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Synthesis of a novel series of imines containing nitrogen heterocycles as promising antibacterial and antifungal agents

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Two biologically potential nitrogen heterocycles quinoline and 2-pyridone have been incorporated to synthesize imine series of 6-amino-1-((2,6-dichloroquinolin-3-yl)methyleneamino)-4-(aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**3a-m**). IR, ^{1}H and ^{13}C NMR, and mass spectrometry have accomplished structural evaluation. All these compounds have been screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* as well as antifungal activity against *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*. From the screened results, it has been observed that the compounds **3b** and **3c** are found to have excellent activity against bacterial strains whereas compound **3f** has unveiled excellent activity against fungal strains. SAR study has revealed that *para* and *meta* positions with electron donating functionality are promising sites for antibacterial and antifungal properties respectively.

Keywords: Nitrogen heterocycles, imines, dichloroquinoline, 2-pyridone, antibacterial activity, antifungal activity.

Mortality rate for infants in India is 38 per 1000 live births as per the data of World Bank analysis - 2015. This is further supported by the WHO's 2014 report on global surveillance of antimicrobial resistance[1]. Due to this reason, it is an urgent need to overcome antimicrobial resistance, which inspired us to explore new heterocyclic molecules that can be lead entities.

Imines of various compounds are reported to possess antiproliferative[2], anticonvulsant[3], cytotoxic[4], anti-cancer[5], antifungal and anti-HIV activities[6]. Schiff bases and their metal complexes show very good antibacterial activity against *E. coli* and *B. subtilis*. Nitrogen heterocycles being abundant in nature are of great significance to life because their structural subunits occur in many natural products as well as pharmaceuticals[7]. Due to this, synthesis of nitrogen containing heterocyclic compounds has gained much more importance in Medicinal Chemistry Research. Quinoline heterocycles have displayed interesting physiological activities and have found attractive applications as pharmaceuticals and general synthetic building blocks[8]. In the same way pyridin-2(1*H*)-ones are also known to possess a range of biological activities such as analgesic, antimalarial, anti-inflammatory, anti-HIV, phytotoxic, antitumoral and antiviral[9-17]. A few selected examples of drugs containing 2-pyridone include Amrinone (phosphodiester inhibitor)[18], Ciclopirox (an antifungal agent)[19], Diazaquinomycin A (anticancer)[20], Olprinone (cardiotonic agent)[21] and recently clinically used HIV-1 inhibitors (L-696,229 and L-697,661)[22,23]. It is an important pharmacophore that can form hydrogen bonded structures related to the base-pairing mechanism found in DNA and RNA[24,25].

Meshram G *et al*. synthesized several 3-(1*H*-benzimidazol-2-yl)-2-chloroquinolines and investigated *in vitro* antibacterial activity against *S. aureus* out of which several compounds showed promising activity. From the results of antibacterial screening, it was suggested that as far as the relation between structure and activity were concerned, derivatives of quinoline substituted at 6^{th} and 7^{th} positions were found to display higher dock score than the other substitutes[26]. These observations indicated the direction to develop new dichloroquinoline derivatives incorporated with potent 2-pyridone moiety possessing diverse biological activities that can be lead molecules for future development to get safer and effective biomolecules.

In continuation to our research on bio-active scaffolds[27-30] and to invent some novel antimicrobial agents that can open a new window into the complex task of antibiotic development in future, we have synthesized a novel series of 6-amino-1-

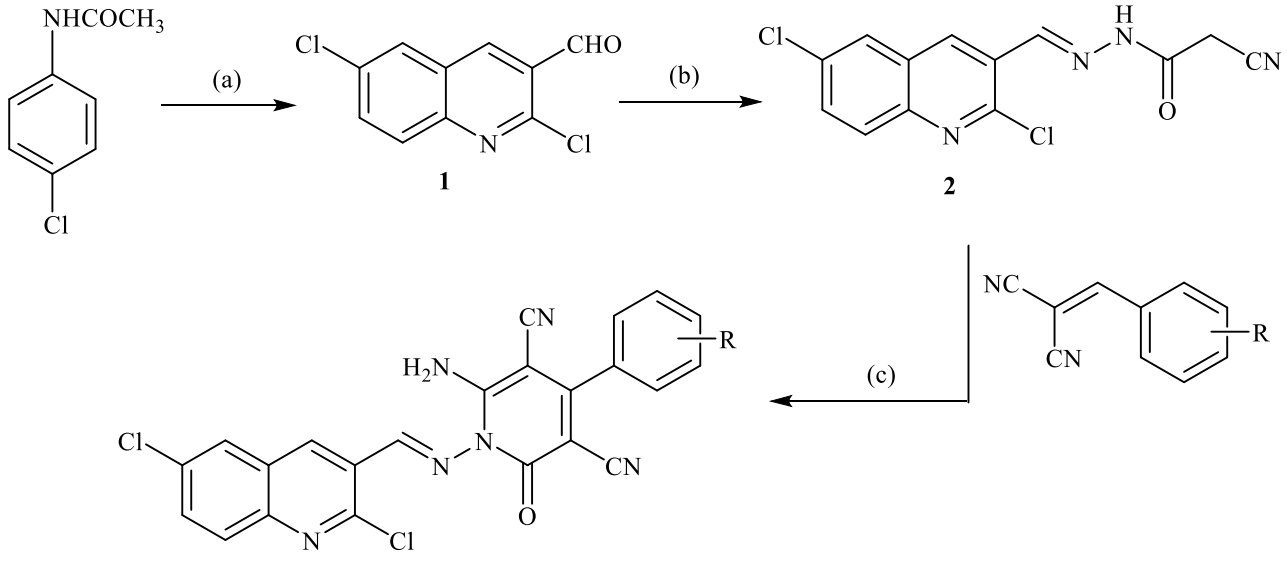
((2,6-dichloroquinolin-3-yl)methyleneamino)-4-(aryl) -2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles (**3a-m**) and screened against several strains of gram negative and gram positive bacteria and fungi.

Results and Discussion

Synthetic route for the preparation of target compounds **3a-m** is described in Scheme I. Vilsmeier Haack reaction was used to prepare the substrate 2,6-dichloroquinoline-3-carbaldehyde **1**. Compound **1** was reacted with 2-cyanoacetohydrazide using 1,4-dioxane as a solvent to obtain intermediate 2-cyano-*N*'-((2,6-dichloroquinolin-3-yl)methylene) acetohydrazide **2** which was cyclized with non-identical 2-arylidenemalononitriles using ethanol as solvent and piperidine as a catalyst to give a series of target imine compounds 6-amino-1-((2,6-dichloroquinolin-3-yl)methyleneamino)-4-(aryl)-2-oxo -1,2-dihydropyridine-3,5-dicarbonitriles **3a-m**.

A plausible mechanism for the synthesis of compounds **3a-m** is depicted in Scheme II. 2-arylidenemalononitriles (**B**) was synthesized by Knoevenagel condensation of various aromatic aldehydes with malononitrile. Hydrazone of dichloroquinoline (**A**) on Michael addition with 2-arylidene-malononitriles (**A**) resulted in intermediate (**C**), which in next step gave intermediate (**D**) by intramolecular nucleophilic cyclization. This was followed by intramolecular electron migration to the nitrogen atom to give targeted imine compounds **3a-m**.

The structural interpretation of all the target compounds **3a-m** was carried out by various spectroscopic techniques. Representative compound **3b** was taken into consideration to discuss spectroscopic data. Appearance of characteristic stretching absorption bands in IR spectra of compound **3b** at 3430 cm^{-1}, 2224 cm^{-1}, 1664 cm^{-1} and 768 cm^{-1} were due to presence of -NH_2,-CN, >C=O and -Cl respectively. ^{1}H NMR spectra gave singlets at δ 8.94 and 2.50 due to presence of aromatic primary amine group in 2-pyridone heterocycle and aromatic -CH_3 group respectively. Presence of imine (CH=N) functionality was indicated by appearance of singlet at δ 8.26, whereas



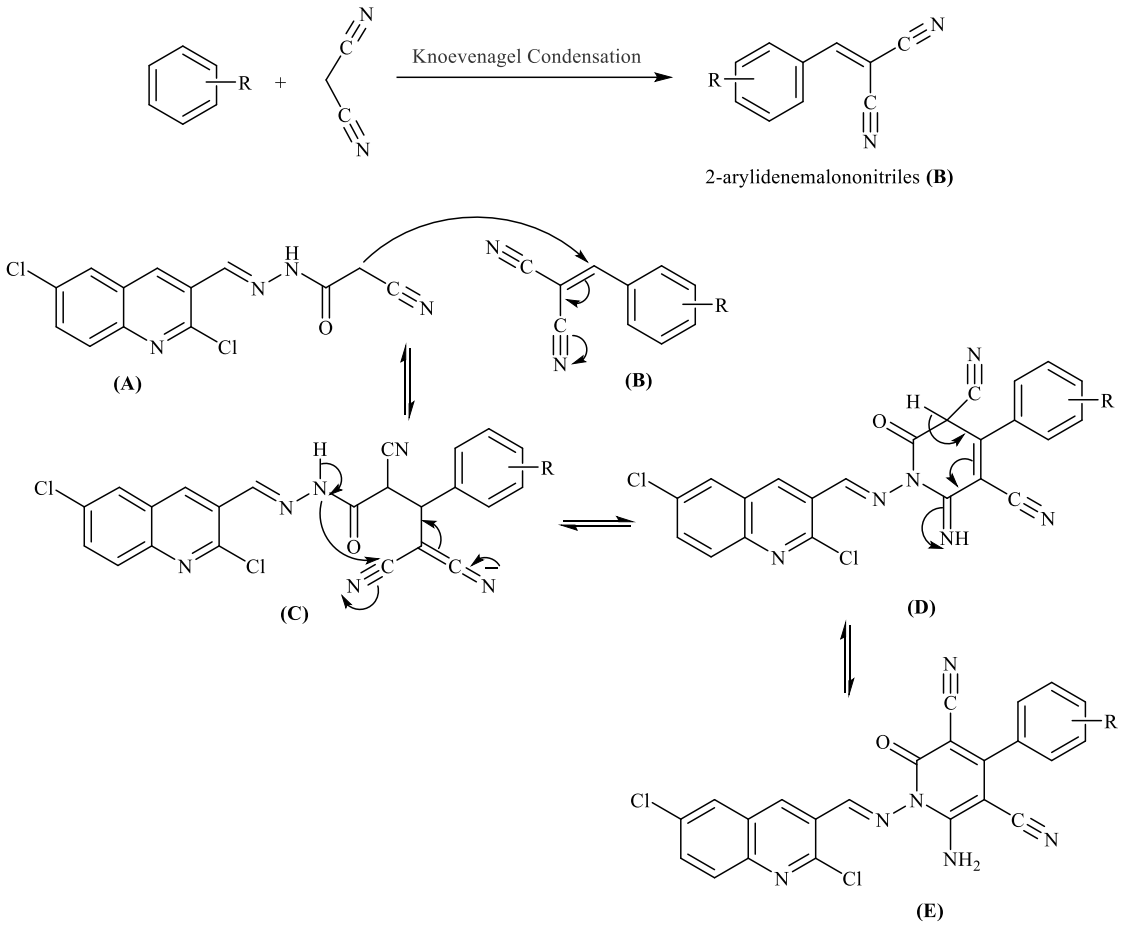
Where, R = **3a** -H, **3b** -4-CH_3, **3c** -4-OCH_3, **3d** -3,4,5-$(OCH_3)_3$, **3e** -2-OH, **3f** -3-OH, **3g** -4-OH, **3h** -4-OH-3-OCH_3, **3i** -3-NO_2, **3j** -4-NO_2, **3k** -4-N,N-$(CH_3)_2$, **3l** -4-F, **3m** -2-Cl

Reagents and conditions:

(a) DMF / $POCl_3$, 100 °C, reflux, 15 h; (b) $NH_2NHCOCH_2CN$, 1,4-dioxane, reflux, 1 h;

(c) Ethanol, Piperidine, 2-arylidenemalononitrile, reflux, 2-3 h.

Scheme I — Synthetic track for the preparation of title compounds **3a-m**



Scheme II — Plausible mechanism for synthesis of imine compounds **3a-m**

eight aromatic protons appeared as multiplet between δ 6.51− 9.20. Furthermore, appearance of characteristic peak in ^{13}C NMR spectra at δ 159.7 and 115.6 pointed out the presence of >C=O and -CN group in pyridone motif. Molecular ion peak at *m/z* 472.1 [M^+] in mass spectroscopic data was in accordance with molecular weight of compound **3b**. All the newly synthesized compounds were screened for their antibacterial and antifungal potential against varied bacterial and fungal strains by conventional broth-dilution method using Ciprofloxacin and Griseofulvin as standard drugs. The results of antimicrobial studies are represented in Table I.

Comparison of antibacterial and antifungal screening data revealed that compounds **3a-m** possessed better antibacterial potential than antifungal potential. Title compounds **3b**, **3c**, **3g** and **3k** displayed superlative antibacterial activity against all bacterial strains with MIC in the range of 12.5-100 µ g/mL. Compounds **3b** and **3c** emerged as outmost potent antibacterial agents with MIC in the range of 12.5-50 µ g/mL against all the bacterial strains, which was 2-4 fold higher than standard drug Ciprofloxacin. Compounds **3b** and **3g** were found to be equipotent to standard drug Ciprofloxacin against gram negative bacteria *S. pyogenes*. Compounds **3g** and **3k** possessed comparable antibacterial activity with MIC values 50-100 µ g/mL against all the bacterial strains. Assessment of antibacterial screening data reflected that substitution design of phenyl ring significantly affects the antibacterial potential. It has been revealed from the bioactivity data that compounds with ring activating functional groups on *para* position of phenyl ring were most potent whereas the presence of electron releasing functional group on *ortho* posit ion of phenyl ring was found to have comparable activity. On the other hand, compound **3f** having -OH group on *meta* position possesses excellent antifungal potential with MIC in the range of 12.5-50 µ g/mL with respect to standard drug Griseofulvin. This outcome of antifungal activity clearly directed that

Table I — Results of antibacterial and antifungal activities of compounds **3a-m**

Entry	-R	Minimum inhibitory concentration (MIC) µg/mL						
		Gram-negative[a]		Gram-positive[b]		Fungi[c]		
		Ec	Pa	Sa	Sp	Ca	An	Ac
3a	-H	250	500	500	500	1000	>1000	>1000
3b	-4-CH_3	12.5	12.5	12.5	50	>1000	500	500
3c	-4-OCH_3	12.5	12.5	25	25	>1000	100	1000
3d	-3,4,5-$(OCH_3)_3$	200	250	>1000	>1000	1000	>1000	>1000
3e	-2-OH	500	200	200	500	>1000	1000	>1000
3f	-3-OH	200	250	500	500	12.5	50	50
3g	-4-OH	100	100	100	50	1000	500	500
3h	-4-OH-3-OCH_3	500	250	200	150	>1000	100	500
3i	-3-NO_2	250	500	500	500	1000	500	500
3j	-4-NO_2	200	200	200	250	1000	1000	1000
3k	-4-N,N-$(CH_3)_2$	100	100	100	100	>1000	500	250
3l	-4-F	250	250	150	500	>1000	500	500
3m	-2-Cl	250	250	100	100	1000	500	250
	Ciprofloxacin	25	25	50	50	–	–	–
	Griseofulvin	–	–	–	–	500	100	100

[a]Ec: *Escherichia coli* MTCC 443; Pa: *Pseudomonas aeruginosa* MTCC 1688;
[b]Sa: *Staphylococcus aureus* MTCC 96; Sp: *Staphylococcus pyogenes* MTCC 442;
[c]Ca: *Candida albicans* MTCC 227; An: *Aspergillus niger* MTCC 282; Ac: *Aspergillus clavatus* MTCC 1323.

compound **3f** which was designed with ring activating substituent on *meta* position was found to be a most potent antifungal agent. In addition, compounds **3c** and **3h** were found to be as potent as Griseofulvin against *A. niger*.

Structure activity relationship

By equating, the data of antibacterial and antifungal screening it can be evaluated that compounds **3a-m** possessed better antibacterial potential than antifungal potential. Results of antimicrobial activity of compounds **3a-m** confer that inductive effect and position of substituent present in phenyl ring of 2-pyridone heterocycle greatly alter the antimicrobial activity. Compounds **3b** (-CH_3), **3c** (-OCH_3), **3g** (-OH) and **3k** [(N(-$CH_3)_2$] came out as most potent antibacterial entities with MIC in the range of 12.5-100 µ g/mL. The presence of ring activating functional groups on *para* position enhance the antibacterial potential, while ring activating substituents on *meta* position boosted the antifungal potential. The highest antifungal activity was observed when electron releasing functional group was present on *meta* position of phenyl ring (**3f**) while presence of ring deactivating substituent resulted in poor activity.

Biological evaluation

Antibacterial assay

The newly synthesized compounds were screened for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)) and Gram negative bacteria (*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)). Antibacterial activity was measured as per National Committee for Clinical Laboratory Standards (NCCLS) protocol by Mueller-Hinton Broth (Becton-Dickinson, USA)[31-33]. Standard strains were procured from the Institute of Microbial Technology, Chandigarh. Compounds were primarily screened for their antibacterial activity in six sets against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* at different concentrations of 1000, 500 and 250 µ g/mL. The drugs found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 µ g/mL concentrations for secondary screening to test in a second set of dilutions against all microorganisms. Inoculum size for test strain was adjusted to 10^6 CFU/mL (Colony Forming Units per milliliter) by comparing the turbidity (turbidimetric method). Mueller Hinton Broth was used as nutrient medium for growth of bacteria and

dilution of the compound suspension for test bacteria. 2% DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Synthesized compounds were diluted to 1000 µ g/mL concentration, as a stock solution. The control tube containing no antibiotic was immediately subcultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of test organisms. The tubes were then incubated at 37°C for 24 h for bacteria. The suspension of 10 µ g/mL was further inoculated on an appropriate media and growth was noted after 24 and 48 h. The highest dilution (lowest concentration) preventing appearance of turbidity was considered as minimum inhibitory concentration (MIC, µ g/mL) *i.e*. the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. A set of tubes containing only seeded broth and solvent controls were maintained under identical conditions to make sure that the solvent had no influence on strain growth. Dimethyl sulphoxide and sterilized distilled water were used as negative control while chloramphenicol (1 U strength) was used as positive control. The results are greatly affected by the size of inoculum. The test mixture should contain 10^6 CFU/mL of bacteria. The standard drug used in the present study was Ciprofloxacin for evaluating antibacterial activity, which showed 25, 25, 50 and 50 µ g/mL MIC against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* respectively.

Antifungal assay

The same compounds were tested for antifungal activity as primary screening in six sets against *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* at various concentrations of 1000, 500, 200 and 100 µ g/mL as shown in Table I. Results were recorded in the form of primary and secondary screening. The synthesized compounds were diluted to 1000 µ g/mL concentration, as a stock solution. Those synthesized compounds, which were found to be active in primary screening, were further tested in a second set of dilution against all fungi. Griseofulvin was used as a standard drug for antifungal activity, which showed 500, 100 and 100 µ g/mL MIC against *C. albicans*, *A. niger* and *A. clavatus* respectively. DMSO (2%) and sterilized distilled water were used as negative control, while Griseofulvin (1 U strength) was used as positive control. For fungal growth, in the present protocol, Sabourauds dextrose broth at 28°C was used under aerobic condition for 48 h.

Experimental Section

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Melting points were taken in open glass capillary tubes using a Toshniwal melting point apparatus and are uncorrected. TLC on silica gel plates (Merck, 60, F_{254}) was used for checking homogeneity and reaction monitoring. Column chromatography over silica gel (Merck, 70-230 mesh and 230-400 mesh ASTH for flash chromatography) was applied when necessary to isolate and purify the reaction products. Elemental analysis (% CHN) was carried out on a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Perkin-Elmer FT-IR spectrophotometer in KBr. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz and ^{13}C NMR spectra on Varian Mercury 400 at 100 MHz in $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer. Anhydrous reactions were carried out in oven-dried glass wares in nitrogen atmosphere and Büchi Rotavapor instrument was used for distillation purpose.

Preparation of 2,6-dichloroquinoline-3-carbaldehyde, 1

Synthesis of 2,6-dichloroquinoline-3-carbaldehyde **1** was achieved by reported literature method[34].

Preparation of 2-cyano-*N′*-((2,6-dichloroquinolin-3-yl)methylene)acetohydrazide, 2

2-Cyanoacetohydrazide (0.01 mol) was added drop-wise to the solution of compound **1** in 1,4-dioxane with stirring. The resulting mixture was refluxed for 1 h and cooled to RT. The separated solid was filtered and purified by recrystallization from a mixture of chloroform and methanol. Yield 88%. m.p.193-95°C. IR (KBr): 3421 (-NH, -CONH-), 3155 (C-H, aromatic), 2371 (CN), 1710 (C=O stretching, -CONH-), 770 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 1H, >NH), 8.20 (s, 1H, CH=N-), 7.6-9.0 (m, 4H, Ar-H), 3.30 (s, 2H, $>CH_2$); LCMS: *m/z* 306 (M^+). Anal. Calcd for $C_{13}H_8Cl_2N_4O$: C, 50.84; H, 2.63; N, 18.24. Found: C, 50.72; H, 2.70; N, 18.30%.

General procedure for preparation of 6-amino-1-((2,6-dichloroquinolin-3-yl)methyleneamino)-4-(aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles, 3a-m

A mixture of compound **2** (0.01 mol), corresponding 2-benzylidenemalononitrile (0.01 mol) and 2 drops of piperidine in ethanol (99.9%, 50 mL) was refluxed for 2-3 h. The mixture was then cooled down to RT and the crystals formed were filtered, dried and purified by recrystallization from aqueous dimethylformamide.

6-Amino-1-((2,6-dichloroquinolin-3-yl) methyl-eneamino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile, 3a: Yield 62%. m.p.222-24°C. IR (KBr): 3428 (-NH, NH_2), 3045 (C-H, aromatic), 2215 (CN), 1665 (>C=O stretching, cyclic amide), 768 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (s, 2H, Ar-NH_2), 8.26 (s, 1H, Ar-CH=N-), 6.67-9.10 (m, 9H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 160.2, 159.7, 152.7, 147.6, 143.5, 136.3, 132.4, 132.2, 129.3, 128.7, 128.4, 128.2, 127.8, 125.6, 124.2, 115.8, 115.5, 76.7; LCMS: *m/z* 458.0 (M^+). Anal. Calcd for $C_{23}H_{12}Cl_2N_6O$: C, 60.15; H, 2.63; N, 18.30. Found: C, 60.00; H, 2.55; N, 18.04%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-2-oxo-4-*p*-tolyl-1,2-dihydropyridine-3,5-dicarbonitrile, 3b: Yield 61%. m.p.226-28°C. IR (KBr): 3430 (-NH, NH_2), 3041, 2925, 2852 (C-H, aromatic), 2224 (CN), 1664 (>C=O stretching, cyclic amide), 768 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.94 (s, 2H, Ar-NH_2), 8.26 (s, 1H, Ar-CH=N-), 6.51-9.20 (m, 8H, Ar-H), 2.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 160.1, 159.7, 152.9, 147.4, 143.3, 137.8, 136.6, 134.5, 132.7, 132.5, 129.7, 129.5, 128.9, 127.3, 125.6, 124.0, 115.6, 76.7, 21.1; LCMS: *m/z* 472.1 (M^+). Anal. Calcd for $C_{24}H_{14}Cl_2N_6O$: C, 60.90; H, 2.98; N, 17.76. Found: C, 60.59; H, 2.77; N, 17.55%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3c: Yield 63%. m.p.229-31°C. IR (KBr): 3426 (-NH,NH_2), 3043 (C-H, aromatic), 2900, 2835 (C-H, OCH_3), 2220 (CN), 1662 (>C=O stretching, cyclic amide), 766 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 2H, Ar-NH_2), 8.25 (s, 1H, Ar-CH=N-), 6.53-9.25 (m, 8H, Ar-H), 3.37 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 160.4, 159.9, 152.5, 147.8, 143.3, 136.1, 132.6, 132.5, 130.1, 129.7, 127.7, 125.6, 125.2, 124.4, 116.0, 115.6, 114.4, 76.7, 56.2; LCMS: *m/z* 488.1 (M^+). Anal. Calcd for $C_{24}H_{14}Cl_2N_6O_2$: C, 58.91; H, 2.88; N, 17.18. Found: C, 58.71; H, 2.76; N, 16.90%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-2-oxo-4-(3,4,5-trimethoxyphenyl)-1,2-dihydropyridine-3,5-dicarbonitrile, 3d: Yield 58%. m.p.243-45°C. IR (KBr): 3432 (-NH, NH_2), 3040 (C-H, aromatic), 2915, 2844 (C-H, OCH_3), 2219 (CN), 1666 (>C=O stretching, cyclic amide), 770 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 2H, Ar-NH_2) 8.24 (s, 1H, Ar-CH=N-), 6.66-9.01 (m, 6H, Ar-H), 3.33 (s, 9H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 160.2, 159.6, 153.0, 152.9, 147.9, 143.7, 138.6, 136.1, 132.5, 132.4, 129.7, 127.5, 126.8, 125.2, 124.0, 115.9, 115.5, 105.0, 76.9, 60.6, 56.0; LCMS: *m/z* 548.1 (M^+). Anal. Calcd for $C_{26}H_{18}Cl_2N_6O_4$: C, 56.84; H, 3.30; N, 15.30. Found: C, 56.75; H, 3.00; N, 15.10%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(2-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile, 3e: Yield 59%. m.p.239-41°C. IR (KBr): 3499 (-OH, Ar-OH), 3432 (-NH, NH_2), 3050 (C-H, aromatic), 2218 (CN), 1664 (>C=O stretching, cyclic amide), 761 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (s, 2H, Ar-NH_2), 8.25 (s, 1H, Ar-CH=N-), 6.59-9.20 (m, 8H, Ar-H), 5.69 (s, 1H, Ar-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 160.3, 159.5, 158.6, 152.9, 148.0, 143.5, 136.9, 132.8, 132.7, 130.0, 129.5, 129.1, 127.9, 125.6, 124.4, 121.5, 121.2, 117.8, 115.3, 115.1, 76.5; LCMS: *m/z* 474.0 (M^+). Anal. Calcd for $C_{23}H_{12}Cl_2N_6O_2$: C, 58.12; H, 2.54; N, 17.68. Found: C, 58.01; H, 2.49; N, 17.50%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(3-hydroxyphenyl)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3f: Yield 57%. m.p.247-49°C. IR (KBr): 3490 (OH, Ar-OH), 3430 (-NH, NH_2), 3050 (C-H, aromatic), 2215 (CN), 1665 (>C=O stretching, cyclic amide), 765 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (s, 2H, Ar-NH_2), 8.25 (s, 1H, Ar-CH=N-), 6.59-9.20 (m, 8H, Ar-H), 5.68 (s, 1H, Ar-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 160.3, 159.5, 158.6, 152.9, 148.0, 143.5, 136.9, 134.0, 132.8, 132.7, 130.0, 129.5, 127.9, 125.6, 124.4, 121.5, 115.8, 115.3, 115.1, 112.0, 76.5; LCMS: *m/z* 474.0 (M^+). Anal. Calcd for $C_{23}H_{12}Cl_2N_6O_2$: C, 58.12; H, 2.54; N, 17.68. Found: C, 58.00; H, 2.45; N, 17.45%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(4-hydroxyphenyl)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3g: Yield 60%. m.p. 252-54°C. IR (KBr): 3489 (OH, Ar-OH), 3433 (-NH, NH_2), 3049 (C-H, aromatic), 2220 (CN), 1664 (>C=O

stretching, cyclic amide), 760 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.97 (s, 2H, Ar-NH_2), 8.26 (s, 1H, Ar-CH=N-), 7.08-9.22 (m, 8H, Ar-H), 5.62 (s, 1H, Ar-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 160.5, 159.4, 157.9, 153.0, 148.0, 143.5, 136.9, 132.6, 132.5, 131.2, 129.5, 127.5, 125.4, 125.2, 124.3, 115.6, 115.3, 76.2; LCMS: *m/z* 474.0 (M^+). Anal. Calcd for $C_{23}H_{12}Cl_2N_6O_2$: C, 58.12; H, 2.54; N, 17.68. Found: C, 57.98; H, 2.33; N, 17.55%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile, 3h: Yield 61%. m.p. 250-52°C. IR (KBr): 3479 (OH, Ar-OH), 3431 (-NH, NH_2), 3040 (C-H, aromatic), 2910, 2850 (C-H, OCH_3), 2217 (CN), 1667 (>C=O stretching, cyclic amide), 771 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 2H, Ar-NH_2), 8.24 (s, 1H, Ar-CH=N-), 6.63-9.15 (m, 7H, Ar-H), 5.54 (s, 1H, Ar-OH), 3.80 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 160.6, 159.8, 152.5, 149.5, 147.7, 147.1, 143.5, 136.3, 132.3, 132.6, 130.7, 129.5, 127.7, 126.4, 125.3, 124.0, 116.6, 115.6, 115.7, 112.3, 76.5, 56.5; LCMS: *m/z* 504.1 (M^+). Anal. Calcd for $C_{24}H_{14}Cl_2N_6O_3$: C, 57.05; H, 2.79; N,16.63. Found: C, 57.09; H, 2.75; N, 16.69%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(3-nitrophenyl)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3i: Yield 62%. m.p. 249-51°C. IR (KBr): 3431 (-NH, NH_2), 3048 (C-H, aromatic), 2218 (CN), 1333, 1531 (NO_2), 1663 (>C=O stretching, cyclic amide), 764 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.94 (s, 2H, Ar-NH_2), 8.26 (s, 1H, Ar-CH=N-), 6.93-9.24 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 160.0, 159.5, 152.9, 147.7, 143.1, 136.7, 135.2, 133.4, 132.7, 132.6, 129.7, 129.5, 127.6, 125.6, 124.1, 123.0, 120.0, 115.8, 115.3, 76.5; LCMS: *m/z* 503.0 (M^+). Anal. Calcd for $C_{23}H_{11}Cl_2N_7O_3$: C, 54.78; H, 2.20; N, 19.44. Found: C, 54.56; H, 2.01; N, 19.22%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(4-nitrophenyl)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3j: Yield 60%. m.p. 255-57°C. IR (KBr): 3428 (-NH, NH_2), 3045 (C-H, aromatic), 2215 (CN), 1350, 1530 (NO_2), 1663 (>C=O stretching, cyclic amide), 769 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 2H, Ar-NH_2), 8.25 (s, 1H, Ar-CH=N-), 7.08-9.10 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 160.6, 159.3, 152.8, 147.6, 147.1, 143.6, 138.5, 136.9, 132.5, 132.6, 130.0, 129.3, 127.7, 125.6, 124.0, 123.6, 115.9, 115.7, 76.3; LCMS: *m/z* 503.0 (M^+). Anal. Calcd for $C_{23}H_{11}Cl_2N_7O_3$: C, 54.78; H, 2.20; N, 19.44%. Found: C, 54.57; H, 2.00; N, 19.23%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(4-(dimethylamino)phenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile, 3k: Yield 60%. m.p.240-42°C. IR (KBr): 3432 (-NH, NH_2), 3041 (C-H, aromatic), 2815 (C-H, -$N(CH_3)_2$), 2216 (CN), 1668 (>C=O stretching, cyclic amide), 773 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 2H, Ar-NH_2) 8.24 (s, 1H, Ar-CH=N-), 6.66-9.05 (m, 8H, Ar-H), 3.00 (s, 6H, Ar-$N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 160.4, 159.6, 153.0, 152.7, 147.9, 143.5, 136.1, 132.5, 132.4, 130.5, 129.7, 127.5, 126.6, 125.1, 124.0, 115.9, 115.5, 111.9, 76.7, 41.5; LCMS: *m/z* 501.1 (M^+). Anal. Calcd for $C_{25}H_{17}Cl_2N_7O$: C, 59.77; H, 3.41; N, 19.52. Found: C, 59.75; H, 3.50; N, 19.44%.

6-Amino-1-((2,6-dichloroquinolin-3-yl)methyl-eneamino)-4-(4-fluorophenyl)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3l: Yield 61%. m.p. 221-23°C. IR (KBr): 3429 (-NH, NH_2), 3044 (C-H, aromatic), 2222 (CN), 1664 (>C=O stretching, cyclic amide), 768 (C-Cl), 560 cm^{-1} (C-F); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 2H, Ar-NH_2), 8.26 (s, 1H, Ar-CH=N-), 6.80-9.10 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 162.2, 160.4, 159.5, 152.8, 147.5, 143.8, 136.9, 132.4, 132.3, 129.3, 128.1, 128.0, 127.7, 125.6, 124.0, 115.6, 115.2, 115.1, 76.5; LCMS: *m/z* 476.0 (M^+). Anal. Calcd for $C_{23}H_{11}Cl_2FN_6O$: C, 57.88; H, 2.32; N, 17.61. Found: C, 57.66; H, 2.12; N, 17.50%.

6-Amino-4-(4-chlorophenyl)-1-((2,6-dichloro-quinolin-3-yl)methyleneamino)-2-oxo-1,2-dihydro-pyridine-3,5-dicarbonitrile, 3m: Yield 69%. m.p.227-29°C. IR (KBr) 3430 (-NH, NH_2), 3041 (C-H, aromatic), 2214 (CN), 1664 (>C=O stretching, cyclic amide), 765 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.97 (s, 2H, Ar-NH_2), 8.26 (s, 1H, Ar-CH=N-), 6.87-9.19 (m, 8H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 160.3, 159.6, 152.4, 147.6, 143.8, 136.8, 133.3, 132.8, 132.6, 130.4, 130.0, 129.1, 128.3, 127.3, 125.6, 124.0, 115.6, 115.1, 76.5; LCMS: *m/z* 492.0 (M^+). Anal. Calcd for $C_{23}H_{11}Cl_3N_6O$: C, 55.95; H, 2.25; N, 17.02. Found: C, 55.76; H, 2.11; N, 16.77%.

Conclusion

Target imine compounds **3a-m** with structural blending of 2,6-dichloroquinoline and 2-pyridone

were synthesized and screened for their antimicrobial potential to unfold the identity of new bioactive molecules with broad spectrum antimicrobial property. Observation of activity profile of all the newly synthesized compounds reflected that the presence of ring activating functional groups like $-CH_3$, $-OCH_3$ and -OH on *para* position came out as most potent antibacterial agent, whereas presence of ring activating substituent on *meta* position of phenyl ring of 2-pyridone entity enhanced the antifungal potential. Compounds **3b** ($-CH_3$) and **3c** ($-OCH_3$) came out as the most potent antibacterial agent, while compound **3f** (-OH) with electron donating functional group on *meta* position was found to be most potent antifungal agent. Antimicrobial activity was not boosted by presence of electron withdrawing group either on *ortho*, *meta* or *para* position and also was not enhanced by presence of electron releasing group on *ortho* position. Structure activity relation study revealed that *para* and *meta* positions with electron donating functionality is favorable for antibacterial and antifungal properties respectively. These effective derivatives are ideally suited for further exploration to obtain more efficacious antimicrobial agents.

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