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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of antiinflammatory activity of substituted chalcone derivatives

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Abstract In an effort to develop potent antiinflammatory agents, a series of substituted chalcone derivatives was synthesized and evaluated for antiinflammatory activity through monitoring of their ability to inhibit xylene-induced ear edema in mice. Some of the tested compounds exhibited significant activity, and compounds 3f [(*E*)-1-(2,4-dihydroxyphenyl)-3-(4-dimethylamino)phenyl)prop-2-en-1-one] and 3h [(*E*)-3-(4-chlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one] showed the highest antiinflammatory activity (62 and 68% inhibition, respectively, 2 h before administration), comparable with or even slightly more potent than the

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reference drug ibuprofen (53%). Furthermore, the structure–activity relationship of these substituted chalcone derivatives was demonstrated.

Keywords Antiinflammatory · Chalcone derivatives · Ibuprofen · Synthesis

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are useful tools in the treatment of acute and chronic inflammation, pain, and fever. However, long-term clinical usage of NSAIDs is associated with significant side effects including gastrointestinal lesions, bleeding, and nephrotoxicity (Hallas *et al*., 1995; Mccarthy, 1998; Raskin, 1999). Therefore, the discovery of new and safer antiinflammatory drugs represents a challenging goal for such a research area (Van and Botting, 1995). Because resistance to antiinflammatory drugs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent, and less toxic antiinflammatory agents.

Chalcone derivatives, among the large families of plant constituents, have various therapeutic benefits including antioncogenic, antiinflammatory, analgesic, antiulcerative, antiviral, antibacterial, antifungal, and antimalarial properties (Bekhit *et al*., 2001; Hiseh *et al*., 1998; Kumar *et al*., 2003; Liu *et al*., 2001; Lopez *et al*., 2001; Murakami *et al*., 1991; Viana *et al*., 2003; Wu *et al*., 2003). Furthermore, it is reported that 2′,5′-dihydroxychalcones derivatives possess antiinflammatory activity (Won *et al*., 2005), and 3,4-dihydroxychalcones is reported to be a 5- or 12-lipoxygenase and cyclooxygenase inhibitor (Sogawa *et al*., 1993).

Ballesteros *et al.* (1995) reported that two synthetic 2′-hydroxychalcones exerted topical antiinflammatory effects in mice. Lee *et al.* (2006) reported 2′,4′,6′-tris(methoxy)chalcone to be an antiinflammatory compound that reduces nitric oxide (NO) production by inhibiting inducible NO synthase expression. The findings suggest that some chalcones may be promising antiinflammatory agents.

In our previous studies (Guan *et al*., 2005), 1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxyphenyl) prop-2-en-1-one and 1,3-bis(4-hydroxyphenyl)prop-2-en-1-one were tested for their protective effects. Because the two compounds have a chalcone structure, they were assumed to possess antiinflammatory activity. Therefore, in view of the observations, we designed and synthesized a series of 2′,4′-dihydroxychalcones or 4′-hydroxychalcones and varied the substitution of the B ring to the screen for their antiinflammatory effects in vivo. We discuss their structure–activity relationships.

Materials and methods

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Infrared (IR) spectra were recorded (in KBr) on an FT-IR1730 (Bruker Biospin,

Switzerland), and ^{1}H-NMR spectra were measured on an AV-300 (Bruker Biospin, Switzerland). All chemical shifts are given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, Shelton, USA). Elemental analyses were performed on a 204Q CHN (Pekin-Elmer, Shelton, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer (Hanau, Hessen Land, Germany). All other chemicals were of analytical grade. The synthesis of 1-(2,4-dihydroxyphenyl)ethanone (Fan, 1992) (1) and 1-(4-hydroxyphenyl)ethanone (Fan, 1992) (2) was as previously reported.

General procedure for the preparation of (E)-1-(2, 4-dihydroxyphenyl)-3-phenylprop-2-en-1-one (3)

In a round-bottomed flask, substituted benzaldehyde (1.0 g, 6 mmol) and 1-(2,4-dihydroxyphenyl)ethanone (0.46 g, 3 mmol) were dissolved in ethylene glycol. Boric acid (0.70 g, 11.3 mmol) was added, and the mixture was refluxed at 120°C for 6 h. Solvents were removed under reduced pressure, and the residue was extracted three times with diethyl ether 30 mL. The diethyl ether layer was washed three times with water (30 mL × 3) and dried over anhydrous $MgSO_4$. After removal of the solvents, the product was purified by silica gel column chromatography (petroleum benzine: acetic ether = 2:1).

(E)-1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxyphenyl)prop-2-en-1-one (3a) Yield: 32%; mp: 213–215°C. IR (KBr) cm^{-1}: 3370 (–OH str.), 1685 (C=O str.), 3045 (Ar C–H str.), 1590, 1480, 1430 (C=C ring str), 1223 (C–O str.), 749 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 6.30$ (d, $J = 15$ Hz, 1H, =CH), 6.82–6.92 (m, 3H, $–C_6H_3$), 7.25–7.28 (m, 3H, $–C_6H_3$), 7.63 (d, $J = 15$ Hz, 1H, =CH), 10.58 (s, 1H, –OH), 13.60 (s, 1H, –OH); MS m/z: 273 (M+1). Anal. for $C_{15}H_{12}O_5$: Calc. C: 66.17, H: 4.44. Found C: 66.01, H: 4.15.

(E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (3b) Yield: 32.5%; mp: 180–182°C. IR (KBr) cm^{-1}: 3384 (–OH str.), 1685 (C=O str.), 3042 (Ar C–H str.), 1591, 1482, 1430 (C=C ring str), 1222 (C–O str.), 750 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 6.72$–6.97 (m, 3H, $–C_6H_3$), 7.31 (d, $J = 15$ Hz, 1H, =CH), 7.73–7.79 (m, 4H, $–C_6H_4$), 8.09 (d, $J = 15$ Hz, 1H, =CH), 10.10 (s, 1H, –OH), 10.64 (s, 1H, –OH); MS m/z: 257 (M+1). Anal. for $C_{15}H_{12}O_4$: Calc. C: 70.31, H: 4.72. Found C: 70.21, H: 4.56.

(E)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (3c) Yield: 56%; mp: 192–194°C. IR (KBr) cm^{-1}: 3384 (–OH str.), 1690 (C=O str.), 3041 (Ar C–H str.), 1590, 1481, 1432 (C=C ring str), 1223 (C–O str.), 748 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 3.32$ (s, 3H, 3-OCH_3), 6.56–6.89 (m, 3H, $–C_6H_3$), 7.52 (d, $J = 15$ Hz, 1H, =CH), 7.70–7.79 (m, 3H, $–C_6H_4$), 8.14 (d, $J = 15$ Hz, 1H, =CH), 10.55 (s, 1H, –OH), 10.67 (s, 1H, –OH); MS *m/z*: 287 (M+1]). Anal. for $C_{16}H_{14}O_5$: Calc. C: 67.13, H: 4.93. Found C: 67.05, H: 4.88.

(E)-1-(2, 4-dihydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (3d) Yield: 38%; mp: 166–168°C. IR (KBr) cm^{-1}: 3388 (–OH str.), 1690 (C=O str.), 3041 (Ar C–H str.), 1590, 1481, 1432 (C=C ring str), 1223 (C–O str.), 748 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 3.87$ (s, 3H, 4-OCH_3), 6.44–6.95 (m, 3H, –C_6H_3), 7.43 (d, $J = 15$ Hz, 1H, =CH), 7.85–8.06 (m, 4H, –C_6H_4), 7.91 (d, $J = 15$ Hz, 1H, =CH), 12.70 (s, 1H, –OH), 13.50 (s, 1H, –OH); MS *m/z*: 271 (M+1). Anal. for $C_{16}H_{14}O_4$: Calc. C: 71.10, H: 5.22. Found C: 70.98, H: 5.13.

(E)-3-(benzo[d][1,3]dioxol-5-yl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (3e) Yield: 39%; mp: 168–170°C. IR (KBr) cm^{-1}: 3384 (–OH str.), 1690 (C=O str.), 3045 (Ar C–H str.), 1590, 1480, 1435 (C=C ring str), 1223 (C–O str.), 748 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 2.56$ (s, 2H, 3,4-OCH_2O), 6.88–6.97 (m, 3H, –C_6H_3), 6.95 (d, $J = 15$ Hz, 1H, =CH), 7.18–7.44 (m, 3H, –C_6H_4), 7.42 (d, $J = 15$ Hz, 1H, =CH), 12.70 (s, 1H, –OH), 13.46 (s, 1H, –OH); MS *m/z*: 285 (M+1). Anal. for $C_{16}H_{12}O_5$: Calc. C: 67.60, H: 4.25. Found C: 67.55, H: 4.18.

(E)-1-(2, 4-dihydroxyphenyl)-3-(4-dimethylamino)phenyl)prop-2-en-1-one (3f) Yield: 53%; mp: 180–182°C. IR (KBr) cm^{-1}: 3378(–OH str.), 1690 (C=O str.), 3045 (Ar C–H str.), 1590, 1480, 1435 (C=C ring str), 1223 (C–O str.), 748 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 3.29$ [s, 6H, 4-$N(CH_3)_2$], 6.44–6.89 (m, 3H, –C_6H_3), 6.70 (d, $J = 15$ Hz, 1H, =CH), 7.78–7.89 (m, 4H, –C_6H_4), 8.09(d, $J = 15$ Hz, 1H, =CH), 10.61 (s, 1H, –OH), 11.62 (s, 1H, –OH); MS m/z: 284 (M+1). Anal. for $C_{17}H_{17}NO_3$: Calc. C: 72.07, H: 6.05, N 4.94. Found C: 72.01, H: 5.90, N 4.79.

(E)-1-(2,4-dihydroxyphenyl)-3-p-tolylprop-2-en-1-one (3g) Yield: 46%; mp: 184–186°C. IR (KBr) cm^{-1}: 3374 (–OH str.), 1691 (C=O str.), 3045 (Ar C–H str.), 1590, 1482, 1434 (C=C ring str), 1221 (C–O str.), 749 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$):$\delta = 2.16$ (s, 3H, –CH_3), 6.75–6.87 (m, 3H, –C_6H_3), 6.75 (d, $J = 15$ Hz, 1H, =CH), 7.25–7.48 (m, 4H, –C_6H_4), 7.41 (d, $J = 15$ Hz, 1H, =CH), 11.70 (s, 1H, –OH), 12.36 (s, 1H, –OH); MS *m/z*: 255 (M+1). Anal. for $C_{16}H_{14}O_3$: Calc. C: 75.57, H: 5.55. Found C: 75.46, H: 5.40.

(E)-3-(4-chlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (3h) Yield: 35.8%; mp: 156–158°C. IR (KBr) cm^{-1}: 3374 (–OH str.), 1692 (C=O str.), 3042 (Ar C–H str.), 1591, 1482, 1435 (C=C ring str), 1221 (C–O str.), 749 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 6.42$–6.51 (m, 3H, –C_6H_3), 7.47 (d, $J = 15$ Hz, 1H, =CH), 7.66–7.89 (m, 4H, –C_6H_4), 8.05 (d, $J = 15$ Hz, 1H, =CH), 12.71 (s, 1H, –OH), 13.20 (s, 1H, –OH); MS *m/z*: 275 (M+1). Anal. for $C_{15}H_{11}ClO_3$: Calc. C: 65.58, H: 4.04. Found C: 65.46, H: 3.95.

(E)-1-(2,4-dichlorophenyl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one (3i) Yield: 54%; mp: 154–156°C. IR (KBr) cm^{-1}: 3480 (–OH str.), 1693 (C=O str.), 3045 (Ar C–H str.), 1590, 1484, 1435 (C=C ring str), 1223 (C–O str.), 749 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 6.47$–6.52 (m, 3H, –C_6H_3), 7.45 (d, $J = 15$ Hz, 1H, =CH), 7.64–7.80 (m, 3H, –C_6H_4), 8.21 (d, $J = 15$ Hz,

1H, =CH), 12.71 (s, 1H, –OH), 13.25 (s, 1H, –OH); MS *m/z*: 309 (M+1). Anal. for $C_{15}H_{10}Cl_2O_3$: Calc. C: 58.28, H: 3.26. Found C: 58.15, H: 3.08.

(E)-1-(2,4-dihydroxyphenyl)-3-(2-nitrophenyl)prop-2-en-1-one (3j) Yield: 37%; mp: 200–202°C. IR (KBr) cm^{-1}: 3384 (–OH str.), 1692 (C=O str.), 3043 (Ar C–H str.), 1591, 1480, 1435 (C=C ring str), 1223 (C–O str.), 750 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 6.36$–6.42 (m, 3H, $–C_6H_3$), 7.86 (d, $J = 15$ Hz, 1H, =CH), 7.78–8.04 (m, 4H, $–C_6H_4$), 8.06 (d, $J = 15$ Hz, 1H, =CH), 10.70 (s, 1H, –OH), 10.84 (s, 1H, –OH); MS *m/z*: 286 (M+1). Anal. for $C_{15}H_{11}NO_5$: Calc. C: 63.16, H: 3.89, N: 4.91. Found C: 63.02, H: 3.78. N: 4.84.

(E)-1-(2, 4-dihydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (3k) Yield: 38%. mp 210–212°C. IR (KBr) cm^{-1}: 3388 (–OH str.), 1690 (C=O str.), 3043 (Ar C–H str.), 1592, 1481, 1435 (C=C ring str), 1223 (C–O str.), 749 (C–H out of plane bending). ^{1}H-NMR ($CDCl_3$): $\delta = 7.73$–7.79 (m, 3H, $–C_6H_3$), 7.93 (d, $J = 15$ Hz, 1H, =CH), 8.31–8.43 (m, 4H, $–C_6H_4$), 8.24 (d, $J = 15$ Hz, 1H, =CH), 10.66 (s, 1H, –OH), 12.58 (s, 1H, –OH). MS: *m/z* 286 [M+1]. Anal. Calcd. For $C_{15}H_{11}NO_5$: C 63.16, H 3.89, N 4.91. Found: C 63.01, H 3.75. N 4.82.

General Procedure for the preparation of (E)-1-(4-dihydroxyphenyl)-3-phenylprop-2-en-1-one (4)

In a round-bottomed flask, substituted benzaldehyde (1.0 g, 6 mmol) and 1-(4-hydroxyphenyl)ethanone (0.46 g, 3 mmol) were dissolved in ethylene glycol. Boric acid (0.70 g, 11.3 mmol) was added, and the mixture was refluxed at 120°C for 6 h. The solvents were removed under reduced pressure, and the residue was extracted three times with diethyl ether 30 mL. The diethyl ether layer was washed three times with water (30 mL × 3) and dried over anhydrous $MgSO_4$. After removal of the solvents, the product was purified by silica gel column chromatography (petroleum benzine: acetic ether = 2:1).

(E)-1,3-bis(4-hydroxyphenyl)prop-2-en-1-one (4a) Yield: 36%; mp: 196–198°C. IR (KBr) cm^{-1}: 3388 (–OH str.), 1691 (C=O str.), 3045 (Ar C–H str.), 1590, 1484, 1432 (C=C ring str), 1222 (C–O str.), 748 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 6.89$–6.95 (m, 4H, $–C_6H_4$), 7.33 (d, $J = 15$ Hz, 1H, =CH), 7.59–7.87 (m, 4H, $–C_6H_4$), 7.99 (d, $J = 15$ Hz, 1H, =CH), 9.67 (s, 1H, –OH), 9.95 (s, 1H, –OH); MS *m/z*: 241 (M+1). Anal. for $C_{15}H_{12}O_3$: Calc. C: 74.99, H: 5.03. Found C: 74.86, H: 4.90.

(E)-3-(4-dimethylamino)phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (4b) Yield: 48%; mp: 182–184°C. IR (KBr) cm^{-1}: 3384 (–OH str.), 1693 (C=O str.), 3041 (Ar C–H str.), 1590, 1482, 1433 (C=C ring str), 1222 (C–O str.), 747 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 2.98$ [s, 6H, 4-$N(CH_3)_2$], 6.87–6.93 (m, 4H, $–C_6H_4$), 7.35 (d, $J = 15$ Hz, 1H, =CH), 7.93–8.12 (m, 4H, $–C_6H_4$), 7.82 (d, $J = 15$ Hz, 1H, =CH), 9.75 (s, 1H, –OH); MS *m/z*: 268 (M+1). Anal. for $C_{17}H_{17}NO_2$: Calc. C: 76.38, H: 6.41, N: 5.24. Found C: 76.26, H: 6.30, N: 5.13.

(E)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (4c) Yield: 39%; mp: 216–218°C. IR (KBr) cm^{-1}: 3384 (–OH str.), 1690 (C=O str.), 3043 (Ar C–H str.), 1590, 1484, 1435 (C=C ring str), 1221 (C–O str.), 749 (C–H out of plane bending); ^{1}H-NMR ($CDCl_3$): $\delta = 3.79$ (s, 3H, 3-OCH_3), 6.89–7.01 (m, 3H, –C_6H_3), 7.23 (d, $J = 15$ Hz, 1H, =CH), 7.35–7.86 (m, 4H, –C_6H_4), 7.97 (d, $J = 15$ Hz, 1H, =CH), 9.74 (s, 1H, –OH), 10.65 (s, 1H, –OH); MS *m/z*: 271 (M+1). Anal. for $C_{16}H_{14}O_4$: Calc. C: 71.10, H: 5.22. Found C: 71.01, H: 5.09.

Pharmacology

The antiinflammatory activity was evaluated by an in-vivo inhibition assay monitoring xylene-induced ear edema. All tested compounds were homogenized with 0.5% sodium carboxymethylcellulose (CMC-Na) and administered orally to kunming mice (body weight, 20–25 g; 10 animals per group). Control mice received the vehicle only (0.5% sodium carboxymethylcellulose, 0.2 mL/20 g).

At a specified later time, 20 μL of xylene was applied to the surface of the right ear of each mouse by a micropipette. The mice were killed 30 min later, and a cylindrical plug (diameter, 7 mm) was excised from each of the treated and untreated ears. Edema was quantified by the difference in weight between the two plugs. The antiinflammatory activity was expressed as a percentage of edema reduction compared with the CMC-Na-administered control group.

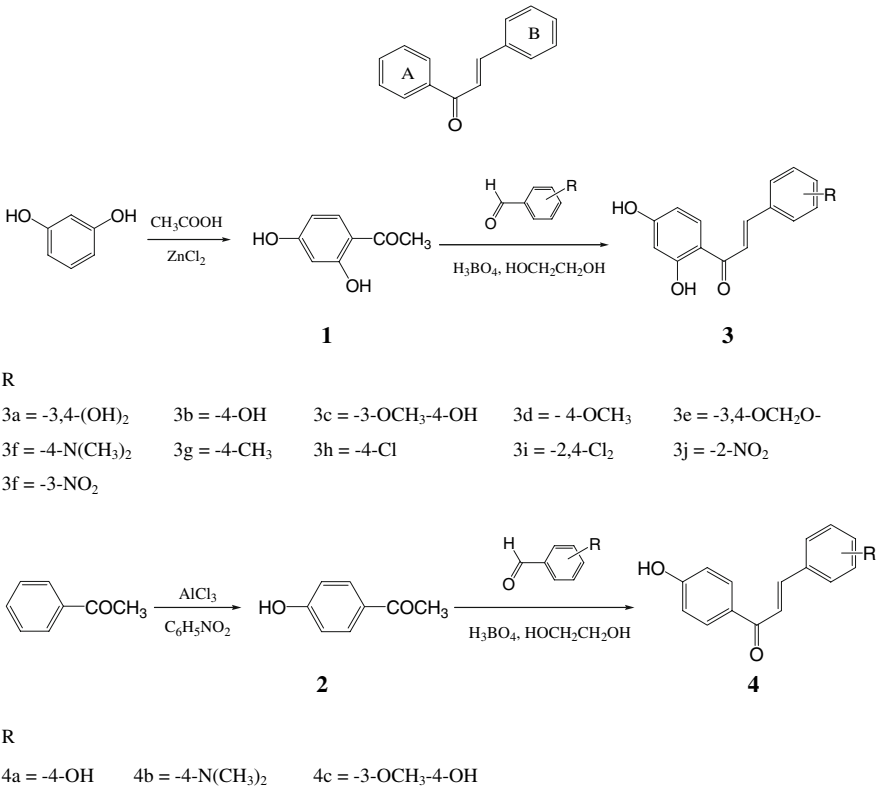
The NSAID drug ibuprofen was tested in parallel as an activity reference. Edema values, expressed as mean ± standard deviation, were compared statistically using Student's *t* test. A *p* value less than 0.05 was adopted as the test of significance.

Results and discussion

The synthesis of the target compounds was accomplished according to the reaction sequence illustrated in Scheme 1. Compound 1 was synthesized using resorcine and acetic acid in the presence of zinc chloride as the catalyst in a good yield (Fan, 1992). Compound 2 was obtained with nitrobenzene through Fries rearrangement in the presence of aluminium trichloride as the catalyst (Fan, 1992). Derivatives 3 and 4 were prepared by Claisen-Schmidt condensation with compounds 1 and 2, respectively.

As shown in Table 1, the evaluation indicated that the 13 synthetic compounds showed antiinflammatory activity at a dose of 200 mg/kg administered orally 2 h before the inflammatory agent xylene. Among the synthesized compounds, 3f [(*E*)-1-(2,4-dihydroxyphenyl-3-(4-dimethylamino)phenyl)prop-2-en-1-one] and 3h [(*E*)-3-(4-chlorophenyl)-1-(2, 4-dihydroxyphenyl)prop-2-en-1-one] showed the highest ear inflammation inhibition rate: 62.55% and 68.17%, respectively.

First, compared with the substituted 4′-hydroxychalcone derivatives, the antiinflammatory activity of the substituted 2′,4′-dihydroxychalcone derivatives was stronger than that of 4′-hydroxychalcone. As shown in Table 1, compound 3f (inhibition rate, 62.55%) showed a stronger inhibitory effect than compound 4b



Scheme 1 The synthesis route of compounds 3a–j and 4a–c

(inhibition rate, 17.27%). Similarly, compounds 3b and 3c (inhibition rate, 14.86 and 25.20%, respectively) rather than compounds 4a and 4c (inhibition rate, 5.42 and 7.83%, respectively) resulted in greater antiinflammatory activity. It seemed that the increase of the hydroxyl group on the chalcone A ring could influence the inhibitory effect on antiinflammatory activity, but the potency depended on the variation in the substituent of the B ring.

Second, for the substituted 2′,4′-dihydroxychalcone derivatives 3a–g, all except one of these compounds (3g) had the antiinflammatory activities. Compound 3f exhibited significantly greater activity than the reference drug ibuprofen. The position of the substituted group on the phenyl ring greatly influenced the antiinflammatory activity, with an activity order of –4-$N(CH_3)_2$ > –4-OCH_3 > –3–OCH_3-4-OH > –3,4-OCH_2O– > –4-OH > –3,4-$(OH)_2$.

On the other hand, when the substitution was a member of atom Cl, the position of atom Cl on the phenyl ring may have influenced the antiinflammatory activity, with the potency order of the two Cl-substituted derivatives being 4-Cl > 2,4-Cl_2, in which the compound 3h exhibited the most antiinflammatory activity. The substitution was a member of the nitro group, with the potency order of the two NO_2-substituted derivatives being 3-NO_2 > 2-NO_2. These results indicated that the

Table 1 Antiinflammatory activity of compounds 3a–k and 4a–c administrated orally

Compound	R	Dose (mg/kg)	No. of mice	Edema mean ± SD (mg)	Inhibition rate (%)
CMC-Na	–	–	10	9.96 ± 0.53	–
Ibuprofen	–	200	10	4.63 ± 0.48***	53.51
3a	–3,4-$(OH)_2$	200	10	8.53 ± 0.52***	14.36
3b	–4-OH	200	10	8.48 ± 0.37***	14.86
3c	–3-OCH_3-4-OH	200	10	7.45 ± 0.24***	25.20
3d	–4-OCH_3	200	10	4.84 ± 0.32***	51.41
3e	–3,4-OCH_2O–	200	10	7.77 ± 0.53***	21.99
3f	–4-$N(CH_3)_2$	200	10	3.73 ± 0.41***	62.55
3g	–4-CH_3	200	10	10.92 ± 0.64	–
3h	–4-Cl	200	10	3.17 ± 0.33***	68.17
3i	–2,4-Cl_2	200	10	7.37 ± 0.70***	26.04
3j	–2-NO_2	200	10	7.95 ± 0.39***	20.18
3k	–3-NO_2	200	10	6.97 ± 0.48***	30.02
4a	–4-OH	200	10	9.42 ± 0.34***	5.42
4b	–4-$N(CH_3)_2$	200	10	8.24 ± 0.51***	17.27
4c	–3-OCH_3-4-OH	200	10	9.18 ± 0.36**	7.83

** $p < 0.01$

*** $p < 0.001$ compared with the CMC-Na (control) group

character of the substitution on the B ring had a significant influence on the antiinflammatory activity.

Based on the results of the screening shown in Table 1, two outstanding derivatives, 3f and 3h, were chosen to be evaluated in the screening shown in the Table 2. The dose still was 200 mg/kg administered orally, but multiple intervals (0.5 h, 1 h, 2 h, 3 h, 4 h, and 24 h) for xylene application were assessed. As the interval lengthened, the antiinflammatory activity of compounds 3f and 3h first increased, then declined. The peak activity was observed at the 2-h interval. Compound 3f showed stronger activity than compound 3h at all time points except the 2-h point. Compound 3f showed significantly greater activity than the reference drug ibuprofen 0.5 h after administration but comparable (1 h, 24 h) or lower (3 h, 4 h) activity at other time points, indicating its quick absorption and potential for acute antiinflammatory action. Compound 3h showed an activity level similar to that of the reference drug at all time points except at the 1-h time point, when it had lower activity than ibuprofen.

Finally, as seen in Table 3, the ear inflammation inhibition rate of compounds 3f, 3h, and the reference drug ibuprofen (at lower doses of 100 and 50 mg/kg administered 2 h before xylene application) were evaluated and compared. Compound 3f showed effects similar to those of ibuprofen at the two lower doses, whereas compound 3h possessed stronger antiinflammatory activity than ibuprofen at 100 mg/kg.

Table 2 Antiinflammatory activity of compounds 3f and 3h administered at different times before the xylene application

Time (h)	Dose (mg/kg)	Inhibition (%)		
		3f	3h	Ibuprofen
0.5	200	41.67[a]	30.21	32.54
1	200	45.81	32.52[a]	41.62
2	200	62.55[b]	68.17	53.51
3	200	27.52[a]	33.56	34.51
4	200	25.29[b]	32.26	30.10
24	200	21.25	21.46	20.59

[a] $p < 0.01$ compared with ibuprofen at the corresponding dose

[b] $p < 0.01$

Table 3 Antiinflammatory activity of compounds 3f and 3h at different doses

Time (h)	Dose (mg/kg)	Inhibition (%)		
		3f	3h	Ibuprofen
2	200	62.55[a]	68.17	53.51
2	100	32.93	48.98[b]	33.03
2	50	15.12	18.57	19.78

[a] $p < 0.01$

[b] $p < 0.001$ compared with ibuprofen at the corresponding dose

Conclusion

In conclusion, for a series of antiinflammatory compounds, the substitution of 2′, 4′-dihydroxychalcones or 4′-hydroxychalcons was synthesized, and the resulting antiinflammatory activity was evaluated by an in vivo test. Two of the compounds, 3f and 3h, exhibited antiinflammatory activity comparable with that of the reference drug ibuprofen.

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