Asian Journal of Chemistry; Vol. 32, No. 5 (2020), 1151-1157

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22583

Synthesis, Characterization, Biological Screening, ADME and Molecular Docking Studies of 2-Phenyl Quinoline-4-Carboxamide Derivatives

P. RAGHURAMA SHETTY[1], G. SHIVARAJA[1], G. KRISHNASWAMY[1], K. PRUTHVIRAJ[1], VIVEK CHANDRA MOHAN[2] and S. SREENIVASA[1,*]

[1]Department of Studies and Research in Organic Chemistry, Tumkur University, Tumakuru-572103, India
[2]Department of Biotechnology, Siddaganga Institute of Technology, Tumakuru-572103, India

*Corresponding author: E-mail: drsreenivasa@yahoo.co.in

Received: 10 December 2019; *Accepted*: 23 January 2020; *Published online*: 29 April 2020; AJC-19849

In this work, some 2-phenyl quinoline-4-carboxamide derivatives **(5a-j)** were synthesized *via* base catalyzed Pfitzinger reaction of isatin and acetophenone followed by C-N coupling reaction using $POCl_3$ and assessed them for their *in vitro* antimicrobial and anticancer activity. The structure of newly synthesized compound were established by FT-IR, ^1H & ^{13}C NMR and Mass spectrometric analysis. The synthesized carboxamides were subjected to preliminary *in vitro* antibacterial activity as well as for antifungal activity. Results of antibacterial activity were compared with standard antibacterial (ciprofloxocin) and antifungal (fluconozole). Among the tested compounds, **5d, 5f** and **5h** exhibited promising activity with zone of inhibition ranging from 10 to 25 mm. Further, the anticancer activity determined using MTT assay against two cancer cell lines. Compounds **5b**, **5d**, **5f** and **5h** showed good anticancer activity among all the other derivatives. In order to correlate the *in vitro* results, *in silico* ADME and Molecular docking studies were carried out for **(5a-j)**. ADME properties results showed that all the compounds obey rule of Five rule except **5a, 5e** and **5g** compound. Molecular docking studies of the synthesized compounds showed good binding affinity through hydrogen bond interactions with key residues on active sites as well as neighboring residues within the active site of chosen target proteins *viz*. antibacterial, antifungal and anticancer. Comparison of both results of *in silico* as well as *in vitro* investigation suggests that the synthesized compounds may act as potential antimicrobial as well as anticancer agents.

Keywords: Pfitzinger reaction, 2-Phenyl-quinoline-4-carboxamide, Bioactivity, *in silico* ADME, Molecular docking studies.

INTRODUCTION

Among the important class of nitrogen-containing heterocycles, quinoline is one of the ubiquitous and privileged structural motifs that occur in bioactive natural products and pharmaceutically active therapeutic agents. The recent estimation reveals that the average nitrogen atoms in a drug molecule rose to 3.0 from 0.7 atoms. Quinoline skeleton are associated with a range of biological and pharmaceutical activities such as antibacterial [1,2], antifungal [3], anthelmintic [4], antileismanial [5], antiviral [6], anticancer [7], anti-inflammatory effects [8], tubulin polymerization inhibitors [9], antioxidant [10], antimalerial [11], HIV integrate inhibitors [12]. Several quinoline analogues have anticancer activity against HeLa (human cervix cancer cell line) and MDA-MB-435 (human breast cancer cell line) [13]. Furthermore, literature survey revealed that the presence of an aryl ring at the second position of quinolinec-4-carboxylic acid derivatives [14] exhibited good antibacterial activity and they are most suitable for further modifications to obtain more effective antimicrobial agents as shown in Fig. 1.

Additionally, several drug molecules containing quinoline motif are known to possess wide variety of pharmacological activities as shown in Fig. 2. All of these stimulated us to focus on further structural modification of quinoline-4-carboxylic acid derivatives in order to find novel molecules with potential antimicrobial as well as anticancer activity.

EXPERIMENTAL

All chemicals were purchased from Merck India, Spectrochem and Sigma-Aldrich. The solvent and the chemicals used were LR grade. The purity of the compound was confirmed using TLC silica gel plate and purification by column chromatography.

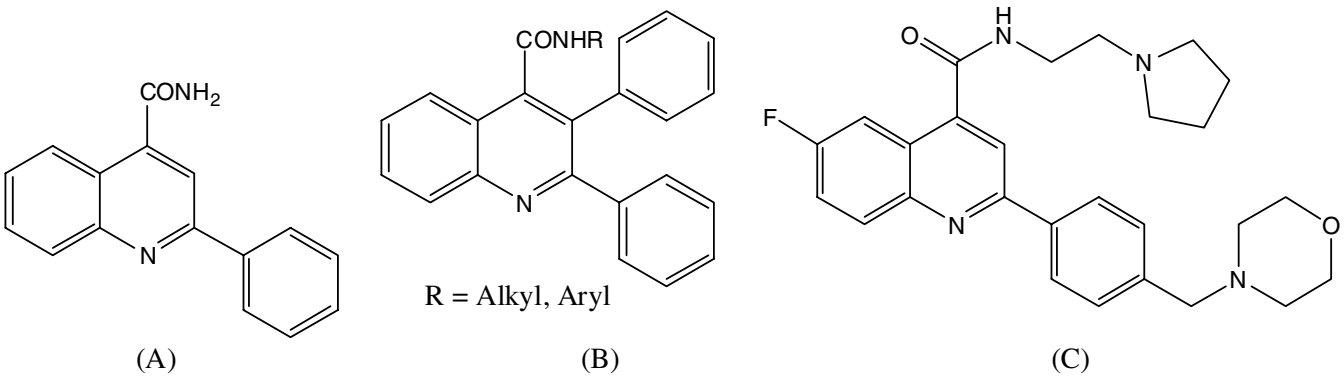
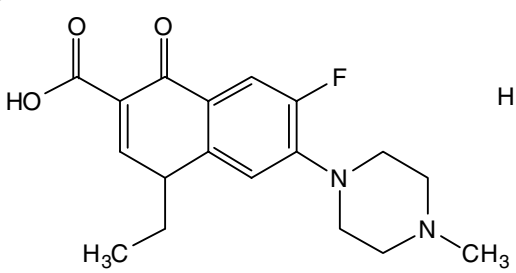
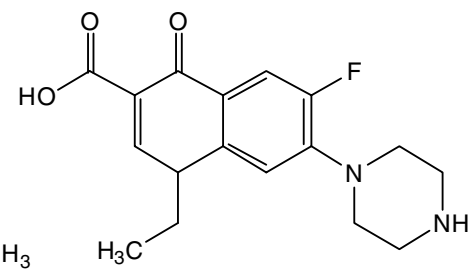


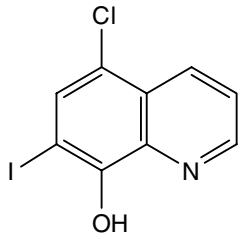
Fig. 1. Structure of 2-aryl-quinoline-4-carboxylic acid derivatives with antimicrobial activities



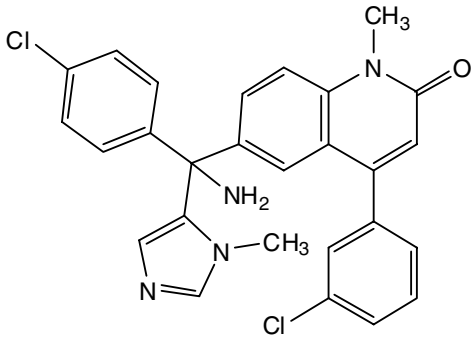
Pefloxacin
(antibacterial)



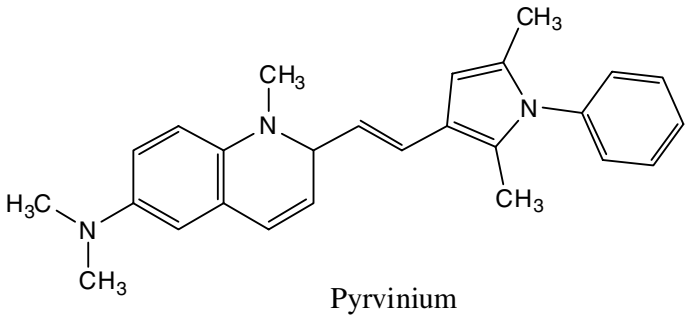
Ciprofloxacin
(antibacterial)



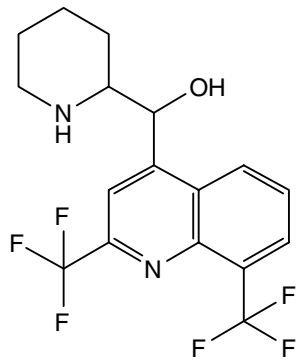
Clioquinol
(antifungal)



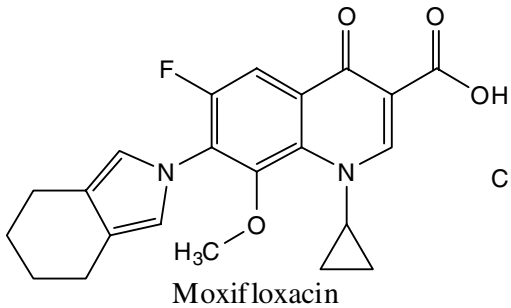
Tipifarnib
(anticancer)



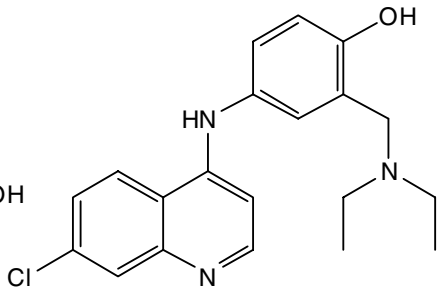
Pyrvinium
(anthelmintic)



Mefloquin
(antimalerial)



Moxifloxacin
(antibiotic)



Amodiaquin
(antimalerial)

Fig. 2. Pharmacological drugs containing quinoline moiety

Melting points were determined by open capillary method. FT-IR spectra was recorded on Jasco FT-IR Spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on 399.65 MHz and 100.40 MHz, respectively using $CDCl_3$ and DMSO-d_6 as solvent. Chemical shift values were reported in ppm. The LC-MS was recorded using Waters Alliance 2795 separation module and the Waters Micromass LCT mass detector.

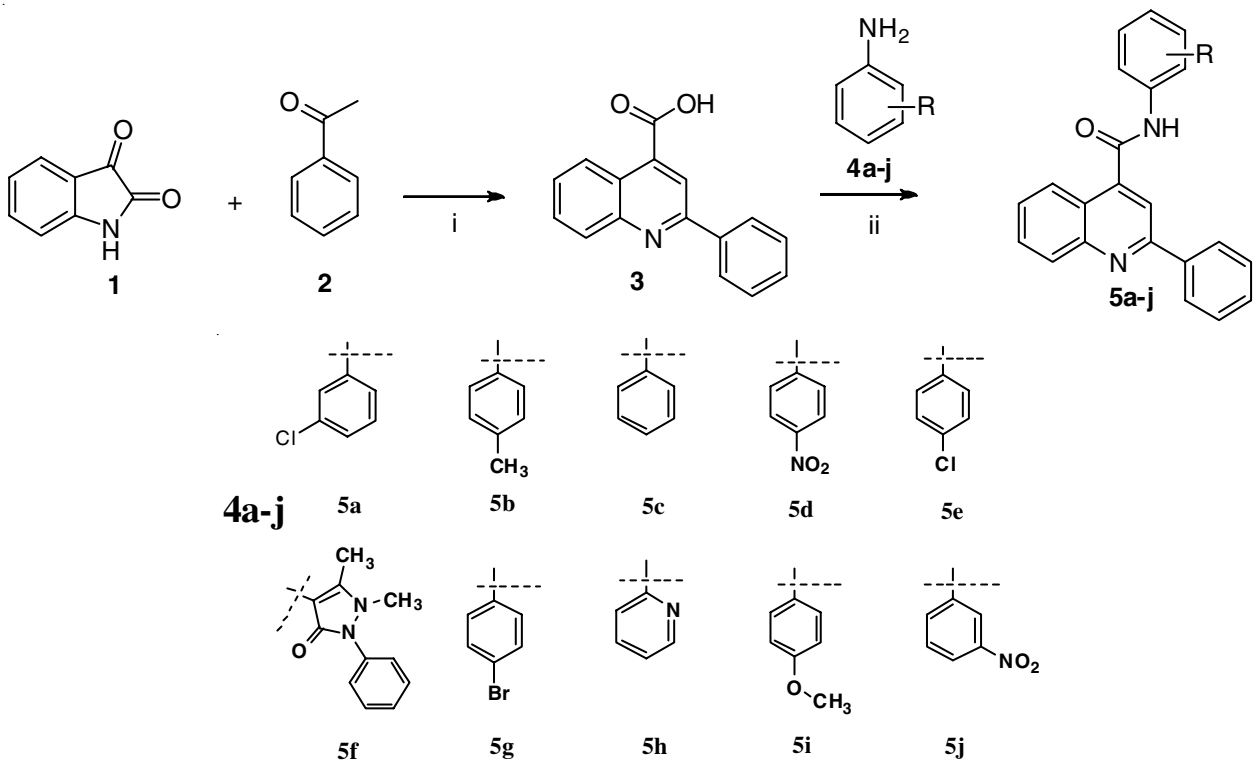
Step-1: Synthesis of compound 2-phenyl quinoline-4-carboxylic acid (3): Isatin (1.47 g, 10.0 mmol) in 33% alc. NaOH (15 mL), acetophenone (1.2 g, 10.0 mmol), ethanol (15 mL) was taken in a round bottom flask. The reaction mixture was stirred and refluxed for 12 h. The progress of the reaction was monitored by TLC (ethyl acetate:petroleum ether, 7:3 v/v). After the completion of the reaction, the reaction mixture was cooled and poured onto ice-water, then acidified with 10% HCl to achieve pH 2.0-3.0. The precipitate was filtered, washed with water and dried. The acid was recrystallized from ethanol [15].

Step-2: Synthesis of compound 2-phenyl quinoline-4-carboxamides (5a-j): 2-Phenyl quinoline-4-carboxylic acid (0.73 g, 3 mmol) was taken in round bottom flask, to this $POCl_3$ (5 mL) was added slowly. After 2 h of reflux, substituted aromatic primary amines (**4a-j**) (0.32 g, 3 mmol) were added. Reaction mixture was stirred and heated under reflux for 8 h. The reaction progress was monitored by TLC (silica gel, ethyl acetate/petroleum ether 7:3). Upon completion of the reaction crushed ice was added to the reaction mixture slowly; the precipitate obtained was filtered, washed with dil. HCl followed by water and sodium carbonate solution and finally dried (**Scheme-I**).

2-Phenyl-*N*-(3-chlorophenyl)-quinoline-4-carboxamide (5a): Yield: 76%, m.p.: 176-178 °C, m.f. (m.w.): $C_{22}H_{15}N_2OCl$ (358.82); IR (KBr, ν_{max}, cm^{-1}): 3426 (NH *str.*), 1675 (CO *str.*), 3116 (aromatic CH *str.*), 2901 (aromatic CH *str.*), 1586 (C=N *str.*), 1416 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.20 (s, 1H, Ar-H), 8.14-8.12 (d, *J* = 4.8 Hz, 1H, Ar-H), 8.08-8.06 (m, 2H, Ar-H), 7.91 (s, 1H, NH), 7.81 (s, 1H, Ar-H), 7.73-7.69 (t, 2H, Ar-H), 7.52-7.48 (t, 1H, Ar-H), 7.46-7.42 (t, 4H, Ar-H), 7.25 (s, 1H, Ar-H), 7.23-7.21 (d, *J* = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.82, 158.16, 152.03, 148.37, 145.4, 144.17, 139.2, 138.29, 135.6, 130.90, 130.76, 129.35, 129.19, 128.34, 127.6, 125.92, 123.75, 122.0, 119.7, 118.54.

2-Phenyl-*N*-(*p*-tolyl)-quinoline-4-carboxamide (5b): Yield: 65%, m.p.: 186-189 °C, m.f. (m.w.): $C_{23}H_{18}N_2O$ (338.40); IR (KBr, ν_{max}, cm^{-1}): 3305 (NH *str.*), 1649.50 (CO *str.*), 3021 (aro-matic CH *str.*), 2914 (aromatic CH *str.*), 1585 (C=N *str.*), 1447 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.20-8.18 (d, *J* = 4.8 Hz, 2H, Ar-H), 8.12-8.11 (d, *J* = 4.8 Hz, 2H, Ar-H), 7.97 (s, 1H, Ar-H), 7.74-7.72 (t, *J* = 4.9 Hz, 1H, Ar-H),7.65-7.63 (m, 4H, Ar-H), 7.56-7.52 (t, *J* = 5.1 Hz, 1H, Ar-H), 7.46-7.45 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.25-7.22 (d, *J* = 8.0 Hz, 2H, Ar-H), 2.38 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 165.46, 156.04, 148.30, 142.91, 138.15, 135.20, 134.80, 130.3, 129.8, 128.8, 127.38, 124.93, 122.97, 116.15, 21.00.

2-Diphenylquinoline-4-carboxamide (5c): Yield: 56%, m.p.: 183-185 °C, m.f. (m.w.): $C_{22}H_{16}N_2O$ (324.37); IR (KBr, ν_{max}, cm^{-1}): 3422.06 (NH *str.*), 1675.24 (CO *str.*), 3059 (aromatic CH *str.*), 2976.95 (aromatic CH *str.*), 1592 (C=N *str.*), 1491 (C=C *str.*) ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.16 (s, 2H, Ar-H), 8.08-8.06 (dd, *J* = 5.6 Hz, 3H, Ar-H), 7.95 (s, 2H, Ar-H), 7.75-7.71 (m, 2H, Ar-H), 7.57-7.53 (m, 2H, -NH & Ar-H), 7.42-7.34 (m, 5H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$,



Scheme-I: Synthetic route for the preparation of 2-phenyl quinoline-4-carboxamides (**5a-j**); Reagents and conditions: (i) EtOH, 33% NaOH, 80 °C, Reflux 12 h; (ii) $POCl_3$, 70 °C, Reflux 8 h

δ ppm): 165.60, 154.40, 148.16, 142.67, 137.96, 137.85, 130.34, 129.92, 129.26, 128.8, 127.39, 127.29, 125.10, 124.90, 122.86, 120.20, 116.10.

2-Phenyl-*N*-(4-nitrophenyl)-quinoline-4-carboxamide (5d): Yield: 84%, m.p.: 185-187 °C, m.f. (m.w.): $C_{22}H_{15}N_3O_3$ (369.37); IR (KBr, ν_{max}, cm^{-1}): 3438.8 (NH *str.*), 1688.1 (CO *str.*), 3027.6 (aromatic CH *str.*), 2933.03 (aromatic CH *str.*), 1586.6 (C=N *str.*), 1510 (C=C *str.*); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.02 (s, 1H, NH), 8.13-8.12 (d, *J* = 8 Hz, 1H, Ar-H), 8.08-8.02 (m, 3H, Ar-H), 7.84-7.75 (m, 5H, Ar-H), 7.61-7.57 (m, 1H, Ar-H), 7.45-7.43 (d, *J* = 7.00 Hz, 2H, Ar-H), 7.23-7.21 (t, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 165.60, 152.13, 147.97, 144.49, 143.26, 138.19, 131.01, 130.07, 129.26, 129.19, 129.10, 128.48, 128.23, 127.59, 125.55, 123.49, 122.01, 116.33.

2-Phenyl-*N*-(4-chlorophenyl)-quinoline-4-carboxamide (5e): Yield: 69%, m.p.: 172-174 °C, m.f. (m.w.): $C_{22}H_{15}N_2OCl$ (358.82); IR (KBr, ν_{max}, cm^{-1}): 3428.00 (NH *str.*), 1635.24 (CO *str.*), 3090.0 (aromatic CH *str.*), 2901 (aromatic CH *str.*), 1564 (C=N *str.*), 1416.0 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.18 (s, 1H, NH), 8.12 (s, 1H, Ar-H), 8.0-8.7.95 (m, 4H, Ar-H), 7.75 (s, 1H, Ar-H), 7.73-7.70 (t, *J* = 4.8 Hz, 1H, Ar-H), 7.56-7.50 (t, *J* = 5.1 Hz, 1H, Ar-H), 7.42-7.38(d, *J* = 8.0 Hz, 2H, Ar-H), 7.34-7.31 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.20-7.18 (d, *J* = 7.6 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.2, 158.3, 148.57, 145.17, 136.9, 135.3, 131.90, 131.76, 130.8, 129.2, 129.1, 128.04, 127.92, 127.4, 125.5, 121.6, 119.54.

2-Phenyl-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-quinoline-4-carboxamide (5f): Yield: 70%, m.p.: 185-187 °C, m.f. (m.w.): $C_{27}H_{22}N_4O_2$ (434.49); IR (KBr, ν_{max}, cm^{-1}): 3412 (NH *str.*), 1640 (CO *str.*), 2930 (aromatic CH *str.*), 2847 (aromatic CH *str.*), 1586.6 (C=N *str.*), 1557 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 11.9 (s, N-H, 1H), 8.56-8.54 (d, *J* = 8 Hz, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 8.13-8.09 (d, *J* = 2 Hz, 1H, Ar-H), 8.07-7.97 (d, *J* = 2 Hz, 1H, Ar-H), 7.85-7.66 (m, 2H, Ar-H), 7.64-7.48 (t, 1H, Ar-H), 7.46-7.4 (t, 1H, Ar-H), 7.36 (t, 1H, Ar-H), 7.34-7.30 (d, 2H, Ar-H), 6.93-6.52 (m, 2H, Ar-H), 3.097 (s, N-CH_3, 3H), 2.422 (s, CH_3-H, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 173.92, 157.14, 152.81, 148.75, 145.81, 139.19, 133.97, 129.64, 129.47, 128.81, 127.45, 126.63, 126.30, 124.04, 120.33, 117.43, 113.26, 77.02, 29.64.

2-Phenyl-*N*-(4-bromophenyl)-quinoline-4-carboxamide (5g): Yield: 82%, m.p.: 164-166 °C, m.f. (m.w.): $C_{22}H_{15}N_2OBr$ (403.27); IR (KBr, ν_{max}, cm^{-1}): 3396 (NH *str.*), 1645 (CO *str.*), 3096 (aromatic CH *str.*), 2804 (aromatic CH *str.*), 1576 (C=N *str.*), 1402 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.72 (d, *J* = 8 Hz, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 8.05-8.01 (d, *J* = 1 Hz, 1H, Ar-H), 8.02-7.97 (d, *J* = 3 Hz, 1H, Ar-H), 7.83 (d, 1H, Ar-H), 7.73-7.59 (t, 1H, Ar-H), 7.62-7.54 (d, 5H, Ar-H), 7.37 (d, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.1, 156.3, 145.53, 136.4, 132.03, 132.0, 129.98, 128.8, 128.08, 127.7, 127.36, 127.24, 125.5, 121.8, 121.4, 119.1, 116.9.

2-Phenyl-N-(pyridin-2-yl)-quinoline-4-carboxamide (5h): Yield: 58%, m.p.: 145-147 °C, m.f. (m.w.): $C_{21}H_{15}N_3O$ (325.36); IR (KBr, ν_{max}, cm^{-1}): 3480 (NH *str.*), 1662.24 (CO *str.*), 3098 (aromatic CH *str.*), 2891 (aromatic CH *str.*), 1536 (C=N *str.*), 1408 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.75 (d, *J* = 8 Hz, 1H, Ar-H), 8.45 (d, 1H, Ar-H), 8.05-8.00 (d, *J* = 4 Hz, 1H, Ar-H), 8.02-7.95 (d, *J* = 2 Hz, 1H, Ar-H), 7.83-7.62 (m, 2H, Ar-H), 7.59 (s, 1H, Ar-H), 7.57 (d, *J* = 7.6, 1H, Ar-H), 7.42-7.40 (t, 1H, Ar-H); 7.11 (d, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 164.18, 159.38, 152.3, 147.02, 145.53, 136.7, 134.9, 129.9, 128.9, 128.76, 128.08, 127.59, 127.52, 127.2, 125.5, 121.9, 119.1, 118.9, 115.2.

2-Phenyl-*N*-(3-methoxyphenyl)-quinoline-4-carboxamide (5i): Yield: 64%, m.p.: 193-195 °C, m.f. (m.w.): $C_{23}H_{18}N_2O_2$ (354.40); IR (KBr, ν_{max}, cm^{-1}): 3408 (NH *str.*), 1625.2 (CO *str.*), 3196 (aromatic CH *str.*), 2896 (aromatic CH *str.*), 1558 (C=N *str.*), 1402 (C=C *str.*); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.72-8.32 (d, *J* = 8Hz, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 8.05-8.00 (d, *J* = 2 Hz, 1H, Ar-H), 8.02-7.95 (d, *J* = 2Hz, 1H, Ar-H), 7.83-7.62 (m, 2H, Ar-H), 7.61-7.59 (t, 1H, Ar-H), 7.42-7.40 (t, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 165.8, 157.38, 151.8, 142.5, 133.9, 130.4, 128.9, 128.7, 128.43, 127.8, 127.1, 127.0, 124.8, 122.6, 121.89, 121.7, 119.1, 114.4, 50.4.

2-Phenyl-*N*-(3-nitrophenyl)-quinoline-4-carboxamide (5j): Yield: 57%, m.p.: 181-183 °C, m.f. (m.w.): $C_{22}H_{15}N_3O_3$ (369.37); IR (KBr, ν_{max}, cm^{-1}): 3412 (NH *str.*), 1640.1 (CO *str.*), 2930 (aromatic CH *str.*), 2847 (aromatic CH *str.*), 1586.6 (C=N *str.*), 1557 (C=C *str.*); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 11.09 (s, 1H, NH), 8.23-8.20 (d, *J* = 8 Hz, 1H, Ar-H), 8.08-8.06 (m, 3H, Ar-H), 7.82-7.72 (m, 5H, Ar-H), 7.63-7.59 (m, 1H, Ar-H), 7.43-7.40 (d, *J* = 7.00 Hz, 2H, Ar-H), 7.25-7.22 (t, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 164.60, 150.13, 148.97, 143.49, 143.26, 137.10, 135.4, 131.05, 130.01, 129.29, 129.21, 129.12, 128.38, 128.18, 127.48, 125.42, 123.46, 122.13, 119.4, 116.27.

Antimicrobial activity: Synthesized 2-phenyl quinoline-4-carboxamides **(5a-j)** were separately tested against one Gram-positive (*S. aureus*), one Gram-negative bacteria (*E.coli*) and two mold fungi *viz*. *Candida albicans* and *Aspergillus flavus* by agar well diffusion method [16]. The microbial inoculum is spread across the entire agar plate surface. After the medium had solidified, holes with a diameter of 6 mm was punched aseptically by a sterile cork-borer and a concentration of 50 µg/50 µL doses of the test compounds **5a-j**, standard ciprofloxacin for antibacterial and fluconazole for antifungal (10 µg/mL) along with negative control DMSO was introduced into the well separately. Then the plates were incubated at 37 °C for 36 h for bacteria and at 28 °C for 72 h for fungal strains. After the incubation period is over, zone of inhibition diameter for each well is measured in mm. The experiment was performed in triplicates and the average values were reported.

Anticancer activity: Anticancer activity of the compounds **5a-j** was carried out by using MTT assay [17,18] against two cancer lines A549 lung cancer cell line and MCF-7 breast cancer cell lines. The test was carried out in accordance with the literature procedure [19,20]. In this assay, the yellow coloured 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is reduced by mitochondrial succinate dehydrogenase to an insoluble, coloured formazan product that is dark purple in colour. The cells are then treated with an organic

solvent (such as DMSO, isopropanol, *etc.*) that solubilizes the cells and releases formazan which was measured spectrophotometrically (570 nm). Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viable cells.

After the treatment, the solutions in the wells were discarded and 100 µL of freshly prepared MTT (1 mg/mL PBS) was added to each well. The plates were shaken gently and incubated for 4 h at 37 °C in 5% CO_2 atmosphere. After 4 h, the supernatant was removed and the formazan crystals formed in the cells were solubilized by addition of 100 µL of DMSO. The absorbance was read using a micro-plate reader (Bio-Tek, ELX-800 MS) at 570 nm. The assay was done in triplicate for each compound.

The growth inhibition was calculated using the formula below and result were reported in percentage:

$$\text{Growth inhibition (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Molecular docking studies

Ligand preparation: The chemical structures of the synthesized compounds were drawn using Chemdraw [21]. Chemistry at Harvard Molecular Mechanics (CHARMm) was used to perform the ligand optimization and Macro Molecular Force Field (MMF) followed by energy minimization protocol [22]. The drug likeliness was evaluated using the Lipinski rule of 5 *via* Lipinski drug filter protocol [23,24].

Protein selection: 3D structures of Bacterial target proteins in *S. aureus* (PDB ID: 2XCT) [25], Fungal *Candida albicans* (PDB ID: 1IYL) [26] and cancer protein (PDB ID: 3OG7) [27] were retrieved from the Protein Data Bank (PDB) website www.rcsb.org. Co-crystallized ligand, water molecules and metal ions were removed from the target structures to obtain clean protein. The resulting structures were optimized classically using CHARMm force field implemented in the DS 3.5, minimized with conjugate gradient energy minimization protocol and by convergence energy minimization (0.001 kcal/mol), respectively [28], that readied the structures for docking and simulations. Active site of the ensemble has been defined as the collection of residues within 10.0 Å of the bound inhibitor and comprised the union of all ligands of the ensemble. All atoms located less than 10.0 Å from any ligand atom were considered.

A flexible docking approach was employed for molecular docking studies using the Lead IT [28] software in which targets were considered as receptor proteins.

Active site residues in *S. aureus* (PDB ID: 2XCT) are Val30, Ala33, Asp39, Lys42, Arg47, Asn57, Ala67, Val85, Ile112, Asn148, Ala175 and Gln267 were selected for molecular docking studies.

Active site residues in *Candida albicans* (PDB ID: 1IYL) Arg48, Arg92, Tyr100, Val51, Phe66, Arg70 and Gly101, were selected for molecular docking studies.

Active site residues in (PDB ID: 3OG7), GLN461, ARG462, ILE463, GLY464, SER465, PHE468, THR470, VAL471, TYR472, LYS473, ASP479, VAL480, MET484 and LEV485 were selected for molecular docking studies.

RESULTS AND DISCUSSION

2-Phenyl quinoline-4-carboxamide derivatives **(5a-j)** were synthesized by the reaction of chosen aromatic primary amines with 2-phenyl quinoline-4-carboxylic acid by using conventional method as shown in **Scheme-I** in good yield (56-86%). The structures of the all the compounds was established by both physico-chemical (melting point) as well as spectral studies (FTIR, ^{1}H & ^{13}C NMR and Mass). Primary evidence for the formation of amide derivative was obtained from the infrared spectrum of compounds **5a-j,** which showed absence of stretching due to OH group and presence of absorption band in the range of 3400-3100 cm^{-1} due to NH-stretching and 1688-1650 cm^{-1} due to a carbonyl group. ^{1}H NMR spectrum of **5a-j** showed a singlet at δ 8.54-8.52 ppm indicating NH proton confirms the coupling of acid with amines. The formation is further evident from the ^{13}C NMR spectra which showed signal at 165 ppm due to carbonyl carbon, signals in the range of 133.5 to 148.0 ppm and 118.0-130.7 ppm due to quinoline and aromatic carbon, respectively.

Biological activity: The antibacterial screening of compounds **5a-j** reveals that the compound **5d** (nitro substituent at *para* position), compound **5f** (antipyrine group) and compound **5j** (nitro group at *meta* position) have shown significant activity compared with standard drug ciprofloxacin, but compound **5a** (chloro group at *meta* position), compound **5b** (methyl group at *para* position), compound **5c** (unsubstituted), **5e** (chloro group at *para* position), **5g** (bromo group at *para* position), **5h** (Unsubstituted pyridine ring) and **5i** (methoxy group at *para* position) have shown moderate activity. The results are tabulated in Table-1.

From the antifungal activity results it is evident that compound **5d** (nitro substituent at *para* position), **5f** (antipyrine group) and compound **5j** (nitro group at *meta* position) have shown significant activity compared with standard drug fluconazole but remaining compounds were moderately active against tested fungal strains. The results are tabulated in Table-1.

From the results of anticancer activity of Schiff bases **5b, 5d**, **5f** and **5h** exhibited anticancer activity whereas compounds **5a**, **5c, 5e, 5i** and **5j** showed weak activity. Among the tested compound **5b** (methyl group at *meta* position) and compound **5d** (nitro group at *para* position) were emerged as potent agents with IC_{50} 54.04 µM and 77.29 µM, respectively against A549 cell line and also against MCF-7 cell lines with IC_{50} 66.37 µM and 60.56 µM, respectively as tabulated in Table-1.

Computational study

ADME properties: The physical properties and the ADME parameters (absorption, distribution, metabolism and excretion) of 2-phenyl quinolie-4-carboxamides were computed using the freely accessible web server Swiss ADME (http://swissadme.ch/index.php#undefined). The results of *in silico* ADME properties of **5a-j** are listed in Table-2. The molecular weight (MW), the number of hydrogen bond acceptors (nHBA), donors (nHBD), the number of rotatable bonds (nRB) and the topological polar surface area (TPSA) for all the compounds were in accordance with the Lipinski's rule of five. The lipophilicity property (expressed as MLogP ≤ 4.15) was in the range for all the compounds.

TABLE-1
in vitro BIOLOGICAL ACTIVITY DATA OF 2-PHENYLQUINOLINE-4-CARBOXAMIDES (**5a-j**)

Compound	Antibacterial activity		Antifungal activity		Anticancer activity	
	S. aureus	*E. coli*	*A. niger*	*C. albicans*	A549	MCF-7
5a	17.22 ± 0.32	14.38 ± 0.56	12.00 ± 0.12	11.50 ± 0.11	107.06 ± 1.1	105.12 ± 0.9
5b	15.80 ± 0.37	12.08 ± 0.33	14.50 ± 0.16	11.21 ± 0.18	54.04 ± 1.5	66.37 ± 2.3
5c	10.92 ± 0.65	14.18 ± 0.42	14.00 ± 0.19	11.00 ± 0.19	139.63 ± 0.69	206.55 ± 1.8
5d	18.09 ± 0.52	19.45 ± 0.25	22.45 ± 0.14	24.12 ± 0.12	77.29 ± 2.1	60.56 ± 1.6
5e	14.22 ± 0.23	16.11 ± 0.41	11.50 ± 0.12	14.00 ± 0.15	62.18 ± 1.3	71.38 ± 1.2
5f	19.28 ± 0.15	21.78 ± 0.16	22.56 ± 0.15	19.12 ± 0.19	81.41 ± 2.3	77.53 ± 1.2
5g	15.15 ± 0.42	12.32 ± 0.22	11.00 ± 0.19	15.00 ± 0.17	No inhibition	134.98 ± 2.0
5h	20.15 ± 0.18	25.72 ± 0.36	12.23 ± 0.13	15.50 ± 0.16	85.32 ± 1.4	91.01 ± 2.5
5i	12.95 ± 0.29	17.38 ± 0.23	11.24 ± 0.19	13.23 ± 0.16	152.88 ± 1.2	181.12 ± 1.5
5j	20.80 ± 0.16	22.65 ± 0.17	19.50 ± 0.12	17.50 ± 0.19	56.76 ± 0.66	105.77 ± 1.0
Ciprofloxacin	26.00 ± 0.16	25.00 ± 0.33	–	–	–	–
Fluconozole	–	–	26.00 ± 0.16	25.00 ± 0.33	–	–
Cisplatin	–	–	–	–	84.28 ± 0.4	57.33 ± 1.2

TABLE-2
in silico ADME PROPERTIES OF 2-PHENYL QUINOLINE-4-CARBOXAMIDES (**5a-j**)

Compound	MW	MLog P	nHBA	nHBD	nRB	TPSA	nViolations
Rule	< 500.00	≤ 4.15	≤ 10	≤ 5	≤ 10	< 160 Å^2	0
5a	358.82	4.24	2	1	4	41.99	1
5b	338.40	3.97	2	1	4	41.99	0
5c	324.38	3.75	2	1	4	41.99	0
5d	369.37	2.68	4	1	5	87.81	0
5e	358.82	4.24	2	1	4	41.99	1
5f	434.49	3.95	3	1	5	68.92	0
5g	403.27	4.34	2	1	4	41.99	1
5h	325.36	3.09	3	1	4	54.88	0
5i	354.40	3.39	3	1	5	51.22	0
5j	369.37	2.68	4	1	5	87.81	0

Molecular docking studies: To understand the mechanism of biological activity of newly synthesized compounds, molecular modelling and docking studies were performed on X-ray crystal structure of target protein PDB ID: 2XCT for antibacterial activity, *N*-myristoyltranferase PDB ID: 1IYL as a target protein to study the antifungal activity and target protein PDB ID: 3OG7 was used for anticancer study. The default spherical Matcher placement method was used for docking. GBVI/WSA dG scoring function which estimates the free energy of binding of the ligand from a given pose was used to rank the final pose. The ligand-enzyme complex with lowest docking score and binding pattern of compounds were selected. The antibacterial, antifungal and anticancer docking data of pyrazole carboxamides **5a-j** are tabulated in Table-3. Bind pose of the active compounds are shown in Fig. 3.

TABLE-3
DOCKING ENERGY (kcal/mol) OF 2-PHENYL QUINOLINE-4-CARBOXAMIDES (**5a-j**)

Compound	Lead IT score		
	PDB ID:2XCT	PDB ID:1IYL	PDB ID:3OG7
5a	-18.27	Lead IT score	-25.76
5b	-19.482	-12.46	-26.391
5c	-19.054	-11.723	-25.364
5d	-24.413	-12.533	-26.207
5e	-19.742	-14.462	-25.39
5f	-21.05	-10.283	-30.443
5g	-19.282	-13.554	-25.488
5h	-23.335	-11.908	-27.794
5i	-16.962	-12.277	-24.647
5j	-24.031	-10.708	-28.332

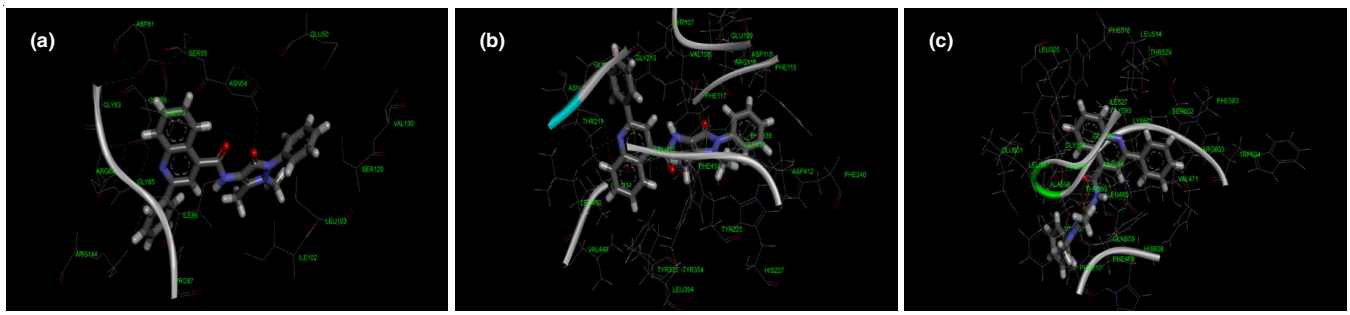


Fig. 3. Binding pose of the title compound **5f** with target protein PDB ID:2XCT (a); PDB ID:1IYL (b); and PDB ID: 3OG7 (c)

Structure-activity relationship (SAR) studies: Structure-activity relation correlates the biological activity and chemical structure of the molecule. In general, the activity of the compound is inûuenced by the electronic structure, size, shape, molecular arrangements and electron donating/withdrawing groups, *etc*. Our initial strategy was to identify the key sub unit required for activity such as quinoline (nitrogen heterocycles - antimicrobial and anticancer agents) which allows its derivatives to readily interact with a wide variety of enzymes and receptors in organisms. Further essential substituents like -CH_3, -OCH_3 (electron donating), -Br, -Cl (halogen) and -NO_2 (electron withdrawing)] groups were varied at *meta* and *para*-position of the phenyl ring to acquire the optimum results. The results suggest that the following assumptions about the structural activity relationship, it is evident, that in a group of compounds having -H, 4-CH_3, 4-OCH_3 substituents on phenyl ring **5d, 5h** and **5j** were essentially influencing the antimicrobial and anticancer activity. In particular, the compounds substituted with halogens and nitro group on phenyl ring **5a, 5d, 5e** and **5h** were found to be the most active compounds in *in vitro* antimicrobial and cytotoxicity.

Conclusion

The present study reports the successful synthesis, characterization, cytotoxic, antimicrobial and pharmacokinetic study of new quinoline carboxamide derivatives. Attempts have been made to predict *in silico* ADME of synthesized molecules. All the compounds were found to be in the acceptable range except three compounds. Two of the tested compounds **5b** and **5d** were effective against A549 with IC_{50} 54.04 µM and 77.29 µM, respectively against A549 cell line and also against MCF-7 cell lines with IC_{50} 66.37 and 60.56 µM, respectively. The results suggest that these compounds may serve as lead chemical entities for further modification in the search for new classes of potential antimicrobial and anticancer agents.

ACKNOWLEDGEMENTS

The authors are thankful to Tumkur University, Tumakuru for providing the laboratory facility to carry out this research work. The authors also thankful to Department of Biotechnology, SIT, Tumakuru for providing Docking software to carry out Docking studies and Sri Siddaganga College of Pharmacy for providing facility to carry out *in vitro* study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Bishnoi, A.K. Tiwari, S. Singh, A. Sethi, C.M. Tripathi and B. Banerjee, *Med. Chem. Res.*, **22**, 3527 (2013); https://doi.org/10.1007/s00044-012-0333-2
2. M.I. Mohamed, N.G. Kandile and H.T. Zaky, *J. Heterocycl. Chem.*, **54**, 35 (2015); https://doi.org/10.1002/jhet.2529
3. R. Musiol, J. Jampilek, V. Buchta, L. Silva, H. Niedbala, B. Podeszwa, A. Palka, K. Majerz-Maniecka, B. Oleksyn and J. Polanski, *Bioorg. Med. Chem.*, **14**, 3592 (2006); https://doi.org/10.1016/j.bmc.2006.01.016
4. B. Kalluraya and S. Sreenivasa, *Il Farmaco*, **53**, 399 (1998); https://doi.org/10.1016/S0014-827X(98)00037-8
5. M.A.S. Abdelwahid, T. Elsaman, M.S. Mohamed, S.A. Latif, M.M. Mukhtar and M.A. Mohamed, *J. Chem.*, **2019**, 2859637 (2019); https://doi.org/10.1155/2019/2859637
6. X. Xu, J. Wang and Q. Yao, *Bioorg. Med. Chem. Lett.*, **25**, 241 (2015); https://doi.org/10.1016/j.bmcl.2014.11.065
7. J.T. Madak, A. Bankhead, C.R. Cuthbertson, H.D. Showalter and N. Neamati, *Pharmacol. Therap.*, **195**, 111 (2019); https://doi.org/10.1016/j.pharmthera.2018.10.012
8. S.A. El-Feky, Z.K. Abd El-Samii, N.A. Osman, J. Lashine, M.A. Kamel and H.Kh. Thabet, *Bioorg. Chem.*, 58, 104 (2014); https://doi.org/10.1016/j.bioorg.2014.12.003
9. L. Zhu, K. Luo, K. Li, Y. Jin and J. Lin, *Bioorg. Med. Chem.*, **25**, 5939 (2017); https://doi.org/10.1016/j.bmc.2017.09.004
10. M.A. Massoud, S.A. El Bialy, W.A. Bayoumi and W.M. El Husseiny, *Heterocycl. Commun.*, **20**, 81 (2014); https://doi.org/10.1515/hc-2013-0163
11. S.S. Bharadwaj, B. Poojary, S.M. Kumar, K. Byrappa, G.S. Nagananda, A.K. Chaitanya, K. Zaveri, N.S. Yarla, Y. Shiralgi, A.K. Kudva and B.L. Dhananjayad, *New J. Chem.*, **41**, 8568 (2017); https://doi.org/10.1039/C6NJ03913H
12. A.M. El-Agrody and A.M. El-Agrody, *ARKIVOC*, 134 (2011)l https://doi.org/10.3998/ark.5550190.0012.b12
13. O. Afzal, S. Kumar, M.R. Haider, R. Kumar, M. Jaggi and S. Bawa, *Eur. J. Med. Chem.*, **97**, 871 (2015); https://doi.org/10.1016/j.ejmech.2014.07.044
14. X. Wang, X. Xie, Y. Cai, X. Yang, J. Li, Y. Li, W. Chen and M. He, *Molecules*, **21**, 340 (2016); https://doi.org/10.3390/molecules21030340
15. Y. Erugu, B. Sangepu, B. Gandu, G. Anupoju and V.R. Jetti, *World J. Pharm. Pharm. Sci.*, **3**, 1612 (2014).
16. H.M. Berman, T. Battistuz, T.N. Bhat, W.F. Bluhm, P.E. Bourne, K. Burkhardt, Z. Feng, G.L. Gilliland, L. Iype, S. Jain, P. Fagan, J. Marvin, D. Padilla, V. Ravichandran, B. Schneider, N. Thanki, H. Weissig, J.D. Westbrook and C. Zardecki, *Acta Crystallogr. D Biol. Crystallogr.*, **58**, 899 (2002); https://doi.org/10.1107/S0907444902003451
17. V. Lamour, L. Hoermann, J.M. Jeltsch, P. Oudet and D. Moras, *J. Biol. Chem.*, **277**, 18947 (2002); https://doi.org/10.1074/jbc.M111740200.
18. W.S. El-serwy, N.A. Mohamed, E.M. Kassem, K. Mahmoud and M.M. Mounier, *Iran. J. Pharm. Res.*, **15**, 179 (2016).
19. F. Denizot and R. Lang, *J. Immunol. Methods*, **89**, 271 (1986); https://doi.org/10.1016/0022-1759(86)90368-6
20. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983); https://doi.org/10.1016/0022-1759(83)90303-4
21. K. Wingen, J.S. Schwed, K. Isensee, L. Weizel, A. •ivkovic, D. Odazic and H. Stark, *Bioorg. Med. Chem. Lett.*, **24**, 2236 (2014); https://doi.org/10.1016/j.bmcl.2014.03.098
22. G. Wu, D.H. Robertson, C.L. Brooks III and M. Vieth, *J. Comput. Chem.*, **24**, 1549 (2003); https://doi.org/10.1002/jcc.10306
23. C.A. Lipinski, *Drug Discov. Today Technol.*, **1**, 337 (2004); https://doi.org/10.1016/j.ddtec.2004.11.007
24. C.A. Lipinski, F. Lombardo, B.A. Dominy and P.J. Feeney, *Adv. Drug Deliv. Rev.*, **46**, 3 (2001); https://doi.org/10.1016/S0169-409X(00)00129-0
25. B.D. Bax, P.F. Chan, D.S. Eggleston, A. Fosberry, D.R. Gentry, F. Gorrec, I. Giordano, M.M. Hann, A. Hennessy, M. Hibbs, J. Huang, E. Jones, J. Jones, K.K. Brown, C.J. Lewis, E.W. May, M.R. Saunders, O. Singh, C.E. Spitzfaden, C. Shen, A. Shillings, A.J. Theobald, A. Wohlkonig, N.D. Pearson and M.N. Gwynn, *Nature*, **466**, 935 (2010); https://doi.org/10.1038/nature09197
26. R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. iyashiro, T.D. Penning, K. Seibert, P.C. Isakson and W.C. Stallings, *Nature*, **384**, 644 (1996); https://doi.org/10.1038/384644a0
27. V. Chandramohan, A. Kaphle, M. Chekuri, S. Gangarudraiah and G.B. Siddaiah, *Adv. Virol.*, **2015**, 972067 (2015); https://doi.org/10.1155/2015/972067
28. R.B. Ravelli, B. Gigant, P.A. Curmi, I. Jourdain, S. Lachkar, A. Sobel and M. Knossow, *Nature*, **428**, 198 (2004); https://doi.org/10.1038/nature02393