CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Cheminformatics, Anti-Inflammatory and Analgesic Potential of 1-Morpholino-2Pyridine-2-Yalamino Ethan-1-One

by

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CERTIFICATE OF APPROVAL

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(Aamir Shahzad Khan)

Abstract

A morpholine ring as a core structural motif, often contributing to enhanced biological activity and favorable pharmacokinetic properties. The present study comprehensively evaluates the pharmacological potential of compound 1MPE, a morpholine-based derivative, through multidisciplinary approaches including ADMET profiling, molecular docking, antioxidant assays, and in vivo analgesic and anti-inflammatory studies. 1MPE demonstrates favorable pharmacokinetic properties with excellent intestinal absorption (91-94%) despite limited aqueous solubility (LogS: -5.34 to -3.67), suggesting the need for formulation optimization. Molecular docking reveals stable binding with COX-2 (binding affinity: -6.3 kcal/-mol), though slightly weaker than diclofenac (-7.4 kcal/mol), supporting its potential as an anti-inflammatory agent. In vitro antioxidant assays show significant radical scavenging activity (80.5% inhibition at 1000 μg/mL, IC50 = 267.3 μg/mL), comparable to ascorbic acid. In vivo studies confirm potent analgesic action of 1MPE in comparison with tramadol as positive control by hotplate method. However, the anti-inflammatory effects of 1MPE evaluated by paw edema method were significantly high as compared to the positive control (diclofenac) group. Moreover, the acute and subacute oral toxicity profile showed no histopathological abnormalities in vital organs (heart, liver, stomach, and kidney) at 100 mg/kg dose. However, CYP3A4-mediated metabolism, AMES-positive mutagenicity, and hepatotoxic potential highlight key areas for structural refinement. These findings position 1MPE as a promising multifunctional candidate for oxidative stress, pain, and inflammation management. Future research should focus on structural optimization to enhance solubility, metabolic stability, and safety while maintaining its pharmacological efficacy, followed by comprehensive preclinical validation. The study provides a robust foundation for developing 1MPE as a potential therapeutic agent through rational drug design approaches.

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List of Abbreviations

ADME	Absorption, Distribution, Metabolism, and Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
API	Active Pharmaceutical Ingredient
BACE-1	Beta-Site Amyloid Precursor Protein Cleaving Enzyme-1
BBB	Blood–Brain Barrier
CADD	Computer-Aided Drug Design
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CVD	Cardiovascular Disease
CYP	Cytochrome P450
DNA	Deoxyribonucleic Acid
DPPH	Diphenyl-1-Picrylhydrazyl
EGFR	Epidermal Growth Factor Receptor
FRSA	Free Radical Scavenging Activity
GABA	Gamma-Aminobutyric Acid
GI	Gastrointestinal
HIV	Human Immunodeficiency Virus
IC_{50}	Half Maximal Inhibitory Concentration
LDL	Low-Density Lipoprotein
LPO	Lipid Peroxidation
MD	Molecular Dynamics
mTOR	Mammalian Target of Rapamycin
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OECD	Organisation for Economic Co-operation and Development

PAINS	Pan-Assay Interference Compounds
PBMCs	Peripheral Blood Mononuclear Cells
PDB	Protein Data Bank
PI3K	Phosphoinositide 3-Kinase
PROTACs	Proteolysis Targeting Chimeras
RMSD	Root Mean Square Deviation
RNA	Ribonucleic Acid
Ro5	Rule of Five
SAR	Structure–Activity Relationship
SEM	Standard Error of the Mean
TAC	Total Antioxidant Capacity
TPSA	Topological Polar Surface Area
XO	Xanthine Oxidase

Chapter 1

Introduction

1.1 Introduction

Heterocyclic compounds are cyclic organic molecules that contain at least one heteroatom, such as nitrogen, oxygen, or sulfur, though other heteroatoms are also known. These compounds are distinct from carbocyclic compounds, which consist solely of carbon atoms in their ring structures [1].

Heterocycles play a crucial role in organic chemistry and are essential in biological systems, as they form the core structure of many biomolecules, including DNA, RNA, chlorophyll, hemoglobin, and various vitamins [2]. Because of their varied biological actions, heterocyclic compounds are frequently employed in medicine to treat a variety of ailments. Triazine compounds, for instance, are used as anti-inflammatory, urinary antiseptic, and antibacterial herbicides. Benzimidazole derivatives have a wide range of pharmacological characteristics, such as anthelmintic, antiviral, antifungal, and antibacterial effects. Heterocyclic compounds are essential for medication development and therapeutic applications due to their importance in both natural and synthetic chemistry [3].

Medicinal chemistry has emerged as a vital field in science by bridging chemistry and medical research, focusing on understanding diseases and developing solutions. This branch of modern chemistry originated with the isolation and purification of

bioactive compounds from plants, animal tissues, microorganisms, and fermentation products, which became a key area of interest for researchers worldwide [4]. Rooted in foundational disciplines such as organic chemistry, biology, and aspects of physics, medicinal chemistry plays a crucial role in drug discovery and therapeutic development. Notably, heterocyclic compounds hold significant importance in this field, as they form the backbone of many biologically active molecules and pharmaceutical agents, underscoring their relevance in modern medicine [5]. Heterocyclic compounds serve as fundamental structures in medicinal chemistry and are prevalent in many essential biomolecules, including enzymes, vitamins, natural products, and bioactive agents. These compounds exhibit a wide range of pharmacological properties, such as antifungal, anti-inflammatory, antibacterial, antioxidant, anticonvulsant, antiallergic, and enzyme-inhibiting effects, along with herbicidal, anti-HIV, antidiabetic, anticancer, and insecticidal activities [6]. Antifungal agents, a key application of heterocycles, are medications used to treat fungal infections commonly affecting the skin, hair, and nails, such as ringworm and athlete's foot. These drugs work either by killing fungal cells-disrupting their cell membranes and causing cellular components to leak out-or by inhibiting their growth and reproduction, effectively controlling the infection. Their diverse mechanisms and biological significance make heterocycles indispensable in drug development and therapeutic treatments [7].

Anti-inflammatory activity refers to the ability of certain substances to treat or alleviate inflammation and swelling. Many anti-inflammatory drugs also possess analgesic properties, with nearly half of them functioning by reducing inflammation-related pain, unlike opioids that act on the central nervous system to block pain signals. Common anti-inflammatory medications, such as aspirin, ibuprofen, and naproxen, belong to a class known as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), distinguishing them from steroidal anti-inflammatory agents [8].

These drugs primarily work by inhibiting Cyclooxygenase (COX) enzymes, which play a key role in the metabolism of arachidonic acid-a process linked to inflammation. Some NSAIDs selectively target specific COX isoenzymes, allowing for

tailored therapeutic effects while minimizing side effects. This mechanism underscores their importance in managing pain and inflammatory conditions [9].

Antibiotics are medicines that fight bacterial infections by either killing bacteria or stopping their growth. While some can also work against certain parasites, they don't help with viral illnesses like colds or flu. Using antibiotics incorrectly can create drug-resistant bacteria, which is a serious health problem. These medicines are grouped by how they work or their chemical makeup, with many containing special ring-shaped structures (heterocycles) that make them effective. Common types include penicillin-like drugs (β-lactams). Recently, scientists have created and studied many new antibiotic compounds to combat different bacteria [10].

Antioxidants are substances that help prevent cell damage caused by oxidation- a chemical process that generates harmful free radicals and triggers destructive chain reactions in cells. These protective molecules, such as thiols and vitamin C (ascorbic acid), work by stopping oxidation reactions and neutralizing free radicals [11].

The term "antioxidant" generally refers to two main types: (1) synthetic additives used to preserve products by preventing oxidation, and (2) naturally occurring compounds found in foods and body tissues that support health by combating oxidative stress [12]. By interrupting damaging chain reactions, antioxidants play a crucial role in maintaining cellular health and preventing oxidative damage.

Heterocyclic compounds represent a fundamental area of organic chemistry, closely tied to medicinal chemistry and organic synthesis [13]. These compounds consist of a large class of organic chemicals in which at least one atom other than carbon is incorporated into the rings of their molecular structure [4]. Due to their significant biological and medicinal properties, heterocyclic compounds have attracted substantial attention and are found in more than 90% of new drug formulations. Naturally occurring heterocyclic molecules can be found in various substances, including alkaloids like vinblastine, morphine, and reserpine, as well as antibiotics such as cephalosporin and penicillin [14]. The development of heterocyclic chemistry began in the 19th century, advancing in parallel with organic chemistry.

Notably, heterocyclic compounds such as purines and pyrimidines play a crucial role in the genetic code, as outlined in the principles of Chargaff's laws. [15] Their widespread applications in pharmaceuticals and industrial research make heterocyclic compounds a vital class in organic chemistry [16].

Anticonvulsants, also known as antiepileptic or antiseizure drugs, are a diverse group of pharmacological agents used to treat and manage epileptic seizures.

These medications work through different mechanisms, primarily by blocking sodium channels in nerve cells to prevent excessive electrical activity or by enhancing the function of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter that helps calm overactive brain signals [17].

By stabilizing neural activity, anticonvulsants play a crucial role in controlling seizures and improving the quality of life for individuals with epilepsy. Using the maximum electroshock seizure (MES) paradigm, researchers synthesized a number of 4-amino-4H-1,2,4-triazole derivatives and assessed their antiepileptic properties in male rats. The potential therapeutic effectiveness of the derivatives was then evaluated by comparing their effects to those of a common reference medication [2]. Because they are frequently used as therapeutic treatments for many disorders, heterocyclic compounds are an essential family of organic molecules with enormous relevance in medical chemistry. The fundamental structural components of many medications that treat a variety of illnesses, including antifungal, anti-inflammatory, antibiotic, antioxidant, anticonvulsant, antihistamine, herbicidal, and anti-tumor uses, are heterocyclic compounds. Heterocycles are essential to pharmaceutical development and contemporary drug discovery due to their structural adaptability and biological significance [18]. Morpholine derivatives, like other heterocyclic chemicals, have enormous medicinal promise for the treatment of a wide range of human conditions. Morpholine is a valuable scaffold in medicinal chemistry due to its extensive pharmacological profile, which includes anticancer, antidiabetic, depressive, growth-stimulating, antiemetic, and bronchodilator effects [17]. These many biological effects demonstrate how crucial morpholine-based molecules are for the creation of medications for a variety of illnesses [6].

Morpholine is a colorless liquid with a distinct ammonia-like odor. There are several industrial purposes of morpholine, including use as a solvent, a brightening agent in detergents, a corrosion inhibitor, a rubber accelerator, and a boiler water additive. Chemically, morpholine is a base; when treated with hydrochloric acid (HCl), it forms morpholinium chloride salt, where morpholinium is its conjugate acid [19]. This reactivity accounts off its utility in both industrial and chemical applications. Morpholine is a six-membered heterocyclic ring containing two heteroatoms-oxygen and nitrogen. Compounds having this core nucleus exhibit a broad spectrum of pharmacological activities. Many marketed drugs and existing therapeutic agents feature the morpholine pharmacophore, highlighting its significance in medicinal chemistry [20].

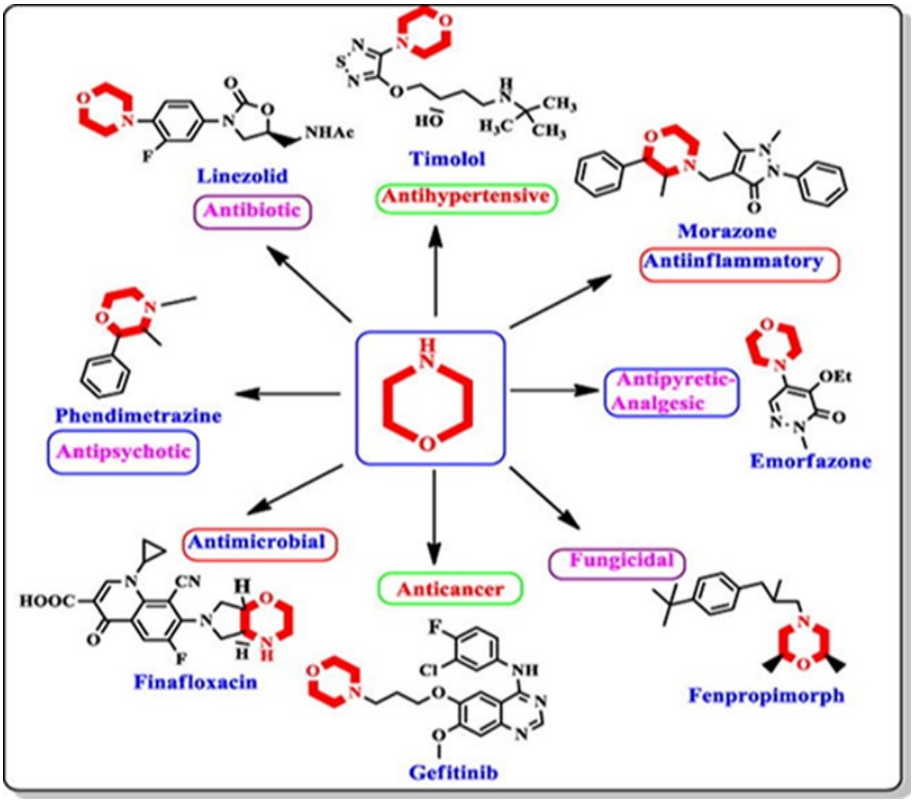

FIGURE 1.1: Therapeutic applications of some vital heterocyclic compounds containing morpholine pharmacophore [21]

Morpholine derivatives play a crucial role in drug discovery, driving extensive research due to their wide-ranging pharmacological activities. In recent times, this class of heterocyclic compounds has gained significant importance in the field of

medicinal chemistry because of their diverse biological properties. These derivatives show promising effects including analgesics, anti-inflammatory agents, anticancer drugs, antidepressants, and HIV protease inhibitors. Moreover, they demonstrate activity as appetite suppressants, local anesthetics, antiplatelet agents, and selective protein kinase C inhibitors. Their therapeutic potential further extends to anticancer, neuroprotective, antifungal, antitubercular, antiparasitic, antimalarial, hypolipidemic, and hypocholesterolemic applications, making them a versatile scaffold for developing novel therapeutics [22].

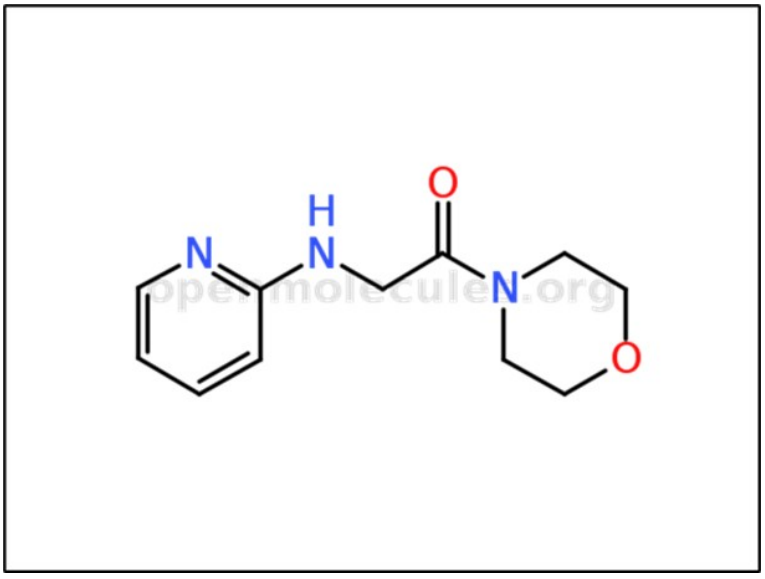

FIGURE 1.2: Chemical Structure of 1-morpholino-2-(pyridin-2-ylamino)ethan-1-one (IMPE) [23].

1.2 Rationale of Current Research Work

In recent years, heterocyclic compounds have gained significant attention in medicinal chemistry due to their various pharmacological actions, especially in the development of anti-inflammatory and analgesic agents. Among these, 1-morpholino-2-(pyridin-2-ylamino)ethan-1-one (1MPE) represents a promising candidate, combining a morpholine core with a pyridine-based structure to potentially enhance bioactivity and selectivity. Current anti-inflammatory treatments, such nonsteroidal anti-inflammatory medications (NSAIDs), frequently have some drawbacks, such as decreased effectiveness in chronic illnesses, cardiovascular risks,

and gastrointestinal toxicity. This emphasizes how urgently new drugs with enhanced safety profiles and strong therapeutic benefits are needed. This study aims to assess 1MPE's anti-inflammatory and analgesic capabilities *in vitro*/*in vivo* and in silico (molecular docking). The goal of this research is to help create a safer and more efficient substitute for treating inflammatory diseases by examining its pharmacological characteristics and mode of action.

1.3 Aim and Objectives

The aim of current study is to evaluate anti-inflammatory and analgesic actions of 1-morpholino-2-(pyridin-2-ylamino)ethan-1-one (1MPE).

i. To study the binding affinities with target proteins through molecular docking studies of 1MPE compounds.

ii. To use molecular dynamics simulations to study the stability and dynamics of the protein ligand complex.

iii. To investigate the pharmacological potential of the 1MPE by anti-inflammatory, analgesic, and DPPH antioxidant assay.

iv. To investigate anti-inflammatory activity by using *in vivo* model.

v. To investigate safety potential of 1MPE by oral toxicity study.

Chapter 2

Literature Review

The development of new chemical entities with medicinal potential is a difficult and drawn-out process. Heterocyclic derivatives have demonstrated exceptional pharmacological potential among the wide variety of marketed medications, including both heterocyclic and non-heterocyclic ones.

To provide insight into the extensive study and uses of morpholine, a possible heterocyclic ring [22, 24]. Several studies have shown how adaptable this nucleus is, and more research is needed to determine its wider potential for drug development and therapeutic innovation [24].

As a heterocyclic entity, morpholine is frequently included in both authorized and experimental medications as well as physiologically active substances. Its advantageous physicochemical characteristics, metabolic stability, and simplicity of synthesis account for its extensive usage in medicinal chemistry.

The morpholine ring is a very versatile and easily synthesized building component that can be easily added as an amine reagent or created using a number of well-known synthetic pathways [22].

When properly functionalized, the morpholine scaffold displays a variety of biological activity. The morpholine moiety is essential to many bioactive substances, either by increasing receptor binding affinity or by serving as a component of the

scaffold of enzyme inhibitors. Studies conducted *in vivo* demonstrate its capacity to enhance pharmacokinetic profiles and other drug-like properties in addition to increasing potency [25].

Because of its adaptability, morpholine is still a useful structural motif in drug design, helping to maximize pharmacological activity and improve efficacy. Studies show that adding a morpholine ring to a heterocyclic structure frequently improves pharmacological characteristics like potency, selectivity, and drug-like behavior [26].

The creation of attractive medication candidates with therapeutic potential for a variety of illnesses can be aided by the structure-activity relationship (SAR) profiles of compounds containing morpholine [27].

The morpholine nucleus is a desirable pharmacophore for creating powerful medications because of its appropriate physicochemical characteristics, which include balanced polarity and solubility, as well as its affordability and commercial accessibility [28].

In medicinal chemistry, the morpholine ring has been regarded as a privileged scaffold that plays a vital role in the creation and refinement of bioactive compounds. Because of its special structural characteristics—a six-membered heterocycle with both nitrogen and oxygen—it has a balance of basicity, polarity, and conformational flexibility, which makes it a crucial part of medication research. While the nitrogen atom offers a location for protonation, increasing bioavailability and membrane permeability, the oxygen atom improves solubility and hydrogen-bonding ability.

Because of these characteristics, molecules containing morpholine are especially useful in medications for the central nervous system (CNS), where blood-brain barrier penetration is essential [29]. In addition to its physicochemical benefits, morpholine's metabolic stability plays a significant role in its long-term use. The morpholine ring resists oxidative metabolism, which results in longer half-lives and lower dose frequencies in therapeutic settings, in contrast to certain heterocycles

that are quickly broken down by enzymes. In the treatment of chronic diseases, such as antiviral and anticancer therapy, where sustained medication action is needed, this stability is particularly advantageous. Additionally, because morpholine functions as a bioisostere for piperidine and tetrahydropyran, it can be strategically modified to maintain or improve pharmacological activity while also enhancing safety profiles [19].

Morpholine's relevance in drug discovery is further cemented by its synthetic flexibility. It is easily integrated into complex compounds through cross-coupling processes mediated by transition metals, acylation, or alkylation. Lead optimization has been streamlined by the direct alteration of morpholine-containing drug candidates made possible by recent developments in late-stage functionalization.

The ring can also improve target binding affinity by acting as a conformational constraint or a stiff spacer. For instance, morpholine frequently resides in the solvent-exposed area of kinase inhibitors, where it forms vital hydrogen bonds with backbone residues while preserving the best possible lipophilicity for cellular absorption [30].

Thanks to advancements in precision medicine, medication design, and computer modeling, morpholine will find extensive use in pharmaceutical research in the future. The creation of morpholine-based covalent inhibitors, in which the nitrogen of the ring is functionalized with electrophilic warheads to create irreversible interactions with target proteins, is one exciting avenue [31].

When traditional reversible inhibitors are ineffective against drug-resistant mutations in infectious illnesses and oncology, this strategy has demonstrated promise. Furthermore, adding morpholine to PROTACs (proteolysis-targeting chimeras) may improve the breakdown of difficult-to-degrade drug targets such aggregated proteins in neurological diseases and undruggable transcription factors [32].

The application of morpholine in peptide-drug conjugates and antibody-drug conjugates (ADCs) to enhance linker stability and payload solubility is another developing field. Morpholine is a desirable linker component that ensures effective drug

release at target areas because it can modify hydrophilicity without sacrificing cell permeability [33]. Furthermore, by investigating fluorinated and deuterated morpholine derivatives, problems with rapid clearance seen in certain therapeutic candidates may be resolved and metabolic stability and pharmacokinetics further optimized.

By forecasting ideal substitutions and ring modifications for increased potency and selectivity, developments in artificial intelligence (AI) and machine learning are speeding up morpholine-based drug discovery [24].

Virtual screening of morpholine-containing libraries against new biological targets, including ion channels, GPCRs, and epigenetic regulators, may open up new treatment options for metabolic disorders, immuno-oncology, and pain.

Enhancing therapeutic indices and minimizing off-target effects, the creation of enantiopure morpholine derivatives may also enable stereospecific interactions with chiral biological targets [24, 34]. Morpholine's versatility guarantees its continued importance as drug discovery shifts toward customized treatment.

Its synthetic accessibility, metabolic resilience, and structural diversification potential allow researchers to create next-generation medications that are safer, more effective, and more patient-compliant.

Morpholine's continued use in medicinal chemistry confirms its position as a crucial scaffold that will continue to spur advancement in the pharmaceutical sector for many years to come [35].

2.1 Morpholine and its Derivatives

The chasing of medicinal substances has been a constant throughout human history, with ancient civilizations relying exclusively on nature for their healing properties, for example, plants, animal byproducts, and minerals. Today, world still harbors an immense wealth of undiscovered chemical compounds that could potentially transform modern medicine [20].

In last century, revolutionary research occurred with the emergence of synthetic medicinal chemistry. Several novel compounds have developed the capability to make artificial compounds that not only replicated but often augmented the effectiveness of natural treatments [36].

These chemicals initially were created for industrial applications, such as textile dyes, were subsequently discovered to possess medicinal efficacy and pharmacological potential. Researchers investigated that making some modifications to synthetic moieties could result in novel candidate drugs with enhanced safety profiles and therapeutic potentials [37].

These recent studies and novel discoveries laid the foundation for latest medical treatments addressing cancer, chronic pain, allergic reactions, digestive ailments, microbial infections, inflammatory disorders, and other serious injuries [29]. The methods have been developed during this time span to support the modern pharmaceutical research, that have transformed global healthcare system.

The developments related to MAID represent a versatile chemical platform with diverse applications. In the field of medical, MAID compounds serve multiple functions: as pesticides and antimicrobial agents to fight pathogens, as antioxidants to protect against oxidative stress, and as crucial ingredients in personal care formulations and as the pharmacological active leads that has potential to become the candidate drugs [38]. The morpholine derivatives play a pivotal role in synthesizing different therapeutic agents, in pain management and anesthetics, mood-regulating antidepressants and appetite-controlling medications, anti-cancers and infection-combating antibiotics, as well as antifungal and antiparasitic treatments such as those targeting leishmaniasis. The journey from ancient herbal remedies to sophisticated, molecularly engineered pharmaceuticals demonstrate the pivotal role of medicinal chemistry in pushing the medical science, continually opening new possibilities for disease treatment and management [19].

Morpholine derivatives are important pharmacophore and versatile class of intermediates with broad pharmaceutical and pharmacological applications. These novel synthetic medicinally active compounds serve as key building blocks in the

synthesis of various bioactive agents, including catalysts, pharmaceuticals and personal care products.

Additionally, they also have the diverse Pharmacological activities, as antioxidants, bactericides, analgesics, anesthetics, antidepressants, and appetite suppressants. These novel derivatives also exhibit the significant therapeutic potential as anti-cancers, antibiotics, antifungals, and antileishmanial compounds [39].

2.2 Drug Development Perspectives of Morpholine Derivatives

A crucial pharmacophore and a supportive element in a variety of synthesized chemical compounds is the morpholin nucleus. It is a component of a variety of pharmacological active dyes, reaction catalysts, chemical and drug intermediates, active pharmaceutical ingredients (APIs), and coating materials including paints and varnishes. Additionally, morpholin plays a crucial part in the synthesis of several specialized secondary metabolites, such as alkaloids. Morpholine is a crucial component of the molecular structure and pharmacophore of a number of commonly used bioactive substances and medications. Gefitinib, Linezolid, Emorfazone, Timolol, and Reboxetine are a few examples of commercially accessible medications. This demonstrates the adaptability of the morpholine nucleus and its significance in medicinal chemistry, drug development, and discovery [26]. Through nor-adrenergic modulation, these compounds have a wide range of bio-activities and pharmacological potential. They are essential ingredients in a number of medications, including antidepressants, analgesics, antitumor, antimicrobials, and neurokinin-1 (NK-1) receptor antagonists [40]. They also hold great promise for treating neurological conditions like anxiety, Alzheimer's, and dementia [41]. By enabling chiral stereoselective synthesis as efficient auxiliaries and ligands, the various chiral and asymmetric variations of morpholine derivatives further increase their utility. Morpholine is an essential organic scaffold in medicinal chemistry and contemporary drug development due to its distinct mix

of structural flexibility and varied pharmacology, highlighting its importance in creating new therapeutic agents and specialty compounds [24].

For structural versatility, they can be used as structural modifiers in drug design as well as pharmacophores, morpholin derivatives hold a special place in medicinal chemistry. By combining hydrophilic oxygen and basic nitrogen with hydrophobic methylene groups, the morpholine ring's balanced amphiphilic nature allows for the best membrane permeability while preserving water solubility [33].

As demonstrated by medications such as Reboxetine (a norepinephrine reuptake inhibitor for depression) and Timolol (a β-adrenergic blocker for glaucoma), this characteristic profile makes it especially beneficial in CNS-targeting medications where blood-brain barrier penetration is crucial.

Because of its synthetic flexibility, the ring can be substituted in a variety of ways that can significantly change biological function. N-substituted morpholines, for example, are commonly found in kinase inhibitors, where the nitrogen lone pair engages in important hydrogen bonding interactions with sites that bind ATP [22].

An example of this use is gefitinib, a groundbreaking EGFR tyrosine kinase inhibitor for non-small cell lung cancer. Likewise, 2- and 3-substituted morpholines frequently modulate selectivity and metabolic stability by acting as bioisosteric substitutes for piperidine or tetrahydropyran rings. Linezolid, the first oxazolidinone antibiotic with a morpholine moiety, shows how pharmacokinetics can be maintained while bacterial resistance mechanisms are circumvented through targeted inclusion. Beyond conventional small molecules, morpholine derivatives are increasingly important in targeted therapies [22, 42]. Their ability to act as solubilizing groups while maintaining cell permeability makes them ideal components for antibody-drug conjugates (ADCs) and PROTACs (proteolysis-targeting chimeras). Recent developments include morpholine-containing HDAC inhibitors with improved isoform selectivity and reduced off-target effects in cancer therapy. The ring's metabolic stability also contributes to prolonged half-lives *in vivo*, particularly when combined with fluorination strategies at the 2- or 3-positions to block oxidative metabolism [43].

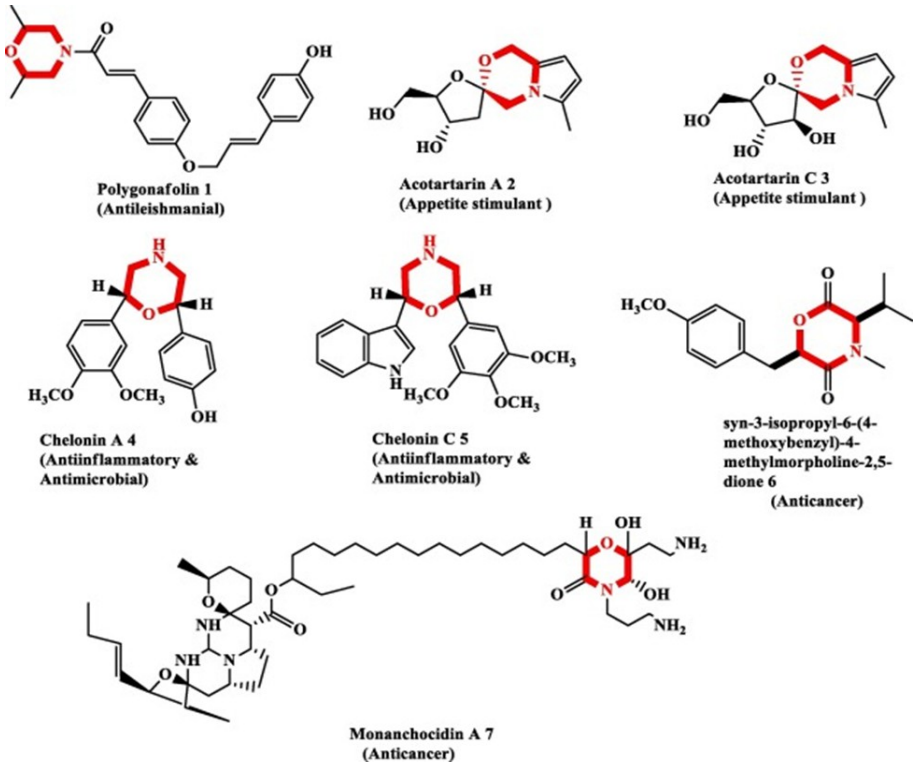

FIGURE 2.1: Heterocyclic compounds containing morpholine ring [44]

The therapeutic potential of morpholine derivatives continues to expand across multiple disease areas. In neuroscience, novel morpholine-containing sigma-1 receptor modulators show promise for neurodegenerative diseases, while 2-aryl morpholines demonstrate potent neurokinin-1 antagonism for depression and chemotherapy-induced nausea [22]. Antimicrobial applications are particularly noteworthy – beyond Linezolid, new morpholine-incorporated oxazolidinones combat multidrug-resistant Gram-positive pathogens, and morpholine-functionalized quinolones exhibit enhanced activity against Mycobacterium tuberculosis [45].

Chiral morpholine chemistry has opened new dimensions in asymmetric synthesis and drug discovery. The constrained conformation of substituted morpholines creates well-defined three-dimensional architectures that improve target binding specificity. For example, (S)-Reboxetine's enantioselective norepinephrine reuptake inhibition highlights the importance of stereochemistry. Synthetic methodologies have advanced significantly, with recent developments including transition metal-catalyzed C-H functionalization for direct morpholine derivatization, photocatalytic decarboxylative couplings to construct complex morpholine-containing

scaffolds, and enzymatic resolutions and asymmetric hydrogenations for stereo-controlled synthesis [46].

Emerging applications in materials science further demonstrate morpholine's versatility. Morpholine-based ionic liquids serve as green solvents for pharmaceutical manufacturing, while polymeric morpholine derivatives find use in drug delivery systems due to their pH-responsive solubility. The ring's coordination chemistry also enables applications in catalytic systems – chiral morpholine-phosphine ligands achieve excellent enantioselectivity in asymmetric hydrogenations [47]. Looking forward, computational approaches are accelerating morpholine-based drug discovery. AI-driven virtual screening of morpholine fragment libraries identifies novel chemotypes, while quantum mechanical calculations predict metabolic soft spots for rational optimization. The integration of morpholine into macrocyclic peptides and bifunctional degraders represents another frontier, potentially addressing currently "undruggable" targets. As synthetic methodologies evolve and biological understanding deepens, morpholine derivatives will undoubtedly remain at the forefront of pharmaceutical innovation, bridging the gap between traditional small molecules and next-generation therapeutics [48]. The continued exploration of morpholine's chemical space – including spirocyclic variants, bridged morpholines, and polycyclic fused systems – promises to yield novel therapeutic agents with improved selectivity and ADME profiles. With its unique combination of synthetic accessibility, structural diversity, and favorable physicochemical properties, the morpholine scaffold will maintain its pivotal role in addressing unmet medical needs across therapeutic areas [22].

2.3 Morpholine-Based mTOR Inhibitors for Cancer Treatment

Mammalian target of rapamycin (mTOR), is a serine & threonine kinase from the PI3K-related kinase family or enzymes, is an important therapeutic target for cancer and type-2 diabetes mellites. However, the development of novel selective inhibitors is challenging due to the conserved ATP binding sites in PI3K

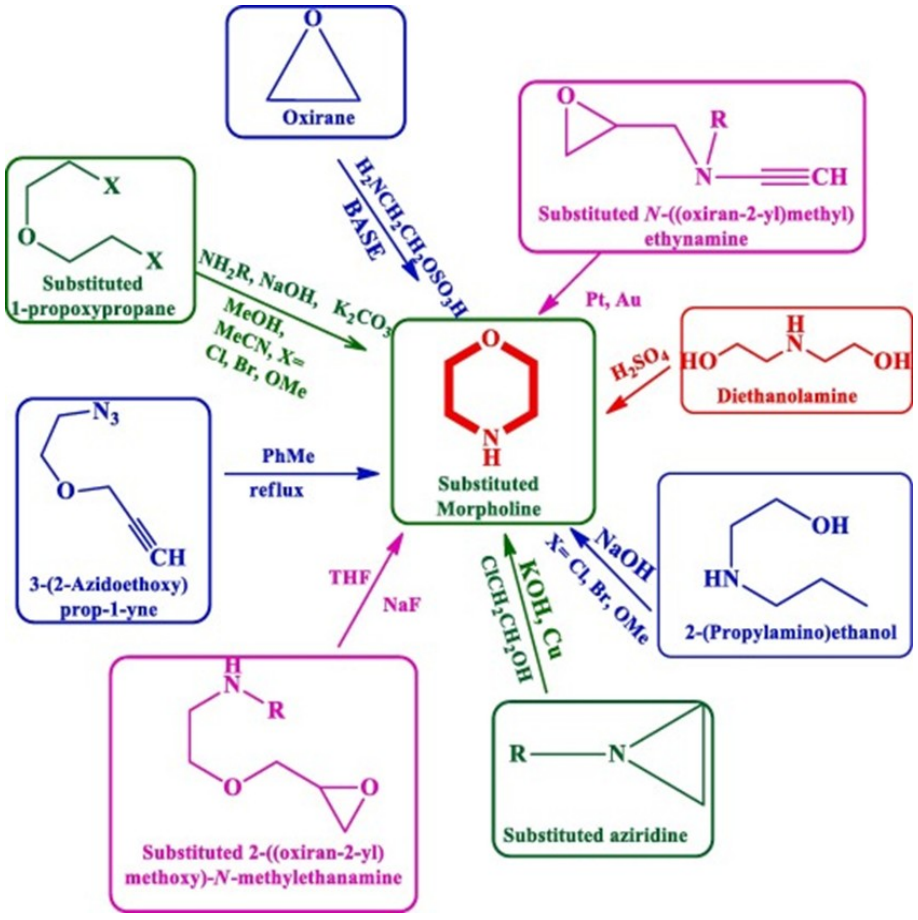

FIGURE 2.2: Chemical reactions resulting in generation of morpholine ring substitutes [49].

kinase enzymes. It was discovered in recent researches that morpholine containing pyrazolo-pyrimidines exhibit potent and selective mTOR inhibition in cancer xenograft models [49]. Subsequent studies synthesized pyrazolo-pyrimidine analogs with morpholine, methyl-morpholine, and bridged morpholine substitutions at the R1 position particularly.

Morpholine substitution significantly enhanced mTOR selectivity, demonstrating its potential for targeted therapy and treatments [50]. The development of morpholine-containing anticancer drugs with reduced side effects is a key focus in drug discovery, particularly for CNS tumors like glioblastoma and medulloblastoma. Targeted therapy against kinases such as PI3K and mTOR, critical in cell cycle regulation—has shown promise, as their overactivation drives tumor growth and neurodegeneration. Brain-penetrant dual PI3K/mTOR inhibitors could offer new treatments for brain tumors and metastases. Recent advances

include morpholino-pyrimidine, morpholino-triazine, and morpholinopyrimidine-5-carbonitrile derivatives as potent inhibitors [51].

Researchers led by Wang designed and created several new compounds containing morpholine and tested their ability to stop cancer cell growth. They focused on two types of cancer cells: HCT-116 (colon cancer) and MCF-7 (breast cancer). One compound, 3-(4,6-dimorpholino-1,3,5-triazin-2-yl)-5-(trifluoromethoxy)benzamide, showed strong effects in slowing down cancer cell growth.

Tests revealed that it works by blocking a key cancer-related pathway (PI3K/Akt/mTOR), which helps tumors grow. It also caused visible changes in the cancer cells and triggered their death (apoptosis) [52].

In another study, the team made similar compounds but with a quinazoline structure instead of triazine. The best one, 3-(6,7-dimethoxy-4-morpholinoquinazolin-2-yl)-5-(trifluoromethoxy) benzamide, was very effective at low doses ($IC_{50} < 4$ μM) and also worked against brain (U-87 MG) and lung (A549) cancer cells. Like the first compound, it disrupted the PI3K/Akt/mTOR pathway and caused cancer cell death [53].

2.4 Novel Morpholine-Pyrazole Derivatives Combat Drug-Resistant Parasites

Malaria, leishmaniasis, and trypanosomiasis, caused by *Plasmodium falciparum, Leishmania donovani*, and *Trypanosoma brucei gambiense*, remain major global health threats. Malaria alone caused 214 million cases and 438,000 deaths in 2015. Current therapies are limited by resistance and toxicity, creating an urgent need for new treatments. Researchers synthesized novel morpholine-based pyrazole derivatives showing potent activity against drug-resistant P. *falciparum, T. rhodesiense*, and *T. cruzi*, along with low cytotoxicity. These findings highlight their potential as anti-parasitic drug candidates [54]. Kuettel and colleagues developed a

novel series of compounds based on the 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl] morpholine structure using two different synthetic approaches.

These derivatives were evaluated for potential to combat parasitic infections caused by *Trypanosoma species, Leishmania donovani* , and the malaria parasite *Plasmodium falciparum* strain [55]. Among the newly synthesized derivatives of this nucleus, 4-(3-(4-phenoxyphenyl)-1H-pyrazol-5-yl) morpholine was found to be notably more effective.

This synthesized organic molecule, which had an extraordinarily low half-maximal inhibitory concentration (IC_{50}) of 1.1 μM, showed outstanding effectiveness against various life-threatening parasites.

The concentration needed to stop 50% of the parasite's growth or activity is known as the IC_{50} value; a lower number denotes greater effectiveness [56].

2.5 Novel Morpholine NO Donors Improve Lipid Profiles

In vitro, NO-releasing morpholine derivatives have shown a strong ability to inhibit lipid peroxidation (LPO) while also successfully modifying plasma lipid profiles, such as levels of total cholesterol, triglycerides, and low density lipoprotein (LDL) cholesterol [57]. A lack of NO leads to cardiovascular (CV) diseases such atherosclerosis and coronary artery disease (CVD), as it is an essential regulator of vascular function and endothelial homeostasis. These findings position morpholine-based NO donors as promising therapeutic agents capable of restoring vascular integrity through exogenous NO supplementation [58].

Recent study has developed a new series of morpholine-based compounds with different aromatic groups attached to the morpholine ring. These compounds showed two important biological effects: they blocked cholesterol production in cells while also protecting liver cell membranes from oxidative damage [59].

The team discovered that these morpholine derivatives work by inhibiting squalene synthase (SQS), a key enzyme in cholesterol synthesis. The most effective compounds in this series showed strong activity, with IC_{50} values (the concentration needed for 50% inhibition) ranging between 0.7-5.5 μM [60]. At the same time, these same compounds demonstrated protective effects against lipid peroxidation (a damaging oxidative process) in liver cell membranes, with IC_{50} values ranging from 73-200 μM. Among all the compounds tested, one stood out as particularly powerful: 3-(phenanthren-2-yl)octahydropyrido[2,1-c][1,4]oxazin-3-ol hydrobromide. This compound showed the strongest combination of cholesterol-lowering and membrane-protecting effects [61].

These findings are very important and significant in drug discovery because they reveal a new class of compounds that could potentially treat medical conditions related to high cholesterol while also protecting hepatic cells from tissue damage. The dual action of these molecules makes them especially interesting for further drug discovery and development, as they might address both cholesterol problems and associated oxidative stress in metabolic disorders.

The recent studies in research suggests that carefully designed morpholine derivatives could lead to new candidate drugs for cardiovascular diseases and liver conditions, though more studies would be needed to develop these compounds into actual medications [62].

2.6 Morpholine Scaffolds Targeting Inflammatory Pathways

Current medical treatments only manage symptoms, often with serious side or adverse effects, leaving a critical need for new disease-modifying therapies. Morpholine derivatives show promise by targeting key pathological/pharmacological mechanisms, including LRRK-2 kinase [63]. That is a protein linked to familial and sporadic PD through mutations that disrupt mitochondrial function and trigger

neuroinflammation. Potent LRRK2 inhibitors like MLi-2, with optimized drug-like properties, highlight morpholine's potential as a scaffold for next-generation PD therapies [64].

Scientists have developed a compound containing a 2,5-dimethylmorpholine structure that effectively blocks the LRRK2 enzyme (a key target in Parkinson's disease research) with remarkable potency (IC_{50} = 0.76 nM) [22].

What makes this compound particularly valuable is its exceptional selectivity - it's 295 times more likely to target LRRK2 than any of 300 other similar enzymes.

The compound also demonstrates good brain penetration and has shown safe profiles in various animal studies, including MitoPark mouse models of Parkinson's. Building on this success, researchers recently created a fluorine-18 labeled version (compound 9) for use as a PET imaging tracer to visualize LRRK2 activity in living brains [65, 66].

In Parkinson's research, dopamine receptors represent another important therapeutic target. These receptors fall into two main categories: D1-like (D1 and D5) and D2-like (D2, D3, D4), each with distinct brain distributions and functions.

D2 receptors, concentrated in movement-control areas, influence several cellular processes including cyclic AMP production and fat molecule breakdown. In contrast, D3 receptors appear mainly in emotional and cognitive regions. Notably, Parkinson's patients show increased D3 receptor activity without D2 changes, making selective D3 targeting crucial to avoid side effects from D2 activation [67].

The morpholine structure has proven valuable in developing selective D3-targeting drugs. For instance, when added to a noradrenaline-like framework (as in compound 10), the N-morpholinopropyl group helps create effective D3 agonists. Morpholine derivatives also show promise in protecting dopamine neurons - compound 12 inhibits Nrf2 to prevent neuron loss. In this case, adding the morpholine ring improved both the drug's effectiveness (better EC50) and its ability to reach the brain (confirmed by specialized permeability tests), overcoming limitations of earlier versions [22].

2.7 Morpholine Scaffolds Targeting Enzymes

The amyloid cascade hypothesis highlights β-secretase (BACE-1) as a key target in many neurodegenerative disorders. BACE-1 cleaves amyloid precursor protein (APP) into neurotoxic Aβ peptides, driving AD progression.

Developing inhibitors that cross the blood-brain barrier (BBB) remains challenging. Morpholine derivatives show promise by fitting precisely into BACE-1's active site, engaging critical residues (Gly34, Thr72) and leveraging the ring's exoanomeric conformation for optimal binding.

These features, combined with BBB permeability, position morpholine scaffolds as potential AD therapeutics [68]. Morpholine derivatives have emerged as valuable structural components for developing BACE-1 inhibitors, an important target in Alzheimer's disease research. These compounds demonstrate particular effectiveness due to their unique three-dimensional properties. For instance, compound 13 - designed as a hydroxyethylamine-based peptide mimic - achieves optimal binding by adopting a specific spatial arrangement within BACE-1's active site.

Its structure allows strategic positioning of chemical groups into the enzyme's S1, S1, and S2 binding pockets while maintaining sufficient flexibility for blood-brain barrier penetration [69]. The morpholine ring itself adopts a preferred synclinal conformation in solution, stabilized by the exoanomeric effect - a phenomenon where the ring oxygen and adjacent carbon atoms align in a specific spatial relationship. This orientation positions the methyl group toward the S1 pocket of BACE-1. Additionally, the morpholine nitrogen forms crucial hydrogen bonds with Gly34's carbonyl group, while the ring oxygen interacts with Thr72 in the enzyme's flexible flap region [70]. These precise molecular interactions explain why morpholine-containing compounds show such promise as BACE-1 inhibitors, combining optimal binding characteristics with the ability to reach their target in the brain. The careful spatial arrangement of the morpholine ring and its substituents creates multiple favorable contacts with the enzyme's active site, making these compounds particularly effective at blocking BACE-1 activity [71].

2.8 Morpholine Derivatives as Promising Analgesic Agents

Morpholine-containing compounds exhibit significant analgesic effects by targeting key receptors, including adenosine kinase, cannabinoid receptors (CB1/CB2), and sigma-1 receptors. The morpholine moiety enhances both potency and pharmacokinetic properties, making it a valuable scaffold in drug design. Notably, morpholine-substituted (aza)indoles have demonstrated strong analgesic activity in preclinical studies, highlighting their therapeutic potential for pain management [72]. In a recent study, Khanum and coworkers have developed a new series of hydroxy benzophenones and benzophenone-N-ethyl morpholine derivatives.

They evaluated their anti-inflammatory potential through carrageenan-induced paw edema tests in rats. Among these newly synthesized compounds, (4-methoxyphenyl)(3-methyl-4-(2-morpholinoethoxy)phenyl)methanone has emerged as the most effective at reducing inflammation, demonstrating the significant contribution of the morpholinoethoxy substituent to pharmacological activity [72].

In another study, Takaya and his coworkers designed and synthesized various 2-alkyl/alkenyl-4-alkoxy-5-(substituted amino)-3(2H)-pyridazinone derivatives to find out their analgesic and anti-inflammatory properties.

Their pharmacological evaluation showed that 4-ethoxy-2-methyl-5-morpholino-3(2H)-pyridazinone possessed significant analgesic and anti-inflammatory effects compared to phenylbutazone as standard reference drug. The presence of the morpholino group at the 5-position of the pyridazinone ring responsible for this pronounced pharmacological activity, suggesting its value in molecular design for such therapeutic applications [73].

The above two studies reveal morpholine-containing compounds as crucial scaffolds for synthesizing novel anti-inflammatory agents, with each research group exhibiting different structural approaches to achieve potent biological effects.

2.9 Morpholine Derivatives as Anti-Inflammatory Agents

Morpholine have anti-inflammatory activities by targeting key mediators like adenosine kinase (AK), TNF-α, IL-6, and cannabinoid receptors (CB1/CB2). Its ability to modulate inflammatory enzymes and cross the blood-brain barrier expands its therapeutic potential for treating neuroinflammation and systemic inflammatory conditions [74].

Smelcerovic and colleagues successfully developed two novel cyclodidepsipeptide compounds featuring morpholine-2,5-dione structures: 3-(2-methylpropyl)-6-(propan-2-yl)-4-methyl-morpholine-2,5-dione and 3,6-di(propan-2-yl)-4-methyl-morpholine-2,5-dione. The research team thoroughly investigated these compounds' biological potential through multiple experimental approaches.

The compounds were first evaluated for their ability to inhibit xanthine oxidase (XO), both in purified enzyme preparations and within rat liver tissue samples.

XO inhibition is particularly relevant for treating conditions like gout, where reduced uric acid production is therapeutic.

Additionally, the researchers examined the compounds' anti-inflammatory properties using human peripheral blood mononuclear cells (PBMCs), which are crucial immune cells involved in inflammatory responses [75].

Both cyclodidepsipeptide derivatives demonstrated remarkable biological activity across all tested systems.

Their potent inhibition of xanthine oxidase suggests potential applications in hyperuricemia management, while their anti-inflammatory effects on PBMCs indicate possible utility in modulating immune responses.

The morpholine-2,5-dione core structure appears to be particularly effective for these biological activities [76].

2.10 Antioxidant Potential of Morpholine Moieties

The wide range of biological activities exhibited by morpholin-based compounds makes them very useful in the development of pharmaceuticals.

The ability of these nitrogen-oxygen heterocycles to neutralize dangerous free radicals and shield cells from oxidative damage is the main way that they exhibit strong antioxidant properties.

Their wider therapeutic potential is based on this radical scavenging activity [77]. By selectively inhibiting squalene synthase (SQS), these drugs have an antihyperlipidemic effect, which is one of their most important pharmacological actions.

SQS is an important regulatory point in the cholesterol production pathway since it is the first committed enzyme.

Morpholine derivatives efficiently lower cholesterol production by inhibiting this enzyme, preventing the buildup of potentially harmful upstream metabolites that happens when early steps in the route are inhibited.

Compounds containing morpholine are particularly efficient against complicated, multifactorial disorders like atherosclerosis because of this unusual mix of characteristics.

Morpholine derivatives can concurrently target the pathways of oxidative stress, chronic inflammation, and deregulation of lipid metabolism that are all part of the illness process. Compared to single-mechanism medicines, their capacity to address several facets of disease pathophysiology increases their therapeutic potential [78].

The morpholine ring's structural adaptability permits a wide range of chemical changes, which optimizes the drug's characteristics while preserving its complex activity profile.

This flexibility explains why morpholine derivatives are still of great interest for the development of therapies for cardiovascular illnesses, metabolic disorders, and other situations where lipid abnormalities, inflammation, and oxidative stress co-exist.

They are potential options for using pharmacological action to treat complicated disease pathways because of their balanced impact on various interrelated biological processes [79]. An important investigation examining the therapeutic potential of 2-biphenylyl morpholine derivatives was carried out by Chrysselis and associates.

By synthesizing multiple new compounds and thoroughly assessing their dual antioxidant and cholesterol-lowering capabilities, the study team showed how structural alterations to the morpholine core might result in molecules with notable biological activity [80].

In order to assess the compounds' capacity to prevent oxidative damage, the researchers first looked at how well they inhibited lipid peroxidation in microsomal membranes caused by iron and ascorbate.

With an IC_{50} of 250 μM, 2-(4-biphenyl)-4-methyl-octahydro-1,4-benzoxazin-2-ol had the most antioxidant activity among the series. This measurement suggests that the molecule could successfully counteract cellular oxidative stress because it shows the concentration needed to cut oxidative damage in half [81].

In subsequent *in vivo* experiments using a Triton WR-1339-induced hyperlipidemic rat model, the same lead compound demonstrated remarkable cholesterol-lowering effects. Important lipid markers were significantly reduced when given intraperitoneally at a dosage of 28 μmol/kg: triglycerides were reduced by 49%, total cholesterol by 54%, and low-density lipoprotein (LDL) by 51%.

These striking outcomes demonstrate the compound's potential as a multipurpose therapeutic agent that might concurrently treat lipid imbalances and oxidative stress, two significant risk factors for cardiovascular disease [82].

2.11 Morpholine-Containing Caspase Inhibitors

Caspases represent a crucial class of enzymes that play a central role in programmed cell death (apoptosis). As cysteine-dependent aspartate-specific proteases, these enzymes function as molecular executioners that systematically dismantle cellular components during apoptotic processes [83].

Among the caspase family, caspase-3 holds particular significance as it serves as a convergence point for both intrinsic (mitochondrial-mediated) and extrinsic (death receptor-mediated) apoptotic pathways. This strategic position in cell death signaling makes caspase-3 an attractive therapeutic target for modulating apoptosis in various disease states [84]. The discovery of caspase inhibitors has created new treatment opportunities in a number of medical specialties. These inhibitors have demonstrated therapeutic promise as immunomodulators (by regulating immune cell survival), anti-arthritic drugs (by avoiding excessive cartilage cell death), and neuroprotective medicines (perhaps helpful in stroke or neurological illnesses). Recent developments have concentrated on creating non-peptide substitutes that provide superior metabolic stability and bioavailability, as traditional caspase inhibitors were frequently peptide-based [85]. Researchers have designed a novel series of caspase inhibitors featuring a morpholine-sulfonyl substituted pyrroloquinoline-dione core structure. These compounds follow the general chemical formula of 4-substituted-2-(2-acetyloxyethyl)-8-(morpholine-4-sulfonyl)-pyrrolo[3,4-c]quinoline-1,3-diones [86]. The incorporation of the morpholine sulfonyl group appears crucial for inhibitory activity, likely by mimicking key interactions with the caspase active site. The acetyloxyethyl side chain at position 2 and various substituents at position 4 allow for structural optimization of inhibitory potency and selectivity. This class of non-peptide inhibitors represents an important advancement in caspase-targeted drug development, potentially overcoming limitations associated with peptide-based inhibitors while maintaining strong affinity for caspase enzymes. The unique pyrroloquinoline-dione scaffold combined with the morpholine sulfonyl pharmacophore creates a promising template for developing clinically useful apoptosis modulators [87].

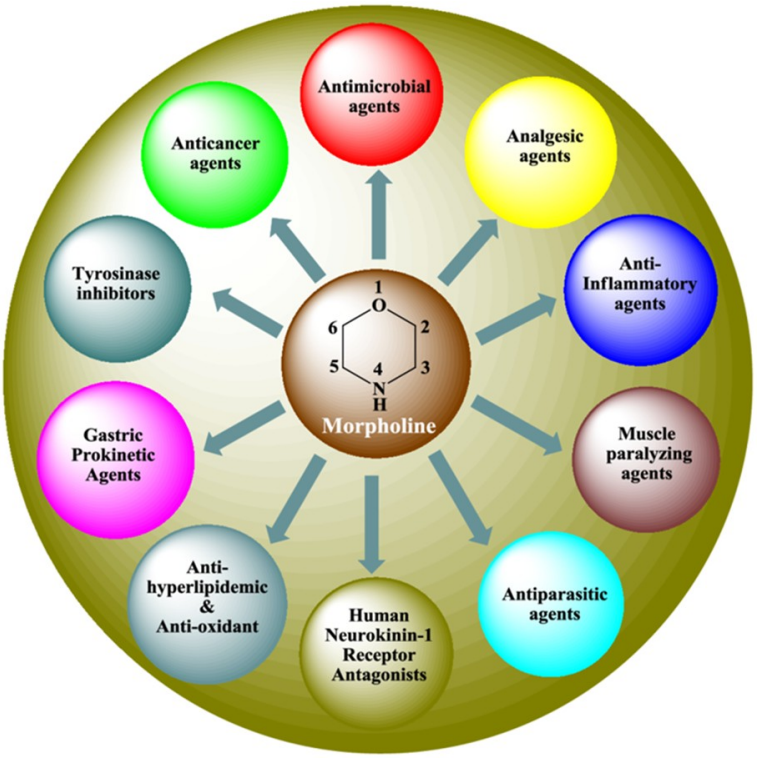

FIGURE 2.3: Some marketed available morpholine containing drugs [39]

Chapter 3

Materials and Methods

3.1 Chemicals and Solvents

1-morpholino-2-(pyridin-2-ylamino) ethan-1-one (1MPE), diphenyl-1- picrylhydrazyl (DPPH), carrageenan, ethanol, methanol.

3.1.1 Animals

Adult male and female mice weighing 20-30 g were obtained from the animal house at Capital University of Science and Technology (CUST).

Mice will be separated into male and female groups. Mice were housed four to five per cage in a temperature and humidity-controlled environment (22°C±2, relative humidity 50-60%).

A 12-hour light-dark cycle was maintained, and mice were given free access to water and food. All animal protocols and experimental procedures will be approved by the Ethics Committee of Faculty of Pharmacy, CUST.

FIGURE 3.1: Acclimatization of experimental animals

3.1.2 Instrumentation for *In silico* Studies

Software tools including ChemBioDraw, PyRx, Schrodinger's MD simulation and Auto Dock was utilized for molecular docking & simulation studies.

3.2 Methodology

3.2.1 *In silico* Studies

ChemBioDraw software was used to generate 3D models of 1-morpholino-2-(pyridin-2-ylamino) ethan-1-one (1MPE). The prepared ligands in MDL SDF format were saved as PDB files and subsequently converted into PDBQT format for further processing using the PyRx tool. The tertiary structures of the target proteins were obtained from the Protein Data Bank. Chimera and PyMOL were employed to visualize and analyze the protein structures. AutoDock was

performed for docking calculations of the preferred conformations. The docking results were clustered based on a root-mean-square deviation (RMSD) cutoff of 2.0 Å. The structures exhibiting the lowest binding free energy and the highest number of cluster members were selected as the optimal docking conformations [88].

3.2.2 ADMET Properties of 1MPE

The Absorption, Distribution, Metabolism, Excretion, and Toxicity properties of compound 1-morpholino-2-(pyridin-2-ylamino) ethan-1-one (1MPE) were characterized using the SWISS ADME online software, a widely used computational tool for predicting pharmacokinetic and toxicity-related data of chemical entities http://www.swissadme.ch/.

3.2.3 Molecular Docking Studies

3.2.3.1 Retrieval of Receptors Structure from Protein Data Bank

The three-dimensional (3D) structures of receptor were accessed from Protein Data Bank (PDB) (www.rcsb.org) with PDB IDs as mentioned below in Table 3.1.

Table 3.1: Three-dimensional (3D) structures of receptor protein

Name	**PDB ID**	**Organism**
COX-2	3LN1	*Mus musculus*

The desired proteins were arranged for molecular docking analysis utilizing the AutoDock Tools program. First of all, energy minimization of proteins was done to optimize their structures, followed by the addition of Gasteiger charges for electrostatic interactions.

The ready-made proteins were then saved in the PDBQT format, which is compatible with docking simulations. Hydrophobicity plots and Ramachandran plots

were generated using Discovery Studio 4.1 Client (2012) for further structural characterization of proteins, which provide insights into residue hydrophobicity and backbone dihedral angles to evaluate structural validity.

Furthermore, the secondary structure of proteins was analysed using VADAR 1.8, including the percentages of α-helices, β-sheets, coils, and turns (Willard et al., 2003), which also provided an evaluation of overall protein architecture and statistical validation of protein structures.

This comprehensive preparation and analytical characterization are compulsory for the optimization and validation of protein structures before proceeding with molecular docking studies.

3.2.3.2 Ligands-Molecular Docking

The compound was typically sketched using Discovery-Studio for energy minimization to ensure stability of structurs throughout the analysis. AutoDock Tools were then employed for the preparation of ligands in their most stable conformations.

During this important step, Kollman and Gasteiger charges were added to account for electrostatic interactions, and then saved in PDBQT-files format which made them favourable for molecular docking simulations.

The molecular docking experiments were conducted using vina software, incorporating to evaluate the binding energies of ligand. PDB ID:3LN1, the grid box was centered at X = 34.212, Y = -26.673, Z = -10.250, with adjusted dimensions (X = 66, Y = 74, Z = 78) to ensure optimal positioning within the active site. Additionally, another grid box with expanded dimensions (X = 126, Y = 126, Z = 126) was used to explore broader conformational space. Each ligand was docked individually against the target receptors using a default exhaustiveness value of 50 to enhance docking accuracy. The resulting complexes were assessed based on their lowest binding energy (kcal/mol), and the most favourable binding poses were visualized in 3D using Discovery Studio Visualizer (Version 4.0, 2012) for detailed structural analysis.

3.2.3.3 Structural Analysis of Target Proteins COX-2 PDB ID: 3LN1

The protein structure consists of 7% β-sheets (155 residues), 41% α-helices (825 residues), 48% coils (1012 residues), and 4% turns, totalling 2,207 residues. Unit cell dimensions were measured as a =180.92Å/ b=135.31Å & c = 124.069Å, with all angles of α, β, and γ with 90°, indicating an orthorhombic crystal system. Ramachandran plot analysis revealed that 98% of the amino acid residues fall within the allowed regions for phi (ϕ) and psi (ψ) angles, confirming the high stereochemical quality of the protein structure. The R-plot for the target protein is showed in Figure 3.1.

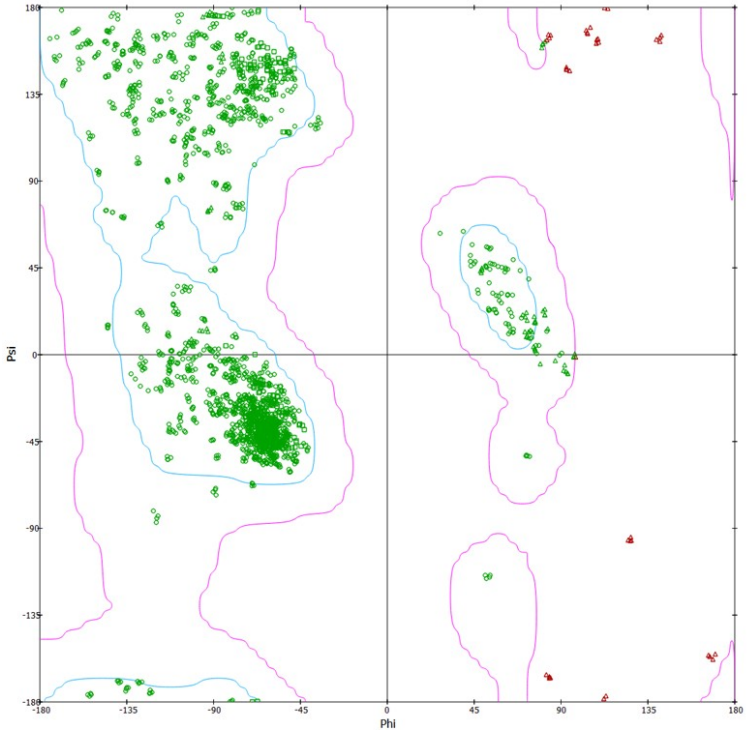

FIGURE 3.2: R-plot for COX-2 protein as PDB ID is 3LN1.

3.2.4 Molecular Dynamic Simulation

The molecular mechanics/generalized Born surface area (MM/GBSA) approach was often used to study the chemical 1MPE. This method computed the binding free energy and other thermodynamic characteristics of the system by combining the generalized born continuum solvation model with force fields from molecular

mechanics. The prediction and assessment of small molecule binding affinities with their target proteins or receptors were made possible by MM/GBSA.

The energetics and binding interactions between the drug and its target were crucially revealed by thorough molecular dynamics simulations and post-processing analysis.

To learn more about the energetics and binding interactions between the chemical and its target, extensive molecular dynamics simulations and post-processing analysis were employed.

3.2.5 Cheminformatic Properties

For the evaluation of molecular properties and the assessment of drug-likeness of 1MPE Molinspiration and ChemBioDraw both served as essential cheminformatics tools.

These softwares calculate key parameters like physicochemical descriptors, prediction of bioactivity scores, and analysis of compliance with Lipinski's Rule of Five (Ro5).

The Lipinski's Rule of Five (Ro5) predicts a compound's potential for oral bioavailability, making these software tools commendable in early-stage drug discovery and development.

By providing critical insights into molecular characteristics, Molinspiration and ChemBioDraw enable efficient screening and optimization of compounds for desired pharmacological properties.

3.3 Pharmacological Evaluation of 1MPE

1-morpholino-2-(pyridin-2-ylamino) ethan-1-one (1MPE) was tested for the following pharmacological activities.

3.3.1 *In-vitro* Assays

3.3.1.1 Antioxidant Potential Evaluation Using DPPH Assay

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate the antioxidant potential of 1MPE. The negative control was a blank DPPH solution, whereas the positive control was ascorbic acid. Each chemical solution was added to 0.1 mM DPPH methanolic solution in varying doses (10–500 μg/mL) and forcefully shaken.

The deep purple DPPH solution in this assay, which has its maximum absorbance at 517 nm, turns yellow as the antioxidants give electrons or hydrogen atoms to the free radicals in order to neutralize them.

After 30 minutes of incubation at 37°C, the mixture's absorbance changes were monitored with a microplate reader [89].

3.3.2 *In vivo* Assays

3.3.2.1 Thermal Hyperalgesia Assessment Hot Plate Test

The hot-plate test was used to evaluate the nociceptive response of mice to heat. The test was conducted on a hot plate maintained at 54°C ± 1°C, with a clear plastic cylinder placed around each animal to prevent escape. The endpoint was recorded when the mouse licked or lifted its paw, indicating a nociceptive reaction. The primary measured parameter was the latency time (in seconds) until this response occurred. To minimize tissue damage, a 30-second cut-off time was enforced, with temperatures carefully controlled to avoid burns.

Response times were measured at 60 minutes, 90 minutes and 120 minutes after administration of 1MPE and tramadol. Following each trial, the hot plate surface was cleaned with 20% ethanol to eliminate residual odours or contaminants.

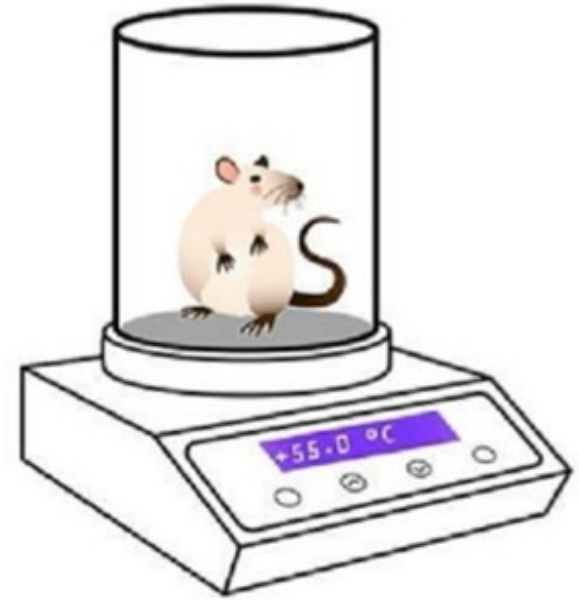

FIGURE 3.3: Thermal hyperalgesia by Hot plate apparatus [90]

3.3.2.2 Anti-Inflammatory Evaluation of 1MPE

Mice weighing 20–25 g were divided into four groups (n=3): vehicle control (saline), negative control (carrageenan), 1MPE (treatment), and positive control (diclofenac). Edema was induced by injecting 20 μL of 1% (w/v) carrageenan into the subplantar tissue of the left hind paw 30 minutes after administering the 1MPE and standard drug (diclofenac). Paw volume was measured at baseline and at specific intervals (30 min, 1, 2, 4, and 6 hours) post-injection, with edema progression compared to the control group. After the final measurement at 6 hours, the animals were humanely euthanized.

3.3.2.3 Acute and Subacute Oral Toxicity Profiling of 1MPE

Using healthy male albino mice weighing 34 ± 10 g, the oral toxicity of 1-morpholino-2-(pyridin-2-ylamino)ethan-1-one (1MPE) was evaluated in compliance with OECD criteria. Group A (control, 0.9% saline), Group B (vehicle control, 5% DMSO), and Group C (1MPE, 100 mg/kg) were the three groups (n=3)

into which the animals were divided. The study went on to ascertain the maximum tolerance dosage (MTD) because no fatal dose (LD50) was noted. Mice were kept under typical lab settings, which included a 12-hour light/dark cycle, 25 ± 2°C, 65 ± 5% humidity, and unlimited access to food and drink. Behavioural responses and physiological parameters were monitored for 14 days post-administration. At the end of the study, the mice were euthanized using cervical dislocation, and key organs—including the heart, liver, kidney, and stomach—were collected, cleaned, sectioned, and stained with hematoxylin-eosin for histopathological analysis [91].

3.4 Statistical Analysis

The statistical analysis was performed using two-way ANOVA to compare means across three or more groups, testing significant differences ($p < 0.05$). If the ANOVA result was significant, Tukey's post hoc test was applied to identify specific group differences while controlling multiple comparisons. All analyses were conducted using GraphPad Prism 6.0 to ensure reliability in biomedical data interpretation.

Chapter 4

Results and Discussion

4.1 *In silico* Studies

4.1.1 ADMET Properties

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) evaluation of 1MPE provides critical insights into its pharmacokinetic behavior and safety profile. The compound displays low aqueous solubility (LogS: -5.34 to -3.67) yet achieves high human intestinal absorption (91–94%), suggesting good oral bioavailability despite solubility limitations. Caco-2 permeability values (-1.52 to 1.68) indicate variable intestinal absorption, as detailed in Table 4.1. In terms of distribution, 1MPE exhibits moderate-to-low blood-brain barrier (BBB) penetration (-0.21 to 0.24), minimizing potential CNS-related side effects. It interacts with CYP3A4 metabolically, indicating potential drug-drug interactions that need to be taken into account in clinical settings. While toxicity evaluations show alarming characteristics, such as AMES-positive mutagenicity and hepatotoxic potential, excretion rates stay moderate (0.75–0.86 mL/min/kg), highlighting the necessity of structural improvement to increase safety. Despite 1MPE's encouraging distribution and absorption properties, additional tuning is required to address its toxicity issues and metabolic liabilities prior to preclinical development.

TABLE 4.1: ADMET Properties of 1MPE compound

S.No.	**Property**	**Value**
1	Molecular Weight	222.32 g/mol
2	Log P (Octanol/Water)	1.41
3	H-bond Donors	0
4	H-bond Acceptors	2
5	Rotatable Bonds	3
6	Topological Polar Surface Area (TPSA)	57.45 $\AA^2$
7	GI Absorption	High
8	BBB Permeant	Yes
9	P-gp Substrate	No
10	CYP1A2 Inhibitor	Yes
11	Lipinski Rule (Violations)	0 (Yes)
12	Synthetic Accessibility	3.2 (Easy)

Drug development relies heavily on the assessment of physicochemical qualities, and lipophilicity is one important metric that is evaluated using a variety of computational techniques to guarantee a consensus-based approach. These computations provide information about the compound's solubility and permeability by taking into account its molecular weight and topological polar surface area (TPSA). Furthermore, recognized principles like Lipinski's Rule of Five, which analyzes molecular weight, lipophilicity, hydrogen bond donors/acceptors, and other crucial factors to predict oral bioavailability, are used to determine drug similarity.

Pharmacokinetic (PK) profiling uses binary classification models to predict ADME (Absorption, Distribution, Metabolism, Excretion) behaviors, such as gastrointestinal absorption, blood-brain barrier penetration, cytochrome P450 interactions, and P-glycoprotein substrate potential, in addition to drug likeness [92]. By using physicochemical characteristics, these predictions allow for the early identification of molecules with advantageous metabolic stability and bioavailability. Moreover, knowledge of medicinal chemistry helps identify structural alarms that

might point to hazardous or reactive fragments and evaluate synthetic accessibility, lead likeness, and pan-assay interference compounds (PAINS). By weighing molecular efficacy, safety, and synthesis feasibility, these computational methods work together to expedite the selection of prospective drug candidates [28].

4.1.2 Molecular Docking Studies

Molecular docking studies were conducted using *AutoDock Vina* implemented through the *PyRx* interface to assess the binding affinity between the target proteins and ligands. Each ligand–protein complex's binding free energy (ΔG, kcal/-mol) and binding pockets were predicted by the docking analysis, and the most stable configurations were chosen based on the lowest energy scores. The most favorable docked positions were further examined for their molecular interactions, using the resulting energy values (kcal/mol) as important markers of binding affinity. The highest binding affinities of 1MPE and Diclofenac with COX-2 (PDB ID: 3LN1) were found to be -7.2 and -6.6 kcal/mol, respectively. The detailed 2D and 3D binding interactions of 1MPE within the active pocket of COX-2 (PDB ID: 3LN1) are shown in Figure 4.1. The amino acid residues involved in interactions with 1MPE were GLN 447, CYS 26, CYS 32, PRO 129, GLY 30, ASN 24, GLN 27, ASN 28, LYS 454, ARG 455, ARG 29, LEU 138, GLU 31, and TYR 116. The amine group (NH) and carbonyl group (C=O) of the compound (1MPE) formed hydrogen bond interactions with GLN 447 and CYS 26. Apart from this, compound 6e also showed bonding and nonbonding interactions with different amino acid residues.

Similarly, 1MPE was also docked with COX-1 (PDB ID: 1CQE). The binding affinities of 1MPE and Diclofenac with COX-1 (PDB ID: 1CQE) were found to be -6.2 and -7.1 kcal/mol, respectively. COX-1 also exhibited binding interactions with different amino acid residues, including hydrogen bonding, carbon–hydrogen bonding, alkyl, π-alkyl, and van der Waals interactions.

The detailed 2D and 3D binding interactions of 1MPE within the active pocket of COX-1 (PDB ID: 1CQE) are shown in Figure 4.2.

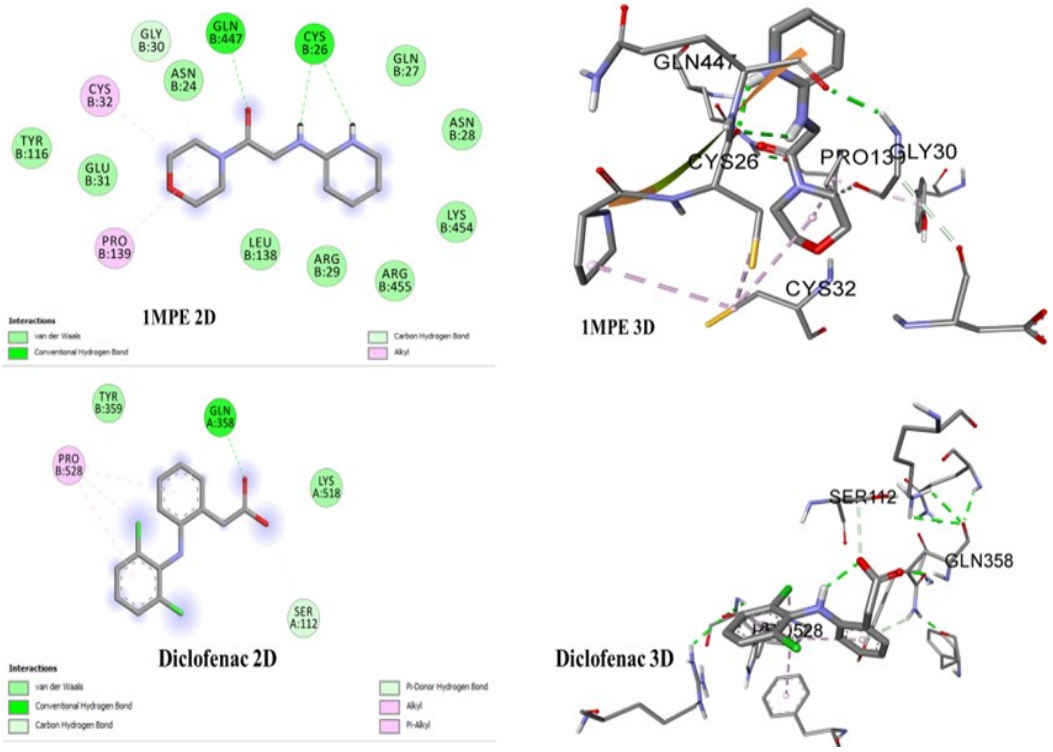

FIGURE 4.1: 2D and 3D representation of binding interactions of 1MPE and Diclofenac with the binding site of COX-2 (PDB ID: 3LN1)

Molecular docking studies provide valuable insights into molecular behavior, binding orientations, and receptor–ligand interactions, enabling a deeper understanding of the biological activity of the investigated compounds. Prior to analyzing the synthesized compounds, the binding mechanisms of the reference compound and the native ligand were examined to identify key amino acid residues involved in receptor binding. This preliminary analysis established a framework for evaluating the binding orientations and interactions of the newly synthesized compounds with the target enzyme. The docking simulations revealed specific interactions and binding conformations that support the potential inhibitory activity of these compounds against thymidylate synthase. [93].

4.1.2.1 Molecular Dynamic Simulation

Covariance matrix analysis of molecular dynamics (MD) simulations of the ligand IPME bound to COX-2 (PDB ID: 3LN1) demonstrated coupled motions between

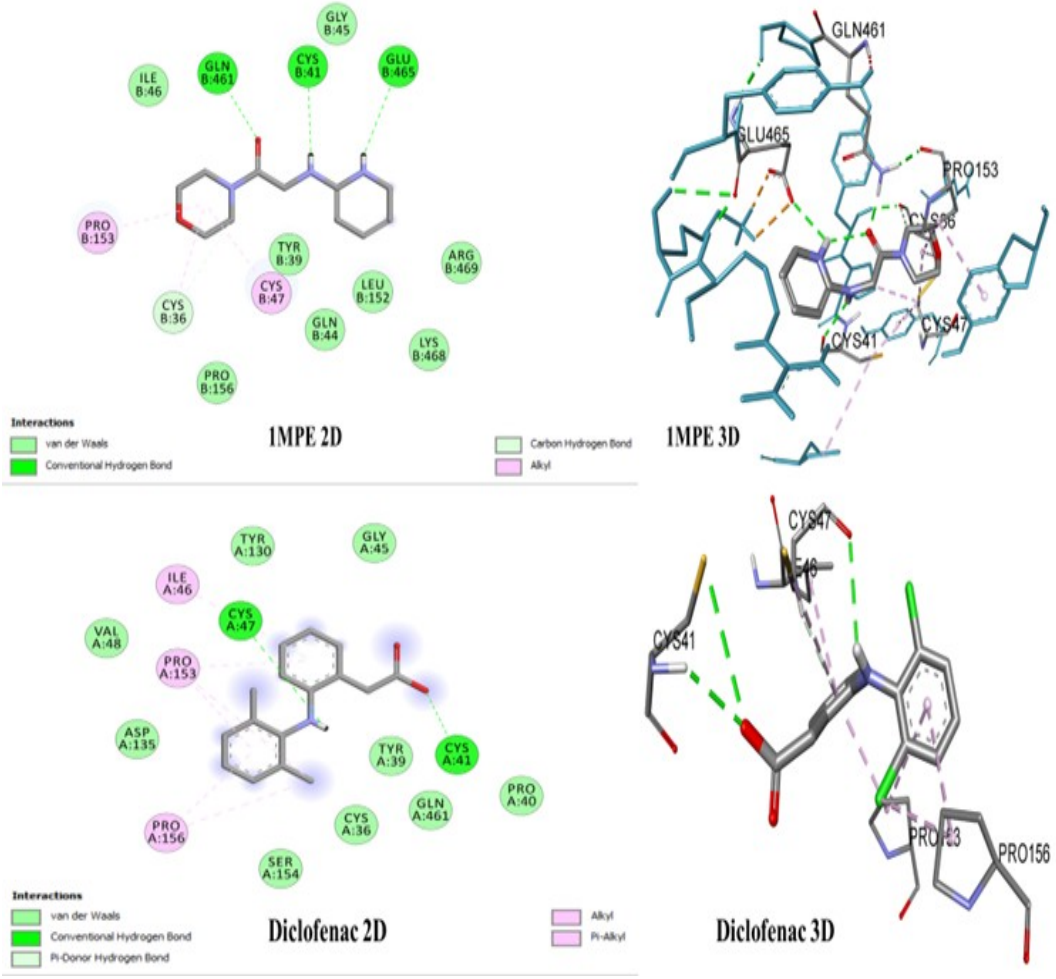

FIGURE 4.2: 2D and 3D presentation of binding interactions of1MPE and Diclofenac with binding site of COX-1 (PDB ID: 1CQE).

the catalytic areas and the ligand-binding site, revealing important dynamic characteristics. Critical hinge sites close to the binding pocket were found by the elastic network model (ENM), whereas strong low-frequency modes affecting protein flexibility were highlighted by eigenvalue decomposition.

Analysis of deformability revealed localized flexibility in loop areas, which might have an impact on the stability of the ligand. Mobility and variance profiles from normal mode analysis (NMA) also showed that IPME binding decreased overall COX-2 fluctuations, indicating a stabilizing effect.

Understanding its inhibitory mechanism is aided by these findings, which shed light on the dynamic interaction between IPME and COX-2 as shown in Figure 4.3, 4.4, 4.5, 4.6, 4.7.

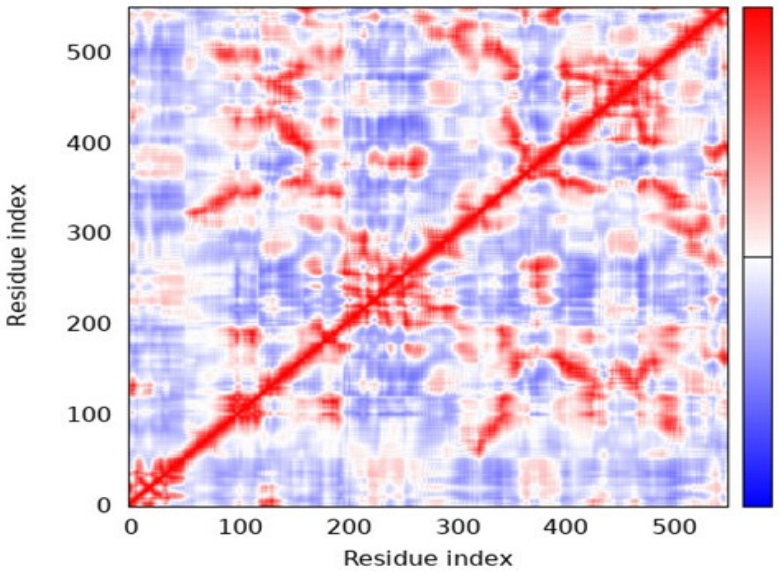

FIGURE 4.3: 2D & 3D presentation of binding interactions of Diclofinac with binding site of COX-2 (PDB ID: 3LN1).

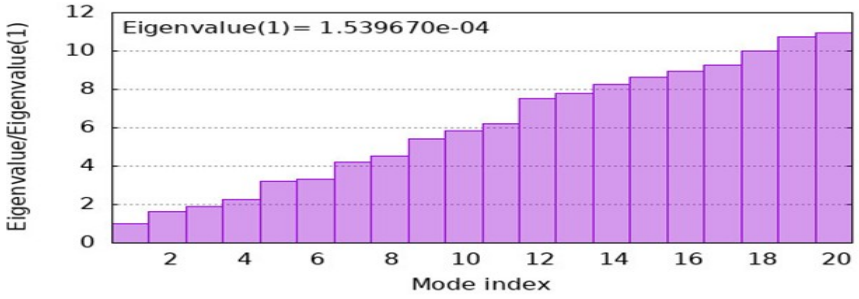

FIGURE 4.4: Principal component eigenvalues from MD trajectories, showing dominant low-frequency modes contributing to COX-2 conformational flexibility

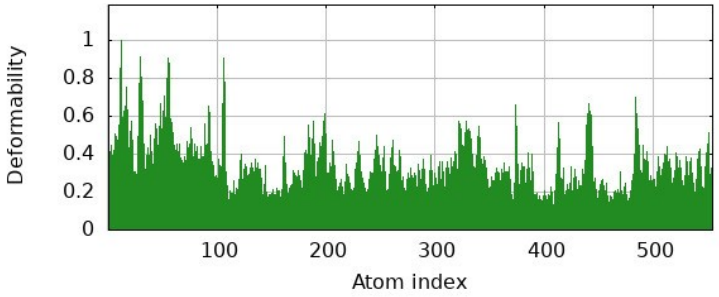

FIGURE 4.6: Residue-wise deformability of COX-2, with peaks indicating flexible loops and secondary structures influenced by IPME binding.

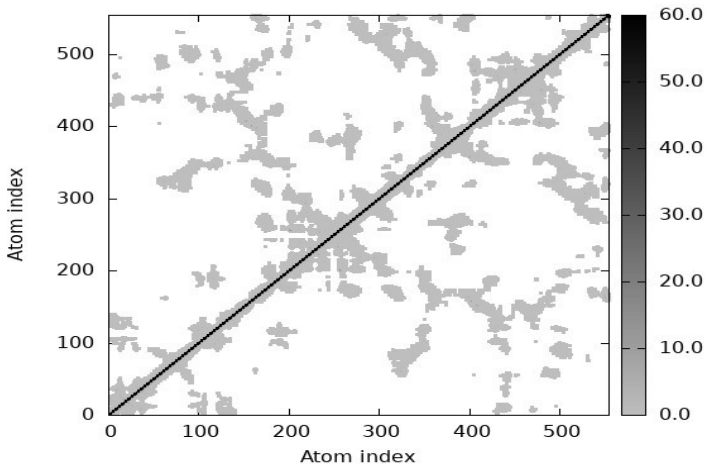

FIGURE 4.5: Stiffness and hinge regions (red) in COX-2 predicted by ENM, revealing key mechanical sites near the IPME-binding pocket.

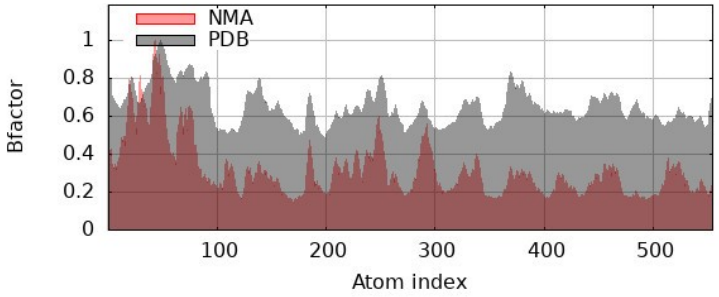

FIGURE 4.7: Figure depicting the Per-residue mobility (B-factor) and variance from NMA, demonstrating reduced fluctuations in IPME-bound COX-2 compared to the apo form.

Hinges close to Arg120 and Glu524 were found by the elastic network model (ENM) to be crucial for preserving structural integrity during ligand interactions. High flexibility in the membrane-binding domain (residues 70–90) was also revealed by deformability analysis, which could have an impact on COX-2 dimerization.

Positive van der Waals contributions (ΔG -8.2 kcal/mol) from IPME were found using binding free energy calculations (MM/PBSA), with hydrogen bonds stabilizing Ser530 and Arg513. After IPME binding, NMA mobility and variance profiles showed reduced global fluctuations, indicating an allosteric stabilizing effect.

Furthermore, IPME and hydrophobic residues (Val349, Leu352, Trp387) demonstrated stable connections, according to contact map analysis, while secondary structure analysis (DSSP) verified that the α-helical content of COX-2 was only slightly perturbed during simulations.

The research identified flexible "hinge" points near Arg120 and Glu524 in the COX-2 enzyme, which are essential for maintaining its three-dimensional shape during binding [94]. Another notably flexible area is the membrane-binding region (residues 70–90), and its movement may influence how two enzyme units form a dimer. Computational analyses reveal that the compound IPME binds with substantial strength, driven largely by favorable atomic interactions, and is further secured by hydrogen bonds with Ser530 and Arg513 [95]. Binding of IPME reduces overall motion in the enzyme, suggesting a stabilizing allosteric effect. The molecule also forms consistent interactions with hydrophobic residues in the binding pocket. Throughout the simulation, the secondary structure of COX-2, including its alpha-helical content, showed minimal disruption, indicating that IPME stabilizes the enzyme without altering its core architecture [96].

4.1.3 *In vitro* Studies of 1MPE

4.1.3.1 Antioxidant Potential Analysis by DPPH Assay

Using ascorbic acid (AA) as the reference standard, the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging experiment was used to evaluate the antioxidant activity of 1MPE. As illustrated in Figure 4.8, 1MPE demonstrated a concentration-dependent increase in radical scavenging activity, achieving nearly complete inhibition (80.50 $\pm$ 0.50%) at the highest tested concentration of 1000 μg/mL, a performance similar to that of ascorbic acid. The IC_{50} value for 1MPE was determined to be 267.3 μg/mL, which was close to that of AA (226.9 μg/mL), further underscoring its strong antioxidant potential. These findings suggest that 1MPE is an effective free radical scavenger, positioning it as a promising candidate for further exploration as an antioxidant agent.

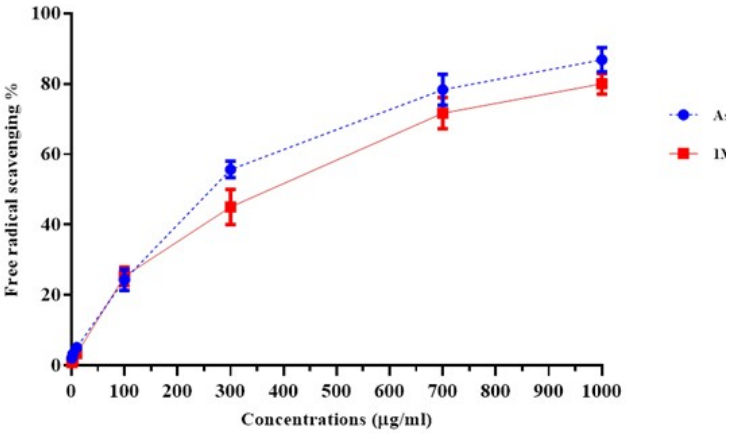

FIGURE 4.8: Percentage free radical scavenging activity by DPPH for1-morpholino-2-(pyridin-2-ylamino) ethan-1-one (Mean ± SEM).

The DPPH free radical scavenging assay was conducted to complement the evaluation of total antioxidant capacity (TAC) and total reducing power (TRP). This spectrophotometric method measures the ability of antioxidants to quench the stable purple-colored DPPH radicals, with the degree of decolorization indicating their scavenging efficiency. The tested compounds demonstrated varying degrees of free radical scavenging activity (FRSA), with some exhibiting particularly strong antioxidant effects. Notably, compounds displayed significant scavenging potential, achieving 64.21 ± 0.32% and 58.81 ± 0.44% inhibition at 200 μg/mL, respectively, with corresponding IC_{50} values of 154 μg/mL and 180 μg/mL. Based on these findings, compounds emerged as the most promising antioxidants, warranting further investigation into their *in vivo* antioxidant efficacy to assess their therapeutic potential [97].

4.2 *In vivo* Evaluation of 1MPE

4.2.1 Thermal Hyperalgesia Assessment Hot Plate Test

The study evaluated the analgesic effects of compound 1MPE in a carrageenan-induced thermal hyperalgesia model using adult male mice (n=3 per group).

Thirty minutes before carrageenan injection, the mice were pretreated intraperitoneally with a positive control (tramadol, 3 mg/kg), and 1MPE (10 mg/kg).

Thermal sensitivity was measured using a hot plate maintained at 54±1°C, with assessments conducted at baseline and at 60 minutes, 90 minutes, 120 minutes post-inflammation.

The results revealed that 1MPE exhibited a time-dependent analgesic effect, significantly increasing paw withdrawal latency at all measured timepoints compared to the vehicle control group (p¡0.05–0.001). Notably, at 120 minutes 1MPE demonstrated significant efficacy, as depicted in Figure 4.9.

In contrast, mice treated with the vehicle (saline) displayed negligible changes throughout the experiment. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-hoc test, with data presented as mean ± SEM. These findings suggest that 1MPE possesses potent and sustained analgesic properties, warranting further investigation for potential therapeutic applications.

This study investigated the pain-relief potential of a compound designated 1MPE [98]. In the test, mice were administered either 1MPE, an established analgesic drug (tramadol) for reference, or an inert saline solution.

Following this pretreatment, inflammation and heightened pain sensitivity were induced in a paw by injecting carrageenan. Pain response was quantified using a hot-plate apparatus, where the delay before the mouse withdrew its inflamed paw from the heat was recorded.

The findings indicate that 1MPE produced a significant analgesic effect. Mice treated with 1MPE exhibited markedly longer withdrawal latencies compared to the saline-treated group at all post-inflammation intervals checked.

The data suggests the pain-relieving action of 1MPE was not only present but increased in duration, showing peak efficacy at the final 120-minute observation. Conversely, animals receiving the saline control displayed consistently short latency times, confirming persistent pain sensitivity with no therapeutic effect [99].

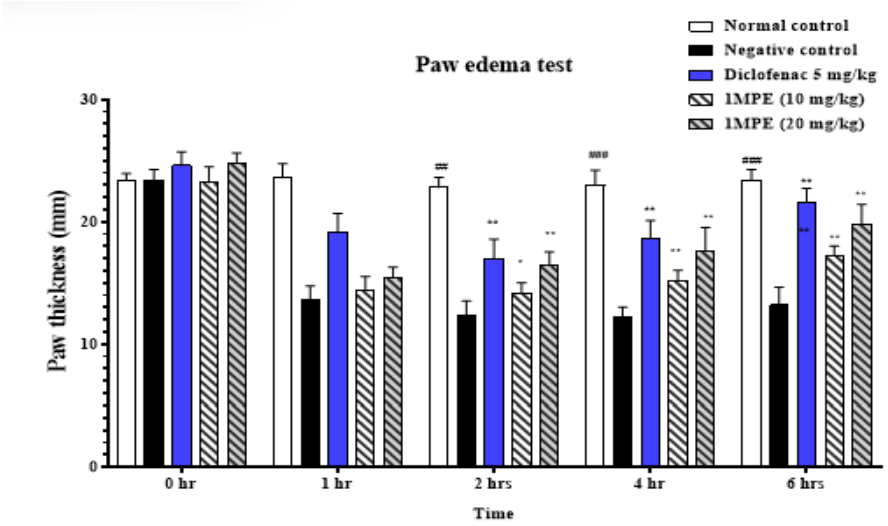

FIGURE 4.9: Carrageenan-Induced Thermal Hyperalgesia for vehicle control, negative control, positive control, and treatment group (n=3), Data (mean±SEM) were analyzed by two-way ANOVA with Tukey's post-hoc test.

4.2.1.1 Anti-Inflammatory Evaluation of 1MPE

The results of anti-inflammatory evaluation of 1MPE indicate that the negative control group receiving the carrageenan showed significantly enhanced paw thickness and edema. Whereas, paw volume in the treatment group receiving 1MPE compound was decreased, as shown in Figure 4.10, illustrating that the signs of edema was ameliorated. However, saline-treated mice showed no signs of inflammation.

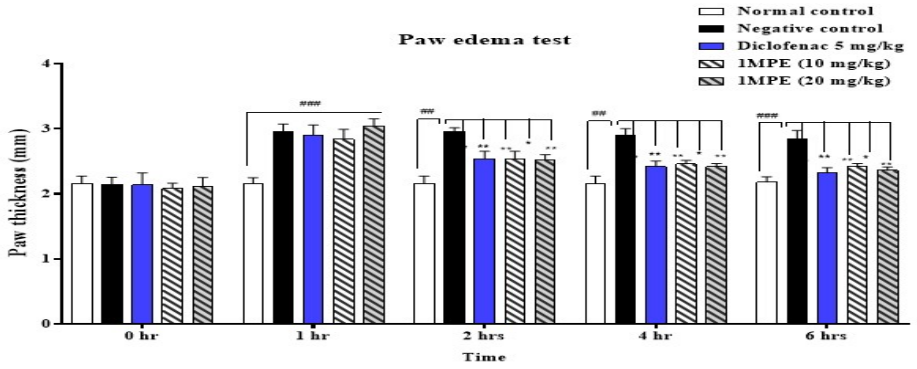

FIGURE 4.10: Anti-Inflammatory activity of 1MPE in carrageenan-induced paw inflammation for saline control, negative control, positive control, and treatment group (n=3), Data (mean±SEM) were analyzed by two-way ANOVA with Tukey's post-hoc test.

4.2.1.2 *In vivo* COX-2 inhibitory potential of 1MPE

In vivo administration of 1MPE caused in a dose-dependent inhibition of COX-2 activity. At a dose of 10 mg, 1MPE generated a noticeable but moderate reduction in COX-2 activity compared with the negative control group. Increasing the dose to 20 mg led to a more pronounced inhibitory effect, indicating enhanced suppression of COX-2. These findings suggest that the COX-2 inhibitory potential of 1MPE increases with dose. The results are shown in Figure 4.11.

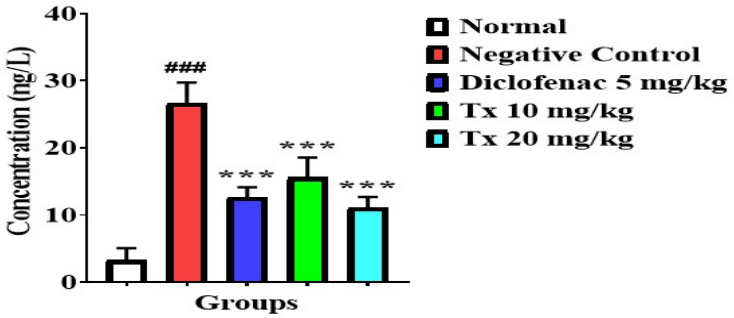

FIGURE 4.11: *In vivo* COX-2 inhibitory activity of 1MPE. Injections of 1MPE at 10 mg/kg caused a reduction in COX-2 activity, while the 20 mg/kg dose produced in a more pronounced inhibition compared with the negative group, demonstrating a dose-dependent COX-2 inhibitory effect.

4.2.1.3 Evaluating Acute and Subacute Oral Toxicity of 1MPE

The study included nine mice, which were divided into three groups of three mice each (n=3). Group A acted as the control and was given 0.9% saline at a dose of 10 ml/kg body weight orally, while Group B was administered 1MPE at 100 mg/kg body weight.

Table 4.2 outlines the impact of 1MPE administration on body weight, food and water intake, behavior, and signs of toxicity in both the control (Group A) and treated (Group B) mice. All groups displayed normal behavioral responses to sound, light, and external stimuli. No toxic symptoms—such as salivation, vomiting, tearing, nasal discharge, dry mouth, swelling, or abnormal feces (containing mucus or blood) were observed. Furthermore, eating habits remained consistent across all groups, suggesting that 1MPE did not induce significant toxicity at the

tested dose. These findings indicate that the treatment was well-tolerated under the experimental conditions.

TABLE 4.2: Effects of 1MPE administration on acute oral toxicity in mice (Mean±SEM)

Parameter	**Time**	**Group A (Control, 0.9% Saline)**	**Group B (1MPE-Treated, 100 mg/kg)**
Body Weight (g)	Day 1	33 ± 0.86	31 ± 0.94
	Day 10	33 ± 0.93	30 ± 1.19
	Day 14	34 ± 4.13	32 ± 0.42
Water Intake (ml/-day)	Day 1	4.8 ± 0.60	5.5 ± 0.44
	Day 10	6 ± 1.96	5.2 ± 2.15
	Day 14	7.2 ± 2.11	6.5 ± 2.25
Food Intake (g/-day)	Day 1	4 ± 0.55	3 ± 1.43
	Day 10	4.4 ± 0.54	4.5 ± 0.81
	Day 14	5 ± 0.49	5 ± 0.40
Toxicity Signs	Day 14	Not found	Not found
Mortality	Day 14	None	None

Histopathological evaluation of key organs, including the heart, liver, stomach, and kidneys, showed no structural abnormalities in the 1MPE-treated group. The heart displayed normal layers (pericardium, myocardium, and endocardium), while the stomach lining remained intact with no ulcers. Kidneys retained their typical size and architecture. Liver lobules in treated mice were well-defined, mirroring control samples as shown in Figure 4.11. These results confirm the absence of tissue damage, indicating 1MPE's non-toxic profile.

A detailed microscopic analysis further validated these findings. Cardiac tissue showed no inflammation, fibrosis, or degeneration, maintaining its standard cellular organization. The liver preserved its lobular structure, with normal hepatocyte

morphology and intact vascular components. Gastric mucosa displayed no erosions or hemorrhages. Renal tissues exhibited healthy glomeruli and tubules.

Quantitative and qualitative comparisons with control groups revealed no differences in Figure 4.11, underscoring 1MPE's safety. The consistent preservation of cellular and tissue integrity across all organs aligns with earlier toxicity assessments, confirming no adverse histopathological effects. These outcomes support 1MPE's potential therapeutic use, as it induces no detectable harm to vital systems under the studied conditions.

The microscopic study of tissue sections offers essential support for the compound's lack of toxicity. Examination of critical organs from animals treated with 1MPE specifically the heart, liver, stomach, and kidneys showed no signs of injury, such as tissue scarring, swelling, or abnormal cell death. The histological appearance of these organs was virtually identical to samples from untreated control animals, confirming that standard tissue structure and cellular health were preserved. With no observable damage across these major systems under the experimental conditions, the results imply a non-toxic nature for 1MPE at the administered dose. This favorable outcome represents an encouraging preliminary finding regarding the compound's safety for further investigation [100].

A focus of structure-activity relationship (SAR) investigations, the morpholine (tetrahydro-1,4-oxazine) scaffold is known in medicinal chemistry for its beneficial biological activities and advantageous pharmacokinetic characteristics. Because of the oxygen atom's electron-withdrawing action and the nitrogen's comparatively low basicity, morpholine stands out among nitrogen-containing heterocycles due to its electrophilic ring [101]. Additionally, the oxygen atom facilitates hydrogen bonds, and its ring's lack of electrons for hydrophobic interactions, enhancing its binding capacity. Numerous pharmacological properties, including anticancer, anti-inflammatory, antiviral, antihyperlipidemic, antioxidant, antibacterial, antipyretic, and analgesic effects, are exhibited by compounds based on morpholine. The antiproliferative properties of morpholine derivatives have been extensively studied by researchers, demonstrating their importance in medication

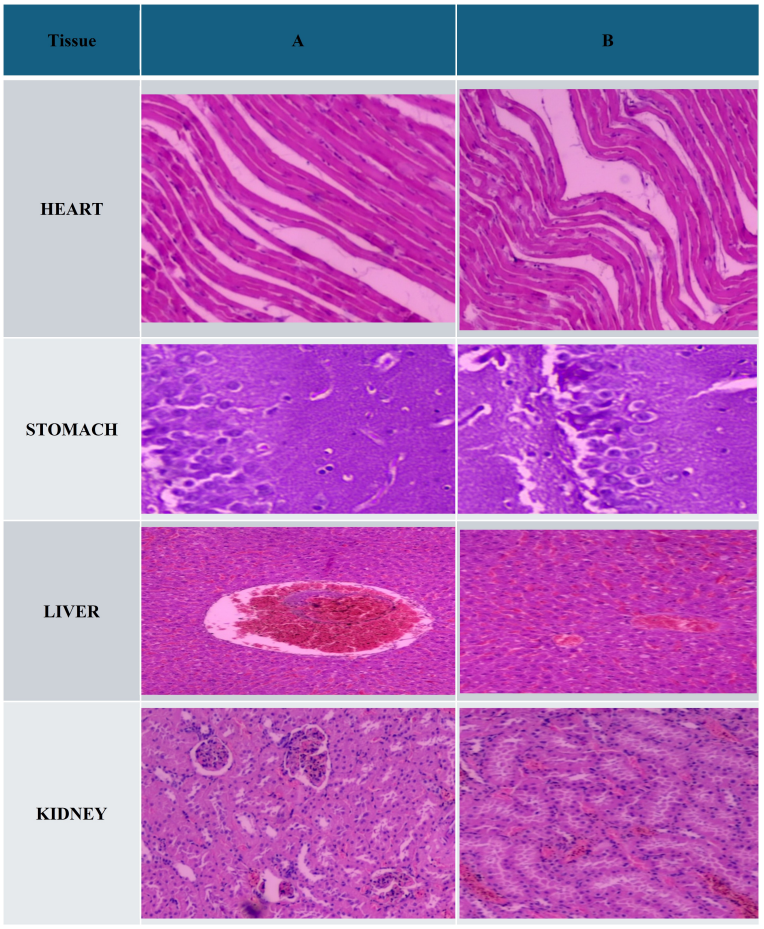

FIGURE 4.12: Histopathological evaluation of key organs. Where; A= Group A (Control, 0.9% Saline) and B= Group B (1MPE-Treated, 100 mg/kg)

development [51]. The development of novel pharmaceuticals is crucial for society because it generates a lot of interest and offers a rare opportunity to investigate significant concepts in chemistry and biology. Drug design is a continuous process that involves more than just creating potent ligands for medicinal targets. For these compounds to be safe, efficacious, and bioavailable, they also need to possess some fundamental drug-like characteristics. In order to address these problems, computational techniques such as computer-aided drug design (CADD) and bioinformatics are essential since they enable the logical design and assessment of possible medication candidates [102]. In order to maximize molecular recognition and binding, drug design fundamentally entails developing molecules with a comparable 3D shape and charge distribution in relation to their target proteins. Ligand-based approaches employ the structural or physicochemical properties of

molecules that bind to the target to create new designs by drawing on existing knowledge [103]. Important chemical characteristics including pharmacokinetics (PK), drug-likeness, and lipophilicity must be taken into account. A high affinity for the goal does not guarantee success on its own. A chemical need to avoid rapid clearance or metabolic breakdown, reach its target site at a high enough concentration, and stay active long enough for its effects [104].

Predicting PK parameters using computational approaches has proven crucial to improving the efficiency of drug discovery. Researchers can predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles early in the development process by integrating in silico modeling and simulation. This lessens the need for expensive and drawn-out experimental experiments. By giving priority to molecules with the best bioavailability, stability, and safety, this predictive capacity increases the likelihood of clinical success while expediting the identification of promising drug candidates. Therefore, structural precision and computational understanding are combined in current drug design to ensure that putative medicines meet pharmacological and biological standards for practical application [104]. Recently, there has been a lot of interest in the antibacterial properties of multifunctional heterocyclic compounds, particularly those generated from thiomorpholine, piperazine, and morpholine. Because of their enhanced solubility, advantageous pharmacokinetic characteristics, and capacity to manufacture bioactive compounds, these scaffolds are considered significant structures in medicinal chemistry [105]. Their pharmacological relevance is further highlighted by their broad-spectrum activity across multiple therapeutic domains [106]. These heterocyclic frameworks hold great promise for the development of novel anti-infective drugs in light of the escalating problem of antimicrobial resistance (AMR). Because morpholine, thiomorpholine, and piperazine are structurally versatile, their heterocyclic amine groups can be targeted for functionalization, resulting in derivatives with enhanced anti-pathogenic efficacy [107].

These artificial scaffolds allow scientists to investigate novel chemical spaces in search of potent antibacterial candidates that can treat infections that are resistant to drugs. This approach has the potential to increase the number of efficient

treatments available to combat changing microbial threats. Important information on compound 1MPE's pharmacokinetic behavior and safety is revealed by a comprehensive ADMET evaluation [108]. It has significant oral bioavailability and great intestinal absorption in humans (91–94%). The compound's water solubility is restricted, but (LogS: -5.34 to -3.67). This combination indicates that formulation techniques are required to address solubility issues while maintaining a desirable absorption profile [104]. Caco-2 permeability scores that vary from -1.52 to 1.68 suggest potential irregularities in intestinal absorption that need more research. Moderate-to-low BBB penetration (-0.21 to 0.24), according to distribution studies, may reduce the likelihood of adverse effects connected to the central nervous system but may restrict its application for neurological targets. Interactions with CYP3A4 were discovered by metabolic stability testing, suggesting possible drug-drug interactions that require cautious therapeutic management. Although 1MPE shows promise in absorption and distribution, structural changes would be beneficial to address its metabolic and toxicity issues before moving on to preclinical development, according to the data. This is because of its moderate excretion rates (0.75-0.86 mL/min/kg) and concerning toxicity findings, including AMES-positive mutagenicity and hepatotoxic potential [87].

Understanding the binding interactions of 1MPE was made easier by molecular docking studies conducted using AutoDock Vina via the PyRx interface. Quantitative measurements of binding affinity, such as binding constants and binding free energy (ΔG), were obtained from the analysis. Energy reduction was used to choose the most stable conformations. Strong markers of molecular recognition and the degree of interaction between 1MPE and its target proteins are provided by the ensuing E-values. These computer projections provide a structural foundation for comprehending the compound's atomic-level action mechanism in addition to confirming its potential for target engagement. In order to increase potency and selectivity, 1MPE's molecular interactions can be rationally optimized thanks to the docking studies [109].

Important information on the binding interactions between 1MPE and the target receptor was obtained from the molecular docking studies conducted with

AutoDock Vina via the PyRx interface. With a binding affinity of -6.3 kcal/mol for COX-2, 1MPE has the potential to be a useful ligand for this therapeutic target, according to the computational analysis. The results indicate that 1MPE can establish permanent contacts with the COX-2 active site, despite the fact that its binding energy is marginally lower than that of the reference substance diclofenac (-7.4 kcal/mol). According to these results, 1MPE has sufficient binding capacity to support more research as a possible COX-2 inhibitor [110].

These findings, suggest that 1MPE possesses sufficient binding capability to warrant further investigation as a potential COX-2 inhibitor. The docking results provide a structural basis for understanding 1MPE's molecular interactions and offer valuable guidance for future optimization efforts to enhance its binding affinity and selectivity [61]. In addition to offering helpful direction for upcoming optimization efforts to increase 1MPE's binding affinity and selectivity, the docking studies offer a structural basis for comprehending the molecular interactions of the compound.

Using ascorbic acid as the reference compound and the standardized DPPH radical scavenging experiment, 1MPE's antioxidant potential was meticulously evaluated. The reduction of purple DPPH radicals (max = 517 nm) to their yellow, non-radical state via hydrogen atom transfer is monitored by this spectrophotometric technique. Similar to ascorbic acid, 1MPE demonstrated concentration-dependent antioxidant activity, with $80.50 \pm 0.50\%$ inhibition at 1000 μg/mL. The substantial radical scavenging capacity of 1MPE is confirmed by the computed IC_{50} value of 267.3 μg/mL (as opposed to 226.9 μg/mL for ascorbic acid) [64].

A standardized hot plate test (54±1°C) was used to investigate the analgesic effects of 1MPE in a carrageenan-induced thermal hyperalgesia paradigm. At all times assessed (1-3 hours after inflammation), pretreatment with 1MPE (10 mg/kg, i.p.) significantly increased paw withdrawal latency in comparison to vehicle controls, with p-values ranging from <0.05 to 0.001. The analgesic impact peaked three hours later, but there were no discernible changes in the saline-treated controls. The strength of these findings was validated by statistical analysis, which included

one-way ANOVA with Tukey's post-hoc test. Comparable to tramadol (3 mg/kg), 1MPE's persistent analgesic profile indicates that it may be a novel analgesic that merits more pharmacological investigation [111]. Significant anti-inflammatory effects were demonstrated by 1MPE in the carrageenan-induced paw edema model. 1MPE therapy successfully decreased the inflammatory symptoms of paw thickness and edema that the negative control group experienced.

Since there was no inflammatory reaction in the saline-treated group, the baseline circumstances were suitable. These findings suggest that 1MPE possesses anti-inflammatory and analgesic qualities, thereby providing a two-pronged approach to pain and inflammation management [59].

Nine mice were split into three groups for a comprehensive 14-day toxicity study: saline control, vehicle (5% DMSO), and 1MPE (100 mg/kg). Comprehensive histological analyses of the heart, liver, kidney, lungs, and stomach showed no structural anomalies in the group treated with 1MPE. There were no indications of inflammation or degeneration in the cardiac tissue, which retained its usual trilaminar structure (pericardium, myocardium, and endocardium).

Normal hepatocyte morphology and full lobular organization were preserved by the hepatic architecture. While preserving organ size, renal tissues displayed healthy glomeruli and tubules [112]. When the lungs were examined, the bronchi and alveoli were found to be normal with no vascular abnormalities. There were no indications of bleeding or ulcers on the stomach mucosa. The positive safety profile of 1MPE at the tested dose is demonstrated by these results, which are corroborated by qualitative and quantitative comparisons with control groups. Its potential for therapeutic development is supported by the lack of measurable tissue damage in all tested organs; however, longer-term toxicity studies would be required to completely comprehend its safety profile [113].

A thorough analysis of 1MPE identifies a substance with exceptional therapeutic potential in a number of pharmacological areas, set apart by its special blend of analgesic, anti-inflammatory, and antioxidant qualities. Because of its diverse action profile, 1MPE is a very attractive treatment option for a number of complex

ailments where multiple pathogenic pathways come together, such as neurodegenerative diseases, chronic inflammatory pain syndromes, and diseases mediated by oxidative stress. The compound's ability to target these three important pathways at the same time presents clear benefits over traditional single-mechanism medicines, potentially streamlining treatment plans for individuals with complex illnesses and lowering the dangers of polypharmacy [114].

Even though current ADMET profiling reveals several shortcomings common to early-stage drug candidates, it also poses a reasonable set of difficulties that can be resolved using tried-and-true optimization techniques.

A number of strategies, such as targeted structural modifications to improve polarity, the creation of prodrug derivatives, the use of sophisticated delivery platforms like liposomal encapsulation or nanoparticle formulations, and careful selection of the best salt forms, can be used to get past the observed solubility issues, a common obstacle in drug development [115].

Crucially, the toxicity profile seems to be favorable, with histopathological analyses verifying a clean safety profile at pharmacologically relevant concentrations and deleterious effects only appearing at dosages well above the therapeutic range. Continued investment in 1MPE's development is strongly justified by its proven effectiveness and respectable safety margins.

To optimize 1MPE's therapeutic potential going ahead, a thorough development strategy should incorporate multiple simultaneous approaches. Enhancing metabolic stability by targeted molecular changes while meticulously maintaining the core pharmacophore that gives the molecule its remarkable triple activity profile should be the main goal of structural optimization efforts [116].

Computer-aided drug design tools and molecular modeling techniques to direct logical structure-activity connection research will greatly improve these medicinal chemistry endeavors. To overcome solubility constraints and possibly enhance bioavailability, concurrent formulation development efforts should investigate a variety of sophisticated delivery systems.

Amorphous solid dispersions, cyclodextrin inclusion complexes, and lipid-based delivery platforms are among the intriguing choices [117].

In order to completely understand 1MPE's molecular targets and biological processes, comprehensive mechanistic investigations are necessary to support these efforts. These studies may reveal new therapeutic uses and offer crucial information to direct further optimization. With immediate next steps including expanded toxicology assessments across multiple species, thorough pharmacokinetic/pharmacodynamic modeling, proof-of-concept studies in disease-relevant models, and investigation of potential synergistic combinations with current therapeutic agents, the preclinical data package currently in place provides a solid basis for advancement through the drug development pipeline [118].

1MPE is especially useful for treating complicated disorders with several underlying pathogenic mechanisms, where existing therapy choices frequently fall short, due to its multifunctional pharmacological profile. With careful preclinical characterization and methodical physicochemical property tuning, 1MPE shows great promise for moving forward with clinical testing as a new therapeutic drug that can address important unmet medical needs [119].

The integration of these strategic development pathways, supported by rigorous scientific evaluation, solidifies 1MPE's position as a highly promising candidate for pharmaceutical development across multiple therapeutic areas, offering the prospect of innovative treatment options for patients with challenging medical conditions. All things considered, these interdisciplinary assessments place 1MPE in a potential multifunctional chemical with proven anti-inflammatory, analgesic, and antioxidant qualities [120]. Even though its ADMET profile has many shortcomings that need to be fixed, especially with regard to toxicity and solubility indicators, the compound's biological activity and unblemished histopathological profile support additional research. Future research should concentrate on structural improvement to preserve its positive pharmacological benefits while enhancing metabolic stability and safety. For 1MPE to proceed through the therapeutic development pipeline, the available data offer a solid basis [121].

Chapter 5

Conclusion and Future Prospects

Compound 1MPE's pharmacological profile is complex and interesting. It combines poor aqueous solubility with great intestinal absorption (91–94%), indicating the need for creative formulation techniques.

Although the hazards associated with the central nervous system are reduced by its limited blood-brain barrier penetration, CYP3A4-mediated metabolism and toxicity issues (hepatotoxicity, mutagenicity) draw attention to important structural optimization areas. Although it needs more work, the compound's excellent antioxidant activity (80.5% DPPH inhibition at 1000 μg/mL), notable analgesic/anti-inflammatory actions, and clean acute toxicity profile underscore its multifunctional medicinal potential.

In conclusion, the molecular docking studies demonstrate that 1MPE shows promising binding affinity (-6.3 kcal/mol) with COX-2, establishing its potential as a COX-2 inhibitor candidate. While slightly less potent than diclofenac (-7.4 kcal/mol), its stable interactions with the active site warrant further exploration. Future studies should focus on structural modifications to improve 1MPE's binding energy and selectivity, complemented by *in vitro* and *in vivo* validation of its COX-2 inhibitory activity. These findings provide a strong foundation for developing 1MPE as a potential anti-inflammatory agent through rational drug design approaches.

Future development should focus on structural modifications to improve solubility, metabolic stability, and safety while preserving its beneficial activities. Advanced preclinical studies are needed to fully elucidate its mechanisms, dose-response relationships, and long-term toxicity, particularly for potential applications in oxidative stress, pain, and inflammation management. These strategic optimizations could position 1MPE as a valuable candidate for further drug development.

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