Synthesis of novel isoxazolo[2″,3″:1′,2′]pyrimido[4′,5′:4,5]pyrimido-[6,1-b]quinazoline-8-ones and their in vitro anticancer and antimicrobial activities

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Synthesis of novel isoxazolo[2″,3″:1′,2′]pyrimido[4′,5′:4,5]pyrimido[6,1-b]quinazoline-8-ones 6a-j, has been achieved by reaction of 5-amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanides 4, with dimethyl formamide dimethylacetal followed by treatment with anthranilic acids in situ in one-pot. The key intermediate, viz., 5-amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide 4 is obtained by reaction of 3-amino-5-methyl isoxazole with aromatic aldehydes and malononitrile by a three-component one-pot process. The newly synthesized compounds 6a-j have been evaluated for their in vitro anticancer and antimicrobial activity. Compounds 6e and 6f exhibit potent anticancer and antimicrobial activity comparable to that of standard drugs.

Keywords: Isoxazolo[2″,3″:1′,2′]pyrimido[4′,5′:4,5]pyrimido[6,1-b]quinazoline-8-ones, one-pot synthesis, three-component reaction, anticancer activity, antimicrobial activity

Quinazolines and condensed pyrimidines show a wide spectrum of biological activities and have been exhaustively reviewed. Pyrido [2,3-d] pyrimidines are considered to be bioisosteres of quinazoline. The concept of bioisosterism has been exploited by medicinal chemists as an approach to drug design. It is a strategy for rational design of new drugs, applied with a lead compound as special process of molecular modification1. The bioisosteres of quinazolines and pyridopyrimidines has given rise to a number of compounds exhibiting potent pharmacological actions like antibacterials2, EGFr and C-erbB-2 inhibitory activity3, kinase inhibitory activity4, and phosphodiesterase-5-inhibitory activity5-8.

Similarly, isoxazole derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. A large number of isoxazole derivatives exhibited antibacterial9, antifungal10, anticonvulsant11, analgesic12, and anticancer13 activities. Attracted by these impressive scaffolds viz., pyrimido quinazolines and isoxazole derivatives and their pharmacological properties, it was decided to set out to develop a synthetic route to prepare novel isoxazolo[2″,3″:1′,2′]pyrimido-[4′,5′:4,5]-pyrimido[6,1-b]quinazoline-8-ones. As a sequel to the work on the synthesis of pharmacologically active fused isoxazole derivatives14-18, herein is reported the synthesis, in vitro anticancer and antimicrobial activity sof novel series of isoxazolo [2″,3″:1′,2′]pyrimido [4′,5′:4,5]-pyrimido[6,1-b]quinazoline-8-ones.

Results and Discussion
The synthesis of title compounds 6a-j was accomplished by synthetic sequence shown in Scheme 1. The three-component reaction of 3-amino-5-methylisoxazole 1 (purchased from Sigma-Aldrich), substituted aromatic aldehyde 2 and malononitrile 3 in presence of p-toluene sulphonic acid (pTSA), a Lewis acid catalyst, in ethanol furnished novel 5-amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanides 4 in good yields. Compound 4 was treated with dimethyl formamide-dimethyl acetal (DMF-DMA) to produce N,N-dimethyl formamide derivative 5, which was then treated with anthranilic acid in situ to furnish the corresponding novel isoxazolo [2″,3″:1′,2′]pyrimido[4′,5′:4,5]pyrimido[6,1-b]quinazoline-8-ones 6.

Ten new derivatives of isoxazolo[2″,3″:1′,2′]-pyrimido[4′,5′:4,5]pyrimido[6,1-b]quinazoline-8-ones 6a-j were reported. The structures of the newly synthesized compounds 4a-j and 6a-j were confirmed by analytical and spectral data (IR, 1H and 13C NMR, and MS).

IR spectra of 4 exhibited absorption bands at 3430, 3410 cm⁻¹ due to NH functional group stretching vibration, whereas CN functional group shown
absorption band at 2210 cm\(^{-1}\). In \(^1\)H NMR spectra of 4 pyrimidine ring CH proton appeared as a sharp singlet at \(\delta 5.12\), and isoxazole ring proton appeared as a singlet at \(\delta 6.12\). A broad peak at \(\delta 8.21\), which is \(\text{D}_2\text{O}\) exchangable, is assigned to NH\(_2\) protons. \(^{13}\)C NMR spectra of 4 exhibited CN and ArCH carbon signals at \(\delta 82.07\) and 50.29 confirming the cyclization. The mass spectra of 4 displayed the molecular ion \([M^+]\) peak at \(m/z\) 252 confirming cyclization. The IR spectra of 6 showed prominent absorption band at 1670 cm\(^{-1}\) due to C=O functional group. The absence of NH\(_2\) and CN functional group absorption bands in 6, which are present in its precursor 4, clearly indicates the formation of title compound 6 by cyclization. Rest of the signals are in agreement with the proposed structure. \(^{13}\)C NMR spectra of 6 displayed C=O carbon at \(\delta 191.43\) confirming cyclization. The mass spectra of 6 displayed the molecular ion \([M^+]\) peak at \(m/z\) 415, which is in agreement with the assigned structure. Data from the elemental analyses further confirmed the assigned structures of 4a-j and 6a-j.

**Anticancer activity**

The newly synthesized isoxazolo[2''3'':1',2']-pyrimido[4',5':4,5]pyrimido[6,1-b]quinazoline-8-ones 6a-j were evaluated against human cancer cell lines Hela, MCF-7 and NCI-H460 for their *in vitro* anti cancer activity, according to MTT assay method\(^{19,20}\) using Cisplatin (DDP) as a reference drug. The human cell cultures HeLa (cervical cancer), MCF-7 (breast cancer) and NCI-H460 (lung cancer) cell lines, were obtained from National Center for Cell Sciences (NCCS), Pune, India. These cells were grown in recommended media supplemented with10% FBS, 1% L-Glutamine and 1% penicillin-streptomycin amphotericin B in a 5% CO\(_2\) humidified atmosphere at 37°C. Cells were seeded in 5 cm\(^2\) tissue culture flasks (Tarsions, India) at 25,0000 cells/flask in a total volume of 9 mL. When confluent, all the cells were
trypsinized (using Trypsin-EDTA, Himedia, Mumbai, India) and seeded in 96-well plates (Tarsions, India). The cell suspension of 1x10³ cells/mL was prepared in complete growth medium. Stock solutions of the compounds 6a-j were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 mg/mL of gentamycin to obtain working test solution of required concentrations (having <1% DMSO). The 100 µL of cell suspension was added to each well of the 96-well plates. The test materials in complete growth medium (100 µL) were added after 4 h incubation to the wells containing cell suspension. After 48 h of treatment with different concentrations of the test compounds, the cells were incubated with MTT (2.5 mg/mL) for 2 h. The medium was then removed, and 100 µL of DMSO was added to each well to dissolve formazan crystals, which is the metabolite of MTT. After thoroughly mixing, the plate was read at 490 nm for optical density that is directly correlated with cell quantity. The cytotoxic effects of the compounds were calculated as percentage inhibition in cell growth as per the formula. % cytotoxicity = \[1 - \left(\frac{O.D. \text{ in sample well}}{O.D. \text{ in control well}}\right) \times 100\].

The results are presented in Table I. IC₅₀ values were based on dose-response curves (IC₅₀ values, defined as the concentration corresponding to 50% growth inhibition). From Table I, it is clear that some of the compounds showed excellent activity against tumor cells. The compounds 6e and 6f are the most cytotoxic towards all cancer cell lines. This enhanced activity of 6e and 6f may be due to the presence of electron releasing methyl and methoxy substituents on the benzene ring, besides isoxazolo[2',3':1,2']pyrimido [4',5':4,5]pyrimido[6,1-b] quinazoline-8-one nucleus. Compounds 6d, 6g, 6j exhibited moderate to good anticancer activity against three different cell lines, and not selectively towards any particular cell line. The presence of electron withdrawing chloro and bromo groups on benzene ring (6b, 6c, 6h and 6i) did not influence the anticancer activity much, and the compounds showed only moderate activity. Compound 6a did not show significant activity in all the tested cell lines. Among all the tested compounds 6a-j, it is interesting to note that compounds 6e and 6f are most cytotoxic towards all the three cancer cell lines.

### Antibacterial activity

Antibacterial activity of 6a-j in acetone was performed by broth dilution method using nutrient agar against Gram-negative bacteria *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Chromobacterium violaceum* and Gram-positive bacteria *Bacillus subtilis*, *Bacillus sphaericus* and *Staphylococcus aureus* at 100 µg/mL concentration. The minimum inhibitory concentration (MIC) study was carried out by broth dilution method²¹. Ciprofloxacin was used as standard drug for comparison. The ready-made nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and test tubes were autoclaved at pressure of 15 p.s.i. for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound was dissolved in acetone and concentration of 100 µg/mL of the test compound was added in the first test tube, which was then serially diluted. A fixed volume of 0.5 mL overnight culture was added in all the test tubes and was incubated at 37°C for 24 h. After 24 h, these tubes were taken out for turbidity measurement.

The results of antibacterial screening (Table II) reveal that the compounds 6a-j displayed better activity and were more active than the standard drug Ciprofloxacin. Compounds 6e and 6f possessing methyl and methoxy groups as substituent on benzene ring showed better activity. However, the degree of inhibition varied both with the test compound as well as with the bacteria used in the present investigation. In conclusion, almost all the compounds 6a-j, exhibited the maximum activity by inhibiting growth

### Table I — Anticancer activity of 6a-j on human cancer cell lines

<table>
<thead>
<tr>
<th>Compd</th>
<th>HeLa</th>
<th>MCF-7</th>
<th>NCI-H460</th>
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<tr>
<td>6a</td>
<td>62.30±2.6</td>
<td>61.34±2.5</td>
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<tr>
<td>6b</td>
<td>37.32±2.2</td>
<td>47.21±2.6</td>
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<tr>
<td>6c</td>
<td>39.46±3.4</td>
<td>38.29±2.8</td>
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<tr>
<td>6d</td>
<td>28.36±2.0</td>
<td>30.28±2.4</td>
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<tr>
<td>6e</td>
<td>8.61±1.2</td>
<td>10.93±1.3</td>
<td>10.93±1.3</td>
</tr>
<tr>
<td>6f</td>
<td>8.42±2.6</td>
<td>11.51±2.0</td>
<td>11.51±2.0</td>
</tr>
<tr>
<td>6g</td>
<td>21.60±2.3</td>
<td>20.22±2.5</td>
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<tr>
<td>6h</td>
<td>44.51±2.5</td>
<td>52.47±2.2</td>
<td>52.47±2.2</td>
</tr>
<tr>
<td>6i</td>
<td>49.57±2.1</td>
<td>32.17±2.4</td>
<td>32.17±2.4</td>
</tr>
<tr>
<td>6j</td>
<td>24.60±2.3</td>
<td>23.45±2.3</td>
<td>23.45±2.3</td>
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<tr>
<td>Cisplatin (DDP)</td>
<td>3.42±0.1</td>
<td>5.05±0.4</td>
<td>5.05±0.4</td>
</tr>
</tbody>
</table>

³Values are expressed as mean±SEM

Cytotoxicity as IC₅₀ for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.

²¹Data represent the mean ±SEM values of three independent determinations.
of all the six bacteria to a greater extent in comparison with standard drug Ciprofloxacin. The antibacterial activity of 6e and 6f is promising compared with standard drug Ciprofloxacin, and they can be exploited for the formulation of bacteriocide after detailed study.

**Antifungal activity**

Antifungal activity of 6a-j was performed by the agar cup bioassay method using Flucanazole as the standard. The compounds were tested for their antifungal activity against five test organisms, *Aspergillus niger, Chrysosporium tropicum, Rhizopus oryzae, Fusarium moniliforme* and *Curvularia lunata* using agar cup bioassay method at 100 µg/mL concentration. For the antifungal assay, the ready-made potato dextrose agar medium (Himedia, 39 g) was suspended in distilled water (1000 mL) and heated until it dissolved completely. The medium and Petri dishes were autoclaved at pressure of 15 p.s.i. for 20 min. The medium was poured into sterile petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone (100 µg/mL). Agar inoculation cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, test solution 100 (µg/mL) was added. Controls were maintained with acetone and Flucanazole (100 µg/mL). The treated and the controls were kept at RT for 72-96 h. Inhibition zones were measured and diameter was calculated in millimeter. Three to four replicates were maintained for each treatment.

The antifungal activity results (Table III) indicated that compounds 6a-j are significantly toxic towards
all the five fungi and they are lethal even at 100 μg/mL concentration. Compounds 6e and 6f exhibited high antifungal activity which may be due to presence of methyl and methoxy groups as substituents on benzene ring, besides the presence of isoxazolo[2,3-b]pyrimidino[4,5':4,5]pyrimido[6,1-b]quinazoline-8-one nucleus. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi. The antifungal activity of these compounds was compared with the standard drug Flucanazole, and they were found to be more toxic, when compared with standard drug. In conclusion, almost all the compounds, 6a-j are highly toxic towards the fungi under investigation, and they are lethal even at 100 μg/mL concentration in comparison with standard drug Flucanazole at the same concentration. It is noteworthy that the compounds 6e and 6f exhibited maximum activity, hence they may be exploited for control of wilt diseases of different crops as fungicides after detailed study.

Experimental Section

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F254 silica gel plates. Visualization was carried out by exposure to iodine vapour. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer. 1H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. 13C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in δ (ppm) with tetramethyl silane as an internal standard. Mass spectral measurements were carried out by EI method on a Jeol JMC-300 spectrometer at 70 eV. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

General procedure for the synthesis of 5-amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanides, 4a-j.

To a vigorously stirred solution of aromatic aldehyde 2 (1 mmol) and malononitrile 3 (1 mmol) in ethanol (20 mL), 3-amino-5-methylisoxazole 1 (1 mmol) was added and the contents were refluxed while stirring for 2 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured on to crushed ice, the seperated solid was filtered, and purified by recrystallization from ethyl acetate to obtain pure compounds 5-amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanides 4.

5-Amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4a. Orange solid; yield 65%, m.p.172-74°C. IR (KBr): 3430, 3410 (NH), 2210 cm⁻¹ (CN); 1H NMR (300 MHz, CDCl₃): δ 2.28 (s, 3H, CH₃), 5.12 (s, 1H, CH), 6.12 (s, 1H, isoaxazole-CH), 6.98-7.53 (m, 5H, Ar-H), 8.21 (s, 2H, NH₂, D₂O exchangeable); 13C NMR (75MHz, CDCl₃): δ 12.83, 50.29, 78.35, 82.07, 118.43, 126.63, 127.51, 127.85, 128.09, 128.21, 142.87, 165.11, 166.23, 187.29; EI-MS: m/z 253 [M+H]⁺. Anal. Calcd for C₁₁H₁₂N₄O: C, 66.65; H, 4.79; N, 22.21. Found: C, 66.61; H, 4.76; N, 22.18.

5-Amino-7-(2-chlorophenyl)-2-methyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4b. Yellow solid; yield 68%, m.p. 168-70°C. IR (KBr): 3435, 3426 (NH₂), 2218 cm⁻¹ (CN); 1H NMR (300 MHz, CDCl₃): δ 2.24 (s, 3H, CH₃), 5.19 (s, 1H, CH), 6.09 (s, 1H, isoaxazole-CH), 7.10-7.63 (m, 4H, Ar-H), 8.25 (s, 2H, NH₂, D₂O exchangeable); 13C NMR (75MHz, CDCl₃): δ 12.91, 50.34, 78.41, 82.02, 119.02, 126.72, 127.42, 127.78, 128.29, 128.67, 143.13, 165.57, 166.71, 187.39; EI-MS: m/z 287 [M+H]⁺. Anal. Calcd for C₁₁H₁₁ClN₄O: C, 58.65; H, 3.87; N, 19.54. Found: C, 58.61; H, 3.84; N, 19.50%.

5-Amino-7-(2-bromophenyl)-2-methyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4c. Brown solid, yield 73%, m.p. 175-77°C. IR (KBr): 3434, 3431 (NH₂), 2224 cm⁻¹ (CN); 1H NMR (300 MHz, CDCl₃): δ 2.27 (s, 3H, CH₃), 5.20 (s, 1H, CH), 6.16 (s, 1H, isoaxazole-CH), 6.93-7.44 (m, 4H, Ar-H), 8.21 (s, 2H, NH₂, D₂O exchangeable); 13C NMR (75MHz, CDCl₃): δ 12.76, 50.46, 78.64, 82.21, 119.38, 126.81, 127.24, 127.78, 128.11, 128.75, 142.87, 165.23, 166.56, 186.89; EI-MS: m/z 331[M+H]**. Anal. Calcd for C₁₁H₁₁BrN₄O: C, 50.77; H, 3.35; N, 16.92. Found: C, 50.73; H, 3.32; N, 16.89%.

5-Amino-7-(2-hydroxyphenyl)-2-methyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4d. Yellow solid, yield 74%, m.p. 165-67°C. IR (KBr): 3445 (OH), 3428, 3422 (NH₂), 2216 cm⁻¹ (CN); 1H NMR (300 MHz, CDCl₃): δ 2.30 (s, 3H, CH₃), 5.28 (s, 1H, CH), 6.23 (s, 1H, isoaxazole-CH), 7.11-7.64 (m, 4H, Ar-H), 8.29 (s, 2H, NH₂, D₂O exchangeable), 8.51 (s, 1H, OH, D₂O exchangeable); 13C NMR (75 MHz, CDCl₃): δ 12.83, 49.67, 79.12, 82.56, 120.08, 126.62, 127.11, 127.92, 128.34, 128.87, 143.14, 166.29, 166.83, 187.25; EI-MS: m/z 269 [M+H]**. Anal. Calcd for C₁₁H₁₀N₄O₂: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.64; H, 4.48; N, 20.85%.
5-Amino-2-methyl-7-(4-methylphenyl)-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4e. Pale yellow solid, yield 71%, m.p. 171-73°C. IR (KBr): 3435, 3430 (NH$_2$), 2229 cm$^{-1}$ (CN); $^1$H NMR (300 MHz, CDCl$_3$): δ 2.25 (s, 3H, CH$_3$), 2.54 (s, 3H, Ar-CH$_3$), 5.25 (s, 1H, Ar-H), 6.21 (s, 1H, isoxazole-CH), 7.12-7.64 (m, 4H, Ar-H), 8.27 (s, 2H, NH$_2$, D$_2$O exchangeable); $^{13}$C NMR (75MHz, CDCl$_3$): δ 12.91, 24.26, 51.13, 64.47, (4H, 5.77; N, 23.68%). Found: C, 67.65; H, 5.30; N, 21.04. 

5-Amino-7-(4-methoxyphenyl)-2-methyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4f. Yellow solid, yield 78%, m.p. 180-82°C. IR (KBr): 3439, 3430 (NH$_2$), 2235 cm$^{-1}$ (CN); $^1$H NMR (300 MHz, CDCl$_3$): δ 2.28 (s, 3H, CH$_3$), 3.69 (s, 3H, Ar-OCH$_3$), 5.29 (s, 1H, CH), 6.28 (s, 1H, isoxazole-CH), 6.89-7.48 (m, 4H, Ar-H), 8.21 (s, 2H, NH$_2$, D$_2$O exchangeable); $^{13}$C NMR (75MHz, CDCl$_3$): δ 12.82, 51.13, 64.47, 78.92, 82.29, 119.67, 126.82, 127.28, 127.88, 128.56, 130.13, 143.02, 165.78, 167.23, 185.76; EI-MS: m/z 267 [M+H]$^+$. Anal. Calcd for C$_{15}$H$_{14}$N$_2$O: C, 67.65; H, 5.27; N, 21.01%.

5-Amino-7-(4-methoxyphenyl)-2-methyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4g. Pale yellow solid, yield 69%, m.p. 171-73°C. IR (KBr): 3441, 3438 (NH$_2$), 2211 cm$^{-1}$ (CN); $^1$H NMR (300 MHz, CDCl$_3$): δ 2.23 (s, 3H, CH$_3$), 5.17 (s, 1H, CH), 6.20 (s, 1H, isoxazole-CH), 7.04-7.53 (m, 3H, Ar-H), 8.29 (s, 2H, NH$_2$, D$_2$O exchangeable); $^{13}$C NMR (75MHz, CDCl$_3$): δ 12.95, 51.43, 78.89, 82.49, 120.11, 126.72, 127.37, 128.35, 128.59, 129.89, 143.29, 166.32, 167.58, 187.69; ESI-MS: m/z 409 [M+H]$^+$. Anal. Calcd for C$_{19}$H$_{19}$Br$_3$N$_2$O: C, 41.01; H, 2.46; N, 13.66. Found: C, 41.00; H, 2.42; N, 13.63%.

5-Amino-7-(1, 3-benzodioxol-5-yl)-2-methyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide, 4j. Yellow solid, yield 66%, m.p. 188-90°C. IR (KBr): 3425, 3420 (NH$_2$), 2220 cm$^{-1}$ (CN); $^1$H NMR (300 MHz, CDCl$_3$): δ 2.28 (s, 3H, CH$_3$), 5.12 (s, 2H, OCH$_3$O), 5.21 (s, 1H, CH), 6.23 (s, 1H, isoxazole-CH), 7.12-7.67 (m, 3H, Ar-H), 8.23 (s, 2H, NH$_2$, D$_2$O exchangeable); $^{13}$C NMR (75MHz, CDCl$_3$): δ 12.91, 51.56, 78.78, 82.53, 101.58, 121.32, 126.87, 127.43, 128.57, 128.79, 130.12, 143.43, 166.54, 167.67, 187.79; EI-MS: m/z 297[M+H]$^+$. Anal. Calcd for C$_{19}$H$_{14}$N$_2$O$_3$: C, 60.81; H, 4.08; N, 18.91. Found: C, 60.79; H, 4.05; N, 18.87%.

General procedure for the synthesis of 2-methyl-14-phenyl-8H,14H-isoxazolo[2',3':1',2']pyrimido[4',5':4,5]pyrimido[6,1-b]quinazolin-8-ones, 6a-j. To a stirred solution of 5-amino-2-methyl-7-phenyl-7H-isoxazolo[2,3-a]pyrimidin-6-yl cyanide 7 (1 mmol), in ethanol (20 mL), acetic acid (0.5 mL) and DMF-DMA (1.5 mmol) were added sequentially at ambient temperature. The contents were refluxed for 2 h and anthranilic acid (1 mmol) was then added, and the reaction was continued for another 2 h under reflux. After completion of the reaction (monitored by TLC), the reaction mixture was poured to it, and the reaction was continued for another 2 h from ethyl acetate. The resulting precipitate was filtered, washed with cold ethanol and recrystallized from ethyl acetate.

2-Methyl-14-phenyl-8H,14H-isoxazolo[2',3':1',2']pyrimido[4',5':4,5]pyrimido[6,1-b]quinazolin-8-one, 6a. Brown solid, yield 60%, m.p. 210-12°C. IR (KBr): 1670 cm$^{-1}$ (C=O); $^1$H NMR (300 MHz, CDCl$_3$): δ 2.30 (s, 3H, CH$_3$), 4.98 (s, 1H, CH), 6.20 (s, 1H, isoxazole-CH), 6.98-7.50 (m, 9H, Ar-H), 7.95 (s, 1H, pyrimidine ring CH); $^{13}$C NMR (75MHz, CDCl$_3$): δ 12.61, 46.79, 82.11, 115.34, 119.45, 121.83, 126.51, 127.19, 127.34, 127.81, 128.55, 128.87, 132.78, 133.38, 143.87, 144.11, 148.23, 163.75, 163.89, 182.21, 191.43; EI-MS: m/z
14-(2-Chlorophenyl)-2-methyl-8H,14H-isoxazolo-[2',3';1',2']pyrimido[4',5':4,5]pyrimido [6,1-b]quinazolin-8-one, 6b. Yellow solid, yield 68%, m.p. 218-20°C. IR (KBr): 1680 cm⁻¹ (C=O); ¹H NMR (300 MHz, CDCl₃): δ 2.31 (3H, CH₃), 4.91 (s, 1H, CH), 6.26 (s, 1H, isoxazole-CH), 7.10-7.65 (m, 8H, Ar-H), 7.89 (s, 1H, pyridine ring CH); ¹³C NMR (75MHz, CDCl₃): δ 12.56, 46.87, 82.43, 115.67, 119.59, 122.45, 126.68, 127.34, 127.68, 128.11, 128.78, 129.05, 129.21, 132.86, 133.54, 143.91, 144.36, 148.47, 163.98, 164.23, 183.01, 192.56; EI-MS: m/z 416 [M+H]⁺. Anal. Calcd for C₂₂H₁₅N₃O₂: C, 63.54; H, 3.39; N, 16.84. Found: C, 63.50; H, 3.36; N, 16.82%.

14-(2-Bromophenyl)-2-methyl-8H, 14H-isoxazolo-[2',3';1',2']pyrimido[4',5':4,5]pyrimido [6,1-b]quinazolin-8-one, 6c. Yellow solid, yield 65%, m.p. 228-30°C. IR (KBr): 1685 cm⁻¹ (C=O); ¹H NMR (300 MHz, CDCl₃): δ 2.28 (3H, CH₃), 4.96 (s, 1H, CH), 6.29 (s, 1H, isoxazole-CH), 7.07-7.55 (5m, 8H, Ar-H), 7.91 (s, 1H, pyridine ring CH); ¹³C NMR (75MHz, CDCl₃): δ 12.41, 46.81, 82.58, 115.79, 120.21, 122.58, 126.72, 127.59, 127.75, 128.32, 128.89, 129.17, 129.42, 132.94, 133.67, 144.13, 144.48, 148.79, 164.21, 164.57, 183.76, 193.32; EI-MS: m/z 416 [M+H]⁺. Anal. Calcd for C₂₂H₁₅N₃O₂: C, 57.41; H, 3.07; N, 15.22. Found: C, 57.38; H, 3.03; N, 15.19%.

14-(2-Hydroxyphenyl)-2-methyl-8H, 14H-isoxazolo-[2',3';1',2']pyrimido[4',5':4,5]pyrimido [6,1-b]quinazolin-8-one, 6d. Pale yellow solid, yield 61%, m.p. 233-39°C. IR (KBr): 3455 (OH), 1675 cm⁻¹ (C=O); ¹H NMR (300 MHz, CDCl₃): δ 2.27 (3H, CH₃), 4.91 (s, 1H, CH), 6.30 (s, 1H, isoxazole-CH), 6.97-7.49 (m, 8H, Ar-H), 7.90 (s, 1H, pyridine ring CH), 8.24 (s, 1H, OH, D₂O exchangeable); ¹³C NMR (75MHz, CDCl₃): δ 12.67, 46.61, 82.45, 115.56, 120.34, 122.71, 126.85, 127.69, 127.89, 128.21, 128.92, 129.26, 129.56, 133.12, 133.72, 144.21, 144.62, 148.82, 164.28, 182.42, 192.87; EI-MS: m/z 398 [M+H]⁺. Anal. Calcd for C₂₂H₁₅N₃O₂: C, 66.49; H, 3.80; N, 17.62. Found: C, 66.45; H, 3.77; N, 17.59%.

2-Methyl-14-(4-methylphenyl)-8H, 14H-isoxazolo-[2',3';1',2']pyrimido[4',5':4,5]pyrimido [6,1-b]quinazolin-8-one, 6e. Pale yellow solid, yield 66%, m.p. 225-27°C. IR (KBr): 1690 cm⁻¹ (C=O); ¹H NMR (300 MHz, CDCl₃): δ 2.31 (3H, CH₃), 2.35 (3H, Ar-CH₃), 4.98 (s, 1H, CH), 6.28 (s, 1H, isoxazole-CH), 7.10-7.67 (m, 8H, Ar-H), 7.93 (s, 1H, pyridine ring CH); ¹³C NMR (75MHz, CDCl₃): δ 12.82, 24.23, 46.91, 82.58, 115.62, 120.41, 122.84, 126.91, 127.58, 127.93, 128.28, 129.02, 129.36, 129.69, 133.21, 133.82, 144.37, 144.72, 148.92, 164.42, 164.72, 183.55, 192.68; EI-MS: m/z 396 [M+H]⁺. Anal. Calcd for C₂₂H₂₁N₃O₂: C, 69.86; H, 4.33; N, 17.17. Found: C, 69.82; H, 4.29; N, 17.16%.
14-(2,6-Dibromophenyl)-2-methyl-8H, 14H-isoxazolo[2''\ 3''\:1',2']pyrimido[4',5':4,5] pyrimido-[6,1-b]quinazolin-8-one, 6i. Pale yellow solid, yield 64%, m.p. 248-50°C. IR (KBr): 1680 cm\(^{-1}\). 1H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 2.29 (s, 3H, CH\(_3\)), 4.95 (s, 1H, CH), 6.28 (s, 1H, pyrimidine ring CH); 13C NMR (75MHz, CDCl\(_3\)): δ 12.91, 46.75, 81.43, 114.56, 121.78, 122.72, 127.23, 127.62, 128.35, 128.62, 130.21, 130.34, 130.59, 133.76, 133.82, 144.21, 145.45, 149.32, 164.72, 164.93, 183.22, 192.78; EI-MS: \(m/z\) 538 [M+H]\(^+\). Anal. Caled for C\(_{22}\)H\(_23\)Br\(_2\)N\(_4\)O\(_2\): C, 43.25; H, 2.62; N, 12.01. Found: C, 43.23; H, 2.61; N, 12.01.

14-(1,3-Benzodioxol-5-yl)-2-methyl-8H, 14H-isoxazolo[2'',3'':1',2']pyrimido[4',5':4,5] pyrimido-[6,1-b]quinazoline-8-ones have been evaluated for their in vitro anticancer and antimicrobial activity. The authors are thankful to the Head, Department of Chemistry, Kakatiya University, Warangal for providing necessary facilities, the Director, Indian Institute of Chemical Technology, Hyderabad for recording spectra. One of the authors (B. Kishore) thanks UGC, New Delhi for financial assistance (JRF). The authors are grateful to Prof. Y. N. Reddy, Department of Pharmacology and Toxicology, Kakatiya University, Warangal for screening in vitro anticancer activity, and Prof. M. V. Rajam, Department of Genetics, University of Delhi, South Campus, New Delhi for screening the antimicrobial activity.

Conclusion
In conclusion, the synthesis of novel isoxazolo[2'',3'':1',2']pyrimido [4',5':4,5]pyrimido[6,1-b]quinazoline-8-ones has been achieved from readily accessible starting materials in moderate to good yields in one-pot. The newly synthesized novel isoxazolo[2'',3'':1',2']pyrimido[4',5':4,5]pyrimido[6,1-b]quinazoline-8-ones have been evaluated for their in vitro anticancer and antimicrobial activity. Compounds 6e and 6f exhibited significant activity. Thus, they may be considered as future drug candidates and by doing a simple modification in the structure, a new potent analogue can be generated with desired activity with good efficacy. It also requires future attempts to reveal the exact mechanism of action and structure-activity relationship.

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References