Bioorganic Chemistry 69 (2016) 48–63

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

# Novel 1,3,4-oxadiazole/oxime hybrids: Synthesis, docking studies and investigation of anti-inflammatory, ulcerogenic liability and analgesic activities

Heba S. Abd-Ellah [a], Mohamed Abdel-Aziz [a], Mai E. Shoman [a,*], Eman A.M. Beshr [a], Tamer S. Kaoud [a,b], Al-Shaimaa F.F. Ahmed [c]

[a] Medicinal Chemistry Department, Faculty of Pharmacy, Minia University, Minia 61519, Egypt
[b] Division of Medicinal Chemistry, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, USA
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Minia University, Minia 61519, Egypt

ARTICLE INFO

*Article history:*
Received 18 July 2016
Revised 13 September 2016
Accepted 16 September 2016
Available online 19 September 2016

*Keywords:*
1,3,4-Oxadiazole
Nitric oxide
Anti-inflammatory
Analgesic
NSAIDs
COX inhibitors
Ibuprofen

ABSTRACT

A novel group of 1,3,4-oxadaiazoles, a group known for their anti-inflammatory activity, is hybridized with nitric oxide (NO) releasing group, oxime, for its gastro-protective action and potential synergistic effect. The synthesized hybrids were evaluated for their anti-inflammatory, analgesic, antioxidant and ulcerogenic activities. Most of the tested compounds showed excellent anti-inflammatory activity with compound **8e** being more active than indomethacin. They also showed moderate analgesic activity but no antioxidant one. The ability of the synthesized compounds to inhibit COX-1 and COX-2 is studied and the prepared compounds were able to inhibit both COXs non-selectively with $IC_{50}$s of 0.75–70.50 μM. Docking studies revealed the mode of interaction of the tested compounds into the empty pocket of the isozymes. All of the synthesized compounds interact with COXs active site with energy scores comparable to that of ibuprofen. All compounds showed a safer profile on the stomach tissue integrity compared to conventional NSAIDs. The designed strategy was applied to ibuprofen to introduce ibuprofen/oxadiazole/NO hybrid. The synthesized ibuprofen hybrid is a promising alternative to ibuprofen having similar anti-inflammatory activity but with safer GIT profile.



## 1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs for treating inflammation, pain, and fever through inhibition of cyclooxygenases (COXs) [1,2]. COXs are the key enzymes responsible for prostaglandin $H_2$ biosynthesis which is the precursor for the inflammatory mediators; prostaglandins, thromboxanes, and prostacyclins [2,3]. COXs are present in two major isoforms COX-1 and COX-2 [3,4]. COX-1 is constitutively synthesized, present in most tissues and plays an important role in physiological homeostasis, while COX-2 is synthesized in response to inflammatory stimuli and present in the site of inflammation [5,6]. In long-term use, NSAIDs are frequently associated with systemic and local gastrointestinal (GI) side effects [7,8]. The systemic side effects are due to non-selective inhibition of COXs, while the free carboxylic functional group is responsible for the local GI tract irritation [9].

Different strategies are adopted to obtain an anti-inflammatory agent devoid of these side effects. Replacing the free carboxylic functional group with different heterocyclic bioisosters such as 1,3,4 oxadiazole [10], 1,2,4-triazole [11,12], and 1,3,4 thiadiazole [12] decreases the gastric upset and enhances the anti-inflammatory activity. Also, selective COX-2 inhibitors such as celecoxib [13], rofecoxib [14], etoricoxib [15], and valdecoxib [16] preserve the cytoprotective prostaglandins that contribute to physiological homeostasis and decrease GI and renal toxicities [17]. Unfortunately, rofecoxib and valdecoxib recently showed serious cardiovascular adverse effects and have been withdrawn from the market [18]. Another promising strategy was applied through designing NSAIDs-hybrids that release nitric oxide (NO) as a gastro-protective mediator that decreases the GI tract damage induced by the parent drug [19]. The NO-NSAIDs hybrids have comparable anti-inflammatory and analgesic activity with more safety profile [20,21]. The mount of nitric oxide released control either it shows pro-inflammatory or anti-inflammatory effects. In low concentrations, NO produced by endothelial nitric oxide synthase (eNOS) has anti-inflammatory activity due to inhibition of the adhesion and migration of inflammatory cells [22]. In contrast,

* Corresponding author.
*E-mail addresses:* Shoman_mai@yahoo.com, mai_shoman@mu.edu.eg (M.E. Shoman).

http://dx.doi.org/10.1016/j.bioorg.2016.09.005
0045-2068/

overproduction of NO by inducible nitric oxide synthase (iNOS) leads to leukocyte infiltration in inflamed tissue and increases vascular permeability [23]. Additional beneficial effects of NO are to keep the integrity of gastric mucosa through increasing mucosal blood flow and enhancing the resistance of the mucosal cell to ulceration [24,25]. NO can also provide the same protective functions of prostaglandins [26]. It increases the mucus and bicarbonate secretion that serve as mucosal defence against injury and helps in ulcer healing [27]. NO is considered an important signalling molecule in cardiovascular system. It prevents platelet aggregation, adhesion of leukocyte to endothelial cells and also preserves vascular physiology [28,29].

Based on aforementioned information, herein, we report the design and synthesis of novel 1,3,4-oxadiazole/oxime hybrids with the oxadiazole ring offering an important pharmacophore that has a promising anti-inflammatory [30,31] and analgesic [32] activities. It also acts as a bioisoster for the free carboxylic group in conventional NSAIDs. Hybridization with oxime group; a NO donating group is also designed for potential synergistic and gastroprotective effect and also to minimize any potential cardiovascular side effects. Additionally, incorporating the designed 1,3,4-oxadiazole/oxime hybrid into ibuprofen is also applied to examine the potential synergistic anti-inflammatory effect and minimize ulcerative profile of the parent ibuprofen.

## 2. Results and discussion

### 2.1. Chemistry

The designed 2-(5-Phenyl-1,3,4-oxadiazol-2-yl)thio)-N-(4-acetylphenyl)acetamides **7a-e** were prepared starting with the esterification of benzoic acid derivatives using usual Fischer esterification [33] followed by hydrazide formation **5a-e**. The hydrazide formed is cyclized using carbon disulfide and potassium hydroxide to yield 1,3,4-oxadiazoles **6a-e** (Scheme 1). The ketonic intermediates **7a-e** are formed through coupling of 1,3,4-oxadiazole derivatives with *N*-(4-acetylphenyl)-2-bromoacetamide **2**. Hybrids of NO releasing compounds **8a-e** are obtained via conversion of the ketone group into an oxime one through a condensation reaction of compounds **7a-e** with hydroxylamine hydrochloride (Scheme 1). Formation of the designed oximes **8a-e** were confirmed through the appearance of a singlet signal of OH group in the offset region (11.09–11.11 ppm) in $^{1}$H NMR and also through shift of ketonic C=O group from (196.79–197.01) to C=N at (152.57–162.76) ppm in $^{13}$C NMR.

A similar procedure was employed to synthesize an ibuprofen/oxadiazole/oxime hybrid **14** (Scheme 2). Ibuprofen/oxadiazole hybrid was synthesized through the cyclization of the corresponding hydrazide **11** using the same previously described procedure. Reaction of oxadiazole with *N*-(4-acetylphenyl)-2-bromoaceta mide **2** gave the corresponding ketone **13**, which is further reacted with $NH_2OH{\cdot}HCl$ to yield the oxime **14**. Formation of the oxime was also confirmed through the appearance of a singlet signal of OH group in the offset region (11.09 ppm) in $^{1}$H NMR and also through shift of the ketonic C=O from (196.95) to C=N at (152.47) ppm in $^{13}$C NMR.

### 2.2. Nitric oxide release

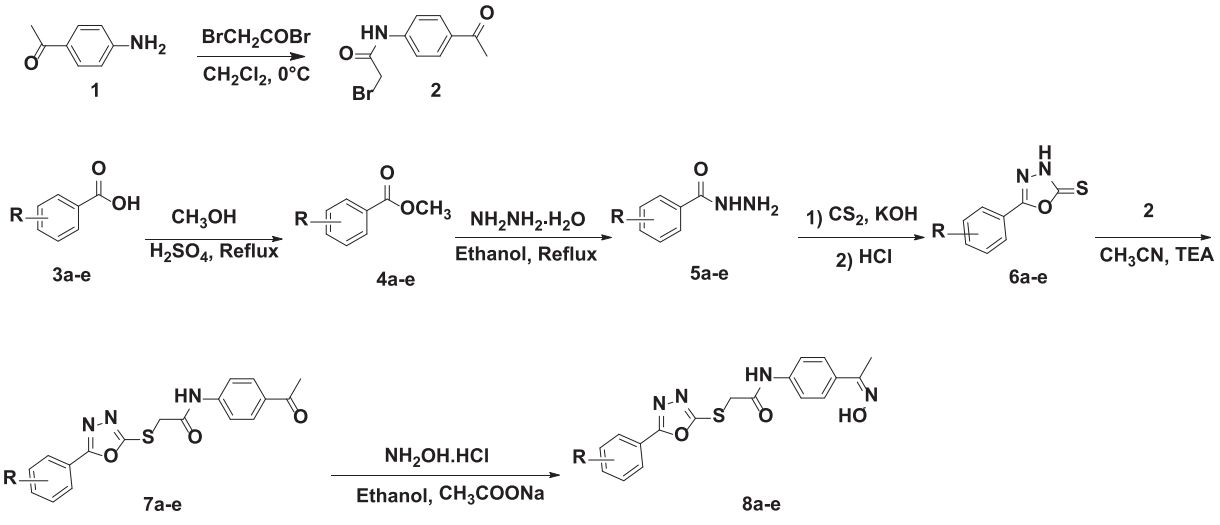
The amount of nitric oxide released from the synthesized compounds **7a**, **8a-e** and **14** was measured using Griess colorimetric method [34] after incubation in either phosphate buffer (pH 7.4) or HCl (0.1 N, pH 1) for 1 h. The amount of NO was measured relative to NO released from standard $NaNO_2$ solution, calculated as percentage of NO released (mol/mol) and listed in Table 1. The results revealed the ability of the tested oximes **8a-e** and **14** to release small amounts of NO at phosphate buffer of pH 7.4 after 1 h (0.37–5.46 mol/mol, Table 1), while the parent ketone **7a** did not show any amount of NO at the specified conditions confirming that oxime group is the only source for NO in the designed structures.

On the other hand, there is no release of NO at pH 1 and this may support the fact that NO-donating moieties (oximes) are weakly hydrolysed in the gastric lumen and confirms that the suggested gastro-protective action of NO is mediated systemically [11,35].

### 2.3. Biological evaluation

#### 2.3.1. Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds **7a-e**, **8a-e**, **-13**, and **14** was tested using carrageenan-induced rat paw oedema method described by Winter et al. [36]. Compounds were




**Scheme 1.** Synthesis of substituted *N*-(4-(1-(hydroxyimino)ethyl)phenyl)-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio) acetamides **8a-e**.