Brief Report

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Synthesis and antiproliferative assay of 1,3,4-oxadiazole and 1,2,4-triazole derivatives in cancer cells

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ABSTRACT: A series of new 1,3,4-oxadiazole and 1,2,4-triazole derivatives were synthesized. The structures were confirmed by IR, ^{1}H-NMR, and MS. The compounds were evaluated for their antiproliferative activity against K562 (human erythromyeloblastoid leukemia cell line), MDA-MB-231 (human breast adenocarcinoma cell line), HT29 (human colon adenocarcinoma grade II cell line) and HepG2 (human hepatocellular liver carcinoma cell line) *in vitro*. The result showed that 7 compounds displayed inhibitory activities against K562 with the inhibition rate more than 50%. Especially, compound 5f exhibited the most potent activity against K562 with 85% inhibition ratio and could be used as lead compound to search new 1,3,4-oxadiazole derivatives as antiproliferative agent.

Keywords: Synthesis, 1,3,4-oxadiazole, 1,2,4-triazole, antitumor

1. Introduction

It was observed from the literature that 1,3,4-oxadiazole ring is associated with many types of biological properties such as anti-inflammatory (*1*), antibacterial (*2*), antifungal (*3*), antitumor (*4*), antiviral (*5*), hypoglycemic (*6*), anticonvulsant (*7*), analgesic (*8*), herbicidal (*9*), and insecticidal activities (*10*). Another 1,2,4-triazole ring also shows broad-spectrum bioactivities, *e.g.*, preparation their derivatives as agrochemical (*11*), medicinal fungicides (*12*), virucides (*13*), anticancer drugs (*14*), antimicrobial anti-inflammatory (*15*), anticonvulsant (*16*), antihypertensive (*17*), and plant growth regulators (*18*).

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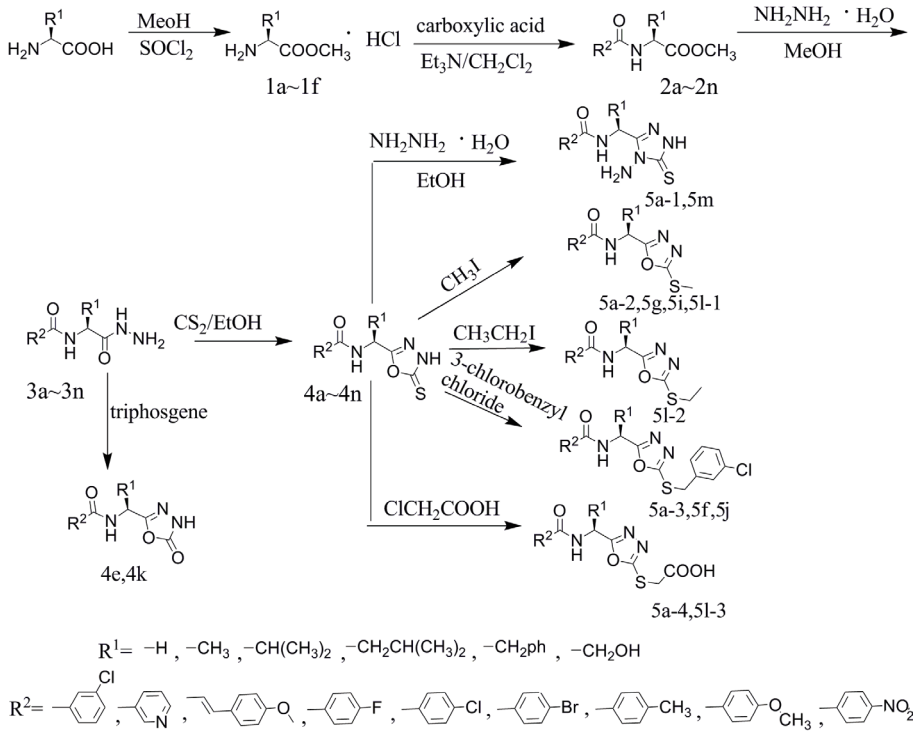
Chronic myelogenous leukaemia (CML) is a haematological malignancy caused by a chromosomal rearrangement that generates a fusion protein, BCR-ABL, with deregulated tyrosine kinase activity. K562 is human erythromyeloblastoid leukemia cell line and can specific express BCR-ABL. Imatinib is an inhibitor of BCR-ABL tyrosine kinase, function through competitive inhibition at the ATP-binding site of the enzyme, which leads to the inhibition of tyrosine phosphorylation of proteins involved in BCR-ABL signal transduction. It shows a high specificity for BCR-ABL, the receptor of platelet-derived growth factor, and c-kit tyrosine kinases, and is well tolerated. Imatinib has significant activity against CML which treatment with α interferon had failed (*19*).

In view of above mentioned facts and an attempt to achieve new potent antitumor agents with good bioavailability and low toxicity, herein, we described the synthesis and the structure-activity relationship (SAR) of a series of new 1,3,4-oxadiazole and 1,2,4-triazole derivatives as potential antitumor agents. Docking simulations were performed using the X-ray crystallographic structure of the ABL complexed with imatinib to explore the binding modes at the active site.

2. Materials and Methods

2.1. *Chemicals*

Target compounds were synthesized *via* the route outlined in Scheme 1. Firstly, amino acids (glycine, L-alanine, L-valine, L-leucine, L-phenylalanine, and L-serine) were reacted with MeOH to yield amino acids methyl ester (*20*), then coupled with carboxylic acids to obtain amide (**2a~2n**) and hydrazinolysised to give hydrazinyl-substituted amide (**3a~3n**) (*21*). (1) Cycling with carbon disulphide yielded the corresponding 1,3,4-oxadiazole derivatives (**4a~4n**) (*22*). (a) Reacting with hydrazine hydrate obtained 1,2,4-triazole derivatives (**5a-1**,**5n**) (*23*). (b) Reacting with methyl iodide, ethyl iodide, 3-chlorobenzyl chloride or chloroacetic acid obtained thioethers (**5a-2**, **5h**, **5j**, **5m-1**; **5m-2**; **5a-3**, **5g**, **5k**). (2) Cycling **3e**, **3k** with



Scheme 1. The synthesis of target compounds.

triphosgene obtained new 1,3,4-oxadiazole derivatives (**4e**, **4k**) (*24*). The structures of the target compounds which were evaluated for antiproliferative activity were confirmed by IR, ^{1}H-NMR, and MS. All of them gave satisfactory analytical and spectroscopic data, which were in full accordance with depicted structures.

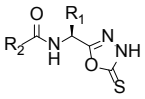
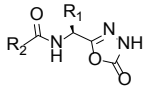
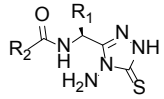
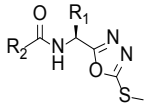
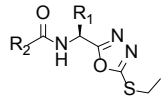
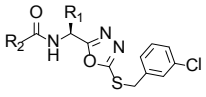
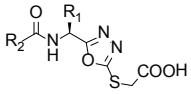
2.2. *Antiproliferation assay (in vitro)*

The antiproliferative activity of 1,3,4-oxadiazole and 1,2,4-triazole derivatives was assessed by means of MTT method (*25*). Imatinib mesylate was used as the positive control. To determine cell proliferation, K562 (human erythromyeloblastoid leukemia cell line), MDA-MB-231 (human breast adenocarcinoma cell line), HT29 (human colon adenocarcinoma grade II cell line), and Hep G2 (human hepatocellular liver carcinoma cell line) were individually plated at density of 1.5×10^4 cells/well, 8.0×10^3 cells/well, 8.0×10^3 cells/well, 4.0×10^3 cells/well, respectively, in 96-well plates at 37°C in 5% CO_2 atmosphere. For MDA-MB-231 cell lines, HT29 cell lines and Hep G2 cell lines, after 24 h of culture, the medium in the wells was replaced with the fresh medium containing compounds of certain concentration (1.0×10^{-4} mol/L). Three wells were tested in parallel for each synthesized compounds. After 48 h, 20 μL of MTT dye solution (5 mg/mL in phosphate buffer, pH7.4) was added to each well and incubated for 4 h at 37°C in 5% CO_2 for exponentially growing cell and 10 min for steady-state confluent cells. The formazan crystals were solubilized with 150 μL of dimethyl sulfoxide (DMSO) and the solution was vigorously mixed to dissolve the reacted dye. For K562 cell lines, the medium containing compounds of certain concentration (1.0×10^{-4} mol/L) was added. After 48 h, 10 μL of MTT dye solution (5 mg/mL in phosphate buffer, pH7.4) was added to each well and incubated for 4 h at 37°C in 5% CO_2 for exponentially growing cell. Then a mixed solution (10% SDS, 5% isobutanol, 0.012 mol/L HCl) was added to each well for 12 h. The absorbance of each well was read on a microplate reader (BTR-600 intruments) at 490 nm. The inhibition ratio on the tumor cells growth by the drugs was determined by the following formula: inhibition ratio on the tumor cells growth = (1 – (experimental group OD value/control group OD value)) × 100%.

3. Results and Discussion

All the synthesized 1,3,4-oxadiazole and 1,2,4-triazole derivatives were evaluated for their antiproliferative activity against K562, MDA-MB-231, HT29, and Hep G2 in Table 1. Preliminary result showed that 7 compounds displayed inhibitory activities against K562 with the inhibition rate more than 50%. Structure-activity relationship of the derivatives inhibition the proliferation of K562 was studied. Comparing 1,2,4-triazole and 5-thioxo-1,3,4-oxadiazole analogues (**5a-1**, **5m**, **4a~4n**), 1,2,4-triazole rings had no significant effect on the antiproliferative activity, while 5-oxo-1,3,4-oxadiazole ring (**4e**, **4k**) leaded to increase in activity. Among alanine analogues (**4a**, **4c**, **4d**, **4n**), compounds **4c** and **4n** showed better activity than others, which indicated that introduction vinylogy and nitro groups to benzene ring might improve the activity. Compounds **5l-1**, **5l-2**, and **5f** displayed excellent antiproliferative activity, which indicated that the substituent attached to

Table 1. Antiproliferative assay of target compounds and imatinib mesylate (rate%)

Structure	Compounds	R_1	R_2	K562	MDA-MB-231	HT29	Hep G2
	4a	$-CH_3$		27	21	15	13
	4b	$-CH(CH_3)_2$		30	17	21	24
	4c	$-CH_3$		57	12	17	15
	4d	$-CH_3$		30	20	11	17
	4f	$-CH_2CH(CH_3)_2$		36	11	15	10
	4g	$-CH_2ph$		42	12	19	13
	4h	$-CH_2OH$		37	18	14	16
	4i	$-CH(CH_3)_2$		32	15	18	20
	4j	$-CH_2CH(CH_3)_2$		57	12	13	12
	4n	$-CH_3$		54	10	16	24
	4e	-H		63	21	23	16
	4k	-H		48	23	25	15
	5a-1	$-CH_3$		40	12	14	12
	5m	$-CH_3$		13	11	9	14
	5a-2	$-CH_3$		12	18	17	7
	5g	$-CH_2ph$		46	16	32	29
	5i	$-CH(CH_3)_2$		36	11	16	20
	5l-1	$-CH(CH_3)_2$		59	20	29	22
	5l-2	$-CH(CH_3)_2$		60	13	17	19
	5a-3	$-CH_3$		17	14	22	23
	5f	$-CH_2CH(CH_3)_2$		85	32	23	12
	5j	$-CH_2CH(CH_3)_2$		34	13	16	14
	5a-4	$-CH_3$		7	12	5	9
	5l-3	$-CH(CH_3)_2$		9	8	11	7
	Imatinib mesylate			93	92	95	94

the 5-position of 1,3,4-oxadiazole ring had significant effect on activity, while carboxyl group made compounds almost lose antiproliferative activity. Compound **5f** was the most potent of all the target compounds with 85% inhibition ratio. Compared with **4i**, compound **4j** with a bulky substituent which connected with chiral carbon was more potent activity. Substituent in benzene ring greatly influenced the activity. In addition, all of target compounds show low activity toward MDA-MB-231, HT29, and Hep G2. In above four types of tumor cells, only the K562 can specific express ABL, and the variant tumor cells showed different sensitivity to anticancer drugs. So the target compounds exhibited K562 selectivity.

In effort to elucidate the possible mechanism which the target compounds displayed antiproliferative activity toward K562 and guide further structure-activity relationship studies, the preferred pharmacophore docking studies were carried out *via* the autodock program. The interaction of **5f** with active site of ABL structure complexed with imatinib (PDB: 3K5V) is shown in Figures 1 and 2. Compounds **5f**, as imatinib, has been shown to recognize efficiently active-site of ABL. The carbonyl group can form hydrogen bond with Asp400 with the distance of 2.81 Å. The nitrogen atom of amide bond forms hydrogen bond with Asp400 and Glu305 with the distance of 2.96 Å and 2.78 Å, respectively. The hydrophobic parts of compound are in contact with nonpolar surface areas of ABL such as Lys290, Ala399, Phe401, Ile312, Lys304, Val308, and Phe378.

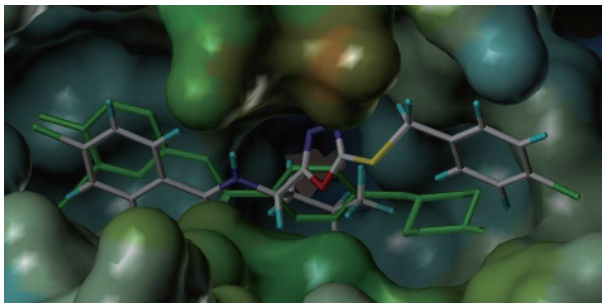


Figure 1. Molecular docking of compound 5f with active-site of ABL. Imatinib is represented as green sticks. Compound **5f** is represented as a tube with colored atoms.

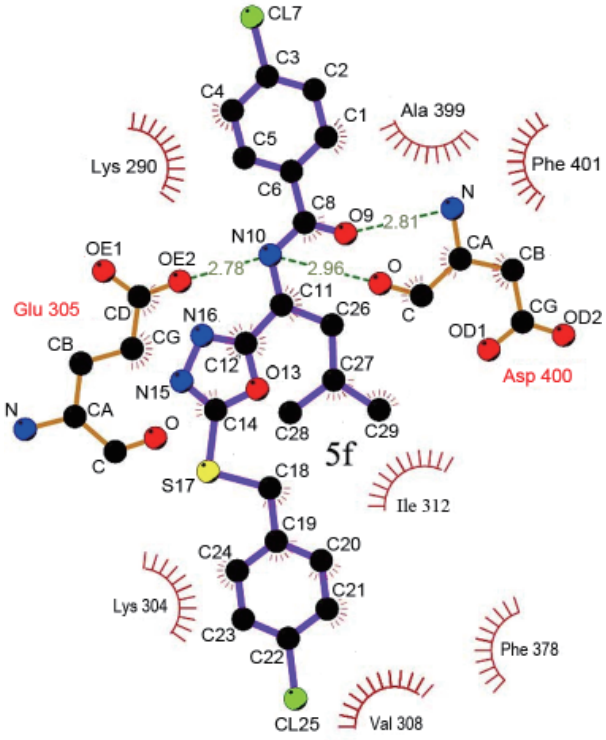


Figure 2. Diagram (LIGPLOT) of the hydrogen bonds and hydrophobic interactions of the compound 5f with active-site residues in ABL.

4. Conclusion

We designed and synthesized a series of new 1,3,4-oxadiazole and 1,2,4-triazole derivatives. Some of them showed moderate potency against K562. Especially, compound **5f** exhibited the best inhibitory activity and could be used as lead compound to search new 1,3,4-oxadiazole derivatives as antiproliferative agents.

Acknowledgements

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Appendix

Chemistry: general procedures

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. The solvents of $SOCl_2$, methanol and carbonic dichloride have been distilled before use. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light. Melting points were determined using WRS-1B melting point apparatus and are uncorrected. The IR spectra were recorded by means of KBr pellet technique on a SHIMADZU FTIR-8400 spectrometer. Mass spectra were recorded on a ZQ-4000/2695 equipment in a negative electron spray ionization (ESI) mode. ^{1}H-NMR was recorded on a Bruker AV-600 MHz spectrometer at room temperature, and chemical shifts were measured using TMS as internal standard. Significant ^{1}H-NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet) number of protons.

1. General procedure for compounds 1a-f

The compounds (**1a-f**) were prepared from the corresponding amino acids (glycine, L-alanine, L-valine, L-leucine, L-phenylalanine, L-serine). MeOH (200 mL) was cooled in ice-bath and $SOCl_2$ (18 mL, 0.25 mol) was added dropwise. The reaction mixture was stirred at 0°C for 1 h and then appropriate amino acid (0.2 mol) was added, stirred for another 1 h at 0°C, followed by 24 h at room temperature and then evaporated in vacuum to dryness, recrystallized from appropriate solvent.

*Glycine methyl ester hydrochloride (**1a**)* White solid; yield: 98%; mp: 175.8-176.8°C; IR(KBr) 3,395, 2,885, 2,685, 2,635, 1,747, 1,585, 1,439, 1,261, 880 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6,) δ 8.504(s, 3H, NH_3Cl), 3.792(s, 2H, CH_2), 3.735(s, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 122.80.

2. General procedure for compounds 2a-n

To a stirred solution of the compounds **1a-f** (0.02 mol) in CH_2Cl_2 (80 mL) triethylamine (0.04 mol, 5.6 mL) was added, reacted at 0°C for 0.5 h. A solution of carboxylic acid (0.02 mol) in CH_2Cl_2 (40 mL) was added, then DCC (0.022 mol, 4.54 g) in CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred for 2 h at 0°C, then allowed to warm to room temperature for 48 h. The white precipitate (DCU) was removed. The solution was washed with saturated $NaHCO_3$ (30 mL, ×3), 10% hydrochloric acid (30 mL, ×3), distilled water (30 mL, ×2) in turn, dried with magnesium sulfate anhydrous, filtered. The solvent was evaporated in vacuo, and the residue was dissolved in ethyl acetate, filtered. The ethyl acetate solution was evaporated in vacuo. The residue was recrystallized from appropriate solvent.

*Methyl 2-(4-chlorobenzamido)acetate (**2e**)* White solid; yield: 67%; mp: 110.0-112.0°C; IR (KBr) 3,275, 3,090, 3,028, 2,990, 2,947, 2,851, 1,751, 1,651, 1,435, 853 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.072(t, J = 5.7 Hz, 1H, NH), 7.893(m, 2H, ArH), 7.578(m, 2H, ArH), 4.02(d, J = 2.7 Hz, 2H, CH_2), 3.659(s, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 225.33.

3. General procedure for compounds 3a-n

The reaction mixture of compounds **2a-n** (10 mmol) and 80% hydrate hydrazine (20 mmol, 1 mL) in methanol was refluxed at 85°C overnight, then cooled, evaporated in vacuo. The residue was recrystallized from absolute EtOH.

*N-(2-hydrazinyl-2-oxoethyl)-4-chlorobenzamide (**3e**)* White solid; yield: 90%; mp: 214.7-216.5°C; IR (KBr) 3,479, 3,364, 3,302, 3,190, 1,643, 1,547, 1,277, 845 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6,) δ 9.140(s, 1H, NH), 8.818(t, J = 5.7 Hz, 1H, NH), 7.895(m, 2H, ArH), 7.551(d, J = 8.4 Hz, 2H, ArH), 4.217(s, 2H, CH_2), 3.825(d, J = 6 Hz, 2H, NH_2); ESI-MS: m/z $[M - H]^-$ 225.42.

4. General procedure for compounds 4a-d, 4f-j, 4l-n

The reaction mixture of compounds **3a-d**, **3f-j**, **3l-n** (0.01 mol), KOH (0.01 mol, 0.56 g), carbon disulfide (0.03 mol, 1.8 mL), and ethanol (80 mL) was heated under reflux until the evolution of H_2S ceased (~12 h). Ethanol was distilled off under reduced pressure and the residue was dissolved in water and then acidified with dilute hydrochloric acid (10%). The precipitate was filtered, washed with water, dried, and recrystallized from ethanol.

*(S)-N-[1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl]-3-chlorobenzamide (**4a**)* White solid; yield: 85%; mp: 163.1-163.3°C; IR (KBr) 3,244, 3,059, 2,943, 2,770, 2,723, 2,554, 1,643, 1,508, 1,061, 806, 725, 683, 656 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.539(br, 1H, oxadiazole NH), 9.206(d, J = 7.8 Hz, 1H, NH), 7.94(d, J = 1.8Hz, 1H, ArH), 7.856(d, J = 1.8 Hz, 1H, ArH), 7.661(m, 1H, ArH), 7.550(t, J = 7.8 Hz, 1H, ArH), 5.252(m, 1H, CH), 1.546(d, J = 7.2 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 281.32.

*(S)-N-[2-methyl-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)propyl]nicotinamide (**4b**)* White solid; yield: 83%; mp: 163.1-163.3°C; IR (KBr) 3,298, 3,271, 2,966, 1,643, 1,539, 1,342, 710 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.582(br, 1H, oxadiazole NH), 9.227(d, J = 7.8 Hz, 1H, NH), 9.03(d, J = 2.4 Hz, 1H, ArH), 8.747(dd, J_1 = 1.2Hz, J_2 = 3.6Hz, 1H, ArH), 8.229(m, 1H, ArH), 7.544(dd, J_1 = 4.8 Hz, J_2 = 3.0 Hz, 1H, ArH), 4.953(t, J = 8.4 Hz, 1H, CH), 2.308(m, 1H, CH), 1.033(d, J = 6.6 Hz, 3H, CH_3), 0.949(d, J = 6.6 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 276.46.

*(S)-N-[1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl) ethyl]-4-methoxylcinnamamide (**4c**)* White solid; yield: 75%, mp: 173.7-175.8°C; IR(KBr) 3,263, 3,071, 2,932, 2,766, 2,341, 1,651, 1,504, 1,173, 826 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.506(br, 1H, oxadiazole NH), 8.722(d, J = 7.8Hz, 1H, NH), 7.538(d, J = 9.0 Hz, 2H, ArH), 7.44(d, J = 15.6 Hz, 1H, CH), 6.989(d, J = 9.0 Hz, 2H, ArH), 6.48(d, J = 16.2 Hz, 1H, CH), 5.114(t, J = 7.5 Hz, 1H, CH), 3.792(s, 3H, CH_3), 1.466(d, J = 7.2 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 303.51.

*(S)-N-[1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl) ethyl]-4-fluorobenzamide (**4d**)* White solid; yield: 80%; mp 172.4-172.6°C; IR (KBr) 3,256, 3,028, 2,978, 2,874, 2,704, 2,611, 1,604, 1,504, 1,150, 814 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.517(br, 1H, oxadiazole NH), 9.1(d, J = 7.8 Hz, 1H, NH), 7.959(m, 2H, ArH),7.312(m, 2H, ArH), 5.232(t, J = 7.2 Hz, 1H, CH), 1.533(d, J = 7.2 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 265.29.

*(S)-N-[3-methyl-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)butyl]-4-chlorobenzamide (**4f**)* White solid; yield: 85%; mp: 155.8-156.3°C; IR (KBr) 3,251, 3,058, 2,960, 2,930, 2,870, 2,766, 2,615, 1,628, 1,485, 1,169, 1,150, 841 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.536(br, 1H, oxadiazole NH), 9.111(d, J = 7.8 Hz, 1H, NH), 7.916(d, J = 8.4 Hz, 2H, ArH), 7.589(d, J = 8.4 Hz, 2H, ArH), 5.214(m, 1H, CH), 2.514(t, J = 1.8 Hz, 1H, CH), 1.916(m, 1H, CH_2), 1.763(m, 1H, CH_2), 0.929(dd, J_1 = 6.6Hz, J_2 = 10.2 Hz, 6H, 2 × CH_3); ESI-MS: m/z $[M - H]^-$ 323.54.

*(S)-N-[2-phenyl-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl]-4-chlorobenzamide (**4g**)* White solid; yield: 82%; mp: 224.2-224.3°C; IR (KBr) 3,244, 3,028, 2,920, 2,766, 2,596, 1551, 1,161, 841 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 14.569(br, 1H, oxadiazole NH), 9.226(d, J = 7.8 Hz, 1H, NH), 7.822(dd, J_1 = 1.2 Hz, J_2 = 4.8 Hz, 2H, ArH), 7.562(dd, J_1 = 1.2Hz, J_2 = 4.8 Hz, 2H, ArH), 7.295(m, 4H, ArH), 7.211(d, J = 7.8 Hz, 1H, ArH), 5.383(m, 1H, CH), 3.262(m, 2H, CH_2); ESI-MS: m/z $[M - H]^-$ 357.52.

*(S)-N-[2-hydroxy-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl]–4-chlorobenzamide (**4h**)* White solid; yield: 80%; mp: 104.8-105.9°C; IR (KBr) 3,564, 3,422, 3,290, 3,055, 2,874, 2,762, 1,656, 1,481, 1,169, 822 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.558(br, 1H, oxadiazole NH), 9.08(d, J = 7.8 Hz, 1H, NH), 7.925(m, 2H, ArH), 7.586(m, 2H, ArH), 5.326(br, 1H, OH), 5.15(dd, J_1 = 6.6 Hz, J_2 = 7.2 Hz, 1H, CH), 3.859(ddd, J_1 = 6.6 Hz, J_2 = 4.2 Hz, J_3 = 15 Hz, 2H, CH_2); ESI-MS: m/z $[M - H]^-$ 297.47.

*(S)-N-[2-methyl-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)propyl]-4-bromobenzamide (**4i**)* White solid; yield: 87%; mp: 214.5-214.6°C; IR (KBr) 3,263, 3,036, 2,951, 2,909, 2,870, 2,731, 1,651, 1,481, 1,142, 845 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.573(br, 1H, oxadiazole NH), 9.106(d, J = 8.4 Hz, 1H, NH), 7.842(m, 2H, ArH), 7.723(m, 2H, ArH), 4.923(t, J = 8.1Hz, 1H, CH), 2.312(m, 1H, CH), 1.017(d, J = 6.6 Hz, 3H, CH_3), 0.939(d, J = 6.6 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 355.39.

*(S)-N-[3-methyl-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)butyl]-4-bromobenzamide (**4j**)* White solid; yield: 88%; mp: 160.8-161.0°C; IR (KBr) 3,260, 3,058, 2,990, 2,870, 2,743, 2,619, 1,628, 1,497, 1,169, 837 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 14.537(br, 1H, oxadiazole NH), 9.115(d, J = 8.4 Hz, 1H, NH), 7.844(d, J = 8.4 Hz, 2H, ArH), 7.73(d, J = 9.0 Hz, 2H, ArH), 5.215(m, 1H, CH), 1.905(m, 1H, CH), 1.759(m, 1H, CH_2), 1.684(d, J = 6.0 Hz, 1H, CH_2), 0.929(dd, J_1 =

6.6 Hz, J_2 = 10.2 Hz, 6H, 2 × CH_3); ESI-MS: m/z [M − H]$^-$ 369.42.

*(S)-N-[1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl]-4-nitrobenzamide (**4n**)* Yellow solid; yield: 81%; mp: 189.6-190.0°C; IR (KBr) 3,344, 3,098, 2,951, 2,746, 1,647, 1,489, 1,150, 845 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 14.557(br, 1H, oxadiazole NH), 9.429(d, *J* = 7.8 Hz, 1H, NH), 8.378(m, 2H, ArH), 8.126(m, 2H, ArH), 5.278(t, *J* = 7.2 Hz, 1H, CH), 1.559(d, *J* = 7.2 Hz, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 292.41.

5. General procedure for compounds 4e, 4k

To an ice-bath solution of compounds **3e**, **3k** (5 mmol) in 20 mL of water, a solution of triphosgene (5.5 mmol, 1.63 g) in 5 mL of toluene was added dropwise. The mixture was reacted for 12 h at 25°C. White solid was formed which was dissolved by adding dropwise of NaOH (10%). The organic phase was eliminated and the water phase was acidified by dilute hydrochloric acid. The white solid formed was filtered, washed with water, dried and recrystallied from EtOH.

*N-[(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-4-chlorobenzamide (**4e**)* White solid; yield: 90%; mp: 208.6-208.8°C; IR (KBr) 3,283, 3,047, 2,990, 1,747, 1,643, 1,535, 1,092, 845, 760 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 12.272(br, 1H, oxadiazole NH), 9.188(t, *J* = 5.4 Hz, 1H, NH), 7.903(d, *J* = 9.0 Hz, 2H, ArH), 7.582(d, *J* = 9.0 Hz, 2H, ArH), 4.394(d, *J* = 5.4 Hz, 2H, CH_2); ESI-MS: m/z [M − H]$^-$ 251.43.

*N-[(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-4-methylbenzamide (**4k**)* White solid; yield: 90%; mp: 180.0-180.1°C; IR (KBr) 3,325, 3,109, 3,036, 2,970, 2,924, 2,789, 1,778, 1,643, 1,535, 1,215, 837 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 12.254(br, 1H, oxadiazole NH), 9.011(s, 1H, NH), 7.786(d, *J* = 8.4 Hz, 2H, ArH), 7.3(d, *J* = 7.8 Hz, 2H, ArH), 4373(d, *J* = 5.4 Hz, 2H, CH_2), 2.359(s, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 231.40.

6. General procedure for compounds 5a-1,5m

A mixture of compounds **4a**, **4m** (5 mmol) in absolute ethanol (20 mL) and 80% hydrate hydrazine (10 mmol, 0.5 mL) was refluxed at 85°C for 8 h. The mixture was cooled and evaporated in vacuum. The residue was recrystallized from absolute EtOH.

*(S)-N-[1-(4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)ethyl]-3-chlorobenzamide (**5a-1**)* White solid; yield: 85%; mp: 218.2-218.7°C; IR (KBr) 3,275, 1,639, 1,535, 1,123, 895, 779, 756 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 13.610(s, 1H, triazole NH), 9.008(d, *J* = 7.2 Hz, 1H, NH), 7.944(t, *J* = 1.8 Hz, 1H, ArH), 7.838(d, *J* = 2.4Hz, 1H, ArH), 7.621(m, 1H, ArH), 7.509(t, *J* = 7.8 Hz, 1H, ArH), 5.593(s, 2H, NH_2), 5.262(t, *J* = 7.2 Hz, 1H, CH), 1.517 (d, *J* = 7.2 Hz, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 295.64.

*(S)-N-[1-(4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)ethyl]-4-methoxybenzamide (**5m**)* White solid; yield: 77%; mp: 239.6-239.9°C; IR (KBr) 3,279, 3,132, 3,044, 2,955, 1,632, 1,535, 1,177, 845 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 13.572(s, 1H, triazole NH), 8.699(d, *J* = 7.2 Hz, 1H, NH), 7.869(d, *J* = 8.4 Hz, 1H, NH), 6.991(d, *J* = 9.0 Hz, 2H, ArH), 5.589(s, 2H, NH_2), 5.251(m, 1H, CH), 3.808(s, 3H, CH_3), 1.506(d, *J* = 7.2 Hz, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 291.64.

7. General procedure for compounds 5a-2, 5g, 5i, 5l-1, 5l-2, 5a-3, 5f, 5j

To a stirred solution containing the compounds **4a**, **4f**, **4g**, **4i**, **4j**, **4l** (5 mmol) and KOH (5 mmol, 0.28 g) in 50% aqueous ethanol (20 mL), a solution of iodomethane (10 mmol, 0.6223 mL), iodoethane (10 mmol, 0.8 mL) or 3-chlorobenzyl chloride (10 mmol, 1.268 mL) in ethanol (5 mL) was added dropwise. After reacting at room temperature for 8 h, the solid product was collected by filtration, washed with water and recrystallized from EtOH.

*(S)-N-[1-(5-(methylthio)-1,3,4-oxadiazol-2-yl)ethyl]-3-chlorobenzamide (**5a-2**)* White solid; yield: 92%; mp: 120.0-120.2°C; IR (KBr) 3,248, 1,643, 1,539, 1,119, 775, 710, 683 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 9.223(d, J=7.8Hz, 1H, NH), 7.934(t, J=2.1Hz, 1H, ArH), 7.845(d, J=7.8Hz, 1H, ArH), 7.649(m, 1H, ArH), 7.536(t, J=7.8Hz, 1H, ArH), 5.378(m, 1H, CH), 2.691(s, 3H, CH_3), 1.586(d, J =7.2Hz, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 295.36.

*(S)-N-[1-(5-(methylthio)-1,3,4-oxadiazol-2-yl)-2-phenylethyl]-4-chlorobenzamide (**5g**)* White solid; yield: 82%; mp: 146.0-146.2°C; IR (KBr) 3,437, 3,287, 1,643, 1,535, 1,481, 1,323, 1,153, 1,092, 841, 702 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 9.249(d, *J* = 7.8 Hz, 1H, NH), 7.809(m, 2H, ArH), 7.55(m, 2H, ArH), 7.31(d, *J* = 7.2 Hz, 2H, ArH), 7.269(t, *J* = 7.8 Hz, 2H, ArH), 7.195(t, *J* = 7.2 Hz, 1H, ArH), 5.51(m, 1H, CH), 3.299(m, 2H, CH_2), 2.679(s, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 371.55.

*(S)-N-[2-methyl-1-(5-methylthio-1,3,4-oxadiazol-2-yl)propyl]-4-bromobenzamide (**5i**)* White solid; yield: 91%; mp: 161.0-161.7°C; IR (KBr) 3,290, 2,962, 2,874, 2,349, 1,655, 1,531, 1,477, 1,173, 845 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.107(d, *J* = 8.4 Hz, 1H, NH), 7.83(dd, J_1 = 1.8 Hz, J_2 = 4.8 Hz, 2H, ArH), 7.709(dd, J_1 = 1.8 Hz, J_2 = 4.8 Hz, 2H, ArH), 5.069(t, *J* = 8.4 Hz, 1H, CH), 2.697(s, 3H, CH_3), 2.335(m, 1H, CH), 1.019(d, *J* = 6.6 Hz, 3H, CH_3), 0.901(d, *J* = 6.6 Hz, 3H, CH_3); ESI-MS: m/z [M − H]$^-$ 369.37.

*(S)-N-[2-methyl-1-(5-methylthio-1,3,4-oxadiazol-2-yl)propyl]-4-methylbenzamide (**5l-1**)* White solid; yield: 84%; mp: 122.8-122.8°C; IR (KBr) 3,294, 3,020, 2,955, 2,870, 1,651, 1,531, 1,477, 1,169, 841 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 8.925(d, *J* = 7.8 Hz, 1H, NH), 7.798(d, *J* =

8.4 Hz, 2H, ArH), 5.072(t, *J* = 8.4 Hz, 1H, CH), 2.699(s, 3H, CH_3), 2.360(s, 4H, CH, CH_3), 1.023(d, *J* = 6.6 Hz, 3H, CH_3), 0.902(d, *J* = 7.2 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 303.48.

*(S)-N-[1-(5-ethylthio-1,3,4-oxadiazol-2-yl)-2-methylpropyl]-4-methylbenzamide (**5l-2**)* White solid; yield: 82%; mp: 111.5-111.7°C; IR (KBr) 3,279, 2,966, 2,928, 1,632, 1,543, 1,474, 1,142, 841 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 8.928(d, *J* = 8.4 Hz, 1H, NH), 7.795(d, *J* = 8.4 Hz, 2H, ArH), 7.289(d, *J* = 7.8 Hz, 2H, ArH), 5.076(t, *J* = 8.4 Hz, 1H, CH), 3.233(q, *J* = 7.2 Hz, 2H, CH_2), 2.346(m, 4H, CH, CH_3), 1.365(t, *J* = 7.2 Hz, 3H, CH_3), 1.023(d, *J* = 7.2 Hz, 3H, CH_3), 0.903(d, *J* = 6.6 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 317.85.

*(S)-N-[1-(5-(3-chlorobenzylthio)-1,3,4-oxadiazol-2-yl)ethyl]-3-chlorobenzamide (**5a-3**)* White solid; yield: 90%; mp: 146.0-148.0°C; IR (KBr) 3,240, 3,051, 2,993, 2,936, 1,643, 1,543, 1,331, 1,123, 860, 799, 714, 679 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 9.111(d, *J* = 7.8 Hz, 1H, NH), 7.934(t, J = 1.8 Hz, 1H, ArH), 7.846(d, *J* = 7.8 Hz, 1H, ArH), 7.65(m, 1H, ArH), 7.541(m, 2H, ArH), 7.389(m, 1H, ArH), 7.316(m, 2H, ArH), 5.384(m, 1H, CH), 4.483(s, 2H, CH_2), 1.577(d, *J* = 7.2 Hz, 3H, CH_3); ESI-MS: m/z $[M - H]^-$ 405.50.

*(S)-N-[1-(5-(3-chlorobenzylthio)-1,3,4-oxadiazol-2-yl)-3-methylbutyl]-4-chlorobenzamide (**5f**)* White solid; yield: 88%; mp: 99.0-99.1°C; IR (KBr) 3,445, 3,275, 3,067, 2,955, 2,924, 2,874, 1,643, 1,473, 1,173, 799, 729, 683 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.1(d, *J* = 7.8 Hz, 1H, NH), 7.9(dd, J_1 = 1.8 Hz, J_2 = 4.8 Hz, 2H, ArH), 7.572(d, *J* = 7.8 Hz, 2H, ArH), 7.512(d, *J* = 1.8 Hz, 1H, ArH), 7.377(m, 1H, ArH), 7.314(m, 2H, ArH), 5.35(m, 1H, CH), 4.475(s, 2H, CH_2), 1.923(m, 1H, CH), 1.772(m, 1H, CH_2), 1.637(m, 1H, CH_2), 0.917(dd, J_1 = 6.6 Hz, J_2 = 12 Hz, 6H, 2 × CH_3); ESI-MS: m/z $[M - H]^-$ 447.61.

*(S)-N-[1-(5-(3-chlorobenzylthio)-1,3,4-oxadiazol-2-yl)-3-methylbutyl]-4-bromobenzamide (**5j**)* White solid; yield: 88%; mp: 129.4-129.6°C; IR (KBr) 3,329, 2,962, 2,874, 1,651, 1,528, 1,481, 1,142, 845 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.118(d, *J* = 7.8 Hz, 1H, NH), 7.831(d, *J* = 8.4 Hz, 2H, ArH), 7.715(d, *J* = 8.4 Hz, 2H, ArH), 7.518(s, 1H, ArH), 7.382(d, *J* = 7.2 Hz, 1H, ArH), 7.315(m, 2ArH), 5.353(m, 1H, CH), 4.478(s, 2H, CH_2), 1.929(m, 1H, CH), 1.772(m, 1H, CH_2), 1.645(m, 1H, CH_2), 0.918(dd, J_1 = 6.0 Hz, J_2 = 12 Hz, 6H, 2 × CH_3); ESI-MS: m/z $[M - H]^-$ 493.52.

8. General procedure for compounds 5a-4, 5l-3

The compounds **4a**, **4l** (5 mmol), dissolved in NaOH solution (10%, 10 mL), was added dropwise to a solution of chloroacetic acid (10 mmol, 0.945 g), which was previously neutrallized with saturated Na_2CO_3 solution(10 mL). The mixture was stirred for 12 h at room temperature, then acidified by dilute hydrochloric acid (1 mol/L). The precipitate was filtered, washed with water, dried and recrystallized from MeOH/H_2O.

*(S)-N-[1-(5-carboxymethylthio-1,3,4-oxadiazol-2-yl)-ethyl]-3-chlorobenzamide (**5a-4**)* White solid; yield: 78%; mp: 164.5-166.6°C; IR (KBr) 3,256, 3,113, 2,997, 2,831, 1,778, 1,647, 1,539, 1,335, 706 cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 12.269(br, 1H, OH), 9.077(d, *J* = 7.8 Hz, 1H, NH), 7.930(t, *J* = 1.8 Hz, 1H, ArH), 7.843(d, *J* = 7.8 Hz, 1H, ArH), 7.645(m, 1H, ArH), 7.535(t, *J* = 7.8 Hz, 1H, ArH), 5.088(m, 1H, CH), 1.475(d, *J* = 7.2 Hz, 3H, CH_3); ESI-MS: m/z $[M + H]^+$ 340.75.

*(S)-N-[1-(5-carboxymethylthio-1,3,4-oxadiazol-2-yl)-2-methylpropyl]-4-methylbenzamide (**5l-3**)* White solid; yield: 76%; mp: 160.0-162.0°C; IR (KBr) 3,287, 3,117, 3,032, 2,966, 2,936, 2,827, 1,774, 1,639, 1,539, 1,381, 1,161, 930, 833cm^{-1}; ^{1}H-NMR(600 MHz, DMSO-d_6) δ 12.291(br, 1H, OH), 8.792(d, *J* = 8.4 Hz, 1H, NH), 7.8(d, *J* = 8.4 Hz, 2H, ArH), 7.289(d, *J* = 8.4 Hz, 2H, ArH), 4.731(t, *J* = 8.7 Hz, 1H, CH), 2.361(s, 3H, CH_3), 2.245(m, 1H, CH), 0.96(dd, J_1 = 6.6 Hz, J_2 = 27 Hz, 6H, 2 × CH_3); ESI-MS: m/z $[M - H]^-$ 347.56.