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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological potentials of some new 1,3,4-oxadiazole analogues

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Abstract In continuation of our research to explore new antiproliferative agents, we report herein the synthesis and antiproliferative activity of two new series of *N*-(substituted phenyl)-5-aryl-1,3,4-oxadiazol-2-amine (**4a–j**) and *N*-{[5-aryl-1,3,4-oxadiazol-2-yl]methyl}-substituted aniline (**4k–t**) analogs. The antiproliferative activity of fifteen compounds (**4a–h**, and **4n**) was tested against nine different panels of nearly 60 NCI human cancer cell lines. *N*-(2-Methoxyphenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (**4b**) and 4-{5-[(2-Methoxyphenyl)amino]-1,3,4-oxadiazol-2-yl} phenol (**4c**) showed maximum antiproliferative activity among the series with a mean growth percents (GPs) of 45.20 and 56.73, respectively. The compound **4b** showed significant percent growth inhibitions (GIs) on nearly 47 cancer cell lines and were found to have higher sensitivity towards HL-60(TB), MDA-MB-435, OVCAR-3, and K-562 with percent GIs (GIs) of 109.62, 105.90, 91.94, and 88.30, respectively. Similarly the compound, **4c** showed significant percent GIs on nearly 42 cancer cell lines and were found to have higher sensitivity towards UO-31, MDA-MB-435, KM12, and K-562 with %GIs of 84.31, 80.52, 78.65, and 77.06, respectively. Both the compounds **4b** and **4c** showed better antiproliferative activity than the standard drug Imatinib while the antiproliferative activity of compound **4b** was found to be nearly comparable to the standard drug 5-flurouracil (5-FU). The antiproliferative activity of five compounds (**4o-s**) was tested against the breast cancer cell lines (MCF-7 and MDA-MB-231) as per Sulforhodamine B assay (SRB assay). *N*-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-methylaniline (**4p**) was found to have significant antiproliferative activity against MCF-7 and MDA-MB-231 with GI_{50} of 12.9 and 59.3 μM, respectively. Further, the free radical scavenging activity results were significant for the most active compounds, **4b** ($IC_{50} = 21.07$ μM) and **4c** ($IC_{50} = 15.58$ μM). The docking studies was also carried against tubulin enzyme and the most active compound (**4b**) showed good interaction with the residues Lys254, Ala250, Cys241, Val318, Ala316, Asn258, and Lys352 present in the hydrophobic cavity of tubulin.

Keywords Antiproliferative activity · Antioxidants · One dose assay · SRB assay · Oxadiazoles · Imatinib

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Introduction

Cancer is one among the leading causes of death and taking second position after cardiovascular disorder (Noolvi et al. 2011). With more than 100 types of different cancer, it became a serious global public health problem (Siegel et al. 2012). More or less 14 million new cases of cancer and 8.2 million cancers' related death tolls were reported in the year 2012. It is envisaged that the new cases of cancer will rise up from 14 million to 22 million in the next two decades (WHO Cancer statistics). Chemotherapy is the major strategy to treat cancers, despite its drawbacks of limited efficacy, safety and selectivity mainly on cancer cells. Higher costs, toxicity, emergence of drug resistant cancer and genotoxicity are other complications of chemotherapy (Aydemir and Bilaloglu 2003). Therefore development of synthetic compounds of medicinal importance is a major focus for scientists and researchers across the world. In the present work two series of oxadiazoles were synthesized and fifteen oxadiazole analogs (**4a–n** and **4t**) were evaluated for their antiproliferative activity on nine different panels of nearly 60 NCI cancer cell lines. The antiproliferative activity of the remaining five oxadiazoles (**4o–s**) was evaluated on two breast cancer cell lines (MCF-7 and MDA-MB-231) because breast cancer is the leading cause of cancer related deaths among the female worldwide (WHO Cancer statistics).

Last few decades witnessed the development of anticancer drugs from chemically synthesized compounds. The heterocyclic 1,3,4-oxadiazoles being good bioisosteres of amides and esters, can interact with the receptors by forming hydrogen bonding and perhaps increase the biological profile significantly (Zhang et al. 2014). A number of potential activities of oxadizoles were reported earlier (Vaidya et al. 2016; Salahuddin et al. 2017). Biological potentials of oxadiazoles were also reported in the literatures as antitubercular (Dhumal et al. 2016), anticancer (Abdel-Aziz et al. 2016; Agarwal and Singh et al. 2016; Ahsan 2016), antioxidant (Mihailovic et al. 2017), antimicrobial (Mohamed et al. 2015), anticonvulsant (Tabatabai et al. 2013), anti-HIV (Khan et al. 2012), anti-inflammatory (Rathore et al. 2017) agents, and many more. Earlier we reported the anticancer activity of some newer oxadiazoles (Ahsan 2016; Ahsan et al. 2014, 2016, 2017; Agarwal and Singh et al. 2016). Encouraged by all these facts we synthesized and report herein the antiproliferative and free radical scavenging activities of some new oxadiazoles (**4a–t**). The oxadiazole linked aryl core of IMC-038525, IMC-094332, 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT), and NSC 777948 was taken to design the title compounds (**4a-k**).The design of series one oxadiazoles (**4a-k**) is shown in Fig. 1 (Ahsan et al. 2014; Tuma et al. 2010; Lukas et al. 2014; Rzeski et al. 2007). The methylene linkage (–CH2–) was introduced in the series two oxadiazoles (**4k–s**) to alter the biological profile. The structure of oxadiazoles (**4k–s**) was based on the structures of our previous reported work which contained the core aryl linked oxadiazole of IMC-039525, with an incorporation methylene linkage. The oxadiazoles NSC 781633 and NSC 783624 showed promising antiproliferative activity and had an inhibitory action on tubulin (Fig. 2) (Ahsan et al. 2014, 2017). The formation of free radicals by oxidative stress induce damage to the biological macromolecules and lead to many health related disorders such as cancer, inflammation, cardiovascular and neurodegenerative disorders (Pham-Huy et al. 2008; Birben et al. 2012). Since cancer and imbalance of oxidative stress share aspects of their underlying pathophysiology therefore, some antioxidants are equally effective antiproliferative agents. Hence the antioxidant potentials of all the oxadiazoles (**4a–t**) were also evaluated by the DPPH assay.

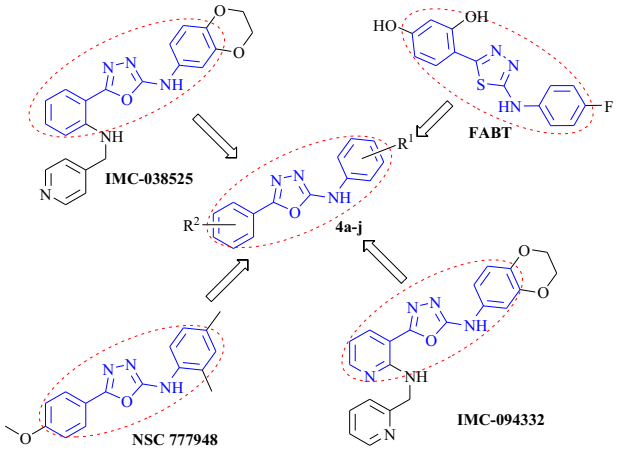


Fig. 1 Design of oxadiazole analogs (**4a–j**) on the basis of reported antiproliferative oxadiazoles

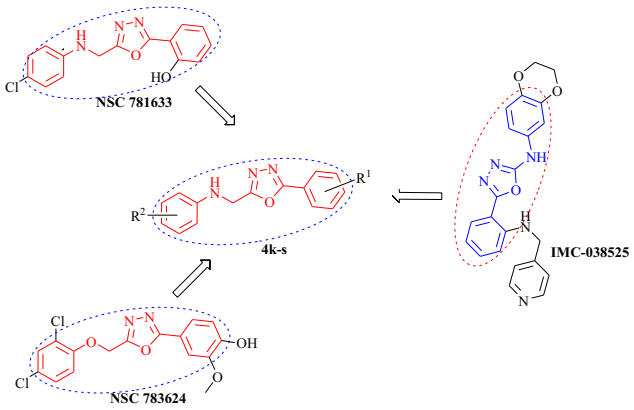


Fig. 2 Design of oxadiazole analogs (**4k–s**) on the basis of reported antiproliferative oxadiazoles

Materials and methods

Experimental

All the chemicals were procured from SD fine, CDH and chemcodyes and were used without being further purified. The open tube capillary method was used to record the melting points and is uncorrected. Fourier transform infrared (FT-IR), nuclear magnetic resonance (NMR), and mass spectral data were recorded on Bruker Alpha-E spectrometer, Bruker AC 400 MHz spectrometer and Bruker Esquire LCMS, respectively. The microanalysis was carried out on Perkin-Elmer 2400 Elemental Analyzer.

Method for the synthesis of substituted phenyl urea (2a–b)

A solution sodium cyanate (0.05 ml; 3.25 g) in hot water was added to a solution of aromatic aniline (0.05 mol) in 10 ml glacial acetic acid and 75 ml hot water with continuous stirring on magnetic stirrer to obtain precipitate of substituted phenyl urea (**2a–b**) which was further recrystallized with hot water (Agarwal and Singh et al. 2016; Ahsan 2016).

Method for the synthesis of substituted N*-(substituted phenyl)hydrazinecarboxamide (3a–b)*

N-(Substituted phenyl)hydrzinecarboxamide (**3a–b**) was obtained by refluxing substituted phenyl urea (**2a–b**) (0.04 mol) and hydrazine hydrate (0.08 mol; ~4 ml) in ethanol for 24 h as per the reported method (Agarwal and Singh et al. 2016; Ahsan 2016).

Method for the synthesis of ethyl[(substituted phenyl) amino]acetate (2c–e)

Ethyl[(substituted phenyl)amino]acetate (**2c–e**) was obtained by stirring a mixture of aromatic aniline (**1**) (0.1 mol), ethylbromoacetate (0.2 mol; ~33.0 ml) and anhydrous potassium carbonate (5 g) suspended in acetone at 80 °C for 6 h, as a creamy solid. This method of synthesis is somewhat faster than the reported method mentioned earlier in which ethylchloroacetate was taken (Agarwal and Singh et al. 2016; Ahsan 2016; Finger et al. 1965).

Method for the synthesis of 2-[(substitutedphenyl)amino] acetohydrazide (3c–e)

2-[(Substituted phenyl)amino]acetohydrazide (**3c–e**) was obtained by refluxing a mixtures of ethyl[(substituted phenyl)amino]acetate (**2c–e**) (0.05 mol) and hydrazine hydrate (0.10 mol; ~ 5 ml) in ethanol for 22 h as creamy solid as per the reported method (Agarwal and Singh et al. 2016; Ahsan 2016; Finger et al. 1965).

General method for the synthesis of 2,5-disubstituted-1,3,4-oxadiazole analogs (4a–t)

An equimolar mixture of substituted phenyl semicarbazide (0.005 mol) (**3a–b**)/2-[(substituted phenyl)amino]acetohydrazide (0.005 mol) (**3c–e**) and aromatic aldehydes (0.005 mol) in ethanol-water system (1:2, v/v) solvent was refluxed for 10–12 h with addition of 20 mol% solution of $NaHSO_3$ (Sangshetti et al. 2011). The progress of the reaction was monitored throughout by preparatory thin layer chromatography (TLC silica gel 60 F_{254}) using eluent n-hexane/ethyl acetate/formic acid (5:4:1) and benzene/acetone (8:2). The reaction mixture was worked up as per the reported protocol by pouring into the crushed ice, to obtain the final compounds *N*-(substituted phenyl)-5-aryl-1,3,4-oxadiazol-2-amine (**4a–j**) and *N*-{[5-aryl-1,3,4-oxadiazol-2-yl]methyl}-substituted aniline (**4k–t**).

N-(2-Methoxyphenyl)-5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (**4a**) Yield: 62%; creamy solid; mp 160–162 °C; infraed (IR) (KBr): 3216, 1517, 1256, 788 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.83 (3H, s, OCH_3), 6.81–7.05 (4H, m, ArH, H-3, H-4, H-5 H-6), 7.99–8.21 (4H, m, ArH, H-2′, H-3′, H-5′, H-6′), 8.49 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.33 (C=N, C-5), 159.01 (C=N, C-2), 152.11 (C, C-4′), 147.03 (C, C-2), 132.61 (C, C-1), 132.01 (C, C-1′), 129.12 (CH, C-2′, C-6′), 121.81 (CH, C-3′, C-5′), 121.61 (CH, C-5), 119.81 (CH, C-4), 118.91 (CH, C-6), 115.11 (CH, C-3), 55.24 (OCH_3); anal. calc. for $C_{15}H_{12}FN_3O_2$: C, 63.15; H, 4.24; N, 14.73; found: C, 63.18; H, 4.23; N, 14.75%. EIMS $m/z = 286.00$ $[M + 1]^+$.

N-(2-Methoxyphenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (**4b**) Yield: 88%; creamy solid; mp 210–212 °C; IR (KBr): 3214, 1511, 1251, 698 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.81 (3H, s, OCH_3), 6.62–6.88 (4H, m, ArH, H-3, H-4, H-5, H-6), 7.46 (2H, d, $J = 8.1$ Hz, ArH, H-3′, H-5′), 7.61 (2H, d, $J = 8.1$ Hz, ArH, H-2′, H-6′), 7.78 (1H, s, ArNH), ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.59 (C=N, C-5), 132.58 (C=N, C-2), 147.21 (C, C-2), 130.00 (C, C-1), 129.07 (C, C-4′), 128.99 (CH, C-3′, C-5′), 121.85 (CH, C-1′), 120.59 (CH, C-5), 120.38 (CH, C-4), 119.09 (CH, C-6), 118.11 (CH, C-3), 110.72 (CH, C-2′, C-6′), 55.62 (OCH_3); anal. calc. for $C_{15}H_{12}ClN_3O_2$: C, 59.71; H, 4.01; N, 13.93; found: C, 59.75; H, 4.04; N, 13.95%. EIMS $m/z = 302.00$ $[M]^+$, 304.01 $[M + 2]^+$.

4-{5-[(2-Methoxyphenyl)amino]-1,3,4-oxadiazol-2-yl}phenol (**4c**) Yield: 74%; creamy solid; mp 176–178 °C; IR (KBr): 3412, 3214, 1513, 1252 cm^{-1}; ^{1}H NMR (400 MHz,

DMSO-d_6): δ 3.85 (3H, s, OCH_3), 6.81–6.97 (4H, m, ArH, H-3, H-4, H-5, H-6), 7.01 (2H, d, $J = 8.0$ Hz, ArH, H-3′, H-5′), 7.43 (2H, d, $J = 8.0$ Hz, ArH, H-2′, H-6′), 7.77 (1H, s, ArNH), 10.72 (1H, s, ArOH), ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.57 (C=N, C-5), 152 (C=N, C-2), 147.01 (C, C-2), 132.51 (C, C-4′), 130.09 (C, C-1), 128.06 (CH, C-2′, C-6′), 121.99 (CH, C-5), 119.09 (CH, C-4), 118.31 (CH, C-1′), 117.81 (CH, C-6), 116.57 (CH, C-3′, C-5′), 115.39 (CH, C-3), 55.63 (OCH_3); anal. calc. for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83; found: C, 63.63; H, 4.61; N, 14.85%. EIMS $m/z = 284.00$ $[M]^+$.

N-(2-Methoxyphenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (**4d**) Yield: 72%; creamy solid; mp 166–168 °C; IR (KBr): 3218, 1519, 1251 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.77 (3H, s, OCH_3, C-4′), 3.81 (3H, s, OCH_3, C-2), 6.84–7.03 (4H, m, ArH, H-3, H-4, H-5, H-6), 7.60 (2H, d, $J = 7.9$ Hz, ArH, H-3′, H-5′), 7.79 (2H, d, $J = 7.9$ Hz, ArH, H-2′, H-6′), 7.93 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.51 (C=N, C-5), 152.11 (C=N, C-5), 147.12 (C, C-2), 132.59 (C, C-1), 130.11 (C, C-4′), 129.09 (CH, C-2′, C-6′), 121.94 (CH, C-5), 119.06 (CH, C-4), 118.51 (C, C-1′), 117.82 (CH, C-6), 115.59 (CH, C-3), 114.31 (CH, C-3′, C-5′), 56.24 (OCH_3, C-4′), 55.61 (OCH_3, C-2); anal. calc. for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13; found: C, 64.67; H, 5.11; N, 14.10%. EIMS $m/z = 298.10$ $[M]^+$.

N-(2,5-Dimethoxyphenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine (**4e**) Yield: 82%; white solid; mp 216–218 °C; IR (KBr): 3216, 1512, 1256, 698 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (3H, s, OCH_3, C-5), 3.86 (3H, s, OCH_3, C-2), 6.53 (1H, d, $J = 8.0$ Hz, ArH, H-4), 6.94 (1H, d, $J = 8.0$ Hz, ArH, H-3), 7.52 (2H, d, $J = 8.3$ Hz, ArH, H-3′, H-5′), 7.67 (2H, d, $J = 8.3$ Hz, ArH, H-2′, H-6′), 7.78 (1H, s, ArH, H-6), 7.95 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.31 (C=N, C-5), 152.15 (C=N, C-2), 142.03 (C, C-5), 139.60 (C, C-2), 134.02 (C, C-1), 133.07 (C, C-4′), 128.97 (CH, C-3′, C-5′), 128.53 (CH, C-2′, C-6′), 127.98 (C, C-1′), 111.30 (CH, C-3), 105.86 (CH, C-4), 104.88 (CH, C-6), 56.47 (OCH_3, C-5), 55.24 (OCH_3, C-2); anal. calc. for $C_{16}H_{14}ClN_3O_3$: C, 57.93; H, 4.25; N, 12.67; found: C, 57.96; H, 4.23; N, 12.65%. EIMS $m/z =$ 332.00 $[M]^+$, 334.02 $[M + 2]^+$.

N-(2,5-Dimethoxyphenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine (**4f**) Yield: 77%; creamy solid; mp 168–170 °C; IR (KBr): 3215, 1517, 1253 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (3H, s, OCH_3, C-5), 3.78 (3H, s, OCH_3, C-4′), 3.86 (3H, s, OCH_3, C-2), 6.51 (1H, d, $J = 7.9$ Hz, ArH, H-4), 6.94 (1H, d, $J = 8.7$ Hz, ArH, H-3), 7.02 (2H, d, $J = 7.4$ Hz, ArH, H-3′, H-5′), 7.60 (2H, d, $J = 7.9$ Hz, ArH, H-2′, H-6′), 7.81 (1H, s, ArH, H-6), 7.90 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.31 (C=N, C-5), 152.15 (C=N, C-2), 151.33 (C, C-4′), 150.11 (C, C-5), 142.03 (C, C-1), 139.60 (C, C-2), 133.07 (CH, C-2′, C-6′), 128.57 (CH, C-3′, C-5′), 118.53 (CH, C-1′), 116.31 (CH, C-3), 114.30 (CH, C-4), 100.86 (CH, C-6), 56.47 (OCH_3, C-5), 55.91 (OCH_3, C-4′), 55.24 (OCH_3, C-2); anal. calc. for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84; found: C, 62.41; H, 5.21; N, 12.85%. EIMS $m/z = 328.10$ $[M]^+$.

4-{5-[(2,5-Dimethoxyphenyl)amino]-1,3,4-oxadiazol-2-yl} phenol (**4g**) Yield: 58%; creamy solid; mp 160–162 °C; IR (KBr): 3409, 3213, 1514, 1257 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.67 (3H, s, OCH_3, C-5), 3.85 (3H, s, OCH_3, C-2), 6.50 (1H, d, $J = 8.7$ Hz, ArH, H-3), 6.83 (2H, d, $J = 8.4$ Hz, ArH, H-3′, H-5′), 6.94 (1H, d, $J = 8.8$ Hz, ArH, H-4), 7.66 (2H, d, $J = 8.3$ Hz, ArH, H-2′, H-6′), 7.81 (1H, s, ArH, H-6), 7.85 (1H, s, ArNH), 10.73 (1H, s, ArOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.33 (C=N, C-5), 152.10 (C=N, C-2), 151.31 (C, C-4′), 150.13 (C, C-5), 142.01 (C, C-1), 139.62 (C, C-2), 133.17 (C, C-1′), 128.77 (CH, C-2′, C-6′), 118.57 (CH, C-3), 116.32 (CH, C-3′, C-5′), 114.31 (CH, C-4), 100.86 (CH, C-6), 56.47 (OCH_3, C-5), 55.24 (OCH_3, C-2); anal. calc. for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41; found: C, 61.36; H, 4.81; N, 13.45%. EIMS $m/z = 313.10$ $[M]^+$.

N-(2,5-Dimethoxyphenyl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-amine (**4h**) Yield: 69%; creamy solid; mp 178–180 °C; IR (KBr): 3212, 1511, 1255 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (3H, s, OCH_3, C-2), 3.77 (3H, s, OCH_3, C-4′), 3.82 (6H, s, OCH_3, C-5, C-3′), 6.51 (1H, d, $J = 5.7$ Hz, ArH, H-4), 6.94 (1H, d, $J = 8.8$ Hz, ArH, H-3), 7.01 (1H, d, $J = 8.4$ Hz, ArH, H-5′), 7.13 (1H, d, $J = 6.6$ Hz, ArH, H-6′), 7.31 (1H, s, ArH, H-6), 7.84 (1H, s, ArH, H-2′), 7.93 (1H, s ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.31 (C=N, C-5), 152.15 (C=N, C-2), 151.12 (C, C-5), 142.03 (C, C-3′), 139.60 (C, C-2), 134.02 (C, C-4′), 133.07 (C, C-1), 128.97 (CH, C-6′), 128.53 (C, C-1′), 127.98 (CH, C-5′), 112.36 (CH, C-3), 111.30 (CH, C-2′), 105.86 (CH, C-4), 104.88 (CH, C-6), 56.47 (OCH_3, C-5), 56.46 (OCH_3, C-3), 55.56 (OCH_3, C-4′), 55.24 (OCH_3, C-2); anal. calc. for $C_{18}H_{19}N_3O_5$: C, 60.50; H, 5.36; N, 11.76; found: C, 60.53; H, 5.33; N, 11.77%. EIMS $m/z =$ 358.10 $[M]^+$.

4-{5-[(2,5-Dimethoxyphenyl)amino]-1,3,4-oxadiazol-2-yl}-2-methoxyphenol (**4i**) Yield: 72%; creamy solid; mp 180–182 °C; IR (KBr): 3214, 1513, 1251 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (6H, s, OCH_3, C-2, C-3′), 3.82 (3H, s, OCH_3, C-5), 6.50 (1H, d, $J = 8.0$ Hz, ArH, H-4), 6.81 (1H, d, $J = 8.3$ Hz, ArH, H-3), 6.94 (1H, d, $J = 8.8$ Hz, ArH, H-5′), 7.01 (1H, d, $J = 7.6$ Hz, ArH, H-6′), 7.27

(2H, s, ArH, H-6, H-2′), 7.84 (1H, s, ArNH) 10.51 (1H, s, ArOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.34 (C=N, C-5), 152.36 (C=N, C-2), 148.57 (C, C-5), 148.01 (C, C-3′), 141.82 (C, C-4′), 141.41 (C, C-2), 128.77 (C, C-1), 125.51 (CH, C-1′), 121.26 (CH, C-6′), 115.48 (CH, C-5′), 111.25 (CH, C-2′), 108.24 (CH, C-3), 105.54 (CH, C-4), 104.62 (CH, C-6), 56.43 (OCH_3, C-5, C-3′), 55.22 (OCH_3, C-2); anal. calc. for $C_{17}H_{17}N_3O_5$: C, 59.47; H, 4.99; N, 12.24; found: C, 59.47; H, 4.97; N, 12.25%. EIMS m/z = 344.10 $[M]^+$.

N-(2,5-Dimethoxyphenyl)-5-(2,3,4-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (**4j**) Yield: 72%; creamy solid; mp 208–210 °C; IR (KBr): 3213, 1514, 1258 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (6H, s, OCH_3, C-2, C-4′), 3.81 (3H, s, OCH_3, C-5), 3.84 (6H, s, OCH_3, C-3′, C-5′), 6.51 (1H, d, J = 7.1 Hz, ArH, H-4), 6.95 (1H, d, J = 8.8 Hz, ArH, H-3), 6.99 (2H, s, ArH, H-2′, H-6′), 7.84 (1H, s, ArH, H-6), 7.88 (1H, s, ArNH), ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.33 (C=N, C-5), 152.11 (C=N, C-2), 142.03 (C, C-5), 139.61 (C, C-2), 134.01 (C, C-4′), 133.06 (C, C-3′, C-5′), 128.91 (C, C-1), 128.57 (C, C-1′), 127.99 (CH, C-3), 111.31 (CH, C-4), 105.76 (CH, C-6), 104.78 (CH, C-2′, C-6′), 56.55 (OCH_3, C-4′), 56.45 (OCH_3, C-5), 56.24 (OCH_3, C-3′, C-5′), 55.24 (OCH_3, C-2); anal. calc. for $C_{19}H_{21}N_3O_6$: C, 58.91; H, 5.46; N, 10.85; found: C, 58.93; H, 5.43; N, 10.87%. EIMS m/z = 387.10 $[M]^+$.

N-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methoxyaniline (**4k**) Yield: 76%; creamy solid; mp 118–120 °C; IR (KBr): 3213, 1518, 1258, 699 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (3H, s, OCH_3), 4.48 (2H, s, CH_2), 7.21–7.58 (4H, m, ArH, H-3, H-4, H-5, H-6), 7.63 (2H, d, J = 7.9 Hz, ArH, H-3′, H-5′), 7.84 (2H, d, J = 7.9 Hz, ArH, H-2′, H-6′), 8.68 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.55 (C=N, C-5), 152.11 (C=N, C-2), 144.21 (C, C-2), 132.53 (C, C-1), 130.01 (C, C-4′), 129.09 (CH, C-3′, C-5′), 128.99 (CH, C-2′, C-6′), 121.89 (CH, C-5), 120.58 (C, C-1′), 120.34 (CH, C-4), 119.07 (CH, C-3), 118.11 (CH, C-6), 56.24 (OCH_3), 52.16 (CH_2, $ArNHCH_2$); anal. calc. for $C_{19}H_{21}N_3O_5$: C, 60.86; H, 4.47; N, 13.31; found: C, 60.83; H, 4.50; N, 13.33%. EIMS m/z = 316.00 $[M]^+$, 318.10 $[M + 2]^+$.

N-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-2-methoxyaniline (**4l**) Yield: 72%; creamy solid; mp 126–128 °C; IR (KBr): 3219, 1517, 1253 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (3H, s, OCH_3, C-4′), 3.83 (3H, s, OCH_3, C-2), 4.46 (2H, s, CH_2), 7.23–7.56 (4H, m, ArH, H-3, H-4, H-5, H-6), 7.61 (2H, d, J = 8.1 Hz, ArH, H-3′, H-5′), 7.68 (2H, d, J = 8.1 Hz, ArH, H-2′, H-4′), 8.72 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.63 (C=N, C-5), 152.11 (C=N, C-2), 152.04 (C, C-4′), 130.15 (C, C-1), 129.09 (CH, C-2′, C-6′), 128.97 (CH, C-5), 128.08 (C, C-1′), 121.81 (CH, C-4), 120.59 (CH, C-3′, C-5′), 120.36 (CH, C-3), 114.16 (CH, C-6), 56.24 (OCH_3, C-2), 55.64 (OCH_3, C-4′), 52.14 (CH_2, $ArNHCH_2$); anal. calc. for $C_{19}H_{21}N_3O_5$: C, 65.58; H, 5.50; N, 13.50; found: C, 65.55; H, 5.53; N, 13.53%. EIMS m/z = 311.40 $[M]^+$.

N-{[5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-2,5-dimethoxyaniline (**4m**) Yield: 76%; creamy solid; mp 112–114 °C; IR (KBr): 3215, 1517, 1253 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.68 (3H, s, OCH_3, C-2), 3.77 (3H, s, OCH_3, C-4), 3.83 (6H, s, OCH_3, C-5, C-3′), 4.18 (2H, s, CH_2), 6.53 (1H, d, J = 7.7 Hz, ArH, H-4), 6.93 (1H, d, J = 7.7 Hz, ArH, H-3), 7.01 (1H, d, J = 8.1 Hz, ArH, H-5′), 7.13 (1H, d, J = 8.1 Hz, ArH, H-6′), 7.33 (1H, s, ArH, H-6, H-2′), 7.88 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.30 (C=N, C-5), 152.18 (C=N, C-2), 151.11 (C, C-5), 142.05 (C, C-3′), 139.61 (C, C-2), 134.06 (C, C-4′), 133.17 (C, C-1), 128.91 (CH, C-3), 128.43 (CH, C-6′), 127.99 (CH, C-1′), 112.31 (CH, C-2′), 111.35 (CH, C-3), 105.81 (CH, C-4), 104.86 (CH, C-6), 56.46 (OCH_3, C-2, C-5), 56.22 (OCH_3, C-3′, C-4′), 52.14 (CH_2, $ArNHCH_2$); anal. calc. for $C_{19}H_{21}N_3O_5$: C, 61.45; H, 5.70; N, 11.31; found: C, 61.43; H, 5.74; N, 11.33%. EIMS m/z = 372.04 $[M]^+$.

N-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-methylaniline (**4n**) Yield: 89 %; light brown solid; mp 140–142 °C; IR (KBr): 3216, 1519, 1252, 695 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, $ArCH_3$), 4.16 (2H, s, CH_2), 6.52 (2H, d, J = 8.4 Hz, ArH, H-2, H-6), 6.89 (2H, d, J = 5.7 Hz, ArH H-3, H-5), 7.48 (2H, d, J = 8.4 Hz, ArH, H-3′, H-5′), 7.73 (2H, d, J = 8.3 Hz, ArH, H-2′, H-6′), 7.97 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.41 (C=N, C-5), 152.22 (C=N, C-2), 144.60 (C, C-1), 134.41 (C, C-4′), 129.91 (CH, C-3, C-5), 129.64 (CH, C-3′, C-5′), 128.91 (CH, C-2′, C-6′), 126.82 (C, C-4), 124.47 (C, C-1′), 113.86 (CH, C-3, C-5), 51.72 (CH_2, $ArNHCH_2$), 24.31 (CH_3, $ArCH_3$); anal. calc. for $C_{16}H_{14}ClN_3O$: C, 64.11; H, 4.71; N, 14.02; found: C, 64.15; H, 5.68; N, 14.05%. EIMS m/z = 300.01 $[M]^+$, 302.00 $[M + 2]^+$.

4-(5-{[(4-Methylphenyl)amino]methyl}-1,3,4-oxadiazol-2-yl)phenol (**4o**) Yield: 76%; light brown solid; mp 112–114 °C; IR (KBr): 3414, 3219, 1516, 1257 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, ArH), 4.16 (2H, s, CH_2), 6.32 (2H, d, J = 8.0 Hz, ArH, H-2, H-6), 6.79 (2H, d, J = 8.1 Hz, ArH, H-3′, H-5′), 6.85 (2H, d, J = 8.0 Hz, ArH, H-3, H-5), 7.33 (2H, d, J = 8.1 Hz, ArH, H-2′, H-6′), 7.95 (1H, s, ArNH), 11.31 (1H, s, ArOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.41 (C=N, C-5), 158.53 (C=N, C-2), 152.21 (C, C-4′), 144.59 (C, C-1), 129.91 (CH, C-3, C-5), 128.93 (CH, C-2′, C-6′), 126.74 (C, C-4), 118.91 (C, C-1′),

116.72 (CH, C-3′, C-5′), 113.50 (CH, C-2, C-6), 51.72 (CH_2, ArNHCH_2), 24.33 (CH_3, ArCH_3); anal. calc. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94; found: C, 68.36; H, 5.35; N, 14.92%. EIMS $m/z = 282.09$ $[M]^+$.

N-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-methylaniline (**4p**) Yield: 72%; light brown solid; mp 158–160 °C; IR (KBr): 3217, 1519, 1259 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, ArCH_3), 3.78 (3H, s, OCH_3), 4.13 (2H, s, CH_2), 6.47 (2H, d, $J = 7.9$ Hz, ArH, H-2, H-6), 6.87 (2H, d, $J = 7.9$ Hz, ArH, H-3, H-5), 6.98 (2H, d, $J = 8.8$ Hz, ArH, H-3′, H-5′), 7.64 (2H, d, $J = 8.8$ Hz, ArH, H-2′, H-6′), 7.93 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.40 (C=N, C-5), 160.51 (C, C-4′), 152.22 (C=N, C-2), 144.63 (C, C-1), 129.92 (CH, C-3, C-5), 128.51 (CH, C-2′, C-6′), 126.81 (C, C-4), 118.60 (C, C-1′), 114.71 (CH, C-3′, C-5′), 113.54 (CH, C-2, C-6), 56.62 (C, OCH_3), 51.71 (CH_2, ArNHCH_2), 24.31 (CH_3, ArCH_3); anal. calc. for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23; found: C, 69.17; H, 5.78; N, 14.21%. EIMS $m/z =$ 295.80 $[M]^+$.

N-{[5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-methylaniline (**4q**) Yield: 67%; light brown solid; mp 70–72 °C; IR (KBr): 3219, 1513, 1249 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, ArCH_3), 3.77 (3H, s, OCH_3, C-4′), 3.79 (3H, s, OCH_3, C-3′), 4.12 (2H, s, CH_2), 6.31 (2H, d, $J = 8.1$ Hz, ArH, H-2, H-6), 6.85 (2H, d, $J =$ 8.1 Hz, ArH, H-3, H-5), 6.72 (1H, d, $J = 8.0$ Hz, ArH, H-6′), 6.88 (1H, s, ArH, H-1′), 6.93 (1H, d, $J = 8.0$ Hz, ArH, H-5′), 7.98 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.42 (C=N, C-5), 150.51 (C, C-3′), 149.90 (C, C-4′), 144.71 (C, C-1), 129.94 (CH, C-3, C-5), 126.91 (C, C-4), 120.80 (CH, C-6′), 119.79 (C, C-1′), 115.94 (CH, C-5′), 113.42 (CH, C-2, C-6), 112.31 (CH, C-1′), 56.62 (OCH_3, C-3′, C-4′), 51.73 (CH_2, ArNHCH_2), 24.34 (CH_3, ArCH_3); anal. calc. for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91; found: C, 66.42; H, 5.90; N, 12.93%. EIMS $m/z = 326.09$ $[M]^+$.

2-Methoxy-4-(5-{[(4-methylphenyl)amino]methyl}-1,3,4-oxadiazol-2-yl)phenol (**4r**) Yield: 62%; light brown solid; mp 120–122 °C; IR (KBr): 3408, 3216, 1511, 1256 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, ArCH_3), 3.80 (3H, s, OCH_3), 4.14 (2H, s, CH_2), 6.40 (2H, d, $J = 7.9$ Hz, ArH, H-2, H-6), 6.68 (1H, d, $J = 8.8$ Hz, ArH, H-5′), 6.82 (1H, s, ArH, H-2′), 6.85 (2H, d, $J = 7.9$ Hz, ArH, H-3, H-5), 6.87 (1H, d, $J = 8.8$ Hz, ArH, H-6′), 7.95 (1H, s, ArNH), 11.31 (1H, s, ArOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.41 (C=N, C-5), 152.71 (C=N, C-5), 151.22 (C, C-3′), 145.71 (C, C-4′), 144.82 (C, C-1), 129.92 (CH, C-3, C-5), 126.93 (C, C-4), 121.30 (CH, C-6′), 119.81 (CH, C-1′), 117.90 (CH, C-5′), 113.43 (CH, C-2, C-6), 112.31 (CH, C-1′), 56.62 (OCH_3, C-3′), 51.73 (CH_2, ArNHCH_2), 24.32 (CH_3, ArCH_3); anal. calc. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50; found: C, 65.55; H, 5.53; N, 13.53%. EIMS $m/z = 312.10$ $[M]^+$.

N-{[5-(2,3,4-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}-4-methylaniline (**4s**) Yield: 78%; light brown solid; mp 110–112 °C; IR (KBr): 3219, 1517, 1259 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, ArCH_3), 3.79 (3H, s, OCH_3, C-4′), 3.81 (6H, s, OCH_3, C-3′, C-5′), 4.17 (2H, s, CH_2), 6.50 (2H, d, $J = 8.8$ Hz, ArH, H-2, H-6), 6.87 (2H, d, $J = 8.8$ Hz, ArH, H-3, H-5), 6.96 (2H, s, ArH), 7.90 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.61 (C=N, C-5), 152.72 (C=N, C-2), 151.60 (C, C-3′, C-5′), 144.71 (C, C-1), 139.92 (C, C-4′), 129.89 (CH, C-3, C-5), 126.82 (C, C-4), 120.91 (C, C-1′), 113.83 (CH, C-2, C-6), 104.91 (CH, C-2′, C-6′), 56.52 (OCH_3, C-4′), 56.25 (OCH3, C-3′, C-5′), 51.62 (CH_2, ArNHCH_2), 24.31 (CH_3, ArCH_3); anal. calc. for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82; found: C, 6sza 4.19; H, 5.98; N, 11.85%. EIMS $m/z = 356.10$ $[M]^+$.

N-{[5-(Furan-2-yl)-1,3,4-oxadiazol-2-yl]methyl}-4-methylaniline (**4t**) Yield: 67%; light brown solid; mp 108–110 °C; IR (KBr): 3218, 1513, 1254 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.12 (3H, s, ArCH_3), 4.13 (2H, s, CH_2), 6.31 (2H, d, $J = 8.0$ Hz, ArH, H-2, H-6), 6.39–6.43 (3H, s, ArH, H-3′, H-4′, H-5′), 6.85 (2H, d, $J = 8.0$ Hz, ArH, H-3, H-5), 7.92 (1H, s, ArNH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.92 (C=N, C-5), 152.71 (C=N, C-2), 147.90 (C, C-1′), 144.42 (C, C-1), 142.81 (CH, C-3′), 129.91 (CH, C-3, C-5), 126.41 (C, C-4), 113.42 (CH, C-2, C-6), 107.93 (CH, C-5′), 105.52 (CH, C-4′), 51.62 (CH_2, ArNHCH_2), 24.32 (CH_3, ArCH_3); anal. calc. for $C_{19}H_{21}N_3O_4$: C, 65.87; H, 5.13; N, 16.46; found: C, 65.89; H, 5.11; N, 16.46%. EIMS $m/z = 256.11$ $[M]^+$.

Antiproliferative activity

The antiproliferative activity of the oxadiazoles was carried out as per the standard protcols reported elsewhere (http://dtp.nci.nih.gov; Boyd and Paull 1995; Monks et al. 1991; Shoemaker 2006; Vichai and Kirtikara 2006). The method for the evaluation of antiproliferative activity was provided as supplementary information.

Molecular docking studies

The present molecular studies were carried out using Maestro 8.5 (Schrodingers LLC) installed in RHEL 5.0 platform. Maestro 10.1 (Academic version) was utilized for docking interpretation. 3-D X-ray crystallographic tubulin

complexed with colchicine as ligand (PDB: 1SA0) at resolution 3.58 Å with *r* value 0.233 (obs.) was obtained from the protein data bank (https://www.rcsb.org/) (Ravelli et al. 2004; Wallace et al. 1995).

DPPH free radical scavenging assay

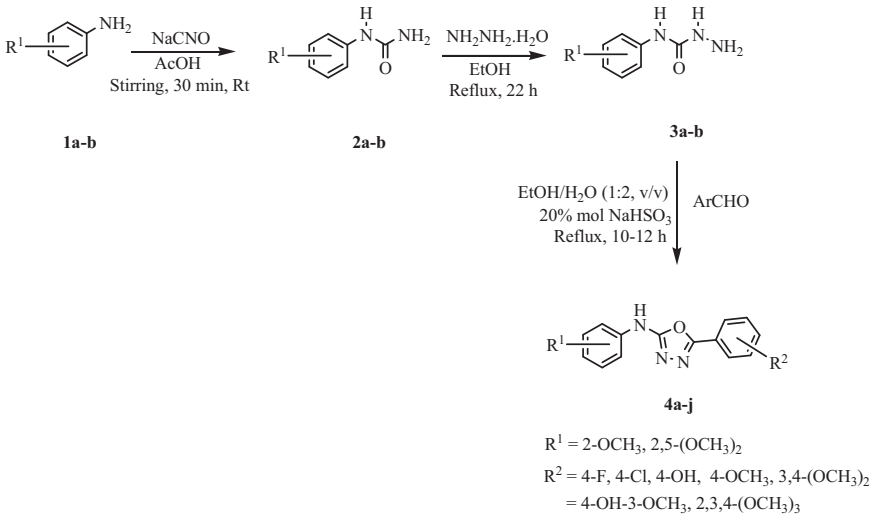
The antioxidant activity of compounds was assessed by DPPH free radical assay (Koleva et al. 2002). This assay is based on the theory that hydrogen donors are antioxidants. DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a commercially available stable free radical with purple color (absorbed at 517 nm) which turns into yellow after accepting hydrogen from the antioxidants.

Results and discussion

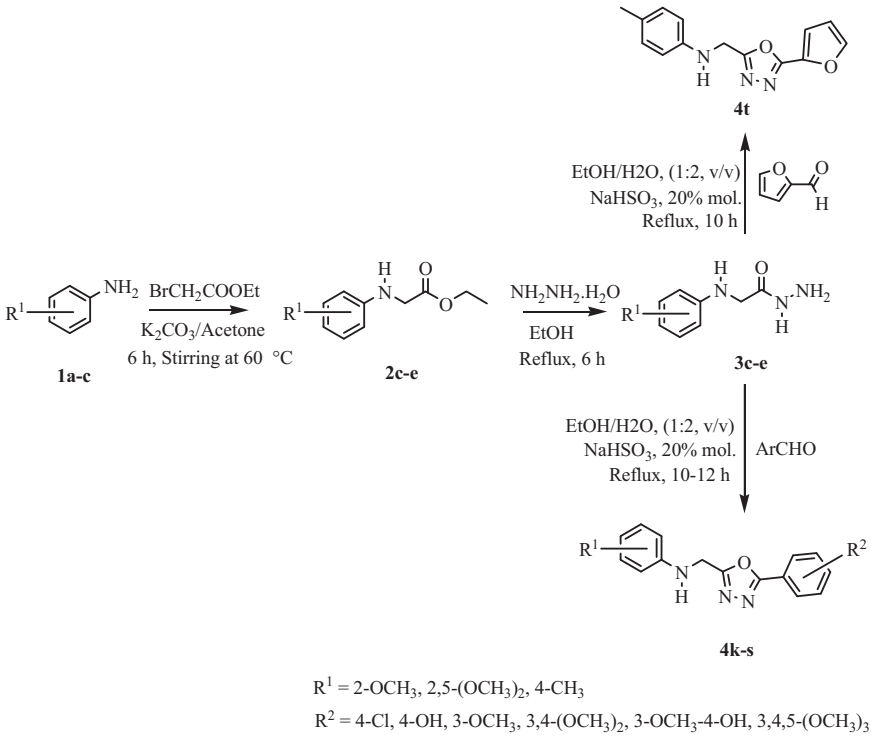
Chemistry

The synthetic routes for the synthesis of oxadiazole analogs (**4a–t**) are outlined in the Schemes 1 and 2. The oxadiazoles (**4a–j** and **4k–t**) were prepared starting from substituted aniline (**1a–b**) by adopting two different routes. For the

Scheme 1 Synthetic route for the preparation of *N*-(substituted phenyl)-5-aryl-1,3,4-oxadiazol-2-amine analogs (**4a-j**)



Scheme 2 Synthetic route for the preparation of *N*-(substituted phenyl)[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl amine analogs (**4k-t**)

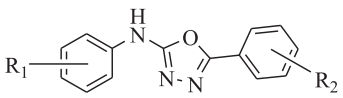


series one oxadizoles (**4a–j**), the intermediate substituted phenyl urea (**2a–b**) was prepared by stirring a solution of substituted anilines (**1a–b**) in glacial acetic acid and warm water with a solution of sodium cyanate in hot water, while the intermediate substituted *N*-(substituted phenyl)hydrazine-carboxamide (**3a–b**) was prepared by refluxing a mixture of substituted phenyl urea (**2a–b**) and hydrazine hydrate in ethanol for 24 h, as per the reported method (Agarwal and Singh et al. 2016; Ahsan 2016; Ahsan et al. 2017; Azam et al. 2010). For series two oxadiazoles (**4k–t**), the intermediates ethyl[(substituted phenyl)amino]acetate (**2c–e**) was obtained by stirring and refluxing a mixture of substituted aniline (**1a–c**) and bromoethylacetate in acetone for 6 h with an addition of K_2CO_3, while 2-[(substituted phenyl)amino]acetohydrazide (**3c–e**) was obtained by refluxing ethyl [(substituted phenyl)amino]acetate (**2c–e**) and hydrazine hydrate in ethanol for 22 h as per the reported method (Agarwal and Singh et al. 2016; Ahsan 2016; Ahsan et al. 2017; Finger et al. 1965). The preparation of ethyl[(substituted phenyl)amino]acetate (**2c–e**) from bromoethylacetate was found to be less time consuming as compared to the earlier reported method (Agarwal and Singh et al. 2016; Ahsan 2016; Ahsan et al. 2017). In the final step for both series oxadiazoles were synthesized by refluxing an equimolar quantities of *N*-(substituted phenyl) hydrazinecarboxamide (**3a-b**)/ 2-[(substituted phenyl) amino]acetohydrazide (**3c–e**) and aromatic aldehyde for 10–12 h in ethanol-water system (1:2, v/v) solvent using 20 mol% $NaHSO_3$ to afford the synthesis of *N*-(substituted phenyl)-5-aryl-1,3,4-oxadiazol-2-amine analogs (**4a–j**)/ *N*-(substituted phenyl)[5-aryl-1,3,4-oxadiazol-2-yl]methyl amine analogs (**4k–t**) (Sangshetti et al. 2011). The progress of reaction was examined throughout by TLC using eluent benzene/acetone (8:2) and *n*-hexane/ethyl acetate/formic acid (5:4:1). The yields of the final compounds were ranging between 58 and 89% after recrystallization with ethanol. The physical constants of oxadiazole analogues (**4a-t**) is given in Table 1. The structure of the final oxadiazole analogs (**4a–t**) was confirmed by IR, NMR, and mass spectral data. The IR spectra of the oxadiazoles (**4a–t**) afforded characteristic oxadiazole stretching (C–O–C) at 1247–1261 cm^{-1}, while C=N stretching was observed at 1511–1519 cm^{-1}. Similarly, the characteristics NH and OH stretching were observed at band 3212–3219 and 3407–3415 cm^{-1}, respectively. The number of protons, multiplicity (singlet/doublet/multiplet) and coupling constant (*J* value in Hz) was observed by recording ^1H NMR in DMSO-d_6 at 400 MHz, using tetramethyl silane (TMS) as an internal standard. The ^1H NMR showed a singlet at δ 2.12–2.14 ppm for the corresponding methyl group (–CH_3); a singlet at δ 3.67–3.86 ppm for the corresponding methoxy group (OCH_3), a singlet at δ 4.11–4.19 ppm corresponding to methylene (–CH_2–) linkage for the compounds (**4k–t**); a singlet at 7.84–7.98 ppm for the corresponding aromatic NH (ArNH) and a singlet at 10.72–11.31 ppm for the corresponding phenolic (OH) group. The aromatic protons (ArH) were observed as singlet/doublet/multiplet depending on the nature of protons at δ 6.31–7.84 ppm. The coupling constants (*J* value in Hz) were also calculated for the doublet peak of aromatic protons. The nature of the carbon atoms was characterized and verified by ^{13}C NMR, recorded in DMSO-d_6 at 100 MHz. The mass spectra of the compounds showed molecular ion peak M^+, $(M^+ + 1)$ and $(M^+ + 2)$.

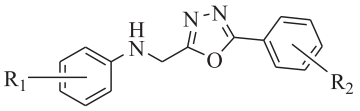
Antiproliferative activity evaluation

Fifteen oxadiazole analogs (**4a-n** and **4t**) were evaluated for their antiproliferative activity on nine different panels (leukemia, non small lung cell cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer) of nearly 59 cancer cell lines as per the standard NCI US protocol (http://dtp.nci.nih.gov; Boyd and Paull 1995; Monks et al. 1991; Shoemaker 2006). The antiproliferative activity in the form of growth percents (GPs) and percent growth inhibitions (%GIs) against the six most sensitive cell lines is given in Table 2. The compounds **4a**, **4d**, **4f**, **4g**, **4h**, **4j,** **4k**, **4l**, **4m** and **4n** showed comparatively less antiproliferative activity, however the compound, **4g** showed moderate antiproliferative activity against SNB-75, KM12, SF-539, and UO-31 with %GIs between 50.61 and 40.58. The compound **4e** showed significant %GIs on 8 cancer cell lines including U251, OVCAR-4, NCI-H460, 786-O, SF295, ACHN, HCT-116, and OVCAR3 with %GIs between 97.45 and 68.18. The compound, **4i** showed significant %GIs on 6 cancer cell lines including A498, TK-10, SNB-75, IGROV1, UO-31, and with %GIs between 93.82 and 79.63. The compound, **4c** showed significant %GIs on nine cancer cell lines including UO-31, MDA-MB-435, KM12, K-562, A498, SR, MCF7, NCI-H322M, and RPMI-8229 with %GIs between 84.31 and 68.98. The compound, **4b** showed significant %GIs on 16 cancer cell lines including HL-60(TB), MDA-MB-435, OVCAR-3, K-562, MDA-MB-468, HT29, NCI-H460, NCI-H522, MCF7, RPMI-8226, SR, NCI/ADR-RES, SW-620, M14, KM12, and MOLT-4 with %GIs between 109.62 and 70.76. The anticancer activity of compounds **4b** **4c**, **4e** and **4i** on 59 cancer cell lines is given in Table 3. The compound which showed GIs of ≥ 68% (i.e. GP of ≤ 32) was considered to be significantly active towards that particular cell lines and shown as bold figures in Table 2 and Table 3 (Corona et al. 2009). The antiproliferative activity in terms of average percent GIs for the compounds, **4b**, **4c**, **4e**, **4i**, 5-Fluorouracil (5-FU) and Imatinib was calculated against each panel of cancer cell lines and is given in Table 4. The compound, **4b** showed

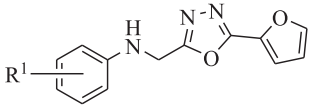
Table 1 The physical constants of oxadiazole analogs (**4a–t**)



4a-j



4k-s



4t

S. no.	Compounds	R^1	R^2	R_f*	% Yield	Mp (°C)
1	**4a**	2-Methoxy-	4-Fluoro-	0.77[a]	62	160–162
2	**4b**	2-Methoxy-	4-Chloro-	0.71[a]	88	210–212
3	**4c**	2-Methoxy-	4-Hydroxy-	0.78[a]	74	196–198
4	**4d**	2-Methoxy-	4-Methoxy-	0.88[a]	72	166–168
5	**4e**	2,5-Dimethoxy-	4-Chloro-	0.72[a]	82	216–218
6	**4f**	2,5-Dimethoxy-	4-Methoxy-	0.86[a]	77	168–170
7	**4g**	2,5-Dimethoxy-	4-Hydroxy-	0.89[a]	58	160–162
8	**4h**	2,5-Dimethoxy-	3,4-Dimethoxy-	0.80[a]	69	178–180
9	**4i**	2,5-Dimethoxy-	4-Hydroxy-3-methoxy-	0.86[a]	72	180–182
10	**4j**	2,5-Dimethoxy-	3,4,5-Trimethoxy-	0.82[a]	72	208–210
11	**4k**	2-Methoxy-	4-Chloro-	0.76[b]	76	118–120
12	**4l**	2-Methoxy-	4-Methoxy-	0.85[b]	72	126–128
13	**4m**	2,5-Dimethoxy-	3,4-Dimethoxy-	0.83[b]	77	112–114
14	**4n**	4-Methyl-	4-Chloro-	0.84[b]	89	140–142
15	**4o**	4-Methyl-	4-Hydroxy-	0.78[b]	76	112–114
16	**4p**	4-Methyl-	4-Methoxy-	0.80[b]	72	158–160
17	**4q**	4-Methyl-	3,4-Dimethoxy-	0.74[b]	67	70–72
18	**4r**	4-Methyl-	4-Hydroxy-3-methoxy-	0.81[b]	62	120–122
19	**4s**	4-Methyl-	2,3,4-Trimethoxy-	0.82[b]	78	110–112
20	**4t**	4-Methyl-	2-Furyl-	0.77[b]	67	108–110

*Mobile phase

[a] Benzene:acetone (8:2)

[b] n-Hexane:ethyl acetate:formic acid (5:4:1)

Table 2 The antiproliferative activity of oxadiazole analogs (**4a-t**)

Compound/NSC Code	Cancer cell lines assay in single dose assay 10 μM concentration				
	Mean GP	Range of GP	The most sensitive cell lines	GP	% GI
4a NSC 791191	96.50	75.73 to 108.84	MCF7 (breast cancer)	75.73	24.27
			NCI-H522 (non-small cell lung cancer)	77.30	22.70
			EKVX (non-small cell lung cancer)	84.92	15.08
			UO-31 (renal cancer)	85.73	14.27
			SR (leukemia)	85.74	14.26
			A549/ATCC (non-small cell lung cancer)	86.37	13.63
4b NSC 791192	45.20	−9.62 to 91.06	HL-60(TB) (leukemia)	**−9.62**	**109.62**
			MDA-MB-435 (melanoma)	**−5.90**	**105.90**
			OVCAR-3 (ovarian cancer)	**8.06**	**91.94**
			K-562 (leukemia)	**11.70**	**88.30**
			MDA-MB-468 (breast cancer)	**13.98**	**86.02**

Table 2 continued

Compound/NSC Code	Cancer cell lines assay in single dose assay 10 μM concentration				
	Mean GP	Range of GP	The most sensitive cell lines	GP	% GI
			HT29 (colon cancer)	**16.05**	**83.95**
4c NSC 791194	56.73	15.69 to 104.42	UO-31 (renal cancer)	**15.69**	**84.31**
			MDA-MB-435 (melanoma)	**19.48**	**80.52**
			KM12 (colon cancer)	**21.35**	**78.65**
			K-562 (leukemia)	**22.94**	**77.06**
			A498 (renal cancer)	**26.74**	**73.26**
			SR (leukemia)	**27.70**	**72.30**
4d NSC 793124	98.56	80.01 to 112.66	UO-31 (renal cancer)	80.01	19.99
			A549/ATCC (non-small cell lung cancer)	84.33	15.67
			NCI-H522 (non-small cell lung cancer)	85.43	14.57
			RXF 393 (renal cancer)	86.56	13.44
			T-47D (breast cancer)	88.31	11.69
			UACC-62 (melanoma)	90.02	9.98
4eNSC 790149	71.78	2.55 to 108.04	U251 (CNS cancer)	**2.55**	**97.45**
			OVCAR-4 (ovarian cancer)	**10.46**	**89.54**
			NCI-H460 (non-small cell lung cancer)	**18.81**	**81.19**
			786-O (renal cancer)	**26.44**	**73.56**
			SF295 (CNS cancer)	**29.20**	**70.8**
			ACHN (renal cancer)	**30.78**	**69.22**
4fNSC 790150	104.27	82.37 to 122.94	TK-10 (renal cancer)	82.37	17.63
			NCI-H522 (non-small cell lung cancer)	82.78	17.22
			786-O (renal cancer)	85.06	14.94
			HT29 (colon cancer)	88.75	11.25
			T-47D (breast cancer)	89.26	10.74
			MOLT-4 (leukemia)	92.64	7.36
4g NSC 790152	95.10	49.39 to 119.70	SNB-75 (CNS cancer)	49.39	50.61
			KM12 (colon cancer)	57.72	42.28
			SF-539 (CNS cancer)	59.19	40.81
			UO-31 (renal cancer)	59.42	40.58
			SN12C (renal cancer)	68.69	31.31
			ACHN (renal cancer)	74.69	25.31
4h NSC 790151	101.75	87.93 to 120.77	HT29 (colon cancer)	87.93	12.07
			K-562 (leukemia)	89.77	10.23
			HL-60(TB) (leukemia)	91.68	8.32
			NCI-H23 (non-small cell lung cancer)	92.11	7.89
			T-47D (breast cancer)	92.62	7.38
			TK-10 (renal cancer)	92.72	7.28
4i NSC 790153	65.37	6.18 to 117.08	A498 (renal cancer)	**6.18**	**93.82**
			TK-10 (renal cancer)	**14.79**	**85.21**
			SNB-75 (CNS cancer)	**15.23**	**84.77**
			IGROV1 (ovarian cancer)	**19.95**	**80.05**
			UO-31 (renal cancer)	**20.28**	**79.72**
			KM12 (colon cancer)	**20.37**	**79.63**
4j NSC 790159	98.20	76.60 to 121.35	NCI-H522 (non-small cell lung cancer)	76.60	23.40
			T-47D (breast cancer)	82.21	17.79
			MOLT-4 (leukemia)	84.11	15.89
			HT-29 (colon cancer)	86.76	13.24

Table 2 continued

Compound/NSC Code	Cancer cell lines assay in single dose assay 10 μM concentration				
	Mean GP	Range of GP	The most sensitive cell lines	GP	% GI
			MALME-3M (melanoma)	88.80	11.20
			SR (leukemia)	89.62	10.38
4k NSC 791196	97.87	72.89 to 117.98	UO-31 (renal cancer)	72.89	27.11
			HOP-62 (non-small cell lung cancer)	87.28	12.72
			PC-3 (prostate cancer)	87.57	12.43
			LOX IMVI (melanoma)	88.64	11.36
			NCI-H522 (non-small cell lung cancer)	89.23	10.77
			SNB-75 (CNS cancer)	89.31	10.69
4l NSC 793123	96.28	66.12 to 115.74	UO-31 (renal cancer)	66.12	33.88
			NCI-H522 (non-small cell lung cancer)	74.69	25.31
			SR (leukemia)	84.89	15.11
			MOLT-4 (leukemia)	86.06	13.96
			UACC-62 (melanoma)	86.41	13.59
			MDA-MB-231/ATCC (breast cancer)	87.74	12.26
4m NSC 791285	95.03	75.99 to 112.04	UO-31 (renal cancer)	75.99	24.01
			A549/ATCC (non-small cell lung cancer)	80.94	19.06
			SNB-75 (CNS cancer)	85.04	14.96
			MCF7 (breast cancer)	85.05	14.95
			HS 578T (breast cancer)	85.48	15.52
			HOP-62 (non-small cell lung cancer)	86.21	13.79
4n (**NSC** 790154)	102.16	73.34 to 120.65	UO-31 (renal cancer)	73.34	26.66
			SNB-75 (CNS cancer)	79.54	20.46
			NCI/ADR-RES (ovarian cancer)	87.70	12.30
			MCF-7 (breast cancer)	88.84	11.16
			NCI-H226 (non-small cell lung cancer)	89.51	10.49
			MDA-MB-231/ATCC (breast cancer)	100.72	−0.72
4o	76.0	55.5 to 96.5	MCF-7 (breast cancer)	55.5	44.5
			MDA-MB-231 (breast cancer)	96.5	3.5
4p	57.7	41.2 to 74.2	MCF-7 (breast cancer)	41.2	58.8
			MDA-MB-231 (breast cancer)	74.2	25.8
4q	99.85	46 to 107.7	MCF-7 (breast cancer)	46.0	54.0
			MDA-MB-231 (breast cancer)	107.7	−7.7
4r	85.35	51.3 to 119.4	MCF-7 (breast cancer)	51.3	48.7
			MDA-MB-231 (breast cancer)	119.4	−19.4
4s	84.65	45.1 to 124.2	MCF-7 (breast cancer)	45.1	54.9
			MDA-MB-231 (breast cancer)	124.2	−24.2
4t (**NSC** 790155)	98.46	68.32 to 127.33	UO-31 (renal cancer)	68.32	31.68
			SNB-75 (CNS cancer)	79.56	20.44
			HOP-92 (non-small cell lung cancer)	80.24	19.76
			KM12 (colon cancer)	82.41	17.59
			T-47D (breast cancer)	82.93	17.07
			IGROV1 (ovarian cancer)	86.57	13.43
Imatinib NSC 759854	94.56	52.9 to 122.8	HT29 (colon cancer)	52.9	47.1
			HOP-92 (non-small cell lung cancer)	56.3	43.7
			MDA-MB-468 (breast cancer)	70.9	29.1
			SF-539 (CNS cancer)	75.5	24.5
5-Fluorouracil	42.21	−19.6 to 95.5	SF-539 (CNS cancer)	**−19.6**	**119.6**

Table 2 continued

Compound/NSC Code	Cancer cell lines assay in single dose assay 10 μM concentration				
	Mean GP	Range of GP	The most sensitive cell lines	GP	% GI
			HCC-2998 (colon cancer)	**−17.8**	**117.8**
			A498 (renal cancer)	**−16.3**	**116.3**
			HS 578T (breast cancer)	**−10.8**	**110.8**
			MCF7 (breast cancer)	**11.5**	**88.5**
			NCI-H460 (non-small cell lung cancer)	**13.0**	**87.0**

The compound showed percent growth inhibition ≥ 68%, is active for that particular cell line (bold figure). The compounds **4a–n** and **4t** were evaluated on NCI 60 cancer cell line while compounds **4o–s** was evaluated on two breast cancer cell lines. The data of one dose assay for Imatinib and 5-FU was taken from the NCI database compound ID NSC 759854 and NSC 19893 (https://dtp.cancer.gov/dtpstandard/servlet/MeanGraphSummary)

GP growth percent, *%GI* percent growth inhibition

Table 3 NCI: DTP, The percent growth inhibition (% GI) of testing compounds (**4b**, **4c**, **4e**, and **4i**) over the full panel of tumor cell lines at a single dose (10 μM)

Panel	Cell lines	4b	4c	4e	4i
Leukemia	CCRF-CEM	62.03	55.88	−8.04	11.43
	HL-60(TB)	**109.62**	36.97	5.25	12.67
	K-562	**88.30**	**77.06**	16.5	67.67
	RPMI-8226	**80.50**	**68.98**	9.58	24.97
	MOLT-4	**70.76**	57.54	11.53	39.22
	SR	**80.27**	**72.30**	20.35	47.65
Non-small cell lung cancer	A549/ATCC	65.61	36.90	55.26	24.47
	EKVX	49.22	53.77	17.5	17.52
	HOP-62	52.59	37.33	53.28	22.37
	HOP-92	8.94	25.73	0.03	14.88
	NCI-H226	26.36	30.52	6.17	22.89
	NCI-H23	36.12	63.88	25	29.25
	NCI-H322M	29.54	**70.46**	43.06	13.08
	NCI-H460	**83.90**	16.1	**81.19**	17.24
	NCI-H522	**83.35**	16.65	10.08	38.92
Colon cancer	COLO 205	64.69	−4.42	0.88	−17.08
	HCC-2998	18	14.78	−4.08	9.44
	HCT-116	65.91	31.38	**68.88**	36.18
	HCT-15	66.94	50.47	4.04	53.49
	HT29	**83.95**	0.05	51.06	0.99
	KM12	**76.06**	**78.65**	33.6	**79.63**
	SW-620	**79.14**	40.99	13.76	21.41
CNS cancer	SF-268	39.49	40.30	44.65	31.19
	SF-295	59.71	39.65	**70.80**	−0.15
	SF-539	42.94	63.59	49.27	50.09
	SNB-19	53.55	27.3	41.64	30.05
	SNB-75	44.94	61.23	40.62	**84.77**
	U251	65.58	43.81	**97.45**	34.93
Melanoma	LOX IMVI	48.29	45.02	26.19	56.68
	MALME-3M	53.72	24.86	33.15	20.09
	M14	**78.25**	46.1	6.87	25.36
	MDA-MB-435	**105.9**	**80.52**	15.37	15
	SK-MEL-2	65.63	18.57	3.13	3.73
	SK-MEL-28	35.95	23.18	−1.58	−8.31
	SK-MEL-5	62.37	34.88	−4.17	28.37
	UACC-257	34.18	7.85	−3.96	−15.27
	UACC-62	48.21	28.84	2.47	3.3
Ovarian cancer	IGROV1	47.25	43.12	18.92	**80.05**
	OVCAR-3	**91.94**	34.52	**68.18**	29.45
	OVCAR-4	31.74	39.79	**89.54**	36.86
	OVCAR-5	13.47	19.04	17.57	29.97
	OVCAR-8	39.85	29.40	14.53	16.43
	NCI/ADR-RES	**79.22**	49.08	28.15	31.26
	SK-OV-3	52.29	50.35	9.28	46.65
Renal cancer	786-O	24.24	39.74	**73.56**	63.31
	A498	54.71	**73.26**	4.02	**93.82**
	ACHN	27.82	54.98	**69.22**	62.05
	RXF 393	25.43	59.26	9.57	47.34
	SN12C	40.45	49.07	9.87	66.04
	TK-10	31.07	36.44	64.01	**85.21**
	UO-31	30.5	**84.31**	48.16	**79.72**
Prostate cancer	PC-3	43.31	38.7	11.6	25.15
	DU-145	27.71	36.86	30.03	48.58
Breast cancer	MCF7	**82.88**	**71.32**	8.36	22.26
	MDA-MB-231/ATCC	36.5	44.17	14.4	30.56
	HS 578T	35.49	60.87	48.65	60.43
	BT-549	43.08	26.15	18.07	20.63
	T-47D	67.58	61.07	40.14	56.06
	MDA-MB-468	**86.02**	51.00	25.75	28.32
Mean	–	54.80	43.27	28.22	34.63

The compound showed percent growth inhibition ≥ 68%, is active for that particular cell line (bold figure)

better antiproliferative activity than the standard drugs 5-FU and Imatinib on leukemia, melanoma and ovarian cancer. Similarly the compound **4e** and **4i** showed better antiproliferative activity the standard drugs 5-FU and Imatinib

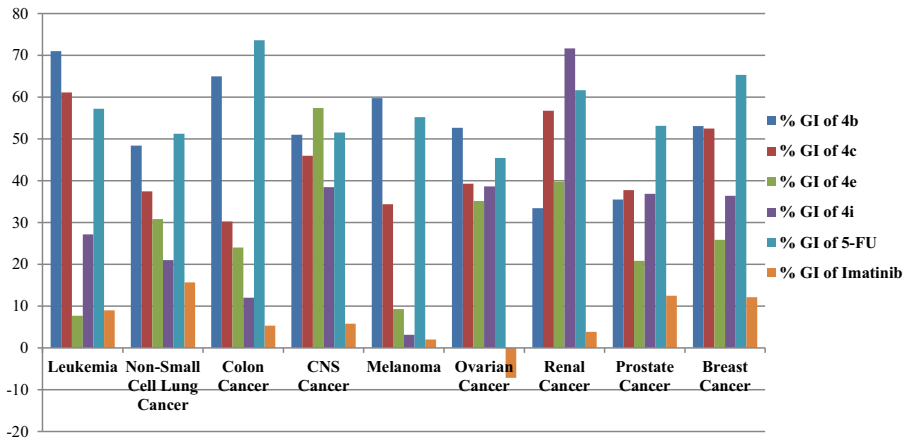


Fig. 3 The calculated average % GI of **4b**, **4c**, **4e**, **4i**, 5-Fluorouracil and Imatinib at 10 μM drug concentration

Table 4 The average percent growth inhibitions (GIs) of **4b**, **4c**, **4e**, **4i**, 5-Fluorouracil and Imatinib

Panel	% GI of 4b	% GI of 4c	% GI of 4e	% GI of 4i	% GI of 5-FU	% GI of Imatinib
Leukemia	**71.01**	61.12	7.73	27.19	57.23	9
Non-small cell lung cancer	48.41	37.47	30.86	21.04	**51.23**	15.68
Colon cancer	64.96	30.27	24.02	12.01	**73.63**	5.34
CNS cancer	51.04	45.98	**57.41**	38.48	51.57	5.80
Melanoma	**59.76**	34.37	9.29	3.15	55.2	2.02
Ovarian cancer	**52.66**	39.32	35.17	38.67	45.44	−7.15
Renal Cancer	33.46	56.73	39.77	**71.64**	61.69	3.86
Prostate cancer	35.51	37.78	20.82	36.87	**53.15**	12.50
Breast cancer	53.11	52.52	25.89	36.38	**65.34**	12.15

Bold figure shows higher activity

GP growth percent, *%GI* percent growth inhibition

Table 5 The percent growth control of oxadiazole analogs (**4o-s**) at different molar concentrations

Compound	MCF-7				MDA-MB-231			
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
4o	82.5	92.8	55.5	−8.5	124.9	112.6	96.5	30.5
4p	69.9	61.2	41.2	−23.1	122.9	101.9	74.2	15.1
4q	73.9	80.4	46.0	36.8	121.6	116.5	107.7	150.2
4r	63.0	80.7	51.3	19.4	120.7	121.6	124.2	74.0
4s	62.9	80.5	45.1	16.9	123.7	122.1	119.4	65.0
ADR	10.9	33.6	−50.7	−56.4	40.0	37.3	−21.9	−29.4

ADR adriamycin

on CNS cancer and renal cancer, respectively. The percent GIs of the compounds **4b**, **4c**, **4e, 4i** and the standard drugs Imatinib and 5-FU was compared and the results are shown in Fig. 2. The compounds **4b**, **4c**, **4e** and **4i** showed better percent GIs than the standard drug imatinib on nearly 49, 47, 40 and 44 cancer cell lines among the common 50 cancer cells. The antiproliferative activity of the compounds, **4e** and **4i** was found to be less, while the antiproliferative activity of the compound, **4c** was found to be moderate when compared with the standard drug 5-FU. The antiproliferative activity of the compound, **4b** (mean GP = 45.20) was found to be comparable to that of the standard drug 5-FU (mean GP = 42.21). The compound, **4b** showed better percent GIs than 5-FU, on 24 cancer cell lines. The antiproliferative activity of **4b**, **4c**, **4e**, **4i**, Imatinib and 5-FU in terms of percents GIs is shown in Fig. 3. The data of one dose assay for Imatinib and 5-FU was taken from the NCI database compound ID NSC 759854 and NSC 19893, respectively for comparison study (https://dtp.cancer.gov/dtpstandard/servlet/MeanGraphSummary). Rest of the compounds (**4o-s**) were evaluated for antiproliferative activity on the breast cancer cell lines (MCF-7 and MDA-

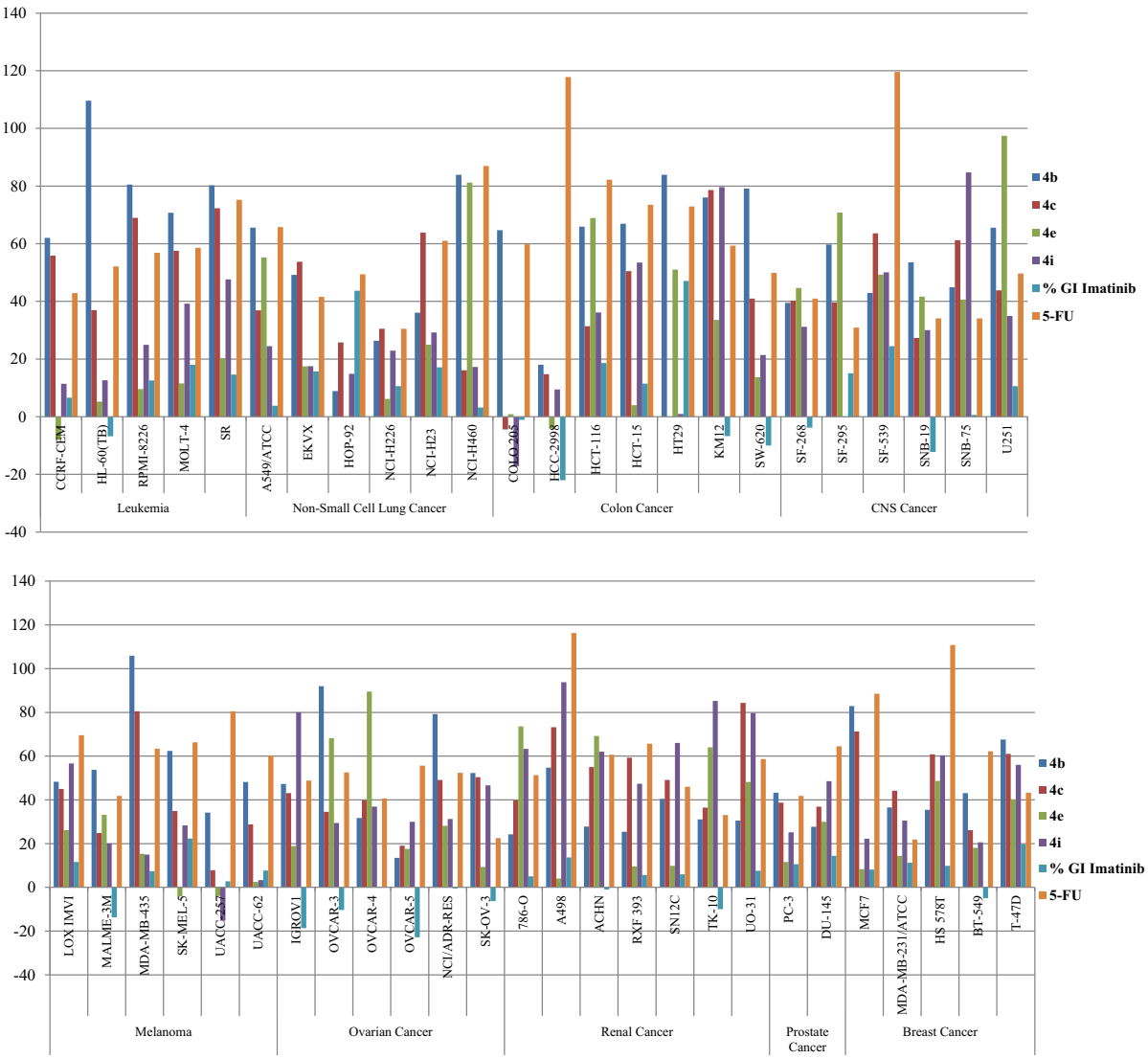


Fig. 4 The comparison of % GI shown by compounds, **4b**, **4c, 4e, 4i, 5-FU** and Imatinib against the NCI human cancer cell lines in common at 10 μM

Table 6 LC_{50}, TGI, and GI_{50} of quinoline analogs (**4o–s**) against two breast cancer cell lines

Compound	Drug concentrations calculated from graph (μM)					
	MCF-7			MDA-MB-231		
	LC_{50}	TGI	GI_{50}	LC_{50}	TGI	GI_{50}
4o	>100	89.0	33.9	>100	>100	76.0
4p	>100	71.6	12.9	>100	>100	59.3
4q	>100	>100	55.3	NE	NE	>100
4r	>100	>100	35.2	>100	>100	>100
4s	>100	>100	30.5	>100	>100	>100
ADR	82.9	2.7	<0.1	>100	39.6	<0.1

ADR adriamycin, GI_{50} is the concentration of drug that results in reduction of 50% protein increase, LC_{50} is the concentration of drug that results in reduction of 50% of cells, TGI is the concentration of drug that results in total growth inhibition

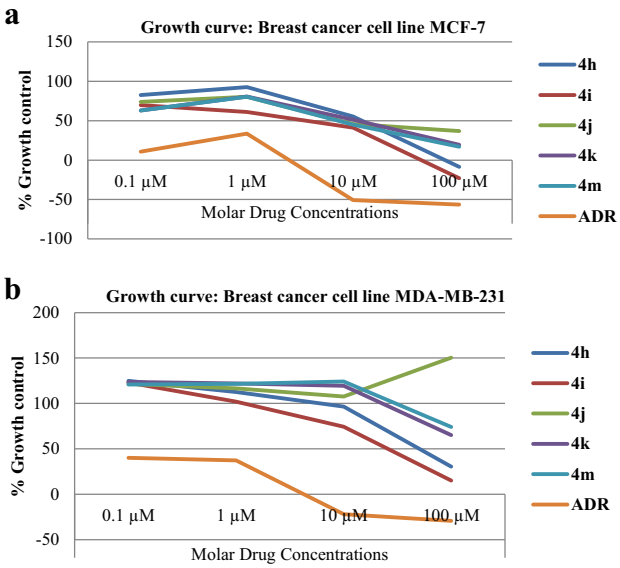
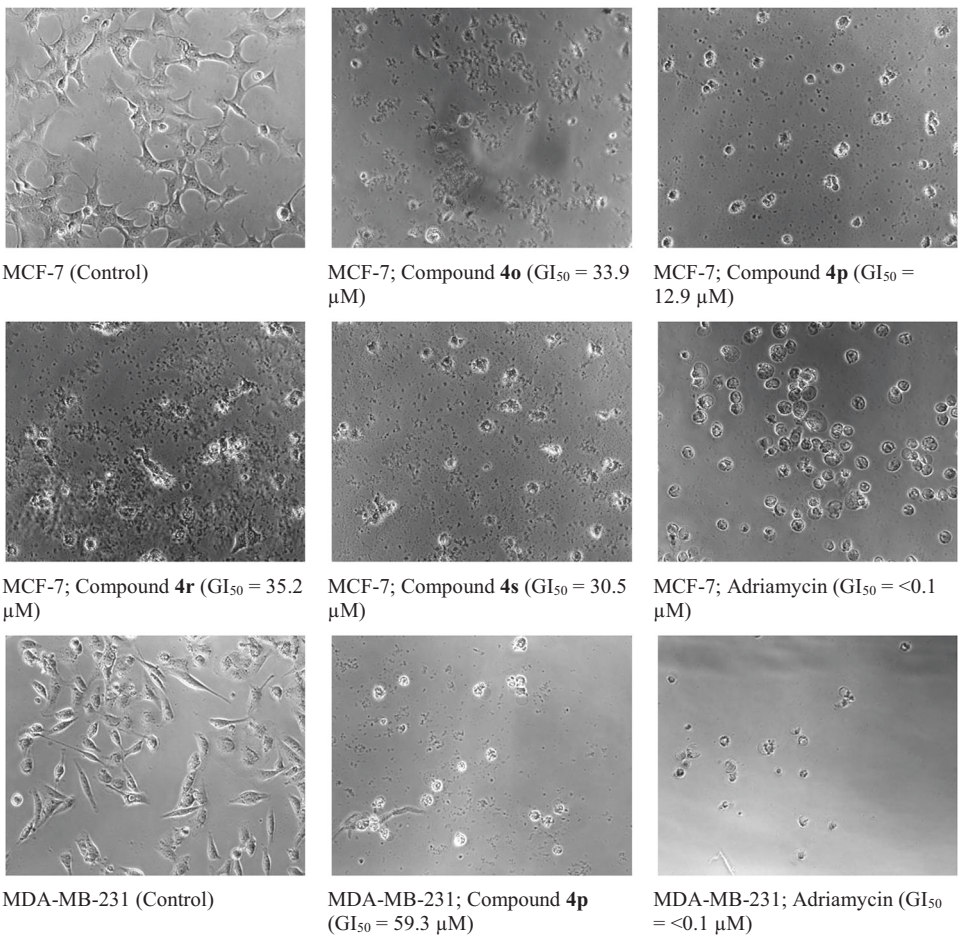


Fig. 5 **a** Growth curve of oxadiazole analogs on MCF-7 at molar drug concentrations. **b** Growth curve of oxadiazole analogs on MDA-MB-231 at molar drug concentrations

MB-231) as per the sulforhodamine B assay (SRB assay) (Vichai and Kirtikara 2006). The compounds, **4o-s** were tested at four different drug concentrations (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4}) and percent growth control was recorded for each compounds is given in Table 5 while the growth control curves on breast cancer cell line at molar drug concentrations are given in Fig. 4a (MCF-7) and **4b** (MDA-MB-231). Three dose related parameters, LC_{50}, TGI and GI_{50} were also calculated for each compound (**4o-s**) (Table 6). The GI_{50} was found to be 12.9 to 55.3 µM on MCF-7 cancer cell line while GI_{50} was found to be 59.3 to > 100 µM on MDA-MB-231. The compound **4p** demonstrated significant antiproliferative activity with GI_{50} of 12.9 and 59.3 µM on MCF-7 and MDA-MB-231 cancer cell lines, respectively. The LC_{50} was observed to be > 100 µM for both the cancer cell lines and the TGI values were ranging from 71.6 to > 100 µM for MCF-7 and > 100 µM for MDA-MB-231 cancer cell lines (Fig. 5). The images of growth control on breast cancer cell lines (MCF-7 and MDA-MB-231) for some the compounds (**4o-s**) are given in Fig. 6. The introduction of methylene group can alter the

Fig. 6 The images of growth control on MCF-7 and MDA-MB-231 for some of the compounds



MCF-7 (Control)

MCF-7; Compound **4o** (GI_{50} = 33.9 µM)

MCF-7; Compound **4p** (GI_{50} = 12.9 µM)

MCF-7; Compound **4r** (GI_{50} = 35.2 µM)

MCF-7; Compound **4s** (GI_{50} = 30.5 µM)

MCF-7; Adriamycin (GI_{50} = <0.1 µM)

MDA-MB-231 (Control)

MDA-MB-231; Compound **4p** (GI_{50} = 59.3 µM)

MDA-MB-231; Adriamycin (GI_{50} = <0.1 µM)

biological activity but not always promising as shown in the present investigation, however further investigations are in progress in our laboratory to establish this fact.

The antiproliferative data was taken into consideration to establish the structure activity relationship (SAR). The oxadiazole linked aryl nucleus with 4-chloro substitution showed maximum anticancer activity and followed by 4-hydroxy, 3-methoxy-4-hydroxy, 3,4-dimethoxy, 2,3,4-trimethoxy, 4-hydroxy and 4-methoxy substitutions on phenyl ring. The order of pharmacological activity followed as 4-Cl > 3-OCH_3-4-OH, > 3,4-$(OCH_3)_2$ > 2,3,4-$(OCH_3)_3$ > 4-OH > 4-OCH_3. Also 2-methoxy substitution showed comparatively higher activity than 2,5-dimethoxy substitution and 4-methyl substitution on the amino phenyl ring.

In vitro free radical scavenging activities

Since cancer and imbalance of oxidative stress share aspects of their underlying pathophysiology therefore, some antioxidants are equally effective antiproliferative agents (Rahal et al. 2014; Milkovic et al. 2014). Therefore, the antioxidant potential of the all the synthesized compounds was also evaluated by the DPPH assay and compared with the standard ascorbic acid (Koleva et al. 2002). Among the twenty compounds tested, three compounds **4c** (R_1 = 2-OCH_3, R_2 = 4-OH), **4g** (R_1 = 2,5-$(OCH_3)_2$, R_2 = 4-OH) and **4o** (R_1 = 4-CH_3, R_2 = 4-OH) showed promising free radical scavenging activities having IC_{50} values of 15.58 ± 0.91, 18.65 ± 0.65, and 20.32 ± 1.60 µM, respectively compared to standard ascorbic acid (12.91 ± 0.66 µM). These results clearly demonstrate that the substitutions on the distal phenyl rings greatly influenced the antioxidant potential of oxadiazole analogs. The increased free radical-scavenging activity of aforesaid compounds might be due to the hydrogen-donating ability of phenolic compounds and the stability of phenoxyl radicals formed after the dehydrogenation. In vitro free radical scavenging activities of oxadiazole analogues (**4a-s**) is given in Table 7 and shown in Fig. 7.

Molecular docking studies

Tubulin is one of the important and attractive targets of anticancer drugs. Many of the anticancer drugs like colchicine, combretastatins, vincristine, vinblastine etc. are inhibitors of tubulin polymerization. The oxadiazole analogs reported in the present investigation were designed based on the structure of tubulin inhibitor IMC-038525 hence tubulin was chosen as putative target and molecular docking against tubulin was carried out for these oxadiazoles. In our previous investigation some of the oxadiazoles moderately inhibited the tubulin (Ahsan et al. 2017). Other investigation also reported tubulin as a potential target for oxadiazole analogs (Abdel-Aziz et al. 2016; Kamal et al. 2016; Ouyang et al. 2006). The most active compound (**4b**) showed good interaction with the residues Lys254, Ala250, Cys241, Val318, Ala316, Asn258, and Lys352 present in the hydrophobic cavity of tubulin. Similarly the compound, **4c** showed good interaction with the residues Ala250, Cys241, Val318, Ala316, Asn258, Leu265, and Lys352, the compound, **4e** showed good interaction with the residues Ala250, Cys241, Leu255, Lys254, Val318, Ala316, Asn258, Thr353, Ala317, and Lys352, and compound, **4i** showed good interaction with the residues Cys241, Val318, Ala316, Asn258, Leu255, Thy353, Lys254, and Lys352.

Table 7 In vitro free radical scavenging activities of oxadiazole analogs (**4a–s**)

S. no.	Compounds	Free radical scavenging activity IC_{50} (µM)
1	4a	35.67 ± 2.38
2	4b	21.07 ± 1.30
3	4c	15.58 ± 0.91
4	4d	28.25 ± 1.46
5	4e	47.27 ± 1.14
6	4f	55.46 ± 2.00
7	4g	18.65 ± 0.65
8	4h	31.47 ± 1.53
9	4i	23.23 ± 1.90
10	4j	49.85 ± 1.92
11	4k	26.30 ± 1.14
12	4l	42.24 ± 1.16
13	4m	22.48 ± 1.49
14	4n	37.58 ± 1.68
15	4o	20.32 ± 1.60
16	4p	57.14 ± 1.90
17	4q	32.06 ± 1.71
18	4r	26.54 ± 1.44
19	4s	35.92 ± 0.69
20	4t	44.61 ± 1.77
21	Ascorbic acid	12.91 ± 0.66

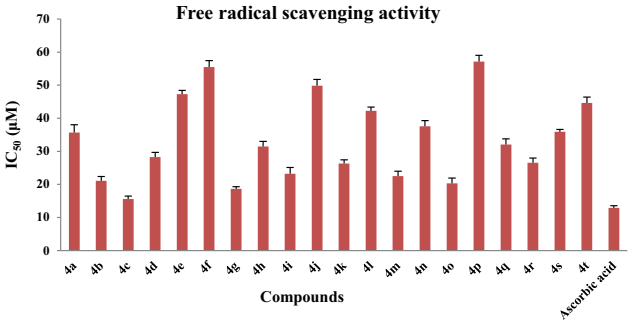


Fig. 7 In vitro free radical scavenging activities in micro molar concentrations (µM) of oxadiazole analogs (**4a–t**)

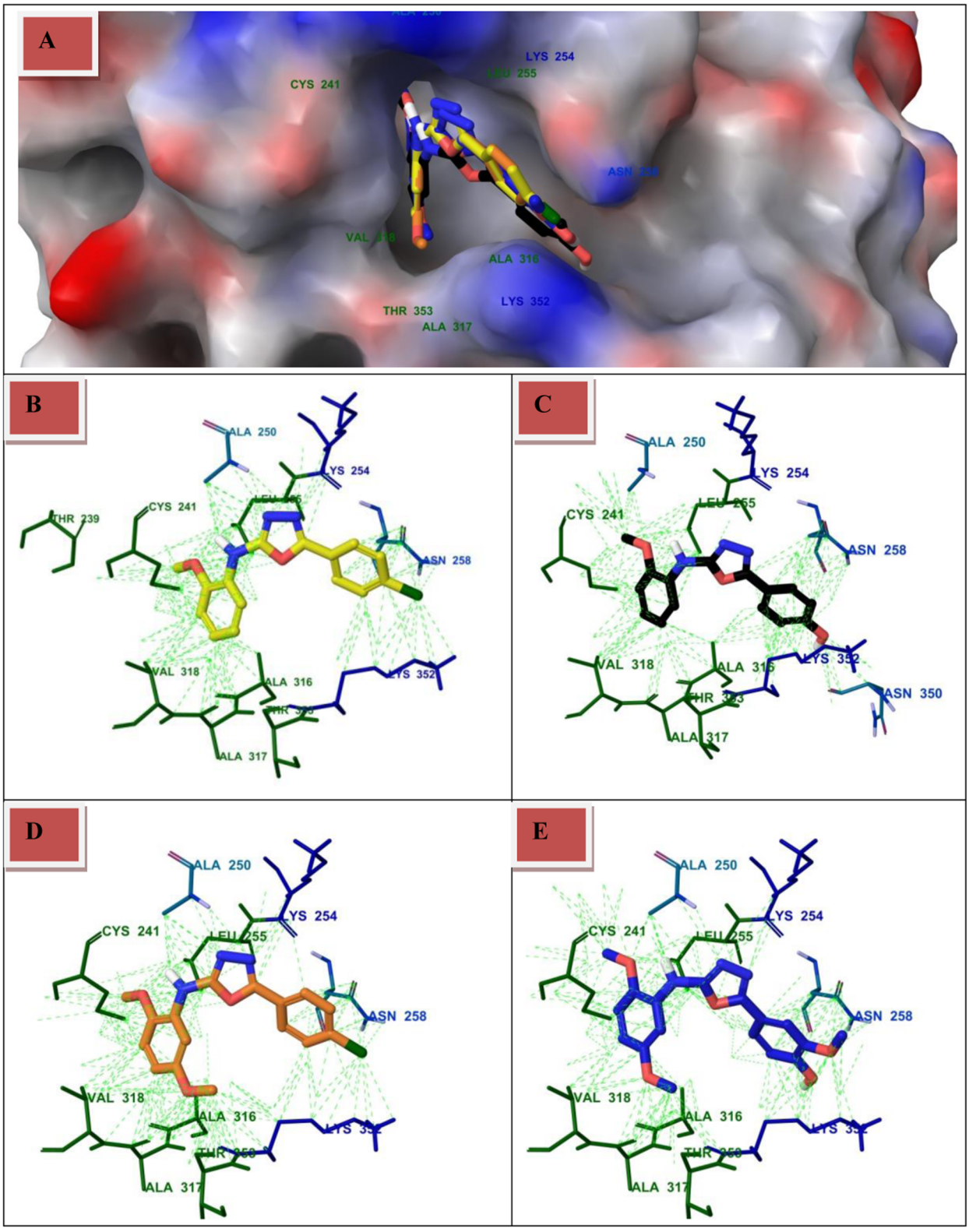


Fig. 8 An overview of compounds **4b** and **4c**, with the tubulin at colchicine binding site (**a**) and the binding mode of the compounds **4b** (**b**), **4c** (**c**), **4e** (**d**), and **4i** (**e**)

Table 8 The molecular properties prediction and molecular docking scores of oxadiazole analogs (**4a–t**)

S. no.	Compound	MW	HBA	HBD	Log *P*	NROTB	Lipinski's violation	Docking score
1	**4a**	285.28	5	1	4.09	4	0	−6.241
2	**4b**	301.73	5	1	4.61	4	0	−5.254
3	**4c**	283.29	6	2	3.45	4	0	−6.332
4	**4d**	297.31	6	1	3.99	5	0	−6.512
5	**4e**	331.76	5	1	3.83	5	0	−5.500
6	**4f**	327.12	6	1	3.27	6	0	−7.006
7	**4g**	313.11	6	2	2.86	5	0	−6.571
8	**4h**	357.13	7	1	3.18	7	0	−7.010
9	**4i**	343.12	7	2	2.83	6	0	−6.524
10	**4j**	387.14	8	1	3.15	8	0	−5.813
11	**4k**	315.76	5	1	3.30	5	0	−6.224
12	**4l**	311.34	6	1	2.68	6	0	−5.897
13	**4m**	371.39	8	1	2.31	8	0	−6.964
14	**4n**	299.08	3	1	3.98	4	0	−6.502
15	**4o**	281.12	4	2	3.00	4	0	−6.245
16	**4p**	295.13	4	1	3.35	5	0	−6.517
17	**4q**	325.14	5	1	3.32	5	0	−5.630
18	**4r**	311.13	5	2	2.97	5	0	−6.435
19	**4s**	355.15	6	1	3.29	7	0	−5.450
20	**4t**	331.13	4	1	4.30	5	0	−5.536
21	IMC-038525	–	–	–	–	–	–	−6.302

MW molecular weight, *HBA* hydrogen bond acceptors, *HBD h*ydrogen bond donors, *Log P* logarithm of partition coefficient between n-butanol and water, *NROTB* number of rotatable bonds

An overview and the binding mode of the compounds **4b**, **4c**, **4e**, and **4i** are shown in Fig. 8. The SP docking scores of the compounds **4b**, **4c**, and IMC-038525 were found to be −5.254, −6.332, and −6.302, respectively. The docking score of the compound, **4b** was found to be higher than the remaining compounds and IMC-038525. The docking scores of all the ligands are given in Table 8.

Molecular properties prediction

Many of the drugs failed to reach the market due to their low bioavailability, lipophilicity, solubility and pharmacokinetic properties. The bioavailability can be improved by increasing lipophilicity, reduced molecular flexibility, low polar surface area etc. The membrane permeability and bioavailability are more often associated with some of the basic molecular descriptors including partition co-efficient (Log $P \leq 5$), molecular weight (MW ≤ 500), and hydrogen bond donors (≤5)/acceptors (≤10) etc (Refsgaard et al. 2005; Ertl et al. 2000; Ahsan et al. 2011). The numbers of rotatable bonds are important for conformational changes in the molecule and should not be more than ten. All the oxadiazoles (**4a–t**) were subjected to computational studies for the predication of ADME properties. The Lipinski's rule of five was also calculated for each of the compounds, which states that a good bioavailability can be achieved for molecules having the Log *P* value ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptor ≤ 10, number of hydrogen bond donor ≤ 5 (Lipinski et al. 2001). In the present investigations the molecular weights of the oxadiazoles were ranging between 281.12 and 387.14 (MW < 500), the Log *P* values ranging between 2.31 and 4.61 (Log $P \leq 5$), the hydrogen bond acceptors ranging between 5 and 8 (≤10), and hydrogen bond donors ranging between 1 and 2 (≤5). The numbers of rotatable bonds were ranging between 4 and 8 (≤10). Hence none of the compounds (**4a–t**) showed any violations of Lipinski's rule of five making them potentially promising agents. The calculated molecular properties predictions are given in Table 8.

Conclusion

Two new series of oxadiazole analogs were synthesized in satisfactory yield. Fifteen oxadiazole analogs (**4a–n** and **4t**) were evaluated for their antiproliferative activity as per the NCI US standard protocol, while the remaining five compounds, (**4o–s**) were evaluated for antiproliferative activity on two breast cancer cell lines as per Sulforhodamine B assay. Some of the oxadiazoles (compounds **4b**, **4c**, **4e**, and

4i) showed significant antiproliferative activity in one dose assay at 10 μM. The compounds **4b**, **4c**, **4e**, and **4i** showed better antiproliferative activity that of the standard drug Imatinib, however only the antiproliferative activity of compound **4b** was found to be nearly similar than the standard drug 5-Fluorouracil. The compound **4p** showed significant antiproliferative activity with GI_{50} of 12.9 μM (MCF-7) and 59.3 μM (MDA-MB-231). The antioxidant activity of the compound **4c** was found to be significant. All these analogs could be modified to increase the biological profile. Further investigation in this area is going on our research laboratory. All these information could be of great help in the anticancer drug discovery.

Supporting information summary

The experimental section containing synthetic procedures and characterization data for all compounds synthesized in this work, as well as the NMR and mass spectra. The procedure to evaluate the antiproliferative activity as well as the antiproliferative activity screening data is provided as Supporting information.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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