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ARTICLE

Synthesis, Docking Studies and Biological Evaluation of Novel *N*-(2-(3-fluorophenyl)-quinolin-5-yl)benzamide Derivatives as Potent Anti-breast Cancer Agents

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ABSTRACT

A novel series of quinoline benzamide derivatives have been synthesized and screened for their anticancer activity against a breast cancer cell line (MDA-MB-231) by MTT assay method. All the synthesized compounds were confirmed by spectral characterization *viz*. FTIR, ^{1}H & ^{13}C NMR and MS. All the molecules demonstrated potency less than 35 µM and were better than standard cisplatin but not comparable to doxorubicin. Compound **3i** (IC_{50} = 6.86 µM) exhibited better promising anti-breast cancer activity among various synthesized molecules and in addition, docking of compound **3i** into kinesin spindle protein (KSP) active site was performed in order to predict the affinity and the orientation at the enzyme active site.

KEYWORDS

Quinoline benzamides, Anti-breast cancer agent, Docking, MDA-MB-231.

INTRODUCTION

Among all the pharmacologically important heterocyclic compounds, quinoline and its analogues have attracted great interest in the scientific community because of its wide diversity in biological activities. Various quinoline derivatives have been synthesized and reported for different activities like anti-prion [1], antimalarial [2], analgesic [3], antidepressant [4], anti-oxidant [5], antitumour [6], antiviral [7], Alzheimer's disease [8], antimicrobial [9-12], anticancer [13-15], anthelminthic [16], anti-HIV [17], anti-inflammatory [18], as anti-Trypanosoma cruzi agents [19] and as sun-screening agents [20].

In fact, particularly quinoline bearing amide moiety is an interesting privileged structure in medicinal chemistry as it is covering a surprisingly wide pharmacological spectrum. For instance, some novel quinoline benzamide derivatives exhibited higher cytotoxic activity against breast cancer cell line MCF7 compared to doxorubicin [21]. Some quinoline amide derivatives were found to be good inhibitors of VEGFR-2 [22]. Quinoline-8-benzamide derivatives were used for selective inhibition of human tankyrases [23]. 2-Aryl-quinoline-4-carboxamides were also reported as prolyl-t-RNA synthetase

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inhibitor [24]. Motivated by the afore-mentioned literature, we synthesized a novel series of quinoline benzamide derivatives and evaluated them for anticancer activity (triple-negative breast cancer cell line MDA-MB-231) by MTT assay in order to study their structure-activity relationship (SAR) in a hope to obtain compounds with remarkable activities.

EXPERIMENTAL

All commercial chemicals and solvents were of LR-grade and AR-grade and used without further purification. Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} plates, with visualization under UV light. Melting points were determined with PEW-340MP melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Bruker 400 MHz and ^{13}C NMR spectra on Bruker 75 and 100 MHz AVANCE instruments, respectively and *J* values in Hertz and chemical shifts (δ) in ppm were reported relative to internal standard tetramethylsilane (TMS). FTIR spectra (ν, cm^{-1}) using KBr discs were recorded on Perkin-Elmer FTIR spectrophotometer. The mass spectra (MS) were measured with Thermo Finnigan-TSQ Quarter Ultra (triple Quad). The purity of all the compounds was determined by HPLC (Waters 2695 Alliance) using Kromasil C_{18} column (250 mm × 4.5 mm, 5 µ), with mobile phase containing ACN and buffer (0.01 M ammonium acetate + 0.5% triethylamine, pH 5.0 adjusted with acetic acid).

Synthesis of 2-(3-fluorophenyl)-5-nitroquinoline (1): A solution of 2-chloro-5-nitroquinoline (2.4 mmol, 1 eq.) and 3-fluorophenylboronic acid (3.6 mmol, 1.5 eq.) in 1:1 mixture of toluene/ethanol was degassed under reduced pressure and flushed with nitrogen. To this suspension anhydrous caesium carbonate (4.8 mmol, 2 eq.) and *tetrakis*(triphenylphosphine) palladium (0) (0.12 mmol, 0.05 eq.) was added and the system was degassed again. The reaction mixture was heated under reflux for 8 h. Completion of the reaction was monitored by TLC in ethyl acetate-petroleum ether (2:8). The reaction mixture was then allowed to cool to room temperature and filtered through celite. The filter cake was washed with ethyl acetate and the organic layer of the filtrate was separated, washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The resulting residue was purified by silica gel (100-200 mesh) flash column chromatography (10% ethyl acetate/petroleum ether) to obtain compound **1**. Orange solid; Yield 78%; mp 140-142 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.94 (d, *J* = 9.2 Hz, 1H), 8.45-8.50 (m, 3H), 8.13-8.19 (m, 2H), 7.96-8.00 (t, 1H), 7.62-7.67 (m, 1H), 7.38-7.43 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 164.45, 158.64, 153.72, 148.64, 142.13, 138.86, 135.64, 131.02, 129.57, 126.78, 124.08, 121.98, 121.87, 118.64, 114.45; IR (KBr, ν_{max}, cm^{-1}): 3080, 1597, 1533, 1462, 1348, 1232, 1047, 828, 785, 688; MS (APCI): *m/z* 267.30 $[M-H]^-$; HPLC: 98.03%.

Synthesis of 2-(3-fluorophenyl)quinolin-5-amine (2): To a solution of compound **1** (2.1 mmol,1 eq.) in ethyl acetate, stannous chloride dihydrate (5.3 mmol, 2.5 eq.) was added and the mixture was stirred at room temperature for 12 h. Completion of the reaction was monitored by TLC in ethyl acetate-petroleum ether (4:6). The reaction mixture was poured into water and neutralized with saturated $NaHCO_3$ solution. The aqueous mixture was extracted with ethyl acetate, the combined organic phases were dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by silica gel (100-200 mesh) flash column chromatography (20% ethyl acetate/petroleum ether) to obtain compound **2**. Brown solid; Yield 52%; mp 160-162 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 8.91 (d, *J* = 8.8 Hz, 1H), 8.43-8.48 (m, 1H), 8.31 (d, *J* = 8.8 Hz, 2H), 8.02-8.05 (m, 1H), 7.63 (t, 1H), 7.30-7.44 (m, 2H), 6.96 (t, 1H), 6.42 (s, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 161.03, 156.34, 148.60, 147.21, 131.56, 131.30, 130.04, 129.74, 128.95, 126.96, 125.92, 121.96, 118.11, 117.80, 114.68; IR (KBr, ν_{max}, cm^{-1}): 3413, 3329, 1613, 1549, 1465, 1358, 1265, 1128, 867, 773, 692; MS (APCI): *m/z* 239.30 $[M+H]^+$; HPLC: 96.41%.

General procedure for the synthesis of target compounds (3a-j): To a solution of compound **2** (1.1 mmol, 1eq.) in THF, respective acid chloride (1.7 mmol, 1.5 eq.) and sodium hydride (1.24 mmol, 1.1 eq.) was added at 0 ºC and the reaction mixture was then stirred at room temperature for 1 h. Completion of the reaction was monitored by TLC in ethyl acetate-petroleum ether (4:6). The reaction mixture was then poured into ice cold water and extracted with ethyl acetate, the combined organic phases were dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by silica gel (100-200 mesh) flash column chromatography (20% ethyl acetate/petroleum ether) to obtain target compounds **3a-j**.

***N*-(2-(3-fluorophenyl)quinolin-5-yl)benzamide (3a):** White solid; yield 78%; m.p. 246-248 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.84 (s, 1H), 8.39 (t, 2H), 8.20 (d, *J* = 8.2 Hz, 2H), 7.84 -7.90 (m, 3H), 7.70 (t, 1H), 7.56-7.63 (m, 3H), 7.44 (t, 2H), 7.20 (t, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 166.45, 164.03, 156.03, 148.72, 146.06, 141.82, 141.64, 138.13, 136.88, 134.23, 133.01, 130.20, 129.05, 128.65, 127.67, 126.33, 123.92, 119.02, 118.78, 116.98, 116.52, 114.58; IR (KBr, ν_{max}, cm^{-1}): 3224, 1644, 1525, 1486, 1348, 1285, 1190, 815, 790, 692; MS (APCI): *m/z* 343.30 $[M+H]^+$; HPLC: 100%.

***N*-(2-(3-Fluorophenyl)quinolin-5-yl)-2-methoxybenzamide (3b):** Brown solid; Yield 77%; m.p. 178-180 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.45 (s, 1H), 8.58 (d, *J* = 8.8 Hz, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.11-8.18 (m, 2H), 7.97 (d, *J* = 7.6 Hz, 2H), 7.83 (d, *J* = 7.2 Hz, 2H), 7.56-7.65 (m, 2H), 7.37 (t, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.13 (t, 1H), 4.05 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.32, 164.29, 161.32, 155.14, 153.78, 150.56, 148.93, 148.79, 148.57, 147.08, 132.08, 132.00, 131.36, 130.44, 129.93, 126.90, 126.00, 124.07, 117.80, 114.72, 114.47, 114.20, 56.51; IR (KBr, ν_{max}, cm^{-1}): 3286, 1670, 1553, 1478, 1319, 1263, 1184, 815, 790, 693; MS (APCI): *m/z* 373.40 $[M+H]^+$; HPLC: 99.04%.

***N*-(2-(3-Fluorophenyl)quinolin-5-yl)-3-methoxybenzamide (3c):** White solid; yield 79%; m.p. 214-216 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.57 (s, 1H), 8.47 (d, *J* = 9.2 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 1H), 8.10-8.16 (m, 2H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.84 (t, 1H), 7.69 (t, 2H), 7.59-7.64 (m, 2H), 7.50 (t, 1H), 7.37 (m, 1H), 7.20-7.22 (m, 1H), 3.87 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 166.04, 163.96, 161.54, 159.27, 154.56, 147.90, 140.92, 140.85, 135.57, 134.10, 133.69, 130.95, 130.87, 129.63, 127.37, 123.85, 123.46, 123.27, 118.34, 117.72, 116.61, 116.40, 55.37; IR (KBr, ν_{max}, cm^{-1}): 3233, 1646,

1599, 1487, 1234, 1177, 792, 753, 687; MS (APCI): *m/z* 371.1 $[M-H]^-$; HPLC: 98.58%.

***N*-(2-(3-Fluorophenyl)quinolin-5-yl)-4-methoxybenzamide (3d):** White solid; yield 80%; m.p. 190-192 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.68 (s, 1H), 8.35 (t, 2H), 8.17 (t, 2H), 7.84 (t, 1H), 7.73 (d, *J* = 8.8Hz, 2H), 7.53-7.67 (m, 4H), 6.97 (d, *J* = 8.4 Hz, 2H), 3.76 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 166.26, 163.57, 161.32, 158.76, 153.78, 150.56, 148.93, 148.79, 148.57, 147.20, 132.38, 132.26, 131.16, 130.44, 129.93, 127.20, 126.00, 124.71, 117.80, 114.89, 114.71, 114.47, 56.71; IR (KBr, ν_{max}, cm^{-1}): 3230, 1646, 1531, 1488, 1234, 1177, 1034, 793, 753, 688; MS (APCI): *m/z* 373.30 $[M+H]^+$; HPLC: 99.80%.

2-Chloro-*N*-(2-(3-fluorophenyl)quinolin-5-yl)benzamide (3e): Brown solid; yield 72%; m.p. 220-222 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.76 (s, 1H), 8.68 (d, *J* = 9.2 Hz, 1H), 8.27 (d, *J* = 9.2 Hz, 1H), 8.11-8.18 (m, 2H), 8.00-8.03 (m, 1H), 7.83-7.87 (m, 2H), 7.76-7.78 (m, 1H), 7.61-7.65 (m, 2H), 7.50-7.59 (m, 2H), 7.34-7.39 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 165.21, 161.85, 159.16, 151.16, 148.03, 141.72, 138.16, 135.33, 130.58, 130.36, 129.22, 128.85, 128.46, 128.28, 127.13, 123.58, 123.36, 118.43, 117.52, 116.64, 116.42, 114.92; IR (KBr, ν_{max}, cm^{-1}): 3262, 1657, 1525, 1398, 1263, 1123, 818, 789, 692; MS (APCI): *m/z* 376.16 $[M+H]^+$; HPLC: 98.64%.

3-Chloro-*N*-(2-(3-fluorophenyl)quinolin-5-yl)benzamide (3f): Brown solid; yield 74%; m.p. 246-248 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.58 (s, 1H), 8.48 (d, *J* = 7.6 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 8.11-8.17 (m, 2H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.85 (t, 1H), 7.70 (t, 2H), 7.60-7.65 (m, 2H), 7.51 (t, 1H), 7.36-7.40 (m, 1H), 7.21-7.24 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 166.07, 163.99, 159.30, 154.59, 147.93, 140.95, 140.88, 135.60, 134.12, 133.71, 130.98, 130.90, 129.66, 127.40, 123.89, 123.49, 123.40, 118.38, 116.55, 113.76, 113.54, 112.90; IR (KBr, ν_{max}, cm^{-1}): 3233, 1646, 1530, 1486, 1347, 1274, 1097, 818, 794, 695; MS (APCI): *m/z* 376.08 $[M+H]^+$; HPLC: 98.09%.

4-Chloro-*N*-(2-(3-fluorophenyl)quinolin-5-yl)benzamide (3g): Grey solid; yield 78%; m.p. 248-250 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.70 (s, 1H), 8.36 (d, *J* = 7.2 Hz, 1H), 8.34 (s, 1H), 8.17 (t, 2H), 7.84 (t, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.53-7.68 (m, 4H), 6.98 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 167.04, 164.96, 159.27, 154.56, 149.63, 147.90, 141.92, 141.85, 136.57, 134.10, 133.69, 130.72, 130.61, 129.63, 127.37, 123.96, 123.54, 123.27, 118.34, 116.95, 116.87, 114.40; IR (KBr, ν_{max}, cm^{-1}): 3267, 1647, 1593, 1485, 1273, 1177, 841, 790, 692; MS (APCI): *m/z* 376.20 $[M+H]^+$; HPLC: 99.63%.

***N*-(2-(3-Fluorophenyl)quinolin-5-yl)-2-nitrobenzamide (3h):** Brown solid; yield 80%; m.p. 216-218 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.78 (s, 1H), 8.58 (s, 1H), 8.40 (d, *J* = 8.8 Hz, 2H), 8.13-8.17 (m, 4H), 8.08 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.72 (t, 1H), 7.58-7.64 (m, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.35 (t, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 165.12, 162.96, 156.08, 147.38, 141.50, 141.25, 134.68, 131.74, 130.20, 129.83, 129.20, 127.58, 127.55, 127.51, 125.12, 123.07, 122.96, 118.45, 117.42, 116.40, 116.11, 113.90; IR (KBr, ν_{max}, cm^{-1}): 3258, 1638, 1534, 1402, 1264, 814, 791, 690; MS (APCI): *m/z* 388.30 $[M+H]^+$; HPLC: 98.32%.

***N*-(2-(3-Fluorophenyl)quinolin-5-yl)-3-nitrobenzamide (3i):** Brown solid; yield 81%; m.p. 250-252 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.60 (s, 1H), 8.49 (d, *J* = 7.2 Hz, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 8.11-8.18 (m, 2H), 8.05 (d, *J* = 8.0 Hz, 1H), 7.85 (t, 1H), 7.71 (t, 2H), 7.61-7.66 (m, 2H), 7.51 (t, 1H), 7.36-7.41 (m, 1H), 7.21-7.24 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 166.18, 163.10, 159.46, 154.70, 148.04, 141.26, 141.02, 136.12, 134.22, 133.82, 131.12, 131.02, 129.78, 127.52, 124.04, 123.58, 123.40, 120.22, 118.46, 116.73, 116.52, 113.94; IR (KBr, ν_{max}, cm^{-1}): 3242, 1674, 1598, 1529, 1352, 1265, 1141, 816, 795, 724; MS (APCI): *m/z* 388.20 $[M+H]^+$; HPLC: 100%.

***N*-(2-(3-Fluorophenyl)quinolin-5-yl)-4-nitrobenzamide (3j):** Yellow solid; yield 78%; m.p. 240-242 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 10.72 (s, 1H), 8.38 (d, *J* = 6.8 Hz, 1H), 8.36 (s, 1H), 8.18 (t, 2H), 7.86 (t, 1H), 7.75 (d, *J* = 8.4 Hz, 2H), 7.55-7.70 (m, 4H), 6.99 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 166.12, 163.07, 158.48, 151.87, 147.50, 141.25, 136.74, 134.79, 131.17, 129.72, 129.25, 127.64, 127.54, 127.49, 124.57, 124.33, 122.96, 119.25, 118.42, 116.64, 116.54, 114.25; IR (KBr, ν_{max}, cm^{-1}): 3287, 1647, 1600, 1540, 1486, 1347, 1277, 1111, 851, 791, 693; MS (APCI): *m/z* 388.10 $[M+H]^+$; HPLC: 97.38%.

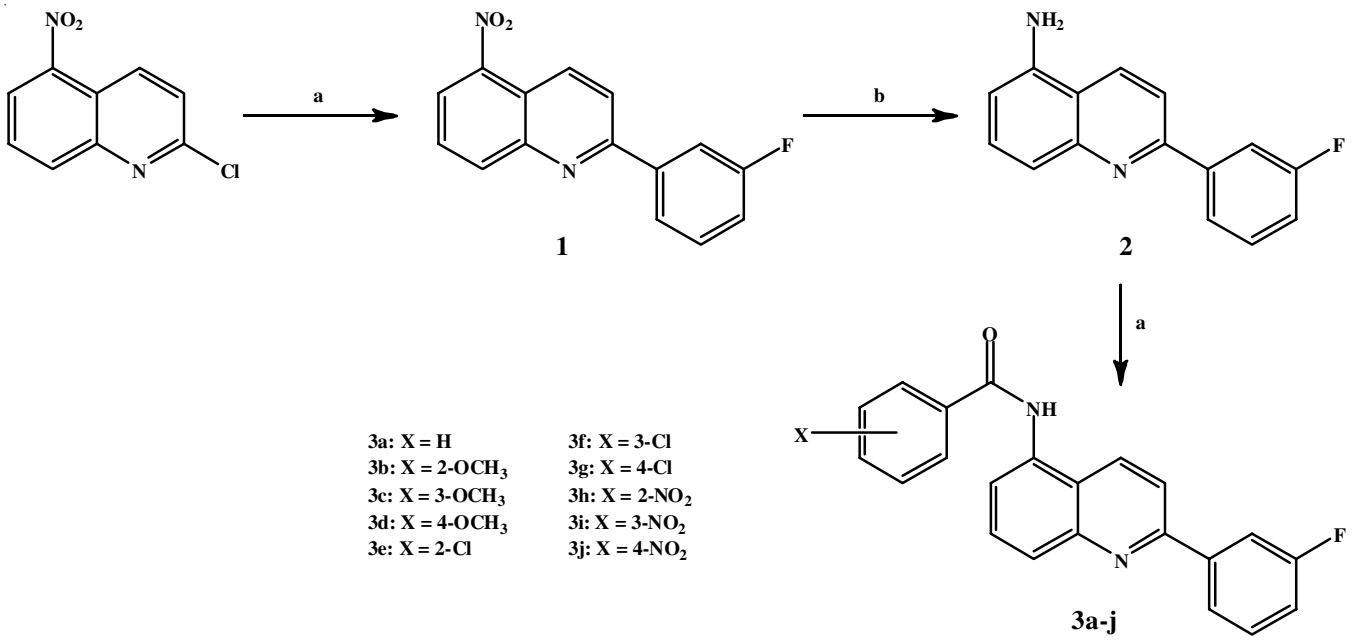
Anti-cancer activity: Cancer cell line MDA-MB-231 (breast adenocarcinoma) was purchased from National Centre for Cell Sciences, Pune, India. 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), tris-HCl were obtained from SRL (Mumbai, India), Fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco′s modified eagle′s medium (DMEM) and trypsin-EDTA were obtained from CellClone (Delhi, India), and the antibiotics *viz.* cisplatin and doxorubicin hydrochloride from Hi-Media Lab. Ltd. (Mumbai, India).

Anti-cancer assay: Briefly, cells were grown in DMEM media supplemented with fetal bovine serum (FBS) 10% (50 µg/mL) and penicillin-streptomycin (50 µg/mL) at 37 ºC, CO_2 (5%) and air (95%). Cells were seeded (1×10^4 cells/well) in each of the 96-well plate for different concentration of synthesized compounds ranging from 0.01 to 100 µM. After incubation, 6 concentrations (triplicate) of test compounds (prepared in DMSO) were added to the cells and incubated at 37 ºC and 5% CO_2 for 48 h. A MTT solution (20 µL of 5 mg/mL) was then added to each well. Plate was further incubated for a period of about 4 h, the supernatant was removed and 200 µL per well DMSO was added to solubilize formazan crystals. Plate was incubated for 10 min and absorbance was measured at 540 nm.

Molecular docking: All the molecular modelling studies described herein were performed on HP Laptop (Intel® Core™i7-5500T CPU @ 2.40 GHz, RAM 4 GB) running Windows 8.1 64-bit Home Basic Operating System. Schrödinger Small-Molecule Drug Discovery Suite Release 2018-1 and the products included therein were used for performing various molecular modelling operations described above.

RESULTS AND DISCUSSION

In present work, a series of ten novel quinoline benzamide derivatives (**3a-j**) were synthesized from 2-chloro-5-nitroquinoline in three steps as shown in **Scheme-I**. The first step is classical Suzuki coupling of 2-chloro-5-nitroquinoline with 1.5 equiv.



Reagents and conditions: (a) 3-fluorophenyl boronic acid, Cs_2CO_3, $Pd(PPh_3)_4$, toluene/ethanol (1:1), reflux, 12 h (b) $SnCl_2{\cdot}2H_2O$, EtOAc, room temperature, 12 h (c) respective acid chloride, NaH, THF, 0 °C → room temperature, 1 h

Scheme-I: Synthesis of quinoline benzamide derivatives

of 3-fluorophenylboronic acid in the presence of *tetrakis*-(triphenylphosphine)palladium (0) and Cs_2CO_3 in 1:1 mixture of toluene/ethanol as solvent under thermal heating to give compound **1**. A key intermediate **2** was synthesized *via* reduction of compound **1** with $SnCl_2{\cdot}2H_2O$ in the presence of ethyl acetate as solvent at room temperature. Finally, all target compounds (**3a-j**) were obtained by coupling of compound **2** with respective acid chlorides using sodium hydride in THF solvent stirred at room temperature for 1 h.

Biological evaluation: All target compounds (**3a-j**) were evaluated against MDA-MB-231 (breast adenocarcinoma) using MTT assay (colorimetric method). Cisplatin and doxorubicin·HCl were used as positive controls and the IC_{50} values were reported in μM. The results were shown in Table-1. It was observed that IC_{50} values of compounds were found to be in the range of 6.86-33.92 μM. All the derivatives exhibited more or less similar potency and trends were observed with varied substituent nature and position. Compounds **3d** (4-OCH_3), **3g** (4-Cl) and **3j** (4-NO_2) possessed higher cytotoxicity and the activity was reduced as the substituent′s (X = -OCH_3, Cl) shifted to 2 and 3 position except for compound **3i**. It can be concluded from the above results that 4th position substituent (X) possessed superior potency than 2nd and 3rd position. Compound **3i** (3-NO_2) was found to be the best molecule (IC_{50} = 6.86 μM) among all analogues (**3a-j**). All the molecules demonstrated potency less than 35 μM and were better than cisplatin but not comparable to doxorubicin. Further modification of compound **3i** can lead to a improve potency, selectivity and other pharmacokinetic parameters, which may be helpful to develop potent anticancer drug.

Molecular docking studies: In order to investigate the potential molecular targets of the hit molecule **3i** and to provide a preliminary data for the molecular/cellular biology, a target 'go fishing' experiments using PharmMapper were carried out. The PharmMapper is an open-source used for screening molecules through a number of pharmacophore databases (Target Bank, Binding DB, Drug Bank and potential drug target database). The present study combines computational analyses with wet-lab to provide logical base for the anticancer effect of this hit molecule and can be useful for the exploration of the proposed molecular target to treat cancer.

TABLE-1
ANTICANCER ACTIVITY OF QUINOLINE BENZAMIDE DERIVATIVES (**3a-3j**)

Compound	X	IC_{50} ± SD (μM)[a]
3a	H	19.32 ± 3.33
3b	2-OCH_3	24.12 ± 2.33
3c	3- OCH_3	21.19 ± 0.50
3d	4- OCH_3	19.37 ± 0.35
3e	2-Cl	33.92 ± 0.83
3f	3-Cl	20.02 ± 0.77
3g	4-Cl	18.91 ± 1.78
3h	2-NO_2	21.81 ± 1.74
3i	3-NO_2	6.86 ± 0.66
3j	4-NO_2	19.12 ± 1.64
Doxorubicin·HCl		0.64 ± 0.04
Cisplatin		47.95 ± 1.26

[a]Results are mean of triplicate analysis
IC_{50} values of compounds against Cancer cell line MDA-MB-231 (breast adenocarcinoma)

One target was selected from PharmMapper displaying highest fitting score with the hit molecule (**3i**) (Table-2). To identify potential interactions of the hit molecule, molecular docking studies were performed using XP mode in the GLIDE module with default settings. The X-ray structure of hIMPDH2 was retrieved from the protein data bank (PDB ID: 1JR1) and optimized by using OPLS2005 forcefield. The hit molecule (**3i**) was prepared and optimized using LigPrep module as implemented in Schrödinger small-molecule drug discovery

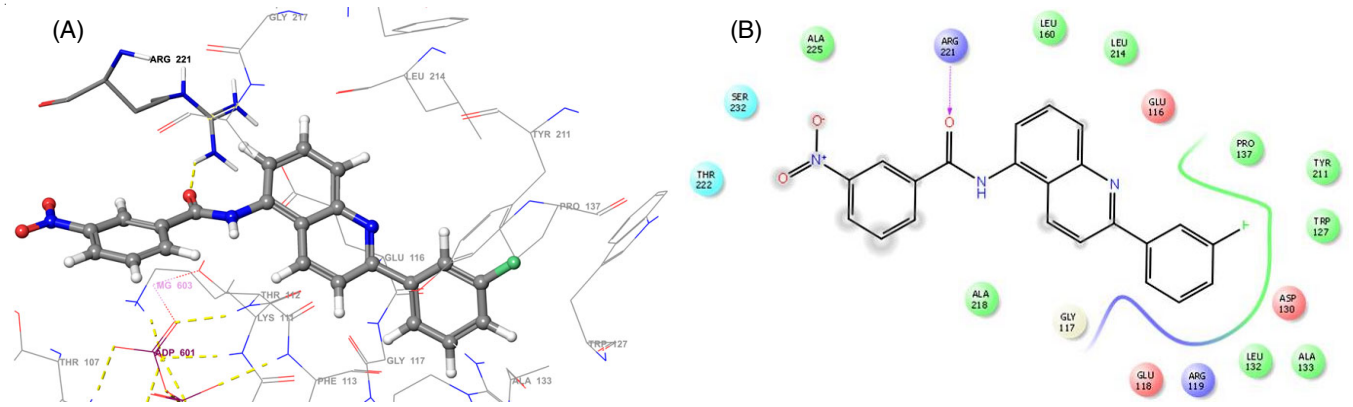


Fig. 1. Molecular docking of hit **3i** in the binding sites of kinesin spindle protein (KSP) (PDB ID 2UYI) - (A) 3D interaction diagram of **3i** and (B) 2D interaction diagram of **3i**

TABLE-2
DOCKING ANALYSIS OF THE HIT COMPOUND (**3i**)

Macromolecule	PDB ID	XP_GScore **3i**	Glide_Emodel **3i**
Kinesin Spindle Protein (KSP)	2UYI	-3.638	-64.888

suite. Receptor grid was generated and the docking studies were performed according to the standard protocol. Individual docked poses were inspected manually to observe the binding interactions of ligands with the selected molecular target.

Compound **3i** showed interaction with the active site amino acid of kinesin spindle protein (KSP) (PDB ID: 2UYI) (Fig. 1). Compound **3i** also displayed hydrogen bonding interaction between Arg 221 and amide carbonyl group were observed in kinesin spindle protein (KSP).

Conclusion

The present study attempts the synthesis of a novel series of quinoline benzamide derivatives and subsequent SAR investigations. Based on the analysis done during the study, It is concluded that all the compounds showed significant anti-cancer activity as compared to standard cisplatin but not comparable to doxorubicin against a breast cancer cell line (breast adenocarcinoma, MDA-MB-231) and the variations were observed with varied substituent (X) nature and position. Among various synthesized molecules, compound **3i** exhibited better promising antibreast cancer activity. Docking calculations revealed that hydrogen bonding interactions played crucial role in demonstrating biological activity.

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