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Research Article

Synthesis and Antiproliferative Activity of Some Quinoline and Oxadiazole Derivatives

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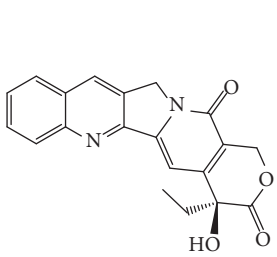
In continuance of our search for newer antiproliferative agents we report herein the synthesis and antiproliferative studies of two series (**5a–j** and **10a–c**) of heterocyclic compounds. All the new compounds were characterized by IR, NMR, and mass spectral data. The antiproliferative activity of 10 compounds (**5a–j**) was carried out on HeLa (cervix cancer cell line) and MDA-MB-435 (melanoma) and LC_{50}, TGI, and GI_{50} were calculated, while the antiproliferative activity of 3 compounds (**10a–c**) was carried out against nine different panels of nearly 60 cell lines (NCI-60) according to the National Cancer Institute (NCI US) Protocol at 10 μM. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)-3-(4-methoxylphenyl)urea (**5j**) was found to have antiproliferative activity with GI_{50} of 35.1 μM against HeLa (cervix cancer cell line) and 60.4 μM against MDA-MB-435 (melanoma), respectively. The compounds **10a**, **10b**, and **10c** showed antiproliferative activity with comparatively higher selectivity towards HOP-92 (Non-Small Cell Lung Cancer) with percent growth inhibitions (GIs) of 34.14, 35.29, and 31.59, respectively.

1. Introduction

Cancer is a genetic disease that is caused by changes to gene that control the way our cell functions. In all types of cancers some of the body's cells begin to divide without stopping and spread into the surrounding tissues. There are more than 100 types of cancer [1]. A total of 1,658,370 new cancer cases and 589,430 cancer deaths are projected to occur in the United States in 2015 [2]. Despite the availability of improved drugs and targeted cancer therapies, it is expected that the new cases of cancer will jump to 19.3 million worldwide by 2025 [3]. The types of cancer treatment include surgery, chemotherapy, radiation therapy, immunotherapy, targeted therapy, and hormonal therapy. The cancer patients are treated either with single therapy or with combinations of more than one therapy depending on the type of cancer's advancement [1]. The therapeutic applications of antiproliferative drugs are restricted due to their toxic potentials, resistance, and genotoxicity [4]. The demand for relatively more effective and safer agents for the treatment of cancer is today's need.

Heterocyclic quinoline nucleus occurs in nature and biologically active substances displaying broad therapeutic applications [5]. Several quinoline analogues were reported as anticancer agents [6–13]. The structure of some of the quinoline anticancer drug is shown in Figure 1 [6]. The biological potential of quinoline inspired us to explore further the quinoline derivatives. The quinoline derivatives showed promising antiproliferative activity against HeLa (human cervix cancer cell line) and MDA-MB-435 (melanoma) cell lines [6, 7, 14]. Hence we selected HeLa and MDA-MB-435 to test the *in vitro* antiproliferative activity of quinoline derivatives (**5a–j**) reported here in the present investigation. Three-dose response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each of the experimental agents. Similarly the



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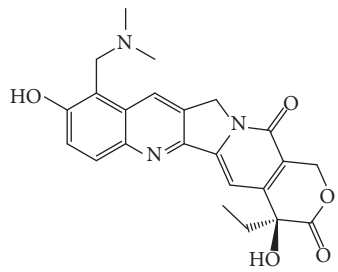

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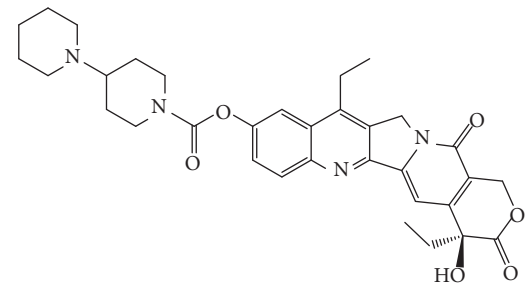


Irinotecan

Figure 1: Some of the quinoline containing anticancer drugs.

biological prospects of five member oxadiazoles as anticancer [15, 16], antitubercular [17], anticonvulsant [18], antimicrobial [19], anti-HIV [20], and anti-inflammatory [21] inspired us to go on further with the exploration of this moiety. Zibotentan, an endothelin receptor A (ET_A) antagonist, is an anticancer agent which contains 1,3,4-oxadiazole ring [22]. A series of oxadiazoles (**10a–c**) were synthesized and evaluated for their antiproliferative activity against 60 cell lines according to the National Cancer Institute (NCI US) Protocol at 10 μM drug concentration and percent growth inhibition (GI) was reported.

2. Materials and Methods

2.1. General. The chemicals were procured from Merck, Mumbai, and S. D. Fine Chemicals, Mumbai (India). Melting points were determined by open tube capillary method and are uncorrected. The completion of reaction was monitored throughout by thin layer chromatography (TLC) using mobile phase benzene/methanol (1 : 4) and cyclohexane/acetone (1 : 4) and the spots were located under iodine vapours or UV light. IR spectra were obtained on a Shimadzu 8201 PC, FT-IR spectrometer (KBr pellets). ^{1}H NMR spectra were recorded on a Bruker AC 400 MHz spectrometer using TMS as internal standard in DMSOd_6. Mass spectra were recorded on a Bruker Esquire LCMS using ESI and elemental analyses were performed on Perkin-Elmer 2400 Elemental Analyzer.

*2.2. Procedure for the Synthesis of 7-Hydroxy-4-methyl-2H-chromen-2-one (**3**).* A solution of resorcinol (**1**) (0.1 mol; 11.01 g) in ethyl acetoacetate (**2**) (0.1 mol; 13.01 g ~13 mL) was added slowly into the concentrated H_2SO_4 (previously cooled to 5°C) and stirred and the temperature was maintained below 10°C for 0.5 h. The reaction mixture was then poured into the crushed ice, filtered, washed, and dried to obtain 7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**) Yield 78%; Mp 192-193°C (reported) [23], 194–196°C (found).

*2.3. Procedure for the Synthesis of 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)urea/thiourea (**5a-b**).* Equimolar quantity of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**) (0.005 mol; 0.88 g) and semicarbazide/thiosemicarbazide (0.005 mol) was dissolved in ethanol and was refluxed for 4–8 h at 200°C and the reaction mixture was then kept overnight. The reaction was monitored throughout by thin layer chromatography (TLC) using benzene/acetone (1 : 4) as mobile phase. Finally the product was separated, dried, and recrystallized with methylated spirit.

*2.3.1. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)urea (**5a**).* Yield 70%; Mp 142–144°C; IR (KBr) cm^{-1}: 3404 (OH), 3202 (NH), 1685 (C=O); ^{1}H NMR (400 MHz; DMSO-d_6) ppm: 2.11 (3H, s, CH_3), 5.42 (2H, s, NH_2), 6.31 (1H, s, CH), 6.18 (1H, s, ArH), 6.38 (1H, d, J = 6.1 Hz, ArH), 7.08 (1H, d, J = 6.0 Hz, ArH), 9.02 (1H, s, CONH) 10.46 (1H, s, OH); Mass (m/z) 233 (M^+); Cacld/Anal. [C (56.65) 56.59, H (4.75) 4.78, N (18.02) 18.05].

*2.3.2. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)thiourea (**5b**).* Yield 68%; Mp 112–114°C; IR (KBr) cm^{-1}: 3414 (OH), 3201 (NH), 1286 (C=S); ^{1}H NMR (400 MHz; DMSO-d_6) ppm: 2.12 (3H, s, CH_3), 5.40 (2H, s, NH_2), 6.31 (1H, s, CH), 6.28 (1H, s, ArH), 6.29 (1H, d, J = 6.1 Hz, ArH), 7.05 (1H, d, J = 6.0 Hz, ArH), 8.32 (1H, s, CSNH), 10.41 (1H, s, OH); Mass (m/z) 249 (M^+); Cacld/Anal. [C (53.00) 53.05, H (4.45) 4.42, N (16.86) 16.85].

*2.4. Procedure for the Synthesis of 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-substituted Phenyl Urea (**5c–j**).* Equimolar quantity of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**) (0.005 mol; 0.88 g) and substituted phenyl semicarbazide (0.005 mol) was dissolved in ethanol and was refluxed for 4–8 h at 200°C and the reaction mixture was then kept overnight. Finally the product was separated, dried, and recrystallized with methylated spirit. The reaction was monitored throughout by thin layer chromatography (TLC) using benzene/acetone (1 : 4) as mobile phase. The substituted phenyl semicarbazide was synthesized as per the reported method [24].

*2.4.1. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-phenylurea (**5c**).* Yield 80%; Mp 150–152°C; IR (KBr) cm^{-1}: 3424 (OH), 3018 (NH), 1675 (C=O); ^{1}H NMR (400 MHz; DMSO-d_6) ppm: 2.11 (3H, s, CH_3), 5.90 (1H, s, NH), 6.31 (1H, s, CH), 6.38–7.05 (8H, m, ArH), 8.39 (1H, s, CONH), 10.49 (1H, s,

OH); Mass (m/z) 309.1 (M^+); Cacld/Anal. [C (66.01) 66.04, H (4.89) 4.90, N (13.58) 13.49].

*2.4.2. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(2,4-dimethylphenyl)urea (**5d**).* Yield 70%; Mp 130–132°C; IR (KBr) cm^{-1}: 3434 (OH), 3010 (NH), 1670 (C=O); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.11 (3H, s, CH_3), 2.19 (3H, s, CH_3), 2.34 (3H, s, CH_3), 5.91 (1H, s, NH), 6.10 (1H, s, CH), 6.68–6.95 (6H, m, ArH), 8.29 (1H, s, CONH), 10.51 (1H, s, OH); Mass (m/z) 338.1 (M^+); Cacld/Anal. [C (67.64) 67.59, H (5.68) 5.70, N (12.46) 12.47].

*2.4.3. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(2-chlorophenyl)urea (**5e**).* Yield 65%; Mp 118–120°C; IR (KBr) cm^{-1}: 3421 (OH), 3110 (NH), 1679 (C=O), 695 (C-Cl); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.13 (3H, s, CH_3), 5.92 (1H, s, NH), 6.33 (1H, s, CH), 6.28–7.45 (7H, m, ArH), 8.72 (1H, s, CONH), 10.49 (1H, s, OH); ^{13}C NMR (100 MHz; DMSO-d_6) ppm: 160.7, 158.3, 153.7, 148.8, 141.5, 134.9, 130.7, 129.1, 128.9, 128.1, 127.3, 125.9, 123.5, 120.7, 113.8, 106.6, 97.5, 15.6; Mass (m/z) 343.0 (M^+)345.1 (M+2)$^+$; Cacld/Anal. [C (59.40) 59.45, H (4.10) 4.08, N (12.22) 12.25].

*2.4.4. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(4-methylphenyl)urea (**5f**).* Yield 59%; Mp 134–136°C; IR (KBr) cm^{-1}: 3409 (OH), 3112 (NH), 1682 (C=O); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.11 (3H, s, CH_3), 2.33 (3H, s, CH_3), 5.91 (1H, s, NH), 6.34 (1H, s, CH), 6.18–7.09 (7H, m, ArH), 8.91 (1H, s, CONH), 10.52 (1H, s, OH); ^{13}C NMR (100 MHz; DMSO-d_6) ppm: 159.9, 158.1, 156.3, 153.0, 147.9., 141.6, 128.9, 128.1, 122.5, 120.1, 114.9, 112.9, 105.3, 97.9, 18,2, 15.5; Mass (m/z) 323.2 (M^+); Cacld/Anal. [C (66.86) 66.82, H (5.30) 5.35, N (13.00) 13.04].

*2.4.5. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(2-methylphenyl)urea (**5g**).* Yield 73%; Mp 140–142°C; IR (KBr) cm^{-1}: 3410 (OH), 3108 (NH), 1680 (C=O); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.14 (3H, s, CH_3), 2.34 (3H, s, CH_3), 5.90 (1H, s, NH), 6.31 (1H, s, CH), 6.19–7.08 (7H, m, ArH), 8.96 (1H, s, CONH), 10.48 (1H, s, OH); Mass (m/z) 323.2 (M^+); Cacld/Anal. [C (66.86) 66.83, H (5.30) 5.34, N (13.00) 13.03].

*2.4.6. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(4-fluorophenyl)urea (**5 h**).* Yield 64%; Mp 136–138°C; IR (KBr) cm^{-1}: 3411 (OH), 3108 (NH), 1678 (C=O), 785 (C-F); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.33 (3H, s, CH_3), 5.90 (1H, s, NH), 6.35 (1H, s, CH), 6.24–7.07 (7H, m, ArH), 8.92 (1H, s, CONH), 10.59 (1H, s, OH); ^{13}C NMR (100 MHz; DMSO-d_6) ppm: 158.9, 156.1, 153.9, 148.7, 147.9, 141.3, 128.9, 127.1, 122.3, 120.1, 114.9, 112.9, 106.3, 97.9, 15.5; Mass (m/z) 327.3 (M^+), 329.1 (M+2)$^+$; Cacld/Anal. [C (62.38) 62.35, H (4.31) 4.34, N (12.84) 12.85].

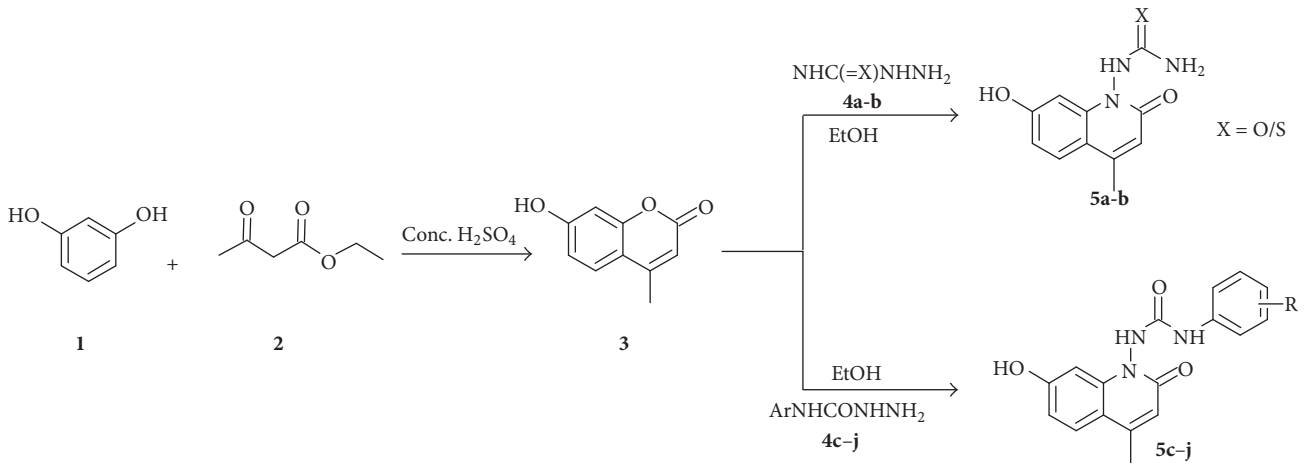
*2.4.7. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(4-bromophenyl)urea (**5i**).* Yield 66%; Mp 126-126°C; IR (KBr) cm^{-1}: 3411 (OH), 3102 (NH), 1672 (C=O), 694 (C-Br); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.31 (3H, s, CH_3), 5.91 (1H, s, NH), 6.35 (1H, s, CH), 6.28–7.25 (7H, m, ArH), 8.82 (1H, s, CONH), 10.54 (1H, s, OH); Mass (m/z) 387.1 (M^+), 389.3 (M+2)$^+$; Cacld/Anal. [C (52.60) 52.63, H (3.63) 3.64, N (10.82) 10.84].

*2.4.8. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2H)-yl)-3-(4-methoxylphenyl)urea (**5j**).* Yield 72%; Mp 166–168°C; IR (KBr) cm^{-1}: 3401 (OH), 3099 (NH), 1678 (C=O); ^1H NMR (400 MHz; DMSO-d_6) ppm: 2.33 (3H, s, CH_3), 3.81 (3H, s, OCH_3), 5.90 (1H, s, NH), 6.34 (1H, s, CH), 6.25–7.09 (7H, m, ArH), 8.88 (1H, s, CONH), 10.55 (1H, s, OH); ^{13}C NMR (100 MHz; DMSO-d_6) ppm: 160.9, 158.2, 156.4, 153.9, 148.7, 141.7, 128.3, 128.1, 122.6, 120.9, 114.5, 113.9, 106.3, 97.9, 56.2, 15.8; Mass (m/z) 339.3 (M^+); Cacld/Anal. [C (63.71) 63.73, H (5.05) 5.08, N (12.38) 12.35].

*2.5. Procedure for the Synthesis of Ethyl(pyridine-2-ylamino)acetate (**8**).* 2-Aminopyridine (**6**) (0.1 mol; 9.41 g) and ethylchloroacetate (**7**) (0.2 mol; ~24 mL) were taken in a round bottom flask and suspended in 80–100 mL acetone and 10 g anhydrous potassium carbonate was added to the mixture. The mixture was refluxed for 24 h on sand bath with vigorous stirring and then cooled and the excess solvent removed under reduced pressure. The residual mass was triturated with ice water to remove potassium carbonate and extracted with ethylacetate (3 × 50 mL) and the ethylacetate layer was washed with 10% sodium hydroxide solution (3 × 30 mL) followed by water (3 × 30 mL) and then dried over anhydrous sodium sulphate and evaporated to dryness to obtain ethyl(pyridin-2-ylamino)acetate (**8**) as brown solid. Yield 81%; Mp 94–96°C (reported) [25], 90–92°C (found).

*2.6. Procedure for the Synthesis of 2-(Pyridin-2-ylamino)acetohydrazide (**9**).* Ethyl(pyridine-2-ylamino)acetate (**8**) (0.075 mol; 12.46 g) and hydrazine hydrate (0.15 mol; ~7.5 mL) were refluxed in ethanol for 16 h on water bath. The two-third volume of reaction mixture was removed under reduced pressure and then poured into crushed ice to obtain 2-(pyridin-2-ylamino)acetohydrazide (**9**) as brown solid. Yield 85%; Mp 100–102°C (reported) [25], 104°C (found).

*2.7. General Procedure for the Synthesis of N-{[5-Aryl-1,3,4-oxadiazol-2-yl]methyl} Pyridin-2-amine Analogues (**10a–c**).* 2-(Pyridin-2-ylamino)acetohydrazide (**9**) (0.001 mol; 0.166 g) and aromatic aldehydes (0.001 mol) were refluxed 10–12 h using 20 mol% $NaHSO_3$ and ethanol-water system (1 : 2, v/v) solvent [26]. After completion of reaction the excess solvent was removed and the concentrate was poured into the crushed ice filter, washed with water, dried, and recrystallized with absolute ethanol to obtain the final product *N*-{[5-aryl/alkyl-1,3,4-oxadiazol-2-yl]methyl} pyridin-2-amine analogues (**10a–c**). The completion of reaction was monitored throughout by thin layer chromatography (TLC) using mobile phase benzene/methanol (1 : 4) and cyclohexane/acetone (1 : 4) and the spots were located under iodine vapours or UV light.



Scheme 1: Protocol for the synthesis of quinoline analogues (**5a–j**).

*2.7.1. N-{[5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**10a**).* Yield 79%; Mp 198–200°C; IR (KBr) cm^{-1}: 3192 (NH), 1531 (C=N), 1153 (C-O-C), 764 (C-Cl); ^{1}H NMR (400 MHz; DMSO-d_6) ppm: 3.32 (2H, s, CH_2), 7.54–7.56 (2H, d, J = 5.7 Hz, ArH), 7.56–7.58 (2H, d, J = 6.3 Hz, ArH), 7.87–7.93 (4H, m, pyridine), 8.70 (1H, s, NH); Mass (m/z) 286 (M^+), 288 $(M+2)^+$; Cacld/Anal. [C (58.62) 58.65, H (3.89) 3.87, N (19.51) 19.54].

*2.7.2. N-{[5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**10b**).* Yield 80%; Mp 150–152°C; IR (KBr) cm^{-1}: 3199 (NH), 1541 (C=N), 1165 (C-O-C); ^{1}H NMR (400 MHz; DMSO-d_6) ppm: 3.32 (2H, s, CH_2), 3.80 (3H, s, OCH_3), 7.02–7.04 (2H, d, J = 6.6 Hz, ArH), 7.32–7.34 (2H, d, J = 6.1 Hz, ArH), 7.78–7.83 (4H, m, pyridine), 8.62 (1H, s, NH); ^{13}C NMR (100 MHz; DMSO-d_6) ppm: 162.1, 160.7, 152.3, 148.9, 138.3, 134.5, 128.5, 118.6, 114.8, 113.6, 109.7, 56.2, 51.5; Mass (m/z) 282 (M^+); Cacld/Anal. [C (63.78) 63.82, H (5.03) 5.00, N (19.87) 19.85].

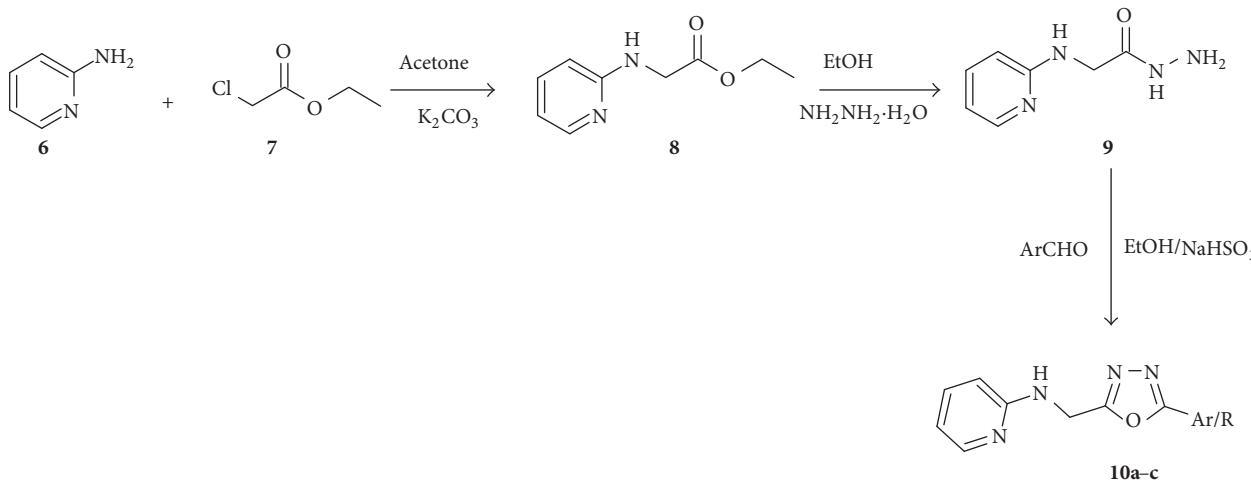
*2.7.3. N-{[5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine (**10c**).* Yield 82%; Mp 160–162°C; IR (KBr) cm^{-1}: 3194 (NH), 1537 (C=N), 1166 (C-O-C); ^{1}H NMR (400 MHz; DMSO-d_6) ppm: 3.32 (2H, s, CH_2), 3.80 (6H, s, OCH_3), 7.04–7.06 (2H, d, J = 6.3 Hz, ArH), 7.34–7.36 (2H, d, J = 6.3 Hz, ArH), 7.46–7.57 (4H, m, pyridine), 8.62 (1H, s, NH); Mass (m/z) 312 (M^+); Cacld/Anal. [C (61.56) 61.53, H (5.13) 5.16, N (17.91) 17.94].

2.8. In Vitro Antiproliferative Activity. Antiproliferative activity of the ten compounds (**5a–j**) was evaluated in two different human cell lines (HeLa and MDA-MB-435) using the sulforhodamine B (SRB) protocol [27, 28], while the antiproliferative screening compounds (**10a–c**) were carried out on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers cell lines, nearly 60 in number according to the reported NCI US protocol [29–32].

Three-dose response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each of the experimental agents. Growth inhibition of 50% (GI_{50}) was calculated from $100 \times [(T_i - T_z)/(C - T_z)] = 50$, which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by sulforhodamine B, SRB staining) in control cells during the drug incubation. The total growth inhibition (TGI) was calculated from $T_i = T_z$, which was the drug concentration resulting in total growth inhibition and signified the cytostatic effect. The LC_{50} was calculated from $100 \times [(T_i - T_z)/(C - T_z)] = -50$, which was the drug concentration resulting in a net loss of cells following treatment which indicated the concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning.

3. Results and Discussion

3.1. Chemistry. The synthetic protocol of quinoline analogues (**5a–j**) is summarized in Scheme 1. In the initial step solution of resorcinol (**1**) (0.1 mol; 11.01 g) in ethyl acetoacetate (**2**) (0.1 mol; 13.01 g ~13 mL) was added slowly into the concentrated H_2SO_4 (previously cooled to 5°C) and stirred and the temperature was maintained below 10°C for 0.5 h to obtain the intermediate 7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**). In the subsequent step an equimolar quantity of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**) (0.005 mol; 0.88 g) and semicarbazide/thiosemicarbazide/substituted phenyl semicarbazide (0.005 mol) in ethanol (20 mL) was refluxed for 4–8 h at 200°C to obtain 1-(7-hydroxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)urea/thiourea (**5a-b**) and 1-(7-hydroxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)-3-substituted phenyl urea (**5c–j**). The reaction was monitored throughout by thin layer chromatography (TLC) using benzene/acetone (1 : 4) as mobile phase. The substituted phenyl semicarbazide used in the final step was synthesized as per the reported method [21]. The yields of the final compounds (**5a–j**) were ranging from 59% to 80% after recrystallization with methylated spirit. 2,5-Disubstituted-1,3,4-oxadiazole analogues



Scheme 2: Protocol for the synthesis of *N*-{[5-aryl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**10a–c**).

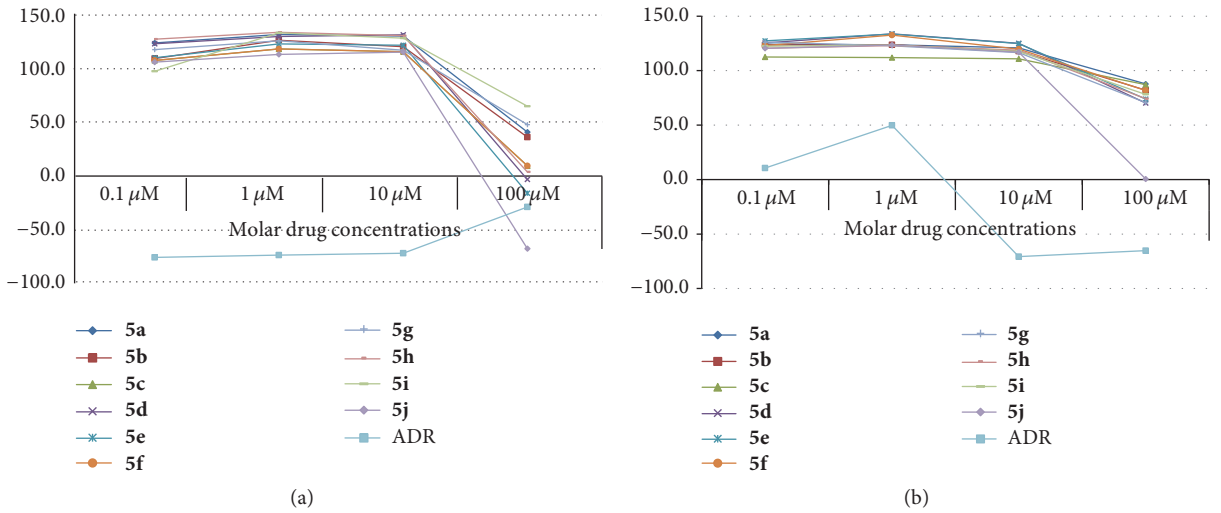


Figure 2: (a) Growth curve of quinoline analogues (**5a–j**) HeLa (human cervix cancer cell line) at molar concentrations. (b) Growth curve of quinoline analogues (**5a–j**) MDA-MB-435 (melanoma) at molar concentrations.

(**10a–c**) described in this study were synthesized as per the synthetic protocol summarized in Scheme 2. In the initial step 2-aminopyridine (**6**) (0.1 mol; 9.41 g) and ethylchloroacetate (**7**) (0.2 mol; ~24 mL) were taken in a round bottom flask and suspended in 80–100 mL acetone and 10 g anhydrous potassium carbonate was added to the mixture. The mixture was refluxed for 24 h on sand bath with vigorous stirring to obtain intermediate semisolid ethyl(pyridine-2-ylamino)acetate (**8**). In the subsequent step compound **8** was refluxed with hydrazine hydrate in ethanol for 8–12 h to obtain 2-(pyridine-2-ylamino)acetohydrazide (**9**) as brown semisolid. In the final step compound **9** was refluxed with aromatic aldehydes for 12–14 h using 20 mol% $NaHSO_3$ and ethanol-water system (1 : 2, v/v) solvent to obtain *N*-{[5-aryl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**10a–c**). The oxadiazole analogues were synthesized as per the reported method [23]. The yields of the title compounds were ranging between 79% and 82% after recrystallization with absolute ethanol. The completion of reaction was monitored throughout by thin layer chromatography (TLC) using mobile phase benzene/acetone (1 : 4), benzene/methanol (1 : 4), and cyclohexane/acetone (1 : 4). The purity of the synthesized compounds was checked by elemental analysis. Both the analytical and spectral data of the compounds were in full agreement with the proposed structure.

3.2. In Vitro Antiproliferative Activity. 10 compounds (**5a–j**) were evaluated for antiproliferative activity on HeLa (human cervix cancer cell line) and MDA-MB-435 (melanoma) at four different molar drug concentrations (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M) and the growth percent was recorded. The cytotoxic result was less at first three concentrations but 10^{-4} M concentration produced strong cytotoxicity ranging between −66.9 and 61.2 percent growth against HeLa and

Table 1: LC_{50}, TGI, and GI_{50} of quinoline analogues (**5a–j**) against HeLa and MDA-MB-435 cancer cell lines.

Compound	Drug concentrations calculated from graph (μM)					
	Human cervix cancer cell line HeLa			Melanoma MDA-MB-435		
	LC_{50}	TGI	GI_{50}	LC_{50}	TGI	GI_{50}
5a	>100	>100	87.0	>100	>100	>100
5b	>100	>100	80.6	>100	>100	>100
5c	>100	>100	73.20	>100	>100	>100
5d	>100	97.28	58.9	>100	97.28	>100
5e	>100	88.17	50.6	>100	88.17	>100
5f	>100	>100	59.9	>100	>100	>100
5g	>100	>100	93.0	>100	>100	>100
5 h	>100	>100	62.7	>100	>100	>100
5i	>100	>100	>100	>100	>100	>100
5j	91.33	63.19	**35.1**	>100	>100	**60.4**
ADR	54.42	<0.1	<0.1	70.6	1.7	<0.1

ADR = adriamycin, positive control compound.
GI_{50} value of $\leq 10^{-6}$ M (i.e., 1 μmolar) is considered to demonstrate activity.

between 0.6 and 87.8 percent growth against MDA-MB-435 (Figures 2(a) and 2(b)). The compound **5j** showed maximum cytotoxicity with −66.9 and 0.6 percent growths against HeLa and MDA-MB-435, respectively. The cytotoxicity of compound **5j** was found to be higher than the standard drug, adriamycin, at 10^{-4} M concentration against HeLa. Further three parameters (GI_{50}, TGI, and LC_{50}) were calculated for all the quinoline derivatives. The GI_{50} recorded were ranging between 35.1 and >100 μM against HeLa, while only the compound **5j** registered GI_{50} of 60.4 μM against MDA-MB-435 and rest of the compounds showed GI_{50} of >100 μM. The LC_{50} recorded was found to be >100 μM for both the cell lines, except for the compound **5j** which showed LC_{50} of 91.33 μM against HeLa. The compounds **5j**, **5e,** and **5d** showed TGI of 63.19, 88.17, and 97.28 μM, respectively, against HeLa, while compounds **5e** and **5d** showed TGI of 63.19 and 88.17 μM, respectively, against MDA-MB-435. The GI_{50}, TGI, and LC_{50} were recorded for the quinoline derivatives (**5a–j**) and are shown in Table 1. The value of GI_{50} was taken into consideration to establish the structure activity relationship (SAR) of the synthesized compounds. The quinoline having 2,4-dimethyl substitution in phenyl ring was found to be more favorable than 4-methyl and 2-methyl substitution, while 2-chloro substitution was found to be more favorable than 4-fluoro and 4-bromo substitutions. The 4-methoxy substitution on phenyl ring showed significant antiproliferative activity. The order of antiproliferative activity followed with substitution on phenyl ring as 4-OCH_3 > 2-Cl > 2,4-$(CH_3)_2$ > 4-CH_3 > 2-CH_3. The images of growth control of MDA-MB-435 and HeLa cancer cell lines by some of the quinoline analogues (**5a–j**) and adriamycin are shown in Figures 3(a) and 3(b).

Further since quinoline derivatives were found to inhibit epidermal growth factor receptor tyrosine kinase (EGFR-TK) [33]. A molecular docking study implying epidermal growth factor receptor tyrosine kinase (EGFR-TK) was carried out to observe the binding mode of new quinoline analogues (**5a–j**) on the active site of EGFR-TK. The molecular docking protocol is the same as reported earlier by our research group [34]. Three different binding modes (green, yellow, and grey) were observed by ligands (**5a–j**) as shown in the Figure 4. The binding mode of compounds **5c**, **5d**, **5f**, **5 h**, **5i,** and **5j** (green ligands) with the active site of EGFR-TK showed interaction with backbone H-bonding of hydroxyl group with Met793 and side chain H-bonding of NH with Asp855 (**5f**, **5i,** and **5j**). The binding mode of compounds **5b** (yellow ligands) with the active site of EGFR-TK showed backbone H-bonding of hydroxy group with Met793 and side chain H-bonding of terminal amine with Thr854. The binding mode of compounds **5a**, **5e**, and **5g** (grey ligands) with the active site of EGFR-TK showed backbone H-bonding of NH group with Arg841 and side chain H-bonding of hydroxyl and aryl NH group with Asp855 and Asn842, respectively, while showing π-π stacking with Phe723 (compound **5e**), π-cationic interaction of substituted phenyl ring with Arg841 (compound **5g**). The compound **5j** showed hydrophobic interaction with Met793, Leu792, Ala743, Gly796, Met766, Leu788, Leu777, and Lys745, backbone H-bonding of hydroxyl group with Met793, and side chain H-bonding of NH with Asp855. The binding mode of interaction with EGFR-TK is given in Figure 5.

Three compounds (**10a–c**) were tested for antiproliferative activity on leukemia, melanoma, lung, colon, CNS, ovarian, prostate, and breast cancer cell lines (nearly 60 cell lines) as per the NCI US protocol and carried out at Nation Cancer Institute, USA. The compound **10b** showed maximum activity with growth percent (GP) of 94.33 followed by compound **10c** (GP = 95.12) and **10a** (GP = 96.37). The compound **10a** showed maximum selectivity towards HOP-92, MCF7, SNB-75, T-47D, PC-3, and UO-31 with percent GI of 34.14, 21.22, 20.52, 15.39, 14.97, and 13.57, respectively. The compound **10b** showed maximum selectivity towards

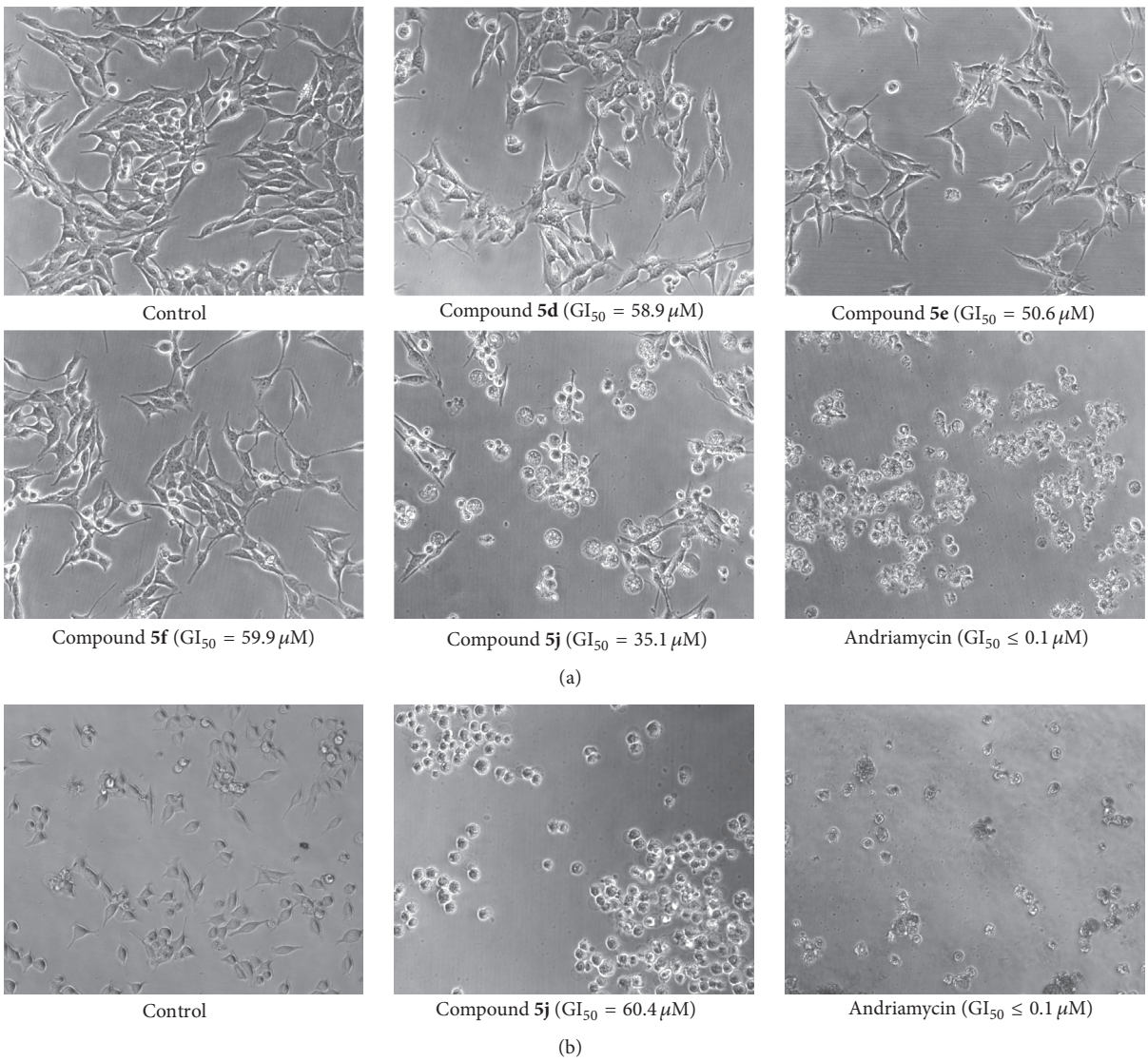


Figure 3: (a) Images of growth control of MDA-MB-435 cancer cell line by quinoline (**5a–j**) and adriamycin. (b) Images of growth control of HeLa cancer cell line by quinoline (**5a–j**) and adriamycin.

HOP-92, CCRF-CEM, HOP-62, PC-3, T-47D, A498, and UO-31 with percent GI of 35.29, 24.42, 23.38, 22.27, 22.00, 19.53, and 19.53, respectively, while compound **10c** showed maximum selectivity towards HOP-92, PC-3, HOP-62, SNB-75, T-47D, and UO-31 with percent GI of 31.59, 25.76, 23.61, 23.04, 21.47, and 19.48 respectively. The antiproliferative activity is given in Figure 6. The compounds **10a**, **10b**, and **10c** showed maximum selectivity towards HOP-92 (Non-Small Cell Lung Cancer). The maximum percent GI was recorded on HOP-92 by compound **10b**. No clear cut structure activity relationship (SAR) was observed with antiproliferative data; however 4-methoxyphenyl substitution on position 5 of oxadiazole ring showed significant result than 3,4-dimethoxyphenyl and 4-chlorophenyl substitution. Earlier we have reported the synthesis of oxadiazole derivatives from pyrimidine-2-amine that showed efficiently binding to the active site of EGFR-TK [35]. We can conclude here that EGFR-TK could also be target of the oxadiazoles (**10a–c**) reported here in the present investigation.

4. Conclusion

All the quinoline (**5a–j**) and oxadiazole (**10a–c**) derivatives were synthesized in satisfactory yields. The compound **5j** showed antiproliferative activity among quinoline derivatives with GI_{50} of 35.1 μM against HeLa (cervix cancer cell line) and 60.4 μM against MDA-MB-435 (melanoma), respectively. The structure activity relationship established showed that 4-methoxy substitution was found to be more favorable than 2-chloro and 2,4-dimethyl substitution in the phenyl ring. Similarly the compound **10b** expressed maximum antiproliferative activity on human cancer cell lines at 10 μM concentration. EGFR-TK could be the potential target of the quinoline and oxadiazole derivatives reported here.

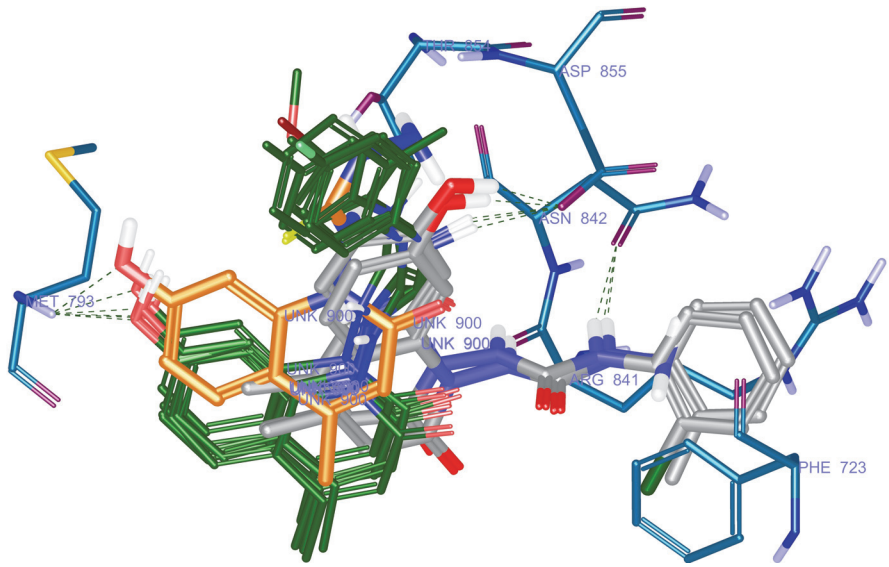


Figure 4: The binding modes of quinoline derivatives (**5a–j**) with the active site of EGFR-TK.

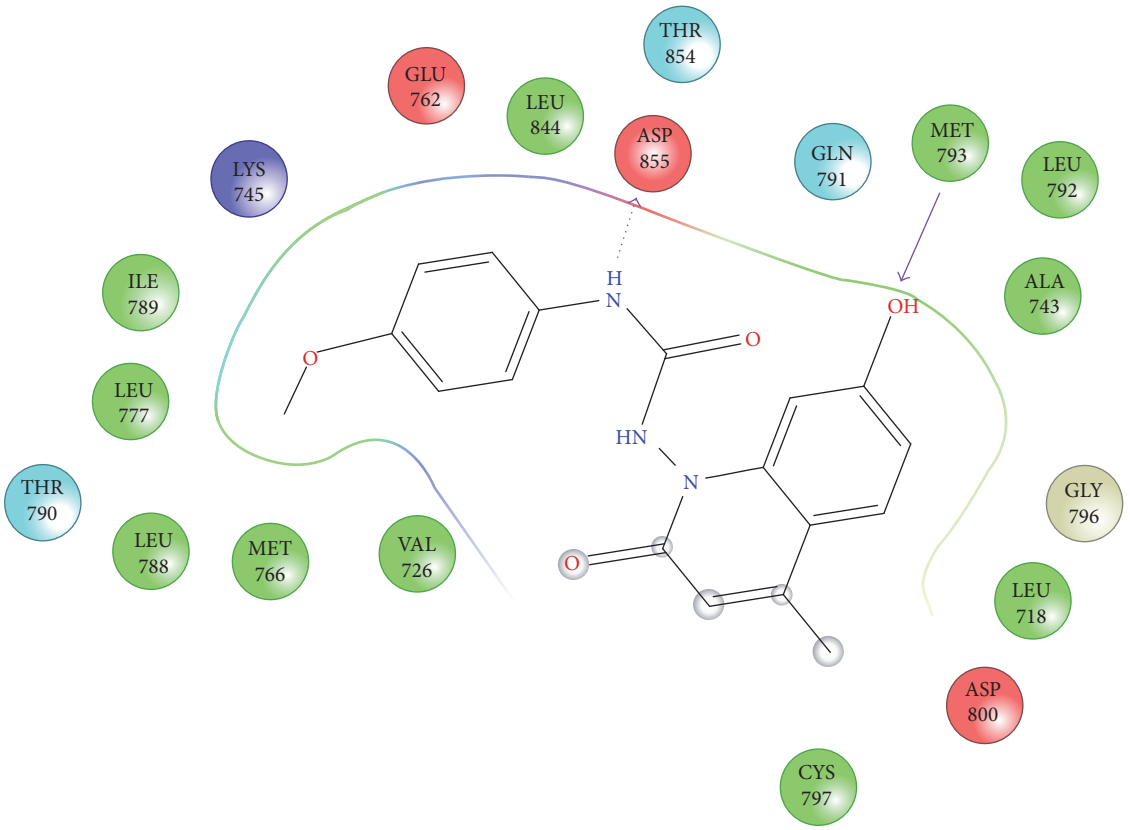


Figure 5: The binding modes of quinoline derivative, **5j** with the active site of EGFR-TK.

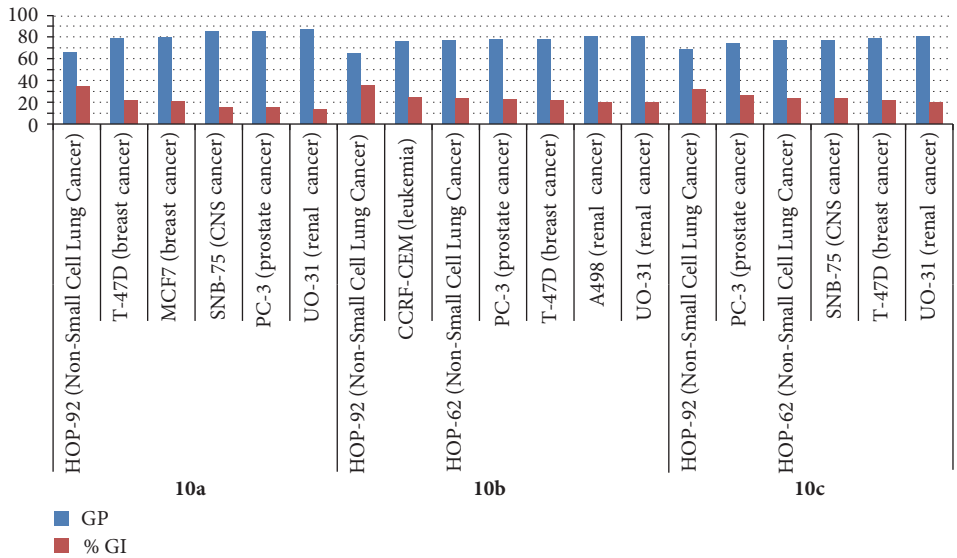


Figure 6: *In vitro* antiproliferative activity of *N*-{[5-aryl-1,3,4-oxadiazol-2-yl]methyl}pyridin-2-amine analogues (**10a–c**) at 10 μM drug concentration.

Disclosure

Part of the work was presented at 1st International Electronic Conference on Medicinal Chemistry, 2015 (doi: 10.3390/ecmc-1-A033 and doi: 10.3390/ecmc-1-A029).

Competing Interests

The authors confirm that this article's content has no conflict of interests.

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