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Remedial effects of novel 2,3-disubstituted thiazolidin-4-ones in chemical mediated inflammation

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ABSTRACT

Three thiazolidin-4-one derivatives were synthesized, purified and characterized by chromatographic and spectroscopic methods. In the *in vitro* assays, these compounds inhibited reactive oxygen species (ROS), nitrite and cytokine generation in RAW 264.7 murine macrophages and whole blood. These derivatives attenuated carrageenan-induced acute inflammation in rats. The most effective compound **4C** possessed identical anti-inflammatory action at two doses (50 and 100 mg/kg). Further, the effect of compound **4C** on locally induced inflammatory mediators was investigated in carrageenan-induced air pouch inflammation in rats. In this model, compound **4C** inhibited the cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-6 (systemic and local). Additionally, compound **4C** was able to reduce locally elevated prostaglandin-E_2 (PGE_2). Inhibition of leukocyte infiltration by compound **4C** was correlated with reduced locally released myeloperoxidase (MPO). To conclude, compound **4C** corrected the inflammatory condition by negative effect on cytokine (TNF-α, IL-6) network and prostaglandin-E_2 generation.

1. Introduction

Tissue injury or insult resulting from a disturbed homeostatic environment ventures towards an uncontrolled inflammatory state which is the hallmark of several diseases like rheumatoid arthritis, atherosclerosis, metabolic disorders, obesity, etc. [1]. Inflammatory mediators play a major role in propagation of inflammatory disorders. Unchecked inflammation disturbs the balance between protective and destructive physiological mechanisms of the human body. Nonsteroidal anti-inflammatory drugs (NSAIDs) modulate the inflammatory disorder by reducing prostaglandins synthesis through cyclo-oxygenase (COX) isoenzyme inhibition. However, these drugs have the propensity to cause gastrointestinal and cardiovascular toxicity owing to their direct inhibition of COX-1 and COX-2 isoenzymes, respectively [2–4]. Further, among the checkpoints to control the inflammation [5], targeting immune cells-derived proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 has been found to improve the inflammatory conditions like rheumatoid arthritis [6,7]. Till date, only biologics, i.e. monoclonal antibodies targeting TNF-α, e.g. infliximab, adalimumab and IL-6, e.g. tocilizumab have been successful against chronic inflammatory conditions. However, these biologics are very expensive. They also pose problems of hypersensitivity in certain individuals [6]. Hence, the discovery of novel small molecules having inhibitory effects on pro-inflammatory mediators is still a viable option.

The therapeutic potential of thiazolidin-4-one moiety has attracted several groups of researchers to develop novel derivatives with significant biological activity. Substitutions on this ring resulted in a class of compounds with diverse actions against microbial [8,9] and viral infections [10,11], metabolic disorders [12–14] and inflammation [9,15]. A few 1,3-disubstituted thiazolidin-4-one molecules have been developed and reported as TNF-α antagonist [16]. Previous work from our laboratory has reported the anti-inflammatory activity of N-[2-(3,5-di-tert-butyl-4-hydroxyphenyl)-4-oxo-thiazolidin-3yl]-nicotinamide in animal models of inflammation [15]. These finding led us to develop a few more derivatives with the aim of targeting pro-inflammatory

Abbreviations: COX, cyclo-oxygenase; DCFH-DA, dichlorofluorescein diacetate; DMEM, Dulbecco's minimum essential media; DMSO, dimethyl sulfoxide; DPI, diphenylene iodonium; FBS, fetal bovine serum; IL-6, interleukin-6; L-NAME, L-N^G-nitroarginine methyl ester; LPS, lipopolysaccharide; MPO, myeloperoxidase; p.o., per oral; PBS, phosphate buffer saline; PGE_2, prostaglandin-E_2; RAW 264.7 cells, murine macrophages; ROS, reactive oxygen species; S.E.M., standard error of mean; TNF-α, tumor necrosis factor-α.

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mediators especially TNF-α and IL-6, thereby correlating the effect of compounds on chemical mediated inflammation.

2. Materials and methods

2.1. Chemicals and instruments

All chemical used were purchased from Sigma–Aldrich Co. LLC, St. Louis, MO, USA and Merck KGaA, Darmstadt, Germany. Melting points of the synthesized compounds were determined using Toshniwal capillary melting point apparatus and are uncorrected. Thin layer chromatography was carried out on silica gel pre coated plates (Merck $60F_{254}$) and the spots were visualized under UV lamp (254 or 366 nm) and/or iodine vapor. The absorption spectra, IR spectra were recorded using UV/Vis spectrometer (Model UV-1800, Shimadzu Co., Kyoto, Japan) and IR spectrometer (Model FTIR-8300, Shimadzu Co., Kyoto, Japan) using KBr pellets, respectively. ^{1}H NMR was recorded at 400 MHz (Brucker), $CDCl_3$ as solvent. Mass spectrum was recorded using LC–MS (Model LC-MS-2010A, Shimadzu Co., Kyoto, Japan) (ESI, Mobile phase – methanol:water; 90:10). NMR and Mass spectra were obtained for final compounds and were consistent with assigned structures. Purity of the compounds was established on RP-HPLC unit (Shimadzu Co., Kyoto, Japan) with UV detector (274 nm) using a Hichrom C_{18} (250 × 4.6 mm i.d., 5 μm) column with acetonitrile:aqueous solution (pH 4.0) [65:35] as mobile phase and a flow rate of 1.0 ml/min with a run time of 10 min and column temperature of 30 °C.

2.1.1. General synthetic procedure for p-chlorophenoxyacetic acid derivatives

p-Chlorophenoxyacetic acid was synthesized from p-chlorophenol (0.05 mol) and monochloroacetic acid (0.05 mol). p-Chlorophenoxyacetic acid (0.05 mol) on esterification with ethanol gave ethyl p-chlorophenoxyacetate. Ethyl p-chlorophenoxyacetate (0.1 mol) on reaction with hydrazine hydrate (0.1 mol) gave p-chlorophenoxyacetic acid hydrazide. Schiff's bases were prepared by reaction between p-chlorophenoxyacetic acid hydrazide (0.01 mol) with various aromatic aldehydes (0.01 mol) in presence of catalytic amounts of glacial acetic acid. Three thiazodin-4-ones were synthesized by the method reported by Surrey (Fig. 1) [17]. In brief, the Schiff's base (0.01 mol) and thioglycolic acid/thiolactic acid (0.02 mol) were refluxed in dry benzene. The water formed during cyclization was removed azeotropically. The completion of the reaction was checked chromatographically. After the completion of the reaction, the solvent was distilled off under reduced pressure. The reaction mixture was poured into water containing sodium bicarbonate solution. The solid separated was filtered, dried and recrystallized from methanol. The recrystallized solid was again purified with neutral alumina as stationary phase and acetone:methanol (10:90) as the mobile phase. The purity of all synthesized compounds was checked by HPLC and found to be >95% pure. The structure of the synthesized compounds is as indicated in Table 1.

*2.1.1.1. N-(2-(4-Bromophenyl)-4-oxothiazolidin-3-yl)-2-(4-chlorophenoxy)acetamide [Compound **2L**].* Yield = 92%. m.p. 141–143 °C; IR (KBr); 3448 (—NH), 3051 (Ar, C—H, Str), 2982, 1714 & 1674 (—C═O of —CH_2—C═O & —CONH) 1587 (C═C, Str), 1230 (C—O, Str), 1176, 1058 (C—Cl), 964, 829 & 673 (C—S—C) cm^{-1}, respectively. ^{1}H NMR ($CDCl_3$); δ = 3.05 (s, 2H, CH_2), 3.78 (s, 2H, —CH_2—CO), 5.64 (s, 1H, CH), 6.72 (m, 4H, Aromatic), 7.15 (m, 4H, Aromatic), 7.84 (br s, 1H, NH) ppm; MS (ESI): *m*/*z* = 442.

*2.1.1.2. 2-(4-Chlorophenoxy)-N-(5-methyl-4-oxo-2-(pyridin-2-yl) thiazolidin-3-yl)acetamide [Compound **4C**].* Yield = 74%. m.p. 139–140 °C; IR (KBr); 3159 (—NH), 2966, 1732 & 1691 (—C═O of —CH_2—C═O & —CONH) 1587 (C═C, Str), 1496 (C═N), 1342 (—CH_3), 1238 (—C—O, Str), 1180, 1060 (C—Cl), 997, 829, 754 & 661 (C—S—C) cm^{-1}, respectively; ^{1}H NMR ($CDCl_3$); δ = 1.45 (s, 3H, —CH_3), 3.89 (s, 2H, —CH_2), 6.10 (s, 1H, —CH), 6.52 (m, 4H, Pyridine), 6.90 (m, 4H, Ar), 8.45 (br s, 1H, —NH) ppm; MS (ESI): *m*/*z* = 378.

*2.1.1.3. 2-(4-Chlorophenoxy)-N-(2-(4-methoxyphenyl)-5-methyl-4-oxothiazolidin-3-yl) acetamide [Compound **4D**].* Yield = 75%. m.p. 114–115 °C; IR (KBr); 3281 (—NH), 2935, 1735 & 1683 (—C═O of —CH_2—C═O & —CONH), 1587 (C═C, Str), 1383 (—CH_3), 1244 (C—O, Str), 1165, 1064 (C—Cl), 964, 821,744 & 636 (C—S—C) cm^{-1}, respectively; ^{1}H NMR ($CDCl_3$); δ = 3.24 (s, 3H, —CH_3), 3.97 (s, 3H, —CH—CH_3), 5.94 (s, 1H, —CH), 6.66 (m, 4H, Ar), 7.15 (m, 4H, Ar′), 8.15 (br s, 1H, —NH) ppm; MS (ESI): *m*/*z* = 407.

2.2. In vitro screening assays

2.2.1. Cell culture maintenance

Cell lines derived from murine macrophages (RAW 264.7) were procured from National Centre for Cell Science (NCCS), Pune, Maharashtra, India. The cells were maintained in 25 cm^2 tissue culture flasks in DMEM complete culture medium (90% DMEM and 10% FBS) with 1% penicillin–streptomycin, at 37 °C in a CO_2 incubator (NU-5510E, NuAire Inc., Plymouth, MN, USA), at an atmosphere of 95% air and 5% CO_2. The viability of cells was determined by Trypan blue dye exclusion. Cells with >90% viable were used for the experiments. All *in vitro* studies were performed as three independent experiments in triplicates (*n* = 3).

2.2.2. Cytotoxicity assay

Cytotoxicity of the test compounds was assessed as previously described method using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [18]. In brief, the test compounds **2L**, **4C** and **4D** (10–500 μM, *n* = 3) were added to the respective well seeded with 5 × 10^4 cells/well, in a sterile 96-well plate. After 24 h incubation with compounds, 100 μl of MTT reagent (1 mg/ml in sterile PBS, pH 7.4) was added to all the wells and incubated at 37 °C for another 4 h. Following 4 h of incubation, the plate was inverted to remove the contents and 100 μl of 100% DMSO was added to each well to solubilize the formazan crystals, which are formed due to the reduction of tetrazolium salt by mitochondrial dehydrogenase in viable cells. The optical density was measured at 540 nm using 96-well microplate reader (ELx800, BioTek Instruments Inc., Winooski, VT, USA). Percentage viability was calculated by the formula, i.e. % cell viability = [(OD of test − OD of blank)/(OD of control − OD of blank)] × 100 and the concentration–response curve was plotted by linear regression to determine the IC_{50} of the compounds.

2.2.3. Reactive oxygen species (ROS) and nitrite inhibition assay

In vitro anti-inflammatory potential of test compounds was assessed using the established method with suitable modifications [19]. In brief, vehicle/standard [L-NAME (200 μM) and DPI (5 μM)]/Test compounds i.e. **2L**, **4C** and **4D** (3.125–300 μM) were added to the respective wells (*n* = 3) seeded with 5 × 10^4 cells/well in a 96-well black plate. After 2 h incubation, LPS (10 μg/ml, *Escherichia coli* 0111:B4; Sigma–Aldrich Co. LLC, St. Louis, MO, USA) was added to each well except the media control. The supernatant was collected after 20 h incubation and used for nitrite estimation using Griess reagent [20]. For the intracellular ROS estimation, dichlorofluorescein diacetate (DCFH-DA) (100 μM) was added to the well after removing the supernatant and then incubated for 1 h. After incubation, wells were rinsed with fresh Hank's balanced salt solution and the fluorescence intensity was measured using