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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of piperazinyl benzothiazole/benzoxazole derivatives coupled with 1,3,4-oxadiazole-2-thiol: novel hybrid heterocycles as anticancer agents

M. S. R. Murty · B. Ramalingeswara Rao · Mohana Rao Katiki · Lekshmi R. Nath · Ruby John Anto

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Abstract The synthesis of a series of substituted 2-(piperazin-1-yl)benzothiazole/benzoxazole coupled with 1,3,4-oxadiazole-2-thiol pharmacophore (**8a–t**) is described using a three carbon spacer (Jones and Helm, Drugs 69:1903–1910, 2009). The structures of the compounds were confirmed by NMR and mass spectral data. All the synthesized compounds have been evaluated for their cytotoxicity towards five human cancer cell lines of different origins, viz. MCF-7 (Breast), HeLa (Cervical), $HepG_2$ (Liver), A431 (Skin) and A549 (Lung), and IC_{50} values were determined. Among the compounds tested, **8j** and **8t** displayed maximum cytotoxic activity. A431 was the most sensitive cell line against the compounds studied, followed by MCF7, A549, $HepG_2$ and HeLa.

Keywords Piperazine · Benzothiazole · Benzoxazole · 1,3,4-Oxadiazole · Anticancer · MTT assay

Introduction

Cancer is a serious disease that can affect almost every tissue lineage in the human body (Varmus, 2006) and poses great challenges to medical science. Hence, there is need for the discovery and development of novel antitumor drug molecules which could effectively inhibit proliferative pathways. Antiproliferative and cytotoxic drugs play a major role in cancer therapy, whether used alone or in combination with other treatments such as surgery, radiation and biological therapy. Nowadays, combination chemotherapy using drugs with different mechanisms of action is being adopted to treat cancer so that the toxicity of treatment can be minimized. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of cancer.

Long-chain arylpiperazine (LCAP) derivatives represent one of the important class of therapeutic agents in medicinal chemistry. Generally, arylpiperazine moiety is a good template for many different biological targets. In recent years, extensive research has been focused on developing novel piperazine derivatives to improve anticancer activities. Among these attempts, modifying the piperazine moiety with some functional groups such as introducing an alkyl chain constituted by two- to four carbon atoms linked to the $N-1$ of piperazine moiety and to a terminal fragment usually containing an amide or imide function were shown to be important, and the resulting compounds (**I**, **II**, **III**) have proved to exhibit high affinity for $5\text{-}HT_{1A}$ receptor (Modica *et al.*, 2000a, b). A large number of studies have been devoted to explore the role of the terminal part in ligand–receptor interaction; therefore, several structural modifications have been carried out in the terminal fragment. In this respect, the synthesis of a series arylpiperazinyl alkylthio benzheterocycles as potent and selective $5\text{-}HT_{1A}$ serotonin receptor ligands have been reported (Maria *et al.*, 2008). Our research group has been interested in the synthesis of arylpiperazine derivatives as anticancer agents. Recently, we reported the synthesis of pyridazin-3(2H)-one derivatives in which the nitrogen

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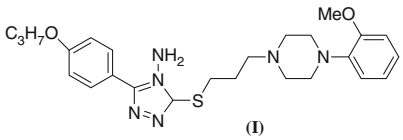
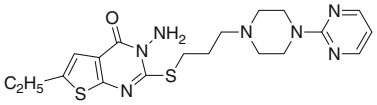
($N-2$) is substituted with different piperazines with a common three carbon spacer (Murty *et al.*, 2012). Some of these compounds showed good values in the micromolar range; in particular, compound (**IV**) exhibited good activity over HeLa (Cervical).



(I)



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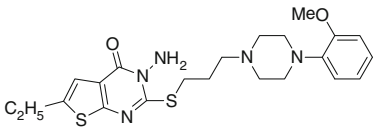

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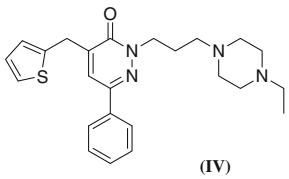
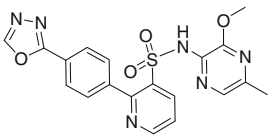


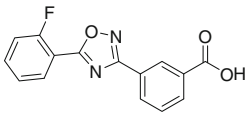
(IV)

On the other hand, benzothiazole/benzoxazole derivatives are known for different biological properties, including antimycobacterial (Klimesova *et al.*, 2009), antimalarial (Burger and Sawhney, 1968), anticonvulsant (Chakole *et al.*, 2005), antihelmintic (Jayachandran *et al.*, 2003), analgesic (Siddiqui *et al.*, 2004), anti-inflammatory (Shafi *et al.*, 2012), antidiabetic (Diaz *et al.*, 2008) and antitumor (Shi *et al.*, 1996) activities. In recent years, extensive research has focused on developing novel benzothiazole derivatives to improve antitumor activities. Among these attempts, modifying the benzothiazole moiety with some functional groups, such as benzenesulfonamide (Kamal *et al.*, 2008), imidazole (Trapani *et al.*, 2001) and aryl (Song *et al.*, 2008) were shown to be important and the resulting compounds had an inhibitory effect against certain cancer cell lines. However, most investigators focused on designing new benzothiazole compounds by substituting benzothiazole-2-thiol as a functional group (Mazaahir *et al.*, 2010). 1,3,4-Oxadiazoles is also a class of heterocycle which have attracted significant interest in medicinal chemistry and they have a wide range of biological activities. These compounds have biological activities such as antimicrobial (Karegoudar *et al.*, 2008; Mathew *et al.*, 2007), antitumoral (Ibrahim, 2009; Zhang *et al.*, 2005), diabetes (Jones *et al.*, 2009), obesity (Lee *et al.*, 2008), inflammation (Unangst *et al.*, 1992) and analgesic (Cottrell *et al.*, 2004) activities.

Even in drug discovery and development, a number of compounds containing an oxadiazole moiety are in late stage of clinical trials, e.g. zibotentan (**V**), an anticancer agent (James *et al.*, 2009) and ataluren (**VI**) for the treatment of cystic fibrosis (Jones *et al.*, 2009). Raltegravir (**VII**), an antiretroviral drug for the treatment of HIV infection (Summa *et al.*, 2008) contain oxadiazole motif, has been launched on to the market-place. Recently, oxadiazoles have been used as replacements for carbonyl-containing compounds such as esters, amides carbamates, and hydroxamic esters (Patani and LaVoie, 1996; Warmus *et al.*, 2008; McBriar *et al.*, 2008). Further, 1,3,4-oxadiazole heterocycles are very good bioisosters of amide and ester functionalities with substantial improvement in biological activity by participating in hydrogen-bonding interactions with different receptors (Guimaraes *et al.*, 2005; Rahman *et al.*, 2005).



(V)



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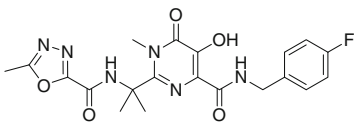
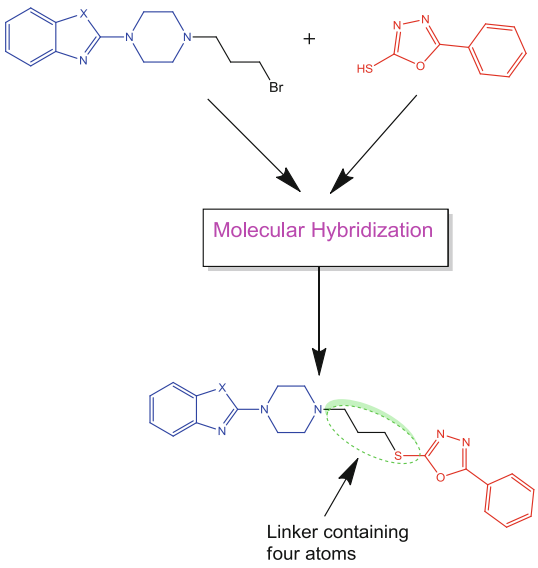


(VII)

A major interest in our group is the design, synthesis and evaluation of new antiproliferative compounds as anticancer agents. As a part of our continuous search for potential bioactive molecules for anticancer activity, a series of hybrid compounds were synthesized that comprise the piperazine, benzothiazole/benzoxazoles and 1,3,4-oxadiazole heterocyclic ring systems in a single molecule. Such hybridization was designed to investigate the effect of structural variation on the anticancer activity. Hence, in continuation of our research in this direction, we synthesized novel hybrid molecules and evaluated them for anticancer activity in random screening approach. Since it was reported in several papers that a length of four atoms is suitable for the linker (Lopez-Rodríguez *et al.*, 1997, 1999) the linker was initially fixed to this length (3C spacer) and

compound modification focused on the other two parts of the lead structure.

Thus, based on the aforementioned results, we hypothesized that integrating piperazinyl benzoxazole/benzothiazole moiety in substituted 1,3,4-oxadiazol-2-thiol scaffold (**VIII**) may lead to novel potential anticancer agents with broad biological activity profile and improved pharmacokinetic properties.



(VIII)

The title compounds **8a–t** can be divided from a structural point of view in three principal parts that may be responsible for pharmacological activity: (i) A pharmacophoric portion constituted by a substituted 2-(piperazin-1-yl)benzothiazole/benzoxazole, (ii) A terminal fragment constituted by a substituted 1,3,4-oxadiazol-2-thiol moiety and (iii) A three carbon spacer between these two substructures.

In the present study, 2-(piperazin-1-yl)benzothiazole/benzoxazoles (**3a–b**) were coupled with some 1,3,4-oxadiazole-2-thiol (**7a–j**) to produce the 20 new hybrid derivatives (**8a–t**). The newly synthesized compounds (**8a–t**) were subjected to evaluate the cell growth inhibitory activities (IC_{50}) in cultures of five different cancer cells using MTT assay and represented in Fig. 1. Most of the compounds were able to induce inhibitory activities against the proliferation of at least three cancer cell lines, while some compounds showed moderate activity.

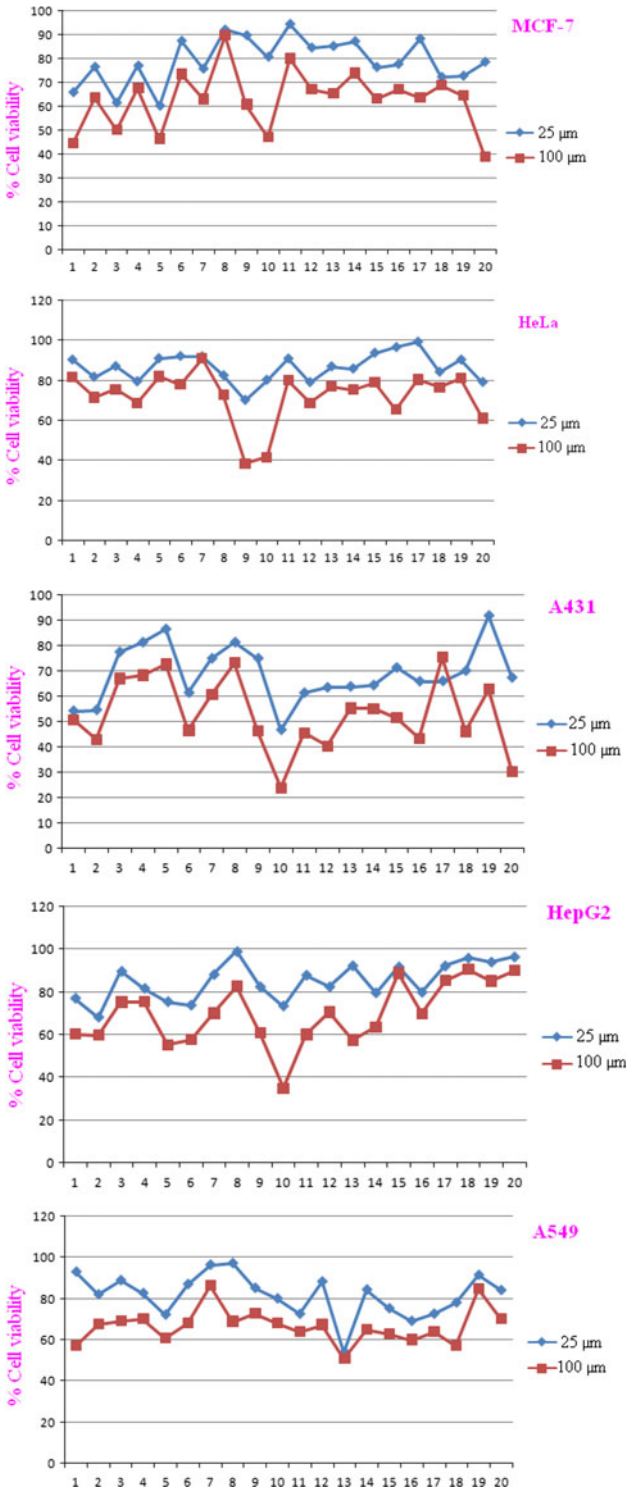


Fig. 1 Dose-dependent effect of substituted 2-(piperazin-1-yl)benzothiazole/benzoxazoles coupled with some 1,3,4-oxadiazo-2-thiol analogs **8a–t** on five cancer cell lines

Experimental

All the reagents were obtained from commercial sources. MTT was obtained from Sigma Chemicals (St. Louis, MO, USA). Melting points were determined on a Buchi capillary melting point apparatus. The NMR (300 and 400 MHz) spectra were recorded on Varian Gemini and Bruker Avance spectrometers. Chemical shifts are

expressed in ppm down field with tetramethylsilane (TMS) as an internal standard. HRMS of the compounds were recorded on high resolution QSTAR XL hybrid MS/MS system, applied bio systems under electron spray ionization method conditions preparing sample solutions in methanol.

Chemistry

*General procedure for the synthesis of 2-(piperazin -1-yl)benzo[d]oxazole/thiazole (**2a–b**)*

2-Chloro benzoxazole/benzothiazole (10 mmol, 1 equiv) was added in one portion to a solution of piperazine (60 mmol, 6 equiv) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, diluted with CH_2Cl_2 (20 mL), quenched by the addition of saturated $NaHCO_3$ (aq) (50 mL), washed with brine (50 mL), dried over Na_2SO_4 and concentrated in vacuo to provide crude product. The crude product was used directly or purified by crystallization using methanol to yield pure 2-(piperazin-1-yl)benzo[d]oxazole/thiazole.

*General procedure for the synthesis of 2-(4-(3-bromo propyl)piperazin-1-yl)benzo[d]oxazole/thiazole (**3a–b**)*

Activated zinc powder (10 mmol) is added to a solution of piperazine (10 mmol), 1,3-dibromopropane (10 mmol) in THF (10 mL) and stirred at room temperature for 2 h. After completion of the reaction, the mixture is filtered and the solid is washed with solvent ether (30 mL). The combined filtrate was treated with 10 % $NaHCO_3$ (10 mL), water (20 mL), dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography to yield pure bromopropylpiperazinylbenzo[d]oxazole/thiazole (**3a–b**).

*2-(4-(3-Bromopropyl)piperazin-1-yl)benzo[d]oxazole (**3a**)*

The compound was prepared as per the general procedure mentioned above purified and isolated as colourless solid; yield 73 %; mp: 114–116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.10–2.25 (m, 2H), 2.60–2.85 (m, 6H), 3.30–3.51 (m, 4H), 3.68 (t, 2H, $J = 7.2$ Hz), 7.30 (m, 2H), 7.52 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 45.1 ($2CH_2$), 51.7 ($2CH_2$), 108.3 (CH), 116.0 (CH), 120.3 (CH) 123.6 (CH), 143.4 (C), 148.9 (C), 162.5 (C).

*2-(4-(3-Bromopropyl)piperazin-1-yl)benzo[d]thiazole (**3b**)*

The compound was prepared as per the general procedure mentioned above purified and isolated as colourless solid; yield 78 %; Mp: 125–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.08–2.23 (m, 2H), 2.58–2.80 (m, 6H), 3.30- 3.51 (m, 4H), 3.68 (t, 2H, $J = 6.8$ Hz), 7.32 (m, 2H), 7.61 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 45.4 ($2CH_2$), 51.5 ($2CH_2$), 117.3 (CH), 118.5 (CH), 120.3 (CH) 123.6 (CH), 133.4 (C), 152.9 (C), 169.2 (C).

*General procedure for the synthesis of substituted-1,3,4-oxadiazole-2-thiol (**7a–j**)*

Substituted aromatic acids (1 mmol) were first converted into corresponding esters using catalytic amount of concentrated H_2SO_4 in methanol. To a solution of appropriate ester (1 mmol) in methanol a solution of hydrazine hydrate (3.0 mmol) was added, and the mixture is refluxed for about 2–4 h; evaporate the solvent to dryness and recrystallize the solid with ethanol. To the solution of hydrazide derivative in methanol, CS_2 (3.0 mmol) and KOH (1.5 mmol) was added. The resulting mixture was refluxed to get the substituted-1,3,4-oxadiazole-2-thiol derivatives.

Compound **7a**: mp: 169–171 °C; IR (KBr): 1,245 (C=S), 1,618 (C=N), 3,073 cm^{-1} (=C–H of Ar); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.75 (s, 3H), 7.08–7.21 (m, 3H, Ar–H), 7.59–7.64 (m, 1H, Ar–H), 7.70 (d, 1H, $J = 7.0$, Ar–H), 7.82 (d, 1H, $J = 7.0$, Ar–H), 7.95 (s, 1H, Ar–H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 56.2 (CH_3), 113.3 (2CH), 115.5 (2CH), 156.4 (C), 160.4 (C), 174.3 (C).

*General procedure for the synthesis of substituted phenyl-1,3,4-oxadiazol-2-ylthiopropylpiperazinyl benzo[d]oxazole/thiazole (**8a–t**)*

A mixture of 5-substituted-1,3,4-oxadiazole-2-thiol (3.0 mmol) and KF-Al_2O_3 (4.5 mmol) in dry acetonitrile (15 mL) was stirred for 20 min under N_2 atmosphere. Bromopropylpiperazin-yl-benzo[d]oxazole/thiazole (3.2 mmol) was added to the above mixture and stirred for about 4 h. After the completion of reaction (confirmed by TLC), the solvent was evaporated and cold water was added to the reaction mixture and stirred for 30 min. Extract the organic compound with ethyl acetate. The ethyl acetate layer is dried over anhydrous sodium sulphate. The compound was purified by column chromatography on silica eluting with EtOAc *n*-hexane.

*2-(4-(3-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio) propyl)piperazin-1-yl)benzo[d]oxazole (**8a**)*

Mp: 154–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.03–2.12 (m, 2H), 2.54–2.62 (m, 6H), 3.36 (t, 2H, $J = 7.0$ Hz), 3.68–3.74 (m, 4H), 3.86 (s, 3H), 6.93–6.99 (m, 3H), 7.11 (t, 1H, $J = 7.6$ Hz), 7.19 (d, 1H, $J = 7.6$ Hz), 7.30 (d, 1H, $J = 7.6$ Hz), 7.91 (s, 1H) 7.92 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 26.2$ (CH_2), 30.4 (CH_2), 45.4 ($2CH_2$), 52.2 ($2CH_2$), 55.3 (CH_3), 56.4 (CH_2), 108.6 (2CH), 114.4 (2CH), 116.1 (2CH), 120.6 (CH), 123.9 (2CH), 125.3 (CH), 142.9 (C),

148.6 (C), 162.1 (2C), 163.6 (C), 165.6 (C). HRMS: cal. for $C_{23}H_{25}N_5O_3S$ 452.17509, found 452.17484.

*2-(4-(3-(5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-ylthio) propyl)piperazin-1-yl)benzo[d]oxazole (**8b**)*

Mp: 165–167 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04–2.18 (m, 2H), 2.55–2.65 (m, 6H), 3.44 (t, 2H, $J = 7.3$ Hz), 3.63–3.70 (m, 4H), 7.08 (t, 1H, $J = 7.2$ Hz), 7.30 (t, 1H, $J = 7.5$ Hz), 7.55 (d, 1H, $J = 7.4$ Hz), 7.60 (d, 1H, $J = 7.4$ Hz), 8.17–8.24 (m, 2H), 8.34–8.40 (m, 2H). ^{13}C NMR ($CDCl_3$) $\delta = 26.2$ (CH_2), 30.8 (CH_2), 46.1 ($2CH_2$), 52.6 ($2CH_2$), 55.9 (CH_2), 108.6 (CH), 113.6 (CH), 115.0 (2CH), 119.5 (2CH), 123.2 (2CH), 124.3 (2CH), 142.4 (C), 147.3 (C), 161.8 (C), 162.6 (C), 165.6 (C). HRMS: cal. for $C_{22}H_{22}N_6O_4S$ 466.13679 found 466.13671.

*2-(4-(3-(5-p-tolyl-1,3,4-oxadiazol-2-ylthio)propyl) piperazin-1-yl)benzo[d]oxazole (**8c**)*

Mp: 123–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04–2.18 (m, 2H) 2.42 (s, 3H) 2.50–2.70 (m, 6H) 3.38 (t, 2H, $J = 7.0$ Hz) 3.69–3.79 (m, 4H) 6.99 (t, 1H, $J = 7.7$ Hz) 7.14 (t, 1H, $J = 7.5$ Hz) 7.19–7.36 (m, 4H,) 7.86 (s, 1H,) 7.89 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 21.3$ (CH_3), 26.2 (CH_2), 30.5 (CH_2), 48.2 ($2CH_2$), 52.3 ($2CH_2$), 56.4 (CH_2), 119.0 (2CH), 120.6 (CH), 121.3 (2CH), 126.0 (CH), 126.7 (2CH), 129.7 (CH), 130.7 (C), 142.1 (C), 152.6 (C), 164.0 (C), 165.8 (C), 168.7 (C). HRMS: cal. for $C_{23}H_{25}N_5O_2S$ = 436.18017 found 436.18023.

*2-(4-(3-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio) propyl)piperazin-1-yl)benzo[d]oxazole (**8d**)*

Mp: 138–140 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.05–2.19 (m, 2H), 2.55–2.71 (m, 6H), 3.40 (t, 2H, $J = 6.8$ Hz), 3.69–3.82 (m, 4H), 6.99 (t, 1H, $J = 7.5$ Hz), 7.13 (t, 1H, $J = 7.5$ Hz), 7.22 (d, 1H, $J = 7.5$ Hz), 7.32 (d, 1H, $J = 7.5$ Hz), 7.46 (s, 1H), 7.49 (s, 1H), 7.92–7.99 (m, 2H). ^{13}C NMR ($CDCl_3$) $\delta = 25.2$ (CH_2), 31.6 (CH_2), 46.8 ($2CH_2$), 55.3 ($2CH_2$), 56.5 (CH_2), 110.7 (CH), 114.2 (CH), 115.3 (CH), 120.6 (2CH), 123.5 (CH), 127.8 (2CH), 128.2 (2CH), 132.4 (C), 152.7 (C), 164.5 (C), 161.3 (C), 169.5 (C). HRMS: cal. for $C_{22}H_{22}ClN_5O_2S$ 456.12555 found 456.12510.

*2-(4-(3-(5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-ylthio) propyl)piperazin-1-yl)benzo[d]oxazole (**8e**)*

Mp: 157–159 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.05–2.14 (m, 2H), 2.54–2.63 (m, 6H), 3.39 (t, 2H, $J = 7.1$ Hz), 3.69–3.76 (m, 4H), 6.97 (m, 1H), 7.12 (t, 1H, $J = 7.6$ Hz), 7.16–7.22 (m, 3H), 7.31 (d, 1H $J = 7.6$ Hz), 7.99–8.06 (m, 2H). ^{13}C NMR ($CDCl_3$) $\delta = 26.3$ (CH_2), 30.5 (CH_2), 48.3 ($2CH_2$), 55.3 ($2CH_2$), 56.5 (CH_2), 108.7 (CH), 116.5 (2CH), 119.0 (CH), 120.6 (CH), 126.0 (CH), 128.9 (CH), 130.7 (2CH), 152.7 (C), 164.5 (2C), 166.3 (2C), 168.7 (C). HRMS: cal. for $C_{22}H_{22}O_2N_5FS$ 440.15510, found 440.15449.

*2-(4-(3-(5-(4-Bromophenyl)-1,3,4-oxadiazol-2-ylthio) propyl)piperazin-1-yl)benzo[d]oxazole (**8f**)*

Mp: 121–123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01–2.14 (m, 2H), 2.51–2.63 (m, 6H), 3.40 (t, 2H, $J = 7.6$ Hz), 3.66–3.75 (m, 4H), 6.95–7.03 (m, 1H), 7.12 (t, 1H, $J = 7.5$ Hz), 7.20 (d, 1H, $J = 8.3$ Hz), 7.30 (d, 1H, $J = 7.5$ Hz), 7.62 (s, 1H), 7.65 (s, 1H), 7.86 (s, 1H), 7.89 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 26.2$ (CH_2), 30.4 (CH_2), 45.5 ($2CH_2$), 52.3 ($2CH_2$), 56.5 (CH_2), 108.7 (CH), 116.2 (2CH), 120.6 (CH), 122.4 (2CH), 126.2 (CH), 127.9 (CH), 132.3 (2CH), 142.9 (C), 148.6 (C), 162.0 (C), 164.8 (C). HRMS: cal. for $C_{23}H_{27}N_5O_2Br$ 502.07275 found 502.07264.

*2-(4-(3-((5-(4-(tert-Butoxy)phenyl)-1,3,4-oxadiazol-2-yl) thio)propyl)piperazin-1-yl)benzo[d]oxazole (**8g**)*

Mp: 160–162 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ:2.02–2.18 (m, 2H), 2.50–2.65 (m, 6H), 3.37 (t, 2H, $J = 6.9$ Hz), 3.67–3.75 (m, 4H), 4.00 (t, 2H, $J = 6.5$ Hz), 6.90–7.01 (m, 3H), 7.11 (t, 1H, $J = 7.5$ Hz), 7.20 (d, 1H, $J = 7.7$ Hz), 7.30 (d, 1H, $J = 7.7$ Hz), 7.89 (s, 1H), 7.92 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 26.2$ (CH_2), 26.6 ($3CH_3$), 30.4 (CH_2), 50.8 (CH_2), 50.8 (CH_2), 52.2 (CH_2), 52.2 (CH), 87.7 (C), 109.4 (CH), 113.7 (CH), 114.5 (2CH), 115.2 (2CH), 117.7 (CH), 123.8 (CH), 124.8 (CH), 143.6 (C), 148.4 (C), 155.3 (C), 160.3 (C), 166.5 (C). HRMS: cal. for $C_{26}H_{31}N_5O_3S$ 493.21324 found 493.21305.

*4-(5-(3-(4-(Benzo[d]oxazol-2-yl)piperazin-1-yl) propylthio)-1,3,4-oxadiazol-2-yl)phenol (**8h**)*

Mp: 154–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ:2.03–2.15 (m, 2H), 2.50–2.65 (m, 6H), 3.37 (t, 2H, $J = 6.9$ Hz), 3.67–3.75 (m, 4H), 4.00 (t, 2H, $J = 6.5$ Hz), 6.90–7.01 (m, 3H), 7.11 (t, 1H, $J = 7.5$ Hz), 7.20 (d, 1H, $J = 7.7$ Hz), 7.30 (d, 1H, $J = 7.7$ Hz), 7.89 (s, 1H), 7.92 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 26.2$ (CH_2), 30.3 (CH_2), 48.3 (CH_2), 52.0 (CH_2), 60.4 (CH_2), 110.6 (CH), 115.5 (2CH), 116.4 (2CH), 118.7 (2CH), 123.8 (CH), 124.8 (CH), 142.6 (C), 149.4 (C), 160.3 (C), 161.7 (C), 167.5 (C). HRMS: cal. for $C_{22}H_{23}N_5O_3S$ 437.15124 found 437.15096.

*2-(4-(3-((5-Phenyl-1,3,4-oxadiazol-2-yl)thio)propyl) piperazin-1-yl)benzo[d]oxazole (**8i**)*

Mp: 145–147 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01–2.11 (q, 2H) 2.46–2.58 (m, 6H), 3.46 (t, 2H, $J = 6.9$ Hz),

3.65–3.78 (m, 4H), 7.04–7.12 (m, 1H), 7.36–7.42 (m, 2H), 7.46–7.63 (m, 3H), 7.94–8.10 (m, 2H). ^{13}C NMR ($CDCl_3$) δ = 25.4 (CH_2), 28.1 (CH_2), 47.5 (CH_2), 52.7 (CH_2), 55.8 (CH_2), 117.2 (CH), 120.6 (CH), 121.3 (CH), 125.9 (CH), 126.1 (CH), 126.5 (CH), 132.5 (CH), 150.6 (C), 165.4 (C), 164.1 (C), 167.9 (C). HRMS: cal. for $C_{22}H_{23}N_5O_2S$ 422.1635 found 422.16378.

*2-(5-(3-(4-(Benzo[d]oxazol-2-yl)piperazin-1-yl) propylthio)-1,3,4-oxadiazol-2-yl)-N-phenylacetamide (**8j**)*

Mp: 175–177 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ:1.95–2.08 (m, 2H), 2.48–2.61 (m, 6H), 3.70 (t, 2H, J = 5.3 Hz), 3.65–3.74 (m, 4H), 4.85 (d, 2H, J = 6.0 Hz), 7.02 (t, 1H, J = 7.5 Hz), 7.16 (t, 1H, J = 7.4 Hz), 7.23–7.30 (m, 2H), 7.35 (d, 1H, J = 7.5 Hz), 7.39–7.48 (m, 2H), 7.49–7.56 (m, 1H), 7.81–7.89 (m, 2H). ^{13}C NMR ($CDCl_3$) δ = 25.8 (CH_2), 29.5 (CH_2), 30.2 (CH_2), 34.6 (CH_2), 48.2 ($2CH_2$), 51.9 ($2CH_2$), 56.2 (CH_2), 108.6 (CH), 115.9 (CH), 120.6 (CH), 123.8 (2CH), 127.2 (2CH), 128.4 (CH), 131.8 (CH), 132.9 (CH), 142.6 (C), 148.4 (C), 161.8 (C), 164.7 (C), 165.2 (C), 168.5 (C=O). HRMS: cal. for $C_{24}H_{26}N_6O_3S$ 478.17865 found 478.17859.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**8k**)*

Mp: 131–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.04–2.12 (q, 2H), 2.54–2.64 (m, 6H), 3.37 (t, 2H, J = 6.7 Hz), 3.63–3.69 (m, 4H), 3.87 (s, 3H), 6.98–7.00 (m, 2H) 7.07 (t, 1H, J = 7.7 Hz), 7.29 (t, 1H, J = 7.7 Hz), 7.55 (d, 1H, J = 7.7 Hz), 7.60 (d, 1H, J = 7.7 Hz), 7.93 (s, 1H), 7.95 (s, 1H). ^{13}C NMR ($CDCl_3$) δ = 26.2 (CH_2), 30.4 (CH_2), 48.2 (CH_2), 52.2 (CH_2), 55.3 (CH_2), 56.4 (CH_2), 114.4 (CH), 116.1 (CH), 119.0 (CH), 120.6 (CH), 121.3 (CH), 125.3 (CH), 125.9 (CH), 128.3 (CH), 130.6 (CH), 162.1 (C), 163.6 (C), 165.6 (C), 168.6 (C). HRMS: cal. for $C_{23}H_{25}N_5O_2S_2$ 468.15224, found 468.15182.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**8l**)*

Mp: 144–146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01–2.15 (q, 2H), 2.52–2.67 (m, 6H), 3.44 (t, 2H, J = 6.5 Hz), 3.59–3.71 (m, 4H), 7.03–7.11 (m, 1H), 7.24–7.31 (m, 1H), 7.45–7.64 (m, 1H), 8.21 (s, 1H), 8.22 (s, 1H), 8.37 (s, 1H), 8.39 (s, 1H). ^{13}C NMR ($CDCl_3$) δ = 26.2 (CH_2), 30.4 (CH_2), 45.3 ($2CH_2$), 52.1 ($2CH_2$), 55.2 (CH_2), 109.2 (CH), 113.6 (CH), 115.5 (2CH), 119.2 (2CH), 123.4 (CH), 124.3 (CH), 142.9 (C), 147.8 (C), 161.1 (C), 163.8 (C), 166.7 (C). HRMS: cal. for $C_{22}H_{22}N_6O_3S_2$ 483.12342, found 483.12328.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-p-tolyl-1,3,4-oxadiazole (**8m**)*

Mp: 159–161 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.05–2.13 (m, 2H), 2.42 (s, 3H), 2.56–2.65 (m, 6H), 3.38 (t, 2H, J = 7.2 Hz), 3.63–3.70 (m, 4H), 7.07 (t, 1H, J = 7.6 Hz), 7.27–7.32 (m, 3H, J = 7.5 Hz), 7.55 (d, 1H, J = 8.6 Hz), 7.59 (d, 1H, J = 7.6 Hz), 7.88 (s, 1H), 7.90 (s, 1H). ^{13}C NMR ($CDCl_3$) δ = 21.6 (CH_2), 26.3 (CH_2), 30.4 (CH_2), 48.2 ($2CH_2$), 52.3 ($2CH_2$), 56.4 (CH_2), 119.0 (CH), 120.6 (CH), 121.3 (CH), 125.9 (CH), 126.5 (CH), 129.7 (CH), 130.7 (CH), 142.1 (C), 152.6 (C), 164.0 (C), 165.8 (C), 168.7 (C). HRMS: cal. for $C_{23}H_{25}N_5OS_2$ 451.15502, found 451.1496.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-(4-chlorophenyl)-1,3,4-oxadiazole (**8n**)*

Mp: 155–157 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.03–2.13 (q, 2H), 2.54–2.69 (m, 6H), 3.40 (t, 2H, J = 6.7 Hz), 3.62–3.71 (m, 4H), 7.05–7.12 (m, 1H), 7.27–7.34 (m, 1H), 7.47 (s, 1H), 7.50 (s, 1H), 7.60 (d, 1H, J = 7.5 Hz), 7.93 (s, 1H) 7.96 (s, 1H).^{13}C NMR ($CDCl_3$) δ = 25.9 (CH_2), 30.1 (CH_2), 47.2 (CH_2), 55.3 (CH_2), 56.5 (CH_2), 108.7 (CH), 116.5 (CH), 116.2 (CH), 119.0 (CH), 120.6 (CH), 121.4 (CH), 126.0 (CH), 128.8 (CH), 128.9 (CH), 130.7 (CH), 152.7 (C), 164.5 (C), 166.3 (C), 168.7 (C). HRMS: cal. for $C_{22}H_{22}ClN_5OS_2$ 472.10076, found 472.10065.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-(4-fluorophenyl)-1,3,4-oxadiazole (**8o**)*

Mp: 133–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.06–2.16 (q, 2H), 2.54–2.64 (m, 6H), 3.39 (t, 2H, J = 7.2 Hz), 3.62–3.69 (m, 4H), 6.99–7.07 (m, 1H), 7.14–7.30 (m, 3H), 7.60 (d, 1H, J = 8.3 Hz), 7.55 (d, 1H, J = 8.3 Hz), 7.98–8.06 (m, 2H). ^{13}C NMR ($CDCl_3$) δ = 26.3 (CH_2), 30.4 (CH_2), 48.3 (CH_2), 52.3 (CH_2), 56.4 (CH_2), 116.2 (CH), 116.5 (CH), 119.0 (CH), 120.6 (CH), 121.4 (CH), 125.9 (CH), 128.8 (CH), 128.9 (CH), 130.7 (CH), 152.7 (C), 164.5 (C), 164.8 (C), 166.3 (C), 168.7 (C). HRMS: cal. for $C_{22}H_{22}FN_5OS_2$ 456.12871, found 456.12867.

*2-((3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propyl)thio)-5-(4-bromophenyl)-1,3,4-oxadiazole (**8p**)*

Mp: 143–145 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.05–2.16 (m, 2H), 2.53–2.64 (m, 6H), 3.51 (t, 2H, J = 7.6 Hz), 3.66–3.75 (m, 4H), 6.95–7.03 (m, 1H), 7.12 (t, 1H, J = 7.5 Hz), 7.20 (d, 1H, J = 8.3 Hz), 7.30 (d, 1H, J = 7.5 Hz), 7.44 (s, 1H), 7.56 (s, 1H), 7.72 (s, 1H), 7.78 (s, 1H). ^{13}C NMR ($CDCl_3$) δ = 26.0 (CH_2), 30.9 (CH_2), 45.5 (CH_2), 52.3 (CH_2), 56.5 (CH_2), 108.7 (CH), 118.2 (CH), 121.6 (CH), 123.4 (CH), 125.2 (CH), 128.6 (CH),

131.7 (CH), 142.9 (C), 148.6 (C), 161.6 (C), 163.8 (C). HRMS: cal. for $C_{22}H_{22}BrN_5OS_2$ 516.03842, found 516.03836.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-(4-tert-butoxyphenyl)-1,3,4-oxadiazole (**8q**)*

Mp: 135–137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04–2.17 (m, 2H), 2.55–2.72 (m, 6H), 3.40 (t, 2H, $J = 7.2$ Hz), 3.64–3.71 (m, 4H), 4.05 (t, 2H, $J = 7.0$ Hz), 6.90–7.01 (m, 3H), 7.09 (t, 1H, $J = 7.5$ Hz), 7.18 (d, 1H, $J = 6.7$ Hz), 7.32 (d, 1H, $J = 7.5$ Hz), 7.78 (s, 1H) 7.80 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 26.8$ ($3CH_3$), 26.2 (CH_2), 51.3 (CH_2), 51.3 (CH_2), 52.0 (CH_2), 52.0 (CH_2), 60.4 (CH_2), 86.0 (CH_2), 114.9 (CH), 114.9 (CH), 115.5 (2CH), 117.7 (CH), 118.3 (CH), 121.8 (CH), 124.5 (CH), 125.3 (CH), 130.8 (CH), 153.2 (C), 157.4 (C), 168.0 (C), 164.5 (C). HRMS: cal. for $C_{26}H_{31}N_5O_2S_2$ 510.18823, found 510.18816.

*4-(5-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl) propylthio)-1,3,4-oxadiazol-2-yl)phenol (**8r**)*

Mp: 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ:1.99–2.13 (q, 2H), 2.55–2.64 (m, 6H), 2.86 (s, 1H), 3.37 (t, 2H, $J = 7.5$ Hz), 3.62–3.69 (m, 4H), 6.92 (s, 1H), 6.95 (s, 1H), 7.08 (m, 1H), 7.56 (d, 1H, $J = 7.7$ Hz), 7.60 (d, 1H, $J = 8.6$ Hz), 7.87 (s, 1H), 7.97 (s, 1H). ^{13}C NMR ($CDCl_3$) $\delta = 28.0$ (CH_2), 34.1 (CH_2), 51.3 (CH_2), 52.0 (CH_2), 60.4 (CH_2), 116.4 (CH), 116.4 (CH), 116.3 (CH), 116.3 (CH), 118.3 (CH), 118.7 (CH), 121.8 (CH), 124.5 (CH), 125.3 (CH), 130.8 (CH), 153.2 (C), 158.5 (C), 164.5 (C), 168.0 (C). HRMS : cal. for $C_{22}H_{23}N_5O_2S_2$ 454.13267, found 446.13249.

*2-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-5-phenyl-1,3,4-oxadiazole (**8s**)*

Mp: 121–123 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.03–2.15 (q, 2H), 2.54–2.64 (m, 6H), 3.40 (t, 2H, $J = 6.9$ Hz), 3.62–3.70 (m, 4H), 7.04–7.12 (m, 1H), 7.46–7.63 (m, 5H), 7.98–8.05 (m, 2H). ^{13}C NMR ($CDCl_3$) $\delta = 26.3$ (CH_2), 30.4 (CH_2), 48.3 (CH_2), 52.3 (CH_2), 56.4 (CH_2), 119.0 (CH), 120.6 (CH), 121.3 (CH), 125.9 (2CH), 128.9 (CH), 131.5 (CH), 152.6 (C), 164.4 (C), 165.6 (C), 168.6 (C). HRMS: cal. for $C_{22}H_{23}N_5OS_2$ 438.14100, found 438.14092.

*2-(5-(3-(4-(Benzo[d]thiazol-2-yl)piperazin-1-yl)propylthio)-1,3,4-oxadiazol-2-yl)-N-phenylacetamide (**8t**)*

Mp: 184–186 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.06–2.15 (q, 2H), 2.54–2.69 (m, 6H), 3.40 (t, 2H, $J = 6.7$ Hz), 3.62–3.71 (m, 4H), 4.63 (s, 2H), 7.05–7.12 (m, 1H), 7.27–7.34 (m, 1H), 7.47 (s, 1H), 7.50 (s, 1H), 7.60 (d, 1H, $J = 7.5$ Hz), 8.23–8.26 (m, 2H). ^{13}C NMR ($CDCl_3$) $\delta = 26.0$ (CH_2), 29.5 (CH_2), 30.3 (CH_2), 34.8 (CH_2), 48.0 ($2CH_2$), 52.2 ($2CH_2$), 56.3 (CH_2), 118.9 (CH), 120.6 (CH), 121.4 (CH), 125.9 (2CH), 127.1 (CH), 128.5 (CH), 129.6 (CH), 130.6 (CH), 132.0 (CH), 133.0 (CH), 152.5 (C), 164.6 (2C), 165.4 (C), 167.4 (C), 168.6 (C=O). HRMS: cal. for $C_{24}H_{26}N_6O_2S_2$ 494.15788, found 494.15776.

Biology

Maintenance of the cells

The human cancer cells of various origin (HeLa: Cervix, MCF-7: Breast, A549: Lung, $HepG_2$: Colon, Skin: A431) were procured from National Centre for Cell sciences, Pune, and maintained in DMEM containing 10 % FBS with antibiotics and antimycotics at 37 °C in a CO_2 incubator.

MTT assay

The cytotoxic activity of the compounds was assessed by standard MTT assay in different cancer cells at 72 h of drug administration as described earlier (Smitha *et al.*, 2005). This assay measures the percentage viability of the cells in response to different concentrations of the compounds. Active mitochondrial dehydrogenases of living cells convert the water soluble yellow tetrazolium salt to an insoluble purple formazan. The intensity of colour developed is an indicator of the percentage of viable cells present. In brief, cells (3,000/well) were plated in 96-well plates and kept overnight at 37 °C after which, the cells were incubated with and without various concentrations of the compounds (25, 50, 100 and 250 μM). Curcumin was used as the positive control. At the end of the incubation, medium was removed and fresh medium containing 20 % MTT solution (2 mg/mL in 1× PBS) was added to each well. After 2 h, 0.1 mL of the extraction buffer (20 % SDS and 50 % DMF) was added, and the optical density was measured at 570 nm using a plate reader (Bio-Rad) after 1 h and compared with the untreated control. The percentage of inhibition of cell viability was determined with reference to the untreated control. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} concentrations were calculated by the respective regression analysis.

Results and discussion

Chemistry

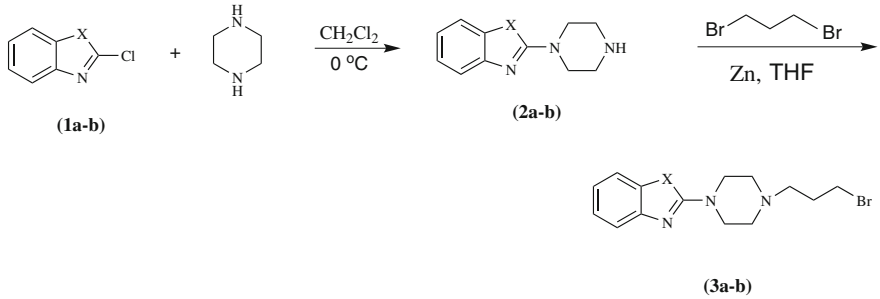
In the present article, a series of novel 5-substituted-1,3,4-oxadiazole-2-thiol containing piperazinyl benzoxazole/

benzothiazole **8a–t** compounds were synthesized by integrating piperazinyl benzothiazole/benzoxazoles **3a–b** with 5-substituted-1,3,4-oxadiazole-2-thiol. The synthetic route for the preparation of target compounds is summarized in Schemes 1, 2, 3.

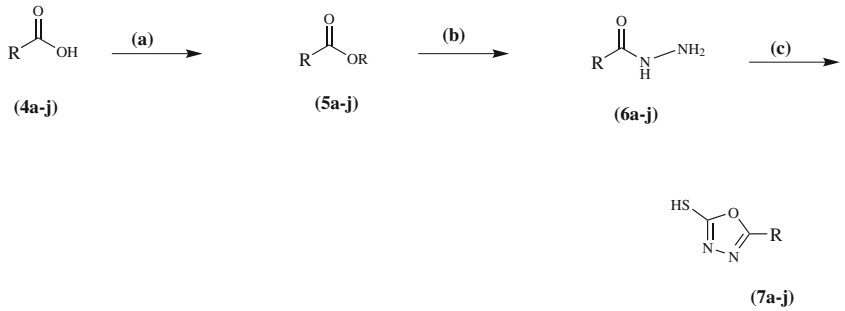
Initially, the compounds **3a–b** were synthesized by the reaction of 2-Chloro benzoxazole/benzothiazole with piperazine, which on subsequent alkylation with 1,3-dibromopropane to yield **3a–b** (Scheme 1).

On the other hand, 5-substituted-1,3,4-oxadiazole-2-thiol (**7a–j**) were prepared starting with the appropriate carboxylic acid which is converted to the corresponding ester **5a–j**. The compounds **5a–j** were hydrazinolyzed using hydrazine hydrate in methanol at 100 °C to get aryl hydrazides **6a–j**. The compounds **6a–j** on reaction with carbon disulphide in ethanolic potassium hydroxide solution under reflux condition yielded corresponding 5-substituted-1,3,4-oxadiazole-2-thione **7a–j** (Scheme 2). These compounds exist in keto-enol form as shown below.

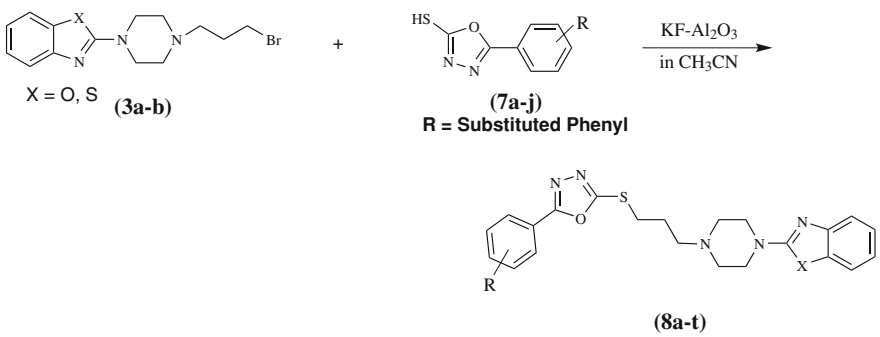
The compounds **7a–j** were then treated with piperazinyl benzothiazole/benzoxazoles **3a–b** using KF-Al_2O_3 and acetonitrile as solvent at 80 °C to afford the final target compounds (**8a–t**; Scheme 3). The formation of *S*-alkylated



Scheme 1 Synthetic pathway of 2-(4-(3-bromopropyl)piperazin-1-yl)benzo[d]oxazole/thiazole (**3a–b**)

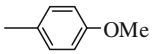
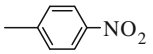
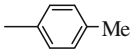
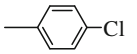
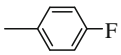
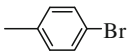
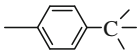
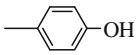
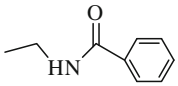
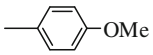
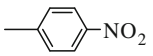
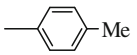
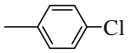
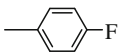
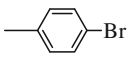
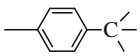
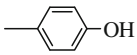
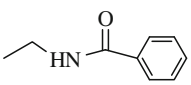


Scheme 2 Reagents and conditions: (*a*) H_2SO_4, MeOH, rt (*b*) NH_2NH_2. H_2O, MeOH, reflux (*c*) CS_2, KOH, EtOH



Scheme 3 Coupling strategy of 2-(4-(3-bromopropyl)piperazin-1yl)benzo[d]oxazole/thiazole and 5-substituted-1,3,4-oxadiazole-2-thiol (**8a–t**)

Table 1 The chemical structure of piperazinyl benzoxazole/thiazole derivatives **8a–t**

Compound[a]	R	X	% Yield[b]
8a		O	86
8b		O	88
8c		O	79
8d		O	87
8e		O	90
8f		O	82
8g		O	70
8h		O	72
8i		O	85
8j		O	88
8k		S	69
8l		S	70
8m		S	85
8n		S	90
8o		S	93
8p		S	82
8q		S	79
8r		S	74
8s		S	87
8t		S	80

[a] All the compounds were characterized by ^{1}H NMR, IR and mass spectroscopy

[b] Isolated and optimized yields

products were confirmed by the absence of C=S characteristic peak at 174.3 ppm in ^{13}C NMR spectrum. Further, the structures of final target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and Mass spectral analysis. All the obtained compounds are in good yield with high purity. The structures and isolated yields of the synthesized molecules are tabulated in Table 1.

In vitro anticancer screening

The newly synthesized compounds, **8a–t** were tested for in vitro biological screening for their cytotoxicity towards cancer cell lines of various origin by MTT assay. The compounds were tested against five human cancer cell lines, namely cervical (HeLa), breast (MCF 7), colon (HCT116), skin (A375), lung (A549) and liver (HepG2) cell lines and the results were presented in the Fig. 1 indicate the percentage cytotoxic activity (dose dependent) of the synthesized compounds at concentrations ranging from 25 to 100 μM. The relationship between fraction of surviving cells for different cell lines and drug concentration was plotted and the response parameter IC_{50}, which is the concentration required for 50 % inhibition of cell viability, was calculated. The IC_{50} values of the test compounds are shown in Table 2.

Table 2 Cytotoxic activity (IC50, μM) of compounds **8a–t** against five human cancer cell lines

Compound	MCF 7	HeLa	A431	HepG2	A549
8a	52.8	209.9	52.7	66.1	93.5
8b	90.1	113.9	*46.9*	67.2	90.8
8c	60.4	132.9	100.0	154.1	106.3
8d	99.7	101.1	89.6	120.7	126.8
8e	52.3	196.3	135.7	83.8	75.7
8f	115.0	186.7	57.8	67.5	123.2
8g	94.9	269.4	65.8	120.7	263.4
8h	260.4	99.5	116.5	502.0	180.6
8i	117.2	67.02	57.6	101.5	107.5
8j	52.7	63.9	*36.9*	88.9	102.0
8k	171.2	165.2	56.2	133.9	90.3
8l	117.8	97.8	*46.5*	93.3	96.6
8m	106.8	179.3	61.6	96.7	53.9
8n	116.0	177.3	69.2	87.7	110.0
8o	84.1	134.2	67.0	234.0	71.7
8p	96.3	243.4	51.6	103.4	62.5
8q	89.3	216.6	67.0	201.9	71.6
8r	81.9	139.0	54.7	364.4	62.1
8s	76.9	229.3	93.4	216.8	256.9
8t	39.0	78.1	55.9	325.1	88.7
Curcumin	26.0	17.0	22.0	16.0	22.0

The compounds that showed good activity are shown in italics

The obtained data revealed that most of the synthesized compounds showed potent anticancer activity against A431 cell line. Most of the compounds showed IC_{50} values less than 100 in MCF-7 cell line, out of which, **8a**, **8e**, **8j** and **8t** are more cytotoxic compared to others. For these compounds, IC_{50} values are in the range of 39.0–52.7 μM (Table 2). Similarly, in A431 cells, the IC_{50} values for **8b**, **8j** and **8l** were found to be in the range of 36.9–46.9 μM, whereas most of the compounds showed IC_{50} values between 50 and 90 μM, which indicates that these compounds are also equally important lead compounds. The present study revealed that among all the tested compounds most of the compounds showed good results in A431 cell line compared to other cell lines. The results of anticancer activity showed that compounds with a 2-*N* phenyl acetamide or 4-nitrophenyl on the oxadiazole ring showed good anticancer activity among all the synthesized compounds.

Many anticancer drugs are effective against MCF-7 and A549 cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS) and damaging DNA (Leong *et al.*, 2003). Chemotherapeutic agents such as doxorubicin and mitoxantrone cause cytotoxicity by generating ROS (Mizutani, 2007). Hence, like the cytotoxic drugs, the synthesized compounds may act as effective anticancer drug by similar mechanism.

In the MTT dose-dependent study (Fig. 1), data revealed that, out of all the synthesized compounds, there was substantial increase in cytotoxicity of the compounds **8j** and **8t** over most cell lines with increasing exposure to drug concentration. The inhibition shown by **8j** and **8t** could be attributed to the presence of (O=C–NH) group on the oxadiazole moiety.

Conclusions

The aim of the present study was to synthesize novel substituted phenyl-1,3,4-oxadiazol-2-ylthiopropylpiperazin ylbenzo[d]oxazole/thiazole carrying different groups at position 2 to examine the effect of the substitution at position 1 on the antiproliferative activity. The newly synthesized compounds were tested for in vitro antiproliferative activity against five cell lines from different origin. Some of the test compounds showed potent activity, especially compounds **8j** (A431) and **8t** [*N*-phenylacetamide] (MCF7). Compound **8j** displayed the highest activity in A431, whereas the compounds **8b** and **8p** also showed a comparable activity against A431 cell line, while most of the compounds showed moderate activity. Compound **8t** showed most potent activity among the synthesized compounds in MCF-7 cell line. Compounds **8a**, **8e** and **8j** are also found to have almost similar activity with that of **8t**. So, to conclude, the compounds were more

cytotoxic towards A431 followed by MCF7, A549, HepG2 and HeLa. The reason behind this variation in sensitivity of the cancer cells towards the compounds is yet to be studied.

From these findings, we were able to identify a few active molecules which are capable of inhibiting the growth of human cancer cell lines, in vitro. In short, our findings might be beneficial as leads for designing new compounds with potential antitumor activity. However, further studies are necessary to evaluate the signal transduction pathways induced by these compounds. A further study in this respect is under progress.

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