Introduction

A large number of organo-sulfur compounds occur in living and non living objects. They belong to open chain, alicyclic, aromatic and heterocyclic types of compounds containing sulfur atom or atoms as a part of chain/ring or both in the structure. Isolation, identification and applications of these organo-sulfur compounds are useful in scientific, technical and pharmaceutical industrial growth. During last three decades organo-sulfur chemistry developed at a much faster pace than any other branches of organic chemistry. Among the sulfur containing heterocyclic compounds, a lot of research in the field of 1,3,4-thiadizoles has been reported. Some salient features regarding chemical reactivity, synthetic pathways and biological interest of 1,3,4-thiadizoles are discussed briefly as background information.

Chemistry and biological activity of 1,3,4-thiadizoles

Alireza et al.³ have reported series of 2-(5-nitro-2-furyl) and 2-(5-nitro-2-thienyl)-5-substituted-1,3,4-thiadiazoles. The novel 5-substituted-1,3,4-thiadiazoles were evaluated against *Leishmania major promastigotes* using 3H-thymidine incorporation. Most of the compounds showed better activity than the reference drug that is sodium stibogluconate (Pentostam).

![Chemical Structures](image)

Sara et al.⁴ have reported series of 1-[1,2,4-triazol-3-yl] and 1-[1,3,4-thiadiazol-2-yl]-3-methylthio-6,7-dihydrobenzo[c]thiophen-4(5H)ones. New 1,3,4-thiadiazole derivatives were demonstrate in vitro antimicrobial activity.
Some of 1,3,4-thiadiazole compounds exhibited a good activity against *Staphylococcus aureus*, and *Bacillus subtilis*.

Foroumadi *et al.* have reported series of 2-(1-methyl-5-nitroimidazol-2-yl)-5-(1-piperazinyl, 1-piperidinyl and 1-morpholinyl)-1,3,4–thiadiazoles. The synthesized 1,3,4–were evaluated for their *in vitro* leishmanicidal activity against *Leishmania major* promastigotes.

All The compounds showed better leishmanicidal activity than the reference drug pentostam. Compound containing piperazine ring was the most active compound (IC$_{50}$ = 0.19 μM).

Joanna *et al.* have reported series N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles. Novel 1,3,4-thiadiazoles were evaluated for their antiproliferative activities.

The *in vitro* cytotoxic activity was evaluated against the four human cell lines: SW707 (rectal), HCV29T (bladder), A549 (lung), and T47D (breast). The compound 2-(2,4-
dichlorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole showed highest antiproliferative activity than the standard cisplatin.

Alireza-Foroumadi et al.\(^7\) have reported series of 2- and 3-[5-(nitroaryl)-1,3,4-thiadiazol-2-ylthio, sulfinyl and sulfonyl] propionic acid alkyl esters. The synthesized 1,3,4-thiadiazoles were screened for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv using the BACTEC 460 radiometric system.

![Chemical Reaction](image)

The result of antitubercular activity revealed that the compound like propyl 3-[5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-ylthio]propionate was the most active one.

Silvia.S. et al.\(^8\) have reported series of N-[5-oxo-4-(arylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-amide derivatives. All compounds were tested for their analgesic and anti-inflammatory activities.

![Chemical Reaction](image)

Talat et al.\(^9\) have reported series of 7-[4-(5-amino-1,3,4 thiaizole-2-sulfonyl)]-1-piperazinyl fluoroquinolonic derivatives.
All the compounds were evaluated for their preliminary in vitro antibacterial activity against some Gram-positive and Gram-negative bacteria and some of the compounds were screened for antitubercular activity against Mycobacterium tuberculosis H37Rv strain by broth dilution assay method.

Matthew. et al.\textsuperscript{10} have reported synthesis and ADAMTS- 5 inhibitors of 5’-phenyl-3’H-spiro[indoline-3,2’-[1,3,4]thiadiazol]-2-one derivatives. The some of the compounds showed selectivity at sub-micromolar concentration.

Adnan et al.\textsuperscript{11} have reported synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. Several derivatives exhibited good activities against the tested Gram-positive bacteria Bacillus subtilis.
Kucukguzel et al.\textsuperscript{12} have reported three novel series of 2',4'-difluoro-4-hydroxybiphenyl-3-carboxylic acid derivatives namely 4-substituted-1,2,4-triazoline-3-thiones, 2-substituted-1,3,4-thiadiazoles and 2-substituted-1,3,4-oxadiazoles. All newly synthesized compounds were screened for their antimicrobial, antiviral and anti-inflammatory activity.

Cai-Jun et al.\textsuperscript{13} have reported synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole.
The synthesis of sulfoxide derivatives with \( \text{H}_2\text{O}_2 \) in the presence of catalytic amount of ammonium molybdate in an ionic liquid has several advantages such as fast reaction rate and good yield. Some of the tested compounds displayed good antifungal activity.

Mohammad et al.\(^{14}\) have prepared a new series of 1,3,4-thiadiazole-2-thione derivatives. The synthesized compounds were assayed for the inhibition of three physiologically relevant carbonic anhydrate isozymes. Compound like 4-(4,5-dihydro-5-thioxo-1,3,4-thiadiazol-2-yl)-1-(5-nitro-2-oxoindolin-3-ylidene)semicarbazide showed better inhibition of the tumor with KI value of 1.25 \( \mu \text{M} \).

Canan et al.\(^{15}\) have reported Synthesis and antioxidant properties of novel \( N \)-methyl-1,3,4-thiadiazol-2-amine derivatives.

Javad et al.\(^{16}\) have reported the synthesis and \textit{in vitro} anti-\textit{Helicobacter pylori} activity of N-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholine related compounds.
The anti-\textit{H. pylori} activity of target compounds along with commercially available antibiotics such as metronidazole and amoxicillin was evaluated by comparing the inhibition zone diameters determined by the paper disc diffusion bioassay.

Mina \textit{et al.}\textsuperscript{17} have reported the synthesis and anti-leishmanial activity of nitroheteroaryl-1,3,4-thiadiazole compounds including 1-[5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-yl]-4-arylpiperazines and 1-[5-(5-nitrothiophen-2-yl)-1,3,4-thiadiazol-2-yl]-4-arylpiperazines.

Most of the synthesized compounds exhibited potent anti-leishmanial activity against both \textit{promastigote} and \textit{amastigote} forms of \textit{Leishmania major} at non-cytotoxic concentrations. In
general, 5-nitrofuran derivatives were more active than the corresponding 5-nitrothiophene analogues.

Ardeshir et al.\textsuperscript{18} have reported a series of 5-(nitroaryl)-1,3,4-thiadiazoles bearing certain sulfur containing alkyl side chain similar to pendent residue in tinidazole molecule. All synthesized compounds were evaluated against \textit{Helicobacter pylori} using disk diffusion method.

\[
\begin{align*}
\text{Ar} & \text{S} & \text{NH}_2 \\
\text{NaNO}_2 & \text{HCl} & 0 \degree \text{C} \\
\text{ClC}_2\text{H}_4\text{SO}_2\text{C}_6\text{H}_5 & \text{KOH} & \text{C}_2\text{H}_5\text{OH} \\
\text{Ar} & \text{S} & \text{SH} \\
\text{R} & = & \text{C}_6\text{H}_5, \text{NO}_2\text{-C}_6\text{H}_4, \text{di-NO}_2\text{-C}_6\text{H}_4
\end{align*}
\]

The synthesized compounds were also evaluated for their antibacterial, antifungal and cytotoxic effects. Study of the structure–activity relationships of this series of compounds indicated that both the structure of the nitroaryl unit and the pendent group on 2-position of 1,3,4-thiadiazole ring dramatically impact the anti-\textit{H.pylori} activity.

GuoGang et al.\textsuperscript{19} have reported the synthesis and in vitro enzyme inhibition assay of 1,3,4-thiadiazole scaffold compounds. These new compounds have potent inhibitory activities toward APN with IC\textsubscript{50} values in the micromol range.

\[
\begin{align*}
\text{R}_1 \text{N} & \text{N} - \text{S} & \text{NH}_2 \\
\text{N-hydroxysuccinimide} & \text{DCC} \\
\text{R}_2 \text{N} & \text{N} - \text{O} & \text{N} - \text{H} & \text{N} & \text{N} & \text{O} & \text{N} & \text{H} \\
\text{R}_1 & = & \text{H}, \text{F}, \text{Cl}, \text{Br}, \text{CH}_3, \text{OCH}_3, \text{NO}_2 \\
\text{R}_2 & = & \text{H}, \text{OH}, \text{OCH}_3
\end{align*}
\]

Marco et al.\textsuperscript{20} have reported synthesis and tyrosine kinase inhibitors of substituted benzoylamino-2-[(4-benzyl) thio]-1,3,4-thiadiazoles derivatives. Molecular docking
simulations on the Abl tyrosine kinase were conducted in order to rationalize the SAR of the synthesized inhibitors.

The most active compound identified from the enzymatic screening was (N-(5(4-fluorobenzylthio)-1,3,4-thiadiazol-2-yl)-4-fluorobenzamide showed interesting inhibitory activity on Imatinib-sensitive murine myeloid 3B clone and Bcr-Abl-independent Imatinib-resistant leukemia cells.

Fang et al. have reported synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moiety.
This method describes that formation of sulfoxide derivatives by mCPBA oxidation in dichloromethane has several advantages such as fast reaction rate and good yield. Some of the synthesized compounds displayed good antifungal activity.

Harish et al.\textsuperscript{22} have reported the synthesis, anti-inflammatory and analgesic activity of a series of 1,3,4-oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yl oxy acetic acid derivatives.

The compounds possessing potent anti-inflammatory activity were further tested for their analgesic, ulcerogenic and antioxidant activities.

Samir et al.\textsuperscript{23} have reported the synthesis and trypanocidal profile of thirteen new 1, 3, 4-thiadiazole-2-arylhydrazone derivatives. Megazol is a highly active compound against \textit{Trypanosoma cruzi}, and has become a core structure for the design of new trypanocidal agents.
Varsha et al.\textsuperscript{24} have reported synthesis of new 3-[5-substituted phenyl-1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-one derivatives. The all synthesized compounds were evaluated for anticonvulsant, sedative hypnotic and CNS depression activities.

Mohammad et al.\textsuperscript{25} have reported the a number of new imine derivatives of 5-amino-1, 3, 4-thiadiazole-2-thiol derivatives. The synthesized compounds were evaluated for their anti-depressant activity, using imipramine as reference drug.

Two compounds namely 5-[[1-(4-chlorophenyl)-3-(4-methoxy-phenyl)prop-2-en-1-ylidene]amino]-5-benzylthio-1, 3,4-thiadiazole and 5-[[1-(4-chlorophenyl)-3-(4-dimethyl-aminophenyl)prop-2-en-1-ylidene]amino]-5-benzylthio-1,3,4-thiadiazole have shown significant anti-depressant activity, which decreased immobility time by 77.99\% and 76.26\%
compared to the standard imipramine (82%). All the compounds in the series have passed neurotoxicity tests.

Ansari et al.\textsuperscript{26} have reported the efficient synthesis of novel azetidin-2-ones. Thus, condensation of 5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiazolid-2-amine with various aromatic aldehydes afforded 5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-N-[(substituted) phenylmethylidene]-1,3,4-thiazolid-2-amine which on cycloaddition with chloroacetyl chloride in the presence of triethylamine catalyst yielded 3-chloro-1-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiazolid-2-yl]-4-(substituted) phenylazetidin-2-one.

\[
\begin{align*}
\text{H}_2\text{SO}_4 & \quad \text{NH}_3 \\
\text{NH}_2\text{NHCSNH}_2 & \\
\text{ClICH}_2\text{COCCl}_2 & \\
\text{NH}_3 & \\
\text{H} & \\
\text{Cl} & \\
\text{O} & \\
\text{S} & \\
\text{N} & \\
\text{Ar} & \\
\text{NH}_2 & \\
\text{NHCSNH}_2 & \\
\text{Et}_3\text{N} & \\
\text{THF} & \\
\text{R} & \\
\end{align*}
\]

Xing-Hai et al.\textsuperscript{27} have reported the synthesis of cyclopropanecarboxamide and tested for antifungal activity. The preliminary bioassays indicated that some compounds are comparable to the commercial fungicides.
To further explore the comprehensive structure-activity relationship on the basis of fungicidal activity data, comparative molecular field analysis (CoMFA) was performed, and a statistically reliable model with good predictive power was achieved.

Sherif et al.\textsuperscript{28} have reported the synthesis of antipyrene containing 1,3,4-thiadiazole derivatives. The newly synthesized compounds were evaluated for their anti-inflammatory activity using two different screening protocols; namely, the formalin-induced paw edema and the turpentine oil-induced granuloma pouch bioassays, using diclofenac sodium as a reference standard.

The ulcerogenic effects and acute toxicity (ALD\textsubscript{50}) values of these compounds were also determined. Meanwhile, the analgesic activities of the same compounds were evaluated using the rat tail withdrawal technique. Additionally, the synthesized compounds also evaluated for their in vitro antimicrobial activity.

Padmavathi et al.\textsuperscript{29} have reported a new class of oxadiazoles was prepared by treating aminosulfonylacetic acids with different carboxylic acid hydrazides.
Interconversion of oxadiazoles to thiadiazoles is carried out with thiourea. The compounds are screened for antimicrobial and antioxidant activities.

Nuray et al.\(^3\) have reported a series of 2-alkyl/aryl amino-5-((6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl) methyl)-1,3,4-thiadiazoles.

All the compounds were tested for antibacterial and antifungal activities. The antimicrobial activities of the compounds were assayed by the micro broth dilution technique. The compounds were also evaluated for antitubercular activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294). The results revealed that some of the compounds exhibited promising antimicrobial activities.

Imtiaz et al.\(^3\) have reported new series of 4,5-disubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and 2,5-disubstituted-1,3,4-thiadiazoles. The 2,5-disubstituted-1,3,4-thiadiazoles were synthesized by dehydrative cyclization of hydrazinecarbothioamide derivatives by refluxing in 4 N aqueous sodium hydroxide and by overnight stirring with polyphosphoric acid, respectively.
The synthesized compounds were screened for their antioxidant and urease inhibition activities. N-(2,4-Dimethylphenyl)-5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine showed excellent antioxidant activity than the standard drug whereas 4-(2,4-dimethylphenyl)-5-(3-nitrophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione and 4-(2,3-dimethylphenyl)-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione exhibited potent urease inhibitory activities.

Andre et al. have reported the synthesis of 1,3,4-thiadiazole derivatives. The synthesized compounds were screened for their anti-Toxoplasma gondii and antimicrobial activities.

The most of the tested compounds show excellent anti-Toxoplasma gondii activity when compared to hydroxyurea and sulfadiazine. In addition it was also shown that most of the compounds in this study have a better performance against intracellular tachyzoites. The results for antimicrobial activity evaluation showed weak antibacterial and antifungal activities for all the tested molecules, when compared with the standard drugs.
chloramphenicol and rifampicin for antibacterial activity; nistatin and ketoconazole for antifungal activity.

Dalip *et al.* have reported a series of 5-(3-indolyl)-2-substituted-1,3,4-thiadiazoles. The synthesized compounds were evaluated for their cytotoxicity against six human cancer cell lines. The reaction of indole-3-carboxylic acid with aryl or heteroaryl hydrazides afforded the *N,N*-diacylhydrazines, which upon treatment with Lawesson’s reagent resulted in the formation of indolyl-1,3,4-thiadiazoles in good yields. Indolyl-1,3,4-thiadiazole with 4-benzyloxy-3-methoxyphenyl and 5-bromo indolyl substituents is the most active in suppressing the growth of cancer cells.

\[
\begin{align*}
\text{R} & = \text{H, Br} \\
R_1 & = \text{C}_6\text{H}_5, 4-\text{Cl-C}_6\text{H}_4, 2,3-\text{di-OCH}_3\text{C}_6\text{H}_3
\end{align*}
\]

Jinhwa *et al.* have reported the structure–activity relationship studies on a series of diarylpyrazolyl thiadiazoles identified cannabinoid-1 receptor antagonists with excellent potency and selectivity. Based on its exceptional *in vivo* efficacy in animal models and its favorable pharmacokinetic and toxicological profiles, 2-((4-(1H-1,2,4-triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1H-pyrazol-3-yl)-5-tert-butyl-1,3,4-thiadiazole was selected as a preclinical candidate for the treatment of obesity.
Nida et al.\textsuperscript{35} have reported the long-chain alkenoic acid hydrazides on reaction with phenylthiocyanate gave their corresponding thiosemicarbazides, which on further refluxing with acetic anhydride yielded corresponding 1,3,4-thiadiazoles.

The synthesized thiadiazoles have been screened for antibacterial and antifungal activities. The investigation of antimicrobial screening revealed that most of the compounds showed good antibacterial and antifungal activities.

Surya et al.\textsuperscript{36} have reported a series of thiadiazole derivatives. The novel thiadiazole compounds were evaluated protein kinase inhibitor
In view of above facts in present work we have designed and synthesized number of 2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-substituted-1,3,4-thiadiazol-2-yl) acetamide derivatives and investigated the benefit of such hybridization on the anticipated synergistic biological activity. The structures of the newly synthesized compounds were confirmed by IR, NMR and elemental analysis. All compounds were tested for antimicrobial activity by twofold serial dilution technique and cytotoxic activity by MTT assay.
**Present work:**

The synthetic work carried out during present investigation has been described in the following scheme.

\[
\text{Ar-COOH} + \text{H-N=N=N-H} \xrightarrow{(a)} \text{Ar-S-N=N-H} \quad (1\ a-n)
\]

\[
\text{O} - \text{C}=\text{O} + \text{H-N=N=S-H} \xrightarrow{(b)} \text{O}-\text{C}=\text{O} \xrightarrow{(c)} \text{O}-\text{Cl} \quad (2)
\]

\[
\text{Ar-S-N=N-H} + \text{O}-\text{C}=\text{O} \xrightarrow{(d)} \text{Ar-S-N=N=S}=\text{O} \quad (3)
\]

**Scheme 1.** Reagents: (a) POCl₃; (b) HCl; (c) SOCl₂, dioxane; (d) Triethyl amine, dioxane.
Results and discussion

Chemistry

2,4-thiazolidinedione-5-acetic acid, prepared from thiourea and maleic anhydride in presence of concentrated hydrochloric acid. 2-amino-5-substituted-1,3,4-thiadiazol were prepared from substituted aryl acid with thiosemicarbazide in presence of dehydrating agent phosphorus oxychloride (POCl₃). 2-(2,4-dioxothiazolidin-5-yl)acetyl chloride was prepared from the 2,4-thiazolidinedione-5-acetic acid and thionylchloride (SOCl₂), without isolation further reacted with 5-substituted-2-amino-1,3,4-thiadiazols as scheme 1.

The IR spectra of 2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-substituted-1,3,4-thiadiazol-2-yl)acetamides (A 1-14) exhibited a absorption band in the region of 3566-3233 cm⁻¹ attributable to the stretching vibration of NH, and around 1690 cm⁻¹ to the carbonyl carbon. The 2,4-thiazolidinedione-5-acetic acid and their derivatives contain diastereotopic protons of methylene group, that is why the fragment CH₃H²CHₓ is presented in ¹H NMR spectra as ABX spin system, which appears as doublet of doublet at ~3.05 to 3.48 ppm, and ~ 4.85 ppm with coupling constant JAB= 15.01-17.76 Hz, JAX=6.9-10.75 Hz, JBX=3.4-5.5 Hz. High value of JAB agreed with the data of Takahashi (“carbonyl effect”) for structurally related 2-thioxo-4-thiazolidinone-acetic acids.³⁷

Antimicrobial activity

The synthesized compounds were tested for their in vitro antibacterial activity against the Gram-positive Staphylococcus aureus (ATCC25923), Enterococcus faecalis (ATCC35550), Gram-negative Escherichia coli (ATCC35218), Pseudomonas aeruginosa (ATCC25619) baceria and antifungal activity against Candida albicans (ATCC2091), Aspergillus flavus (NCIM No. 524), Aspergillus niger (ATCC6275), and Cryptococcus neoformans (Clinical isolate). The MIC values were determined by using the twofold serial dilution technique³⁸ in Mueller-Hinton broth and Sabouraud dextrose agar for the antibacterial
and antifungal assays, respectively. Ciprofloxacin was used as the reference antibacterial agents; Ketoconazole was used as the reference antifungal agents. All the biological results of the tested compounds are given in Table 2a. The combined data were reported that the synthesized compounds (A 1-14) showing MIC values between 64 and 4 µg/ml concentration.

All compounds were found to be active against Gram-positive bacteria especially *Staphylococcus aureus* at 4-32 µg/ml concentration. Compound A1 which has no substitution at the *para* position of phenyl ring attached to 5th position of the thiadiazole moiety did not promote much activity against the tested microbial strains, whereas compounds A 6 and A7 which have electron donating –CH₃ group at the *para* position of phenyl ring and –OCH₃ group at different position of the phenyl ring exhibits moderate activity. The introduction of -Cl, -Br groups at the *para* position of phenyl ring were found to enhance the antibacterial potency significantly. Thus the compounds A2 and A3 active against Gram-positive especially *Staphylococcus aureus* at 4-8 µg/ml concentration, while A4 which has electron withdrawing –F functional group substituted at the *para* position of phenyl ring exerted excellent antibacterial activity by inhibiting the growth of all the tested bacterial strains at 4-8 µg/ml.

Compounds like A11, A12, and A13 showed moderate antibacterial activity against all the tested bacterial strains. Antifungal results indicated that compounds A2, A3, and A4 exhibit good activity against all the tested fungal strains.

**Cytotoxic activity**

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] cell proliferation assay was used to evaluate cytotoxic activity of the synthesized compounds (A 1-14) against four human cancer cell lines including HeLa (cervical carcinoma), HT29 (colorectal cancer), A549 (lung cancer), MCF-7 (breast adenocarcinoma) cell lines. The inhibition of the cell proliferation was determined 24 h after cells were exposed to the tested compounds. The
IC$_{50}$ (the concentration that causes 50% growth inhibition) values were determined and summarized in Table 2b. Among the fourteen compounds A10 which was 3,4,5-trimethoxyphenyl, exhibited the good inhibitory activity against HeLa, HT29, A549 and MCF-7 cell lines, with the inhibitory concentration (IC$_{50}$) values of 33, 35, 30 and 36 µM, respectively. Compounds A7, A8 and A9 showed moderate cytotoxic activities against all tested human tumor cell lines with IC$_{50}$ values of 35-60 µM. In general, structures with donor substituents showed activity, the position of substituents appears to play an important role in activity too.

**Drug Likeliness**

In order to predict the drug likeliness of the synthesized compounds on the guidelines of Lipinski rule of 5 (Molecular weight ≤500, Log P ≤5, HBD≤5 and HBA≤10) study was carried out using Pallas software$^{40}$ the result are given in Table 2c. The relevance of the synthesized molecules with respect to Lipinski rule of five is as follows.

Molecular weight of the compound is important in drug action, if the molecular weight is increases beyond a limit, the bulkiness of the compounds also increases, which will affect the drug action (affect the drug receptor/DNA interactions). Molecular weight of compounds lie between 326.29 and 424.28 show that it follows Lipinski rule of 5. So the bulkiness of the compounds is in optimum limit for the action.

Pharmacokinetic property optimization is a rather complex undertaking that is likely to require changes in those molecular determinants that are responsible for binding affinity and specificity like hydrogen bonds. Hydrogen bond accepotor (HBA) and Hydrogen bond donor (HBD) groups in the compound optimize the drug receptor interaction. Number of hydrogen bond acceptors (≤10) and hydrogen bond donors (≤5) in the proposed compounds obeys the Lipinski rule of 5, so it may have good absorption or permeability properties through the biological membrane.
Dissolution is highly interdependent influences of aqueous solubility, ionizability (pKa) and lipophilicity (log P). Furthermore, log P is a crucial factor governing passive membrane partitioning, influencing permeability opposite to its effect on solubility. The log P values of the synthesized compounds lie in between 0.45 and 2.22.

**Conclusion**

We report the synthesis of a series of 2,4-thiazolidinedione-5-acetic acid amides in good yields and are characterized by their physical and analytical data. The antibacterial and antifungal studies carried out to by twofold serial dilution technique. The preliminary structural-activity relationship study showed that 4-fluorophenyl and 3,4,5-trimethoxyphenyl ring are important for activity and selectivity of 2,4-thiazolidinedione-5-acetic acid amides. Moreover, the cytotoxic activity of this series suggests that 2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl)acetamide A10 offers a novel template for development of a new class of antimicrobial agent and 2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-fluorophenyl-1,3,4-thiadiazol-2-yl)acetamide A4 offers a novel template for development of a new class of anticancer agent.
Experimental

All the chemicals were obtained from commercial suppliers and used without further purification. Melting point was determined by electrothermal melting point apparatus and is uncorrected. All reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel (60GF-254) plates and visualized with UV light. Column chromatography was performed on silica gel (200-300 mesh). Infra red (IR) spectra were recorded on using KBr disk on a Nicolet MX-1 FTIR spectrophotometer, $^1$H and $^{13}$C-NMR spectra were recorded on AMX-400, Bruker-400 liquid-state NMR spectrometer using tetramethylsilane (TMS) as internal standard (chemical shift in δ ppm). Elemental analysis was carried out using a Perkin Elmer 2400-CHN Analyzer. Spectral analysis was carried out at Sophisticated Analytical Instruments Facility (SAIF), division of Indian Institute of Science, Bangalore, India.

**General procedure for synthesis of 5-substituted-1,3,4-thiadiazol-2-amine (1a-m)**

A stirring mixture of benzoic acid (50 mmol), thiosemicarbazide (50 mmol) and phosphorus oxychloride (15 ml) was heated at 75 °C for 1 h. After cooling to room temperature, water was added; the reaction mixture was further refluxed for 4 h. After cooling, the mixture was basified to pH 7 by the dropwise addition of 50% potassium hydroxide solution under stirring. Thus obtained precipitate was filtered and recrystallized from ethanol.

**5-phenyl-1,3,4-thiadiazol-2-amine (1a)** This compound was obtained as white solid in a yield of 78%; m.p.: 225-227 °C; IR (KBr, ν, cm$^{-1}$): 3590, 3126 (ν$_{NH}$), 1620 (ν$_{C=N}$), 690(ν$_{C=S}$); $^1$H NMR (400 MHz, δ, ppm, DMSO-$d_6$): 7.76 (s, 2H), 7.5 (m, 5H).
5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (1b) This compound was obtained as white solid in a yield of 75%; m.p.: 212-214 °C; IR (KBr, ν, cm⁻¹): 3345, 3156 (vNH), 1626 (νC=N), 685 (νC=S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.50 (d, J =6.56 Hz, 2H), 7.47 (d, J =6.23 Hz, 2H), 7.44 (s, 2H).

5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine (1c) This compound was obtained as white solid in a yield of 74%; m.p.: 225-226 °C; IR (KBr, ν, cm⁻¹): 3394, 3136 (vNH), 1626 (νC=N), 665 (νC=S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.78-7.82 (dd, J₁ =8.42 Hz, J₂ =16.63 Hz, 4H), 7.44 (s, 2H).

5-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (1d) This compound was obtained as white solid in a yield of 80%; m.p.: 232 °C; IR (KBr, ν, cm⁻¹): 3294, 3156 (vNH), 1633 (νC=N), 682 (νC=S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.79-7.84 (dd, J₁ =6.36 Hz, J₂ =8.46 Hz, 2H), 7.42 (s, 2H), 7.28-7.34 (m, 2H).

5-(2-chlorophenyl)-1,3,4-thiadiazol-2-amine (1e) This compound was obtained as white solid in a yield of 82%; m.p.: 228-230 °C; IR (KBr, ν, cm⁻¹): 3384, 3136 (vNH), 1643 (νC=N), 672 (νC=S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.97-8.01 (m, 2H), 7.57-7.60 (m, 1H), 7.44-7.47 (m, 1H), 7.42 (s, 2H).

5-(4-methylphenyl)-1,3,4-thiadiazol-2-amine (1f) This compound was obtained as white solid in a yield of 88%; m.p.: 212 °C; IR (KBr, ν, cm⁻¹): 3578, 3143 (vNH), 1651 (νC=N), 681 (νC=S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.60-7.63 (d, J =9.96 Hz, 2H), 7.31 (s, 2H), 7.26 (d, J =7.93 Hz, 2H), 2.32 (s, 3H, CH₃).
5-(3-methoxyphenyl)-1,3,4-thiadiazol-2-amine (1g) This compound was obtained as light yellow solid in a yield of 84%; m.p.: 220 °C; IR (KBr, ν cm⁻¹): 3448, 3155 (ν(NH)), 1650 (ν(C=N)), 1231 (ν(CH-O-C)), 676 (ν(C-S)); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.25-7.35 (m, 3H), 6.97-7.01 (dd, 1H), 7.40 (s, 2H), 3.79 (s, 3H, OCH₃).

5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (1h) This compound was obtained as light yellow solid in a yield of 80%; m.p.: 196 °C; IR (KBr, ν cm⁻¹): 3548, 3126 (ν(NH)), 1660 (ν(C=N)), 1243 (ν(CH-O-C)), 670 (ν(C-S)); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.62 (d, J=7.83 Hz, 2H), 7.50 (d, J=8.01 Hz, 2H), 7.27 (s, 2H), 3.53 (s, 3H, OCH₃).

5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol-2-amine (1i) This compound was obtained as white solid in a yield of 79%; m.p.: 210 °C; IR (KBr, ν cm⁻¹): 3578, 3156 (ν(NH)), 1640 (ν(C=N)), 1258 (ν(CH-O-C)), 670 (ν(C-S)); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 8.65-8.69 (m, 3H), 7.61 (s, 2H), 3.71 (s, 3H, OCH₃), 3.70 (s, 3H, OCH₃).

5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-amine (1j) This compound was obtained as greyish solid in a yield of 90%; m.p.: 206 °C; IR (KBr, ν cm⁻¹): 3590, 3126 (ν(NH)), 1620 (ν(C=N)), 1266 (ν(CH-O-C)), 690 (ν(C-S)); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.85 (s, 2H), 6.95 (s, 2H, NH₂), 3.83 (s, 6H, OCH₃), 3.80 (s, 3H, OCH₃).

5-(2-nitrophenyl)-1,3,4-thiadiazol-2-amine (1k) This compound was obtained as yellow solid in a yield of 73%; m.p.: 242 °C; IR (KBr, ν cm⁻¹): 3577, 3132 (ν(NH)), 1667 (ν(C=N)), 676 (ν(C-S)); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 8.41-8.48 (m, 1H), 8.27 (d, J=8.84 Hz, 2H), 8.01 (m, 1H), 7.70 (s, 2H).
5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine (1I) This compound was obtained as light yellow solid in a yield of 71%; m.p.: 219 °C; IR (KBr, ν cm⁻¹): 3532, 3151 (vNH), 1637 (vC=N), 651 (vC-S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.99 (d, J = 8.14 Hz, 2H), 7.87 (m, 2H), 7.70 (s, 2H).

5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (1m) This compound was obtained as yellow solid in a yield of 78%; m.p.: 260 °C; IR (KBr, ν cm⁻¹): 3568, 3147 (vNH), 1658 (vC=N), 656 (vC-S); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 8.29 (d, J = 8.92 Hz, 2H), 7.98 (d, J = 8.92 Hz, 2H), 7.69 (s, 2H).

Synthesis of 5-(trifluoromethyl)-1,3,4-thiadiazol-2-amine (1n) A mixture of Trifluoroacetic acid anhydride (50 mmol), thiosemicarbazide (50 mmol) was heated at 40 °C on a water bath for 1 h. The reaction mixture was cooled, diluted with water and made alkaline with ammonia; the crystalline precipitate was recrystallized with ethanol gave white crystals in a yield of 81%; m.p.: 226 °C; IR (KBr, ν cm⁻¹): 3468 (vNH), 1647 (vC=N); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 7.96 (s, 2H).

General procedure for synthesis of 2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-substituted-1,3,4-thiadiazol-2-yl)acetamide (A 1-14)

A mixture of thiourea (25 mmol) and maleic anhydride (25 mmol) in 15 ml of concentrated hydrochloric acid was refluxed for 5 h. After cooling down to room temperature, the precipitate was separated by filtration, washed with cooled water, and recrystallized from water to yield 2,4-dioxothiazolidine-5-acetic acid (2).

A mixture of 2,4-dioxothiazolidine-5-acetic acid (50 mmol) and 1.2 g of thionyl chloride in 5 ml of dioxane was heated under reflux for 5 h. cooled, and treated with 15 ml of
hexane. The precipitate was separated by filtration and used without further purification. To a
solution of corresponding 5-substituted-1,3,4-thiadiazol-2-amine (10 mmol) and 1 ml of
triethylamine in 10 ml of anhydrous dioxane was added the solution of acid chloride (50
mmol) in 10 ml of same solvent. The mixture was heated for 10 min at 100 °C, cooled, and
diluted with 100 ml of water. The precipitate was separated by filtration and recrystallized
with appropriate solvent.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-phenyl-1,3,4-thiadiazol-2-yl) acetamide (A1)
White colour solid; yield 66%; m.p.: 256 °C; IR (KBr, ν, cm⁻¹): 3325, 3255 (νNH), 2881, 1699
(vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.90 (s, 1H), 12.10 (s, 1H), 7.95-7.92 and
7.51 (m, 5H), 4.80 (m, 1H), 3.42 (dd, J =17.11 Hz, J =4.71 Hz, 1H), 3.29 (dd, J =16.12 Hz, J =4.32 Hz,
1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 175.63, 172.44, 168.46, 162.07, 157.96, 130.63,
130.06, 129.32, 126.92, 46.21, 36.90; Anal. Calcd. for C₁₃H₁₀N₄O₃S₂: C, 46.70; H, 3.01; N,
16.76; Found: C, 46.65; H, 3.00; N, 16.84%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-chlorophenyl-1,3,4-thiadiazol-2-yl) acetamide
(A2) Light brown colour solid; yield 59%; m.p.: 261 °C; IR (KBr, ν, cm⁻¹): 3566, 3498 (νNH),
2966, 1690 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.89 (s, 1H), 12.12 (s, 1H), 7.55 (d, J =6.71 Hz, 2H), 7.51 (d, J =7.26 Hz, 2H), 4.78 (m, 1H), 3.41 (dd, J =15.53 Hz, J =4.61 Hz, 1H), 3.27
(dd, J =17.13 Hz, J =4.82 Hz, 1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 170.03, 168.21,
166.53, 162.13, 153.19, 133.23, 129.74, 128.17, 126.89, 50.12, 34.09; Anal. Calcd. for
C₁₃H₉ClN₄O₃S₂: C, 42.34; H, 2.46; N, 15.19; Found: C, 42.26; H, 2.40; N, 15.29%.
2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-bromophenyl-1,3,4-thiadiazol-2-yl) acetamide (A3) Brown colour solid; yield 61%; m.p.: 247°C; IR (KBr, v, cm⁻¹): 3362, 3271 (vNH), 2942, 1693 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.99 (s, 1H), 12.13 (s, 1H), 7.78-7.74 (m, 4H), 4.81 (m, 1H), 3.39 (dd, J = 16.52 Hz, J = 4.71 Hz, 1H), 3.08 (dd, J = 17.11 Hz, J = 4.36 Hz, 1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 169.87, 167.01, 164.21, 162.09, 153.12, 135.11, 129.89, 128.45, 126.61, 51.18, 34.41; Anal. Calcd. for C₁₃H₉BrN₄O₃S₂: C, 37.78; H, 2.20; N, 13.56; Found: C, 37.70; H, 2.18; N, 13.63%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-fluorophenyl-1,3,4-thiadiazol-2-yl) acetamide (A4) Light yellow solid; yield 55%; m.p.: 239°C; IR (KBr, v, cm⁻¹): 3434, 3246 (vNH), 2963, 1698 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.90 (s, 1H), 12.10 (s, 1H), 7.71 (d, 2H), 7.67 (d, 2H), 4.83 (m, 1H), 3.33 (dd, J = 17.41 Hz, J = 4.43 Hz, 1H), 3.06 (dd, J = 15.23 Hz, J = 4.62 Hz, 1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 171.23, 167.12, 165.13, 163.15, 151.45, 132.34, 129.78, 128.11, 126.69, 50.72, 33.09; Anal. Calcd. for C₁₃H₉FN₄O₃S₂: C, 44.31; H, 2.57; N, 15.90; Found: C, 44.23; H, 2.51; N, 15.98%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-2-chlorophenyl-1,3,4-thiadiazol-2-yl) acetamide (A5) White solid; yield 62%; m.p.: 231°C; IR (KBr, v, cm⁻¹): 3421, 3233 (vNH), 2982, 1690 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.89 (s, 1H), 12.00 (s, 1H), 8.01-7.92 (m, 4H), 4.81 (m, 1H), 3.43 (dd, J = 17.14 Hz, J = 4.20 Hz, 1H), 3.19 (dd, J = 15.51 Hz, J = 4.34 Hz, 1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 168.97, 165.86, 164.34, 161.56, 151.875, 136.31, 133.32,
129.59, 128.38, 127.78, 126.28, 50.11, 35.29; Anal. Calcd. for C_{13}H_{9}ClN_{4}O_{3}S_{2}: C, 42.34; H, 2.46; N, 15.19; Found: C, 42.24; H, 2.39; N, 15.28%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-methylphenyl-1,3,4-thiadiazol-2-yl) acetamide (A6) Light yellow solid; yield 58%; m.p.: 260 °C; IR (KBr, ν, cm\(^{-1}\)): 3372, 3259 (ν\(\text{NH}\)), 2961, 1697 (ν\(C=O\)); \(^1\)H NMR (400 MHz, δ, ppm, DMSO-\(d_6\)): 12.89 (s, 1H), 12.00 (s, 1H), 7.62 (d, \(J=7.4\) Hz, 2H), 7.52 (d, \(J=6.7\) Hz, 2H), 4.82 (m, 1H), 3.39 (dd, \(J=16.87\) Hz, \(J=4.69\) Hz, 1H), 3.08 (dd, \(J=15.54\) Hz, \(J=4.81\) Hz, 1H), 2.63 (s, 3H, CH\(_3\)); \(^{13}\)C NMR (100 MHz, δ, ppm, DMSO-\(d_6\)): 169.65, 167.21, 165.28, 161.64, 151.73, 131.19, 130.11 129.37, 128.21, 51.02, 35.12 29.15.

Anal. Calcd. for C\(_{14}\)H\(_{12}\)N\(_4\)O\(_3\)S\(_2\): C, 48.26; H, 3.47; N, 16.08; Found: C, 48.27; H, 3.41; N, 16.20%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-3-methoxyphenyl-1,3,4-thiadiazol-2-yl) acetamide (A7) Light brown solid; yield 54%; m.p.: 237 °C; IR (KBr, ν, cm\(^{-1}\)): 3357, 3241 (ν\(\text{NH}\)), 2979, 1689 (ν\(C=O\)); \(^1\)H NMR (400 MHz, δ, ppm, DMSO-\(d_6\)): 12.87 (s, 1H), 12.21 (s, 1H), 7.35-7.32 (m, 3H), 7.16-7.9 (m, 1H), 4.83 (m, 1H), 3.31 (dd, \(J=17.34\) Hz, \(J=4.23\) Hz, 1H), 3.05 (dd, \(J=15.71\) Hz, \(J=4.78\) Hz, 1H); \(^{13}\)C NMR (100 MHz, δ, ppm, DMSO-\(d_6\)): 171.76, 168.12, 165.97, 161.78, 151.61, 132.87, 130.12, 129.23, 128.31, 127.13, 126.44, 56.28, 50.34, 34.45; Anal. Calcd. for C\(_{14}\)H\(_{12}\)N\(_4\)O\(_4\)S\(_2\): C, 46.15; H, 3.32; N, 15.37; Found: C, 46.11; H, 3.24; N, 15.45%.
2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-methoxyphenyl-1,3,4-thiadiazol-2-yl) acetamide

(A8) White solid; yield 69%; m.p.: 249°C; IR (KBr, ν, cm⁻¹): 3561, 3315 (vNH), 2955, 1698 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 13.00 (s, 1H), 12.15 (s, 1H), 7.59 (d, 2H), 7.52 (d, 2H), 4.78 (m, 1H), 3.43 (dd, J =16.21 Hz, J =4.78 Hz, 1H), 3.07 (dd, J =15.12 Hz, J =4.65 Hz, 1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 170.29, 168.81, 165.24, 163.11, 153.32, 130.11, 129.24, 128.22, 125.41, 54.21, 49.89, 34.31; Anal. Calcd. for C₁₄H₁₂N₄O₄S₂: C, 46.15; H, 3.32; N, 15.37; Found: C, 46.10; H, 3.26; N, 15.43%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-(-3,4-dimethoxyphenyl)-1,3,4-thiadiazol-2-yl) acetamide (A9) White solid; yield of 62%; m.p.: 241°C; IR (KBr, ν, cm⁻¹): 3448 3278 (vNH), 2957, 1698 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.97 (s, 1H), 12.21 (s, 1H), 7.31-7.27 (m, 3H), 4.78 (m, 1H), 3.83 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃). 3.41 (dd, J =17.26 Hz, J =4.47 Hz, 1H), 3.12 (dd, J =15.44 Hz, J =4.53 Hz, 1H); ¹³C NMR (100 MHz, δ, ppm, DMSO-d₆): 171.33, 169.28, 165.11, 162.47, 152.31, 130.79, 129.66, 127.38, 126.12, 124.21, 57.37, 51.45, 49.23, 33.97; Anal. Calcd. for C₁₅H₁₄N₄O₅S₂: C, 45.68; H, 3.58; N, 14.20; Found: C, 45.69; H, 3.53; N, 14.31%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-(-3,4,5-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl)acetamide (A10) Light yellow solid; yield 52%; m.p.: 260°C; IR (KBr, ν, cm⁻¹): 3412, 3253 (vNH), 2966, 1700 (vC=O); ¹H NMR (400 MHz, δ, ppm, DMSO-d₆): 12.81 (s, 1H), 12.14 (s, 1H), 7.21 (d, 2H), 4.81 (m, 1H), 3.87 (s, 6H, 2OCH₃), 3.71 (s, 3H, OCH₃), 3.38 (dd, J =16.43 Hz, J =4.32 Hz, 1H).
Hz, 1H), 3.27 (dd, J =15.23 Hz, J =4.6 Hz, 1H); $^{13}$C NMR (100 MHz, δ, ppm, DMSO-$d_6$): 170.23, 169.67, 166.71, 163.67, 152.39, 131.28, 129.57, 128.49, 125.38, 108.34, 57.34, 50.89, 33.29; Anal. Calcd. for C$_{16}$H$_{16}$N$_4$O$_6$S$_2$: C, 45.28; H, 3.80; N, 13.20; Found: C, 45.26; H, 3.76; N, 13.23%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-2-nitrophenyl-1,3,4-thiadiazol-2-yl) acetamide (A11) Light yellow solid; yield 56%. m.p.: 248 °C; IR (KBr, ν, cm$^{-1}$): 3423, 3304 (ν$_{NH}$), 2946, 1688 (ν$_{C=O}$); $^1$H NMR (400 MHz, δ, ppm, DMSO-$d_6$): 12.92 (s, 1H), 12.18 (s, 1H), 7.72-7.69 (m, 4H), 4.44 (m, 1H), 3.42 (dd, J =16.37 Hz, J =4.23 Hz, 1H), 3.08 (dd, J =17.13 Hz, J =4.47 Hz, 1H). $^{13}$C NMR (100 MHz, δ, ppm, DMSO-$d_6$): 170.87, 168.78, 164.25, 163.49, 150.87, 142.31, 130.78, 128.11, 126.65, 125.12, 121.43, 50.72, 32.28; Anal. Calcd. for C$_{13}$H$_9$N$_5$O$_5$S$_2$: C, 41.16; H, 2.39; N, 18.46; Found: C, 41.15; H, 3.33; N, 18.53%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-3-nitrophenyl-1,3,4-thiadiazol-2-yl) acetamide (A12) Light yellow solid; yield 50%. m.p.: 241°C; IR (KBr, ν, cm$^{-1}$): 3453, 3219 (ν$_{NH}$), 2982, 1697 (ν$_{C=O}$); $^1$H NMR (400 MHz, δ, ppm, DMSO-$d_6$): 13.12 (s, 1H), 12.13 (s, 1H), 7.78 (d, 2H), 7.74-7.72 (m, 2H), 4.31 (m, 1H), 3.41 (dd, J =16.41 Hz, J =4.38 Hz, 1H), 3.21 (dd, J =15.43 Hz, J =4.78 Hz, 1H); $^{13}$C NMR (100 MHz, δ, ppm, DMSO-$d_6$): 171.91, 167.57, 163.41, 162.12, 151.13, 141.54, 130.38, 128.87, 126.11, 124.42, 51.76, 34.81; Anal. Calcd. for C$_{13}$H$_9$N$_5$O$_5$S$_2$: C, 41.16; H, 2.39; N, 18.46; Found: C, 41.14; H, 3.34; N, 18.54%.
2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-4-nitrophenyl-1,3,4-thiadiazol-2-yl)acetamide

(A13) Bright yellow solid; yield 54%; m.p.: 256 °C; IR (KBr, v, cm\(^{-1}\)): 3418, 3312 (\(v_{\text{NH}}\)), 2944, 1691 (\(v_{\text{C=O}}\)); \(^1\)H NMR (400 MHz, \(\delta\), ppm, DMSO-\(d_6\)): 12.92 (s, 1H), 12.14 (s, 1H), 8.27 (d, \(J = 7.2\) Hz, 2H), 7.98 (d, \(J = 6.23\) Hz, 2H), 4.12 (m, 1H), 3.42 (dd, \(J = 15.83\) Hz, \(J = 4.62\) Hz, 1H), 3.13 (dd, \(J = 16.41\) Hz, \(J = 4.23\) Hz, 1H); \(^{13}\)C NMR (100 MHz, \(\delta\), ppm, DMSO-\(d_6\)): 169.24, 167.98, 165.46, 161.59, 150.22, 130.54, 128.43, 126.63, 125.65, 52.43, 34.21; Anal. Calcd. for \(\text{C}_{13}\text{H}_9\text{N}_5\text{O}_5\text{S}_2\): C, 41.17; H, 2.39; N, 18.46; Found: C, 41.17; H, 3.35; N, 18.50%.

2-(2,4-dioxo-1,3-thiazolidin-5-yl)-N-(5-trifluoromethyl-1,3,4-thiadiazol-2-yl)acetamide

(A14) White solid; yield 49%; m.p.: 198 °C; IR (KBr, v, cm\(^{-1}\)): 3417, 3319 (\(v_{\text{NH}}\)), 1689 (\(v_{\text{C=O}}\)); \(^1\)H NMR (400 MHz, \(\delta\), ppm, DMSO-\(d_6\)): 13.00 (s, 1H), 12.23 (s, 1H), 4.33 (m, 1H), 3.43 (dd, \(J = 17.38\) Hz, \(J = 4.78\) Hz, 1H), 3.11 (dd, \(J = 15.33\) Hz, \(J = 4.23\) Hz, 1H); \(^{13}\)C NMR (100 MHz, \(\delta\), ppm, DMSO-\(d_6\)): 170.45, 168.33, 165.27, 156.85, 150.18, 121.44, 50.12, 34.21; Anal. Calcd. for \(\text{C}_8\text{H}_3\text{N}_4\text{O}_3\text{S}_2\): C, 29.45; H, 1.54; N, 17.17; Found: C, 29.40; H, 1.51; N, 17.56%.
### Table 2a. *In vitro* Antimicrobial activity of A1-A14 expressed as Minimum inhibitory concentration (MIC) in μg/ml

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<th>S.a</th>
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<td>8</td>
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<td>A7</td>
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<td>32</td>
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<td>8</td>
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Table 2b. *In vitro* cytotoxicity profile of thiazolidine-2,4-dione derivatives (A1-14) against selected human cancer cell lines IC\textsubscript{50} (µM)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>HeLa</th>
<th>HT29</th>
<th>A549</th>
<th>MCF7</th>
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<tbody>
<tr>
<td>A1</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>62</td>
<td>78</td>
<td>80</td>
<td>80</td>
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<tr>
<td>A2</td>
<td>4-Cl.C\textsubscript{6}H\textsubscript{4}</td>
<td>62</td>
<td>64</td>
<td>60</td>
<td>68</td>
</tr>
<tr>
<td>A3</td>
<td>4-Br.C\textsubscript{6}H\textsubscript{4}</td>
<td>52</td>
<td>60</td>
<td>68</td>
<td>72</td>
</tr>
<tr>
<td>A4</td>
<td>4-F.C\textsubscript{6}H\textsubscript{4}</td>
<td>50</td>
<td>42</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
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<td>2-Cl.C\textsubscript{6}H\textsubscript{4}</td>
<td>60</td>
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<tr>
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<td>50</td>
<td>58</td>
<td>46</td>
<td>52</td>
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<tr>
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<td>54</td>
<td>56</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
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<td>45</td>
<td>42</td>
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<td>50</td>
<td>68</td>
<td>72</td>
<td>78</td>
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<tr>
<td>A12</td>
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<td>68</td>
<td>72</td>
<td>78</td>
<td>67</td>
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<tr>
<td>A13</td>
<td>4-NO\textsubscript{2}.C\textsubscript{6}H\textsubscript{4}</td>
<td>50</td>
<td>60</td>
<td>68</td>
<td>72</td>
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<tr>
<td>A14</td>
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</table>

Cell lines include cervical carcinoma (HeLa); colorectal cancer (HT29); lung cancer (A549); breast adenocarcinoma (MCF-7).
Table 2c. Drug Likeliness of A 1-14

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>LogP</th>
<th>HBD</th>
<th>HBA</th>
<th>Rule-5 violation</th>
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<tbody>
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<td>7</td>
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<tr>
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<td>2</td>
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<tr>
<td>A3</td>
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<td>2.22</td>
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<tr>
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<tr>
<td>A8</td>
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<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>A12</td>
<td>379.39</td>
<td>1.34</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>A13</td>
<td>379.39</td>
<td>1.34</td>
<td>2</td>
<td>10</td>
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<tr>
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<td>0.45</td>
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<td>7</td>
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</tr>
</tbody>
</table>

MW: molecular weight; LogP: calculated using software; HBD: hydrogen bond donors; HBA: hydrogen bond acceptor; Lipinski rule of 5: (Molecular weight ≤500, Log P ≤5, HBD ≤5 and HBA ≤10) study was carried out using Pallas and molinspiration software.
**In vitro assay for antimicrobial activity**

The cultures were obtained from Mueller-Hinton broth for all the bacterial strains after 24 h of incubation at 37 ± 1 °C. Fungi were maintained in Sabouraud dextrose broth after incubation for 24 h at 25 ± 1 °C. Testing was carried out in Mueller-Hinton broth and Sabouraud dextrose broth at pH 7.4 and the twofold serial dilution technique was applied. The final inoculums size was 10⁵ CFU/ml for the antibacterial assay and 10⁴ CFU/ml for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37±1 °C and after incubation for 48 h at 25 ± 1°C for antifungal assay, the tube with no growth of microorganism was recorded to represent the MIC expressed in µg/ml. Every experiment in the antibacterial and antifungal assays was performed in triplicate.

**In vitro assay for cytotoxic activity**

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system. Test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the drugs. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100µM MTT reagent (1 mg/ml) for 5h at 37°C. The suspension was placed on microvibrator for 10 min and absorbance was recorded by the ELISA reader. The experiment was performed in triplicate.

Spectral analytical data for all the compounds are described in experimental part of this chapter. Some representative spectra are given in the following sections.
Spectrum No. 1: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2a in DMSO-$d_6$

Spectrum No. 2: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2b in DMSO-$d_6$
Spectrum No.3: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2c in DMSO-$d_6$.

Spectrum No.4: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2d in DMSO-$d_6$. 
Spectrum No.5: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2e in DMSO-$d_6$

Spectrum No.6: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2f in DMSO-$d_6$
Spectrum No.7: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2g in DMSO-$d_6$

Spectrum No.8: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2h in DMSO-$d_6$
Spectrum No. 9: $^1$H NMR (400 MHz, $\delta$, ppm) of compound $2i$ in DMSO-$d_6$

Spectrum No. 10: $^1$H NMR (400 MHz, $\delta$, ppm) of compound $2j$ in DMSO-$d_6$
Spectrum No.11: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2k in DMSO-$d_6$

Spectrum No.12: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2l in DMSO-$d_6$
Spectrum No. 13: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2m in DMSO-$d_6$.

Spectrum No. 14: $^1$H NMR (400 MHz, $\delta$, ppm) of compound 2n in DMSO-$d_6$. 
Spectrum No. 15: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A1 in DMSO-$d_6$

Expanded spectrum of compound A1 in DMSO-$d_6$
Spectrum No. 16. $^{13}$C NMR (100 MHz, $\delta$, ppm) of compound A1 in DMSO-$d_6$

Spectrum No. 17: IR (KBr, $\nu$, cm$^{-1}$) of compound A1
Spectrum No.18: Mass of compound A1

Spectrum No.19: $^1$H NMR (400 MHz, δ, ppm) of compound A2 in DMSO-$d_6$
Spectrum No. 20: Spectrum No. IR (KBr, \( \nu \), cm\(^{-1} \)) of compound A2

Spectrum No. 21: \(^1\)H NMR (400 MHz, \( \delta \), ppm) of compound A3 in DMSO-\( d_6 \)
Spectrum No.22:  IR (KBr, $\nu$, cm$^{-1}$) of compound A3

Spectrum No.23:  $^1$H NMR (400 MHz, $\delta$, ppm) of compound A4 in DMSO-$d_6$
Spectrum No.24: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A6 in DMSO-$d_6$

Spectrum No.25: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A7 in DMSO-$d_6$
Spectrum No. 26: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A8 in DMSO-$d_6$

Spectrum No. 27: IR (KBr, $\nu$, cm$^{-1}$) of compound A8
Spectrum No. 28: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A9 in DMSO-$d_6$

Spectrum No. 29: IR (KBr, $\nu$, cm$^{-1}$) of compound A9
Spectrum No. 30: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A10 in DMSO-$d_6$

Spectrum No. 31: IR (KBr, $\nu$, cm$^{-1}$) of compound A10
Spectrum No. 32: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A11 in DMSO-$d_6$

Spectrum No. 33: IR (KBr, $\nu$, cm$^{-1}$) of compound A11
Spectrum No. 34: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A12 in DMSO-$d_6$.

Spectrum No. 35: IR (KBr, $\nu$, cm$^{-1}$) of compound A12.
Spectrum No. 36: $^1$H NMR (400 MHz, δ, ppm) of compound A13 in DMSO-$d_6$

Spectrum No. 37: IR (KBr, ν, cm$^{-1}$) of compound A13
Spectrum No. 38: $^1$H NMR (400 MHz, $\delta$, ppm) of compound A14 in DMSO-$d_6$
References


Chapter-II

*Chem. 2009, 17, 882.*


[40] Pallas 3.7.1.2, ADME-Tox software, CompuDrug International Inc. U.S.A.