Bioorganic & Medicinal Chemistry Letters 26 (2016) 5732–5735

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Beatriz Sepulveda [a], Cristina Quispe [b], Mario Simirgiotis [c], Alfredo Torres-Benítez [d], Johanna Reyes-Ortíz [d], Carlos Areche [e,*], Olimpo García-Beltrán [d,*]

[a] Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andres Bello, Quillota 980, Viña del Mar, Chile
[b] Facultad de Ciencias de la Salud, Instituto de Etnofarmacología (IDEA), Universidad Arturo Prat. Casilla 121, Iquique, Chile
[c] Instituto de Farmacia, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia 5090000, Chile
[d] Facultad de Ciencias Naturales y Matemáticas, Universidad de Ibagué, Carrera 22 Calle 67, Ibagué, Colombia
[e] Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 15 August 2016
Revised 18 October 2016
Accepted 19 October 2016
Available online 22 October 2016

Keywords:
Coumarins
Gastroprotective
Ulcer
Heterocycles

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]

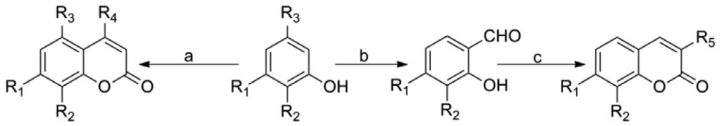
Coumarins are a small group of molecules whose structures contain the 2*H*-chromen-2-one or 1-benzopyran-2-one cores. Many coumarins have numerous pharmacological applications as anticoagulants, anti-inflammatory, antipyretics, antibacterials, antihelminthics, and also as photoprotectors.[5] The first 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]

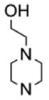
In the course of our studies on gastroprotective drugs, we report here the synthesis of twenty coumarins (**1**–**20**) and their gastroprotective effect in mice. In addition, we discuss the mode of gastroprotective action of **6** and **16**, including the involvement of prostaglandins (PGs), nitric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

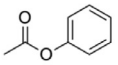
Some twenty coumarins (Schemes 1–3) were synthetized to disclosure structure activity relationship (SAR) based on the gastroprotective effect on the model of HCl/EtOH-induced gastric lesions in mice (Table 1).[8,9] All coumarins have been reported previously and were prepared through known synthetic 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.

* Corresponding authors. Tel.: +56 2 29787218; fax: +56 2 22713888.
E-mail addresses: areche@uchile.cl (C. Areche), jose.garcia@unibague.edu.co (O. García-Beltrán).

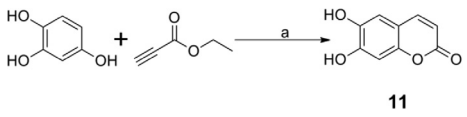
http://dx.doi.org/10.1016/j.bmcl.2016.10.056
0960-894X/



	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.



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%).

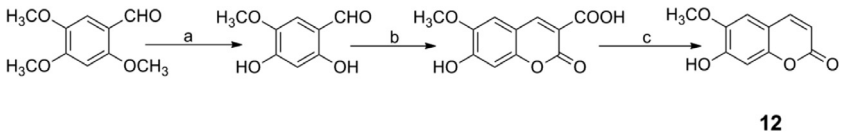
Regarding dihydroxycoumarins **8**–**12**, the highest gastroprotective activity was observed for compounds **10** (76%) and **12** (61%). Indeed, catechol moiety increases the gastroprotective effects for both compounds. There were no differences (compounds **8** and **9**) if a chlorine or methyl group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



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.