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Gastroprotective activity of synthetic coumarins: Role of endogenous prostaglandins, nitric oxide, non-protein sulfhydryls and vanilloid receptors

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ABSTRACT

Natural or synthetic coumarins showed gastroprotective and antiulcer activity in animal models. In this study, we have synthetized twenty coumarins using classic methods to evaluate their gastroprotective effects on the ethanol/HCl-induced gastric lesion model in mice at 20 mg/kg. Among the coumarins synthetized, compounds **6** and **10** showed the greatest gastroprotective activity being as active as lansoprazole at 20 mg/kg and reducing gastric lesions by 75 and 76%, respectively. Then, in a second experiment, compounds **6** and **10** were re-evaluated in order to understand the possible mode of gastroprotective activity. Regarding coumarin **6**, the protective effect was reduced by pre-treatment of the mice with *N*-ethylmaleimide and l-NAME suggesting that sulfhydryl compounds and endogenous nitric oxide are involved in its gastroprotective activity. While for coumarin **10** the effect was reduced by pre-treatment with indomethacin suggesting that prostaglandins are positively involved in its gastroprotective activity.

Peptic ulcer develops when an imbalance occurs between the defensive and aggressive factors.[1] Defensive factors include mucus, bicarbonate, prostaglandins, mucosal blood flow, nitric oxide, sulfhydryls and growth factors, while aggressive factors include hydrochloride acid, pepsins, bile acids, hypoxia, smoking and alcohol. Up to date, around one in five persons suffer from ulcers associated to stress, diet and certain types of drugs. Medicines used in the treatment of gastric ulcers are mainly antiacids, H_2-receptor antagonists and proton pump inhibitors and when the gastric ulcer is produced by *Helicobacter pylori*, antibiotics are included as well. However, side effects produced by occidental medicine shows the need for looking new antiulcer agents.[1–4]

Coumarins are a small group of molecules whose structures contain the 2*H*-chromen-2-one or 1-benzopyran-2-one cores. Many coumarins have numerous pharmacological applications as anticoagulants, anti-inflammatory, antipyretics, antibacterials, antihelminthics, and also as photoprotectors.[5] The first coumarin was isolated in 1820, and synthesized for the first time in 1868.[6] Several synthetic routes have been published, being the most representative those developed by Perkin, Pechmann, Knoevenagel, Reformatsky, Wittig and Heck.[7]

In the course of our studies on gastroprotective drugs, we report here the synthesis of twenty coumarins (**1**–**20**) and their gastroprotective effect in mice. In addition, we discuss the mode of gastroprotective action of **6** and **16**, including the involvement of prostaglandins (PGs), nitric oxide (NO), sulfhydryl compounds (SHs) and vanilloid receptors (VR).

Some twenty coumarins (Schemes 1–3) were synthetized to disclosure structure activity relationship (SAR) based on the gastroprotective effect on the model of HCl/EtOH-induced gastric lesions in mice (Table 1).[8,9] All coumarins have been reported previously and were prepared through known synthetic routes. The compounds **1**–**11** were synthetized through Pechmann condensation with modifications[7b,10] (Schemes 1 and 2), while the compounds **12**–**20** were synthetized through Knoevenagel condensation (Scheme 3).[7d,11] The synthesis and characterization of the coumarins can be found in Supporting information.

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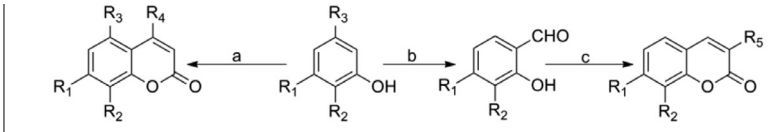
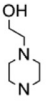
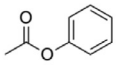
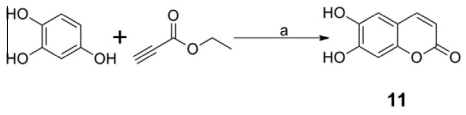


	R_1	R_2	R_3	R_4	R_5
1	OH	H	H	H	H
2	OH	H	H	Cl	H
3	OH	H	H	OH	H
4	OH	H	H	$NHCH_3$	H
5	OH	H	H	COOH	H
6	OH	H	H		H
7	OH	H	H		H
8	OH	H	OH	H	H
9	OH	H	OH	Cl	H
10	OH	OH	H	Cl	H

	R_1	R_2	R_3	R_4	R_5
13	H	H	H	H	$COOCH_2CH_3$
14	H	OH	H	H	$COOCH_2CH_3$
15	H	OCH_3	H	H	$COOCH_2CH_3$
16	H	H	H	H	$COCH_3$
17	H	OH	H	H	$COCH_3$
18	OH	H	H	H	COOH
19	OH	H	H	H	
20	OH	H	H	H	$COCH_3$

Scheme 1. Reagents and conditions: (a) H_2SO_4, respective ethylacetoacetate, rt, 6 h; (b) $POCl_3$, ACN, or the corresponding benzaldehyde; (c) respective ethylacetoacetate or malonate, piperidine, EtOH, reflux, 4 h.



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Scheme 2. Reagents and conditions: (a) $ZnCl_2$, MW, 10 min, 400 W.

Table 1 shows the effect of the synthetic coumarins **1**–**20** at 20 mg/kg. The greatest gastroprotective activity was displayed by compounds **6**, and **10**, which resulted as active as lansoprazole at 20 mg/kg and reduced gastric lesions by 75% and 76%, respectively. The gastroprotective activity of the coumarins **3**, **11**, **14** and **15** did not differ statistically from the control. As for the rest of coumarins, the gastroprotective activity were found over the range 15–68%.

In the case of the 7-hydroxycoumarins **1**–**7**, series where exists substitution at C-4, a significant increase in the gastroprotective activity was observed for compound **6** bearing a morpholine moiety (77%). The effect of a piperazine group in **7** (65%) was similar to that of the compound **5** (68%) but less active than lansoprazole (74%). The presence of an OH group (compound **3**) at C-4, produced a significant decrease in the gastroprotective effect (16%).

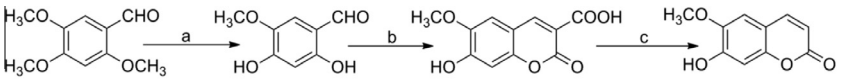
Regarding dihydroxycoumarins **8**–**12**, the highest gastroprotective activity was observed for compounds **10** (76%) and **12** (61%). Indeed, catechol moiety increases the gastroprotective effects for both compounds. There were no differences (compounds **8** and **9**) if a chlorine or methyl group is placed at C-4.

In the case of the coumarins **13**–**17**, bearing a carbonyl group at C-3, the highest gastroprotective activity was observed for compound **16** (66%). The ester moiety decreases the gastroprotective effects in compounds **13**–**15**, while the effect of a carboxyl group was lower than compound **16** (41%).

Finally, for the 7-hydroxycoumarins **18**–**20** with substitutions at C-3 the activity collapsed when an acetyl group is present. Regarding the carboxyl (60%) and ester (63%) groups, the gastroprotective activity was lower than lansoprazole (74%).

The best gastroprotective compounds were **6** and **10**, so we selected these compounds for further experiments. The possible mode of gastroprotective action by **6** and **10** on the gastric lesions induced by HCl/EtOH in mice pretreated with Indometacin[9,12] (10 mg/kg, s.c.), *N*-ethylmaleimide[9,12] (NEM, 10 mg/kg, s.c.), N^G-nitro-L-arginine methyl ester[9,12] (L-NAME, 70 mg/kg, i.p.) or ruthenium red[9,12] (RR, 3.5 mg/kg, s.c.) at an oral dose of 20 mg/Kg is shown in Table 2.

Endogenous PGs are involved in the mechanism of gastroprotection induced by mild irritants, and necrotizing agents. In this sense, PGs inhibit the gastric acid secretion, stimulate release of



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Scheme 3. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2, rt, 24 h; (b) malonic acid, phenylamine, pyridine, rt, 24 h; (c) pyridine/ethylene glycol (1:1.1); (d) K_2CO_3, acetone, reflux, 2 h.

Table 1
Gastroprotective effect of synthetic coumarins 1–20 at 20 mg/kg on HCl/EtOH-induced gastric lesions in mice

Compound	*n*	Lesion index (mm)	% lesion reduction
1	7	28.4 ± 1.3*	31**
2	7	17.6 ± 1.1*	57**
3	7	34.6 ± 1.3	16**
4	7	21.1 ± 1.4*	49**
5	7	13.4 ± 1.1*	68**
6	7	10.3 ± 1.2*	75
7	7	14.6 ± 1.1*	65**
8	7	22.9 ± 1.1*	45**
9	7	24.1 ± 1.6*	42**
10	7	10.0 ± 1.0*	76
11	7	34.3 ± 1.5	17**
12	7	16.1 ± 1.1*	61**
13	7	26.9 ± 1.2*	35**
14	7	35.3 ± 1.9	15**
15	7	43.4 ± 1.2	0**
16	7	14.0 ± 0.8*	66**
17	7	24.6 ± 1.2*	41**
18	7	16.6 ± 1.1*	60**
19	7	15.3 ± 1.0*	63**
20	7	35.3 ± 1.5	15**
Lansoprazole	7	10.8 ± 1.3*	74
Control	7	41.4 ± 2.8	—

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.

Table 2
Effect of the coumarins (**6** and **10**) on the appearance of gastric lesions induced by HCl/EtOH (po) in indomethacin-, NEM-, L-NAME- and RR-pretreated mice

Treatment	Dose (mg/kg)	Lesion index (mm)
Control	—	38.8 ± 1.6
IND	30	41.1 ± 1.5
NEM	10	40.1 ± 2.1
L-NAME	70	35.7 ± 1.1
RR	3.5	39.8 ± 1.8
6	20	10.3 ± 1.2*
IND + **6**	30 + 20	13.6 ± 1.0*
NEM + **6**	10 + 20	28.8 ± 1.1
L-NAME + **6**	70 + 20	34.7 ± 1.5
RR + **6**	3.5 + 20	14.4 ± 1.8*
10	20	10.0 ± 1.0*
IND + **10**	30 + 20	42.0 ± 1.2
NEM + **10**	10 + 20	12.4 ± 1.0*
L-NAME + **10**	70 + 20	11.0 ± 0.8*
RR + **10**	3.5 + 20	12.6 ± 1.2*
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.

mucus and bicarbonate and increase blood flow on gastric mucosal.[13] In the present study, PGs are not involved in the gastroprotective action of **6**, because the activity of this compound was not reduced by pretreatment with IND (an inhibitor of the PG synthesis). While for compound **10**, PGs seem to be involved in the gastroprotective effect of **10**, because IND reduced the activity of this compound.

Endogenous sulfhydryls such as glutathione play an important role in the protection of the gastric mucosa. 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.[14] In this study, a pretreatment with NEM (an SH blocker) reduced the gastroprotective activity of **6**, suggesting that the protective effect of this coumarin is related to the participation of endogenous SHs. Furthermore, the mode of gastroprotective action of compound **10** is not involving endogenous SHs because pretreatment with NEM did not reduced the gastroprotective effect of **10**.

NO in the gastrointestinal tract play a role in the health, defense and repair of the gastric mucosa.[3,15] 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 (an inhibitor of NO synthase) attenuated the gastroprotective activity of **6**. This finding suggests that endogenous NO have participation in the protective effect of this coumarin. The gastroprotective effect of **10** with L-NAME was similar to **10** without L-NAME. This fact suggests that endogenous NO have null participation in the protective effect of **10**.

Capsaicin-sensitive sensory neurons via VR on the gastrointestinal tract participate in gastric defense mechanisms by regulating the gastric motility, acid secretion and gastric blood flow. This action is promoted by calcitonin gene-related peptide (CGRP) and stimulation of gastric mucus and bicarbonate.[4,16] In this study, pretreatment with ruthenium red (a vanilloid receptor antagonist), did not reduce the lesion index suggesting that the mechanism of gastroprotection of **6** and **10** have no relationship with capsaicin-sensitive sensory neurons via VR.

In the last decades, many coumarins have been isolated from natural sources and synthetized from simple precursors.[17] Coumarins showed numerous biological activities such as anti-inflammatory, antiplatelet, anticancer, antibacterial, anti-obesity, antiviral, antifungal, antioxidants, analgesic, anticonvulsivant, antihyperlipidemic, gastroprotective, antiulcerogenic, antiParkinson and neuroprotective activities.[17–19]

Several coumarins were reported to be protective for the induced lesions of gastric mucosa in different animal models. Among them, esculin[20] at doses of 25 and 50 mg/kg protected the gastric mucosa against ethanol and indomethacin, while its gastroprotective mechanism include stimulation of prostaglandins, nitric oxide synthesis, opening of K_{ATP} channels, reduction of free radicals, and modulation of antioxidant enzyme systems. In 2015, Choi et al.[21] reported the gastroprotective activity of scoparone derivatives and showed that 5,6,7-trimethoxycoumarin and 6,7,8-trimethoxycoumarin had a greater protection than rebamipide (a standart drug), and suggested that the presence of methyl group at position C-5 or C-8 of scoparone improves the gastroprotective effects. Carvallo et al.[22] reported an antiulcerogenic study of a coumarin (2*H*-1-benzopyran-2-one) isolate from *Mikania laevigata* and suggested that this coumarin had anti-secretory activity mediated by the parasympathetic system. Furthermore, Reyes-Chilpa et al.[23] isolated and tested two coumarins known as mammea A/BA and mammea C/OC and suggested that their gastroprotective properties are in part related to the inhibition of H^+, K^+-ATPase gastric enzyme (proton pumps).

The carbonate dehydratases or carbonic anhydrases (CA) are a family of very important zinc-containing metalloenzymes useful in the maintenance of several physiological processes including homeostasis, carbon dioxide and bicarbonate transportation, and electrolytic balance.[24] The enzymes interconvert carbon dioxide and bicarbonate to maintain base–acid balance in blood and other liquids and tissues. There are at least five distinct CA families (α, β, γ, δ and ε). The cytosolic isozymes CA II and CA VI are important agents in the production of bicarbonate in the saliva and neutralization of stomach acid.[24] Indeed, several CA inhibitors have been reported[25–27] for the treatment of several ailments including epilepsy, glaucoma, Alzheimer's disease, obesity, microbial infection, cáncer and osteoporosis.[24,28] Therefore, CA inhibitors might be used for generating new candidates for treatment of gastric and duodenal ulcers.

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Even though the gastroprotective properties are well established, the mechanism of action are not fully understood, and seems to be related to stimulation of the defensive factors rather than inhibition of the aggressive factors.[1] In summary, we reported here the gastroprotective activity of twenty synthetic coumarins. Compounds **6** and **10** exerted the best gastroprotective activity. The mode of gastroprotective action for compound **6** was explained through the participation of endogenous SHs and endogenous NO. Finally, compound **10** exerted its mode of action through the participation of prostaglandins.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.10.056.

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