Synthesis and biological evaluation of some novel quinoline based pyrimidine derivatives

N C Desai*, G M Kotadiya & D V Vaja
Division of Medicinal Chemistry, Department of Chemistry (DST-FIST Sponsored & UGC NON-SAP), Mahatma Gandhi Campus, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364 002, India
E-mail: dnisheeth@rediffmail.com

Received 6 June 2017; accepted (revised) 16 November 2017

A rational approach has been adopted for the synthesis of novel series of 3-((6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidin-2-yl)thio)propanenitriles 5a-o. The synthesized compounds have been evaluated for their in vitro antimicrobial activity. Compounds 5j-m display broad spectrum antibacterial activity against all the bacterial strains. Moreover, compound 5m is found to be the most potent antibacterial and antifungal agent. Further, the results of preliminary MTT cytotoxicity studies on HeLa cells suggest that potent antimicrobial activity of 5j-m is accompanied by low level of cytotoxicity.

Keywords: Quinoline clubbed pyrimidines, antibacterial activity, antifungal activity, cytotoxicity, MIC

Now a days antibiotics are in great demand due to the broad spectrum of biological activities. There are a huge range of antibiotics that have been used for different types of microbial diseases. Treatment with antibiotics also produces some toxic effect and side effects on other organs of the body. Due to this reason, more effective and less toxic antibiotics are the burning need of the day. Generally, commercially available drugs such as Enoxacin, sulfadiazine, clioquinol, etc. contain pyrimidine and quinoline based heterocyclic scaffolds. In particular, quinolines have exceptional antibacterial activity against MTB due to their excellent oral bioavailability and an ability to penetrate the macrophages14. Moreover, they are known to possess antibiotic, antimalarial, anticancer, anti-inflammatory, antihypertensive, tyrokinase PDGF-RTK inhibition and anti-HIV properties. It is interesting to note that quinoline is a core pharmacophore in the recently developed antimicrobial drugs, viz. Trovafloxacin, Levofloxacin and Merlofoxine.

On the other hand, pyrimidine framework was the base of many bioactive molecules such as antitubercular, antibacterial, antitumor, anti-inflammatory, antifungal and antileishmanial agents. Consequently, synthetic methodologies for synthesis of novel pyrimidines or pyrimidine fused compounds are of particular usefulness to organic and medicinal chemists. In addition, pyrimidines are potential inhibitors of dihydrofolate reductase (DHFR), a promising drug target for the development of anti-infective agents. Although DHFR does not represent a novel target, there is still a large scope for the development of DHFR inhibitors, particularly with respect to mycobacteria.

As an extension of our previous work, we reached the synthesis of new endeavours towards the development of anti-infective agents. It was envisaged that the design and synthesis of new prototypes which include advantage of dual pharmacophore of quinoline and pyrimidine in single molecular framework is worth the attempt. The new derivatives have some structural features that led us to consider them as viable candidates to evaluate as antimicrobial agents.

Results and Discussion

A novel series 3-((6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidin-2-yl)thio)propanenitriles 5a-o presented in this work was prepared through the synthetic path established in Scheme I. The starting 2-chloro-6-methoxyquinoline-3-carbaldehyde 1 was equipped through reported method. The chalcones 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(aryl)prop-2-en-1-ones 3a-o were synthesized through the Claisen-Schmidt condensation of equimolar amounts of 2-
chloro-6-methoxyquinoline-3-carbaldehyde 1 and acetophenone derivatives 2a-o in agreement with the method designated in the literature.23 Intermediates 3a-o commenced cyclization with thiourea in presence of ethanolic potassium hydroxide to furnish quinolyl dihydropyrimidines recognized as 6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidine-2-thiols 4a-o in accordance with method illustrated in literature.24 The structures of 4a-o were characterized by IR spectra which exhibited the characteristic absorption bands at 2550-2600 and 3400-3417 cm\(^{-1}\) for S-H and N-H stretching respectively, beside withdrawal of carbonyl group stretching due to its sharing in cyclization. \(^1\)H NMR spectrum of compounds 4a-o showed beside the predictable aromatic signals, \(i.e.\) singlet at \(\delta 9.60-9.69\), doublet at \(\delta 6.10-6.20\) and a doublet at \(\delta 5.10-5.20\) for protons of NH and the proton attached to olefinic carbon and asymmetric carbon respectively. In the final step, intermediates 4a-o were reacted with acrylonitrile in pyridine followed by neutralization with hydrochloric acid to furnish the respective targeted 3-((6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidin-2-yl)thio)propanenitriles 5a-o.

The structures of the final compounds 5a-o were evidenced by IR, NMR and mass spectroscopic techniques in which IR spectrum displayed fading of S-H stretching in intermediates 4a-o. Additionally, absorption band appearing between 2318-2354 cm\(^{-1}\) was assigned to >CN group and its \(^1\)H NMR spectra revealed two triplets at \(\delta 3.09-3.17\) and 3.41-3.49 for methylene protons attached to cyanide group and S atom respectively, besides the vanishing of -SH group singlet. Furthermore, \(^13\)C NMR spectrum of compound 5a displayed, along with the aromatic signals, four signals at \(\delta 166.4, 119.0, 54.5\) and 47.7 due to pyrimidine ring carbon attached to S, cyanide group carbon and two methylene carbons dedicated to cyanide group and S atom respectively. The mass spectrum of 5a observed molecular ion peak at \(m/z\) 435.07 [M\(^+\) + 181\%], in agreement with its proposed structure. All the newly synthesized compounds were evaluated for their \textit{in vitro} antibacterial and antifungal activity (MIC) by conventional broth microdilution method.25,26 Bacterial strains \textit{Staphylococcus aureus} MTCC 96 and \textit{Streptococcus pyogenes} MTCC 442 as Gram-positive, \textit{Escherichia coli} MTCC 443 and \textit{Pseudomonas aeruginosa} MTCC 1688 as Gram-negative and \textit{Candida albicans} MTCC 227, \textit{Aspergillus niger} MTCC 282 and \textit{Aspergillus clavatus} MTCC 1323 as fungal strains were used. The results of the antimicrobial studies are presented in Scheme I — Synthetic pathway of compounds 5a-o.
Table I and Ciprofloxacin and Griseofulvin were used as standard drugs. Compounds 5j-m displayed broad spectrum antibacterial activity (MIC = 125 - 12.5 µg/mL) against both gram-positive and gram-negative bacteria as compared with ciprofloxacin. Compound 5m was found to be 2-fold more active against all the bacterial strains (MIC = 12.5 µg/mL), 5l exhibited same effect against E. coli and P. aeruginosa (MIC = 25 µg/mL), while compound 5k exhibited equivalent activity against E. coli and P. aeruginosa (MIC = 25 µg/mL) as compared to the standard drug. Moreover, compounds 5j and 5m displayed MIC at 100 µg/mL against E. coli, P. aeruginosa, S. aureus and S. pyogens, respectively. High antibacterial potency of 5j-m may be attributed to the presence of substituents such as methoxy and hydroxy at 3rd and 4th position of phenyl ring attached to pyrimidine ring.

Moreover, in vitro antifungal activity results indicated that compound 5m having methoxy group at 4th position of phenyl ring was found to be the most potent activity (MIC = 62.5 µg/mL) against A. niger, while compounds 5k and 5l exhibited least potency against A. niger, A. clavatus and C. albicans.

Cytotoxicity studies confirm the toxicity of drugs. In vitro cytotoxicity of the titled compounds 5a-o was considered by means of the IC_{50} value, according to the NCCLS recommendations, the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] analysis method. This series was evaluated against human cervical cancer cell line (HeLa) by the MTT colorimetric test. In Table II cytotoxicity results indicate that the derivatives 5c, 5d, 5h and 5j showed no toxicity at a concentration of 100 µM (IC50 > 100 µM), while other derivatives exhibited moderate toxicity against HeLa cell lines. None of the tested

<table>
<thead>
<tr>
<th>Compd</th>
<th>-R</th>
<th>Gram positive bacteria^a</th>
<th>Gram negative bacteria^b</th>
<th>Fungi^c</th>
<th>Minimum inhibitory concentration (MIC) µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>-H</td>
<td>Sa 500</td>
<td>Sp 500</td>
<td>Ec 250</td>
<td>Pa 250</td>
</tr>
<tr>
<td>5b</td>
<td>-3-Cl</td>
<td>1000</td>
<td>500</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>5c</td>
<td>-4-Cl</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>5d</td>
<td>-3-F</td>
<td>1000</td>
<td>&gt;1000</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>5e</td>
<td>-4-F</td>
<td>500</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>5f</td>
<td>-3-CH3</td>
<td>250</td>
<td>250</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5g</td>
<td>-4-CH3</td>
<td>125</td>
<td>250</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5h</td>
<td>-3-NO2</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>5i</td>
<td>-4-NO2</td>
<td>1000</td>
<td>&gt;1000</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>5j</td>
<td>-3-OH</td>
<td>125</td>
<td>125</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5k</td>
<td>-4-OH</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>5l</td>
<td>-3-OCH3</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>5m</td>
<td>-4-OCH3</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>5n</td>
<td>-3-Br</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>5o</td>
<td>-4-Br</td>
<td>1000</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>–</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>500</td>
</tr>
</tbody>
</table>

^a Sa.: Staphylococcus aureus MTCC 96; S.p.: Staphylococcus pyogenes MTCC 442; ^b E.c.: Escherichia coli MTCC 443; P.a.: Pseudomonas aeruginosa MTCC 1688; ^c C.a.: Candida albicans MTCC 227; A.n.: Aspergillus niger MTCC 282; A.c.: Aspergillus clavatus MTCC 1323.
compounds showing any significant cytotoxic effects on HeLa cell line, signifying that the compounds had potential for their in vivo use as antimicrobial agents.

**Structure activity relationship**

Results of antimicrobial activity of final compounds clearly suggested that compounds 5a-o demonstrated better activity than its intermediates 3a-o and 4a-o. The substitution pattern of both scaffolds quinoline and pyrimidine were cautiously selected to bestow different electronic environment on the molecules. The antimicrobial activity was considerably affected by substitution pattern on the phenyl ring attached pyrimidine moiety and the most active compounds sustained inductively electron donating substituents (methoxy and hydroxyl) at para or meta positions of the phenyl ring. In contrast, the existence of electron withdrawing groups on the phenyl ring witnessed a substantial decrease in antimicrobial activity for compounds 5d, 5e, 5h and 5i. It was observed from the activity data that the presence of inductive electron releasing substituents at para position (5k > 5j, 5m > 5l) of the phenyl ring were responsible for effective activity than meta substituted derivatives. In case of antibacterial activity, some analogues of this series were found to have even more potency than the standard drug ‘ciprofloxacin’ while some of them exhibited comparable potency. Compounds 5j-m bearing hydroxyl and methoxy groups were found to be most potent antibacterial agents, whereas compounds 5k-m were evaluated as the most potent antifungal agents. Out of them, compound 5m substituted with electron releasing methoxy group at para position demonstrated the highest antibacterial activity with twofold higher MIC (12.5 µg/mL) against Gram negative and MIC (25 µg/mL) against Gram positive bacterial strains. Moreover, as per activity results, it was observed that the introduction of pyrimidine ring was also responsible for enhancing biological potency.

**Biological assay**

**In vitro antimicrobial assay**

Antibacterial studies of newly synthesized compounds 5a-o were carried out against the representative panel of Gram-positive (Staphylococcus aureus (MTCC-96), Streptococcus pyogenes (MTCC-442) and Gram-negative (Escherichia coli (MTCC-443), Pseudomonas aeruginosa (MTCC-1688) bacteria using conventional broth dilution method. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh. The activity of compounds was determined as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller Hinton Broth (Becton Dickinson, USA). Primary screening was done first for antibacterial activity in six sets against E. coli, S. aureus, P. aeruginosa and S. pyogenes at different concentrations of 1000, 500 and 250 µg/mL. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, 12.5 and 6.25 µg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculum size for test strain was adjusted to 10⁶ CFU/mL (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Mueller Hinton Broth was used as a nutrient medium to grow and dilute the compound suspension for test organisms. 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 µg/mL concentration, as stock solution. The control tube containing no antibiotic was immediately subcultured [before inoculation] by spreading a loopful evenly over quarter of a plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 37°C and the growth was monitored visually and spectrophotometrically. Ten µg/mL suspensions were further inoculated on an appropriate media and growth was noted after 24 h and 48 h. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC) i.e. the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Solvent had no influence on strain growth. The result of this was greatly affected by the size of inoculum. The test mixture contained 10⁶ CFU/mL organisms. DMSO and sterilized distilled water were used as negative control while chloramphenicol antibiotic (1 µ strength) was used as positive control. Standard drug used in the present study was ‘Ciprofloxacin’ for evaluating antibacterial activity which showed 50 µg/mL and 25 µg/mL MIC against E. coli, P. aeruginosa, S. aureus and S. pyogenes respectively.

The newly prepared compounds 5a-o were screened for their antifungal activity as primary screening in six
sets against C. albicans, A. niger and A. clavatus at various concentrations of 1000, 500, 250 µg/mL. The primary active compounds were similarly diluted to obtain 200, 125, 100, 6.25, 50, 25 and 12.5 µg/mL concentrations for secondary screening to test in a second set of dilution against all fungi.

The antifungal activity of each compound was compared with griseofulvin as a standard drug, which showed 500 and 100 µg/mL MIC against C. albicans, A. niger and A. clavatus respectively. For fungal growth, in the present protocol, we have used Sabourauds dextrose broth at 28°C in aerobic condition for 48 h. DMSO and sterilized distilled water were used as negative control while griseofulvin was used as positive control.

In vitro cytotoxicity assay
In vitro cytotoxicity of most active compounds 5j-m was evaluated against human cervical cancer cell line (HeLa) by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay\(^2,28\), which measures the reduction of tetrazolium bromide salt into a formazan dye by mitochondrial dehydrogenases in treated versus untreated cells. The IC\(_{50}\) values obtained for these compounds are shown in Table II. Cytotoxicity results discovered that the derivatives 5i and 5m caused no toxicity at concentration of 100 µM (IC\(_{50}\) > 100 µM), while other derivatives showed moderate toxicity against HeLa cell lines. It was confirmed that none of the tested compounds exhibited any substantial cytotoxic effects on HeLa cell lines, suggesting that compounds were potential candidates for antimicrobial agents.

Experimental Section
All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Melting points were determined on an electro thermal melting point apparatus by open capillary method and are reported uncorrected. TLC on silica gel plates (Merck, 60, F254) was used for reaction monitoring. Column chromatography on 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. Yields refer to purified products and are not optimized. Elemental analysis (% C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Shimadzu IR Prestige-21 (CE) FT-IR spectrophotometer in KBr and frequencies are reported in cm\(^{-1}\). \(^1\)H and \(^13\)C NMR spectra were run on Bruker Avance II 400 MHz in DMSO-\(d_6\) as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported as δ (ppm) values. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer.

General procedure for the synthesis of 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(aryl)prop-2-en-1-ones, 3a-o
Synthesis of 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(aryl)prop-2-en-1-ones 3a-o was achieved by reported methods\(^29\).

General procedure for the synthesis of 6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidine-2-thiols, 4a-o
A mixture of 3-(2-chloro-6-methoxyquinolin-3-yl)-1-(aryl)prop-2-en-1-ones 3a-o (0.01 mol) and thiourea (0.01 mol) in ethanolic potassium hydroxide (1 g in 15 mL) was heated under reflux for 7-8 h. The volume of the reaction mixture was reduced to half of its original volume, diluted with ice cold water, then acidified with dilute acetic acid and kept overnight. The solid precipitates thus obtained were filtered and washed with water and purified by recrystallization from absolute alcohol.

General procedure for the synthesis of 3-(6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidin-2-ylthio)propanenitriles, 5a-o
A mixture of compounds 6-(2-chloro-6-methoxyquinolin-3-yl)-4-aryl-1,6-dihydropyrimidine-2-thiols 4a-o (0.01 mol) and acrylonitrile (0.02 mol) in 15 mL pyridine was refluxed for 18-20 h. Then, reaction mixture was neutralized with cold dilute hydrochloric acid and the solid product was filtered off and purified by recrystallization from methanol.

3-(6-(2-Chloro-6-methoxyquinolin-3-yl)-4-phenyl-1,4,5,6-tetrahydropyrimidin-2-ylthio)propenitrile, 5a: Off-white. Yield 62%. m.p.209-12°C. IR (KBr): 3400 (N-H stretching, pyrimidine ring), 3140 (C-H stretching, nitrile group), 2950 (C-H stretching, alkyl chain), 2835 (C-H stretching, -OCH\(_3\)), 2318 (-C\(\equiv\)N), 2240 (-C=O), 1536 (>C=N- stretching) , 1490 (>C=C< stretching), 1270 (-C-N amide I), 1196 (-C-O), 1100 (-C-N amide II), 750 cm\(^{-1}\).\(^1\)H NMR (300 MHz, DMSO-\(d_6\)): δ 9.60 (s, 1H, H-N=C\(^{=2}\) amine), 7.20-8.19 (m, 9H, Ar-H), 5.15 (d, 1H, H-C=C-N), 3.44 (t, 2H, -CH\(_2\)-S), 3.15 (t, 2H, -CH\(_2\)-CN);
3.84 (s, 3H, -OCH3) - OCH3 (C-H stretching, alkyl chain), 2825 (C-H stretching, ring), 3192 (C-H stretching, aromatic ring), 2948 (C-H stretching, pyrimidine ring), 3413 (N-H stretching, pyrimidine thio)propenitrile, 5c 

IR (KBr): 3413 (N-H stretching, pyrimidine thio)propenitrile, 5c

M.p. 195-198°C. IR (KBr): 3409 (N-H stretching, pyrimidine ring), 3185 (C-H stretching, aromatic ring), 2965 (C-H stretching, alky chain), 2835 (C-H stretching, -OCH3), 2314 (-C≡N); 3185 (C-H stretching, aromatic ring), 2965 (C-H stretching, alkyl chain), 2839 (C-H stretching, -OCH3), 2338 (-C≡N)

δ 9.69 (s, 1H, H-N< 2° amine), 7.24-8.17 (m, 8H, Ar-H), 6.13 (d, 1H, H-C=N-C), 5.19 (d, 1H, H-C-NH-), 3.84 (s, 3H, -OCH3), 3.41 (t, 2H, -CH2-S), 3.13 (t, 2H, -CH2-CN); 13C NMR (100 MHz, DMSO-d6): δ 166.7, 157.6, 149.1, 142.6, 141.9, 138.0, 137.9, 135.4, 134.4, 131.7, 130.3, 128.3, 128.6, 126.1, 124.3, 122.6, 119.7, 114.6, 105.0, 55.3, 47.3, 48.1; LCMS: m/z 471.4 (M+). Anal. Calcd for C23H18Cl2N2O3: C, 63.51; H, 3.87; N, 11.94. Found: C, 58.91; H, 3.96; N, 11.95%.

3-(4-(2-Chloro-6-methoxyquinolin-3-yl)-1,4,5,6-tetrahydropyrimidin-2-yl)thiopropenitrile, 5c: Off-white. Yield 61%. m.p. 195-98°C. IR (KBr): 3409 (N-H stretching, pyrimidine ring), 3185 (C-H stretching, aromatic ring), 2965 (C-H stretching, alky chain), 2835 (C-H stretching, -OCH3), 2314 (-C≡N); 3185 (C-H stretching, aromatic ring), 2969 (C-H stretching, alkyl chain), 2839 (C-H stretching, -OCH3), 2338 (-C≡N)

δ 9.69 (s, 1H, H-N< 2° amine), 7.22-8.14 (m, 8H, Ar-H), 6.16 (d, 1H, H-C=N-C), 5.19 (d, 1H, H-C-NH-), 3.80 (s, 3H, -OCH3), 3.41 (t, 2H, -CH2-S), 3.15 (t, 2H, -CH2-CN); 13C NMR (100 MHz, DMSO-d6): δ 166.4, 162.7, 157.6, 149.6, 142.7, 141.7, 137.0, 135.4, 132.7, 131.7, 128.7 (2), 122.4, 119.4, 115.7 (2), 114.4, 105.9, 55.3, 54.1, 48.6, 47.6; LCMS: m/z 454.1 (M+). Anal. Calcd for C23H18Cl2N2O3: C, 60.99; H, 4.01; N, 12.37. Found: C, 60.93; H, 4.10; N, 12.42%

3-(4-(2-Chloro-6-methoxyquinolin-3-yl)-1,4,5,6-tetrahydropyrimidin-2-yl)thiopropenitrile, 5f: Light yellow. Yield 67%. m.p. 223-25°C. IR (KBr): 3408 (N-H stretching, pyrimidine ring), 3198 (C-H stretching, aromatic ring), 2945 (C-H stretching, alky chain), 2924 (C-H stretching, -CH3), 2839 (C-H stretching, -OCH3), 2338 (-C≡N)

δ 9.68 (s, 1H, H-N< 2° amine), 7.21-8.18 (m, 8H, Ar-H), 6.14 (d, 1H, H-C=N-C), 5.17 (d, 1H, H-C-NH-), 3.87 (s, 3H, -OCH3), 3.46 (t, 2H, -CH2-S), 3.12 (t, 2H, -CH2-CN); 13C NMR (100 MHz, DMSO-d6): δ 166.1, 157.6, 149.4, 142.6, 141.9, 137.9, 135.9, 134.1, 133.5, 131.7, 128.4, 128.6 (2), 127.9, 122.6, 119.4, 114.0, 105.0, 55.9, 54.1, 48.6, 47.2; LCMS: m/z 471.6 (M+). Anal. Calcd for C23H18Cl2N2O3: C, 58.85; H, 3.87; N, 11.94. Found: C, 58.93; H, 3.92; N, 11.90%.

3-(4-(2-Chloro-6-methoxyquinolin-3-yl)-1,4,5,6-tetrahydropyrimidin-2-yl)thiopropenitrile, 5g: Light yellow. Yield 68%. m.p. 200-203°C. IR (KBr): 3407 (N-H stretching, pyrimidine ring), 3187 (C-H stretching, aromatic ring), 2965 (C-H stretching, alky chain), 2837 (C-H stretching, -OCH3), 2330 (-C≡N stretching, nitrile group), 1545 (>C=N- stretching), 1484 (>C≡C- stretching), 1045 (C-F stretching), 740 cm⁻¹ (C-Cl stretching); 1H NMR (300 MHz, DMSO-d6): δ 9.70 (s, 1H, H-N< 2° amine), 7.22-8.12 (m, 8H, Ar-H), 6.14 (d, 1H, H-C=N-C), 5.14 (d, 1H, H-C-NH-), 3.82 (s, 3H, -OCH3), 3.44 (t, 2H, -CH2-S-), 3.12 (t, 2H, -CH2-CN); 13C NMR (100 MHz, DMSO-d6): δ 166.4, 162.1, 157.6, 149.6, 142.4, 141.3, 138.4, 134.7, 131.0, 127.9, 122.6, 122.7, 119.4, 114.4, 114.8, 111.5, 105.6, 55.7, 54.9, 48.0, 47.4; LCMS: m/z 454.5 (M+). Anal. Calcd for C23H18Cl2N2O3: C, 60.99; H, 4.01; N, 12.37. Found: C, 60.93; H, 4.10; N, 12.42%.
3-((6-(2-Chloro-6-methoxyquinolin-3-yl)-4-(p-tolyl)-1,4,5,6-tetrahydropyrimidin-2-yl)(thio)-propennitrile, 5g: Cremish yellow. Yield 63%. m.p.250-52°C. IR (KBr): 3412 (N-H stretching, pyrimidine ring), 3178 (C-H stretching, aromatic ring), 2945 (C-H stretching, alkyl chain), 2930 (C-H stretching, -CH$_3$), 2849 (C-H stretching, -OCH$_3$), 2347 (-C≡N stretching, nitrile group), 1553 (>C≡N- stretching), 1481 (>C≡C< stretching), 758 cm$^{-1}$ (C-Cl stretching); 1$^H$ NMR (300 MHz, DMSO-$d_6$): δ 9.63 (s, 1H, H-N<2° amine), 7.26-8.15 (m, 8H, Ar-H), 6.14 (d, 1H, H-C≡C-N), 5.20 (d, 1H, H-NC=N), 3.81 (s, 3H, -OCH$_3$), 3.49 (t, 2H, -CH$_2$-S), 3.18 (t, 2H, -CH$_2$-CN), 2.30 (s, 3H, -CH$_3$); 1$^3$C NMR (100 MHz, DMSO-$d_6$): δ 166.2, 157.9, 149.6, 142.6, 141.9, 137.0, 135.4, 133.9, 131.4, 128.4 (2), 126.0 (2), 122.4, 119.4, 114.6, 105.4, 55.8, 54.9, 48.9, 47.1, 21.6; LCMS: m/z 450.1 (M$^+$). Anal. Calcd for C$_{23}$H$_{23}$Cl$_2$N$_2$O$_2$: C, 54.60; H, 3.33; N, 12.55%

3-((6-(2-Chloro-6-methoxyquinolin-3-yl)-4-(3-nitrophenyl)-1,4,5,6-tetrahydropyrimidin-2-yl)(thio)-propennitrile, 5h: Light yellow. Yield 69%. m.p.234-46°C. IR (KBr): 3410 (N-H stretching, pyrimidine ring), 3168 (C-H stretching, aromatic ring), 2936 (C-H stretching, alkyl chain), 2852 (C-H stretching, -OCH$_3$), 2342 (-C≡N stretching, nitrile group), 1535 (N-O, -NO$_2$), 1550 (>C≡C- stretching), 1478 (>C≡C< stretching), 798 cm$^{-1}$ (C-Cl stretching); 1$^H$ NMR (300 MHz, DMSO-$d_6$): δ 9.66 (s, 1H, H-N<2° amine), 7.20-8.14 (m, 8H, Ar-H), 6.17 (d, 1H, H-C≡C-N), 5.18 (d, 1H, H-C-NH-), 3.82 (s, 3H, -OCH$_3$), 3.49 (t, 2H, -CH$_2$-S), 3.18 (t, 2H, -CH$_2$-CN); 1$^3$C NMR (100 MHz, DMSO-$d_6$): δ 166.4, 157.4, 149.6, 147.6, 142.6, 141.0, 137.4, 137.0, 135.1, 132.8, 131.4, 129.7, 128.7, 123.2, 122.9, 120.4, 119.4, 114.8, 105.9, 55.3, 48.6, 47.0; LCMS: m/z 462.1 (M$^+$). Anal. Calcd for C$_{23}$H$_{23}$Cl$_2$N$_2$O$_2$: C, 57.56; H, 3.78; N, 12.48. Found: C, 64.30; H, 4.80; N, 12.55%

3-((6-(2-Chloro-6-methoxyquinolin-3-yl)-4-(3-hydroxyphenyl)-1,4,5,6-tetrahydropyrimidin-2-yl)(thio)propennitrile, 5i: Brown. Yield 69%. m.p.239-41°C. IR (KBr): 3615 (O-H stretching, -OH), 3405 (N-H stretching, pyrimidine ring), 3178 (C-H stretching, aromatic ring), 2960 (C-H stretching, alkyl chain), 2847 (C-H stretching, -OCH$_3$), 2340 (-C≡N stretching, nitrile group), 1560 (>C≡N- stretching), 1475 (>C≡C< stretching), 789 cm$^{-1}$ (C-Cl stretching); 1$^H$ NMR (300 MHz, DMSO-$d_6$): δ 9.65 (s, 1H, H-N<2° amine), 7.25-8.18 (m, 8H, Ar-H), 6.12 (d, 1H, H-C≡C-N), 5.37 (s, 1H, -OH), 5.14 (d, 1H, H-C-NH-), 3.84 (s, 3H, -OCH$_3$), 3.48 (t, 2H, -CH$_2$-S), 3.13 (t, 2H, -CH$_2$-CN); 1$^3$C NMR (100 MHz, DMSO-$d_6$): δ 166.1, 158.4, 157.1, 149.6, 142.4, 141.0, 138.4, 137.0, 135.7, 131.8, 130.7, 128.4, 1220, 119.4, 115.6, 114.9, 112.8, 105.7, 55.3, 48.6, 47.0; LCMS: m/z 452.1 (M$^+$). Anal. Calcd for C$_{23}$H$_{23}$Cl$_2$N$_2$O$_2$: C, 61.26; H, 4.25; N, 12.42. Found: C, 61.29; H, 4.39; N, 12.52%

3-((6-(2-Chloro-6-methoxyquinolin-3-yl)-4-(4-hydroxyphenyl)-1,4,5,6-tetrahydropyrimidin-2-yl)(thio)propennitrile, 5j: Dark brown. Yield 65%. m.p.270-72°C. IR (KBr): 3642 (O-H stretching, -OH), 3408 (N-H stretching, pyrimidine ring), 3167 (C-H stretching, aromatic ring), 2954 (C-H stretching, alkyl chain), 2854 (C-H stretching, -OCH$_3$), 2334 (-C≡N stretching, nitrile group), 1572 (>C≡N- stretching), 1472 (>C≡C< stretching), 775 cm$^{-1}$ (C-Cl stretching); 1$^H$ NMR (300 MHz, DMSO-$d_6$): δ 9.64 (s, 1H, H-N<2° amine), 7.23-8.19 (m, 8H, Ar-H), 6.13 (d, 1H, H-C≡C-N), 5.36 (s, 1H, -OH), 5.11 (d, 1H, H-C-NH-), 3.83 (s, 3H, -OCH$_3$), 3.45 (t, 2H, -CH$_2$-S), 3.18 (t, 2H, -CH$_2$-CN); 1$^3$C NMR (100 MHz, DMSO-$d_6$): δ 166.1, 157.4, 157.6, 149.9, 142.6, 141.0, 137.9, 135.9, 131.0, 129.6, 128.4, 127.4 (2), 122.4, 119.4, 115.0 (2), 114.4, 105.0, 55.4, 48.0, 47.6; LCMS: m/z 452.1 (M$^+$). Anal. Calcd for C$_{23}$H$_{23}$Cl$_2$N$_2$O$_2$: C, 61.26; H, 4.28; N, 12.42. Found: C, 61.36; H, 4.36; N, 12.50%

3-((6-(2-Chloro-6-methoxyquinolin-3-yl)-4-(3-methoxyphenyl)-1,4,5,6-tetrahydropyrimidin-2-yl)(thio)propennitrile, 5k: Brown. Yield 64%. m.p.216-19°C. IR (KBr): 3410 (N-H stretching,
pyrimidine ring), 3162 (C-H stretching, aromatic ring), 2978 (C-H stretching, alkyl chain), 2832 (C-H stretching, -OCH₃), 2345 (>C=N stretching, nitrile group), 1567 (>C=N stretching), 1460 (>C=C< stretching), 798 cm⁻¹ (C-Cl stretching); ¹H NMR (300 MHz, DMSO-d₆): δ 9.64 (s, 1H, H-N< 2° amine), 7.21-8.20 (m, 8H, Ar-H), 6.21 (d, 1H, H=C=C-N), 5.15 (d, 1H, H-C=NH-), 3.83 (s, 6H, -OCH₃), 3.44 (t, 2H, -CH₂-S-), 3.17 (t, 2H, -CH₂-CN); ¹³C NMR (100 MHz, DMSO-d₆): δ 166.5, 160.2, 157.0, 149.6, 142.7, 141.0, 137.9, 137.4, 135.7, 131.0, 129.0, 128.7, 122.4, 119.3, 118.7, 114.9, 113.0, 110.4, 105.9, 55.0 (2), 54.7, 48.6, 47.9; LCMS: m/z 466.1 (M⁺). Anal. Calcd for C₂₂H₂₄BrClN₂O₅S: C, 53.76; H, 3.53; N, 10.90. Found: C, 53.65; H, 3.66; N, 10.85%.

3-((6-(2-Chloro-6-methoxyquinolin-3-yl)-4-(3-bromophenyl)-1,4,5,6-tetrahydropyrimidin-2-yl)thio)propenitrile, 5m: Yellow. Yield 61%. m.p.252-55°C. IR (KBr): 3414 (N-H stretching, pyrimidine ring), 3178 (C-H stretching, aromatic ring), 2941 (C-H stretching, alkyl chain), 2823 (C-H stretching, -OCH₃), 2341 (>C=N stretching, nitrile group), 1545 (>C=N-stretching), 1480 (>C=C-stretching), 721 cm⁻¹ (C-Cl stretching); ¹H NMR (300 MHz, DMSO-d₆): δ 9.65 (s, 1H, H-N< 2° amine), 7.22-8.20 (m, 8H, Ar-H), 6.19 (d, 1H, H-C≡C-N), 5.16 (d, 1H, H-C≡C-NH-), 3.84 (s, 6H, -OCH₃), 3.41 (t, 2H, -CH₂-S-), 3.16 (t, 2H, -CH₂-CN); ¹³C NMR (100 MHz, DMSO-d₆): δ 166.4, 159.0, 157.7, 149.5, 142.7, 141.9, 137.8, 135.1, 131.6, 129.7, 128.4, 127.1 (2), 122.0, 119.5, 114.4 (2), 105.4, 55.7 (2), 54.6, 48.4, 47.7; LCMS: m/z 516.0 (M⁺). Anal. Calcd for C₂₂H₁₂BrClN₂O₅S: C, 53.76; H, 3.53; N, 10.90. Found: C, 53.82; H, 3.63; N, 10.98%.

Conclusion

The central focus of the present work was the development of new medicinally active moieties based on quinoline clubbed pyridine structural fragment. We have synthesized compounds 5a-o by conventional methods with enlargement in yield of reactions. Among the fifteen new derivatives 5a-o, analogues 5k-5m displayed considerable inhibition against nearly all of the tested bacteria and fungi. Out of these analogues, 5l and 5m possessing electron donating group such as methoxy at the meta and para position have been identified as the most potent antibacterial agents with relatively low cytotoxicity. Also, para positioning of methoxy group in derivative 5m resulted in the most potent antifungal activity. The importance of this type of work lies in the design and development of new valuable, less toxic and pharmaceutically effective antimicrobial agents.

Supplementary Information

Supplementary information is available in the website http://nopr.nisc.ac.in/handle/123456789/60.

Acknowledgements

Authors are thankful to the University Grants Commission, New Delhi and Department of Science & Technology, New Delhi for financial support under the NON-SAP and DST-FIST programs respectively. One of the authors, G M Kotadiya is thankful to...
UGC, New Delhi for providing ‘Research Fellowships in Science to Meritorious Students’.

References