European Journal of Medicinal Chemistry 56 (2012) 120–126

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Benzofuranones as potential antinociceptive agents: Structure–activity relationships

Cleiton José Gonçalves [a], Andrey Sávio Lenoir [a], Pâmela Padaratz [b], Rogério Corrêa [a,b], Rivaldo Niero [a,b], Valdir Cechinel-Filho [a,b], Fátima de Campos Buzzi [a,b,*]

[a] Núcleo de Investigações Químico-Farmacêuticas (NIQFAR)/CCS, Universidade do Vale do Itajaí-UNIVALI, Caixa Postal 360, CEP 88.302-202 Itajaí, Santa Catarina, Brazil
[b] Programa de Pós-graduação em Ciências Farmacêuticas, Universidade do Vale do Itajaí-UNIVALI, 88.302-202 Itajaí, Santa Catarina, Brazil

ARTICLE INFO

Article history:
Received 19 May 2012
Received in revised form
8 August 2012
Accepted 9 August 2012
Available online 16 August 2012

Keywords:
Benzofuranone
Antinociceptive properties
Topliss method

ABSTRACT

This work evaluates the antinociceptive properties of benzofuranones using chemically induced models of pain and the hot plate test. All the compounds exhibited significant antinociceptive activity, with 3-[2-(4-chlorophenyl)-2-oxoetil]-2-benzofuran-1(3*H*)-one (**3d**) being the most active. According to the application of the Topliss method, the $2\pi-\pi^2$ parameter was the preponderant one, indicating that the hydrophobicity (π) seems to be more involved in the antinociceptive activity. Based on the table of other possible substituents proposed by Topliss, three derived from compound **3d** were tested. 3-[2-(3-methoxyphenyl)-2-oxoetil]-2-benzofuran-1(3*H*)-one (**3g**) showed greater antinociceptive activity with better pharmacokinetic properties predicted. These results show the efficiency of the Topliss Method as a research tool for the discovery of potential candidate molecules for a new antinociceptive drug.

1. Introduction

Chalcones are a group of plant-derived polyphenolic compounds belonging to the flavonoids family that can also be obtained by synthetic route. This class has a wide variety of biological properties, including antinociceptive and anti-inflammatory [1–3], antitumoral [4] antibacterial and antifungal [3,5], and antileishmanial [6] properties, among others [7–9]. Chalcone-containing plants have long been used as medical treatments in Asia, Africa and South America. Several pure chalcones were approved for clinical use or tested in humans, such as Metochalcone, marketed as a choleretic drug, and Sofalcone, marketed as an anti-ulcer and mucoprotective drug [10,11]. Benzofuranones are a group of substances that can be obtained by synthesis from several starting products, including chalcones. Several compounds belonging to this class exhibit a broad spectrum of biological properties, such as antinociceptive, cytotoxic, antibacterial and antioxidant properties [12–15]. In previous studies, we have demonstrated that a new benzofuranone, denoted 3-[2-(2-hydroxy-4, 6-dimethoxyphenyl)-2-oxoethyl]-2-benzofuran-1(3*H*)-one, was more potent as an antinociceptive than the original chalcone and some clinically used drugs [14]. In the present study, we have extended our observations, synthesizing some benzofuranones and evaluating their possible antinociceptive effects. In addition, the Topliss method was used to obtain more potent substances.

2. Materials and methods

2.1. Chemistry

The melting points were determined by a Microquímica WG APF-301 apparatus, and are uncorrected. IR spectra were recorded in KBr using a Perkin Elmer 16 PC-FT spectrometer. The ^{1}H NMR and ^{13}C spectra were recorded in the solvent indicated, with a Bruker WM 300 MHz spectrometer, with TMS as internal standard. The purity of the synthesized substances was monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} pre-coated aluminum sheets (Merck®) with several solvent systems of different polarities.

The compounds were synthesized from carboxybenzaldehyde and different acetophenones (Fig. 1). Initially the acetophenones (0.92 mmol) were dissolved in ethanol (25 ml) and mixed in a solution of NaOH 50% (*p*/*v*; 2.5 mmol). The carboxybenzaldehyde (0.95 mmol) was then added. The reaction was heated for 14 h, then acidified with HCl at 50% until complete precipitation. The product was recrystallized in ethanol (10 ml) and filtered in a Buchner

* Corresponding author. Núcleo de Investigações Químico-Farmacêuticas (NIQFAR)/CCS, Universidade do Vale do Itajaí (UNIVALI), Caixa Postal 360, CEP 88302-202, Itajaí, Santa Catarina, Brazil. Tel.: +55 47 3341 7855; fax: +55 47 3341 7601.
E-mail address: fcamposbuzzi@univali.br (F.de Campos Buzzi).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.08.015

funnel. The residue was purified on a silica gel column (eluent: hexane–ethyl acetate).

*2.1.1. 3-(2-Oxo-2-phenylethyl)-2-benzofuran-1(3H)-one (**3a**)*

Colorless crystals, yield 53%, mp: 146.5–147.5 °C, IR (KBr, cm^{-1}): 3062 (CH–Ar), 1771 (C=O–C), 1682 (C=O) and 1212 (C–O). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 7.51–8.01 (m, 9H, H-ring A and B), 6.11 (dd, 1H, J = 6.0, 3.0 Hz, H-8), 3.81 (dd, 1H, J = 12.0; 6.0 Hz; H-9α), 3.78 (dd, 1H, J = 12.0; 6.0 Hz; H-9β). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 196.5 (C-10), 169.9 (C-7), 149.9–122.9 (C=C-ring A and B), 77.2 (C-8), 42.7 (C-9), Anal. Calc. for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.15; H, 4.81.

*2.1.2. 3-[2-(4-Metoxyphenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3b**)*

Colorless, yield 68%; mp: 121–122 5 °C, IR (KBr, cm^{-1}): 1759 (C=O–C), 1676 (C=O) and 1216 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 6.92 (d, 2H, J = 9.0 Hz, H-2′ and H-6′), 7.94 (d, 2H, J = 9.0 Hz, H-3′ and H-5′), 7.65–7.50 (m, 4H, H-ring-B), 6.15 (dd, 1H, J = 12.0; 6.0 Hz, H-8), 3.71 (dd, 1H, J = 15.0; 6.0 Hz, H-9α), 3.34 (dd, 1H, J = 15.0; 6.0 Hz, H-9β), 3.87 (s, 3H, O–CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 194.3 (C-10), 170.1 (C-7), 149.8–122.8 (C=C-ring A and B), 76.5 (C-8), 55.4 (OCH_3), 43.2 (C-9). Anal. Calc. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.31; H, 5.03.

*2.1.3. 3-[2-(4-Methylphenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3c**)*

Colorless, yield 64%, mp: 149.1–150.1 °C, IR (KBr, cm^{-1}): 1761 (C=O–C), 1673 (C=O) and 1295 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.34 (d, 2H, J = 9.0 Hz, H-2′ and H-6′), 7.95 (d, 2H, J = 9.0 Hz, H-3′ and H-5′), 7.41–7.85 (m, 4H, H-ring-B), 6.15 (dd, 1H, J = 12.0; 6.0 Hz, H-8), 3.72 (dd, 1H, J = 12.0; 6.0 Hz, H-9α), 3.74 (dd, 1H, J = 12.0; 6.0 Hz, H-9β), 2.40 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 196.0 (C-10), 170.1 (C-7), 150.7–123.3 (C=C-ring A and B), 77.7 (C-8), 43.6 (Ar–CH_3), 43.4 (C-9). Anal. Calc. for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.65; H, 5.25.

*2.1.4. 3-[2-(4-Chlorophenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3d**)*

White powder, yield 45%, mp: 147.5–148.6 °C, IR (KBr, cm^{-1}): 3061 (CH–Ar), 1754 (C=O–C), 1679 (C=O) and 1217 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.45 (d, 4H, J = 9.0 Hz, H-2′ and H-6′), 7.90 (d, 2H, J = 9.0 Hz, H-3′ and H-5′), 7.58–7.67 (m, 4H, H-ring-B), 6.10 (dd, 1H, J = 12.0; 6.0 Hz, H-8), 3.71 (dd, 1H, J = 15.0; 6.0 Hz, H-9α), 3.35 (dd, 1H, J = 15.0; 6.0 Hz, H-9β). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 194.8 (C-10), 170.0 (C-7), 149.5–122.7 (C=C-ring A and B), 76.6 (C-8), 43.6 (C-9). Anal. Calc. for $C_{16}H_{11}ClO_3$: C, 67.06; H, 3.87. Found: C, 67.12; H, 3.82.

*2.1.5. 3-[2-(3,4-Dichlorophenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3e**)*

White powder, yield 78%, mp: 192.3–192.4 °C, IR (KBr, cm^{-1}): 1751 (C=O–C), 1682 (C=O) and 1215 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.95 (d, 2H, J = 9.0, H-3′ and H-5′), 7.85 (d, 2H, J = 9.0 Hz, H-2′ and H-6′), 7.62–7.83 (m, 4H, H-ring-B), 6.10 (dd, 1H, J = 12.0; 6.0 Hz, H-8), 3.80 (dd, 1H, J = 15.0; 6.0 Hz, H-9α), 3.86 (dd, 1H, J = 15.0; 6.0 Hz, H-9β). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 194.8 (C-10), 169.7 (C-7), 150.5–122.2 (C=C-ring A and B), 76.8 (C-8), 43.8 (C-9). Anal. Calc. for $C_{16}H_{10}Cl_2O_3$: C, 59.84; H, 3.12. Found: C, 59.82; H, 3.12.

*2.1.6. 3-[2-(3,4-Dimethoxyphenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3f**)*

White powder, yield 55%, mp: 152.0–152.7 °C. IR (KBr, cm^{-1}): 3087 (CH–Ar), 1760 (C=O–C), 1670 (C=O) and 1259 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.89 (d, 1H, J = 9.0 Hz, H-5′), 7.66 (dd, 1H, J = 6.0, 2.0 Hz, H-6′), 7.53 (d, 1H, J = 2.0 Hz, H-2′), 7.53–7.59 (m, 4H, H-ring-B), 6.18 (dd, 1H, J = 12.0; 6.0 Hz, H-8), 3.96 (s, 6H, 2× OCH_3), 3.75 (dd, 1H, J = 15.0; 6.0 Hz, H-9α), 3.36 (dd, 1H, J = 15.0; 6.0 Hz, H-9β). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 194.4 (C-10), 170.1 (C-7), 153.9–110.3 (C=C-ring A and B), 76.4 (C-8), 56.1 (2× OCH_3), 43.2 (C-9). Anal. Calc. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.32; H, 5.10.

*2.1.7. 3-[2-(3-Methoxyphenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3g**)*

White powder, yield 57%, mp: 134.2–135.8 °C. IR (KBr, cm^{-1}): 1765 (C=O–C), 1682 (C=O) and 1255 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.92 (d, 1H, J = 7.5 Hz, H-4′), 7.66 (dd, 1H, J = 7.5; 2.0 Hz, H-6′), 7.36–7.56 (m, 4H, H-ring-B), 7.50 (d, 1H, J = 2.0 Hz, H-2′), 6.15 (dd, 1H, J = 9.0; 2.0 Hz, H-8), 3.86 (s, 3H, OCH_3), 3.74 (dd, 1H, J = 12.0; 6.0 Hz, H-9α), 3.38 (dd, 1H, J = 12.0; 6.0 Hz, H-9β). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 195.8 (C-10), 170.1 (C-7), 159.9–112.2 (C=C-ring A and B), 76.6 (C-8), 55.4 (OCH_3), 43.7 (C-9). Anal. Calc. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.30; H, 5.35.

*2.1.8. 3-[2-(4-Bromophenyl)-2-oxoethyl]-2-benzofuran-1(3H)-one (**3h**)*

White powder, yield 43%, mp: 147.0–149.9 °C. IR (KBr, cm^{-1}): 1749 (C=O–C), 1682 (C=O) and 1215.92 (C–O). ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.82 (dd, 2H, J = 9.0 Hz, H-2′ and H-6′), 7.98 (dd, 2H, J = 9.0 Hz, H-3′ and H-5′), 7.58–7.85 (m, 4H, H-ring-B), 6.15 (dd, 1H, J = 18.0; 9.0 Hz, H-8), 3.76 (d, 2H, J = 9.0 Hz, H-9α and H-9β). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 195.4 (C-10), 169.6 (C-7), 150.2–123.1 (C=C-ring A and B), 77.0 (C-8), 43.3 (C-9). Anal. Calc. for $C_{16}H_{11}BrO_3$: C, 58.03; H, 3.35. Found: C, 58.11; H, 3.33.

2.2. Biological assays

2.2.1. Animals

Male Swiss mice (25–35 g) were used, housed at 22 ± 2 °C under a 12 h light/12 h dark cycle, with access to food and water *ad libitum*. The experiments were performed during the light phase of the cycle. The animals were acclimatized to the laboratory for at least 2 h before testing, and were used once throughout the experiments. All the experiments reported in this study were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigation of experimental pain in conscious animals [16]. The number of mice (6–8 per group) and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments.

2.2.2. Drugs

The following substances were used: acetic acid, formalin, L-glutamic acid, capsaicin (Calbiochem, San Diego, CA, USA), morphine hydrochloride and naloxone (Merck, Darmstadt, Germany). All synthetic compounds, as well as the reference drugs, were dissolved in Tween 80 (E. Merck), plus 0.9% of NaCl solution, with the exception of the capsaicin, which was dissolved in ethanol. The final concentration of Tween 80 and ethanol did not exceed 5% and did not cause any effect *per se*.

2.2.3. Writhing test

Abdominal constriction was induced in mice by intraperitoneal injection of acetic acid (0.6%), as described by Collier et al. [17] with minor modifications. The animals were pre-treated intraperitoneally (0.5 at 30 mg kg^{-1}, 30 min before) for all compounds, and orally (30, 60 and 100 mg kg^{-1}, 1 h before) with compound **3g**. The control animals received a similar volume of saline solution (10 ml kg^{-1}).

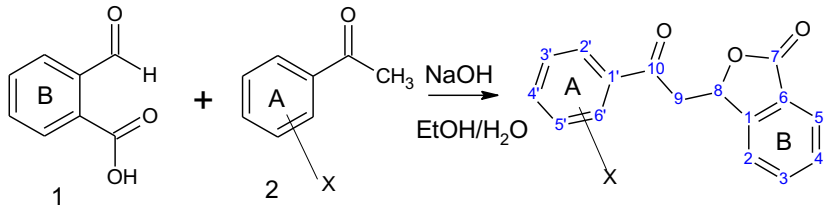


Fig. 1. General route for preparation of benzofuranone derivatives.

The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions between the control animals and the mice pre-treated with the compounds.

2.2.4. Formalin-induced nociception

The observation chamber was a glass cylinder of 20 cm in diameter, with a mirror at a 45° angle to allow clear observation of the animals' paws. The mice were treated with 0.9% saline solution (i.p.), or compound **3g** (10, 30 and 60 mg kg^{-1}, i.p.), 30 min before formalin injection. Each animal was placed in the chamber for 5 min before treatment, in order to acclimatize to the new environment. The formalin test was carried out as described by Hunskaar and Tjolsen [18,19], with minor modifications. Twenty microliters of a 2.5% formalin solution (0.92% formaldehyde) in 0.9% saline solution were injected intraplantarly into the right hind paw. The animal was then returned to the chamber, and the amount of time spent licking the injected paw was considered as indicative of pain. Two distinct phases of intensive licking activity were identified: an early acute phase and a late or tonic phase (0–5 and 15–30 min after formalin injection, respectively).

2.2.5. Capsaicin-induced nociception

The procedure used was similar to that described previously [20]. After the adaptation period, 20 ml of capsaicin (1.6 μg/paw) was injected intraplantarly into the right hind paw. The animals were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The animals were treated with the compound **3g** via i.p. (3, 6 and 10 mg kg^{-1}) 30 min prior to capsaicin injection, respectively. The control animals received a similar volume of saline, intraperitoneally.

2.2.6. Glutamate-induced nociception

The animals were treated with the compound **3g** via i.p. (3, 10 and 30 mg kg^{-1}) 30 min before glutamate injection, respectively. A volume of 20 μl of glutamate solution (30 μmol/paw), was injected intraplantarly under the surface of the right hind paw as described previously [21]. After injection with glutamate, the animals were individually placed in glass cylinders of 20 cm in diameter and observed from 0 to 15 min. The time spent licking and biting the injected paw was timed with a chronometer and considered indicative of pain.

2.2.7. Hot-plate test

The hot-plate test was used to measure response latencies, according to the method described by Eddy and Leimback [22]. The mice were treated with saline solution, morphine (10 mg kg^{-1}, *s.c.*) or the compound **3g** (10, 30 and 60 mg kg^{-1}, i.p.), and placed individually on a hot plate maintained at 56 ± 1 °C. The time between placing the animal on the hot plate and the occurrence of either licking of the hind paws, shaking the paw or jumping off the surface was recorded as response latency. Mice with baseline latencies of more than 20 s were eliminated from the study and the cut-off time for the hot-plate latencies was set at 30 s. The animals were treated 30 min before the assay. In order to correct for individual differences in latencies, the antinociceptive data were converted to percentage maximum possible effect (%MPE).

2.2.8. Statistical analysis

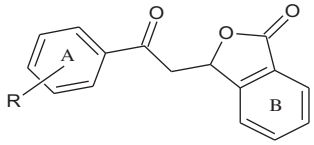
The results are presented as mean ± S.E.M., except for the ID values (i.e. the dose of the compounds reducing the nociceptive response by 50%, relative to the control value), which are reported as geometric means, accompanied by their respective 95% confidence limits. The ID_{50} value was determined by linear regression from individual experiments using linear regression GraphPad software (GraphPad software, San Diego, CA). The statistical significance of the differences between the groups was detected by ANOVA, followed by Dunnett's multiple comparison tests. *P*-values of less than 0.05 ($P < 0.05$) were considered indicative of significance.

3. Results

3.1. Chemistry

The synthesis of the target compounds **3a**–**h** were prepared in only one step, in similar form to that previously studied by our research group [14]. Generally, these conditions are used to obtain chalcones, but when using the carboxybenzaldehyde as the starting material and different acetophenones in the presence of alkali, the expected chalcones are not obtained. In this reaction, the only product is 3-phenacylphthalides, also known as benzofuranones, which are produced in good yield. The spectral data of the

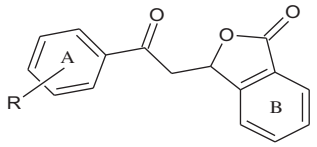
Table 1
Comparison of the antinociceptive activity of first generation benzofuranones and reference drugs (AAS and PAR) in the acetic acid-induced pain model.



Substituents	Code	DI_{50} (μmol kg^{-1})	MI (%)
H	**3a**	63.5 (44.5–90.6)	74.8
4-OCH_3	**3b**	52.7 (47.2–65.7)	69.5
4-CH_3	**3c**	42.5 (36.0–13.3)	88.4
4-Cl	**3d**	33.7 (29.2–39.0)	97.7
3,4-Cl_2	**3e**	34.6 (26.2–45.8)	90.7
	AAS[a]	133.0 (73.0–243.0)	83.0
	PAR[a]	125.0 (104.0–250.0)	88.0

[a] F. de Campos-Buzzi et al [2].

Table 2
Comparison of the antinociceptive activity of second generation benzofuranones and **3d** from the first series in the acetic acid-induced pain model (10 mg kg^{-1}, i.p).



Substituent (R)	Code	MI (%)
4-Cl	**3d**	73.5
3,4-OCH_3	**3f**	49.4
3-OCH_3	**3g**	93.5
4-Br	**3h**	50.8

synthesized compounds in the present investigation were in accordance with their assigned structures.

3.2. Biological assays

In the writhing test, the first generation of compounds caused 69.5 at 97.7% of inhibition at 30 mg kg^{-1} with dose-dependent antinociceptive effect, with ID_{50} values ranging of 33.7 (29.2–39.0) at 63.5 (44.5–90.6) μmol kg^{-1} (Table 1). Three more representative compounds were thus selected in accordance with the Topliss method: 3,4-OCH_3 (**3f**), 3-OCH_3 (**3g**); 4-Br (**3h**) and were evaluated in the same model at 10 mg kg^{-1} (Table 2). These results were determinants for selecting the substituent 3-OCH_3 (**3g**), due to its considerable improvement in the antinociceptive activity. Compound **3g** presented an ID_{50} value of 9.6 (7.8–11.9) μmol kg^{-1}, i.p. and an ID_{50} value of 257.1 (250.7–242.3) μmol kg^{-1} by the oral route (Fig. 2).

The formalin-test revealed that it inhibited the dose-dependent manner the first phase of pain with a 38.3% of inhibition at 212.5 μmol kg^{-1} and the second phase with a more significant inhibition with a ID_{50} value of 174.3 (166.3–182.6) μmol kg^{-1}. Formalin induced edema was also inhibited in a dose-dependent manner (Fig. 3).

The administration of compound **3g** produced dose-dependent attenuation of capsaicin-induced neurogenic pain (Fig. 4), with ID_{50} value of 63.7 (59.2–68.5) μmol kg^{-1}, and maximal inhibition of 62.0%.

The results presented in Fig. 5 show that compound **3g**, given intraperitoneally, caused significant inhibition of glutamate-induced nociception, with a mean ID_{50} value of 66.5 (58.3–75.8) μmol/kg and maximal inhibition of 60.3%.

In the hot plate test, the animals did not show an increase in the threshold of pain perception compared with the control group and the pre-test carried out 24 h previously (Fig. 6).

4. Discussion

This study initially focused on the evaluation of a benzofuranone series based on the Topliss manual method, using the acetic acid-induced writing test. These compounds were obtained with greater ease than previously reported. The literature data revealed that 3-alkylphthalides are synthesized by reacting 2-bromo benzaldehyde with 1,3 dicarbonyl compounds under carbon monoxide in the presence of a catalytic conditions of $PdCl_2(PPh_3)_2$ [23]. Although one-pot synthesis was reported to obtained 3-phenacylphthalides, this reaction was performed in the presence of trifluoroacetic acid [24]. The method used in our studies uses more brand conditions than those reported in the literature, and similar conditions used to synthesis of chalcones. For this reason, we suggest that the chalcone intermediary is formed mechanistically. However, the rearrangement occurs between the α,β-unsaturated moiety and the carboxyl group to produce the corresponding benzofuranones.

According to the ID_{50} values calculated, the potency ranking for this first generation of active compounds was: 4-Cl > 3,4-Cl_2 > 3-CH_3 > 4-OCH_3 > H. Analyzing the influence of the electronic parameter of the substituent groups in relation to the inhibition of constrictions caused by acetic acid, it was observed that the compounds with electron-acceptor substituents were more effective than the electron-donors or without substituents. All the compounds were about 2–4-fold more active than the standard drugs, such as acetylsalicylic acid and acetaminophen, used for comparison.

Besides the electronic parameter, the lipophilic parameter should also be influencing the pharmacological activity, and on comparison with the Topliss manual method, $2\pi-\pi^2$ was determined as the dominant parameter for selecting the second set of derivatives.

Three benzofuranones were therefore synthesized as representative, from among those suggested by the Topliss method: 4-Br; 3-OCH_3; 3,4-OCH_3 and were evaluated in the writhing test at 10 mg kg^{-1}. Compound **3g** (3-OCH_3) was the most effective, causing inhibition of 93.5%. Since it caused pronounced antinociceptive action in this preliminary assay, it was then evaluated in more detail in different classical models of pain in mice. Compound **3g** was evaluated as an antinociceptive agent, initially using the same methodology i.e. the acetic acid-induced writhing test in mice, administered intraperitoneally.

In comparison with the first generation of benzofuranones classical according to Topliss (**3a**–**e**) the ID_{50} were calculated in order to evaluate the potency of this derivative which was about 3.5 times more active than compound **3d**. This demonstrated that the structural modification improved the activity, being about 14-fold more active than the standard drugs, such as acetylsalicylic acid and acetaminophen.

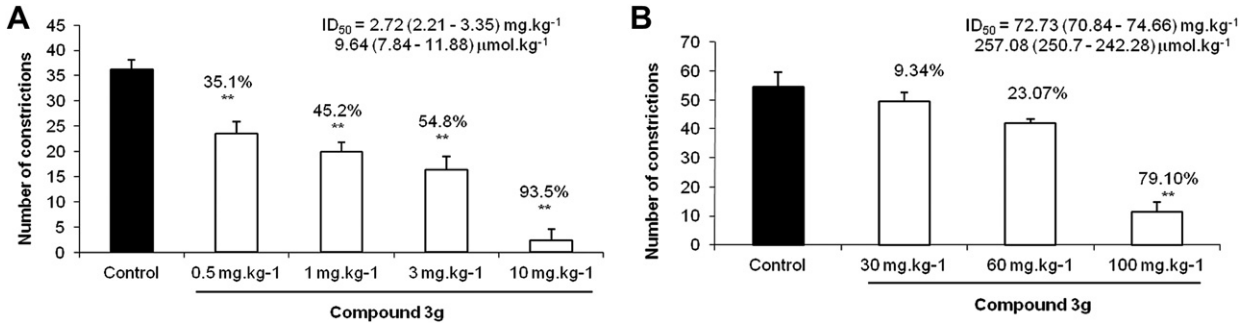


Fig. 2. Effect of compound **3g** (0.5, 1.0, 3.0 and 10 mg kg^{-1}), given intraperitoneally, (A) and (30, 60 and 100 mg kg^{-1}), given orally (B) against 0.6% acetic acid-induced abdominal constrictions in mice. Each column represents the mean s.e.m. of six to eight experimental values. $^{*}p < 0.05$; $^{**}p < 0.01$.

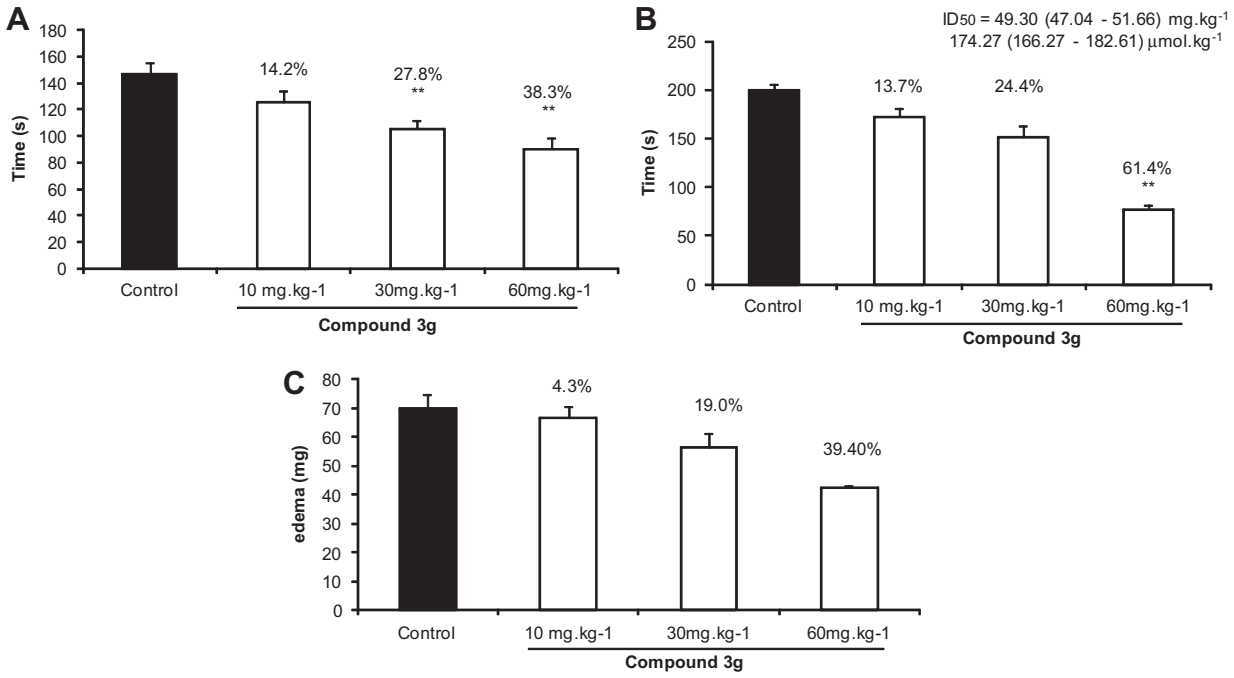


Fig. 3. Effect of compound **3g** (10, 30 and 60 mg kg^{-1}), given intraperitoneally, against the first (0–5 min) phase (A) and against the second (15–30 min) phase (B) and edema (C) in a formalin test in mice. Each column represents the mean s.e.m. of six to eight experimental values. $^{*}p < 0.05$; $^{**}p < 0.01$.

When evaluated by the oral route, compound **3g** inhibited abdominal constrictions by 79.1% at 100 mg/kg, being 2-times more active than the standard drugs and demonstrating good oral absorption, which is important for a drug candidate. Although the writhing test is a non-specific model (i.e. anticholinergic and antihistaminic and other agents also show activity in this test), the fact that compound **3g** significantly reduces the number of abdominal constrictions induced by 0.6% acetic acid solution suggests an antinociceptive potential, since this model is widely used for analgesic screening and involves local peritoneal receptors (cholinergic and histamine receptor) and the acetylcholine and histamine mediators [25].

Compound **3g** was also analyzed in the formalin-induced pain test, a reported behavior model characterized by the first phase (neurogenic), which is evoked by direct formalin stimulation of the nerve endings followed by substance P release, and the second phase, mainly due to a subsequent inflammation reaction in the peripheral tissue [21,25,26].

The biphasic control of pain, which reflects different pathological processes, enables the elucidation of the possible mechanism involved in analgesia [26]. Centrally acting drugs, such as opioids, inhibit both phases of pain by equally involving the effect produced by prostaglandins released at this level in response to inflammation [26,27]. Some nonsteroidal anti-inflammatory drugs (NSAIDs) are only peripheral acting drugs, such as acetylsalicylic acid, and therefore reduce nociception only in the late phase, while another, mefenamic acid, had a central site of action, as well as a peripheral one, and affected both phases, although the second phase response was inhibited by lower doses of these drugs than the first phase response [27,28]. Compound **3g** inhibited the first and second phase of the formalin test, suggesting an involvement at both central and peripheral levels, being much more active in the second phase.

Compound **3g** (10 mg/kg, i. p.) was also analyzed in the capsaicin test, confirming the direct evidence of the antinociceptive effect of this compound on neurogenic pain, since acute neurogenic

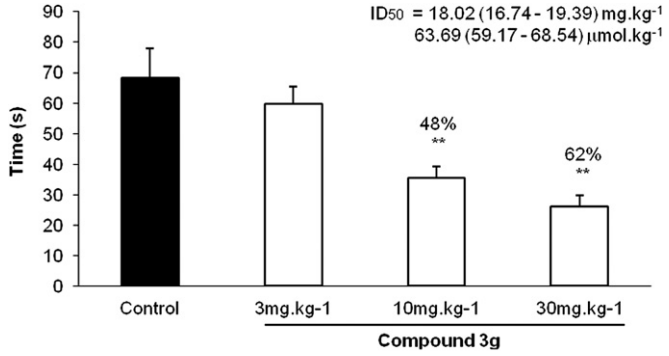


Fig. 4. Effect of compound **3g** (3, 10 and 30 mg kg^{-1}) on licking/biting response induced by intraplantarly injection of capsaicin in mice. Each group represents the mean of six to eight experiments. $^{**}p < 0.01$, compared with the corresponding control value.

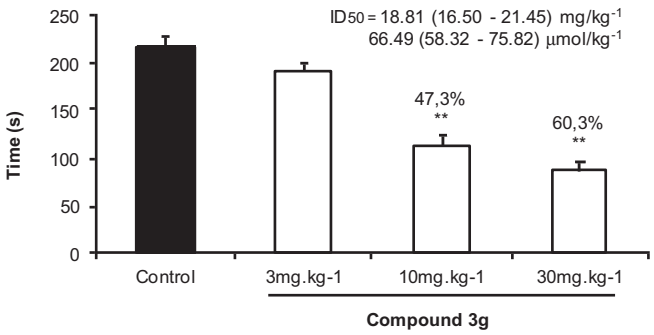


Fig. 5. Effect of compound **3g** (3, 10 and 30 mg kg^{-1}) on licking/biting response induced by intraplantarly injection of glutamate in mice. Each group represents the mean of six to eight experiments. $^{**}p < 0.01$, compared with the corresponding control value.

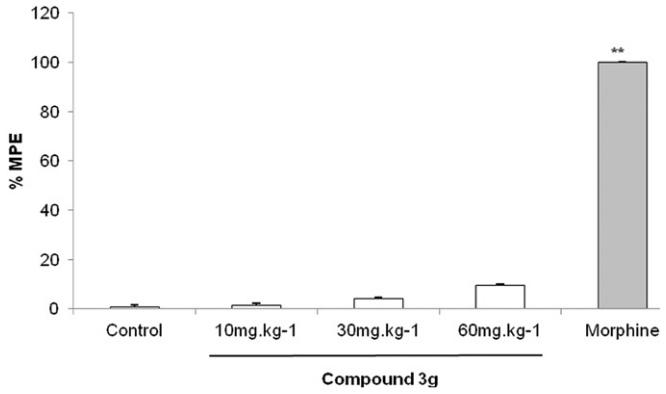


Fig. 6. Effect of compound **3g** (10, 30 and 60 mg kg^{-1}, i.p.) and morphine (5 mg kg^{-1}, *s.c.*) on the nociceptive effect in the hot plate test. Each column represents the mean s.e.m. of six to eight experimental values. $^{*}p < 0.05$; $^{**}p < 0.01$.

inflammation can be evoked by intradermal injection of capsaicin. Inflammation triggered by substances released from sensory terminals is referred to as neurogenic inflammation. The substances that contribute to neurogenic inflammation include substance P, which results in plasma extravasation and calcitonin gene-related peptide that triggers vasodilation [29]. These results suggest a possible involvement of this compound with the antagonism of the vanilloid receptor.

Of great interest are the findings that demonstrate that the antinociception caused by **3g** could involve, at least in part, its ability to interact with excitatory amino acids, as demonstrated by the fact that it caused significant inhibition of hyperalgesia induced by intraplantar injection of glutamate in mice. According to Beirith and co-workers [21], intraplantar injection of glutamate into the mouse hind paw results in rapid onset and short duration nociception, the response of which is largely mediated by both *N*-methyl-D-aspartate (NMDA) and non-NMDA (α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate (AMPA), kainite (KA) and metabotropic) receptors and by the glycine modulatory site.

It is known that NSAIDs usually do not increase the pain threshold in normal tissues in the way local anesthetics and narcotics do. Likewise, the results obtained for compound **3g** in the hot plate test did not show any significant activity.

In recent years, estimate potential for drug have rapidly and significantly increased in drug discovery. A computational study for prediction of ADME properties of all molecules **3**(**a**–**h**) was performed in a web-based application denoted as Molinspiration [30] and is presented in Table 1 to assess the bioavailability of the synthesized compounds. Initially we calculated the parameters according to the Lipinski's "rule of five", which has been widely used as a filter for substances that could likely be further developed in drug design programs. This rule follows some principles: poor absorption of permeation is more likely when there are more than five H-bonds donors, the H-bond acceptors, the molecular weight (MW) is greater than 500 and the calculated log *P* is greater than five [31]. Molecules violating more than one of these rules may have problems with bioavailability. According to Lipinski' Rule the synthesized compounds presented in this study do not show any violation, these compounds being, theoretically, defined as druglike.

The Human Intestinal Absorption (HIA) and penetration in Blood-Brain Barrier (BBB) of the studied compounds were predicted by using a PreADMET [32] HIA is one of the most important ADME properties. It is also one of the key steps during the drugs transporting to their targets. Due to the diverse pathways of drugs absorption, powerful descriptors related to carrier-mediated transport and first-pass metabolism are needed for building a useful prediction model for human oral bioavailability [33].

Topological polar surface area (TPSA), a surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines. TPSA and volume are inversely proportional to %HIA [34].

Although HIA is considered as one of the important components which influence the bioavailability, there are some differences in the experimental values by compounds or their metabolisms. Yee [35] consider three categories: Poorly absorbed (0–20%), moderately absorbed (20–70%) and well absorbed (70–100%). All the studied compounds predicted by PreADMET presented % HIA over 97%, being considered well absorbed.

The TPSA also is related with the blood–brain barrier crossings. Prediction Blood–Brain Barrier (BBB) penetration means predicting whether compounds pass across the blood–brain barrier. This is crucial in pharmaceutical sphere because CNS-inactive compounds do not should pass across it in order to avoid of CNS side effects. Such evaluation also can present some differences in the experimental values by compounds or their metabolisms. According to Ma et al. [36], high absorption to CNS occur when BBB is more than 2.0; middle absorption is in the range 2.0–0.1 and low absorption is less than 0.1.

With exception the compound **3a** which exhibited a BB value 2.98, being the only compounds of series considered CNS-active, the other compounds were classified as middle to low absorption to

Table 3
Pharmacokinetic properties important for ADME prediction of compounds **3a**–**h**.

Code	MW[a]	Volume (A^3)	TPSA (A^2)[b]	NROTB[c]	HBA[d]	HBD[e]	log *P*[f]	%HIA[g]	BBB[h]	Violations
3a	252.27	225.19	43.38	3	3	0	2.96	97.91	2.98	0
3b	282.29	250.74	52.61	4	4	0	3.01	98.07	0.18	0
3c	266.30	241.75	43.38	3	3	0	3.41	97.82	0.04	0
3d	286.71	238.73	43.38	3	3	0	3.64	97.61	0.18	0
3e	321.16	252.26	43.38	3	3	0	4.24	97.65	0.15	0
3f	312.32	276.28	61.84	5	5	0	2.61	98.50	0.05	0
3g	282.29	250.74	52.61	4	4	0	2.99	98.07	0.02	0
3h	331.16	243.08	43.38	3	3	0	3.77	97.61	0.18	0

[a] MW, molecular weight.
[b] TPSA, topological polar surface area.
[c] NROTB, number of rotatable bonds.
[d] HBA, number of hydrogen bond donors.
[e] HBD, number of hydrogen bond acceptors.
[f] log *P*, logarithm of compound partition coefficient between *n*-octanol and water.
[g] %HIA, percentage of Human intestinal absorption.
[h] BBB, blood–brain barrier penetration.

CNS. It is important emphasize that the 3-[2-(3-methoxyphenyl)-2-oxoetil]-2-benzofuran-1(3*H*)-one (**3g**), which showed greater antinociceptive activity than the compound 3-[2-(4-chlorophenyl)-2-oxoetil]-2-benzofuran-1 (3*H*)-one (**3d**), confirmed the efficiency of the Topliss Method as a research tool for the discovery of candidate molecules for a new antinociceptive drug. In addition it (**3g**) presented better characteristics pharmacokinetics such as the absorption intestinal and less side effects according data indicated in Table 3.

In conclusion, the use of the Topliss approach led to the synthesis of a novel benzofuranones series with potential antinociceptive activity and the discovery of a promising molecule (**3g**) with better pharmacokinetic properties predicted. This compound (**3g**) demonstrated spinal and supraspinal antinociception when assessed against both formalin- and capsaicin-induced neurogenic pain, as well as in glutamate-induced hyperalgesia in mice. The precise mechanism underlying the antinociceptive action of compound **3g** has yet to be determined, but it is unlikely to be associated with an opioid-like effect. Finally, it is interesting to observe that compound **3g** was more potent than some clinically used drugs, and experiments are currently underway to look for other possible pharmacological properties.

Acknowledgments

We are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Apoio à Pesquisa Científica e Tecnológica do Estado de Santa Catarina (FAPESC) and Universidade do Vale do Itajaí (UNIVALI) for their financial support.

References

[1] R. Corrêa, M.A.S. Pereira, D. Buffon, L. Santos, V. Cechinel-Filho, A.R.S. Santos, R.J. Nunes, Antinociceptive properties of chalcones. Structure-activity relationships, Arch. Pharm. 334 (2001) 332–334.
[2] F. Campos-Buzzi, J.P. Campos, P.P. Tonini, R. Corrêa, R.A. Yunes, P. Boeck, V. Cechinel-Filho, Antinociceptive effects of synthetic chalcones obtained from xanthoxyline, Arch. Pharm. 339 (2006) 361–365.
[3] Z. Nowakowska, A review of anti-infective and anti-inflammatory chalcones, Eur. J. Med. Chem. 42 (2007) 125–137.
[4] M. Cabrera, M. Simoens, G. Falchi, M.L. Lavaggi, O.E. Piro, E.E. Castellano, A. Vidal, A. Azqueta, A. Monge, A.L. de Ceráin, G. Sagrera, G. Seoane, H. Cerecetto, M. González, Synthetic chalcones, flavanones, and flavones as antitumoral agents: biological evaluation and structure-activity relationships, Bioorg. Med. Chem. 15 (2007) 3356–3367.
[5] D. Batovska, S. Parushev, A. Slavova, V. Bankova, I. Tsvetkova, M. Ninova, H. Najdenski, Study on the substituents' effects of a series of synthetic chalcones against the yeast *Candida albicans*, Eur. J. Med. Chem. 42 (2007) 87–92.
[6] P. Boeck, B. Falcão, P.C. Leal, R.A. Yunes, V. Cechinel-Filho, E.C. Torres-Santos, B. Rossi-Bergmann, Synthesis of chalcone analogues with increased antileishmanial activity, Bioorg. Med. Chem. 14 (2006) 1538–1545.
[7] C. Kontogiorgis, M. Mantzanidou, D. Hadjipavlou-Litina, Chalcones and their potential role in inflammation, Mini Rev. Med. Chem. 8 (2008) 1224–1242.
[8] A.M. Katsori, D. Hadjipavlou-Litina, Chalcones in cancer: understanding their role in terms of QSAR, Curr. Med. Chem. 16 (2009) 1062–1081.
[9] A.M. Katsori, D. Hadjipavlou-Litina, Recent progress in therapeutic applications of chalcones, Expert Opin. Ther. Pat. 21 (2011) 1575–1596.
[10] J.R. Dimmock, D.W. Elias, M.A. Beazely, N.M. Kandepu, Bioactivities of chalcones, Curr. Med. Chem. 6 (1999) 1125–1149.
[11] L. Ni, Q.M. Meng, J.A. Siroski, Recent advances in therapeutic chalcones, Expert Opin. Ther. 14 (2004) 1669–1691.
[12] K. Terasawa, Y. Sugita, I. Yokoe, S. Fujisawa, H. Sakagami, Cytotoxic activity of 2-aminomethylene-3(2*H*)-benzofuranones against human oral tumor cell lines, Anticancer Res. 21 (2001) 3371–3375.
[13] N. Hadj-esfandiari, L. Navidpour, H. Shadnia, M. Amini, N. Samadi, M.A. Faramarzi, A. Shafie, Synthesis, antibacterial activity, and quantitative structure–activity relationships of new (*Z*)-2-(nitroimidazolylmethylene)-3(2*H*)-benzofuranone derivatives, Bioorg. Med. Chem. Lett. 17 (2007) 6354–6363.
[14] P. Padaratz, M. Fracasso, F. Campos-Buzzi, R. Corrêa, R. Niero, F. Delle Monache, V. Cechinel-Filho, Antinociceptive activity of a new benzofuranone derived from a chalcone, Basic Clin. Pharmacol. Toxicol. 105 (2009) 257–261.
[15] X.Q. Zhu, J. Zhou, C.H. Wang, X.T. Li, S. Jing, Actual structure, thermodynamic driving force, and mechanism of benzofuranone-typical compounds as antioxidants in solution, J. Phys. Chem. B 115 (2011) 3588–3603.
[16] T.B. Schnaide, C. de Souza, Aspectos éticos da experimentação animal, Rev. Bras. Anestesiol. 53 (2003) 278–285.
[17] H.D.J. Collier, L.C. Dinneen, C.A. Johnson, C. Schneider, The abdominal constriction response and its suppression by analgesic drugs in the mouse, Br. J. Pharmacol. 32 (1968) 295–310.
[18] S. Hunskaar, O.B. Fasmer, K. Hole, Formalin test in mice, a useful technique for evaluating mild analgesics, J. Neurosci. Methods 14 (1985) 69–76.
[19] A. Tjolsen, O.G. Berge, S. Hunskaar, J.H. Rosland, K. Hole, The formalin test: an evaluation of the method, Pain 51 (1992) 5–17.
[20] T. Sakurada, K. Katsumata, K. Tan-No, S. Sakurada, K. Kisara, The capsaicin test in mice for evaluating tachykinin antagonists in the spinal cord, Neuropharmacology 31 (1992) 1279–1285.
[21] A. Beirith, A.R.S. Santos, J.B. Calixto, Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw, Brain Res. 924 (2002) 219–228.
[22] N.B. Eddy, D. Leimback, Synthetic analgesics. II. Dithienylbutenyl and dithienylbutilamines, J. Pharmacol. Exp. Ther. 107 (1953) 385–393.
[23] D.Y. Lee, C.S. Cho, L.H. Jiang, X. Wu, S.C. Shim, D.H. Oh, Palladium-Catalysed synthesis of 3-alkylphtalides via carbonylative cyclization of o-bromobenzaldehyde with 1,3-dicarbonyl compounds, Syn. Commun. 27 (1997) 3449–3455.
[24] M.V. Paradkar, S.Y. Gadre, T.A. Pujari, P.P. Khandekar, V.B. Kumbhar, One-pot synthesis of 3-phenacylphthalides, Syn. Commun. 35 (2005) 471–474.
[25] C. Meyre-Silva, R.A. Yunes, V. Schlemper, F. Campos-Buzzi, V. Cechinel Filho, Analgesic potential of marrubiin derivatives, a diterpene present in *Marrubium vulgare* (Lamiaceae), Il Fármaco 60 (2005) 321–326.
[26] M. Shibata, T. Okhubo, H. Takahashi, R. Inoki, Modified formalin test: characteristics biphasic pain response, Pain 38 (1989) 346.
[27] S. Hunskaar, K. Hole, The formalin test in mice: dissociation between inflammatory and non-inflammatory, Pain 30 (1987) 103.
[28] J.H. Rosland, A. Tjolsen, B. Machle, K. Hole, The formalin test in mice, effect of formalin concentration, Pain 42 (1990) 235.
[29] Q. Lin, X. Zou, Y. Ren, J. Wang, L. Fang, D. Willis, Involvement of peripheral neuropeptide y receptors in sympathetic modulation of acute cutaneous flare induced by intradermal capsaicin, Neuroscience 123 (2004) 337–347.
[30] http://www.molinspiration.com/cgi-bin/properties.
[31] C.L. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Del. Rev. 46 (2001) 3–26.
[32] http://preadmet.bmdrc.org/preadmet/index.php.
[33] A.P. Beresford, H.E. Selick, M.H. Tarbit, The emerging importance of predictive ADME simulation in drug discovery, Drug Discov. Today 7 (2002) 109–116.
[34] P. Ertl, B. Rohde, P. Selzer, Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties, J. Med. Chem. 43 (2000) 3714–3717.
[35] S. Yee, In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man–fact or myth, Pharm. Res. 14 (1997) 763–766.
[36] X. Ma, C. Chen, J. Yang, Predictive model of blood-brain barrier penetration of organic compounds, Acta Pharmacol. Sínica 26 (2005) 500–512.