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ORIGINAL PAPER

Design, synthesis, and evaluation of the anticancer properties of a novel series of carboxamides, sulfonamides, ureas, and thioureas derived from 1,2,4-oxadiazol-3-ylmethyl-piperazin-1-yl substituted with pyrazolo[1,5-*a*]pyrimidine derivatives

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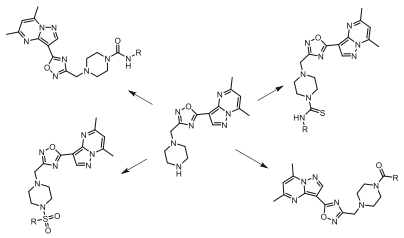
Abstract A series of novel carboxamides, sulfonamides, ureas, and thioureas derived from 1,2,4-oxadiazol-3-yl-methyl-piperazin-1-yl substituted with pyrazolo[1,5-*a*]pyrimidine analog were designed and synthesized. The newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI–MS, and IR and were tested for their in vitro antiproliferative activity by MTT assay. Out of these twenty derivatives, five compounds showed good anticancer activity against HeLa cell line. These are superior with less than 10 μg/cm^3 of IC_{50} when compared to the marketed anticancer drug paclitaxel with 30 μg/cm^3 of IC_{50} against Hela cell line.

Graphical abstract



Keywords Carboxamide · Sulfonamide · Urea · Thiourea · Pyrazolo[1,5-*a*]pyrimidine · 1,2,4-Oxadiazole · Piperazine · Anticancer activity

Introduction

Pyrazole represents one of the most active classes of heterocyclic compounds, since many of them possesses a wide range spectrum of biological activities like potential inhibitors of HIV-1 [1], analgesic drugs [2, 3], antihypertensive agents [4], anticancer activity [5, 6]. Also, compounds containing substituted pyrimidine derivatives are of significant biological importance and used as antibacterial [7], antifungal [8], antitumor [9], antiviral [10], anti-inflammatory [11], and antihypertensive [12] agents.

In general, pyrazole derivatives are utilized for the synthesis of other fused heterocyclic systems. Among these, pyrazolopyrimidines which mimic structurally with biogenic purines [13, 14] have considerable chemical and pharmacological importance and are bioisosters to triazolothienopyrimidines [15], imidazoquinazolines [16], and pyrimidoquinazolines [17]. Many analogs of pyrazolo[1,5-

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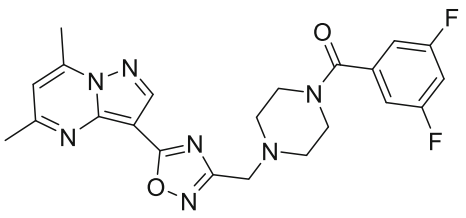
a]pyrimidine are associated with diverse pharmacological activities [18–22] like tuberculostatic [23], antimicrobial activities [24], neuroleptic [25], CNS depressant [26], and antihypertensive [27]. Moreover, pyrazolopyrimidines have useful properties as antimetabolite in purine biochemical reactions.

Derivatives of 1,2,4-oxadiazole constitute an important family of heterocyclic compounds [28]. Since many of them exhibit a remarkable biological activity [29, 30] and find wide usage as dyes, photosensitive electrical materials, polymer precursors, and stabilizers, the synthesis and transformations have received great attention for a long time.

In particular, 2-aryl-5-substituted 1,2,4-oxadiazoles have been reported to show antibacterial [31], antifungal [32], analgesic, anti-inflammatory [33], and hypoglycemic activities. Also 1,2,4-oxadiazoles are well-known compounds with promising physiological activities [34, 35]. 1,2,4-Oxadiazole rings occur widely in biologically active synthetic compounds and are often used in drug discovery as good bioisosters of amides and esters [28, 36, 37]. Furthermore, they have been reported to have agonist for cortical muscarinic receptors [38–41], benzodiazepine [42, 43], 5-HT_{1D} (5-hydroxytryptamine) receptors [29], and as antagonists for 5-HT_3 [30] or histamine H_3 receptors [44]. They showed activity against several breast and colorectal cancer cell lines [45–47].

A number of piperazine derivatives have been shown to possess a variety of pharmacological properties like antihistamanic [48], analgesic [49], antiinflammatory [50], anti-HIV [51], antimalarial [52], antitubercular [53], and antimicrobial activity [54]. Hence, piperazine is found to be an important structural feature in some synthetic drugs such as prazosin, lidoflazine, and urapidil.

Prompted by these observations and as a continuation of our ongoing research program in the synthesis of biologically active molecules, we envisaged the synthesis of novel molecules based on the three lead pharmacophores realized viz., pyrazolo[1,5-*a*]pyrimidine, 1,2,4-oxadiazole, and piperazine. In this communication, we disclose the synthesis of a novel series of carboxamides, sulfonamides, ureas, and thioureas derived from 1,2,4-oxadiazol-3-ylmethyl-piperazin-1-ylsubstituted with pyrazolo[1,5-*a*]-pyrimidine analog with representative example, i.e., compound **12l** as shown in Fig. 1, showing good growth inhibition against HeLa cell line.



12l

Fig. 1 Chemical structure of compound **12l** which showed good antiproliferative activity

Results and discussion

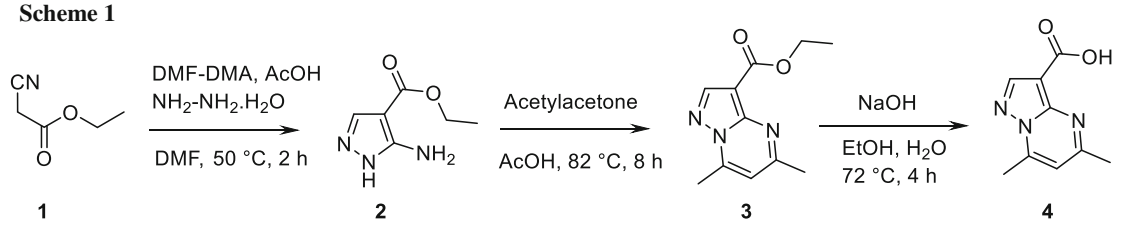
Chemistry

Synthesis of a series of novel carboxamides, sulfonamides, ureas, and thioureas derived from 1,2,4-oxadiazol-3-ylmethyl-piperazin-1-yl substituted with pyrazolo[1,5-*a*]-pyrimidine derivative involves four consecutive schemes. First part involves the synthesis of 5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid as shown in Scheme 1. This involves the synthesis of ethyl 5-amino-1*H*-pyrazole-4-carboxylate (**2**) as the initial counterpart and in general, it was carried out by two steps protocol [55, 56]. But we have carried out the synthesis by a single pot reaction from the commercially available ethyl cyanoacetate (**1**), dimethylformamide dimethylacetal, and hydrazine hydrate in acetic acid medium under mild heating with dimethylformamide as solvent. The ethyl 5-amino-1*H*-pyrazole-4-carboxylate (**2**) was converted to the ethyl 5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylate (**3**) with acetyl acetone in acetic acid medium at 82 °C in good yield [57]. After having synthesized the known compound **3**, the 5,7-dimethylpyrazolo[1,5-*a*]-pyrimidine-3-carboxylic acid (**4**) was synthesized by hydrolyzing the ester derivative **3** with sodium hydroxide in ethanol and water medium under reflux condition.

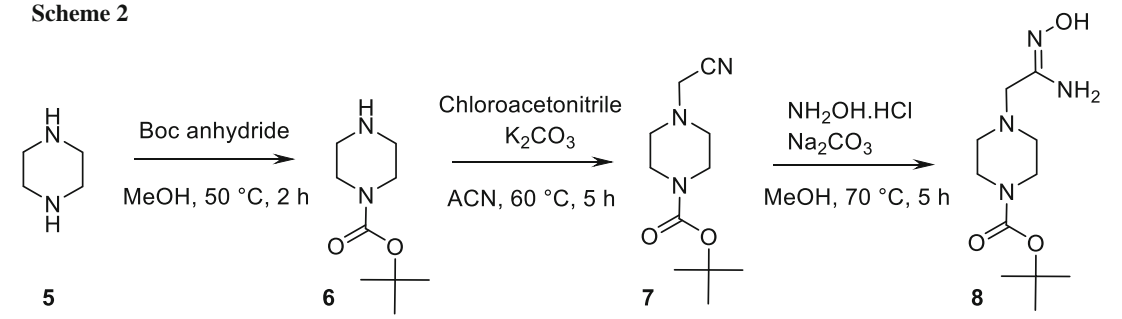
Second part involves the synthesis of *tert*-butyl 4-(*N*′-hydroxycarbamimidoyl)piperazine-1-carboxylate (**8**) in three steps as shown in Scheme 2. The piperazine-1-carboxylic acid *tert*-butyl ester (**6**) was prepared by heating piperazine (**5**) with BOC-anhydride in methanol at 50 °C for 2 h [58]. The *tert*-butyl 4-(cyanomethyl)piperazine-1-carboxylate (**7**) was synthesized by reaction with piperazine-1-carboxylic acid *tert*-butyl ester and chloroacetonitrile with potassium carbonate in acetonitrile at 60 °C for 5 h. After having synthesized the nitrile derivative, *tert*-butyl 4-(*N*′-hydroxycarbamimidoyl)piperazine-1-carboxylate (**8**) was prepared by reacting *tert*-butyl 4-(cyanomethyl)piperazine-1-carboxylate (**7**) with hydroxylamine hydrochloride in the presence of sodium carbonate at reflux temperature with methanol for 5 h.

The third part involves the synthesis of scaffold, 5,7-dimethyl-3-(3-piperazin-1-ylmethyl-1,2,4-oxadiazol-5-yl)pyrazolo[1,5-*a*]pyrimidine (**11**) as shown in Scheme 3. This involves the synthesis of 4-[2-(5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxamido)-2-(hydroxyimino)-ethyl]piperazine-1-carboxylic acid *tert*-butyl ester (**9**)

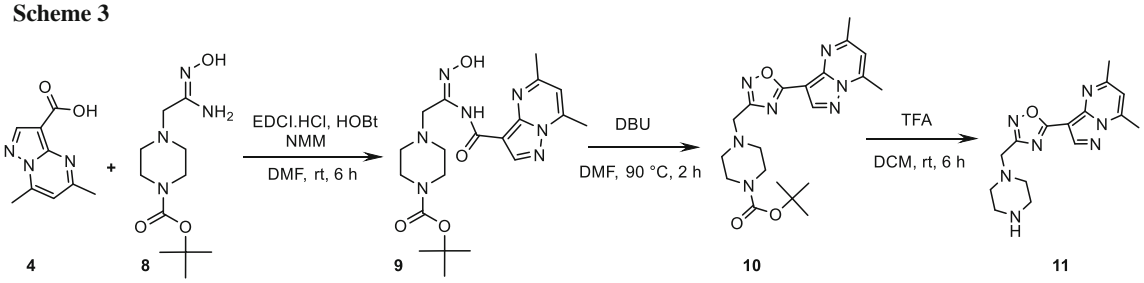
Scheme 1



Scheme 2



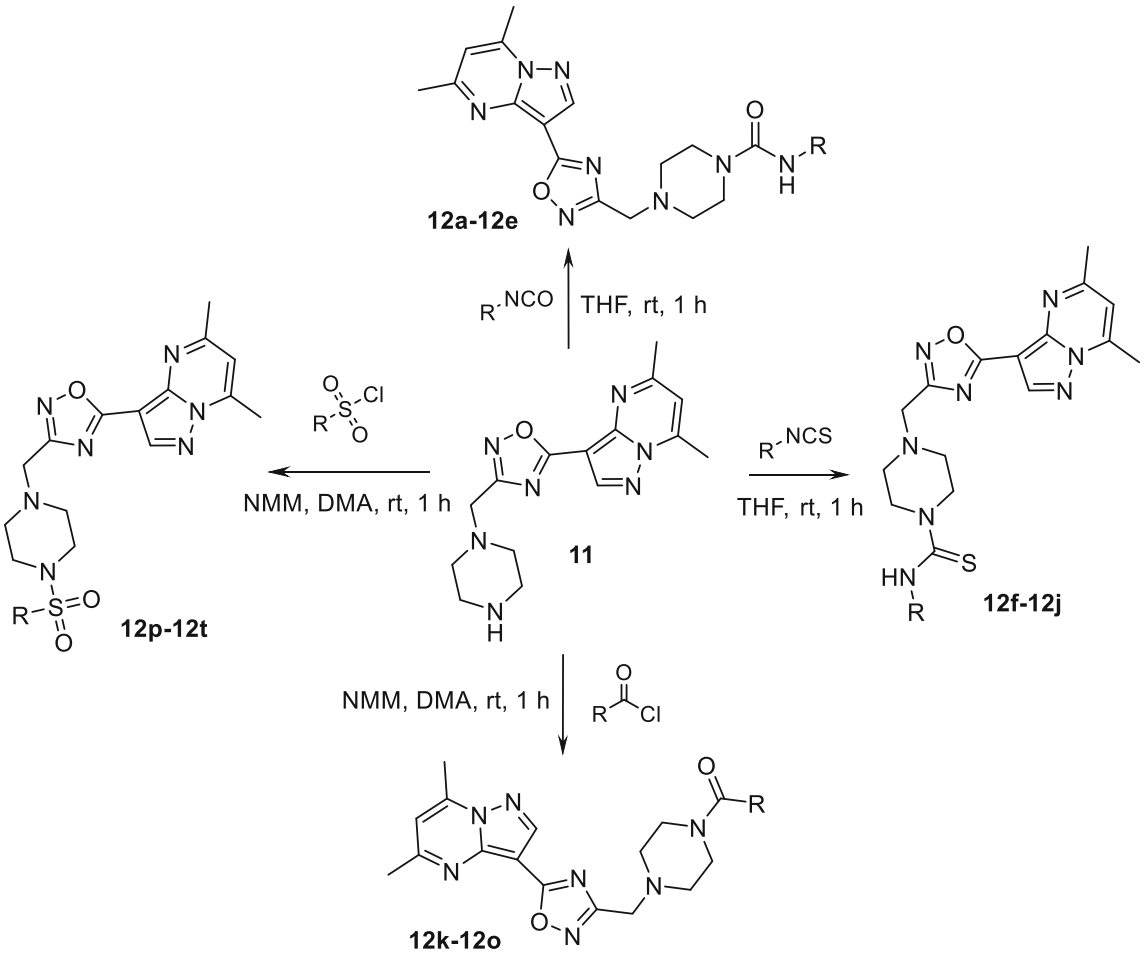
Scheme 3



derived from 5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**4**) and *tert*-butyl 4-(*N*′-hydr oxycarbamimidoyl)piperazine-1-carboxylate (**8**) using EDCI•HCl and HOBt with *N*-methylmorpholine in dimethylformamide solvent at room temperature for 6 h. Cyclization of this amide derivative was initially tried by heating with *N*,*N*′-dicyclohexylcarbodiimide (DCC) in ethanol at 80 °C. But the isolation involved the purification by column chromatography due to the formation of dicyclohexylurea (DCU) from the coupling agent owing to lower yields. We then employed 1,8-diazabicycloundec-7-ene (DBU) as the dehydrating agent. So, the amide intermediate was further cyclised to the corresponding 1,2,4-oxadiazole, 4-[5-(5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)-1,2,4-oxadiazol-3-yl)methyl]piperazine-1-carboxylic acid *tert*-butyl ester (**10**) by heating at 90 °C with DBU in *N*,*N*-dimethylformamide (DMF) solvent for 2 h. In order to synthesize novel compounds from above intermediate, we have prepared the corresponding free amine, 5,7-dimethyl-3-(3-piperazin-1-ylmethyl-1,2,4-oxadiazol-5-yl)pyrazolo[1,5-*a*]pyrimidine (**11**) by deprotecting BOC group of piperazine ring from the cyclized 1,2,4-oxadiazole moiety. This step was carried out using trifluoroacetic acid as the reagent in dichloromethane solvent at room temperature. The trifluoroacetate salt obtained was made basic with saturated potassium carbonate solution and extracted with dichloromethane:methanol (100:5). The crude product obtained after evaporation was recrystallised from ethyl acetate:hexane (1:8) medium at room temperature to get the scaffold as off white solid.

The fourth part involves the synthesis of target molecules **12a–12t** as shown in Scheme 4. The free amine obtained after BOC cleavage was derivatized into four series of final target molecules. First series involves the synthesis of urea derivatives **12a–12e** obtained by treating amine and the corresponding isocyanates in THF medium

Scheme 4



at room temperature. The second series involves the synthesis of thiourea derivatives **12f**–**12j** obtained by reacting amine and the corresponding isothiocyanates in THF medium at room temperature. The third series involves the synthesis of carboxamide derivatives **12k**–**12o** obtained by the reacting amine and corresponding acid chlorides with *N*-methylmorpholine as base in dimethylacetamide as solvent at room temperature. The fourth series involves the synthesis of sulfonamide derivatives **12p**–**12t** obtained by reacting amine and the corresponding sulfonyl chlorides with *N*-methylmorpholine as base in dimethylacetamide as solvent at room temperature.

The structures for all the above compounds were confirmed by spectral studies. The structure of the amide compound, 4-[2-(5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxamido)-2-(hydroxyimino)ethyl]piperazine-1-carboxylic acid *tert*-butyl ester (**9**) was elucidated by its IR, ^{1}H and ^{13}C NMR, and LC–MS analyses. The ^{1}H NMR spectrum of **9** showed singlet at $\delta = 10.69$ ppm, for one proton which corresponds to the amide NH proton. Compound **10** was elucidated by its IR, ^{1}H and ^{13}C NMR, and LC–MS analyses. The ^{1}H NMR of compound **10** did not show the peak for $-NH_2$ group of carboximidamide, thereby confirming the oxadiazole ring formation. The ^{13}C NMR showed a signal at 170.87 ppm confirming the presence of oxadiazole ring in the molecule. Compound **11** was elucidated by its IR, ^{1}H and ^{13}C NMR, and LC–MS analyses. The ^{1}H NMR of free amine scaffold did not show the peak for BOC group of piperazine, thereby confirming the free piperazine ring in the molecule. The IR spectrum showed peak at 3327 cm^{-1} which attributed to the –N–H stretch vibration of the amine from the piperazine ring.

The results of all the newly synthesized compounds **12a**–**12t** were confirmed by ^{1}H NMR, ^{13}C NMR, LC–MS, and IR analyses and given in the "Experimental" section. The structures of urea derivatives **12a**–**12e** were confirmed by the following spectral studies. In general, the FT-IR spectrum of **12a** contained a broad band at 1631 cm^{-1} which was attributed to the carbonyl stretching vibration of the corresponding urea derivative. The ^{1}H

NMR spectrum of **12a** contained a broad singlet with a chemical shift of 6.37 ppm which was consistent with the proton of the –NH from the urea link. ^{13}C NMR spectrum of **12a** contained a signal at 158.98 ppm which was consistent with the carbonyl group from the urea link. The structure of compound **12a** was further confirmed by mass spectrometry which gave a molecular ion peak with an *m/z* value of 433.4 for [M+H] which was consistent the molecular formula $C_{22}H_{24}N_8O_2$. The structures of thiourea derivatives **12f–12j** were confirmed by the following spectral studies. In general, the FT-IR spectrum of **12i** contained a broad band at 1629 cm^{-1} which was attributed to the C=S stretching vibration of the corresponding thiourea derivative. The ^{1}H NMR spectrum of **12i** contained a broad singlet with a chemical shift of 6.72 ppm which was consistent with the proton of the –NH from the thiourea link. The ^{13}C NMR spectrum of **12i** contained a signal at 181.63 ppm which was consistent with the thiocarbonyl group from the thiourea link. The structure of compound **12i** was further confirmed by mass spectrometry which gave a molecular ion peak with an *m/z* value of 429.4 for [M+H] which was consistent the molecular formula $C_{20}H_{28}N_8OS$.

The structures of carboxamide derivatives **12k–12o** were confirmed by the following spectral studies. In general, the FT-IR spectrum of **12o** contained a broad band at 1654 cm^{-1} which was attributed to the carbonyl stretching vibration of the corresponding carboxamide derivative. The ^{1}H NMR spectrum of **12o** contained a sharp singlet with a chemical shift of 7.78 ppm which was consistent with the protons of the 2,4,6-trichlorobenzene ring from the carboxamide link. The ^{13}C NMR spectrum of **12o** contained a signal at 162.20 ppm which was consistent with the carbonyl group from the carboxamide group. The structure of compound **12o** was further confirmed by mass spectrometry which gave a molecular ion peak with an *m/z* value of 522.1 for [M+H], which was consistent the molecular formula $C_{22}H_{20}Cl_3N_7O_2$. Finally, the structures of sulfonamide derivatives **12p–12t** were confirmed by the following spectral studies. In general, the FT-IR spectrum of **12p** contained a broad band at 1346 cm^{-1}, which was attributed to the sulfonyl antisymmetric stretching vibration of the corresponding sulfonamide derivative. The ^{13}C NMR spectrum of **12p** contained two signals at 155.88 and 159.22 ppm with a coupling constant of $J = 250.50$ Hz which were consistent with the fluorine group from the sulfonamide derivative. The structure of compound **12p** was further confirmed by mass spectrometry which gave a molecular ion peak with an *m/z* value of 506.4 for [M+H] which was consistent the molecular formula $C_{21}H_{21}ClFN_7O_3S$. The spectral data generated in the current study were in good agreement with the assigned structures of all the novel molecules synthesized in this series.

Anticancer evaluation

All compounds were screened for their in vitro anti-cancer activity against representative human cervical cancer cell line called HeLa. Paclitaxel was used as a reference standard. The data generated from this study Table 1 showed that some of the target compounds exhibit good potency in inhibiting the growth of HeLa cell line. Compounds **12b**, **12e**, **12k**, **12l**, **12m**, and **12r** were showed good anti proliferative activity against HeLa cell line. Interestingly, among these compounds, the in vitro anticancer activity of compounds **12b**, **12e**, **12k**, **12l**, and **12r** are superior to the marketed anti-cancer drug paclitaxel. However, some of the synthesized compounds are less potent when compared to paclitaxel.

The anticancer activity of these novel compounds **12a–12t** suggested that introduction of the urea derivatives, carboxamides, and sulfonamides were increased the antiproliferative activity when compared to the thiourea derivatives. In addition to these observations, we have concluded that the optimum anticancer activity observed for compounds containing one or more halogen derivatives.

Conclusion

A series of novel carboxamides, sulfonamides, ureas, and thioureas derived from pyrazolo[1,5-a]pyrimidine scaffold were designed and successfully synthesized. The synthesis involves the preparation of both 5,7-dimethylpyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid and 4-(*N*-hydroxycarbamimidoylmethyl)piperazine-1-carboxylic acid *tert*-butyl ester. The coupling reaction between both leads to get the corresponding amide 4-{2-[(5,7-dimethyl-1,3-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonyl)amino}-2-(hydroxyimino)ethyl]piperazine-1-carboxylic acid *tert*-butyl ester. This amide was further cyclized to the corresponding heterocyclic 4-[5-(5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid *tert*-butyl ester with DBU in DMF heating condition. The BOC deprotection was carried out with trifluoroacetic acid in dichloromethane medium to get the scaffold 5,7-dimethyl-3-(3-piperazin-1-ylmethyl-1,2,4-oxadiazol-5-yl)-pyrazolo[1,5-*a*]pyrimidine. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI–MS, and IR. The new compounds were tested for their in vitro antiproliferative activity by MTT assay. Out of these twenty derivatives, compounds **12b**, **12e**, **12k**, **12l**, and **12r** showed good anticancer activity against HeLa cell line. These are superior with less than 10 μg/cm^3 of IC_{50} when compared to the marketed anticancer drug paclitaxel with 30 μg/cm^3 of IC_{50} against Hela cell line.

Table 1 Anticancer activity of compounds **12a–12t**

Compound	Concentration/ μG	OD at 492 nm	% of cell lysis as observed	*IC*50
11	10	0.632	No lysis	30 μg
	20	0.510	25	
	30	0.495	50	
12	10	0.680	No lysis	30 μg
	20	0.646	25	
	30	0.630	50	
12a	10	0.690	25	>30 μg
	20	0.682	25	
	30	0.640	50	
12b	10	0.362	50	>10 μg
	20	0.340	75	
	30	0.311	100	
12c	10	0.802	No lysis	No activity
	20	0.790	No lysis	
	30	0.786	No lysis	
12d	10	0.540	25	30 μg
	20	0.486	>25	
	30	0.465	50	
12e	10	0.281	100	<10 μg
	20	0.262	100	
	30	0.211	100	
12f	10	0.721	No lysis	>30 μg
	20	0.740	25	
	30	0.789	>25	
12g	10	0.521	25	20 μg
	20	0.428	50	
	30	0.410	>50	
12h	10	0.781	No lysis	No activity
	20	0.721	No lysis	
	30	0.681	No lysis	
12i	10	0.516	25	20 μg
	20	0.415	50	
	30	0.411	>50	
12j	10	0.562	>25	20 μg
	20	0.428	50	
	30	0.416	50	
12k	10	0.281	75	<10 μg
	20	0.224	100	
	30	0.211	100	
12l	10	0.320	75	<10 μg
	20	0.316	>75	
	30	0.312	>75	
12m	10	0.408	50	10 μg
	20	0.388	75	
	30	0.341	>75	
12n	10	0.712	No lysis	>30 μg
	20	0.682	25	
	30	0.620	50	
12o	10	0.745	No lysis	30 μg
	20	0.660	25	
	30	0.412	50	
12p	10	0.490	50	20 μg
	20	0.432	50	
	30	0.420	>50	
12q	10	0.632	No lysis	30 μg
	20	0.510	25	
	30	0.496	50	
12r	10	0.281	75	<10 μg
	20	0.261	>75	
	30	0.200	100	
12s	10	0.802	No lysis	No activity
	20	0.790	No lysis	
	30	0.786	No lysis	
12t	10	0.782	No lysis	30 μg
	20	0.626	25	
	30	0.424	50	
Control	10	0.862	No lysis	
Paclitaxel	10	0.512	No lysis	30 μg
	20	0.484	25	
	30	0.401	50	

Experimental

Chemicals were obtained from Sigma-Aldrich Co. Final purifications were carried out using Merck silica gel 60–120 mesh size. TLC experiments were performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and $KMnO_4$. Melting points were determined using Büchi B-540. All ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-300 and Bruker AM-400 (300 and 400 MHz for ^{1}H NMR and 75 and 100 MHz for ^{13}C NMR), Bruker BioSpin Corp., Germany. Molecular weights of unknown compounds were checked by LC MS 6200 series Agilent Technology. Chemical shifts are reported in ppm (δ) with reference to internal standard TMS. The signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. IR for all the synthesized compounds was recorded using a Bruker Alpha FT-IR spectrometer using a diamond ATR single reflectance module (24 scans).

5-Amino-1H-pyrazole-4-carboxylic acid ethyl ester (**2**, $C_6H_9N_3O_2$)

To a solution of 25 g of ethyl cyanoacetate (**1**, 0.221 mol) in 25 cm^3 dimethylformamide was added 37.5 cm^3 glacial acetic acid (0.077 mol) followed by 47.40 g of *N*,*N*-dimethylformamide dimethylacetal (0.397 mol) drop wise at room temperature and stirred for 1 h. To the resultant pale yellow color solution was added 25 cm^3 hydrazine hydrate (0.42 mol) drop wise at 0 °C and the reaction medium was stirred for 2 h 50 °C. After completion of the reaction, the reaction medium was diluted with 2.5 dm^3 of water and extracted with 2 × 700 cm^3 of ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure to obtain the crude product as pale brown liquid. The crude product was further purified by column chromatography over silica gel, eluted with chloroform/methanol (95:5, v/v) to afford the compound **2** as a white solid (29 g, 85 %). M.p.: 106.7–108.9 °C; TLC: $R_f = 0.22$ ($CHCl_3$–MeOH 8:2); IR (ATR): $\bar{\nu} = 3193$ (–NH), 2972 (=C–H), 1662 (ester –C=O), 1551 (–C–C), 1496 (ester –C–O), 1337 (C–N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.23$ (3H, t, $J = 8$ Hz, CH_3), 4.15 (2H, q, $J = 8$ Hz, OCH_2CH_3), 5.98 (2H, bs, $ArNH_2$), 7.45 (1H, bs, ArH), 12.04 (1H, bs, pyrazole NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 14.9$, 59.1, 94.0, 139.9, 151.8, 164.2 ppm; LC–MS: $m/z = 156.7$ $[M+H]^+$.

Ethyl 5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-carboxylate (**3**)

This compound was prepared as per the reported literature [59] and obtained as a pale yellow solid (34 g, 98 %). M.p.: 102.5–105.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.30$ (3H, t, $J = 8$ Hz, CH_3), 2.58 (3H, s, $ArCH_3$), 2.71 (3H, s, $ArCH_3$), 4.27 (2H, q, $J = 8$ Hz, OCH_2CH_3), 7.12 (1H, s, ArH), 8.53 (1H, s, ArH) ppm.

5,7-Dimethylpyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**4**)

This compound was prepared as per the reported literature [57, 59] and obtained as off white solid (25 g, 96 %). M.p.: 176.8–179.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.56$ (3H, s, $ArCH_3$), 2.70 (3H, s, $ArCH_3$), 7.09 (1H, s, ArH), 8.49 (1H, s, ArH) ppm, 12.24(1H, bs, acid) which is consistent with literature values.

Piperazine-1-carboxylic acid tert-butyl ester (**6**, $C_9H_{18}N_2O_2$)

To a solution of 30 g of piperazine (**5**, 0.348 mol) in 120 cm^3 of methanol was added a solution of 83.54 g di-*tert*-butyl carbonate (0.382 mol) in 90 cm^3 of methanol drop wise over a period of 45–60 min at 0 °C under nitrogen atmosphere. The reaction mixture was heated to 50 °C for 2 h. After completion of the reaction, the reaction mixture was concentrated completely under reduced pressure to remove methanol. The crude material obtained was purified by column chromatography on silica gel eluted with chloroform/methanol (100:4, v/v) to afford compound **6** as off white solid (35.6 g, 55 %). M.p.: 45.5–46.8 °C; TLC: $R_f = 0.35$ ($CHCl_3$–MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.38$ (9H, s, CH_3), 2.29–2.39 (1H, m, NH), 2.60 (4H, t, $J = 4.95$ Hz, $N(CH_2)_2$, 3.20 (t, 4H, $J = 5.4$ Hz) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$, 45.9 (two peaks), 78.9, 154.4 ppm; MS: $m/z = 187.3$ $[M+H]^+$.

4-(Cyanomethyl)piperazine-2-carboxylic acid tert-butyl ester (**7**)

This compound was prepared following the protocol mentioned in the literature [60] and obtained as an off white solid (32 g, 88 %). M.p.: 91.5–93.7 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.40$ (9H, s, CH_3), 2.41 [4H, t, $J = 5.1$ Hz, $N(CH_2)_2$], 3.35 [t, 4H, $J = 5.4$ Hz, $N(CH_2)_2$], 3.76 (2H, s, CH_2) ppm; and values were in correlation with reported literature.

4-(N-Hydroxycarbamimidoylmethyl)piperazine-1-carboxylic acid tert-butyl ester (**8**)

This compound was prepared following the protocol mentioned in the literature [61] and obtained as off white solid (20 g, 70 %). M.p.: 236.6–238.6 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.39$ (9H, s, CH_3), 2.30 [4H, t, $J = 4.8$ Hz, $N(CH_2)_2$], 2.83 (2H, s, CH_2), 3.38 [4H, t, $J = 8.1$ Hz, $N(CH_2)_2$], 5.24 (2H, s, NH_2), 8.89 (1H, s, OH) ppm and values were in correlation with reported literature.

4-[2-(5,7-Dimethylpyrazolo[1,5-a]pyrimidine-3-carboxamido)-2-(hydroxyimino)ethyl]piperazine-1-carboxylic acid tert-butyl ester (**9**, $C_{20}H_{29}N_7O_4$)

To a solution of 13 g of compound **4** (0.067 mol) and 18.44 g of compound **8** (0.071 mol) in 65 cm^3 of dimethylformamide was added 17.98 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.094 mol) followed by 14.39 g of 1-hydroxybenzotriazole (0.094 mol) and 4 g of *N*-methylmorpholine (0.033 mol) at room temperature under nitrogen atmosphere. The resultant reaction mixture was stirred for 6 h at room temperature. After completion of the reaction, reaction mixture was quenched with drop-wise addition of 650 cm^3 of ice-cold water and stirred for further 30 min at room temperature. The solid precipitated out was filtered, washed with 100 cm^3 of cold water and dried under vacuum at 50 °C for 1 h to afford compound **9** as an off white solid (22 g, 75 %). M.p.: 164.3–167.1 °C; TLC: $R_f = 0.28$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3490$ (amide –NH), 2981 (=C–H), 2812 (–OH), 1712 (ester –C=O), 1685 (C=N), 1630 (amide –C=O), 1550 (–C–C), 1476 (ester –C–O), 1475 (amide –C–O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.39$ (9H, s, CH_3), 2.38–2.40

[4H, m, $N(CH_2)_2$], 2.60 (3H, s, CH_3), 2.73 (3H, s, CH_3), 3.06 (2H, s, CH_2), 3.34–3.36 [4H, m, $N(CH_2)_2$], 6.52 (1H, s, NH), 6.91 (1H, s, OH), 7.15 (1H, s, pyrimidine H), 8.71 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 25.0, 28.5 (three peaks), 43.0, 52.7 (two peaks), 57.0, 79.2, 101.0, 111.0, 146.6, 147.4, 147.5, 154.2, 156.9, 159.6, 163.0 ppm; LC–MS: $m/z = 432.0$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (**10**, $C_{20}H_{27}N_7O_3$)

To a solution of 20 g of compound **9** (0.046 mol) in 100 cm^3 of dimethyl formamide, 8.3 cm^3 of DBU (0.055 mol) was added drop wise at room temperature under nitrogen atmosphere. The resultant reaction mixture was stirred at 90 °C for 2 h. After completion of reaction, the reaction mixture was quenched with drop-wise addition of 1 dm^3 of ice-cold water and stirred for further 30 min at room temperature. The solid precipitated out was filtered, washed with 200 cm^3 of cold water and dried under vacuum at 50 °C for 1 h to afford compound **10** as off white solid (15.9 g, 83 %). M.p.: 166.0–168.3 °C; TLC: $R_f = 0.35$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 2969$ (=C–H), 1678 (ester –C=O), 1556 (–C–C), 1427 (ester –C–O), 1323 (–C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.39$ (9H, s, CH_3), 2.51–2.59 [4H, m, $N(CH_2)_2$], 2.64 (3H, s, CH_3), 2.74 (3H, s, CH_3), 3.07 (2H, s, CH_2), 3.34–3.36 [4H, m, $N(CH_2)_2$], 7.22 (1H, s, pyrimidine H), 8.84 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 28.5 (three peaks), 52.5 (two peaks), 52.6 (two peaks), 79.2, 95.2, 111.6, 145.2, 146.5, 147.5, 154.2, 163.5, 167.4, 170.8 ppm; LC–MS: $m/z = 414.4$ $[M+H]^+$.

5,7-Dimethyl-3-(3-piperazin-1-ylmethyl-1,2,4-oxadiazol-5-yl)pyrazolo[1,5-a]pyrimidine (**11**, $C_{15}H_{19}N_7O$)

To a solution of 15 g of compound **10** (0.036 mol) in 150 cm^3 of dichloromethane was added 45 cm^3 of trifluoroacetic acid drop wise at 0 °C under nitrogen atmosphere. The reaction mixture was slowly allowed to raise the temperature to 25 °C and stirred for 6 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in water, basified with 75 cm^3 of saturated potassium carbonate solution and extracted with 2 × 150 cm^3 of dichloromethane/methanol (100:5, v/v). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated completely to obtain the crude product as pale yellow solid. The crude material was further purified by recrystallization with ethyl acetate/hexane (1:8, v/v) at room temperature to afford compound **11** as an off white solid (8.5 g, 75 %). M.p.: 147.8–150.9 °C; TLC: $R_f = 0.11$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3327$ (–NH), 2958 (=C–H), 1556 (–C–C), 1323 (–C–N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 2.42$–2.49 [4H, m, $N(CH_2)_2$], 2.63 (3H, s, CH_3), 2.67–2.69 [4H, m, $N(CH_2)_2$], 2.76 (3H, s, CH_3), 3.63 (2H, s, CH_2), 7.21 (1H, s, pyrimidine H), 8.84 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.9$, 24.9, 45.8 (two peaks), 53.2, 54.1 (two peaks), 95.2, 111.5, 145.1, 146.4, 147.4, 163.3, 167.4, 170.6 ppm; LC–MS: $m/z = 314.3$ $[M+H]^+$.

*Synthesis of substituted urea derivatives **12a–12e** (general procedure)*

To a solution of compound **11** (1.00 mmol) in 4 cm^3 of tetrahydrofuran, corresponding isocyanates (1.05 mmol) were added drop wise at 0 °C under nitrogen atmosphere. The reaction medium was stirred at the same temperature for 30 min and then allowed to stir at room temperature for 1 h under nitrogen atmosphere. After completion of reaction, the reaction mixtures were concentrated under reduced pressure and purified on silica gel column chromatography with chloroform/methanol (100:2, v/v) to afford corresponding urea derivatives **12a–12e**.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid phenylamide (**12a**, $C_{22}H_{24}N_8O_2$)

From 0.100 g of compound **11** (0.319 mmol) and 0.040 g of phenyl isocyanate (0.335 mmol), compound **12a** was obtained as an off white solid (0.12 g, 85 %) after chromatography on a silica gel column with chloroform/methanol (100:2, v/v). M.p.: 189.2–192.2 °C; TLC: $R_f = 0.43$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3301$ (–NH), 2920 (=CH), 1631 (amide –C=O), 1541 (–C–C), 1437 (amide –C–O), 1363 (Ar–C–N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.74$ (3H, s, $ArCH_3$), 2.76–2.83 [4H, m, $N(CH_2)_2$], 2.84 (3H, s, $ArCH_3$), 3.59–3.69 [4H, m, $N(CH_2)_2$], 3.90 (2H, s, $–CH_2–$), 6.37 (1H, bs, urea NH), 6.84 (1H, s, pyrimidine H), 7.04 (1H, t, $J = 7.2$ Hz, ArH), 7.26–7.34 (2H, m, ArH), 7.34 (2H, d, $J = 8.4$ Hz, ArH), 8.72 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.9$, 24.9, 44.1, 52.4, 52.7, 95.2, 111.6, 121.0, 121.5, 140.3, 143.0, 145.1, 146.5, 147.4, 155.2, 163.4, 167.3, 170.8 ppm; LC–MS: $m/z = 433.4$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid [4-(trifluoromethoxy)phenyl]amide (**12b**, $C_{23}H_{23}F_3N_8O_3$)

From 0.100 g of compound **11** (0.319 mmol) and 0.068 g of 4-(trifluoromethoxy)phenyl isocyanate (0.335 mmol), compound **12b** was obtained as an off white solid (0.14 g, 87 %) after chromatography on a silica gel column with chloroform/methanol (100:2, v/v). M.p.: 179.6–182.4 °C; TLC: $R_f = 0.38$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3333$ (–NH), 2921 (=CH), 1634 (amide –C=O), 1535 (–C–C), 1463 (amide –C–O), 1359 (Ar–C–N), 1281 (C–O–C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.65$–2.71 [4H,

m, $N(CH_2)_2$], 2.73 (3H, s, $ArCH_3$), 2.83 (3H, s, $ArCH_3$), 3.61–3.55 [4H, m, $N(CH_2)_2$], 3.86 (2H, s, $–CH_2–$), 6.43 (1H, bs, urea NH), 6.84 (1H, s, pyrimidine H), 7.13 (2H, d, $J = 8.4$ Hz, ArH), 7.35–7.39 (2H, m, ArH), 8.72 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 15.9$, 23.9, 43.0 (two peaks), 51.4, 51.6 (two peaks), 94.2, 110.5, 115.8, 118.3, 120.9,123.4 (q, $^1J_{C-F} = 253.6$ Hz), 118.4, 120.6, 139.3, 141.9, 144.1, 145.5, 146.4, 154.1, 162.4, 166.3, 169.8 ppm; LC–MS: $m/z = 517.3$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid cyclopentylamide (**12c**, $C_{28}H_{21}N_8O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.037 g of cyclopentyl isocyanate (0.335 mmol), compound **12c** was obtained as an off white solid (0.12 g, 89 %) after chromatography on a silica gel column with chloroform/methanol (100:2, v/v). M.p.: 115.5–118.2 °C; TLC: $R_f = 0.40$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3446$ (NH), 2967 (=CH), 1643 (amide –C=O), 1551 (–C–C), 1442 (amide –C–O), 1336 (Ar–C–N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.31–1.46$ (4H, m, cyclopentane CH_2), 1.58–1.60 (2H, m, cyclopentane CH_2), 1.72–1.77 (2H, m, cyclopentane CH_2), 2.45–2.48 [4H, m, $N(CH_2)_2$], 2.64 (3H, s, ArCH3), 2.76 (3H, s, $ArCH_3$), 3.28–3.30 [4H, m, $N(CH_2)_2$], 3.71 (2H, s, $–CH_2–$), 3.85–3.89 (1H, m, cyclopentyl CH), 6.21 (1H, d, $J = 6.8$ Hz, urea NH), 7.22 (1H, s, pyrimidine H), 8.34 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 16.5$, 25.2 (two peaks), 28.7, 32.9 (two peaks), 43.7 (two peaks), 52.3, 52.5, 52.7 (two peaks), 95.2, 111.5, 145.1, 146.4, 147.4, 157.6, 163.4, 167.4, 170.8 ppm; LC–MS: $m/z = 425.4$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid m-tolylamide (**12d**, $C_{23}H_{26}N_8O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.045 g of 3-methylphenyl isocyanate (0.335 mmol), compound **12d** was obtained as an off white solid (0.13 g, 90 %) after chromatography on a silica gel column with chloroform/methanol (100:2, v/v). M.p.: 164.9–167.4 °C; TLC: $R_f = 0.42$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3301$ (–NH), 3064 (–CH), 2921 (=CH), 1632 (amide –C=O), 1546 (–C–C), 1428 (amide –C–O), 1318 (Ar–C–N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 2.24$ (3H, s, $ArCH_3$), 2.56 [4H, t, $J = 4.4$ Hz, $N(CH_2)_2$], 2.64 (3H, s, $ArCH_3$), 2.77 (3H, s, $ArCH_3$), 3.46 [4H, t, $J = 4.4$ Hz, $N(CH_2)_2$], 3.76 (2H, s, $–CH_2–$), 6.74 (1H, d, $J = 7.6$ Hz, urea NH), 7.09 (1H, t, $J = 7.6$ Hz, ArH), 7.22–7.24 (2H, m, ArH), 7.27 (1H, s, ArH), 8.43 (1H, s, pyrimidine H), 8.85 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.8$, 21.5, 22.4, 44.0, 52.3, 52.6, 95.1, 111.4, 117.0, 120.5, 122.7, 128.4, 137.6, 140.7, 145.0, 146.3, 147.3, 155.2, 163.2, 167.2, 170.7 ppm; LC–MS: $m/z = 447.3$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carboxylic acid (3,4-dichlorophenyl)amide (**12e**, $C_{22}H_{22}Cl_2N_8O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.063 g of 3,4-dichlorophenyl isocyanate (0.335 mmol), compound **12e** was obtained as off white solid (0.15 g, 92 %) after chromatography on a silica gel column with chloroform/methanol (100:2, v/v). M.p.: 196.7–198.7 °C; TLC: $R_f = 0.38$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 337$ (–NH), 3065 (=CH), 1624 (amide –C=O), 1550 (–C–C), 1477 (amide –C–O), 1378 (Ar–C–N), 818 (–C–Cl) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 2.56–2.60$ [4H, m, $N(CH_2)_2$], 2.63 (3H, s, $ArCH_3$), 2.77 (3H, s, $ArCH_3$), 3.41–3.51 [4H, m, $N(CH_2)_2$], 3.76 (2H, s, $–CH_2–$), 7.21 (1H, s, pyrimidine H), 7.45 (2H, d, $J = 12$ Hz, ArH), 7.82 (1H, s, ArH), 8.80 (1H, s, urea NH), 8.83 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 41.4, 46.0, 52.1, 52.2, 52.81, 95.2, 111.6, 129.1, 131.8, 134.2, 135.1, 144.2, 145.0, 146.0, 146.6, 147.4, 159.1, 163.4, 167.2, 170.8 ppm; LC–MS: $m/z = 502.9$ $[M+H]^+$.

*Synthesis of substituted thiourea derivatives **12f–12j** (general procedure)*

To a solution of compound **11** (1.00 mmol) in 4 cm^3 of tetrahydrofuran, corresponding isothiocyanates (1.05 mmol) were added drop wise at 0 °C under nitrogen atmosphere. The reaction medium was stirred at the same temperature for 30 min and then allowed to stir at room temperature for 1 h under nitrogen atmosphere. After completion of reaction, the reaction mixture was concentrated under reduced pressure and purified on silica gel column chromatography with chloroform/methanol (100:3, v/v) to afford corresponding urea derivatives **12f–12j**.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carbothioic acid phenylamide (**12f**, $C_{22}H_{24}N_8OS$)

From 0.100 g compound **11** (0.319 mmol) and 0.045 g of phenyl isothiocyanate (0.335 mmol), compound **12f** was obtained as an off white solid (0.12 g, 82 %) after chromatography on a silica gel column with chloroform/methanol (100:3, v/v). M.p.: 199.2–201.7 °C; TLC: $R_f = 0.44$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3296$ (–NH), 2921 (=CH), 1631 (thiourea –C=S), 1533 (–C–C), 1441 (thiourea –C–S), 1320 (Ar–C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.52–2.52$ [4H, m, $N(CH_2)_2$], 2.64 (3H, s, $ArCH_3$), 2.77 (3H, s, $ArCH_3$), 3.79 (2H, s, $–CH_2–$), 3.89–3.99 [4H, m, $N(CH_2)_2$], 7.09–7.11 (1H, m, ArH), 6.23 (1H, s, thiourea NH), 7.28 (4H, d, $J = 3.6$ Hz, ArH), 8.85 (1H, s, pyrimidine H), 9.32 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 16.9$, 24.7,

46.0, 53.4, 54.0, 95.3, 111.3, 123.8, 128.9, 129.7, 130.8, 132.0, 135.0, 145.1, 146.4, 147.4, 163.3, 167.4, 171.1, 179.8 ppm; LC–MS: $m/z = 449.3$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carbothioic acid isopropylamide (**12g**, $C_{19}H_{26}N_8OS$)

From 0.100 g compound **11** (0.319 mmol) and 0.034 g of isopropyl isothiocyanate (0.335 mmol), compound **12g** was obtained as a white solid (0.11 g, 84 %) after chromatography on a silica gel column with chloroform/methanol (100:3, v/v). M.p.: 175.0–177.5 °C; TLC: $R_f = 0.41$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3335$ (–NH), 3010 (–CH), 2966 (–CH_3), 2866 (=CH), 1631 (thiourea –C=S), 1531 (–C–C), 1447 (thiourea –C–S), 1346 (Ar–C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.12$ (6H, d, $J = 6.3$ Hz, RCH_3), 2.51–2.59 [4H, m, $N(CH_2)_2$], 2.64 (3H, s, $ArCH_3$), 2.77 (3H, s, $ArCH_3$), 3.75 (2H, s, –CH_2–), 3.79–3.88 [4H, m, $N(CH_2)_2$], 4.48–4.54 (1H, s, R_2CH), 7.22 (1H, s, pyrimidine H), 7.31 (1H, d, $J = 7.8$ Hz, thiourea NH), 8.84 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.8$, 22.3 (two peaks), 24.8, 47.3, 47.4, 52.1, 52.3, 95.1, 111.4, 145.0, 146.3, 147.3, 163.2, 167.1, 170.6, 180.6 ppm; LC–MS: $m/z = 415.4$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carbothioic acid cyclohexylamide (**12h**, $C_{22}H_{30}N_8OS$)

From 0.100 g compound **11** (0.319 mmol) and 0.047 g of cyclohexyl isothiocyanate (0.335 mmol), compound **12h** was obtained as an off white solid (0.13 g, 88 %) after chromatography on a silica gel column with chloroform/methanol (100:3, v/v). M.p.: 186.1–188.5 °C; TLC: $R_f = 0.39$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3309$ (–NH), 3080 (–CH), 2924 (=CH), 1632 (thiourea –C=S), 1525 (–C–C), 1443 (thiourea –C–S), 1350 (Ar–C–N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.00$–1.12 (2H, m, cyclohexane CH_2), 2.25–3.00 (4H, m, cyclohexane CH_2), 2.62–2.71 (2H, m, cyclohexane CH_2), 2.81–2.89 (2H, m, cyclohexane CH_2), 2.52–2.59 [4H, m, $N(CH_2)_2$], 2.64 (3H, s, $ArCH_3$), 2.73 (3H, s, $ArCH_3$), 3.71 (2H, s, –CH_2–), 3.75–3.83 [4H, m, $N(CH_2)_2$], 4.11–4.20 (1H, m, cyclohexyl CH), 7.19 (1H, s, pyrimidine H), 7.23 (1H, d, $J = 7.2$ Hz, thiourea NH), 8.80 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.9$, 24.9, 25.5, 25.7, 32.5, 47.4, 52.1, 52.4, 54.9, 95.2, 111.5, 145.1, 146.5, 147.4, 163.4, 167.1, 170.8, 180.7 ppm; LC–MS: $m/z = 455.4$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carbothioic acid tert-butylamide (**12i**, $C_{20}H_{28}N_8OS$)

From 0.100 g compound **11** (0.319 mmol) and 0.039 g of *tert*-butyl isothiocyanate (0.335 mmol), compound **12i** was obtained as an off white solid (0.12 g, 86 %) after chromatography on a silica gel column with chloroform/methanol (100:3, v/v). M.p.: 144.3–145.9 °C; TLC: $R_f = 0.45$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3393$ (–NH), 3069 (–CH), 2920 (–CH_3), 2852 (=CH), 1629 (thiourea –C=S), 1534 (–C–C), 1463 (thiourea –C–S), 1334 (Ar–C–N) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.44$ [9H, s, –$C(CH_3)_3$], 2.51–2.59 [4H, m, $N(CH_2)_2$], 2.63 (3H, s, $ArCH_3$), 2.76 (3H, s, $ArCH_3$), 3.69–3.85 [6H, m, –CH_2–, $N(CH_2)_2$], 6.72 (1H, s, thiourea NH), 7.21 (1H, s, pyrimidine H), 8.82 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 14.4$, 24.9, 29.4 (three peaks), 47.6, 52.1, 52.6 (two peaks), 53.6, 95.2, 111.5, 145.1, 146.4, 147.4, 163.4, 167.2, 170.8, 181.6 ppm; LC–MS: $m/z = 429.4$ $[M+H]^+$.

4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazine-1-carbothioic acid propylamide (**12j**, $C_{19}H_{26}N_8OS$)

From 0.100 g compound **11** (0.319 mmol) and 0.034 g of *n*-propyl isothiocyanate (0.335 mmol), compound **12j** was obtained as an off white solid (0.12 g, 87 %) after chromatography on a silica gel column with chloroform/methanol (100:3, v/v). M.p.: 154.5–156.4 °C; TLC: $R_f = 0.46$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3348$ (–NH), 3002 (–C–H), 2926 (=CH), 1605 (thiourea –C=S), 1533 (–C–C), 1440 (thiourea –C–S), 1326 (Ar–C–N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 0.84$ (3H, t, $J = 8$ Hz, –CH_3), 1.49–1.58 (2H, m, –CH_2–), 2.50–2.52 [4H, m, $N(CH_2)_2$], 2.63 (3H, s, $ArCH_3$), 2.76 (3H, s, $ArCH_3$), 3.40 (2H, t, $J = 10$ Hz, –CH_2–), 3.74 (2H, s, –CH_2–), 3.78–3.82 [4H, m, $N(CH_2)_2$], 7.21 (1H, s, pyrimidine H), 7.69 (1H, t, $J = 3$ Hz, thiourea NH), 8.82 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 11.6$, 16.8, 22.2, 24.8, 47.3, 47.4, 52.1, 52.3, 95.1, 111.4, 145.0, 146.3, 147.3, 163.3, 167.1, 170.7, 181.6 ppm; LC–MS: $m/z = 416.2$ $[M+H]^+$.

*Synthesis of substituted carboxamides **12k–12o** (general procedure)*

To a solution of the compound **11** (1.00 mmol) in 3 cm^3 of dimethylacetamide was added *N*-methylmorpholine (5.00 mmol) followed by drop wise addition of corresponding acid chlorides (1.30 mmol) at 0 °C under nitrogen atmosphere. The reaction medium was stirred at the same temperature for 10 min and then allowed to stir at room temperature for 1 h under nitrogen atmosphere. After completion of reaction, water was added to the reaction mixture and stirred for 10 min. The solid precipitated out was filtered, washed with water and dried under vacuum to afford the corresponding carboxamides **12k**–**12o**.

(3,4-Dichlorophenyl){4-[5-(5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazin-1-yl}methanone (**12k**, $C_{22}H_{21}Cl_2N_7O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.087 g of 3,4-dichlorobenzoyl chloride (0.414 mmol), compound

12k was obtained as off white solid (0.14 g, 89 %) after recrystallization with water. M.p.: 224.4–226.9 °C; TLC: $R_f = 0.38$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 2911$ (=CH), 1628 (–C=O), 1547 (–C–C), 1440 (–C–O), 1379 (Ar–C–N), 827 (–C–Cl) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.55$–2.65 [4H, m, $N(CH_2)_2$], 2.62 (3H, s, $ArCH_3$), 2.77 (3H, s, $ArCH_3$), 3.59–3.69 [4H, m, $N(CH_2)_2$], 3.75 (2H, s, $–CH_2–$), 7.21 (1H, s, pyrimidine H), 7.38 (1H, dd, $J = 4$ Hz, 8 Hz, ArH), 7.76–7.70 (2H, m, ArH), 8.83 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.8, 41.8, 46.1, 52.5, 52.7, 95.0, 111.4, 128.6, 129.0, 131.7, 134.0, 144.8, 145.0, 146.5, 146.9, 147.4, 158.6, 162.8, 166.7, 171.0 ppm; LC–MS: $m/z = 486.4$ $[M+H]^+$.

(3,5-Difluorophenyl){4-[5-(5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazin-1-yl}methanone (**12l**, $C_{22}H_{21}F_2N_7O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.073 g of 3,5-difluorobenzoyl chloride (0.414 mmol), compound **12l** was obtained as a white solid (0.13 g, 87 %) after recrystallization with water. M.p.: 198.9–201.2 °C; TLC: $R_f = 0.37$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 2927$ (=CH), 1627 (–C=O), 1542 (–C–C), 1434 (–C–O), 1378 (Ar–C–N), 1124 (–C–F) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.48$–2.53 [4H, m, $N(CH_2)_2$], 2.63 (3H, s, $ArCH_3$), 2.76 (3H, s, $ArCH_3$), 3.51–3.65 [4H, m, $N(CH_2)_2$], 3.76 (2H, s, $–CH_2–$), 7.15 (2H, d, $J = 4.8$ Hz, ArH), 7.21 (1H, s, pyrimidine H), 7.34 (1H, t, $J = 9$ Hz, ArH), 8.82 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 41.9, 47.3, 52.3, 52.7, 95.2, 105.4 (d, $^2J_{C\text{-}F} = 26$ Hz), 105.6 (d, $^2J_{C\text{-}F} = 26$ Hz), 110.8 (d, $^2J_{C\text{-}F} = 26$ Hz), 111.6, 139.8 (d, $^3J_{C\text{-}F} = 8$ Hz), 145.2, 146.5, 147.5, 162. 6 (d, $^1J_{C\text{-}F} = 249$ Hz), 163.5, 164.1 (d, $^1J_{C\text{-}F} = 249$ Hz), 166.7, 167.3, 170.8 ppm; LC–MS: $m/z = 454.4$ $[M+H]^+$.

{4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazin-1-yl}[3-(trifluoromethyl)phenyl]methanone (**12m**, $C_{23}H_{22}F_3N_7O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.086 g of 3-(trifluoromethyl)benzoyl chloride (0.414 mmol), compound **12m** was obtained as off white solid (0.14 g, 88 %) after recrystallization with water. M.p.: 185.4–187.6 °C; TLC: $R_f = 0.40$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3085$ (–ArH), 1627 (–C=O), 1542 (–C–C), 1441 (–C–O), 1335 (Ar–C–N), 1023 (–C–F) cm^{-1}; ^1H NMR (300 MHz, DMSO–d_6): $\delta = 2.52$–2.48 [4H, m, $N(CH_2)_2$], 2.62 (3H, s, $ArCH_3$), 2.76 (3H, s, $ArCH_3$), 3.55–3.69 [4H, m, $N(CH_2)_2$], 3.76 (2H, s, $–CH_2–$), 7.21 (1H, s, pyrimidine H), 7.68 (2H, d, $J = 6.3$ Hz, ArH), 7.73 (1H, s, ArH), 7.87 (1H, t, $J = 5.7$ Hz, ArH), 8.82 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.9$, 24.8, 41.9 (two peaks), 47.3 (two peaks), 52.2, 95.2, 111.5, 124.3 (q, $^1J_{C\text{-}F} = 270.75$ Hz), 124.0 (q, $^3J_{C\text{-}F} = 7.5$ Hz), 126.6 (q, $^3J_{C\text{-}F} = 6.8$ Hz), 129.5 (q, $^2J_{C\text{-}F} = 75.8$ Hz), 129.9, 131.3, 137.3, 145.1, 146.5, 147.4, 163.4, 167.2, 167.8, 170.8 ppm; LC–MS: $m/z = 486.3$ $[M+H]^+$.

(3,5-Dimethylphenyl){4-[5-(5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazin-1-yl}methanone (**12n**, $C_{24}H_{27}N_7O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.070 g of 3,5-dimethylbenzoyl chloride (0.414 mmol), compound **12n** was obtained as off white solid (0.12 g, 85 %) after recrystallization with water. M.p.: 176.6–179.4 °C; TLC: $R_f = 0.39$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3077$ (–CH), 2915 (=CH), 1627 (–C=O), 1537 (–C–C), 1463 (–C–O), 1377 (Ar–C–N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.28$ (6H, s, $ArCH_3$), 2.59–2.48 [4H, m, $N(CH_2)_2$], 2.63 (3H, s, $ArCH_3$), 2.76 (3H, s, $ArCH_3$), 3.51–3.65 [4H, m, $N(CH_2)_2$], 3.75 (2H, s, $–CH_2–$), 6.96 (2H, s, ArH), 7.06 (1H, s, ArH), 7.21 (1H, s, pyrimidine H), 8.83 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 21.2 (two peaks), 24.9, 47.5, 52.4, 52.6, 95.2, 111.6, 124.9, 131.1, 136.3, 138.0, 145.1, 146.5, 147.4, 163.4, 167.3, 169.6, 170.8 ppm; LC–MS: $m/z = 446.4$ $[M+H]^+$.

[4-[5-(5,7-Dimethylpyrazolo[1,5-a]pyrimidin-3-yl)-1,2,4-oxadiazol-3-ylmethyl]piperazin-1-yl](2,4,6-trichlorophenyl)methanone (**12o**, $C_{22}H_{20}Cl_3N_7O_2$)

From 0.100 g compound **11** (0.319 mmol) and 0.101 g of 2,4,6-trichlorobenzoyl chloride (0.414 mmol), compound **12o** was obtained as a white solid (0.15 g, 90 %) after recrystallization with water. M.p.: 187.2–189.3 °C; TLC: $R_f = 0.44$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 2956$ (=CH), 1654 (–C=O), 1545 (–C–C), 1441 (–C–O), 1326 (Ar–C–N), 857 (–C–Cl) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.53$–2.54 (2H, m, $NCH_2–$), 2.60–2.62 (2H, m, $NCH_2–$), 2.63 (3H, s, $ArCH_3$), 2.76 (3H, s, $ArCH_3$), 3.19 (2H, t, $J = 5$ Hz, $NCH_2–$), 3.67 (2H, t, $J = 5$ Hz, $NCH_2–$), 3.77 (2H, s, $–CH_2–$), 7.21 (1H, s, pyrimidine H), 7.78 (2H, s, ArH), 8.83 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 41.5, 46.1, 52.2, 52.2, 52.8, 95.2, 111.6, 128.7 (two peaks), 131.9 (two peaks), 134.1, 135.0, 145.1, 146.5, 147.5, 162.2, 163.5, 167.2, 170.8 ppm; LC–MS: $m/z = 522.1$ $[M+H]^+$.

*Synthesis of substituted sulfonamides **12p–12t** (general procedure)*

To a solution of compound **11** (1.00 mmol) in 3 cm^3 of dimethylacetamide was added *N*-methylmorpholine (5.00 mmol) followed by drop wise addition of corresponding sulfonyl chlorides (1.30 mmol) at 0 °C under nitrogen atmosphere. The reaction medium was stirred at the same temperature for 10 min and then allowed to stir at room temperature for 1 h under nitrogen atmosphere. After

completion of reaction, water was added to the reaction mixture and stirred for 30 min at room temperature. The solid precipitated out was filtered, washed with water and dried under vacuum at 50 °C for 1 h to afford the corresponding sulfonamides **12p**–**12t**.

3-{3-[4-(4-Chloro-3-fluorobenzenesulfonyl)piperazin-1-ylmethyl]-1,2,4-oxadiazol-5-yl}-5,7-dimethylpyrazolo[1,5-a]pyrimidine (**12p**, $C_{21}H_{21}ClFN_7O_3S$)

From 0.100 g compound **11** (0.319 mmol) and 0.095 g of 4-chloro-3-fluorobenzenesulfonyl chloride (0.414 mmol), compound **12p** was obtained as an off white solid (0.13 g, 85 %) after recrystallization with water. M.p.: 202.0–204.1 °C; TLC: $R_f = 0.46$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3087$ (=CH), 1548 (–C–C), 1346 (sulfonyl –S=O), 1328 (Ar–C–N), 1055 (–C–F), 817 (–C–Cl) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.52$–2.60 [4H, m, $N(CH_2)_2$], 2.61 (3H, s, $ArCH_3$), 2.74 (3H, s, $ArCH_3$), 2.92–3.01 [4H, m, $N(CH_2)_2$], 3.71 (2H, s, –H_2–), 7.20 (1H, s, pyrimidine H), 7.58 (1H, d, $J = 8$ Hz, ArH), 7.78 (1H, d, $J = 8$ Hz, ArH), 7.87 (1H, t, $J = 16$ Hz, ArH), 8.78 (1H, s, pyrazole H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 16.9$, 24.8, 46.1 (two peaks), 51.5 (two peaks), 51.8, 95.1, 111.5, 116.5 (d, $^2J_{C\text{-}F} = 23.25$ Hz), 125.1 (d, $^3J_{C\text{-}F} = 3.75$ Hz), 125.5 (d, $^2J_{C\text{-}F} = 18$ Hz), 132.3, 136.2 (d, $^3J_{C\text{-}F} = 6$ Hz), 145.1, 146.4, 147.4, 157.5 (d, $^1J_{C\text{-}F} = 250.50$ Hz), 163.4, 167.2, 170.8 ppm; LC–MS: $m/z = 506.4$ $[M+H]^+$.

3-{3-[4-(Cyclopropanesulfonyl)piperazin-1-ylmethyl]-1,2,4-oxadiazol-5-yl}-5,7-dimethylpyrazolo[1,5-a]pyrimidine (**12q**, $C_{18}H_{23}N_7O_3S$)

From 0.100 g compound **11** (0.319 mmol) and 0.058 g of cyclopropylsulfonyl chloride (0.414 mmol), compound **12q** was obtained as a white solid (0.11 g, 83 %) after recrystallization with water. M.p.: 236.5–238.2 °C; TLC: $R_f = 0.48$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3094$ (–CH), 2975 (=CH), 1629 (sulfonyl –S=O), 1540 (–C–C), 1443 (sulfonyl –C–O), 1374 (Ar–C–N), 1329 (–SO_2) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 0.91$–0.99 (4H, m, cyclopropane CH_2), 2.57–2.62 [4H, m, $N(CH_2)_2$], 2.63 (3H, s, $ArCH_3$), 2.68–2.71 (1H, m, cyclopropyl CH), 2.76 (3H, s, $ArCH_3$), 3.15–3.22 [4H, m, $N(CH_2)_2$], 3.77 (2H, s, –CH_2–), 7.22 (1H, s, pyrimidine H), 8.83 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 12.4$, 16.9, 24.7, 45.7, 48.8, 52.2, 53.8, 95.5, 111.4, 144.9, 146.2, 147.29, 158.9, 163.0, 167.2, 170.6 ppm; LC–MS: $m/z = 418.3$ $[M+H]^+$.

3-{3-[4-(2-Fluorobenzenesulfonyl)piperazin-1-ylmethyl]-1,2,4-oxadiazol-5-yl}-5,7-dimethylpyrazolo[1,5-a]pyrimidine (**12r**, $C_{21}H_{22}FN_7O_3S$)

From 0.100 g compound **11** (0.319 mmol) and 0.081 g of 2-fluorobenzenesulfonyl chloride (0.414 mmol), compound **12r** was obtained as an off white solid (0.13 g, 86 %) after recrystallization with water. M.p.: 199.4–201.3 °C; TLC: $R_f = 0.44$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 2832$ (=CH), 1631 (sulfonyl –S=O), 1541 (–C–C), 1439 (sulfonyl –C–O), 1352 (Ar–C–N), 1323 (–SO_2), 937 (–C–F) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.52$–2.60 [4H, m, $N(CH_2)_2$], 2.61 (3H, s, $ArCH_3$), 2.74 (3H, s, $ArCH_3$), 3.02–3.12 [4H, m, $N(CH_2)_2$], 3.72 (2H, s, –CH_2–), 7.20 (1H, s, pyrimidine H), 7.40–7.50 (2H, m, ArH),7.72–7.78 (2H, m, ArH), 8.79 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 45.9, 51.8, 52.0, 95.1, 111.6, 118.0 (d, $^2J_{C\text{-}F} = 22$ Hz), 124.2 (d, $^2J_{C\text{-}F} = 14$ Hz), 125.6 (d, $^4J_{C\text{-}F} = 3$ Hz), 131.2 (d, $^3J_{C\text{-}F} = 8$ Hz), 131.3, 136.5 (d, $^3J_{C\text{-}F} = 8$ Hz), 145.1, 146.5, 147.5, 158. 7 (d, $^1J_{C\text{-}F} = 253$ Hz), 163.4, 167.2, 170.8 ppm; LC–MS: $m/z = 472.3$ $[M+H]^+$.

5,7-Dimethyl-3-{3-[4-(naphthalene-1-sulfonyl)piperazin-1-ylmethyl]-1,2,4-oxadiazol-5-yl}pyrazolo[1,5-a]pyrimidine (**12s**, $C_{25}H_{25}N_7O_3S$)

From 0.100 g compound **11** (0.319 mmol) and 0.094 g of 1-naphthylsulfonyl chloride (0.414 mmol), compound **12s** was obtained as an off white solid (0.13 g, 81 %) after recrystallization with water. M.p.: 210.6–212.9 °C; TLC: $R_f = 0.45$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3081$ (–CH), 2922 (=CH), 1631 (sulfonyl –S=O), 1540 (–C–C), 1437 (sulfonyl –C–O), 1381 (Ar–C–N), 1322 (–SO_2) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.54$–2.60 [4H, m, $N(CH_2)_2$], 2.66 (3H, s, $ArCH_3$), 2.74 (3H, s, $ArCH_3$), 3.05–3.15 [4H, m, $N(CH_2)_2$], 3.67 (2H, s, –CH_2–), 7.19 (1H, s, pyrimidine H), 7.62–7.73 (3H, m, ArH), 8.08 (1H, d, $J = 8$ Hz, ArH), 8.13 (1H, d, $J = 4$ Hz, ArH), 8.27 (1H, d, $J = 8$ Hz, ArH), 8.65 (1H, d, $J = 12$ Hz, ArH), 8.75 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 45.8, 51.9, 52.0, 95.2, 111.6, 125.1, 127.4, 128.6, 129.5, 130.7, 132.4, 134.4, 135.1, 145.2, 146.5, 147.5, 163.5, 167.2, 170.8 ppm; LC–MS: $m/z = 504.2$ $[M+H]^+$.

3-{3-[4-(4-Chlorobenzenesulfonyl)piperazin-1-ylmethyl]-1,2,4-oxadiazol-5-yl}-5,7-dimethylpyrazolo[1,5-a]pyrimidine (**12t**, $C_{21}H_{22}ClN_7O_3S$)

From 0.100 g compound **11** (0.319 mmol) and 0.087 g of 4-chlorobenzenesulfonyl chloride (0.414 mmol), compound **12t** was obtained as a white solid (0.14 g, 87 %) after recrystallization with water. M.p.: 207.6–210.6 °C; TLC: $R_f = 0.46$ ($CHCl_3$–MeOH 9:1); IR (ATR): $\bar{\nu} = 3083$ (ArH), 1548 (–C–C), 1443 (sulfonyl –S–O), 1348 (sulfonyl –S=O), 835 (–C–Cl) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.56$–2.60 [4H, m, $N(CH_2)_2$], 2.61 (3H, s, $ArCH_3$), 2.74 (3H, s, $ArCH_3$), 2.90–3.03 [4H, m, $N(CH_2)_2$], 3.71 (2H, s, –CH_2–), 7.19 (1H, s, pyrimidine H), 7.70 (2H, d, $J = 8.8$ Hz, ArH), 7.73 (2H, d, $J = 8.4$ Hz, ArH), 8.79 (1H, s, pyrazole H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 17.0$, 24.9, 46.2, 51.6, 51.9, 95.2, 111.6, 129.9, 130.0,

134.2, 138.7, 145.2, 146.5, 147.5, 163.5, 167.2, 170.8 ppm; LC–MS: $m/z = 489.1$ $[M+H]^+$.

Anticancer activity

All compounds were screened for their in vitro anti-cancer activity against representative human cancer cell line (HeLa cell line) by MTT assay. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent (e.g., dimethylsulfoxide, isopropanol) and then released solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of these cells.

The 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was made a solution in such a way that 10 mg was dissolved in 10 cm^3 of Hank's balanced solution. The cell lines were maintained in 96 wells microtiter plate containing MEM media supplemented with 10 % heat inactivated fetal calf serum (FCS), containing 5 % of mixture of gentamycin, penicillin (100 Units/cm^3) and streptomycin (100 μg/cm^3) in presence of 5 % CO_2 at 37 °C for 3–4 days. Then after, remove the supernatant and replace MEM media with Hank's balanced solution and the cells were incubated overnight. The in vitro growth inhibitions of test compounds were assessed by calorimetric or spectrophotometric method. This helps to determine the conversion of MTT into formazan blue by living cells. Remove the supernatant from the plate, add fresh Hank's balanced salt solution and treated with different concentration of compound (approx diluted with DMSO). The marketed anticancer drug paclitaxel was tested as a reference compound in the assay. The control group contains only DMSO. After 24 h of incubation at 37 °C in a humidified atmosphere of 5 % CO_2, the medium was replaced with MTT solution (100 mm^3, 5 mg/cm^3 in MEM medium) for further 4 h. The supernatant was carefully aspirated and the precipitated crystals of Formazan blue were solubilized by adding DMSO (200 mm^3) and optical density was measured at wavelength of 570 nm using LISA microplate reader. The results were represented out in triplicates for each concentration. Concentration at which the optical density (OD) of treated cells was reduced by 50 % with respect to the untreated control. Calculation of the percentage of lyses of cells was done by comparing the OD of sample to that of the control and also by microscopic analysis.

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