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Novel amalgamation of phthalazine–quinolines as biofilm inhibitors: One-pot synthesis, biological evaluation and in silico ADME prediction with favorable metabolic fate

Zahid Zaheer [a,*], Firoz A. Kalam Khan [a], Jaiprakash N. Sangshetti [a], Rajendra H. Patil [b], K. S. Lohar [c]

[a] Dr. Rafiq Zakaria Campus, Y.B. Chavan College of Pharmacy, Aurangabad 431001, M.S., India
[b] Department of Biotechnology, Savitribai Phule Pune University, Pune 411007, M.S., India
[c] Materials Research Laboratory, Srikrishna Mahavidyalaya Gunjoti, Omerga, Osmanabad 413 613, M.S., India

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ABSTRACT

A facile and highly efficient one-pot synthesis of phthalazine–quinoline derivatives is reported via four component reaction of phthalic anhydride, hydrazine hydrate, 5,5-dimethyl 1,3 cyclohexanedione and various quinoline aldehydes using $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles as a catalyst. The synthesized compounds have been evaluated for anti-biofilm activity against *Pseudomonas aeruginosa* and *Candida albicans*. The compounds **12a** (IC_{50} = 30.0 μM) and **12f** (IC_{50} = 34.5 μM) had shown promising anti-biofilm activity against *P. aeruginosa* and *C. albicans*, respectively, when compared with standards without affecting the growth of cells (and thus behave as anti-quorum sensing agents). Compounds **12a** (MIC = 45.0 μg/mL) and **12f** (MIC = 57.5 μg/mL) showed significant potent antimicrobial activity against *P. aeruginosa* and *C. albicans*, respectively. Thus, the active derivatives were not only potent biofilm inhibitors but also efficient antimicrobial agents. In silico ADME and metabolic site prediction studies were also held out to set an effective lead candidate for the future antimicrobial drug discovery initiatives.

The treatment of microbial infections still remains a very challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens.[1] A number of life threatening infections caused by multi-drug resistant microbial pathogens have reached an alarming level in the hospitals and the community.[2] Treatment is further hampered when the microbes are growing in biofilms.[3] Biofilms are defined as conglomerations of microbial cells protected by a self-synthesized extrapolymeric substance (EPS).[4] By forming biofilm, microorganisms are protected inside, and show an increased resistance to antimicrobial agents including antibiotics. As a consequence infections caused by microbial biofilms are generally chronic and very difficult to eradicate.[5]

The formation of biofilm is quorum sensing (QS) mediated phenomenon. The QS is an inter-cell communication system aided by released chemical signals when cell density reaches a critical concentration.[6] In particular, *Pseudomonas aeruginosa* is the Gram-negative bacterium responsible for biofilm growth has attracted considerable attentions, as this pathogen are able to form biofilms in lungs, kidney, urinary tract, causing inflammations and septic shock in patients. Thus, *P. aeruginosa* is used as model organism to study bacterial biofilm inhibition.[7] *Candida albicans* is an opportunistic fungal that can cause superficial and systemic infections in immunocompromised patients.[8] It has been demonstrated that Candida species are important cause of morbidity and mortality in hospitalized patients by promoting blood stream infections, with an elevated mortality (20–60%).[9] *C. albicans* is able to promote the transition from budding yeast form to filamentous form, producing biofilms, which is a critical step in colonization and can determine the fugal virulence. Of particular clinical significance, it has been demonstrated that biofilms formed by *C. albicans* are generally resistant to different antifungal drugs.[10] Therefore, the development of more effective antimicrobial therapies; especially against infections associated with biofilm formation is highly required.

Nitrogen containing heterocyclic compounds constitutes a huge group of organic compounds playing a key role in drug discovery because of their biological properties. Phthalazine framework is a key structural fragment of many heterocyclic compounds showing a broad spectrum of biological activities like antidiabetic,[11] anticancer,[12] antihypertensive,[13] anticonvulsant,[14] antiparasitic,[15] as well as antimicrobial[16] activities. Quinoline ring is another important nitrogen containing heterocycle and exhibits potent biological

* Corresponding author. Tel./fax: +91 240 2381129.
E-mail address: zahidzresearch@gmail.com (Z. Zaheer).

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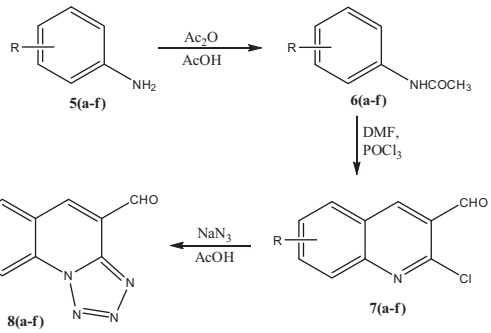
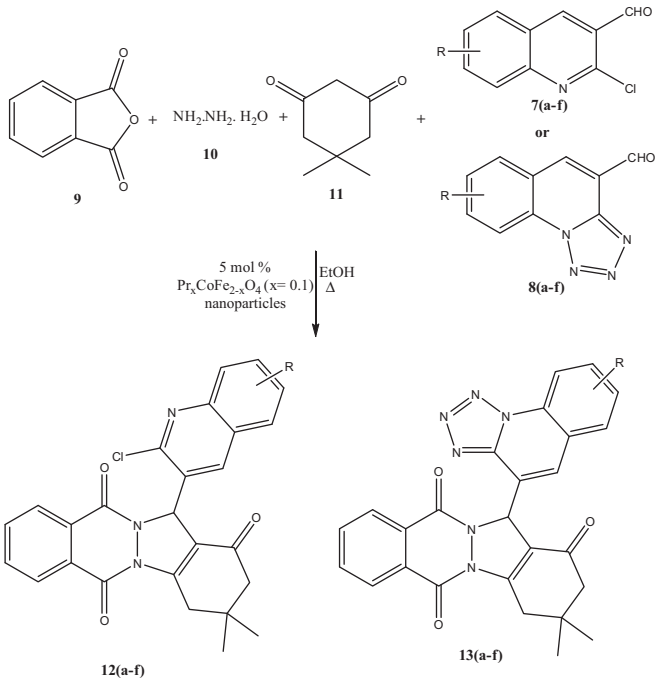
activities like analgesic,[17] antiallergenic,[18] anti-alzheimer,[19] anti-cancer,[20] antileishmanial[21] and antimicrobial[22] activities. Structures of some reported antimicrobial agents bearing phthalazine and quinoline rings are presented in Figure 1. Based on the importance of the two scaffolds, it was proposed to conceive both the scaffolds in a single molecule to have promising activity. Taking into account all of the aforementioned, and as a part of our ongoing effort towards identifying novel bioactive compounds,[23,24] we decided to explore ways of novel amalgamation of phthalazine and quinoline nucleus and the study of their effects on inhibition of *P. aeruginosa* and *C. albicans* biofilm. The synthesized compounds, that is, phthalazine–quinoline derivatives were also evaluated for antibacterial against *P. aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* and antifungal activity against *C. albicans*. We have used in silico method to predict ADME properties to suggest the suitability of any of the new compounds for further drug development, particularly with respect to oral activity. We have also performed the in silico study to analyze the metabolic site prediction of phthalazine–quinoline derivatives.

The quinoline aldehydes **7**(**a**–**f**) and **8**(**a**–**f**) were synthesized using various amines **5**(**a**–**f**) as per reported method (Scheme 1).[25] One-pot four component synthesis has been employed for synthesis of phthalazine–quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**) as presented in Scheme 2. These compounds were obtained via reaction of phthalic anhydride **9** (0.1 mmol), hydrazine hydrate **10** (0.1 mmol), 5,5-dimethyl 1,3-cyclohexanedione **11** (0.1 mmol) and various quinoline aldehydes (0.1 mmol) **7**(**a**–**f**) and **8**(**a**–**f**) in ethanol using 5 mol % of $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles as a novel magnetically recoverable and reusable catalyst. $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles were prepared and characterized by our group[26] (Supporting data, Fig. S1). With the increasing demand for 'green chemistry', the use of efficient and recoverable supported heterogeneous catalysts becomes one of the most important topics of research in synthetic organic chemistry, material science, and engineering. One of the most attractive alternatives to catalyst are use magnetic nanoparticles (MNPs), which have witnessed increasing popularity due to their high surface areas and improved dispersability in the reaction medium.[27] Specifically, MNPs catalysts can be recovered using an external magnet due to the paramagnetic character. This makes the removal and recycling of the catalyst much easier than filtration and centrifugation. Inspired by the utilization of $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles as magnetically recoverable and reusable catalyst and as a part of our continuous interest in the field of multicomponent reactions,[28] herein we wish to report an efficient one-pot four component synthesis of novel phthalazine–quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**) in high yields and short reaction times by using $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles as a green, robust and easily recoverable catalyst (Scheme 2).



Scheme 1. Synthesis of some quinoline aldehydes **7**(**a**–**f**) and **8**(**a**–**f**).



Scheme 2. One-pot four components synthesis of titled phthalazine-quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**).

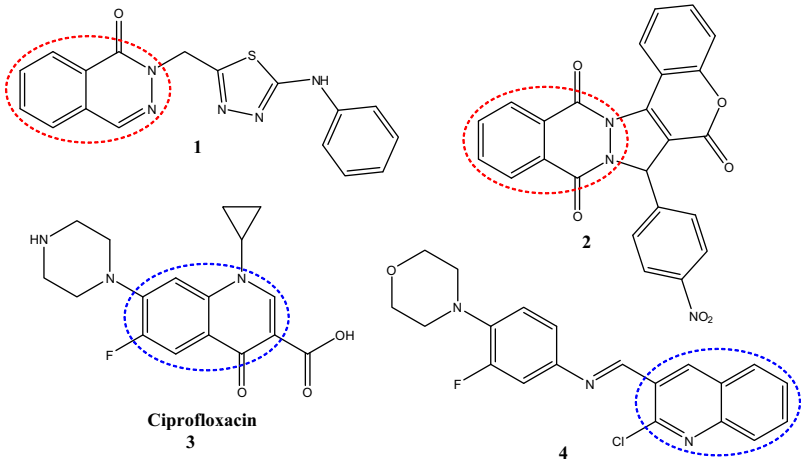
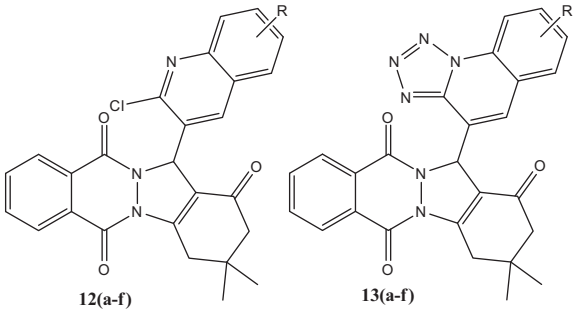


Figure 1. Structures of some antimicrobial agents reported in literatures.

Table 1
Physical data for phthalazine–quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**)



Entry	R	Molecular formula	Time (min)	Yield (%)	R_f value	Mp (°C)
12a	H	$C_{26}H_{20}ClN_3O_3$	45	94	0.54	254–256
12b	6-CH_3	$C_{27}H_{22}ClN_3O_3$	50	86	0.52	222–224
12c	7-CH_3	$C_{27}H_{22}ClN_3O_3$	45	92	0.58	238–240
12d	8-CH_3	$C_{27}H_{22}ClN_3O_3$	60	89	0.49	230–232
12e	6-OCH_3	$C_{27}H_{22}ClN_3O_4$	55	90	0.45	266–268
12f	7-OCH_3	$C_{27}H_{22}ClN_3O_4$	60	88	0.42	280–282
13a	H	$C_{26}H_{20}N_6O_3$	50	92	0.51	232–234
13b	6-CH_3	$C_{27}H_{22}N_6O_3$	50	90	0.46	218–220
13c	7-CH_3	$C_{27}H_{22}N_6O_3$	60	85	0.48	214–216
13d	8-CH_3	$C_{27}H_{22}N_6O_3$	65	89	0.50	228–230
13e	6-OCH_3	$C_{27}H_{22}N_6O_4$	50	93	0.55	244–246
13f	7-OCH_3	$C_{27}H_{22}N_6O_4$	55	84	0.46	220–222

To optimize the reaction conditions, we studied a model reaction of phthalic anhydride **9**, hydrazine hydrate **10**, 5,5-dimethyl 1,3-cyclohexanedione **11** and aldehyde **7a** in ethanol to synthesize compound **12a**. We screened the $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles at various loads such as 0, 5, 10, 15 and 20 mol % (Supporting data, Table S1). When no catalyst was added for model reaction, there was only trace amount (yield ~10%) of product obtained after 180 min. The use of 5 mol % nanoparticles gave the compound **12a** with 94% yield in short reaction time (45 min). The increase in amount of catalyst from 5 mol % to 10, 15 and 20 mol % did not show any change in yield and time of reaction (Supporting data, Table S1). Therefore, 5 mol % of the catalyst $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles was assumed to ensure the best yield (94%) in short reaction time (45 min). Thus, our results make the process under study more attractive and interesting from the viewpoint of economy and simplicity. The recovery and reusability of the catalyst was investigated in this reaction for model reaction (**12a**). After completion of reaction (monitored by TLC), catalyst recycling was achieved by fixing the catalyst magnetically at the bottom of the flask with a strong magnet, after which the solution was taken off with a pipette, the solid washed twice with acetone and the fresh substrates with solvent was introduced into the flask, allowing the reaction to proceed for the next run. The catalyst was consecutively reused three times without any noticeable loss of its catalytic activity (Cycle number and yield of **12a**: 1, 94%; 2, 94%; 3, 93%). So the catalyst was found to be recyclable and reusable.

We further expanded our series and synthesized 12 novel phthalazine–quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**). The isolated yields of synthesized compounds were in the range of 84–94% and reactions were completed in about 45–65 min (monitored by TLC). Melting points were determined in open capillary tubes and are uncorrected. The physical data for the compounds are presented in Table 1. The formation of synthesized compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis (Supplementary data) and data suggested for proposed structures.

The synthesized phthalazine–quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**) were evaluated for anti-biofilm activity to explore a possible role of phthalazine–quinoline derivatives in inhibiting/impeding the formation of biofilm in *P. aeruginosa* and *C. albicans*. Most of the microbes acquire the resistance to antimicrobial drugs by virtue of the synthesis of an extracellular matrix (biofilm). So, an anti-biofilm approach is the promising approach to tackle the aforementioned problem. The biofilm inhibition study was performed using crystal violet assay retention method using ciprofloxacin and fluconazole as standard.[29] This method is based on hypothesis that higher the biofilm formation greater the extent of absorption of crystal violet and, thus less is the effectiveness of the compounds. The IC_{50} value (concentration that decreased biofilms by 50%) of synthesized compounds is presented in Table 2.

The synthesized compounds **12**(**a**–**f**) and **13**(**a**–**f**) had shown good biofilm inhibition activity (IC_{50} range = 30.0–73.5 μM) against *P. aeruginosa* strain. Compounds **12a** (IC_{50} = 30 μM), **12b** (IC_{50} = 46.5 μM) and **13e** (IC_{50} = 46.5 μM) were more active than standard ciprofloxacin (IC_{50} = 47 μM). The structure–activity relationship (SAR) revealed that the compounds showed varied biofilm inhibition activity depending upon the various substituents present on quinoline ring (–*R*). Compound **12a** (IC_{50} = 30.0 μM) without any substitution on 2-chloroquinoline ring showed most potent biofilm inhibition activity among the synthesized compounds. Substitution on 2-chloroquinoline ring led to decrease in biofilm inhibition of *P. aeruginosa*. Compounds **12b** (IC_{50} = 46.5 μM) with 6-mehyl substitution on 2-chloroquinoline ring showed equipotent activity when compared with standard ciprofloxacin (IC_{50} = 47.0 μM). The replacement of 6-methyl **12b** group with 6-methoxy **12e** (IC_{50} = 73.5 μM) further led to decrease in biofilm inhibition activity. Compound **12c** (IC_{50} = 51.0 μM) with 7-methyl substitution on 2-chloroquinoline ring showed significant biofilm inhibition activity when compared with standard ciprofloxacin. Further, replacement of 7-methy group **12c** with 7-methoxy **12f** (IC_{50} = 58.5 μM) led to decrease in activity. Compound **12d** (IC_{50} = 66.0 μM) with 8-methyl group substitution on 2-chloroquinoline ring showed decrease in biofilm inhibition activity when compared with compounds **12b** (6-methyl) and **12c** (7-methyl).

The introduction of the tetrazole ring into a molecule of an organic substrate quite often leads to an increase in the prolongation of drug action and this is not accompanied by an increase in

Table 2
In vitro biofilm inhibition and antimicrobial activities of phthalazine–quinoline derivatives

Entry	Antibacterial activity				Antifungal activity	
	P. aeruginosa biofilm inhibition (IC_{50} μM)	MIC values in μg/mL			*C. albicans* biofilm inhibition (IC_{50} μM)	MIC values in μg/mL
		P. aeruginosa	*B. subtilis*	*S. aureus*		*C. albicans*
12a	30.0	45.0	148.5	178.5	79.5	125.0
12b	46.5	150.0	157.5	77.5	51.0	175.0
12c	51.0	185.0	87.5	70.0	49.5	112.5
12d	66.0	120.0	142.5	67.5	37.5	50.0
12e	73.5	205.0	57.5	77.5	36.0	55.0
12f	58.5	110.0	98.5	102.0	34.5	57.5
13a	66.0	68.5	100.0	85.0	49.5	112.5
13b	64.5	67.5	157.5	225.0	70.5	67.5
13c	51.0	102.0	157.5	112.0	67.5	55.0
13d	61.5	84.5	170.0	91.5	61.5	175.0
13e	46.5	67.5	187.5	225.0	81.0	195.0
13f	72.0	250.0	168.0	195.0	43.5	220.0
Ciprofloxacin	47.0	50.0	50.0	25.0	—	—
Fluconazole	—	—	—	—	40.0	50.0

Experiments were performed in triplicates and compared to DMSO-treated controls; standard errors were all within 10% of the mean.

acute toxicity.[30] Inspired by this fact, we introduced the tetrazole in quinoline ring and synthesized the 6 tetrazole derivatives **13** (**a**–**f**). Compound **13a** (IC_{50} = 66.0 μM) without any substitution on quinoline ring showed decrease activity when compared with compound **12a**. Introduction of 6-methyl group **13b** (IC_{50} = 64.5 - μM) on quinoline ring did not show any significant change in biofilm inhibition activity. The replacement of 6-methyl **13b** with 6-methoxy **13e** (IC_{50} = 46.5 μM) showed increase in activity with 1.5 fold and also equipotent activity when compared with standard ciprofloxacin (IC_{50} = 47.0 μM). Compound **13c** (IC_{50} = 51.0 μM) with 7-methyl group at quinoline ring showed significant biofilm inhibition activity when compared with standard ciprofloxacin. When we replaced the 7-methyl **13c** with 7-methoxy **13f** (IC_{50} = 72.0 μM), the biofilm inhibition activity was reduced by 1.5 fold. The introduction of 8-methyl group **13d** (IC_{50} = 61.5 μM) led to decrease in activity when compared with standard ciprofloxacin.

We have also evaluated for biofilm inhibition activity against *C. albicans*. The synthesized compounds had shown good to moderate activity. Compounds **12d** (IC_{50} = 37.5 μM), **12e** (IC_{50} = 36.0 μM) and **12f** (IC_{50} = 34.5 μM) were found to be show potent biofilm inhibition activity against *C. albicans* when compared with standard fluconazole (IC_{50} = 40.0 μM). The compound **13f** (IC_{50} = 43.5 μM) had shown significant biofilm inhibition activity when compared with standard fluconazole. Compound **12a** (IC_{50} = 79.5 μM) without any substitution on 2-chloroquinoline ring showed no significant biofilm inhibition activity when compared with standard fluconazole (IC_{50} = 40.0 μM). The introduction of electron donating groups like $-CH_3$ and $-OCH_3$ led to increase in biofilm inhibition activity. Compound **12b** (IC_{50} = 51.0 μM) with 6-methyl group on 2-chloroquinoline ring showed increase activity when compared with compound **12a**. Replacement of 6-methyl **12b** with 6-methoxy **12e** (IC_{50} = 36.0 μM) showed increase in biofilm inhibition activity by 1.5 fold. Introduction of 7-methyl group **12c** (IC_{50} = 49.5 μM) did not show any significant change in activity when compared with compound **12b**. Replacement of 7-methyl **12c** with 7-methoxy **12f** (IC_{50} = 34.5 μM) led to most potent biofilm inhibition activity against *C. albicans* among the synthesized compounds. Introduction of 8-methyl group **12d** (IC_{50} = 37.5 μM) on 2-chloroquinoline ring also showed potent activity when compared with standard fluconazole. Among tertrazole derivatives **13**(**a**–**f**), only compounds **13a** (IC_{50} = 49.5 μM) without any substitution and **13f** (IC_{50} = 43.5 μM) with 7-methoxy substitution on quinoline ring had shown significant biofilm inhibition activity when compared with standard fluconazole (IC_{50} = 40.0 μM). All other synthesized compounds **13b** (IC_{50} = 70.5 μM), **13c** (IC_{50} = 67.5 μM), **13d** (IC_{50} = 61.5 μM) and **13e** (IC_{50} = 81.0 μM) showed less activity when compared with fluconazole.

Having identified the leads with potent anti-biofilm activity and to better understand the biofilm inhibition by compounds, we performed the field emission scanning electron microscopy (FESEM) analysis of most active compounds **12a** (biofilm inhibition of *P. aeruginosa*) and **12f** (biofilm inhibition of *C. albicans*). In absence of compounds, cells of *P. aeruginosa* and *C. albicans* were seen as enmeshed-covered structures, and cells were enclosed, a typical of biofilm structures. When the cells of *P. aeruginosa* and *C. albicans* were subjected to inhibitory concentration of compounds **12a** and **12f**, respectively, there was a prominent decrease in the biofilm formation and cells were seen as spatially distributed. More importantly, the numbers of the planktonic cells (cells in suspension) were not affected, suggesting that the inhibition of the biofilm in *P. aeruginosa* and *C. albicans* are QS mediated (Fig. 2).[29]

The main problem associated to biofilm infections is the dissemination of biofilm cells into the systemic circulation. In order to colonize new surfaces and to prevent density-mediated starvation within the mature biofilm, the cells detach and disseminate.[31] Dispersal is accomplished by shedding, detachment, or shearing. This may result in bloodstream infection, depending on the host immune system and bioburden of cells released. Single cells released by shedding are susceptible to antibiotics and can be controlled by antimicrobial therapy and/or the host's immune system. However, those released in clumps retain antimicrobial resistance and may embolize at a distant anatomic site to develop metastatic infections such as endocarditis or osteomyelitis.[32] Therefore, we have evaluated these compounds for antibacterial activity against two Gram-positive bacteria, namely *B. subtilis* (NCIM-2063), and *S. aureus* (NCIM-2901) and one Gram-negative bacterium, namely *P. aeruginosa* (NCIM-2036) and antifungal activity against *C. albicans* (NCIM-3471). Interestingly, our results demonstrated that most potent biofilm inhibitors **12a** (MIC = 45.0 μg/mL) and **12f** (MIC = 57.5 μg/mL) showed also a significantly potent antimicrobial activity against *P. aeruginosa* and *C. albicans*, respectively, when compared with standards (Table 2).

Minimum inhibitory concentration (MIC) values for antibacterial activity were determined using standard agar method[33] using ciprofloxacin as standard. Dimethyl sulfoxide was used as solvent control. From activity data (Table 2), the synthesized compounds **12**(**a**–**f**) and **13**(**a**–**f**) had exhibited moderate to good antibacterial activity. Compound **12a** (MIC = 45.0 μg/mL) showed better activity against *P. aeruginosa* when compared with standard ciprofloxacin

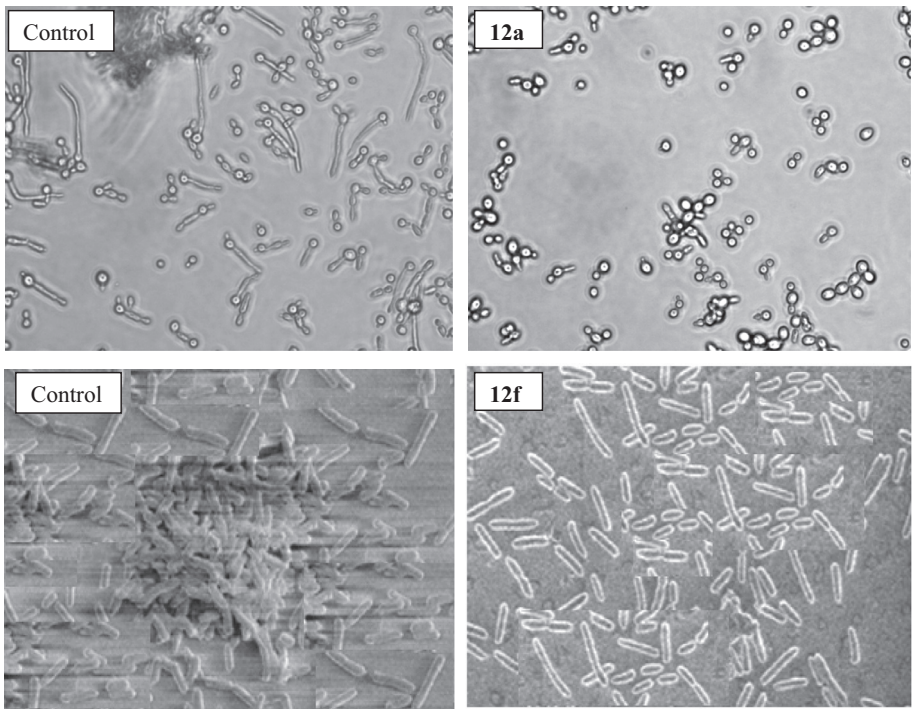


Figure 2. (a) Upper panel: inhibition of *P. aeruginosa* biofilm (FESEM images). FESEM analysis of *P. aeruginosa* biofilm (control) shows bunches of cells surrounded by biofilm. However, in presence of compound **12a** individual cells were observed, indicating an inhibition of biofilm formation; (b) lower panel: Inhibition of *C. albicans* biofilm (FESEM images). FESEM analysis of *C. albicans* biofilm (control) shows bunches of cells surrounded by biofilm. However, in presence of compound **12f** individual cells were observed, indicating an inhibition of biofilm formation.

(MIC = 50.0 μg/mL). All the other synthesized compounds (MIC range = 57.5–250.0 μg/mL) had shown less activity than standard ciprofloxacin against all the tested bacterial strains. Compounds **13a** (MIC = 68.5 μg/mL), **13b** (MIC = 67.5 μg/mL) and **13e** (MIC = 67.5 μg/mL) against *P. aeruginosa* and compound **12e** (MIC = 57.5 μg/mL) against *B. subtilis* showed comparable antibacterial activity when compared with standard ciprofloxacin (MIC = 50.0 μg/mL). Compound **13a** (MIC range = 68.5–100.0 μg/mL) can be said to have broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The structure–activity relationship (SAR) studies for antibacterial activity revealed that unsubstituted 2-chloroquinoline ring **12a** (MIC = 45.0 μg/mL) is good for antibacterial activity against *P. aeruginosa* (Gram-negative). Introduction of substituents like methyl and methoxyl on 2-chloroquinoline ring led to decrease antibacterial activity against *P. aeruginosa*. Among tetrazole series **13**(**a**–**f**), unsubstituted quinoline ring **13a** (MIC = 68.5 μg/mL) had shown improved activity against *P. aeruginosa*. Introduction of 6-methyl **13b** (MIC = 67.5 μg/mL) and 6-methoxy **13e** (MIC = 67.5 μg/mL) have comparable antibacterial activity with ciprofloxacin (MIC = 50.0 μg/mL) against *P. aeruginosa*. Introduction of 6-methoxy **12e** (MIC = 57.5 μg/mL) had shown significant against *B. subtilis* when compared with standard ciprofloxacin (MIC = 50.0 μg/mL). Introduction of electron donation group like, methyl **12b** (MIC = 77.5 μg/mL), **12c** (MIC = 70.0 μg/mL), and **12d** (MIC = 67.5 μg/mL) and methoxyl **12e** (MIC = 77.5 μg/mL), and **12f** (MIC = 102.0 μg/mL) on 2-chloroquinoline ring had shown the improved activity against *S. aureus* when compared with without any substitution on 2-chloroquinoline ring **12a** (MIC = 178.5 μg/mL).

Minimum inhibitory concentration (MIC) values for antifungal activity against *C. albicans* were determined using standard agar method[33] using fluconazole as standard. Dimethyl sulfoxide was used as solvent control The results of in vitro antifungal activity (Table 2) showed that synthesized compounds **12**(**a**–**f**) and **13**(**a**–**f**) have moderate to good activity. Comparison of antifungal activity of compounds with that of antifungal drug fluconazole (MIC = 50.0 μg/mL), showed that compound **12d** (MIC = 50.0 μg/mL) had same antifungal profile against *C. albicans*. Compounds **12e** (MIC = 55.0 μg/mL), **12f** (MIC = 57.5 μg/mL), **13b** (MIC = 67.5 μg/mL) and **13c** (MIC = 55.0 μg/mL) had shown comparable antifungal activity when compared with fluconazole. All the other synthesized compounds, that is, **12a** (MIC = 125.0 μg/mL), **12b** (MIC = 175.0 μg/mL), **12c** (MIC = 112.5 μg/mL), **13a** (MIC = 112.5 μg/mL), **13d** (MIC = 175.0 μg/mL), **13e** (MIC = 195.0 μg/mL) and **13f** (MIC = 220.0 μg/mL) were found less active with fluconazole. Structure–activity relationship of synthesized compounds revealed that scaffold containing phthalazine and quinoline shows considerable antifungal activity. As observed through data analysis, the introduction of 6-methoxy **12e** (MIC = 55.0 μg/mL) and 7-methoxy **12f** (MIC = 57.5 μg/mL) group on 2-chloroquinoline nucleus led to increase in antifungal activity when compared with compounds with 6-methyl **12b** (MIC = 175.0 μg/mL) and 7-methyl **12c** (MIC = 112.5 μg/mL) substituent on 2-chloroquinoline nucleus. Introduction of 8-methyl group on 2-chloroquinoline nucleus led to the most active antifungal compound **12d** (MIC = 50.0 μg/mL) amongst synthesized compounds. Among the tetrazole series **13**(**a**–**f**), introduction of 6-methyl **13b** (MIC = 67.5 μg/mL) and 7-methyl **13c** (MIC = 55.0 μg/mL) on quinoline ring led to increase in antifungal activity when compared with compound **13a** (MIC = 112.5 μg/mL) without any substitution on quinoline. Replacement of methyl group with methoxyl group **13d** (MIC = 175.0 μg/mL) and **13f** (MIC = 220.0 μg/mL) led to decrease in antifungal activity.

Late phase recognition of depressed pharmacokinetics of drugs can cause vast loss in the drug development process, thus, initial evaluation of ADME (Adsorption, Distribution, Metabolism, Excretion and Toxicity) is an only alternative for an effective drug development process. Therefore, a computational study of synthesized

Table 3
In silico physicochemical pharmacokinetic parameters important for good oral bioavailability of phthalazine–quinoline derivatives

Entry	% ABS	TPSA (A^2)	MV	MW	miLog*P*	*n*-ON acceptors	*n*-OHNH donors	Lipinski's violations
Rule	–	–	–	<500	⩽5	<10	<5	⩽1
12a	83.48	73.97	386.29	457.92	4.08	6	0	0
12b	83.48	73.97	402.85	471.94	4.50	6	0	0
12c	83.48	73.97	402.85	471.94	4.50	6	0	0
12d	83.48	73.97	402.85	471.94	4.48	6	0	0
12e	80.29	83.21	411.84	487.94	4.11	7	0	0
12f	80.29	83.21	411.84	487.94	4.11	7	0	0
13a	73.06	104.17	393.80	464.49	2.77	9	0	0
13b	73.06	104.17	410.36	478.51	3.19	9	0	0
13c	73.06	104.17	410.36	478.51	3.19	9	0	0
13d	73.06	104.17	410.36	478.51	3.17	9	0	0
13e	69.87	113.41	419.35	494.51	2.80	10	0	0
13f	69.87	113.41	419.35	494.51	2.80	10	0	0

% ABS: percentage absorption, TPSA: topological polar surface area, MV: molecular volume, MW: molecular weight, miLog*P*: logarithm of partition coefficient of compound between *n*-octanol and water, *n*-ON acceptors: number of hydrogen bond acceptors, *n*-OHNH donors: number of hydrogen bonds donors.

compounds **12**(**a**–**f**) and **13**(**a**–**f**) was performed for assessment of ADME properties and value obtained is depicted in Table 3. We had analyzed various pharmaceutically relevant properties like, topological polar surface area (TPSA), molecular volume (MV) and Lipinski's rule of five using Molinspiration online property calculation toolkit.[34] Absorption (% ABS) was calculated by: % ABS = 109 − (0.345 × TPSA).[35] From all these parameters, it can be observed that all the synthesized compounds exhibited good % absorption (69.77–83.48%). The most active compounds **12a** and **12f** showed 83.48% and 80.29% absorption, respectively. As per Lipinski's rule-of-five, A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: log*P* (octanol–water partition coefficient) ⩽5, molecular weight ⩽500, number of hydrogen bond acceptors ⩽10 and number of hydrogen bond donors ⩽5.[36] None of the compounds violated Lipinski's rule of five and thus showing possible utility of series for developing the compound with drug like properties. The compounds which show the topological polar surface area (TPSA) ⩽140 Å are expected to have proper oral bioavailability. TPSA is a parameter used to predict transport properties of drugs and used for passive molecular transport of drug molecules.[37] The compounds showed TPSA value ranges between 73.97 and 113.41 Å which showed good cell permeability. The results of this in silico ADME prediction analysis suggest that the synthesized compounds follow the criteria for orally active drug and thus represent a pharmacologically active framework that should be considered on progressing further potential hits.

MetaPrint2D-React, a metabolic product predictor developed by Unilever Cambridge, Centre for Molecular Science Informatics, University of Cambridge, UK. It is a tool for predicting the sites of a molecule based on historic metabolic data, described by circular fingerprints that are most likely to undergo Phase I metabolism, anchored in their similarity to known sites of metabolism and sites that are known not to be metabolized. The MetaPrint2D data is generated through processing of the transformations found in the Symyx Metabolite database. For each transformation, the differences between the structure of the reactant and product are identified: groups added or eliminated, bonds broken or made and bonds whose order has changed. With the intention of simplify the results, only Phase I additions (defined as the addition of a single oxygen atom; covering hydroxylation, oxidation and epoxidation), and eliminations (e.g., dealkylation, ester and amide hydrolysis) are engaged. For an addition, the atom neighboring the added oxygen is marked as a reaction center. In the case of elimination, a bond gets broken, and both atoms connected by the bond are considered to be reaction centers.[38] Most active compounds **12a** (Fig. 3) and **12f** (Fig. 4) were analyzed through web server of MetaPrint2D-React for prediction of possible metabolic pathways and the predicted site of metabolism (only Human).[39] The color highlighting an atom indicates its NOR (Normalized

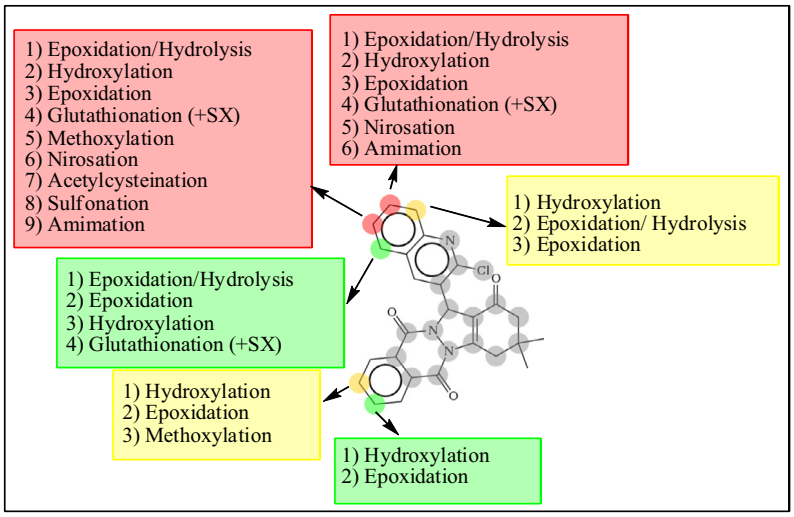


Figure 3. Possible ways of metabolic deactivation by MetaPrint-2D React of compound **12a**.

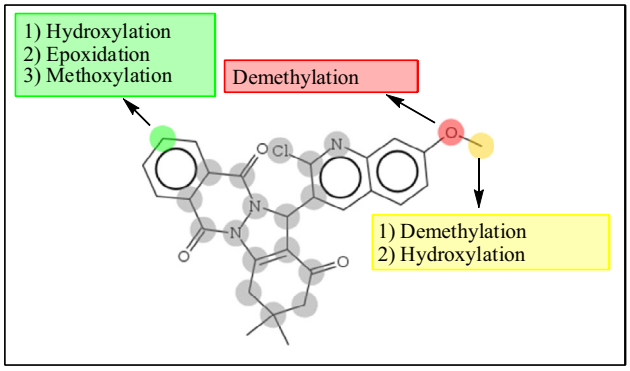


Figure 4. Possible ways of metabolic deactivation by MetaPrint-2D React of compound **12f**.

Occurrence Ratio). This NOR indicates the relative likelihood of each atomic site in a molecule being a center of metabolism, while making no prediction as to the absolute likelihood of the molecule undergoing metabolic transformation.

The NOR ratio for **12a** was observed as Red $0.66 \leqslant NOR \leqslant 1.00$, Orange $0.33 \leqslant NOR < 0.66$, Green $0.15 \leqslant NOR < 0.33$, White (No color) $0.00 \leqslant NOR < 0.15$, Gray Little/no data. The NOR ratio for **12f** was observed as Red $0.66 \leqslant NOR \leqslant 1.00$, Orange $0.33 \leqslant NOR < 0.66$, Green $0.15 \leqslant NOR < 0.33$, White (No color) $0.00 \leqslant NOR < 0.15$, Gray Little/no data. A high NOR indicates a more frequently reported site of metabolism in the metabolite database. Whereas, the NOR does not show how likely a molecule is to be metabolized, but quite the relative probability of metabolism occurring at a particular site in the molecule, taking it is metabolized. It was indicated that skeleton of the target compounds were less prone to metabolic deactivation.

In conclusion, synthesis and biological activities of novel phthalazine–quinoline derivatives **12**(**a**–**f**) and **13**(**a**–**f**) have been presented. Use of 5 mol % of $Pr_xCoFe_{2-x}O_4$ ($x = 0.1$) nanoparticles as a catalyst helps in efficient one-pot four component synthesis of phthalazine–quinoline derivatives, therefore proving its advantage. Good yields and reusability of catalyst imparts further advantage of using it in the reaction. Thus, our results made the process under study more attractive and interesting from the viewpoint of economy and simplicity. Newly synthesized compounds were evaluated for anti-biofilm activity and compounds **12a** ($IC_{50} = 30.0$ μM) and **12f** ($IC_{50} = 34.5$ μM) had shown promising activity against *P. aeruginosa* and *C. albicans*, respectively. FESEM analysis of compounds **12a** and **12f** revealed that compounds had inhibited the biofilm without affecting planktonic cells growth. This is important because when the growth is not affected, there is no selective pressure for the development of resistant microbes. Compounds have been also evaluated for antibacterial and antifungal activity and compounds **12a** (MIC = 45.0 μg/mL) and **12f** (MIC = 57.5 μg/mL) showed significant potent antimicrobial activity against *P. aeruginosa* and *C. albicans*, respectively, when compared with standards. In silico ADME and metabolic sites prediction studies of synthesized compounds indicated that compounds had potential to develop as good oral drug like candidate. Thus, suggesting that the compounds from the present series can serve as important gateway for the design and development of new good oral drug-like anti-biofilm agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.057.

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