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Original article

Synthesis, antitumour and antimicrobial activities of new peptidyl derivatives containing the 1,3-benzodioxole system

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Abstract

Two series of 5 and 6-substituted 1,3-benzodioxole peptidyl derivatives were synthesized and evaluated as antitumour and antimicrobial agents. The compounds that could be conveniently prepared in a few steps processes from natural safrole have been characterised by IR and ^{1}H-NMR spectroscopy. In vivo antitumor activity tests showed that some of the compounds were able to inhibit carcinoma S-180 tumour growth in mice. The in vitro antimicrobial activity of all compounds revealed that they are able to promote the growth of some organisms, including *Bacillus subtilis*.

Keywords: 1,3-Benzodioxole; Safrole; Peptidyl derivatives; Antitumour agents

1. Introduction

Safrole, from sassafras oil (*Ocotea pretiosa Mer., Lauraceae)*, is an abundant Brazilian natural product that demonstrates interesting functionality and chemical reactivity suggesting its use as an efficient and versatile natural *synton* [1–6]. In 1983 the FDA reported that, safrole or sassafras, the extract or the oil, was an ingredient in 113 over-the counter drug formulations generally for topical application, but occasionally for oral administration [7]. However, carcinogenic and other toxicological effects to this product have been pointed out [8–10].

The methylenedioxy unit, present in safrole, can be identified in the clinical antitumour agents etoposide and teniposide [11] and lignan lactone podophylotoxin [12].

A major problem in chemotherapy today is the severe host toxicity exhibited by most of the anticancer drugs due to their poor selectivity toward cancer cells. Recently, antitumour drugs with reduced toxicity have been developed by linking them to small peptides or amino acid residues [13–17]. The role of the conjugated peptide in these prodrugs is not to physically locate the prodrug on tumour cells, but to serve as a substrate for designated enzymes that are produced and secreted preferentially by tumour cells [18]. In fact some peptides that selectively target tumour blood vessels have been identified. These remarks indicates that it may be possible to develop highly selective chemotherapy strategies that are based on the selective expression of receptors in tumour vasculature [19].

In view of the available information on structure–activity relationships, we decided to synthesize derivatives, considering the potential of ring system from safrole and amino acid residues. We undertook the synthesis of two series of compounds exploring five and six positions in aromatic ring 1,3-benzodioxole nucleus [20].

Safrole **(1)** was initially converted to 5-allyl-6-nitro-benzo [1,3]dioxole. This compound was then used to obtain the intermediate product 6-allyl-benzo [1,3]dioxole-5-yl-amine **(3)** and (6-nitro-benzo [1,3]dioxole-5-yl) acetic acid **(5)**. The condensation reactions with amino acids were accomplished by employing the classical method of peptide synthesis. DCC and HOBt were used to prepare the two new series of

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peptidyl derivatives of safrole. The structures of the compounds were determined by spectroscopic methods (IR and ^{1}H-NMR).

Antimicrobial screening, acute toxicity and antitumour activities were performed. Peptidyl derivatives and the intermediate compound, 6-allyl-benzo [1,3]dioxole-5-yl-amine **(3)**, were able to induce the growth of some bacteria which are important in phytopathogenic control in agriculture.

The acute toxicity of the *N*-(6-allyl-1,3-benzodioxol-5-yl)-*N*-(*tert*-butoxy carbonyl)phenylalaninamide **(4e)** derivative was evaluated and revealed a LD50 at 250 mg/kg in mice. The antitumour activity of our compounds was checked against sarcoma 180. Peptidyl derivatives of leucine, valine, glycine and glutamic acid significantly reduced the growth of the tumour at 35 mg/kg. All of the compounds that were tested revealed low toxicity when compared to intermediary 6-allyl-benzo [1,3]dioxole-5-yl-amine **(3)**, that was toxic/lethal at the same dose.

2. Chemistry

The general synthetic route that was chosen for the preparation of peptidyl derivatives of safrole included two stages. Initially, our synthetic strategy was based on the selective and classical nitration with nitric acid in acetic acid, on the five position of the 1,3-benzodioxole moiety [20].

The derivatives of the series **4a–h**, were prepared, from the functionalised nitro safrole, by reduction in mild conditions using iron in powder and NH_4Cl in ethanol/water to afford the amino derivative [21]. The synthetic routes were applied on appropriately protected lipophilic (glycine, phenylalanine, alanine, valine, leucine), acid (aspartic and glutamic acid) and basic (lysine) amino acids. The amino group in the side chain of lysine was protected by a fluorenylmethyloxycarbonil (Fmoc) group. The expected peptidyl derivatives **4a–f** have been synthesized by condensation with 6-allyl-benzo [1,3]dioxole-5-ylamine **(3)** to α-amino acids using diciclohexylcarbodiimide and 1-hydroxybenzotriazole to activate the carboxylic acid function of the amino acid to generate an active ester in situ [22–24]. The peptidyl derivatives were obtained at 25–95% yield.

The next step was the synthesis of compounds **6a–d**. Among all possible methods for oxidation of 5-allyl-6-nitro-benzo [1,3]dioxole **(2)**, a heterogeneous two-phase water–benzene system in permanganate and a phase-transfer catalyst is one of the simplest and most convenient routes [25]. We have tested several phase-transfer agents. Treatment of **(2)** with permanganate in benzene water and acetic acid medium with tetrabutylammonium tetrafluorborate produced the intermediate **(5)** with 25% yield. In the same conditions, benzyldimethyltetradecylammonium chloride dihydrate was used as phase-transfer agent. The desired carboxylic acid was obtained in 60%. Our attention was next turned to the over-oxidation product resulting from a loss of two carbons in permanganate oxidation of olefins. The use of an acetic acid medium suppresses (but does not completely inhibit) the over-oxidation product. The acetic acid's function (soluble in water, benzene, and pentane) may merely reflect its solubility in the organic phase and rapid destruction of the OH- formed during the disproportionation of manganese (VI) intermediates [26]. An additional function is the destruction of any excess of permanganate and prevention of further oxidation of the initially formed carboxylic acid [27]. In fact, the desired (6-nitro-benzo [1,3]dioxole-5-yl) acetic acid **(5)** was obtained, but it was contaminated with 6-nitrobenzo [1,3]dioxole-5-carboxylic acid (5%). This made it necessary to purify it **(5)** before amino acid condensation.

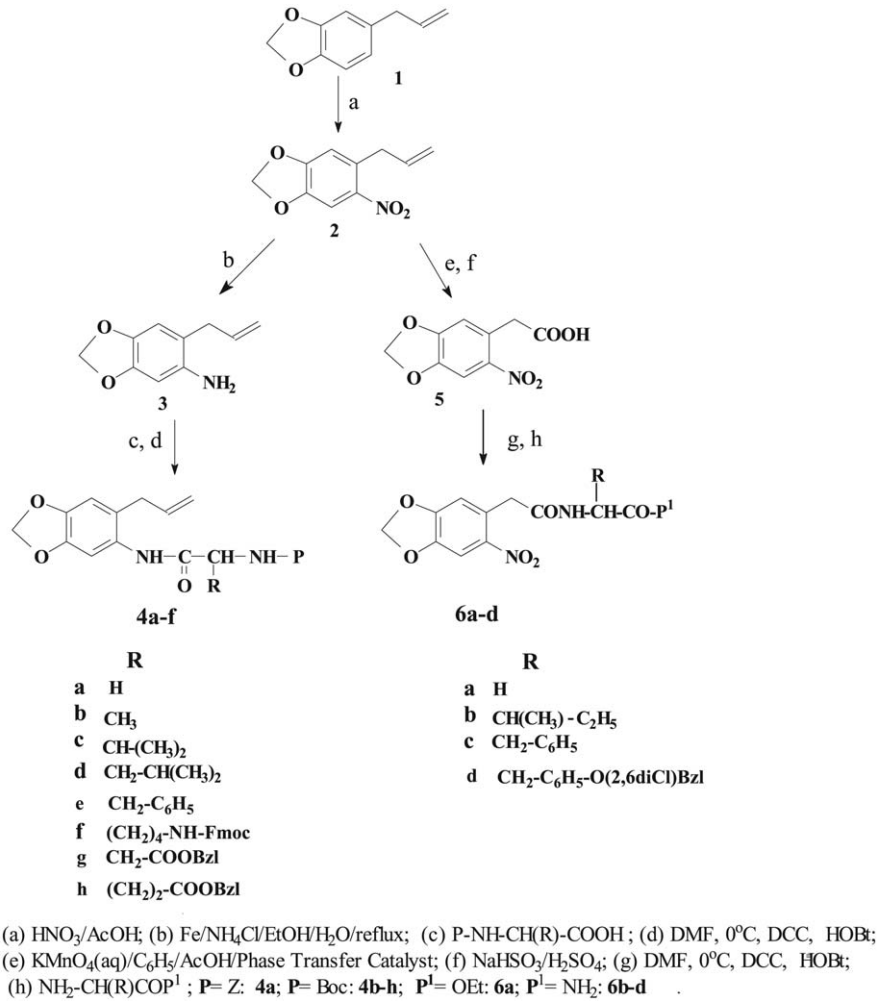
For this series, the synthetic routes were applied on glycine methyl ester, phenylalaninamide, leucinamide and tyrosinamide. The side chain of tyrosinamide residue was protected by a 2,6-dichloro-benzyl group. The expected aminoacyl derivatives **6a–d** were synthesized by condensation with (6-nitro-benzo [1,3]dioxole-5-yl) acetic acid **(5)** to α-amino acids using the same procedure used to the series **4a–h**. The safrole derivatives were obtained at 18 in 99% yield. The obvious synthetic route planned to achieve the new safrole derivative compounds **4a–h** and **6a–d** is shown in Fig. 1.

The formation of **4a–h** and **6a–d**, was supported by IR and ^{1}H-NMR spectral data. The common characteristic signals to all compounds are described here. The IR spectra showed a characteristic N–H stretching vibration around 3193 and 3387 cm^{-1}, overlap C=O stretching, amide I band around 1627 and 1671 cm^{-1}. Asymmetrical C–O–C stretching band around 1200–1275 cm^{-1} and symmetrical stretching near 1020–1075 cm^{-1}. The ^{1}H-NMR spectra of compounds showed the same characteristic at (Δ) 5.75–5.91 ppm attributed to ethylenic proton, at 4.97–4.99 and 5.03–5.05 ppm, corresponding to methylenic protons. Another representative signal occurs at 5.98–6.21 ppm was attributed to the methylenedioxy group. A characteristic doublet around 6.91–8.60 ppm for the NH indicating a coupling constant with CH chiral. In the **4a–h** series, two signals occurring between 6.70 and 6.74 ppm appeared 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. In the **9a–d** series, an aromatic hydrogen pattern near to nitro group appeared as two signals occurring between 7.53 and 7.72 ppm. Another aromatic proton occurring between 6.77 and 7.06 ppm. The NH_2 signal appears as two singlets at about 7.35–7.42 and 7.00–7.09 ppm.

3. In vitro and in vivo results and discussions

3.1. Antimicrobial activity

The antimicrobial activity of compounds were determined in vitro by the disc diffusion method [28] against some strains of *Staphylococcus aureus* (DAUFPE 02), *Bacillus subtilis* (DAUFPE 16), *Escherichia coli* (DAUFPE 224),



(a) HNO_3/AcOH; (b) Fe/NH_4Cl/EtOH/H_2O/reflux; (c) P-NH-CH(R)-COOH ; (d) DMF, 0°C, DCC, HOBt; (e) $KMnO_4$(aq)/C_6H_5/AcOH/Phase Transfer Catalyst; (f) $NaHSO_3$/H_2SO_4; (g) DMF, 0°C, DCC, HOBt; (h) NH_2-CH(R)COP^1 ; **P**= Z: **4a**; **P**= Boc: **4b-h**; $\mathbf{P^1}$= OEt: **6a**; $\mathbf{P^1}$= NH_2: **6b-d** .

Fig. 1.

Klebsiella pneumoniae (DAUFPE 396), *Salmonella enteritidis* (DAUFPE 414), *Pseudomonas aeruginosa* (DAUFPE 39), *Shigella sonei* (DAUFPE 413), *Sarcinea lutea* (DAUFPE 100). All the strains came from the collection of the Department of Antibiotics (UFPE-BR). One strain, *Candida* spp., that was isolated from immune suppressed patients was also used.

All compounds of the series **4a–f**, promoted an increase in the growth of the micro-organisms *P. aeruginosa*, *Shigella sonei*, *B. subtilis*, *Staphylococcus aureus* and *K. pneumoniae*. All compounds of series **6a–d** promoted an increase in the growth of the micro-organism *E. coli.*

In this way, it was verified that our products did not reveal antimicrobial activity. However, the literature tells us that some micro-organisms like *B. subtilis*, *Pseudomonas* spp, are being studied as rhyzobacteria, acting in the promotion of plant growth. The interest in this kind of alternative method for the control of phytopathogens has been increasing because they do not require the use of many chemical products that elevate the manufacturing costs of conventional plant protection products and cause environmental damage. As examples, the innoculation of carrots with *B. subtilis* has resulted in a tax increase of 48% [29], 37% on the peanuts that are inoculated with *B. subtilis* [30] and 33% on the pea production that are inoculated with *Pseudomonas fluorescens* [31].

The present work, suggests that the peptidyl derivatives of safrole could offer an exciting new method to promote *B. subtilis* growth for agricultural use.

3.2. Behavioral effects and in vivo acute toxicity

The acute toxicity test of *N*-(6-allyl-1,3-benzodioxol-5-yl)-*N*-(*tert*-butoxy carbonyl)phenylalaninamide **(4e)**, was realised through intraperitoneal way, on the concentrations: 5, 50, 100, 250 and 500 mg/kg of the animal weight. All animals were monitored continuously for 72 h after dosing for signs of toxicosis.

In the present study, we verified that the effects were not noticed until the dose of 250 mg/kg, presenting acute toxicity with cumulative action, irreversible and lethal hydric retention of two animals. This group of animals presented augmented hearty reactions. Two animals presented cardio-respiratory failure and then death. There was one death 50 min after the administration and other 20 h later. Higher doses present toxicity, but in lower level. Clinical signals

included motor uncoordination, hyperactivity, tremors, decrease of muscle tone, prostração and labored brating. There was only one death in the group that received the dose of 500 mg/kg.

These results lead us to conclude that the dose of 25 mg/kg (10% of the acute toxicity) will be the ideal for evaluation of the antitumour activity. However, in an attempt to find a better therapeutic response, we began with a dose of 35 mg/kg.

3.3. In vivo antitumour activity

The antitumour activity of compounds **4a–h** and intermediary compound 6-allyl-benzo(1,3)dioxol-5-ylamine (**3**) was tested against sarcoma S-180 in six Swiss albino mice (25–28 g) for each tested product. The pre-clinical trial began 48 h after the implantation of the tumour and longed for 8 days of treatment, with the dose of 35 mg/kg/day, using the intraperitoneal way.

All the products of the series **4a–h** were able to inhibit the growth of the tumour mass on the assays. The products **4b** (leucine), **4c** (valine), **4h** (glutamic acid) and **4e** (glycine) derivatives, presented an antitumour profile with reduction of the tumour mass in 66.44%; 83.48%; 71.49%; 77.49%, respectively. The other tested products **4a** (phenylalanine), **4d** (lysine), **4f** (alanine) and **4g** (aspartic acid) derivatives were less active, with inhibition rates of 37.30%; 37.56%; 52.17% and 51.78%, respectively. Concerning compound 6-allyl-benzo(1,3)dioxol-5-ylamine **(3)**, this product was very toxic, with cumulative and lethal action. Six animals were tested with this product, just one did not die.

This data clearly demonstrates that the linking of the 1,3-benzodioxole nucleus from safrole to an amino acid moiety reduced the intrinsic toxicity. Six tested products of this series were able to reduce the tumour in 50% or more.

Our results are according to the literature shown in all cases, were that the drugs were linked to amino acids or peptide fragments as a delivery system the resulting prodrug was less toxic [13–17]. The reduced toxicity of the peptidyl derivatives of safrole, in comparison to its prototype (intermediary 3), could indicate an effective enzymatic cleavage process selective to the tumour cell.

4. Experimental

4.1. Chemistry

^{1}H-NMR spectra were recorded on a Varian UNITY plus-300 MHz NMR spectrophotometer using DMSO-d_6 as solvent as tetramethylsilane as an internal standard. IR spectra were obtained on using KBr pellets. Microanalyses for C, H, N were performed in a EA1110 apparatus and were within ±0.4% of the required theoretical values. The melting points were determined using a Thomas Hoover apparatus and the results are given without correction. Thin-layer chromatography was carried out on silica gel plates having fluorescence indicator F_{254} (0.2 mm, E. Merck); the spots were visualised with UV light, and by spraying with a 2% ethanol solution of ninhydrin or charging reagent. Column chromatography was performed on silica using Kiesegel 60 (230–400 mesh, E. Merck). All reagents used in the present work were of analytical grade.

*4.1.1. 5-Allyl-6-nitro-benzo [1,3]dioxole **(2)***

To a stirred mixture of 0.062 mol of 5-allyl-benzo [1,3]dioxole, 0.062 mol of acetic acid under 5 °C, 0.062 mol of concentrated nitric acid in 1.5 ml of acetic acid was slowly added. After 2 h, the mixture was taken up in 100 ml of water and then extracted with three 50 ml 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 8.9 g (70%), of a yellow oil. ^{1}H-NMR (Δ ppm): 3.8 (d, 2H, $CH_2$8); 4.98 (dd, 1H, $CH_2$10a), 5.05 (dd, 1H, $CH_2$10b), 5.97 (m, 1H, CH9); 6.00 (s, 2H, $CH_2$2), 6.70 (s, 1H, Ar-7), 6.74 (s, 1H, Ar-4). Anal. CHN $C_{10}H_9O_4N$.

*4.1.2. 6-Allyl-benzo [1,3]dioxole-5-ilamine **(3)***

A mixture of **2** (0.017 mol), iron powder (0.095 mol) and NH_4Cl (0.01 mol) in 150 ml of EtOH:H_2O (2:1) was refluxed for 1 h. The hot mixture was filtered through Celite and concentrated under reduced pressure. 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. ^{1}H-NMR (Δ ppm): 3.10 (d, 2H, $CH_2$8); 4.52 (s, 2H, NH_2); 4.98 (dd, 1H, $CH_2$10a), 5.03 (dd, 1H, $CH_2$10b), 5.88 (m, 1H, CH9); 5.96 (s, 2H, $CH_2$2), 6.31 (s, 1H, Ar-4), 6.49 (s, 1H, Ar-7). Anal. CHN $C_{10}H_{11}O_2N$.

*4.1.3. General procedure for the synthesis of peptidyl derivatives from safrole (**4a–h**)*

To a stirred solution of 2.5 mmol of the N-protected amino acid in dimethylformamide under 0 °C, 6.8 mmol of the diciclohexylcarbodiimide, 5.1 mmol of hydroxybenzotriazole and 1.7 mmoles of 6-allyl-benzo [1,3]dioxole-5-ylamine **(3)** was added and was allowed to warm to room temperature. After that, the reaction mixture was filtered, and the filtrate was treated with ethyl acetate. The organic phase washed with $NaHCO_3$, water and aqueous NaCl and dried (Na_2SO4) and concentrated. The residue was treated with hexane and filtered.

*4.1.3.1. N-(6-Allyl-1,3-benzodioxol-5-yl)-N-(benzyloxy carbonyl)glycinamide **(4a)**.* Yield 95.45%; m.p. 118–120 °C; TLC R_f = 0.46 (3:2 hexane/ethyl acetate); I.R. (cm^{-1}): 1040 *N*C–O–C sym, 1245 *N*C–O–C assym, 1167 *N*C–C(=O)–O, 3338 *N*N–H, 1672 *N*C=O amide, 1685 *N*C=O carbamate, 1736 *N*C=O ester; ^{1}H-NMR (Δ ppm): 1.37 (s, 9H, $(CH_3)_3$), 2.49 (dd, J = 2.1 Hz, J = 6.3 Hz, 1H, $CH_2\beta$), 2.51 (dd, J = 2.1 Hz, J = 6.0 Hz, 1H, $CH_2\beta$), 3.20 (d, J = 6.60 Hz, 2H,

$CH_2$8), 4.43 (ddd, *J* = 8.69 Hz, *J* = 6.30 Hz, *J* = 6.00 Hz, 1H, CHα), 4.98 (dd, *J* = 2.69 Hz, *J* = = 17.09 Hz, 1H, $CH_2$10a), 5.05 (dd, *J* = 2.69 Hz, *J* = 9.89 Hz, 1H, $CH_2$10b), 5.12 (s, 2H, CH_2α″) 5.75-5.86 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.70 (s, 1H, Ar-4), 6.79 (s, 1H, Ar-7), 7.27 (d, *J* = 8.69 Hz, 1H, NH-14), 7.35 (s, 5H, Ar-*m, p* and *o*), 9.25 (s, 1H, NH-11). Anal. CHN $C_{20}H_{20}O_5N_2$.

4.1.3.2. N-(6-Allyl-1,3-benzodioxol-5-yl)-N-(tert-butoxy carbonyl)alanynamide ***(4b)***. Yield 42.85%; m.p. 93–95 °C; TLC R_f = 0.44 (7:3 hexane/ethyl acetate); I.R. (cm^{-1}): 1037 *N*C–O–C sym, 1250 *N*C–O–C assym, 1172 *N*C–C(=O)–O, 3341 *N*N–H, 1659 *N*C=O amide, 1684 *N*C=O carbamate, 1730 *N*C=O ester; ^{1}H-NMR (*Δ* ppm): 1.38 (s, 9H, $(CH_3)_3$), 1.82–1.92 (m, 1H, CH_2β), 1.98–2.04 (m, 1H, CH_2β), 2.45 (broad, 2H, CH_2γ), 3.20 (d, *J* = 6.89 Hz, 2H, $CH_2$8), 4.07–4.09 (m, 1H, CHα), 4.96 (dd, *J* = 1.79 Hz, *J* = 17.09 Hz, 1H, $CH_2$10a), 5.04 (dd, *J* = 1.79 Hz, *J* = 10.19 Hz, 1H, $CH_2$10b), 5.09 (s, 2H, CH_2α″), 5.76–5.89 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.73 (s, 1H, Ar-4), 6.86 (s, 1H, Ar-7), 7.11 (d, *J* = 7.49 Hz, 1H, NH-14), 7.32-7.37 (m, 5H, Ar-*m, p* and *o*), 9.14 (s, 1H, NH-11). Anal. CHN $C_{18}H_{24}O_5N_2$.

4.1.3.3. N-(6-Allyl-1,3-benzodioxol-5-yl)-N-(tert-butoxy carbonyl)valynamide ***(4c)***. Yield 24.27%; m.p. 203–205 °C; TLC R_f = 0.74 (3:2 hexane/ethyl acetate); I.R. (cm^{-1}): 1043 *N*C–O–C sym, 1248 *N*C–O–C assym, 1171 *N*C–C(=O)–O, 3322 *N*N–H, 1653 *N*C=O amide, 1690 *N*C=O carbamate; ^{1}H-NMR (*Δ* ppm): 0.89 (d, *J* = 6.89 Hz, 3H, $CH_3\gamma_1$), 0.92 (d, *J* = 6.89 Hz, 3H, $CH_3\gamma_2$),1.39 (s, 9H, $(CH_3)_3$), 1.99–2.02 (m, 1H, CHβ), 3.22 (d, *J* = 6.60 Hz, 2H, $CH_2$8), 3.77 (dd, *J* = 5.70 Hz, *J* = 7.79 Hz, 1H, CHα), 4.97 (dd, *J* = 1.20 Hz, *J* = 16.79 Hz, 1H, $CH_2$10a), 5.05 (dd, *J* = 1.20 Hz, *J* = 10.19 Hz, 1H, $CH_2$10b), 5.77–5.91 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.73 (s, 1H, Ar-4), 6.81 (s, 1H, Ar-7), 6.91 (d, *J* = 7.79 Hz, 1H, NH-14), 9.18 (s, 1H, NH-11). Anal. CHN $C_{20}H_{28}O_5N_2$.

4.1.3.4. N-(6-Allyl-1,3-benzodioxol-5-yl)-N-(tert-butoxy carbonyl)leucynamide ***(4d)***. Yield 46.4%, m.p. 211–212 °C; TLC R_f = 0.87 (3:2 hexane/ethyl acetate I.R. (cm^{-1}): 1042 *N*C–O–C sym, 1250 *N*C–O–C assym, 1172 *N*C–C(=O)–O, 3363 *N*N–H, 1649 *N*C=O amide, 1691 *N*C=O carbamate; ^{1}H-NMR (*Δ* ppm): 0.82 (d, *J* = 6.90 Hz, 3H, $CH_3\Delta$), 0.85 (d, *J* = 6.60 Hz, 3H, $CH_3\Delta$), 0.90 (t, *J* = 6.60 Hz, 2H, CH_2β), 1.37 (s, 9H, $(CH_3)_3$), 1.43–1.53 (m, 1H, CHγ), 3.20 (d, *J* = 5.09 Hz, 2H, $CH_2$8), 4.05–4.13 (m, 1H, CHα), 4.99 (dd, *J* = 2.02 Hz, *J* = 17.09 Hz, 1H, $CH_2$10a), 5.06 (dd, *J* = 2.02 Hz, *J* = 9.90 Hz, 1H, $CH_2$10b), 5.79–5.88 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.73 (s, 1H, Ar-4), 6.84 (s, 1H, Ar-7), 7.04 (d, *J* = 8.09 Hz, 1H, NH-14), 9.11 (s, 1H, NH-11). Anal. CHN $C_{21}H_{30}O_5N_2$.

4.1.3.5. N-(6-Allyl-1,3-benzodioxol-5-yl)-N-(tert-butoxy carbonyl)phenylalaninamide ***(4e)***. Yield 36.6%, m.p. 110–113 °C; TLC R_f = 0.53 (7:3 hexane/ethyl acetate); I.R. (cm^{-1}): 1044 *N*C–O–C sym, 1219 *N*C–O–C assym, 1169 *N*C–C(=O)–O, 3323 *N*N–H, 1658 *N*C=O amide, 1690 *N*C=O carbamate; ^{1}H-NMR (*Δ* ppm): 1.33 (s, 9H, $(CH_3)_3$), 2.84 (dd, *J* = 13.2 Hz, *J* = 9.6 Hz, 1H, CH_2β), 3.01 (dd, *J* = 13.49 Hz, *J* = 5.69 Hz, 1H, CH_2β), 3.15 (d, *J* = 6.89 Hz, 2H, $CH_2$8), 4.27–4.32 (m, 1H, Hα), 4.97 (dd, *J* = 1.8 Hz, *J* = 16.8 Hz, 1H, $CH_2$10a), 5.05 (dd, *J* = 1.8 Hz, *J* = 9.90 Hz, 1H, $CH_2$10b), 5.75–5.88 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.72 (s, 1H, Ar-4), 6.73 (s, 1H, Ar-7), 7.13 (d, *J* = 8.40 Hz, 1H, NH-14), 7.29–7.30 (m, 5H, Ar-*m*, *p* and *o* prótons), 9.23 (s, 1H, NH-11). Anal. CHN $C_{24}H_{28}O_5N_2$.

4.1.3.6. N-(6-Allyl-1,3-benzodioxol-5-yl)- N^6-(9H-fluoren-9-ylmetoxicarbonylamino) N-(tert-butoxy carbonyl)lysinamide ***(4f)***. Yield 92.60%; m.p. 115–117 °C; TLC R_f = 0.51 (3:2 hexane/ethyl acetate); I.R. (cm^{-1}): 1027 *N*C–O–C sym, 1262 *N*C–O–C assym, 1165 *N*C–C(=O)–O, 3317 *N*N–H, 1654 *N*C=O amide, 1689 *N*C=O carbamate; ^{1}H-NMR (*Δ* ppm): 1.14–1.23 (m, 2H, CH_2γ), 1.36–1.37 (m, 2H, Hβ), 1.38 (s, 9H, $(CH_3)_3$), 1.44–1.47 (m, 2H, HΔ), 2.96–3.00 (m, 2H, Hϵ), 3.20 (d, *J* = 6.30 Hz, 2H, $CH_2$8), 3.27 (s, 1H, CHβ′), 3.99–4.02 (m, 1H, CHα), 4.98 (dd, *J* = 1.79 Hz, *J* = 17.09 Hz, 1H, $CH_2$10a), 5.05 (dd, *J* = 1.79 Hz, *J* = 10.19 Hz, 1H, $CH_2$10b), 5.08 (s, 2H, CH_2α′) 5.79–5.88 (m, 1H, CH9), 5.97 (s, 2H, $CH_2$2), 6.64 (broad s, 1H, NH-ζ), 6.73 (s, 1H, Ar-4), 6.85 (s, 1H, Ar-7), 6.99 (d, *J* = 8.39 Hz, 1H, NH-14), 7.39–7.34 (m, 4H, Ar-γ′), 7.44–7.49 (m, 4H, Ar-γ′), 9.12 (s, 1H, NH-11). Anal. CHN $C_{31}H_{32}O_6N_2Cl_2$.

4.1.3.7. N-(6-Allyl-1,3-benzodioxol-5-yl)-O^4-benzyl ester-N-(tert-butoxy carbonyl)aspartic 1-amide ***(4g)***. Yield 30.65%; m.p. 110–112 °C; TLC R_f = 0.46 (3:2 hexane/ethyl acetate) I.R. (cm^{-1}): 1040 *N*C–O–C sym, 1219 *N*C–O–C assym, 1147 *N*C–C(=O)–O, 3263 *N*N–H, 1666 *N*C=O amide, 1744 *N*C=O ester; ^{1}H-NMR (*Δ* ppm): 3.21 (d, *J* = 6,30 Hz, 2H, $CH_2$8), 3.78 (d, *J* = 6.30 Hz, 2H, CH_2α), 4.99 (dd, *J* = 1.80 Hz, *J* = 16.79 Hz, 1H, $CH_2$10a), 5.03 (dd, *J* = 1.80 Hz, *J* = 10.19 Hz, 1H, $CH_2$10b), 5.05 (s, 2H, CH_2α′), 5.77–5.86 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.74 (s, 1H, Ar-4), 6.89 (s, 1H, Ar-7), 7.31–7.37 (m, 5H, Ar-β′), 7.57 (t, *J* = 6.30 Hz, 1H, NH-14), 9.15 (s, 1H, NH-11). Anal. CHN $C_{26}H_{30}O_7N_2$.

N-(6-Allyl-1,3-benzodioxol-5-yl)-O^4-benzyl ester-*N*-(*tert*-butoxy carbonyl)glutamic **(4h)**

Yield 31.35%; m.p. 75–78 °C; TLC R_f = 0.70 (7:3 hexane/ethyl acetate); I.R. (cm^{-1}): 1038 *N*C–O–C sym, 1215 *N*C–O–C assym, 1167 *N*C–C(=O)–O, 3324 *N*N–H, 1659 *N*C=O amide, 1690 *N*C=O carbamate; ^{1}H-NMR (*Δ* ppm): 1.25 (d, *J* = 7.49 Hz, 3H, CH_3β), 1.38 (s, 9H, $(CH_3)_3$), 3.20 (d, *J* = 6.89 Hz, 2H, $CH_2$8), 4.07–4.12 (m, 1H, CHα), 5.03 (dd, *J* = 2.20 Hz, *J* = 17.09 Hz, 1H, $CH_2$10a), 5.05 (dd, *J* = 2.20 Hz, *J* = 10,19 Hz, 1H, $CH_2$10b), 5.77–5.91 (m, 1H, CH9), 5.98 (s, 2H, $CH_2$2), 6.73 (s, 1H, Ar-4), 6.86 (s, 1H, Ar-7), 7.06 (d, *J* = 6.59 Hz, 1H, NH-14), 9.07 (s, 1H, NH-11). Anal. CHN $C_{27}H_{32}O_7N_2$.

*4.1.4. (6-Nitro-benzo [1,3]dioxole-5-yl) acetic acid **(5)***

The aqueous $KMnO_4$ (0.135 mol) in about 150 ml of water was stirred and cooled out in an ice bath. A solution of compound **2** (0.024 mol), benzyldimethyltetradecylammonium chloride dihydrate (0.0012 mol), and 30 ml of acetone, 150 ml of benzene, and 30 ml of acetic acid was added in one portion. After 2 h, stirring continued without any further addition of ice to the bath for 70 h. A total of 40 g of $NaHSO_3$ was added to the cooled reaction mixture followed by the slow addition of 70 ml of solution of aqueous H_2SO_4 (25 g of concentrated H_2SO_4 in 100 ml of water). Two clear layers resulted. The layers were separated and the organic layer was washed once with a 100 ml of portion of water. The organic layer was dried over anhydrous sodium sulfate, the drying agent was removed by filtration, and the solvent was removed on a rotary evaporator. The crude product was chromatographed on silica gel with 5% ethyl acetate in hexane to give 3.25 g (60%), of a yellow solid. m.p. 180–182 °C. ^{1}H-NMR (Δ ppm): 3.9 (s, 2H, CH_28); 6.23 (s, 2H, CH_22); 7.12 (s, 1H, Ar-7); 7.69 (s, 1H, Ar-4); 12.48 (s, 1H, COOH). Anal. CHN $C_9H_7O_6N$.

*4.1.5. General procedure for the synthesis of peptidyl derivatives of safrole (**6a**–**d**)*

To a stirred solution of 2.5 mmol of the N-protected amino acid in dimethylformamide under 0 °C, 6.8 mmol of the diciclohexylcarbodiimide, 5.1 mmol of hydroxybenzotriazole and 1.7 mmoles of 6-alyl-benzo [1,3]dioxole-5-ilamine (**3**) was added and was allowed to warm at room temperature. After that, the reaction mixture was filtered, and the filtrate was treated with ethyl acetate. The organic phase was washed with the $NaHCO_3$ water, aqueous NaCl and dried (Na_2SO4) and concentrated. The residue was treated with hexane and filtered. The safrole derivatives **4a**–**d**, were obtained as white crystals.

*4.1.5.1. Ethyl N-[(6-nitro-1,3-benzodioxol-5-yl) acetyl] glycinate **(6a)**.* Yield 99%; m.p. 130–133 °C; TLC R_f = 0.83 (ethyl acetate); I.R. (cm^{-1}): 3324 NN–H amide 2[a], 1628 *N*C=O amide, 1524 *N*Ar–NO_2 assym, 1260 *N*Ar–NO_2 sym, 1.739 *N*C=O ester; ^{1}H-NMR (Δ ppm): 1.19 (t, *J* = 7.19 Hz, 3H, $CH_3\beta$), 3.82 (d, *J* = 5,7 Hz, 2H, $CH_2\alpha'$); 3.85 (s, 2H, $CH_2\alpha$), 4.08 (q, *J* = 7.19 Hz, 2H, $CH_2\alpha''$); 6.21 (s, 2H, CH_22), 7.06 (s, 1H, Ar-7), 7.61 (s, 1H, Ar-4), 8.33 (broad, 1H, NH-10). Anal. CHN $C_{13}H_{14}O_7N_2$.

*4.1.5.2. N-[(6-nitro-1,3-benzodioxol-5-yl) acetyl]leucinamide **(6b)**.* Yield 17.80%; m.p. 175–176 °C; TLC R_f = 0.30 (1:1 hexane/ethyl acetate); I.R. (cm^{-1}): 3326 *N*N–H amide 2[a], 1629 *N*C=O amide, 1574 *N*Ar–NO_2 assym, 1244 *N*Ar–NO_2 sym, 3387 *N*N–H assym, 3199 *N*N–H sym; ^{1}H-NMR (Δ ppm): 0.79 (dd, *J* = 7.49 Hz, *J* = 6.8 Hz, 3H, $CH_3\Delta$), 0.83 (d, *J* = 5.89 Hz, 3H, $CH_2\gamma$); 1.02–1.09 (m, 1H, $CH_2\gamma_2$), 1.18–1.30 (m, 1H, $CH_2\gamma_2$), 1.59–1.73 (m, 1H, CHβ), 3.80 (d, *J* = 15.89 Hz, 1H, $CH_2\alpha$), 3.91 (d, *J* = 16.19 Hz, 1H, $CH_2\alpha$), 4.11 (dd, *J* = 6.59 Hz, *J* = 9.00 Hz, 1H, $CH\alpha'$), 6.21 (s, 2H, CH_22), 7.03 (s, 1H, NH_2-13), 7.05 (s, 1H, Ar-7), 7.35 (s, 1H, NH_2-13), 7.62 (s, 1H, Ar-4), 7.98 (d, *J* = 9.29 Hz, 1H, NH-10). Anal. CHN $C_{15}H_{19}O_6N_3$.

*4.1.5.3. N-[(6-nitro-1,3-benzodioxol-5-yl) acetyl]phenylalaninamide **(6c)**.* Yield 76.40%; m.p. 158–160 °C; TLC R_f = 0.40 (1:1 hexane/ethyl acetate); I.R. (cm^{-1}): 3320 *N*N–H amide 2[a], 1627 *N*C=O amide, 1527 *N*Ar–NO_2 assym, 1330 *N*Ar–NO_2 sym, 3320 *N*N–H assym, 3196 *N*N–H sym; ^{1}H-NMR (Δ ppm): 2.80 (dd, *J* = 13.80 Hz, *J* = 4.50 Hz, 1H, $CH_2\beta$), 3.01 (dd, *J* = 13.80 Hz, *J* = 4.80, 1H, $CH_2\beta$); 3.73 (d, *J* = 16.20 Hz, 1H, $CH_2\alpha$), 3.82 (d, *J* = 16.20 Hz, 1H, $CH_2\alpha$); 4.43 – 4.41 (m, 1H, $CH_2\alpha'$); 6.20 (s, 2H, CH_22), 6.88 (s, 1H, Ar-7), 7.0 (s, 1H, NH_2-13); 7.29 – 7.21 (1H do NH_2-13, 5H do Ar-*m, p* and *o*); 7.57 (s, 1H, Ar-4), 8.07 (d, *J* = 7.80 Hz, 1H, NH-10). Anal. CHN $C_{18}H_{17}O_6N_3$.

*4.1.5.4. N-[(6-nitro-1,3-benzodioxol-5-yl) acetyl]-O[4]-(2,6-dichlorophenyl)tyrosinamide **(6d)**.* Yield 46.15%; m.p. 150–153 °C; TLC R_f = 0.80 (ethyl acetate); I.R. (cm^{-1}): 3326 *N*N–H amide 2[a], 3326 *N*N–H assym, 3193 *N*N–H sym, 1628 *N*C=O amide, 1532 *N*Ar–NO_2 assym, 1243 *N*Ar–NO_2 sym; ^{1}H-NMR (Δ ppm): 2.85 (dd, *J* = 9.10 Hz, *J* = 14.10 Hz, 1H, $CH_2\beta$), 3.06 (dd, *J* = 5.10 Hz, *J* = 14.10, 1H, $CH_2\beta$); 3.35–3.34 (m, 2H, $CH_2\alpha$), 4.61–4.54 (m, 1H, $CH\alpha'$); 5.21 (s, 2H, $CH_2\alpha''$); 6.25 (s, 2H, CH_22), 6.77 (s, 1H, Ar-7), 6.97 (d, *J* = 8.70 Hz, 2H, Ar-γ); 7.09 (s, 1H, NH_2-13); 7.22 (d, *J* = 8.70 Hz, 2H, Ar-γ); 7.42 (s, 1H, NH_2-13); 7.53 (s, 1H, Ar-4), 7.56–7.45 (3H, Ar - β′), 8.60 (s, 1H, NH-10). Anal. CHN $C_{25}H_{21}O_7N_3Cl_2$.

4.2. Biology

4.2.1. Antimicrobial activity

The antimicrobial activity was determined in vitro by disc diffusion method against *Staphylococcus aureus, Sarcina lutea, B. subtilis, Streptococcus faecalis, 381, Staphylococcus aureus, 466 Staphylococcus aureus, E. coli, K. pneumoniae, Shigella sonei, Salmonella enteritides, P. aeruginosa and Candida albicans* from Departamento de Antibióticos da Universidade Federal de Pernambuco collection. It is dispensed into Petri dishes to yield a uniform depth of 6 mm. Overnight cultures were grown in adequate broth and temperature, and diluted to obtain an opacity equivalent to 0.5 on the Mc Farland scale. The drugs are weighed and dissolved in acetone to give concentrations equal to 2 mg/ml. The discs were placed in the surface of culture medium after the complete evaporation of acetone. The plates are incubated for 18 h at adequate temperature.

4.2.2. Toxicity tests

Thirty-six male Swiss adult white mice (ca. 25–28 g) were divided into six groups and privately fed 20 h before injections. The preliminary phase dosages are 500, 250, 100, 50, 5 mg/kg to compound **4e**. It was diluted in Tween 80 (two crops) and saline (0.9%). The single intraperitoneal injec-

tions are given and the general effects were observed during 60 min and continued about 72 h after injection. The control group, receiving solvent only.

4.2.3. Antitumor activity

Compounds were diluted in Tween 80 (two crops) and saline (0.9%). Females, 20–25 g, from Departamento de Antibióticos da Universidade Federal de Pernambuco, were divided into six groups. Transplants of tumour fragments, about 1.5–2 mm of Sarcoma S-180 are made subcutaneously in lateral thoracic wall of six mice; starting 48 h later, injections (35 mg/kg) of test compounds are made i.p. once daily for 8 days. After treatment, tumours were removed and measured. The efficacy of treatment was estimated by the ellipsoid formula, and V of control group was taken in calculations for 100%.

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