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ARTICLE

Design and synthesis of oxaprozin-1,3,4-oxadiazole hybrids as potential anticancer and antibacterial agents

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Abstract

In the present study, we report design, synthesis and screening of new novel 5-substituted-2-mercapto-1,3,4-oxadiazole analogues appended to oxaprozin for their in vitro anticancer and antibacterial activity. The synthesised compounds were characterized using various spectroscopic techniques. Furthermore, the structure of **5b** (2-(2-[4,5-diphenyloxazol-2-yl]ethyl)-5-(ethylthio)-1,3,4-oxadiazole) was unequivocally confirmed by X-ray analysis. Among the series **5c** (2-(2-[4,5-diphenyloxazol-2-yl]ethyl)-5-(propylthio)-1,3,4-oxadiazole) showed most promising anticancer activity against A549 cancer cell line and all the reported analogues manifested satisfactory safety profiles against human normal cell line HEK293T. The products exhibited good antibacterial activity and among the tested **5j** (2-(2-[4,5-diphenyloxazol-2-yl]ethyl)-5-([4-fluorobenzyl]thio)-1,3,4-oxadiazole) exhibited most potent.

1 | INTRODUCTION

Cancer, the uncontrolled division or abnormal growth of cells and spread or invade to other parts of the body, thereby posing severe health consequences. Cancer remains the second major challenging disorder for the cause of morbidity and mortality around the world and is a global challenge in the current scenario.[1] In 2012, approximately 8.2 million tumor related deaths and 14.1 million new cancer cases are enrolled.[2] Howbeit, the current chemotherapy methods are insufficient to meet the expected results and prompted unsatisfactory results.[3] Another sector of global diseases is ruled by notorious multidrug-resistant Gram-negative pathogenic and Gram-positive bacteria which are causing life-threatening infections in living models thus posing acquired resistance to many existing drugs, emphasizing the desperate need for the "miracle agents"[4] to treat these microorganisms. Therefore, discovery and development of new effective drug candidates has attracted great attention among pharmaceutical and chemistry researchers in recent years.

1,3,4-oxadiazole constitutes an important privileged heterocyclic structure highlighting two nitrogens and an oxygen atom.[5] This pharmacophore has gained greater attention over years due to exhibition of wide range of biological profiles[6] owing to the existence of —N═C—O— toxophoric linkage.[3] Development of several techniques for the facile construction of the scaffold had captured acceleration.[7] Over the last decade, approximately 686 patents

have been filed on oxadiazole pharmacophore and its importance[8] 2,5-Disubstituted oxadiazole subsidiaries have been encompassed with promising pharmacological profiles such as anticancer,[9] antitubercular,[10] antimicrobial,[11] antifungal,[12] antiepileptic,[13] analgesic,[14] antitumour,[15] ulcerogenic,[16] hypoglycaemic,[17] central nervous system,[18] antidepressant,[19] hypnotic[20] and sedative properties.[21] Few compounds viz., zibotentan, ataluren, and raltegravir (Figure 1) comprising oxadiazole as core nucleus are in the late stages of clinical trials substantiating the valuable privileges of the scaffold. Furthermore, oxadiazoles are great bioisosters of carbonyl-containing groups such as amides, esters, hydroxamic and carbamates which improve hydrogen bond interactions with various receptors thereby increasing the biological responses to a remarkable extent.[3] Aside from their latent potential medicinal applications, they have also captured attention in material sciences due to electron transporting and hole blocking abilities.[22]

On the other hand, oxaprozin (Figure 2), one of the leading non-steroidal anti-inflammatory drug (NSAID) is a global market drug used for the therapeutic management of pain, osteoarthritis, ankylosing spondylitis, chronic inflammatory disease and bursitis.[23] It acts by suppressing the prostaglandin (PG) biosynthesis in which COX-1 and COX-2 are the key intermediates thereby controlling the production of arachidonic acid.[24] However, its therapeutic usage is restricted due to the development of side effects such as gastrointestinal injury (GI), peptic ulceration and aperture due to the presence of corrosive carboxylic group as well as considerable damage to renal system.[25] Albeit several strategies have been proposed, none of them completely address these symptoms and thus became the focal point of the present research.

Additionally, it was established from the collected data, that the oxaprozin and its derivatives as well elicit anticancer[26] and antibacterial profiles[26a] through a COX-2 independent mechanism.[27] Bozic et al have reported that transition metal complexes of oxaprozin demonstrated for significant antiproliferative activity against human colon (HCT-116) and breast (MDA-231) cancer cells.[28] Earlier, Babic et al. have documented on oxaprozin hydrogels as potential antibacterial agents.[23] Recently, we have reported etodolac (a non-steroidal anti-inflammatory drug) based 1,2,3-triazole hybrids and etodolac-pyridazinones as potential anti-cancer properties.[26d, e] To the continuation of our current research programme[29] for the identification of dormant heterocyclic compounds, herein, we have designed the synthesis of hybrid template **A** (Figure 2) by considering the following points and to ascertain their anticancer and antibacterial activities. (a) To mask the side effects of oxaprozin carboxylic motif and convert it to its 1,3,4-oxadiazole bioisoster for safer profiles. (b) Retain the hydrophobic interactions of oxaprozin.[23] (c) To add the other vacant position in oxadiazole with thio alkyl group for the enhancement of lipophilicity and pharmacokinetic properties by formation of C—S bond which aids in penetration of the analogues across the cell wall.[30]

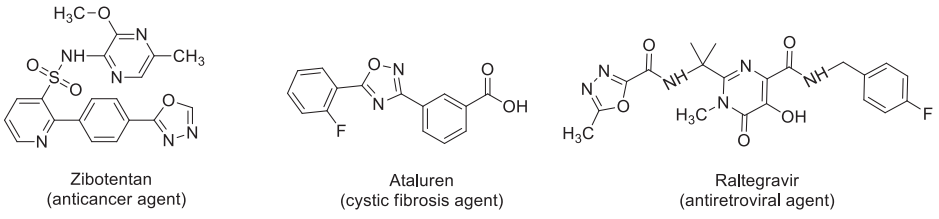


FIGURE 1 Representative bioactive compounds featuring oxadiazole motif

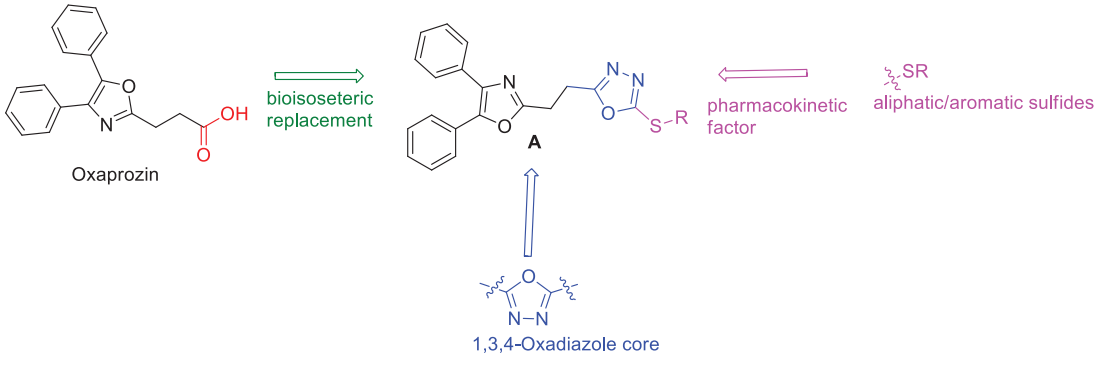


FIGURE 2 Design strategy of hybrid template A based on oxaprozin and 1,3,4-oxadiazole

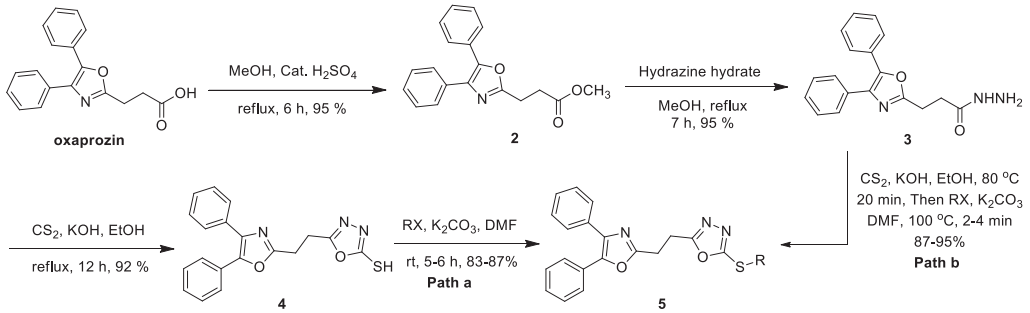
2 | RESULTS AND DISCUSSION

Among the variety of available methods[31] the synthesis of previously unreported compounds (**5a-p**) was accomplished by employing conventional (Scheme 1, **path a**) as well as microwave irradiation methods (Scheme 1, **path b**). Briefly, the compound **3** was prepared in a stepwise manner which involves esterification of the carboxylic group of Oxaprozin (**1**) followed by the reaction with hydrazine to give acid hydrazide **3**.[32] Intramolecular cyclization of **3** under strong basic conditions with carbon disulfide furnished the key intermediate **4** in good yield.[9] Finally, the reaction of **4** with appropriate electrophilic halides gave the title compounds **5a-p** in excellent yields.[10] Microwave irradiation (MWI) technique has offered a paradigm shift in the target synthesis due to its peculiar direct "in core" heating of the reaction mixture thus diminishing the reaction times.[33] Moreover, it offers myriad advantages such as acceleration of sluggish transformations, environmentally sustainable conditions, higher yields and strongly promote the cyclocondensation reaction.[34] Under non-classical reaction condition (MWI), the reaction rates were faster than classical heating conditions (Table 1).

As a representative example, synthesis of **5a** was accomplished in 6 hours by traditional methods whereas under MW condition, it was achieved in 3 minutes (**5a**, Table 1). The structures of compounds **5a-p** were supported by NMR, IR and HRMS spectral analysis. On the ^{1}H NMR timescale, disappearance of $NHNH_2$ proton signals in **3** at δ 4.72 ppm and 4.77 ppm and appearance of a signal at δ 11.31 ppm (SH) asserted **4**. The ^{13}C NMR peaks at δ 162.51 (C-2) ppm and 160.51 (C-5) ppm indicates the formation of 1,3,4-oxadiazole core. Compound **4** IR spectrum displayed absorption bands at υ_{max} 1624 cm^{-1} (C═N), 1155 cm^{-1} (—N—N═C—) and 1059 cm^{-1} (C—O—C) evident to —C—O—C— linkage and —N—N═C— in oxadiazole frame work. Manifestation of IR signals between υ_{max} 2771-2924 cm^{-1} established the formation of **5a-p** due to the presence of S-CH_2. Furthermore, the structure of compound **5b** was verified by a single crystal X-ray diffraction studies (Figure 3).[35]

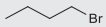
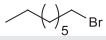
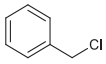
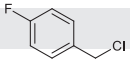
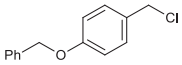
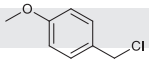
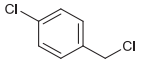
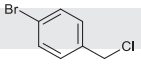
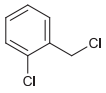
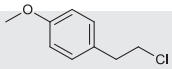
2.1 | Biological evaluation

In vitro cytotoxicity of all synthesized compounds was assessed by employing MTT colorimetric assay[36] against prostate (PC3) and lung (A549) cancer cell lines (Table 2). The anticancer drug Doxorubicin was used as a reference compound in the present study. The results revealed that the compounds **5a**, **5b** and **5h** showed higher cytotoxicity against PC3 cells. All of the tested compounds were more potent than the parent scaffold oxaprozin except **5j** for the A549 cells. The compound **5c** exhibited promising cytotoxicity whereas the compounds **5a**, **5e** and **5h** displayed moderate to good cytotoxicity against A549 cells. From the results, the following SAR studies can be inferred. Homologation from methyl (**5a**) to ethyl (**5b**) in turns to n-propyl (**5c**) and then to n-butyl (**5d**) decreased the cytotoxicity profile for PC3 cell line and similar pattern was not observed for A549 cell line indicating the selectivity of synthesized compounds to a particular cell line. However, increment in activity was observed when carbon chain was increased from methyl to ethyl as in the case of 1-methyl-4-methoxybenzene (**5l**) and 1-ethyl-4-methoxybenzene (**5p**) for both the cell lines. Introduction of unsaturation (n-propyl (**5c**) and allyl (**5g**)) led to the decrement of cytotoxicity for both cell lines and further increment of unsaturation that is, propargylation (**5h**) led to improvement of activity. For the analogues phenyl (**5i**), 4-fluorobenzene (**5j**), 4-chlorobenzene (**5m**) and 4-bromobenzene (**5n**), the cytotoxicity trend followed the pattern of lesser the -I effect of the substituent (electronic effect) higher the activity against A549 cell line. It is well recorded that cytotoxic medications mitoxantrone and doxorubicin display cytotoxicity against A549 cells by the generation of reactive oxygen species, therefore causing apoptosis through the outflow of caspase-3.[3] Hence, it is trusted



SCHEME 1 Synthesis of oxaprozin-1,3,4-oxadiazole derivatives

TABLE 1 Reaction conditions for the formation of products 5a-p

Compounds	R-X	Microwave		Conventional	
		Time (min)	Yield (%)[a]	Time (h)	Yield (%)[a]
5a		3	94	6	85
5b		4	94	6	83
5c		3	94	6	84
5d		3	93	5	85
5e		3	90	5	82
5f		2	95	5	85
5g		2	94	5	83
5h		3	94	5	85
5i		3	93	6	87
5j		4	92	6	85
5k		3	89	5	85
5l		2	95	5	85
5m		3	94	6	85
5n		3	94	6	83
5o		2	95	6	84
5p		2	87	5	82

[a]Isolated yield.

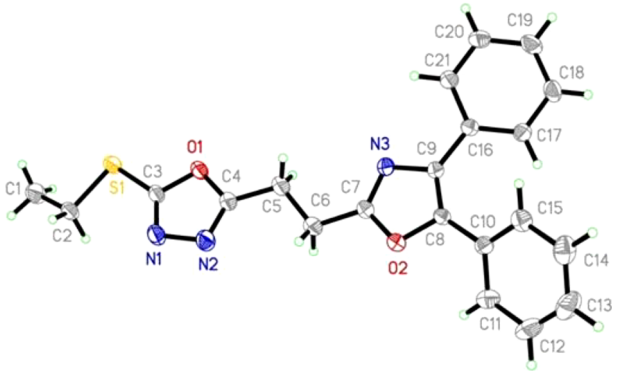


FIGURE 3 Single-crystal structure of compound 5b

that these new derivatives may act as effective anticancer agents by the similar mechanism discussed above. The dose response study revealed that the compound **5c** showed consistent growth of inhibition against A549 cells (Figure 4). Furthermore, the synthesized derivatives were also tested on HEK293T human normal cells to study their toxicity profile and are found to exhibit mediocre safety profiles.

Additionally, the newly synthesized analogues **5a-p** were screened for their in vitro antibacterial activity (Table 2) against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) by Agar well diffusion method.[37,38] As anticipated and from the bacterial investigations, it is obvious that all the tested derivatives showed enhanced bacterial inhibition than the oxaprozin core. Most of them displayed better antibacterial activity largely against Gram-negative strains. Among the tested series **5**, compound **5j** displayed potent antibacterial activity against all the strains under study except *K. pneumoniae* and **5c** showed good activity against *K. pneumoniae* strain. Among the homologues **5a-d**, the activity order is compound containing odd no of carbons that is, methyl (**5a**), n-propyl (**5c**)

demonstrated higher activity than derivatives with even no. of carbons ethyl (**5b**), n-butyl (**5d**) except for the *K. pneumoniae* strain. For the structures allyl (**5g**), propargyl (**5h**) increment of unsaturation led to decrement of activity for Gram-positive strain and increment for Gram-negative species except *K. pneumoniae*. For the series, the halogenated analogues 4-fluorobenzene (**5j**), 4-chlorobenzene (**5m**), 4-bromobenzene (**5n**), 2-chlorobenzene (5**o**) demonstrated higher activity for all the tested strains than non-halogenated derivatives 1-methyl-4-methoxybenzene (**5l**), 1-ethyl-4-methoxybenzene (**5p**) which might be due to better pharmacodynamics owing to the presence of halogens. Pharmacokinetic and physicochemical properties is due to optimum lipophilicity, and electronegativity of the halogen bond.[29a]

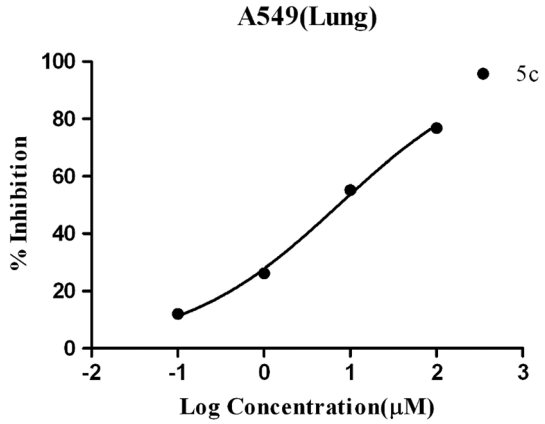


FIGURE 4 Dose response curve of the most active compound 5c on A549 cell line

3 | CONCLUSION

A simple and efficient protocol for the synthesis of novel hybrids of 1,3,4-oxadiazole appended to oxaprozin, evaluation of cytotoxicity as well as antibacterial activity have been documented in the present communication. Among the series, **5c** has displayed superior anticancer and antibacterial activities against A549 and *Klebsiella pneumonia* respectively. **5h** has exhibited good anticancer activity against both cell lines. All these compounds have elicited mediocre to good safer profiles against HEK293T normal cell lines.

TABLE 2 In vitro cytotoxic and antibacterial activities of the designed compounds 5a-p

	Cytotoxicity % of inhibition at 25 μM			Zone of inhibition (Diameter in mm) at 0.4 mg/50 μL			
Compounds	**PC3**	**A549**	**HEK293T**	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***	***E. coli***
5a	22.44	45.33	23.63	14 ± 0.23	17 ± 0.38	18 ± 0.42	14 ± 0.29
5b	21.84	31.38	34.71	12 ± 0.13	18 ± 0.55	15 ± 0.37	13 ± 0.21
5c	08.04	65.04	33.74	15 ± 0.35	20 ± 0.89	19 ± 0.58	17 ± 0.46
5d	02.30	23.28	52.63	13 ± 0.22	15 ± 0.35	13 ± 0.28	-
5e	12.27	45.71	21.06	15 ± 0.34	17 ± 0.42	17 ± 0.38	16 ± 0.36
5f	00.93	21.23	20.30	13 ± 0.24	14 ± 0.32	19 ± 0.65	17 ± 0.86
5g	05.54	30.68	09.30	17 ± 0.54	17 ± 0.47	13 ± 0.26	14 ± 0.32
5h	32.29	45.71	42.67	15 ± 0.35	16 ± 0.45	17 ± 0.62	15 ± 0.43
5i	18.10	21.19	29.58	17 ± 0.54	19 ± 0.98	19 ± 0.89	20 ± 1.02
5j	04.18	14.83	16.45	18 ± 0.59	17 ± 0.63	20 ± 0.88	22 ± 1.22
5k	11.06	23.17	01.70	17 ± 0.32	16 ± 0.48	19 ± 0.89	21 ± 0.93
5l	11.64	17.86	−03.40	15 ± 0.29	16 ± 0.58	14 ± 0.19	15 ± 0.42
5m	18.90	18.03	20.14	16 ± 0.36	16 ± 0.42	17 ± 0.59	18 ± 0.98
5n	18.14	20.98	23.62	16 ± 0.29	18 ± 0.67	16 ± 0.31	19 ± 0.86
5o	19.45	18.68	21.18	17 ± 0.19	17 ± 0.31	18 ± 0.42	19 ± 0.79
5p	12.48	19.26	5.67	15 ± 0.27	15 ± 0.35	16 ± 0.48	17 ± 0.65
Oxaprozin	17.21	17.23	20.06	12 ± 0.16	10 ± 0.12	11 ± 0.19	12 ± 0.17
Doxorubicin	—[a]	—[b]	—[c]	—	—	—	—
Ciprofloxacin	—	—	—	34 ± 0.15	28 ± 0.20	32 ± 0.15	31 ± 0.30

Note: All the values of the average of experiments done in triplicates.

[a]Doxorubicin IC_{50} is 2.1 μM.

[b]Doxorubicin IC_{50} is 3.3 μM.

[c]Doxorubicin IC_{50} is 1.2 μM.

4 | EXPERIMENTAL

4.1 | General

All the reactions were carried under inert nitrogen atmosphere employing dry solvents. Precoated TLC silica gel plates (Kieselgel 60 F254, Merck) were used for monitoring reactions. Purification was performed by column chromatography using silica gel (particle size 100-200 mesh, Merck). Melting points were determined in open capillary tubes on cintex melting point apparatus and are uncorrected. A monowave 300 mas 24 (option) (Anton paar) microwave was used to carry out the microwave reactions in 30 mL microwave process vials with temperature control by infrared detection temperature sensor. IR (KBr) spectra were recorded on a Perkin-Elmer 400 FTIR spectrometer (υ_{max} in cm^{-1}) or a Varian 670-IR FT-IR spectrometer (ATR) in the frequency range of 600 to 4000 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR)/Varian Mercury 400 MHz/500 MHz spectrometer. Proton chemical shifts are presented in δ ppm regarding TMS. *J* values are presented in Hz. Mass spectra were recorded using Jeol SX-102 spectrometer. High-resolution mass spectra (HRMS) were recorded with an Agilent Technologies 6510 Q-TOF spectrometer.

4.2 | Synthesis of methyl 3-(4,5-diphenyloxazol-2-yl)propanoate (2)

To a solution of oxaprozin (5 g, 17 mmol) in methanol (30 mL), few drops of conc. H_2SO_4 were added and the resulting mixture was refluxed for 6 hours. After completion of the reaction as indicated by TLC, excess methanol was distilled. The obtained reaction mass was diluted with 10% sodium bicarbonate solution and extracted with ethyl acetate and dried over sodium sulphate and reduced under pressure. The crude product was purified by recrystallization from ethanol to furnish the title compound **2** as white solid in 95% yield. mp: 56-58°C; Rf: 0.48 (ethyl acetate/hexane, 20/80); IR (KBr, υ_{max} in cm^{-1}): 3049 (C—H of aromatic), 2946 (C—H of aliphatic), 1734 (C═O), 1590 (C═N), 1496 (C═C), 1060 (C—O—C), 763, 700; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.64 (dd, J = 8.2 Hz, 2H, Ar-H), 7.56 (dd, J = 8.2 Hz, 2H, Ar-H), 7.26-7.38 (m, 6H, Ar-H), 3.74 (s, 3H, OCH_3), 3.2 (t, J = 7.4 Hz, 2H, CH_2), 2.92 (t, J = 7.4 Hz, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 173.02, 162.17, 145.07, 138.54, 136.05, 133.49, 132.07, 129.05, 128.97, 128.64, 60.87, 29.90, 23.03; ESI-Ms m/z 308.0 $(M + H)^+$.

4.3 | Synthesis of 3-(4,5-diphenyloxazol-2-yl)propanehydrazide (3)

To the solution of **2** (4.70 g, 15 mmol) in absolute alcohol (50 mL) was added hydrazine hydrate (1.6 mL, 30 mmol) and refluxed for about 7 hours. Excess ethanol was distilled off and the reaction mass was added in to water and extracted with ethyl acetate (2 × 30 mL). The combined organic layer was dried over sodium sulphate and evaporated under reduced pressure. The obtained crude product was purified by recrystallization from ethanol to furnish **3** as white solid in 95% yield. mp: 108-110°C; Rf: 0.50 (ethyl acetate/hexane, 70/30); IR (KBr, υmax in cm-1): 3324, 3285 (NH/NH2), 3034 (C—H of aromatic), 2921 (C—H of aliphatic), 1659 (C═O), 1513 (C═N), 1437 (C═C), 1060 (C—O—C), 762, 692; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.60 (dd, J = 8.2 Hz, 2H, Ar-H), 7.55 (dd, J = 8.2 Hz, 2H, Ar-H), 7.31-7.38 (m, 6H, Ar-H), 4.72 (bs, 1H, NH, D_2O exchangeable), 4.77 (bs, 2H, NH_2, D_2O exchangeable), 3.20 (t, J = 7.4 Hz, 2H, CH2),2.72 (t, J = 7.4 Hz, 2H, CH2); ^{13}C NMR (100 MHz, CDCl3) δ ppm: 175.36, 162.22, 145.05, 138.69, 135.05, 132.49, 130.07, 129.53, 128.97, 128.64, 29.90, 23.03; ESI-Ms m/z 308.0 (M + H) + .

4.4 | Synthesis of 5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole-2-thiol (4)

Mixture of compound **3** (4.23 g, 14 mmol), potassium hydroxide (0.92 g, 16 mmol) and carbon disulphide (1.73 mL, 27 mmol) in ethanol (50 mL) was stirred under reflux for 12 hours, till the evolution of H_2S gas was ceased. The progress of the reaction was monitored by TLC and excess solvent was distilled. The residue mass was poured over crushed ice, added 10% hydrochloric acid till the P^H of the solution is 5. The precipitated crude product thus obtained was filtered, washed with water, dried and recrystallized from ethanol to afford the compound **4** as white solid in 92% yield. mp: 162-164°C; Rf: 0.42 (ethyl acetate/hexane, 40/60); IR (KBr, υ_{max} in cm^{-1}): 3078 (C—H of aromatic), 2924 (C—H of aliphatic), 2772 (SH), 1624, 1510 (C═N), 1322, 1155, 1059 (C—O—C), 766, 799; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 11.31 (bs, 1H, SH), 7.55-7.63 (m, 4H, Ar-H), 7.26-7.38 (m, 6H, Ar-H), 3.27-3.37 (m, 4H, CH_2CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm: 162.51, 160.51, 146.05, 135.09, 131.65, 128.74, 128.41, 128.05, 126.50, 24.09, 23.29; ESI-Ms m/z 350.0 $(M + H)^+$.

4.5 | General procedure for synthesis of title compounds (5a-p)

4.5.1 | Conventional synthetic route[10]

To a solution of compound **4** (1.0 eq) in DMF (5 vol), respected alkyl/substituted benzyl halide (1.2 eq) was added followed by K_2CO_3 (1.5 eq) and stirred at ambient temperature for 5 to 6 hours. The reaction mass was diluted with water and extracted with EtOAc (2 X 20 mL). Purification of the crude compounds has been done by using 100-200 silica gel mesh to yield the title compounds (**5a-p**) in 83-87% yields.

4.5.2 | Microwave one-pot synthetic route[34a–c]

Alternately, we tested whether the reaction occurs in diminished time by heating the above reaction mixture at 80°C in DMF (5 vol). Howbeit, the reactants were successfully turned in to products within 1 to 2 hours. Next, we thought of diminishing the reaction time further, by seeking the help of Microwave where heat transfers to those components of the reaction mixture, which are susceptible to microwave polarization, and raises the temperature of sample much faster than conventional methods.[34d, e] Mixture of compound **3** (1.0 eq), carbon disulphide (1.2 eq) and potassium hydroxide (1.2 eq) in ethanol (5 vol) were taken in a closed vessel and irradiated under microwave (MW) at 80°C for 20 minutes. After completion of the reaction as indicated by TLC, excess ethanol solvent was distilled off. To the above reaction mixture, respective alkyl/substituted benzyl halides (1.2 eq) in DMF (5 vol) were added followed by K_2CO_3 (1.2 eq) and it was again irradiated under MW at 100°C for 2-4 minutes. After completion of the reaction, the reaction crude was diluted with water and extracted with ethyl acetate (2 × 20 mL). The combined organic layer was dried over sodium sulphate and evaporated under reduced pressure. The obtained crude product was recrystallized from ethanol to furnish **5a-p** in 87-95%.

4.6 | 2-(2-(4,5-diphenyloxazol-2-yl)ethyl)-5-(methylthio)-1,3,4-oxadiazole (5a)

White solid; mp: 80-82°C; Rf: 0.35 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3054 (C—H of aromatic), 2926 (C—H of aliphatic), 2863 (SCH_2), 1582 (C═N), 1486 (C═C), 1168, 1059 (C—O—C), 763, 695; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 7.59 (dd, J = 8.2 Hz, 4H, Ar-H), 7.42-7.31 (m, 6H, Ar-H), 3.50-3.30 (m, 4H, CH_2), 2.70 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 166.33, 165.41, 160.63, 145.77, 135.21, 133.13, 132.26, 129.93, 129.04, 128.90, 128.77, 128.69, 128.61, 128.18, 127.96, 127.90, 126.51, 25.02, 23.03, 14.55; ESI-Ms m/z 364.0 $(M + H)^+$.

4.7 | 2-(2-(4,5-diphenyloxazol-2-yl)ethyl)-5-(ethylthio)-1,3,4-oxadiazole(5b)

White solid; mp: 88°C to 90°C; Rf: 0.40 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3052 (C—H of aromatic), 2927 (C—H of aliphatic), 2863 (SCH_2), 1576 (C═N), 1452(C═C), 1269, 1111 (C—O—C), 763, 698; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.2 Hz, 2H, Ar-H), 7.56 (dd, J = 8.2 Hz, 2H, Ar-H), 7.40-7.30 (m, 6H, Ar-H), 3.45-3.35 (m, 4H, CH_2), 3.23 (q, J = 7.3 Hz, 2H, SCH_2),1.45 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 166.14, 164.63, 160.57, 145.70, 135.16, 132.23, 128.73, 128.62, 128.54, 128.11, 127.84, 126.46, 26.89, 25.01, 22.99, 14.68; HRMS: (ESI *m/z*) for $C_{21}H_{20}O_2N_3S$ calcd: 378.1270, found: 378.1276 $(M + H)^+$; Crystal Data: $C_{21}H_{19}N_3O_2S$ (M = 377.45): orthorhombic, space group Pbca (no. 61), a = 10.130(2) Å, b = 7.9144(17) Å, c = 46.917(10) Å, V = 3761.3(14) $Å^3$, Z = 8, T = 294.15 K, μ (MoKα) = 0.193 mm^{-1}, *Dcalc* = 1.333 g/mm^3, 40 979 reflections measured ($3.472 \leq 2\Theta \leq 56.668$), 4640 unique ($R_{int}$ = 0.0288) which were used in all calculations. The final R_1 was 0.0622 ($I > 2\sigma[I]$) and wR_2 was 0.1670 (all data). CCDC 1471510 contains supplementary Crystallographic data for the structure. [These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; email: deposit@ccdc.cam.ac.uk]].

4.8 | 2-(2-(4,5-diphenyloxazol-2-yl)ethyl)-5-(propylthio)-1,3,4-oxadiazole (5c)

White solid; mp: 90°C to 92°C; Rf: 0.43 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3058 (C—H of aromatic), 2964 (C—H of aliphatic), 2871 (SCH_2), 1581 (C═N), 1482 (C═C), 1152, 1061 (C—O—C), 765, 695; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.2 Hz, 2H, Ar-H), 7.56 (dd, J = 8.2 Hz, 2H, Ar-H), 7.40-7.29 (m, 6H, Ar-H), 3.44-3.33 (m, 4H, CH_2), 3.19 (t, J = 7.3 Hz, 2H, SCH_2), 1.81 (m, 2H, $SCH_2\underline{CH_2}$), 1.03 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 165.96, 164.65, 160.45, 145.52, 134.99, 132.08, 129.28, 128.75, 128.58, 128.46, 128.38, 127.95, 127.67, 126.29, 34.22, 24.81, 22.81, 22.50, 12.94; ESI-Ms m/z 392.0 $(M + H)^+$.

4.9 | 2-(butylthio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5d)

Light yellow solid; mp: 78°C to 80°C; Rf: 0.48 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3053 (C—H of aromatic), 2928 (C—H of aliphatic), 2861 (SCH_2), 1578 (C═N), 1478 (C═C), 1148, 1058 (C—O—C), 762, 695; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.2 Hz, 4H, Ar-H), 7.41-7.29 (m, 6H, Ar-H), 3.98 (t, J = 7.3 Hz, 2H, SCH_2), 3.50-3.32 (m, 4H, CH_2), 2.24-2.12 (m, 4H, SCH_2—$\underline{CH_2}$—$\underline{CH_2}$), 1.64 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 166.18, 164.95, 160.67, 145.76, 135.22, 132.28, 128.79, 128.68, 128.60, 128.17, 127.90, 126.52, 32.24, 31.23, 25.02, 23.02, 21.72, 13.50; HRMS: (ESI m/z) for $C_{23}H_{24}O_2N_3S$ calcd: 406.1583, found: 406.1585 $(M + H)^+$.

4.10 | 2-(2-(4,5-diphenyloxazol-2-yl) ethyl)-5-(octylthio)-1,3,4-oxadiazole (5e)

White solid; mp: 85°C to 87°C; Rf: 0.53 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3002 (C—H of aromatic), 2966 (C—H of aliphatic), 2924 (SCH_2), 1734 (C═N), 1689 (C═C), 1220, 1093 (C—O—C); ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.63 (dd, J = 8.2 Hz, 4H, Ar-H), 7.36-7.26 (m, 6H, Ar-H), 3.41-3.37 (m, 4H, CH_2), 3.21 (t, J = 7.3 Hz, 2H, SCH_2), 1.79-1.75 (m, 2H, $SCH_2C\underline{H_2}$), 1.42-1.27 (m, 13H, $CH_2[CH_2]_4CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 166.16, 164.97, 160.70, 145.77, 135.20, 132.25, 128.77, 128.67, 128.18, 127.91, 126.51, 32.55, 31.76, 29.71, 29.22, 28.98, 28.59, 25.01, 23.02, 22.63, 14.10; ESI-Ms m/z 462.0 $(M + H)^+$.

4.11 | 2-(2-(4,5-diphenyloxazol-2-yl) ethyl)-5-(isobutylthio)-1,3,4-oxadiazole (5f)

Yellow solid; mp: 80°C to 82°C; Rf: 0.40 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3003 (C—H of aromatic), 2924 (C—H of aliphatic), 2784 (SCH_2), 1686 (C═N), 1495 (C═C), 1225, 1093 (C—O—C), 903, 782; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.2 Hz, 4H, Ar-H), 7.41-7.28 (m, 6H, Ar-H), 3.47-3.30 (m, 4H, CH_2), 3.12 (d, J = 6.7 Hz, 2H, SCH_2), 2.13-1.95 (m, 1H, CH), 1.03 (d, J = 6.7 Hz, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:165.96164.65, 160.45, 145.52, 134.99, 132.08, 129.28, 128.75, 128.58, 128.46, 128.38, 127.95, 127.67, 126.29, 34.22, 30.72, 24.81, 22.50, 22.81, 12.94; HRMS: (ESI m/z) for $C_{23}H_{24}O_2N_3S$ calcd: 406.1573, found: 406.1576 $(M + H)^+$.

4.12 | 2-(allylthio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5g)

Yellow solid; mp: 76°C to 78°C; Rf: 0.43 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3059 (C—H of aromatic), 2926 (C—H of aliphatic), 2856 (SCH_2), 1583 (C═N), 1481 (C═C), 1161, 1059 (C—O—C), 761, 691; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.63 (dd, J = 8.2 Hz, 4H, Ar-H), 7.44-7.30 (m, 6H, Ar-H), 6.10-5.86 (m, 1H, $C\underline{H}$═CH_2), 5.35 (d, J = 17.3 Hz, 1H, CH═$C\underline{H_2}$), 5.19 (d, 1H, J = 9.8 Hz, 1H, CH═$C\underline{H_2}$), 3.84 (d, J = 6.7 Hz, 2H, SCH_2), 3.31-3.49 (m, 4H, CH_2); ^{13}C NMR (125 MHz, CDCl3) δ ppm: 166.45, 164.06, 160.61, 145.78, 135.22, 132.27, 131.70, 128.69, 128.61, 128.18, 127.91, 126.53, 119.72, 35.16, 24.98, 23.03; HRMS: (ESI m/z) for $C_{22}H_{20}O_2N_3S$ calcd: 390.1270, found: 390.1268 $(M + H)^+$.

4.13 | 2-(2-(4,5-diphenyloxazol-2-yl) ethyl)-5-(prop-2-yn-1-ylthio)-1,3,4 oxadiazole (5h)

White solid; mp: 77°C to 79°C; Rf: 0.40 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3073 (C—H of aromatic), 2923 (C—H of aliphatic), 2771 (SCH_2), 2274 (C≡C), 1624 (C═N), 1509 (C═C), 1154,1058 (C—O—C), 764, 698; ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.0 Hz, 4H, Ar-H), 7.42-7.30 (m, 6H, Ar-H), 3.98 (s, 2H, CH_2), 3.33-3.51 (m, 4H, CH_2—CH_2), 2.29 (s, 1H, C ≡ C—H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.85, 162.98, 160.54, 145.80, 135.23, 132.25, 128.76, 128.70, 128.62, 128.19, 127.90, 126.53, 76.76, 72.99, 24.95, 23.03, 21.02; HRMS: (ESI m/z) for $C_{22}H_{18}O_2N_3S$ calcd: 388.1114, found: 388.1114 $(M + H)^+$.

4.14 | 2-(benzylthio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5i)

Light brown solid; mp: 90°C to 92°C; Rf: 0.46 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3056 (C—H of aromatic), 2926 (C − H of aliphatic), 2855 (SCH_2), 1581 (C═N), 1483 (C═C), 1152, 1052 (C—O—C), 766, 699; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.8 Hz,2H, Ar-H), 7.56 (dd, J = 8.8 Hz, 2H, Ar-H), 7.41 (dd, J = 8.8 Hz, 2H, Ar-H), 7.39-7.27 (m, 9H, Ar-H), 4.44 (s, 2H, SCH_2), 3.44-3.33 (m, 4H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.68, 167.38, 166.35, 160.57, 145.75, 135.46, 133.34, 132.25, 129.09, 129.12, 128.78, 128.76, 128.66, 128.58, 128.55, 128.52, 128.14, 128.07,

127.87, 127.84, 126.49, 36.75, 24.90, 23.00; ESI-Ms m/z 440.6 $(M + H)^+$.

4.15 | 2-(2-(4,5-diphenyloxazol-2-yl) ethyl)-5-([4-fluorobenzyl]thio)-1,3,4-oxadiazole (5j)

White solid; mp: 78°C to 80°C; Rf: 0.43 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3051 (C—H of aromatic), 2928 (C—H of aliphatic), 2864 (SCH_2), 1584 (C═N), 1481 (C═C), 1232, 1154 (Ar-F), 1056, 1019 (C—O—C), 762, 694; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.62 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.56 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.43-7.30 (m, 8H, Ar-H), 7.00 (t, $J = 8.8$ Hz, 2H, Ar-H), 4.41 (s, 2H, SCH_2), 3.46-3.33 (m, 4H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.94, 175.01, 166.44, 164.02, 163.41, 135.20, 132.21, 131.40, 131.37, 130.86, 130.80, 128.69, 128.58, 128.16, 127.86, 126.55, 126.51, 123.19, 122.94, 122.68, 115.77, 35.96, 24.92, 22.98; HRMS: (ESI *m/z*) for $C_{26}H_{21}O_2N_3FS$ calcd: 458.1333, found: 458.1317 $(M+ H)^+$.

4.16 | 2-((4-[benzyloxy] benzyl) thio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5k)

White solid; mp: 88°C to 90°C; Rf: 0.43 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3047 (C—H of aromatic), 2925 (C—H of aliphatic), 2854 (SCH_2), 1578 (C═N), 1484 (C═C), 1247, 1029 (C—O—C), 747, 693; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 7.62 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.56 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.48-7.27 (m, 13H, Ar-H), 6.91 (d, $J = 8.5$ Hz, 2H, Ar-H), 5.03 (s, 2H, OCH_2), 4.40 (s, 2H, SCH_2), 3.45-3.30 (m, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 166.23, 164.26, 160.53, 158.54, 145.68, 136.69, 135.15, 132.20, 130.33, 128.70, 128.60, 128.53, 128.08, 127.95, 127.81, 127.59, 127.38, 126.44, 115.01, 69.96, 36.34, 24.91, 22.94; ESI-Ms m/z 546.0 $(M + H)^+$.

4.17 | 2-(2-(4,5-diphenyloxazol-2-yl) ethyl)-5-([4-methoxybenzyl]thio)-1,3,4-oxadiazole (5l)

Light yellow solid; mp: 90°C to 92°C; Rf: 0.43 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3043 (C—H of aromatic), 2925 (C—H of aliphatic), 2849 (SCH_2), 1575 (C═N), 1473 (C═C), 1146, 1060 (C—O—C), 764, 686; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.62 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.56 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.45-7.31 (m, 8H, Ar-H), 6.61 (d, $J = 8.5$ Hz, 2H, Ar-H), 5.12 (s, 2H, SCH_2), 3.90 (s, 3H, OCH_3), 3.45-3.30 (m, 4H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 166.32, 164.39, 160.62, 159.41, 145.77, 135.23, 132.27, 130.40, 128.77, 128.69, 128.62, 128.18, 127.90, 127.37, 126.51, 114.17, 55.30, 36.41, 24.99, 23.02; HRMS: (ESI *m/z*) for $C_{27}H_{24}O_3N_3S$ calcd: 470.1532, found: 470.1528 $(M + H)^+$.

4.18 | 2-([4-chlorobenzyl]thio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5m)

White solid; mp: 84°C to 86°C; Rf: 0.46 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3063 (C—H of aromatic), 2928 (C—H of aliphatic), 2838 (SCH_2), 1675 (C═N), 1485 (C═C), 1163, 1093 (C—O—C), 704, 646; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.61 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.57 (dd, $J = 8.2$ Hz, 2H, Ar-H), 7.42-7.30 (m, 10H, Ar-H), 4.46 (s, 2H, SCH_2), 3.42-3.34 (m, 4H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 167.25, 163.41, 160.57, 158.55, 146.76, 135.46, 132.55, 131.52, 130.50, 129.56, 129.21, 129.34, 128.55, 128.33, 128.21, 127.07, 127.05, 126.49, 114.54, 34.75, 24.91, 23.07; ESI-Ms m/z 474.0 $(M + H)^+$, 476.0 $(M + 2 + H)^+$.

4.19 | 2-([4-bromobenzyl] thio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5n)

Light brown solid; mp: 90°C to 92°C; Rf: 0.44 (ethylacetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3054 (C—H of aromatic), 2927 (C—H of aliphatic), 2845 (SCH_2), 1585 (C═N), 1476 (C═C), 1157, 1059 (C—O—C), 762, 691; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.60 (dd, $J = 8.2$ Hz, 4H, Ar-H), 7.47-7.29 (m, 8H, Ar-H), 6.69 (d, $J = 8.5$ Hz, 2H, Ar-H), 4.44 (s, 2H, SCH_2), 3.41-3.32 (m, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 166.22, 165.26, 161.53, 158.51, 146.68, 137.62, 135.12, 132.21, 130.32, 128.71, 128.65, 128.07, 127.95, 127.84, 127.69, 127.38, 126.44, 125.53, 114.01, 36.35, 23.91, 21.45; ESI-Ms m/z 518.0 $(M + H)^+$, 520.0 $(M + 2 + H)^+$.

4.20 | 2-([2-chlorobenzyl]thio)-5-(2-(4,5-diphenyloxazol-2-yl)ethyl)-1,3,4-oxadiazole (5o)

White solid; mp: 84°C to 86°C; Rf: 0.42 (ethylacetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3063 (C − H of aromatic), 2930 (C − H of aliphatic), 2849 (SCH_2), 1703 (C═N), 1586 (C═C), 1160, 1053 (C—O—C), 759, 700; ^1H NMR (500 MHz, $CDCl_3$) δ ppm: 7.64 (dd, $J = 8.3$ Hz, 2H,

Ar-H), 7.54 (dd, J = 8.2 Hz, 2H, Ar-H), 7.41-7.27 (m, 10H, Ar-H), 5.39 (s, 2H, -SCH_2), 3.21 (t, J = 7.4 Hz, 2H, CH_2), 2.98 (t, J = 7.4 Hz, 2H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm:167.38, 166.35, 160.57, 145.75, 135.46, 129.12, 129.09, 128.92, 128.87, 128.78, 128.76, 128.66, 128.58, 128.55, 128.49, 128.14, 128.11, 128.07, 36.75, 24.96, 23.02; ESI-Ms m/z 474.0 $(M + H)^+$, 476.0 $(M + 2 + H)^+$.

4.21 | 2-(2-(4,5-diphenyloxazol-2-yl) ethyl)-5-([4-methoxyphenethyl]thio)-1,3,4-oxadiazole (5p)

Light yellow solid; mp: 90°C to 92°C; Rf: 0.44 (ethyl acetate/hexane, 30/70); IR (KBr, υ_{max} in cm^{-1}): 3055 (C—H of aromatic), 2927 (C—H of aliphatic), 2845 (SCH_2), 1580 (C═N), 1447 (C═C), 1146, 1024 (C—O—C), 760, 692; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.62 (dd, J = 8.2 Hz, 2H, Ar-H), 7.56 (dd, J = 8.2 Hz, 2H, Ar-H), 7.40-7.29 (m, 6H, Ar-H), 7.14 (d, J = 8.6 Hz, 2H, Ar-H), 6.84 (d, J = 8.6 Hz, 2H, Ar-H), 3.79 (s, 3H, OCH_3), 3.46-3.34 (m, 6H, CH_2—CH_2 and overlapping with —S$\underline{CH_2}$—CH_2), 3.04 (t, J = 7.8 Hz, 2H, —SCH_2—$\underline{CH_2}$); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 167.08, 163.68, 161.78, 158.41, 145.31, 134.84, 132.31, 131.48, 130.07, 129.39, 129.35, 129.10, 128.75, 128.66, 127.78, 126.79, 114.25, 55.44, 34.52, 33.91, 24.63, 22.59; ESI-Ms m/z 484.0 $(M + H)^+$.

5 | BIOLOGY

5.1 | In vitro anticancer activity

In vitro anticancer activity of the test compounds was studied using MTT colorimetric assay[36] as per ATCC protocol. Cell lines that were used for testing in vitro cytotoxicity included PC-3 derived from human prostate adenocarcinoma cells (ATCC No. CRL-1435), A549 derived from human lung carcinoma cells (ATCC No. CCL-185) and HEK 293 derived from human embryonic kidney cells (non-cancerous; ATCC No. CRL-1573) which were procured from American Type Culture Collection, Manassas, VA, USA. Lung cancer cell line A549 was maintained in DMEM medium supplemented with 10% new born calf serum, along with 1% non-essential amino acids, 0.2% sodium bicarbonate, 1% sodium pyruvate and 1% antibiotic mixture (10, 000 U penicillin and 10 mg streptomycin per mL). PC-3, HEK 293 were maintained in RPMI-1640 supplemented with 10% new born calf serum (NBCS), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM-glutamine. Cell lines were maintained at 37°C in a humidified 5% CO_2 incubator (Thermo scientific). Cell lines were processed by initial trypsinization to detach the adhered cells and followed by centrifugation to get cell pellet. Fresh media was added to the pellet to make a cell count using haemocytometer and plate 100 μL of media with cells ranging from 5,000 to 6,000 per well in a 96-well plate. The plate was incubated overnight in CO_2 incubator for the cells to adhere and regain its shape. After 24 hours cells were treated with the test compounds at 25 μM diluted using the media to deduce the percentage inhibition on cancer cells and human normal cells. The cells were incubated for 48 hours to assay the effect of the test compounds on different cell lines. Zero hour reading was noted down with untreated cells and also control with 1% DMSO to subtract further from the 48 hours reading. After 48 hours incubation, cells were treated by MTT (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) dissolved in PBS (5 mg/mL) and incubated for 3-4 hours at 37°C. The formazan crystals thus formed were dissolved in 100 μL of DMSO and the viability was measured at 540 nm on a multimode reader (Spectra max).

5.2 | In vitro antibacterial activity

A collection of four organisms including Gram-positive and Gram-negative organisms were used for this study. Clinical isolates such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* were obtained from Microbiology laboratory of Osmania Medical Hospital, Hyderabad. All strains were tested for purity by standard microbiological methods. The bacterial stock cultures were maintained on Mueller Hinton Agar (MHA) slants and stored at 4°C. Agar-well diffusion method[37,38] was employed for evaluation of antibacterial activity. The bacterial strains were reactivated from stock cultures by transferring into Mueller Hinton Broth (MHB) and incubating at 37°C for 18 hours. A final inoculum containing 10^6 colony forming units (1×10^6 CFU/mL) was added aseptically to MHA medium and poured into sterile Petri dishes. Different test extracts at a concentration of 0.4 mg/50 μL were added to wells (8 mm in diameter) punched on agar surface. Plates were incubated overnight at 37°C and diameter of inhibition zone (DIZ) around each well was measured in mm. Experiments were performed in triplicates. Antibiotics such as Ciprofloxacin at a concentration of 0.005 mg/mL were used as positive reference to determine sensitivity of microorganisms tested. DMSO was used as Negative control.

5.3 | X-ray Crystallography

X-ray data for the compounds were collected at room temperature using a Bruker Smart Apex CCD diffractometer

with graphite monochromated MoKα radiation (λ = 0.71073 Å) with ω-scan method.[39] Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Integration and scaling of intensity data were accomplished using SAINT program.[39] The structure was solved by direct methods using SHELXS97[40] and refinement was carried out by full-matrix least-squares technique using SHELXL97.[40] Anisotropic displacement parameters were included for all non-hydrogen atoms. All H atoms were positioned geometrically and treated as riding on their parent C atoms [C—H = 0.93-0.97 Å and U_{iso} (H) = 1.5 U_{eq} (C) for methyl H or 1.2 U_{eq} (C) for other H atoms]. The methyl groups were allowed to rotate but not to tip.

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