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Artigo

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Silva, A. T. M.; Pereira, V. V.; de Almeida, L. T. G.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Dias, D. F.; de Moreira, M. E. C.; Silva, R. R.; Duarte, L. P.*

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Síntese e Atividade Biológica de Ésteres do Borneol

Resumo: Ésteres do borneol têm sido considerados como promissores agentes anti-inflamatórios e antimicrobianos. Neste trabalho, oito ésteres do borneol (**1-8**) foram sintetizados utilizando o método DIC/DMAP ou $SOCl_2$ e os produtos caracterizados por meio de técnicas espectroscópicas. Seis desses compostos são inéditos e o método DIC/CMAP foi mais rápido e resultou em rendimentos de reação elevados. Os compostos **1-8** foram submetidos ao ensaio antiproliferativo *in vitro* utilizando linhagens de células normais e tumorais e à avaliação da atividade antiedematogênica. O composto **6** [(1S,2R,4S)-1,7,7-trimetilbiciclo[2.2.1]heptan-2-il 3,4,5-trimetoxibenzoato] apresentou promissora atividade citotóxica contra várias linhagens de células tumorais e **7** [(1S,2R,4S)-1,7,7-trimetilbiciclo[2.2.1]heptan-2-il benzoato] foi efetivo na redução da resposta edematogênica em todos os períodos de tempo avaliados.

Palavras-chave: Ésteres do borneol; atividade antiproliferativa; atividade antiedematogênica.

Abstract

Borneol derivatives have been regarded as promising anti-inflammatory and antimicrobial agents. In this work, eight borneol esters (**1**-**8**) were synthesized using DIC/DMAP or $SOCl_2$ method and the products characterized by means of spectroscopic techniques. Six of them are new compounds and the DIC/DMAP method was faster and resulted in high reaction yields. Compounds **1**-**8** were subjected to *in vitro* antiproliferative assay using normal and tumor human cell lines and to antioedematogenic activity evaluation. Compound **6** [(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 3,4,5-trimethoxybenzoate] presented a promisor cytotoxic activity against various tumor cell lines and **7** [(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl benzoate] was effective in the reduction of oedematogenic response in all period of time evaluated.

Keywords: Borneol ester; antiproliferative activity; antioedematogenic activity.

* Universidade Federal de Minas Gerais, Departamento de Química, Belo Horizonte, CEP 31270-901, Brazil.
lucienir@ufmg.br
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Synthesis and Biological Activity of Borneol Esters

Aline T. M. e Silva,[a] Vinicius V. Pereira,[a,b] Laila Thayanne G. de Almeida,[b] Ana Lúcia T. G. Ruiz,[c] João Ernesto de Carvalho,[c,d] Danielle F. Dias,[e] Maria Eliza C. de Moreira,[e] Roqueline R. Silva,[f] Lucienir P. Duarte[b,*]

[a] Universidade Federal de Minas Gerais, Faculdade de Farmácia, CEP 31270-901 Belo Horizonte-MG, Brazil.

[b] Universidade Federal de Minas Gerais, Departamento de Química, CEP 31270-901 Belo Horizonte-MG, Brazil

[c] Universidade Estadual de Campinas, Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, CEP 13148-218, Paulínia-SP, Brazil.

[d] Universidade Estadual de Campinas, Faculdade de Ciências Farmacêuticas, C.P. 6029, CEP 13083-859, Campinas-SP, Brazil.

[e] Universidade Federal de Alfenas, Laboratório de Fitoquímica e Química Medicinal, CEP 37130-000, Alfenas-MG, Brazil.

[f] Universidade Federal dos Vales do Jequitinhonha e Mucuri, Departamento de Química, CEP 39100-000 Diamantina-MG, Brazil.

* lucienir@ufmg.br

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1. Introduction

Borneol, a bicyclic monoterpenoid alcohol, is used in food, cosmetics and also in the traditional medicine to treat painful and inflammatory conditions.[1] This natural product is frequently found as constituent of essential oils from numerous families of plants (*e.g,* Asteraceae, Lamiaceae,

Valerianaceae and others).[2] The essential oil of *Ampelopsis megalophylla* a species of the Vitaceae family was analyzed by GC-MS and presented borneol as the major constituent (10.8%).[3] Furthermore, two cinnamic esters of borneol were identified for the first time in *Cistus* genus.[4]

Previous investigation of borneol and its ester derivatives have showed their antimicrobial and anti-inflammatory properties.[5,6] Caffeic acid bornyl ester was previously isolated from the chloroform extract of *Valeriana wallichii* rhizome and presented antileishmanial activity. Other 27 borneol derivatives were synthesized and the cinnamic acid bornyl ester showed the best activity against *Leishmania major* and *Leishmania donovani* promastigotes (IC_{50}= 39.6 and 15.6 µM, respectively) with cytotoxicity in acceptable levels.[7]

Antitrypanosomal activity was reported for two synthetic borneol derivatives: trimethoxy-benzoate and benzoate bornyl ester. These compounds presented an antiproliferative effect on the epimastigote forms of the parasite *Trypanosoma cruzi* ($IC_{50/72h}$= 28.9 and 49.5 µM, respectively) and both compounds were more selective against epimastigotes than HEp-2 cells. Thus, bornyl benzoate derivatives can be a potential chemotherapeutic agents against *T. cruzi* infections.[5]

Bornyl salicylate is another example of borneol derivative that presents anti-inflammatory effect in topical use. Recent study evaluated the effect of bornyl salicylate in experimental models of acute inflammation and signs of acute toxicity were not observed in male and female mice. Moreover, treatment with bornyl salicylate was effective in the reduction of paw edema in early and late phases, suggesting an anti-inflammatory effect related with the decrease of pro-inflammatory mediators.[6] In a recent report was described the synthesis of some natural product esters with improved biological activity.[8] Keeping this in mind, the present work aimed to synthesize eight borneol ester derivatives and evaluate their antiproliferative and antioedematogenic properties, that were not described yet.

2. Experimental

2.1. Chemistry

2.1.1. Materials and methods

Commercially reagent grade chemicals were used as received without additional purification. (-)-Borneol was purchased at 95% purity from Sigma-Aldrich. All reactions were monitored by thin layer chromatography (TLC) processes. Column chromatographic purifications were performed using silica gel 60 (70–230 Mesh). The IR spectra were carried out on a Perkin Elmer – Spectrum One (ATR, 4000-400 cm^{-1}) spectrometer. The ^{1}H and ^{13}C spectra were recorded on a Bruker AVANCE DPX-200 spectrometry at 200 and 50 MHz, respectively, using CDCl3 as solvent. Chemical shifts are reported in parts per million (δ ppm) using tetramethylsilane (TMS) as internal standard. Melting points were determined on a Microquímica MQAPF 301 hot plate apparatus and are uncorrected.

2.1.2. Synthesis of borneol ester derivatives using DIC/DMAP

(–)-Borneol (1 mmol) and the appropriate organic acid (5 mmol) with catalytic amount of 4-dimethylaminopyridine (DMAP) were dissolved in dichloromethane (6 mL) and cooled in an ice bath. The mixture was stirred for an additional 10 min and then diisopropylcarbodiimide (DIC) (5 mmol) was added. The mixture was removed from the ice bath and was magnetically stirred at room temperature until esterification was complete.[9] The progress of the reaction was monitored by TLC using as eluent a mixture of chloroform and n-hexane in different proportions. After finishing, the reaction

mixture was evaporated in vacuum to dryness. The residue was purified by means of column chromatography on silica gel using hexane/chloroform (1:1, V/V) as eluent. All the synthesized compounds were oils, except **6** and **8**, which were solids.

2.1.3. Synthesis of borneol ester derivatives using $SOCl_2$

A solution of 2.0 mL (27.5 mmol) of thionyl chloride and 1 mmol of each acid were added in a round bottom flask. After heating the reaction mixture for 3 h in reflux, thionyl chloride excess was removed under vacuum. The remaining residue was dissolved in toluene (2 mL) and 1 mmol of (–)-borneol was added. This mixture was stirred in reflux and the reaction was monitored by TLC using as eluent a mixture of chloroform and n-hexane in different proportions. $NaHCO_3$ aqueous solution (5%) was slowly added when the reaction was finished. This last mixture was extracted with chloroform (3× 25 mL) and the organic phase was washed again with 30 mL of $NaHCO_3$ aqueous solution (5%) and then with water (2× 20 mL). The organic phases were pooled and dried with anhydrous $NaSO_4$. Organic solvent was removed using a rotary evaporator and the residue was purified by silica gel column chromatographic using as eluent a mixture of chloroform and n-hexane in different proportions.

2.2. Biological activities

2.2.1. Antiproliferative assay

The human cancer cell lines MCF-7 (human breast adenocarcinoma), NCI-ADR/RES (drug resistant ovarian tumor), 786-0 (renal cell adenocarcinoma), OVCAR-3 (human ovarian carcinoma), HT-29 (colon adenocarcinoma), K562 (leukemia) were kindly provided by Frederick Cancer Research & Development Center–National Cancer Institute–Frederick, MA, USA. HaCat cell line (immortalized human keratinocytes) was kindly donated by Dr. Ricardo Della Coletta, FOP–Unicamp.

Stock cell cultures were grown in medium containing RPMI 1640, supplemented with 5% fetal bovine serum. Experimental cultures were supplemented also with peniciline:streptomicine (10 µg/mL:10 UI/mL). Cells (100 µL cells/well, inoculation density from 3-6 x 10^4 cell/mL) in 96-well microtiter plates were exposed to different sample concentrations (0.25 to 250 µg/mL, 100 µL/well) in DMSO/RPMI 1640 at 37 °C, 5% of CO_2 in air during 48 h. DMSO final concentrations (≤ 0.25%) in culture medium did not affect cell viability. Cells were fixed with 50% trichloroacetic acid and cell proliferation was evaluated by spectrophotometric quantification (540 nm) of cellular protein content, by means of sulforhodamine B assay. Doxorubicin (DOX) (0.025-25 µg/mL) was used as positive control.

Three measurements were obtained at the beginning of incubation (time zero, T_0) and 48h *post*-incubation for compound-free (C) and tested (T) cells. Cell proliferation was determined according to the equation $100x[(T-T_0)/C-T_0]$, for $T_0<T\leq C$, and $100x[(T-T_0)/T_0]$, for $T\leq T_0$. The concentration-response curve for each cell line was plotted and, from these curves, GI_{50} (concentration causing 50% growth inhibition) and TGI (concentration that promotes total growth inhibition) were determined by means of non-linear regression analysis using Origin 8.0 software.[10,11]

2.2.2. Antioedematogenic assay

Borneol and its ester derivatives (**1**-**8**) were submitted to the antioedematogenic bioassay. This assay was conducted at the Laboratory of Physiology, Universidade Federal de Alfenas (UNIFAL, MG, Brazil). To

perform the anti-inflammatory assay (paw edema induced by carrageenan), borneol and its ester derivatives were suspended in carboxymethylcellulose solution (CMC, 0.5% w/V). The CMC was only used as thickener, since borneol esters are not water soluble and to avoid use organic solvents that might cause other type of damage to animals. Indomethacin solubilized in Tris buffer and saline in 1:1 ratio was used as positive control and CMC as negative control.[12]

The animals used were adult male Swiss mice (25-35 g), which were obtained from the UNIFAL vivarium (UNIFAL Ethics Committee, protocol 488/2013). They were treated with commercial feed and water *ad libitum*, guaranteed its adaptation for 7 days at room temperature of 23 ± 2 °C with 12 h dark/light cycle in the appropriate polypropylene maintenance cycle boxes. They were deprived of food for 12 h prior to the experiment and at the end of the experiments the animals were sacrificed by halothane inhalation.

Evaluation of the anti-inflammatory activity via paw edema test was induced by injection of 40 µL of carrageenan (2% w V^{-1}) solubilized in sterile saline and administered into the subplantar region of the right hind paw of male mice (n = 8). One hour before injection of carrageenan, the animals were treated with the samples (oral via) at doses of 20 mg kg^{-1}, or indomethacin (10 mg kg^{-1}), or CMC vehicle (10 mL kg^{-1}). The volume of the right paw of the animal was determined by immersing the tibio-tarsal region using a plethysmometer before carrageenan administration and one, two, three and four hours after receiving the carrageenan.[13]

Results were processed using GraphPad® 5.0 software to determine mean ± standard error of mean (SEM). Analysis of variance met assumptions of the method followed by Scott-Knott multiple comparisons test was conducted. Significant results were assumed for $p<0.05$ in all calculations. To facilitate comparisons among the tested samples, the results were expressed as % of oedema inhibition.

3. Results and discussion

3.1. Synthesis

The structures and general synthesis of borneol ester derivatives are illustrated in Fig. 1. Eight borneol analogs were obtained by two different methods. In all cases, DIC/DMAP method furnished high yields (54 to 84%). The $SOCl_2$ method also produced compounds **1**-**8**, but the yields were moderate (7 to 44%). The compounds **1**-**5** and **8** were news. All the esters were characterized by infrared (ATR), ^{1}H and ^{13}C NMR spectra. Assignments of the signals are based on the chemical shifts and intensity patterns. The structures of the compounds **1**-**8** were confirmed by the presence of an intense absorption at 1708–1774 cm^{-1} that is characteristic of a carbonyl ester group. ^{1}H and ^{13}C NMR spectra of all the compounds were obtained and the presence of signals between δ_H 4.85–5.27 ppm (H-2) and δ_C 164.7–174.3 ppm (C=O), respectively, confirmed the achievement of expected borneol esters.

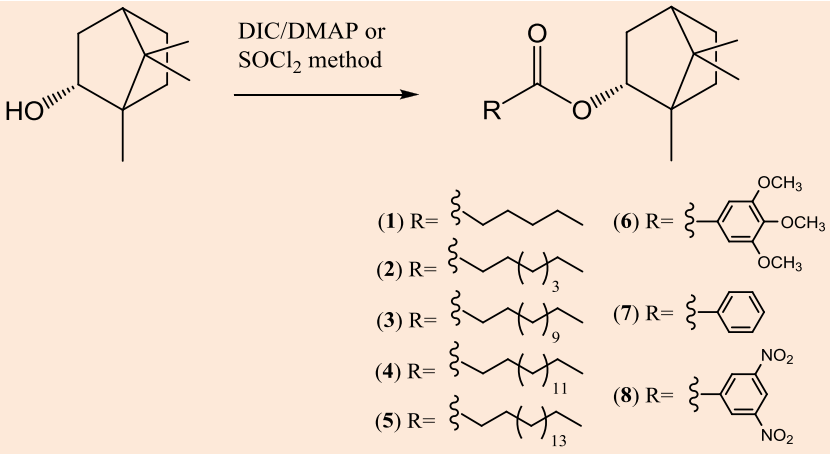

Figure 1. Synthesis of borneol ester derivatives

3.2. Characterization

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl hexanoate (**1**): yield: 62% by DIC/DMAP and 36% by $SOCl_2$; clear oil; IR (ATR, cm^{-1}): 1160, 1175, 1782, 2873, 2954; ^1H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.83 (s, 3H), 0.87 (s, 3H), 0.91-0.93 (m, 6H), 0.99 (d, *J*= 3.4 Hz, 1H), 1.16-1.35 (m, 6H), 1.56-1.78 (m, 4H), 1.87-2.01 (m, 1H), 2.27-2.43 (m, 3H), 4.85-4.92 (ddd, J_1= 2.2 Hz, J_2= 3.4 Hz, J_3= 10.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.5 (CH_3), 13.9 (CH_3), 18.8 (CH_3), 19.7 (CH_3), 22.3 (CH_2), 24.8 (CH_2), 27.1 (CH_2), 28.0 (CH_2), 31.3 (CH_2), 34.7 (CH_2), 36.8 (CH_2), 44.9 (CH), 47.7 (C), 48.7 (C), 79.5 (CH), 174.2 (CO).

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl octanoate (**2**): yield: 79% by DIC/DMAP and 44% by $SOCl_2$; clear oil; IR (ATR, cm^{-1}): 1160, 1175, 1782, 2873, 2954; ^1H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.83 (s, 3H), 0.87-0.91 (m, 9H), 0.98 (d, *J*= 3.4 Hz, 1H), 1.29 (br s, 10H), 1.60-1.76 (m, 4H), 1.87-2.01 (m, 1H), 2.27-2.43 (m, 3H), 4.85-4.92 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.5 (CH_3), 14.1 (CH_3), 18.9 (CH_3), 19.7 (CH_3), 22.6 (CH_2), 25.2 (CH_2), 27.1 (CH_2), 28.1 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 31.7 (CH_2), 34.8 (CH_2), 36.8 (CH_2), 44.9 (CH), 47.8 (C), 48.7 (C), 79.6 (CH), 174.3 (CO).

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl myristate (**3**): yield: 82% by DIC/DMAP and 17% by $SOCl_2$; clear oil; IR (ATR, cm^{-1}): 1159, 1177, 1784, 2853, 2923; ^1H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.83 (s, 3H), 0.87-0.91 (m, 9H), 0.98 (d, *J*= 3.4 Hz, 1H), 1.26 (br s, 22H), 1.61-1.69 (m, 4H), 1.87-2.01 (m, 1H), 2.27-2.43 (m, 3H), 4.85-4.92 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.5 (CH_3), 14.1 (CH_3), 18.8 (CH_3), 19.7 (CH_3), 22.7 (CH_2), 25.1 (CH_2), 27.1 (CH_2), 28.0 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 ($4CH_2$), 31.9 (CH_2), 34.7 (CH_2), 36.8 (CH_2), 44.9 (CH), 47.7 (C), 48.7 (C), 79.5 (CH), 174.2 (CO).

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl palmitate (**4**): yield: 73% by DIC/DMAP and 36% by $SOCl_2$; clear oil; IR (ATR, cm^{-1}): 1159, 1177, 1734, 2853, 2922; ^1H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.83 (s, 3H), 0.87-0.90 (m, 9H), 0.98 (d, *J*= 3.4 Hz, 1H), 1.26 (br s, 26H), 1.60-1.69 (m, 4H), 1.87-2.00 (m, 1H), 2.27-2.43 (m, 3H), 4.85-4.92 (ddd, J_1= 2.0 Hz, J_2= 3.0 Hz, J_3= 10.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.5 (CH_3), 14.1 (CH_3), 18.8 (CH_3), 19.7 (CH_3), 22.7 (CH_2), 25.1 (CH_2), 27.1 (CH_2), 28.0 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 ($5CH_2$), 31.9 (CH_2), 34.7 (CH_2), 36.8 (CH_2), 44.9 (CH), 47.7 (C), 48.7 (C), 79.5 (CH), 174.2 (CO).

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl stearate (**5**): yield: 65% by DIC/DMAP and 41% by $SOCl_2$; clear oil; IR (ATR, cm^{-1}): 1159, 1175, 1734, 2852, 2922; ^{1}H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.83 (s, 3H), 0.87-0.91 (m, 9H), 0.98 (d, *J*= 3.4 Hz, 1H), 1.26 (br s, 30H), 1.59-1.76 (m, 4H), 1.87-2.01 (m, 1H), 2.27-2.43 (m, 3H), 4.85-4.92 (ddd, J_1= 2.2 Hz, J_2= 3.4 Hz, J_3= 9.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.5 (CH_3), 14.1 (CH_3), 18.8 (CH_3), 19.7 (CH_3), 22.7 (CH_2), 25.1 (CH_2), 27.1 (CH_2), 28.0 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.7 (8CH_2), 31.9 (CH_2), 34.7 (CH_2), 36.8 (CH_2), 44.9 (CH), 47.7 (C), 48.7 (C), 79.5 (CH), 174.2 (CO).

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 3,4,5-trimethoxybenzoate (**6**): yield: 54% by DIC/DMAP and 35% by $SOCl_2$; white solid; m.p. 91-93 °C; IR (ATR, cm^{-1}): 767, 873, 1122, 1228, 1708, 2836, 2952; ^{1}H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.92 (s, 6H), 0.98 (s, 3H), 1.12 (d, *J*= 13.6 Hz, 1H), 1.26-1.47 (m, 2H), 1.75-1.81 (m, 2H), 2.05-2.16 (m, 1H), 2.42-2.54 (m, 1H), 3.92 (s, 9H), 5.07-5.12 (m, 1H), 7.32 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.6 (CH_3), 18.9 (CH_3), 19.7 (CH_3), 27.4 (CH_2), 28.0 (CH_2), 36.9 (CH_2), 44.9 (CH), 47.8 (C), 49.1 (C), 56.2 (2CH_3), 60.9 (CH_3), 80.6 (CH), 106.7 (2CH), 125.9 (C), 142 (C), 152.9 (2C), 166.4 (CO). ^{13}C NMR spectral data are in accordance with data reported in the literature.[5]

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl benzoate (**7**): yield: 59% by DIC/DMAP and 26% by $SOCl_2$; clear oil; IR (ATR, cm^{-1}): 708, 978, 1112, 1270, 1451, 1714, 2879, 2953 cm^{-1}; ^{1}H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.92 (s, 6H), 0.97 (s, 3H), 1.08-1.17 (dd, J_1= 3.4 Hz, J_2= 13.6 Hz, 1H), 1.27-1.46 (m, 2H), 1.74-1.86 (m, 2H), 2.07-2.21 (m, 1H), 2.40-2.56 (m, 1H), 5.08-5.16 (ddd, J_1= 2.0 Hz, J_2= 3.0 Hz, J_3= 9.8 Hz, 1H), 7.41-7.60 (m, 3H), 8.07 (d, *J*= 6.8 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.6 (CH_3), 18.9 (CH_3), 19.7 (CH_3), 27.4 (CH_2), 28.1 (CH_2), 36.9 (CH_2), 44.9 (CH), 47.8 (C), 49.1 (C), 80.5 (CH), 128.3 (2CH), 129.5 (2CH), 130.8 (C), 132.7 (CH), 166.8 (CO). ^{13}C NMR spectral data are in accordance with data reported in the literature.[5]

(1S,2R,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 3,5-dinitrobenzoate (**8**): yield: 84% by DIC/DMAP and 7% by $SOCl_2$; white solid; m.p. 145-146 °C; IR (ATR, cm^{-1}): 718, 719, 822, 913, 1343, 1541, 1723, 2879, 2956; ^{1}H RMN (200 MHz, $CDCl_3$) δ_H (ppm): 0.95 (s, 6H), 0.99 (s, 3H), 1.12-1.21 (dd, J_1= 3.4 Hz, J_2= 13.8 Hz, 1H), 1.36-1.58 (m, 2H), 1.80-1.88 (m, 2H), 2.01-2.14 (m, 1H), 2.46-2.62 (m, 1H), 5.20-5.27 (m, 1H), 9.15 (d, *J*= 2.0 Hz, 2H), 9.23 (t, *J*= 2.1 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δc (ppm): 13.6 (CH_3), 18.8 (CH_3), 19.7 (CH_3), 27.4 (CH_2), 28 (CH_2), 36.7 (CH_2), 44.8 (CH), 48.1 (C), 49.2 (C), 83.1 (CH), 129.3 (2CH), 122.2 (CH), 134.5 (C), 148.6 (2C), 162.7 (CO).

3.3. Biological activities

3.3.1. Antiproliferative assay

In vitro antiproliferative property of borneol and its esters **1**-**8** was investigated in seven human tumor cell lines [breast (MCF-7), ovarian (NCI-ADR/RES, OVCAR-03), renal (786-0), colon (HT-29) and leukemia (K-562)] and one human normal cell line (HaCat, human keratinocytes). Borneol and its derivatives (**1**-**8**) were assayed in concentrations of 0.25-250 µg/mL and doxorubicin (DOX, 0.25-250 µg/mL) was used as positive control. Cell proliferation was determined by spectrophotometric measurements using sulforhodamine B as a protein-binding. An effective concentration, eliciting 50% growth inhibition (GI_{50}) was determined after 48 h of cell treatment (see Table 1).

Compounds **3**, **4**, **5** and **8** do not present relevant effect against any of the cell lines

assayed (GI_{50}>250 µg/mL). These compounds had only cytostatic activities against all cell lines in the concentrations studied. The borneol derivatives **2, 6** and **7** were more selective against K562; however, compoud **7** was 9-fold more potent to cancer cell line (K562) than human normal cell (HaCat). In fact, the bornyl ester **6** was the most potent ester derivative for all cell lines evaluated. Notably**,** the compound **6** was 2.4-fold more potent than the reference drug DOX in inhibiting the growth of adryamycin-resistant ovarian (NCI-ADR/RES) and less selective than DOX to human normal cell (HaCat).

Table 1. Antiproliferative activity (GI_{50}, µg/mL) of borneol and its ester derivatives (**1**-**8**) on human cell lines

Cell lines[a]	DOX[b]	Borneol	1	2	3	4	5	6	7	8
MCF-7	0.044	>250	76.4	29.2	>250	>250	>250	18.1	25.3	>250
NCI-ADR/RES	6.7	>250	250	25.7	>250	>250	>250	2.8	26.1	>250
786-0	0.092	>250	>250	29.4	>250	>250	>250	3.1	25.3	>250
OVCAR-3	0.27	5.3	19.7	25.4	>250	>250	>250	3.0	26.6	>250
HT29	0.26	111.5	>250	108.4	>250	>250	>250	8.5	12.1	>250
K562	0.31	83.7	10.2	9.5	>250	>250	>250	2.6	2.8	>250
HaCat	0.040	>250	17.0	26.6	>250	>250	>250	4.8	25.9	>250

[a]Tumor cell lines: MCF-7 (mammary); NCI-ADR/RES (drug resistant ovary); 786-0 (kidney); OVCAR-3 (ovary); HT-29 (colon); K562 (leukemia). Normal cell lines: HaCat (immortalized keratinocytes); [b]Doxorubicin: positive control

The percentage values for the growth inhibition of the cell proliferation for the three most active compounds, at four different concentrations are listed in the Table 2. Compound **2**, **6** and **7** presented low cell growth inhibition at 0.25 and 2.5 µg/mL. However, in the concentration of 25 µg/mL, compound **6** showed relevant cytostatic activity for MCF-7 and OVCAR-3 and low cytotoxic activity for 786-0. In the same concentration, **2** and **7** present lower cytostatic activity for all cell lines. Compounds **2**, **6** and **7** were cytotoxic to MCF-7, NCI-ADR/RES, 786-0, OVCAR-3, HaCat cell lines when used at 250 µg/mL. Only the compound **7** was cytostatic against HT29 at 250 µg/mL.

Comparing activity of borneol and compound **7** against NCI-ADR/RES, MCF-7 and 786-0 cells, borneol was inactive (GI_{50}>250 µg/mL). In this case, the introduction of phenyl ring increased the antiproliferative activity 4-fold. The antiproliferative activity results of the compounds **6** and **7**, allows inferring that the presence of phenyl group contributes to the action against the cell growth. Furthermore, the presence of electron-donating groups in compound **6**, methoxyl substituents, suggests an improvement of the antiproliferative activity in up to 9-fold.

Table 2. Antiproliferative activity of **2**, **6** and **7** on human cell lines as percentage of cell growth

Cell lines	**2** (μg/mL)				**6** (μg/mL)				**7** (μg/mL)			
	0.25	**2.5**	**25**	**250**	**0.25**	**2.5**	**25**	**250**	**0.25**	**2.5**	**25**	**250**
MCF-7	13	18	20	-81	21	27	89	-65	15	19	48	-82
NCI-ADR/RES	22	25	25	-86	24	40	83	-11	20	35	42	-88
786-0	14	20	20	-92	30	35	-2	-64	28	30	48	-90
OVCAR-3	30	39	47	-96	31	35	90	-100	15	27	38	-94
HT29	14	24	27	63	21	27	65	90	18	25	52	-4
K562	27	34	45	95	31	41	76	84	35	39	67	97
HaCat	24	28	36	-100	25	27	82	-100	22	26	42	-75

[a]Tumor cell lines: MCF-7 (mammary); NCI-ADR/RES (drug resistant ovary); 786-0 (kidney); OVCAR-3 (ovary); HT-29 (colon); K562 (leukemia). Normal cell lines: HaCat (immortalized keratinocytes); [b]Doxorubicin: positive control.

3.3.2. Antioedematogenic assay

This assay demonstrates anti-inflammatory effect of borneol esters in an experimental model of acute inflammation induced by carrageenan. Carrageenan is a polysaccharide present in red algae Rhodophyceae widely used to induce acute inflammatory response in experimental animals, since it induces the release of various inflammatory mediators such as histamine, bradykinin, prostaglandin, and superoxide anions.[14] The use of carrageenan as irritant for inducing inflammation in rat paw was introduced by Winter et al.,[15] making it one of the most popular methods for drug testing and evaluation of novel anti-inflammatory therapies.[16]

Previous work shows carrageenan promoting a long-lasting inflammatory response in two phases when injected into rat paw. The first step results in a rapid production of various inflammatory mediators such as histamine, serotonin, and bradykinin. The second phase occurs with the release of prostaglandins and nitric oxide, with a peak at the third hour produced by inducible isoforms of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS), respectively.[17] Thus, antioedematogenic study was conducted during four hours aiming to understand how the borneol and its ester derivatives could act in the inflammatory process.

Table 3 shows paw volumes followed by the percent inhibition of borneol esters (**1**-**8**) and indomethacin (positive control) in carrageenan-induced paw oedema. Indomethacin treatment at a dose of 10 mg kg^{-1} throughout the experimental period significantly inhibited hind paw swelling ($p<0.05$), with maximal inhibition of 53% and maximal effect after 4 h.

Table 3. Antioedematogenic activity induced by borneol and its ester derivatives (**1**-**8**)

Treatment	Paw volume in mL (% of oedema inhibition)			
	1st hour	2nd hour	3rd hour	4th hour
Vehicle	0.049±0.004[a]	0.058±0.007[b]	0.067±0.006[b]	0.065±0.010[a]
Borneol	0.038±0.005[a] (23)	0.051±0.005[b]	0.055±0.006[a]	0.055±0.004[a] (15)
1	0.027±0.007[a] (45)	0.046±0.007[b]	0.049±0.006[a]	0.053±0.007[a] (18)
2	0.026±0.006[a] (47)	0.029±0.006[a]	0.041±0.005[a]	0.043±0.05[a] (33)
3	0.026±0.005[a] (47)	0.034±0.006[a]	0.051±0.003[a]	0.046±0.005[a] (29)
4	0.029±0.007[a] (41)	0.038±0.007[a]	0.037±0.009[a]	0.034±0.010[a] (47)
5	0.028±0.010[a] (43)	0.052±0.010[b] (9)	0.038±0.008[a]	0.053±0.009[a] (18)
6	0.032±0.004[a] (34)	0.058±0.008[b] (0)	0.065±0.009[b]	0.049±0.010[a] (25)
7	0.025±0.007[a] (49)	0.022±0.005[a]	0.040±0.007[a]	0.024±0.004[a] (63)
8	0.031±0.005[a] (36)	0.033±0.006[a]	0.048±0.006[a]	0.042±0.008[a] (35)
Indomethacin	0.030±0.006[a] (39)	0.031±0.005[a]	0.041±0.004[a]	0.031±0.008[a] (53)

The means followed by the same letter do not differ significantly by the Scott-Knott test ($p<0.05$).

At the first hour, borneol ester derivatives (**1**-**8**) reduced the oedematogenic process (from 34% to 47%), while indomethacin inhibited 39%. In the second hour, compounds **2** (49%), **3** (41%), **7** (63%), **8** (43%) and indomethacin (46%) were more effective in inhibiting the inflammatory process. At the third hour, compounds **4** (44%), **5** (43%), **7** (41%) and positive control (39%) were capable of reducing the oedema. At the fourth hour only compounds **4** (47%), **7** (63%) and indomethacin (53%) exhibited a relevant inhibitory effect.

Borneol and compound **6** did not inhibit inflammatory process in any time analyzed. However, the hydrophobic chain esters (compounds **1**-**5**) have reduced the oedema with very similar inhibitory percentages, indicating that chain length did not influence their anti-inflammatory property. Compound **7** was effective in inhibiting different stages of inflammation evaluated (1 h: 49%; 2 h: 63%; 3 h: 41% and 4 h: 63%) and presented inhibition values greater than the positive control. The oral treatment with compound **7** was effective in reducing the oedematogenic response evoked by carageenan in two phases. This reduction may be caused by inhibition of intracellular signalling pathways involved in mediating the inflammatory response.

Anti-inflammatory activity is a known property of terpenes.[18] To this biological effect have been attributed to different mechanisms of action such as: inhibition of elastase, inhibition of cyclooxygenase and lipoxygenase activities, and inhibition of complement activity, probably via inhibition of C3-convertase of the classical complement pathway.[19]

4. Conclusion

Eight borneol ester derivatives (**1**-**8**) were synthesized with high yields using the DIC/DMAP method and subjected to antiproliferative and antioedematogenic evaluation. Compounds **2**, **6** and **7** showed a pronounced cytostatic activity against all cell lines assayed, revealing to be promising models for the development of alternative drugs that may be used in the treatment of

cancer. Moreover, compound **7** showed potential antioedematogenic activity and could be useful models to assist the development of new anti-inflammatory drugs.

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