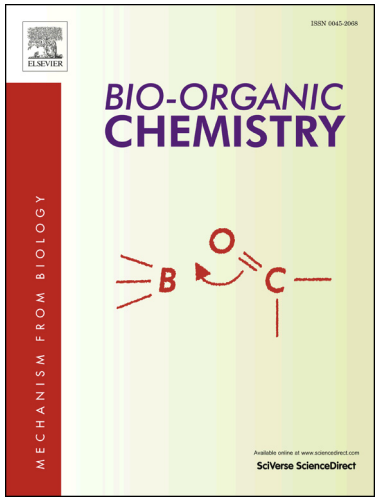


Novel 1,3,4-Oxadiazole/Oxime Hybrids: Synthesis, Docking Studies and Investigation of Anti-inflammatory, Ulcerogenic liability and Analgesic activities

Heba S. Abd-Ellah, Mohamed Abdel-Aziz, Mai E. Shoman, Eman A.M. Beshr, Tamer S. Kaoud, Al-Shaimaa F.F. Ahmed

PII: S0045-2068(16)30136-5
DOI: http://dx.doi.org/10.1016/j.bioorg.2016.09.005
Reference: YBIOO 1951

To appear in: *Bioorganic Chemistry*

Received Date: 18 July 2016
Revised Date: 13 September 2016
Accepted Date: 16 September 2016

Please cite this article as: H.S. Abd-Ellah, M. Abdel-Aziz, M.E. Shoman, E.A.M. Beshr, T.S. Kaoud, A.F.F. Ahmed, Novel 1,3,4-Oxadiazole/Oxime Hybrids: Synthesis, Docking Studies and Investigation of Anti-inflammatory, Ulcerogenic liability and Analgesic activities, *Bioorganic Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bioorg.2016.09.005

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.

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 0f 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.

Key words: 1,3,4-Oxadiazole, Nitric Oxide, Anti-inflammatory, Analgesic, NSAIDs, COX inhibitors, ibuprofen.

*Address correspondence to:

Dr. Mai E. Shoman
Department of Medicinal chemistry
Faculty of Pharmacy, Minia University, Egypt
Email: Shoman_mai@yahoo.com, mai_shoman@mu.edu.eg
Phone: +20 11116757931; Fax: +20 862369075

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, 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 gastro-protective 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

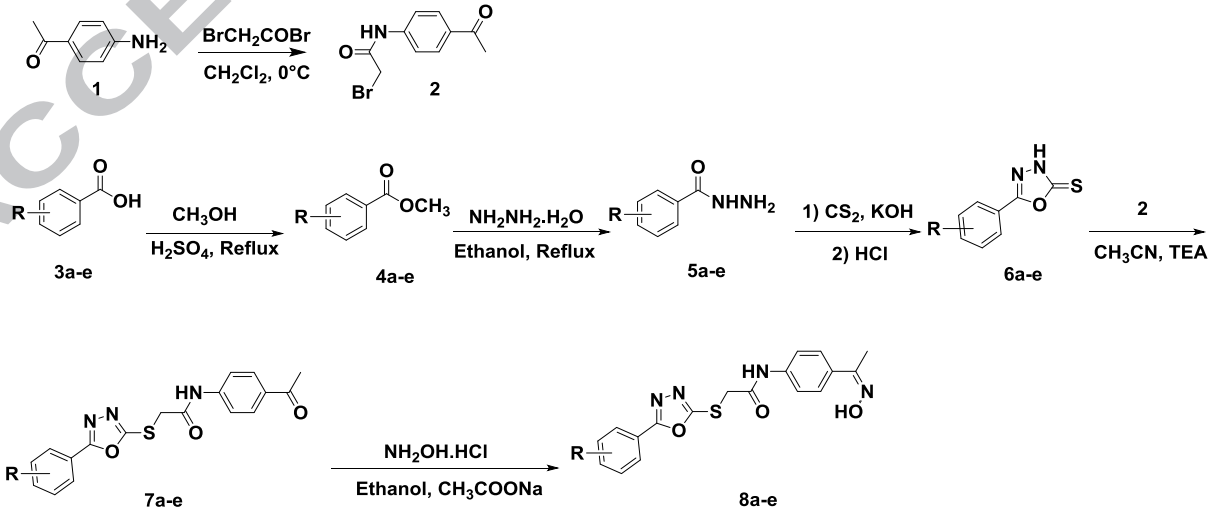
synergistic anti-inflammatory effect and minimize ulcerative profile of the parent ibuprofen.

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.

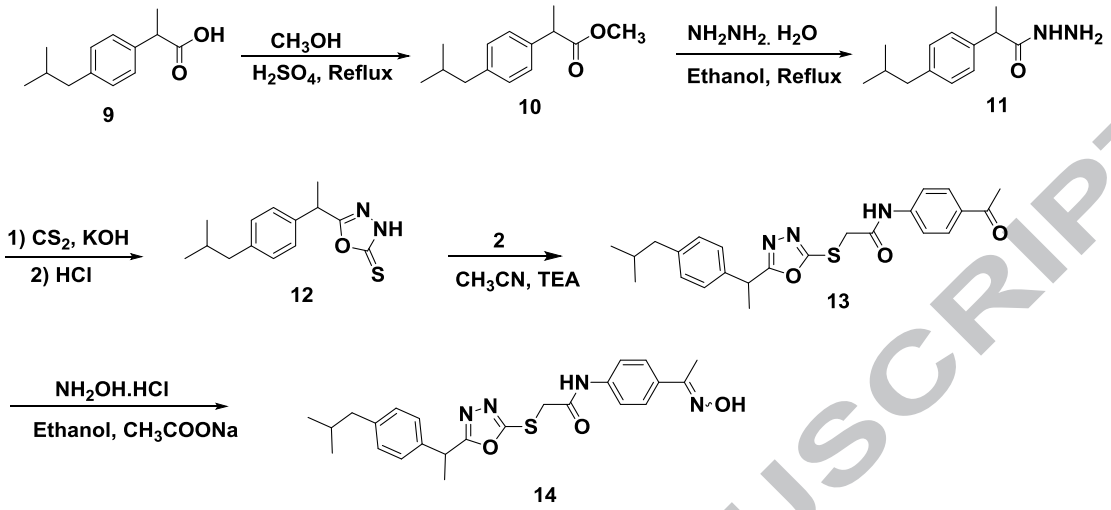
A similar procedure was employed to synthesize an ibuprofen/oxadiazole/oxime hybrid **14 (Scheme 2**). Ibuprofen/oxadiazole hybrid was synthesized through the cyclization of the corresponding hydrazide **11** using the same previously described procedure. Reaction of oxadiazole with *N*-(4-acetylphenyl)-2-bromoacetamide **2** gave the corresponding ketone **13**, which is further reacted with $NH_2OH.HCl$ 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.



R= a, H; b, 4-Cl; c, 4-methoxy; d, 3,4 dimethoxy or e, 3,4,5 trimethoxy

Scheme 1: Synthesis of substituted *N*-(4-(1-(hydroxyimino)ethyl)phenyl)-2-((5-

phenyl-1,3,4-oxadiazol-2-yl)thio) acetamides **8a-e**.



Scheme 2: Synthesis of *N*-(4-(1-(hydroxyimino)ethyl)phenyl)-2-((5-(1-(4-isobutyl phenyl)ethyl)-1,3,4-oxadiazol-2-yl)thio)acetamide **14**.

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 administered via the intraperitoneal route in equimolar doses to (0.05 mol, 17mg/Kg) of the standard drug (indomethacin), 30 min before carrageenan injection at the right hind paw of adult albino male rats. Mean changes in

the paw oedema thickness were recorded every hour for 4 hours after carrageenan injection. The anti-inflammatory activity was calculated as the percentage of reduction in oedema thickness induced by carrageenan and was determined using the following formula:

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

Where V_R represents the mean right hind paw thickness and V_L represents the mean left hind paw thickness. $(V_R - V_L)_{control}$ represents the mean increase in paw thickness in the control groups of rats. $(V_R - V_L)_{treated}$ represents the mean increase in paw thickness in rats treated with the tested compounds.

Results are expressed as % mean ± standard error of mean (SEM) and listed in **Table 1**. Results revealed that most of the synthesized compounds have significant anti-inflammatory activity compared to that of indomethacin and most of them reach their maximum activity at 3-4 h interval. In series **I**, ketones **7a-e**, compound **7a** had excellent anti-inflammatory activity with % inhibition of 100.00 while compounds **7b**, **7c** and **7e** showed comparable activity to indomethacin with % inhibition of 86.67, 83.33 and 83.33, respectively. Compound **7d** had good anti-inflammatory activity (70 % reduction in inflammation, **Table 1**). Thus compounds **7a** elicited promising anti-inflammatory activity of 120 % potency relative to indomethacin at 4 h. In series **I,** unsubstituted phenyl ring had the highest anti-inflammatory activity among the tested derivatives. Addition of electron withdrawing group decreased the anti-inflammatory activity from 100 % to 86 %. Addition of electron donating group also decreased the anti-inflammatory activity from 100 % to 78 %.

In series **II**, oximes **8a-e**, compounds **8e** and **8c** have excellent anti-inflammatory activity with % inhibition of 96.67 and 96.66, respectively while compounds **8a** and **8d** have comparable anti-inflammatory activity to indomethacin with % inhibition of 87.50 and 83.33, respectively. Compounds **8b** exhibited moderate anti-inflammatory activity with 64.17 % reduction in inflammation (**Table 1**). Compounds **8a, 8c** and **8e** elicited promising anti-inflammatory activity of 105 %, 115 %, and 116 % potency relative to indomethacin at 4 h. In series **II**, addition of electron withdrawing group decreased the anti-inflammatory activity from 87.50 % to 64.17 % while the presence of monomethoxy and trimethoxy groups increased the anti-inflammatory activity from 87.50 % to 96.66 % and 96.67 %, respectively. Addition of dimethoxy group decreases the anti-inflammatory activity from 87.50 % to 83.33 %.

The results obtained from measuring the oedema thickness are confirmed by weighing granuloma formed in right hind paw at 4 h. Percentage of inhibition in granuloma weight relative to control was calculated and listed in **Table 1**. Compounds **7a, 7b, 8a, 8c** and **8e** have the highest anti-inflammatory activity of % inhibition in granuloma weight of 81.33, 72.14, 77.57, 82.49, and 84.17 relative to 66.96 % of indomethacin.

Addition of NO releasing group to ketones **7a-e** did not uniformly increase the observed anti-inflammatory activity of the tested ketones suggesting a minor role for NO in such activity. The observed activity was hypothetically explained by the ability of these compounds to inhibit COXs. This hypothesis was tested using *in vitro* inhibition assays and docking of the tested compounds on both COX-1 and COX-2. Inhibition assays and docking studies are discussed later in sections **2.3.5** and **2.4**.

Transforming the free carboxylic group of ibuprofen into 1,3,4-oxadiazole derivative **13** caused a slight increase in the anti-inflammatory activity for the first 3 h (from 75 % into 100 % in the second hour and from 77 to 86 at the third one). The anti-inflammatory activity of compound **13** decreased over the fourth hour. Addition of oxime moiety into compound **14** decreased the activity into 70 % at the third hour. So compound **13** is considered an alternative to ibuprofen but probably with a shorter duration of action.

Histological examination of the granuloma of the right hind paw was done for rats sacrificed at 4h post evaluation of anti-inflammatory activity of oxime **8e**, indomethacin, and control to confirm the elicited anti-inflammatory activity for the tested compounds. Carrageenan untreated granuloma showed non-intact tissues with signs of neutrophil infiltration and tissue injury (**Fig. 1D**). Tissues of rats treated with indomethacin were more intact with fewer sites of oedema and infiltration of inflammatory cells (**Fig. 1C**). Sections of Compound **8e** treated rats showed minimal injury and appeared as healthy as normal tissue sections (**Fig. 1A** and **B**). The use of NO donating oxime provided healthier tissues than those treated with indomethacin and this might be attributed to the release of NO and its protective effect on the tissues examined. Compound **8e** had an excellent anti-inflammatory profile with safer effect on tissues.

Table 1. Percentage of NO released from compounds 7a, **8a-e**, and **14** using Griess colorimetric method and % of oedema inhibition exhibited by compounds 7**a-e**, **8a-e**, **13**, **14**, ibuprofen, and indomethacin using carrageenan induced paw oedema method.

Compound No.	NO release (% mol/mol)	Oedema inhibition (% mean ± standard error, n=5)				Potency [a] (%)	Granuloma weight inhibition (% mean ± standard error)
		1 h	2 h	3 h	4 h	4 h	4 h
Control	0	0	0	0	0	0	0
7a	0	66.66±0.07****	86.66±8.16**	93.33±6.66***	100.00±5.27***	120.00	81.33±±1.40
7b	-----	60.00±4.08***	93.33±4.08***	86.67±8.16**	86.67±6.23***	104.01	72.14±1.87
7c	-----	45.83±4.16**	62.50±4.16**	79.19±7.97**	83.33±0.05***	100.00	68.93±1.44
7d	-----	53.33±4.81****	66.67±8.27***	60.00±7.28***	70.00±5.21****	84.00	61.89±1.90
7e	-----	66.67±0.06****	75.00±4.81**	79.17±7.97**	83.33±9.62*	100.00	69.95±2.84
8a	2.88	58.33±4.81**	66.67±0.08****	91.67±4.81**	87.50±7.97**	105.00	77.57±1.74
8b	4.73	54.17±7.97*	66.67±6.80**	66.67±0.05****	64.17±2.50***	77.00	60.65±0.35
8c	5.46	66.66±7.45**	70.00±8.16**	86.66±6.23***	96.66±6.23***	115.99	82.49±0.88
8d	5.38	45.83±7.97*	58.33±8.33*	75.00±4.81**	83.33±6.80**	100.00	64.74±3.91
8e	4.24	60.00±6.66**	76.67±4.08***	100±0.05****	96.67±6.23***	116.00	84.17±1.32
13	-----	63.33±3.33***	100±0.10****	88.89±4.08***	76.67±4.08***	92.01	63.12±1.93
14	0.37	33.33±7.45	75.00±6.66**	70.00±6.23**	60.00±8.49*	72.00	55.06±4.91
Ibuprofen[b]	------	60.00±8.49*	75.00±6.66***	77.78±6.23***	77.78±6.23***	104.01	76.74±2.08
Indomethacin[c]	------	54.33±5.46***	66.67±7.27***	86.66±7.27***	83.33±5.14****	100.00	66.96±1.21

[a] Potency was expressed as % of edema inhibition of the tested compounds relative to % of edema inhibition of indomethacin at 4 h.
[b] Ibuprofen dose = 10 mg/Kg, [c] Indomethacin dose = 17 mg/Kg
*Significantly different from control group at p= 0.05, ** significantly different from control group at p= 0.01, *** significantly different from control group at p= 0.001, and **** significantly different from control group at p= 0.0001.

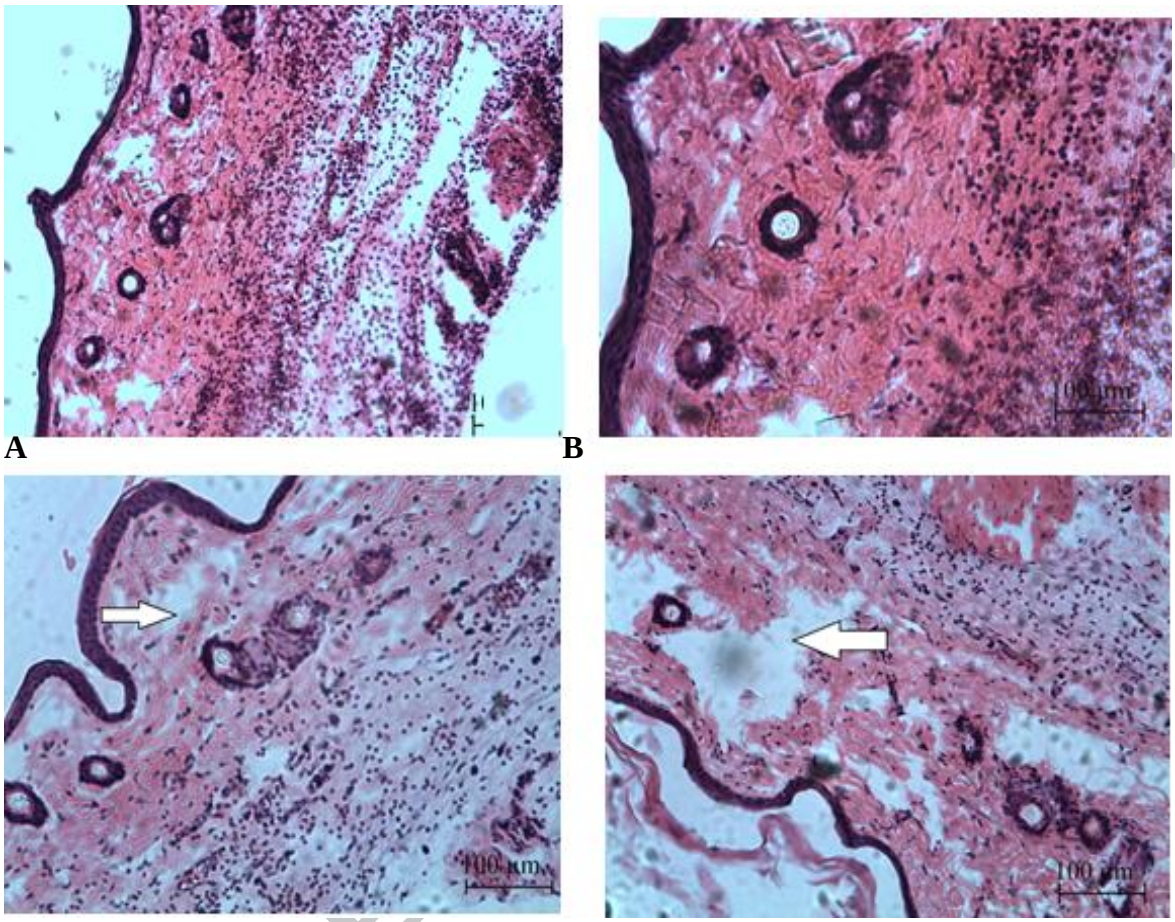


Fig. 1. Microscopic features (hemoxylene stain) of carrageenan induced oedema formed in the right hind paw of **A**) rats treated with compound **8e** (10x); **B**) rats treated with compound **8e** (20x); **C**) rats treated with indomethacin; **D**) untreated control rats. White arrows indicate sites of tissue injury.

2.3.2. Analgesic activity

Analgesic activity of compounds **7a-e**, **8a-e**, **13**, **14**, indomethacin, and paracetamol was evaluated using tail flick method [37]. Tested compounds were administered intraperitoneally in a dose similar to that used in anti-inflammatory screening (0.05 mol, equimolar to 17 mg/Kg indomethacin). Paracetamol was used as a reference drug and administered in two doses (0.05 mol, 0.50 mol). Results were expressed as a percentage of increase in the latency time of the treated group relative to untreated control group and listed in **Table 2**. Paracetamol administered in equimolar dose to the tested compounds (0.05 mol, 7.2 mg/Kg) did not show any significant analgesic activity (19.99 %). The test was repeated using a dose reported in similar studies (0.50 mol, 72 mg/Kg) [37], and the analgesic activity was found to be (123.90 %). Most of drug treated groups have shown significant increase in tail flick latency time

compared to control. Compound **8b** had excellent analgesic activity with 196.30 % increase in tail flick latency time compared to 123.90 % of paracetamol. Compounds **7b**, **7a**, **8a**, and **8e** exhibited good analgesic activity with mean percentage of 130.60 %, 118.30 % and 102.50 %, 97.63 %, respectively. The rest of the compounds show weak analgesic activity (36.93-80.34 %, **Table 2**). Analysing data revealed that both unsubstituted and *p*-Cl substitution is favoured for analgesic activity. The role of NO in such activity is not confirmed, as NO donating oximes did not introduce a uniform increase in the measured activities.

The good analgesic activity of the tested compounds along with their ability to reduce inflammation probably suggests that COX inhibition is their mode of action (see sections **2.3.5** and **2.4**).

Since the tail flick method is measuring central analgesic activity [38], the ability of the synthesized compounds to penetrate blood brain barrier (BBB) might affect their action. Lipophilicity and molecular size of drugs are important factors affecting the penetration of drugs into central nervous system (CNS) [39]. Compounds with moderate lipophilicity are better to penetrate BBB since very lipophilic compounds tend to be highly protein bound and bind to lipid membranes[40]. Also, the entry of a compound into the cerebrospinal fluid (CSF) is proportional to the square root of the molecular mass, so the penetration of large hydrophilic compounds into the CSF is low [41]. Results mentioned earlier suggested that analgesic activity is favoured with *p*-Cl and unsubstituted derivatives. This might be partially explained by the higher lipophilicity and smaller molecular size of such derivatives. Compound **8e**, with best anti-inflammatory activity, was expected to have a higher analgesic activity. The decrease in lipophilicity (log p 3.17, **Table 2**) and large molecular mass (**Table 2**) of such compound might hinder its passage into the CSF and decrease its central analgesic activity. Compounds **13** and **14** with the highest value of log p (5.76 and 6.06, respectively) have low analgesic activity that may also be explained by their large size and inability to diffuse into the BBB. While compound **8b**, with low anti-inflammatory activity, has moderate lipophilicity (log p = 4.16, **Table 2**), its smaller size might contribute to a higher diffusion rate through the bilayer membrane and hence a better analgesic activity.

2.3.3. Measurement of lipid peroxidation level

Lipid peroxidation was used as a measure for anti-oxidant activity. It was determined in serum via measuring thiobarbituric acid reactive species (TBARS; sometimes referred to as malondialdehyde, MDA) as a marker of oxidative stress [42] according to the method described by Buege and Aust [43].

Lipid peroxidation products including MDA, 4-hydroxynonenal, and others react with thiobarbituric acid (TBA) to form a pink colored adduct which is measured colourmetrically at λ_{max} 532nm. This method was used to evaluate the antioxidant activity of the synthesized compounds **7a-e**, **8a-e** as well as indomethacin. Level of

TBARS of serum of rats sacrificed 4 h post drug administration. Results were calculated as the concentration of TBARS in nmol/mL (**Table 2)**. They showed that samples obtained from animals treated with compound **7a** had lower amount of TBARS indicating a slight reduction in lipid peroxidation process and a slight antioxidant activity (18 %, **Table 2**). The rest of compounds caused a slight increase in the oxidative stress, (**Table 2**). NSAIDs are reported to increase the serum level of reactive oxygen species (ROS) [44], therefore the observed increase in oxidative stress might support the assumption that the tested compounds reveal their anti-inflammatory and analgesic activity via COX inhibition.

2.3.4. Ulcerogenic liability

The synthesized compounds **7a-e**, **8a-e**, **13**, **14**, indomethacin, and ibuprofen were assessed for their ulcerogenic liability. Results were obtained from the post mortem studies of rats sacrificed 4 h after anti-inflammatory evaluation. All synthesized compounds exhibited excellent safe profile compared to that of indomethacin (UI =35). These finding is supported with the histological examination of the gastric mucosa of compound **8e** (**Fig. 2A**), which shows normal histology of rat gastric wall while indomethacin increased vascular congestion, infiltration of inflammatory cells and sloughing in mucosal layer as signs of mucosal ulcer. The high safety profile is probably due to bioisosteric replacement of carboxylic function group of conventional NSAIDs with 1,3,4-oxadiazole ring and additional protective effects from NO released from compounds **8a-e**. Compound **13** showed an intact stomach lining compared to ibuprofen (UI= 20), so it is considered a safer alternative to ibuprofen with comparable activity. However, adding a nitric oxide donating group in to compound **14** enhanced safety profile on stomach lining with slightly weaker anti-inflammatory action.

Table 2. Analgesic activity, Log p , square root of molecular mass, level of TBARS, and ulcer index of compounds **7a-e**, **8a-e**, **13**, **14**, indomethacin, and paracetamol.

Compound No.	Analgesic activity[a] (% mean ± standard error, n=5)	Log P[b]	Square root of molecular mass	Thiobarbituric acid reactive species[c] (concentration of TBARS in nmol/mL, n=5)	Ulcer index (mean ± standard error)
Control	0	-----	------	4.52±0.02	9±0.15
7a	118.30±2.98	3.16	18.79	3.68±0.05	3±0.02
7b	130.60±6.02	3.72	19.67	5.64±0.02	3±0.05
7c	68.51±7.65	3.03	19.57	4.84±0.01	4±0.18
7d	41.89±4.85	2.90	20.32	5.26±0.05	2±0.10
7e	68.41±3.31	2.78	21.05	4.74±0.02	5±0.05
8a	102.50±6.80	3.55	19.19	6.09±0.05	1±0.04
8b	196.30±8.84	4.16	20.05	5.86±0.03	2±0.10
8c	80.34±6.65	3.42	19.95	5.17±0.02	Zero±0.01
8d	36.93±2.84	3.29	20.69	5.46±0.01	zero±0.10
8e	97.63±8.53	3.17	21.40	5.19±0.02	zero±0.05
13	65.47±4.58	5.76	20.91	4.97±0.05	3± 0.15
14	2.37±3.33	6.06	21.26	6.08±0.04	zero±0.07
Indomethacin[d]	143.70±9.35	3.75	18.90	6.06±0.02	35± 0.35
Paracetamol[e]	19.99±4.42	0.55	12.29	Nd	Nd
Paracetamol[f]	123.90±9.45	0.55	12.29	Nd	Nd

[a] Analgesic activity using tail flick method and calculated as % of increase in latency time compared to control.

[b] Log p calculated by Crippen's fragmentation, ChemBioDraw Ultra 14.00.

[c] Lipid peroxidation level measured as the concentration of TBARS and expressed in nmol/mL

[d] Indomethacin dose = 17 mg/Kg; [e]paracetamol dose 7.2 mg/Kg ; [f]paracetamol dose 72 mg/Kg
Nd not determined.

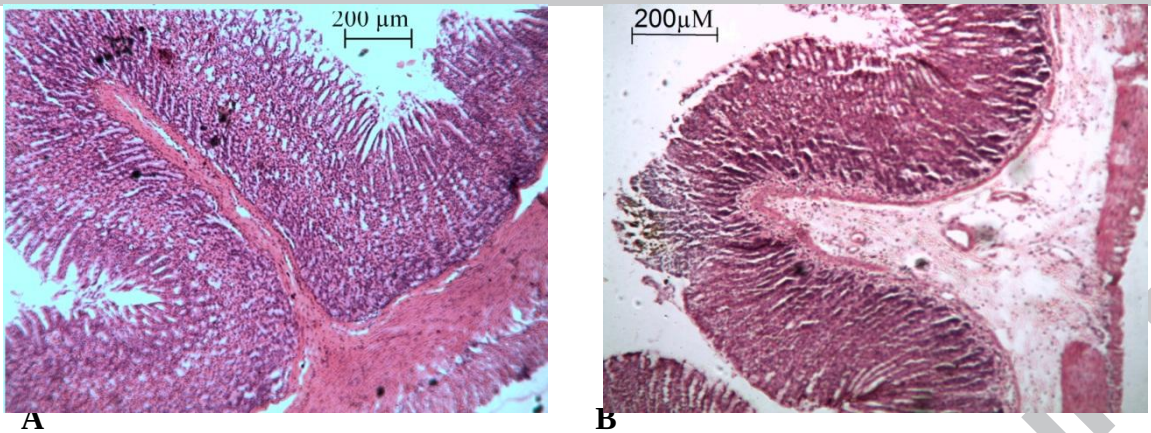


Fig. 2. Histological examination of the stomach lining for rats treated with **A**) compound **8e** (4x); **B**) Indomethacin (4x).

2.3.5. *In vitro* COX inhibition assay.

Compounds **7a-b**, **8c**, **8e** and **13;** compounds with the highest anti-inflammatory activity; were selected for examining their ability to inhibit both COXs. Their effect is studied on both ovine COX-1 (using SC-560 as a reference) [45] and on human recombinant COX-2 (using Dup-697 as a reference) [46] using florescent inhibitor screening assay kit (Cayman Chemical, Ann Arbor, MI, USA). The results were recorded, dose response curves were built (**Fig. 3**) and the concentration required to produce 50% inhibition of either COX-1 or 2 (IC_{50}) is calculated.

All the examined compounds exhibited a low IC_{50} values on both COXs supporting that the described activity occurred though COX inhibition. They exhibited low inhibition to COX-1 compared to indomethacin (IC_{50} of 0.1 µM) [47] while they were potent inhibitors against COX-2 compared to indomethacin (IC_{50} of 24.60 µM) [48]. No significant difference was observed in IC_{50}s measured on both COX-1 and 2 indicating no selectivity on either isozymes. The presence of an electron withdrawing group (*p*-cl) in compound **7b** increased the measured IC_{50} to 10 µM. Also the presence of the long alkyl group from the ibuprofen structure in compound **13** dramatically decreased the ability of the compound to inhibit the enzyme. The presence of small electron donating methoxy group in compound **8c** achieved the best COX-1 enzyme inhibition. Introduction of a trimethoxy function group (compound **8e**) resulted in the best COX-2 enzyme inhibition and that may attributed to the bulky nature of trimethoxy group which may make it a better fit on the allosteric pocket of COX-2 active site (see docking at section **2.4**).

Moreover, both functional groups used (ketones and oximes) showed good inhibition of both COXs suggesting a minimal role of NO in the measured activities.

Table 3. *In vitro* COX-1 and COX-2 enzyme inhibition for compounds **7a, b, 8c, 8e** and **13** compared to SC-560 and Dup-697.

Compound	COX-1 IC_{50} (μM)[a] (mean ± SEM, n=3)	COX-2 IC_{50} (μM)[a] (mean ± SEM, n=3)	SI[b]
7a	1.2±0.15	7.1±1.4	0.17
7b	10.3±1.4	8.9±1.0	1.15
8c	0.98±0.25	3.3±0.21	0.3
8e	2.12±0.28	0.75±0.10	2.8
13	70.3±9.1	70.5±10.9	1
SC-560	0.066±0.008	----	---
DuP-697	----	0.07±0.01	-

[a] Values in the table represent IC_{50} (μM) and % inhibition at saturation

[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

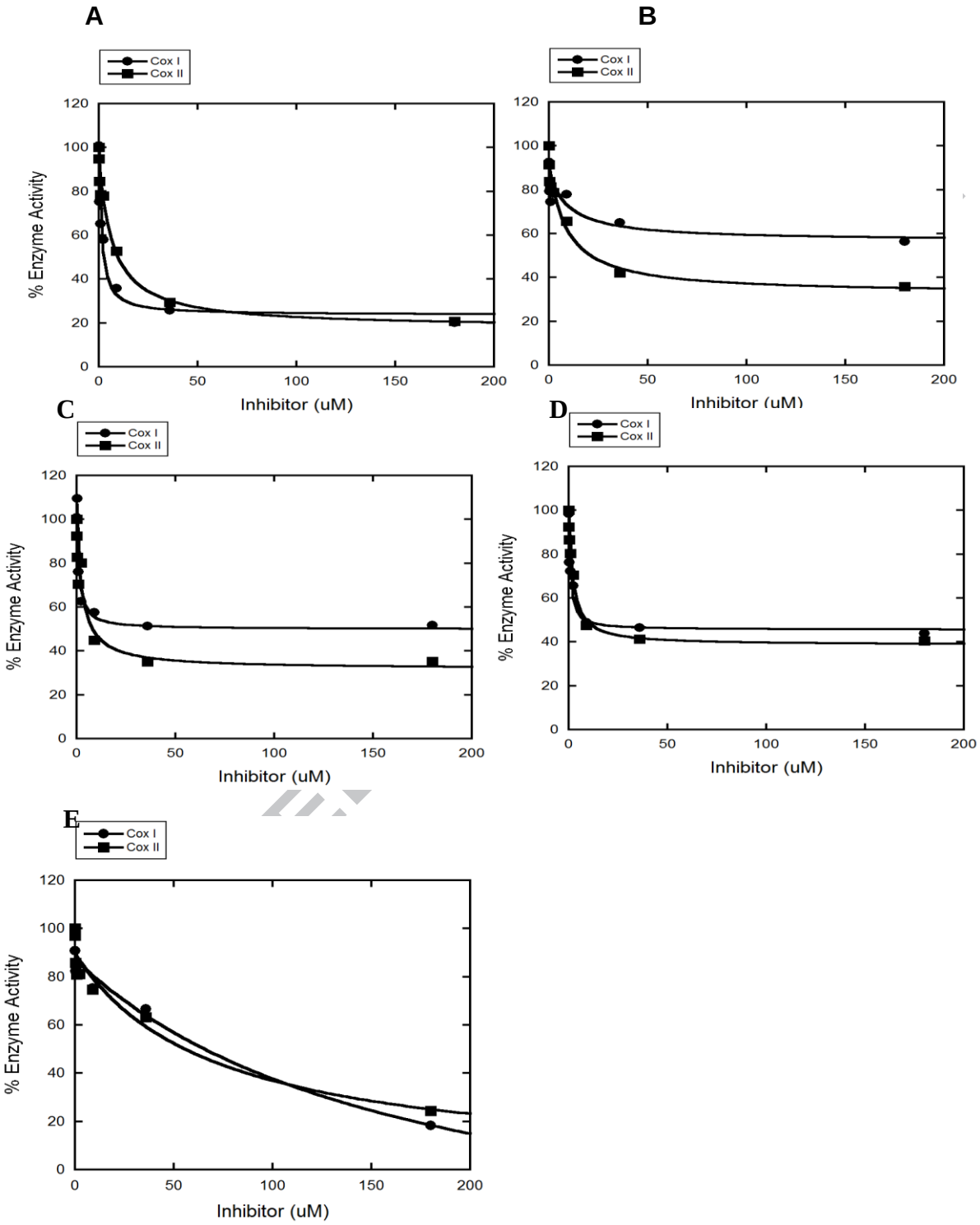


Fig. 3. The effect of compounds **7a** (A), **7b** (B), **8c** (C), **8e** (D) and **13** (E) on the activity of ovine COX-1 (•) and human recombinant COX-2 (■).The results are expressed as percent of control in the absence of inhibitor (100 % maximum activity).

2.4. Docking studies

Docking of the synthesized compounds **7a-e**, **8a-e**, **13**, **14**, and ibuprofen was done on both COX-1 and COX-2 enzymes to suggest the possible binding modes between the compounds and the enzymes active site. Docking experiments were done using MOE 2014 software. Target compounds were constructed into the builder interface of MOE program, the energy was minimized until a RMSD (root mean square deviations) gradient of 0.01 Kcal/mol and RMS (Root Mean Square) distance of 0.1 Å with MMFF94X (Merck molecular force field 94x) force-field and the partial charges were automatically calculated. X-ray crystallographic structure of the ligand-enzyme complex were downloaded from protein data bank (www.rcsb.org); COX-1 enzyme (pdb: 1eqg) and COX-2 enzyme (pdb: 1cx2).

Enzymes were prepared for docking studies by deleting the ligand, adding hydrogen atoms, checking the atom connection and type with automatic correction. Then the potential of the receptor was fixed and docking of the designed compounds is done into the 3D structure of the catalytic site of COX-1 and COX-2 enzymes. The obtained poses were studied and the poses showed best ligand-enzyme interactions were selected and stored for energy calculations.

Table 4 lists the docking energy scores for the synthesized compounds and ibuprofen (the specific substrate for COX-1), and detailed interactions formed from the tested compounds with the amino acid residues in the empty pocket of both isozymes. All of the synthesized compounds gave energy scores from 10.01 to 14.52 Kcal/mol relative to that of ibuprofen (9.58 and 5.67, **Table 4**) supporting that COX inhibition is a reasonable mechanism explaining the analgesic and anti-inflammatory activities observed with those compounds. All the synthesized compounds have higher energy scores at COX-2 active site (10.55-12.59 Kcal/mol) compared to that of ibuprofen (5.67 Kcal/mol).

It is obvious from the results that there is no significant difference was observed in energy scores obtained for interaction with COX-1 and COX-2 suggesting no selectivity on either isozymes. The same result was shown before in *in vitro* COX inhibition assays. (section **2.3.5**)

There is no significant difference between energy scores of all the tested compounds and ibuprofen, so energy scores could not be used in explaining differences observed in activities. Detailed study of the binding interactions might offer an explanation for such differences. **Table. 4**, shows the number and modes of interactions formed from the tested compounds with the amino acid residues in the empty pocket of both isozymes.

Compound **8e** with the highest anti-inflammatory activity, showed the best affinity to COX-2 receptor (energy score -12 Kcal/mol, **Table 4,** IC_{50} = 0.75 μM, **Table 3**) compared that of ibuprofen and the other examined structures. Compound **8e** forms the highest amount of interaction with the active site of COX-2. It forms two hydrogen bonds with Glu524, two hydrogen bonds with both Arg120 and Tyr385, and one hydrophobic interaction with Arg513, **Fig. 4B, 5B**.

It also shows the highest amount of interaction with the COX-1 enzyme active site, **Table. 4**. It forms 3 hydrogen bonds with Arg120, Tyr355 and Arg83, **Fig. 4A, 5A**. The strong binding stabilize the complex formed between compound **8e** and the enzymes and those two amino acids Arg 120 and Tyr 355 are reported to play an important role in the binding of drugs into COX-1 active site [49],[50].

In series **I,** compounds 7**a-e**, compound **7a** and **7b** (with high anti-inflammatory activity) have the highest amount of interactions with the empty cavities of COX-1 and COX-2, **Table 4, supplementary Information**. Compounds **7c**, **7d**, and **7e** show lower amount of interaction with COXs active site in accordance with anti-inflammatory data of this group. In series **II,** compounds **8a-e**, compounds **8e** and **8c** (with high anti-inflammatory activity) have the highest number of interactions of COXs active site, **Table 4** and **supplementary information**. Compounds **8a** and **8d** showed lower number of interactions **Table 4** and supplementary information. Compound **13** has higher amount of interaction relative to the corresponding oxime **14**. Compound **14**, having low anti-inflammatory activity, forms only one hydrogen bond with Arg 120 in the pocket of COX-1, **Fig. 6A**, **7A** and one with Arg 513 in the pocket of COX-2, **Fig. 6B, 7B**.

Table 4: Types of interactions and Energy scores for the complexes formed from compounds **7a-e, 8a-e**, **13** and **14** and the active sites of both COX-1 and COX-2.

Compound	Types of interactions		Energy scores	
	COX-1	COX-2	COX-1	COX-2
Ibuprofen	- Two hydrogen bonding with Arg120 (3.60 and 3.83ºA).	- Two hydrogen bonding with Arg120 (2.84 and 3.30ºA). -One hydrogen bonding with Tyr 355 (2.76 ºA)	-9.58	-5.67
7a	-One hydrogen bonding with Arg83 (3.17 ºA) - One hydrogen bonding with Arg120 (2.76 ºA).	-One hydrogen bonding with Arg 120 (2.90ºA) -One hydrogen bonding with Glu 524(2.94 ºA) - One hydrophobic interaction with Arg 513 (4.51ºA).	-11.50	-11.13
7b	-One hydrogen bonding with Arg83 (3.17 ºA) - One hydrogen bonding with Arg120 (2.71ºA).	-One hydrogen bonding with Tyr355 (2.81ºA) -One hydrogen bonding with Tyr385 (3.07 ºA) - One hydrophobic interaction with Ala527 (3.85ºA).	-11.37	-10.90
7c	-One hydrogen bonding with Arg83 (3.11ºA) - One hydrogen bonding with Arg120 (2.73ºA).	-One hydrogen bonding with Arg 513 (3.43ºA) -One hydrogen bonding with Glu 524(3.48 ºA)	-11.79	-11.24
7d	- One hydrogen bonding with Arg120 (2.89ºA).	-One hydrogen bonding with Arg120 (2.68 ºA) - One hydrophobic interaction with Ala527 (4.05ºA).	-11.68	-11.82
7e	- One hydrogen bonding with Arg120 (2.57ºA). -One hydrophobic interaction with Tyr355 (3.52 ºA).	-One hydrogen with Arg 513 (3.21 ºA). -One hydrogen with Lys83 (2.94 ºA).	-11.54	-10.92

8a	-One hydrogen bonding with Arg 120 (2.78 ºA) - One hydrogen bonding with Arg83 (3.14 ºA).	-One hydrogen bonding with Ser 530 (3.29 ºA). -One hydrogen bonding with His 90 (3.06 ºA).	-11.38	-12.59
8b	-One hydrogen bonding with Met 522 (2.54 ºA). -One hydrophobic interaction with Ala 527 (4.42 ºA).	-One hydrogen bonding with Tyr 385 (3.08 ºA)	-11.49	-10.55
8c	-One hydrogen bonding with Met522 (2.72 ºA) -One hydrogen bonding with Arg120 (3.46 ºA). - One hydrogen bonding with Arg83 (2.77 ºA).	-One hydrogen bonding with Glu524 (2.93 ºA) . -One hydrogen with Arg120 (2.97 ºA). -One hydrogen with Tyr385 (3.19 ºA). -One hydrophobic interaction with Arg513 (4.52 ºA).	-11.76	-10.75
8d	-One hydrogen bonding with Arg120 (2.79 ºA) - One hydrogen bonding with Arg83 (2.75ºA).	-One hydrogen bonding with Arg120 (2.81 ºA) - One hydrogen bonding with Lys83 (2.92ºA).	-12.41	-11.02
8e	-One hydrogen bonding with Tyr355 (3.20 ºA) -One hydrogen bonding with Arg120 (3.13 ºA). - One hydrogen bonding with Arg83 (3.68 ºA)	- Two hydrogen bonding with Glu524 (2.91 and 3.11 ºA) . -One hydrogen with Arg120 (3.03 ºA). -One hydrogen with Tyr385 (3.03 ºA). -One hydrophobic interaction with Arg513 (4.23 ºA).	-14.52	-12.56
13	-Two hydrogen bonding with Arg120 (2.74 and 2.74 ºA).	-One hydrogen bonding with Glu524 (2.50 ºA) . -One hydrogen with Arg513 (3.00 ºA). -One hydrogen with Lys83 (3.30 ºA).	-10.80	-11.02
14	-One hydrogen bonding with Arg120 (2.65ºA).	-One hydrophobic interaction with Arg513 (4.4 ºA)	-10.01	-11.81

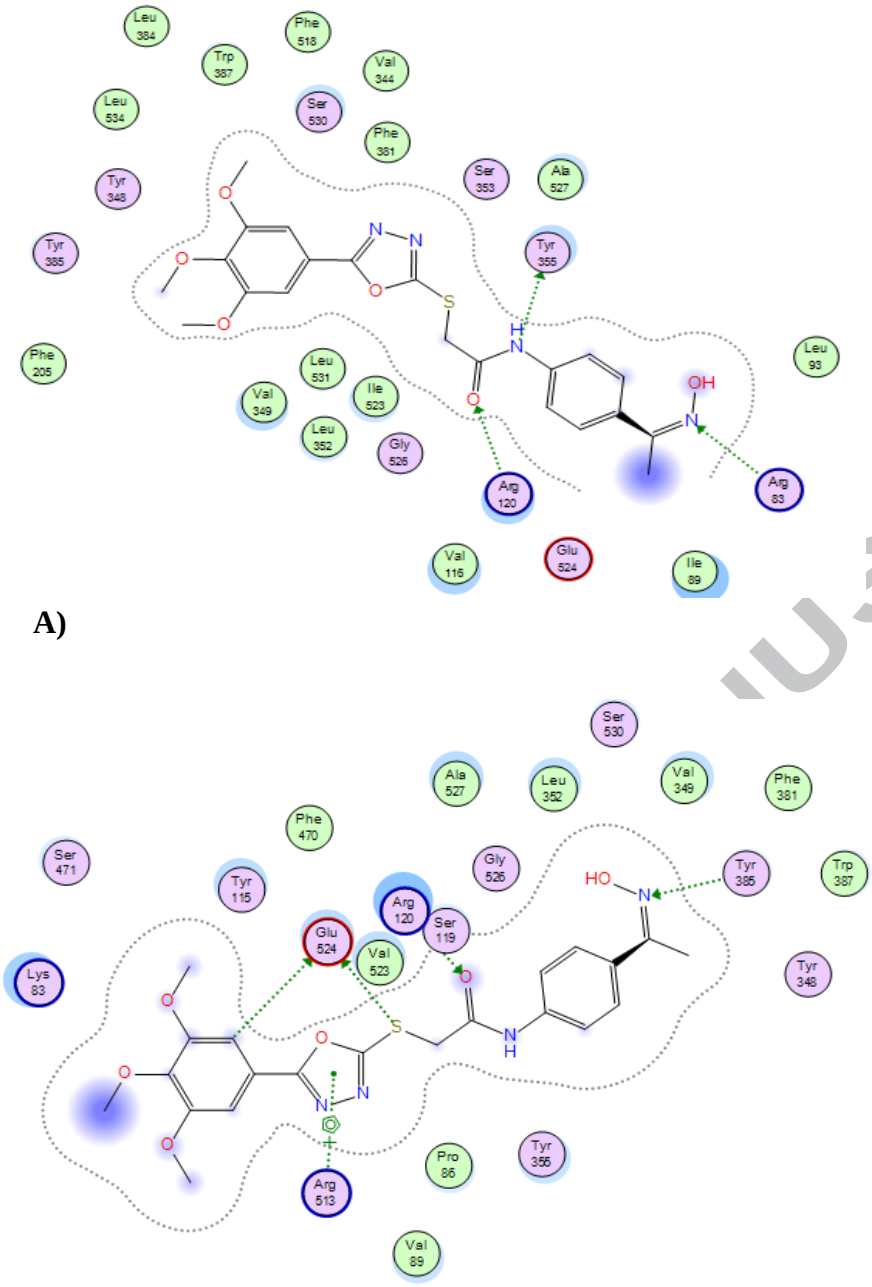


Fig. 4. 2D drawings of the compound **8e** interacted with both active sites of **A**) COX-1 and **B**) COX-2 showing interactions with different amino acid residues found in the active site.

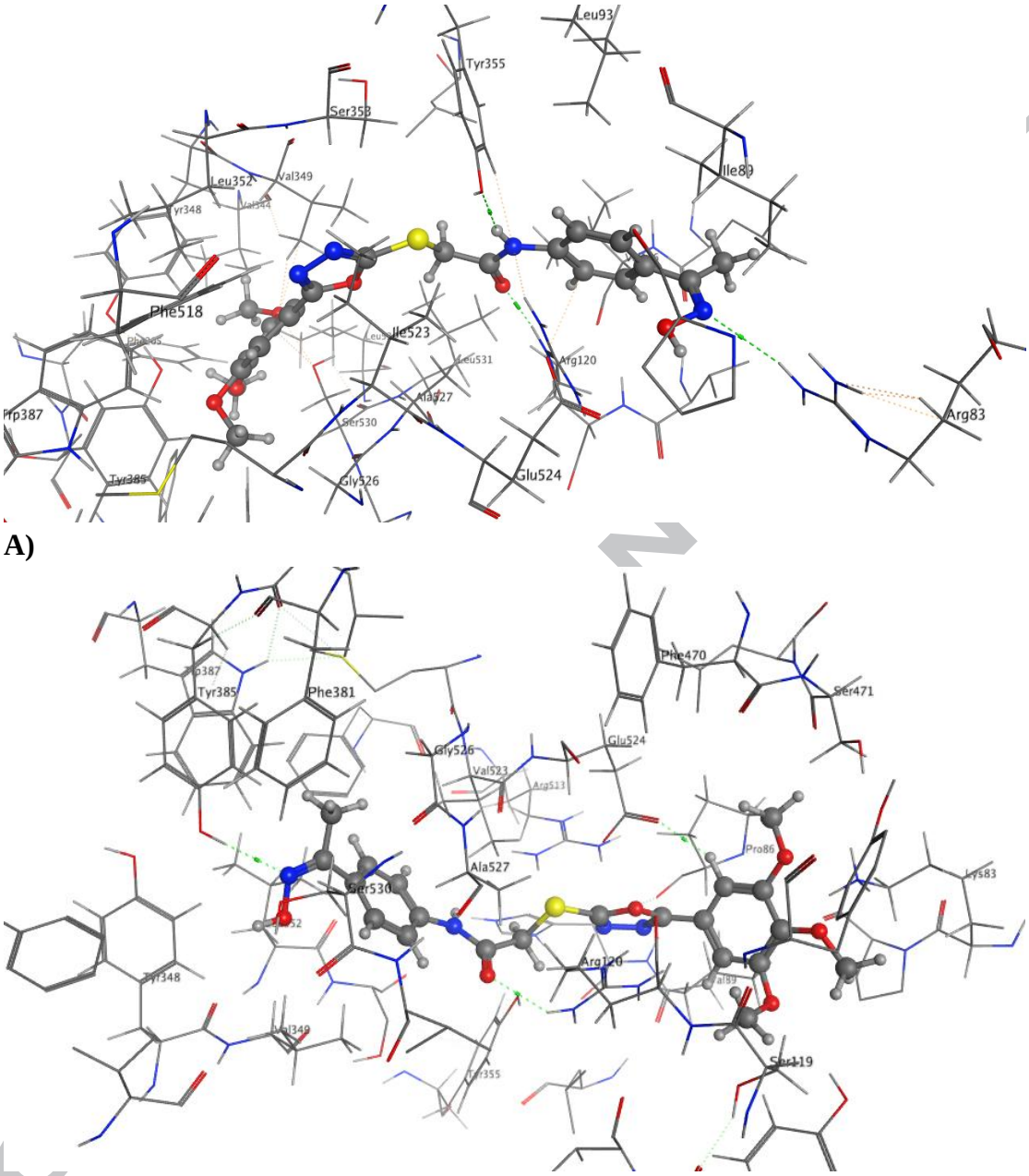
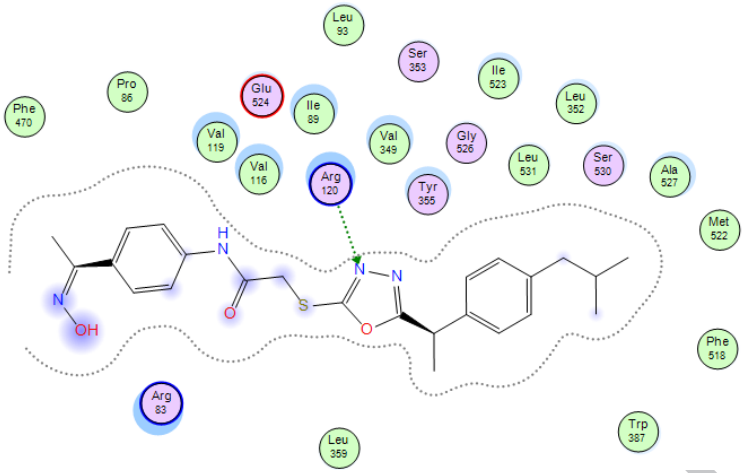
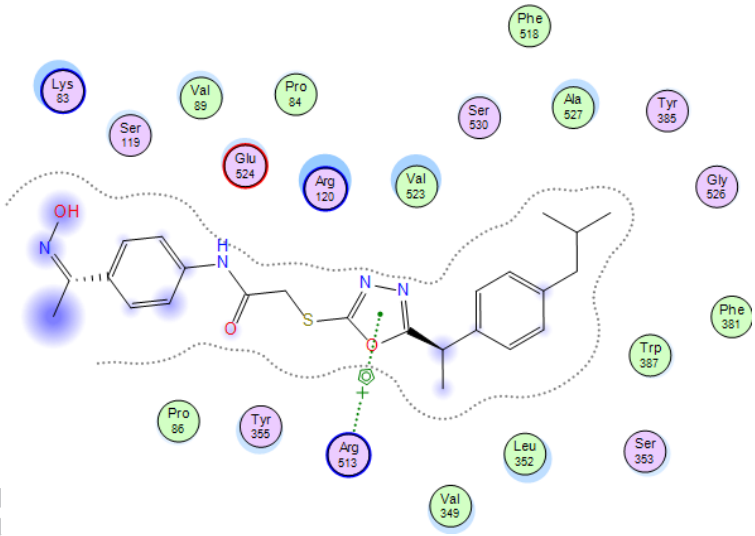


Fig. 5. 3D drawings of the compound **8e** interacted with both active sites of **A**) COX-1 and **B**) COX-2 showing interactions with different amino acid residues found in the active site.

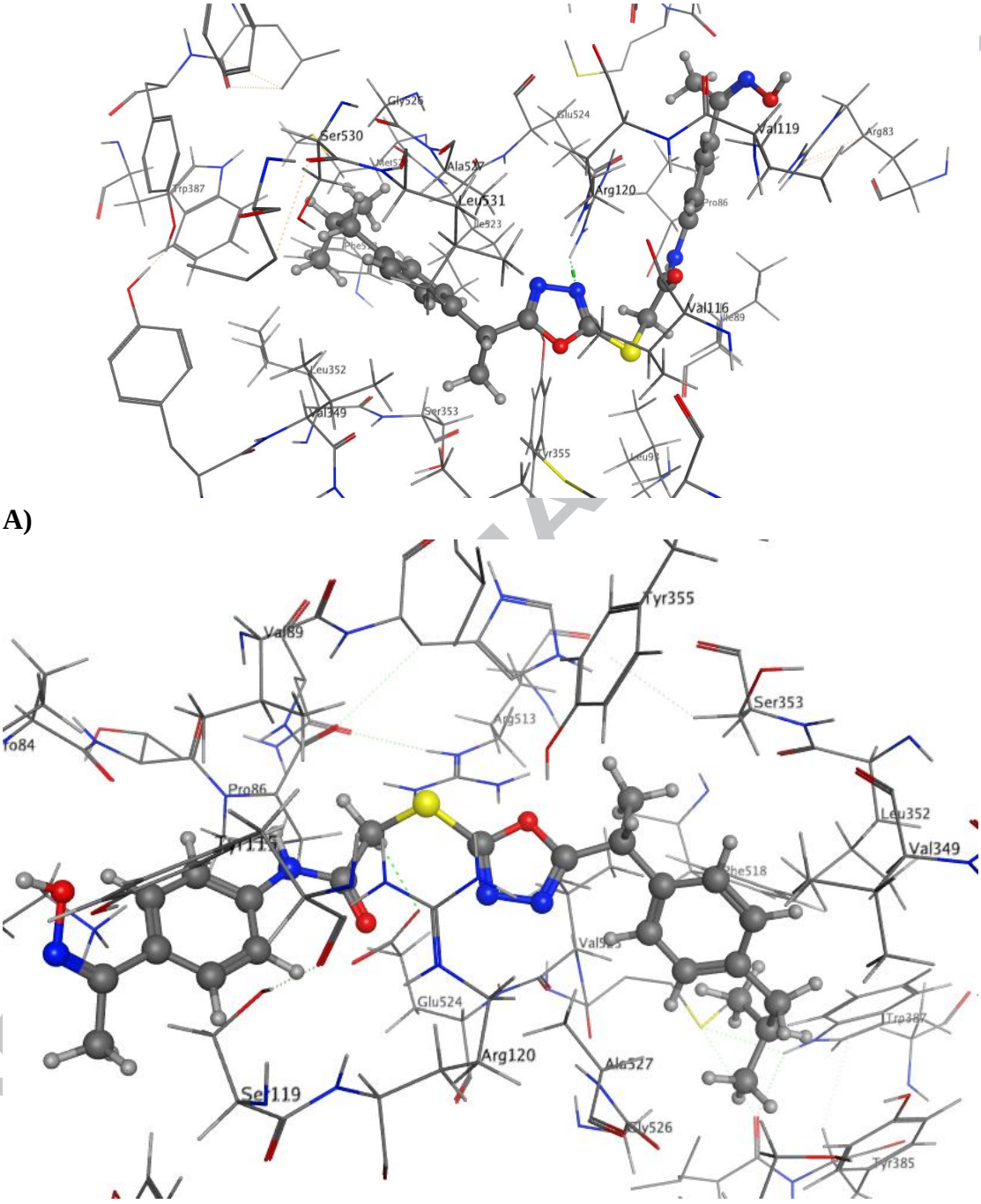


A)



B)

Fig. 6. 2D drawings of the compound **14** interacted with both active sites of **A)** COX-1 and **B)** COX-2 showing interactions with different amino acid residues found in the active site.



A)

B)

Fig. 7. 3D drawings of the compound **14** interacted with both active sites of **A)** COX-1 and **B)** COX-2 showing interactions with different amino acid residues found in the active site.

3. Experimental

3.1. Chemistry

Chemicals and solvents used were of analytical grade. Progress of the reactions was monitored by thin layer chromatography with ethyl acetate/methylene chloride (1:1) as the mobile phase on pre–coated Merck silica gel 60 F254 aluminum sheets. Melting points were determined on Stuart electro-thermal melting point apparatus and were uncorrected. IR spectra were recorded on Nicolet iS5 (ATR) FT-IR spectrometer at Minia University. ^{1}H NMR spectra were recorded on Bruker Avance III 400 MHz and ^{13}C spectra were recorded on Bruker AG, Switzerland, 100 MHz . High resolution mass spectra were collected via Thermo Scientific Q Exactive™ Orbitrap mass spectrometer.

3.1.1. Synthesis of *N*-(4-acetylphenyl)-2-bromoacetamide 2 [51]**.**
3.1.2. Substituted methyl benzoate 4a-e [52,33]**.**
3.1.3. Substituted benzohydrazides 5a-e [53,33]**.**
3.1.4. Substituted 1,3,4-oxadiazole-2(*3H*)-thione 6a-e.
3.1.4.1. Substituted 1,3,4-oxadiazole-2(*3H*)-thione 6a-c [54]**.**
3.1.4.2. 5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazole-2(*3H*)-thione 6d.
A mixture of **5d** (11.90 g, 0.05 mol), potassium hydroxide (3 g, 0.05 mol), carbon disulfide (0.17 mol, 10 mL) in absolute ethanol (50 mL) was heated under reflux with stirring for 12 h. The solvent was evaporated under vaccum. The residue was dissolved in water and acidified with hydrochloric acid (10 %). The formed precipitate was filtered, washed with water, dried, and recrystallized from absolute ethanol to give yellowish white solid Yellowish white solid (7.10 g, 60 % yield); mp 237-238 °C; IR (KBr, cm^{-1}): 3246 (NH), 1621 (C=N); ^{1}H NMR (DMSO-d$_6$) δ: 8.00 (s, 1H, NH), 7.42 (d, 1H, J = 8.4 Hz, Ar-H), 7.28 (s, 1H, Ar-H), 7.10 (d, 1H, *J* = 8.4 Hz, Ar-H), 3.80 (s, 6H, 2-OCH$_3$); ^{13}C NMR (DMSO-d$_6$) δ: 177.33, 160.73, 152.27, 149.29, 130.47, 119.88, 114.74, 112.12, 55.80, 55.70.

3.1.4.3. 5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazole-2(*3H*)-thione 6e [55**,** 56]**.**
3.1.5. General procedure for the synthesis of compounds 7a-e.
To a stirred mixture of the appropriate oxadiazole **6a-e** (1.00 mmol) and compound **2** (0.26 g, 1.00 mmol) in acetonitrile (30 mL), triethylamine (0.12 g, 1.20 mmol) was added. The mixture was stirred at room temperature and the immediately formed precipitate was filtered off, washed with methanol, and recrystallized from absolute ethanol.

3.1.5.1. 2-(5-Phenyl-1,3,4-oxadiazol-2-ylthio)-*N*-(4-acetylphenyl)acetamide7a [57]**.**
White solid (0.14 g, 56% yield); mp 200-201°C; reported 184-188°C.
3.1.5.2. 2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-acetylphenyl) acetamide 7b [58]**.**
Yellowish white solid (0.20 g, 54% yield); mp 218-219°C; reported 211-212 °C.
3.1.5.3. 2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-acetylphenyl) acetamide 7c.
Yellowish white solid (0.13 g, 65 % yield); mp 206-207°C; IR (KBr, cm^{-1}): 3258 (NH), 1685 (COCH$_3$), 1666 (CONH); ^{1}H NMR (DMSO-d$_6$) δ: 10.79 (s, 1H, NH), 7.96 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.89 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.73 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.12 (d, 2H, *J* = 8.9 Hz, Ar-H), 4.37 (s, 2H, CH$_2$), 3.85 (s, 3H, OCH$_3$),

2.54 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 197.01, 166.11, 165.63, 162.93, 162.55, 143.38, 132.57, 130.07, 128.72, 118.94, 118.76, 115.36, 56.01, 37.35, 26.93; HRMS: m/z calculated for $C_{19}H_{16}N_3O_4S$ [M-1]$^-$: 382.08670, found: 382.08722.

3.1.5.4. 2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-acetylphenyl) acetamide 7d.

Yellow solid (0.23 g, 56 % yield); mp 208-209°C; IR (KBr, cm^{-1}): 3311 (NH), 1686 (COCH$_3$), 1664 (CONH); ^{1}H NMR (DMSO-d_6) δ: 11.01 (s, 1H, NH), 7.93 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.76 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.52 (dd, 1H, *J* = 8.5 and 1.5 Hz, Ar-H), 7.41 (d, 1H, *J* = 1.5 Hz, Ar-H), 7.10 (d, 1H, *J* = 8.5 Hz, Ar-H), 4.41 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.52 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 196.79, 165.90, 165.43, 162.69, 152.04, 149.24, 143.17, 132.18, 129.67, 120.10, 118.57, 115.32, 112.10, 109.12, 55.75, 55.68, 36.86, 26.42; HRMS: m/z calculated for $C_{20}H_{18}N_3O_5S$ [M-1]$^-$: 412.09726, found: 412.09787.

3.1.5.5. 2-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-acetyl phenyl)acetamide 7e.

White solid (0.24 g, 55 % yield); mp 187-188°C; IR (KBr, cm^{-1}): 3311 (NH), 1681 (COCH$_3$), 1658 (CONH); ^{1}H NMR (DMSO-d_6) δ: 10.88 (s, 1H, NH), 7.95 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.74 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.21 (s, 2H, Ar-H), 4.40 (s, 2H, CH_2), 3.83 (s, 6H, $2OCH_3$), 3.74 (s, 3H, OCH_3), 2.54 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 197.00, 166.08, 165.60, 163.60, 153.93, 143.42, 141.05, 132.56, 130.04, 118.92, 118.59, 104.27, 60.70, 56.62, 37.37, 26.92; HRMS: m/z calculated for $C_{21}H_{20}N_3O_6S$ [M-1]$^-$: 442.10783, found: 442.10840.

3.1.6.General procedure for the synthesis of compounds 8a-e.

A mixture of the appropriate ketone derivatives **7a-e** (1.00 mmol), hydroxylamine hydrochloride (0.35 g, 5.00 mmol) and anhydrous sodium acetate (0.08 g, 1.00 mmol) in absolute ethanol (30 mL) was heated under reflux for 10-30 min. and then left to cool to room temperature. The separated solid was filtered off, washed with dil. ammonia solution (10 %) and then with distilled water, dried, and recrystallized from aqueous ethanol affording the corresponding oximes **8a-e**.

3.1.6.1. *N*-(4-(1-(Hydroxyimino)ethyl)phenyl)-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)acetamide 8a.

Yellowish white solid (0.15 g, 42 % yield); mp 210-211°C; IR (KBr, cm^{-1}): 3253 (OH, NH), 1659 (CONH); ^{1}H NMR (DMSO-d_6) δ: 11.09 (s, 1H, OH), 10.54 (s, 1H, NH), 8.04 -7.87 (m, 2H, Ar-H), 7.71-7.46 (m, 7H, Ar-H), 4.35 (s, 2H, CH_2), 2.12 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 165.40, 165.25, 163.60, 152.57, 139.15, 132.43, 132.23, 129.6, 126.54, 126.30, 123.11, 119.01, 36.85, 11.34; HRMS: m/z calculated for $C_{18}H_{15}N_4O_3S$ [M-1]$^-$: 367.08703, found: 367.08734.

3.1.6.2. *N*-(4-(1-(Hydroxyimino)ethyl)phenyl)-2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8b.

Yellowish white solid (0.26 g, 62 % yield); mp 231-232°C; IR (KBr, cm^{-1}): 3381 (OH, NH), 1681 (CONH), 1636 (C=N). ^{1}H NMR (DMSO-d_6) δ: 11.11 (s, 1H, OH), 10.56 (s, 1H, NH), 7.97 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.65-7.61 (m, 6H, Ar-H), 4.37 (s, 2H, CH_2), 2.13 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 165.43, 164.89, 164.16, 152.82, 139.42, 137.23, 132.72, 130.08, 128.65, 126.61, 122.33, 119.32, 37.32, 11.84; HRMS: m/z calculated for $C_{18}H_{14}ClN_4O_3S$ [M-1]$^-$: 401.04806, found: 401.04883.

3.1.6.3. *N*-(4-(1-(Hydroxyimino)ethyl)phenyl)-2-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8c.

Yellowish white solid (0.20 g, 52 % yield); mp 220-221°C; IR (KBr, cm^{-1}): 3286 (OH, NH), 1664 (CONH), 1612 (C=N); ^{1}H NMR (DMSO-d_6) δ: 11.11 (s, 1H, OH), 10.54

(s, 1H, NH), 7.90 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.67-7.59 (m, 4H, Ar-H), 7.12 (d, 2H, *J* = 8.8 Hz, Ar-H), 4.35 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 165.33, 165.26, 162.70, 162.26, 152.53, 139.14, 132.39, 128.38, 126.27, 118.97, 115.42, 114.98, 55.55, 36.84, 11.32; HRMS: m/z calculated for $C_{19}H_{17}N_4O_4S$ [M-1]$^-$: 397.09760, found: 397.09796.

3.1.6.4. *N*-(4-(1-(Hydroxyimino)ethyl)phenyl)-2-((5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8d.

Yellow solid (0.20 g, 47 % yield); mp 198-199°C; IR (KBr, cm^{-1}): 3307 (OH, NH), 1655 (CONH); ^{1}H NMR (DMSO-d_6) 11.10 (s, 1H, OH), 10.86 (s, 1H, NH), 7.65 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.61 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.53 (dd, 1H, *J* = 8.4 Hz and 1.5 Hz, Ar-H), 7.43 (d, 1H, *J* = 1.5 Hz, Ar-H), 7.11 (d, 1H, *J* = 8.5 Hz, Ar-H), 4.38 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.12 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 165.94, 165.42, 165.43, 162.76, 152.52, 149.25, 139.29, 132.31, 126.20, 120.11, 118.95, 115.34, 112.12, 109.14, 55.75, 55.69, 36.78, 11.32; HRMS: m/z calculated for $C_{20}H_{19}N_4O_5S$ [M-1]$^-$: 427.10816, found: 427.10867

3.1.6.5. *N*-(4-(1-(Hydroxyimino)ethyl)phenyl)-2-((5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 8e.

White solid (0.17 g, 46 % yield); mp 227-228°C; IR (KBr, cm^{-1}): 3289 (OH, NH), 1662 (CONH); ^{1}H NMR (DMSO-d_6) δ: 11.11 (s, 1H, OH), 10.59 (s,1H,NH), 7.62 (s, 4H, Ar-H), 7.21 (s, 2H, Ar-H), 4.37 (s, 2H, CH_2), 3.83 (s, 6H, 2OCH_3), 3.74 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ: 165.59, 165.49, 163.66, 153.93, 152.82, 141.05, 139.46, 132.72, 126.60, 119.30, 118.61, 104.27, 60.71, 56.61, 37.34, 11.84; HRMS: m/z calculated for $C_{21}H_{21}N_4O_4S$ [M-1]$^-$: 457.11818, found: 457.11954.

3.1.7. Methyl 2-(4-isobutylphenyl)propanoate 10 [59]**.**

3.1.8. 2-(4-Isobutylphenyl)propanehydrazide 11 [60]**.**

3.1.9. 5-(1-(4-Isobutylphenyl)ethyl)-1,3,4-oxadiazole-2(3*H*)-thione 12 [60]**.**

3.1.10. Synthesis of *N*-(4-acetylphenyl)-2-((5-(1-(4-isobutylphenyl)ethyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 13.

Triethylamine (0.12 g, 1.20 mmol) was added to oxadiazole **12** (0.44 g, 1.00 mmol) and compound **2** (0.26 g, 1.00 mmol) in acetonitrile (30 mL). The mixture was stirred; a precipitate is immediately formed, filtered off, washed, and recrystallized from absolute ethanol. to give a yellow solid of **13** (0.20 g, 47 % yield); mp 71-73°C; IR (KBr, cm^{-1}): 3460 (NH), 1675 (COCH$_3$, CONH); ^{1}H NMR (DMSO-d_6), δ: 10.76 (s, 1H, NH), 7.95 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.71 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.16 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.06 (d, 2H, *J* = 7.6 Hz, Ar-H) 4.41 (q, 1H, *J* = 7.0 Hz, CH-CH$_3$), 4.30 (s, 2H, CH$_2$-S), 3.06 (d, 2H, *J* = 7.2 Hz, CH$_2$-Ar), 2.50 (s, 3H, CH$_3$CO), 2.37 (d, 3H, *J* = 7.0 Hz, CH$_3$-CH), 1.79-1.73 (m, 1H, CH(CH$_3$)$_2$), 0.83 (d, 6H, *J* = 6.4 Hz, (CH$_3$)$_2$CH); ^{13}C NMR (DMSO-d_6) δ: 196.95, 170.36, 165.88, 163.48, 143.42, 140.66, 138.07, 132.50, 129.42, 129.34, 127.45, 118.90, 40.62, 37.18, 36.49, 29.98, 27.32, 22.62, 19.77; HRMS: m/z calculated for $C_{24}H_{26}N_3O_3S$ [M-1]$^-$: 436.17004, found: 436.16946.

3.1.11. Synthesis of *N*-(4-(1-(hydroxyimino)ethyl)phenyl)-2-((5-(1-(4-isobutyl-phenyl)ethyl)-1,3,4-oxadiazol-2-yl)thio)acetamide 14.

A mixture of compound **13** (0.44 g, 1.00 mmol), hydroxylamine hydrochloride (0.35 g, 5.00 mmol) and anhydrous sodium acetate (0.08 g, 1.00 mmol) in absolute ethanol (30mL) was heated under reflux for 10-30 min. and then left to cool. The separated solid was filtered off, washed with dil. ammonia solution (10%) and distilled water,

dried, and recrystallized from aqueous ethanol to give a white precipitate in (0.80 g, 60 % yield); mp 158-160 °C; IR (KBr, cm^{-1}): 3383 (OH, NH), 1662 (CONH); ^{1}H NMR (DMSO-d_6), δ: 11.09 (s, 1H, OH), 10.51 (s, 1H, NH), 7.63-7.57 (m, 4H, Ar-H), 7.16 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.07 (d, 2H, *J* = 8.7 Hz, Ar-H), 4.42 (q, 1H, *J* = 7.2 Hz, CH-CH_3), 4.26 (s, 2H, CH_2-S), 2.38 (d, 2H, *J* = 6.8 Hz, CH_2-Ar), 2.13 (s, 3H, CH_3-C=NOH), 1.79-1.75 (m, 1H, CH), 1.59 (d, 3H, *J* = 7.2 Hz, CH_3-CH), 0.83 (d, 6H, *J* = 6.4 Hz, $(CH_3)_2$CH); ^{13}C NMR (DMSO-d_6) δ: 170.14, 165.22, 163.35, 152.47, 140.52, 139.41, 138.23, 132.85, 129.57, 127.26, 126.36, 119.13, 44.55, 36.87, 36.34, 29.85, 22.62, 19.85, 12.10; HRMS: m/z calculated for $C_{24}H_{27}N_4O_3S$ $[M-1]^-$: 451.18093, found: 451.18066.

3.2. Nitric oxide release

The amount of nitric oxide released from the tested compounds of different forms: starting ketone **7a**, NO-donating oximes **8a-e**, and **14** was measured using Griess colorimetric method [34]. A solution of the appropriate compound (20 µL) in dimethylsulfoxide (DMSO) was added to 2 mL of 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) with MeOH, containing 5×10^{-4} M of L-cysteine. The final concentration of drug was 10^{-4} M. After 1 h at 37 ºC, 1 mL of the reaction mixture was treated with 250 µL of Griess reagent [sulfanilamide (2 g), N-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid (10 mL) in distilled water (final volume: 100 mL)]. After 10 min at room temperature, the absorbance was measured at λ_{max} 546 nm. Sodium nitrite standard solutions (10-80 nmol/mL) were used to construct the calibration curve. The same procedure was repeated using different solutions of the test compounds under the same conditions using 0.1 N HCl of pH 1 instead of phosphate buffer of pH 7.4. The results were expressed as amount of NO released relative to a theoretical maximum release of 1 mol NO/mol of test compound.

3.3. Biological evaluation

3.3.1. Screening of anti-inflammatory activity

Anti-inflammatory activity of the different compounds was tested using the carrageenan-induced paw oedema method described by winter *et al*, [36]. Briefly, male albino rats were randomly assigned to different groups (5 rats each). Rats received either control (vehicle) or equimolar doses of the test compounds by an intraperitoneal injection. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 mL of 1% solution of carrageenan into the plantar side of the right hind paw while the left paw was used as a control. The size of the paw oedema was recorded every hour for 4 h to determine the decrease in oedema thickness. At the end of the experiment, rats were sacrificed and the weights of the right and left paws were measured. Carrageenan-injected and control paws were also processed for Histopathological examination. The difference between the average values of weights of the left paws and the corresponding right ones were calculated and compared to standard drugs.

3.3.2. Screening of analgesic activity

Analgesic activity of the different compounds was tested using the standard tail flick method [37]. Briefly, male albino rats were randomly assigned to different groups. Tail flick analgesimeter (Analgesy-meter LE7106, Panlab, Harvard apparatus) was used to determine the reaction time for each rat before and after the administration of the test compound. Radiant heat was applied to the lower third of the rat tail and the time in which the rat tries to escape was recorded as the reaction time. Reaction times were recorded thirty minutes after intraperitoneal administration of equimolar doses of the test compounds. The difference between the average values of reaction time for each group and the corresponding pretest values were calculated and compared to standard drugs.

3.3.3. Measurement of lipid peroxidation level

Antioxidant activity was evaluated through determination of thiobarbituric acid reactive species (TBARS; sometimes referred to as malondialdehyde, MDA). Aliquots of 0.5 mL of serum of sacrificed albino male rats were added to standard centrifuge tubes. Then distilled water (0.5 mL) was added to each test tube. A mixture of thiobarbituric acid/trichloroacetic acid/HCl (15% TBA, 0.2 N HCl and 0.37 % TCA, 2 mL) was added to the centrifuge tube, then tubes were covered with aluminium foil and incubated in a boiling water bath for 15 minutes, allowed to cool, and centrifuged for 15 minutes at 5000 rpm. The supernatant was filtered and the intensity of the pink colored adduct was measured in the supernatants at λ_{max} = 532 nm. Concentration of TBARS was calculated from standard calibration curve and expressed as nmol/ml.

3.3.4. Ulcerogenic liability

The synthesized compounds **7a–e**, **8a–e**, **13**, **14**, indomethacin, and ibuprofen were evaluated for their ulcerogenic liability according to the reported procedure [61]. Ulcerogenic potential was evaluated 4 h post intraperitoneal administration of the compounds under investigation. The stomachs were removed, collected, opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. Examination of mucosal layer was done using magnifying lens to detect macroscopically visible lesions. The number of lesions if any was counted and recorded. Ulcers were classified into levels, level I, in which ulcer area is less than 1 mm^2, level II, in which ulcer area in the range of 1-3 mm^2, and level III, in which ulcer area is more than 3 mm^2, and this rated according their areas in mm^2. The following parameters were calculated according to the following formula:

Ulcer index (UI) = 1×(number of ulcers level I) + 2 (number of ulcers level II) + 3 (number of ulcers level III).

3.3.5. *In vitro* COX inhibition assay

COX fluorescent inhibitor screening assay kit (catalog number 700100, Cayman chemical, Ann Arbour, MI, USA) has been employed to investigate the isozymes

specifity of some of the synthesized compounds **7a**, **7b**, **8c**, **8e**, and **13** following the procedure suggested by manufacturer. Briefly, in 96- well plate, either ovine COX-1 or human re-combinant COX-2 has been incubated with different concentrations of each tested compound in presence of the assay buffer (100mM Tris-HCl, pH 8.0), heme and the fluorometric substrate 10-acetyl-3,7-dihydroxyphenoxazine (ADHP) for 20 min at room temperature. The reaction started after addition of arachidonic acid solution for 2 min at room temperature. Fluorescence of resorufin that is produced by the reaction of between PGG2 and the fluorometric substrate were analysed. Dup-697 (selective COX-2 inhibitor) and SC-560 (selective COX-1 inhibitor) were used as reference compounds. The measured fluorescence intensity is proportional to the amount of PGG2 present in each well during the reaction. IC_{50} (µM) that corresponds to the concentration of the inhibitor that causes 50 % inhibition of COX-1 or COX-2 activity was calculated from the dose response curve of inhibition (triplicate determinations). Dose-response curves of data conforming to inhibition were fitted to:

$$V_0 = V' - \left[V' \frac{i}{i + (IC_{50})}\right] + V\infty$$

Where: V_0 is the observed rate; I is the concentration of inhibitor I; V' is the observe rate in the absence of the inhibitor; V_∞ is the observed rate constant at saturating the inhibitor, I; IC_{50} is the concentration that leads to half the maximal change in V_0.

3.3.6. Statistical analysis

The results of all the four methods namely, anti-inflammatory activity, ulcerogenic liability, analgesic activity, and antioxidant activity were expressed as mean ± SEM. Results were statistically analysed by using ANOVA (two-way classification analysis). A probability value of less than 0.05 ($P < 0.05$) was considered to be statistically significant.

3.4. Docking studies.

Docking of the synthesized compounds **7a-e**, **8a-e**, **13**, **14** and ibuprofen was done on both COX-1 and COX-2 enzymes to suggest the possible mechanism of their action and obtain the possible binding modes between the compounds and the enzymes active site. Docking experiments were done using MOE 2014 software. Target compounds were constructed into the builder interface of MOE program, the energy was minimized until a RMSD (root mean square deviations) gradient of 0.01 Kcal/mol and RMS (Root Mean Square) distance of 0.1 Å with MMFF94X (Merck molecular force field 94x) force-field and the partial charges were automatically calculated. X-ray crystallographic structure of the ligand-enzyme complex was

downloaded from protein data bank (www.rcsb.org); COX-1 enzyme (pdb: 1eqg) and COX-2 enzyme (pdb: 1cx2).

Enzymes were prepared for docking studies by deleting the ligand, adding hydrogen atoms, checking the atom connection and type with automatic correction. Then the potential of the receptor was fixed and docking of the designed compounds is done into the 3D structure of the catalytic site of COX-1 and COX-2 enzymes. The obtained poses were studied and the poses showed best ligand-enzyme interactions were selected and stored for energy calculations.

4. Conclusion

New series of 1,3,4-oxadiazole NO/hybrids were synthesized (**8a-e**) including oxadiazole NO hybrid of ibuprofen **14**. Most of the synthesized compounds exhibited promising anti-inflammatory activity compared to that of indomethacin. Compounds **7a**, **7b**, **8a**, **8c**, and **8e** exhibited significant inhibition in paw oedema thickness in carrageenan induced paw oedema. The best activity was obtained with compound **8e** (96.67% reduction in oedema). Analgesic activity was also evaluated using tail flick method. Compounds showed moderate to good analgesic activity compared to indomethacin and paracetamol. Since the tail flick method is measuring central analgesic activity, the ability of the tested compounds to penetrate BBB will affect their activity. Compound **8e** with best anti-inflammatory activity showed low analgesic activity that was explained by its large molecular size that hindered its passage to the brain. Compound **8b**, with moderate lipophilicity and small molecular size, was better as an analgesic agent. The tested compounds devoid of any antioxidant activity that excluded the antioxidant pathway from explaining the postulated anti-inflammatory action. Nitric oxide released from compound **8a-e** and **14** was found to be not essential for anti-inflammatory activity but it plays critical role in preserving the safety profile of these compounds. It offered better healing to the granuloma tissue and best integrity for stomach lining. Collectively, data suggested that COX inhibition is a reasonable mechanism for the actions exerted by the tested compounds. In vitro assays suggested a non-selective inhibition of the compounds on both COX-1 and COX-2 isozymes. The presence of bulky group on the phenyl ring favours COX-2 inhibition. Though the tested compounds were non-selective COX inhibitors, no ulcerogenicity was observed and this might be attributed to the absence of COOH functionality and the presence of NO donating group that is known for its gastroprotective actions. Docking studies supported such results. All compounds were fitted in both the active site of COX-1 and COX-2 to form stable complexes with energy scores comparable to ibuprofen. The number, type of interaction, and significance of amino acids residues interacting with the tested compounds were suggested to identify the strength of the anti-inflammatory activity. Compounds with higher number of interactions with Arg 120, Tyr 355, such as compound **7a**, **7b**, **8c**, **8e** and **13** showed higher anti-inflammatory activity. In conclusion, the synthesized oxadiazole NO/hybrids offer promising GIT-friendly alternatives to conventional

NSAIDs and should be considered as future goal for medicinal chemists working in the area of anti-inflammatory agents.

5. Acknowledgement.

Research funded by Faculty of Pharmacy, Minia University, Minia, Egypt. Appreciation for Dr. Safwat Rabea, Faculty of Pharmaceutical Sciences, The University of British Columbia and Dr. Taha Farouk, Osaka university for spectroscopic data analysis. Appreciation for teaching assistants in pharmacology and toxicology department, Minia university for helping in biological evaluations.

6. Conflict of interest.

Authors declare that there is no conflict of interest in the presented research**.**

7- References.

[1] C.K.S. Ong, P. Lirk, C.H. Tan, R.A. Seymour, An avidence-based update on nonsteroidal anti-Inflammatory drugs, Clin. Med. Res. 5 (2007) 19–34.

[2] C.J. Smith, Y. Zhang, C.M. Koboldt, J. Muhammad, B.S. Zweifel, A. Shaffer, J.J. Talley, J.L. Masferrer, K. Seibert, P.C. Isakson, Pharmacological analysis of cyclooxygenase-1 in inflammation, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13313–13318.

[3] S. Viveka, Dinesha, P. Shama, G.K. Nagaraja, S. Ballav, S. Kerkar, Design and synthesis of some new pyrazolyl-pyrazolines as potential anti-inflammatory, analgesic and antibacterial agents, Eur. J. Med. Chem. 101 (2015) 442–451.

[4] A.G. Banerjee, N. Das, S.A. Shengule, R.S. Srivastava, S.K. Shrivastava, Synthesis, characterization, evaluation and molecular dynamics studies of 5, 6-diphenyl-1,2,4-triazin-3(2*H*)-one derivatives bearing 5-ubstituted 1,3,4-oxadiazole as potential anti–inflammatory and analgesic agents, Eur. J. Med. Chem. 101 (2015) 81–95.

[5] K.R.A. Abdellatif, M.A. Abdelgawad, H.A.H. Elshemy, S.S.R. Alsayed, Design, synthesis and biological screening of new 4-thiazolidinone derivatives with promising COX-2 selectivity, anti-inflammatory activity and gastric safety profile, Bioorganic Chem. 64 (2016) 1–12.

[6] B.J. Al-Hourani, S.K. Sharma, J.Y. Mane, J. Tuszynski, V. Baracos, T. Kniess, M. Suresh, J. Pietzsch, F. Wuest, Synthesis and evaluation of 1,5-diaryl-substituted tetrazoles as novel selective cyclooxygenase-2 (COX-2) inhibitors, Bioorg. Med. Chem. Lett. 21 (2011) 1823–1826.

[7] M.C. Allison, A.G. Howatson, C.J. Torrance, F.D. Lee, R.I. Russell, Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs, N. Engl. J. Med. 327 (1992) 749–754.

[8] F. Halter, K.D. Rainsford, S.P. Sirko, A. Schmassmann, NSAID-Induced mucosal injury: analysis of gastric toxicity with new generation NSAIDs: ulcerogenicity compared with ulcer Healing, Yale J. Biol. Med. 70 (1997) 33–43.

[9] M. Gund, P. Gaikwad, N. Borhade, A. Burhan, D.C. Desai, A. Sharma, M. Dhiman, M. Patil, J. Sheikh, G. Thakre, S.G. Tipparam, S. Sharma, K.V.S. Nemmani, A. Satyam, Gastric-sparing nitric oxide-releasable “true” prodrugs of aspirin and naproxen, Bioorg. Med. Chem. Lett. 24 (2014) 5587–5592.

[10] S. Bala, S. Kamboj, V. Saini, D.N. Prasad, S. Bala, S. Kamboj, V. Saini, D.N. Prasad, Anti-Inflammatory, analgesic evaluation and molecular docking studies of *N*-Phenyl anthranilic acid-based 1,3,4-Oxadiazole analogues, J. Chem. J. Chem. 2013, 2013 (2013) e412053.

[11] G.E.-D.A.A. Abuo-Rahma, M. Abdel-Aziz, E.A.M. Beshr, T.F.S. Ali, 1,2,4-Triazole/oxime hybrids as new strategy for nitric oxide donors: Synthesis, anti-inflammatory, ulceroginicity and antiproliferative activities, Eur. J. Med. Chem. 71 (2014) 185–198.

[12] K.A. Metwally, S.H. Yaseen, E.-S.M. Lashine, H.M. El-Fayomi, M.E. El-Sadek, Non-carboxylic analogues of arylpropionic acids: Synthesis, anti-inflammatory activity and ulcerogenic potential, Eur. J. Med. Chem. 42 (2007) 152–160.

[13] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang, P.C. Isakson, Synthesis and Biological Evaluation of the 1,5-Diarylpyrazole Class of Cyclooxygenase-2 Inhibitors: Identification of 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib), J. Med. Chem. 40 (1997) 1347–1365.

[14] M.J. Langman, D.M. Jensen, D.J. Watson, S.E. Harper, P.L. Zhao, H. Quan, J.A. Bolognese, T.J. Simon, Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs, JAMA. 282 (1999) 1929–1933.

[15] A. Dallob, C.J. Hawkey, H. Greenberg, N. Wight, P. De Schepper, S. Waldman, P. Wong, L. DeTora, B. Gertz, N. Agrawal, J. Wagner, K. Gottesdiener, Characterization of etoricoxib, a novel, selective COX-2 inhibitor, J. Clin. Pharmacol. 43 (2003) 573–585.

[16] J.J. Talley, D.L. Brown, J.S. Carter, M.J. Graneto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Zhang, B.S. Zweifel, K. Seibert, 4-[5-Methyl-3-phenylisoxazol-4-yl]- benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2, J. Med. Chem. 43 (2000) 775–777.

[17] L. Laine, The gastrointestinal effects of nonselective NSAIDs and COX-2-selective inhibitors, Semin. Arthritis Rheum. 32 (2002) 25–32.

[18] S.X. Sun, K.Y. Lee, C.T. Bertram, J.L. Goldstein, Withdrawal of COX-2 selective inhibitors rofecoxib and valdecoxib: impact on NSAID and gastroprotective drug prescribing and utilization, Curr. Med. Res. Opin. 23 (2007) 1859–1866.

[19] R. Kodela, M. Chattopadhyay, K. Kashfi, NOSH-Aspirin: A novel nitric oxide-hydrogen sulfide-releasing hybrid: A new class of anti-inflammatory pharmaceuticals, ACS Med. Chem. Lett. 3 (2012) 257–262.

[20] F. Stefano, E. Distrutti, Cyclo-oxygenase (COX) inhibiting nitric oxide donating (CINODs) drugs: a review of their current status, Curr. Top. Med. Chem. 7 (2007) 277-282.

[21] S. Fiorucci, L. Santucci, P. Gresele, R.M. Faccino, P. Del Soldato, A. Morelli, Gastrointestinal safety of NO-aspirin (NCX-4016) in healthy human volunteers: a proof of concept endoscopic study, Gastroenterology. 124 (2003) 600–607.

[22] F.S. Laroux, K.P. Pavlick, I.N. Hines, S. Kawachi, H. Harada, S. Bharwani, J.M. Hoffman, M.B. Grisham, Role of nitric oxide in inflammation, Acta Physiol. Scand. 173 (2001) 113–118.

[23] G. Nagy, J.M. Clark, E.I. Buzás, C.L. Gorman, A.P. Cope, Nitric oxide, chronic inflammation and autoimmunity, Immunol. Lett. 111 (2007) 1-5.

[24] J.L. Wallace, M.J. Miller, Nitric oxide in mucosal defense: a little goes a long way, Gastroenterology. 119 (2000) 512–520.
[25] K. Takeuchi, T. Yasuhiro, Y. Asada, Y. Sugawa, Role of nitric oxide in pathogenesis of aspirin-induced gastric mucosal damage in rats, Digestion. 59 (1998) 298–307.
[26] J.L. Wallace, Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself?, Physiol. Rev. 88 (2008) 1547–1565. doi:10.1152/physrev.00004.2008.
[27] A. Szlachcic, G. Krzysiek-Maczka, R. Pajdo, A. Targosz, M. Magierowski, K. Jasnos, D. Drozdowicz, S. Kwiecien, T. Brzozowski, The impact of asymmetric dimethylarginine (ADAMA), the endogenous nitric oxide (NO) synthase inhibitor, to the pathogenesis of gastric mucosal damage, Curr. Pharm. Des. 19 (2013) 90–97.
[28] K. Bian, M.-F. Doursout, F. Murad, Vascular system: role of nitric oxide in cardiovascular diseases, J. Clin. Hypertens. Greenwich Conn. 10 (2008) 304-310.
[29] J. Loscalzo, G. Welch, Nitric oxide and its role in the cardiovascular system, Prog. Cardiovasc. Dis. 38 (1995) 87–104.
[30] A. Kumar Singh, M. Lohani, R. Parthsarthy, synthesis, characterization and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives, Iran. J. Pharm. Res. IJPR. 12 (2013) 319-323.
[31] B. Sahoo, S. Dinda, B. Kumar, J. Panda, P. Brahmkshatriya, Design, green synthesis, and anti-Inflammatory activity of schiff base of 1,3,4-oxadiazole analogues, Lett. Drug Des. Discov. 11 (2013) 82-89.
[32] D. Dewangan, K.T. Nakhate, D.K. Tripathi, P. Kashyap, H. Dhongde, Synthesis, Characterization and Screening for Analgesic and Anti-inflammatory activities of 2,5-disubstituted 1,3,4-oxadiazole derivatives, anti-Inflammatory, anti-allergy agents Med. Chem. 14 (2015) 138–145.
[33] Z. Chen, W. Xu, K. Liu, S. Yang, H. Fan, P.S. Bhadury, D.-Y. Huang, Y. Zhang, Synthesis and antiviral activity of 5−(4Chlorophenyl)-1,3,4-Thiadiazole Sulfonamides, Molecules. 15 (2010) 9046–9056.
[34] J. Sun, X. Zhang, M. Broderick, H. Fein, Measurement of nitric oxide production in biological systems by using Griess reaction assay, Sensors. 3 (2003) 276–284.
[35] M.E. Shoman, M. Abdel-Aziz, O.M. Aly, H.H. Farag, M.A. Morsy, Synthesis and investigation of anti-inflammatory activity and gastric ulcerogenicity of novel nitric oxide-donating pyrazoline derivatives, Eur. J. Med. Chem. 44 (2009) 3068–3076.
[36] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs, Proc. Soc. Exp. Biol. Med. Soc. Exp. Biol. Med. N. Y. N. 111 (1962) 544–547.
[37] R.N. Kishore, N. Anjaneyulu, M.N. Ganesh, J.V. Desai, R. Thomas, S.D. Kamat, D.V. Kamat, Comparison of analgesic activity of paracetamol and metamizole and their combination in animal model (Albino Mice), J. Adv. Pharm. Res. 3 (2012) 1-4.
[38] T.A. Wani, D. Kumar, R. Prasad, P.K. Verma, K.K. Sardar, S.K. Tandan, D. Kumar, analgesic activity of the ethanolic extract of Shorea robusta resin in experimental animals, Indian J. Pharmacol. 44 (2012) 493–499. doi:10.4103/0253-7613.99322.
[39] H. Pajouhesh, G.R. Lenz, Medicinal chemical properties of successful central nervous system drugs, NeuroRx. 2 (2005) 541–553.

[40] R. Nau, F. Sörgel, H. Eiffert, Penetration of drugs through the Blood-cerebrospinal fluid/Blood-Brain Barrier for treatment of central nervous system infections, Clin. Microbiol. Rev. 23 (2010) 858–883.
[41] F. Sörgel, H.W. Prange, others, Pharmacokinetic optimisation of the treatment of bacterial central nervous system infections, Clin. Pharmacokinet. 35 (1998) 223–246.
[42] S. Gaweł, M. Wardas, E. Niedworok, P. Wardas, [Malondialdehyde (MDA) as a lipid peroxidation marker], Wiad. Lek. Wars. Pol. 1960. 57 (2004) 453–455.
[43] J.A. Buege, S.D. Aust, Microsomal lipid peroxidation, Methods Enzymol. 52 (1978) 302–310.
[44] H. Li, M. Hortmann, A. Daiber, M. Oelze, M.A. Ostad, P.M. Schwarz, H. Xu, N. Xia, A.L. Kleschyov, C. Mang, A. Warnholtz, T. Münzel, U. Förstermann, Cyclooxygenase-2 selective and nonselective nonsteroidal anti-Inflammatory drugs induce oxidative stress by up-regulating vascular NADPH Oxidases, J. Pharmacol. Exp. Ther. 326 (2008) 745–753.
[45] M.G. Perrone, P. Vitale, P. Malerba, A. Altomare, R. Rizzi, A. Lavecchia, C. Di Giovanni, E. Novellino, A. Scilimati, Diarylheterocycle core ring features effect in selective COX-1 inhibition, ChemMedChem. 7 (2012) 629–641.
[46] J.K. Gierse, S.D. Hauser, D.P. Creely, C. Koboldt, S.H. Rangwala, P.C. Isakson, K. Seibert, Expression and selective inhibition of the constitutive and inducible forms of human cyclo-oxygenase., Biochem. J. 305 (1995) 479–484.
[47] J.L. Johnson, J. Wimsatt, S.D. Buckel, R.D. Dyer, K.R. Maddipati, Purification and characterization of prostaglandin H synthase-2 from sheep placental cotyledons, Arch. Biochem. Biophys. 324 (1995) 26–34.
[48] J. Barnett, J. Chow, D. Ives, M. Chiou, R. Mackenzie, E. Osen, B. Nguyen, S. Tsing, C. Bach, J. Freire, Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system, Biochim. Biophys. Acta. 1209 (1994) 130–139.
[49] L.J. Marnett, S.W. Rowlinson, D.C. Goodwin, A.S. Kalgutkar, C.A. Lanzo, Arachidonic acid oxygenation by COX-1 and COX-2 mechanisms of catalysis and inhibition, J. Biol. Chem. 274 (1999) 22903–22906.
[50] C.A. Harman, M.V. Turman, K.R. Kozak, L.J. Marnett, W.L. Smith, R.M. Garavito, Structural Basis of Enantioselective Inhibition of Cyclooxygenase-1 by S-α-Substituted Indomethacin Ethanolamides, J. Biol. Chem. 282 (2007) 28096–28105. doi:10.1074/jbc.M701335200.
[51] H. Xie, D. Ng, S.N. Savinov, B. Dey, P.D. Kwong, R. Wyatt, A.B. Smith, W.A. Hendrickson, Structure-activity relationships in the binding of chemically derivatized CD4 to gp120 from human immunodeficiency virus, J. Med. Chem. 50 (2007) 4898–4908.
[52] N.N. Karade, V.H. Budhewar, A.N. Katkar, G.B. Tiwari, Oxidative methyl esterification of aldehydes promoted by molecular and hypervalent (III) iodine, Arkivoc. 11 (2006) 162–167.
[53] J.R. Maxwell, D.A. Wasdahl, A.C. Wolfson, V.I. Stenberg, Synthesis of 5-aryl-2H-tetrazoles, 5-aryl-2H-tetrazole-2-acetic acids, and [(4-phenyl-5-aryl-4H-1,2,4-triazol-3-yl)thio]acetic acids as possible superoxide scavengers and antiinflammatory agents, J. Med. Chem. 27 (1984) 1565–1570.
[54] D.A. Charistos, G.V. Vagenas, L.C. Tzavellas, C.A. Tsoleridis, N.A. Rodios, Synthesis and a UV and IR spectral study of some 2-aryl-Δ-^{2}-1,3,4-oxadiazoline-5-thiones, J. Heterocycl. Chem. 31 (1994) 1593–1598.

[55] A. Saeed, M. Akram, A. Rauf, M. Bolte, 5-(3,4,5-Trimeth-oxy-phen-yl)-1,3,4-oxadiazole-2(3H)-thione, Acta Crystallogr. Sect. E Struct. Rep. Online. 66 (2010) o1911.

[56] C.-J. Chen, B.-A. Song, S. Yang, G.-F. Xu, P.S. Bhadury, L.-H. Jin, D.-Y. Hu, Q.-Z. Li, F. Liu, W. Xue, P. Lu, Z. Chen, Synthesis and antifungal activities of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives, Bioorg. Med. Chem. 15 (2007) 3981–3989.

[57] H.A.Elsherief, A.G.Ghattas, A.M.Mahmoud, A.E.Abdel-Rahman, Synthesis of some new 2-(N-substituted carboxamidomethyl thio)-5-phenyl-1,3,4-oxadiazoles and related compounds, J. Indian Chem. Soc. 58 (1981) 1173–1176.

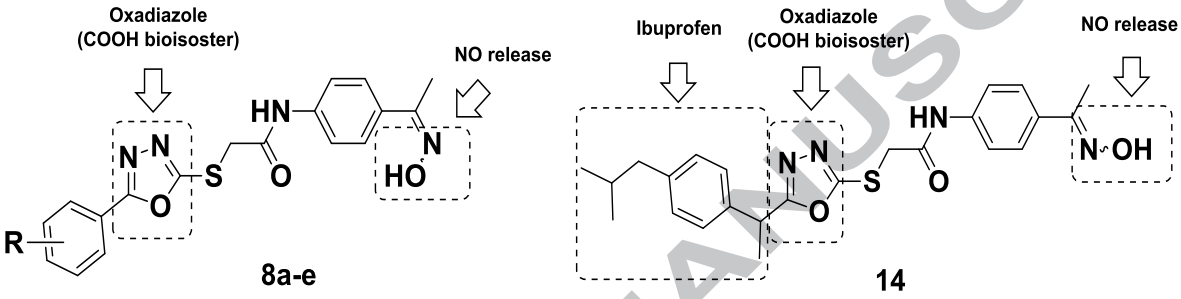
[58] A.G. Ghattas, H.A. El-Sherief, A.E. Abdel Rahman, A.M. Mahmoud, Synthesis of some new heterocyclic 1,3,4-oxadiazoles with antibacterial activity, Pharm. 37 (1982) 410–412.

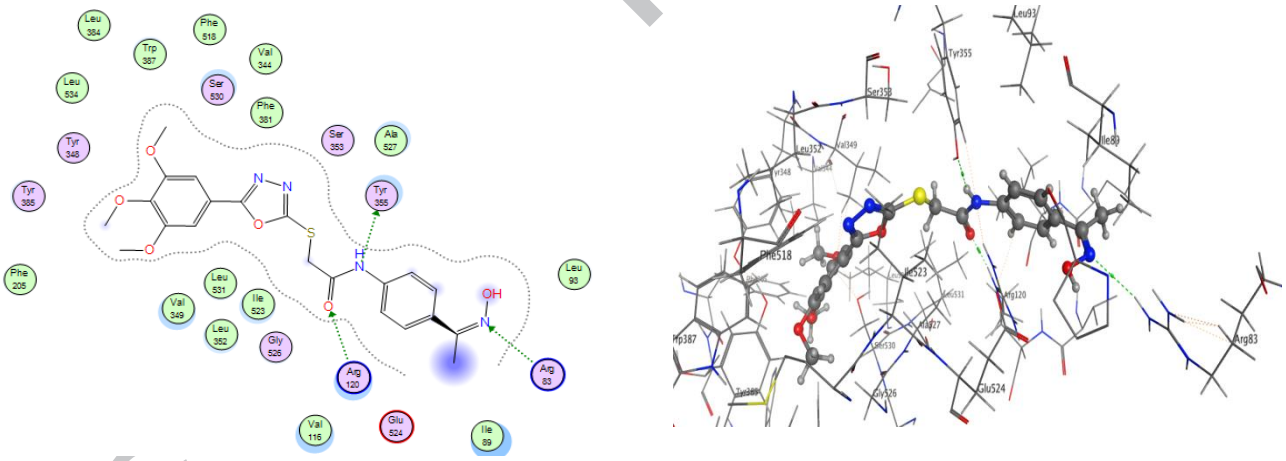
[59] V. Sharma, M.S.Y. Khan, Prodrugs and mutual prodrugs: synthesis of some new pyrazolone and oxadiazole analogues of a few non-steroidal anti-inflammatory drugs, Pharm.- Int. J. Pharm. Sci. 58 (2003) 99–103.

[60] K. Manjunatha, B. Poojary, P.L. Lobo, J. Fernandes, N.S. Kumari, Synthesis and biological evaluation of some 1,3,4-oxadiazole derivatives, Eur. J. Med. Chem. 45 (2010) 5225–5233.

[61] S.F. de Andrade, M. Lemos, E. Comunello, V.F. Noldin, V.C. Filho, R. Niero, Evaluation of the antiulcerogenic activity of Maytenus robusta (Celastraceae) in different experimental ulcer models, J. Ethnopharmacol. 113 (2007) 252–257.

Graphical abstract





2D and 3D drawings of the compound **8e** (96.67 % inhibition in Paw edema**)** interacted with both active site of COX-1

Highlights

- Synthesis of new 1,3,4-oxadiazole/ nitric oxide hybrids
- Investigation of nitric oxide release
- Biological evaluation including anti-inflammatory activity, analgesic , antioxidant activity and ulcerogenic liability.
- Investigating possible mechanism of action through *in vitro* COX inhibition assays
- Docking studies on the empty active site of COX-1 and COX-2 enzymes