European Journal of Medicinal Chemistry 89 (2015) 304–309

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and evaluation of new indole-based chalcones as potential antiinflammatory agents

Ahmet Özdemir [a, *], Mehlika Dilek Altıntop [a], Gülhan Turan-Zitouni [a], Gülşen Akalın Çiftçi [b], İpek Ertorun [c], Özkan Alataş [c], Zafer Asım Kaplancıklı [a]

[a] Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470 Eskişehir, Turkey
[b] Anadolu University, Faculty of Pharmacy, Department of Biochemistry, 26470 Eskişehir, Turkey
[c] Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Biochemistry, 26480 Eskişehir, Turkey

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form
14 October 2014
Accepted 18 October 2014
Available online 18 October 2014

Keywords:
Chalcone
Indole
Cyclooxygenase

ABSTRACT

In the present work, new indole-based chalcone derivatives were obtained *via* the reaction of 5-substituted-1*H*-indole-3-carboxaldehydes/1-methylindole-3-carboxaldehyde with appropriate acetophenones. The synthesized compounds were investigated for their *in vitro* COX-1 and COX-2 inhibitory activity. The most effective COX inhibitors were also evaluated for their *in vivo* antiinflammatory and antioxidant activities in LPS induced sepsis model. Furthermore, the CCK-8 assay was carried out to determine cytotoxic effects of all compounds against NIH/3T3 mouse embryonic fibroblast cells. 3-(5-Bromo-1*H*-indol-3-yl)-1-(4-cyanophenyl)prop-2-en-1-one (**6**) can be considered as a non-selective COX inhibitor (COX-1 IC_{50} = 8.1 ± 0.2 μg/mL, COX-2 IC_{50} = 9.5 ± 0.8 μg/mL), whereas 3-(5-methoxy-1*H*-indol-3-yl)-1-(4-(methylsulfonyl)phenyl)prop-2-en-1-one (**1**) inhibited only COX-1 (IC_{50} = 8.6 ± 0.1 μg/mL). According to *in vivo* studies, these compounds also displayed antiinflammatory and antioxidant activities.

1. Introduction

Cyclooxygenase (COX) enzymes have attracted a great deal of interest as important targets for drug discovery due to their essential role in prostaglandin biosynthesis [1–5].

Inhibition of COX enzymes is a promising approach for pharmacologic intervention in inflammation. Among therapeutic agents in clinical use today, nonsteroidal anti-inflammatory drugs (NSAIDs) exert their therapeutic action by inhibiting COX enzymes. The long-term use of NSAIDs may also lead to severe gastrointestinal side effects, which limit the use of these drugs. The adverse effects accompanying the use of nonselective NSAIDs arise from the reduction of the levels of protective prostaglandins in the gastrointestinal (GI) tract due to the inhibition of COX-1. Although selective COX-2 inhibitors cause less GI adverse effects than nonselective NSAIDs, their use in the treatment is also limited due to their serious cardiovascular effects [1–7].

Chalcones (1,3-diaryl-2-propen-1-ones) are considered as precursors of open chain flavonoids and isoflavonoids present in edible plants. Naturally occurring chalcones and their synthetic analogs have attracted a great deal of interest due to their synthetic and biological importance in medicinal chemistry and considerable research on them in relation to inflammation has been accomplished [8–19].

Indoles have been the subject of intense investigation in both academia and industry owing to their wide range of biological activity [20–23]. A considerable number of natural products and currently available drugs carry an indole moiety. Indomethacin, a nonselective COX inhibitor, is one of the most widely used NSAIDs bearing an indole moiety. Many researchers have carried out considerable research for the synthesis and biological evaluation of indole derivatives (in particular indomethacin analogs) as COX inhibitors [24–34].

In an effort to develop potent COX inhibitors, herein we described the synthesis of a new series of indole-based chalcones and focused on their *in vitro* COX inhibitory effects. We also evaluated *in vivo* antiinflammatory and antioxidant activities of the most effective COX inhibitors. Furthermore, all compounds were evaluated for their cytotoxicity against NIH/3T3 mouse embryonic fibroblast cells.

* Corresponding author.
E-mail address: ahmeto@anadolu.edu.tr (A. Özdemir).

http://dx.doi.org/10.1016/j.ejmech.2014.10.056
0223-5234/

2. Results and discussion

The synthesis of the target compounds (**1**–**9**) was carried out as outlined in Scheme 1. The base-catalyzed Claisen–Schmidt condensation of 5-substituted-1*H*-indole-3-carboxaldehydes/1-methylindole-3-carboxaldehyde with appropriate acetophenones afforded chalcone derivatives (**1**–**9**). The spectral data and elemental analysis results of the synthesized compounds (**1**–**9**) were in agreement with the proposed structures.

Colorimetric COX (ovine) Inhibitor Screening Assay was carried out to evaluate their ability to inhibit COX-1 and COX-2 (Table 1).

The most potent COX-1 inhibitor was found as compound **6** (IC_{50} = 8.1 ± 0.2 μg/mL) followed by compounds **1** (IC_{50} = 8.6 ± 0.1 μg/mL), and **8** (IC_{50} = 9.7 ± 0.4 μg/mL) when compared with indometacin (IC_{50} = 0.7 ± 0.2 μg/mL). Among these derivatives, compound **6** bearing 5-bromo-1*H*-indol-3-yl and 4-cyanophenyl moieties also showed COX-2 inhibitory effect with an IC_{50} value of 9.5 ± 0.8 μg/mL when compared with indometacin (IC_{50} = 10.0 ± 4.2 μg/mL). IC_{50} values of other derivatives were >10 μg/mL.

According to the CCK-8 assay, the cytotoxic doses of compounds **1** (IC_{50} = 32.3 ± 6.7 μg/mL) and **6** (IC_{50} = 51.6 ± 15.3 μg/mL) for NIH/3T3 cells were higher than their effective doses (Table 2).

The *in vitro* data suggest that compounds **1** and **6** have the potential to be potent antiinflammatory compounds, therefore, they were further evaluated for their *in vivo* antiinflammatory and antioxidant activity.

The activity of liver function marker enzymes in control and experimental groups was presented in Table 3. Aspartate aminotransferase (AST) activity was increased in rats with LPS induced sepsis (LPS group) compared to control rats ($p < 0.05$). Furthermore, compounds **1**, **6** and indometacin (93.8, 98.8, and 92.5 U/L, respectively) significantly decreased AST levels compared to LPS group (148.9 U/L) ($p < 0.05$). As a result, compounds **1** and **6** therapies reverted the AST activity to near normal on 5 mg/kg/day concentration.

On the other hand, LPS group's alanine aminotransferase (ALT) activity was also increased compared to controls. But this increase was not statistically significant ($p > 0.05$). Compounds **1**, **6** and indometacin (60.9, 62.4, and 67.6 U/L, respectively) decreased ALT levels compared to LPS group (70.3 U/L). But these decreases were also not statistically significant.

Inflammation and oxidative stress play important roles in sepsis [35]. The initially elevated inflammatory marker as high sensitive C-reactive protein (hsCRP) proved the severity of sepsis [36]. The elevation of CRP levels shows that systemic inflammation has developed. In our study, the hsCRP levels were significantly increased in LPS group compared to controls ($p < 0.001$). Compounds **1** and **6** therapies significantly decreased hsCRP levels compared to LPS group ($p < 0.001$). These decreases were similar to hsCRP levels caused by indometacin therapy (Table 4).

Vascular leakage and recruitment of circulating polymorphonuclear cells (PMNs) to the site of injury are seen in response to sepsis or tissue injury. These events represent the early

Table 1
In vitro COX-1 and COX-2 inhibitory effects of chalcone derivatives (**1**–**9**).

Compound	R	R′	R″	IC_{50} (μg/mL)	
				COX-1	COX-2
1	H	OCH_3	SO_2CH_3	8.6 ± 0.1	>10
2	H	OCH_3	CN	>10	>10
3	H	Cl	SO_2CH_3	>10	>10
4	H	Cl	CN	>10	>10
5	H	Br	SO_2CH_3	>10	>10
6	H	Br	CN	8.1 ± 0.2	9.5 ± 0.8
7	CH_3	H	SO_2CH_3	>10	>10
8	CH_3	H	CN	9.7 ± 0.4	>10
9	CH_3	H	$NHCOCH_3$	>10	>10
Indometacin				0.7 ± 0.2	10.0 ± 4.2

Table 2
Cytotoxicity of chalcone derivatives (**1**–**9**) against NIH/3T3 mouse embryonic fibroblast cells.

Compound	IC_{50} (μg/mL)
1	32.3 ± 6.7
2	20.0 ± 7.0
3	21.0 ± 16.5
4	33.3 ± 2.9
5	17.3 ± 5.5
6	51.6 ± 15.3
7	350.0 ± 86.6
8	210.0 ± 69.2
9	96.6 ± 25.2

Table 3
Effects of compounds on ALT and AST levels.

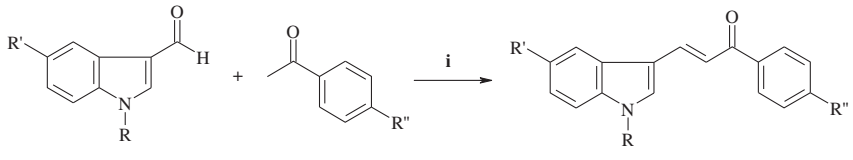
Groups	ALT (U/L)	AST (U/L)
Control	59.5 ± 6.2	89.6 ± 7.6
LPS (1 mg/kg)	70.3 ± 7.9	148.9 ± 41.3$^{###}$
Compound **1** (5 mg/kg/day)	60.9 ± 8.7	93.8 ± 20.7***
Compound **6** (5 mg/kg/day)	62.4 ± 7.9	98.8 ± 14.7**
Indometacin (5 mg/kg/day)	67.6 ± 5.7	92.5 ± 10.1***

Values are given as mean ± SD. Significance against control values, ###$p < 0.001$; Significance against LPS values, $^{**}p < 0.01$; $^{***}p < 0.001$. One-way ANOVA, post-hoc Tukey test, $n = 8$.

Table 4
Effects of compounds on hsCRP and MPO levels.

Groups	hsCRP (mg/L)	MPO (U/L)
Control	1.0 ± 0.4	0.4 ± 0.1
LPS (1 mg/kg)	1.9 ± 0.3$^{###}$	4.2 ± 1.8$^{###}$
Compound **1** (5 mg/kg/day)	1.0 ± 0.3***	0.6 ± 0.2***
Compound **6** (5 mg/kg/day)	0.6 ± 0.2***	0.8 ± 0.3***
Indometacin (5 mg/kg/day)	1.1 ± 0.6**	0.6 ± 0.2***

Values are given as mean ± SD. Significance against control values, ###$p < 0.001$; Significance against LPS values, $^{**}p < 0.01$; $^{***}p < 0.001$. One-way ANOVA, post-hoc Tukey test, $n = 8$.



1-9

Scheme 1. The synthetic route for the preparation of the chalcone derivatives (**1**–**9**). Reagents and conditions: (**i**) NaOH, ethanol, rt, 10 h.