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# Gastroprotective activity of *ent*-beyerene derivatives in mice: Effects on gastric secretion, endogenous prostaglandins and non-protein sulfhydryls

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ABSTRACT

Seventeen compounds (**2**–**18**) synthetized from the diterpenoid *ent*-beyer-15-en-18-ol (**1**) isolated from aerial part of *Baccharis tola* were tested for their gastroprotective activity on the model of HCl/EtOH-induced gastric lesions in mice. Furthermore cytotoxicity test toward fibroblasts and AGS cells were performed. The results showed that compound **1** ($ED_{50}$ = 50 mg/kg), **2**, **6** and **13** were the most active regarding gastroprotective activity. Compounds **8**–**10** and **17**–**18** showed the lowest cytotoxicity toward fibroblasts and AGS cells. Regarding to mode of gastroprotective action, the effect elicited by **6** (50 mg/kg) was reversed by Indomethacin but not by *N*-ethylmaleimide, $N^G$-nitro-L-arginine methyl ester or ruthenium red, which suggests that prostaglandins are involved in the mode of gastroprotective action of **6**.


The genus *Baccharis* is represented in Chile by 48 species.[1] Several of them are used in traditional medicine to protect stomach and liver, restore blood circulation, reduce inflammatory process and cure ulcers, burns and skin wounds.[2] In particular, *Baccharis tola* has been reported to be a producer of acetophenones, coumarines, diterpenoids, triterpenoids and flavonoids.[3,4] In the course of our investigation of medicinal plants from Chile, we report here the gastroprotective effect and cytotoxicity of *ent*-beyer-15-en-18-ol and several *ent*-beyerene derivatives (**2**–**18**). In addition, we discuss the mode of gastroprotective action of **6**, including the involvement of prostaglandins (PGs), nitric oxide (NO), sulfhydryl compounds (SHs) and vanilloid receptors (VR).

From the dichloromethane extract of *B. tola*,[5] *ent*-beyer-15-en-18-ol (**1**) was isolated[6] (Fig. 1). The effect of *ent*-beyer-15-en-18-ol (**1**) on the model of HCl/EtOH-induced gastric lesions[7,8] in mice[9] is shown in Figure 2. An oral administration of **1** at 12.5, 25, 50 and 100 mg/kg ($ED_{50}$ = 50 mg/kg) inhibited the appearance of gastric lesions in a dose-dependent manner compared with the control group ($P$ <0.01). The inhibition displayed by **1** at 50 mg/kg, *p.o.* (53%) was similar to that observed with lansoprazole (57%), while the strongest effect was observed at 100 mg/kg (79%). Taken into account its gastroprotective activity, we decided to prepare lipophilic analogs of **1** (Fig. 1) containing a side chain of fatty acids and cinnamic acids semi synthesized thorough esterification reactions.

Seventeen esters were prepared by standard methods using DCC as coupling agent.[8] In this way, we obtained the compounds **2**-**18**. All proton and carbon resonances were assigned by $^1$H, $^{13}$C, DEPT, HHCOSY, HMQC and HMBC experiments.[10]

Table 1 shows the effect of the semisynthetic derivatives **2**–**18** at 50 mg/kg. The greatest gastroprotective activity was displayed by compounds **2**, and **6**, which resulted as active as lansoprazole at 20 mg/kg and reduced gastric lesions by 68 and 77%, respectively. The gastroprotective activity of compounds **3**–**5** and **8**–**11** did not differ statistically from the control. As for compounds **7** and **12**–**18**, the gastroprotective active were found over the range 24–52%.

In the case of the cinnamic esters **2**–**12**, a significant increase in the gastroprotective activity was observed for compound **6** bearing a chlorine at C-3′ (77%). The effect of chlorine at C-4′ (42%) was similar to that of the parent compound (**1**). Regarding the nitro group (**3**–**5**) and methoxy group (**8**–**10**), the effect was lower than the parent compounds **1**. Furthermore, a significant decrease in the

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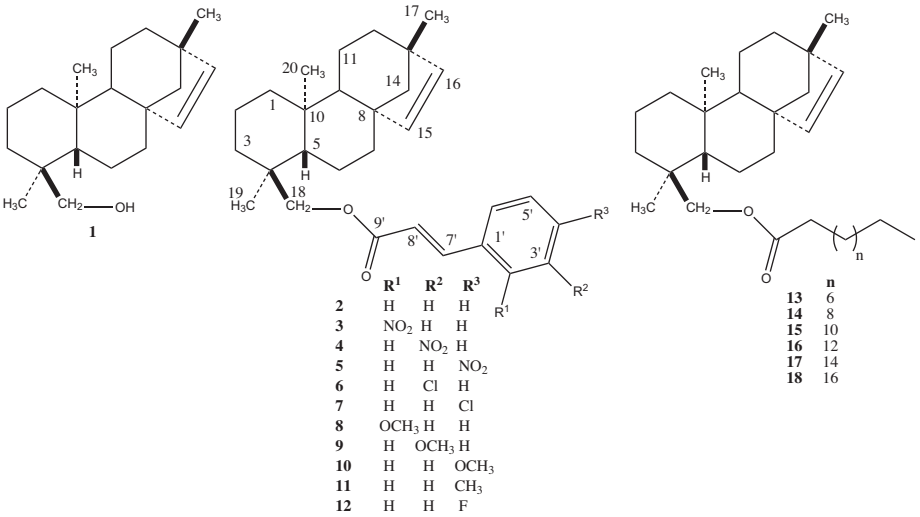


**Figure 1.** The structures of compounds **2**–**18**.

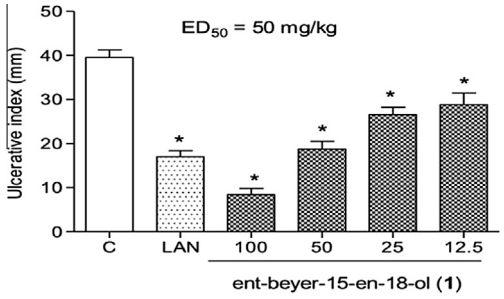


**Figure 2.** Effects of **1** (12.5, 25, 50 and 100 mg/kg) and lansoprazole (20 mg/kg) on the model of HCl/EtOH-induced gastric lesions in mice. Results are expressed as mean ± SEM, $n = 7$. Analysis of variance followed by Dunnett's test. $^{*}P < 0.01$ compared with control group.

**Table 1**
Gastroprotective effect of **1** and the semisynthetic derivatives **2**–**18** at 50 mg/kg on HCl/EtOH-induced gastric lesions in mice and cytotoxicity towards AGS cells and human fibroblasts

| Compound | *n* | Lesion index (mm) | % lesion reduction | Cytotoxicity $IC_{50}$ (μM) | |
|---|---|---|---|---|---|
| | | | | AGS | Fibroblasts |
| **1** | 7 | 17.7 ± 1.8* | 50* | 19 ± 2 | 31 ± 4 |
| **2** | 7 | 11.6 ± 1.3* | 68 | 30 ± 3 | 45 ± 4 |
| **3** | 7 | 31.0 ± 1.4 | 14** | 25 ± 4 | 38 ± 5 |
| **4** | 7 | 35.4 ± 1.7 | 2** | 29 ± 4 | 44 ± 3 |
| **5** | 7 | 36.7 ± 2.1 | 0** | 35 ± 5 | 61 ± 4 |
| **6** | 7 | 8.4 ± 1.2* | 77** | 12 ± 2 | 15 ± 1 |
| **7** | 7 | 20.8 ± 1.6* | 42** | 18 ± 1 | 29 ± 2 |
| **8** | 7 | 33.4 ± 1.3 | 7** | 151 ± 13 | 311 ± 9 |
| **9** | 7 | 34.6 ± 1.8 | 4** | 165 ± 18 | 289 ± 12 |
| **10** | 7 | 32.0 ± 1.7 | 11** | 230 ± 11 | 378 ± 14 |
| **11** | 7 | 32.9 ± 1.5 | 8** | 39 ± 5 | 49 ± 6 |
| **12** | 7 | 27.5 ± 1.0* | 24** | 58 ± 6 | 69 ± 4 |
| **13** | 7 | 17.1 ± 0.9* | 52** | 21 ± 2 | 33 ± 3 |
| **14** | 7 | 17.7 ± 1.6* | 51** | 33 ± 2 | 52 ± 4 |
| **15** | 7 | 19.3 ± 1.2* | 46** | 38 ± 3 | 61 ± 5 |
| **16** | 7 | 19.4 ± 0.6* | 46** | 64 ± 4 | 88 ± 6 |
| **17** | 7 | 20.1 ± 1.1* | 44** | 228 ± 11 | 359 ± 15 |
| **18** | 7 | 23.8 ± 1.0* | 34** | 321 ± 10 | 581 ± 13 |
| Lansoprazole | 7 | 12.6 ± 1.5* | 65 | 149 ± 9 | 291 ± 12 |
| Control | 7 | 35.9 ± 0.9 | — | — | — |

The results are expressed as mean ± SEM $^{*}P < 0.01$; significantly different compared with the control and $^{**}P < 0.01$ significantly different compared with lansoprazole (ANOVA followed by Dunnett's test), *n* = number of mice.

gastroprotective effect was observed for compounds bearing methyl group **11** and fluorine group **12**.

In the case of the fatty acid esters **13**–**18**, the effect of **13**–**14** and **15**–**16** was similar to that of the parent compound (**1**). No clear relationship was observed between number of carbons in the ester lateral chain and the gastroprotective activity.

The best gastroprotective effect was displayed by **6**, so this compound was chosen for further experiments to explain the possible mode of gastroprotective action. Table 2 shows the effects of **6** on the gastric lesions induced by HCl/EtOH in mice pretreated with Indomethacin (10 mg/kg, s.c.), *N*-ethylmaleimide (NEM, 10 mg/kg, s.c.), $N^{G}$-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg, ip) or ruthenium red (RR, 3.5 mg/kg, s.c.) at an oral dose of 50 mg/kg.

Endogenous sulfhydryls such as glutathione play an important role in the protection of the gastric mucosa. In this sense glutathione is known to protect the integrity and permeability of the cell membrane and may act as antioxidants, scavengers of free radicals, maintenance of immune function, regulation of protein synthesis and degradation, and the maintenance protein structure.[11,12] In this study, pretreatment with NEM[13] (an SH blocker) have not reduced the gastroprotective activity of **6**, suggesting that the protective effect of this semi-synthetic diterpenoid is not involving the participation of endogenous SHs.

Endogenous PGs are known to be implicated in the mechanism of gastroprotection induced by mild irritants, and necrotizing

**Table 2**
Effect of *ent-beyer-15-en-18-yl-3-chlorocinnamate* (**6**) on the appearance of gastric lesions induced by HCl/EtOH (*p.o.*) in Indomethacin-, NEM-, L-NAME- and RR-pretreated mice

| Treatment | Dose (mg/kg) | Lesion index (mm) |
|---|---|---|
| Control | — | 42.0 ± 1.9 |
| IND | 10 | 39.9 ± 1.8 |
| NEM | 10 | 41.9 ± 2.1 |
| L-NAME | 70 | 42.7 ± 2.8 |
| RR | 3.5 | 40.5 ± 2.3 |
| **6** | 50 | 12.1 ± 2.5* |
| IND + **6** | 10 + 50 | 38.9 ± 2.1 |
| NEM + **6** | 10 + 50 | 15.2 ± 2.1* |
| L-NAME + **6** | 70 + 50 | 13.1 ± 2.1* |
| RR + **6** | 3.5 + 50 | 17.0 ± 1.9* |
| Carbenoxolone | 100 | 13.1 ± 2.9* |

Results are expressed as mean ± SEM, $n = 7$. Analysis of variance followed by Dunnett's test. $^{*}P < 0.01$ compared with the respective control.

agents. In this sense, PGs inhibit the gastric acid secretion, stimulate release of mucus and bicarbonate and increase blood flow on gastric mucosal.[14–17] In the present study, PGs are proved to be involved in the gastroprotective action of **6**, because the activity of this compound was reduced by pretreatment with IND[18] (an inhibitor of the PG synthesis).

NO in the gastrointestinal tract play a role in the health, defense and repair of the gastric mucosa.[19] Furthermore, it has been demonstrated that NO participates in gastric defense by regulating the gastric mucosal blood flow, angiogenesis and gastric mucus secretion. In this study, pretreatment with L-NAME[20] (an inhibitor of NO synthase) did not attenuate the gastroprotective activity of **6**. This finding suggests that endogenous NO have little participation in the protective effect of this compound.

Capsaicin-sensitive sensory neurons via VR on the gastrointestinal tract participate in gastric defense mechanisms by regulating the gastric motility, acid secretion, gastric blood flow through the action of calcitonin gene-related peptide (CGRP) and stimulation of gastric mucus and bicarbonate.[21,22] In this study, pretreatment with ruthenium red[23] (a vanilloid receptor antagonist), did not reduce the lesion index suggesting that the mechanism of gastroprotection of **6** have no relationship with capsaicin-sensitive sensory neurons via VR.

Table 1 shows cytotoxic activity[24] of the compounds **1**–**18** toward AGS cells and fibroblasts. Among the studied compounds, the analogs **8**–**10** and **17**–**18** as well as lansoprazole were less cytotoxic than *ent*-beyer-15-en-18-ol. Compounds **1**–**7** and **11**–**16** were the most cytotoxic against fibroblasts and AGS cells.

Compounds **2** and **6** were the most active in the gastroprotective effect but they also showed high cytotoxicity. Compounds **8**–**10** were inactive while compounds **17**–**18** displayed gastroprotective effects in this model but with much lower cytotoxicity than the parent compound.

It is well known that the impairment of the balance between aggressive and defensive factors in the gastric mucosa could lead to gastric ulcers.[26] Several terpenoids were reported to be beneficial and protective for the induced lesions of gastric mucosa in different animal models.[26–28] Even though the gastroprotective properties are well established, their mechanism of action seems to be related to stimulation of the defensive factors rather than inhibition of the aggressive factors.[26–28] In summary, we reported here the gastroprotective activity of a natural diterpenoid and a series of novel lipophilic *ent*-beyer-15-en-18-ol analogs. Compound **6** exerted the best gastroprotective activity and its mode of action was explained through the participation of prostaglandins.

## Acknowledgments

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## References and notes

1. Marticorena, C. *Gayana Bot.* **1985**, *42*, 32.
2. Wickens, G. E. *Opera Bot.* **1993**, *121*, 291.
3. San-Martin, A.; Rovirosa, J.; Becker, R.; Castillo, M. *Phytochemistry* **1985**, *1980*, 19.
4. San-Martin, A.; Rovirosa, J.; Castillo, M. *Phytochemistry* **1983**, *22*, 1461.
5. *Baccharis tola* (Asteraceae) was collected in January 2012, in the III region of Chile (Copiapo, Chile). The plant was identified by Dr. Garcia O., Biology Department, University of Chile. A voucher (N° 3599) was deposited at the Herbarium of the University of Chile, Chile.
6. The aerial parts of *B. tola* (3 kg) were extracted with dichloromethane for 7 days (3x5L), affording 110 g of a dark syrup. This crude was chromatographed on a Silica gel column (1.0 kg) and eluted with mixtures of *n*-hexane and EtOAc. Fractions of 200 mL were collected and combined based upon TLC monitoring. After repeated CC using *n*-hexane and EtOAc as mobile phase was isolated *ent*-beyer-15-en-18-ol (**1**, 3.0 g) as the main diterpenoid.
7. The gastroprotective activity of the diterpenes **1**–**18** was assessed in the HCl/EtOH-induced lesion model as described previously (Ref. 8). Mice were randomly distributed into groups of seven animals each and fasted for 24 h with free access to water prior to the experiment. Fifty min after oral administration of the diterpenoids (12.5, 25, 50 and 100 mg/kg for **1** and 50 mg/kg for **2**–**18**), lansoprazole (20 mg/kg) or 12% Tween 80 (10 mL/kg), all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric lesion induction. The derivative **6** was assessed at 50 mg/kg in a second experiment to evaluate its possible mode of action using carbenoxolone (100 mg/kg) as a gastroprotective drug. Animals were sacrificed 1 h after the administration of HCl/EtOH, and the stomachs were excised and inflated by injection of saline (1 mL). The ulcerated stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. Gastric damage visible to the naked eye was observed in the gastric mucosa as elongated black-red lines, parallel to the long axis of the stomach similar to the HCl/EtOH-induced lesions in rats. The length (mm) of each lesion was measured, and the lesion index was expressed as the sum of the length of all lesions.
8. Areche, C.; Rodriguez, J.; Razmilic, I.; Yañez, T.; Theoduloz, C.; Schmeda-Hirschmann, G. *J. Pharm. Pharmacol.* **2007**, *59*, 289.
9. Animals were purchased from the Instituto de Salud Pública de Chile, Santiago. Swiss albino mice weighing 30 ± 3 g were fasted for 24 h prior to the experiment. The animals were fed on certified Champion diet with free access to water under standard conditions of 12 h dark-light period, 50% relative humidity and 22 °C room temperature. The protocols were approved by the Animal Use and Care Committee of the Universidad de Chile that follows the recommendations of the Canadian Council on Animal Care and with the ethical guidelines for investigations in conscious animal.
10. NMR data for compound **2–18**.
*ent-Beyer-15-en-18-ylcinnamate* (**2**): $^1$H NMR (400 MHz, $CDCl_3$): 5.71 (1H, d, *J* = 5.6 Hz, H-15), 5.48 (1H, d, *J* = 5.6 Hz, H-16), 4.02 (1H, d, *J* = 11.0 Hz, H-18), 3.83 (1H, d, *J* = 11.0 Hz, H-18), 1.01 (3H, s, H-17), 0.92 (3H, s, H-19), 0.82 (3H, s, H-20), 7.70 (1H, d, *J* = 15.9 Hz, H-7′), 7.57–7.55 (2H, m, H-2′, H-6′), 7.41–7.39 (3H, m, H-3′, H-4′, H-5′), 6.48 (1H, d, *J* = 15.9 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.8 (C-1), 17.8 (C-2), 36.0 (C-3), 37.2 (C-4), 50.1 (C-5), 20.1 (C-6), 37.0 (C-7), 48.9 (C-8), 52.8 (C-9), 36.7 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.1 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 73.2 (C-18), 17.7 (C-19), 15.5 (C-20), 134.4 (C-1′), 128.0 (C-2′, C-6′), 128.8 (C-3′ C-5′), 130.2 (C-4′), 144.5 (C-7′), 118.3 (C-8′), 167.0 (C-9′). HRMS: calculated for $C_{29}H_{38}O_2$: 418.2872, found: 418.2868.
*ent-Beyer-15-en-18-yl-2-nitrocinnamate* (**3**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.9 Hz, H-15), 5.46 (1H, d, *J* = 5.9 Hz, H-16), 4.01 (1H, d, *J* = 10.8 Hz, H-18), 3.83 (1H, d, *J* = 10.8 Hz, H-18), 0.99 (3H, s, H-17), 0.91 (3H, s, H-19), 0.80 (3H, s, H-20), 8.15 (1H, d, *J* = 15.9 Hz, H-7′), 8.05 (1H, dd, *J* = 8.3, 1.9 Hz, H-3′), 7.69–7.66 (2H, m, H-4′,H-5′), 7.41 (1H, dd, *J* = 7.09, 1.6 Hz, H-6′), 6.39 (1H, d, *J* = 15.9 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.7 (C-2), 36.0 (C-3), 37.1 (C-4), 50.1 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.7 (C-9), 36.6 (C-10), 20.1 (C-11), 33.0 (C-12), 43.6 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 73.2 (C-18), 17.7 (C-19), 15.4 (C-20), 132.8 (C-1′), 149.1 (C-2′), 124.8 (C-3′), 129.1 (C-4′), 135.8 (C-5′), 128.9 (C-6′), 141.2 (C-7′), 119.8 (C-8′), 166.4 (C-9′). HRMS: calculated for $C_{29}H_{37}NO_4$: 463.2723, found: 463.2711.
*ent-Beyer-15-en-18-yl-3-nitrocinnamate* (**4**): $^1$H NMR (400 MHz, $CDCl_3$): 5.68 (1H, d, *J* = 5.6 Hz, H-15), 5.45 (1H, d, *J* = 5.6 Hz, H-16), 4.01 (1H, d, *J* = 10.7 Hz, H-18), 3.81 (1H, d, *J* = 10.7 Hz, H-18), 0.98 (3H, s, H-17), 0.90 (3H, s, H-19), 0.79 (3H, s, H-20), 8.40 (1H, s, H-2′), 8.23 (1H, d, *J* = 8.3 Hz, H-4′), 7.84 (1H, d, *J* = 7.6 Hz, H-6′), 7.71 (1H, d, *J* = 15.9 Hz, H-7′), 7.59 (1H, dd, *J* = 7.6, 8.3 Hz, H-5′), 6.60 (1H, d, *J* = 15.9 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.5 (C-1), 17.7 (C-2), 35.9 (C-3), 37.1 (C-4), 49.9 (C-5), 20.1 (C-6), 36.9 (C-7), 48.8 (C-8), 52.7 (C-9), 36.6 (C-10), 20.1 (C-11), 33.0 (C-12), 43.5 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 73.5 (C-18), 17.7 (C-19), 15.4 (C-20), 136.1 (C-1′), 122.3 (C-2′), 149.6 (C-3′), 124.4 (C-4′), 129.9 (C-5′), 133.8 (C-6′), 141.6 (C-7′), 121.4 (C-8′), 166.2 (C-9′). HRMS: calculated for $C_{29}H_{37}NO_4$: 463.2723, found: 463.2717.
*ent-Beyer-15-en-18-yl-4-nitrocinnamate* (**5**): $^1$H NMR (400 MHz, $CDCl_3$): 5.70 (1H, d, *J* = 5.6 Hz, H-15), 5.47 (1H, d, *J* = 5.6 Hz, H-16), 4.03 (1H, d, *J* = 10.8 Hz, H-18), 3.84 (1H, d, *J* = 10.8 Hz, H-18), 1.00 (3H, s, H-17), 0.92 (3H, s, H-19), 0.81 (3H, s, H-20), 8.26 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 7.72 (1H, d, *J* = 16.1 Hz, H-7′), 7.71 (2H, d, *J* = 16.1 Hz, H-3′- H-5′), 6.60 (1H, d, *J* = 16.1 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.7 (C-2), 36.1 (C-3), 37.1 (C-4), 50.1 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.8 (C-9), 36.6 (C-10), 20.1 (C-11), 33.0 (C-12), 43.4 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 73.4 (C-18), 17.7 (C-19), 15.5 (C-20), 141.5 (C-1′), 128.9 (C-2′, C-6′), 126.4 (C-3′, C-5′), 149.9 (C-4′), 143.1 (C-7′), 120.3 (C-8′), 166.5 (C-9′). HRMS: calculated for $C_{29}H_{37}NO_4$: 463.2723, found: 463.2712.
*ent-Beyer-15-en-18-yl-3-chlorocinnamate* (**6**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.6 Hz, H-15), 5.46 (1H, d, *J* = 5.6 Hz, H-16), 4.00 (1H, d, *J* = 10.8 Hz, H-18), 3.81 (1H, d, *J* = 10.8 Hz, H-18), 1.00 (3H, s, H-17), 0.90 (3H, s, H-19), 0.80 (3H, s, H-20), 7.61 (1H, d, *J* = 16.1 Hz, H-7′), 7.54 (1H, br s, H-2′), 7.41 (1H, br d, *J* = 7.1 Hz, H-6′), 7.36 (1H, dd, *J* = 8.1, 1.7, H-4′), 7.32 (1H, dd, *J* = 8.1, 7.1 Hz, H-5′), 6.47 (1H, d, *J* = 16.1 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 50.0 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.7 (C-9), 36.7 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.0 (C-14), 135.1 (C-

15), 136.4 (C-16), 24.9 (C-17), 73.3 (C-18), 17.7 (C-19), 15.5 (C-20), 136.2 (C-1′), 127.7 (C-2′), 134.8 (C-3′), 130.1 (C-4′), 130.1 (C-5′), 126.3 (C-6′), 142.9 (C-7′), 119.7 (C-8′), 166.6 (C-9′). HRMS: calculated for $C_{29}H_{37}ClO_2$: 452.2482, found: 452.2468.

*ent-Beyer-15-en-18-yl-4-chlorocinnamate* (**7**): $^1$H NMR (400 MHz, $CDCl_3$): 5.68 (1H, d, *J* = 4.8 Hz, H-15), 5.45 (1H, d, *J* = 4.8 Hz, H-16), 3.99 (1H, d, *J* = 10.8 Hz, H-18), 3.81 (1H, d, *J* = 10.8 Hz, H-18), 0.99 (3H, s, H-17), 0.90 (3H, s, H-19), 0.80 (3H, s, H-20), 7.63 (1H, d, *J* = 16.1 Hz, H-7′), 7.47 (2H, d, *J* = 8.1 Hz, H-2′, H-6′), 7.35 (2H, d, *J* = 8.1 Hz, H-3′, H-5′), 6.43 (1H, d, *J* = 16.1 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 50.1 (C-5), 20.1 (C-6), 36.9 (C-7), 48.8 (C-8), 52.7 (C-9), 36.6 (C-10), 20.1 (C-11), 33.1 (C-12), 43.5 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 73.3 (C-18), 17.7 (C-19), 15.5 (C-20), 132.9 (C-1′), 129.1 (C-2′, C-6′), 129.2 (C-3′ C-5′), 136.0 (C-4′), 143.0 (C-7′), 118.9 (C-8′), 166.7 (C-9′). HRMS: calculated for $C_{29}H_{37}ClO_2$: 452.2482, found: 452.2479.

*ent-Beyer-15-en-18-yl-2-methoxycinnamate* (**8**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.6 Hz, H-15), 5.46 (1H, d, *J* = 5.6 Hz, H-16), 4.00 (1H, d, *J* = 10.8 Hz, H-18), 3.81 (1H, d, *J* = 10.8 Hz, H-18), 1.00 (3H, s, H-17), 0.90 (3H, s, H-19), 0.80 (3H, s, H-20), 7.99 (1H, d, *J* = 16.1 Hz, H-7′), 7.49 (1H, dd, *J* = 7.8, 1.6 Hz, H-3′), 7.40 (1H, dd, *J* = 8.01, 1.9 Hz, H-6′), 6.97-6.89 (2H, m, H-4′, H-5′), 6.41 (1H, d, *J* = 16.1 Hz, H-8′), 3.89 (3H, s, $OCH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.5 (C-1), 17.7 (C-2), 36.0 (C-3), 37.1 (C-4), 49.9 (C-5), 20.1 (C-6), 36.9 (C-7), 48.8 (C-8), 52.9 (C-9), 36.5 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 72.9 (C-18), 17.7 (C-19), 15.4 (C-20), 129.5 (C-1′), 159.8 (C-2′), 116.6 (C-3′), 132.1 (C-4′), 122.8 (C-5′), 128.6 (C-6′), 143.2 (C-7′), 117.9 (C-8′), 166.3 (C-9′), 55.1 ($OCH_3$). HRMS: calculated for $C_{30}H_{40}O_3$: 448.2977, found: 448.2975.

*ent-Beyer-15-en-18-yl-3-methoxycinnamate* (**9**): $^1$H NMR (400 MHz, $CDCl_3$): 5.68 (1H, d, *J* = 5.6 Hz, H-15), 5.45 (1H, d, *J* = 5.6 Hz, H-16), 3.99 (1H, d, *J* = 10.8 Hz, H-18), 3.80 (1H, d, *J* = 10.8 Hz, H-18), 0.99 (3H, s, H-17), 0.90 (3H, s, H-19), 0.79 (3H, s, H-20), 7.65 (1H, d, *J* = 16.1 Hz, H-7′), 7.29 (1H, t, *J* = 7.8 Hz, H-5′), 7.13 (1H, d, *J* = 7.8 Hz, H-6′), 7.05 (1H, br s, H-2′), 6.92 (1H, dd, *J* = 8.1, 1.9 Hz, H-4′), 6.44 (1H, d, *J* = 16.1 Hz, H-8′), 3.82 (3H, s, $OCH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 50.0 (C-5), 20.1 (C-6), 36.9 (C-7), 48.8 (C-8), 52.7 (C-9), 36.6 (C-10), 20.1 (C-11), 33.1 (C-12), 43.5 (C-13), 61.0 (C-14), 135.0 (C-15), 136.3 (C-16), 24.9 (C-17), 73.1 (C-18), 17.7 (C-19), 15.5 (C-20), 135.7 (C-1′), 112.9 (C-2′), 159.8 (C-3′), 115.9 (C-4′), 129.8 (C-5′), 120.7 (C-6′), 144.4 (C-7′), 118.5 (C-8′), 166.9 (C-9′), 55.2 ($OCH_3$). HRMS: calculated for $C_{30}H_{40}O_3$: 448.2977, found: 448.2973.

*ent-Beyer-15-en-18-yl-4-methoxycinnamate* (**10**): $^1$H NMR (400 MHz, $CDCl_3$): 5.70 (1H, d, *J* = 5.6 Hz, H-15), 5.46 (1H, d, *J* = 5.6 Hz, H-16), 3.99 (1H, d, *J* = 10.8 Hz, H-18), 3.81 (1H, d, *J* = 10.8 Hz, H-18), 1.0 (3H, s, H-17), 0.90 (3H, s, H-19), 0.80 (3H, s, H-20), 7.65 (1H, d, *J* = 16.1 Hz, H-7′), 7.49 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.34 (1H, d, *J* = 16.1 Hz, H-8′), 3.83 (3H, s, $OCH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 50.1 (C-5), 20.1 (C-6), 36.9 (C-7), 48.8 (C-8), 52.7 (C-9), 36.6 (C-10), 20.1 (C-11), 33.1 (C-12), 43.5 (C-13), 61.0 (C-14), 135.1 (C-15), 136.3 (C-16), 24.9 (C-17), 73.0 (C-18), 17.7 (C-19), 15.4 (C-20), 127.1 (C-1′), 129.6 (C-2′, C-6′), 114.2 (C-3′ C-5′), 161.3 (C-4′), 144.1 (C-7′), 115.7 (C-8′), 167.3 (C-9′), 55.2 ($OCH_3$). HRMS: calculated for $C_{30}H_{40}O_3$: 448.2976, found: 448.2973.

*ent-Beyer-15-en-18-yl-4-methylcinnamate* (**11**): $^1$H NMR (400 MHz, $CDCl_3$): 5.71 (1H, d, *J* = 5.6 Hz, H-15), 5.48 (1H, d, *J* = 5.6 Hz, H-16), 4.00 (1H, d, *J* = 10.8 Hz, H-18), 3.82 (1H, d, *J* = 10.8 Hz, H-18), 1.01 (3H, s, H-17), 0.91 (3H, s, H-19), 0.81 (3H, s, H-20), 7.67 (1H, d, *J* = 16.1 Hz, H-7′), 7.46 (2H, d, *J* = 8.1 Hz, H-2′, H-6′), 7.21 (2H, d, *J* = 8.1 Hz, H-3′, H-5′), 6.43 (1H, d, *J* = 16.1 Hz, H-8′), 2.39 (1H, s, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 50.1 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.8 (C-9), 36.7 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.1 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 73.1 (C-18), 17.7 (C-19), 15.5 (C-20), 131.7 (C-1′), 128.1 (C-2′, C-6′), 129.6 (C-3′ C-5′), 140.6 (C-4′), 144.5 (C-7′), 117.2 (C-8′), 167.3 (C-9′), 21.4 ($CH_3$). HRMS: calculated for $C_{30}H_{40}O_2$: 432.3028, found: 432.3025.

*ent-Beyer-15-en-18-yl-4-fluorocinnamate* (**12**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.6 Hz, H-15), 5.46 (1H, d, *J* = 5.6 Hz, H-16), 3.99 (1H, d, *J* = 11.0 Hz, H-18), 3.81 (1H, d, *J* = 11.0 Hz, H-18), 0.99 (3H, s, H-17), 0.90 (3H, s, H-19), 0.80 (3H, s, H-20), 7.65 (1H, d, *J* = 15.9 Hz, H-7′), 7.54 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 7.08 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.39 (1H, d, *J* = 15.9 Hz, H-8′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 50.1 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.8 (C-9), 36.6 (C-10), 20.1 (C-11), 33.1 (C-12), 43.5 (C-13), 61.1 (C-14), 135.0 (C-15), 136.4 (C-16), 24.8 (C-17), 73.2 (C-18), 17.7 (C-19), 15.5 (C-20), 130.7, 130.6 (C-1′), 129.9, 129.8 (C-2′, C-6′), 116.0, 115.8 (C-3′ C-5′), 165.0, 162.5 (C-4′), 143.1 (C-7′), 118.1 (C-8′), 166.9 (C-9′). HRMS: calculated for $C_{29}H_{37}FO_2$: 436.2768, found: 436.2758.

*ent-Beyer-15-en-18-ylcaproate* (**13**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.6 Hz, H-15), 5.46 (1H, d, *J* = 5.6 Hz, H-16), 3.90 (1H, d, *J* = 10.7 Hz, H-18), 3.62 (1H, d, *J* = 10.7 Hz, H-18), 1.00 (3H, s, H-17), 0.84 (3H, s, H-19), 0.79 (3H, s, H-20), 2.32 (2H, t, *J* = 7.4, H-2′), 1.24-1.33 (14H, m, H-3′-H-9′), 0.88 (3H, t, *J* = 6.7, H-10′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.7 (C-1), 17.7 (C-2), 36.0 (C-3), 37.1 (C-4), 49.8 (C-5), 20.1 (C-6), 37.0 (C-7), 48.8 (C-8), 52.7 (C-9), 36.6 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 72.7 (C-18), 17.7 (C-19), 15.5 (C-20), 173.9 (C-1′), 34.5 (C-2′), 25.1 (C-3′), 29.2-29.6 (C-4′-C-7′), 31.9 (C-8′), 22.6 (C-9′), 14.1 (C-10′). HRMS: calculated for $C_{30}H_{50}O_2$: 442.3811, found: 442.3809.

*ent-Beyer-15-en-18-yllaurate* (**14**): $^1$H NMR (400 MHz, $CDCl_3$): 5.70 (1H, d, *J* = 5.7 Hz, H-15), 5.46 (1H, d, *J* = 5.7 Hz, H-16), 3.89 (1H, d, *J* = 10.8 Hz, H-18), 3.62 (1H, d, *J* = 10.8 Hz, H-18), 1.00 (3H, s, H-17), 0.85 (3H, s, H-19), 0.79 (3H, s, H-20), 2.32 (2H, t, *J* = 7.5, H-2′), 1.23–1.34 (18H, m, H-3′-H-11′), 0.88 (3H, t, *J* = 6.8, H-12′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 35.9 (C-3), 37.1 (C-4), 49.7 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.8 (C-9), 36.5 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.1 (C-14), 135.0 (C-15), 136.5 (C-16), 24.9 (C-17), 72.8 (C-18), 17.7 (C-19), 15.5 (C-20), 173.9 (C-1′), 34.6 (C-2′), 25.2 (C-3′), 29.2-29.6 (C-4′-C-9′), 31.9 (C-10′), 22.7 (C-11′), 14.1 (C-12′). HRMS: calculated for $C_{32}H_{54}O_2$: 470.4124, found: 470.4121.

*ent-Beyer-15-en-18-ylmyristate* (**15**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.6 Hz, H-15), 5.46 (1H, d, *J* = 5.6 Hz, H-16), 3.89 (1H, d, *J* = 10.8 Hz, H-18), 3.62 (1H, d, *J* = 10.8 Hz, H-18), 1.00 (3H, s, H-17), 0.84 (3H, s, H-19), 0.78 (3H, s, H-20), 2.32 (2H, t, *J* = 7.4, H-2′), 1.26-1.36 (22H, m, H-3′-H-13′), 0.88 (3H, t, *J* = 6.7, H-12′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 49.7 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.8 (C-9), 36.5 (C-10), 20.0 (C-11), 33.1 (C-12), 43.6 (C-13), 61.1 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 72.7 (C-18), 17.7 (C-19), 15.5 (C-20), 173.9 (C-1′), 34.6 (C-2′), 25.1 (C-3′), 29.2-29.6 (C-4′-C-11′), 31.9 (C-12′), 22.6 (C-13′), 14.1 (C-14′). HRMS: calculated for $C_{34}H_{58}O_2$: 498.4437, found: 498.4435.

*ent-Beyer-15-en-18-ylpalmitate* (**16**): $^1$H NMR (400 MHz, $CDCl_3$): 5.69 (1H, d, *J* = 5.6 Hz, H-15), 5.47 (1H, d, *J* = 5.6 Hz, H-16), 3.90 (1H, d, *J* = 10.7 Hz, H-18), 3.63 (1H, d, *J* = 10.7 Hz, H-18), 1.00 (3H, s, H-17), 0.85 (3H, s, H-19), 0.79 (3H, s, H-20), 2.33 (2H, t, *J* = 7.5, H-2′), 1.26–1.36 (26H, m, H-3′-H-15′), 0.89 (3H, t, *J* = 6.8, H-16′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.8 (C-2), 36.0 (C-3), 37.1 (C-4), 49.7 (C-5), 20.1 (C-6), 36.9 (C-7), 48.9 (C-8), 52.8 (C-9), 36.5 (C-10), 20.0 (C-11), 33.1 (C-12), 43.6 (C-13), 61.2 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 72.7 (C-18), 17.7 (C-19), 15.5 (C-20), 174.0 (C-1′), 34.6 (C-2′), 25.2 (C-3′), 29.2-29.7 (C-4′-C-13′), 31.9 (C-14′), 22.7 (C-15′), 14.1 (C-16′). HRMS: calculated for $C_{36}H_{62}O_2$: 526.4750, found: 526.4746.

*ent-Beyer-15-en-18-ylestereate* (**17**): $^1$H NMR (400 MHz, $CDCl_3$): 5.68 (1H, d, *J* = 5.6 Hz, H-15), 5.45 (1H, d, *J* = 5.6 Hz, H-16), 3.88 (1H, d, *J* = 10.8 Hz, H-18), 3.61 (1H, d, *J* = 10.8 Hz, H-18), 1.00 (3H, s, H-17), 0.84 (3H, s, H-19), 0.79 (3H, s, H-20), 2.32 (2H, t, *J* = 7.5, H-2′), 1.26–1.36 (30H, m, H-3′-H-17′), 0.88 (3H, t, *J* = 6.8, H-18′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.7 (C-2), 35.9 (C-3), 37.0 (C-4), 49.8 (C-5), 20.1 (C-6), 37.1 (C-7), 48.9 (C-8), 52.8 (C-9), 36.5 (C-10), 20.1 (C-11), 33.1 (C-12), 43.6 (C-13), 61.0 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 72.7 (C-18), 17.7 (C-19), 15.5 (C-20), 173.9 (C-1′), 34.5 (C-2′), 25.2 (C-3′), 29.1-29.7 (C-4′-C-15′), 31.9 (C-16′), 22.6 (C-17′), 14.1 (C-18′). HRMS: calculated for $C_{38}H_{66}O_2$: 554.5063, found: 554.5061.

*ent-Beyer-15-en-18-ylarachidate* (**18**): $^1$H NMR (400 MHz, $CDCl_3$): 5.68 (1H, d, *J* = 5.6 Hz, H-15), 5.45 (1H, d, *J* = 5.6 Hz, H-16), 3.89 (1H, d, *J* = 10.7 Hz, H-18), 3.62 (1H, d, *J* = 10.7 Hz, H-18), 1.00 (3H, s, H-17), 0.85 (3H, s, H-19), 0.79 (3H, s, H-20), 2.33 (2H, t, *J* = 7.5, H-2′), 1.24–1.36 (34H, m, H-3′-H-19′), 0.89 (3H, t, *J* = 6.8, H-20′). $^{13}$C NMR (100 MHz, $CDCl_3$): 38.6 (C-1), 17.7 (C-2), 36.0 (C-3), 37.1 (C-4), 49.7 (C-5), 20.1 (C-6), 36.9 (C-7), 48.8 (C-8), 52.8 (C-9), 36.6 (C-10), 20.0 (C-11), 33.1 (C-12), 43.5 (C-13), 61.1 (C-14), 135.1 (C-15), 136.4 (C-16), 24.9 (C-17), 72.7 (C-18), 17.7 (C-19), 15.5 (C-20), 173.9 (C-1′), 34.5 (C-2′), 25.2 (C-3′), 29.1-29.7 (C-4′-C-17′), 31.9 (C-18′), 22.7 (C-19′), 14.1 (C-20′). HRMS: calculated for $C_{40}H_{70}O_2$: 582.5376, found: 582.5374.


11. Szabo, S. *Gastroenterology* **1984**, *87*, 228.
12. Szabo, S.; Nagy, L.; Plebani, M. *Clin. Chim. Acta* **1992**, *206*, 95.
13. To investigate the involvement of sulfhydryl compounds (SHs) in the gastroprotective effect of **6**, NEM s.c. (10 mg/kg, an SH blocker was dissolved in saline) was injected 30 min before administration of **6** or vehicle (NEM-treated) (Refs. 13,14). Fifty min after oral administration of **6** (50 mg/kg) or vehicle, all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric lesion induction. Animals were sacrificed 1 h after the administration of HCl/EtOH, and the stomachs were excised and inflated by injection of saline (1 mL). The gastric mucosal lesions were induced and the length of gastric lesions was measured as described above.
14. Matsuda, H.; Pongpiriyadacha, Y.; Morikawa, T.; Kashima, Y.; Nakano, K.; Yoshikawa, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 477.
15. Areche, C.; Theoduloz, C.; Yañez, T.; Souza-Brito, A. R. M.; Barbastefano, V.; Ferreira, A.; Rodriguez, J. A. *J. Pharm. Pharmacol.* **2008**, *60*, 1.
16. Robert, A. *Prostaglandins* **1981**, *21*, 89.
17. Wallace, J. L.; Miller, M. J. S. *Gastroenterology* **2000**, *199*, 512.
18. To investigate the involvement of endogenous prostaglandins in the gastroprotective effect of **6**, Indomethacin *s.c.* (30 mg/kg, an inhibitor of the prostaglandin synthesis was dissolved in 5% $NaHCO_3$) was injected 30 min before administration of **6** or vehicle (IND-treated) (Refs. 13,14). Fifty min after oral administration of **6** (50 mg/kg) or vehicle, all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric lesion induction. Animals were sacrificed 1 h after the administration of HCl/EtOH, and the stomachs were excised and inflated by injection of saline (1 mL). The gastric mucosal lesions were induced and the length of gastric lesions was measured as described above.
19. Wallace, J. L.; Ma, L. *Exp. Biol. Med.* **2001**, *226*, 1003.
20. To investigate the involvement of endogenous nitric oxide (NO) in the gastroprotective effect of **6**, L-NAME ip (70 mg/kg, an inhibitor of NO synthase was dissolved in saline) was injected 30 min before administration of **6** or vehicle (L-NAME-treated) (Refs. 13,14). Fifty min after oral administration of **6** (50 mg/kg) or vehicle, all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric lesion induction. Animals were sacrificed 1 h after the administration of HCl/EtOH, and the stomachs were excised and inflated by injection of saline (1 mL). The

gastric mucosal lesions were induced and the length of gastric lesions was measured as described above.
21. Abdel-Salam, O. M. E.; Czimmer, J.; Debreceni, A.; Szolcsányi, J.; Mózsik, G. *J. Physiol.-Paris* **2001**, *95*, 105.
22. Szolcsányi, J.; Bartho, L. *J. Physiol.-Paris* **2001**, *95*, 181.
23. To investigate the involvement of vanilloid receptor in the gastroprotective effect of **6**, RR *s.c.* (3.5 mg/kg, a vanilloid receptor antagonist was dissolved in saline) was injected 30 min before administration of **6** or vehicle (RR-treated) (Refs. 13,14). Fifty min after oral administration of **6** (50 mg/kg) or vehicle, all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/EtOH) for gastric lesion induction. Animals were sacrificed 1 h after the administration of HCl/EtOH, and the stomachs were excised and inflated by injection of saline (1 mL). The gastric mucosal lesions were induced and the length of gastric lesions was measured as described above.
24. The cytotoxic assay expressed as cell viability was conducted using MTT assay method.[25] Cells at a density of $3 \times 10^4$ of MRC fibroblasts or AGS cells were placed in 96-well culture dishes. Compounds **1**–**18** were assayed at concentrations ranging from 0 up to 500 μM. The cells were incubated at 37 °C in a humidified $CO_2$ incubator for 24 h. After incubation, several concentrations of the compounds dissolved in DMSO were added. Each compound was tested in quadruplicate and repeated three times. After 48 h incubation, the assay was stopped by adding MTT reagent [3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and the incubation continued for the next 4 h before the addition of MTT stop solution containing sodium dodecyl sulfate (SDS). The incubation continued for other 24 h., and the optical density was measured using a microplate reader at 550 nm. $IC_{50}$ values were obtained from the plotted graph between percentage live cells compared to control.
25. Areche, C.; Rodriguez, J.; Razmilic, I.; Yanez, T.; Schmeda, G. *J. Pharm. Pharmacol.* **2007**, *59*, 289.
26. Lewis, D. A.; Hanson, D. In *Progress in Medicinal Chemistry*; Ellis, G. P., West, G. B., Eds., 3rd ed.; Elsevier Science: Amsterdam, 1991; pp 201–231.
27. Tundis, R.; Loizzo, M. R.; Bonesi, M.; Menichini, F.; Conforti, F.; Statti, G.; Menichini, F. *Nat. Prod. Commun.* **2008**, *3*, 2129.
28. Awaad, A. S.; El-meligy, R. M.; Soliman, G. A. J. *Saudi Chem. Soc.* **2013**, *17*, 101.