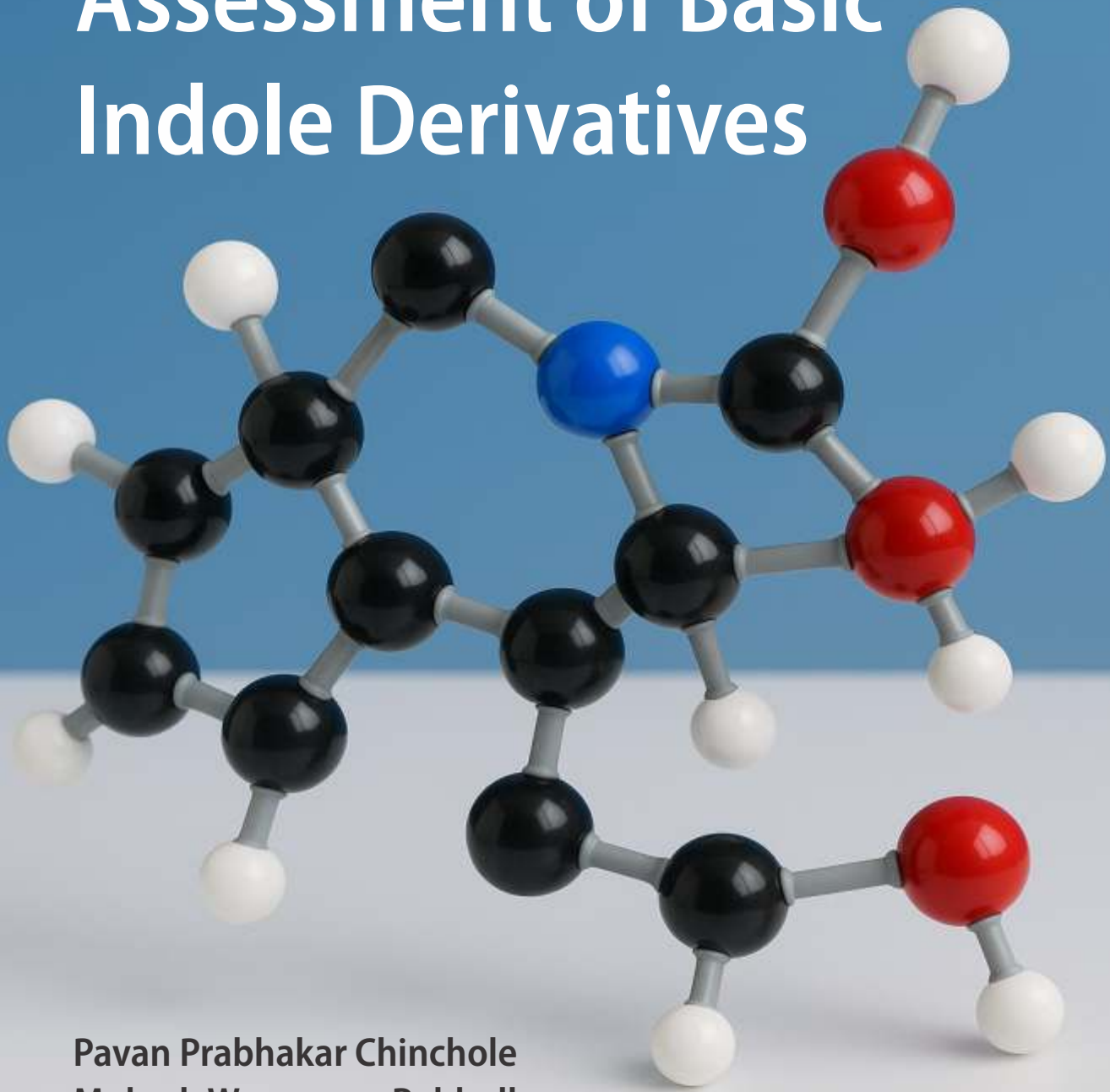
In-Vitro Biological Assessment of Basic Indole Derivatives



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DeepScience

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Preface

This book provides an in-depth study of the synthesis, characterization, and biological evaluation of newly designed Schiff bases derived from N-benzyl isatin. This book primarily focuses on addressing inflammation and pain, two significant concerns in therapeutic research. Through a carefully structured synthetic strategy, we developed a series of derivatives and validated their structures using advanced analytical techniques such as FT-IR spectroscopy, ^{1}H-NMR, UV-Visible spectrometry, and Thin Layer Chromatography. Their anti-inflammatory efficacy was assessed through an in-vitro protein denaturation method, offering important preliminary insights into their biological potential. This study bridges the fields of synthetic organic chemistry and pharmacological evaluation, highlighting the importance of interdisciplinary approaches in modern drug discovery. A detailed examination of the relationship between structural modifications and biological activity forms a core part of this research.

We believe this book will serve as a valuable reference for researchers, students, and professionals in medicinal chemistry, pharmaceutical sciences, and related disciplines. We are deeply grateful to all those who supported and encouraged this work. It is our hope that the findings presented here will inspire further research and spark new ideas in the field of drug design and development.

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Chapter 1: Introduction

1.0 Introduction

The current study focuses on synthesizing various isatin derivatives substituted with benzyl groups, specifically at the 3-position of the isatin ring, through the formation of Schiff bases. The synthesized compounds were evaluated for their analgesic and anti-inflammatory potential using an in vitro protein denaturation assay.

Isatin and its derivatives hold significant pharmaceutical value due to their wide range of biological activities, including antimicrobial, analgesic, anti-inflammatory, and anticonvulsant properties. Drugs such as Indomethacin, which contains an indole nucleus, and Phenylbutazone are prominent examples of commonly used non-steroidal anti-inflammatory drugs (NSAIDs). The presence of the indole core plays a crucial role in the inhibition of cyclooxygenase (COX) enzymes. Although Indomethacin primarily inhibits the COX-1 isoform, structural modifications to the indole nucleus can alter its selectivity, potentially favoring COX-2 inhibition.

Cyclooxygenase enzymes (COX-1 and COX-2) are essential in converting arachidonic acid into key pro-inflammatory mediators such as prostaglandins, prostacyclins, and thromboxanes. NSAIDs achieve their therapeutic effects primarily by blocking the activity of these enzymes.

1.1 Inflammation:

Inflammation is one of the protective consequences related to tissue homeostasis which involve the sequential biochemical reaction in response to cellular injury. This biochemical reaction results in the synthesis of mediator of inflammation. Mediators formed may be the component of metabolism, immune system or endocrine (hormone) system. Tissue injury decides extend of inflammation. Generally the word **“itis”** is

suffixes after the injured body part to which inflammation occurs, for example "nephritis" indicating that inflammation of kidney.

1.1.1 Cardinal characteristics:

Roman scientist **Aulus*Cornelius Celsus 20 A.D*.** were focused the four cardinal sign and symptoms of inflammation. The magnitude of the injury define the way to express the inflammation as follows,

Tumor: Tumor termed for Swelling. Collection of extra vascular fluid along with the inflammatory mediators migrating into damaged part results in the formation of edema and is the mechanism for Swelling

1. Rubor : Due to dilation of small blood vessels within the injured area tissue appears to be red termed as hyperemia.

2. Calor: Blood flow increases due to dilation of small blood vessels within the injured part. This increases the temperature as supply of warm blood to the area.

3. Dolor: Chemical mediators of acute inflammation, especially bradykinin and different type of prostaglandins are released from biogenic pathway that are responsible for the inflammatory edema leads to the stretching and distortion of tissues and pain termed as Dolor.

4. functiolaesa: If the pain is continuous and unaffordable movement of inflamed area may be inhibited. Similarly severe swelling may lead to immobilize that area. This simply explained as loss of function of affected area. In the IInd century A.D., Roman physician Galen, proposed a fifth sign as *functiolaesa* (disturbance of function). Virchow (1821-1902) afterword, added this fifth sign of inflammation as consequence of inflammation, and features were described in written work '**Celsus**'.

1.1.2 Causes of Inflammation:

1. Microbial infections: Microbial infection is one of the causes of the inflammation. Viruses, bacteria, protozoa, fungi and various parasites included microbes. After intracellular multiplication of viruses may lead to death of individual cells as cell not functioning properly and cause explosion of the cell (cytolytic). In contrast bacteria release specific toxins. For example anthrax toxins or tetanus toxin are produced specifically for export (exotoxins) whereas endotoxins in Gram negative bacteria are cell identifier and just part of the cell walls.

2. Hypersensitivity reactions: As a result of altered state of immunologic responsiveness, hypersensitivity reaction occurs that may cause an excessive or inappropriate immune reaction that may damages the tissues.

3. Physical agents, corrosive and chemicals irritant: Inflammation may occur through tissue damage provoke by burns or excessive frostbite, ultraviolet or other ionizing radiation, physical trauma. Inflammation may occur through direct tissue damage when come in contact with Corrosive chemicals (acids, alkalis, oxidizing agents).

4. Tissue necrosis: As result of inadequate blood flow, Death of tissues occurs from lack of oxygen or nutrients (infarction). The edge of a recent infarct often acts as a potent inflammatory stimulus that shows an acute inflammatory response.

1.1.3 Type of Inflammation:

1. Acute inflammation

2. Chronic inflammation

1. Acute inflammation: With increase in blood flow in the affected tissue , it becomes reddened and swollen due to edema fluid. In early stages of inflammation, these changes occur as the result of vascular response in affected part. A rapid response is designed to deliver inflammatory mediators such as plasma proteins and leukocytes to sites of injury.

In acute inflammatory response, the vascular events involve three main processes:

1. Increased in vascular blood flow (hemodynamics)

2. Change vascular permeability and

3. Formation of the edema form fluid exudates

2. Chronic inflammation: Depending upon persistence of the causative stimulus to inflammation in the tissue, chronic inflammation is prolonged duration from few days' weeks to several months, or even indefinitely. Chronic inflammation caused by repetitive use of NSAIDs, a weakened immunity or an irregular nerve supply. Chronic inflammation leads to tissue damage and is accompanied by simultaneous effect of repair mechanism. The exact nature, magnitude as well as duration of chronic inflammation is invariable depends on the response between the causative stimulus and the response by the body to resolve it.

As compare to acute inflammation, chronic inflammation does not have as much cardinal sign and symptoms of inflammation such as rubor (redness) or calor (heat) also fluid exudates does not so grossly in appearance.

1.1.4 Components of Inflammation

Cellular Components (inflammatory cells) and chemical mediators interplay complex cascade in the biogenesis of inflammation. Hypersensitivity illustrated that two phases of response to the allergen. In early-phase response degranulation of mast cell occurs with the release of histamine (inflammatory mediators) similarly in a late-phase response inflammatory cells are migrated towards the affected area.

A) Cellular Components

1. Endothelial cells
2. Circulating WBC
3. Connective tissues:
    - Lymphocytes
    - Mast cells
    - Tissue macrophages
    - Fibroblasts

B) Chemical Mediators

1. Histamine: Histamine, chemically known as β-imidazolylethylamine, plays a crucial role by acting on various cell types, including those in the endocrine and exocrine systems, smooth muscle, neurons, blood components, and immune cells. Upon activation, histamine functions as a powerful vasodilator, induces smooth muscle contraction, enhances vascular permeability, and stimulates the secretion of respiratory mucus and gastric acid. Moreover, histamine is involved in complex anti-inflammatory mechanisms through H2-receptor activity. It does so by inhibiting the release of lysosomal enzymes from human neutrophils, reducing IgE-mediated histamine release from peripheral leukocytes, and activating suppressor T-lymphocytes.

2. Leukotrienes: Leukotrienes, which share similar biological effects with histamine, are important chemical mediators in the inflammatory response. These compounds are synthesized and secreted by several types of immune cells involved in inflammation, including mast cells, basophils, eosinophils, neutrophils, and monocytes. Among the various leukotrienes, LTD_4 and LTE_4 are classified as slow-reacting substances that contribute significantly to anaphylactic reactions. Pharmacological investigations have shown that H1-receptor antagonists can inhibit leukotriene release, potentially through mechanisms involving the antagonism of G-protein-coupled receptors.

3. Prostaglandins: Prostaglandins are the mediators of different allergic effect evoke by the activation of the mast cell these includes platelet aggregation and degranulation, pain and sensations of pruritus, smooth muscle contractility, vascular permeability etc.

Prostaglandins are synthesized from arachidonic acid through an enzymatic process involving cyclooxygenase, an enzyme associated with the endoplasmic reticulum in mast cells. When human lung mast cells are immunologically activated—either through IgE receptor engagement or by the action of a calcium ionophore—they generate different prostaglandin derivatives as a component of the body's inflammatory reaction.

4. Kinins: During the inflammatory process, powerful peptide hormones known as kinins are newly synthesized and released into body fluids and tissues. They are produced through the enzymatic cleavage of α2-globulins—specifically high and low molecular weight kininogens—by different enzymes, resulting in the formation of kinins via plasma and tissue kallikreins.

5. Platelet activating factor: Cells involved in the inflammatory response—such as mast cells, neutrophils, eosinophils, and macrophages—synthesize a group of ether-linked phospholipids collectively known as Platelet-Activating Factor (PAF). The biosynthesis of PAF follows a two-step pathway, where the intermediate compound 1-O-alkyl-2-acylglyceryl-3-phosphocholine (lyso-PAF) is generated through the enzymatic hydrolysis of 1-O-alkyl-2-acyl-glyceryl-3-phosphorylcholine by phospholipase A2. Lyso-PAF is finally acylated to PAF by an acetyltransferase enzyme. Platelets are basophil derived mediator and released after the platelet activation in rabbit so term suggested as such Platelet activating factor (PAF) for that phospholipids.

6. Cytokines and Chemokines: Specific types of cytokines and chemokines play a direct role in activating nociceptive sensory neurons, contributing significantly to the development of pain. Certain inflammatory cytokines are also associated with contralateral hyperalgesia and allodynia, as they are involved in central sensitization triggered by nerve injury or inflammation.

Cytokines are protein molecules secreted by activated macrophages, and many of them are key players in promoting the inflammatory response. These are commonly referred to as proinflammatory cytokines. Notable among them—interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)—are actively involved in the onset and persistence of pathological pain.

The classification of cytokines is often based on their cellular origin: *lymphokines* are produced by lymphocytes, *monokines* by monocytes, *chemokines* are distinguished by their chemotactic properties, and *interleukins* are released by one type of leukocyte to act on another. Cytokines can exert their effects in different ways—on the same cell that

secretes them (autocrine action), on nearby cells (paracrine action), or on distant cells throughout the body (endocrine action).

1.2 Pain (Analgesia):

Among the various modern neural scientists, Sherrington, in 1900,defined pain as vital protective reflex as adjunct with psychical state. The International Association for the Study of Pain (IASP) describes the Pain as "an unnecessary sensory as well as emotional experience related with potential or actual tissue damage". Pain broadly classify into the various categories as according to type (nociceptive, inflammatory, neuropathic), the duration (acute or chronic) and on basis of severity (mild, moderate, severe)[10].

Over the past two decades, significant research has expanded our understanding of the neurobiology of pain, revealing that chronic pain, often associated with peripheral nerve damage or tissue inflammation, is supported by a highly complex and adaptable pain signaling system. According to Type and Duration Pain is classified into the different categories.

1.2.1 Pain According to Type:

1. Nociceptive Pain

2. Inflammatory Pain

3. Neuropathic Pain

1. Nociceptive Pain: Nociceptive pain occurs when nociceptors in intact or previously unaffected skin, internal organs, and other tissues are activated, leading to the typical sensation of acute pain. This pain is transmitted through specialized primary afferent nerve fibers.

2. Inflammatory Pain: Inflammatory pain refers to the pain that arise from the inflamed tissue such as arthritis, hypersensitivity following sensitization of peripheral nerve terminals.

3. Neuropathic Pain: According to the International Association for the Study of Pain (IASP), neuropathic pain is defined as pain resulting from dysfunction or damage to nerve fibers, typically in the peripheral nervous system. When this pain is associated with damage to the central nervous system (CNS), it is often inaccurately referred to as central pain.

Examples of peripheral neuropathic pain include post-herpetic neuralgia (pain following shingles), HIV-AIDS-related neuropathy, painful diabetic neuropathy, and chemotherapy-induced neuropathic pain. In contrast, common forms of central neuropathic pain include post-stroke pain, pain caused by multiple sclerosis, and pain resulting from spinal cord injury.

Pain According to Duration

1.Acute Pain:

2. Chronic Pain

1.Acute Pain: The International Association for the Study of Pain (IASP) defines acute pain as "pain of likely brief duration, with a recent onset, typically associated with injuries or illnesses."

Acute pain typically unfolds in two stages. The initial phase, which lasts only seconds, acts as an alert, signaling the body in response to potentially harmful stimuli. The second phase, known as the subchronic phase, can last from hours to days and serves as a protective mechanism. During this phase, the body guards the injured area to support healing and recovery. Acute pain is often considered an adaptive response with a crucial physiological role, such as following surgery, burns, trauma, or a heart attack (myocardial infarction).

2. Chronic Pain: The IASP has described the chronic pain as “pain that lasting for long periods of time. In chronic pain frequently there may not be any clearly identifiable cause and commonly persists beyond the time of healing of an injury”. As the pain state has become the disease, requiring treatment and confers no physiological advantage, it is often regarded as a maladaptive response. Such type of pain is regarded as Persistent pain.

Persistent pain can involve different types, including nociceptive pain, inflammatory pain, and neuropathic pain.

1.3 Biosignaling Apparatus in Pain:

When tissue is exposed to harmful or potentially harmful stimuli, it generates signals known as nociceptive or pain signals. These signals are detected by nociceptors located within the damaged tissue, and the resulting impulses are transmitted to the cell bodies found in the dorsal root ganglia. Nociceptive signals then travel through primary afferent nerve fibers toward the outer layers of the spinal cord’s dorsal horn. From there, second-

order neurons relay these signals through the spinothalamic tracts to specific regions of the brain. In response, the brain may activate descending inhibitory pathways, which help reduce the intensity of the pain, making it more tolerable.

1.4 Cyclooxygenase (COX):

Cyclooxygenase (COX), also referred to as Prostaglandin-endoperoxide synthase (PTGS), consists of two main isoforms: COX-1 and COX-2. COX-1 is primarily located in the gastrointestinal tract, whereas COX-2 is found in activated cells. The cyclooxygenase enzyme plays a key role in converting polyunsaturated fatty acids into prostaglandins (PGs) and thromboxane (THx). Its primary function is to catalyze the conversion of arachidonic acid into prostaglandin H2, which serves as a precursor for prostanoids, molecules with diverse and potent biological activities.

COX gained significant attention starting in 1971 due to John Vane's research, which highlighted the anti-inflammatory effects of non-steroidal anti-inflammatory drugs (NSAIDs) in inhibiting COX enzymes and, consequently, prostaglandin synthesis. COX-2 inhibitors specifically prevent the conversion of arachidonic acid into prostaglandin H2.

The existence of the COX-2 enzyme was confirmed by Dr. Dan Simmons at Brigham University in 1991. Following its discovery, compounds such as DuP-697 and NS-398 became key leads in the development of COX-2 inhibitors. The development of COX-2 selective agents has been supported by successful clinical trials demonstrating their superior safety and efficacy compared to traditional NSAIDs. Celecoxib (Celebrex), one of the first selective COX-2 inhibitors, was approved for use in the USA in 1999 and later marketed in the UK in May 2000 by Pfizer. Another COX-2 inhibitor, rofecoxib (Vioxx), was introduced around the same time in both the USA and the UK. Based on DuP-697, Celecoxib and rofecoxib were among the first COX-2 inhibitors to reach the market. Additionally, other selective COX-2 inhibitors, including valdecoxib, parecoxib, and mavacoxib, were developed by John Talley and his research team.

COX-1, a glycoprotein consisting of 599 amino acids, is encoded by a gene located on chromosome. It is crucial for maintaining homeostasis by regulating various cellular processes, from cell proliferation to angiogenesis. On the other hand, COX-2, with 604 amino acids, is regulated by growth factors and cytokines such as interleukins and tumor necrosis factors, especially during inflammation. It is located on chromosome 1 and has an inhibitor binding site that is 25% larger than that of COX-1. COX-1 and COX-2 are about 60% structurally similar, but COX-2 has a different C-terminal and an altered binding site for NSAIDs, which makes it a preferred target for selective inhibition.

COX-3 is a variant of COX-1, predominantly found in the brain and spinal cord. The exact role of COX-3 is still unclear, but there is evidence suggesting it may be involved in pain sensitivity. This is supported by the proposed mechanism of acetaminophen (paracetamol) action, as it has been recently suggested that acetaminophen selectively inhibits COX-3.

1.5 Classification of NSAID

Non-steroidal anti-inflammatory drugs can be categorized in various ways as by

- Mode of action (effect on the COX enzymes)
- Chemical structure,
- Non-selective or selective COX-II inhibitors.

1.Non-selective COX-II inhibitors

- Salicylates: diflunisal , aspirin
- Acetates: indomethacin, diclofenac,sulindac
- Fenamates: mefenamic acid
- Pyrazolones: Phenylbutazone
- Oxicams: piroxicam
- Propionates: ketoprofen, ibuprofen, naproxen

2. Selective COX-II inhibitors

- More COX-II selective: nimesulide meloxicam
- Coxibs derivatives: celecoxib, etorcoxib, valdecoxib

3.On the basis of half-life, NSAIDs are also classified as

Drug of Short to medium half-life (< 6 hours): ibuprofen, aspirin, diclofenac, ketoprofen, Indomethacin

Drug of Long half-life (> 10 hours): naproxen, diflunisal, phenylbutazone, sulindac,Piroxicam.

Mechanism of action

The principal stage is the transformation of Arachidonic Acid to the hydroperoxyendoperoxide, PGG_{26}. The biosynthesis of prostanoids happens in three phases:

(a) The release of arachidonic acid (AA) from membrane phospholipids through the action of phospholipase A_2.

(b) The conversion of AA by cyclooxygenase in a bifunctional process, producing unstable PGG_2, which is rapidly transformed into PGH_2.

c) Translation of PGH_2 to definite prostanoids via the feat of precise isomerases.

1.6 A Schiff's base:

Ugo Schiff, recognized as one of the pioneers of modern chemistry, was born on April 26, 1834, in Frankfurt, into a prosperous Jewish merchant family. He developed a strong passion for various branches of chemistry, including physical and analytical chemistry, organic and inorganic chemistry, as well as mineralogy and the study of natural substances. He prepared and studies the organic base popularly known as Schiff bases by reacting the aldehydes or ketones with the amine. Unlike the organic bases, Schiff's bases are not base but the designation of these compounds has persisted up to the present time. Ugo Schiff studied reviews and published a book entitled as **"A New Series of Organic Bases" ("Eineneue Reiheorganischer Basen")**

Schiff's bases are generally synthesized from the reaction between the aldehydes and ketones with amine resulting in the formation of the compound known as Imines or azomethines. Schiff bases, Imines or azomethines are compounds that are represented by the general empirical formula $R_2R_1C{=}NR$. The substituent R_2 and R_3 represents alkyl, heteroaryl, aryl, hydrogen while R^1 at the *N*-imino (C=N) may be alkyl, heteroaryl, aryl, hydrogen or metallo (usually Si, Al, B, Sn).

A Schiff's base is a primary or secondary nitrogen fused with an aldehydes or ketones in which the C=O group is modified to C=N-R group. The condensation of an aldehyde or ketone with a primary amine takes place according to the following scheme (Fig 1.2):

Where an alkyl or an aryl group may be shown by R. Heterocyclic or aryl substituent containing Schiff's bases are substantially more readily synthesized and more stable, while those which contain aliphatic or alkyl substituent are relatively unstable. Schiff's base derived from aliphatic aldehyde undergoes polymerization as it is very unstable in nature, while those conjugates obtained from the aromatic aldehyde are more stable than other Schiff's bases.

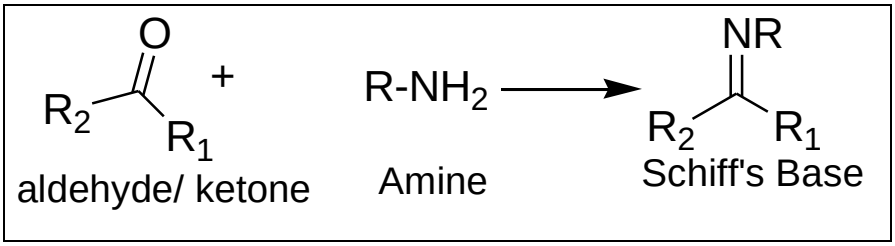

Fig. 1.2 Formation of Schiff Base

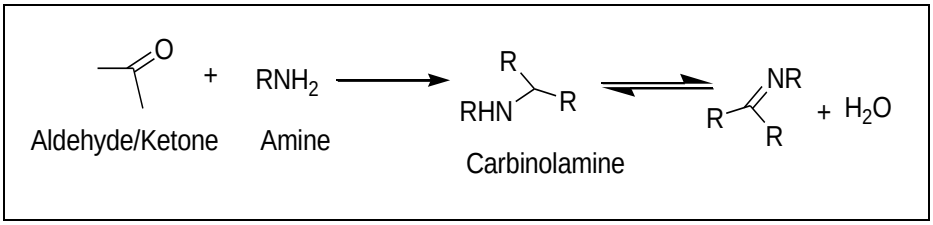

Fig.1. 3 Reversible reaction of Schiff's base synthesis

The synthesis of a Schiff's base from an ketones or aldehydes is catalyze by acid or bases or upon heating as acid / base catalyst provide driving force for completion in forward direction otherwise reaction is a reversible (Fig. 1.3).

Completion of the reaction ends with the separation of the product or removal of the water, or formation of both. In the presence of the aqueous acid or bases many Schiff's bases may be hydrolyzed back to their aldehydes or ketones. Another route for the synthesis of Schiff base is the addition of the neucleophile to the carbonyl group, for instance neucleophile is the amines. Such type of reaction is carried out with the formation of the carbinolamine as an intermediate, after condensation of aldehydes or ketones with amines. Finally loss of water from the unstable carbinolamine takes places in the presence of catalyst acid or base (Fig. 1.4).

Typically Schiff base formation is divided into the two steps first is the formation of the carbinolamine and second subsequent dehydration of carbinolamine. The second step, formation dehydration of carbinolamine is the rate limiting step hence it requires acids as catalyst to provide forward driving force for completion of reaction. If the concentration of acid as catalyst is too high, it may protonated the amines and amines become non-neucleophilic in nature. Such reaction is pulled towards the left and carbinolamine formation is restricted. Therefore mildly acidic pH is prime requirement for maximum yield.

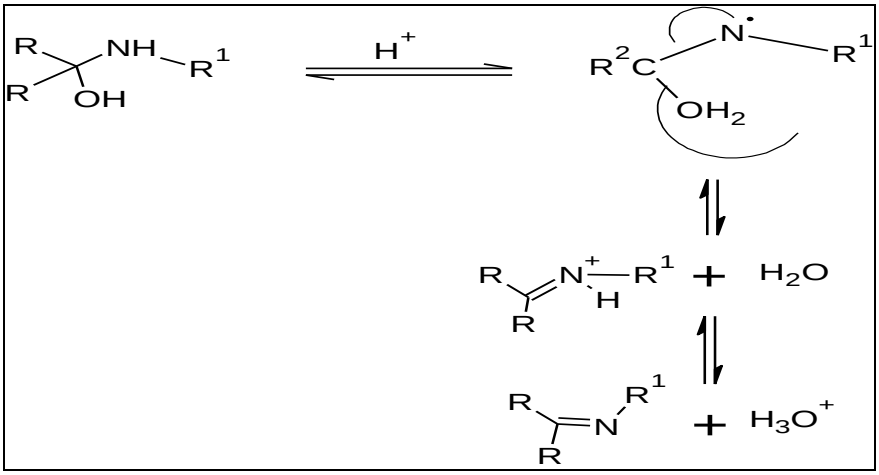

Fig. 1.4 Acid Catalyzed Schiff's Base Formation

The dehydration reaction of carbinolamines is somewhat analogous to the E2 elimination of alkyl halides that catalyzed by base. Formation of the Schiff's base proceeds in two steps i.e. addition followed by elimination through an anionic intermediate.

There are several reactions based Schiff's base mechanism that may lead to the formation of the imines or azomethines. Some of the examples cited below

Reaction of Aldehydes and Ketones with Amines

Addition of Organometallic Reagents to Cyanides

Reaction of Phenols and Phenol-Ethers with Nitriles

Reaction of Metal Amides

1.7 Indole 2, 3 Dione (Isatin):

Several review articles were published and outcome has been patented during recent years for isatin as a privileged molecule. Chemically isatin is defined as 1H-indole-2, 3-Dione, synthetically versatile substrates, where that can be used for the preparation of variety of biologically active derivatives. Isatin, naturallyoccuring in mammalian tissue, act as a biochemical processes modulator for example it is intermediate in the process of melanin formation in human being, in Bufofrogs as a secretary component from the parotid gland, isatin also been found in plants of the genus Isatis in Couroupitaguianensis Aubland in Calanthe discolor LINDL.

1.7.1 Structure activity relationship:

Various reviewer proposed relationship for pharmacological activity with structural modification of isatin (Fig. 1.5).

Substitution atC-5 is by electronegative groups leads to formation of more active compound.

Nitro- substitution at C-5 increases the anticancer activity, where as presence of a methoxy group responsible for the cytotoxicity.

Any Substitution at 5, 6, and 7 the CNS activity.

N- Alkylation and acylation can be done on position 1st.

If substituted phenyl ring is substituted at 3rd position than it enhances antimicrobial and anti inflammatory activity.

At 3rd position, formation of Schiff based on reaction with aromatic amine results in analgesic, antimicrobial compound.

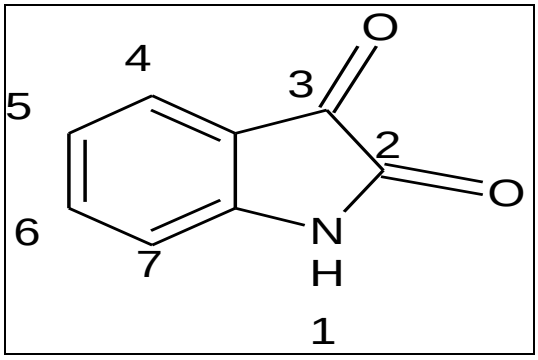

Fig. 1.5 Indole 2, 3 Dione (Isatin)

1.7.2 Synthesis of isatin:

Sand Meyer procedure: This is routine method for synthesis of isatin.In this procedure Sand Meyer synthesized isonitrosoacetanilide by reacting aniline with choral hydrate and hydroxylamine hydrochloride in the presence of sodium sulfates solution in water, which on treatment with concentrated sulfuric acid yields (75%) isatin. (Fig.1.6)

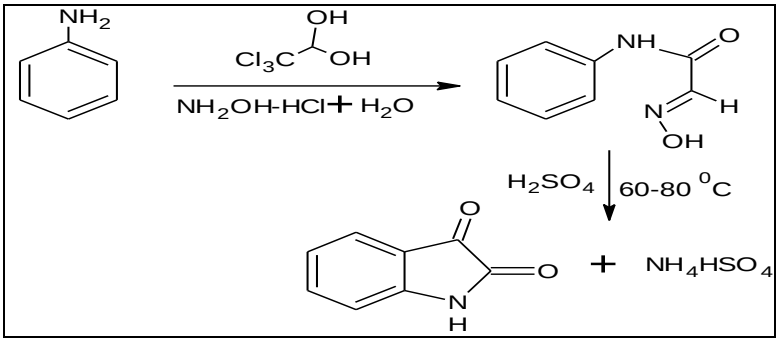

Fig.1.6 Synthesis of isatin

The Martinet procedure: In this method 3-(3-hydroxy-2-oxindole) carboxylic acid derivative subjected to oxidative decarboxylation yields isatin. A 3-(3-hydroxy-2-oxindole) carboxylic acid synthesized from reaction between aromatic amino compound and an oxomalonate ester or its hydrate in the presence of an acid. (Fig.1.7)

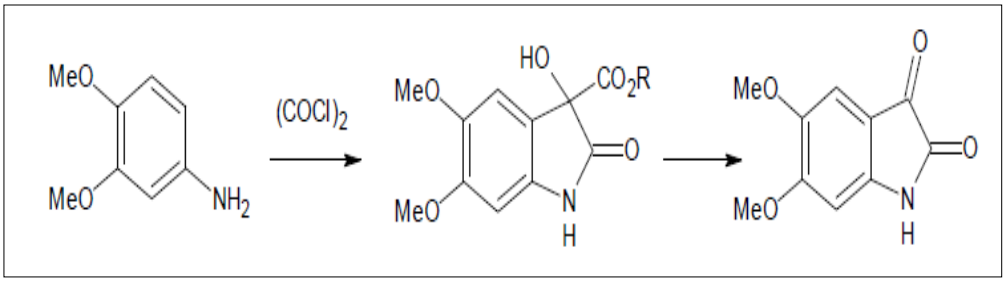

Fig.1.7 The Martinet procedure

The Stolle procedure: This procedure involves cyclization of chlorooxalylanilide, an intermediate which form by the reaction of Anilines with oxalyl chloride, in the presence of a Lewis acid, usually BF3.Et2O or aluminum chloride. **(Fig.1.8)**

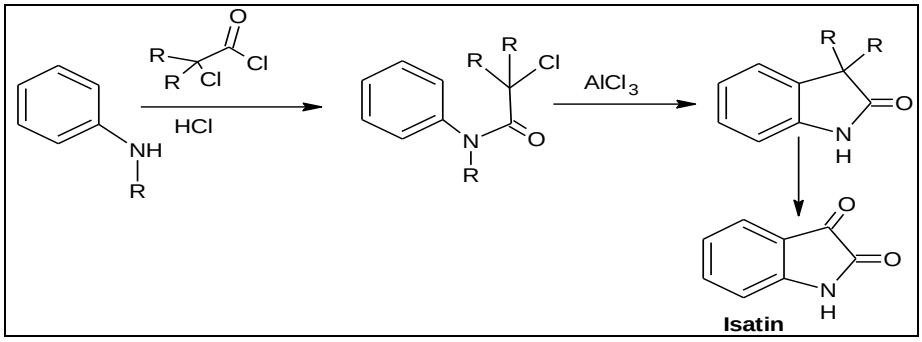

Fig.1.8 The Stolle procedure

The Gassman Reaction: This method involves the synthesis and subsequent oxidation of an intermediate 3- methylthio-indole 2-one. (**Fig.1.9)**

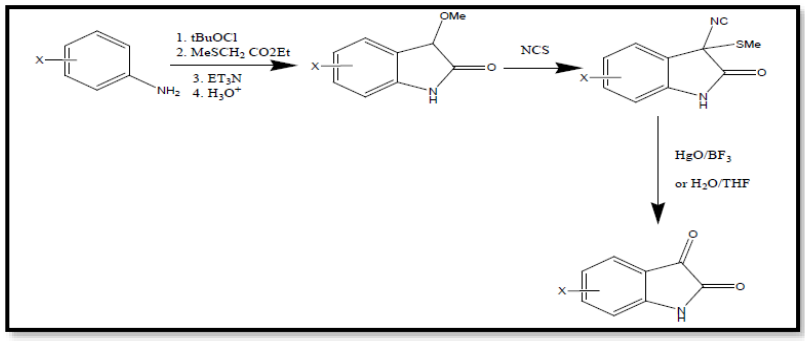

Fig.1.9 The Gassman Reaction

Metalation of anilide: This is a regioselective mechanism in which meta-substituted anilines is use to obtain 4-substituted isatin. This method described the ortho-metalation (DoM) of N-(t-butoxycarbonyl)-anilines and N-pivaloyl-. The intermediate a-keto esters obtained after reaction is deprotected and cyclized to form isatin.(**Fig.1.10)**

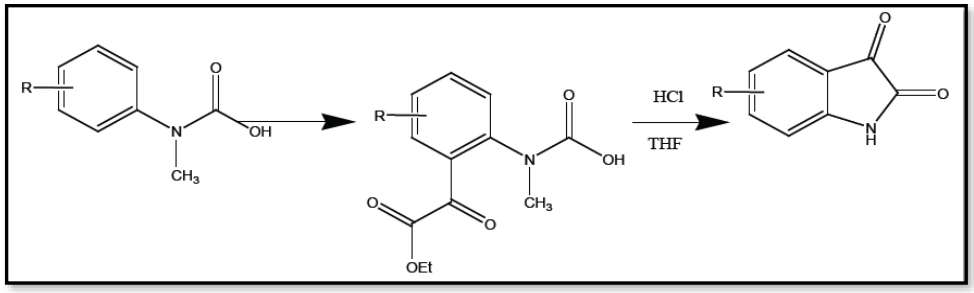

Fig.1.10 Metalation of anilide

Drug Likeness study:

Biological activity (BA) is related to molecular property is mathematically calculated from the following equation

Log (BA) = a log P + b σ + Es + d

Where Log (BA) biological activity, σ electronic parameter, Es steric parameter, a, b, c and d are the numerical values.

Selection of suitable molecular descriptors for correctly predicting the drug likeness of an analogue is of prime importance for drug design[5]. Methods for drug-likeness prediction include from simple technique like Lipinski's "rule of five" to Molinspiration software. Lipinski's "rule of five" predicts drug-likeness on the basis of various molecular descriptors MiLog P, *n* violations, molecular mass, *n* rotb No. hydrogen bond donors and No. hydrogen bond acceptor, Bioactivity score.

1. Milog P <5 shows good permeability across cell membrane.
2. TPSA below 160 $Å^2$ TPSA is related to hydrogen bonding potential of compound
3. *n* violations =1 or <0 related to the binding properties,
4. molecular mass <500 Dalton,
5. *n*rotb< 5 Number of rotatable bonds measures molecular flexibility.
6. No. H- bond donors ≤ 5 (The sum of OHs and NHs)
7. No. H-bond acceptor ≤ 10 (The sum of Os and Ns).
8. Bioactivity score: For molecules, the probability is if the bioactivity score is (>0), then it is active, if (-5.0-0.0) then moderately active, if (< -5.0) then inactive.

The pharmacological effectiveness of a molecule, when considered as a ligand, primarily depends on its physicochemical characteristics. These molecular traits, often referred to as molecular descriptors, play a crucial role in determining the ligand's pharmacodynamics and pharmacokinetics, ultimately influencing its absorption, distribution, metabolism, and excretion (ADME) within biological systems. In 1997, Christopher A. Lipinski proposed a theory explaining the connection between molecular properties and a molecule's potential as a drug candidate. Key descriptors such as log P (partition coefficient), molecular weight (MW), topological polar surface area (TPSA), and the number of hydrogen bond donors and acceptors in a molecule form the basis of what is known as Lipinski's Rule of Five, or Pfizer's Rule of Five (RO5). Molecular descriptor data were gathered using online platforms like Molinspiration and the Supercomputing Facility for Bioinformatics and Computational Biology at IIT, New Delhi. The collected data are presented in Table 6.1.

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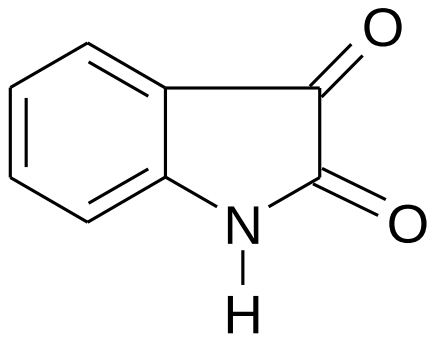
Chapter 2: Review of literature

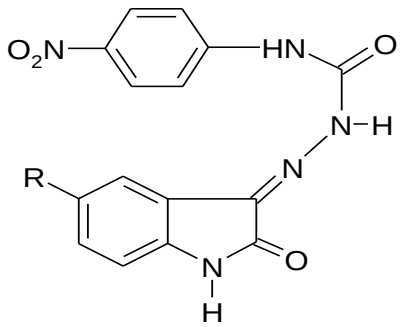
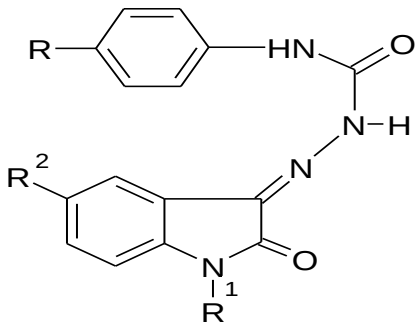
2.0 Review of literature

Isatin (1H-indole-2,3-dione) was initially discovered by Erdman and Laurent in 1841 through the oxidation of indigo using nitric and chromic acids. Its broad synthetic versatility has led to significant scientific interest in exploring its biological and pharmacological activities, as well as those of its derivatives. Naturally present in the human body, isatin exhibits a range of pharmacological effects, including anticonvulsant, tuberculostatic, and analgesic properties. Extensive literature has been reviewed, with particular emphasis on evaluating its anticonvulsant, analgesic, and anti-inflammatory activities.



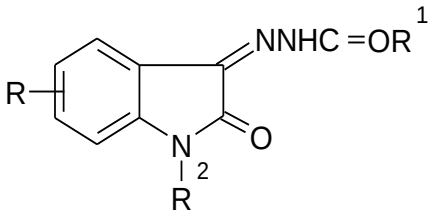
(1H-indole-2, 3-Dione)

2. In 2008, Pandeya and colleagues developed a series of semicarbazones featuring P-nitro phenyl substitutions and evaluated their anticonvulsant properties using MES, scPTZ, and scSTY models. The results showed that all synthesized compounds exhibited activity in both the scPTZ and MES tests. Notably, two of the compounds demonstrated effectiveness in the MES test at a dosage of 100 mg/kg.

Semithiocarbazone derivatives of isatin

3. In 2002, Pandeya and colleagues developed a range of N-methyl/acetyl-5-(un)substituted isatin-3-semicarbazone derivatives. Among these, compounds bearing 4-bromo and 2-chloro substituents (R = 4-Br and 2-Cl) demonstrated significant activity, showing effectiveness in MES, scPTZ, and scSTY-induced seizure models. Additionally, their work involved the synthesis of halo-substituted isatin semicarbazones to investigate the influence of hydrogen bonding on anticonvulsant properties.



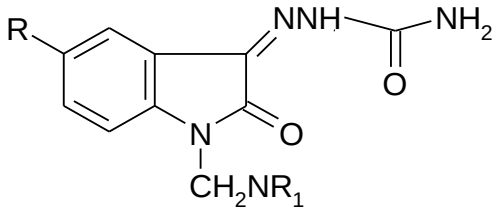
(Halo substituted isatin semicarbazones.)

R^1=-CH_2O-C_6H_5, 1-CH_2O-(4Br)-C_6H_4,

R^2 = $COCH_3$ $CH_2C_6H_5$, R= H, 4-Cl, 5-Cl, 6-Cl.

4. Yogeeswari and colleagues synthesized seven different series of substituted aryl semicarbazones and assessed their anticonvulsant properties using the MES and scPTZ-induced seizure threshold models. A detailed structure–activity relationship (SAR) analysis was conducted, focusing on variations in both the aryl ring substituents and modifications at the carbimino terminal. The general trend in anticonvulsant activity, based on substitutions on the primary aryl group, followed the order: 4-fluoro > 2-bromo = 3-bromo = 4-chloro > 4-methyl > 4-bromo > 3-cyano > 3-methyl. Notably, a majority of the synthesized compounds demonstrated effectiveness in both MES and scPTZ tests. Among them, the semicarbazones bearing a 4-fluorophenyl group exhibited the strongest anticonvulsant effects.

5. Pandeya and Sriram developed Schiff bases by reacting isatin and its derivatives with trimethoprim, along with their corresponding N-Mannich bases. The resulting compounds demonstrated notable antibacterial activity, exhibiting Minimum Inhibitory Concentration (MIC) values between 10 and 25 µg/mL against pathogens such as Vibrio cholerae, Shigella boydii, Enterobacter faecalis, and Edwardsiella tarda. Additionally, certain compounds displayed effectiveness against Salmonella typhi and Vibrio cholerae.

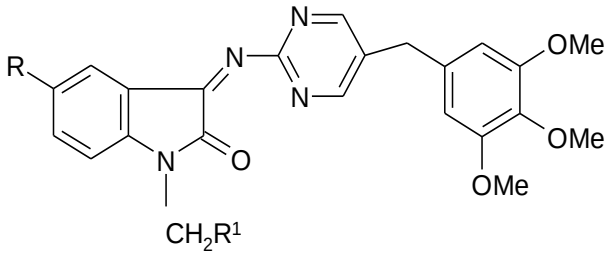


R = H, CH_3, Br;

R_1 = piperidino, morpholino, diethylamino,

3-methyl piperidino

6. P. Yogeeswari and colleagues developed a novel compound, identified as 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[[N4-[3'-(4'-amino-5' trimethoxybenzylpyrimidin-2'-yl)imino-1'-(5-methylisatinyl)]methyl]-N1-piperazinyl]-3-quinoline carboxylic acid. This molecule demonstrated remarkable broad-spectrum chemotherapeutic activity, showing significant effectiveness against HIV, HCV, Mycobacterium tuberculosis, and a range of other pathogenic bacteria.

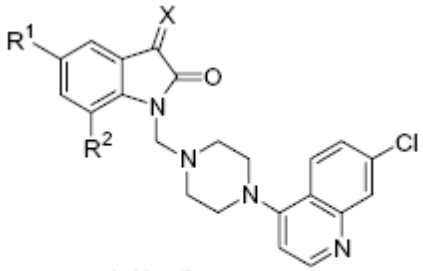


Schiff bases of isatin and its derivatives with trimethoprim and their N-Mannich bases

Where, R= H, CH_3; R^1= $N(CH_3)_2$, $N(C_2H_5)$, morpholino, piperidino, pyrrolidino

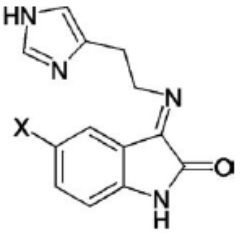
7. Idan Chiyanzu and colleagues developed and synthesized a novel series of 4-aminoquinoline derivatives inspired by the natural compound isatin, aiming to evaluate

their biological activity against three different strains of the malaria parasite Plasmodium falciparum. These newly designed compounds demonstrated anti-plasmodial activity, with IC_{50} values ranging from 1.3 to 0.079 μM against the chloroquine-sensitive D10 strain, and from 2.0 to 0.050 μM against two chloroquine-resistant strains, K1 and W2. To explore potential biological targets for these derivatives within P. falciparum, selected molecules were further assessed for their inhibitory effects on the parasite's cysteine protease, falcipain-2.



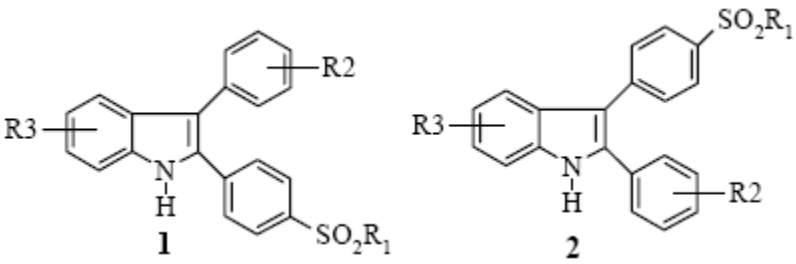
1; X = O
2; X = NNHC(S)NH_2

8. Ashraf H. Abadi and colleagues reported the synthesis of various 3-substituted-2-oxoindole analogues by reacting isatin or its halogenated derivatives with selected amino acids or histamine under neutral conditions. The resulting imino compounds were evaluated for their ability to inhibit three serine/threonine kinases: CDK1/cyclin B, CDK5/p25, and GSK3α/β. Most histidine-based derivatives demonstrated significant kinase inhibition within the low micromolar concentration range. In contrast, histamine-derived compounds exhibited weaker inhibitory effects against CDK1/cyclin B and CDK5/p25 and showed no activity against GSK3α/β. These findings suggest that modulation of the carboxyl group could serve as a strategy to enhance selectivity among this class of kinases.



23 X= H
24 X= Cl
25 X= Br

9. Isatin (1H-indole-2,3-dione) serves as a highly adaptable scaffold for the development of new antiviral compounds. Research has shown that isatin-β-thiosemicarbazone (1H-indole-2,3-dione-3-thiosemicarbazone) and its N-Mannich base derivatives exhibit antiviral activity against a range of viruses. Among them, N-methylisatin-β-thiosemicarbazone, commonly known as methisazone, became the first antiviral medication to gain clinical approval. Both methisazone and isatin-β-thiosemicarbazone have proven effective particularly against poxvirus infections.



1 **2**

	R1	R2	R3
1	CH3	H	H
2	NH2	H	H

10. Wen Hui HU et. al, Phenyl sulfone-based 2,3-diarylindole derivatives were specifically designed and identified as selective COX-2 inhibitors. Additionally, an efficient synthetic method was established to produce these novel inhibitors. Recently, Searle and Merck & Co., Inc. have introduced Celecoxib[8] and Rofecoxib[9] to the market as a new class of NSAIDs, with the pharmacophore of these drugs being recognized as phenyl sulfone-containing cis-1,2-diaryl-alkenes or their structural analogs.[42]

11. Maria Eline Matheus et.al, Isatin is a highly adaptable molecule known for its wide range of biological activities. Researchers have explored the potential of isatin derivatives to inhibit the lipopolysaccharide/interferon-γ-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins, along with their ability to suppress the production of prostaglandin E2 (PGE2), nitric oxide (NO), and tumor necrosis factor-alpha (TNF-α), as well as their capacity to scavenge NO. Isatin compounds were found to reduce TNF-α production and inhibit the expression of iNOS and COX-2 proteins, leading to decreased levels of NO and PGE2. These findings suggest that isatin and its derivatives may serve as promising inhibitors of iNOS and COX-2, with potential applications as anti-inflammatory and anticancer agents.

12. N.Mushtaq et.al, Several acylated azaindole compounds have been reported to exhibit analgesic and anti-inflammatory activities. In addition, various indole derivatives have been synthesized as cyclooxygenase inhibitors and have proven to be effective as anti-inflammatory agents. One indole derivative designated as indomethacin has demonstrated a high degree of anti-inflammatory and analgesic activity.

13 Le Anh Vu and colleagues published a review discussing the history of drug design, highlighting key stages of the in silico drug design approach involved in discovering new molecular entities. They outlined the complete process of a virtual design campaign, beginning with target selection, ligand identification, and evaluation of the target's structure. Their review also covered receptor theory, molecular docking studies, virtual high-throughput screening, potential challenges in choosing methods for ligand design, and the evaluation of the ligand's effectiveness.

14. Soma Mandal and colleagues offer a review on the current understanding of drug design, focusing on the approach of rational drug design. The drug development process is costly, complex, and time-consuming, necessitating the use of computational tools and methodologies to speed up progress. The current structure-based drug design (SBBD) approach remains incomplete, as many drugs developed using ligand-based methods have shown unpredictable ADME (absorption, distribution, metabolism, and excretion) processes in biological systems. This review discusses the integration of multidisciplinary approaches, such as gene expression analysis and computational chemistry, to fully capitalize on the development of successful drugs.

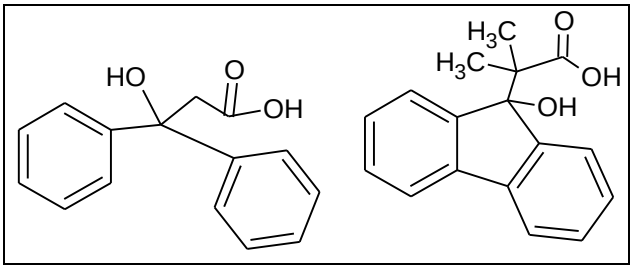
15. Sunita Pandey et al. discussed Current advances and new mindset in relation to computer-aided drug design. The importance of Computer aided approaches is to identify the new lead compounds using the various methodologies such as structure based and ligand based pharmacophore modeling, molecular mechanics and dynamics, molecular modeling, virtual screening. They also discuss the utility and role of Chemo

informatics and Bioinformatics in the modern and digital era of the drug discovery and development.

16. Chia-Hsien Lee et al. Reviewed Ligand-Based rational drug design for an ATP Synthase-β Subunit Inhibitor. Ligand-based drug design based on either the quantitative structure-activity relationship (QSAR) method or pharmacophore model. Since they were constructed pharmacophore models for ATP synthase-β ligands are potentially useful drugs in cancer therapy that pave the way for novel inhibitor designs. Enterostatin and Bathophenanthroline-metal chelates molecules were designed manually and confirmed carefully using the Discovery Studio package software. Data pertaining with these known ligands of the ATP synthase β subunit were obtained from the PubChem database.

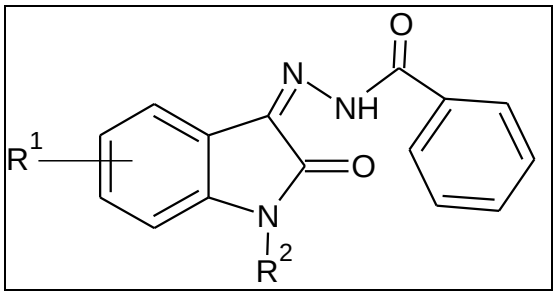
17. David J. Huggins et al. discussed the rational approaches important to improve the selectivity in drug design. Selectivity strategies based on five principles such as shape, flexibility, electrostatics, hydration and allostery that can be employed to gain binding selectivity for a given target: (1) Altering the charges on the ligand to specifically target the desired receptor while avoiding interaction with the decoy; (2) Targeting an allosteric site on the receptor that is present in the target but absent in the decoy; (3) Eliminating a high-energy water molecule in the target that is not found in the decoy; (4) Inducing a structural clash with the decoy receptor, which it cannot resolve, while the target receptor can accommodate the interaction; (5) Facilitating a structural rearrangement that allows binding to the target receptor, but not to the decoy receptor, preventing the decoy from forming a similar interaction; and (6) Targeting a receptor conformation that is accessible in the target but not in the decoy.

18. Sanda P. Dilber et al., conducted a study involving the two-step synthesis and molecular docking of six diastereomeric analogues of 3-hydroxy-2-methyl-3-(4-biphenylyl)butanoic acids. Docking experiments were performed using the AutoDock v4.0.1 software, targeting the three-dimensional active sites of the COX-1 (PDB ID: 1EQG) and COX-2 (PDB ID: 1CX2) enzymes. The synthesized compounds, belonging to the β-hydroxy-β-arylpropanoic acid class—structurally related to nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen—were evaluated for their anti-inflammatory potential and ulcerogenic effects. Oral administration studies revealed that all derivatives demonstrated notable anti-inflammatory properties. Notably, 2-(9-(9-hydroxyfluorenyl))-2-methylpropanoic acid (compound 5) and 3-hydroxy-3,3-diphenylpropanoic acid (compound 3) exhibited the most potent anti-inflammatory effects without inducing significant gastric damage, showing comparable efficacy to ibuprofen.



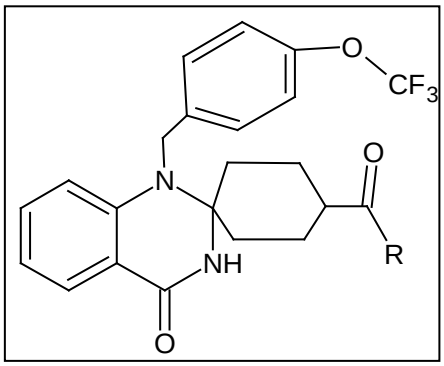
Diastereomeric 3-Hydroxy-2- Methyl-3- (4-Biphenylyl) Butanoic Acids

19. Ravi Jarapula et al. Synthesis New Isatin Derivatives that subjected to *in-vivo* Anti-Inflammatory Activity and Molecular Docking Studies.2-hydroxybenzohydrazide condensed with substituted isatin to synthesis novel derivatives of 2-hydroxy-N-(2-oxoindolin-3-ylidene) benzohydrazide which later confirmed with spectral characterization by FT-IR, 1H-NMR, and mass spectra. Further, molecular docking studies were also carried out for the catalytic site of COX-2 enzymes (PDB ID: 3LN1) and COX- 1 (PDB ID: 3N8Y). The compounds were screened by carrageenan induced paw edema method for *in-vivo* anti-inflammatory activity. The results obtained were comparable with standard COX-2 inhibitor Celecoxib. The screened analogues have shown mild-to-moderate activity. The compounds **VIIc** and **VIId** found to be good anti-inflammatory and docking results comparable with standard COX-2 inhibitor Celecoxib.



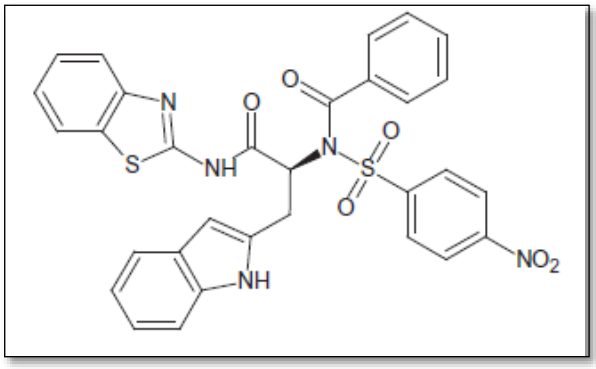
2-hydroxy-N-(2-oxoindolin-3-ylidene) benzohydrazide

20. Subba Poojari et al. screened the novel spiro-piperidine quinazolinone derivatives for Anti-inflammatory, antibacterial and molecular docking studies. The Carrageenan-induced rat Paw oedema method using ibuprofen as a reference drug to compare the anti-inflammatory activities of the synthesized compounds. **Molecular docking study** of lead com-pounds **4e**, **4g**, **5e**, **5f**, **6a** and **6f performed using HEX 8** *software* into the 3D catalytic sites in the COX-2 (pdb code: 1CX2) and COX-1 (pdb code: 1EQG)enzymes. This results found that compounds **6a** and **6f** have significant binding activities of score 58.36 and 57.67 kJ mol−1, respectively.(6a = 3-F-Ph and 6f = Uracil-5).



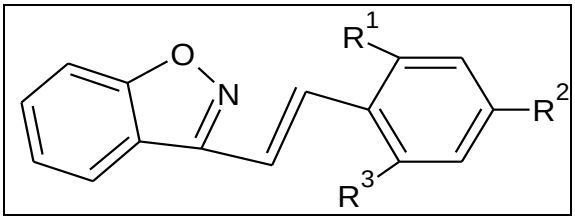
Spiro-Piperidine Quinazolinone Derivatives

21. David Izuchukwu Ugwu et al. prepared of twelve novel derivatives of benzothiazole bearing benzenesulphonamide and carboxamide and compared anti-inflammatory and analgesic activity, molecular docking and *in-vivo* studies with Celecoxib as standard. Studies of the physicochemical parameter of new derivatives indicate that they will not have oral bioavailability problems. Molecular operating environment (MOE) was used to dock the compound with 1CX2 as target. Derivatives 17c found to be most active in relation to anti-inflammatory and analgesic activity and highest binding energy (–12.50 kcal/mol).



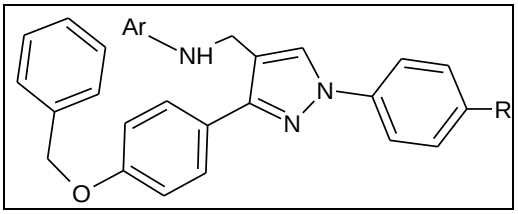
Benzothiazole bearing benzenesulphonamide and carboxamide derivatives

22. S. S. kumar et al. designed 1,2-benzisoxazole derivatives were analyzed in silico using Chemsketch software, and their molecular docking was studied to assess their analgesic and anti-inflammatory properties. The results of these molecular docking studies demonstrated that all the compounds exhibited strong binding interactions with both COX-2 and the nicotinic acetylcholine receptor. Among them, compounds 4a and 4c displayed the highest binding scores (-7.46 and -7.21, respectively) with the nicotinic acetylcholine receptor and showed the most significant analgesic effects.



1, 2-Benzisoxazole derivatives

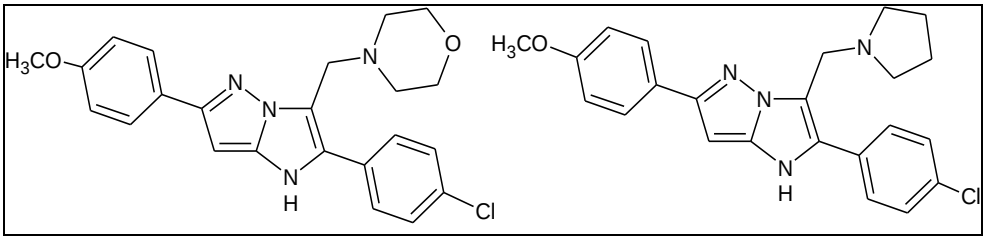
23. Md Jahangir Alam et al. published an article about designing, synthesis, and evaluation of pharmacological activity of a new series of hybrid pyrazole derivatives.



Hybrid pyrazole derivatives

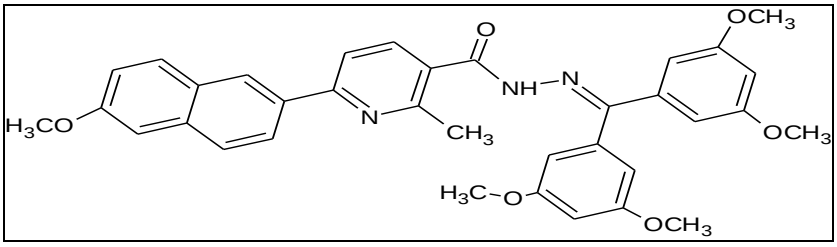
Some compounds were selected on the basis of *in-vivo* studies for assessment of their *in-vitro* inhibitory action against COX1/2 and TNFα. Docking studies of the potent compounds **5s** and **5u** were also carried out to determine possible interactions within the binding site of COX1 (PDB ID 3KK6) which showed high docking scores of-12.24 and-12.907 compared to Celecoxib, with a -9.924 docking score.

24. A. H. Shridhar et al., synthesized a series of novel fused 1H-imidazo[1,2-b]pyrazole derivatives and assessed their anti-inflammatory and analgesic activities. The biological findings were further supported by molecular docking studies targeting COX-1 and COX-2 enzymes. The study demonstrated that compounds 6b, 6c, 7b, and 7c exhibited strong binding affinities toward the active site residues of COX-2 (PDB ID: 1CX2) and COX-1 (PDB ID: 1EQG). The primary objective of the investigation was to establish an efficient and practical synthetic route for biologically active 2-(4-haloaryl-substituted)-3-morpholinomethyl-6-(phenyl-substituted)-1H-imidazo[1,2-b]pyrazole derivatives, as well as 2-(4-haloaryl-substituted)-3-(pyrrolidin-1-yl)methyl-6-(phenyl-substituted)-1H-imidazo[1,2-b]pyrazole derivatives. All synthesized compounds underwent evaluation for their analgesic and anti-inflammatory effects, and the results were analyzed in relation to structure-activity relationship (SAR) studies. Among the compounds tested, 6b and 7c showed the most promising anti-inflammatory and analgesic activities.



2-(4-halo aryl substituted)-3-morpholinomethyl-6-(phenyl substituted)-1H-imidazole [1, 2-b] pyrazole derivatives

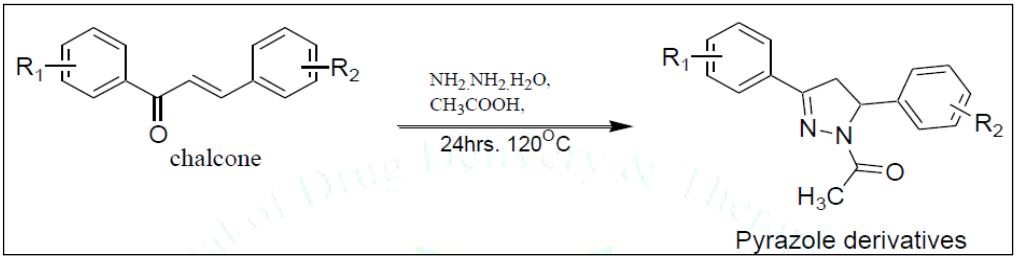
25. Mardia T. El Sayed et al., synthesized a series of Naprosyn derivatives following established synthetic procedures. The structures of the newly prepared compounds were confirmed through various spectroscopic methods, including elemental analysis, infrared (IR) spectroscopy, proton (^1H) and carbon (^13C) nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry. The biological activities of these compounds were evaluated both in vitro and in vivo. In vitro assessments were conducted strictly following the NCI protocol, involving antitumor screening against 60 different cell lines, as well as evaluating COX-1 and COX-2 inhibition activities. In vivo testing employed the rat carrageenan-induced paw edema model to measure anti-inflammatory effects. Among the tested compounds, compound 13b demonstrated outstanding activity, achieving 100% inhibition and complete edema recovery, along with significant inhibition of both COX enzymes. Furthermore, molecular docking studies and molecular dynamics simulations were conducted, providing strong support for the experimental findings.



Naprosyn

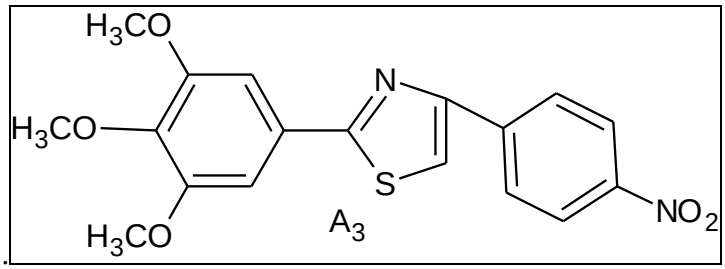
26. Mishra K. et al., concentrated on developing novel pyrazole-ethanone linked compounds through a two-step synthetic approach aimed at enhancing biological activity. Initially, an intermediate chalcone was synthesized, which subsequently underwent a cyclization reaction in the second step to form the final pyrazole derivative incorporating an ethanone group. The cyclization process proceeded via a proton transfer mechanism. The resulting derivatives were subjected to various identification and

characterization techniques, including melting point determination, solubility analysis, thin layer chromatography, and spectral studies using UV, IR, and NMR spectroscopy.



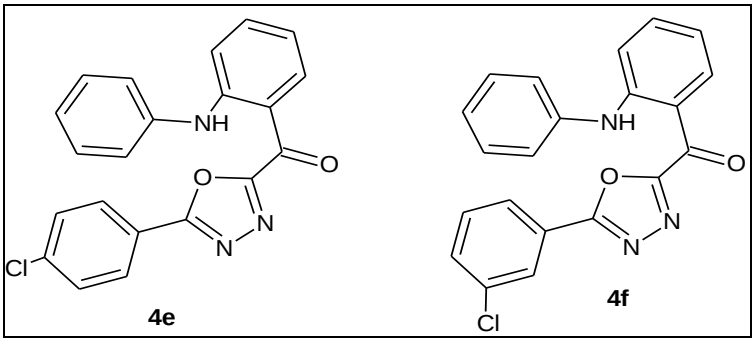
Pyrazole ethanone linked compounds

27. Smaranda Dafina Oniga et al., explored the potential binding interactions of a series of previously developed 2-(trimethoxyphenyl)-thiazoles with cyclooxygenase (COX) enzymes, aiming to identify safer alternatives to conventional NSAIDs. Their study involved in-vitro evaluation of COX inhibitory activity against ovine COX-1 and human recombinant COX-2. To further understand the interaction patterns between the inhibitors and the active sites of both COX isoforms, molecular docking analyses were conducted. All tested compounds exhibited strong inhibitory effects on both isoforms, with compound A3 displaying a particularly favorable COX-2 selectivity index compared to meloxicam. Docking simulations suggested that compound A3 established hydrophobic interactions with residues such as Leu352, Val349, Leu359, Phe518, Gly526, and Ala527, alongside hydrogen bonding with critical active site residues including Tyr355, Arg120, Ser530, Trp387, and Met522. Additionally, the pharmacokinetic properties predicted through computational methods highlighted compound A3 as a promising lead candidate. The findings confirmed that these newly designed molecules effectively inhibit COX, aligning with earlier in-vivo anti-inflammatory studies, and position compound A3 as a potential candidate for further development as a next-generation NSAID.



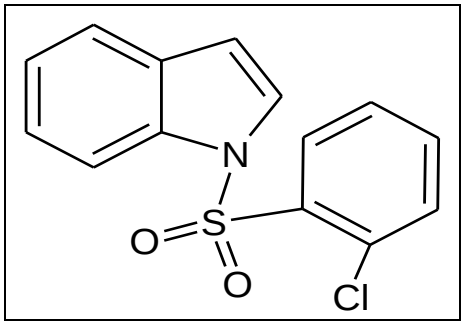
2-(trimethoxyphenyl)-thiazoles

28. Suman Bala et al., developed a new series of N-phenyl anthranilic acid-derived 1,3,4-oxadiazoles (4a–h) and evaluated their anti-inflammatory and analgesic properties. Molecular docking studies were also conducted to investigate their interaction with the cyclooxygenase-2 (COX-2) enzyme (PDB ID: 1CX2). The anti-inflammatory potential was assessed using the carrageenan-induced rat paw edema model, while analgesic effects were measured through the tail immersion method. To minimize gastric side effects typically associated with free carboxylic groups, the compounds were structurally modified by incorporating a 1,3,4-oxadiazole heterocyclic moiety, which demonstrated favorable binding with the COX-2 receptor. Among the synthesized derivatives, compounds 4e and 4f exhibited the most promising COX-2 inhibitory activity.



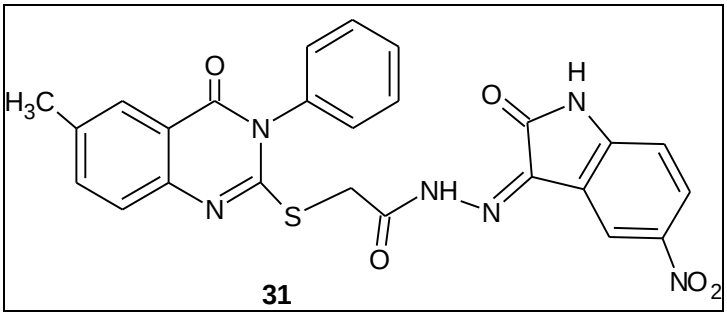
N-phenyl anthranilic acid-based 1, 3, 4-oxadiazoles

29. Vibha C Patil et al., conducted molecular docking studies on a series of 1-substituted (phenyl) sulfonyl-1H-indole derivatives using Accelrys' Discovery Studio, targeting the active site of cyclooxygenase-2 (COX-2, PDB ID: 1CX2). Their analysis focused on examining the inhibitors' conformations, protein interactions, and hydrogen bonding patterns. The results indicated that these 1-substituted (phenylsulfonyl) indole compounds exhibited a strong binding affinity toward the COX-2 enzyme. The CDocker Energy and CDocker Interaction energy was calculated for 5 compounds, compound no 1 shows maximum interaction energy (-28.243). In conclusion, N-protection by the above compounds of 1-substituted (phenyl) sulfonyl-1H-indole could be used as a successful pharmacophore to synthesize the drug candidates for a class of COX-2 indole derivatives. The docking study was demonstrated that the binding interactions exhibited by compounds signify the importance of specific amino acid residues in the active site of COX-2 protein.



Substituted (phenyl) sulfonyl-1H-indole derivatives

30. Adel S. El-Azab et al., developed a new class of quinazolinone derivatives featuring isatin groups and evaluated their antitumor properties against the MDA-MB-231 breast cancer cell line and the LOVO colon cancer cell line. Several compounds—specifically 20, 21, 22, 23, 25, 27, 28, 29, 30, 31, 32, 33, and 34—demonstrated strong antitumor effects, with IC_{50} values ranging from 10.38 to 38.67 μM against MDA-MB-231 cells and 9.91 to 15.77 μM against LOVO cells. In comparison, the standard drugs 5-fluorouracil and erlotinib exhibited IC_{50} values of 70.28 μM and 22.24 μM, and 15.23 μM and 25.31 μM, respectively, in these cell lines. To further investigate the cytotoxic potential, compound 31 underwent EGFR-TK inhibition assays and apoptosis induction studies at a concentration of 10 μM, serving as a representative candidate from the synthesized series. Additionally, a molecular docking analysis was conducted for compound 31 and compared with erlotinib, revealing that compound 31 exhibited a binding mode closely resembling that of erlotinib toward the EGFR kinase enzyme.

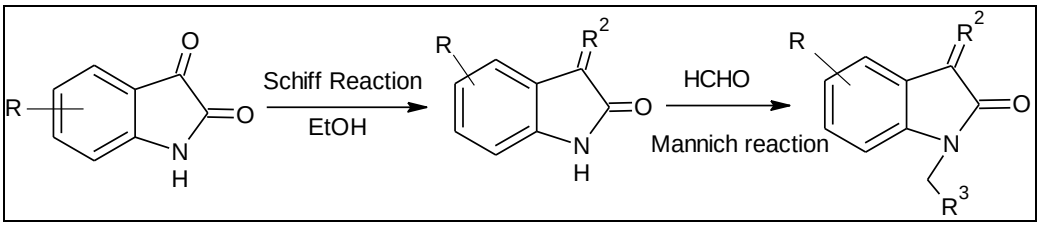


31

Quinazolinone compounds

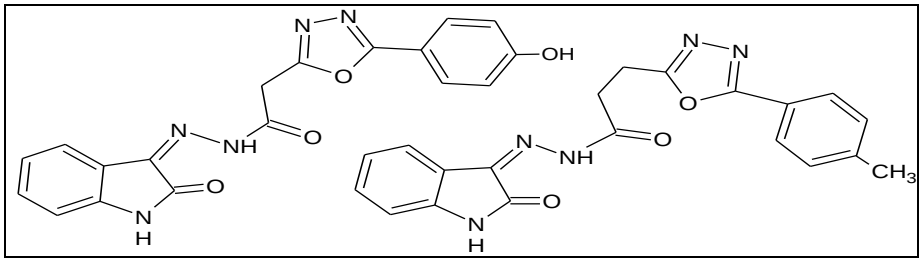
31. R.A. Hajare and colleagues developed a series of Schiff bases derived from 5-substituted-1H-indole-2,3-dione by refluxing ethanol with various aromatic primary amines and hydrazine. These Schiff bases were subsequently used to synthesize a range of Mannich bases in the presence of formaldehyde. The chemical structures of the synthesized compounds were confirmed through FT-IR, 1H-NMR, 13C, and mass spectrometry analyses. The designed molecules were further evaluated through

molecular docking studies and their findings were correlated with in-vivo anticonvulsant activity.



5-substituted-1H indole-2, 3 Dione

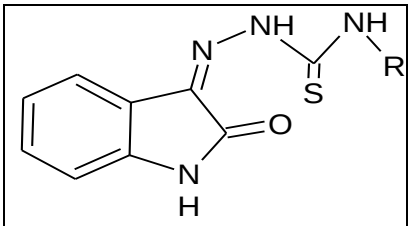
32. Deweshri Kerzare et al., designed and synthesized a series of novel oxadiazolyl-2-oxoindolinylidene propane hydrazides, representing amide-linked hybrids of oxadiazole and indole structures. These compounds were evaluated for their anti-inflammatory and analgesic properties. The synthesis involved a five-step procedure that successfully produced fifteen derivatives, identified as 3-(5-substituted-1,3,4-oxadiazol-2-yl)-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]propane hydrazides. Both in-vitro and in-vivo assessments highlighted compounds 50 and 51 as particularly potent, with severity indices of 0.35 and 0.56, respectively, indicating strong analgesic potential. Structure-activity relationship (SAR) analysis showed that hydroxy and methyl substitutions on the phenyl ring system significantly enhanced biological activity, achieving 84.11% and 83.17% inhibition, respectively, closely matching the reference drug's inhibition rate of 85.84%. Additionally, molecular docking studies supported these findings, with a notable docking score of −4.44 for the active compounds. Overall, the SAR studies affirm the potential of these derivatives as promising lead candidates for further therapeutic development.



Oxadiazolyl-2-oxoindolinylidene propane hydrazides

33. J. Haribabu and colleagues developed a novel series of isatin-based thiosemicarbazones by reacting benzyl isatin with various unsubstituted and substituted thiosemicarbazides (compounds 1–5). The structures of the newly synthesized compounds were verified using a combination of techniques, including elemental analysis, FT-IR, UV-Visible spectroscopy, mass spectrometry, and both 1H and 13C NMR spectroscopy. Additionally, the three-dimensional structures of compounds 1, 3,

and 4 were elucidated through single-crystal X-ray diffraction analysis. Evaluation of the antioxidant properties of these thiosemicarbazone analogues revealed a strong free radical scavenging ability, along with notable anti-hemolytic activity. Furthermore, in silico molecular docking studies, conducted using Schrodinger-Maestro software, assessed the anti-inflammatory and anti-tuberculosis potential of these derivatives.



R=H,CH_3, C_2H_5, C_6H_{11}, C_6H_5

Substituted thiosemicarbazides

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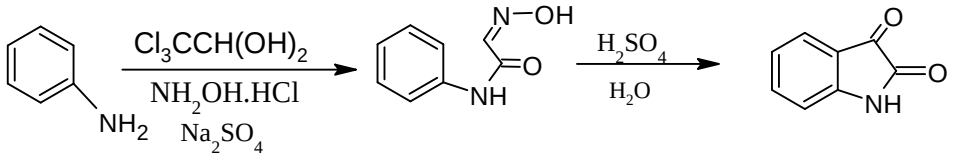
Chapter 3: Plan of works

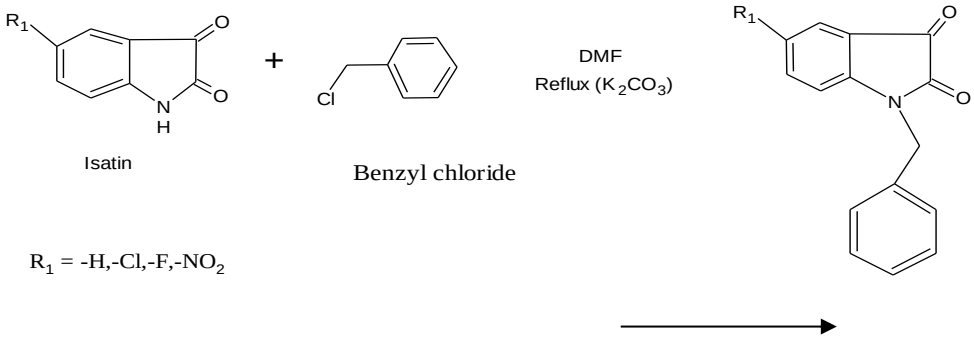
3.0 Plan of work

Literature survey evident to the plan synthesis of Isatin derivatives. The schematic representation of the route of synthesis is as follows:

Step I: Synthesis of isatin

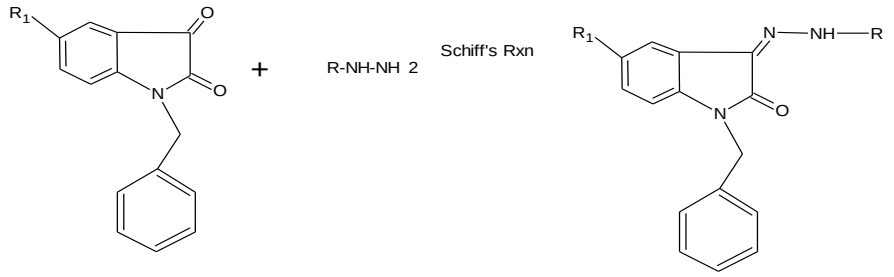


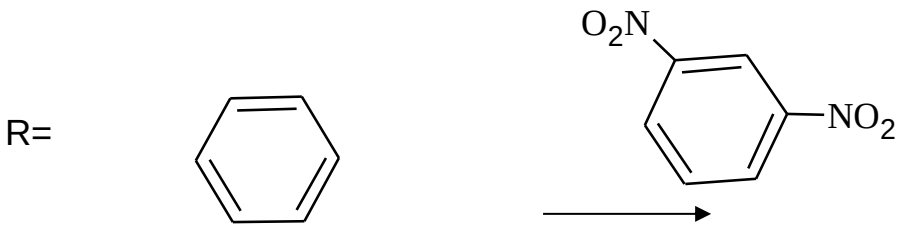
Step II: N-Benzylation of isatin



R_1 = -H,-Cl,-F,-NO_2

Step III: Schiff's bases of N-benzyl isatin:





3.2 Physico-Chemical Studies:

1. Melting point [m.p].
2. Thin Layer Chromatography [TLC].
3. Rotational and Vibrational Spectra [FT-IR]
4. Proton Nuclear magnetic resonance spectra [1H-NMR].
5. U.V. Spectral study.

3.3. Preliminary Pharmacological Screening:

1. Study of Analgesic and Anti-inflammatory activity.

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Chapter 4: Experimental works

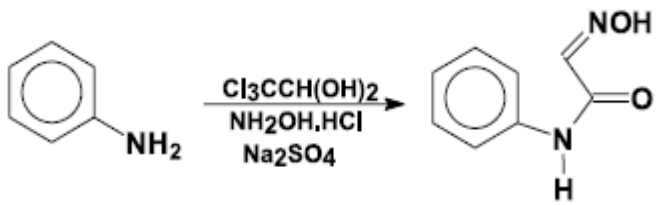
4.1 Material and methods:

All reagents utilized in the experiments were of laboratory grade and were purified according to established protocols. The melting points were determined using the open capillary tube technique and are reported without any corrections. The purity and homogeneity of the synthesized compounds were verified by thin-layer chromatography (TLC) on silica gel G-coated plates, using a benzene:ethanol (9:1) solvent mixture. Spot detection was carried out using iodine vapor. FT-IR spectra were recorded on a SHIMADZU-8101A infrared spectrometer with KBr discs, while ^1H-NMR spectra were obtained in $CDCl_3$ using a BRUCKER spectrometer. UV-Visible spectra were measured using instruments from Thermo Electron Corporation.

4.2 Methods for preparation of Isatin and its halo-derivatives:

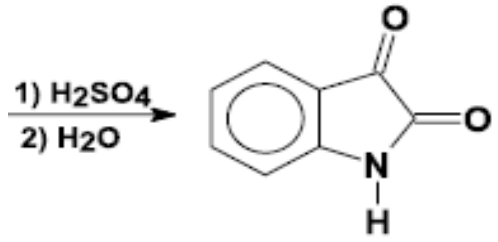
Step I: Synthesis of Isonitrosoacetanilide (INA):

In a round-bottom flask (RBF), dissolve 9 g (0.05 mol) of chloral hydrate in 86 ml of water. To this solution, add 7 g of sodium sulfate, followed by a solution prepared by dissolving 3.1 g (0.03 mol) of aniline or its 4-halo-substituted derivatives in 30 ml of water containing 4–5 ml of hydrochloric acid (to enhance amine solubility). Next, add an aqueous solution of hydroxylamine, prepared in 50 ml of water, into the flask. Heat the mixture to approximately 45°C to ensure complete dissolution of any solid, then bring the solution to a vigorous boil for 1–2 minutes. Allow the mixture to cool to room temperature. The resulting solid is then collected by filtration. This method results in a yield of 80%.



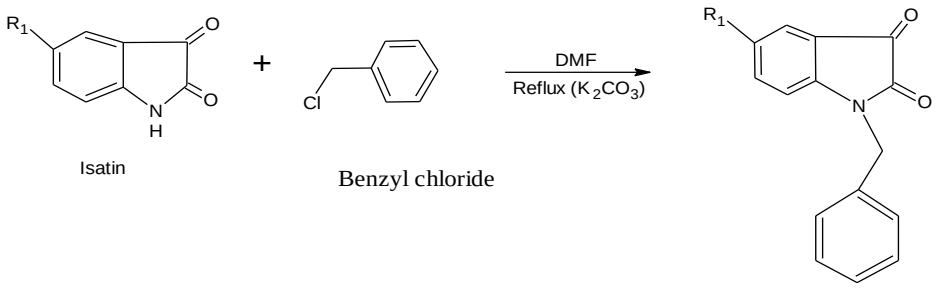
Step II: Synthesis of Isatin:

Heat 3 ml of concentrated sulfuric acid to 70°C. While stirring vigorously, slowly add 1 g of isonicotinic acid (INA) to the heated acid. Increase the temperature to 80°C and hold it for 1–2 minutes. Allow the reaction mixture to cool to room temperature. Once cooled, add 3–4 pieces of ice or an equivalent amount of crushed ice, approximately 3 to 4 times the volume of the reaction mixture, while stirring continuously. The reaction produces a product with a yield of 72%.



4.3 Methods of preparation of 5-substituted N-benzyl isatin derivatives:

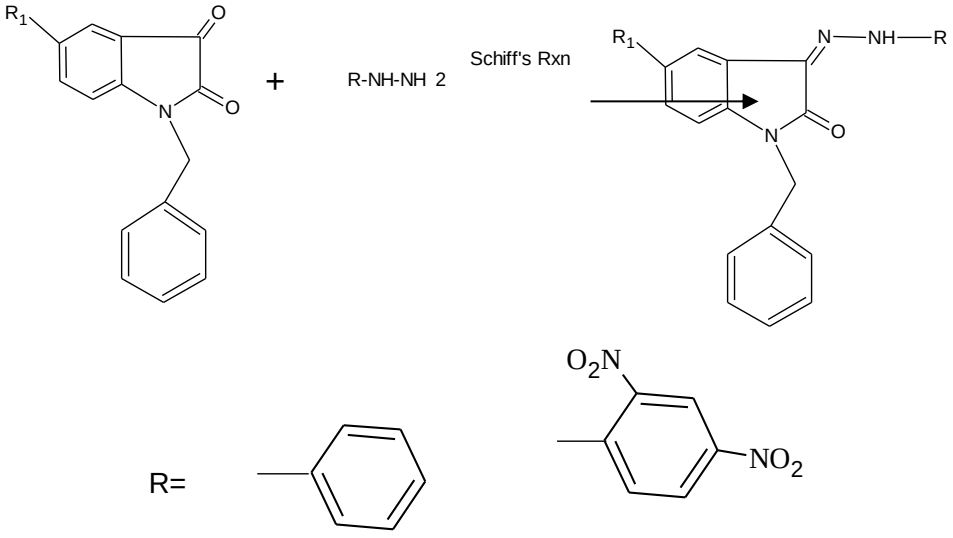
In a round-bottom flask (RBF), combine equimolar amounts of isatin and benzyl chloride. Add 20 ml of dimethylformamide along with potassium carbonate to the mixture. After gently mixing, reflux the reaction for 2 hours. Once completed, allow the mixture to cool and slowly pour it into 100 ml of ice-cold water. The resulting orange precipitate is collected by filtration, washed thoroughly with water, dried, and then recrystallized using ethanol. M.P. is 120^0C-130^0C.



R = H, Cl, F, NO_2

4.4 Methods of preparation of Schiff's bases of 5-substituted N-benzyl isatin derivatives:

An equimolar amount of N-benzyl derivatives and amines was mixed in 20 ml of absolute ethanol within a 250 ml round-bottom flask. A few drops of glacial acetic acid were subsequently added. The resulting mixture was refluxed for 2–3 hours, with progress monitored to ensure the reaction's completion. Afterward, the mixture was left undisturbed for 24 hours, followed by filtration and purification through recrystallization using ethanol.



4.5. Preliminary Pharmacological Screening:

4.5.1 Protein albumin denaturation method for Anti-inflammatory activity [72,73,74]

The anti-inflammatory potential of all synthesized analogues was assessed through an in vitro procedure, adapting the method originally reported by Muzushima and Kabayashi with minor modifications. The evaluation employed the albumin denaturation inhibition assay. For each test, a reaction mixture totaling 10.0 ml was prepared, comprising 0.4 ml of freshly obtained hen's egg albumin, 5.8 ml of phosphate-buffered saline (pH 6.4), and 2.0 ml of the test compound solutions at various concentrations, ultimately reaching a final concentration of 50 µg/ml. An equivalent volume of double-distilled water served as the control. The prepared mixtures were incubated at 37±2°C for 15 minutes, followed by heating at 70°C for 5 minutes. After allowing the samples to cool, the absorbance of the control was measured at 660 nm. Indomethacin (IND) was utilized as the reference standard at final concentrations of 50 and 100 µg/ml, with absorbance readings taken under the same conditions. The inhibition of protein denaturation was then calculated using the following formula:

Percentage inhibition = ($Ab_{control} - Ab_{sample}$) X 100/ $Ab_{control}$

$Ab_{control}$ = Absorbance of control

Ab_{sample} = Absorbance of sample

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Chapter 5: Material and methods

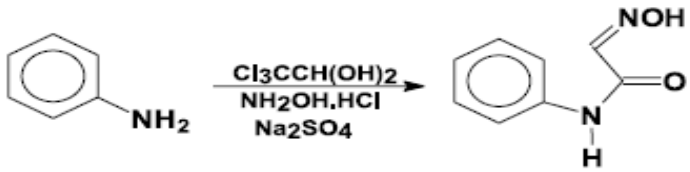
5.1 Material and methods

All chemicals utilized were of laboratory grade and underwent purification following standard procedures. Melting points were measured using the open capillary tube method and are reported without correction. The purity and uniformity of the synthesized compounds were confirmed through thin-layer chromatography (TLC) conducted on glass plates coated with silica gel G, employing a benzene:ethanol (9:1) solvent system. Spots were detected by exposure to iodine vapor. FT-IR spectra were obtained with a SHIMADZU-8101A infrared spectrometer using KBr discs, ^1H-NMR spectra were recorded in $CDCl_3$ using a BRUCKRS instrument, and UV spectra were analyzed using a THERMO ELECTRONIC CORPORATION spectrophotometer.

5.2 Methods for preparation of Isatin and its halo-derivatives

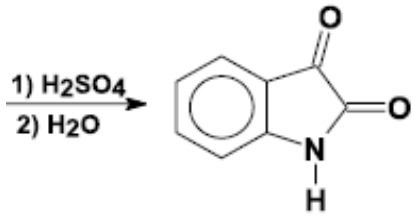
Step I: Synthesis of Isonitrosoacetanilide (INA):

In a round-bottom flask (RBF), dissolve 9 g (0.05 moles) of chloral hydrate in 86 ml of water. To this solution, add 7 g of sodium sulfate along with a solution prepared by dissolving 3.1 g (0.03 moles) of aniline (or its 4-halo substituted derivatives) in 30 ml of water containing 4–5 ml of hydrochloric acid (used to aid the dissolution of amines). Subsequently, introduce a hydroxylamine solution prepared in 50 ml of water into the flask. Heat the mixture to 45 °C to fully dissolve any precipitate, then bring it to a vigorous boil for 1–2 minutes. Allow the mixture to cool to room temperature. The resulting precipitate is collected by filtration, yielding approximately 80%.



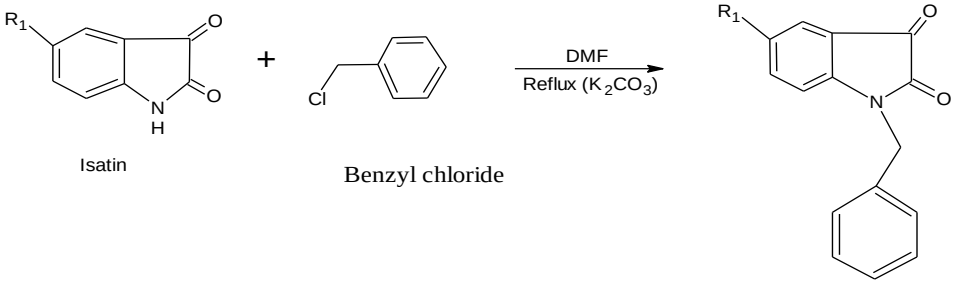
Step II: Synthesis of Isatin:

Heat 3 ml of concentrated sulfuric acid to 70°C. Gradually add 1 g of INA while stirring vigorously. Increase the temperature to 80°C and maintain it for 1–2 minutes. Allow the mixture to cool to room temperature. After cooling, add 3–4 pieces of ice or an equivalent amount of crushed ice—approximately 3–4 times the volume of the mixture—while continuing to stir vigorously. The process yields a 72% product.



5.3 Methods of preparation of 5-substituted N-benzyl isatin derivatives

In a round-bottom flask (RBF), an equimolar amount of isatin and benzyl chloride is combined. To this mixture, 20 mL of dimethylformamide and potassium carbonate are added. After gently mixing, the reaction mixture is refluxed for two hours. Upon completion, the mixture is cooled and then poured into 100 mL of ice-cold water. The resulting orange-colored precipitate is collected, thoroughly washed with water, dried, and finally recrystallized using ethanol. M.P. is 120^0C-130^0C.



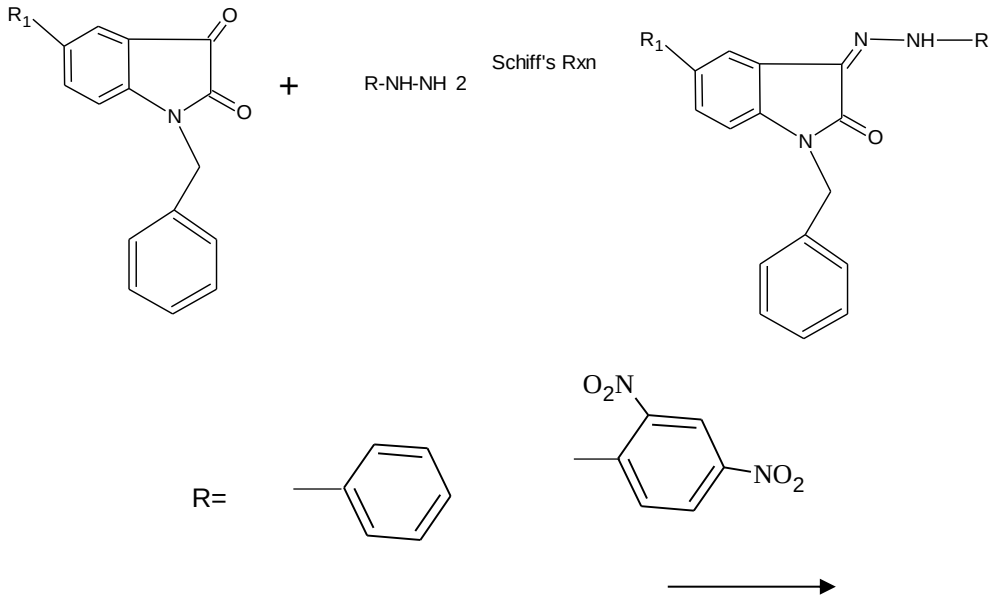
Isatin

Benzyl chloride

R = H, Cl, F, NO_2

5.4 Methods of preparation of Schiff's bases of 5-substituted N-benzyl isatin derivatives:

An equimolar amount of N-benzyl derivatives and amines was combined in 20 ml of absolute ethanol within a 250 ml round-bottom flask. A few drops of glacial acetic acid were then added to the mixture. The reaction mixture was refluxed for 2–3 hours, with periodic monitoring to confirm the completion of the reaction. Upon completion, the mixture was allowed to stand undisturbed for 24 hours, after which it was filtered and the resulting product was recrystallized using ethanol.



5.5. Preliminary Pharmacological Screening:

5.5.1 Protein albumin denaturation method for Anti-inflammatory activity 72,73,74

All the synthesized analogues were assessed for their anti-inflammatory potential through an in vitro method, following the general protocol outlined by Muzushima and Kabayashi with slight modifications. In this assay, the ability of the compounds to prevent albumin denaturation was evaluated. A 10.0 ml reaction system was set up, comprising 0.4 ml of freshly obtained hen's egg albumin, 5.8 ml of phosphate-buffered saline (PBS) adjusted to pH 6.4, and 2.0 ml of the test compound solutions at various concentrations, reaching a final concentration of 50 µg/ml. For the control sample, an equivalent volume of double-distilled water was used in place of the test solution. The reaction mixtures were incubated at 37 ± 2 °C for 15 minutes, followed by heating at 70 °C for 5 minutes. After allowing the samples to cool, the absorbance was recorded

at 660 nm, using the vehicle solution as the blank. Indomethacin (IND), tested at final concentrations of 50 µg/ml and 100 µg/ml, served as the standard and was processed similarly. The inhibition of protein denaturation was calculated using the following formula:

Percentage inhibition = ($Ab_{control} - Ab_{sample}$) X 100/ $Ab_{control}$

$Ab_{control}$ = Absorbance of control

Ab_{sample} = Absorbance of sample

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Chapter 6: Results and discussion

6.6 Results and discussion

The synthesized compounds were analyzed through FT-IR spectroscopy using a KBr disc with a SHIMADZU-8101A infrared spectrometer, along with ^{1}H-NMR spectroscopy in $CDCl_3$. The reliability of the developed method was confirmed by evaluating the physical characteristics of the obtained compounds.

6.1. N-benzyl 3-[(phenylimino)-hydrazono]- isatin (P-01)

Yield 70%, m.p.2600C, Rf value 0.6, I.R (cm-1) C=N1612, C=O1662, C-H 785, C-NH 3088; 1H-NMR (1H) =N-NH δ 12.82, (4-H) δ 7.6, (2 Ar-H) N-CH2 δ 6.6 , UV(λmax,nm) 247,365.

6.2. N-benzyl 3-[(2, 4-dinitrophenylimino)-hydrazono]- isatin (P-02)

Yield 70%, m.p. >3000C Rf value 0.53, I.R (cm-1)C-NO2 1315,C=N1608, C=O1722, C-H 785,C-NH 3325; 1H-NMR (1H) =N-NH δ 12.82, (4-H) δ 7.6, (2 Ar-H) N-CH2 δ 6.6, UV(λmax,nm) 230, 371.

6.3. 3-[(phenylimino)-hydrazono]- isatin (P-03)

Yield 76%,m.p.>2200C,Rf 0.46value, I.R (cm-1) C=N1618, C=O1686, C-H 748,C-NH 3161, 1H-NMR (1H) =N-NH δ 12.7, (4-H) δ 7.8, (4 Ar-H) δ 7.3, UV(λmax,nm) 258, 381.

6.4. 3-[(2,4-dinitro-phenylimino)-hydrazono]-isatin (P-04)

Yield 80%,m.p. >2900C, Rf value0.5, I.R. (cm-1) C-NO2 1313, C=N 1612, C=O1730, C-H 740, C-NH 3316 , 1H-NMR (1H) =N-NH δ 12.15, (4-H) δ 8.5, (2 Ar-H) δ 7.4 , UV(λmax,nm) 254,380.

6.5. N-benzyl 3-[(phenylimino)-hydrazono]-5-Chloro- isatin (P-05)

Yield 40%, m.p. >300^0C Rf value0.7, I.R. ($cm^{-1)}$ C-Cl 640,C=N1676, C=O 1718, C-H 735 , ^{1}H-NMR (1H) =N-NH δ 12.8, (3-H) δ 7.6 , (4 Ar-H) δ 7.3, (2H) N-CH_2 δ 6.6 , UV(λmax,nm) 248,259.

6.6. 3-[(phenylimino)-hydrazono]-5chloro- isatin (P-06)

Yield 70%, m.p. >300^0C Rf value 0.53, I.R ($cm^{-1)}$ C-Cl 633,C=N1618, C=O1678, C-H 754,C-NH 3438, ^{1}H-NMR (1H) =N-NH δ 11.14, (3-H) δ 7.5 , (4 Ar-H) δ 7.3, UV(λmax,nm) 248,259.

6.7. 3-[(2,4-dinitro-phenylimino)-hydrazono]-5Chloro- isatin (P-07)

Yield 80%, m.p. >350^0C Rf value 0.52, I.R ($cm^{-1)}$ C-Cl 493,C=N1613, C=O1733, C-H 721,C-NH 3373 , ^{1}H-NMR (1H) =N-NH δ 9.4, (3-H) δ 8.3, (3 Ar-H) δ 7.3, UV(λmax,nm) 257,370.

6.8. 3-[(phenylimino)-hydrazono]-5 Fluro- isatin (P-08)

Yield 45%, m.p. 270^0C Rf value 0.9, I.R ($cm^{-1)}$ C-F1041 ,C=N 1599, C=O1678, C-H 779,C-NH 3165, ^{1}H-NMR (1H) =N-NH δ 12.7, (3-H) δ 7.6, (4Ar-H) δ 7.3, UV(λmax,nm) 249,385.

6.9. 3-[(2,4-dinitro-phenylimino)-hydrazono]-5 Fluro- isatin (P-09)

Yield 50%, m.p. >350^0C Rf value 0.51, I.R($cm^{-1)}$ C-F 1007, C-NO_2 493,C=N1616, C=O1741, C-H 740, C-NH 3306, ^{1}H-NMR (1H) =N-NH δ 12.7, (3-H) δ 7.6, (2Ar-H) δ 7.3, UV(λmax,nm) 252,360.

6.10. 3-[(phenylimino)-hydrazono]-5 Nitro- isatin (P-10)

Yield 72%, m.p. 300^{0}C Rf value 0.9, I.R(cm^{-1}) C-NO_2 1315, C=N 1593, C=O1682, C-H 758,C-NH 3109, ^{1}H-NMR (1H) =N-NH δ 12.7,(3-H) δ7.6, (2 Ar-H) δ 7.3, UV(λmax,nm) 262,365.

6.11. 3-[(2,4-dinitro-phenylimino)-hydrazono]-5 Nitro- isatin (P-11)

Yield 80%, m.p. >3500C Rf value 0.5, I.R (cm-1) C-NO2 1336,C=N1616, C=O1722, C-H 787,C-NH 3400, 1H-NMR (1H) =N-NH δ 9.4, (3-H) δ 8.5, (3 ArH) δ 7.5, UV(λmax,nm) 271,368.

All the results obtained are expressed statistically as Mean + S.D. by using ONE WAY ANOVA followed by Dunnett's t-test. The Graph Pad Prism 5 was used for the statistical analysis. The compound showing p≤ 0.05 as value of significance considered as active compound biologically. (Table No.1)

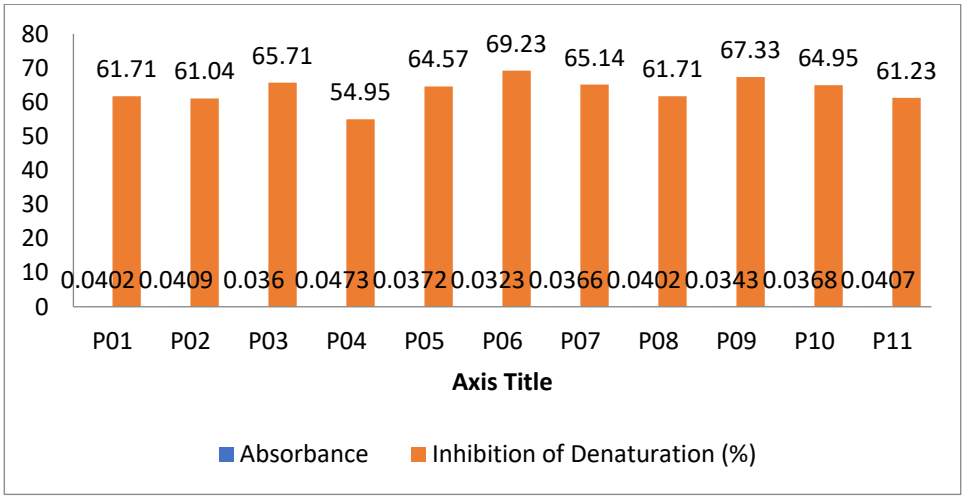
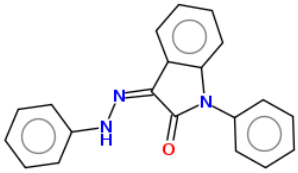


Fig.2 % inhibition by Protein denaturation method
Values are expressed as mean ± S.D. (N=5)
GroupsP01, P06, P09 compared to Indomethacin

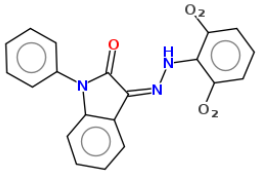
Table no.1: Table No.8.10 In Vitro Anti-inflammatory activity

Sr. No.	Compound	Absorbance[a]	Inhibition of Denaturation (%) (Mean±SD)
1.	P01	0.0402	61.71±0.40
2.	P02	0.0409	61.04±0.25
3.	P03	0.0360	65.71±0.43
4.	P04	0.0473	54.95±0.26
5.	P05	0.0372	64.57±0.41
6.	P06	0.0323	69.23±0.38
7.	P07	0.0366	65.14±1.54
8.	P08	0.0402	61.71±0.36
9.	P09	0.0343	67.33±0.31
10.	P10	0.0368	64.95±1.42
11.	P11	0.0407	61.23±0.25

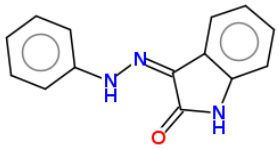
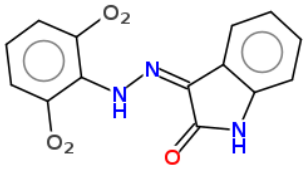
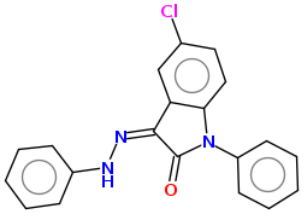
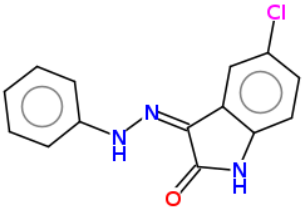
Drug likeness Properties of Some Synthesized Derivatives: (Molinspiration property engine v2022.08)

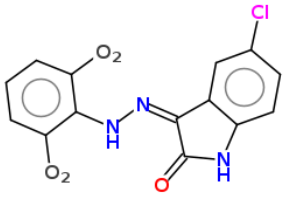
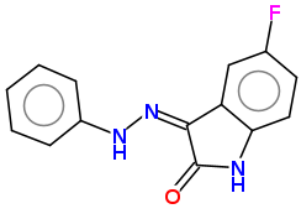
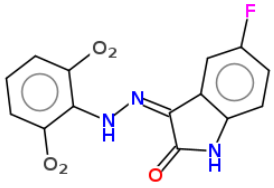
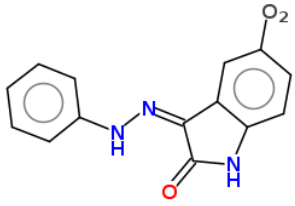


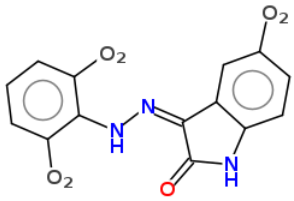
miLogP	4.34
TPSA	46.40
natoms	24
MW	313.36
nON	4
nOHNH	1
nviolations	0
nrotb	3
volume	283.45



miLogP	2.50
TPSA	46.40
natoms	26
MW	311.34

	nON 4 nOHNH 1 nviolations 0 nrotb 3 volume 302.83
	miLogP 3.00 TPSA 57.25 natoms 18 MW 237.26 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 211.66
	miLogP 1.16 TPSA 57.25 natoms 20 MW 235.25 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 231.04
	miLogP 4.99 TPSA 46.40 natoms 25 MW 347.81 nON 4 nOHNH 1 nviolations 0 nrotb 3 volume 296.99
	miLogP 3.65 TPSA 57.25 natoms 19 MW 271.71 nON 4 nOHNH 2 nviolations 0 nrotb 2

	volume 225.19
	miLogP 1.81 TPSA 57.25 natoms 21 MW 269.69 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 244.57
	miLogP 2.17 TPSA 57.25 natoms 19 MW 255.25 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 216.59
	miLogP 0.33 TPSA 57.25 natoms 21 MW 253.24 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 235.97
	miLogP 2.10 TPSA 57.25 natoms 19 MW 236.25 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 221.35

	miLogP 0.26 TPSA 57.25 natoms 21 MW 234.24 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 240.73

6. Conclusion

The literature review highlights the significant role of indoles (heterocyclic compounds) in both naturally occurring substances and synthetic agents, recognizing them as a crucial class with a variety of pharmacological effects, including antimicrobial, antifungal, antitubercular, antiviral, antimalarial, cytotoxic, and anti-inflammatory properties. Inflammation, often accompanied by pain and fever, is the body's nonspecific defense mechanism responding to tissue injury. It is a multifaceted process involving both biochemical and immunological factors. During inflammation, local alterations take precedence, with changes in the connective tissue of the affected area, such as vascular modifications and the formation of fluid exudates. A key event in this process is the accumulation of a large number of phagocytic cells at the site of inflammation. Tissue damage caused by the introduction of a foreign antigen, physical trauma, or exposure to certain chemicals activates a complex inflammatory response. This process can involve fluid stasis and the accumulation of various cellular and non-cellular immune components. Previous studies have demonstrated that diaryl substitutions at the second and third positions exhibit anti-inflammatory effects. Therefore, the synthesis of N-benzyl isatin with a substitution at the third position was undertaken to enhance analgesic and anti-inflammatory activity. A compound featuring N-benzyl-3-substituted isatin was synthesized using an appropriate synthetic pathway and subsequently tested for its analgesic and anti-inflammatory effects.

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