1.1. General introduction to heterocyclic compounds

Heterocyclic compounds constitute the largest and most varied family of organic compounds, and can have a variety of structure. These structures can be acyclic or cyclic. The cyclic systems containing only carbon atoms are called as carbocyclic and if at least one atom other than carbon forms a part of the ring system then it is designated as a heterocyclic compound [1, 2]. Though a number of heteroatoms are known to be part of the heterocyclic rings, the most common heteroatoms are nitrogen, oxygen or sulphur. Enormous number of heterocyclic compounds is known and this number is increasing rapidly. Heterocyclic compounds may be classified into aliphatic and aromatic. The aliphatic heterocyclics are the cyclic analogues of amines, ethers, thioethers, amides, etc., and their properties are particularly influenced by the presence of strain in the ring. The aromatic heterocyclic compounds, in contrast, are those which have heteroatoms in the ring and behave in a manner similar to benzene in some of their properties. These compounds follow the Huckels rule which states that cyclic conjucted and planar systems having \((4n+2)\) π electron are aromatic.

Several nitrogen and sulfur containing heterocyclic compounds namely triazoles [3], thiazoles [4], oxadiazoles [5], pyrimidines and isoxazoles [6] display important biological activities. Hence the syntheses of new compounds incorporating these moieties with known pharmacological properties have gained impetus in recent years. The piperazine nucleus is capable of binding to multiple receptors with high affinity and therefore piperazine has been classified as a privileged structure [7]. Various compounds such as alkaloids, antibiotics, essential amino acids, the vitamins, hemoglobin, the hormones, a large number of synthetic drugs and dyes contain heterocyclic ring systems. Knowledge of heterocyclic chemistry is useful in biosynthesis and in drug metabolism as well. There are a large number of synthetic heterocyclic compounds with additional important applications, and many are valuable intermediates in synthesis. Some heterocyclic compounds are shown below.
In medicinal chemistry they are commonly used as templates to design biologically active agents [8, 9]. Heterocyclic chemistry is a vast and expanding area of chemistry because of the obvious applications of compounds derived from heterocyclic rings in pharmacy, medicine, agriculture and other fields. Moreover, in the past 20 years the drug-discovery process has undergone extraordinary changes and high-throughput biological screening of potential drug candidates has led to an ever increasing demand for novel drug-like compounds [10]. The pharmacological activity of drugs depends mainly on interaction with their biological targets, which have a complex three-dimensional structure, and molecular recognition is guided by the nature of the intermolecular interactions.

Compounds with heterocyclic ring are inextricably woven into the most basic biochemical processes of life. From thousands of compounds thus synthesized, only one or two may reach to therapeutically useful form. Though, in the early stages of development, an accurate evaluation is not achieved, a low cost screening which adequately appraises the potency of a compound is of great economical importance. Most biologically active compounds are heterocyclic organic compounds which have a ring structure containing atoms such as sulfur, oxygen or nitrogen in addition to carbon, as part of the ring. There are large number of synthetic heterocyclic compounds with important practical applications, as dyestuffs, copolymers, solvents, photographic sensitizers and developers, as antioxidants and vulcanization accelerators in the rubber industry, and many are valuable intermediates in synthesis. There are also a vast number of pharmacologically active heterocyclic compounds, many of which are in regular clinical use. Some of these are natural products, for example antibiotics such as penicillin and cephalosporin, alkaloids such as vinblastine, ellipticine, morphine and reserpine.
1.1.1. Introduction to triazoles

The search for new agent is one of the most challenging tasks to the medicinal chemist. The synthesis of high nitrogen containing heterocyclic systems has been increasing over the past decade because of their utility in various applications such as propellants, explosives, pyrotechnics and especially chemotherapy. In recent years, the chemistry of triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. The derivatization of triazole is considered to be based on the phenomenon of bioisosterism in which replacement of oxygen of oxadiazole nucleus with nitrogen atom yields triazole analogue. There are two possible isomers of triazole (1, 2) depending on the position of nitrogen atom in the ring and are numbered as shown below.

![Triazole isomers](image)

1, 2, 3-Triazole          1, 2, 4-Triazole

Out of the two triazoles, 1,2,4-triazole have drawn great attention to medicinal chemists from two decades due to its wide variety of activity [11], low toxicity, good pharmacokinetic and pharmacodynamics profiles. 1,2,4-Triazole derivatives usually exist in solid form. 3,4,5-Substituted 1,2,4-triazole derivatives melt with thermolysis at high temperature when heated at 316 °C for 30 minutes [12]. 1,2,4-Triazole derivatives are readily soluble in polar solvents and only slightly soluble in nonpolar solvents, however, the solubility in non-polar solvents can be increased by substitution on the nitrogen atom. Due to annular prototropic tautomerism, 1,2,4-triazoles without substituents on the ring nitrogen atoms, a priori can exist in three forms. Analysis of the crystal data [13] showed that with rare exceptions [14], unsymmetrical 3,5-disubstituted 1,2,4-triazoles exists in the solid state as tautomer bearing an electron substituent at position 3 and an electron-donor substituent at position 5. The precise experimental data on the annular tautomerism in 1,2,4-triazoles in solution are limited [15]. Tautomeric form with the hydrogen atom at N-1 adjacent to carbon atom bearing relatively less electronegative group was found to predominate in the solution.
Among the substituted 1,2,4-triazoles, 3-mercapto-1,2,4-triazoles exists in two tautomeric forms (3, 4), because the labile hydrogen may be attached either to the nitrogen or to the sulfur atom. It exhibits thione-thiol tautomeric forms shown below.

1, 2, 4-Triazole derivatives undergo Mannich reaction (Mannich reaction is a 3-component condensation reaction involving an active hydrogen containing formaldehyde and a secondary amine). The amino methylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [16]. Amino group at position 4 of 1,2,4-triazole derivative undergoes reaction with 4-methoxybenzaldehyde [17] and eliminate water molecule with the formation of Schiff base. Compounds having Schiff base structure may exist as E/Z geometrical isomers about the –N=CH- double bond. Compounds containing imine bond are present in higher percentage in dimethyl-d₆ sulfoxide solution in the form of geometrical E isomer about –N=CH- double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. It is known that 1,2,4-triazole and 4,5-dihydro-1H triazol-5-one rings have weak acidic properties and so some 1,2,4-triazole and 4, 5-dihydro-1H derivatives were titrated potentiometrically with tetrabutylammonium hydroxide in non-aqueous solvents such as acetonitrile, isopropyl alcohol and N, N-dimethylformamide, and the half-neutralization potential values and the corresponding pKa values of the compounds were determined [18].
Triazoles are potential bioactive agents due to their wide spectrum of therapeutic importance. Drug molecules having 1,2,4-triazole nucleus with good activity are listed under,

- Fluconazole
- Triazophane
- Triadimentol
- Fluorimazole
- Azaconazole
- Amitrole
Literature survey reveals that various 1,2,4-triazole derivatives display wide spectrum of biological activities. 3-Amino-1,2,4-triazole was the first 1,2,4-triazole to be manufactured on large scale from aminoguanidine formate [19]. A number of biological activity such as antimicrobial [20], anti-inflammatory [21], anticonvulsant [22], anticancer [23], antimycobacterial [24], antioxidant [25] and antimalarial [26] have been associated with N-substituted triazoles attached with different heterocyclic nuclei. It has been noticed continuously over the years that interesting biological activities were associated with triazole derivatives.

Jubie et al. [27] have synthesized some novel ciprofloxacin analogues as antimicrobial agents. Ciprofloxacin have been incorporated to the new series of Schiff bases of 1,2,4-triazole via Mannich reaction. The new compounds have been evaluated in vitro for their antimicrobial activity against B. subtilis, K. pneumoniae, and P. aeruginosa at 10 µg/ml concentration. All the compounds showed in vitro gram positive and gram negative activity and generally comparable or superior to that of reference ciprofloxacin.

Bijul lakshman et al. [28] synthesized twenty eight derivatives of 4-amino-5-substituted aryl-3-mercapto-1,2,4-triazoles and these compounds have been tested in vitro against Rhizoctonia solani, Sclerotium rolfsii, Fusarium oxysporum, Pythium aphanidermatum, Puccinia reconditeand Bipolaris sorokiana.

Veena Vani Ktla et al. [29] synthesized novel 1,2,4-triazole derivatives by cyclization of 1-(2-(3-chloro-4-methyl-2-oxo-2H-chromen-7-yloxy)-4-substituted phenyl thiosemicarbazide in alkaline medium. The newly synthesized compounds were characterized by 1H NMR, IR and Mass spectral data. Further, all the compounds were screened for their antibacterial activity against Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa, and antifungal activity against Aspergillus Niger and
Aspergillus flaves using fluconazole as standard drug. The screening results revealed that, most of the compounds were found to exhibit significant antimicrobial activity. Tomasz Plech et al. [30] described a fast and efficient method for the synthesis of some 1,4-disubstituted thiosemicarbazide derivatives. The reaction of 3-chlorobenzoic acid hydrazide with various aryl isothiocyanates gave thiosemicarbazide derivatives in good yield. The cyclization of compounds in the presence of 2% NaOH resulted in the formation of compounds containing 1,2,4-triazole ring. A series of new Mannich bases related to the structure 1,2,4-triazole has also been synthesized. An attempt was made to clarify the influence of nature/position of substituents on antibacterial activity of compounds described. A variety of novel s-triazoles and their Mannich bases were prepared as well. Some derivatives showed promising antimicrobial activity, especially against Gram-positive bacteria. Monika et al. [31] synthesized derivatives of the 9-substituted-3-aryl-5H,13aH-quinolino[3,2-f][1,2,4]triazolo[4,3-b][1,2,4]triazepines from the 5-aryl-3,4-diamo-no-1,2,4-triazols and 2-chloro-3-formyl quinolones using catalytic amount of p-TsOH and N,N-dimethylformamide as an energy transfer medium using microwave, and the synthesized compounds were screened for antifungal activity against Aspergillus flaves, Aspergillus niger, Rhizopus species and Pencillum notatum species by paper disc technique against 500 and 1000 µg/ml concentrations.

Aniket et al. [32] synthesized Schiff’s bases of 5-mercapto-3-(3-pyridyl)-4H-1,2,4-triazole-4-yl-thiosemicarbazide by microwave assisted method. The synthesized compounds have been evaluated in vitro for their antibacterial, antifungal and anticonvulsant activities. Mali et al. [33] synthesized 5-(N-substituted carboxamidomethylthio)-3-(3′-pyridyl)-1,2,4-triazole and their antifungal activity was carried out against C. albicans and A. niger at the concentrations of 50 and 100 µg/mL using Fluconazole as the standard, and in vitro antitubercular activity was done at 50 µg/mL against Mycobacterium tuberculosis H37 Rv. A series of new coumarin based 1,2,4-triazoles were synthesized and evaluated for antimicrobial activity in vitro against Gram-positive bacteria (Staphylococcus aureus, MRSA, Bacillus subtilis and micrococcus luteus), and Gram-negative bacteria (Escheichia coli, Proteus vulgaris, Salmonella typi and Shigella dysenteriae) as well as fungi (Candida albicans, Sacchoromyces cerevisiae and Aspergillus fumigatus) by two fold serial dilution techniques [34].

Siddiqui et al. [35] synthesized some 4-{[1-(aryl)methylidene]-amino}-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole starting from isonicotinic acid hydrazide, potassium
hydroxide and carbon disulphide, and screened for analgesic and antipyretic activities. Analgesic activity evaluated by tail-flick method in rats at a dose of 25 mg/kg and antipyretic activity was evaluated using Brewer’s yeast-induced pyrexia in rats. Fever was induced by subcutaneously administered 20 ml/kg of 20 % aqueous suspension of Brewer’s yeast in normal saline, below the nape of the neck and rectal temperature was recorded with a clinical thermometer. Aspirin (300 mg/kg) was used as a standard drug for comparing the antipyretic action of compounds. Singha et al. [36] synthesized a series of potential bioactive 4-amino-5-mercapto-3-aryl-1,2,4-triazoles according the literature methods. The synthesized compounds were characterized by spectroscopy and evaluated their anticancer activity against EAC (Ehrlich Ascites Carcinoma). Compounds were given at a dose of 25 mg/kg body weight intraperitoneally. Groups were found to reduce tumor volume, viable cell count and increase the tumor weight (%) inhibition, ascites cells (%) inhibition and non-viable cell count and increase in life span. All the compounds exhibited the significant anticancer activity compared to control and some of the compounds were found to be most potent.

A series of [1,2,4]triazolo[1,5-a]pyridines were synthesized and characterized by Wang et al [37]. The in vitro antiproliferative activity was evaluated by MTT against three human cancer cell lines, HCT-116, U-87 MG and MCF-7. The SAR of target compounds was preliminarily discussed. Some of the compounds with potent antiproliferative activity were tested for their effects on the AKT and p-AKT473. The anticancer effect was evaluated in mice bearing sarcoma S-180 model. The results suggest that the title compounds are potent anticancer agents. Ying-Chao Duan et al. [38] synthesized a series of novel 1,2,3-triazolethiosemicarbazide hybrids and their antiproliferative activity was evaluated against four human cancer lines. The results showed that number of hybrids exhibited potent activity in selected human cancer cell lines. Among them few compounds showed broad spectrum anticancer activity with IC₅₀ values ranging from 0.76 to 20.84 nm. Evidences of cell cycle arrest and apoptosis induction were obtained for the most effective compounds.

Olcay Bekircan et al. [39] synthesized a series of 4-arylideneamino-4H-1,2,4-triazoles and 4-(1-aryl)ethylidene-4H-1,2,4-triazoles by the treatment of 4-amino-1,2,4-triazole with certain aldehydes and ketones. Compounds have been reduced with NaBH₄ to yield corresponding 4-arylmethylamino-4H-1,2,4-triazoles and 4-(1-aryl)ethylamino-4H-1, 2, 4-triazoles. The chalcones were reacted with thiourea in the presence of KOH in ethanol,
which led to the formation of dihydropyrimidine derivatives as a beneficial antimicrobial, anticonvulsant and anticancer agents by Khanage et al [40]. All the synthesised compounds were screened for their in vitro antimicrobial activity by agar well method and their anticonvulsant activity by the MES model. Anticancer activity of two newly synthesised heterocycles was evaluated against 60 cell lines of different human tumor at a single dose of $10^{-5}$ M.

Number of articles were found for the anticonvulsant potential of 1,2,4-triazole where substitution on 2,3,5 positions were done. Recently, anticonvulsant activity of clubbed thiazolidinone-barbituric acid and thiazolidinone-triazole derivatives has been reported by Shiradkar et al [41]. 3-(2-Chloroacetyl)-2-arylimino-5-[(Z)-arylmethylidene]-1,3-thiazolan-4-ones on treatment with 5-(1-phenoxyethyl)-4H-1,2,4-triazole-3-thiol in identical conditions provided a set of bulkier derivatives which have also shown the anticonvulsant potential. Pradeep Goyal et al [42] synthesized some new derivatives of 3-substituted-4H-1,2,4-triazoles. All the synthesised compounds were evaluated for anti-inflammatory activity and acute toxicity. Most of the compounds showed potent and significant results compared to standard ibuprofen.

Krzysztof Sztanke et al. [43] reported the synthesis of ethyl 1-(7-phenyl-2H-3, 5, 6, 7-tetrahydroimidazo [2,1-c][1,2,4]triazol-3-yl)formate. The influence of ethyl 1-(7-phenyl-2H-3,5,6,7-tetrahydro-imidazo[2,1-c][1,2,4]triazol-3-yl)formatan on human adenovirus 5 (Ad-5) and human enterovirus (Echo-9) replication has been investigated. The activity against the selected DNA (Ad-5) and RNA (Echo-9) viruses and the cytotoxicity towards normal GMK (Green Monkey Kidney) cells were also determined.

1,2,4-Triazoles were used as analytical reagents for the determination of boron [44], antimony [45] and cobalt [46]. Other triazoles find many synthetic uses as halogenating agents [47] or as activating polymeric reagents [48]. It has been noticed that, modification on triazole moiety displayed valuable biological activities, and can be utilized as potent therapeutic agents in future. Thus, the quest to explore many more modifications on triazole moiety needs to be continued for the use of mankind.

1.1.2. Introduction to oxadiazoles

Oxadiazole is a heterocyclic compound containing an oxygen atom and two nitrogen atoms in a five-membered ring. It is derived from furan by substitution of two methylene
groups (=CH) with two pyridine type nitrogens (-N=). There are four known isomers, 1,3,4-oxadiazole (5), 1,2,4-oxadiazole (6), 1,2,3-oxadiazole (7) and 1,2,5-oxadiazole (8). However, 1,3,4-oxadiazole and 1,2,4-oxadiazole are better known, and more widely studied because of their many important chemical and biological properties, but 1,2,3-isomer is unstable and reverts to the diazoketone tautomer.

1,3,4-oxadiazole is thermally stable aromatic molecule [50], and it has become an important construction motif for the development of new drugs. Oxadiazole rings have been introduced into drug discovery programs for several different purposes. In some cases, they have been used as an essential part of the pharmacophore, favorably contributing to ligand binding. In other cases, oxadiazole moieties have been shown to act as a flat, aromatic linker to place substituent’s in the appropriate orientation, as well as modulating the molecular properties by positioning them in the periphery of the molecules. Oxadiazoles display interesting hydrogen bond acceptor properties, and it will be shown that the regioisomers display significantly different hydrogen bonding potentials. Compounds containing 1,3,4-oxadiazole cores have a broad spectrum of biological activity including antibacterial, antifungal, analgesic, anti-inflammatory, antiviral, anticancer, antihypertensive, anticonvulsant and anti-diabetic properties. During the last decades, synthesis and transformations of five member heterocyclic compounds has received considerable attention and importance due to their remarkable and wide variety of applications. 2,5-Disubstituted-1,3,4-oxadiazole and its derivatives constitute an important family of heterocyclic compounds. Due to their remarkable unique properties, 1,3,4-oxadiazole and its derivatives have been frequently employed in drug synthesis, various commercial and industrial applications. The 1,3,4-oxadiazole ring carrying substitution in an appropriate position and substituent with a nucleophilic center are excellent precursors for further synthesis of heterocyclic compounds. For example, ring rearrangements for the synthesis of five and six membered heterocycles. 5-Substituted-2-mercapto-1,3,4-oxadiazoles are interesting and
important class of compounds. They have general formula (9) ($R =$ alkyl or aryl group). These compounds are known to exist in tautomeric thiol (9) and thione (10) forms.

In recent past, extensive study of 1,3,4-oxadiazole derivatives show diverse biological activities. Substituted 1,3,4-oxadiazoles and 5-substituted-2-mercapto-1,3,4-oxadiazoles are of considerable pharmaceutical interest. They display remarkable biological activities. For instance, 2-amino-1,3,4-oxadiazoles have been reported as muscle relaxants [51], analgesic [52-58], anti-inflammatory [59-62], anticonvulsive [63-68] and diuretic properties. Whereas derivatives of 2-mercapto-1,3,4-oxadiazoles have been reported to exhibit antimicrobial [69-70] and anticancer [71] activities. As part of interest in heterocyclics derived from Schiff bases that have been explored for developing pharmaceutically important molecules, 2-azetidinones, 4-thiazolidinones and fused thiazolidinones have played a pivotal role in medicinal chemistry. Moreover, they have been studied extensively because of their ready accessibility, diverse reactivity and broad spectrum of biological activity.

Some examples of compounds containing the 1,3,4-oxadiazole unit currently used in clinical medicine are: Raltegravir (11), antiretroviral drug and zibotentan (12), are anticancer agents. Vadrin a leprostatic drug, eudromil a hypnotic drug, nesapil (13) used in anti-arrhythmic therapy, furamizole (14), a nitrofuran derivative has strong antibacterial activity, tiodazosin (15), an antihypertensive drug and ataluren (16) used for the treatment of cystic fibrosis.
A wide variety of 1,3,4-oxadiazole derivatives have been synthesized and evaluated their broad spectrum of biological activity. Farshori et al. [72] synthesized 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles and tested for in vitro antimicrobial activities by disc diffusion method. Among the synthesized compounds, few compounds were found to be more active against the tested strain and were compared with griseofulvin as standard drug. 5-(3,4,5-Trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives were synthesized and tested for their antifungal activity against fungal strain [73]. Among the tested compounds, few compounds exhibiting promising antifungal activity even better than that of the commercial fungicide hymexazol.

Mishra et al. [74] synthesized a series of oxadiazole derivatives and tested for their antimicrobial activity by cup and plate method. Among the tested compounds few showed promising antibacterial activity against Gram +ve bacteria and Gram –ve bacteria when compared to standard drugs ofloxacin and levofloxacin. A series of novel unsymmetrical 2,5-disubstituted 1,3,4-oxadiazoles [75] have been synthesized and the final compounds were tested for their antibacterial and antifungal activities. Among the tested compounds, few showed maximum antibacterial activity and were compared with ciprofloxacin as standard drug. Arora et al. [76] synthesized a series of 2,5-disubstituted oxadiazole based
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chalcone derivatives and their antimicrobial activity by cup-plate method and compared with vancomycin as standard drug. Some compounds showed promising result and were considered for further studies. A series of 5-(1-benzylamino-2-(substituted phenyl)vinyl)-2-amino-1,3,4-oxadiazole derivatives were synthesized by Rajitha et al [77]. Antimicrobial activity of these compounds was tested by using filter paper disc. These compounds showed moderate activity.

A series of novel 1,3,4 oxadiazole derivatives were synthesized by Vipul et al. [78], and screened for antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli. Biological results indicated that the synthesized compounds showed a broad spectrum activity against tested microorganisms. Patel et al. [79] synthesized 2-benzylsulfanyl-nicotinic acid based 1,3,4-oxadiazoles and these were showed promising activity against Escherichia coli compared to ampicillin. A series of novel 2-[5-(substituted phenyl)-[1,3,4] oxadiazol-2-yl]-benzoxazoles have been synthesized [80], and their antimicrobial activity has been evaluated against various bacteria and fungi, and almost all compounds showed good activity against bacteria and fungi.

Afshin Zarghi et al. [81] reported a new series of 2-substituted-5-[2-(2-halobenzyloxy)phenyl]-1,3,4-oxadiazoles. The anticonvulsant activity of all the synthesized compounds was evaluated against maximal electroshock induced seizures (MES) and subcutaneous pentylenetetrazole (sc PTZ) induced seizure models in mice. The size and nature of groups at C-2 position of 1,3,4-oxadiazole ring are very important on anticonvulsant activity in both PTZ and MES models. In addition, the size of electron withdrawing substituent at ortho position of benzylxy moiety is also important for their anticonvulsant effects. A series of isonicotinic acid hydrazide incorporated derivatives of 1,3,4-oxadiazoles has been synthesized by Sadaf Jamal Gilani et al [82]. 1,3,4-Oxadiazole ring systems were active in MES test at a dose of 300 mg/kg indicative of their ability to protect the seizure spread. The compounds showed protection at a dose of 100 mg/kg after 0.5 h. These compounds also showed protection after 4h but at a higher dose of 300 mg/kg.

A new series of 2-substituted-5-{2-[(2-halobenzyl)thio]phenyl}-1,3,4-oxadiazoles was synthesized by Afshin Zarghi et al [83]. The designed compounds contain the main essential pharmacophore for binding to the benzodiazepine receptors. The structure-activity relationship study of these compounds indicated that the introduction of an amino
group at position 2 of 1,3,4-oxadiazole ring and a fluoro substituent at the ortho position of the benzylthio moiety had the best anticonvulsant activity. Anticonvulsant effects of active compounds were antagonized by flumazenil, a benzodiazepine antagonist, which establishes the involvement of benzodiazepine receptors in these effects.

A series of novel \( N^1\)-(5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl)-\( N^4\)-[1-(4-substitutedphenyl)(phenyl)methanone]-semicarbazones were designed and synthesized on the basis of semicarbazone based pharmacophoric model to meet the structural requirements necessary for anticonvulsant activity by Harish Rajak et al [84]. The anticonvulsant activity of the compounds was investigated using maximal electroshock seizure (MES), subcutaneous pentylenetrazole (scPTZ) and subcutaneous strychnine (scSTY) models. A novel semicarbazone based 2,5-disubstituted 1,3,4-oxadiazoles possessing four vital structural features hydrophobic aryl ring system, hydrogen binding domain, electron donor moiety and distal aryl ring required for anticonvulsant activity are disclosed. The aryl semicarbazones have been found to possess anticonvulsant activity through GABA mediation.

A novel series of 3-[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2 phenylquinazoline-4(3H)-ones [85] have been synthesized and screened for their anticonvulsant and neurotoxic activities. After i.p. injection to mice at doses of 30, 100, and 300 mg/kg body weight and they were examined in the maximal electroshock induced seizures (MES) and subcutaneous pentylenetrazole (scPTZ) induced seizure models in mice. The compounds showed protection at a dose of 30,100 and 300 mg/kg after 0.5 h and 4.0 h. Two novel series of (Z)-\( N\)-[5-(4-substituted)phenyl-1,3,4-oxadiazol-2-yl]-2-[(Z)-3,7-dimethylocta-2,6-dien-1-ylidene]hydrazinecarboxamide and 5-(4-substituted)phenyl-\( N\)-[(Z)-3,7-dimethylocta-2,6-dien-1-ylidene]-1,3,4-oxadiazol-2-amine [86] have been synthesized and screened for their anticonvulsant and neurotoxic activities. After i.p. injection to mice at doses of 30,100 and 300 mg/kg body weight, 2,5-disubstituted-1,3,4-oxadiazole analogues were examined in the maximal electroshock induced seizures (MES) and subcutaneousmetrazole (ScMET) induced seizure models in mice. Amongst all the compounds, some compounds exhibited activity at 100 mg/kg body weight at 0.5 and 4 h in ScMET method. The neurotoxicity was assessed by rotorod method and some of the compounds were found to be safe at maximum administered dose.
Tabatabai et al. [87] synthesized 2-(2-phenoxy)phenyl-1,3,4-oxadiazole derivatives. Anticonvulsant activity of the synthesized compounds was determined by pentylenetetrazole-induced lethal convulsion test and showed that the introduction of an amino substituent in position 5 of 1,3,4-oxadiazole ring generates considerable effect. The results are in agreement with SAR of benzodiazepine receptor ligands since the elimination of electronegative substituent in position 2 of phenoxy ring or position 4 of phenyl ring reduces the anticonvulsant activity. A series of new 1,2,4-oxadiazole derivatives [88] containing 3,4-dihydro-2H-chromen-2-amine moiety were synthesized and screened for their anticonvulsant properties. Few of the compounds exhibited excellent anticonvulsant activity as compared to the standard drug diazepam. A series of novel 1,3,4-oxadiazole derivatives of phthalimide [89] were synthesized and evaluated for their anticonvulsant and neurotoxicity studies. All the compounds were active in MES screen and less neurotoxic than phenytoin. Compounds having methoxy substitution at para position of the distal aryl ring emerged as most promising anticonvulsant agent with low neurotoxicity.

A series of new 1,3,4-oxadiazole and 1,2,4-triazole derivatives were synthesized by Yan et al. [90]. The compounds were evaluated for their antiproliferative activity against K562 (human erythromyeloblastoid leukemia cell line), MDA-MB-231 (human breast adenocarcinoma cell line), HT29 (human colon adenocarcinoma grade II cell line) and HepG2 (human breast adenocarcinoma cell line) in vitro. The results showed that 7 compounds displayed inhibitory activities against K562 with the inhibition rate more than 50%. Especially, few compounds exhibited the most potent activity against K562 with 85% inhibition ratio and could be used as lead compounds to search newer 1,3,4-oxadiazole derivatives as antiproliferative agents. Luo et al. [91] synthesized a series of novel 1,3,4-oxadiazole derivatives based on benzisoselenazolane and tested for antiproliferative activity in vitro against the human cancer cell lines, SSMC-7721 (human liver cancer cell), MCF-7 (human breast cancer cell) and A549 (human lung cancer cell). All the compounds obtained exhibited antiproliferative activity and showed selective cytotoxicity against different cancer cells.

Samir et al. [92] explained the synthetic strategies and characterization of some novel 1,3,4-oxadiazole derivatives carrying different pharmacophores and heterocyclic rings that are relevant to potential antitumor and cytotoxic activities. The antitumor activity of the newly synthesized compounds was evaluated according to the protocol of the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel.
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assay. The results revealed that five compounds displayed promising \textit{in vitro} antitumor activity in the 4-cell lines assay. Incorporating a thiazole ring to 1,3,4-oxadiazole skeleton resulted in better antitumor activity than those displayed by the pyrazole and thiophene ring systems. The results of the anticancer screening revealed that five compounds were found to exhibit variable degrees of anticancer activity against the four cell lines used.

Pushpan \textit{et al.} [93] designed and synthesized a novel combinatorial library of S-substituted-1,3,4-oxadiazole bearing \textit{N}-methyl-4-(trifluoromethyl) phenyl pyrazole moiety and tested for \textit{in vitro} cytotoxic activity by MTT assay. Amongst the tested compounds, few compounds showed the most promising anticancer activity with IC50 value of 15.54 Mm in MCF-7 cells compared to doxorubicin as standard drug. Three dimensional quantitative structure activity relationship (3D QSAR) study by means of partial least square regression (PLSR) method was performed on a series of 3-(aryl)-N-(aryl)-1,2,4-oxadiazole-5-amines as antiproliferative agents using molecular design suite by Sanmati \textit{et al} [94]. This study was performed with 20 compounds using sphere exclusion (SE) algoitham and manual selection method used for the division of the data set into training and test set. The molecular field analysis (MFA) contour plots provided further understanding of the relationship between structural features of the substituted oxadiazole derivatives and their activities, which should be applicable to design newer potential antiproliferative agent.

1.1.3. Introduction to pyrimidines

Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring (17). It is isomeric with two other forms of diazine.

Pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases, the ring electrons become less energetic and electrophilic aromatic substitution gets more difficult while nucleophilic aromatic substitution gets easier. Pyrimidines can also be prepared within the laboratory by organic synthesis. One
method is the classic Biginelli reaction. Three nucleobases found in nucleic acids (cytosine, thymine, and uracil) are pyrimidine derivatives [95].

Pyrimidines and its derivatives are integral part of DNA and RNA, and found to be associated with diverse biological activities. The substituted pyrimidines are complex molecules because of natural substituents. Uracil and thymine may be considered to contain neutral urea unit or acidic imide moiety. Thymine is also referred to as 5-methyluracil. The metabolism of these pyrimidines is unique and important to understand both biochemical utilization of these compounds and drug metabolism of pyrimidine derivatives. Uracil is converted into a useful uridylic acid needed for the synthesis of RNA. Thymine is metabolized by conjugation via salvage pathway with PRPP to the thymine ribosyl-5-phosphate. This form of thymidylic acid can be utilized in specific RNA molecule. In a similar manner cytosine is conjugated with PRPP to yield cytosine-5-monophosphate or cytidylic acid. Pyrimidine is the most important member of all the diazines as this ring system occurs widely in living organisms [96-100]. Pyrimidine and its derivatives have gained prominence because of their potential pharmaceutical values. Several pyrimidine derivatives play vital role in many physiological actions. They are among those molecules that make life possible as being some of the building blocks of DNA and RNA.

Pyrimidine is considered to be a resonance hybrid of the charged and uncharged canonical structures and its resonance energy has been found to be less than benzene or pyridine [101-104]. The naturally occurring pyrimidine derivative was first isolated by Gabrial and Colman in 1870, and its structure was confirmed in 1953 as 5-β-D-glucopyranoside of divicine. Pyrimidines and their derivatives are considered to be important for drugs and agricultural chemicals. The use of pyrimidines is critical to successful treatment of various diseases. Many pyrimidine derivatives used for thyroid drugs and leukaemia are shown below.
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The pyrimidine moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. In the last few years, the therapeutic interest of pyrimidine derivatives in pharmaceutical and medicinal fields has been given great attention to the medicinal chemist. Literature survey reveals that pyrimidine derivatives are well known to have antimicrobial [105-107], antimalarial [108], anticancer [109-112], anti-inflammatory, analgesic [113] and antimycobacterial activities [114-116]. In recent years, the extensive studies have been focused on pyrimidine derivatives because of their diverse chemical reactivity, accessibility and wide range of biological activities.

An efficient method has been described by Shantaram et al. [117] for the synthesis of 6-(substituted aryl)-4-(3,5-diphenyl-1H-1,2,4-triazol-1-yl-1,6-dihydropyrimidine-2-thiol as beneficial antimicrobial, anticonvulsant and anticancer agents. Compounds were screened for their in vitro antimicrobial activity by agar well method and their anticonvulsant activity by the MES model. Anticancer activity of the two newly synthesized heterocycles was evaluated at National Cancer Institute (NCI), USA against 60 cell lines of different human tumor at a single dose of 10^{-5} M. Ten novel dihydropyrimidine analogues have been synthesized, characterized and found to be promising antibacterial and anticonvulsant agents.

Li et al. [118] synthesized a novel series of 7-substituted-[1,2,4]triazole[4,3-f]pyrimidine derivatives as potential anticonvulsant agents. The anticonvulsant activity was evaluated by the maximal electroshock (MES) test, and their neurotoxicities were
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evaluated by the rotarod neurotoxicity test. The pharmacological results showed that the compound 7-(4-chlorophenoxy)-[1,2,4]triazolo[4,3-f]pyrimidine was among the most active agent with median effective dose (ED$_{50}$) value of 34.7 mg/kg, median toxicity dose (TD$_{50}$) of 262.9 mg/kg, and providing a protective index value of 7.6. Few compounds also showed oral activity against MES-induced seizures and lower oral neurotoxicity. A series of N-(4,6-substituted diphenylpyrimidine-2-yl)semicarbazones were synthesized by Alam et al [119] and tested for their anticonvulsant activity against the two seizure models, maximal electroshock seizure (MES) and subcutaneously pentylenetetrazole (scPTZ). All the synthesized compounds possesses the four essential pharmacophoric elements for good anticonvulsat activity. Most of the compounds displayed good anticonvulsant activity with lesser neuroxicity. To assess the unwanted effects of compounds on liver, estimation of enzymes and proteins were carried out.

Jiang et al. [120] synthesized several new 7-substituted-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidines through incorporating triazole moiety into the pyrimidine ring, which are expected to have the synergistic effects in dealing with the epilepsy. Their anticonvulsant activities were measured through the Maximal Electroshock Seizure (MES) test. Among the compounds tested, 7-(heptyloxy)-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine showed potent anticonvulsant activity which was weaker than carbamazepine, but better than valproate. Carbamazepine and valproate were considered as positive control drugs. Wang et al [121] synthesized a series of 5-alkoxytetrazolo[1,5-c]thieno[2,3-e]pyrimidine derivatives and their anticonvulstant and antidepressant activities were evaluated. Pharmacological tests showed that, four of the synthesized compounds had weak anticonvulsant activity, while most of the compounds had excellent antidepressant activity. The most active compound was 5-(2,4-dichlorobenzol oxy)tetrazolo[1,5-c]thieno[2,3-e]pyrimidine which decreased the immobility time by 51.61 % at a dose of 100 mg/kg. The results of open-field of this compound indicated that it had no significant effects on the locomotor activity compared with the control group at the dose assayed in the forced swimming tests. This means that the antidepressant activity detected in the FST for the compound is not the result of central nervous system stimulant properties, and further confirms its antidepressant–like effect.

Free radicals are well known for playing a dual role in our body—deterious as well as beneficial. It includes a metabolic pathway for its generation. Oxidative stress in our body occurs due to excessive generation of free radicals and reduced level of antioxidants, but at low concentration, these radicals help to perform normal physiological function of the
body. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Bano et al. [122] review shows the current tendency in the pyrimidine synthesis and revealed the pyrimidine core to be a very potent moiety which can be a rich source for the synthesis of new compounds having desirable antioxidant activity.

Abu-Hashem et al. [123] synthesized a series of pyrimidine derivatives and were subjected for their antioxidant activity. Out of the synthesized compounds, two compounds manifested potent antioxidant activity by lipid peroxidation assay. The authors concluded that thiazolopyrimidine derivatives incorporated with carbahydrazide, amino, pxadiazol and other moieties possess potencial antioxidant activities. Gressler et al. [124] synthesized a series of 4-trifluoro-methyl-2-(5-arytl-3-styryl-1H-puazol-1 yl)-pyrimidine derivatives and were screened for their in vitro antioxidant activity. The antioxidant activity was evaluated using the DPPH and HRP/H2O2 chemiluminescence assay methods. The DPPH antioxidant assay measures the hydrogen-donating capacity of the molecules in the sample. On the other hand, the chemiluminescence method is based on the light emission produced by a chemical reaction. Mahesh et al. [125] carried out the synthesis of pyrimidine derivatives and were investigated for their in vitro antioxidant activity. The results revealed that, some of the tested compounds showed potent antioxidant activity. Among the compounds few have shown moderate activity. The remaining compounds were found to be weakly active.

1.2. Introduction to biological activity

1.2.1. Antibacterial activity

Diseases caused by microbial infection are a serious menace to the health of human beings, and often have connection to some other diseases whenever the body system gets debilitated. In order to combat these diseases, a large number of drugs are available in clinical practice ranging from natural product antibacterial to tailor-made antibacterial drugs. Developing antimicrobial drugs and maintaining their potency in opposition to resistance by different classes of microorganisms as well as a broad spectrum of antimicrobial activity are some of the major concern of research in this area. The health problem demands to search and synthesize a new class of antimicrobial compounds which are effective against pathogenic microorganisms, and develop resistance to the antibiotics used in the current regime [126]. The increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem. During the 20th century, vaccines for
bacterial toxins and many other common acute viral infections were developed and made widely available. There are thirty vaccines that are mainly given prophylactically to prevent or minimize diseases by agents infectious to human. The World Health Organization (WHO) estimates that sixty percent of hospital-acquired (nosocomial) infections are drug-resistant [127-129].

Various different classes of antibacterial [130, 131] and antifungal agents [132] have been discovered. Although, since the discovery of several synthetic and semi-synthetic antibacterial sulfa drugs, nitrofuranes, penicillins, cephalosporins, tetracyclines, macrolides and oxazolidinones, and antifungal agents such as fluconazole, ketoconazole and miconazole, including amphotericin B, there has been much progress in this field. Despite advances in antibacterial and antifungal therapies, many problems remain to be solved for most antimicrobial drugs available. The extensive use of antibiotics has led to the appearance of multi-drug resistant microbial pathogens [133]. This highlights the incessant need for the development of new classes of antimicrobial agents, and alteration of known drugs in such a way that would allow them to retain their physiological action, but reducing their resistance to the pathogen. The design of novel chemotherapeutic agents is particularly beneficial due to their dissimilar mode of action which can avoid cross resistance to known drugs.

Antibacterial drug discovery research accompanied by clinical development has historically been conducted by large pharmaceutical companies. Although the earliest antibiotics were first identified in academic laboratories such as those of Alexander Fleming (Penicillium notatum) [134] and Selman Waksman (Streptomyces griseus) [135], the pharmaceutical companies were responsible for successful strain optimization, compound scale-up, formulation and clinical development activities that allowed anti-infective drug research to gain prominence as a viable area for corporate investment.

Microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: (a) Antibiotics which are natural substances produced by certain groups of microorganisms and (b) Chemotherapeutic agents which are chemically synthesized. A hybrid substance is a semi synthetic antibiotic wherein a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds originally discovered as products of microorganisms were synthesized entirely by chemical means. They might be referred
to as synthetic antibiotics to distinguish them from the chemotherapeutic agents. The most important property of an antimicrobial agent, from a host point of view, is its selective inhibition, i.e., the agent acts in some way that inhibits or kills bacterial pathogens but has little or no toxic effect on the host. This implies that the biochemical processes in the bacteria are in some way different from those in the animal cells, and that the advantage of this difference can be taken in chemotherapy [136]. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MIC values can be determined by a number of standard test procedures.

Antimicrobial susceptibility testing methods are divided into two types based on the principle applied in each system. They include disc diffusion method (Stokes method and Kirby-Bauer method) and dilution method (Broth dilution and Agar dilution). The most commonly employed method is disc diffusion method. Antimicrobial agents are any chemical or biological agents that either destroy or inhibit the growth of microorganisms. Some antibacterial agents are:

(a) Ampicillin: Ampicillin is a beta-lactam antibiotic that has been used extensively to treat bacterial infections since 1961. It demonstrated the activity against Gram-negative organisms such as *H. influenzae*, *Coliforms* and *Proteus species*. Ampicillin was the first member of so-called broad spectrum penicillins, subsequently introduced by Beecham. Ampicillin is part of the aminopenicillin family and is roughly equivalent to its successor, amoxicillin in terms of spectrum and level of activity.

(b) Ciprofloxacin: Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a second generation fluoroquinolone antibacterial. It kills bacteria by interfering with the enzymes that cause DNA to rewind after being copied, which stops DNA and protein synthesis.

(c) Gentamicin: Gentamicin is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative bacteria. However, gentamicin is not used for *Neisseria gonorrhoeae*, *Neisseria meningitidis* or *Legionella pneumophila*. It was synthesized by *Micromonospora*, a genus of Gram-positive bacteria widely present in the environment (water and soil).
(d) **Penicillin**: Penicillin constitutes one of the most important groups of antibiotics. These are bactericidal, and act by interfering with the synthesis of bacterial peptidoglycan cell wall. Penicillin-G and Penicillin-V have a useful antimicrobial spectrum against *Streptococci, Pneumococci, Gonococci, and Meningococci*.

(e) **Rifampicin**: Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is active against Gram-positive bacteria as well as many Gram-negative species. It enters phagocytic cells and kills intercellular microorganisms including the tubercle bacillus.

(f) **Sulfadiazine**: Sulfadiazine is a sulfonamide antibiotic. It eliminates bacteria that cause infections by stopping the production of folic acid inside the bacterial cell, and is commonly used to treat urinary tract infections (UTIs). In combination, sulfadiazine and pyrimethamine can be used to treat toxoplasmosis, a disease caused by *Toxoplasma gondii*.

(g) **Streptocycline**: Streptocycline (bacteromycin) is an antibiotic formulation recommended for effective control of bacterial diseases of plants.

1.2.2. **Antifungal activity**

The incidence of fungal infections has increased significantly in the past two decades [137]. The first generation antifungal inhibitors of CYP51 have revolutionized the treatment of some serious fungal infections. Triazoles have been the leading agents for the control of fungal diseases of humans and animals for over 20 years [138, 139]. According to this, azole derivatives are currently the most widely studied class of antifungal agents. Nystatin is a polyene-macrolide antifungal antibiotic produced by *Streptomyces noursei* that was discovered and developed in 1950 [140]. Toxicity problems prevented its use as a systemic agent, but recently developed liposomal delivery technologies have made it an attractive candidate for the treatment of severe systemic fungal infections [141]. This has prompted new investigations of its antifungal properties and spectrum as well as its physicochemical and pharmacokinetic characteristics [142]. The development of resistance among pathogens to routinely used pesticides demands that a renewed effort should be made to seek antimicrobial agents which are effective against pathogenic microbes [143]. Oral candidiasis is a common oral lesion caused by overgrowth of fungal species in the *Candida* (genus). Among many species, *Candida albicans* is the most important microorganism implicated in fungal infection [144]. Almost all antifungal agents currently
used in human mycoses target the ergosterol biosynthetic pathway, an important component of fungal membranes.

Fungal infections vary widely with respect to clinical picture and mainly include superficial mycoses involving infections to skin, hair, mucous membranes and nails. Fungal infections are being recognized with increasing frequency as an important cause of both morbidity and mortality. Some antifungal drugs could also help to kill fungi which infect the human body. Many of the drugs currently available have undesirable side effects and might be toxic. It is very important to explore additional sources for substances with potential antifungal activity, which could possibly have different modes of activity or affect different sites in the fungal cells. In view of widespread resistant strains of microorganism, there is an urgent need for the development of new antimicrobial agents to treat the patients infected with multidrug-resistant bacteria and fungi. Some antifungal agents are:

(a) **Nystatin**: Nystatin is a polyene antifungal drug to which many molds and yeast infections are sensitive, including *Candida*. It is now widely available for the topical treatment of localized fungal infections.

(b) **Amphotericin B**: Amphotericin B is a polyene antifungal drug, often used intravenously for systemic fungal infections. Two amphotericins, Amphotericin A and Amphotericin B are known, but only B is used clinically because it is significantly more active *in vivo*. Amphotericin A is almost identical to Amphotericin B but has little antifungal activity.

(c) **Hamycin**: Hamycin is a polyene antimycotic organic compound. It is a heptaene antifungal compound rather similar in chemical structure to amphotericin B except that it has an additional aromatic group bonded to the molecule. It is obtained from a strain of streptomycyes bacteria growing in soil i.e., *Streptomyces pimprina*.

**1.2.3. Antioxidant activity**

Oxygen, an element indispensable for life, can under certain circumstances, adversely affect the human body. It is produced by plants during photosynthesis, and is necessary for aerobic respiration in animals. The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). Free radical (FR) is a chemical species or an atom or a molecule that has one or more unpaired
electrons in its valence shell and is capable of existing independently. Free radical contains an odd number of electrons which makes it unstable, short lived and highly reactive. Therefore, it reacts quickly with other compounds in order to capture the needed electron to gain stability. Generally, free radical attacks the nearest stable molecule, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction cascade resulting in disruption of a living cell [145, 146].

The most common free radicals are derivatives of oxygen like superoxide free radical anion \( \text{O}_2^\cdot^- \), hydroxyl free radical (OH·), lipid peroxyl (LO·), lipid alk oxyl (LOO·) and lipid peroxide (LOOH) as well as non-radical derivatives such as hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and singlet oxygen \( \left( ^1\text{O}_2 \right) \) which are collectively known as ROS. These free radicals/reactive oxygen species are produced mainly from two important sources in the biological system, i.e., cellular metabolism like mitochondrial electron transport chain, endoplasmic reticulum oxidation, NADPH oxidase, xanthine oxidase, prostaglandin synthesis, reduced riboflavin, nitric oxide synthetase, reperfusion injury, cytochrome P\textsubscript{450}, activated neutrophils and phagocytic cells and environmental sources like drugs, pesticides, transition metals, tobacco smoke, alcohol, radiations and high temperature. Owing to the great potentiality of free radicals to react with various compounds by electron transfer, proton transfer, H-atom abstraction or addition reaction, they are considered responsible for a series of undesired processes such as aging, material degradation, food deterioration and many diseases [147].

Free radical and ROS production in the animal cell is inevitable. Normally, there is an equilibrium between a free radical/reactive oxygen species formation and endogenous antioxidant defense mechanisms, but if this balance is disturbed, it can produce oxidative stress [148]. There are two basic categories of antioxidants namely synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines) or carotenoids as well as ascorbic acid [149, 150].

The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines [151]. These antioxidants act usually both through chain transfer and chain termination. The first step of the reactive radical’s termination by this type of antioxidants is hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate [152]. Small amounts of antioxidants are added into most synthetic polymers
to prevent or retard oxidation and to increase the service lifetimes of the products [153]. Free radicals and active oxygen species have been related with cardiovascular and inflammatory diseases, and even with a role in cancer and ageing [154]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants [155].

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma while lipid-soluble antioxidants protect cell membranes from lipid peroxidation [156]. These compounds may be synthesized in the body or obtained from the diet [157]. The amount of protection provided by any antioxidant will depend on its concentration, its reactivity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts. The relative importance and interactions between these different antioxidants is a very complex question with the various metabolites and enzyme systems having synergistic and interdependent effects on one another [158].

Phenolic derivatives are one of the groups of antioxidants that have been studied by many research groups. A great number of examples have been described in the literature, such as caffeic acid and its analogues which are known to have antiviral and antiatherosclerotic properties [159], resveratrol with known anticancer and heart protecting effects [160] and olive oil phenols, particularly hydroxyl tyrosol which inhibits human low-density lipoprotein (LDL) oxidation (a critical step in atherosclerosis) [161] inhibits platelet aggregation [162] and exhibits anti-inflammatory [163] and anticancer properties [164]. Phenols have been utilized extensively for food preservation.

Various analytical methods have been developed to measure the radical scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, the superoxide anion radical (O2), the hydroxyl radical (OH) or the peroxyl radical (ROO). These methods are used to measure the antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant. There are other methods which determine the resistance of lipid or lipid emulsions to oxidation in the presence of the antioxidant being tested.
DPPH is a well-known radical and a scavenger for other radicals. DPPH has two major applications in laboratory research. (a) To monitor the chemical reactions involving radicals and (b) The number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the electron paramagnetic resonance signal of the DPPH. The molecule of DPPH (18) is characterized as a stable free radical by virtue of the delocalization of the electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour which is characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (19) with the loss of this violet colour. Representing the DPPH radical by \( \dot{Z} \) and the donor molecule by \( AH \), the primary reaction is

\[
\dot{Z} + AH = ZH + \dot{A}
\]

where \( ZH \) is the reduced form and \( \dot{A} \) is free radical produced in this step. The latter radical will then undergo further reactions which control the overall stoichiometry, i.e., the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant.

The above reaction is therefore intended to provide the link with the reactions taking place in an oxidizing system such as the autoxidation of a lipid or other unsaturated substance and the activity of \( Z \) is suppressed by \( AH \). At the present time, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate, tert-butyl hydroquinone, Vitamin E and Vitamin C.

### 1.2.4. Anticonvulsant activity

Epilepsy is a major neurological disorder affecting a large section of people both male and female throughout the world. Currently available drugs for the treatment of epilepsy are symptomatically effective in only 60-70 % of patients. Every year
approximately 2,50,000 new cases are added to this figure. Epilepsy also poses a considerable economic burden on society. The direct costs of epilepsy vary significantly depending on the severity of the disease and the response to treatment. The known potential causes of epilepsy include brain tumors, infections, traumatic head injuries, perinatal insults, developmental malformations, cerebrovascular diseases, febrile seizures and status epilepticus [165]. Many patients have seizures that are resistant to the available medical therapies. Although 70 – 80 % of epileptics are currently controlled by a variety of drugs, seizure protection is often accompanied by numerous side effects including drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, hirsutism and megaloblastic anemia [166].

Epileptic seizures can be generalized (generalized epileptic seizure), originating in both hemispheres of the brain simultaneously or partial (focal seizures), originating in one or more parts of one or both hemispheres, most commonly the temporal lobe. With generalized seizures, consciousness is always impaired or lost. Consciousness may be maintained in partial seizures but partial seizures may become generalized seizures in a process referred to as secondary generalization, at which point consciousness is lost. In patients, the types of epilepsy or epileptic syndrome are further classified according to features such as the type of seizure, etiology, age of onset and electroencephalogram. Epilepsy or epileptic syndromes can be either idiopathic (etiology or cause is unknown) with a presumed genetic basis or symptomatic (acquired). The pharmacological strategies for the treatment of epilepsy are aimed at suppressing the initiation or propagation of seizures rather than the underlying processes that lead to epilepsy. Some epileptic patients are unresponsive to current antiepileptic drug treatment, and for this reason the major goal in epilepsy research has been to develop drugs with greater anticonvulsant efficacy and less toxicity than existing drugs [167]. There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally-induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically epilepsy-prone animals [168, 169].

The anticonvulsants are a diverse group of pharmaceuticals used in the treatment of epileptic seizures. Anticonvulsants are also increasingly being used in the treatment of bipolar disorder, since many seem to act as mood stabilizers. The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure. Failing this, an effective anticonvulsant would prevent the spread of the seizure within the brain and offer
protection against possible excitotoxic effects that may result in brain damage. Some studies have site that anticonvulsants themselves are linked to lower IQ in children [170]. Anticonvulsants are more accurately called antiepileptic drugs (AEDs), sometimes referred to as antiseizure drugs. While an anticonvulsant is a fair description of AEDs, it neglects to differentiate the difference between convulsions and epilepsy. Convulsive non-epileptic seizures are quite common and these types of seizures will not have any response to an antiepileptic drug. In epilepsy, an area of the cortex is typically hyperirritable that can often be confirmed by completing an electroencephalogram (EEG).

Conventional AEDs such as phenobarbital, primidone, phenytoin, carbamazepine, ethosuximide and benzodiazepine are widely used, but exhibit an unfavourable side effect profile and failure to adequately control seizures. These include older ‘first generation’ drugs such as carbamazepine, phenobarbital, valproic acid and newer second generation drugs such as lamotrigine, vigabatrin, tiagabine, topiramate, gabapentin and levetiracetam [171]. These newer drugs have proven to be effective in reducing seizure whilst their therapeutic efficacy is overcome by some undesirable side effects such as headache, nausea, hepatotoxicity, anorexia, ataxia, drowsiness, gastrointestinal disturbances and hirsutism [172]. These observations affirm the further scope and need for the development of newer agents. The long-established AEDs control seizures in 50 % of patients developing partial seizures and in 60-70 % of those developing generalized seizures [173-176].

The selection of an antiepileptic drug for treatment is predicated on its efficacy for the specific type of seizures, tolerability and safety [177, 178]. Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy. A review on new structural entities having anticonvulsant activity has recently appeared [179]. The first strategy is the identification of new targets through better understanding of molecular mechanisms of epilepsy. Another way is to modify already existing drugs and formulations. AEDs belong to many different chemical classes of compounds including hydantoines, iminostilbenes, barbiturates, benzodiazepines, valproate, imides, oxazolidine-2,3-diones, sulphonamides and miscellaneous agents [180]. The efficacy of AEDs is due to the main activities which include interaction with ion channels or neurotransmitter systems [181-183]. Currently available AEDs can be broadly classified into four categories, (1) those whose main action relates to the inhibition of sustained repetitive firing through blockage of voltage-dependent sodium channels and consequent inhibition and release of
excitatory neurotransmitters (phenytoin, carbamazepine, oxcarbazepine) (2) those which enhance GABA-ergic transmission (benzodiazepines, barbiturates, vigabatrin, tiagabine) (3) those stabilizing thalamic neurons through inhibition of T-type calcium channels (ethosuximide) and (4) those possessing a combination of the above actions, often coupled with additional mechanisms (valproic acid, gabapentin, lamotrigine, topiramate, zonisamide, felbamate). However, this classification has limited value because the majority of AEDs possess more than one mechanism of action, which may account for their efficacy, and it is also the fact that some of the clinically used drugs have not been linked with a specific site of brain, and the exact mechanism of many AEDs remain unknown [184, 185].

The new AEDs and anticonvulsant agents have been reviewed during last few years [186]. The chemical diversity and various mechanisms of action of anticonvulsants make it difficult to find a common way of identifying new drugs. Novel anticonvulsant agents are discovered through conventional screening and/or structure modification rather than a mechanism driven design. Therefore, drug identification is usually conducted via \textit{in vivo} screening tests on the basis of seizure type rather than etiology. Phenytoin (20) is still one of the most commonly used antiepileptic drugs. Today, in addition to phenytoin, several AEDs are widely used in the treatment of the various forms of epilepsy [187]. Some of the other standard drugs include ethosuximide, valproic acid and various benzodiazepines have been used for anticonvulsant activity.

![Chemical Structure](image)

Many of the compounds presented in the review of the literature have been tested according to the procedure established by the Antiepileptic Drug Development (ADD) Program [188]. The anticonvulsant screening project use for its initial screening procedure includes two major convulsant tests, the maximal electroshock seizures (MES) and the subcutaneous pentylenetetrazole (scPTZ) as well as a toxicity screen (rotorod in mice, positional sense and gait in rats). The MES is a model for generalized tonic-clonic seizures. The behavioral and electrographic seizures generated in this model are consistent with the
human disorder \[189\]. This model identifies those compounds which prevent the spread of seizures. The scPTZ seizure test is a model which primarily identifies compounds that raise seizure threshold. The behavioral seizure produced is not typical of absence epilepsy but clonic in nature. Like other rodent models of absence seizures, PTZ-induced seizures are potentiated by \(\gamma\)-aminobutyric acid (GABA) agonist. With some minor exceptions, the pharmacological profile of the scPTZ seizure model is consistent with the human condition \[190\]. All clinically active anticonvulsants have been found to be protective in at least one of these two tests.

### 1.2.5. Neurotoxicity

Neurotoxicity is concerned with the adverse changes in the structure or function of the nervous system. A neurotoxin is considered to be a substance which elicits a pathological response primary or specifically on the nervous system. The complexity of the nervous system results in a broad range of potential targets and adverse squeal, since the activity of the nervous system balance between all the various organs in the body.

The importance of the potential impact of chemicals on human neurological function has been recognized by the organization for Economic Co-operation and Development (OECD). The deleterious effects of chemicals may not become clinically evident for some time following exposure, aptly called the silent period. Such latent toxicity has been defined as ‘persistent morphological or biochemical injury’ which means clinically unapparent \[191\]. Several hypotheses have been proposed to explain the asymptomatic period prior to clinical expression of neurotoxic injury. Exposure to neurotoxic chemicals may cause cell death of a subpopulation of neuronal cells, but the total number of cells lost is insufficient to cause adverse effects because of the reserve capacity regarding this fraction. Alternatively, toxic exposure may cause subethal injury to neuronal cells in critical areas of the brain such as the substantia nigra which leads to a progressive loss of function \[192\]. Initially this can be compensated for, although overtime loss of function arises due to the lack of plasticity of the brain \[191\]. Such hypothesis also exists for developmental neurotoxicity, exposure to chemicals or infections during the appropriate gestational age can result in fewer dopaminergic neurons. Although no clinical signs may be evident in early years, the subject may be predisposed to parkinson traits later on in life. This has lead to particular concern about exposure to low levels of environmental chemicals and effects on neurobehavioral/development in children and on the development of neurodegenerative
diseases in the adult. Neurodegenerative diseases are of great concern, bearing in mind the aging of the population. Alzheimer’s disease and Parkinson’s disease are the most common neurodegenerative diseases.

1.2.5.1. In vivo models of neurotoxicity

A multidisciplinary approach is required in order to adequately assess potential neurotoxic effects of compounds due to the diverse function of the nervous system. Many effects may be measured using neurophysiological (e.g. electroencephalography measurement of evoked potentials), neuropathological (e.g. microscopy, histochemistry, immunohistochemistry) or behavioural techniques [193].

The Functional Observational Battery (FOB) is a standardized screening battery for assessing many aspects of behaviour and neurological functions in rodents and is designed to detect and quantify major overt behavioural, physiological and neurological signs [194]. The tests have been validated with many known neurotoxic chemicals and are often used in conjunction with other measures of toxicity [195]. The FOB comprises a number of tests to identify specific deficits in motor and sensory functions by measuring neuromuscular, sensory and autonomic functions.

1.2.5.2. In vitro models of neurotoxicity

To address problems associated with increasing cost and time required for neurotoxicity testing, the large number of chemicals in commercial use that have not been investigated and animal welfare issues, considerable effort is being directed at the development of in vitro alternatives. These may be considered as part of the tiered system with which to identify potential neurotoxic chemicals, although such approaches have not been validated as replacements for animal studies. Several in vitro models are commonly used in neurotoxicity evaluations, including synaptic fractions, primary cultures of rat astrocytes, rat cerebellar granule neurones, primary motor neurones of dissociated cultures of murine spinal cord, rat brain region organ cultures and hippocampal slices [196].

1.2.6. Anticancer activity

Cancer is a class of diseases in which a group of cells display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues) and sometimes metastasis (spread to other locations in the body via lymph or
blood). These three malignant properties of cancer differentiate them from benign tumors which are self-limited and do not invade or metastasize. Worldwide, about 7.6 million people die from cancer every year [197]. Research on the anticancer drug discovery has significant attention over the last few years. The term cancer chemoprevention coined by Sporn and co-workers [198] in 1976 has been defined as a strategy for reducing cancer mortality by the prevention, delay or reversal of cancer by pharmaceutical agents capable of mediating the process of carcinogenesis [199]. The introduction of nitrogen mustard in 1940s can be considered the origin of antineoplastic chemotherapy targeting all tumor cells [200].

A need for effective anti-cancer therapeutic agents, as well as a well-defined pharmacokinetic property of the drug was felt. Induction of apoptosis is another indicator for drug activity in cancer cells [201]. Recently, many chemotherapeutic compounds have been shown to have antiproliferative effects by inhibiting cells cycle at certain checkpoints. Furthermore, apoptosis provides a number of clues with respect to effective anticancer therapy, and many chemotherapeutic agents reportedly exert their antitumor effects by inducing apoptosis in cancer cells [202]. Considerable attention has been devoted to the sequence of events with respect to the apoptotic cell death and its role in mediation of the lethal effects of the diverse antineoplastic agents. These studies have become a focus of interest in cancer chemotherapy to shed light on the mechanism of action of candidate drugs.

Leukaemia or leukemia is a cancer of the blood or bone marrow and is characterized by an abnormal proliferation (production by multiplication) of blood cells, usually white blood cells (leukocytes). Leukemia is a broad term covering a spectrum of diseases. In turn, it is part of the even broader group of diseases called hematological neoplasms. Chronic myelogenous (or myeloid) leukemia (CML) also known as chronic granulocytic leukemia (CGL) is a cancer of the white blood cells. It is a disease of hematopoietic stem cells characterized by hyper proliferation of often immature cells of the myeloid, megakaryocytic and erythroid lineages. The Bcr-abl protein activates multiple signalling pathways that promote cell proliferation, block apoptosis and decrease cell adhesion, thereby blocking maturation and causing release of immature cells into the blood. After several years, CML turns into blast crisis, a rapidly lethal disease resembling acute leukemia. CML is treated by chemotherapy, interferon therapy and allogeneic stem cell
transplantation. Imatinib mesylate (Gleevec or Glivec), which directly targets a molecular abnormality in certain types of cancer (CML, gastrointestinal stromal tumors).

New cancer targeted therapies that make use therapeutic antibodies or small molecules have made treatment more tumors specific and less toxic. Nevertheless, there remain several challenges to the treatment of cancer, including drug resistance, cancer stem cells and high tumor interstitial fluid pressure. Cell birth and death rates determine adult body size and the rate of growth in reaching the appropriate size. In some adult tissues, cell proliferation occurs continuously as a constant tissue-renewal strategy. Identification of novel, efficient, selective and less toxic anticancer agents remains an important and challenging goal in medicinal chemistry. The past two decades have seen a dramatic change in cancer treatment paradigms. Similarly, cell cycle-mediated apoptosis is also gaining importance because certain compounds are believed to function via this pathway [203]. In the recent years, the number of the anticancer drugs such as 5-fluorouracil (5-FU) [204, 205], doxorubicin [206], palcitaxol [207], methotrexate [208], camptothecin [209], cytarabine [210], cis-platinum [211], taxotere [212] and combrestatin [213] have been reported as clinical anticancer drugs.

Cytotoxicity assay is widely used in in vitro toxicology studies. The lactate dehydrogenase (LDH) leakage of cytotoxicity or cell viability following exposure to toxic substances. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a water soluble tetrazolium salt which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. The formazan product is impermeable to the cell membranes assay, protein assay, neutral red assay and methyl tetrazolium (MTT) assay are the most commonly employed for the detection and therefore it accumulates in healthy cells. The MTT assay was tested for its validity in various cell lines [214]. More recent evidence suggests that reduction of MTT can also be mediated by nicotinamide adenine dinucleotide (NADH) within the cells and out of mitochondria [215]. Further modification of the initial protocol was proposed [216] in order to improve the repeatability and the sensitivity of the assay.

1.3. Scope of the present work

Heterocycles containing triazole, oxadiazole and pyrimidine groups were proven to be biologically very potent and selective. A wide spectrum of pharmacological activities has been reported for these compounds. In view of the above facts, triazole, oxadiazole and
pyrimidine derivatives were synthesized and characterized by different spectral studies. These derivatives were screened for their biological activity. It is expected that these would result in highly potent and selective pharmaceutical agents. Chapter II describes the synthesis of new fluorinated schiff bases derived from 1,2,4-triazoles and their antiproliferative activity. New compounds were structurally characterized by $^1$H NMR, $^{13}$C NMR, LC-MS, FT-IR and elemental analyses. The synthesized compounds showed good antiproliferative activity. Chapter III describes the synthesis, characterization and *in vitro* antimicrobial evaluation of new 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-c]pyrimidines. A series of new 5-chloro-8-bromo-3-aryl-1,2,4-triazolo[4,3-c]pyrimidines have been accomplished in excellent yields by the oxidative cyclization of pyrimidinehydrazines of various aryl aldehydes with iodobenzene diacetate in methanol. The chemical structures of the synthesized compounds were confirmed by elemental analysis, LC-MS, FT-IR $^1$H NMR and $^{13}$C NMR spectral studies. Chapter IV describes the synthesis 1,3,4-oxadiazoles bearing 5-chloro-2-methoxyphenyl moiety by the reactions of acid hydrazide with different aromatic carboxylic acid in the presence of phosphorous oxychloride. New compounds were structurally characterized by different spectral studies and showed good antimicrobial activity. Chapter V describes the synthesis of some pyrimidine derivatives and their biological activity. New compounds were structurally characterized by different spectral studies. The synthesized compounds showed good anticonvulsant and antioxidant activity. Chapter VI describes the synthesis of $N$-[[5-aryl-1,3,4-oxadiazole-2-yl]methyl]-4-methoxyaniline derivatives and their anticonvulsant activity. A series of new of $N$-[[5-aryl-1,3,4-oxadiazole-2-yl]methyl]-4-methoxyaniline have been accomplished in excellent yields by the intramolecular oxidative cyclization of E)-2-(arylbenzylidene)-2-[[4-methoxyphenyl]amino]acetohydrazides of various aryl aldehydes with iodobenzene diacetate in methanol. The anticonvulsant activity of the nine newly synthesized 2,5-disubstituted-1,3,4-oxadiazoles was evaluated by MES induced seizure in rats. Few compounds were exhibited good anticonvulsant activity. Chapter-VII describes the synthesis and *in vitro* antiproliferative activity of 2,5-disubstituted-1,3,4-oxadiazoles containing trifluoromethyl benzene sulfonamide moiety. The antiproliferative action of the synthesized compounds was tested against four different cell lines. Chapter VIII describes the synthesis and *in vitro* antiproliferative activity of new S-alkylated bis-1,2,4-triazole derivatives. New compounds were evaluated for their antiproliferative effect using the MTT assay method against four human cancer cell lines. Hence, there is a need for further investigations to clarify the features underlying the biological activities of these new
heterocyclic derivatives. Further studies on this line are under progress. A detailed survey of literature revealed that the compounds synthesized and reported in this thesis have not been carried out earlier by any other workers.
References


Chapter I


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