

**SYNTHESIS AND BIOLOGICAL
SCREENING OF NOVEL
ARYLOXYACETIC ACID
ANALOGS**

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ABSTRACT:

A series of 5-Bromo- 2-formyl phenoxyacetyl amino acids and peptides have been synthesized by coupling of the 5-Bromo- 2-formyl phenoxyacetic acid with amino acid/methyl esters/dipeptides/ tripeptides using DCC as coupling agent and NMM as base. The structures were elucidated FTIR and ¹HNMR .The newly synthesized compounds were evaluated for their antibacterial, antifungal and anthelminitic activities. The compounds (**2, 6, 11 and 13**) were found to exhibit potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* (gram positive bacteria) and *Escherichia coli* (gram negative) bacterias. The compounds (**5, 6 and 13**) were found to exhibit potent antifungal activity against *Candida albicans* and *Aspergillus niger*. The moderate to good anthelminitic activity was shown by the synthesized compounds (**7 and 13**) against *Eudrilus* species.

Keywords: Phenoxyacetic acid, amino acids, antibacterial, antifungal and anthelminitic.

Phenoxyacetic acid is among the most vital moieties which are associate with potent antidiabetic (Rival et al 2004), antimycobacterial (Yar et al 2007), diuretic (Lebedev et al 1985, Woltersdorf et al 1976, Bicking et al 1976), anti-inflammatory (Kunsch et al 2005, Shokol et al 2005), antibiotic (Grardin et al 1995), anti-obesity (Kiso et al 1999), diagnostic (Ohmomo et al 1989), inhibition of platelet aggregation (Meanwell et al 1993, Seiler et al 1994) activities. The review of literature has suggested that incorporation of amino acids and peptides into aromatic and heterocyclic congeners have resulted in compounds with potent bioactivities.

Introducing an amino acid or peptide into aromatic compounds can increase the potency, decrease the toxicity and prolong its action. Among aromatics, phenolic compounds have wide range of activities. Further phenoxylation the resulting compound phenoxyacetic acid is obtained, which is well known for their biological potential. Thus keeping in view the biological potency of phenoxyacetic acids as well as taking advantage of biodegradability and biocompatibility of a novel series of substituted phenoxyacetic acid derivatives of amino acids and peptides have been synthesized with an anticipation to get potent agents with

good therapeutic efficacy with negligible side effects.

The study of IR and ¹HNMR spectrum gives us most of the required information of certain vibrational bands or characteristic groups present in the molecule. The IR spectrum of newly synthesized compounds showed characteristic bands in the region 3329-3318, 1654-1642, 1537-1532 cm⁻¹ which can be assigned as N-H stretching, C=O stretching and N-H bend respectively. In their ¹HNMR spectra, a singlet appeared in the range 8.33-8.36 ppm corresponding to the CO-NH proton.

The compounds were found to exhibit potent antimicrobial and moderate anthelmintic activity in comparison to standard drugs against the same concentration.

Results and discussion

Antibacterial Activity:

The synthesized peptide derivatives were screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* using modified Kirby-Bauer disc diffusion method (DMF as a solvent). The test samples were tested at the concentrations 25, 50, 100 µg/ml. The petri plates inoculated with bacterial cultures were incubated at 37°C for 18 hrs. The diameters obtained for the test sample were compared with that produced by the standard drug ciprofloxacin. The results are shown in Table 2.

Antifungal Activity:

The synthesized peptide derivatives were screened for antifungal activity against *Candida albicans* and *Asperigillus niger*. DMSO is used as negative control. The test samples were tested at the concentrations 25, 50, 100 µg/ml. The petri plates inoculated with fungal cultures were incubated at 25°C for 48 hrs. Diameters of the zone of inhibition were calculated in triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug griseofulvin. The results are shown in Table 2.

Anthelmintic Activity:

The anthelmintic activity was carried out against earthworms *Eudrilus* species by Garg and Atal method at 2 mg/ml concentration. Suspension of samples was prepared by triturating synthesized cyclic peptide (200 mg) with Tween 80 (0.5 %) and distilled water. Suspension of the standard drug albendazole was prepared with the same concentration in a similar way. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50°C) which stimulated the movement. The results were shown in Table 3.

The results of biological activities revealed that newly synthesized peptide derivative 73 at 50µg/ml concentration exhibited highest zone of inhibition against *Staphylococcus aureus* and 75 at 50µg/ml concentration exhibited highest zone of inhibition against *Candida albicans*. Moreover, other compounds

showed moderate antimicrobial activities against tested organisms. Comparison of anthelmintic activity data revealed that peptide derivative 75 was found to exhibit potent anthelmintic activity and other peptide derivatives showed good to moderate activity.

Experimental

Melting points were determined and uncorrected. The amino acids, di-tert-butyl pyrocarbonate (Boc_2O), 5-Bromosalicylaldehyde, DCC and NMM were obtained from Spectrochem Limited, Himedia laboratories Limited Mumbai and Sd-fine-chem Limited, Mumbai, India. The IR spectra were recorded on a Perkin Elmer Fourier transform infrared spectrophotometer using KBr pellets. The ^1H NMR spectra were recorded on the Bruker Avance II-400 NMR spectrometer using CDCl_3 as the solvent. The purity of all the compounds was controlled by TLC on silica gel G plates. Chloroform:Methanol (9:1 v/v) was used as developing solvent system and dark brown spots were detected on exposure to iodine vapours in a tightly closed chamber. The physical data of synthesized compounds is listed in Table 1. The scheme of synthesis is given in Scheme 1.

Synthesis of Boc amino acids (1-3):

L-Leucine (1.31gm, 10mmol) was dissolved in 10 ml of sodium hydroxide (1 mol L^{-1}) and 10 ml of i-propanol. di-tert.butylpyrocarbonate (3 ml, 13 mmol) in 5 ml of i-propanol was added followed by 10 ml of sodium hydroxide (1 mol L^{-1}) to the resulting solution. The

solution was stirred at room temperature for 2 hr, washed with 10 ml of light petroleum ether (b.p. $40\text{-}60^\circ\text{C}$), acidified to p H 3.0 with 1 mol L^{-1} sulphuric acid and finally extracted with chloroform (3 x 20 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure to give crude product. The crude product was purified by recrystallization from methanol and ether at 0°C to get pure Boc-Leucine (1). Similarly, Boc-Serine (2) and Boc-Alanine (3) were prepared by stirring di-tert.butylpyrocarbonate (3 ml, 13 mmol) with Boc-Serine (1.05gm, 10 mmol) and Boc-Alanine (0.89gm, 10 mmol) respectively.

Synthesis of L-amino acid methyl ester hydrochlorides (4-6) :

Thionyl chloride (0.73mL, 10 mmol) was slowly added to methanol (50 mL) at 0°C and 1.15 gm of L- Proline (10 mmol) was added to the above solution. The resulting mixture was refluxed for 9 hrs at 110°C . Methanol was evaporated and the residue was triturated with ether at 0°C until excess dimethyl sulphite was removed. The crude product was purified by recrystallization from methanol and ether at 0°C to get L-proline methyl ester hydrochloride (4). Similarly, L-leucine methyl ester hydrochloride (5) and L-tryptophan methyl ester hydrochloride (6) was prepared by refluxing 1.31 gm of L-leucine (10 mmol) and 2.04 gm of L-tryptophan with 50 ml methanol in the

presence of 0.73 ml of thionyl chloride (10 mmol).

Synthesis of Boc-dipeptide methyl esters (7-9) :

To a mixture of 1.65 gm compound **4** (10 mmol) in 20 ml of chloroform, 2.3 ml of N- methylmorpholine (21mmol) was added at 0°C. The reaction mixture was stirred for 15 min. 2.31gm compound **1** (10mmol) in 20 ml chloroform and 2.1gm of DCC (10mmol) were added under stirring to the above mixture. After 36 hrs, the reaction mixture was filtered and the residue was washed with 30 ml of chloroform and added to the filtrate. The filtrate was washed with 5% sodium hydrogen carbonate and saturated sodium chloride solution (25 ml each). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and petroleum ether (b.p. 40-60°C) followed by cooling at 0°C to get Boc-Leu-Pro-OMe (**7**). Similarly Boc-Ser-Leu-OMe (**8**) and Boc-Ala-Pro-OMe (**9**) were prepared by stirring compounds **2** and **3** with amino acid methyl ester hydrochlorides **5** and **4**, respectively in the presence of DCC and NMM.

Deprotection of dipeptides at carboxyl end (8a, 9a) :

To a solution of 3.32 gm of compound **8** (10 mmol) in 36 ml of THF/H₂O (1:1), 0.36 gm lithium hydroxide (15mmol) was added at 0°C. The mixture was stirred at room temperature for 1 hr, and

acidified to ¹ pH 3.5 with 0.5 mol L⁻¹ H₂SO₄. The aqueous layer was extracted with diethyl ether (3 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was recrystallized from methanol and ether to get Boc-Ser-Leu-OH (**8a**). Similarly compound **9** was hydrolyzed under alkaline conditions to obtain Boc-Ala-Pro-OH (**9a**).

Deprotection of dipeptide at amino end (7a):

Compound **7** (3.42 gm, 10mmol) was dissolved in 15 ml of chloroform and treated with 2.28 gm of trifluoroacetic acid (20 mmol). The resulting solution was stirred at room temperature for 1 hr and washed with 25 ml of saturated sodium hydrogen carbonate solution. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by recrystallization from mixture of chloroform and light petroleum ether (b.p. 40-60°C) to get pure Leu-Pro-OMe (**7a**).

Synthesis of Boc-tripeptide methyl esters (10, 11) :

To synthesize Boc-Ser-Leu-Trp-OMe (**10**), 3.18 gm of dipeptide unit **8a** (10 mmol) was coupled with 2.54 gm of amino acid methyl ester hydrochloride **6** (10 mmol) in the presence of DCC and NMM following the same procedure as adopted for the synthesis of Boc-dipeptide methyl esters 7-9. Similarly Boc-Ala-Pro-Pro-OMe (**11**) was

prepared by coupling 2.86 gm of deprotected dipeptide unit 9a and 1.65 gm of amino acid methyl ester hydrochloride 4 using DCC as the coupling agent and NMM as the base.

Deprotection of tripeptides at amino end (10a, 11a):

Compound **10** (5.18 gm, 10mmol) was dissolved in 15 ml of chloroform and treated with 2.28 gm of trifluoroacetic acid (20 mmol). The resulting solution was stirred at room temperature for 1 hr and washed with 25 ml of saturated sodium hydrogen carbonate solution. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by recrystallization from mixture of chloroform and light petroleum ether (b.p. 40-60°C) to get pure Ser-Leu-Trp-OMe (**10a**). Similarly Ala-Pro-Pro-OMe (**11a**) was prepared by stirring compound **11** with 2.28 gm of trifluoroacetic acid (20 mmol).

Synthesis of free acid

Sodium hydroxide (0.89gm, 22.4mmol) in 25 ml water was slowly added with stirring to 2.01gm of 5-bromo-2-hydroxyaldehyde (10mmol) and 0.94gm of chloroacetic acid (10mmol). The mixture was heated on heating mantle to remove all the liquid and the residue was treated with 30 ml water. The mixture was cooled and filtered and clear solution was acidified with dilute hydrochloric acid. The aqueous layer was extracted with diethyl ether (2 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate.

The crude product was recrystallized from ethanol and purified by recrystallization from ethanol-water (1:1) to get 5-Bromo-2-formyl-phenoxyacetic acid (**12**).

Synthesis of 5-Bromo-2-formyl-phenoxyacetyl amino acid and peptide methyl esters

L-Proline methyl ester hydrochloride (1.65 gm, 10mmol) was dissolved in Tetrahydrofuran (75 mL). To this, 2.3 ml of N-methylmorpholine (21mmol) was added at 0°C and the reaction mixture was stirred for 15 min. 2.01gm of compound **12** (10mmol) in tetrahydrofuran and 2.1gm dicyclohexylcarbodiimide (10mmol) were added under stirring to the above mixture. After 36 hrs, the reaction mixture was filtered and the residue was washed with 30 ml of tetrahydrofuran and added to the filtrate. The filtrate was washed with 5% NaHCO₃ and saturated NaCl solution (25 ml each). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated in vacuum. The crude product was recrystallized from mixture of chloroform and n-hexane followed by cooling at 0°C to get 5-Bromo-2-formyl-phenoxyacetyl-proline methyl ester (**13**). Similarly, 5-Bromo-2-formyl-phenoxyacetyl-leucyl-proline methyl ester (**14**), 5-Bromo-2-formyl-phenoxyacetyl-seryl-leucyl-tryptophan methyl ester (**15**) and 5-Bromo-2-formyl-phenoxyacetyl-alanyl-prolyl-proline methyl ester (**16**) were prepared by stirring compound **7a**, **10a** and **11a**

with compound **12** respectively in the presence of DCC and NMM.

Deprotection of 5-Bromo-2-formyl-phenoxyacetyl-alanyl-prolyl-proline methyl ester at carboxyl end (16a) :

To a solution of 4.39 gm of compound **16** (10 mmol) in 36 ml of THF/H₂O (1:1), 0.36 gm lithium hydroxide (15mmol) was added at 0°C. The mixture was stirred at room temperature for 1 hr, and acidified to ¹ pH 3.5 with 0.5 mol L H₂SO₄. The aqueous layer was extracted with diethyl ether (3 x 25 ml). Combined organic extracts were dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was recrystallized from methanol and ether to get 5-Bromo-2-formyl-phenoxyacetyl-alanyl-prolyl-proline (**16a**).

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Table 1 : Physical data of the synthesized compounds

Compound No.	Physical state	Yield (%)	M.P (°C)	R _F	Molecular formula
12.	Pale yellow solid	74.0	86-87	0.81	C ₉ H ₇ O ₄ Br
13.	Brown solid	68.2	72-75	0.94	C ₁₅ H ₁₆ NO ₅ Br
14.	Semisolid mass	70.7	-	0.61	C ₂₁ H ₂₇ N ₂ O ₆ Br
15.	Semisolid mass	63.2	-	0.62	C ₃₀ H ₃₅ N ₄ O ₈ Br
16.	Semisolid mass	74.3	-	0.78	C ₂₃ H ₂₈ N ₃ O ₇ Br
16a.	Yellow brown solid	67.3	73-75	0.80	C ₂₂ H ₂₆ N ₃ O ₇ Br

Table 2: Antimicrobial activity data of synthesized compounds

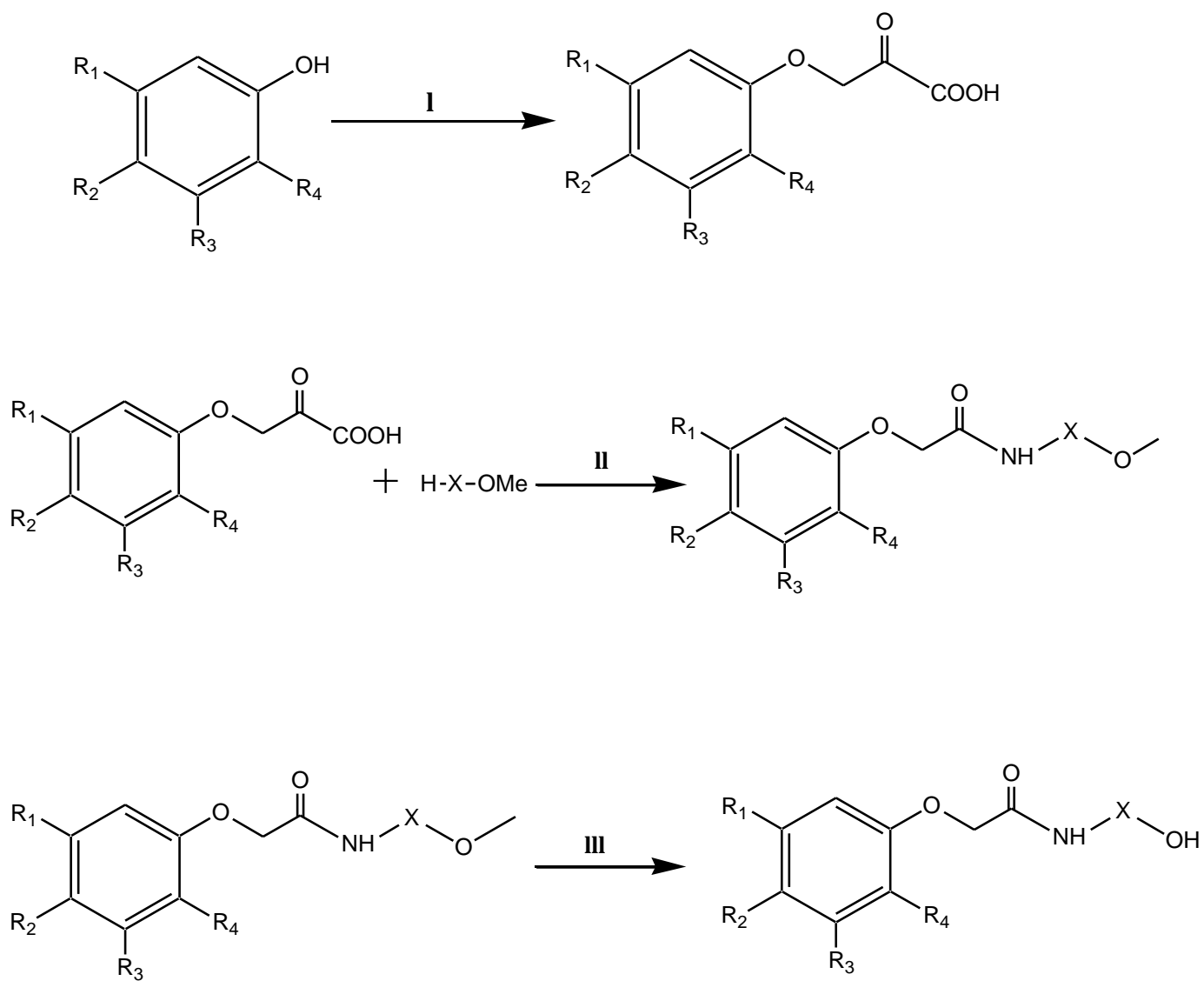
Compound No.	Zone of inhibition in mm				
	Bacterial strains			Fungal strains	
	<i>B. subtilus</i>	<i>S. aureus</i>	<i>E.coli</i>	<i>C. albicans</i>	<i>A. niger</i>
12	13.00	12.67	10.33	14.33	13.33
13	9.33	5.33	11.67	11.67	5.67
14	10.30	10.33	10.67	6.33	6.67
15	12.67	20.67	11.33	8.00	13.33
16	17.30	12.67	10.67	16.67	11.33
16a	20.00	13.67	11.33	20.33	14.67
Control	-	-	-	-	-
Ciprofloxacin	17.67	17.33	18.33	-	-
Griseofulvin	-	-	-	16.67	15.33

Table 3: Anthelmintic activity data of synthesized compounds

<i>Eudrilus species</i>		
Compound No.	Mean paralyzing time	Mean death time
12	14.15±1.62	28.18±1.52
13	15.54±0.28	30.87±0.24
14	11.73±0.90	23.82±1.34
15	10.32±0.51	21.64±2.63
16	9.66±0.34	18.13±1.00
16a	4.67±0.43	9.72±0.83
Control	-	-
Albendazole	10.89±0.48	22.78±0.56

Table 4: Spectral data of synthesized compounds

Compound No.	IR (cm ⁻¹)	¹ HNMR δ (ppm)
12.	3310 (-OH, str), 1719 (-COOH, str), 1305 (-C-O, str), 1247 (C-O-C, asym), 1070 (C-O-C, sym)	10.92 (1H, s, -OH), 4.50 (2H, s, -OCH ₂)
13.	3329 (-NH, str), 1654 (-C=O, str), 1537 (-NH, bend)	-
14.	3322 (-NH, str), 1648 (-C=O, str), 1533 (-NH, bend)	8.33 (2H, s, -CO-NH)
15.	3325 (-NH, str), 1653 (-C=O, str), 1534 (-NH, bend)	8.36 (3H, s, -CO-NH)
16.	3318 (-NH, str), 1642 (-C=O, str), 1532 (-NH, bend)	8.31 (3H, s, -CO-NH)
16a	3328 (-OH, str), 1718 (-COOH, str), 1326 (-C-O, str)	10.75 (1H, s, -OH)



Scheme

$R_1 = Br$; $R_2 = H$; $R_3 = H$; $R_4 = CHO$ (**12-16a**)

I = $ClCH_2COOH$, $NaOH$, RT 1hr, **II** = DCC , NMM , RT 36hrs, **III** = $LiOH$, $THF:H_2O$ (1:1) RT 1hr

$X = Pro$ (**13**), $Leu-Pro$ (**14**), $Ser-Leu-Trp$ (**15**), $Ala-Pro-Pro$ (**16, 16a**)