Bioorganic & Medicinal Chemistry 21 (2013) 3839–3849

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

New nitric oxide donating 1,2,4-triazole/oxime hybrids: Synthesis, investigation of anti-inflammatory, ulceroginic liability and anti-proliferative activities

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ARTICLE INFO

Article history:
Received 29 January 2013
Revised 30 March 2013
Accepted 8 April 2013
Available online 18 April 2013

Keywords:
1,2,4-Triazole
Nitric oxide donors
Anti-inflammatory
Gastric ulceration
Antiproliferative

ABSTRACT

A series of novel nitric oxide (NO) donating triazole/oxime hybrids was prepared and evaluated for their anti-inflammatory activity. Most of the tested compounds showed significant anti-inflammatory activity using carrageenan-induced rat paw edema method compared to indomethacin. Calculation of the ulcer indices and histopathological investigation indicated that the prepared NO-donating oximes exhibited less ulcerogenicity compared to their intermediate ketones and indomethacin. The NO-donating oxime **6i** revealed significant activity against renal cancer A498 cell lines with 50.52 cell growth inhibition.

1. Introduction

Conventional NSAIDs are the most commonly prescribed medications all over the world. Although the free carboxylic group in most of these compounds is critical for their anti-inflammatory activity,[1] it is responsible for the potential unwanted gastrointestinal discomfort associated with these compounds.[2] Modification of the carboxyl function by the less ulcerogenic bioisosters such as triazoles and oxadiazoles may help to minimize the gastrointestinal upset.[3] Amir et al. reported that cyclization of carboxyl group of diclofenac into the 1,2,4-triazole analogues (Fig. 1) increases both the anti-inflammatory and the analgesic activities with reduction of ulcerogenic liability compared to the parent diclofenac.[4] One of the most important strategies used to overcome NSAIDs side effects is designing nitric oxide-donating NSAIDs (NO-NSAIDs), which are capable of generating the radical biomediator and gastro protective NO.[5,6] It was reported that NO plays several physiological functions in the digestive system[7] such as; increasing the mucosal blood flow[8] which results in enhancement of the mucosal resistance to ulceration,[9] preventing adherence of leukocytes to the vascular endothelium[10] and modulating gastroduodenal secretion of both mucus[11] and bicarbonate.[12] Moreover, NO can profoundly influences the mucosal immune system[7] and increases the ability of ulcerated mucosal cells to undergo healing and repair.[13] Also, the vasodilatation effect of NO is known to spare the renal system through increasing the mucosal blood flow.[14] On the other hand, NO and reactive oxygen species exert multiple modulating effects on inflammation and play a key role in the regulation of immune responses.[15,16]

Additionally, NO can prevent tumor cells from metastasizing and assists macrophage to kill tumor cells.[17] Several targets have been reported for the combination between NO and cancer therapy including; synergistic effect,[18,19] increasing the influx of the anticancer therapy,[20] increasing the efficiency of cytostatic therapy and retardation of drug resistance to anticancer agents.[21]

1,2,4-Triazole derivatives represent an interesting class of heterocyclic compounds, they possess many biological activities such as antimicrobial,[22,23] anti-tubercular,[24] anti-inflammatory,[3,4,25–27] analgesic[4] and anticancer[28–32] activities. Additionally, it was reported that alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazole derivatives exhibits high anti-inflammatory activity with low acute toxicity.[33]

NO-NSAIDs are considered promising anticancer agents, in vitro and in vivo studies indicated that NCX 4040 (Fig. 1) shows a promising anticancer activity, compared to its parent aspirin.[34] Moreover, the NO–profen hybrid (Fig. 1) exhibits significant antiproliferative activity against PC-3 cells. Additionally, several reports indicated that oximation of the carbonyl group in some compounds enhances the anticancer activity several folds compared to their corresponding ketones.[35,36]

Promoted with the above-mentioned studies and as a continuation of our research interest in the synthesis and biological

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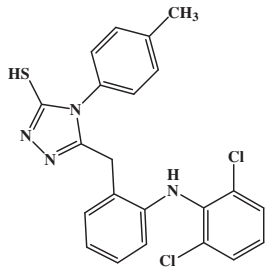
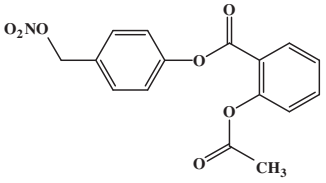
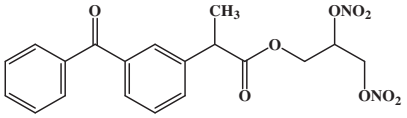
http://dx.doi.org/10.1016/j.bmc.2013.04.022



1,2,4-triazole analogues of diclofenac.



NCX 4040



Ketoprofen-NO hybrid

Figure 1. The structure of 1,2,4-triazole analogues of diclofenac, NCX 4040 and ketoprofen–NO hybrids.

evaluation of NO-NSAIDs derivatives.[37–40] The aim of the present study is gathering the two bioactive entities, the less acidic 1,2,4-triazole-3-thiol and oxime as a NO donor in one compact structure for the purpose of synergism and/or minimizing the expected ulcerogenic side effects. The prepared triazole/NO hybrids are evaluated for their anti-inflammatory activity using carrageenan-induced rat paw edema and compared to the well-known NSAID, indomethacin. Calculation of ulcer indices and histopathological investigation were carried out to assess the beneficial effects of the NO in decreasing ulcer formation. The prepared triazole/NO hybrids were also evaluated for their antiproliferative activity using different cancer cell lines.

2. Results and discussion

2.1. Chemistry

4-*R*-5-Aryl-4*H*-1,2,4-triazole-3-thiol derivatives **4a–l** were synthesized as outlined in Scheme IA according to the reported procedure.[24]

Coupling of 1,2,4-triazole-3-thiol derivatives **4a–l** with phenacyl bromide was achieved in acetonitrile in the presence of TEA afforded the corresponding ketone intermediates **5a–l** in 62–85% yield. Heating at reflux of the ketone intermediates **5a–l** with hydroxylamine HCl in ethanol gave the corresponding oximes **6a–l** in 56–93% yields. The chemical structure of the prepared compounds was elucidated on the basis of their IR, ^{1}H NMR, ^{13}C NMR, mass spectra as well as the elemental analyses.

A characteristic feature of the ^{1}H NMR spectra for oximes **6a–l** is the appearance of a downfield singlet at δ 8.08–11.84 ppm corresponding to the hydroxyl group. The high downfield shifted OH proton may be attributed to the expected intramolecular hydrogen bonding with the sulfur atom. The CH_2 protons appears upfield shifted by δ 0.28–0.63 ppm compared to the CH_2 protons of the corresponding ketones that may be attributed to the low electronegativity of N atom relative to O atom. The ^{13}C NMR spectra of oximes **6d**, **6f**, **6h**, **6k** and **6l** showed the disappearance of the ketonic carbonyl due to its conversion to ketoxime group (C=N–OH). A characteristic feature of the mass spectra of the oximes **6a–l** is the appearance of a very weak abundance for the molecular ion peaks from 0.1% to 11.5% of the respective base peak. Kallury and Rao[41] reported that the abundances of some oximes are very low (less than 4%) of the corresponding base peak.

2.2. Measurement of nitric oxide release

The NO releasing properties of the prepared NO-donating oximes **6a–l** were assessed. The produced nitrite which is a convenient index of nitric oxide production trend was determined in both phosphate buffer of pH 7.4 and 0.1 M HCl buffer of pH 1 by using Griess colorimetric method. The reaction was carried out in the presence of *N*-acetylcysteine as a source of the SH group. The amount of NO released from the tested compounds, was measured relative to NO released from standard sodium nitrite solution and calculated as amount of NO released (mol/mol) and listed in Table 1. The results of measurement of NO release revealed that the NO-donating oximes **6a–l** release NO at pH of 7.4 after 5 h. Compound **6g** that contains 3,4-dimethoxyphenyl moiety released the highest amount of NO among this group (0.25 mol/mol). The results also indicated that NO-donating compounds were not able to release NO at pH 1, which may support the fact that these compounds are weakly hydrolyzed in the gastric lumen and confirms that the suggested gastroprotective action of NO is mediated systemically.[42]

The data of Table 1 indicated that the released amount of NO from oximes **6a–l** was relatively small compared to the previously reported data from other NO-donating hybrids[37–39] and this may be attributed to the expected intramolecular hydrogen bonding between the oxime OH group and the sulfur atom (Fig. 2).

2.3. Biological investigations

2.3.1. Screening of anti-inflammatory activity

The synthesized compounds **5a–l** and **6a–l** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema in rats described by Winter et al.,[43] The tested compounds and the reference drug indomethacin were administered orally at a dose level of 0.28 mmol/kg, 30 min before carrageenan injection at the right hind paw of Albino male rats. The thickness of both paws was measured at different time intervals of 1, 2, 3, 4 and 5 h after carrageenan injection. The anti-inflammatory activity of the tested compounds and indomethacin was calculated as the percentage decrease in edema thickness induced by carrageenan and was determined using the following formula:

$$\% \text{ of edema inhibition} = \frac{(V_R - V_L)_{control} - (V_R - V_L)_{treated}}{(V_R - V_L)_{control}} \times 100$$