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Full Length Research Paper

Synthesis and antimicrobial screening of peptidyl derivatives of bromocoumarins/methylimidazoles

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In this investigation, it was of interest to synthesize novel series of peptide analogs of 3-bromocoumarins and 2-methylimidazoles. Substituted coumarinyl-oxyacetic acids 1, 2 were synthesized by alkylation of corresponding hydroxycoumarins with chloroacetic acid. The imidazolyl derivatives 13, 14 were prepared by interaction of 2-methylimidazole with substituted/unsubstituted anthranilic acids. Coupling of compounds 1, 2, 13 and 14 with different amino acid methyl ester hydrochlorides, dipeptide, tripeptide and tetrapeptide methyl esters afforded novel coumarino/imidazolopeptide derivatives 3-10 and 15-22. Selected peptide ester derivatives were further hydrolyzed by using lithium hydroxide to afford corresponding acid derivatives 11, 12 and 23, 24. The antibacterial and antifungal effects of synthesized peptide derivatives were studied against eight pathogenic microbes. Among the tested compounds, 19, 22 and its hydrolyzed analog 24 exhibited good antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Trichophyton mentagrophytes* and *Microsporum audouinii*, and compounds 8, 10 and 12 displayed good antifungal activity against *Candida albicans* with minimum inhibitory concentration of 6.25 g/ml.

Key words: Bromocoumarin, methylimidazole, anthranilic acid, peptide coupling, antibacterial activity, antifungal activity.

INTRODUCTION

During the past five decades, natural, semi-synthetic or synthetic antimicrobial agents have been used with great success against the life threatening diseases. At the onset of new millennium, rapid emergence of antibiotic resistant pathogens led researchers to continue their efforts to develop new classes of antimicrobials (Stephensen, 2001). Out of these, research lines aim to exploit the therapeutic potential of a variety of natural antimicrobial peptides and their synthetic analogs, for the development of antimicrobials with a mechanism of action different from those of conventional antibiotics (Hancock, 1997) . Most common and repeating amino acid units from structures of natural and synthetic antimicrobial peptides (Dahiya, 2008a; 2008b; 2007; Dahiya and Kumar, 2008; 2007) were selected and combined together in form of di/tri/tetrapeptides which were further coupled with bioactive heterocyclic moieties with an anticipation to get novel potent compounds of biological interest. Moreover, past literature is enriched with several findings proving biological potential of N- and O-containing heterocycles. Many coumarin and imidazole derivatives are known to possess diverse bioactivities such as antimicrobial and other pharmacological activities (Abou-Melha et al., 2008; Sardari et al., 1999; Khabnadideh et al., 2003; Hadizadeh et al., 2008; Amini et al., 2008; Lee et al., 2006). In addition, there are lot of reports in literature indicating antimicrobial potential of substituted benzoic acid analogs (Shahid et al., 2005). As alkylation has been proved as vital organic reaction to afford bioactive antimicrobial congeners, 2-(2-oxo-2*H*-4-chromenyloxy)acetic acid 1 and 2-(3-bromo-2-oxo-2*H*-4-chromenyloxy)acetic acid 2 were synthesized from corresponding hydroxycoumarins in alkaline medium. Further, prompted from the antimicrobial properties of substituted imidazoles and benzoic acids, these two vital moieties were combined together into single nuclei to yield 2-(2-methyl-1*H*-5-imidazolyl)benzoic acid 13 and

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5-iodo-2-(2-methyl-1*H*-5-imidazolyl)benzoic acid 14. Thus, keeping in view the biological potential of amino acids/peptides and further, taking advantage of biodegradability and biocompatibility of peptides and in an expectation to get novel potent congeners with potential antimicrobial effects, the present investigation was aimed at the synthesis of four novel series of coumarino/imidazolopeptides, 2- (2-oxo-2H-4-chromenyloxy)acetyl amino acids/peptides 3—6, 2-(3-bromo-2-oxo-2H-4-chromenyloxy)acetyl amino acids/peptides 7—10, 2-(2-methyl-1H -5-imidazolyl)benzoyl amino acids/peptides 15—18 and 5-iodo-2-(2-methyl-1H-5-imidazolyl)benzoyl amino acids/peptides 19—22. Selected coumarinopeptides and imidazolopeptides were further hydrolyzed to get corresponding acid derivatives 11, 12, 23 and 24. The potential antibacterial and antifungal activities of all the newly synthesized compounds were also studied.

MATERIALS AND METHODS

General experimental part

Melting points were determined by open capillary method and are uncorrected. L-Amino acids, di -tert-butyldicarbonate (Boc_2O), dicyclohexylcarbodiimide (DCC), trifluoroacetic acid (TFA), triethylamine (TEA) and *N*-methylmorpholine (NMM) were purchased from Spectrochem Limited (Mumbai, India). 1-Ethyl- 3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) and diisopropylcarbodiimide (DIPC) were procured from RANKEM RFCL Limited (New Delhi, India). The chemical structures of all newly synthesized compounds were elucidated by means of spectral as well as elemental analysis. The IR spectra were run on Shimadzu 8700 FTIR spectrophotometer using a thin film supported on KBr pellets or utilizing chloroform. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC NMR spectrometer using $CDCl_3$ as solvent and TMS as internal standard. The mass spectra of the representative compounds were recorded on JMS-DX 303 Mass spectrometer (Jeol, Tokyo, Japan) operating at 70 eV by electron impact ionization technique (EI MS). Optical rotation of synthesized peptide derivatives was measured on automatic polarimeter in a 2 dm tube at 25 °C using sodium D-light and methanol as solvent. Elemental analyses of all compounds were performed on Vario EL
III elemental analyzer. Purity of all synthesized compounds was checked by TLC on precoated silica gel G plates utilizing chloroform / methanol as developing solvent system in different ratios (9:1 / 8:2 v/v).

General method for preparation of di/tri/tetrapeptide intermediates

L-Amino acid methyl ester hydrochloride/peptide methyl ester (10 mmol) was dissolved in $CHCl_3$ (20 ml) . To this, triethylamine (TEA) (2.8 ml, 20 mmol) was added at 0 °C and the reaction mixture was stirred for 15 minutes. Boc-L-amino acid/peptide (10 mmol) in $CHCl_3$ (20 ml) and dicyclohexylcarbodiimide (DCC) (2.1 g, 0.01 mol) were added with stirring. After 24 h, the reaction mixture was filtered and the residue was washed with $CHCl_3$ (30 ml) and added to the filtrate. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether (b.p. 40-60°C) followed by cooling at 0°C.

^{t}Butyloxycarbonyl-histidinyl-isoleucine methyl ester: Yield 87%; $[\alpha]_D$ −34.5° (*c*, 0.3 in MeOH); R_f - 0.84; IR ($CHCl_3$) *v* cm^{-1}: 3479, 3125, 1751, 1639, 1535, 1385, 1365, 1268, 929. ^{1}H-NMR ($CDCl_3$) *δ*: 9.10 (1H, br. s, NH, imz), 7.58-7.56 (1H, d, *J*=7.9 Hz, H-b, imz), 6.56 (1H, br. s, NH), 6.38 (1H, br. s, NH), 6.27 (1H, s, H-d, imz), 4.92—4.87 (1H, m, H-a, his), 4.22—4.18 (1H, dd, *J*=4.85 Hz, 3.2 Hz, H- , ile), 3.53 (3H, s, OCH_3), 2.97—2.95 (2H, d, *J*=4.6 Hz, H-b), 2.05—1.94 (1H, m, H-β), 1.72—1.66 (2H, q, H-), 1.52 (9H, s, Butyl-t), 0.94—0.88 (6H, m, H- ′ & H-). *Anal*. Calcd for $C_{18}H_{30}N_4O_5$: C, 56.53; H, 7.91; N, 14.65. Found: C, 56.55; H, 7.88; N, 14.66.

^{t}Butyloxycarbonyl-histidinyl-tryptophan methyl ester: Yield 77%; mp 66-67 °C; $[\alpha]_D$ –18.4° (*c*, 0.35 in MeOH); R_f - 0.72; IR (KBr) *v* cm^{-1}: 3483, 3477, 3129, 2828, 1748, 1642, 1539, 1388, 1362, 1270, 928, 682. ^{1}H-NMR ($CDCl_3$) *δ*: 9.02 (2H, br. s, NH, imz & indole), 7.63—7.61 (1H, d, *J*=7.95 Hz, H-b, imz), 7.51—7.49 (1H, d, *J*=7.85 Hz, H-b′, indole), 7.16—7.08 (4H, m, H-d′–g′), 6.67 (1H, br. s, NH), 6.58 (1H, br. s, NH), 6.30 (1H, s, H-d), 4.93—4.89 (1H, m, H- , his), 4.88—4.83 (1H, m, H- ′, try), 3.52 (3H, s, OCH_3), 3.26—3.15 (4H, m, H- & H- ′), 1.55 (9H, s, Butyl-t). *Anal*. Calcd for $C_{23}H_{29}N_5O_5$: C, 60.65; H, 6.42; N, 15.37. Found: C, 60.67; H, 6.42; N, 15.36.

^{t}Butyloxycarbonyl-tryptophanyl-alanine methyl ester: Yield 73%; $[\alpha]_D$ –19.7° (*c* , 0.35 in MeOH); R_f - 0.59; IR ($CHCl_3$) *v* cm^{-1}: 3489, 3482, 3129, 1743, 1647, 1640, 1536, 1532, 1389, 1370, 1267. ^{1}H-NMR ($CDCl_3$) *δ*: 8.95 (1H, br. s, NH, indole), 7.53 (1H, d, *J*=7.6 Hz, H-d, indole), 7.11—7.03 (3H, m, H-e–g), 6.68 (1H, br. s, NH), 6.56 (1H, br. s, NH), 6.22 (1H, d, *J*=7.7 Hz, H- b, indole), 4.99—4.95 (1H, m, H- , try), 4.45—4.39 (1H, m, H- ′, ala), 3.61 (3H, s, OCH_3), 3.39—3.37 (2H, d, *J*=5.6 Hz, H-), 1.54 (9H, s, Butyl-t), 1.27—1.25 (3H, d, *J*=5.9 Hz, H- ′). *Anal*. Calcd for $C_{20}H_{27}N_3O_5$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.69; H, 6.96; N, 10.77.

^{t}Butyloxycarbonyl-prolyl-proline methyl ester: Yield 77%; $[\alpha]_D$ +53.9 ° (*c*, 0.35 in MeOH); R_f - 0.82; IR ($CHCl_3$) *v* cm^{-1}: 2997, 2993, 2990, 1746, 1664, 1659, 1388, 1369, 1267. ^{1}H-NMR ($CDCl_3$) *δ*: 4.16—4.13 (1H, t, *J*=6.65 Hz, H- , pro-1), 4.12—4.09 (1H, t, *J*=6.7 Hz, H- ′, pro-2), 3.64 (3H, s, OCH_3), 3.61—3.58 (2H, t, *J*=7.2 Hz, H-), 3.22—3.19 (2H, t, *J* =7.15 Hz, H- ′), 2.59—2.54 (2H, q, H-), 2.07—1.95 (6H, m, H- ′, H- & H- ′), 1.49 (9H, s, Butyl-t) . *Anal*. Calcd for $C_{16}H_{26}N_2O_5$: C, 58.88; H, 8.03; N, 8.58. Found: C, 58.90; H, 8.02; N, 8.59.

^{t}Butyloxycarbonyl-tryptophanyl-alanyl-tryptophan methyl ester: Yield 79%; mp 109-111°C; $[\alpha]_D$ −122.6° (*c*, 0.35 in MeOH); R_f - 0.71; IR (KBr) *v* cm^{-1}: 3489, 3486, 3122, 1744, 1717, 1642, 1640, 1537, 1533, 1389, 1371, 1272, 758. ^{1}H-NMR ($CDCl_3$) *δ*: 8.98 (2H, br. s, NH, indole), 8.72 (1H, br. s, NH), 7.52—7.50 (1H, d, *J*=7.45 Hz, H-d, indole-1), 7.49—7.47 (1H, d, *J*=7.75 Hz, H- b′, indole-2), 7.16—7.05 (6H, m, H-e–g & e′–g′), 7.04—7.02 (1H, d, *J*=7.3 Hz, H- d′, indole-2), 6.83 (1H, br. s, NH), 6.58 (1H, br. s, NH), 6.22—6.20(1H, d, *J*=7.7 Hz, H-b, indole-1), 4.83—4.78 (1H, m, H- , try-1), 4.45—4.38 (1H, m, H- , ala), 4.22—4.16 (1H, m, H-′, try-2), 3.54 (3H, s, OCH_3), 3.25—3.18 (4H, m, H-β & H-β′), 1.54 (9H, s, Butyl-t), 1.49—1.47 (3H, d, *J* =5.85 Hz, H-β, ala). *Anal*. Calcd for $C_{31}H_{37}N_5O_6$: C, 64.68; H, 6.48; N, 12.17. Found: C, 64.66; H, 6.49; N, 12.20.

^{t}Butyloxycarbonyl-phenylalanyl-valyl-tyrosine methyl ester: Yield 85%; mp 77-78 °C; $[\alpha]_D$ –39.6° (*c*, 0.35 in MeOH); R_f - 0.58; IR (KBr) *v* cm^{-1}: 3372, 3122, 3119, 1743, 1727, 1642, 1639, 1530,

1528, 1389, 1386, 1360, 1357, 1273, 689. ^{1}H-NMR ($CDCl_3$) δ: 8.42 (1H, br. s, NH), 7.53—7.48 (2H, m, H-m, phe), 6.94—6.90 (1H, t, *J*=6.15 Hz, H-p), 6.89—6.84 (2H, dd, *J*=8.5 Hz, 4.25 Hz, H-o, tyr), 6.83 (1H, br. s, NH), 6.82—6.78 (2H, dd, *J*=8.75 Hz, 4.2 Hz, H-o, phe), 6.77—6.73 (2H, dd, *J*=8.6 Hz, 4.75 Hz, H-m, tyr), 6.56 (1H, br. s, NH), 5.96 (1H, br. s, OH), 4.49—4.44 (1H, dd, *J*=4.55 Hz, 3.8 Hz, H- ′ , val), 4.43—4.39 (1H, m, H- , phe), 3.66—3.60 (1H, m, H-′′, tyr), 3.55 (3H, s, OCH_3), 3.06—2.97 (4H, m, H- & H- ′′), 2.09—2.01 (1H, m, H- ′), 1.56 (9H, s, Butyl-t), 1.07—1.05 (6H, d, *J*=4.55 Hz, H- ′). *Anal* . Calcd for $C_{29}H_{39}N_3O_7$: C, 64.31; H, 7.26; N, 7.76. Found: C, 64.29; H, 7.28; N, 7.75.

^{t}Butyloxycarbonyl-phenylalanyl-glycyl-phenylalanine methyl ester: Yield 81%; mp 99-100 °C; $[\alpha]_D$ +11.2° (*c*, 0.35 in MeOH); R_f - 0.79; IR (KBr) *v* cm^{-1}: 3125, 3116, 1747, 1643, 1640, 1634, 1539, 1535, 1386, 1359, 1269. ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, br. s, NH), 7.52—7.48 (2H, m, H-m, phe-1), 7.15—7.11 (1H, t, *J*=6.2 Hz, H-p′, phe-2), 7.01—6.95 (3H, m, H-p & H-m′), 6.85—6.80 (4H, m, H-o & H-o′), 6.65 (1H, br. s, NH), 6.29 (1H, br. s, NH), 4.80—4.76 (1H, m, H- , phe-1), 4.06—4.04 (2H, d, *J*=5.2 Hz, H- , gly), 3.95—3.91 (1H, m, H- ′, phe-2), 3.53 (3H, s, OCH_3), 2.97—2.92 (4H, m, H- & H- ′), 1.55 (9H, s, Butyl-t). *Anal*. Calcd for $C_{32}H_{33}N_5O_5$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.56; H, 6.89; N, 8.72.

^{t}Butyloxycarbonyl-glycyl-valyl-glycine methyl ester: Yield 89%; mp 111-112 °C; $[\alpha]_D$ +52.9° (*c*, 0.35 in MeOH); R_f - 0.77; IR (KBr) *v* cm^{-1}: 3124, 3119, 1742, 1648, 1640, 1637, 1537, 1529, 1389, 1384, 1364, 1361, 1271, 588. ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, br. s, NH), 6.80 (1H, br. s, NH), 6.32 (1H, br. s, NH), 4.68—4.64 (1H, dd, *J*=4.6 Hz, 3.85 Hz, H- ′, val), 4.06—4.04 (2H, d, *J*=5.2 Hz, H- , gly-2), 3.73—3.71 (2H, d, *J*=5.15 Hz, H- , gly-1), 3.62 (3H, s, OCH_3), 1.52 (9H, s, Butyl-t), 1.51—1.46 (1H, m, H- ′), 0.97—0.95 (6H, d, *J*=4.6 Hz, H- ′). *Anal*. Calcd for $C_{15}H_{27}N_3O_6$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.15; H, 7.89; N, 12.15.

^{t}Butyloxycarbonyl-phenylalanyl-threonyl-glycyl-leucine methyl ester: Yield 74%; $[\alpha]_D$ −27.8° (*c*, 0.35 in MeOH); R_f - 0.52; IR ($CHCl_3$) *v* cm^{-1}: 3126, 3120, 3115, 1742, 1719, 1649, 1646, 1637, 1539, 1532, 1388, 1383, 1362, 1359 (C−H_{bend}, propyl-i), 1326 (O−H_{def}), 1268. ^{1}H-NMR ($CDCl_3$) δ: 8.34 (1H, br. s, NH), 8.06 (1H, br. s, NH), 7.52—7.47 (2H, m, H-m, phe), 6.93—6.90 (1H, t, *J*=6.2 Hz, H-p), 6.86—6.82 (2H, dd, *J*=8.7 Hz, 4.15 Hz, H-o), 6.65 (1H, br. s, OH), 6.56 (1H, br. s, NH), 6.07 (1H, br. s, NH), 4.56—4.42 (1H, m, H- , phe), 4.45—4.41 (1H, dd, *J*=4.45 Hz, 3.7 Hz, H- ′, thr), 3.82—3.80 (2H, d, *J*=5.15 Hz, H- , gly), 3.79—3.74 (1H, m, H-β′), 3.60 (3H, s, OCH_3), 3.51—3.46 (1H, m, H- ′′, leu), 2.98— 2.96 (2H, d, *J* =5.65 Hz, H-β), 1.55 (9H, s, Butyl-t), 1.51—1.42 (3H, m, H-β′′ & H- ′′), 1.30—1.28 (3H, d, *J*=4.85 Hz, H- ′), 0.94— 0.92 (6H, d, *J*=6.3 Hz, H- ′′). *Anal*. Calcd for $C_{27}H_{42}N_4O_8$: C, 58.89; H, 7.69; N, 10.17. Found: C, 58.92; H, 7.68; N, 10.19.

^{t}Butyloxycarbonyl-histidinyl-glycyl-histidinyl-alanine methyl ester : Yield 78%; $[\alpha]_D$ +17.3° (*c*, 0.25 in MeOH); R_f - 0.58; IR ($CHCl_3$) *v* cm^{-1}: 3483, 3479, 3129, 3122, 1740, 1646, 1642-1637, 1539, 1536-1531, 1389, 1370, 1269. ^{1}H-NMR ($CDCl_3$) δ: 9.46 (1H, br. s, NH), 9.10 (2H, br. s, NH, imz-1 & imz-2), 7.95 (1H, br. s, NH), 7.67 (1H, s, H-d′, imz-2), 7.58—7.56 (1H, d, *J*=7.9 Hz, H-b), 7.39—7.37 (1H, d, *J*=7.95 Hz, H-b′), 6.95 (1H, br. s, NH), 6.58 (1H, br. s, NH), 6.28 (1H, s, H-d, imz-1), 4.77—4.72 (1H, m, H- , his-1), 4.51—4.46 (1H, m, H- ′, his-2), 4.11—4.09 (2H, d, *J*=5.15 Hz, H- , gly), 3.81—3.75 (1H, m, H- ′′, ala), 3.62 (3H, s, OCH_3), 2.98—2.91 (4H, m, H- & H- ′), 1.53 (9H, s, Butyl -t), 1.30—1.28 (3H, d, *J*=5.9 Hz, H- ′′). *Anal*. Calcd for $C_{23}H_{34}N_8O_7$: C, 51.68; H, 6.41; N, 20.96. Found: C, 51.66; H, 6.44; N, 20.95.

^{t}Butyloxycarbonyl-prolyl-alanyl-glycyl-proline methyl ester: Yield 76%; $[\alpha]_D$ –111.3° (*c*, 0.25 in MeOH); R_f - 0.76; IR ($CHCl_3$) *v* cm^{-1}: 3123, 3120, 2999-2991, 1744, 1659, 1647-1641, 1538, 1532, 1387, 1368, 1267. ^{1}H-NMR ($CDCl_3$) δ: 8.45 (1H, br. s, NH), 6.89 (1H, br. s, NH), 4.46—4.39 (1H, m, H- , ala), 4.12—4.09 (1H, t, *J*=6.65 Hz, H- ′ , pro-1), 3.91—3.87 (1H, t, *J* =6.7 Hz, H- ′′, pro-2), 3.85—3.83 (2H, d, *J*=5.15 Hz, H- , gly), 3.62 (3H, s, OCH_3), 3.38—3.35 (2H, t, *J*=7.15 Hz, H- ′), 3.23—3.19 (2H, t, *J*=7.2 Hz, H- ′′), 2.56—2.52 (2H, q, H- ′), 2.05—1.98 (4H, m, H- ′′ & H- ′′), 1.95—1.89 (2H, m, H- ′), 1.51—1.49 (3H, d, *J*=5.85 Hz, H-), 1.48 (9H, s, Butyl-t) . *Anal*. Calcd for C_{21} $H_{34}N_4O_7$: C, 55.49; H, 7.54; N, 12.33. Found: C, 55.47; H, 7.55; N, 12.35.

^{t}Butyloxycarbonyl-threonyl-alanyl-histidinyl-proline methyl ester: Yield 86%; $[\alpha]_D$ –77.4° (*c*, 0.25 in MeOH); R_f - 0.68; IR ($CHCl_3$) *v* cm^{-1}: 3484, 1744, 1656, 1642, 1638, 1539, 1534, 1422, 1388, 1367, 1329, 1269. ^{1}H-NMR ($CDCl_3$) δ: 9.61 (1H, br. s, NH), 9.32 (1H, br. s, NH), 7.68—7.66 (1H, d, *J*=7.9 Hz, H-b′, imz), 7.50 (1H, s, H-d, imz), 7.05 (1H, br. s, NH), 7.93 (2H, br. s, NH & OH), 4.58—4.53 (1H, m, H- , his), 4.46—4.40 (1H, m, H- , ala), 4.17—4.14 (1H, t, *J*=3.9 Hz, H- ′, thr), 3.93—3.89 (1H, t, *J*=6.75 Hz, H- ′′, pro), 3.62 (3H, s, OCH_3), 3.53—3.47 (1H, m, H-β′), 3.41—3.37 (2H, t, *J*=7.2 Hz, H- ′ ′), 2.99—2.97 (2H, d, *J*=5.55 Hz, H-), 2.07—1.99 (4H, m, H- β′′ & H- ′′), 1.56 (9H, s, Butyl-t), 1.49—1.47 (3H, d, *J*=5.85 Hz, H- , ala), 1.31—1.29 (3H, d, *J*=4.95 Hz, H- ′). *Anal*. Calcd for $C_{24}H_{38}N_6O_8$: C, 53.52; H, 7.11; N, 15.60. Found: C, 53.50; H, 7.12; N, 15.59.

General method for preparation of 2-(3-unsubstituted/substituted-2-oxo-2H-4-chromenyloxy)acetic acids (1, 2)

Sodium hydroxide (0.9 g, 22.4 mmol) was dissolved in 20 ml of water and the alkaline solution was slowly added to another solution of 4-hydroxycoumarin/3-bromo- 4-hydroxycoumarin (1.62 g/2.41 g, 10 mmol) and chloroacetic acid (0.94 g, 10 mmol) in 20 ml of water with stirring. The reaction mixture was heated to remove all the liquid and the residue was treated with 35 ml of water. The mixture was cooled, filtered and acidified with dilute HCl. The aqueous layer was extracted with diethylether (2 × 25 ml) and combined organic extracts were dried over anhydrous Na_2SO_4. The crude product was crystallized from equimolar mixture of EtOH–H_2O.

2-(2-Oxo-2H-4-chromenyloxy)acetic acid (1): Yield 72%; mp 201-203 °C; IR (KBr) *v* cm^{-1}: 3298—2508 (O–H_{str}), 1722 (C=O_{str}, -lactone), 1709 (C=O_{str}, COOH), 1240, 1037 (C–O–C_{str}). ^{1} H-NMR ($CDCl_3$) δ: 7.92 (1H, br. s, COOH), 7.66—7.64 (1H, d, *J*=6.9 Hz, H-5), 7.54—7.51 (1H, t, *J*=8.25 Hz, H-7), 7.35—7.31 (2H, m, H-6 & H-8), 5.85 (1H, s, H-3), 4.63 (2H, s, OCH_2). ^{13}C-NMR ($CDCl_3$) δ: 178.7 (C=O), 169.4 (C-4), 163.2 (C=O, - lactone), 155.3 (C-2′), 130.6 (C-7), 127.2 (C-6), 124.8 (C-5), 119.2 (C-3′), 117.5 (C-8), 91.5 (C-3), 69.8 (OCH_2). *Anal*. Calcd for $C_{11}H_8O_5$: C, 60.01; H, 3.66. Found: C, 59.98; H, 3.68.

2-(3-Bromo-2-oxo-2H-4-chromenyloxy)acetic acid (2): Yield 69%; mp 178-179 °C; IR (KBr) *v* cm^{-1}: 3304—2496, 1719 (C=O_{str}, -lactone), 1707 (C=O_{str}, COOH), 1243, 1035 (C–O–C_{str}), 682 (C–Br_{str}). ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, br. s, COOH), 7.84—7.82 (1H, d, *J*=6.85 Hz, H-5), 7.65—7.62 (1H, t, *J*=5.3 Hz, H-7), 7.38—7.33 (2H, m, H-6 & H-8), 4.68 (2H, s, OCH_2). ^{13}C-NMR ($CDCl_3$) δ: 177.9 (C=O), 163.3 (C-4), 160.8 (C=O, -lactone), 152.6 (C-2′), 133.2 (C-7), 126.6 (C-6), 122.6 (C-5), 118.0 (C-3′), 117.2 (C-8), 91.2 (C-3), 71.2 (OCH_2). *Anal*. Calcd for $C_{11}H_7BrO_5$: C, 44.18; H, 2.36. Found: C, 44.20; H, 2.35.

General method for preparation of 5- unsubstituted/substituted-2-(2-methyl-1H-5-imidazolyl) benzoic acids (13, 14)

A mixture of anthranilic acid/5-iodoanthranilic acid (6.86 g/13.2 g, 50 mmol), dilute hydrochloric acid (15%, 30 ml) and water (45 ml) was heated to get a clear solution. After cooling to 0 °C, the solution was diazotized by addition of sodium nitrite solution (30%, 12 ml). To the filtrate of diazotized salt solution, 2-methylimidazole (4.1 g, 50 mmol) and aqueous cupric chloride solution (1.3 g in 5 ml water) were added with stirring followed by slow addition of 25 ml of water, maintaining the temperature between 5-10 °C. Stirring was continued for 6 h and finally reaction mixture was kept overnight in the refrigerator. The separated solid was collected by filtration and washed with cold water. The crude product was recrystallized from acetone.

2-(2-Methyl-1H-5-imidazolyl)benzoic acid (13) : Yield 77%; mp 141-143 °C; IR (KBr) *v* cm^{-1}: 3487 (N–H_{str}), 3306—2498 (O–H_{str}), 1696 (C=O_{str}). ^{1}H-NMR ($CDCl_3$) δ: 9.57 (2H, br. s, N<u>H</u> & COO<u>H</u>), 7.74—7.69 (2H, m, H-3 & H-5, benzoic acid moiety (bza)), 7.63—7.59 (2H, m, H-4 & H-6, bza), 6.85 (1H, s, H-d, imidazole moiety (imz)), 2.68 (3H, s, C<u>H</u>$_3$). ^{13}C- NMR ($CDCl_3$) δ: 162.7 (C=O), 158.3 (C-b, imz), 145.7 (C-e), 137.2 (C-4, bza), 136.8 (C-d), 135.3 (C-2), 133.5 (C-6), 128.7 (C-5), 126.6 (C-1), 125.1 (C-3), 18.6 (<u>C</u>H$_3$, imz). *Anal*. Calcd for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.32; H, 4.99; N, 13.88.

5-Iodo-2-(2-methyl-1H-5-imidazolyl)benzoic acid (14): Yield 74%; mp 192-193 °C; IR (KBr) *v* cm^{-1}: 3489 (N–H_{str}), 3302—2495 (O–H_{str}), 1698 (C=O_{str}), 587 (C–I_{str}) . ^{1}H-NMR ($CDCl_3$) δ: 9.59 (2H, br. s, N<u>H</u> & COO<u>H</u>), 8.15 (1H, s, H-6, bza), 7.84—7.82 (1H, d, *J*=7.25 Hz, H-4), 6.99—6.97 (1H, d, *J*=7.5 Hz, H-3), 6.40 (1H, s, H-d, imz), 2.66 (3H, s, C<u>H</u>$_3$). ^{13}C-NMR ($CDCl_3$) δ: 163.0 (C=O), 158.5 (C-b, imz), 146.2 (C-e), 143.3 (C-4, bza), 142.5 (C-6), 137.0 (C-d), 135.7 (C-2), 129.6 (C-1), 128.3 (C-3), 99.4 (C- 5), 18.9 (<u>C</u>H$_3$, imz). *Anal*. Calcd for $C_{11}H_9IN_2O_2$: C, 40.27; H, 2.76; N, 8.54. Found: C, 40.26; H, 2.79; N, 8.55.

General procedure for synthesis of 2-(2-oxo-2H-4-chromenyloxy)acetyl / 2-(3-bromo-2-oxo-2H-4-chromenyloxy)acetyl amino acid/peptide ester derivatives (3-6, 7-10)

To a mixture of amino acid methyl ester hydrochloride/di/tri/tetra-peptide methyl ester (10 mmol) in DMF (50 ml), 2.3 ml of NMM was added at 0 °C with stirring. Compound 1 / 2 (2.2 g / 3.0 g, 10 mmol) in DMF (50 ml) and DIPC (1.26 g, 10 mmol) were added to the above mixture and stirring was done for 24 h. After 24 h, the reaction mixture was filtered. To the filtrate, water is added in equal proportions and aqueous layer was washed with ether (3 × 50 ml). The organic layer was separated and dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The product obtained was dissolved in chloroform, washed with 10% HCl, saturated $NaHCO_3$ solution and water (25 ml each) followed by evaporation in vacuum. Crude product was crystallized from a mixture of ethyl acetate and petroleum ether.

2-(2-Oxo-2H-4-chromenyloxy)acetyl tyrosine methyl ester (3): Yield 67%; mp 154-155 °C; $[\alpha]_D$ +24.3° (*c*, 0.25 in MeOH); R_f - 0.77; IR (KBr) *v* cm^{-1}: 3372 (O–H_{str}), 3125 (N–H_{str}, amide), 1742 (C=O_{str}, ester), 1719 (C=O_{str}, -lactone), 1642 (C=O_{str}, amide), 1535 (N–H_{def}, amide), 1273, 1224 (C–O_{str}, ester and phenolic), 1235, 1044 (C–O–C_{str}). ^{1}H-NMR ($CDCl_3$) δ: 7.89—7.87 (1H, d, *J* =6.85 Hz, H-5), 7.56—7.52 (1H, t, *J* =5.3 Hz, H-7), 7.29—7.23 (2H, m, H-6 & H-8), 6.92—6.87 (2H, dd, *J*=8.55 Hz, 8.25 Hz, H-o, tyr), 6.81—6.77 (2H, dd, *J*=8.6 Hz, 4.9 Hz, H-m), 6.17 (1H, br. s, N<u>H</u>), 5.98 (1H, br. s, O<u>H</u>), 5.95 (1H, s, H-3), 4.65 (2H, s, OC<u>H</u>$_2$), 4.62—4.58 (1H, m, H-), 3.57 (3H, s, OC<u>H</u>$_3$), 2.82—2.80 (2H, d, *J*=4.7 Hz, H-β) . *Anal*. Calcd for $C_{21}H_{19}NO_7$: C, 63.47; H, 4.82; N, 3.52. Found: C, 63.45; H, 4.80; N, 3.55.

2-(2-Oxo-2H- 4-chromenyloxy)acetyl histidinyl-isoleucine methyl ester (4) : Yield 73%; $[\alpha]_D$ −102.7° (*c*, 0.25 in MeOH); R_f - 0.61; IR ($CHCl_3$): *v* cm^{-1}: 3485, 3129, 3122, 1745, 1717, 1645, 1641, 1538, 1532, 1269, 1235, 1044. ^{1}H-NMR ($CDCl_3$) δ: 9.12 (1H, br. s, N<u>H</u>, imz), 8.39 (1H, br. s, N<u>H</u>), 7.91—7.89 (1H, d, *J*=6.9 Hz, H-5), 7.65 (1H, s, H-d, imz), 7.55—7.51 (1H, t, *J*=8.25 Hz, H-7), 7.39—7.37 (1H, d, *J*=7.95 Hz, H-b), 7.27—7.22 (2H, m, H-6 & H-8), 6.72 (1H, br. s, N<u>H</u>), 5.97 (1H, s, H-3), 5.02—4.98 (1H, m, H-a, his), 4.68 (2H, s, OC<u>H</u>$_2$), 3.85—3.80 (1H, dd, *J*=4.9 Hz, 3.25 Hz, H- , ile), 3.49 (3H, s, OC<u>H</u>$_3$), 2.78—2.76 (2H, d, *J*=4.55 Hz, H-b), 2.02—1.98 (1H, m, H-β), 1.68—1.63 (2H, q, H-), 0.95—0.89 (6H, m, H- ′ & H-). *Anal*. Calcd for $C_{24}H_{28}N_4O_7$: C, 59.50; H, 5.82; N, 11.56. Found: C, 59.48; H, 5.85; N, 11.53.

2-(2-Oxo-2H-4-chromenyloxy)acetyl tryptophanyl- alanyl-tryptophan methyl ester (5) : Yield 79%; mp 82-83 °C; $[\alpha]_D$ −62.1° (*c*, 0.35 in MeOH); R_f - 0.82; IR (KBr) *v* cm^{-1}: 3489, 3482, 3125, 1749, 1719, 1647, 1643, 1539, 1534, 1274, 1239, 1041. ^{1}H-NMR ($CDCl_3$) δ: 8.95 (2H, br. s, N<u>H</u>, indole), 8.36 (1H, br. s, N<u>H</u>), 8.12 (1H, br. s, N<u>H</u>), 7.90—7.88 (1H, d, *J* =6.9 Hz, H-5), 7.56—7.52 (1H, t, *J*=5.3 Hz, H- 7), 7.49—7.47 (1H, d, *J*=7.8 Hz, H-b′, indole-2), 7.27—7.22 (2H, m, H-6 & H-8), 7.21—7.19 (1H, d, *J*=7.75 Hz, H-b, indole-1), 7.18—7.16 (1H, d, *J*=7.4 Hz, H- d, indole-1), 7.15—7.06 (6H, m, H-e–g & e′ –g′), 7.03—7.01 (1H, d, *J*=7.35 Hz, H-d′ , indole-2), 6.78 (1H, br. s, N<u>H</u>), 5.95 (1H, s, H-3), 4.95—4.91 (1H, m, H- , try-1), 4.67 (2H, s, OC<u>H</u>$_2$), 4.32—4.27 (1H, m, H- , ala), 4.23—4.17 (1H, m, H- ′, try-2), 3.57 (3H, s, OC<u>H</u>$_3$), 3.26—3.19 (4H, m, H-β & H-β′), 1.50—1.48 (3H, d, *J*=5.9 Hz, H-β , ala) . MS *m/z*: 677 (M^+), 662, 646, 618, 460, 432, 389(100), 361, 203, 175, 161, 130, 116, 59, 31, 15. *Anal*. Calcd for $C_{37}H_{35}N_5O_8$: C, 65.57; H, 5.21; N, 10.33. Found: C, 65.55; H, 5.20; N, 10.32.

2-(2-Oxo-2H-4- chromenyloxy)acetyl phenylalanyl -threonyl-glycyl-leucine methyl ester (6): Yield 69%; $[\alpha]_D$ −19.7° (*c*, 0.35 in MeOH); R_f - 0.69; IR ($CHCl_3$): *v* cm^{-1}: 3129, 3122-3117, 1746, 1721, 1646, 1644, 1639, 1538, 1533; 1385, 1363 (C−H_{bend}, propyl-i), 1329 (O−H_{def}), 1271, 1237, 1044. ^{1}H-NMR ($CDCl_3$) δ: 8.38 (1H,br. s, N<u>H</u>), 8.15 (1H, br. s, N<u>H</u>), 7.92—7.90 (1H, d, *J*=6.85 Hz, H-5), 7.82 (1H, br. s, N<u>H</u>), 7.55—7.51 (1H, t, *J*=8.25 Hz, H-7), 7.28—7.23 (2H, m, H-6 & H-8), 7.20—7.16 (2H, m, H-m, phe), 7.01—7.98 (1H, t, *J*=6.15 Hz, H-p), 6.85—6.81 (2H, dd, *J*=8.75 Hz, 4.1 Hz, H-o), 6.05 (1H, br. s, N<u>H</u>), 5.98 (1H, s, H-3), 4.69 (2H, s, OC<u>H</u>$_2$), 4.52 (1H, br. s, O<u>H</u>), 4.40—4.36 (1H, m, H- , phe), 4.18—4.14 (1H, dd, *J* =4.5 Hz, 3.75 Hz, H- ′, thr), 3.92—3.87 (1H, m, H-β′), 3.81—3.79 (2H, d, *J*=5.2 Hz, H- , gly), 3.62 (3H, s, OC<u>H</u>$_3$), 3.52—3.47 (1H, m, H- ′′, leu), 2.90—2.88 (2H, d, *J*=5.6 Hz, H-β), 1.50—1.43 (3H, m, H-β′′ & H- ′′), 1.25—1.23 (3H, d, *J*=4.9 Hz, H-′), 0.95—0.93 (6H, d, *J*=6.25 Hz, H- ′ ′). MS *m/ z*: 652 (M^+), 637, 621, 593, 508, 480, 451(100), 423, 350, 322, 203, 175, 161, 91, 66, 59, 57, 43, 42, 31, 16, 15. *Anal* . Calcd for $C_{33}H_{40}N_4O_{10}$: C, 60.73; H, 6.18; N, 8.58. Found: C, 60.75; H, 6.18; N, 8.60.

***2-(3-Bromo-2-oxo-2H-4- chromenyloxy)acetyl proline methyl ester (7)*:** Yield 71%; mp 97-99 °C; $[\alpha]_D$ +4.7° (*c*, 0.25 in MeOH); R_f - 0.56; IR (KBr) *v* cm^{-1}: IR (KBr) *v* cm^{-1}: 2998, 2993 (C–H_{str}, pro), 1752, 1721; 1659 (C=O_{str}, *tert* amide), 1273, 1246, 1032, 680. ^{1}H-NMR ($CDCl_3$) δ: 7.93—7.91 (1H, d, *J*=6.9 Hz, H-5), 7.55—7.52 (1H, t, *J*=8.25 Hz, H-7), 7.28—7.22 (2H, m, H-6 & H-8), 4.62 (2H, s, OC<u>H</u>$_2$), 3.95—3.92 (1H, t, *J*=6.65 Hz, H-), 3.65 (3H, s, OC<u>H</u>$_3$), 3.44—3.41 (2H, t, *J*=7.15 Hz, H-), 2.05—1.98 (4H, m, H- & H-). *Anal*. Calcd for $C_{17}H_{16}BrNO_6$: C, 49.78; H, 3.93; N, 3.41. Found: C, 49.75; H, 3.95; N, 3.42.

2-(3-Bromo-2-oxo-2H-4-chromenyloxy)acetyl histidinyl-tryptophan methyl ester (8): Yield 75%; mp 126-127 °C; $[\alpha]_D$ –44.9° (*c*, 0.25 in MeOH); R_f - 0.65; IR (KBr) *v* cm^{-1}: 3484, 3478, 3128, 3120, 1749, 1724, 1644, 1640, 1537, 1531, 1272, 1243, 1036, 684. ^{1}H-NMR ($CDCl_3$) δ: 9.05 (2H, br. s, NH, imz & indole), 8.40 (1H, br. s, NH), 7.92—7.90 (1H, d, *J*=6.9 Hz, H-5), 7.66 (1H, s, H-d, imz), 7.56—7.53 (1H, t, *J*=5.3 Hz, H-7), 7.50—7.48 (1H, d , *J*=7.8 Hz, H- b′, indole), 7.38—7.36 (1H, d, *J*=7.95 Hz, H-b), 7.26—7. 23 (2H, m, H-6 & H-8), 7.14—7.05 (4H, m, H-d′ –g′), 6.92 (1H, br. s, NH), 4.77—4.73 (1H, m, H- , his), 4.32 (2H, s, OCH_2), 4.22—4.17 (1H, m, H- ′, try), 3.56 (3H, s, OCH_3), 3.02—2.98 (4H, m, H- & H- ′). *Anal*. Calcd for $C_{29}H_{26}BrN_5O_7$: C, 54.73; H, 4.12; N, 11.00. Found: C, 54.76; H, 4.15; N, 10.98.

2-(3-Bromo-2- oxo-2H- 4-chromenyloxy)acetyl phenylalanyl-valyl- tyrosine methyl ester (9): Yield 77%; mp 109-110 °C; $[\alpha]_D$ –12.4° (*c*, 0.35 in MeOH); R_f - 0.78; IR (KBr) *v* cm^{-1}: 3375, 3125, 3118, 1745, 1722, 1646, 1642, 1533, 1529, 1388, 1362, 1270, 1244, 1227, 1032, 682. ^{1}H-NMR ($CDCl_3$) δ: 8.38 (1H, br. s, NH), 7.99 (1H, br. s, NH), 7.94—7.92 (1H, d, *J*=6.85 Hz, H-5), 7.67—7.64 (1H, t, *J*=5.3 Hz, H-7), 7.29—7.24 (2H, m, H-6 & H-8), 7.19—7.15 (2H, m, H-m, phe), 7.02—7.99 (1H, t, *J*=6.2 Hz, H-p), 6.92—6.88 (2H, dd, *J*=8.55 Hz, 8.25 Hz, H-o, tyr), 6.86 (1H, br. s, NH), 6.84—6.80 (2H, dd, *J*=8.8 Hz, 4.1 Hz, H-o, phe), 6.79—6.75 (2H, dd, *J*=8.55 Hz, 4.85 Hz, H-m, tyr), 5.98 (1H, br. s, OH), 4.68 (2H, s, OCH_2), 4.37—4.33 (1H, m, H- , phe), 4.14—4.10 (1H, dd, *J*=4.6 Hz, 3.85 Hz, H- ′, val), 3.67—3.62 (1H, m, H- ′′, tyr), 3.53 (3H, s, OCH_3), 2.99—2.94 (4H, m, H- & H- ′′), 2.07—2.03 (1H, m, H- ′), 1.05—1.03 (6H, d, *J*=4.6 Hz, H- ′). MS *m*/*z*: 722 (M^+), 707, 691, 663, 528, 429(100), 401, 282, 254, 240, 107, 93, 91, 79, 66, 59, 43, 42, 31, 16, 15. *Anal*. Calcd for $C_{35}H_{36}BrN_3O_9$: C, 58.18; H, 5.02; N, 5.82. Found: C, 58.15; H, 4.99; N, 5.85.

2-(3-Bromo-2-oxo-2H-4-chromenyloxy)acetyl histidinyl-glycyl-histidinyl-alanine methyl ester (10) : Yield 73%; $[\alpha]_D$ +57.9° (*c*, 0.35 in MeOH); R_f - 0.71; IR ($CHCl_3$) *v* cm^{-1}: 3488, 3485, 3128, 3123, 1742, 1718, 1648, 1643-1639, 1538, 1535-1532, 1273, 1238, 1035, 686. ^{1}H-NMR ($CDCl_3$) δ: 9.49 (1H, br. s, NH), 9.12 (2H, br. s, NH, imz-1 & imz-2), 8.25 (1H, br. s, NH), 7.96—7.94 (1H, d, *J*=6.9 Hz, H-5), 7.82 (1H, br. s, NH), 7.69—7.66 (1H, t, *J*=8.25 Hz, H-7), 7.65 (1H, s, H- d, imz-1), 7.62 (1H, s, H-d′, imz-2), 7.39—7.35 (2H, dd, *J* =7.95 Hz, 7.9 Hz, H-b & H-b′), 7.30—7.25 (2H, m, H-6 & H-8), 6.93 (1H, br. s, NH), 4.69 (2H, s, OCH_2), 4.62—4.58 (1H, m, H- , his-1), 4.53—4.49 (1H, m, H- ′, his- 2), 3.87—3.85 (2H, d, *J*=5.2 Hz, H- , gly), 3.79—3.72 (1H, m, H- ′′, ala), 3.59 (3H, s, OCH_3), 2.99—2.93 (4H, m, H- & H- ′′), 1.29—1.27 (3H, d, *J*=5.85 Hz, H- ′′). MS *m*/*z*: 716 (M^+ + 1), 715 (M^+), 700, 684, 656, 613, 585, 476(100), 448, 419, 391, 282, 254, 240, 81, 79, 67, 59, 31, 30, 15. *Anal*. Calcd for $C_{29}H_{31}BrN_8O_9$: C, 48.68; H, 4.37; N, 15.66. Found: C, 48.66; H, 4.40; N, 15.65.

General procedure for synthesis of 2-(2-methyl-1H-5-imidazolyl)benzoyl / 5-iodo-2-(2-methyl-1H-5-imidazolyl)benzoyl amino acid/peptide ester derivatives (15-18, 19-22)

Amino acid methyl ester hydrochloride/di/tri/tetrapeptide methyl ester (10 mmol) was dissolved in 60 ml of tetrahydrofuran (THF). To this, 2.8 ml of TEA was added at 0 °C and the reaction mixture was stirred for 15 min. Compound 13 / 14 (2.0 g / 3.3 g, 10 mmol) in THF (60 ml) and EDC.HCl (1.92 g, 10 mmol) were added to the above mixture with stirring. After 36 h, the reaction mixture was filtered and the residue was washed with 25 ml of THF. Filtrate was washed with 5% $NaHCO_3$ and saturated NaCl solutions (15 ml each). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and *n*-hexane followed by cooling at 0°C.

2-(2-Methyl-1H-5-imidazolyl)benzoyl threonine methyl ester (15): Yield 86%; $[\alpha]_D$ –112.5° (*c*, 0.25 in MeOH); R_f - 0.82; IR ($CHCl_3$) *v* cm^{-1}: 3482, 3128, 1744, 1645, 1536, 1328, 1268. ^{1}H-NMR ($CDCl_3$) δ: 7.74—7.63 (4H, m, H-3–6, bza), 7.59 (1H, s, H-d, imz), 7.06 (1H, br. s, NH), 5.45 (2H, br. s, OH & NH, imz), 4.62—4.58 (1H, m, H- , thr), 4.52—4.48 (1H, dd, *J*=4.45 Hz, 3.8 Hz, H-), 3.59 (3H, s, OCH_3), 2.66 (3H, s, CH_3), 1.32—1.30 (3H, d, *J*=4.85 Hz, H- ′). *Anal*. Calcd for $C_{16}H_{19}N_3O_4$: C, 60.56; H, 6.03; N, 13.24. Found: C, 60.59; H, 6.06; N, 13.25.

2-(2-Methyl-1H-5-imidazolyl)benzoyl tryptophanyl-alanine methyl ester (16): Yield 89%; $[\alpha]_D$ –7.6° (*c*, 0.25 in MeOH); R_f - 0.76; IR ($CHCl_3$) *v* cm^{-1}: 3485, 3479, 3126, 3122, 1746, 1646, 1642, 1539, 1535, 1269. ^{1}H-NMR ($CDCl_3$) δ: 8.26 (2H, br. s, NH, imz & indole), 8.10 (1H, br. s, NH), 7.93—7.91 (1H, d, *J*=7.35 Hz, H-6, bza), 7.70—7.67 (1H, t, *J*=6.9 Hz, H-4), 7.61 (1H, s, H-d, imz), 7.53—7.49 (2H, m, H-3 & H-5), 7.25—7.23 (1H, d, *J*=7.75 Hz, H-b, indole), 7.19—7.12 (4H, m, H-d–g), 6.95 (1H, br. s, NH), 5.13—5.08 (1H, m, H- , try), 3.79—3.74 (1H, m, H- ′ , ala), 3.57 (3H, s, OCH_3), 3.18—3.16 (2H, d, *J*=5.5 Hz, H-), 2.64 (3H, s, CH_3), 1.28—1.26 (3H, d, *J*=5.85 Hz, H- ′). *Anal*. Calcd for $C_{26}H_{27}N_5O_4$: C, 65.95; H, 5.75; N, 14.79. Found: C, 65.96; H, 5.72; N, 14.82.

2-(2-Methyl-1H-5-imidazolyl)benzoyl phenylalanyl -glycyl-phenylalanine methyl ester (17): Yield 94%; mp 121-123 °C; $[\alpha]_D$ +2.8° (*c*, 0.35 in MeOH); R_f - 0.64; IR (KBr) *v* cm^{-1}: 3482, 3123, 3118, 1744, 1647, 1643, 1639, 1538, 1532, 1271. ^{1}H-NMR ($CDCl_3$) δ: 8.55 (1H, br. s, NH), 8.22 (1H, br. s, NH), 7.97—7.95 (1H, d, *J*=7.4 Hz, H-6, bza), 7.72—7.69 (1H, t, *J*=6.85 Hz, H- 4), 7.65 (1H, br. s, NH, imz), 7.60 (1H, s, H-d, imz), 7.55—7.51 (2H, m, H-3 & H-5), 7.22—7.17 (2H, m, H-m, phe-1), 7.13—7.10 (1H, t, *J*=6.15 Hz, H-p′, phe-2), 7.05 (1H, br. s, NH), 7.02—6.96 (3H, m, H-p & H-m′), 6.87—6.82 (4H, m, H-o & H-o′), 4.46—4.41 (1H, m, H- , phe-1), 3.97—3.92 (1H, m, H- ′, phe-2), 3.79—3.77 (2H, d, *J*=5.15 Hz, H- , gly), 3.56 (3H, s, OCH_3), 2.94—2.89 (4H, m, H- & H- ′), 2.67 (3H, s, CH_3). MS *m*/*z*: 567 (M^+), 552, 536, 508, 389, 361, 332(100), 304, 185, 157, 82, 77, 66, 59, 31, 30, 15. *Anal*. Calcd for $C_{32}H_{33}N_5O_5$: C, 67.71; H, 5.86; N, 12.34. Found: C, 67.69; H, 5.88; N, 12.37.

2-(2-Methyl-1H-5-imidazolyl)benzoyl prolyl-alanyl -glycyl-proline methyl ester (18): Yield 82%; $[\alpha]_D$ –54.7° (*c*, 0.35 in MeOH); R_f - 0.59; IR ($CHCl_3$) *v* cm^{-1}: 3478, 3126, 3122, 2998-2992, 1742, 1658, 1645-1642, 1536, 1531, 1267. ^{1}H-NMR ($CDCl_3$) δ: 8.76 (1H, br. s, NH), 7.93—7.91 (1H, d, *J*=7.35 Hz, H-6, bza), 7.74—7.71 (1H, t, *J*=6.9 Hz, H-4), 7.62 (1H, br. s, NH, imz), 7.54—7.49 (2H, m, H-3 & H-5), 7.43 (1H, s, H-d, imz), 6.52 (1H, br. s, NH), 4.72—4.67 (1H, m, H- , ala), 4.33—4.30 (1H, t, *J*=6.7 Hz, H- ′, pro- 1), 3.95—3.93 (2H, d, *J*=5.2 Hz, H- , gly), 3.90—3.87 (1H, t, *J*=6.65 Hz, H- ′′, pro-2), 3.65 (3H, s, OCH_3), 3.58—3.55 (2H, t, *J*=7.2 Hz, H- ′), 3.39— 3.36 (2H, t, *J*=7.15 Hz, H- ′′), 2.70—2.66 (2H, q, H- ′), 2.65 (3H, s, CH_3), 2.04—1.96 (6H, m, H- ′′, H- ′ & H- ′′), 1.48—1.46 (3H, d, *J*=5.9 Hz, H-). MS *m*/*z*: 539 (M^+ + 1), 538 (M^+), 523, 507, 479, 410, 382, 353(100), 325, 282, 254, 185, 157, 82, 77, 59, 42, 31, 30, 15. *Anal*. Calcd for $C_{27}H_{34}N_6O_6$: C, 60.21; H, 6.36; N, 15.60. Found: C, 60.19; H, 6.35; N, 15.62.

5-Iodo-2-(2-methyl-1H-5-imidazolyl)benzoyl histidine methyl ester (19): Yield 96%; mp131-133 °C;$[\alpha]_D$ –15.3 °(*c*, 0.25 in MeOH); R_f - 0.79; IR (KBr) *v* cm^{-1}: 3485, 3479, 3128, 1739, 1643, 1539, 1267, 589. ^{1}H-NMR ($CDCl_3$) δ: 8.35 (2H, br. s, NH, imz), 8.21 (1H, s, H-6, bza), 7.92—7.90 (1H, d, *J*=7.3 Hz, H-4), 7.75—7.73 (1H, d, *J*=7.9 Hz, H-b′, imz), 7.60 (1H, s, H-d′), 7.47—7.45 (1H, d, *J*=7.45

Hz, H-3), 7.10 (1H, br. s, NH), 6.79 (1H, s, H-d, imz), 4.85—4.81 (1H, m, H-), 3.57 (3H, s, OCH_3), 3.03—3.01 (2H, d, *J*=5.45 Hz, H-), 2.65 (3H, s, CH_3). *Anal*. Calcd for C_{18} $H_{18}IN_5$ O_3: C, 45.11; H, 3.79; N, 14.61. Found: C, 45.14; H, 3.77; N, 14.58.

5-Iodo- 2-(2-methyl -1H-5-imidazolyl)benzoyl prolyl-proline methyl ester (20): Yield 89%; $[\alpha]_D$ +17.8° (*c*, 0.25 in MeOH); R_f - 0.66; IR ($CHCl_3$) *v* cm^{-1}: 3487, 2998-2992, 1744, 1662, 1658, 1269, 585. ^{1}H-NMR ($CDCl_3$) δ: 8.39 (1H, s, H-6, bza), 7.96—7.94 (1H, d, *J*=7.25 Hz, H-4), 7.65 (1H, br. s, NH, imz), 7.36—7.34 (1H, d, *J*=7.5 Hz, H-3), 6.96 (1H, s, H-d, imz), 4.40—4.37 (1H, t, *J*=6.7 Hz, H- , pro-1), 4.26—4.23 (1H, t, *J*=6.65 Hz, H- ′, pro-2), 3.75—3.72 (2H, t, *J*=7.15 Hz, H-), 3.62 (3H, s, OCH_3), 3.58—3.55 (2H, t, *J*=7.2 Hz, H- ′), 2.72—2.68 (2H, q, H-), 2.67 (3H, s, CH_3), 2.05—1.96 (6H, m, H- , H-β′ & H- ′). *Anal*. Calcd for $C_{22}H_{25}IN_4O_4$: C, 49.27; H, 4.70; N, 10.45. Found: C, 49.25; H, 4.69; N, 10.48.

5-Iodo- 2-(2-methyl -1H-5-imidazolyl)benzoyl glycyl-valyl-glycine methyl ester (21): Yield 87%; mp 170-171 °C; $[\alpha]_D$ +61.4° (*c*, 0.35 in MeOH); R_f - 0.60; IR (KBr) *v* cm^{-1}: 3485, 1747, 1644, 1641-1638, 1539, 1527, 1387, 1362, 1274, 589. ^{1}H-NMR ($CDCl_3$) δ: 9.36 (1H, br. s, NH), 9.05 (1H, br. s, NH), 8.45 (1H, s, H-6, bza), 7.94—7.92 (1H, d, *J*=7.3 Hz, H-4), 7.63 (1H, br. s, NH, imz), 7.33—7.31 (1H, d, *J*=7.45 Hz, H-3), 6.84 (1H, s, H-d, imz), 6.32 (1H, br. s, NH), 4.08— 4.06 (2H, d, *J*=5.2 Hz, H- , gly-1), 3.99—3.95 (1H, dd, *J*=4.55 Hz, 3.9 Hz, H- ′, val), 3.94—3.92 (2H, d, *J*=5.15 Hz, H- , gly-2), 3.65 (3H, s, OCH_3), 2.68 (3H, s, CH_3), 1.57—1.52 (1H, m, H- ′), 0.98—0.96 (6H, d, *J*=4.55 Hz, H- ′). MS *m*/*z*: 556 (M^+ + 1), 555 (M^+), 540, 524, 496, 467, 439, 368(100), 340, 311, 283, 203, 126, 82, 59, 43, 42, 31, 30, 15. *Anal*. Calcd for $C_{21}H_{26}IN_5O_5$: C, 45.42; H, 4.72; N, 12.61. Found: C, 45.45; H, 4.75; N, 12.60.

5-Iodo-2-(2-methyl-1H-5-imidazolyl)benzoyl threonyl-alanyl-histidinyl-proline methyl ester (22): Yield 92%; $[\alpha]_D$ –109.5° (*c*, 0.35 in MeOH); R_f - 0.83; IR ($CHCl_3$) *v* cm^{-1}: 3488, 3483, 1751, 1659, 1643, 1639, 1538, 1532, 1328, 1267, 587. ^{1}H-NMR ($CDCl_3$) δ: 9.33 (1H, br. s, NH), 8.56 (1H, br. s, NH), 8.38 (1H, s, H-6, bza), 8.10 (1H, br. s, NH), 7.95—7.93 (1H, d, *J*=7.25 Hz, H-4), 7.80 (3H, br. s, NH & OH), 7.69—7.67 (1H, d, *J*=7.85 Hz, H- b′, imz), 7.48 (1H, s, H- d′, imz), 7.32—7.29 (1H, d, *J*=7.5 Hz, H-3), 7.48 (1H, s, H-d, imz), 4.57—4.49 (2H, m, H- , his & H- ′, thr), 4.05—4.02 (1H, m, H- , ala), 3.92—3.89 (1H, t, *J* =6.7 Hz, H- ′′, pro), 3.78—3.74 (1H, m, H-β′), 3.63 (3H, s, OCH_3), 3.41—3.38 (2H, t, *J*=7.15 Hz, H- ′′), 2.98—2.96 (2H, d, *J*=5.5 Hz, H-), 2.66 (3H, s, CH_3), 2.05—1.98 (4H, m, H-β ′′ & H- ′′), 1.30—1.28 (3H, d, *J*=4.9 Hz, H- ′), 1.24—1.22 (3H, d, *J*=5.85 Hz, H- , ala). MS *m*/ *z*: 748 (M^+), 733, 717, 689, 620, 592, 483, 455, 412(100), 384, 311, 283, 203, 126, 82, 81, 67, 59, 42, 31, 17, 15. *Anal*. Calcd for $C_{30}H_{37}IN_8O_7$: C, 48.14; H, 4.98; N, 14.97. Found: C, 45.17; H, 4.96; N, 14.99.

General procedure for hydrolysis of amino acid / peptide methyl ester derivatives of 2-(2-oxo-2H-4-chromenyloxy)acetic acid (1) / 2-(3-bromo-2-oxo-2H-4-chromenyloxy)acetic acid (2) / 2-(2-methyl-1H-5-imidazolyl)benzoic acid (13) / 5-iodo-2-(2-methyl-1H-5-imidazolyl) benzoic acid (14)

To a solution of the amino acid / peptide methyl esters 3 / 8 / 17 / 22 (10 mmol) in THF:H_2 O (1:1, 36 ml), LiOH (0.36 g) was added at 0 °C. The mixture was stirred at RT for 1 h and then acidified to pH 3.5 with 1 N H_2SO_4. The aqueous layer was extracted with Et_2O (3 × 25 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude products were crystallized from methanol and ether to get hydrolyzed peptide derivatives 11, 12, 23 and 24.

2-(2-Oxo-2H-4-chromenyloxy)acetyl tyrosine (11): Yield 90%; mp100-102 °C; $[\alpha]_D$ +78.1° (*c*, 0.5 in MeOH); R_f - 0.51; IR (KBr): *v* cm^{-1}: 3375, 3296-2499, 3122, 1721, 1706, 1644, 1532, 1235, 1226, 1044. ^{1}H-NMR ($CDCl_3$) δ: 8.98 (2H, br. s, OH, COOH & tyr), 7.90—7.88 (1H, d, *J*=6.9 Hz, H-5), 7.52—7.49 (1H, t, *J*=8.25 Hz, H-7), 7.28—7.22 (2H, m, H-6 & H-8), 7.19—7.15 (2H, dd, *J*=8.6 Hz, 8.25 Hz, H-o), 6.80—6.76 (2H, dd, *J*=8.55 Hz, 4.85 Hz, H-m), 6.45 (1H, br. s, NH), 5.97 (1H, s, H-3), 4.87—4.83 (1H, m, H-), 4.62 (2H, s, OCH_2), 2.85—2.83 (2H, d, *J*=4.65 Hz, H- β). *Anal*. Calcd for $C_{20}H_{17}NO_7$: C, 62.66; H, 4.47; N, 3.65. Found: C, 62.63; H, 4.48; N, 3.68.

2-(3-Bromo-2-oxo-2H- 4-chromenyloxy)acetyl histidinyl-tryptophan (12): Yield 88%; mp 153-154 °C; $[\alpha]_D$ –21.2° (*c*, 0.5 in MeOH); R_f - 0.81; IR (KBr) *v* cm^{-1}: 3488, 3473, 3307-2497, 3125-3122, 1722, 1705, 1642, 1638, 1535, 1530, 1241, 1033, 688. ^{1}H-NMR ($CDCl_3$) δ: 10.02 (3H, br. s, NH & OH), 7.96—7.94 (1H, d, *J*=6.85 Hz, H-5), 7.85 (1H, br. s, NH), 7.65 (1H, s, H-d, imz), 7.62—7.59 (1H, t, *J*=8.25 Hz, H- 7), 7.55—7.53 (1H, d , *J*=7.75 Hz, H-b′, indole), 7.39—7.37 (1H, d, *J*=7.95 Hz, H-b), 7.29—7.25 (2H, m, H-6 & H-8), 7.22 (1H, br. s, NH), 7.19—7.09 (4H, m, H-d′–g′), 5.76—5.72 (1H, m, H- , his), 4.69 (2H, s, OCH_2), 4.48—4.42 (1H, m, H- ′, try), 3.01—2.96 (4H, m, H- & H- ′). *Anal*. Calcd for $C_{28}H_{24}BrN_5O_7$: C, 54.03; H, 3.89; N, 11.25. Found: C, 54.05; H, 3.89; N, 11.22.

2-(2-Methyl-1H-5-imidazolyl)benzoyl phenylalanyl- glycyl-phenylalanine (23): Yield 92%; mp 147-148 °C; $[\alpha]_D$ +1.9° (*c*, 0.5 in MeOH); R_f - 0.86; IR (KBr) *v* cm^{-1}: 3486, 3299-2504, 3125, 3119, 1703, 1645, 1641, 1638, 1539, 1533. ^{1} H-NMR ($CDCl_3$) δ: 9.82 (2H, br. s, NH & OH), 8.26 (1H, br. s, NH), 8.10 (1H, br. s, NH), 7.95—7.93 (1H, d, *J*=7.35 Hz, H- 6, bza), 7.71—7.68 (1H, t, *J*=6.9 Hz, H-4), 7.62 (1H, s, H- d, imz), 7.56—7.52 (2H, m, H-3 & H-5), 7.20—7.15 (2H, m, H-m, phe-1), 7.12—7.05 (3H, m, H-o′ & H-p′, phe-2), 7.02—6.95 (3H, m, H-p & H-m′), 6.84—6.80 (2H, dd, *J* =8.75 Hz, 4.1 Hz, H-o), 6.56 (1H, br. s, NH), 4.68—4.64 (1H, m, H- , phe-1), 4.21—4.16 (1H, m, H- ′, phe- 2), 3.80—3.78 (2H, d, *J*=5.2 Hz, H- , gly), 2.98—2.92 (4H, m, H- & H- ′), 2.66 (3H, s, CH_3). MS *m*/*z*: 553 (M^+), 536, 508, 389, 361, 332(100), 304, 185, 157, 82, 77, 66, 45, 30, 17, 15. *Anal*. Calcd for $C_{31}H_{31}N_5O_5$: C, 67.26; H, 5.64; N, 12.65. Found: C, 67.29; H, 5.62; N, 12.66.

5-Iodo-2-(2-methyl-1H- 5-imidazolyl)benzoyl threonyl-alanyl-histidinyl-proline (24): Yield 86%; $[\alpha]_D$ –71.9° (*c*, 0.5 in MeOH); R_f - 0.64; IR ($CHCl_3$) *v* cm^{-1}: 3489, 3485, 3297-2498, 2998-2994, 1707, 1658, 1641, 1637, 1539, 1530, 1329, 586. ^{1}H-NMR ($CDCl_3$) δ: 9.32 (1H, br. s, NH), 8.58 (1H, br. s, NH), 8.45 (4H, br. s, NH & OH), 8.39 (1H, s, H-6, bza), 8.09 (1H, br. s, NH), 7.94—7.92 (1H, d, *J*=7.3 Hz, H-4), 7.68—7.66 (1H, d, *J*=7.85 Hz, H-b′, imz), 7.49 (1H, s, H-d′, imz), 7.35—7.33 (1H, d, *J*=7.45 Hz, H-3), 6.85 (1H, s, H-d, imz), 5.14—5.10 (1H, m, H- , his), 4.52—4.49 (1H, m, H- ′, thr), 4.10—4.02 (2H, m, H- , ala & H- ′′, pro), 3.79—3.73 (1H, m, H-β′), 3.42—3.37 (2H, t, *J*=7.2 Hz, H- ′′), 2.96—2.94 (2H, d, *J*=5.45 Hz, H-), 2.67 (3H, s, CH_3), 2.04—1.96 (4H, m, H-β′′ & H- ′′), 1.31— 1.29 (3H, d, *J*=4.85 Hz, H- ′), 1.23—1.21 (3H, d, *J*=5.9 Hz, H- , ala). MS *m*/*z*: 734 (M^+), 717, 689, 620, 592, 483, 455, 412(100), 384, 311, 283, 203, 126, 82, 81, 67, 45, 42, 17, 15. *Anal*. Calcd for $C_{29}H_{35}IN_8O_7$: C, 47.42; H, 4.80; N, 15.25. Found: C, 47.42; H, 4.79; N, 15.28.

Antibacterial activity assay

All newly synthesized peptide derivatives were evaluated for their antimicrobial potential against two Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus epidermidis* and two Gram-negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at 50—6.25 μg/ml concentration by using modified Kirby-Bauer disc diffusion method (Bauer et al., 1966). MIC values of test compounds were determined by Tube Dilution Technique. All the synthesized compounds were dissolved separately to prepare a

Table 1. Antibacterial activity data of compounds 3-24.

Compounds	Diameter of zone of inhibition (ZI) in mm			
	Bacterial strains			
	B. subtilis	***S. epidermidis***	***P. aeruginosa***	***K. pneumoniae***
3	14(12.5)	19(6.25)	18(12.5)	15(12.5)
4	20(12.5)	27(6.25)	21(6.25)	19(6.25)
5	19(12.5)	28(6.25)	16(25)	16(12.5)
6	14(12.5)	15(12.5)	20(12.5)	18(12.5)
7	11(12.5)	17(6.25)	17(25)	20(12.5)
8	17(12.5)	22(6.25)	15(6.25)	16(6.25)
9	15(25)	19(6.25)	19(12.5)	14(25)
10	16(12.5)	24(6.25)	21(6.25)	20(6.25)
15	14(25)	17(25)	18(6.25)	20(6.25)
16	17(12.5)	22(6.25)	21(6.25)	21(6.25)
17	15(25)	18(50)	20(6.25)	18(12.5)
18	11(50)	14(12.5)	21(12.5)	20(6.25)
19	16(12.5)	20(6.25)	26(6.25)	28(6.25)
20	13(50)	16(25)	22(6.25)	24(6.25)
21	15(25)	13(25)	23(6.25)	22(6.25)
22	18(12.5)	22(6.25)	26(6.25)	26(6.25)
11	16(12.5)	22(6.25)	19(12.5)	21(12.5)
12	18(12.5)	25(6.25)	21(6.25)	18(6.25)
23	19(25)	23(50)	22(6.25)	23(12.5)
24	19(12.5)	24(6.25)	27(6.25)	28(6.25)
Control[b]	—	—	—	—
Gatifloxacin	20(12.5)	28(6.25)	24(6.25)	25(6.25)

[a] Values in bracket are MIC values (μg/ml) [b] DMF '—' indicates 'no activity'

stock solution of 1 mg/ml using DMF. Stock solution was aseptically transferred and suitably diluted with sterile broth medium to contain seven different concentrations of each test compound ranging from 200- 3.1 g ml^{-1} in different test tubes. All the tubes were inoculated with one loopful of one of the test bacterium. The process was repeated with different test bacteria and different samples. Tubes inoculated with bacterial cultures were incubated at 37˚C for 18 h and the presence/absence of growth of the bacteria was observed. From these results, MIC of each test compound was determined against each test bacterium. A spore suspension in sterile distilled water was prepared from 5 days old culture of the test bacteria growing on nutrient broth media. About 20 ml of the growth medium was transferred into sterilized petri plates and inoculated with 1.5 ml of the spore suspension (spore concentration – 6×10^4 spores ml^{-}). Filter paper disks of 6 mm diameter and 2 mm thickness were sterilized by autoclaving at 121˚C (15 psig) for 15 min. Each petri plate was divided into five equal portions along the diameter to place one disc. Three discs of test sample were placed on three portions together with one disc with reference drug - gatifloxacin and a disk impregnated with the solvent (DMF) as negative control. The petri plates inoculated with bacterial cultures were incubated at 37˚C for 18 h. Diameters of the zones of inhibition (in mm) were measured and the average diameters for test sample were calculated of triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug. The results of antibacterial studies are presented in Table 1.

Antifungal activity assay

Serial plate dilution method was used for the evaluation of antifungal activity against diamorphic fungal strain *C. albicans* and three other fungal strains, including *A. niger* and two cutaneous fungal strains *M. audouinii* and *T. mentagrophytes* at 50—6.25 μg/ml concentration (Khan, 1997). MIC values of test compounds were determined by employing the same technique as used for antibacterial studies using DMSO instead of DMF and tubes inoculated with fungal cultures were incubated at 37˚C for 48 h. After incubation, the presence/absence of growth of the fungi was observed and MIC of test compounds was determined against each test fungus. A spore suspension in normal saline was prepared from culture of the test fungi on sabouraud's broth media. After transferring growth medium, petri plates were inoculated with spore suspension. After drying, wells were made using an agar punch and test samples, reference drug - griseofulvin and negative control (DMSO) were placed in labeled wells in each petri plate. The petri plates inoculated with fungal cultures were incubated at 37˚C for 48 h. Antifungal activity was determined by measuring the diameter of the inhibition zone for triplicate sets. Activity of each compound was compared with reference standard. The results of antifungal studies are given in Table 2.

RESULTS AND DISCUSSION

Chemistry

3-Bromo-4 -hydroxycoumarin and 3 -iodoanthranilic acid were prepared by halogenation of 4-hydroxycoumarin and anthranilic acid respectively, according to the literature

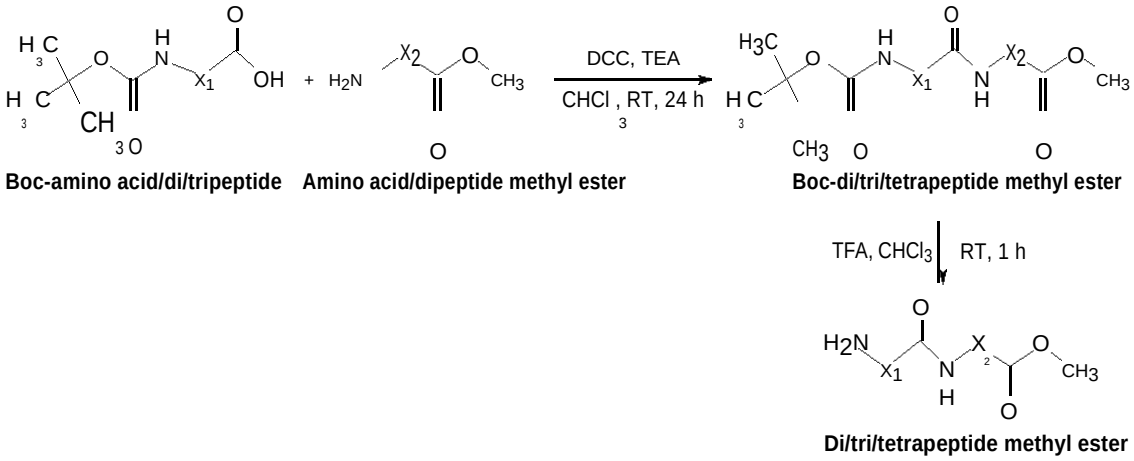
Table 2. Antifungal activity data of compounds 3-24.

Compounds	Diameter of zone of inhibition (ZI) in mm			
	Fungal strains			
	C. albicans	*M. audouinii*	*A. niger*	*T. mentagrophytes*
3	12(12.5)	11(6.25)	10(12.5)	15(12.5)
4	15(6.25)	14(6.25)	14(12.5)	17(6.25)
5	16(6.25)	14(6.25)	15(12.5)	18(6.25)
6	11(12.5)	12(12.5)	11(12.5)	13(6.25)
7	18(6.25)	14(6.25)	14(12.5)	17(6.25)
8	24(6.25)	15(6.25)	16(12.5)	19(6.25)
9	18(6.25)	14(6.25)	15(12.5)	18(6.25)
10	25(6.25)	16(6.25)	18(12.5)	18(6.25)
15	12(6.25)	11(12.5)	16(50)	14(12.5)
16	15(6.25)	14(6.25)	14(12.5)	17(6.25)
17	11(12.5)	12(6.25)	17(25)	13(6.25)
18	14(25)	10(25)	12(12.5)	11(6.25)
19	15(6.25)	21(6.25)	13(12.5)	25(6.25)
20	17(12.5)	19(6.25)	12(25)	18(6.25)
21	13(6.25)	18(6.25)	16(25)	17(6.25)
22	16(6.25)	20(6.25)	14(12.5)	24(6.25)
11	15(12.5)	14(6.25)	13(12.5)	18(12.5)
12	27(6.25)	16(6.25)	16(12.5)	21(6.25)
23	17(12.5)	17(6.25)	19(50)	18(6.25)
24	18(6.25)	22(6.25)	16(12.5)	27(6.25)
Control[b]	-	-	-	-
Griseofulvin	20(6.25)	17(6.25)	18(12.5)	22(6.25)

[a] Values in bracket are MIC values (μg/ml) [b] DMSO '—' indicates 'no activity'

procedures (Singh et al., 2006). Dipeptides Boc-L-His-L-Ile-OMe, Boc-L-His-L-Trp-OMe, Boc-L- Trp- L-Ala-OMe and Boc-L- Pro-L- Pro-OMe were prepared by coupling Boc-amino acids with the respective amino acid methyl ester hydrochlorides using dicyclohexylcarbodiimide (DCC) as coupling agent and *N*-methylmorpholine (NMM) as base (Bodanszky and Bodanszky, 1984). Similarly, tripeptides Boc-L-Trp-L-Ala-L-Trp-OMe, Boc-L-Phe-L-Val-L-Tyr-OMe, Boc-L-Phe-Gly-L-Phe-OMe, Boc-Gly-L-Val-Gly-OMe and tetrapeptides Boc-L-Phe-L-Thr-Gly-L-Leu-OMe, Boc-L-His-Gly-L-His-L-Ala-OMe, Boc-L-Pro-L-Ala-Gly-L-Pro-OMe, Boc-L-Thr-L-Ala-L-His-L-Pro-OMe were synthesized by coupling Boc-dipeptides with respective amino acid methyl ester hydrochlorides/dipeptide methyl esters in alkaline conditions. Prior to coupling with compounds 1, 2, 13 and 14, all di/tri/tetrapeptides were deprotected at amino terminal (Dahiya, 2008b) using trifluoroacetic acid (TFA) to afford L-His-L-Ile-OMe, L-His-L-Trp-OMe, L-Trp-L-Ala-OMe, L-Pro-L-Pro-OMe, L-Trp-L-Ala-L-Trp-OMe, L-Phe- L-Val- L-Tyr-OMe, L-Phe-Gly-L-Phe-OMe, Gly-L-Val-Gly- OMe, L-Phe-L-Thr-Gly-L-Leu-OMe, L-His-Gly-L-His-L-Ala-OMe, L-Pro-L-Ala-Gly-L-Pro-OMe and L-Thr-L-Ala-L-His-L-Pro-OMe (Scheme A) . Compounds 1, 2 were synthesized by interaction of 4-hydroxycoumarin/3-bromo-4-hydroxycoumarin with monochloroacetic acid in alkaline conditions whereas compounds 13, 14 were synthesized by reaction of 2- methylimidazole with diazotized solution of anthranilic acid/5-iodoanthranilic acid. Finally, coupling of compounds 1, 2, 13 and 14 with amino acid methyl ester hydrochlorides/peptide methyl esters using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl)/diisopropylcarbodiimide (DIPC) and NMM/triethylamine (TEA) afforded several peptidyl derivatives 3—10, 15—22. Moreover, amino acid/peptide derivatives 3, 8, 17 and 22 were hydrolyzed using lithum hydroxide (LiOH) to yield corresponding acid derivatives 11, 12, 23 and 24 (Scheme B and C).

Synthesis of all peptide derivatives 3—10, 15—22 was accomplished in good yields using EDC.HCl and DIPC as coupling agents and NMM/TEA as bases. Presence of medium to strong bands at 3306—2495 cm^{-1} and 1709— 1696 cm^{-1} in IR spectra of compounds 1, 2, 13 and 14 clearly indicated presence of free carboxylic group. This fact was further supported by appearance of broad singlets at 9.59—7.92 ppm in ^{1}H NMR spectra and singlets at 178.7—163.0 ppm in ^{13}C NMR spectra of compounds 1, 2, 13 and 14. IR spectra of compounds



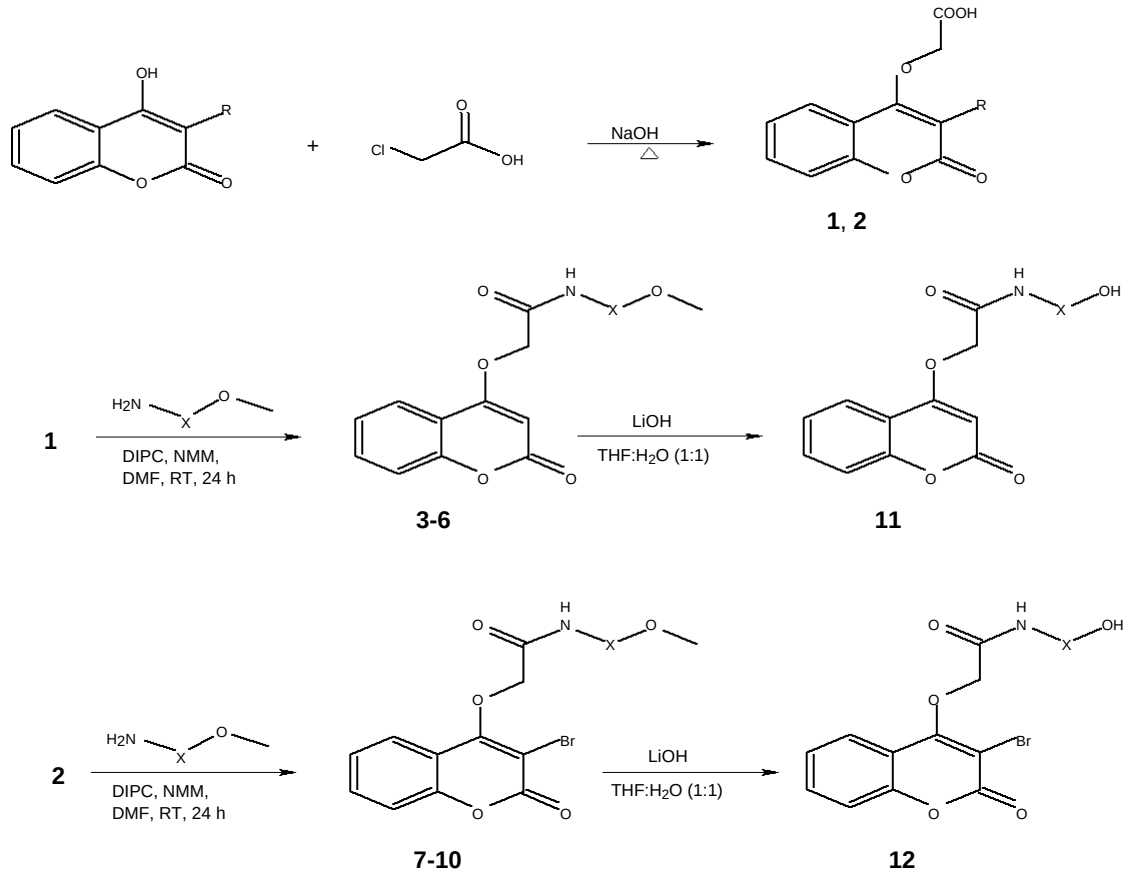
Boc-amino acid/di/tripeptide **Amino acid/dipeptide methyl ester** **Boc-di/tri/tetrapeptide methyl ester**

Di/tri/tetrapeptide methyl ester

X_1 = His, Trp, Pro, Trp-Ala, Phe-Val, Phe-Gly, Gly-Val, Phe-Thr-Gly, His-Gly-His, Pro-Ala-Gly, Thr-Ala-His

X_2 = Ile, Trp, Ala, Pro, Tyr, Phe, Gly, Gly-Leu, His-Ala, Gly-Pro, His-Pro

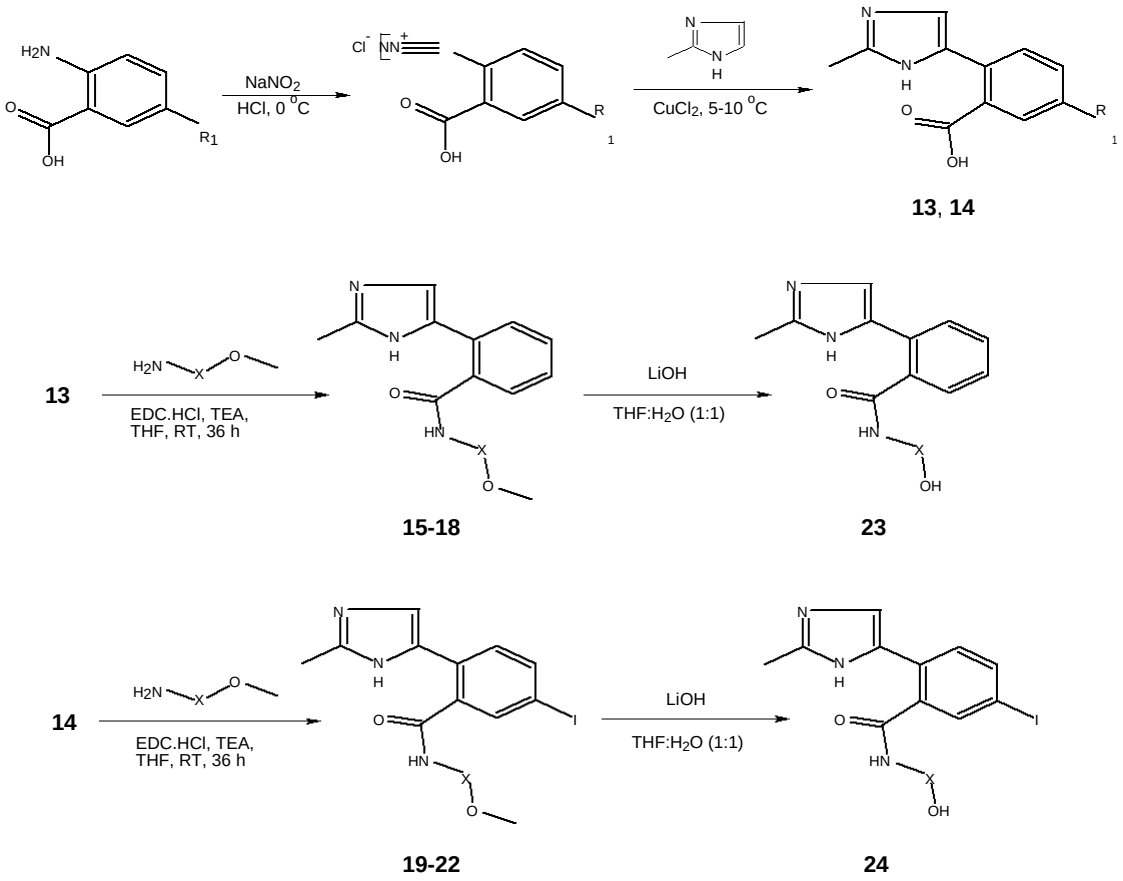
Scheme A. Synthetic pathway for intermediate peptide methyl esters.



R = H (**1**), Br (**2**)

X = L-Tyr (**3**, **11**), L-His-L-Ile (**4**), L-Trp-L-Ala-L-Trp (**5**), L-Phe-L-Thr-Gly-L-Leu (**6**) X = L-Pro (**7**), L-His-L-Trp (**8**, **12**), L-Phe-L-Val-L-Tyr (**9**), L-His-Gly-L-His-L-Ala (**10**)

Scheme B. Synthetic pathway for novel coumarinopeptides 3-12.



R_1 = H (**13**), I (**14**)

X = L-Thr (**15**), L-Trp-L-Ala (**16**), L-Phe-L-Gly-L-Phe (**17**, **23**), L-Pro-L-Ala-Gly-L-Pro (**18**) X = L-His (**19**), L-Pro-L-Pro (**20**), Gly-L-Val-Gly (**21**), L-Thr-L-Ala-L-His-L-Pro (**22**, **24**)

Scheme C. Synthetic pathway for novel imidazolopeptides 15-24.

3—10 and 15—22 showed amide I (C=O_{str}) and amide II (N−H_{def}) bands at 1662—1638 cm^{-1} and 1539—1527 cm^{-1} indicating formation of peptide bonds and accomplishment of the coupling reaction. This fact was further confirmed by appearance of broad singlets at 9.49—6.05 ppm (for imino proton of CO–N<u>H</u> moiety) in ^{1}H NMR spectra of the synthesized compounds. Presence of bromo group in peptide analogs of compound 2 was indicated by appearance of medium bands at 688—680 cm^{-1} (C−Br $_{str}$) in their IR spectra whereas presence of iodo group in peptide derivatives of compound 14 was indicated by appearance of bands of medium intensity at 589—585 cm^{-1} (C−I_{str}) in respective IR spectra. Structures of hydrolyzed derivatives 11, 12, 23 and 24 were confirmed by appearance of strong bands at 1707— 1703 cm^{-1} (C=O_{str}, <u>CO</u>OH) in IR spectra, broad singlets at 10.02—8.45 ppm (for hydroxyl proton of CO <u>OH</u>) in ^{1}H NMR spectra and disappearance of medium to strong bands at 1751—1742 cm^{-1} (C=O_{str}, ester) and 1273— 1267 cm^{-1} (C−O_{str}, ester) in IR spectra and singlets at 3.63—3.56 ppm (for three protons of O<u>CH$_3$</u>) in ^{1}H NMR spectra of compounds 3, 8, 17 and 22. Furthermore, mass spectra of peptide ester derivatives showed easily distinguishable R–C O^+ ion peaks at M^+ – 31 along with [CH_3O^+] and [CH_3OCO^+] fragment ion peaks at m/z values 31 and 59 respectively. Hydrolyzed peptide derivatives showed characteristics peaks at M^+ – 17 and M^+ – 45 in their mass spectra by loss of OH and COOH along with [$COOH^+$] fragment ion peak at m/z value 45 in their mass spectra. The newly synthesized peptide analogs were also analyzed for C, H and N content and the results revealed the variation by a factor of ± 0.03 from calculated values.

Pharmacology

Almost all the synthesized compounds were found to exhibit moderate to good bioactivity against pathogenic bacteria, dermatophytes and *C. albicans*. Analysis of

Table 3. ANOVA table for bacterial species.

Source of variation	Degree of freedom	Sum of square	Mean square
Treatments (between columns)	3	325.24	108.41
Residuals (within columns)	76	998.65	13.140

Table 4. ANOVA table for fungal species.

Source of variation	Degree of freedom	Sum of square	Mean square
Treatments (between columns)	3	131.05	43.683
Residuals (within columns)	76	1000.9	13.170

antimicrobial activity data of compounds 1, 2 and 13, 14 revealed that introduction of bromo group at position-3 of coumarin nucleus resulted increase in bioactivity against Gram-positive bacterium *S. epidermidis* and pathogenic fungi *C. albicans* and dermatophytes whereas introduction of iodo group at position-5 of the benzoic acid moiety of compound 13 resulted increase in antimicrobial activity against all tested microorganisms except Gram-positive bacterium *B. subtilis*. Coumarinopeptides 3—10 were found to be effective towards *B. subtilis* and *S. epidermidis* whereas imidazolopeptides 15—22 displayed good activity towards *P. aeruginosa* and *K. pneumoniae*. Moreover, bromocoumariopeptide analogs 7—10 displayed slightly improved antifungal activity especially against *C. albicans*, in comparison to non-bromocoumarinopeptides 3—6 whereas iodoimidazolopeptides 19—22 exhibited improved antibacterial activity against Gram-negative bacteria as well as antidermatophyte activity when compared to non-iodoimidazolopeptide derivatives 15—18. The killing mechanism of these peptide derivatives may involve the interaction with and disruption of microbial membranes through a relatively nonspecific mechanism, largely based on differences in membrane lipid composition. Comparison of biological activity data further, proved that hydrolyzed peptide derivatives possessed slightly increased antibacterial and antifungal activity in comparison to corresponding methyl ester derivatives. Compounds 19, 22 and 24 were found to possess potent antimicrobial activity against Gram-negative bacteria and dermatophytes *M. audouinii* and *T. mentagrophytes*, comparable to gatifloxacin /griseofulvin whereas compounds 4 and 5 displayed better bioactivity against Gram-positive bacteria.

Furthermore, compounds 8, 10 and 12 showed potent antifungal activity against *C. albicans* comparable to reference drug – griseofulvin. However, compound 10 displayed significant level of antifungal activity against *A. niger* (Tables 1 and 2). Antibacterial and antifungal data were subjected to one way ANOVA with post test (GraphPad InStat). As the value of F_{calc} is 8.250 and 3.317 for bacterial and fungal species respectively, and the F_{tab} is 2.768, the significant difference exists between the values of zone of inhibition of species tested (Tables 3 and 4).

Conclusion

Present investigation describes successful synthesis of novel peptide derivatives in good yields. For peptide coupling, EDC.HCl proved to be a better coupling agent in comparison to DIPC, providing 10—12% extra yields. Coupling of compounds 1, 2 13 and 14 with amino acids and di/tri/tetrapeptides yielded novel heteropeptide congeners with good antimicrobial activity. Gram-negative bacteria were found to be more sensitive towards imidazolopeptides whereas Gram-positive bacteria were more sensitive towards newly synthesized coumarinopeptide derivatives. Greater antimicrobial activity was found in peptide derivatives having histidine and tryptophan constituents in their amino acid chains. No significant change in antimicrobial activity was observed upon hydrolysis of methyl ester group in amino acid side chain of synthesized compounds. Among tested compounds, 4, 5, 8, 10, 19, 22 and hydrolyzed analogs 12 and 24 exhibited good antimicrobial activity. On passing toxicity tests, these compounds may prove novel candidates for clinical studies and may be potential antibacterial and antifungal agents of future.

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