

Research Article

Design, Synthesis and Biological Screening of 2, 4-
Disubstituted QuinolinesIlango K^{1*}, Valentina P¹, Subhakar K¹ and
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603203, Tamil Nadu, India**Received:** June 13, 2015; **Accepted:** September 02,
2015; **Published:** September 04, 2015**Abstract**

A series of 2,4 disubstituted quinoline derivatives were synthesized through multi-step reactions. Aniline was reacted with benzaldehyde and pyruvic acid to form 2-Phenylquinolin-4-carboxylic acid 1 and converted to 2-Phenylquinolin-4-carbonyl chloride 2, which on subsequent reaction with substituted amines gave 2-Phenylquinoline-4-substituted Phenylcarboxamide 3. The newly synthesized title compounds were evaluated for their antibacterial and antifungal activities against *B. subtilis*, *K. pneumonia*, *E. coli*, *S. aureus*, *A. niger* as well as anticancer activity. Preliminary results indicated that most of the 2,4 Diphenyl quinoline derivatives demonstrated good antibacterial and antifungal activities. Among the newly synthesized compounds, N-2-Diphenyl quinolin-4-carboxamide 3a and N-p-Tolylquinolin-4-carboxamide 3f were found to possess maximum anticancer activity. It was concluded that two bulky aryl groups at 2 and 4 positions enhances the activity of synthesized quinoline derivatives. The compounds N-p-Tolylquinolin-4-carboxamide 3f and 2-Phenyl-N-(pyridin-2-yl) quinolin-4-carboxamide 3j were found to possess maximum activity against the tested strains of *B. subtilis*, *K. pneumonia*, *E. coli*, *S. aureus* and *A. niger*. It can be concluded that two bulky aryl groups at 2 and 4 positions enhances the activity of quinoline derivatives.

Keywords: 2, 4-disubstituted quinolines; Anticancer; Antibacterial; Antifungal**Introduction**

Heterocyclic compounds play an important role in designing new classes of structural entities of medicinal importance with potentially new mechanisms of action. These heterocyclic compounds are well known to possess diverse pharmacological properties, viz. antimicrobial, anticancer, anticonvulsant, antimalarial activities etc. Quinoline nucleus occurs in several natural compounds and pharmacologically active substances displaying a broad range of biological activity. Quinolines exhibits wide range of activity such as antiprion [1], antimicrobial [2-5], antibacterial [6], antitubercular [7-9] and anticancer activities [9-12]. Quinoline ring plays an important role in new anti-cancer agents development as their derivatives have shown excellent results through different mechanism of action such as growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation of nuclear receptor responsiveness [13-15]. A review on anticancer potential of bioactive heterocyclic quinoline was published recently revealing the significance of quinoline [16]. Figure 1 illustrates promising structures of quinoline as biologically active nucleus.

Our interest was mainly focused on 2, 4-disubstituted quinolines due to its wide range of biological activities. Recently we found that C-2 quinoline derivatives possessing good antibacterial and antifungal activities. We identified structural similarities between our series of compounds and reported structures as antimicrobials [15]. So it was thought logically to screen this potential moiety for antibacterials as well as antifungal. In continuation to our ongoing work on quinoline derivatives, we here in report 2, 4-disubstituted quinolines as antimicrobials hitherto unreported.

Materials and Methods**General**

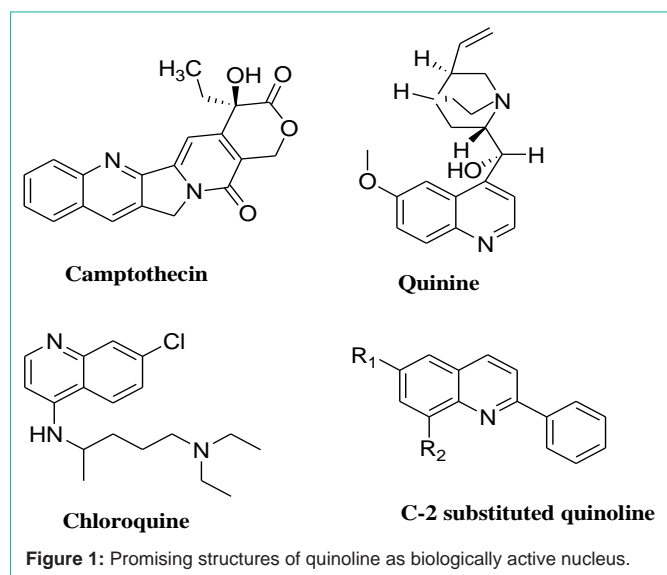
Melting points of newly synthesized compounds were determined using VEEGO VMP-D. IR spectra were recorded in (KBr) on a Perkin Elmer FTIR, ¹H-NMR on Bruker Avance, 400 MHz and Mass spectra on LC-MSD-Tranp-SL 2010A SHIMADZU. Purity of synthesized compounds were checked by precoated TLC aluminium sheets-silica gel 60 F254 (0.2mm).

General procedure for the synthesis of 2-Phenylquinolin-4-substituted phenyl carboxamide (3a-3j)

Aniline (0.01mol), benzaldehyde (0.01mol) and pyruvic acid (0.01 mol) was refluxed in ethanol (20 ml) for 3 hours. The product formed was filtered and recrystallized from ethanol. 1 (0.01mol) was treated with phosphorus pentachloride (0.01 mol) and refluxed. The intermediate 2 (0.01 mol) was reacted with various amines (0.01 mol) at 10-15°C in presence of ethanol. The product was isolated by filtration and recrystallized from ethanol to yield 3a-3j (Table 1).

Representative data: *N*-2-Diphenylquinolin-4-carboxamide (3a): pale white crystals; Yield 78%; mp 164-165°C; ¹H-NMR δ (ppm): 7-8.0 (15H, m, Ar-H), 8.89 (1H, s, NH); FT-IR: 3311 (NH of CONH), 3060 (Ar-H), 1675 (C=O), 1599 (C=N), 1453 (C=C), 1445 (C-N) cm⁻¹; C₂₂H₁₆N₂O (324.2); MS: m/z: 324 (M⁺), 163 (base peak), 130, 122, 114.

N-Benzyl-2-phenylquinolin-4-carboxamide (3b): white crystals; Yield 76%; mp 166-168°C; ¹H-NMR δ (ppm): 4.1- 4.3 (2H, s, CH₂), 7-7.9 (15H, m, Ar-H), 8.89 (1H, s, NH); FT-IR: 3311 (NH of



CONH), 3060 (Ar-H), 2868 (C-H of CH₂), 1675 (C=O), 1599 (C=N), 1453 (C=C), 1445 (C-N) cm⁻¹; C₂₃H₁₈N₂O (338.3); MS: m/z: 338 (M⁺), 174 (base peak), 142, 138, 130.

N-(4-Nitrophenyl)-2-phenylquinolin-4-carboxamide (3c): Pale yellow crystals; Yield 81%; mp 172-174°C; ¹H-NMR δ (ppm): 7-7.9 (14H, m, Ar-H), 8.8 (1H, s, NH); FT-IR: 3311 (NH of CONH), 3060 (Ar-H), 1675 (C=O), 1599 (C=N), 1544 (Ar-NO₂), 1453 (C=C), 1405 (C-N) cm⁻¹; C₂₂H₁₅N₃O₃ (369.4); MS: m/z: 369 (M⁺), 184 (base peak), 142, 130, 126.

N-(3-Nitrophenyl)-2-phenylquinolin-4-carboxamide (3d): Deep brown crystals; Yield 82%; mp 176-178°C; ¹H-NMR δ (ppm): 7-7.9 (14H, m, Ar-H), 8.8 (1H, s, NH); FT-IR: 3311 (NH of CONH), 3060 (Ar-H), 1675 (C=O), 1599 (C=N), 1544 (Ar-NO₂), 1453 (C=C), 1405 (C-N) cm⁻¹; C₂₂H₁₅N₃O₃ (369.4); MS: m/z: 369 (M⁺), 184 (base peak), 142, 130, 126.

N-(2-Nitrophenyl)-2-phenylquinolin-4-carboxamide (3e): Yellow crystals; Yield 74%; mp 178-180°C; ¹H-NMR δ (ppm): 7-7.9 (14H, m, Ar-H), 8.8 (1H, s, NH); FT-IR: 3311 (NH of CONH), 3060 (Ar-H), 1675 (C=O), 1599 (C=N), 1544 (Ar-NO₂), 1453 (C=C), 1405 (C-N) cm⁻¹; C₂₂H₁₅N₃O₃ (369.4); MS: m/z: 369 (M⁺), 184 (base peak), 142, 130, 126.

2-Phenyl-N-p-tolylquinolin-4-carboxamide (3f): Yellow brown crystals; Yield 76%; mp 178-180°C; ¹H-NMR δ (ppm): 2.5-2.7 (3H, s, CH₃), 7-7.9 (14H, m, Ar-H), 8.8 (1H, s, NH); FT-IR: 3311 (NH of CONH), 3060 (Ar-H), 2916 (C-H of CH₃), 1673 (C=O), 1599 (C=N), 1455 (C=C), 1406 (C-N) cm⁻¹; C₂₃H₁₈N₂O (338.2); MS: m/z: 338 (M⁺), 174 (base peak), 138, 120, 116.

Biological experiments

The newly synthesized compounds 3a-j were screened for antibacterial activity against freshly cultured strains of *E. coli* (EC), *S. aureus* (SA), *K. pneumonia* (KP) and *B. subtilis* (BS) using sterile nutrient agar media and antifungal activity against of *A. niger* (AN) using Sabeurad's agar medium. Disk diffusion method was used at a concentration of 3mg per mL using DMF as solvent. The results were recorded in duplicate using ciprofloxacin and ketoconazole at

Table 1: Anticancer activity-sensitivity testing 3a-3j.

Sample code	Concentration	Live cells	Dead cells	Total cells	% Viability	% Mortality
3a	100 µg/mL	5	19	24	20.83	79.16
3b	100 µg/mL	9	15	24	39.73	61.32
3c	100 µg/mL	11	10	21	52.38	47.61
3d	100 µg/mL	10	11	21	47.61	52.38
3e	100 µg/mL	7	17	24	30.21	69.79
3f	100 µg/mL	5	16	21	23.80	76.19
3g	100 µg/mL	--	--	--	--	--
3h	100 µg/mL	6	14	20	30	70
3i	100 µg/mL	5	15	20	29.9	71.0
3j	100 µg/mL	5	11	16	31.25	68.75
Ara-c	10 µg/mL	4	21	25	7.83	92.46

a concentration of 1 mg per mL as standards. The target compounds 3a-3j were screened for anticancer activity by Trypan blue dye exclusion technique.

Results and Discussion

Chemistry

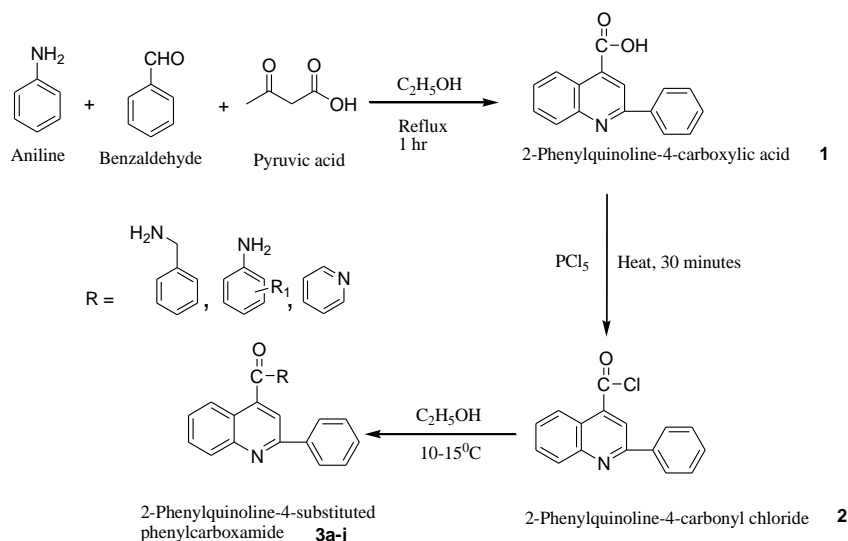
2-Phenylquinolin-4-carboxylic acid 1 was prepared by the reaction of aniline, benzaldehyde and pyruvic acid in ethanol under reflux condition. The aniline underwent nucleophilic addition across the carbonyl carbon of benzaldehyde and resulted in the formation of imine, which on reaction with pyruvic acid formed 1. The acid function of 1 was converted into acid chloride by reacting it with phosphorus pentachloride forming 2-Phenylquinolin-4-carbonyl chloride 2. The active acid chloride was treated with variety of aromatic amines to yield the target compounds 2-Phenylquinolin-4-aryl substituted carboxamide 3a-3j (scheme-1). The aromatic amines were bearing EDG, EWG, Benzyl, Pyridyl, as well as halogen atoms such as Cl and Br.

Biological activity

The newly synthesized compounds 3a-3j were screened for anticancer activity by Trypan blue dye exclusion technique. The viable tumor cell count (trypan blue test) was carried out with Neubauer Hemocytometer. Equal volume of cells (20µL) and equal volume (20 µL) of trypan blue solution and the cells are carried in modified Neubauer's chamber. Dead cells are stained with blue while live cells are unstained, followed by determination of percentage viability and percentage mortality. The results were recorded in duplicate using Ara-c at a concentration of 10 µg per ml as standards. Among newly tested (Cytarabine) compounds, nine compounds showed % mortality above 50. However three compounds 3a, 3f and 3i were found to have 70 % mortality when compared with Ara-c against the tumour cells. All the newly tested compounds showed antibacterial and antifungal activities. Among newly synthesized derivatives, compounds 3f and 3j were found to possess good antibacterial and antifungal activities when tested against *E. coli*, *S. aureus*, *B. subtilis*, *K. pneumonia* and *A. niger* (Table 2).

Conclusions

From the result of anticancer testing the compounds 3a-3j, it can be concluded that the incorporation of two bulky aryl groups at 2



Scheme 1: Synthesis of 2-phenylquinolin-4-arylsubstituted carboxamide derivatives (3a-3j).

Table 2: Antimicrobial activity-sensitivity testing of 3a-3j.

Compound code	Zone of inhibition in mm				
	Antibacterial activity				Antifungal activity
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i> ,	<i>K. pneumonia</i>	<i>A. niger</i>
3a	16	16	16	16	18
3b	17	17	15	17	16
3c	18	18	16	15	15
3d	16	16	17	16	18
3e	17	18	16	15	16
3f	18	16	14	15	15
3g	16	17	15	16	14
3h	16	17	15	16	14
3i	15	17	17	18	16
3j	18	17	18	19	18
Ciprofloxacin	22	24	22	22	--
Ketoconazole	--	--	--	--	24

and 4 positions plays an important role in activity. The incorporation of amido group in quinoline derivatives enhanced their anticancer and antimicrobial activities. The presence of electron donating and electron withdrawing groups on the two aryl rings did not make any significant difference in their activity. However three compounds showed good potency 3a, 3f and 3j. Further studies to acquire more information about structure activity relationship are in progress in our laboratory.

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