Synthesis and anti-bacterial evaluation of 4-aryloxymethyl carbostyrils derived from substructures and degradation products of Vancomycin

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Vancomycin has been used as an antibiotic selectively against Gram-positive bacteria; however in the past decade they have grown resistant against it. The present work describes synthesis of a series of 4-aryloxymethyl carbostyrils derived from the reaction of 4-bromomethyl carbostyrils with degradation products of Vancomycin (ethyl gallate and ethyl ester of N-benzoyle tyrosine ethyl ester). Further, gallate ethers 4a-d and tyrosine ethers 5a-d have been found to be selectively active against Gram-positive bacteria.

Keywords: Ethyl gallate, N-benzoyle tyrosine ethyl ester, antibacterial activity, gram-positive, 4-bromomethyl carbostyril

Extensive plant origin\(^1\) and structural diversity of biological significance\(^2\) are the two salient features of coumarins. Coumarins and 1-aza coumarins (carbostyrils) are isosteric heterocyclic systems possessing a close biosynthetic relation as revealed by their metabolism in plants\(^3\). 4-Aryloxymethyl coumarins and 1-aza coumarins have been found to exhibit anti-microbial\(^4\), anti-inflammatory\(^5\) and interesting photo-physical properties\(^6\). 1-Aza-coumarins or carbostyrils serve as potent inhibitors of bacterial DNA gyrase, which are involved in cell growth\(^7\). 3-Amino carbostyrils have been reported to behave as potassium (Maxi-K) channel openers for neural protection\(^8\). Coumarin and 1-aza coumarin derivatives, with substituents at 4\(^{th}\) allylic position have exhibited promising anti-bacterial activity\(^9\). Fluorinated aryloxymethyl carbostyrils have shown potent anti-inflammatory and anti-microbial activity\(^10\).

Hydroxy coumarins like scopoletin and gallic acid have been found to occur in Pelargonium sidoides, Pelargonium reniforme\(^11\) and other plant species, exhibiting a range of biological activities\(^12\). Ester conjugates of 7-hydroxy coumarin with gallic acid have been found to be anti-proliferative against human cancer cell lines\(^13\), methyl gallates with bisaryl ether linkage and tyrosine moieties are common substructures to the anti-biotics of vancoomycin family\(^14\). Naturally occurring bromotyrosine derivatives have been found to possess anti-microbial effect on the methicillin resistant S. aureus (MRSA)\(^15\).

Further continuing with our previously reported work\(^16\), it was planned to employ two of the degradation products of Vancomycin viz. tyrosine ethyl ester and ethyl gallate and react them with 4-bromomethyl 1-azacarbostyril (Figure 1). The steps involved in these are represented in the Scheme I.

Present work

Work carried out during the present investigation has been described in Scheme I. The synthetic sequence of reactions leading to the title compounds was initiated with preparation of 4-bromomethyl-1-aza-coumarins (carbostyrils) 2 which were synthesized by bromination of acetoacetanilides in acetic acid and subsequent cyclisation of the intermediate 1 in sulphuric acid.

1 M of ethyl gallate 3 along with 1.5 M of anhydrous potassium carbonate in ethanol was allowed to stir for 30 min. To this equimolar quantity of substituted 4-bromomethyl-carbostyril (1 M) 2 was added, the stirring was continued for 24 h. The reaction was monitored using TLC. It was then quenched in crushed ice and neutralized with 1:1 HCl, the precipitate separated out immediately; it was dried and purified by recrystallization from appropriate solvents to give ethers 4a-d.

1 M of commercially available N-benzoyletyrosine ethyl ester 3a was reacted with 4-bromomethyl
carbostyril along with 1.5 M of anhydrous potassium carbonate in ethanol to obtain ethers 5a-d.

Anhydrous potassium carbonate abstracts proton from phenolic –OH to give resonance stabilized phenoxide anion, which then reacts with 4-bromomethyl-carbostyril to give the desired compounds.

Results and Discussion

Formation of products 4 and 5 is well supported by spectroscopic analysis. In case of compound 4b (R = 6-Cl) IR spectrum exhibited two bands at 1668 cm\(^{-1}\) and a broad band around 3425 cm\(^{-1}\) due to amide carbonyl and –OH stretching bands of gallate.
moiety respectively. The compound was brown in colour and melts at 202-206°C with a yield of about 70%. Formation of ethers 4b was further confirmed by 1H NMR, wherein the O-CH$_2$ protons appear as a singlet at δ 5.19 which is characteristic of 4-aryloxymethyl carbostyril (10). The C$_3$H of carbostyril was observed at δ 6.6. Aromatic protons appeared in the range δ 7.2-7.5. Methyl and methylene protons of ethoxy appeared as a triplet at δ 1.3 and quartet at δ 4.3 with $J$ = 7.0 Hz. The downfield D$_2$O exchangeable signals at δ 11.9 and 11.8 corresponded to the two phenolic −OH protons and a singlet at δ 10.1 corresponded to the lactam NH. Molecular ion peak at m/z 389 (M$^+$, 30%), 391 (M+2, 9.9%) in the EI-MS confirmed the proposed structure.

Formation of product 5b was confirmed by spectral analysis. IR spectrum exhibited three prominent bands at 3425, 1668 and 1716 cm$^{-1}$ due to NH, amide and ester carbonyls respectively. The compound was white in colour and melted at 186-88°C with a yield of around 58%. In 1H NMR spectrum, the signals observed at δ 5.3 (s), 4.1 (q), (J = 7.2 Hz) and δ 3.2 (m) are due to O-CH$_2$-Ar, O-CH$_2$ (ethoxy) and Ph-CH$_2$ protons respectively. The three proton signals at δ 1.1 (t) $J$ = 7.2 Hz correspond to the CH$_3$ of ethoxy group. Aromatic protons (12H), appear in the range of δ 7.0-7.9. The −NH proton appeared as a doublet at δ 8.7 with a coupling constant of $J$ = 7.2 Hz, whereas the vicinal C-H proton appeared at δ 4.6 as a multiplet. A singlet at δ 11.8 corresponded to lactam NH. The molecular ion peak in EI-MS was not observed, instead a peak at m/z 384 (15%) and 386 (4.9%) indicated the loss of C$_6$H$_3$-CO–NH moiety. Compounds synthesized along with their spectral data are given in the Experimental Section. Physical properties of other compounds have been listed in Table I.

**Anti-bacterial screening**

All the synthesized compounds were screened for their antibacterial activity against Gram Positive (E. faecalis and S. aureus) and Gram negative (P. aeruginosa and E. coli) bacteria with Ciprofloxacin as the standard. As per literature reports, Gram positive bacteria have become resistant to Vancomycin in recent years but by the linkage of carbostyril moiety with the degradation products of Vancomycin viz., an ether linkage, has specifically inhibited the growth of Gram positive bacteria whereas they were moderately active against Gram negative bacteria. Among all the compounds, 4b, 4d, 5c and 5d were ten times more active than Ciprofloxacin with MIC value of 0.2 µg/mL. Compounds 4c and 5a showed MIC value of 0.4 and 0.8 µg/mL respectively.

On the other hand, all the compounds showed excellent activity against S. aureus with MIC ranging from 0.2-0.4 µg/mL. The details are represented in Table II. These results are in conjunction with results on ethers of 4-bromomethyl coumarin with degradation products of vancomycin which was reported earlier by our lab$^{16}$.

**Experimental Section**

The melting points were determined by open capillary method and are uncorrected. IR spectra (KBr disc) were recorded on Nicolet-5700 FT-IR spectrometer. 1H NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-$d_6$ as solvent and TMS as internal standard. The chemical shifts are expressed in δ (ppm). Mass spectra were recorded using Shimadzu GCMS-QP2010S mass spectrometer. The elemental analyses were carried out using Hereaus CHN rapid analyser. Homogeneity of the compounds was checked by TLC. All the chemicals purchased were of analytical grade and were used without further purification unless otherwise stated.

### Table I — Physical properties of compounds in series 4 and 5

<table>
<thead>
<tr>
<th>Compd</th>
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<th>Crystallisation</th>
<th>m.p. (°C)</th>
<th>Yield (%)</th>
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<td>4a</td>
<td>H</td>
<td>Dioxane</td>
<td>244-46</td>
<td>68</td>
</tr>
<tr>
<td>4b</td>
<td>6-Cl</td>
<td>Dioxane</td>
<td>202-206</td>
<td>70</td>
</tr>
<tr>
<td>4c</td>
<td>7-Cl</td>
<td>DMF</td>
<td>174-76</td>
<td>71</td>
</tr>
<tr>
<td>4d</td>
<td>8-CH$_3$</td>
<td>DMF</td>
<td>224-26</td>
<td>75</td>
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<tr>
<td>5a</td>
<td>H</td>
<td>Ethanol</td>
<td>152-54</td>
<td>55</td>
</tr>
<tr>
<td>5b</td>
<td>6-Cl</td>
<td>Ethanol</td>
<td>186-88</td>
<td>58</td>
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<tr>
<td>5c</td>
<td>7-Cl</td>
<td>Ethanol</td>
<td>203-206</td>
<td>62</td>
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<tr>
<td>5d</td>
<td>8-CH$_3$</td>
<td>Ethanol</td>
<td>206-208</td>
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### Table II — Antibacterial and antifungal activity of compounds in series 4 and 5

<table>
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<th>Compd</th>
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<th>Gram-negative</th>
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<td></td>
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<td>P. aeruginosa</td>
<td>E. coli</td>
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<tr>
<td>4a</td>
<td>H</td>
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<td>--</td>
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<tr>
<td>4b</td>
<td>6-Cl</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>4c</td>
<td>7-Cl</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>4d</td>
<td>8-CH$_3$</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>5a</td>
<td>H</td>
<td>0.4</td>
<td>0.8</td>
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<tr>
<td>5b</td>
<td>6-Cl</td>
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<td>0.2</td>
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<td>5c</td>
<td>7-Cl</td>
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<tr>
<td>5d</td>
<td>8-CH$_3$</td>
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<td>0.2</td>
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<tr>
<td>Ciprofloxacin</td>
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Synthesis of substituted 4-bromomethyl-1-azacoumarins (carbostyrils), 2

The required substituted 4-bromomethyl-1-azacoumarins have been synthesized by bromination of acetoacetanilides and cyclising the intermediate 1 in sulphuric acid to give 4-bromomethyl-carbostyril18.

Synthesis of ethyl gallate, 3

It was synthesized using known procedure19.

General procedure for the synthesis of ethyl 3,5-dihydroxy-4-(2-oxo-1,2-dihydroquinolin-4-yl)methoxy)benzoates, 4

A mixture of ethyl gallate 3 (0.001 M) and of anhydrous potassium carbonate (0.001 M) were stirred for 30 min in dry ethanol (30 mL). To this, substituted 4-bromomethyl carbostyril 2 was added and stirring was continued for 24 h, the reaction was monitored using TLC. After completion, the reaction mixture was quenched in crushed ice and neutralized using 1:1 HCl. The separated solid was filtered and washed with dilute HCl. The compound was dried and purified by recrystallization from appropriate solvent.

Ethyl 3,5-dihydroxy-4-(2-oxo-1,2-dihydroquinolin-4-yl) methoxy)benzoate, 4a: Dioxane, m.p.244-46°C. Yield 68%. IR (KBr): 1655 (C=O), 3425 (-OH) 1740 cm−1. 1H NMR (DMSO-d6, 400 MHz, TMS): δ 1.3 (t, 3H, J = 7.2 Hz, O-CH3), 4.2 (q, 2H, O-CH2-CH3, J = 7.2 Hz), 5.19 (s, 2H, O-CH2-CH3, J = 7.2 Hz), 6.2 (s, 1H, O-CH), 6.6 (s, 1H, C3-H),7.1-7.8 (m, 5H, Ar-H), 9.9 (s, 1H, NH Lactam), 11.7 (s, 1H, OH, D2O Exchangeable). Anal. Calcd for C19H17NO7: C, 64.21; H, 4.80; N, 3.89%. Found: C, 64.21; H, 4.80; N, 3.89%.

Ethyl 4-(6-chloro-2-oxo-1,2-dihydroquinolin-4-yl)methoxy)-3,5-dihydroxybenzoate, 4b: Dioxane, m.p.202-206°C. Yield 70%. IR (KBr): 1668 (C=O), 3425 (-OH), 1739 cm−1 (Ester C=O); 1H NMR (DMSO-d6, 400 MHz, TMS): δ 1.3 (t, 3H, J = 7.2 Hz, O-CH3), 4.3 (q, 2H, O-CH2-CH3, J = 7.2 Hz), 5.19 (s, 2H, O-CH2-CH3, J = 7.2 Hz), 6.2 (s, 1H, O-CH), 6.6 (s, 1H, C3-H),7.1-7.8 (m, 5H, Ar-H), 10.1 (s, 1H, NH Lactam), 11.9 (s, 1H, OH, D2O Exchangeable); 11.9 (s, 1H, OH, D2O Exchangeable); EI-MS: m/z 355 (M+, 10%). Anal. Calcd for C19H17NO7: C, 64.22; H, 4.82; N, 3.94. Found: C, 64.21; H, 4.80; N, 3.89%.

Ethyl 4-(6-chloro-2-oxo-1,2-dihydroquinolin-4-yl)methoxy)-3,5-dihydroxybenzoate, 4d: DMF. m.p.224-26°C. Yield 75%. IR (KBr): 1659 (C=O), 3414 (-OH), 1743 cm−1 (Ester C=O); 1H NMR (DMSO-d6, 400 MHz, TMS): δ 1.4 (t, 3H, J = 7.2 Hz, O-CH3-C2H5, 2.4 (s, 3H, -CH3), 4.2 (q, 2H, O-CH2-CH3, J = 7.2 Hz), 5.4 (s, 2H, O-CH3), 6.2 (s, 1H, C3-H),7.1-7.8 (m, 5H, Ar-H). 9.9 (s, 1H, NH Lactam), 10.7 (s, 1H, OH, D2O Exchangeable), 10.8 (s, 1H, OH, D2O Exchangeable). Anal. Calcd for C23H19NO6: C, 65.03; H, 5.18; N, 3.79. Found: C, 64.99; H, 5.13; N, 3.74%.

General procedure for the synthesis of ethyl 2-benzamido-3-(4-((2-oxo-1,2-dihydroquinolin-4-yl)methoxy)phenyl)propanoates, 5

A mixture of N-benzoyl tyrosine ethyl ester 3a (0.001 M) and anhydrous potassium carbonate (0.001 M) were stirred for 30 min in dry ethanol (30 mL). Substituted 4-bromomethyl carbostyril 2 was added and stirring was continued for 24 h, the reaction was monitored using TLC. After completion, the reaction mixture was quenched in crushed ice and neutralized using 1:1 HCl. The separated solid was filtered and washed with dilute HCl. The compound was dried and purified by recrystallization from appropriate solvent.

Ethyl 2-benzamido-3-(4-((2-oxo-1,2-dihydroquinolin-4-yl)methoxy)phenyl)propanoate, 5a: Ethanol, m.p.152-54°C. Yield 55%. IR (KBr): 1665 (amide C=O), 3423 (-NH), 1724 cm−1 (Ester, C=O); 1H NMR (DMSO-d6, 400 MHz, TMS): δ 1.1 (t, 3H, J = 7.2 Hz, O-CH3-C2H5), 3.1 (m, 2H, Ph-CH2), 4.2 (q, 2H, O-CH2-CH3, J = 7.2 Hz), 4.5 (t, 1H, Ph-CH2-CH3, J = 7.2 Hz), 5.2 (s, 2H, O-CH3), 6.2 (s, 1H, C3-H), 7.0-7.8 (m, 13H, Ar-H), 8.7 (d, 1H, NH-CO, J = 7.2Hz, D2O Exchangeable), 11.0 (s, 1H, NH Lactam); EI-MS: m/z 470 (M+, 5%). Anal. Calcd for C29H25NO6: C, 71.47; H, 5.57; N, 5.95. Found: C, 71.42; H, 5.53; N, 5.95%.

Ethyl-2-benzamido-3-(4-((6-chloro-2-oxo-1,2-dihydroquinolin-4-yl)methoxy)phenyl)propanoate, 5b: Ethanol; m.p.186-88°C. Yield 58%. IR (KBr):
1668 (amide C=O), 3425 (-NH) 1715 cm$^{-1}$ (ester C=O); \textsuperscript{1}H NMR (DMSO-$d_6$, 400 MHz, TMS): $\delta$ 1.1 (t, 3H, O-CH$_2$-CH$_3$, $J$ = 7.2 Hz ), 3.2 (m, 2H, Ph-CH$_2$), 4.1 (q, 2H, O- CH$_2$-CH$_3$, $J$ = 7.2 Hz ), 4.6 (m, 1H, Ph-CH$_2$-CH$_2$), 5.3 (s, 2H, O-CH$_2$), 6.6 (s, 1H, C$_4$-H), 7.0-7.9 (m, 12H, Ar-H), 8.7 (d, 1H, Ar-H), 9.7 (d, 2H, NH), 11.8 (s, 1H, lactam NH); EI-MS: (M$^+$-C$_6$H$_5$CONH)$^+$ m/z 384 (15%) and 386 (4.9%). Anal. Calcd for C$_{25}$H$_{25}$ClN$_2$O$_5$: C, 66.60; H, 4.99; N, 5.55. Found: C, 66.58; H, 4.94; N, 5.51%.

Ethyl-2-benzamido-3-(4-6((7-chloro-2-oxo-1,2-dihydroquinolin-4yl)methoxy)phenyl) propanoate, 5c:

Ethanol; m.p.203-205°C. Yield 62%. IR (KBr): 1654 (amide C=O); \textsuperscript{1}H NMR (DMSO-$d_6$, 400 MHz, TMS): $\delta$ 1.15 (t, 3H, O-CH$_2$-CH$_3$, $J$ = 7.2 Hz ), 3.1 (m, 2H, Ph-CH$_2$), 4.11 (q, 2H, O- CH$_2$-CH$_3$, $J$ = 7.2 Hz), 4.62 (t, 1H, Ph-CH$_2$-CH, $J$ = 7.2 Hz), 5.31 (s, 2H, O-CH$_2$), 6.6 (s, 1H, C$_4$-H), 7.0-7.9 (m, 12H, Ar-H), 8.79 (d, 1H, NH CO, $J$ = 7.2Hz, D$_2$O Exchangeable), 11.2 (s, 1H, NH Lactam); EI-MS: (M$^+$-C$_6$H$_5$CONH)$^+$ m/z 384 (15%) and 386 (4.9%). Anal. Calcd for C$_{25}$H$_{25}$ClN$_2$O$_5$: C, 66.60; H, 4.99; N, 5.55. Found: C, 66.58; H, 4.95; N, 5.50%.

Ethyl-2-benzamido-3-(4-6((8-methyl-2-oxo-1,2-dihydroquinolin-4yl)methoxy)phenyl) propanoate, 5d:

Ethanol; m.p.203-205°C. Yield 58%. IR (KBr): 1654 (amide C=O), 3427 (-NH), 1720 cm$^{-1}$ (C=O); \textsuperscript{1}H NMR (DMSO-$d_6$, 400 MHz, TMS): $\delta$ 1.2 (t, 3H, O-CH$_2$-CH$_3$, $J$ = 7.2 Hz ), 2.4 (s,3H,-CH$_3$), 3.3 (m, 2H, Ph-CH$_2$), 4.3 (q, 2H, O- CH$_2$-CH$_3$, $J$ = 7.2 Hz), 4.7 (t, 1H, Ph-CH$_2$-CH, $J$ = 7.2 Hz), 5.3 (s, 2H, O-CH$_2$), 6.6 (s, 1H, C$_4$-H), 7.0-7.8 (m, 12H, Ar-H), 8.7 (d, 1H, NH CO, $J$ = 7.2Hz, D$_2$O Exchangeable), 10.8 (s, 1H, NH Lactam); EI-MS: (M$^+$-C$_6$H$_5$CONH)$^+$ m/z 364 (5%) and 368 (1.5%). Anal. Calcd for C$_{25}$H$_{25}$ClN$_2$O$_5$: C, 66.60; H, 4.99; N, 5.55. Found: C, 66.58; H, 4.95; N, 5.50%.

Anti-microbial screening
The anti-bacterial activity of the synthesized compounds were performed in vitro against (i) Gram-positive bacteria: \textit{E. faecalis} (ATCC no.35550), \textit{S. aureus} (ATCC no. 12598) (ii) Gram-negative bacteria: \textit{E. coli} (ATCC No. 25922), \textit{P. aeruginosa} (ATCC No.25619) by broth dilution methods$^{15}$. The MIC determination of the tested compounds was carried out in comparison with Ciprofloxacin. The anti-fungal activity was performed against these standard strains: \textit{C. albicans} (ATCC no.2091) and \textit{A. niger} (ATCC no. 9029). The MIC determination of the tested compounds was carried out in comparison with Fluconazole by broth dilution method$^{17}$. Nine dilutions of each drug were prepared with BHI (brain heart infusion) for MIC. In the initial tube 20 µL of drug was added into the 380 µL of BHI broth. Then from the initial tube 200 µL was transferred to the first tube containing 200 µL of BHI broth. This was considered as 10$^{-1}$ dilution. From 10$^{-3}$ diluted tube 200 µL was transferred to second tube to make 10$^{-2}$ dilution. The serial dilution was repeated up to 10$^{-9}$ dilution for each drug. From the maintained stock cultures of required organisms, 5 µL was taken and added into 2 mL of BHI broth. In each serially diluted tube 200 µL of above culture suspension was added. The tubes were incubated for 24 h at 37°C in the incubator and observed for turbidity.

Conclusion
The present investigation has shown that, introduction of substructures and degradation products of Vancomycin viz. gallic and tyrosine esters at allylic position in carbostyrils leads to molecules with enhanced degree of specificity in their antimicrobial activity against Gram-positive bacteria. Vancomycin, which was traditionally used against Gram-positive bacteria is now ineffective.

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References