CHAPTER-I

Introduction

1.1. Introduction to $N$-containing heterocyclic compounds and their pharmacological importance:

The present research topic is “Synthesis and Biological Activity of Benzodiazepines, Dihydropyrimidinones, Quinoxalin-amines and Their Derivatives”. In the present research work, our aim is to design and synthesis of the biologically active novel molecules and their basic synthesis, suitable catalyst for commercialization of the process and the investigation on anti-neuroinflammatory studies are important tasks. Chapter-I describes an introduction to 1,5-Benzodiazepines (BDPs), 3,4-Dihydropyrimidin–2-ones (DHPMs), 3,4-Dihydro quinoxalin-2-amine derivatives and their pharmacological importance. We have presented a brief review on the synthesis and biological activity of the above compounds.

Among the various organic compounds heterocyclic compounds (1) are very important in pharmaceutical sectors. These compounds consist at least one carbon atom and at least one element other than carbon, such as nitrogen, sulfur or oxygen within a ring structure. Among all the heterocyclic compounds, $N$-containing heterocyclic compounds are used more widely because they have been proven to possess high biological activity. Pyridine is an example for simple heterocyclic compound. $N$-Containing hetero compounds with two ‘N’ in six
membered rings are called Diazines and in seven membered rings are called Diazepine.

A number of $N$-Containing Heterocyclic compounds possess a wide spectrum of applications in various fields like medicine, industry, pharmacology and analytical chemistry. Some of this derivatives also acts as antibacterial, anticancer, antimicrobial, antiviral, antihelmenthetic, insecticidal, and Herbicidal compounds. These are important class of bioactive molecules and widely used as anticonvulsant, antifungal, antitubercular, antimalarial, anti-hypertensive and anti-inflammatory agents. Some of the examples are Omeprazole, EsOmeprazole, Lansomeprazole, Olanazopine, and Temocapril.

**Pharmacological importance of some $N$-containing heterocyclic compounds:** The presence of $N$-containing heterocyclic moiety in drugs has been approved for human therapeutic applications. Various examples of $N$-containing heterocyclic moiety containing compounds along with their pharmacological activity are listed in Table.1.1.
Table 1.1. Pharmacological activity of some N-containing heterocyclic compounds:

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Class</th>
<th>Generic name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antibacterial</td>
<td>Aztreonam, Cefoxitin, Cefepime, Cefotan, Cephalosporins, Meropenem, Pencillin, Vancomycin</td>
</tr>
<tr>
<td>2</td>
<td>Anticancer</td>
<td>Actinomycin, Capacitabine, Carboplatin, Docetaxel, Gemcitabine, Imatinib, Mitomycin, Doxorubicin Liposomal,</td>
</tr>
<tr>
<td>3</td>
<td>Anticonvulsant</td>
<td>Carbamazepine, Clonazepam, Diazepam, Felbamate, Paramethadione</td>
</tr>
<tr>
<td>4</td>
<td>Antifungal</td>
<td>Amphotericin, Caspofungin, Flucytosine, Griseofluvin, Itraconazole, Isconazole, Ketoconazole, Nystatin, Terbinafine, Voriconazole</td>
</tr>
<tr>
<td>5</td>
<td>Antihypertensive</td>
<td>Amlodipine, Candesartan, Captopril, Diltiazem, Nifedipine, Temocapril</td>
</tr>
<tr>
<td>6</td>
<td>Antiinflammatory</td>
<td>Aspirin, Ibuprofen, Paracetamol</td>
</tr>
<tr>
<td>7</td>
<td>Antimalarial</td>
<td>Artemisinin &amp; derivatives, Chloroquine, Clindamycin, Hydroxy chloroquine, Pyrimethamine, Quinine &amp; related agents</td>
</tr>
<tr>
<td>8</td>
<td>Antiviral</td>
<td>Acyclovir, Lamivudine, Oseltanvir (Tamiflu), Zanamivir, Zidovudine</td>
</tr>
</tbody>
</table>

Among the different N-containing heterocyclic compounds, 1,5-Benzodiazepines, 3,4-Dihydropyrimidinones and 3,4-Dihydro
quinoxalin-2-amine derivatives are exhibiting the prominent role in the drugs and pharmaceutical fields.

1.2. Introduction to benzodiazepines:

A benzodiazepine (2) is an example for psychoactive drug. In 1930 Austrian scientist Leo Sternback was discovered benzodiazepines while working for the Roche Company, but not known until Hoffman launched Librium© in 1957. Chlordiazepoxide (Librium) was discovered in 1954 by the Leo Sternbach which is used to cure anxiety. Experimental results revealed that the compound had anxiolytic, hypnotic, and muscle relaxant effects. Later it was marketed as Librium in the market and many benzodiazepine compounds further which were introduced.8

The search for new and efficient methods for the synthesis of pure compounds has been an active area of research in organic synthesis. We herein highlight only some selected important reactions of benzodiazepines with different reagents and under different reaction conditions as reported by different authors.9 Different reagents such as BF₃-etherate, polyphosphoric acid, NaBH₄, MgO/POCl₃, Yb(OTf)₃, Ga(OTf)₃, lead nitrate, L-proline, acetic acid under microwave conditions, molecular iodine, and in ionic liquids have been also used for the synthesis of benzodiazepines.⁹a-⁹k Recently the synthesis of benzodiazepines was also reported using different solid acid catalysts.
such as sulfated zirconia, $\text{Al}_2\text{O}_3/\text{P}_2\text{O}_5$, $\text{Ag}_3\text{PW}_{12}\text{O}_{40}$, PVP-$\text{FeCl}_3$, and zeolite catalysts.\textsuperscript{9l-9p} To the best of our knowledge, nobody reported on the synthesis of benzodiazepines using silica gel supported sulfuric acid and AlKIT-5 materials as catalysts in the open literature so far.

Benzodiazepines are bicyclic heterocyclic compounds possessing a benzene nucleus fused to a seven-member ring containing two nitrogen atoms at different positions in the ring, and thus have been classified into six groups depending on the position of nitrogen atom in the seven member ring. Many modifications and substitutions have been made in both rings by employing different methods to obtain a large number of compounds of varied types of biological activities. The information regarding their chemical, biological importance and drugs based on the skeleton of benzodiazepines is scattered in the literature.\textsuperscript{9q}

The benzodiazepines are a class of psychoactive drugs considered minor tranquilizers with varying hypnotic, anxiolytic, anticonvulsant, muscle relaxant and sedative properties.\textsuperscript{10} Benzodiazepines are used to control alcohol withdrawal, insomnia, agitation, anxiety, seizures, and muscle spasms. These BDPs also can be used to control the tension and anxiety before certain medical procedures such as or dental work or endoscopies.\textsuperscript{11} BDPs acts as sedatives in low doses and as hypotics in high doses.
1.3. Pharmacological importance of Benzodiazepines:

BDPs are safe, effective drugs, good therapeutics, potent anticonvulsants and possessing life-saving and anti-anxiety properties. BDPs can be administrated via ingestion, in tablet or capsule form. Librium©, Ativan©, and Valium©, are sometimes dispensed intravenously, but midazolam© is used exclusively as an intravenous medication. Ativan© used as tablet and which can be dissolved under the tongue.

Mechanism of action: BDPs affect a key neurotransmitter in the brain called gamma-amino butyric acid (GABA). GABA has an inhibitory effect on motor neurons, thus the presence of GABA slows or stops neuronal activity. BDPs can enhance the activity of GABA, effectively slowing nerve impulses throughout the body. Benzodiazepine receptors are two types in the human nervous system one causes the anti-anxiety effect, and another one elicits the sedative effect.

The BDPs can control the depressing to stimulating properties of the central nervous system via GABA\textsubscript{A} receptor, which is prevalent inhibitory receptor within the brain. BDPs binds with the subset of GABA\textsubscript{A} receptors are called as Benzodiazepine receptors (BzR). The GABA\textsubscript{A} receptor consist of five subunits, and these are two α's, two β's, and one γ (α₂β₂γ) and these are further divided into α\textsubscript{1-6}, β\textsubscript{1-3}, and γ\textsubscript{1-3}. GABA\textsubscript{A} receptors have different properties with different activities
with respect to clinical and pharmacological effects. BDPs bind on the GABA$_A$ receptor at the interface of α and γ subunits. The alpha subunits with a histidine amino acid residue, (i.e., α$_{1-3}$, and α$_5$ containing GABA$_A$ receptors) is required for the binding of BDPs. BDPs are not binding with the GABA$_A$ receptors containing α$_4$ and α$_6$ subunits, which contain an arginine instead of a histidine residue. The GABA$_A$ receptor other sites also bind with neurosteroids, barbiturates and certain anesthetics.$^{12}$ The benzodiazepine ligand locks the BzR with higher affinity.

Different BDPs can have different affinities for BzRs depends on different types of subunits. For example, BDPs with high activity at the α$_1$ are associated with sedation, with higher affinity for GABA$_A$ receptors containing α$_2$ and α$_3$ subunits have good anti-anxiety activity.$^{13}$ BDPs also bind to glial cell membranes.$^{14}$

Some of the BDPs are full BzR agonists, producing anxiolytic and sedating properties. Compounds with inverse agonists showed potent convulsant activities. Compounds that inhibit the binding of agonists to the receptor are called BzR antagonists. Some compounds are exhibiting either partial agonists or partial antagonists.$^{15}$ BDPs anticonvulsant properties may be due to binding to sodium channels rather than benzodiazepine receptors.$^{16}$ Peripheral benzodiazepine receptors (PBRs) also can be seen in non nervous-system tissues. These PBRs are not attached to GABA$_A$ receptors. PBRs are found in various tissues such as liver, heart, adrenal, testis, lymphatic and
Peripheral benzodiazepine receptor functions on immune cells are oxidative bursts by neutrophils, inhibition of macrophage secretion of cytokines, and inhibition of the proliferation of lymphoid cells.\textsuperscript{19}

\textbf{Table 1.2. Some of the different benzodiazepine drugs by class:}

<table>
<thead>
<tr>
<th>S.No</th>
<th>By class</th>
<th>Generic name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti convulsant\textsuperscript{20}</td>
<td>Clobazam, Clonazepam, Clorazepate, Diazepam, Lorazepam, Midazolam, Carbaazepine</td>
</tr>
<tr>
<td>2</td>
<td>Anxiolytics\textsuperscript{21}</td>
<td>Alprazolam, Bromazepam, Chlordiazepoxide, Clonazepam, Diazepam, Lorazepam, Medazepam, Nordazepam, Oxazepam, Prazepam, Temazepam</td>
</tr>
<tr>
<td>3</td>
<td>Insomnia\textsuperscript{22}</td>
<td>Diazepam, Brotizolam, Flunitrazepam, Estazolam, Loprazolam, Phenazepam, Lormetazepam, Nitrazepam, Triazolam, Midazolam, Nimetazepam, Temazepam</td>
</tr>
<tr>
<td>4</td>
<td>Muscular disorder\textsuperscript{23}</td>
<td>Clonazepam, Lorazepam, Temazepam</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol dependence\textsuperscript{24}</td>
<td>Diazepam and Chlordiazoxide</td>
</tr>
<tr>
<td>6</td>
<td>Acute mania\textsuperscript{25}</td>
<td>Clonazepam, Lorazepam</td>
</tr>
</tbody>
</table>

Some of the commonly used benzodiazepine derivatives as drugs are Alprazolam, Bromazepam, Clobazam, Clonazepam, Diazepam, Estazolam, Flunitrazepam, Flumazenil, Loprazolam, Midazolam,
Nitrazepam, Oxazepam, Prazepam, Triazolam and Temazepam. Among all the drugs Clobazam and Clonazepam are used to treat epilepsy, Nitrazepam and Temazepam are powerful anticonvulsant agents. Lorazepam and diazepam are used for seizure control.
Structures of some benzodiazepine derivatives as drugs: The benzodiazepine (BDP) structural motifs are present in various compounds with established biological activity, some of which are commercially available drugs.
BDPs medications are short-acting and long-acting which differ from each other in how fast they take effect and how long the effects last. A short-acting benzodiazepine is cleared from the body in a short period of time, whereas long-acting benzodiazepines may either accumulate in the bloodstream or take a much longer period of time to leave the body. These BDPs administrated based on dependence, potential for tolerance and abuse. Shorter-acting BDPs used to manage insomnia, include flurazepam, estazolam, temazepam, quazepam and triazolam. BDPs with longer durations of action include clorazepate, alprazolam, diazepam, chlordiazepoxide, lorazepam, halazepam, prazepam and oxazepam. Flumazenil has an imidazobenzodiazepine structure and which is a benzodiazepine receptor antagonist. Flumazenil chemical name is ethyl 8-fluoro-5,6-dihydro -5-methyl -6-oxo-4H-imidazo [1,5-a](1,4) benzodiazepine-3-carboxylate.

1.4. Introduction to Dihydropyrimidin–2-ones (DHPMs):

Heterocyclic aromatic compound, Pyrimidine (18) is similar to benzene and pyridine, which consist of two nitrogen atoms at 1 and 3 positions of the six-membered ring. Pyrimidine is isomeric with two other forms of diazine. Other names are 1,3-diazen, \( m \)-diazen. Pyrimidiones are derivatives of pyrimidine which have at least one additional keto (\( =O \)) group. They are the basis of many other biological molecules, including: Barbiturates, such as metharbital (19) and Nucleobases, such as cytosine (20).
The major nucleic acid bases are five, Out of the five major bases three are pyrimidine derivatives such as Cytosine (20) which is found in DNA and RNA, Uracil (21) in RNA and Thymine (22) in DNA. Since these bases are playing an important role in DNA and RNA, the synthesis of these derivatives is very important task for synthetic organic chemists. Hence the Polyfunctionalized dihydropyrimidines (DHPMs) synthesis is challenging and an important task for organic chemists.

Pyrimidines or pyrimidinones can be prepared within the laboratory using different methods. Among the different methods Biginelli method is most popular one.26 The other methods are rely on condensation of carbonyls with amines for example the synthesis of 2-Thio-6-methyluracil using ethylacetoacetate and thiourea.27

The Biginelli reaction28,29,30,41a is an acid-catalyzed, three-component reaction between a β-ketoester 23, an aldehyde 24 and urea 25 constitutes a rapid and facile synthesis of dihydropyrimidinones 26, which are interesting compounds with a potential for pharmaceutical application.
In 1891, Pietro Biginelli developed this reaction and which can be catalyzed by Bronsted acids or by Lewis acids such as boron trifluoride.31 Several workers are published Biginelli compounds using solid-phase protocols.32-33 DHPMs are widely used in the pharmaceutical industry as calcium channel blockers,34 anti-hypertensive agents and α<sub>1a</sub>-antagonists. The mechanism involves the series of bimolecular reactions which leads for the formation of dihydropyrimidinone.35

Sweet in 1973 proposed the mechanism, which is the aldol condensation of ethylacetoacetate 27 with aryl aldehyde 28 which forms the carbenium ion 29. Then urea was interacted by nucleophilic addition to give the intermediate 30, which dehydrates to give the final desired product 31.36
Biginelli reaction often gives only moderate yields, in particular when aliphatic or ortho-substituted aromatic aldehydes are employed. In 1987, Atwal et al. reported a modification to the Biginelli reaction that consistently generated higher yields.

In 1997, Kappe modified Sweet mechanism and the first step begins with rate determining nucleophilic addition by the urea to the aldehyde. The condensation step is catalyzed by the addition of acid, resulting in the formation of imine nitrogen (electrophile). The β-ketoester then adds to the imine bond and consequently the ring is closed by the nucleophilic attack by the amine on to the carbonyl group. This final step completes second condensation and results in the formation of Biginelli product.
Dihydropyrimidinones (DHPMs) are well known for their wide range of bioactivities and their applications in the field of pharmaceutical research. The first synthesis of dihydropyrimidinones was reported by Biginelli in 1893. Later the multifunctionalized dihydropyrimidines or Biginelli compounds synthesis was drastically increased since its heterocyclic system of remarkable pharmacological efficiency. Since then several reviews on synthesis and chemical properties of pyrimidinones have been published.

The search for new and efficient methods for the synthesis of pure compounds has been an active area of research in organic synthesis. We herein highlight only some selected important reactions of DHPMs with different reagents and under different reaction conditions as reported by different authors. Several workers have been reported the synthesis of DHPMs including classical conditions with microwave irradiation and by using Lewis acids as well as protic acids as promoters such as Conc.HCl, BF₃.OEt₂, PPE, KSF clay, InCl₃, LaCl₃, lanthanide triflate, H₂SO₄, ceric ammonium nitrate (CAN), Mn(OAc)₃, ion-exchange resin, 1-n-butyl-3-methylimidazolium tetrafluoroborate (BMIImBF₄), BiCl₃, LiClO₄, InBr₃, FeCl₃, ZrCl₄, Cu(OTf)₂, Bi(OTf)₃, LiBr, ytterbium triflates, NH₄Cl, MgBr₂, SiO₂/NaHSO₄, DDQ, and other reagents have been found to be effective. However, this so-called
Biginelli reaction often suffers from low yields practically in case of substituted aromatic and aliphatic aldehydes. Even though high yields could be achieved by following complex multi-step procedures, these methods lack the simplicity of original one-pot Biginelli protocol. Therefore, Biginelli reaction continues to attract the attention of researchers for the discovery of milder and efficient procedures for the synthesis of dihydropyrimidinones. Unfortunately, there has been no report available on the synthesis of DHPMs using DDQ and AlKIT-5 materials as catalyst in the open literature so far.

1.5. Pharmacological importance of dihydropyrimidin-2-ones (DHPMs):

Biginelli compounds exhibits a diverse range of biological activities. Polyfunctionalized dihydropyrimidinones (DHPMs) represent a heterocyclic system of remarkable pharmacological efficiency and many exhibit anti-viral,\textsuperscript{43a} anti-tumor,\textsuperscript{41c,f} anti-bacterial,\textsuperscript{43b} and anti-inflammatory\textsuperscript{18c} properties. Aryl-substituted 3,4-dihydropyrimidin-2(1H)-one and their derivatives are important class of substances in organic and medicinal chemistry.

As early as 1930 simple derivatives such as 38 were patented as agents for the protection of wool against moths.\textsuperscript{44} Later, nittractin 39, was developed for antiviral activity,\textsuperscript{43a} which has excellent activity against the viruses of the trachoma group.\textsuperscript{45} The same compound 39 also exhibits modest antibacterial activity.\textsuperscript{43b}
Dihydropyrimidinone 37 and some of its analogs were screened as antitumor agents and found to be active against e.g. Walker carcinosarcoma in rats and mice.\textsuperscript{46} Pyrimidine-5-carboxamides of type 40 are reported to possess anti-carcinogenic activity.\textsuperscript{47} Anti-inflammatory,\textsuperscript{43c} analgesic,\textsuperscript{43c} and blood platelet aggregation inhibitory activity\textsuperscript{48} was found in a number of derivatives. 1,4-Dihydropyrimidine 41 is useful as platelet-activating factor antagonist.\textsuperscript{49} Other Biginelli compounds were shown to inhibit the uptake of adenosine by thrombocytes.\textsuperscript{50}

Since 1986 the number of publications and patents dealing with the cardiovascular activity of dihydropyrimidines has grown rapidly. Simple modifications of the aromatic ring are reported to give substances with only moderate cardiovascular activity, e.g. 38,\textsuperscript{51} 43,\textsuperscript{52} or 44.\textsuperscript{53} S-Alkyl-1,4-dihydropyrimidines, e.g. 45, were shown to be potent calcium channel blockers,\textsuperscript{54} but they do not show any significant antihypertensive activity in \textit{vivo}.\textsuperscript{54}
Among the most potent derivatives are Biginelli compounds bearing an ester group at N-3 (46), thereby closely resembling the nifedipine (42) structure.\(^5\) Further modification of the substituent at N-3 finally led to the development of orally effective long-lasting antihypertensive agents 47.\(^6\) Resolution of enantiomers of 47 demonstrated that the desired biological activity solely resides in the (R)-enantiomer, which is similar in potency and duration to the long-lasting dihydropyrididine, amlodipine 48.\(^6\)

A general structure-activity relationship of dihydropyrimidine calcium channel blockers has been presented.\(^3\) Apart from their use as antihypertensive agents, dihydropyrimidine calcium channel blockers are also of interest as agents for treating anxiety\(^5\) and optic nerve dysfunction.\(^5\)

Some of the DHPMs are acting as drugs. These are Monastrol, (R)-SQ 32,926, Nitractin, Capacitabine, Lamivudine, Zidovudine,

**Structures of some of the dihydropyrimidinones as drugs:**

The dihydropyrimidinone (DHPM) structural motifs are present in various compounds with established biological activity, some of which are commercially available drugs.
**Monastrol (49):** 4-(3-hydroxyphenyl)-2-thione derivative **49** called monastrol\(^{59a}\) as a novel cell-permeable, mitosis inhibitor molecule for the development of new anticancer drugs. Monastrol **49** has been identified as a compound that specifically affects the cell-division (mitosis) by a new mechanism which does not involve tubulin targeting. It was discovered by Stuart Schreiber and Tim Mitchison. Monastrol was shown to inhibit kinesin-5, a motor protein. Monastrol binds to a long loop that is specific to the Eg5 kinesin family, and allosterically inhibits ATPase activity of the kinesin.\(^{59b}\)

**(R)-SQ 32926 (50):** A practical and short synthesis of the enantiomerically pure dihydropyrimidone antihypertensive agent (R)-SQ 32926.\(^{60}\)

**Nitractin (39):** Biginelli compounds leading to the development of nitractin that has excellent activity against the virus of trachoma group\(^{45}\) the same compounds also exhibit antibacterial activity.

**Lamivudine (51):** (2',3'-dideoxy-3'-thiacytidine). The common name is 3TC, which are reverse transcriptase inhibitor (nRTI) and a potent nucleoside analog. Lamivudine is cytidine analogue can inhibit HIV reverse transcriptase (I&II types). DNA polymerase was inhibited by the triphosphate form 3TC. The lower dose of Lamivudine\(^{61}\) has been used for treatment of chronic hepatitis B than for treatment of HIV. The seroconversion of e-antigen positive hepatitis B, the liver histology staging can be improved by this drug. 3TC, is administered orally, which can rapidly absorbed and can cross the blood-brain barrier.
Lamivudine is taken along with zidovudine combination, which is highly synergistic.

**Zidovudine (52) or azidothymidine (AZT):** Zidovudine is a thymidine nucleoside analogue; reverse transcriptase inhibitor (NRTI), an antiretroviral drug used for the treatment of HIV, sold as Retrovis and Retrovir. AZT use was a major breakthrough in AIDS therapy in the 1990s that significantly altered the course of the illness and helped destroy the notion that HIV/AIDS was an instant death sentence. AZT slows HIV spread significantly, but does not stop it entirely. This allows HIV to become AZT resistant over time, and for this reason AZT is usually used in conjunction with other NRTIs and anti-viral drugs. In this form, AZT is used as an ingredient in Combivir and Trizivir, among others.

**Telbivudine (53):** Telbivudine is a synthetic thymidine nucleoside analogue; it is the L-isomer of thymidine and which is an antiviral drug used for the treatment of hepatitis B infection. Clinical trials have shown it to be significantly more effective than lamivudine or adefovir, and less likely to cause resistance.\(^{62}\)

**Apricitabine (54):** It is an experimental nucleoside reverse transcriptase inhibitor (NRTI) against HIV. It is structurally related to lamivudine and emtricitabine, and like these, is an analogue of cytidine.
**Capecitabine (55):** Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. It is a prodrug, that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. Activation of capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR), to form 5-fluorouracil.

**Emtricitabine (56):** It is used for the treatment of HIV infection in adults and children. It is a nucleoside reverse transcriptase inhibitor (NRTI). It is also sold in a fixed-dose combination with tenofovir under the name Truvada. A fixed-dose triple combination of tenofovir, emtricitabine, and efavirenz was approved by the U.S. Food and Drug Administration under the brand name Atripla.

**Icilin (57):** Icilin is a synthetic super-agonist of the transient receptor potential M8 ion channel. Although structurally not related with menthol it produces an extreme sensation of cold both in humans and animals. It is almost 200 times more potent than menthol and 2.5 times more efficacious. Despite their similar effects icilin activates the TRPM8 receptor in a different way. Icilin is effective in an experimental model of itch. Recently it is used as a research tool for the study of TRP channels.
**Lopinavir (58):** It is an antiretroviral of the protease inhibitor class.\(^{65a, b}\) It is sold by Abott laboratories as Kaletra (high-income countries) and Aluvia (low-income countries), both of which represent a co-formulation with a sub-therapeutic dose of ritonavir, as a component of combination therapy to treat AIDS/HIV. The Kaletra formulation has also been used successfully as monotherapy in some studies. As of 2006, lopinavir/ritonavir forms part of the preferred combination for first-line therapy recommended by U.S. Department of Health and Human services (DHHS).\(^{65c}\) It is available as capsules, tablets and oral solution.

**1.6. Introduction to quinoxaline derivatives:** Quinoxaline (59), is also called as benzopyrazine, which is heterocyclic compound contains pyrazine ring and benzene ring. They are similar like quinazolines. Other names are Benzoparadiazine, 1,4-Benzodiazine, Phenopiazine, Phenpiaze, Quinazine, Chinoxalin. Among the various classes of \(N\)-containing heterocyclic compounds, the synthesis and chemistry of quinoxalines having much attention from last 10 years.\(^{66, 67}\) Quinoxalines display a broad spectrum of biological activity.\(^{68, 69, 70}\) This has contributed to their usefulness in combinatorial drug discovery libraries.\(^{71–76}\) Quinoxalines plays a significant role as a basic skeleton for the design of different antibiotics such as echinomycin, actinomycin, and levomycin. It has been reported that these compounds inhibit the growth of gram-positive bacteria, and are
active against various transplantable tumors.\textsuperscript{77,78} The quinoxaline ring is also a constituent of many pharmacologically and biologically active compounds such as insecticides, fungicides, herbicides, and anthelminitics.\textsuperscript{79,80}

Multicomponent Reactions\textsuperscript{81} (MCRs) are different type of reaction, in which 2 or more reactants react to form final compound, where basically all the reactants or most of the atoms contribute to the newly formed compound. The resulting compound is clearly dependent on the reaction conditions such as temperature, solvent, concentration, catalyst, kind of starting reactants, and functional groups. Such considerations are of particular importance in correlation with the propose and innovation of novel MCRs. In recent years, MCRs have become important tools in modern preparative synthetic chemistry. MCRs have emerged as valuable tools for the preparation of structurally diverse chemical libraries of drug-like heterocyclic compounds.\textsuperscript{82-84} Isocyanide-based MCRs\textsuperscript{81} are especially important in this area. The MCR of cyclohexyl isocyanide, an aromatic aldehyde and \textit{o}-phenylenediamine in the presence of a catalytic amount of ferric perchlorate in acetonitrile was complete within 2 h to afford \textit{N}-cyclohexyl-3-aryl-quinoxaline-2-amines in good yields.

A number of synthetic strategies have been reported for the synthesis of quinoxaline derivatives.\textsuperscript{85-90} The mainly used method is the condensation of an aryl 1,2-diamine with a 1,2-dicarbonyl compound in refluxing ethanol or acetic acid for 2–12 h, and this
typically gives yields of 34–70%. They can be formed by condensing o-diamines with 1,2-diketones. The main substance of the group, quinoxaline, results when glyoxal is condensed with 1,2-diaminobenzene. Substituted derivatives are formed when α-chloroketones, α-ketonic acids, and α-aldehyde alcohols are used in place of diketones. In one report, 2-iodoxybenzoic acid (IBX) used as a catalyst in the reaction of benzil with 1,2-diaminobenzene. Unfortunately, the quinoxaline amine derivatives are not much reported in the literature so far.

1.7. Pharmacological importance of quinoxaline derivatives:

Some of the quinoxalines exhibit a wide variety of biological activities including anti-viral, anti-bacterial, anti-inflammatory, antiprotozoal, anti-cancer (colon cancer therapies), anti-depressant, anti-HIV, kinase inhibitors, and anti-tumoral. These are used in the field of agriculture as herbicides, fungicides and insecticides. Also some antibiotics like echinomycin, actinoleutin and levomycin having quinoxaline moiety, which are known to inhibit the growth of gram positive bacteria and they are active against various transplantable tumors. And also, quinoxaline derivatives are used in dyes, efficient electron luminescent materials, organic semi conductors, chemically controllable switches, building blocks for the synthesis of anion receptors, cavitands, and dehydroannulenes. They also serve as useful rigid subunits in macrocyclic receptors in molecular recognition.
Some of the quinoxalines as drugs:

Some of the quinoxalines as drugs are tetracycline, oxytetracycline, chlortetracycline, oleomphocycline, monomycin, kanamycin, neomycin, polymyxin, erythromycin, streptomycin, levomycin, sintomycin, penicillin, bicillin-3, spectomycin, furazolidone, furidin.

Structures of some of the quinoxalines as drugs:

The quinoxaline structural motifs are present in various compounds with established biological activity, some of which are commercially available drugs.
Echinomycin\textsuperscript{106} (60): It is a peptide antibiotic. It intercalates into DNA at two specific sites, thereby blocking the binding of hypoxia inducible factor 1 alpha (HIF1alpha).

Brimonidine\textsuperscript{107} (61): It is a drug used to treat open-angle glaucoma or ocular hypertension. Brand name for brimonidine is Alphagan, also used to induce miosis for people suffering from poor night vision after Lasik or PRK surgery.
Varenicline\textsuperscript{108 (62)}: It is a prescription medication used to treat smoking addiction. Varenicline is a nicotinic receptor partial agonist.

Licostin\textsuperscript{109 (63)}: It is an excitatory amino acid antagonists. Licostin, ACEA 1021, is an anionic NMDA antagonist for the glycine site that is being developed for cerebral protection in stroke.

Quinoxin\textsuperscript{110 (64)}: It is a new growth-promoting agent.

Cyadox\textsuperscript{111 (65)}: It is a veterinary drug mainly used as an effective antimicrobial promoter in animal husbandry. It was reported that the other quinoxaline-1,4-dioxide compounds had phototoxicity, but only few data are reported on phototoxicity of cyadox. It was performed to evaluate the phototoxicity of cyadox on skin. Severe erythema, oedema and necrosis of auricles were observed on olaquindox group with irradiation. The phototoxicity of cyadox was mild and reversible, which demonstrated a good safety profile of cyadox in terms of phototoxicity.

Olaquindox\textsuperscript{112 (66)}: It is an antibiotic belonging to the quinoxaline group. Some antibiotics and synthetic chemotherapeutics of quinoxaline and nitrofuran groups have a practical value among the agents stimulating weight gain and feeding efficiency of farm animals commonly referred to as nutritive, ergotropic agents or growth promoters.

Carbadox\textsuperscript{113 (67)}: It is a drug that combats bacterial infection in swine, particularly swine dysentary.

Caroverine\textsuperscript{114 (68)}: It is a drug used as a spasmylytic and \textit{otoneuroprotective} (inner ear protective) agent. It acts as an \textit{N}-type
calcium channel blocker, competitive AMPA receptor antagonist, and non-competitive NMDA receptor antagonist.\textsuperscript{114} It also has potent antioxidant effects.

**Panadiplon\textsuperscript{115} (69):** It is an anxiolytic drug with a novel chemical structure that is not closely related to other drugs of this type. It has a similar pharmacological profile to the benzodiazepine family of drugs, but with mainly anxiolytic properties and relatively little sedative or amnestic effect, and so is classified as a nonbenzodiazepine anxiolytic.\textsuperscript{115}

Panadiplon acts as a high-affinity GABA\textsubscript{A} receptor partial agonist,\textsuperscript{116} but despite showing a useful effects profile of a potent anxiolytic with little sedative effects, panadiplon was discontinued from clinical development for use in humans after showing evidence of liver damage in both animals and human trials.\textsuperscript{117} Panadiplon however continues to be used in animal research, mainly as a subtype-selective reference drug to compare other GABA\textsubscript{A} agonists against.\textsuperscript{118}

**Triostin A\textsuperscript{119}:** It is used as a antibacterial agent. Its structure is same as echnomycin. Triostin antibiotics, having a cyclic peptide with disulphide bridge, have been prepared by growing Streptomyces triostinicus in the presence of inorganic [35S]-sulphate. Triostin A has been shown to behave in all respects similarly to the authentic natural product and to enable a much more sensitive radiochemical adaptation of the solvent-partition method for determining antibiotic binding to DNA. By this means, binding isotherms at low, biologically
relevant levels (down to one antibiotic molecule per gene) have been measured.

Since these quinoxalines are pharmacologically active, we have synthesized novel quinoxalines and studied the antineuro-inflammatory activity for these compounds.

1.8. Literature survey on biological activity of \(N\)-containing heterocyclic compounds such as Benzodiazepines, Dihydropyrimidinones, Quinoxalin-amines and Their Derivatives:

1.8.1. Introduction: Many \(N\)-Containing heterocyclic compounds are widely acting as drugs. Among those BDPS, DHPMs and quinoxaline derivatives are playing very predominant role in pharmaceutical industry. In the present work, the anti-neuroinflammatory effect for all synthesized \(N\)-containing heterocyclic compounds is performed. We have reported a new class of \(N\)-containing heterocyclic compounds that showing the anti-neuroinflammatory activity in microglia cells.

Microglia is one kind of glial cells that are the resident macrophages of the brain and spinal cord and the main function is immune defense in the central nervous system (CNS). Microglia constitutes 20% of the total glial cell population within the brain. Microglia (astrocytes) are distributed throughout the brain and spinal cord\(^{120,121}\). Microglial cells within the CNS involves into various tasks like immune response and maintaining homeostasis. Microglia are regularly excavating the CNS for damaged neurons, infectious agents,
and plaques.\textsuperscript{122} The spinal cord and brain are very important and called "immune privileged" organs and these two are separated by the blood-brain barrier, which prevents most infections from reaching the vulnerable nervous tissue. Infectious agents will damage the sensitive neural tissue and Microglial cells will not allow the infectious agents into brain or cross the blood-brain barrier. Microglia is extremely sensitive to even small pathological changes in the CNS.\textsuperscript{123} Some of the antibodies cross the blood brain barrier due to their large size, in such case microglia must be able to recognize foreign bodies, swallow them, and act as antigen-presenting cells or activating T-cells.

**History:** During histology or cell staining experiments in the 1880s microglial cells were discovered by Franz Nissl and F. Robertson and showed those microglia’s are related to macrophages. Victor Babeş noted the activation of microglia and formation of ramified microglial clusters and he found variety of viral brain infections.\textsuperscript{124} Pio del Rio-Hortega, first named the cells “microglia” in 1920. So that Rio-Hortega recognized as the “Father of Microglia.” \textsuperscript{125,126} Later, in 1988, Hickey and Kimura showed that perivascular microglial cells are bone-marrow derived, and express high levels of MHC class II proteins used for antigen presentation.

**Origin:** Microglial cells differentiate in the bone marrow from hematopoietic stem cells, the progenitors of all blood cells. During hematopoiesis process, some of the stem cells differentiate into monocytes and travel from the bone marrow to the brain, and further
differentiate into microglia. Monocytes also can differentiate in the peripheral systems into macrophages and myeloid dendritic cells.

Microglia use phagocytic and cytotoxic mechanisms to destroy foreign materials like macrophages in the body. Microglia and macrophages responsible for immune response and can act as antigen presenting cells, as well as promoting inflammation and homeostatic mechanisms within the body by secreting cytokines and other signaling molecules. Microglia in their downregulated form, lack the MHC class I/MHC class II proteins, IFN-γ cytokines, CD45 antigens, and other receptors required to act in the antigen-presenting, phagocytic and cytotoxic roles that hallmark normal macrophages. The microglia continuously and rapidly proliferate in order to increase their numbers. Some times the blood-brain barrier will be weaken, due to extreme infection, and microglia will be replaced with haematogenous, bone-marrow derived cells, called myeloid progenitor cells and macrophages. After infection has decreased then microglia will play good role for the recovery and regrowth.

**Types:** Microglial cells are extremely plastic, and plasticity is required to fulfill the vast variety of immunological functions that microglia perform, as well as to maintain homeostasis within the brain.

**Ameboid:** This type of microglial are scavenger cells and found in “Fountains of Microglia.” Ameboid shape microglial can move freely throughout the neural tissue and able to phagocytose debris, but do not fulfill the same antigen-presenting and inflammatory roles as
Activated microglia. Ameboid microglia are especially prevalent during the rewiring and development of the brain. ¹²²,¹³⁰,¹³¹

**Ramified:** This type of microglial cell is commonly found at strategic locations throughout the entire brain and spinal cord in the absence of foreign material or dying cells. This “resting” form of microglia is composed of a small cellular body and long branching processes. The branches are very sensitive to small changes and are constantly moving and surveying the surrounding area. The purpose of constantly moving is to detect and fight infection. ¹²⁸,¹³¹

**Activated:** Microglia move from their ramified form to active phagocytic form and can be activated by a variety of factors such as glutamate receptor agonists, pro-inflammatory cytokines, cell necrosis factors, lipopolysaccharide, and changes in extracellular potassium (indicative of ruptured cells). Activated microglia can involve in the various functions such as expression of immunomolecules, secretion of cytotoxic factors, secretion of recruitment molecules, and secretion of pro-inflammatory signaling molecules. Microglia also undergoes rapid proliferation in order to increase their numbers for the protection. Microglial with different morphologies in different pathological states exhibit undetectable changes. ¹²²,¹²⁸,¹³¹,¹³²

**Phagocytic:** Activated phagocytic microglia are highly immune responsive form of microglia and also interact with astrocytes and neural cells to fight off the infection as early as possible with minimal
damage to the healthy brain cells.\textsuperscript{122,128} Phagocytic microglia travel to the site of the injury, engulfs the offending material, and secretes pro-inflamatory factors to proliferate more cells.

**Gitter cells:** Microglial cell’s phagocytosis will give the infectious material, which is called glitter cells. After engulfing a certain amount of material, the phagocytic microglia becomes unable to phagocytose any further materials. The resulting residual cellular mass is known as a granular corpuscle, named for its ‘grainy’ appearance. These tissues stained to reveal gitter cells.\textsuperscript{133}

**Perivascular:** “Perivascular” microglia is mainly found encased within the walls of the basal lamina. They perform normal microglial functions, but unlike normal microglia they are replaced by bone marrow derived precursor cells on a regular basis and express MHC class II antigens regardless of the outside environment. Perivascular microglia also reacts strongly to macrophage differentiation antigens.\textsuperscript{122} Perivascular microglia involves in endothelial cell proliferation which allows new vessels formation and repair of damaged vessels.\textsuperscript{127}

**Juxtavascular:** Juxtavascular microglia can be distinguished mainly by their location and found making direct contact with the basal lamina wall of blood vessels but are not found within the walls. They express MHC class II proteins even at low levels of inflammatory cytokine activity. Juxtavascular microglia do not exhibit rapid
turnover or replacement with myeloid precursor cells like other microglia."\textsuperscript{122}

**Microglia Functions:** The following are some of the major known functions by these cells.

**Scavenging:** Microglial cells are highly sensitive to even small changes in their environment and action is carried out in the ameboid and resting states. Microglial cells act as “housekeepers”.\textsuperscript{128} If they find any foreign material, apoptotic cells, damaged cells, DNA fragments, neural tangles, or plaques it will activate immediately and phagocytose the material or cell. Microglial cells can remove unwanted excess cellular matter during developmental wiring of the brain.

**Phagocytosis:** Microglia, phagocytosis, mainly involves in engulfing of various unwanted foreign materials. Generally Engulfed matter consist of cellular debris, lipids, apoptotic cells, and invading viruses, bacteria, or other foreign materials in the inflamed state. After completion of phagocytic activity by microglial cell and changes into a relatively non-reactive gitter cell.

**Cytotoxicity:** Microglia can release a various cytotoxic substances during phagocytosis. Microglia in respiratory burst process can secretes huge amounts of H$_2$O$_2$ and NO and these chemicals can directly damage cells and lead to neuronal cell death. Proteases secreted by microglia catabolise specific proteins causing direct
cellular damage, while cytokines like IL-1 promote demyelination of neuronal axons.

**Antigen presentation:** Due to the lack of MHC class I/II proteins in resident non-activated microglia which can act as poor antigen presenting cells. After activation they rapidly uptake MHC class I/II proteins and quickly become efficient antigen presenters. T-cells cross the blood-brain barrier during inflammation, and then directly bind to microglia cells in order to receive antigens. After this process T-cells go on to fulfill a variety of functions such as formation of immunomemories, pro-inflammatory recruitment, secretion of cytotoxic materials, and direct attacks on the plasma membranes of foreign cells.\textsuperscript{122,128}

**Synaptic stripping:** In 1968 Blinzinger and Kreutzberg observed this, and in which microglia removes the branches from nerves near damaged tissue in spinal lesions by post-inflammation and which leads for regrowth and remapping of damaged neural circuitry.\textsuperscript{122}

**Promotion of repair:** Microglia undergoes several steps to promote regrowth of neural tissue by post-inflammation and these are recruitment of neurons, secretion of anti-inflammatory cytokines, astrocytes to the damaged area, synaptic stripping, and formation of gitter cells. The regrowth of microglial cells and remapping would be slower in the CNS resident areas and almost impossible in many of the vascular systems surrounding the brain and eyes.\textsuperscript{122,127}
**Extracellular signaling:** The role of the large part of microglial cells in the brain is promoting inflammation in infected or damaged tissue and maintaining homeostasis in non-infected regions. Microglia accomplishes this through extracellular signaling molecules which allow them to communicate with other microglia, astrocytes, nerves, T-cells, and myeloid progenitor cells. The cytokine INF-γ can be used to activate microglial cells and after becoming activated with INF-γ, microglia also releases more INF-γ into the extracellular space. This INF-γ activates more microglia and starts a cytokine induced activation cascade rapidly activating all nearby microglia. T-cells and dendritic cells are recruited to the site of injury through the microglial production of the chemotactic molecules like MDC, IL-8, and MIP-3β. Finally, prostanoids and PGE$_2$ help to prevent chronic inflammation by inhibiting microglial pro-inflammatory response and down regulating Th1 (T-helper cell) response.$^{128}$

**Role in chronic neuroinflammation:** The word *neuroinflammation* has come to stand for chronic, central nervous system (CNS) specific, inflammation-like glial responses that may produce neurodegenerative symptoms such as plaque formation, excessive tau phosphorylation and dystrophic neurite growth.$^{134}$ Neuroinflammation is unique from inflammation in other organs, and can seen some similar mechanisms such as the localized production of chemoattractant molecules to the site of inflammation.$^{135}$ Neuroinflammations are acute and chronic and acute neuroinflammation is generally caused by some neuronal
injury after which microglia migrates to the injured site engulfing dead cells and debris. When microglia is activated they changes into amoeboid shape and they increase their gene expression and which leads for the production of numerous potentially neurotoxic mediators.

In chronic neuroinflammation, microglia remain activated for an extended period during which the production of mediators is sustained longer than usual and this increase in mediators contributes to neuronal death. The following list of the numerous substances that are secreted when microglia are activated.

**Cytokines:** Cytokines play an important role in neurodegeneration when microglia remain in a sustained activated state. Microglia activate the proinflammatory cytokines IL-1α, IL-1β and TNF-α in the CNS. Direct injection of the cytokines IL-1α, IL-1β and TNF-α into the CNS result in local inflammatory responses and neuronal degradation.

**Chemokines:** Chemokines are cytokines that stimulate directional migration of inflammatory cells in vitro and in vivo. Chemokines are proinflammatory and therefore contribute to the neuroinflammation process. Chemokines are divided into four main subfamilies: C, CC, CXC, and CX3C. Chemokines source is Microglial cell and express the monocyte chemoattractant protein-1 (MCP-1) chemokine in particular. Other inflammatory cytokines like IL-1β and TNF-α, as well as bacterial-derived lipopolysaccaride (LPS) may stimulate
Microglia to produce MCP-1, MIP-1α, and MIP-1β. Microglia can express CCR3, CCR5, CXCR4, and CX3CR1 in vitro.

**Proteases:** The secretion of proteolytic enzymes takes place when microglia is activated and these potentially involves in many functions. Some of the proteases degrade both the neuronal cells and extracellular matrix and that are in the neighborhood of the microglia releasing these compounds. These proteases include; cathepsins B, L, and S, the matrix metalloproteinases MMP-1, MMP-2, MMP-3, and MMP-9, and the metalloprotease-disintegrin ADAM8 plasminogen which forms outside microglia and degrades the extracellular matrix. Both Cathepsin B, MMP-1 and MMP-3 have been found to be increased in Alzheimer's disease (AD) and cathepsin B is increased in multiple sclerosis (MS). Elastase, another protease, could have large negative effects on the extracellular matrix.

**Amyloid precursor protein (APP):** This protein was synthesized by Microglia which is in response to excitotoxic injury. Plaques result from abnormal proteolytic cleavage of membrane bound APP. Microglia can be stimulated by Amyloid plaques to produce neurotoxic compounds such as cytokines, excitotoxin, nitrite oxide and lipophylic amines, which all cause neural damage.

**Aging:** Microglia activation causes extra load on the anabolic and catabolic machinery of the cells causing activated microglia to die sooner than non-activated cells. In order to compensate microglial
loss over time, microglia undergo mitosis and bone marrow derived progenitor cells migrate into the brain via the meninges and vasculature.\textsuperscript{134} Microglial activation may cause DNA damage and another factor might be the accumulation of advanced glycation endproducts, which accumulate with aging.\textsuperscript{137} These proteins are strongly resistant to proteolytic processes and promote protein cross-linking.\textsuperscript{137}

**Role of microglia in neurodegeneration:** Neurodegenerative disorders are characterized by progressive cell loss in specific neuronal populations.\textsuperscript{135} When the cells become chronically activated in progressive neurodegenerative disorders, then many of the normal trophic functions of glia may be lost or overwhelmed. In such case activated glia play destructive roles by direct and indirect inflammatory attack.\textsuperscript{135} The following are different prominent examples of microglial cells' role in neurodegenerative disorders.

**Alzheimer's disease (AD):** AD is a progressive, neurodegenerative disease where the brain develops tangled fiber bundles (neurofibrillary tangles) and abnormal clumps (amyloid plaques).\textsuperscript{138} Many activated microglia over-expressing IL-1 in the brains of Alzheimer patients that are distributed with both αβ plaques and neurofibrillary tangles.\textsuperscript{137} and this over expression of IL-1 leads to excessive tau phosphorylation that is related to tangle development in Alzheimer’s disease.\textsuperscript{137} Activated microglia are found to be associated with amyloid deposits in the brains of Alzheimer’s patients.\textsuperscript{135} and interact with β-amyloid
plaques through cell surface receptors that are linked to tyrosine kinase based signaling cascades that induce inflammation.\textsuperscript{135} When microglia interact with the β-amyloid it leads to the conversion of the microglia into an activated cell and results in the secretion of cytokines and other proteins that are neurotoxic.\textsuperscript{135}

**Treatment:** Alzheimer’s disease can be controlled by using Non-steroidal anti-inflammatory drugs (NSAIDs).\textsuperscript{135} The treatment with NSAIDs lowers the risk of AD by 55\%, delays disease onset, attenuates symptomatic severity and slows the loss of cognitive abilities.\textsuperscript{135}

**Parkinson’s disease:** Parkinson’s disease occurs when dopamine-producing neurons in the brain do not function properly.\textsuperscript{139} The area of the brain affected by Parkinson’s is called the substantia nigra and this is due to the neurons either become impaired or die.\textsuperscript{139} The substantia nigra has one of the highest concentrations of microglia in the brain.\textsuperscript{135}

Parkinson’s disease is due to the activated microglial cells have been found around extraneuronal and releases neuromelanin from impaired dopaminergic neurons.\textsuperscript{140} Henrik Wilms found that neuromelanin acts as a chemoattractant for microglial cells and induces morphological transformation of microglia cells to an activated state.\textsuperscript{140} Neuromelanin also induces synthesis of proinflammatory microglial molecules. Wei Zhang stated, aggregated α-synuclein, the major components of Lewy bodies in patients with
Parkinson’s disease or dementia with Lewy bodies, activated microglia leading to enhance dopaminergic neurotoxicity.\textsuperscript{141}

**Role in viral infections:**

**Human Immunodeficiency Virus (HIV):** Mononuclear phagocytes infection with HIV-1 is an important element in the development of HIV-associated dementia complex (HAD).\textsuperscript{142} Microglial cells with brain cell type only can productively infects with the virus.\textsuperscript{142} Brain microglia can releases neurotoxic mediators and can play an important role in the pathogenesis of HIV-1.\textsuperscript{142} HIV-1 can enter the microglial cell via CD4 receptors and chemokine co-receptors such as CCR3, CCR5, CXCR4 and CCR5. IL-4 and IL-10 enhance the entry and replication of HIV-1 in microglia through the up-regulation of CD4 and CCR5 expression, respectively. The chemokines CCL5/RANTES, CCL3/MIP-1\textalpha, CCL4/MIP-1\textbeta, all of which bind to CCR5, are inhibitory to HIV-1 replication in microglial cells, apparently by their ability to block viral entry.\textsuperscript{142}Infected microglia consist of viral particles intracellularly.\textsuperscript{142}

HIV-1 infected microglial cells actively secrete both endogenous neurotoxins such as TNF-\textalpha, IL-1\textbeta, CXCL8/IL-8, glutamate, quinolinic acid, platelet activating factor, eicosanoids, and NO as well as the neurotoxic viral proteins Tat, gp120, and gp41.”\textsuperscript{142} Microglias are the main target of HIV-1 in the brain. When microglias are activated by HIV-1 or viral proteins, they secrete or induce other cells to secrete
neurotoxic factors and this process is accompanied by neuronal dysfunction (HAD).\textsuperscript{142}

**Role in bacterial infections:** The outer membrane of a gram-negative bacterial cell is Lipopolysaccharide (LPS). LPS can activate microglia in vitro and stimulates microglia to produce cytokines, chemokines, and prostaglandins.\textsuperscript{142}

**Streptococcus pneumoniae:** *Streptococcus pneumoniae* is the most common cause of bacterial meningitis and which is localized to the subarachnoid space while cytokines and chemokines are produced inside the blood brain barrier.\textsuperscript{142} Microglia interact with streptococcus then activates microglia to produce nitric oxide which is neurotoxic.\textsuperscript{143} The inflammatory response, triggered by microglia, may cause intracerebral edema.\textsuperscript{142}

**Role in parasitic infections:**

**Plasmodium falciparum:** Malaria in humans is due to *Plasmodium falciparum* parasite.\textsuperscript{142} Cerebral malaria (CM) is serious complication of malaria\textsuperscript{142} and it occurs when red blood cells break through the blood brain barrier causing microhemorrhages, ischemia and glial cell growth.\textsuperscript{142} Recent research has indicated that microglia play a major role in the pathogenesis of CM.\textsuperscript{142}

**As a target to treat neuroinflammation:**
**Inhibition of activation:** The inhibition of microglial activation can control the neuroinflammation. Microglia activated by diverse stimuli but they are dependent on activation of mitogen-activated protein kinase (MAPK). Recently, minocycline a tetracycline derivative has shown down-regulation of microglial MAPK. Another promising treatment is CPI-1189, which induces cell death in a TNF α-inhibiting compound that also down-regulates MAPK.

**Inhibition of amyloid deposition:** Inhibitors of amyloid deposition include the enzymes responsible for the production of extracellular amyloid such as β-secretase and γ-secretase inhibitors. Currently the γ-secretase inhibitors are in phase II clinical trials as a treatment for Alzheimer’s disease but they have immunosuppressive properties, which could limit their use. Another strategy involves increasing the antibodies against a fragment of amyloid.

**Regulation of chemokine receptor:** The chemokine receptor, CX3CR1, is expressed by microglia in the CNS. Fractalkine (CX3CL1) is the exclusive ligand for CX3CR1 and is made as a transmembrane glycoprotein from which a chemokine can be released. Further studies into how CX3CR1 regulates microglial neurotoxicity could lead to new therapeutic strategies for neuroprotection.

**Inhibition of cytokine synthesis:** Central and peripheral cytokine synthesis and action can be inhibited by using anti-inflammatory
steroids called as Glucocorticosteroids (GCS). According to the recent studies lovastatin and sodium phenylacetate were found to inhibit TNF-α, IL-1β, and IL-6 in rat microglia. This shows that the mevalonate pathway plays a role in controlling the expression of cytokines in microglia and may be important in developing drugs to treat neurodegenerative diseases.

**Nitric oxide synthases (NOSs):** These are eukaryotic enzymes that catalyze the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule, having a vital role in many biological processes. NOS are an enzyme in the body that contributes to transmission from one neuron to another, to the immune system and to dilating blood vessels. Synthesis of nitric oxide (NO) takes place from the terminal nitrogen atom of arginine in the presence of NADPH and dioxygen (O₂). NOS are the only known enzyme that binds flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH₄) and calmodulin. NO activates Glanylate cyclase, which induces smooth muscle relaxation by:

- Increased intracellular cGMP, which inhibits calcium entry into the cell, and decreases intracellular calcium concentrations.
- Activation of K⁺ channels, which leads to hyperpolarization and relaxation.
- Stimulates a cGMP-dependent protein kinase that activates myosin light chain phosphatase, the enzyme that dephosphorylates myosin light chains, which leads to smooth muscle relaxation.
Classification: Different members of the NOS family are encoded by separate genes.\textsuperscript{146} NOS is one of the most regulated enzymes in biology. There are three known isoforms, two are constitutive (cNOS) and the third is inducible (iNOS).\textsuperscript{147} Cloning of NOS enzymes indicates that, cNOS include both brain constitutive (NOS1) and endothelial constitutive (NOS3), the third is the inducible (NOS2) gene.\textsuperscript{148} Recently, NOS activity has been demonstrated in several bacterial species, including such notorious pathogens as Bacillus anthraces and Staphylococcus aureus.\textsuperscript{149,150} Bacterial NOS (bNOS) has been shown to protect bacteria against oxidative stress, diverse antibiotics, and host immune response.\textsuperscript{151,152} The various forms of NO synthase have been classified as follows:

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene(s)</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal NOS (nNOS or NOS1)</td>
<td>NOS1</td>
<td>• nervous tissue</td>
<td>cell communication</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• skeletal muscle type II</td>
<td></td>
</tr>
<tr>
<td>Inducible NOS (iNOS or NOS2)</td>
<td>NOS2A, NOS2B, NOS2C</td>
<td>• immune system</td>
<td>immun defence against pathogens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• cardiovascular system</td>
<td></td>
</tr>
<tr>
<td>Endothelial NOS (eNOS or NOS3 or cNOS)</td>
<td>NOS3</td>
<td>• endothelium</td>
<td>vasodilation</td>
</tr>
<tr>
<td>Bacterial NOS (bNOS)</td>
<td>multiple</td>
<td>• various Gram(+) species</td>
<td>defense against oxidative stress, antibiotics, immune attack</td>
</tr>
</tbody>
</table>
**nNOS:** Neuronal NOS (nNOS) produces NO in nervous tissue in both the central and peripheral nervous system. nNOS also involves in cell communication and which is associated with plasma membranes. nNOS function can be inhibited by N-propyl-L-arginine (NPA). This form of the enzyme is specifically inhibited by 7-nitroindazole.\textsuperscript{153}

**iNOS:** As opposed to the critical calcium-dependent regulation of constitutive NOS enzymes (nNOS and eNOS), iNOS has been described as calcium-insensitive, likely due to its tight non-covalent interaction with calmodulin (CaM) and Ca\textsuperscript{2+}. While evidence for ‘baseline’ iNOS expression has been elusive, IRF-1 and NF-κB-dependent activation of the inducible NOS promoter supports an inflammation mediated stimulation of this transcript.

From a functional perspective, it is important to recognize that high-output iNOS occurs in an oxidative environment, and this high levels of NO can react with superoxide to form peroxynitrite and which leads for cell toxicity. These properties may define the roles of iNOS in host immunity, enabling its participation in anti-microbial and anti-tumor activities as part of the oxidative burst of macrophages.\textsuperscript{154}

**eNOS:** Endothelial NOS (eNOS), also called as nitric oxide synthase 3 (NOS3), generates NO in blood vessels and is involved with regulating vascular function. A constitutive Ca\textsuperscript{2+} dependent NOS provides a basal release of NO. eNOS is associated with plasma membranes, surrounding cells and the membranes of Golgi bodies within cells.
Nitric oxide synthase produces NO by catalysing a five-electron oxidation of guanidino nitrogen of L-arginine (L-Arg). Oxidation of L-Arg to L-citrulline occurs via two successive monooxygenation reactions producing \( N^\alpha \)-hydroxy-L-arginine (NOHLA) as an intermediate. To synthesize 1 mole of NO, 2 mol of \( O_2 \) and 1.5 mol of NADPH are consumed.

\[
\text{L-Arg + NADPH + H}^+ + \text{O}_2 \rightarrow \text{NOHLA + NADP}^+ + \text{H}_2\text{O}
\]

\[
\text{NOHLA + } \frac{1}{2} \text{ NADPH + } \frac{1}{2} \text{ H}^+ + \text{O}_2 \rightarrow \text{L-Citrulline + } \frac{1}{2} \text{ NADP}^+ + \text{NO + H}_2\text{O}
\]

**Structure:** NOSs can be dimeric, calmodulin-containing cytochrome p450-like hemoprotein or calmodulin-dependent that combines reductase and oxygenase catalytic domains in one dimer, bear both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and carry out a 5'-electron oxidation of non-aromatic amino acid arginine with the aid of tetrahydrobiopterin.\textsuperscript{155}

The first nitric oxide synthase was found in neuronal tissue (NOS1 or nNOS); the endothelial NOS (eNOS or NOS3) is third one. In NOS1
and NOS3, physiological concentrations of Ca$^{2+}$ in cells regulate the binding of calmodulin to the "latch domains", thereby initiating electron transfer from the flavins to the heme moieties. In contrast, calmodulin remains tightly bound to the inducible and Ca$^{2+}$-insensitive isoform (iNOS or NOS2) even at a low intracellular Ca$^{2+}$ activity, acting essentially as a subunit of this isoform. Nitric oxide may itself regulate NOS expression and activity. Specifically, NO has been shown to play an important negative feedback regulatory role on NOS3, and therefore vascular endothelial cell function.

This process is known as S-nitrosation/S-nitrosylation and shown to reversibly inhibit NOS3 activity in vascular endothelial cells. In addition to NOS3, both NOS1 and NOS2 have been found to be S-nitrosated, but the evidence for dynamic regulation of those NOS isoforms by this process is less complete. In addition, both NOS1 and NOS2 have been shown to form ferrous-nitrosyl complexes in their heme prosthetic groups that may act partially to self-inactivate these enzymes under certain conditions. The rate-limiting step for the production of nitric oxide may well be the availability of L-arginine in some cell types. This may be particularly important after the induction of NOS2.

**Biological functions of Nitric oxide:** NO is one of the few gaseous signaling molecules known and which is a key vertebrate biological messenger, playing a role in a variety of biological processes. Nitric oxide, known as the 'endothelium-derived relaxing factor', or 'EDRF',
is biosynthesized endogenously from arginine and oxygen by various nitric oxide synthase (NOS) enzymes and by reduction of inorganic nitrate. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive with a few seconds of life time and which diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signaling molecule.\textsuperscript{156} Nitroglycerin and amyl nitrite serve as vasodilators because they are converted to nitric oxide in the body. Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways.\textsuperscript{157} Sildenafil citrate, trade name \textit{Viagra}, stimulates erections primarily by enhancing signaling through the nitric oxide pathway in the penis.

Phagocytes (monocytes, macrophages, and neutrophils) also can generate the Nitric oxide as part of the human immune response. Phagocytes are armed with inducible nitric oxide synthase (iNOS), which is activated by interferon-gamma (IFN-γ) as a single signal or by tumor necrosis factor (TNF) along with a second signal.\textsuperscript{158} On the converse, transforming growth factor-beta (TGF-β) provides a strong inhibitory signal to iNOS, whereas interleukin-4 (IL-4) and IL-10
provide weak inhibitory signals. In this way the immune system may regulate the armamentarium of phagocytes that play a role in inflammation and immune responses. Nitric oxide secreted as an immune response is as free radicals and is toxic to bacteria; the mechanism for this includes DNA damage\textsuperscript{159,160,161} and degradation of iron sulfur centers into iron ions and iron-nitrosyl compounds.\textsuperscript{162} However, many bacterial pathogens have evolved mechanisms for nitric oxide resistance.\textsuperscript{163} Nitric oxide also can serve as an \textit{inflammometer} in conditions like asthma.

An important biological reaction of nitric oxide is S-nitrosylation, the conversion of thiol groups, including cysteine residues in proteins, to form S-nitrosothiols (RSNOs). S-Nitrosylation is a mechanism for dynamic, post-translational regulation of most or all major classes of protein.

\textbf{Mechanism of action:} Several mechanisms are available to explain the NO affect on the biology of living cells. These include oxidation of iron-containing proteins such as ribonucleotide reductase and aconitase, activation of the soluble guanylate cyclase, ADP ribosylation of proteins, protein sulfhydryl group nitrosylation, and iron regulatory factor activation.\textsuperscript{164} NO has been demonstrated to activate NF-κB in peripheral blood mononuclear cells, an important transcription factor in iNOS gene expression in response to inflammation.\textsuperscript{165} It was found that NO acts through the stimulation of the soluble guanylate cyclase, which is a heterodimeric enzyme with
subsequent formation of cyclic GMP. Cyclic GMP activates protein kinase G, which causes phosphorylation of myosin light chain phosphatase, and therefore inactivation of myosin light-chain kinase, and leads ultimately to the dephosphorylation of the myosin light chain, causing smooth muscle relaxation.\textsuperscript{166}

**Use in pediatric intensive care:** Nitric oxide/oxygen blends are used in critical care to promote capillary and pulmonary dilation to treat primary pulmonary hypertension in neonatal patients\textsuperscript{167,168} post meconium aspiration and related to birth defects. These are often a last-resort gas mixture before the use of extracorporeal membrane oxygenation (ECMO). Nitric oxide therapy has the potential to significantly increase the quality of life and in some cases, save the lives of infants at risk for pulmonary vascular disease.\textsuperscript{169}

**Pharmacology:** Nitric oxide is considered as anti-anginal drug which causes vasodilation, and can help with ischemic pain known as angina by decreasing the cardiac workload. By dilating the veins there is less blood returned to the heart per cycle.\textsuperscript{170} This decreases the amount of volume that the heart has to pump. To prevent or treat acute chest pain (ACS), nitroglycerin pills are used sublingually (under the tongue). The nitroglycerin reacts with a sulfhydryl group (–SH) to produce nitric oxide, which eases the pain by causing vasodilation.
1.8.2. Literature survey on Anti-neuroinflammatory activity of 1,5-benzodiazepine derivatives: Many benzodiazepine derivatives are highly active from the reported literature.\textsuperscript{171} A variety of the central depressant effects of 1,4-benzodiazepines have been reported. Shimamoto, Takaori and Nakajima \textit{et al.} also have reported the potent sedative, taming, muscle relaxant and anti-convulsive properties of 1-alkyl carbamoyl-1,4-benzodiazepin-2-ones synthesized by Usui \textit{et al.} Most of the chemical studies in this area have been devoted to syntheses of the derivatives of 1,4-benzodiazepines with relatively simple substituents at 1, 2 or 3 position or in phenyl ring as exemplified by chlordiazepoxide, diazepam, oxazepam and nitrazepam. On the other hand, Meguro and Kuwada in this Chemical Laboratories have synthesized a number of novel 6-phenyl-4H-s-triazolo-[43,a] [1,4] benzodiazepines with a tricyclic ring system.

1,4-benzodiazepines report deals with the anticonvulsive, muscle relaxant, sedative and taming effects of these novel compounds in small experimental animals. In these respects, it was proved that several derivatives including 8-chloro-6-phenyl-4H-s-triazolo-[43,a] [1,4] benzodiazepine (D-40TA) and 1-methyl-8-chloro-6-phenyl-4H-s-triazolo[4,3,a] [1,4] benzodiazepine (D-65MT) were more effective in the central depression than diazepam and nitrazepam. Structure-activity relation ship of s-triazolo 1,4-benzodiazepines in central nervous depressant action were reported earlier.\textsuperscript{171} Many 1,4-benzodiazepines are highly active from the above reported literature. Encouraged by
these results, we examined several 1,5-benzodiazepines for their anti-neuroinflammatory studies. To our knowledge, nobody has done these studies until now.

1.8.3. Literature survey on Anti-neuroinflammatory activity of DHPMs derivatives: Many DHPMs are highly active from the above reported literature. DHPMs in central nervous depressant action were reported earlier. Encouraged by these results, we examined several DHPMs for their anti-neuroinflammatory studies. DHPMs compounds significantly may inhibit the enzyme activity of inducible NO synthase (iNOS) without changes of iNOS protein expression and NO scavenging activity. These compounds may exhibit the anti-neuroinflammatory effect by suppressing iNOS. Further studies are required to elucidate precise mechanisms underlying the anti-inflammatory activity of active compounds. To our knowledge, nobody has done these studies until now.

1.8.4. Literature survey on Anti-neuroinflammatory activity of Quinoxaline derivatives: Many Quinoxaline derivatives are highly active from the reported literature. Encouraged by these results; we examined several Quinoxaline derivatives for their anti-neuroinflammatory studies. These compounds may exhibit the anti-neuroinflammatory effect by suppressing iNOS. These compounds significantly may inhibit the enzyme activity of inducible NO synthase (iNOS) without changes of iNOS protein expression and NO scavenging
activity. As per our literature survey, nobody has done these studies in the open literature so far.

1.9. Conclusions: Pharmacological importance of BDPs, DHPMs and Quinoxalin-2-amine derivatives prompted us to develop various synthetic methods for novel derivatives. In the present thesis, a review of the relevant literature is undertaken using Scopus, SciFinder Scholar and google search, in order to assess this more accurately and to provide a contextual framework. Although several strategies and methodologies have been applied to achieve conveniently the synthesis of these compounds, further research must, however, be undertaken in order to design and develop efficient, practical, and scalable synthetic routes to some of these compounds and their analogues for biological and preclinical studies. The challenge for prospective research in this area of synthetic organic chemistry involves the optimization of known procedures on the one hand, and the development of new useful synthetic approaches on the other. In particular, future work should be directed to develop effective processes involving different reaction conditions and employing different catalyst, which should be designed to eliminate/reduce the use and generation of hazardous substances, whenever possible, the utilization of the atom-economy concept of all materials used in the process which should be conducted at favorable condition. It also important to note that good strategies for the catalyst–product
separation and the catalyst recycling should be established for industrial application.

Thus, synthesis and pharmacological importance of these compounds are reviewed and keeping the pharmacological importance of the various $N$-containing compounds in the view, we have synthesized the different compounds using various catalysts and all synthesized compounds antineuro-inflammatory activity studied in the present study. As per our literature survey, there has been no report available on the synthesis of BDPs, DHPMs and Quinoxalin-2-amine derivatives using silica gel supported sulfuric acid, Dichloro Dicyano Quinine (DDQ), $p$-Toulene sulfonic acid (PTSA) and mesoporous aluminosilicate (AlKIT-5) catalysts and similarly no reports observed on antineuroinflammatory studies of these compounds till now.

References:


Moore, K.W.; Carling, R.W.; Street, L.J.; Castro, J.L; Ragan, C, I.; Dawson, G.R.; Whiting, P.J. “Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA_A receptor α₁ subtype” *Nature neuroscience*, 3, 2000, 587-592.


potentials of spinal cord neurons in cell culture” *J. Pharmacol Exp. Ther.* 244, **1988**, 789-795.

17. Woods, M.J.; Williams, D.C. “Multiple forms and locations for the peripheral-type benzodiazepine receptor” *Biochemical Pharmacol.* 52, **1996**, 1805-1814.


31. Hu, E.H.; Sidler, D.R.; Dolling, U.H. “Unprecedented Catalytic Three Component One-Pot Condensation Reaction: An Efficient Synthesis of 5-Alkoxy carbonyl-4-aryl-3,4-dihydropyrimidin-


37. O'Reilly, B.C.; Atwal, K. S. “Synthesis of Substituted 1,2,3,4-Tetrahydro-6-methyl-2-oxo-5-pyrimidinecarboxylic Acid Esters: The Biginelli Condensation Revisited” Heterocycles 26, 1987, 1185-1188 & 1189-1192.


   [Fortschr. Teerfarbenfabr. Verw. Industriezweige (Friedlander, E.;
   Ed.) 25, 1932, 2590-2591.

   229.

   Abstr. 1971, 75, 47266e]

   Abstr. 102, 1985, 132067].


   Abstr. 114, 1991, 143437f]

50. Siliniece, G.; Kimenis, A. Farmakol. Neirotropnykh, Sredstv. 1978,
   135-138. [Chem. Abstr. 92, 1980, 69368t]

51. Ertan, M.; Balkan, A., Sarac, S.; Uma, S.; Renaud, J. F. Rolland,
   Y. “Synthesis and Calcium Antagonistic Activity of Some New 2-
   Thioxo-1,2,3,4-tetrahydropyrimidine Derivatives” Arch. pharm.
   324, 1991, 135-139.

52. Kastron, V.V.; Vitolima, R.; Khanina, E.L.; Duburs, G., Kimenis,
   A., Kondratenko, N.V.; Popov, V.I.; Yagupol’skii, L.M.;
   1989, 18547h].


56. Atwal, K.S; Swanson, B.N., Unger, S.E.; Floyd, D.M.; Moreland, S.; Hedberg, A.; O'Reilly, B.C. “Dihydropyrimidine calcium channel blockers. 3. 3-Carbamoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl- 5-pyrimidinecarboxylic acid esters as orally effective antihypertensive agents” *J. Med.Chem.* 34, **1991**, 806-811.


158. Gorczyniski, Stanely, Clinical Immunology. Landes Bioscience; Austin, TX. ISBN 1570596255


4-aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic acid esters as potent mimics of dihydropyridines” *J. Med. Chem.* 33, **1990**, 2629-2635.


