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Short communication

Synthesis and cytotoxic activity of *N*-substituted thiosemicarbazones of 3-(3,4-methylenedioxy)phenylpropanal

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Abstract

Five new *N*-substituted thiosemicarbazones of 3-(3,4-methylenedioxy)phenylpropanal were synthesized. Safrole, a natural product obtained from sassafras oil (*Ocotea pretiosa*), was oxidized to alcohol using BH_3–THF and H_2O_2, followed by oxidation to aldehyde using pyridinium dichromate (PDC) and condensation with five *N*-substituted derivatives of thiosemicarbazide. Tests were performed to evaluate the cytotoxic activity with continuous chain KB cells (epidermoide carcinoma of the floor of the mouth). Compounds **5** and **6** showed IC_{50} values of 1.5 and 4.6 μg/ml, respectively.

Keywords: Safrole; Thiosemicarbazones; Cytotoxic activity

1. Introduction

The thiosemicarbazones continue to be a class of compounds with a broad spectrum of therapeutic activities. Several members of this class have antineoplastic [1], anti-inflammatory [2], tuberculostatic [3,4] and antiviral, including anti-HIV [5,6], activities. The cytotoxic activity against cancer cells has also been investigated [7].

Motivated by such a broad spectrum of therapeutic activities we synthesized a set of new thiosemicarbazones. During previous studies we have observed that the thiosemicarbazone moiety and an aromatic ring are important for biological activity.

To obtain these new compounds, we utilized safrole obtained by vacuum distillation of sassafras oil, which was extracted from *Ocotea pretiosa*, very common in the southern region of Brazil. Safrole's chemical structure and its abundance have interested researchers to use it as raw material in the synthesis of biologically active molecules.

The allyl side chain of the safrole was oxidized with BH_3–THF and H_2O_2 to give the alcohol (**2**) [8]. The alcohol obtained was oxidized with pyridinium dichromate (PDC) to give the aldehyde (**3**), as described by Corey and Schmidt [9]. The thiosemicarbazones of the 3-(3,4-methylenedioxy)phenylpropanal (**4–8**) were obtained by condensation of the aldehyde with five thiosemicarbazide derivatives in ethanol and a catalytic amount of acetic acid (Scheme 1).

The compounds synthesized were evaluated against continuous chain cells KB (epidermoide carcinoma of the floor of the mouth) [10].

2. Experimental

2.1. Chemistry

The melting points of the synthesized compounds were determined by a capillary method in a Thomas Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AMX400 400 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane as the internal reference. Chemical shifts are reported in parts per million (δ), and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). IR spectra were recorded on a Nicolet 510 FT-IR spectrometer in KBr pellets. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, USA, and the results for the indicated elements

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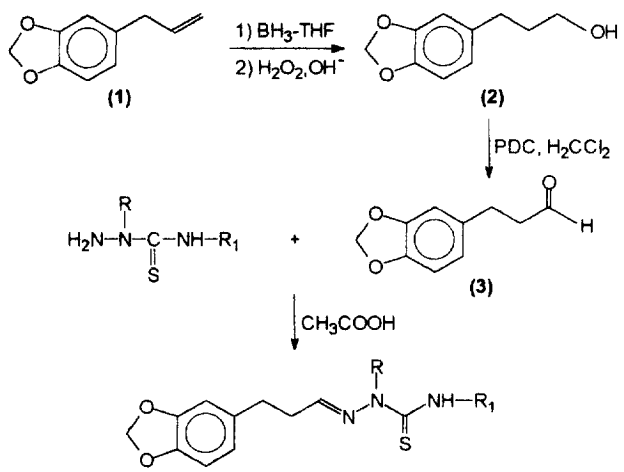

(4): R=H, R_1=H; (5): R=H, R_1=CH_3; (6): R=H, R_1=CH_2CH_3; (7): R=H, R_1=C_6H_5; (8): R=CH_3, R_1=H

Scheme 1.

were within ±0.3% of the calculated values. All reactions were monitored using thin layer chromatography (TLC) on Analtech, 200 mm silica gel GF plates.

2.1.1. 3-(3,4-Methylenedioxy)phenylpropanol (2)

An anhydrous tetrahydrofuran (THF) solution (109 ml, 109 mmol) of borane–THF complex was stirred at 0–5°C under nitrogen atmosphere. Then a solution of safrole (8.91 g, 55 mmol) in 150 ml of anhydrous THF was added. The reaction mixture was stirred at room temperature for 5 h, cooled to 0°C, treated with 34 ml solution of THF–water (1:1), 42 ml of 2N sodium hydroxide solution and 34 ml of 30% hydrogen peroxide solution. Each addition required 10 minutes. The turbid mixture was stirred for 2.5 h, chilled again, and treated portionwise with 110 ml of saturated aqueous solution of sodium thiosulfate to decompose the excess peroxide. Sodium chloride was added to saturate the aqueous layer, which was separated and extracted with ethyl acetate. The organic phase was dried with anhydrous sodium sulfate, then evaporated under vacuum to give a clear and viscous oil, which was purified by flash chromatography using ethyl acetate and hexane (1:1) as eluant. Yield: 3.30 g (37%).

2.1.2. 3-(3,4-Methylenedioxy)phenylpropanal (3)

In a round flask previously dried at 250°C for 2 h, PDC (10 g) and 300 ml of anhydrous dichloromethane were added. The resulting suspension was stirred at room temperature and under nitrogen atmosphere for 40 minutes. Then, 3.19 g of alcohol **2** were added, and the reaction mixture was stirred for 24 h. Upon completion of the oxidation as indicated by TLC, 150 ml of a mixture of diethyl ether and n-pentane (1:1) were added, then the reaction mixture was stirred for 1 h, filtered and treated with 450 ml of 5% sodium hydroxide solution. The organic phase was dried with anhydrous sodium sulfate and evaporated under vacuum. A dark-coloured and viscous oil was obtained which was used in the next step without further purification. Yield: 2.59 g (82%).

Table 1
Physicochemical data of the thiosemicarbazones **4–8**

Compound	R	R_1	Formula [a]	M.P. (°C)	Yield (%)
4	H	H	$C_{11}H_{13}O_2N_3S$	127–28	43
5	H	CH_3	$C_{12}H_{15}O_2N_3S$	119–20	30
6	H	C_2H_5	$C_{13}H_{17}O_2N_3S$	84–85.5	47
7	H	C_6H_5	$C_{17}H_{17}O_2N_3S$	169–69.5	46
8	CH_3	H	$C_{12}H_{15}O_2N_3S$	163–64	35

[a] The analytical results for C, H and N were within ±0.3% of the calculated values.

2.1.3. General procedure for obtaining the thiosemicarbazones (4–8)

A ethanolic solution containing 427 mg (2.4 mmol) of the aldehyde was added to a solution containing 2.0 mmol of thiosemicarbazide in ethanol, followed by addition of five drops of glacial acetic acid. The reaction mixture was refluxed with constant stirring. The reaction was monitored by TLC, then at the end of the reaction the precipitated crystals were filtered, washed with water and dried under vacuum. The purification was done with the help of chromatography on a column of silica gel, utilizing ethyl acetate and hexane (2:3) as an eluting system. The characteristics of the thiosemicarbazones are collected in Table 1. The IR spectra exhibited the following bands (cm^{-1}): 3327–3424 (N–H); 1543–1603 (C=N); 1240–1246 (C=S); 1435–1442 (N–CS–N) and 1035–1039 (C–O-ϕ). The ^1H NMR spectra were consistent with the described structures and, because of the lack of unusual features, only the spectra of two isomeric thiosemicarbazones **5** and **8** are described as examples.

5: δ 11.07 (s, 1H, NH, D_2O exchangeable); 8.06 (q, 1H, J=4.4 Hz, NH, D_2O exchangeable); 7.41 (t, 1H, J=5 Hz, CH); 6.82–6.66 (m, 3H, aromatic-H); 5.96 (s, 2H, O–CH_2–O); 2.93 (d, 3H, J=4.5 Hz, CH_3); 2.72 (t, 2H, J=7.5 Hz, CH_2); 2.50–2.43 (m, 2H, CH_2).

8: δ 8.19 (s, 1H, NH_2, D_2O exchangeable); 7.84 (s, 1H, NH_2, D_2O exchangeable); 7.18 (t, 1H, J=4.5 Hz, CH); 6.84–6.68 (m, 3H, aromatic-H); 5.96 (s, 2H, O–CH_2–O); 3.55 (s, 3H, CH_3); 2.78 (t, 2H, J=7.5 Hz, CH_2); 2.60–2.49 (m, 2H, CH_2).

2.2. Biology

The tissue culture method with continuous chain KB cells in the exponential growth phase was used for the determination of the cytotoxic activity of the thiosemicarbazones of 3-(3,4-methylenedioxy)phenylpropanal.

A MEM (minimal essential medium) [11], enriched with 10% of fetal bovine serum, 1% of L-glutamine and 1% of antibiotics (penicillin and streptomycin) was utilized for cultivation of cells and to perform the tests. Adriamycin was used as a standard. The methodology utilized for the cytotoxicity test is in accordance with the 'Protocol for Screening Chemical Agents and Natural Products' [12].

Table 2
Results of the cytotoxic test performed with continuous chain KB cells

Compound	4	5	6	7	8	Standard
R	H	H	H	H	CH_3	
R_1	H	CH_3	C_2H_5	C_6H_5	H	adriamycin
IC_{50} (μg/ml)	>10	1.5	4.6	>10	>10	≤1.0

2.2.1. Determination of the cytotoxic activity

KB cells were maintained as monostratified cultures for a 24 h assay after which they were removed from the surface medium using 0.25% (wt./vol.) trypsin and diluted to 3×10^4 cells/ml. 0.9 ml of the cell suspension was spread on a culture plate with 0.1 ml of dimethylsulfoxide solution of the compound to be tested with the following concentrations: 10, 5, 2.5 and 1.25 μg/ml. Three test plates were prepared for each concentration. The plates were incubated for 72 h at 37°C in an atmosphere containing 5% of CO_2. The cytotoxic activity was evaluated through percentage inhibition of the treated growth related to the control. The cell proliferation was measured by protein concentration [13], which permitted calculation of IC_{50}.

3. Results and discussion

The results of the cytotoxic evaluation in vitro showed that compound **5** inhibited 50% of cellular growth of KB cells at a concentration of 1.5 μg/ml and compound **6** at 4.6 μg/ml. The remaining compounds were inactive up to a concentration of 10 μg/ml (Table 2).

In accordance with the NCI (National Cancer Institute), compounds presenting an IC_{50} equal to or less than 4 μg/ml, such as compound **5**, are considered to be satisfactory for animal studies.

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