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Synthesis and biological activity of some new leucine-enkephalin analogues

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Abstract. The opioid pentapeptide leucine-enkephalinamide and eleven of its analogues have been synthesised by the solid phase technique employing mostly 9-fluoroenylmethyloxycarbonyl amino acid active esters in the presence of 1-hydroxybenzotriazole. Both the conventional chloromethylated copolystyrene-2% divinylbenzene resin as well as *p*-alkoxybenzyl alcohol resin were employed and it was observed that yields were uniformly better with the latter resin. The analogues were made by affecting single or multiple replacements of amino acids involving positions 1,2 and 5. Some of the analogues were found to be more potent than morphine in the guinea pig ileum assay.

Keywords. Fmoc-amino acid active esters; *p*-alkoxylbenzyl alcohol resin; merrifield resin; enkephalin analogues; synthesis; activity.

Introduction

The importance of Tyr^1 in the opioid pentapeptides leucine- and methionine-enkephalins, Tyr^1-Gly^2-Gly^3-Phe^4-Leu/Met^5, has been well recognised (Morley, 1980). Its replacement by other amino acids generally leads to loss of biological activity. Further, the shortening of the side chain which happens when it is replaced by L-*p*-hydroxyphenylglycine resulted in virtual loss of activity (Dutta *et al.,* 1977). The consequences of replacing Tyr^1 by D-*p*-hydroxyphenylglycine have now been investigated. Substitution of Gly^2 in the enkephalins by D-amino acids generally leads to enhanced activity (Morley, 1980); hence, 5 analogues with D-amino acids in this position have been made by us. In two other analogues D-amino acids replace both Tyr^1 and Gly^2. Three more analogues have been prepared by replacement of Gly^2 and Leu/Met^5.

Experimental

All the amino acids used, except glycine, are of L-configuration unless otherwise specified. Melting points were determined using Leitz-Wetzlar melting point apparatus

Abbreviations used: Fmoc, 9-Fluorenylmethyloxycarbonyl; OTcp, trichlorophenyl ester; HOBt, 1-hydroxybenzotriazole; D-Gly(*p*-OH-phenyl), *p*-hydroxyphenylglycine; Nle, norleucine; AcOH, acetic acid; NEt_3, triethylamine; Et_2 NH, diethylamine; DMF, dimethylformamide.

and are uncorrected. Thin layer chromatography was carried out on silica gel G plates using the solvent system, chloroform: methanol: acetic acid (40:5:5), and the R_f value is designated as R_f(CMA).Fmoc-amino acids were prepared by the method of Chang *et al.* (1980). Fmoc-amino acid active esters were prepared by methods similar to those used for the corresponding *tert*-butyloxycarbonyl amino acids. The coupling and deprotection steps during the synthesis were monitored by Kaiser's test (Kaiser *et al.*, 1970).

Fmoc -Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-*resin*

Fmoc-Leu (7·06g, 20 mmol) was reacted with p-alkoxybenzyl alcohol resin (12 g, prewashed with dichloromethane) using dicyclohexylcarbodiimide (4·12 g, 20 mmol) and *p*-dimethylaminopyridine (2·45 g, 20 mmol) in 4 : 1 dichloromethane/dimethyl formamide (240 ml) according to Chang's procedure (Chang *et al.,* 1980) to afford the title peptide resin; yield 13·5 g; leucine content: 0·41 mmol/g of resin (Meienhofer *et al.,* 1979)

Fmoc-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-*resin*

Fmoc-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (13·5 g) was subjected to the following cycles using 120 ml solvent for each operation.

CH_2Cl_2 Wash, 3×2 min.
Deblocking with 55% piperdine in CH_2Cl_2, 1 × 40 min.
CH_2Cl_2 wash, 3 × 2 min.
DMF wash, 2 ×2 min.
Dioxane: Water (2:1) wash, 3 × 5 min.
DMF wash, 2 × 2 min.
CH_2Cl_2 wash, 2×2 min.
DMF wash, 2×2 min.
Coupling with Fmoc-Phe-OTcp (3 equiv.)/HOBt (1 equiv.) in DMF (45 ml), 60 min.
DMF wash, 2×2 min.
Isopropanol wash, 2×2 min.

Fmoc-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-*resin*

The procedure described in the previous preparation was repeated to introduce the glycine unit using Fmoc-Gly-OTcp (7·9 g, 16·6 mmol) and 1-hydroxybenzotriazole (0·74 g, 5·5 mmol) in DMF (45 ml).

BOC-Tyr-Gly-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (I), BOC-Tyr-D-Ala-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (II), BOC-Tyr-D-Val-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (III), BOC-D-Gly(p-OH-phenyl)-Gly-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (IV), BOC-D-Gly(p-OH-phenyl)-D-Ala-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (V), and BOC-D-Gly(p-OH-phenyl)-D-Val-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (VI)

Using portions of the tripeptide resin, Fmoc-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-

C_6H_4-Resin, and performing the same cycle of operations that was employed for the introduction of Gly and Phe but using appropriate amino acid active esters, the protected peptide resins (I to VI) were obtained.

BOC-Leu-OCH_2-*C_6H_4-Resin and* BOC-Nle-OCH_2-C_6H_4-*resin*

To a suspension of Merrifield resin (2 g) in ethanol (8 ml), BOC-Leu (or BOC-Nle) (0·65 g, 2·8 mmol) and triethylamine (0·392 ml, 2·8 mmol) were added and the mixture was stirred slowly at 90° under anhydrous conditions for 24 h. The esterified resin was collected and washed successively with ethanol, water, ethanol, dichloromethane and methanol and dried over P_2O_5 *in vacuo*. Yield 2·35 g. The leucine (and norleucine) content on the resin was estimated to be 0·45mmol/g of resin (Stewart and Young, 1969).

BOC-Tyr-D-Gly(p-OH-phenyl)-Gly-Phe-Leu-OCH_2-C_6H_4-Resin (VII), BOC-Tyr-D-Ser-Gly-Phe-Leu-OCH_2-C_6H_4-Resin (VIII), BOC-Tyr-D-Phe-Gly-Phe-Leu-OCH_2-C_6H_4-Resin (IX), BOC-Tyr-D-Ser-Gly-Phe-Nle-OCH_2-C_6H_4-Resin (X), BOC-Tyr-D-Phe-Gly-Phe-Nle-OCH_2-C_6H_4-Resin (XI), and BOC-Tyr-D-Met-Gly-Phe-Nle-OCH_2-C_6H_4-Resin (XII)

BOC-Leu-OCH_2-C_6H_4-Resin and BOC-Nle-OCH_2-C_6H_4-Resin were taken in separate solid phase vessels and peptide synthesis was carried out using a manual procedure. All washings and reactions were carried out using 10–12 ml portions of solvents per gram of resin. Each cycle of the synthesis consisted of the following operations.

CH_2Cl_2 wash, 2×2min.
DMF wash, 2 × 2 min.
Deprotection with 50% diethylamine in DMF 1 × 120 min. (The BOC group of the first amino acid was deprotected using 1N HCl/AcOH for 30 min., with AcOH (3 × 2 min.) wash preceding and succeeding the deprotection step, and neutralization with 10% NEt_3 -DMF for l0min.)
DMF wash, 2 × 2 min.
Dioxane: Water (2:1) wash, 2× 5 min.
DMF wash, 3×2 min.
CH_2Cl_2 wash, 3×2 min.
DMF wash, 2×2 min.
Coupling with 3 equiv. of Fmoc-amino acid active ester/1 equiv. HOBt in DMF. [Fmoc-D-Ser (3 equiv.) was added in the presence of DCC (2 equiv.)/HOBt (1 equiv.) in DMF. The last amino acid Tyr was incorporated using BOC-Tyr-OTcp (3 equiv.)/HOBt (1 equiv.)]
DMK wash,
Isopropanol wash. } three times successively
CH_2Cl_2 wash, 2 min. each.

After the incorporation of the third amino acid, each of the peptide resins (Fmoc-Gly-Phe-Leu-OCH_2-C_6H_4-Resin and Fmoc-Gly-Phe-Nle-OCH_2-C_6H_4-Resin) was dried and divided into 3 equal parts and the synthesis was continued to incorporate the last two amino acids.

The time required for the completion of coupling of each amino acid is given below.

Amino acid residue	Time in min.
Fmoc-Phe-OTcp	60
Fmoc-Gly-OTcp	60
Fmoc-D-Gly-(p-OH-phenyl)-OTcp	75
Fmoc-D-Ser (DCC/HOBt)	180
Fmoc-D-Phe-OTcp	60
Fmoc-D-Met-OTcp	120
BOC-Tyr-OTcp	75

BOC-Tyr-Gly-Gly-Phe-Leu-NH_2 (XIII), BOC-Tyr-D-Ala-Gly-Phe-Leu-NH_2 (XIV), BOC-Tyr-D-Val-Gly-Phe-Leu-NH_2 (XV), BOC-D-Gly(p-OH-phenyl)-Gly-Gly-Phe-Leu-NH_2 (XVI), BOC-D-Gly-(p-OH-phenyl)-D-Ala-Gly-Phe-Leu-NH_2 (XVII), BOC-D-Gly(p-OH-phenyl)-D-Val-Gly-Phe-Leu-NH_2 (XVIII), BOC-Tyr-D-Gly(p-OH-phenyl)-Gly-Phe-Leu-NH_2 (XIX), BOC-Tyr-D-Ser-Gly-Phe-Leu-NH, (XX), BOC-Tyr-D-Phe-Gly-Phe-Leu-NH_2 (XXI), BOC-Tyr-D-Ser-Gly-Phe-Nle-NH_2 (XXII), BOC-Tyr-D-Phe-Gly-Phe-Nle-NH_2 (XXIII) and BOC-Tyr-D-Met-Gly-Phe-Nle-NH_2 (XXIV)

Each of the protected peptide resins (XIII to XXIV) was allowed to swell in absolute methanol (50 ml/g of resin) for 2 h. The suspension was cooled to –10° and saturated with dry ammonia with stirring. The mixture was stirred for 3 h at –10° and 72 h at 0°. The resin was filtered off and washed with hot methanol. The combined filtrate was evaporated to dryness. The residue was again evaporated with additional amounts of methanol to expel ammonia completely. The residue was purified using ethyl acetate and methanol/ether. The yields of the title peptides are given in the scheme mentioned later.

Tyr-Gly-Gly-Phe-Leu-NH_2 (XXV), Tyr-D-Ala-Gly-Phe-Leu-NH_2 (XXVI), Tyr-D-Val-Gly-Phe-Leu-NH_2 (XXVII), D-Gly(p-OH-phenyl)-Gly-Gly-Phe-Leu-NH_2 (XXVIII), D-Gly(p-OH-phenyl)-D-Ala-Gly-Phe-Leu-NH_2 (XXIX), D-Gly(p-OH-phenyl)-D-Val-Gly-Phe-Leu-NH_2 (XXX), Tyr-D-Gly(p-OH-phenyl)-Gly-Phe-Leu-NH_2 (XXXI), Tyr-D-Ser-Gly-Phe-Leu-NH_2 (XXXII), Tyr-D-Phe-Gly-Phe-Leu-NH_2 (XXXIII), Tyr-D-Ser-Gly-Phe-Nle-NH_2 (XXXIV), Tyr-D-Phe-Gly-Phe-Nle-NH_2 (XXXV) and Tyr-D-Met-Gly-Phe-Nle-NH_2 (XXXVI)

For the removal of BOC group, each of the protected peptides (XIII to XVIII) was dissolved in trifluoroacetic acid (3·0 ml per 100 mg of peptide) and allowed to stand at room temperature for 40 min. Each of the other protected peptides (XIX to XXIV) was dissolved in 98 % formic acid (2·0 ml per l00 mg peptide) in the presence of anisole (10 %, v/v) and allowed to stand at room temperature for 3 h. The acid was removed *in vacuo.* The residue was dissolved in anhydrous methanol and precipitated with dry ether. The hygroscopic solid thus obtained was dissolved in methanol (50 ml for 100 mg peptide) and treated with Amberlite IRA-400 till the solution attained a pH of 7·5. The

resin was filtered off and washed with methanol. The combined filtrate was concentrated under reduced pressure and dried *in vacuo* over P_2O_5 to afford the peptides (XXIV to XXXVI), whose yields are shown in the scheme given later.

The physical constants of the peptides are listed below. (Amino acid analysis and elemental analysis (C, H and N) of the peptides gave satisfactory results).

Peptides		M.P. (°C)	$[\alpha]_D^{25}$ (C 1, DMF)	R_f(CMA)
BOC-Tyr-D-Ala-Gly-Phe-Leu-NH_2	(XIV)	137–139	−14°	0.71
BOC-Tyr-D-Val-Gly-Phe-Leu-NH_2	(XV)	200–202	−19°	0.68
BOC-D-Gly-(p-OH-phenyl)-Gly-Gly-Phe-Leu-NH_2	(XVI)	211–213	−21°	0.66
BOC-D-Gly-(p-OH-phenyl)-D-Ala-Gly-Phe-Leu-NH_2	(XVII)	209–211	−48°	0.69
BOC-D-Gly(p-OH-phenyl)-D-Val-Gly-Phe-Leu-NH_2	(XVIII)	236–239	−42°	0.67
BOC-Tyr-D-Gly(p-OH-phenyl)-Gly-Phe-Leu-NH_2	(XIX)	164–166	−31°	0.53
BOC-Tyr-D-Ser-Gly-Phe-Leu-NH_2	(XX)	156–158	−15°	0.42
BOC-Tyr-D-Phe-Gly-Phe-Leu-NH_2	(XXI)	168–170	−7°	0·59
BOC-Tyr-D-Ser-Gly-Phe-Nle-NH_2	(XXII)	140–142	−16°	0·39
BOC-Tyr-D-Phe-Gly-Phe-Nle-NH_2	(XXIII)	178–180	−9°	0.63
BOC-Tyr-D-Met-Gly-Phe-Nle-NH_2	(XXIV)	183–185	−12°	0.57

Results

For the synthesis of these analogues the solid phase technique (Merrified, 1963) was employed using either the conventional Merrifield resin (chloromethylated copolystyrene-2 % divinylbenzene) or *p*-alkoxybenzyl alcohol resin (Wang, 1973). For building the peptide chain Fmoc-amino acid active esters were generally employed in the presence of 1-hydroxybenzotriazole (Khan and Sivanandaiah, 1976; Sivanandaiah and Gurusiddappa, 1981). However, the first amino acid to be attached to Merrifield resin was a BOC-amino acid and not Fmoc-amino acid, as triethylamine employed for this reaction would effect partial cleavage of Fmoc group. This problem does not arise in the case of *p*-alkoxybenzyl alcohol resin. Consequently, the first amino acid was attached by employing Fmoc-amino acid. For the introduction of the final amino acid in all these analogues, BOC-amino acid active esters were employed. If Fmoc-protection had been employed instead of BOC, there would have been partial deprotection during the next stage, i.e., ammonolysis. The general scheme of synthesis using *p*-alkoxybenzyl alcohol resin is outlined below.

Fmoc- Leu ↓ +HOH_2C-C_6H_4–OCH_2-C_6H_4-Resin
DCC and *p*-dimethylaminopyridine

Fmoc- Leu- OCH_2-C_6H_4 – OCH_2-C_6H_4-Resin
↓ 1) 55% piperidine in CH_2Cl_2
2) Fmoc-Phe-OTcp (3 equiv.) and
HOBt (1 equiv.); 60 min

Fmoc-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin

↓ 1) 55 % piperidine in CH_2Cl_2
2) Fmoc-Gly-OTcp (3 equiv.) and HOBt (1 equiv.); 60 min

Fmoc-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin

↓ 1) 55 % piperidine in CH_2Cl_2
2) Fmoc-Gly-OTcp (3 equiv.) and HOBt (1 equiv.)
or
Fmoc-D-Ala-OTcp (3 equiv.) and HOBt (1 equiv.)
or
Fmoc-D-Val-OTcp (3 equiv.) and HOBt (1 equiv.)

Fmoc-Gly-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin
or
Fmoc-D-Ala-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin
or
Fmoc-D-Val-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin

↓ 1) 55 % piperidine in CH_2Cl_2
2) BOC-Tyr-OTcp (3 equiv.) and HOBt (1 equiv.)
or
BOC-D-Gly(p-OH-phenyl)-OTcp (3 equiv.) and HOBt (1 equiv.)

BOC-Tyr-Gly-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (I)
or
BOC-Tyr-D-Ala-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (II)
or
BOC-Tyr-D-Val-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (III)
or
BOC-D-Gly(*p*-OH-phenyl)-Gly-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (IV)
or
BOC-D-Gly(*p*-OH-phenyl)-D-Ala-Giy-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (V)
or
BOC-D-Gly(*p*-OH-phenyl)-D-Val-Gly-Phe-Leu-OCH_2-C_6H_4-OCH_2-C_6H_4-Resin (VI)

When the conventional Merrifield resin was employed, the following modifications were made in the general scheme. In the first step, BOC-amino acid was reacted with the resin at 90° in the presence of triethylamine and BOC-protection from the product was removed by treatment with 1N HCl in glacial acetic acid. The remaining steps were virtually the same as in the earlier scheme except that the cheaper diethylamine was used for deprotection of Fmoc group instead of the more expensive piperidine. But the duration of deprotection extends to nearly 2h. Further, it was observed that introduction of D-Ser was incomplete with its Fmoc-active ester/HOBt. Therefore, Fmoc-D-Ser in the presence of DCC/HOBt was employed to effect complete reaction. The general scheme is outlined below.

BOC-Leu/BOC-Nle + $ClCH_2$-C_6H_4-Resin

↓ NEt_3

BOC-Leu-OCH_2-C_6H_4-Resin
or
BOC-Nle-OCH_2-C_6H_4-Resin

↓ 1) IN HCl/AcOH
2) Net3
3) Fmoc-Phe-OTcp (3 equiv.) and HOBt (1 equiv.)

Fmoc-Phe-Leu-OCH_2-C_6H_4-Resin
or
Fmoc-Phe-Nle-OCH_2-C_6H_4-Resin

↓ 1) 50% Et_2 NH in DMF, 2h
2) Other steps as in the previous scheme; for duration of coupling see experimental

BOC-Tyr-D-Gly (*p*-OH-phenyl)-Gly-Phe-Leu-OCH_2-C_6H_4-Resin (VII)
or
BOC-Tyr-D-Ser-Gly-Phe-Leu-OCH_2-C_6H_4-Resin (VIII)
or
BOC-Tyr-D-Phe-Gly-Phe-Leu-OCH_2-C_6H_4-Resin (IX)
or
BOC-Tyr-D-Ser-Gly-Phe-Nle-OCH_2-C_6H_4-Resin (X)
or
BOC-Tyr-D-Phe-Gly-Phe-Nle-OCH_2-C_6H_4-Resin (XI)
or
BOC-Tyr-D-Met-Gly-Phe-Nle-OCH_2-C_6H_4-Resin (XII)

($D\text{-}Ser^2$ was introduced using 3 equiv. of Fmoc-D-Ser in the presence of DCC (2 equiv.)/HOBt (1 equiv.)

The protected pentapeptides were then released from the resins by ammonolysis as indicated below.

Peptide Resins (I to XII)

↓ NH3-CH3OH

		Yield (%)
BOC-Tyr-Gly-Gly-Phe-Leu-NH_2	(XIII)	76·4
BOC-Tyr-D-Ala-Gly-Phe-Leu-NH_2	(XIV)	73
BOC-Tyr-D-Val-Gly-Phe-Leu-NH_2	(XV)	75
BOC-D-Gly-(*p*-OH-phenyl)-Gly-Gly-Phe-Leu-NH_2	(XVI)	68
BOC-D-Gly(*p*-OH-phenyl)-D-Ala-Gly-Phe-Leu-NH_2	(XVII)	69
BOC-D-Gly(*p*-OH-phenyl)-D-Val-Gly-Phe-Leu-NH_2	(XVIII)	71
BOC-Tyr-D-Gly-(*p*-OH-phenyl)-Gly-Phe-Leu-NH_2	(XIX)	48
BOC-Tyr-D-Ser-Gly-Phe-Leu-NH_2	(XX)	62
BOC-Tyr-D-Phe-Gly-Phe-Leu-NH_2	(XXI)	51
BOC-Tyr-D-Ser-Gly-Phe-Nle-NH_2	(XXII)	64

BOC-Tyr-D-Phe-Gly-Phe-Nle-NH_2	(XXIII)	52
BOC-Tyr-D-Met-Gly-Phe-Nle-NH_2	(XXIV)	63

(The yields reported are after purification of the crude product).

The BOC group was cleaved from the protected peptides by treatment with either trifluoroacetic acid or formic acid.

Peptides (XIII to XVIII)
↓ 1) Trifluoroacetic acid
↓ 2) Amberlite IRA-400

		Yield (%)
Tyr-Gly-Gly-Phe-Leu-NH_2	(XXV)	70
Tyr-D-Ala-Gly-Phe-Leu-NH_2	(XXVI)	78
Tyr-D-Val-Gly-Phe-Leu-NH_2	(XXVII)	81
D-Gly(*p*-OH-phenyl)-Gly-Gly-Phe-Leu-NH_2	(XXVIII)	75
D-Gly(*p*-OH-phenyl)-D-Ala-Gly-Phe-Leu-NH_2	(XXIX)	72
D-Gly(*p*-OH-phenyl)-D-Val-Gly-Phe-Leu-NH_2	(XXX)	76

Peptides (XIX to XXIV)
↓ 98 % Formic acid-anisole
↓ Amberlite IRA-400

		Yield (%)
Tyr-D-Gly(*p*-OH-phenyl)-Gly-Phe-Leu-NH_2	(XXXI)	81
Tyr-D-Ser-Gly-Phe-Leu-NH_2	(XXXII)	78
Tyr-D-Phe-Gly-Phe-Leu-NH_2	(XXXIII)	82
Tyr-D-Ser-Gly-Phe-Nle-NH_2	(XXXIV)	76
Tyr-D-Phe-Gly-Phe-Nle-NH_2	(XXXV)	81
Tyr-D-Met-Gly-Phe-Nle-NH_2	(XXXVI)	77

The peptides were assayed for their ability to inhibit the electrically induced contractions of the guinea pig ileum (Kosterlitz and Watt, 1964), and their activities are listed below·

Name of the compound		$IC_{50} \times 10^{-9}$ M	Relative potency
Morphine sulphate		4·15	1·00
Tyr-Gly-Gly-Phe-Leu-NH_2	(XXV)	19·40	0·21
Tyr-D-Ala-Gly-Phe-Leu-NH_2	(XXVI)	1·92	2·10
Tyr-D-Val-Gly-Phe-Leu-NH_2	(XXVII)	14·80	0·28
D-Gly(*p*-OH-phenyl)-Gly-Gly-Phe-Leu-NH_2	(XXVIII)	Inactive	0·00
D-Gly-(*p*-OH-phenyl)-D-Ala-Gly-Phe-Leu-NH_2	(XXIX)	Inactive	0·00
D-Gly(*p*-OH-phenyl)-D-Val-Gly-Phe-Leu-NH_2	(XXX)	Inactive	0·00
Tyr-D-Gly(*p*-OH-phenyl)-Gly-Phe-Leu-NH_2	(XXXI)	1·90	2·18
Tyr-D-Ser-Gly-Phe-Leu-NH_2	(XXXII)	15·96	0·26
Tyr-D-Phe-Gly-Phe-Leu-NH_2	(XXXIII)	1·74	2·38
Tyr-D-Ser-Gly-Phe-Nle-NH_2	(XXXIV)	1·33	3·10
Tyr-D-Phe-Gly-Phe-Nle-NH_2	(XXXV)	9·02	0·46
Tyr-D-Met-Gly-Phe-Nle-NH_2	(XXXVI)	0·49	8·44

(Bioassays were performed at the Government College of Pharmacy, Bangalore)

Discussion

The synthetic experiments reveal that superior yields are obtained when Fmoc group is used for N-protection along with *p*-alkoxybenzyl alcohol resin as solid support; further, purification of the final peptides was also easier.

Biological assays prove that replacement of Tyr^1 by D-Gly(*p*-OH-phenyl) residue leads to inactive peptides thereby emphasising the precise requirements of this position, a conclusion drawn by earlier investigators also (Morley, 1980). Further, in conformity with the earlier observation (Morley, 1980) we have found that replacement of Gly^2 by D-amino acids generally leads to increased activity, the exceptions being peptides XXVII and XXXII wherein either the branched amino acid D Val or D-Ser with a side chain alcoholic function is present in this position. However, the presence of a phenolic hydroxyl in the aromatic side chain does not seem to affect the activity (compare activities of XXXI and XXXIII). Though the presence of $D\text{-}Ser^2$ in peptide XXXII leads to decreased activity, the replacement of Leu^5 in this peptide by Nle residue (peptide XXXIV) enhances the activity significantly. The most potent analogue prepared by us is peptide XXXVI in which Gly^2 and Leu^5 have been replaced by D-Met and Nle respectively.

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