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Original article

1,2,4-Triazole/oxime hybrids as new strategy for nitric oxide donors: Synthesis, anti-inflammatory, ulceroginicity and antiproliferative activities

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ABSTRACT

A series of novel nitric oxide (NO) donating triazole/oxime hybrids was prepared and evaluated for their anti-inflammatory activity and antiproliferative 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 ketone intermediates and indomethacin. The NO-donating oximes **7i** and **7k** achieved remarkable cell growth inhibition activity against most of the tested cell lines. Compound **7k** was found to be with high selectivity against CNS subpanel with selectivity ratio of 11.99 at GI_{50} level.

1. Introduction

1,2,4-Triazole derivatives represent an interesting class of heterocyclic compounds, they possess many biological activities such as antimicrobial [1,2], anti-tubercular [3], anti-inflammatory [4–10], analgesic [10] and anticancer activities [11–15]. In recent decades, NO has attracted a tremendous interest in a broad field of basic and applied research as one of the most significant physiological signaling molecule in the body. 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 gastroprotective NO [16,17]. It was reported that NO plays several physiological functions in the digestive system [18] such as; increasing the mucosal blood flow [19] which results in enhancement of the mucosal resistance to ulceration [20], preventing adherence of leukocytes to the vascular endothelium [21] and modulating gastroduodenal secretion of both mucus [22] and bicarbonate [23]. Moreover, NO can profoundly influences the mucosal immune system [18] and increases the ability of ulcerated mucosal cells to undergo healing and repair [24]. Also, the vasodilatation effect of NO is known to spare the renal system through increasing the mucosal blood flow [25]. Additionally, it was reported that alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazole derivatives exhibits high anti-inflammtnqh_0009;atory activity with low acute toxicity [26]. Y.-P. Hou et al. [11] reported that the triazole derivative I (Fig. 1) exhibited potent inhibitory activity against HEPG2 cancer cell line growth. X. Ouyang et al. [12] reported that the triazole derivative II (Fig. 1) exhibited 100% inhibition of tubulin polymerization in vitro and induced G2/M arrest of A431 human cancer cells with EC_{50} similar to Combretastatin A_4.

The application of NO donors as cancer therapeutics is a new venue; the literature provides evidence that metabolic NO release mediates the cytotoxic activities against different cancer cell lines [27–30]. Moreover, NO can prevent tumor cells from metastasizing and assist macrophage to kill tumor cells [31]. Several targets have been reported for the combination between NO and cancer therapy including either synergistic effect between the anticancer drugs and NO [32,33], increasing the influx of the anticancer therapy by NO into intracellular compartments [34], or increasing the efficiency of cytostatic therapy and retard the development of drug resistance to anticancer agents [35].

NO-NSAIDs are considered promising anticancer agents, in vitro and *in vivo* studies indicated that NCX 4040 (Fig. 2) shows a promising anticancer activity, compared to its parent aspirin [36]. Moreover, the NO-ketoprofen hybrid (Fig. 2) exhibits significant

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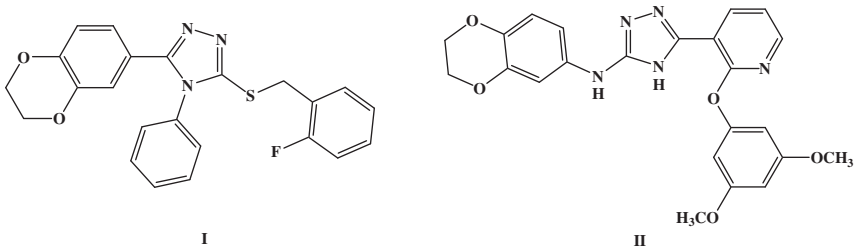


I II

Fig. 1. Structure of some 1,2,4-triazole derivatives that have strong cell growth inhibition.

anti-proliferative 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 [37,38].

Promoted with the above-mentioned studies and as a continuation of our research interest in the field of synthesis and biological evaluation of NO-NSAIDs hybrids [39–41]. 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. A new linker has been designed aiming for improvement of the amount of NO released from these hybrids compared to our recent research work; by avoiding the expected hydrogen bonding in the previous linker [42]. 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 was carried out to assess the beneficial effects of the NO in decreasing ulcer formation. The prepared triazole/NO hybrids were also evaluated for their anti-proliferative activity using different cancer cell lines in order to investigate the possibility of contributing synergistically to their potential antiproliferative effect.

2. Results and discussion

2.1. Chemistry

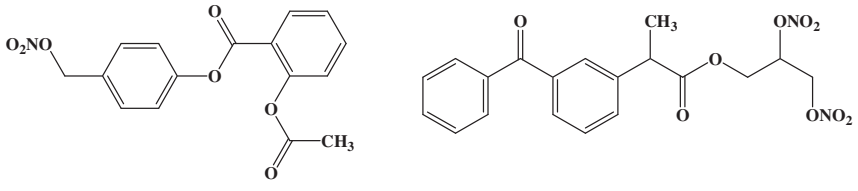
4-Allyl/ethyl/phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives **4a**–**l** were synthesized as outlined in Scheme 1 according to the reported procedure [5].

The synthesis of the target compounds 1-phenyl-2-((4-allyl/ethyl/phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio)ethanone oxime **7a**–**l** is illustrated in Scheme 2.

N-(4-Acetylphenyl)-2-bromoacetamide **5** was prepared in high yield according to the reported procedure [43] through treatment of *p*-aminoacetophenone with bromoacetyl bromide in the presence of potassium carbonate. Heating at reflux of the 1,2,4-triazole-3-thiol derivatives **4a**–**l** with *N*-(4-acetylphenyl)-2-bromoacetamide **5** in acetonitrile in the presence of TEA afforded the corresponding ketone intermediates **6a**–**l** in 62–85% yield. In the ^{1}H NMR spectra for compounds **6a**–**l**; three singlet peaks are common and appeared at δ 10.68–10.70 ppm related to (NH) proton, at δ 4.19–4.24 ppm related (S–$\underline{CH_2}$–CO) and at δ 2.49 ppm which related to (CO–$\underline{CH_3}$). The target NO releasing oxime derivatives **7a**–**l** were prepared in high yield by heating at reflux ketone intermediates **6a**–**l** and hydroxylamine hydrochloride in ethanol. 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 **7a**–**l** is the appearance of downfield singlets in the range of δ 7.76–11.10 ppm, related to the hydroxyl group. The CH_3 protons appeared to be more upfield shifted by 0.23–0.42 ppm than the CH_3 protons of the corresponding ketones due to the low electronegativity of N atom of the oxime relative to O atom of the ketone. The ^{13}C NMR spectra of compounds **7d**, **7g** and **7j** showed one carbonyl group of the amide at δ 166.41–166.72 ppm and disappearance of the ketonic carbonyl due to its conversion to ketoxime group (C=N–OH) which appear at δ 155.98–160.89 ppm. A characteristic feature of the mass spectra of the oximes **7a**–**l** is the appearance of a very weak abundance for the molecular ion peaks from 0.1 to 17% of the respective base peak. Kallury and Rao [44] 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 **7a**–**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 N 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 **7a**–**l** release NO at pH of 7.4 after 4 h. Compound **7g** that contains 3,4-dimethoxyphenyl moiety released the highest amount of this group (0.43 mol/mol). The results of NO



NCX 4040 Ketoprofen-NO hybrid

Fig. 2. NCX 4040 and ketoprofen–NO hybrids.