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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of ester derivatives of indomethacin as selective COX-2 inhibitors

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Abstract The ester derivatives of indomethacin were prepared by condensing indomethacin with an equimolar quantity of an appropriate alcoholic compound in anhydrous dichloromethane in the presence of DCC and DMAP. Spectral studies comprising of IR, ^{1}H NMR, mass, and microanalysis were performed in order to confirm their structures. In vivo anti-inflammatory studies were carried out using carrageenan rat paw edema method and in vivo ulcerogenic studies by ulcer index method for the panel of synthesized compounds. Out of eleven compounds, the compound **IIc** displayed moderate anti-inflammatory activity with no observable ulcerogenic effect when compared to indomethacin. Furthermore, compound **IIc**, indomethacin and celecoxib were tested at a concentration of 20 μM against COX-1 and COX-2 enzymes by colorimetric COX inhibitor screening assay. Compound **IIc** was found to be active against COX-2 and COX-1 enzymes exhibiting 62.0 and 12.9% inhibition, respectively.

Keywords Indomethacin esters · COX-2 inhibitors · Colorimetric COX inhibitor screening assay

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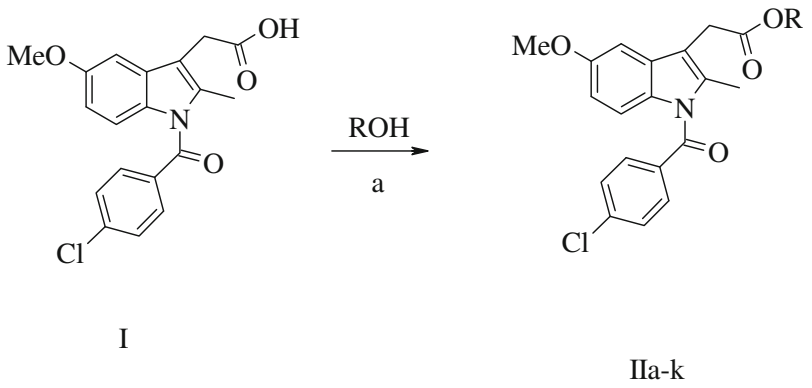
Introduction

NSAIDs are used extensively to alleviate inflammation, pain, rheumatoid arthritis, and osteoarthritis. Long-term regimens of NSAIDs have been greatly shortened due to their gastrointestinal side effects (Laine, 2001). These adverse effects are driving the research toward development of NSAIDs with minimal, if any, gastrointestinal side effects. Vane proposed that the mechanism-of-action of classical NSAIDs viz., aspirin, ibuprofen, diclofenac, mefenamic acid, indomethacin, sulindac, and picroxicam is through their inhibition of prostaglandin biosynthesis (Vane, 1971). We have therefore directed our efforts to develop safer NSAIDs over conventional NSAIDs. In addition, the structures of flurbiprofen (Bayly *et al.*, 1999), indomethacin (Kalgutkar *et al.*, 2000), meclofenamic acid (Kalgutkar *et al.*, 2002), ketrolac (Black *et al.*, 1996; Bhandari *et al.*, 2007; Mishra *et al.*, 2008), etc. have been modified with selective COX-2 inhibitory activity. These modified structures were found to have similar or moderate efficacy to that of selective COX-2 inhibitors, but with greater gastrointestinal safety. Recently, several research groups independently tried to modify the structure of Indomethacin (Kalgutkar *et al.*, 2000; Wood *et al.*, 2001; Khanna *et al.*, 2006), which has led to clinical trials of L-761, 000, as a potent selective COX-2 inhibitor (Leblanc *et al.*, 1996). This is encouraged us to attempt to synthesize safer indomethacin esters via modifications of the carboxylic group.

Chemistry

The indomethacin derivatives were prepared according to the synthetic route illustrated in Scheme 1. The indomethacin esters were prepared from condensation between

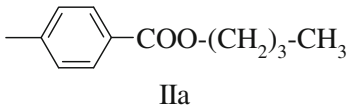
Scheme 1 Synthesis of indomethacin esters



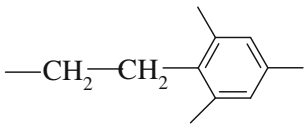
I IIa-k

Reagents and conditions: (a) N,N'-Dicyclohexyl carbodiimide (DCC), Dimethyl amino pyridine (DMAP), Dichloromethane, 5h

R=

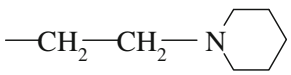


IIa

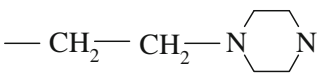


IIb

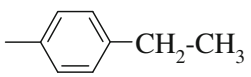
IIc



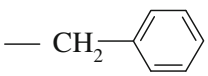
IId



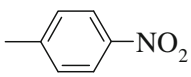
IIe



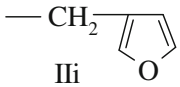
IIf



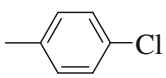
IIg



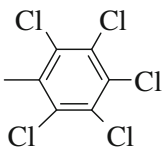
IIh



IIi



IIj



IIk

indomethacin and hydroxyl group containing alcoholic compounds in the presence of DCC and DMAP (Kalgutkar *et al.*, 2000). The synthesized compounds were evaluated for their in vivo anti-inflammatory activity, gastrointestinal toxicity, and COX-1 and COX-2 enzyme inhibitory activities. All the final compounds were chemically characterized by melting point, infrared (IR) spectra, nuclear magnetic resonance (NMR) spectra, mass spectra, and elemental analysis.

Experiment

Melting points were determined by the open capillary method using a Besto melting point apparatus without

correction. Indomethacin was procured from Sun Pharma, Gujarat (India). All other chemicals and solvents were purchased from Lancaster and Merck, respectively, except anhydrous methylene chloride which was purchased from Sigma-Aldrich. Precoated TLC plates from Merck India were used to follow the course of reactions. Developed plates were visualized by iodine vapor. Silica gel (60–120 mesh) was used for column chromatography and purchased from Spectrochem Pvt. Ltd. (Mumbai). IR spectra were recorded on a Perkin-Elmer 237 spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker DRX300 (300 MHz FT NMR) instrument. Mass spectra were determined on a Joel SX-102 spectrometer. Carbon, hydrogen, and nitrogen analysis were performed on CHN analyses Carlo Erba 1108, Heracus at the Central Drug Research Institute (CDRI), Lucknow.

Synthesis of indomethacin esters

A reaction mixture containing indomethacin (0.003 mol) in 6 ml of anhydrous dichloromethane (CH_2Cl_2) was treated with DCC (2.76 mmol), DMAP (252 μmol), and appropriate alcohol (0.003 mol). After stirring at room temperature for 5 h, the reaction mixture was filtered and the filtrate was concentrated in a vacuum. The residue was diluted with water (~30 ml) and extracted with ethyl acetate (2 × 30 ml). The combined organic solution was washed with 5% acetic acid (2 × 30 ml), 1 N sodium hydroxide (2 × 30 ml) and water (~100 ml), dried in sodium sulphate, filtered and the solvent was removed under a vacuum. The product was obtained as a solid or oil. This procedure was used to synthesize all of the compounds (**IIa**–**k**). Physical data of compounds are given in Table 1.

*[4{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl 1H-indol-3-yl] acetoxy}-benzoic acid butyl ester (**IIa**)*

Yield 90.6%; mp 68–71°C; ^{1}H NMR ($CDCl_3$) δ 0.94–0.99 (m, 3H, CH_3), 1.43–1.50 (m, 2H, CH_2), 1.69–1.76 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 3.92 (s, 2H, –CH_2–COO–), 4.26–4.34 (m, 2H, COO–CH_2), 6.69–6.71 (d, 2H, $J = 7.5$ Hz, Ar–H), 6.72–6.90 (m, 2H, Ar–H), 7.04 (s, 1H, Ar–H), 7.13–7.16 (d, 1H, $J = 8.7$ Hz, Ar–H), 7.46–7.49 (d, 1H, $J = 8.1$ Hz, Ar–H), 7.66–7.69 (d, 2H, $J = 8.1$ Hz, Ar–H), 8.04–8.06 (d, 2H, $J = 8.4$ Hz, Ar–H); IR (KBr) 1738 cm^{-1} (C=O, ester); FAB–MS *m/z* 535 $[M + H]^+$; Anal. cald. for $C_{30}H_{28}ClNO_6$; C, 67.48; H, 5.29; N, 2.62; Found: C, 67.50:, H, 5.27; N,2.64.

Table 1 Anti-inflammatory activity of indomethacin esters in carrageenan induced rat paw edema

Group ($N = 5$)	0 h	1.5 h	3 h	% Inhibition
Control	1.00 ± 0.057	1.60 ± 0.10	1.66 ± 0.07	–
IIa	0.92 ± 0.01	1.51 ± 0.08	1.53 ± 0.08	7.83
IIb	0.93 ± 0.04	1.42 ± 0.07	1.51 ± 0.12	9.04
IIc	0.98 ± 0.03	1.22 ± 0.05	1.33 ± 0.08[b]	19.88
IId	1.01 ± 0.03	1.42 ± 0.06	1.54 ± 0.09	7.23
IIe	1.00 ± 0.01	1.63 ± 0.14	1.67 ± 0.13	0
IIf	1.04 ± 0.02	1.52 ± 0.04	1.62 ± 0.04	2.41
IIg	1.00 ± 0.09	1.49 ± 0.29	1.60 ± 0.26	3.61
IIh	0.95 ± 0.09	1.47 ± 0.12	1.58 ± 0.13	4.81
IIi	0.98 ± 0.08	1.53 ± 0.23	1.77 ± 0.18	0
IIj	0.92 ± 0.89	1.47 ± 0.25	1.71 ± 0.30	0
IIk	1.00 ± 0.07	1.52 ± 0.17	1.66 ± 0.20	0
Indomethacin	1.03 ± 0.01	1.27 ± 0.06	1.43 ± 0.06[a]	13.86

Each value is the mean ± SEM in five rats

[a] $P < 0.05$ compared to control

[b] $P < 0.01$ compared to control

*[1-(4-Chloro-benzoyl)-5-methoxy-2methyl 1H-indol-3-yl] acetic acid 2-(2,4,6-trimethyl-phenyl)-ethyl ester (**IIb**)*

Yield 88%; mp = 98–100°C; ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 9H, CH_3), 2.32 (s, 3H, CH_3), 2.93–2.98 (m, 2H, CH_2), 3.66 (s, 2H, CH_2COO), 3.82 (s, 3H, OCH_3), 4.16–4.21 (m, 2H, $COOCH_2$), 6.66–6.69 (d, 2H, $J = 8.7$ Hz, Ar–H), 6.82–6.93 (m, 2H, Ar–H), 7.26 (s, 1H, Ar–H), 7.46–7.49 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.65–7.68 (d, 2H, $J = 8.1$ Hz, Ar–H); IR (KBr) 1729 cm^{-1} (C=O, ester); FAB–MS *m/z* 505 $[M + H]^+$; Anal. cald. For $C_{30}H_{30}ClNO_4$; C, 71.49; H, 6.00; N, 2.78; Found: C, 71.50; H, 6.10; N, 2.80.

*4{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl 1H-indol-3-yl] acetoxy} benzoic acid ethyl ester (**IIc**)*

Yield 74.0%; ^{1}H NMR($CDCl_3$) δ 1.05–1.13 (m, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.08 (s, 2H, –CH_2COO), 3.77 (s, 3H, –OCH_3), 4.28–4.35 (m, 2H, –$COOCH_2$–), 6.59–6.63 (d, 2H, $J = 9.00$ Hz, Ar–H), 7.10 (m, 2H, Ar–H), 7.34–7.41 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.55–7.58 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.83–7.86 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.07–8.10 (d, 1H, $J = 8.4$ Hz, Ar–H); IR (KBr) 1742 cm^{-1} (C=O, ester); FAB–MS *m/z* 507 $[M + H]^+$; Anal. cald. for $C_{28}H_{24}ClNO_6$; C, 66.47; H, 4.78; N, 2.77; Found: C, 66.50; H, 4.78; N, 2.80.

*[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H indol-3-yl] acetic acid-2-piperidin-1-yl ethyl ester (**IId**)*

Yield 69.7%; mp = 130–132°C; ^{1}H NMR($CDCl_3$) δ 1.11–1.90 (m, 10H, Piperidyl), 2.37 (s, 3H, CH_3), 3.20 (s, 2H, –CH_2COO–), 3.84 (s, 3H, –OCH_3), 3.98 (m, 2H, –COOCH_2–), 3.98 (m,2H, –COOCH_2), 6.75–6.78 (d, 2H, J = 8.4 Hz, Ar–H), 7.13–7.16 (2H, d, J = 8.4 Hz, Ar–H), 7.28–7.32 (m, 2H, Ar–H), 7.87 (s,1H, Ar–H); IR (KBr) 1742 cm^{-1} (C=O, ester); FAB-MS *m/z* 470 $[M + H]^+$; Anal. cald. for $C_{26}H_{29}ClN_2O_4$; C, 66.59; H, 6.23; N, 5.97; Found: C, 66.61; H, 6.19; N, 5.91.

*[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H indol-3-yl] acetic acid-2 piperazin-1-yl ethyl ester (**IIe**)*

Yield 67.0%; mp = 128–130°C; ^{1}H NMR($CDCl_3$) δ 1.07–1.90 (m, 8H, piperazinyl, CH_2), 2.00 (s, 1H, NH), 2.37 (s, 3H, CH_3), 3.61–3.66 (m, 2H, CH_2COO–), 3.54 (m, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.98–4.01 (m, 2H, –CO-OCH_2), 6.74–6.78 (d, J = 8.2 Hz, 2H, Ar–H), 7.13–7.15 (d, J = 8.7 Hz, 2H, Ar–H), 7.27–7.33 (m, 2H, Ar–H), 7.85 (s, 1H, Ar–H); IR (KBr) 1769 cm^{-1} (–C=O, ester); FAB-MS *m/z* 471 $[M + H]^+$, Anal. cald. for $C_{25}H_{28}ClN_3O_4$; C, 63.89; H, 6.01; N, 8.94; Found: C, 63.91; H, 6.10; N, 8.96.

*[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl] acetic acid 4-ethyl phenyl ester (**IIf**)*

Yield 87.0%; mp = 128–130°C; ^{1}H NMR($CDCl_3$) δ 1.19–1.26 (m, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.59–2.64 (m, 2H, –CH_2CH_3), 3.61–3.66 (m, 2H, CH_2) 3.84 (s, 3H, –OCH_3), 6.68–6.71 (d, 2H, J = 8.1 Hz, Ar–H), 6,89–6.97 (m, 1H, Ar–H), 7.06 (1H, s, Ar–H), 7.15–7.18 (d, 2H, J = 8.4 Hz, Ar–H), 7.36 (s, 1H, Ar–H), 7.46–7.49 (d, 2H, J = 8.4 Hz, Ar–H), 7.66–7.70 (d, 2H, J = 8.4 Hz, Ar–H); IR (KBr) 1752 cm^{-1} (C=O, ester); FAB-MS *m/z* 463 $[M + H]^+$; Anal. cald. for $C_{27}H_{24}ClNO_4$; C, 70.20; H, 5.24; N, 3.03; Found: C, 70.94; H, 5.69; N, 2.99.

*[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl 1H-indol-3-yl] acetic acid benzyl ester (**IIg**)*

Yield 72%; ^{1}H NMR($CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.71 (s, 2H, CH_2–COO–), 3.77 (s, 3H, –OCH_3), 5.36 (s, 2H, CH_2–C_6H_5), 6.75–6.78 (d, J = 8.7 Hz, 1H, Ar–H), 6.70 (s, 1H, Ar–H), 7.14–7.17 (d, J = 8.7 Hz, 1H, Ar–H), 7.31–7.42 (m, 6H, Ar–H), 7.63–7.66 (d, J = 8.4 Hz, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 7.99–8.02 (d, J = 8.4 Hz, 1H, Ar–H); IR (KBr) 1752 cm^{-1} (–C=O, ester); FAB-MS *m/z* 448 $[M + H]^+$, Anal. cald. for $C_{26}H_{22}ClNO_4$; C, 69.72; H, 4.95; N, 3.13; Found: C, 69.89; H, 4.95; N, 3.11.

*[1-(4-Chloro-benzoyl)-5-methoxy-2methyl-1H-indol-3-yl]-acetic acid 4-nitro phenyl ester (**IIh**)*

Yield 78%; ^{1}H NMR($CDCl_3$) δ 2.48 (s, 3H, CH_3), 3.71 (s, 2H, –CH_2–COO–), 3.95 (s, 3H, OCH_3), 6.69–6.72 (d, J = 9.3 Hz, 2H, Ar–H), 6.86–6.89 (d, J = 9.3 Hz, 2H, Ar–H), 7.03 (s, 1H, Ar–H), 7.47–7.50 (m, 2H, Ar–H), 7.47–7.50 (d, J = 7.8 Hz, 2H, Ar–H), 8.24–8.27 (d, J = 8.4 Hz, 2H, Ar–H); FAB-MS *m/z* 479 $[M + H]^+$; IR (KBr) 1740 cm^{-1} (–C=O, ester); Anal. cald. for $C_{25}H_{19}ClN_2O_6$; C, 62.70; H, 4.00; N, 5.85; Found: C, 62.80; H, 4.10; N, 5.90.

*1-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]–acetic acid furan-2-yl methyl ester (**IIi**)*

Yield 84%; ^{1}H NMR($CDCl_3$) δ2.35 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 3.80 (s, 2H, CH_2-CO), 3.99 (s, 3H, OCH_3), 6.36–6.39 (d, J = 8.4 Hz, 2H, Ar–H), 6.65–6.68 (d, J = 8.7 Hz, 2H, Ar–H), 6.86–6.96 (m, 2H, Ar–H), 7.38–7.48 (m, 2H, Ar–H), 7.64–7.66 (d, J = 8.4 Hz, 2H, Ar–H); IR (KBr) 1680 cm^{-1} (C=O, ester); FAB-MS *m/z* 439 $[M + H]^+$; Anal. cald. for $C_{24}H_{20}ClNO_5$; C, 65.83; H, 4.60; N, 3.20; Found: C, 65.90; H, 3.10; N, 3.27.

*[1-(4-Chloro-benzoyl)-5-methoxy-2methyl 1H-indol-3-yl] acetic acid 4-chloro-phenyl ester (**IIj**)*

Yield 76%; ^{1}H NMR($CDCl_3$) δ 2.45 (s, 3H, CH_3), 3.84 (s, 2H, –CH_2–CO–)3.97 (s, 3H, OCH_3), 6.78 (d, J = 9.3 Hz, 2H, Ar–H), 6.89–6.92 (d, J = 9.0 Hz, 2H, Ar–H), 7.06 (s, 1H, Ar–H), 7.10–7.32 (m, 2H, Ar–H), 7.45–7.48 (d, J = 8.1 Hz, 2H, Ar–H), 7.65–7.68 (d, J = 8.1 Hz, 2H, Ar–H); IR (KBr) 1750 cm^{-1} (–C=O, ester); FAB-MS *m/z* 469 $[M + H]^+$; Anal. cald. for $C_{25}H_{19}Cl_2NO_4$; C, 64.11; H, 4.09; N, 2.99; Found: C, 64.44; H, 4.09; N, 2.89.

*[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H- indol-3-yl] acetic acid pentachlorophenyl ester (**IIk**)*

Yield 58%; ^{1}H NMR($CDCl_3$) δ2.46 (s, 3H, CH_3), 3.84 (s, 2H, –CH_2CO–), 4.12 (s, 3H, OCH_3), 6.68–6.71 (d, J = 9.0 Hz, 1H, Ar–H), 7.47–7.49 (d, J = 7.8 Hz, 2H, Ar–H), 7.65–7.68 (d, J = 7.8 Hz, 2H, Ar–H), 7.65–7.68 (d, J = 7.8 Hz, 2H, Ar–H); IR (KBr) 1680 cm^{-1} (–C=O, ester); FAB-MS *m/z* 607 $[M + H]^+$; Anal. cald. for $C_{25}H_{15}C_{l6}NO_4$; C, 49.54; H, 2.49; N, 2.31; Found: C, 49.58; H, 2.49; N, 2.39.

Pharmacology

Pharmacological tests were carried out on adult male Sprague–Dawley rats (100–150 g) to evaluate the anti-

inflammatory and ulcerogenic properties of the panel of indomethacin derivatives. Animals were obtained from the Animal House, Central Drug Research Institute (CDRI), Lucknow, India. The animals were housed 5 per cage and kept on standard laboratory diet with free access to water.

Carrageenan-induced rat paw edema method

The inhibitory activity of the compounds on carrageenan induced rat paw edema was performed according to method of (Winter and Porter 1957). Sprague–Dawley rats of either sex, weighing between 100–150 g, and fasted overnight were taken and divided into thirteen groups with five animals in each group. These animals were humanely restrained and 100 µl of 1% w/v carrageenan solution in sterile normal saline (0.9% w/v NaCl) was injected into the sub plantar site of the right hind footpad by inserting the needle bevel down through the callus at an angle nearly parallel to the footpad. The paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) at 0, 1.5, and 3 h after carrageenan injection. Indomethacin and its esters suspended in 2% gum acacia were administrated at 10 mg/kg orally, as a standard and test, respectively. The percentage inhibition of edema was calculated using the formula, Percent edema inhibition $= [1-(V_t/V_c)] \times 100$ where, V_t and V_c are mean edema volume in the drug treated and control groups, respectively. The differences were compared using one-way ANOVA followed by Dunnett's test. P values of 0.05 were considered significance.

Ulcerogenic activity

Eight hours after the oral administration of indomethacin and test compounds, the rats were sacrificed under ether anesthesia. Their stomachs were removed, opened through greater curvature, washed under running water, and fixed in saline solution. The degree of ulcerogenicity was determined by viewing the gastric epithelial ulceration with a magnascope at five magnifications and rated by ulcer score. Ulcer score was used to grade the incidence and severity of the lesions such as (i) Shedding of epithelium—10 (ii) Petechial and frank hemorrhages—20 (iii) One or more ulcers—30 (iv) More than two ulcers—40 (v) Perforated ulcers—50 (srivastava *et al.*, 1991).

Colorimetric COX inhibitor screening assay

The percentage of COX-1 and COX-2 inhibition was measured using the Colorimetric COX (ovine) Inhibitor Screening Assay Kit, Catalog no. 760111 supplied by Caymen Chemicals, USA. The Colorimetric COX (ovine) Inhibitor Screening Assay utilizes the peroxidase component of cyclooxygenase. The peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. The preparation of reagents was followed the instructions given with the assay kit (Catalog no. 760111).

Procedure

(1) Background wells—160 µl of Assay Buffer and 10 µl of heme were added to 96-well plates.
(2) The 100% Initial activity wells—150 µl of Assay Buffer, 10 µl of heme, and 10 µl of enzyme (COX-1 or COX-2) were added.
(3) Inhibitor wells—150 µl of assay buffer, 10 µl of heme, and 10 µl of enzyme (COX-1 or COX-2) were added.
(4) The 10 µl of inhibitors (20 µM) were added to the inhibitor wells and 10 µl of solvent (DMSO) was added to the 100% Initial activity wells and background wells.
(5) The plate was shaken for a few seconds and incubated for 5 min at 25°C.
(6) The 20 µl of colorimetric substrate solution (TMPD) was added to all the wells.
(7) The 20 µl of arachidonic acid was added to all the wells.
(8) The plate was shaken for a few seconds and incubated for 5 min at 25°C.
(9) The absorbance was measured at 590 nm using the Bioteck instrument.

Calculations

The calculations were carried out in the following manner:

(1) The average absorbance of all the samples was determined.
(2) The absorbance of the background wells was subtracted from absorbance's of the 100% Initial activity and the inhibitor wells
(3) Percent inhibition = (100% Initial activity−Inhibitor wells/100% Initial activity) × 100

Results and discussion

The anti-inflammatory effects were estimated for the panel of synthesized compounds and indomethacin using the carrageenan induced rat paw edema method. The results are shown in Table 1. Out of eleven compounds, compound **IIc** showed enhanced anti-inflammatory activity (19.88%) at 10 mg/kg over indomethacin (13.86%) as a

Table 2 Ulcer index for compound **IIc** and indomethacin

No.	Compound	Ulcer index
1	Compound IIc	0
2	Indomethacin	$5 \times 10 = 50$

Table 3 Inhibitory activity of the compound **IIc**, indomethacin and celecoxib against COX-2 and COX-1 enzymes

No.	Compound	COX-2 inhibitory activity (%)	COX-1 inhibitory activity (%)
1	Compound IIc[a]	62.0	12.9
2	Indomethacin[a]	76.0	71.0
3	Celecoxib[a]	95.3	2.0

[a] The mean of the duplicate values for each sample was taken

reference drug. Other compounds, such as **IIa**, **IIb**, and **IId** showed moderate activity (7.83, 9.04, and 7.23%). Compounds **IIe**, **IIi**, **IIj**, and **IIk** inhibited the edema in carrageenan-induced rat- paw edema model at 0 and 1.5 h. Unfortunately, these four compounds lost their anti-inflammatory activity at the end of 3 h. This data indicates that compounds containing non-polar rings along with optimum number of alkyl groups showed improved anti-inflammatory activity. Conversely, compounds containing polar ring or non-polar rings lost their anti-inflammatory activity together with electronegative groups.

Compound **IIc** was therefore subjected for further evaluation to check its ulcerogenic property. It was observed from the present study that compound **IIc** did not promote observable ulceration in the stomachs of rats. However, the reference compound indomethacin promoted ulcers in the stomachs of rats. A total of five areas of shedding of epithelium were observed in rats administrated with indomethacin. The results are given in Table 2. Furthermore, it was analyzed that the ulceration can be reduced via masking the free carboxylic group into an ester derivative.

Further, colorimetric COX inhibitor screening assay was employed to characterize compound **IIc** together with reference compounds. Compound **IIc**, indomethacin, and celecoxib were tested at a concentration of 20 μM in the assay. Compound **IIc** was found to be active against COX-2 and COX-1 enzymes exhibiting 62.0 and 12.9% inhibition, respectively (Table 3). However, indomethacin has produced 76 and 71% inhibition against COX-2 and COX-1 enzymes, respectively. It can thus be concluded that compound **IIc** displays more selective inhibition against COX-2 enzyme than COX-1 enzyme.

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