SYNOPSIS

The thesis entitled **"**DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF TETRAZOLE CONTAINING PIPERAZINE, QUINOLONE AND QUINAZOLINONE DERIVATIVES & DEVELOPMENT OF NEW SYNTHETIC METHODOLOGIES**"** has been divided into six chapters.

CHAPTER I: This chapter describes the "Introduction and biological importance of heterocycles (tetrazoles), molecular hybridization, and recoverable and recyclable catalysts"

CHAPTER II:

This chapter describes the regioselective synthesis and biological evaluation of tetrazole appendage *N*-substituted piperazine derivatives

CHAPTER III:

This chapter describes the synthesis and preliminary antiproliferative activity of novel 4-substituted phenylsulfonyl piperazines appendage tetrazole moiety

CHAPTER IV:

This chapter describes the synthesis of novel tetrazole containing hybrid ciprofloxacin and pipemidic acid analogues and preliminary biological evaluation of their antibacterial and antiproliferative activity

CHAPTER V:

This chapter describes the design, synthesis of novel quinazolinone-tetrazole hybrids by using recyclable nano-$CuFe_2O_3$ catalyst in aqueous medium and their preliminary anticancer activity

CHAPTER VI: This chapter describes the novel green protocols for the synthesis of benzaozels

CHAPTER I

Chemistry of heterocyclic compounds is one of the most complex and intriguing branch of organic chemistry. Heterocyclic compounds are widely distributed in nature and are essential for life. They play a vital role in the metabolism of all living cells. There are vast numbers of pharmacologically active heterocyclic compounds, many of which are a regular clinical use. The majority of the compounds we are familiar with as natural drugs, synthetic drugs, dyes, luminophores, pesticides and herbicides are also heterocyclic in nature. Each of these natural and synthetic heterocyclic compounds can and do participate in chemical reactions in the human body. Moreover, all biological processes are expressed through chemical reactions. Such fundamental manifestations of life as the provision of energy, transmission of nerve impulses, sight, metabolism and transfer of genetic information are all based on chemical interactions involving participation of many heterocyclic compounds, such as vitamins, enzymes, coenzymes, ATP, DNA, RNA and serotonin. Heterocycles are chemically more flexible and better able to carter the needs of biochemical systems. Moreover, the important practical application of heterocycles in the field of industries as additives or modifiers including cosmetics, reprography, information, storage, plastics, solvents and vulcanization accelerators. In short, heterocyclic chemistry is the branch of chemistry dealing with synthesis, properties and applications of heterocycles.

Heterocycles have enormous potential as the most promising molecules as lead structures for the design of new drugs. Development of new approaches towards the construction of heterocyclic moieties, employing efficient and atom economical routes, remains an important goal. The nitrogen, oxygen and sulphur containing heterocycles play a vital role in drug discovery to identify new chemical entities (NCEs) of immense therapeutic potential. Certain possible modifications on the heterocyclic ring by the addition of diverse substituents may lead to new products with better biological profiles.

In the present thesis work, we explore the synthesis and biological properties of novel tetrazole based heterocyclic derivatives and new methods for the synthesis of benzazole (benzoxazole/benzothiazole/benzimidazole) derivatives.

Tetrazole

Tetrazole is a heterocyclic compound containing a carbon atom and four nitrogen atoms in a five-membered ring. Tetrazoles are not found in nature. The simplest tetrazole is CN_4H_2. It is white to pale yellow crystalline solid with weak characteristic odour. The tetrazole family has attracted a great deal of attention due to the wide spectrum of their pharmaceutical and agrochemical activities. Tetrazole also possess interesting synthetic versatility and possible binding sites for interaction with various receptors and these are a typical bioisosteric replacement system for carboxylic acids. Therefore, these molecules have been used as positive inotropic agents for the treatment of congestive heart failure, potassium channel activators, antiasthmatics, an anti-histaminic agent (Azelastine) and a phosphodiesterase inhibitor (Zordoverine).

Molecular Hybridization

Molecular hybridization is a molecular modification approach to obtain multiple-ligands/compounds with pharmacokinetic advantages over concomitant administration of two different drugs and this is a new concept in drug design and development based on the combination of pharmacophoric moieties of different bioactive substances to produce a new hybrid compound with improved affinity and efficacy, when compared to the parent drugs. Additionally, this strategy can result in compounds presenting modified selectivity profile with different and/or dual modes of action and hence reduced the undesired side effects. The molecular hybridization of more than one biolabile moiety is an organized and chief technique to expand the vicinity of medicinal chemistry research. It involves the combination of separate pharmacophoric groups of analogous activity into one compound, which results into the substantial changes in the biological activity. Molecular hybridization is a structural modification strategy useful in the design of new optimized ligands and prototypes with new molecular architectures composed of two or more known bioactive derivatives, through the adequate fusion of these sub-unities.

Recoverable and recyclable catalysts

The challenging task in chemistry is to develop practical methods, reaction conditions and the use of materials based on the principles of green chemistry. The concept of ‘‘Green Chemistry’’ has emerged as one of the guiding principles of

environmentally benign synthesis. Conventionally, heterogeneous catalysis is favoured over homogeneous catalysis for a large number of applications in both fundamental research and industrial applications due to its ease of handling, simple workup and regenerability. Recently, nano iron oxide and copper ferrite catalyst have been efficiently used as environmentally friendly solid catalysts for various organic transformations.

CHAPTER-II

Regioselective synthesis and preliminary biological evaluation of tetrazole appendage N-substituted piperazine derivatives

Chemistry

The synthesis of nitrogen containing five-membered heterocyclic systems play a vital role in drug discovery to identify new chemical entities (NCEs) of immense therapeutic potential. Tetrazoles, in particular 5-mercapto tetrazoles are the most privileged structures that are widely explored for their range of pharmacological properties. Further, the presence of a sulfur atom at 5^{th} position of tetrazole ring has been reported to show a broad spectrum of biological properties. The main idea of synthesizing novel tetrazole containing piperazine derivatives is to study the cytotoxicity of the products by varying the substituents on tetrazole moiety as well as on cyclic amine moiety as these additional groups may enhance the biological activity. Tetrazole containing piperazine derivatives can be divided from a structural point of view in three principal parts that may be responsible for pharmacological activity (Figure 1)

(i) A pharmacophoric portion constituted by a substituted 5-thio- tetrazole moiety
(ii) A terminal fragment constituted by a cyclic amine moiety and
(iii) A three carbon spacer between these two substructures.

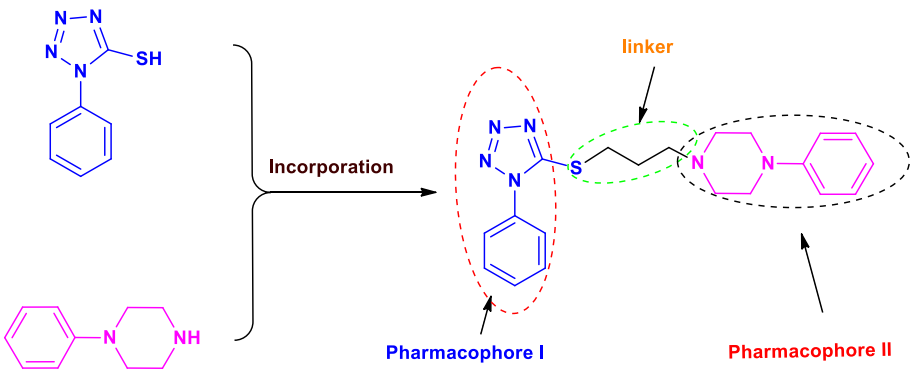


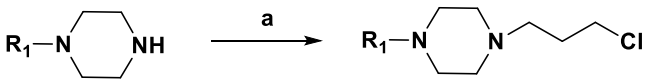
Figure 1: Rational concept to the synthesis of **6-35.**

A series novel 1-(4-substituted)-4-(3-((1-(substituted)-1*H*-tetrazol-5-yl)thio)propyl)piperazine derivatives were prepared and the synthetic route for the preparation of target compounds (**6-35**) is summarized in Scheme 2. Initially, Substituted isothiocyanates (**2a-g**) were prepared from anilines (**3a-g**) following the procedure shown in Scheme 2. As depicted in Scheme 2, reaction between substituted isothiocyanates (**4a-g**) and sodium azide in water provided 1- substituted-1*H*-tetrazole-5-thiol (**5a-g**) in good yield. The mercapto tetrazole (**5a-g**) formed can exist in thione-thiol tautomeric forms shown in Scheme 2 (**I** and **II**).

On the other hand, 1-(3-chloropropyl)-4-(4-substituted)piperazines (**2a–f**) were prepared from substituted piperizines (**1a-f**) with 1-bromo-3-chloropropane using K_2CO_3 and acetonitrile as a solvent (Scheme 1). The novel target compounds 1-(4-substituted)-4-(3-((1-(substitued)-1*H*-tetrazol-5-yl)thio)propyl)piperazine **(6-35)** were prepared by reaction with 1-substituted-1*H*-tetrazole-5-thiol (**5a–g)** and 1-(3-chloropropyl)-4-(4-substituted)piperazines (**2a–f)** using KF-Al_2O_3 and ethanol as solvent at 80 ^{0}C to afford the final target compounds (**6–35**, Scheme 2). The formation of *S*-alkylated products (**6–35)** were confirmed by the presence of *S*–$\underline{C}H_2$ characteristic peak appeared at δ 39.5–40.7 ppm in ^{13}C NMR and appearance of signal as a triplet for the *S*-C$\underline{H}_2$ group at δ 3.39–3.47 ppm in ^{1}H NMR spectrum, which is typical for connectivity. All the synthesized compounds were characterized by NMR, ^{13}CNMR and ESI/MS spectral data.

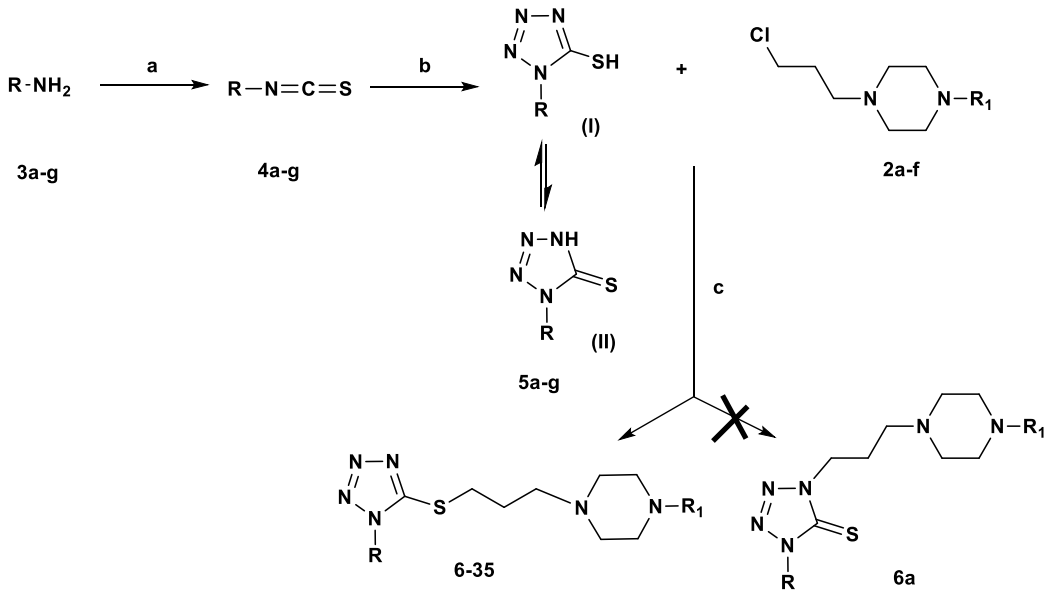
Thus, under the present reaction condition compound **6** is formed as the only product in a regioselective manner and the compound **6a** is not formed (Scheme 2). Theoretically two tautomeric forms are possible for the synthesized 1-substituted-1*H*-

tetrazole-5-thiol derivatives (Figure 2). Considering **26** as an example, we investigated the tautomerism of these mercapto tetrazole through single X-ray crystal diffraction analysis. Crystallographic data for **26** have been deposited with the Cambridge Crystallographic Data Centre with the deposition number CCDC 954133. As shown in ORTEP diagram (Figure 3), the compound **26** exhibits an S–H tautomer.



Reagents and conditions: a) 1-bromo-3-chloropropane, K_2CO_3, Acetonitrile, rt, 12 h.

Scheme 1: Preparation of 1-(3-chloropropyl)-4-substituted piperazines (**2a-d**)



Reagents and conditions: a) i) CS_2 , ET_3N, THF,1 h, ii) TsCl, 1 h, rt b) NaN_3 , H_2O, 80 °C. c) KF-Al_2O_3, ethanol, reflux, 4 h

Scheme 2: synthesis of 1-(4-substituted)-4-(3-((1-(substituted)-1*H*-tetrazol-5-yl)thio)propyl)piperazine derivatives (**6-35**)

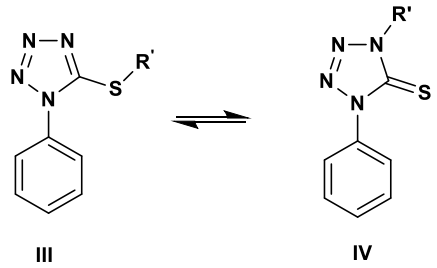


Figure 2. Tautomeric forms for the 1-substituted -1*H*-tetrazole-5-thiol

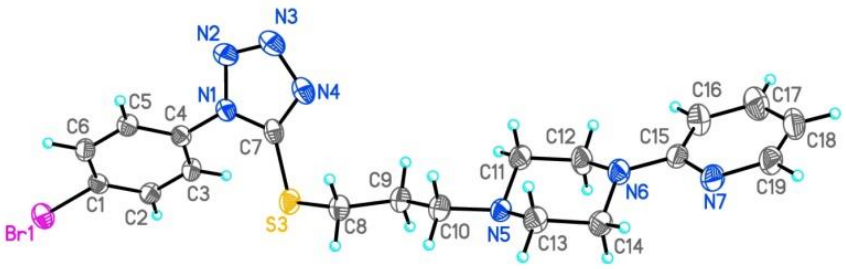


Figure 3: The molecular structure of **26** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radius. There are two molecules in the asymmetric unit (Z′=2) of the crystal structure, however, only one molecule is shown for clarity.

Biology

In vitro anti-cancer activity screening

All the title compounds **6-35** were screened for their *in vitro* anticancer activity against two cancer cell lines DU-145 and HeLa (Cervical) by using MTT bioassay, IC_{50} values of the compounds were reported at 48 h of drug administration. Doxorubicin was used as a positive control. After treatment of DU-145 and HeLa (Cervical) cell lines for 48 h with compounds **6-35** in the range of concentration 9.2 to >100 µg /mL. The cytotoxicity of the compounds was found to be commonly concentration-dependent. The Compound **28** was found as the significant potent compound against DU-145 and HeLa cell lines with IC_{50} values of 9.2 and 19.9 µg/mL compare to standard doxorubicin had IC_{50} values of 1.5 and 0.9 µg /mL against DU-145 and HeLa cells lines, respectively. Moreover, compounds **13**, **15**, **19**, **24**, **25**, **26**, showed good cytotoxic activity DU-145 and HeLa cell lines. Furthermore, compounds **13**, **25** showed good activity against DU-

145. The two compounds **33** and **35** did not show activity against DU-145 and HeLa cell lines. The results in DU-145 and HeLa cell lines demonstrated that after **28**, compound **25** was found to be the second significant potent compound. The tetrazole moiety with different subtituents was applied to investigate cytotoxicity *i.e*., alkyl, phenyl, benzyl, substituted phenyl groups (Electron-withdrawing (Br) and electron-donating (OMe) were introduced on the tetrazole). On the other hand, substituted piperazines were employed for enhance the cytotoxic effects. According to the substituent difference alkyl, phenyl, benzyl groups on tetrazole showed moderate to good activity. But the Electron-withdrawing (Br) substituted phenyl tetrazole of all target compounds shown enhanced the anticancer activity against DU-145 and HeLa cell lines, while the electron-donating group (OMe) cause a poor enhancement in potency. Probably, the increase in electron-withdrawing activity of phenyl substituted tetrazole is beneficial for activity. The utilization an (OMe) electron-donating phenyl substituted tetrazole cause a poor enhancement in potency.

***In vitro* antimicrobial screening**

The antimicrobial activities of the target compounds were determined against a panel of pathogenic bacterial strains, including *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Bacillus subtilis* MTCC 121, *Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 2453 and *Klebsiella planticola* MTCC 530 and *Candida albicans* MTCC 3017. None of the compounds showed significant activity against antibacterial strains tested. Interestingly some of the compounds displayed more potent activity against *Candida albicans* MTCC 3017 compare with standard Miconozole showed in Figure **4**. Among them the compounds **14, 15, 16, 17** and **25**, showed more potent ativity against *Candida albicans* MTCC 3017 compare with standard Miconozole, while the compounds **13**, **26**, **29** showed equipotent with standard Micanozole. The remaining compounds did not show activity against *Candida albicans* MTCC 3017. The phenyl group attached tetrazole compounds showed maximum MIC values compare to other groups attached to tetrazole.

CHAPTER-III

Synthesis and Preliminary Antiproliferative Activity of Novel 4-Substituted Phenylsulfonyl Piperazines Appendage Tetrazole Moiety

The sulfonamides have gained renewed attention in recent years as medicinally important compounds exhibiting diversified pharmacological activities. Among the family of sulfonamide derivatives arylsulfonylpiperazine derivatives represent an important class of therapeutic agents of interest in medicinal chemistry. The pharmacological potency of tetrazole as well as arylsulfonylpiperazines analogues has drawn our attention to synthesize the compounds containing both these moieties in a single molecular frame work (Figure 4). As a part of our continuous search for potential bioactive molecules for anti-cancer activity, a series of hybrid compounds were synthesized that comprise the arylsulfonylpiperazine and tetrazole heterocyclic ring system in a single molecule. Such hybridization was designed to investigate the effect of structural variation on the antiproliferative activity.

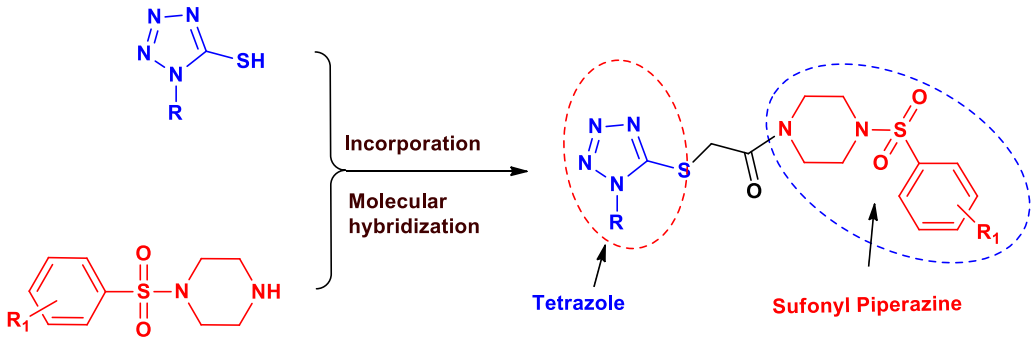
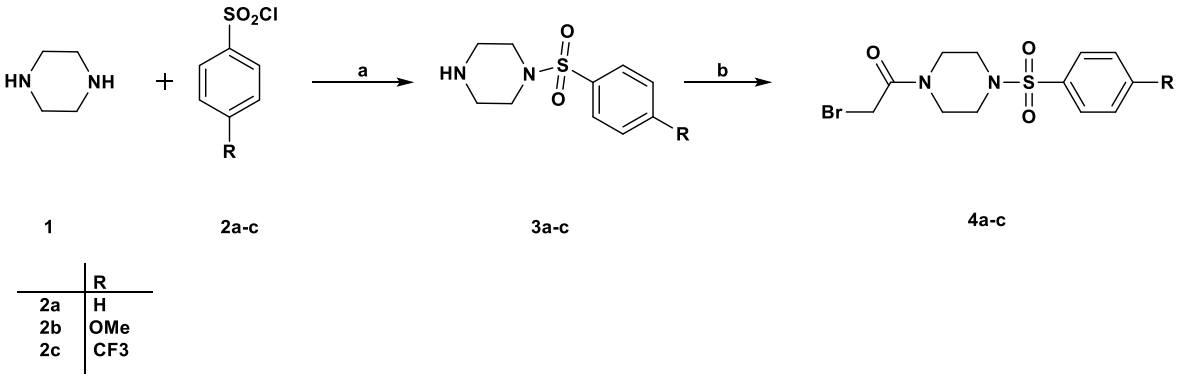


Figure 4: Rational concept to the synthesis of **7a-x**

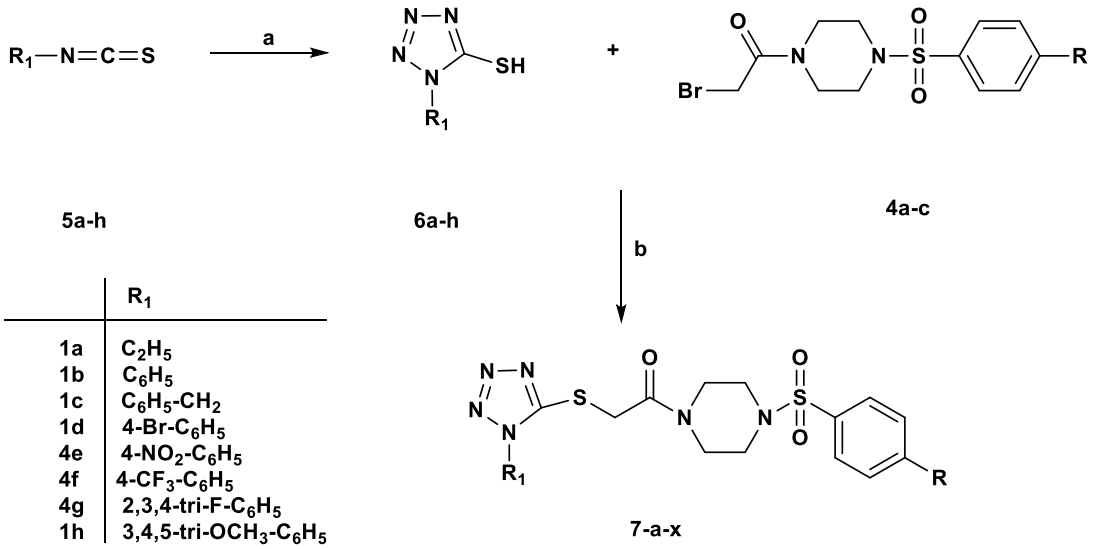
The synthetic route for the preparation of target compounds (**7a-x**) is outlined in Scheme 3 and Scheme 4. The 1-substituted-1*H*-tetrazole-5-thiol coupled with substituted phenyl sulfonyl piperazine acetamide derivatives were synthesized by a converging synthesis route that requires the preparation of the 4-substituted phenyl sulfonyl piperazines and 1-substituted-1*H*-tetrazole-5-thiol precursors independently that can be subsequently coupled together. The synthesis of 4-substitutedphenylsulfonyl piperazines (**3a–c**) were prepared by starting with the appropriate arylsulfonylchloride (**2**) reacted

with simple piperazine in CH_2Cl_2 under catalyst free conditions at 0 °C**.** The unreacted piperazine was removed by treating with saturated aq. $NaHCO_3$ solution to afford the required product. 2-bromo-1-(4-(substitutedsulfonyl)piperazin-1-yl)ethanone (**4a–c**) was synthesized in good yield by simple acylation involving reaction of 4-substitutedphenylsulfonyl piperazines and 2-bromoacetyl bromide in THF solvent at 0 ºC.

On the other hand, 1-substituted 5-mercaptotetrazoles (**6a–h)** derivatives were obtained by the reaction of NaN_3 with substitutedphenyl or alkyl isothocyanates (**5a–h)** in water at 80 ºC**,** further, these compounds were treated with 2-bromo-1-(4-(substituted sulfonyl)piperazin-1-yl)ethanone (**4a–c)** to afford the final target compounds yields ranging from 79 to 88% (Table 1, scheme 3.14**)**. The formation of *S*-alkylated products (**7a–x)** were confirmed by the presence of *S*–$\underline{C}H_2$ characteristic peak appeared at δ 41.2–41.7 ppm in ^{13}C NMR and appearance of signal as a singlet for the methylene group at δ 3.39–3.47 ppm in ^{1}H NMR spectrum, which is typical for connectivity. The spectroscopic data of all the newly synthesized compounds are in full accordance with their depicted structures. The detailed general synthesis procedure of the compounds is mentioned in the experimental section.



Scheme 3: Preparation of 2-bromo-1-(4-(substitutedsulfonyl)piperazin-1-yl)ethanone (**4a–c**)



	R_1
1a	C_2H_5
1b	C_6H_5
1c	C_6H_5-CH_2
1d	4-Br-C_6H_5
4e	4-NO_2-C_6H_5
4f	4-CF_3-C_6H_5
4g	2,3,4-tri-F-C_6H_5
1h	3,4,5-tri-OCH_3-C_6H_5

Reagents and conditions: a) NaN_3 , H_2O, 80 °C. b) Et_3N, ethanol, reflux, 4 h

Scheme 4: Preparation of 2-((1-substituted phenyl-1*H*-tetrazol-5-yl)thio)-1-(4-(substituted phenylsulfonyl)piperazin-1-yl)ethanone (**7a–x**).

Biology:

Antiproliferative activity:

The novel target compounds (**7a–x)** were evaluated for their *in vitro* inhibition of human cancer cell lines *viz* cervix (SiHa), breast (MDA-MB-231) and pancreatic carcinoma (PANC-1) (SIHA, MDA-MB-231 and PANC cell lines were obtained from American Type culture collection) using the sulforhodamine B (SRB) assay method. Tamoxifen and DMSO were used as positive and negative controls, respectively. The GI_{50} values (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth). It was observed that all the compounds are possessed antiproliferative activities against these cell lines in a concentration dependent manner and was found to be moderate to potent active than reference drug Tamoxifen.

Among the tested cell lines, compounds such as **7d**, **7e** and **7n** showed 1–2 fold greater activity than reference drug (GI_{50} = 0.12 µM), as evidenced by GI_{50} values of 0.071–0.098 µM against SiHa cancer cell line. On the other hand, compounds **7a, 7c, 7e** and **7n** showed potent activity (GI_{50} = 0.193–0.239 µM) than reference drug (GI_{50} = 0.24

μM) against MIDA-MB-231 cancer cell line, whereas the compounds **7h, 7s** and **7m** showed potent to equal activity (GI_{50} = 0.072–0.15 μM) than reference drug (GI_{50} = 0.15 μM) against PANC-1 cancer cell line

The different subtituents (*i.e* alkyl, phenyl, benzyl, Electron-withdrawing (Br, CF_3, F, NO_2) and electron-donating (OCH_3) on phenyl ring) were applied on tetrazole moiety as well as different subtituents (CF_3, OCH_3) were employed on sulfonylpiperazines to investigate antiproliferative activity. However the interesting inhibitory behavior of these compounds is relatively dependent on electronic nature of substituents on the tetrazole and sulfonylpeparazine hybrids (**7a–x**). The overall results of antiproliferative activity indicated that the compounds bearing methoxy group (OCH_3) sulfonylpeparazine attached to the tetrazole which were having unsubstituted phenyl (**7d, 7e**) and substituted electron withdrawing (NO_2, F) groups (**7n, 7s, 7t**) showed 50% inhibition at a concentrations ranges from 0.071- 0.22 μM against various tested cancer cell lines. From the obtained results it revealed that compounds bearing methoxy group (OCH_3) sulfonylpeparazine attached to the tetrazole which were having unsubstituted phenyl and substituted electron withdrawing (NO_2, F) groups contributed to the promising antiproliferative activity.

CHAPTER-IV

Synthesis of novel tetrazole containing hybrid ciprofloxacin and pipemidic acid analogues and preliminary biological evaluation of their antibacterial and antiproliferative activity

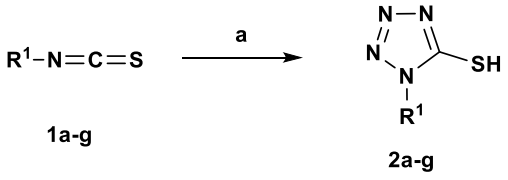
In view of the previous rationale and in continuation of an ongoing program aiming at attempt to design and develop new potential antibacterial and anticancer agents, a hybrid pharmacophoric approach was adopted in which modification at C-7 position at quinolone core moiety and substituted tetrazole were hybridized in one structure hoping to synergize better biological activity potential and increase lipophilicity of both groups. The present work explicates the synthesis of a new series of 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(3-((1-substituted-1*H*-tetrazol-5-yl)thio)propanoyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**5a–g**) and 8-ethyl-5-oxo-2-(4-(3-((1-substituted-1*H*-tetrazol-5-yl)thio)propanoyl)piperazin-1-yl-5,8-dihydropyrido[2,3-d]pyrimidine-6-

carboxylic acid (**8a–g**) derivatives. The validity of this design was assessed through preliminary *in vitro* antibacterial and antiproliferative study of the target compounds

Chemistry

The target compounds **5a–g** and **8a–g** were obtained in a two-step synthesis as depicted in scheme **1** and **2**. The synthesis of 1-substituted 5-mercaptotetrazoles **2a–g** derivatives were obtained by the reaction of NaN_3 with substitutedphenyl or alkylisothiocyanates **1a–g** (Scheme 5) in water at 80 °C. On the other hand, the compounds ciprofloxacin **3** and pipemidic acid **6** were treated with 3-chloropropionyl chloride and triethylamine in dichloromethane yielded compounds 7-(4-(3-chloropropanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **4** and 2-(4-(3-chloropropanoyl)piperazin-1-yl)-8-ethyl-5-oxo-5,8-dihydropyrido[2,3-d]pyrimidine-6-carboxylic acid **7** in 75% with excess of ether wash followed by column chromatography.

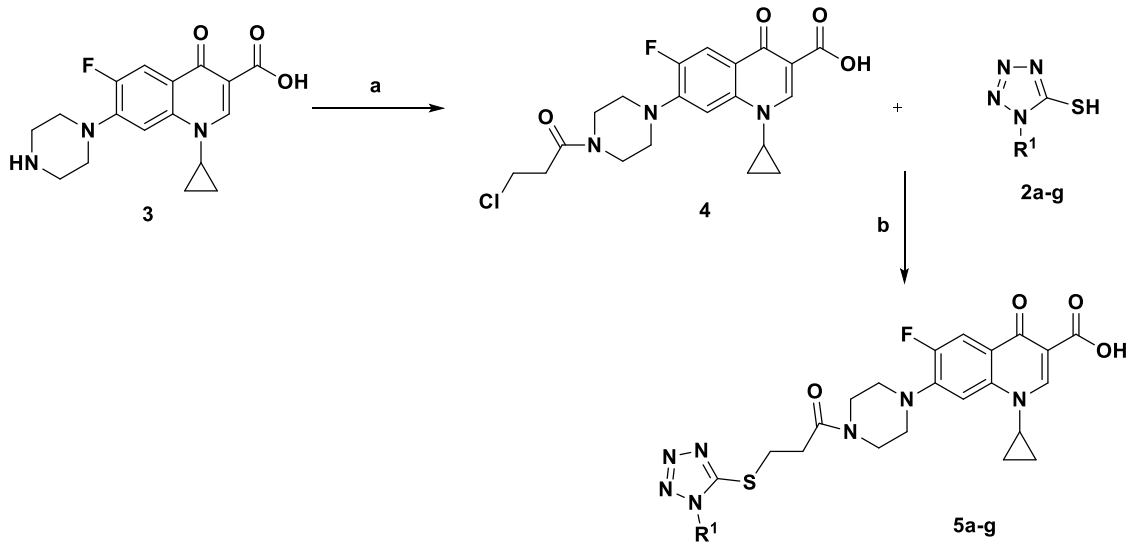
The target compounds ciprofloxacin derivatives **5a**–**g** and pipemidic acid derivatives **8a**–**g** were prepared by *S*- alkylation of 1-substituted 5-mercaptotetrazoles with compound **4** and **7** using triethylamine in ethanol under reflux conditions to afford the final compounds in yields ranging from 82 to 92%, the overall reaction sequence described in Scheme **6**. The target compounds were identified by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The NMR data for compounds **5a**–**g** and **8a**–**g** showed the characteristic pattern for final compounds: the characteristic peak *S*-C$\underline{H}_2$ appeared as a triplet range at δ = 3.2–3.6 ppm whereas ^{13}CNMR data for target compounds **5a**–**g** and **8a**–**g** showed the characteristic peak *S*-$\underline{C}H_2$ appeared range at δ = δ 34.2–36.5 ppm.



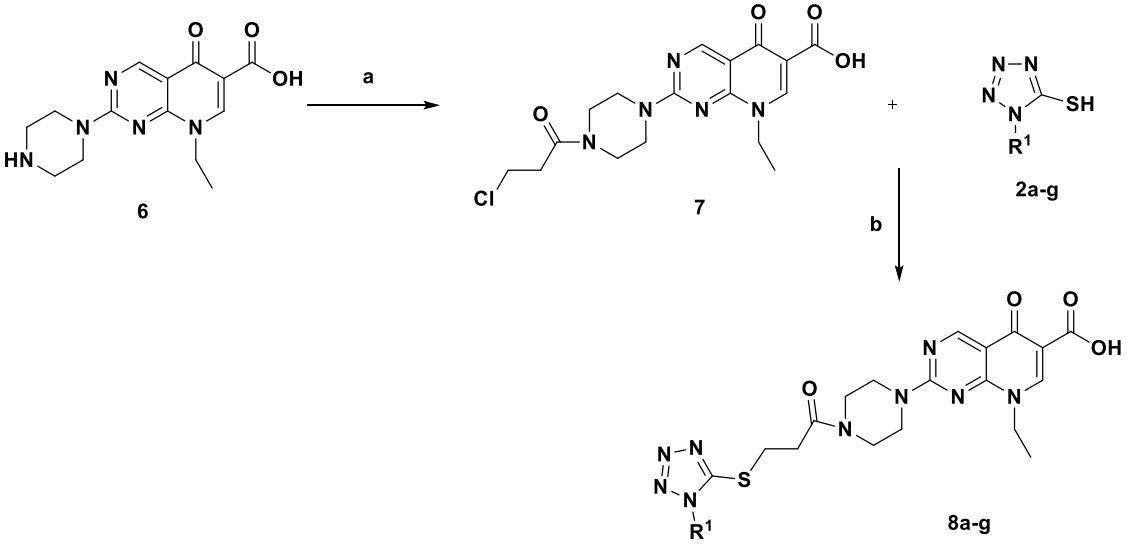
R^1 = ethyl, phenyl, benzyl, 4-Br-phenyl, 4-CF_3-phenyl, 2,3,4-tri-F-phenyl, 2,4,5-tri-OCH_3-phenyl

Reagents and conditions: a) NaN_3, H_2O, 80°C, 3-4 h.

Scheme 5: Synthesis of 1-substituted 5-mercaptotetrazoles



Reagents and Conditions: a) 3-Chloropropionyl chloride, Dry CH_2Cl_2,Et_3N, 1 h, b) Ethanol, Et_3N, 3-4 h, 80°C.



Reagents and Conditions: a) 3-Chloropropionyl chloride, Dry CH_2Cl_2,Et_3N, 1 h, b) Ethanol, Et_3N, 3-4 h, 80°C.

Scheme 6: Synthesis of tetrazole containing ciprofloxacin **5a-g** and pipemidic acid **8a-g** derivatives

Biological Activity

Antibacterial activity

A total of fourteen novel analogues (**5a**–**g** and **8a**–**g)** were synthesized and tested for antibacterial activity against six microorganisms, out of which three are (*Bacillus subtilis*, *Bacillus megaterium*, *Micrococcus luteus*) Gram positive and other three (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*) are Gram-negative bacterial strains compared with ciprofloxacin, streptomycin B and pipemidic acid. The zone of inhibition of all tested compounds **5a–g** and **8a–g** showed similar activity compared to reference drugs. The antimicrobial activity profile of the synthesized compounds revealed that they could be divided into active and moderately active based on antibacterial data against Gram-positive and Gram-negative bacterial strains. Though the activity profile of active compounds **5a**–**g** was effective on almost all tested strains, one of the bacterial strains, *P. aeruginosa*, was resistant to compounds (**5a**–**c** and **8a**–**g**); however, it showed to undergo moderate inhibition by compounds **5d**–**g**. Among all examined compounds the best activity profile was noticed by compound **5e** and **5f.** Compound **8g** did not display zone of inhibition activity against all the tested the bacterial strains. From the obtained the results showed that compounds having an electron-withdrawing group (tri fluoromethyl, tri fluoro, bromine) at the tetrazole ring of ciprofloxacin derivatives demonstrated a good zone of inhibition against all the bacterial strains compared to the standard. The remaining compounds were characterized by a smaller zone of inhibition against all the bacterial strains. In view of the the preliminary zone of growth inhibition data, only the ciprofloxacin derivatives **5a**–**g** were further analysed for their minimal inhibitory concentrations (MIC µg/mL) at the selected microbial strains.

Generally, MIC is the minimum concentration of drug that required arresting the growth of the bacterium. In the present study, the MIC of compounds, **5a–g** was determined by using tube dilution method. In general, most of the target compounds **5a**–**g** has exhibited considerable antibacterial activity against all the tested Gram-positive and Gram-negative bacterial strains except the compounds **5a, 5b** and **5c** were not exhibited antibacterial activity against Gram-negative strain *P.aeruginosa*. All the compounds **5a**–**g** showed similar promising activity compared MIC (15.6 µg/mL) compared to reference

ciprofloxacin (7.8 µg/mL), streptomycin B (15.6 µg/mL) and pipemidic acid (31.2 µg/mL).

Antiproliferative activity

In addition to antibacterial activity, the target hybrid compounds **5a**–**g** and **8a**–**g** were screened for their *in vitro* antiproliferative activity against a panel of three different human cancer cell lines i.e cervix (SiHa), breast (MDA-MB-231) and pancreatic carcinoma cell lines by using sulforhodamine B (SRB) assay method. Tamoxifen and DMSO were used as positive and negative controls, respectively. The GI_{50} values (GI_{50} = molar concentration of the compound that inhibits 50 % net cell growth) were reported. The results were exhibited for the antiproliferative activity of the synthesized ciprofloxacin analogues **5a**–**g** and pipemidic acid analogues **8a**–**g** after 48 h incubation. Among the 14 compounds synthesized, 5 against SiHa cancer cell line, 11 against MDA-MB-231 and 1 against PANC-1 cancer cell lines exhibited greater inhibition growth than reference drug Tamoxifen. Among the tested cell lines, compounds such as **5c**, **5d**, **8c**, **8d** and **8f** showed 2 fold greater activity than reference drug (GI_{50} = 0.12 µM), as evidenced by GI_{50} values of 0.06–0.08 µM against SiHa cancer cell line. On the other hand, compounds **5a, 5c**–**5g, 8a, 8b, 8d–8f** showed 3-10 fold greater activity (GI_{50} = 0.08–0.02 µM) than reference drug (GI_{50} = 0.24 µM) against MIDA-MB-231 cancer cell line, whereas the only compound **8d** showed 2 fold greater activity (GI_{50} = 0.07 µM) than reference drug (GI_{50} = 0.15 µM) against PANC-1 cancer cell line. Among the tested compounds, **8d** showed the most potent activity against the tested all cancer cell lines.

From the Structure–activity relationship (SAR) point of view, different substituents were employed on the tetrazole moiety attached to ciprofloxacin (**5a**–**g**) and pipemidic acid (**8a**–**g**) derivatives to investigate antiproliferative activity. Tetrazole moiety has different substituents at position 1, namely ethyl, benzyl, phenyl and substituted phenyl ring such as 4-bromo, 4-trifluoro methane, 2, 3, 4-trifluro and 3, 4, 5-trimethoxy phenyl groups. The resulting compounds were tested for the growth inhibition effect. Further, the target compounds are divided into two series: one is the group (a-c) with aliphatic, aromatic and benzylic substituents at the tetrazole where a π-electron cloud is absent, conjugated or not conjugated with tetrazole; another group is the one with aromatic

substitution, namely derivatives (d-g) to be compared with the corresponding b (phenyl) as reference.

Compounds **5b** and **8b** bearing unsubstituted phenyl moiety on tetrazole ring showed growth inhibition (GI_{50} values) at concentrations ranges from 0.085–1.15 µM against various tested cancer cell lines, whereas the compounds (**5d**–**g** and **8d**–**g**) bearing different substituents on phenyl moiety attached tetrazole ring showed enhanced growth inhibition (GI_{50} values) at concentrations ranges from 0.066-1.3 µM against various tested cancer cell lines. Presence of substituents on phenyl ring of tetrazole overall enhanced the activity of the tested compounds. Particularly, compounds **5d** and **8d** bearing bromine group (electron withdrawing group) on phenyl ring of tetrazole attached to the both ciprofloxacin and pipemidic acid showed enhancement of growth inhibition activity against all the tested cancer cell lines. Electron releasing properties seem detrimental for the pipemidic acid system (**8g**), but not for the ciprofloxacin derivatives where **5g** is even more active than unsubstituted phenyl derivative **5b**, and where electron withdrawing and electron releasing group don't make large differences. Considering the type of tetrazole substituent (derivatives a-c), 1-benzyltetrazole analogues **5c** and **8c** show a potent activity against SiHa and MDA-MB-231 cell lines in the case of ciprofloxacin derivatives and against SiHa cell line in the case of pipemidic acid derivatives. Considering the type of substituent at the aryltetrazole moiety (derivatives d-g in comparison with the unsubstituted phenyl derivative b), the p-bromo derivatives **5d** and **8d** show potent activity against SiHa and MDA-MB-231 cell lines the first one and against SiHa and PANC-1 cell lines followed the second one. What have in common the benzyl (c) and p-bromophenyl (d) derivatives, is a polarizable (that can undergo an induced polarization) electronic system at a certain distance from the tetrazole moiety that hypothetically can be important for a receptor interaction

CHAPTER-V

Design, Synthesis of Novel Quinazolinone-Tetrazole Hybrids by using Recyclable Nano-$CuFe_2O_3$ Catalyst in Aqueous Medium and their Preliminary Anticancer Activity

In view of the synthetic and therapeutic importance, quinazolinones scaffold would serve as a privileged structure due to their prevalence in anticancer agents and other biologically active molecules. Prompted by the literature development in the field of quinazolinone and tetrazole groups and as a part of our continuous efforts to search for new biologically active compounds, we have planned to join the two active moieties with an intention to afford better biological activity. With this proof of concept, we are here in reporting the pharmacological activity 3-substituted phenyl-2-((3-((1-substituted-1*H*-tetrazol-5-yl)thio)propyl)thio)quinazolin-4(3*H*)-one hybrids for their anticancer activity. The synthetic route for the novel quinazolinone-tetrazole analogous was shown in Scheme 7 and Scheme 8.

Initially, 2-mercapto-3-substituted phenethylquinazolin-4(3*H*)-one (**5a–c**) as a key intermediate was achieved by the reaction of anthranilicacid and substituted substitutedphenyl isothiocyanates (**4a–c**) using nano-$CuFe_2O_3$ in water medium afford 85-90 % yield. Then, the compounds (**5a–c**) were reacted with with 1-bromo-3-chloropropane under nano-$CuFe_2O_3$ catalyst in water solvent to give *S*-alkylated quinazolinone core intermediate (**6a–c**), and these compounds were subsequently treated with 1- Substituted -1H-tetrazole-5-thiol (**2a–g**) afforded final target compounds (**7a–u**) under same reaction conditions (Scheme **8**).

On the other hand, 1-substituted 5-mercaptotetrazole (2**a–g)** derivatives were synthesized by the reaction of NaN_3 with isocyanates (**1a–g)** in water yielded in 92–95% (Scheme **7**). The target compounds were identified by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The NMR data for compounds (**7a–u**) showed the characteristic pattern for final compounds the characteristic peak -*S*-C$\underline{H}_2$- appeared as a triplet range at δ = 3.2–3.6 ppm and*S*–$\underline{C}H_2$ characteristic peak appeared at δ 41.2–41.7 ppm in ^{13}C NMR. Further, the structure of the compound **7b** was also confirmed by X-ray crystallographic analysis and the ORTEP diagram is represented below Figure 5.

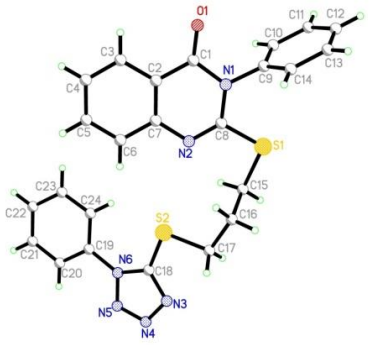
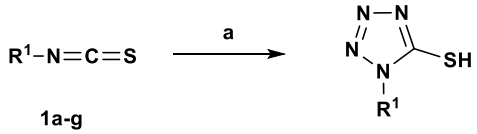


Figure 5: The molecular structure of **7b** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radius

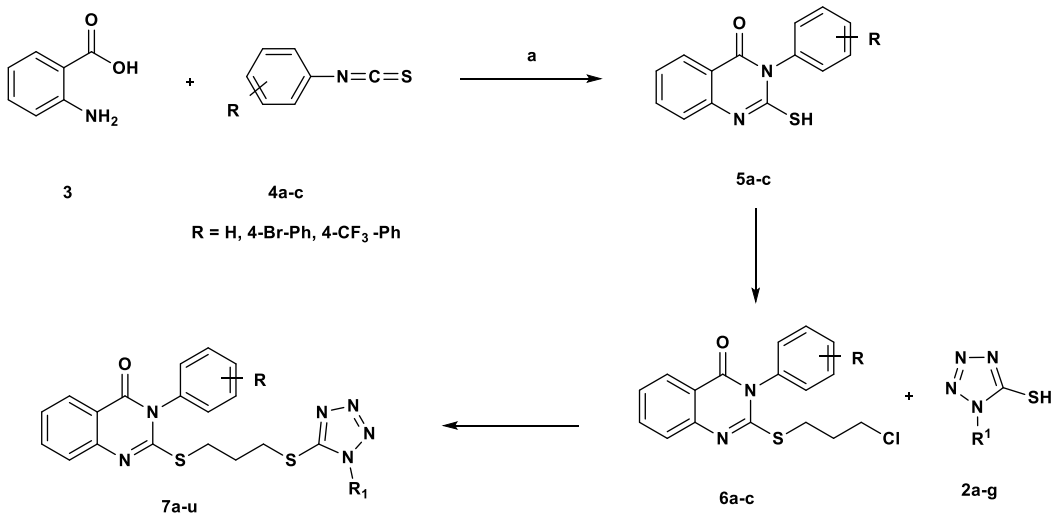


1a-g 2a-g

R^1 = ethyl, phenyl, benzyl, 4-Br-phenyl, 4-CF_3-phenyl, 2,3,4-tri-F-phenyl, 3,4,5-tri-OCH_3-phenyl

Reagents and conditions: a) NaN_3, H_2O, 80°C, 3-4 h.

Scheme 7: Preparation of 1-substituted 5-mercaptotetrazole (**2a-g**)



Reagentsand condotions: a) nano-$CuFe_2O_3$, H_2O, rt, 3-4 h, b) 1-Bromo-3-chloropropane, nano-$CuFe_2O_3$, H_2O, rt, 4-6 h, c) nano-$CuFe_2O_3$, H_2O, rt, 3-4 h.

Scheme 8: Preparation of 3-substituted phenyl-2-((3-((1-substituted-1H-tetrazol-5-yl)thio)propyl)thio)quinazolin-4(3H)-one (**7a–u**).

Biology

***In vitro* anti-cancer screening**

The *in vitro* antitumor activity of the newly synthesized compounds **7a–u** were evaluated against a panel of three human cancer cell lines, *viz* SIHA (cervical carcinoma), MD-AMB-231(Breast carcinoma), Hepg2 (Human liver carcinoma) and non-cancerous cell line HEK-293 (Human embryonic kidney) by employing MTT method. Etoposide was chosen as a reference drug due to its availability and widespread use. Each experiment was repeated at least three times and the obtained results (IC_{50} values) were presented. The title compounds showed moderate to good anticancer activity of the above three cancer cell lines in different extents (IC_{50} values 8.15–38.82 μM), and exhibited broad spectrum anticancer activity.

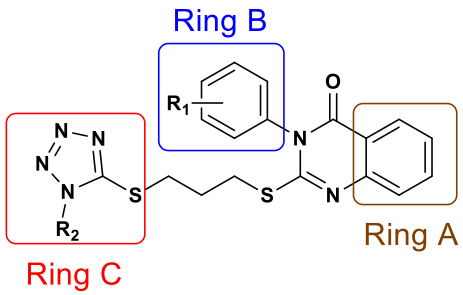


Figure 6: Structure of quinazolinone-tetrazole hybrid is given. The sites for structure diversification under ring B (R_1) and ring C (R_2) are highlightedby the colored boxes.

The title compounds consisted of 3 rings (A, B, and C) with different substituents employed on B and C rings, while A ring unchanged. Particularly, the tetrazole consisting C ring were modified with substituents like $–C_2H_5$, –Ph, –Substituted Ph (–Br, $–CF_3$, –F, and $–OCH_3$,) and –Bz groups, while B ring were modified with substituents like, –H, –Br, and $–CF_3$ groups (Figure 6). Compounds **7o–u** possess 4-CF_3 group on ring B showed enhancement in growth inhibition activity over compounds **7a–g** and **7h–n** possess like simple phenyl and Br groups on ring B, especially, in MDA-MB-231 cancer cell line with IC_{50} values range of 8.16-19.47 μM compare to standard drug. Moreover, the compounds possess the similar substituents like, phenyl (**7b**), 4-bromo phenyl (**7k**) and 4-trifluoromethyl phenyl (**7s**) on both B-ring and C-ring had lesser anticancer activity, whereas compounds **7d**, **7e**, **7i**, **7p** and **7r** possess alternate combination of substituents

(like, simple phenyl group on B-ring , while –Br group on C-ring (**7d**) and *vise versa* (**7i**)) on both B-ring and C-ring also showed significant anticancer activity, but this statement is exempted in the case of compound **7l** showed good activity against all tested cancer cell lines. Among the all tested compounds, **7f, 7m, 7o, 7r** and **7t** showed significant potent activity against all the tested cancer cell lines compared to reference drug.

Moreover, the representative compounds **7f**, **7m** and **7t** with the fluoro group on the C-ring of tetrazole exhibited significant potent activity in breast cancer MDA-MB 231 cells with IC_{50} values of 10.3 μM, 9.13 μM and 9.86 μM, respectively. Compound **7g**, bearing trimethoxy on the C-ring showed considerable potent activity in MDA-MB-231 cell line with IC_{50} value of 16.01 μM. In comparison, same compound **7g** showed lesser activity in SiHa and HepG2 cell lines with IC_{50} value of 29.51 μM in, 23.7 μM, respectively. In addition, other compounds **7n** and **7u** bearing trimethoxy on the C-ring showed decrease in growth inhibition activity against all tested cancer cell lines. Interestingly, the representative compound **7o** with the ethyl group on the C-ring exhibited potent activity in breast cancer MDA-MB-231 cells with an IC_{50} value of 8.16 μM, whereas the same compound **7o** exhibited considerable potent activity with IC_{50} values of 23.78 μM and 18.56 μM in SiHa and HepG2 cells, respectively. Thus the overall finding cytotoxicity results indicated that the analogues with halogen substituents ($–CF_3$, –Br, –F) demonstrated significant growth inhibitory effects compared to electron-positive ($–OCH_3$, $–C_2H_5$, –Bz) and phenyl substituted congeners. This may be attributed to the presence of huge electron densities around the halogen. Furthermore, all the target compounds tested on noncancerous HEK-293 cell line showed that least inhibitory effect with IC_{50} values close to or above 40 μM, indicating that these compounds have a minimal toxicity towards normal cells. Flow cytometric analysis revealed that compounds **7f, 7m, 7o, 7r** and **7t** arrested the cell growth in the G1 phase in MD-AMB-231 cell line.

CHAPTER-VI

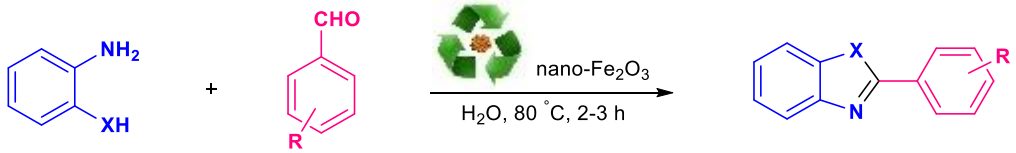
PART A

Synthesis of benzimidazoles/benzothiazoles by using recyclable, magnetically separable nano-Fe_2O_3 in aqueous medium

Benzimidazole/benzothiazole moieties are important scaffolds in pharmaceutical applications, and these moieties appear in many drugs encompassing a broad range of activities. Benzimidazole/benzothiazole derivatives were associated with a wide variety of medicinal, biological activities such as antifungal, antiviral, antibacterial, anticancer, anti-inflammatory, antiulcer, antihypertensive, antihistaminic, anticonvulsant, and antiparkinsonian activities.

On the other hand, nanochemistry is an emerging research field in modern science. The nanoscale catalysts can provide higher surface areas and lower coordinating sites, which are responsible for the higher catalytic activity. In recent years, magnetic nanoparticles (MNPs) have gained attention as a useful group of heterogeneous catalysts and in view of their recovery.

Inspired by these findings, herein, we report a new method Herein, we report an efficient protocol for the synthesis of benzimidazole/benzothiazole derivatives by a two-component reaction, involving 1,2-diamino benzene/2- amino thiophenol and substituted aromatic aldehydes for the first time promoted by recyclable iron oxide nanoparticles in aqueous medium (Scheme 9).



X=NH, S R= H, NO_2, Cl, CH_3, OCH_3, OC_2H_5

Scheme 9: Synthesis of benzimidazole/benzothiazole derivatives

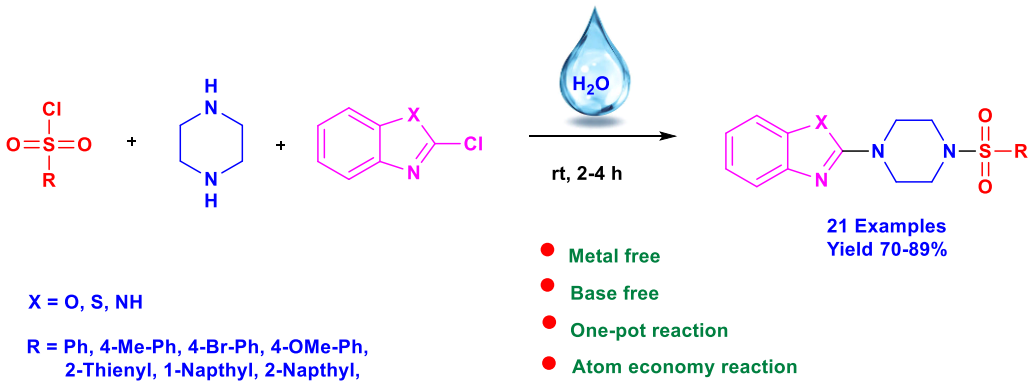
The reaction of 1,2-diamino benzene/2- amino thiophenol and substituted aromatic aldehydes were performed for the preparation of benzimidazole/benzothiazole. The

aldehydes bearing either electron-withdrawing or electron-donating groups reacted satisfactorily to furnish the corresponding 2-substituted benzimidazole/benzothiazole.

PART B

Aqueous, one-pot, three-component reaction for efficient synthesis of 2-[4-(arylsulfonyl)piperazin-1-yl]-1,3-benzothiazole, -1*H*-benzimidazole, or -1,3-benzoxazole derivatives

Multicomponent reactions (MCRs) are convergent reactions, in which three or more starting materials react to give a highly complex product in one-pot. Typically, purification of products resulting from MCRs is also simple since all the organic reagents employed are consumed and are incorporated into the target compound. In addition, MCRs obey the principles of green chemistry and, consequently, this kind of approach has become a powerful strategy for the synthesis of new chemical entities. Furthermore, MCRs can provide cost-effective methods because they do not involve protection/deprotection steps and because they minimize the number of purification processes. The usefulness of MCRs is even greater if they provide access to "privileged medicinal scaffolds" like benzazole. Benzazole (benzoxazole, benzothiazole, or benzimidazole) motifs are important scaffolds in pharmaceutical research, and their derivatives show diverse biological properties such as antibacterial, anticancer, HIV-1 reverse transcriptase inhibition. In our continued search for the development of novel environmentally benign protocols for the construction of heterocyclic frameworks, we report a simple, one-pot, three-component protocol for the synthesis of 2-[4-(substituted arenesulfonyl)piperazin-1-yl]-1,3-benzothiazole, -1*H*-benzimidazole, and -1,3-benzoxazole scaffolds (Scheme 10).



Scheme 10: Synthesis of 2-[4-(substituted arenesulfonyl)piperazin-1-yl]-1,3-benzothiazole, -1*H*-benzimidazole, and -1,3-benzoxazole

In an attempt to expand the scope of this protocol, experiments were conducted with various substituted sulfonyl chlorides, 2-chlorobenzazoles, and piperazine. This protocol was observed to be compatible with a broad range of substituted arenesulfonyl chlorides bearing either electron-withdrawing or electron-donating groups.