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A novel templates of piperazinyl-1,2-dihydroquinoline-3-carboxylates: Synthesis, anti-microbial evaluation and molecular docking studies

Saleha Banu [a], Rajitha Bollu [a], Mohammad Naseema [a], P. Mary Gomedhika [a], Lingaiah Nagarapu [a,*], K. Sirisha [b,c], C. Ganesh Kumar [b,c], Shravan Kumar Gundasw [d]

[a] Organic Chemistry Division II (CPC), CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, Telangana, India
[b] Medicinal Chemistry and Biotechnology Division (MCB), CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500 007, India
[c] Academy of Scientific and Innovative Research (AcSIR), New Delhi, India
[d] Bioinformatics Division, PGRRCDE, Osmania University, Hyderabad 500007, India

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ABSTRACT

A series of piperazinyl-1,2-dihydroquinoline carboxylates were synthesized by the reaction of ethyl 4-chloro-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylates with various piperazines and their structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR and mass spectral analysis. All the synthesized compounds were screened for their *in vitro* antimicrobial activities. Further, the *in silico* molecular docking studies of the active compounds was performed to explore the binding interactions between piperazinyl-1,2-dihydroquinoline carboxylate derivatives and the active site of the Staphylococcus aureus (CrtM) dehydrosqualene synthase (PDB ID: 2ZCQ). The docking studies revealed that the synthesized derivatives showed high binding energies and strong H-bond interactions with the dehydrosqualene synthase validating the observed antimicrobial activity data. Based on antimicrobial activity and docking studies, the compounds **9b** and **10c** were identified as promising antimicrobial lead molecules. This study might provide insights to identify new drug candidates that target the S. aureus virulence factor, dehydrosqualene synthase.

N-Heterocyclic compounds remain an attractive topic from both fundamental organic chemistry and medicinal chemistry point of view. Among all heterocycles, the synthesis of quinolones has received much attention from medicinal chemists due to a wide variety of pharmacological properties attributed to this compound class itself. They have been reported to show typical antibacterial activity, and also displayed a typical diverse biological profiles such as anti-tumor, anti-tubercular, anti-HIV, antibacterial, anti-malarial activities, and the biological properties continue to expand[1–4] as shown in Fig. 1. The majority of quinolones in clinical use are fluoroquinolones which are one of the most useful and versatile antibacterial agents, where several candidates are already in clinical use such as Ciprofloxacin, Norfloxacin and Ofloxacin.[5] They have emerged as one of the dominant classes of chemotherapeutic drugs for the treatment of various bacterial infections in both community and hospital settings.[6] However, since these compounds became available for clinical use, resistance among Methicillin-resistant *Staphylococcus aureus* has been observed in different parts of the world.[7–9] Molecular hybridization, which is based on the incorporation of two or more pharmacophores into a single molecule, may provide novel candidates having complimentary activities and/or multiple pharmacological targets and/or one part can counterbalance the side effects caused by another part.[10] Modifications in the basic structure of quinolones[11] have increased their antibacterial spectrum and potency, as well as improving bioavailability, making quinolones useful agents for the treatment of urinary, systemic and respiratory tract infections. Obviously, this strategy represents an encouraging approach on the development of new agents with potential therapeutic application (Fig. 2).[12–18]

As a part of our efforts to develop new biologically active molecules,[19] we describe the synthesis and antimicrobial evaluation of quinolones bearing piperazines at C-4 position as shown in Scheme 1. All the derivatives were further screened for *in vitro* antimicrobial activities. In this context, we herein report the synthesis of piperazine linked 1,2-dihydroquinoline carboxylate hybrids in good to excellent yields as depicted in Fig. 3.

Synthesis of intermediates and target compounds was accomplished according to the steps illustrated in Scheme 1. The first synthetic step involved *N*-alkylation of isatoic anhydride with iodoalkanes to obtain the corresponding *N*-alkyl isatoic anhydrides **2** and **3**. Condensation of diethyl malonate and *N*-alkyl isatoic

* Corresponding author.
E-mail address: nagarapu@iict.res.in (L. Nagarapu).

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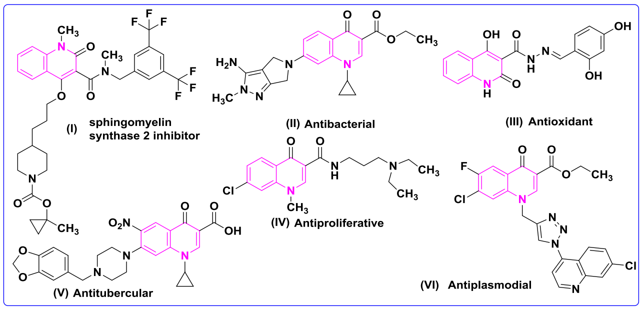


Fig. 1. Representative examples of biologically active quinolone-based compounds.

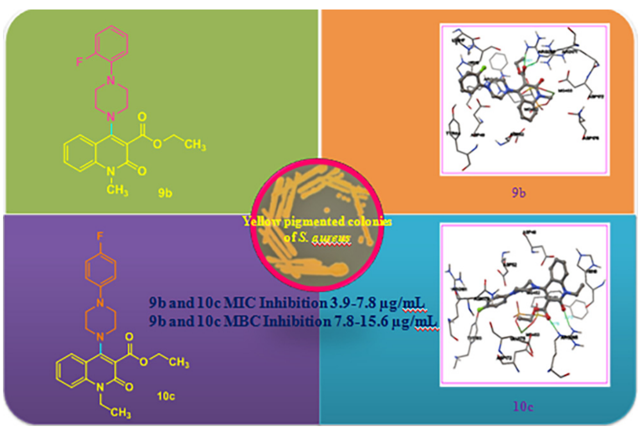
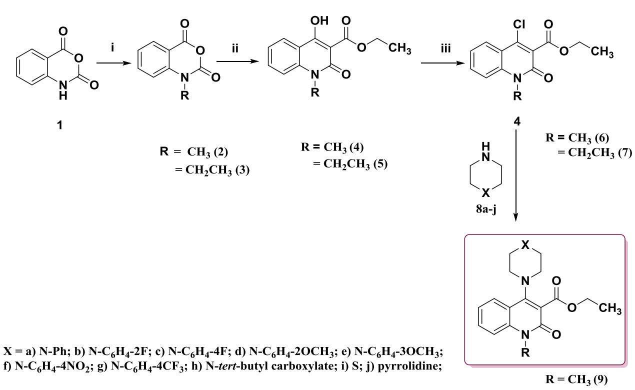


Fig. 2. Design strategy for ethyl-2-oxo-4-(piperazin-1-yl)-1,2-dihydroquinoline-3-carboxylate hybrids.



Scheme 1. Synthesis of ethyl-2-oxo-4-(piperazin-1-yl)-1,2-dihydroquinoline-3-carboxylate hybrids **9a–j** and **10a–j**. *Reagents and conditions:* i) RI, NaH, dry DMF, 0 °C-RT, 5 h ii) Diethyl malonate, NaH, dry DMF, 120 °C, reflux, 16 h, iii) $POCl_3$, reflux, 3 h.

anhydrides in the presence of sodium hydride in dry dimethylformamide led to compounds **4** and **5** in good yields (78%–72%).[20] The corresponding **4** and **5** were further converted into chloro derivatives using phosphoryl chloride under thermal condition for 3 h under inert atmosphere.[21] (Scheme 1).

All the synthesized compounds (**9a–j** and **10a–j**) were characterized by using ^{1}H NMR, ^{13}C NMR, HR-Mass and IR spectroscopic methods. Spectral data of all synthesized compounds were in good agreement with the proposed structures. In ^{1}H NMR spectra, the characteristic triplet signals appeared for piperazine protons at δ

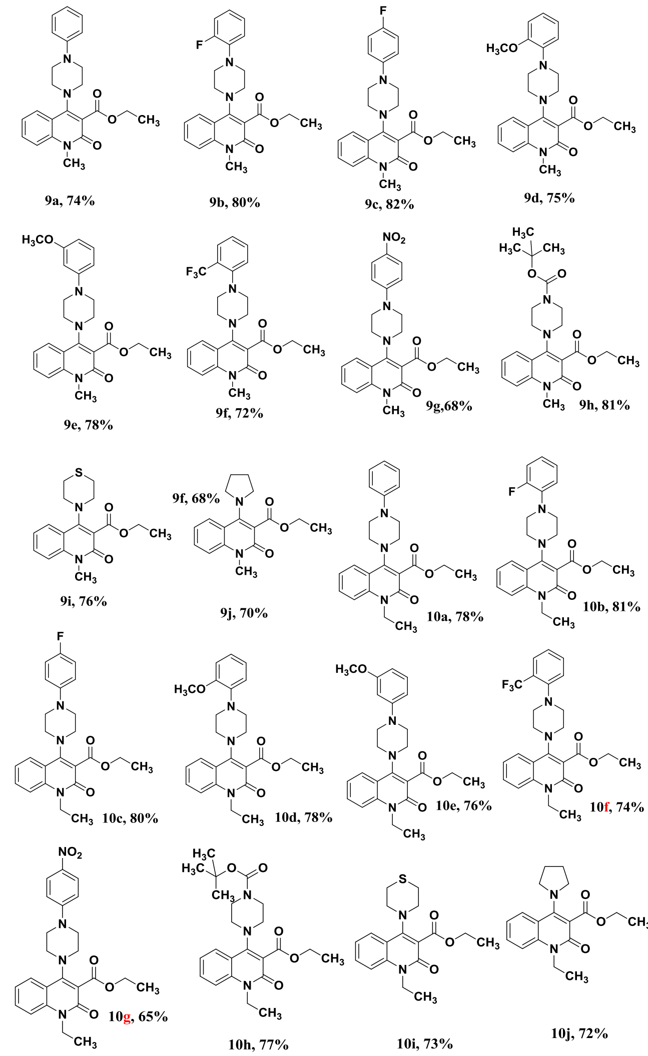


Fig. 3. Newly synthesized ethyl-2-oxo-4-(piperazin-1-yl)-1,2-dihydroquinoline-3-carboxylatederivatives **9a–j** and **10a–j.**

3.10–3.85 ppm. The structures for all these compounds were further confirmed by HRMS analysis. For instance, **9a** displayed a molecular ion peak at *m/z* 392.19687 $[M+H]^+$ suggesting the molecular formula of $C_{23}H_{25}N_3O_3$. Additionally, the IR spectra for the target compounds **9a–j** and **10a–j** exhibited characteristic absorption bands at 1635–1648 cm^{-1}, 1080–1360 cm^{-1} and 2924–2982 cm^{-1} which corresponded to C=O, C—N and C—H_3 respectively (Fig. 4).

The synthesized hybrids **9a–j** and **10a–j** were evaluated for their *in vitro* antimicrobial activity against Gram positive bacterial strains such as *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96, *Staphylococcus aureus* MLS-16 MTCC 2940, *Micrococcus luteus* MTCC 2470, Gram-negative bacterial strains such as *Escherichia coli* MTCC 739, *Klebsiella planticola* MTCC 530, *Pseudomonas aeruginosa* MTCC 2453 and a fungal strain *Candida albicans* MTCC 3017, and the results to this regard are tabulated in Table 1. Ciprofloxacin and Miconazole were used as standard controls for the bacterial and fungal strains, respectively. The compounds **9b** and **10c** exhibited promising and broad spectrum antimicrobial activity against all the test pathogens except for *Klebsiella planticola* MTCC 530 and *Pseudomonas aeruginosa* MTCC 2453 with MIC values ranging from 3.9 to 7.8 μg/mL. Further, the compounds **9b** and **10c** exhibited a MIC value of 3.9 μg/mL against *Candida albicans*

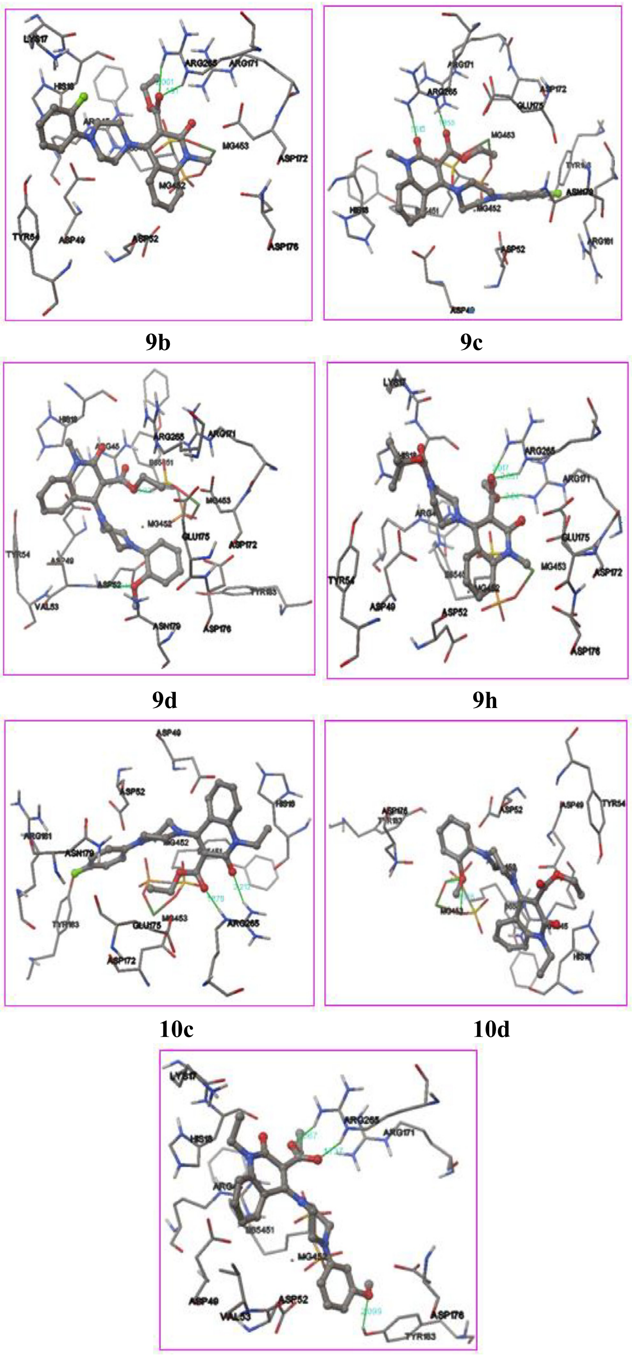


Fig. 4. Docking of all the lead compounds with active site of *Staphylococcus aureus* dehydrosqualene synthase (PDB ID: 2ZCQ).

MTCC 3017 which was found to be lower than that of the standard drug Miconazole (MIC value 7.8 μg/mL). Hence, from a structure-activity relationship (SAR) perspective, it was observed that the synthesized compounds **9b** and **10c** having 2-F and 4-F substituents respectively, attached to the dihydroquinoline scaffold exhibited electron withdrawing properties which plausibly may be contributing to the antibacterial and anti-*Candida* activities. Further, the compounds **9c**, **9d**, **9h**, **10b**, **10d**, **10e** and **10h** exhibited a MIC value of 3.9 μg/mL against *Staphylococcus aureus* MTCC 96. While, the compounds **9c**, **9f** and **10f** exhibited antifungal activity against *Candida albicans* MTCC 3017 with MIC value of 7.8 μg/mL. From the above data, it can be inferred that the substituents attached to the phenyl ring of piperazines, exhibiting strong electron donating and/or electron withdrawing properties may have contributed to the antibacterial and anti-*Candida* activities. Furthermore, all the synthesized compounds were also evaluated for the minimum bactericidal concentration (MBC) and the results to this regard are tabulated in Table 2. In this case too, the compounds **9b** and **10c** were found to be promising and exhibited broad spectrum of antimicrobial activity except for *Pseudomonas aeruginosa* MTCC 2453.

Dehydrosqualene synthase (CrtM) of *Staphylococcus aureus* is involved in the synthesis of golden carotenoid pigment staphyloxanthin.[22] This pigment functions as a virulence factor in *S. aureus* and also acts as an antioxidant which protects the *S. aureus* against oxidative stress due to host immune defense by reactive oxygen species and neutrophils and enables its survival within the host cell.[23–25] In the search for the next generation of antibiotics, recent efforts have targeted virulence rather than essential gene functions as an antimicrobial target.[26] A team of investigators, including structural biologists, chemists, and microbiologists, discovered recently that inhibition of the *S. aureus* dehydrosqualene synthase reduced the bacterial survival during infections, offering a proof-of-principle for such a virulence-targeted approach.[22–26] Considering the above facts, molecular docking studies were accomplished to explore the binding interactions between the lead compounds and the active site of *Staphylococcus aureus* dehydrosqualene synthase (PDB ID: 2ZCQ).

Molecular docking is the most extensively used method for the calculation of protein–ligand interactions. AutoDock ver. 4.2 uses binding free energy assessment to assign the best binding conformation. Docking of all the lead compounds into the binding site of the dehydrosqualene synthase protein and estimating the binding affinity of the complex is a significant part of the structure based drug design process. The structural interactions between PDB with 9 inhibitors were docked separately. Docking studies are commonly performed for predicting binding modes to proteins and their binding energies of ligands. X,Y,Z coordinates of PDB were selected by using SPDBV. Binding energy and <DELTA>G bind value exists on the basis of Hydrogen bond, Hydrophobic and Van der Wall interactions.

Experimental activities and predicted values by Lamarckian Genetic Algorithm dockings of the 9 compounds are summarized in Table 3. The synthesized compounds selected for molecular docking have some collective structural features. All the lead compounds showed good binding energy and also exhibited interactions and better lower free energy values, indicating more thermodynamically favored interaction. The compounds **9b** and **9c** exhibited binding energies of −6.96 kcal/mol and −8.05 kcal/mol, respectively, with two interacting Arg265. Compound **9d** interacted with Arg52 with binding energy of −6.42 kcal/mol and **9h** interacted with Arg171 and two Arg265 with binding energy of −7.21 kcal/mol. Compound **10c** interacted with two Arg265, **10d** interacted with the magnesium (Mg) ion, MG453 and **10e** interacted with Tyr183 and two Arg265 with binding energies of −7.04 kcal/mol, −6.62 kcal/mol and −6.55 kcal/mol, respectively. Further, in case of compounds **10b** and **10h**, there are no hydrogen bond interactions, but these two compounds are showing binding energy and <DELTA>G on the basis of Hydrophobic and Van der Walls interactions.

In conclusion, we have synthesized a series of novel piperazinyl-1,2-dihydroquinoline carboxylates (**9a–j** and **10a–j**) and their structures were characterized by corresponding spectral analyses. All the synthesized compounds have been investigated for their antimicrobial activity. The antimicrobial results indicated that compounds **9b** and **10c** were promising and exhibited broad spectrum antimicrobial activity. Molecular docking studies with *Staphylococcus aureus* dehydrosqualene synthase (CrtM) revealed

Table 1
Antimicrobial activity of the synthesized ethyl -2-oxo-4-(piperazin-1-yl)-1,2-dihydroquinoline-3-carboxylate hybrids **9a–j** and **10a–j.**

Test compounds	Minimum inhibitory concentration (μg/mL)							
	Bacillus subtilis MTCC 121	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940	*Micrococcus luteus* MTCC 2470	*Klebsiella planticola* MTCC 530	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
9a	–	–	–	–	–	–	–	–
9b	3.9	3.9	7.8	3.9	–	3.9	–	3.9
9c	–	3.9	–	–	–	–	–	7.8
9d	–	3.9	–	–	–	–	–	–
9e	–	–	–	–	–	–	–	–
9f	–	–	–	–	–	–	–	7.8
9g	–	–	–	–	–	–	–	–
9h	–	3.9	–	–	–	–	–	–
9i	–	–	–	–	–	–	–	–
9j	–	–	–	–	–	–	–	–
10a	–	–	–	–	–	–	–	–
10b	–	3.9	–	–	7.8	–	–	–
10c	3.9	3.9	3.9	3.9	3.9	3.9	–	3.9
10d	–	3.9	–	–	–	–	–	–
10e	–	3.9	–	7.8	–	–	–	–
10f	7.8	–	–	–	–	7.8	–	7.8
10g	–	–	–	–	–	–	–	–
10h	–	3.9	–	–	–	–	–	–
10i	–	–	–	–	–	–	–	–
10j	–	–	–	7.8	–	–	–	–
Miconazole (Standard)	–	–	–	–	–	–	–	7.8
Ciprofloxacin (Standard)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–

Table 2
Minimum Bactericidal / Fungicidal Concentration (MBC / MFC) of the synthesized compounds **9a–j** and **10a–j**.

Test compounds	Minimum bactericidal/fungicidal concentration (μg/mL)							
	Bacillus subtilis MTCC 121	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* MLS-16 MTCC 2940	*Micrococcus luteus* MTCC 2470	*Klebsiella planticola* MTCC 530	*Escherichia coli* MTCC 739	*Pseudomonas aeruginosa* MTCC 2453	*Candida albicans* MTCC 3017
9a	–	–	–	–	–	–	–	–
9b	7.8	7.8	15.6	7.8	–	7.8	–	7.8
9c	–	7.8	–	–	–	–	–	15.6
9d	–	7.8	–	–	–	–	–	–
9e	–	–	–	–	–	–	–	–
9f	–	–	–	–	–	–	–	15.6
9g	–	–	–	–	–	–	–	–
9h	–	7.8	–	–	–	–	–	–
9i	–	–	–	–	–	–	–	–
9j	–	–	–	–	–	–	–	–
10a	–	–	–	–	–	–	–	–
10b	–	7.8	–	–	7.8	–	–	–
10c	7.8	7.8	7.8	7.8	7.8	7.8	–	7.8
10d	–	7.8	–	–	–	–	–	–
10e	–	7.8	–	15.6	–	–	–	–
10f	15.6	–	–	–	–	15.6	–	15.6
10g	–	–	–	–	–	–	–	–
10h	–	7.8	–	–	–	–	–	–
10i	–	–	–	–	–	–	–	–
10j	–	–	–	15.6	–	–	–	–
Miconazole (Standard)	–	–	–	–	–	–	–	7.8
Ciprofloxacin (Standard)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	–

that the synthesized derivatives showed high binding energies and strong H-bond interactions with the dehydrosqualene synthase validating the observed antimicrobial activity results. Meanwhile, compounds **9c**, **9d**, **9h**, **10c**, **10d**, **10e**, **10f** and **10h** displayed promising activities against the tested pathogenic strains. Consequently, such type of compounds would represent a promising class for future development of a new class of antimicrobial agents that deserves further investigation and derivatization.

Table 3
Molecular docking studies of all lead compounds with the active site of *Staphylococcus aureus* dehydrosqualene synthase (PDB ID: 2ZCQ).

Test compounds	Interacting amino acids	Binding energy, ΔG (kcal/Mol)	Dissociation constant (kI) (μM)
9b	Arg265(2)	−6.96	7.87
9c	Arg265(2)	−8.05	1.26
9d	Arg52	−6.42	19.82
9h	Arg265(2), Arg171	−7.21	5.19
10b	–	−6.93	8.33
10c	Arg265(2)	−7.04	6.89
10d	Mg453	−6.62	14.05
10e	Tyr183, Arg265 (2)	−6.55	15.71
10h	–	−6.41	20.16

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.03.007.

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