Bioorganic & Medicinal Chemistry 20 (2012) 195–206

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis, anti-inflammatory activity and ulcerogenic liability of novel nitric oxide donating/chalcone hybrids

Gamal El-Din A.A. Abuo-Rahma [a], Mohamed Abdel-Aziz [a,*], Mai A.E. Mourad [a], Hassan H. Farag [b]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt
[b] Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, 71526 Assiut, Egypt

ARTICLE INFO

Article history:
Received 6 September 2011
Revised 1 November 2011
Accepted 7 November 2011
Available online 13 November 2011

Keywords:
Chalcones
Nitric oxide donors
Anti-inflammatory
Gastric ulceration
Histopathology

ABSTRACT

A group of novel nitric oxide (NO) donating chalcone derivatives was prepared by binding various amino chalcones with different NO donating moieties including; nitrate ester, oximes and furoxans. Most of the prepared compounds showed significant anti-inflammatory activity using carrageenan-induced rat paw edema method compared with indomethacin. The prepared compounds exhibited more protection than indomethacin in regard to gastric toxicity. Histopathological investigation confirmed the beneficial effects of the NO releasing compounds in reducing ulcer formation. The incorporation of the NO-donating group into the parent chalcone derivatives caused a moderate increase in the anti-inflammatory activity with a marked decrease in gastric ulcerations compared to their parent chalcone derivatives.

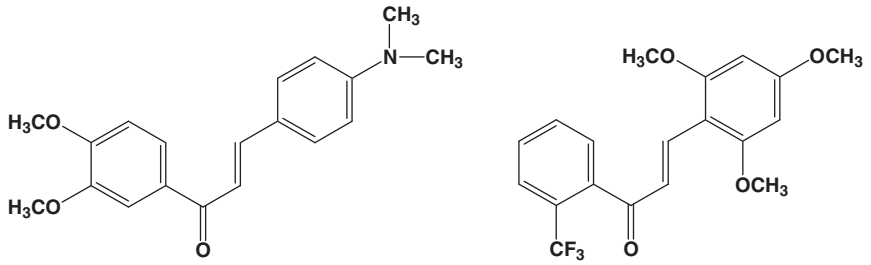
1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most useful clinical therapies for the treatment of pain, fever, and inflammation.[1] The major mechanism by which NSAIDs exert their anti-inflammatory activity through inhibition of cyclooxygenase-derived prostaglandin synthesis, which is also responsible for the gastrointestinal,[2–6] renal,[7–9] and hepatic side effects,[10] that are observed mainly in chronic use of NSAIDs. Therefore, the challenge still exists for the pharmaceutical industry to develop, effective anti-inflammatory agents with enhanced safety profile. One of the most important strategies used to overcome NSAIDs side effects is to design nitric oxide-donating NSAIDs (NO–NSAIDs) which are capable of generating the radical biomediator and gastroprotective agent NO.[11,12] NO contributes to the modulation of several key physiological functions in the digestive system,[13] it has the ability to increase the mucosal blood flow,[14] resulting in enhanced mucosal resistance to ulceration,[15] NO also prevents adherence of leukocytes to the vascular endothelium,[16] it is known to modulate gastroduodenal secretion of mucus,[17] and bicarbonate,[18] NO can profoundly influence the mucosal immune system,[13] it also increases the ability of mucosal cells to undergo healing and repair of the existing ulcers.[19] NO is also known to spare the renal system mainly through stimulating the renal blood flow.[20] Moreover, naturally occurring and synthetic chalcone derivatives are of current interest because of their diverse pharmacological activities such as anti-inflammatory,[21–23] antimicrobial,[24,25] antifungal,[26,27] antiviral,[28] antituberculosis,[29] antimalarial,[30–33] antioxidant,[34–36] antimitotic,[37] antileishmanial,[38] antiplatelet,[39] and anticancer activities.[40–43] Herencia et al.,[44–47] tested a series of chalcone derivatives for possible anti-inflammatory effect, chalcone I (Fig. 1) was significantly active as a scavenger of superoxide anion generated by stimulated human neutrophils or by the hypoxanthine/xanthine oxidase system. Chalcone I inhibited also the inducible NO synthase (iNOS) expression through a superoxide-dependent mechanism in stimulated mouse peritoneal macrophages and protected cells against oxidant stress. Additionally chalcone I significantly reduced tumor necrosis factor-α (TNF-α) levels, a crucial mediator of the anti-inflammatory process, especially in chronic inflammatory conditions. 2,4,6-Trimethoxy-2′-tifluoromethyl chalcone (II),[48] (Fig. 1) inhibited the production prostaglandin E_2 in lipopolysaccharaide-stimulated RAW 264.7 macrophage cells.

Promoted with the above-mentioned studies and as a continuation of our research interest in the synthesis and biological activities of novel NO–NSAIDs derivatives,[49,50] the present study aimed at gathering the two bioactive entities (chalcone and NO) in one compact structure for the purpose of synergism and/or decreasing the expected ulcerogenic side effects. The prepared NO–chalcone hybrids were evaluated for their anti-inflammatory activity using carrageenan-induced rat paw edema and compared to a well-known NSAID, indomethacin. The ability of the prepared compounds to induce gastric toxicity has been evaluated. Moreover,

* Corresponding author. Tel.: +20 103311327; fax: +20 86 2369075.
E-mail address: abulnil@hotmail.com (M. Abdel-Aziz).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.11.012



Chalcone I Chalcone II

Figure 1. Structures of chalcones I and II.

histopathological investigation has been carried out to asses the beneficial effects of the NO releasing chalcone hybrids in decreasing ulcer formation.

2. Results and discussion

2.1. Chemistry

Chalcone derivatives **1a–d** were synthesized by a base catalyzed Claisen–Schmidt condensation of 4-aminoacetophenone with substituted benzaldehyde **1a–c** or furfural **1d**.[51–53] Treatment of compounds **1a–d** with bromoacetyl bromide in the presence of potassium carbonate afforded the corresponding 2-bromo-*N*-{4-[3-arylacryloyl]phenyl}acetamides **2a–d** in high yields (Scheme 1). Conversion of the bromo-derivatives **2a–d** into the target titled NO-donating nitrate esters **3a–d** has occurred in moderate yields (52–62%), upon heating the bromo-derivatives **2a–d** with silver nitrate in acetonitrile via simple nucleophilic attack of nitrate ion on the carbon atom of the CH_2Br group (Scheme 1). It is worth noting that the ^1H NMR spectra of the novel nitrate ester derivatives **3** compared to their corresponding bromo-derivatives **2** showed that replacement of a bromine atom by a nitrate group clearly caused a remarkable downfield effect on the observed chemical shift values of the methylene protons by δ 1.12 ppm. Mass spectra showed a prominent peak at [M^+-46(NO_2)], this phenomenon is in agreement with that reported.[54]

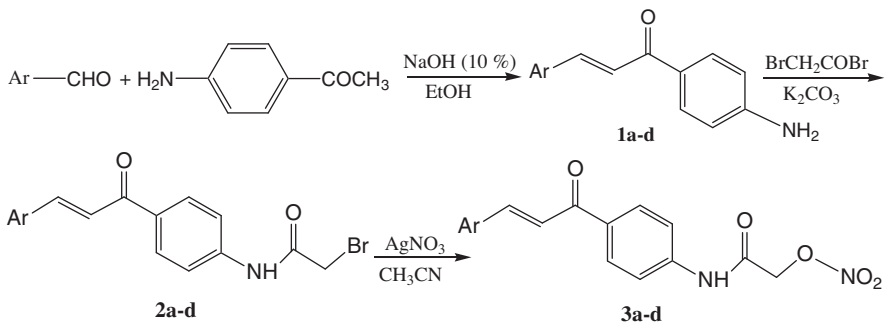
Acylation of the amino chalcone derivatives **1a–d** with 2-bromopropionic acid through mixed anhydride intermediate using ethyl chloroformate in the presence of triethylamine afforded the methylated analogues **4a–d** in moderate yields (Scheme 2). Heating at reflux of compounds **4a–d** with silver nitrate in acetonitrile for 12 h yielded the corresponding NO-donating methylated analogues **5a–d** in moderate yields. In compounds **5a–d**, it is worth to mention that *CH*ONO_2 proton is more downfield shifted by δ 0.17 ppm than the corresponding *CH_2*ONO_2, because its position attached to tertiary carbon. Also, the chemical shift value of the *CH*-ONO_2 showed a downfield shift by δ 0.90–0.74 ppm, caused by the deshielding effect of the nitrate group, comparable with the data of their corresponding bromo-derivatives **4a–d**.

The reaction of bromo-derivatives **2a–d** with *p*-hydroxyacetophenone in the presence of potassium carbonate was successfully applied to prepare the ketones **6a–d**. Heating at reflux of compounds **6a–d** with hydroxylamine HCl in EtOH yielded the target ketoxime derivatives **7a–d** (Scheme 3). In the ^1H NMR spectra of oximes **7a** and **7b**, the CH_3 protons appeared upfield shifted by δ 0.47–0.40 ppm than the CH_3 protons of the corresponding ketones due to the low electronegativity of the neighboring nitrogen atom relative to oxygen atom. Mass spectrum for the oxime **7a** indicated that the base peak recorded at an abundant $[M-15]^+$ ion, which was attributed to the result of a process occurring prior to ionization in analogy with other oximes,[55] due to the loss of oxygen and abstraction of hydrogen. Kallury and Rao,[56] reported that the abundances of some oximes are very low (less than 4%) of the respective base peaks, which further support the respective mass results.

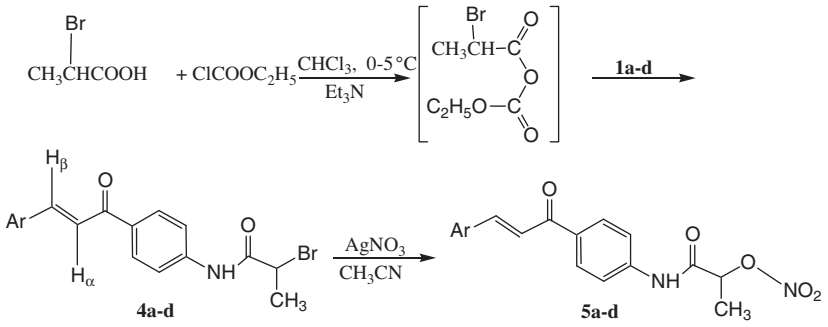
The synthesis of novel chalcone hybrids formed by linking different chalcone derivatives to the furoxan substructure **8** is outlined in Scheme 4. Synthesis of 3-phenyl-4-furoxanmethanol **8** was done by treatment of cinnamyl alcohol with sodium nitrite in glacial acetic acid.[57] Coupling of furoxan derivative **8** with bromo-derivatives **2a–d** in the presence of anhydrous potassium carbonate in acetone afforded the desired NO-donating furoxan derivatives **9a–d** in moderate yields.

2.2. Measurement of nitric oxide release

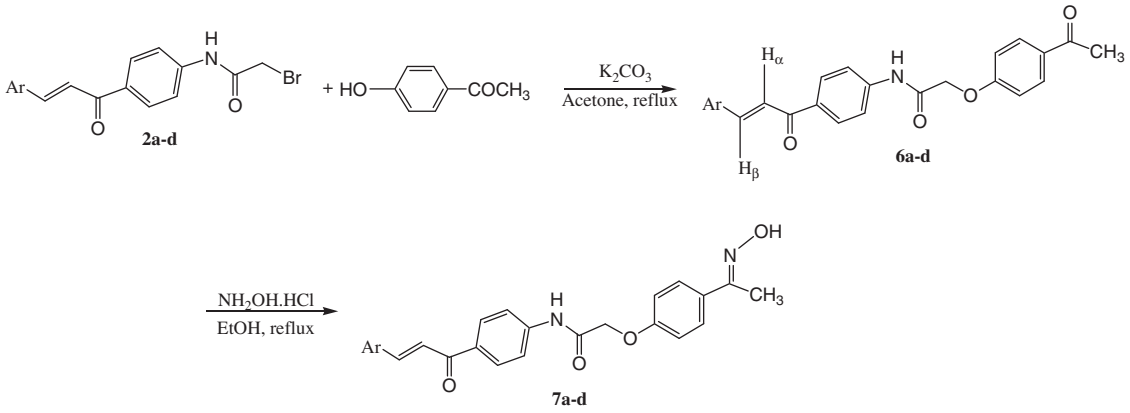
The NO releasing properties of the prepared NO donors including; NO-donating nitrate esters **3a–d**, their methylated analogues **5a–d**, NO-donating oximes **7a–d** and NO-donating furoxans **8** and **9a–d** were assessed. The produced nitrite which is a convenient index of nitric oxide production trend was determined in both



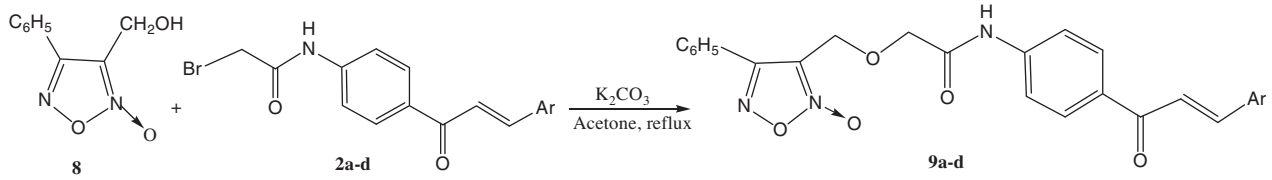
Scheme 1. Synthesis of *N*-{4-[3-arylacryloyl]phenyl}-2-nitroxyacetamides **3a–d**. Ar: (a) 4-Cl–C_6H_4; (b) 4-CH_3O–C_6H_4; (c) 3′,4′-OCH_2O–C_6H_3; (d) 2-furanyl.



Scheme 2. Synthesis of *N*-{4-[3-arylacryloyl]phenyl}-2-nitrooxypropionamides **5a–d**. Ar: (a) 4-Cl–C_6H_4; (b) 4-CH_3O–C_6H_4; (c) 3′,4′-OCH_2O–C_6H_3; (d) 2-furanyl.



Scheme 3. Synthesis of *N*-{4-[3-arylacyloyl]phenyl}-2-[4-(1-hydroxyiminoethyl)phenoxy]acetamides **7a–d**. Ar: (a) 4-Cl–C_6H_4; (b) 4-CH_3O–C_6H_4; (c) 3′,4′-OCH_2O–C_6H_3; (d) 2-furanyl.



Scheme 4. Synthesis of *N*-[4-(arylacryloyl)phenyl]-2-(2-oxy-4-phenylfurazan-3-ylmethoxy)acetamides **9a–d**. Ar: (a) 4-Cl–C_6H_4; (b) 4-CH_3O–C_6H_4; (c) 3′,4′-OCH_2O–C_6H_3; (d) 2-furanyl.

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 tested compounds could only release NO at pH 7.4. Nitrate esters **3a–d** achieved maximum NO release after 5 h, while the methylated analogues **5a–d** did after 3 h. The amount of NO released from compounds **5a–d** was lower than that released from compounds **3a–d**. This might be attributed to the steric effect of the methyl group in the propionic acid spacer present in compounds which may decrease the dissociation of the nitrite group giving lower amount of NO compared to the acetic acid spacer present in compounds **3a–d**.[49] NO-donating oximes **7a–d** gave the maximum amount of NO released after 4 h, while NO-donating furoxans **8** and **9a–d** exhibited maximum NO release after 5 h. On contrary, the tested compounds were not able to release NO at pH 1 which suggest that these compounds are weakly hydrolyzed in the gastric lumen and this confirms that the suggested gastro protective action of NO is mediated systemically.[58]

2.3. Biological investigations

2.3.1. Screening of anti-inflammatory activity

The synthesized compounds **1a–d**, **2a–d**, **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–d**, **8** and **9a–d** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema method in rats described by Winter et al.[59] The tested compounds and indomethacin (reference drug) were administered orally at a dose level of 100 mg/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:

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 **3a–9d** in the presence of 0.5 mM *N*-acetylcysteine in phosphate buffer of pH 7.4

Compd No.	Amount of NO released (mol/mol) ± SEM					
	1h	**2h**	**3h**	**4h**	**5h**	**6h**
3a	0.32 ± 0.015	0.37 ± 0.012	0.54 ± 0.030	0.73 ± 0.035	0.75 ± 0.034	0.73 ± 0.035
3b	0.27 ± 0.052	0.31 ± 0.026	0.48 ± 0.022	0.66 ± 0.022	0.68 ± 0.019	0.59 ± 0.021
3c	0.39 ± 0.021	0.45 ± 0.030	0.60 ± 0.031	0.64 ± 0.037	0.73 ± 0.035	0.67 ± 0.040
3d	0.15 ± 0.010	0.28 ± 0.011	0.37 ± 0.014	0.59 ± 0.019	0.63 ± 0.041	0.59 ± 0.033
5a	0.13 ± 0.009	0.16 ± 0.014	0.46 ± 0.026	0.35 ± 0.023	0.28 ± 0.009	0.16 ± 0.013
5b	0.11 ± 0.005	0.12 ± 0.010	0.37 ± 0.019	0.17 ± 0.012	0.15 ± 0.014	0.14 ± 0.010
5c	0.10 ± 0.003	0.14 ± 0.009	0.40 ± 0.025	0.28 ± 0.016	0.15 ± 0.012	0.12 ± 0.011
5d	0.09 ± 0.005	0.11 ± 0.004	0.38 ± 0.021	0.13 ± 0.010	0.11 ± 0.008	0.09 ± 0.002
7a	0.13 ± 0.017	0.19 ± 0.013	0.22 ± 0.016	0.41 ± 0.028	0.37 ± 0.028	0.36 ± 0.026
7b	0.11 ± 0.009	0.12 ± 0.006	0.14 ± 0.011	0.30 ± 0.013	0.16 ± 0.013	0.15 ± 0.012
7c	0.14 ± 0.011	0.16 ± 0.013	0.25 ± 0.017	0.31 ± 0.020	0.26 ± 0.021	0.24 ± 0.018
7d	0.10 ± 0.004	0.12 ± 0.007	0.12 ± 0.006	0.28 ± 0.011	0.14 ± 0.010	0.13 ± 0.011
8	0.35 ± 0.024	0.51 ± 0.031	0.68 ± 0.034	0.72 ±0.041	0.94 ± 0.048	0.68 ± 0.040
9a	0.55 ± 0.031	0.98 ± 0.074	1.22 ± 0.091	1.295 ± 0.099	1.50 ± 0.095	0.87 ± 0.051
9b	0.11 ± 0.005	0.27 ± 0.012	0.47 ± 0.027	0.482 ± 0.021	0.52 ± 0.017	0.30 ± 0.025
9c	0.50 ± 0.024	0.71 ± 0.050	0.86 ± 0.038	0.890 ± 0.057	1.26 ± 0.081	0.75 ± 0.048
9d	0.16 ± 0.010	0.23 ± 0.018	0.40 ± 0.015	0.499 ± 0.033	0.65 ± 0.025	0.54 ± 0.037

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

where V_R represents the mean right 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.

The results listed in Table 2 show the percentage of edema inhibition induced by carrageenan for all of the tested compounds and indomethacin versus time in h. Most of the tested compounds showed a significant anti-inflammatory activity against carrageenan-induced paw edema in rats (p <0.01). The reference drug, indomethacin showed an inhibitory activity of 78% against carrageenan-induced paw edema after 3 h, which is the time required reaching the maximum activity for most of the tested compounds, then the activity decreased gradually for the next 2 h for the majority of the synthesized compounds. The synthesized compounds exhibited considerable anti-inflammatory activity relative to indomethacin that increased significantly to a maximum after 3 h. The nitrate ester **3a** exhibited 69% anti-inflammatory activity which represents 89% of indomethacin activity after 3 h. The nitrate ester **3b**–**d** showed an anti-inflammatory activity of 53%, 61% and 48%, respectively, which is equal to 69%, 79% and 61% of indomethacin activity, respectively. Moreover, the anti-inflammatory activity of the NO-donating methylated analogues **5a–d** was 62%, 50%, 59% and 54%, respectively, after 3 h that represents 80%, 65%, 77% and 69% of indomethacin activity, respectively. The anti-inflammatory activity of the NO-donating oxime derivatives **7a–d** was also evaluated and the results showed that the anti-inflammatory activity of compounds **7a–d** was 66%, 46%, 59% and 49% after 3 h, respectively, that represents 85%, 59%, 77% and 64% of indomethacin activity, respectively. For NO-donating furoxan derivatives **8** and **9a–d**, the results showed that compounds **8**, **9a** and **9c** exhibited the strongest anti-inflammatory activity among the synthesized compounds with 69%, 75% and 72% of edema inhibition, respectively, after 4 h, which is equal to 84%, 91% and 87% of indomethacin activity, respectively. Furoxans **9b** and **9d** exhibited 56% and 59% of edema inhibition, respectively, after 3 h, which represents 73% and 76% of indomethacin activity, respectively.

The anti-inflammatory activity of the bromoacetyl derivatives **2a–d** and **4a–d** did not significantly decrease compared to their starting chalcones **1a–d** (Table 2). The NO-donating nitrate esters **3a–d** showed significant increase in the anti-inflammatory activity when compared to their starting chalcones **1a–d** and bromoacetyl derivatives **2a–d**. The same results were obtained upon comparing the anti-inflammatory activity of the NO-donating methylated analogues **5a–d**, oximes **7a–d** and furoxans **9a–d** with their corresponding chalcones **1a–d** and their bromoacetyl derivatives **4a–d**. This might suggest that NO potentiate the activity of the synthesized compounds. The role of NO was also confirmed by the higher anti-inflammatory activity achieved by the NO-donating furoxan derivatives **9a–d** and the NO-donating nitrate esters **3a–d** compared to the NO-donating methylated analogues **5a–d** and NO-donating oxime derivatives **7a–d** (Table 2) that was coupled with high NO release rates (Table 1) for the aforementioned compounds.

2.3.2. Screening of ulcerogenic liability

The in vivo ulcerogenic liability was evaluated for the synthesized compounds **1a–d**, **2a–d**, **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–d**, **8** and **9a–d** relative to indomethacin according to the reported procedure.[60] Ulcers were classified into levels, level I, ulcer area less than 1 mm^2, level II, ulcer area is 1–3 mm^2 and level III, ulcer area more than 3 mm^2, and the following parameters were calculated:

1- 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 = $100-(UI_{treated} \times 100/UI_{protype})$

where, UItreated: 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 **1a–d**, **2a–d**, **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–d**, **8** and **9a–d** were calculated and were listed in Table 2 as (mean ± S.E.). The results of ulcerogenic liability revealed that indomethacin caused significant ulcerogenic toxicity with UI of 45.5, whereas an equal dose of the majority of the synthesized compounds exhibited much lower UI. The NO-donating nitrate esters **3a–d** and NO-donating methylated analogues **5a–d** exhibited lower ulcerogenicity relative to indomethacin, with UIs of 2.5, 5.8, 3 and 7 for the nitrate esters **3a–d**, and 3.5, 8, 4 and 5.7 for NO-donating esters **5a–d** (Fig. 2A). The NO-donating oximes **7a–d** also exhibited lower toxicity relative to indomethacin where the UIs were 2.2, 5, 4 and 4.8, respectively (Fig. 2B).

Table 2
Anti-inflammatory activity at different time intervals and ulcer indices of compounds **1a–d**, **2a–d**, **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–d**, **8** and **9a–d** using carrageenan-induced paw edema in rats

Compd No.	% of edema inhibition (% mean ± SEM)					Ulcer index (UI) (Mean ± SEM)
	1h	**2h**	**3h**	**4h**	**5h**	
Control	0	0	0	0	0	0.5 ± 0.06
1a	41 ± 1.55***	50 ± 2.07***	60 ± 1.18***	58 ± 2.62***	54 ± 2.20***	18 ± 1.04**
1b	42 ± 1.28***	48 ± 2.62***	51 ± 2.31***	48 ± 1.16***	40 ± 1.13***	22.5 ± 0.28**
1c	32 ± 2.17***	44 ± 1.12***	55 ± 1.24***	50 ± 1.24***	47 ± 1.20***	20 ± 0.86***
1d	33 ± 1.80***	38 ± 1.07***	42 ± 1.98***	33 ± 2.53***	30 ± 2.24***	29 ± 2.78**
2a	44 ± 1.72***	52 ± 1.47***	59 ± 2.10***	59 ± 2.75***	54 ± 2.51***	21.4 ± 0.95***
2b	37 ± 1.07***	42 ± 1.23***	46 ± 2.44***	41 ± 1.94***	34 ± 2.32***	28 ± 1.32**
2c	40 ± 1.16***	45 ± 1.63***	53 ± 3.41***	48 ± 2.31***	46 ± 1.73***	24 ± 1.73**
2d	30 ± 1.12***	37 ± 2.16***	41 ± 2.60***	33 ± 1.20***	25 ± 1.16***	25.8 ± 0.96**
3a	42 ± 2.38***	57 ± 2.11***	69 ±1.71***	67 ± 1.08***	60 ± 3.69***	2.5 ± 0.28***
3b	33 ± 1.26***	49 ± 2.02***	53 ± 2.05***	51 ± 1.57***	46 ± 1.80***	5.8 ± 0.47***
3c	38 ± 2.16***	45 ± 1.47***	61 ± 1.89***	55 ± 1.47***	54 ± 2.13***	3 ± 0.28***
3d	34 ± 1.41***	36 ± 1.56***	48 ± 1.52***	40 ± 2.03***	39 ± 2.16***	7 ± 0.50***
4a	39 ± 1.81***	45 ± 2.25***	59 ± 3.54***	54 ± 2.23***	50 ± 3.09***	16.7 ± 0.90***
4b	39 ± 1.38***	40 ± 3.08***	40 ± 1.65***	38 ± 1.86***	31 ± 1.18***	30.1 ± 1.49**
4c	37 ± 1.69***	43 ± 1.49***	56 ± 2.71***	53 ± 4.47***	50 ± 1.64***	18 ± 1.0***
4d	30 ± 1.70**	37 ± 1.79***	42 ± 2.39***	36 ± 2.44***	34 ± 2.06***	25.3 ± 2.0**
5a	45 ± 2.13***	55 ± 2.22***	62 ± 1.43***	61 ± 2.32***	56 ± 1.12***	3.5 ± 0.28***
5b	43 ± 1.81***	46 ± 1.71***	50 ± 1.70***	43 ± 1.52***	35 ± 1.06***	8 ± 0.76***
5c	45 ± 1.44***	40 ± 2.41***	59 ± 2.06***	54 ± 1.90***	53 ± 2.89***	4 ± 0.17***
5d	41 ± 1.60***	48 ± 3.05***	54 ± 2.60***	49 ± 1.08***	49 ± 3.05***	5.7 ± 0.51***
6a	43 ± 1.74***	52 ± 3.10***	61 ± 3.41***	58 ± 1.53***	55 ± 2.13***	8.1 ± 0.55***
6b	28 ± 1.60**	35 ± 1.59***	42 ± 2.38***	33 ± 1.68***	30 ± 0.84**	6 ± 0.58***
6c	37 ± 1.45***	43 ± 2.08***	57 ± 3.13***	53 ± 2.46***	50 ± 1.52***	11.2 ± 0.72***
6d	28 ± 1.25**	32 ± 1.96***	45 ± 1.84***	39 ± 3.63***	36 ± 1.61***	5 ± 0.76***
7a	39 ± 2.05***	54 ± 2.76***	66 ± 2.60***	55 ± 3.79***	49 ± 1.81***	2.2 ± 0.11***
7b	33 ± 1.66***	41 ± 1.52***	46 ± 2.92***	42 ± 1.27***	37 ± 1.41***	5 ± 0.28***
7c	40 ± 3.45***	49 ± 1.68***	59 ± 3.68***	52 ± 1.29***	44 ± 2.10***	4 ± 0.23***
7d	36 ± 2.76***	44 ± 1.88***	49 ± 1.66***	48 ± 1.45***	42 ± 2.85***	4.8 ± 0.47***
8	40 ±1.20***	53 ± 1.64***	62 ± 1.28***	69 ± 3.67***	67 ± 3.62***	1.5 ± 0.12***
9a	38 ± 1.96***	48 ± 1.37***	66 ± 2.50***	75 ± 3.17***	62 ± 2.01***	1 ± 0.05***
9b	30 ± 1.74***	42 ± 2.42***	56 ± 1.42***	55 ± 2.13***	49 ± 1.40***	2 ± 0.11***
9c	34 ± 2.14***	55 ± 3.84***	62 ± 1.73***	72 ± 3.59***	70 ± 1.40***	1.3 ± 0.10***
9d	30 ± 1.31**	45 ± 2.33***	59 ± 2.15***	58 ± 2.02***	49 ± 2.70***	2.5 ± 0.23***
Indomethacin	56 ± 0.78***	70 ± 2.23***	78 ± 1.14***	83 ± 2.36***	87 ± 1.47***	45.5 ± 1.75

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. ($n = 4$), $^{**}p < 0.01$, $^{***}p < 0.001$, significant difference from control group.

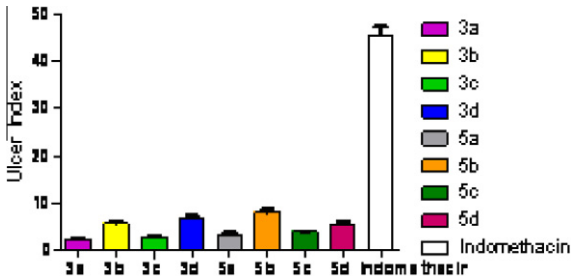


Figure 2A. UI of compounds **3a–d**, **5a–d** compared to indomethacin expressed as mean ± S.E.

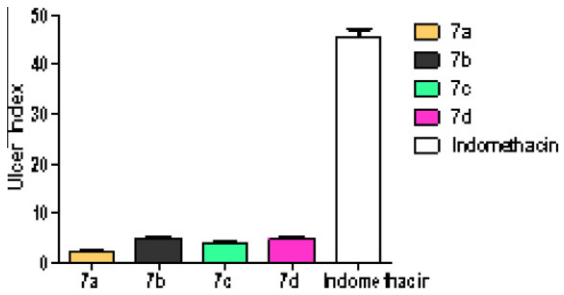


Figure 2B. UI of compounds **7a–d** compared to indomethacin expressed as mean ± S.E.

The cure ratio of the target NO-donating derivatives **3a–d**, **5a–d**, **7a–d** and **9a–d** compared to their starting chalcone derivatives **1a–d** and their intermediates **2a–d**, **4a–d**, **6a–d** and **8** has been calculated and the results revealed that the NO-donating nitrate esters **3a–d** achieved 74–86% reduction of ulcers than their starting chalcones **1a–d** and 73–88% reduction of ulcers than their corresponding bromoacetyl intermediates **2a–d**. On the other hand, the NO-donating methylated analogues **5a–d** exhibited 64–81% reduction of ulcers than their starting chalcones **1a–d** and 73–79% reduction of ulcers than their corresponding bromoacetyl intermediates **4a–d**. The NO-donating oximes **7a–d** exhibited 78–88% reduction of ulcers than their starting chalcones and 81–90% reduction of ulcers than their corresponding bromoacetyl intermediates **2a–d** and 4–73% reduction of ulcers than their corresponding ketone intermediates **6a–d**. Additionally, the results revealed that NO-donating furoxans **9a–d** exhibited the highest cure ratio in comparison with the other synthesized NO-donors (Fig. 2C). NO-donating furoxans **9a–d** exhibited 91–94% reduction of ulcers compared to their starting chalcones **1a–d** and 90–95% reduction of ulcers compared to their corresponding bromoacetyl intermediates **2a–d** and these results are in a good agreement with the NO release data (Table 1). The decreased gastric toxicity of the targeted NO-chalcone hybrids **3a–d**, **5a–d**, **7a–d** and **9a–d** compared to their starting chalcones **1a–d** and their intermediates **2a–d**, **4a–d**, **6a–d** and **8** can be attributed to the release of NO that increases mucosal blood flow resulting in enhanced mucosal resistance to ulceration,[61,62] and/or an enhanced ability of the intact

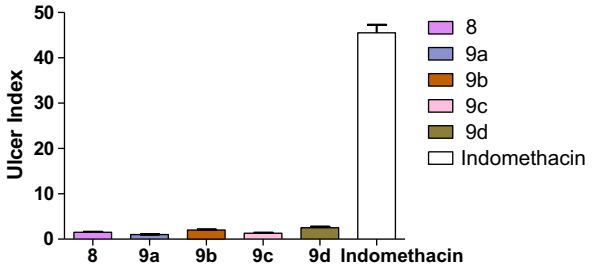


Figure 2C. UI of compounds **8** and **9a–d** compared to indomethacin expressed as mean ± S.E.

NO-donating derivatives to cross the gastric mucosal lining prior to the subsequent release of NO and starting chalcone derivatives (Fig. 2).[63]

2.3.3. Histopathological investigation

Different stomach sections of the ulcers including the control and the treated groups were stained by standard hematoxylin and eosin stains. The produced slides were subjected to microscopical examination and pictures were picked for these slides.

The control (Fig. 3A) showed no lesions and characterized by the presence of continuous mucosal layer. Indomethacin (Fig. 3B) exhibited marked loss of mucosal membrane at the areas of ulceration where certain areas of fundic glands were totally degenerated and cellular details were lost. Capillary inflammatory cells were also found and apoptotic glandular epithelial cells could be detected. The stomach sections of the ulcers treated with NO-donating nitrate ester **3a** showed normal morphology for the fundic glands and the results are in consistence with the previous results.[49,50] The edema was very minimal and also the vasodilatation of blood capillaries was not marked and this was confirmed by the low UI of 2.5. Changes in the structure of gastric mucosa including loss of mucosal membrane at the areas of ulceration and loss of the morphology of certain glandular structures was observed by the bromoacetyl derivative **2a** associated with high UI of 21.4. In comparison to compound **3a,** the NO-donating methylated analogue **5a** showed less protective effect against the ulcerative changes that affect the gastric mucosa with partial loss of mucosal membrane at the areas of ulceration with UI of 3.5. Partial healing of the ulcers with remnants of the structural damage was still found induced by the NO-donating oxime **7c** associated with low UI of 4. The ketonic intermediate **6c** 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 the UI of 11.2. Complete protective effect against ulcers was induced by the NO-donating furoxan **9a** (Fig. 3C) where complete healing of ulcers was observed with absence of capillary inflammatory cells, the ulcerative damage of the gastric mucosa was markedly decreased which was approved by the very low UI of 1.

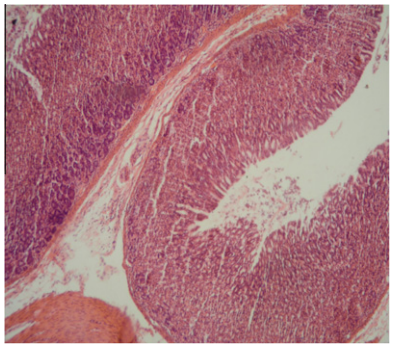


Figure 3A. Photomicrograph of the mucosa of fundic stomach of control.

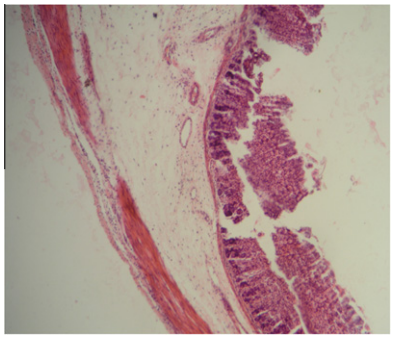


Figure 3B. Photomicrograph of the mucosa of fundic stomach of indomethacin.

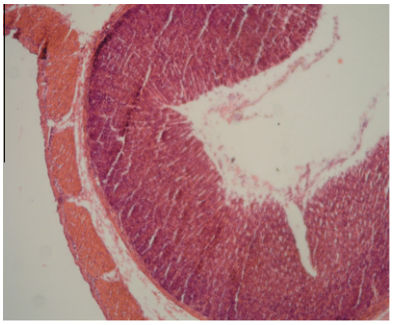


Figure 3C. Photomicrograph of mucosa of fundic stomach of furoxan **9a**.

3. Conclusions

A group of novel chalcone–NO hybrids was prepared and characterized by different spectroscopic and elemental analysis techniques. Most of the synthesized compounds showed significant anti-inflammatory activity using carrageenan-induced rat paw edema method. The NO-donating furoxans exhibited maximum amount of NO released among the synthesized NO-donating hybrids. The prepared chalcone/NO hybrids either nitrate ester; oxime or furoxan derivatives showed pronounced gastroprotective activity that might attribute to the release of NO. Histopathological examination of some intermediates and their NO hybrids indicated that the NO donating moiety reduced greatly the incidence of gastric ulceration. In conclusion the use of hybrid molecules containing NO-donating moieties looks as a promising approach to improve the safety of NSAIDs without altering their effectiveness.

4. Experimental section

4.1. Chemistry

Reactions were monitored by TLC: Pre-coated plastic sheets, 0.2 mm silica gel with fluorescent indicator (Macherey–Nagel). Column chromatography: Silica Gel 60 (Merck) for column chromatography. Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. IR spectra were recorded as KBr disks on a Shimadzu IR-408 spectrophotometer. ^{1}H NMR spectra were carried out on Bruker apparatus at DRX400 MHz, AV300 MHz, DPX 200 MHz (Braunschweig University, Germany); Geol EX-270 MHz spectrometers (National Research Center, Dokki, Giza, Egypt); using TMS as internal

reference. Chemical shifts (δ values are given in parts per million (ppm) using $CDCl_3$ (7.29) or DMSO-d_6 (2.5) as solvents and coupling constants (J) in Hertz. 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: Finnigan MAT 4515 and Jeol JMS 600 spectrometers. Elemental analysis was performed on Perkin–Elmer 2400 CHN Elemental analyzer, Faculty of Science, Cairo University, Egypt.

4.1.1. General procedure for the synthesis of 1-(4-aminophenyl)-3-arylprop-2-en-1-one 1a–d

p-Aminoacetophenone (0.1 mol) and the aldehydes (0.1 mol) were dissolved in a minimum amount of methanol, then aqueous NaOH (0.25 mol, 10%) was added. The reaction mixture was stirred at rt until a precipitate was formed (within 4–24 h). The precipitate was filtered off and washed thoroughly with distilled water and cold methanol (2×20 mL). The product was recrystallized from the appropriate solvent. The structure of chalcone derivatives **1a–d** was confirmed by mp and IR spectroscopy.

1-(4-Aminophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (**1a**). Mp 159–160 °C [Ref. 51; 160 °C].

1-(4-aminophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**1b**). Mp 113–114 °C [Ref. 53; 114–115 °C].

1-(4-aminophenyl)-3-(1,3-benzodioxol-5-yl)prop-2-en-1-one (**1c**). Mp 197–198 °C [Ref. 53; 199 °C].

1-(4-aminophenyl)-3-(2-furyl) prop-2-en-1-one (**1d**). Mp 119–120 °C [Ref. 52; 118–119 °C].

4.1.2. General procedure for the synthesis of 2-bromo-*N*-{4-[3-arylacryloyl] phenyl}acetamides 2a–d

To a stirred mixture of chalcones **1a–d** (6.30 mmol) in dichloromethane (20 mL) and potassium carbonate (1.302 mmol) in 100 mL water cooled in an ice bath, bromoacetyl bromide (1.856 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, and at rt overnight. The reaction mixture was extracted with (2×60 mL) dichloromethane, and the organic layer was washed with distilled water (2×40 mL), dried over anhydrous sodium sulphate, filtered, evaporated on a rotary evaporator and the residue was recrystallized from appropriate solvent.

4.1.3. 2-Bromo-*N*-{4-[3-(4-chlorophenyl)acryloyl]phenyl}acetamide (2a)

Pale yellow crystals (ethanol) in (1.81 g, 76% yield); mp 190–192 °C; IR (KBr) ν_{max} = 3300 (NH), 1675 (*CO*–NH), 1655 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.78 (s, 1H, NH), 8.19 (d, 2H, J = 8.18 Hz, ArH), 7.98 (d, 1H, J = 15.58 Hz, H_β), 7.94 (d, 2H, J = 8.18 Hz, ArH), 7.79 (d, 2H, J = 8.18 Hz, ArH), 7.72 (d, 1H, J = 15.58 Hz, H_α), 7.53 (d, 2H, J = 8.18 Hz, ArH), 4.11 (s, 2H, CH_2Br); EI-MS (70 eV) m/z (%) 381 (M^++4, 31), 379 (M^++2, 92), 377 (M^+, 59), 350 (20), 298 (100), 256 (18), 242 (33), 229 (31), 165 (56), 137 (29), 120 (47), 101 (31). Anal. Calcd for $C_{17}H_{13}BrClNO_2$ (376.98): C, 53.92; H, 3.46; N, 3.70. Found: C, 54.26; H, 3.63; N, 4.00.

4.1.4. 2-Bromo-*N*-{4-[3-(4-methoxyphenyl)acryloyl]phenyl}acetamide (2b)

Pale orange crystals (chloroform) in (1.85 g, 79% yield); mp 160–162 °C; IR (KBr) ν_{max} = 3295 (NH), 1695 (*CO*–NH), 1685 (*CO*–C=C), 1595 cm^{-1} (C=C); ^1H NMR (200 MHz, $CDCl_3$, δ = ppm) δ = 10.75 (s, 1H, NH), 8.04 (d, 2H, J = 8.44 Hz, ArH), 7.79 (d, 1H, J = 15.60 Hz, H_β), 7.70 (d, 2H, J = 8.43 Hz, ArH), 7.60 (d, 2H, J = 8.44 Hz, ArH), 7.41 (d, 1H, J = 15.60 Hz, H_α), 6.94 (d, 2H, J = 8.44 Hz, ArH), 4.05 (s, 2H, CH_2Br), 3.84 (s, 3H, OCH_3); EI-MS (70 eV) m/z (%) 375 (M^++2, 71), 374 (M^++1, 32), 373 (M^+, 74), 294 (100), 257 (13), 252 (11), 240 (41), 234 (20), 161 (43), 133 (24), 120 (38), 112 (11), 91 (16). Anal. Calcd for $C_{18}H_{16}BrNO_3$ (373.03): C, 57.77; H, 4.31; N, 3.74. Found: C, 57.49; H, 4.57; N, 3.99.

4.1.5. *N*-[4-(3-Benzo[1,3]dioxol-5-ylacryloyl)phenyl]-2-bromoacetamide (2c)

Yellow crystals (methanol) in (1.99 g, 82% yield); mp 202–203 °C; IR (KBr) ν_{max} = 3340 (NH), 1670 (br, *CO*–NH, *CO*–C=C), 1596 cm^{-1} (C=C); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.72 (s, 1H, NH), 8.18 (d, 2H, J = 8.03 Hz, ArH), 7.81 (d, 1H, J = 15.30 Hz, H_β), 7.75 (d, 2H, J = 8.04 Hz, ArH), 7.60 (d, 1H, J = 1.83 Hz, ArH), 7.51 (d, 1H, J = 15.30 Hz, H_α), 7.29 (dd, 1H, J = 8.04 Hz, J = 1.83 Hz, ArH), 6.93 (d, 1H, J = 8.03 Hz, ArH), 6.10 (s, 2H, OCH_2O), 4.08 (s, 2H, CH_2Br); EI-MS (70 eV) m/z (%) 389 (M^++2, 57), 388 (M^++1, 35), 387 (M^+, 60), 359 (8), 309 (27), 308 (100), 266 (25), 240 (11), 238 (19), 180 (17), 175 (21), 152 (14), 145 (37), 120 (31), 91 (28). Anal. Calcd for $C_{18}H_{14}BrNO_4$ (387.01): C, 55.69; H, 3.63; N, 3.61. Found: C, 55.75; H, 3.39; N, 3.80.

4.1.6. 2-Bromo-*N*-[4-(3-furan-2-ylacryloyl)phenyl]acetamide (2d)

Pale yellow crystals (ethanol) in (1.57 g, 75% yield); mp 138–139 °C; IR (KBr) ν_{max} = 3460 (NH), 1695 (*CO*–NH), 1682 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (200 MHz, $CDCl_3$, δ = ppm) δ = 10.75 (s, 1H, NH), 8.45–6.43 (m, 9H: 3furanyl, 4ArH, –CH=CH–), 4.23 (s, 2H, CH_2Br). Anal. Calcd for $C_{15}H_{12}BrNO_3$ (333.00): C, 53.91; H, 3.62; N, 4.19. Found: C, 53.79; H, 3.25; N, 4.49.

4.1.7. General procedure for the synthesis of *N*-{4-[3-arylacryloyl]phenyl}-2-nitrooxyacetamides 3a–d

A solution of the appropriate bromoacetyl derivatives **2a–d** (2.7 mmol) in dry acetonitrile (5 mL) was treated portion wise with a solution of silver nitrate (0.68 g, 4 mmol) in dry acetonitrile (10 mL). The mixture was heated under reflux temperature for 12 h. The formed precipitate was filtered off, and the filtrate was evaporated till dryness. The residue was dissolved in dichloromethane, washed with distilled water (2×25 mL) and brine (2×25 mL), dried over sodium sulphate, filtered and the organic layer was evaporated till dryness, and the residue was recrystallized from the appropriate solvent.

4.1.8. *N*-{4-[3-(4-Chlorophenyl)acryloyl]phenyl}-2-nitrooxyacetamide (3a)

Yellow crystals (methanol) in (0.53 g, 55% yield); mp 180–181 °C; IR (KBr) ν_{max} = 3350 (NH), 1655 (*CO*–NH), 1648 (*CO*–C=C), 1605 (C=C), 1275 cm^{-1} (ONO_2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.79 (s, 1H, NH), 8.20 (d, 2H, J = 8.15 Hz, ArH), 7.98 (d, 1H, J = 15.44 Hz, H_β), 7.94 (d, 2H, J = 8.15 Hz, ArH), 7.77 (d, 2H, J = 8.15 Hz, ArH), 7.73 (d, 1H, J = 15.44 Hz, H_α), 7.54 (d, 2H, J = 8.15 Hz, ArH), 5.28 (s, 2H, CH_2); EI-MS (70 eV) m/z (%) 362 (M^++2, 4), 360 (M^+, 10), 314 (49), 313 (100), 284 (37), 256 (27), 241 (19), 228 (36), 176 (30), 164 (59), 146 (28), 136 (51), 110 (36), 101 (37). Anal. Calcd for $C_{17}H_{13}ClN_2O_5$ (360.05): C, 56.60; H, 3.63; N, 7.77. Found: C, 56.86; H, 4.05; N, 7.45.

4.1.9. *N*-{4-[3-(4-Methoxyphenyl)acryloyl]phenyl}-2-nitrooxyacetamide (3b)

Yellow crystals (acetonitrile) in (0.57 g, 59% yield); mp 144–145 °C; IR (KBr) ν_{max} = 3395 (NH), 1672 (*CO*–NH), 1660 (*CO*–C=C), 1600 (C=C), 1254 cm^{-1} (ONO_2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.77 (s, 1H, NH), 8.17 (d, 2H, J = 8.12 Hz, ArH), 8.86 (d, 1H, J = 15.40 Hz, H_β), 7.82 (d, 2H, J = 8.12 Hz, ArH), 8.76 (d, 2H, J = 8.12 Hz, ArH), 7.75 (d, 1H, J = 15.40 Hz, H_α), 7.03 (d, 2H, J = 8.12 Hz, ArH), 5.28 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3); EI-MS (70 eV) m/z (%) 356 (M^+,3), 309 (100), 308 (27), 299 (12), 280 (13), 254 (11), 252 (33), 237 (13), 224 (23), 161 (40), 135 (14),

133 (20), 120 (12), 108 (8). Anal. Calcd for $C_{18}H_{16}N_2O_6$ (356.10): C, 60.67; H, 4.53; N, 7.86. Found: C, 61.09; H, 4.45; N, 7.39.

4.1.10. *N*-[4-(3-Benzo[1,3]dioxol-5-ylacryloyl)phenyl]-2-nitooxyacetamide (3c)

Yellow crystals (acetonitrile) in (0.62 g, 62% yield); mp 160–162 °C; IR (KBr) ν_{max} = 3290 (NH), 1690 (*CO*–NH), 1665 (*CO*–C=C), 1595 (C=C), 1250 cm^{-1} (ONO_2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.78 (s, 1H, NH), 8.19 (d, 2H, *J* = 8.06 Hz, ArH), 7.83 (d, 1H, *J* = 15.38 Hz, H_β), 7.76 (d, 2H, *J* = 8.06 Hz, ArH), 7.67 (d, 1H, *J* = 15.38 Hz, H_α), 7.66 (d, 1H, *J* = 1.88 Hz, ArH), 7.33 (dd, 1H, *J* = 8.06 Hz, *J* = 1.88 Hz, ArH), 7.00 (d, 1H, *J* = 8.06 Hz, ArH), 6.12 (s, 2H, OCH_2O), 5.28 (s, 2H, OCH_2ONO_2); EI-MS (70 eV) *m*/*z* (%) 324 (M^+−46, 25), 323 (100), 322 (40), 295 (10), 293 (20), 268 (15), 266 (38), 238 (15), 176 (12), 175 (26), 149 (11), 147 (24), 146 (15), 120 (17), 104 (10). Anal. Calcd For $C_{18}H_{14}N_2O_7$ (370.08): C, 58.38; H, 3.81; N, 7.56. Found: C, 58.76; H, 3.62; N, 7.82.

4.1.11. *N*-[4-(3-Furan-2-ylacryloyl)phenyl]-2-nitrooxyacetamide (3d)

Dark brown crystals (methanol) in (0.44 g, 52% yield); mp > 300 °C; IR (KBr) ν_{max} = 3370 (NH), 1671 (*CO*–NH), 1652 (*CO*–C=C), 1598 (C=C), 1251 cm^{-1} (ONO_2); ^1H NMR (200 MHz, $CDCl_3$, δ = ppm) δ = 10.79 (s, 1H, NH), 8.14–8.42 (m, 9H: 3furanyl, 4ArH, –CH=CH–), 5.35 (s, 2H, CH_2). Anal. Calcd for $C_{15}H_{12}N_2O_6$ (316.07): C, 56.96; H, 3.82; N, 8.86. Found: C, 56.60; H, 4.00; N, 8.80.

4.1.12. General procedure for the synthesis of 2-bromo-*N*-{4-[3-arylacryloyl]phenyl}propionamides 4a–d

To a stirred solution of 2-bromopropionic acid (1.53 g, 10 mmol) in 50 mL chloroform cooled at 0 °C, triethylamine (1.0 g, 10 mmol) was added, followed by ethyl chloroformate (1.08 g, 10 mmol) in drop wise manner. Stirring was continued for further 1 h at 0 °C, then chalcones **1a–d** (10 mmol) were added in portion wise manner, and the reaction mixture was then stirred for additional 12 h at rt Chloroform (2 × 40 mL) was then added, and the organic layer was separated, washed with distilled water (2 × 40 mL), 5% sodium bicarbonate (2 × 40 mL), 1 N hydrochloric acid (2 × 40 mL), distilled water (2 × 40 mL) and finally with brine (2 × 40 mL). The organic layer was then dried over sodium sulphate, filtered, evaporation of the organic layer and the obtained crude product was then recrystallized from the appropriate solvent.

4.1.13. 2-Bromo-*N*-{4-[3-(4-chlorophenyl)acryloyl]phenyl}propionamide (4a)

White crystals (methanol) in (2.82 g, 72% yield); mp 188–190 °C; IR (KBr) ν_{max} = 3279 (NH), 1670 (br, *CO*–NH, *CO*–C=C), 1595 cm^{-1} (C=C); ^1H NMR (200 MHz, $CDCl_3$, δ = ppm) δ = 10.70 (s, 1H, NH), 8.09 (d, 2H, *J* = 8.20 Hz, ArH), 7.71 (d, 1H, *J* = 15.20 Hz, H_β), 7.59 (d, 2H, *J* = 8.17 Hz, ArH), 7.43 (d, 2H, *J* = 8.20 Hz, ArH), 7.30 (d, 1H, *J* = 15.20 Hz, H_α), 7.01 (d, 2H, *J* = 8.17 Hz, ArH), 4.55 (q, 1H, *J* = 7.11 Hz, CHBr), 1.98 (d, 3H, *J* = 7.11 Hz, CH_3); EI-MS (70 eV) *m*/*z* (%) 395 (M^++4, 12), 393 (M^++2, 54), 391 (M^+, 44), 312 (100), 302 (26), 267 (28), 229 (36), 204 (17), 165 (52), 137 (34), 120 (42), 101 (39). Anal. Calcd for $C_{18}H_{15}BrClNO_2$ (391.00): C, 55.06; H, 3.85; N, 3.57. Found: C, 55.30; H, 3.46; N, 3.46.

4.1.14. 2-Bromo-*N*-{4-[3-(4-methoxyphenyl)acryloyl]phenyl}propionamide (4b)

Buff crystals (methanol) in (2.55 g, 66% yield); mp 142–143 °C; IR (KBr) ν_{max} = 3287 (NH), 1655 (*CO*–NH), 1645 (*CO*–C=C), 1589 cm^{-1} (C=C); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.68 (s, 1H, NH), 8.11 (d, 2H, *J* = 8.80 Hz, ArH), 7.84 (d, 2H, *J* = 8.80 Hz, ArH), 7.79 (d, 1H, *J* = 15.53 Hz, H_β), 7.68 (d, 1H, *J* = 15.53 Hz, H_α), 7.64 (d, 2H, *J* = 8.80 Hz, ArH), 7.02 (d, 2H, *J* = 8.80 Hz, ArH), 4.78 (q, 1H, *J* = 7.08 Hz, CHBr), 3.83 (s, 3H, OCH_3), 1.79 (d, 3H, *J* = 7.08 Hz, CH_3); EI-MS (70 eV) *m*/*z* (%) 389 (M^++2, 1), 388 (M^++1, 2), 387 (M^+, 1), 325 (100), 310 (5), 297 (15), 296 (36), 280 (6), 279 (11), 251 (9), 224 (15), 192 (9), 161 (21), 133 (10), 120 (7), 92 (8). Anal. Calcd for $C_{19}H_{18}BrNO_3$ (387.05): C, 58.78; H, 4.67; N, 3.61. Found: C, 58.75; H, 4.39; N, 3.81.

4.1.15. *N*-[4-(3-Benzo[1,3]dioxol-5-ylacryloyl)phenyl]-2-bromopropionamide (4c)

Pale yellow powder (chloroform) in (2.77 g, 69% yield); mp 158–159 °C; IR (KBr) ν_{max} = 3292 (NH), 1665 (*CO*–NH), 1650 (*CO*–C=C), 1595 (C=C) cm^{-1}; ^1H NMR (DMSO-d_6, δ = ppm) δ = 10.69 (s, 1H, NH), 8.21–6.97 (m, 9 H: 7 ArH, –CH=CH–), 6.12 (s, 2H, CH_2), 4.74 (q, 1H, *J* = 6.69 Hz, CHBr), 1.78 (d, 3H, *J* = 6.67 Hz, CH_3); EI-MS (70 eV) *m*/*z* (%) 403 (M^++2, 20), 402 (M^++1, 67), 401 (M^+, 33), 339 (100), 327 (66), 310 (41), 394 (17), 238 (42), 207 (45), 192 (45), 175 (59), 153 (28), 120 (41), 117 (26). Anal. Calcd for $C_{19}H_{16}BrNO_4$ (401.03): C, 56.73; H, 4.01; N, 3.48. Found: C, 57.19; H, 4.25; N, 3.49.

4.1.16. 2-Bromo-*N*-[4-(3-furan-2-ylacryloyl)phenyl]propionamide (4d)

Dark brown crystals (ethanol) in (2.26 g, 65% yield); mp 121–122 °C; IR (KBr) ν_{max} = 3297 (NH), 2927 (Aliph.–CH), 1671 (*CO*–NH), 1655 (*CO*–C=C), 1596 (C=C) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, δ = ppm) δ = 10.82 (s, 1H, NH), 8.35–6.52 (m, 9H: 3furanyl, 4ArH, –CH=CH–), 4.55 (q, 1H, *J* = 6.95 Hz, CHBr), 1.86 (d, 3H, *J* = 6.95 Hz, CH_3). Anal. Calcd for $C_{16}H_{14}BrNO_3$ (347.02): C, 55.19; H, 4.05; N, 4.02. Found: C, 55.00; H, 4.10; N, 3.79.

4.1.17. General procedure for the synthesis of *N*-{4-[3-arylacryloyl]phenyl}-2-nitrooxypropionamides 5a–d

The titled compounds were prepared according to the general procedure used in the synthesis of nitrate esters **3a–d**, using the same molar ratios.

4.1.18. *N*-{4-[3-(4-Chlorophenyl)acryloyl]phenyl}-2-nitrooxypropionamide (5a)

Pale yellow crystals (acetonitrile) in (0.62 g, 61% yield); mp 192–193 °C; IR (KBr) ν_{max} = 3310 (NH), 1650 (*CO*–NH, *CO*–C=C), 1595 (C=C), 1225 (ONO_2) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.89 (s, 1H, NH), 8.20 (d, 2H, *J* = 8.40 Hz, ArH), 7.94 (d, 2H, *J* = 8.40 Hz, ArH), 7.80 (d, 2H, *J* = 8.40 Hz, ArH), 7.78 (d, 1H, *J* = 16.01 Hz, H_β), 7.53 (d, 2H, *J* = 8.40 Hz, ArH), 7.35 (d, 1H, *J* = 16.01 Hz, H_α), 5.52 (q, 1H, *J* = 6.91 Hz, $CHONO_2$), 1.54 (d, 3H, *J* = 6.91 Hz, CH_3); EI-MS (70 eV) *m*/*z* (%) 376 (M^++2, 4), 374 (M^+, 9), 328 (100), 284 (10), 267 (50), 256 (15), 239 (23), 231 (9), 204 (65), 190 (23), 164 (52), 155 (16), 136 (20), 110 (8), 101 (96). Anal. Calcd for $C_{18}H_{15}ClN_2O_5$ (374.07): C, 57.69; H, 4.03; N, 7.47. Found: C, 58.03; H, 4.45; N, 7.17.

4.1.19. *N*-{4-[3-(4-Methoxyphenyl)acryloyl]phenyl}-2-nitrooxypropionamide (5b)

Pale yellow crystals (acetonitrile) in (0.56 g, 56% yield); mp 144–145 °C; IR (KBr) ν_{max} = 3321 (NH), 2998 (Aliph.–CH), 1676 (*CO*–NH), 1654 (*CO*–C=C), 1598 (C=C), 1220 cm^{-1} (ONO_2); ^1H NMR (400 MHz, $CDCl_3$, δ = ppm) δ = 10.78 (s, 1H, NH), 8.02 (d, 2H, *J* = 8.09 Hz, ArH), 7.79 (d, 1H, *J* = 15.42 Hz, H_β), 7.60 (d, 2H, *J* = 8.09 Hz, ArH), 7.52 (d, 2H, *J* = 8.09 Hz, ArH), 7.43 (d, 1H, *J* = 15.42 Hz, H_α), 6.94 (d, 2H, *J* = 8.09 Hz, ArH), 5.51 (q, 1H, *J* = 7.11 Hz, $CHONO_2$), 3.86 (s, 3H, OCH_3), 1.61 (d, 3H, *J* = 7.12 Hz, CH_3); EI-MS (70 eV) *m*/*z* (%) 324 (M^+−46, 100), 310 (7), 296 (47), 252 (15), 237 (8), 224 (64), 209 (9), 180 (14), 165 (22), 161 (41), 153 (16), 146 (19), 133 (29), 118 (19), 101 (10), 90 (31). Anal. Calcd

for $C_{19}H_{18}N_2O_6$ (370.12): C, 61.62; H, 4.90; N, 7.56. Found: C, 61.33; H, 5.12; N, 7.50.

4.1.20. *N*-[4-(3-Benzo[1,3]dioxol-5-ylacryloyl)phenyl]-2-nitooxypropionamide (5c)

Yellowish-green crystals (acetonitrile) in (0.69 g, 67% yield); mp 165–166 °C; IR (KBr) ν_{max} = 3310 (NH), 2850 (Aliph.–CH), 1662 (*CO*–NH), 1650 (*CO*–C=C), 1600 (C=C), 1250 cm^{-1} (ONO_2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.79 (s, 1H, NH), 8.19 (d, 2H, J = 8.11 Hz, ArH), 7.83 (d, 1H, J = 15.53 Hz, H_β), 7.78 (d, 2H, J = 8.11 Hz, ArH), 7.67 (d, 1H, J = 15.53 Hz, H_α), 7.65 (s, 1H, ArH), 7.33 (dd, 1H, J_o = 8.11 Hz, J_m = 1.85 Hz, ArH), 7.00 (d, 1H, J = 8.11 Hz, ArH), 6.12 (s, 2H, OCH_2O), 5.49 (q, 1H, J = 7.07 Hz, $CHONO_2$), 1.54 (d, 3H, J = 7.07 Hz, CH_3); EI-MS (70 eV) m/z (%) 338 (M^+−46, 20), 310 (10), 239 (13), 238 (68), 224 (8), 181 (12), 175 (10), 165 (12), 152 (32), 146 (41), 120 (30), 117 (18), 95 (11), 89 (100). Anal. Calcd for $C_{19}H_{16}N_2O_7$ (384.10): C, 59.38; H, 4.20; N, 7.29. Found: C, 59.00; H, 4.25; N, 7.01.

4.1.21. *N*-[4-(3-Furan-2-ylacryloyl)phenyl]-2-nitrooxypropionamide (5d)

Brown crystals (acetonitrile) in (0.52 g, 58% yield); mp 200–202 °C; IR (KBr) ν_{max} = 3306 (NH), 2922 (Aliph.–CH), 1660 (*CO*–NH), 1647 (*CO*–C=C), 1597 (C=C), 1225 cm^{-1} (ONO_2); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.72 (s, 1H, NH), 8.22–6.65 (m, 9H: 3furanyl, 4ArH, –CH=CH–), 5.45 (q, 1H, J = 7.15 Hz, $CHONO_2$), 1.82 (d, 3H, J = 7.15 Hz, CH_3). Anal. Calcd for $C_{16}H_{14}N_2O_6$ (330.09): C, 58.18; H, 4.27; N, 8.48. Found: C, 58.48; H, 4.48; N, 8.75.

4.1.22. General procedure for the synthesis of 2-(4-acetylphenoxy)-*N*-{4-[3-arylacryloyl]phenyl}acetamides 6a–d

To a solution of *p*-hydroxyacetophenone (0.68 g, 5 mmol), bromoacetyl derivatives **2a–d** (5 mmol) in 100 mL acetone, anhydrous potassium carbonate (0.50 g, 3.60 mmol) was added. The reaction mixture was heated on a water-bath for 12 h. The solvent was then evaporated, and the obtained residue was washed with distilled water and recrystallized from the appropriate solvent.

4.1.23. 2-(4-Acetylphenoxy)-*N*-{4-[3-(4-chlorophenyl)acryloyl]phenyl}acetamide (6a)

Pale yellow crystals (chloroform) in (1.52 g, 70% yield); mp 224–226 °C; IR (KBr) ν_{max} = 3410 (NH), 1690 (br, *CO*–NH, *CO*–CH_3), 1675 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.60 (s, 1H, NH), 8.21 (d, 2H, J = 8.71 Hz, ArH), 8.02 (d, 1H, J = 15.73 Hz, H_β), 7.99 (d, 2H, J = 8.71 Hz, ArH), 7.94 (d, 2H, J = 8.71 Hz, ArH), 7.86 (d, 2H, J = 8.73 Hz, ArH), 7.25 (d, 1H, J = 15.70 Hz, H_α) 7.55 (d, 2H, J = 8.71 Hz, ArH), 7.14 (d, 2H, J = 8.73 Hz, ArH), 4.92 (s, 2H, CH_2), 2.53 (s, 3H,, CH_3); EI-MS (70 eV) m/z (%) 435 (M^++2, 31), 433 (M^+, 100), 418 (8), 405 (12), 375 (33), 360 (17), 332 (8), 296 (51), 270 (87), 257 (88), 238 (37), 192 (44), 165 (90), 137 (75), 121 (96), 102 (51). Anal. Calcd for $C_{25}H_{20}ClNO_4$ (433.11): C, 69.20; H, 4.65; N, 3.23. Found: C, 68.87; H, 4.86; N, 3.40.

4.1.24. 2-(4-Acetylphenoxy)-*N*-{4-[3-(4-methoxyphenyl)acryloyl]phenyl}acetamide (6b)

Pale yellow crystals (methanol) in (1.57 g, 73% yield); mp 216–218 °C; IR (KBr) ν_{max} = 3398 (NH), 1678 ((br, *CO*–NH, *CO*–CH_3), 1655 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.55 (s, 1H, NH), 8.16 (d, 2H, J = 8.68 Hz, ArH), 7.97 (d, 2H, J = 8.68 Hz, ArH), 7.88–7.79 (m, 5H: 4 ArH, H_β), 7.70 (d, 1H, J = 15.68 Hz, H_α) 7.12 (d, 2H, J = 8.76 Hz, ArH), 7.02 (d, 2H, J = 8.76 Hz, ArH), 4.90 (s, 2H, CH_2), 4.19 (s, 3H, OCH_3), 2.50 (s, 3H, CH_3); EI-MS (70 eV) m/z (%) 430 (M^++1, 23), 429 (M^+, 100), 401(8), 293 (22), 266 (18), 207 (8), 161 (24), 121 (16). Anal. Calcd for $C_{26}H_{23}NO_5$ (429.16): C, 72.71; H, 5.40; N, 3.26. Found: C, 72.55; H, 5.23; N, 3.34.

4.1.25. 2-(4-Acetylphenoxy)-*N*-[4-(3-benzo[1,3]dioxol-5-ylacryloyl)phenyl]acetamide (6c)

Yellow powder (chloroform) in (1.66 g, 75% yield); mp 244–246 °C; IR (KBr) ν_{max} = 3408 (NH), 1692 (*CO*–NH), 1675 (*CO*–CH_3), 1649 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.57 (s, 1H, NH), 8.20 (d, 2H, J = 8.73 Hz, ArH), 7.98 (d, 2H, J = 8.73 Hz, ArH), 7.86 (d, 1H, J = 15.42 Hz, H_β), 7.84 (d, 2H, J = 8.78 Hz, ArH), 7.69 (d, 1H, J = 15.42 Hz, H_α), 7.68 (d, 1H, J_o = 1.15 Hz, ArH), 7.36 (dd, 1H, J_o = 1.15, J_m = 8.78 Hz, ArH), 7.15 (d, 2H, J = 8.78 Hz, ArH), 7.03 (d, 1H, J = 8.78 Hz, ArH), 6.10 (s, 2H, OCH_2O), 4.92 (s, 2H, CH_2), 2.53 (s, 3H, CH_3); EI-MS (70 eV) m/z (%) 444 (M^++1, 24), 443 (M^+, 100), 415 (8), 400 (4), 307 (44), 266 (20), 250 (8), 214 (8), 185 (5). Anal. Calcd for $C_{26}H_{21}NO_6$ (443.14): C, 70.42; H, 4.77; N, 3.16. Found: C, 70.64; H, 4.55; N, 3.25.

4.1.26. 2-(4-Acetylphenoxy)-*N*-[4-(3-furan-2-ylacryloyl)phenyl]acetamide (6d)

Pale brown crystals (acetone) in (1.28 g, 66% yield); mp 218–220 °C; IR (KBr) ν_{max} = 3415 (NH), 1691 (*CO*–NH), 1672 (*CO*–CH_3), 1652 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.65 (s, 1H, NH), 8.22–6.65 (m, 13H: 3furanyl, 8ArH, –CH=CH–), 4.92 (s, 2H, CH_2), 2.60 (s, 3H, CH_3); EI-MS (70 eV) m/z (%) 390 (M^++1, 23), 389 (M^+, 100), 361 (4), 335 (6), 296 (41), 254 (6), 226 (12), 198 (5), 160 (4), 140 (20), 121 (32). Anal. Calcd for $C_{23}H_{19}NO_5$ (389.13): C, 70.94; H, 4.92; N, 3.60. Found: C, 71.12; H, 4.69; N, 3.45.

4.1.27. General procedure for the synthesis of *N*-{4-[3-arylacryloyl]phenyl}-2-[4-(1-hydroxyiminoethyl phenoxy] acetamides 7a–d

A mixture of equimolar amounts of the appropriate ketone derivatives **6a–d** (2.3 mmol) and hydroxylamine hydrochloride in absolute ethanol (30 mL) was heated under reflux for 20 h and then left to cool. The separated solid was filtered, washed with dil. ammonia solution and distilled water, dried, and recrystallized from the appropriate solvent.

4.1.28. *N*-{4-[3-(4-Chlorophenyl)acryloyl]phenyl}-2-[4-(1-hydroxyiminoethyl)phenoxy]acetamide (7a)

Yellow crystals (methanol) in (0.92 g, 89% yield); mp 200–202 °C; IR (KBr) ν_{max} = 3600–3100 (OH), 3400 (NH), 1690 (*CO*–NH), 1662 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 11.04 (s, 1H, OH), 10.52 (s, 1H, NH), 8.18 (d, 2H, J = 8.18 Hz, ArH), 7.95–7.16 (m, 10H: 8ArH, –CH=CH–), 7.03 (d, 2H, J = 6.41 Hz, ArH), 4.81 (s, 2H, CH_2), 2.13 (s, 3H, CH_3); EI-MS (70 eV) m/z (%) 448 (M^+, 38), 450 (M^++2, 13), 433 (100), 417 (97), 404 (62), 369 (15), 297 (95), 279 (36), 270 (75), 257 (42), 191 (22), 165 (86), 121 (72). Anal. Calcd for $C_{25}H_{21}ClN_2O_4$ (448.12): C, 66.89; H, 4.72; N, 6.24. Found: C, 66.74; H, 4.60; N, 6.39.

4.1.29. 2-[4-(1-Hdroxyiminoethyl)phenoxy]-*N*-{4-[3-(4-methoxyphenyl)acryloyl]phenyl}acetamide (7b)

Buff crystals (methanol) in (0.92 g, 90% yield); mp 198–200 °C; IR (KBr) ν_{max} = 3700–3200 (OH), 3398 (NH), 2923 (Aliph.–CH), 1685 (*CO*–NH), 1679 (*CO*–C=C), 1592 cm^{-1} (C=C); ^1H NMR (270 MHz, DMSO-d_6, δ = ppm) δ = 11.03 (s, 1H, OH), 10.53 (s, 1H, NH), 8.14–7.01 (m, 14H: 12 ArH, –CH=CH–), 4.88 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 2.10 (s, 3H, CH_3); EI-MS (70 eV) m/z (%) 444 (M^+, 3), 429 (100), 401 (8), 414 (8), 398 (4), 294 (22), 279 (10), 269 (12), 252 (13), 207 (7), 161 (22), 133 (14), 121 (15), 107

(10). Anal. Calcd for $C_{26}H_{24}N_2O_5$ (444.17): C, 70.26; H, 5.44; N, 6.30. Found: C, 70.40; H, 5.33; N, 6.35.

4.1.30. *N*-[4-(3-Benzo[1,3]dioxol-5-ylacryloyl)phenyl]-2-[4-(1-hydroxyiminoethyl)phenoxy]acetamide (7c)

Yellow powder in (0.96 g, 91% yield); mp > 300 °C; IR (KBr) ν_{max} = 3700–3300 (OH), 3408 (NH), 1670 (*CO*–NH), 1651 (*CO*–C=C), 1600 cm^{-1} (C=C); ^1H NMR (insoluble in NMR deuterated solvents); EI-MS (70 eV) *m*/*z* (%) 458 (M^+, 5), 443 (100), 415 (10), 400 (4), 307 (44), 280 (18), 266 (11), 250 (8), 213 (10), 185 (8), 175 (11), 145 (17), 121 (17). Anal. Calcd for $C_{26}H_{22}N_2O_6$ (458.15): C, 68.11; H, 4.84; N, 6.11. Found: C, 68.35; H, 4.70; N, 6.37.

4.1.31. *N*-[4-(3-Furan-2-ylacryloyl)phenyl]-2-[4-(1-hydroxyiminoethyl)phenoxy]acetamide (7d)

Pale yellow powder in (0.82 g, 88% yield); mp 270–272 °C; IR (KBr) ν_{max} = 3600–3200 (OH), 3389 (NH), 2925 (Aliph.–CH), 1668 (*CO*-NH), 1652 (*CO*–C=C), 1596 cm^{-1} (C=C); ^1H NMR (insoluble in NMR deuterated solvents); EI-MS (70 eV) *m*/*z* (%) 405 (M^++1, 7), 404 (M^+, 4), 389 (100), 361 (7), 335 (13), 296 (41), 254 (5), 226 (16), 212 (5), 198 (8), 160 (9), 140 (20), 121 (33). Anal. Calcd for $C_{23}H_{20}N_2O_5$ (404.14): C, 68.31; H, 4.98; N, 6.93. Found: C, 68.59; H, 5.23; N, 6.80.

4.1.32. Synthesis of 3-phenyl-4-furoxanmethanol (8)

To a stirred solution of cinnamyl alcohol (6.7 g, 0.05 mol) in 10 mL glacial acetic acid heated at 70 °C, a solution of sodium nitrite (6.9 g, 0.1 mol) in 5 mL water was added in a drop-wise manner. The reaction mixture was stirred at rt for 2 h. The organic layer was extracted with dichloromethane, washed with water (2 × 40 mL), dried over anhydrous calcium chloride and evaporated on a rotary evaporator giving an oily brown product, which was purified by column chromatography using pentane/ethylacetate (4:1) as an eluent to give the titled compound (3.84 g, 40%) as pale yellow crystals, mp 67 °C.

4.1.33. General procedure for the synthesis of *N*-[4-(arylacryloyl)phenyl]-2-(2-oxy-4-phenylfurazan-3-ylmethoxy)acetamides 9a–d

To a solution of 3-phenyl-4-furoxanmethanol **8** (0.192 g, 1 mmol), bromoacetyl derivatives **2a–d** (1 mmol) in 15 mL acetone, anhydrous potassium carbonate (0.138 g, 1 mmol) was added. The reaction mixture was heated on a water-bath for 12 h. The solvent was evaporated, and the residue was washed with distilled water and recrystallized from the appropriate solvent.

4.1.34. *N*-{4-[3-(4-Chlorophenyl)acryloyl]phenyl}-2-(2-oxy-4-phenylfurazan-3-ylmethoxy)acetamide (9a)

Yellow crystals (chloroform) in (0.39 g, 79% yield); mp > 250 °C; IR (without solvent) ν_{max} = 3359 (NH), 3063 (Ar –CH), 1700 (*CO*–NH), 1657 (*CO*–C=C), 1592 (furoxan), 1269 cm^{-1} (O–CH_2); ^1H NMR (200 MHz, DMSO-d_6, δ = ppm) δ = 10.73 (s, 1H, NH), 8.28–7.29 (m, 15H: 13H, ArH, –CH=CH–), 4.19 (s, 2H, Ar–*CH_2*–O), 4.05 (s, 2H, O–*CH_2*–CO); EI-MS (70 eV) *m*/*z* (%) 491 (M^++2, 33), 489 (M^+, 100), 379 (42), 342 (10), 298 (88), 283 (6), 257 (28), 240 (10), 257 (22), 240 (10), 229 (23), 193 (11), 178 (10), 165 (30), 137 (17), 120 (33). Anal. Calcd for $C_{26}H_{20}ClN_3O_5$ (489.11): C, 63.74; H, 4.11; N, 8.58. Found: C, 63.50; H, 4.10; N, 8.33.

4.1.35. *N*-{4-[3-(4-Methoxyphenyl)acryloyl]phenyl}-2-(2-oxy-4-phenylfurazan-3-ylmethoxy)acetamide (9b)

Yellow powder (chloroform) in (0.27 g, 55% yield); mp 182–184 °C; IR (without solvent) ν_{max} = 3368 (NH), 3004 (Ar –CH), 1686 (*CO*–NH), 1653 (*CO*–C=C), 1589 (furoxan), 1254 cm^{-1} (O–CH2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.54 (s, 1H, NH), 8.24–6.99 (m, 15H: 13H, ArH, –CH=CH–), 4.75 (s, 2H, Ar–*CH_2*–O), 4.68 (s, 2H, O–*CH_2*–CO), 3.83 (s, 3H, OCH_3); EI-MS (70 eV) *m*/*z* (%) 486 (M^++1, 4), 485 (M^+, 12), 469 (12), 455 (5), 366 (6), 350 (13), 311 (4), 264 (10), 252 (20), 237 (13), 224 (8), 161 (24), 133 (15), 103 (100). Anal. Calcd for $C_{27}H_{23}N_3O_6$ (485.16): C, 66.80; H, 4.78; N, 8.66. Found: C, 67.00; H, 4.50; N, 8.29.

4.1.36. *N*-[4-{3-Benzo[1,3]dioxol-5-ylacryloyl)phenyl]-2-(2-oxy-4-phenylfurazan-3-ylmethoxy)acetamide (9c)

Pale yellow powder (DMF) in (0.33 g, 67% yield); mp 148–150 °C; IR (without solvent) ν_{max} = 3357 (NH), 2908 (Aliph. –CH), 1700 (*CO*–NH), 1659 (*CO*–C=C), 1590 (furoxan), 1243 cm^{-1} (O–CH_2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.45 (s, 1H, NH), 8.25–6.93 (m, 14H: 12H, ArH, –CH=CH–), 6.15 (s, 2H, OCH_2O), 4.65 (s, 2H, Ar–*CH_2*–O), 4.55 (s, 2H, O–*CH_2*–CO); EI-MS (70 eV) *m*/*z* (%) 499 (M^+, 16), 483 (18), 453 (5), 397 (4), 380 (7), 364 (19), 323 (28), 293 (24), 267 (52), 238 (21), 209 (7), 180 (10), 175 (20), 145 (36), 103 (100). Anal. Calcd for $C_{27}H_{21}N_3O_7$ (499.14): C, 64.93; H, 4.24; N, 8.41. Found: C, 65.25; H, 3.86; N, 8.30.

4.1.37. *N*-[4-(3-Furan-2-ylacryloyl)phenyl]-2-(2-oxy-4-phenylfurazan-3-ylmethoxy)acetamide (9d)

Pale yellow powder (DMF) in (0.24 g, 53% yield); mp > 250 °C; IR (without solvent) ν_{max} = 3326 (NH), 3096 (Ar–CH), 1655 (br, C=O), 1586 (furoxan), 1227 cm^{-1} (O–CH_2); ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 10.71 (s, 1H, NH), 8.39–6.62 (m, 14H, 9 ArH, 3 Furanyl, –CH=CH–), 4.55 (s, 2H, Ar–*CH_2*–O), 4.46 (s, 2H, O–*CH_2*–CO); EI-MS (70 eV) *m*/*z* (%) 446 (M^++1, 8), 445 (M^+, 12), 413 (100), 385 (8), 369 (9), 291 (4), 237 (4), 219 (7), 225 (10), 213 (11), 197 (14), 160 (15), 132 (41), 121 (16), 105 (12), 77 (13). Anal. Calcd for $C_{24}H_{19}N_3O_6$ (445.13): C, 64.72; H, 4.30; N, 9.43. Found: C, 64.35; H, 4.45; N, 9.06.

4.2. Nitric oxide release

4.2.1. Measurement[64]

Different solutions of the tested compounds **3a–d**, **5a–d**, **7a–d**, **8** and **9a–d** (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 **1a–d**, **2a–d**, **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–d**, **8** and **9a–d** and reference drug (indomrthacin) in carboxy methyl cellulose (CMC) solution (0.5% w/v in

water) was administered orally in a dose level of (100 mg/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 sacrified 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.[65] 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.

Acknowledgement

Authors introduce their great thanks to Dr. Ahmed A. Mourad, Pharmacology Department, Faculty of Pharmacy, Minia University for his valuable help in measuring the anti-inflammatory activity and ulcerogenic liability. Authors also introduce their great thanks to Prof. Dr. Entesar Ali Saber and Dr. Sohaa Abdel-Kawi, Histology Department, Faculty of Medicine, Minia University for their great help in performing the histopathological investigation.

References and notes

1. Singh, G.; Triadafilopoulos, G. *J. Rheumatol.* **1999**, 18.
2. Botting, R. M. *J. Therm. Biol.* **2006**, *3*, 208.
3. Naesdal, J.; Brown, K. *Drug Saf.* **2006**, *29*, 119.
4. Cryer, B. *Am. J. Gastroenterol.* **2005**, *100*, 1694.
5. Lazzaroni, M.; Bianchi, P. G. *Aliment. Pharmacol. Ther.* **2004**, *20*, 48.
6. James, M. W.; Hawkey, C. J. *Br. J. Clin. Pharmacol.* **2003**, *56*, 146.
7. Schneider, V.; Levesque, L. E.; Zhang, B.; Hutchinson, T.; Brophy, J. M. *Am. J. Epidemiol.* **2006**, *164*, 881.
8. Mounier, G.; Guy, C.; Berthoux, F.; Beyens, M. N.; Ratrema, M.; Ollagnier, M. *Therapie* **2006**, *61*, 255.
9. Zadrazil, J. *Vnitr. Lek.* **2006**, *52*, 686.
10. Adebayo, D.; Bjarnason, I. *Postgrad. Med. J.* **2006**, *82*, 186.
11. Velázquez, C. A.; Praveen Rao, P. N.; Citro, M. L.; Keefer, L. K.; Knaus, E. E. *Bioorg. Med. Chem.* **2007**, *15*, 4767.
12. 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.
13. Wallace, J. L.; Miller, M. *J. Gastroenterol.* **2000**, *119*, 512.
14. Takeuchi, K.; Yasuhiro, T.; Asada, Y.; Sugawa, Y. *Digestion* **1998**, *59*, 298.
15. Holzer, P.; Pabst, M. A.; Lipp, I. T.; Peskar, B. M.; Livingston, E. H.; Guth, P. H. *Gastroenterology* **1990**, *98*, 838.
16. 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.
17. Brown, J. F.; Keates, A. C.; Hanson, P. J.; Whittle, B. J. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1993**, *265*, G418.
18. Holm, M.; Johansson, B.; Petterssson, A.; Fandrisk, L. *Acta Physiol. Scand.* **1998**, *162*, 461.
19. Iwata, F.; Leung, F. W. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1995**, *268*, G153.
20. Fujihara, C. K.; Malheiros, D. M.; Donato, J. L.; Poli, A.; De Nucci, G.; Zatz, R. *Am. J. Physiol. Renal Physiol.* **1998**, *274*, F573.
21. Lee, S. H.; Seo, G. S.; Kim, J. Y.; Jin, X. Y.; Kim, H. D.; KimSohn, D. H. *Eur. J. Pharmacol.* **2006**, *532*, 178.
22. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
23. Rajendra, P. Y.; Srinivasa, R. A.; Rambabu, R. *Asian J. Chem.* **2009**, *21*, 907.
24. Tomar, V.; Bhattacharjee, G.; Kamaluddina; Kumar, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5321.
25. Nowakowska, Z.; Kedzia, B.; Shroeder, G. *Eur. J. Med. Chem.* **2008**, *43*, 707.
26. Lopez, S. N.; Castelli, M. V.; Zacchino, S. A.; Dominguez, J. N.; Lobo, G.; Charris-Charris, J.; Cortes, J. C. J.; Ribas, J. C.; Devia, C.; Rodriguez, A. M.; Enriz, R. D. *Bioorg. Med. Chem.* **2001**, *9*, 1999.
27. Lahtchev, K. L.; Batovska, D. I.; Parushev, P., St; Ubiyvovk, V. M.; Sibirny, A. A. *Eur. J. Med. Chem.* **2008**, *43*, 2220.
28. Trivedi, J. C.; Bariwal, J. B.; Upadhyay, K. D.; Naliapara, E. D.; Joshi, S. K.; Pannecouque, C. C.; Clercq, E. D.; Shah, A. K. *Tetrahedron Lett.* **2007**, *48*, 8472.
29. Lin, Y. M.; Zhou, Y.; Flavin, M. T.; Zhow, L. M.; Nie, W.; Chen, F. C. *Bioorg. Med. Chem.* **2002**, *10*, 2795.
30. Dominguez, J. N.; León, C.; Rodrigues, J.; Domínguez, N. G.; Gut, J.; Rosenthal, P. J. *IL Farmaco.* **2005**, *60*, 307.
31. Motta, L. F.; Gaudio, A. C.; Takahata, Y. *Int. Elect. J. Mol. Des.* **2006**, *5*, 555.
32. Dominguez, J. N.; Charris, J. E.; Lobo, G.; Gamboan, N.; Dominguez, N. G.; Moreno, M. M.; Riggione, F.; Sanchez, E.; Olson, J.; Rosenthal, P. J. *Eur. J. Med. Chem.* **2001**, *36*, 555.
33. Liu, M.; Wilairat, P.; Go, M. L. *J. Med. Chem.* **2002**, *45*, 1735.
34. Yayli, N.; Ucuncu, O.; Aydin, E.; Gok, Y.; Yasar, A.; Baltaci, C.; Yildirim, N.; Kucuk, M. *J. Photbio. A: Chem.* **2005**, *169*, 229.
35. Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J. *Bioorg. Med. Chem.* **2008**, *16*, 4286.
36. Gacche, R. N.; Dhole, N. A.; Kamble, S. G.; Bandgar, B. P. *Enz. Inhib. Med. Chem.* **2008**, *23*, 28.
37. Ducki, S.; Forrest, R.; Hadfield; Kendall, A.; Lawrence, R. J.; McGown, A. T.; Rennison, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051.
38. Boeck, P.; Falco, C. A. B.; Leal, P. C.; Yunes, R. A.; Filho, V. C.; Torres-Santos, E. C.; Rossi-Bergmann, B. *Bioorg. Med. Chem.* **2006**, *14*, 1538.
39. Zhao, L.; Jin, H.; Sun, L.; Piaoa, H.; Quana, Z. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5027.
40. Won, S. J.; Liu, C. T.; Tsao, L. T.; Weng, J. R.; Ko, H. H.; Wang, J. P.; Lin, C. N. *Eur. J. Med. Chem.* **2005**, *40*, 103.
41. Achanta, G.; Modzelewska, A.; Feng, L.; Khan, S. R.; Huang, P. *Mol. Pharmacol.* **2006**, *70*, 426.
42. Modzelewska, A.; Pettit, C.; Achanta, G.; Davidson, N. E.; Huang, P.; Khan, S. R. *Bioorg. Med. Chem.* **2006**, *14*, 3491.
43. Ducki, S. *IDrugs* **2007**, *10*, 42.
44. Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Dominguez, J. N.; Charris, J. E.; Lobo, G. M.; Alcaraz, M. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1169.
45. Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Guillen, I.; Dominguez, J. N.; Charris, J. E.; Lobo, G. M.; Alcaraz, M. J. *Free Radic. Biol. Med.* **2001**, *30*, 43.
46. Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Guillen, I.; Dominguez, J. N.; Charris, J. E.; Lobo, G. M.; Alcaraz, M. J. *FEBS Lett.* **1999**, *453*, 129.
47. Herencia, F.; Lopez-Garcia, M. P.; Ubeda, A.; Ferrandiz, M. L. *Nitric Oxide* **2002**, *6*, 242.
48. Rojas, J.; Dominguez, J. N.; Ferrandiz, M. L. *Eur. J. Pharmacol.* **2003**, *465*, 183.
49. Shoman, M. E.; Abdel-Aziz, M.; Aly, O. M.; Farag, H. H.; Morsy, M. A. *Eur. J. Med. Chem.* **2009**, *44*, 3068.
50. Abdel-Hafez, M. N.; El-SM; Abuo-Rahma, A. A.; Gel-D; Abdel-Aziz, M.; Radwan, M. F.; Farag, H. H. *Bioorg. Med. Chem.* **2009**, *17*, 3829.
51. Fengping, Y.; Yangping, P.; Gonghua, S.; Jizong, L. *J. Chem. Res.* **2005**, 311.
52. Nakaya, K.; Funabiki, K.; Shibata, K.; Muramatsu, H.; Matsul, M. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2961.
53. Ansari, F. L.; Umbreen, S.; Hussain, L.; Makhmoor, T.; Nawaz, A. S.; Lodhi, M. A.; Khan, S. N.; Shamsun, N.; Shaheen, F.; Choudhary; Atta.-ur.-Rahman, M. I. *Chem. Biodivers.* **2005**, *2*, 487.
54. Silverstein, R. M; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; Wiley: New York, 1991.
55. Bowie, J. W.; Simons, B. K.; Donaghue, P. F.; Kallury, P. K. M. R. *Tetrahedron* **1969**, *25*, 3696.
56. Kallury, R. K. M. R.; Rao, P. L. K. M. *Org. Mass Spectr.* **1977**, *12*, 411.
57. Gasco, A. M.; Fruttero, R.; Sorba, G.; Gasco, A. *Liebigs Ann. Chem.* **1991**, 1211.
58. 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.

59. Winter, C. A.; Risely, E. A.; Nuss, G. W. *Proc. Soc. Biol. Med.* **1962**, *111*, 544.
60. De Andrade, S. F.; Lemosa, M.; Comunello, E.; Noldin, V. F.; Filho, V. C.; Niero, R. *J. Ethnopharmacol.* **2007**, *113*, 252.
61. Perini, R.; Fiorucci, S.; Wallace, J. L. *Can. J. Gastroenterol.* **2004**, *18*, 229.
62. Bastaki, S. M.; Wallace, J. L. *Can. J. Gastroenterol.* **1999**, *13*, 123.
63. Velázquez, C. A.; Praveen Rao, P. N.; Citro, M. L.; Keefera, L. K.; Knaus, E. *Bioorg. Med. Chem.* **2007**, *15*, 4767.
64. Tsikas, D. *J. Chromatogr., B* **2007**, *851*, 51.
65. Gartner, L. P.; Haitt, J. L. *Color Textbook of Histology*, 2nd ed.; W. B. Saunders Company: Philadelphia, London, New York, 2001.