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Synthesis, pharmacological screening and in silico studies of new class of Diclofenac analogues as a promising anti-inflammatory agents

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ABSTRACT

A novel series of 5-[2-(2,6-dichlorophenylamino)benzyl]-3-(substituted)-1,3,4-oxadiazol-2(3*H*)-thione (**4a**–**k**) derivatives have been synthesized by the Mannich reaction of 5-[2-(2,6-dichlorophenylamino)benzyl]-1,3,4-oxadiazol-2(3*H*)-thione (**3**) with an appropriately substituted primary/secondary amines, in the presence of formaldehyde and absolute ethanol. Structures of these novel compounds were characterized on the basis of physicochemical, spectral and elemental analysis. The title compounds (**4a**–**k**) were screened for *in vivo* acute anti-inflammatory and analgesic activities at a dose of 10 mg/kg b.w. Compound **4k** exhibited the most promising and significant anti-inflammatory profile while compounds **4a**, **4d**, **4e**, **4i**, and **4j** showed moderate to good inhibitory activity at 2nd and 4th h, respectively. These compounds were also found to have considerable analgesic activity (acetic acid induced writhing model) and antipyretic activity (yeast induced pyrexia model). In addition, the tested compounds were also found to possess less degree of ulcerogenic potential as compared to the standard NSAIDs. Compounds that displayed promising anti-inflammatory profile were further evaluated for their inhibitory activity against cyclooxygenase enzyme (COX-1/COX-2), by colorimetric COX (ovine) inhibitor screening assay method. The results revealed that the compounds **4a**, **4e**, **4g** and **4k** exhibited effective inhibition against COX-2. In an attempt to understand the ligand–protein interactions in terms of the binding affinity, docking studies were performed using Molegro Virtual Docker (MVD-2013, 6.0) for those compounds, which showed good anti-inflammatory activity. It was observed that the binding affinities calculated were in agreement with the IC_{50} values.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most preferred class of drugs in use for the treatment of various pathological conditions such as pain, fever, inflammatory diseases and rheumatoid arthritis.[1] The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme *Cyclooxygenases* (COXs) and *tromboxane synthase* with a varying degree of selectivity.[2] It is well known that COX exists in two isoforms, COX-1 and COX-2, which are regulated differently.[3] COX-1 provides cytoprotection in the gastrointestinal (GI) tract whereas inducible COX-2 mediates inflammation.[4] COX-2 is an attractive target for medicinal chemists as it is expressed only in few normal tissues and is greatly upregulated in inflamed tissues as well as many premalignant and malignant tumors. Since, most of the NSAIDs in the market show greater selectivity for COX-1 than COX-2[5] chronic use of NSAIDs, including Diclofenac may elicit appreciable gastro-intestinal (GI) irritation, bleeding and ulceration.[6] The GI damage from NSAIDs is generally attributed to two factors, local irritation by the carboxylic acid moiety, common to most NSAIDs and (topical effect)

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decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining the GI health and homeostasis.[7] Inflammation is known not only as a symptom of great deal of common diseases but also as an early phase of some serious diseases such as cancer, cardiac vascular diseases and Alzheimer's dementia. Therefore, the challenge still exists for the pharmaceutical industry to develop, effective anti-inflammatory agents with enhanced safety profile.

One of the most important strategies used to overcome NSAIDs side effects is to modify the vital carboxylic acid group, yet maintaining the efficacy of the drug. It has been reported that the derivatization of the carboxylic functional group of some NSAIDs, for example Flufenamic and Meclofenamic acids with a tetrazole group, not only retained COX inhibitory activity of the parent drug, but also introduced *5-lipooxygenase* (5-LOX) inhibition. These drugs also showed an increased anti-inflammatory activity with reduced ulcerogenic effect.[8,9] Several other heterocyclic compounds, including pyrroles, thiazoles, imidazoles, thiadiazoles, di-*tert*-butylphenyloxadiazoles and substituted 1,3,4-oxadiazole derivatives have been studied and proved to be potent COX/5-LOX inhibitors.[10-14]

Diclofenac is one of most widely used anti-inflammatory drug, which suffers from a common toxicity of GI irritation, due to non-selective inhibition of COX enzymes.[15] From literature, it has been observed that the Diclofenac moiety has been extensively exploited by the organic and medicinal chemist to synthesize potential therapeutic agents such as antibacterial, antitumor and antituberculosis etc.[16–18] Further, Bhandari et al.,[19] have reported the synthesis and pharmacological evaluations (anti-inflammatory, analgesic and ulcerogenicity) of novel S-substituted phenacyl-1,3,4-oxadiazol-2-thione and Schiff bases of Diclofenac. Recently, El-Henawy[20] reported the synthesis and molecular docking studies of some novel Diclofenac derivatives containing phenylalanine moiety as selective COX-2 inhibitors.

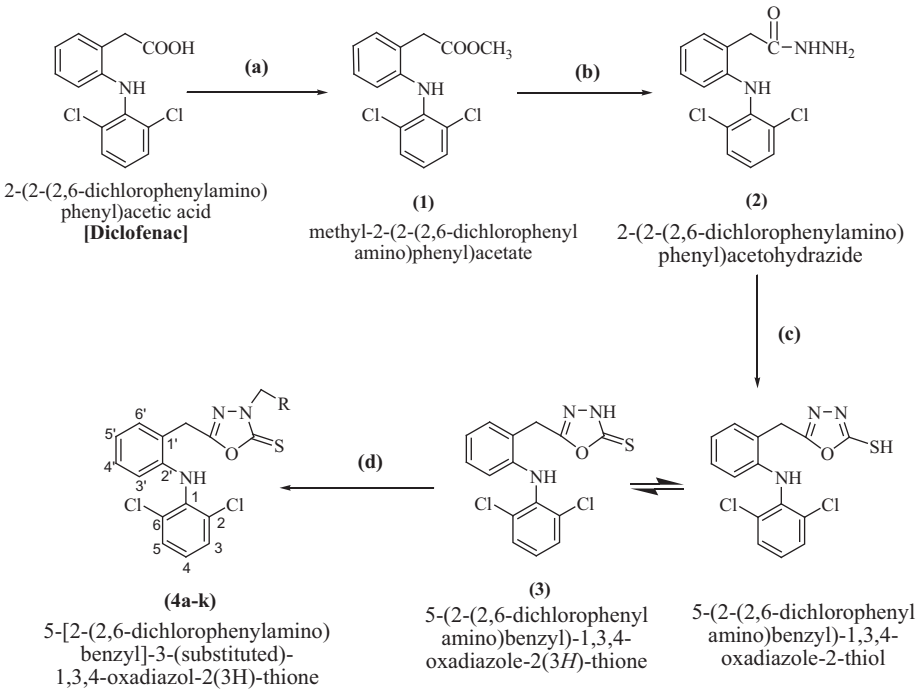
1,3,4-Oxadiazole is an imperative scaffold since several of these derivatives are known to be associated with multiple biological activities such as antiallergic, antibacterial, anticancer, antitubercular and immunosuppressive.[21–27] It has been reported in literature that certain compounds bearing 1,3,4-oxadiazole nucleus possess significant anti-inflammatory activity with reduced ulcerogenic effect.[28–30] The substituted 1,3,4-oxadiazole derivatives serve both as biomimetic and reactive pharamacophores, which are of considerable pharmaceutical interest. Based on the above mentioned studies and in continuation of our attempts[31–34] to develop novel therapeutic anti-inflammatory agents, we herein report the synthesis of a novel series of Mannich base of 1,3,4-oxadiazole derivatives (**4a**–**k**). These compounds have displayed a promising anti-inflammatory and analgesic profile with a significant reduction in their ulcerogenic effect.

2. Results and discussion

2.1. Synthetic and spectral studies

The synthesis of 5-[2-(2,6-dichlorophenylamino)benzyl]-3-(substituted methyl)-1,3,4-oxadiazol-2(3*H*)-thione (**4a**–**k**) derivatives were achieved through convenient and efficient synthetic route as outlined in Scheme 1. The methyl ester of Diclofenac (**1**) was prepared by esterification of 2-[(2,6-dichloroanilino)phenyl]acetic acid (Diclofenac) in absolute methanol and few drops of concd H_2SO_4 under reflux for 8 h. Methyl ester of Diclofenac (**1**) was reacted with hydrazine hydrate (99%) in methanol (anhydrous) and refluxed for 20 h to afford 2-[2-(2,6-dichlorophenylamino)phenyl]acetohydrazide (**2**), which was further treated with carbon disulphide and potassium hydroxide in methanol (anhydrous) to give 5-[2-(2,6-dichlorophenylamino)benzyl]-1,3,4-oxadiazol-2(3*H*)-thione (**3**). Finally, the title compounds (**4a**–**k**) were synthesized in good yield by reacting 5-[2-(2,6-dichlorophenylamino)benzyl]-1,3,4-oxadiazol-2(3*H*)-thione (**3**) with various appropriately substituted primary/secondary amines in formaldehyde. This reaction is an example of the Mannich reaction.[35]

Structures of compound **3** and its Mannich base derivatives (**4a**–**4k**) were established on the basis of their physicochemical (see Table 1), spectral (UV, IR, ^{1}H NMR, ^{13}C NMR) and elemental



Scheme 1. Synthesis of a novel series of 5-[2-(2,6-dichlorophenylamino)benzyl]-3-(substituted)-1,3,4-oxadiazol-2(3*H*)-thione (**4a**–**k**) derivatives. Reagents and Conditions: (a) methanol, concd H_2SO_4, reflux, 2 h; (b) hydrazine hydrate (99%), absolute methanol, reflux, 20 h; (c) carbon disulphide, KOH, methanol, reflux, steam bath, 12 h; (d) primary/secondary amine, formaldehyde, ethanol, gentle reflux, 10–15 h.