Antinociceptive Activity and Preliminary Structure-Activity Relationship of Chalcone-Like Compounds

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Chalcones belong to a class of α,β-unsaturated aromatic ketones which occur abundantly in nature, especially in plants. They are promising and interesting compounds due to their vast applications in pharmaceuticals, agriculture and industry. Several studies have shown that these compounds exert important biological activities in different experimental models. The present work deals with the antinociceptive activity, evaluated against the writhing test, of three series of chalcone-like compounds obtained by the Claisen-Schmidt condensation, using different aldehydes and substituted acetophenones. The results reveal that the compounds synthesized show a significant antinociceptive effect compared with nonsteroidal drugs such as aspirin, paracetamol and diclofenac. They also show that the electronic demand of the substituents is the dominant factor of the biological activity.

Key words: Chalcones, Claisen-Schmidt Reaction, Antinociceptive Activity

Introduction

The present work deals with the antinociceptive activity, evaluated against the writhing test, of three series of chalcone-like compounds obtained by the Claisen-Schmidt condensation, using different aldehydes and substituted acetophenones. The Claisen-Schmidt reaction has been used to synthesize carbonyl compounds due to the operational simplicity, easy work-up, good yield and eco-friendly nature (Thirunarayanan, 2007). This reaction is very useful for the formation of carbon-carbon bonds in the synthesis of chalcones.

Chalcones consist of a group of compounds which exhibit promising biological activities (Popova *et al.*, 2001; Ni *et al.*, 2004; Yunes *et al.*, 2006). In plants, chalcones are precursors of flavones (Dewick, 1997; Usman *et al.*, 2006), *i.e.* compounds which are abundant in edible plants (Achanta *et al.*, 2006). The chemical structure of chalcones (1,3-diphenyl-2-propen-1-ones) consists of two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system (Corrêa *et al.*, 2001).

Previous studies carried out by our research group have revealed that some simple synthetic chalcones, including those derived from natural products, like xanthoxyline (Boeck *et al.*, 2006), exhibit a pronounced antinociceptive effect on mice in the writhing test. We have verified that the introduction of different substituent groups in the chalcone aromatic rings causes drastic changes in the measured biological activity. This behaviour may be explained by the involvement of electronic and steric parameters (Corrêa *et al.*, 2001; Campos-Buzzi *et al.*, 2007).

Material and Methods

General chemical procedures

The chalcone-like compounds were synthesized by an adaptation of the general method of the Claisen-Schmidt condensation (Corrêa *et al.*, 2001; Pinto *et al.*, 2000; Mamolo *et al.*, 1999; Batovska *et al.*, 2007; Campos-Buzzi *et al.*, 2007).

The method foresees an equimolar mixture of benzaldehydes and acetophenones dissolved in water and ethanol, in the presence of 10 % sodium hydroxide. The solution was kept under mechanical stirring for 24 h or until the point at which it was no longer possible to stir the mixture (due to product precipitation). In order to confirm the effective termination of the reaction, the product formation was analyzed by thin layer chromato-

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graphy. The products were percolated and washed several times with cold distilled water, until the wash waters presented a neutral pH value. The isolated products were dried under reduced pressure in the presence of phosphorus pentoxide. In some cases, depending on the nature of the substratum, it was necessary to use higher energy conditions, through the heating of the reactional mixture with a reflux apparatus. In cases where there was no precipitation, the mixture was submitted to an extraction process with various solvents. The solutions obtained were dried with anhydrous magnesium sulfate, and solvent was evaporated under reduced pressure. All solids obtained were purified, where necessary, by the usual methods such as recrystallization or column chromatography on SiO_2 eluted with gradients of hexane/ethyl acetate. The solvents and reagents were purchased from Sigma-Aldrich® Co. (St. Louis, MO, USA) and Merck® KGaA (Darmstadt, Germany).

The compounds obtained, in all the series, were characterized by their melting point using a Micro-Química APF-01 apparatus (Micro-Química, Florianópolis, SC, Brazil) and were uncorrected. Microanalysis (CHN) was performed with a Perkin Elmer PE 2400 Series II CHNS/O analyzer (Perkin-Elmer, Norwalk, CT, USA). Additional characterization was performed by Fourier transform infrared (FT-IR) spectroscopy using a BOMEM-MB 100 spectrometer (BOMEM, St. Jean Baptiste, QB, Canada) with KBr disks, and ^1H and ^{13}C nuclear magnetic resonance spectroscopy using a Bruker AC-300 apparatus (Bruker, Karlsruhe, Germany). The compounds were dissolved in deuterated solvents with tetramethylsilane (TMS) as the internal standard.

Physicochemical and NMR data for the 21 analogues can be obtained by directly contacting the authors.

All derivatives **1**–**21** were synthesized according to the general method described above and also displayed in Fig. 1.

*Cinnamaldehyde series (compounds **1** to **7**)*

(2*E*,4*E*)-1,5-(Diphenyl)-penta-2,4-dien-1-one (**1**); (2*E*,4*E*)-1-(3,4-dichlorophenyl)-5-phenyl-penta-2,4-dien-1-one (**2**); (2*E*,4*E*)-1-(4-methylphenyl)-5-phenyl-penta-2,4-dien-1-one (**3**); (2*E*,4*E*)-1-(4-methoxy-phenyl)-5-phenyl-penta-2,4-dien-1-one (**4**); (2*E*,4*E*)-1-(4-nitrophenyl)-5-phenyl-penta-2,4-dien-1-one (**5**); (2*E*,4*E*)-1-(4-bromophenyl)-5-phenyl-penta-2,4-dien-1-one (**6**); (2*E*,4*E*)-1-(4-chlorophenyl)-5-phenyl-penta-2,4-dien-1-one (**7**).

*2-Pyridine carboxaldehyde series (compounds **8** to **14**)*

(2*E*)-1-(Phenyl)-3-pyridin-2-yl-propenone (**8**); (2*E*)-1-(3,4-dichlorophenyl)-3-pyridin-2-yl-propenone (**9**); (2*E*)-1-(4-methylphenyl)-3-pyridin-2-yl-propenone (**10**); (2*E*)-1-(4-methoxyphenyl)-3-pyridin-2-yl-propenone (**11**); (2*E*)-1-(4-nitrophenyl)-3-pyridin-2-yl-propenone (**12**); (2*E*)-1-(4-bromophenyl)-3-pyridin-2-yl-propenone (**13**); (2*E*)-1-(4-chlorophenyl)-3-pyridin-2-yl-propenone (**14**).

*Salicylaldehyde series (compounds **15** to **21**)*

(2*E*)-1-(Phenyl)-3-(2-hydroxyphenyl)-propen-1-one (**15**); (2*E*)-1-(3,4-dichlorophenyl)-3-(2-hydroxyphenyl)-propen-1-one (**16**); (2*E*)-1-(4-methylphenyl)-3-(2-hydroxyphenyl)-propen-1-one (**17**); (2*E*)-1-(4-methoxyphenyl)-3-(2-hydroxyphenyl)-propen-1-one (**18**); (2*E*)-1-(4-nitrophenyl)-3-(2-hydroxyphenyl)-propen-1-one (**19**); (2*E*)-1-(4-bromophenyl)-3-(2-hydroxyphenyl)-propen-1-one (**20**); (2*E*)-1-(4-chlorophenyl)-3-(2-hydroxyphenyl)-propen-1-one (**21**).

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 only 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 investigations of experimental pain in conscious animals (Zimmerman, 1983).

Acetic acid-induced writhing

Abdominal constriction was induced in mice by intraperitoneal injection of acetic acid (0.6 %), as described by Collier *et al.* (1968) with minor modifications (Campos-Buzzi *et al.*, 2002, 2007; Costa *et al.*, 2007). The animals were pre-treated intraperitoneally with all the studied compounds (1 to 600 μmol/kg, 30 min before). The reference drugs were tested in the same manner. The control animals received a similar volume of saline solution (10 mL/kg). 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.

Statistical analysis

The biological results are presented as mean ± S.E.M., except for the ID_{50} values (the dose of each tested compound 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} values were determined by linear regression from individual experiments using the GraphPad® software (San Diego, CA, USA). The statistical significance of the differences for the comparison between the groups and the control was detected by ANOVA, followed by Dunnett's multiple comparison test. *P*-values of less than 0.05 ($P < 0.05$) were considered indicative of significance. The graph with multiple scatter fits shown in Fig. 2 was produced using the software Sigmaplot® 10.0 (Systat Software Inc., San Jose, CA, USA).

Results and Discussion

This work presents the antinociceptive effects on mice in the writhing test of some chalcone-like compounds (**1**–**21**) that were obtained by the Claisen-Schmidt condensation of cinnamaldehyde, 2-pyridine carboxaldehyde and salicylaldehyde with acetophenones (Fig. 1).

The majority of the synthesized compounds was obtained with good yields, and all of them were satisfactorily characterized by spectroscopic techniques (^1H and ^{13}C NMR and FT-IR spectroscopy) and microanalysis. The compounds that presented small yields were those which were difficult to isolate from the reactional mixture.

The derivatives of 2-pyridine carboxaldehyde, **8**–**14**, were obtained in markedly slower reactions. The isolated products were purified by column chromatography, due to the presence of impurities, which were not eliminated by recrystallization techniques.

The hydroxylated chalcones derived from salicylaldehyde, **15**–**21**, were obtained in reactions that required more energetic conditions. In these compounds, the increased electron density of the aromatic ring due to the *p*-hydroxy group decreases the carbon eletrophilicity of the carbonyl group, making it difficult for the carbanion to attack the eletrophilic centre.

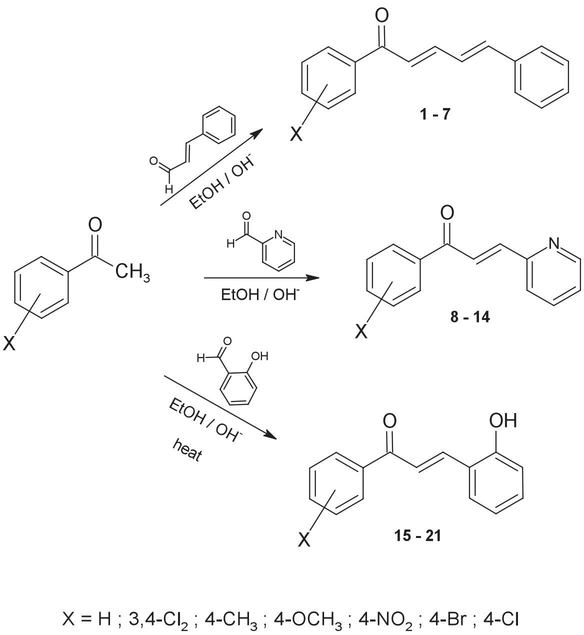


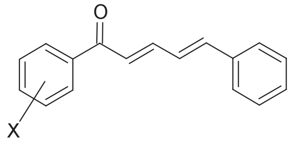
Fig. 1. Synthesis of chalcone-like compounds.

The chalcone molecule is easily recognizable by FT-IR spectroscopy due to the presence of characteristic functional groups, like carbonyl, and the conjugated olefinic unsaturation. In the NMR spectra, the main contribution is the recognition of the molecular stereochemistry, since all the synthesized compounds present the coupling constant (*J*) from olefinic hydrogen (H_α-H_β) coupling, situated in the range of 15–16 Hz, that indicates *E* configuration (Pavia *et al.*, 1996; Ram *et al.*, 2000).

The antinociceptive potential of all the compounds and some reference drugs was determined using the writhing test in mice. In this assay the maximum inhibition (MI), promoted by the synthesized compounds, was determined by means of the algic impulse obtained through the induction of abdominal constrictions promoted by diluted acetic acid. The inhibitory dose of 50 % of the nociceptive response, relative to the control value (ID_{50}), was also determined (Tables I–III).

Although the writhing test is a nonspecific model (*e.g.* anticholinergic and antihistaminic and other agents show activity), it is widely used for analgesic screening. It involves local peritoneal receptors (cholinergic and histamine receptors) and the acetylcholine and histamine mediators which are generally

Table I. Effect of compounds **1** to **7** and the reference drugs in the writhing test using intraperitoneal injection of diluted acetic acid (0.6 %) in mice.



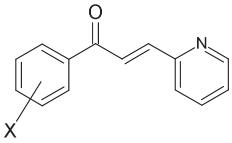
Compound	X	σ	ID_{50} [μM]	log $1/ID_{50}$	MI (%)
1	H	0.00	27.60 (33.80– 23.30)	4.56	92 ± 2.0
2	3,4-Cl_2	0.52	10.80 (17.30– 5.40)	4.97	100
3	4-CH_3	−0.14	34.30 (47.40– 23.10)	4.46	88 ± 4.0
4	4-OCH_3	−0.28	52.20 (66.70– 38.40)	4.28	82 ± 1.0
5	4-NO_2	0.81	12.30 (15.80– 9.70)	4.91	98 ± 2.0
6	4-Br	0.26	17.60 (28.90– 8.00)	4.75	96 ± 4.0
7	4-Cl	0.23	16.75 (29.60– 9.80)	4.78	96 ± 2.0
Aspirin[a]	–	–	133.20 (73.00–243.10)	–	83 ± 2.0
Paracetamol[b]	–	–	125.00 (104.00–150.00)	–	88 ± 1.0
Diclofenac[c]	–	–	38.00 (29.50– 49.00)	–	93 ± 7.0

[a] Acetyl salicylic acid (A.A.S.), 2-(acetyloxy) benzoic acid.

[b] Acetaminophen, *N*-(4-hydroxyphenyl) acetamide.

[c] Sodium diclofenac, sodium {2-[(2,6-dichlorophenyl)amino]phenyl} acetate.

Table II. Effect of compounds **8** to **14** and the reference drugs in the writhing test using intraperitoneal injection of diluted acetic acid (0.6 %) in mice.

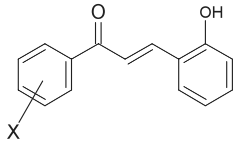


Compound	X	σ	ID_{50} [μM]	log $1/ID_{50}$	MI (%)
8	H	0.00	36.60 (31.00– 43.40)	4.44	89 ± 1.0
9	3,4-Cl_2	0.52	22.20 (19.30– 26.40)	4.65	94 ± 2.0
10	4-CH_3	−0.14	51.00 (45.30– 58.00)	4.29	80 ± 2.0
11	4-OCH_3	−0.28	64.30 (61.60– 69.00)	4.19	83 ± 2.0
12	4-NO_2	0.81	26.90 (22.30– 32.40)	4.57	92 ± 4.0
13	4-Br	0.26	33.70 (21.60– 47.30)	4.47	86 ± 4.0
14	4-Cl	0.23	31.80 (22.75– 43.28)	4.50	92 ± 2.0
Aspirin	–	–	133.20 (73.00–243.10)	–	83 ± 2.0
Paracetamol	–	–	125.00 (104.00–150.00)	–	88 ± 1.0
Diclofenac	–	–	38.00 (29.50– 49.00)	–	93 ± 7.0

related to prostanoids by the increase of the levels of PGE2 and PGF2a in peritoneal fluids, as well as to products of lipoxygenase (Vongtau *et al.*, 2004).

The results summarized in Tables I–III reveal that the compounds synthesized show a significant antinociceptive effect when compared with non-steroidal drugs such as aspirin, paracetamol and diclofenac, based on the acetic acid-induced abdominal contortion model. All the synthesized compounds were more potent than aspirin and paracetamol; for example, compounds **2** and **5** were more than ten times more potent than these two drugs. Regarding diclofenac, just five of the twenty-one derivatives prepared showed lower po-

Table III. Effect of compounds **15** to **21** and the reference drugs in the writhing test using intraperitoneal injection of diluted acetic acid (0.6 %) in mice.



Compound	X	σ	ID_{50} [μM]	log $1/ID_{50}$	MI (%)
15	H	0.00	34.30 (22.30– 47.80)	4.46	90 ± 3.0
16	3,4-Cl_2	0.52	16.70 (8.300– 27.10)	4.78	97 ± 1.0
17	4-CH_3	–0.14	48.20 (42.20– 56.30)	4.32	84 ± 2.0
18	4-OCH_3	–0.28	64.00 (56.50– 73.10)	4.19	82 ± 2.0
19	4-NO^2	0.81	19.00 (15.10– 23.80)	4.72	89 ± 4.0
20	4-Br	0.26	24.70 (18.60– 31.90)	4.61	95 ± 3.0
21	4-Cl	0.23	28.10 (22.13– 34.80)	4.55	94 ± 1.0
Aspirin	–	–	133.20 (73.00–243.10)	–	83 ± 2.0
Paracetamol	–	–	125.00 (104.00–150.00)	–	88 ± 1.0
Diclofenac	–	–	38.00 (29.50– 49.00)	–	93 ± 7.0

tency: compounds **4**, **10**, **11**, **17** and **18**. Structural observation of these compounds shows that they have electron-donor substituents in common: 4-CH_3 (in compounds **10** and **17**) and 4-OCH_3 (in compounds **4**, **11** and **18**).

In terms of effectiveness, the results show that most of the synthesized compounds presented the same maximum inhibition ranges as those observed for the standard drugs. The three series highlight, in particular, compounds **2**, **9** and **16**, which showed greater effectiveness than the three reference drugs and have, in common, an electron-withdrawing substituent (3,4-Cl_2).

Analyzing the data displayed in Tables I–III and despite the reduced number of compounds in each series, it is clear that the electronic demand of the substituents is the dominant factor for the activity. This can be better verified by the graph in Fig. 2, which correlates the biological activity (log $1/ID_{50}$) with the physicochemical parameter (Hammett constant σ). This graph shows that the three series exhibit the same behaviour.

Additionally, the structure-activity relationship indications can be analyzed by Topliss' criteria. This manual method, which consists of a nonstatistical and noncomputerized model, is based on the synthesis of five derivatives which contain the substituents: H, 4-Cl, 3,4-Cl_2, 4-CH_3 and 4-OCH_3. The compounds thus substituted were chosen because they are easy to obtain synthetically and they present varied sterical and electronic characteristics. The results of the biological activity tests were analyzed according to the parameters π (hydrophobic constant), σ (Hammett constant), *E* (steric effect), and $\pi-\sigma$; $\pi-2\sigma$; $\pi-3\sigma$; $\pi+\sigma$, ... (π- and σ-related). A proposed potency order for various parameter dependencies (Table IV) is compared with the quantitative sequential order of biological activity of the five derivatives synthesized. At this point, the method identifies which structural parameters are involved in obtaining the biological effect (Topliss, 1977; Rando *et al.*, 2002; Yunes *et al.*, 2002; Costa, *et al.*, 2007).

In the present work, the three series showed dependency on the same parameter, according to the criteria defined by Topliss. It is the Hammett constant σ, which is directly linked to the property of molecular electronic demand (Table V).

The conclusion, based on Topliss' manual method, is according to what is observed in Fig. 2, which correlates log $1/ID_{50}$ with σ, and shows the same behaviour occurring in the three proposed series.

The next step in this work will be to test a larger series of these chalcone-like compounds, with a wider chemical diversity, including other derivatives proposed by Topliss (proposed derivatives in the other steps of the manual method), as well as a quantitative evaluation of the structure-activity relationship. Another objective will be to evaluate the "drugability" of these compounds through Lipinski's parameters (Lipinski *et al.*, 2001; Rando *et al.*, 2002; Keller *et al.*, 2006).

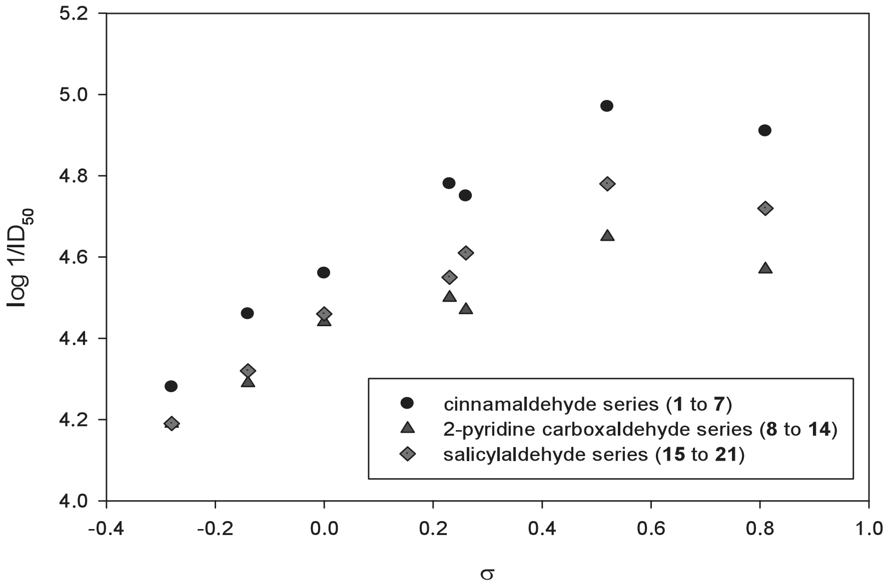


Fig. 2. Biological activity (log $1/ID_{50}$) versus Hammett constant (σ) for the three chalcone-like compound series.

Table IV. Topliss' potency order for various parameter dependencies (Topliss, 1977).

Substituent	Parameters									
	π	$2\pi - \pi^2$	σ	$-\sigma$	$\pi + \sigma$	$2\pi - \sigma$	$\pi - \sigma$	$\pi - 2\sigma$	$\pi - 3\sigma$	E_4^a
3,4-Cl_2	1	1–2	1	5	1	1	1–2	3–4	5	2–5
4-Cl	2	1–2	2	4	2	2–3	3	3–4	3–4	2–5
4-CH_3	3	3	4	2	3	2–3	1–2	1	1	2–5
4-OCH_3	4–5	4–5	5	1	5	4	4	2	2	2–5
H	4–5	4–5	3	3	4	5	5	5	3–4	1

[a] Unfavourable steric effect from 4-substitution.

Table V. Observed potency order for the three synthesized compound series.

Substituent	Potency order		
	Cinnamaldehyde series	2-Pyridine carboxaldehyde series	Salicylaldehyde series
3,4-Cl_2	1	1	1
4-Cl	2	2	2
4-CH_3	4	4	4
4-OCH_3	5	5	5
H	3	3	3

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Achanta G., Modzelewska A., Feng L., Khan S. R., and Hvang P. (2006), A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. Mol. Pharmacol. **70**, 426–433.

Batovska D., Parushev St., Slavova A., Bankova V., Tsvetkova I., Ninova M., and Najdenski H. (2007), Study on the substituents' effects of a series of synthetic chalcones against the yeast *Candida albicans*. Eur. J. Med. Chem. **42**, 87–92.

Boeck P., Falcão C. A. B., Leal P. C., Yunes R. A., Cechinel-Filho V., Torres-Santos E. C., and Rossi-Bergmann B. (2006), Synthesis of chalcone analogues with increased antileishmanial activity. Bioorg. Med. Chem. **14**, 1538–1545.

Campos-Buzzi F., Corrêa R., De Souza M. M., Yunes R. A., Nunes R. J., and Cechinel-Filho V. (2002), Studies on new cyclic imides obtained from aminophenazone with analgesic properties. Arzneimittelforschung – Drug Res. **52**, 455–461.

Campos-Buzzi F., Padaratz P., Meira A. V., Corrêa R., Nunes R. J., and Cechinel-Filho V. (2007), 4-Acetamidochalcone derivatives as potential antinociceptive agents. Molecules **12**, 896–906.

Collier H. D. J., Dinnin L. C., Johnson C. A., and Schneider C. (1968), The abdominal response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. **32**, 295–310.

Corrêa R., Pereira M. A. S., Buffon D. E., Santos L., Cechinel-Filho V., Santos A. R. S., and Nunes R. J. (2001), Antinociceptive properties of chalcones. Structure activity relationships. Archiv. Pharm. **10**, 332–334.

Costa B. B. C., Corrêa R., Souza M. M., Pretto J. B., Ardenghi J. V., Campos-Buzzi F., and Cechinel Filho V. (2007), Antinociceptive effects of tetrahydrophthalimides and related compounds. Z. Naturforsch. **62c**, 201–206.

Dewick P. M. (1997), Medicinal Natural Products: A Biosynthetic Approach. Wiley & Sons Ltd., Chichester, England.

Keller T. H., Pichota A., and Yin Z. (2006), A practical view of 'drugability'. Curr. Opin. Chem. Biol. **10**, 357–361.

Lipinski C. A., Lombardo F., Dominy B. W., and Feeney P. J. (2001), Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. **46**, 3–26.

Mamolo M. G., Falagiani V., Vio L., and Banfi E. (1999), Synthesis and antimycobacterial activity of some N^1-[1-[3-aryl-1-(pyridine-2-, 3-, or 4-yl)-3-oxo]propyl]-2-pyridinecarboxamidazones. Farmaco **54**, 761–767.

Ni L., Meng Q. M., and Siroski J. A. (2004), Recent advances in therapeutic chalcones. Exp. Opin. **14**, 1669–1691.

Pavia D. L., Lampman G. M., and Kriz G. S. (1996), Introduction to Spectroscopy – A Guide for Students of Organic Chemistry, 2nd ed. Saunders College Publishing, Philadelphia, USA.

Pinto D. C. G. A., Silva A. M. S. S., Levai A., Cavaleiro J. A. S., Patonay T., and Elguero J. (2000), Synthesis of 3-benzoyl-4-styryl-2-pyrazolines and their oxidation to the corresponding pyrazoles. Eur. J. Org. Chem. **14**, 2593–2599.

Popova M., Bankova V., Spassov S., Tsvetkova I., Naydenski C., Silva M. V., and Tsartsarova M. (2001), New bioactive chalcones in propolis from El Salvador. Z. Naturforsch. **56c**, 593–596.

Ram V. J., Saxena A. S., Srivastava S., and Chandra S. (2000), Oxygenated chalcones and bischalcones as potential antimalarial agents. Bioorg. Med. Chem. Lett. **10**, 2159–2161.

Rando D. G., Sato D. N., Siqueira L., Malvezzi A., Leite C. Q. F., Amaral A. T., Ferreira E. I., and Tavares L. C. (2002), Potential tuberculostatic agents. Topliss application on benzoic acid [(5-nitro-thiophen-2-yl)-methylene]-hydrazide series. Bioorg. Med. Chem. **10**, 557–560.

Thirunarayanan G. (2007), Synthesis, characterization and correlation analysis in styryl 6-methoxy-2-naphthyl ketones. J. Korean Chem. Soc. **51**, 115–124.

Topliss J. G. (1977), A manual method for applying the Hansch approach to drug design. J. Med. Chem. **20**, 463–469.

Usman H., Hakim E. H., Harlim T., Jalaluddin M. N., Syah Y. M., Achmad S. A., and Takayama H. (2006), Cytotoxic chalcones and flavanones from the tree bark of *Cryptocarya costata*. Z. Naturforsch. **61c**, 184–188.

Vongtau H. O., Abbah J., Ngazal I. E., Kunle O. F., Chindo B. A., Otsapa P. B., and Gamaniel K. S. (2004), Anti-nociceptive and anti-inflammatory activities of the methanolic extract of *Parinari polyandra* stem bark in rats and mice. J. Ethnopharmacol. **90**, 115–121.

Yunes R. A., Heizen V. E. F., Cechinel Filho V., and Lazzarotto M. (2002), From the manual method of Topliss to a modified quantitative method. Arzneimittelforschung – Drug Res. **52**, 125–132.

Yunes R. A., Chiaradia L. D., Leal P. C., Cechinel Filho V., Torres-Santos E. C., Falcão C. A.B., and Rossi Bergmann B. (2006), Chalcones as new drugs against leishmaniasis. Curr. Trends Med. Chem. **4**, 47–56

Zimmerman M. (1983), Ethical guidelines for investigations of experimental pain in conscious animals. Pain **16**, 109–110.