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

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ABSTRACT

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

1. Introduction

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

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

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

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

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

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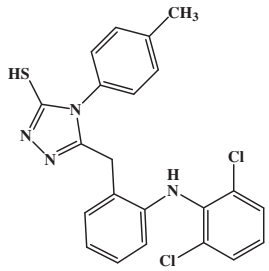
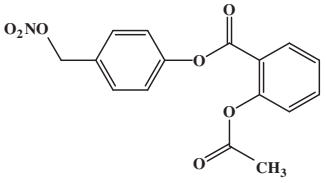
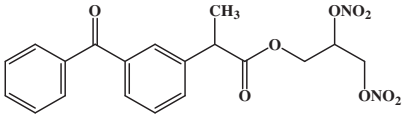


1,2,4-triazole analogues of diclofenac.



NCX 4040



Ketoprofen-NO hybrid

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

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

2. Results and discussion

2.1. Chemistry

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

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

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

2.2. Measurement of nitric oxide release

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

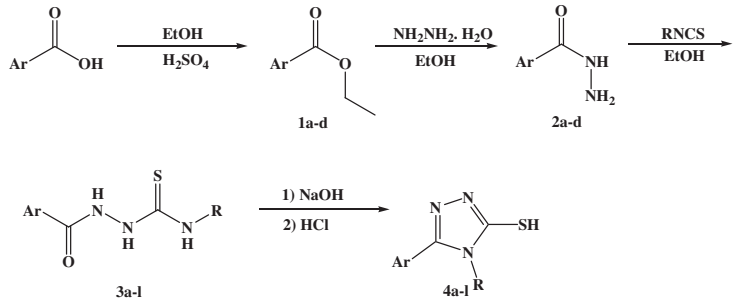
The data of Table 1 indicated that the released amount of NO from oximes **6a–l** was relatively small compared to the previously reported data from other NO-donating hybrids[37–39] and this may be attributed to the expected intramolecular hydrogen bonding between the oxime OH group and the sulfur atom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

2.3. Biological investigations

2.3.1. Screening of anti-inflammatory activity

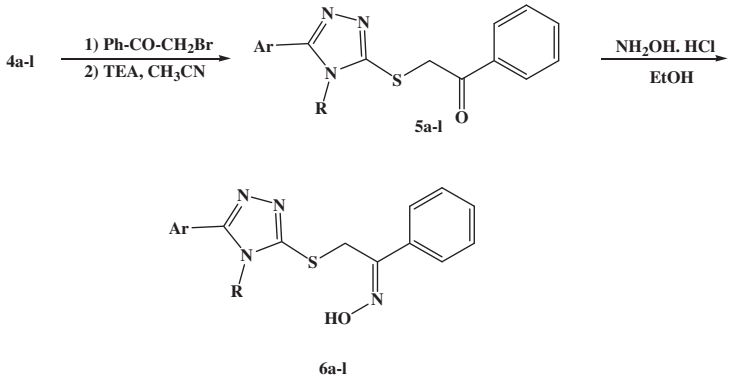
The synthesized compounds **5a–l** and **6a–l** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema in rats described by Winter et al.,[43] The tested compounds and the reference drug indomethacin were administered orally at a dose level of 0.28 mmol/kg, 30 min before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

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



Compound	R	Ar	Compound	R	Ar
1a, 2a	---	Ph	**3e, 4e**	Et	Ph
1b, 2b	---	4-OCH_3-Ph	**3f, 4f**	Et	4-OCH_3-Ph
1c, 2c	---	3,4-di-OCH_3-Ph	**3g, 4g**	Et	3,4-di-OCH_3-Ph
1d, 2d	---	3,4,5-tri-OCH_3-Ph	**3h, 4h**	Et	3,4,5-tri-OCH_3-Ph
3a, 4a	allyl	Ph	**3i, 4i**	Ph	Ph
3b, 4b	allyl	4-OCH_3-Ph	**3j, 4j**	Ph	4-OCH_3-Ph
3c, 4c	allyl	3,4-di-OCH_3-Ph	**3k, 4k**	Ph	3,4-di-OCH_3-Ph
3d, 4d	allyl	3,4,5-tri-OCH_3-Ph	**3l, 4l**	Ph	3,4,5-tri-OCH_3-Ph

Scheme IA. Synthesis of 4-*R*-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives **4a–l**. The synthesis of the target compounds 1-phenyl-2-((4-*R*-5-aryl-4*H*-1,2,4-triazol-3-yl)thio)ethanone oxime **6a–l** is illustrated in Scheme IB.



Compound	R	Ar	Compound	R	Ar
5a, 6a	allyl	Ph	**5g, 6g**	Et	3,4-Di-OCH_3-Ph
5b, 6b	allyl	4-OCH_3-Ph	**5h, 6h**	Et	3,4,5-Tri-OCH_3-Ph
5c, 6c	allyl	3,4-Di-OCH_3-Ph	**5i, 6i**	Ph	Ph
5d, 6d	allyl	3,4,5-Tri-OCH_3-Ph	**5j, 6j**	Ph	4-OCH_3-Ph
5e, 6e	Et	Ph	**5k, 6k**	Ph	3,4-Di-OCH_3-Ph
5f, 6f	Et	4-OCH_3-Ph	**5l, 6l**	Ph	3,4,5-Tri-OCH_3-Ph

Scheme IB. Synthesis of 1-phenyl-2-((4-*R*-5-aryl-4*H*-1,2,4-triazol-3-yl)thio)ethanone oxime **6a–l**.

where V_R represents the mean right paw thickness and V_L represents the mean left paw thickness.

$(V_R - V_L)$ control represents the mean increase in paw thickness in the control group of rats.

$(V_R - V_L)$ treated represents the mean increase in paw thickness in rats treated with the tested compounds.

Results in Table 2 show the percentage of edema inhibition induced by carrageenan for the ketone intermediates **5a–l**, oxime derivatives **6a–l** and indomethacin versus time in hours. Most of the tested compounds showed a significant anti-inflammatory activity against carrageenan-induced paw edema in rats ($p < 0.01$). Indomethacin showed an inhibitory activity of 83% against carrageenan-induced paw edema after 4 h, which is the time required to reach the maximum activity for most of the tested compounds, then the activity decreased in the next hour. Compounds **5a–l** exhibited from 63% to 73% anti-inflammatory activity after the fourth hour representing 80%, 81%, 87%, 80%, 76%, 84%, 88%, 87%, 82%, 82%, 78% and 88% of indomethacin activity, respectively.

Furthermore, oxime derivatives **6a–l** exhibited high anti-inflammatory activity after the fourth hour ranging from 73% to 82% representing 93%, 92%, 94%, 88%, 88%, 94%, 99%, 95%, 95%, 96%, 98% and 92% of indomethacin activity, respectively.

Table 1
Amount of NO released ($n = 4$, number of reaction mixtures assayed for each compound) determined by Griess reagent using 0.1 mM of the tested compounds **6a–l** in the presence of 0.5 mM *N*-acetylcysteine in phosphate buffer of pH 7.4

Compound number	Amount of NO released (mol/mol)					
	1 h	2 h	3 h	4 h	5 h	6 h
6a	0.06 ± 0.010	0.10 ± 0.099	0.13 ± 0.031	0.16 ± 0.095	0.22 ± 0.048	0.17 ± 0.091
6b	0.09 ± 0.091	0.12 ± 0.024	0.12 ± 0.010	0.15 ± 0.027	0.15 ± 0.040	0.14 ± 0.048
6c	0.09 ± 0.048	0.15 ± 0.074	0.17 ± 0.099	0.17 ± 0.012	0.24 ± 0.099	0.13 ± 0.081
6d	0.08 ± 0.017	0.10 ± 0.013	0.12 ± 0.016	0.13 ± 0.028	0.17 ± 0.028	0.17 ± 0.026
6e	0.06 ± 0.005	0.08 ± 0.010	0.12 ± 0.019	0.13 ± 0.012	0.14 ± 0.014	0.13 ± 0.010
6f	0.08 ± 0.003	0.11 ± 0.009	0.14 ± 0.025	0.19 ± 0.016	0.14 ± 0.012	0.12 ± 0.011
6g	0.06 ± 0.005	0.13 ± 0.004	0.2 ± 0.021	0.24 ± 0.010	0.25 ± 0.008	0.20 ± 0.002
6h	0.11 ± 0.024	0.12 ± 0.031	0.12 ± 0.034	0.21 ± 0.041	0.18 ± 0.048	0.14 ± 0.040
6i	0.06 ± 0.031	0.10 ± 0.074	0.12 ± 0.091	0.13 ± 0.099	0.15 ± 0.095	0.09 ± 0.051
6j	0.08 ± 0.005	0.08 ± 0.012	0.09 ± 0.027	0.15 ± 0.099	0.15 ± 0.017	0.12 ± 0.025
6k	0.15 ± 0.024	0.17 ± 0.050	0.18 ± 0.038	0.19 ± 0.057	0.23 ± 0.081	0.17 ± 0.048
6l	0.07 ± 0.010	0.11 ± 0.018	0.12 ± 0.015	0.14 ± 0.033	0.16 ± 0.025	0.15 ± 0.037

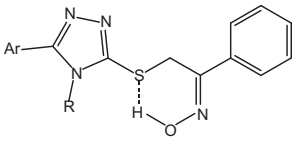


Figure 2. Expected intramolecular hydrogen bonding between the oxime OH group and the sulfur atom.

Several conclusions could be deduced from the above mentioned results; slight improvement for the anti-inflammatory activity was achieved by the oxime derivatives **6a–l** compared to their ketone intermediates **5a–l** that may attributed to the synergistic effect of NO and/or the enhanced physicochemical properties of oxime derivatives compared to their corresponding ketones. Compounds **6g** (R = ethyl, Ar = 3,4-di-OCH_3-Ph) and **6k** (R = Ph, Ar = 3,4-di-OCH_3-Ph) exhibited the highest anti-inflammatory activity among the tested compounds that may explain the impact of the presence of the 3,4-di-OCH_3-Ph moiety on the anti-inflammatory activity of the tested compounds which is in agreement with the scope of this work. The results also showed that there is no great difference between allyl, ethyl and phenyl substituents on the triazole nucleus on the anti-inflammatory activity of these compounds.

2.3.2. Screening of ulcerogenic liability

The ulcerogenic liability was evaluated according to the reported procedure[44] for the synthesized compounds **5a–l** and **6a–l** relative to indomethacin. Ulcerogenic liability was evaluated in rats after oral administration of the tested compounds and the reference indomethacin at a dose level of 0.28 mmol/kg suspended in 0.5% aqueous CMC. 0.5% Aqueous CMC was used as a control.

Ulcers were classified into levels, level I, in which the ulcer area is less than 1 mm^{-2}, level II, in which ulcer area is in the range from 1 to 3 mm^2 and level III, in which the ulcer area more than 3 mm^2, where the following parameters were calculated:[45]

Table 2
The anti-inflammatory activity at different time intervals for the ketone intermediates **5a–l** and their corresponding oximes **6a–l** using carrageenan-induced paw edema in rats

Compound number	% of edema inhibition (% mean ± SEM)				
	1 h	2 h	3 h	4 h	5 h
Control	0	0	0	0	0
5a	43 ± 1.16***	58 ± 1.55***	70 ± 3.39***	66 ± 0.99***	66 ± 1.48***
5b	49 ± 4.38***	53 ± 4.70***	63 ± 4.16***	67 ± 2.82***	61 ± 4.10***
5c	61 ± 3.49***	63 ± 0.42***	64 ± 1.85***	72 ± 4.77***	62 ± 2.78***
5d	49 ± 2.75***	62 ± 3.87***	66 ± 2.79***	66 ± 2.03***	60 ± 0.59***
5e	52 ± 2.81***	55 ± 2.44***	61 ± 2.26***	63 ± 1.49***	51 ± 2.72***
5f	53 ± 1.42***	60 ± 2.89***	67 ± 1.88***	70 ± 3.50***	61 ± 5.34***
5g	42 ± 0.19***	55 ± 1.46***	61 ± 3.36***	73 ± 5.35***	69 ± 3.87***
5h	29 ± 1.02**	41 ± 3.03***	49 ± 0.69***	72 ± 3.04***	59 ± 1.66***
5i	53 ± 3.36***	60 ± 0.40***	64 ± 3.94***	68 ± 4.89***	63 ± 1.83***
5j	52 ± 1.17***	57 ± 5.63***	58 ± 2.42***	68 ± 2.36***	61 ± 3.37***
5k	56 ± 4.92***	57 ± 0.83***	59 ± 1.75***	65 ± 1.27***	59 ± 5.09***
5l	44 ± 2.47***	51 ± 2.92***	68 ± 1.89***	73 ± 3.00***	72 ± 3.11***
6a	58 ± 1.36***	60 ± 2.57***	65 ± 2.29***	77 ± 4.31***	68 ± 1.84***
6b	61 ± 1.07***	66 ± 2.26***	67 ± 2.74***	76 ± 1.37***	57 ± 0.56***
6c	53 ± 4.36***	57 ± 3.05***	58 ± 2.93***	78 ± 1.26***	69 ± 1.06***
6d	61 ± 1.20***	68 ± 1.81***	70 ± 3.29***	73 ± 4.29***	77 ± 0.24***
6e	65 ± 1.93***	69 ± 2.04***	72 ± 4.38***	73 ± 2.80***	71 ± 1.23***
6f	69 ± 1.70***	72 ± 1.35***	75 ± 3.72***	78 ± 2.21***	71 ± 0.30***
6g	61 ± 1.83***	67 ± 1.36***	71 ± 2.81***	82 ± 0.92***	71 ± 3.14***
6h	63 ± 2.72***	67 ± 1.23***	69 ± 4.76***	79 ± 1.79***	74 ± 0.92***
6i	60 ± 1.70***	63 ± 0.76***	65 ± 1.93***	79 ± 2.71***	75 ± 1.62***
6j	58 ± 0.22***	63 ± 1.76***	63 ± 3.29***	80 ± 1.74***	73 ± 1.48***
6k	55 ± 4.36***	61 ± 2.42***	67 ± 0.99***	81 ± 1.69***	78 ± 3.53***
6l	61 ± 2.38***	61 ± 2.81***	64 ± 2.47***	76 ± 2.84***	68 ± 2.65***
Indomethacin	51 ± 0.78***	67 ± 1.36***	74 ± 2.17***	83 ± 1.41***	87 ± 0.47***

Note. One way ANOVA test was applied to determine the significance of the difference between the control group and rats treated with the tested compounds. ($n = 4$), $^{**}p < 0.01$, $^{***}p < 0.001$, significant difference from control group.

1. The ulcer index (UI) was calculated as follows:
   1 × (number of ulcers level I) + 2 × (number of ulcers level II) + 3 × (number of ulcers level III), etc. . . . . ..
2. Cure ratio = 100 − ($UI_{treated}$ × 100/$UI_{protype}$)

where, $UI_{treated}$: means the average of the UI of the groups of rats treated with the NO-donating derivatives; $UI_{protype}$: means the average of the UI of the groups of rats treated with the starting and the intermediate derivatives.

The UI of compounds **5a–l** and **6a–l** were calculated and listed in Table 3 (mean ± SEM). The results revealed that indomethacin caused significant ulcerogenic toxicity with UI of 63.45, whereas an equal dose of most of the synthesized compounds exhibited much safer UI compared to indomethacin. For example, the ketone derivatives **5a–l** exhibited UI ranging from 2.44 to 9.58, while their corresponding NO-donating oxime analogues **6a–l** exhibited a very low significant ulcerogenic liability compared to indomethacin and their starting ketone derivatives with UI ranging from of 1.14 to 7.57 (Table 3 and Figs. 3A–C).

The cure ratio of the target NO-donating derivatives **6a–l** relative to their ketone intermediates **5a–l** was calculated and listed in Table 3. The results revealed that the NO-donating oxime derivatives **6a–g** and **6i–k** achieved a great reduction of ulcers than their corresponding ketone intermediates **5a–g** and **5i–k**, respectively. On the other hand, compounds **6h** and **6l** showed a negative cure ratio of −7.02% and −4.35%, respectively, than their corresponding ketone intermediate **5h** and **5l**.

Several conclusions could be deduced from the above mentioned results; all the tested compounds achieved a very low significant ulcerogenic liability compared to indomethacin that may be attributed to the beneficial effect of the triazole nucleus as less ulcerogenic bioisoster of the carboxyl function. The oxime derivatives produce lower UI than their corresponding ketone that may be attributed to the beneficial effect of the NO and the triazole nucleus. The NO donating oxime **6b** (R = allyl, Ar = 4-OCH_3-Ph) showed the lowest UI (1.14) among all the synthesized oximes **6a–l**. The decreased gastric toxicity of the targeted NO–triazole hybrids **6a–l** compared to their starting ketone intermediates **5a–l** may be attributed to the release of NO that increases mucosal blood flow resulting in enhanced mucosal resistance to ulceration.[46,47]

2.3.3. Histopathological investigation

After assessment of gastric mucosal ulcerogenicity and determination of UI, stomach sections of the ulcers for the control and the treated groups were stained by standard hematoxylin and eosin

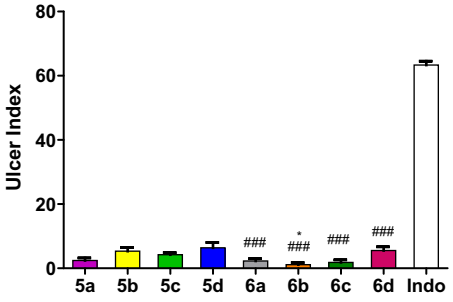


Figure 3A. UI of compounds **5a–d** and **6a–d** compared to indomethacin (Indo) expressed as mean ± SEM.

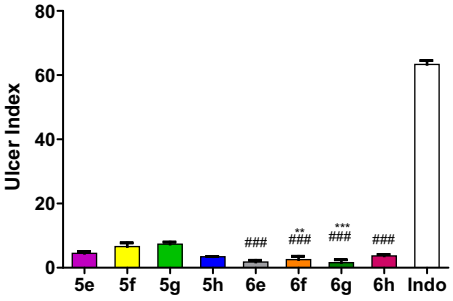


Figure 3B. UI of compounds **5e–h** and **6e–h** compared to indomethacin (Indo) expressed as mean ± SEM.

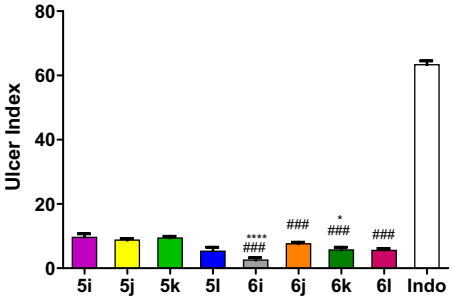


Figure 3C. UI of compounds **5i–l** and **6i–l** compared to indomethacin (Indo) expressed as mean ± SEM.

Table 3
Ulcer indices of compounds **5a–l** and **6a–l** compared to indomethacin expressed as mean ± SEM and cure ratio (%) of oximes relative to its starting ketones

Compound number	Ulcer index (UI) (mean ± SEM)	Compound number	Ulcer index (UI) (mean ± SEM)	Cure ratio (%) of oximes relative to its ketones intermediates
Control	0.60 ± 0.05			
5a	2.44 ± 0.82***	**6a**	2.30 ± 0.67***	5.74
5b	5.34 ± 1.16***	**6b**	1.14 ± 0.65***	78.65
5c	4.26 ± 0.62***	**6c**	1.82 ± 0.81***	57.28
5d	6.39 ± 1.67***	**6d**	5.52 ± 1.15***	13.62
5e	4.49 ± 0.62***	**6e**	1.78 ± 0.54***	60.36
5f	6.59 ± 1.17***	**6f**	2.53 ± 1.05***	61.61
5g	7.36 ± 0.65***	**6g**	1.58 ± 0.90***	78.53
5h	3.42 ± 0.10***	**6h**	3.66 ± 0.46***	−7.02
5i	9.58 ± 1.13***	**6i**	2.53 ± 0.70***	73.59
5j	8.74 ± 0.53***	**6j**	7.57 ± 0.54***	13.39
5k	9.41 ± 0.54***	**6k**	5.71 ± 0.82***	39.32
5l	5.29 ± 1.24 ***	**6l**	5.52 ± 0.63***	−4.35
Indomethacin	63.45 ± 2.75			

Note. One way ANOVA test was applied to determine the significance of the difference between the control group and rats treated with the tested compounds. ($n = 4$), ***$p < 0.001$, significant difference from control group.

stain. The produced slides were subjected to microscopical examination and pictures were picked for these slides (Figs. 4A–D).

The control group (Fig. 4A) showed no lesions and characterized by continuous mucosal layer while the one treated with indomethacin (Fig. 4B) showed that gastric mucosa was decreased in thickness with marked loss of mucosal membrane at the areas of ulceration. Lamina propria showed capillary vasodilatation associated with accumulation of edema fluid and acute inflammatory cell infiltrating its whole thickness and apparently destroying the surface epithelium and the epithelial lining of gastric pits. Severe atrophic gastritis and gland atrophy and was noticed. Apoptotic glandular epithelial cells could be detected.

The group treated with NO-donating oxime **6g** (Fig. 4D) indicated partial healing of the ulcer but remnants of the structural damage were still found and not completely regenerated that also indicated by the low UI where it was 1.58. On the other hand, the group treated with its ketone derivative **5g** (Fig. 4C) exhibited marked capillary dilatation in the lumina propria just below the fundic glands, capillary inflammatory cells were also found and edema fluid leads to flattening of the above mucosal membrane which also confirmed by its high UI as it was 7.36.

In conclusion, the histopathological investigation confirms the previously mentioned ulcerogenic liability results of the tested compounds and supports the effect of NO as a gastroprotective agent through increasing the mucosal blood flow, mucus and bicarbonate secretion.

2.3.4. Screening of antiproliferative activity

Compounds **6c**, **6e**, **6g**, **6i**, **6j**, **6k** and **6l** were selected by the National Cancer Institute (NCI) according to the protocol of the Drug Evaluation Branch of the National Cancer Institute, Bethesda, USA for in vitro anticancer screening. Primary in vitro one dose anticancer assay was performed in full NCI 60 cell lines derived from nine tumor subpanels, including leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancer cell lines. The selected compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye sulforhodamine B (SRB). Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells.

The NO-donating oxime **6c** achieved cell growth inhibition of 27.70 and 22.07, respectively, against both leukemia HL-60(TB) and renal cancer UO-31, while the NO-donating oxime **6e** exhibited moderate cell growth inhibition activity against non-small cell lung cancer HOP-92 and renal cancer A498 cell lines with cell growth inhibition of 37.52 and 30.67, respectively. In addition, the NO-donating oxime **6i** exhibited moderate cell growth inhibition activity against both renal cancer A498 and breast cancer MDA-MB-231/ATCC cell lines with cell growth

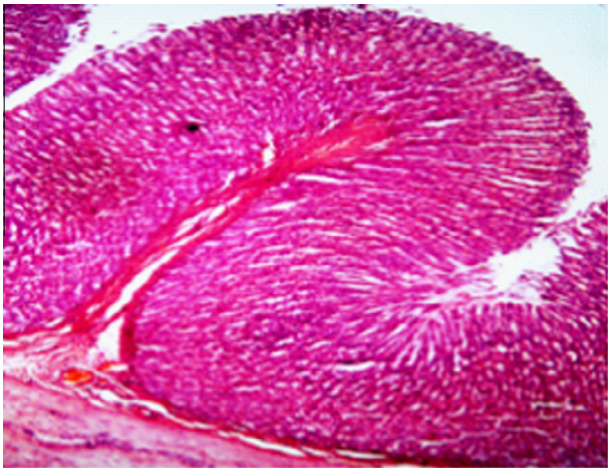


Figure 4A. Photomicrograph of the mucosa of fundic stomach of control.

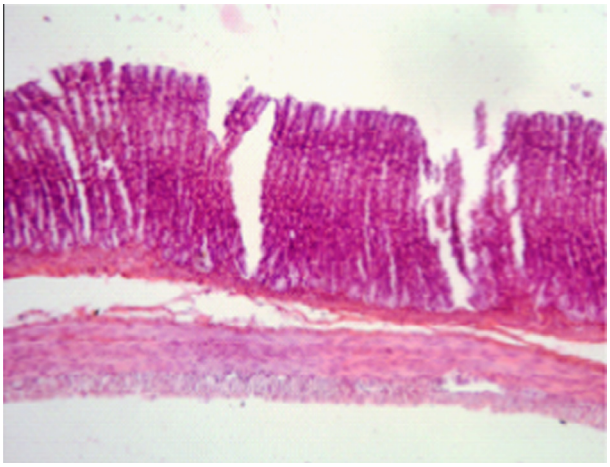


Figure 4B. Photomicrograph of the mucosa of fundic stomach of indomethacin.

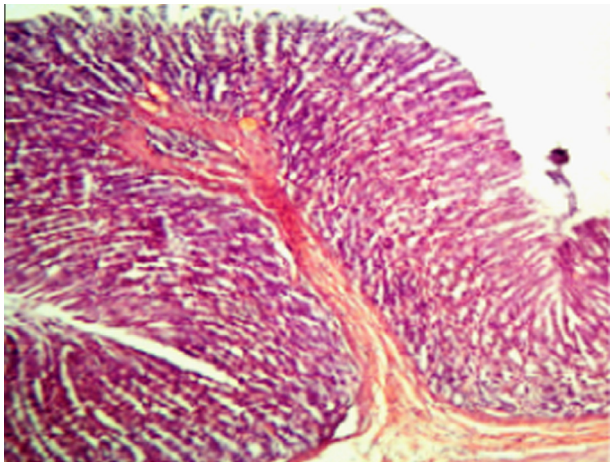


Figure 4C. Photomicrograph of the mucosa of fundic stomach of compound **5g**.

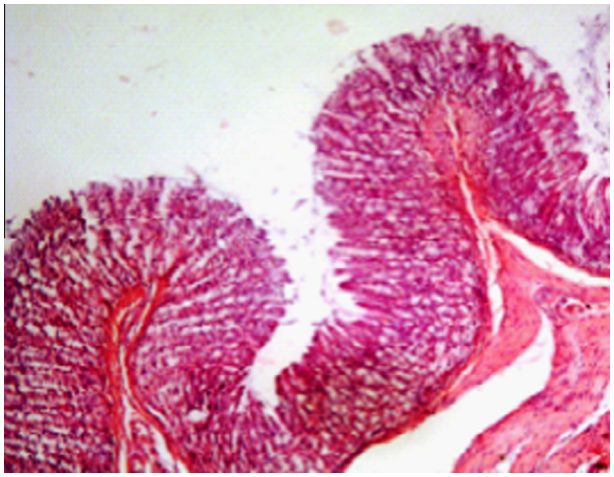


Figure 4D. Photomicrograph of the mucosa of fundic stomach of compound **6g**.

inhibition of 50.52 and 31.56, respectively and weak cell growth inhibition activity against both leukemia K-562 and renal cancer UO-31 cell lines with cell growth inhibition of 25.39 and 22.60, respectively. Compound **6j** exhibited cell growth inhibition of 29.72, 26.55, 24.12 and 26.15 against leukemia MOLT-4, RPMI-8226, SR and renal cancer A498 cell lines, respectively. The NO-donating oxime **6k** exhibited moderate cell growth inhibition activity against leukemia HL-60(TB), RPMI-8226 cell lines with cell growth inhibition of 31.76 and 30.60, respectively. Additionally, compound **6l** exhibited 26.43 and 25.17, 21.04 and 30.48, respectively, against leukemia RPMI-8226, SR, renal cancer CAKI-1 and UO-31 cell lines. From the above mentioned results, it is clear that the *N*-allyl and *N*-ethyl substituted triazoles exhibited weak antiproliferative activity while the *N*-phenyl substituted triazoles revealed from moderate to weak activity against most of the tested cell lines. It is obvious that the unsubstituted 1,2-diphenyl triazole **6i** exhibited the most significant activity among the tested compounds against renal cancer A498 cell lines.

3. Conclusions

A series of novel triazole–NO hybrids was prepared and characterized by different spectroscopic techniques and elemental analysis. Most of the synthesized compounds showed significant anti-inflammatory activity using carrageenan-induced rat paw edema method. The NO-donating oximes **6a**–**l** could release NO at pH 7.4 where the maximum amount was released after 5 h. The prepared NO-donating oximes hybrids showed pronounced gastroprotective activity better than their corresponding ketones that might be attributed to the release of NO. Histopathological examination indicated that the NO donating moiety reduced greatly the incidence of gastric ulceration. Additionally, the oxime **6i** revealed the most significant activity against renal cancer A498 cell lines with 50.52 cell growth inhibition. In summary, the use of hybrid molecules containing NO-donating moieties looks as a promising approach to improve the safety of NSAIDs without altering their effectiveness and may be a challenge in the field of anticancer therapy.

4. Experimental section

4.1. Chemistry

Reactions were monitored by TLC, pre-coated plastic sheets, 0.2 mm silica gel F_{254} with fluorescent indicator (Macherey-Nagel). Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. IR spectra were recorded on Nicolet iS5 (ATR) FT-IR spectrometer. ^{1}H NMR spectra were run on JEOL JNM-GX-300 spectrometer (300 MHz), JEOL JNM-GX-400 spectrometer (400 MHz), JEOL JNM-GX-500 spectrometer (500 MHz) and JEOL JNM-LA-400 FT-spectrometer (400 MHz). ^{13}C NMR spectra were recorded on a JEOL JNM-GX-300 spectrometer (75 MHz) or JEOL JNM-GX-400 spectrometer (100 MHz) or JEOL JNM-GX-500 spectrometer (125 MHz) using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) using $CDCl_3$ (7.29 for proton and 76.98 for carbon), CD_3OD (3.31 and 4.87 for protons and 49.00 for carbon) or DMSO-d_6 (2.50 for proton) as solvents and coupling constants (J) in Hz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet; br s, broad singlet. EI-MS was performed on JEOL JMS 600 spectrometers and Micromass LCT mass spectrometer which was recorded in the positive ion mode. Elemental analyses were recorded on Perkin Elmer 2400 CHN, Microanalytical unit, Faculty of Science, Cairo University, Egypt.

4.1.1. Substituted ethylbenzoate 1a–d, benzohydrazides 2a–d, 2-Benzoyl-*N*-Rthiosemicarbazides 3a–l and 4-*R*-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives 4a–l was prepared according to the reported procedure[24]

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4.1.2. General procedure for the synthesis of 1-phenyl-2-((4-*R*-5-aryl-4*H*-1,2,4-triazol-3-yl)thio)ethanone 5a–l

An equimolar mixture of **4a**–**l**, phenacyl bromide (1 mmol) and TEA (1.2 mmol) in acetonitril (50 mL) was heated at reflux for 4–8 h. The reaction mixture was evaporated to dryness. The residue was crystallized from aqueous ethanol affording the pure products **5a**–**l**. The structure of compounds **5a**–**l** was confirmed by mp, IR and ^{1}H NMR spectroscopy.

4.1.2.1. 2-(4-Allyl-5-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-1-phenylethanone 5a. White crystals (ethanol) in (0.244 g, 73% yield); mp 106–107 °C; FT-IR (ν_{max}): 3058 (CH aromatic), 2904 (CH aliphatic), 1686 (C=O), 1595 (C=C), 1576 cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 4.65–4.66 (m, 2H, NCH_2), 4.85 (d, 1H, CH=CH_2, J_{trans} = 17.6 Hz), 4.98 (s, 2H, SCH_2), 5.24 (d, 1H, CH=CH_2, J_{cis} = 10.4 Hz), 5.94–6.03 (m, 1H, CH=CH$_2$), 7.53–7.70 (m, 8H, Ar-H), 8.03 (d, 2H, J = 7.6 Hz, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 40.85 (CH$_2$), 46.51 (CH$_2$), 117.17 (=CH$_2$), 126.88 (CH), 128.10 (CH), 128.39 (CH), 128.79 (CH), 128.94 (CH), 130.07 (CH), 132.40 (CH), 133.72 (C), 135.27 (C), 150.45 (C), 155.13 (C), 193.27 (C=O); FAB-MS (%) 336.2 (M+1, 100).

4.1.2.2. 2-[4-Allyl-5-(4-methoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5b. White crystals (ethanol) in (0.296 g, 81% yield); mp 114–115 °C; FT-IR (ν_{max}): 3062 (CH aromatic), 2914, 2837 (CH aliphatic), 1682 (C=O), 1612 (C=C), 1580 (C=N), 1250 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.80 (s, 3H, OCH_3), 4.62–4.63 (m, 2H, NCH_2), 4.84 (d, 1H, CH=CH_2, J_{trans} = 17.6 Hz), 4.95 (s, 2H, SCH_2), 5.23 (d, 1H, CH=CH_2, J_{cis} = 10.8 Hz), 5.93–6.03 (m, 1H, CH=CH$_2$), 7.08 (d, 2H, J = 8.8 Hz, Ar-H), 7.80–7.53 (m, 5H, Ar-H), 8.04 (d, 2H, J = 8.8 Hz, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 40.82 (CH$_2$), 46.42 (CH$_2$), 55.29 (CH$_3$), 114.37 (CH), 117.07 (=CH$_2$), 119.06 (C), 128.39 (CH), 128.79 (CH), 129.61 (CH), 132.51 (CH), 133.72 (CH), 135.27 (C), 149.92 (C), 154.80 (C), 160.52 (C), 193.32 (C=O); FAB-MS (%) 366.2 (M+1, 100).

4.1.2.3. 2-[4-Allyl-5-(3,4-dimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5c. White crystals (ethanol) in (0.328 g, 83% yield); mp 123–124 °C; FT-IR (ν_{max}): 3062 (CH aromatic), 2938, 2837 (CH aliphatic), 1680 (C=O), 1596 (C=C), 1534 (C=N), 1264 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.64–4.65 (m, 2H, NCH_2), 4.88 (d, 1H, CH=CH_2, J_{trans} = 17.6 Hz), 4.95 (s, 2H, SCH_2), 5.26 (d, 1H, CH=CH_2, J_{cis} = 10.8 Hz), 5.97–6.06 (m, 1H, CH=CH$_2$), 7.00–7.15 (m, 3H, Ar-H), 7.54–7.70 (m, 3H, Ar-H), 8.03 (d, 2H, J = 8.4 Hz, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 40.82 (CH$_2$), 46.57 (CH$_2$), 55.54 (CH$_3$), 55.57 (CH$_3$), 111.47 (CH), 111.78 (CH), 117.06 (=CH$_2$), 119.08 (C), 120.81 (CH), 128.44 (CH), 128.84 (CH), 132.70 (C), 133.78 (CH), 135.30 (CH), 148.73 (C), 150.03 (C), 150.20 (C), 155.17 (C), 193.33 (C=O); FAB-MS (%) 396.2 (M+1, 100).

4.1.2.4. 2-[4-Allyl-5-(3,4,5-trimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5d. White crystals (ethanol) in (0.323 g, 76% yield); mp 73–74 °C; FT-IR (ν_{max}): 3061 (CH aromatic), 2937, 2833 (CH aliphatic), 1680 (C=O), 1586 (C=C), 1534 (C=N), 1125 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.71 (s, 3H, OCH_3), 3.79 (s, 6H, 2 OCH_3), 4.67–4.68 (m, 2H, NCH_2), 4.92 (d, 1H, CH=CH_2, J_{trans} = 18.8 Hz), 4.97 (s, 2H, SCH_2), 5.28 (d, 1H, CH=CH_2, J_{cis} = 10 Hz), 6.01–6.07 (m, 1H, CH=CH$_2$),

6.87 (s, 2H, Ar-*H*), 7.55–7.71 (m, 3H, Ar-*H*), 8.03 (d, 2H, *J* = 8.4 Hz, Ar-*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 40.80 (*C*H_2), 46.73 (*C*H_2), 56.05 (*C*H_3), 60.15 (*C*H_3), 105.64 (*C*H), 117.15 (=*C*H_2), 122.14 (*C*), 128.47 (*C*H), 128.89 (*C*H), 132.80 (*C*), 133.83 (*C*H), 135.33 (*C*H), 138.81 (*C*), 150.30 (*C*), 153.12 (*C*), 155.17 (*C*), 193.32 (*C*=O); EI-MS (70 eV) *m*/*z* (%) 425 ($M^{+\cdot}$, 0.2), 347 (20), 307 (53), 292 (25), 194 (15), 193 (17), 105 (100), 77 (40). Anal. Calcd for $C_{22}H_{23}N_3O_4S$ (425.14): C, 62.10; H, 5.45; N, 9.88. Found: C, 61.95; H, 5.27; N, 9.64.

4.1.2.5. 2-(4-Ethyl-5-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-1-phenylethanone 5e[48]. White crystals (ethanol) in (0.200 g, 62% yield); mp 135–136 °C; FT-IR (ν_{max}): 3064 (CH aromatic), 2977, 2918 (CH aliphatic), 1688 (C=O), 1594 (C=C), 1578 (C=N).

4.1.2.6. 2-[4-Ethyl-5-(4-methoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenyl-ethanone 5f[48]. White crystals (ethanol) in (0.279 g, 79% yield); mp 83–84 °C; FT-IR (ν_{max}): 3060 (CH aromatic), 2839 (CH aliphatic), 1680 (C=O), 1612 (C=C), 1596 (C=N), 1249 (C–O).

4.1.2.7. 2-[4-Ethyl-5-(3,4-dimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5g. White crystals (ethanol) in (0.264 g, 69% yield); mp 121 °C; FT-IR (ν_{max}): 3060 (CH aromatic), 2837 (CH aliphatic), 1680 (C=O), 1596 (C=C), 1533 (C=N), 1255 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.22 (t, 3H, N-CH$_2$-C*H*$_3$, *J* = 7.2 Hz), 3.79 (s, 3H, OC*H*$_3$), 3.82 (s, 3H, OC*H*$_3$), 4.02 (q, 2H, N-C*H*$_2$-CH$_3$, *J* = 7.2 Hz), 4.98 (s, 2H, SC*H*$_2$), 7.09–7.15 (m, 3H, Ar-*H*), 7.54–7.70 (m, 3H, Ar-*H*), 8.03 (d, 2H, *J* = 8.4 Hz, Ar-*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 16.71 (–CH$_2$*C*H$_3$), 38.30 (–*C*H$_2$CH$_3$), 40.87 (–S*C*H$_2$), 51.17 (CH$_3$), 57.52 (CH$_3$), 113.91 (*C*H), 114.11 (*C*H), 121.25 (*C*), 123.45 (*C*H), 130.54 (*C*H), 130.92 (*C*H), 135.90 (*C*H), 137.61 (*C*), 151.46 (*C*), 152.26 (*C*), 153.00 (*C*), 157.60 (*C*), 195.42 (*C*=O); FAB-MS (%) 384.2 (M+1, 100).

4.1.2.8. 2-[4-Ethyl-5-(3,4,5-trimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5h. White crystals (ethanol) in (0.331 g, 80% yield); mp 82–83 °C; FT-IR (ν_{max}): 3060 (CH aromatic), 2937 (CH aliphatic), 1680 (C=O), 1585 (C=C), 1486 (C=N), 1124 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.24 (t, 3H, N-CH$_2$-C*H*$_3$, *J* = 7.2 Hz), 3.72 (s, 3H, OC*H*$_3$), 3.82 (s, 6H, 2 OC*H*$_3$), 4.04 (q, 2H, N-C*H*$_2$-CH$_3$, *J* = 7.2 Hz), 4.99 (s, 2H, SC*H*$_2$), 6.87 (s, 2H, Ar-*H*), 7.55–7.71 (m, 3H, Ar-*H*), 8.04 (d, 2H, *J* = 8 Hz, Ar-*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 15.07 (–CH$_2$*C*H$_3$), 38.65 (–*C*H$_2$CH$_3$), 40.60 (–S*C*H$_2$), 56.80 (*C*H$_3$), 60.14 (*C*H$_3$), 105.94 (*C*H), 122.49 (*C*), 128.44 (*C*H), 128.86 (*C*H), 133.78 (*C*H), 135.33 (*C*), 138.78 (*C*), 149.49 (*C*), 153.15 (*C*), 154.90 (*C*), 193.27 (*C*=O); FAB-MS (%) 414.2 (M+1, 100).

4.1.2.9. 2-(4,5-Diphenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-1-phenylethanone 5i[49]. White crystals (ethanol) in (0.316 g, 85% yield); mp 183–184 °C ([Ref. 48; 181 °C]); FT-IR (ν_{max}): 3056 (CH aromatic), 2914 (CH aliphatic), 1676 (C=O), 1594 (C=C), 1578 (C=N).

4.1.2.10. 2-[5-(4-Methoxyphenyl)-4-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5j[50]. White crystals (ethanol) in (0.31 g, 77% yield); mp 159–160 °C; FT-IR (ν_{max}): 3057 (CH aromatic), 2916 (CH aliphatic), 1678 (C=O), 1609 (C=C), 1595 (C=N), 1253 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.71 (s, 3H, OC*H*$_3$), 4.92 (s, 2H, SC*H*$_2$), 6.89 (d, 2H, *J* = 8.8 Hz, Ar-*H*), 7.26 (d, 2H, *J* = 8 Hz, Ar-*H*), 7.40–7.70 (m, 8H, Ar-*H*), 8.02 (d, 2H, *J* = 7.2 Hz, Ar-*H*).

4.1.2.11. 2-[5-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5k. White crystals (ethanol) in (0.285 g, 66% yield); mp 168–169 °C; FT-IR (ν_{max}): 3056 (CH aromatic), 2915 (CH aliphatic), 1676 (C=O), 1595 (C=C), 1534 (C=N), 1260 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.51 (s, 3H, OC*H*$_3$), 3.72 (s, 3H, OC*H*$_3$), 4.92 (s, 2H, SC*H*$_2$), 6.88–6.92 (m, 3H, Ar-*H*), 7.42–7.71 (m, 8H, Ar-*H*), 8.02 (d, 2H, *J* = 8.4 Hz, Ar-*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 40.26 (–S*C*H$_2$), 56.65 (*C*H$_3$), 56.93 (CH$_3$), 112.87 (*C*H), 113.07 (*C*H), 120.16 (*C*), 122.71 (*C*H), 129.40 (*C*H), 130.04 (*C*H), 130.49 (*C*H), 131.71 (*C*H), 131.84 (*C*), 135.47 (*C*H), 135.72 (*C*H), 137.00 (*C*), 150.16 (*C*), 151.94 (*C*), 153.02 (*C*), 156.29 (*C*), 194.82 (*C*=O); FAB-MS (%) 332.2 (M+1, 15).

4.1.2.12. 2-[5-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone 5l. White crystals (ethanol) in (0.328 g, 71% yield); mp 152–153 °C; FT-IR (ν_{max}): 3058 (CH aromatic), 2937, 2835 (CH aliphatic), 1681 (C=O), 1587 (C=C), 1487 (C=N), 1124 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.52 (s, 3H, OC*H*$_3$), 3.62 (s, 6H, 2 OC*H*$_3$), 4.94 (s, 2H, SC*H*$_2$), 6.61 (s, 2H, Ar-*H*), 7.42–7.71 (m, 8H, Ar-*H*), 8.02 (d, 2H, *J* = 7.2 Hz, Ar-*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 41.56 (–SCH$_2$), 56.63 (*C*H$_3$), 61.16 (*C*H$_3$), 106.84 (*C*H), 122.71 (*C*), 129.04 (*C*H), 129.21 (*C*H), 129.73 (*C*H), 130.11 (*C*H), 131.42 (*C*H), 131.59 (*C*H), 135.07 (*C*H), 135.55 (*C*), 136.82 (*C*), 140.48 (*C*), 153.32 (*C*), 154.41 (*C*), 155.98 (*C*), 194.45 (*C*=O); FAB-MS (%) 462.2 (M+1, 100).

4.1.3. General procedure for the synthesis of 1-phenyl-2-((4-*R*-5-aryl-4*H*-1,2,4-triazol-3-yl)thio)ethanone oxime 6a–l

A mixture of equimolar amounts of the appropriate ketone derivatives **5a–l** (0.3 mmol) and hydroxylamine hydrochloride in absolute ethanol (30 mL) was heated under reflux for 8–12 h and then left to cool. The separated solid was filtered, washed with dil. ammonia solution and distilled water, dried, and recrystallized from aqueous ethanol affording the pure novel products **6a–l**.

4.1.3.1. 2-(4-Allyl-5-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-1-phenylethanone oxime 6a. White crystals (ethanol) in (0.097 g, 92% yield); mp 145–146 °C; FT-IR (ν_{max}): 3600–3100 (br, OH), 3021 (CH aromatic), 2849 (CH aliphatic), 1620 (C=C), 1570 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 4.43 (s, 2H, SC*H*$_2$), 4.52–4.53 (m, 2H, N-C*H*$_2$), 4.70 (d, 1H, CH=C*H*$_2$, J_{trans} = 16.8 Hz), 5.13 (d,1H, CH=C*H*$_2$, J_{cis} = 10.8 Hz), 5.81–5.90 (m, 1H, C*H*=CH$_2$), 7.37–7.68 (m, 10H, Ar-*H*), 11.83 (s, 1H, O*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 26.45 (–SCH$_2$), 46.41 (*C*H$_2$), 116.98 (=*C*H$_2$), 125.89 (*C*H), 126.99 (*C*H), 128.21(*C*H), 128.53 (*C*H), 128.95 (*C*H), 129.15 (*C*H), 129.35 (*C*H), 130.17 (*C*), 132.59 (*C*), 134.31 (*C*), 152.19 (*C*), 155.50 (*C*); EI-MS (70 eV) *m*/*z* (%) 350 ($M^{+\cdot}$, 0.3), 340 (18), 339 (23), 338 (82), 242 (27), 218 (25), 217 (61), 202 (45), 131 (32), 104 (80), 103 (100), 77 (49), 76 (22). Anal. Calcd for $C_{19}H_{18}N_4OS$ (350.12): C, 65.12; H, 5.18; N, 15.99. Found: C, 64.81; H, 4.96; N, 16.06.

4.1.3.2. 2-[4-Allyl-5-(4-methoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6b. White crystals (ethanol) in (0.097 g, 85% yield); mp 141–142 °C; FT-IR (ν_{max}): 3500–3000 (br, OH), 3015 (CH aromatic), 2838 (CH aliphatic), 1612 (C=C), 1580 (C=N), 1252 cm^{-1} (C–O); ^{1}H NMR (300 MHz, $CDCl_3$, δ = ppm) δ = 3.85 (s, 3H, OC*H*$_3$), 4.40–4.42 (m, 2H, NC*H*$_2$), 4.59 (s, 2H, SC*H*$_2$), 4.92 (d, 1H, CH=C*H*$_2$, J_{trans} = 17.1 Hz), 5.24 (d, 1H, CH=C*H*$_2$, J_{cis} = 10.5 Hz), 5.77–5.86 (m, 1H, C*H*=CH$_2$), 6.98 (d, 2H, *J* = 8.4 Hz, Ar-*H*), 7.34–7.37 (m, 3H, Ar-*H*), 7.52 (d, 2H, *J* = 8.4 Hz, Ar-*H*), 7.66–7.69 (m, 2H, Ar-*H*); EI-MS (70 eV) *m*/*z* (%) 380 ($M^{+\cdot}$, 0.4), 284 (10), 247 (100), 232 (54), 214 (18), 134 (80), 133 (59), 119 (17), 103 (45), 77 (22); ^{13}C NMR (75 MHz, $CDCl_3$, δ = ppm) δ = 26.48 (–SCH$_2$), 46.38 (*C*H$_2$), 55.35 (*C*H$_3$), 114.37 (*C*H), 114.41 (*C*H), 116.93 (=*C*H$_2$), 119.00 (*C*H), 125.88 (*C*H), 128.52 (*C*H), 129.14 (*C*H), 129.74 (*C*H), 132.63 (*C*), 134.32 (*C*), 149.87 (*C*),

152.21 (*C*), 155.15 (*C*), 160.61 (*C*); Anal. Calcd for $C_{20}H_{20}N_4O_2S$ (380.13): C, 63.14; H, 5.30; N, 14.73. Found: C, 63.14; H, 5.61; N, 14.64.

4.1.3.3. 2-[4-Allyl-5-(3,4-dimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6c. White crystals (ethanol) in (0.069 g, 56% yield); mp 183 °C; FT-IR (ν_{max}): 3450–3000 (br, OH), 3139 (CH aromatic), 2838 (CH aliphatic), 1607 (C=C), 1534 (C=N), 1265 cm^{-1} (C–O); ^{1}H NMR^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 3.89 (s, 3H, O*CH*$_3$), 3.93 (s, 3H, O*CH*$_3$), 4.43–4.45 (m, 2H, N*CH*$_2$), 4.60 (s, 2H, S*CH*$_2$), 4.94 (d, 1H, CH=C*H*$_2$, J_{trans} = 17.1 Hz), 5.26 (d, 1H, CH=C*H*$_2$, J_{cis} = 10.5 Hz), 5.79–5.88 (m, 1H, C*H*=CH$_2$), 7.08–7.18 (m, 3H, Ar-*H*), 7.35–7.38 (m, 3H, Ar-*H*), 7.66–7.69 (m, 2H, Ar-*H*), 8.31 (br s, 1H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 26.51 (–S*C*H$_2$), 46.45 (–*C*H$_2$), 55.59 (2 *C*H$_3$), 111.52 (*C*H), 111.75 (*C*H), 116.87 (=*C*H$_2$), 119.18 (*C*H), 120.93 (*C*H), 125.90 (*C*H), 128.54 (*C*H), 128.57 (*C*H), 129.16 (*C*H), 132.83 (*C*), 134.34 (*C*), 148.70 (*C*), 149.85 (*C*), 150.22 (*C*), 152.27 (*C*), 155.34 (*C*); EI-MS (70 eV) *m*/*z* (%) 410 ($M^{+\cdot}$, 0.3), 405 (18), 404 (21), 276 (100), 261 (24), 164 (44), 163 (54), 104 (19), 103 (42), 77 (27). Anal. Calcd for $C_{21}H_{22}N_4O_3S$ (410.14): C, 61.44; H, 5.40; N, 13.65. Found: C, 61.08; H, 5.19; N, 13.36.

4.1.3.4. 2-[4-Allyl-5-(3,4,5-trimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6d. White crystals (ethanol) in (0.12 g, 91% yield); mp 159–160 °C; FT-IR (ν_{max}): 3600–3100 (br, OH), 3138 (CH aromatic), 2837 (CH aliphatic), 1587 (C=C, C=N), 1126 cm^{-1} (C–O); ^{1}H NMR (300 MHz, $CDCl_3$, δ = ppm) δ = 3.83 (s, 6H, 2 O*CH*$_3$), 3.87 (s, 3H, O*CH*$_3$), 4.44–4.45 (m, 2H, N*CH*$_2$), 4.60 (s, 2H, S*CH*$_2$), 4.95 (d, 1H, CH=C*H*$_2$, J_{trans} = 17.4 Hz), 5.28 (d, 1H, CH=C*H*$_2$, J_{cis} = 10.5 Hz), 5.81–5.99 (m, 1H, C*H*=CH$_2$), 6.80 (s, 2H, Ar-*H*), 7.32–7.73 (m, 5H, Ar-*H*), 9.93 (br s, 1H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$, δ = ppm) δ = 25.92 (–S*C*H$_2$), 46.83 (*C*H$_2$), 56.26 (*C*H$_3$), 60.92 (*C*H$_3$), 105.81 (*C*H), 117.96 (=*C*H$_2$), 121.81 (*C*), 126.30 (*C*H), 128.55 (*C*H), 129.52 (*C*), 131.80 (*C*H), 133.83 (*C*), 139.70 (*C*), 151.46 (*C*), 153.46 (*C*), 156.00 (*C*). EI-MS (70 eV) *m*/*z* (%) 440 ($M^{+\cdot}$, 1.5), 422 (15), 357 (19), 344 (21), 343 (100), 328 (28), 327 (88), 311 (23), 292 (21), 193 (30), 150 (27), 104 (29), 103 (42), 91 (20), 77 (74). Anal. Calcd for $C_{22}H_{24}N_4O_4S$ (440.15): C, 59.98; H, 5.49; N, 12.72. Found: C, 59.74; H, 5.62; N, 13.02.

4.1.3.5. 2-(4-Ethyl-5-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-1-phenylethanone oxime 6e. White crystals (ethanol) in (0.090 g, 89% yield); mp 166–167 °C; FT-IR (ν_{max}): 3400–3100 (br, OH), 3056 (CH aromatic), 2913, 2848 (CH aliphatic), 1600 (C=C, C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.08 (t, 3H, N-CH$_2$-C*H*$_3$, *J* = 7.2 Hz), 3.90 (q, 2H, N-C*H*$_2$-CH$_3$, *J* = 7.2 Hz), 4.44 (s, 2H, S*CH*$_2$), 7.37–7.68 (m, 10H, Ar-*H*), 11.84 (s, 1H, O*H*); EI-MS (70 eV) *m*/*z* (%) 338 ($M^{+\cdot}$, 0.5), 325 (67), 205 (53), 204 (52), 177 (33), 131 (34), 104 (100), 103 (73), 77 (57). Anal. Calcd for $C_{18}H_{18}N_4OS$ (338.12): C, 63.88; H, 5.36; N, 16.56. Found: C, 64.03; H, 5.29; N, 16.86.

4.1.3.6. 2-[4-Ethyl-5-(4-methoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6f. White crystals (ethanol) in (0.087 g, 79% yield); mp 142–143 °C; FT-IR (ν_{max}): 3500–3000 (br, OH), 3051 (CH aromatic), 2838 (CH aliphatic), 1612 (C=C), 1600 (C=N), 1252 cm^{-1} (C–O); ^{1}H NMR (300 MHz, $CDCl_3$, δ = ppm) δ = 1.16 (t, 3H, N-CH$_2$-C*H*$_3$, *J* = 7.2 Hz), 3.84 (q, 2H, N-C*H*$_2$-CH$_3$, *J* = 7.2 Hz), 3.85 (s, 3H, O*CH*$_3$), 4.60 (s, 2H, S*CH*$_2$), 7.08 (d, 2H, *J* = 8.8 Hz, Ar-*H*) 7.53–7.80 (m, 5H, Ar-*H*), 8.03 (d, 2H, *J* = 7.2 Hz, Ar-*H*), 10.66 (s, 1H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$, δ = ppm) δ = 11.92 (–CH$_2$*C*H$_3$), 26.41 (–S*C*H$_2$), 38.22 (*C*H$_2$CH$_3$), 56.64 (*C*H$_3$), 106.50 (*C*H), 120.09 (*C*H), 121.83 (*C*), 127.14 (*C*H), 132.32 (*C*H), 133.72 (*C*H), 139.28 (*C*), 140.49 (*C*), 154.21 (*C*), 156.96 (*C*), 167.12 (*C*). EI-MS (70 eV) *m*/*z* (%) 368 ($M^{+\cdot}$, 0.1), 235 (100), 210 (83), 161 (34), 134 (59), 133 (67), 104 (46), 103 (70), 90 (39), 77 (51). Anal. Calcd for $C_{19}H_{20}N_4O_2S$ (368.13): C, 61.94; H, 5.47; N, 15.21. Found: C, 62.09; H, 5.81; N, 15.27.

4.1.3.7. 2-[4-Ethyl-5-(3,4-Dimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6g. White crystals (ethanol) in (0.079 g, 66% yield); mp 176–177 °C; FT-IR (ν_{max}): 3300–3000 (br, OH), 3020 (CH aromatic), 2975, 2832 (CH aliphatic), 1607 (C=C), 1536 (C=N), 1257 cm^{-1} (C–O); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 1.22 (t, 3H, N-CH$_2$-C*H*$_3$, *J* = 7.2 Hz), 3.89 (q, 2H, N-C*H*$_2$-CH$_3$, *J* = 7.2 Hz), 3.91 (s, 3H, O*CH*$_3$), 3.93 (s, 3H, O*CH*$_3$), 4.61 (s, 2H, S*CH*$_2$), 6.93–7.14 (m, 3H, Ar-*H*), 7.33–7.38 (m, 3H, Ar-*H*), 7.67–7.71 (m, 2H, Ar-*H*), 8.74 (br s, 1H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 15.13 (–CH$_2$*C*H$_3$), 26.43 (–S*C*H$_2$), 39.50 (–CH$_2$*C*H$_3$), 55.57 (*C*H$_3$), 55.60 (*C*H$_3$), 111.76 (*C*H), 115.80 (*C*H), 119.53 (*C*H), 121.04 (*C*), 125.86 (*C*H), 128.52 (*C*H), 129.14 (*C*), 134.34 (*C*H), 148.76 (*C*), 148.97 (*C*), 150.14 (*C*), 152.26 (*C*), 155.00 (*C*). EI-MS (70 eV) *m*/*z* (%) 398 ($M^{+\cdot}$, 0.4), 246 (100), 218 (50), 204 (70), 104 (89), 77 (43). Anal. Calcd for $C_{20}H_{22}N_4O_3S$ (398.14): C, 60.28; H, 5.56; N, 14.06. Found: C, 60.50; H, 5.81; N, 14.09.

4.1.3.8. 2-[4-Ethyl-5-(3,4,5-Trimethoxyphenyl)-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6h. White crystals (ethanol) in (0.108 g, 84% yield); mp 162–163 °C; FT-IR (ν_{max}): 3500–3100 (br, OH), 3136 (CH aromatic), 2835 (CH aliphatic), 1586 (C=C, C=N), 1126 cm^{-1} (C–O); ^{1}H NMR (300 MHz, $CDCl_3$, δ = ppm) δ = 1.24 (t, 3H, N-CH$_2$-C*H*$_3$, *J* = 7.2 Hz), 3.72 (s, 3H, O*CH*$_3$), 3.82 (s, 6H, 2 O*CH*$_3$), 4.04 (q, 2H, N-C*H*$_2$-CH$_3$, *J* = 7.2 Hz), 4.63 (s, 2H, S-C*H*$_2$), 6.72 (s, 2H, Ar-*H*), 7.30–7.68 (m, 5H, Ar-*H*), 10.39 (s, 1H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$, δ = ppm) δ = 15.57 (–CH$_2$*C*H$_3$), 26.79 (–S*C*H$_2$), 39.85 (–CH$_2$*C*H$_3$), 56.23 (*C*H$_3$), 60.95 (*C*H$_3$), 105.82 (*C*H), 122.14 (*C*), 126.20 (*C*H), 128.54 (*C*H), 129.46 (*C*H), 133.83 (*C*), 139.47 (*C*), 150.74 (*C*), 153.44 (*C*), 154.05 (*C*), 155.60 (*C*). EI-MS (70 eV) *m*/*z* (%) 428 ($M^{+\cdot}$, 11.5), 409 (8), 309 (14), 295 (100), 279 (26), 252 (13), 135 (89), 103 (91), 77 (9). Anal. Calcd for $C_{21}H_{24}N_4O_4S$ (428.15): C, 58.86; H, 5.65; N, 13.07. Found: C, 58.86; H, 5.81; N, 13.21.

4.1.3.9. 2-(4,5-Diphenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-1-phenylethanone oxime 6i. White crystals (ethanol) in (0.10 g, 86% yield); mp 175–176 °C; FT-IR (ν_{max}): 3500–3000 (br, OH), 3054 (CH aromatic), 2850 (CH aliphatic), 1590 (C=C), 1496 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 4.40 (s, 2H, S*CH*$_2$), 7.66–7.32 (m, 15H, Ar-*H*), 11.80 (s, 1H, O*H*); ^{13}C NMR (75 MHz, DMSO-d_6, δ = ppm) δ = 25.57 (–S*C*H$_2$), 125.90 (*C*H), 126.66 (*C*H), 127.79 (*C*H), 127.97 (*C*H), 128.57 (*C*H), 128.60 (*CH*), 129.19 (*C*H), 129.84 (*C*), 129.87 (*C*H), 130.06 (*C*), 133.86 (*C*), 134.29 (*C*H), 151.09 (*C*), 151.91 (*C*), 154.72 (*C*). EI-MS (70 eV) *m*/*z* (%) 386 ($M^{+\cdot}$, 0.6), 378 (17), 361 (4), 253 (100), 252 (64), 237 (17), 180 (10), 104 (27), 103 (47), 77 (57). Anal. Calcd for $C_{22}H_{18}N_4OS$ (386.12): C, 68.37; H, 4.69; N, 14.50. Found: C, 68.55; H, 5.04; N, 14.43.

4.1.3.10. 2-[5-(4-Methoxyphenyl)-4-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6j. White crystals (ethanol) in (0.116 g, 93% yield); mp 184–185 °C; FT-IR (ν_{max}): 3500–3000 (br, OH), 3052 (CH aromatic), 2837 (CH aliphatic), 1611 (C=C), 1496 (C=N), 1253 cm^{-1} (C–O); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 3.77 (s, 3H, O*CH*$_3$), 4.62 (s, 2H, S*CH*$_2$), 6.79 (d, 2H, *J* = 9 Hz, Ar-*H*), 7.12–7.15 (m, 2H, Ar-*H*), 7.30–7.49 (m, 8H, Ar-*H*), 7.71–7.74 (m, 2H, Ar-*H*), 8.08 (br s, 1H, O*H*); ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 25.61 (–S*C*H$_2$), 55.24 (*C*H$_3$), 114.05 (*C*H), 118.88 (*C*H), 125.89 (*C*H), 127.82 (*C*H), 128.53 (*C*H), 129.16 (*C*), 129.43 (*C*H), 129.87 (*C*H), 129.98 (*C*), 133.99 (*C*),

134.30 (*C*H), 150.53 (*C*), 151.92 (*C*), 154.59 (*C*), 160.26 (*C*). EI-MS (70 eV) *m*/*z* (%) 399 ($M^{+\cdot}-H_2O$, 0.1), 315 (33), 282 (100), 266 (34), 133 (52), 103 (44), 77 (52). Anal. Calcd for $C_{23}H_{20}N_4O_2S$ (416.13): C, 66.33; H, 4.84; N, 13.45. Found: C, 65.97; H, 4.91; N, 13.68.

4.1.3.11. 2-[5-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6k. White crystals (ethanol) in (0.116 g, 87% yield); mp 182–183 °C; FT-IR (ν_{max}): 3500–3100 (br, OH), 3144 (CH aromatic), 2837 (CH aliphatic), 1606 (C=C), 1534 (C=N), 1258 cm^{-1} (C–O) cm^{-1} (C–O); ^{1}H NMR (400 MHz, $CDCl_3$, CD_3OD, δ = ppm) δ = 3.66 (s, 3H, O*C*H_3), 3.84 (s, 3H, O*C*H_3), 4.64 (s, 2H, S*C*H_2), 6.69–7.73 (m, 13H, Ar-*H*), 8.77 (br s, 1H, O*H*); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 27.57 (–S*C*H_2), 56.69 (*C*H_3), 56.91 (*C*H_3), 112.31 (*C*H), 112.34 (*C*H), 119.53 (*C*H), 122.72 (*C*), 127.46 (*C*H), 128.89 (*C*H), 129.67 (*C*H), 130.53 (*C*H), 131.20 (*C*H), 131.36 (*C*H), 135.17 (*C*), 135.62 (*C*), 149.94 (*C*), 151.78 (*C*), 153.20 (*C*), 154.06 (*C*), 156.59 (*C*). EI-MS (70 eV) *m*/*z* (%) 446 ($M^{+\cdot}$, 1.2), 316 (100), 315 (77), 298 (65), 103 (47), 77 (50). Anal. Calcd for $C_{24}H_{22}N_4O_3S$ (446.14): C, 64.56; H, 4.97; N, 12.55. Found: C, 64.92; H, 5.24; N, 12.27.

4.1.3.12. 2-[5-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-[1,2,4]triazol-3-ylsulfanyl]-1-phenylethanone oxime 6l. White crystals (ethanol) in (0.107 g, 75% yield); mp 180–181 °C; FT-IR (ν_{max}): 3600–3100 (br, OH), 3054 (CH aromatic), 2837 (CH aliphatic), 1588 (C=C), 1489 (C=N), 1125 cm^{-1} (C–O); ^{1}H NMR (500 MHz, $CDCl_3$, δ = ppm) δ = 3.57 (s, 6H, 2 O*C*H_3), 3.81 (s, 3H, O*C*H_3), 4.66 (s, 2H, S*C*H_2), 6.62 (s, 2H, Ar-*H*), 7.17–7.73 (m, 10H, Ar-*H*), 9.47 (br s, 1H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$, δ = ppm) δ = 25.66 (–S*C*H_2), 55.80 (*C*H_3), 60.87 (*C*H_3), 105.19 (*C*H), 126.41 (*C*), 127.54 (*C*H), 128.58 (*C*H), 129.63 (*C*H), 129.96 (*C*H), 129.99 (*C*), 133.81 (*C*), 134.22 (*C*), 139.10 (*C*), 152.97 (*C*), 154.30 (*C*), 154.81 (*C*). EI-MS (70 eV) *m*/*z* (%) 476 ($M^{+\cdot}$, 3), 343 (14), 327 (21), 251 (14), 232 (14), 169 (18), 135 (21), 103 (100), 77 (78). Anal. Calcd for $C_{25}H_{24}N_4O_4S$ (476.15): C, 63.01; H, 5.08; N, 11.76. Found: C, 62.85; H, 5.27; N, 11.73.

4.2. Nitric oxide release measurement[51]

Different solutions of the tested compounds **6a–l** (20 μL) in DMF 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 *N*-acetylcysteine. The final concentration of drug was 10^{-4} M. After 1 h at 37 °C, 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 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 the percentage of NO released relative to a theoretical maximum release of 1 mol NO/mol of test compound.

4.3. Biological evaluation

4.3.1. Anti-inflammatory activity

The experiments were performed on adult male albino rats, weighing (120–140 g), obtained from the animal house, Minia University. The animals were housed in stainless steel cages, divided into groups of four animals each and deprived of food but not water 24 h before the experiment. The anti-inflammatory activity of the compounds under investigation was studied using carrageenan. A suspension of the tested compounds **5a–l** and **6a–l** and reference drug (indomrthacin) in carboxy methyl cellulose (CMC) solution (0.5% w/v in water) was administered orally at a dose level of 0.28 mmol/kg. Control animals were similarly treated with CMC solution (0.5% w/v in water). After 30 min, 0.1 mL of freshly prepared 1% carrageenan solution in normal saline was injected into the subplantar region of the right hind paw of rats according to the method of Winter et al. An equal volume of saline was injected into the left hind paw of each rat. The right paw thickness was measured by a Vernier celiper (SMIEC) directly before and after 1, 2, 3, 4 and 5 h intervals after carrageenan injection. The anti-inflammatory activity of the tested compounds and indomethacin was calculated as the percentage decrease in edema thickness induced by carrageenan.

4.3.2. Ulcerogenic liability

After measuring the anti-inflammatory activity the rats were sacrificed by decapitation. The stomachs were removed, collected, opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage for each stomach was examined with a magnifying lens for the presence of macroscopically visible lesions. The number of lesions in each stomach, if any, was counted and recorded. Ulcers were classified into levels, level I, in which the ulcer area is less than 1 mm^{-2}, level II, in which ulcer area is in the range from 1–3 mm^2 and level III, in which the ulcer area more than 3 mm^2 and this rated according to their areas in mm^2.

The data are expressed as mean ± SEM, *one way* ANOVA test was applied to determine the significance of the difference between the control group and rats treated with the tested compounds.

4.3.3. Histopathological investigation

The histological slides were prepared according to the reported procedures for examination of ulcers under light microscope.[52] Identify site of the slide on which the section was applied by scratching wax around section with a needle. Dewax hydrated sections by using graded alcohols to water. Slides were stained with haematoxylin for 5–7 min, washed with tap water until sectioning for 5 min and immersed for 5–10 s in solution of (1% HCl in 70% alcohol), then washed well with tap water for 10–15 min followed by staining with 1% Eosin for 10 min, washed with running tap water for 1–5 min. The slide was then dehydrated using alcohols, cleaned by using xylene, covered by glass cover using Canda balsam then examined under microscope (magnification ×400).

4.3.4. Antiproliferative activity

The methodology of the NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov). Briefly, the primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, SRB. Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

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Supplementary data

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References and notes

1. Lombardino, G. *Non Steroidal Antiinflammatory Drugs*; John Wiley & Sons: New York, 1985.
2. Soll, A. H.; Weinstein, W. M.; Kurata, J.; McCarthy, D. *Ann. Intern. Med.* **1991**, *114*, 307.
3. Kumar, H.; Javed, S. A.; Khan, S. A.; Amir, M. *Eur. J. Med. Chem.* **2008**, *43*, 2688.
4. Amir, M.; Shikha, K. *Eur. J. Med. Chem.* **2004**, *39*, 535.
5. Velázquez, C. A.; Praveen Rao, P. N.; Citro, M. L.; Keefer, L. K.; Knaus, E. E. *Bioorg. Med. Chem.* **2007**, *15*, 4767.
6. Bhandari, V. S.; Parikh, K. J.; Bothara, G. K.; Chitre, S. T.; Lokwani, K. D.; Devale, L. T.; Modhave, S. N.; Pawar, S. V.; Panda, S. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 520.
7. Wallace, J. L.; Miller, M. J. *Gastroenterology* **2000**, *119*, 512.
8. Takeuchi, K.; Yasuhiro, T.; Asada, Y.; Sugawa, Y. *Digestion* **1998**, *59*, 298.
9. Holzer, P.; Pabst, M. A.; Lipp, I. T.; Peskar, B. M.; Livingston, E. H.; Guth, P. H. *Gastroenterology* **1990**, *98*, 838.
10. Wallace, J. L.; Vergnolle, N.; Muscara, M. N.; Asfaha, S.; Chapman, K.; McKnight, W.; Del Soldato, P.; Morelli, A.; Fiorucci, S. *Gastroenterology* **1999**, *117*, 557.
11. Brown, J. F.; Keates, A. C.; Hanson, P. J.; Whittle, B. J. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1993**, *265*, G418.
12. Holm, M.; Johansson, B.; Petterssson, A.; Fandrisk, L. *Acta Physiol. Scand.* **1998**, *162*, 461.
13. Iwata, F.; Leung, F. W. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1995**, *268*, G153.
14. Fujihara, C. K.; Malheiros, D. M.; Donato, J. L.; Poli, A.; De Nucci, G.; Zatz, R. *Am. J. Physiol. Renal Physiol.* **1998**, *274*, F573.
15. Guzik, T. J.; Korbut, R.; Adamek-Guzik, T. *J. Physiol. Pharmacol.* **2003**, *54*, 469.
16. T., Akaike; Maeda, H. In *Nitric Oxide Biology and Pathology*; Ignarro, L. J., Ed.; Academic Press: San Diego, 2001; p 733.
17. Wink, D. A.; Vodovotz, Y.; Laval, J.; Dewhirst, M. W.; Mitchell, J. B. *Carcinogenesis* **1998**, *19*, 711.
18. Cai, T. B.; Tang, X.; Nagorski, J.; Brauschweiger, P. G.; Wang, P. G. *Bioorg. Med. Chem.* **2003**, *11*, 4971.
19. Wink, D. A.; Cook, J. A.; Christodoulou, D.; Krishna, M. C.; Pacelli, R.; Kim, S.; DeGraff, W.; Gamson, J.; Vodovotz, Y.; Russo, A.; Mitchell, J. B. *Nitric Oxide* **1997**, *1*, 88.
20. Jia, L.; Schweizer, J.; Wang, Y.; Cerna, C.; Wong, H.; Revilla, M. *Biochem. Pharmacol.* **2003**, *66*, 2193.
21. Konovalova, N. P.; Goncharova, S. A.; Volkova, L. M.; Rajewskaya, T. A.; Eremenko, L. T.; Korolev, A. M. *Nitric Oxide* **2003**, *8*, 59.
22. Jubie, S.; Dhanabal, S. P.; Kalirajan, R.; Gowramma, B.; Gomathyx, B.; Sankar, S.; Elango, K., et al *J. Pharm. Res.* **2010**, *3*, 511.
23. Eswaran, S.; Adhikari, A. V.; Shetty, N. S. *Eur. J. Med. Chem.* **2009**, *44*, 4637.
24. Kucukguzel, I.; Kucukguzel, S. G.; Rollas, S.; Kiraz, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1703.
25. Goyal Pradeep, K. B. A.; Rana, A. C.; Jain, C. B. A. *J. Pharm. Clin. Res.* **2010**, 3.
26. Maxwell, J. R.; Wasdahl, D. A.; Wolfson, A. C.; Stenberg, V. I. *J. Med. Chem.* **1984**, *27*, 1565.
27. Navidpour, L.; Shafaroodi, H.; Abdi, K.; Amini, M.; Ghahremani, M. H.; Dehpour, A. R.; Shafiee, A. *Bioorg. Med. Chem.* **2006**, *14*, 2507.
28. Hou, Y. P.; Sun, J.; Pang, Z. H.; Lv, P. C.; Li, D. D.; Yan, L.; Zhang, H. J.; Zheng, E. X.; Zhao, J.; Zhu, H. L. *Bioorg. Med. Chem.* **2011**, *19*, 5948.
29. Ouyang, X. H.; Chen, X. L.; Piatnitski, E. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5154.
30. Ngan, E. S. W.; Hashimoto, Y.; Ma, Z. Q.; Tsai, M. J.; Tsai, S. Y. *Oncogene* **2003**, *22*, 734.
31. Park, H.; Bahn, Y. J.; Ryu, S. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4330.
32. Abou-Seri, S. M. *Eur. J. Med. Chem.* **2010**, *45*, 4113.
33. Labanauskas, L.; Kalcas, V.; Udrenaite, E.; Gaidelis, P.; Brukštus, A.; Daukšas, V. *Pharmazie* **2001**, *56*, 617.
34. Lombardino, G. *Non-Steroidal Antiiflammatory Drugs*; John Wiley & Sons: New York, 1985.
35. Shanab, K.; Pongprom, N.; Wulz, E.; Holzer, W.; Spreitzer, H.; Schmidt, P.; Aicher, B.; Muller, G.; Gunther, E. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6091.
36. Thongthoom, T.; Promsuwan, P.; Yenjai, C. *Eur. J. Med. Chem.* **2011**, *46*, 3755.
37. Shoman, M. E.; Abdel-Aziz, M.; Aly, O. M.; Farag, H. H.; Morsy, M. A. *Eur. J. Med. Chem.* **2009**, *44*, 3068.
38. Abdel-Hafez, M. N. El-SM.; Abuo-Rahma, G. A.; Abdel-Aziz, M.; Radwan, M. F.; Farag, H. H. *Bioorg. Med. Chem.* **2009**, *17*, 3829.
39. Abuo-Rahma, G. A.; Abdel-Aziz, M.; Mourad, A. E. M.; Farag, H. H. *Bioorg. Med. Chem.* **2012**, *20*, 195.
40. Mourad, A. E. M.; Abdel-Aziz, M.; Abuo-Rahma, G. A.; Farag, H. H. *Eur. J. Med. Chem.* **2012**, *54*, 907.
41. Kallury, R. K. M. R.; Rao, P. L. K. M. *Org. Mass Spectrom.* **1977**, *12*, 411.
42. Lolli, M. L.; Cena, C.; Medana, C.; Lazzarato, L.; Morini, G.; Coruzzi, G.; Manarini, S.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2001**, *44*, 3463.
43. Winter, C. A.; Risely, E. A.; Nuss, G. W. *Proc. Soc. Biol. Med.* **1962**, *111*, 544.
44. Cocco, M. T.; Congiu, C.; Onnis, V.; Morelli, M.; Felipo, V.; Cauli, O. *Bioorg. Med. Chem.* **2004**, *12*, 4169.
45. De Andrade, S. F.; Lemosa, M.; Comunello, E.; Noldin, V. F.; Filho, V. C.; Niero, R. *J. Ethnopharmacol.* **2007**, *113*, 252.
46. Perini, R.; Fiorucci, S.; Wallace, J. L. *Can. J. Gastroenterol.* **2004**, *18*, 229.
47. Bastaki, S. M.; Wallace, J. L. *Can. J. Gastroenterol.* **1999**, *13*, 123.
48. Andersen, Henrik Sune, Kampen, Gita Camilla Tejlgaard, Christensen, Inge Thoger, Mogensen, John Patrick, Larsen, Annette Rosendal, PCT Int. Appl. 2004, WO 2004089367 A1 20041021.
49. Babichev, F. S.; Kovtunenko, V. A.; Tyltin, A. K.; Lelyukh, I. G. *Khimiya Geterotsiklicheskikh Soedinenii* **1977**, *8*, 1132.
50. Iradyan, M. A.; Iradyan, N. S.; Grigoryan, R. T. *Hayastani Kimiakan Handes* **2011**, *64*, 105.
51. Tsikas, D. *J. Chromatogr., B* **2007**, *851*, 51.
52. Gartner, L. P.; Haitt, J. L. *Color Textbook of Histology*, 2nd ed.; W. B. Saunders Company: Philadelphia, London, New York, 2001.