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Syntheses and biological evaluation of indole-2 and 3-carboxamides: new selective cyclooxygenase-2 inhibitors

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A series of indol-2 and 3-carboxamides were prepared and evaluated for their ability to inhibit cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1). Substitution on indol nitrogen with benzyl and p-fluorobenzyl group of indole-2 carboxamides **8**, **10**, **11** provides fairly active COX-2 enzyme inhibitors.

1. Introduction

Considering that COX-2 was only discovered in the last decade, it is remarkable that COX-2 inhibitors have been developed and prepared for the commercial market so fast. It is also surprising that there has been so much success in the development of COX-2 selective inhibitors, considering the similarities of COX-1 and COX-2 enzyme active sites.

Recently many projects were initiated to develop selective COX-2 enzyme inhibitors with little or no gastric side effects [1, 2]. Working on the basic of the receptor active site, Black *et al.* [1] found that increasing the size of the indomethacin nucleus to produce a compound which would still fit into the COX-2 active site but not into the COX-1 active site, would generate the desired selectivity. After setting up several structure-activity relationship (SAR) studies of COX-1 and COX-2 enzyme active sites, Kalgutkar et al. designed several amide and ester derivatives of indomethacin [2]. The results suggest that conversion of NSAIDs to amide or ester derivatives may be a very useful approach for the generation of novel and efficacious COX-2 selective inhibitors. On the other hand, the facile nature of this strategy is evident from the observation that a single chemical derivatization of the carboxylate moiety generates an impressive array of potent and highly selective COX-2 inhibitors.

Some studies related to indole carboxylic acid esters and amide derivatives were started by our laboratory a few years ago. The receptor docking studies were performed to investigate the docking mode of some compounds by using the Dock 4.0 program. It was predicted that some N-substituted indole-2 and 3-carboxylic acid ester and amide derivatives would show good binding capability to the enzyme active site. Based on our findings and literature reports, we designed new N-substituted indole-2 and 3-carboxamides. Here, we describe the synthesis and antiinflammatory evaluation of our target selective COX-2 enzyme inhibitors.

2. Investigations and results

Well-established methodology was used to synthesise amide derivatives of indole-2 and 3-carboxylic acids as indicated in Schemes 1, 2 and 3. In this study we synthesized 1-benzyl and substituted benzyl carboxylic acids by the method of Murakami et al. [3]. Indole-2 and 3-carboxylic amides were prepared using $SOCl_2$ as a carboxyl activator. To synthesize the N-phenyl indole 2-carboxylic acid derivative, Ullmann's condensation was used efficiently [4, 5]. The catalyst used for the arylation was copper (II) oxide and DMF was the best reaction solvent to obtain good yield. The structures of all compounds were established by NMR, IR and MS. Elemental analyses of target amides indicated purity in the range of 40%. Some physicochemical properties and spectral data for the products are given in Table 1.

Scheme 1

COOH — a → COOMe **1** + Br–CH$_2$–C$_6$H$_4$–X (X= H, F) — b → **2** X= H, **3** X= F — c → COOH **4** X= H, **5** X= F — d → CONHR

6 X= H, R= 2-chloropyridyl
7 X= F, R= 2-chloropyridyl
8 X= H, R= thiazolyl
9 X= F, R= thiazolyl
10 X= H, R= phenyl
11 X= F, R= phenyl

Reagents: (a) HCl in MeOH, reflux; (b) NaH, DMF, RT; (c) 10% NaOH, MeOH, 65 °C, AcOH; (d) 1. $SOCl_2$, benzene, reflux, 2. corresponding amines, $CHCl_3$, pyridine, Rt

Scheme 2

COOH a COOMe Br + X b COOMe N

12 X= H, F

13 X= H
14 X= F

c

CONHR COOH

d

17 X= H, R= 2-chloropyridyl
18 X= F, R= 2-chloropyridyl
19 X= H, R= thiazolyl
20 X= F, R= thiazolyl
21 X= H, R= phenyl
22 X= F, R= phenyl

15 X= H
16 X= F

Reagents: (a) HCl in MeOH, reflux; (b) NaH, DMF, RT; (c) 10% NaOH, MeOH, 65 °C, AcOH; (d) 1. $SOCl_2$, benzene, reflux, 2. corresponding amines, $CHCl_3$, pyridine, Rt

The human whole blood assay, originally developed by Patrignani et al. [6], is considered to be the most biologically relevant way to assess the inhibition of the cyclooxygenase isoenzymes, COX-1 and COX-2, by a test compound. In this assay, platelets stimulated by calcium ionophore are believed to be the main source of COX-1, whereas monocytes stimulated with LPS are thought to be the source of COX-2. COX-1 activity is determined by the production of thromboxane B_2 (TXB_2), while COX-2 activity is determined by the production of prostaglandin E_2 (PGE_2).

3. Discussion

Among the derivatives, synthesized **8**, **10** and **11** are highly potent compounds comparable with rofecoxib and celecoxib. The activity and selectivity index of target compounds are shown in Table 2. Replacement of benzyl group **8** with p-fluoro benzyl group **9** of indol-2 thiazolyl amides provide very high potency and selectivity. In contrast, p-fluoro benzyl substituted indole-2 benzamide **11** was found to be more active then its benzyl substituted analog **10**. On the other hand, N-phenyl substitued compounds **24**, **25**, **26** did not show any inhibitory effects at concentrations up to 50 μM. This suggest that N-substitution at the indole ring plays a very important role in activity. It is noteworthy that an N-benzyl or p-fluoro benzyl group is necessary for selective COX-2 enzyme inhibitory activity. This can be explained by the binding capability of the benzyl group to the enzyme active site compaired with the phenyl group which has no activity at all.

Recent studies have mostly concerned indole-3 acetic acid esters and amides. Some derivatives have been found to be very highly potent and selective compounds [2]. Despite these good activity results with indole-3 acetic acid derivatives, notable selective COX-2 inhibition has also been found with indole-2 carboxylic acid derivatives. It is noteworthy that indole-2-carboxylic acid derivatives could be an important class of compounds to investigate as selective COX-2 inhibitors.

Compounds **8** can be remarkable for lack of their gastrointestinal toxicity (see Table 2), because of the very good selectivity profile of 0.02 (COX-2/COX-1) for COX-2 enzyme inhibition compared with rofexocib (0.08) and celecoxib (0.07). Based on the literature 0.01 is reported as a good selectivity index when evaluating the selectivity of COX-2 enzyme inhibitors [7].

Scheme 3

COOH a COOH b CONHR

23

24 **R=** 2-chloropyridyl, X= H
25 R= thiazolyl, X= H
26 R= phenyl, X= H

Reagents: (a) benzyl bromide, CuO, anhydrous K_2CO_3, reflux; (b) 1. $SOCl_2$, benzene, reflux, 2. corresponding amines, pyridine, $CHCl_3$, RT

Table 1: Physical and spectral data of compounds 1–26

Compd.	X	R_2	M.P. (°C)	Yield (%)	IR (KBr, cm^{-1})	UV (λ_{max}, ε)		Formula	MASS (FAB)	^{1}H NMR
1	H	OMe	152	96	1697,05			$C_{10}H_9NO_2$	175.0612	7.52–6.77 (m, 5 H, aromatic), 2.81 (s, 3 H, COOMe)
2	H	OMe	86	42	1708,62			$C_{17}H_{15}NO_2$	265.1113	7.72–6.87 (m, 10 H, aromatic), 5.83 (s, 2 H, CH$_2$Ph), 2.87 (s, 3 H, COOMe)
3	F	OMe	91	48	1716,34			$C_{17}H_{14}FNO_2$	283.1021	7.75–6.77 (m, 9 H, aromatic), 5.72 (s, 2 H, CH$_2$Ph), 2.55 (s, 3 H, COOMe)
4	H	H	193	82	1667,16			$C_{16}H_{13}NO_2$	251.0912	11.05 (s, COOH), 7.72–6.87 (m, 10 H, aromatic), 5.83 (s, 2 H, CH$_2$Ph)
5	F	H	179	75	1684,52			$C_{16}H_{12}FNO_2$	269.0922	11.01 (s, COOH), 7.75–6.77 (m, 9 H, aromatic), 5.72 (s, 2 H, CH$_2$Ph)
6	H	Cl N	129	50	1683,55	302.5	2496.98	$C_{21}H_{16}N_3O$	362.1058	8.65 (s, 1 H, NH), 8.98–7.35 (m, 13 H, aromatic), 6.03 (s, 2 H, CH$_2$Ph)
7	F	Cl N	155	45	1691,27	302.0	2583.28	$C_{21}H_{15}ClFN_3O$ · 0.9 C_6H_6 · 0.5 MeOH	380.0979	8.45 (s, 1 H, NH), 8.85–6.66 (m, 12 H, aromatic), 5.69 (s, 2 H, CH$_2$Ph)
8	H	N S	206	42	1666,20	315.5	2913.00	$C_{19}H_{15}N_3OS$ · 1.1 C_6H_6 · 0.01 EtOH	334.1015	12.53 (s, 1 H, NH), 7.88–7.08 (m, 12 H, aromatic), 6.04 (s, 2 H, CH$_2$Ph)
9	F	N S	214	40	1664,27	315.5	2660.80	$C_{19}H_{14}FN_3OS$	352.0933	12.64 (s, 1 H, NH), 8.05–6.94 (m, 11 H, aromatic), 5.87 (s, 2 H, CH$_2$Ph)
10	H		140	70	1683,55	300.5	1721.06	$C_{22}H_{18}N_2O$	327.1497	9.91 (s, 1 H, NH), 7.55–6.94 (m, 15 H, aromatic), 5.82 (s, 2 H, CH$_2$Ph)
11	F		134	67	1668,12	300.5	1592.99	$C_{22}H_{17}FN_2O$	345.1414	10.28 (s, 1 H, NH), 7.84–6.91 (m, 14 H, aromatic), 5.87 (s, 2 H, CH$_2$Ph)
12	H	OMe	145	86	1666,20			$C_{10}H_9NO_2$	175.0615	7.54–6.71 (m, 5 H, aromatic), 2.75 (s, 3 H, COOMe)
13	H	OMe	98	64	1692,33			$C_{17}H_{15}NO_2$	265.1127	7.52–6.77 (m, 10 H, aromatic), 5.81 (s, 2 H, CH$_2$Ph), 2.87 (s, 3 H, COOMe)
14	F	OMe	78	56	1696,09			$C_{17}H_{14}FNO_2$	283.1083	7.72–6.71 (m, 9 H, aromatic), 5.79 (s, 2 H, CH$_2$Ph), 2.55 (s, 3 H, COOMe)
15	H	H	202	83	1651,73			$C_{16}H_{13}NO_2$	251.0923	11.09 (s, COOH), 7.68–6.72 (m, 10 H, aromatic), 5.78 (s, 2 H, CH$_2$Ph)
16	F	H	198	79	1652,70			$C_{16}H_{12}FNO_2$	269.0918	11.08 (s, COOH), 7.66–6.53 (m, 9 H, aromatic), 5.69 (s, 2 H, CH$_2$Ph)
17	H	Cl N	165	56	1646,91	298.0 260.0	2367.31 1559.10	$C_{21}H_{16}N_3O$	362.1087	8.54 (s, 1 H, NH), 8.40–7.40 (m, 13 H, aromatic), 5.61 (s, 2 H, CH$_2$Ph)
18	F	Cl N	178	48	1644,02	299.0 261.0	2035.45 1297.87	$C_{21}H_{15}ClFN_3O$	380.0982	8.47 (s, 1 H, NH), 9.14–7.17 (m, 12 H, aromatic), 5.52 (s, 2 H, CH$_2$Ph)
19	H	N S	203	47	1653,66	306.5 275.0	2534.17 1310.43	$C_{19}H_{15}N_3OS$	334.1027	11.46 (s, 1 H, NH), 8.53–7.02 (m, 12 H, aromatic), 5.51 (s, 2 H, CH$_2$Ph)
20	F	N S	212	43	1664,27	306.5 274.5	1805.11 888.98	$C_{19}H_{14}FN_3O$ · 0.45 $CHCl_3$	352.0910	11.71 (s, 1 H, NH), 8.37–7.01 (m, 11 H, aromatic), 5.41 (s, 2 H, CH$_2$Ph)
21	H		176	75	1640,16	299.0 259.5	1177.91 1009.24	$C_{22}H_{18}N_2O$	327.1497	9.92 (s, 1 H, NH), 8.09–7.99 (m, 15 H, aromatic), 5.36 (s, 2 H, CH$_2$Ph)
22	F		185	73	1638,23	297.5 259.5	2473.30 2159.83	$C_{22}H_{17}FN_2O$	345.1422	9.69 (s, 1 H, NH), 8.32–6.77 (m, 14 H, aromatic), 5.43 (s, 2 H, CH$_2$Ph)
23	H	H	176	42	1670,00			$C_{15}H_{11}NO_2$	237.08	11.05 (s, COOH), 7.72–6.87 (m, 10H aromatic)
24	H	Cl N	131	42	1683,55			$C_{20}H_{14}ClN_3O$	347.17	8.97 (d, 1 H, NH), 8.68–7.12 (m, 13 H, aromatic)
25	H	N S	212	48	1666,20			$C_{18}H_{13}N_3OS$	319.37	12.04 (s, 1 H, NH), 7.74–6.98 (m, 12 H, aromatic)
26	H		140	71	1683,55			$C_{21}H_{16}N_2O$	312.14	9.13 (s, 1 H, NH), 7.44–6.45 (m, 15 H, aromatic)

Table 2: Activity of compounds 6–11, 17–22 and 24–26

Compd.	HBW COX-2 % Inhibition (μM) or IC_{50} μM[a]	HBW COX-1 % Inhibition (μM) or IC_{50} μM[a]	Selectivity COX-2/ COX-1[b]
6	46% ± 5.0 (10 μM)	ND	ND
7	7% ± 5.6 (10 μM)	ND	ND
8	1.6 ± 1.5	60.9 ± 10.2	0.02
9	42.7% ± 10.5 (10μM)	49% ± 15.7 (100μM)	ND
10	2.0 ± 1.2	21% ± 5.2 (100μM)	ND
11	1.1 ± 0.1	14% ± 7.2 (100μM)	ND
17	1% ± 0.5 (10 μM)	ND	ND
18	15% ± 1.0 (10 μM)	ND	ND
19	0% ± 0.5 (10 μM)	ND	ND
20	18% ± 3.1 (10 μM)	ND	ND
21	5% ± 4.1 (10 μM)	ND	ND
22	44% ± 6.3 (10 μM)	37% ± 10.8 (10 μM)	1.1892
24	5% (10 μM)	ND	ND
25	5% (10 μM)	ND	ND
26	5% (10 μM)	ND	ND
Rofecoxib	0.76 ± 0.33	11.4 ± 0.8	0.07
Celecoxib	1.1 ± 0.2	14.2 ± 4.4	0.08

[a] Data are indicated as IC_{50} (μM) or percentage of inhibition at 10 μM ± SEM (n = 3).
[b] The ratio of IC_{50} (COX-2) : IC_{50} (COX-1)
ND = Non Determined

4. Experimental

Indole-2 and 3-carboxylic acids, bromobenzene, benzyl bromide, p-fluorobenzyl bromide, anh. potassium carbonate, 2-chloro-3-amino pyridine, and potassium bromide were purchased from Aldrich chemical company, and sodium hydroxide, anh. magnesium sulphate, hydrogen chloride, ethanol, methanol, ethyl acetate, hexanes, thiazolyl-(2)-amine, and aniline from Merck.
M.p.'s were measured with a capillary apparatus (Buchi SMP 20) and uncorrected. The ^1H NMR spectra were recorded on a Bruker 400 AMX spectrometer at 400 MHz, with Me_4Si as internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br s (broad singlet). MS were recorded on a Micromass Autospec high resolution mass spectrometer. IR spectra were measured on an FT-IR spectrometer. Elemental analyses were taken on a Leco-932 CHNS-O analyser. CC was performed on silica gel 60 (Merck). All the results were in an acceptable range.
Biological material: human blood obtained from healthy volunteers. LPS (from *Escherichia coli*, ref. 0127:B8, DIFCO). A23187 calcium ionophore (Sigma).
PGE_2 and TXB_2 EIA (enzyme immunoassay) kits (Amersham).

4.1. *Methyl indole-2-carboxylate* (**1**)

Indole-2-carboxylic acid (25 g, 0.15 mol) was dissolved in % 10 HCl in MeOH and refluxed at 65 °C for 1 h. The solvent was evaporated under vacuum and neutralized by K_2CO_3. Crystallization by ethanol to give pale white crystals. M.p. 152 °C. Yield: 96%.

4.2. *1-Benzyl methyl indole-2-carboxylate* (**2**)

Compound **1** (24 g, 0.14 mol) and 4.4 g (0.18 mol)% 50 NaH were dissolved in 10 ml of DMF and stirred at room temperature for 30 min. 12 ml (0.10 mol) of benzyl bromide were added dropwise and stirred at room temperature for 48 h. Then the reaction mixture was poured into ice-water. Neutralization with acetic acid gave an oily compound which was crystallized by ethanol: water to give pale yellow crystals. M.p. 86 °C. Yield: 42%.

4.3. *1-(p-Fluorobenzyl) methyl indole-2-carboxylate* (**3**)

Compound **3** was synthesized as described for compound **2**. 2.7 g of white compound was obtained. M.p. 91 °C. Yield: 48%.

4.4. *1-Benzyl indole-2-carboxylic acid* (**4**)

Compound **2** (15 g, 0.56 mol) was dissolved in 50 ml of MeOH and 50 ml of 10% NaOH solution were added. The reaction mixture was stirred at 65 °C for 2 h. The reaction was cooled down to room temperature and neutralized by AcOH to give a white precipitate. Crystallization with MeOH: water to give white crystals. M.p. 193 °C. Yield: 82%.

4.5. *1-(p-Fluorobenzyl) indole-2-carboxylic acid* (**5**)

Compound **5** was synthesized as described for compound **4**. 1.8 g of white compound was obtained. M.p. 179 °C. Yield: 75%.

4.6. *Synthesis of N-substituted indole-2-carboxamide derivatives* **6–11**

1-Benzyl and *p*-fluorobenzyl indole-2-carboxylic acids were refluxed in 5 ml benzene with 2.5 ml $SOCl_2$ for 2 h at 80 °C. Then the solvent and excess amount of $SOCl_2$ were evaporated by coevaporation with toluene (3 × 10 ml). The residue was dissolved in 10 ml chloroform and corresponding amine and pyridine derivatives in the same equivalents than carboxylic acids (1.2 mmol) were added after which the mixture was stirred at RT overnight. The solvent was evaporated to dryness to give crude compounds which were purified by silicagel CC (hexane: EtOAc = 8 : 2). All spectral and physical data are given in Table 1.

4.7. *Synthesis of compounds* **12–22**

Indole-3-carboxylic acid derivatives **12–22** were synthesized using the same methods as for their corresponding indol-2 derivatives. All spectral and physical data are given in Table 1.

4.8. *1-Phenyl indol-2-carboxylic acid* **23**

Indole-2-carboxylic acid (8 g, 0.05 mol) was dissolved in 10 ml of DMF and 7 g of anh. K_2CO_3, 0.25 g CuO and 5.2 ml (0.05 mol) bromobenzene were added. The reaction mixture was refluxed for 24 h at 154 °C. At the end of the reaction the mixture was cooled and added to ice-water. The water layer was washed with $CHCl_3$ (3 × 100 ml). The water layer was acidified with conc. HCl and allowed to stand overnight. The precipitated N-phenyl indole-2-carboxylic acid was filtered off and purified by crystallization from MeOH : H_2O. M.p. 176 °C. Yield: 42%.

4.9. *Synthesis of 1-phenyl indol-2-carboxamides* **24–26**

Compounds **24–26** were synthesized using the same method as described for compounds **6–11**. All spectral and physical data are given in Table 1.

4.10. *COX-1 activity in human whole blood*

The blood was obtained from healthy volunteers who had not taken NSAIDs in the previous week. The blood was heparinised (20 U/ml) and distributed in 0.5 ml aliquots to tubes containing vehicle or the test compound. Each drug was evaluated at five of six different concentrations in triplicate determinations. The samples were incubated at 37 °C with gentle shaking for 40 min. Calcium ionophore (5 μl) was added for a further 20 min and the reaction was then stopped by submerging the tubes in a cold bath and centrifuging at 13000 rpm for 10 min at 4 °C. Levels of TXB_2 in the supernatants were determined by EIA.

4.11. *COX-2 activity in human whole blood*

The blood was heparinised (20 U/ml) and distributed in 0.5 aliquots in tubes containing 10 μg/ml of LPS together with vehicle or test compound. Each drug was evaluated at five or six different concentrations in triplicate determinations. The samples were incubated in a bath at 37 °C for 24 h; during this time COX-2 was induced in mononuclear cells. The reaction was stopped by submerging the tubes in a cold bath and centrifuging at 13000 rpm for 10 min at 4 °C. Levels of PGE_2 in the supernatant were determined by EIA.

Acknowledgements: This research was partially supported by a grant from the Turkish Scientific and Technical Research Institute (SBAG-AYD-341). The activity tests were performed at Almirall Prodesfarma S.A., Research Center, Barcelona, Spain. The authors thank María I. Crespo for her collaboration.

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Received May 26, 2001
Accepted September 13, 2001

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