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Novel 2,4-dichlorophenoxy acetic acid substituted thiazolidin-4-ones as anti-inflammatory agents: Design, synthesis and biological screening

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ABSTRACT

A library of fourteen 2-imino-4-thiazolidinone derivatives (**1a-1n**) has been synthesized and evaluated for *in vivo* anti-inflammatory activity and effect on *ex-vivo* COX-2 and TNF–α expression. Compounds **1k** (5-(2,4-dichloro-phenooxy)-acetic acid (3-benzyl-4-oxo-thiazolidin-2-ylidene)-hydrazide) and **1m** (5-(2,4-dichloro-phenooxy)-acetic acid (3-cyclohexyl-4-oxo-thiazolidin-2-ylidene)-hydrazide) exhibited *in vivo* inhibition of 81.14% and 78.80% respectively after 5 h in comparison to indomethacin which showed 76.36% inhibition of inflammation without causing any damage to the stomach. Compound **1k** showed a reduction of 68.32% in the level of COX-2 as compared to the indomethacin which exhibited 66.23% inhibition of COX-2. The selectivity index of compound **1k** was found to be 29.00 in comparison to indomethacin showing selectivity index of 0.476. Compounds **1k** and **1m** were also found to significantly suppress TNF-α concentration to 70.10% and 68.43% in comparison to indomethacin which exhibited 66.45% suppression.

Introduction

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[1] It is a protective attempt by the organism to remove the injurious stimuli there by initiating the healing process. Non-Steroidal anti-Inflammatory drugs (NSAIDs) such as indomethacin, ibuprofen, naproxen, and fenbufen are most commonly used for the treatment of inflammation, pain, fever and arthritis.[2] However NSAIDs are associated with adverse effects such as gastrointestinal disorders, ulceration and kidney damage.[3] Therefore the development of new drugs with potent anti-inflammatory activity and reduced side effects is still a great challenge.

4-Thiazolidinones are an important class of compounds which have been found to exhibit, anti-inflammatory,[4–8] analgesic,[9] anti-HIV,[10] anticancer,[11] anti-bacterial,[12] anti-fungal,[13] anti-tubercular,[14] anti-histaminic activities[15] and have also been found out to act as tumor necrosis factor-α antagonists.[16] Of particular importance in the anti-inflammatory potential of thiazolidinones containing compounds.[17] Sharma et al.[18] reported a series of thiazolidinone derivatives and found 2-hydroxyphenyl thiazolidinones as a potential anti-inflammatory and analgesic agent. Ali et al.[6] also reported a series of 4-thiazolidinone derivatives and found 2-imino-4-thiazolidinons as a potent anti-inflammatory as well as TNF-α antagonists. In addition, thiazolidinone has been considered as an effective lead scaffold for anti-inflammatory COX-2 inhibitors. Ottana et al.[19] evaluated 3,3-(1,2-ethanediyl) bis[2-(4-methoxyphenyl)-thiazolidin-4-one] as a new COX-2 inhibitor and showed its ability to attenuate the carrageenan-induced lung injury in experimental models. Considering the biological importance of 4-thiazolidinones as anti-inflammatory agents, we herein report the synthesis of novel 2,4-dichlorophenoxy acetic acid based 4-thiazolidinone derivatives. The synthesized molecules have been subjected to *in vivo* anti-inflammatory evaluation. In order to explore the mechanistic aspects of activity, the synthesized molecules were subjected to *in silico* molecular docking studies with respect to COX-2 as well as TNF-α target. All the synthesized compounds were also evaluated for their *ex-vivo* COX-2 and TNF-α inhibition. The synthesized compounds have been further screened for their anti-nociceptive potential and also evaluated for lipid peroxidation and gastric risk.

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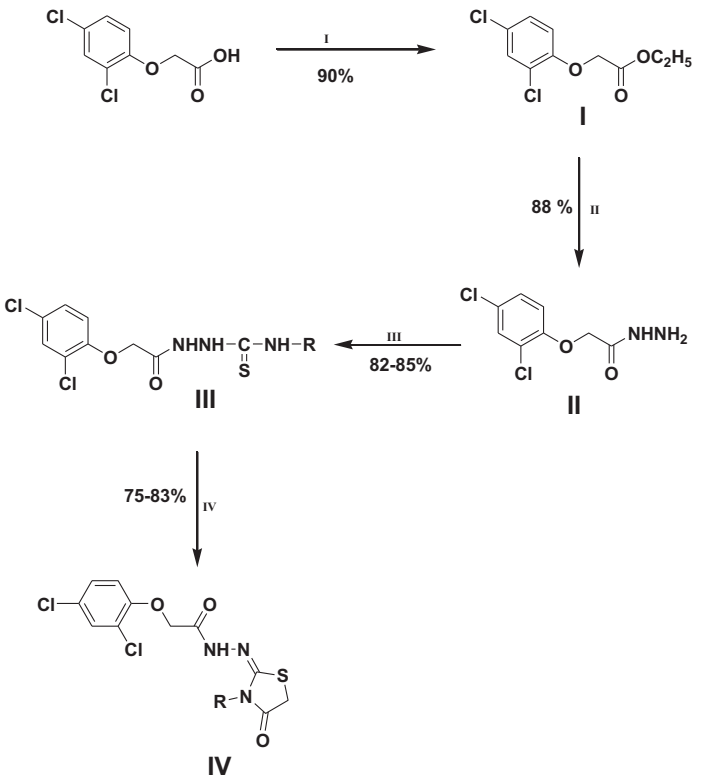
In the present study, the library of 5-(2,4-dichloro-phenoxy)-acetic acid 3-(4-substituted-phenyl-4-oxo-thiazolidin-2-ylidene)-hydrazides were prepared by the condensation of substituted thiosemicarbazides with ethyl chloroacetate in the presence of sodium acetate (Scheme 1).

The formation of the synthesized compounds (**1a-1n**) was confirmed by IR, ^{1}H, ^{13}C NMR and mass spectrometry studies (Supplementary data). The ^{1}H NMR data showed the appearance of NH protons as singlets in the range of δ 8.72–10.65 along with CH_2 (thiazolidinone ring) protons appearing as singlets at δ 3.88–4.25 in the ^{1}H NMR spectra. In the ^{13}C NMR spectra, CH_2 carbon of thiazolidinone ring appeared at δ 31.89–32.98. Other peaks were observed at appropriate values. Further confirmatory evidence was obtained from their mass spectra.

All the synthesized compounds (1a-1n) were evaluated for their *in vivo* anti-inflammatory activity by carrageenan induced rat paw edema model. The results of anti-inflammatory activity are shown in Table 1. Standard drugs indomethacin and celecoxib are well known anti-inflammatory drugs which are already available in the market, so we used these two drugs as standard drugs for comparing anti-inflammatory activities of synthesized compounds. The compounds **1k** and **1m** showed 81.14% and 78.80% inhibition, respectively which was better than the standard drugs indomethacin and celecoxib (76.31% inhibition and 77.67%, respectively) after 5 h. The compounds **1d** (72.22%), **1h** (68.12%), **1j** (68.02%) and **1c** (64.00%) showed anti-inflammatory activity comparable to the standard drug indomethacin.

In order to determine their mode of action, all the synthesized compounds (**1a-1n**) were docked against COX-2 target (PDB No. 3LN1). All docking runs were carried out using Maestro (Schrodinger). However, only nine molecules (**1a**, **1c**, **1d**, **1e**, **1g**, **1h**, **1k**, **1l** and **1n**) could be docked against the target protein. The docking scores of ligands **1h** and **1k** were found to be −7.5 and −8.4 respectively. The molecular binding and interactions of ligands **1h** and **1k** in 3D and 2D space are shown in Fig. 1. Molecule **1h** interacted through H-bonding with the ARG-499 residue, whereas molecule **1k** was found to exhibit hydrophobic interactions and π-π stacking with TYR-341 and ARG-106 residues. The docking score of reference ligands indomethacin and celecoxib were found to be −7.06 and −11.57 respectively. Reference drug celecoxib binds into the binding pocket with specific interactions including H-bond formation with residues like LEU-338, PHE-504 and ARG-499, whereas indomethacin exhibits hydrophobic interactions with target protein as shown in Fig. 1. The glide score and binding energy of all the nine docked molecules have been summarized in Table 2. Although compound 1 m exhibited significant *in vivo* inhibition of inflammation but it could not be docked against COX-2 target. In 1 m molecule, cylcohexyl ring is directly attached to oxo-thiazolidin-ylidene ring, resulting in a rigid bulky structure having restricted rotation. All these factors hinder docking of the molecule into the binding pocket of COX-2 target.

Intrigued by the docking results, *ex-vivo* COX-2 activity of the nine compounds (**1a**, **1c**, **1d**, **1e**, **1g**, **1h**, **1k**, **1l** and **1n**) was determined. Compound **1k** was found to show a higher suppression 68.32% of COX-2 enzyme as compared to the standard drug indomethacin which exhibited 66.23% inhibition but the suppression was slightly less than the other reference drug *viz* celecoxib which showed 72.96% COX-2 suppression. Other compounds showed



Reagents and Conditions: (I) Absolute alcohol, sulphuric acid refluxes; (II) Hydrazine hydrate, absolute alcohol, refluxes; (III) Absolute alcohol, isothiaocyanates, reflux; (IV) ethyl chloroacetate, absolute alcohol, sodium acetate, reflux.

Scheme 1. Protocol for synthesis of title compounds.