Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(14):94-99
(http://www.derpharmachemica.com/archive.html)

Synthesis, Cyclooxygenase Inhibition, Anti-inflammatory Evaluation and Gastric Liability of Some Novel Indole Derivatives as Selective COX-2 Inhibitors

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ABSTRACT

A new group of (4-substitutedphenyl)(3-((2-(4-substitutedphenyl)hydrazono)methyl)-1H-indol-1-yl)-methanone derivatives 13a-f as indomethacin analogs was synthesized through N-benzoylation of indole-3-cabaldehyde with the appropriate benzoyl fragment followed by reaction with substituted phenylhydrazine. All the synthesized compounds were evaluated in vitro for Cyclooxygenase (COX-1/COX-2) inhibitory activity and in vivo for their anti-inflammatory activity in comparison with the parent drug indomethacin. Compounds 13a, b, d, e which, contain SO_2Me or SO_2NH_2 group as a pharmacophore of COX-2, exhibited the most anti-inflammatory and selectivity actives so, they were more evaluated by calculating their ED_{50}% doses and ulcerogenic indices to ensure their gastric safety margin relative to indomethacin.

Keywords: NSAIDS, Cyclooxygenase, Indomethacin, Anti-inflammatory activity, Ulcerogenicity

INTRODUCTION

Nonsteroidal Anti-inflammatory Drug (NSAIDs) is among the most widely used therapeutics. Through their anti-inflammatory, anti-pyretic and analgesic activities, they represent a choice treatment in various inflammatory diseases, especially arthritis, as well as relieving the pains of everyday life [1,2]. Their activity usually arises from inhibition of Cyclooxygenase (COX) enzyme, which mediates the bioconversion of arachidonic acid to inflammatory Prostaglandins (PGs) and Thromboxane's (TXs) [3,4]. Cyclooxygenase enzyme exists in two distinct isoforms, a constitutive form (COX-1) and an inducible one (COX-2). COX-1 is expressed constitutively in many tissues and PGs produced by COX-1 mediate the "housekeeping" functions such as cytoprotection of gastric mucosa, regulation of renal blood flow and platelet aggregation. In contrast, COX-2 is not detected in most normal tissues, but its expression is rapidly induced during pathological processes such as inflammation and various cancer types [5-7]. Despite of their activity, many of NSAIDs, such as aspirin (1), ibuprofen (2) and indomethacin (3), have pronounced side effects such as gastrointestinal and renal toxicity resulting from the inhibition of gastro protective PGs synthesized through COX-1 pathway [8,9]. Thus, it was though those more selective COX-2 inhibitors would have reduced side effects. Based upon a number of selective COX-2 inhibitors such as celecoxib (4), rofecoxib (5) and valdecoxib (6) were developed as safer NSAIDs with improved gastric safety profile. However, the recent market removal of some COXIBs such as rofecoxib and valdecoxib due to their adverse cardiovascular side effects clearly encourages the researchers to explore and evaluate alternative templates with COX-2 inhibitory activity [10-12].

In recent studies, novel series of indomethacin analogs 7a-f [13] and 8a-h [14] were synthesized which were approved as good COX-2 selective inhibitors (Figures 1 and 2). So, these results encouraged us to continue the research on such type of compounds. We now describe the synthesis, *in vitro* evaluation as COX-1/COX-2 inhibitors, *in vivo* anti-inflammatory (AI) activity, and ulcerogenic liability for a new series of N-substituted indole derivatives as indomethacin analogs 13a-f in which, (i) the $–CH_2COOH$ moiety in position 3 of indomethacin was replaced with an aromatic moiety containing phenyl hydrazine substituted with COX-2 pharmacophore, SO_2Me in 11a, d or SO_2NH_2 in 11b, e or with methyl group in 11c, f to evaluate the effect of these groups on COX selectivity and anti-inflammatory activity, (ii) the chlorobenzoyl moiety of indomethacin in position 1, which is important for anti-inflammatory activity, is maintained in 11d, 11e, 11f or replaced with benzoyl in 10a, 10b, 10c and (iii) methyl group at position 2 and methoxy group at position 5 was removed in all compounds.

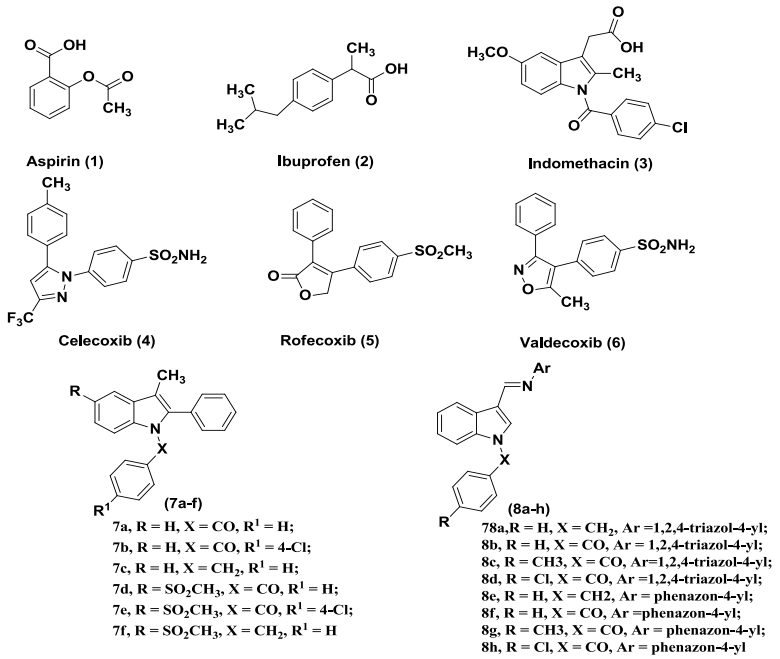


Figure 1: Chemical structures of some traditional NSAIDs (1-3), some selective COX-2 inhibitor drugs (4-6) and reported indomethacin analogs (7a-f and 8a-h)

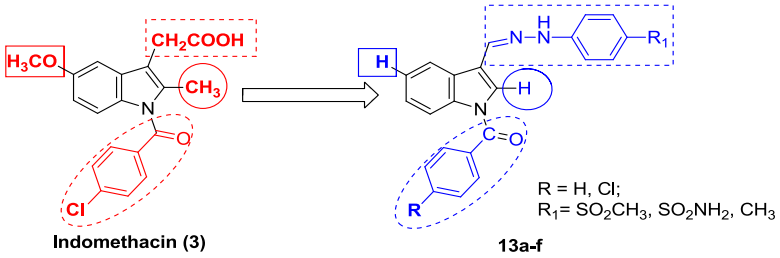


Figure 2: Chemical structures of indomethacin (3) and the designed N-substituted indole derivatives (13a-f)

MATERIALS AND METHODS

Instrument and reagents

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on KBr plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker Avance III 400 MHz spectrophotometer, Faculty of Pharmacy, Beni-Suef University, Egypt in Deuterated Dimethyl Sulfoxide (DMSO-d_6) with Tetramethylsilane (TMS) as the internal standard, where J (coupling constant) values are estimated in Hertz (Hz) and chemical shifts were recorded in ppm on δ scale. Mass Spectra (MS) were recorded on Hewlett Packard 5988 spectrometer. Microanalyses for C, H and N were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the regional center for mycology and Bio-technology, Al-Azhar University, Egypt. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-120 mesh). All other reagents, indole (9) and *p*-tolylhydrazine hydrochloride (12c) were purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

Chemistry

Indole-3-carboxaldehyde (10) [15,16], 1-benzoyl-1*H*-indole-3-carbaldhyde (11a) [17]; 1-(4-chlorobenzoyl)-1*H*-indole-3-carbaldhyde (11b) [18]; (4-methylsulphonylphenyl) hydrazine hydrochloride (12a) [19] and (4-aminosulphonylphenyl) hydrazine hydrochloride (12b) [19] were prepared according to the reported procedures.

General procedure for the synthesis of (4-substitutedphenyl) (3-((2-(4-substitutedphenyl)-hydrazono) methyl)-1H-indol-1-yl)-methanone (13a-f)

A mixture of 11a or 11b (0.3 g, 1 mmol) and the appropriate phenyl hydrazine hydrochloride derivative 12a-c (1.2 mmol) in absolute ethanol (10 ml) and glacial acetic acid (1 ml) was refluxed for 5-7 h (monitored by TLC). The precipitate that formed on hot was filtered off, dried and recrystallized from 95% ethanol to afford 13a-f.

(3-((2-(4-(methylsulfonyl)phenyl)hydrazono)methyl)-1H-indol-1-yl)(Phenyl)-methanone (13a)

Yellow solid; Yield 72%; mp. 191-193°C; IR (KBr) 3298 (NH), 3059, 3024 (CH aromatic), 2924, 2854 (CH aliphatic), 1685 (C=O), 1597 (C=N), 1323, 1138 (SO_2) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=3.12 (s, 3H, SO_2CH_3), 7.22 (d, 2H, *J*=8.8Hz, phenyl H-3, H-5), 7.48 (d, 2H, *J*=9.2Hz, phenyl H-2, H-6), 7.64 (d, 2H, *J*=8Hz, indole H-5, H-6), 7.71 (s, 1H, indole H-2), 7.76 (d, 2H, *J*=8.8Hz, benzoyl H-3, H-5), 7.81-7.86 (m, 3H, benzoyl H-2, H-4, H-6), 8.17 (s, 1H, CH=N), 8.33 (d, 1H, *J*=8.8Hz, indole H-7), 8.46 (d, 1H, *J*=8.8Hz, indole H-4), 10.93 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ=44.78 (SO_2CH_3), 111.78, 116.35, 118.28, 122.94, 125.19, 126.16, 127.70, 129.31, 129.49, 129.61, 129.66, 130.06, 132.75, 134.24, 136.25, 136.86, 149.62(CH=N), 168.58(C=O); MS *m/z* (ES+) 417.48 (M^+). Anal. Calcd for $C_{23}H_{19}N_3O_3S$: C, 66.17; H, 4.59; N, 10.07. Found: C, 65.89; H, 4.71; N, 10.34.

4-(2-((1-benzoyl-1H-indol-3-yl)methylene)hydrazinyl)benzenesulfonamide (13b)

Brown solid; Yield 65%; mp. 211-213°C; IR (KBr) 3429, 3321 (NH_2), 3290 (NH), 3116, 3062 (CH aromatic), 2924, 2854 (CH aliphatic), 1662 (C=O), 1597 (C=N), 1327, 1153 (SO_2) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=7.08 (s, 2H, NH_2, D_2O exchangeable), 7.16 (d, 2H, *J*=8.8Hz, phenyl H-2, H-6), 7.35 (d, 2H, *J*=7.6Hz, phenyl H-3, H-5), 7.44-7.62 (m, 5H, indole H-2, H-5, H-6, benzoyl H-3, H-5), 7.71-7.77 (m, 3H, benzoyl H-2, H-4, H-6), 8.14 (s, 1H, CH=N), 8.32 (d, 1H, *J*=9.2Hz, indole H-7), 8.44 (d, 1H, *J*=9.2Hz, indole H-4), 10.74 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ=44.78 (SO_2CH_3), 111.35, 116.29, 118.25, 122.96, 125.14, 126.18, 126.74, 128.01, 129.36, 129.48, 129.74, 131.60, 133.07, 133.64, 135.14, 136.19, 148.12(CH=N), 167.59(*C*=O); MS *m/z* (ES+) 418.47 (M^+). Anal. Calcd for $C_{22}H_{18}N_4O_3S$: C, 63.14; H, 4.34; N, 13.39. Found: C, 63.25; H, 4.46; N, 13.61.

Phenyl(3-((2-(p-tolyl)hydrazono)methyl)-1H-indol-1-yl)methanone (13c)

Yellow solid; Yield 80%; mp. 146-148°C; IR (KBr) 3298 (NH), 3028 (CH aromatic), 2954, 2912, 2854 (CH aliphatic), 1662 (C=O), 1543 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=2.23 (s, 3H, CH_3), 7.00 (d, 2H, *J*=7.6Hz, phenyl H-2, H-6), 7.08 (d, 2H, *J*=8Hz, phenyl H-3, H-5), 7.47 (d, 2H, *J*=7.6Hz, indole H-5, H-6), 7.63 (d, 2H, *J*=7.6Hz, benzoyl H-3, H-5), 7.71-7.77 (m, 2H, benzoyl H-4, indole H-2), 7.80 (d, 2H, *J*=7.6Hz, benzoyl H-2, H-6), 8.02 (s, 1H, CH=N), 8.32 (d, 1H, *J*=6.8Hz, indole H-7), 8.45 (d, 1H, *J*=6.8Hz, indole H-4), 10.19 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ=20.75 (*C*H_3), 112.24, 116.34, 119.06, 122.98, 125.00, 125.99, 127.46, 128.04, 128.14, 129.26, 129.59, 130.10, 132.03, 132.60, 134.34, 136.84, 143.65(CH=N), 168.49(*C*=O); MS *m/z* (ES+) 353.42 (M^+). Anal. Calcd for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89. Found: C, 77.89; H, 5.86; N, 12.04.

(4-chlorophenyl)(3-((2-(4-(methylsulfonyl)phenyl)hydrazono)methyl)-1H-indol-1-yl)methanone (13d)

Buff powder; Yield 81%; mp. 202-204°C; IR (KBr) 3302 (NH), 3093, 3051 (CH aromatic), 2924, 2839 (CH aliphatic), 1678 (C=O), 1593 (C=N), 1327, 1091 (SO_2) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=3.12 (s, 3H, SO_2CH_3), 7.22 (d, 2H, *J*=8.8Hz, phenyl H-3, H-5), 7.49 (d, 2H, *J*=9.2Hz, phenyl H-2, H-6), 7.70 (d, 2H, *J*=8.4Hz, indole H-5, H-6), 7.76 (d, 2H, *J*=8.8Hz, benzoyl H-3, H-5), 7.84-7.89 (m, 3H, benzoyl H-2, H-6, indole H-2), 8.16 (s, 1H, CH=N), 8.31 (d, 1H, *J*=8.8Hz, indole H-7), 8.42 (d, 1H, *J*=8.8Hz, indole H-4), 10.95 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ=44.82 (SO_2CH_3), 111.80, 116.62, 118.40, 123.05, 125.27, 126.54, 127.91, 129.45, 129.58, 129.87, 130.00, 130.14, 132.95, 134.52, 136.28, 137.00, 149.65 (CH=N), 168.78 (C=O); MS *m/z* (ES+) 451.93 (M^+). Anal. Calcd for $C_{23}H_{18}ClN_3O_3S$: C, 61.13; H, 4.01; N, 9.30. Found: C, 61.42; H, 4.18; N, 9.56.

4-(2-((1-(4-chlorobenzoyl)-1H-indol-3-yl)methylene)hydrazinyl)benzene-sulfonamide (13e)

Reddish brown solid; Yield 78%; mp. 140-142°C; IR (KBr) 3436, 3332 (NH_2), 3294 (NH), 3089, 3051 (CH aromatic), 2924, 2850 (CH aliphatic), 1678 (C=O), 1593 (C=N), 1329, 1153 (SO_2) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=7.08 (s, 2H, NH_2, D_2O exchangeable), 7.16 (d, 2H, *J*=8.8Hz, phenyl H-2, H-6), 7.49 (d, 2H, *J*=8.8Hz, phenyl H-3, H-5), 7.69-7.72 (m, 3H, indole H-2, H-5, H-6), 7.84-7.87 (m, 4H, benzoyl H-2, H-3, H-5, H-6), 8.13 (s, 1H, CH=N), 8.33 (d, 1H, *J*=9.2Hz, indole H-7), 8.43 (d, 1H, *J*=9.2Hz, indole H-4), 10.77 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ=111.47, 116.37, 118.42, 123.02, 125.38, 126.24, 126.85, 128.05, 129.40, 129.52, 129.88, 131.60, 133.12, 133.71, 135.23, 136.22, 148.27 (CH=N), 167.82 (C=O); MS *m/z* (ES+) 452.91 (M^+). Anal. Calcd for $C_{22}H_{17}ClN_4O_3S$: C, 58.34; H, 3.78; N, 12.37. Found: C, 58.62; H, 3.91; N, 12.48.

(4-chlorophenyl)(3-((2-(p-tolyl)hydrazono)methyl)-1H-indol-1-yl)methanone (13f)

Yellow solid; Yield 76%; mp. 192-194°C; IR (KBr) 3286 (NH), 3032 (CH aromatic), 2939, 2912, 2854 (CH aliphatic), 1654 (C=O), 1543 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ=2.23 (s, 3H, CH_3), 7.00 (d, 2H, *J*=8Hz, phenyl H-2, H-6), 7.07 (d, 2H, *J*=8Hz, phenyl H-3, H-5), 7.46 (d, 2H, *J*=7.6Hz, indole H-5, H-6), 7.68-7.73 (m, 3H, benzoyl H-3, H-5, indoleH-2), 7.83 (d, 2H, *J*=8.4Hz, benzoyl H-2, H-6), 8.00 (s, 1H, CH=N), 8.34 (d, 1H, *J*=6.8Hz, indole H-7), 8.45 (d, 1H, *J*=7.2Hz, indole H-4), 10.22 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ=20.75 (*C*H_3), 112.24, 116.37, 119.25, 123.01, 125.10, 126.06, 127.49, 128.06, 128.14, 129.37, 130.11, 131.55, 131.94, 133.23, 136.84, 137.36, 143.61(CH=N), 167.53(*C*=O); MS *m/z* (ES+) 387.86 (M^+). Anal. Calcd for $C_{23}H_{18}ClN_3O$: C, 71.22; H, 4.68; N, 10.83. Found: C, 70.96; H, 4.60; N, 11.09.

Biological evaluation

Experimental animals

Adult male Wister albino rats (120-150 g) were obtained from the animal house, (Nahda University, Beni-Suef, Egypt) were used throughout the study and were kept at controlled conditions (temperature 27 ± 2°C, humidity 60 ± 10%) and a 12/12 h light/dark cycle. The animals were housed in stainless steel cages, divided into groups of four animals each and deprived of food not water 24 h before the experiment. All procedures relating to animal care and treatments were conducted in accordance with protocols approved by the Research Ethical Committee of Faculty of Pharmacy Beni-Suef University (2017-Beni-Suef, Egypt).

COX-1/COX-2 inhibition colorimetric assay

The ability of tested compounds listed in Table 1 was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to the previous reported method [20,21]. This assay directly measures PGF2α that was produced by stannous chloride reduction of COX derived PGH2 by enzyme immunoassay.

Carrageenan-induced rat paw edema assay

The anti-inflammatory activity of newly synthesized indomethacin derivatives was evaluated by using carrageenan-induced rat paw edema test [22]. Rats were divided into 9 groups (4 animals per each group) then, they were administered with a suspension of vehicle, tested compounds or indomethacin in 10% DMSO at a dose of 10 mg/kg orally (one group per one compound). After 30 min, the rats received 100 µl of carrageenan (1% in saline) subcutaneously on the sub plantar region of the left hind paw. The left paw thickness was measured after 1, 3 and 6 h after carrageenan injection. The right hind paw served as a reference of non-inflamed paw for comparison. Results are expressed as percentage decrease in edema thickness induced by carrageenan. Compounds 13a, 13b, 13d, 13e and indomethacin were experimented for calculating ED50 values by using at least three doses and the paw thickness was measured after 3 h after carrageenan injection.

Ulcerogenic liability

The most potent Compounds 13a, 13b, 13d, 13e and indomethacin were experimented for their ulcerogenic liability according to the reported method [23]. Rats were divided into 6 groups of 5 animals each, and then were fasted for about 18h before drug administration. The 4 tested compounds and indomethacin as a reference drug were given orally at a dose of 10 mg/kg suspended in 10% DMSO while, remaining group received DMSO as a control negative group. Treatment was continued once daily for 3 successive days in all groups. At fourth day, 1 h after the last dose, animals were sacrificed under general anesthesia and stomachs were removed, collected, opened along the greater curvature, washed with distilled water and rinsed with saline. The gastric mucosa of each stomach was examined for the presence of lesions by using magnifying lens (10X). The number of mucosal lesions which appeared as red spots was counted, and their severity was determined and graded from 0-4. The following parameters were calculated:

1. % Incidence/10=[Number of rats showing ulcer of any grade divided by total number of rats in the group × 100]/10.
2. Average number of ulcer=Number of ulcer in the group/ total number of rats in the group.
3. Average severity =∑ [each ulcer multiplied by its score of severity/number of ulcer in the group.
4. Ulcer index=the sum of the above three parameters.

(% Incidence/10+Average number of ulcer+Average severity); Ulcer index value was compared to that of indomethacin.

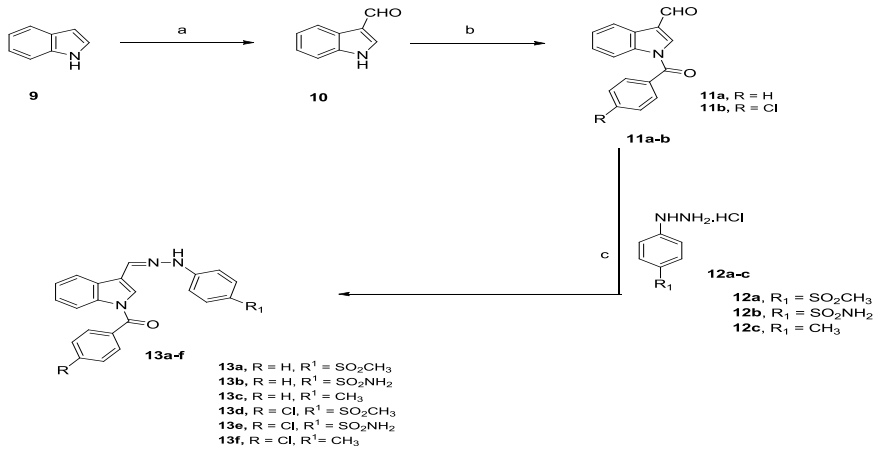
RESULTS AND DISCUSSIONS

Chemistry

The synthesis of the new compounds (4-substitutedphenyl)(3-((2-(4-substitutedphenyl)hydrazono)methyl)-1H-indol-1-yl)methanone derivatives was achieved through using the reaction sequence illustrated in Scheme 1. The starting material indole-3-carbaldehyde 10 was prepared in a good yield (70%) via Vilsmeier reaction and then reacted with benzoyl or *p*-chlorobenzoyl chloride in dry Dimethylformamide (DMF) under basic condition using NaH to give compounds 11a-b. Compounds 11a-b were allowed to react with 4-methylsulfonylphenylhydrazine hydrochloride 12a, 4-aminosulphonylphenylhydrazine hydrochloride 12b or 4-methylphenylhydrazine hydrochloride 12c in absolute ethanol under reflux conditions to give target compounds 13a-f in good yields (65-80%).

All the newly synthesized compounds 13a-f have been characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra, and elemental analyses. The IR spectra of these compounds showed the appearance of a sharp singlet peak at 3302-3286 cm^{-1} corresponding to NH group, two sharp peaks at 1654-1685 and 1543-1597 cm^{-1} corresponding to C=O and C=N respectively. While, compounds such as 13a, b, d, e exhibited two sharp peaks at 1323-1329 and 1138-1153 cm^{-1} corresponding to SO_2, in addition to a forked peak at 3436-3429 and 3332-3321 cm^{-1} corresponding to NH_2 for compounds 13b, e.

Also, ^{1}H-NMR spectra for indole derivatives showed singlet peak at δ=3.12 or 7.08 or 2.23 corresponding to SO_2CH_3 for compounds 13a, d or SO_2NH_2 for compounds 13b, e or CH_3 for compounds 13c, f. Additionally, all compounds exhibited two singlet peaks at δ=8.00-8.17 and 10.19-10.95 corresponding to CH=N and NH respectively. Finally, ^{13}C-NMR spectra showed peak at δ=44.78-44.82 corresponding to SO_2CH_3 for compounds 13a, d, peak at δ=20.75 for CH_3 for compounds 13b, e and absence of aliphatic carbons for compounds 13c, f. Two other peaks appeared at δ=143.61-149.65 and 167.53-168.78 corresponding to CH=N and C=O for all final compounds.



Reagents and reaction conditions: (a) $POCl_3$, dry DMF, reflux, 8 h; **(b)** appropriate acid chloride, NaH, DMF, RT, overnight; **(c)** abs.EtOH, gl.acetic acid, refux, 5-7 h.

Scheme 1: (4-substitutedphenyl)(3-((2-(4-substitutedphenyl)hydrazono)methyl)-1H-indol-1-yl)methanone derivatives

***In vitro* cyclooxygenase (COX) inhibition assay**

The *in vitro* COX-1/COX-2 isozymes inhibition studies for the new indomethacin analogs 13a-f revealed a reversal of COX selectivity profile compared to indomethacin. The newly synthesized compounds showed relatively weak inhibition of COX-1 subtype with IC_{50} values 6.7-10.1 μM while, they were highly potent inhibitors of COX-2 subtype with IC_{50} values 0.19-0.53 μM consequently compounds 13a-f were highly COX-2 selective with COX-2 selectivity indexes (S.I. 12.64-53.16) in comparison with standard indomethacin (COX-1 IC_{50}=0.039 μM, COX-2 IC_{50}=0.49 μM and COX-2 S.I.=0.079) (Table 1).

Data from Table 1 revealed that (i) all tested compounds 13a-f exhibited more potent inhibition for COX-2 than COX-1, (ii) compounds having the SO_2Me or SO_2NH_2 as COX-2 pharmacophore (13a, d with S.I. 37.83 and 51.11 respectively and 13b, e with S.I. 35.42 and 53.16 respectively) were more potent inhibitors of COX-2 than the corresponding analogs containing CH_3 (13c-f with S.I. 12.64 and 14.79 respectively) and that confirms the importance of SO_2Me for COX-2 selectivity, (iii) compounds having chloro benzoyl moiety at indole N (13d, e, f with S.I. 51.11, 53.16, 14.79 respectively) exhibited higher potency for COX-2 than that having benzoyl one (13a, b, c with S.I. 37.83, 35.43, 12.64 respectively) and (iv) within all compounds 13a-f, compounds 13e was the most potent COX-2 inhibitors and the most COX-2 selective and it was about 650 folds more COX-2 selective than indomethacin (COX-2 IC_{50}=0.49 µM, S.I.=0.079).

Table 1: *In vitro* COX-1 and COX-2 inhibition for compounds 13a-f and reference drug (Indomethacin)

Compounds	COX inhibition (IC_{50} µM)[a]		Selectivity Index[b]
	COX-1	COX-2	
13a	8.7	0.23	37.83
13b	8.5	0.24	35.42
13c	6.7	0.53	12.64
13d	9.2	0.18	51.11
13e	10.1	0.19	53.16
13f	7.1	0.48	14.79
Indomethacin	0.039	0.49	0.079

[a]The concentration of test compound produce 50% inhibition of COX-1, COX-2 enzyme, the results are the mean of two value obtained by assay of enzyme kits obtained from (Cayman Chemicals Inc., Ann Arbor, MI, USA) where the deviation from the mean is<10% of the mean value; [b]Selectivity index (COX-1 IC_{50}/COX-2 IC_{50})

***In vivo* anti-inflammatory activity**

The anti-inflammatory activity of the prepared indomethacin derivatives 13a-f was evaluated using carrageen-induced rat paw edema test in comparison to indomethacin as a reference drug. Each compound was administered orally (10 mg/kg) immediately prior to induction of inflammation by carrageenan subcutaneous injection. The anti-inflammatory activity was then calculated based on paw-thickness changes at 1, 3 and 6 h after carrageenan injection as presented in Table 2.

A comparable study of the anti-inflammatory activity of the test compounds relative to indomethacin as a reference drug at different time intervals indicated that; after 1 h, the indomethacin derivatives (13a-f) showed an intermediate edema inhibition activity between 41.6-58.7% and compounds 13d, e were the most potent derivatives (56.7, 58.7% edema inhibition for 13d and 13e) in comparison with indomethacin (56% edema inhibition). After 3 h, 13a-f showed a remarkable increase in edema inhibition percentage activities 60.6-87.2% and compound 13e was also the most potent derivatives (87.2% edema) in comparison with indomethacin (86.7% edema inhibition). After 6 h, all compounds showed a little increase in edema inhibition percentage activities 65.8-91.5%, while indomethacin showed a much increase in edema inhibition percentage activity 95.1%.

The results, seen in Table 2, were consistent with the *in vitro* results and in a similar manner to *in vitro* data, the *in vivo* data indicated the same conclusions; (i) the presence SO_2Me or SO_2NH_2 moiety (13a, d and 13b, e) increases the anti-inflammatory activity for this class of compounds, (ii) 4-chlorobenzoyl is favorable over unsubstituted benzoyl for substitution at indole N, (iii) also, within all compounds 13a-f, the most potent COX-2 inhibitor and the most COX-2 selective (13e) was the most potent anti-inflammatory derivative after 3 h of carrageenan injection (91.5% edema inhibition) in comparison with indomethacin (86.7% edema inhibition).

Moreover, ED_{50} values for the most four potent derivatives (13a, 13b, 13d and 13e) were calculated after 3 h from drug administration in comparison with reference drug indomethacin. The four derivatives (13a, 13b, 13d and 13e) showed good anti-inflammatory activities (ED_{50}=0.6, 1.05, 0.48 and 0.22 mg/kg respectively) in comparison with indomethacin ($ED_{50=}$0.4 mg/kg). The most COX-2 selective derivative (13e, about 650 folds more COX-2 selective than indomethacin) was the most potent anti-inflammatory derivative (ED_{50}=0.22 mg/kg=approximately 1.8x potency of indomethacin).

Table 2: % Inhibition of tested compounds (13a-f) at 1, 3, 6 h after carrageenan injection in comparison with indomethacin

Comp	Oedema thickness (mm) ± SEM (oedma inhibition %)[a]			ED_{50} (mg/kg)[b]
	1 h (% inhibition)	3 h (% inhibition)	6 h (% inhibition)	
Control	2.118 ± 0.025	2.215 ± 0.028	1.878 ± 0.029	-
Indomethacin	0.933 ± 0.027 (55.96%)	0.295 ± 0.033 (86.68%)	0.050 ± 0.015 (95.07%)	0.40
13a	0.960 ± 0.014 (54.67%)	0.408 ± 0.023 (81.58%)	0.260 ± 0.027 (86.16%)	0.60
13b	1.013 ± 0.025 (52.17%)	0.400 ± 0.021 (81.94%)	0.215 ± 0.028 (88.55%)	1.05
13c	1.238 ± 0.024 (41.55%)	0.878 ± 0.026 (60.63%)	0.643 ± 0.026 (65.76%)	NDc
13d	0.918 ± 0.017 (56.66%)	0.300 ± 0.012 (86.46%)	0.160 ± 0.014 (91.48%)	0.48
13e	0.875 ± 0.012 (58.69%)	0.280 ± 0.019 (87.24%)	0.176 ± 0.010 (90.63%)	0.22
13f	1.200 ± 0.017 (43.34%)	0.818 ± 0.021 (63.07%)	0.573 ± 0.034 (69.49%)	ND[c]

[a]Data analyzed by one way ANOVA, (n=4), P<0.05, all were significant from control; [b]ED_{50} values are determined at 3 h after oral administration of compounds and expressed in mg/Kg; [c]ND=Not Determined

Ulcerogenic liability test

The most potent anti-inflammatory compounds (13a, 13b, 13d and 13e) were tested for their ulcerogenic liability in comparison with indomethacin (Table 3). The results revealed that, all tested compounds exhibited lower ulcerogenic liability (Ulcer index=8.87, 6.40, 6.97 and 3.9 respectively) in comparison with indomethacin (Ulcer Index=20.2). 13e (the most COX-2 selective derivative with about 650 folds more COX-2 selective than indomethacin and the most potent derivative has approximately 1.8x potency of indomethacin) was also the least ulcerogenic derivative (Ulcer Index=3.9) which approximately 1/5 ulcerogenic liability of indomethacin). The tested compounds (13a, 13b, 13d and 13e) were characterized by the presence of a SO_2Me or SO_2NH_2 moiety (COX-2 pharmacophore) and absence of an acidic center, in contrast to indomethacin which having an acidic center and devoid of a COX-2 pharmcophore moiety. Consequently, these compounds possess more selectivity to COX-2 isozyme and exhibited an excellent gastric safety profile compared to indomethacin which caused a great damage on gastric membrane that could be attributed to the high affinity to COX-1 over COX-2.

Table 3: Ulcer index of tested compounds (13a, b, d, e) in with indomethacin as a reference drug

Compound No.	% Incidence	Average no of ulcer	Average severity	ulcer index
13a	6	1.2	1.67	8.87[a]
13b	4	1.0	1.4	6.4[a]
13d	4	1.4	1.57	6.97[a]
13e	2	0.6	1.30	3.9[a]
Indomethacin	8	9.2	3	20.2

[a]Statistical analysis using one-way ANOVA followed by post hook (Tukey's test) for multiple pair-wise comparison between different compounds and respective indomethacin at significance level P<0.01

DECLARATION OF INTEREST

The authors have declared no conflict of interest.

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