SYNTHESIS OF PEPTIDYL BENZODIOXOLE DERIVATIVES FROM SAFROLE, A POTENTIAL CLASS OF ANTITUMOR DRUGS

Kezia Peixoto da Silva, Ivone A. de Souza, Antonio Rodolfo de Faria, Dalci Jose Brondani & Ana Cristina Lima Leite*

LABSINFA - Departamento de Ciencias Farmaceuticas - Universidade Federal de Pernambuco. Rua Prof. Artur Sa S/N. CDU. 50740-520. Recife. Pernambuco - Brasil
aclb *a* npd.ufpe.br

Abstract: As part of a preliminary study on novel antitumor drugs, the synthesis of a new class of peptidyl compounds, using natural safrole is described. The 1,3-benzodioxole group was initially converted into a 6-alyl-benzo[1,3]dioxole-5-ilamine. The peptidyl derivatives were prepared by condensation of amino moiety with appropriately protected α-amino acids. The structures of this new class of compounds was determined by IR and NMR spectral data.

Introduction

Safrole is an abundant Brazilian natural product from sassafraz oil *(ocotea pretiosa Mer., Lauraceae)*, which presents interesting functionality and chemical reactivity that suggests its use as an efficient and versatile natural *synton*[1-6] The methylenedioxy unit from safrole, has been identified in some clinical antitumor agents like etoposide and teniposide[7]. As part of a research program with the objective of synthesizing bioactive compounds by the use of abundant and inexpensive starting material, we have thus developed a synthetic sequence dealing with the synthesis of the safrole derivatives[8].

On the other hand, it has been identified some peptides that home specifically to tumor blood vessels[9]. On this basis, It has been shown that therapeutically active agents linked to small peptides or amino acid residues, reduce toxicity and enhance their therapeutic effects[10-13]. It is supposed that an amino acid transport system in the cell can be exploited to deliver the active nucleus to target cells for the drug activity. Considering the potential of ring system from safrole we undertook the synthesis of peptidyl compounds 4a-h employing the coupling reagents used in peptide synthesis. This paper reports reports the strategy of synthesis of these new compounds and

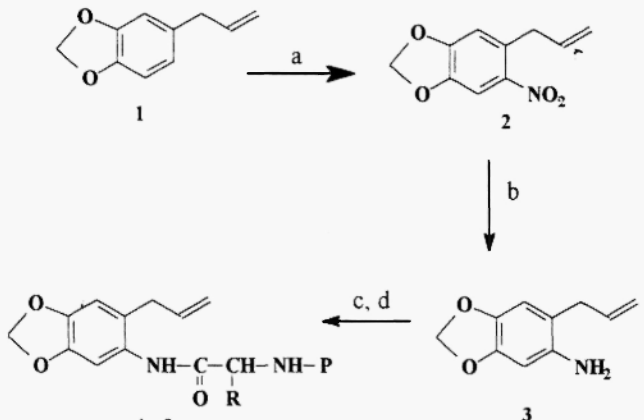
the verification of their structures by spectroscopic means. The compounds described herein constitute a set of potentially useful building blocks in the synthesis of modified peptides.

Synthesis of the compounds

Our synthetic strategy was based on the selective and classical nitration with nitric acid in acetic acid, on the 5 position on the 1,3-benzodioxole moiety. The nitro group was reduced in mild conditions using iron powder and NH_4Cl in ethanol/water to afford the amino derivative.

The peptidomimetic derivatives were synthesized from Boc-protected L-amino acids. The synthetic routes were applied on appropriately protected lipophilic (glycine, phenylalanine, alanine, valine, leucine) and basic (lysine) amino acids. The amino group in side chain of lysine was protected by a fluorenylmethyloxycarbonil (Fmoc) group. The expected peptidyl derivatives **4a-h** have been synthesized by condensation with 6-allyl-benzo[1,3]dioxole-5-ilamine **(3)** with α-aminoacids using diciclohecylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) to activate the carboxylic acid function in order to generate an active ester *in situ*[14-16] **(Scheme I)**. The safrole derivatives were obtained at 25 to 95% yield.

Scheme I

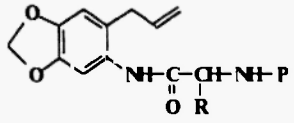


(a) HNO_3,AcOH; (b) Fe,NH_4Cl/EtOH/H_2O,reflux; (c) P-NH-CH(R)-COOH ; (d) DMF, 0°C, DCC, HOBt; **P**= Boc: **4a-e**; **P**= Z: **4f**

The structure of **4a-f**, was proved by IR and ^{1}H-NMR spectral data. **(Table I)**. The IR spectra showed a characteristic N-H stretching vibration around 3263 and 3363 cm-1, overlap C=O stretching, amide I band around 1649 and 1671 cm^{-1}. Asymmetrical C-O-C stretching band around 1262 cm^{-1} and symmetrical streching near 1026 cm^{-1}. The ^{1}H-NMR spectra of compounds showed the same characteristic at (δ) 5.75-5.91 ppm attributed to methinic proton, at 4.97-4.99 and 5.03-5.05 ppm, corresponding to methilenic protons. Another representative signal occurred at 5.98 ppm and was attributed to the methylenedioxy group. Two signals occurring between 6.70 and 6.74 ppm as two singlets indicating a typical para-aromatic hydrogen pattern. The NH linked to aromatic ring showed a singlet around 9.07 and 9.25 ppm. For the NH a characteristic dublet around 6.91-7.57 ppm has shown

a coupling constant with CH quiral. Table I, report ^{1}H-NMR from amino acid moiety.

Table I: IR and ^{1}H-NMR spectral data from amino acid moiety of compounds **4a-h**[a]



	R
4a	CH_2-C_6H_5
4b	CH_2-CH-$(CH_3)_2$
4c	CH-$(CH_3)_2$
4d	$(CH_2)_4$-NH-Boc
4e	CH_3
4f	H

Comp.	IR, cm^{-1}				^{1}H NMR (DMSO-d_6)
	C-O-C		NH	CO	δ
4a	1044	1219	3323	1658	1.33 (s. 9H. $(CH_3)_3$). 2.84/3.01 (dd. J = 13.2Hz/13.49Hz. J= 9.6Hz/5.69Hz .1H. $CH_2\beta$). 4.27-4.32 (m. 1H. Hα). 7.29-7.30 (m. 5H. Ar-*m. p and o*);
4b	1042	1250	3363	1649	0.82 (d. J = 6.90Hz. 3H. $CH_3\delta$). 0.85 (d. J = 6.60Hz. 3H. $CH_3\delta$). 0.90 (t. J = 6.60Hz. 2H. $CH_2\beta$). 1.37 (s. 9H. $(CH_3)_3$). 1.43-1.53 (m. 1H. CHγ). 4.05-4.13 (m. 1H. CHα);
4c	1043	1248	3322	1653	0.89(d. J = 6.89 Hz. 6H. $CH_3\gamma$). 0.92(d. J = 6.89 Hz. 6H. $CH_3\gamma$). 1.39 (s. 9H. $(CH_3)_3$). 1.99-2.02 (m. 1H. CHβ);
4d	1027	1262	3317	1654	1.14-1.23(m. 2H. $CH_2\gamma$). 1.38 (s. 9H. $(CH_3)_3$). 1.44-1.47 (m. 2H. Hδ). 1.36-1.37(m. 2H. Hβ). 2.96-3.0 (m. 2H. Hε). 3.20 (d. J = 6.30Hz. 2H. $CH_2\delta$). 3.27 (s. 1H. CHβ). 3.99-4.02 (m. 1H. CHα).7.39-7.34 (m. 4H. Ar-γ). 7.44-7.49 (m. 4H. Ar-γ);
4e	1040	1219	3263	1666	1.25(d. J = 7.49Hz. 3H. $CH_3\beta$). 1.38 (s. 9H. $(CH_3)_3$). 4.07-4.12 (m. 1H. CHα);
4f	1038	1215	3324	1659	3.78 (d. J = 6.30Hz. 2H. $CH_2\alpha$). 7.31-7.37 (m. 5H. Ar-β). 5.05 (s. 2H. $CH_2\alpha$).

[a] P= Boc: **4a-e**. P=Z: **4f**.

Conclusions

The compounds synthesized in the present work represent a novel class of peptidil compounds of safrole derivatives. The reactions producing 6-allyl-benzo[1,3]dioxole having a peptide moiety are versatile and can be performed starting from most of the natural amino acids. We are presently employing the same procedure in the preparation of others derivatives. The peptidil compounds were tested in vivo in experimental animal tumor system. The antitumor activity has shown promising preliminary results. These results will be reported in up coming.

Experimental

All melting points were determined 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 and tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was carried out on silica gel plates having fluorescent 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. All reagents used in the present work were of analytical grade.

5-alil-6-nitro-benzo[1,3]dioxole (2): To a stirred mixture of 0,062mol of 5-alil-benzo[1,3]dioxole, 0,062mol of acetic acid under 5°C, 0,062mol of concentrated nitric acid in 1,5 mL of acetic acid was

slowly added. After 2h., the mixture was taken up in 100 mL of water and then extracted with three 50mL portions of ethyl acetate. The organic phase was washed with water, filtered and dried (Na_2SO_4). The residue was chromatographed on silica gel with 2% ethyl acetate in hexane to give 8,9g (70%) of a yellow oil. ^1H NMR ($CDCl_3$) ($DMSO_{d6}$):

6-alyl-benzo[1,3]dioxole-5-ilamine (3): A mixture of **2** (0,017 mol), iron powder (0,095mol) and NH_4Cl (0,01mol) in 150 mL of EtOH:H_2O (2:1) was refluxed for 1 h. The hot mixture was filtered through Celite and concentrated *in vacuo*. The residue was diluted with H_2O and extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated to give **3** (80%) as a light brown solid. ^1H NMR ($DMSO_{d6}$).

General Procedure for the Synthesis of safrole derivatives Containing a peptide Moiety (4a-h): To a stirred solution of 2,5mmol of the N-protected amino acid in dimethylformamide under 0°C, 6,8mmol of the diciclohexylcarbodiimide, 5,1 mmol of hydroxybenzotriazole and 1,7 mmoles of 2-amino-safrole (**3**) was added and was left to warm up to room temperature. After that, the reaction mixture was filtered, and the filtrate was treated with ethyl acetate. The organic phase was washed with $NaHCO_3$, water, aqueous NaCl and dried (Na_2SO4) and concentrated. The residue was treated with hexane and filtered.

Acknowledgements

The financial support from the "Conselho Nacional de Desenvolvimento Cientifico e Tecnologico" (CNPq), the "Fundação de Amparo a Ciência do Estado de Pernambuco" (FACEPE) is gratefully acknowledged. The authors thank the Departamento de Quimica Fundamental, Universidade Federal de Pernambuco (UFPE) for recording the ^1H-NMR and IR spectra of all compounds.

References

1- A.S. Lages, K.C.M. Silva,; A.L.P. Miranda, C.A.M. Fraga, E.J. Barreiro, Bioorg. Med. Chem. Lett., **8**(2): 183-188 (1998).
2- P.R.R. Costa, Quimica Nova, **23**(3): 357-369 (2000).
3- E.J. Barreiro, C.A.M. Fraga, Quimica Nova, **22**(5): 744-759 (1999).
4- P.R.R. Costa, C.C. Lopes, An. Acad. Brasil. Ciênc., **54**(4): 758-759 (1982).
5- E.J.; Barreiro, P.R.R. Costa, P.R.V.R. Barros, W.M. Queiroz, J. Chem. Research **(S)**, 102-103 (1982).
6- L.G. French, J. Chem. Educ., **72**(6): 484-491, 1995. *In*: Chem. Abs., v. 122, 111109v, (1995)
7- G. L. Chen, L. Yang, T. C. Rowe, B. D. Halligan, K. Tewey, L. Liu, J. Biol. Chem., 259, 13560-13566 (1984).
8- Abstracted in part, from Thesis of K. P. da SILVA (Universidade Federal de Pernambuco), PE, BR, in preparation.
9- W. Arap, R. Pasqualini, E. Ruoslahti, Science, **279**: 377-380 (1998).
10- P.K. Chakravarty, P.L. Carl, M. J. Weber, J.A. Katzenellenbogen, J. Med. Chem., **26:** 633-638 (1983).
11- P.K. Chakravarty, P. L. Carl, M. J. Weber, J. A. Katzenellenbogen, J. Med. Chem., **26**: 638-644 (1983).
12- Haines, D. Fuller, R. Ahmad, S. Vistica, D. and Marquez, V. J. Chem. Soc, **30**, p. 542-7 (1987).
13- A. Trouet, A. Passioukov, K. Derpoorten, A.M. Fernandez,; J. Abarca-Quinones, R. Baurain, T. J. Lobl, C. Oliyai, D. Shochat, V. Dubois, Cancer Research, **61**(7): 2843-2846 (2001).
14- M. Bodanszky– Peptide Chemistry *A Practical Textbook*. New York: Springer-Verlag, 1988. 200p.
15- J. Jones - The Chemical Synthesis of Peptides. New York: Oxford University Press, 1994. 229p.
16- Bodanszky, M. – Principles of Peptide Synthesis. New York: Springer-Verlag, 1984. 307p.

Received on July 7, 2001