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Original Article

Synthesis, Molecular Docking and Biological Evaluation of New Quinoline Analogues as Potent Anti-breast Cancer and Antibacterial Agents

Shrimant V. Rathod*[, a], Kailas W. Shinde[a,b], Prashant S. Kharkar[c], Chetan P. Shah[c]

[a]Depatment of Chemistry, Bhavan's Hazaimal Somani College, Mumbai University, Mumbai, India, [b]Department of Chemistry, Wilson College, Mumbai University, Mumbai, India, [c]Depatment of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, Narsee Monjee Institute of Management Studies University, Mumbail, India.

Abstract

A series of new class of quinoline analogues were synthesized from isatin through two steps in good yields. All compounds were further evaluated for their anticancer activity against triple-negative breast cancer cell line (MDA-MB-231) using 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. All synthesized compounds were confirmed by spectral characterization viz FT-IR, MS, ^{1}H-NMR, and ^{13}C-NMR. Results indicated that *in vitro* anticancer evaluation, IC50 values of all target compounds were in the range of 11.50-37.99 μM and compound 4h showed better promising anti-breast cancer activity among all synthesized derivatives. *In vitro* antibacterial evaluation, compounds 4d, 4f, 4h, and 4j showed moderate antibacterial activity among all derivatives. Molecular docking analysis demonstrated good interaction of compound 4h with the active site residue of Human Carbonic Anhydrase I, Protein Kinase A, and Kinesin Spindle Protein (KSP).

Keywords: Antibacterial, Anticancer, Docking, MDA-MB-231, Quinoline analogues, Synthesis.

Corresponding Authors: Shrimant V. Rathod, Depatment of Chemistry, Bhavan's Hazaimal Somani College, Mumbai University, Mumbai, India.
Tel: +91-9892992024
Email: shrees.rathod@gmail.com
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1. Introduction

Breast cancer is one of the most commonly diagnosed cancers causing the highest number of cancer-related deaths among women. Worldwide, there were 2.1 million newly diagnosed breast cancer cases in 2018, accounting for almost 1 in 4 cancer cases in women. In 2018 alone, it was estimated that 627000 women died from breast cancer

(approx. 11.6% of all cancer deaths in women) [1]. We urgently need newer therapeutic agents to curb the deadly disease and its variants, such as triple-negative breast cancer.

On the similar lines, antimicrobial resistance is precarious to practical eradication and treatment of an escalating range of diseases caused by microbes (bacteria, fungi, viruses, and others). The effective treatment of infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite several antibiotics and chemotherapeutics in our armamentarium, the emergence of old and new antibiotic-resistant bacterial strains in the last decade or so led to a substantial need for new classes of antimicrobial agents, in general, and antibacterials, in particular [2].

Several heterocyclic compounds or privileged structures are known for their key role in the field of medicinal chemistry, biochemistry as well as other area of sciences. A large number of drugs contain heterocyclic cores. Extensive literature review indicated that among all pharmacologically important heterocyclic compounds, quinoline and its derivatives represent as one of the important classes with as key role in therapeutically important agents. Quinoline moiety has increasingly attracted the attention of synthetic chemists. It is found in a large variety of natural products and synthetically useful molecules having diverse biological activities. Quinoline compounds have evoked considerable attention in recent years in view of their wide range of pharmacological properties such as antimalarial [3, 4], antitubercular [5], anti-inflammatory [6], antifungal [7], antiproliferative [8] and antimicrobial [9]. Also, the anticancer and antibacterial activities of numerous quinoline derivatives have been studied and are well documented in the literature [10-21].

In order to further expand the scope of quinoline derivatives as privileged medicinal scaffolds and a substantial need of the discovery of new chemical entities (NCEs) of potential biological interest, we attempted the synthesis and biological evaluation of new quinoline derivatives for possible applications as potential therapeutic agents. Motivated by the afore-mentioned literature, we synthesized a new class of quinoline analogues bearing carboxamide functionality and evaluated them for anticancer (triple-negative breast cancer cell line MDA-MB-231) by MTT assay and antibacterial (Gram-positive bacteria-*Staphylococcus aureus 6538p* and *Bacillus subtilis* and Gram-negative bacteria-*Escherichia coli* and *Pseudomonas aeruginosa)* activities by agar well diffusion method. Here, we present our efforts in the design, synthesis and biological evaluation of quinoline derivatives as potential anticancer and antibacterial agents.

2. Materials and Methods

2.1. Materials

2.1.1. Chemistry

All commercial chemicals and solvents (LR- or AR-grade) were purchased from commercial vendors such as Sigma-Aldrich,

VWR, sd-fine chemicals and others, and were used without further purification, unless otherwise mentioned and/or required. 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 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 discs 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 X 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).

2.1.2. 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) , 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) , antibiotics from Hi-Media Laboratories Ltd. (Mumbai, India).

2.1.3. Antibacterial Activity

The Gram-positive organisms viz. *Bacillus subtilis* and *Staphylococcus aureus 6538p* and Gram-negative organisms viz. *Pseudomonas aeruginosa* and *Escherichia coli* cultures were obtained from neighbouring hospitals and pathological laboratories located in Mumbai.

2.1.4. Molecular Docking

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

2.2. Methods

2.2.1. Chemistry

In the present work, a novel series of quinoline derivatives (**4a-4j**) was synthesized from isatin in two steps (Figure 1). Compounds **4a** and compound **4g** were already reported as anti-tubercular agent and tubulin polymerization inhibitor, respectively [22, 23]. The key intermediate **3** was synthesized by following the literature method [24]. The first step involved the condensation of isatin (**1**) and acetophenone (**2**) in the presence of hydroalcoholic KOH at 80°C to yield 2-phenylquinoline-4-carboxylic acid (**3**). Finally,

target compounds (**4a-4j**) were obtained by coupling of the corresponding acid chloride formed by refluxing intermediate **3** with $SOCl_2$ at 80°C for 5 h, with respective amines using NaH in THF with stirring at RT for 1 h.

2.2.2. Procedure for the synthesis of 2-phenylquinoline-4-carboxylic Acid (3)

A mixture of isatin (**1**) (1 mmol, 1 eq.) and KOH (5 mmol, 5 eq.) in 1:1 mixture of EtOH-water was stirred at RT for 15-30 min. The mixture was then acidified to pH 2-3 with conc. HCl and acetophenone (**2**) (1 mmol, 1 eq.) was added. The resulting mixture was stirred at 80°C for 12-13 h and precipitate was obtained. The reaction progress was monitored by TLC. After reaction completion, the precipitate was filtered, washed with water and recrystallized from a suitable solvent such as EtOH to obtain pure compound (**3**).

White solid; Yield 77%; m.p. 210-212 °C; ^{1}H-NMR (DMSO-d_6, 300MHz, δ ppm): 14.35 (brs, 1H, COOH), 8.87 (d, *J* = 7.8 Hz, 1H, quinoline), 8.22 (s, 1H, quinoline), 8.15 (d, *J* = 9.0 Hz, 1H, quinoline), 7.85-7.89 (m, 2H, quinoline), 7.78-7.81 (m, 1H, aromatic), 7.65-7.69 (m, 1H, aromatic), 7.52-7.57 (m, 2H, aromatic), 7.44-7.47 (m, 1H, aromatic); ^{13}C-NMR (DMSO-d_6, 75MHz, δ ppm): 167.52, 158.89, 147.20, 140.38, 135.16, 133.57, 132.26, 132.16, 131.47, 131.16, 128.52, 126.76, 125.00, 124.89, 124.71, 121.57; IR (KBr) ν_{max}/cm^{-1}: 3263, 1716, 1659, 1525, 1398, 1215, 818, 751, 692; MS (APCI): *m/z* 250.20 $[M+H]^+$; HPLC: 98.93%.

2.2.3. General Procedure for the Synthesis of Target Compounds (4a-4j)

A mixture of 2-phenylquinoline-4-carboxylic acid (**3**) (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 acid chloride (0.9 mmol, 1 eq.) in THF, respective amine (1.5 mmol, 1.5 eq.) and NaH (1.0 mmol, 1.1 eq.) was added at 0^{o}C and the reaction mixture was then stirred at RT for 1 h. Completion of the reaction was monitored by TLC. The reaction mixture was then poured into ice-cold water and extracted with EtOAc. 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% EtOAc/petroleum ether) to obtain target compounds (**4a-4j**).

2.2.3.1. Synthesis of N, 2-Diphenylquinoline-4-Carboxamide (4a)

Light yellow solid; Yield 72%; m.p. 194-196 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 10.82 (s, 1H, NH), 8.37 (t, 2H, quinoline), 8.18 (d, *J* = 8.4 Hz, 2H, quinoline), 7.82-7.88 (m, 4H, aromatic), 7.68 (t, 1H, quinoline), 7.54-7.62 (m, 3H, aromatic), 7.42 (t, 2H, aromatic), 7.18 (t, 1H, aromatic); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 165.28, 155.81, 147.90, 143.03, 138.83, 138.12, 130.29, 129.94, 129.61, 128.90, 128.81, 127.61, 127.32, 125.07, 124.13, 123.25,

123.08, 121.62, 121.54, 120.31, 119.97, 116.80; IR (KBr) ν_{max}/cm^{-1}: 3243, 1677, 1598, 1547, 1355, 1257, 879, 756, 696; MS (APCI): *m/z* 325.40 $[M+H]^+$; HPLC: 100%.

2.2.3.2. Synthesis of N-(2-fluorophenyl)-2-Phenylquinoline-4-Carboxamide (4b)

Yellow solid; Yield 71%; m.p. 164-166 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 10.69 (s, 1H, NH), 8.37 (d, *J* = 7.2 Hz, 1H-aromatic, 2H-quinoline), 8.18-8.24 (m, 2H, quinoline), 7.85-7.94 (m, 2H, aromatic), 7.70 (t, 1H, quinoline), 7.53-7.62 (m, 3H, aromatic), 7.29-7.39 (m, 3H, aromatic); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 166.10, 160.92, 158.57, 149.27, 147.56, 141.40, 137.37, 134.10, 132.69, 130.35, 129.91, 129.15, 128.92, 128.85, 126.37, 124.05, 123.90, 123.77, 121.34, 121.11, 119.45, 116.27; IR (KBr) ν_{max}/cm^{-1}: 3263, 1676, 1595, 1542, 1355, 1199, 757, 695; MS (APCI): *m/z* 341.10 $[M-H]^-$; HPLC: 99.48%.

2.2.3.3. Synthesis of N-(3-fluorophenyl)-2-Phenylquinoline-4-Carboxamide (4c)

White solid; Yield 70%; m.p. 216-218 °C; ^{1}H-NMR (DMSO-d_6, 300MHz, δ ppm): 10.71 (s, 1H, NH), 8.37 (s, 1H, quinoline), 8.18-8.26 (m, 2H-aromatic, 2H-quinoline), 7.84-7.93 (m, 1H-aromatic, 1H-quinoline), 7.70 (t, 1H-aromatic, 1H-quinoline), 7.58 (t, 3H, aromatic), 7.33-7.37 (m, 2H, aromatic); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 165.82, 161.76, 157.92, 149.15, 146.71, 140.21, 138.92, 136.25, 132.25, 131.91, 130.25, 129.91, 129.59, 127.98, 127.65, 127.31, 125.15, 123.27, 120.25, 118.89, 116.54, 116.22; IR (KBr) ν_{max}/cm^{-1}: 3184, 1684, 1613, 1549, 1355, 1244, 1128, 867, 757, 699; MS (APCI): *m/z* 343.20 $[M+H]^+$; HPLC: 98.71%.

2.2.3.4. Synthesis of N-(4-fluorophenyl)-2-Phenylquinoline-4-Carboxamide (4d)

White solid; Yield 78%; m.p. 222-224 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 10.88 (s, 1H, NH), 8.38 (d, *J* = 7.6 Hz, 1H-aromatic, 2H-quinoline), 8.18 (d, *J* = 8.4 Hz, 2H, quinoline), 7.83-7.88 (m, 3H, aromatic), 7.68 (t, 1H, quinoline), 7.55-7.61 (m, 3H, aromatic), 7.27 (t, 2H, aromatic); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 165.54, 161.21, 158.52, 148.98, 146.51, 141.21, 138.27, 135.15, 133.25, 132.91, 131.27, 129.61, 129.39, 127.98, 127.65, 127.31, 126.15, 125.97, 121.75, 120.29, 116.74, 116.52; IR (KBr) ν_{max}/cm^{-1}: 3242, 1679, 1616, 1553, 1356, 1212, 1152, 837, 755, 697; MS (APCI): *m/z* 343.20 $[M+H]^+$; HPLC: 98.41%.

2.2.3.5. Synthesis of N-(2-methoxyphenyl)-2-Phenylquinoline-4-Carboxamide (4e)

Brown solid; Yield 78%; m.p. 160-162 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 10.43 (s, 1H, NH), 8.45 (d, *J* = 9.2 Hz, 1H, quinoline), 8.29 (d, *J* = 7.2 Hz, 2H, aromatic), 8.17 (d, *J* = 8.8 Hz, 1H, quinoline), 8.10 (d, *J* = 8.8 Hz, 2H, quinoline), 8.00 (d, *J* = 8.4 Hz, 1H, aromatic), 7.81 (t, 1H, quinoline), 7.67 (d, *J* = 7.2 Hz, 1H, aromatic), 7.52-7.60 (m, 3H, aromatic), 7.12 (d, *J* = 8.4 Hz, 2H, aromatic), 3.88 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6, 75MHz, δ ppm): 165.16, 158.81, 156.73, 149.62, 148.56, 147.34, 140.38, 138.56, 135.16, 133.57, 132.08, 131.47, 131.16,

129.31, 124.84, 124.34, 121.22, 118.80, 116.57, 116.26, 112.71, 112.47, 56.62; IR (KBr) ν_{max}/cm^{-1}: 3299, 1673, 1596, 1529, 1354, 1258, 1117, 1031, 810, 754, 698; MS (APCI): *m/z* 353.20 $[M-H]^-$; HPLC: 97.61%.

2.2.3.6. Synthesis of N-(3-Methoxyphenyl)-2-Phenylquinoline-4-Carboxamide (4f)

Brown solid; Yield 75%; m.p. 152-154 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 10.79 (s, 1H, NH), 8.35 (d, *J* = 8.4 Hz, , 1H-aromatic, 2H-quinoline), 8.15-8.18 (m, 2H, quinoline), 7.83-7.87 (m, 1H, quinoline), 7.65-7.69 (m, 1H, aromatic), 7.51-7.60 (m, 4H, aromatic), 7.38 (d, *J* = 8.4 Hz, 1H, aromatic), 7.31 (t, 1H, aromatic), 6.74-6.77 (m, 1H, aromatic), 3.78 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 165.31, 159.54, 155.81, 147.89, 142.98, 139.98, 138.11, 130.31, 129.95, 129.62, 128.91, 127.38, 127.32, 125.05, 123.14, 121.54, 118.25, 116.89, 116.78, 112.22, 109.61, 105.75, 55.06; IR (KBr) ν_{max}/cm^{-1}: 3056, 1675, 1610, 1544, 1354, 1250, 1158, 861, 753, 690; MS (APCI): *m/z* 353.10 $[M-H]^-$; HPLC: 96.14%.

2.2.3.7. Synthesis of N-(4-Methoxyphenyl)-2-Phenylquinoline-4-Carboxamide (4g)

White solid; Yield 82%; m.p. 218-220 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 10.67 (s, 1H, NH), 8.37 (d, *J* = 7.2 Hz, 2H, quinoline), 8.32 (s, 1H, quinoline), 8.17 (t, 2H, aromatic), 7.85 (t, 1H, aromatic), 7.73 (d, *J* = 8.8 Hz, 2H, quinoline), 7.67 (t, 1H, aromatic), 7.54-7.60 (m, 3H, aromatic), 6.98 (d, *J* = 8.8 Hz, 2H, aromatic), 3.77 (s, 3H, OCH_3); ^{13}C-NMR (DMSO-d_6, 100MHz, 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, 116.65, 116.52, 55.04; IR (KBr) ν_{max}/cm^{-1}: 3304, 1683, 1589, 1527, 1349, 1247, 1179, 1030, 825, 769, 689; MS (APCI): *m/z* 355.20 $[M+H]^+$; HPLC: 100%.

2.2.3.8. Synthesis of N-(2-Nitrophenyl)-2-Phenylquinoline-4-Carboxamide (4h)

Yellow solid; Yield 72%; m.p. 180-182 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 11.29 (s, 1H, NH), 8.27 (m, 2H-aromatic, 1H-quinoline), 8.26 (m, 2H, quinoline), 8.16 (m, 1H, quinoline), 7.70 (m, 1H-aromatic, 1H-quinoline), 7.57 (m, 3H, aromatic), 7.53 (m, 3H, aromatic); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 165.31, 158.54, 149.59, 145.15, 140.21, 137.92, 133.27, 130.25, 129.91, 129.59, 128.98, 128.65, 128.31, 127.92, 125.15, 123.27, 123.15, 121.27, 121.15, 118.54, 118.22, 116.76; IR (KBr) ν_{max}/cm^{-1}: 3317, 1691, 1590, 1548, 1347, 1279, 1151, 774, 749, 690; MS (APCI): *m/z* 370.32 $[M+H]^+$; HPLC: 99.66%.

2.2.3.9. Synthesis of N-(3-Nitrophenyl)-2-Phenylquinoline-4-Carboxamide (4i)

Yellow solid; Yield 74%; m.p. 264-266 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 11.30 (s, 1H, NH), 8.88 (d, *J* = 1.6 Hz, 1H, aromatic), 8.45 (s, 1H, quinoline), 8.39 (d, *J* = 6.8 Hz, 2H, quinoline), 8.15-8.23 (m, 2H-aromatic, 1H-quinoline), 8.04-8.07 (m, 1H, aromatic), 7.86-7.90 (m, 1H, quinoline), 7.68-7.75 (m, 2H, aromatic), 7.55-7.62 (m, 3H,

aromatic); ^{13}C-NMR (DMSO-d_6, 100MHz, δ ppm): 165.38, 159.62, 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, 105.82; IR (KBr) ν_{max}/cm^{-1}: 3275, 1691, 1631, 1543, 1526, 1354, 1109, 848, 752, 676; MS (APCI): *m/z* 370.10 $[M+H]^+$; HPLC: 99.30%.

2.2.3.10. Synthesis Of N-(4-Nitrophenyl)-2-Phenylquinoline-4-Carboxamide (4j)

Yellow solid; Yield 68%; m.p. 264-266 °C; ^{1}H-NMR (DMSO-d_6, 400MHz, δ ppm): 11.40 (s, 1H, NH), 8.31 (m, 2H, aromatic), 8.28 (m, 2H-aromatic, 1H-quinoline), 8.02 (d, *J* = 8.0 Hz, 3H, quinoline), 7.85 (m, 1H, quinoline), 7.55 (m, 1H, aromatic), 7.52 (m, 4H, aromatic); ^{13}C-NMR (DMSO-d_6, 75MHz, δ ppm): 165.63, 156.73, 155.77, 153.46, 147.91, 142.40, 138.14, 130.28, 129.92, 129.61, 128.90, 127.37, 127.31, 127.07, 126.96, 126.23, 125.31, 125.14, 124.04, 118.48, 116.43, 116.26; IR (KBr) ν_{max}/cm^{-1}: 3198, 1689, 1595, 1555, 1343, 1259, 1189, 860, 758, 695; MS (APCI): *m/z* 370.40 $[M+H]^+$; HPLC: 98.08%.

2.2.4. MTT Assay

MTT assay was performed in order to determine anticancer activity of all target compounds against MDA-MB-231 (breast adenocarcinoma). Briefly, cells were grown in DMEM media supplemented with fetal bovine serum (FBS) 10% (50 µg/mL) and penicillin-streptomycin (50 µg/ml) at 37ºC, CO_2 (5%) and air (95%). Cells were seeded ($1x10^4$ 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°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).

2.2.5. Agar Well Diffusion Assay

The antibacterial activity of all final compounds was checked by agar well diffusion method. The compounds were diluted to obtain final concentration of 32µg/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 millimetres. 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.

2.2.6. Molecular Docking

Three targets were selected from PharmMapper displaying highest fitting score with the hit molecule (**4h**) (Table 3). 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 force field. The hit molecule was prepared and optimized using LigPrepmodule as implemented in Schrodinger Small-Molecule Drug Discovery 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 targets (Table 3).

3. Results and Discussion

3.1. Biological Evaluation

3.1.1. Anticancer Activity

All target compounds (**4a-4j**) were evaluated as anti-breast cancer agents 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 all tested compounds were found to be in the range of 11.50-37.99 µM and variations were observed when the substitution was varied. Compound **4d** (4-F) showed higher potency which decreased as the strongly electron-withdrawing substituent was shifted to 2- (**4b**) and 3- (**4c**) positions. Compound **4h** (2-NO_2) was found to be the best molecule (IC_{50} = 11.50 µM) among all analogues and the activity reduced as the very strongly-electron-withdrawing substituent shifted to 3- (**4i**) and 4- (**4j**) positions. The compound, having strongly electron-donating -OCH_3 substituent **4**(**e-g**), showed similar activity. It was revealed from the above results that, substituent (X) at 4 position exhibited superior activity than at 2 and 3 position. All compounds exhibited potency less than 50 µM and were better than standard cisplatin but not comparable to doxorubicin.

3.1.2. Antibacterial Activity

All target compounds (**4a-4j**) were screened against Gram-positive bacteria (*Staphylococcus aureus 6538p* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by agar well diffusion method. Streptomycin was used as a standard drug and zones of inhibition (mm) were noted. The results were shown in Table 2.

From antibacterial activity data, it was confirmed that all compounds showed less potency compared to standard streptomycin. Compounds **4d**, **4f**, **4h** and **4j** showed moderate antibacterial activity against all the tested organisms. Compounds **4b**, **4e**, **4g** and **4i** were active against only Gram-positive bacteria (*Staphylococcus aureus 6538p* and *Bacillus subtilis*). Compounds **4a** and **4c** did not exhibit any antibacterial activity.

3.2. Molecular Docking Studies

In order to investigate the potential molecular targets of the hit molecule (**4h**) (Table 3) and to provide a preliminary data for the molecular/cellular biology, the authors carried out a target 'go fishing' experiment using PharmMapper [18]. 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 data provided logical base for the anticancer effects of this hit molecule and can be useful for the exploration of the proposed molecular target(s) to treat cancer.

Compound **4h** showed interaction with the active site residue of Human Carbonic Anhydrase I (PDB ID: 1CZM), Protein Kinase A (PDB ID: 2F7X) and Kinesin Spindle Protein (KSP) (PDB ID: 2UYI) (**Figure 2**). Compound **4h** displayed π–π stacking with His64 and His94 in Human Carbonic Anhydrase I and additional interactions with Phe91 (π–π stacking) and H-bonding interaction between Gln92 and amide carbonyl group were observed. In Protein Kinase A receptor, compound **4h** showed π–π stacking with Phe54 and H-bonding interactions with Lys72. Also in Kinesin Spindle Protein (KSP) receptors, the compound **4h** showed hydrogen bonding interaction between Glu116 and NH of amide.

4. Conclusion

A novel series of quinoline analogues was synthesized, characterized and evaluated for anti-breast cancer and antibacterial activities. All compounds showed potency less than 50 µM against breast adenocarcinoma cell line, MDA-MB-231 and a systematic structure-activity relationship trends were observed with varied substituent nature and position. Compound **4h** exhibited better promising anti-breast cancer activity among various synthesized molecules. It was also revealed that, all compounds showed less antibacterial activity as compared to standard streptomycin. Compounds **4d**, **4f**, **4h** and **4j** exhibited moderate antibacterial activity against all tested organisms. Molecular docking results provided additional insight into the good interaction of compound **4h** with the active site amino acid of Human Carbonic Anhydrase I, Protein Kinase A and Kinesin Spindle Protein (KSP).

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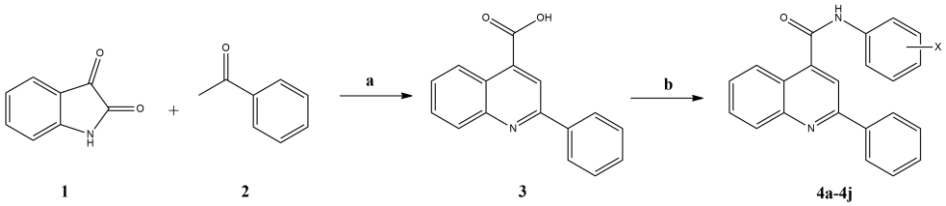
Figures



[a]Reagents and conditions: (a) KOH, EtOH, 80°C, reflux, 12-13 h (b) i) $SOCl_2$, 80°C, reflux, 5 h ii) substituted aniline, NaH, THF, 0°C → R.T., 1 h.

Figure 1. Synthesis of novel 2-phenyl-quinoline-4-carboxamide derivatives.

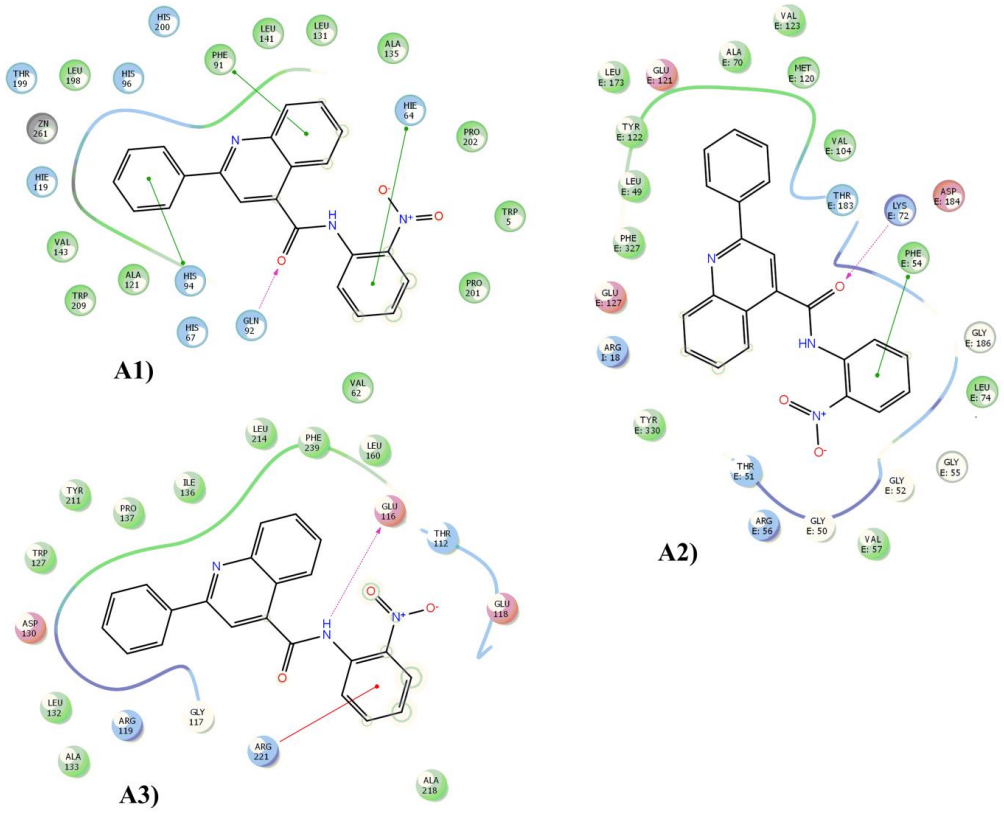


Figure 2. 2D interaction diagram of molecular docking of hit **4h** in the binding sites of macromolecular targets - A1) **4h** docked in the binding site of Human Carbonic Anhydrase I (PDB ID 1CZM). A2) **4h** docked in the binding site of Protein Kinase A (PDB ID 2F7X). A3) **4h** docked in the binding site of Kinesin Spindle Protein (KSP) (PDB ID 2UYI). Grey dotted lines represent hydrogen bonding interaction and green or red solid line indicates π–π stacking interaction.

Tables:

Table 1. Anticancer activity of novel quinoline analogues (**4a-4j**).

Compd. No.	X	IC_{50} ± SD (µM)[a]
4a	H	37.99±1.54
4b	2-F	35.69±2.49
4c	3-F	24.72±1.43
4d	4-F	16.45±0.72
4e	2-OCH_3	22.08±1.01
4f	3- OCH_3	23.03±2.25
4g	4- OCH_3	19.83±0.69
4h	2-NO_2	11.50±0.98
4i	3-NO_2	22.21±1.12
4j	4-NO_2	34.18±2.35
Doxorubicin.HCl	-	0.64±0.04
Cisplatin	-	47.95±1.26

Table 2. Antibacterial activity of novel quinoline analogues (4a-4j).

Compd. No.	Zone of inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	S.aureus 6538p	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
4a	-	-	-	-
4b	8	6	-	-
4c	-	-	-	-
4d	12	10	8	9
4e	12	11	-	-
4f	13	12	10	8
4g	10	12	-	-
4h	13	14	10	10
4i	10	11	-	-
4j	15	16	9	10
Streptomycin	20	22	22	24

- No Inhbition

Results are mean of triplicate analysis

Table 3. Result of docking analysis of the hit compound (4h).

Macromolecule	PDB ID	XP_GScore	Glide_Emodel
		4h	4h
Human Carbonic Anhydrase I	1CZM	-4.278	-55.676
Protein Kinase A	2F7X	-6.655	-65.507
Kinesin Spindle Protein (KSP)	2UYI	-5.715	-70.240