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Antinociceptive properties of chalcones. Structure-activity relationships

Eleven chalcones were prepared and tested as antinociceptive agents using the writhing test in mice. Some compounds, given intraperitoneally, caused potent and dose-related antinociception, being several times more active than some reference drugs. The results evidenced that some physico-chemical parameters are involved in the pharmacological activity. 3,4-Dichlorochalcone (**2**) was the most effective compound, and was also studied in another model of pain in mice, the formalin test. Here it inhibited only the inflammatory pain (second phase), being equipotent to the reference drugs.

Key Words: Chalcones; Antinociception; Writhing test; Mice

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Introduction

Chalcones or 1,3-diaryl-2-propen-1-ones are natural or synthetic compounds belonging to the flavonoid family. They exhibit different kinds of biological activities, such as antimicrobial, anticancer, antiprotozoal, antiulcer, antiinflammatory, among others [1–4], and thus comprise a class with important therapeutic potential. Previous studies performed by Cechinel Filho and co-workers (1996) have demonstrated that some simple chalcones derived from xanthoxylin exert antinociceptive and antioedematogenic action in mice, and were more potent than some well-known antiinflammatory and analgesic drugs[5]. Such experimental observation and the fact that most of the actual analgesic drugs cause undesired effects and are not useful in all cases[6] encourage us to synthesize other chalcones with possible antinociceptive properties. Thus, we have prepared 11 compounds using a classical condensation reaction (base-catalyzed) of substituted acetophenones and benzaldehydes which were evaluated as antinociceptive agents by using the writhing test in mice. The most active compound was also analysed in the formalin-induced pain test. Furthermore, we have preliminarily investigated the structure-activity relationships of these compounds. The results of some reference drugs were included for comparison.

Results and discussion

The scheme shows the general synthetic procedure used. All the compounds were obtained in good yields (70–98%) and readily characterized by conventional spectral data. Inspection of the ^{1}H NMR spectra suggested that the chalcones were geometrically pure and configured *trans* ($J_{H\alpha\text{-}H\beta}$ = 15–16 Hz)[4].

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The results summarized in Table 1 indicate that several chalcones exhibited potent antinociceptive effect when assessed by the writhing test in mice. Chalcone **4**, which does not contain substituent groups in either aromatic ring, gave a calculated ID_{50} value of 99.7 μmol/kg, being equipotent to aspirin and paracetamol, two standard drugs widely employed in clinical practice. The introduction of different substituent groups in the aromatic rings caused drastic changes in activity. The choice of halogen atoms or methyl group on the rings A and B of chalcones was made in order to verify the influence of some physico-chemical parameters, e.g. hydrophobicity, electronic, and steric effects.

In general, the introduction of substituent groups in the ring A of chalcones produces more active compounds. The two most potent compounds, chalcones **2** and **11** containing two or one chloro atom attached in the ring A, showed

Table 1. Effect of chalcones and some reference drugs given intraperitoneally against acetic acid-induced abdominal constriction (writhing test) in mice.

Compound	ID_{50} (μmol/kg)	MI[a] (%)
1	45.5 (23.3–81.5)[b]	90 ± 4.0
2	9.0 (5.0–15.8)	91 ± 4.0
3	16.8 (7.3–38.4)	92 ± 3.0
4	99.7 (44.2–224.6)	75 ± 5.0
5	108.3 (64.9–176.9)	65 ± 5.0
6	16.0 (11.8–21.8)	97 ± 2.0
7	35.5 (28.8–43.6)	54 ± 3.0
8	113.4 (76.7–167.8)	80 ± 1.0
9	113.9 (72.3–180.6)	95 ± 2.0
10	137.8 (103.1–184.2)	88 ± 3.0
11	13.0 (8.8–19.5)	93 ± 1.0
Aspirin[c]	133.2 (73.0–243.1)	83 ± 2.0
Paracetamol[c]	125.0 (104.0–150.0)	88 ± 1.0
Diclofenac[d]	38.0 (29.5–49.0)	93 ± 7.0

[a] maximal inhibition; [b] 95% confidence limit; [c] from reference [7]; [d] from reference [12]. Each group represents the mean of five to seven animals.

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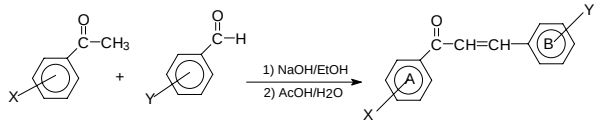

Scheme

ID_{50} values of 9.0 and 13.0 μmol/kg, and maximal inhibition of 91 and 93%, respectively. They were about 14-fold more active than aspirin and paracetamol and about 4-fold more potent than diclofenac in the writhing test. It should be noted that the introduction of a methyl group (chalcone **10**) instead of halogen atoms in the ring A considerably decreased the antinociceptive potency, suggesting that electron-withdrawing groups but not electron-donor groups increase the antinociceptive effect. This hypothesis may be supported by the activity of compound **1**, which was about 3-fold more active than compound **10**. However, other additional studies are required to confirm such observation. Although both bromine or chloro atoms present in the position 4 of ring A improve the pharmacological effect, the latter seems to be more effective, indicating that a steric parameter is also involved in the antinociceptive activity of these compounds. In addition, preliminary evaluation of the hydrophobicity by using the periodic box method by Hyper Chem indicated that this parameter is not related with the antinociceptive activity of these compounds.

In view of the interesting antinociceptive activity of chalcone **2**, it was analysed by the formalin-induced pain test, which defines two distinct periods of response, i.e., "early response (neurogenic pain)" and "late response (inflammatory pain)". It dose-dependently prevented only the late phase of the formalin test, like the standard drugs. The calculated ID_{50} value was 111.5 (50.5–243.7) μmol/kg with maximal inhibition of 82%, being equipotent to aspirin and acetaminophen, which presented ID_{50} of approximately 120 μmol/kg in relation to the second phase of the formalin test [7].

The mechanism by which the chalcones exert antinociceptive action still remains undetermined, but chemical and pharmacological studies are currently in progress in our laboratories and the results will be published elsewhere.

Considering that only a few authors have demonstrated the antinociceptive activity of chalcones and the level of current interest in discovering other medicinal agents to treat pain processes, we believe that some compounds, such as chalcones **2** and **11**, might be further used as models to obtain new more potent and/or selective analgesic drugs.

Acknowledgements

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Experimental

Synthesis: general procedures

Compounds **1–11** were obtained by reaction of acetophenone and benzaldehyde (1:1) in the presence of sodium hydroxide and ethanol and further addition to cool diluted acetic acid, according the methodology previously described[8]. The respective products were purified by recrystallization or column chromatography over silica gel. All the compounds were synthesized in good yields (55–98%) and characterized by ^{1}H NMR, IR and microanalyses. The purity of these compounds was determined by TLC using several solvent systems of different polarity.

Physico-chemical data of synthesized compounds

Compound (**1**): ^{1}H-NMR (DMSO-d_6, ppm): 7.27 (d, 1H, CH=CH), 7.38 (d, 1H, CH=CH, *J* = 15.63), 7.51–7.75 (m, 9H, Ar); FT-IR (KBr disk, cm^{-1}): 1660 (ν -C=O), 1605 (ν -C=C-); mp (°C): 155–160; Yield: 54.8%.

Compound (**2**): ^{1}H-NMR (DMSO-d_6 , ppm): 7.27 (d, 1H, CH=CH), 7.39 (d, 1H, CH=CH, *J* = 15.70), 7.50–7.93 (m, 8H, Ar); FT-IR (KBr disk, cm^{-1}): 1661 (ν -C=O), 1605 (ν -C=C-); mp (°C): 96–100; Yield: 68%.

Compound (**3**): ^{1}H-NMR (DMSO-d_6, ppm): 3.01 (s, 6H, N-$(CH_3)_2$), 7.28 (d, 1H, CH=CH), 7.41 (d, 1H, CH=CH, *J* = 15.71), 7.51–7.75 (m, 7H, Ar); FT-IR (KBr disk, cm^{-1}): 1649 (ν -C=O), 1612 (ν -C=C-); mp (°C): 124–126; Yield: 85%.

Compound (**4**): ^{1}H-NMR (DMSO-d_6, ppm): 6.38 (d, 1H, CH=CH), 6.89 (d, 1H, CH=CH, *J* = 15.72), 7.25–7.76 (m, 10 H, Ar); FT-IR (KBr disk, cm^{-1}): 1661 (ν -C=O), 1605 (ν -C=C-); mp (°C): 119–122; Yield: 77%.

Compound (**5**):^{1}H-NMR (DMSO-d_6, ppm): 7.28 (d, 1H, CH=CH), 7.42 (d, 1H, CH=CH, *J* = 15.71), 7.47–7.87 (m, 8H, Ar); FT-IR (KBr disk, cm^{-1}): 1657 (ν -C=O), 1605 (ν -C=C-), mp (°C): 160–163; Yield: 89%.

Compound (**6**): ^{1}H-NMR (DMSO-d_6, ppm): 7.29 (d, 1H, CH=CH), 7.43 (d, 1H, CH=CH, *J* = 15.69), 7.54–7.94 (m, 7H, Ar); FT-IR (KBr disk, cm^{-1}): 1661 (ν -C=O), 1602 (ν -C=C-); mp (°C): 120–123; Yield: 96%.

Compound (**7**): ^{1}H-NMR (DMSO-d_6, ppm): 2.43 (s, 3H, CH_3), 7.51 (d, 1H, CH=CH), 7.41 (d, 1H, CH=CH, *J* = 15.70), 7.44–7.80 (m, 8H, Ar); FT-IR (KBr disk, cm^{-1}): 1657 (ν -C=O), 1605 (ν -C=C-); mp (°C): 159–162; Yield: 80%.

Compound (**8**): ^{1}H-NMR (DMSO-d_6, ppm): 7.28 (d, 1H, CH=CH), 7.42 (d, 1H, CH=CH, *J* = 15.71), 7.25–7.76 (m, 9H, Ar); FT-IR (KBr disk, cm^{-1}): 1658 (ν -C=O), 1604 (ν -C=C-); mp (°C): 122–125; Yield: 74%.

Compound (**9**): ^{1}H-NMR (DMSO-d_6, ppm): 7.28 (d, 1H, CH=CH), 7.43 (d, 1H, CH=CH, *J* = 15.69), 7.54–7.75 (m, 8H, Ar); FT-IR (KBr disk, cm^{-1}): 1657 (ν -C=O), 1604 (ν -C=C-); mp (°C): 168–170; Yield: 97%.

Compound (**10**): ^{1}H-NMR (DMSO-d_6,ppm): 2.42 (s, 3H, CH_3), 7.93 (d, 1H, CH=CH), 7.37 (d, 1H, CH=CH, *J* = 15.65), 7.43–7.79 (m, 9H, Ar); FT-IR (KBr disk, cm^{-1}): 1656 (ν -C=O), 1596 (ν -C=C-); mp (°C): 95.6–100; Yield: 96%.

Compound (**11**): ^{1}H-NMR (DMSO-d_6, ppm): 7.28 (d, 1H, CH=CH); 7.39 (d, 1H, CH=CH, *J* = 15.72), 7.47–7.78 (m, 9H, Ar); FT-IR (KBr disk, cm^{-1}): 1661 (ν -C=O), 1606 (ν -C=C-); mp (°C): 85–92; Yield: 98%.

Pharmacological analysis: evaluation of antinociceptive activity

Writhing test

Male Swiss mice (25–30 g) were used. The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%), was carried out according to the procedures described previously[9,10] with minor modifications. The animals were pretreated with chalcones intraperitoneally 30 min before the acid acetic injection. Control animals received a similar volume of 0.9% NaCl (10 ml kg^{-1} , i.p.). All experiments were carried out at 23 ± 2 °C. The pairs of mice were placed in separate boxes and the number of abdominal constrictions of the abdominal muscles together with a stretching, were cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal contractions between control animals and mice pretreated with the compound studied.

Formalin-induced test

The procedure used was essentially similar to that previously described [11,12]. Animals (25–30 g) from the same strain were slightly anaesthetized with ether, except when used to analyse the first phase of formalin-induced pain, and 20 µl of 2.5% (0.92% formaldehyde) made up PBS (phosphate buffered solution, containing: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) was injected s.c. under the plantar surface of the left hindpaw with a Hamilton syringe. Animals were acclimatized to the laboratory for at least 24 h before the experiments. Two mice (control and treated) were simultaneously observed from 0 up to 30 min following formalin injection. The initial nociceptive scores normally peaked after 5 min (first phase, representing the neurogenic pain), and 15–30 min after formalin injection (second phase, representing the inflammatory pain)[11]. The animals were treated with saline 0.9% (10 ml/kg, i.p.) or with compound **2** 60 min before formalin injection. After intraplantar irritant application, the animals were immediately placed into a glass cylinder (20 cm diameter). The time spent by animals licking or biting the injected paw was timed with a chronometer and was considered indicative of pain.

Statistical analysis

The results are presented as mean ± s.e.m., and the statistical significance between the groups was analysed by means of an analysis of variance followed by Dunnett's multiple comparison test. *P* values less than 0.05 were considered as indicative of significance. The ID_{50} values (the dose of the compound that reduced responses by 50% in relation to control values) were estimated by graphical interpolation from individual experiments. ID_{50}'s are presented as mean values and 95% confidence interval. MI is the maximum inhibition at higher dose used.

References

[1] R.J. Anto, K. Sukumaran, G. Kuttan, M.N.A. Rao, V. Subbaraju, R. Kuttan, *Cancer Lett.* **1995,** *97*, 33–37.

[2] J.R. Dimmock, D.W. Elias, M.A. Beazely, N.M. Kandepu, *Curr. Med. Chem.* **1999,** *6*, 1125–1149.

[3] H.K. Hisieh, T.H. Lee, J.P. Wang, J.J. Wang, C.H. Lin, *Pharm. Res.* **1997,** *15*, 39–46.

[4] V.J. Ram, A.S. Saxena, S. Srivastava, S. Chandra, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2159–2161.

[5] V. Cechinel Filho, Z.R. Vaz, L. Zunino, J.B. Calixto, R.A. Yunes, *Eur. J. Med. Chem.* **1996,** *31*, 833–839.

[6] J.B. Calixto, A. Beirith, J. Ferreira, A.R.S. Santos, V. Cechinel Filho, R.A. Yunes, *Phytother. Res.* **2000,** *14*, 401–418.

[7] V. Cechinel Filho, Z.R. Vaz, L. Zunino, J.B. Calixto, R.A. Yunes, *Arzneim. Forsch./Drug Res.* **2000,** *50*, 281–285.

[8] J.M. Guider, T.H. Simpson, D.B. Thomas, *J. Chem. Soc.* **1955,** 170–173.

[9] R.F. Collier, H.O.J. Dinnen, C.A. Johnson, C. Schneider, *Br. J. Pharmacol.* **1968,** *32*, 295–310.

[10] M.M. Souza, P. Kern, A.E.O. Floriani, V. Cechinel Filho, *Phytother. Res.* **1998,** *12*, 279–281.

[11] S. Hunskaar, O.B. Fasmer, K. Hole, *J. Neurosci. Methods* **1985,** *14,* 69–76.

[12] R.A.P. De Jesus, V. Cechinel Filho, A.E. Oliveira, V. Schlemper, *Phytomedicine* **2000,** *7, 111–115.*

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