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Original Research Article

Synthesis of some new propanamide derivatives bearing 4-piperidinyl-1,3,4-oxadiazole, and their evaluation as promising anticancer agents

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Abstract

Purpose: *To sequentially synthesize piperidine-4-carboxylic acid ethyl ester-appended 1,3,4-oxadiazole hybrids and to evaluate them as anticancer agents.*
Methods: *Ethyl 1-[(4-methylphenyl)sulfonyl]-4-piperidinecarboxylate (**1**) was synthesized from 4-methylbenzenesulfonylchloride (a) and ethyl 4-piperidinecarboxylate (b). Compound (**1**) was converted into ethyl 1-[(4-methylphenyl)sulfonyl]-4-piperidine carbohydrazides (**2**) and 5-{1-[(4-methylphenyl)sulfonyl]-4-piperidinyl}-1,3,4-oxadiazole-2-thiol (**3**) respectively. A variety of aryl amine (**4a-l**) were treated with 2-bromopropionylbromide to synthesize an array of propanamide (**5a-l**). Finally, 5-{1-[(4-methylphenyl)sulfonyl]-4-piperidinyl}-1,3,4-oxadiazole-2-thiol (**3**) and propanamides (**5a-l**) were reacted to synthesize target compounds (**6a-l**). Purity compounds **6a-l** was confirmed by spectroscopic techniques like (^{1}H-NMR), (^{13}C-NMR) and EI-MS. To determine their anticancer potential, the change in absorbance of mixture and cell line before and after incubation was determined.*
Results: *All the compounds **6a-l** were successfully synthesized in 73-85 % yield. Compounds **6h**, **6j** and **6e** have low IC_{50} (±SD) values of 20.12 ± 6.20, 10.84 ± 4.2 and 24.57 ± 1.62 µM to act as strong anticancer agents relative to doxorubicin (0.92 ± 0.1 µM) used as a reference.*
Conclusion: *The synthesized propanamide derivatives bearing 4-piperidinyl-1,3,4-oxadiazole are potential anticancer agents, but further studies, especially in vivo, are required to ascertain their therapeutic usefulness.*

Keywords: *Ethyl isonipecotate, Propanamides, 1,3,4-Oxadiazole, Anti-cancer activity*

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INTRODUCTION

Heterocyclic compounds have gained great interest by chemists in the quest for new significant therapeutic and pharmacological agents [1]. Nitrogen and oxygen based azoles are well known and identified as bioactive agents in field of pharmacy. Azoles are having great

extent of pharmacological applications because of heteroatoms providing active sites for inter action. Oxadizole and triazole are members of the azole family having wide range of biological applications.1,3,4-oxadiazole derivatives are reported as strong antibacterial [2], anti-depressant [3], anti-proliferate [4], anti-HIV [5], anti-inflammatory [6], anti-mitotic [7], hypoglycemic [8], anti-cancer [9], herbicidal [10], muscle rel*ax*ant [11], anticonvulsant [12], insecticidal [13], anti-fungal [14] and plant growth regulator agents [15]. The current work is the continuity of our efforts to add some new compounds in the field of synthetic chemistry having various modifications in the structural frame work. In the same year we have reported acetamides derivatives based on azinane bearing 1,3,4-oxadiazole as active anti-bacterial agents [16]. The current study was to evaluate the effect of propanamide bearing three carbons in place of acetamides bearing two carbons. The synthesis of targeted compounds **6a-l** is significant, as it has different heterocyclic systems submerged in one unit in order to enhance their biological applications, in the evaluation of anticancer activity.

EXPERIMENTAL

General

The progress and completion of reactions was checked by TLC on pre-coated silica gel plates by UV_{254} lamp along with ethyl acetate and n-hexane (different polarity solvent system). Griffin and George apparatus was employed to calculate the melting points of compounds **6a-l** [16]. The presence of various functional groups in compounds **6a-l** were identified by IR technique using Jasco-320-A spectrophotometer. With the help of Bruker spectrometers, ^{1}H-NMR (at 600 MHz in MeOD) and ^{13}C-NMR (at 400 MHz in MeOD) spectra were recorded. Mass spectral analysis was attained by JMS-HX-110 spectrometer.

Synthesis of ethyl 1-[(4-methylphenyl)sulfonyl]-4-piperidinecarboxylate (1)

Ethyl 4-piperidinecarboxylate (**b**: 0.04 mol) was added into round bottom flask having 20 ml distilled water. The mixture was agitated for 10 minutes followed by the addition of 4-methylbenzenesulfonylchloride (**a**: 0.04 mol) gradually. A volume of 5 % Na_2CO_3 solution was used to maintain the pH of solution up to 9-10 until the completion of reaction. As the reaction completed, the product in the form of precipitates was attained by addition of distilled water.

Synthesis of ethyl 1-[(4-methylphenyl)sulfonyl]-4-piperidine carbohydrazide (2)

Compound **1** (0.019 mol) was dissolved in 20 ml of methanol and refluxed with hydrazine hydrate for 4 hours. TLC was performed throughout the reaction to monitor the reaction completion. At the completion of reaction, the mixture was cooled at room temperature. The precipitates of target compound **2** were acquired by the water addition followed by the filtration and washing.

Synthesis of 5-{1-[(4-methylphenyl)sulfonyl]-4-piperidinyl}-1,3,4-oxadiazole-2-thiol (3)

Compound **2** (0.02 mol) and KOH (0.02 mol) were dissolved in methanol and refluxed with carbon disulphide (0.02mol) for 5 hours. TLC was performed after every to check the progress of reaction. At the completion of reaction, distt. H_2O and dil. HCl (to adjust pH to 4–5) were used to acquire the precipitates of titled product. Precipitates were filtered, washed and dried at room temperature.

General procedure for synthesis of *N*-aryl-2-bromopropanamides (5a–l)

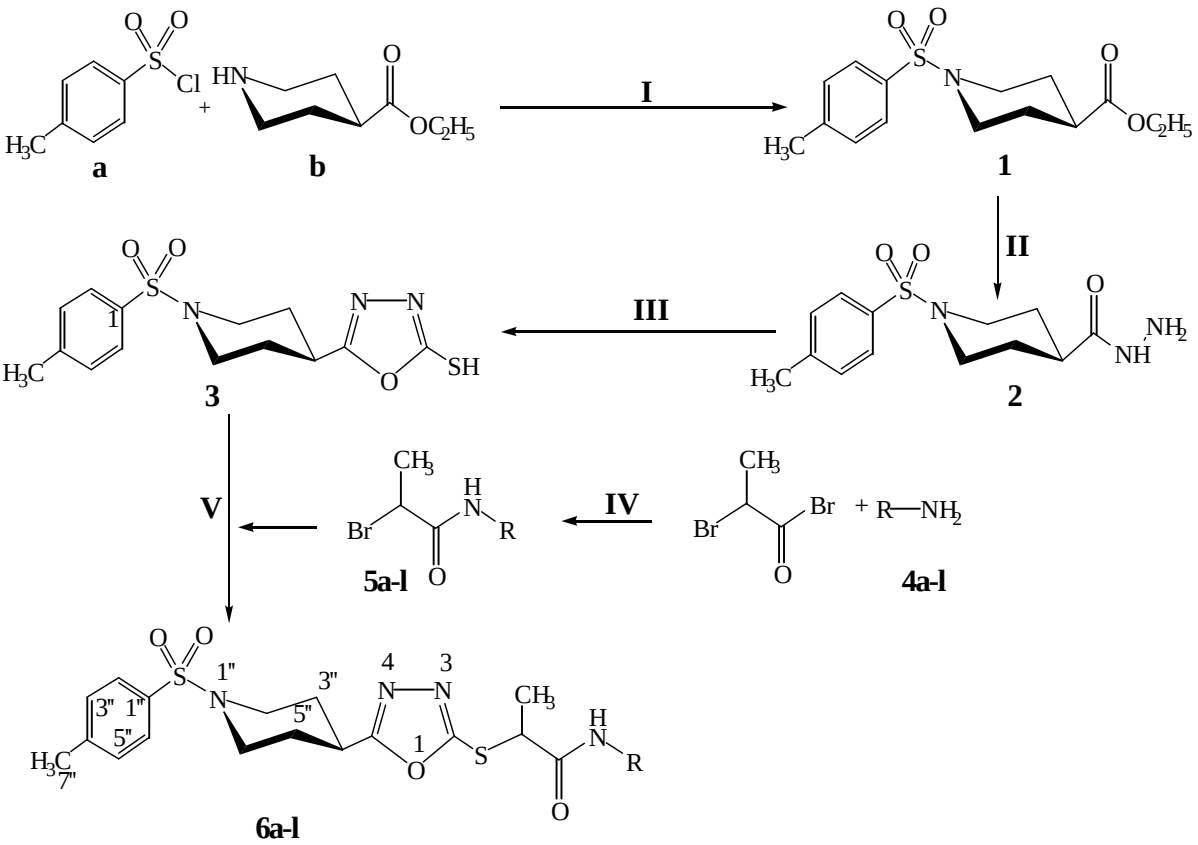
A mixture of 2-bromopropionyl bromide and aryl amines (**4a-l**; 0.02 mol) was stirred for 1 hour in aqueous medium by following reported method [17]. The pH was adjusted to 9-10 by addition of 10% Na_2CO_3 solution. The reaction mixture was stirred vigorously to attain the precipitates. The obtained product was filtered and dried.

General procedure for the synthesis of different *N*-(substituted)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6a-l)

Compound **4** (0.006 mol) was stirred with lithium hydride in the presence of DMF for 30 minutes *N*-substituted propanamides **(5a-l)** were subsequently added. The reaction mixture stirred for 4 hours at the room temperature. Reaction progress was monitored by TLC. Following the addition of cold distilled water, the target compounds **(6a-l)** were obtained in the form of precipitates that were filtered, washed and dried.

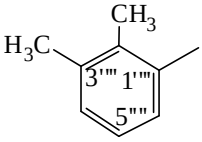
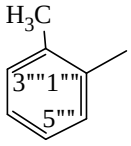
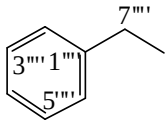
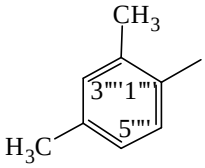
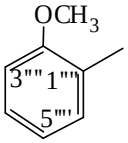
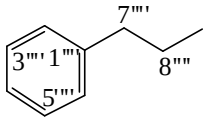
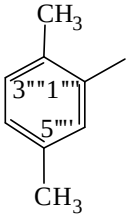
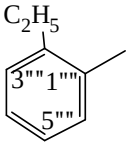
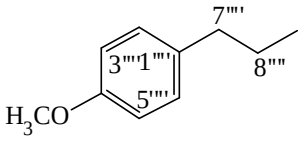
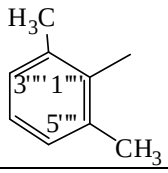
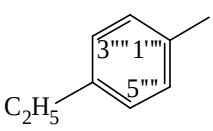
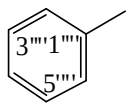
Anticancer activity assay

The anticancer activity (*in vitro*) was evaluated using the reported method for MTT assay [18]. For the anticancer potential, the change in absorbance of mixture and cell line before and after incubation was determined.



Scheme 1: Protocol for the synthesis of *N*-(substituted)-2-[[5-[1-[(4-methylphenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide. **Reagents & conditions:** (**I**) 10 % sodium carbonate solution, water. (**II**) Hydrazine, Ethanol. (**III**) Carbon disulphide, potassium hydroxide, ethanol. (**IV**) 10 % sodium carbonate solution, water, (**V**) Dimethyl formamide, lithium hydride.

Table 1: Different *N*-substituted alkyl/aryl/aralkyl groups

Comp	R	Comp	R	Comp	R
6a		**6e**		**6i**	
6b		**6f**		**6j**	
6c		**6g**		**6k**	
6d		**6h**		**6l**	

Statistical analysis

Calculations were performed in triplicate and statistical analysis was processed by Microsoft Excel 2000. The results are displayed as mean ± SEM with 85% CL. The results for 50% inhibitory concentration (IC_{50}) were obtained at different dilutions (µg/well) and analyzed by EZ-Fit software (Perrella Scientific Inc, Amherst, USA).

RESULTS

The methodology of all *N*-substituted propanamide derivatives of 1,3,4-oxadiazole have been summarized in scheme-1. The MTT assay is at best used for initial screening and the activities were from moderate to high.

***N*-(2,3-dimethylphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6a)**

Pink amorphous solid; Yield: 80 %; M.P.: 95-97 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass.: 514.66 g/mol; IR (KBr, $\nu_{max,}$ cm^{-1}): 3448, 3040, 1605, 1567, 1325, 616; ^{1}H-NMR: 7.70 (d, *J* = 8.1 Hz, 2H, H-2'' & H-6''), 7.55 (d, *J* = 7.8 Hz, 1H, H-6'''), 7.46 (d, *J* = 9.10 Hz, 2H, H-3'' & H-5''), 7.21-8.01 (m, 1H, H-5'''), 7.11-8.92 (m, 1H, H-4'''), 4.67-4.61 (m, 1H, CH-2''''), 3.74-2.55 (m, 4H, H-2' & H-6'), 2.90-2.80 (m, 1H, H-4'), , 2.46 (s, 3H, CH_3-7''), 2.37 (s, 3H, CH_3-7'''), 2.32 (s, 3H, CH_3-8'''), 2.23-1.78 (m, 4H, H-3' & H-5'), 1.73-1.70 (m, 3H, CH_3-3''''); ^{13}C-NMR: 170.56 (C-1''''), 170.04 (C-5), 168.19 (C-2), 146.10 (C-4''), 145.39 (C-3'''), 139.07 (C-1''), 134.51 (C-1'''), 129.90 (C-3'' & C-5''), 128.97 (C-2'' & C-6''), 129.06 (C-2'''), 128.16 (C-4'''), 126.96 (C-5'''), 122.32 (C-6'''), 46.72 (C-2' & C-6'), 41.43 (C-2''''), 33.46 (C-4'), 29.64 (C-3' & C-5'), 21.48 (C-7''), 20.57 (C-8'''), 19.42 (C-3''''), 13.93 (C-7'''): EIMS (m/z): 514 $[M]^+$ (<1 %), 266 (1.5 %), 155 (29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(2,4-dimethylphenyl)-2-[[5-[1-[(4-methylphenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6b)**

White amorphous solid; Yield: 82 %; M.P.: 87-89 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass: 514.66 g/mol; IR (KBr, υ_{max}, cm^{-1}): 3447, 3042, 1604, 1566, 1323, 617; ^{1}H-NMR: 7.70 (d, *J* = 8.3 Hz, 2H, H-2'' & H-6''), 7.46 (d, *J* = 7.9 Hz, 2H, H-3'' & H-5''), 7.40 (d, *J* = 8.1 Hz, 1H, H-6'''), 7.065-7.062 (br.s, 1H, H-5'''), 7.03-7.01 (m, 1H, H-3'''), 4.60 (s, 1H, H-2''''), 3.74-2.80 (m, 4H, H-2' & H-6'), 2.91-2.85 (m, 1H, H-4'), 2.46 (s, 3H, H-7''), 2.30 (s, 3H, H-7'''), 2.25 (s, 3H, H-8'''), 2.16-1.85 (m, 4H, H-3' & H-5'), 1.50-1.52 (m, 3H, H-3''''); ^{13}C-NMR: 170.5 (C-1''''), 163.6 (C-5), 163.4 (C-2), 145.4 (C-4''), 136.1 (C-1''), 132.4 (C-1'''), 132.0 (C-2'''), 131.9 (C-4'''), 131.0 (C-3'' & C-5''), 129.2 (C-2'' - C-6''), 127.98 (C-5'''), 127.9 (C-3'''),123.8 (C-6'''), 46.4 (C-2' & C-6'), 42.6 (C-2''''), 33.4 (C-4'), 29.7 (C-3' & C-5'), 21.4 (C-7''), 20.9 (C-8'''), 19.4 (C-3''''), 17.8 (C-7'''); EIMS: m/z: 514 $[M]^+$ (<1%), 266 (1.5 %), 155 $[C_7H_7O_2S]^+$, (29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(2,5-dimethylphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6c)**

Light brown amorphous solid; Yield: 85 %; M.P.: 150-152 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass: 514.66 g/mol. IR (KBr, υ_{max}, cm^{-1}): 3446, 3043, 1602, 1565, 1323, 614; ^{1}H-NMR: 7.70 (d, *J* = 9.2 Hz, 2H, H-2'' & H-6''), 7.42 (d, *J* = 9.5 Hz, 2H, H-3'' & H-5''), 7.42 (s, 1H, H-6'''), 7.11 (d, *J* = 8.8 Hz, 1H, H-3'''), 6.93 (d, *J* = 8.5 Hz, 1H, H-4'''), 4.60 (br.s, 1H, CH-2''''), 3.74-2.58 (m, 4H, H-2' & H-6'), 2.91-2.86 (m, 1H, H-4'), 2.50 (s, 3H, H-7''), 2.31 (s, 3H, H-7'''), 2.25 (s, 3H, H-8'''), 2.17-1.87 (m, 4H, H-3' & H-5'), 1.50 (br.s, 3H, H-3''''); ^{13}C-NMR: 170.9 (C-1''''), 163.7 (C-5), 163.1 (C-2), 145.39 (C-4''), 138.2 (C-1''), 137.2 (C-1'''), 135.1 (C-5'''), 132.0 (C-2'''), 131.9 (C-3'' & C-5''), 129.8 (C-2'' & C-6''), 129.2 (C-4'''), 127.6 (C-3'''), 123.7 (C-6'''), 46.5 (C-2' & C-6'), 42.6 (C-2''''), 33.4 (C-4'),29.6 (C-3' & C-5'), 21.4 (C-8'''), 21.0 (C-7''), 19.4 (C-3''''), 17.4 (C-7'''); EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155(29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(2,6-dimethylphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6d)**

Light yellow amorphous solid; Yield: 80 %; M.P.: 160-162 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass: 514.66 g/mol. IR (KBr, υ_{max}, cm^{-1}): 3444, 3040, 1607, 1568, 1323, 613; ^{1}H-NMR: 8.06 (d, *J* = 8.0 Hz, 2H, H-2'' & H-6''), 8.02 (d, *J* = 9.0 Hz, 2H, H-3'' & H-5''), 7.04 (br.s, 3H, H-3''', H-4''' & H-5'''), 4.86 (br.s, 1H, H-2''''), 3.68-2.50 (m, 4H, H-2' & H-6'), 2.84-2.78 (m, 1H, H-4'), 2.43 (s, 3H, H-7''), 2.20 (s, 6H, H-7''' & H-8'''), 2.10-1.817 (m, 4H, H-3' & H-5'), 1.30 (br.s, 3H, H-3''''); ^{13}C-NMR: 172.1 (C-1''''), 170.1 (C-5), 168.2 (C-2),144.4 (C-4''), 138.3 (C-1'''), 137.2 (C-1''), 131.9 (C-2''' & C-6'''), 130.8 (C-3'' & C-5''), 129.3 (C-2'' & C-6''), 126.4 (C-3''' & C-5'''), 125.8 (C-4'''),42.6 (C-2''''), 42.9 (C-2' & C-6'), 32.9 (C-4'), 29.0 (C-3' & C-5'), 21.4 (C-7''), 19.4 (C-3''''), 17.4 (C-7''' - C-8'''); EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155(29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(2-methylphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6e)**

Light grey amorphous solid; Yield: 80 %; M.P.: 133-135 °C; Molecular formula: $C_{24}H_{28}N_4O_4S_2$; Molecular mass: 500.63 g/mol; IR (KBr, υ_{max}, cm^{-1}): 3446, 3041, 1601, 1564, 1322, 618: ^{1}H-NMR: 8.70 (d, *J* = 9.1 Hz, 2H, H-2'' & H-6''), 8.46 (d, *J* = 7.9 Hz, 2H, H-3'' & H-5''), 7.60 (d, *J* = 7.9 Hz, 1H, H-6'''), 7.24-7.19 (m, 2H, H-4''' & H-5'''), 7.09-7.06 (m, 1H, H-3'''), 4.62 (br.s, 1H, H-2''''), 3.74-2.58 (m, 4H, H-2' & H-6'), 2.89-2.85 (m, 1H, H-4'), 2.46 (s, 3H, H-7''), 2.30 (s, 3H, H-7'''), 2.17-1.86 (m, 4H, H-3' & H-5'), 1.60 (br.s, 3H, CH_3-3''''): ^{13}C-NMR: 170.6 (C-1''''), 163.7 (C-5), 163.0 (C-2), 145.4 (C-4''), 137.6 (C-1''), 134.5 (C-1'''), 131.8 (C-2'''), 131.1 (C-3'''), 130.8 (C-3'' & C-5''), 129.8 (C-2'' & C-6''), 128.7 (C-5'''), 125.9 (C-4'''), 123.1 (C-6'''), 46.5 (C-2' & C-6'), 42.6 (C-2''''), 33.4 (C-4'), 29.6 (C-3' & C-5'), 21.5 (C-7''), 19.4 (C-3''''), 17.9 (C-7'''); EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155(29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(2-methoxyphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6f)**

White amorphous ; Yield: 84 %; M.P.: 116-118 °C; Molecular formula: $C_{24}H_{28}N_4O_5S_2$; Molecular mass: 516.63 g/mol: IR (KBr, υ_{max}, cm^{-1}): 3449, 3041, 1602, 1562, 1321, 615: ^{1}H-NMR: 7.71 (d, *J* = 9.2 Hz, 2H, H-2'' & H-6''), 7.46 (d, *J* = 9.4 Hz, 2H, H-3'' & H-5''), 7.90 (dd, *J* = 6.3, 1.4 Hz, 1H, H-6'''), 7.05-7.04 (m, 1H, H-3'''), 7.03-7.01 (m, 1H, H-5'''), 6.98-6.95 (m, 1H, H-4'''), 4.60 (br.s, 1H, H-2''''), 3.92 (s, 3H, H-7'''), 3.81-2.60 (m, 4H, H-2' & H-6'), 2.92-2.87 (m, 1H, H-4'), 2.46(s, 3H, H-7''), 2.18-1.87 (m, 4H, H-3' & H-5'), 1.50 (s, 3H, H-3''''): ^{13}C-NMR: 163.5 (C-1''''), 162.18 (C-5), 150.1 (C-2), 148.3 (C-2'''), 145.2 (C-4''), 135.5 (C-1''), 131.9 (C-3'' & C-5''), 129.8 (C-2'' & C-6''), 128.6 (C-1'''), 124.5 (C-4'''), 121.8 (C-6'''), 119.6 (C-5'''), 111.8 (C-3'''), 56.3 (C-7'''),46.8 (C-2' & C-6'),42.6 (C-2''''), 33.4 (C-4'), 29.6 (C-3' & C-5'), 21.0 (C-7''), 19.3 (C-3''''): EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155(29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(2-ethylphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6j)**

Yellowish white amorphous solid; Yield: 88 %; M.P.: 150-152 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass: 514.66 g/mol: IR (KBr, υ_{max}, cm^{-1}): 3446, 3049, 1601, 1566, 1325, 611: ^{1}H-NMR: 7.67 (d, *J* = 9.4 Hz, 2H, H-2'' & H-6''), 7.53 (d, *J* = 8.0 Hz, 1H, H-6'''), 7.42 (d, *J* = 8.1 Hz, 2H, H-3'' & H-5''), 7.24 (d, *J* = 7.6 Hz, 1H, H-3'''), 7.21 (dt, *J* = 6.3, 2.1 Hz, 1H, H-5'''), 7.13 (dt, *J* = 7.4 & 2.2 Hz, 1H, H-4'''), 4.60 (br.s, 1H, CH-2''''), 3.69-2.81 (m, 4H, H-2' & H-6'), 2.87-2.81 (m, 1H, H-4'), 2.68 (m, 2H, H-7'''), 2.43(s, 3H, CH_3-7''), 2.13-1.84 (m, 4H, H-3' & H-5'), 1.99-1.61 (m, 3H, H-8'''), 1.27 (br.s, 3H, CH_3-3''''): ^{13}C-NMR: 170.5 (C-1''''), 170.1 (C-5), 168.1 (C-2), 145.2 (C-1'''), 144.7 (C-4''), 137.6 (C-1''), 138.8 (C-3'' & C-5''), 129.3 (C-2'' & C-6''), 126.5 (C-2'''), 127.7 (C-3'''), 127.4 (C-5'''), 124.5 (C-4'''), 118.1 (C-6'''), 42.6 (C-2''''), 42.9 (C-2' & C-6'), 32.7 (C-4'), 29.0 (C-3' & C-5'), 23.5 (C-7'''), 21.4 (C-7''), 19.4 (C-3''''), 12.9 (C-8'''): EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155 (29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(4-ethylphenyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6h)**

Dark yellow amorphous solid; Yield: 80 %; M.P.: 155-157 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass: 514.66 g/mol: IR (KBr, umax, cm-1): 3445, 3042, 1604, 1568, 1321, 610: ^{1}H-NMR: 7.68 (d, *J* = 8.1 Hz, 2H, H-2'' & H-6''), 7.43 (d, *J* = 9.0 Hz, 2H, H-3'' & H-5''), 7.35 (d, *J* = 7.9 Hz, 2H, H-2''' & H-6'''), 7.15 (d, *J* = 9.1 Hz, 2H, H-3''' & H-5'''), 4.51 (br.s, 1H, CH-2''''), 3.71-2.53 (m, 4H, H-2' & H-6'), 2.86-2.85 (m, 1H, H-4'), 2.43 (s, 3H, H-7''), 2.14-1.85 (m, 4H, H-3' & H-5'), 1.51-1.49 (m, 2H, H-7'''), 1.49-1.46 (m, 3H, H-8'''), 1.00-1.01 (m, 3H, H-3''''): ^{13}C-NMR: 171.5 (C-1''''), 172.0 (C-5), 169.0 (C-2), 144.7 (C-4''), 143.9 (C-4'''), 142.0 (C-1'''), 136.7 (C-1''), 138.8 (C-3'' & C-5''), 128.3 (C-2'' & C-6''), 128.5 (C-3''' & C-5'''), 118.9 (C-2''' & C-6'''), 42.6 (C-2''''), 42.9 (C-2' & C-6'),32.9 (C-4'), 29.1 (C-3' & C-5'), 28.0 (C-7'''), 21.4 (C-7''), 19.4 (C-3''''), 15.8 (C-8'''): EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155 (29.1 %), 91 (18.1 %), 82 (23.7 %).

***N*-(phenylmethyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6i)**

Yellowish white amorphous solid; Yield: 73 %; M.P.: 60-63 °C; Molecular formula: $C_{24}H_{28}N_4O_4S_2$; Molecular mass: 500.63 g/mol: IR (KBr, υ_{max}, cm^{-1}): 3444, 3046, 1602, 1565, 1329, 612: ^{1}H-NMR: 7.70 (d, *J* = 8.7 Hz, 2H, H-2'' & H-6''), 7.45 (d, *J* = 9.1 Hz, 2H, H-3'' & H-5''), 7.31-7.26 (m, 5H, H-2''' to H-6'''), 4.33-4.29 (m, 1H, H-2''''), 3.78-2.36 (m, 4H, H-2' & H-6'), 2.91-2.80 (m, 1H, H-4'), 2.55 (s, 2H, H-7'''), 2.48 (s, 3H, H-7''), 2.33-1.86 (m, 4H, H-3' & H-5'), 1.60-1.58 (s, 3H, H-3''''): ^{13}C-NMR: 171.0 (C-1''''), 170.0 (C-5), 168.9 (C-2), 144.6 (C-4''), 137.9 (C-1'''), 136.6 (C-1''), 131.8 (C-3'' & C-5''), 128.6 (C-3''' & C-5'''), 128.3 (C-2'' & C-6''), 127.7 (C-2''' & C-6'''), 127.4

(C-4'''), 44.2 (C-7'''), 42.9 (C-2' & C-6'), 42.2 (C-2''''), 32.9 (C-4'), 29.0 (C-3' & C-5'), 21.5 (C-7''), 19.3 (C-3''''): EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155(29.1 %), 91 (18.1 %), 82 (23.7 %).

_N_-(2-phenylethyl)-2-[[5-[1-[(4-methyl phenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6j)

Light brown amorphous solid; Yield: 78 %; M.P.: 65-67 °C; Molecular formula: $C_{25}H_{30}N_4O_4S_2$; Molecular mass: 514.66 g/mol: IR (KBr, υ_{max}, cm^{-1}): 3440, 3047, 1606, 1566, 1329, 611: ^{1}H-NMR: 7.70 (d, *J* = 9.2 Hz, 2H, H-2'' & H-6''), 7.46 (d, *J* = 9.0 Hz, 2H, H-3'' & H-5''), 7.27-7.09 (m, 5H, H-2''' to H-6'''), 4.13-4.06 (m, 1H, H-2''''), 4.05-3.95 (m, 2H, H_2-8'''), 3.79-2.45 (m, 4H, H-2' & H-6'), 3.05-3.00 (m, 2H, H-7'''), 2.98-2.94 (m, 1H, H- 4'), 2.46(s, 3H, H-7''), 2.35-1.1.84 (m, 4H, H-3' & H-5'), 1.44 (d, *J* = 7.4 Hz, 3H, H-3''''): ^{13}C-NMR: 171.3 (C-1''''), 170.0 (C-5),168.9 (C-2), 144.8 (C-4''),136.6 (C-1''), 133.8 (C-1'''), 131.8 (C-3'' & C-5''), 128.7 (C-2''' & C-6'''), 128.6 (C-3''' & C-5'''), 128.3 (C-2'' & C-6''), 127.9 (C-4'''), 42.9 (C-2' & C-6'), 41.7 (C-2''''), 41.5 (C-8'''), 35.8 (C-7'''), 32.9 (C-4'), 29.0 (C-3' & C-5'), 21.4 (C-7''), 19.22 (C-3''''): EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155 (29.1 %), 91(18.1 %), 82 (23.7 %).

_N_-[2-(4-methoxyphenyl)ethyl]-2-[[5-[1-[(4-methylphenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6k)

White amorphous solid; Yield: 89 %; M.P.: 92-94 °C; Molecular formula: $C_{26}H_{32}N_4O_5S_2$; Molecular mass: 544.68 g/mol: IR (KBr, υ_{max}, cm^{-1}): 3447, 3042, 1606, 1566, 1328, 614: ^{1}H-NMR: 7.70 (d, *J* = 9.3 Hz, 2H, H-2'' & H-6''), 7.46 (d, *J* = 7.9 Hz, 2H, H-3'' & H-5''), 7.15 (d, *J* = 8.5 Hz, 2H, H-2''' & H-6'''), 6.83 (d, *J* = 9.6 Hz, 2H, H-3''' & H-5'''), 3.98-3.91 (m, 1H, CH-2''''), 3.80-2.90 (m, 4H, H-2' & H-6'), 3.78 (s, 3H, O-$\underline{CH_3}$), 3.45-3.41 (m, 2H, H-8'''), 2.98-2.95 (m, 1H, H-4'), 2.46 (s, 3H, H-7''), 2.45-2.42 (m, 2H, H-7'''), 2.34-1.86 (m, 4H, H-3' & H-5'), 1.44 (s, 3H, CH_3-3''''): ^{13}C-NMR: 171.1 (C-1''''), 170.0 (C-5), 168.1 (C-2), 158.5 (C-4'''), 144.5 (C-4''), 136.4 (C-1''), 132.0 (C-3'' & C-5''), 130.6 (C-1'''), 130.0 (C-2''' & C-6'''), 129.3 (C-2'' & C-6''), 114.5 (C-3''' & C-5'''), 55.3 (C-7'''), 42.9 (C-2' & C-6'), 41.7 (C-2''''), 41.4 (C-8'''), 32.9 (C-4'),29.0 (C-3' & C-5'), 21.3 (C-7''), 19.3 (C-3''''): EIMS (*m/z*): 514 $[M]^+$ (<1%), 266 (1.5 %), 155 (29.1 %), 91 (18.1 %), 82 (23.7 %).

_N_-phenyl-2-[[5-[1-[(4-methylphenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide (6l)

Greyish white amorphous solid; Yield: 80 %; M,P.: 110-112 °C; Molecular formula: $C_{23}H_{26}N_4O_4S_2$; Molecular mass: 486.60 g/mol: IR (KBr, υ_{max}, cm^{-1}): 3444, 3043, 1604, 1563, 1322, 618: ^{1}H-NMR: 7.68 (d, *J* = 9.0 Hz, 2H, H-2'' & H-6''), 7.57 (d, *J* = 9.0 Hz, 2H, H-3'' & H-5''), 7.48-7.43 (m, 2H, H-2''' & H-6'''), 7.37-7.28 (m, 2H, H-3''' & H-5'''), 7.02-7.00 (m, 1H, H-4'''), 4.60 (s, 1H, CH-2''''), 3.69-2.53 (m, 4H, H-2' & H-6'), 2.88-2.85 (m, 1H, H-4'), 2.43 (s, 3H, H-7''), 2.22-2.16 (m, 2H, H_{eq}-3' & H_{eq}-5'), 1.92-1.83 (m, 2H, H_{ax}-3' & H_{ax}-5'), 1.55 (d, *J* = 4.0 Hz, 3H, CH_3-3''''): ^{13}C-NMR: 170.2 (C-5),169.1 (C-1''''), 168.7 (C-2), 144.7 (C-4''), 137.9 (C-1'''), 136.6 (C-1''), 130.8 (C-3'' & C-5''), 128.6 (C-3''' & C-5'''), 128.3 (C-2'' & C-6''), 127.7 (C-2''' & C-6'''), 127.40 (C-4'''), 42.99 (C-2' & C-6'),42.68 (C-2''''), 32.97 (C-4'), 29.06 (C-3' & C-5'), 21.45 (C-7''), 19.48 (C-3''''): EIMS (*m/z*): 486 $[M]^+$ (<1%), 266 (1.5 %), 155 (29.1 %), 91 (18.1 %), 82 (23.7 %).

Anticancer activity

The results are given as % inhibition and IC_{50} ± SD in Table 2.

DISCUSSION

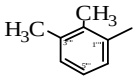
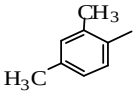
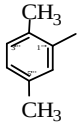
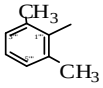
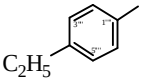
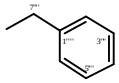
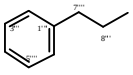
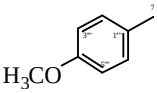
Compound **6c**, light brown amorphous solid, having melting point 150-152 °C with 85 % yield was selected randomly for single molecule discussion to explain the structural elucidation of synthesized compound. Deduction of molecular formula and molecular mass was done through EIMS spectrum and molecular ion peak at *m/z* = 514 respectively.

The presence of the main functional groups was confirmed by considering the absorption peaks presented by IR spectrum. Existence of N-H was verified by the peak at 3448cm^{-1}. The signals appeared at ν_{max} 3040 cm^{-1} and 1567 cm^{-1} confirmed the presence of C-H and C=C groups of aromatic ring. The presence of C=N stretching of oxadiazole ring, -SO_2 and C-S was verified by the peaks appeared at 1605, 1325 and 616 cm^{-1} respectively. Verification of numbers of carbon atoms were done by the help of ^{13}C-NMR spectrum. Four methyl groups and eight quaternary carbons were appeared at 21.08(C-7''), 19.48 (C-3''''), 17.48 (C-7'''), 21.48 (C-8'''), and170.9 (C-1''''), 163.7 (C-5), 163.1 (C-2), 145.39 (C-4''), 137.68 (C-1''), 137.25 (C-1'''), 134.52 (C-5'''), 131.69 (C-2''') respectively.

Aromatic carbons were verified by the peaks at 130.90 (C-3'' & C-5''), 128.86 (C-2'' & C-6''), 129.20 (C-4'''), 126.66 (C-3'''), 123.76 (C-6''') while the signal for carbons of piperidine ring and carbonyl group were appeared at 46.53 (C-2' & C-6'), 33.45 (C-4'), 29.65 (C-3' & C-5') and 170.8 (C-1'''').^{1}H-NMR spectrum presented doublets as

7.69 (d, *J* = 9.2 Hz, 2H, H-2'' & H-6''), 7.46 (d, *J* = 9.5 Hz, 2H, H-3'' & H-5'') for 4-methylbenzenesulfonyl group and one singlet and two doublets at of aromatic ring of amide group at 7.42 (s, 1H, H-6'''), 7.11 (d, *J* = 7.8 Hz, 1H, H-3'''), 6.93 (d, *J* = 8.5 Hz, 1H, H-4'''). The signals for four methyl groups were appeared at 2.50 (s, 3H, H-7''), 2.31 (s, 3H, H-7'''), 2.25 (s, 3H, H-8''') and 1.50 (br.s, 3H, H-3'''') and for piperidine ring at3.74-3.58 (m, 4H, H-2' & H-6'), 2.91-2.86 (m, 1H, H-4'), 2.17-1.87 (m, 4H, H-3' & H-5'), to confirm their presence.

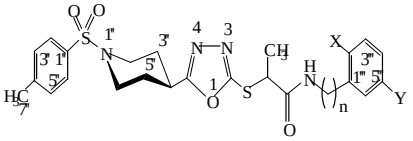
Table 2: Inhibition and IC_{50} data for anti-cancer activity of synthesized compounds

Compound	R	*Inhibition (%)*	*IC_{50} ± SD (µg/mL)*
6a		65.98	41.21±1.16
6b		50.82	50.00±1.23
6c		50.11	50.00±1.25
6d		42.11	-
6e		89.50	24.57±1.62
6f		55.91	50.00±1.28
6g		05.19	-
6h		74.19	20.12±6.20
6i		54.45	50.00±1.31
6j		86.53	10.84±4.2
6k		55.29	50.00±1.21
6l		54.34	-
Doxorubicin		***89.19***	***0.92±0.1***

The detailed spectral studies made us able to deduce the name of discussed compound **6c** as *N*-(2,5-dimethylphenyl)-2-[[5-[1-[(4-methylphenyl)sulfonyl]-4-piperidinyl]-1,3,4-oxadiazol-2-yl]thio]-propanamide. By following the same criteria, all the other synthesized compounds were interpreted according to their spectral data and available information.

All the compounds were screened for their anticancer activity and were found to possess moderate to high anticancer potential. Compounds **6h**, **6j** and **6e** having high anticancer potential, 74.19, 86.53 and 89.50 % measured in terms of % inhibition, compared to doxorubicin (89.19 %**)** used as reference. The ascending order of anticancer activity of compounds **6h**, **6j** and **6e** was due to the different substituted alkyl groups at aromatic ring of propanamides. Compound **6h**, **6j** both have aromatic ring of propanmides substituted with bulky ethyl groups which might be responsible for their lower anticancer activity when compared to compound **6e** having substitution of methyl group at *ortho* position of aromatic ring [19,20].

The substituted ethyl groups showed hydrophobic characteristics and reduced the interaction between substrate and compounds **6h**, **6j** while this character was less effective and less dominant for compound **6e.** Compound **6e** with impressive good inhibition requires further studies for possible development and addition to existing anticancer agents in the pharmaceutical industry. MTT assay is an initial screening and other assays for *in vitro* and *in vivo* will be required to support the anticancer potential. Figure-1 has explained the structural features and anticancer potential of the compounds **6e**, **6h** and **6j**.



6e: n=0, X=CH_3, Y=H: Methyl group at ortho position responsible for higher anticancer potential
6h: n=0, X=H, Y=C_2H_5: Ethyl, a bulky group at para position slightly reduces anticancer potential
6j: n=C_2H_4, X=H, Y=H: Ethyl, a bulky group at position 1'' extremely reduces anticancer potential

Figure-1: Structure of compounds **6e**, **6h** and **6j** showing best anticancer potential

CONCLUSION

All the compounds have been synthesized with good yield and their structures corroborated using spectroscopic techniques and mass spectroscopy). The compounds have moderate to high anticancer impact. Compounds **6h**, **6j** and **6e** have good potentials as anticancer agents. However among these three compounds, compound **6e** was outstanding and possess

even better anticancer potential when compared to the Doxorubicin as standard. Therefore compound **6e** should be further investigated.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Dabhi T, Shah V, Parikh A. Studies on 1,3,4-Oxadiazoles: Preparation and antimicrobial activity of 2-aercapto-/carboxy-methylthio-5-('-arylaminosulfophenyl)-1,3,4-oxadiazoles. Indian Journal of Pharmaceutical Sciences 1992; 54: 98-154.*
2. *Khalid H, Aziz-ur-Rehman, Abbasi MA, Malik A, Rasool S, Nafeesa K, Ahmad I. Afzal S. Synthesis, spectral analysis and anti-bacterial study of N-substituted derivatives of 2-(5-(1-(phenylsulfonyl)piperidin-4-yl)-1,3,4-Oxadiazol-2-ylthio)acetamide. J Saudi ChemSoc 2013; Doi:http://dx.doi.org/10.1016/j.jscs.2013.05.001.*
3. *Ameen S, Akhtar MS, Seo HK, Shin HS. An electrochemical sensing platform based on hollow mesoporous ZnO nanoglobules modified glassy carbon electrode: Selective detection of piperidine chemical. ChemEng J. 2015; 270: 564-571.*
4. *Omar FA, Mahfouz NM, Rahman MA. Design, synthesis and antiinflammatory activity of some 1,3,4-oxadiazole derivatives. Eur J Med Chem 1996; 31: 819-825.*
5. *Amir M, Shahani S. Synthesis and antiinflammatory activity of naphthylmethyl oxadiazoles, thiadiazoles and triazoles. Indian J Heterocycl Chem 1998; 8(2):107-109.*
6. *Hazarika J, Kataky J. Synthesis and anti-inflammatory activity of naphthylmethyloxadiazoles, thiadiazoles and riazoles. Indian J Heterocycl Chem 1988; 8: 83-84.*
7. *Yang CR, Zang Y, Jacob MR, Khan SI, Zhang YJ, Li XC. Antifungal activity of C-27 steroidal saponins. Antimicrob Agents Chemother 2006; 50: 1710-1714.*
8. *Varma R, Bajpai V, Kapil A. 4-Heterocyclic aminomethyl-2-(3-nitro-4-benzyloxy phenyl)-1,3,4-oxadiazole-5-thiones and their antileishmanial activity. Indian J Heterocycl Chem 1999; 8(4): 281-284.*
9. *Kaspady M, Narayanaswamy VK, Raju M, Rao GK. Synthesis, antibacterial activity of 2,4-Disubstituted oxazoles and thiazoles as bioisosteres. Lett Drug Des Discov 2009; 6: 21-28.*
10. *Vitnik VD, Vitnik ZJ. The spectroscopic (FT-IR, FT-Raman, ^{13}C, ^{1}H NMR and UV) and NBO analyses of 4-bromo-1-(ethoxycarbonyl)piperidine-4-carboxylic acid. Spectrochimica Acta Part A: Mole Biom Spec 2015; 138: 1-12.*
11. *Tan TM, Chen Y, Kong KH, Bai J, Li Y, Lim SG, Ang H, Lam Y. Synthesis and the biological evaluation of 2-benzenesulfonylalkyl-5-substituted-sulfanyl-[1,3,4]-oxadiazoles as potential anti-hepatitis B virus agents. Antivir Res 2006; 71: 7-14.*
12. *Koparır M, Çetin A, Cansız A. 5-Furan-2yl [1, 3, 4] oxadiazole-2-thiol, 5-furan-2yl-4H [1, 2, 4] triazole-3-thiol and their thiol-thione tautomerism. Molecules. 2005; 10(2):475-80.*
13. *Aziz-ur-Rehman, Fatima A, Abbas N, Abbasi MA, Khan KM, Ashraf M, Ahmad I, Ejaz SA. Synthesis, characterization and biological screening of 5-substituted-1,3,4-oxadiazole-2yl-N-(2-methoxy-5-chlorophenyl)-2-sulfanyl acetamide. Pak J Pharm Sci 2013; 26: 345-352.*
14. *Liu F, Luo XQ, Song BA, Bhadury PS, Yang S, Jin LH. Synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1, 3, 4-thiadiazole and 1, 3, 4-oxadiazole moiety. Bioorg Med Chem 2008;16(7):3632-3640.*
15. *Rajak H, Agarawal A, Parmar P, Thakur BS, Veerasamy R, Sharma PC, et al. 2, 5-Disubstituted-1, 3, 4-oxadiazoles/thiadiazole as surface recognition moiety: Design and synthesis of novel hydroxamic acid based histone deacetylase inhibitors. Bioorg med chem lett.2011; 21(19): 5735-5738.*
16. *Iqbal J, Aziz-ur-Rehman, Abbasi MA, Siddiqui SZ, Rasool S, Nafeesa K, Khan SG, Shah SAA. Synthesis of N-substituted acetamide derivatives of azinane-bearing 1,3,4 oxadiazole nucleus and screening for antibacterial activity. Trop J Pharm Res 2017; 16 (2): 429-437.*
17. *Aziz-ur-Rehman, Gul S, Abbasi MA, Nafeesa K, Siddiqa A, Khan KM, Shahid M, Subhani Z. N-Substituted Derivatives of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl-2-sulfanyl acetamide as Valuable Bioactive Compounds. J Chem Soc Pak 2014; 36(3): 503-511.*
18. *Shamsuzzaman, Siddiqui T, Alam MG, Dar AM. Synthesis, characterization and anticancer studies of new steroidal oxadiazole, pyrrole and pyrazole derivatives. J Saudi Chem Soc 2015; 19: 387-391.*
19. *Sun J, Ren SZ, Lu XY, Li JJ, Shen FQ, Xu C, Zhu HL. Discovery of a series of 1,3,4-oxadiazole-2(3H)-thione derivatives containing piperazine skeleton as potential FAK inhibitors. Bioorg Med Chem 2017; 25: 2593–2600.*

20. Constantinos GN, Livadiotou D, Tsiaras V, Tzitzikas TZ, Samatibou E. The indoleacetic acid in IMCRs: A three-component Ugi reaction involving TosMIC. Tetrahedron 2016; 72: 5149-5156.