Synthesis of Novel Amide Containing Schiffs Bases of 5-(4-Chloro-Phenyl)-Furan-2-Carboxaldehyde: Their In Vivo Anti-Inflammatory, Antioxidant and Antinociceptive Activities with Ulcerogenic Risk Evaluation

Saqlain Haidera, Mohammad Sarwar Alam*, Hinna Hamida, Sadiq Umarb, Deepak Kumarc, Syed Nazreena

aDepartment of Chemistry, Faculty of Science, Jamia Hamdard (Hamdard University) New Delhi -110 062, India
bDepartment of Toxicology, Faculty of Science, Jamia Hamdard(Hamdard University) New Delhi -110062, India
cDepartment of Pharmaceutical Chemistry, Sikkim Central University, Sikkim-737136, India

ABSTRACT

A library of eighteen amide containing Schiffs bases has been synthesized and screened for their anti-inflammatory, antioxidant and antinociceptive activities. The compound 2 (COX-1 IC₅₀ = 63.23 µM; COX-2 IC₅₀ = 1.80 µM; SI = 35.12) exhibited potent selective COX-2 inhibition as compared to indomethacin (COX-1 IC₅₀ = 3.60 µM; COX-2 IC₅₀ = 7.50 µM; SI = 0.48). The compounds 2 and 7 reduced the COX-2 level to 7.5 ± 0.35 nmole/min/ml and 6.8 ± 0.32nmole/min/ml respectively. The compounds 6 exhibited reduced the TNF-α level to 3.36 ± 0.18pg/ml. The compounds 2, 6, 7, 13 and 17 did not induce any gastric ulceration in comparison to the standard drug indomethacin.

*Corresponding Author: Mohammad Sarwar Alam, E-mail: mmslam@jamiahamdard.ac.in
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**Introduction**

Polysubstituted furans are the pioneers involved in the synthesis of natural and synthetic products [1]. The electron rich system of the furan ring favours its participation in chemical reactions and interactions with different enzymes [2]. Natural products containing furan nucleus have been found to exhibit potent biological activities like antitumor [3], antispasmodic [4] and anti-feeding [5]. Many synthetic furan derivatives have been used in the pharmaceutical industries due to their remarkable properties. Some commonly used furan containing drugs include Cefuroxime (antibacterial), Nitrofurantoin (antibiotic) and Ranitidine (antiulcer) [6]. The furan derivatives have also been found to exhibit anti-inflammatory activity [7] and antioxidant activities [8]. Furan derivatives have also been reported to possess various biological activities such as antihyperglycemic, analgesic, antibacterial and antifungal [9]. Benzofuran-5-carbonyl derivative has been found to exhibit significantly potent antitumor activity [10]. Furan containing chalcones have been reported to exhibit strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* [11]. Furan derivatives are also known to exhibit an immense potential as herbicidal agents [12]. Compounds containing amide linkage have been found to exhibit different activities like anti-inflammatory [13], antitumor [14], antitumor [15], antimicrobial [16] and antinociceptive [17]. The amide-containing Schiff bases have been the focus of attraction of the coordination chemists throughout the world [18-20]. The great diversity in the magnetic, optical and biological properties of this class of compounds is due to the presence of strong donor sites like carbonyl oxygen atom and imine nitrogen atom and their ability to coordinate with metal cations [21]. Heterocyclic compounds bearing amide linkage and Schiff base have been reported to exhibit a broad spectrum of biological activities like anti-inflammatory [22], antidiabetic [23] and antibacterial [24]. Drugs like indomethacin, diclofenac sodium and aspirin have been reported to suppress pain and inflammation [25]. The anti-inflammatory efficacy of these drugs is suppressed by their side effects like gastric ulceration due to the presence of the carboxylic acid group in their structure [26]. Therefore in this study we have protected the carboxylic acid group of (2,4-Dichloro-benzoylamino)-acetic acid by reacting it with 5-(4-Chloro-phenyl)-furan-2-carboxaldehyde. Keeping in view the pharmacological importance of the furan nucleus and the gastric intolerance of the carboxylic acid group the present work has been designed by introducing the amide and the imine linkage between the 5-(4-Chloro-phenyl)-furan-2-carboxaldehyde and (2-Dichloro-benzoylamino)-acetic acid so as to develop novel effective anti-inflammatory molecules with lesser side effects like gastric ulceration. The nonsteroidal anti-inflammatory drugs available in the market have certain adverse effects like ulceration and gastric hemorrhage [27] associated with them. A prolonged consumption of these drugs may cause gastric injuries [28]. The pro-inflammatory cytokines like TNF-α, NO and IL-1β play an imperative role in the inflammatory reactions like tissue destruction, shock and organ failure [29-31]. The aim of this work is to develop new molecules with a potential to inhibit the target specific over expression of the proinflammatory cytokines such as TNF-α, NO and IL-1β without inducing any gastric ulceration.

**Materials and Methods**

**Animals**

Albino Wistar rats of either sex (150–200 g) were obtained from Central Animal House, Hamdard University, New Delhi. The animals were kept in cages at the room temperature and fed with food and water ad libitum. Fourteen hours before the start of the experiment the animals were sent to lab and fed only with water ad libitum. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA).
5-(4-Chloro-phenyl)-furan-2-carboxaldehyde as anti-inflammatory agents

Chemicals

Celecoxib, carrageenan, potassium chloride and carboxymethyl cellulose along with the other chemicals used in the different experiments were purchased from Sigma–Aldrich Chemicals Pvt. Limited, Bangalore, India.

Anti-inflammatory activity

All the synthesized compounds have been tested for their in vivo anti-inflammatory activity using carrageenan-induced hind paw edema method. The rat paw edema was induced by subcutaneous injection of 0.1 ml of 1% freshly prepared saline solution of carrageenan into the right hind paw of rats [32]. The standard drug, celecoxib (0.05 mmole/Kg) was given orally as a positive control. The control group was administered orally with 0.9% of 0.1 ml of saline solution only. The test groups were administered orally with equimolar dosage of the synthesized compounds as the standard drug, 1 h before the administration of carrageenan. The paw volumes were measured using plethysmometer at interval of 3 h and 5 h [33].

Measurement of IL-1β, TNF-α and COX-2

Levels of the proinflammatory cytokines (IL-1β and TNF-α) [34] and COX-2 [35] in the serum have been determined by using commercially available ELISA kits (eBioscience and Cayman, USA). Assays have been performed in duplicate in accordance to the manufacturer’s guidelines. Cytokine concentrations were expressed as picograms of antigen per millilitre of protein.

Measurement of Nitric oxide (NO) level

Animals were sacrificed and their hind paw tissues were washed with PBS (pH 7.4) and placed on ice as described earlier [36]. 50µL of the sample was added to 100 µL of Griess reagent and reaction mixture was incubated for about 5-10 minutes at room temperature and protected from light. The optical density was measured at 540 nm in microplate reader according to the reagent manufacturer’s protocol. Calculations were done after generating a standard curve for sodium nitrite in the same buffer as used for preparation of homogenate.

Estimation of thiobarbituric acid reactive substances (TBARS)

The assay of TBARS was done according to earlier method [37] adapted to microtiter plates by bringing the final volume to 150 µL. In brief, hind paw tissue homogenate was prepared in 0.15 M KCl (5% w/v homogenate) and aliquots of 30 µL were incubated for 0°C and 37°C for 1hr. Subsequently, 60 µL of 28% w/v TCA was added and the volume was made up to 150 µL by adding 60 µL of distilled water followed by centrifugation at 3000xg for 10 min. The supernatant (125 µL) was taken and color development was achieved by addition of 25 µL of 1% w/v TBA dissolved in 0.05 N NaOH and kept in boiling water bath for 15 min. The absorbance was read at 532 nm in a plate reader (Bio-Rad, U.S.A). The result was expressed in µmoles TBARS formed/hr/g tissue using a molar extinction coefficient of 1.56×10⁵ M⁻¹ cm⁻¹.

Reduced glutathione (GSH)

GSH level was measured using the method described earlier [38]. Homogenized hind paw tissue (10% w/v in phosphate buffer pH 7.4) was deproteinized by adding an equal volume of 10% TCA and was allowed to stand at 4°C for 2 h. The contents were centrifuged at 2000xg for 15 min. 50 µL supernatant was added to 200 µL of 0.4 M Tris buffer (pH 8.9) containing 0.02 M EDTA (pH 8.9) followed by the addition 20 µL of 0.01M DTNB. The absorbance was read in a microplate reader at 412 nm and results are expressed as µg GSH/g tissue using a molar extinction coefficient of 13.6×10³ M⁻¹ cm⁻¹.

Antinociceptive activity

Writhing test

The writhing test in mice was carried out using the method of Koster [39]. The writhes were
induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). The standard drug i.e. celecoxib was given orally at a dose 0.05 mmoles/Kg of body weight. The test compounds were administered orally at an equimolar dosage to groups of six animals each, 30 min before chemical stimulus. The numbers of muscular contractions were counted over a period of 20 min after acetic acid injection. The data represents the total number of writhes observed during 20 min and is expressed as writhing numbers.

Ulcerogenic activity

The test compounds having anti-inflammatory & analgesic activities comparable with the indomethacin were further tested for their ulcerogenic risk evaluation \[40\]. This was done at three times higher dose in comparison to the dose used for anti-inflammatory activity, i.e. 0.15 mmoles/Kg body weight of celecoxib and the test compounds were used. Each group had three animals which were later sacrificed after five hours of oral drug administration.

 Experimental

Chemistry

All the chemicals and reagents used in this study were purchased from Merck (India), Spectrochem and Sigma Aldrich (India). All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan); IR spectra were recorded as potassium bromide pellets on a Perkin Elmer 1650 spectrophotometer (USA), \(^1\)H and \(^{13}\)C NMR spectra were measured with a BRUKER spectrometer at 300 and 75 MHz, respectively and chemical shifts were expressed as ppm against TMS as internal reference. Mass spectra were recorded on 70eV (EI Ms-QP 1000EX, Shimadzu, Japan) and Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06e 0.20 mm). The completion of all the reactions was monitored by TLC and the formation of the intermediates and the final products was confirmed from spectroscopic data. Glycine was dissolved in 5 % sodium hydroxide and the solution was stirred at room temperature for 5-10 min. 2,4-dichlorobenzoyl chloride was added drop wise to the above solution and the reaction mixture was stirred for 30 min. This reaction mixture was neutralized by HCL and white solid obtained was filtered and crystallized in warm CCl\(_4\). The (2,4-Dichloro-benzoylamino)-acetic acid (II) thus obtained was refluxed with 5-(4-Chloro-phenyl)-furan-2-carboxaldehyde (I) in presence of CH\(_3\)COONa and (CH\(_3\)CO)\(_2\)O for two hours. The 4-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(2,4-dichloro-phenyl)-4H-oxazol-5-one (III) thus obtained was crystallized in methanol. The 4-[5-(4-Chloro-phenyl)-furan-2-ylmethylene]-2-(2,4-dichloro-phenyl)-4H-oxazol-5-one was refluxed (III) with NH\(_2\).NH\(_2\).H\(_2\)O in ethanol for five hours. The 2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-hydrazinocarbonyl-vinyl]-benzamide (IV) thus obtained was crystallized in methanol. 2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-hydrazinocarbonyl-vinyl]-benzamide (IV) was reacted with different aromatic aldehydes in presence of absolute ethanol for four hours resulting in the formation of eighteen new Schiff bases (Scheme 1).
5-(4-Chloro-phenyl)-furan-2-carboxaldehyde as anti-inflammatory agents

Scheme 1. a) CH₃COONa, (CH₃CO)₂O / Reflux / 2h; b) NH₃·H₂O, C₂H₅OH, reflux, 5h; c) Ar-CHO, C₂H₅OH, reflux, 4h

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(4-fluoro-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (1)

Brown powder; yield 74% ; m. p. 190-191°C; IR (KBr): ν (cm⁻¹) 3135, 3123, 1680, 1425, 1190; ¹H NMR (300 MHz, DMSO): δ 6.99 (s, 1H, H-1), 7.07 (s, 1H, H-3), 7.17 (s, 1H, H-2), 7.28-7.42 (m, 4H, Ar-H), 10.23 (s, 1H, NH-6); ¹³C NMR (75MHz, DMSO): δ 109.59, 116.19, 116.48, 117.78, 126.11, 126.68, 127.65, 128.86, 129.25, 129.61, 129.72, 130.04, 131.42, 132.29, 133.06, 134.63, 135.75, 146.86, 149.88, 153.81, 161.57, 161.92, 165.21; ESI-MS: 556 (M+1); Anal. Calcd. for C₂₇H₁₇Cl₂FN₃O₃: C, 58.24; H, 3.08; N, 7.55. Found C, 58.22; H, 3.10; N, 7.57.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(4-methoxy-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (2)

White powder; yield 79% ; m. p. 203-204°C; IR (KBr): ν (cm⁻¹) 3647, 3292, 1679, 1481, 1303, 1185; ¹H NMR (300 MHz, DMSO): δ 6.34 (s, 1H, H-3), 6.86-6.89 (m, 2H, H-2, H-3), 7.01 (d, 1H, J=8.7 Hz, Ar-H), 7.37-7.49 (m, 4H, Ar-H), 7.95 (s, 1H, H-4), 9.02 (s, 1H, NH-6), 11.44 (s, 1H, NH-5); ¹³C NMR (75MHz, DMSO): δ 55.70, 107.87, 110.30, 114.65, 114.78, 125.23, 125.31, 127.16,
N-[1-(4-Bromo-benzylidene-hydrazinocarbonyl)-2-[5-(4-chloro-phenyl)-furan-2-yl]-vinyl]-2,4-dichloro-benzamide (3)

Creamish yellow powder; yield 75%; m. p. 210-211°C; IR (KBr): v (cm⁻¹) 3786, 3650, 1691, 1591, 1353, 1226; ¹H NMR (300 MHz, DMSO): δ 6.99-7.05 (m, 2H, H-1 & H-2), 7.30 (d, 1H, J=3.6 Hz, H-3), 7.42 (d, 2H, J=8.1 Hz, Ar-H), 7.63-7.80 (m, 9H, Ar-H), 8.38 (s, 1H, H-4), 10.26 (s, 1H, NH-H), 11.61 (s, 1H, NH-5); ¹³C NMR (75MHz, DMSO): δ 109.62, 116.23, 117.48, 117.88, 126.21, 126.48, 127.75, 129.36, 129.45, 129.61, 129.82, 132.24, 132.52, 132.89, 132.26, 133.42, 135.35, 145.66, 146.31, 149.21, 153.62, 162.47, 162.62, 164.31, 167.23; ESI-MS: 614 (M)+, 616 (M+2)⁺; Anal. Calcd. for C₂₂H₁₉BrClN₃O₅: C, 52.50; H, 2.77; N, 6.80. Found C, 52.48; H, 2.79; N, 6.79.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(3-nitro-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (4)

Yellow powder; yield 70%; m. p. 189-190°C; IR (KBr): v (cm⁻¹) 3651, 3621, 1698, 1529, 1353, 1096; ¹H NMR (300 MHz, DMSO): δ 7.01 (s, 1H, H-1), 7.19 (d, 2H, J=3.3 Hz, H-2, H-3), 7.42 (d, 2H, J=8.1 Hz, Ar-H), 7.68 (d, 4H, J=7.8 Hz, Ar-H), 7.72-7.80 (m, 2H, Ar-H), 8.15 (d, 1H, J=7.5 Hz, Ar-H), 8.26 (d, 2H, J=7.8 Hz, Ar-H), 8.54 (s, 1H, H-4), 10.30 (s, 1H, NH-H), 11.92 (s, 1H, NH-5); ¹³C NMR (75MHz, DMSO): δ 109.65, 116.73, 117.98, 121.35, 124.63, 126.13, 126.47, 127.69, 128.83, 129.27, 130.05, 130.92, 131.36, 132.35, 133.09, 133.78, 134.61, 134.61, 135.76, 136.72, 148.73, 149.79, 153.88, 165.25, 171.20; ESI-MS: 582 (M)+; Anal. Calcd. for C₂₂H₁₇Cl₃N₃O₅: C, 55.55; H, 2.94; N, 9.60. Found C, 55.53; H, 2.91; N, 9.58.

N-[1-(Benzylidene-hydrazinocarbonyl)-2-[5-(4-chloro-phenyl)-furan-2-yl]-vinyl]-2,4-dichloro-benzamide (5)

White powder; yield 78%; m. p. 178-179°C; IR (KBr): v (cm⁻¹) 3660, 1195, 1156, 1027; ¹H NMR (300 MHz, DMSO): δ 7.00 (d, 1H, J=3.3 Hz, H-2), 7.08 (s, 1H, H-1), 7.17 (d, 1H, J=3.3 Hz, H-3), 7.40-7.44 (m, 5H, Ar-H), 7.69-7.83 (m, 7H, Ar-H), 8.43 (s, 1H, H-4), 10.27 (s, 1H, H-6), 11.67 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 109.60, 116.53, 117.84, 126.11, 126.68, 127.52, 128.86, 129.27, 130.05, 130.49, 131.43, 132.29, 133.06, 134.64, 134.83, 135.76, 148.00, 149.89, 153.79, 161.57, 162.25; ESI-MS: 537 (M)+; Anal. Calcd. for C₂₂H₁₉ClN₃O₅: C, 60.19; H, 3.37; N, 7.80. Found C, 60.17; H, 3.35; N, 7.78.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(3,4-dimethoxy-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (6)

White powder; yield 68%; m. p. 192-193°C; IR (KBr): v (cm⁻¹) 3637, 1580, 1384, 1332, 1256, 1137; ¹H NMR (300 MHz, DMSO): δ 3.81 (s, 6H, 2xAr-OCH₃), 6.98-7.05 (m, 3H, H-1, H-2, H-3), 7.17-7.21 (m, 2H, Ar-H), 7.34-7.43 (m, 3H, Ar-H), 7.67 (d, 3H, J=8.4Hz, Ar-H), 7.80 (s, 2H, Ar-H), 8.33 (s, 1H, H-4), 10.24 (s, 1H, H-6), 11.51 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 57.45, 58.12, 109.48, 114.76, 116.54, 117.76, 126.25, 126.27, 126.35, 127.40, 128.27, 128.42, 129.54, 130.15, 130.65, 131.24, 132.69, 133.21, 134.34, 134.74, 135.45, 148.21, 149.32, 153.54, 161.68, 162.25, 165.32; ESI-MS: 597 (M)+; Anal. Calcd. for C₂₂H₁₇Cl₃N₃O₅: C, 58.16; H, 3.70; N, 7.02. Found C, 58.18; H, 3.71; N, 7.04.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(4-dimethylamino-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (7)

Yellow powder; yield 73%; m. p. 188-189°C; IR (KBr): v (cm⁻¹) 3828, 3687, 1662, 1154, 1074; ¹H NMR (300 MHz, DMSO): δ 2.98 (s, 6H, N(CH₃)₂), 6.76 (d, 2H, J=7.8 Hz, H-2, H-3), 6.96-7.03 (m, 2H, Ar-H), 7.17 (s, 1H, H-1), 7.41 (d, 2H, J=8.1Hz, Ar-H), 7.53 (d, 2H, J=8.1 Hz, Ar-H), 7.65-7.79 (m, 5H, Ar-H).
2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(2-hydroxy-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (8)

White powder; yield 66%; m. p. 207-208°C; IR (KBr): ν (cm⁻¹) 3655, 3621, 1696, 1535, 1390; ¹H NMR (300 MHz, DMSO): δ 6.90 (s, 1H, H-1), 7.00 (d, 2H, J=3.0 Hz, Ar-H), 7.12-7.19 (m, 2H, H-2, H-3), 7.28-7.33 (m, 1H, Ar-H), 7.41 (d, 2H, J=8.1 Hz, Ar-H), 7.54 (d, 1H, J=7.2 Hz, Ar-H), 7.66 (d, 3H, J=7.8 Hz Ar-H), 7.81-7.87 (m, 2H, Ar-H), 8.61 (s, 1H, H-4), 10.27 (s, 1H, H-6), 11.24 (s, 1H, Ar-ÖH), 11.90 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 109.53, 116.76, 117.99, 119.08, 119.71, 126.01, 127.55, 128.70, 129.13, 129.78, 131.40, 131.70, 133.23, 133.97, 134.39, 135.68, 144.80, 146.99, 153.81, 157.77, 161.22, 165.17; ESI-MS: 554 (M+1)⁺; Anal. Calcd. for C₂₉H₂₆Cl₂N₆O₅: C, 58.45; H, 3.27; N, 7.57. Found C, 58.43; H, 3.25; N, 7.56.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(4-methyl-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (9)

White powder; yield 75%; m. p. 182-183°C; IR (KBr): ν (cm⁻¹) 3284, 3025, 1580, 1717, 1656, 1544, 1375, 1157; ¹H NMR (300 MHz, DMSO): δ 2.35 (s, 3H, Ar-CH₃), 6.98 (d, 1H, J=3.3 Hz, H-2), 7.06 (s, 1H, H-1), 7.16 (d, 1H, J=3.6 Hz, H-3), 7.27 (d, 2H, J=7.5 Hz, Ar-H), 7.41 (d, 2H, J=8.1 Hz, Ar-H), 7.60-7.78 (m, 7H, Ar-H), 8.38 (s, 1H, H-4), 10.21 (s, 1H, H-6), 11.54 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 21.49, 109.59, 116.44, 117.73, 126.11, 127.50, 127.65, 128.87, 129.25, 129.90, 130.04, 131.41, 132.12, 133.04, 134.66, 135.72, 140.31, 148.08, 149.91, 153.76, 161.47, 165.20; ESI-MS: 551 (M)⁺; Anal. Calcd. for C₂₇H₂₀Cl₂N₄O₅: C, 60.83; H, 3.65; N, 7.60. Found C, 60.81; H, 3.63; N, 7.62.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(3-ethoxy-2-hydroxy-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (10)

Yellow powder; yield 65%; m. p. 212-213°C; IR (KBr): ν (cm⁻¹) 3318, 1645, 1367, 1197, 1154; ¹H NMR (300 MHz, DMSO): δ 1.35 (t, 3H, J=6.9 Hz, CH₃), 4.07 (q, 2H, J=6.9 Hz), 6.82-6.87 (m, 1H, H-1), 7.01 (d, 2H, J=7.2 Hz, H-2, H-3), 7.13-7.19 (m, 3H, Ar-H), 7.40 (d, 2H, J=8.1 Hz, Ar-H), 7.66 (d, 3H, J=7.8 Hz, Ar-H), 7.80-7.88 (m, 2H, Ar-H), 8.62 (s, 1H, H-4), 10.27 (s, 1H, H-6), 10.92 (s, 1H, Ar-ÖH), 11.89 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 15.22, 64.44, 109.64, 115.77, 117.00, 118.13, 119.52, 121.45, 126.13, 127.67, 128.82, 129.25, 129.90, 130.10, 131.54, 132.38, 133.10, 134.49, 135.82, 147.53, 147.97, 148.52, 149.83, 153.93, 161.32, 165.29; ESI-MS: 597 (M)⁺; Anal. Calcd. for C₂₉H₂₆Cl₂N₆O₅: C, 58.16; H, 3.70; N, 7.02. Found C, 58.18; H, 3.72; N, 7.04.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(2-nitro-benzylidene-hydrazinocarbonyl)-vinyl]-benzamide (11)

Yellow powder; yield 71%; m. p. 185-186°C; IR (KBr): ν (cm⁻¹) 3678, 3631, 1693, 1094; ¹H NMR (300 MHz, DMSO): δ 7.02 (d, 1H, J=3.6 Hz, H-2), 7.09 (s, 1H, H-1), 7.19 (d, 1H, J=3.3 Hz, H-3), 7.42 (d, 2H, J=8.1 Hz, Ar-H), 7.65-7.80 (m, 7H, Ar-H), 8.07-8.14 (m, 2H, Ar-H), 8.81 (s, 1H, H-4), 10.28 (s, 1H, H-6), 12.02 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 109.66, 116.91, 118.02, 125.11, 126.15, 127.69, 128.63, 128.83, 129.28, 130.07, 131.06, 131.33, 132.28, 133.10, 134.12, 134.59, 135.76, 148.71, 149.80, 153.90, 162.25, 164.24; ESI-MS: 582 (M)⁺; Anal. Calcd. for C₂₉H₁₈Cl₂N₄O₅: C, 55.55; H, 2.94; N, 9.60. Found C, 55.51; H, 2.92; N, 9.58.

2,4-Dichloro-N-[1-(2-chloro-benzylidene-hydrazinocarbonyl)-2-[5-(4-chloro-phenyl)-furan-2-yl]-vinyl]-benzamide (12)

Yellowish green powder; yield 73%; m. p. 206-207°C; IR (KBr): ν (cm⁻¹) 3691, 3629, 1385, 1095, 1048; ¹H NMR (300 MHz, DMSO): δ 7.00 (d, 1H, J=3.3 Hz, H-2), 7.08 (s, 1H, H-1), 7.18 (d, 1H, J=3.3
2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(3,4,5-trimethoxy-benzylidene-hydrazinocarbonyl]-vinyl]-benzamide (13)

White powder; yield 70%; m. p. 213-214°C; IR (KBr): ν (cm⁻¹) 3725, 1677, 1412, 1359, 1293, 1127; ¹H NMR (300 MHz, DMSO): δ 3.71 (s, 3H, 1xAr-CH₃), 3.84 (s, 6H, 2xAr-CH₃), 7.00-7.02 (m, 4H), 7.18 (d, 1H, J=3.0 Hz), 7.42 (d, 2H, J=7.8 Hz), 7.66-7.69 (m, 3H, Ar-H), 7.80 (s, 2H, Ar-H), 8.34 (s, 1H, H-4), 10.27 (s, 1H, H-6), 11.64 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 56.30, 60.47, 104.67, 109.49, 117.70, 125.98, 127.57, 128.74,129.15, 129.92, 130.19, 131.25, 132.11, 132.29, 134.56, 147.89, 149.73, 153.55, 161.35, 165.53; ESI-MS: 627 (M⁺)⁺; Anal. Calcd. for C₇₈H₇₂Cl₃N₁₀O₁₂: C, 57.30; H, 3.85; N, 6.68. Found C, 57.27; H, 3.83; N, 6.67.

2,4-Dichloro-N-[1-(4-chloro-benzylidene-hydrazinocarbonyl)-2-[5-(4-chloro-phenyl)-furan-2-yl]-vinyl]-benzamide (14)

White powder; yield 76%; m. p. 211-212°C; IR (KBr): ν (cm⁻¹) 3331, 3059, 1691, 1463, 1140, 1147; ¹H NMR (300 MHz, DMSO): δ 6.90 (d, 1H, J=3.3 Hz, H-2), 7.06 (s, 1H, H-1), 7.17 (d, 1H, J=3.3 Hz, H-3), 7.41 (d, 2H, J=8.1 Hz, Ar-H), 7.52 (d, 2H, J=7.8 Hz, Ar-H), 7.66-7.78 (m, 7H, Ar-H), 8.40 (s, 1H, H-4), 10.25 (s, 1H, H-6), 11.70 (s, 1H, H-5); ¹³C NMR (75MHz, DMSO): δ 109.59, 117.89, 126.11, 127.66, 128.84, 129.13, 129.25, 129.39, 130.04, 131.41, 132.26, 133.06, 133.75, 134.59, 146.71, 149.82, 153.85, 162.33, 165.45; ESI-MS: 572 (M⁺)⁺; Anal. Calcd. for C₇₈H₇₂Cl₃N₁₀O₁₂: C, 57.55; H, 2.94; N, 9.60. Found C, 55.53; H, 2.95; N, 9.62.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(thiophen-2-ylmethylenehydrazinocarbonyl)-vinyl]-benzamide (17)

Yellowish powder; yield 77%; m. p. 221-222°C; IR (KBr): ν (cm⁻¹) 3231, 3231, 1676, 1422, 1390; ¹H NMR (75MHz, DMSO): δ 109.59, 117.89, 126.11, 127.66, 128.84, 129.13, 129.25, 129.39, 130.04, 131.41, 132.26, 133.06, 133.75, 134.59, 146.71, 149.82, 153.85, 162.33, 165.45; ESI-MS: 572 (M⁺)⁺; Anal. Calcd. for C₇₈H₇₂Cl₃N₁₀O₁₂: C, 55.55; H, 2.94; N, 9.60. Found C, 55.53; H, 2.95; N, 9.62.
5-(4-Chloro-phenyl)-furan-2-carboxaldehyde as anti-inflammatory agents

NMR (300 MHz, DMSO): δ 6.97 (d, 1H, J=3.6 Hz, H-2), 7.12-7.14 (m, 2H, H-1, H-3), 7.22-7.26 (m, 3H, Ar-H), 7.42 (d, 2H, J=4.2 Hz, Ar-H), 7.66-7.71 (m, 3H, Ar-H), 8.16 (d, 2H, J=8.4 Hz, Ar-H), 8.68 (s, 1H, H-4), 10.11 (s, 1H, H-6), 11.50 (s, 1H, H-5); 13C NMR (75MHz, DMSO): δ 109.55, 118.30, 118.72, 125.90, 126.36, 128.33, 128.70, 128.97, 129.10, 129.39, 130.47, 131.23, 132.94, 137.26, 139.65, 143.54, 148.54, 150.30, 153.92, 162.23, 165.33; ESI-MS: 544 (M+1)+; Anal. Calcd. for C25H16Cl3N3O3S: C, 55.11; H, 2.96; N, 7.71; S, 5.89. Found C, 55.13; H, 2.94; N, 7.69; S, 5.91.

2,4-Dichloro-N-[2-[5-(4-chloro-phenyl)-furan-2-yl]-1-(1H-indol-3-ylmethylene-hydrazinocarbonyl)-vinyl]-benzamide (18)

Red powder; yield 69%; m. p. 224-225°C; IR (KBr): ν [cm-1] 3233, 3084, 1678, 1435, 1323, 1124; 1H NMR (300 MHz, DMSO): δ 7.09-7.27 (m, 3H, H-1, H-2), 7.37 (d, 1H, J=3.6 Hz, H-3), 7.50-7.70 (m, 7H, Ar-H), 7.89 (d, 2H, J=8.4 Hz, Ar-H), 8.01-8.08 (m, 2H, Ar-H), 8.36 (d, 2H, J=8.4 Hz, Ar-H), 9.36 (s, 1H, H-6), 11.91 (s, 1H, H-5); 13C NMR (75MHz, DMSO): δ 111.27, 111.50, 114.71, 121.81, 121.99, 122.92, 123.51, 124.53, 126.48, 127.85, 128.46, 129.06, 129.70, 131.75, 134.61, 135.19, 137.27, 137.78, 151.10, 155.92, 157.01, 162.78; ESI-MS: 577 (M+1)+; Anal. Calcd. for C29H16Cl3N4O5: C, 60.28; H, 3.31; N, 9.70. Found C, 60.26; H, 3.29; N, 9.68.

Results

In vivo anti-inflammatory activity

All the synthesized compounds have been tested for their in vivo anti-inflammatory activity by carrageenan-induced hind paw edema model (Figure 1). The results obtained show that the compound 13 exhibited potent anti-inflammatory activity with 62.00% (p<0.001) and 52.00% (p<0.001) inhibition after 3hr and 5hr as compared to indomethacin which showed 67.00% (p<0.001) and 62.00% (p<0.001) inhibition after 3hr and 5hr respectively. The compound 7 exhibited 59.00% (p<0.001) inhibition at 3hr post-carrageenan and 39.00% (p<0.05) inhibition 5hr post-carrageenan administration as compared to indomethacin. Whereas the compounds 2 and 6 showed a time-dependent decrease in the inhibition of inflammation (55.00% and 54.00% inhibition at 3hr post-carrageenan and 42.00% and 49.00% inhibition at 5hr post-carrageenan respectively). The compound 17 showed significant anti-inflammatory activity with 55.00% (p<0.001) and 47.00% (p<0.01) inhibition after 3hr and 5hr respectively.
The structure activity relationship of the synthesized compounds has been analysed on the basis of the nature of the substituents on the aromatic ring of the hydrazone linkage. The compounds having electron donating groups exhibited more potent anti-inflammatory activity as compared to those having electron withdrawing groups on the aromatic ring. The compounds 2, 6, 7 and 13 having electron releasing (-OMe and -NMe$_2$) exhibited more potent activity than those containing electron withdrawing -NO$_2$ group 4, 11 and 16. The compounds 17 and 18 containing heterocyclic rings on the hydrazone linkage exhibited moderate activity. The para substituted halogen containing compounds showed a reduction in the activity with decrease in size (Br>Cl>F) of the halogen i.e 3>14>1.

**In vivo COX Assay**

The compounds exhibiting significant in vivo anti-inflammatory activity were screened for their in vivo anti-inflammatory activity by biochemical selective COX-2 inhibitory assay. Compounds 2, 6, 7, 13 and 17 showed significant COX-2 inhibition as compared to the standard drug indomethacin (Figure 2). The carrageenan induced edema group exhibited a high level of COX-2 i.e. 12.8 ± 0.34 nmole/min/ml. The standard drug indomethacin suppressed the increase in COX-2 level to 7.6 ± 0.16 nmole/min/ml. The compounds 2 and 7 reduced the COX-2 level to 7.5 ± 0.35 nmole/min/ml and 6.8 ±0.32 nmole/min/ml in comparison to the standard drug indomethacin. The COX-2 selectivity of these compounds and their gastric liability may be related to their selective COX-2 inhibition (Table 1).The compound 2 (COX-1 $IC_{50}$ = 63.23 µM; COX-2 $IC_{50}$ = 1.80 µM; SI = 35.12) exhibited potent selective COX-2 inhibition as compared to indomethacin (COX-1 $IC_{50}$ = 3.60 µM; COX-2 $IC_{50}$ = 7.50 µM; SI = 0.48). The COX-1/COX-2 Selective Index (SI value) of the compounds 6, 7, 13 and 17 shows the selective nature of these compounds towards COX-2 inhibition as compared to indomethacin.
5-(4-Chloro-phenyl)-furan-2-carboxaldehyde as anti-inflammatory agents

![Graph showing anti-inflammatory activity](image)

**Fig. 2.** *In vivo* anti-inflammatory activity by COX-2 assay.

**Table 1.** Selective COX-2 inhibitory activity of the schiffs base.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(µM) (COX-1)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(µM) (COX-2)</th>
<th>Selectivity index COX-1/COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>63.23</td>
<td>1.80</td>
<td>35.12</td>
</tr>
<tr>
<td>6</td>
<td>58.22</td>
<td>5.79</td>
<td>10.05</td>
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<tr>
<td>7</td>
<td>51.76</td>
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<td>13</td>
<td>72.43</td>
<td>12.34</td>
<td>5.86</td>
</tr>
<tr>
<td>17</td>
<td>53.82</td>
<td>15.60</td>
<td>3.45</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3.60</td>
<td>7.50</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Values are the means ± SEM from three independent experiments using COX assay kits (Cayman Chemicals Inc., Ann Arbor, MI, USA).

**In vivo TNF-α Assay**

The compounds showing significant *in-vivo* anti-inflammatory activity were further screened for their *in-vivo* TNF-α activity (Figure 3). The standard drug indomethacin suppressed the level of TNF-α to 3.4 ± 0.60 pg/ml from 6.8 ± 0.18pg/ml in the carrageenan injected group. The compounds 6 exhibited potent anti TNF-α activity and reduced the TNF-α level to 3.36 ± 0.18pg/ml. The compound 7 showed an inhibition of 3.4 ± 0.21 pg/ml which was comparable to the standard drug indomethacin.
In vivo IL-1β Assay

The compounds exhibiting significant in vivo anti-inflammatory activity have been screened for their in vivo anti-inflammatory activity by measuring the IL-1β levels in the serum of the animals (Figure 4). Administration of the selected active compounds 2, 6, 7, 13 and 17 suppressed the increase in the level of IL-1β significantly when compared with the edema group in which the level of IL-1β was found to be 5.98 ± 0.16 pg/ml. The compounds 2 and 7 exhibited a reduction of 2.98 ± 0.17 pg/ml and 2.60 ± 0.17 pg/ml respectively in the level of IL-1β in comparison to indomethacin which showed a reduction of 2.87 ± 0.17 pg/ml.

![Graph showing IL-1β levels](image)

Fig. 3. In vivo anti-inflammatory activity by TNF-α assay.

![Graph showing IL-1β levels](image)

Fig. 4. In vivo anti-inflammatory activity by IL-1β assay.
**5-(4-Chloro-phenyl)-furan-2-carboxaldehyde as anti-inflammatory agents**

**In vivo Nitric oxide (NO) Assay**

Analysis of nitrite estimation is summarized in **Figure 5**. A significant increase in level of nitrite was observed in carrageenan induced edema group (9.5 ± 0.18 μmol/mg) as compared to control group (3.94 ± 0.18 μmol/mg). All synthesized compounds declined the increase in the nitrite level significantly as compared to the edema group. **Compound 2** significantly suppressed the increase in the NO level to 5.16 ± 0.12 μmol/mg in comparison to indomethacin which showed a reduction of 5.60 ± 0.16 μmol/mg.

![Fig. 5. In vivo anti-inflammatory activity by nitric oxide assay.](image)

**In vivo antioxidant activity**

The effect of the active compounds **2, 6, 7, 13** and **17** on TBARS (Thiobarbituric acid reactive substances) level was measured to demonstrate the oxidative damage on lipid (**Figure 6**). A significant increase in TBARS level was observed in carrageenan induced edema group (7.9 ± 0.27 nmole) when compared with the control group (3.4 ± 0.17 nmole). The level of TBARS was suppressed to 5.80 ± 0.18 nmole by the compound **16** whereas indomethacin reduced the TBARS level to 6.18 ± 0.15 nmole.
Figure 6. Effect of the active compounds on TBARS.

Figure 7 shows the changes in GSH levels evaluated in the joints of the experimental groups. A marked decrease in GSH was observed in the joint of carrageenan induced (0.63 ± 0.04 µGSH/g tissue) edema rats. However the treatment with compound 17 (0.94 ± 0.04 µGSH/g tissue) significantly inhibited the decrease in GSH as compared to indomethacin (0.88± 0.02µGSH/g tissue).

Fig. 7. Effect of the active compounds on GSH.
In vivo antinociceptive activity

The compounds showing significant in vivo anti-inflammatory activity have been further screened for their in vivo antinociceptive potential by the writhing test method (Figure 8). It was found that the compounds 6 and 17 exhibited 55.78% and 51.47% inhibition respectively in comparison to indomethacin which showed 56.00% inhibition.

Ulcerogenic study

The compounds showing potential in vivo anti-inflammatory and in vivo antinociceptive activity were further tested for their gastric ulceration activity (Figure 9). When compared with indomethacin, compounds 2, 6, 7, 13 and 17 did not induce any gastric ulceration and rupture of the gastric mucosal layer. Hence the gastric tolerance towards the test compounds (2, 6, 7, 13 and 17) was better than that of indomethacin.
Discussion

A focused library of eighteen compounds has been synthesized. All the synthesized compounds have been screened for their in vivo anti-inflammatory. During the inflammatory reactions, large amounts of the proinflammatory mediators are generated which affect the immune system by suppressing the proliferation of T and B cells, as well as cytokine synthesis [41]. Blockade of these molecules results in a reduction of disease severity and bone resorption [42-44]. Proinflammatory cytokines IL-1β, NO and TNF-α as well as COX-2 have an important role in the perpetuation of chronic inflammation and tissue damage during progression of inflammatory disorder [45]. There is a significant increase in the level of TNF-α, IL-1β, NO and COX-2 in carrageenan induced edema rats as compared to the control group animals. The active compounds showing significant in vivo anti-inflammatory activity have therefore been further screened for their in-vivo COX-2, TNF-α, IL-1β and NO inhibitory potential. The anti-inflammatory
activity has been finally screened by using in vivo Nitric oxide (NO) assay. Nitric oxide (NO) is an important signaling molecule, produced as part of the inflammatory response from activated cells and macrophages [46, 47]. An increase in the NO level has been previously reported in synovial fluids of patients suffering from rheumatoid arthritis [48]. In the present study, increased NO levels have been detected in carrageenan group similar to those previously reported in synovial fluids of patients with rheumatoid arthritis. In vivo antioxidant activity has been carried out because lipid peroxidation has been implicated in the pathogenesis of cancer, atherosclerosis, degenerative diseases, and inflammatory arthritis [49]. During lipid peroxidation, lipid peroxyl radicals are produced that can lead to cell membrane damage. Matrix degradation arising from cytokine-stimulated chondrocytes has been shown to be primarily due to lipid peroxidation [50]. Free radical production that occurs during development of arthritis in the articular cartilage leads to decreased GSH (Glutathione) and SOD (Super oxide dismutase) levels as a result of their consumption during oxidative stress and cellular lysis [51-53]. The concentration of GSH was evaluated to estimate endogenous defense against hydrogen peroxide formation. Finally the in vivo antinociceptive activity and gastric risk evaluation of the active compounds has been carried out. The anti-nociceptive activity of the active compounds has been evaluated using chemical method of nociception. Acetic acid induced writhing test is used for detecting both central and peripheral analgesia. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE2 and PGF2α and their levels are increased in the peritoneal fluid of the acetic acid induced mice [54].

Conclusion

We have synthesized a focused library of eighteen amide containing Schiff's base. The compounds 2, 6, 7, 13 and 17 exhibited potent anti-inflammatory and antinociceptive activity. The compound 2 (COX-1 IC₅₀ = 63.23 µM; COX-2 IC₅₀ = 1.80 µM; SI = 35.12) exhibited potent selective COX-2 inhibition as compared to indomethacin (COX-1 IC₅₀ = 3.60 µM; COX-2 IC₅₀ = 7.50 µM; SI = 0.48). The SI values of the other molecules shows that these molecules can be considered as potent anti-inflammatory agents as predicted by their COX-1/COX-2 selective index. The results of in vivo TNF-α activity indicated that the compound 6 exhibited a more potent TNF-α inhibition than the standard drug Indomethacin. The compound was 2 to exhibit potent reduction in the level of NO as compared to indomethacin. The compound 7 exhibited better suppression of IL-1β level than the standard drug Indomethacin. The compounds 2, 6, 7, 13 and 17 also exhibited potent antioxidant activity. The histopathological studies showed that the compounds 2, 6, 7, 13 and 17 did not induce any gastric ulceration and exhibited better gastric tolerance than indomethacin.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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