Indian Journal of Chemistry
Vol. 60B, September 2021, pp. 1215-1222

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[d], K Aruna[e] & Darshana A Raut[e]

[a] Department of Chemistry, Bhavan's Somani and Hazarimal College, Mumbai 400 007, India
[b] Department of Chemistry, Wilson College, Mumbai 400 007, India
[c] Department of Pharmaceutical Quality Assurance, SPTM, NMIMS, Mumbai 400 056, India
[d] Department of Pharmaceutical Chemistry, SPTM, NMIMS, Mumbai 400 056, India
[e] Department of Microbiology, Wilson College, Mumbai 400 007, India

E-mail: shrees.rathod@gmail.com

Received 12 January 2020; accepted (revised) 18 August 2021

A new class of quinoline analogues have been synthesized from isatin through two steps in good yields. They have been further evaluated for 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*). All synthesized compounds have been confirmed by spectral characterization *viz*. FT-IR, MS, HPLC, ^{1}H and ^{13}C NMR. Among them, compound **4h** exhibits promising anti-breast cancer activity whereas compounds **4d**, **4f**, **4h** and **4j** exhibit moderate antibacterial activity against all the tested organisms. Molecular docking analysis demonstrates the interaction of compound **4h** with the active site amino acid of Human Carbonic Anhydrase I, Protein Kinase A and Kinesin Spindle Protein (KSP).

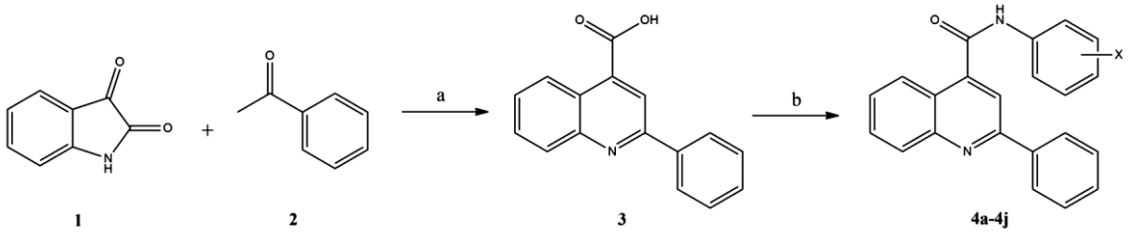
Keywords: Antibacterial, anticancer, docking, MDA-MB-231, quinoline analogues, synthesis

Breast cancer is the most commonly diagnosed cancer and also causes the greatest number of cancer-related deaths among women. Worldwide, there will be about 2.1 million newly diagnosed female breast cancer cases in 2018, accounting for almost 1 in 4 cancer cases among women. In 2018, it is estimated that 6,27,000 women died from breast cancer - that is approximately 11.6% of all cancer deaths among women[1].

Antibacterial resistance is precarious to practical eradication and treatment of an escalating range of diseases caused by bacteria. Also, the treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades leads to a substantial need for new classes of antibacterial agents[2].

Quinolines are important pharmacophores which 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]. The quinoline moiety has increasingly attracted the attention of synthetic chemists. The anticancer and antibacterial activity of numerous quinoline derivatives has been studied and well documented in the literature[10-21].

Based on this trend, it was expected in the present work that the incorporation of substituents on the quinoline skeleton could produce derivatives with enhanced biological properties. In order to further expand the scope of quinoline derivatives as privileged medicinal scaffold, we have been engaged in anticancer and antibacterial evaluation of new quinoline analogues so as to develop novel molecules which could possibly find applications as potential anticancer and antibacterial agents. Thus, a new class of quinoline derivatives bearing carboxamide functionality were conveniently synthesized and screened for their anticancer activity (breast cancer cell line MDA-MB-231) and antibacterial activity (Gram-positive bacteria- *Staphylococcus aureus* 6538p and *Bacillus subtilis* and Gram-negative bacteria- *Escherichia coli* and *Pseudomonas aeruginosa*).



Reagents and conditions: (a) KOH, EtOH, 80°C, reflux, 12-13 h, (b) phenyl boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene/ethanol (1:1), reflux, 12 h, i) DMF, $POCl_3$, 100°C, 4 h, ii) respective amine, NaH, THF, 0°C → RT, 1 h.

Scheme I — Synthesis of quinoline analogues

Result and Discussion

Chemistry

In the present work, a series of quinoline analogues **4a-4j** were synthesized from isatin in two steps as shown in Scheme I. Compound **4a** and compound **4g** were already reported as antitubercular agent and tubulin polymerization inhibitor respectively[22,23]. The key intermediate **3** was synthesized by the following method described in literature[24]. The first step involved the condensation of isatin **1** and acetophenone **2** in the presence of aqueous solution of ethanol and potassium hydroxide at 80°C to give 2-phenylquinoline-4-carboxylic acid **3**. Finally, the target compounds **4a-4j** were obtained by coupling of acid chloride formed by refluxing intermediate **3** with $POCl_3$ in DMF at 100°C for 4 h with respective amines using sodium hydride in THF solvent stirred at room temperature for 1 h.

Biological evaluation

Anticancer activity

All the compounds 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 I.

During analysis, it was observed that the IC_{50} values of all tested compounds were found to be in the range of 37.99-11.50 μM. and variations were observed when the substituent's (X) changed the positions. Compound **4d** (4-F) demonstrated higher activity and the potency was decreased as the substituent (fluoro group) was shifted to 2 (**4b**) and 3 (**4c**) position, while the compound **4h** (2-NO_2) was found to be the best molecule (IC_{50} = 11.50 μM) among all analogues and the activity was reduced as the substituent was moved to 3 (**4i**) and 4 (**4j**) position. The compounds having -OCH_3 substituent **4 (e-g)** demonstrated almost same activity. It can be revealed from the above results that, the substituent (X) at 4 position exhibited superior activity than at 2 and 3 position. All the molecules demonstrated potency less than 50 μM and were better than cisplatin but not comparable to doxorubicin. Further optimization of Compound **4h** may give rise to potent candidates to develop a new anticancer drug.

Table I — Anticancer activity of qunoline analogues **4a-j**

Compd	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

[a] Results are mean of triplicate analysis

IC_{50} values of compounds against Cancer cell line MDA-MB-231 (breast adenocarcinoma)

Antibacterial activity

All the compounds were evaluated against Gram-positive bacteria (*Staphylococcus aureus 6538p and Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*). Streptomycin was used as a standard drug and zones of inhibition (mm) were noted. The results are shown in Table II.

From antibacterial activity data, it was confirmed that all the compounds showed less potency compare to standard streptomycin. Among all the synthesized analogues, the compounds **4d**, **4f**, **4h** and **4j** exhibited moderate antibacterial activity against all the tested organisms. The compounds **4b**, **4e, 4g** and

4i are active against only Gram-positive bacteria (*Staphylococcus aureus 6538p and Bacillus subtilis*). The compound **4a** and **4c** did not exhibit any antibacterial activity.

Molecular docking studies

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

Three targets were selected from PharmMapper displaying highest fitting score with the hit molecule **4h** (Table III). 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 4**h** 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 III).

Compound **4h** showed interaction with the active site amino acid of Human Carbonic Anhydrase I (PDB ID: 1CZM), Protein Kinase A (PDB ID: 2F7X) and Kinesin Spindle Protein (KSP) (PDB ID: 2UYI) (Figure 1). Compound **4h** displayed π–π stacking with Hie64 and His94 in Human Carbonic Anhydrase I and additional interactions with Phe91 (π–π stacking) and hydrogen bonding interaction between Gln92 and amide carbonyl group were observed.

In Protein Kinase A receptor, compound **4h** Showed π–π stacking with Phe 54 and hydrogen bonding interaction with Lys 72. Also in Kinesin Spindle Protein (KSP) receptors, the compound **4h** showed hydrogen bonding interaction between Glu 116 and NH of amide.

Table II — Antibacterial activity of qunoline analogues **4a-j**

Compd	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 III — Result of docking analysis of the hit compound **4h**

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

Experimental Section

Organic Chemistry

Materials and Methods

All commercial chemicals and solvents are of LR-grade and AR-grade and were used without further purification. The thin layer chromatography 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 and ^{13}C NMR spectra were recorded with Bruker 300 MHz and Bruker 100 MHz ADVANCE instrument respectively and *J* values are in Hertz and chemical shifts (δ) are reported in ppm relative to internal tetramethylsilane. FTIR spectra (ν in 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 column Kromasil C18, solvent acetonitrile & buffer (0.01 M ammonium acetate + 0.5% triethylamine, pH 5.0 with acetic acid).

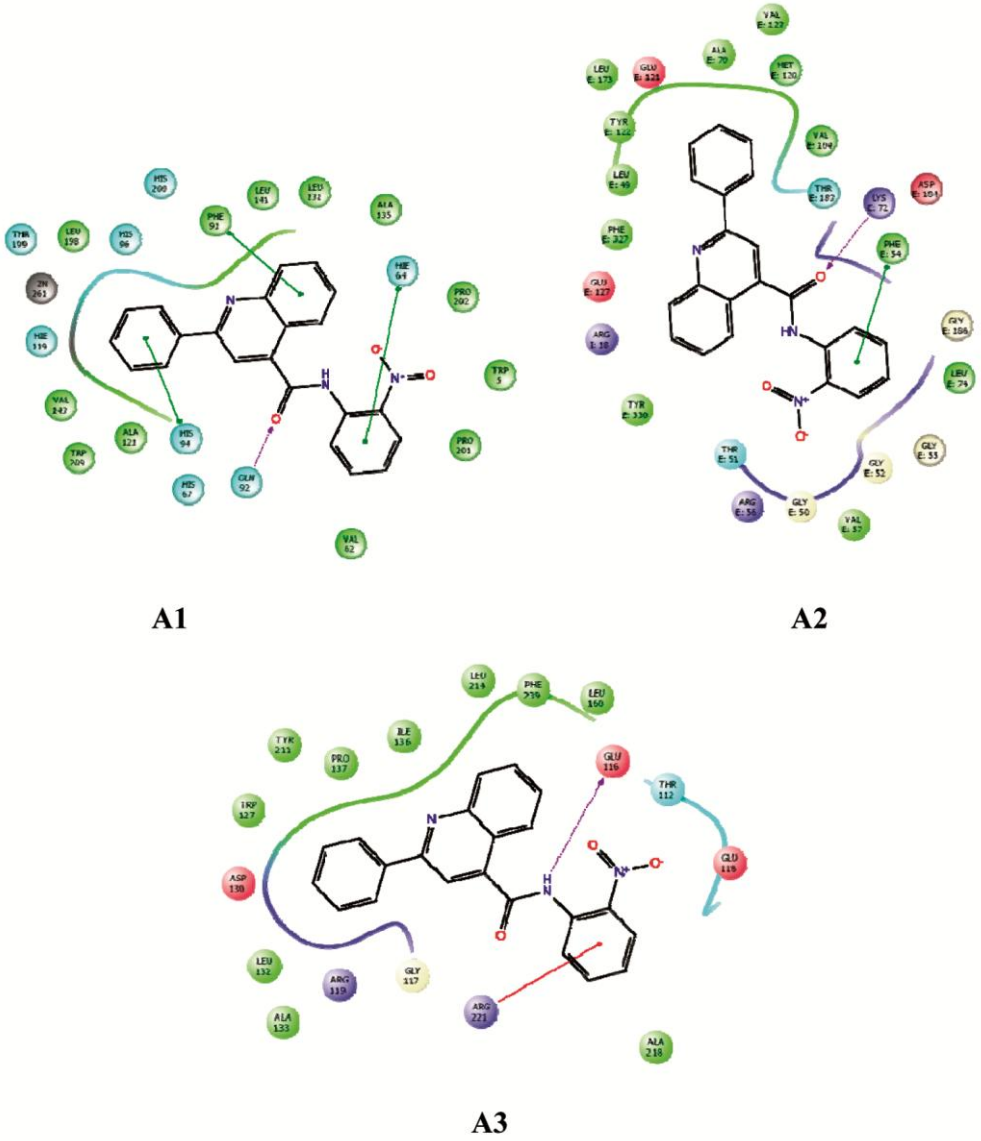


Figure 1 — 2D interaction diagram of molecular docking of hit **4h** in the binding sites of macromolecular targets – (**A1**) **4h** docked in the binding site of Human Carbonic Anhydrase I (PDB ID 1CZM). (**A2**) **4h** docked in the binding site of Protein Kinase A (PDB ID 2F7X). (**A3**) **4h** docked 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.

General Procedure and Analytical Data

Procedure for the synthesis of 2-phenylquinoline-4-carboxylic acid, 3

A mixture of isatin **1** (147 mg, 1 mmol) and 250 mg potassium hydroxide in 5 ml ethanol-water was stirred at room temperature for 15-30 min. The mixture was then acidified to pH 2-3 with 0.38 ml concentrated hydrochloric acid, and acetophenone **2** (224 mg, 1 mmol) was added. The resulting mixture was stirred at 80°C for 12-13 h and a precipitate appeared. The reaction progress was monitored by TLC. After the starting material had vanished, the precipitate was filtered, washed with water and recrystallized to obtain the pure compound **3**.

White solid; Yield 77%; mp 210–212 °C; ^{1}H NMR (DMSO-d_6, 300MHz, δ ppm): 14.35 (brs, 1H), 8.87 (d, *J* = 7.8 Hz, 1H), 8.22 (s, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 7.85-7.89 (m, 2H), 7.78-7.81 (m, 1H), 7.65-7.69 (m, 1H), 7.52-7.57 (m, 2H), 7.44-7.47 (m, 1H); ^{13}C NMR (DMSO-d_6, 300MHz, 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) νmax/cm^{-1}: 3263, 1716, 1659, 1525, 1398, 1215, 818, 751, 692; MS (APCI): *m/z* 250.20 $[M+H]^+$; HPLC: 98.93%.

General procedure for the synthesis of compounds 4a-j

To a solution of 2-phenylquinoline-4-carboxylic acid **3** (2.0 mmol, 1eq.) in DMF, $POCl_3$ (20 mmol, 10 eq.) was added at 0°C. The reaction mixture was stirred at 100°C for 4 h. Completion of the reaction was monitored by TLC. The reaction mass was diluted with ice cold water, a pale yellow solid precipitates out was filtered and dried at suction pump.

To a solution of 2-phenylquinoline-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.) was added at 0^{o}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 **4a-4j.**

Synthesis of N,N-2-diphenylquinoline-4-carboxamide, 4a

Light yellow solid; Yield 72%; mp 194-196 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 10.82 (s, 1H), 8.37 (t, 2H), 8.18 (d, *J* = 8.4 Hz, 2H), 7.82-7.88 (m, 4H), 7.68 (t, 1H), 7.54-7.62 (m, 3H), 7.42 (t, 2H), 7.18 (t, 1H); ^{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) νmax/cm^{-1}: 3243, 1677, 1598, 1547, 1355, 1257, 879, 756, 696; MS (APCI): *m/z* 325.40 $[M+H]^+$; HPLC: 100%.

Synthesis of N-(2-fluorophenyl)-2-phenylquinoline-4-carboxamide, 4b

Yellow solid; Yield 71%; mp 164-166 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 10.69 (s, 1H), 8.37 (d, *J* = 7.2 Hz, 3H), 8.18-8.24 (m, 2H), 7.85-7.94 (m, 2H), 7.70 (t, 1H), 7.53-7.62 (m, 3H), 7.29-7.39 (m, 3H); ^{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) νmax/cm^{-1}: 3263, 1676, 1595, 1542, 1355, 1199, 757, 695; MS (APCI): *m/z* 341.10 $[M-H]^-$; HPLC: 99.48%.

Synthesis of N-(3-fluorophenyl)-2-phenylquinoline-4-carboxamide, 4c

White solid; Yield 70%; mp 216-218 °C; ^{1}H NMR (DMSO-d_6, 300MHz, δ ppm): 10.71 (s, 1H), 8.37 (s, 1H), 8.18-8.26 (m, 4H), 7.84-7.93 (m, 2H), 7.70 (t, 2H), 7.58 (t, 3H), 7.33-7.37 (m, 2H); ^{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) νmax/cm^{-1}: 3184, 1684, 1613, 1549, 1355, 1244, 1128, 867, 757, 699; MS (APCI): *m/z* 343.20 $[M+H]^+$; HPLC: 98.71%.

Synthesis of N-(4-fluorophenyl)-2-phenylquinoline-4-carboxamide, 4d

White solid; Yield 78%; mp 222–224 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 10.88 (s, 1H), 8.38 (d, *J* = 7.6 Hz, 3H), 8.18 (d, *J* = 8.4 Hz, 2H), 7.83-7.88 (m, 3H), 7.68 (t, 1H), 7.55-7.61 (m, 3H), 7.27 (t, 2H); ^{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) νmax/cm^{-1}: 3242, 1679, 1616, 1553, 1356, 1212, 1152, 837, 755, 697; MS (APCI): *m/z* 343.20 $[M+H]^+$; HPLC: 98.41%.

Synthesis of N-(2-methoxyphenyl)-2-phenylquinoline-4-carboxamide, 4e

Brown solid; Yield 78%; mp 160-162 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 10.43 (s, 1H), 8.45 (d, *J* = 9.2 Hz, 1H), 8.29 (d, *J* = 7.2 Hz, 2H), 8.17 (d, *J* = 8.8 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 2H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.81 (t, 1H), 7.67 (d, *J* = 7.2 Hz, 1H), 7.52-7.60 (m, 3H), 7.12 (d, *J* = 8.4 Hz, 2H), 3.88 (s, 3H); ^{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) νmax/cm^{-1}: 3299, 1673, 1596, 1529, 1354, 1258, 1117, 1031, 810, 754, 698; MS (APCI): *m/z* 353.20 $[M-H]^-$; HPLC: 97.61%.

Synthesis of N-(3-methoxyphenyl)-2-phenylquinoline-4-carboxamide, 4f

Brown solid; Yield 75%; mp 152-154 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 10.79 (s, 1H), 8.35 (d, *J* = 8.4 Hz, 3H), 8.15-8.18 (m, 2H), 7.83-7.87 (m, 1H), 7.65-7.69 (m, 1H), 7.51-7.60 (m, 4H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.31 (t, 1H), 6.74-6.77 (m, 1H), 3.78 (s, 3H); ^{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) νmax/cm^{-1}: 3056, 1675, 1610, 1544, 1354, 1250, 1158, 861, 753, 690; MS (APCI): *m/z* 353.10 $[M-H]^-$; HPLC: 96.14%.

Synthesis of N-(4-methoxyphenyl)-2-phenylquinoline-4-carboxamide, 4g

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

Synthesis of N-(2-nitrophenyl)-2-phenylquinoline-4-carboxamide, 4h

Yellow solid; Yield 72%; mp 180-182 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 11.29 (s, 1H), 8.27 (m, 3H), 8.26 (m, 2H), 8.16 (m, 1H), 7.70 (m, 2H), 7.57 (m, 3H), 7.53 (m, 3H); ^{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) νmax/cm^{-1}: 3317, 1691, 1590, 1548, 1347, 1279, 1151, 774, 749, 690; MS (APCI): *m/z* 370.32 $[M+H]^+$; HPLC: 99.66%.

Synthesis of N-(3-nitrophenyl)-2-phenylquinoline-4-carboxamide, 4i

Yellow solid; Yield 74%; mp 264-266 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 11.30 (s, 1H), 8.88 (d, *J* = 1.6 Hz, 1H), 8.45 (s, 1H), 8.39 (d, *J* = 6.8 Hz, 2H), 8.15-8.23 (m, 3H), 8.04-8.07 (m, 1H), 7.86-7.90 (m, 1H), 7.68-7.75 (m, 2H),7.55-7.62 (m, 3H); ^{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) νmax/cm^{-1}: 3275, 1691, 1631, 1543, 1526, 1354, 1109, 848, 752, 676; MS (APCI): *m/z* 370.10 $[M+H]^+$; HPLC: 99.30%.

Synthesis of N-(4-nitrophenyl)-2-phenylquinoline-4-carboxamide, 4j

Yellow solid; Yield 68%; mp 264-266 °C; ^{1}H NMR (DMSO-d_6, 400MHz, δ ppm): 11.40 (s, 1H), 8.31 (m, 2H), 8.28 (m, 3H), 8.02 (d, *J* = 8.0 Hz, 3H), 7.85 (m, 1H), 7.55 (m, 1H), 7.52 (m, 4H); ^{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) νmax/cm^{-1}: 3198, 1689, 1595, 1555, 1343, 1259, 1189, 860, 758, 695; MS (APCI): *m/z* 370.40 $[M+H]^+$; HPLC: 98.08%.

Biology

Anti-cancer activity

Material

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).

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°C, 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)

Antibacterial activity

Material

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.

Antibacterial assay

The Antibacterial activity of all quinoline derivatives was checked by agar well diffusion method. The synthesized 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 hr 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 diffused 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. Solvent and growth controls were kept and streptomycin was used as a standard drug and zones of inhibition (mm) were noted.

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 [49] and the products included therein were used for performing various molecular modelling operations described above.

Conclusions

In summary, new class of quinoline analogues were prepared and also revealed their SAR information. Based on the analysis done, we can conclude that all the compounds showed significant anticancer activity against a breast cancer cell line (breast adenocarcinoma, MDA-MB-231) and the trends were observed with variations in the substituents (X). The substituent (X) is favoured 4 position more than 2 and 3 position to exhibit superior potency. Compound **4h** exhibited better promising anticancer activity among various synthesized molecules. Molecular modelling results provided additional insight into the interaction of compound **4h** with the active site amino acid of Human Carbonic Anhydrase I, Protein Kinase A and Kinesin Spindle Protein (KSP). These current results will help us to further optimize and develop new drug candidates in future.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

Acknowledgements

Authors are thankful to Dr. Sandip Gavade and Dr. Anand Burange for their help in recordings spectral data. Authors are also thankful to Dr. Hina Shaikh and Dr. Ashish Keche for their valuable support that has helped in this work.

References

1 Bray F, Ferlay J, Soerjomataram I, Siegel R L, Torre L A & Jemal A, *CA: Cancer J Clin*, 68 (2018) 394.
2 Inca S Z, Selma S, Semra C & Kevser E, *Bioorg Med Chem*, 14 (2006) 8582.
3 Ekoue-Kovi K, Yearick K, Iwaniuk D P, Natarajan J K, Alumasa J, Dios A C, Roepe P D & Wolf C, *Bioorg Med Chem*, 17 (2009) 270.
4 Chibale K, Moss J R, Blackie M, Schalkwyk D & Smith P J, *Tetrahedron Lett*, 41 (2000) 6231.
5 Desai P K, Desai P, Machhi D, Desai C M & Patel D, *Indian J Chem*, 35B (1996) 871.
6 Roma G, Di Braccio M, Grossi G, Mattioli F & Ghia M, *Eur J Med Chem*, 35 (2000) 1021.
7 Vargas L Y, Castelli M V, Kouznetsov V V, Urbina J M, Lopez S N, Sortino M, Enriz R D, Ribas J C & Zacchino S, *Bioorg Med Chem*, 11 (2003) 1531.
8 Broch S, Aboab B, Anizon F & Moreau P, *Eur J Med Chem*, 45 (2010) 1657.
9 Kidwai M, Bhushan K R, Sapra P, Saxena R K & Gupta R, *Bioorg Med Chem*, 8 (2000) 69.
10 Adana A K, Mirza Y, Aneja K R & Prakash O, *Eur J Med Chem*, 38 (2003) 533.
11 Yu X Y, Hill J M, Yu G, Yang Y, Kluge A F, Keith D, Finn J, Gallant P, Silverman J & Lim A, *Bioorg Med Chem Lett*, 11 (2001) 541.
12 Ghorab M M & Alsaid M S, *Acta Pharm*, 65 (2015) 271.
13 Yang Y, Shi L, Zhou Y, Li H Q, Zhu Z W & Zhu H L, *Bioorg Med Chem Lett*, 20 (2010) 6653.

14 Narwal M, Venkannagari H & Lehtio L, *J Med Chem*, 55 (2012) 1360.
15 Matsuno K, Masuda Y, Uehara Y, Sato H, Muroya A, Takahashi O, Yokotagawa T, Furuya T, Okawara T, Otsuka M, Ogo N, Ashizawa T, Oshita C, Tai S, Ishii H, Akiyama Y & Asai A, *ACS Med Chem Lett*, 1 (2010) 371.
16 Wang X, Xie X, Cai Y, Yang X, Li J, Li Y, Chen W & He M, *Molecules*, 21 (2016) 340.
17 Benzerka S, Bouraiou A, Bouacida S, Roisnel T, Bentchouala C, Smati F & Belfaitah A, *Lett Org Chem*, 10 (2013) 94.
18 Labuschagne A, Lall N & Mphahlele M J, *Int Arabic J Antimicrob Agents*, 3 (2013) 2.
19 Benzerka S, Bouraiou A, Bouacida S, Roisnel T, Bentchouala C, Smati F & Belfaitah A, *Lett Org Chem*, 9 (2012) 309.
20 Aboutorabzadeh S M, Mosaffa F, Hadizadeh F & Ghodsi R, *Iran J Basic Med Sci*, 21 (2018) 1.
21 Ghodsi R, Azizi E, Ferlin M G, Pezzi V & Zarghi A, *Lett Drug Des Discovery*, 13 (2016) 89.
22 Zimichev A V, Zemtsova M N, Kashaev A G & Klimochkin Y N, *Pharm Chem J*, 45 (2011) 217.
23 Zhu L, Luo K, Li K, Jin Y & Lin J, *Bioorg Med Chem*, 25 (2017) 5939.
24 Erugu Y, Sangepu B, Gandu B, Anupoju G & Jetti V R, *World J Pharm Pharm Sci*, 3 (2014) 1612.