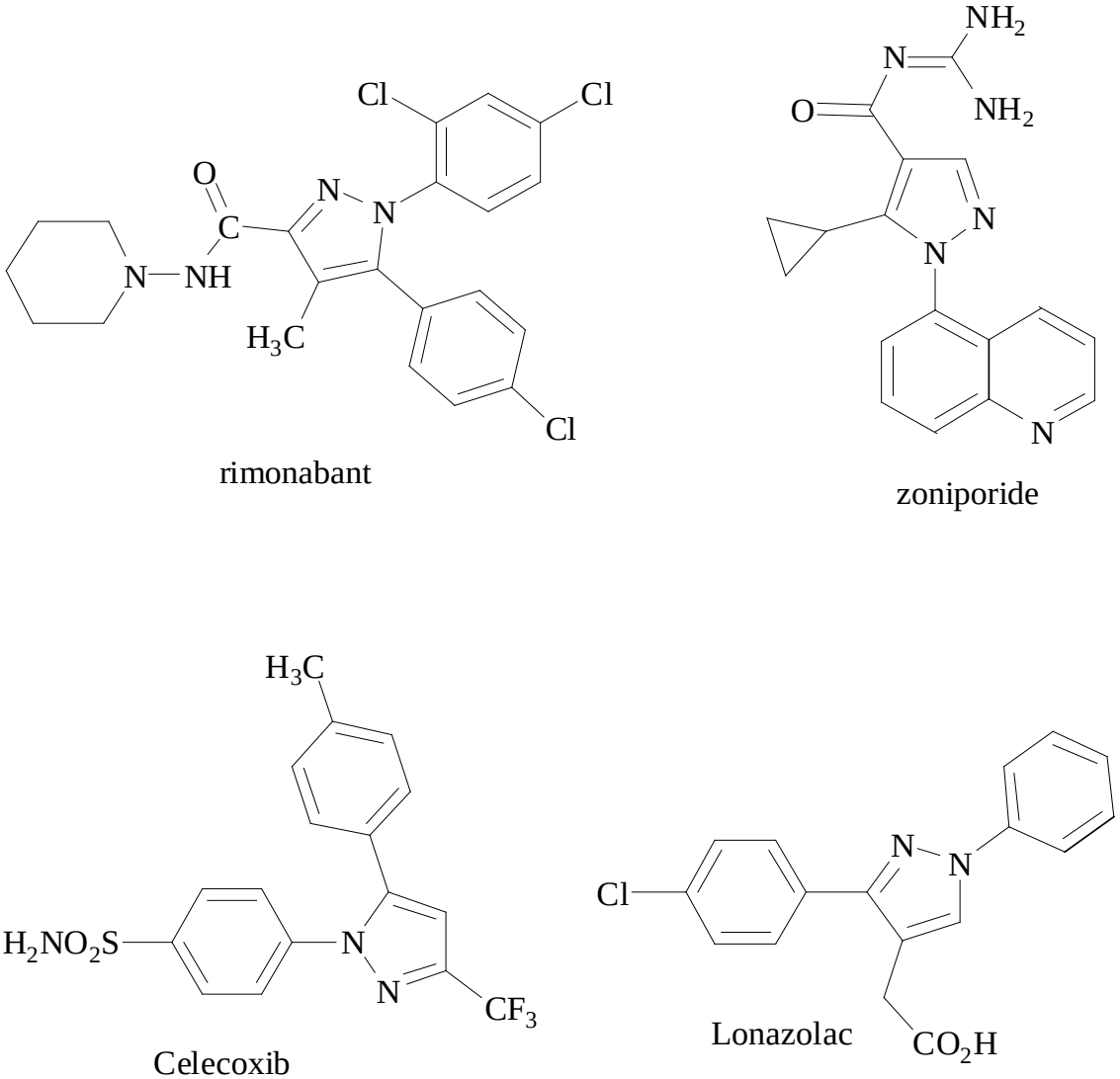


Figure 2.24: Clinical drugs molecule synthesized by Mannich reaction

2.4. Green Chemistry

Green chemistry is a process where by some new chemical products are developed in a manner to reduce or eliminate the use and generation of hazardous substances. [59-60]

Green chemistry concept was initiated in the years 90's. The major aim is to reduce pollution by using green solvents (solvents that are free from toxicity). The idea was introduced gradually in Europe and in some parts of the United States of America under control of Environmental Protection Agency (EPA) which played a vital role for introducing new idea called green solvent. [61]

Waste management agencies were created to reduce waste generation during chemical processes and the use of non-toxic solvents or free of catalysts where possible. [62] The essential aspects that was adopted in green chemistry play major role in elimination or reduction of the use of toxic chemical substances such as (solvents, reagents, preservatives, additives for pH adjustment and others), reducing energy consumption and increasing safety for the operators as well as environmental. Microwave reactors could be used to replace traditional heating methods as they reduce waste and time needed thus less environmental damage. [63]

2.4.1. Microwave Assisted Organic Synthesis

Microwaves are electromagnetic waves having a wavelength of $1cm^{-1}$ corresponding to frequencies in the range of 0.3-300 GHz. Microwaves lie between infrared (IR) and radio wave region in the electromagnetic spectrum.

Microwave assisted organic synthesis has emerged as opportunity to carry out synthesis that is historically not feasible by time. The technique involves clean, simple, efficient, fast and economically desirable, in synthesis. This technique 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. [64-68]

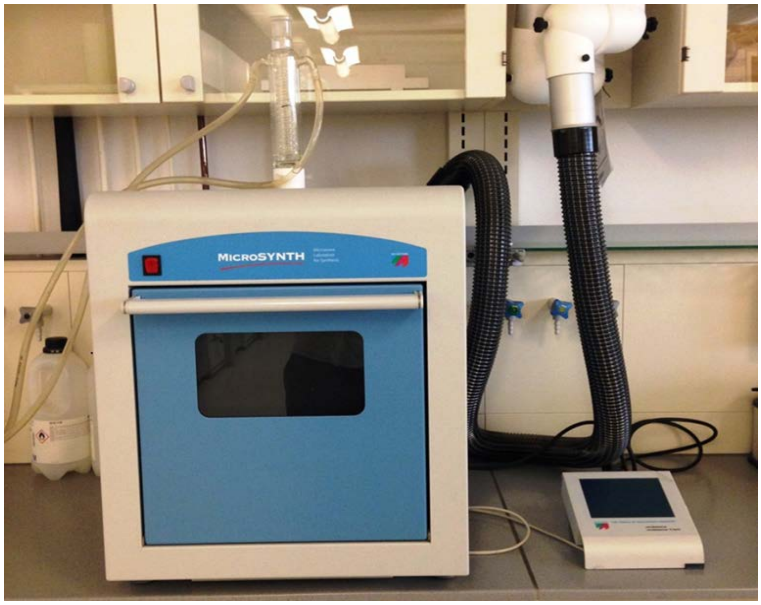


Figure 2.25: Example of Microwave Reactor

2.4.1.1. Conventional Heating vs Microwave Heating

It has been reported that, energy efficiency is higher with microwaves than with conventional heating in many ways. [69-71]

Table 1: Differences between reflux and microwave heating methods

MICROWAVE HEATING SYNTHESIS	CONVENTIONAL HEATING SYNTHESIS
1. Shorter reaction time (within munites)	1. Long reaction time (within hours)
2. Safe from explossion and environmental friendly	2. Risk in handling which may led to explosion not environmental friendly
3. Energy pass in non-contact heating via zreaction vessel and uniformly distributed within molecule	3. Direct heat contact to reaction vessel before pass across to the molecule.
4. Less by-product with maximum yield production	4. Less production yield with side product
5. Temperature and frequency occur in higher proportion	5. Temperature gradiant and absent of frequency

2.4.1.2. Solvent Effect in Microwave Assisted Organic Synthesis

Every solvent and reagent absorb microwave energy differently. They each have a different degree of polarity within the molecule. Therefore, will be affected either more or less by the changing microwave field. A solvent that is more polar, for example, will have a stronger dipole to cause more rotational movement in an effort to align with the changing field. A compound that is less polar, however, will not be as disturbed by the changes of the field and, therefore, will not absorb much microwave energy. The polarity of the solvent is not the only factor to determine the true absorbance of microwave energy, but it does provide a good frame of reference. Other factors such as, loss tangent (δ) and dielectric constant are also 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 tangent and is expressed in term of tangent value:

$\tan\delta = \acute{\varepsilon}/\check{\varepsilon}$ ..... (1)

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

The higher the tangent value the better is the solvent at absorbing microwave energy and thus better heat is generated. Table 2 below shows the loss tangent values of some pure common solvents at room temperature. [71]

Table 2: Dielectric constant and loss tangent values of some common solvents

Solvent	**Dielectric constant (ε)**	**Loss of tangent (tanδ) / 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

3. MATERIALS AND METHODS

3.1. Materials

All reactions were carried out using standard laboratory equipment and standard laboratory glassware. All the chemicals, 2-benzoxazolone, 3-methylpiperidine, methanol and 37% formaldehyde, benzene, chloroform, ethanol, cyclohexane were purchased from Sigma Aldrich Company which was used directly without further purification.

3.1.2. Microwave Reaction

Microwave irradiation was carried out in microwave oven (Micro SYNTH, Milestone, Italy).

3.2 Methods

3.2.1. General Method For Reflux Reaction (Method A)

200 mg (0.0015 mol) of benzoxazolinone and 0.2 mL (0.0015mol) of 3-methylpiperidine were dissolved in 8 mL of methanol in 50 mL round bottom flask. 0.2 mL of 37% (w/v) Formalin solution was mixed with 2 mL of methanol and then poured into the reaction mixture. The solution was refluxed in a water bath for 60 min. The resulting precipitate was filtered off, washed with methanol, dried and purified by crystallization using cyclohexane.

3.2.2. General Method For Microwave Reaction (Method B)

400 mg (0.0030 mol) of benzoxazolinone and 0.4 mL (0.0030 mol) of 3-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 solution was placed in a microwave reactor and irradiated at 150 W for 3 min, then at 100 W at 65 ^{0}C for 5 min. After the mixture was cooled down to room temperature, it was poured into crushed ice upon which a precipitate was formed. The resulting precipitate was filtered off, washed with methanol, dried and purified by crystallization using cyclohexane.

3.2.3. Thin Layer Chromatography

Thin layer chromatography (TLC) is a technique that used for identification of compounds in a mixture and determining their purity. The reaction was conducted using silica gel plate (0. 2 mm) thickness as stationary phase and view the spot under UV-Vis light fluorescent indicator at 254 nm wavelenght.

Three different mobile phases (Mp) solution were prepared (liquid solvent) belowed:

Mp1- Benzene to Methanol ratio: **9:1**

Mp2- Benzene to Methanol ratio: **5:1**

Mp3- Ethylacetate to Hexane: **1:2**

The benzoxazolinone and synthesized compound were dissolved in two different test tubes containing chloroform. The silica gel plate were marked (spotted) with the two different prepared chloroform solution which was placed into the different prepared mobile phases chambers. The silica gel plates were allowed for munites to reach the desired height, which later was removed from solvent and the covered distance were marked. The silica gel was allowed to dried. After drying the spots were determined under UV light at 254 nm. The R_f -values were calculated using the below equation;

$$Rf = \frac{distance\ Traveled\ by\ the\ sample}{distance\ Traveled\ by\ the\ solvent}$$

3.3. Spectroscopy

3.3.1. Fourier Transform Infra-Red (FT-IR)

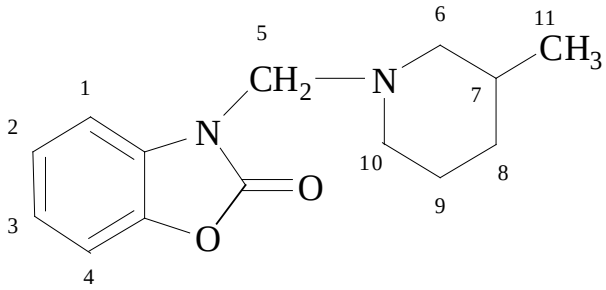
The FT-IR spectra of the product was recorded on Agilent Carry 630 Spectrometer at Ankara University, Central Instrumental Analysis Laboratory, Faculty of Pharmacy.

3.3.2. Proton Nuclear Magnetic Resonance (^{1}H-NMR)

The ^{1}H-NMR spectra of the product was recorded on a Mercury Varian 400 MHz Spectrometer where deuterated solvent of dimethyl sulfoxide (DMSO-d_6) was used. The test was conducted at Ankara University, Central Instrumental Analysis Laboratory, Faculty of Pharmacy. Chemical shift (δ) values were reported in parts per million (ppm).

4. RESULTS AND DISCUSSION

4.1. Results



3-[(3-Methylpiperidin-1-yl)Methyl]Benzoxazol-2-one

Figure 4.1: Structure of 3-[(3-methylpiperidin-1-yl) methyl]-2-benzoxazolinone

The above compound was synthesized by two methods (Reflux method A and Microwave method B) mentioned in the experimental part. [71]

Reflux Method A

Appearance: Product was obtained as pale yellow crystals.

Yield (%): The percentage yield of the final product was calculated to be: 53 %. (197 mg)

Mp: The melting point of the product: 151 ℃

TLC Result: The mobile phases of Mp1, Mp2 and Mp3 were calculated with Rf value; 0.31, 0.31 and 0.38 respectively.

Microwave Method B

Appearance: Product was obtained as pale yellow crystals.

Yield (%): The percentage yield of the final product was calculated to be: 58 %. (430 mg)

Mp: The melting point of the product:151 ℃

TLC Result: the mobile phases of Mp1, Mp2 and Mp3 were calculated with Rf value; 0.32, 0.32 and 0.38 respectively.

Fourier Transforms Infra-Red (FT-IR) Spectroscopy (IR V_{max}):

FT-IR showed absorption band at 2806-2931 cm^{-1} (C-H stretch), 1750 cm^{-1} carbonyl group (C=O stretch).

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR in CD_3SOCD_3; ppm)

^{1}H-NMR showed chemical shift at 7.0-7.4 ppm (4H, m, Ar-H); 4.6 ppm (2H, s, N-CH_2-N) 3.3-1.4 ppm (9H, m, protons of pip. peaks); and 0.8 ppm (3H, s, H^{10}).

4.2. Discussion

Reaction Pathway of the Compound

The compound was synthesized via Mannich reaction where the 3rd position (N) of benzoxazolinone was substituted with 3-methylpiperidine using methanol as solvent in the presence of formalin. The synthesis pathway of the reaction is given below.

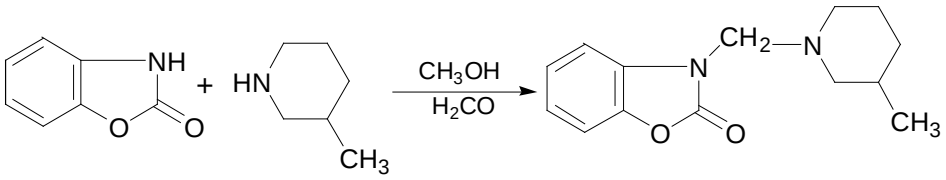
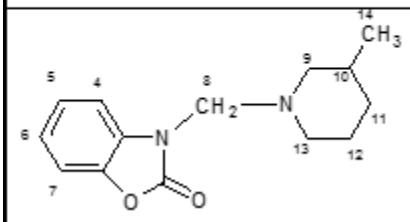


Figure 4.2: General synthesis of 3-(3-methylpiperidinyl) methyl-2-benzoxazolinone

The reaction of this compound was carried out by two different synthesis method: reflux and microwave assisted organic synthesis. Microwave irradiation method was completed within 5 minutes while using conventional reflux method, it takes about 60 mins for the same reaction to be completed.

Table 3: Summary of microwave and reflux methods of yield and melting point

Structure of the compound	Reaction Condition	Reaction Time	Melting Point °C	Yield (%)
	Microwave Irradiation	5 mins	151.0	58
	Reflux heating	60 mins	151.0	53

The structures of the synthesized compound were confirmed by Fourier Transform Infra-Red (FT- IR) and Proton Nuclear Resonance (^{1}H NMR) Spectroscopy. The melting point was used to determine the purity of the compound, while thin layer chromatography was used to check the progress of the reaction.

Fourier Transforms Infra-Red (FT-IR) Spectroscopy

The FT-IR spectra of synthesized compound shows the absence of N-H stretch which is reported to come around 3100-3400 cm^{-1}, which indicates that the reaction have actually taken place at position 3 of 2(*3H)*-benzoxazolinone. The strong (C=O) stretch band of 2*(3H)*-benzoxazolone are visible at 1750 cm^{-1}. The (C-H) stretch for the product also appear at 2806-2931 cm^{-1} as expected.

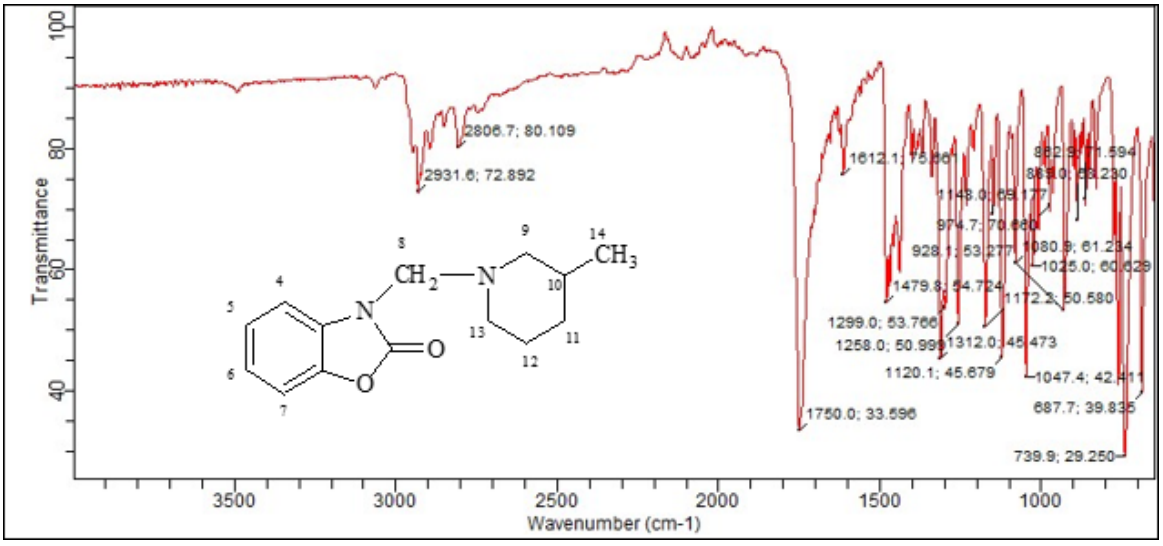


Figure 4.3: IR spectrum of 3-[(3-methylpiperidin-1-yl)methyl]-2-benzoxazolinone

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

^{1}H-NMR spectrum of the synthesized compound show peaks at expected chemical shifts. Investigations of ^{1}H-NMR spectrum reveal the presence of aromatic peaks as multiplets between 7.0 to 7.4 ppm which are similar to the literature. The methylene bridge-proton signal of the compound observed as a singlet signal at 4.6 ppm. The neighbouring methyl protons of 3-methylpiperidine peak signals were observed at chemical shifts between 3.3-0.8 ppm respectively at upfield. The values of the peaks on the ^{1}H-NMR of the compound corresponded to the number of protons in the proposed structure of the compounds.

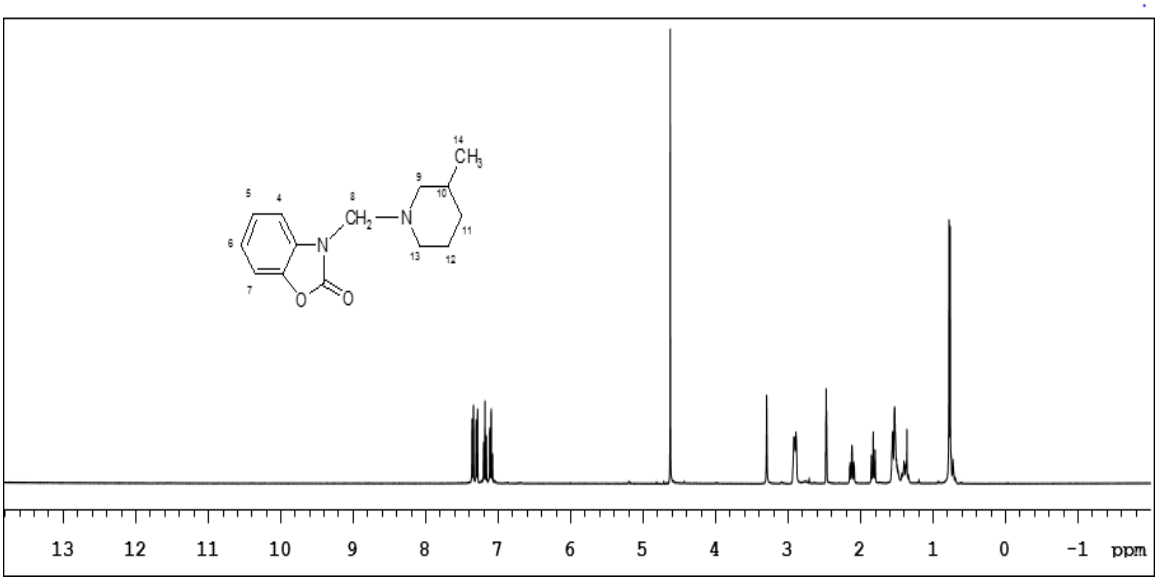


Figure 4.4: ^{1}H-NMR spectrum of 3-[(3-methylpiperidin-1-yl)methyl]-2-benzoxazolinone

5. CONCLUSION

This study shows the synthesis of 3-[(3-methylpiperidin-1-yl) methyl]-2-benzoxazolinone using two different reaction methods namely, microwave irradiation and reflux reaction method. The the reaction time of microwave synthesis method was much shorter (5 mins) with 58 % yield when compared to reflux method in (60 mins) with 53 %. The faster reaction time is a desirable advantage in the synthesis of drug molecules in the field of pharmaceutical sciences.

The synthesized compound was reported from the recent literature review to possess anti-inflammatory activity.

Further researchs can be focused on studying the effect of 2,5 and 6-methyl piperidine substitution on benzoxazolinone or increase of the alkyl chain length on piperidine derivative. Alternatively, structure-activity relationship can be investigated by using various piperidine derivatives with electron withdrawing and electron donating substituents.

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