



RESEARCH ARTICLE

SYNTHESIS AND ANTIMICROBIAL SCREENING OF SOME NOVEL HALOGENATED PHENOXYACETYL AMINO ACID AND PEPTIDE ANALOGS

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In present study, a novel series of trisubstituted phenoxyacetyl amino acids and peptide derivatives was synthesized by coupling 2-(2,6-dibromo-4-formylphenoxy)acetic acid with amino acid methyl ester hydrochlorides/peptide methyl esters using DIPC as coupling agent and TEA as base. The structures were elucidated by IR, ¹H NMR, ¹³C NMR and MS spectral data as well as elemental analysis. The newly synthesized peptide derivatives were evaluated for antibacterial and antifungal potential against pathogenic microorganisms. Compounds I_g, I_k and I_n displayed potent antibacterial activity against gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and compounds I_c, I_h, I_i and I_j, I_m were found to exhibit potent antifungal activity against pathogenic *Candida albicans* and dermatophytes, as compared to standard drugs ciprofloxacin and griseofulvin.

Key words: Aryloxyacetic acid, 3,5-Dibromo-4-hydroxybenzaldehyde, Peptides, Antimicrobial activity.

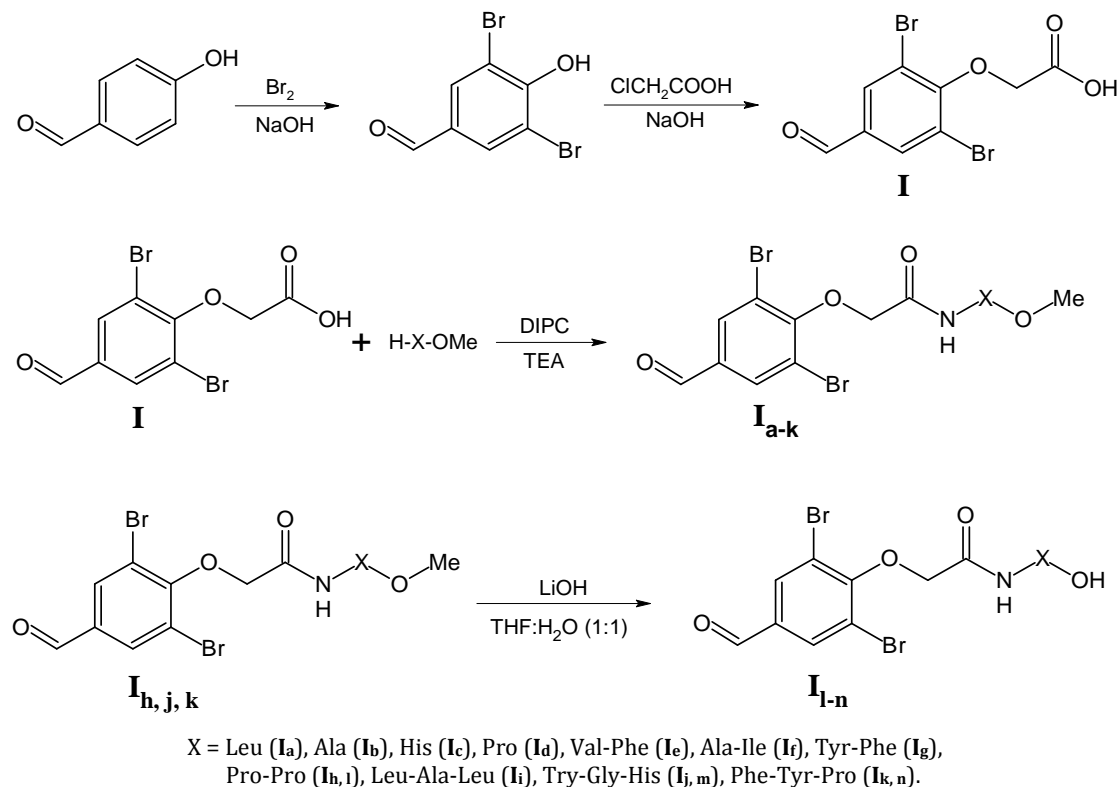
INTRODUCTION

Phenoxy acetic acid is the most vital moiety which is concerned with potent antimicrobial activity. Much work has been done on synthesis of potent phenoxy acetic acid derivatives with diverse bioactivities (Takeda *et al* 1998; Shaharyar *et al* 2006; Shahar Yar *et al* 2009) but less reports have been received regarding coupling of phenoxy acetic acids with peptides. The literature is enriched with several reports indicating incorporation of amino acids and peptides into the aromatic and heterocyclic moieties have resulted in compounds with potent bioactivities (Belagali *et al* 2001; Himaja *et al* 2002; 2003; Poojary *et al* 2003). Thus, keeping in view biopotency of phenoxyacetic acids and further, in continuation of our work on synthesizing potent peptide derivatives of aroylbenzoic acid, furoic acid, aryloxyacetic acid, coumarin, quinoxaline, quinazolinone, benzimidazole imidazole, (Dahiya and Pathak,

2006a; 2006b; 2006c; Dahiya *et al* 2006a; 2006b; Dahiya and Pathak, 2007; Dahiya and Kaur, 2007a; 2007b; 2008; Dahiya, 2008a, 2008b; Dahiya *et al* 2008a; 2008b; Dahiya and Kumar, 2008; Dahiya and Bansal, 2008; Dahiya *et al* 2010), a novel series of halogenated phenoxy acetyl amino acids and peptides was synthesized with an anticipation to get the novel compounds with more therapeutic efficacy. 2-(2,6-Dibromo-4-formylphenoxy) acetic acid (I) was prepared by the interaction of 3,5-dibromo-4-hydroxybenzaldehyde with chloroacetic acid in presence of alkali (Manchand *et al* 1990). Dipeptides Boc-Ala-Ile-OMe, Boc-Tyr-Phe-OMe and tripeptides Boc-Leu-Ala-Leu-OMe, Boc-Try-Gly-His-OMe and Boc-Phe-Tyr-Pro-OMe were prepared from amino acid methyl esters and Boc-amino acids using dicyclohexylcarbodiimide (DCC) as the coupling agent. 2-(2,6-Dibromo-4-formylphenoxy)acetyl amino acid methyl esters

I_{a-d}, di/tripeptides esters **I_{e-h}** and **I_{i-k}** were prepared by coupling compound **I** with respective amino acid methyl ester hydrochlorides and dipeptide/ tripeptide methyl esters. Furthermore, compounds **I_h**, **I_j** and **I_k** were hydrolyzed to get corresponding free acids

I_{l-n} (**Scheme 1**). Structures of all newly synthesized compounds were confirmed by IR, ¹H/¹³C NMR and mass spectra. Elemental analysis of the newly synthesized compounds was performed for carbon, hydrogen and nitrogen content.



Scheme 1. Synthetic pathway for 2-(2,6-dibromo-4-formylphenoxy)acetyl peptide analogs

All the synthesized compounds were screened for *in vitro* antimicrobial activity against gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, cutaneous fungi *Microsporium audouinii* and *Trichophyton mentagrophytes*, diamorphic fungi *Candida albicans* using modified Kirby-Bauer disk diffusion method (Bauer *et al* 1966). The results are shown in **Table 1**.

EXPERIMENTAL

Materials and Methods

Melting points were determined by open capillary method and are uncorrected. L-Amino acids, di-*tert*-butylpyrocarbonate (Boc), diisopropylcarbodiimide (DIPC), trifluoroacetic acid (TFA) and triethylamine (TEA) were obtained from Spectrochem Limited, Mumbai, India. *p*-Hydroxybenzaldehyde was procured from HIMEDIA Lab, Mumbai. IR spectra were recorded on Shimadzu 8700 fourier transform

infrared spectrophotometer using a thin film supported on KBr pellets for all synthesized compounds. ¹H NMR and ¹³C NMR spectra were recorded on Bruker AC NMR spectrometer (300 MHz) using CDCl₃ as solvent and TMS as internal standard. Mass spectra were recorded on Jeol JMS DX 303 Mass spectrometer operating at 70 eV by considering Cl atomic wt. 36. Elemental analyses of all compounds were performed on Elementar vario EL III. Purity of all the compounds was checked by TLC on precoated silica gel G plates.

Preparation of peptides

Amino acid methyl ester hydrochloride (0.01 mol) was dissolved in CH₂Cl₂ (25 ml). To this, *N*-methylmorpholine (NMM) (0.021 mmol, 2.3 ml) was added at 0°C followed by stirring of reaction mixture for 15 min. Boc-amino acid/dipeptide (0.01 mol) in CH₂Cl₂ (25 ml) and DCC (0.01 mol, 2.1 g) were added with stirring. After 24 h, the reaction mixture was filtered and the residue

Table 1. Antimicrobial activity data of compounds **I_{a-n}**

Compound	Zone of Inhibition (mm)						
	<i>B. subt.</i>	<i>E. coli</i>	<i>S. aure.</i>	<i>P. aeru.</i>	<i>M. audo.</i>	<i>T. ment.</i>	<i>C. albi.</i>
I_a	9(6)	12(25)	–	14(12.5)	9(12.5)	12(6)	10(6)
I_b	10(12.5)	11(25)	10(25)	16(6)	–	–	9(12.5)
I_c	13(6)	12(12.5)	11(25)	18(6)	11(12.5)	11(6)	22(6)
I_d	9(12.5)	10(25)	–	13(12.5)	10(6)	10(12.5)	11(12.5)
I_e	15(6)	15(12.5)	17(12.5)	19(6)	9(6)	13(6)	13(6)
I_f	17(6)	13(12.5)	15(12.5)	21(6)	10(6)	13(6)	–
I_g	16(6)	20(12.5)	16(12.5)	27(6)	11(6)	11(6)	13(6)
I_h	15(6)	14(12.5)	16(12.5)	21(6)	10(6)	12(6)	21(6)
I_i	–	10(12.5)	–	17(6)	14(6)	16(6)	16(6)
I_j	10(6)	12(12.5)	11(12.5)	17(6)	17(6)	21(6)	16(6)
I_k	12(6)	20(12.5)	13(12.5)	26(6)	13(6)	16(6)	16(6)
I_l	17(6)	16(12.5)	18(12.5)	23(6)	12(6)	15(6)	24(6)
I_m	12(6)	15(12.5)	14(12.5)	20(6)	19(6)	24(6)	18(6)
I_n	16(6)	22(12.5)	17(12.5)	28(6)	17(6)	18(6)	18(6)
Control	–	–	–	–	–	–	–
Standard I	20(6)	19(12.5)	20(12.5)	25(6)	–	–	–
Standard II	–	–	–	–	17(6)	20(6)	20(6)

Standard I – Ciprofloxacin; Standard II – Griseofulvin; *B. subt.* – *Bacillus subtilis*; *E. coli* – *Escherichia coli*; *S. aure.* – *Staphylococcus aureus*; *P. aeru.* – *Pseudomonas aeruginosa*; *M. audo.* – *Microsporium audouinii*; *T. ment.* – *Trichophyton mentagrophytes*; *C. albi.* – *Candida albicans*

was washed with CH₂Cl₂ (25 ml) and added to the filtrate. The filtrate was washed with 5% NaHCO₃ and saturated NaCl solutions. The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated in vacuum. The crude product was recrystallized from a mixture of chloroform and petroleum ether followed by cooling at 0°C. For protecting the amino group of L-amino acids, di-*tert*-butylpyrocarbonate (Boc) was utilized whereas the carboxyl group of L-amino acids was protected by esterification with methanol. Peptides were prepared by modified Bodanzsky method (Bodanzsky and Bodanzsky, 1984). Moreover, trifluoroacetic acid was used for the removal of Boc group and ester group was removed by alkaline hydrolysis with LiOH.

^tButyloxycarbonyl-alanyl-isoleucine methyl ester

(Semisolid mass, Yield 2.85 g, 89.6%); IR (CHCl₃): 3122 (m, -NH str, amide), 2962, 2958 (m, -CH str, asym, CH₃), 2871, 2849, (m, -CH str, sym, CH₃ and CH₂), 2825 (m, -CH str, OCH₃), 1752 (s, -C=O str, ester), 1642, 1637 (s, -C=O str, 2° amide), 1539, 1535 (m, -NH bend, 2° amide), 1389, 1372 (m, -CH bend, ^tButyl group), 1279 (s, C–O str, ester), 932 (w, CH₃ rocking, *tert*-butyl group) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 6.69

(1H, br. s, -NH), 6.51 (1H, br. s, -NH), 4.53-4.48 (1H, m, α-H, Ala), 4.22-4.19 (1H, t, *J* = 8.65 Hz, α-H, Ile), 3.53 (3H, s, OCH₃), 2.08-2.03 (1H, m, β-H, Ile), 1.72-1.65 (2H, m, γ-H's, Ile), 1.58-1.56 (3H, d, *J* = 5.2 Hz, α-H's, Ala), 1.52 (9H, s, ^tButyl group), 0.95-0.92 (3H, t, *J* = 7.75 Hz, δ-H's, Ile), 0.89-0.87 (3H, d, *J* = 5.95 Hz, γ'-H's, Ile); Anal. Calcd. For C₁₅H₂₈N₂O₅: C, 56.94; H, 8.92; N, 8.85. Found: C, 56.95; H, 8.95; N, 8.82%.

^tButyloxycarbonyl-tyrosinyl-phenylalanine methyl ester

(Semisolid mass, Yield 3.7 g, 83.7%); IR (CHCl₃): 3369 (m, -OH str, Tyr), 3128, 3123 (m, -NH str, amide), 3066 (w, -CH str, arom. Ring), 2922 (m, -CH str, asym, CH₂), 2850 (m, -CH str, sym, CH₂), 2825 (m, -CH str, OCH₃), 1743 (s, -C=O str, ester), 1642, 1638 (s, -C=O str, 2° amide), 1585, 1479 (m, skeletal bands, arom. rings), 1536, 1529 (m, -NH bend, 2° amide), 1390, 1370 (m, -CH bend, ^tButyl group), 1271 (s, C–O str, ester), 1224 (s, C–O str, phenolic), 825, 715, 694 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 172.3 (-C=O, Tyr), 169.2 (-C=O, Phe), 155.9 (p-C, Tyr), 152.4 (-C=O, Boc group), 138.5 (γ-C, Phe), 134.1 (2C, m-C's, Tyr), 130.6 (2C, o-C's, Tyr), 128.8 (2C, o-C's, Phe), 128.1 (2C, m-C's, Phe), 126.5 (γ-C, Tyr), 125.3 (p-C, Phe), 79.9 (α-

C, ^tButyl group), 54.3 (α -C, Tyr), 53.9 (α -C, Phe), 51.8 (-OCH₃), 38.5 (β -C, Tyr), 37.2 (β -C, Phe), 29.2 (3C, β -C's, ^tButyl group) ppm; Anal. Calcd. For C₂₄H₃₀N₂O₆: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.17; H, 6.85; N, 6.32%.

^tButyloxycarbonyl-leucyl-alanyl-leucine methyl ester

(Semisolid mass, Yield 3.82 g, 89%); IR (CHCl₃): 3122, 3117 (m, -NH str, amide), 2967, 2925 (m, -CH str, asym, CH₃ and CH₂), 2872, 2851, 2849 (m, -CH str, sym, CH₃ and CH₂), 2826 (m, -CH str, OCH₃), 1749 (s, -C=O str, ester), 1643, 1638 (s, -C=O str, 2° amide), 1536, 1532 (m, -NH bend, 2° amide), 1392, 1371 (m, -CH bend, ^tButyl group), 1385, 1368 (s, -CH bend, isopropyl group), 1268 (s, C-O str, ester), 925 (w, CH₃ rocking, isopropyl group) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 8.11 (1H, br. s, -NH), 6.79 (1H, br. s, -NH), 6.51 (1H, br. s, -NH), 4.46-4.41 (1H, m, α -H, Ala), 4.21-4.16 (1H, m, α -H of Leu-1), 3.63 (3H, s, OCH₃), 3.57-3.52 (1H, m, α -H of Leu-2), 1.94-1.91 (2H, t, J = 7.9 Hz, β -H's, Leu-1), 1.59-1.52 (1H, m, γ -H of Leu-1), 1.48-1.41 (3H, m, β - and γ -H's, Leu-2), 1.50-1.48 (3H, d, J = 5.15 Hz, α -H's, Ala), 1.52 (9H, s, ^tButyl group), 1.03-1.01 (6H, d, J = 6.15 Hz, δ -H's, Leu-1), 0.95-0.93 (6H, d, J = 6.2 Hz, δ -H's, Leu-2) ppm; Anal. Calcd. For C₂₁H₃₉N₃O₆: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.74; H, 9.12; N, 9.77%.

^tButyloxycarbonyl-phenylalanyl-tyrosinyl-proline methyl ester

(Semisolid mass, Yield 4.75 g, 88%); IR (CHCl₃): 3366 (m, -OH str, Tyr), 3129-3124 (m, -NH str, amide), 3069, 3066-3062 (w, -CH str, arom. rings), 2997, 2989 (m, -CH str, cyclic CH₂ and CH), 2923, 2852 (m, -CH str, asym and sym, CH₂), 2827 (m, -CH str, OCH₃), 1746 (s, -C=O str, ester), 1675, 1641-1634 (s, -C=O str, 3° and 2° amide), 1589, 1476 (m, skeletal bands, arom. rings), 1537, 1533-1527 (m, -NH bend, 2° amide), 1389, 1368 (m, -CH bend, ^tButyl group), 1274 (s, C-O str, ester), 1222 (s, C-O str, phenolic), 828, 711, 698 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 175.8 (-C=O, Phe), 169.3 (-C=O, Pro), 167.7 (-C=O, Tyr), 154.1 (p-C, Tyr), 150.9 (-C=O, Boc group), 133.6 (γ -C, Phe), 132.3 (γ -C, Tyr), 130.5 (2C, m-C's, Phe), 129.6 (2C, o-C's, Tyr), 128.8 (p-C, Phe), 128.3 (2C, o-C's, Phe), 126.5 (2C, m-C's, Tyr), 79.3 (α -C, ^tButyl group), 58.8 (α -C, Pro), 54.2 (-OCH₃), 52.1 (α -C, Phe), 48.6 (α -C, Tyr), 45.9 (δ -C, Pro), 37.7 (β -C, Phe), 36.4 (β -C, Tyr), 28.4 (β -C, Pro), 27.9 (3C, β -C's,

^tButyl group), 24.6 (γ -C, Pro) ppm; Anal. Calcd. For C₂₉H₃₇N₃O₇: C, 64.55; H, 6.91; N, 7.79. Found: C, 64.52; H, 6.93; N, 7.78%.

Preparation of 2-(2,6-dibromo-4-formylphenoxy)acetic acid (I)

3,5-Dibromo-4-hydroxy benzaldehyde (0.024 mol, 6.72 g) was dissolved in 33% NaOH solution (25 ml). To the above mixture, 50% chloroacetic acid solution (25 ml) was added. The reaction mixture was refluxed on gently boiling water bath for 1 h. After cooling, water (50 ml) was added and whole mixture was acidified to congo red with dil. HCl and finally extracted with ether (3 \times 50 ml). Ethereal extract was washed with water (50 ml) and shaken twice with 5% Na₂CO₃ solution (2 \times 35 ml). Crude acid was precipitated out by acidifying the Na₂CO₃ extract with dil. HCl. The separated solid was collected by filtration, washed with cold water and finally crystallized from aqueous ethanol to get the title compound. (Offwhite crystals, m.p. 126-127°C, Yield 7.22 g, 89%); IR (KBr): 3295-2499 (m/br, -OH str, -COOH), 3072 (w, -CH str, phenoxy ring), 2925 (m, -CH str, asym, CH₂), 2825, 2720 (m, -CH str, -CHO), 1713 (m, -C=O str, -COOH), 1702 (s, -C=O str, -CHO), 1597, 1483 (m, skeletal bands, phenoxy ring), 1266 (s, C-O-C str, asym), 1128, 1123 (m, C-Br str), 1062 (s, C-O-C str, sym), 883, 802 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.75 (1H, s, -CHO), 7.97 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 7.02 (1H, s, -COOH), 4.52 (2H, s, -OCH₂-) ppm; ¹³C NMR (CDCl₃, 300 MHz): δ 192.9 (-C=O, -CHO), 172.3 (-C=O, -COOH), 159.7 (C₁, phenoxy ring), 138.9 (C₄, phenoxy ring), 135.6 (2C, C₃ and C₅, phenoxy ring), 115.3 (2C, C₂ and C₆, phenoxy ring), 68.8 (-OCH₂-) ppm; Anal. Calcd. For C₉H₆Br₂O₄: C, 31.99; H, 1.79. Found: C, 31.97; H, 1.80%.

General procedure for preparation of 2-(2,6-dibromo-4-formylphenoxy)acetyl amino acid and peptide methyl esters (I_{a-k})

To a mixture of L-amino acid methyl ester hydrochloride/di/tripeptide methyl ester (0.01 mol) in DMF (50 ml), TEA (2.8 ml) was added at 0°C with stirring. Compound I (3.4 g, 0.01 mol) in DMF (50 ml) and DIPC (1.26 g, 0.01 mol) were added to the above mixture and stirring was done for 24 h. The reaction mixture was filtered and water was added in equal proportions to the filtrate. Finally, the aqueous layer was washed with ether (3 \times 50 ml) in three proportions. The organic layer was separated and dried over

anhydrous Na₂SO₄, filtered and evaporated in vacuum. The product obtained was dissolved in chloroform, washed with 10% HCl, saturated NaHCO₃ solution and water (20 ml each) followed by evaporation in vacuum. Crude product was crystallized from a mixture of ethyl acetate and petroleum ether.

2-(2,6-Dibromo-4-formylphenoxy)acetyl leucine methyl ester (I_a)

(Pale yellow solid, m.p. 171-172°C, Yield 3.95 g, 84%); IR (KBr): 3121 (m, -NH str, amide), 3072 (w, -CH str, phenoxy ring), 2962 (m, -CH str, asym, CH₃), 2855, 2851 (m, -CH str, sym, CH₂), 2825, 2720 (m, -CH str, -CHO), 1742 (m, -C=O str, ester), 1702 (s, -C=O str, -CHO), 1644 (s, -C=O str, 2° amide), 1597, 1483 (m, skeletal bands, phenoxy ring), 1535 (m, -NH bend, 2° amide), 1388, 1369 (s, -CH bend, isopropyl group), 1272 (s, C-O str, ester), 1264 (s, C-O-C str, asym), 1128, 1123 (m, C-Br str), 1062 (s, C-O-C str, sym), 833 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.74 (1H, s, -CHO), 8.23 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 6.36 (1H, br. s, -NH), 4.63 (2H, s, -OCH₂-), 4.25-4.19 (1H, m, α-H, Leu), 3.62 (3H, s, OCH₃), 1.46-1.39 (1H, m, γ-H's, Leu), 1.27-1.24 (2H, t, J=7.9 Hz, β-H's, Leu), 0.95-0.93 (6H, d, J=6.2 Hz, δ-H's, Leu) ppm; ¹³C NMR (CDCl₃, 300 MHz): δ 193.4 (-C=O, -CHO), 179.8 (-C=O, ester), 176.3 (-C=O, -OCH₂CO-), 163.3 (C₁, phenoxy ring), 138.5 (C₄, phenoxy ring), 134.9 (2C, C₃ and C₅, phenoxy ring), 116.8 (2C, C₂ and C₆, phenoxy ring), 69.0 (-OCH₂-), 52.2 (-OCH₃), 50.8 (α-C, Leu), 43.5 (β-C, Leu), 27.2 (γ-C, Leu), 25.7 (2C, δ-C, Leu) ppm; Anal. Calcd. For C₁₆H₁₉Br₂NO₅: C, 41.32; H, 4.12; N, 3.01. Found: C, 41.29; H, 4.11; N, 3.04%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl alanine methyl ester (I_b)

(Yellow solid, m.p. 102-103°C, Yield 3.84 g, 90%); IR (KBr): 3127 (m, -NH str, amide), 3069 (w, -CH str, phenoxy ring), 2966 (m, -CH str, asym, CH₃), 2871, 2855 (m, -CH str, sym, CH₃ and CH₂), 2826, 2723 (m, -CH str, -CHO), 1745 (m, -C=O str, ester), 1704 (s, -C=O str, -CHO), 1640 (s, -C=O str, 2° amide), 1596, 1482 (m, skeletal bands, phenoxy ring), 1538 (m, -NH bend, 2° amide), 1270 (s, C-O str, ester), 1262 (s, C-O-C str, asym), 1129, 1122 (m, C-Br str), 1067 (s, C-O-C str, sym), 835 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.74 (1H, s, -CHO), 8.23 (2H, singlet overlapped over

singlet, m-H's, phenoxy ring), 6.36 (1H, br. s, -NH), 4.63 (2H, s, -OCH₂-), 4.25-4.19 (1H, m, α-H, Leu), 3.62 (3H, s, OCH₃), 1.46-1.39 (1H, m, γ-H's, Leu), 1.27-1.24 (2H, t, J=7.9 Hz, β-H's, Leu), 0.95-0.93 (6H, d, J=6.2 Hz, δ-H's, Leu) ppm; Anal. Calcd. For C₁₃H₁₃Br₂NO₅: C, 36.91; H, 3.10; N, 3.31. Found: C, 36.89; H, 3.12; N, 3.32%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl histidine methyl ester (I_c)

(Yellow crystals, m.p. 225°C, Yield 4.2 g, 85.2%); IR (KBr): 3486 (m, -NH str, heteroarom. ring), 3124 (m, -NH str, amide), 3068 (w, -CH str, phenoxy ring), 2966, 2919 (m, -CH str, asym, CH₃ and CH₂), 2852, 2848 (m, -CH str, sym, CH₂), 2826, 2723 (m, -CH str, -CHO), 1747 (m, -C=O str, ester), 1701 (s, -C=O str, -CHO), 1647 (s, -C=O str, 2° amide), 1592-1588, 1489-1484 (m, skeletal bands, arom. rings), 1533 (m, -NH bend, 2° amide), 1276 (s, C-O str, ester), 1268 (s, C-O-C str, asym), 1129, 1124 (m, C-Br str), 1066 (s, C-O-C str, sym), 834, 712 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 192.5 (-C=O, -CHO), 176.2 (-C=O, -OCH₂CO-), 174.6 (-C=O, ester), 160.9 (C₁, phenoxy ring), 140.6 (C₂, imz ring), 139.0 (C₄, phenoxy ring), 132.2 (2C, C₃ and C₅, phenoxy ring), 128.8 (C₄, imz ring), 121.1 (2C, C₂ and C₆, phenoxy ring), 115.5 (C₄, imz ring), 67.7 (-OCH₂-), 52.7 (α-C, His), 50.8 (-OCH₃), 21.9 (β-C, His) ppm; Anal. Calcd. For C₁₆H₁₅Br₂N₃O₅: C, 39.29; H, 3.09; N, 8.59. Found: C, 39.31; H, 3.10; N, 8.57%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl proline methyl ester (I_d)

(Yellowish-brown solid, m.p. 158-159°C, Yield 3.8 g, 83.9%); IR (KBr): 3077 (w, -CH str, phenoxy ring), 2997-2989 (m, -CH str, cyclic CH₂ and CH), 2858 (m, -CH str, sym, CH₂), 2833 (m, -CH str, OCH₃), 2821, 2725 (m, -CH str, -CHO), 1746 (m, -C=O str, ester), 1707 (s, -C=O str, -CHO), 1673 (s, -C=O str, 3° amide), 1595, 1484 (m, skeletal bands, phenoxy ring), 1270 (s, C-O str, ester), 1262 (s, C-O-C str, asym), 1129, 1121 (m, C-Br str), 1065 (s, C-O-C str, sym), 838 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.71 (1H, s, -CHO), 8.20 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 4.66 (2H, s, -OCH₂-), 4.05-4.02 (1H, t, J=6.8 Hz, α-H, Pro), 3.67 (3H, s, OCH₃), 4.05-4.02 (2H, t, J=7.15 Hz, δ-H's, Pro), 2.05-1.97 (4H, m, β- and γ-H's, Pro) ppm; Anal. Calcd. For C₁₅H₁₅Br₂NO₅: C, 40.12; H, 3.37; N, 3.12. Found: C, 40.15; H, 3.35; N, 3.11%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl valyl-phenylalanine methyl ester (I_e)

(Yellow crystals, m.p. 109-110°C, Yield 4.82 g, 80%); IR (KBr): 3128-3123 (m, -NH str, amide), 3077, 3065 (w, -CH str, arom. rings), 2969, 2921 (m, -CH str, asym, CH₃ and CH₂), 2855, 2849 (m, -CH str, sym, CH₂), 2824, 2726 (m, -CH str, -CHO), 1745 (m, -C=O str, ester), 1706 (s, -C=O str, -CHO), 1643, 1639 (s, -C=O str, 2° amide), 1590-1587, 1488-1483 (m, skeletal bands, arom. rings), 1536, 1532 (m, -NH bend, 2° amide), 1387, 1366 (s, -CH bend, isopropyl group), 1273 (s, C-O str, ester), 1265 (s, C-O-C str, asym), 1126, 1122 (m, C-Br str), 1068 (s, C-O-C str, sym), 835, 710, 692 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 191.6 (-C=O, -CHO), 173.3 (-C=O, -OCH₂CO-), 170.4 (-C=O, ester), 169.2 (-C=O, Val), 160.7 (C₁, phenoxy ring), 139.3 (C₄, phenoxy ring), 136.6 (γ-C, Phe), 133.6 (2C, C₃ and C₅, phenoxy ring), 128.9 (2C, o-C's, Phe), 126.7 (2C, m-C's, Phe), 125.4 (p-C, Phe), 118.3 (2C, C₂ and C₆, phenoxy ring), 65.9 (-OCH₂-), 60.3 (α-C, Val), 55.7 (α-C, Phe), 53.1 (-OCH₃), 38.2 (β-C, Phe), 33.5 (β-C, Val), 33.5 (2C, γ-C's, Val) ppm; Anal. Calcd. For C₂₄H₂₆Br₂N₂O₆: C, 48.18; H, 4.38; N, 4.68. Found: C, 48.15; H, 4.36; N, 4.69%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl alanyl-isoleucine methyl ester (I_f)

(Semisolid mass, Yield 4.75 g, 88%); IR (CHCl₃): 3125-3119 (m, -NH str, amide), 3071 (w, -CH str, phenoxy ring), 2866-2962 (m, -CH str, asym, CH₃), 2874, 2869, 2851, 2847 (m, -CH str, sym, CH₃ and CH₂), 2836 (m, -CH str, OCH₃), 2824, 2721 (m, -CH str, -CHO), 1744 (m, -C=O str, ester), 1701 (s, -C=O str, -CHO), 1647-1642 (s, -C=O str, 2° amide), 1593, 1482 (m, skeletal bands, phenoxy ring), 1537, 1534 (m, -NH bend, 2° amide), 269 (s, C-O str, ester), 1259 (s, C-O-C str, asym), 1127, 1123 (m, C-Br str), 1066 (s, C-O-C str, sym), 836 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.74 (1H, s, -CHO), 8.23 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 8.12 (1H, br. s, -NH), 7.05 (1H, br. s, -NH), 4.95-4.89 (1H, m, α-H, Ala), 4.68 (2H, s, -OCH₂-), 3.85-3.82 (1H, t, J=8.6 Hz, α-H, Ile), 3.53 (3H, s, OCH₃), 2.03-1.97 (1H, m, β-H, Ile), 2.03-1.97 (2H, m, γ-H, Ile), 1.27-1.25 (3H, d, J=5.15 Hz, α-H's, Ala), 0.97-0.94 (3H, t, J = 7.8 Hz, δ-H's, Ile), 0.88-0.86 (3H, d, J = 5.9 Hz, γ'-H's, Ile) ppm; MASS: *m/z* (relative intensity) 537 (M⁺+1, 2.1), 536 (M⁺, 6.8), 521 (14.2), 505 (44.5), 477 (19.6), 392 (bp, 100), 364 (11.7), 320 (70.2), 292 (35.5), 278 (30.9), 262 (14.8), 234 (7.7), 105

(5.9), 86 (10.9), 79 (6.9), 59 (13.8), 57 (6.8), 44 (13.2), 31 (9.1), 29 (8.2), 15 (5.6); Anal. Calcd. For C₁₉H₂₄Br₂N₂O₆: C, 42.56; H, 4.51; N, 5.22. Found: C, 42.58; H, 4.49; N, 5.23%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl tyrosinyl-phenylalanine methyl ester (I_g)

(Yellow solid, m.p. 197-198°C, Yield 6.68 g, 77.8%); IR (KBr): 3369 (m, -OH str, phenolic), 3129, 3125-3121 (m, -NH str, amide), 3075-3066 (w, -CH str, arom. rings), 2969, 2925-2921 (m, -CH str, asym, CH₃ and CH₂), 2857-2852 (m, -CH str, sym, CH₂), 2827, 2722 (m, -CH str, -CHO), 1742 (m, -C=O str, ester), 1701 (s, -C=O str, -CHO), 1642-1637 (s, -C=O str, 2° amide), 1595-1586, 1489-1482 (m, skeletal bands, arom. rings), 1535, 1531 (m, -NH bend, 2° amide), 1270 (s, C-O str, ester), 1263 (s, C-O-C str, asym), 1224 (s, C-O str, phenolic), 1129, 1123 (m, C-Br str), 1069 (s, C-O-C str, sym), 835, 822, 714, 690 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.72 (1H, s, -CHO), 8.21 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 7.94 (1H, br. s, -NH), 7.16 (1H, t, J=6.2 Hz, p-H, Phe), 7.05-6.96 (4H, m, m-H's, Phe and Tyr), 6.91-6.89 (2H, dd, J=8.45, 5.2 Hz, o-H's, Tyr), 6.86-6.84 (2H, dd, J=8.75, 4.15 Hz, o-H's, Phe), 6.08 (1H, br. s, -NH), 5.95 (1H, br. s, -OH), 4.66 (2H, s, -OCH₂-), 4.59-4.54 (1H, m, α-H, Tyr), 3.96-3.91 (1H, m, α-H, Phe), 3.57 (3H, s, OCH₃), 2.87-2.79 (4H, m, β-H's, Phe and Tyr) ppm; Anal. Calcd. For C₂₈H₂₆Br₂N₂O₇: C, 50.78; H, 3.96; N, 4.23. Found: C, 50.76; H, 3.99; N, 4.22%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl prolyl-proline methyl ester (I_h)

(Semisolid mass, Yield 4.45 g, 85%); IR (CHCl₃): 3079 (w, -CH str, phenoxy ring), 2999-2986 (m, -CH str, cyclic CH₂ and CH), 2856 (m, -CH str, sym, CH₂), 2832 (m, -CH str, OCH₃), 2822, 2728 (m, -CH str, -CHO), 1746 (m, -C=O str, ester), 1705 (s, -C=O str, -CHO), 1675, 1669 (s, -C=O str, 3° amide), 1597, 1485 (m, skeletal bands, phenoxy ring), 1269 (s, C-O str, ester), 1261 (s, C-O-C str, asym), 1128, 1122 (m, C-Br str), 1068 (s, C-O-C str, sym), 839 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 192.2 (-C=O, -CHO), 172.9 (-C=O, ester), 169.4 (-C=O, -OCH₂CO-), 168.1 (-C=O, Pro-1), 162.4 (C₁, phenoxy ring), 139.9 (C₄, phenoxy ring), 135.5 (2C, C₃ and C₅, phenoxy ring), 121.3 (2C, C₂ and C₆, phenoxy ring), 66.9 (-OCH₂-), 59.7, 57.3 (2C, C-α, Pro-1 and Pro-2), 53.9 (-OCH₃), 47.8, 43.6 (2C, C-δ, Pro-1 and Pro-2), 29.8, 27.2 (2C, C-β, Pro-1 and Pro-2), 25.5, 24.7 (2C, C-γ, Pro-2 and Pro-1) ppm;

Anal. Calcd. For $C_{20}H_{22}Br_2N_2O_6$: C, 43.98; H, 4.06; N, 5.13. Found: C, 43.96; H, 4.05; N, 5.15%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl leucyl-alanyl-leucine methyl ester (I_i)

(Yellow crystals, m.p. 189-190°C, Yield 5.7 g, 87%); IR (KBr): 3127, 3123 (m, -NH str, amide), 3068 (w, -CH str, phenoxy ring), 2868-2963 (m, -CH str, asym, CH₃), 2873-2868, 2853, 2846 (m, -CH str, sym, CH₃ and CH₂), 2825, 2721 (m, -CH str, -CHO), 1745 (m, -C=O str, ester), 1706 (s, -C=O str, -CHO), 1644-1638 (s, -C=O str, 2° amide), 1592, 1486 (m, skeletal bands, phenoxy ring), 1539, 1533 (m, -NH bend, 2° amide), 1388, 1364 (s, -CH bend, isopropyl group), 1268 (s, C-O str, ester), 1261 (s, C-O-C str, asym), 1127, 1122 (m, C-Br str), 1065 (s, C-O-C str, sym), 836 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.75 (1H, s, -CHO), 8.55 (1H, br. s, -NH), 8.23 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 8.16 (1H, br. s, -NH), 6.77 (1H, br. s, -NH), 4.67 (2H, s, -OCH₂-), 4.42-4.37 (1H, m, α-H, Leu-1), 4.24-4.19 (1H, m, α-H, Ala), 3.62 (3H, s, OCH₃), 3.57-3.53 (1H, m, α-H, Leu-2), 1.81-1.78 (2H, t, *J*=7.85 Hz, β-H's, Leu-1), 1.55-1.43 (2H, m, γ-H's, Leu-1 and Leu-2), 1.29-1.26 (2H, t, *J*=7.9 Hz, β-H's, Leu-2), 1.26-1.24 (3H, d, *J*=5.15 Hz, α-H's, Ala), 0.99-0.97 (6H, d, *J*=6.15 Hz, δ-H's, Leu-1), 0.95-0.93 (6H, d, *J*=6.2 Hz, δ-H's, Leu-2) ppm; Anal. Calcd. For $C_{25}H_{35}Br_2N_3O_7$: C, 46.24; H, 5.43; N, 6.47. Found: C, 46.21; H, 5.45; N, 6.49%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl tryptophanyl-glycyl-histidine methyl ester (I_j)

(Yellow solid, m.p. 240-41°C, Yield 5.88 g, 80%); IR (KBr): 3489, 3485 (m, -NH str, heteroaromatic rings), 3129-3122 (m, -NH str, amide), 3077, 3069 (w, -CH str, arom. rings), 2969, 2923-2917 (m, -CH str, asym, CH₃ and CH₂), 2853-2847 (m, -CH str, sym, CH₂), 2829, 2725 (m, -CH str, -CHO), 1742 (m, -C=O str, ester), 1708 (s, -C=O str, -CHO), 1648-1643 (s, -C=O str, 2° amide), 1595-1589, 1487-1482 (m, skeletal bands, aromatic rings), 1536 (m, -NH bend, 2° amide), 1273 (s, C-O str, ester), 1265 (s, C-O-C str, asym), 1126, 1121 (m, C-Br str), 1065 (s, C-O-C str, sym), 839, 715, 709 (s, -CH bend, oop, aromatic rings) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 193.3 (-C=O, -CHO), 177.9 (-C=O, Gly), 175.8 (-C=O, ester), 173.6 (-C=O, -OCH₂CO-), 169.6 (-C=O, Try), 162.8 (C₁, phenoxy ring), 144.4 (C₂, imz ring), 140.2 (C₄, phenoxy ring), 137.6 (C₂, indole ring), 135.5 (2C, C₃ and C₅,

phenoxy ring), 129.2 (C₃, indole ring), 125.8 (C₄, imz ring), 124.4 (C₂, indole ring), 123.9 (C₅, indole ring), 121.4 (C₆, indole ring), 119.2 (C₄, indole ring), 118.9 (2C, C₂ and C₆, phenoxy ring), 115.5 (C₅, imz ring), 112.2 (C₃, indole ring), 110.8 (C₇, indole ring), 70.5 (-OCH₂-), 59.8 (α-C, Try), 54.2 (α-C, His), 51.1 (-OCH₃), 48.3 (α-C, Gly), 23.6 (β-C, Try), 21.9 (β-C, His) ppm; Anal. Calcd. For $C_{29}H_{28}Br_2N_6O_7$: C, 47.56; H, 3.85; N, 11.47. Found: C, 47.55; H, 3.88; N, 11.45%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl phenylalanyl-tyrosinyl-proline methyl ester (I_k)

(Semisolid mass, Yield = 4.8 g, 95%); IR (CHCl₃): 3366 (m, -OH str, phenolic), 3127, 3122 (m, -NH str, amide), 3069-3065 (w, -CH str, arom. rings), 2998-2986 (m, -CH str, cyclic CH₂ and CH), 2928-2923 (m, -CH str, asym, CH₂), 2855-2851 (m, -CH str, sym, CH₂), 2825, 2720 (m, -CH str, -CHO), 1741 (m, -C=O str, ester), 1704 (s, -C=O str, -CHO), 1677, 1644-1639 (s, -C=O str, 3° and 2° amide), 1593-1587, 1489-1485 (m, skeletal bands, aromatic rings), 1533, 1529 (m, -NH bend, 2° amide), 1269 (s, C-O str, ester), 1261 (s, C-O-C str, asym), 1227 (s, C-O str, phenolic), 1125, 1122 (m, C-Br str), 1066 (s, C-O-C str, sym), 833, 826, 716, 692 (s, -CH bend, out of plane (oop), aromatic rings) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.76 (1H, s, -CHO), 8.24 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 7.99 (1H, br. s, -NH), 7.93 (1H, br. s, -NH), 7.22-7.16 (4H, m, m-H's, Phe and Tyr), 7.02 (1H, t, *J*=6.15 Hz, p-H, Phe), 6.93-6.91 (2H, dd, *J*=8.5, 5.15 Hz, o-H's, Tyr), 6.85-6.83 (2H, dd, *J*=8.7, 4.2 Hz, o-H's, Phe), 5.97 (1H, br. s, -OH), 4.63 (2H, s, -OCH₂-), 4.40-4.35 (2H, m, α-H's, Tyr and Phe), 3.93-3.89 (1H, t, *J*=6.75 Hz, α-H, Pro), 3.65 (3H, s, OCH₃), 3.41-3.38 (2H, t, *J*=7.2 Hz, δ-H's, Pro), 2.94-2.89 (4H, m, β-H's, Phe and Tyr), 2.04-1.96 (4H, m, β- and γ-H's, Pro) ppm; MASS: *m/z* (relative intensity) 760 (M⁺+1, 2.4), 759 (M⁺, 7.3), 744 (15.4), 728 (30.7), 700 (12.3), 631 (bp, 100), 603 (15.7), 468 (69.8), 440 (35.4), 320 (75.4), 292 (36.2), 278 (30.4), 262 (16.2), 234 (8.1), 184 (8.5), 159 (21.7), 136 (13.7), 120 (11.9), 105 (6.3), 93 (7.4), 91 (6.4), 79 (6.4), 77 (8.9), 70 (14.2), 59 (14.5), 31 (9.4), 17 (3.6), 15 (5.1); Anal. Calcd. For $C_{33}H_{33}Br_2N_3O_8$: C, 52.19; H, 4.38; N, 5.53. Found: C, 52.22; H, 4.40; N, 5.52%.

General procedure for preparation of 2-(2,6-dibromo-4-formylphenoxy)acetyl dipeptides and tripeptides (I_{1-n})

To a solution of the peptide methyl ester (0.01 mol) in THF : H₂O (1:1, 36 ml), LiOH (0.36 g, 0.015 mol) was added at 0 °C. The mixture was stirred at RT for 1 h and then acidified to pH 3.5 with 1 N H₂SO₄. The aqueous layer was extracted with Et₂O (3 × 20 ml). The combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was crystallized from methanol and ether to get title compounds.

2-(2,6-Dibromo-4-formylphenoxy)acetyl prolyl-proline (I_i)

(Semisolid mass, Yield 4.75 g, 89.3%); IR (CHCl₃): 3323-2535 (m/br, -OH str, -COOH), 3076 (w, -CH str, phenoxy ring), 2997-2985 (m, -CH str, cyclic CH₂ and CH), 2855 (m, -CH str, sym, CH₂), 2835 (m, -CH str, OCH₃), 2821, 2729 (m, -CH str, -CHO), 1713 (m, -C=O str, -COOH), 1702 (s, -C=O str, -CHO), 1676, 1664 (s, -C=O str, 3° amide), 1595, 1487 (m, skeletal bands, phenoxy ring), 1427 (m, C-O-H bend, -COOH), 1265 (s, C-O-C str, asym), 1126, 1120 (m, C-Br str), 1069 (s, C-O-C str, sym), 836 (s, -CH bend, oop, phenoxy ring) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 191.9 (-C=O, -CHO), 180.4 (-C=O, -COOH), 169.7 (-C=O, -OCH₂CO-), 165.5 (-C=O, Pro-1), 161.8 (C₁, phenoxy ring), 139.2 (C₄, phenoxy ring), 135.9 (2C, C₃ and C₅, phenoxy ring), 120.6 (2C, C₂ and C₆, phenoxy ring), 67.3 (-OCH₂-), 59.9, 58.5 (2C, C-α, Pro-1 and Pro-2), 46.6, 43.8 (2C, C-δ, Pro-1 and Pro-2), 29.6, 28.4 (2C, C-β, Pro-1 and Pro-2), 26.9, 23.7 (2C, C-γ, Pro-2 and Pro-1) ppm; Anal. Calcd. For C₁₉H₂₀Br₂N₂O₆: C, 42.88; H, 3.79; N, 5.26. Found: C, 42.85; H, 3.81; N, 5.29%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl tryptophanyl-glycyl-histidine (I_m)

(Yellow crystals, m.p. 199-201°C, Yield 6.72 g, 93.6%); IR (KBr): 3488, 3483 (m, -NH str, heteroarom. rings), 3327-2539 (m/br, -OH str, -COOH), 3132-3126 (m, -NH str, amide), 3071, 3065 (w, -CH str, arom. rings), 2965, 2922-2915 (m, -CH str, asym, CH₃ and CH₂), 2851-2845 (m, -CH str, sym, CH₂), 2826, 2722 (m, -CH str, -CHO), 1714 (m, -C=O str, -COOH), 1701 (s, -C=O str, -CHO), 1649-1644 (s, -C=O str, 2° amide), 1592-1585, 1488-1484 (m, skeletal bands, arom. rings), 1534 (m, -NH bend, 2° amide), 1425 (m, C-O-H bend, -COOH), 1267 (s, C-O-C str, asym), 1123, 1119 (m, C-Br str), 1069 (s, C-O-C str, sym), 837, 711, 707 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹³C NMR (CDCl₃, 300 MHz): δ 193.1 (-C=O, -CHO), 178.4 (-C=O, -COOH), 177.6 (-C=O, Gly), 173.3 (-C=O, -OCH₂CO-), 168.9 (-C=O, Try),

162.4 (C₁, phenoxy ring), 143.9 (C₂, imz ring), 139.6 (C₄, phenoxy ring), 137.2 (C₂, indole ring), 135.7 (2C, C₃ and C₅, phenoxy ring), 129.2 (C₄, imz ring), 128.8 (C₃, indole ring), 123.8 (C₂, indole ring), 122.7 (C₅, indole ring), 120.9 (C₆, indole ring), 119.0 (C₄, indole ring), 118.3 (2C, C₂ and C₆, phenoxy ring), 115.8 (C₅, imz ring), 111.7 (C₃, indole ring), 110.5 (C₇, indole ring), 69.9 (-OCH₂-), 58.2 (α-C, Try), 53.8 (α-C, His), 48.1 (α-C, Gly), 22.7 (β-C, Try), 21.3 (β-C, His) ppm; MASS: *m/z* (rel. int.) 719 (M⁺+1, 0.6), 718 (M⁺, 4.9), 701 (12.8), 673 (10.2), 564 (55.9), 536 (61.4), 507 (bp, 100), 479 (47.3), 320 (79.3), 292 (38.5), 278 (29.9), 262 (15.7), 234 (7.8), 184 (9.5), 159 (22.4), 130 (16.2), 116 (8.1), 110 (19.5), 105 (6.3), 81 (16.8), 79 (6.7), 67 (5.9), 45 (9.5), 30 (11.6), 17 (4.2); Anal. Calcd. For C₂₈H₂₆Br₂N₆O₇: C, 46.82; H, 3.65; N, 11.70. Found: C, 48.79; H, 3.66; N, 11.68%.

2-(2,6-Dibromo-4-formylphenoxy)acetyl phenylalanyl-tyrosinyl-proline (I_n)

(Semisolid mass, Yield 6.8 g, 91.3%); IR (CHCl₃): 3369 (m, -OH str, phenolic), 3326-2532 (m/br, -OH str, -COOH), 3128, 3121 (m, -NH str, amide), 3067-3063 (w, -CH str, arom. rings), 2999-2983 (m, -CH str, cyclic CH₂ and CH), 2926-2922 (m, -CH str, asym, CH₂), 2857-2853 (m, -CH str, sym, CH₂), 2823, 2722 (m, -CH str, -CHO), 1711 (m, -C=O str, -COOH), 1702 (s, -C=O str, -CHO), 1673, 1647-1638 (s, -C=O str, 3° and 2° amide), 1591-1588, 1487-1482 (m, skeletal bands, arom. rings), 1532, 1527 (m, -NH bend, 2° amide), 1424 (m, C-O-H bend, -COOH), 1259 (s, C-O-C str, asym), 1229 (s, C-O str, phenolic), 1126, 1121 (m, C-Br str), 1065 (s, C-O-C str, sym), 831-825, 712, 695 (s, -CH bend, oop, arom. rings) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 9.74 (1H, s, -CHO), 8.26 (2H, br. s, -OH and -COOH), 8.19 (2H, singlet overlapped over singlet, m-H's, phenoxy ring), 7.98 (1H, br. s, -NH), 7.94 (1H, br. s, -NH), 7.20-7.15 (4H, m, m-H's, Phe and Tyr), 7.06 (1H, t, *J*=6.2 Hz, p-H, Phe), 6.91-6.89 (2H, dd, *J*=8.45, 5.2 Hz, o-H's, Tyr), 6.84-6.82 (2H, dd, *J*=8.65, 4.15 Hz, o-H's, Phe), 4.95-4.90 (1H, m, α-H, Tyr), 4.65 (2H, s, -OCH₂-), 4.39-4.34 (1H, m, α-H, Phe), 4.10-4.07 (1H, t, *J*=6.7 Hz, α-H, Pro), 3.38-3.35 (2H, t, *J*=7.15 Hz, δ-H's, Pro), 2.95-2.89 (4H, m, β-H's, Phe and Tyr), 2.05-1.97 (4H, m, β- and γ-H's, Pro) ppm; Anal. Calcd. For C₃₂H₃₁Br₂N₃O₈: C, 51.56; H, 4.19; N, 5.64. Found: C, 51.53; H, 4.22; N, 5.65%.

Biological activity studies

Antibacterial screening

In present study, modified Kirby-Bauer disc diffusion method (Bauer *et al* 1966) was utilized for the evaluation of antibacterial activity against two gram-negative and two gram-positive bacteria. MIC values of test compounds were determined by tube dilution technique. All the synthesized compounds were dissolved separately to prepare a stock solution of 1 mg/ml using DMF. Stock solution was aseptically transferred and suitably diluted with sterile broth medium to have seven different concentrations of each test compound ranging from 200 to 3.1 $\mu\text{g/ml}$ in different test tubes. All the tubes were inoculated with one loopful of one of the test bacteria. 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 five-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 ciprofloxacin and a disk impregnated with the solvent (DMF) as negative control. Test sample and reference drugs were tested at the concentration of 10 $\mu\text{g/ml}$. The petri plates inoculated with bacterial cultures were incubated at 37°C for 18 h. Diameters of the zones of inhibition (mm) were measured and the average diameters for test sample were calculated in triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug.

Antifungal screening

Serial plate dilution method (Khan, 1997) was employed for the evaluation of antifungal activity against three fungal strains, including dermatophytes. 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 and negative control (DMSO) were placed in labeled wells in each petri plate. Test samples and reference drug, griseofulvin were tested at 10 $\mu\text{g/ml}$ concentration. 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 griseofulvin as standard drug.

RESULTS AND DISCUSSION

Synthesis of all newly synthesized compounds was accomplished with good yields. All the synthesized compounds were found to exhibit moderate to good antimicrobial activity against pathogenic microorganisms. DIPC was found to be a good coupling agent both economically as well as yield wise. IR spectra of synthesized compounds showed characteristic amide I and amide II bands of the $-\text{CO}-\text{NH}-$ moiety and MS spectra showed molecular ion peak (M^+) at m/z values which were in consistent with the molecular formulas. Comparison of the antimicrobial activity data suggested that dipeptide ester derivatives are more potent antibacterial agents than amino acid methyl ester derivatives and tripeptide ester derivatives whereas tripeptide derivatives are more potent antifungal agents than amino acid and dipeptide ester derivatives. Furthermore, derivatives of amino acids and peptides were found to be more potent antimicrobial agents than their corresponding ester derivatives.

CONCLUSION

Among synthesized peptide analogs, compounds $I_{g,k}$ and I_n were found to be more potent than standard drug - ciprofloxacin, against gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. Compounds $I_{c,h}$ and I_l were found to be more potent than standard drug - griseofulvin, against pathogenic fungus *Candida albicans*. In addition, I_j and I_m displayed potent bioactivity against pathogenic dermatophytes.

On passing toxicity tests, these peptide derivatives may prove good candidate for

clinical studies and can be new antibacterial and antifungal agents of future.

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