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Original article

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

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ABSTRACT

A series of novel nitric oxide (NO) donating triazole/oxime hybrids was prepared and evaluated for their anti-inflammatory activity and antiproliferative activity. Most of the tested compounds showed significant anti-inflammatory activity using carrageenan-induced rat paw edema method compared to indomethacin. Calculation of the ulcer indices and histopathological investigation indicated that the prepared NO-donating oximes exhibited less ulcerogenicity compared to their ketone intermediates and indomethacin. The NO-donating oximes **7i** and **7k** achieved remarkable cell growth inhibition activity against most of the tested cell lines. Compound **7k** was found to be with high selectivity against CNS subpanel with selectivity ratio of 11.99 at GI_{50} level.

1. Introduction

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

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

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

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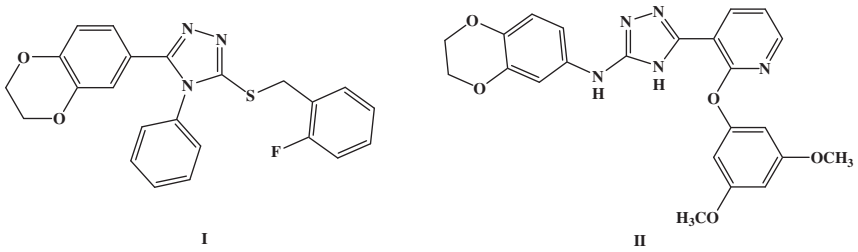


Fig. 1. Structure of some 1,2,4-triazole derivatives that have strong cell growth inhibition.

anti-proliferative activity against PC-3 cells. Additionally, several reports indicated that oximation of the carbonyl group in some compounds enhances the anticancer activity several folds compared to their corresponding ketones [37,38].

Promoted with the above-mentioned studies and as a continuation of our research interest in the field of synthesis and biological evaluation of NO-NSAIDs hybrids [39–41]. The aim of the present study is gathering the two bioactive entities, the less acidic 1,2,4-triazole-3-thiol and oxime as a NO donor in one compact structure for the purpose of synergism and/or minimizing the expected ulcerogenic side effects. A new linker has been designed aiming for improvement of the amount of NO released from these hybrids compared to our recent research work; by avoiding the expected hydrogen bonding in the previous linker [42]. The prepared triazole/NO hybrids are evaluated for their anti-inflammatory activity using carrageenan-induced rat paw edema and compared to the well-known NSAID, indomethacin. Calculation of ulcer indices and histopathological investigation was carried out to assess the beneficial effects of the NO in decreasing ulcer formation. The prepared triazole/NO hybrids were also evaluated for their anti-proliferative activity using different cancer cell lines in order to investigate the possibility of contributing synergistically to their potential antiproliferative effect.

2. Results and discussion

2.1. Chemistry

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

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

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

2.2. Measurement of nitric oxide release

The NO releasing properties of the prepared NO-donating oximes **7a**–**l** were assessed. The produced nitrite, which is a convenient index of nitric oxide production trend, was determined in both phosphate buffer of pH 7.4 and 0.1 N HCl buffer of pH 1 by using Griess colorimetric method. The reaction was carried out in the presence of *N*-acetylcysteine as a source of the SH group. The amount of NO released from the tested compounds, was measured relative to NO released from standard sodium nitrite solution and calculated as amount of NO released (mol/mol) and listed in Table 1. The results of measurement of NO release revealed that the NO-donating oximes **7a**–**l** release NO at pH of 7.4 after 4 h. Compound **7g** that contains 3,4-dimethoxyphenyl moiety released the highest amount of this group (0.43 mol/mol). The results of NO

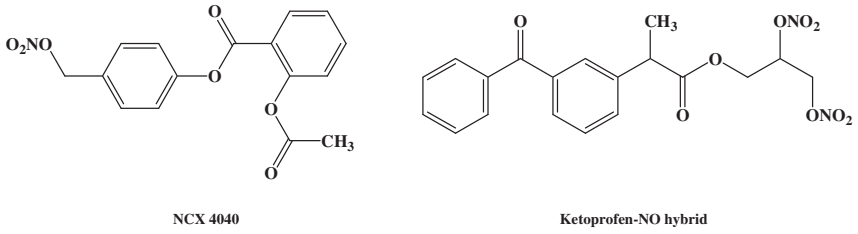
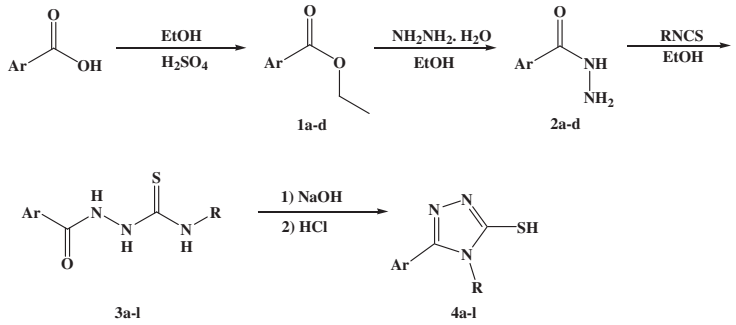


Fig. 2. NCX 4040 and ketoprofen–NO hybrids.



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 1. Synthesis of 4-allyl/ethyl/phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives **4a**–**l**.

release indicated that the target compounds released several folds amount of NO compared to the previously prepared NO-triazole hybrids [42].

The result 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 [45].

2.3. Biological investigations

2.3.1. Screening of anti-inflammatory activity

The synthesized compounds **6a**–**l** and **7a**–**l** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema in rats described by Winter et al. [46]. 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:

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

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

$(V_R - V_L)_{\text{control}}$ represents the mean increase in paw thickness in the control group of rats.

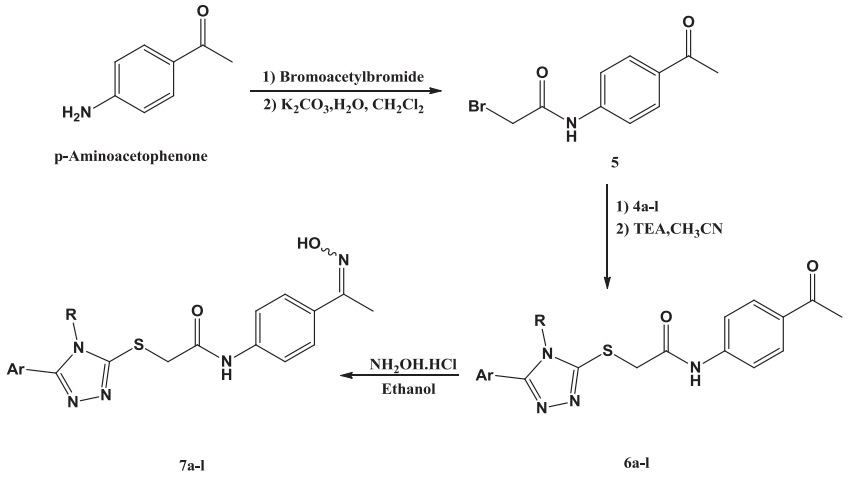
$(V_R - V_L)_{\text{treated}}$ represents the mean increase in paw thickness in rats treated with the tested compounds.

Results in Table 2 show the percentage of edema inhibition induced by carrageenan for the ketone intermediates **6a**–**l**, oxime derivatives **7a**–**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 **6a**–**l** exhibited from 62% to 70% anti-inflammatory activity after the fourth hour representing from 74% to 85% of indomethacin activity.

Furthermore, oxime derivatives **7a**–**l** exhibited high anti-inflammatory activity after the fourth hour ranging from 67% to 79% representing 81%–96% of indomethacin activity, respectively. Several conclusions could be deduced from the above mentioned results; slight improvement for the anti-inflammatory activity was achieved by the oxime derivatives **7a**–**l** compared to their ketone intermediates **6a**–**l** that may attributed to the synergistic effect of NO and/or change of the physichochemical properties of the final oximes **7a**–**l** than their corresponding ketone intermediates **6a**–**l**. Compounds **7c** (R = ethyl, Ar = 3,4-di-OCH_3–Ph) and **7k** (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 ulcerogenicity

The ulcerogenicity was evaluated according to the reported procedure [47] for the synthesized compounds **6a**–**l** and **7a**–**l** relative to indomethacin. Ulcerogenicity 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.



Compound	R	Ar	Compound	R	Ar
6a, 7a	Allyl	Ph	**6g, 7g**	Et	3,4-di-OCH_3-Ph
6b, 7b	Allyl	4-OCH_3-Ph	**6h, 7h**	Et	3,4,5-tri-OCH_3-Ph
6c, 7c	Allyl	3,4-di-OCH_3-Ph	**6i, 7i**	Ph	Ph
6d, 7d	Allyl	3,4,5-tri-OCH_3-Ph	**6j, 7j**	Ph	4-OCH_3-Ph
6e, 7e	Et	Ph	**6k, 7k**	Ph	3,4-di-OCH_3-Ph
6f, 7f	Et	4-OCH_3-Ph	**6l, 7l**	Ph	3,4,5-tri-OCH_3-Ph

Scheme 2. Preparation of *N*-(4-(1-(hydroxyimino)ethyl)phenyl)-2-[(4-allyl/ethyl/phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetamide **7a**–**l**.

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 [48]:

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 **6a**–**l** and **7a**–**l** were calculated and listed in Table 3 (mean ± SEM). The results revealed that indomethacin caused significant ulcerogenic toxicity with UI of 63.34, whereas an equal dose of most of the synthesized compounds exhibited much safer UI compared to indomethacin. For example, the ketone derivatives **6a**–**l** exhibited UI ranging from 2.45 to 10.52, while their corresponding NO-donating oxime analogs **7a**–**l** exhibited a very low significant ulcerogenicity compared to both indomethacin and their ketone intermediates with UI ranging from of 0.25–3.43 (Table 3 and Fig. 3A–C).

The cure ratio of the target NO-donating oximes **7a**–**l** relative to their ketone intermediates **6a**–**l** was calculated and listed in Table 3. The results revealed that the NO-donating oximes **7a**–**l** achieved a great reduction of ulcers than their corresponding ketone intermediates **6a**–**l**. Several conclusions could be deduced from the above mentioned results; all the tested compounds

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**7a**–**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
7a	0.15 ± 0.015	0.20 ± 0.012	0.22 ± 0.030	0.24 ± 0.035	0.16 ± 0.034	0.15 ± 0.035
7b	0.15 ± 0.052	0.17 ± 0.026	0.23 ± 0.022	0.32 ± 0.022	0.23 ± 0.019	0.16 ± 0.021
7c	0.13 ± 0.021	0.16 ± 0.030	0.32 ± 0.031	0.35 ± 0.037	0.27 ± 0.035	0.26 ± 0.040
7d	0.11 ± 0.010	0.15 ± 0.011	0.19 ± 0.014	0.24 ± 0.019	0.18 ± 0.041	0.16 ± 0.033
7e	0.11 ± 0.009	0.17 ± 0.014	0.20 ± 0.026	0.21 ± 0.023	0.14 ± 0.009	0.12 ± 0.013
7f	0.16 ± 0.005	0.20 ± 0.010	0.24 ± 0.019	0.29 ± 0.012	0.24 ± 0.014	0.23 ± 0.010
7g	0.15 ± 0.003	0.19 ± 0.009	0.25 ± 0.025	0.43 ± 0.016	0.36 ± 0.012	0.32 ± 0.011
7h	0.12 ± 0.005	0.13 ± 0.004	0.14 ± 0.021	0.22 ± 0.010	0.15 ± 0.008	0.14 ± 0.002
7i	0.11 ± 0.017	0.15 ± 0.013	0.20 ± 0.016	0.23 ± 0.028	0.14 ± 0.028	0.16 ± 0.026
7j	0.13 ± 0.009	0.17 ± 0.006	0.24 ± 0.011	0.29 ± 0.013	0.24 ± 0.013	0.23 ± 0.012
7k	0.15 ± 0.011	0.20 ± 0.013	0.23 ± 0.017	0.39 ± 0.020	0.35 ± 0.021	0.31 ± 0.018
7l	0.11 ± 0.004	0.14 ± 0.007	0.16 ± 0.006	0.25 ± 0.011	0.15 ± 0.010	0.14 ± 0.011

Table 2
The anti-inflammatory activity at different time intervals for the ketone intermediates **6a**–**l** and their corresponding oximes **7a**–**l** using carrageenan-induced paw edema in rats intermediates **6a**–**l** and their corresponding oximes **7a**–**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
6a	48 ± 1.43***	52 ± 2.41***	54 ± 2.89***	70 ± 2.61***	63 ± 2.45***
6b	50 ± 1.04***	51 ± 1.53***	52 ± 2.74***	68 ± 3.21***	66 ± 1.18***
6c	56 ± 2.41***	57 ± 3.13***	58 ± 1.89***	70 ± 3.22***	64 ± 2.46***
6d	48 ± 1.94***	54 ± 1.12***	55 ± 3.42***	68 ± 2.61***	64 ± 1.49***
6e	50 ± 1.84***	53 ± 1.59***	56 ± 1.41***	69 ± 0.45***	66 ± 2.86***
6f	51 ± 2.48***	54 ± 3.54***	57 ± 2.22***	65 ± 1.86***	62 ± 0.42***
6g	56 ± 1.64***	59 ± 0.84***	62 ± 1.45***	69 ± 2.91***	66 ± 0.86***
6h	46 ± 1.93***	50 ± 1.71***	54 ± 1.84***	68 ± 1.61***	64 ± 0.21***
6i	41 ± 1.70***	49 ± 2.25***	62 ± 2.60***	67 ± 2.44***	63 ± 1.64***
6j	29 ± 1.69**	42 ± 1.49***	58 ± 3.13***	63 ± 1.06***	60 ± 2.97***
6k	28 ± 1.38**	43 ± 3.08***	51 ± 2.46***	62 ± 4.47***	55 ± 1.86***
6l	49 ± 1.81***	54 ± 2.13***	55 ± 1.90***	68 ± 1.12***	63 ± 2.03***
7a	61 ± 1.15***	63 ± 1.70***	73 ± 2.31***	73 ± 3.06***	71 ± 1.33***
7b	54 ± 2.31***	59 ± 1.27***	63 ± 1.28***	75 ± 1.15***	72 ± 1.47***
7c	53 ± 1.14***	56 ± 3.06***	70 ± 0.76***	79 ± 0.31***	79 ± 2.31***
7d	49 ± 0.51***	53 ± 1.17***	62 ± 1.27***	72 ± 2.54***	64 ± 1.39***
7e	54 ± 1.25***	57 ± 2.11***	65 ± 4.17***	77 ± 0.15***	66 ± 0.35***
7f	54 ± 1.34***	58 ± 3.15***	60 ± 2.16***	73 ± 2.56***	65 ± 3.19***
7g	53 ± 1.45***	65 ± 3.25***	66 ± 0.45***	75 ± 2.27***	73 ± 2.65***
7h	29 ± 1.73**	49 ± 1.02***	70 ± 1.98***	70 ± 1.47***	61 ± 3.45***
7i	50 ± 1.65***	52 ± 1.89***	63 ± 2.79***	67 ± 0.28***	66 ± 2.34***
7j	45 ± 1.47***	56 ± 2.73***	57 ± 2.84***	69 ± 1.92***	58 ± 0.73***
7k	57 ± 1.52***	63 ± 2.92***	77 ± 3.02***	79 ± 0.62***	76 ± 2.32***
7l	59 ± 2.43***	62 ± 1.70***	66 ± 0.74***	75 ± 2.03***	70 ± 3.33***
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.

achieved a very low significant ulcerogenicity compared to indomethacin that may be attributed to the beneficial effect of the triazole nucleus as less ulcerogenic bioisostere of the carboxyl function. The oxime derivatives produce lower UI than their corresponding ketones that may be attributed to the synergistic effect of the NO and the triazole nucleus. The NO donating oxime **7c** (R = allyl, Ar = 3,4-di-OCH_3–Ph) achieved high anti-inflammatory activity after 4 h (79%) and in the same time exhibited lower ulcerogenicity (0.25) among the synthesized oximes **7a**–**l**. The decreased gastric toxicity of the targeted NO-triazole hybrids **7a**–**l** compared to their starting ketone intermediates **6a**–**l** may be attributed to the release of NO that increases mucosal blood flow resulting in enhanced mucosal resistance to ulceration [49,50].

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 stain. The produced slides were subjected to microscopical examination and pictures were picked for these slides (Fig. 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 was noticed. Apoptotic glandular epithelial cells could be detected.

The group treated with NO-donating oxime **7c** (Fig. 4D) showed no ulcers. The histological appearance of gastric mucosa showed improvement e.g. disappearance of the capillary dilatation, edema and leukocytes infiltration. The gastric glands restore their normal arrangement and this was confirmed by its low UI, which was 0.25. In contrast the group treated with its ketone derivative **6c** (Fig. 4C) exhibited changes in the structure of gastric mucosa through the loss of mucosal membrane at the areas of ulceration and the morphology of certain glandular structures were lost.

In conclusion, the histopathological investigation confirms the previously mentioned ulcerogenicity results of the tested compounds and supports the effect of NO as a gastroprotective agent

Table 3
Ulcer indices of compounds **6a**–**l** and **7a**–**l** compared to indomethacinexpressed as mean ± SEM and cure ratio (%) of oximes relative to its starting ketones 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			
6a	9.30 ± 1.19***	**7a**	0.64 ± 0.05***	93.12
6b	8.66 ± 1.28***	**7b**	2.69 ± 0.55***	68.94
6c	2.45 ± 0.46***	**7c**	0.25 ± 0.04***	89.80
6d	6.49 ± 1.09***	**7d**	0.75 ± 0.06***	88.44
6e	7.12 ± 0.58***	**7e**	1.18 ± 0.10***	83.43
6f	10.52 ± 0.55***	**7f**	1.04 ± 0.11***	90.11
6g	3.60 ± 1.06***	**7g**	3.43 ± 0.56***	4.72
6h	3.34 ± 1.67***	**7h**	0.83 ± 0.05***	75.15
6i	3.38 ± 0.64***	**7i**	0.95 ± 0.14***	71.89
6j	5.53 ± 1.19***	**7j**	1.61 ± 0.39***	70.89
6k	8.64 ± 1.26***	**7k**	1.11 ± 0.48***	87.15
6l	4.38 ± 0.42***	**7l**	2.21 ± 1.19***	49.54
Indomethacin	63.34 ± 1.19			

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.

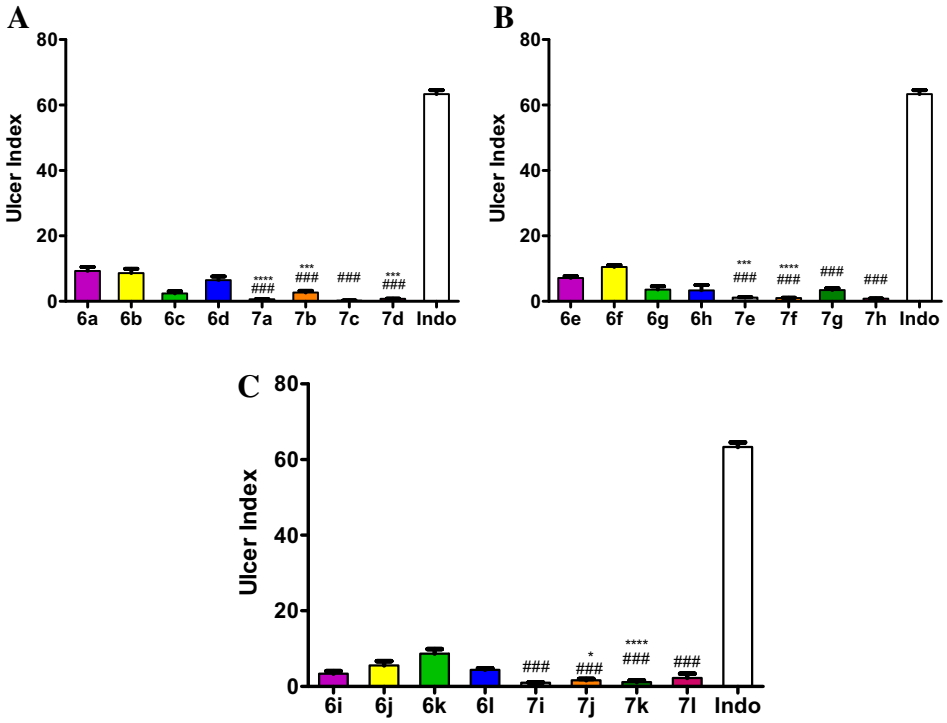


Fig. 3. A. UI of compounds **6a**–**d** and **7a**–**d** compared to indomethacin (Indo) expressed as mean ± SEM. B. UI of compounds **6e**–**h** and **7e**–**h** compared to indomethacin (Indo) expressed as mean ± SEM. C. UI of compounds **6i**–**l** and **7i**–**l** compared to indomethacin (Indo) expressed as mean ± SEM.

through increasing the mucosal blood flow, mucus and bicarbonate secretion.

2.3.4. Screening of antiproliferative activity

Compounds **7c**, **7e**, **7g**, **7i** and **7k** were selected by the National Cancer Institute (NCI) according to the protocol of the Drug Evaluation Branch of the National Cancer Institute [51] 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 **7i** exhibited remarkable cell growth inhibition activity against non-small cell lung cancer HOP-92, Colon cancer HCT-116, ovarian cancer SK-OV-3, and breast cancer BT-549. A complete cell death was recorded for the non-small cell lung cancer NCI-H522 cell line where the growth percent was −34.71. A moderate cell growth inhibition was achieved against colon cancer COLO 205, CNS cancer SF-539, SNB-75 and melanoma SK-MEL-5 cell lines (Table 3).

The NO-donating oxime **7k** achieved remarkable cell growth inhibition activity against most of the tested cell lines including non-small cell lung cancer NCI-H522, CNS cancer SF-268, SF-295, U251, ovarian cancer SK-OV-3, renal cancer RXF 393 cell lines. A complete cell death was recorded for the non-small cell lung cancer HOP-62, HOP-92 and CNS cancer SNB-75 where the growth percent were −26.44, −8.56 and −16.53, respectively. Compound **7k** revealed moderate cell growth inhibition against CNS cancer SF-539, melanoma SK-MEL-2, ovarian cancer OVACR-8, renal cancer TK-10, breast cancer MDA-MB-231/ATCC and HS 578T cell lines (Table 4).

Compounds **7c**, **7e** and **7g** exhibited a weak cell growth inhibition against most of the tested cell lines. The obtained results indicate that the NO-donating oximes **7i** and **7k** exhibited the highest ability to inhibit the proliferation of different cancer cell lines (Table 4) compared to others **7c**, **7e** and **7g**. The presence of N(4)−phenyl is essential for the antiproliferative activity of the test compounds over ethyl or allyl (compounds **7i** and **7k** has the highest ability to inhibit the proliferation of different cancer cell lines over compounds **7c**, **7e** and **7g**). In the group of N(4)−phenyl triazole derivatives, the presence of 3,4-dimethoxyphenyl substituents on C(5) of triazole nucleus is preferable over the unsubstituted phenyl moiety (NO-donating oxime **7k** has significant antiproliferative activity against different cancer cell lines over **7i**).

2.3.5. In vitro five dose full NCI 60 cell panel assay

Compound **7k** was selected for advanced five dose testing against the full panel of 60 human tumor cell lines. All the 60 cell lines representing nine tumor subpanels were incubated at five different concentrations (0.01, 0.1, 1, 10, and 100 μM). The outcomes were used to create log concentration versus % growth inhibition curves and three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition (TGI) and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h. Compound **7k** exhibited remarkable antiproliferative activity against most of the tested cell lines representing nine different subpanels. Compound **7k** showed high activity against most of the tested cell lines with GI_{50} ranging from 2.06 to >100 μM (Table 5). The criterion for selectivity of a

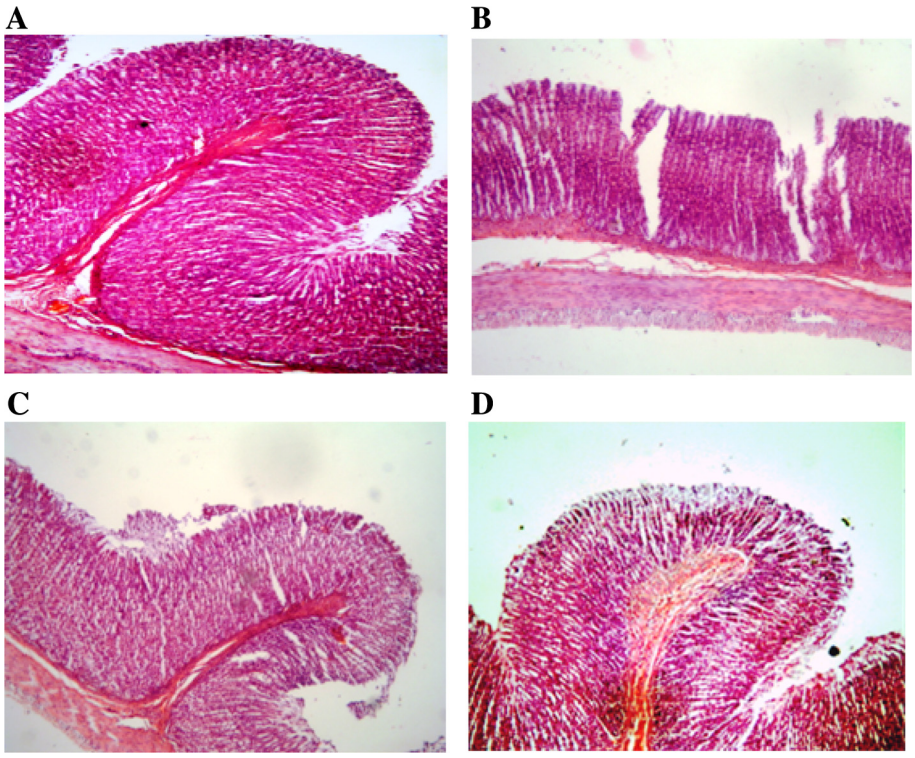


Fig. 4. A. Photomicrograph of the mucosa of fundic stomach of control. B. Photomicrograph of the mucosa of fundic stomach treated with indomethacin. C. Photomicrograph of the mucosa of fundic stomach treated with compound **6c**. D. Photomicrograph of the mucosa of fundic stomach treated with compound **7c**.

compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) (μM) by their individual subpanel MID (μM). Ratios between 3 and 6 refer to moderate selectivity; ratios >6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated nonselective [52]. In this context, compound **7k** was found to have broad-spectrum antitumor activity against most of the tumor subpanels tested with selectivity ratios ranging between 0.51 and 11.99 at the GI_{50} level. Compound **7k** was found to be broad-spectrum antitumor activity against most of the tested tumor subpanels cell lines with high selectivity against CNS subpanel with selectivity ratio of 11.99 at GI_{50} level (Table 5). Compound **7k** exhibited a moderate selectivity against non-small lung subpanel with selectivity ratio of 2.55 at GI_{50} level (Table 5).

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 **7a**–**l** could release NO at pH 7.4 where the maximum amount was released after 4 h. The prepared NO-donating oximes hybrids showed pronounced gastroprotective activity better than their corresponding ketone intermediates that may be attributed to the release of NO. Histopathological examination indicated that the NO donating moiety reduced greatly the incidence of gastric ulceration. The NO-donating oximes **7i** and **7k** achieved remarkable cell growth inhibition activity against most of the tested cell lines. Compound **7k** was found to be highly selective against CNS subpanel with a selectivity ratio of 11.99 at GI_{50} level. 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 antiproliferative 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; bs, broad singlet. EI-MS was performed on JEOL JMS 600 spectrometers. 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*-allyl/ethyl/phenylthiosemicarbazides **3a**–**l** and 4-allyl/ethyl/phenyl-5-aryl-4H-1,2,4-triazole-3-thiol derivatives **4a**–**l**

Substituted ethylbenzoate **1a**–**d**, benzohydrazides **2a**–**d**, 2-Benzoyl-*N*-allyl/ethyl/phenylthiosemicarbazides **3a**–**l** and 4-Allyl/

ethyl/phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives **4a**–**l** was prepared according to the reported procedure [5].

*4.1.2. Synthesis of N-(4-acetylphenyl)-2-bromoacetamide **5***

To a stirred mixture of *p*-aminoacetophenone (6.30 mmol) in dichloromethane (20 ml) and potassium carbonate (1.30 mmol) in 100 ml water cooled in an ice bath, bromoacetyl bromide (1.86 g, 9.20 mmol) in 30 ml dichloromethane was added in a dropwise manner with stirring over 30 min. Stirring was continued for 2 h at 0 °C, then at room temperature overnight. The reaction mixture was extracted with (2 × 50 ml) dichloromethane, and the organic layer was washed with distilled water (2 × 50 ml), dried over anhydrous sodium sulfate, filtered, evaporated on a rotary evaporator and the residue was recrystallized from 95% ethanol to give 1.53 g (95%) of compound **5**, m.p. = 155–156 °C.

Table 4
Single concentration mean graph growth percent of nine different cancer cell types for compounds **7i** and **7k** cell types for compounds **7i** and **7k**.

Panel	Cell line	Growth percent for compound **7i**	Growth percent for compound **7k**
Leukemia	CCRF-CEM	92.03	98.47
	HL-60(TB)	90.42	77.33
	K-562	95.84	105.08
	MOLT-4	95.18	91.71
	RPMI-8226	91.16	91.56
	SR	106.36	95.41
Non-small cell lung cancer	A549/ATCC	81.91	70.71
	EKVX	91.89	71.08
	HOP-62	64.67	−26.44
	HOP-92	30.06	−8.56
	NCI-H226	72.38	81.87
	NCI-H23	76.56	68.75
	NCI-H322M	66.70	75.74
	NCI-H460	−34.71	102.05
	NCI-H522	53.29	35.30
Colon cancer	COLO 205	91.14	99.10
	HCC-2998	28.61	94.02
	HCT-116	97.47	69.23
	HCT-15	84.04	98.60
	HT29	73.30	91.21
	KM12	83.11	106.14
	SW-620	99.81	114.80
CNS cancer	SF-268	92.20	30.60
	SF-295	52.61	39.07
	SF-539	97.20	43.04
	SNB-19	45.78	75.80
	SNB-75	59.23	−16.53
	U251	63.61	34.38
Melanoma	LOX IMVI	82.92	80.07
	MALME-3M	91.75	61.84
	M14	100.10	89.91
	MDA-MB-435	118.22	103.56
	SK-MEL-2	97.74	57.60
	SK-MEL-28	48.64	87.31
	SK-MEL-5	94.49	94.63
	UACC-257	81.85	91.79
	UACC-62	76.73	82.83
Ovarian cancer	IGROV1	81.42	70.90
	OVCAR-3	87.28	82.00
	OVCAR-4	86.16	103.49
	OVCAR-5	84.71	97.05
	OVCAR-8	90.76	42.75
	NCI/ADR-RES	39.26	90.56
	SK-OV-3	80.55	4.74
Renal cancer	786-0	92.72	64.48
	A498	63.71	91.78
	ACHN	85.65	75.54
	CAKI-1	99.90	87.36
	RXF-393	72.45	32.35
	SN12C	76.21	71.81
	TK-10	73.73	41.08
	UO-31	90.62	104.17
Prostate cancer	PC-3	104.11	109.75
	DU-145	67.90	88.28
Breast cancer	MCF7	62.48	69.24
	MDA-MB-231/ ATCC	20.85	47.86
	HS 578T	79.56	42.83
	BT-549	75.35	65.18
	T-47D	92.03	67.95
	MDA-MB-468	90.42	78.51

*4.1.3. General procedure for the synthesis of N-(4-acetylphenyl)-2-[(4-allyl/ethyl/phenyl-5-aryl-4H-1,2,4-triazol-3-yl)thio]acetamide **6a**–**l***

An equimolar mixture of **4a**–**l**, compound **5** (1 mmol) and TEA (1.2 mmol) in acetonitrile (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 white products **6a**–**l**. The structure of compounds **6a**–**l** was confirmed by mp, R_f, IR and ^{1}H NMR spectroscopy.

*4.1.3.1. N-(4-Acetylphenyl)-2-(4-allyl-5-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl)- acetamide **6a**.* White crystals (ethanol) in (0.345 g, 88% yield); mp 196–197 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.63; FT-IR (ν_{max}): 3239 (br, NH), 1678 (CO–NH, CO–CH_3), 1597 (C=C), 1538 cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CH_3), 4.21 (s, 2H, SCH_2), 4.62–4.63 (m, 2H, NCH_2), 4.81 (d, 1H, CH=CH_2, J_{trans} = 17.6 Hz), 5.21 (d, 1H, CH=CH_2, J_{cis} = 10.8 Hz), 5.92–5.99 (m, 1H, CH=CH_2), 7.51–7.59 (m, 5H, Ar–H), 7.68 (d, 2H, J = 8 Hz, Ar–H), 7.91 (d, 2H, J = 8 Hz, Ar–H), 10.68 (s, 1H, NH).

*4.1.3.2. N-(4-Acetylphenyl)-2-[4-allyl-5-(4-methoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide **6b**.* White crystals (ethanol) in (0.376 g, 89% yield); mp 208–209 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.63; FT-IR (ν_{max}): 3256 (br, NH), 1673 (CO–NH, CO–CH_3), 1595 (C=C), 1536 (C=N), 1250 cm^{-1} (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 3.78 (s, 3H, OCH_3), 4.19 (s, 2H, SCH_2), 4.60–4.61 (m, 2H, NCH_2), 4.81 (d, 1H, CHCH3CH_2, J_{trans} = 17.2 Hz), 5.20 (d, 1H, CHCH3CH_2, J_{cis} = 10.8 Hz), 5.91–6.00 (m, 1H, CH=CH_2), 7.05 (d, 2H, J = 8.8 Hz, Ar–H), 7.51 (d, 2H, J = 8.4 Hz, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8.4 Hz, Ar–H), 10.68 (s, 1H, NH).

*4.1.3.3. N-(4-Acetylphenyl)-2-[4-allyl-5-(3,4-dimethoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide **6c**.* White crystals (ethanol) in (0.384 g, 85% yield); mp 171–172 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.61; FT-IR (ν_{max}): 3258 (br, NH), 1674 (CO–NH, CO–CH3), 1595 (C=C), 1535 (C=N), 1266 cm^{-1} (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 3.74 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.19 (s, 2H, SCH_2), 4.63–4.64 (m, 2H, NCH_2), 4.85 (d, 1H, CH=CH_2, J_{trans} = 17.2 Hz), 5.23 (d, 1H, CH=CH_2, J_{cis} = 10.8 Hz), 5.94–6.03 (m, 1H, CH=CH_2), 7.05–7.12 (m, 3H, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8.8 Hz, Ar–H), 10.68 (s, 1H, NH).

*4.1.3.4. N-(4-Acetylphenyl)-2-[4-allyl-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]- triazol-3-ylsulfanyl]acetamide **6d**.* White crystals (ethanol) in (0.434 g, 90% yield); mp 196–197 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.58; FT-IR (ν_{max}): 3247 (br, NH), 1672 (CO–NH, CO–CH_3), 1600, 1585 (C=C), 1547 (C=N), 1120 cm^{-1} (C–O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 3.70 (s, 3H, OCH_3), 3.78 (s, 6H, 2 OCH_3), 4.23 (s, 2H, SCH_2), 4.68–4.69 (m, 2H, NCH_2), 4.91 (d, 1H, CH=CH_2, J_{trans} = 17.2 Hz), 5.27 (d, 1H, CH=CH_2, J_{cis} = 10.8 Hz), 5.99–6.08 (m, 1H, CH=CH_2), 6.86 (s, 2H, Ar–H), 7.70 (d, 2H, J = 8.8 Hz, Ar–H), 7.93 (d, 2H, J = 8.8 Hz, Ar–H), 10.70 (s, 1H, NH).

Table 5
NCI in vitro testing results of compound **7k** at five dose level in μM.

Panel	Cell line	GI_{50}			TGI	LC_{50}
		Conc. per cell line	Subpanel MID[b]	Selectivity ratio (MID[a]/MID[b])	Conc. per cell line	
Leukemia	CCRF-CEM	>100	>100	0.51	>100	>100
	HL-60(TB)	>100			>100	>100
	K-562	>100			>100	>100
	MOLT-4	>100			>100	>100
	RPMI-8226	>100			>100	>100
	SR	>100			>100	>100
Non-small cell lung cancer	A549/ATCC	9.73	19.99	2.55	>100	>100
	HOP-62	2.06			6.80	>100
	HOP-92	2.12			6.42	>100
	NCI-H226	7.28			>100	>100
	NCI-H23	12.90			>100	>100
	NCI-H322M	4.41			>100	>100
	NCI-H460	>100			>100	>100
	NCI-H522	21.4			>100	>100
Colon cancer	COLO 205	>100	87.33	0.58	>100	>100
	HCC-2998	>100			>100	>100
	HCT-116	11.3			>100	>100
	HCT-15	>100			>100	>100
	HT29	>100			>100	>100
	KM12	>100			>100	>100
	SW-620	>100			>100	>100
CNS cancer	SF-268	3.07	4.26	11.99	20.3	>100
	SF-295	3.39			37.7	>100
	SF-539	4.77			38.8	>100
	SNB-19	6.43			>100	>100
	U251	3.62			19.6	>100
Melanoma	LOX IMVI	>100	79.96	0.64	>100	>100
	MALME-3M	17.20			>100	>100
	M14	97.4			>100	>100
	MDA-MB-435	>100			>100	>100
	SK-MEL-2	5.04			28.8	>100
	SK-MEL-28	>100			>100	>100
	SK-MEL-5	>100			>100	>100
	UACC-257	>100			>100	>100
	UACC-62	>100			>100	>100
Ovarian cancer	IGROV1	3.74	45.96	1.11	29.4	>100
	OVCAR-3	7.87			>100	>100
	OVCAR-4	>100			>100	>100
	OVCAR-5	>100			>100	>100
	OVCAR-8	7.36			>100	>100
	NCI/ADR-RES	>100			>100	>100
	SK-OV-3	2.73			13.7	>100
Renal cancer	786-0	14.90	33.22	1.54	>100	>100
	A498	2.72			46.1	>100
	ACHN	8.08			>100	>100
	CAKI-1	>100			>100	>100
	RXF-393	2.56			7.11	>100
	SN12C	26.20			>100	>100
	TK-10	11.30			52.70	>100
	UO-31	>100			>100	>100
Prostate cancer	PC-3	>100	62.45	0.82	>100	>100
	DU-145	24.90			>100	>100
Breast cancer	MCF7	>100	24.59	2.08	>100	>100
	MDA-MB-231/ATCC	4.26			23.8	>100
	HS 578T	3.75			>100	>100
	BT-549	13.70			>100	>100
	T-47D	9.45			>100	>100
	MDA-MB-468	16.4			>100	>100
MID[a]			51.07			

[a] MID: Average sensitivity of all cell lines in μM.
[b] MID: Average sensitivity of all cell lines of a particular subpanel in μM.

4.1.3.5. N-(4-Acetylphenyl)-2-(4-ethyl-5-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl)-acetamide ***6e****.* White crystals (ethanol) in (0.331 g, 87% yield); mp 190–191 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.41; FT-IR (ν_{max}): 3239 (br, NH), 1666 (CO–NH, CO–CH_3), 1596 (C=C), 1539 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.19 (t, 3H, N–CH_2–CH_3, J = 7.2 Hz), 2.49 (s, 3H, CH_3), 3.99 (q, 2H, N–CH_2–CH_3, J = 7.2 Hz), 4.23 (s, 2H, SCH_2), 7.49–7.60 (m, 5H, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8.8 Hz, Ar–H), 10.69 (s, 1H, NH).

4.1.3.6. N-(4-Acetylphenyl)-2-[4-ethyl-5-(4-methoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***6f****.* White crystals (ethanol) in (0.353 g, 86% yield); mp 199–200 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.36; FT-IR (ν_{max}): 3243 (br, NH), 1675 (CO–

NH, CO–CH_3), 1596 (C=C), 1539 (C=N), 1250 cm^{-1} (C–O); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.18 (t, 3H, N–CH_2–CH_3, J = 7 Hz), 2.49 (s, 3H, CO–CH_3), 3.79 (s, 3H, OCH_3), 3.97 (q, 2H, N–CH_2–CH_3, J = 7 Hz), 4.21 (s, 2H, SCH_2), 7.06 (d, 2H, J = 8.8 Hz, Ar–H), 7.52 (d, 2H, J = 8 Hz, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8.8 Hz, Ar–H), 10.69 (s, 1H, NH).

4.1.3.7. N-(4-Acetylphenyl)-2-[5-(3,4-dimethoxyphenyl)-4-ethyl-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***6g***. White crystals (ethanol) in (0.374 g, 85% yield); mp 172–173 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.31; FT-IR (ν_{max}): 3243 (br, NH), 1675 (CO–NH, CO–CH_3), 1595 (C=C), 1535 (C=N), 1269 cm^{-1} (C–O); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.19 (t, 3H, N–CH_2–CH_3, J = 7.0 Hz), 2.49 (s, 3H, CO–CH_3), 3.76 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.99 (q, 2H, N–CH_2–CH_3, J = 7.0 Hz), 4.21 (s, 2H, SCH_2), 7.06–7.12 (m, 3H, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8 Hz, Ar–H), 10.69 (s, 1H, NH).

4.1.3.8. N-(4-Acetylphenyl)-2-[4-ethyl-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]- triazol-3-ylsulfanyl]acetamide ***6h***. White crystals (ethanol) in (0.423 g, 90% yield); mp 215–216 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.28; FT-IR (ν_{max}): 3246 (br, NH), 1675 (CO–NH, CO–CH_3), 1600, 1586 (C=C), 1546 (C=N), 1128 cm^{-1} (C–O); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.22 (t, 3H, N–CH_2–CH_3, J = 6.8 Hz), 2.49 (s, 3H, CO–CH_3), 3.69 (s, 3H, OCH_3), 3.79 (s, 6H, 2 OCH_3), 4.02 (q, 2H, N–CH_2–CH_3, J = 6.8 Hz), 4.24 (s, 2H, S–CH_2), 6.84 (s, 2H, Ar–H), 7.69 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8.4 Hz, Ar–H), 10.70 (s, 1H, NH).

4.1.3.9. N-(4-Acetylphenyl)-2-(4,5-diphenyl-4H-[1,2,4]triazol-3-ylsulfanyl)-acetamide ***6i***. White crystals (ethanol) in (0.377 g, 88% yield); mp 245–246 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.67; FT-IR (ν_{max}): 3243 (br, NH), 1685 (CO–NH), 1661 (CO–CH_3), 1595 (C=C), 1534 cm^{-1} (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 4.22 (s, 2H, SCH_2), 7.30–7.53 (m, 10H, Ar–H), 7.68 (d, 2H, J = 8.8 Hz, Ar–H), 7.91 (d, 2H, J = 8.8 Hz, Ar–H), 10.70 (s, 1H, NH).

4.1.3.10. N-(4-Acetylphenyl)-2-[5-(4-methoxyphenyl)-4-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***6j***. White crystals (ethanol) in (0.398 g, 87% yield); mp 256–257 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.63; FT-IR (ν_{max}): 3244 (br, NH), 1680 (CO–NH), 1664 (CO–CH_3), 1610 (C=C), 1539 (C=N), 1245 cm^{-1} (C–O); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 3.68 (s, 3H, OCH_3), 4.19 (s, 2H, SCH_2), 6.86 (d, 2H, J = 8.8 Hz, Ar–H), 7.24 (d, 2H, J = 8.8 Hz, Ar–H), 7.37–7.53 (m, 5H, Ar–H), 7.68 (d, 2H, J = 8.8 Hz, Ar–H), 7.91 (d, 2H, J = 8.4 Hz, Ar–H), 10.70 (s, 1H, NH).

4.1.3.11. N-(4-Acetylphenyl)-2-[5-(3,4-dimethoxyphenyl)-4-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***6k***. White crystals (ethanol) in (0.44 g, 90% yield); mp 219–220 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.61; FT-IR (ν_{max}): 3179 (br, NH), 1674 (CO–NH, CO–CH_3), 1595 (C=C), 1535 (C=N), 1255 cm^{-1} (C–O); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 3.48 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.19 (s, 2H, SCH_2), 6.83–6.88 (m, 3H, Ar–H), 7.39–7.54 (m, 5H, Ar–H), 7.68 (d, 2H, J = 8 Hz, Ar–H), 7.91 (d, 2H, J = 8 Hz, Ar–H), 10.70 (s, 1H, NH).

4.1.3.12. N-(4-Acetylphenyl)-2-[4-phenyl-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***6l***. White crystals (ethanol) in (0.462 g, 89% yield); mp 229–230 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.61; FT-IR (ν_{max}): 3172 (br, NH), 1675 (CO–NH, CO–CH_3), 1589 (C=C), 1538 (C=N), 1122 cm^{-1} (C–O); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CO–CH_3), 3.49 (s, 6H, 2 OCH_3), 3.59 (s, 3H, OCH_3), 4.21 (s, 2H, SCH_2), 6.59 (s, 2H, Ar–H), 7.44–7.55 (m, 5H, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.91 (d, 2H, J = 8.4 Hz, Ar–H), 10.70 (s, 1H, NH).

4.1.4. General procedure for the synthesis of N-(4-(1-(hydroxyimino)ethyl)phenyl)-2-((4-allyl/ethyl/phenyl-5-aryl-4H-1,2,4-triazol-3-yl)thio)acetamide ***7a–l***

A mixture of equimolar amounts of the appropriate ketones **6a–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 off, washed with dil. ammonia solution (10%) and distilled water, dried, and recrystallized from aqueous ethanol affording the pure white products **7a–l**.

4.1.4.1. 2-(4-Allyl-5-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl)-N-[4-(1-hydroxyimino-ethyl)phenyl]acetamide ***7a***. White crystals (ethanol) in (0.11 g, 90% yield); mp 194–195 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.48; FT-IR (ν_{max}): 3500–3000 (br, OH, NH), 1685 (CO–NH), 1598 (C=C), 1534 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.07 (s, 3H, HO–N=C–CH_3). 4.15 (s, 2H, SCH_2), 4.61–4.62 (m, 2H, NCH_2), 4.79 (d, 1H, CH=CH_2, J_{trans} = 17.2 Hz), 5.19 (d, 1H, CH=CH_2, J_{cis} = 10.8 Hz), 5.89–5.97 (m, 1H, CH=CH_2), 7.48–7.57 (m, 9H, Ar–H), 10.40 (s, 1H, NH), 11.04 (s, 1H, OH); EI-MS (70 eV) m/z (%): 408 (M^+, 4), 257 (48), 217 (41), 216 (59), 202 (45), 201 (33), 184 (100), 150 (97), 104 (39), 103 (51). Anal. Calcd for $C_{21}H_{21}N_5O_2S$ (407.14): C, 61.90; H, 5.19; N, 17.19; S, 7.87. Found: C, 61.95; H, 5.24; N, 16.95; S, 7.65.

4.1.4.2. 2-[4-Allyl-5-(4-methoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7b***. White crystals (ethanol) in (0.108 g, 82% yield); mp 219–220 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.43; FT-IR (ν_{max}): 3200–3000 (br, OH, NH), 1647 (CO–NH), 1596 (C=C), 1536 (C=N), 1179 (C–O); ^1H NMR (400 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 2.25 (s, 3H, HO–N=C–CH_3), 3.87 (s, 3H, OCH_3), 4.03 (s, 2H, SCH_2), 4.55–4.56 (m, 2H, NCH_2), 5.09 (d, 1H, CH=CH_2, J_{trans} = 17.2 Hz), 5.38 (d, 1H, CH=CH_2, J_{cis} = 10.4 Hz), 5.89–5.96 (m, 1H, CH=CH_2), 7.02 (d, 2H, J = 8.8 Hz, Ar–H), 7.57 (d, 4H, J = 8.8 Hz, Ar–H), 7.67 (d, 2H, J = 8.8 Hz, Ar–H), 10.77 (s, 1H, NH). Anal. Calcd for $C_{22}H_{23}N_5O_3S$ (437.15): C, 60.39; H, 5.30; N, 16.01; S, 7.33. Found: C, 60.51; H, 5.35; N, 16.31; S, 7.23.

4.1.4.3. 2-[4-Allyl-5-(3,4-dimethoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7c***. White crystals (ethanol) in (0.119 g, 85% yield); mp 140–141 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.41; FT-IR (ν_{max}): 3700–3000 (br, OH, NH), 1689 (CO–NH), 1599 (C=C), 1535 (C=N), 1266 (C–O); ^1H NMR (400 MHz, $CDCl_3$ + CD_3OD, δ = ppm) δ = 2.25 (s, 3H, HO–N=C–CH_3), 3.91 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.96 (s, 2H, SCH_2), 4.57–4.58 (m, 2H, NCH_2), 5.11 (d, 1H, CH=CH_2, J_{trans} = 17.4 Hz), 5.39 (d, 1H, CH=CH_2, J_{cis} = 10.5 Hz), 5.91–6.00 (m, 1H, CH=CH_2), 6.97 (d, 2H, J = 8.4 Hz, Ar–H), 7.14–7.26 (m, 3H, Ar–H), 7.58 (d, 2H, J = 8.4 Hz, Ar–H), 7.67 (d, 2H, J = 9 Hz, Ar–H), 10.74 (s, 1H, NH). EI-MS (70 eV) m/z (%): 467 (M^+, 17), 311 (96), 247 (70), 232 (43), 214 (59), 161 (61), 150 (82), 134 (50), 133 (100), 118 (49), 107 (50), 100 (49), 77 (54). Anal. Calcd for $C_{23}H_{25}N_5O_4S$: (467.16) C, 59.08; H, 5.39; N, 14.98; S, 6.86. Found: C, 59.10; H, 5.72; N, 15.17; S, 6.63.

4.1.4.4. 2-[4-Allyl-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7d***. White crystals (ethanol) in (0.113 g, 76% yield); mp 189–190 °C; R_f value using $CHCl_3$:CH_3OH (9:1) as an eluent was 0.39; FT-IR (ν_{max}): 3600–3000 (br, OH, NH), 1677 (CO–NH), 1588 (C=C), 1533 (C=N), 1125 cm^{-1} (C–O); ^1H NMR (300 MHz, $CDCl_3$, δ = ppm) δ = 2.24 (s, 3H, HO–N=C–CH_3), 3.84 (s, 6H, 2 OCH_3), 3.89 (s, 3H, OCH_3), 4.02 (s,

2H, SCH$_2$), 4.58–4.59 (m, 2H, NCH$_2$), 5.12 (d, 1H, CH=CH$_2$, J_{trans} = 17.4 Hz), 5.42 (d, 1H, CH=CH$_2$, J_{cis} = 10.5 Hz), 5.94–6.03 (m, 1H, CH=CH$_2$), 6.85 (s, 2H, Ar–H), 7.54 (d, 2H, J = 8.4 Hz, Ar–H), 7.62 (d, 2H, J = 8.7 Hz, Ar–H), 9.59 (bs, 1H, OH), 10.64 (s, 1H, NH). ^{13}C NMR (75 MHz, CDCl$_3$, δ = ppm) δ = 11.97 (*C*H$_3$), 36.57 (*C*H$_2$), 47.28 (*C*H$_2$), 56.23 (*C*H$_3$), 60.97 (*C*H$_3$), 105.60 (*C*H), 118.74 (*C*H$_2$), 119.45 (*C*H), 120.93 (*C*), 126.53 (*C*H), 130.88 (*C*), 132.41 (*C*H), 138.94 (*C*), 139.83 (*C*), 152.87 (*C*), 153.59 (*C*), 155.10 (*C*), 156.49 (*C*), 166.62 (*C*O). EI-MS (70 eV) m/z (%): 497 (M$^+$, 8), 318 (52), 284 (61), 277 (67), 261 (56), 224 (42), 186 (47), 150 (100), 149 (69), 105 (78), 83 (42), 69 (41), 55 (47). Anal. Calcd for $C_{24}H_{27}N_5O_5S$ (497.17): C, 57.93; H, 5.47; N, 14.08; S, 6.44. Found: C, 58.22; H, 5.56; N, 14.33; S, 6.31.

4.1.4.5. 2-(4-Ethyl-5-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl)-N-[4-(1-hydroxy-iminoethyl)phenyl]acetamide ***7e***. White crystals (ethanol) in (0.101 g, 85% yield); mp 224–225 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.33; FT-IR (ν_{max}): 3300–3100 (br, OH, NH), 1667 (CO–NH), 1600 (C=C), 1537 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.21 (t, 3H, N–CH$_2$–CH$_3$, J = 7.4 Hz), 2.10 (s, 3H, HO–N=C–CH$_3$), 4.02 (q, 2H, N–CH$_2$–CH$_3$, J = 7.4 Hz), 4.21 (s, 2H, SCH$_2$), 7.54–7.63 (m, 9H, Ar–H), 10.47 (s, 1H, NH), 11.10 (s, 1H, OH). EI-MS (70 eV) m/z (%): 396 (M$^+$, 6), 378 (100), 297 (49), 265 (68), 150 (46), 133 (63), 77 (57). Anal. Calcd for $C_{20}H_{21}N_5O_2S$ (395.14): C, 60.74; H, 5.35; N, 17.71; S, 8.11. Found: C, 61.04; H, 5.50; N, 17.48; S, 7.86.

4.1.4.6. 2-[4-Ethyl-5-(4-methoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7f***. White crystals (ethanol) in (0.101 g, 79% yield); mp 254–255 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.27; FT-IR (ν_{max}): 3700–3100 (br, OH, NH), 1673 (CO–NH), 1599 (C=C), 1534 (C=N), 1247 (C–O); ^{1}H NMR (400 MHz, CDCl$_3$ + CD$_3$OD, δ = ppm) δ = 1.37 (t, 3H, N–CH$_2$–CH$_3$, J = 7.4 Hz), 2.24 (s, 3H, HO–N=C–CH$_3$), 3.88 (s, 3H, OCH$_3$), 4.00 (q, 2H, N–CH$_2$–CH$_3$, J = 7.2 Hz), 4.07 (s, 2H, SCH$_2$), 7.05 (d, 2H, J = 8.8 Hz, Ar–H), 7.54 (d, 2H, J = 8.8 Hz, Ar–H), 7.57 (d, 2H, J = 8.4 Hz, Ar–H), 7.67 (d, 2H, J = 8.8 Hz, Ar–H), 10.81 (s, 1H, NH). EI-MS (70 eV) m/z (%): 425 (M$^+$, 3), 344 (12), 343 (100), 328 (16), 327 (36), 307 (33), 295 (29), 235 (19), 219 (17), 150 (29), 133 (14). Anal. Calcd for $C_{21}H_{23}N_5O_3S$ (425.15): C, 59.28; H, 5.45; N, 16.46; S, 7.54. Found: C, 59.33; H, 5.70; N, 16.43; S, 7.51.

4.1.4.7. 2-[5-(3,4-Dimethoxyphenyl)-4-ethyl-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7g***. White crystals (ethanol) in (0.126 g, 92% yield); mp 225–226 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.23; FT-IR (ν_{max}): 3450–3000 (br, OH, NH), 1685 (CO–NH), 1598 (C=C), 1535 (C=N), 1255 (C–O); (300 MHz, CDCl$_3$, δ = ppm) δ = 1.37 (t, 3H, N–CH$_2$–CH$_3$, J = 7.2 Hz), 2.23 (s, 3H, HO–N=C–CH$_3$), 3.90 (s, 3H, OCH$_3$), 3.93 (s, 3H, OCH$_3$), 3.98 (q, 2H, N–CH$_2$–CH$_3$, J = 7.2 Hz), 4.04 (s, 2H, SCH$_2$), 6.95–7.17 (m, 3H, Ar–H), 7.53 (d, 2H, J = 9 Hz, Ar–H), 7.61 (d, 2H, J = 9 Hz, Ar–H), 9.58 (bs, 1H, OH), 10.66 (s, 1H, NH). ^{13}C NMR (75 MHz, CDCl$_3$, δ = ppm) δ = 11.99 (*C*H$_3$), 15.29 (*C*H$_3$), 36.42 (*C*H$_2$), 40.14 (*C*H$_2$), 55.97 (*C*H$_3$), 56.01 (*C*H$_3$), 111.04 (*C*H), 111.81 (*C*H), 118.55 (*C*H), 119.46 (*C*H), 120.86 (*C*), 126.54 (*C*H), 132.41 (*C*), 138.94 (*C*), 149.32 (*C*), 150.79 (*C*), 151.76 (*C*), 155.16 (*C*), 155.98 (*C*), 166.72 (*C*O). EI-MS (70 eV) m/z (%): 455 (M$^+$, 0.2), 410 (69), 392 (62), 391 (37), 264 (100), 237 (60), 164 (43), 163 (86), 103 (70). Anal. Calcd for $C_{22}H_{25}N_5O_4S$ (455.16): C, 58.01; H, 5.53; N, 15.37; S, 7.04. Found: C, 58.30; H, 5.59; N, 15.32; S, 6.81.

4.1.4.8. 2-[4-Ethyl-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7h***. White crystals (ethanol) in (0.137 g, 94% yield); mp 219 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.20; FT-IR (ν_{max}): 3500–3000 (br, OH, NH), 1680 (CO–NH), 1589 (C=C), 1532 (C=N), 1123 cm^{-1} (C–O); (400 MHz, CDCl$_3$ + CD$_3$OD, δ = ppm) δ = 1.40 (t, 3H, N–CH$_2$–CH$_3$, J = 6.8 Hz), 2.24 (s, 3H, HO–N=C–CH$_3$), 3.92 (s, 6H, 2 OCH$_3$), 3.93 (s, 3H, OCH$_3$), 4.03 (q, 2H, N–CH$_2$–CH$_3$, J = 7.2 Hz), 4.06 (s, 2H, SCH$_2$), 6.84 (s, 2H, Ar–H), 7.57 (d, 2H, J = 8.8 Hz, Ar–H), 7.66 (d, 2H, J = 8.8 Hz, Ar–H), 10.71 (s, 1H, NH). Anal. Calcd for $C_{23}H_{27}N_5O_5S$ (485.17): C, 56.89; H, 5.60; N, 14.42; S, 6.60. Found: C, 56.58; H, 5.46; N, 14.60; S, 6.40.

4.1.4.9. 2-(4,5-Diphenyl-4H-[1,2,4]triazol-3-ylsulfanyl)-N-[4-(1-hydroxyimino-ethyl)phenyl]acetamide ***7i***. White crystals (ethanol) in (0.116 g, 87% yield); mp 213–215 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.53; FT-IR (ν_{max}): 3600–3000 (br, OH, NH), 1670 (CO–NH), 1598 (C=C), 1535 (C=N); (400 MHz, DMSO-d_6, δ = ppm) δ = 2.10 (s, 3H, HO–N=C–CH$_3$), 4.21 (s, 2H, SCH$_2$), 7.34 (s, 5H, Ar–H), 7.41–7.62 (m, 9H, Ar–H), 10.49 (s, 1H, NH), 11.10 (s, 1H, OH). EI-MS (70 eV) m/z (%): 443 (M$^+$, 0.3), 404 (22), 403 (42), 388 (100), 387 (87), 370 (24), 150 (22), 133 (24), 121 (75), 106 (15). Anal. Calcd for $C_{24}H_{21}N_5O_2S$ (443.14): C, 64.99; H, 4.77; N, 15.79; S, 7.23. Found: C, 65.18; H, 4.96; N, 15.45; S, 7.11.

4.1.4.10. N-[4-(1-Hydroxyiminoethyl)phenyl]-2-[5-(4-methoxyphenyl)-4-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***7j***. White crystals (ethanol) in (0.125 g, 88% yield); mp 181–182 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.47; FT-IR (ν_{max}): 3300–3050 (br, OH, NH), 1677 (CO–NH), 1599 (C=C), 1535 (C=N), 1237 (C–O); (400 MHz, CDCl$_3$ + CD$_3$OD, δ = ppm) δ = 2.25 (s, 3H, HO–N=C–CH$_3$), 3.78 (s, 3H, OCH$_3$), 3.95 (s, 2H, SCH$_2$), 6.81 (d, 2H, J = 8.8 Hz, Ar–H), 7.33–7.24 (m, 2H, Ar–H), 7.34 (d, 2H, J = 8.8 Hz, Ar–H), 7.49–7.56 (m, 3H, Ar–H), 7.59 (d, 2H, J = 8.8 Hz, Ar–H), 7.67 (d, 2H, J = 8.8 Hz, Ar–H), 8.01 (bs, 1H, OH), 10.64 (s, 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$ + CD$_3$OD, δ = ppm) δ = 11.96 (*C*H$_3$), 36.27 (*C*H$_2$), 55.11 (*C*H$_3$), 114.01 (*C*H), 117.70 (*C*), 119.30 (*C*H), 126.52 (*C*H), 126.98 (*C*H), 129.53 (*C*H), 130.07 (*C*H), 130.24 (C), 132.62 (*C*), 133.27 (*C*), 138.62 (*C*), 152.82 (*C*), 155.16 (*C*), 160.89 (*C*), 166.41 (*C*O). EI-MS (70 eV) m/z (%): 474 (M$^+$, 0.1), 307 (18), 291 (25), 150 (100), 133 (43), 118 (29), 93 (20), 92 (33), 65 (18). Anal. Calcd for $C_{25}H_{23}N_5O_3S$ (473.15): C, 63.41; H, 4.90; N, 14.79; S, 6.77. Found: C, 63.50; H, 5.04; N, 14.57; S, 6.49.

4.1.4.11. 2-[5-(3,4-Dimethoxyphenyl)-4-phenyl-4H-[1,2,4]triazol-3-ylsulfanyl]-N-[4-(1-hydroxyiminoethyl)phenyl]acetamide ***7k***. White crystals (ethanol) in (0.113 g, 75% yield); mp 214–215 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.43; FT-IR (ν_{max}): 3450–3150 (br, OH, NH), 1680 (CO–NH), 1603 (C=C), 1544 (C=N), 1260 (C–O); (400 MHz, CDCl$_3$ + CD$_3$OD, δ = ppm) δ = 2.25 (s, 3H, HO–N=C–CH$_3$), 3.69 (s, 3H, OCH$_3$), 3.86 (s, 3H, OCH$_3$), 3.95 (s, 2H, SCH$_2$), 6.76–7.02 (m, 3H, Ar–H), 7.28–7.55 (m, 5H, Ar–H), 7.68 (d, 2H, J = 8.8 Hz, Ar–H), 7.68 (d, 2H, J = 8.6 Hz, Ar–H), 10.63 (s, 1H, NH). Anal. Calcd for $C_{26}H_{25}N_5O_4S$ (503.16): C, 62.01; H, 5.00; N, 13.91; S, 6.37. Found: C, 61.93; H, 5.23; N, 13.93; S, 6.30.

4.1.4.12. N-[4-(1-Hydroxyiminoethyl)phenyl]-2-[4-phenyl-5-(3,4,5-trimethoxy- phenyl)-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide ***7l***. White crystals (ethanol) in (0.134 g, 84% yield); mp 227–228 °C; R_f value using CHCl$_3$:CH$_3$OH (9:1) as an eluent was 0.40; FT-IR (ν_{max}): 3600–3100 (br, OH, NH), 1671 (CO–NH), 1593 (C=C), 1536 (C=N), 1126 cm^{-1} (C–O); (400 MHz, CDCl$_3$ + CD$_3$OD, δ = ppm) δ = 2.26 (s, 3H, HO–N=C–CH$_3$), 3.62 (s, 6H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 3.96 (s, 2H, SCH$_2$), 6.64 (s, 2H, Ar–H), 7.29–7.57 (m, 5H, Ar–H), 7.60 (d, 2H, J = 8.8 Hz, Ar–H), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.76 (bs, 1H, OH), 10.56 (s, 1H, NH). EI-MS (70 eV) m/z (%): 515 (M$^+$ – H$_2$O, 0.2), 327 (57), 311 (83), 224 (45), 150 (100), 133 (81), 108 (47), 92 (54), 65 (23). Anal. Calcd for $C_{27}H_{27}N_5O_5S$ (533.17): C, 60.77; H, 5.10; N, 13.12; S, 6.01. Found: C, 61.00; H, 5.05; N, 13.36; S, 5.85.

4.2. Nitric oxide release measurement [53]

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 **6a**–**l** and **7a**–**l** and reference drug (indomethacin) 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 caliper (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. Screening of ulcerogenicity

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 to 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 [54]. 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 Canada balsam then examined under microscope.

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. The cytotoxic and/or growth inhibitory effects of the most active selected compound were tested in vitro against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. Using the seven absorbance measurements [time zero (Tz), control growth in the absence of drug (*C*), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: $[(Ti - Tz)/(C - Tz)] - 100$ for concentrations for which $Ti > Tz$, and $[(Ti - Tz)/Tz] - 100$ for concentrations for which $Ti < Tz$. Three dose–response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] - 100 = 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in TGI was calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/Tz] - 100 = -50$. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The log GI_{50}, log TGI, and log LC_{50} were then determined, defined as the mean of the logs of the individual GI_{50}, TGI, and LC_{50} values. The lowest values are obtained with the most sensitive cell lines. Compound having log GI_{50} values -4 and <-4 was declared to be active.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.11.006.

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