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Synthesis, molecular docking, and biological evaluation of novel 2-(3-chlorophenyl) quinoline-4-carboxamide derivatives as potent anti-breast cancer and antibacterial agents

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ABSTRACT

Objectives: The study aims to synthesize and to evaluate the anti-breast cancer and antibacterial activity of some novel quinoline-4-carboxamide derivatives. **Materials and Methods:** A series of novel quinoline analogs 6a-6j with varied substituent (X = H, F, OCH_3, NO_2) were synthesized from aniline through multi-step reactions in good yields. All analogues were confirmed by spectral characterization, namely, FT-IR, MS, ^{1}H-NMR, and ^{13}C-NMR. All the molecules were evaluated for their anticancer activity against a breast cancer cell line, MDA-MB-231 by MTT assay, and antibacterial activity against Gram-positive bacteria (Staphylococcus aureus 6538p and Bacillus subtilis) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using agar well diffusion method. **Results:** All the compounds exhibited significant anticancer activity as compared to standard cisplatin but not comparable to doxorubicin HCl. Compound **6j** exhibited better promising anti-breast cancer activity. All the compounds showed less antibacterial activity as compared to standard streptomycin and compound **6h** was found to be the best molecule among them. Molecular docking showed the interaction of compound **6j** with the active site amino acid of human carbonic anhydrase I, protein kinase A, and kinesin spindle protein. **Conclusion:** Based on the results, compounds **6h** and **6j** can be further optimized to develop potent antibacterial and anticancer drug.

Keywords: Antibacterial, anticancer, docking, MDA-MB-231, quinoline carboxamide

INTRODUCTION

Quinoline, nitrogen containing heterocyclic compound, is one of the most attractive scaffold with a wide range of biodynamic properties. In medicinal chemistry, quinoline and their derivatives have been very well known for their therapeutic applications such as analgesic,[1] antileishmanial,[2] antimicrobial,[3-5] antimalarial,[6] antioxidant,[7,8] anti-inflammatory,[9] anti-HIV,[10] and antidepressant.[11]

In addition, quinoline compounds play an important role in anticancer drug development as they have shown excellent results through different mechanisms of action. A number of quinoline derivatives have been reported till date for their anticancer activity.[12,13] Some C-2-substituted quinolines showed good activity against human cancer cell lines (MCF-7, H-460, and SF-268).[14] 6-Substituted-2-(3-phenoxyphenyl)-4-phenylquinoline derivatives are highly potent to cancer cells.[15] The anti-cancer activity of the benzo[h]quinolines was evaluated on cultured human skin cancer (G361), lung cancer (H460), breast cancer (MCF7), and colon cancer (HCT116) cell lines and they showed potential cytotoxicity against these human cancer cell lines by oxidative stress-mediated DNA damage.[16] Further studies have been investigated that the incorporation of amide group in quinoline derivatives enhanced their anticancer activities. 2-Phenyl-quinoline-4-carboxamides

were found to possess maximum anticancer activity.[17] Some quinoline-4-carboxamides were also reported as STAT3 inhibitors which provide a new therapeutic approach for cancer treatment.[18] Some quinoline-2-carboxamides were also evaluated for their antiproliferative effects on the cancer cell lines (HEP-3B and A-375).[19] Quinoline-3-carboxamides were found as potent EGFR inhibitors with cytotoxic activity on MCF-7 breast cancer cell line.[20] The Cu(II)-quinoline carboxamide complexes were also found to exhibit cytotoxicity against murine leukemia P-388 and human leukemia HL-60 cell lines and were more potent than cisplatin.[21] Furthermore, reports on the antibacterial activity of the quinoline derivatives are impressive.[22,23]

In the light of these facts, we planned to synthesize a novel series of quinoline derivatives bearing a carboxamide moiety and to evaluate their anticancer activity against a breast cancer cell line (MDA-MB-231) and antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* 6538p and *Bacillus subtilis*) and Gram-negative bacteria (Escherichia *coli* and *Pseudomonas aeruginosa*). The structure activity relationship (SAR) studies were also performed for all the bioactivities taken into account.

MATERIALS AND METHODS

Materials

Chemistry

All commercial chemicals and solvents are of LR-grade and AR-grade and were used without further purification. The 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 are uncorrected. ^{1}H-NMR spectra were recorded on Bruker 200, 300, and 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). FT-IR spectra (ν in cm^{-1}) using KBr disks were recorded on Perkin-Elmer FT-IR 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).

Anticancer 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) and Tris-HCl were obtained from SRL (Mumbai, India), fetal bovine serum (FBS), phosphate-buffered saline, Dulbecco's modified eagle's medium (DMEM), and Trypsin-EDTA were obtained from Cell Clone (Delhi, India), antibiotics from HiMedia Laboratories Ltd. (Mumbai, India).

Antibacterial activity

The Gram-positive organisms, namely, B. *subtilis* and S. *aureus* 6538p and Gram-negative organisms, namely, *P. aeruginosa* and *E. coli* cultures were obtained from neighboring hospitals and pathological laboratories located in Mumbai.

Molecular docking

Hardware and Software: All the molecular modeling 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. Schrodinger Small-Molecule Drug Discovery Suite Release 2018-1 and the products included therein were used for performing various molecular modeling operations.

Methods

Chemistry

In the present work, a series of novel quinoline carboxamide derivatives **(6a-6j)** were synthesized from aniline through multi-step reactions in good to moderate yields.

*Procedure for the synthesis of 3-oxo-N-phenylbutanamide **(1)***

Aniline (4.9 mL, 53.6 mmol, 1 eq.) and ethyl acetoacetate (6.9 mL, 53.6 mmol, 1 eq.) were taken together in a 250 mL round bottom flask. The reaction mixture was then refluxed at 160°C for 36 h continuously. At the end of the reaction period, 100 mL of hot water was added to the flask and the contents were heated to boiling. The mixture was then filtered; the filtrate was cooled till the white crystals appeared. The crystals were then retrieved, dried in air, recrystallized from toluene to obtain the compound **1.**

White crystalline solid; Yield 80 %; mp 83–86°C; ^{1}H-NMR (DMSO-d_6, 200 MHz, δ ppm): 9.13 (s, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.31 (t, J = 8.0 Hz, 2H), 7.10 (m, 1H), 3.57 (s, 2H), 2.30 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ ppm): 205.11, 163.50, 137.46, 128.94, 124.54, 120.15, 49.81, 31.15; MS (ESI): m/z $[M+H]^+$ = 178.32; HPLC: 97.58%.

*Procedure for the synthesis of 4-Methylquinoline-2(1H)-one **(2)***

In a 100 mL conical flask, 10 g of polyphosphoric acid was taken and heated up to 100 °C on the magnetic heater with stirrer. Compound, 3-oxo-N-phenylbutanamide **(1)** (1 g, 5.6 mmol, 1 eq.) was then added in the flask and stirred continuously for 3 h. During the reaction period, temperature was maintained between 95 and 100°C. At the end of the reaction period, reaction mixture was poured into ice cold water and stirred till the lumps dissolved completely. This solution was then neutralized with 4N Sodium hydroxide solution. The solution was then cooled till the compound settled. The precipitate was then retrieved, dried in air to obtain the compound **2.**

White amorphous solid; Yield 45 %; mp 224–226°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.35 (s, 1H), 8.19–8.22 (m, 1H), 7.68–7.71 (m, 1H), 7.42–7.47 (m, 1H), 7.18–7.22 (m, 1H), 6.22 (s, 1H), 2.34 (s, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 162.45, 148.64, 140.13, 130.64, 129.27, 127.57, 124.38, 122.08, 118.87, 21.45; MS (ESI): m/z $[M+H]^+$ = 160.18; HPLC: 95.78%.

*Procedure for the synthesis of 2-oxo-1,2-dihydroquinoline-4-carboxylic acid **(3)***

4-Methylquinoline-2(1H)-one (**2**) (1 g, 6.25 mmol, 1 eq.) and 30 mL of water were taken together in a 100 mL round bottom flask. $KMnO_4$ (2.5 g, 15.6 mmol, 2.5 eq.) and NaOH

(0.7 g, 15.6 mmol, 2.5 eq.) was then added in the flask and stirred at room temperature for 3 h and then at 85°C for 2 h continuously. At the end of the reaction period, reaction mixture was taken in 500 mL beaker and 250 mL of hot water was added and the solution was then neutralized with 4N HCl solution. The solution was then cooled till the compound settled. The precipitate was then retrieved, dried in air to obtain the compound **3.**

White amorphous solid; Yield 52 %; mp 242–244°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 13.82 (s, 1H), 10.68 (s, 1H), 8.69–8.72 (m, 1H), 7.60–7.62 (m, 1H), 7.48 (s, 1H), 7.32–7.37 (m, 1H), 7.08–7.12 (m, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 166.45, 162.23, 148.22, 138.01, 132.01, 130.02, 128.05, 126.65, 123.92, 117.82; MS (ESI): m/z $[M+H]^+$ = 190.17; HPLC: 99.86%.

Procedure for the synthesis of 2-Chloroquinoline-4-carboxylic acid **(4)**

To a solution of 2-oxo-1,2-dihydroquinoline-4-carboxylic acid (**3**), (500 mg, 2.6 mmol, 1 eq.) in DMF, freshly-distilled $POCl_3$ (2.5 mL, 26.4 mmol, 10 eq.) was added at 0°C. The reaction mixture was refluxed at 100°C for 4 h. Completion of the reaction was monitored by LC-MS. The reaction mass was diluted with ice cold water, a pale yellow solid precipitated out which was filtered and dried at suction pump to obtain the compound **4.**

Yellow crystalline solid; Yield 67 %; mp 340–344°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 14.05 (s, 1H), 8.69 (d, J = 4.8 Hz, 1H), 8.44–8.48 (m, 1H), 8.18–8.22 (m, 1H), 8.02–8.07 (m, 1H), 7.85–7.89 (m, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 167.27, 151.85, 149.18, 138.68, 132.27, 130.17, 128.58, 126.23, 124.11, 120.72; MS (ESI): m/z $[M+H]^+$ = 207.62, m/z $[(M+2)-H]^+$ = 209.62; HPLC: 95.66%.

Procedure for the synthesis of 2-(3-chlorophenyl)quinoline-4-carboxylic acid **(5)**

A solution of 2-chloroquinoline-4-carboxylic acid (**4**) (2.4 mmol, 1 eq.) and 3-chlorophenylboronic 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 sodium carbonate (4.8 mmol, 2 eq.) and tetrakis(triphenylphosphine) palladium (0) (0.12 mmol, 0.05 eq.) were added and the system was degassed again. The reaction mixture was heated under reflux for 12 h. Completion of the reaction was monitored by TLC in ethyl acetate-petroleum ether (2:8). The reaction mixture was then allowed to cool at 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 (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 the compound **5.**

White crystalline solid; Yield 78%; mp 232–234°C; ^{1}H-NMR (DMSO-d_6, 300 MHz, δ ppm): 14.32 (brs, 1H), 8.72 (d, J = 2.1 Hz, 1H), 8.40 (t, J = 1.8 Hz, 1H), 8.20 (d, J = 7.8 Hz, 2H), 8.09 (d, J = 9.0 Hz, 1H), 7.77–7.81 (m, 2H), 7.67–7.70 (m, 1H), 7.48 (t, J = 8.8 Hz, 1H); ^{13}C-NMR (DMSO-d_6, 75 MHz, δ ppm): 167.56, 154.64, 147.16, 140.25, 138.21, 133.18, 132.96, 132.21, 131.51, 131.06, 130.10, 126.64, 125.34, 125.10, 122.96, 120.51; IR (KBr) ν_{max}/cm^{-1}: 3270, 3063, 1715, 1646, 1533, 1402, 1067, 767; MS (ESI): m/z $[M+H]^+$ = 283.12, m/z $[(M+2)-H]^+$ = 285.12; HPLC: 95.00%.

General procedure for the synthesis of compounds **6a-6j**

A mixture of 2-(3-chlorophenyl)quinoline-4-carboxylic acid (5) (2.0 mmol, 1eq.) and freshly-distilled $SOCl_2$ (20 mmol, 10 eq.) was refluxed at 80 °C for 5 h. The reaction progress was monitored by TLC. After reaction completion, the reaction mixture was evaporated to yield corresponding acid chloride.

To a solution of above solid (2-(3-chlorophenyl)quinoline-4-carboxylic acid chloride), 0.9 mmol, 1 eq. in THF, respective amine (1.5 mmol, 1.5 eq.), and sodium hydride (1.0 mmol, 1.1 eq.) were added at 0 °C 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 (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 the compounds **6a-6j.**

2-(3-chlorophenyl)-N-phenylquinoline-4-carboxamide **(6a)**

Yellow crystalline solid; Yield 80%; mp 160–162°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.82 (s, 1H), 8.36 (s, 2H), 8.17 (d, J = 6.0 Hz, 2H), 7.83 (m, 3H), 7.67 (m, 1H), 7.58 (d, J = 6.4 Hz, 3H), 7.41 (m, 2H), 7.17 (m, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 164.10, 157.45, 148.72, 141.06, 141.02, 138.64, 134.13, 132.86, 130.64, 130.32, 129.95, 129.27, 127.57, 126.78, 124.08, 123.72, 120.98, 118.87, 118.72, 116.98; IR (KBr) ν_{max}/cm^{-1}: 3257, 3060, 1685, 1631, 1571, 1441, 792; MS (ESI): m/z $[M+H]^+$ = 358.17, m/z $[(M+2)-H]^+$ = 360.17; HPLC: 97.41%.

2-(3-chlorophenyl)-N-(2-fluorophenyl) quinoline-4-carboxamide **(6b)**

Yellow amorphous solid; Yield 74 %; mp 170–172°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.68 (s, 1H), 8.36 (d, J = 7.2 Hz, 2H), 8.16–8.22 (m, 1H), 7.84-7.88 (m, 3H), 7.70 (d, J = 6.4 Hz, 2H), 7.54–7.60 (m, 2H), 7.31–7.36 (m, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 165.16, 161.72, 159.12, 149.23, 146.63, 141.72, 138.16, 135.12, 134.85, 132.36, 131.21, 129.22, 128.85, 128.46, 127.13, 125.58, 123.36, 123.28, 122.52, 119.46, 117.32, 116.94; IR (KBr) ν_{max}/cm^{-1}: 3242, 3031, 1664, 1632, 1535, 1385, 1114, 784; MS (ESI): m/z $[M+H]^+$ = 376.14, m/z $[(M+2)-H]^+$ = 378.14; HPLC: 97.95%.

2-(3-chlorophenyl)-N-(3-fluorophenyl) quinoline-4-carboxamide **(6c)**

White amorphous solid; Yield 72%; mp 204–206°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.82 (s, 1H), 8.37 (dd, J_1 = 7.6 Hz, J_2 = 4.4 Hz, 2H), 8.18 (d, J = 8.4 Hz, 2H), 7.82–7.88 (m, 3H), 7.68 (dd, J_1 = 8.0 Hz, J_2 = 7.2 Hz, 1H), 7.54–7.61 (m, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.18 (dd, J_1 = 7.6 Hz, J_2 = 7.2 Hz, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 165.87, 163.19, 159.57, 149.30, 146.65, 140.15, 139.88, 136.40, 134.12, 133.91, 130.98, 130.70, 129.16, 127.40, 125.19, 124.79, 124.48, 122.13, 120.38, 118.73, 116.76, 116.54; IR

(KBr) ν_{max}/cm^{-1}: 3235, 3031, 1684, 1612, 1548, 1441, 1202, 757; MS (ESI): m/z $[M+H]^+$ = 376.10, m/z $[(M+2)-H]^+$ = 378.10; HPLC: 98.14%.

2-(3-chlorophenyl)-N-(4-fluorophenyl) quinoline-4-carboxamide **(6d)**

Yellow amorphous solid; Yield 78%; mp 216–218°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.70 (s, 1H), 8.40 (d, J = 9.6 Hz, 1H), 8.35 (s, 1H), 8.18–8.23 (m, 2H), 7.85-7.91 (m, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.56–7.71 (m, 4H), 7.02 (d, J = 8.4 Hz, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 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; IR (KBr) ν_{max}/cm^{-1}: 3244, 3165, 1680, 1615, 1552, 1459, 1212, 756; MS (ESI): m/z $[M+H]^+$ = 376.27, m/z $[(M+2)-H]^+$ = 378.27; HPLC: 98.12%.

2-(3-chlorophenyl)-N-(2-methoxyphenyl)quinoline-4-carboxamide **(6e)**

Yellow crystalline solid; Yield 81%; mp 160–162°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.07 (s, 1H), 8.43 (d, J = 6.8 Hz, 1H), 8.35 (d, J = 8.4 Hz, 1H), 8.29 (s, 1H), 8.22 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.84 (t, J = 7.6 Hz, 1H), 7.69 (dd, J_1 = 9.2 Hz, J = 7.6 Hz, 1H), 7.52–7.65 (m, 2H), 7.24 (dd, J_1 = 7.6 Hz, J = 7.2 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.03 (dd, J_1 = 8.0 Hz, J = 7.6 Hz, 1H), 3.84 (s, 3H); ^{13}C-NMR (DMSO-d_6, 75 MHz, δ ppm): 164.89, 156.34, 148.60, 147.21, 141.38, 140.38, 138.56, 135.56, 131.16, 130.04, 129.74, 128.95, 126.96, 125.92, 124.84, 121.47, 121.22, 120.80, 117.92, 116.26, 114.68, 112.47, 55.73; IR (KBr) ν_{max}/cm^{-1}: 3325, 3086, 1673, 1631, 1528, 1461, 1254, 1030, 767; MS (ESI): m/z $[M+H]^+$ = 388.12, m/z $[(M+2)-H]^+$ = 390.12; HPLC: 100%.

2-(3-chlorophenyl)-N-(3-methoxyphenyl)quinoline-4-carboxamide **(6f)**

Yellow amorphous solid; Yield 74%; mp 210–212°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.53 (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 = 8.8 Hz, 1H), 7.84 (dd, J_1 = 8.4 Hz, J = 7.6 Hz, 1H), 7.69 (dd, J_1 = 8.0 Hz, J = 6.0 Hz, 2H), 7.59–7.64 (m, 2H), 7.50 (dd, J_1 = 8.0 Hz, J = 7.6 Hz, 1H), 7.35–7.39 (m, 1H), 7.20–7.22 (m, 1H), 3.81 (s, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 166.54, 161.85, 157.54, 155.27, 149.56, 147.90, 142.92, 140.85, 138.57, 134.10, 132.69, 130.95, 130.56, 129.63, 127.37, 125.85, 123.46, 123.27, 118.34, 116.61, 116.27, 112.40, 55.40; IR (KBr) ν_{max}/cm^{-1}: 3178, 3083, 1643, 1599, 1508, 1403, 1203, 1032, 752; MS (ESI): m/z $[M+H]^+$ = 388.28, m/z $[(M+2)-H]^+$ = 390.28; HPLC: 99.30%.

2-(3-chlorophenyl)-N-(4-methoxyphenyl)quinoline-4-carboxamide **(6g)**

Yellow crystalline solid; Yield 78 %; mp 178–180°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.70 (s, 1H), 8.38 (d, J = 7.2 Hz, 1H), 8.34 (s, 1H), 8.19 (dd, J_1 = 7.2 Hz, J = 6.0 Hz, 2H), 7.86 (t, J = 7.6 Hz, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.68 (t, J = 7.6 Hz, 1H), 7.55–7.61 (m, 3H), 6.99 (d, 2H), 3.78 (s, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 165.16, 155.69, 147.82, 142.92, 138.70, 138.02, 134.23, 131.64, 130.16, 129.80, 129.52, 128.76, 128.68, 127.20, 124.98, 124.02, 123.08, 122.02, 121.92, 119.75, 55.22; IR (KBr) ν_{max}/cm^{-1}: 3289, 3080, 1643, 1612, 1527, 1404, 1246, 1030, 769; MS (ESI): m/z $[M+H]^+$ = 388.38, m/z $[(M+2)-H]^+$ = 390.38; HPLC: 99.81%.

2-(3-chlorophenyl)-N-(2-nitrophenyl) quinoline-4-carboxamide **(6h)**

Yellow crystalline solid; Yield 67%; mp 224–226°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 11.25 (s, 1H), 8.35 (d, J = 7.6 Hz, 2H), 8.27 (d, J = 6.8 Hz, 1H), 8.20 (dd, J_1 = 9.6 Hz, J = 8.0 Hz, 1H), 8.07 (d, J = 7.2 Hz, 2H), 7.83–7.88 (m, 2H), 7.71–7.78 (m, 2H), 7.52–7.61 (m, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 165.03, 159.45, 151.03, 148.32, 146.06, 143.82, 141.64, 138.13, 136.58, 134.13, 133.91, 131.20, 129.85, 129.65, 127.67, 126.33, 126.02, 124.02, 122.93, 122.78, 119.54, 118.44; IR (KBr) ν_{max}/cm^{-1}: 3257, 3027, 1691, 1633, 1596, 1501, 1401, 772; MS (ESI): m/z $[M+H]^+$ = 403.40, m/z $[(M+2)-H]^+$ = 405.40; HPLC: 97.22%.

2-(3-chlorophenyl)-N-(3-nitrophenyl) quinoline-4-carboxamide **(6i)**

Yellow amorphous solid; Yield 70%; mp 250–252°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 10.80 (s, 1H), 8.38 (d, J = 8.8 Hz, 2H), 8.34 (s, 1H), 8.16–8.19 (m, 2H), 7.84–7.88 (m, 1H), 7.66–7.70 (m, 1H), 7.52–7.62 (m, 3H), 7.39 (d, J = 9.2 Hz, 1H), 7.32 (dd, J_1 = 8.8 Hz, J = 7.2 Hz, 1H), 6.75–6.78 (m, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 164.87, 155.89, 147.97, 143.23, 141.64, 140.28, 138.22, 134.23, 131.99, 130.45, 130.34, 129.98, 129.65, 128.96, 127.38, 126.98, 125.26, 123.54, 123.38, 121.62, 116.84, 113.98; IR (KBr) ν_{max}/cm^{-1}: 3233, 3091, 1680, 1597, 1527, 1508, 1431, 772; MS (ESI): m/z $[M+H]^+$ = 403.17, m/z $[(M+2)-H]^+$ = 405.17; HPLC: 98.06%.

2-(3-chlorophenyl)-N-(4-nitrophenyl) quinoline-4-carboxamide **(6j)**

Yellow crystalline solid; Yield 72%; mp 246–248°C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 11.41 (s, 1H), 8.45 (s, 1H), 8.34–8.39 (m, 3H), 8.19 (t, J = 9.2 Hz, 2H), 8.09 (d, J = 9.2 Hz, 2H), 7.88 (dd, J_1 = 7.6 Hz, J = 6.8 Hz, 1H), 7.70 (t, J = 7.2 Hz, 1H), 7.53-7.62 (m, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz, δ ppm): 162.62, 155.88, 147.97, 142.98, 140.02, 138.19, 130.38, 129.98, 129.70, 128.98, 127.46, 127.42, 125.12, 123.22, 121.33, 120.92, 118.78, 116.87, 112.29, 109.70; IR (KBr) ν_{max}/cm^{-1}: 3029, 2996, 1690, 1615, 1595, 1505, 1407, 753; MS (ESI): m/z $[M+H]^+$ = 403.13, m/z $[(M+2)-H]^+$ = 405.13; HPLC: 97.07%.

MTT assay

MTT assay was performed as reported previously.[24] Briefly, cells were grown in DMEM media supplemented with FBS 10% (50 μg/mL) and penicillin-streptomycin (50 μg/mL) at 37°C, 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, six concentrations (triplicate) of test compounds (prepared in DMSO) were added to the cells and incubated at 37°C and 5 % CO_2 for 48 h. 20 μL of MTT solution (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. (IC_{50} determination at concentrations: 0.01, 0.1, 1, 10, 50, and 100 μM). The statistical analyses were performed using GraphPad Prism (version 6.0 for Windows; San Diego, CA, USA).

Agar well diffusion assay

Agar well diffusion assay was performed as reported previously.[25] All target compounds were diluted to obtain final concentration of 25 μg/mL using HPLC grade DMSO. The sterile molten Mueller and Hinton agar butt was seeded with 0.4 mL of 24 h old test pathogens (0.1 OD at 540 nm). The seeded NA butt was poured into sterile Petri plates. After solidification of medium, compounds were allowed to diffuse into the punched wells. After incubation at 37°C for 24 h, the resulting zones of inhibition were measured in millimeters. The derivatives showing the maximum zone of inhibition against test pathogens were checked. The experiment was done in triplicates and the result was reported as mean standard deviation. A control was also prepared for the plates in the same way using solvent DMSO and streptomycin was used as a standard drug and zones of inhibition (mm) were noted.

Molecular docking

Three targets were selected from PharmMapper displaying highest fitting score with the hit molecule **6j** [Table 1]. To identify potential interactions of the hit molecule, molecular docking studies were performed using XP mode in the GLIDE module (Schrödinger small-molecule drug discovery suite[26]), with default settings. The X-ray structures of human carbonic anhydrase I (PDB ID 1CZM[27]), protein kinase A (PDB ID 2F7X[28]), and kinesin spindle protein (KSP) (PDB ID 2UYI[29]) were retrieved from the RCSB Protein Data Bank (rcsb.org)[30] and optimized using OPLS2005 force field. The hit molecule **6j** was prepared and optimized using LigPrep module as implemented in Schrodinger small-molecule drug discovery suite. Receptor grid was generated and the docking studies were performed according to the standard protocol.[31] Individual docked poses were inspected manually to observe the binding interactions of ligands with the selected molecular targets [Table 1].

Table 1: Result of docking analysis of the hit compound (**6j**)

Macromolecule	PDB ID	XP_GScore	Glide_Emodel
		6j	6j
Human carbonic anhydrase I	1CZM	–3.897	–55.15
Protein kinase A	2F7X	–6.679	–80.871
Kinesin spindle protein	2UYI	–4.779	–69.427

RESULTS AND DISCUSSION

Chemistry

A series of novel 2-(3-chlorophenyl)quinoline-4-carboxamide derivatives **(6a-6j)** were synthesized in six steps, as shown in Figure 1. The key intermediate **5** was synthesized by following methods described in the literature.[32-35] The first two step involved the condensation of aniline and ethyl acetoacetate at high temperature to give 3-oxo-N-phenylbutanamide **(1)** which on heating at 100°C in the presence of polyphosphoric acid readily cyclized to give 4-methylquinoline-2(1H)-one **(2).** Further, oxidation of **2** in the presence of aqueous solution of the potassium permanganate and sodium hydroxide gave 2-oxo-1,2-dihydroquinoline-4-carboxylic acid **(3)** which was converted to 2-chloroquinoline-4-carboxylic acid **(4)** by reaction with $POCl_3$ in DMF solvent at 100°C for 4 h. The key intermediate, 2-(3-chlorophenyl)-4-quinolinecarboxylic acid (**5**), was synthesized through a classical Suzuki coupling of 4 with 3-chlorophenylboronic acid in the presence of tetrakis(triphenylphosphine) palladium (0) and sodium carbonate in 1:1 mixture of toluene/ethanol as solvent under thermal heating. Finally, the target compounds **6a-6j** were obtained by stirring of acid chloride, formed by refluxing 5 with $SOCl_2$ at 80 °C for 4 h, with respective amines using sodium hydride in THF solvent at room temperature for 1 h.

All target compounds **(6a-6j)** were confirmed and characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, and MS. Compound **6f** was discussed for the structural corroboration

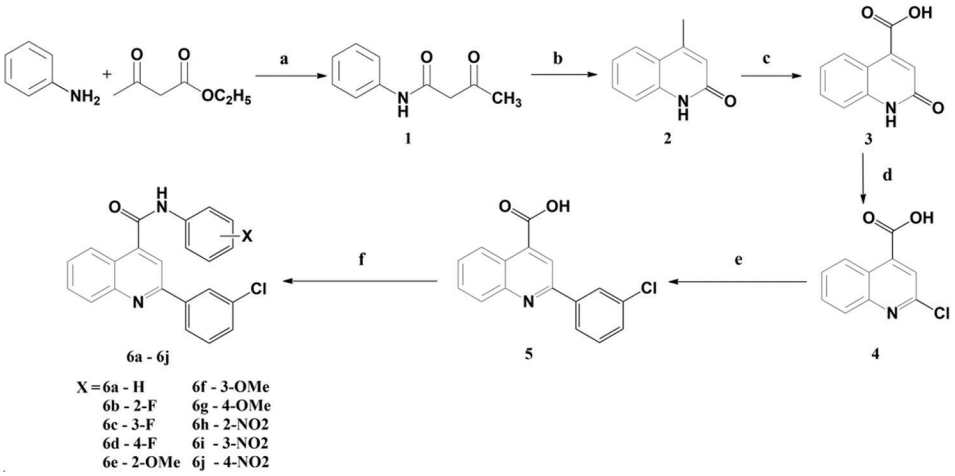

Figure 1: Synthesis of novel quinoline-4-carboxamide derivatives. Reagents and conditions: (a) 160°C, reflux, 36 h (b) polyphosphoric acid, 100°C, reflux, 3 h (c) $KMnO_4$, NaOH, 85°C, reflux, 2 h (d) DMF, $POCl_3$, 100°C, reflux, 4 h (e) 3-chlorophenyl boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene/ethanol (1:1), reflux, 12 h (f) (i) $SOCl_2$, 80°C, reflux, 4 h (ii) respective amine, NaH, THF, 0°C → R.T., 1 h

through spectral studies. ^{1}H-NMR spectrum of compound 6f showed signals at 10.53 (s, 1H, NH of CONH), 8.47 (d, J = 9.2 Hz, 1H, aromatic), 8.22 (d, J = 8.8 Hz, 1H, aromatic), 8.10-8.16 (m, 2H, quinoline), 8.03 (d, J = 8.8 Hz, 1H, aromatic), 7.84 (dd, J_1 = 8.4 Hz, J = 7.6 Hz, 1H, quinoline), 7.69 (dd, J_1 = 8.0 Hz, J = 6.0 Hz, 2H, quinoline), 7.59–7.64 (m, 2H, aromatic), 7.50 (dd, J_1 = 8.0 Hz, J = 7.6 Hz, 1H, aromatic), 7.35–7.39 (m, 1H, aromatic), 7.20–7.22 (m, 1H, aromatic), and 3.81 (s, 3H, OCH_3). ^{13}C-NMR spectrum showed signals at 166.54 (C=O of amide), 161.9 (C-O), 157.5 (C-N), and 155.3 (C-N), remaining in the range of 149.56–112.40 indicated aromatic carbons, 55.40 (OCH_3). The infrared (IR) spectrum showed an absorption bands: 3178 (NH of CONH), 3083 (Ar-H), 1643 (C=O), 1599 (C=N), 1508 (C=C), 1403 (C-N), 1203 (C-O), 1032 (C-O), 752 (C-Cl). The mass spectrum displayed two peaks: $[M-H]^+$ = 388.28 and $[(M+2)-H]^+$ = 390.28. This analysis confirmed the structure of compound **6f.**

Table 2: Anticancer activity of novel quinoline-4-carboxamide derivatives

Compound No.	X	MDA-MB-231[a] IC_{50}±SD (μM)[b]
6a	H	20.65±1.19
6b	2-F	21.70±1.34
6c	3-F	14.94±0.75
6d	4-F	13.89±0.71
6e	2-OMe	46.71±2.12
6f	3-OMe	35.03±1.75
6g	4-OMe	21.48±0.87
6h	2-NO_2	22.18±1.08
6i	3-NO_2	13.16±0.72
6j	4-NO_2	8.24±0.66
Doxorubicin.HCl		0.64±0.04
Cisplatin		47.95±1.26

[a]Breast adenocarcinoma cell line. [b]Results are mean of triplicate analysis

Biological Evaluation

Anticancer activity

All the derivatives 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 are reported in μM. The results are shown in Table 2.

It was observed that the IC_{50} values of compounds were found to be in the range of 46.71–8.24 μM. All the derivatives exhibited more or less similar potency and trends were observed when the substituent (X) was varied through its nature and position. Compound **6d** (4-F), **6g** (4-OCH_3) and **6j** (4-NO_2) possessed higher cytotoxicity and the activity was reduced as the substituent's (X = F, -OCH_3 and NO_2) shifted to two and three position. It can be concluded from the above results that the substituent (X) at four position possessed superior potency than at two and three position. Compound **6j** (4-NO_2) was found to be the best molecule (IC_{50} = 8.24 μM) among all analogs **6a-6j.** All the molecules demonstrated potency less than 50 μM and were better than cisplatin but not comparable to doxorubicin HCl. Compound 6j can be further optimized which may give rise to lead structure to develop potent anticancer drug.

Antibacterial activity

All the compounds were screened against Gram-positive bacteria (*S. aureus* 6538p and *B. subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Streptomycin was used as a standard drug and zones of inhibition (mm) were noted. The results are shown in Table 3.

From antibacterial activity data, it was confirmed that all the compounds showed less potency as compared to standard streptomycin. Among all the synthesized analogs, the compounds **6c, 6d, 6f, 6h, 6i,** and **6j** exhibited moderate antibacterial activity against all the tested organisms except **6d** with no activity against *P. aeruginosa*. Compounds **6c** (3-F) and **6f** (3-OCH_3) exhibited higher potency and the activity was decreased as the substituent's (X = F and -OCH_3) shifted

Table 3: Antibacterial activity of novel quinoline-4-carboxamide derivatives

Compd. No.	Zone of inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	***Staphylococcus aureus* 6538p**	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
6a	-	-	-	-
6b	10	10	-	-
6c	12	10	6	5
6d	13	12	10	-
6e	10	11	-	-
6f	13	12	8	9
6g	10	12	-	-
6h	16	14	11	10
6i	12	11	8	6
6j	12.5	12	10	10
Streptomycin	20	22	22	24

No inhibition. Results are mean of triplicate analysis

to two and four position. Compounds **6b, 6e,** and **6g** were active against only Gram-positive bacteria (*S. aureus* 6538p and *B. subtilis*). Compound **6h** (2-NO_2) was found to be best the molecule among all analogues and the activity reduced as the substituent shifted to 3- **(6i)** and 4- **(6j)** positions. Compound **6a** did not exhibit any antibacterial activity.

Molecular Docking Studies

To investigate the potential molecular targets of the hit molecule **6j** and to provide a preliminary data for the molecular/cellular biology, the authors carried out a "target fishing" computational experiment using PharmMapper.[36] 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 effects of these hit molecules and can be useful for the exploration of the proposed molecular target(s) to treat cancer. Such studies have been previously reported in the literature.[31]

As shown in Table 1, the docking score (XP_GScore) of **6j** with the Protein Kinase A was the highest followed by KSP and

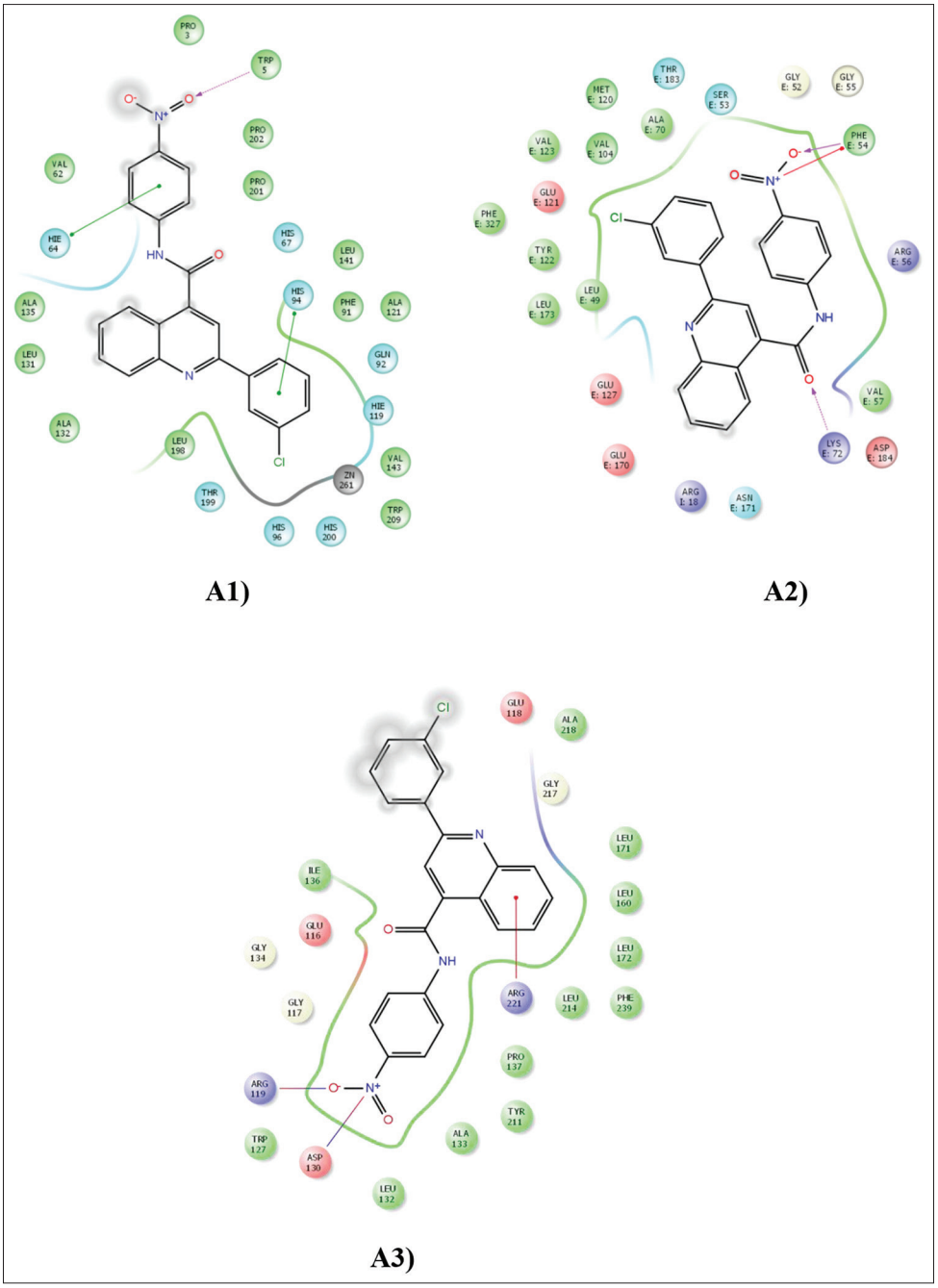

Figure 2: 2D interaction diagram of molecular docking of hit **6j** in the binding sites of macromolecular targets – (A1) **6j** docked in the binding site of human carbonic anhydrase I (PDB ID 1CZM). (A2) **6j** docked in the binding site of protein kinase A (PDB ID 2F7X). (A3) **6j** docked in the binding site of KSP (PDB ID 2UYI). Gray dotted lines represent hydrogen bonding interaction and green or red solid line indicates π–π stacking interaction

Human Carbonic Anhydrase I. The XP_GScore is an empirical scoring function that approximately represents the ligand binding free energy, with contributions from electrostatic, H-bonding, hydrophobic, and Van der Waals energy terms. Higher the score (on the negative side), tighter is the binding due to shape and electrostatic complementarity. On the other hand, E_{model} represents the force field components (electrostatic and Van der Waals energies) with higher weighting, making it better suited for conformer comparison. It may not be much useful for comparing different molecules. On other words, E_{model} is used for selecting the best ligand pose while Gscore is used for raking these best poses against one another. Similar order was observed for the E_{model} as seen with XP_GScore.

Compound **6j** showed interaction with the active site amino acid of Human Carbonic Anhydrase I (PDB ID: 1CZM), Protein Kinase A (PDB ID: 2F7X), and KSP (PDB ID: 2UYI) [Figure 2]. Compound **6j** displayed π-π stacking with His64 and His94 along with H-bonding interaction of aromatic NO_2 group with Trp5 in Human Carbonic Anhydrase I. In Protein Kinase A receptor, compound **6j** displayed hydrogen bonding interaction with Lys72 and Phe54. Furthermore, in KSP receptors, compound 6j displayed the hydrogen bonding interaction between Arg 119 and Asp 130 with nitro group. In addition, the prominent cation-πIn addition, the prominent cation-eraction between Arg 119 and Asp 130 with nitro group.: 1CZM), Protein Kinase A (PDB ID: 2F7X)ing

CONCLUSION

The present study attempts the synthesis of a novel series of quinoline-4-carboxamide derivatives and subsequent SAR investigations. Based on the observation made during the study, we can conclude that all the compounds showed significant anticancer and the trends were observed with variations in the substituent's (X). The substituent's (X) is favored four position more than two and three position to exhibit superior potency. Compound **6j** exhibited better promising anticancer activity among various synthesized molecules. Furthermore, docking study of compound was done and the compound **6j** showed good interaction with the active site amino acid of Human Carbonic Anhydrase I, Protein Kinase A and KSP. It was also revealed that all the compounds showed less antibacterial activity as compared to standard streptomycin. Compounds **6c, 6f, 6h, 6i,** and **6j** exhibited moderate antibacterial activity against all the tested organisms. Compound **6h** was found to be the best molecule among all of them. The current results may be useful in developing potential anti-breast cancer and antibacterial drugs in future after further modification and optimization of the reported molecules.

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CONFLICTS OF INTEREST

I declared that all authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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