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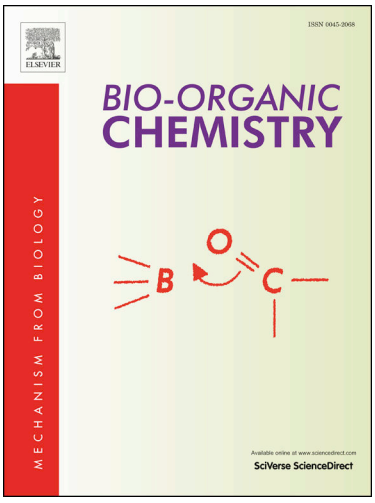

Exploiting oxadiazole-sulfonamide hybrids as new structural leads to combat diabetic complications via aldose reductase inhibition

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Exploiting oxadiazole-sulfonamide hybrids as new structural leads to combat diabetic complications via aldose reductase inhibition

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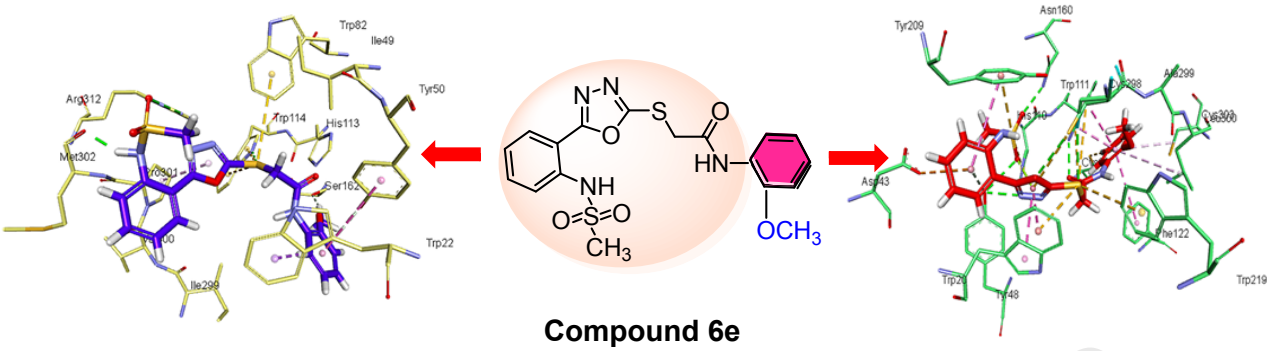
Abstract:

A series of oxadiazole-sulfonamide hybrids was synthesized through multistep reaction and for the formation of targeted thioethers **6(a-l)**, a much facile route was adopted through which S-alkylation was successfully carried out at room temperature. These novel thioethers **6(a-l)** were later screened against aldehyde reductase (ALR1) and aldose reductase (ALR2). Beside the enzyme inhibition studies, the compounds were also tested against cervical cancer cell lines (HeLa). The results suggested the significant inhibition pattern towards ALR2, while few compounds were active against ALR1. The synthesized derivatives have shown weak to moderate cytotoxicity. The most potent inhibitors (**6b**, **6e**, **6f** and **6l**) were selected for molecular docking studies and the binding interactions were reported.

Keywords: Aldose reductase; oxadiazole-sulfonamides; cytotoxicity; docking studies.

Graphical Abstract:



Compound 6e

Potent Dual Inhibitor

Against ALR1: IC_{50} 3.26 ± 0.23 μM

Against ALR2: IC_{50} 3.06 ± 0.48 μM

Introduction

Among the nitrogen/oxygen containing heterocycles, oxadiazole nucleus has become a significant pharmacophore in the medicinal arena. Oxadiazole moiety is present in the structures of many drugs such as nesapidil, furamizole, and raltegravir whereas some other derivatives have also been reported as plant growth regulators [1]. Literature studies expose that compounds bearing 1,3,4-oxadiazole moiety hold noteworthy biological and pharmacological properties such as analgesic [2], anticancer [3], hypoglycemic [4], anti-HIV [5] and antitubercular activities [6]. Oxadiazole derivatives have also been reported as photosensitizers and a few of their derivatives possess liquid crystals properties [7]. On the other hand, sulfonamides are an important class of compounds of paramount significance to humanity. Since sulpha drugs were reported as the first anti-microbial agents, these active molecules have turned into important class of heterocyclic compounds that find their applications in pharmaceutical and agrochemical industries. Various types of pharmacological agents carry sulfonamide moiety which exhibit antitumor [8], anti-carbonic anhydrase [9], hypoglycemic [10], diuretic [11] and anti-HIV activities [12]. Most of the commercially available antibacterial, antihypertensive and hypoglycemic drugs such as topiramate, mafenide, and acetazolamide etc. are sulfonamides in nature [13]. The literature reveals that the combination of oxadiazole and sulfonamide as hybrid molecules could exhibit diverse pharmacological properties (**Fig. 1**) [14-17].

The hybrid structures of oxadiazole and sulfonamide were selected for aldose reductase inhibition because both moieties had previously shown selective inhibition against ALR2 [18]. So oxadiazole and sulfonamide moieties might inhibit the enzyme synergistically. As in previous studies, similar structure oxazolidine possess good inhibitory activities against ALR2 [19]. Other sulfonamide derivatives have also shown inhibitory potential for ALR2 [20-21].

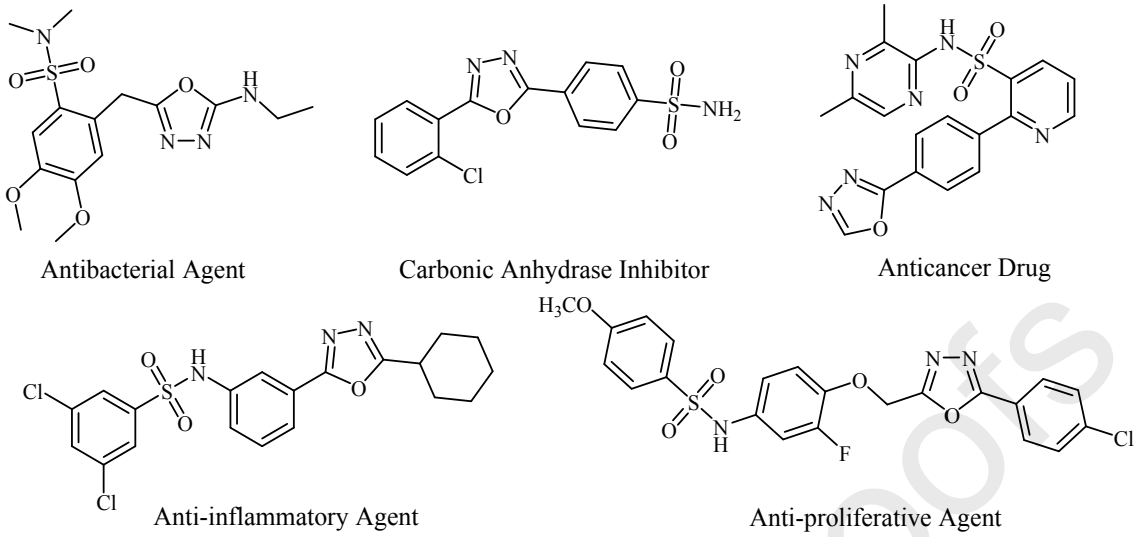


Figure 1. Biologically important oxadiazole-sulfonamide hybrids [14-17]

Aldo-keto reductases comprise an oxidoreductase superfamily having ubiquitous distribution throughout the living organisms, most of them are NADPH dependent and are monomeric proteins present in cytosol. The protein contains variable external loops and conserved substrate binding region, which are responsible for the reduction of carbonyl groups present in various compounds [22-23].

Aldose reductase AKR1B1 (ALR2; AR; EC 1.1.1.21) is responsible for the determination of reaction rate in polyol pathway in which ALR2 converts glucose into sorbitol alongside the parallel conversion of NADPH to $NADP^+$ [24]. Sorbitol dehydrogenase is another enzyme which further converts sorbitol to fructose (**Fig. 2**). The accumulation of sorbitol inside the interior of the cell causes osmotic imbalance which ultimately leads to cell damage. While passing through several intermediates, the end products of fructose metabolism are the advanced glycation end products which lead to the production of reactive oxygen species. Moreover, ALR2 causes NADPH depletion which results in increase in oxidative stress [25]. ALR2 is known as very critical in diabetic patients for their cardiovascular, renal, ocular system and nervous neuropathies [25]. Moreover, AKR1B1 has been found in retina, nervous tissues, kidney, aorta and the tissues in which diabetic complications occur. Aldehyde reductase (ALR1, EC 1.1.1.2) is known for the detoxification of metabolites in liver [26]. The structures of ALR1 and ALR2 have 65% sequence homology, with few differences in their respective active sites [27].

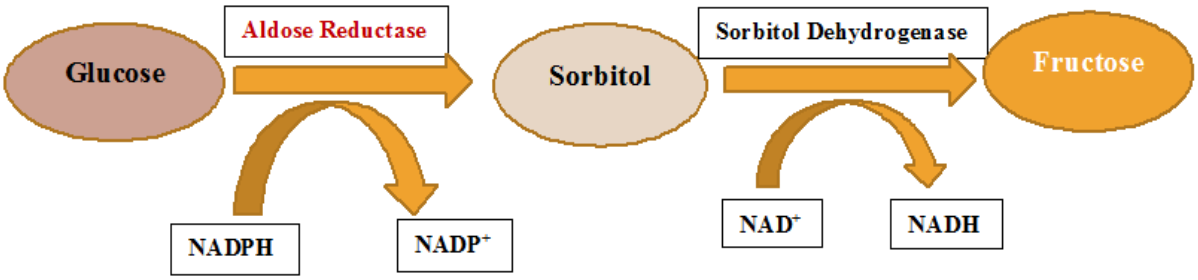


Figure 2. Polyol pathway of glucose metabolism

ALR2 was initially reported to be involved in diabetic complications like neuropathy, nephropathy, retinopathy and atherosclerotic plaque formation [27]. Several research studies suggest that inhibition of ALR2 could be helpful in the prevention of complications associated with diabetes including atherosclerosis, kidney damage, cataract, retinopathy and neuropathy. But on the other hand, increased level of ALR2 may also induce the oxidative stress which could lead to many inflammatory pathologies, tissues dysfunction, cell death and modified intracellular signaling [27-29]. Several cardiac complications including myocardial ischemia, congestive heart failure, cardiac hypertrophy, cardiomyopathy linked with increased generation of reactive oxygen species (ROS) due to over expression of ALR2 are the reported side effects [30].

There are several inhibitors of ALR2 identified in recent years but only few showed enhanced therapeutic advancement and are in clinical trials, while epalrestat is the only ALR2 inhibitor available commercially in market till now. Most of the reported drugs and known inhibitors either show toxicity in the body or have poor blood brain barrier and pharmacological properties. Therefore, new drugs as ALR2 inhibitors with enhanced biological profile are required. The targeted drugs to be used as enzyme inhibitors are good therapeutic design to combat the associated diseases. The novel sulfonamide hybrids with 4-thiadiazoles and 1,3,4-oxadiazoles act as potent antibacterial and antifungal agents [14]. Sulfonamides other than potential carbonic anhydrase inhibitors [15] revealed antidiabetic, anti-inflammatory and anticancer activities when substituted with 2,5-disubstituted-1,3,4-oxadiazole derivatives [16]. Moreover, antiproliferative activity of new 1,3,4-oxadiazole analogues having sulfonamide moiety is also reported [17]. Therefore, these hybrid compounds (having oxadiazole and sulfonamide moieties) were designed and synthesized to investigate their inhibition profile against ALR2 and ALR1. Afterwards the toxicity of the compounds was explored against cancer cell lines. Moreover, the selective inhibitors were docked inside the protein to justify

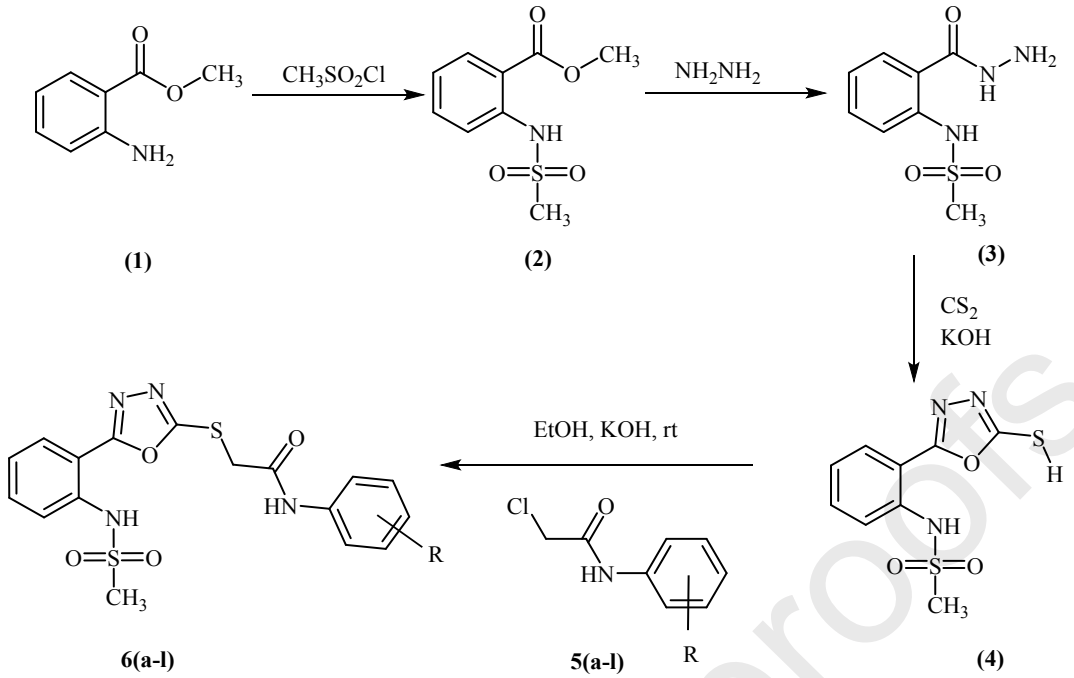
the experimental results so these inhibitors may be a good addition to the field of medicinal chemistry.

Results and discussion

Chemistry

A series of oxadiazole-sulfonamide hybrids **6(a-l)** have been synthesized by using the synthetic route illustrated in **Scheme 1**. The designed route involved the initial synthesis of sulfonamide (**2**) by the reaction of methyl anthranilate (**1**) and methanesulfonyl chloride. Later, hydrazide (**3**) was prepared and further cyclized into corresponding oxadiazole (**4**). On the other side, different chloroacylated anilines **5(a-l)** were synthesized from substituted anilines and chloroacetyl chloride. Finally, S-alkylation was carried out at room temperature in ethanol and the new products **6(a-l)** were obtained in good to excellent yields. The reaction was successfully carried out under basic medium (KOH) through S_N2 mechanistic route. The washings with dil. HCl also helped in neutralizing KOH remains.

For structure elucidation, different spectroscopic methods were employed. In ^{1}H NMR, the singlet of methyl (-SO_2-C<u>H</u>$_3$) protons appeared around 3.10-3.20 ppm whereas another significant singlet of methylene (-S-C<u>H</u>$_2$) appeared around 4.20-4.45 ppm which confirmed the formation of new S-CH_2 linkage. Two important N<u>H</u> singlets also noted around 9.50-10.50 ppm in all compounds **6(a-l)**. Beside other ^{13}C NMR signals, an important methyl carbon (-S-<u>C</u>H_3) has appeared around 37.00 ppm. Unfortunately, methylene carbon (-S-<u>C</u>H_2) has been masked by solvent signal, however, significant carbonyl carbon (-<u>C</u>=O) spotted near to 165.00 ppm in all samples. In addition, FTIR data and elemental analysis also confirmed the product formation.



6a. R= H
6b. R = 2-CH_3
6c. R = 3-CH_3
6d. R = 4-CH_3
6e. R = 2-OCH_3
6f. R = 4-OCH_3
6g. R= 4-I
6h. R = 2-$COOCH_3$
6i. R = 4-$COOC_2H_5$
6j. R = 2-COOH
6k. R = 4-COOH
6l. R = 4-NO_2

Scheme 1. Synthesis of oxadiazole based thioethers.

Biological Activity

Enzyme Inhibition Studies

All the synthesized compounds (**6a-l**) were tested for their potential inhibition against two enzymes of aldo-keto reductase family: ALR2 and partially purified ALR1. Due to the sequence homology (65%) in the structures of both the enzymes, among the screened compounds, some dual inhibitors have been identified. The main purpose of the study was to identify the most potent and selective inhibitors of ALR2, as the enzyme was found to be involved in diabetic complication, inflammatory pathologies, cardiac injuries and different types of cancers. On the other side, the ALR1 is mainly responsible for the detoxification of toxic aldehyde and lipids. Non-selective inhibitors of ALR2 bear some unwanted side effects. The inhibition data obtained from the enzymatic analysis is presented in **Table 1**. Valproic acid and sorbinil were taken as reference standards during the assay. The inhibitory concentration obtained for the valproic acid was 57.4 ± 0.89 µM for the ALR1, whereas, IC_{50} value of sorbinil against ALR2 was found as 3.14 ± 0.02 µM.

Table 1. The aldose and aldehyde reductase inhibition activity and percent scavenging activity (%RSA) of synthetic derivatives **6(a-l)**

Compounds	ALR1[b]	ALR2	%RSA[c]	Selectivity Index[d]
	IC_{50} ± SEM (µM) / %Inhibition			
6a	5.84 ± 0.32	4.26 ± 0.84	18.9 ± 1.07	1.37
6b	4.77 ± 0.47	6.38 ± 0.64%	17.5 ± 0.99	-
6c	5.66 ± 0.87	3.94 ± 0.32	15.2 ± 1.21	1.43
6d	5.13 ± 0.57	3.69 ± 0.21	2.81 ± 0.58	1.39
6e	3.26 ± 0.23	3.06 ± 0.48	5.70 ± 0.43	1.06
6f	14.3 ± 1.29%	2.21 ± 0.73	35.4 ± 1.26	-
6g	-	-	-	-
6h	22.1 ± 1.98%	24.8 ± 1.87%	27.5 ± 2.11	-
6i	-	-	-	-
6j	18.2 ± 1.05%	30.6 ± 2.33%	2.81 ± 0.22	-
6k	13.4 ± 1.14%	8.60 ± 0.38%	21.2 ± 1.51	-
6l	3.99 ± 0.45	3.56 ± 0.02	20.4 ± 1.06	1.12
Valproic acid	57.4 ± 0.89	-	-	-
Sorbinil[a]	-	3.14 ± 0.02	-	-
Ascorbic Acid			81.4 ± 4.12	-

[a]: IC_{50} for sorbinil and valproic acid was taken as reference value; [b]: partially purified preparation containing ALR1; [c]: %radical scavenging activity by DPPH assay; [d]: Defined as IC_{50} [ALR1]:IC_{50} [ALR2]. The compounds were tested at 1 mM end concentration for ALR1, ALR2 and %RSA activity.

Structure-activity Relationship

Figure 3 represents the detailed structure-activity relationship of active scaffolds and substituents attached. A series of thioether derivatives was synthesized and evaluated for their ability to inhibit ALR1 and ALR2. These thioether derivatives contained phenyl ring attached to the nitrogen atom of acetamide. Varying the nature and position of these substituents on this phenyl ring lead to an altered inhibitory activity. Among all the synthesized compounds, ten compounds were screened for their inhibitory potential for ALR1 and ALR2. In this regard, six compounds were identified as potent inhibitors of these enzymes. Only one compound i.e. **6b** was identified as selective inhibitor of ALR1 with an IC_{50} value of 4.77 ± 0.47 µM, whereas,

compound **6f** (IC_{50} = 2.21 ± 0.73 µM) inhibited ALR2, selectively. However, remaining analogues showed dual inhibition of both enzymes. Valproic acid was used as standard inhibitor for ALR1 while sorbinil was employed as standard inhibitor for ALR2

In case of ALR1, six compounds were identified as potent inhibitors with IC_{50} values ranging from 3.26 ± 0.23 to 5.84 ± 0.32 µM. Whereas, % inhibition of four compounds (**6f, 6h, 6j** and **6k**) was less than 50%. The highest activity was shown by compound **6e** (IC_{50} = 3.26 ± 0.23 µM) containing a methoxy group at *ortho* position. Here position of substituent had an important role since the introduction of this methoxy group at *para* position resulted in decreased activity (<50%). However, in case of methyl substitution, change of position resulted in the maintenance of activity. Thus, with respect to position of methyl substitution, following order of activity was observed *ortho*-CH_3 > *meta*-CH_3 > *para*-CH_3. Moreover, compound **6b** was also found to be selective inhibitor of ARL1. Likewise, incorporation of a nitro group at para position (**6l**) lead to good inhibitory activity for ALR1. However, inhibitory activity of compounds containing either carboxyl group or ester was <50%.

On the other hand, six compounds were found to be potent inhibitors of ALR2, with their IC_{50} values in micromolar range (2.21 ± 0.73-6.38 ± 0.64 µM). Only one compound i.e. **6f** was identified as selective inhibitor of ALR2. Here again same pattern of activity was observed as seen in ALR1, although with slight changes. The most potent inhibitory activity was shown by the compounds containing a methoxy group. Although para position was slightly more favorable as compared to *ortho* position, as reflected by the IC_{50} values of **6f** (IC_{50} = 2.21 ± 0.73 µM) and **6e (**IC_{50} =3.06 ± 0.48 µM), respectively. Moreover, **6e** and **6f** are the only compounds whose IC_{50} value is higher as compared to standard i.e. sorbinil (IC_{50} = 3.14 ± 0.02 µM). On the other hand, compounds incorporating a methyl group also had good inhibitory activity. In this regard, *meta* and *para* position was favorable since the introduction of methyl group at *ortho* position resulted in reduced activity i.e. <50%. Similarly, introduction of nitro group also resulted in good inhibitory activity with an IC_{50} value of 3.56 ± 0.02 µM. The compounds **6j** and **6k** did not show any promising inhibition of this enzyme.

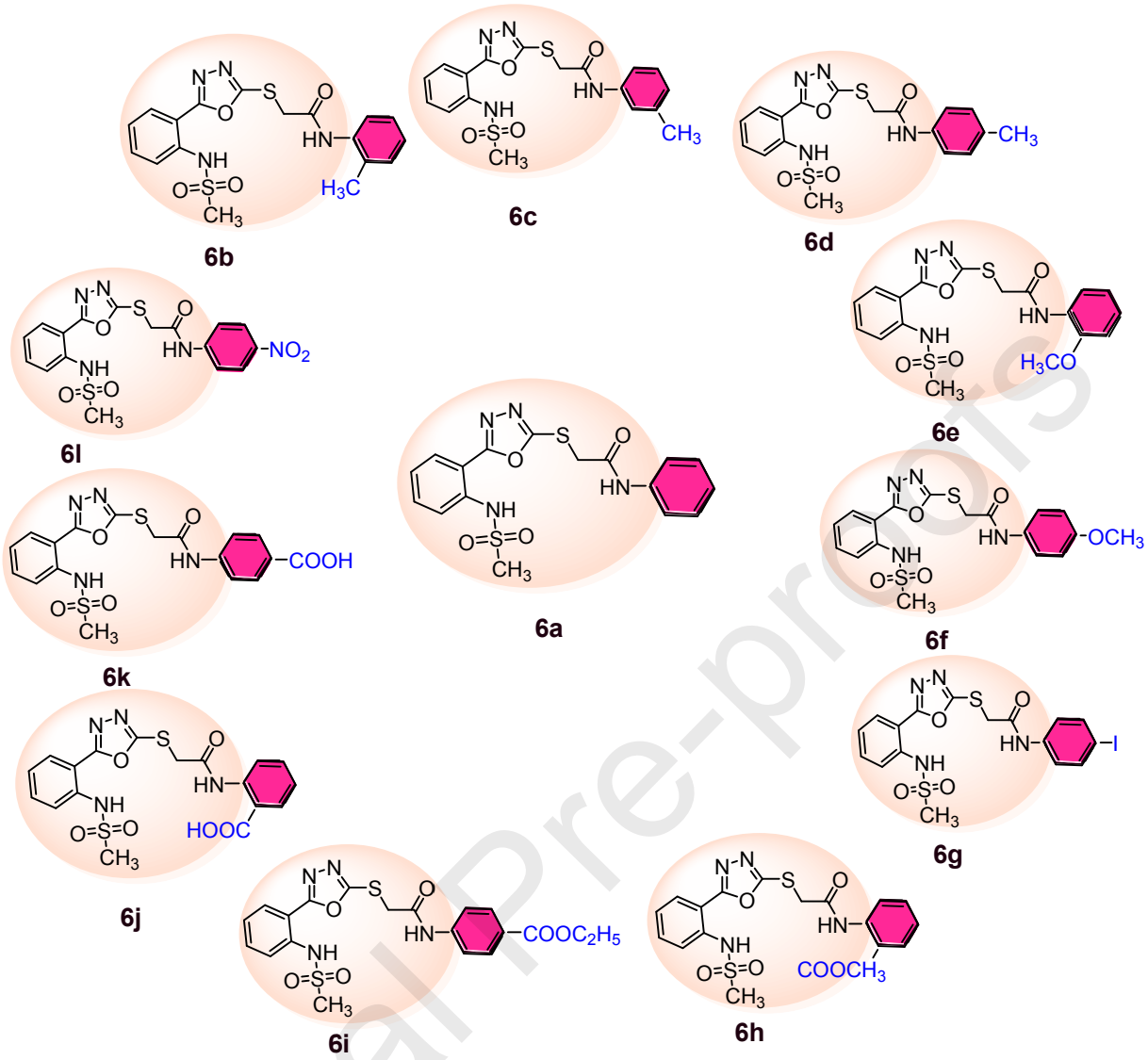
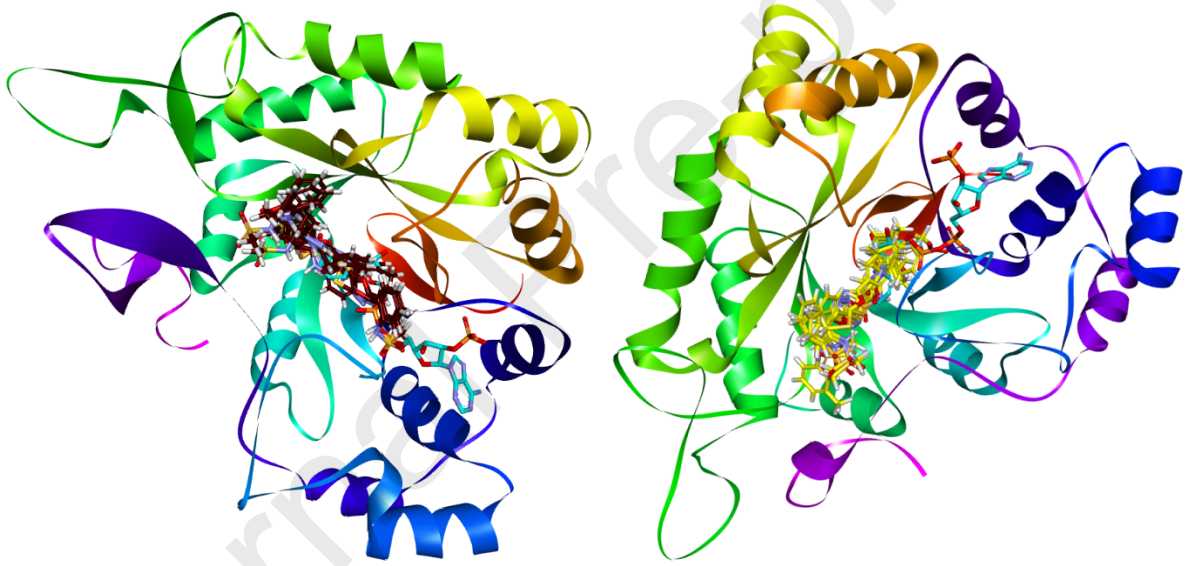


Figure 3. Structure-activity relationship of synthetic compounds **6(a-l)**

Surprisingly, switching to the electron-withdrawing -NO_2 group, as in compound **6l**, has dual and potent inhibitory effects against both the enzymes. This compound showed significant inhibition against ALR1 along with good inhibitory activity against ALR2. Compounds **6g** having 4-iodophenyl and **6i** having 4-ethyl benzoate as substituents were not tested against any of the enzyme. These results pointed out towards the influential effects of electron-donating functionalities that remarkably improve the inhibition potential of molecules.

Docking studies

In order to justify the experimental results, the docking analysis was carried out against the potent and selective inhibitors of ALR2 as well as ALR1. The *in vitro* results clarified that **6b** was potent and selective inhibitor of ALR1, whereas, **6f** was selective as well as potent inhibitor of ALR2. However, some dual inhibitors were found and docking analysis was carried out against those dual inhibitors, **6e** and **6l** in the active site of both the receptors. To investigate the binding poses of selected compounds, the protein structures were selected from protein databank. In case of ALR2, the crystal structure of human ALR2 was available (1US0) and was downloaded [31]. However, human ALR1 crystal structure was not available and porcine ALR1 structure (3FX4) [32] was selected (as human and porcine show about 97% sequence homology) [33-34]. **Fig. 4**(**a**,**b**) depicts the overlap of all the selected compounds docked inside the active site of ALR1 and ALR2, respectively.

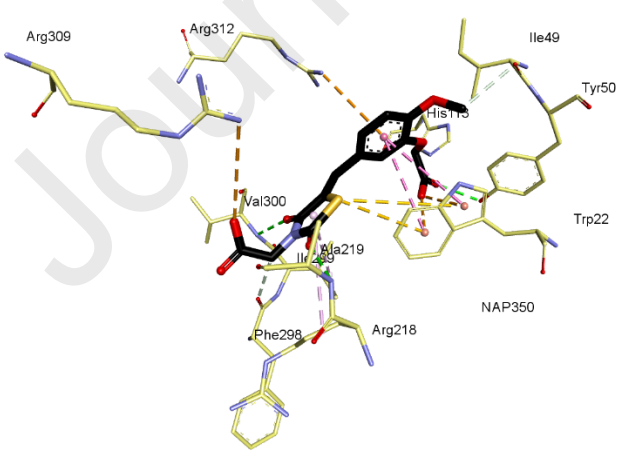


(a) **(b)**

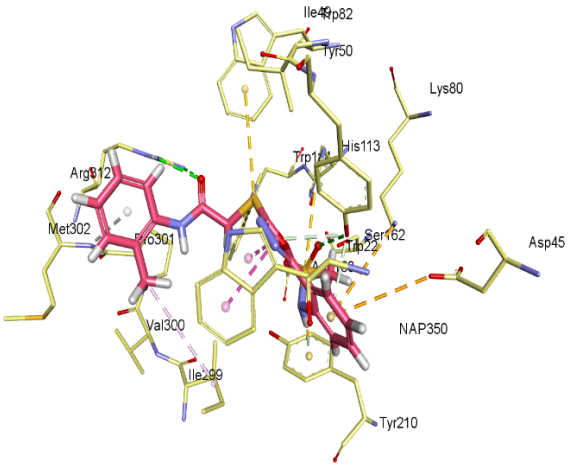
Figure 4. The overlap of all the docked inhibitors (**6a-f** and **6l**) inside the active site of aldehyde reductase **(a)** and aldose reductase **(b)**

To validate the docking studies, the co-crystallized ligands of ALR1 and ALR2 were docked inside the active site after extraction from respective receptor. The re-docking of co-crystallized ligands (FX4401 for 3FX4 and IDD594 for 1US0) were carried out by root mean square deviation of 0.89 and 1.01 Å for ALR1 and ALR2, respectively. The active pockets were selected after analysis of binding interactions of co-crystallized ligands within the active site. The 3D interaction diagrams of co-crystallized ligand, selective inhibitor and dual inhibitors of ALR1 were shown in **Fig. 5**, while the 3D interaction diagrams of co-crystallized ligand, selective inhibitor and dual inhibitors of ALR2 were presented in **Fig. 6**.

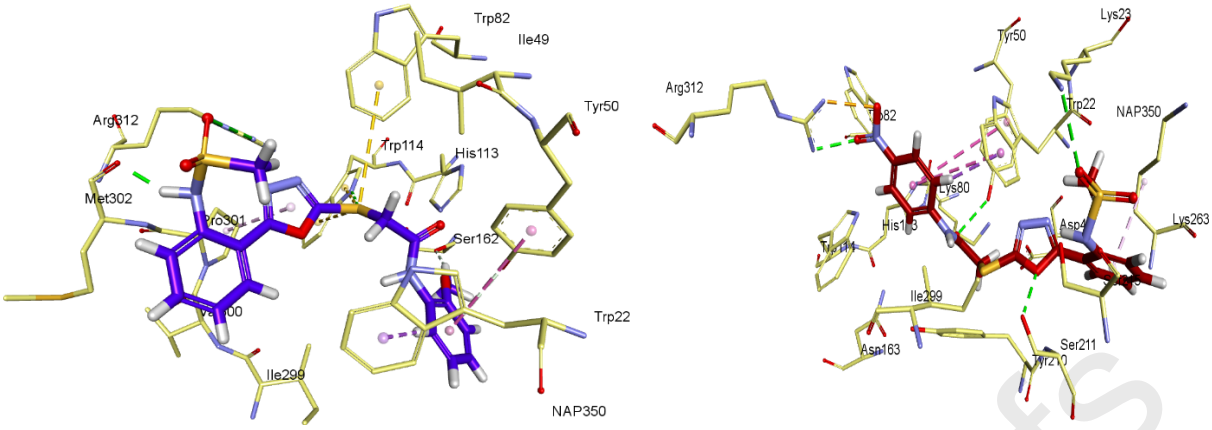
The detailed analysis of ALR1 co-crystallized ligand and the inhibitors showed that Trp22 and Tyr50 were the most important residues showing interaction with the inhibitors within the active site. The cognate ligand presented the same interactions and our selective inhibitor, **6b** was making π-π interactions with Tyr50. In addition to π-π interactions, the hydrogen bonds were noticed between active site amino acid residues and the compound. The dual inhibitors, **6e** and **6l** were making the hydrogen bonds and π-π interactions with important residues. The interaction analysis suggested that the selected compounds showed best fit interaction inside the pocket of ALR1. The bond distances for all the interactions presented in 3D in **Fig. 5** were shown in **Fig. S1**. The cognate ligand showed the bond distance of 2.62 Å with Tyr50. However, the bond distance showed by Trp22 with the phenyl ring was 4.78 and 4.87 Å. The selective inhibitor **6b**, was found at a bond distance of 4.11 and 4.35 Å from Trp22 from 2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide group, while at a distance of 2.85 Å from Tyr50. Similarly, the dual inhibitors showed the interactions with active site residues. The 2-methoxyphenyl group of **6e** was found at 5.44 Å from Tyr50 and 2.54 Å from Trp22. In the compound **6l**, 4-nitrophenyl group was present at a bond distance of 5.74 Å from Try22 and 2.90 Å from Tyr50 inside the active pocket of ALR1. Moreover, the 2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide was found as contributing group for **6b** towards the inhibition, while, 2-methoxyphenyl group of **6e** and 4-nitrophenyl group of **6l** were found to show interactions more than other atoms of the respective compounds. The selected compounds showed the binding interactions necessary for the inhibition of ALR1 within the active site.



(**a**)



(**b**)



(**c**) (**d**)

Figure 5. 3D interaction diagrams of cognate ligand (FX4401) (**a**) and selective inhibitor (**b**; 6b) of ALR1 along with dual inhibitors (**c**: 6e; **d**; 6l) inside the 3FX4

When ALR2 active site residues and interaction analysis of selected compounds were investigated, it was noted that the compounds exhibited the interactions which were reported in the literature [35-36] and are responsible for the inhibitory behavior towards ALR2. In addition to Typ111 and Trp20, His110 and Tyr48 are also important to show interactions and are playing key role towards the inhibition of enzyme. The detailed bond distances of selected compounds with all the active site residues have been provided in **Fig. S2**. However, the bond distance of some of the important residues and their type of interactions are explained here. The co-crystallized ligand showed the hydrogen bonding with Tyr48 at the bond distance of 2.73 Å. However, the selective inhibitor of ALR2, compound **6f** showed the same interaction with Tyr48 at bond distance of 3.47 Å. The dual inhibitors, **6e** and **6l** exhibited 4.36 and 4.03 Å, respectively, with Tyr48. When π-π interactions of Trp20 were considered, the cognate ligand showed 4.68 and 5.26 Å bond distance, while **6e** was 3.98 and 3.76 Å away from Trp20. Similarly, compound **6l** was at 4.53 and 4.80 Å from Trp20. The cognate ligand was at a bond distance of 3.43 and 3.73 Å from Trp111 while selective compound **6f** was at 2.86 Å from the same residue in the active pocket of ALR2. The compound **6f** showed 4.17 and 4.14 Å distance from another important residue of active site, His110 from middle of the compound. The anionic pocket/ catalytic pocket of ALR2 contain His110, Tyr48 and Trp111 [37], therefore, upon binding of negatively charged group (thioacetamide in the case of compound **6f**), flexible adaptation has been noticed and as a result additional hydrophobic pocket was opened. In addition to sulfonamide moiety, here the role of thioacetamide moiety was found which anchor inside the anionic site and was responsible for selective behavior of **6f** towards ALR2 as compared to ALR1 and exhibited hydrogen bonds with the side chains. The interaction analysis

suggested that the compounds were found in best fitted poses inside the active site of ALR2 and therefore the investigated interactions are responsible for the inhibition profile of the compounds.

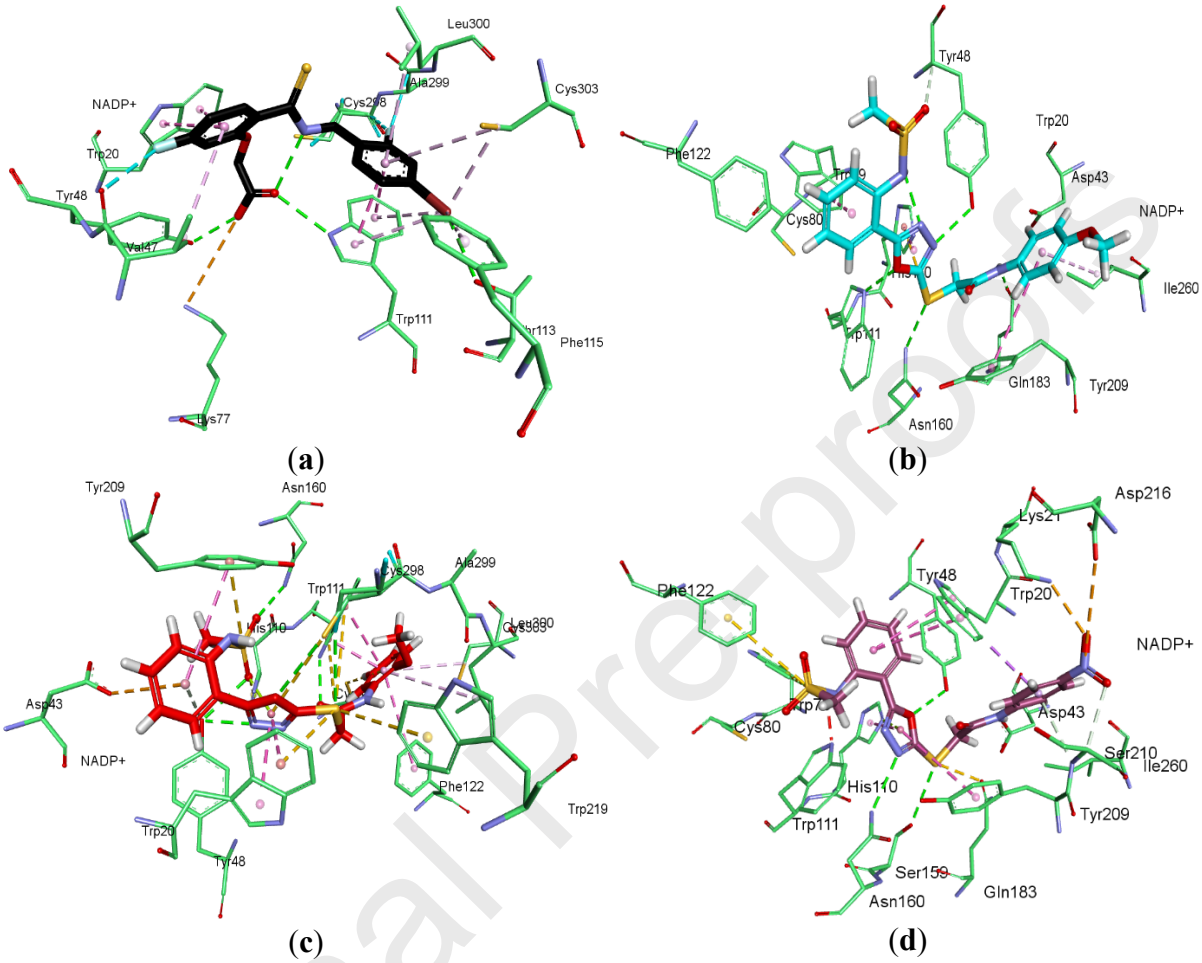


Figure 6. 3D interaction diagrams of cognate ligand (IDD594) (**a**) and selective inhibitor (**b**; 6f) of ALR2 along with dual inhibitors (**c**: 6e; **d**; 6l) inside the 1US0

Molecular Dynamic Simulations

In order to justify the docking analysis, the molecular dynamic simulations were performed for both the enzymes (ALR1 and ALR2). The structure of proteins (apo) were first subjected to MD run of 30 ns and then docked poses of selected ligands were submitted to MD run. The results showed that 3FX4 and the ligand attained stability shown by RMSD value for apo (protein) and holo (Protein + ligand) structures (Figure 7). However, the RMSD plot of ALR2 showed that the trajectories of apo and holo forms showed less fluctuations after 4 ns and both the plots were found stable afterwards (Figure 10). When root-mean-square fluctuation (RMSF) of ALR1 and ALR2 were examined, it was observed that residues were found stable

in both the cases (Figure 8 and 11). When radius of gyration was taken into account for ALR1 and ALR2, it was observed that the range of ALR1 apo and holo protein was 1.8 to 1.9 nm (Figure 9), however, the radius of gyration lies between 1.80 and 1.95 nm in ALR2 (Figure 12).

The results of docking studies revealed that the after binding of potent compounds inside ALR1 and ALR2, the tight complex formation made the complex stable and resulted in lowering of the energy. Our results suggested that docked complex throughout MD trajectories exhibit stable behavior and therefore, increasing the efficacy of docked poses and hence docking results.

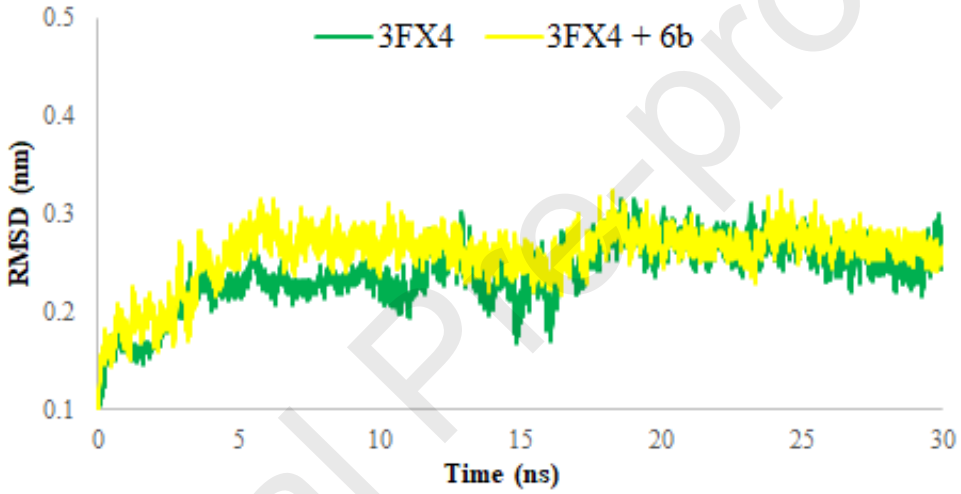


Figure 7. Root Mean Square Deviation (RMSD) of amino acid residues of 3FX4 and protein plus selective compound (**6b**) during 30 ns MD-simulation run

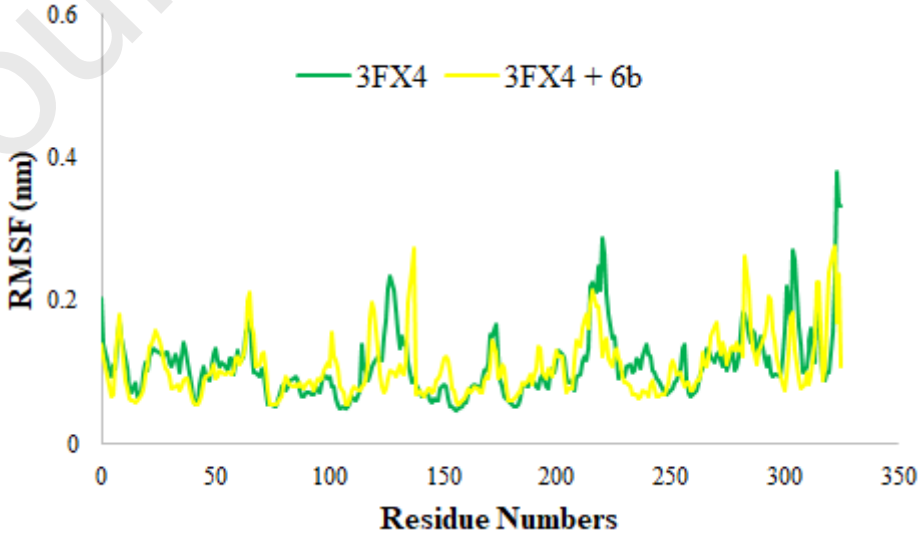


Figure 8. Root Mean Square Fluctuation (RMSF) of amino acid residues of 3FX4 and protein plus selective compound (**6b**) during 30 ns MD-simulation run

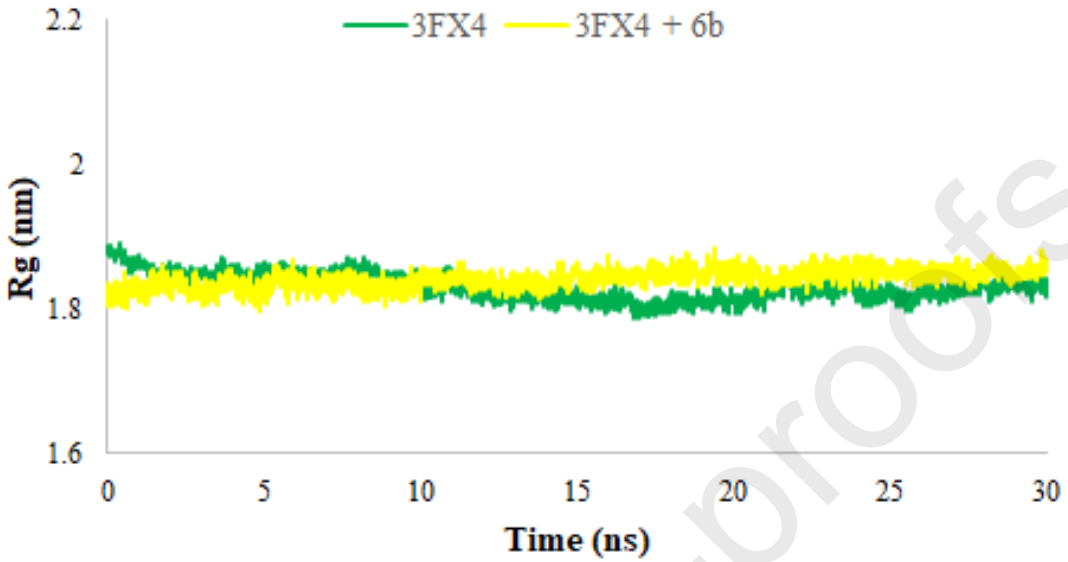


Figure 9. Radius of gyration (Rg) of amino acid residues of 3FX4 and protein plus selective compound (**6b**) during 30 ns MD-simulation run

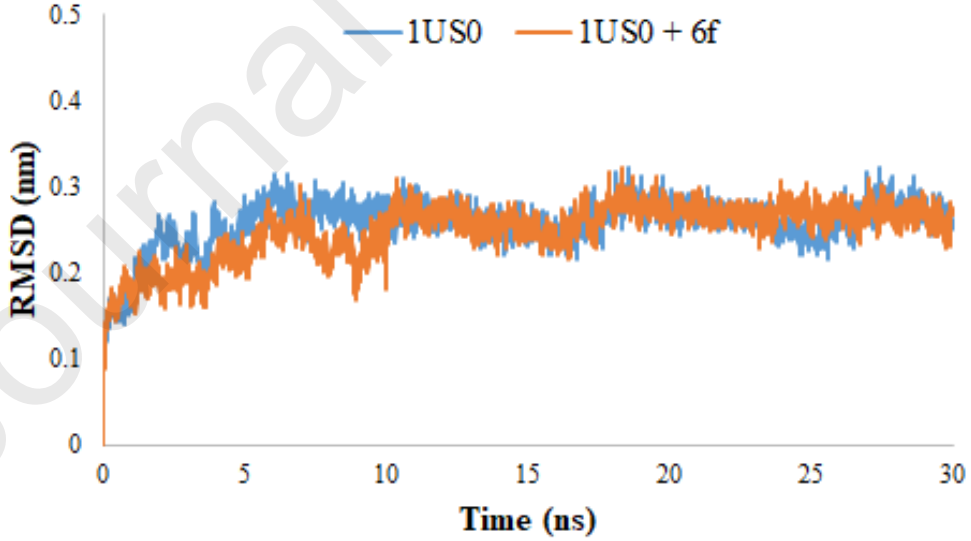


Figure 10. Root Mean Square Deviation (RMSD) of amino acid residues of 1US0 and protein plus selective compound (**6f**) during 30 ns MD-simulation run

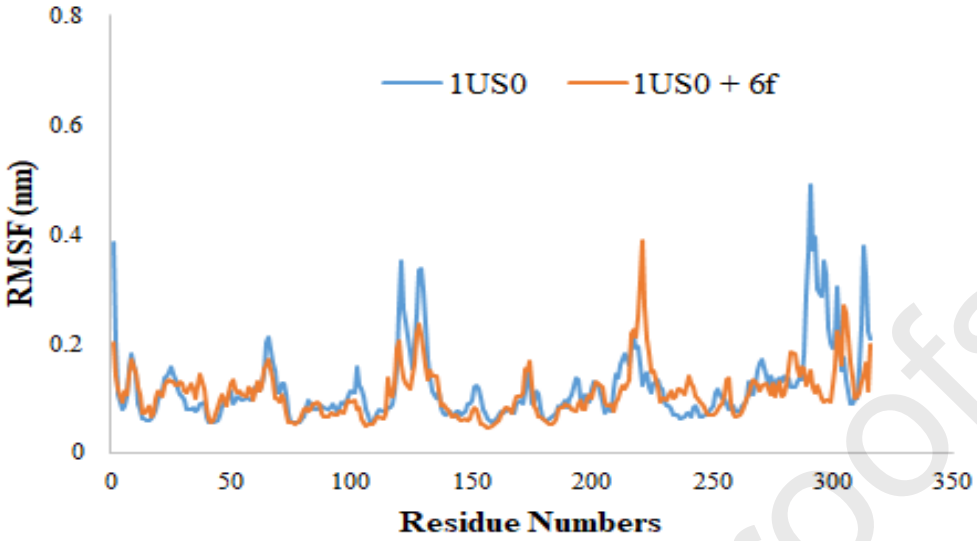


Figure 11. Root Mean Square Fluctuation (RMSF) of amino acid residues of 1US0 and protein plus selective compound (**6f**) during 30 ns MD-simulation run

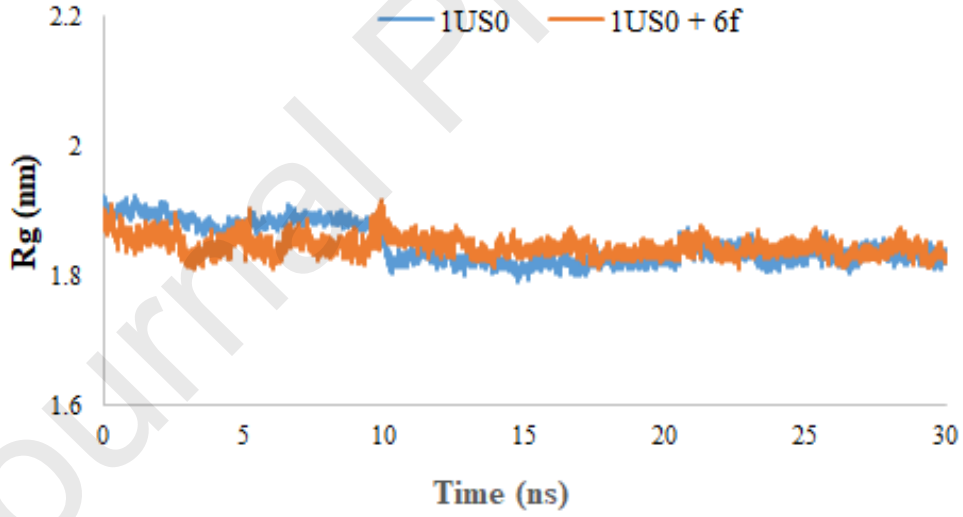


Figure 12. Radius of gyration (Rg) of amino acid residues of 1US0 and protein plus selective compound (**6f**) during 30 ns MD-simulation run

Cytotoxic activity against HeLa cervical carcinoma cells

The cytotoxic activity of **6(a-l)** against cervical carcinoma cells (HeLa) was evaluated using MMT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [38-39]. The

percentage inhibition of all the synthetic derivatives was assessed at 100 μM (**Table 2**). Cisplatin was used as a positive control and exhibited 89.3% growth inhibition at the same concentration level. As cisplatin is known to exhibit highly cytotoxic behavior, most of the derivatives revealed lower cytotoxic activity than cisplatin towards the HeLa cells. The synthetic compounds exhibited %age inhibition against cancer cells in the range of 12.6 – 53.1%. Compounds **6j** and **6k** were found to have more cytotoxic activity than other derivatives and both the derivatives contain carboxylic acid substituent at *ortho* and *para* positions, respectively.

Table 2. Cytotoxic potential of oxadiazole–sulfonamide hybrids **6**(**a-l**) against cervical carcinoma cells HeLa at 100 μM

Codes	%age inhibition ± SEM
6a	29.8 ± 2.31
6b	26.4 ± 1.17
6c	19.2 ± 3.09
6d	21.0 ± 0.52
6e	32.9 ± 3.26
6f	35.8 ± 2.14
6g	-
6h	44.6 ± 0.98
6i	-
6j	49.2 ± 3.35
6k	53.1 ± 3.14
6l	12.6 ± 1.05
Cisplatin	89.3 ± 1.99

DPPH assay (Free radical scavenging assay)

The synthesized analogues were screened for their percent scavenging activity (%RSA) following the previously used protocol with slight modifications [40]. The tested compounds exhibited less percent scavenging activity (**Table 1**), however, the standard, ascorbic acid used

in the experiment showed 81.4% activity. All the compounds revealed less than 40% scavenging activity. Although, the synthesized derivatives were identified as potent inhibitors of ALR1 and ALR2, but unfortunately none of them showed antioxidant activity.

Conclusions

Aldose reductase belongs to aldo-keto super family which converts glucose into sorbitol along the parallel conversion of NADPH to $NADP^+$. The enzyme along with taking part in polyol pathway is also responsible for the antioxidant role in the body. In this way, formation of free radicals may also be targeted by inhibiting the above enzyme. Therefore, a series of compounds was designed to synthesize different oxadiazole-sulfonamide hybrid molecules **6(a-l)** through simple and facile protocol. The synthesized products were tested against ALR1 and ALR2 enzymes. Most of the compounds exhibited good results and among them **6b**, **6e**, **6f** and **6l** were the most prominent. Only one compound i.e. **6b** was identified as selective inhibitor of ALR1 with an IC_{50} value of 4.77 ± 0.47 µM, whereas, compound **6f** (IC_{50} = 2.21 ± 0.73 µM) inhibited ALR2, selectively. The common observation was the presence of electron-donating groups which plausibly enhanced the activity. The docking results also helped in finding ligand-protein interactions. Furthermore, these compounds have also shown weak to moderate cytotoxic activity. Although sulfonamides have been reported to possess some other biological activities, we currently have focused on the aldose reductase inhibition studies. Thus, off-target effects of these compounds have not been evaluated and will be studied as a part of future research.

Material & methods

Chemistry

Melting points were obtained on Gallenkamp melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 on Brücker Avance NMR (300 MHz) on a TMS scale as internal standard. Chemical shifts were recorded in δ (ppm). IR spectra were recorded on Thermo Nicolet FT-IR Spectrophotometer in KBr pellets. Elemental analyses were performed by LECO 630-200-200 TruSpec CHNS micro analyzer and the values are within ± 0.4% of the calculated results. The initial precursors (**2-5**) were prepared by using the literature reported methodologies [41-43].

General procedure for the synthesis of compounds 6(a-l)

To an ethanolic solution (10 mL) of substituted oxadiazole (**4**) (1 mmol, 0.27 g) was added KOH (1 mmol, 0.05 g). The reaction mixture was stirred until KOH was dissolved followed by the addition of acylated aniline (**5a**) (1 mmol, 0.17 g) at room temperature. After completion of the reaction (monitored by TLC), the precipitates formed were filtered off, washed with dilute HCl and then with distilled water and dried.

The similar procedure was followed to prepare other derivatives **6(b-l)**.

2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)-N-phenylacetamide (6a)

White crystalline solid; mp 168 ºC; Yield: 58%; IR (ν_{max}/cm^{-1}; neat): 3352 (N-H), 3143-2940 (C-H), 1691 (C=O), 1601 (C=N), 1546 (C=C), 1327, 1145 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.20 (s, 3H; -CH_3), 4.38 (s, 2H; -CH_2), 7.08 (t, 1H, *J* = 7.4 Hz; Ar-1H), 7.26-7.35 (m, 3H; Ar-2H), 7.58-7.89 (m, 4H; Ar-4H), 7.90 (d, 1H, *J* = 7.3 Hz; Ar-1H), 9.95 (s, 1H; NH), 10.47 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 37.28, 112.34, 119.63, 119.96, 124.19, 124.42, 129.14, 129.35, 133.60, 137.00, 139.10, 163.86 (C-5 Oxd), 164.53 (C-2 Oxd), 165.25 (C=O); Anal. Calcd. For $C_{17}H_{16}N_4O_4S_2$: 50.48; H, 3.99; N, 13.85; S, 15.86 %. Found: C, 50.18; H, 3.78; N, 13.91; S, 15.95 %.

2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)-N-(o-tolyl)acetamide (6b)

White powder; mp 138-139 ºC; Yield: 70%; IR (ν_{max}/cm^{-1}; neat): 3369 (N-H), 3151-2926 (C-H), 1662 (C=O), 1609 (C=N), 1555 (C=C), 1292, 1156 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 2.20 (s, 3H; -CH_3), 3.20 (s, 3H; -CH_3), 4.40 (s, 2H; -CH_2), 7.07-7.23 (m, 3H; Ar-3H), 7.28-7.35 (m, 1H; Ar-1H), 7.41 (d, 1H, *J* = 7.5 Hz; Ar-1H), 7.64-7.69 (m, 2H; Ar-2H), 7.93 (d, 1H, *J* = 7.5 Hz; Ar-1H), 9.75 (s, 1H; NH), 9.95 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 18.22, 37.28, 112.42, 120.05, 124.46, 125.26, 126.01, 126.51, 129.18, 130.87, 132.12, 133.61, 136.24, 137.00, 163.87 (C-5 Oxd), 164.56 (C-2 Oxd), 165.45 (C=O); Anal. Calcd. For $C_{18}H_{18}N_4O_4S_2$: C, 51.66; H, 4.34; N, 13.39; S, 15.32 %. Found: C, 51.82; H, 4.31; N, 13.19; S, 15.51 %.

2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)-N-(m-tolyl)acetamide (6c)

White powder; mp 182 ºC; Yield: 66%; IR (ν_{max}/cm^{-1}; neat): 3353 (N-H), 3147-2927 (C-H), 1687 (C=O), 1610 (C=N), 1556 (C=C), 1343, 1148 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 2.28 (s, 3H; -CH_3), 3.20 (s, 3H; -CH_3), 4.36 (s, 2H; -CH_2), 6.90 (d, 1H, *J* = 7.5 Hz; Ar-1H), 7.20 (t, 1H, *J* = 7.8 Hz; Ar-1H), 7.29 (t, 1H, *J* = 7.3 Hz; Ar-1H), 7.37 (d, 1H, *J* = 8.1 Hz; Ar-1H), 7.43 (s, 1H; Ar-1H), 7.60-7.69 (m, 2H; Ar-2H), 7.90 (d, 1H, *J* = 7.5 Hz; Ar-1H), 9.95 (s,

1H; NH), 10.38 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 21.64, 37.28, 112.31, 116.83, 119.94, 120.13, 124.41, 124.89, 129.14, 129.18, 133.61, 137.01, 138.57, 139.03, 163.86 (C-5 Oxd), 164.53 (C-2 Oxd), 165.18 (C=O); Anal. Calcd. For $C_{18}H_{18}N_4O_4S_2$: C, 51.66; H, 4.34; N, 13.39; S, 15.32 %. Found: C, 51.41; H, 4.29; N, 13.13; S, 15.79 %.

2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)-N-(p-tolyl)acetamide (6d)

White powder; mp 210 ºC; Yield: 64%; IR (ν_{max}/cm^{-1}; neat): 3347 (N-H), 3126-2928 (C-H), 1657 (C=O), 1608 (C=N), 1546 (C=C), 1345, 1148 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 2.25 (s, 3H; -CH_3), 3.20 (s, 3H; -CH_3), 4.37 (s, 2H; -CH_2), 7.13 (d, 2H, J = 8.3 Hz; Ar-2H), 7.30 (t, 1H, J = 7.4 Hz, Ar-1H), 7.48 (d, 2H, J = 8.3 Hz; Ar-2H), 7.60-7.69 (m, 2H; Ar-2H), 7.90 (d, 1H, J = 7.8 Hz; Ar-1H), 9.95 (s, 1H; NH), 10.38 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 20.91, 37.27, 112.31, 119.63, 119.93, 124.41, 129.13, 129.71, 133.15, 133.59, 136.60, 137.00, 163.88 (C-5 Oxd), 164.52 (C-2 Oxd), 164.98 (C=O); Anal. Calcd. For $C_{18}H_{18}N_4O_4S_2$: C, 51.66; H, 4.34; N, 13.39; S, 15.32 %. Found: C, 51.61; H, 4.01; N, 13.55; S, 15.91 %.

N-(2-Methoxyphenyl)-2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide (6e)

Light brown powder; mp 211-212 ºC; Yield: 84%; IR (ν_{max}/cm^{-1}; neat): 3346 (N-H), 3086-2929 (C-H), 1655 (C=O), 1607 (C=N), 1550 (C=C), 1332 & 1144 (S=O), 1250, 1021 (C-O-C); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.10 (s, 3H; -CH_3), 3.79 (s, 3H; -OCH_3), 4.26 (s, 2H; -CH_2), 7.08 (t, 1H, J = 7.5 Hz; Ar-1H), 7.20-7.3 (m, 3H; Ar-3H), 7.47-7.55 (m, 3H; Ar-3H), 7.76 (d, 1H, J = 7.1 Hz; Ar-1H), 10.56 (s, 1H; NH), 11.18 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 33.01, 56.37, 113.16, 119.93, 120.67, 121.20, 123.61, 129.09, 130.45, 131.26, 133.09, 138.95, 155.22, 164.70 (C-5 Oxd), 164.83 (C-2 Oxd), 171.50 (C=O); Anal. Calcd. For $C_{18}H_{18}N_4O_5S_2$: C, 49.76; H, 4.18; N, 12.89; S, 14.76 %. Found: C, 50.01; H, 4.03; N, 13.09; S, 14.35 %.

N-(4-Methoxyphenyl)-2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide (6f)

White powder; mp 157-158 ºC; Yield: 62%; IR (ν_{max}/cm^{-1}; neat): 3306 (N-H), 3043-2931 (C-H), 1672 (C=O), 1608 (C=N), 1558 (C=C), 1323, 1110 (S=O), 1243 & 1058 (O-C-O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.11 (s, 3H; -CH_3), 3.72 (s, 3H; -OCH_3), 4.32 (s, 2H; -CH_2), 6.89 (d, 2H, J = 8.9 Hz; Ar-2H), 7.04-7.28 (m, 2H; Ar-2H), 7.51 (d, 2H, J = 8.9 Hz; Ar-2H), 7.59-7.64 (m, 1H; Ar-1H), 7.83 (d, 1H, J = 8.2 Hz; Ar-1H), 9.98 (s, 1H; NH), 10.32 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 37.20, 55.63 (-OCH_3), 114.42, 119.96, 121.22, 127.20, 129.30,

132.24, 133.41, 155.96, 164.77 (C-5 Oxd); Anal. Calcd. For $C_{18}H_{18}N_4O_5S_2$: C, 49.76; H, 4.18; N, 12.89; S, 14.76 %. Found: C, 49.34; H, 4.55; N, 13.25; S, 14.73 %.

N-(4-Iodophenyl)-2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide (6g)

White powder; mp 116-118 ºC; Yield: 87%; IR (ν_{max}/cm^{-1}; neat): 3295 (N-H), 3040-2992 (C-H), 1677 (C=O), 1629 (C=N), 1339, 1125 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.18 (s, 3H; -CH_3), 4.30 (s, 2H; -CH_2), 7.25-7.31 (m, 1H; Ar-1H), 7.39-7.50 (m, 2H; Ar-2H), 7.56-7.72 (m, 4H; Ar-4H), 7.88 (d, 1H, J = 7.5 Hz; Ar-1H), 9.94 (s, 1H; NH), 10.59 (s, 1H; NH); Anal. Calcd. For $C_{17}H_{15}IN_4O_4S_2$: C, 38.50; H, 2.85; I, 23.93; N, 10.56; S, 12.09 %. Found: C, 38.69; H, 2.95; N, 10.73; S, 12.26.

Methyl 2-(2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamido)benzoate (6h)

White powder; mp 163-166 ºC; Yield: 57%; IR (ν_{max}/cm^{-1}; neat): 3350 (N-H), 3180-2916 (C-H), 1709 (C=O), 1687 (C=O), 1607 (C=N), 1589 (C=C), 1320, 1157 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.20 (s, 3H; -CH_3), 3.80 (s, 3H; -$COOCH_3$), 4.42 (s, 2H; -CH_2), 7.20-7.33 (m, 2H; Ar-2H), 7.61-7.69 (m, 3H; Ar-3H), 7.90-7.93 (m, 2H; Ar-2H), 8.23 (d, 1H, J = 8.1 Hz; Ar-1H), 9.94 (s, 1H; NH), 11.08 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 37.12, 52.89, 112.31, 118.81, 119.98, 121.90, 124.41, 129.14, 131.07, 133.64, 134.47, 137.00, 139.26, 163.48 (C-5 Oxd), 164.69 (C-2 Oxd), 165.86 (C=O), 167.74 (C=O); Anal. Calcd. For $C_{19}H_{18}N_4O_6S_2$: C, 49.34; H, 3.92; N, 12.11; S, 13.87%. Found: C, 49.51; H, 3.99; N, 12.21; S, 13.65 %.

Ethyl 4-(2-((5-(2-(methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamido)benzoate (6i)

Light yellow powder; mp 258-260 ºC; Yield: 86%; IR (ν_{max}/cm^{-1}; neat): 3291 (N-H), 3050-2991 (C-H), 1756 (C=O), 1672 (C=O), 1631 (C=N), 1342, 1117 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 1.31 (t, 3H, J = 7.1 Hz; -CH_3), 2.28 (s, 3H; -CH_3), 4.22-4.39 (m, 4H; 2×CH_2), 7.18-7.51 (m, 6H; Ar-6H), 7.60-7.65 (m, 2H; Ar-2H), 7.73-7.78 (m, 2H; Ar-2H), 7.90-7.95 (m, 2H; Ar-2H), 10.32 (s, 1H; NH), 10.80 (s, 1H; NH); Anal. Calcd. For $C_{20}H_{20}N_4O_6S_2$: C, 50.41; H, 4.23; N, 11.76; S, 13.46 %. Found: C, 50.63; H, 4.43; N, 11.89; S, 13.26 %.

2-(2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamido)benzoic acid (6j)

White powder; mp 216-217 ºC; Yield: 51%; IR (ν_{max}/cm^{-1}; neat): 3352-3116 (N-H & O-H), 3050-2929 (C-H), 1702 (C=O), 1672 (C=O), 1604 (C=N), 1587 (C=C), 1330, 1157 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.19 (s, 3H; -CH_3), 4.39 (s, 2H; -CH_2), 7.13 (t, 1H; *J* = 7.5 Hz; Ar-1H), 7.28 (t, 1H; *J* = 7.8 Hz; Ar-1H), 7.50-7.67 (m, 3H; Ar-3H), 7.88 (d, 1H; *J* = 7.6 Hz; Ar-1H), 7.98 (d, 1H; *J* = 7.0 Hz; Ar-1H), 8.44 (d, 1H; *J* = 8.3 Hz; Ar-1H), 10.06 (s, 1H; NH), 12.53 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 37.36, 112.45, 120.04, 120.10, 123.39, 124.39, 129.23, 131.60, 133.37, 133.56, 137.08, 140.53, 163.59 (C-5 Oxd), 164.64 (C-2 Oxd), 165.50 (C=O), 169.74 (C=O); Anal. Calcd. For $C_{18}H_{16}N_4O_6S_2$: C, 48.21; H, 3.60; N, 12.49; S, 14.30 %. Found: C, 47.99; H, 3.81; N, 12.75; S, 14.51 %.

4-(2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetamido)benzoic acid (6k)

White powder; mp 208 ºC; Yield: 75%; IR (ν_{max}/cm^{-1}; neat): 3486 (O-H), 3248 (N-H), 3114-3004 (C-H), 1685 (C=O), 1665 (C=O), 1608 (C=N), 1588 (C=C), 1326, 1150 (S=O); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.09 (s, 3H; -CH_3), 4.41 (s, 2H; -CH_2), 7.15 (t, 1H; *J* = 7.4 Hz; Ar-1H), 7.52-7.62 (m, 2H; Ar-2H), 7.68 (d, 2H; *J* = 8.5 Hz; Ar-2H), 7.80-7.83 (m, 2H; Ar-1H, NH),7.89 (d, 2H; *J* = 8.5 Hz; Ar-2H), 10.90 (s, 1H; NH) ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 37.34, 112.82, 118.82, 119.99, 124.54, 129.42, 130.73, 133.28, 142.26, 163.41 (C-5 Oxd), 165.29 (C-2 Oxd), 165.84 (C=O), 167.83 (C=O); Anal. Calcd. For $C_{18}H_{16}N_4O_6S_2$: C, 48.21; H, 3.60; N, 12.49; S, 14.30 %. Found: C, 48.56; H, 3.47; N, 12.73; S, 14.19 %.

2-((5-(2-(Methylsulfonamido)phenyl)-1,3,4-oxadiazol-2-yl)thio)-N-(4-nitrophenyl)acetamide (6l)

White powder; mp 226 ºC; Yield: 89%; IR (ν_{max}/cm^{-1}; neat): 3290 (N-H), 3155-2921 (C-H), 1705 (C=O), 1611 (C=N), 1562 (C=C), 1335, 1115 (S=O), 1478 & 1350 (NO_2); ^{1}H NMR (DMSO-d_6, 300 MHz), δ: 3.19 (s, 3H; -CH_3), 4.44 (s, 2H; -CH_2), 7.27-7.32 (m, 1H; Ar-1H), 7.60-7.68 (m, 2H; Ar-2H), 7.84 (d, 2H, *J* = 9.1 Hz; Ar-2H), 7.89 (d, 1H, *J* = 7.9 Hz; Ar-2H), 8.25 (d, 2H, *J* = 9.1 Hz; Ar-2H), 9.92 (s, 1H; NH), 11.07 (s, 1H; NH); ^{13}C NMR (DMSO-d_6, 75 MHz), δ: 37.34, 112.37, 119.28, 119.43, 124.46, 125.59, 129.13, 133.62, 136.99, 143.01, 145.15, 163.71 (C-5 Oxd), 164.60 (C-2 Oxd), 166.40 (C=O); Anal. Calcd. For $C_{17}H_{15}N_5O_6S_2$: C, 45.43; H, 3.36; N, 15.58; S, 14.27 %. Found: C, 45.21; H, 3.63; N, 15.81; S, 14.43 %.

Biological Protocols

The details of biological protocols have been provided in the Supporting Information file.

Financial & competing interest disclosure

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