Full Paper

# Novel Conjugates of Aspirin with Phenolic Acid as Anti-inflammatory Agents Having Significantly Reduced Gastrointestinal Toxicity

**Shan Jiang[1], Ning Ding[2], Wei Zhang[2], Yichun Zhang[1], Meiyu Geng[1], and Yingxia Li[2]**

[1] School of Medicine and Pharmacy, Ocean University of China, Qingdao, P.R. China
[2] Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai, P.R. China

A series of novel conjugates of aspirin with natural phenolic acid antioxidants connected through a diol linker were designed and synthesized as potential bifunctional agents combining antioxidant and anti-inflammatory activity for reducing gastrointestinal toxicity. In general, the conjugates were found to be efficient antioxidants and many of them demonstrated much more potent anti-inflammatory activity than aspirin. Among them, **5a** and **5b** which bear the best anti-inflammatory activity exhibited significantly reduced ulcerogenic potency and toxicity compared to aspirin. However, it is evident that the anti-inflammatory activity of these dual-acting molecules *in vivo*, was not simply consistent with their antioxidant ability *in vitro*.

**Keywords:** Antioxidant / Aspirin / Inflammation / NSAIDs / Ulcerogenicity

Received: September 09, 2009; Accepted: December 01, 2009

DOI 10.1002/ardp.200900217

## Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed drugs in the world due to their anti-inflammation, anti-pyreticosis, anti-thrombosis, and acesodyne properties [1]. However, gastrointestinal (GI) ulceration was found to be the main and most severe adverse effect. It has been reported that 15–35% of all peptic ulcer complications are caused by NSAIDs [2], and the increase of hospitalization and deaths due to GI-related disorders parallels the increased use of NSAIDs [3].

It is believed that the GI toxicity of NSAIDs is caused by their "dual-insult" [4] – the suppression of cyclooxygenase-1 (COX-1) activity and direct irritation of the gastric mucosa (many NSAIDs are acids) [3]. COX-1 is a constitutive isozyme and is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal tract, while COX-2 is inducible and short-lived; its expression is stimulated in response to a pro-inflammatory insult [5]. COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells and in the central nervous system [6]. Classical NSAIDs are not selective and inhibit both COX-1 and COX-2, thus, cause peptic ulceration and dyspepsia. The development of selective COX-2 inhibitors was thought to be a reasonable target for safer NSAIDs [6]. However, serious doubts for a substantial benefit of COX-2 inhibitors have been expressed at the end of last century [7]. Moreover, the withdrawal of several COX-2-selective NSAIDs from the market in the beginning of new century attracted the public concern regarding the cardiovascular safety of the COX-2 inhibitors. To some degree, this result eliminated the benefit of selective inhibition of COX-2; subsequently, it made non-selective NSAIDs more attractive than before in terms of prescriptions. This was supported by the fact that there were significant increases in non-selective NSAID prescriptions after the withdrawal of rofecoxib and valdecoxib from the market [8].

In addition to the systemic effects of NSAIDs on gastric mucosa due to their action on prostaglandin synthesis, the acidic nature of NSAIDs was also considered an important factor to induce GI ulceration, since the acid

**Correspondence:** Yingxia Li, Department of Medicinal Chemistry, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, P.R. China.
**E-mail:** liyx417@fudan.edu.cn
**Fax:** +86 21 519-80127

**Abbreviations:** dicyclohexylcarbodiimide (DCC); 4-(dimethylamino)-pyridin (DMAP); 2,2-diphenyl-1-picrylhydrazyl (DPPH); gastrointestinal (GI); intragastrically (i.g.); malondialdehyde (MDA); tetrahydropyranyl (THP)

group can produce a local action on the gastric epithelium [9]. In this respect, molecular modifications to reduce the acidic irritation of NSAIDs have been proposed and proven to be a useful approach in the past years [9].

Furthermore, reactive oxygen species (ROS) and related free radical reactions are considered to be implicated in numerous pathophysiological conditions, such as inflammation, atherosclerosis, gastric ulceration, neuronal degeneration, cancer, and the aging process [10]. Several antioxidants have been shown to exhibit anti-inflammatory activity [11], also proving that reactive oxygen species play a fundamental role in inflammatory conditions [12].

These findings indicated that the chemical derivatization of known NSAID molecules, which combine anti-inflammatory and antioxidant activities may lead to the development of drugs with an improved therapeutic index. For example, Kourounakis *et al*. [2] reported that the conjugates of several widely used NSAIDs with antioxidant cysteamine and L-cysteine showed better anti-inflammatory and antioxidant activities than the parent compounds as well as the significant reduction of gastrointestinal toxicity [9]. Detsi *et al*. reported a series of novel quinolinone 3-aminoamides and their a-lipoic acid hybrids possess significant anti-inflammatory activity *in vivo*, whereas most of them are potent •OH scavengers and inhibit soybean lipoxygenase *in vitro* [13]. The success of this strategy has been further proven for a number of commercially available NSAIDs, which also possess radical scavenging properties [14].

In view of the above mentioned mechanisms, in this paper, we would like to report preliminary results on the design, synthesis, and pharmacological evaluation of a series of conjugates of aspirin with three natural phenolic antioxidants, *p*-coumaric acid **1a**, ferulic acid **1b**, and caffeic acid **1c**, through diol linker-mediated ester bonds (Fig. 1).

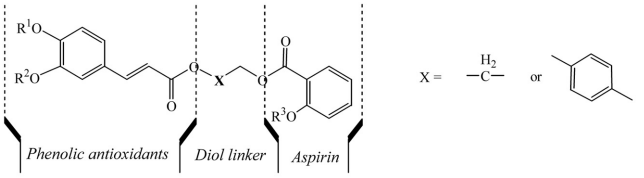



**Figure 1.** Novel conjugates of aspirin with phenolic antioxidants.

**Table 1.** Structures and yields of the conjugates of aspirin with phenolic antioxidants (cf. Scheme 1).

| Compounds | | $R^1$ | $R^2$ | $R^3$ | X | Conditions | Yields (%) |
|---|---|---|---|---|---|---|---|
| 4 | a | Ac | H | - | - | Ref. [16] | - |
| | b | Ac | $OCH_3$ | - | - | Ref. [17] | - |
| | c | Ac | OAc | - | - | Ref. [18] | - |
| | d | THP | H | - | - | Ref. [19] | - |
| | e | THP | $OCH_3$ | - | - | Ref. [20] | - |
| | f | THP | OAc | - | - | Ref. [21] | - |
| 5 | a | Ac | H | - | -$CH_2$- | iv | 67.5 |
| | b | Ac | $OCH_3$ | - | | iv | 64.9 |
| | c | Ac | OAc | - | | iv | 64.4 |
| | d | THP | H | - | | v | - |
| | e | THP | $OCH_3$ | - | | v | - |
| | f | THP | OAc | - | | v | - |
| | g | THP | H | - |  | v | - |
| | h | THP | $OCH_3$ | - | | v | - |
| | i | THP | OTHP | - | | v | - |
| 6 | a | H | H | H | -$CH_2$- | vi | 53.5 |
| | b | H | $OCH_3$ | H | | vi | 48.5 |
| | c | H | OH | H | | vi | 60.1 |
| | d | H | H | Ac | | vii | 60.5 (2 steps) |
| | e | H | $OCH_3$ | Ac | | vii | 77.4 (2 steps) |
| | f | H | OH | Ac | | vii | 58.2 (2 steps) |
| | g | H | H | Ac |  | vii | 61.3 (2 steps) |
| | h | H | $OCH_3$ | Ac | | vii | 86.7 (2 steps) |
| | i | H | OH | Ac | | vii | 58.2 (2 steps) |

## Results and discussion

### Chemistry

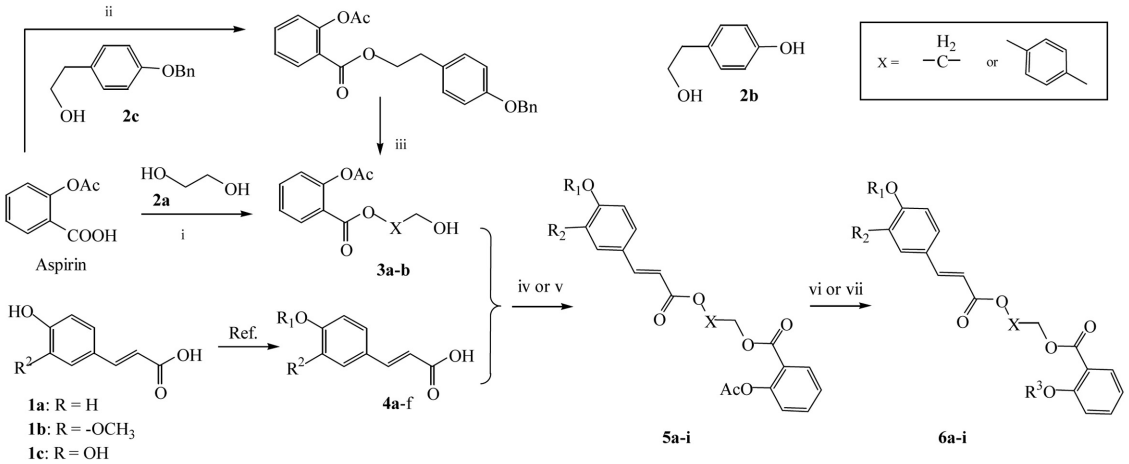
The structures and synthesis of the novel aspirin conjugates are presented in Table 1 and Scheme 1. The synthesis was started from the connection of the carboxylic acid group of aspirin with two diol linkers, glycol ethylene **2a** and 4-(2-hydroxy-ethyl)-phenol **2b**, respectively. The key intermediate **3a** [15] was generated by treating the acid chloride of aspirin directly with excessive **2a**, while compound **3b** was synthesized by coupling aspirin with **2c** in the presence of DCC-DMAP and followed by debenzylation. With the two aspirin derivatives **3a**, **b** in hand, six derivatives **4a–f** [16–21] of the three natural antioxidants **1a–c** bearing different phenolic hydroxyl protecting groups were coupled with **3a**, **b** to provide **5a–i** either through their acid chlorides or promoted by DCC-DMAP directly. Removal of the acetyl group on **5a–c** using 3 M HCl in acetone led to compounds **6a–c** (Scheme 1). When tetrahydropyranyl (THP) ether served as protecting group for the phenolic hydroxyl **5d–i**, the acetyl group on the aspirin part was preserved during the course of THP removal under mild acid conditions. Thus, compounds **6d–i** were provided smoothly.

### Biological activity

#### *Antioxidant activity in vitro*

The antioxidant activity of aspirin conjugates **5a–c** and **6a–i** were examined through two individual investigation according to their $IC_{50}$ values: the inhibition of malondialdehyde (MDA) formation [9, 22] and the stable




**Reagents and conditions**: (i) $SOCl_2$, reflux, 2 h; then excessive **2a**, acetone, $Et_3N$, 55.9%; (ii) **2c**, DCC-DMAP, 52.8%; (iii) Pd/C, $H_2$, 67.7%; (iv) **4a–c**, $SOCl_2$, reflux; then **3a**, $Et_3N$; (v) **4d–f**, DCC, DMAP, **3a** or **3b**, $CH_2Cl_2$; (vi) **5a–c**, 3 M HCl, acetone, reflux; (vii) **5d–i**, 5% HCl, MeOH, r. t.

**Scheme 1.** Synthesis of the conjugates of aspirin with phenolic antioxidants.

**Table 2.** Inhibition of lipid peroxidation, scavenging of 0.2 mM DPPH radical, and inhibition of croton-oil-induced mice-ear swelling by derivatives of aspirin.

| | Compounds | Inhibition of MDA formation $IC_{50}$ (mM) | Elimination of DPPH $IC_{50}$ (mM) | Inhibition on croton-oil-induced mice-ear swelling (%)[j] |
|---|---|---|---|---|
| | **A**[a] | >>8[c] | NT[g] | 22.4** |
| *p-coumaric acid 1a series* | **A + 1a**[b] | >8[d] | >125[h] | 28.3*** |
| | **5a** | ≈8[e] | NT[g] | 43.5*** |
| | **6a** | ≈8[f] | NT[i] | 27.5** |
| | **6d** | 3.9 | NT[i] | 12.2 |
| | **6g** | 1.8 | NT[i] | 30.7*** |
| *ferulic acid 1b series* | **A + 1b**[b] | 4.8 | 0.40 | 4.8 |
| | **5b** | 2.5 | NT[g] | 37.1*** |
| | **6b** | 3.4 | 0.50 | 34.4*** |
| | **6e** | 2.7 | 0.50 | 16.2* |
| | **6h** | 0.9 | 0.65 | 14.5** |
| *caffeic acid 1c series* | **A + 1c**[b] | 3.0 | 0.08 | 9.5 |
| | **5c** | 1.1 | NT[g] | 5.8 |
| | **6c** | 0.6 | 0.10 | 3.0 |
| | **6f** | 1.0 | 0.12 | 22.8*** |
| | **6i** | 0.4 | 0.11 | 8.8 |

a): Acetylsalicylic acid (Aspirin); b): this two compounds were mixed in equal mol; c): inhibition of MDA formation was 6.6% at 8 mM; d): inhibition of MDA formation was 27.2% at 8 mM; e): inhibition of MDA formation was 44.6% at 8 mM; f): inhibition of MDA formation was 50.7% at 8 mM; g): not tested because it had almost no elimination activity even at 125 mM; h): elimination of DPPH was 35.8% at 125 mM; i): not tested because of whose $IC_{50}$ was higher than 125 mM; j): all the tested compounds were given i. g. in 1 mmol/kg.

Each value represents the mean obtained in two independent experiments: *: $P > 0.05$, **: $P < 0.01$, ***: $P < 0.001$ *vs.* control.

free-radical scavenging ability to 2,2-diphenyl-1-picrylhydrazyl (DPPH) [9, 23].

As shown in Table 2, all the aspirin conjugates inhibited MDA formation efficiently with $IC_{50}$ values ranging from 0.4 to 8 mM. It should be noted that all the conjugates are stronger inhibitors of MDA formation ($IC_{50}$ = 8 mM) than aspirin ($IC_{50}$ >> 8 mM) as well as the equal-molar mixtures of aspirin and their corresponding parent antioxidants. Interestingly, those conjugates comprising a caffeic acid moiety (**5c**, **6c**, **6f**, **6i**) exhibited lower $IC_{50}$ values than their corresponding ferulic-acid moiety-containing counterparts (**5b**, **6b**, **6e**, **6h**), and, in turn, the later ones' $IC_{50}$ values were lower than those of *p*-coumaric acid series compounds (**5a**, **6a**, **6d**, **6g**). These results are

**Table 3**. The effects of aspirin, **5a**, and **5b** on gastric ulcer and toxicity.

| Compounds | Gastric ulcer | | | Toxicity | | | |
|---|---|---|---|---|---|---|---|
| | Number of mice | Dose (mmol/kg) | Ulcer score (X ± SD) | Number of mice | Dose (mmol/kg) | Mortality[a)] | Toxicity response[b)] |
| Aspirin | 13 | 4 | 8.62 ± 7.03 | 16 | 4 | 3/16 | 16 |
| **5a** | 12 | 4 | 4.58 ± 3.64 | 12 | 4 | 0 | 2 |
| **5b** | 12 | 4 | 3.83 ± 4.04 | 12 | 4 | 0 | 1 |

a): Dead/total; b): including listless and sluggish behavior.

consistent with their DPPH elimination ability. It is easily to understand that the compounds without free hydroxyls **5a–c** showed almost no free-radical scavenging ability ($IC_{50}$ >> 125 mM). However, those conjugates containing *p*-coumaric acid moiety **1a** and thus bearing one or two free hydroxyls in total (**6a**, **6d**, **6g**) also did not possess DPPH elimination ability ($IC_{50}$ >> 125 mM), whereas ferulic acid **1b** and caffeic acid **1c** containing conjugates (**6b**, **6e**, **6h**, **6c**, **6f**, **6i**) appeared to be strong inhibitors in the DPPH-elimination process.

### *Anti-inflammatory activity*

The anti-inflammatory activity of derivatives **5a–c** and **6a–i** was assessed from their ability to inhibit the croton-oil-induced mice-ear swelling (Table 2). The compounds were administered intragastrically (i.g.) at a dose of 1 mmol/kg and demonstrated various degrees of inhibition of the edema. Obviously enhanced inhibitory activities were observed for **5a** (43.5%), **6g** (30.7%), **5b** (37.1%), and **6b** (34.4%) in comparison to aspirin (22.4%). Co-administration of equimolar mixtures of aspirin and phenolic acid resulted in most cases in a lower anti-inflammatory activity than that of the test compounds in the *p*-coumaric acid **1a** and ferulic acid **1b** series, with the exception of **6a** and **6d**.

However, both mixtures and conjugates of caffeic acid and aspirin (**A + 1c** and **5c**, **6c**, **6i**) showed almost no effect on croton-oil-induced mice-ear swelling except for **6f** (22.8% inhibition). This is not consistent with their antioxidant ability, since caffeic acid series conjugates were the most powerful antioxidants among the test compounds. Further evidence was revealed by the fact that in most cases conjugates bearing a *p*-coumaric acid **1a** moiety inhibited edema more efficiently than those of the ferulic acid **1b** containing counterparts, while both, their MDA inhibition and DPPH-scavenging abilities, were evidently much lower than those of the later ones.

Notably, the obtained data showed the linker part had no influence on the antioxidant and anti-inflammatory activities of the target compounds.

### *Effects of compounds **5a** and **5b** on gastric ulcer and toxicity*

Compounds **5a** and **5b**, which showed the most potent anti-inflammatory activity among the test conjugates, were evaluated *in vivo* for their effects on gastric ulcer and toxicity (Table 3). Administration of aspirin at a dose of 4 mmol/kg in mice resulted in a high incidence of ulcer (8.62 ± 7.03), and this effect was accompanied by 3/16 mortality and 100% toxicity response (including listless and sluggish behavior) of the tested animals. In contrast, the same dose of **5a** and **5b** exhibited a significantly reduced ulcer effect and all animals survived with almost no toxicity response. Evidently, the designed compounds **5a** and **5b** were better than aspirin in respect of ulcerogenic potency and toxicity.

## Conclusion

In conclusion, a series of conjugates of aspirin with natural phenolic antioxidants were designed and synthesized to remain or enhance the analgesic activity but reduce the risk of GI ulceration and toxicity. Among them, **5a** and **5b** were found to be the best anti-inflammatory agents. They were further confirmed to significantly reduce ulcerogenic potency and toxicity in mice in comparison to aspirin. This molecular modifcation may offer a general route to safer anti-inflammatory agents potentially suitable for chronic use. However, it is evident that the anti-inflammatory activity *in vivo* of these dual-acting molecules was not simply consistent with their antioxidant ability *in vitro*. This needs to be further explored in the future.

## Experimental

### Chemistry

Solvents were purified in the usual way. TLCs were performed on precoated Merk silica gel 60 $F_{254}$ plates (Merck, Germany). Flash column chromatography was performed on silica gel (100–200

mesh, Qingdao, China). Melting points were determined with a "Yanaco" apparatus and were uncorrected. $^1$H-NMR and $^{13}$C-NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer (Jeol, Japan) with tetramethylsilane (TMS) as an internal standard, and chemical shifts are recorded in δ values. Mass spectra were obtained on a Waters Q-TOF micro mass spectrometer (Waters).

### *2-Acetoxy-benzoic acid 2-(4-hydroxy-phenyl)-ethyl ester **3b***

To a cold solution of aspirin (2.56 g, 14.2 mmol) and compound **2c** (2.7 g, 11.8 mmol) in $CH_2Cl_2$ (300 mL) were added dicyclohexylcarbodiimide (DCC; 2.93 g, 14.2 mmol) and 4-(dimethylamino)-pyridin (DMAP; 0.15 g, 1.2 mmol). The mixture was stirred for 1 h at 0°C and then overnight at r.t. After the precipitate was filtrated off, the solvent was removed under reduced pressure. The oily residue was then purified by column chromatography to give a white solid in 52.8% yield. The solid was dissolved in EtOAc/petroleum ether (120 mL, 1:1) and hydrogenated over 10% Pd/C (0.44 g) at r.t. for 4 h. After the catalyst was filtrated off and the solvent was removed under reduced pressure, the residue was subjected to silica gel column chromatography to give **3b** as a white solid in 67.7% yield; $R_f$ = 0.1 (EtOAc/petroleum ether, 1:4); $^1$H-NMR ($CDCl_3$) δ: 7.96 (dd, 1H, *J* = 1.6, 7.9 Hz), 7.57–7.54 (m, 1H), 7.30 (td, 1H, *J* = 1.1, 7.9 Hz), 7.13–7.11 (m, 2 H), 7.10 (dd, 1H, *J* = 1.1, 8.2 Hz), 6.79–6.77 (m, 2H), 4.44 (t, 2H, *J* = 7.0 Hz), 2.97 (t, 2H, *J* = 7.0 Hz), 2.31 (s, 3H); $^{13}$C-NMR ($CDCl_3$) δ: 169.8, 164.4, 154.3, 150.7, 133.9, 131.7, 130.1 (2C), 129.8, 126.0, 123.8, 123.3, 115.4 (2C), 65.8, 34.2, 21.0.

### *General procedure for the preparation of **5a–c***

To a cold solution of **3a** (10 mmol) and triethylamine (12 mmol) in anhydrous $CHCl_3$ (20 mL) was added 10 mmol of acid chloride of **4a–c** in 20 mL anhydrous $CHCl_3$. The mixture was stirred for 1 h at 0°C and then 1 h at r.t. After removal of the solvent, the residue was subjected to silica gel column chromatography to give **5a**, **b**, **c** in 67.5%, 64.9%, and 64.4% yield, respectively.

### *2-Acetoxy-benzoic acid 2-[3-(4-acetoxy-phenyl)-acryloyloxy]-ethyl ester **5a***

White solid, m. p.: 70–72°C; $R_f$ = 0.30 (EtOAc/petroleum ether, 1:2); $^1$H-NMR ($CDCl_3$) δ: 8.06 (dd, 1H, *J* = 1.4, 7.7 Hz), 7.71 (d, 1H, *J* = 16.1 Hz), 7.58 (td, 1H, *J* = 1.8, 8.1 Hz), 7.57–7.54 (m, 2H), 7.33 (td, 1H, *J* = 1.1, 7.7 Hz), 7.13–7.11 (m, 3H), 6.42 (d, 1H, *J* = 17.7 Hz), 4.56–4.51 (m, 4H), 2.34 (s, 3H); $^{13}$C-NMR ($CHCl_3$) δ: 169.6, 169.1, 166.5, 164.1, 152.2, 150.8, 144.4, 134.1, 131.9 (2C), 129.3 (2C), 126.1, 123.9, 122.8, 122.1, 117.5, 62.8, 62.2, 21.1, 21.0 (2C). ESI-HRMS (*m*/*z*) calcd. for $C_{22}H_{20}O_8Na$ [M + $Na^+$]: 435.1056. Found: 435.1073.

### *2-Acetoxy-benzoic acid 2-[3-(4-acetoxy-3-methoxy-phenyl)-acryloyloxy]-ethyl ester **5b***

White solid, m. p.: 99–100°C; $R_f$ = 0.20 (EtOAc/petroleum ether, 1:2); $^1$H-NMR ($CDCl_3$) δ: 8.06 (dd, 1H, *J* = 1.9, 8.0 Hz), 7.68 (d, 1H, *J* = 16.1 Hz), 7.58 (td, 1H, *J* = 1.4, 7.3 Hz), 7.33 (td, 1H, *J* = 1.1, 7.7 Hz), 7.14–7.11 (m, 3H), 7.05 (d, 1H, *J* = 8.1 Hz), 6.41 (d, 1H, *J* = 16.1 Hz), 4.56–4.52 (m, 4H), 3.86 (s, 3H), 2.34 (s, 3H), 2.32 (s, 3H); $^{13}$C-NMR ($CDCl_3$) δ: 169.7, 168.8, 166.5, 164.1, 151.4, 150.8, 144.8, 141.6, 134.2, 133.2, 131.9, 126.1, 123.9, 123.3, 122.8, 121.4, 117.6, 111.3, 62.9, 62.2, 55.9, 21.0, 20.6. ESI-HRMS (*m*/*z*) calcd. for $C_{23}H_{22}O_9Na$ [M + $Na^+$]: 465.1162. Found: 465.1171.

### *2-Acetoxy-benzoic acid 2-[3-(3,4-di-acetoxy-phenyl)-acryloyloxy]-ethyl ester **5c***

White solid, m. p.: 86–88°C; $R_f$ = 0.24 (EtOAc/petroleum ether, 4:5); $^1$H-NMR ($CDCl_3$) δ: 8.06 (dd, 1H, *J* = 1.9, 7.7 Hz), 7.66 (d, 1H, *J* = 15.8 Hz), 7.57 (td, 1H, *J* = 1.8, 8.0 Hz), 7.42 (dd, 1H, *J* = 2.2, 8.5 Hz), 7.37 (d, 1H, *J* = 1.9 Hz), 7.33 (td, 1H, *J* = 1.1, 7.7 Hz), 7.22 (d, 1H, *J* = 8.4 Hz), 7.12 (dd, 1H, *J* = 0.8, 8.1 Hz), 6.41 (d, 1H, *J* = 15.8 Hz), 4.56–4.51 (m, 4H), 2.34 (s, 3H), 2.31 (s, 3H), 2.30 (s, 3H); $^{13}$C-NMR ($CDCl_3$) δ: 170.0, 168.1, 168.0, 166.3, 164.1, 150.8, 143.6, 142.4 (2C), 134.2, 133.1, 131.9, 126.5, 126.1, 124.0, 123.9, 122.8 (2C), 118.6, 62.8, 62.3, 21.0, 20.7, 20.6. ESI-HRMS (*m*/*z*) calcd. for $C_{24}H_{22}O_{10}Na$ [M + $Na^+$]: 493.1111. Found: 493.1093.

### *General procedure for the preparation of **6a–c***

A solution of **5a–c** (1.76 mmol) in 45 mL of acetone containing 15 mL of 3 M HCl was refluxed for 45 min, respectively, after which the solvent was removed under reduced pressure. The residual aqueous layer was extracted with EtOAc (80 mL). The organic layer was separated and washed with satd. aqueous $NaHCO_3$ (2 × 20 mL) and water (2 × 20 mL), then dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was subjected to silica gel column chromatography to give **6a**, **b**, **c** in 53.5%, 48.5%, and 60.1% yield, respectively.

### *2-Hydroxy-benzoic acid 2-[3-(4-hydroxy-phenyl)-acryloyloxy]-ethyl ester **6a***

White solid, m. p.: 148–150°C; $R_f$ = 0.29 (EtOAc/petroleum ether, 1:2); $^1$H-NMR (DMSO-$d_6$) δ: 10.42 (s, 1H, -OH), 7.78 (dd, 1H, *J* = 1.6, 7.7 Hz), 7.59 (d, 1H, *J* = 16.0 Hz), 7.56–7.51 (m, 3H), 6.99 (dd, 1H, *J* = 0.8, 8.2 Hz), 6.95 (td, 1H, *J* = 1.1, 8.2 Hz), 6.79–6.77 (m, 2H), 6.43 (d, 1H, *J* = 16.0 Hz), 4.58–4.49 (m, 4H); $^{13}$C-NMR (DMSO-$d_6$) δ: 168.4, 166.5, 160.0, 159.9, 145.3, 135.8, 130.4 (2 C), 130.1, 125.0, 119.4, 117.4, 115.7 (2 C), 113.6, 112.9, 63.3, 61.6. ESI-HRMS (*m*/*z*) calcd. for $C_{18}H_{15}O_6$ [M – H]: 327.0869. Found: 327.0869.

### *2-Hydroxy-benzoic acid 2-[3-(4-hydroxy-3-methoxy-phenyl)-acryloyloxy]-ethyl ester **6b***

Buff solid, m. p.: 121–122°C; $R_f$ = 0.23 ($CHCl_3$); $^1$H-NMR (DMSO-$d_6$) δ: 10.44 (s, 1H, OH), 9.64 (s, 1H, OH), 7.79 (dd, 1H, *J* = 1.4, 7.7 Hz), 7.58 (d, 1H, *J* = 16.1 Hz), 7.53 (td, 1H, *J* = 1.8, 8.8 Hz), 7.33 (d, 1H, *J* = 1.8 Hz), 7.11 (dd, 1H, *J* = 1.9, 8.0 Hz), 7.00–6.94 (m, 2H), 6.78 (d, 1H, *J* = 8.0 Hz), 6.53 (d, 1H, *J* = 15.7 Hz), 4.59–4.48 (m, 4H), 3.81 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$) δ: 168.5, 166.6, 160.1, 149.4, 147.9, 145.6, 135.8, 130.1, 125.5, 123.4, 119.4, 117.4, 115.4, 113.9, 112.9, 111.2, 63.4, 61.6, 55.7. ESI-HRMS (*m*/*z*) calcd. for $C_{19}H_{17}O_7$ [M – H]: 357.0974. Found: 357.0989.

### *2-Hydroxy-benzoic acid 2-[3-(3,4-dihydroxy-phenyl)-acryloyloxy]-ethyl ester **6c***

Buff solid, m. p.: 123–124°C; $R_f$ = 0.13 (MeOH/CHCl3, 1:50); $^1$H-NMR (DMSO-$d_6$) δ: 10.43 (s, 1H, -OH), 9.64 (s, 1H, -OH), 9.15 (s, 1H, -OH), 7.79 (dd, 1H, *J* = 1.4, 7.7 Hz), 7.53 (td, 1H, *J* = 1.4, 7.3 Hz), 7.51 (d, 1H, *J* = 15.8 Hz), 7.05 (d, 1H, *J* = 2.2 Hz), 7.00 (dd, 1H, *J* = 2.2, 8.4 Hz), 6.99 (dd, 1H, *J* = 0.7, 8.0 Hz), 6.95 (td, 1H, *J* = 1.1, 8.0 Hz), 6.76 (d, 1H, *J* = 8.0 Hz), 6.30 (d, 1H, *J* = 16.1 Hz), 4.58–4.48 (m, 4H); $^{13}$C-NMR (DMSO-$d_6$) δ: 168.3, 166.4, 160.0, 148.5, 145.7, 145.5, 125.4, 121.5, 119.4, 117.4, 115.7, 114.9, 113.4, 112.9, 63.3, 61.6. ESI-HRMS (*m*/*z*) calcd. for $C_{18}H_{15}O_7$ [M – H]: 343.0818. Found: 343.0833.

### *General procedure for the preparation of **5d–i***

To a cold mixture of **5d–i** (1.2 eq.) and compound **3a** (or **3b**) (1.0 eq.) in $CH_2Cl_2$ were added DCC (1.2 eq.) and DMAP (0.1 eq.). The mixture was stirred at 0°C for 1 h and then at r.t. overnight. After the precipitate was filtrated off, the solvent was removed under reduced pressure. The residue was used for next reaction without further purification.

### *General procedure for the preparation of **6d–i***

A solution of corresponding **5d–i** in methanol (40 mL) containing five drops of 5% HCl was stirred at r.t. for 2 h. Half of the solvent was removed under reduced pressure and then, 20 mL of water and 150 mL of ether were added to the mixture. The organic portion was separated, washed with water (3 × 20 mL), and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was subjected to silica gel column chromatography to give **6d–i**, respectively.

### *2-Acetoxy-benzoic acid 2-[3-(4-hydroxy-phenyl)-acryloyloxy]-ethyl ester **6d***

Yield: 81.6%; white solid, m.p.: 137–138°C; $R_f$ = 0.12 (EtOAc/petroleum ether, 1:2); $^1$H-NMR (DMSO-$d_6$) δ: 7.96 (dd, 1H, *J* = 1.7, 7.8 Hz), 7.69 (td, 1H, *J* = 1.8, 7.7 Hz), 7.59 (d, 1H, *J* = 15.9 Hz), 7.56–7.55 (m, 2H), 7.42 (td, 1H, *J* = 1.3, 7.6 Hz), 7.25 (dd, 1H, *J* = 1.3, 8.1 Hz), 6.80–6.78 (m, 2H), 6.42 (d, 1H, *J* = 17.7 Hz), 4.51–4.43 (m, 4H), 2.27 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$) δ: 169.1, 166.5, 163.9, 159.9, 150.0, 145.3, 134.5, 131.3, 130.4 (2C), 126.3, 125.0, 124.0, 122.8, 115.8 (2C), 113.6, 63.1, 61.8, 20.7. ESI-HRMS (*m*/*z*) calcd. for $C_{20}H_{18}O_7Na$ [M + $Na^+$]: 393.0950. Found: 393.0932.

### *2-Acetoxy-benzoic acid 2-[3-(4-hydroxy-3-methoxy-phenyl)-acryloyloxy] -ethyl ester **6e***

Yield over two steps: 77.4%; white solid, m.p.: 77–80°C; $R_f$ = 0.20 (EtOAc/petroleum ether, 2:3); $^1$H-NMR (DMSO-$d_6$) δ: 9.65 (s, 1H, OH), 7.97 (dd, 1H, *J* = 1.9, 7.7 Hz), 7.70 (td, 1H, *J* = 1.8, 7.7 Hz), 7.59 (d, 1H, *J* = 15.7 Hz), 7.43 (td, 1H, *J* = 1.1, 7.7 Hz), 7.33 (d, 1H, *J* = 1.8 Hz), 7.25(dd, 1H, *J* = 0.7, 8.0 Hz), 7.12 (dd, 1H, *J* = 2.2, 8.5 Hz), 6.79 (d, 1H, *J* = 8.1 Hz), 6.52 (d, 1H, *J* = 15.7 Hz), 4.51–4.43 (m, 4H), 3.81 (s, 3H), 2.27 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$) δ: 169.1, 166.5, 163.9, 150.0, 149.5, 147.9, 145.6, 134.5, 131.3, 126.3, 125.5, 124.1, 123.4, 122.8, 115.5, 113.9, 111.1, 63.2, 61.8, 55.7. ESI-HRMS (*m*/*z*) calcd. for $C_{21}H_{20}O_8Na$ [M + $Na^+$]: 423.1056. Found: 423.1063.

### *2-Acetoxy-benzoic acid 2-[3-(3,4-di-hydroxy-phenyl)-acryloyloxy]-ethyl ester **6f***

Yield: 94.0%; white solid, m.p.: 79–83°C; $R_f$ = 0.12 (EtOAc/petroleum ether, 2:3); $^1$H-NMR (DMSO-$d_6$) δ: 9.63 (s, 1H, OH), 9.15 (s, 1H, OH), 7.96 (dd, 1H, *J* = 1.4, 7.7 Hz), 7.69 (td, 1H, *J* = 1.4, 8.1 Hz), 7.51 (d, 1H, *J* = 15.7 Hz), 7.42 (td, 1H, *J* = 1.1, 7.7 Hz), 7.25 (dd, 1H, *J* = 1.1, 8.0 Hz), 7.05 (d, 1H, *J* = 1.8 Hz), 7.00 (dd, 1H, *J* = 1.8, 8.0 Hz), 6.75 (d, 1H, *J* = 8.1 Hz), 6.29 (d, 1H, *J* = 15.7 Hz), 4.50–4.42 (m, 4H), 2.27 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$) δ: 169.0, 166.3, 163.8, 149.9, 148.4, 145.6, 145.4, 134.3, 131.2, 126.2, 125.3, 124.0, 122.7, 121.4, 115.6, 114.8, 113.3, 63.0, 61.7, 20.6. ESI-HRMS (*m*/*z*) calcd. for $C_{20}H_{18}O_8Na$ [M + $Na^+$]: 409.0899. Found: 409.0914.

### *2-Acetoxy-benzoic acid 2-{4-[3-(4-hydroxy-phenyl)-acryloyloxy]-phenyl}-ethyl ester **6g***

Yield over two steps: 61.3%; yellow solid, m.p.: 122–124°C; $R_f$ = 0.12 (EtOAc/petroleum ether, 2:5); $^1$H-NMR (DMSO-$d_6$) δ: 10.12 (s, 1H, -OH), 7.88 (dd, 1H, *J* = 1.5, 7.7 Hz), 7.75 (d, 1H, *J* = 15.8 Hz), 7.68 (td, 1H, *J* = 1.4, 8.8 Hz), 7.64 (d, 2H, *J* = 8.4 Hz), 7.41 (td, 1H, *J* = 1.1, 7.3 Hz), 7.36 (d, 2H, *J* = 8.4 Hz), 7.23 (d, 1H, *J* = 8.0 Hz), 7.13 (d, 2H, *J* = 8.5 Hz), 6.83 (d, 2H, *J* = 8.4 Hz), 6.62 (d, 1H, *J* = 16.1 Hz), 4.46 (t, 2H, *J* = 7.0 Hz), 3.03 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H). ESI-HRMS (*m*/*z*) calcd. for $C_{26}H_{22}O_7Na$ [M + $Na^+$]: 469.1263. Found: 469.1282.

### *2-Acetoxy-benzoic acid 2-{4-[3-(4-hydroxy-3-methoxy-phenyl)-acryloyloxy]-phenyl}-ethyl ester **6h***

Yield over two steps: 86.7%, yellow solid, m.p.: 113–114°C; $R_f$ = 0.14 (EtOAc/petroleum ether, 1:2); $^1$H-NMR (DMSO-$d_6$) δ: 9.73 (s, 1H, OH), 7.89 (dd, 1H, *J* = 1.8, 7.7 Hz), 7.74 (d, 1H, *J* = 15.7 Hz), 7.68 (td, 1H, *J* = 1.9, 7.7 Hz), 7.42 (d, 1H, *J* = 1.9 Hz), 7.41 (td, 1H, *J* = 1.1, 7.3 Hz), 7.39–7.36 (m, 2H), 7.24 (dd, 1H, *J* = 1.0, 8.0 Hz), 7.21 (dd, 1H, *J* = 1.8, 8.4 Hz), 7.14–7.13 (m, 2H), 6.82 (d, 1H, *J* = 8.0 Hz), 6.71 (d, 1H, *J* = 15.7 Hz), 4.46 (t, 2H, *J* = 7.0 Hz), 3.84 (s, 3H), 3.03 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$) δ: 169.1, 165.4, 163.9, 150.5, 149.8, 149.2, 148.0, 147.0, 135.3, 134.3, 131.1, 129.9 (2C), 126.3, 125.4, 124.0, 123.7, 123.0, 121.8 (2C), 115.5, 113.4, 111.4, 65.3, 55.7, 33.6, 20.7. ESI-HRMS (*m*/*z*) calcd. for $C_{27}H_{24}O_8Na$ [M + $Na^+$]: 499.1369. Found: 499.1385.

### *2-Acetoxy-benzoic acid 2-{4-[3-(3,4-di-hydroxy-phenyl)-acryloyloxy]-phenyl}-ethyl ester **6i***

Yield over two steps: 81.4%, yellow solid, m.p.: 151–152°C; $R_f$ = 0.17 (EtOAc/petroleum ether, 2:3); $^1$H-NMR (DMSO-$d_6$) δ: 9.72 (s, 1H, OH), 9.21 (s, 1H, OH), 7.88 (dd, 1H, *J* = 1.4, 7.7 Hz), 7.68 (td, 1H, *J* = 1.4, 8.1 Hz), 7.67 (d, 1H, *J* = 15.8 Hz), 7.41 (td, 1H, *J* = 1.1, 7.7 Hz), 7.36 (d, 2H, *J* = 8.4 Hz), 7.24 (dd, 1H, *J* = 1.1, 8.1 Hz), 7.14–7.12 (m, 3H), 7.09 (dd, 1H, *J* = 2.2, 8.5 Hz), 6.80 (d, 1H, *J* = 8.0 Hz), 6.50 (d, 1H, *J* = 15.8 Hz), 4.46 (t, 2H, *J* = 7.0 Hz), 3.03 (t, 2H, *J* = 7.0 Hz), 2.23 (s, 3H); $^{13}$C-NMR (DMSO-$d_6$) δ: 169.0, 165.2, 163.8, 149.9, 149.1, 148.8, 147.0, 145.5, 135.1, 134.2, 131.0, 129.7 (2C), 126.2, 125.3, 123.9, 123.0, 121.8, 121.7 (2C), 115.7, 115.0, 112.8, 65.2, 33.5, 20.6. ESI-HRMS (*m*/*z*) calcd. for $C_{26}H_{23}O_8$ [M + $H^+$]: 463.1393. Found: 463.1411.

## Biology

### *Lipid-peroxidation assay*

The anti-lipid peroxidation activity of the target compounds was measured by determination of thiobarbituric acid (TBA) reactive substances in 96-well plates. 10% liver homogenate from untreated Kunming male mice (18–22 g; obtained from the Department of Experimental Animals, Institute of Hematology, Chinese Academy of Medical Sciences) was prepared as previously described (Sun *et al*., [22a]). The incubation mixture which containing 80 μL of 10% liver homogenate, 10 μL of 25 mmol/L $FeSO_4$, and 10 μL of various concentrations of the test compounds dissolved in 90% DMSO (90% DMSO, 10% water), was incubated at 37°C for 30 min. The reaction was quenched by addition of 100 μL 10% $CCl_3COOH$ and then centrifuged at 3000 rpm for 10 min. 100 μL of the supernate was decanted to another well and 100 μL TBA reagent (containing 0.67% TBA, 10% $CCl_3COOH$) was added to the supernatant. After incubation at 60°C for 30 min, lipid peroxidation was assessed spectrophotometrically (535 nm against 600 nm) ($A_i$). The absorbance of the

control sample without test compound ($A_0$) was measured simultaneously. Each experiment was performed at least twice and the results were expressed as inhibition of malondialdehyde (MDA) formation and calculated according to the following equation:

$$\text{Inhibition of MDA formation } (\%) = \frac{(A_0 - A_i)}{A_0} \times 100 \quad (1)$$

### *Measurement of the reducing activity against the stable radical DPPH*

Various concentrations of the test compounds dissolved in DMSO (analytical grade) were added to an equal volume of ethanolic solution of DPPH (final concentration $2 \times 10^{-4}$ mol/L). The mixtures were then kept at room temperature for 45 min and the absorbance (517 nm) was recorded ($A_i$). The absorbance of the control sample without test compound ($A_0$) and the blank sample with the equal volume of ethanol and the test compounds ($A_j$) were measured simultaneously. Each experiment was performed at least twice. The results were expressed as percentage of DPPH elimination and calculated according to the following equation:

$$\text{elimination of DPPA} = \left(1 - \frac{(A_i - A_j)}{A_0}\right) \times 100\% \quad (2)$$

### *Topical anti-inflammatory activity*

Topical anti-inflammatory activity was evaluated as an inhibition of the croton-oil-induced ear edema in mice. Male Kunming mice (18–22 g) were injected (i.g.) with the tested compounds suspended in 5% Arabic gum (1 mmol/kg) and the control group were given 5% Arabic gum (i.g.). 30 min later, 50 μL of 2% croton oil (2% croton oil, 20% ethanol, 78% ether) was applied to the inner and outer surface of right ear, and the left ear remained untreated. 4 h later, mice were sacrificed and from each ear, a disc (8 mm in diameter) was removed with a metal punch. The edematous response was assessed as the weight difference (in mg) between the plugs of right and left ear and the anti-inflammatory activity was expressed as percentage of edema reduction in treated mice with regard to control mice.

### *Effects on gastric ulcer and toxicity*

Male ICR mice (29–32 g; obtained from the Department of Experimental Animals, Institute of Hematology, Chinese Academy of Medical Sciences) were fasted for 24 h with water *ad libitum* and then given the tested compound (i.g.) suspended in Tween 80 (2 mmol/10 mL) at a final dose of 4 mmol/kg. Fasting was continued for an additional 12 h. In this 12 h, the animals were observed and the number of dead mice was counted. Then, the mice were sacrificed. The stomach was extracted and infused with 3% formaldehyde solution. Subsequently, the stomach was dipped in 3% formaldehyde solution and then dissected along the greater curvature. The gastric lesions score was expressed in mm of lesion and calculated by summing the length of all lesions in a given stomach.

*We thank Dr. Guifang Cheng (Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College) for evaluating the anti-inflammaoty activity, the effects on gastric ulcer and toxicity of the synthesized compounds.*

*The authors have declared no conflict of interest.*

## References

[1] M. C. Allison, A. G. Howatson, C. J. Torrence, F. D. Lee, R. I. Russell, *N. Engl. J. Med.* **1992**, *327*, 749–754.

[2] D. Galanakis, A. P. Kourounakis, K. C. Tsiakitzis, C. Doulgkeris, *et al.*, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3639–3643.

[3] J. Tenenbaum, *Can. J. Gastroenterol.* **1999**, *13*, 119–122.

[4] R. T. Schoen, R. J. Vender, *Am. J. Med.* **1989**, *86*, 449–458.

[5] A. S. Kalgutkar, A. B. Marnett, B. C. Crews, R. P. Remmel, L. J. Marnett, *J. Med. Chem.* **2000**, *43*, 2860–2870.

[6] J. L. Masferrer, B. S. Zweifel, P. T. Manning, S. D. Hauser, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 3228–3232.

[7] J. L. Wallace, *Trends Pharmacol. Sci.* **1999**, *20*, 4–6.

[8] S. X. Sun, K. Y. Lee, C. T. Bertram, J. L. Goldstein, *Curr. Med. Res. Opin.* **2007**, *23*, 1859–1866.

[9] P. N. Kourounakism, K. Tsiakitzis, A. P. Kourounakis, D. Galanakis, *Toxicology* **2000**, *144*, 205–210.

[10] *Free Radicals in Biology and Medicine* (Eds.: B. Halliwell, J. M. C. Gutteridge), 3rd ed., Oxford University Press, Oxford, UK, **1999**, pp. 661–662 and pp. 744–751.

[11] D. Kovala-Demertzi, S. K. Hadjikakou, M. A. Demertzis, Y. Deligiannakis, *J. Inorg. Biochem.* **1998**, *69*, 223–229; B. I. Hirschowitz, C. J. Hawkey, *Am. J. Med.* **2001**, *110 (1A)*, S74–S78.

[12] N. Sugiyama, F. Akahoshi, S. Kuwahara, M. Kajii, Y. Sakaue, *et al.*, *J. Med. Chem.* **1994**, *37*, 1977–1982; J. N. Gashman, *Drugs* **1996**, *52 (Suppl. 5)*, 13–23.

[13] A. Detsi, D. Bouloumbasi, K. C. Prousis, M. Koufaki, *et al.*, *J. Med. Chem.* **2007**, *50*, 2450–2458.

[14] H. Orhan, G. Sahin, *Exp. Toxicol. Pathol.* **2001**, *53*, 133–140.

[15] A. A. Akhrem, V. V. Zharkov, G. V. Zaitseva, I. A. Mikhailopulo, *Tetrahedron Lett.* **1973**, *17*, 1475–1478.

[16] K. Kimura, H. Inoue, S. I. Kohama, Y. Yamashita, Y. Sakaguchi, *Macromol.* **2003**, *36*, 7721–7729.

[17] R. F. Helm, J. Ralph, R. D. Hatfield, *Carbohydr. Res.* **1992**, *229*, 183–194.

[18] S. Ryczek, K. Dettner, C. Unverzagt, *Bioorg. Med. Chem.* **2009**, *17*, 1187–1192.

[19] J. Lub, W. P. M. Nijssen, R. T. Wegh, I. de Francisco, *et al.*, *Liq. Cryst.* **2005**, *32*, 1031–1044.

[20] P. Rummakko, G. Brunow, M. Orlandi, B. Rindone, *Synlett.* **1999**, *3*, 333–335.

[21] Y. C. Zhang, P. T. Chen, H. S. Guan, Y. X. Li, *Chin. J. Chem.* **2005**, *23*, 1523–1529.

[22] S. Sun, B. Xu, Q. Li, R. Li, *Prog. Biochem. Biophys.* **1995**, *22*, 165–169; D. Lapenna, G. Ciofani, D. Festi, M. Neri, *et al.*, *Biochem. Pharmacol.* **2002**, *64*, 1661–1667.

[23] Z. Sroka, W. Cisowski, *Food Chem. Toxicol.* **2003**, *41*, 753; N. Cotelle, J. L. Bernier, J. P. Catteau, J. Pommery, *et al.*, *Free Rad. Biol. Med.* **1996**, *20 (1)*, 35–43.