A NEW SERIES OF 1-PHENYLPYRAZOLE PEPTIDOMIMETICS

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Abstract: A facile and efficient method for the synthesis of a new series of 1-phenylpyrazole peptidomimetics 6a-f is described. Peptide bond formation between the 1-phenylpyrazole unsaturated carboxylic acid and the amino acid residue has been achieved using benzotriazolyloxy-tris-(dimethylamino) phosphonium hexafluorophosphate (BOP) reagent and triethylamine (TEA). This strategy was found to be successful for obtaining the pyrazole peptidomimetics.

Introduction

The pyrazol nucleus in general and its chemistry have caught widespread attention due to outstanding biological activities as antipyretic, analgesic and anti-inflammatory drugs (1a-c). A pyrazole ring system represents a thermally stable, aromatic ring system which resists ring opening. Although very few pyrazole derivatives occur naturally, many are pharmacologically active (2,3). It has been shown that therapeutically active agents linked to small peptides or amino acid residues, reduce toxicity and enhance their therapeutic effects (4,5,6). It is supposed that an amino acid transport system in the cell can be exploited to deliver the pyrazole nucleus to target cells for the drug activity.

Considering the potential of this pyrazole ring system, we undertook the synthesis of phenylpyrazole 6a-f employing the classical method of peptide synthesis. This paper reports the

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strategy of synthesis of small compounds and the verification of their structures by spectroscopic means and elemental analysis. The compounds described herein constitute a set of potentially useful building blocks in the synthesis of modified peptides.

Synthesis of the compounds

To start the synthesis, the formylation of 1-phenylpyrazole 1 at C-4 was carried out using the Vilsmeier-Haack conditions (7). The malonic acid condensation of this aldehyde 2 was realized in pyridine:piperidine resulting in a compound 3.

The peptidomimetic heterocyclic derivatives were synthesized from Boc-protected L-amino acids. The synthetic routes were applied on appropriately protected lipophilic (phenylalanine, alanine, valine, leucine) and acidic (aspartic and glutamic) amino acids. The amino acids were firstly amidified. The carboxylic acid function of 4 was treated with isobutyl chloroformate in the presence of triethylamine and ammonium hydroxyde in order to obtain the desired amide in good yield. We have chosen using the aspartic and glutamic amino acids so the carboxylic acid function of the side chain is protected by a benzyl ester.

The expected peptidimimetic derivatives 6a-f have been synthesized by condensation with 1-phenylpyrazole unsaturated carboxylic acid 3 with 5a-f using benzotriazolyl-N-oxy-tris (dimethilamino)phosphonium hexafluorophosphate (BOP) to activate the carboxylic acid function by the generation of a highly reactive 1-hydroxybenzotriazole ester of the carboxyl component in its anionic form (8a-d) **(scheme)**. The phenylpyrazole derivatives were obtained in 35-70% yield.

All compounds present characteristics of ^{1}H-NMR for NH_2 and NH moiety and the proton linked to quiral carbon. Two singlets for NH_2 broaded has shown nonequivalent protons. For the NH a characteristic duplet has shown a coupling constant with CH quiral. Different chemical shift groups next to chiral center are observed. The phenomenon is more pronounced with bulky substituents. The IR spectra showed a N-H stretching asymmetric, band around 3377 cm^{-1} and symmetric, 3300 cm^{-1}, overlap C=O stretching, amide I band around 1641 cm^{-1}, and C=N stretching band at 1620 cm^{-1}. The analytical data and chemical shifts of the compounds are given in Tables I and II.

Scheme

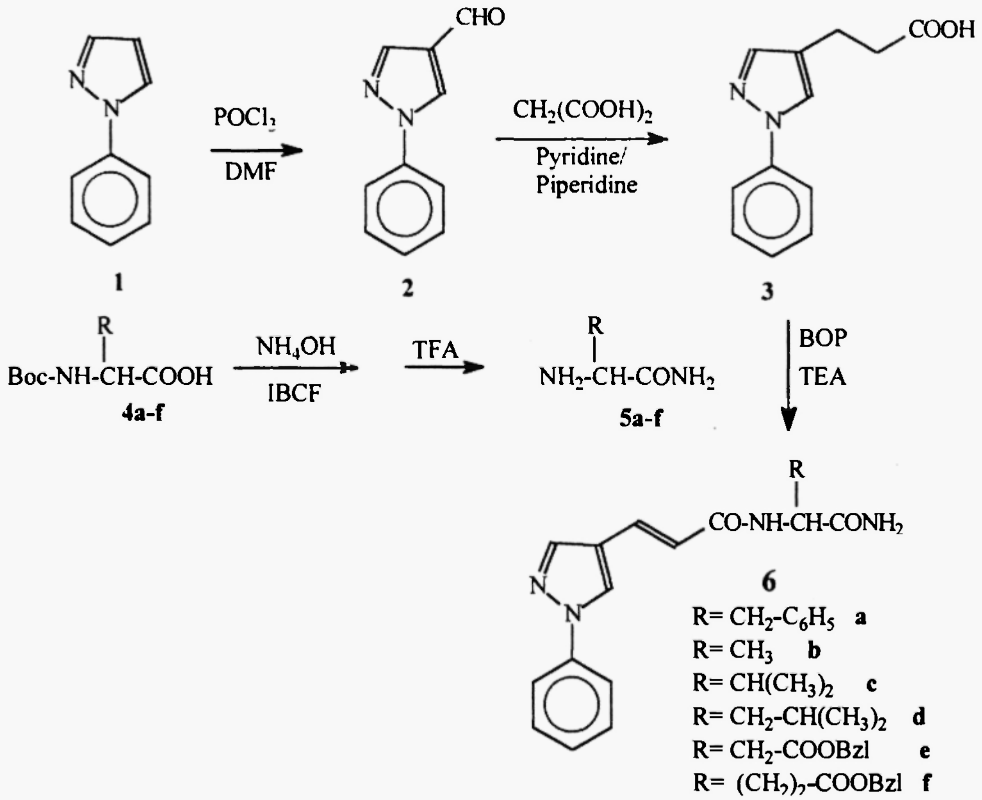

Table I- Analytical data of phenylpyrazole derivatives

Compound	M.p.	Yield	$[\alpha]_D$	R_f[b]	Molecular	Calc.(%)			Found		
	[°C][a]	[%]			Formula	C	H	N	C	H	N
6a	232	63.3	-22,1	0.3A	$C_{21}H_{20}N_4O_2$	76.8	6.13	17.0	76.6	6.11	16.9
6b	162-4	35.6	25.3	0.39B	$C_{15}H_{16}N_4O_2$	71.4	6.39	22.2	71.2	6.4	22.3
6c	252-4	75.3	78,1	0.3A	$C_{17}H_{20}N_4O_2$	72.8	7.19	19.9	72.6	7.2	20.0
6d	197-9	52.45	39,3	0.3C	$C_{18}H_{22}N_4O_2$	73.4	7.53	19.0	73.2	7.54	19.1
6e	168-9	51.36	15,7	0.68B	$C_{23}H_{22}N_4O_4$	77.9	6.25	15.8	77.6	6.2	15.6
6f	157-9	76.42	12,2	0.3C	$C_{24}H_{24}N_4O_4$	78.2	6.56	15.2	78.1	6.55	15.0

[a] Crystallized from Ethyl acetate/ether/ hexane. [b] Solvent systems: A(AcOET), B(AcOET/hexane, 8:2), C(AcOET/hexane, 7:3).

Table II- IR and NMR Spectral data

Compound	IR cm^{-1} (KBr)				^{1}H-NMR δppm (DMSOd$_6$)
	(N-Hasym)	(NHsym)	(C=O amide I)	C=N	
6a	3377	3303	1643	1619	3.36(d, 2H), 4.54-4.57(m, 1H), 7.3-7.9(m, 10H), 4.4(1H), 6.49-6 8.4(d, 2H)
6b	3394	3288	1672	1604	2.5 (2H), 4.31-4.36(q, 1H), 1.25(d, 3H), 3.35(1H), 6.39-6.58(t, 2H), 7.33-8.13(m,5H), 8.77-8.79(d 2H)
6c	3376	3297	1659	1621	1.82-1.89(m,3H), 4.07-4.12(q, 1H), 0.72-0.74(d, 6H), 3.4 6.54(t,2H), 7.16-7.87(m,5H), 8.05-8.1(d, 2H)
6d	3381	3305	1655	1621	1.5(2H), 4.35-4.37(q,1H), 1.58-1.6(t,2H),0.88-0.93(m,1H), 0.85 0.51-0.57(t,2H), 7.3-8.7(m,5H), 8.75-8.77(d, 2H)
6e	3383	3313	1661	1624	2.48(2H), 4.74-4.79(q, 1H), 2.60-2.64(d,2H), 5.12(s,2H), 7.32-7 6.52-6.57(t,2H), 8.40-8.42(d, 2H)
6f	3385	3315	1653	1619	1.66(2H), 4.35-4.37(q,1H), 3.42(1H), 2.39-2.50(m,2H), 2.51-2.54(t,2H), 5.09(s,2H), 8.29-8.32(t,2H)

Conclusions

The compounds synthesized in the present work represent a novel class of pseudopeptides. The reactions producing 1-phenylpyrazole having a pseudopeptide chain are versatile and can be performed starting from most of the natural amino acids. The overall yields of the pseudopeptides are satisfactory and the synthetic sequences are short and facile. The reaction conditions used are mild for the protected groups.

Experimental

All melting points were determined by using a Thomas Hoover apparatus and are incorrected. IR spectra were obtained by using KBr pellets. ^{1}H NMR spectra were measured with a Varian UNITYplus-300 MHz NMR spectrophotometer using DMSO-d$_6$ as solvent as tetramethylsilane like an internal standard. Thin-layer chromatography (TLC) was carried out on silica gel plates having fluorescence indicator F$_{254}$ (0.2 mm, E. Merck); the spots were visualized with UV light, and by spraying with a 2% ethanol solution of ninhydrin or charing reagent. Column chromatography was performed on silica using Kiesegel 60 (230-400 mesh, E. Merck) with ethyl acetate/hexane. Optical rotations were measured at 20°C, using DMSO as solvent in a 10 cm cell, and *c* is expressed in g/100mL. The elemental analyses were performed in a PE-2400 apparatus. Protected amino acids

(Boc and Obzl), were purchased from Bachem (Switzerland) or Propetide (vert le Petit, France). All reagents used in the present work were of analytical grade.

N-Phenylpyrazole 1: Was prepared in 85% yield by the previously described procedure (10).

4-Formyl-1- phenylpyrazole 2: was obtained in 80% yield from **1**, as crystals, mp 83-84° C by using the modified conditions described by Finar[7]. Spectroscopic data were identical to those previously reported (7).

(E)-3-(1-phenylpyrazol-4-yl)prop-2enoic Acid 3: a mixture of malonic acid (12.4 g, 119.2 mmols) and pyridine (30 ml, 58.1 mmoles) was treated with 4-Formyl-1- phenylpyrazole (10 g, 58.1 mmoles) and 1 ml of piperidine. The reaction mixture was heated at 115° C.for 4 hours, and cooled. After acidification, the white solid product was collected by filtration, crystallized from ethanol to give the analytical sample, mp 185-186 ° C(lit 186-7 ° C) (11).

Procedure for the protection of the C-terminus moiety of the amino acids 5a-f: To a cold (-10 0°C) solution of N-Boc-Amino acid (3.0 mmol) in dimethylformamide, were successively added isobutyl chloroformate (3.0 mmol) and ammonium hydroxide dropwise for 20 min. After that, the mixture was stirred at room temperature during 1h. Water was added and the pure product was easily isolated by filtration and dried.

General Procedure for the Synthesis of 1-Phenylpyrazoles Containing Pseudopeptide Moiety 6a-f: To the Boc-Amino acylamide, trifluoroacetic acid was added and was left to stand at room temperature, during half an hour. After that, the mixture was concentrated *in vacuo*, and gave a white powder after trituration with ether. The pure product was easily isolated by filtration and dried. TFA-Amino Acylamide (2.6mmol) was dissolved in DMF (10mL) containing **3** (2.6 mmoles) and BOP (2.6mmol). The solution was cooled in an ice bath, and TEA (0.7 mL) was added to it. The preparation was left to stand for 4 hours at room temperature. Afterwards, a saturated solution of sodium bicarbonate was added under stirring, followed by ethyl acetate (50 mL). The organic layer was washed several times with sodium bicarbonate (50 mL), water (50 mL), 1M citric acid (50 mL), water (50 mL), dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with ethyl acetate-hexane (6a: 5/5, 6b: 7/3, 6c: 8/2), ethyl acetate (6d) and ethyl acetate-methanol 9/1 (6e) as solvent, and gave a white powder after being crystallized with diethyl ether-hexane.

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