SYNTHESIS AND BIOLOGICAL EVALUATION OF MEDICINALLY POTENT HETEROCYCLIC SCAFFOLDS AND THEIR PRECURSORS

THESIS

Submitted in fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

to

The Faculty of Sciences

by

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(Registration No.: 17-YMCA-901001)

Under the supervision of

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April 2024

CANDIDATE'S DECLARATION

I hereby declare that this thesis entitled "**SYNTHESIS AND BIOLOGICAL EVALUATION OF MEDICINALLY POTENT HETEROCYCLIC SCAFFOLDS AND THEIR PRECURSORS**" by **CHANDER**, being submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in **CHEMISTRY** under the Faculty of Sciences, J.C. Bose University of Science and Technology, YMCA, Faridabad, during the academic year 2023-2024, is a bonafide record of my original work carried out under the guidance and supervision of **Dr. SITA RAM**, **ASSISTANT PROFESSOR**, **DEPARTMENT OF CHEMISTRY** and has not been presented elsewhere.

I further declare that the thesis does not contain any part of any work which has been submitted for the award of any degree either in this university or in any other university.

Chander
17-YMCA-901001

CERTIFICATE

This is to certify that this thesis entitled "**SYNTHESIS AND BIOLOGICAL EVALUATION OF MEDICINALLY POTENT HETEROCYCLIC SCAFFOLDS AND THEIR PRECURSORS**" by **CHANDER**, being submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy in **CHEMISTRY** under the Faculty of Sciences, J.C. Bose University of Science and Technology, YMCA, Faridabad, during the academic year 2023-2024, is a bonafide record of work carried out under my guidance and supervision.

I further declare that to the best of my knowledge, the thesis does not contain any part of any work submitted for the award of any degree either in this university or in any other university.

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ABSTRACT

The main objectives of this work are the design, synthesis and characterization of novel heterocyclic compounds and biological evaluation of heterocyclic comopunds as well as their precursors as anti-microbial and anti-oxidant agents. Thesis work starts with the literature review of triazoles and oxadiazoles summarizing their various biological activities such as anti-viral, anti-malarial, anti-microbial and anti-cancer etc. Further, 3-mercapto-1,2,4-triazole derivatives with alkyne, ester, acid and hydrazide functionalizations at sulfur atom were synthesized. In the similar vein, 1,2,3-triazole tethered 1,2,4-triazole derivatives, 1,3,4-oxadiazole incorporated 1,2,4-triazoles, 3,4-diamino-1,2,4-triazoles and their Schiff's base derivatives have also been synthesized. All the target compounds were synthesized utilizing variously substitued aryl hydrazides as starting material using various set of chemical reactions. The synthetic methodologies used for the synthesis result high purity and excellent yields of target compounds. Structures of the newly synthesized target compounds were confirmed by their in-depth characterization using various analytical techniques such as ^{1}H NMR, ^{13}C NMR, IR and HRMS. Biological potential of the synthesized compounds was tested against the growth of three Gram-positive bacterial strains including *Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657* , and *Bacillus cereus ATCC 11770*, four Gram-negative bacterial strains including *Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733* and *Shigella flexneri ATCC 9199* bacterial strains and one fungal strain i.e. *Candida albicans MTCC 183*. Most of the tested compounds possessed good to excellent inhibitory potential against the tested microbes. Further, all the synthesized target compounds have shown good to excellent results during their anti-oxidant evaluation. Cytotoxicity studies of target compounds were also performed against mouse fibroplast cell and plant seed germination cell lines. Results showed that synthesized compounds have no adverse effects on normal plant and animal cell lines. 1,2,3-Triazole tethered 1,2,4-triazole derivatives were tested for their inhibitory potential against a breast cancer cell line (MCF-7 cell line) also using MTT *in vitro* cell proliferation assay. Unfortunately, tested compounds possessed only poor to moderate anti-cancer potential against the tested cancer cell line. The biological activities exhibited by these compounds along with the cytotoxicity results indicated that the synthesized compounds can play an important role in drug discovery and can be used as potential therapeutic candidates for the treatment of various disease.

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LIST OF ABBREVIATIONS

Symbols	Meaning
AcOH	Acetic acid
AZA	Acetazolamide
^{t}BuOH	tert-Butanol
CAs	Carbonic anhydase enzymes
CAIs	Carbonic anhydrase inhibitors
$CuSO_4$	Coppersulfate
$CDCl_3$	Deuterated chloroform
d	Doublet
dd	Doublet of doublets
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DMSO-d_6	Deuterated dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
ex	Exchangeable
h	Hour
hrs	Hours
hCA	Human carbonic anhydrase
HCl	Hydrochloric acid
HI	Hydrogen iodide
HRMS	High resolution mass spectrometry
H_2S	Hydrogen sulfide
Hz	Hertz
I_2	Iodine
IR	Infrasred
J	Coupling constant
K_2CO_3	Potassium carbonate
$KMnO_4$	Potassium permanganate
KOH	Potassium hydroxide
Ki	Inhibition constant
LDA	Lithium diisopropylamide
Lit. m. p.	Literature melting point
m	Multiplet (in NMR) or medium (in IR)

MeOH	Methanol
MHz	Mega hertz
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
NaOH	Sodium hydroxide
nM	Nano molar
NMR	Nuclear magnetic resonance
Obs. m.p.	Observed melting point
s	Singlet (in NMR) or sharp (in IR)
SAR	Structure-activity relationship
t	Triplet
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultraviolet

CHAPTER 1

TRIAZOLES AND OXADIAZOLES AS POTENT BIOLOGICAL AGENTS - A LITERATURE REVIEW

1.1 Introduction

Heterocyclic compounds are the compounds having atleast one of the atoms of cyclic ring as heteroatom (N, O or S) in their structure. These are the most bountiful organic compounds in nature and are remarkably important to living organisms as their structural subunits occur in majority of natural products such as alkaloids, hormones, and vitamins as well as in dyes, herbicides, pharmaceuticals etc. These are receiving increasingly attention in medicinal chemistry owing to their remarkable biological activities [1]. More than 90 % of drugs available in market have heterocyclic nucleus as a part of their structures [2]. Further, nitrogen and oxygen containing heterocyclic compounds are the most important one. These play a crucial role in synthesis of newer drug candidates and are considered as key skeletons in medicinal chemistry. Triazoles are nitrogen containing five membered heterocycles having three nitrogen atoms in the ring. Further, they can be classified as 1,2,3-triazoles and 1,2,4-triazoles based on the position of nitrogen atoms with respect to each other. 1,2,3-Triazoles exists in three tautomeric forms i.e. 1*H*, 2*H* and 4*H*-1,2,3-triazoles while 1,2,4-triazoles exists in two tautomeric forms i.e. 1*H*-1,2,4-triazole and 4*H*-1,2,4-triazole (Fig. 1.1). Numerous triazole fused heterocycles have been synthesized in the literature and used in various clinical drugs. Some of the well-known marketed drugs having triazole moiety in their structure are alprazolam, etizolam, hexaconazole, trapidil, tazobactam etc. [3] (Fig. 1.1). Being isostere of ester, amide, carboxylic acid and other heterocycles, 1,2,3-triazoles readily interact with diverse proteins, enzymes and receptors in organisms through Van der Waals forces,

hydrogen bonding and hydrophobic interactions [4] and possess many biological and pharmacological properties including anti-tubercular [5], anti-fungal [6], anti-tumor [7], anti-bacterial [8], anti-alzheimer [9], anti-viral [10], and anti-malarial [11] activities. Also, 1,2,3-triazole can readily be prepared using 'click' chemistry with copper- or ruthenium-catalyzed azide-alkyne cycloaddition reactions.

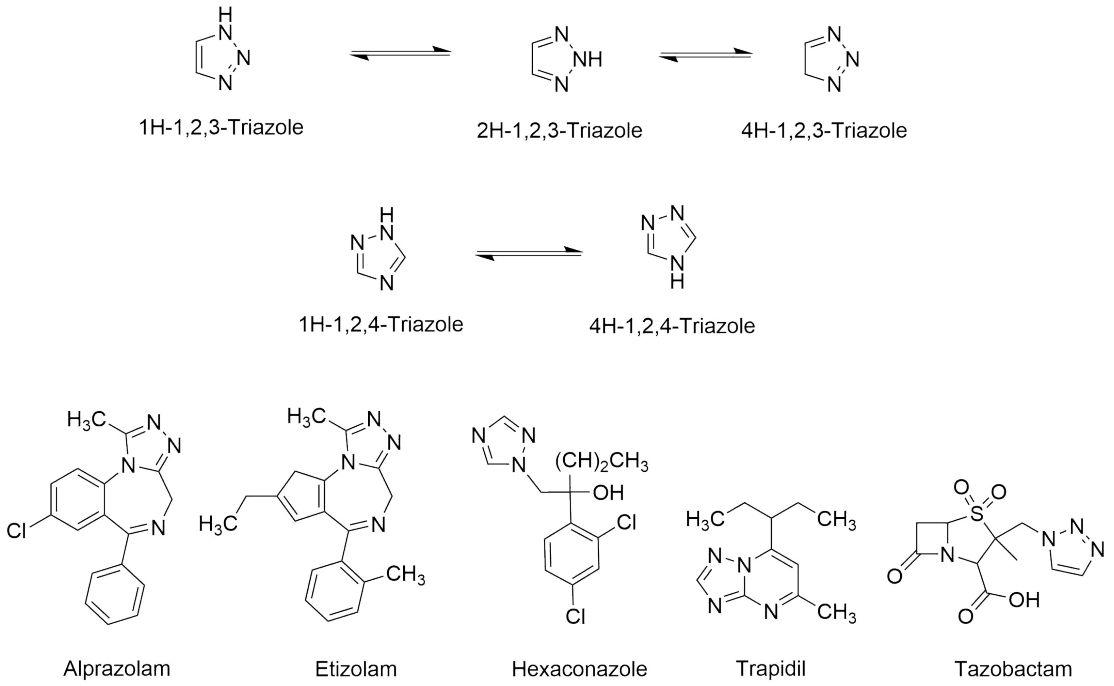


Fig. 1.1 Isomeric forms of 1,2,3-triazole and 1,2,4-triazole and structures of drugs having triazole ring

1,2,4-Triazole is another important class of aza-heterocyclic compounds and its presence can improve the biological profile of molecules through influencing polarity, lipophilicity and hydrogen bonding capacity. Compounds containing 1,2,4-triazole moiety hold intriguing role in the development of new drugs as they possess many pharmacological properties such as anti-viral [12], anti-cancer [8], anti-bacterial [13], anti-fungal [14], anti-tubercular [15] etc. Because of their interesting biological profile, both 1,2,3-triazole and 1,2,4-triazole are privileged scaffolds for the development of novel drug molecules. Oxadiazoles constitute an another important class of biologically potent compounds. These are five membered heterocyclic compounds having two nitrogen atoms and one oxygen atom in their ring structure. Further they can be divided into four categories *viz* 1,2,3-oxadiazoles, 1,2,4-oxadiazoles, 1,2,5-oxadiazoles and 1,3,4-oxadiazoles based on the positions of nitrogen and oxygen atoms in their ring (Fig. 1.2). Drug candidates having oxadiazole nucleus in their skeleton possess multiple biological activities

including anti-cancer, anti-malarial, anti-inflammatory and anti-bacterial etc. [16]. Till date numerous oxadiazole derivatives have been synthesized and evaluated as potent drug agents [17]. A number of marketed drugs contains oxadiazole moiety in their pharmacophoric unit. Zibotentan and Raltegravir are examples of very well-known marketed drugs having oxadiazole nucleus in their skeleton (Fig. 1.2) [18].

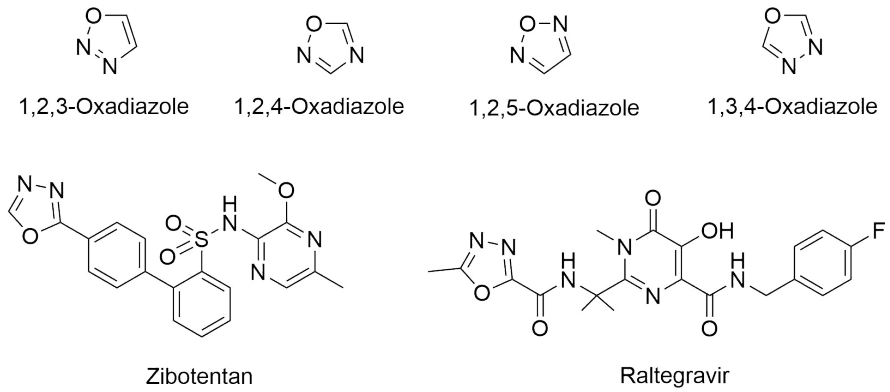


Fig. 1.2 Isomeric forms of oxadiazole and structures of drugs having oxadiazole ring

In addition, the presence of simple sulfonamide moiety in the structure of a molecule leads to a wide array of biological actions. Drugs containing sulfonamide group (known as sulfa drugs) constitute an important class of extensively used pharmaceutical agents possessing anti-bacterial, anti-diabetic, anti-cancer, anti-thyroid, anti-diurectics etc. biological activities [19]. There are more than 30 drugs having sulfonamide group and some of them *viz* sulfamethazine, sulfadiazine, sulfamerazine, sulfafurazole, sulfanilamide, sulfasalazine and sulfadimethoxine etc. are being used for the treatment of several diseases [20]. Owing to the biological potential of 1,2,3-triazole, 1,2,4-triazole and oxadiazole derivatives, a lot of their derivatives have been synthesized and evaluated as potent biological agent. In this chapter we have summarized all the reports on the biological activities of 1,2,3-triazole, 1,2,4-triazole and oxadiazole molecules giving a special attention to the benzenesulfonamide incorporated derivatives of them. Structure-activity relationship studies have also been presented herein.

1.2 Literature Review

1.2.1 1,2,3-Triazoles

Being the privileged class of aza-heterocycles, 1,2,3-triazoles have been well studied in past couple of years and are still continuously seeking researchers' attention due to its unique characteristics such as aromaticity, great chemical stability, strong dipole moment and hydrogen bonding capability etc. [21]. Compounds having 1,2,3-triazole motif in their structure are medicinally potent and possess wide range of biological actions such as anti-inflammatory, anti-platelet, anti-microbial, anti-tubercular, anti-tumor and anti-viral activities etc. [22]. There are numerous reports in literature exploring the synthesis and biological profile of 1,2,3-triazoles having benzenesulfonamide derivatization in them. In this section, we have summarized all such reports on biological actions of benzenesulfonamide containing 1,2,3-triazoles.

Murray *et al*. [23] have reported a series of three newly synthesized glucosyl and galactosyl saccharin conjugates **1** containing 1,2,3-triazole and benzenesulfonamide motif and evaluated them for inhibitory activity against human carbonic anhydrase isoforms hCA II and hCA IX (Fig. 1.3). The tested compounds were found to be selective inhibitors of hCA IX, a membrane bound tumor associated isoform, over ubiquitous cytosolic isoform hCA II exhibiting 22-fold selectivity when compared with the classically used CA inhibitor acetazolamide (AZA). Increased spacing between triazole and carbohydrate motifs from 1C to 2C-units resulted in enhanced inhibitory activity as well as selectivity for hCA IX over the off-target hCA-II isozyme. Kumar *et al.* [24] have reported a series of 4-functionalized 1-aryl-5-aryl/alkyl-1,2,3-triazoles **2** having benzenesulfonamide moiety at N-1 position of triazole ring (Fig. 1.3). Variations with ester, carboxylic acid, carboxamide, hydrazinocarbonyl and hydroxymethyl group was done at C-4 position of triazole and all the target compounds were evaluated for their inhibitory activity against hCA I, hCA II, hCA IV and hCA IX showing significant inhibitory profile against all of the tested isoforms with low nanomolar inhibition constants (Ki). Structure-Activity Relationship (SAR) studies show that compounds having bulkier substitutions at C-5 position were comparatively weaker inhibitors of cytosolic isoform hCA I owing to its smaller active site cavity as compared to that of other isozymes. A series of novel carbohydrate-based benzenesulfonamides **3** was synthesized by Hao *et al*. [25] using the sugar-tail approach and screened for

carbonic anhydrase inhibitory activity against hCA I, hCA II, and hCA IX (Fig. 1.3). 1,2,3-Triazole acted as linker between the benzenesulfonamide and hydrophilic sugar-tail moiety. All the tested compounds exhibited potent inhibitory activity against hCA IX with high selectivity. The target compounds were also reported to be exhibiting moderate anti-proliferative activity against two human cancer cell lines HT-29 and MDA-MB 231 under normoxic as well as hypoxic conditions.

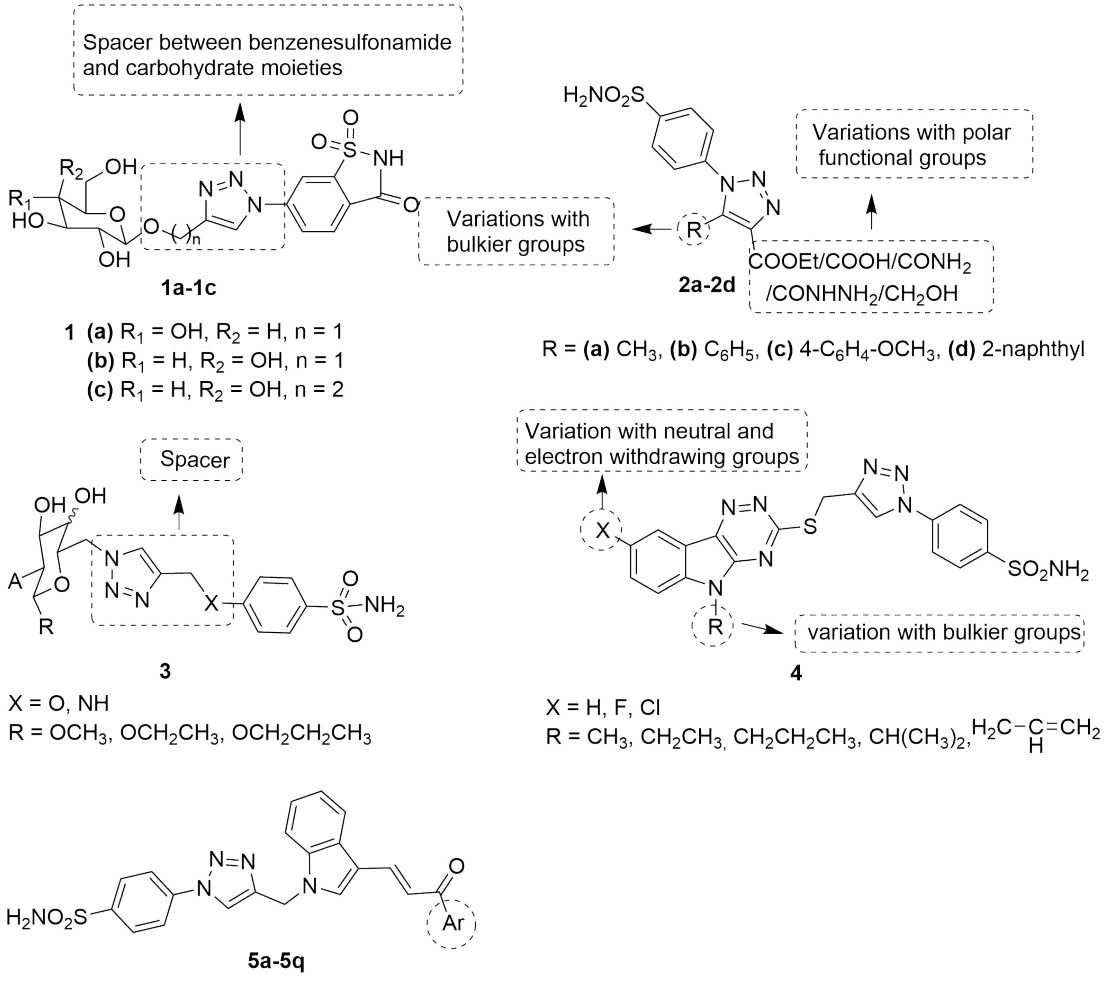


Fig. 1.3 Chemical structures of 1,2,3-triazole derivatives **1-5**

Chinchilli *et al*. [26] recently reported the synthesis and hCA inhibition profile of 1,2,3-triazole linked triazino[5,6-*b*]indole-benzenesulfonamide hybrids **4** against hCA I, hCA

II, hCA IX and hCA XIII (Fig. 1.3). All the compounds exhibited low to medium nanomolar range of Ki values against hCA II and hCA IX with more selectivity over hCA I and hCA XIII. One of the tested compounds containing fluoro and isopropyl group as variation on triazinoindole ring was found to be the most potent inhibitor of hCA II (Ki = 7.7 nM) even better than standard drug AZA.

A series of novel indolylchalcones incorporating benzenesulfonamide-1,2,3-triazole hybrids **5** has been reported recently using click chemistry reactions and shown to exhibit good inhibitory profile against hCA I, hCA II, hCA IX and hCA XII by Singh *et al*. [27] (Fig. 1.3). Most of the newly synthesized compounds exhibited interesting inhibition against hCA I isoform. Compounds **5d** (Ki = 18.8 nM), **5e** (Ki = 54.4 nM) and **5q** (Ki = 38.3 nM) were 13, 6 and 5 times more potent than AZA for hCA I isoform, respectively. Sharma *et al*. [28] have reported a series of hydroxy-trifluoromethylpyrazoline-carbonyl-1,2,3-triazoles **6** and hydrazones **7** containing benzenesulfonamide moiety as selective inhibitors of human carbonic anhydrase isoforms hCA I, hCA II, hCA IX and hCA XII (Fig. 1.4). Most of the compounds were excellent inhibitors against all of the four investigated hCA isoforms having some of them as even better inhibitors than standard reference drug AZA. Compound **6** having 4-fluorophenyl substitution at triazole ring and 2-pyridyl as substitution on pyrazoline ring was the most effective inhibitor of tumor associated isoform hCA IX (Ki = 0.7 nM) about 45-fold better than AZA.

A series of aryl-triazole substituted coumarins **8** was synthesized using click chemistry reactions and evaluated as inhibitors of a panel of four human carbonic anhydrase isoforms hCA I, hCA II, h CA IX and hCA XII by Nocentini *et al*. [29] (Fig. 1.4). All the target compounds showed selective inhibition potential against tumor associated trans memberane isoforms hCA IX and hCA XII (Ki = 4.7-37.8 nM) over the off target cytosolic and ubiquitous isoforms hCA I and hCA II. Kumar *et al*. [30] synthesized a series of 1,2,3-triazole derivatives **9** and **10** substituted at position 5 with -H and $-CF_3$, respectively, containing benzenesulfonamide at N-1 position and screened them for inhibition profile against four human carbonic anhydrase isoforms hCA I, hCA II, hCA IV and hCA IX (Fig. 1.4). Compounds performed better inhibition against isoform hCA I (Ki = 30.1-86.8 nM) when compared to standard drug AZA (Ki = 250 nM). Further, all the compounds have shown moderate inhibition potential against tumor associated hCA IX and glucoma associated isoforms hCA IV. SAR studies show that compounds having electron withdrawing groups on aroyl ring at position-4 of 1,2,3-triazole performed better inhibition profile against all of the tested isoforms. Variation

with heteroaroyl substituents led to better performance of compounds against hCA I and hCA II over hCA IV and IX. A series of benzenesulfonamide-triazole conjugates **11-16** was synthesized and evaluated for their inhibitory potential against hCA I, hCA II, hCA IV and hCA IX by El-Gazzar *et al*. [31] (Fig. 1.4). The final compounds, particularly, triazolopyridine derivatized benzenesulfonamides **15a**, **15c** and **15d** were found to be effective against tumor associated isoform hCA IX. Selected compounds were also tested for anti-proliferative activity against a panel of 57 human tumor cell lines and showed fair range of activity.

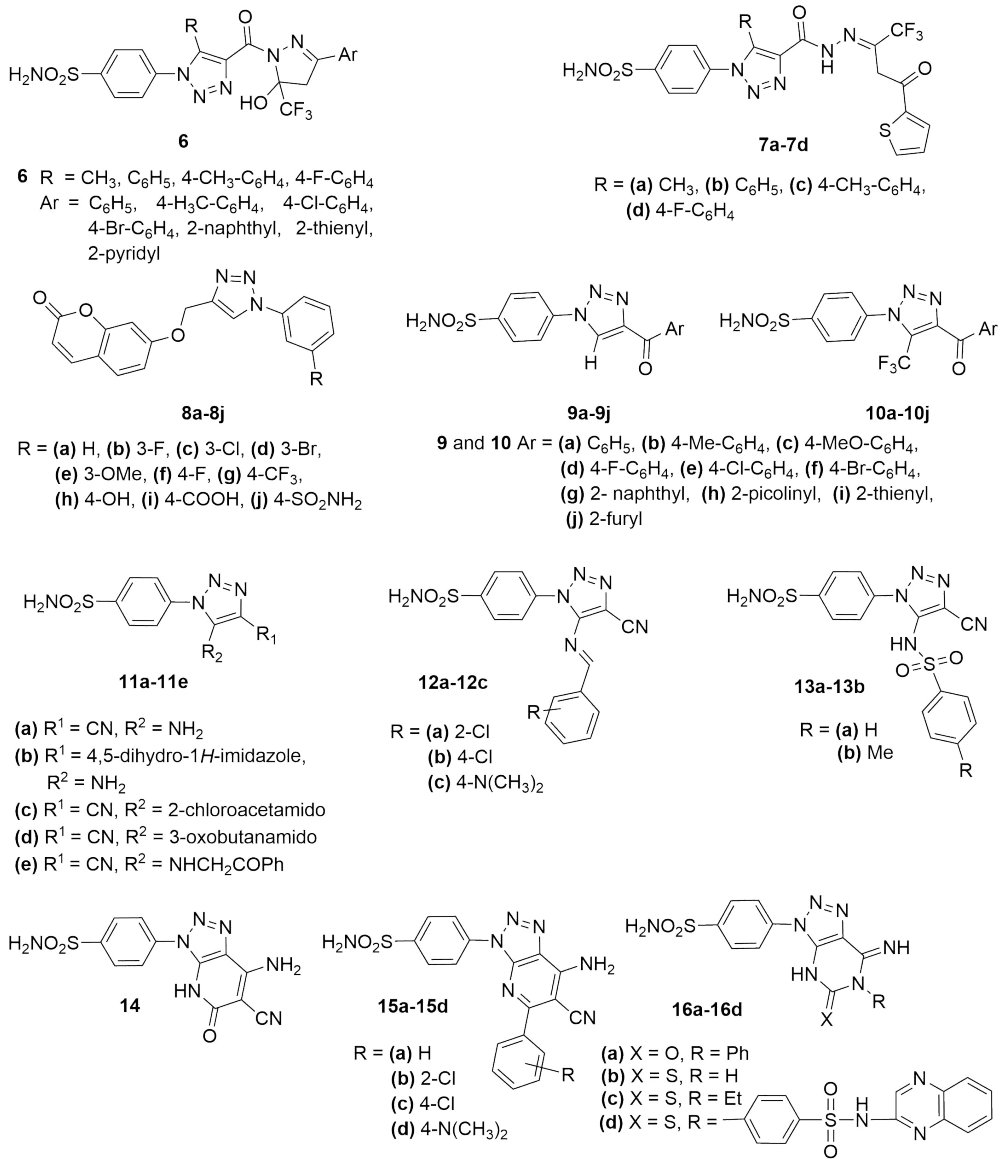


Fig. 1.4 Chemical structures of 1,2,3-triazole derivatives **6-16**

Carta *et al*. [32] have synthesized novel fluorescent benzenesulfonamide containing

triazoles **17**, **18** and **19**, and evaluated them against five physiologically relevant human carbonic anhydrase isoforms hCA I, hCA II, hCA IX, hCA XII and hCA XIV (Fig. 1.5). All the tested compounds were found to be poor inhibitors of hCA I but effective inhibitors of remaining tested isoforms, specifically, hCA XII, even better than AZA.

Synthesis of 2-pyridyl-1,2,3-triazole ligands containing 4-substituted benzenesulfonamide **20** and their rhenium complexes **21**, was reported by Aimene *et al*. [33] (Fig. 1.5). Both, the ligands and their rhenium complexes were evaluated for their inhibitory potential against hCA I, hCA II and hCA IX. Compound **20a** was the most potent inhibitor of all the tested hCA isoforms even 9-fold better than the standard reference drug AZA. Complex compound **21b** was proven to be the most selective against hCA IX among all the four tested compounds making it a promising potential anti-cancer drug candidate.

A series of novel 1,2,3-triazole benzene sulfonamide derivatives **22**, **23** and **24** was synthesized and evaluated as inhibitors against human carbonic anhydrase isoforms hCA I, hCA II, hCA IV and hCA IX by Vats *et al*. [34] (Fig. 1.5). All the reported compounds showed weak inhibition profile against hCA I while moderate against cytosolic isoform hCA II. The tumor associated membrane bound isoform hCA IX was also moderately inhibited by newly synthesized compounds while another membrane bound isoform hCA IV was strongly inhibited (Ki = 4.5 nM-4.3 μM) by some of the compounds. SAR studies reveal that the presence of a phenyl group at C-5 position of 1,2,3-triazole ring enhanced the potency of compounds especially against both hCA IV and hCA IX. Majority of the phenyl substituted compounds were better inhibitors than the standard drug AZA against hCA IV. Manzoor *et al*. [35] synthesized a series of triazole-sulfonamide containing pyrimidines **25** and **26**, and evaluated them as inhibitors of hCA I, hCA II, hCA IX, and hCA XII isoforms (Fig. 1.5). Most of the compounds have been reported to show poor inhibitory activity against hCA I (Ki = 41.5-5583 nM) while moderate to excellent activity against hCA IX and hCA XII (Ki = 1.6-465.7 nM and 0.36-90.5 nM, respectively). Derivatives containing *p*-methoxyphenyl **26e**, *o*-methoxyphenylpiperazine **26i** and ethyl piperazine-1-carboxylate **26m** have shown 16-, 37- and 44- fold inhibition potential against hCA XII, respectively, as compared to the standard drug AZA. However, one of the derivatives containing *p*-chlorophenylpiperazine **26j** was the most efficient and selective inhibitor of both tumor associated trans membrane isoforms hCA IX and hCA XII.

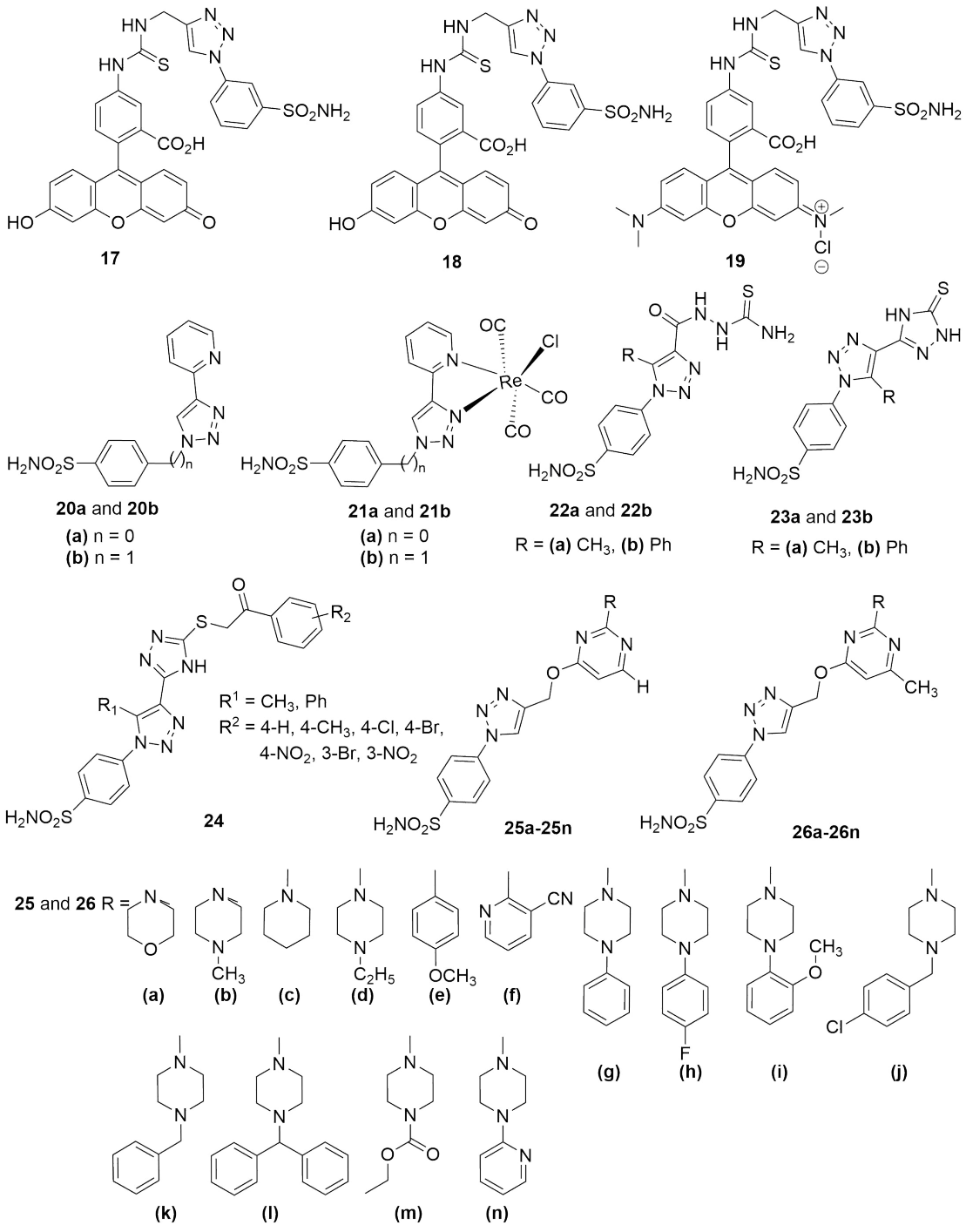


Fig. 1.5 Chemical structures of 1,2,3-triazole derivatives **17-26**

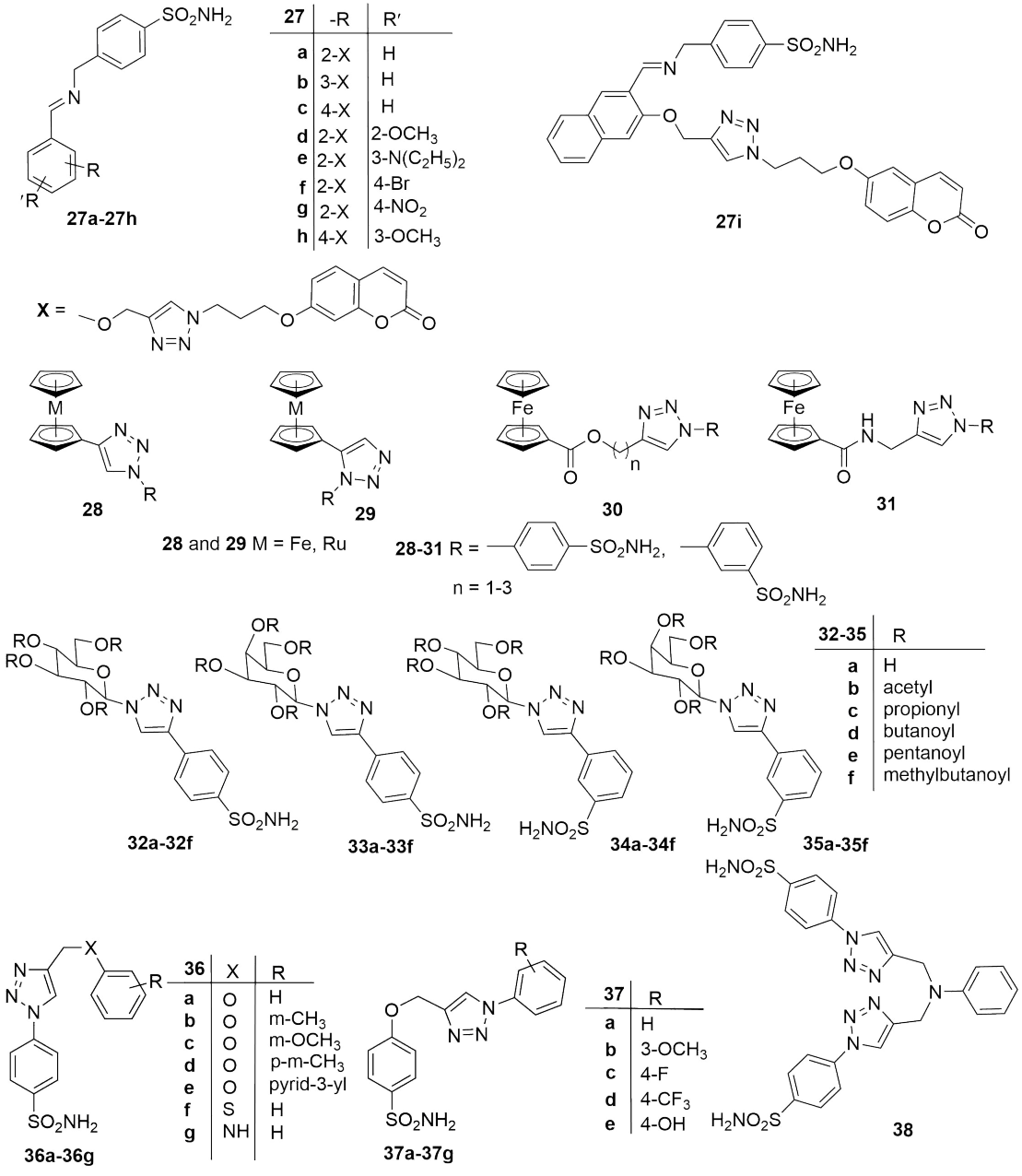
Kurt *et al.* [36] have synthesized coumarin-sulfonamide based 1,2,3-triazole derivatives **27** and evaluated their inhibitory potential against hCA I, hCA II, hCA IX and hCA XII isoforms (Fig. 1.6). Among all the synthesized compounds, **27i** was found to possess highest inhibition potential against hCA IX with Ki value 45.5 nM. In addition, **27i** was also found to be potent inhibitor of cancer cell proliferation. Salmon *et al.* [37]

synthesized a series of metallocene-based triazoles derivatives **28**, **29**, **30** and **31**, and evaluated them as carbonic anhydrase inhibitors against CA isoforms CA I, CA II, CA IX and CA XII (Fig. 1.6). All the compounds showed moderate to good CA inhibition profile, however, ferrocene-based compound **29** containing benzene-3-sulfonamide motif was the most potent inhibitor of hCA IX and hCA XII with Kis of 5.9 and 6.8 nM, respectively. Carroux *et al*. [38] reported the synthesis of a series of 24 novel glycoconjugates **32**, **33, 34** and **35** containing benzenesulfonamide and having 1,2,3-triazole moieties (Fig. 1.6). The CA enzyme inhibition profile for all the compounds was measured. *In vitro* metabolic stability, plasma stability, and plasma protein binding assay for a set of compounds was also measured. Results obtained demonstrate that acyl group protected carbohydrate-based sulfonamides have potential as prodrugs for selectively targeting the extracellular cancer-associated CA enzymes.

Benzenesulfonamide bearing 1,2,3-triazole derivatives **36, 37** and **38** were synthesized and evaluated as inhibitiors of carbonic anhydrases from bacteria *Vibrio cholerae* (VchCAα and VchCAβ) and *Mycobacterium tuberculosis* (β-mtCA3) by Bua *et al*. [39] (Fig. 1.6). Compounds **36** and **38** having benzenesulfonamide directly linked to triazole nucleus possessed low inhibition constants (Ki = 0.72-22.6 nM) against VcchCAα while **37** showed better inhibitory potential against VchCAβ (Ki = 54.8-102.4 nM) and β-mt CA3 (Ki = 28.2-192.5 nM).

Rezki *et al.* [40] have reported the synthesis of benzothiazole and isation-1,2,3-triazole-sulfa drug hybrid derivatives **39** and **40**, respectively, and evaluated their cytotoxic activity against a panel of cancer cell lines (Fig. 1.7). Most of the synthesized derivatives have shown promising activity when compared to reference drug taken. The pyrimidine and methylpyrimidine derivatives of benzothiazole series **39a** and **39b** showed the most potent epidermal growth factor receptor (EGFR) inhibition with IC_{50} values of 103 nM and 104 nM respectively. The newly synthesized compounds were also evaluated for their anti-proliferative activities against MCF-7, HCT-116 and HepG 2 and were found to possessing remarkable activity when compared to reference drug, staurosporine. Synthesis of novel heterocycles containing 1,2,3-triazoles hybridized with pharmacophoric anti-cancer fragments **41-45** and their evaluation as first-in-class simultaneous inhibitors of COX-2, 15-LOX and tumor associated carbonic anhydrase inhibitors was reported by Elzahhar *et al*. [41] (Fig. 1.7). Compounds **44a** and **45** were found to be potent inhibitors of 15-LOX and COX-2 enzymes. Both these compounds were also found to be effective nanomolar and submicromolar inhibitors of

tumor asscociated carbonic anhydrase isoform hCA XII. Almashal *et al*. [42] have synthesized a series of 1,4-disubstituted-1,2,3-triazolethymine derivatives **46** and reported their *in vitro* cytotoxic activity profile against human cancer cell line MDA-MB 231 using MTT assay (Fig. 1.7). Some of the compounds displayed significant cytotoxic activity with IC_{50} values up to 1.81μM. The triazole derivatives were also reported to be possessing potent anti-viral activity against HIV-1 and HIV-2 replications in MT-4 cells.



27	-R	R′
a	2-X	H
b	3-X	H
c	4-X	H
d	2-X	2-OCH_3
e	2-X	3-$N(C_2H_5)_2$
f	2-X	4-Br
g	2-X	4-NO_2
h	4-X	3-OCH_3

32-35	R
a	H
b	acetyl
c	propionyl
d	butanoyl
e	pentanoyl
f	methylbutanoyl

36	X	R
a	O	H
b	O	m-CH_3
c	O	m-OCH_3
d	O	p-m-CH_3
e	O	pyrid-3-yl
f	S	H
g	NH	H

37	R
a	H
b	3-OCH_3
c	4-F
d	4-CF_3
e	4-OH

Fig. 1.6 Chemical structures of 1,2,3-triazole derivatives **27-38**

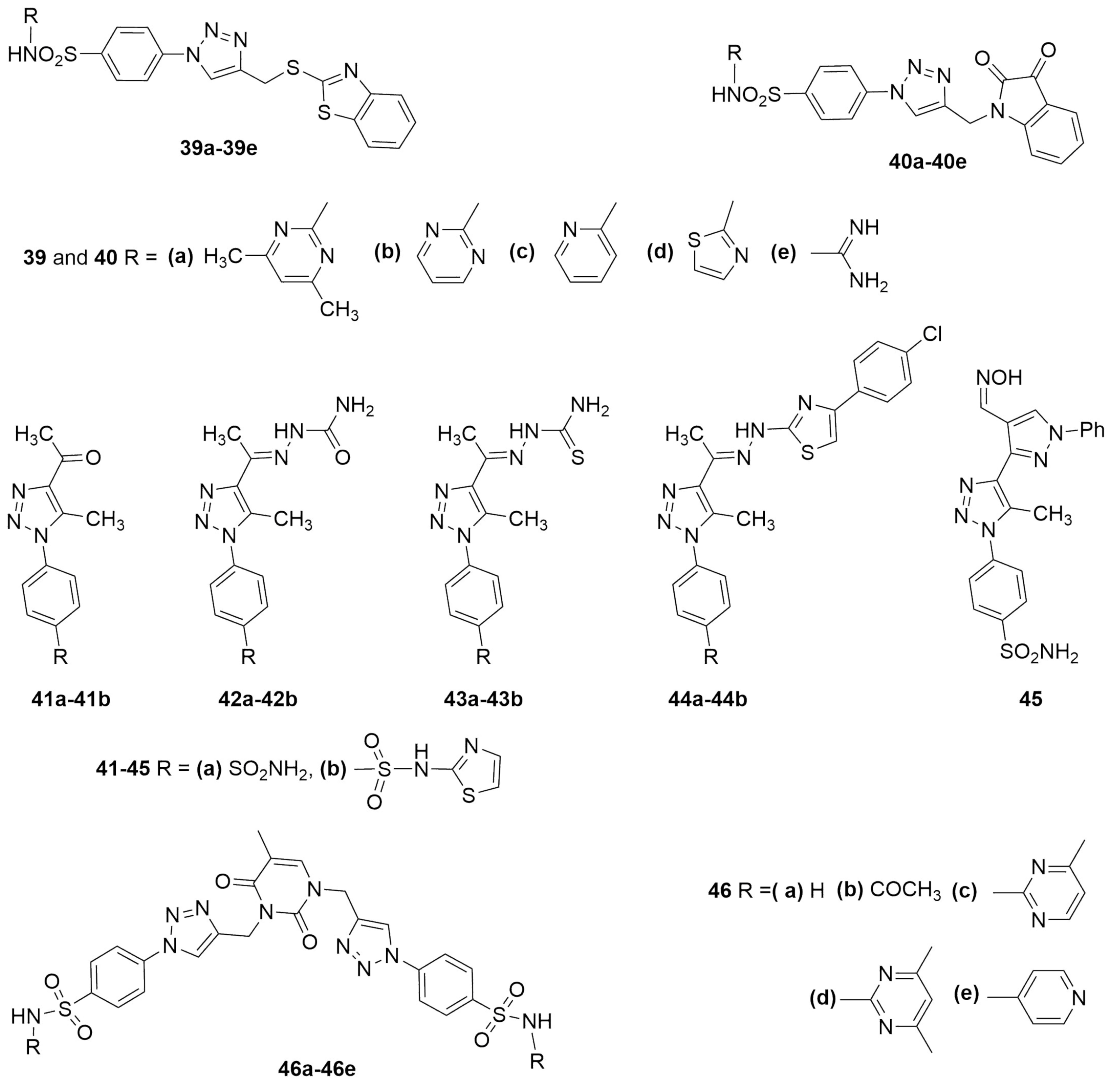


Fig. 1.7 Chemical structures of 1,2,3-triazole derivatives **39-46**

Elgogary *et al*. [43] have synthesized new 1,2,3-triazole derivatives **47**, **48**, **49** and **50**, and evaluated them for anti-tumor activity (Fig. 1.8). All the tested compounds possessed moderate to good anti-cancer activity against MFC-7 cancer cell lines, however, highest activity was obtained for **48a** (having IC_{50} value 12.4 μM) in relation to doxorubicin, a standard reference drug. Replacing the methyl group by amino **48b** led to two-fold decrease in the anti-cancer potential of the compound. A series of 1-(4'-sulfamoylphenyl)-1,2,3-trizole derivatives bearing a 4-dithiocarbamylmethyl moiety **51** was synthesized using click chemistry reaction and evaluated as anti-proliferative agents against MGC-803 (human gastric cancer cell line), EC-109 (human esophageal cancer cell line) and PC-3 (human prostate cancer cell line) using MTT method by Fu *et al.* [7] (Fig. 1.8). Most of the newly synthesized compounds

exhibited moderate to good anti-proliferative activity against all the tested cancer cell lines. SAR studies reveal that there was a significant effect of variations on dithiocarbamate moiety on anti-proliferative activity. tert-Butoxycarbonyl substituted compound **51g** showed the highest activity with IC_{50} value of 2.4 μM, about 10-fold more active than 5-fluorouracil, the standard reference drug used.

Imidazole based mono- and bis-1,4-disubstituted-1,2,3-triazole-sulfonamide derivatives **52** and **53** were reported by Al-blewi *et al*. [44] possessing anti-microbial and anti-proliferative activities **53** (Fig. 1.8). Compound **53a** was found to be most potent anti-microbial agent with MIC values ranging 32-64 μg/mL. The synthesized final molecules were also tested against three aggressive human cancer cell lines PC-3, HepG2, and HEK293 exhibiting moderate anti-proliferative activities with IC_{50} values in the micromolar range (55–106 μM). A series of 1,4-disubstituted 1,2,3-triazoles **54** bearing benzenesulfonamide moiety has been synthesized using terminal alkynes and aromatic azides through Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (Fig. 1.8) [45]. The synthesized compounds were further investigated for *in vitro* anti-microbial activities against *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumonia* and *Enterobacter aerogenes* as bacterial strains and *Candida albicans* and *Aspergillus niger* as fungal strain. A number of targeted compounds exhibited good potency against all of the above-tested microbial strains. Benzensulfonamide derivatized with 1,2,3-triazole derivatives **55** and **56** were synthesized and tested *in vitro* for their anti-bacterial and anti-fungal activities by Wang *et al*. [46] (Fig. 1.8). Compounds **55g**, **55i** and **55e** showed most potent anti-bacterial activity with MIC ranging 32-128 mg/mL against *B. subtilis, E. typhosa, S. aureus, S. dysenteriae,* methicillin-resistant *S.aureus (MRSA), P. aeruginosa* and *E. coli*. All the compounds exhibited moderate activities against fungal strains *C. albicans* and *C. mycoderma*. Bis-arylsulfonamide derivatives **57** were synthesized and evaluated as anti-mycobacterial agents by Wilkinson *et al*. [47] (Fig. 1.8). Compound **57a** exhibited growth inhibition of *Mycobacterium smegmatis* at less than 25 µg/mL but has no such activity against *Staphylococcus aureus* or *Escherichia coli*.

Diaryl-based triazole derivatives **58** and **59** were synthesized and were evaluated for *in vitro* COX-1 and COX-2 inhibition assay [48] (Fig. 1.9). Compound **59** was found to have higher inhibitory activity against COX-2 with IC_{50} of 0.002 μM. A series of 1,4,5-trisubstituted triazoles bearing benzenesulfonamide moiety **60-71** was synthesized and screened for their binding interactions with both COX isozymes and further *in vitro* COX-1 and COX-2 inhibition activities by Bekheit *et al*. [49] (Fig. 1.9). Among all the

synthesized compounds, highest inhibition activities against COX-2 were shown by **62** and **65** with IC_{50} values of 2.618 & 2.92 μM, respectively.

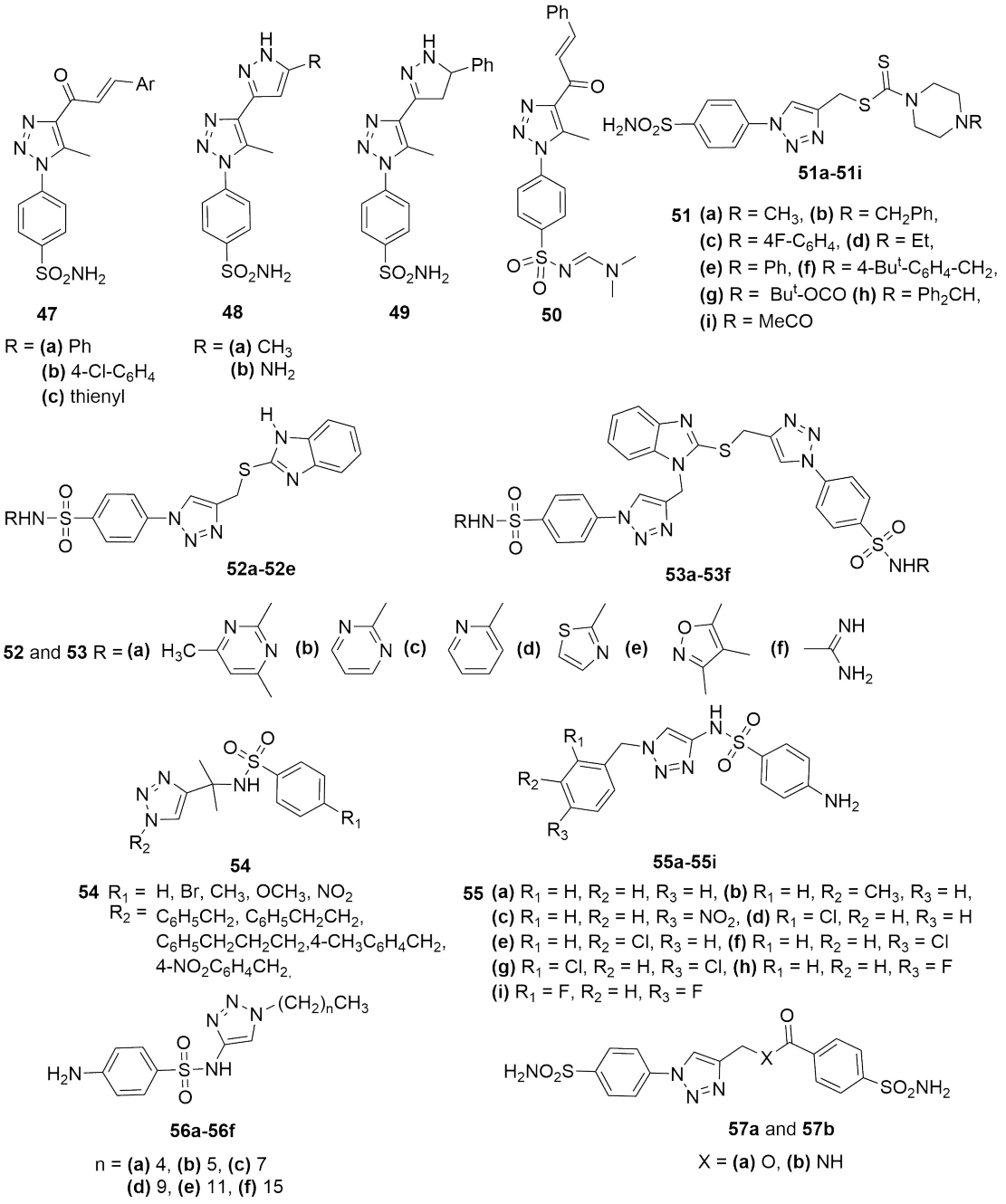


Fig. 1.8 Chemical structures of 1,2,3-triazole derivatives **47-57**

El-Dershaby *et al.* [50] designed and synthesized new selective COX-2 inhibitors by tethering 1,2,3-triazole and benzenesulfonamide moieties to some NSAIDs **72**-**74** (Fig. 1.9). Compounds **73b** and **73j** showed higher *in vitro* COX-2 selectivity and inhibitory activity (IC_{50} = 0.04 μM and S.I. = 329 and 312, respectively) than celecoxib (IC_{50} =

0.05 μM and S.I. = 294).

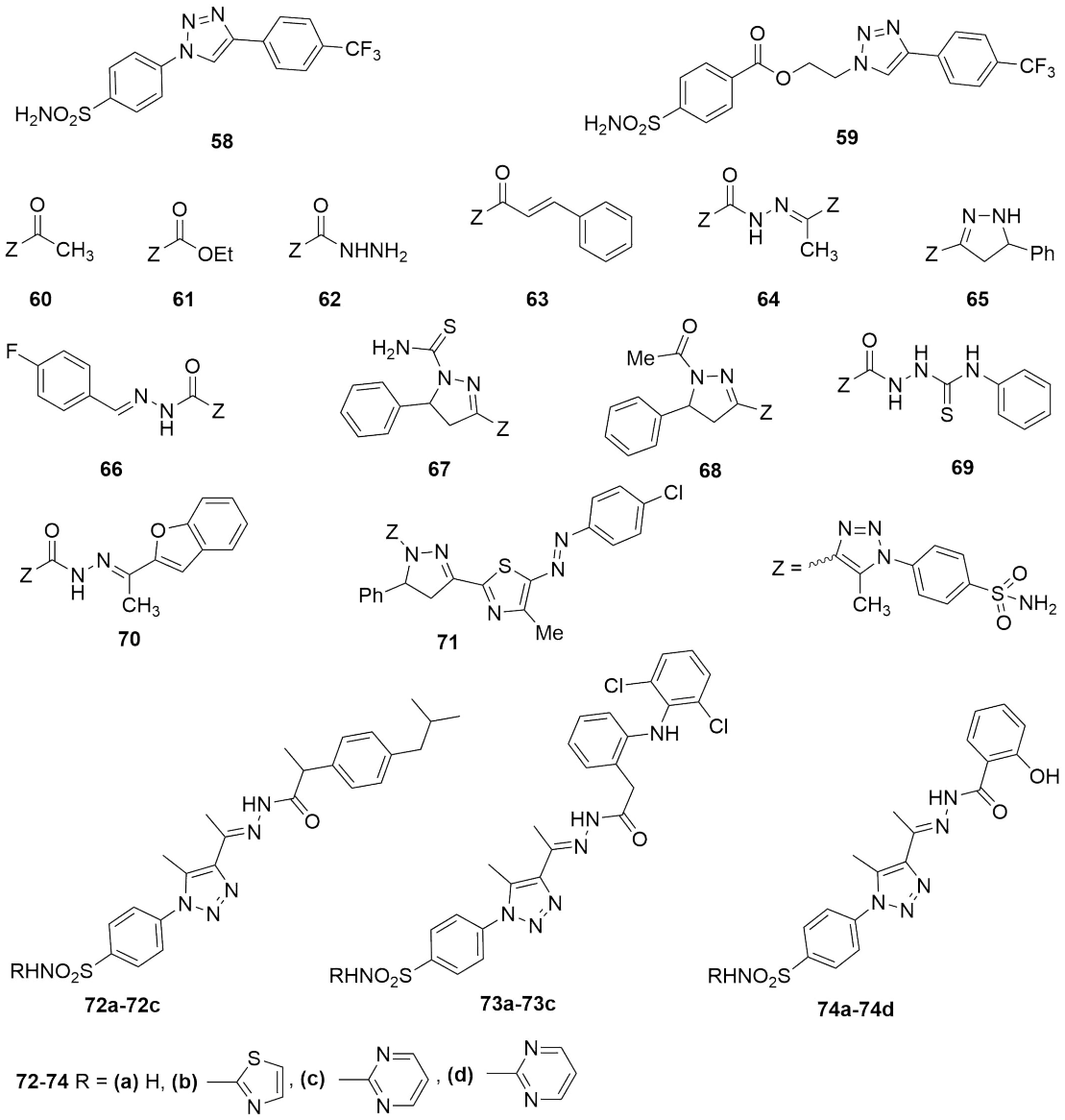


Fig. 1.9 Chemical structures of 1,2,3-triazole derivatives **58-74**

1,2,3-Triazole derivatives containing benzenesulfonamide moiety **75** and **76** were synthesized and evaluated as novel selective inhibitors of leucine-zipper and sterile-α motif Kinase (ZAK) by Yang *et al*. [51] (Fig. 1.10). Among all the tested compounds, **76g** was evaluated as best ZAK inhibitor suppressing Kinase function of ZAK with IC_{50} of 4.0 nM which makes it a promising lead compound for further development. Liu *et al*. [52] have synthesized 5-substituted biphenyl-2-sulfonamide derivatives containing 1,2,4-triazole **77** and evaluated them as angiotensin-II subtype 2 (AT_2) receptor agonists (hypertensive agents) (Fig. 1.10). Compounds **77f** and **77m** displayed significant

activity with IC_{50} values 0.4 nM and 0.5 nM, respectively, for AT_2 receptor. Siliveri *et al*. [53] have synthesized benzene sulfonamide containing 1,2,3-triazoles **78**, **79** and **80** (Fig. 1.10). In docking study, all the compounds showed effective dock scores from 122.165-145.031. Compound **78** bearing 2,4-dimethoxyphenyl group showed highest Libdock score of 145.031 and highest cytotoxic activity with an IC_{50} value of 4.54 μg/mL.

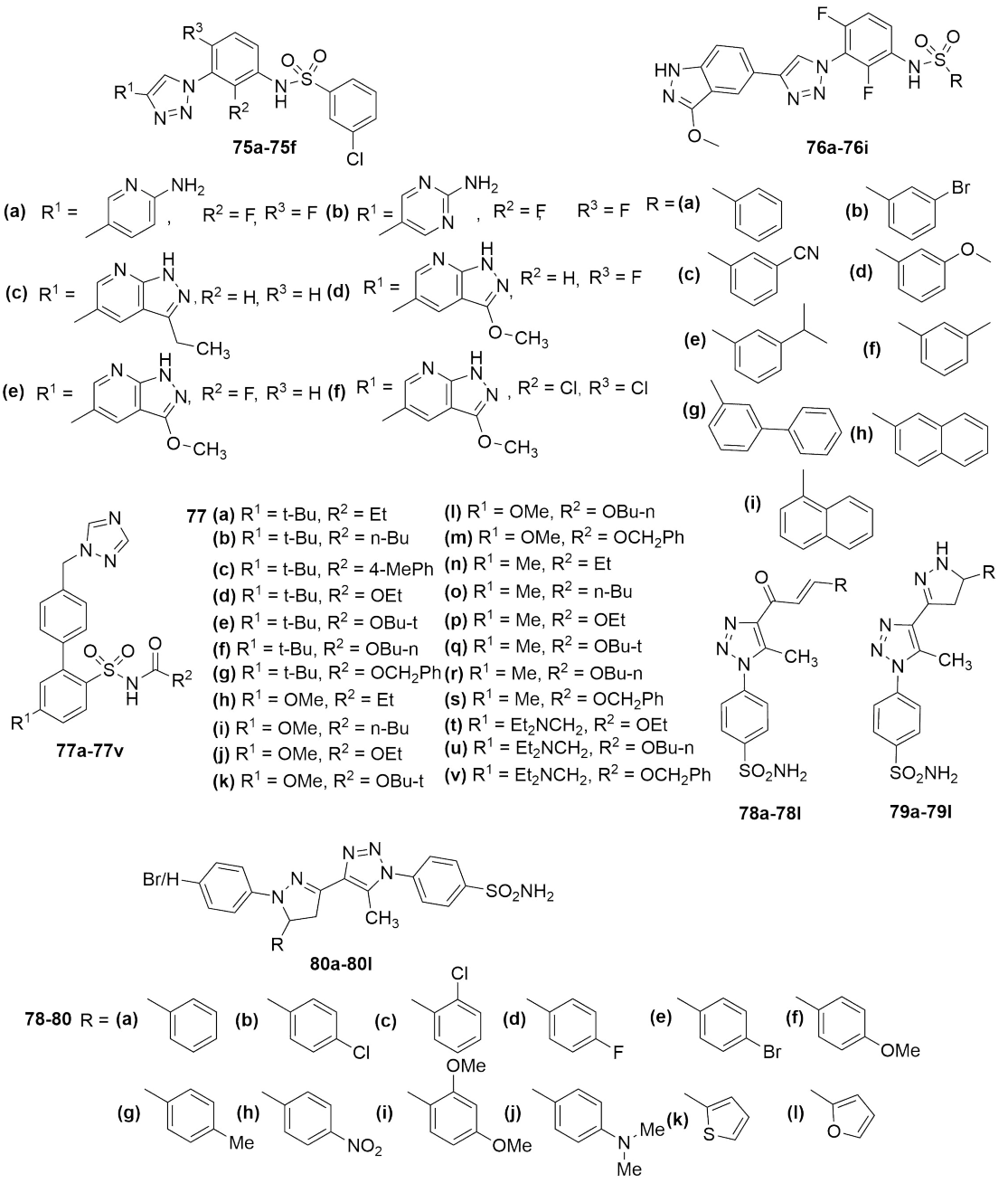


Fig. 1.10 Chemical structures of 1,2,3-triazole derivatives **75-80**

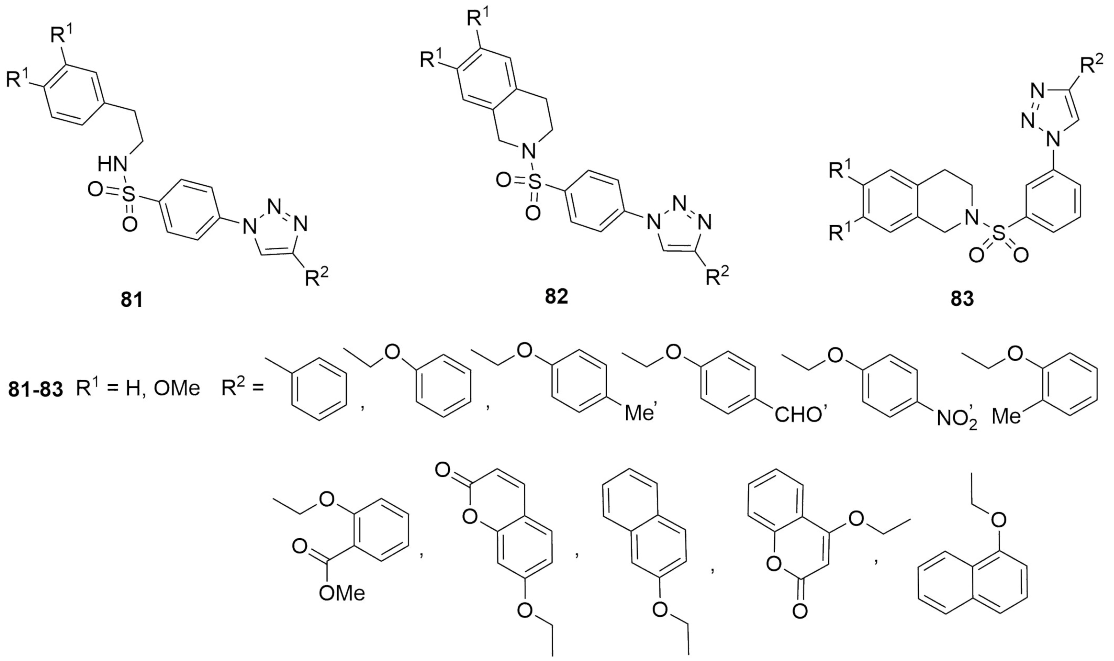


Fig. 1.11 Chemical structures of 1,2,3-triazole derivatives **81-83**

Pingaew *et al*. [54] synthesized a series of benzene sulfonamide containing 1,4-disubstituted-1,2,3-triazoles **81-83** using click chemistry reactions and evaluated them for aromatase inhibitory effects (Fig. 1.11). All the compounds were also explored for their molecular docking study. Most of the compounds showed effective aromatase inhibitory activity (IC_{50} = 1.3-9.4 μM) and the compounds containing 6,7-dimethoxy substituents on the isoquinoline ring displayed the most potent aromatase inhibitory activity (IC_{50} = 0.2 μM). Batra *et al*. [55] synthesized benzenesulfonamide [1,2,3]-triazole hybrids **84** and evaluated their anti-plasmodial potential using radioactive [^{3}H] hypoxanthine incorporation method against *P. falciparum* (3D7) strain (Fig. 1.12). Compound containing electron withdrawing substituents 4-nitrophenoxymethyl on triazole ring and 3,5-dimethylpyrimidy substituted sulfonamide group was most effective with IC_{50} value 6.2 μg/mL and CC_{50} value 7.5 μg/mL against *P. falciparum* (3D7) strain and human *hepatocarcinoma* (HUH7) cells, respectively. Synthesis of a novel series of isooxazoline and pyrazoline incorporated 1,2,3-triazole benzene sulfonamides **85** and **86**, respectively, and their docking simulations to identify binding affinity towards the selected target human protein PI3K α and evaluation for ADMET profiles were reported by Siliveri *et al.* [56] (Fig. 1.12). Among all the docked compounds, **86m** showed the highest Lib Dock score of 137.05 compared with the reference ligand KKR exhibiting a Lib Dock score of 88.35**.**

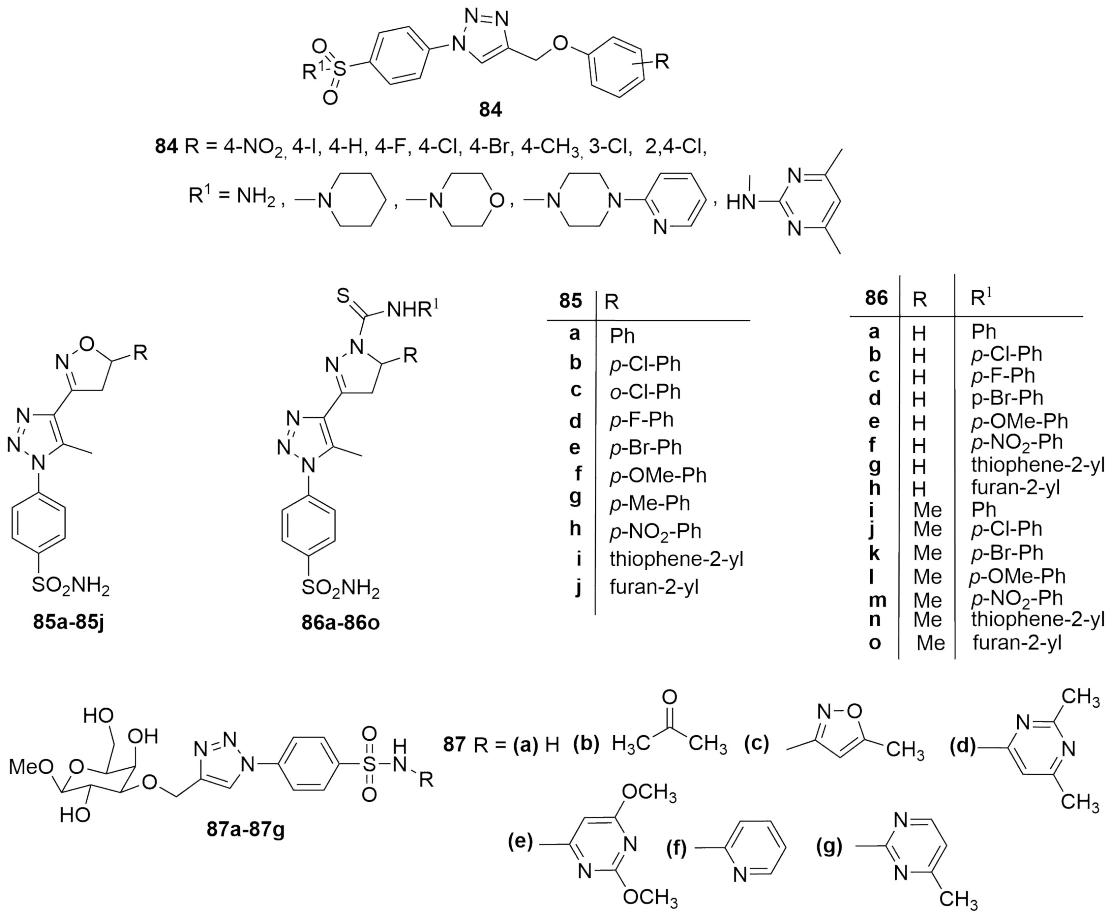


Fig. 1.12 Chemical structures of 1,2,3-triazole derivatives **84-87**

1,2,3-Triazole-linked galactosyl arylsulfonamides **87** were synthesized and investigated as anti-trypanosomal agents by Marchiori *et al*. [57] (Fig. 1.12). Compounds **87c** and **87e** showed most potent inhibition assay against *T. cruzi* cell invasion towards fibroblasts cells. The newly synthesized compounds were also evaluated for their inhibitory activities against *T. cruzi trans-sialidase* and galectin-3 present in the host cells. One of the target compounds **87b** showed highest *T. cruzi trans-sialidase* inhibition in fluorimetric inhibitory assay. A series of 1,2,3-triazole tethered sulfonamide-berberine hybrids containing isoquinoline pharmacophore **88** and **89** were synthesized using click chemistry reactions and evaluated as anti-malarial agents by Batra *et al*. [58] (Fig. 1.13). Most of the compounds displayed significant *in vitro* anti-malarial activity with IC_{50} values in the range of 0.1-20 μg/mL against *P. falciparum* (3D7) and also found to be non-cytotoxic against human prostate cancer cells. Compound containing 4-chlorophenylamino group **88d** was found to be the most significant one with IC_{50} value of 0.1 μg/mL while 4-nitrophenyl substituted sulfonamide **88g** was the least active

compound showing IC_{50} value of 20 μg/mL.

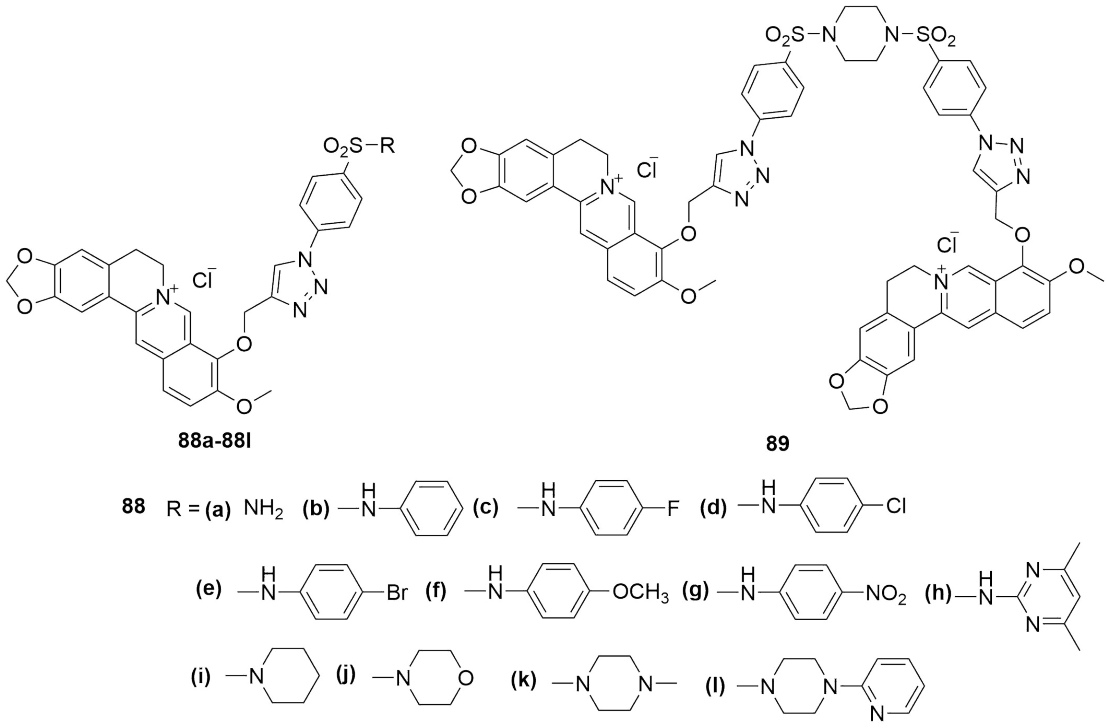


Fig. 1.13 Chemical structures of 1,2,3-triazole derivatives **88-89**

1.2.2 1,2,4-Triazoles

1,2,4-Triazole nucleus have captured considerable attention of medicinal chemists over the past decade due to the associated remarkable pharmacophoric characteristics [59]. This nucleus possess wide range of biological activities such as antibacterial, antifungal, antitumor, anti-inflammatory, anti-tubercular, analgesic, antiviral, anticancer, antimalarial, hypoglycemic, antidepressant, anticonvulsant, antioxidant etc. activities [60]. Over the time, this nucleus has become more and more prevalent in the design of newer drug candidates. In this section, biological importance of 1,2,4-triazoles have been discussed keeping main focus on the benzensulfonamide incorporated analogues of them.

Boechat *et al.* [61] have designed and synthesized 1H-1,2,4-triazol-3-yl benzenesulfonamide derivatives **90** and reported their molecular docking study for anti-malarial activity (Fig. 1.14). In molecular docking studies, compounds containing trifluoromethyl group at 5-position of the triazole ring showed stronger interaction with *P. falsiparum* DHPS enzyme. Said *et al.* [62] have reported the synthesis of benzenesulfonamide containing triazolopyrimidines **91** and triazoles **92**, **93, 94, 95** and

96 (Fig. 1.14) and their inhibitory activity against hCA I, hCA II, hCA IX and hCA XII. Out of the panel of four hCA isoforms tested, hCA IX and XII were efficiently inhibited by all the tested compounds with Ki values 3.3-85 nM and 4.4-105 nM, respectively. Compounds **91d** (containing 4-pyridyl) and **96e** (containing 4-methoxyphenyl) were found to be the most selevtive hCA IX inhibitor over hCA I (100.85 and 210.58 times selectivity, respectively). Both these compounds were also intervened for dock study into the active site of hCA II, hCA IX and hCA XII. Ram *et al.* [63] synthesized three series of benzenesulfonamide containing 1,2,4-triazoles **97**, **98** and **99**, and investigated them as inhibitors against four of the hCA isoforms comprising hCA I, hCA II, hCA IX and hCA XII (Fig. 14). The tested compounds have shown moderate to excellent inhibitory potential with Ki values in the range of 2.8-431 nM and 1.3-63 nM against hCA IX and hCA XII, respectively. SAR studies reveal that compounds containing a nuclear triazole ring **97** and triazole ring fused with six membered thiadiazene **98** were better inhibitors against all the tested isoforms when compared with compound **99** having triazole ring fused with a five member thiadiazole ring. Mustafa *et al*. [64] synthesized a series of celecoxib derivatives **100** and **101** and evaluated them for carbonic anhydrase inhibition activity (Fig. 1.14). All the tested compounds showed increased inhibition activity against the cytosolic isoform hCA I even more than Celecoxib. Compounds **101c**, **101d**, **101f** and **101k** displayed highest potency (Ki = 55.4 to 84.6 nM) while **101b, 101g, 101h** and **101i** displayed a medium potency (Ki = 511.8- 647.2 nM) against hCA II. Compounds **101a, 101e** and **101m** also displayed inhibition (Ki = 2662-808 nM) against hCA II**.**

Swain *et al.* [65] synthesized a series of 3-functionalized benzenesulfonamides incorporating phenyl-1,2,3-triazole derivatives with an amide linker **102** and assayed them as inhibitors of human carbonic anhydrase isoforms hCA I, hCA II, hCA IV and hCA IX (Fig. 1.15). All the synthesized compounds showed inhibition in low to medium nanomolar range except **102k**, **102l**, **102m** and **102o** (containing electron withdrawing substituents on both the phenyl rings) which displayed strong inhibitory activity against three isoforms hCA I, hCA II and hCA IV**.** Al-Sehemi *et al*. [66] has reported the synthesis of fused triazole derivatives **103**, **104** and **105** (Fig. 1.15) using 4-amino-benzenesulfonamide as the starting material. All the synthesized compounds exhibited promising anti-bacterial activity when compared with the standard drug gentamycin.

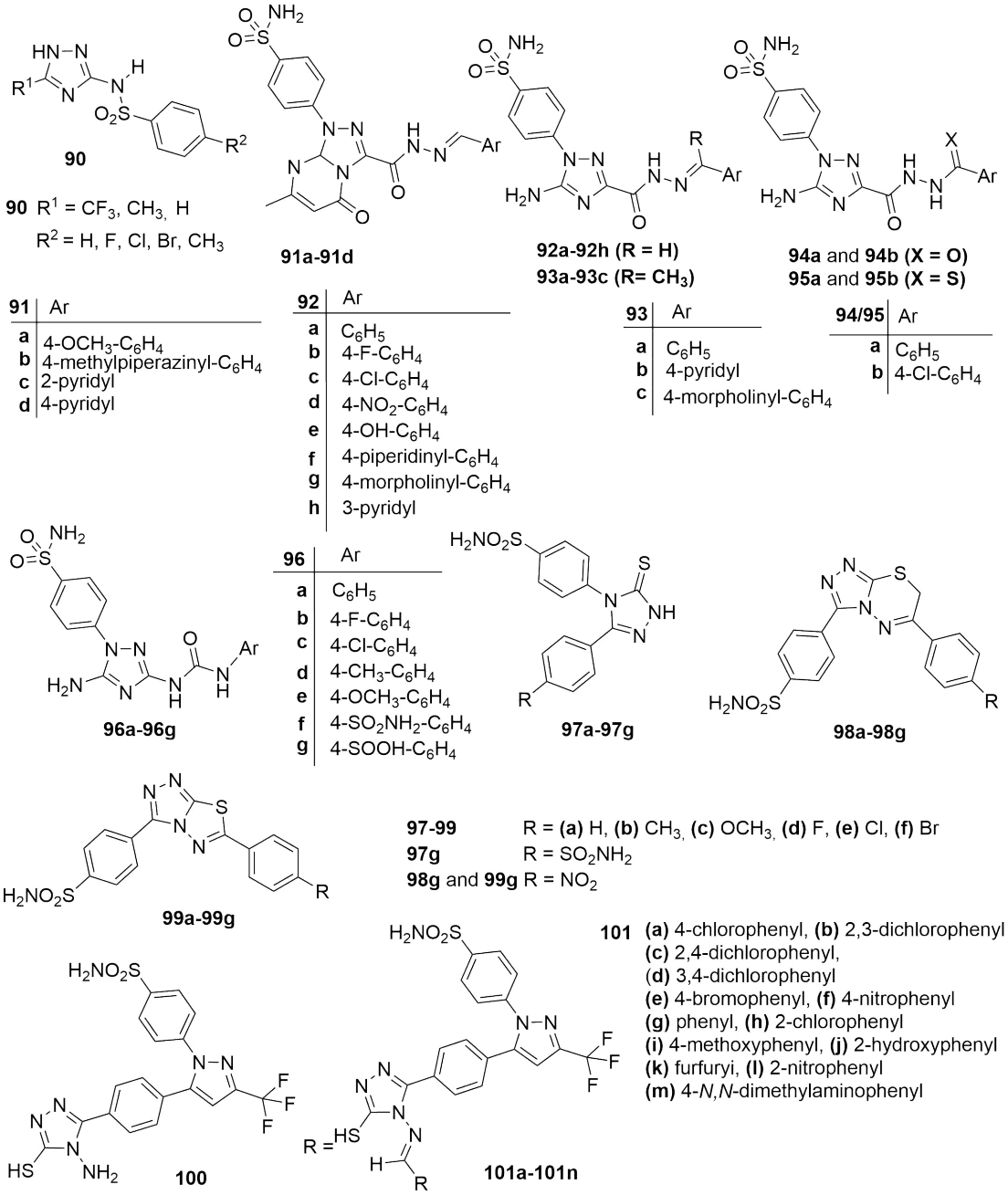


Fig. 1.14 Chemical structures of 1,2,4-triazole derivatives **90-101**

Fluorinated 1,2,4-triazole derivatives carrying benzenesulfonyl urea and thiourea derivatives as well as their cyclic sulfonyl thioureas **106-113** were synthesized and investigated as anti-microbial agents by Faidallah *et al*. [67] (Fig. 1.15). All the tested compounds were found to exhibit mild to moderate activity against *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*.

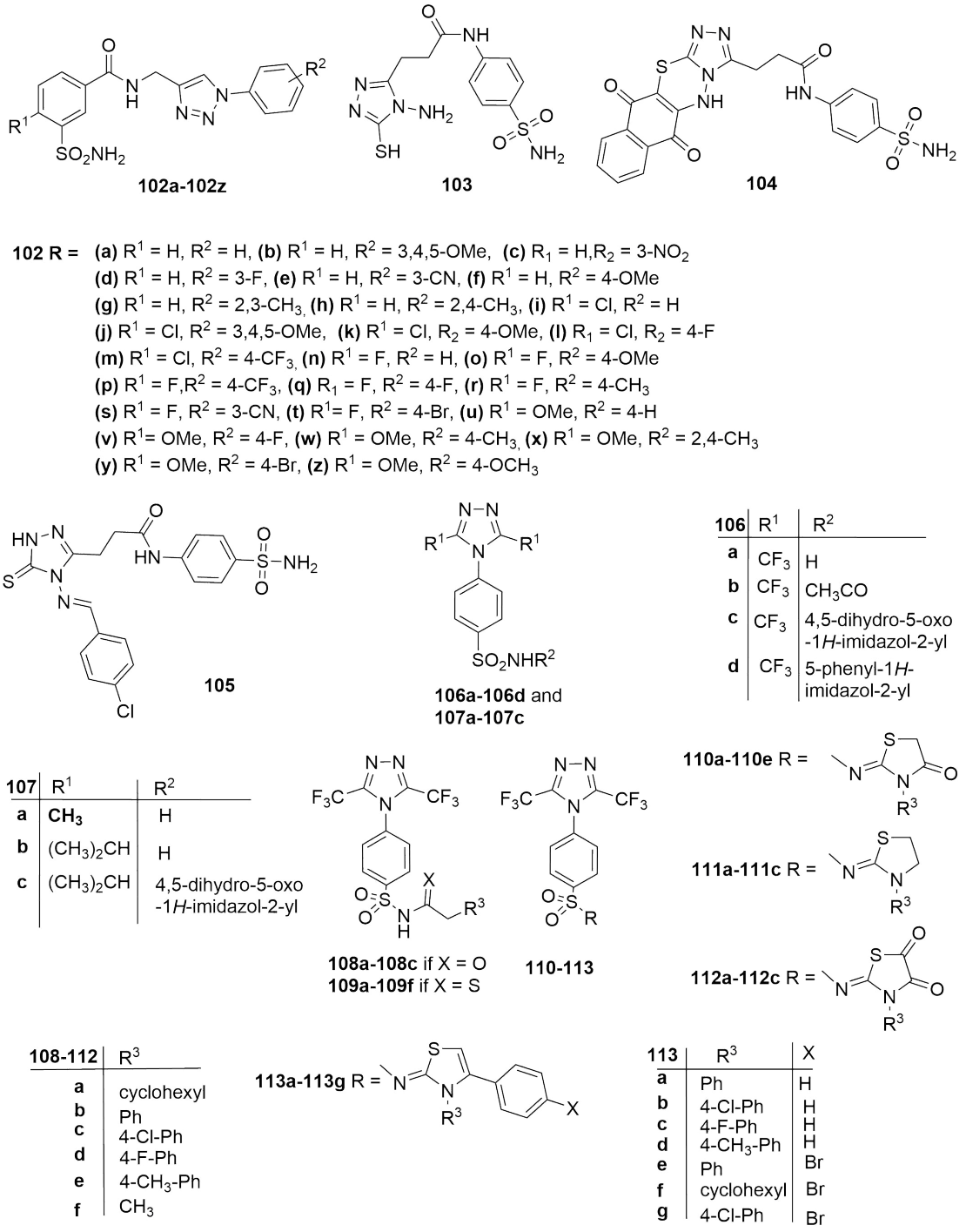


Fig. 1.15 Chemical structures of 1,2,4-triazole derivatives **102-113**

Zoumpoulakis *et al*. [68] have reported the synthesis and anti-microbial evaluation of novel series of 1,2,4-triazoles **114** (Fig. 1.16). All the evaluated compounds exhibited promising bactericidal effect. The SAR studies reveal that presence of aliphatic chain with increased number of carbon atoms enhanced the potency of compounds against anti-bacterial activity. All the target compounds were also investigated for *in vitro* anti-fungal

activity and some of the analogues displayed very promising results as anti-fungal agents even better than the standard drug ketokonazol. Synthesis of a series comprising three benzenesulfonamide bearing thienotriazolopyrimidine derivatives **115** were reported by Hafez *et al*. [69] and evaluated them for anti-bacterial activity using ciprofloxacin as standard drug (Fig. 1.16). Compounds **115b** exhibited highest activity against both Gram-positive and Gram-negative bacteria with MIC values of 1-3 μM**.** All the three compounds were also evaluated for their anti-tumor activity against three human tumor cell lines HepG-2, HT-29 and NCI-H460. Again compound **115b** was found to be the most potent anti-tumor agent.

Synthesis of a series of novel sulfonamide derived compounds **116-120** and their anti-microbial activity was reported by He *et al*. [70] (Fig. 1.16). *In vitro* anti-microbial evaluation results revealed that some of the compounds were either equipotent or better anti-microbial agents than the reference drug. SAR studies show that combination of 1,2,4 triazole and sulfonyl fragments exerted a significance influence on biological activities leading to much better results when compared with chloramphenicol, one of the reference drugs. Desai *et al*. [71] have synthesized a series of 1,2,4 triazole derivatives **121-126** and screened them for anti-microbial activity against *Escherichia coli*, *Bacillus cirroflagellosus*, *Aspergillus niger* and *Colletorichumcapsici* by cup plate method using cotrimoxazole and diflucan as standard drugs (Fig. 1.16). Most of the tested compounds exhibited minimum to moderate anti-bacterial activities, however, all the compounds displayed interesting anti-fungal activities. Ezabadi *et al*. [72] have synthesized a series of benzenesulfonamide bearing triazolo-3-thiones **127** and investigated them for *in vitro* anti-fungal and anti-bacterial activities (Fig. 1.16). All the tested compounds exhibited significant anti-fungal activity against all the micromycetes even greater than the commercial fungicide bifonazole. Anti-bacterial activity profile of all the tested compounds was also moderate to excellent. Zhang *et al*. [73] synthesized two series of benzenesulfonamide containing azole derivatives **128** and **129** and evaluated them for anti-microbial activities (Fig. 1.17). Some of the compounds displayed moderate activity. A series of 4-amino-5-mercapto-4(H)-1,2,4-triazole derivatives **130** having biologically active motif as benzenesulfonamide were synthesized and evaluated for their biological activities by Desai *et al*. [74] (Fig. 1.17). Most of the evaluated compounds exhibited significant anti-fungal activity against *Colletotrichum capsici*, even greater than fluconazole. All of the compounds possessed moderated anti-inflammatory activity and significant analgesic activity, however, moderate anti-tuberculosis activity was shown by

130e and **130f**.

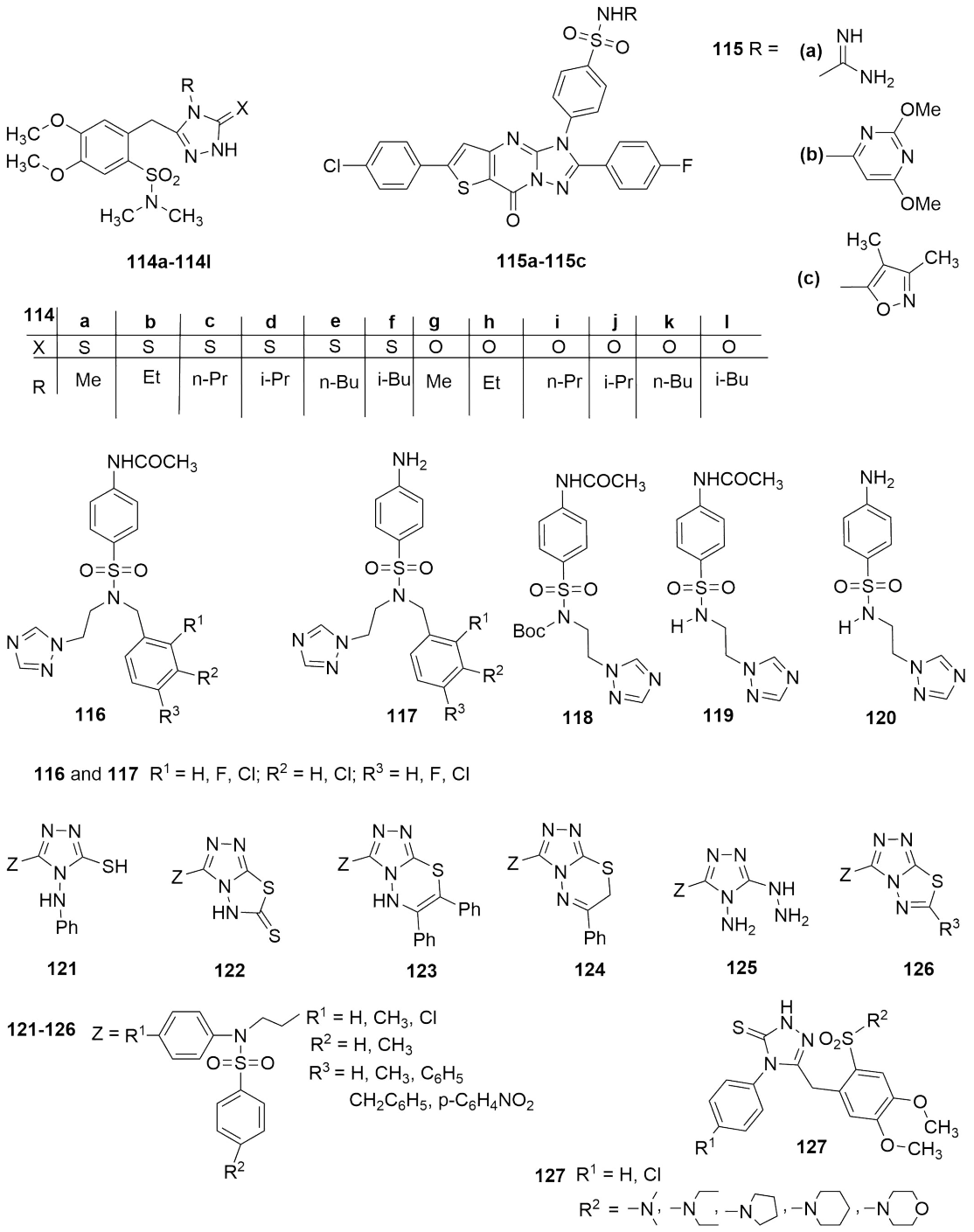


Fig. 1.16 Chemical structures of 1,2,4-triazole derivatives **114-127**

A series of 4-substituted N-(5-amino-1H-1,2,4-triazol-3-yl)pyridine-3-sulfonamides **131** was synthesized and investigated as anti-fungal agents against strains of the genera *Candida*, *Geotrichum*, *Rhodotorula*, and *Saccharomycess* by Szafranski *et al* [75] (Fig. 1.17). Some of the tested compounds displayed better efficacy than fluconazole towards

Rhodotorula mucilaginosa and *Candida albicans* species with MIC values $\leq$ 25 g/mL.

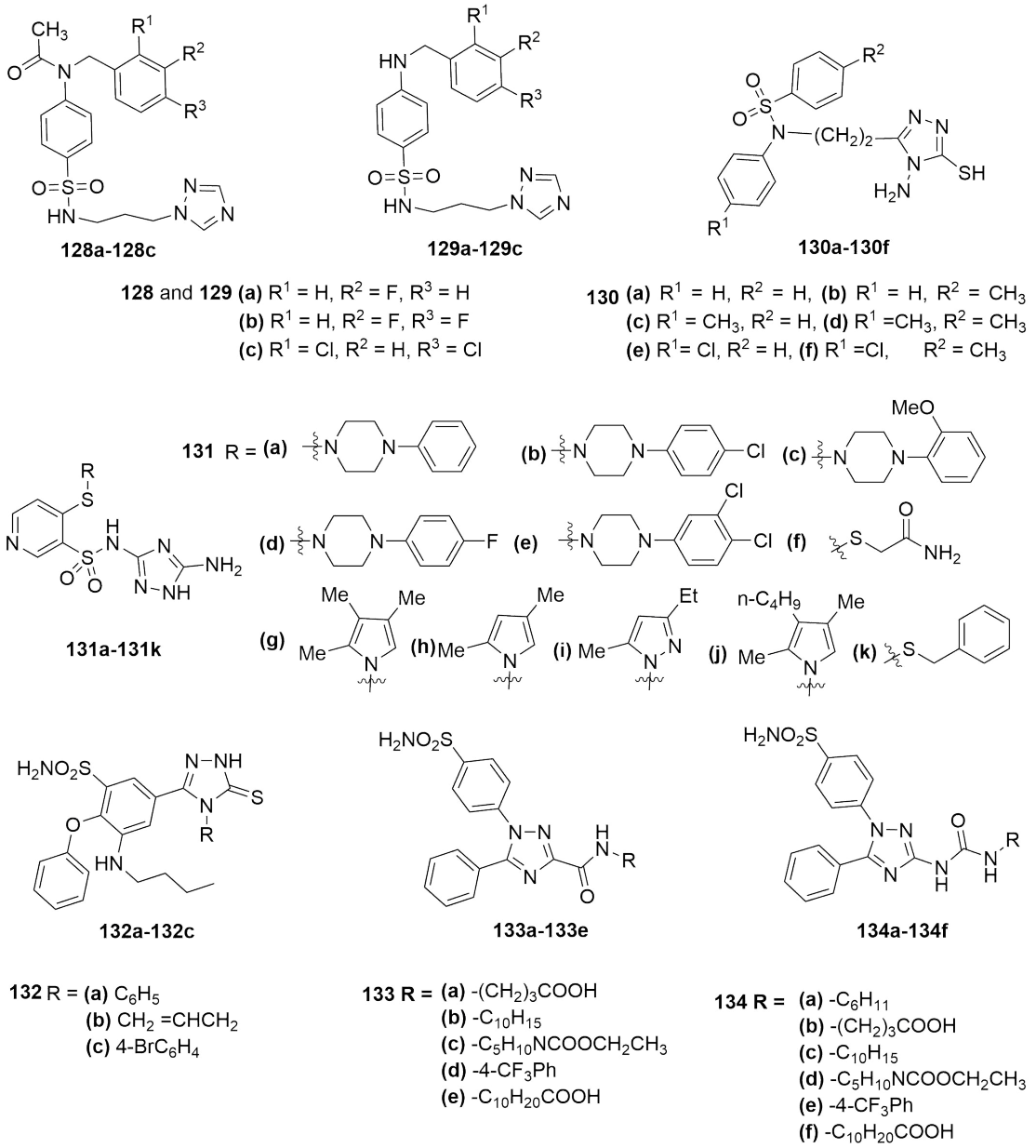


Fig. 1.17 Chemical structures of 1,2,4-triazole derivatives **128-134**

Ibrahim *et al.* [76] have synthesized benzenesulfonamide derivatives based on bumetanide scaffold **132** and evaluated them as COX-2 inhibitors (Fig. 1.17). Benzenesulfonamide bearing triazole moieties **132a** and **132c** were good inhibitors of COX-2 with IC_{50} values 0.28 and 0.17 μM, even 14.4 and 23.7-fold more potent than celecoxib, respectively. Ahmed *et al.* [77] have synthesized urea and amide conjugates of diaryl-1,2,4-triazole **133a-133e** and **134a-134f**, respectively, and evaluated them for inhibition activity against both COX-2 and eSH (Fig. 1.17). Among the evaluated

compounds, **134e** showed the highest inhibition activity. A series of benzenesulfonamide containing thiazolo[3,2-*b*][1,2,4]triazoles **135** and their corresponding acyclic intermediates **136** were synthesized and assessed *in vivo* for their anti-inflammatory and analgesic activities (Fig. 1.18) [78]. Compounds **136b, 136c** and **136d** displayed the most significant anti-inflammatory activity at 2 hrs, 3 hrs and 4 hrs, respectively, after the induction of inflammation while **135a**, **135d, 136a**, **136b** and **136c** displayed significant analgesic properties at 4 hrs.

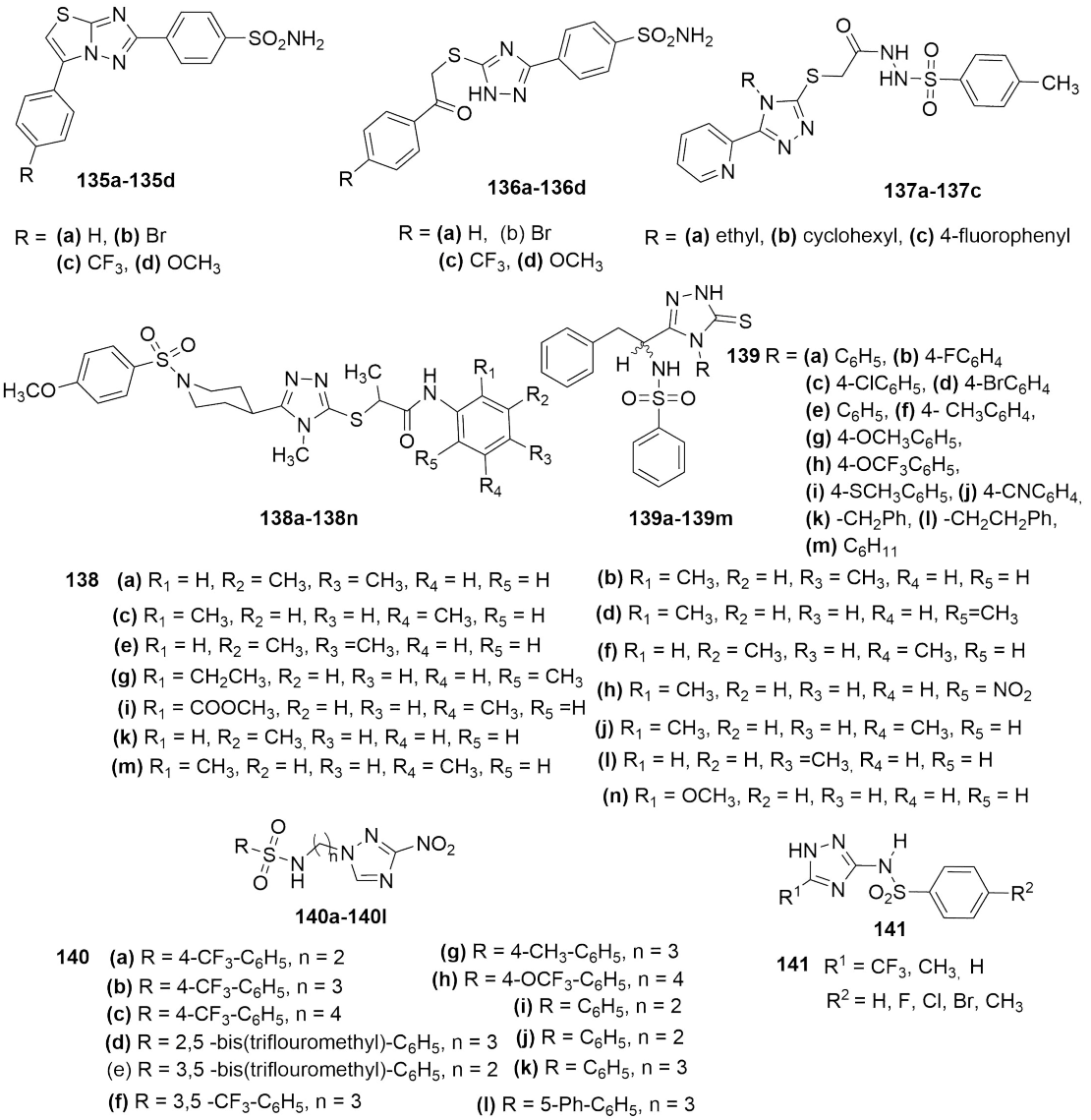


Fig. 1.18 Chemical structures of 1,2,4-triazole derivatives **135-141**

Khalid *et al.* [79] have synthesized benzenesulfonamide derivatives of 1,2,4-triazole **137** and evaluated compounds **137a** and **137b** for anti-platelet and anti-coagulant activities (Fig. 1.18). Both the compounds inhibited arachidonic acid, adenosine diphosphate and

collagen-induced platelets aggregation with IC_{50} values of 121.6, 956.8 and 30.1 for the compound **137a** and 99.9, 519 and 29.97 for the compound **137b**, respectively. 1,2,4-Triazole containing piperidine and propenamide derivatives **138** were synthesized and screened for biological potential against acetylcholinesterase (AChE) and α-glucosidase enzymes by Virk *et al.* [80] (Fig. 1.18). Most of the compounds were found to be active against both the enzymes. In the molecular docking study, compound **138m** showed the maximum inhibition potential against AChE enzyme. 1,2,4-Trizole-3-thiones containing benzenesulfonamide moiety **139** were synthesized and screened for *in vitro* anti-viral activity against a broad variety of DNA and RNA viruses by Basaran *et al*. [81] (Fig. 1.18). Compounds **139c**, **139f**, **139g**, **139h** and **139i** showed excellent activity with (EC_{50} = 1.6-6.5 μM) against influenza A H1N1 compared to reference drug ribavirin (EC_{50} = 8.0 μM). 3-Nitro-1H-1,2,4-triazoles containing differently substituted benzenesulfonamides **140** were synthesized and tested for *in vitro* anti-trypanosomal and anti-leishmanial activities by Papadopoulou *et al*. [82] (Fig. 1.18). Most of the compounds showed significant activity against *T. cruzi* intracellular amastigotes (IC_{50} = 28 nM-3.72 μM) even more potent (up to 56-fold) than the reference drug benznidazole. Boechat *et al*. [83] have designed and synthesized 1H-1,2,4-triazol-3-yl benzenesulfonamide derivatives **141** and reported their molecular docking study for anti-malarial activity (Fig. 1.18). In molecular docking studies, compounds containing trifluoromethyl group at 5-position of the triazole ring showed stronger interaction with *P. falsiparum* DHPS enzyme.

1.2.3 Oxadiazoles

Compounds having oxadiazole ring in their structure constitute another important class of medicinally potent heterocyclic compounds [84]. This nucleus plays a key role in medicinal chemistry due to its broad range of biological activities such as anti-microbial, anti-fungal, anti-convulsat, anti-inflammatory, anti-viral, analgesic and anti-proliferative activities [85]. The multiple biological actions of oxadiazole containing compounds have encouraged the medicinal chemists to synthesize newer drug candidates having oxadiazole ring incorporated in their skeleton. A number of articles on the synthesis of oxadiazole derivatives and their biological evaluation are well documented in literature. In this section, we have summarized the work reported on biological activities of oxadiazole derivatives along with the structure-activity relationship studies.

Slawinski *et al.* [86] have synthesized a series of 2-mercapto benzenesulfonamide containing 1,3,4-oxadiazoles **142** and evaluated them as anti-cancer agents against three cancer cell lines *viz* colon cancer cell HCT-116, breast cancer cell MCF-7 and cervical cancer cell HeLa (Fig. 1.19). Compounds **142s**, **142t**, **142u**, **142v** and **142w** having styryl moiety attached at position 5 of 1,3,4-oxadiazole ring exhibited significant inhibitory potential against two and/or three cancer lines tested.

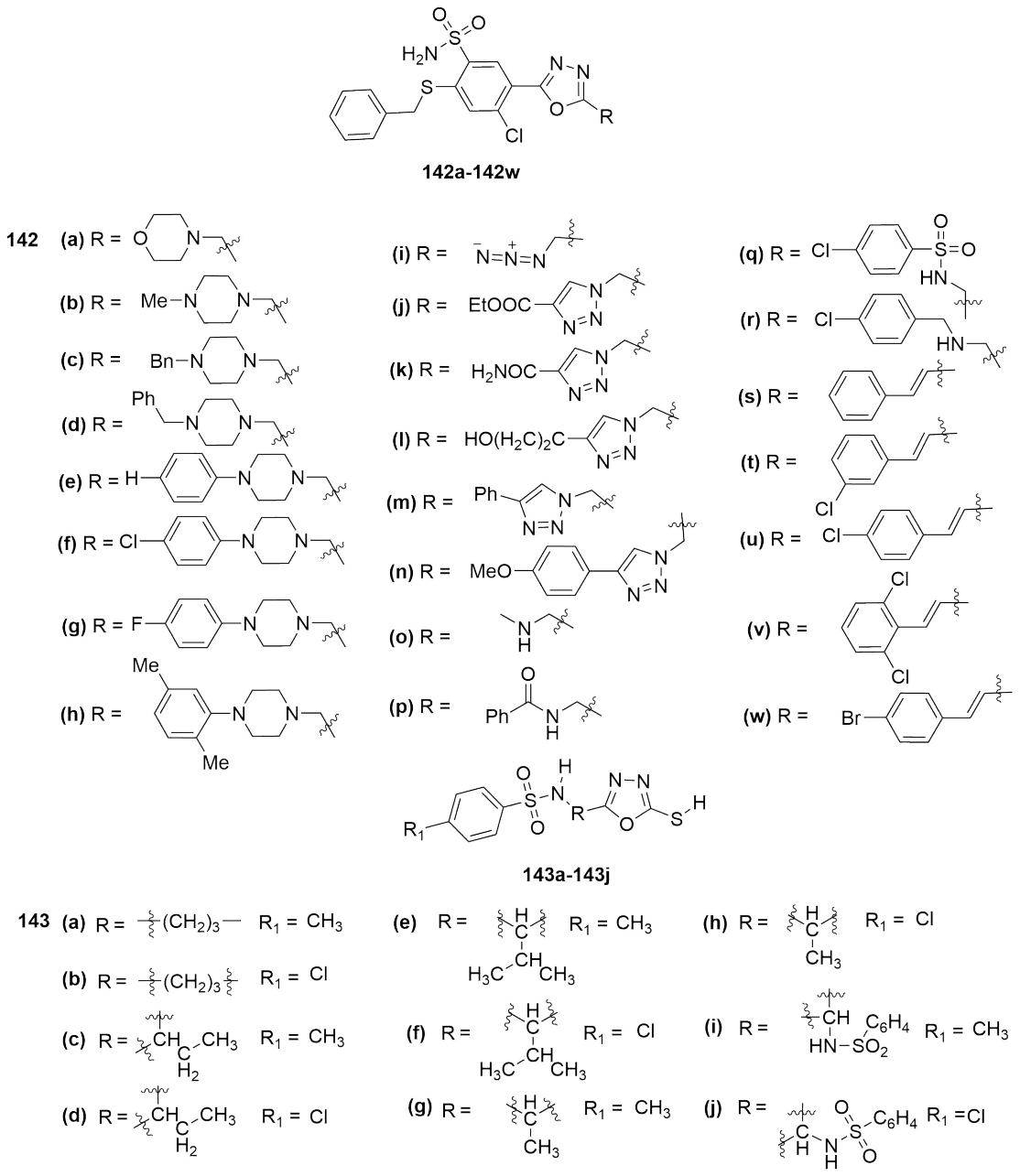


Fig. 1.19 Chemical structures of oxadiazole derivatives **142-143**

Zareef *et al.* [87] have synthesized a series of chiral 1, 3, 4-oxadiazole incorporating

benzenesulfonamide derivatives **143** and evaluated them for anti-malarial activity and inhibition assay against hem polymerization (Fig. 1.19). For anti-malarial activity, mice infected with plasmodium berghei was treated with the sample compounds. Compounds were found to have potential to be used as anti-malarial agents. Further, results depicted that compounds have ability to inhibit hemoglobin degradation also. Two series of benzenesulfonamide containing 1,3,4-oxadizole hybrids **144** and **145** have been synthesized and evaluated for their inhibition potential against four human carbonic anhydrase isoforms *viz* hCA I, II, IX, and XII by Sharma *et al*. [88] (Fig. 1.19). Compounds **144g** and **145j**, possessed excellent inhibition of hCA I, even more than the reference drug Acetazolamide (Ki = 250 nM).

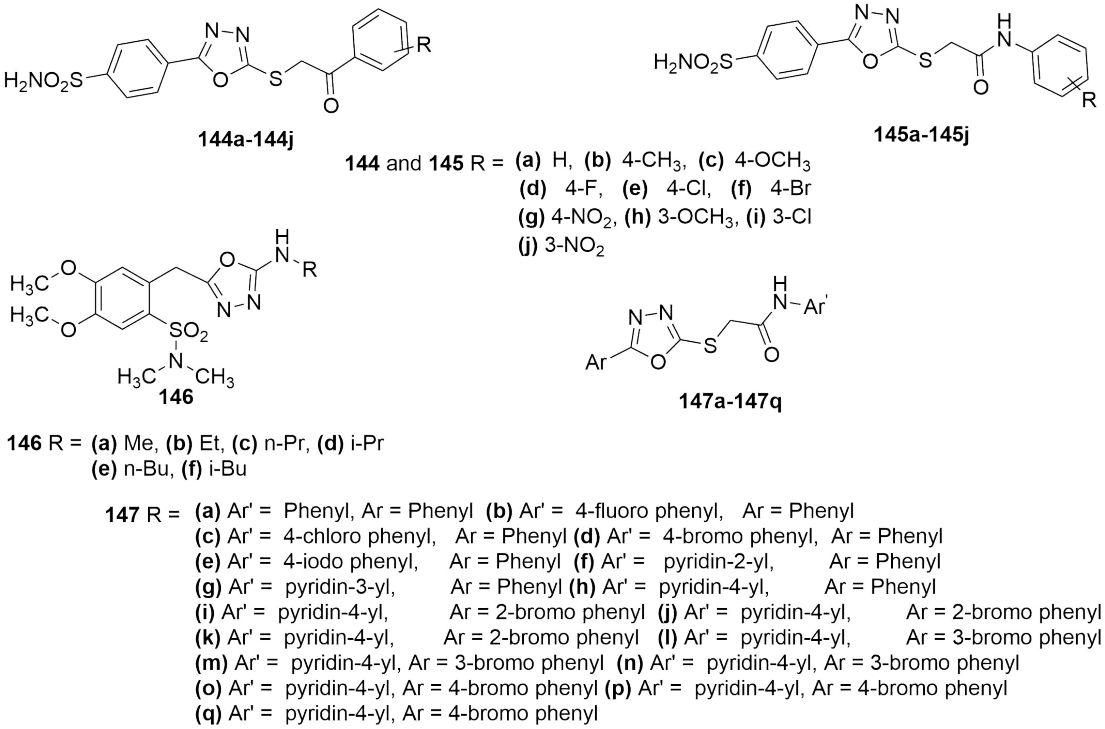


Fig. 1.20 Chemical structures of oxadiazole derivatives **144-147**

Zoumpoulakis *et al*. [68] have reported the synthesis and biological evaluation as anti-microbial agents of series of 1,3,4-oxadiazole derivatives **146** (Fig. 1.20). All the evaluated compounds exhibited promising bactericidal effect. The SAR studies reveal that presence of aliphatic chain with increased number of carbon atoms enhanced the potency of compounds against bacterial strains. All the target compounds were also investigated for their *in vitro* anti-fungal activity and some of them displayed promising results as anti-fungal agents even better than the standard drug, Ketokonazol. A library

of seventeen 1,3,4-oxadiazole derivatives **147** was synthesized and investigated for their *in vitro* carbonic anhydrase potential by Vanjare *et al.* [89] (Fig. 1.20). 3-Pyridine substituted analogue **147g** was found to be the most potent carbonic anhydrase inhibitor (IC_{50} = 0.1 μM) even 11-fold more active than the reference drug, Acetazolamide (IC_{50} = 1.1 ± 0.1 μM). Narella *et al*. [90] have synthesized a series of coumarin-1,3,4-oxadiazole hybrids **148** and evaluated them for inhibition potential against four human carbonic anhydrase isoforms hCA I, hCA II, hCA IX and hCA XII (Fig. 1.21). Results depicted that all the compounds exhibited selective inhibition of hCA IX and hCA XII over hCA I and hCA II isoforms and particularly compounds **148h** and **148p** could be the potential candidates for development of drug in effective treatment of cancer.

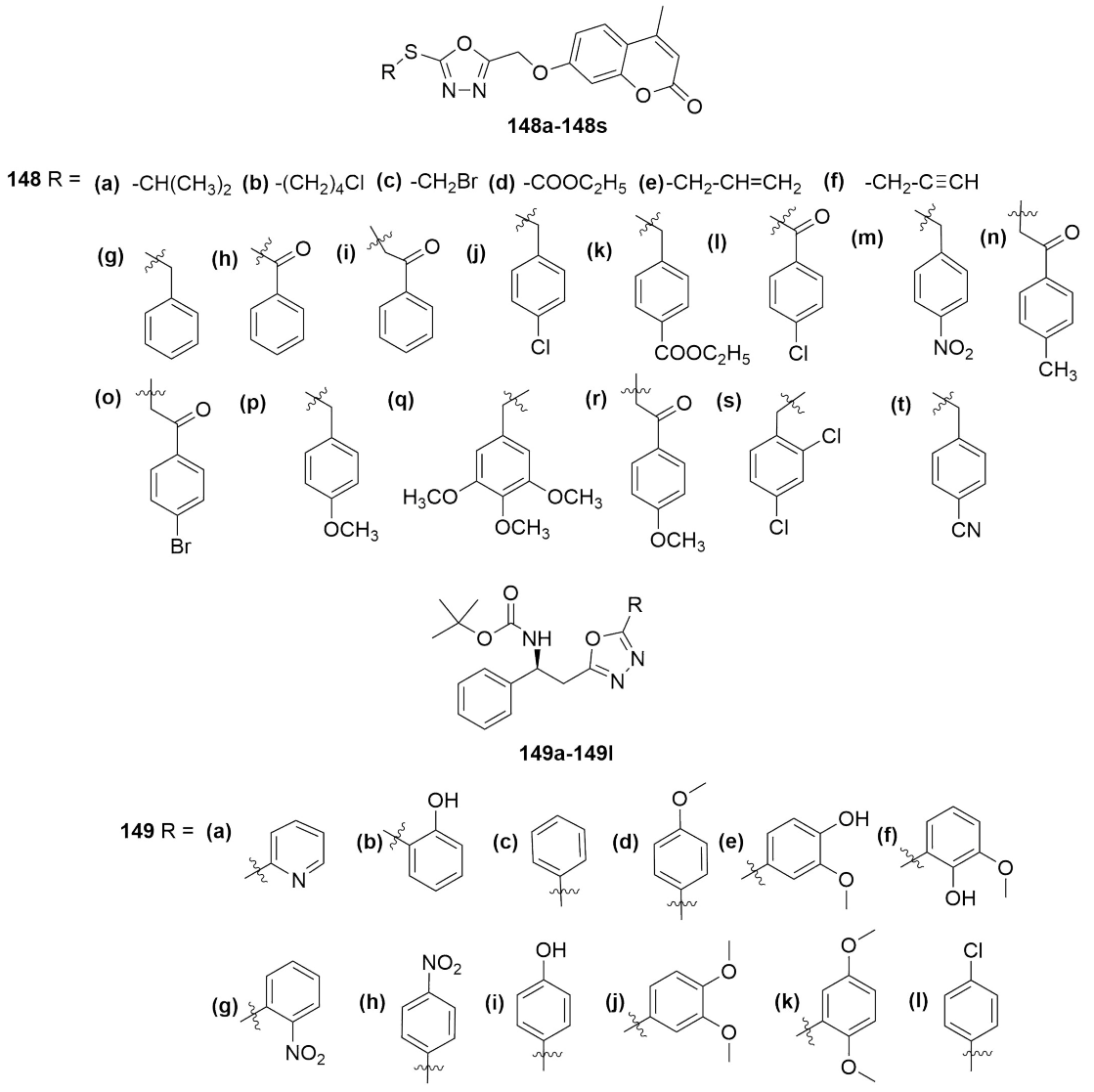
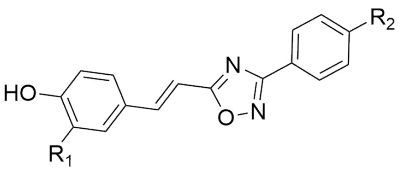


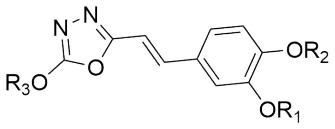
Fig. 1.21 Chemical structures of oxadiazole derivatives **148** -**149**

Rafiq *et al*. [91] have synthesized a series of twelve novel 1,3,4-oxadiazole and 3-phenyl-β-alanine hybrids **149** and evaluated them for inhibitory activity against CA-II (Fig. 1.21). Results showed that compounds **149a** (12.1 µM), **149c** (13.8 µM) and **149f** (20.7 µM) were the most potent agents against CA-II enzyme among all the tested compounds. Sucu *et al*. [92] have synthesized ferulic and caffeic acid-based oxadiazole molecular hybrids **150** and **151** and evaluated their inhibition potential against three different Glioblastoma cell lines (GBM) *viz* LN229, T98G, and U87 and three different cancer cell lines *viz* SKOV3, MCF7, and A549 cell (Fig. 1.22). Compounds **150a** and **151b** exhibited the highest inhibitory activity against all the GBM cell lines tested. Cytotoxicity evaluation showed that compounds have better inhibitory activity against cancer cell lines than GBM cell lines. Hamdani *et al.* [93] have collaborated 1,3,4-oxadiazole and benzenesulfonamide moieties in a single unit to synthesize novel *S*-benzylated and *S*-alkylphthalimidated hybrids **152** (Fig. 1.22) and tested their inhibition potential against dengue virus protease. Among the synthesized hybrids, **152g** (IC_{50} = 13.9 μM) and **152h** (IC_{50} = 15.1 μM) were found to be the most potent inhibitors of dengue virus protease.



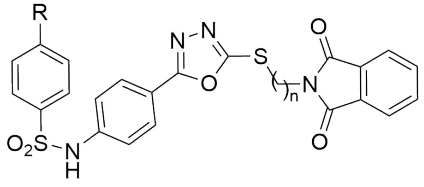
150a-150c

150 **(a)** R_1 = CH_3, R_2 = H
(b) R_1 = H, R_2 = CF_3
(c) R_1 = CH_3, R_2 = CF_3



151a-151f

151 **(a)** R_1 = H, R_2 = H, R_3 = Phenyl
(b) R_1 = H, R_2 = H, R_3 = 2-furyl
(c) R_1 = H, R_2 =CH_3, R_3 = Phenyl
(d) R_1 = H, R_2 = CH_3, R_3 = 2-furyl
(e) R_1 = CH_3, R_2 = H, R_3 = Phenyl
(f) R_1 = CH_3, R_2 = H, R_3 = 2-furyl



152a-152j

152 **(a)** R = CH_3, n = 1, **(b)** R = CH_3, n = 2
(c) R = CH_3, n = 4, **(d)** R = CH_3, n = 6
(e) R = CF_3, n = 1, **(f)** R = CF_3, n = 2
(g) R = CF_3, n = 3, **(h)** R = CF_3, n = 4
(I) R = CF_3, n = 5, **(j)** R = CF_3, n = 6

Fig. 1.22 Chemical structures of oxadiazole derivatives **150-152**

1.3 Conclusion

Triazoles are important motifs in medicinal chemistry owing to their astonishing biological behavior including various biological activities such as anti-tubercular, anti-fungal, anti-tumor, anti-bacterial, anti-alzheimer, anti-viral, anti-malarial etc. Oxadiazoles constitute an another important class of medicinally potent heterocyclic compounds. The presence of this nucleus in the architecture of drug candidate causes multiple biological activities such as anti-inflammatory, anti-viral, anti-microbial, anti-tumor, anti-tubercular, anti-depressant and analgesic activities. In this chapter, we have made a succinct compilation of such reports well documented in literature reflecting the biological importance of these motifs giving a special attention to benzenesulfonamide derivatives of them. As a lot of attention has been made in this area yet there is an enormous space to work. The compilation is done by hoping that it will pave the way for further work in this area and motivates the researchers to work with more enthusiasm in the direction of synthesis of novel drug candidates having triazole and oxadiazole nucleus in their pharmacophoric unit.

CHAPTER 2

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,2,4-TRIAZOLE DERIVATIVES AS ANTI-MICROBIAL AND ANTI-OXIDANT AGENTS

2.1 Motivation for the Current Work

Heterocyclic compounds are the organic compounds having one or more heteroatoms such as nitrogen, oxygen, sulfur, etc. in their ring structure. They constitute a vital class of organic compounds due to their applications in various fields such as medicines [94], polymers [95], dyes [96], pesticides [97], cosmetics [98] etc. These compounds are building blocks of various natural products, hormones, and DNA (the genetic material) [99]. More than 90 % of the marketed drugs used for treatment of various diseases contain heterocyclic ring in their architectures [100]. In the modern scenario, researchers are working toward the synthesis of potent drugs candidates *via* functionalization of heterocyclic rings at various positions [101]. Triazoles are five membered heterocyclic compounds having two carbon atoms and three nitrogen atoms in their ring structures. Based on the position of nitrogen atoms with respect to each other, these are further divided into two categories namely 1,2,3-triazoles and 1,2,4-triazoles [102]. Owing to a wide spectrum of biological actions against various microbes such as bacteria, fungi, viruses, etc. 1,2,4-triazoles hold an intriguing class of biologically important heterocyclic compounds [103]. They are present in the architecture of various natural products [104] and in a number well-known marketed drugs [105] (Fig. 2.1).

There are numerous reports in literature on the synthesis of functionalized 1,2,4-triazoles

and their evaluation as anti-tubercular [106], analgesics [103], anti-oxidants [107], anti-proliferative [108], anti-inflammatory [109], anti-microbial [110], anti-convulsant [111], anti-viral [12], and anti-depressant [112] agents etc.

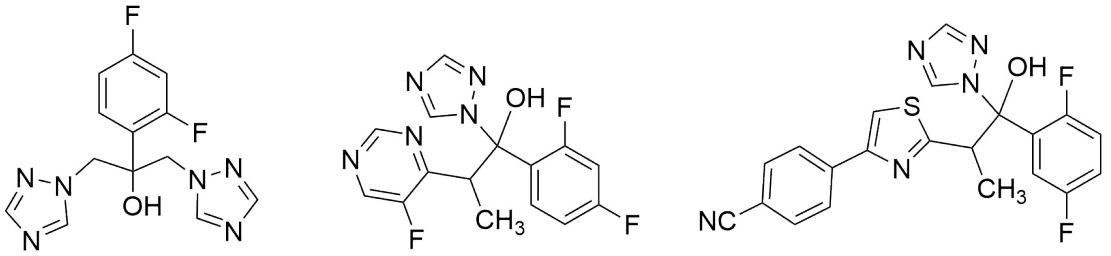


Fig. 2.1 Clinically used drugs having 1,2,4-triazole in their structures

Further, sulfonamide is also an important moiety in medicinal chemistry, presence of which in the pharmacophoric unit of a drug candidate leads to its augmented biological actions [113]. Various heterocyclic molecules having sulfonamide group in their structure have been studied as pharmaceutical agents possessing anti-diabetic [114], anti-microbial [115], anti-cancer [116], anti-thyroid [117], CA inhibition [118], anti-tumor [119] etc. biological activities. A lot of sulfonamide group bearing drugs are in use as medicaments for various disease.

A lot of anti-microbial drugs have been developed and are in use against microbial infections, however, due to over-use and mis-use, microbes have developed resistance to available ones causing a serious health issue affecting health of humans all over the world. Researchers are working toward developing novel anti-microbial agents having different mechanism of action, high efficacy and better selectivity than the available ones [120]. To address the synthesis of novel anti-microbial agents with broad spectrum potential, researchers are fascinated by molecular hybridization approach, wherein, two or more pharmacological moieties are fused in single one. As 1,2,4-triazole and benzenesulfonamide are two important moieties in medicinal chemistry, hybridization of these may generate newer drug candidates with better efficacy and higher potential. Being motivated by the above study coupled with our interest in the synthesis of biologically potent heterocyclic compounds [121–124], we hypothesized a library of benzenesulfonamide incorporated 1,2,4-triazoles with various functionalization on the triazole nucleus. In this chapter, we have reported the synthesis of a series of thirty novel 1,2,4-triazole derivatives having benzenesulfonamide moiety **1a-1g, 2a-2g, 3a-3g**,

4b-4f and **4h** and their biological evaluation as anti-microbial and anti-oxidant agents (Fig. 2.2). Cytotoxicity against mouse fibroblast cell line and plant seed germination cell line was also tested for all the newly synthesized compounds.

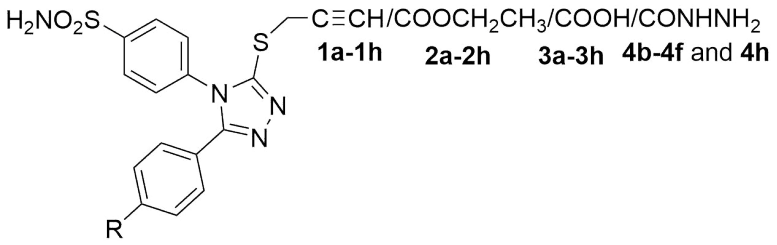


Fig. 2.2 Benzenesulfonamide bearing 1,2,4-triazole derivatives as anti-microbial and anti-oxidant agents

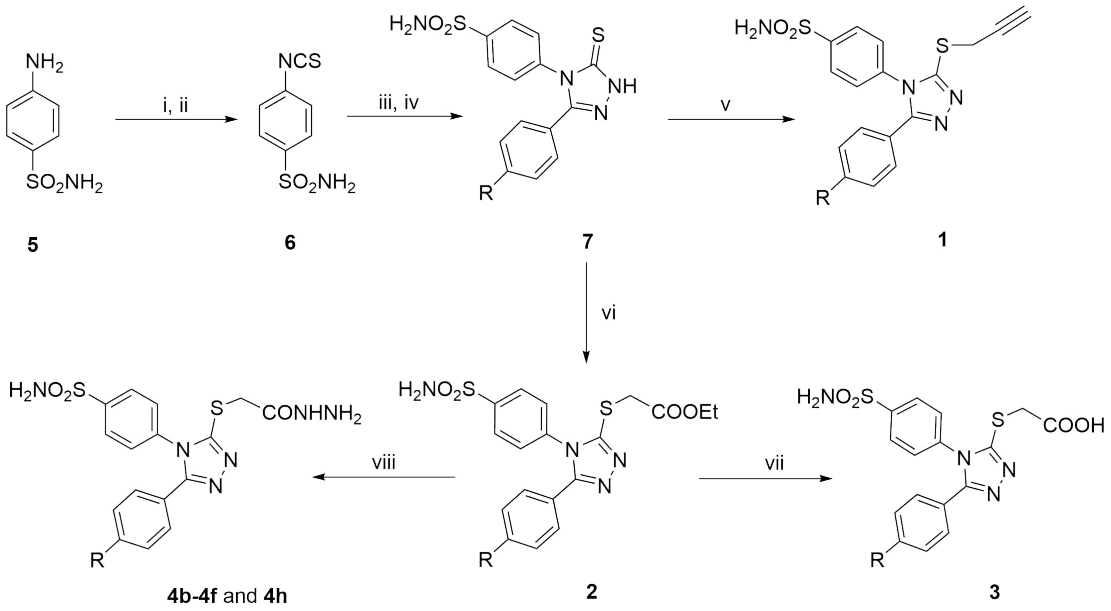
2.2 Results and Discussion

2.2.1 Synthesis overview of benzenesulfonamide bearing 1,2,4-triazole derivatives 1a-1h, 2a-2h, 3a-3h, 4b-4f and 4h

The synthetic route for preparing all the target compounds is outlined in scheme 2.1. The structures of newly synthesized compounds were elucidated based on their spectral (^{1}H NMR, ^{13}C NMR and IR) and HRMS data.

The key intermediates for the preparation of 1,2,4-triazole derivatives **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** were 5-thioxo-1,5-dihydro-4H-1,2,4-triazoles **7a-7h** which were synthesized using literature procedure [125]. The synthetic pathway for **7** involves conversion of 4-aminobenzenesulfonamide **5** to corresponding isothiocyanate **6** *via* a two-step procedure. The first step involves reaction of **5** with carbon disulfide using triethylamine as base in acetone solvent leading to its conversion to the corresponding dithiocarbamate salt which is then desulfurized in the successive step to **6** using molecular iodine and sodium bicarbonate in aqueous ethyl acetate as solvent. Isothiocyanate **6** thus obtained was converted to corresponding triazoles **7** by its condensation with differently substituted aryl hydrazides followed by the base catalyzed cyclization under aqueous condition. Propargylated triazoles **1** were obtained by alkylation of triazoles **7** with propargyl bromide. Alkylation of **7** with ethyl bromoacetate resulted into their corresponding ester derivatives **2** which were further hydrolyzed with 2 % aqueous NaOH resulting into the formation of acids **3**. Refluxing 3-mercapto triazoles **7** in ethanol with hydrazine hydrate led to their conversion to corresponding hydrazides **4**.

Stepwise detailed discussion of the synthesis of target compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** is given below.



Compound 1/2/3/4/7	a	b	c	d	e	f	g	h
R	H	CH_3	OCH_3	F	Cl	Br	NO_2	SO_2NH_2

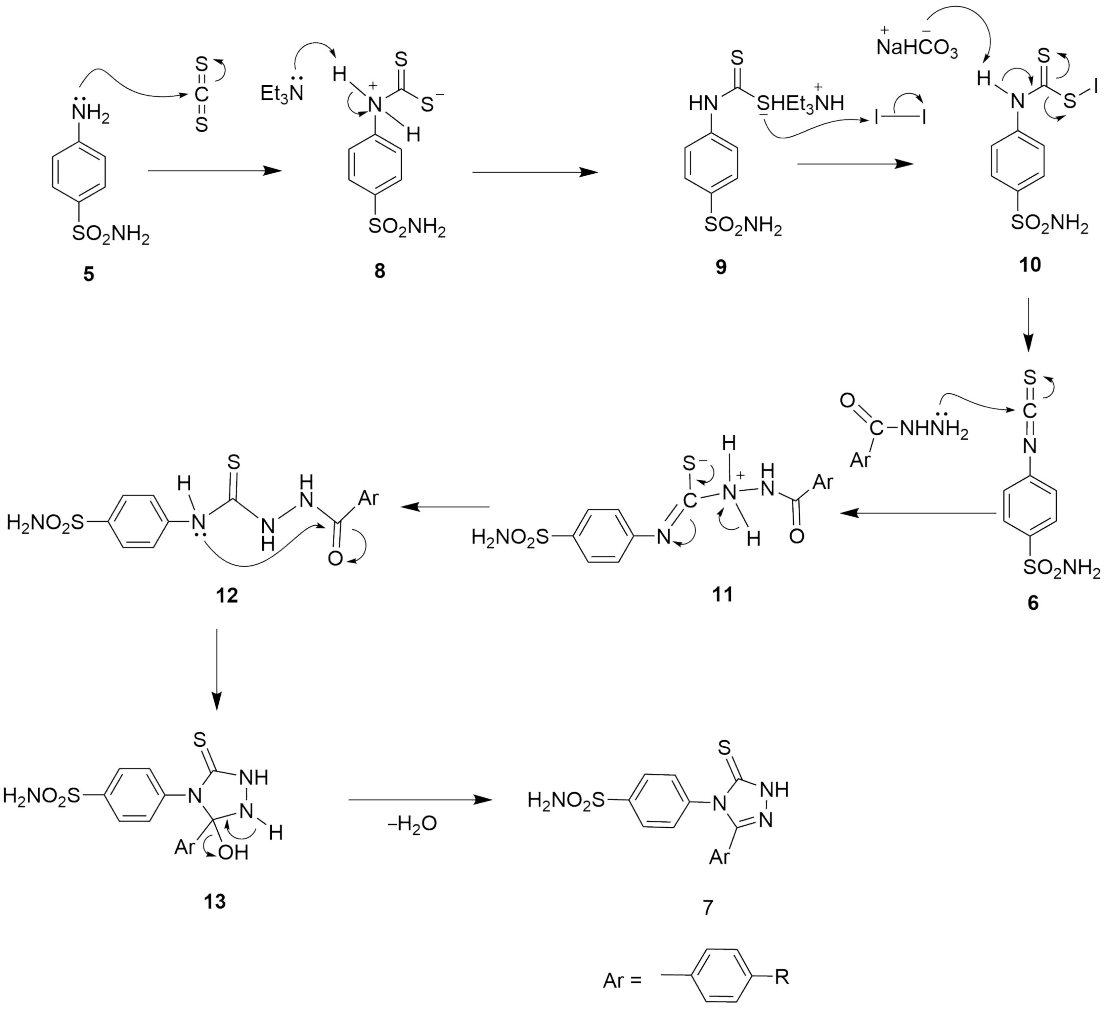
Scheme 2.1 Synthesis of target compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h**

Reagents and conditions: (i) CS_2 , TEA, Acetone; (ii) I_2, $NaHCO_3$, Ethyl acetate/water (1:1); (iii) 4R-$C_6H_4CONHNH_2$, Anhyd. ethanol, reflux; (iv) Aqueous NaOH, reflux; (v) Propargyl bromide, TEA, ethanol, reflux; (vi) Ethyl bromoacetate, ethanol, K_2CO_3, reflux; (vii) 2 % Aqueous NaOH, reflux; (viii) Hydrazine hydrate, ethanol, reflux

2.2.2 Synthesis of 3-mercapto 1,2,4-triazole derivatives 7a-7h

The synthetic pathway for 3-mercapto 1,2,4-triazoles **7** starts with the reaction of 4-amino benzenesulfonamide **5** with carbon disulfide using triethylamine as a base and acetone as solvent (Scheme 2.1 and 2.2). Completion of reaction required continuous stirring for 24 hrs which resulted into the formation of dithiocarbamate salt **9**. The salt **9** was further treated with 2 equivalents of molecular iodine and 2 equivalents of sodium bicarbonate in a mixture of ethyl acetate and water (1:1) as solvent giving corresponding

4-aminosulfonylphenyl isothiocyanate **6**. Isothiocyanate **6** was refluxed with aryl hydrazides in anhyd. ethanol as solvent resulting into the formation of thiosemicarbazides **12**. Finally, the thiosemicarbazides **12** were cyclized by refluxing with aqueous solution of NaOH (2 %) to 3-mercapto-1,2,4-triazoles **7**.



Scheme 2.2 Mechanism of formation of 3-mercapto-1,2,4-triazoles **7**

The mechanism of reaction for formation of isothiocyanate **6** involves nucleophilic attack of primary NH_2 group of **5** on carbondisulfide followed by abstraction of proton by triethyl amine resulting in dithiocarbamate salt **9**. In the next step, sulfur atom of dithiocarbamate salt **9** attacks as nucleophile on iodine resulting into **10**. The intermediate **10** subsequently undergoes desulfurization in the presence of sodium bicarbonate to afford isothiocyanate **6**. In the next step, primary amino group of carbohydrazide attacks as nucleophile on electrophilic carbon atom of **6** resulting into intermediate **11**.

Protonic rearrangement of **11** converts it into hydrazinocarbothiamide **12**. Intramolecular nucleophilic attack of NH group of **12** on carbonyl carbon atom leads to the formation of cyclized 3-mercapto 1,2,4-triazole 7 (Scheme 2.2).

Comparing the melting points with literature values confirmed the formation of compounds **7a-7f** and **7h**, however, structure of hitherto unreported compound **7g** was confirmed by its IR, ^{1}H and ^{13}C spectral data. Sulfonamide group displayed two characteristic bands at 3359 & 3194 cm^{-1} for N-H stretches and other two bands at 1326 & 1154 cm^{-1} for SO_2 stretches in its IR spectrum. In ^{1}H NMR spectrum (Fig. 2.3), a broad exchangeable singlet was obtained at 14.44 ppm corresponding to the ring N-H proton, however, the sulfonamide protons resonated at 7.53 ppm. A signal at approximately 168.8 ppm in ^{13}C NMR spectrum (Fig. 2.4) confirmed the presence of C=S group. HRMS data were also found in accordance with the molecular structure of **7g** (Fig. 2.5).

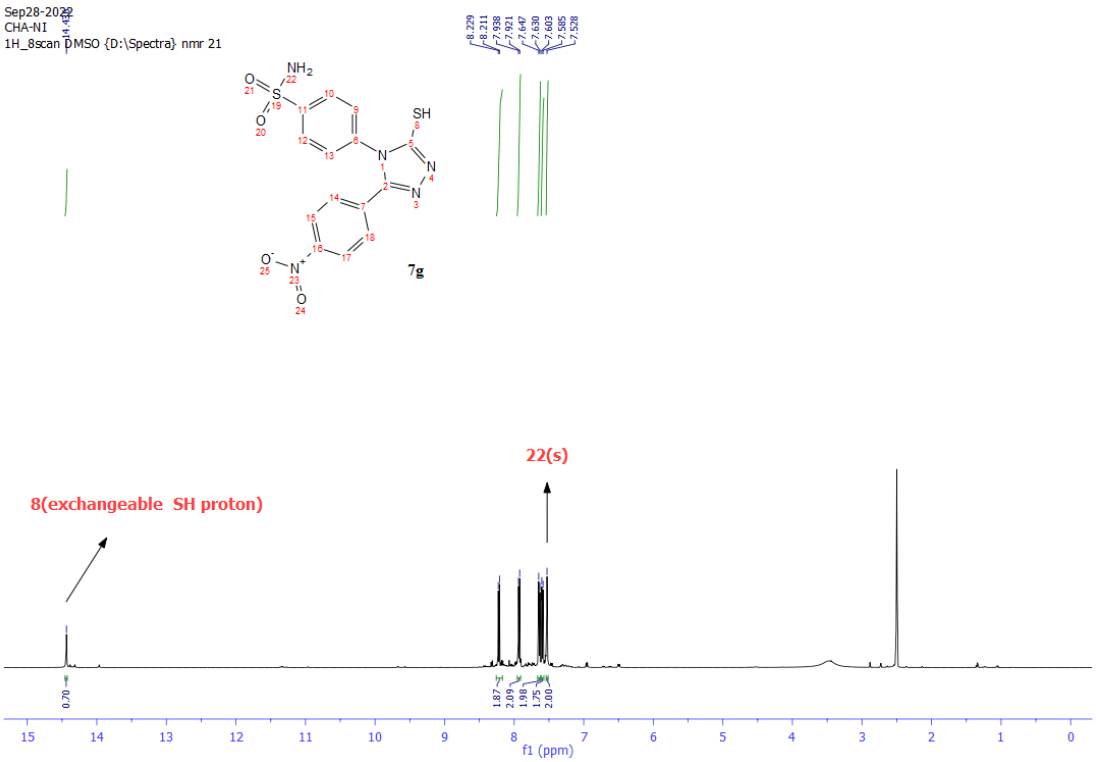


Fig. 2.3 ^{1}H NMR spectrum of compound **7g**

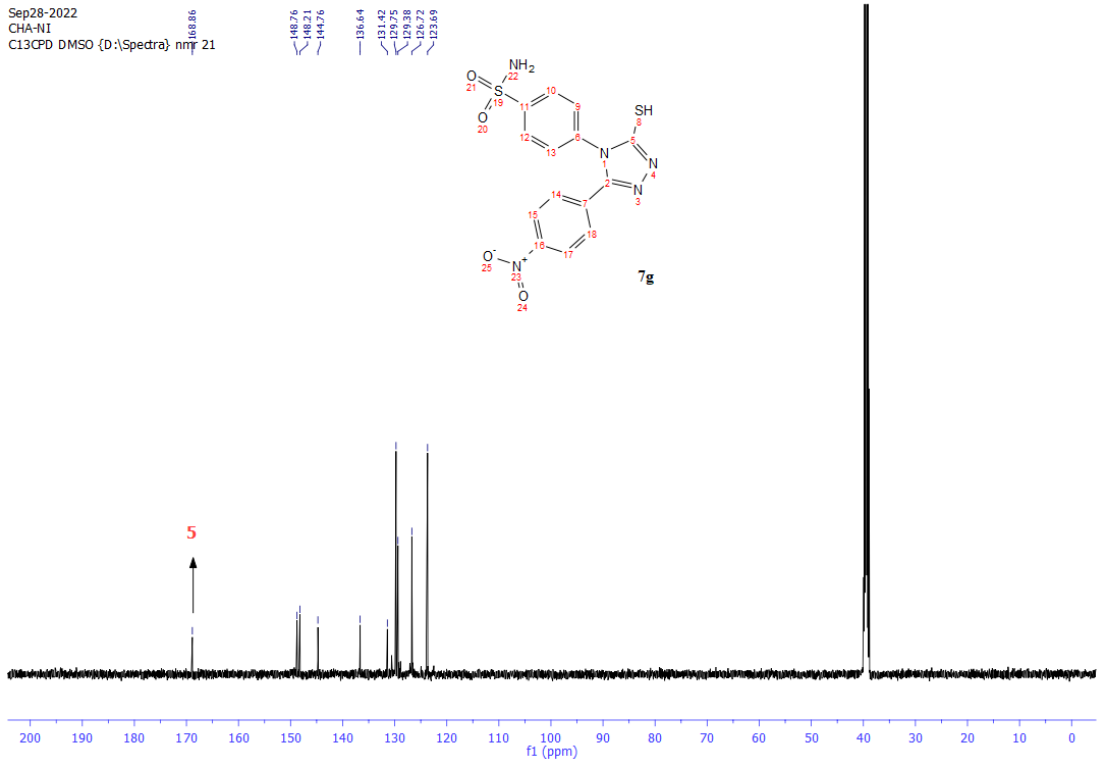


Fig. 2.4 ^{13}C NMR spectrum of compound **7g**

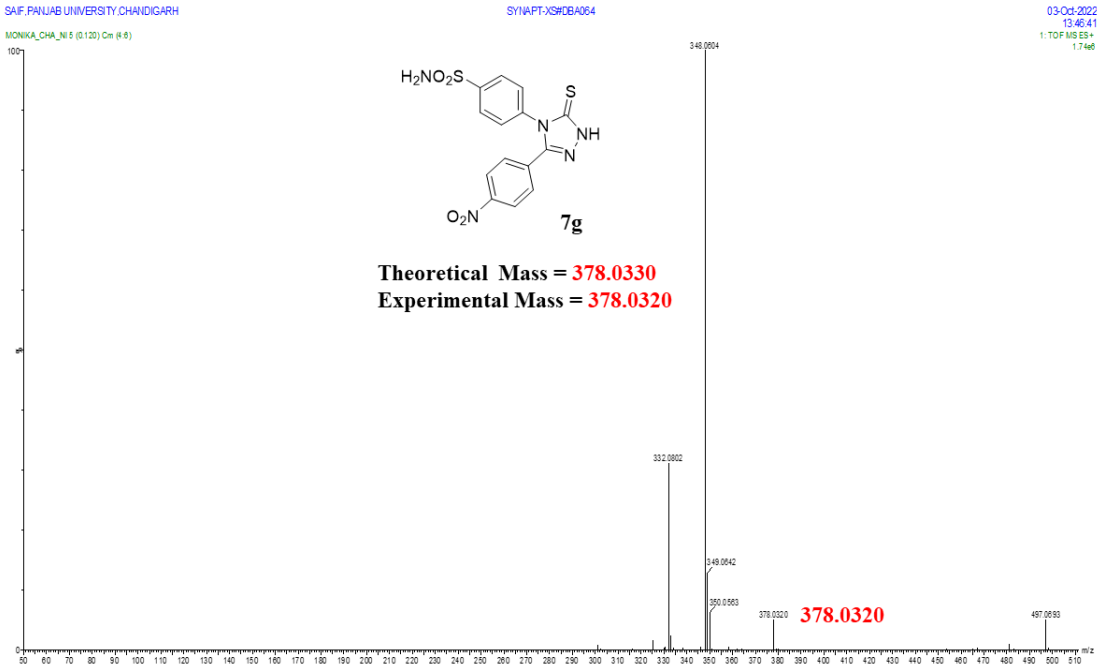
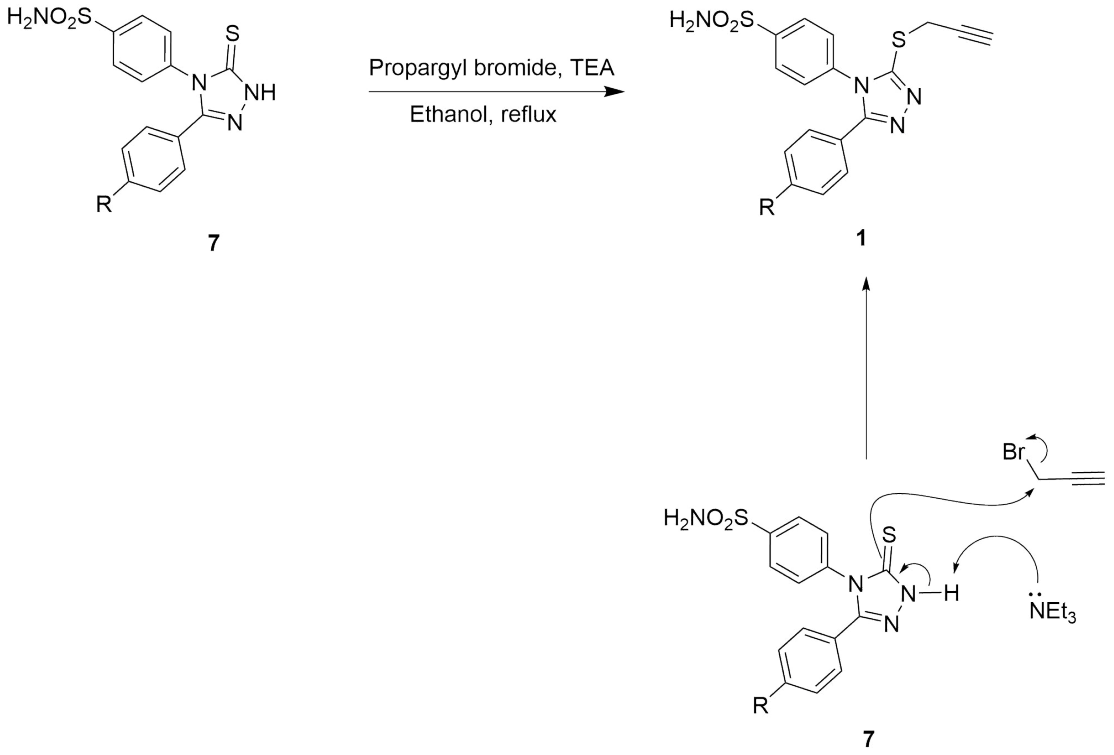


Fig. 2.5 HRMS spectrum of compound **7g**

2.2.3 Synthesis of propargyl derivatives of 1,2,4-triazolothiones 1a-1h

Propargyl derivatives of 1,2,4-triazolothiones **1** were synthesized by refluxing 1,2,4-triazolothiones **7** with propargyl bromide in ethanol solvent in presence of triethylamine as base. Mechanism of the reaction involves the formation of C-S bond by attack of sulfur atom of triazole as a nucleophile on the carbon atom of propargyl bromide bonded to bromine leading to the formation of **1** (Scheme 2.3).



Scheme 2.3 Synthesis of compounds **1** with mechanism

The structures of the novel alkyne derivatives **1** were confirmed by disappearance of exchangeable triazole-ring NH proton in ^{1}H NMR (resonating at approximately 14 ppm) and appearance of two new signals, one corresponding to methylene (-CH_2-) protons resonating at approximately 4.00 ppm as doublet and the other one corresponding to terminal alkyne proton resonating at approximately 3.25 ppm as triplet (Fig. 2.6). The sulfonamide protons resonated at approximately 7.53 ppm as singlet. In ^{13}C NMR (Fig. 2.7), methylene (-CH_2-) carbon resonated at approximately 21.3 ppm while two carbon atoms of alkyne group (-C≡CH) resonated at approximately 74.8 and 79.3 ppm values.

Presence of a sharp band in the range of 3350-3287 cm^{-1} in IR spectra also confirmed the presence of terminal alkyne group in the molecule. HRMS data also confirmed the formation of desired products **1** (Fig. 2.8).

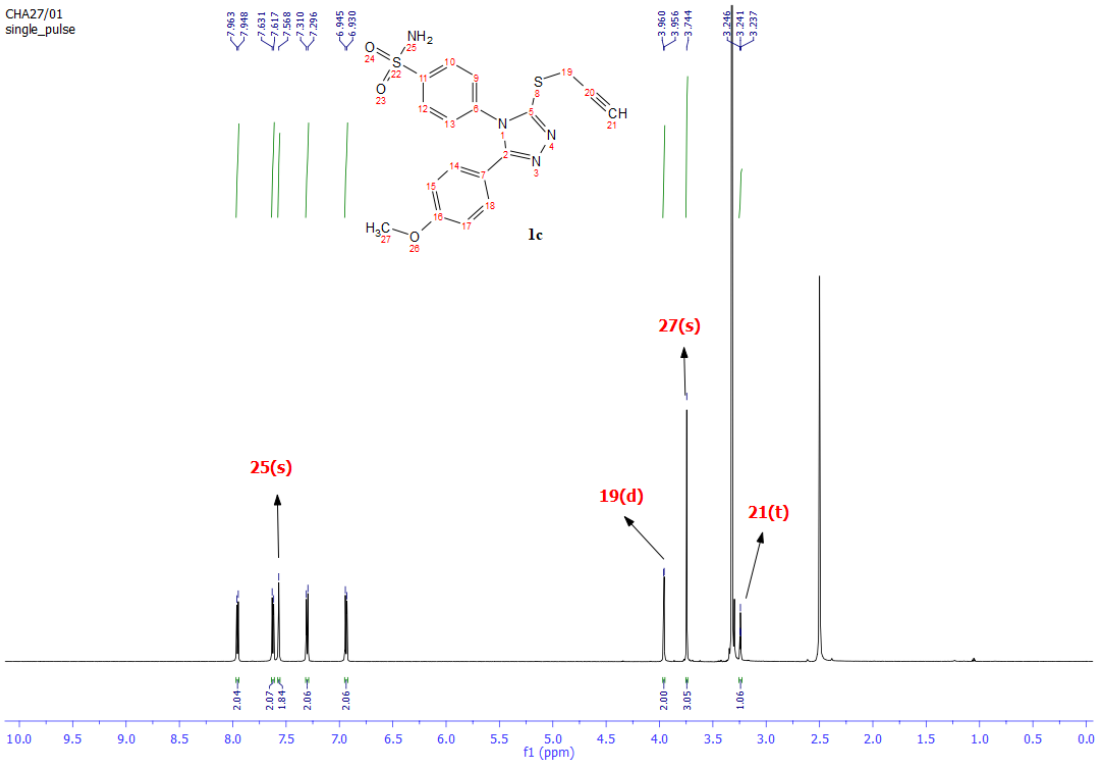


Fig. 2.6 ^{1}H NMR spectrum of compound **1c**

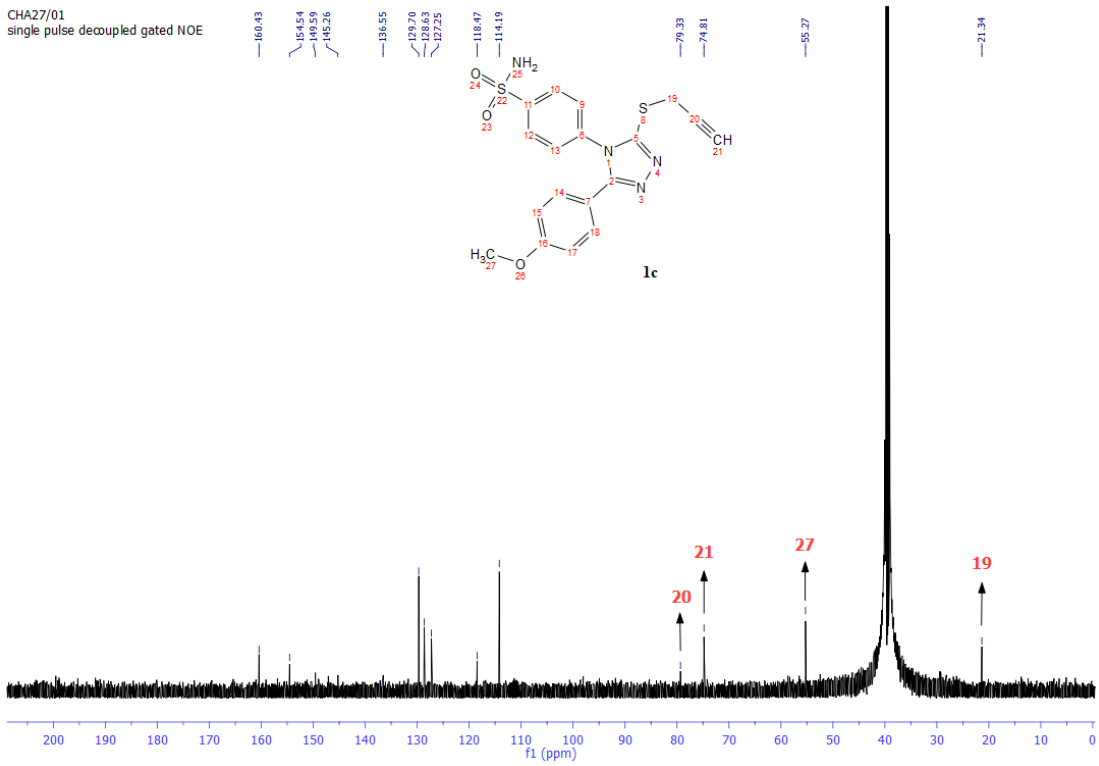


Fig. 2.7 ^{13}C NMR spectrum of compound **1c**

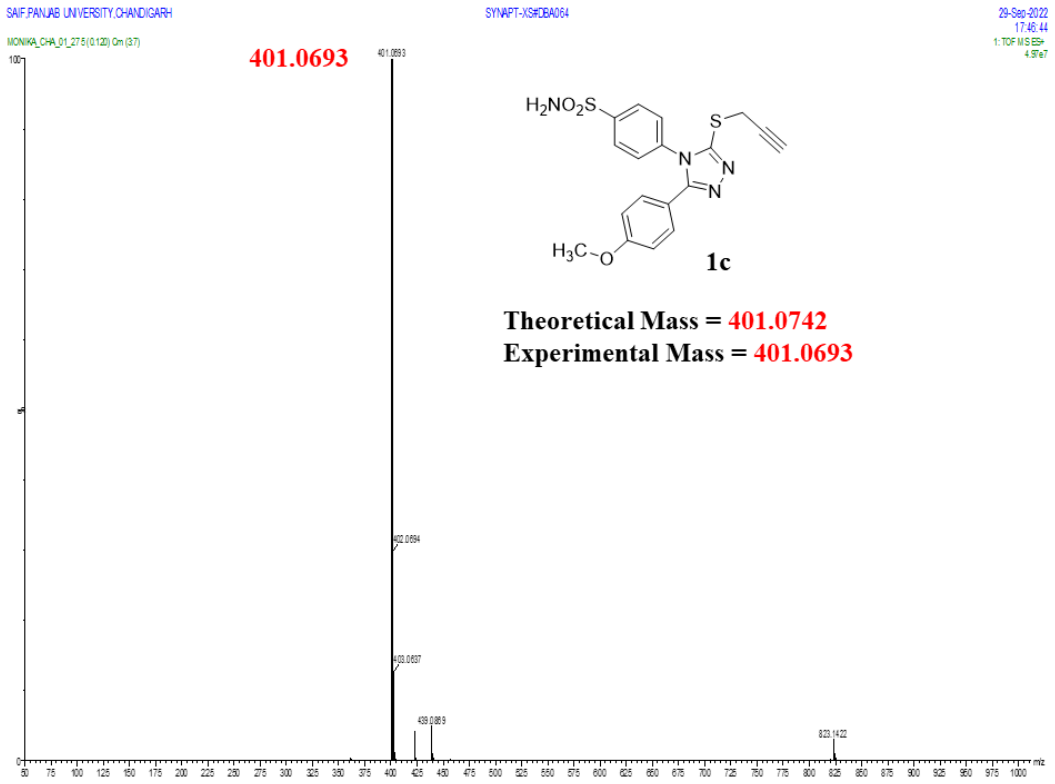
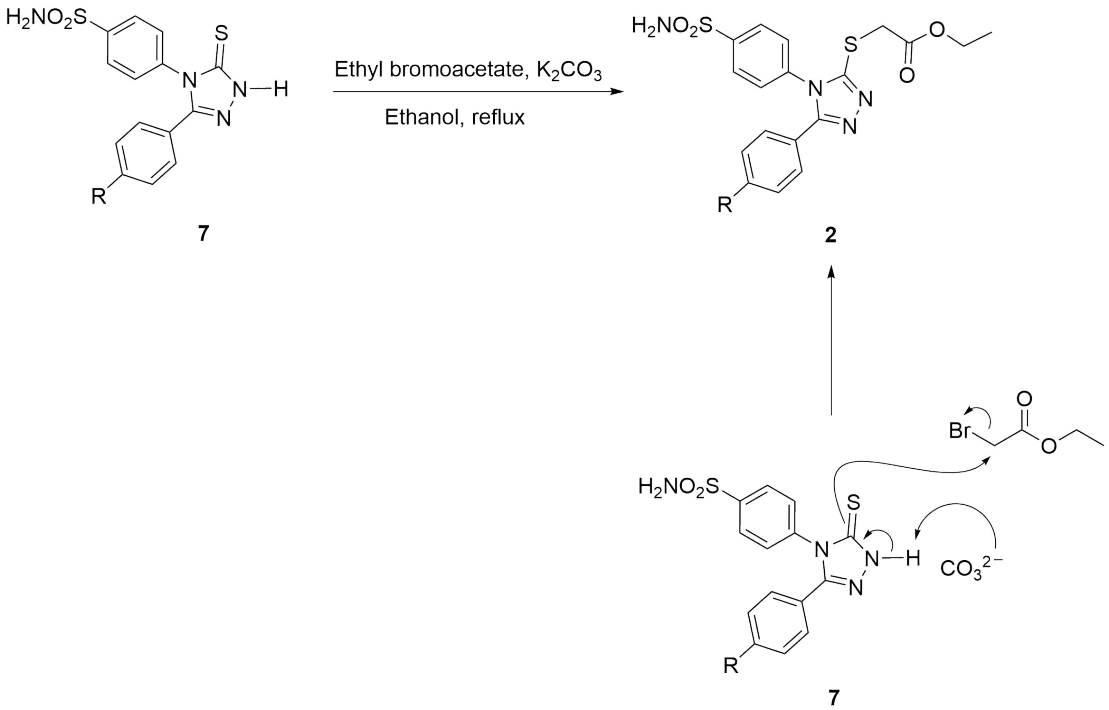


Fig. 2.8 HRMS spectrum of compound **1c**

2.2.4 Synthesis of ester derivatives of 1,2,4-triazolothiones 2a-2h

1,2,4-Triazolothiones **7** were converted to their corresponding ester derivatives **2** by reaction with ethyl bromoacetate under reflux condition in ethanol solvent using K_2CO_3 as base. Reaction mechanism involves C-S bond formation by nucleophilic attack of lone pair of electron of sulfur atom of **7** on the carbonyl carbon atom of ethyl bromoacetate leading to the formation of compounds **2** (Scheme 2.4).

A quartet at 4.10 ppm and triplet at 1.20 ppm in ^{1}H NMR confirmed the presence of ethyl group of ester while methylene protons resonated at 4.00 ppm as singlet (Fig. 2.9). In ^{13}C NMR (Fig. 2.10), methylene (-CH_2-) carbon resonated at approximately 34.3 ppm while carbon atoms of ester group resonated at approximately 168.0 ppm (C=O), 61.3 ppm (OCH_2) and 14.0 ppm (CH_3). A strong band in-between 1747-1707 cm^{-1} in IR spectra further confirmed the presence of ester functionality in the target molecules. HRMS data were also in accordance with the structures of target compounds **2** (Fig. 2.11).



Scheme 2.4 Synthesis of compounds **2** with mechanism

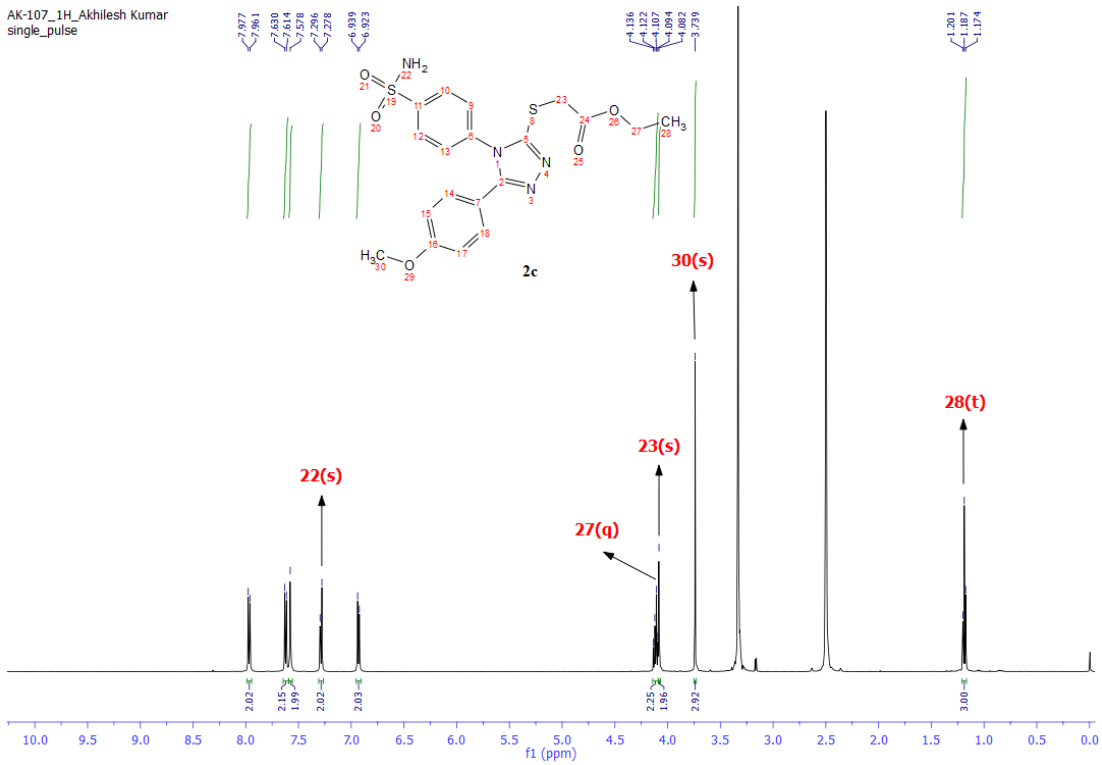


Fig. 2.9 ^{1}H NMR spectrum of compound **2c**

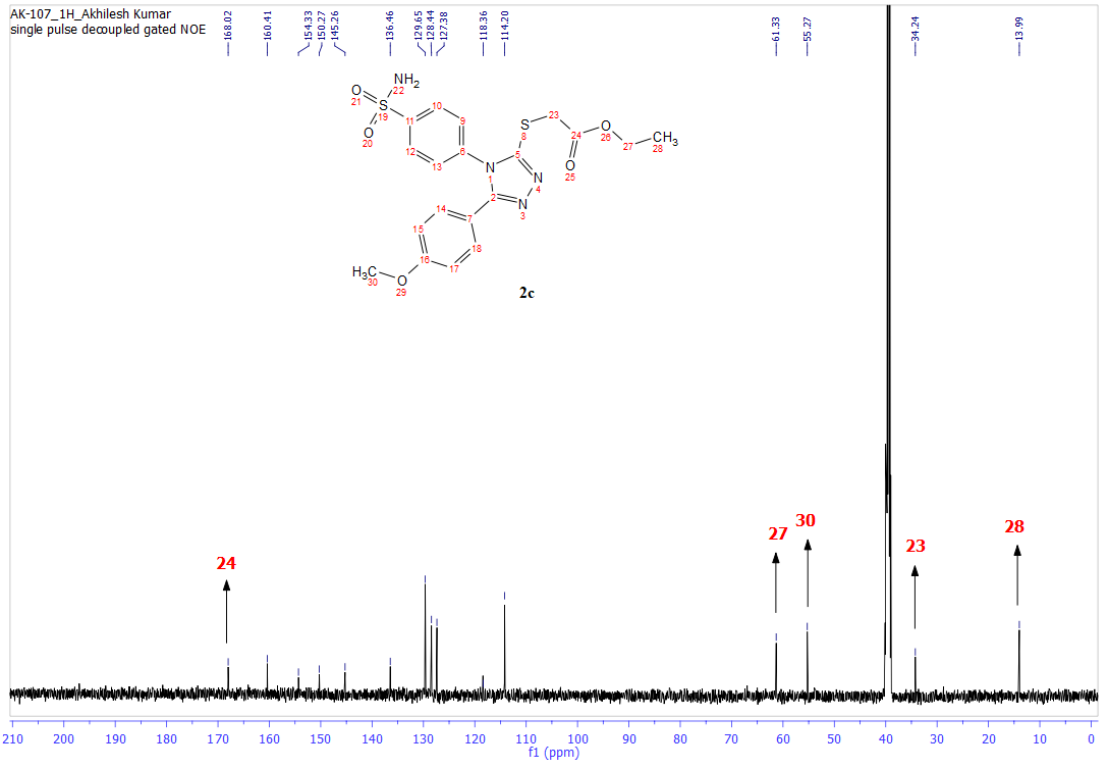


Fig. 2.10 ^{13}C NMR spectrum of compound **2c**

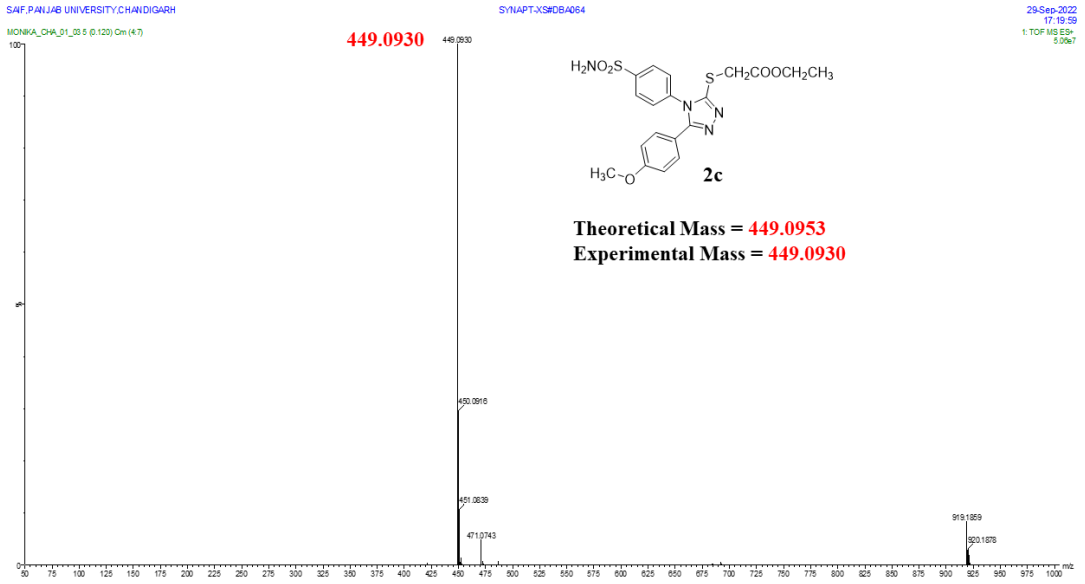
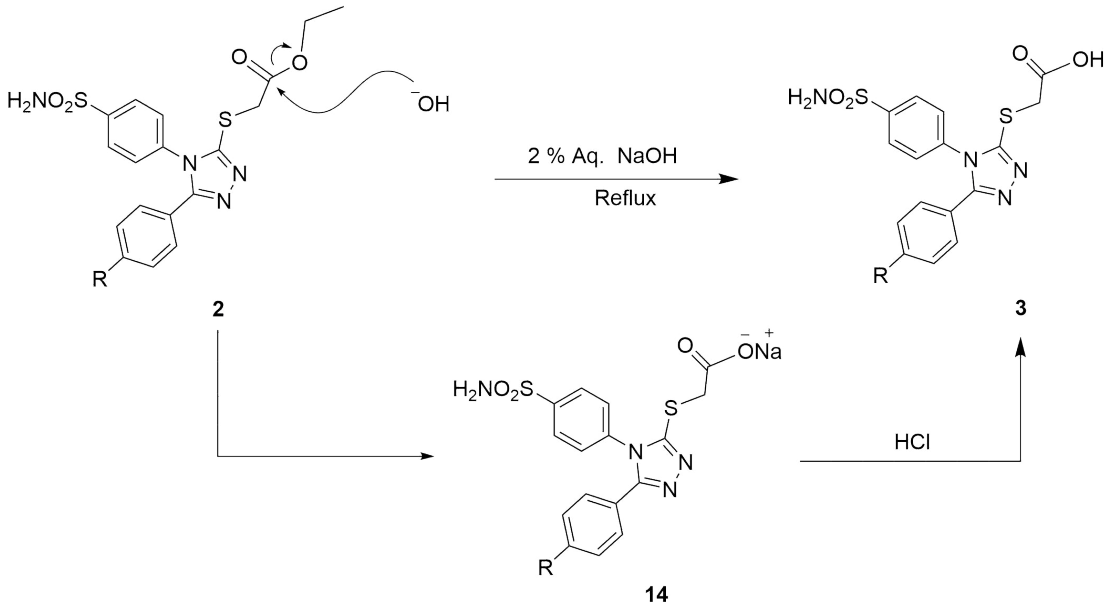


Fig. 2.11 HRMS spectrum of compound **2c**

2.2.5 Synthesis of carboxylic acid derivatives of 1,2,4-triazolothiones 3a-3h

Hydrolysis of compounds **2** was performed in aqueous medium using 2 % aqueous NaOH as base under basic condition. After completion of reaction, neutralization of reaction mixture with dil. HCl solution resulted into corresponding carboxylic acid derivatives **3**. Mechanism of reaction depicted in scheme 2.5 invlolves nucleophilic attack of hydroxide ion on the carbonyl carbon of ester and substitution of ethoxide takes place by hydroxide ion leading to its conversion to **3**.

The structures of final compounds were confirmed by disappearance of a triplet at 1.20 ppm and a quartet at 4.10 ppm in ^{1}H NMR spectra (Fig. 2.12). In ^{13}C NMR (Fig. 2.13), a peak at approximately 169.1 ppm was obtained corresponding to the carbon atom of carboxylic group. A strong band in-between 1738-1704 cm^{-1} in IR spectra corresponding to the C=O bond further confirmed the presence of carboxylic acid group in the target molecules. HRMS data were also confirmed the formation of the target compounds **3** (Fig. 2.14).



Scheme 2.5 Synthesis of compounds 3 with mechanism

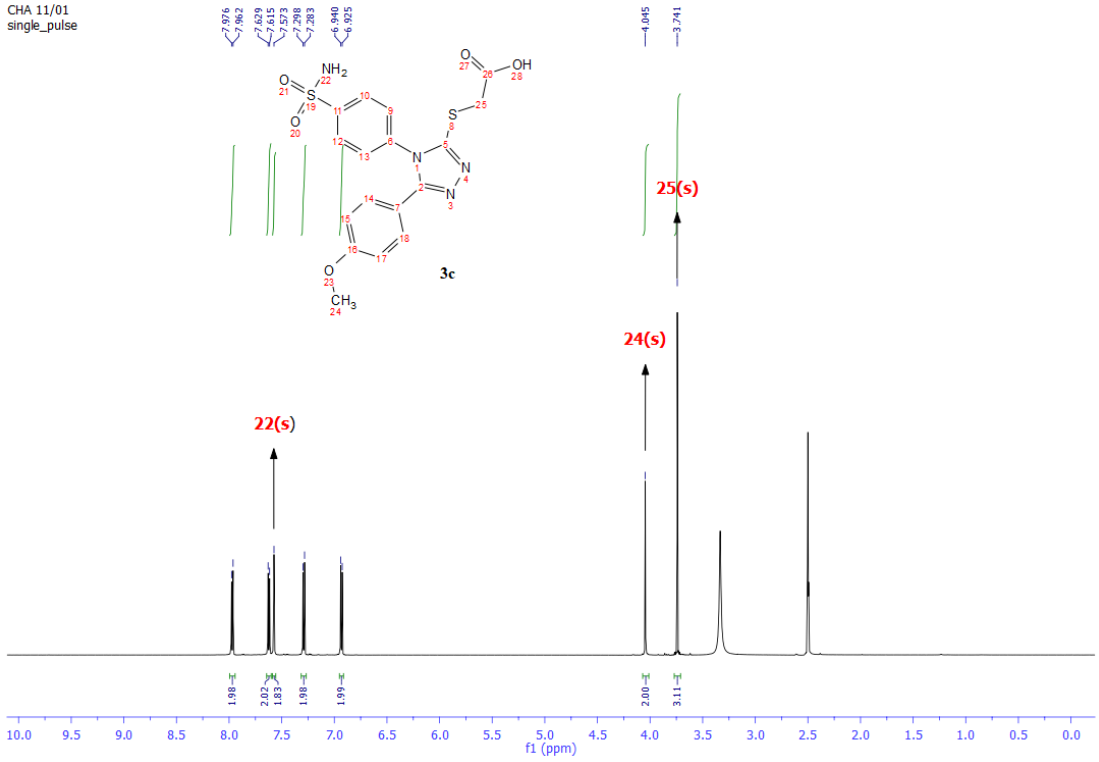


Fig. 2.12 ^{1}H NMR spectrum of compound **3c**

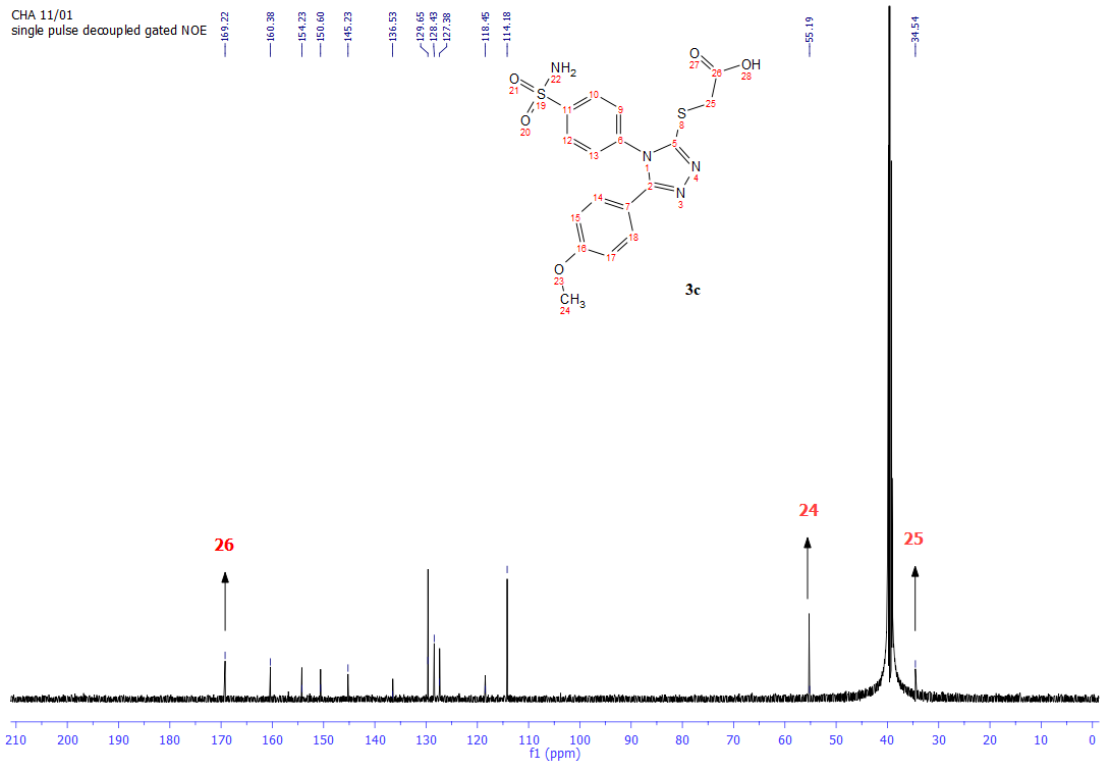


Fig. 2.13 ^{13}C NMR spectrum of compound **3c**

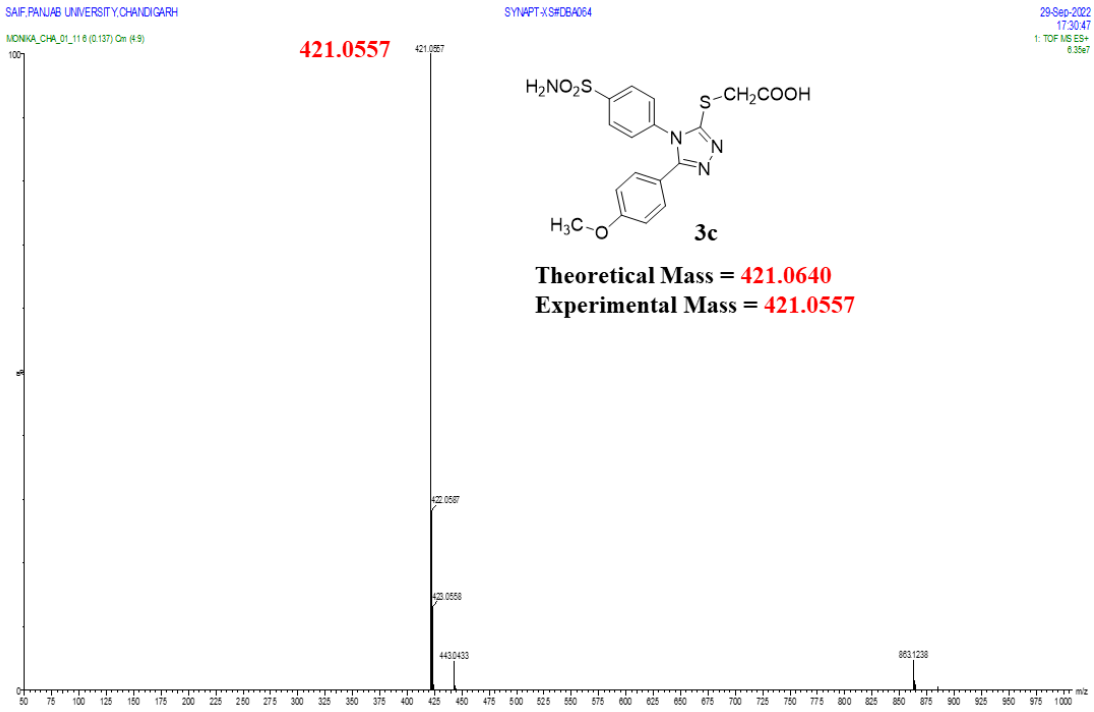
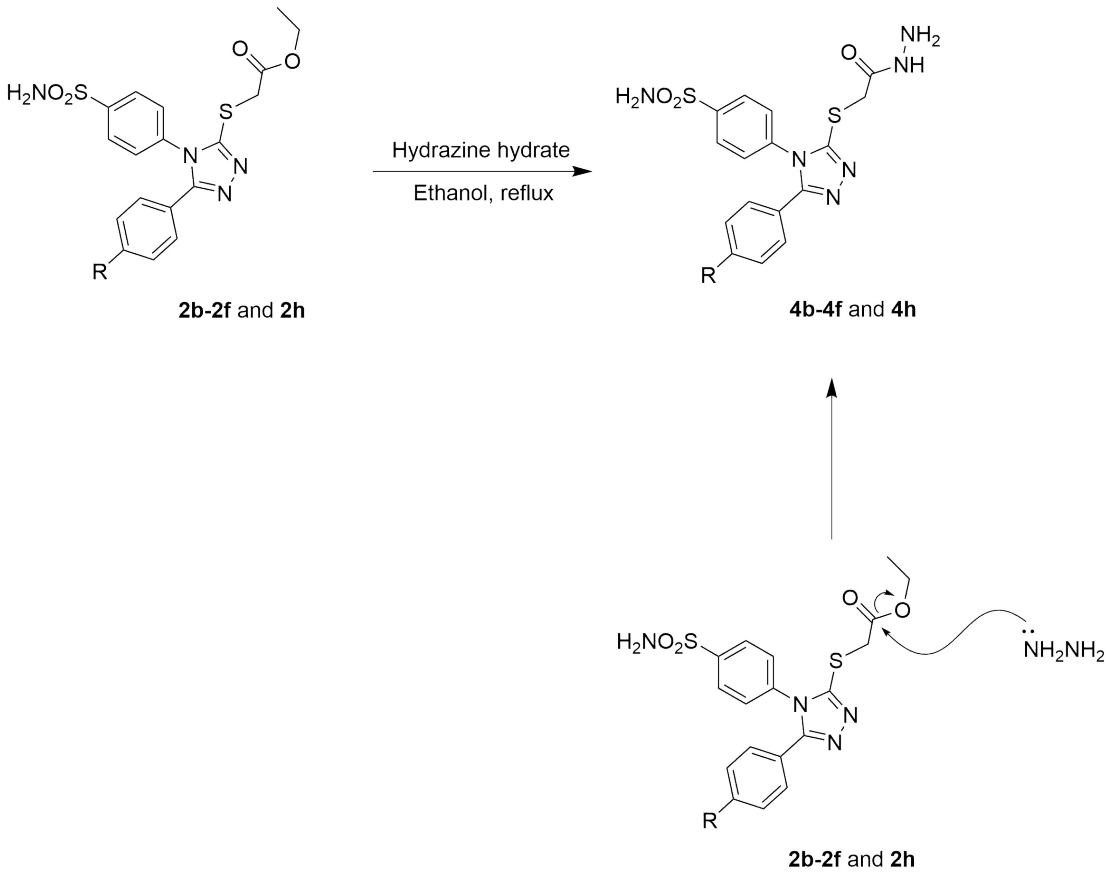


Fig. 2.14 HRMS spectrum of compound **3c**

2.2.6 Synthesis of hydrazide derivatives of 1,2,4-triazolothiones 4b-4f and 4h

The hydrazide derivatives **4** were finally obtained by refluxing ethanolic solution of esters **2**, respectively, with 80 % hydrazine hydrate. The mechanism of reaction involves formation of C-N bond and cleavage of C-O bond when nucleophilic attack of lone pair of nitrogen atom of hydrazine occurs on the carbonyl carbon of ester and substitution of ethoxide takes place by hydrazine leading to the formation of hydrazides compounds **4** (Scheme 2.6). It is pertinent to mention here that hydrazide derivatives **4a** and **4g** from the corresponding esters **2a** and **2g** could neither be obtained as pure products nor be purified even after repeated attempts.



Scheme 2.6 Synthesis of compounds **4b-4f** and **4h** with mechanism

Disappearance of a triplet and a quartet owing to ethyl group of ester in ^{1}H NMR spectra and appearance of broad exchangeable singlets at 9.30 and 4.30 ppm values approximately (Fig. 2.15) confirmed the conversion of esters **2** to hydrazide **4**. In

^{13}C NMR (Fig. 2.16), a peak at approximately 168.3 ppm was obtained for carbon atom of hydrazide group. A strong band in the range of 1674-1662 cm^{-1} in IR spectra corresponding to the C=O bond further confirmed the presence of hydrazide group in the target molecules. HRMS data of compounds also confirmed the formation of target compounds **4a-4f** and **4g** (Fig. 2.17).

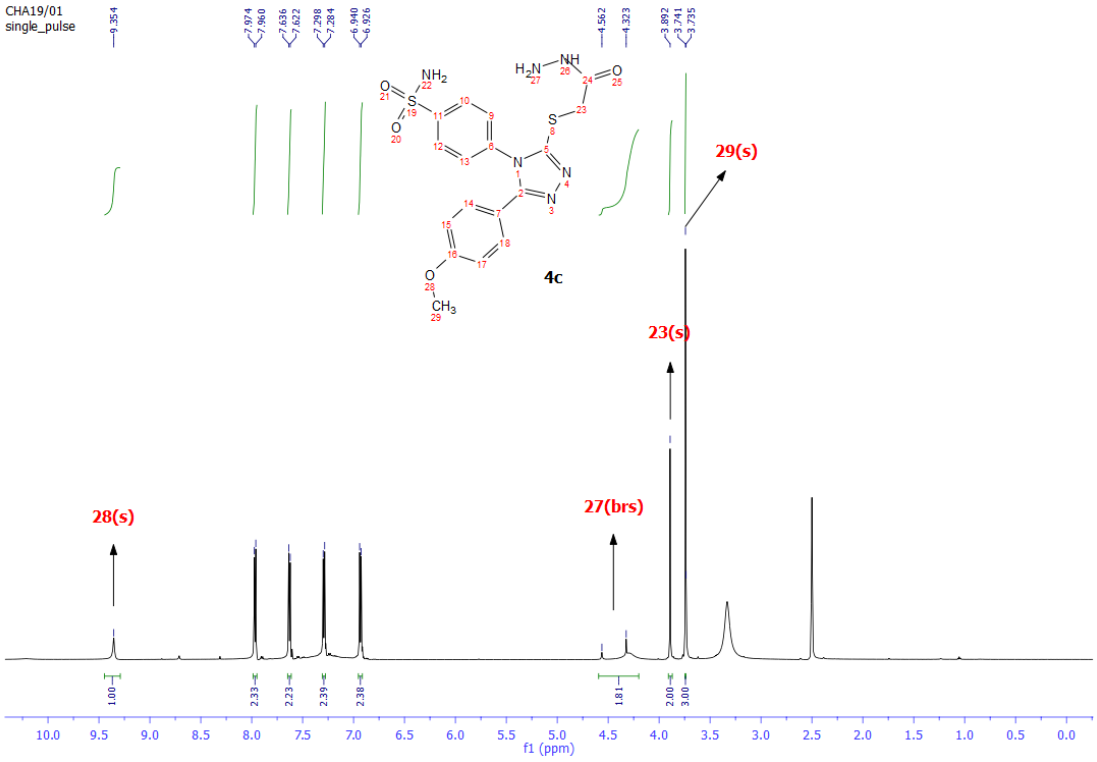


Fig. 2.15 ^{1}H NMR spectrum of compound **4c**

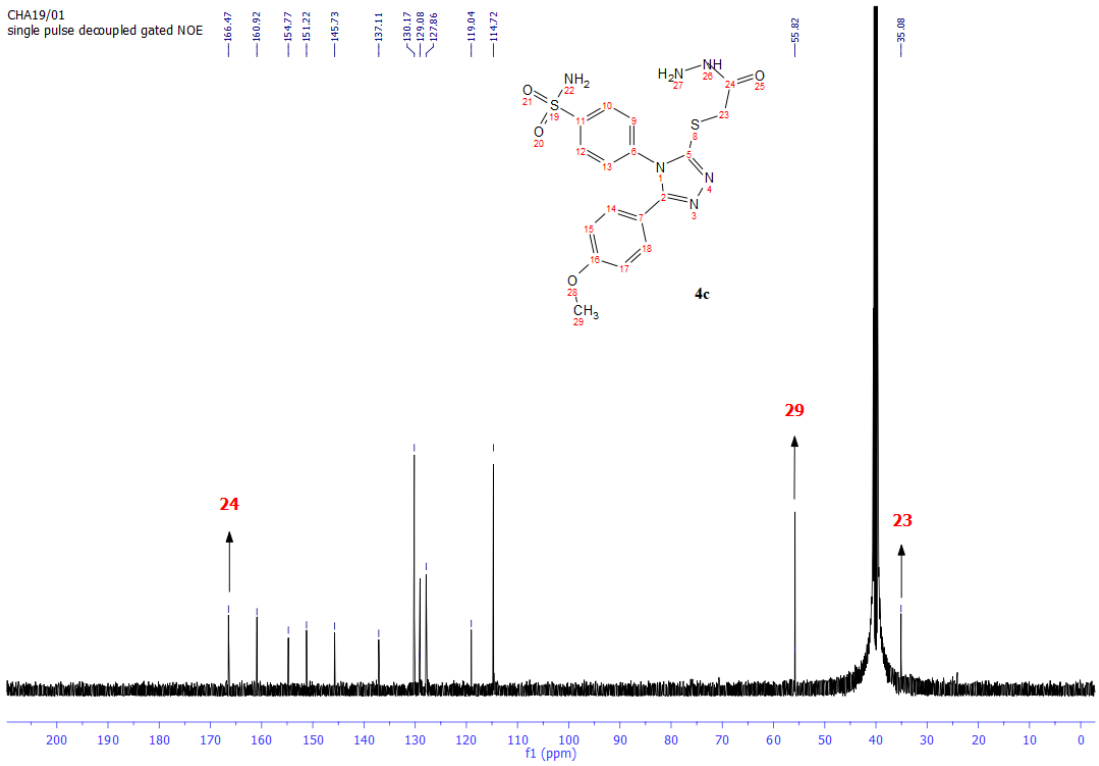


Fig. 2.16 ^{13}C NMR spectrum of compound **4c**

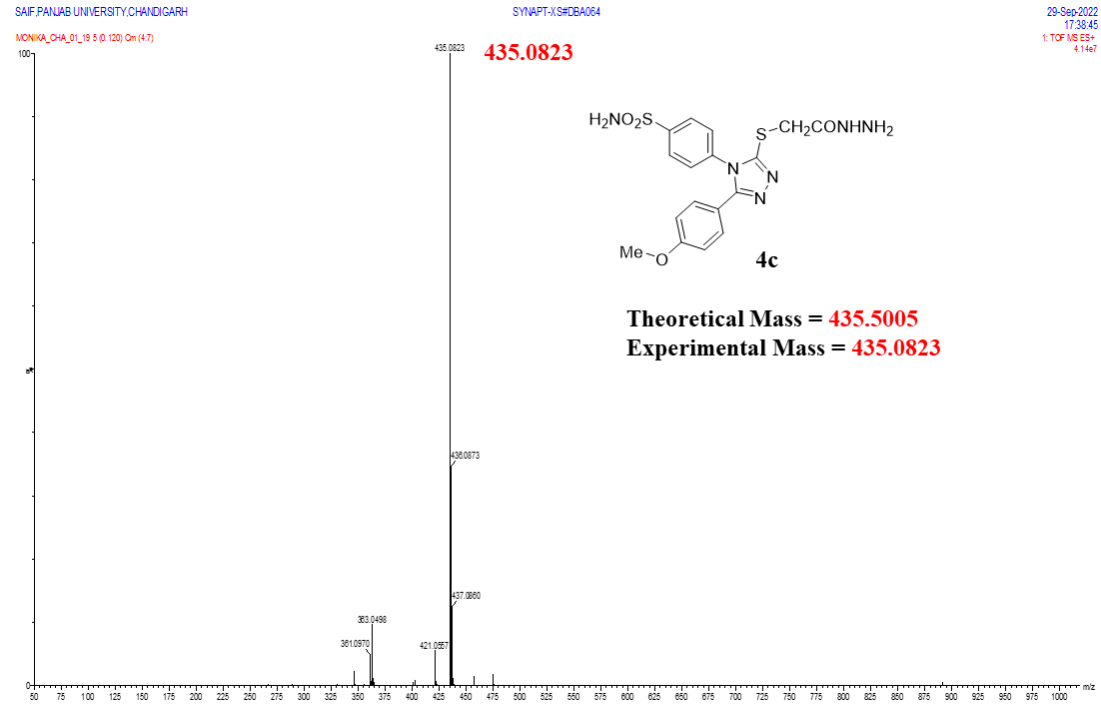


Fig. 2.17 HRMS spectrum of compound **4c**

2.3 Biological Testing Results

2.3.1 Anti-microbial assay

The sample were tested for determination of anti-microbial properties against the diverse range of Gram-negative, Gram-positive and fungal potential human pathogens under *in-vitro* conditions [126]. All the bacterial and fungal strains were cultured in nutrient agar and potato dextrose agar from glycerol stock (20 % v/v) at -20 ºC until utilised in present study. The assay was evaluated against *Staphylococcus aureus ATCC 6538P*, *Listeria monocytogenes MTCC 657*, and *Bacillus cereus ATCC 11770*, *Pseudomonas aeruginosa ATCC 15442*, *Escherichia coli MTCC 143*, *Salmonella typhi MTCC 733*, *Shigella flexneri ATCC 9199*) and fungal strain of *Candida albicans MTCC 183* using double dilution method [127] in Mueller Hinton broth using 96 well ELISA plates. Microbial growth in all the samples and control were incubated for 24 h at 37 ºC for bacterial pathogens and 30 ºC for *Candida albicans MTCC 183*. The estimation or presence of growth was observed at 690 nm in ELISA plate reader. All the observations were recorded as minimum inhibitory concentration (MIC, μg/mL) of tested samples against human pathogens and the standard drugs namely with Amoxicillin (broad spectrum anti-bacterial agent) and Fluconazole (broad spectrum anti-fungal agent) as reference substance.

2.3.2 Anti-oxidant assay

Anti-oxidant activity of the synthesized compounds was measured in terms of free radical scavenging activity using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) stable free radical. DPPH was purchased from Central Drug House (CDH). Ascorbic acid was purchased from Sigma Aldrich. Stock solution of DPPH (0.1 mM) and solutions of target compounds (0.5 mg/mL) were prepared in ethanol. Added 1.0 mL of the DPPH solution to the 2.5 mL solution of target compound and kept it for 30 minutes. Measured the absorbance at 517 nm. Ascorbic acid was taken as reference compound. Experiments were done in triplicates and the mean values were taken into consideration [128]. Lower value of absorbance showing higher anti-oxidant activity. The anti-oxidant activity was expressed as free radical scavenging activity (% RSA) and calculated using the following

formula [129]:

$$\% \text{ RSA} = (A_0 - A_t)/A_0 \times 100$$

where, A_0 = Absorbance of the blank (without compound) and A_t = Absorbance of the sample (with compound).

2.3.3 Results and discussion

A. Anti-microbial activity - results and discussion

All the newly synthesized 1,2,4-triazoles **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** were screened for their *in vitro* anti-microbial activity against three Gram-positive pathogenic bacterial strains (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657,* and *Bacillus cereus ATCC 11770)*, four Gram-negative pathogenic bacterial strains (*Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733, Shigella flexneri ATCC 9199)* and one pathogenic fungal strain (*Candida albicans MTCC 183*) using double dilution method. The results are reported as minimum inhibitory concentration (MIC, µg/mL) values of compounds against tested bacterial and fungal strains and compared with Amoxicillin (anti-bacterial) and Fluconazole (anti-fungal) as the standard reference drugs (Table 2.1). Results revealed that all the final compounds possessed moderate to excellent anti-microbial activities against all the microbial strains tested. Against *L. monocytogenes* and *E. coli* bacteria, all the tested compounds were either equivalent (MIC value 6.25 µg/mL) or double-fold (MIC value 3.12 µg/mL) better inhibitors in comparison with the standard drug used (MIC value 6.25 µg/mL). Compound **3d** was either equivalent or better inhibitor than the standard drug against all bacterial as well as fungal strains. Graphical comparisons of anti-bacterial profile of different variations of tested compounds **1a-1g**, **2a-2g**, **3a-3g**, **4a-4f** and **4g** with reference drugs Amoxicilliln are shown in Fig. 2.18, Fig. 2.19, Fig. 2.20 and Fig. 2.21, respectively, while the graphical comparison of anti-fungal profile of all the tested compounds with standard drug Fluconazole is shown in Fig. 2.22.

Table 2.1 MIC values of compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** against tested bacterial and fungal strains

Compound	Minimum Inhibitory Concentration (MIC in µg/mL)[a]							
	Gram-positive bacterial strain			Gram-negative bacterial strains				Fungal strain
	S.aureus	*L.monocytogenes*	*B.cereus*	*P.aeruginosa*	*E.coli*	*S.typhi*	*S.flexneri*	*C.albicans*
1a	6.25	6.25	12.5	12.5	6.25	12.5	6.25	12.5
1b	12.5	6.25	12.5	12.5	6.25	12.5	6.25	12.5
1c	12.5	6.25	12.5	12.5	6.25	6.25	12.5	12.5
1d	3.12	6.25	3.12	3.12	6.25	3.12	3.12	3.12
1e	3.12	3.12	12.5	3.12	6.25	3.12	6.25	6.25
1f	6.25	6.25	6.25	6.25	6.25	6.25	3.12	6.25
1g	3.12	3.12	12.5	3.12	6.25	3.12	6.25	6.25
1h	3.12	6.25	6.25	6.25	6.25	3.12	6.25	6.25
2a	6.25	6.25	12.5	12.5	6.25	6.25	6.25	12.5
2b	6.25	3.12	12.5	12.5	6.25	6.25	12.5	12.5
2c	12.5	6.25	12.5	12.5	6.25	6.25	6.25	6.25
2d	3.12	6.25	3.12	3.12	6.25	3.12	3.12	3.12
2e	6.25	6.25	6.25	12.5	6.25	6.25	3.12	3.12
2f	6.25	6.25	12.5	12.5	6.25	6.25	6.25	12.5
2g	3.12	6.25	6.25	3.12	6.25	6.25	6.25	6.25
2h	6.25	6.25	6.25	6.25	6.25	6.25	12.5	6.25
3a	6.25	6.25	12.5	6.25	6.25	6.25	6.25	12.5

3b	12.5	6.25	6.25	6.25	6.25	6.25	3.12	6.25
3c	12.5	6.25	12.5	3.12	6.25	6.25	6.25	6.25
3d	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
3e	6.25	3.12	6.25	3.12	6.25	3.12	3.12	6.25
3f	3.12	6.25	6.25	6.25	6.25	12.5	3.12	6.25
3g	6.25	6.25	6.25	3.12	3.12	6.25	12.5	12.5
3h	6.25	6.25	3.12	6.25	6.25	3.12	3.12	6.25
4b	6.25	12.5	12.5	3.12	6.25	6.25	12.5	12.5
4c	6.25	6.25	12.5	6.25	6.25	6.25	6.25	6.25
4d	3.12	6.25	6.25	3.12	6.25	3.12	3.12	3.12
4e	6.25	3.12	6.25	12.5	6.25	6.25	3.12	3.12
4f	6.25	6.25	6.25	3.12	6.25	6.25	3.12	12.5
4h	6.25	6.25	6.25	12.5	6.25	3.12	12.5	12.5
Amoxicillin[b]	3.12	6.25	6.25	3.12	6.25	3.12	3.12	-
Fluconazole[c]	-	-	-	-	-	-	-	3.12

[a]Mean of the three replicates
[b]Amoxicillin was used as positive control for anti-bacterial activity
[c]Fluconazole was used as positive control for anti-fungal activity

Following structure-activity relationship has been drawn from the above data:-

1. Compounds containing 4-fluoro substituted phenyl group at C-5 position of 1,2,4-triazole ring were found to be the most potent anti-microbial agents exhibiting equivalent or two-fold better activities than the standard drug against all bacterial as well as fungal strains.

2. In overall, electron withdrawing groups on phenyl ring at C-5 position of 1,2,4-triazole ring led to enhanced activities of compounds.

3. Compounds having a simple phenyl ring at C-5 position of 1,2,4-triazole or phenyl ring substituted with electron donating group (CH_3 or OCH_3) at *p*-position were

comparatively less potent anti-microbial agents.

4. 1,2,4-triazoles having $-SCH_2COOH$ group at C-2 position were better anti-microbial agents as compared to other triazole derivative reported.

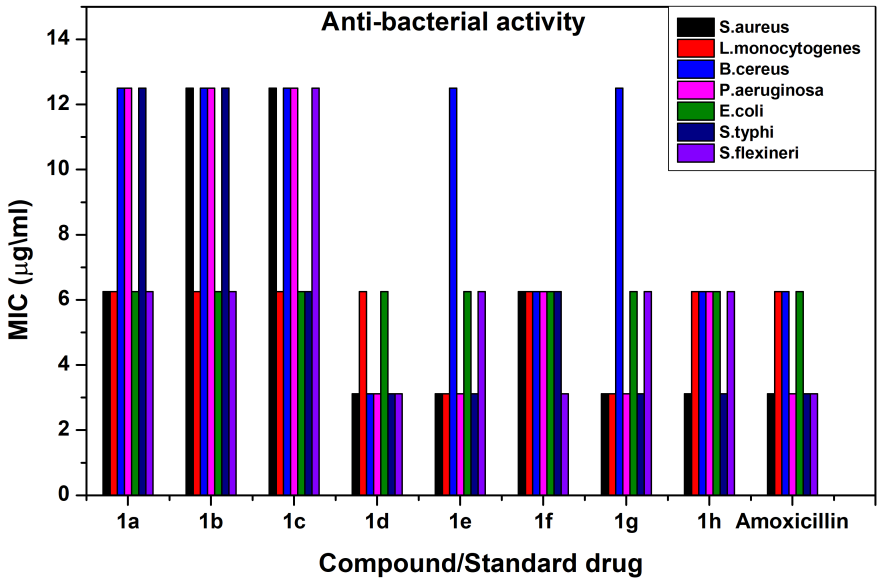


Fig. 2.18 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **1a-1g** and the standard drug Amoxicillin

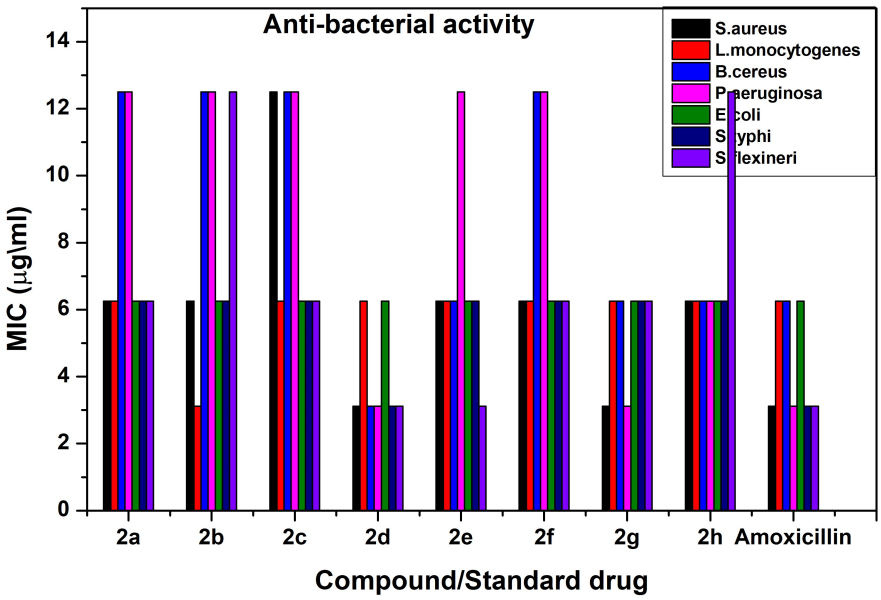


Fig. 2.19 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **2a-2g** and the standard drug Amoxicillin

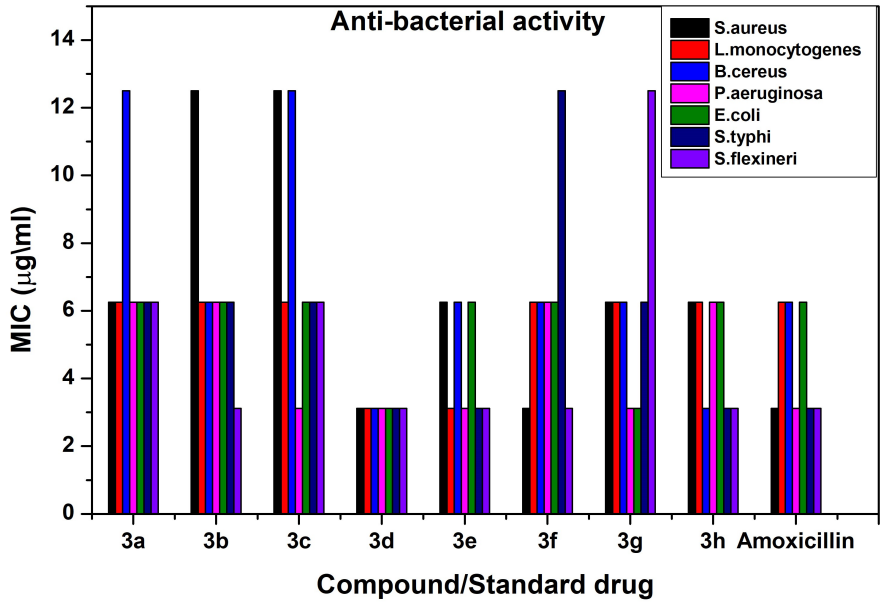


Fig. 2.20 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **3a-3g** and the standard drug Amoxicillin

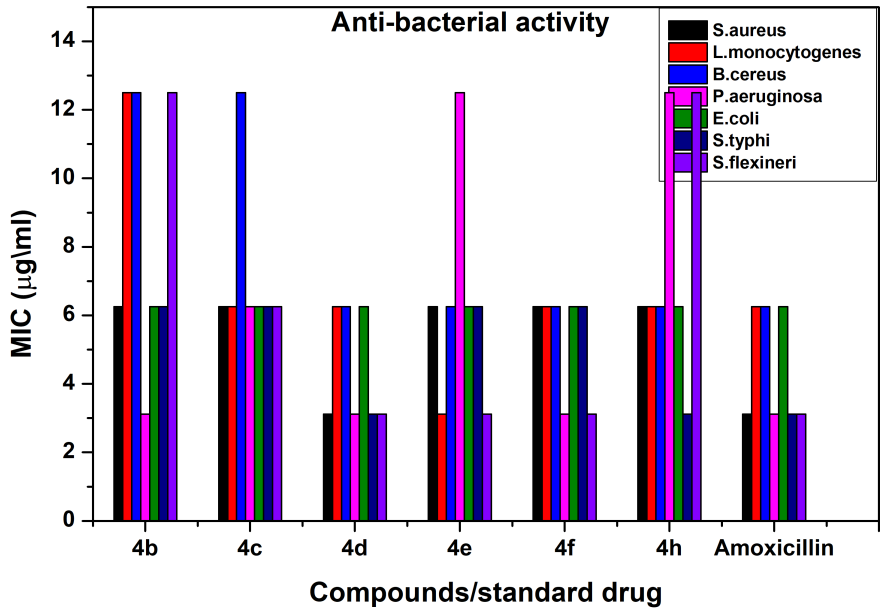


Fig. 2.21 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **4a-4f**, **4h** and standard drug Amoxicillin

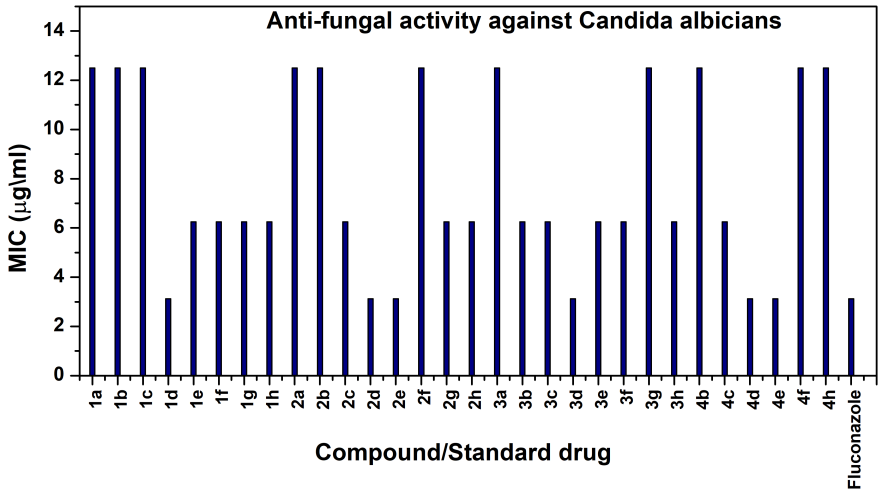


Fig. 2.22 Graphical comparison of anti-fungal activity profile of newly synthesized compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f**, **4h** and the standard drug Fluconazole

B. Anti-oxidant activity - results and discussion

The *in vitro* anti-oxidant activity of all the target 1,2,4-triazoles **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** was performed spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The experiment was done in triplicate for each substance and anti-oxidant activities were taken in terms of % RSA (radical scavenging activity) with respect to reference control values. The results are presented in Table 2.2. summarizing the radical scavenging activities of all the compounds compared to ascorbic acid as standard. Graphical comparison of anti-oxidant profile of tested compounds with ascorbic acid (AA) is shown in Fig. 2.23.

Table 2.2 Anti-oxidant activities of compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** using DPPH method

Compound	Anti-oxidant activity % RSA[a]	Compound	Anti-oxidant activity % RSA[a]
1a	93.56	**3a**	91.57
1b	55.37	**3b**	83.45
1c	47.93	**3c**	88.65
1d	93.57	**3d**	76.23

1e	76.34	**3e**	90.23
1f	89.23	**3f**	74.14
1g	91.35	**3g**	87.34
1h	84.19	**3h**	86.72
2a	93.24	**4b**	81.35
2b	92.76	**4c**	79.43
2c	92.34	**4d**	94.35
2d	86.74	**4e**	76.87
2e	94.12	**4f**	90.87
2f	89.23	**4h**	65.34
2g	93.23	**AA**	97.34
2h	88.34		

[a]Values were mean of three replicates % RSA, showed anti-oxidant potentials of the tested samples

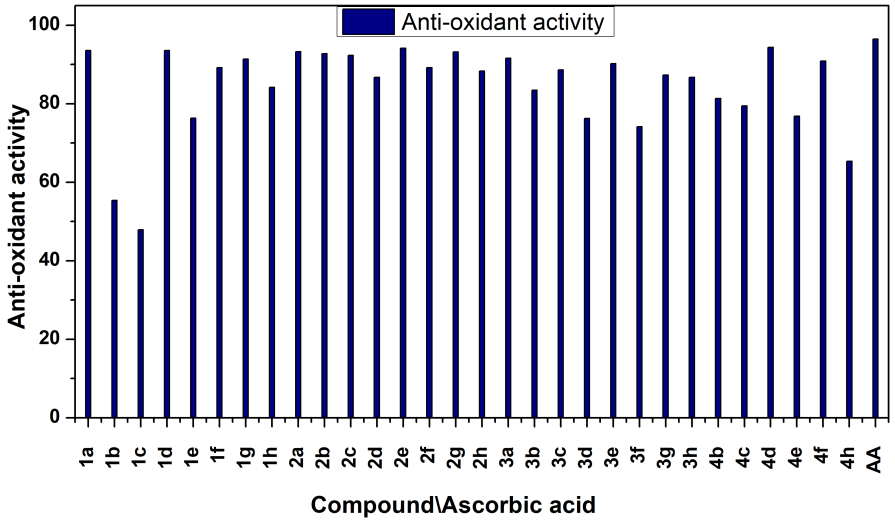


Fig. 2.23 Graphical comparison of anti-oxidant activity profile of newly synthesized compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f**, **4h** and ascorbic acid (AA)

From the above data it has been generalized that

1. The results obtained depicted that most of the tested compounds have exhibited

moderate to excellent anti-oxidant activity.

2. Compound **4d** was found to be the most potent anti-oxidant agent (94.35 %) among the tested compounds followed by **2e**, **1d**, **2a**, **2g**, **1a**, **2b**, **2c**, **3a**, **4f** and **3e** showing activity in the range of 94.12-90.23 %.

3. *p*-Methyl and *p*-methoxy substituted triazole derivatives **1b** and **1c**, respectively, were comparatively poor anti-oxidant agents with scavenging activities 55.37 % and 47.93 %.

2.3.4 Cytotoxicity

All the target compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** were also tested for their cytotoxicity against one mammalian cell line (mouse fibroblast cell line) and plant seed germination (vigna radiate seeds) at very high concentration of 1 mg/mL using MTT assay. The results of the cytotoxic studies against mouse fibroblast cell line are presented in table 2.3. It can be seen from cytotoxicity screening that after 24 hrs of exposure time, all the compounds were found to be safe to normal cells with 89.8-99.8 % cell viability even at much higher doses of the corresponding MICs. Also, all the tested compounds were 100 % safe to plant seed germination. Therefore, the compounds possessed another positive point which is highly desirable in the development of new drug molecules.

Table 2.3 *In vitro* cytotoxic studies of compounds **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** against normal cells at the concentration of 1 mg/mL

Compound	Mouse fibroblast cell Cell Viability %[a]	Compound	Mouse fibroblast cell Cell Viability %[a]
1a	95.2	**3a**	97.3
1b	95.6	**3b**	90.45
1c	94.6	**3c**	91.2
1d	99.8	**3d**	90.16
1e	99.1	**3e**	93.5
1f	99.2	**3f**	89.8

1g	99.1	**3g**	97.4
1h	99.1	**3h**	92.4
2a	93.5	**4b**	96.1
2b	94.3	**4c**	94.8
2c	90.3	**4d**	96.4
2d	94.3	**4e**	93.5
2e	92.6	**4f**	90.2
2f	95.4	**4h**	91.4
2g	95.2	**DMSO**	76.1
2h	93.2		

[a]Mean of three replicates, showed the viability percentage on challenged with the tested compounds as compared to the control case

2.4 Conclusion

A series of benzenesulfonamide bearing 1,2,4-triazoles **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** was successfully synthesized and structures were confirmed by their spectral data (^{1}H NMR, ^{13}C NMR, IR) and HRMS. *In vitro* anti-microbial evaluation showed that most of the synthesized compounds could effectively inhibit growth of all tested Gram-positive and Gram-negative bacterial strains as well as the tested fungal strain. Particularly, compound **3d** showed equivalent or better anti-microbial activity when compared with standard drug Amoxicillin. All the compounds were either equivalent or weaker inhibitors against tested fungal strain when compared with Fluconazole, a reference drug. Synthesized compounds were also screened for anti-oxidant profile using DPPH method and most of them were found moderate to excellent free radical scavengers. Compound **4d** was found to possess highest anti-oxidant activity with control value 94.35 %. All the compounds were also found to be safe against mouse fibroplast cell line and plant seed germination cell line. These results suggested that benzenesulfonamide incorporated 1,2,4-triazole derivatives could be potential candidates

for treatment of bacterial infections and for the development of new potent anti-oxidant agents.

2.5 Experimental Section

All glassware were used after solvent wash and drying in an oven for 12 hrs. Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silicagel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. The infrared (IR) spectra were recorded on FT-IR Perkin Elmer Spectrophotometer, CIL, JCBUST, YMCA, Faridabad. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance Neo 500/600 MHz NMR spectrometer and AVH D 500 AVANCE III HD 500 MHz OneBay NMR spectrometer using tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in δ (ppm). Mass data were recorded on SYNAPT-XS#DBA064 and Agilent 7800 ICP-MS mainframe. The reference values for the residual solvent were taken as δ = 2.50 ppm (DMSO-d_6) for ^{1}H NMR, δ = 39.54 ppm (DMSO-d_6) for ^{13}C NMR. The abbreviations: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet and ex = exchangeable protons are used for NMR assignments.

2.5.1 Synthesis of 4-isothiocyanatobenzenesulfonamide 6

To a well cooled mixture of methanol and triethylamine to 0 ºC was added carbon disulfide dropwise and stirred for 10 minutes. Then amine was added in small lots with constant stirring and allowed the reaction mixture to stir further for 15-16 hrs when triethyl ammonium dithiocarbamate salt was precipitated completely. The solid thus obtained was filtered, washed with diethyl ether and air dried. Subsequently, to stirred and ice-cooled biphasic solvent system of water/ethyl acetate (1:1) was added the dithiocarbamate salt as obtained above followed by sodium bicarbonate (2 equivalents). To this stirring solution was then added iodine (2 equivalents) pinch wise over a period of 15-20 minutes. After the completion of reaction, aqueous solution of sodium thiosulfate was added to neutralize the excess of iodine. Ethyl acetate layer was separated from

aqueous layer, filtered to remove precipitated sulfur, washed with water, dried over anhyd. sodium sulfate and evaporated under reduced pressure yielding pure isothiocyanate **6**. Yield 79 %, Lit. mp 212-214 ºC, Obs. mp 208-210 ºC.

2.5.2 Synthesis of 3-mercapto-1,2,4-triazoles 7a-7h

A mixture of **6** and aromatic acid hydrazide was refluxed in absolute ethanol for 3-4 hrs resulting into precipitation of solid which was filtered, dried and used in next step. The above solid obtained was dissolved in 2 % aqueous NaOH solution and refluxed for 4 hrs. Then cooled the solution to room temperature, added ice to it and neutralized with conc. HCl which resulted into the precipitation of a white solid. The solid was filtered off, washed with water, dried and recrystallized from appropriate solvent affording the pure products **7**. Formation of all the compounds (except **7g**) was confirmed by matching the melting points with the literature.

4-(3-(4-nitrophenyl)-5-thioxo-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (7g)

Yellow solid; Yield: 71 %; mp 284-286 ºC; IR (KBr) ν_{max} 3359, 3030, 1326, 1154; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 14.43 (1H, s, ring NH), 8.22 (2H, d, J = 9.0 Hz, Ar), 7.93 (2H, d, J = 8.5 Hz, Ar), 7.63 (2H, d, J = 8.5 Hz, Ar), 7.59 (2H, d, J = 9.0 Hz, Ar), 7.52 (2H, ex, s, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 168.8, 148.7, 148.2, 144.7, 136.6, 131.4, 129.7, 129.3, 126.7, 123.6; HRMS (ESI) m/z calcd for $C_{14}H_{11}N_5O_4S_2$ $[M+H]^+$ 378.0330, found 378.0320.

2.5.3 General procedure for the synthesis of target compounds 1a-1h

To the solution of compound **7** in ethanol was added propargyl bromide (1.5 equivalents) and triethylamine (1.5 equivalents). The reaction mixture was then refluxed for 3-4 hrs resulting in the formation **1** as pure product as indicated by the TLC. The reaction mixture was allowed to cool at room temperature followed by addition of crushed ice. A solid was precipitated out which was filtered off, washed with excess of water, dried, and recrystallized from ethanol.

4-(3-phenyl-5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (1a)

White solid; Yield: 78 %; mp 190-192 ºC; IR (KBr) ν_{max} 3319, 3282, 3186, 3061, 1573, 1449, 1430, 1345, 1158, 624, 602, 542; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.95 (2H, d, J = 9.0 Hz, Ar), 7.64 (2H, d, J = 9.0 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.42-7.38 (5H, m, Ar), 3.99 (2H, d, J = 2.4 Hz, SCH_2), 3.25 (1H, t, J = 2.4 Hz, ≡CH); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 154.7, 150.1, 145.3, 136.4, 130.0, 128.7, 128.6, 128.2, 127.2, 126.3, 79.3, 74.8, 21.3; HRMS (ESI) m/z calcd for $C_{17}H_{14}N_4O_2S_2$ $[M+H]^+$ 371.0636, found 371.0558.

4-(3-(prop-2-yn-1-ylthio)-5-(*p*-tolyl)-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (1b)

White solid; Yield: 82 %; mp 196-198 ºC; IR (KBr) ν_{max} 3306, 3189, 3252, 3003, 1584, 1479, 1443, 1340, 1162, 829, 725, 624, 544; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.95 (2H, d, J = 9.0 Hz, Ar), 7.61 (2H, d, J = 8.4 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.26 (2H, d, J = 9.0 Hz, Ar), 7.18 (2H, d, J = 8.4 Hz, Ar), 3.97 (2H, d, J = 3.0 Hz, SCH_2), 3.24 (1H, t, J = 3.0 Hz, ≡CH), 2.29 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 151MHz) δ (ppm) 154.7, 149.8, 145.2, 139.8, 136.4, 129.3, 128.5, 128.1, 127.2, 123.4, 79.2, 74.8, 21.3, 20.8; HRMS (ESI) m/z calcd for $C_{18}H_{16}N_4O_2S_2$ $[M+H]^+$ 385.0792, found 385.0854.

4-(3-(4-methoxyphenyl)-5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazol-4-yl) benzenesulfonamide (1c)

White solid; Yield: 82 %; mp 216-218 ºC; IR (KBr) ν_{max} 3321, 3185, 3250, 2972, 2943, 2870, 1614, 1481, 1442, 1345, 1161, 841, 623, 592; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.95 (2H, d, J = 9.0 Hz, Ar), 7.62 (2H, d, J = 8.4 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.30 (2H, d, J = 8.4Hz, Ar), 6.93 (2H, d, J = 9.0 Hz, Ar), 3.95 (2H, d, J = 2.4 Hz, SCH_2), 3.74 (3H, s, Ar-OCH_3), 3.24 (1H, t, J = 2.4 Hz, ≡CH); ^{13}C NMR (DMSO-d_6, 151MHz) δ (ppm) 160.4, 154.5, 149.5, 145.2, 136.5, 129.7, 128.6, 127.2, 118.4, 114.1, 79.3, 74.8, 55.2, 21.3; HRMS (ESI) m/z calcd for $C_{18}H_{16}N_4O_3S_2$ $[M+H]^+$ 401.0742, found 401.0693.

4-(3-(4-fluorophenyl)-5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazol-4-yl) benzenesulfonamide (1d)

White solid; Yield: 78 % ; mp 184-186 ºC; IR (KBr) ν_{max} 3300, 3182, 3250, 2998, 1606, 1534, 1481, 1442, 1343, 1162, 845, 628, 587; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.91 (2H, d, J = 8.4 Hz, Ar), 7.60 (2H, d, J = 8.4, Ar), 7.53 (2H, ex, s, SO_2NH_2), 7.39 (2H, dd, $^3J_{H-H}$ = 7.5 Hz and $^4J_{H-F}$ = 4.5 Hz, Ar), 7.21 (2H, t, $^3J_{H-H}$ & $^3J_{H-F}$ = 9.0 Hz, Ar), 3.94 (2H, d, J = 3.0 Hz, SCH_2), 3.21(1H, t, J = 3.0 Hz, ≡CH); ^{13}C NMR (DMSO-d_6, 151MHz) δ (ppm) 162.0, 153.9, 150.1, 145.3, 136.2, 130.7, 128.5, 127.2, 122.8, 115.9, 79.3, 74.8, 21.2; HRMS (ESI) m/z calcd for $C_{17}H_{13}FN_4O_2S_2$ $[M+H]^+$ 389.0542, found 389.0606.

4-(3-(4-chlorophenyl)-5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazol-4-yl) benzenesulfonamide (1e)

White solid; Yield: 80 %; mp 220-222 ºC; IR (KBr) ν_{max} 3323, 3287, 3180, 2998, 2886, 1598, 1438, 1343, 1343, 1166, 836, 726, 651; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm) 7.96 (2H, d, J = 9.0 Hz, Ar), 7.64 (2H, d, J = 8.4 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.47 (2H, d, J = 9.0 Hz, Ar), 7.38 (2H, d, J = 9.0 Hz, Ar), 3.99 (2H, d, J = 3.0 Hz, SCH_2), 3.24 (1H, t, J = 3.0 Hz, ≡CH); ^{13}C NMR (DMSO-d_6, 151 MHz)δ (ppm) 153.7, 150.4, 145.3, 136.1, 134.9, 129.9, 128.8, 128.5, 127.3, 125.1, 79.2, 74.8, 21.2; HRMS (ESI) m/z calcd for $C_{17}H_{13}ClN_4O_2S_2$ $[M+H]^+$/ $[M+H+2]^+$ 405.0246/407.0246, found 405.0314/407.0282.

4-(3-(4-bromophenyl)-5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (1f)

White solid; Yield: 78 %; mp 230-232 ºC; IR (KBr) ν_{max} 3333, 3180, 3284, 2976, 2871, 1594, 1499, 1436, 1343, 1164, 833, 624, 544; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.96 (2H, d, J = 8.4 Hz, Ar), 7.64 (2H, d, J = 8.4 Hz, Ar), 7.61 (2H, d, J = 8.4 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.31 (2H, d, J = 8.4, Ar), 3.99 (2H, d, J = 2.4 Hz, SCH_2), 3.24 (1H, t, J = 2.4 Hz, ≡CH); ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 153.8, 150.4, 145.3, 136.1, 131.7, 130.1, 128.5, 127.3, 125.5, 123.7, 79.2, 74.8, 21.2; HRMS (ESI) m/z calcd for $C_{17}H_{13}BrN_4O_2S_2$ $[M+H]^+$/ $[M+H+2]^+$ 448.9742/450.9721, found

448.9771/450.9749.

4-(3-(4-nitrophenyl)-5-(prop-2-yn-1-ylthio)-1,5-dihydro-4*H*-1,2,4-triazol-4-yl)-benzenesulfonamide (1g)

Yellowish solid; Yield: 75 %; mp 242-244 ºC; IR (KBr) ν_{max} 3364, 3196, 3274, 2973, 2883, 1734, 1598, 1436, 1346, 1159, 855, 617, 546; ^{1}H NMR (DMSO-d$_6$, 500 MHz) δ (ppm) 8.24 (2H, d, *J* = 8.0 Hz, Ar), 7.98 (2H, d, *J* = 8.0 Hz, Ar), 7.70 (2H, d, *J* = 7.0 Hz, Ar), 7.65 (2H, d, *J* = 8.0 Hz, Ar), 7.58 (2H, ex, s, SO_2NH_2), 4.04 (2H, s, SCH_2), 3.26 (1H, s, ≡CH); ^{13}C NMR (DMSO-d$_6$, 126 MHz) δ (ppm) 153.0, 147.9, 145.4, 135.7, 132.1, 129.3, 128.3, 127.3, 127.0, 123.8, 79.1, 74.7, 21.0; HRMS (ESI) m/z calcd for $C_{17}H_{13}N_5O_4S_2$ $[M+H]^+$ 416.0487, found 416.0440.

4,4'-(5-(prop-2-yn-1-ylthio)-4*H*-1,2,4-triazole-3,4-diyl)dibenzenesulfonamide (1h)

White solid; Yield: 70 %; mp 244-246 ºC; IR (KBr) ν_{max} 3318, 3174, 3273, 3059, 2969, 2895, 1741, 1612, 1432, 1333, 1160,842, 622, 520; ^{1}H NMR (DMSO-d$_6$, 600 MHz) δ (ppm) 7.97 (2H, d, *J* = 9.0 Hz, Ar), 7.81 (2H, t, *J* = 9.0 Hz, Ar), 7.67 (2H, d, *J* = 9.0 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.56 (2H, d, *J* = 9.0 Hz, Ar), 7.44 (2H, ex, s, SO_2NH_2), 4.01 (2H, d, *J* = 3.0 Hz, SCH_2), 3.25 (1H, t, *J* = 3.0 Hz, ≡CH); ^{13}C NMR (DMSO-d$_6$, 151 MHz)δ(ppm) 153.7, 150.8, 145.4, 145.1, 136.0, 129.3, 128.8, 128.5, 127.3, 125.9, 79.3, 74.8, 21.1; HRMS (ESI) m/z Calcd for $C_{17}H_{15}N_5O_4S_3$ $[M+H]^+$ 450.0364, found 450.0320.

2.5.4 General procedure for the synthesis of target compounds 2a-2h

To a clear solution of compound **7** in acetone was added one equivalent of each of ethyl bromoacetate and potassium carbonate. The reaction mixture was refluxed for 6 hrs when TLC showed complete consumption of reactant. After completion of reaction, reaction mixture was cooled and poured into ice cold water resulting into the precipitation of white solid which was then filtered, dried, and crystallized from ethanol.

ethyl-2-((5-phenyl-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (2a)

White solid; Yield: 72 %; mp 166-168 ºC; IR (KBr) ν_{max} 3362, 3215, 3061, 2972, 2946,

2876, 1742, 1475, 1434, 1343, 1165, 838, 783, 701; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 7.92 (2H, d, J = 8.0 Hz, Ar), 7.59 (2H, d, J = 8.0 Hz, Ar), 7.54 (2H, ex, s, SO_2NH_2), 7.38-7.31 (5H, m, Ar), 4.12-4.06 (4H, m, SCH_2 & OCH_2), 1.14 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 168.1, 154.5, 150.9, 145.4, 136.3, 130.1, 128.8, 128.5, 128.2, 127.4, 61.4, 48.6, 34.3, 14.0; HRMS (ESI) m/z calcd for $C_{18}H_{18}N_4O_4S_2$ $[M+H]^+$ 419.0848, found 419.0821.

ethyl-2-((4-(4-sulfamoylphenyl)-5-(*p*-tolyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (2b)

White solid; Yield: 76 %; mp 184-186 ºC; IR (KBr) ν_{max} 3296, 3182, 2986, 2921, 2846, 1728, 1495, 1444, 1345, 1168, 759, 621; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 7.96 (2H, d, J = 7.5 Hz, Ar), 7.61 (2H, d, J = 7.5 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.24 (2H, d, J = 7.0 Hz, Ar), 7.18 (2H, d, J = 7.0 Hz, Ar), 4.12 (2H, q, J = 6.0 Hz, OCH_2), 4.09 (2H, s, SCH_2), 2.28 (3H, s, Ar-CH_3), 1.19 (3H, t, J = 7 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm) 168.0, 154.5, 150.5, 145.2, 139.8, 136.3, 129.2, 128.4, 128.0, 127.3,123.3, 61.3, 34.1, 20.8, 13.9; HRMS (ESI) m/z calcd for $C_{19}H_{20}N_4O_4S_2$ $[M+H]^+$ 433.1004, found 433.1040.

ethyl-2-((5-(4-methoxyphenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3- yl)thio)-acetate (2c)

White solid; Yield: 76 %; mp 180-182 ºC; IR (KBr) ν_{max} 3304, 3184, 3097, 3037, 2991, 1747, 1473, 1441, 1338, 1160, 840, 619; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 7.96 (2H, d, J = 8.0 Hz, Ar), 7.62 (2H, d, J = 8.0 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.28 (2H, d, J = 9.0 Hz, Ar), 6.93 (2H, d, J = 8.0 Hz, Ar), 4.13 (2H, s, SCH_2), 4.09 (2H, q, J = 6.0 Hz, OCH_2), 3.73 (3H, s, Ar-OCH_3), 1.19 (3H, t, J = 6.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 168.0, 160.4, 154.3, 150.2, 145.2, 136.4, 129.6, 128.4, 127.3, 118.3, 114.2, 61.3, 55.2, 34.2, 13.9; HRMS (ESI) m/z calcd for $C_{19}H_{20}N_4O_5S_2$ $[M+H]^+$ 449.0953, found 449.0930.

ethyl-2-((5-(4-fluorophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (2d)

White solid; Yield: 76 %; mp 190-192 ºC; IR (KBr) ν_{max} 3304, 3168, 2982, 2943, 2846,

1725, 1485, 1447, 1350, 1173, 844, 759, 612; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm) 8.00-7.95 (2H, m, Ar), 7.66-7.62 (2H, m, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.42 (2H, dd, $^{3}J_{H-H}$ = 7.5 Hz and $^{4}J_{H-F}$ = 4.5 Hz, Ar), 7.24 (2H, t, $^{3}J_{H-H}$ & $^{3}J_{H-F}$ = 9.0 Hz, Ar),4.15-4.10 (4H, m, SCH_2 & CH_2), 1.19 (3H, t, CH_3); ^{13}C NMR (DMSO-d_6, 151MHz) δ (ppm) 167.9, 163.6, 162.0, 153.6, 150.8, 145.3, 136.1, 130.7, 128.4, 127.4, 122.8, 115.9, 115.8, 61.3, 34.2, 14.0; HRMS (ESI) m/z calcd for $C_{18}H_{17}FN_4O_4S_2$ 437.0753 $[M+H]^+$, found 437.0840.

ethyl-2-((5-(4-chlorophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (2e)

White solid; Yield: 76 %; mp 190-192 ºC; IR (KBr) ν_{max} 3306, 3168, 3044, 2985, 2944, 2893, 1722, 1477, 1443, 1353, 1173, 810, 760, 622; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 7.92 (2H, d, *J* = 8.5 Hz, Ar), 7.57 (2H, d, *J* = 8.5 Hz, Ar), 7.53 (2H, ex, s, SO_2NH_2), 7.23 (2H, d, *J* = 8.5 Hz, Ar), 6.88 (2H, d, *J* = 9.0 Hz, Ar), 4.08-3.99 (4H, m, SCH_2 & OCH_2), 1.14 (3H, *t*, J = 7.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ(ppm) 168.0, 160.4, 154.37, 150.3, 145.2, 136.4, 129.6, 128.4, 127.4, 118.4, 114.2, 61.3, 55.3, 34.2, 30.7, 14.0; HRMS (ESI) m/z calcd for $C_{18}H_{17}ClN_4O_4S_2$ $[M+H]^+$/ $[M+H+2]^+$ 453.0458/455.0428, found 453.0417/455.0413.

ethyl-2-((5-(4-bromophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (2f)

White solid; Yield: 76 %; mp 230-132 ºC; IR (KBr) ν_{max} 3301, 3168, 2990, 2888, 1720, 1474, 1440, 1351, 1173, 836, 758, 622; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 7.97 (2H, d, *J* = 8.4 Hz, Ar), 7.65 (2H, d, *J* = 8.4 Hz, Ar), 7.60 (2H, d, *J* = 8.4 Hz), 7.57 (2H, ex, s, SO_2NH_2), 7.31 (2H, d, *J* = 8.4 Hz, Ar), 4.14-4.10 (4H, m, SCH_2 & OCH_2), 1.19 (3H, t, *J* = 7.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 167.9, 153.6, 151.1, 145.4, 136.0, 131.8, 130.1, 128.3, 127.4, 125.4, 123.6, 61.3, 34.2, 13.9; HRMS (ESI) m/z calcd for $C_{18}H_{17}BrN_4O_4S_2$ $[M+H]^+$/ $[M+H+2]^+$ 496.9953/498.9932, found 497.0016/499.0012.

ethyl-2-((5-(4-nitrophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)ace-

tate (2g)

Yellowish solid; Yield: 76 %; mp 168-170 ºC; IR (KBr) ν_{max} 3291, 3177, 3040, 2988, 2940, 1728, 1440, 1343, 1163, 854, 759, 620; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 8.23 (2H, t, J = 9.0 Hz, Ar), 7.99 (2H, d, J = 8.4 Hz, Ar), 7.69 (2H, d, J = 8.4 Hz, Ar), 7.63 (2H, d, J = 8.4 Hz, Ar), 7.58 (2H, ex, s, SO_2NH_2), 4.17-4.08 (4H, m, SCH_2 & OCH_2), 1.20 (3H, t, J = 7.2 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 151MHz) δ (ppm) 167.9, 152.9, 152.1, 148.0, 145.5, 135.8, 132.2, 129.3, 128.3, 127.5, 123.9, 61.3, 34.1, 14.0; HRMS (ESI) m/z calcd for $C_{18}H_{17}N_5O_6S_2$ $[M+H]^+$ 464.0698, found 464.0674.

ethyl2-((4,5-bis(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetate (2h)

White solid; Yield: 77 %; mp 158-160 ºC; IR (KBr) ν_{max} 3328, 3248, 3100, 2982, 2865, 2844, 1708, 1478, 1452, 1347, 1154, 844, 710, 628; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.98 (2H, d, J = 9.0 Hz, Ar), 7.79 (2H, d, J = 9.0 Hz, Ar), 7.67 (2H, d, J = 8.0 Hz, Ar), 7.58 (2H, ex, s, SO_2NH_2), 7.54 (2H, d, J = 7.5 Hz, Ar), 7.44 (2H, ex, s, SO_2NH_2), 4.14-4.10 (4H, m, SCH_2 & OCH_2), 1.19 (3H, t, J = 6.5 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 167.9, 153.5, 151.5, 145.5, 145.1, 136.0, 129.2, 128.7, 128.4, 127.5, 125.9, 61.4, 34.2, 14.0; HRMS (ESI) m/z calcd for $C_{18}H_{19}N_5O_6S_3$ $[M+H]^+$ 497.0497, found 498.0574.

2.5.5 General procedure for the synthesis of target compounds 3a-3h

To an aqueous suspension of compound **2** was added 2 % NaOH solution and refluxed for four hours resulting in the complete hydrolysis of ester to corresponding acid **3**. After completion of the reaction, the reaction mixture was cooled to room temperature and added to crushed ice followed by neutralization with dilute hydrochloric acid. A white solid was precipitated out which was filtered off, washed with excess of water, dried, and recrystallized from ethanol.

2-((5-phenyl-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3a)

White solid; Yield: 69 %; mp 250-252 ºC; IR (KBr) ν_{max} 3338, 3248, 3076, 2984, 2862, 1707, 1479, 1433, 1345, 1163, 896, 758, 699; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm)

7.96 (2H, d, J = 8.4 Hz, Ar), 7.63 (2H, d, J = 8.4 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.40-7.32 (5H, m, Ar), 4.06 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 169.2, 154.3, 151.1, 145.3, 136.3, 129.9, 128.6, 128.4, 128.1, 127.3, 126.2, 34.5; HRMS (ESI) m/z calcd for $C_{16}H_{14}N_4O_4S_2$ $[M+H]^+$ 391.0535, found 391.0463.

2-((4-(4-sulfamoylphenyl)-5-(*p*-tolyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3b)

White solid; Yield: 68 %; mp 220-222 ºC; IR (KBr) ν_{max} 3374, 3268, 3064, 2976, 2928, 2872, 1738, 1496, 1449, 1343, 1163, 782, 699, 622; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 7.96 (2H, d, J = 8.0 Hz, Ar), 7.61 (2H, d, J = 8.0 Hz, Ar), 7.59 (2H, ex, s, SO_2NH_2), 7.24 (2H, d, J = 7.5 Hz, Ar), 7.18 (2H, d, J = 7.5 Hz, Ar), 4.05 (2H, s, SCH_2), 2.27 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 169.2, 154.4, 150.8, 145.2, 139.7, 136.4, 129.2, 128.4, 128.0, 127.3, 34.5, 20.8; HRMS (ESI) m/z calcd for $C_{17}H_{16}N_4O_4S_2$ $[M+H]^+$ 405.0691, found 405.0739.

2-((5-(4-methoxyphenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3c)

White solid; Yield: 72 %; mp 246-248 ºC; IR (KBr) ν_{max} 3330, 3248, 2978, 2944, 2898, 1705, 1482, 1457, 1343, 1164, 838, 755, 595; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.96 (2H, d, J = 8.4 Hz, Ar), 7.62 (2H, d, J = 8.4 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.29 (2H, d, J = 9.0 Hz, Ar), 6.93 (2H, d, J = 9.0 Hz, Ar), 4.05 (2H, s, SCH_2), 3.74 (3H, s, Ar-OCH_3); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm) 169.2, 160.3, 154.2, 150.6, 145.2, 136.5, 129.6, 128.4, 127.3, 118.4, 114.1, 55.1, 34.5; HRMS (ESI) m/z calcd for $C_{17}H_{16}N_4O_5S_2$ $[M+H]^+$ 421.0640, found 421.0557.

2-((5-(4-fluorophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3d)

White solid; Yield: 72 %; mp 230-232 ºC; IR (KBr) ν_{max} 3390, 3284, 2973, 2869, 1734, 1477, 1433, 1331, 1161, 850, 620; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.97 (2H, d, J = 9.0 Hz, Ar), 7.63 (2H, d, J = 8.4 Hz, Ar), 7.58 (2H, ex, s, SO_2NH_2), 7.42 (2H, dd, $^3J_{H-H}$ = 7.5 Hz and $^4J_{H-F}$ = 4.5 Hz, Ar), 7.24 (2H, t, $^3J_{H-H}$ & $^3J_{H-F}$ = 9.0 Hz, Ar), 4.06 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 151MHz) δ (ppm) 169.1, 163.6, 162.0,

153.6, 151.1, 145.3, 136.1, 130.7, 130.6, 128.4, 127.4, 115.8, 115.9, 34.5, 8.4; HRMS (ESI) m/z Calcd for $C_{16}H_{13}FN_4O_4S_2$ $[M+H]^+$ 409.0440, found 409.0512.

2-((5-(4-chlorophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3e)

White solid; Yield: 72 %; mp 224-226 ºC; IR (KBr) ν_{max} 3325, 3250, 3032, 2981, 1704, 1445, 1307, 1164, 828, 645; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.97 (2H, d, *J* = 8.4 Hz, Ar), 7.64 (2H, d, *J* = 8.4 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.47 (2H, d, *J* = 9.0 Hz, Ar), 7.37 (2H, d, *J* = 8.4 Hz, Ar), 4.07 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 169.2, 153.4, 151.4, 145.3, 136.12, 134.8, 129.9, 128.8, 128.3, 127.4, 125.1, 34.5; HRMS (ESI) m/z calcd for $C_{16}H_{13}ClN_4O_4S_2$ $[M+H]^+$/ $[M+H+2]^+$ 425.0145/427.0115, found 425.0173/427.0118.

2-((5-(4-bromophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3f)

White solid; Yield: 71 %; mp 240-242 ºC; IR (KBr) ν_{max} 3390, 3299, 2986, 2868, 1719, 1474, 1442, 1350, 1173, 835, 760, 619; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.47 (2H, d, *J* = 8.4 Hz, Ar), 7.10-7.08 (6H, m, SO_2NH_2 & Ar), 6.79 (2H, d, *J* = 9.0, Ar), 3.56 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 169.6, 154.0, 152.0, 145.9, 136.6, 132.3, 130.6, 128.8, 127.9, 126.0, 124.1, 35.1; HRMS (ESI) m/z calcd for $C_{16}H_{13}BrN_4O_4S_2$ $[M+H]^+$/ $[M+H+2]^+$ 468.9640/470.9619, found 468.9635/470.9670.

2-((5-(4-nitrophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio) acetic acid (3g)

White solid; Yield: 68 %; mp 210-212 ºC; IR (KBr) ν_{max} 3394, 3265, 3095, 2978, 2888, 1713, 1513, 1445, 1346, 1166, 853, 755, 619; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm): 8.23 (2H, d, *J* = 9.6 Hz, Ar), 7.99 (2H, d, *J* = 8.4 Hz, Ar), 7.69 (2H, d, *J* = 8.4 Hz, Ar), 7.63 (2H, d, *J* = 9.6 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 4.10 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm) 169.1, 152.8, 152.4, 148.0, 145.5, 135.8, 132.2, 129.3, 128.3, 127.5, 123.9, 34.5; HRMS (ESI) m/z calcd for $C_{16}H_{13}N_5O_6S_2$ $[M+H]^+$ 436.0385, found 436.0286.

2-((4,5-bis(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)acetic acid (3h)

White solid; Yield: 74 %; mp 186-188 ºC; IR (KBr) ν_{max} 3326, 3226, 3057, 2989, 2930, 1713, 1496, 1443, 1334, 1161, 848, 763, 622; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 7.98 (2H, d, J = 8.4 Hz, Ar), 7.80 (2H, d, J = 8.4 Hz, Ar), 7.67 (2H, d, J = 8.4 Hz, Ar), 7.58 (2H, ex, s, SO_2NH_2), 7.53 (2H, d, J = 8.4 Hz, Ar), 7.44 (2H, s, SO_2NH_2), 4.08 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 151 MHz,) δ (ppm) 169.2, 153.4, 152.1, 145.4, 145.1, 136.1, 129.3, 128.8, 128.4, 127.5, 126.0, 35.0; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_5O_6S_3$ $[M+H]^+$ 470.0262, found 470.0222.

2.5.6 General procedure for the synthesis of target compound 4b-4f and 4h

To the solution of ester **2** in ethanol was added 80 % hydrazine hydrate solution (6 equivalents) and the resulting reaction mixture was refluxed for 6 hrs. The completion of reaction was confirmed by TLC followed by cooling the reaction mixture at room temperature. A white solid was precipitated out which was filtered, washed, dried and crystallized from ethanol yielding pure compounds **4b–4g** and **4h**.

4-(3-((2-hydrazineyl-2-oxoethyl)thio)-5-(*p*-tolyl)-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (4b)

White solid; Yield: 70 %; mp 250-252 ºC; IR (KBr) ν_{max} 3313, 3278, 3198, 3037, 2922, 2850, 1662, 1482, 1439, 1326, 1160, 836, 754, 617; ^{1}H NMR (DMSO-d_6, 600 MHz) δ (ppm) 9.36 (1H, ex, s, NH), 7.94 (2H, d, J = 7.8 Hz, Ar), 7.59 (2H, d, J = 8.4 Hz, Ar), 7.54 (2H, ex, s, SO_2NH_2), 7.22 (2H, d, J = 7.8 Hz, Ar), 7.15 (2H, d, J = 8.4 Hz, Ar), 4.51 (2H, ex, s, NH_2), 3.88 (2H, s, SCH_2), 2.25 (3H, s, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 151 MHz) δ (ppm) 166.5, 154.9, 151.50, 145.8, 140.4, 137.1, 129.8, 129.0, 128.6, 127.8, 124.0, 35.0, 21.3; HRMS (ESI) m/z calcd for $C_{17}H_{18}N_6O_3S_2$ $[M+H]^+$ 419.50116, found 419.0952.

4-(3-((2-hydrazineyl-2-oxoethyl)thio)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-4-yl)-

benzenesulfonamide (4c)

White solid; Yield: 68 %; mp 225-228 ºC; IR (KBr) ν_{max} 3309, 3254, 3205, 3095, 2840, 1674, 1453, 1403, 1321, 1163, 836, 710, 588; ^{1}H NMR (DMSO-d$_6$, 600 MHz) δ(ppm) 9.35 (1H, ex, s, NH), 7.96 (2H, d, *J* = 8.4 Hz, Ar), 7.62 (2H, d, *J* = 9.6 Hz, Ar), 7.56 (2H, s, ex, SO_2NH_2), 7.29 (2H, d, *J* = 8.4 Hz, Ar), 6.93 (2H, d, *J* = 8.4 Hz, Ar), 4.56 (2H, ex, s, NH_2), 3.89 (2H, s, SCH_2), 3.74 (3H, s, Ar-OCH_3); ^{13}C NMR (DMSO-d$_6$, 151 MHz) δ (ppm)66.4, 160.9, 154.7, 151.2, 145.7, 137.1, 130.1, 129.0, 127.8, 119.0, 114.7, 55.8, 35.0; HRMS (ESI) m/z calcd for $C_{17}H_{18}N_6O_4S_2$ $[M+H]^+$ 435.50056, found 435.0823.

4-(3-(4-fluorophenyl)-5-((2-hydrazineyl-2-oxoethyl)thio)-4*H*-1,2,4-triazol-4-yl)-benzenesulfonamide (4d)

White solid; Yield: 73 %; mp 260-262 ºC; IR (KBr) ν_{max} 3394, 3339, 3269, 3071, 2982, 1674, 1481, 1447, 1300, 1167, 860, 756, 589; ^{1}H NMR (DMSO-d$_6$, 600 MHz) δ (ppm) 9.35 (1H, ex, s, NH), 7.96 (2H, d, *J* = 8.4Hz, Ar), 7.64 (2H, d, *J* = 8.4 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.41 (2H, dd, $^3J_{H-H}$ = 7.5 Hz and $^4J_{H-F}$ = 4.5 Hz, Ar), 7.24 (2H, t, $^3J_{H-H}$ & $^3J_{H-F}$ = 9.0 Hz, Ar), 4.34 (2H, ex, s, NH_2), 3.91 (2H, s, SCH_2); ^{13}C NMR (DMSO-d$_6$, 151 MHz) δ (ppm) 166.4, 162.5, 154.1, 151.7, 145.8, 136.7, 131.5, 131.2, 129.0, 127.8, 123.4, 116.5, 116.3, 35.0; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_6O_3FS_2$ $[M+H]^+$ 423.46504, found 423.0630.

4-(3-(4-chlorophenyl)-5-((2-hydrazineyl-2-oxoethyl)thio)-4*H*-1,2,4-triazol-4-yl)-benzenesulfonamide (4e)

White solid; Yield: 65 %; mp 240-242 ºC; IR (KBr) ν_{max} 3319, 3201, 3097, 2993, 2872, 1669, 1471, 1442, 1331, 1164, 840, 750, 623; ^{1}H NMR (DMSO-d$_6$, 500 MHz) δ (ppm) 10.46 (1H, ex, s, NH), 7.97 (2H, d, *J* = 8.0 Hz, Ar), 7.65 (2H, d, *J* = 8.0 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.47 (2H, d, *J* = 8.5 Hz, Ar), 7.37 (2H, d, *J* = 8.5 Hz, Ar), 4.41 (2H, ex, s, NH_2), 4.10 (2H, s, SCH_2); ^{13}C NMR (DMSO-d$_6$, 126 MHz) δ (ppm) 168.3, 153.2, 151.7, 145.2, 136.0, 134.7, 129.8, 128.7, 128.3, 127.3, 36.1; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_6O_3ClS_2$ $[M+H]^+$/ $[M+H+2]^+$ 439.0414/441.0384, found 439.0409/441.0387.

4-(3-(4-bromophenyl)-5-((2-hydrazineyl-2-oxoethyl)thio)-4*H*-1,2,4-triazol-4-yl)-benzenesulfonamide (4f)

White solid; Yield: 70 %; mp 250-252 ºC; IR (KBr) ν_{max} 3328, 3262, 3049, 2977, 2838, 1662, 1449, 1446, 1328, 1163, 833, 754, 617; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 9.37 (1H, ex, s, NH), 7.97 (2H, d, J = 8.0 Hz, Ar), 7.65 (2H, d, J = 7.5 Hz, Ar), 7.61 (2H, d, J = 7.5 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.30 (2H, d, J = 8.0 Hz, Ar), 4.41 (2H, ex, s, NH_2), 3.92 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 165.7, 153.4, 151.4, 145.2, 136.1, 131.7, 130.3, 130.0, 128.3, 127.3, 62.9, 34.4; HRMS (ESI) m/z calcd for $C_{16}H_{15}N_6O_3BrS_2$ $[M+H]^+$/ $[M+H+2]^+$ 482.9909/484.9888, found 482.9933/484.9913.

4,4′-(5-((2-hydrazineyl-2-oxoethyl)thio)-4*H*-1,2,4-triazole-3,4-diyl)dibenzenesulfonamide (4h)

White solid; Yield: 70 %; mp 270-272 ºC; IR (KBr) ν_{max} 3312, 3272, 3194, 3051, 2970, 1668, 1439, 1327, 1160, 843, 760, 641; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 10.50 (1H, ex, s, NH), 7.99 (2H, d, J = 7.0 Hz, Ar), 7.80 (2H, d, J = 8.0 Hz, Ar), 7.68 (2H, d, J = 7.5 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.55 (2H, d, J = 7.5 Hz, Ar), 4.44 (2H, ex, s, NH_2), 4.13 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 168.3, 162.9, 156.1, 153.1, 151.8, 145.2, 135.9, 128.6, 128.3, 127.3, 125.8, 30.5; HRMS (ESI) m/z calcd for $C_{16}H_{17}N_7O_5S_3$ $[M+H]^+$ 484.05315, found 484.0495.

CHAPTER 3

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,2,3-TRIAZOLE TETHERED 1,2,4-TRIAZOLE DERIVATIVES AS ANTI-MICROBIAL AND ANTI-OXIDANT AGENTS

3.1 Motivation for the Current Work

The increasing incidence of microbial resistance to clinically used anti-biotics coupled with the alarming growth of emerging pathogens has been a serious concern to public health as well as scientific community in last couple of decades [130–132]. Further, the side effects associated with the available drugs are also life-threatening giving rise to the need to develop newer potent drug candidates with novel structural skeletons which might possess new and different mechanism of action in comparison with existing clinical drugs, improved efficacy and lesser side-effects [133–135]. Among the attractive approaches to achieve this goal, combining two different active fragments in one molecule and developing molecular hybrids has been gaining the increased prevalence in recent years [136, 137].

Triazoles are the privileged five-membered azo-heterocycles gaining large interest of researchers due to their astonishing pharmacological behavior [138]. They can further

be classified as 1,2,3-triazoles and 1,2,4-triazoles based on the position of nitrogen atoms whether adjacent or not, respectively. Many contributions in the literature survey reveal that both, 1,2,3- and 1,2,4-triazoles are known to exhibit various pharmacological applications such as analgesic [77], anti-cancer [139], anti-oxidant [140], anti-microbial [141], anti-urease [142], anti-malarial [143], anti-depressant [144], anti-convulsant [145], anti-viral [146], anti-tubercular [147], anti-inflammatory [148] activities etc. Numerous articles are well reported in the literature on functionalization in triazole moiety, proving it as amazing biological entity [149]. A number of clinically used drugs such as alprazolam, etizolam, trapidil, tazobactam etc. have triazole moiety in their structure (Fig. 3.1).

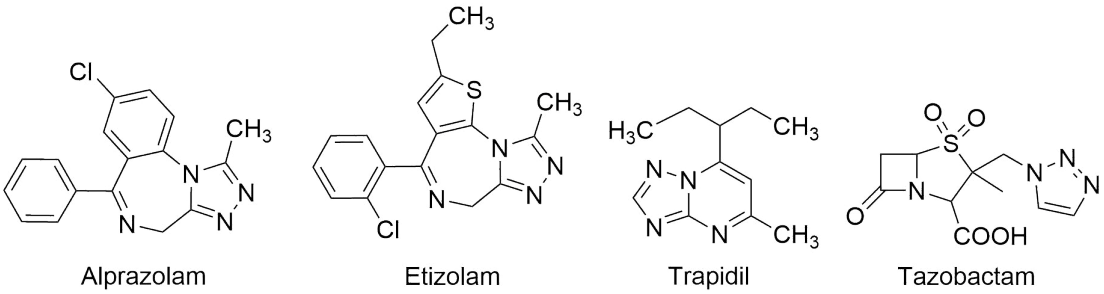


Fig. 3.1 Structures of drugs having triazole ring

Furthermore, sulfonamides constitute a distinctive class of biologically active compounds and have aroused considerable interest in medicinal chemistry for their diversified biological activities such as anti-microbial [150], anti-thyroid [151], anti-tumor [152], anti-bacterial [153], carbonic anhydrase inhibition [154] etc. Based on biological importance of triazoles and sulfonamides coupled with our ongoing interest in the synthesis of benzenesulfonamide incorporating aza-heterocycles as medicinally potent drug molecules [155], we hypothesized two series of novel benzenesulfonamide incorporated hybrids of 1,2,3- and 1,2,4-triazoles. Herein, we have synthesized novel 4-(1,2,4-triazol-3-ylsulfanylmethyl)-1,2,3-triazole derivatives **1a-1g** and **2a-2g** and evaluated them along with **8a-8g** (precursors for synthesis of **1a-1g**) as anti-microbial and anti-oxidant agents (Fig. 3.2). Cytotoxicity studies against mouse fibroblast and plant seed germination cell lines have also been tested for the newly synthesized compounds.

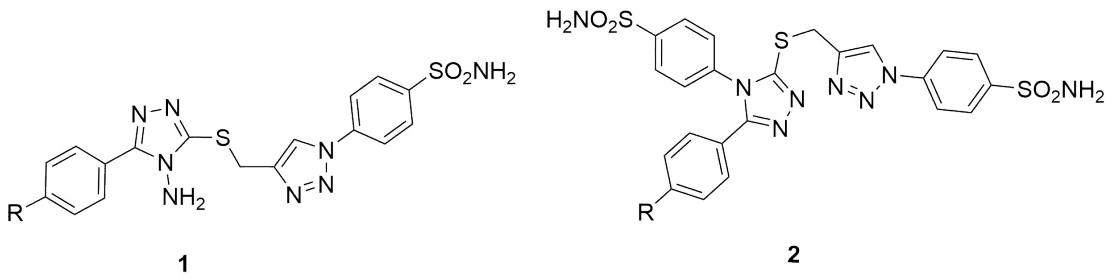


Fig. 3.2 1,2,3-Triazole tethered 1,2,4-triazole derivatives **1** and **2**

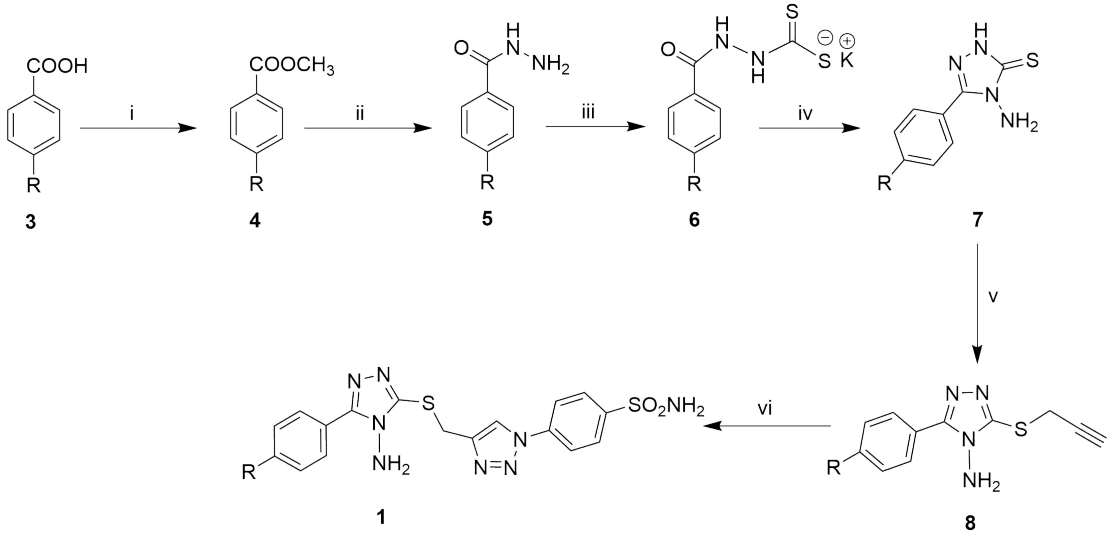
3.2 Results and Discussion

3.2.1 Synthesis overview of 1,2,3-triazole tethered 1,2,4-triazole derivative 1a-1g and 2a-2g

The synthetic routes adopted in the present work for synthesis of 1,2,3-triazole tethered 1,2,4-triazole derivatives **1** and **2** are depicted in scheme 3.1 and scheme 3.2, respectively. The final structures of newly synthesized target compounds were confirmed by their spectral (^{1}H NMR, ^{13}C NMR and IR) and HRMS data.

The key intermediates for preparation of target compounds **1** were aminotriazoles **7** which in turn were prepared from 4-substituted benzoic acids **3** using literature procedure [63] [63]. The synthetic pathway for **7** involves conversion of carboxylic acids **3** to their methyl ester derivatives **4** by refluxing in methanol under acidic condition followed by their subsequent conversion to hydrazide derivatives **5** by reaction with hydrazine hydrate. Aryl hydrazides **5** were then treated with carbon disulfide in presence of potassium hydroxide as base in anhyd. ethanol to yield corresponding dithiocarbamate salts **6** which upon subsequent cyclization with hydrazine hydrate under reflux condition yielded **7**. Aminotriazoles **7** were further S-propargylated by reaction with propargyl bromide in presence of triethylamine as base leading to the formation of **8**. Finally, target compounds **1** were obtained by click reaction of **8** with 4-azidobenzenesulfonamide using catalytic amount of coppersulfate pentahydrate and sodium ascorbate in mixture of tert-butanol and water (1:1) as solvent. For achieving the synthesis of compounds **2**, propargylated 3-mercapto-1,2,4-triazoles **9** were reacted with 4-azido benzene sulfonamide in presence of coppersulfate pentahydrate and sodium ascorbate through click chemistry reaction. propargylated 3-mercapto-1,2,4-triazoles **9** itself were synthesized using the methodology

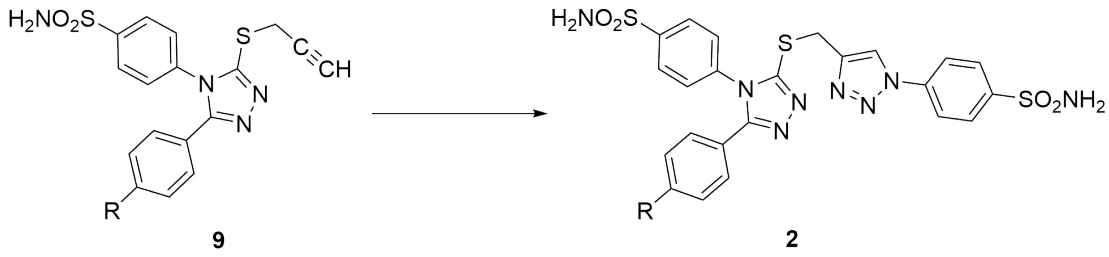
already disscussed in Chapter 2 (Scheme 2.3). Stepwise detailed discussion of the synthesis of target compounds **1a-1g** and **2a-2g** is given below.



Compound 1/3/4/5/6/7/8	a	b	c	d	e	f	g
R	H	CH_3	OCH_3	F	Cl	Br	SO_2NH_2

Scheme 3.1 Synthesis of target compounds **1a-1g**

'**Reagents and conditions:** (i) Methanol, H_2SO_4, reflux; (ii) Hydrazine hydrate, reflux; (iii) CS_2, KOH, Anhyd. ethanol; (iv) Hydrazine hydrate, H_2O, reflux; (v) Propargyl bromide, TEA, ethanol, reflux; (vi) 4-Azidobenzenesulfonamide, $CuSO_4.5H_2O$, ^{t}BuOH : H_2O (1 : 1)



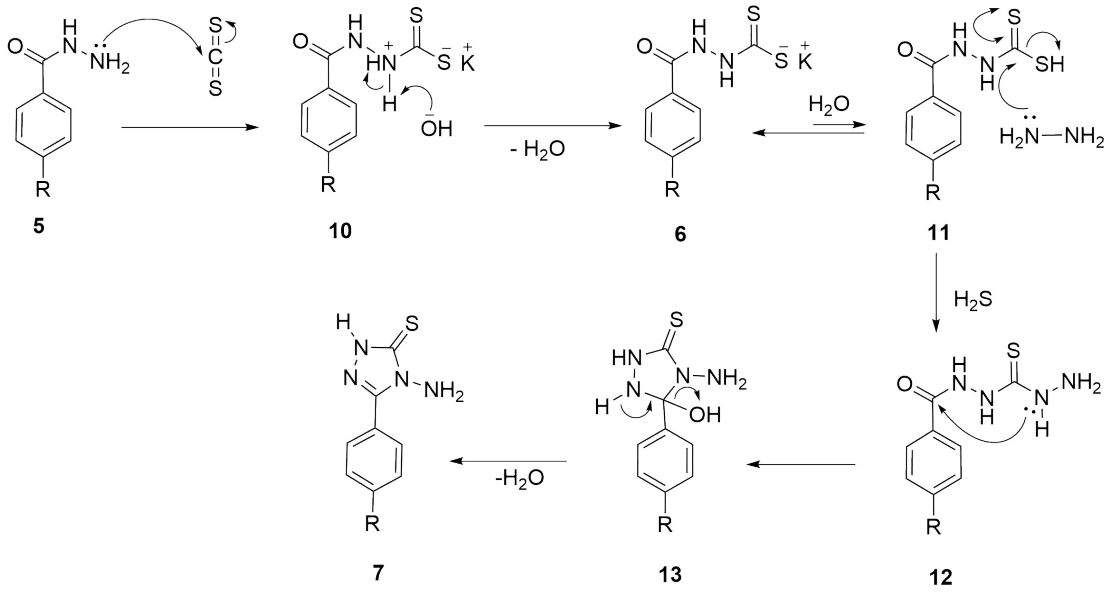
Compound 2/9	a	b	c	d	e	f	g
R	H	CH_3	OCH_3	F	Cl	Br	SO_2NH_2

Scheme 3.2 Synthesis of target compounds **2a-2g**

Reagents and conditions: 4-Azidobenzenesulfonamide, $CuSO_4.5H_2O$, sodium ascorbate, $^tBuOH : H_2O$ (1:1)

3.2.2 Synthesis of aminotriazoles 7a-7g

The synthetic pathway for aminotriazoles **7** starts with the esterification reaction of 4-substituted benzoic acids **3** (Scheme 3.1). Substituted benzoic acids **3** were esterified using methanol and few drops of conc. sulfuric acid. Completion of reaction required refluxing for 8-10 hrs giving corresponding methyl esters **4**. The esters **4** upon subsequent hydrazinolysis with excess of hydrazine hydrate led to the formation of corresponding aryl hydrazides **5**. Aryl hydrazides **5** were further treated with carbon disulfide using potassium hydroxide as inorganic base and anhyd. ethanol as solvent resulting in the formation of corresponding dithiocarbazinate salts **6**. Finally, the dithiocarbazinate salts **6** were cyclized by refluxing with excess of hydrazine hydrate resulting into the formation of aminotriazoles **7** [156]. The mechanism for the complete synthesis of **7** from hydrazides **5** is depicted in scheme 3.3.



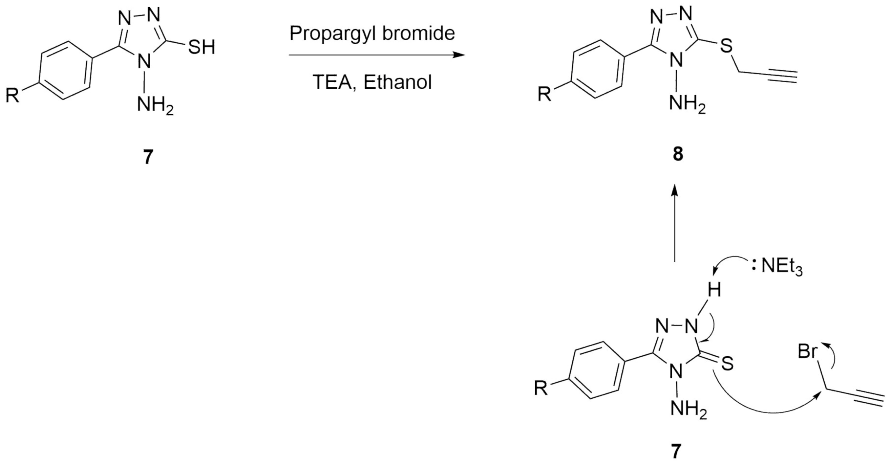
Scheme 3.3 Mechanism for the synthesis of aminotriazoles **7**

In the first step, lone pair of NH_2 group of hydrazide attacks on carbon disulfide followed by abstraction of proton by potassium hydroxide resulting in the formation of dithiocarbazinate salt **6**. The second step involves the nucleophilic attack of hydrazine hydrate on thiocarboxylic acid **11** which exists in minor proportion in equilibrium with **6**

in the aqueous suspension resulting into the formation of thiocarbazides **12**. In the next step, cyclization of thiocarbazide occurs by the nucleophilic attack of nitrogen of thiocarbohydrazide group on the carbonyl carbon giving the intermediates **13** which undergoes cyclocondensation giving cyclized aminotriazoles **7** as products. Comparing the melting points with literature values confirmed the formation of compounds **7**.

3.2.3 Synthesis of propargylated aminotriazoles 8a-8g

The synthesis of propargylated aminotriazoles **8** involves the reaction of **7** with propargyl bromide using triethyl amine (TEA) as a base and ethanol as solvent. Completion of reaction required refluxing for 1 h affording the desired propargylated aminotriazoles **8** as products (Scheme 3.4). The mechanism for the conversion of **7** to **8** starts with the abstraction of a proton by TEA base and subsequent attack of sulfur as nucleophile on the methylene carbon of propargyl bromide to form S-C bond resulting into the formation of propargylated aminotriazoles **8** (Scheme 3.4).

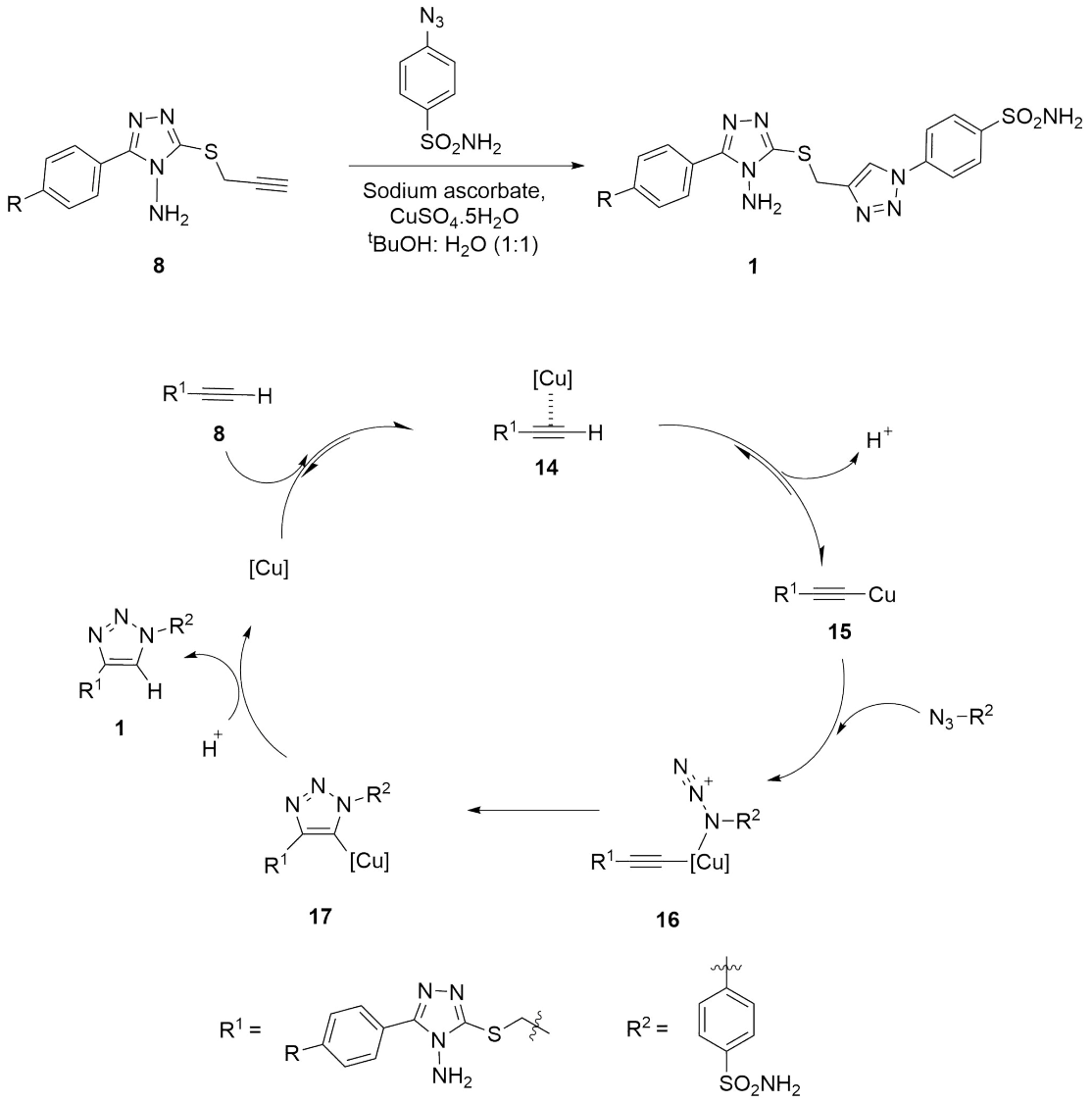


Scheme 3.4 Synthesis of compounds **8** with mechanism

3.2.4 Synthesis of 1,2,3-triazole tethered 1,2,4-triazole derivatives 1a-1g

Novel 1,2,3-triazole tethered 1,2,4-triazole derivatives **1** were constructed *via* Huisgen [3+2] cycloaddition reaction of azide group of 4-azidobenzensulfonamide and alkyne group of **8** in tert-butanol and water (1:1) as solvent system using catalytic amount of sodium ascorbate and $CuSO_4.5H_2O$ (Scheme 3.5). The mechanism of the reaction

begins with the reduction of Cu(II) to Cu(I) in presence of sodium ascorbate which act here as reducing agents. Now, Cu(I) acts as a catalyst and coordinates with the alkyne to form a Cu(I)-acetylide complex **14** which easily converted to **15** with the release of H^+ ion. The azide reacts with a copper salt to generate a reactive copper azide species **16**. The Cu(I)-acetylide complexed with azide **16** undergoes [3+2] cycloaddition leading to the formation of **17**. Subsequent protonolysis of **17** results into the formation of product **1** and regeneration of the Cu(I) catalyst which participate in further cycloaddition reactions [157].



Scheme 3.5 Synthesis of compounds **1** with mechanism

Conversion of **8** to **1** was confirmed by disappearance of singlet approximately at 3.20 ppm for alkynic proton and appearance of another singlet at approximately 8.90 ppm for

1,2,3-triazole ring proton in ^{1}H NMR (Fig. 3.3). Methylene protons (-SCH_2-) resonated at approximately 4.50 ppm as a singlet while broad exchangeable singlet obtained at approximately 7.55 ppm corresponding to sulfonamide protons. In ^{13}C NMR (Fig. 3.4), methylene carbon (-SCH_2-) resonated at approximately 26.0 ppm while methine carbon (=CH-) of 1,2,3-triazole ring resonated at approximately 160.0 ppm. Disappearance of a sharp band in the range of 3350-3287 cm^{-1} for C-H stretch of terminal alkyne (≡C-H) and another band at approximately 2150 cm^{-1} for C≡C stretch in IR spectra supported the conversion of alkynes to 1,2,3-triazoles. Further, HRMS data were also in line with the formation of desired product **1** (Fig. 3.5).

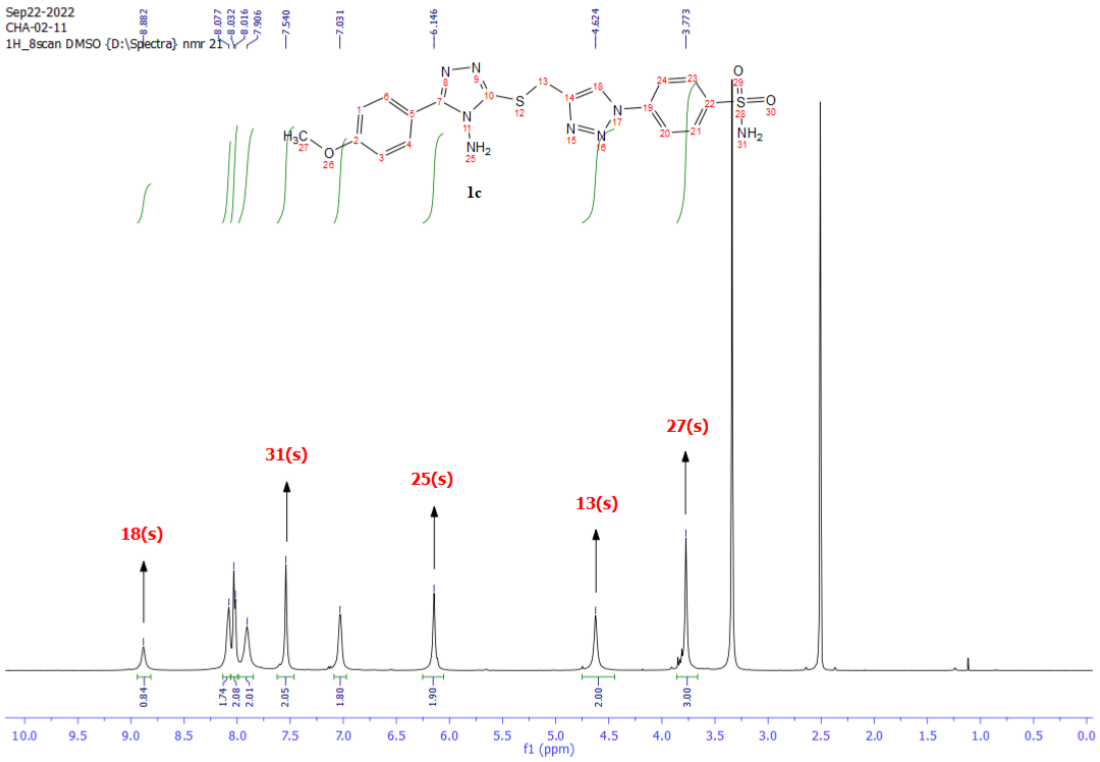


Fig. 3.3 ^{1}H NMR spectrum of compound **1c**

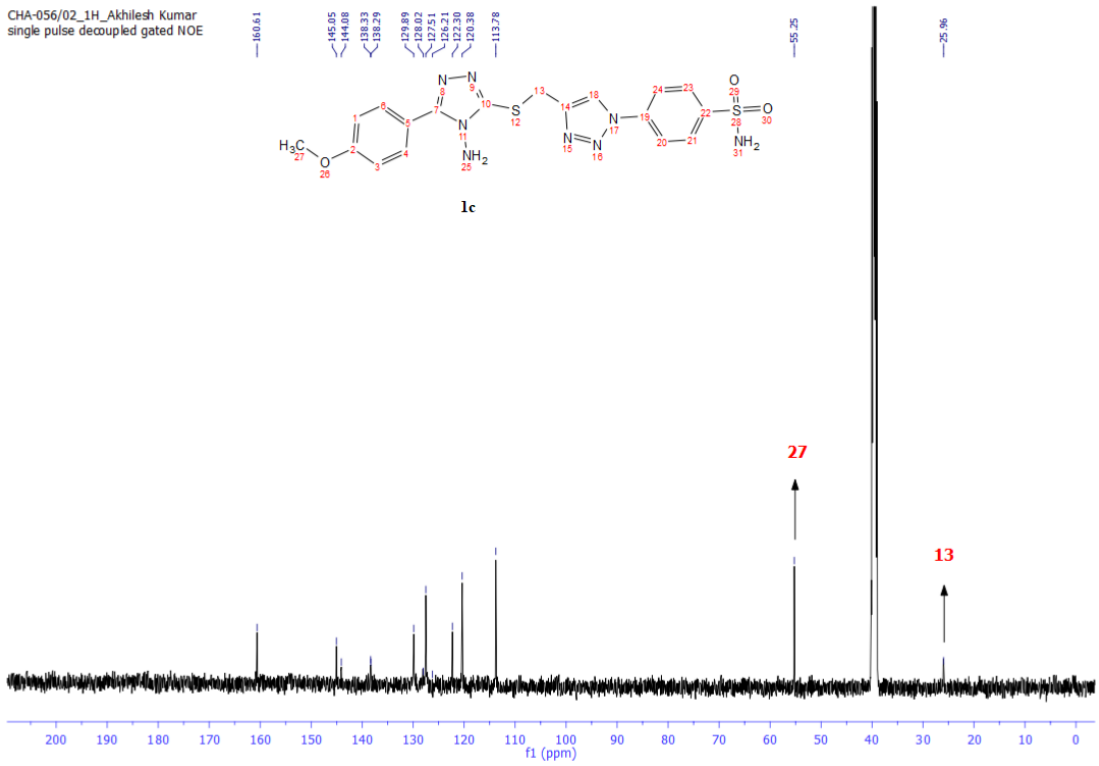


Fig. 3.4 ^{13}C NMR spectrum of compound **1c**

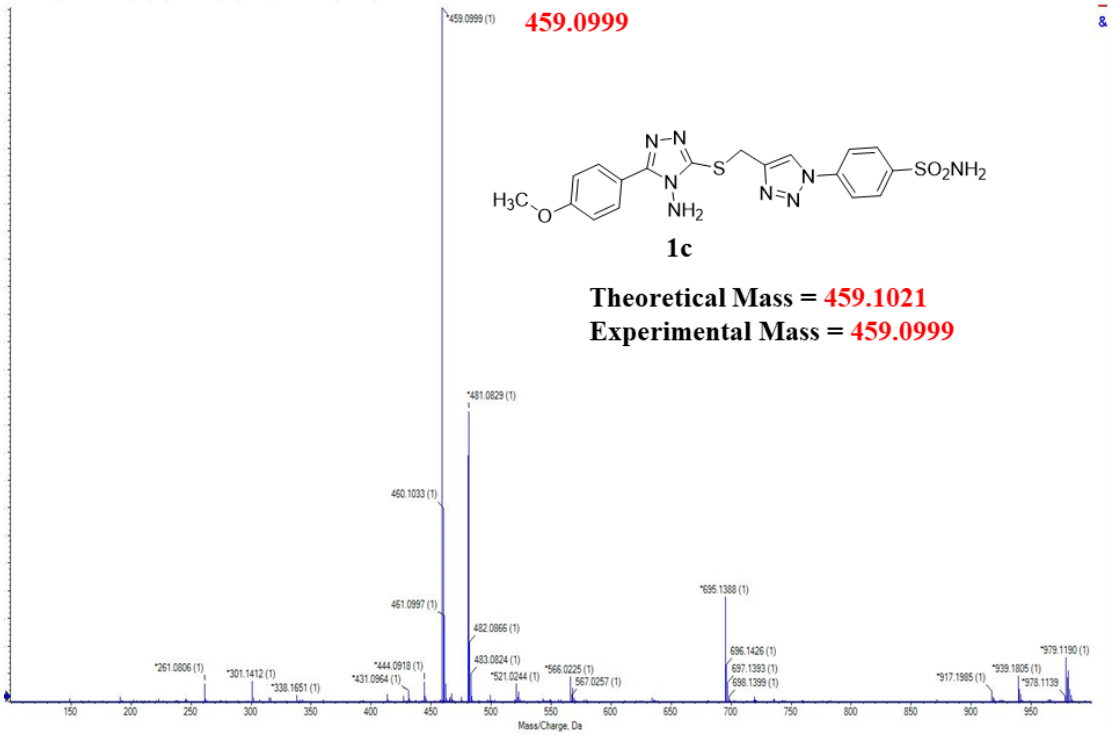
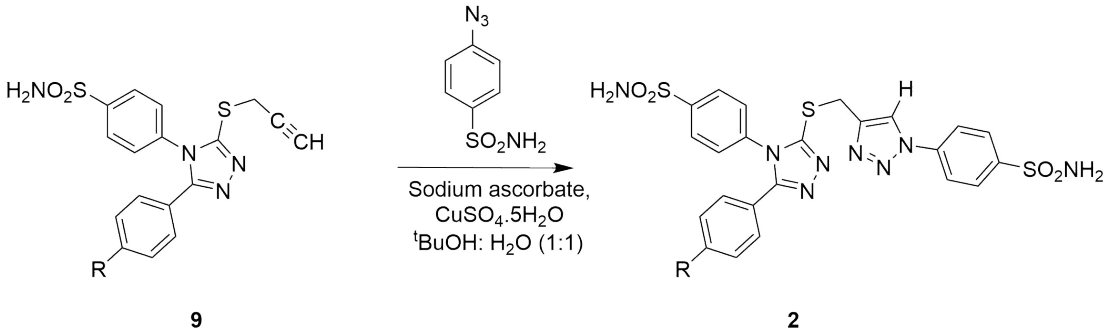


Fig. 3.5 HRMS spectrum of compound **1c**

3.2.5 Synthesis of 1,2,3-triazole tethered 1,2,4-triazole derivatives 2a-2g

For the synthesis of target molecules 1,2,3-triazole tethered 1,2,4-triazole derivatives **2**, propargylated 3-mercapto-1,2,4-triazoles **9** were reacted with azide group of 4-azidobenzenesulfonamide through click chemistry reaction (Scheme 3.6). Completion of reaction required stirring for 3-4 hrs at 70 ºC resulting into the formation of target compounds 1,2,3-triazole tethered 1,2,4-triazole derivatives **2** . Mechanism of the complete conversion of propargylated 3-mercapto-1,2,4-triazoles **9** to target molecules 1,2,3-triazole tethered 1,2,4-triazole derivatives **2** is the same as discussed in the previous scheme 3.5.



Scheme 3.6 Synthesis of compounds **2**

Formation of **2** was confirmed by appearance of a singlet at approximately 8.84-9.10 ppm for one proton (CH proton of triazole ring) and another singlet at approximately 4.50 ppm for methylene protons (-SCH_2-) in ^{1}H NMR (Fig. 3.6). In ^{13}C NMR (Fig. 3.7), methylene carbon (-SCH_2-) resonated at approximately 27.0 ppm while methine carbon (=CH-) of 1,2,3-triazole ring resonated at approximately 160.0 ppm. Disappearance of a sharp band in the range of 3350-3287 cm^{-1} for C-H stretch of terminal alkyne (≡C-H) and another band at approximately 2150 cm^{-1} for -C≡C- stretch in IR spectra also supporting the formation of products. Further, HRMS data were also found in accordance with the structures assigned to the final compounds **2** (Fig. 3.8).

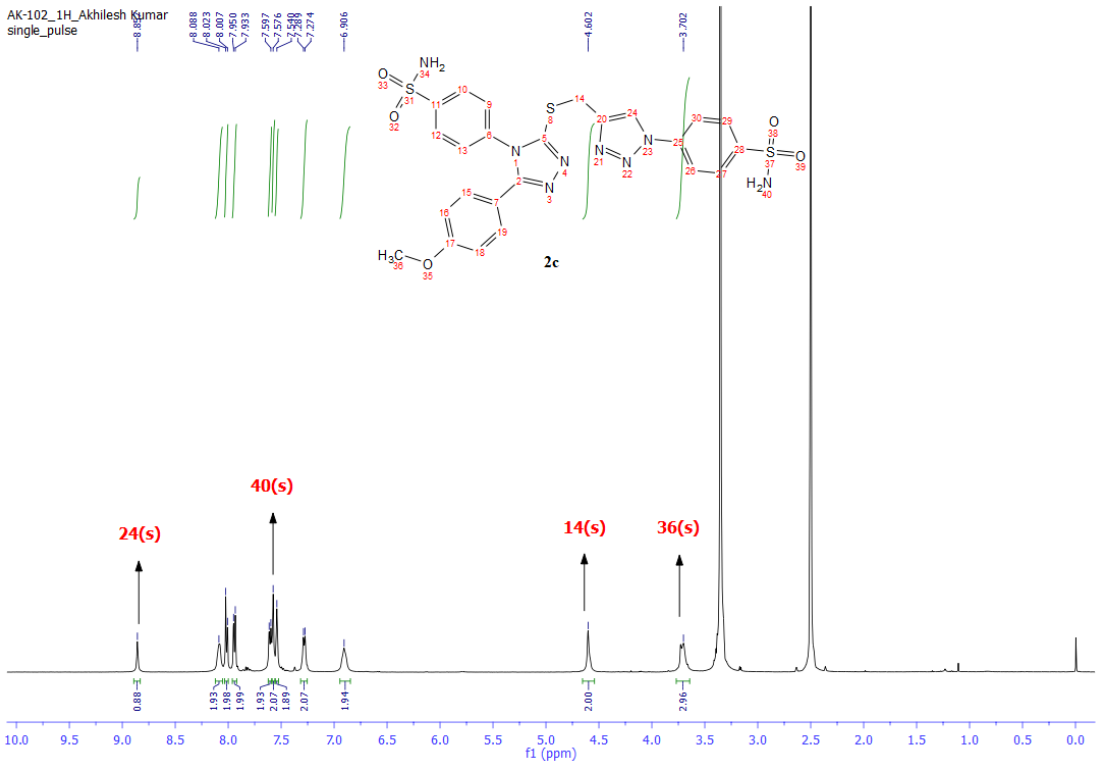


Fig. 3.6 ^{1}H NMR spectrum of compound **2c**

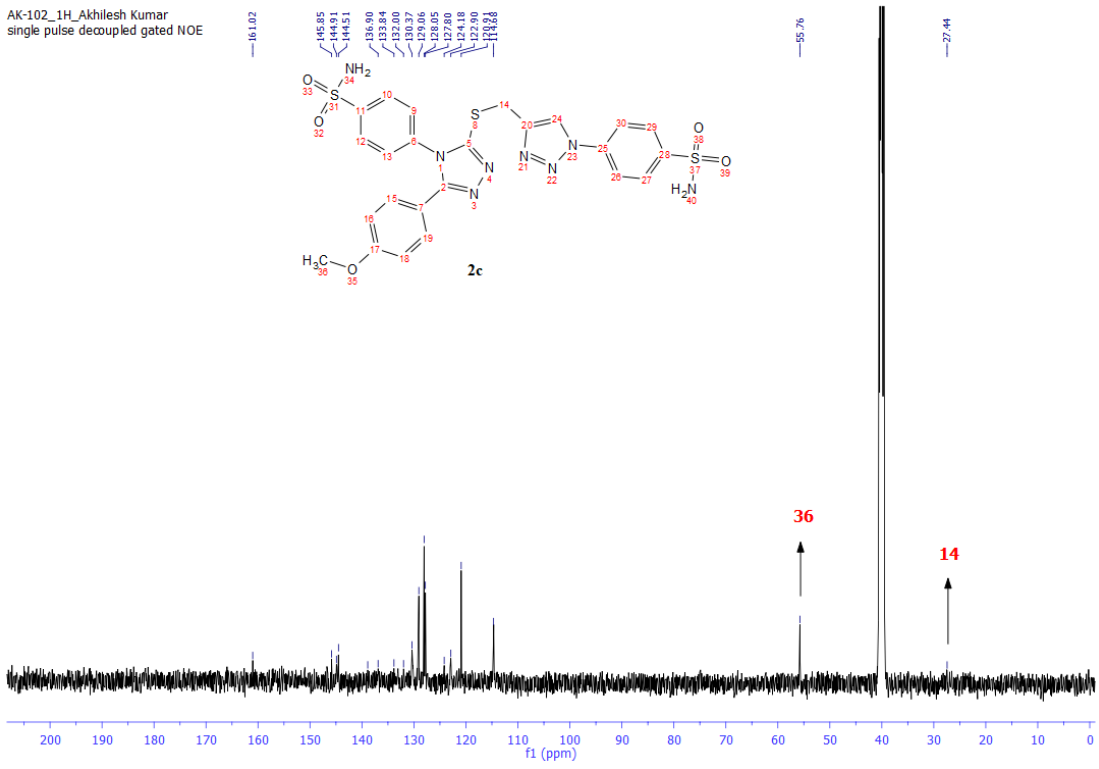


Fig. 3.7 ^{13}C NMR spectrum of compound **2c**

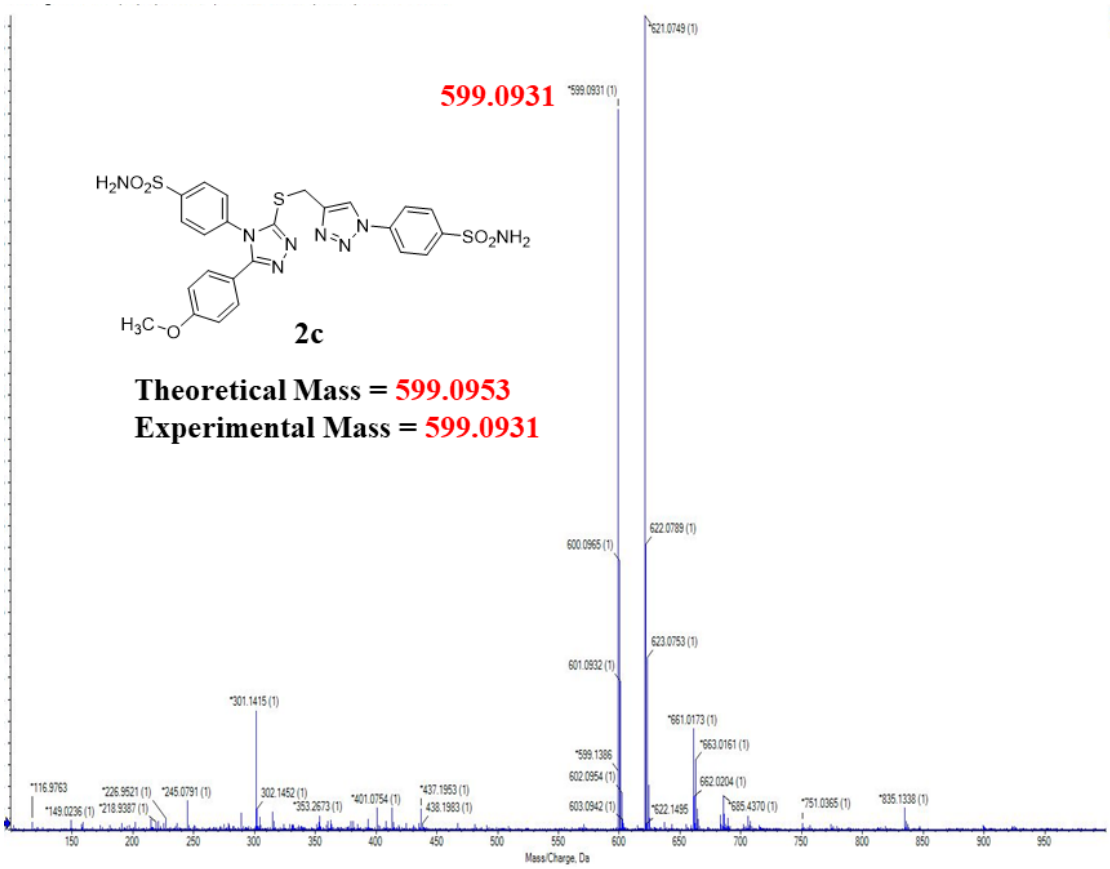


Fig. 3.8 HRMS spectrum of compound **2c**

3.3 Biological Testing Results

3.3.1 Anti-microbial assay

All the newly synthesized fourteen target compounds **1a-1g** and **2a-2g** and the precursors **8a-8g** were screened for their anti-microbial profile using double dilution method against three Gram-positive (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657* , and *Bacillus cereus ATCC 11770)*, four Gram-negative (*Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733* and *Shigella flexneri ATCC 9199)* bacterial strains and one fungal strain (*Candida albicans MTCC 183*). The detailed procedure for anti-microbial assay has already been disscussed in chapter 2.

3.3.2 Anti-oxidant assay

All the newly synthesized fourteen target compounds **1a-1g** and **2a-2g** and the precursors **8a-8g** were screened for their anti-oxidant profile using DPPH method and ascorbic acid as reference. The detailed procedure used for anti-oxidant assay has already been disscussed in chapter 2.

3.3.3 Results and discussion

A. Anti-microbial activity - results and discussion

All the newly synthesized fourteen target compounds **1a-1g**, **2a-2g** and the precursors **8a-8g** were evaluated for their *in vitro* anti-microbial activity against three Gram-positive (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657* , and *Bacillus cereus ATCC 11770)*, four Gram-negative (*Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733* and *Shigella flexneri ATCC 9199)* pathogenic bacterial strains and one pathogenic fungal strain (*Candida albicans MTCC 183*) using double dilution method. The results are reported as minimum inhibitory concentration (MIC, μg/mL) values of compounds against tested bacterial and fungal strains and compared with Amoxicillin (anti-bacterial) and Fluconazole (anti-fungal) as the standard reference drugs (Table 3.1). As depicted from results, all the final compounds have exhibited good to excellent anti-microbial activities against all the microbial strains tested. Against *E. coli* bacterium, all the tested compounds were either equivalent (MIC value 6.25 μg/mL) or double-fold (MIC value 3.12 μg/mL) better inhibitors in comparison with the standard drug used (MIC value 6.25 μg/mL). Compounds **1e**, **1f**, **2e** and **2f** were either equivalent or better inhibitor than the standard drug against all bacterial strains while **1d** and **2d** were most efficient inhibitors of tested fungal strain. In comparison to the final compounds **1a-1g** and **2a-2g**, precursors **8a-8g** were found to have lower inhibition potential against all the microbes tested. Graphical comparison of anti-bacterial activity profile of tested compounds **1a-1g**, **2a-2g** and **8a-8g** with reference drugs Amoxicilliln is shown in Fig. 3.9, Fig. 3.10 and Fig. 3.11, respectively, while graphical comparison of anti-fungal activity profile all the tested compounds with standard drug Fluconazole is shown in Fig. 3.12.

Table 3.1 MIC values of compounds **1a-1g**, **2a-2g** and **8a-8g** against tested bacterial and fungal strains

Compound	Minimum Inhibitory Concentration (MIC in µg/mL)[a]							
	Gram-positive bacterial strain			Gram-negative bacterial strains				Fungal strain
	S.aure us	*L.monocytogenes*	*B.cereus*	*P.aeruginosa*	*E.coli*	*S.typhi*	*S.flexneri*	*C.albicans*
1a	6.25	6.25	12.5	12.5	6.25	12.5	6.25	12.5
1b	12.5	6.25	12.5	12.5	6.25	12.5	6.25	12.5
1c	12.5	6.25	12.5	6.25	6.25	12.5	12.5	12.5
1d	3.12	6.25	12.5	3.12	6.25	6.25	3.12	3.12
1e	3.12	3.12	3.12	3.12	3.12	3.12	3.12	6.25
1f	3.12	6.25	3.12	3.12	6.25	3.12	3.12	6.25
1g	3.12	3.12	12.5	3.12	6.25	3.12	6.25	6.25
2a	6.25	12.5	12.5	12.5	6.25	6.25	6.25	12.5
2b	12.5	3.12	6.25	12.5	6.25	12.5	12.5	12.5
2c	12.5	6.25	12.5	12.5	6.25	6.25	12.5	6.25
2d	3.12	6.25	12.5	3.12	6.25	3.12	6.25	3.12
2e	3.12	6.25	6.25	3.12	3.12	3.12	3.12	6.25
2f	3.12	3.12	3.12	3.12	6.25	3.12	3.12	12.5
2g	3.12	6.25	6.25	3.12	6.25	6.25	6.25	6.25
8a	12.5	12.5	6.25	12.5	6.25	6.25	12.5	6.25
8b	6.25	12.5	12.5	12.5	6.25	12.5	12.5	3.12
8c	12.5	6.25	6.25	6.25	12.5	6.25	12.5	6.25

8d	6.25	12.5	12.5	6.25	6.26	12.5	6.25	12.5
8e	12.5	12.5	6.25	6.25	12.5	3.12	6.25	12.5
8f	6.25	12.5	12.5	12.5	6.25	6.25	12.5	12.5
8g	12.5	6.25	6.25	6.25	6.25	3.12	12.5	12.5
Amoxicillin[b]	3.12	6.25	6.25	3.12	6.25	3.12	3.12	-
Fluconazole[c]	-	-	-	-	-	-	-	3.12

[a]Mean of the three replicates
[b]Amoxicillin was used as positive control for anti-bacterial activity
[c]Fluconazole was used as positive control for anti-fungal activity

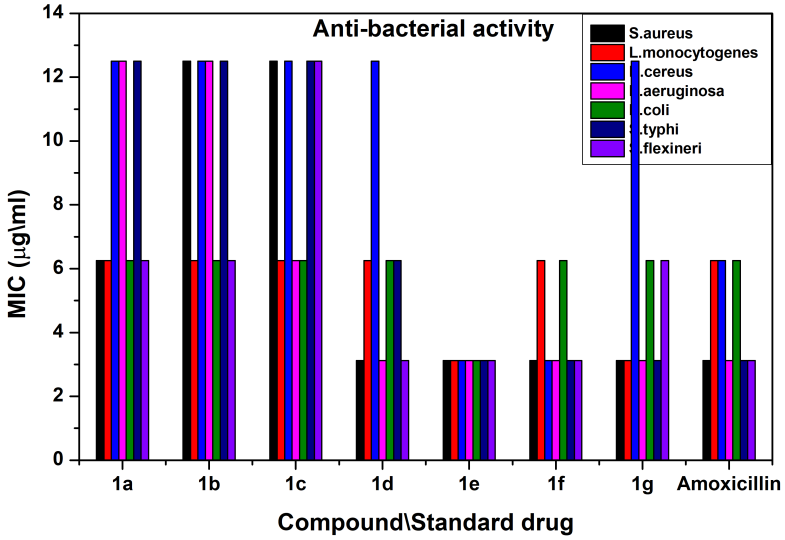


Fig. 3.9 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **1a-1g** and the standard drug Amoxicillin

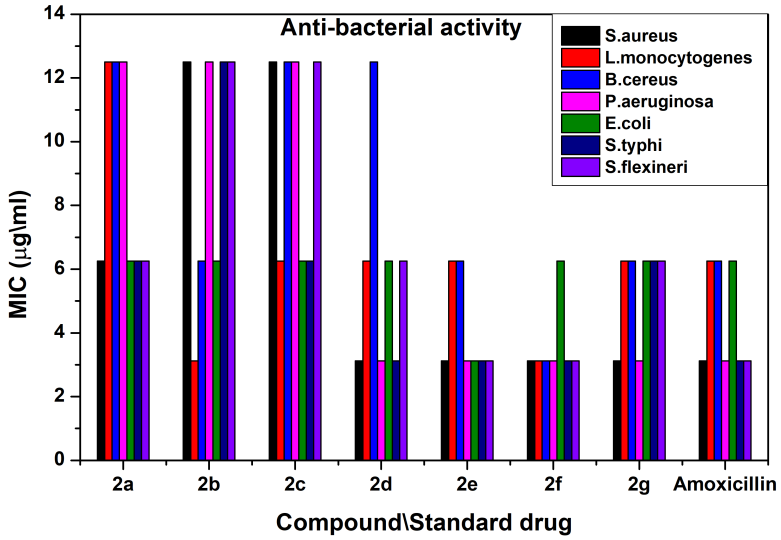


Fig. 3.10 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **2a-2g** and the standard drug Amoxicillin

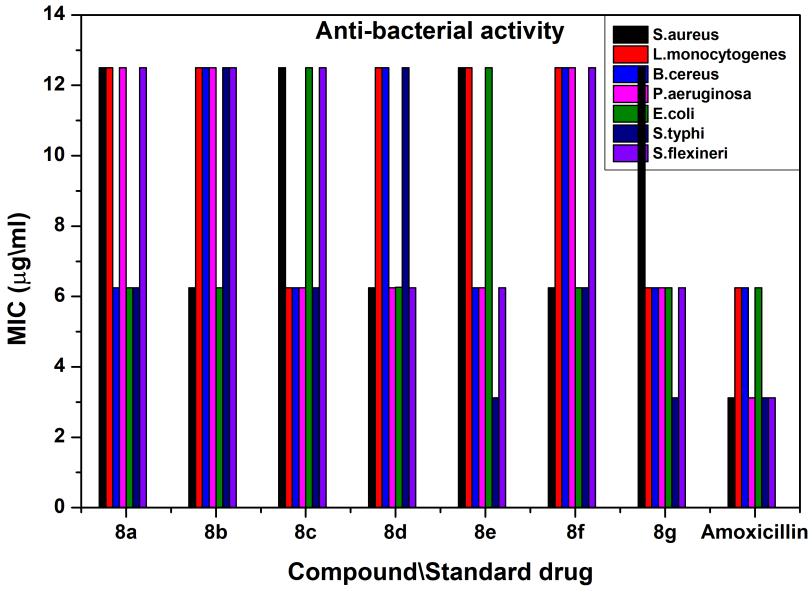


Fig. 3.11 Graphical comparison of anti-bacterial activity profile of precursors **8a-8g** and the standard drug Amoxicillin

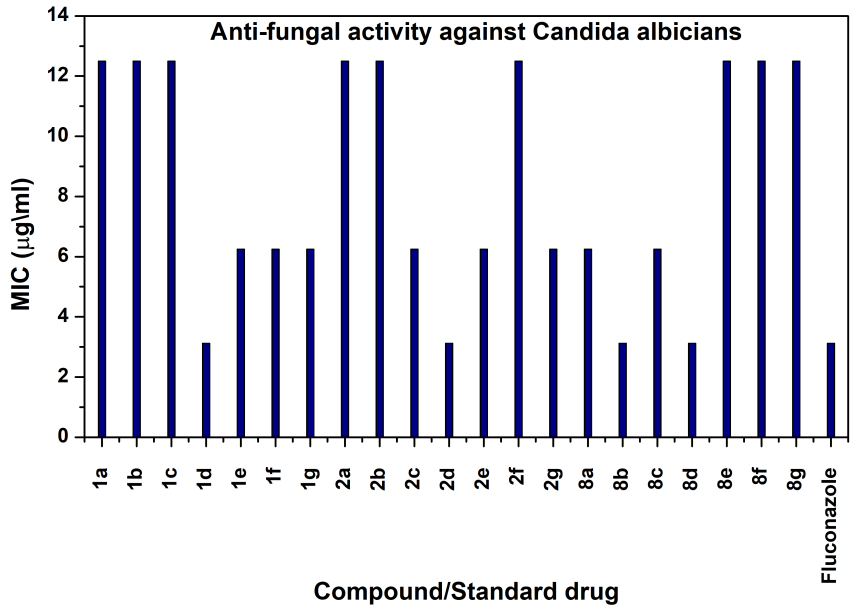


Fig. 3.12 Graphical comparison of anti-fungal activity profile of compounds **1a-1g**, **2a-2g**, **8a-8g** and the standard drug Fluconazole

Following Structure-Activity Relationship has been drawn from the above data:

1. Compounds containing 4-chloro and 4-bromo substituted phenyl group at C-5 position of 1,2,4-triazole ring were found to be exhibiting equivalent or two-fold better activities than Amoxicillin against all bacterial strains tested.

2. In overall, electron withdrawing groups on phenyl ring at C-5 position of 1,2,4-triazole ring led to enhanced activity of compounds against bacterial strains.

3. Compounds having hydrogen or electron donating groups on phenyl ring at C-5 position of 1,2,4-triazole ring were comparatively less potent anti-microbial agents.

4. 4-Fluoro substituted phenyl group at C-5 position of 1,2,4-triazoles led the compounds to exhibit highest anti-fungal activities, even more than the reference drug, Fluconazole.

5. Results depicted in table 3.1 prove that the conversion of precursors **8** to the target compounds **1** resulted in the increased anti-bacterial potential.

6. Among the precursors tested, **8b** possessed highest anti-microbial activity while precursor **8f** possessed lowest anti-microbial activity.

B. Anti-oxidant activity - results and discussion

Anti-oxidant agents are the powerful tools for controlling free radicals which are harmful to living beings as they can cause food spoilage, damage various biological systems and are particularly responsible for aging. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is a spectrophotometric method which is used to test free radical scavenging (anti-oxidant) activities of sample compounds. This test is very well known and widely used to evaluate anti-oxidant activities of new drug candidates. Newly synthesized target compounds **1a-1g**, **2a-2g** and the precursors **8a-8g** were evaluated for their anti-oxidant activities by DPPH free radical scavenging method using ascorbic acid as positive control. The experiments were done in triplicate for each substance and anti-oxidant activities were taken in terms of % RSA (radical scavenging activity) with respect to reference control values. The results are presented in table 3.2 summarizing the radical scavenging activities of all the compounds compared to ascorbic acid as standard. Graphical comparison of anti-oxidant profile of tested compounds with ascorbic acid (AA) is shown in Fig. 3.13.

Table 3.2 Anti-oxidant activities of compounds **1a-1g**, **2a-2g** and **8a-8g** using DPPH method

Compound	Anti-oxidant activity % RSA[a]	Compound	Anti-oxidant activity % RSA[a]
1a	88.72	**2e**	94.32
1b	86.45	**2f**	79.23
1c	87.43	**2g**	86.45
1d	95.34	**8a**	92.55
1e	92.34	**8b**	93.20
1f	94.30	**8c**	91.33
1g	93.56	**8d**	94.33
2a	87.34	**8e**	86.23

2b	88.23	**8f**	85.44
2c	89.23	**8g**	87.62
2d	87.94	**AA**	96.78

[a]Values were mean of three replicates % RSA, showed anti-oxidant potentials of the tested samples

From the above data it has been generalized that:

1. The results obtained depicts that most of the tested compounds have exhibited moderate to excellent anti-oxidant activity (86.45-95.34 %).

2. 4-Fluoro-phenyl substituted compound **1d** was found to be the most potent anti-oxidant agent (95.34 %) among all the tested compounds.

3. Compound **2f** was comparatively poor anti-oxidant agent with 79.23 % scavenging activity.

4. Among the precursors, **8d** possessed highest anti-oxidant activity i.e. 94.33 %.

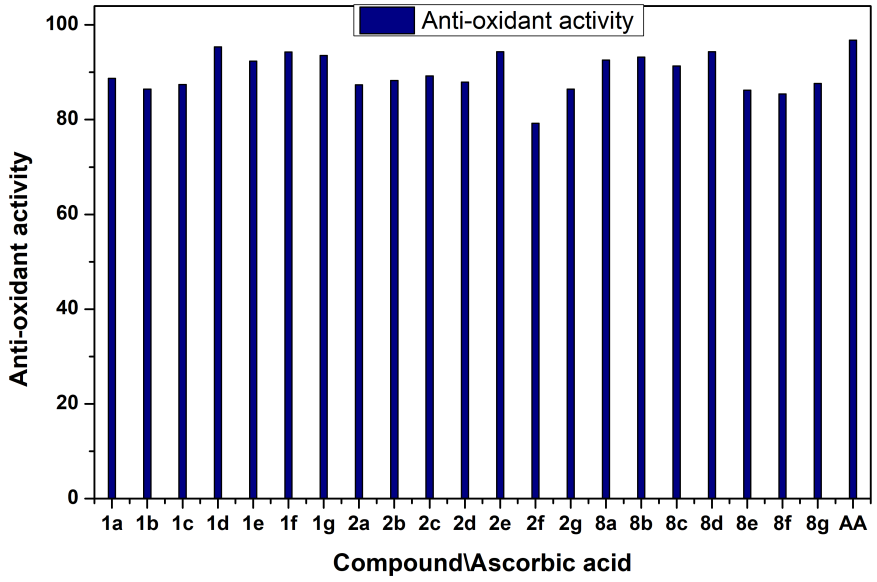


Fig. 3.13 Graphical comparison of anti-oxidant activity profile of compounds **1a-1g**, **2a-2g**, **8a-8g** and ascorbic acid (AA)

3.3.4 Cytotoxicity

Cytotoxicity was also tested for the synthesized compounds **1a-1g**, **2a-2g** and **8a-8g** against one mammalian cell line (mouse fibroblast cell) and one plant seed germination cell line (vigna radiate seeds) at very high concentration of 1 mg/mL using MTT assay. The results of the cytotoxic studies against mouse fibroblast cell line are presented in table 3.3. It can be seen from cytotoxicity screening that after 24 hrs of exposure time, all the compounds were found to be safe to mammalian cells with 89.6-99.5 % cell viability even at much higher doses of the corresponding MICs. Also, all the tested compounds were 100 % safe to plant seed germination.

Table 3.3 *In vitro* cytotoxic studies of compounds **1a-1g**, **2a-2g** and **8a-8g** against normal cells at the concentration of 1 mg/mL

Compound	Mouse fibroblast cell Cell Viability %[a]	Compound	Mouse fibroblast cell Cell Viability %[a]
1a	92.7	**2e**	94.3
1b	89.6	**2f**	91.4
1c	94.8	**2g**	90.9
1d	99.1	**8a**	92.7
1e	98.9	**8b**	89.6
1f	94.2	**8c**	94.8
1g	95.2	**8d**	99.1
2a	92.7	**8e**	98.9
2b	99.5	**8f**	94.2
2c	94.3	**8g**	95.2
2d	98.1	**DMSO**	76.8

[a]Mean of three replicates, showed the viability percentage on challenged with the tested compounds as compared to the control case

3.4 Conclusion

A library of fourteen new derivatives of 1,2,3-triazole tethered 1,2,4-triazoles **1** and **2** was designed and synthesized successfully in moderate to good yields *via* the click chemistry reactions and structures of final compounds were confirmed by their spectral data (^{1}H NMR, ^{13}C NMR and IR) and HRMS data. *In vitro* anti-microbial activity was evaluated for all the newly synthesized compounds **1** and **2** as well as precursors **8** against three Gram-positive and four Gram-negative bacterial strains and one pathogenic fungal strain. All the tested compounds exhibited good to excellent anti-bacterial and anti-fungal activities when compared with reference drugs. Compounds **1e**, **1f**, **2e** and **2f** were found equivalent or better than reference drug as anti-bacterial agent while **1d** and **2d** were most efficient among all the newly synthesized compounds against tested fungal strain. Results showed that the conversion of precursors **8** to target compound **1** and **2** cause higher inhibition potential against bacterial strains tested. Anti-oxidant profile was also tested for all the synthesized compounds and most of them were found moderate to excellent free radical scavengers. Compound **1d** was found to possess highest anti-oxidant activity with 94.35 % control values. Further cytotoxic studies revealed that newly synthesized compounds are safe against plant seed germination cell line as well as mouse fibroblast cell line with 100 % and 89.65-99.56 % cell viability values, respectively. It can be concluded from the results obtained that 1,2,3-triazole tethered 1,2,4-triazole derivatives have the potential for their further exploration as biological agents.

3.5 Experimental Section

All glassware were used after solvent wash and drying in an oven for 12 hrs. Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silicagel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. The infrared (IR) spectra were recorded on FT-IR Perkin Elmer Spectrophotometer, CIL,

JCBUST, YMCA, Faridabad. ^{1}H and ^{13}C NMR data were recorded on Bruker Advance Neo 500 MHz NMR Spectrometer and AVH D 500 AVANCE III HD 500 MHz OneBay NMR Spectrometer in pure DMSO-d_6 using TMS as internal reference. Mass data were recorded on SYNAPT-XS#DBA064 mainframe. The reference values for the residual solvent were taken as δ = 2.50 ppm (DMSO-d_6) for ^{1}H NMR, δ = 39.54 ppm (DMSO-d_6) for ^{13}C NMR. The abbreviations: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet and ex = exchangeable protons are used for NMR assignments.

3.5.1 Synthesis of aminotriazoles 7a-7g

To the solution of carboxylic acid **3** in methanol was added few drops of conc. H_2SO_4 and refluxed for 24 hrs. After complete conversion of carboxylic acid **3** to corresponding ester **4** (as indicated by TLC), hydrazine hydrate was added in excess and reaction mixture was further refluxed for 20 hrs. Cooled the reaction mixture to the room temperature and evaporated the solvent. Washed the solid obtained and recrystallized from ethanol. To the stirring solution of the above obtained solid in ethanol was added carbon disulfide (1.5 equivalents) and potassium hydroxide (1.0 equivalent) at 0 ºC and stirred it for 24 hrs at room temperature. Filtered the salt obtained and washed with diethyl ether. To the solution of the salt in water was added hydrazine hydrate in excess and refluxed for 20 hrs. Cooled the reaction mixture, added ice-cold water and neutralized the excess hydrazine hydrate with glacial acetic acid. Filtered the solid obtained, dried and recrystallized from ethanol to afford the pure product **7**. Formation of all the desired compounds was confirmed by comparing their melting points with literature values.

3.5.2 Synthesis of propargylated aminotriazoles 8a-8g

To the solution of compound **7** in ethanol was added propargyl bromide (1.5 equivalents) and triethylamine (1.5 equivalents). The reaction mixture was then refluxed for 3 to 4 hrs resulting in the formation **8** as pure product. The reaction mixture was allowed to cool at room temperature followed by addition of crushed ice. A solid were precipitated out which was filtered off, washed with excess of water, dried, and recrystallized from ethanol. Formation of desired products were confirmed by comparing their melting point

with literature values.

3.5.3 General method for the synthesis of target compounds 1a-1g and 2a-2g

To the solution of compound **8/9** in tert-butanol and water (1:1) added 4-azido benzenesulfonamide under stirring condition followed by addition of coppersulfate pentahydrate (0.6 equivalent) and sodium ascorbate (0.4 equivalent). Stirred the reaction mixture for 3-4 hrs at 70 ºC. After the completion of reaction, as monitored by TLC, added ice-cold water to the reaction mixture. Filtered the solid obtained and washed with water. Recrystallized the product from ethanol to obtain pure product **1/2**.

4-(4-(((4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)-benzenesulfonamide (1a)

White solid; Yield: 62 %; mp 258-260 ºC; IR (KBr) ν_{max} 3343, 3262, 3131, 2986, 1598, 1509, 1311, 1156, 612, 549; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.89 (1H, s, triazole H), 8.08-8.02 (5H, m, Ar), 7.95 (2H, brs, Ar), 7.55 (2H, ex, s, SO_2NH_2), 7.48 (2H, brs, Ar), 6.20 (2H, s, NH_2) 4.66 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 126 MHz,) δ (ppm) 159.17, 144.96, 143.89, 138.27, 129.83, 128.32, 127.94, 127.41, 126.12, 122.23, 120.31, 25.62; HRMS (ESI) m/z calcd for $C_{17}H_{16}N_8O_2S_2$ $[M+H]^+$ 429.0915, found 429.0894.

4-(4-(((4-amino-5-(*p*-tolyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (1b)

White solid; Yield: 67 %; mp 240-242 ºC; IR (KBr) ν_{max} 3356, 3263, 3138, 3066, 2986, 1598, 1333, 1155, 620, 550; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.85 (1H, s, triazole H), 8.09 (2H, d, J = 8.0 Hz, Ar), 8.01 (2H, d, J = 8.0 Hz, Ar), 7.87 (2H, d, J = 7.5 Hz, Ar), 7.51 (2H, ex, s, SO_2NH_2), 7.31 (2H, d, J = 7.0 Hz, Ar), 6.13 (2H, s, NH_2), 4.60 (2H, s, CH_2S), 2.35 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 160.65, 144.94, 143.93, 139.57, 138.43, 128.98, 127.84, 127.53, 123.64, 122.22, 120.34, 25.78, 20.93; HRMS (ESI) m/z calcd for $C_{18}H_{18}N_8O_2S_2$ $[M+H]^+$ 443.1072, found

443.1053.

4-(4-(((4-amino-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (1c)

White solid; Yield: 71 %; mp 250-252 ºC; IR (KBr) ν_{max} 3363, 3281, 3126, 2943, 1611, 1331, 1155, 611, 547; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.88 (1H, s, triazole H), 8.07 (2H, brs, Ar), 8.02 (2H, d, J = 8.0 Hz, Ar), 7.90 (2H, brs, Ar), 7.54 (2H, ex, s, SO_2NH_2), 7.03 (2H, brs, Ar) 6.14 (2H, s, NH_2), 4.62 (2H, s, CH_2S), 3.77 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 160.61, 145.05, 144.08, 138.33, 138.29, 129.89, 128.02, 127.51, 126.21, 122.30, 120.38, 113.78, 55.25, 25.96; HRMS (ESI) m/z calcd for $C_{18}H_{18}N_8O_3S_2$ $[M+H]^+$ 459.1021, found 459.0999.

4-(4-(((4-amino-5-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (1d)

White solid; Yield: 70 %; mp 210-212 ºC; IR (KBr) ν_{max} 3399, 3383, 3133, 2981, 1598, 1329, 1158, 620, 546; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.78 (1H, s, triazole H), 8.04 (2H, d, J = 17 Hz, Ar), 8.00-7.98 (4H, m, Ar), 7.52 (2H, ex, s, SO_2NH_2), 7.34 (2H, t, $^{3}J_{H-F}$ & $^{3}J_{H-H}$ = 8.0 Hz, Ar), 6.12 (2H, s, NH_2), 4.56 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 164.43, 162.47, 144.20, 139.06, 134.23, 130.75, 130.69, 128.10, 124.43, 123.60, 120.93, 116.25, 116.07, 26.35; HRMS (ESI) m/z calcd for $C_{17}H_{15}FN_8O_2S_2$[M+H]$^+$ 447.0821, found 447.0796.

4-(4-(((4-amino-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (1e)

White solid; Yield: 69 %; mp 230-240 ºC; IR (KBr) ν_{max} 3399, 332, 3138, 3066, 2986, 1598, 1333, 1154, 610, 550; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.86 (1H, s, triazole H), 8.10 (2H, d, J = 8.5 Hz, Ar), 8.03 (2H, d, J = 8.0 Hz, Ar), 8.00 (2H, d, J = 8.5 Hz, Ar), 7.59 (2H, d, J = 7.0 Hz, Ar), 7.52 (2H, ex, s, SO_2NH_2), 6.19 (2H, s, NH_2) 4.60 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 144.83, 143.87, 138.48, 138.29, 130.88, 129.75, 128.07, 127.52, 126.21, 125.80, 121.55, 120.32, 25.71; HRMS (ESI) m/z calcd for $C_{17}H_{15}ClN_8O_2S_2$ $[M+H]^+$/ $[M+H+2]^+$ 463.05262/465.0497, found

463.0 458/465.0446.

4-(4-(((4-amino-5-(4-bromophenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (1f)

White solid; Yield: 74 %; mp 220-222 ºC; IR (KBr) ν_{max} 3365, 3131, 2942, 1598, 1331, 1155, 619, 550, 505; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.89 (1H, s, triazole H), 8.05 (6H, m, Ar), 7.53 (2H, s, SO_2NH_2), 7.33 (2H, m, Ar), 6.18 (2H, s, NH_2), 4.64 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 163.77, 161.80, 143.79, 138.28, 130.14, 130.08, 127.36, 122.98, 122.19, 120.22, 115.50, 115.33, 25.57; HRMS (ESI) m/z calcd for $C_{17}H_{15}BrN_8O_2S_2$ $[M+H]^+/[M+H+2]^+$ 507.0021/509.0001, found 507.0010/508.9988.

4-(4-amino-5-(((1-(4-sulfamoylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-4*H*- 1,2,4-triazol-3-yl)benzenesulfonamide (1g)

White solid; Yield: 61 %; mp 245-247 ºC; IR (KBr) ν_{max} 3365, 3327, 3275, 3148, 3073, 2981, 1596, 1314, 1164, 623, 591; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.86 (1H, s, triazole H), 8.19 (2H, d, *J* = 8.0 Hz, Ar), 8.10 (2H, d, *J* = 8.5 Hz, Ar), 8.01 (2H, d, *J* = 8.5 Hz, Ar), 7.94 (2H, d, *J* = 8.0 Hz, Ar), 7.51 (2H, ex, s, SO_2NH_2), 7.47 (2H, ex, s, SO_2NH_2), 6.23 (2H, s, NH_2), 4.62 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 160.06, 144.78, 143.82, 138.46, 129.76, 128.56, 128.00, 127.48, 125.76, 122.16, 120.27, 25.66; HRMS (ESI) m/z calcd for $C_{18}H_{18}N_8O_2S_2$ $[M+H]^+$ 443.1072, found 443.1053.

4-(4-(((5-phenyl-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (2a)

White solid; Yield: 68 %; mp 210-212 ºC; IR (KBr) ν_{max} 3356, 3278, 3148, 2984, 1596, 1332, 1162, 604, 545; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.83 (1H, s, triazole H), 8.09 (2H, d, *J* = 8.5 Hz, Ar), 8.01 (2H, d, *J* = 8.5 Hz, Ar), 7.93 (2H, d, *J* = 8.0 Hz, Ar), 7.61 (2H, d, *J* = 7.5 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.52 (2H, ex, s, SO_2NH_2), 7.41-7.36 (5H, m, Ar), 4.58 (2H, s, SCH_2); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 160.06, 144.41, 137.96, 136.91, 130.57, 130.12, 129.24, 129.01, 128.69, 128.06, 127.81,

126.80, 122.89, 122.81, 120.93, 120.88, 27.46; HRMS (ESI) m/z calcd for $C_{23}H_{20}N_8O_4S_3$ $[M+H]^+$ 569.0847, found 569.0832.

4-(3-(((1-(4-sulfamoylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-5-(*p*-tolyl)-4*H*-1,2,4-triazol-4-yl)benzenesulfonamide (2b)

White solid; Yield: 70 %; mp 238-240 ºC; IR (KBr) ν_{max} 3334, 3066, 2979, 1328, 1597, 1332, 1159, 609, 550; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.73 (1H, s, triazole H), 8.03 (2H, brs, Ar), 8.00 (2H, brs, Ar), 7.91 (2H, d, J = 7.0 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.53 (4H, s, SO_2NH_2 & Ar), 7.20 (2H, brs, Ar), 7.16 (2H, brs, Ar), 4.53 (2H, s, CH_2S), 2.25 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 145.49, 144.13, 140.54, 138.87, 136.82, 135.08, 131.10, 129.74, 128.88, 128.53, 128.00, 127.68, 123.53, 122.71, 120.84, 27.33, 21.20; HRMS (ESI) m/z calcd for $C_{24}H_{22}N_8O_4S_3$ $[M+H]^+$ 583.1004, found 583.0982.

4-(4-(((5-(4-methoxyphenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)-methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (2c)

White solid; Yield: 65 %; mp 235-237 ºC; IR (KBr) ν_{max} 3344, 3253, 3080, 2979, 1610, 1333, 1159, 617, 547; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.85 (1H, s, triazole H), 8.08 (2H, brs, Ar), 8.01 (2H, d, J = 8.0 Hz, Ar), 7.94 (2H, d, J = 8.5 Hz, Ar), 7.60 (2H, d, J = 8.5 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.54 (2H, ex, s, SO_2NH_2), 7.28 (2H, d, J = 7.5 Hz, Ar), 6.90 (2H, brs, Ar), 4.60 (2H, s, CH_2S), 3.70 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 161.02, 145.85, 144.91, 144.51, 138.89, 136.90, 133.84, 132.00, 130.37, 129.06, 128.05, 127.80, 124.18, 122.90, 120.91, 114.68, 55.76, 27.44; HRMS (ESI) m/z calcd for $C_{24}H_{22}N_8O_5S_3$ $[M+H]^+$ 599.0953, found 599.0931.

4-(4-(((5-(4-fluorophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (2d)

White solid; Yield: 72 %; mp 210-212 ºC; IR (KBr) ν_{max} 3368, 3272, 3138, 3061, 2988, 1596, 1334, 1162, 621, 588, 552; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.82 (1H, s, triazole H), 8.08 (2H, d, J = 9.0 Hz, Ar), 8.00 (2H, d, J = 9.0 Hz, Ar), 7.93 (2H, d, J = 7.5 Hz, Ar), 7.60 (2H, d, J = 80 Hz, Ar), 7.56 (2H, ex, s, SO_2NH_2), 7.52 (2H, ex, s,

SO_2NH_2), 7.42 (2H, dd, $^3J_{H-H}$ & $^4J_{H-F}$ = 7.5 Hz & 5.5 Hz, Ar), 7.23 (2H, t, $^3J_{H-F}$ & $^3J_{H-H}$ = 8.0 Hz, Ar), 4.57 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 161.28, 145.03, 144.28, 140.48, 135.48, 130.81, 130.75, 128.99, 128.56, 127.62, 127.41, 120.45, 116.07, 115.90, 27.25; HRMS (ESI) m/z calcd for $C_{23}H_{19}FN_8O_4S_3$ $[M+H]^+$ 587.0753, found 587.0737.

4-(4-(((5-(4-chlorophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (2e)

White solid; Yield: 68 %; mp 215-217 ºC; IR (KBr) ν_{max} 3346, 3264, 3068, 2979, 1597, 1332, 1162, 621, 543; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.89 (1H, s, triazole H), 8.07 (2H, brs, Ar), 8.02 (2H, d, *J* = 6.0 Hz, Ar), 7.94 (2H, brs, Ar), 7.63 (2H, brs, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.54 (2H, ex, s, SO_2NH_2), 7.43 (2H, brs, Ar), 7.37 (2H, brs, Ar), 4.66 (2H, s); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 161.48, 146.02, 144.70, 138.76, 136.55, 135.70, 131.39, 130.74, 129.36, 129.03, 128.07, 127.87, 125.55, 121.00, 26.28; HRMS (ESI) m/z calcd for $C_{23}H_{19}ClN_8O_4S_3$ $[M+H]^+$/$[M+H+2]^+$ 603.04 58/605.0429, found 603.0443/605.0427.

4-(4-(((5-(4-bromophenyl)-4-(4-sulfamoylphenyl)-4*H*-1,2,4-triazol-3-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (2f)

White solid; Yield: 65 %; mp 222-224 ºC; IR (KBr) ν_{max} 3368, 3272, 3138, 2986, 1596, 1329, 1163, 621, 588; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.88 (1H, s, triazole H), 8.07 (2H, brs, Ar), 8.03 (2H, brs, Ar), 7.95 (2H, d, *J* = 7.0 Hz, Ar), 7.63 (2H, brs, Ar), 7.57 (2H, brs, Ar), 7.54 (2H, ex, s, SO_2NH_2), 7.31 (2H, brs, Ar), 4.65 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 145.35, 144.34, 143.90, 138.17, 135.77, 131.58, 130.21, 128.32, 127.40, 127.21, 123.80, 122.34, 120.29, 26.84; HRMS (ESI) m/z calcd for $C_{23}H_{19}BrN_8O_4S_3$ $[M+H]^+$/$[M+H+2]^+$ 646.9953/648.9933, found 647.0032/648.9984.

4,4′-(5-(((1-(4-sulfamoylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-4*H*-1,2,4-triazole-3,4-diyl)dibenzenesulfonamide (2g)

White solid; Yield: 60 %; mp 230-232 ºC; IR (KBr) ν_{max} 3365, 3284, 3068, 2986, 1596,

1330, 1160, 607, 547; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 8.83 (1H, s, triazole H), 8.08 (2H, d, J = 8.0 Hz, Ar), 8.01 (2H, d, J = 9.0 Hz, Ar), 7.95 (2H, d, J = 8.5 Hz, Ar), 7.78 (2H, d, J = 8.5 Hz, Ar), 7.64 (2H, d, J = 8.0 Hz, Ar), 7.57 (2H, ex, s, SO_2NH_2), 7.54 (2H, d, J = 8.0, Ar), 7.52 (2H, ex, s, SO_2NH_2), 7.44 (2H, brs, Ar), 4.60 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 160.75, 145.96, 145.58, 144.39, 138.98, 136.58, 135.46, 129.83, 129.29, 128.98, 128.06, 127.92, 126.47, 122.86, 120.89, 27.37; HRMS (ESI) m/z calcd for $C_{23}H_{21}N_9O_6S_4$ $[M+H]^+$ 648.05759, found 648.0534.

CHAPTER 4

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,3,4-OXADIAZOLE INCORPORATED 1,2,3-TRIAZOLE DERIVATIVES AS ANTI-CANCER, ANTI-MICROBIAL AND ANTI-OXIDANT AGENTS

4.1 Motivation for the Current Work

Cancer is a complex disease involving abnormal, rapid and uncontrollable cell division in a part of the body with the capacity to spread to the other parts also violating the principles of normal cell division [158] and has been a serious health problem leading to huge number of deaths worldwide [159]. The most common types of cancer in males are colorectal cancer, prostate cancer, stomach cancer and lung cancer etc. while in females are lung cancer, colorectal cancer, breast cancer and cervical cancer etc. [160]. A lot of chemotherapeutic agents have been developed to treat cancer by directly attacking the synthesis of DNA, thereby restricting proliferation, and spread of cancer cells [161]. With the time, cancer cells have developed toxicity and drug resistance

against chemotherapeutic agents due to their over-use and mis-use. Increasing drug resistance make the available drugs less effective causing the need of developing more effective and non-toxic anti-cancer agents [162].

Nitrogen containing heterocycles and their derivatives have continuously been explored as important biological scaffold and are pivotal in medicinal chemistry [163]. Triazoles and oxadiazoles are such a class of heterocycles possessing wide array of biological actions such as anti-urease [164], analgesic [165], anti-cancer [166], anti-microbial [167], anti-malarial [168], anti-convulsant [169], anti-oxidant [170], anti-viral [171], anti-tubercular [172], anti-inflammatory [173], anti-depressant [174] activities etc. In the last few years, these heterocycles have emerged as key scaffolds in the synthesis of anti-cancer agents [175]. Compounds having 1,2,3-triazole in their molecular architectures and exerting anti-cancer effect are well reported in literature [176]. CAI is an example of 1,2,3-triazole containing anti-cancer agents [177] (Fig. 4.1). Furthermore, various reports on anti-micorbial and anti-oxidant activities of 1,2,3-triazoles are also reported in literaure. Triazoles are present in various marketed drugs such as alprazolam, trapidil and tazobactam. Oxadiazole is another important class of heterocyclic compounds possessing anti-cancer activity [178]. The mechanism behind the anti-cancer effect of oxadiazole ring is its potential to inhibit different enzymes, growth factors and kinases [179]. Zibotentan, a very well-known anti-cancer drug, contains 1,3,4-oxadiazole ring in its structure (Fig. 4.1) [180]. Along with the anti-cancer behaviour, these are reported in literature as potent anti-microbial and anti-oxidant agents [144] also and are present in various marketed drugs. Furamizole is a very well known drug having oxadiazole ring in its structure (Fig. 4.1).

Further, sulfonamides are a prominent class of biologically active compounds. Due to the tendency to form hydrogen bonds, sulfonamide group has evoked as isostere of carboxylic group in medicinal chemistry avoiding some drawbacks of carboxylic acid such as limited passive diffusion, metabolic instability and toxicity [181]. Various sulfonamide derivatives have been reported in literature possessing anti-thyroid [182], anti-microbial [183], antitumor [184], carbonic anhydrase inhibition [185] activities. Bosentan, belinostat, amsacrine are some of the examples of sulfonamide containing anti-cancer drugs [181] (Fig. 4.1).

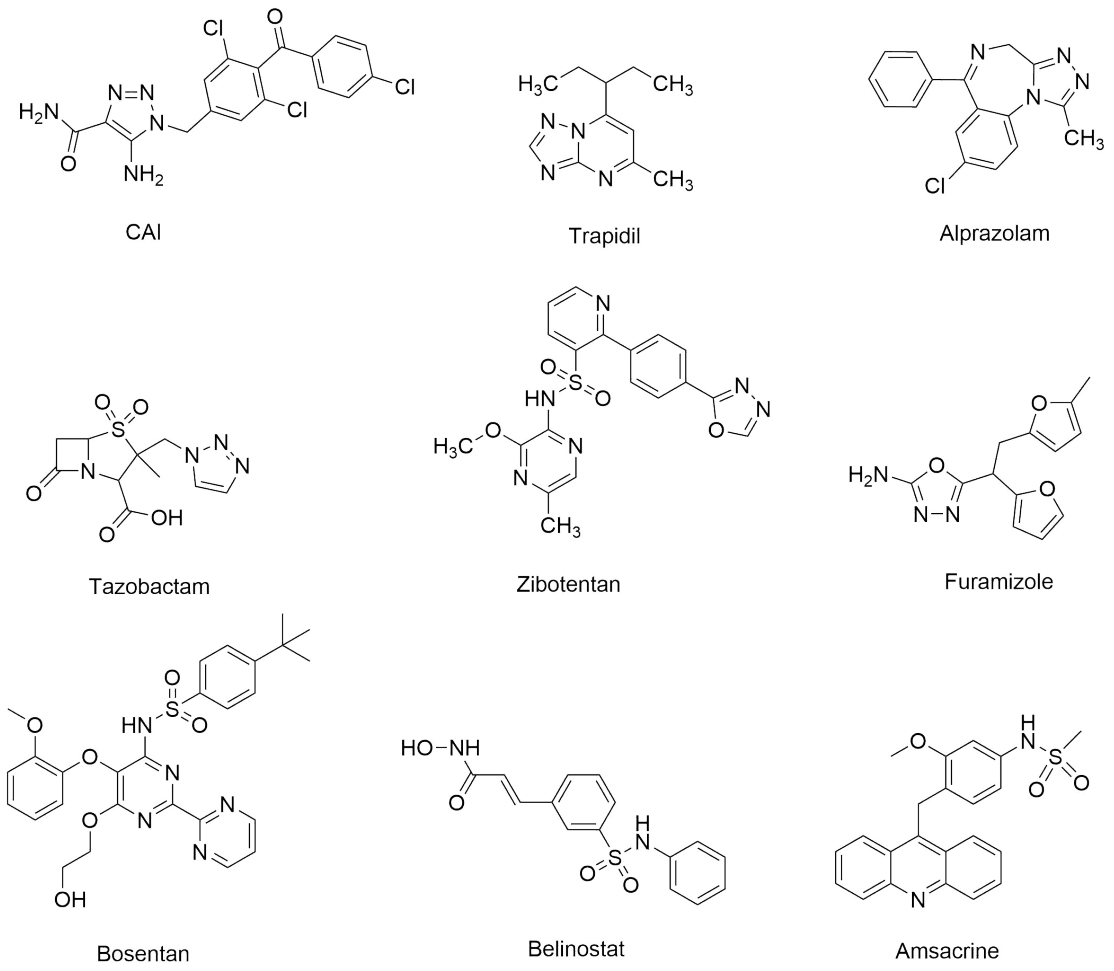


Fig. 4.1 Structure of drugs having triazole/oxadiazole/sulfonamide group

Based on the above studies, we hypothesized that combining structural features of 1,2,3-triazole and 1,3,4-oxadiazole motifs with benzenesulfonamide group may lead to the development of drug candidates with multiple biological activities. In this chapter, we designed and synthesized twenty-one novel benzenesulfonamide incorporating 1,2,3-triazole and 1,3,4-oxadiazole hybrids **1**, **2** and **3** *via* click chemistry. The newly synthesized compounds **1**, **2**, **3** and their precursors **8** were further evaluated as potent anti-cancer agents (Fig. 4.2). Anti-microbial, anti-oxidant and cell cytotoxicity profiles were also tested for the newly synthesized compounds **1**, **2**, **3** and precursors **8**.

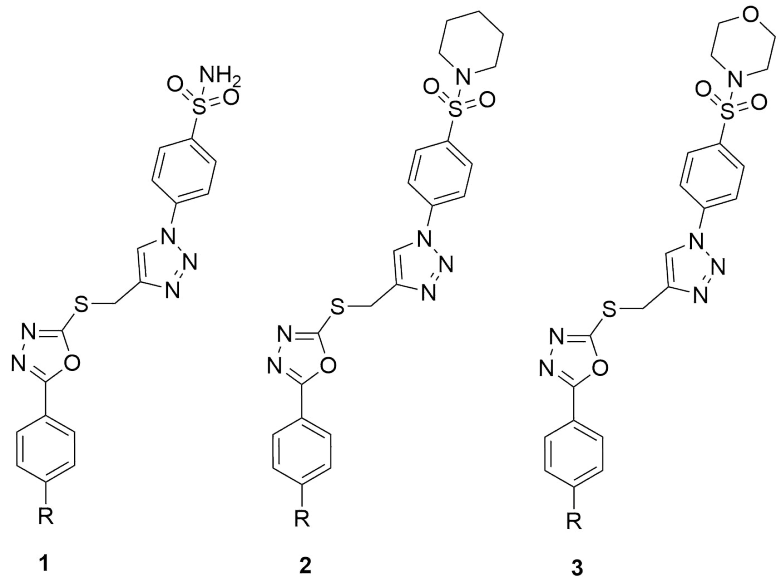


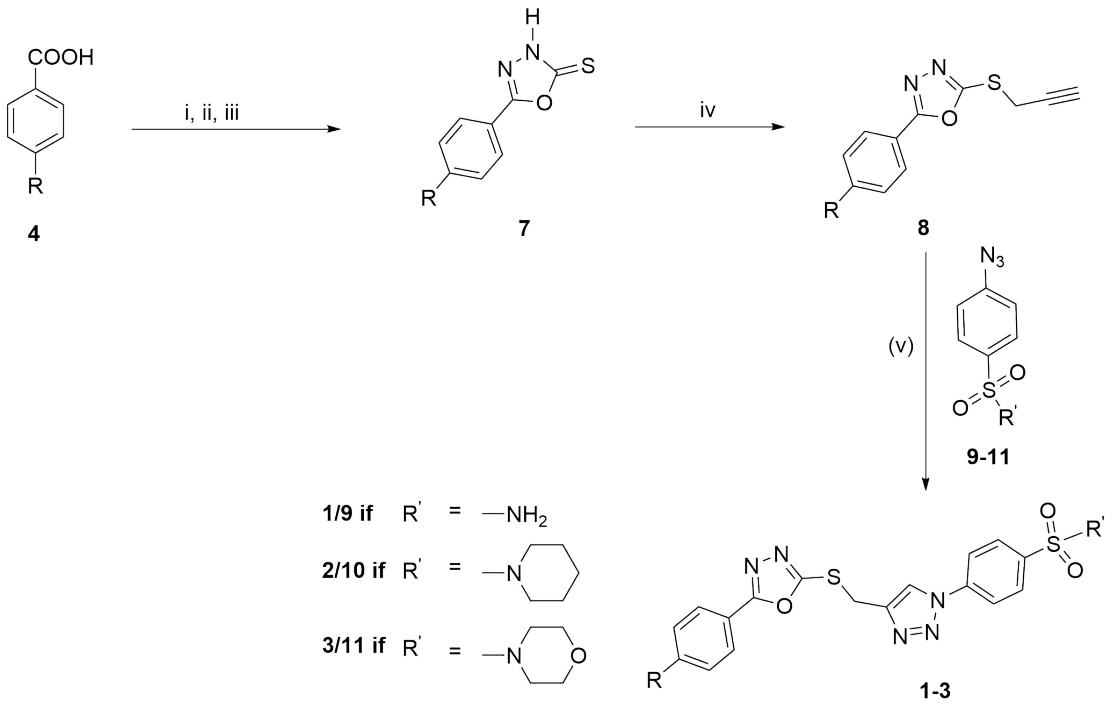
Fig. 4.2 1,3,4-Oxadiazole incorporated 1,2,3-triazole derivatives **1**, **2** and **3**

4.2 Results and Disscusion

4.2.1 Synthesis overview of 1,3,4-oxadiazole incorporated 1,2,3-triazole derivatives 1a-1g, 2a-2g and 3a-3g

The procedure adopted for the synthesis of target compounds **1, 2** and **3** is depicted in scheme 4.1. Structures of all the target compounds were confirmed by their spectral data (NMR, IR and HRMS data).

Key intermediates for the synthesis of target compounds **1**, **2** and **3** were oxadiazoles **7** which were synthesized using a sequence of reactions starting from aryl carboxylic acids **4**. The propargylation of compounds **7** with propargyl bromide in the presence of triethylamine as organic base yielded **8**. Having **8** in hand, target compounds **1, 2** and **3** were obtained using click chemistry reactions of **8** in the presence of coppersulfate pentahydrate and sodium ascorbate with different aromatic azides **9**, **10** and **11**, respectively. Aromatic azides **9**, **10** and **11** were synthesized using the methodology reported by Batra *et al.* [58]. Stepwise detailed discussion of the synthesis of target compounds **1a-1g**, **2a-2g**, and **3a-3g** is given below.



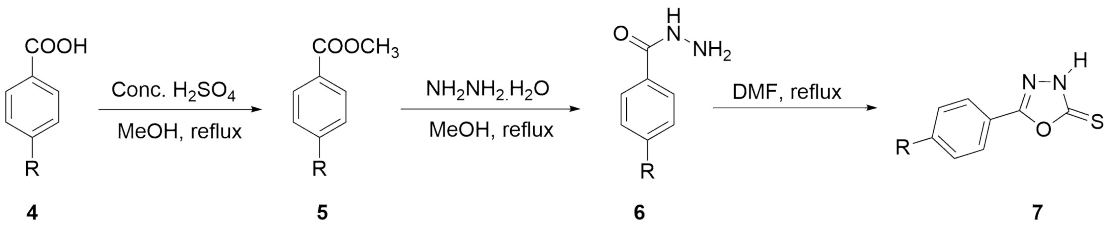
Compound 1/2/3/4/7/8	a	b	c	d	e	f	g
R	H	CH_3	OCH_3	F	Cl	Br	SO_2NH_2

Scheme 4.1 Synthesis of target compounds **1a-1g**, **2a-2g** and **3a-3g**

Reagents and conditions: (i) Methanol, H_2SO_4, reflux; (ii) Hydrazine hydrate, reflux; (iii) Dimethylformamide, CS_2, reflux; (iv) Propargyl bromide, TEA, ethanol, reflux; (v) $CuSO_4.5H_2O$, Sodium ascorbate, $^tBuOH : H_2O$ (1:1)

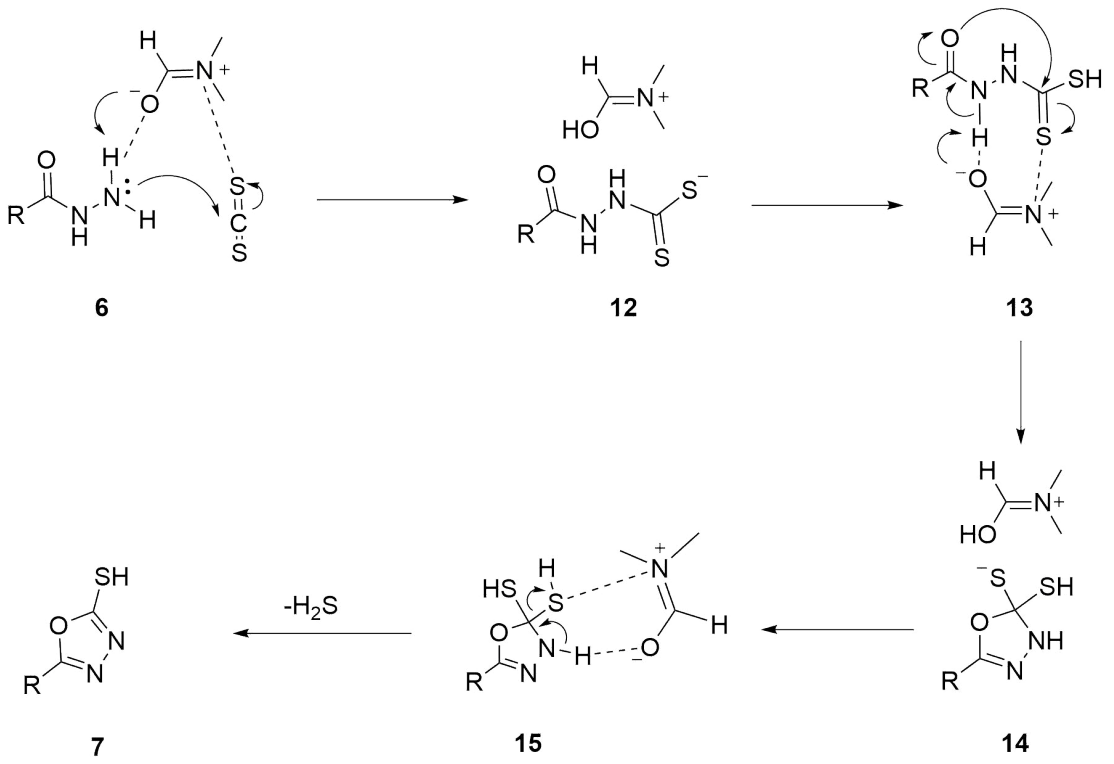
4.2.2 Synthesis of 1,3,4-oxadiazoles 7a-7g

The synthetic pathway for oxadiazoles **7** starts with the esterification of aromatic acids **4**. Aromatic acids **4** upon refluxing in methanol with few drops of conc. sulfuric acid for 8-10 hrs resulted in the formation of methyl esters **5**. Ester derivatives **5** upon refluxing with excess of hydrazine hydrate for 4-5 hrs led to their conversion to corresponding aryl hydrazides **6** as product. Aryl hydrazides **6** were treated with carbondisulfide in DMF for 1 h at room temperature and further refluxed for 2-3 hrs to yield 1,3,4-oxadiazoles **7** (Scheme 4.2).



Scheme 4.2 Synthesis of 1,3,4-oxadiazoles **7**

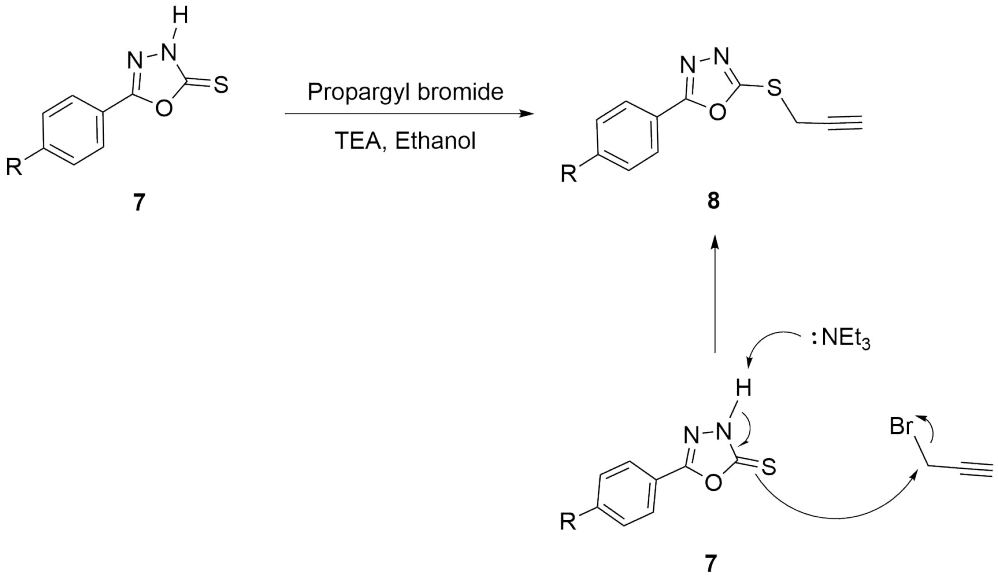
The mechanism involved in the complete conversion of aryl hydrazide derivatives **6** to corresponding 3-mercapto-1,3,4- oxadiazoles **7** is depicted in scheme 4.3. The first step of mechanism involves the nucleophilic attack of lone pair of nitrogen of hydrazide to electrophilic carbon of carbon disulfide followed by the deprotonation by N,N-dimethylformamide (DMF) leading to the formation of dithiocarbazinate salts **12**. The obtained dithiocarbazinate salts **12** get converted to the thiocarboxylic acids **13** by the abstraction of hydrogen from DMF. In the next step, abstraction of hydrogen from **13** by the nucleophilic attack of DMF followed by subsequent cyclization by C-O bond formation occur resulting into the formation of **14**. Now, the intermediate **14** obtained abstracts proton from DMF giving intermediate **15** which upon cyclocondensation results into the formation of corresponding 3-mercapto-1,3,4-oxadiazoles **7** .



Scheme 4.3 Mechanism for the formation of 1,3,4-oxadiazoles **7**

4.2.3 Synthesis of propargylated oxadiazoles 8a-8g

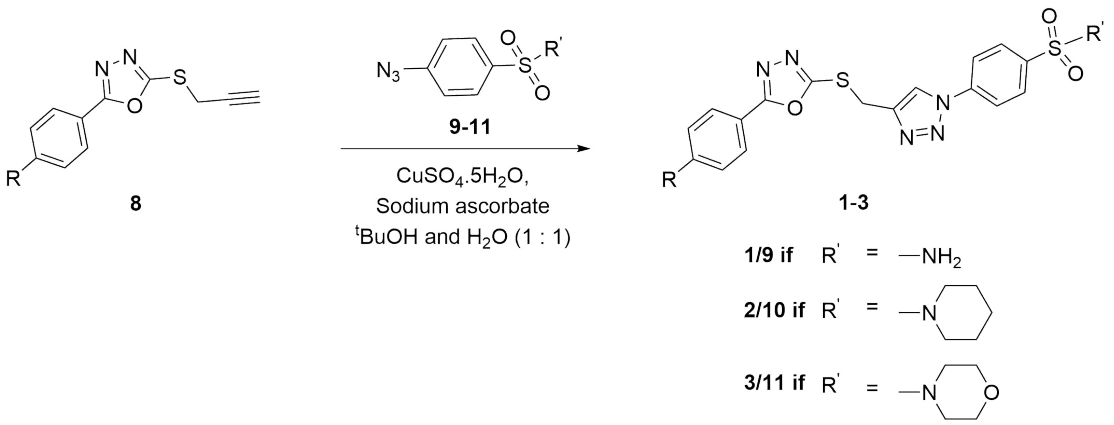
For the synthesis of propargylated oxadiazoles **8**, 1,3,4-oxadiazoles **7** were reacted with propargyl bromide in presence of triethyl amine as a base and ethanol as solvent. Completion of reaction required refluxing the reaction mixture for 2-3 hrs (Scheme 4.4). The mechanism of conversion of oxadiazoles **7** to propargylated oxadiazoles **8** involves the abstraction of a proton by triethylamine base and subsequent C-S bond formation by the nucleophilic attack of sulfur of compounds **7** on the methylene carbon of propargyl bromide giving **8** .



Scheme 4.4 Synthesis of compounds **8** with mechanism

4.2.4 Synthesis of 1,3,4-oxadiazole incorporated 1,2,3-triazole derivatives 1a-1g, 2a-2g and 3a-3g

Novel 1,3,4-oxadiazole incorporated 1,2,3-triazole derivatives **1**, **2** and **3** were synthesized *via* Huisgen [3+2] cycloaddition reaction of alkyne group of propargylated 1,3,4-oxadiazoles **8** with azides **9**, **10** and **11** in water and tert-butanol (1:1) as solvent system using catalytic amount of sodium ascorbate and coppersulfate pentahydrate under stirring condition for 3-4 hrs at 60 ºC (Scheme 4.5). The mechanism for the formation of **1**, **2** and **3** is similar to the mechanism already disscussed in chapter 3 (Scheme 3.5).



Scheme 4.5 Synthesis of compounds **1**, **2** and **3**

Conversion of **8** to target compounds **1**, **2** and **3** were confirmed by disappearance of singlet at approximately 3.21 ppm for alkynic proton and appearance of another singlet at approximately 8.95 ppm for 1,2,3-triazole ring proton in ^{1}H NMR (Fig. 4.3, 4.6 and 4.9). Methylene protons (-SCH_2-) resonated at approximately 4.50 ppm as a singlet while broad exchangeable singlet obtained at approximately 7.55 ppm corresponding to sulfonamide protons. In ^{13}C NMR (Fig. 4.4, 4.7 and 4.10), methylene carbon atom (-SCH_2-) resonated at approximately 25.0 ppm while methine carbon atom (=CH-) of 1,2,3-triazole ring resonated at approximately 160.0 ppm. Disappearance of a sharp band in the range of 3350-3280 cm^{-1} for C-H stretch of terminal alkyne (≡C-H) and another band at approximately 2150 cm^{-1} for C≡C stretch in IR spectra supported the conversion of alkyne to 1,2,3-triazole. Further, HRMS data also confirmed the formation of desired products **1, 2** and **3** (Fig. 4.5, 4.8 and 4.11).

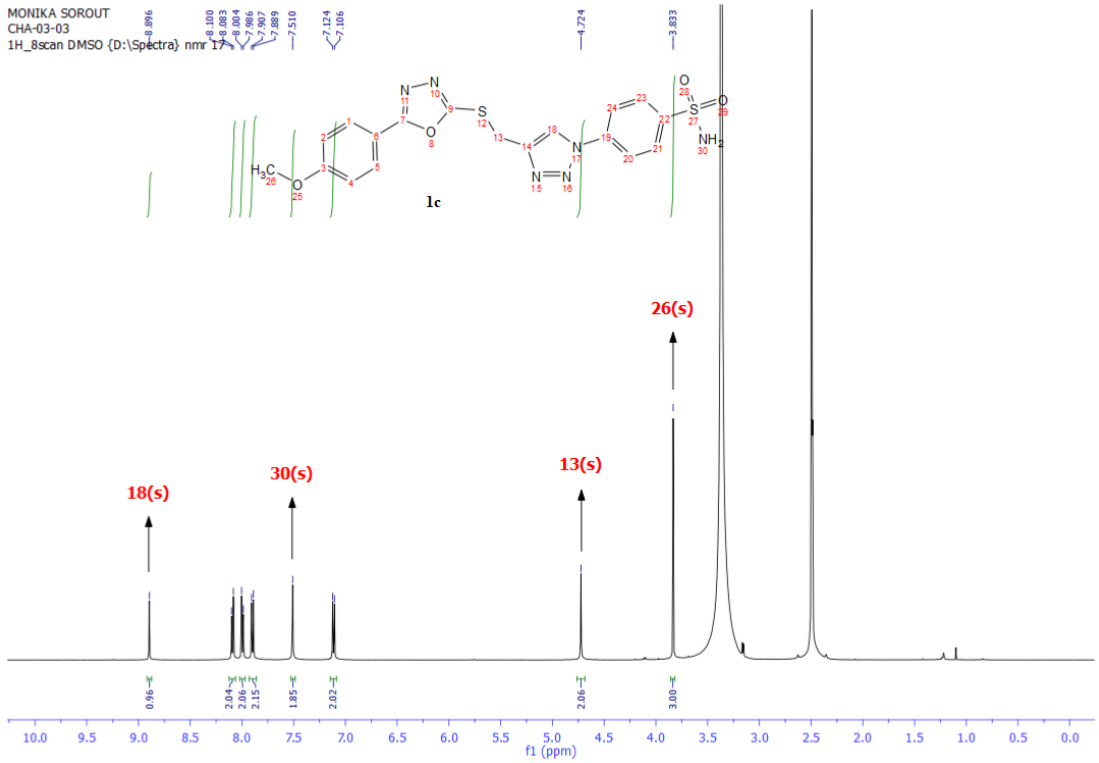


Fig. 4.3 ^{1}H NMR spectrum of compound **1c**

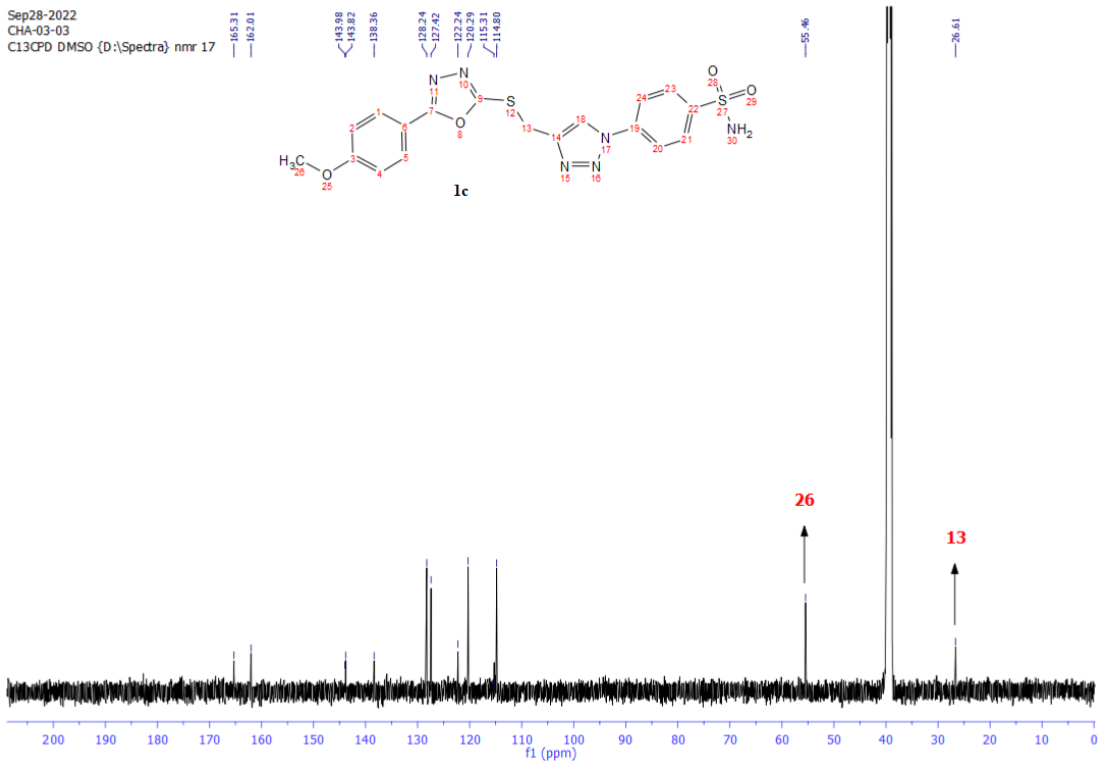


Fig. 4.4 ^{13}C NMR spectrum of compound **1c**

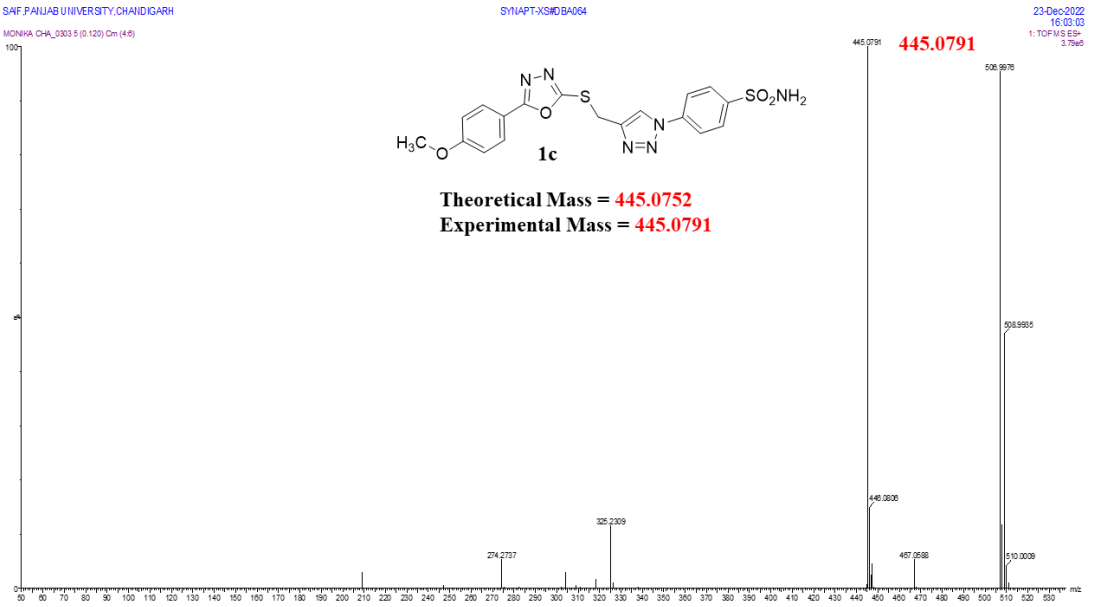


Fig. 4.5 HRMS spectrum of compound **1c**

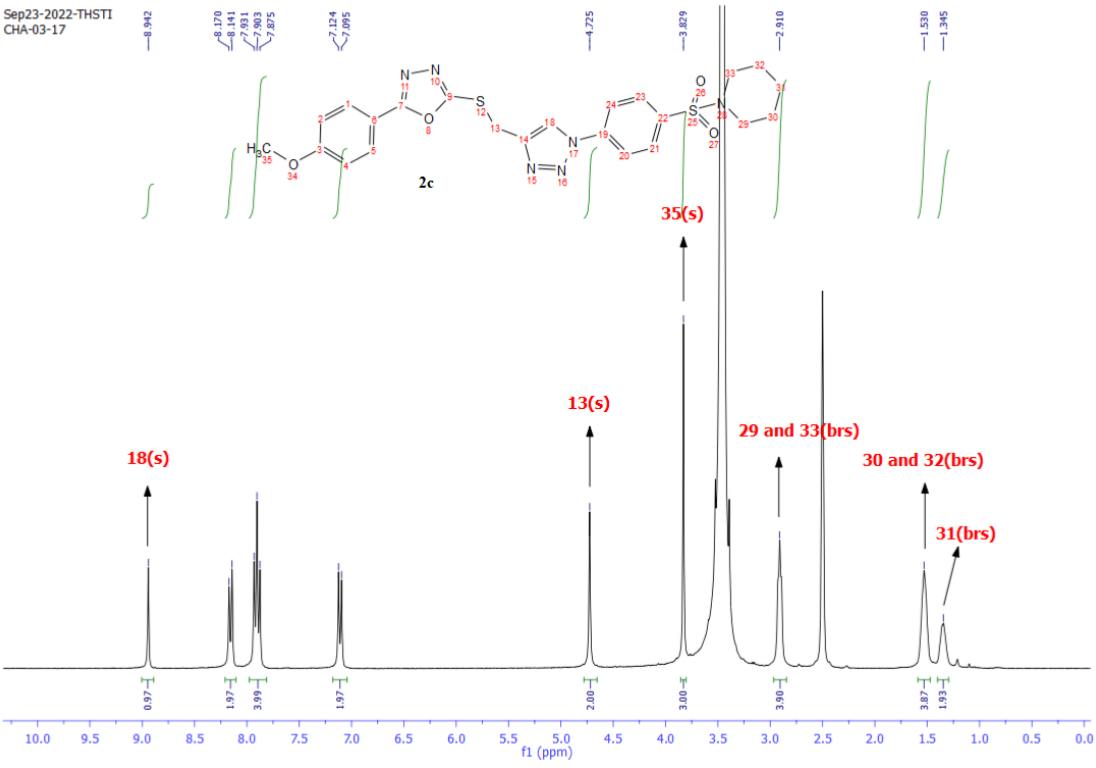


Fig. 4.6 ^{1}H NMR spectrum of compound **2c**

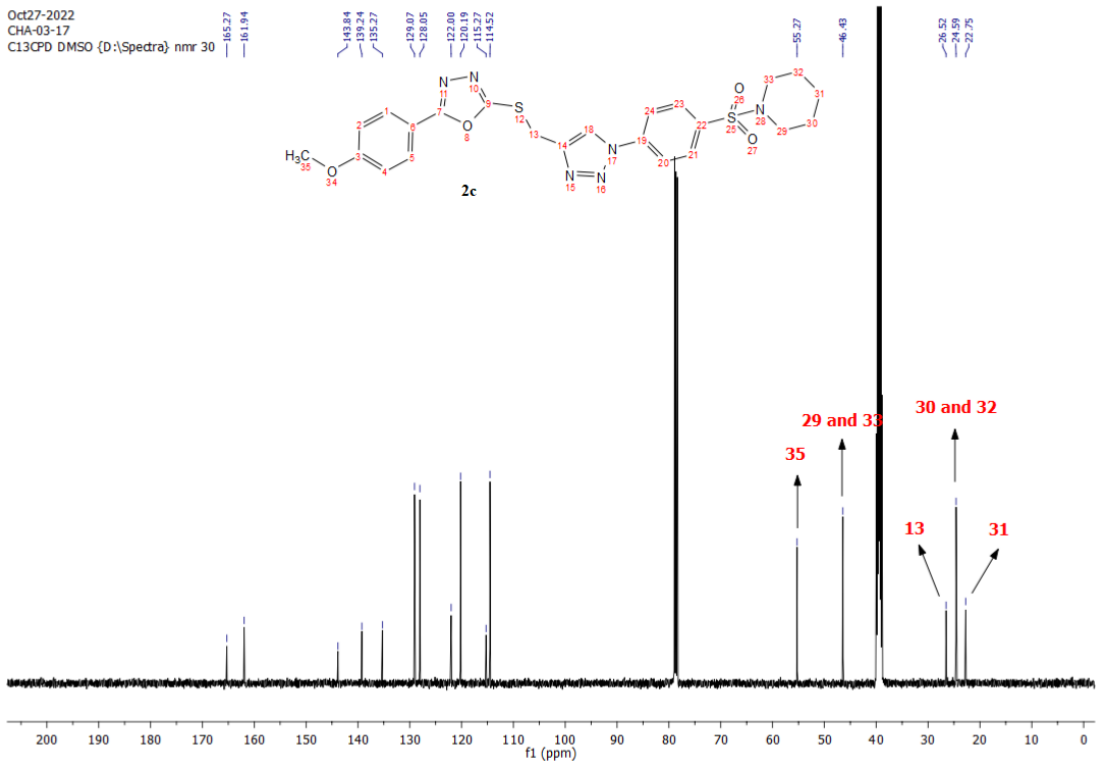


Fig. 4.7 ^{13}C NMR spectrum of compound **2c**

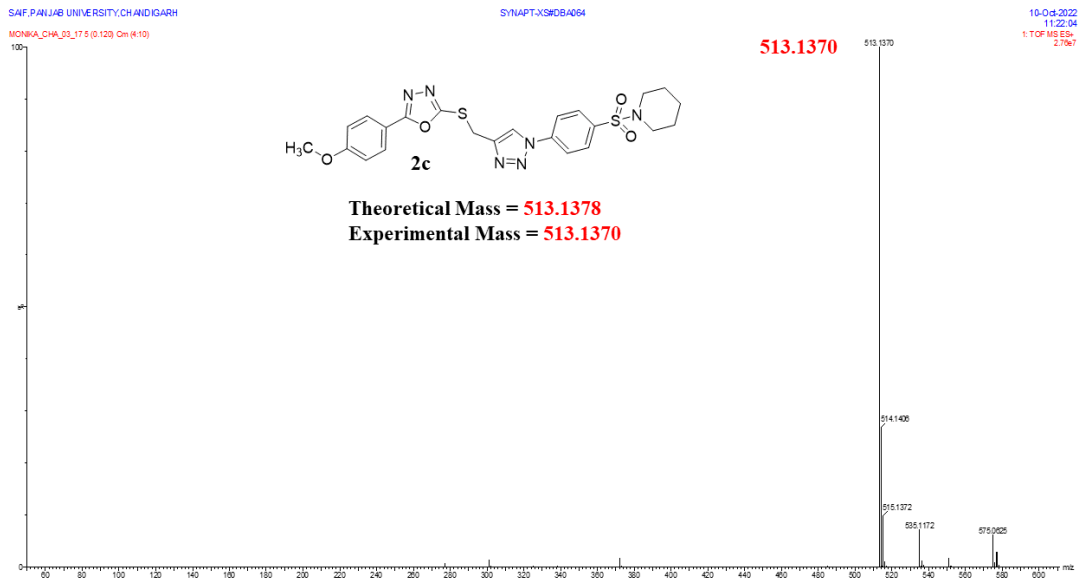


Fig. 4.8 HRMS spectrum of compound **2c**

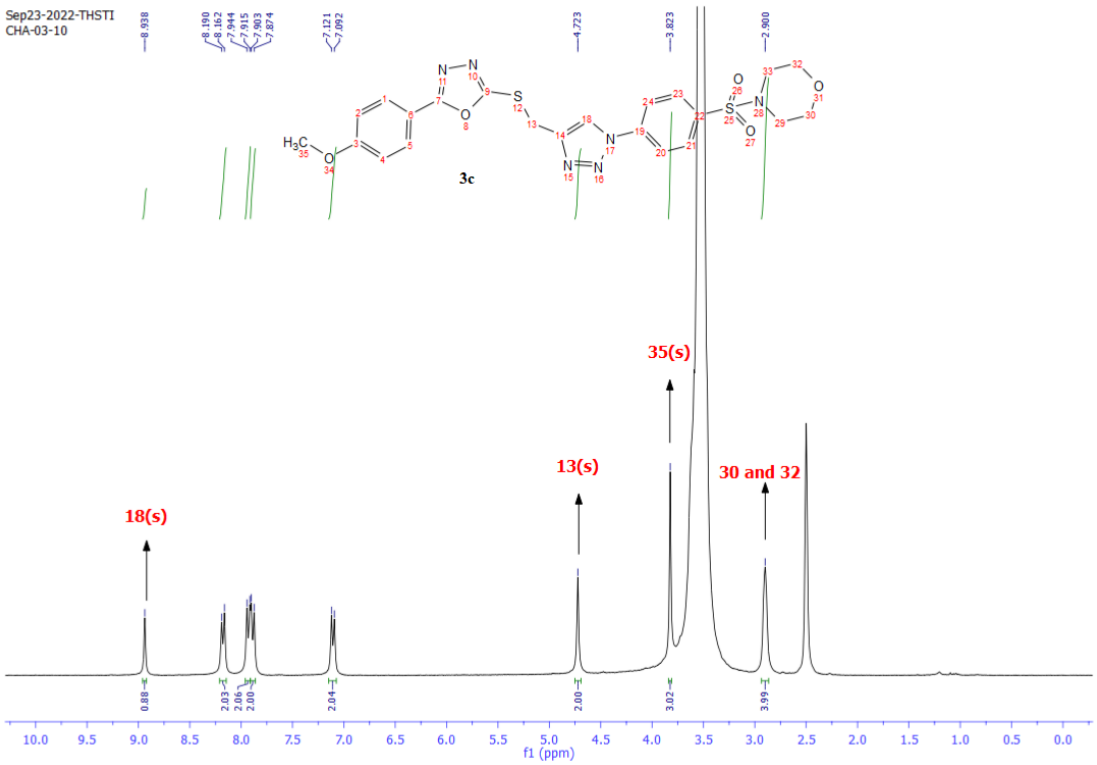


Fig. 4.9 ^{1}H NMR spectrum of compound **3c**

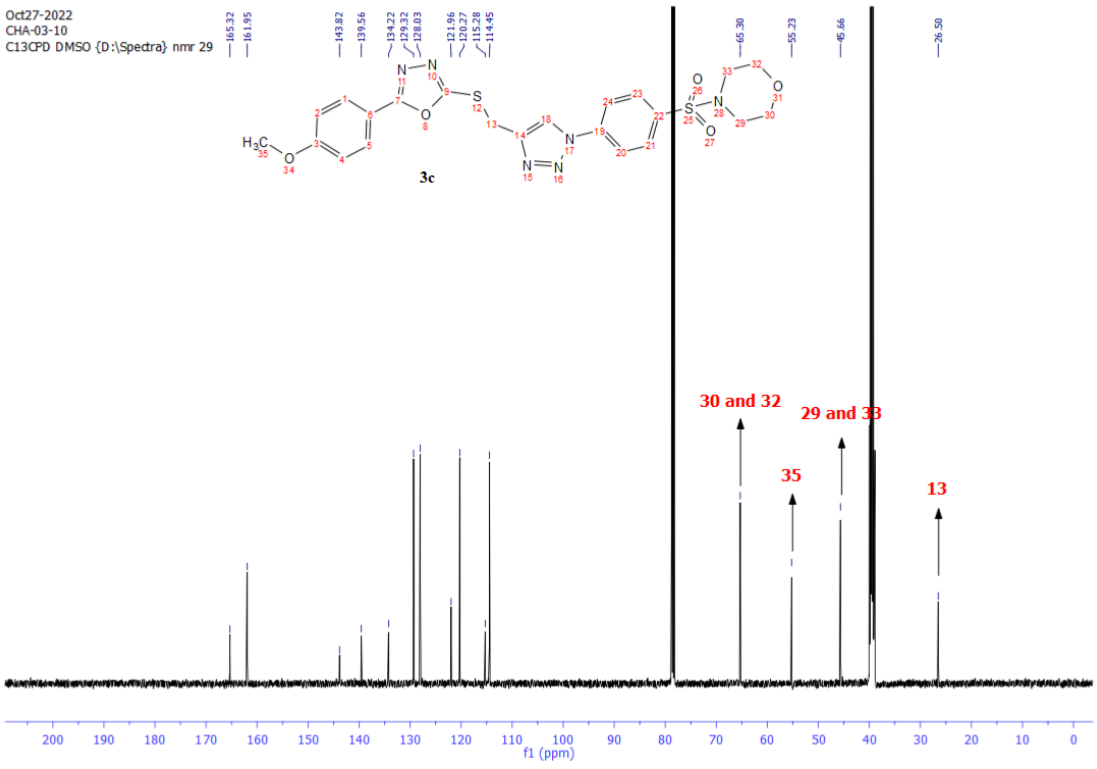


Fig. 4.10 ^{13}C NMR spectrum of compound **3c**

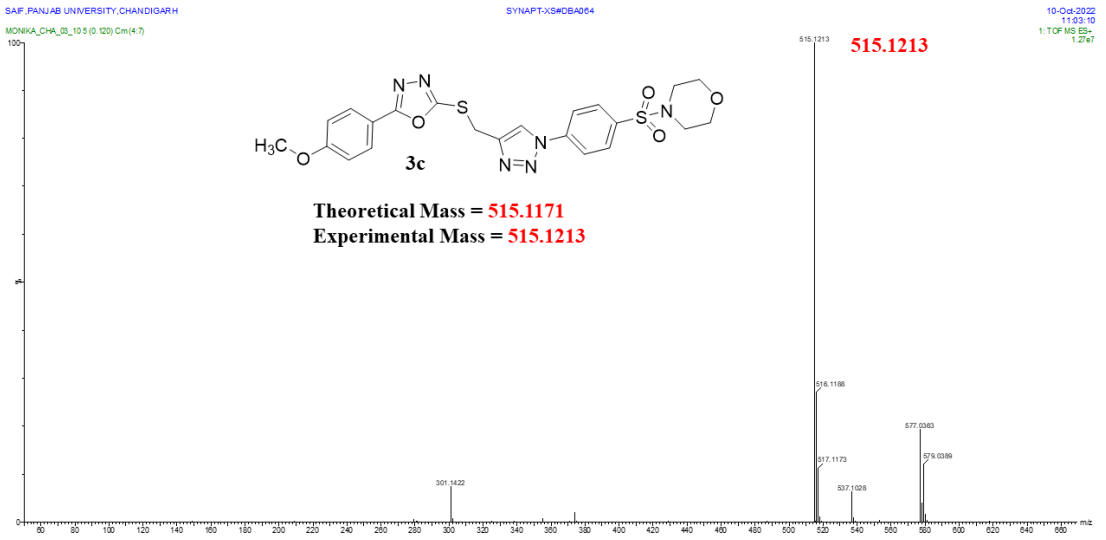


Fig. 4.11 HRMS spectrum of compound **3c**

4.3 Biological Testing Results

4.3.1 Anti-cancer assay

Cytotoxicity analysis of newly synthesized compounds against human breast cancer cell lines (MCF-7) cells was assessed by standard MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) protocol. In brief, MCF-7 cells (1×10^4/well) were seeded in a Roswell Park Memorial Institute series 1640 (RPMI-1640) supplemented with 5 C fetal bovin serum in a 96-well microliter plate at 37 °C for overnight. On the subsequent day, all compounds were added to cells in a separate well and incubated at 37 °C for 24 hrs. The cells were further treated with 50 μL of MTT solution (2 mg/mL) in phosphate buffer saline (PBS) and incubated at 37 °C for 4 hrs. The supernatant was removed, and 100 μL of DMSO was added to dissolve formazan crystals. The % viability of cells was calculated by the ratio of OD-570 of treated cells to the OD-570 of untreated cells. Untreated cells and 10 % DMSO were taken as negative and positive controls, respectively.

4.3.2 Anti-microbial assay

All of the newly synthesized compounds **1a-1g**, **2a-2g**, **3a-3g** and precursors **8a-8g** were screened for their anti-microbial profile using double dilution method against three Gram-positive (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657* , and *Bacillus cereus ATCC 11770)*, four Gram-negative (*Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733* and *Shigella flexneri ATCC 9199)* bacterial strains and one fungal strain (*Candida albicans MTCC 183*). The detailed procedure for anti-microbial assay has already been disscussed in chapter 2.

4.3.3 Anti-oxidant assay

All of the newly synthesized compounds **1a-1g**, **2a-2g**, **3a-3g** and precursors **8a-8g** were screened for their anti-oxidant profile using DPPH method and ascorbic acid as reference. The detailed procedure used for anti-oxidant assay has already been disscussed in chapter 2.

4.3.4 Results and discussion

A. Anti-cancer activity - results and discussion

The synthesized compounds can be divided into three categories **1a-1g**, **2a-2g** and **3a-3g** based on the variation on sulfa group in their molecular architecture. To evaluate the anti-cancer potential of newly synthesized compounds **1a-1g**, **2a-2g** and **3a-3g** and their precursors **8a-8g** , cytotoxicity studies were carried out against Michigan Cancer Foundation (MCF-7) cell line using MTT *in vitro* cell proliferation assay in comparison with Doxorubicin as reference drug. The activities were tested in triplicates and average values were considered (Table 4.1). Unfortunately, among the tested compounds only four compounds **1f**, **2b**, **2c** and **3c** possessed significant anti-cancer activity while all of them possessed highly poor inhibitory effects in comparison to the reference drug used. Graphical comparisons of anti-cancer profile of tested compounds **1a-1g**, **2a-2g**, **3a-3g** and **8a-8g** are shown in Fig. 4.12, Fig. 4.13, Fig. 4.14 and Fig. 4.15, respectively. The graphical comparison of IC_{50} values of compounds **1f**, **2b**, **2c**, **3c** with the standard anti-cancer drug Doxorubicin is shown in Fig. 4.16.

Table 4.1 MIC values of **1a-1g**, **2a-2g**, **3a-3g** and **8a-8g** against MCF-7 cell line

Compound	MIC (μg\mL) against MCF-7 Cell line[a]	Compound	MIC (μg\mL) against MCF-7 Cell line[a]
1a	$>$ 1280	**3b**	$>$ 1280
1b	$>$ 1280	**3c**	161.5
1c	$>$ 1280	**3d**	$>$ 1280
1d	$>$ 1280	**3e**	$>$ 1280
1e	$>$ 1280	**3f**	$>$ 1280
1f	635	**3g**	$>$ 1280
1g	$>$ 1280	**8a**	$>$ 1280
2a	$>$ 1280	**8b**	$>$ 1280
2b	640	**8c**	$>$ 1280
2c	480	**8d**	$>$ 1280
2d	$>$ 1280	**8e**	$>$ 1280
2e	$>$ 1280	**8f**	$>$ 1280
2f	$>$ 1280	**8g**	$>$ 1280
2g	$>$ 1280	**Doxorubicin**	10.5
3a	$>$ 1280		

[a]Mean of three replicates, showed the viability percentage on challenged with the tested compounds as compared to the control case

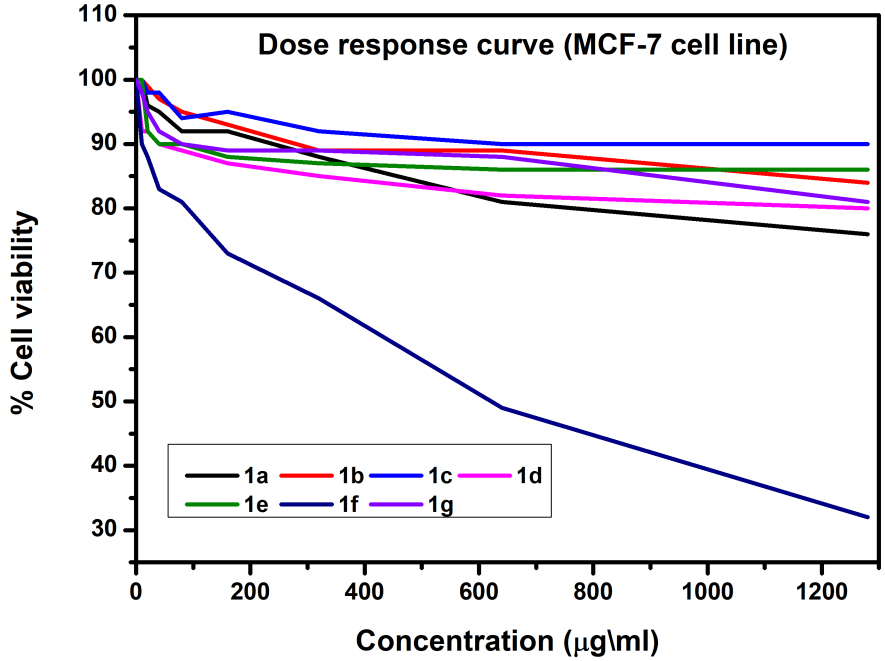


Fig. 4.12 Graphical comparison of anti-cancer activity profile of newly synthesized compounds **1a-1g**

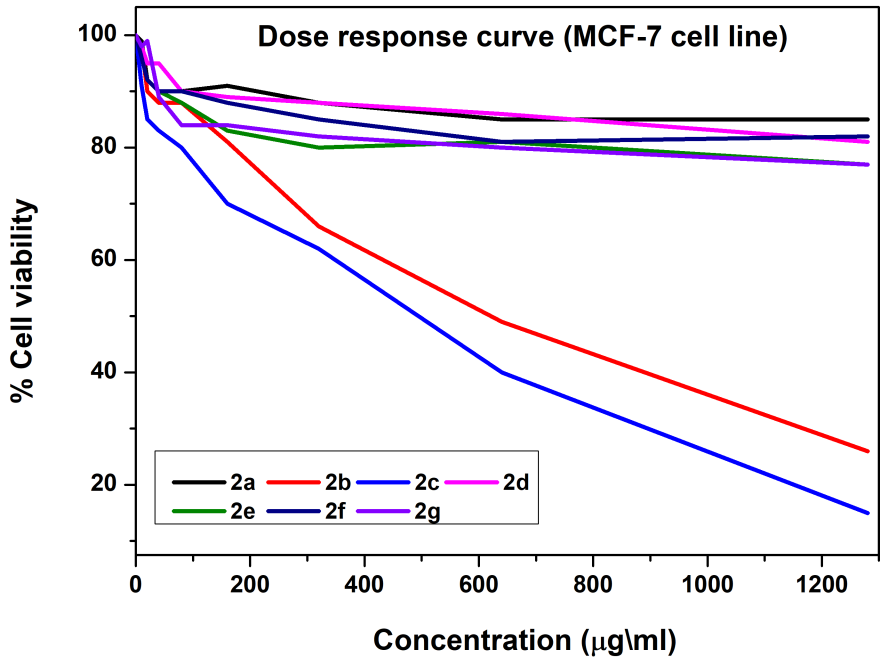


Fig. 4.13 Graphical comparison of anti-cancer activity profile of newly synthesized compounds **2a-2g**

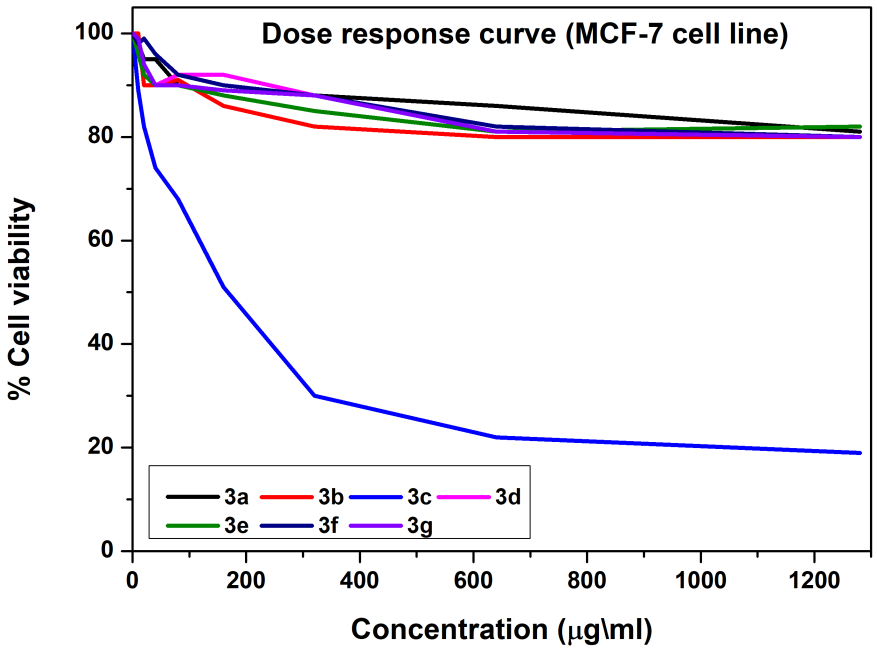


Fig. 4.14 Graphical comparison of anti-cancer activity profile of newly synthesized compounds **3a-3g**

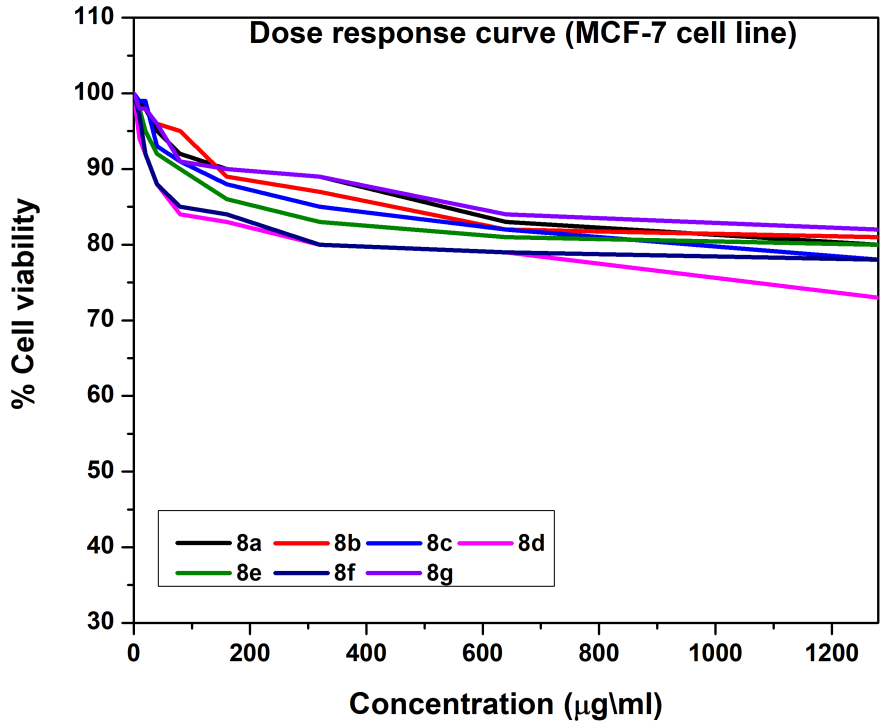


Fig. 4.15 Graphical comparison of anti-cancer activity profile of compounds **8a-8g**

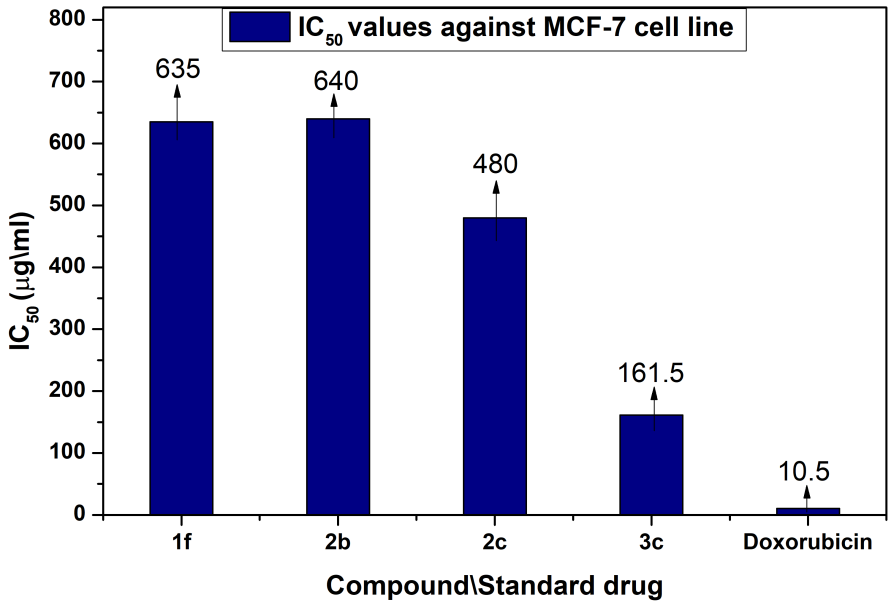


Fig. 4.16 Graphical comparison of MIC values against MCF-7 cell line of **1f**, **2b**, **2c**, **3c** and standard drug Doxorubicin

B. Anti-microbial activity - results and discussion

All of the newly synthesized compounds **1a-1g**, **2a-2g**, **3a-3g** and precursors **8a-8g** were tested *in vitro* against three Gram-positive pathogenic bacterial strains (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657,* and *Bacillus cereus ATCC 11770),* four Gram-negative pathogenic bacterial strains *(Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733,* and *Shigella flexneri ATCC 9199*) and one pathogenic fungal strain (*Candida albicans MTCC 183*) using double dilution method. As shown in the result all the tested compounds showed good to excellent activity as anti-microbial agents (Table 4.2). Results are expressed as minimum inhibitory concentration values (MIC) of the sample required to inhibit the growth of the bacterial and fungal strain and compared with the standard drugs Amoxicillin and Fluconazole as reference drugs, respectively. Graphical comparisons of anti-bacterial profile of tested compounds **1a-1g**, **2a-2g**, **3a-3g**, **4a-4g** and **8a-8f** with reference drug Amoxicilliln are shown in Fig. 4.17, Fig. 4.18, Fig. 4.19 and Fig. 4.20, respectively, while the graphical comparison of anti-fungal profile all tested compounds with standard drug Fluconazole is shown in Fig. 4.21.

Table 4.2 MIC values of compounds **1a-1g**, **2a-2g**, **3a-3g** and **8a-8g** against tested bacterial and fungal strains

Compound	Minimum Inhibitory Concentration (MIC in µg/mL)[a]							
	Gram-positive bacterial strain			Gram-negative bacterial strains				Fungal strain
	S.aure us	*L.monocytogenes*	*B.cereus*	*P.aeruginosa*	*E.coli*	*S.typhi*	*S.flexneri*	*C.albicans*
1a	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
1b	6.25	6.25	6.25	6.25	6.25	6.25	6.25	3.12
1c	6.25	3.12	3.12	6.25	6.25	6.25	3.12	6.25
1d	3.12	6.25	3.12	3.12	6.25	3.12	6.25	12.5
1e	3.12	3.12	6.25	6.25	6.25	6.25	3.12	3.12
1f	3.12	6.25	6.25	6.25	6.25	6.25	6.25	6.25
1g	3.12	3.12	3.12	3.12	3.12	6.25	3.12	3.12
2a	6.25	3.12	3.12	3.12	6.25	6.25	3.12	3.12
2b	3.12	3.12	3.12	3.12	6.25	3.12	6.25	3.12
2c	6.25	6.25	3.12	3.12	3.12	3.12	3.12	3.12
2d	6.25	6.25	3.12	3.12	3.12	6.25	3.12	3.12
2e	6.25	3.12	12.5	12.5	3.12	3.12	12.5	6.25
2f	3.12	3.12	6.25	3.12	3.12	3.12	3.12	6.25
2g	3.12	3.12	6.25	3.12	3.12	3.12	6.25	6.25
3a	3.12	3.12	3.12	6.25	3.12	3.12	3.12	6.25
3b	3.12	3.12	6.25	3.12	3.12	3.12	6.25	3.12
3c	6.25	6.25	6.25	6.25	12.5	12.5	6.25	3.12

3d	3.12	3.12	6.25	6.25	6.25	6.25	6.25	6.25
3e	3.12	6.25	3.12	3.12	3.12	3.12	6.25	6.25
3f	3.12	6.25	6.25	3.12	3.12	3.12	3.12	6.25
3g	3.12	6.25	3.12	6.25	3.12	12.5	3.12	6.25
8a	6.25	6.25	12.5	6.25	6.25	12.5	12.5	12.5
8b	6.25	12.5	12.5	6.25	6.25	12.5	6.25	6.25
8c	12.5	6.25	6.25	6.25	12.5	12.5	12.5	6.25
8d	6.25	3.12	12.5	12.5	6.25	6.25	12.5	6.25
8e	12.5	6.25	12.5	12.5	6.25	6.25	12.5	12.5
8f	12.5	3.12	12.5	12.5	6.25	6.25	6.25	12.5
8g	6.25	6.25	3.12	12.5	12.5	12.5	6.25	12.5
Amoxicillin[b]	3.12	6.25	6.25	3.12	6.25	3.12	3.12	-
Fluconazole[c]	-	-	-	-	-	-	-	3.12

[a]Mean of the three replicates
[b]Amoxicillin was used as positive control for anti-bacterial activity
[c]Fluconazole was used as positive control for anti-fungal activity

The Following generalizations can be made from the obtained data:

1. Results depicts that all the tested compounds have exhibited good to excellent anti-microbial activity against tested microbes.

2. Compound **1a** possessed either equivalent or two-fold better anti-bacterial and anti-fungal activities in comparison with reference drugs.

3. Compound **2f** was either equivalent or two-fold better anti-bacterial agent and weaker anti-fungal agent than the reference drugs.

4. All the newly synthesized compounds **1**, **2** and **3** possessed either equivalent or better anti-microbial activity than the reference drug against *Listeria monocytogenes MTCC 657*, *Bacillus cereus ATCC 11770* and *Escherichia coli MTCC 143* bacterial strains.

5. Compounds **1a**, **1b**, **1e**, **1g**, **2a**, **2b**, **2c**, **2d**, **3b** and **3c** exhibited equivalent while others exhibited weaker anti-fungal activities when compared with the reference drug, Fluconazole.

6. Results showed that compounds **1** have overall better inhibition potential than **2**, **3** and **8**.

7. Against all the bacterial strains, except *L.monocytogenes* and the fungal strain tested, the final compounds **1**, **2** and **3** possessed higher inhibition potential than the precursors **8**.

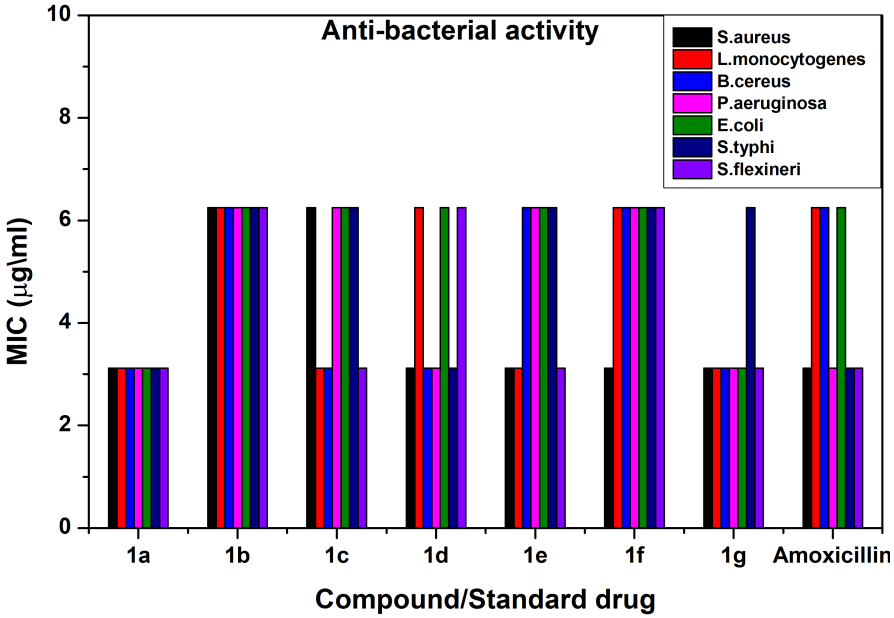


Fig. 4.17 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **1a-1g** and the standard drug Amoxicillin

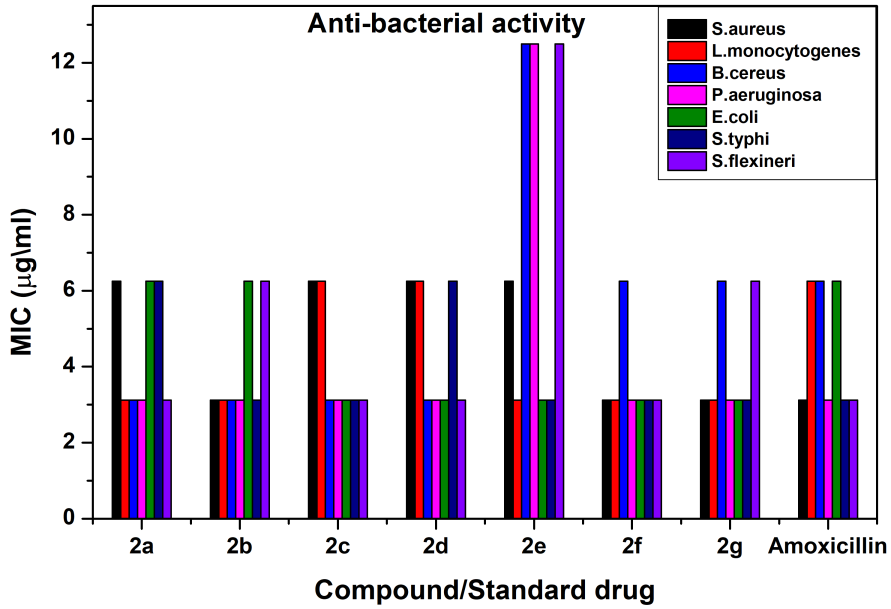


Fig. 4.18 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **2a-2g** and the standard drug Amoxicillin

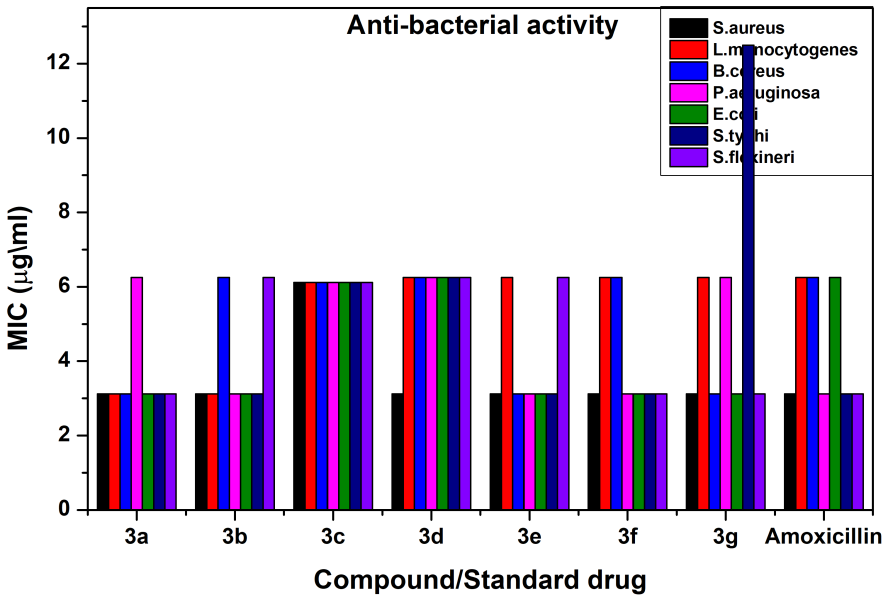


Fig. 4.19 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **3a-3g** and the standard drug Amoxicillin

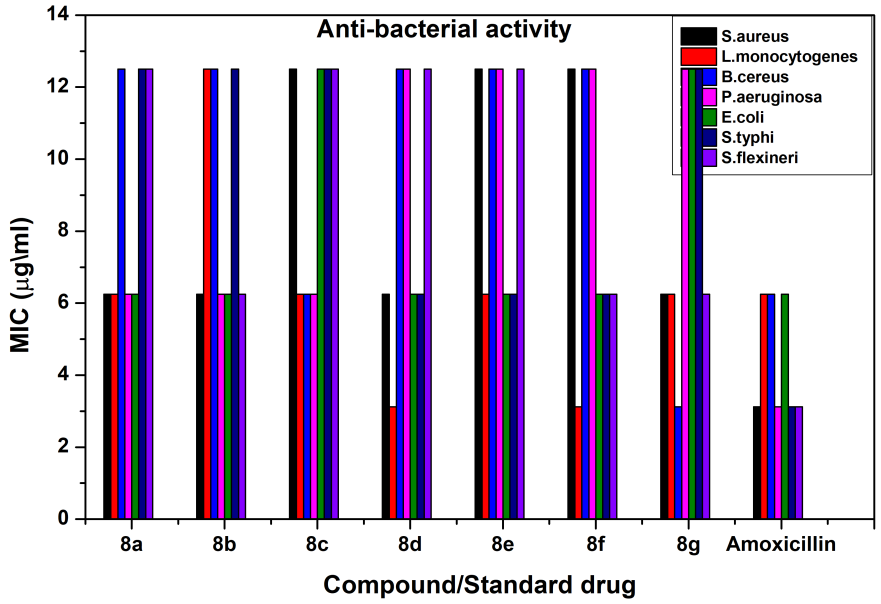


Fig. 4.20 Graphical comparison of anti-bacterial activity profile of compounds **8a-8g** and the standard drug Amoxicillin

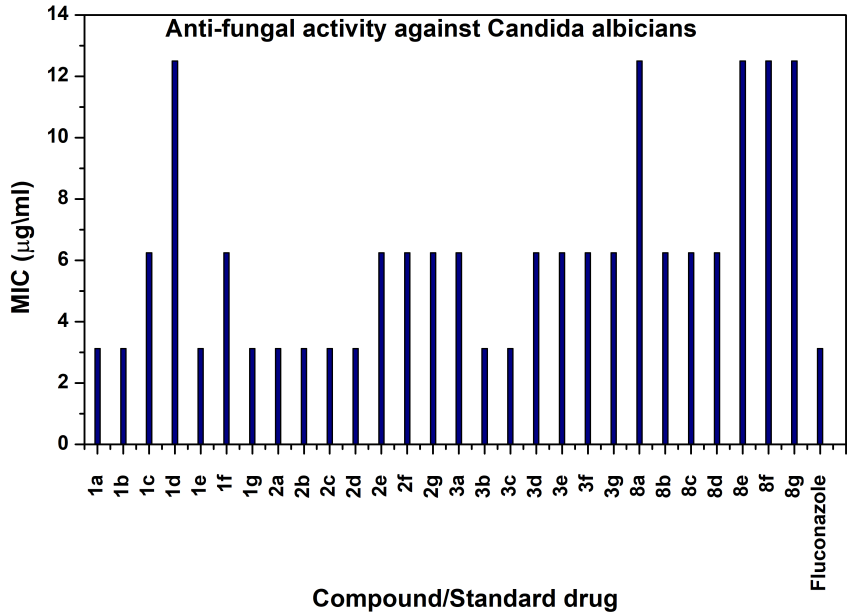


Fig. 4.21 Graphical comparison of anti-fungal activity profile of **1a-1g**, **2a-2g**, **3a-3g**, **8a-8g** and standard drug Fluconazole

C. Anti-oxidant activity - results and discussion

Free radicals are harmful to human, animal and aquatic life as they are involved in a number of human diseases. These are unstable and react with various biological substrates such as lipids, protein, DNA etc. causing cell damage. Here, we have assessed free radical scavenging activity of all the newly synthesized compounds **1a-1g**, **2a-2g**, **3a-3g** and the precursors **8a-8g** spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Anti-oxidant activities of all the tested compounds along with the standard (DMSO) are presented in the table 4.3. Results are depicted in terms of % RSA i.e. radical scavenging activity with respect to reference control values. The experiments were done in triplicates and average values were taken into consideration. Graphical comparison of anti-oxidant profile of tested compounds with ascorbic acid (AA) is shown in Fig. 4.22.

From the obtained data, it can be generalized that:

1. Almost all the tested compounds have exhibited moderate to excellent anti-oxidant activity.

2. Compound **2b** was found to possess highest anti-oxidant activity (96.73 %) among the tested compounds followed by **1a**, **1e**, **1d**, **1g**, **3d**, **2a**, **2d**, **3a**, **1f**, **3f**, **2e** and **3b** showing activity in the range of 96.73-90.21 %.

3. *p*-Methoxy substituted triazole derivative **1c** was comparatively poor anti-oxidant agent with scavenging activity 44.65 %.

4. Results depicted that final compounds **1**, **2** and **3** exhibited higher anti-oxidant potential than the precursors **8**.

Table 4.3 Anti-oxidant activities of compounds **1a-1g**, **2a-2g**, **3a-3g** and **8a-8g** using DPPH method

Compound	Anti-oxidant activity % RSA[a]	Compound	Anti-oxidant activity % RSA[a]
1a	94.56	**3b**	90.21
1b	88.24	**3c**	84.65

1c	79.34	**3d**	91.34
1d	93.43	**3e**	89.56
1e	94.54	**3f**	90.34
1f	90.34	**3g**	91.23
1g	93.21	**8a**	80.52
2a	91.26	**8b**	88.20
2b	96.73	**8c**	89.31
2c	87.45	**8d**	73.43
2d	91.23	**8e**	88.54
2e	90.23	**8f**	80.34
2f	86.45	**8g**	81.20
2g	88.13	**AA**	96.78
3a	91.23		

[a]Values were mean of three replicates % RSA, showed anti-oxidant potentials of the tested samples

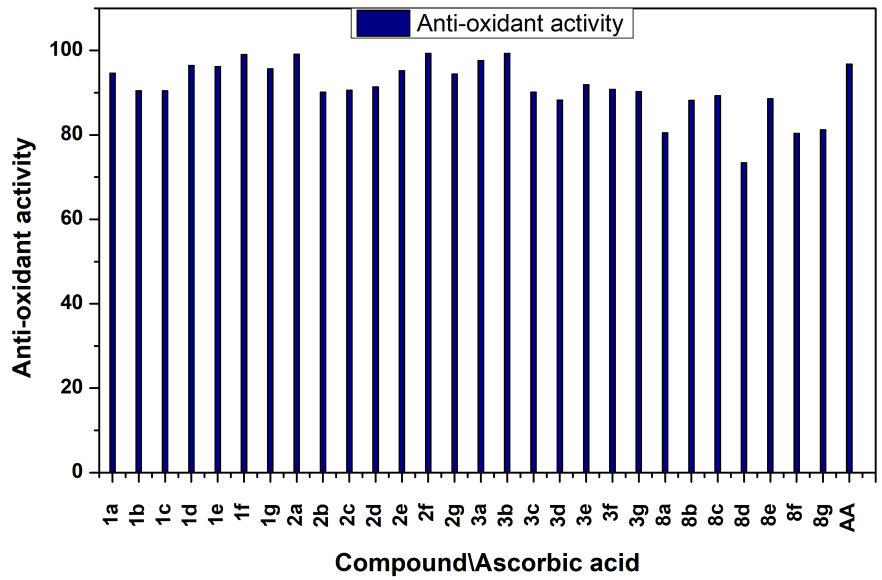


Fig. 4.22 Graphical comparison of anti-oxidant activity profile of compounds **1a-1g**, **2a-2g 3a-3g**, **8a-8g** and ascorbic acid (AA)

4.3.5 Cytotoxicity

Cytotoxicity was tested for all the newly synthesized compounds **1a-1g**, **2a-2g**, **3a-3g** and the precursors **8a-8g** against mouse fibroblast cell line and plant seed germination cell line at very high concentration of 1 mg/mL using MTT assay. It was observed that, all the compounds were safe against mouse fibroblast cell line with 88.23-99.34 % cell viability values even at very high doses of corresponding MICs (Table 4.4). Results also indicate that all the tested compounds are 100 % safe to plant seed germination cell line. Therefore, results of cytotoxicity study reveal that use of all the synthesized molecules **1a-1g**, **2a-2g**, **3a-3g** and **8a-8g** in drug synthesis will not cause any harm to mammalian cell lines and plant seed germination cell lines, which is a highly desirable need.

Table 4.4 *In vitro* cytotoxic studies of compounds **1a-1g**, **2a-2g**, **3a-3g** and **8a-8g** against normal cells at the concentration of 1 mg/mL

Compound	Mouse fibroblast cell Cell Viability %[a]	Compound	Mouse fibroblast cell Cell Viability %[a]
1a	94.65	**3b**	99.34
1b	90.45	**3c**	90.15
1c	90.46	**3d**	88.23
1d	96.45	**3e**	91.90
1e	96.20	**3f**	90.76
1f	99.02	**3g**	90.23
1g	95.65	**8a**	89.90
2a	99.10	**8b**	87.99
2b	90.12	**8c**	88.84
2c	90.56	**8d**	85.23
2d	91.40	**8e**	90.20
2e	95.23	**8f**	98.80
2f	99.34	**8g**	92.54
2g	94.40	**DMSO**	90.23
3a	97.64		

[a]Mean of three replicates, showed the viability percentage on challenged with the

tested compounds as compared to the control case

4.4 Conclusion

We have synthesized a library of twenty-one novel benzenesulfonamide containing 1,2,3-triazole tethered 1,2,4-triazoles **1**, **2** and **3** and evaluated them along with precursors **8** for their inhibition potential against MCF-7 cancer cell line. Among the tested compounds, only four compounds **1f**, **2b**, **2c** and **3c** possessed significant anti-cancer activity which too very poor in comparison to the inhibition potential of standard drug, Doxorubicin. All the newly synthesized compounds **1**, **2** and **3** and the precursors **8** were also evaluated for anti-microbial activity against Gram-positive (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657,* and *Bacillus cereus ATCC 11770)* and Gram-negative (*Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733* and *Shigella flexneri ATCC 9199*) bacterial strains and one fungal strain (*Candida albicans MTCC 183*). Compound **1a** possessed excellent anti-microbial activity with 3.12 μg/ml MIC value which is either equivalent or lower than the MIC values of reference drugs, Amoxicillin (anti-bacterial) and Fluconazole (anti-fungal). Anti-oxidant activity was evaluated for all the newly synthesized compounds **1**, **2**, **3** and precursors **8** using DPPH method. Compound **2b** exhibited excellent anti-oxidant activity (96.73 %) when compared to the ascorbic acid (96.78 %) as reference. Results showed that the final compounds **1**, **2** and **3** have higher anti-cancer, anti-microbial and anti-oxidant potential than the precursors **8**. Cytotoxicity results showed that all the synthesized compounds are safe to normal animal cells and plant seed germination cells.

4.5 Experimental Section

All glassware were used after solvent wash and drying in an oven for 12 hrs. Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silicagel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. The infrared

(IR) spectra were recorded on FT-IR Perkin Elmer Spectrophotometer, CIL, JCBUST, YMCA, Faridabad. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance Neo, 300 MHz and Jeol JNM-ECZ 400S, 400 MHz NMR spectrometer using TMS as internal standard. Chemical shifts are expressed in δ (ppm). Mass data were recorded on Agilent 7800 ICP-MS mainframe. The reference values for the residual solvent were taken as δ = 2.50 ppm (DMSO-d_6) for ^{1}H NMR, δ = 39.54 ppm (DMSO-d_6) for ^{13}C NMR. The abbreviations: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet and ex = exchangeable protons are used for NMR assignments.

4.5.1 Synthesis of 1,3,4-oxadiazoles 7a-7g

To a clear solution of aryl hydrazide **6** (1.0 equivalent) in 20 mL of DMF was added carbon disulfide (3.0 equivalents) dropwise and stirred the reaction mixture for 1 h at room temperature. Excess of DMF was added to the reaction mixture and refluxed for further 2-3 hrs. The completion of reaction was monitored with TLC. After completion of reaction, the reaction mixture was poured in crushed ice when a yellowish solid precipitated out. Filtered the precipitated solid and washed with excess of ice cold water, dried and recrystallized from aqueous ethanol.

4.5.2 Synthesis of propargylated 1,3,4-oxadiazoles 8a-8g

To the solution of compound **7** in ethanol was added propargyl bromide (1.5 equivalents) and triethylamine (1.5 equivalents). Reaction mixture was refluxed for 2-3 hrs resulting in the formation of **8** as pure product as indicated by the TLC. The reaction mixture was allowed to cool at room temperature followed by addition of crushed ice. A solid was precipitated out which was filtered off, washed with excess of water, dried, and recrystallized from ethanol.

4.5.3 General procedure for the synthesis of target compounds 1a-1g, 2a-2g and 3a-3g

To well stirred solution of aromatic azide **9/10/11** (1 equivalent) and propargylated oxadiazole **8** (1.1 equivalents) in tert-butanol and water (1:1), a solution of sodium

ascorbate (0.6 equivalent) in water (2 mL) was added. After 10 minutes, solution of $CuSO_4.5H_2O$ (0.6 equivalent) in water (2 mL) was added and reaction mixture was allowed to stirr at 90 ºC for 6 hrs. Reactions progress was monitored using TLC. After completion of reaction, reaction mixture was poured into crushed ice. The solid obtained was filtered, dried, and then recrystallized from ethanol yielding the pure product **1/2/3**.

4-(4-(((5-phenyl-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzene-sulfonamide (1a)

White solid; Yield: 75 %; mp 220-222 ºC; IR (KBr) ν_{max} 3342, 3260, 3030, 2975, 1311, 1156, 708; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.92 (1H, s, triazole H), 8.10 (2H, d, J = 7.2 Hz, Ar), 7.98 (m, 5H, Ar), 7.59 (3H, s, J = 7.2 Hz, Ar), 7.53 (2H, ex, s, SO_2NH_2), 4.75 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.45, 162.90, 144.04, 143.92, 138.46, 132.09, 129.45, 127.51, 126.44, 123.05, 122.37, 120.39, 26.70; HRMS (ESI) m/z calcd for $C_{17}H_{14}N_6O_3S_2$ $[M+H]^+$ 415.0647, found 415.0616.

4-(4-(((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzene-sulfonamide (1b)

White solid; Yield: 76 %; mp 228-230 ºC; IR (KBr) ν_{max} 3334, 3356, 2982, 1312, 1154, 718; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.90 (1H, s, triazole H), 8.08 (2H, brs, Ar), 8.00 (2H, brs, Ar), 7.84 (2H, d, Ar), 7.53 (2H, ex, s, SO_2NH_2), 7.37 (2H, brs, Ar), 4.73 (2H, s, CH_2S), 2.37 (3H, s, Me); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.62, 162.59, 144.12, 143.97, 142.38, 138.52, 130.06, 127.59, 126.47, 122.42, 120.45, 120.34, 26.75, 21.20; HRMS (ESI) m/z calcd for $C_{18}H_{16}N_6O_3S_2$ $[M+H]^+$ 429.0803, found 429.0827.

4-(4-(((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl-)benzenesulfonamide (1c)

White solid; Yield: 82 %; mp 234-36 ºC; IR (KBr) ν_{max} 3336, 3354, 2984, 1314, 1150, 602; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.89 (1H, s, triazole H), 8.09 (2H, d, J = 6.8 Hz, Ar), 7.99 (2H, d, J = 7.2 Hz, Ar), 7.89 (2H, s, J = 7.2 Hz, Ar), 7.51 (2H, ex, s, SO_2NH_2), 7.11 (s, J = 7.2 Hz, 2H, Ar), 4.72 (2H, s, CH_2S), 3.83 (3H, s, OMe); ^{13}C

NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.31, 162.01, 143.98, 143.82, 138.36, 128.24, 127.42, 122.24, 120.29, 115.31, 114.80, 55.46, 26.61; HRMS (ESI) m/z calcd for $C_{18}H_{16}N_6O_4S_2$ $[M+H]^+$ 445.0752, found 445.0791.

4-(4-(((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)-benzenesulfonamide (1d)

White solid; Yield: 78 %; mp 226-228 ºC; IR (KBr) ν_{max} 3330, 3346, 2982, 1310, 1150, 720; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.92 (1H, s, triazole H), 8.15-7.95 (6H, m, Ar), 7.54 (2H, ex, s, SO_2NH_2), 7.43 (2H, brs, Ar), 4.75 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.20, 163.45, 144.43, 138.97, 129.73, 129.64, 128.03, 122.89, 120.88, 120.25, 117.32, 117.10, 27.20; HRMS (ESI) m/z calcd for $C_{17}H_{13}FN_6O_3S_2$ $[M+H]^+$ 433.0552, found 433.0522.

4-(4-(((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1- yl)-benzenesulfonamide (1e)

White solid; Yield: 80 %; mp 232-234 ºC; IR (KBr) ν_{max} 3320, 3100, 1311, 1156, 710. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.90 (1H, s, triazole H), 8.09 (2H, d, J = 7.2 Hz, Ar), 8.10 - 7.97 (4H, m, Ar), 7.65 (2H, s, J = 7.2 Hz, Ar), 7.53 (2H, ex, s, SO_2NH_2), 4.75 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.74, 163.27, 144.03, 143.98, 138.50, 136.86, 129.68, 128.32, 127.57, 122.41, 121.99, 120.45, 26.72; HRMS (ESI) m/z calcd for $C_{17}H_{13}ClN_6O_3S_2$ $[M+H]^+$ 449.0257, found 449.0185.

4-(4-(((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)benzenesulfonamide (1f)

White solid; Yield: 72 %; mp 240-242 ºC; IR (KBr) ν_{max} 3331, 3352, 2972, 1313, 1154, 712; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.91 (1H, s, triazole H), 8.10 (2H, d, J = 8.8, Ar), 8.00 (2H, d, J = 8.8, Ar), 7.91 (2H, d, J = 8.6, Ar), 7.53 (2H, ex, s, SO_2NH_2), 4.76(2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.84, 163.27, 144.00, 143.97, 138.49, 132.58, 128.41, 127.55, 125.72, 122.40, 122.32, 120.44, 26.70; HRMS (ESI) m/z calcd for $C_{17}H_{13}BrN_6O_3S_2$ $[M+H]^+$ 492.9752, found 492.9746.

4-(4-(((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)-benzenesulfonamide (1g)

White solid; Yield: 68 %; mp 245-247 ºC; IR (KBr) ν_{max} 3331, 3353, 2981, 1314, 1154, 695; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.93 (1H, s, triazole H), 8.40 (2H, d, *J* = 8.4, Ar), 8.23 (2H, d, *J* = 8.4, Ar), 8.10 (2H, d, *J* = 8.0, Ar), 7.53 (2H, ex, s, SO_2NH_2), 4.80 (2H, s, CH_2S); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.35, 164.06, 149.15, 143.91, 138.42, 128.55, 127.78, 127.48, 124.60, 122.40, 120.37, 104.01, 26.64; HRMS (ESI) m/z calcd for $C_{17}H_{13}N_7O_5S_2$ $[M+H]^+$ 460.0497, found 460.0523.

2-phenyl-5-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-thio)-1,3,4-oxadiazole (2a)

White solid; Yield: 76 %; mp 241-243 ºC; IR (KBr) ν_{max} 2980, 1560, 1314, 1153, 710; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.97 (1H, s, triazole H), 8.16 (2H, d, *J* = 8.4 Hz, Ar), 7.96 (2H, d, *J* = 6.8 Hz, Ar), 7.91 (2H, d, *J* = 8.4 Hz, Ar), 7.66-7.55 (3H, m, Ar), 4.76 (2H, s, CH_2S), 2.91 (4H, brs, CH_2N), 1.53 (4H, brs, CH_2), 1.34 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.29, 162.71, 144.01, 139.19, 135.25, 131.92, 129.30, 129.20, 126.29, 122.91, 122.28, 120.41, 46.43, 26.56, 24.53, 22.64; HRMS (ESI) m/z calcd for $C_{22}H_{22}N_6O_3S_2$ $[M+H]^+$ 483.1273, found 483.1299.

2-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-5-(p-tolyl)-1,3,4-oxadiazole (2b)

White solid; Yield: 80 %; mp 250-252ºC; IR (KBr) ν_{max} 2956, 1565, 1314, 1154, 698; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.97 (1H, s, triazole H), 8.16 (2H, d, *J* = 8.0 Hz, Ar), 7.92 (2H, d, *J* = 8.0 Hz, Ar), 7.85 (2H, d, *J* = 7.6 Hz, Ar), 7.38 (2H, d, *J* = 7.6 Hz, Ar), 4.75 (2H, s, CH_2S), 2.91 (4H, brs, CH_2N), 2.38 (3H, s, CH_3), 1.53 (4H, brs, CH_2), 1.35 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.41, 162.34, 142.15, 139.20, 135.26, 129.85, 129.22, 126.27, 122.28, 120.43, 120.17, 46.44, 26.55, 24.54, 22.64, 21.00; HRMS (ESI) m/z calcd for $C_{23}H_{24}N_6O_3S_2$ $[M+H]^+$ 497.1429, found 497.1424.

2-(4-methoxyphenyl)-5-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4-

yl)methyl)thio)-1,3,4-oxadiazole (2c)

White solid; Yield: 83 %; mp: 256-258 ºC; IR (KBr) ν_{max} 2965, 1571, 1312, 1153, 705; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.94 (1H, s, triazole H), 8.15 (2H, d, J = 8.7 Hz, Ar), 7.90 (4H, t, J = 8.4 Hz, Ar), 7.11 (2H, d, J = 8.7 Hz, Ar), 4.72 (2H, s, CH_2S), 3.83 (3H, s, OCH_3), 2.91 (4H, brs, CH_2N), 1.53 (4H, brs, CH_2), 1.34 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.27, 161.94, 143.84, 139.24, 135.27, 129.07, 128.05, 122.00, 120.19, 115.27, 114.52, 73.55, 55.27, 46.43, 26.52, 24.59, 22.75; HRMS (ESI) m/z calcd for $C_{23}H_{24}N_6O_4S_2$ $[M+H]^+$ 513.1378, found 513.1370.

2-(4-fluorophenyl)-5-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)thio)-1,3,4-oxadiazole (2d)

White solid; Yield: 76 %; mp 246-248 ºC; IR (KBr) ν_{max} 2962, 1560, 1315, 1150, 695; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.95 (1H, s, triazole H), 8.15 (2H, d, J = 8.8 Hz, Ar), 7.99 (2H, brs, Ar), 7.92 (2H, d, J = 9.2 Hz, Ar), 7.41 (2H, t, J = 9.2 Hz, Ar), 4.74 (2H, s, CH_2S), 2.88 (4H, brs, CH_2N), 1.50 (4H, brs, CH_2), 1.32 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.61, 162.79, 144.03, 139.22, 135.30, 129.26, 129.13, 122.31, 120.47, 119.64, 116.71, 116.53, 46.48, 26.60, 24.58, 22.68; HRMS (ESI) m/z calcd for $C_{22}H_{21}FN_6O_3S_2$ $[M+H]^+$ 501.1178, found 501.1154.

2-(4-chlorophenyl)-5-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)thio)-1,3,4-oxadiazole

(2e)

White solid; Yield: 78 %; mp 238-240 ºC; IR (KBr) ν_{max} 2968, 1545, 1315, 1148, 710; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.93 (1H, s, triazole H), 8.12 (2H, d, J = 8.4 Hz, Ar), 7.94 (2H, d, J = 8.0 Hz, Ar), 7.88 (2H, d, J = 8.4 Hz,Ar), 7.62 (2H, d, J = 7.6 Hz, Ar), 4.72 (2H, s, CH_2S), 2.88 (4H, brs, CH_2N), 1.50 (4H, brs, CH_2), 1.31 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 163.08, 160.56, 144.11, 139.23, 136.71, 135.32, 129.54, 129.27, 128.17, 122.33, 121.84, 120.49, 46.49, 26.58, 24.58, 22.69; HRMS (ESI) m/z calcd for $C_{22}H_{21}ClN_6O_3S_2$ $[M+H]^+$ 517.0883, found 517.0853.

2-(4-bromophenyl)-5-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4- yl)-methyl)thio)-1,3,4-oxadiazole (2f)

White solid; Yield: 68 %; mp 252-254 ºC; IR (KBr) ν_{max} 2965, 1540, 1310, 1156, 720; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.93 (1H, s, triazole H), 8.13 (2H, d, J = 8.4 Hz, Ar), 7.87 (4H, t, J = 8.0 Hz, Ar), 7.76 (2H, d, J = 8.0 Hz, Ar), 4.72 (2H, s, CH_2S), 2.88 (4H, brs, CH_2N), 1.50 (4H, brs, CH_2), 1.32 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.72, 162.84, 144.01, 135.31, 132.46, 129.26, 128.28, 125.59, 122.33, 122.18, 120.48, 46.48, 26.58, 24.58, 22.68; HRMS (ESI) m/z calcd for $C_{22}H_{21}BrN_6O_3S_2$ $[M+H]^+$ 561.03782, found 561.0407.

2-(4-nitrophenyl)-5-(((1-(4-(piperidin-1-ylsulfonyl)phenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)thio)-1,3,4-oxadiazole (2g)

White solid; Yield: 64 %; mp 258-260 ºC; IR (KBr) ν_{max} 2986, 1542, 1316, 1152, 710; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.98 (1H, s, triazole H), 8.40 (2H, brs, Ar), 8.22 (2H, brs, Ar), 8.17 (2H ,brs, Ar), 7.93 (2H, brs, Ar), 4.81 (2H, s, CH_2S), 2.91 (4H, brs, CH_2N), 1.53 (4H, brs, CH_2), 1.34 (2H, brs, CH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.72, 162.84, 144.01, 135.31, 132.46, 129.26, 128.28, 125.59, 122.33, 122.18, 120.48, 46.48, 26.58, 24.58, 22.68; HRMS (ESI) m/z calcd for $C_{22}H_{21}N_7O_5S_2$ $[M+H]^+$ 528.1123, found 528.1161.

4-((4-(4-(((5-phenyl-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl)morpholine (3a)

White solid; Yield: 64 %; mp 254-256 ºC; IR (KBr) ν_{max} 2976, 1550, 1314, 1152, 702; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.99 (1H, s, triazole H), 8.18 (2H, brs, Ar), 7.94 (4H, brs, Ar), 7.58 (3H, brs, Ar), 4.76 (2H, s, CH_2S), 3.63 (4H, brs, CH_2O), 2.91 (4H, brs, CH_2N); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.34, 162.75, 144.09, 139.51, 134.16, 131.97, 129.51, 129.33, 126.33, 122.92, 122.35, 120.55, 65.18, 45.77, 26.60; HRMS (ESI) m/z calcd for $C_{21}H_{20}N_6O_4S_2$ $[M+H]^+$ 485.10657, found 485.1094.

4-((4-(4-(((5-(*p*-tolyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl)morpholine (3b)

White solid; Yield: 81 %; mp 251-253 ºC; IR (KBr) ν_{max} 2978, 1572, 1310, 1154, 705; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.98 (1H, s, triazole H), 8.20 (2H, d, *J* = 8.0 Hz, Ar), 7.93 (2H, d, *J* = 8.0 Hz, Ar), 7.85 (2H, d, *J* = 7.6 Hz, Ar), 7.38 (2H, d, *J* = 7.6 Hz, Ar), 4.75 (2H, s, CH_2S), 3.63 (4H, brs, CH_2O), 2.91 (4H, brs, CH_2N), 2.38 (3H, s, Me). ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.43, 162.35, 144.09, 142.16, 139.50, 134.16, 129.86, 129.50, 126.28, 122.32, 120.53, 120.19, 65.17, 45.76, 26.58, 21.02; HRMS (ESI) m/z calcd for $C_{22}H_{22}N_6O_4S_2$ $[M+H]^+$ 499.1222, found 499.1190.

4-((4-(4-(((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol -1-yl)phenyl)sulfonyl)morpholine (3c)

White solid; Yield: 76 %; mp 255-257 ºC; IR (KBr) ν_{max} 2984,, 1538 1317, 1156, 698; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.93 (1H, s, triazole H), 8.17 (2H, d, *J* = 8.4 Hz, Ar), 7.92 (2H, d, 8.7 Hz, Ar), 7.88 (2H, d, 8.7 Hz, Ar), 7.10 (2H, d, *J* = 8.7 Hz, Ar), 4.72 (2H, s, CH_2S), 3.82 (3H, s, OMe), 2.90 (4H, brs, CH_2N); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 165.32, 161.95, 143.82, 139.56, 134.22, 129.32, 128.03, 121.96, 120.27, 115.28, 114.45, 65.30, 55.23, 45.66, 26.50; HRMS (ESI) m/z calcd for $C_{22}H_{22}N_6O_5S_2$ $[M+H]^+$ 515.1171, found 515.1213.

4-((4-(4-(((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl-)thio)methyl)-1*H*-1,2,3-triazol- 1-yl)phenyl)sulfonyl)morpholine (3d)

White solid; Yield: 82 %; mp 247-249 ºC; IR (KBr) ν_{max} 2986, 1535, 1314, 1150, 689; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.98 (1H, s, triazole H), 8.19 (2H, d, *J* = 7.2 Hz, Ar), 8.03 (2H, dd, *J* = 8.8, 5.2 Hz, Ar), 7.93 (2H, d, *J* = 8.8 Hz, Ar), 7.43 (2H, t, *J* = 8.8 Hz, Ar), 4.76 (2H, s, CH_2S), 3.63 (4H, t, *J* = 4.4 Hz, CH_2O), 2.91 (4H, t, *J* = 4.4 Hz, CH_2N); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.57, 162.76, 144.03, 139.49, 134.16, 129.49, 129.09, 122.32, 120.53, 119.65, 116.67, 116.49, 65.16, 45.74, 26.56; HRMS (ESI) m/z calcd for $C_{21}H_{19}FN_6O_4S_2$ $[M+H]^+$ 503.0971, found 503.0999.

4-((4-(4-(((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl) thio)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl)morpholine (3e)

White solid; Yield: 77 %; mp 245-247 ºC; IR (KBr) ν_{max} 2984, 1545, 1315, 1152, 700;

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.95 (1H, s, triazole H), 8.16 (2H, d, J = 8.4 Hz, Ar), 7.94 (2H, d, J = 8.4 Hz, Ar), 7.90 (2H, s, J = 8.4 Hz, Ar), 7.62 (2H, d, J = 8.4 Hz, Ar), 4.75 (2H, s, CH_2S), 3.59 (4H, brs, CH_2O), 2.87 (4H, brs, CH_2N); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.59, 163.06, 144.04, 139.50, 136.72, 134.18, 129.51, 128.15, 122.35, 121.84, 120.56, 65.18, 45.76, 26.56; HRMS (ESI) m/z calcd for $C_{21}H_{19}ClN_6O_4S_2$ $[M+H]^+$ 519.0676, found 519.0692.

4-((4-(4-(((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol- 1-yl)phenyl)sulfonyl)morpholine (3f)

White solid; Yield: 75 %; mp 254-256 ºC; IR (KBr) ν_{max} 2986, 1540, 1314, 1152, 715; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.99 (1H, s, triazole H), 8.20 (2H, brs, Ar), 7.92 (4H, brs, Ar), 7.78 (2H, brs, Ar), 4.77 (2H, s, CH_2S), 3.63 (4H, brs, CH_2O), 2.91 (4H, brs, CH_2N); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.71, 163.10, 144.04, 139.51, 134.20, 132.45, 129.53, 128.28, 125.59, 122.36, 122.19, 120.58, 65.20, 45.78, 26.57; HRMS (ESI) m/z calcd for $C_{21}H_{19}BrN_6O_4S_2$ $[M+H]^+$ 563.0170, found 563.0155.

4-((4-(4-(((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)sulfonyl)morpholine (3g)

White solid; Yield: 65 %; mp 243-245 ºC; IR (KBr) ν_{max} 2976, 1545, 1314, 1154, 720; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 8.99 (1H, s, triazole H), 8.40 (2H, brs, Ar), 8.21 (4H, brs, Ar), 7.93 (2H, brs, Ar), 4.80 (2H, s, CH_2S), 3.63 (4H, brs, CH_2O), 2.91 (4H, brs, CH_2N); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 164.21, 163.95, 143.94, 139.49, 134.18, 132.43, 132.38, 129.50, 127.70, 124.53, 122.38, 122.21, 120.55, 65.17, 45.76, 26.55; HRMS (ESI) m/z calcd for $C_{21}H_{19}N_7O_6S_2$ $[M+H]^+$ 530.0916, found 530.0886.

CHAPTER 5

SYNTHESIS AND BIOLOGICAL EVALUATION OF 3,4-DIAMINO-1,2,4-TRIAZOLES AND THEIR SCHIFF'S BASE DERIVATIVES AS ANTI-MICROBIAL AND ANTI-OXIDANT AGENTS

5.1 Motivation for the Current Work

1,2,4-Triazoles are five-membered heterocyclic compounds having two carbon and three nitrogen atoms in their ring structures. Owing to their wide range of applications in various fields such as in agriculture, in material science, in coordination chemistry and in pharmaceuticals etc. these have attracted significant attention of researchers in the past few decades [186]. 1,2,4-Triazoles functionalized with amino groups have been well studied for anti-microbial, anti-tumor, anti-viral, anti-inflammatory, enzyme inhibitory action etc. biological activities [187].

Further, Schiff's base derivatives of 1,2,4-triazoles have also been well reported in literature as potent biological agents [188] exhibiting various biological activities such as anti-microbial, anti-cancer, anti-viral, anti-inflammatory, anti-tumor etc. [189].

Numerous marketed drugs such as nifuroxide (an anti-biotic drug), nitrofurantoin (an anti-microbial drug) and thioacetazone (anti-tubercular drug) etc. (Fig. 5.1) [190] have imine functionality in their structures.

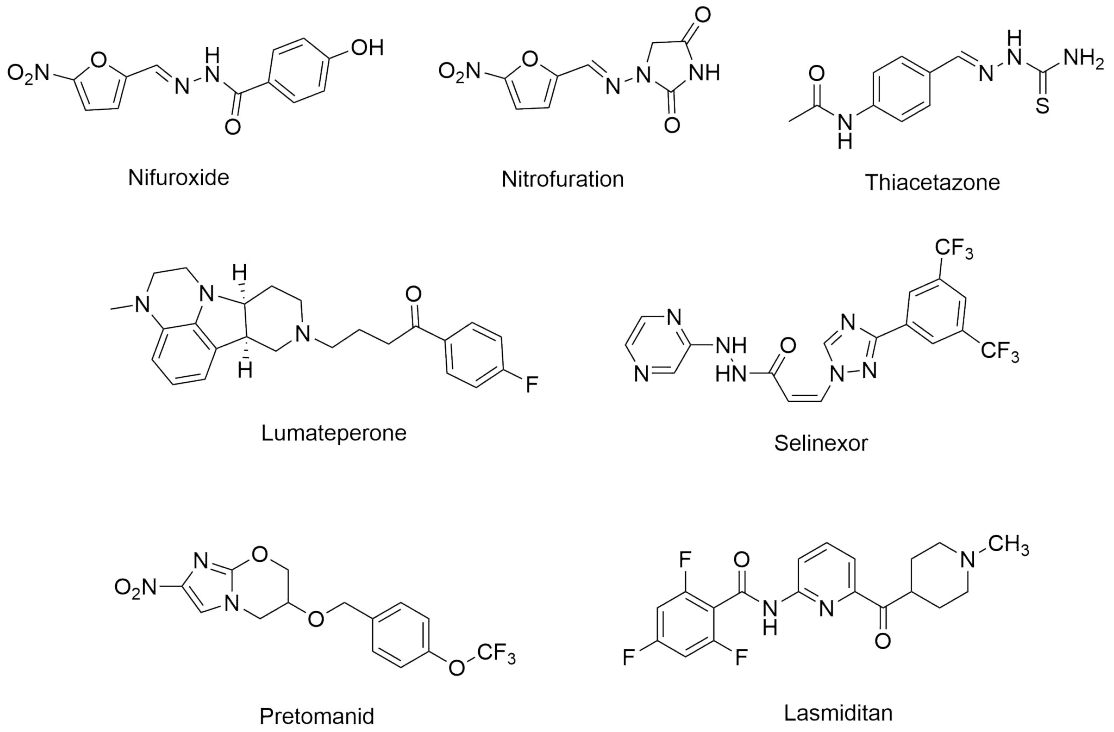
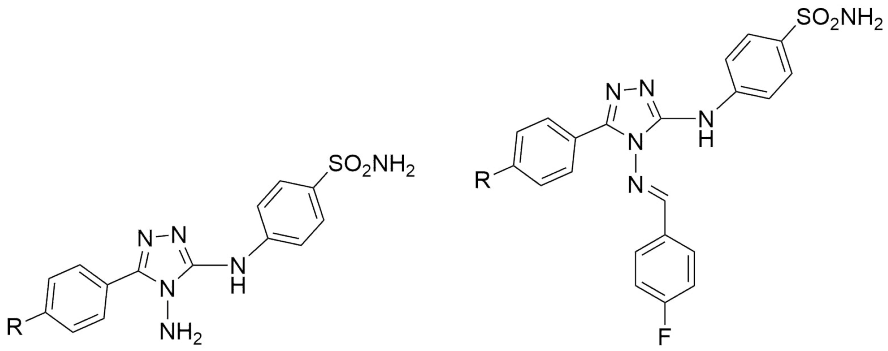


Fig. 5.1 Drugs having Schiff's base derivative/fluorine atom in their structures

Furthermore, fluorinated compounds have gained notable recognition in medicinal chemistry [191]. Due to high electronegativity and small atomic size of fluorine, introduction of fluorine in the structure of a compound can influence its properties such as increased thermal and chemical stability, increased lipophilicity, altered bond strength, intermolecular interactions and solubility [189]. Incorporation of fluorine atom into drug molecules in order to increase receptor binding affinity and metabolic stability of the drug have been well studied in literature [192]. Lumateperone (antipsychotic drug), selinexor (anti-cancer drug), pretomanid (anti-biotic drug) and lasmidita (abortive migrane drug) are the few examples of clinically used fluorinated drugs (Fig. 5.1) [193]. Motivated by the above study, we decided to further explore the research on 3,4-diamino-1,2,4-triazoles and their fluorinated Schiff's base derivatives in order to develop molecular architecture with biological applications in medicinal chemistry. In this chapter, we have given the synthesis of sixteen novel 3,4-diamino-1,2,4-triazoles **1** and their fluorinated Schiff's base derivatives **2** (Fig. 5.2) and their evaluation as

anti-microbial and anti-oxidant agents.



1 2

Fig. 5.2 3,4-Diamino-1,2,4-triazoles **1** and their fluorinated Schiff's base derivatives **2**

5.2 Results and Discussion

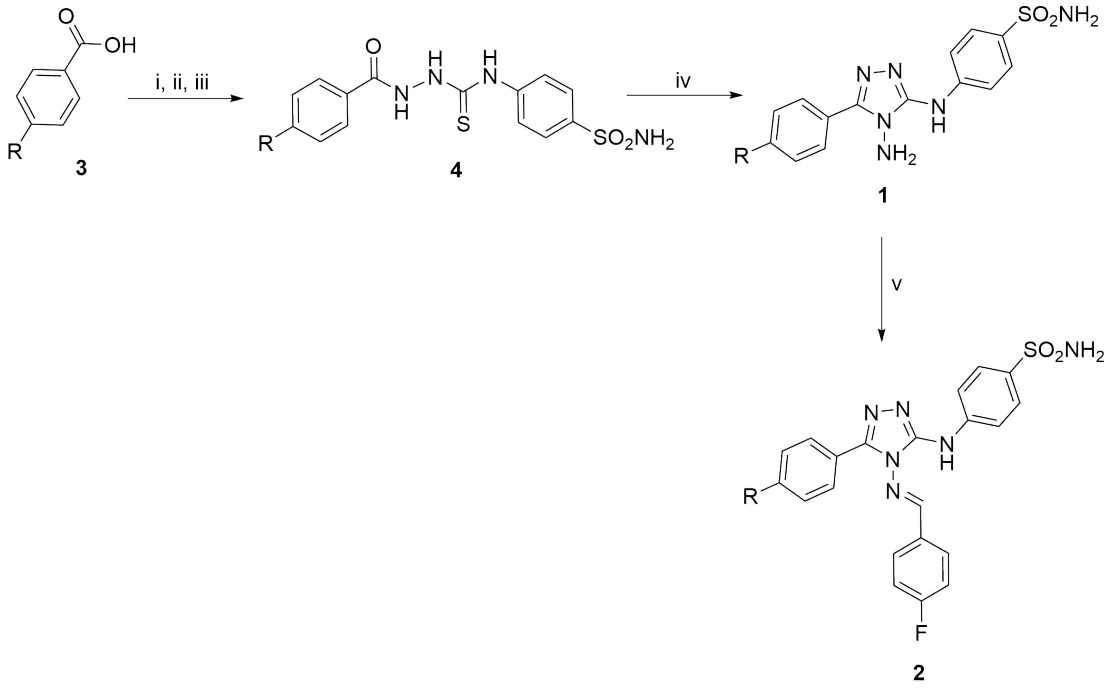
5.2.1 Synthesis overview of 3,4-diamino-1,2,4-triazoles 1a-1h and their Schiff's base derivatives 2a-2h

The route adopted for the synthesis of 3,4-diamino-1,2,4-triazoles **1** and their Schiff's base derivatives **2** is illustrated in scheme 5.1. Methodology starts with the synthesis of thiosemicarbazides **4** as intermediate from variously substituted aryl benzoic acids **3** using the methodology already disscussed in chapter 2 (Scheme 2.1). The reaction of thiosemicarbazides **4** with hydrazine hydrate under refluxing condition in DMSO as solvent resulted into the formation of target compounds **1**. Further, amino triazoles **1** were converted to their Schiff's base derivatives **2** by condensation reaction with 4-fluoro benzaldehyde under refluxing in glacial acetic acid. Final structures of all the target compounds **1** and **2** were confirmed by their spectral (IR, ^{1}H NMR,^{13}C NMR) and HRMS characterization data. Stepwise detailed discussion of the synthesis of target compounds **1a-1h** and **2a-2h** is given below.

5.2.2 Synthesis of 3,4-diamino-1,2,4-triazoles 1a-1h

For the synthesis of target compounds **1**, thiosemicarbazide derivatives **4** were treated with excess of hydrazine hydrate under reflux condition using DMSO as solvent. Mechanism for cyclization of **4** to target compounds **1** is depicted in scheme 5.2. The

first step of the mechanism involves nucleophilic attack of hydrazine hydrate on carbonyl carbon of thiosemicarbazide **4** resulting into intermediate **5** which upon



Compound 1-4	a	b	c	d	e	f	g	h
R	H	CH_3	OCH_3	F	Cl	Br	NO_2	SO_2NH_2

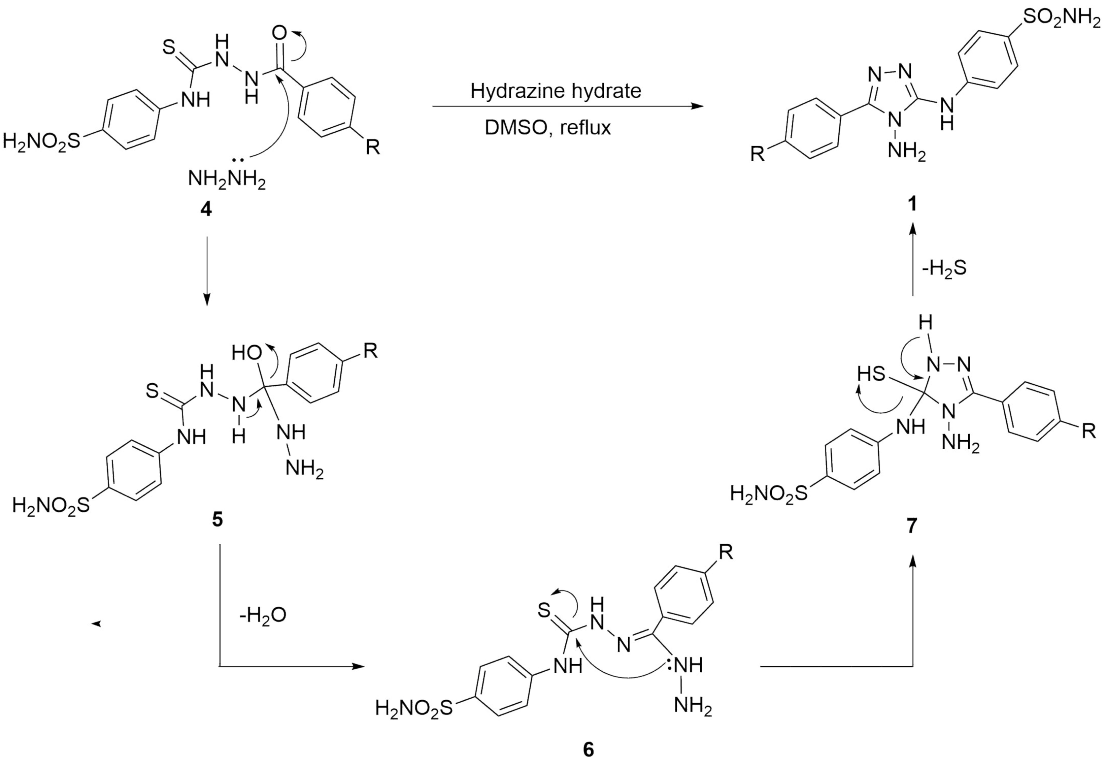
Scheme 5.1 Synthesis of target compounds **1a-1h** and **2a-2h**

Reagents and conditions: (i) Methanol, H_2SO_4, reflux; (ii) Hydrazine hydrate, methanol, reflux; (iii) 4-Isothiocyanato benzenesulfonamide, ethanol, reflux; (iv) Hydrazine hydrate, DMSO, reflux; (v) 4-F-Benzaldehyde, glacial acetic acid, reflux

subsequent loss of water molecule results into the formation of **6**. In the next step, cyclocondensation of thiocarbazone **6** occurs by the nucleophilic attack of nitrogen of carbohydrazide group on the thiocarbonyl carbon followed by removal of hydrogen sulfide molecule from intermediates **7** resulting into cyclized aminotriazoles **1** as products.

Formation of target compounds **1** were confirmed by the characteristic singlet around 6.0 ppm for -NH_2 proton, a singlet around 7.2 ppm for free -SO_2NH_2 group and a

singlet at approximately 9.10 ppm for N-H proton attached with 1,2,4-aminotriazole ring in ^{1}H-NMR (Fig. 5.3). In ^{13}C NMR (Fig. 5.4), peaks assigned to two carbon atoms of triazole ring were obtained at appoximately 124 ppm and 142 ppm. In IR spectra, absorption bands at approximately 3350-3300 cm^{-1} were assigned to the N-H stretch and at approximately 1700-1600 cm^{-1} for C=N bond formation. Molecular ion peaks in HRMS (Fig. 5.5) were also in accordance with the final structures of target compounds **1**.



Scheme 5.2 Synthesis of target compounds **1** with mechanism

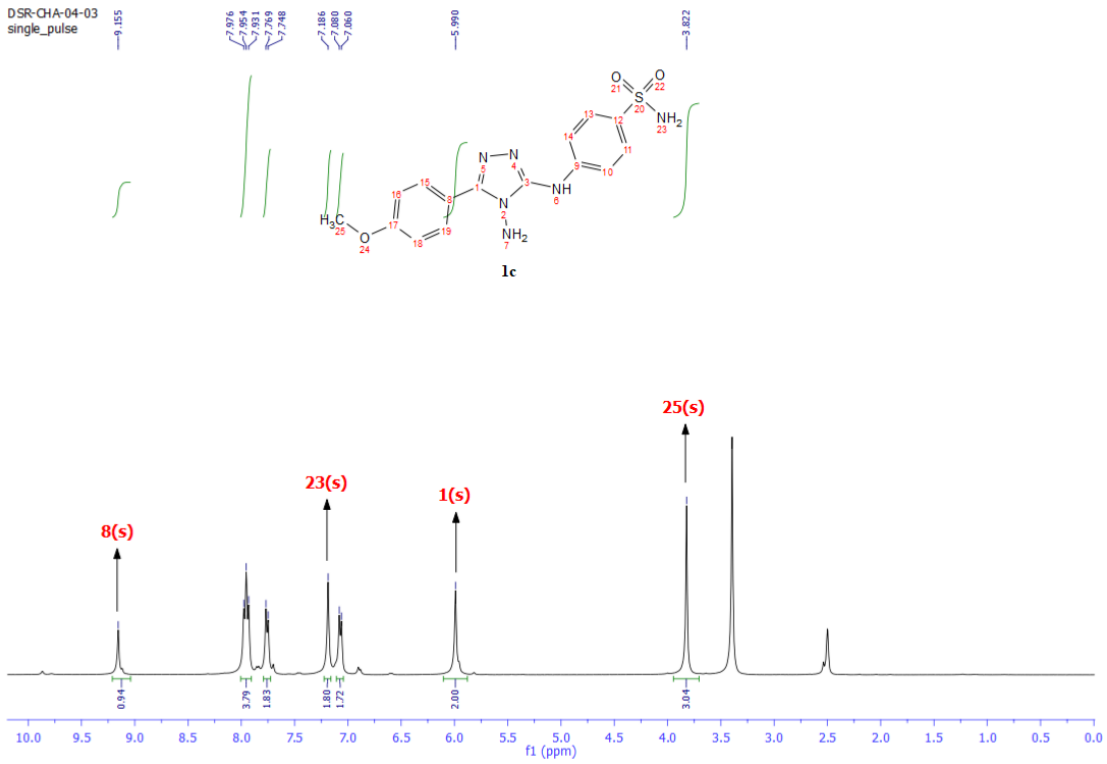


Fig. 5.3 ^{1}H NMR spectrum of compound **1c**

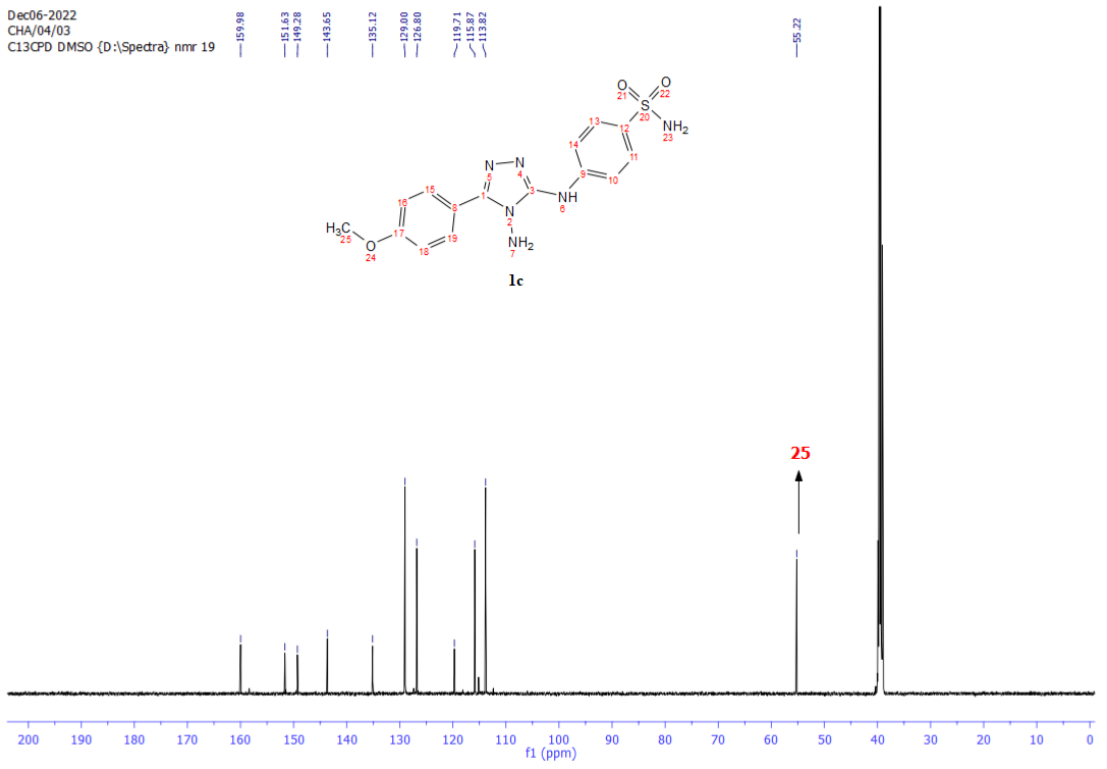


Fig. 5.4 ^{13}C NMR spectrum of compound **1c**

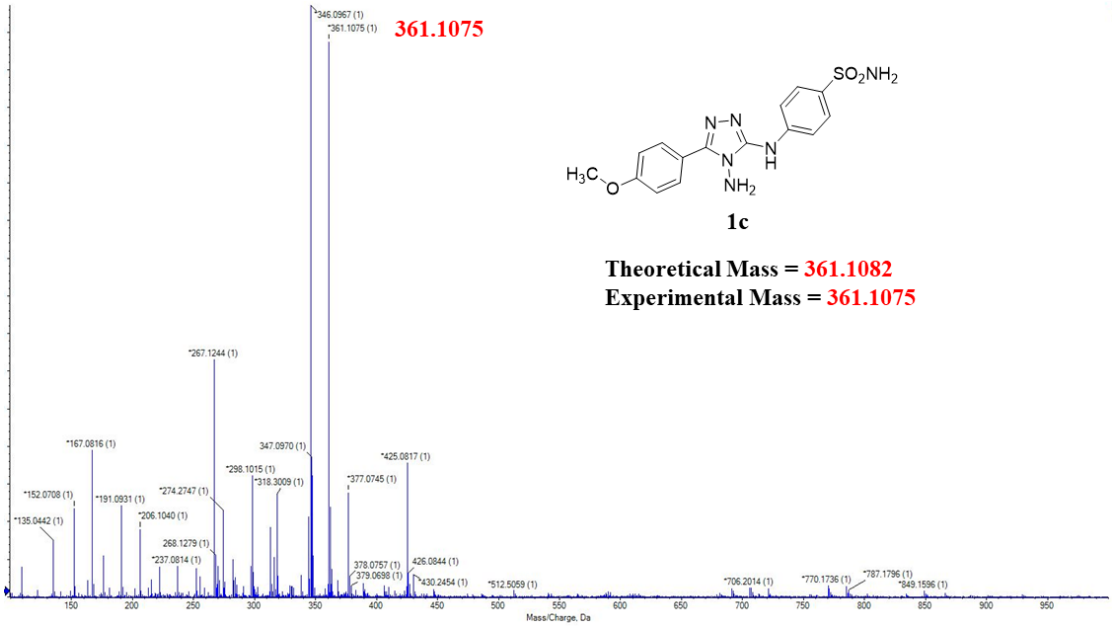
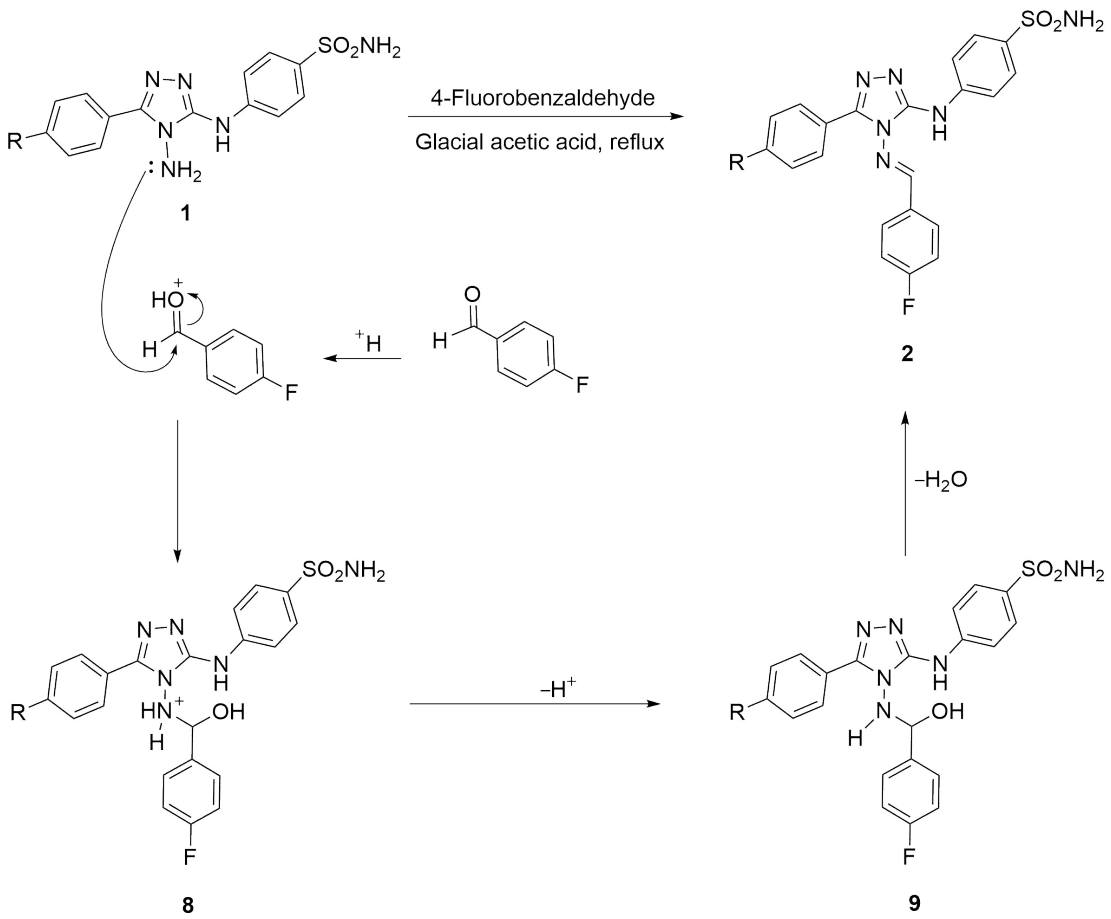


Fig. 5.5 HRMS spectrum of compound **1c**

5.2.3 Synthesis of Schiff's base derivatives 2a-2h

Schiff's base derivatives **2** were conveniently prepared by refluxing 3,4-diamino-1,2,4-triazoles **1** with 4-fluoro-benzaldehyde in glacial acetic acid. Mechanism for this condensation reaction of compounds **1** to Schiff's base derivatives **2** is depicted in scheme 5.3. The first step of mechanism involves nucleophilic attack of amino group of aminotriazole **1** on activated carbonyl group of 4-fluoro benzaldehyde resulting into the formation of intermediate **8**. Finally removal of water molecule from intermediate **8** results into the formation of targeted Schiff's base derivatives **2** [194]. Conversion of compounds **1** to **2** was confirmed by disappearance of singlet appearing at approximately 6.0 ppm for -NH_2 protons of triazole ring and appearance of additional peaks at approximately 8.85 ppm corresponding to imine proton (-CH=N-) in ^{1}H-NMR spectra (Fig. 5.6). In ^{13}C NMR (Fig. 5.7), a peak at approximately 163 ppm was obtained for the carbon atom of imine group. A strong band in-between 1550-1500 cm^{-1} in IR spectra corresponding to the C=N bond further confirmed the formation of Schiff's base derivative in the target molecules. The HRMS data showed molecular ion peaks which were in accordance with the molecular formulae of target compounds **2** (Fig. 5.8).



Scheme 5.3 Synthesis of target compounds **2** with mechanism

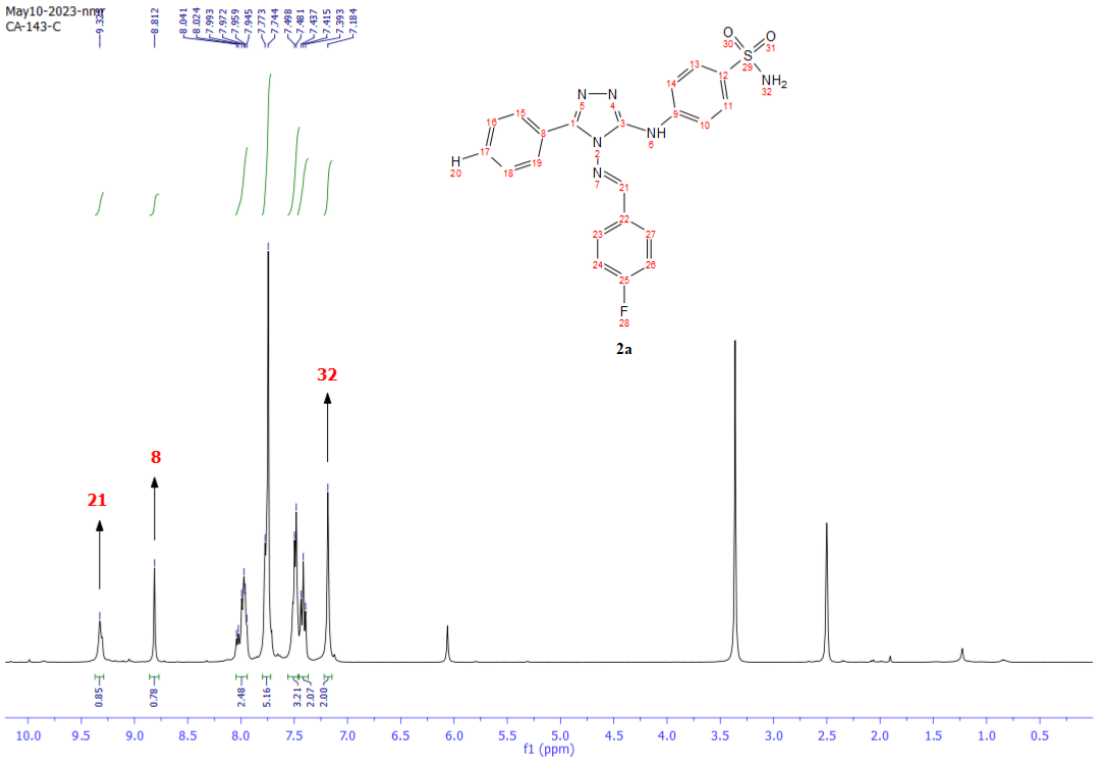


Fig. 5.6 ^{1}H NMR spectrum of compound **2a**

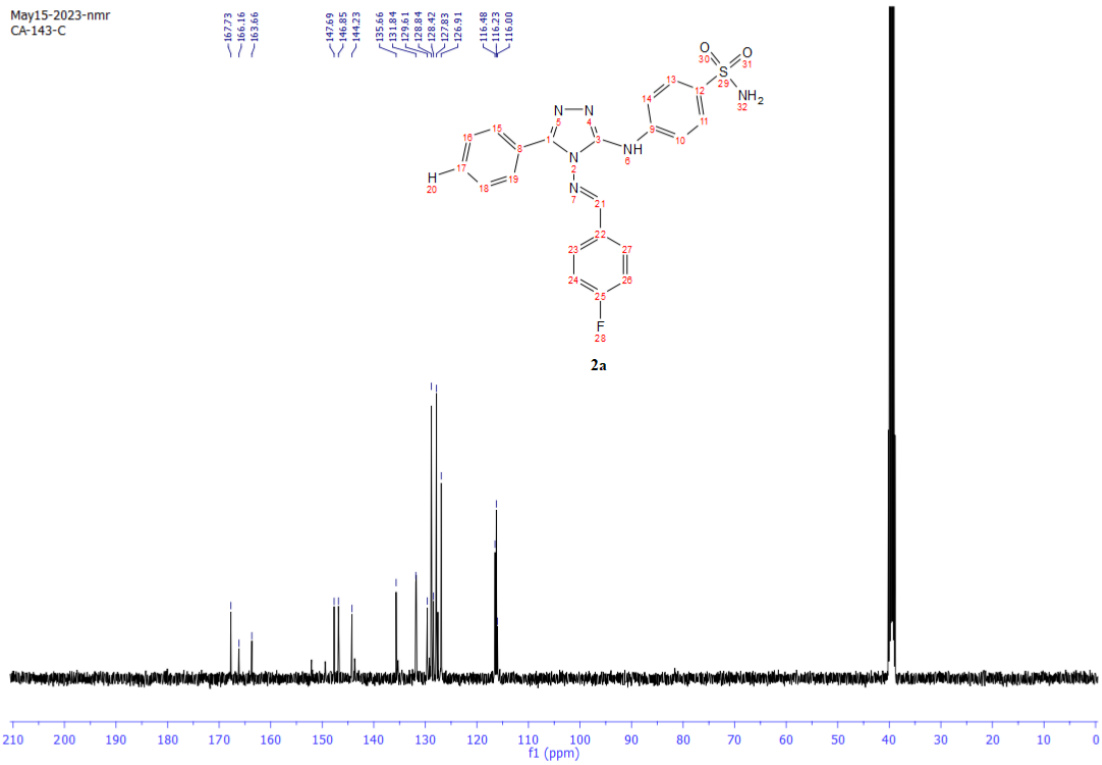


Fig. 5.7 ^{13}C NMR spectrum of compound **2a**

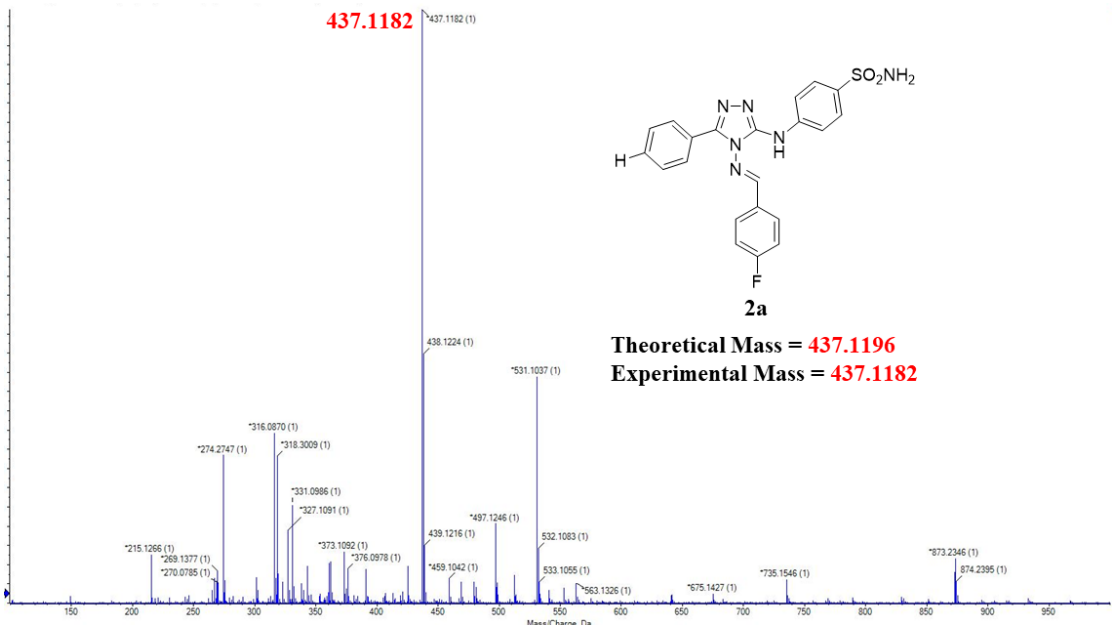


Fig. 5.8 HRMS spectrum of compound **2a**

5.3 Biological Testing Results

5.3.1 Anti-microbial assay

All the newly synthesized compounds **1a-1h** and **2a-2h** were screened for their anti-microbial profile using double dilution method against three Gram-positive (*Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657* , and *Bacillus cereus ATCC 11770)*, four Gram-negative (*Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733* and *Shigella flexneri ATCC 9199)* bacterial strains and one fungal strain (*Candida albicans MTCC 183*). The detailed procedure for anti-microbial assay has already been disscussed in chapter 2.

5.3.2 Anti-oxidant assay

All the newly synthesized compounds **1a-1h** and **2a-2h** were screened for their anti-oxidant profile using DPPH method and ascorbic acid as reference. The detailed procedure used for anti-oxidant assay has already been disscussed in chapter 2.

5.3.3 Results and discussion

A. Anti-microbial activity - results and discussion

All the synthesized target compounds **1a-1h** and **2a-2h** were tested for their potential to inhibit the growth of Gram-positive bacterial strains including *Staphylococcus aureus ATCC 6538 P, Listeria monocytogenes MTCC 657,* and *Bacillus cereus ATCC 11770* and Gram-negative bacterial strains including *Pseudomonas aeruginosa ATCC 15442, Escherichia coli MTCC 143, Salmonella typhi MTCC 733, Shigella flexneri ATCC 9199* and pathogenic fungal strain i.e. *Candida albicans MTCC 183* using double dilution method. Amoxicillin was used as reference drug for anti-bacterial activity while Fluconazole was used as reference drug for anti-fungal activity. All the tested compounds possessed good to excellent anti-bacterial as well as anti-fungal behavior in comparison to the reference drugs. Experiments were done in triplicates and results obtained are depicted in table 5.1. Graphical comparisons of anti-bacterial profile of tested compounds **1a-1h** and **2a-2h** with reference drugs Amoxicillin are shown in Fig. 5.9 and Fig. 5.10 respectively, while graphical comparison of anti-fungal profile all tested compounds with standard drug Fluconazole is shown in Fig. 5.11.

Table 5.1 MIC values of compounds **1a-1h** and **2a-2h** against tested bacterial and fungal strains

Compound	Minimum Inhibitory Concentration (MIC in µg/mL)[a]							
	Gram-positive bacterial strain			Gram-negative bacterial strains				Fungal strain
	S.aureus	*L.monocytogenes*	*B.cereus*	*P.aeruginosa*	*E.coli*	*S.typhi*	*S.flexneri*	*C.albicans*
1a	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
1b	12.5	6.25	12.5	12.5	12.5	12.5	6.25	12.5
1c	12.5	6.25	12.5	6.25	6.25	12.5	12.5	12.5

1d	3.12	6.25	3.12	3.12	3.12	3.12	3.12	6.25
1e	3.12	3.12	6.25	6.25	6.25	3.12	3.12	3.12
1f	3.12	6.25	12.5	6.25	6.25	6.25	3.12	6.25
1g	3.12	3.12	12.5	6.25	6.25	12.5	12.5	3.12
1h	3.12	6.25	6.25	12.5	6.25	6.25	6.25	3.12
2a	12.5	12.5	12.5	12.5	6.25	12.5	12.5	12.5
2b	12.5	12.5	12.5	12.5	12.5	12.5	6.25	12.5
2c	6.25	6.25	12.5	12.5	6.25	6.25	6.25	12.5
2d	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12
2e	3.12	3.12	6.25	3.12	3.12	3.12	3.12	6.25
2f	3.12	6.25	6.25	6.25	6.25	3.12	3.12	6.25
2g	3.12	6.25	6.25	12.5	6.25	6.25	3.12	3.12
2h	6.25	3.12	3.12	3.12	6.25	3.12	3.12	3.12
Amoxicillin[b]	3.12	6.25	6.25	3.12	6.25	3.12	3.12	-
Fluconazole[c]	-	-	-	-	-	-	-	3.12

[a]Mean of the three replicates
[b]Amoxicillin was used as positive control for anti-bacterial activity
[c]Fluconazole was used as positive control for anti-fungal activity

Following Structure-Activity Relationship has been drawn on the basis of the results obtained:

1. Compound **1d** containing 4-fluoro substituted phenyl group at triazole ring exhibited either equivalent or better activity than reference drug, Amoxicillin, against all bacterial strains tested while compound **2d** having two fluoro groups in the structure possessed highest inhibition potential against all bacterial strains tested.

2. Compounds having substitutions with electron withdrawing groups in the structure were found better inhibitors for microbial strains in comparison to the compounds

having substitutions with electron donating groups.

3. Compounds **1e**, **1g**, **1h**, **2d**, **2g** and **2h** were found to have excellent anti-fungal activities, even more than the reference drug, Fluconazole.

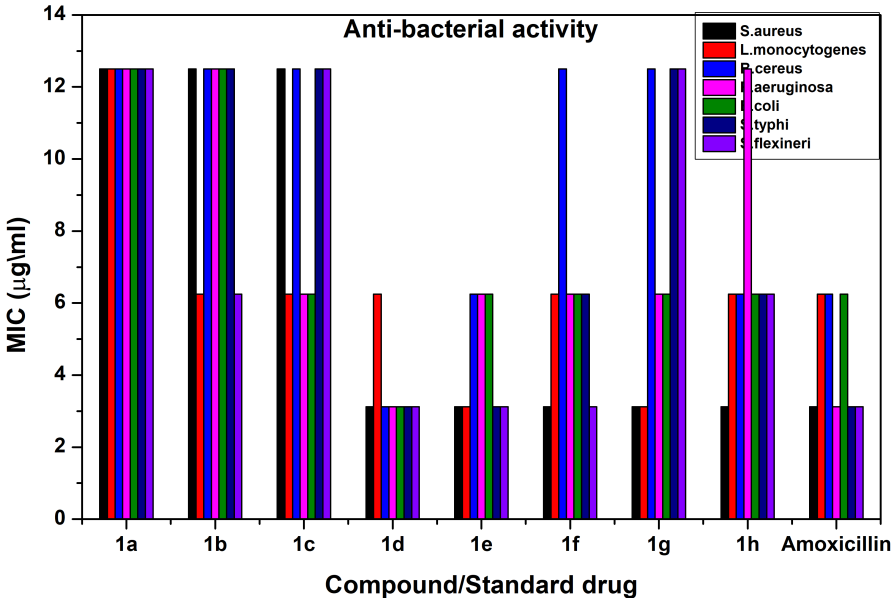


Fig. 5.9 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **1a-1h** and the standard drug Amoxicillin

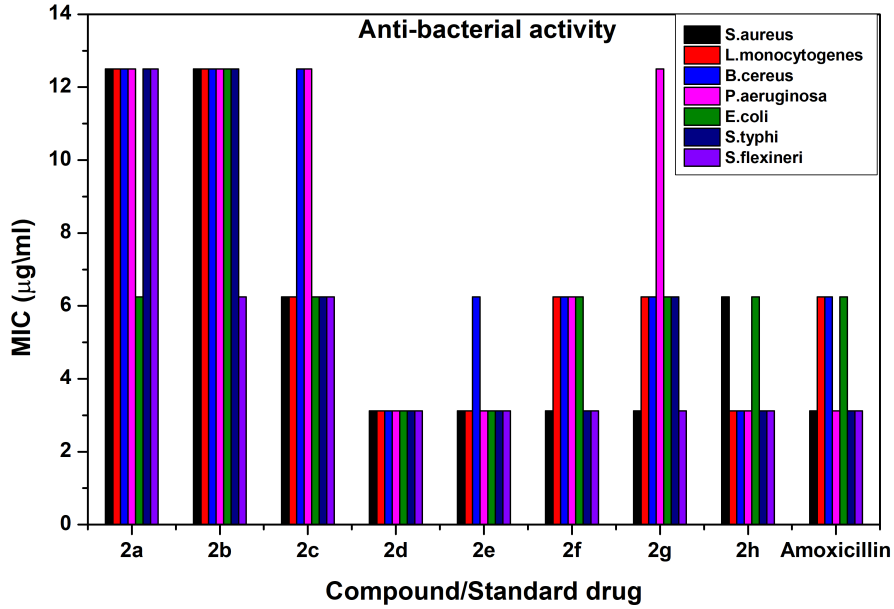


Fig. 5.10 Graphical comparison of anti-bacterial activity profile of newly synthesized compounds **2a-2h** and the standard drug Amoxicillin

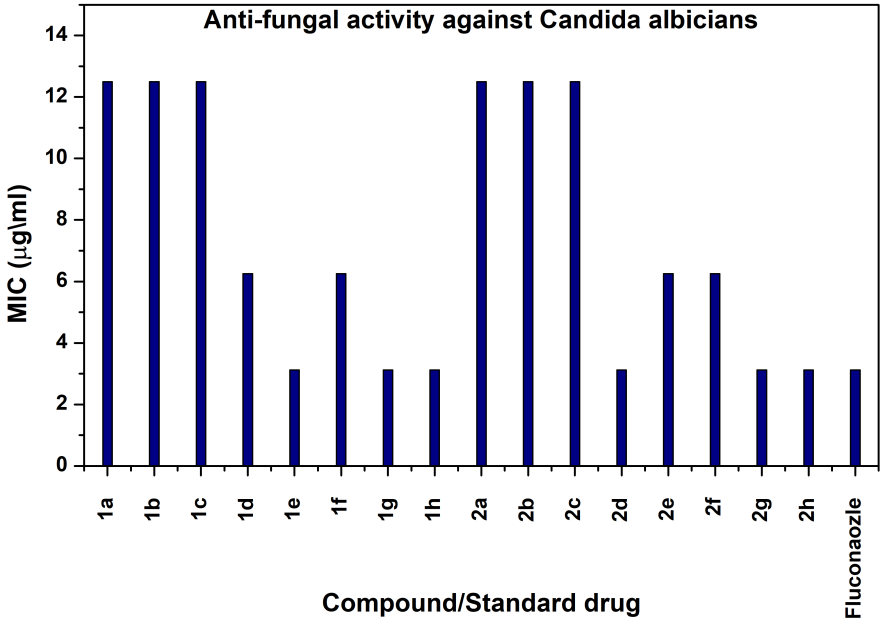


Fig. 5.11 Graphical comparison of anti-fungal activity profile of newly synthesized compounds **1a-1h**, **2a-2h** and the standard drug Fluconazole

B. Anti-oxidant activity - results and discussion

Free radicals are very harmful to the environment and contribute cell damage, aging, illness and many other chronic diseases to humans as well as animals. Anti-oxidant agents are free radical scavengers used to prevent damages caused by free radicals. We have investigated free radical scavenging activity of all the target compounds **1a-1h** and **2a-2h** synthesized in this chapter, using DPPH method with ascorbic acid scavenging activity value as reference. Results obtained reveal that all the tested compounds are moderated to excellent free radical scavengers in comparison to ascorbic acid (Table 5.2). Graphical comparison of anti-oxidant profile of tested compounds with ascorbic acid (AA) is shown in Fig. 5.12.

Following generalization can be drawn from the data obtained:

1. All the compounds were found to be moderate to excellent free radical scavengers with % RSA values ranging 71.12-92.20.

2. Compound **2e** possessed highest free radical scavenging activity with % RSA value 94.41.

Table 5.2 Anti-oxidant activities of compounds **1a-1h** and **2a-2h** using DPPH method

Compound	Anti-oxidant activity % RSA[a]	Compound	Anti-oxidant activity % RSA[a]
1a	85.40	**2b**	71.12
1b	80.20	**2c**	76.60
1c	75.45	**2d**	92.20
1d	90.90	**2e**	94.41
1e	78.56	**2f**	78.28
1f	84.40	**2g**	75.50
1g	81.11	**2h**	87.88
1h	89.34	**AA**	96.70
2a	77.25		

[a]Values were mean of three replicates % RSA, showed anti-oxidant potentials of the tested samples

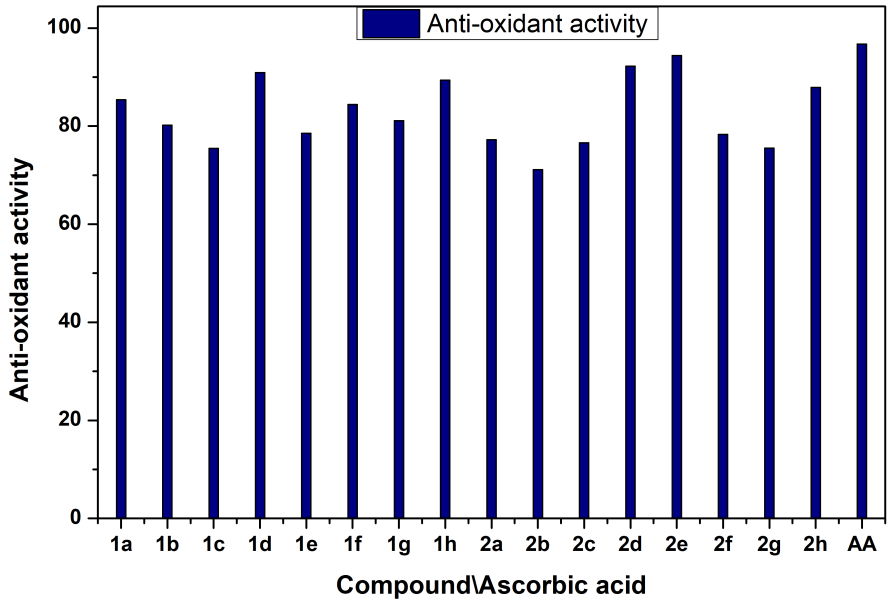


Fig. 5.12 Graphical comparison of anti-oxidant activity profile of compounds **1a-1h**, **2a-2h** and ascorbic acid (AA)

3. Among all, **2b** was found to be the weakest anti-oxidant agent with % RSA value 71.12.

5.3.4 Cytotoxicity

Cytotoxicity against plant seed germination cell line (vigna radiate seeds) and animal cell line (mouse fibroblast cell) was tested for all synthesized target compounds **1a-1h** and **2a-2h** using MTT assay. Cells were exposed overnight to the tested compounds having higher concentrations up to 1 mg/mL. All of the tested compounds were found safe against both the cells tested with 100 % cell viability values for plant seed germination cell line and 81.1-92.2 % for mouse fibroblast cell line (Table 5.3). The result obtained proved that the synthesized compounds are safe to be used during drug discovery.

Table 5.3 *In vitro* cytotoxic studies of compounds **1a-1h** and **2a-2h** against normal cells at the concentration of 1 mg/mL

Compound	Mouse fibroblast cell Cell Viability %[a]	Compound	Mouse fibroblast cell Cell Viability %[a]
1a	82.4	**2b**	88.8
1b	79.2	**2c**	89.9
1c	88.3	**2d**	88.4
1d	87.2	**2e**	87.6
1e	81.1	**2f**	91.2
1f	84.7	**2g**	89.8
1g	90.0	**2h**	87.8
1h	89.34	**DMSO**	76.5
2a	92.2		

[a]Mean of three replicates, showed the viability percentage on challenged with the tested compounds as compared to the control case

5.4 Conclusion

In this chapter, sixteen novel benzenesulfonamide bearing 1,2,4-triazoles **1** and their fluorinated Schiff's base derivatives **2** have been synthesized. Structures of all the synthesized target molecules were confirmed by their ^{1}H NMR, ^{13}C NMR, IR and HRMS data. Inhibition profile against bacterial and fungal strains was tested for all the target compounds **1** and **2**. Three Gram-positive (*Staphylococcus aureus ATCC 6538 P*, *Listeria monocytogenes MTCC 657*, and *Bacillus cereus ATCC 11770*), four Gram-negative (*Pseudomonas aeruginosa ATCC 15442*, *Escherichia coli MTCC 143*, *Salmonella typhi MTCC 733*, and *Shigella flexneri ATCC 9199*) and one fungal strain (*Candida albicans MTCC 183*) were included in the anti-microbial study. Amoxicillin and Fluconazole were used as reference drugs for anti-bacterial and anti-fungal activities, respectively. Results showed that all the tested compounds possessed good to excellent anti-microbial activities while compound **2d** possessed highest inhibition potential against all the bacterial strains tested. Anti-oxidant activity was also tested for all the final compounds **1** and **2** using DPPH method. Compound **2e** was found to exhibit highest anti-oxidant activity (94.41 %). Further, all the final compounds were found to be safe during cytotoxicity studies against mouse fibroblast and plant seed germination cell lines.

5.5 Experimental Section

All glassware were used after solvent wash and drying in an oven for 12 hrs. Melting points were determined in open glass capillaries in an electrical melting point apparatus and are uncorrected. The purity of the compounds was checked by ^{1}H NMR and thin layer chromatography (TLC) on silica gel plates using a mixture of petroleum ether and ethyl acetate as eluent. Iodine or UV lamp was used as a visualizing agent. The infrared (IR) spectra were recorded on FT-IR Perkin Elmer Spectrophotometer, CIL, JCBUST, YMCA, Faridabad. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance Neo 300/500 MHz and Jeol JNM-ECZ 400S 400 MHz NMR Spectrometer using TMS as internal standard. Chemical shifts are expressed in δ ppm. Mass data were recorded

on SYNAPT-XS#DBA064 and Agilent 7800 ICP-MS mainframe. The reference values for the residual solvent were taken as δ = 2.50 (DMSO-d_6) for ^{1}H NMR and δ = 39.54 (DMSO-d_6) for ^{13}C NMR. The abbreviations: s = singlet, d = doublet, dd = doublet of doublet, m = multiplet and ex = exchangeable protons are used for NMR assignments.

5.5.1 General procedure for the synthesis of target compounds 1a-1h

To a clear solution of compound **4** in DMSO solvent was added excess of hydrazine hydrate and refluxed for 24 hrs. The progress of reaction was monitored by TLC. After completion, reaction mixture was cooled to room temperature and poured into crushed ice. Solid was precipiated which was filtered, washed with excess of water and air dried. Finally, the product was recrystallized in ethanol.

4-((4-amino-5-phenyl-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (1a)

White solid; Yield: 75 %; mp 260-262 ºC; IR (KBr) ν_{max} 3342, 3260, 3030, 2975, 1610, 1311, 1156; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.17 (1H, s, NH), 8.02 (2H, d, *J* = 6.8 Hz, Ar), 7.95 (2H, d, *J* = 8.8 Hz, Ar), 7.75 (2H, d, *J* = 8.4 Hz, Ar), 7.69 (1H, brs, Ar), 7.50 (2H, d, *J* = 7.6 Hz, Ar), 7.17 (2H, ex, s, SO_2NH_2), 6.10 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 152.58, 149.97, 144.15, 135.79, 129.78, 128.98, 128.08, 127.88, 127.40, 116.54; HRMS (ESI) m/z calcd for $C_{14}H_{14}N_6O_2S$ $[M+H]^+$ 331.0977, found 331.0952.

4-((4-amino-5-(*p*-tolyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (1b)

White solid; Yield: 68 %; mp 265-267 ºC; IR (KBr) ν_{max} 3340, 3262, 3034, 2975, 1618, 1314, 1154; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) 9.12 (1H, s, NH), 7.93 (2H, d, *J* = 9.0 Hz, Ar), 7.91 (2H, d, *J* = 8.0 Hz, Ar), 7.75 (2H, d, *J* = 8.5 Hz, Ar), 7.32 (2H, d, *J* = 8.0 Hz, Ar), 7.15 (2H, ex, s, SO_2NH_2), 5.98 (2H, s, NH_2), 2.37 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 151.88, 149.45, 143.67, 138.80, 135.23, 128.98, 127.45, 126.85, 124.58, 115.97, 20.95; HRMS (ESI) m/z calcd for $C_{15}H_{16}N_6O_2S$ $[M+H]^+$ 345.1133, found 345.1132.

4-((4-amino-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonam-

ide (1c)

White solid; Yield: 65 %; mp 268-270 ºC; IR (KBr) ν_{max} 3335, 3261, 3034, 2976, 1612, 1312, 1156; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.15 (1H, s, NH), 7.96 (2H, d, *J* = 8.8 Hz, Ar), 7.94 (2H, d, *J* = 9.2 Hz, Ar), 7.75 (2H, d, *J* = 8.4 Hz, Ar), 7.18 (2H, ex, s, SO_2NH_2), 7.07 (2H, d, *J* = 8.0 Hz, Ar), 5.99 (2H, s, NH_2), 3.82 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 159.98, 151.63, 149.28, 143.65, 135.12, 129.00, 126.80, 119.71, 115.87, 113.82, 55.22; HRMS (ESI) m/z calcd for $C_{15}H_{16}N_6O_3S$ $[M+H]^+$ 361.1082, found 361.1075.

4-((4-amino-5-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (1d)

White solid; Yield: 67 %; mp 254-256 ºC; IR (KBr) ν_{max} 3338, 3262, 3032, 2974, 1616, 1316, 1154; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.23 (1H, s, NH), 8.016 (2H, d, *J* = 6.8 Hz, Ar), 7.94 (2H, d, *J* = 8.8 Hz, Ar), 7.75 (2H, d, *J* = 8.8 Hz, Ar), 7.50 (2H, d, *J* = 8.0 Hz, Ar), 7.18 (2H, ex, s, SO_2NH_2), 6.03 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 159.98, 153.63, 149.26, 143.54, 134.14, 129.20, 125.82, 120.74, 115.82, 112.81; HRMS (ESI) m/z calcd for $C_{14}H_{13}FN_6O_2S$ $[M+H]^+$ 348.0804, found 349.0834.

4-((4-amino-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (1e)

White solid; Yield: 62 %; mp 258-260 ºC; IR (KBr) ν_{max} 3342, 3254, 3034, 2974, 1622, 1316, 1156; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 9.20 (1H, s, NH), 8.06 (2H, d, *J* = 8.7 Hz, Ar), 7.93 (2H, d, *J* = 9.0 Hz, Ar), 7.75 (2H, d, *J* = 8.7 Hz, Ar), 7.59 (2H, d, *J* = 8.4 Hz, Ar), 7.17 (2H, ex, s, SO_2NH_2), 6.00 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 152.52, 147.64, 144.62, 135.46, 131.40, 129.65, 127.95, 126.74, 123.77, 115.11; HRMS (ESI) m/z calcd for $C_{14}H_{13}ClN_6O_2S$ $[M+H]^+$/ $[M+H+2]^+$ 365.0587/367.0558, found .365.0577/367.0556.

4-((4-amino-5-(4-bromophenyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (1f)

White solid; Yield: 70 %; mp 253-255 ºC; IR (KBr) ν_{max} 3340, 3250, 3025, 2968, 1630, 1314, 1152; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.27 (1H, s, NH), 7.99 (2H, d, *J* = 7.6 Hz, Ar), 7.92 (2H, d, *J* = 9.0 Hz, Ar), 7.74 (2H, d, *J* = 8.8 Hz, Ar), 7.273-7.017 (4H, m, Ar & SO_2NH_2), 6.03 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 152.32, 148.63, 143.64, 135.44, 131.50, 129.45, 126.94, 126.64, 122.76, 116.11; HRMS (ESI) m/z calcd for $C_{14}H_{13}BrN_6O_2S$ $[M+H]^+$/ $[M+H+2]^+$ 409.0082 /411.0061, found 409.0078/411.0058.

4-((4-amino-5-(4-nitrophenyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (1g)

White solid; Yield: 58 %; mp 270-272 ºC; IR (KBr) ν_{max} 3336, 3246, 3020, 2965, 1625, 1314, 1156; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.19 (1H, s, NH), 8.04 (2H, d, *J* = 8.8 Hz, Ar), 7.86 (2H, d, *J* = 9.2 Hz, Ar), 7.80 (2H, d, *J* = 8.8 Hz, Ar), 7.30 (2H, ex, s, SO_2NH_2), 6.76 (2H, d, *J* = 8.4 Hz, Ar), 6.04 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 151.71, 150.55, 150.36, 144.30, 135.48, 129.10, 127.31, 116.32, 114.78, 113.73; HRMS (ESI) m/z calcd for $C_{14}H_{13}N_7O_4S$ $[M+H]^+$ 376.0828, found 376.0845.

4-(4-amino-5-((4-sulfamoylphenyl)amino)-4*H*-1,2,4-triazol-3-yl)benzenesulfonamide (1h)

White solid; Yield: 65 %; mp 278-280 ºC; IR (KBr) ν_{max} 3336, 3240, 3024, 2960, 1635, 1314, 1154; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.26 (1H, s, NH), 8.21 (2H, d, *J* = 8.8 Hz, Ar), 7.91 (2H, d, *J* = 8.8 Hz, Ar), 7.91 (2H, d, *J* = 8.8 Hz, Ar), 7.74 (2H, d, *J* = 8.8 Hz, Ar), 7.46 (2H, ex, s, SO_2NH_2), 7.16 (2H, ex, s, SO_2NH_2), 6.04 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 152.96, 148.89, 144.81, 143.94, 135.96, 130.81, 128.18, 127.37, 126.22, 116.61; HRMS (ESI) m/z calcd for $C_{14}H_{15}N_7O_4S_2$ $[M+H]^+$ 410.0705, found 410.0701.

5.5.2 General procedure for the synthesis of target compounds 2a-2h

To a clear solution of compound **1** in glacial acetic acid was added 4-fluoro benzaldehyde (1 equivalent) and refluxed the reaction mixture for 2 hrs. Progress of reaction was monitored by TLC. After completion of reaction, the resulting mixture was

poured into crushed ice. A solid was precipitated out which was filtered, washed with excess of cold water, dried and recrystallized in ethanol to obtain pure product.

(E)-4-((4-((4-fluorobenzylidene)amino)-5-phenyl-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (2a)

White solid; Yield: 62 %; mp 276-278 ºC; IR (KBr) ν_{max} 3320, 3234, 3010, 2975, 1615, 1545, 1314, 1153, 1095; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.32 (1H, s, NH), 8.81 (1H, s, CH), 8.04-7.94 (2H, m, Ar), 8.0-7.72 (5H, m, Ar), 7.56-7.46 (4H, m, Ar), 7.41 (2H, t, J = 8.8 Hz, Ar), 7.18 (2H, ex, s, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 126 MHz) δ (ppm) 167.73, 166.16, 163.66, 147.69, 146.85, 144.23, 135.66, 131.84, 129.61, 128.84, 128.42, 127.83, 126.91, 116.48, 116.23, 116.00; HRMS (ESI) m/z calcd for $C_{21}H_{17}FN_6O_2S$ $[M+H]^+$ 437.1196, found 437.1182.

(E)-4-((4-((4-fluorobenzylidene)amino)-5-(p-tolyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (2b)

White solid; Yield: 70 %; mp 310-312 ºC; IR (KBr) ν_{max} 3330, 3250, 2998, 1606, 1534, 1346, 1162, 1090; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.27 (1H, s, NH), 8.78 (1H, s, CH), 7.987 (2H, d, J = 6.8 Hz, Ar), 7.80-7.70 (4H, m, Ar), 7.64 (2H, J = 8.0 Hz, Ar), 7.41 (2H, t, J = 8.8 Hz, Ar), 7.29 (2H, J = 8.4 Hz, Ar), 7.19 (2H, ex, s, SO_2NH_2), 2.33 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 168.42, 164.16, 148.09, 147.31, 144.66, 139.84, 136.05, 132.38, 132.29, 129.93, 128.26, 127.43, 124.44, 117.02, 116.80, 116.70, 21.44; HRMS (ESI) m/z calcd for $C_{22}H_{19}FN_6O_2S$ $[M+H]^+$ 451.1352, found 451.1361.

(E)-4-((4-((4-fluorobenzylidene)amino)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-yl)amino)benzenesulfonamide (2c)

White solid; Yield: 72 %; mp 315-317 ºC; IR (KBr) ν_{max} 3332, 3254, 2990, 1610, 1525, 1342, 1160, 1095; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.22 (1H, s, NH), 8.67 (1H, s, CH), 7.87 (2H, d, J = 7.6 Hz, Ar), 7.71-7.67 (4H, m, Ar), 7.64 (2H, J = 8.0 Hz, Ar), 7.19 (2H, ex, s, SO_2NH_2), 7.11 (2H, t, J = 8.8 Hz, Ar), 7.03 (2H, J = 8.8 Hz, Ar), 3.77 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 169.42, 164.48, 148.29, 147.35, 145.67, 139.84, 136.55, 133.38, 131.29, 129.95, 129.27, 128.43, 126.45, 119.06, 116.94,

116.56, 56.23.; HRMS (ESI) m/z calcd for $C_{22}H_{19}FN_6O_3S$ $[M+H]^+$ 467.1302, found 451.1294.

(E)-4-((4-((4-fluorobenzylidene)amino)-5-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl)-amino)benzenesulfonamide (2d)

White solid; Yield: 65 %; mp 312-314 ºC; IR (KBr) ν_{max} 3335, 3256, 2996, 1615, 1528, 1344, 1164, 1105; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.34 (1H, s, NH), 8.84 (1H, s, CH), 8.08-8.00 (4H, m, Ar), 7.86 (2H, t, *J* = 8.8 Hz, Ar), 7.74 (2H, brs, Ar), 7.27 (2H, brs, Ar), 7.17 (2H, ex, s, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 169.42, 164.48, 148.29, 147.35, 145.67, 139.84, 136.55, 133.38, 131.29, 129.95, 129.27, 128.43, 126.45, 119.06, 116.94, 116.56, 56.23; HRMS (ESI) m/z calcd for $C_{21}H_{16}F_2N_6O_2S$ $[M+H]^+$ 455.1102, found 455.1117.

(E)-4-((5-(4-chlorophenyl)-4-((4-fluorobenzylidene)amino)-4*H*-1,2,4-triazol-3-yl)-amino)benzenesulfonamide (2e)

White solid; Yield: 76 %; mp 318-320 ºC; IR (KBr) ν_{max} 3338, 3250, 2998, 1614, 1520, 1342, 1162, 1101; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.31 (1H, s, NH), 8.82 (1H, s, CH), 7.99 (2H, t, *J* = 7.5, Ar), 7.79 (2H, d, *J* = 8.4 Hz, Ar), 7.73 (2H, d, *J* = 8.4 Hz, Ar), 7.57 (2H, d, *J* = 8.4 Hz, Ar), 7.42 (2H, *J* = 8.8 Hz, Ar), 7.17 (2H, ex, s, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 168.73, 164.22, 148.26, 146.52, 144.55, 136.18, 134.89, 132.48, 132.39, 130.00, 129.52, 127.43, 126.15, 117.04, 116.81; HRMS (ESI) m/z calcd for $C_{21}H_{16}ClFN_6O_2S$ $[M+H]^+$/ $[M+H+2]^+$ 471.0806/473.0777, found 471.0808/473.0792.

(E)-4-((5-(4-bromophenyl)-4-((4-fluorobenzylidene)amino)-4*H*-1,2,4-triazol-3-yl)-amino)benzenesulfonamide (2f)

White solid; Yield: 65 %; mp 325-327 ºC; IR (KBr) ν_{max} 3336, 3255, 2997, 1605, 1524, 1344, 1160, 1104; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.33 (1H, s, NH), 8.84 (1H, s, CH), 7.94 (2H, t, *J* = 6.8, Ar), 7.74 (2H, d, *J* = 8.0 Hz, Ar), 7.72 (2H, d, *J* = 8.0 Hz, Ar), 7.54 (2H, d, *J* = 8.4 Hz, Ar), 7.40 (2H, t, *J* = 8.4 Hz, Ar), 7.16 (2H, ex, s, SO_2NH_2); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 168.54, 164.76, 147.24, 146.36, 144.50, 135.86, 134.84, 132.28, 132.12, 130.10, 128.25, 126.43, 126.10, 116.06, 115.31; HRMS (ESI) m/z calcd for $C_{21}H_{16}BrFN_6O_2S$ $[M+H]^+$/ $[M+H+2]^+$ 514.0223/516.0202, found 514.0228/516.0216.

(E)-4-((4-((4-fluorobenzylidene)amino)-5-(4-nitrophenyl)-4*H*-1,2,4-triazol-3-yl)-amino)benzenesulfonamide (2g)

White solid; Yield: 56 %; mp 306-308 ºC; IR (KBr) ν_{max} 3328, 3246, 2976, 1612, 1522, 1340, 1162, 1084; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.32 (1H, s, NH), 8.86 (1H, s, CH), 8.06 (2H, d, *J* = 8.4, Ar), 7.90 (2H, d, *J* = 8.4 Hz, Ar), 7.82 (2H, d, *J* = 8.4 Hz, Ar), 7.40 (2H, t, *J* = 8.4 Hz, Ar), 7.16 (2H, ex, s, SO_2NH_2), 6.78 (2H, d, *J* = 8.0 Hz, Ar); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 169.12, 165.67, 151.72, 150.24, 144.53, 135.76, 133.82, 132.26, 132.15, 130.10, 129.22, 127.44, 126.10, 116.30, 113.24; HRMS (ESI) m/z calcd for $C_{21}H_{16}FN_7O_4S$ $[M+H]^+$ 482.1047, found 482.1068.

(E)-4-(4-((4-fluorobenzylidene)amino)-5-((4-sulfamoylphenyl)amino)-4*H*-1,2,4-triazol-3-yl)benzenesulfonamide (2h)

White solid; Yield: 65 %; mp 328-330 ºC; IR (KBr) ν_{max} 3312, 3228, 2982, 1614, 1515, 1342, 1164, 1099; ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 9.41 (1H, s, NH), 8.91 (1H, s, CH), 8.13 (2H, d, *J* = 8.4, Ar), 8.05 (2H, d, *J* = 8.4 Hz, Ar), 8.02 (2H, d, *J* = 8.4 Hz, Ar), 7.96 (2H, d, *J* = 8.4 Hz, Ar), 7.61 (2H, ex, s, SO_2NH_2), 7.49 (2H, t, *J* = 8.0 Hz, Ar), 7.23 (2H, ex, s, SO_2NH_2), 6.78 (2H, d, *J* = 8.0 Hz, Ar); ^{13}C NMR (DMSO-d_6, 101 MHz) δ (ppm) 169.25, 165.81, 152.62, 150.24, 144.75, 135.86, 133.87, 132.26, 132.15, 130.80, 128.18, 127.38, 126.20, 116.60, 113.20; HRMS (ESI) m/z calcd for $C_{21}H_{18}FN_7O_4S_2$ $[M+H]^+$ 516.0924, found 516.0929.

CHAPTER 6

CONCLUSION AND FUTURE PERSPECTIVES

Microbial infections have became a serious health problem all over the world. Further, increasing number of pathogenic micobes and over-use of anti-micorbial drugs has led to the development of multi-drug resistance, another serious key factor causing increase in morbidity as well as mortality rate. The latest report of World Health Organisation reveals an increase in resistance to treatment of bacterial infections and alarms that despite of large progress in the field of synthesis of novel anti-microbial agents capable to combat with multi-drug resistance, there is still enormous scope for work in this area. In the fight to overcom multi-drug resistance and develop new options for treatment of microbial infections, we have synthesized 81 novel benzenesulfonamide containing triazole and oxadiazole derivatives as potent anti-microbial agents. The hitherto unreported final compounds were characterized and confirmed for their structures by their spectral (^{1}H NMR, ^{13}C NMR, and IR) and HRMS data and were found sufficiently pure for further evaluation. All the newly synthesized compounds were evaluated against three Gram-positive pathogenic bacterial strains (*Staphylococcus aureus ATCC 6538 P*, *Listeria monocytogenes MTCC 657*, and *Bacillus cereus ATCC 11770*), four Gram-negative pathogenic bacterial strains (*Pseudomonas aeruginosa ATCC 15442*, *Escherichia coli MTCC 143*, *Salmonella typhi MTCC 733*, and *Shigella flexneri ATCC 9199*) and one pathogenic fungal strain (*Candida albicans MTCC 183*). In addition to anti-microbial evaluation, all the compounds were also screened for anti-oxidant activity and cytotoxicity study in order to explore their more biological applications. 21 out of the 81 target compounds, were evaluated for their cytotoxicity against a breast cancer cell line i.e. Michigan Cancer Foundation Cell (MCF-7) also. Further, chapterwise conclusion of the thesis is given as follows.

In Chapter 1, a succinct compilation of the triazole-benzenesulfonamide hybrids and oxadiazoles as potential biological agents has been given. The compilation of such literature reports at one place has driven and motivated us to work further in this area of developing novel drug candidates having triazole and oxadiazole nucleus in their pharmacophoric unit.

Synthesis of a series of thirty novel 1,2,4-triazole derivatives having benzenesulfonamide moiety **1a-1h**, **2a-2h**, **3a-3h**, **4b-4f** and **4h** and their biological evaluation as anti-microbial and anti-oxidant agents as well as cytotoxicity study has been described in Chapter 2. *In vitro* anti-microbial evaluation showed that most of the synthesized compounds could effectively inhibit growth of all tested Gram-positive and Gram-negative bacterial strains as well as the tested fungal strain. Particularly, compound **3d** showed equivalent or better anti-microbial activity when compared with standard drug Amoxicillin. All the compounds were either equivalent or weaker inhibitors against tested fungal strain when compared with Fluconazole, a reference drug. Synthesized compounds were also screened for anti-oxidant profile using DPPH method and most of them were found moderate to excellent free radical scavengers. Compound **4d** was found to possess highest anti-oxidant activity with control value 94.35 %. All the compounds were also found to be safe against mouse fibroplast cell line and plant seed germination cell line. These results suggested that benzenesulfonamide incorporated 1,2,4-triazole derivatives could be potential candidates for treatment of bacterial infections and for the development of new potent anti-oxidant agents.

In chapter 3, we have described the synthesis of a library of fourteen new derivatives of 1,2,3-triazole tethered 1,2,4-triazoles **1** and **2** as potent biological agents. All the tested compounds exhibited good to excellent anti-bacterial and anti-fungal activities when compared with reference drugs. Compounds **1e**, **1f**, **2e** and **2f** were found equivalent or better than reference drug as anti-bacterial agent while **1d** and **2d** were most efficient among all the newly synthesized compounds against tested fungal strain. Results showed that the conversion of precursors **8** to target compound **1** and **2** cause higher inhibition potential against bacterial strains tested. Anti-oxidant profile was also tested for all the synthesized compounds and most of them were found moderate to excellent free radical scavengers. Compound **1d** was found to possess highest anti-oxidant activity with 94.35 % control values. Further cytotoxic studies revealed that newly synthesized compounds are safe against plant seed germination cell line as well as mouse fibroblast cell line with 100 % and 89.65-99.56 % cell viability values, respectively. It can be concluded

from the results obtained that 1,2,3-triazole tethered 1,2,4-triazole derivatives have the potential for their further exploration as biological agents.

Synthesis of a library of twenty-one novel benzenesulfonamide containing 1,2,3-triazole tethered 1,2,4-triazoles **1**, **2** and **3** and their biological evaluation along with precursors **8** is disscussed in chapter 4. In addition, the compounds were also evaluated for their inhibition potenital against MCF-7 cell line. Among the tested compounds, only four compounds **1f**, **2b**, **2c** and **3c** possessed significant anti-cancer activity which too very poor in comparison to the inhibition potential of standard drug, Doxorubicin. Compound **1a** possessed excellent anti-microbial activity with 3.12 μg/ml MIC value which is either equivalent or lower than the MIC values of reference drugs, Amoxicillin (anti-bacterial) and Fluconazole (anti-fungal). Compound **2b** exhibited excellent anti-oxidant activity (96.73 %) when compared to the ascorbic acid (96.78 %) as reference. Results showed that the final compounds **1**, **2** and **3** have higher anti-cancer, anti-microbial and anti-oxidant potential than the precursors **8**. Cytotoxicity results showed that all the synthesized compounds are safe to normal animal cells and plant seed germination cells.

In chapter 5, sixteen novel benzenesulfonamide bearing 1,2,4-triazoles **1** and their fluorinated Schiff's base derivatives **2** have been synthesized and evaluated as anti-microbial and anti-oxidant agents. Results showed that all the tested compounds possessed good to excellent anti-microbial activities while compound **2d** possessed highest inhibition potential against all the bacterial strains tested. Anti-oxidant activity was also tested for all the final compounds **1** and **2** using DPPH method. Compound **2e** was found to exhibit highest anti-oxidant activity (94.41 %). Further, all the final compounds were found to be safe during cytotoxicity studies against mouse fibroblast and plant seed germination cell lines.

As per the results obtained from biological evaluation of synthesized target compounds, all the novel 81 compounds have possessed moderate to excellent anti-microbial and anti-oxidant activities, while 4 of them have significant anti-cancer activity. Non-cytotoxicity of the compounds against growth of plant seed germination cell line and very little cytotoxicity against the growth of mouse fibroblast cell line during *in-vitro* cytotoxicity study positively indicate them as safer drug candidates. The work reported in the thesis involves hybridization of two or more biological motifs which will help the researchers to develop drug candidates with newer mechanism of action and helps to overcome multi-drug resistance.

The future perspective for further work can be summarized as:

1. The compilation made in Chapter 1 is done by hoping that it will pave the way for further work in this area and motivate the researchers to work with more enthusiasm in the direction of synthesis of novel drug candidates having triazole and oxadiazole nuclei in their pharmacophoric unit.

2. The original research work done in further chapters provides novel drug candidates during the discovery of newer drugs with improved results than the available ones.

3. This research will lend a hand to medicinal chemists in designing and synthesizing new triazole-benzenesulfonamide hybrids to develop improved pharmacological entities.

4. Structure-activity-relationship studies has been given for biological results in all the chapters which will help the researchers to understand the impact of the incorporation of different variations in the architecture of a drug molecule.

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LIST OF PUBLICATIONS OUT OF THESIS

List of published papers

Sr. No.	Title of paper	Name of Journal where published	Publisher	ISSN No.	Volume & Issue	Year	Page No.
1.	Novel benzenesulfonamide bearing 1,2,4-triazoles as potent anti-microbial and anti-oxidant agents	Medicinal Chemistry Research	Springer	1054-2523	32 & 3	2023	542-555
2.	Recent advances in triazole-benzenesulfonamide hybrids and their biological activities	Medicinal Chemistry Research	Springer	1054-2523	32 & 5	2023	777-801
3.	A review on molecular iodine catalyzed/mediated multicomponent reactions	Asian Journal of Organic Chemistry	Wiley	2193-5807	12 & 1	2023	e202200616

BRIEF PROFILE OF RESEARCH SCHOLAR

Mr. Chander is pursuing Ph.D. under the guidance of Dr. Sita Ram, Assistant Professor, Department of Chemistry, J.C. Bose University of Science and Technology, YMCA, Faridabad. He has obtained Master's degree in Chemistry from Kurukshetra University, Kurukshetra in 2014 and Bachelor's degree from Aggarwal College Ballabgarh, Faridabad affiliated to Maharshi Dayanand University, Rohtak in 2012. His research interests include design and synthesis of novel heterocyclic compounds with various functionalizations and to evaluate them for inhibitory potential against the growth of various microbes. Over the duration of this thesis work, he has contributed total three peer reviewed journal articles to the scientific community. Mr. Chander is presently working as Assistant Professor in the Department of Chemistry, Aggarwal College Ballabgarh.