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Short communication

# Synthesis and investigation of anti-inflammatory activity and gastric ulcerogenicity of novel nitric oxide-donating pyrazoline derivatives

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ABSTRACT

A group of 3,5-diaryl-2-pyrazoline derivatives were prepared via the reaction of various chalcones with hydrazine hydrate in ethanol. A group of NO-donating-2-pyrazoline derivatives were synthesized by carrying a nitrate ester group or an oxime group onto the prepared pyrazoline derivatives through different spacers. The prepared compounds were evaluated for their anti-inflammatory activity using carrageenan-induced rat paw edema and compared to a well-known NSAID, indomethacin as a reference drug. The ability of the prepared compounds to induce gastric toxicity was also evaluated. Most of the prepared compounds showed significant anti-inflammatory activity at the injected dose (100 mg/kg) but they were safer than indomethacin in regard to gastric toxicity. The incorporation of the NO-donating group into the parent pyrazoline derivatives caused a non-significant reduction in the anti-inflammatory activity while a marked decrease in gastric ulcerations induced by their parent pyrazolines was observed.


## 1. Introduction

Non-steroidal 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 is by the inhibition of cyclo-oxygenase-derived prostaglandin synthesis, which is also responsible for the gastrointestinal [2–6], renal [7–9], and hepatic [10] side effects 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]. NO contributes to the modulation of several key physiological functions in the digestive system [12], it has the ability to increase the mucosal blood flow [13], resulting in enhanced mucosal resistance to ulceration [14], NO also prevents adherence of leukocytes to the vascular endothelium [15], it is known to modulate gastroduodenal secretion of mucus [16] and bicarbonate [17], NO can profoundly influence the mucosal immune system [12], it also increases the ability of mucosal cells to undergo healing and repair of the existing ulcers [18]. NO is also known to spare the renal system mainly through stimulating the renal blood flow [19].

Moreover, 2-pyrazoline derivatives have been reported to exhibit various pharmacological activities such as antimicrobial [20–22], anti-inflammatory [23–25], and antihypertensive [26]. In addition, 1-unsubstituted-3,5-diaryl-2-pyrazolines were reported to exhibit human Acyl CoA cholesterol acyltransferase activity [27] as well as activity of low-density lipoprotein oxidation inhibitors [28]. In addition, 1,3,5-triaryl-2-pyrazolines were reported to possess antidepressant properties [29], and monoamine oxidase (MAO) inhibitory activities [30].

Promoted with the above-mentioned studies, the present study aimed at gathering the two bioactive entities (pyrazoline and NO) into one compact structure and evaluating their biological activities as anti-inflammatory agents with reduced gastric toxicity.

## 2. Chemistry

Chalcones (**6a–d**, Scheme 1) were synthesized by a base catalyzed Claisen–Schmidt condensation reaction of appropriately substituted acetophenones (**1–2**) and substituted aromatic aldehydes (**3–5**) in the presence of 10% NaOH in ethanol. Heating at reflux chalcones (**6a–d**) with hydrazine monohydrate 95% in absolute ethanol afforded the corresponding pyrazolines (**7a–d**). Treatment of pyrazolines (**7a–d**) with bromoacetyl bromide in the presence of $K_2CO_3$ at 0 °C afforded the corresponding 2-bromoacyl pyrazolines (**8a–d**). Other 2-acyl pyrazoline derivatives

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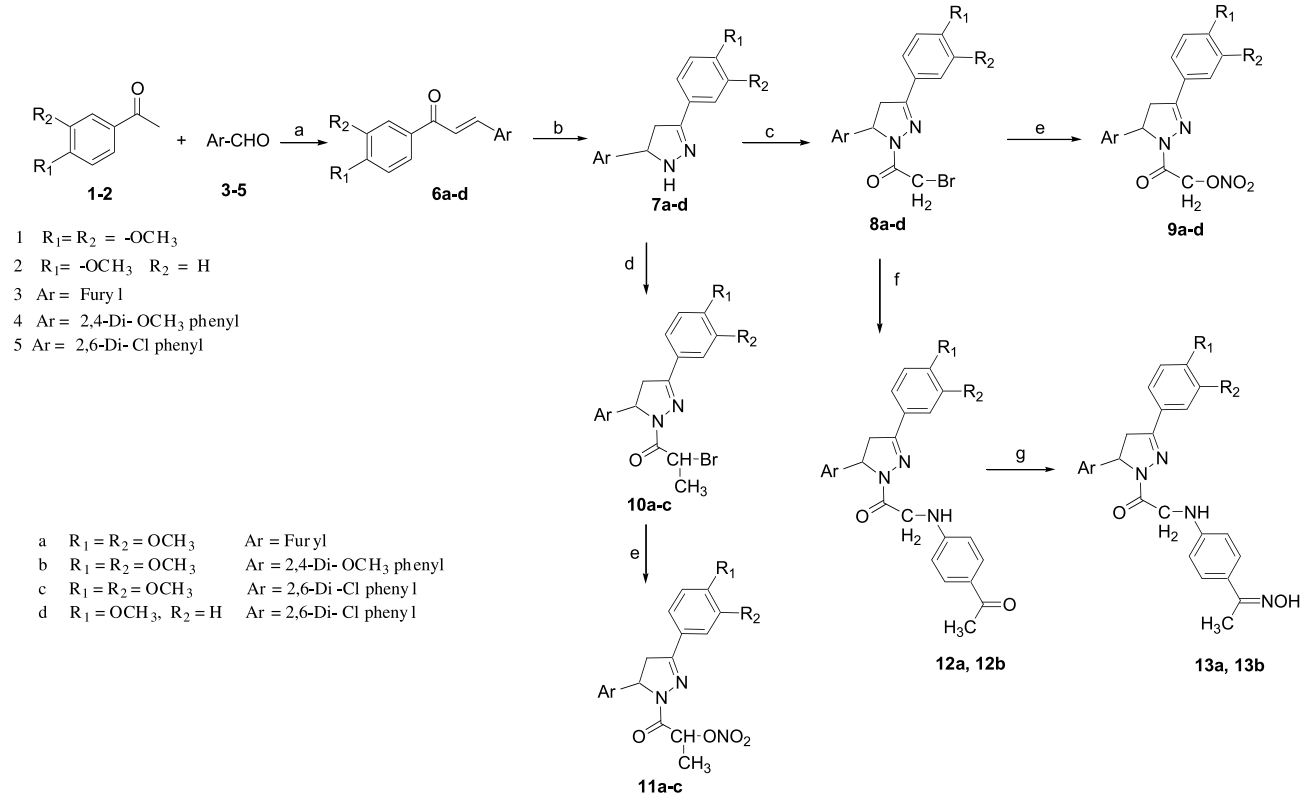



**Scheme 1.** Synthesis of different NO-donating pyrazoline derivatives **9a–d**, **11a–c**, **13a** and **13b**.

(**10a–c**, Scheme 1) were prepared by the reaction of pyrazolines (**7a–c**) with 2-bromopropionic acid mixed anhydride, which was prepared by the reaction of 2-bromopropionic acid with ethyl chloroformate in the presence of triethylamine in equimolar ratio in dry chloroform at −10 °C. The objective nitrate esters (**9a–d** and **11a–c**) were prepared by stirring the corresponding bromoacyl pyrazoline derivatives (**8a–d** and **10a–c**) with silver nitrate in acetonitrile for 15 h at 80 °C, which afforded the corresponding nitrate esters.

Heating at reflux of pyrazoline bromoacyl derivatives **8a** and **8b** with *p*-amino acetophenone in acetone in the presence of $K_2CO_3$ afforded the corresponding coupling products **12a** and **12b**, respectively. Heating at reflux of compounds **12a** and **12b** with hydroxylamine hydrochloride in absolute ethanol gave the corresponding oximes **13a** and **13b**, respectively. The structure of the prepared compounds was confirmed on the basis of their IR, $^1$H NMR, mass spectra and elemental analysis. The reaction sequences are outlined in Scheme 1.

## 3. NO release measurement

The NO-releasing properties of the tested compounds using different forms including: starting derivatives (**7a** and **8a**), NO-donating nitrates (**9a–d** and **11a–c**) and NO-donating oximes (**13a** and **13b**) were assessed in both phosphate buffer of pH 7.4 and pH 1 using 0.1 N HCl with Griess reagent. 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, calculated as the amount of NO (mol/mol) released and are listed in Table 1.

**Table 1**
The amount of NO released from tested compounds **7a**, **8a**, **9a–d**, **11a–c**, **13a** and **13b** in phosphate buffer (pH = 7.4)

| Compound no. | Amount of NO released (mol/mol) ± SEM | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| **7a** | – | – | – | – | – | – |
| **8a** | – | – | – | – | – | – |
| **9a** | 0.126 ± 0.050 | 0.211 ± 0.029 | 0.281 ± 0.037 | 0.483 ± 0.079 | 0.614 ± 0.082 | 0.535 ± 0.100 |
| **9b** | 0.329 ± 0.036 | 0.391 ± 0.020 | 0.433 ± 0.044 | 0.523 ± 0.047 | 0.780 ± 0.088 | 0.588 ± 0.012 |
| **9c** | 0.147 ± 0.031 | 0.395 ± 0.045 | 0.516 ± 0.047 | 0.555 ± 0.080 | 0.738 ± 0.086 | 0.625 ± 0.065 |
| **9d** | 0.195 ± 0.034 | 0.295 ± 0.068 | 0.356 ± 0.015 | 0.451 ± 0.060 | 0.656 ± 0.071 | 0.593 ± 0.074 |
| **11a** | 0.078 ± 0.013 | 0.190 ± 0.021 | 0.398 ± 0.041 | 0.200 ± 0.040 | 0.125 ± 0.012 | 0.087 ± 0.009 |
| **11b** | 0.098 ± 0.023 | 0.160 ± 0.027 | 0.438 ± 0.037 | 0.128 ± 0.028 | 0.065 ± 0.005 | 0.090 ± 0.007 |
| **11c** | 0.123 ± 0.017 | 0.190 ± 0.024 | 0.501 ± 0.027 | 0.255 ± 0.041 | 0.145 ± 0.015 | 0.114 ± 0.012 |
| **13a** | 0.118 ± 0.021 | 0.128 ± 0.023 | 0.138 ± 0.016 | 0.167 ± 0.032 | 0.277 ± 0.021 | 0.267 ± 0.030 |
| **13b** | 0.137 ± 0.030 | 0.114 ± 0.030 | 0.148 ± 0.082 | 0.194 ± 0.043 | 0.302 ± 0.024 | 0.268 ± 0.010 |

## 4. Biological activity

### 4.1. Anti-inflammatory activity

The synthesized new compounds **7a–d**, **8a–d**, **9a–d**, **10a–c**, **11a–c**, **13a** and **13b** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema described by Winter et al. [31]. The tested compounds and reference drug (indomethacin) were administered orally at a dose level of 100 mg/kg, 0.5 h 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 with the following formula [32].

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

where $V_R$ represents the mean right paw thickness, $V_L$ represents the mean left paw thickness, $(V_R - V_L)_{control}$ represents the mean increase in paw thickness in the control group of rats and $(V_R - V_L)_{treated}$ represents the mean increase in paw thickness in rats treated with the tested compounds. The results are listed in Table 2 and shown in Fig. 1A–D.

### 4.2. Ulcerogenic liability

The synthesized compounds **7a–d**, **8a–d**, **9a–d**, **10a–c**, **11a–c**, **13a** and **13b** were evaluated for their ulcerogenic activity according to a reported procedure [33]. Ulcerogenic activity was evaluated in rats after oral administration of the tested compounds and indomethacin at a dose level of 100 mg/kg in 0.5% aqueous CMC, 0.5% aqueous CMC was used as a control.

Ulcers were classified into levels: level I, in which the ulcer area is less than 1 $mm^2$; level II, in which the ulcer area is in the range from 1 to 3 $mm^2$; and level III, in which the ulcer area equals 3 $mm^2$ and more than 3 $mm^2$ and this is rated according to their areas in $mm^2$, and the following parameters were calculated [34]:

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

Where $UI_{treated}$ means the average of ulcer index of groups treated with the NO-donating derivatives and $UI_{protype}$ means the average of ulcer index of groups treated with the starting pyrazoline derivatives. The ulcer indexes of the tested compounds were calculated and listed in Table 2 and Fig. 2A–D.

**Table 2**
The anti-inflammatory activity at different time intervals and ulcer indexes of compounds **7a–d**, **8a–d**, **9a–d**, **10a–c**, **11a–c**, **13a** and **13b** using carrageenan-induced paw edema in rats compared to indomethacin

| Compound no. | Percent of edema inhibition (% mean ± SEM) | | | Ulcer index (mean ± SEM) |
|---|---|---|---|---|
| | 1 h | 2 h | 3 h | |
| Control | 0 | 0 | 0 | 1 ± 0.5 |
| Indomethacin | 58 ± 3.01*** | 67 ± 2.70*** | 82 ± 4.86*** | 43.5 ± 3.5 |
| **7a** | 45 ± 4.61*** | 53 ± 5.40*** | 60 ± 5.53*** | 26.5 ± 1.5* |
| **7b** | 38 ± 0.82*** | 39 ± 2.70*** | 42 ± 2.58*** | 7 ± 0.6** |
| **7c** | 26 ± 4.45* | 49 ± 2.70*** | 58 ± 7.62*** | 3 ± 1.0** |
| **7d** | 54 ± 3.04*** | 58 ± 2.70*** | 60 ± 2.58*** | 31 ± 3.0 |
| **8a** | 38 ± 4.44*** | 46 ± 1.60*** | 51 ± 2.58*** | 13 ± 1.0* |
| **8b** | 53 ± 3.01*** | 67 ± 3.62*** | 71 ± 1.29*** | 30 ± 3.0 |
| **8c** | 48 ± 6.01** | 58 ± 5.26*** | 69 ± 2.58*** | 3 ± 0.5** |
| **8d** | 43 ± 6.90** | 49 ± 2.70** | 47 ± 0.44** | 16 ± 1.0* |
| **9a** | 32 ± 4.32*** | 37 ± 2.34*** | 43 ± 2.14*** | 2 ± 0.3** |
| **9b** | 38 ± 1.65*** | 48 ± 4.77*** | 58 ± 3.95*** | 5 ± 0.5** |
| **9c** | 40 ± 4.61*** | 49 ± 6.03*** | 58 ± 3.87*** | 4 ± 0.3** |
| **9d** | 34 ± 2.49** | 42 ± 4.10*** | 52 ± 2.98*** | 0 ± 0.0** |
| **10a** | 22 ± 1.65* | 36 ± 3.62*** | 42 ± 3.46*** | 10 ± 0.5** |
| **10b** | 58 ± 4.25*** | 63 ± 5.40*** | 69 ± 2.58*** | 23 ± 2.0* |
| **10c** | 51 ± 7.52*** | 56 ± 3.77*** | 60 ± 5.76*** | 10 ± 1.0** |
| **11a** | 35 ± 3.78*** | 39 ± 3.04*** | 42 ± 4.46** | 7 ± 1.0** |
| **11b** | 44 ± 5.51*** | 61 ± 5.80*** | 56 ± 4.94*** | 3 ± 0.1** |
| **11c** | 47 ± 5.62*** | 53 ± 3.82*** | 55 ± 2.30*** | 7 ± 0.0** |
| **13a** | 38 ± 0.82*** | 46 ± 3.63*** | 46 ± 0.44*** | 0 ± 0.0** |
| **13b** | 27 ± 6.01 | 44 ± 3.44** | 46 ± 7.29*** | 2 ± 1.0** |

*Significantly different from control group at $P = 0.05$; **significantly different from control group at $P = 0.01$; ***significantly different from control group at $P = 0.001$.

## 5. Results and discussions

The IR spectra of the prepared pyrazolines (**7a–d**) showed $\nu$ (C=N) stretching at 1611–1599 $cm^{-1}$ due to the ring closure. In addition, absorption bands at 1115–1261 $cm^{-1}$ were attributed to the $\nu$ (C–N) stretching vibrations, which also confirm the formation of the desired pyrazoline ring in all prepared compounds. A sharp band in the region of 3226–3334 $cm^{-1}$ due to the $\nu$ (NH) stretching was also observed. $^1$H NMR spectra recorded for the prepared compounds in $CDCl_3$ clearly supported the proposed structures. Protons of pyrazoline ring in compounds **7a** and **7b** showed a prominent ABX system, with protons Ha, Hb and Hx seen as doublets of doublets at $\delta$ 3.20–3.44, 3.40–3.53 and 5.04–5.27 ppm ($J_{Ha-Hb}$ = 16.50–16.70, $J_{Ha-Hx}$ = 7.30–7.90, $J_{Hb-Hx}$ = 9.90–10.20 Hz), respectively. On the other hand, pyrazolines (**7c** and **7d**) with substituted benzaldehyde carrying highly electronegative groups (2,6-dichloro) made Ha and Hb that appeared to be equivalent so the pattern became AX system. The $CH_2$ protons appeared as doublet at $\delta$ 3.24 and 3.44 ppm while the CH proton appeared as triplet at $\delta$ 5.80 ppm ($J$ = 11.90–12.40 Hz). The protons belonging to the aromatic system and phenyl substituents were observed at the expected chemical shifts and integral values.

Treatment of pyrazolines (**7a–d**) with bromoacetyl bromide in the presence of $K_2CO_3$ afforded the corresponding 2-bromoacyl pyrazolines (**8a–d**), the structure of the prepared compounds was confirmed by the appearance of an additional strong absorption band of (C=O) stretching at 1653–1665 $cm^{-1}$ and disappearance of that of NH stretching in IR spectra, while $^1$H NMR spectra showed an additional two doublet peaks each for one proton of the $CH_2$ protons nearly at $\delta$ 4.3 and 4.4 ppm with coupling constant ($J$ = 11.40–12.00 Hz) due to geminal coupling.

Another form of 2-bromoacyl pyrazolines (**10a–c**) was prepared by the reaction of pyrazolines (**7a–c**) with 2-bromopropionic acid mixed anhydride. The IR spectra of the prepared compounds showed an additional strong absorption band of (C=O) stretching at 1656–1687 $cm^{-1}$ and disappearance of the NH stretching band of pyrazoline. $^1$H NMR spectra showed prominent doublet signals nearly at $\delta$ 1.69 ppm indicating the three protons of $CH_3$, and quartet signals of the CH proton nearly at $\delta$ 5.39 ppm.

The objective nitrate esters (**9a–d** and **11a–c**) were prepared by stirring the corresponding bromoacyl pyrazolines (**8a–d** and **10a–c**) with silver nitrate in acetonitrile. The IR spectra of the prepared compounds showed additional absorption bands at 1536–1653 $cm^{-1}$ due to asymmetric stretching of $NO_2$ group and another strong band at 1256–1276 $cm^{-1}$ due to $NO_2$ group symmetrical stretching. A characteristic downfield shift of the $CH_2$ protons attached to the nitrate ester was observed by nearly 1 ppm to appear at $\delta$ 5.37–6.09 ppm in the $^1$H NMR spectra of the prepared nitrates.

Heating at reflux of bromoacyl pyrazoline derivatives **8a** and **8b** with *p*-amino acetophenone followed by refluxing with hydroxylamine hydrochloride in absolute ethanol gave the corresponding

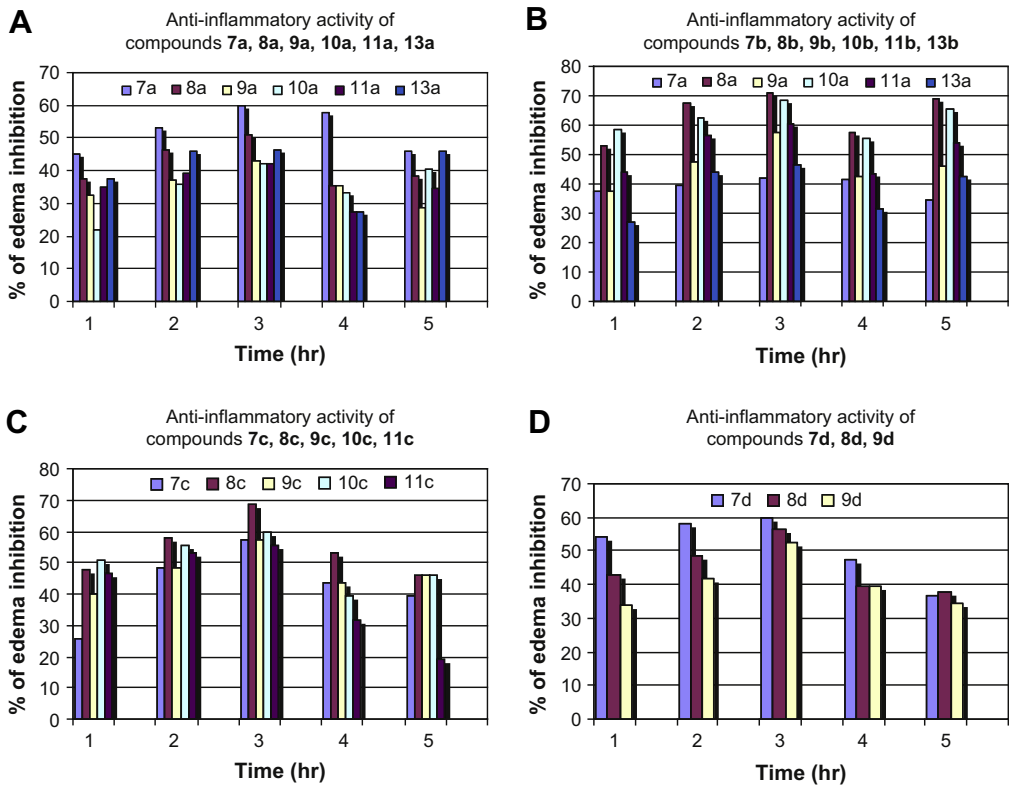
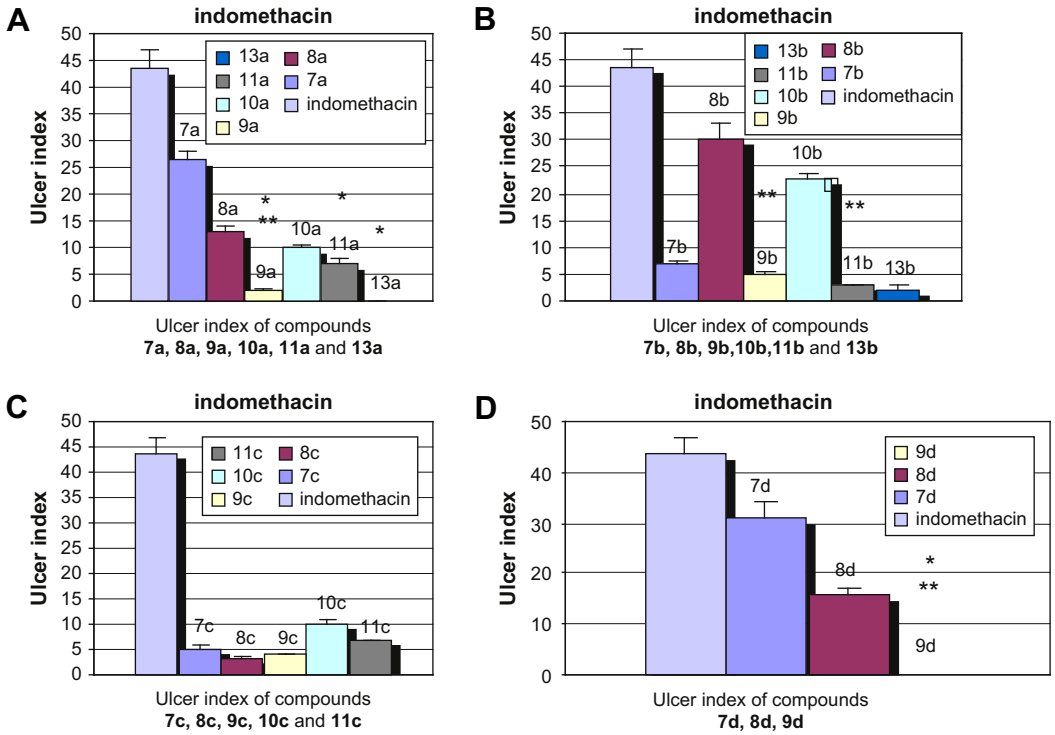



**Fig. 1.** Anti-inflammatory activity of different tested compounds expressed as mean ± S.E.

oximes (**13a** and **13b**). The disappearance of ketonic (C═O) stretching band and appearance of the (C═N) stretching band at 1606 $cm^{-1}$ in addition to the appearance of the(OH) stretching band at 3491 $cm^{-1}$ in the IR spectra confirmed the proposed structure. Mass spectra and elemental analysis are in agreement with the proposed structure of the prepared compounds.

The results of NO-releasing properties of the tested compounds **7a**, **8a**, **9a–d**, **11a–c**, **13a** and **13b** indicated that the starting




* Significantly different fromparent pyrazoline at P < 0.001

** Significantly different fromits acetyl derivative at P < 0.001

**Fig. 2.** Ulcer indexes of different tested compounds compared to indomethacin expressed as mean ± S.E.

pyrazoline derivative **7a** and its bromoacyl derivative **8a** did not release any amount of NO neither at phosphate buffer of pH 7.4 nor at pH 1 and this is a proof that the nitrates and oximes are the main sources of NO release.

Both forms of NO-donating derivatives including nitrate esters (**9a–d** and **11a–c**) and NO-donating oximes **13a** and **13b** were found to release different amounts of NO at phosphate buffer of pH 7.4 (Table 1). For the first group of NO-donating nitrate esters **9a–d**, there was a regular increase in the amount of NO released reaching their maximum after 5 h and then begin to decrease gradually in the sixth hour. The NO-donating oximes **13a** and **13b** were found to release fewer amount of NO compared to the previous nitrate esters **9a–d** reaching their maximum after 5 h. The last group of nitrate esters **11a–c** were found to release a moderate amount of NO reaching their maximum after 3 h and then begin to decrease gradually in the fourth hour. This may be attributed to the electron donating properties of the methyl group in the propionic acid spacer present in compounds **11a–c** which decrease the dissociation of the nitrite group giving lower amount of NO compared to the acetic acid spacer present in compounds **9a–d**. On the other hand there is no release of NO at pH 1 and this may support the fact that NO-donating moieties (nitrates and oximes) are weakly hydrolyzed in the gastric lumen and this confirms that the suggested gastroprotective action of NO is mediated systemically [35,36].

The synthesized new compounds **7a–d**, **8a–d**, **9a–d**, **10a–c**, **11a–c**, **13a** and **13b** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema described by Winter et al. The results show that most of the synthesized compounds showed significant anti-inflammatory activity against carrageenan-induced paw edema in rats after 3 h, which is the time necessary to reach the maximum activity for the tested compounds. The tested pyrazoline derivatives **7a–c** showed considerable anti-inflammatory activity, which is nearly equal to 75% of indomethacin activity. Introduction of an acetyl moiety into compounds **8a–d** and **10a–c** was found to increase the anti-inflammatory activity in most compounds. Compounds **8b** and **10b** induced equipotent activity relative to indomethacin for the first 2 h and reached about 86% after 3 h. Compound **8c** showed 84% of indomethacin activity while compound **10c** showed 86% in the first hour and began to decrease gradually to reach to 73% after 3 h. On the other hand compounds **8a** and **8d** showed inhibitory activity of about 70% of indomethacin after 2 h and began to decrease gradually in the third hour to reach about 58%. The anti-inflammatory activity of the designed NO donors **9a–d**, **11a–c**, **13a** and **13b** indicated that NO donors **9b**, **9c**, **11b** and **11c** showed considerable anti-inflammatory activity, which ranges from 67% to 70% of indomethacin activity. The anti-inflammatory activity of the oxime NO donors **13a** and **13b** was found to be 56% and 59% of indomethacin activity, respectively. These results indicated that the incorporation of NO-donating group into the pyrazoline nucleus had caused a non-significant reduction in the anti-inflammatory activity (Fig. 1A–D).

The synthesized compounds **7a–d**, **8a–d**, **9a–d**, **10a–c**, **11a–c**, **13a** and **13b** were evaluated for their ulcerogenic activity. The results indicated that there is a direct relationship between the anti-inflammatory activity and the ulcerogenic toxicity of the tested compounds. The starting pyrazoline derivatives and their bromoacyl intermediates caused a milder ulcerogenic toxicity compared to indomethacin, while the NO-donating pyrazolines were considered to be safe in regard to gastric toxicity having 0–7 range of ulcer index (Table 2). The percentage inhibition of ulcers (cure ratio) was calculated and revealed that oxime **13a** exhibited 96% and 92% reduction of ulcers induced by its starting pyrazoline derivative **7a** and its bromoacyl intermediate **8a**, respectively, while the nitrate ester **9a** exhibited 92% and 84% reduction of ulcers induced by its starting pyrazoline derivative **7a** and its bromoacyl intermediate **8a**, respectively. On the other hand the NO-donating nitrate **11a** showed 73% reduction of ulcers induced by its starting pyrazoline derivative **7a**, and only 30% reduction of ulcers induced by its bromoacyl intermediate **10a**. Also, oxime **13b** indicated 71% and 93% reduction of ulcers induced by its starting pyrazoline derivative **7b** and its bromoacyl intermediate **8b**, respectively. While NO-donating compound **11b** exhibited 57% and 86% reduction of ulcers induced by its starting pyrazoline derivative **7b** and its bromoacyl intermediate **10b**, respectively. On the other hand the NO-donating derivative **9b** indicated 28% and 83% reduction of ulcers induced by its starting pyrazoline derivative **7b** and its bromoacyl intermediate **8b**, respectively. The NO-donating nitrate **9d** showed 96% and 93% reduction of ulcers induced by its starting pyrazoline derivative **7d** and its bromoacyl intermediate **8d**, respectively (Fig. 2A–D).

The decreased gastric toxicity of the targeted NO-donating derivatives **9a–d**, **11a–c**, **13a** and **13b** relative to their starting compounds **7a–d**, **8a–d** and **10a–c**, could be explained by the release of NO that increases mucosal blood flow resulting in enhanced mucosal resistance to ulceration [37,38] and/or an enhanced ability of the intact NO-donating derivatives to cross the gastric mucosal lining prior to the subsequent release of NO and starting pyrazoline derivatives [11].

In conclusion the use of hybrid molecules containing NO-releasing moieties looks as a promising approach to improve the safety of NSAIDs without altering their effectiveness.

## 6. Experimental

### 6.1. Chemistry

Reactions were monitored by TLC analysis using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) with $F_{254}$ indicator thin layer plates. Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected.

IR spectra were recorded as KBr disks on a Bruker Vector 22 IR spectrophotometer. $^1$H NMR spectra were carried out on 300 MHz Mercury 300BB NMR spectrophotometer, on 200 MHz GEMINI-200 NMR spectrophotometer and on 60 MHz Varian EM-360L NMR spectrophotometer using TMS as an internal reference. Chemical shift ($\delta$) values are given in parts per million (ppm) relative to $CDCl_3$ (7.29) or DMSO-$d_6$ (2.5) 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.

Accurate masses were obtained on Micromass LCT mass spectrometer and on HP mass spectrometer. Elemental analysis was performed on Perkin Elmer 2400 CHN Elemental Analyzer and on Vario EL III, Elemental Analyzer, GmbH. D-63452 Hanau.

#### 6.1.1. Chalcones (*6a–d*)

Chalcones (**6a–d**) were prepared according to reported procedure [39].

#### 6.1.2. General procedure for the preparation of compounds *7a–d* [40]

A mixture of appropriate chalcones **6a–d** (10.0 mmol) and hydrazine monohydrate (95%) (1 g, 20 mmol) was heated at reflux for 6–10 h in 50 mL of absolute ethanol. The solution was left to cool at room temperature and the solid formed was filtered off, washed with water, dried and recrystallized from absolute ethanol.

*6.1.2.1. 5-(2-Furyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1*H*-pyrazole (**7a**).* Pale yellow crystals (1.82 g, 66.8% yield), m.p. 150–151 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3226 (NH), 3045–3004 (Ar-CH), 2960–2830 (Aliph-CH), 1599 (C=N), 1567 (C=C). $^1$H NMR (300 MHz, $CDCl_3$)

δ (ppm): 3.30 (dd, 1H, $J = 16.50$ and 9.86 Hz, $CH_2$ of pyrazoline), 3.40 (dd, 1H, $J = 16.50$ and 7.32 Hz, $CH_2$ of pyrazoline), 3.84 (s, 6H, 2 $OCH_3$), 5.05 (dd, 1H, $J = 9.85$ and 7.40 Hz, CH of pyrazoline), 6.26 (d, 2H, $J = 7.33$ Hz, Ar-H), 6.79 (d, 1H, $J = 8.43$ Hz, Ar-H), 7.04 (d, 1H, $J = 8.43$ Hz, Ar-H), 7.20 (s, 1H, NH), 7.30 (s, 1H, Ar-H), 7.38 (d, 1H, $J = 1.47$ Hz, Ar-H). MS: *m/z* (%): 272 (100) [$M^+$], 271 (12), 154 (26), 137 (14), 136 (18). Anal. calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.10; H, 5.92; N, 10.14.

*6.1.2.2. 4,5-Dihydro-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**7b**).* Pale yellow crystals (2.44 g, 71.5% yield), m.p. 120–121 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3334 (NH), 3076–3002 (Ar-CH), 2960–2834 (Aliph-CH), 1611 (C═N), 1566 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.20 (dd, 1H, $J = 16.68$ and 7.90 Hz, $CH_2$ of pyrazoline), 3.54 (dd, 1H, $J = 16.60$ and 10.05 Hz, $CH_2$ of pyrazoline), 3.76 (s, 3H, $OCH_3$), 3.78 (s, 3H, $OCH_3$), 3.89 (s, 6H, 2 $OCH_3$), 5.27 (dd, 1H, $J = 9.90$ and 8.00 Hz, CH of pyrazoline), 6.40 (d, 1H, $J = 2.57$ Hz, Ar-H), 6.43 (s, 1H, Ar-H), 6.83 (d, 1H, $J = 8.43$ Hz, Ar-H), 7.11 (dd, 1H, $J = 8.25$ and 2.02 Hz, Ar-H), 7.24 (d, 1H, $J = 8.10$ Hz, Ar-H), 7.47 (d, 1H, $J = 2.20$ Hz, Ar-H). MS: *m/z* (%): 342 (100) [$M^+$], 341 (21), 326 (7), 164 (7), 154 (5). Anal. calcd for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.57; H, 6.44; N, 7.79.

*6.1.2.3. 5-(2,6-Dichlorophenyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**7c**).* Yellow crystals (2.42 g, 69% yield), m.p. 130–131 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3291 (NH), 3076–2999 (Ar-CH), 2941–2837 (Aliph-CH), 1599 (C═N), 1568 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.46 (d, 2H, $J = 11.90$ Hz, $CH_2$ of pyrazoline), 3.91 (s, 3H, $OCH_3$), 3.93 (s, 3H, $OCH_3$), 5.80 (t, 1H, $J = 11.91$ Hz, CH of pyrazoline), 6.86 (d, 1H, $J = 8.43$ Hz, Ar-H), 7.03 (dd, 1H, $J = 8.25$ and 2.01 Hz, Ar-H), 7.15 (dd, 1H, $J = 8.80$ and 1.47 Hz, Ar-H), 7.33 (d, 2H, $J = 8.06$ Hz, Ar-H), 7.47 (d, 1H, $J = 2.20$ Hz, Ar-H), 7.60 (br s, 1H, NH). MS: *m/z* (%): 354 (11.0) [M + 4], 352 (72) [M + 2], 350 (100) [$M^+$], 205 (86), 189 (32), 163 (44), 77 (37). Anal. calcd for $C_{17}H_{16}Cl_2N_2O_2$: C, 58.13; H, 4.59; N, 7.98. Found: C, 58.19; H, 4.62; N, 7.87.

*6.1.2.4. 5-(2,6-Dichlorophenyl)-4,5-dihydro-3-(4-methoxyphenyl)-1H-pyrazole (**7d**).* Brown crystals (2.03 g, 63.5% yield), m.p. 120–121 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3264 (NH), 3075–3001 (Ar-CH), 2954–2832 (Aliph-CH), 1602 (C═N), 1561 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.44 (d, 2H, $J = 12.04$ Hz, $CH_2$ of pyrazoline), 3.84 (s, 3H, $OCH_3$), 5.78 (t, 1H, $J = 11.90$ Hz, CH of pyrazoline), 6.90 (d, 2H, $J = 9.10$ Hz, Ar-H), 7.12–7.17 (m, 2H, Ar-H), 7.32 (d, 1H, $J = 8.06$ Hz, Ar-H), 7.63 (d, 2H, $J = 8.80$ Hz, Ar-H), 8.00 (br s, 1H, NH). MS: *m/z* (%): 324 (6) [M + 4], 322 (38) [M + 2], 320 (63) [$M^+$], 175 (100), 133 (66), 77 (25). Anal. calcd for $C_{16}H_{14}Cl_2N_2O$: C, 59.83; H, 4.39; N, 8.72. Found: C, 59.88; H, 4.09; N, 8.50.

### 6.1.3. General procedure for the preparation of compounds **8a–d** [41]

To a stirred solution of **7a–d** (4.20 mmol) in dichloromethane in an ice bath, a solution of $K_2CO_3$ (0.868 g, 6.30 mmol) in 100 mL water was added. To this solution bromoacetyl bromide (0.928 g, 4.60 mmol) in 20 mL of dichloromethane was added in a drop wise manner while stirring over 30 min; the mixture was left for stirring for further 2 h at 0 °C and 24 h at room temperature. The organic layer was separated and the aqueous layer was extracted with (2 × 30 mL) $CH_2Cl_2$, the combined organic layer was washed consequently with distilled water (2 × 20 mL), 1 N HCl (2 × 20 mL) and again with distilled water (2 × 20 mL); the organic layer was dried over anhydrous sodium sulfate, evaporated under reduced pressure and the obtained crude product was recrystallized from absolute ethanol.

*6.1.3.1. 1-(2-Bromoacetyl)-5-(2-furyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**8a**).* Pale brown crystals (1.42 g, 86.2% yield), m.p. 147–148 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3068–3004 (Ar-CH), 2965–2842 (Aliph-CH), 1653 (C═O), 1597 (C═N), 1570 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.48 (dd, 1H, $J = 17.30$ and 5.77 Hz, $CH_2$ of pyrazoline), 3.60 (dd, 1H, $J = 17.30$ and 11.55 Hz, $CH_2$ of pyrazoline), 3.94 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$), 4.30 (d, 1H, $J = 11.54$ Hz, $CH_2$), 4.40 (d, 1H, $J = 11.54$ Hz, $CH_2$), 5.67 (dd, 1H, $J = 11.50$ and 5.70 Hz, CH of pyrazoline), 6.29–7.45 (m, 6H, Ar-H). MS: *m/z* (%): 394 (79) [M + 2], 392 (80) [$M^+$], 271 (100), 243 (27), 128 (30), 121 (23), 77 (20). Anal. calcd for $C_{17}H_{17}BrN_2O_4$: C, 51.92; H, 4.36; N, 7.12. Found: C, 51.92; H, 4.40; N, 7.06.

*6.1.3.2. 1-(2-Bromoacetyl)-4,5-dihydro-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**8b**).* Pale orange powder (1.81 g, 92.5% yield), m.p. 148–149 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3062–2998 (Ar-CH), 2958–2835 (Aliph-CH), 1658 (C═O), 1598 (C═N), 1514 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.07 (dd, 1H, $J = 16.36$ and 5.45 Hz, $CH_2$ of pyrazoline), 3.68 (dd, 1H, $J = 16.30$ and 12.10 Hz, $CH_2$ of pyrazoline), 3.77 (s, 3H, $OCH_3$), 3.80 (s, 3H, $OCH_3$), 3.92 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$), 4.32 (d, 1H, $J = 12.00$ Hz, $CH_2$), 4.43 (d, 1H, $J = 12.00$ Hz, $CH_2$), 5.74 (dd, 1H, $J = 12.00$ and 5.50 Hz, CH of pyrazoline), 6.38–7.44 (m, 6H, Ar-H); MS: *m/z* (%): 464 (35) [M + 2], 463 (9) [M + 1], 462 (38) [$M^+$], 342 (34), 341 (100), 121 (36.5), 139 (28), 93 (25), 77 (30). Anal. calcd for $C_{21}H_{23}BrN_2O_5$: C, 54.44; H, 5.00; N, 6.05. Found: C, 54.47; H, 5.05; N, 5.93.

*6.1.3.3. 1-(2-Bromoacetyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**8c**).* White crystals (1.73 g, 87.5% yield), m.p. 167–168 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3070–3001 (Ar-CH), 2962–2840 (Aliph-CH), 1688 (C═O), 1599 (C═N), 1565 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.45 (dd, 1H, $J = 18.00$ and 9.00 Hz, $CH_2$ of pyrazoline), 3.81 (dd, 1H, $J = 18.00$ and 12.00 Hz, $CH_2$ of pyrazoline), 3.95 (s, 3H, $OCH_3$), 3.98 (s, 3H, $OCH_3$), 4.33 (d, 1H, $J = 11.44$ Hz, $CH_2$), 4.43 (d, 1H, $J = 11.40$ Hz, $CH_2$), 6.25 (dd, 1H, $J = 12.00$ and 9.00 Hz, CH of pyrazoline), 6.85–7.47 (m, 6H, Ar-H). MS: *m/z* (%): 476 (15) [M + 4], 475 (15) [M + 3], 474 (43.0) [M + 2], 473 (26.0) [M + 1], 472 (100) [$M^+$], 471 (17.0) [M − 1], 471 (60) [M − 2], 437 (40), 350 (60), 271 (48), 205 (83), 163 (64), 121 (87), 93 (39), 77 (24). Anal. calcd for $C_{19}H_{17}BrCl_2N_2O_3$: C, 48.33; H, 3.63; N, 5.93. Found: C, 48.39; H, 3.62; N, 5.81.

*6.1.3.4. 1-(2-Bromoacetyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-3-(4-methoxyphenyl)-1H-pyrazole (**8d**).* Yellowish white crystals (1.45 g, 79.0% yield), m.p. 159–160 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3084–2989 (Ar-CH), 2964–2838 (Aliph-CH), 1656 (C═O), 1602 (C═N), 1562 (C═C). $^1H$ NMR (300 MHz, $CDCl_3$) δ (ppm): 3.32 (dd, 1H, $J = 18.00$ and 9.00 Hz, $CH_2$ of pyrazoline), 3.68 (dd, 1H, $J = 18.00$ and 12.55 Hz, $CH_2$ of pyrazoline), 3.85 (s, 3H, $OCH_3$), 4.30 (d, 1H, $J = 12.00$ Hz, $CH_2$), 4.40 (d, 1H, $J = 12.00$ Hz, $CH_2$), 6.21 (dd, 1H, $J = 12.60$ and 9.00 Hz, CH of pyrazoline), 6.90–7.85 (m, 7H, Ar-H). MS: *m/z* (%): 444 (29) [M + 2], 443 (7) [M + 1], 442 (73) [$M^+$], 441 (19) [M − 1], 440 (47), 407 (72), 405 (46), 322 (56), 175 (100), 144 (92), 121 (63), 77 (32). Anal. calcd for $C_{18}H_{15}BrCl_2N_2O_2$: C, 48.90; H, 3.42; N, 6.34. Found: C, 48.87; H, 3.44; N, 6.28.

### 6.1.4. General procedure for the preparation of compounds **10a–c** [42]

To a stirred solution of 2-bromopropionic acid (0.76 g, 5 mmol) in 30 mL dry chloroform at −10 °C, triethylamine (0.5 g, 5 mmol) was added, followed by ethyl chloroformate (0.54 g, 5 mmol) in a drop wise manner, the mixture was stirred for 30 min, and then the corresponding pyrazolines **7a–c** were added. The mixture was stirred for additional 12 h at room temperature, the solvent was evaporated under reduced pressure and the crude product was dissolved in 30 mL chloroform and washed consequently with 1 N HCl (2 × 20 mL), distilled water (2 × 30 mL), 5% $NaHCO_3$ solution (2 × 20 mL), distilled water (2 × 30 mL) and finally with brine

(2 × 30 mL). The organic layer was dried over anhydrous sodium sulfate, and evaporated under reduced pressure; the product was collected and recrystallized from methanol.

*6.1.4.1. 1-(2-Bromopropionyl)-5-(2-furyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**10a**).* Yellowish brown crystals (1.40 g, 69.0% yield), m.p. 135–136 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3111–2965 (Ar-CH), 2937–2837 (Aliph-CH), 1687 (C=O), 1601 (C=N), 1516 (C=C). $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 1.39 (d, 3H, $J$ = 8.40 Hz, $CH_3$), 3.65–3.95 (m, 2H, $CH_2$ of pyrazoline), 4.30 (s, 6H, 2 $OCH_3$), 4.60 (q, 1H, $J$ = 8.40 Hz, CH), 5.95 (dd, 1H, $J$ = 11.00 and 6.20 Hz, CH of pyrazoline), 6.70–8.10 (m, 6H, Ar-H). MS: *m/z* (%): 408 (33) [M + 2], 406 (34) [$M^+$], 344 (64), 272 (100), 255 (52), 243 (62), 122 (34), 77 (24). Anal. calcd for $C_{18}H_{19}BrN_2O_4$: C, 53.08; H, 4.70; N, 6.88. Found: C, 53.10; H, 4.71; N, 7.11.

*6.1.4.2. 1-(2-Bromopropionyl)-4,5-dihydro-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**10b**).* White powder (1.61 g, 67.50% yield), m.p. 208–209 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3066–2998 (Ar-CH), 2968–2838 (Aliph-CH), 1657 (C=O), 1599 (C=N), 1521 (C=C). $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 1.85 (d, 3H, $J$ = 7.80 Hz, $CH_3$), 3.20–3.90 (m, 2H, $CH_2$ of pyrazoline), 4.05 (s, 6H, 2 $OCH_3$), 4.25 (s, 6H, 2 $OCH_3$), 5.70–6.20 (m, 2H, $CH(CH_3)$ and CH of pyrazoline), 7.00–8.20 (m, 6H, Ar-H). MS: *m/z* (%): 478 (22) [M + 2], 476 (26) [$M^+$], 341 (100), 154 (35), 121 (22), 77 (34). Anal. calcd for $C_{22}H_{25}BrN_2O_5$: C, 55.35; H, 5.28; N, 5.87. Found: C, 55.00; H, 5.31; N, 5.73.

*6.1.4.3. 1-(2-Bromopropionyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**10c**).* White crystals (1.75 g, 72.30% yield), m.p. 150–151 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3086–2974 (Ar-CH), 2946–2835 (Aliph-CH), 1656 (C=O), 1602 (C=N), 1515 (C=C). $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.69 (d, 3H, $J$ = 6.80 Hz, $CH_3$), 3.35 (dd, 1H, $J$ = 17.10 and 8.90 Hz, $CH_2$ of pyrazoline), 3.71 (dd, 1H, $J$ = 17.30 and 12.90 Hz, $CH_2$ of pyrazoline), 3.91 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$), 5.39 (q, 1H, $J$ = 6.70 Hz, CH), 6.17 (dd, 1H, $J$ = 13.10 and 8.90 Hz, CH of pyrazoline), 6.83–7.55 (m, 6H, Ar-H). MS: *m/z* (%): 488 (6) [M + 4], 486 (13) [M + 2], 484 (18) [$M^+$], 422 (71), 350 (22), 205 (41), 163 (56), 77 (24), 63 (100). Anal. calcd for $C_{20}H_{19}BrCl_2N_2O_3$: C, 49.41; H, 3.94; N, 5.76. Found: C, 49.43; H, 3.95; N, 6.06.

*6.1.5. General procedure for the preparation of compounds **9a–d** and **11a–c** [43]*

To a stirred solution of the bromoacetyl derivatives **8a–d** and **10a–c** (10.00 mmol) in 20 mL acetonitrile, silver nitrate (6.80 g, 40.00 mmol) was added, the mixture was heated for 13–17 h at 80 °C, the formed precipitate of silver bromide was filtered off. The filtrate was evaporated till dryness, and dissolved in dichloromethane; the organic layer was washed consequently with distilled water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated under reduced pressure and the crude product was recrystallized from methanol.

*6.1.5.1. 5-(2-Furyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1-(2-nitrooxyacetyl)-1H-pyrazole (**9a**).* White crystals (1.18 g, 63% yield), m.p. 155–156 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3119–3020 (Ar-CH), 2967–2841 (Aliph-CH), 1678 (C=O), 1641 ($NO_2$), 1601 (C=N), 1516 (C=C), 1258 ($NO_2$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 3.49 (dd, 1H, $J$ = 16.00 and 12.00 Hz, $CH_2$ of pyrazoline), 3.62 (dd, 1H, $J$ = 16.00 and 8.00 Hz, $CH_2$ of pyrazoline), 3.94 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$), 5.38 (d, 1H, $J$ = 16.00 Hz, $CH_2$), 5.44 (d, 1H, $J$ = 16.00 Hz, $CH_2$), 5.68 (dd, 1H, $J$ = 12.00 and 8.00 Hz, CH of pyrazoline), 6.33 (d, 1H, $J$ = 4.00 Hz, Ar-H), 6.37 (d, 1H, $J$ = 4.00 Hz, Ar-H), 6.90 (d, 1H, $J$ = 8.00 Hz, Ar-H), 7.19 (dd, 1H, $J$ = 8.00 and 4.00 Hz, Ar-H), 7.31 (d, 1H, $J$ = 4.00 Hz, Ar-H), 7.39 (s, 1H, Ar-H). MS: *m/z* (%): 375 (8) [$M^+$], 374 (11) [M − 1], 272 (25), 162 (20), 122 (76), 121 (100), 93 (23), 77 (31). Anal. calcd for $C_{17}H_{17}N_3O_7$: C, 54.40; H, 4.57; N, 11.20. Found: C, 54.33; H, 4.21; N, 10.61.

*6.1.5.2. 4,5-Dihydro-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1-(2-nitrooxyacetyl)-1H-pyrazole (**9b**).* White powder (1.47 g, 65.80% yield), m.p. 153–154 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3079–3000 (Ar-CH), 2962–2836 (Aliph-CH), 1682 (C=O), 1653 ($NO_2$), 1617 (C=N), 1514 (C=C), 1256 ($NO_2$). $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 3.35 (dd, 1H, $J$ = 17.10 and 7.10 Hz, $CH_2$ of pyrazoline), 4.00 (dd, 1H, $J$ = 17.10 and 11.40 Hz, $CH_2$ of pyrazoline), 4.10 (s, 6H, 2 $OCH_3$), 4.30 (s, 6H, 2 $OCH_3$), 5.95 (s, 2H, $CH_2$), 6.20 (dd, 1H, $J$ = 11.40 and 7.10 Hz, CH of pyrazoline), 6.90–8.00 (m, 6H, Ar-H). MS: *m/z* (%): 445 (16) [$M^+$], 398 (25), 342 (38), 192 (100), 163 (26), 121 (24), 77 (18). Anal. calcd for $C_{21}H_{23}N_3O_8$: C, 56.63; H, 5.20; N, 9.43. Found: C, 56.59; H, 4.63; N, 9.28.

*6.1.5.3. 5-(2,6-Dichlorophenyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1-(2-nitrooxyacetyl)-1H-pyrazole (**9c**).* Yellow crystals (1.30 g, 57.70% yield), m.p. 172–173 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3080–3009 (Ar-CH), 2964–2837 (Aliph-CH), 1681 (C=O), 1642 ($NO_2$), 1601 (C=N), 1517 (C=C), 1276 ($NO_2$). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 3.44 (dd, 1H, $J$ = 17.40 and 7.80 Hz, $CH_2$ of pyrazoline), 3.74 (dd, 1H, $J$ = 17.35 and 13.20 Hz, $CH_2$ of pyrazoline), 3.93 (s, 3H, $OCH_3$), 3.97 (s, 3H, $OCH_3$), 5.37 (d, 1H, $J$ = 15.00 Hz, $CH_2$), 5.49 (d, 1H, $J$ = 15.00 Hz, $CH_2$), 6.27 (dd, 1H, $J$ = 13.20 and 7.87 Hz, CH of pyrazoline), 6.86–7.43 (m, 6H, Ar-H). MS: *m/z* (%): 455 (18) [M + 2], 453 (27) [$M^+$], 377 (53), 350 (70), 205 (100). Anal. calcd for $C_{19}H_{17}Cl_2N_3O_6$: C, 50.24; H, 3.77; N, 9.25. Found: C, 50.28; H, 3.75; N, 9.17.

*6.1.5.4. 5-(2,6-Dichlorophenyl)-4,5-dihydro-3-(4-methoxyphenyl)-1-(2-nitrooxyacetyl)-1H-pyrazole (**9d**).* Yellow crystals (1.33 g, 62.80% yield), m.p. 165–166 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3078–3013 (Ar-CH), 2964–2837 (Aliph-CH), 1686 (C=O), 1631 ($NO_2$), 1602 (C=N), 1514 (C=C), 1265 ($NO_2$). $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 3.20–4.00 (m, 2H, $CH_2$ of pyrazoline), 4.20 (s, 3H, $OCH_3$), 5.80 (s, 2H, $CH_2$), 6.70 (dd, 1H, $J$ = 10.00 and 7.00 Hz, CH of pyrazoline), 7.35–8.40 (m, 7H, Ar-H). MS: *m/z* (%): 427 (6) [M + 4], 425 (26) [M + 2], 423 (34) [$M^+$], 349 (41), 321 (24), 200 (81), 121 (52), 93 (8), 76 (100). Anal. calcd for $C_{18}H_{15}Cl_2N_3O_5$: C, 50.69; H, 3.56; N, 9.90. Found: C, 51.07; H, 3.14; N, 9.45.

*6.1.5.5. 5-(2-Furyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1-(2-nitrooxypropionyl)-1H-pyrazole (**11a**).* Yellow crystals (1.36 g, 67.0% yield), m.p. 143–145 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3121–2966 (Ar-CH), 2938–2838 (Aliph-CH), 1686 (C=O), 1636 ($NO_2$), 1601 (C=N), 1517 (C=C), 1263 ($NO_2$). $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 1.76 (d, 3H, $J$ = 6.60 Hz, $CH_3$), 3.60–4.00 (m, 2H, $CH_2$ of pyrazoline), 4.30 (s, 6H, 2 $OCH_3$), 5.80–6.30 (m, 1H, CH of pyrazoline), 6.6 (q, 1H, $J$ = 6.90 Hz, CH), 6.80–8.20 (m, 6H, Ar-H). MS: *m/z* (%): 389 (19) [$M^+$], 272 (26), 163 (33), 122 (100), 77 (30). Anal. calcd for $C_{18}H_{19}N_3O_7$: C, 55.53; H, 4.92; N, 10.79. Found: C, 55.59; H, 5.18; N, 10.69.

*6.1.5.6. 4,5-Dihydro-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1-(2-nitrooxypropionyl)-1H-pyrazole (**11b**).* White powder (1.45 g, 63.20% yield), m.p. 213–214 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3101–3000 (Ar-CH), 2954–2834 (Aliph-CH), 1666 (C=O), 1641 ($NO_2$), 1601 (C=N), 1515 (C=C), 1268 ($NO_2$). $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 1.87 (d, 3H, $J$ = 7.20 Hz, $CH_3$), 3.00–3.80 (m, 2H, $CH_2$ of pyrazoline), 4.10 (s, 6H, 2 $OCH_3$), 4.25 (s, 6H, 2 $OCH_3$), 5.80–6.35 (m, 2H, $CH(CH_3)$ and CH of pyrazoline), 6.80–8.00 (m, 6H, Ar-H). MS: *m/z* (%): 461 (41) [M + 2], 459 (26) [$M^+$], 341 (100), 121 (32), 77 (22). Anal. calcd for $C_{22}H_{25}N_3O_8$: C, 57.51; H, 5.48; N, 9.15. Found: C, 57.53; H, 5.41; N, 9.27.

*6.1.5.7. 5-(2,6-Dichlorophenyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1-(2-nitrooxypropionyl)-1H-pyrazole (**11c**).* White crystals (1.66 g, 71.30% yield), m.p. 185–187 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3097–2990 (Ar-CH), 2935–2838 (Aliph-CH), 1672 (C=O), 1635 ($NO_2$), 1601 (C=N), 1517 (C=C), 1263 ($NO_2$). $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ (ppm): 1.60 (d, 3H, $J = 6.40$ Hz, $CH_3$), 3.35 (dd, 1H, $J = 17.70$ and 8.30 Hz, $CH_2$ of pyrazoline), 3.73 (dd, 1H, $J = 17.60$ and 12.8 Hz, $CH_2$ of pyrazoline), 3.94 (s, 3H, $OCH_3$), 3.97 (s, 3H, $OCH_3$), 6.04 (q, 1H, $J = 6.90$ Hz, CH), 6.26 (dd, 1H, $J = 12.60$ and 8.20 Hz, CH of pyrazoline), 6.9 (d, 1H, $J = 8.60$ Hz, Ar-H), 7.10–7.40 (m, 5H, Ar-H). MS: *m/z* (%): 469 (32) [M + 2], 467 (37) [$M^+$], 422 (16), 377 (59), 205 (100), 200 (80), 163 (57), 77 (24). Anal. calcd for $C_{20}H_{19}Cl_2N_3O_6$: C, 51.30; H, 4.09; N, 8.97. Found: C, 51.89; H, 4.15; N, 8.72.

*6.1.6. General procedure for the preparation of compounds **12a** and **12b** [44]*

To a stirred solution of *p*-amino acetophenone (0.68 g, 5 mmol), bromoacetyl derivatives **8a** and **8b** (5 mmol) in 80 mL acetone, and anhydrous $K_2CO_3$ (0.25 g, 1.80 mmol) were added. The mixture was heated at reflux for about 13–15 h on a water bath, the solvent was evaporated under reduced pressure and the resulting solid was poured into distilled water and then filtered off, washed with distilled water and recrystallized from methanol.

*6.1.6.1. 1-[2-(4-Acetylphenylamino)acetyl]-5-(2-furyl)-4,5-dihydro-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**12a**).* White powder (1.56 g, 70.0% yield), m.p. 184–185 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3369 (NH), 3058–3001 (Ar-CH), 2937–2840 (Aliph-CH), 1662 (C=O), 1601 (C=N), 1520 (C=C). $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 2.70 (s, 3H, $CH_3$), 3.70–4.00 (m, 2H, $CH_2$ of pyrazoline), 4.30 (s, 3H, $OCH_3$), 4.35 (s, 3H, $OCH_3$), 4.70 (s, 2H, $CH_2$), 6.20 (dd, 1H, $J = 12.00$ and 6.00 Hz, CH of pyrazoline), 6.90 (s, 1H, Ar-H), 7.20 (d, 2H, $J = 9.00$ Hz, Ar-H), 7.40–8.10 (m, 5H, Ar-H), 8.50 (d, 2H, $J = 9.00$ Hz, Ar-H). MS: *m/z* (%): 447 (45) [$M^+$], 383 (66), 272 (100), 148 (72), 120 (37), 77 (20).

*6.1.6.2. 1-[2-(4-Acetylphenylamino)acetyl]-4,5-dihydro-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**12b**).* White powder (1.84 g, 71.30% yield), m.p. 107–108 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3388 (NH), 3062–2999 (Ar-CH), 2958–2836 (Aliph-CH), 1658 (C=O), 1598 (C=N), 1515 (C=C). $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ (ppm): 2.70 (s, 3H, $CH_3$), 3.05–4.00 (m, 2H, $CH_2$ of pyrazoline), 4.10 (s, 6H, 2 $OCH_3$), 4.25 (s, 6H, 2 $OCH_3$), 4.70 (s, 2H, $CH_2$), 6.20 (dd, 1H, $J = 12.00$ and 6.00 Hz, CH of pyrazoline), 6.80–8.00 (m, 10H, Ar-H). MS: *m/z* (%): 517 (25) [$M^+$], 462 (35), 341 (100), 309 (44), 178 (33), 77 (10).

*6.1.7. General procedure for the preparation of compounds **13a** and **13b** [45]*

To a solution of **12a** and **12b** (5 mmol) in 20 mL absolute ethanol, hydroxylamine hydrochloride (0.35 g, 5 mmol) was added; the mixture was heated at reflux for 3 h and then left to cool. The obtained precipitate was filtered off, washed with dilute ammonia solution and then with distilled water, dried and recrystallized from aqueous ethanol.

*6.1.7.1. 5-(2-Furyl)-4,5-dihydro-1-[2-(4-(1-hydroxyiminoethyl)phenylamino)acetyl]-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**13a**).* Pale brown crystals (1.69 g, 73.0% yield), m.p. 225–226 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3491 (OH), 3392 (NH), 3080–3004 (Ar-CH), 2964–2838 (Aliph-CH), 1660 (C=O), 1606 (C=N), 1521 (C=C). $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ (ppm): 2.20 (s, 3H, $CH_3$), 3.47 (dd, 1H, $J = 17.40$ and 4.80 Hz, $CH_2$ of pyrazoline), 3.60 (dd, 1H, $J = 17.40$ and 11.40 Hz, $CH_2$ of pyrazoline), 3.95 (s, 3H, $OCH_3$), 4.00 (s, 3H, $OCH_3$), 4.36 (s, 2H, $CH_2$), 5.71 (dd, 1H, $J = 11.50$ and 4.95 Hz, CH of pyrazoline), 6.32–6.36 (m, 2H, Ar-H), 6.55 (d, 2H, $J = 8.70$ Hz, Ar-H), 6.93 (d, 1H, $J = 8.10$ Hz, Ar-H), 7.23–7.31 (m, 2H, Ar-H), 7.45 (s, 1H, Ar-H), 7.50 (d, 2H, $J = 8.70$ Hz, Ar-H). MS: *m/z* (%): 462 (64) [$M^+$], 348 (23), 272 (100), 163 (83), 77 (26). Anal. calcd for $C_{25}H_{26}N_4O_5$: C, 64.92; H, 5.67; N, 12.11. Found: C, 64.90; H, 5.65; N, 11.62.

*6.1.7.2. 4,5-Dihydro-1-[2-(4-(1-hydroxyiminoethyl)phenylamino-acetyl]-5-(2,4-dimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrazole (**13b**).* White powder (1.84 g, 69.50% yield), m.p. 145–146 °C. IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3490 (OH), 3388 (NH), 3056–3012 (Ar-CH), 2960–2835 (Aliph-CH), 1658 (C=O), 1615 (C=N), 1503 (C=C). $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ (ppm): 1.95 (s, 3H, $CH_3$), 3.40–4.00 (m, 2H, $CH_2$ of pyrazoline), 4.20 (s, 6H, 2 $OCH_3$), 4.35 (s, 6H, 2 $OCH_3$), 4.80 (s, 2H, $CH_2$), 6.00–6.30 (m, 1H, CH of pyrazoline), 6.80–9.00 (m, 10H, Ar-H). MS: *m/z* (%): 532 (8) [$M^+$], 437 (18), 418 (40), 341 (48), 79 (100). Anal. calcd for $C_{29}H_{32}N_4O_6$: C, 65.40; H, 6.06; N, 10.52. Found: C, 65.31; H, 5.89; N, 10.71.

*6.2. NO release assay [46]*

A solution of the appropriate compound (20 μL) in dimethylsulfoxide (DMSO) was added to 2 mL of 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) with MeOH, containing $5 \times 10^{-4}$ M of L-cysteine. 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 $\lambda = 546$ nm. Sodium nitrite standard solutions (10–80 nmol/mL) were used to construct the calibration curve. The same procedure was repeated using different solutions of the test compounds under the same conditions using 0.1 N HCl of pH 1 instead of phosphate buffer of pH 7.4.

The results were expressed as amount of NO released relative to a theoretical maximum release of 1 mol NO/mol of test compound.

*6.3. Pharmacology*

*6.3.1. Anti-inflammatory activity*

The experiments were performed on adult male albino rats, weighing 140–200 g and 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 **7a–d**, **8a–d**, **9a–d**, **10a–c**, **11a–c**, **13a** and **13b** and reference drug (indomethacin) 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 treated similarly 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. [31]. 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.

*6.3.2. Ulcerogenic toxicity*

Adult male albino rats weighing 140–200 g were divided into different groups consisting of four animals in each group. Animals were deprived of food but not water 24 h before the experiment. Ulcerogenic activity was evaluated after oral administration of a suspension of the tested compounds **7a–d**, **8a–d**, **9a–d**, **10a–c**,

**11a–c**, **13a** and **13b** or indomethacin in carboxy methyl cellulose (CMC) solution (0.5% w/v in water) in a dose level of 100 mg/kg. Control animals were treated similarly with CMC solution (0.5% w/v in water). After 5 h 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 of 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.

The data are expressed as mean ± S.E., one way ANOVA test was applied to determine the significance of the difference between the different groups of rats treated with the tested compounds or indomethacin.

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