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Original article

# Synthesis and anti-inflammatory evaluation of some new acyl-hydrazones bearing 2-aryl-thiazole

Cristina Mariana Moldovan [a,*], Ovidiu Oniga [a], Alina Pârvu [b], Brînduşa Tiperciuc [a], Philippe Verite [c], Adrian Pîrnău [d], Ovidiu Crişan [e], Marius Bojiţă [f], Raluca Pop [g]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, 41 Victor Babes Street, RO-400010 Cluj-Napoca, Romania
[b] Department of Physiology, Faculty of Medicine, University of Medicine and Pharmacy, Victor Babes Street, Cluj-Napoca, Romania
[c] Department of Analytical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, 22 Gambetta Boulevard, F-76183 Rouen Cedex, France
[d] National Institute for Research and Development of Isotopic and Molecular Technologies, RO-400293 Cluj-Napoca, Romania
[e] Department of Organic Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, 41 Victor Babes Street, RO-400010 Cluj-Napoca, Romania
[f] Department of Drug Analysis, Faculty of Pharmacy, University of Medicine and Pharmacy, 6 Louis Pasteur Street, Cluj-Napoca, Romania
[g] National Institute for Research and Development for Electrochemistry and Condensed Matter, 144 Aurel Păunescu-Podeanu Street, RO-300569, Timişoara, Romania

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ABSTRACT

This work describes recent results from our research program aiming at the synthesis and evaluation of new compounds acting as potential anti-inflammatory drugs. A series of novel acyl-hydrazones bearing 2-aryl-thiazole moiety were synthesized by the condensation between derivatives of 4-[2-(4-methyl-2-phenyl-thiazole-5-yl)-2-oxo-ethoxy]-benzaldehyde and 2, 3 or 4-(2-aryl-thiazol-4-ylmethoxy)-benzaldehyde, respectively and different carboxylic acid hydrazides. The structures of newly synthesized compounds were established by the combined use of IR, $^1$H NMR, mass spectral data and elemental analysis. These compounds were tested in vivo for their anti-inflammatory activity, in an acute experimental inflammation. The acute phase bone marrow response, phagocytes' activity and NO synthesis were evaluated. Compounds **10**, **15**, **17**, **18** and **22** reduced the absolute leukocytes count due to the lower neutrophils percentage. Phagocitary index was decreased by all the compounds. Seven of them reduced the phagocitary activity. Five compounds inhibited NO synthesis, **3**, **4**, **16** and **22** stronger than Meloxicam, the anti-inflammatory reference drug.



## 1. Introduction

Rheumatic diseases are the most prevalent causes of disability in European countries and non-steroidal anti-inflammatory drugs (NSAIDs) are still the most commonly used remedies. Chronic use may cause several serious adverse effects, the most important one being gastric injury and renal complications. GI damage from NSAID is generally attributed to two factors: local irritation by the direct contact of the free carboxylic acid (−COOH) moiety of NSAID with GI mucosal cells (topical effect) and decreased tissue prostaglandin production in tissues [1].

The development of new non-steroidal anti-inflammatory drugs follows different strategies: selective COX-2 inhibition or the inhibition of inducible nitric oxide synthase (iNOS). iNOS contributes to acute and chronic inflammation by producing nitric oxide as a cytotoxic inflammatory mediator [2]. Synthetic approaches have been taken with the aim of improving safety profile and in turn therapeutic window of NSAIDs. Several studies have described the derivatization of the carboxylate function of representative NSAID with less acidic azoles: thiazole [3–8], oxadiazole [9–12], triazole, thiadiazole [2], etc. which resulted in an increased anti-inflammatory activity with reduced ulcerogenicity.

In our attempt to synthesize new, safer and potent agents for treatment of inflammatory diseases, we used 2-aryl-thiazole moiety. This heterocyclic system has found application in drug development for the treatment of inflammation [13]. Fentiazac (2-phenyl-thiazole), Meloxicam (2-methyl-thiazole), Fanetizole (2-amino-thiazole) [14–16] are some examples of thiazole bearing anti-inflammatory products.

A number of hydrazone derivatives have been claimed to possess anti-inflammatory activity [2,17–21]. Several studies presented the broad range of biological activities, including anti-inflammatory, for compounds bearing chromone moiety [22].

* Corresponding author. Present address: 2B Calea Floresti Street, A$_1$B Building, Apartment 25, Cluj-Napoca, Romania. Tel.: +40 745 264393; fax: +40 264 450529.
*E-mail addresses:* cris.moldovan@yahoo.com, cmoldovan@umfcluj.ro (C.M. Moldovan).

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With the aim of obtaining new anti-inflammatory agents, we designed a series of new acyl-hydrazones bearing 2-aryl-thiazole scaffold. The compounds were evaluated for their effect on acute inflammation.

## 2. Chemistry

The conversion of the aldehydes **1**, **5**–**9** (Fig. 1) to the corresponding acyl-hydrazones **3**, **4**, **10**–**18** (Scheme 1), **20**–**22** (Scheme 2) was readily accomplished by the reaction with the respective carboxylic acid hydrazides, in refluxing acetic acid 50%.

The benzaldehyde derivatives **1** and **5**–**9** can be synthesized as described in our previous paper by the etherification of a halogenated component with 2, 3 or 4-hydroxy-benzaldehyde [23], and the hydrazides **2a**, **b** and **19** were prepared according to the literature by refluxing thiazolyl acetic esters with hydrazine hydrate. The purity of the compounds was demonstrated by TLC. All the new compounds **3**, **4**, **10**–**18**, **20**–**22** were characterized by m.p., elemental analysis and spectroscopic data (IR, $^1$H NMR, $^{13}$C NMR, MS).

## 3. Pharmacology

Our study investigated the effects of the newly synthesized compounds in an acute experimental inflammation, induced by the i.m. injection of turpentine oil. Their anti-inflammatory capacity was assessed by evaluating the acute phase bone marrow response, phagocytes' activity and NO synthesis. The acute phase bone marrow response was measured by absolute leukocytes count and leukocytes count expressed as percentage. Phagocytic activity was assessed with the *in vitro* phagocytosis test by calculating two parameters: the phagocytic index (PI) (PI% = phagocytes with at least one phagocyted germ from 200 leukocytes counted) and the phagocytic activity (PA) (PA = number of germs phagocyted by 100 leukocytes) [24–28]. NO synthesis was evaluated measuring nitrates/nitrites concentration [29,30]. The effects of new compounds were compared with those from the inflammation group, and with those from the group treated with Meloxicam as a reference NSAID. A negative control group of healthy rats without any treatment was also used.

## 4. Results and discussion

### 4.1. Chemistry

The structures of all new compounds were confirmed by their spectral (IR, $^1$H NMR, $^{13}$C NMR and mass) and elemental analytical data. The IR spectra exhibited characteristic absorption band at 1675–1649 cm$^{-1}$ and 3446–3161 cm$^{-1}$ due to –CO– and –NH stretching. Each of the new acyl-hydrazones displays a strong and sharp band in the region 1555–1508 cm$^{-1}$ ascribed to $\nu$(C=N) of azomethine group, providing confirmatory evidence for the condensation reaction between aldehyde group and hydrazide. The $^1$H NMR spectra showed characteristic singlets due to NH and N=CH proton at $\delta$ 12.1–11 ppm and $\delta$ 8.41–8.198 ppm, respectively. The C(5)–H in thiazole appeared as one singlet or two singlet peaks (compounds **10**–**18**) at $\delta$ 7.622–7.422 ppm, the protons from the two methylene groups appeared as two singlet signals at $\delta$ 5.4–4.17 ppm and those from methyl group appeared at $\delta$ 2.785–2.34 ppm as singlet peak or two singlet peaks (compound **3**). Other protons appeared in the aromatic regions. $^{13}$C NMR data also supported the structures of acyl-hydrazones.

#### 4.1.1. The structure of the compounds

The resulting double bond –HC=N– following the condensation reaction, contributes to the formation of geometrical isomers *Z* and *E*. One distinctive feature to be remarked is the amide proton resonance which is well downfield of all other peaks. Thus, the $^1$H NMR spectra indicated the chemical shift of the NH proton at $\delta$ 12.1–11 ppm in the form of a singlet peak of an intense signal, assigned to the NH proton of *E* isomer, accompanied by a weak singlet signal, assigned to the NH proton of *Z* isomer, more deshielded.

The proton signals consistent with isomers *Z* and *E* in liquid state were in agreement with ROESY spectra. These showed a stronger interaction between the proton of NH group and that of –CH=, both protons with higher chemical shift (*Z* isomer), compared against the interaction between the same two protons (NH and –CH=N) with lower chemical shift, belonging to the *E* isomer. The formation of both isomers is thermodynamically controlled, fact reflected in NMR spectra, as differences in intensities of NH proton signals, corresponding to both isomers. The NMR spectra showed a difference between the intensities of the two peaks and the predominant formation of one of the isomers. This one must have lower minimum energy stabilization. HyperChem calculation [31], based on the Polak-Ribiere (conjugate gradient) indicated for compound **10**, for example, a minimum energy stabilization of *E* isomer of 58.1345 kcal/mol, with a 0.08947 kcal/A*mol RMS gradient, whereas for *Z* isomer the energy was 62.2176 kcal/mol, with a 0.09077 kcal/A*mol RMS gradient. For all the other compounds, the minimum energy stabilization of *E* isomer was lower than of *Z* isomer. The *ab initio* calculation using Gaussian 03 [32] was possible after the optimization of the geometry for the two isomers. The calculation with this method indicated a total energy for *E* isomer(–2262.493 a.u.), whereas for *Z* isomer (–2262.472 a.u.) and a relative energy of 12.79 kcal/mol, comparing to the energy of *E* isomer, considered 0. This indicates that *E* isomer is more stable.

Considering these observations, we can say that the two isomers may coexist, in liquid state, but the *E* isomer is formed predominantly, this being in agreement with its higher stability and with the literature data [33–35].

Hydrazones display prototropic amido-imido tautomerism. The possibility of this type of tautomerism involving a hydroxyl group was excluded for all the compounds, by the evident absence of typical

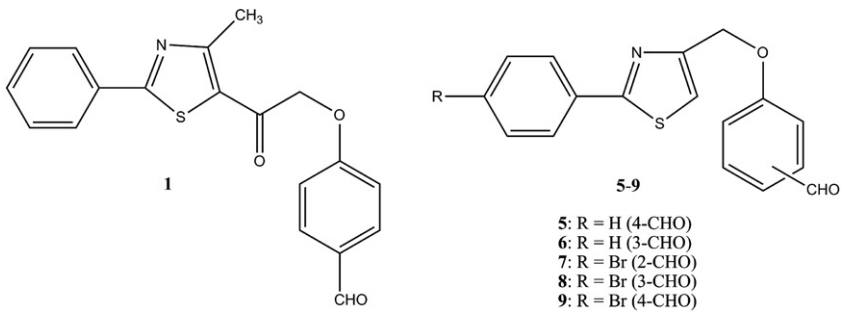
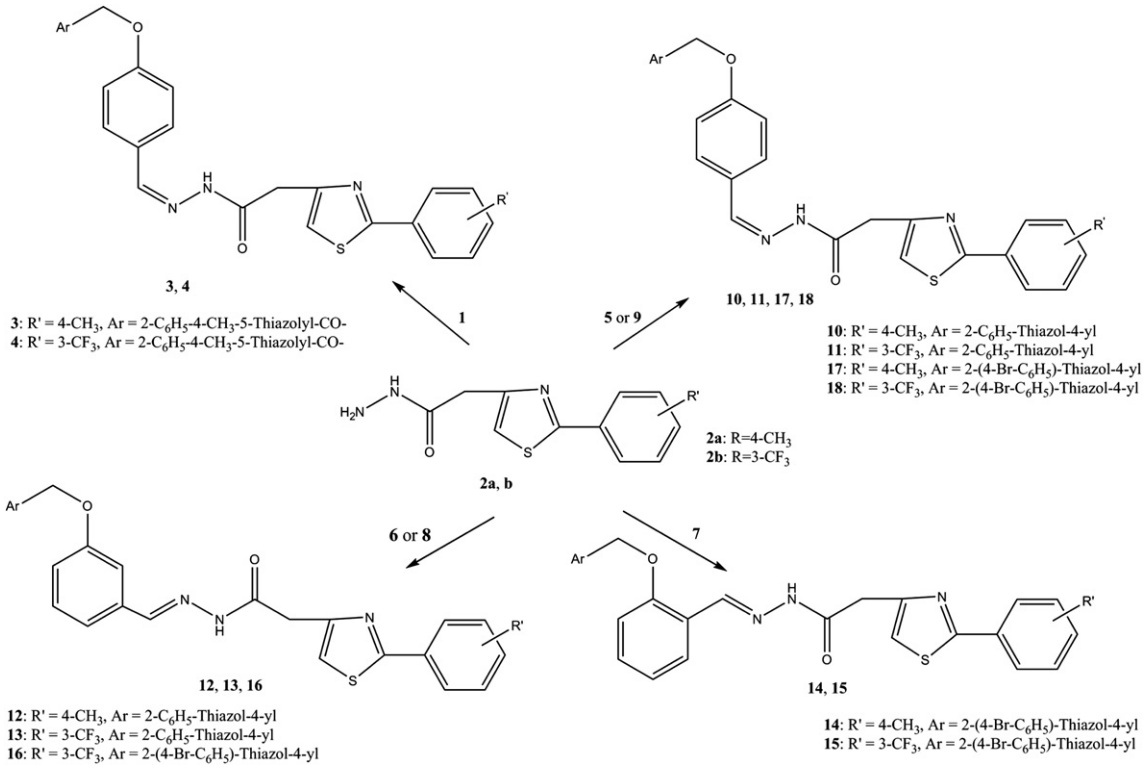



**Fig. 1.** Aldehydes **1** and **5**–**9**.




**Scheme 1.** Synthesis of acyl-hydrazones **3**, **4** and **10**–**18**.

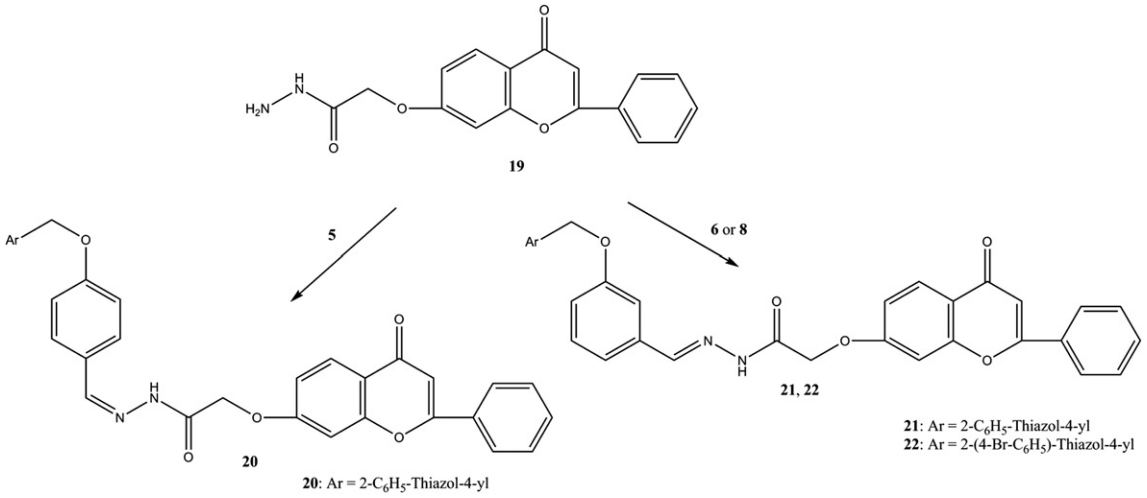
signals of an OH group in IR and $^1$H NMR spectra. The IR absorption of the hydrazone $\nu$(NH) group at 3446–3161 cm$^{-1}$, together with a strong band at about 1675–1649 cm$^{-1}$ for the $\nu$(C═O) group, confirms the amido tautomeric form in solid state.

### 4.2. Anti-inflammatory activity

#### 4.2.1. Acute phase bone marrow response

Acute phase bone marrow response, as a part of the systemic acute phase response, is induced by the early production of pro-inflammatory cytokines IL-1, IL-6 and TNF-$\alpha$, resulting leukocytosis and neutrophilia. After 24 h from turpentine oil administration, it was found that the synthesized compounds **10**, **11**, **15**–**18**, **20** and **22** significantly reduced absolute leukocytes count ($p < 0.001$), **4** and **21** caused an important increase ($p < 0.01$) and **13** and **14** had no significant influence ($p > 0.05$). Comparing the same results with those from the Meloxicam group, we noticed that five compounds (**10**, **11**, **16**–**18**) had a stronger inhibitory activity ($p < 0.01$) (Table 1). Absolute leukocytes count reduction was positively correlated with NO synthesis for substances **11**, **15**, **16** ($r = 0.7$), with PI for substances **17**, **22** ($r = 0.7$), and for PA with substance **18** ($r = 0.7$).

Analyzing neutrophils percentage, we found a very significant reduction in groups **3**, **10**, **13**, **17**, **18** and **22** ($p < 0.001$), a significant decrease in groups **14** and **15** ($p < 0.01$) and a no significant change




**Scheme 2.** Synthesis of acyl-hydrazones **20**–**22**.

**Table 1**
Effects of the compounds on the acute medullar response.

| Compound | Leukocytes[a] (no./mm$^3$) | Neutrophiles[a] (%) | Monocytes[a] (%) | Lymphocytes[a] (%) |
|---|---|---|---|---|
| Control | 4952.7 ± 436.9 | 55.6 ± 1.32 | 7.6 ± 0.45 | 37 ± 2.2 |
| Inflammation | 9280 ± 412.79 | 73.43 ± 1.9 | 2.28 ± 0.76 | 27.71 ± 4.53 |
| Meloxicam | 7660 ± 422.37 | 66.57 ± 4.27 | 2 ± 1.15 | 32.28 ± 4.23 |
| **3** | 8742.85 ± 390.97 | 66 ± 1.63 | 2.43 ± 0.79 | 32.14 ± 1.34 |
| **4** | 10200 ± 556.77 | 78 ± 1.15 | 2 ± 0 | 18 ± 4.16 |
| **10** | 3271.43 ± 393.28 | 52.57 ± 2.5 | 1.86 ± 0.69 | 21.43 ± 3.41 |
| **11** | 5664.28 ± 355.57 | 82.28 ± 2.93 | 2 ± 0.58 | 20.71 ± 3.68 |
| **12** | 8620 ± 488.98 | 73.29 ± 3.77 | 2.86 ± 1.07 | 19.71 ± 4.23 |
| **13** | 9540 ± 333.46 | 61.71 ± 1.8 | 2.4 ± 0.84 | 32.28 ± 3.35 |
| **14** | 9300 ± 310.91 | 67.71 ± 3.9 | 2.86 ± 1.07 | 29.14 ± 4.88 |
| **15** | 7837.14 ± 203.11 | 68.85 ± 2.79 | 2.57 ± 0.79 | 24.71 ± 3.77 |
| **16** | 6914.28 ± 279.45 | 70.86 ± 3.97 | 2.86 ± 1.07 | 23.43 ± 4.28 |
| **17** | 7314.28 ± 481.07 | 68.28 ± 2.43 | 2.71 ± 0.95 | 28.57 ± 2.7 |
| **18** | 7402.85 ± 479.78 | 63.14 ± 3.44 | 3.14 ± 1.07 | 32 ± 4.32 |
| **20** | 8228.57 ± 275.16 | 76 ± 3.83 | 3.71 ± 0.75 | 23.57 ± 4.47 |
| **21** | 12100 ± 314.9 | 71.71 ± 1.8 | 2.6 ± 0.97 | 24 ± 2.83 |
| **22** | 8000 ± 273.86 | 57.43 ± 2.76 | 3.28 ± 0.95 | 39.86 ± 2.48 |

[a] Values are expressed as mean of three experiments ± standard deviation.

in **12**, **16** and **21**. Two of the new molecules, **10** and **22**, showed a more potent inhibitory activity on neutrophils than Meloxicam ($p < 0.001$) (Table 1). Neutrophils count expressed as percentage was positively correlated with NO synthesis for substances **11**, **17**, **20** ($r = 0.7–0.8$), with PI for substance **11** ($r = 0.7$) and with substances **18**, **21** for PA ($r = 0.8$).

Compounds **10**, **17**, **18** and **22** showed an important reduction of total leukocytes count by reducing neutrophils' percentage. Analyzing the chemical structures of these compounds, it can be observed that the substitution of phenyl from position 2 of thiazole with a brome atom in 4, had a good influence on the acute phase bone marrow response (**R** = Br for compounds **17**, **18** and **22**).

On the basis of these data, it could be argued that the compounds with significant inhibitory activity observed in the reduction of acute phase bone marrow response, have systemic anti-inflammatory effects.

*4.2.2. Phagocytosis test*

Phagocytosis is part of the cellular acute phase response from acute inflammations. There are two professional phagocytes: polymorphonuclear (PMN) leukocytes and mononuclear phagocytes. The PMN cells are released from the bone marrow as mature cells, which circulate in the blood before migrating into the tissues where they perform their effector functions for 1 or 2 days. In contrast, mononuclear phagocytes emerge from the marrow as immature cells monocytes, circulate in the blood and then enter tissues and organs of the body where they mature into macrophages. After recognizing the targets, phagocytic cells are activated to ingesting them and destroying with reactive oxidants and hydrolytic enzymes.

In our study, we have investigated phagocytic activity of blood phagocytes for the evaluation of immunomodulating activity of the synthesized agents. All the tested compounds caused a very significant reduction of the PI ($p < 0.001$). Compared to Meloxicam, just seven compounds (**3**, **4**, **11**, **13**, **15**, **20** and **22**) had a stronger inhibitory activity on PI ($p < 0.001$) (Fig. 2). As for the phagocytic activity, it was observed that this parameter was very significantly ($p < 0.001$) reduced by the compounds **3**, **11**, **13**, **15**, **16**, **20** and **22**. Only four compounds (**3**, **11**, **20** and **22**) were more powerful inhibitors of PA than Meloxicam ($p < 0.05$ for **3** and $p << 0.001$ for **11**, **20** and **22**) (Fig. 3). PI reduction was positively correlated with NO synthesis for substances **13**, **15**, **18**, **22** ($r = 0.7$), and with PA for substance **11** ($r = 0.7$).

Compounds **3**, **11**, **13**, **15**, **16**, **20** and **22** had a significant influence on the phagocytosis process, reducing PI and PA, too. These results indicated that the presence of $CF_3$ group in position meta of phenyl determined an important decrease of phagocytary parameters (**X** = F for the compounds **11**, **13**, **15** and **16**). Also, it should be noticed the influence of the chromone group for the compounds **20** and **22**.

*4.2.3. Nitrites and nitrates concentration*

In acute inflammation there is a significant increase of NO synthesis due to the expression of iNOS. This will raise serum nitrates/nitrites concentration, as side metabolites of NO. Meloxicam reduced significantly NO synthesis ($p < 0.001$) (Fig. 4). For the

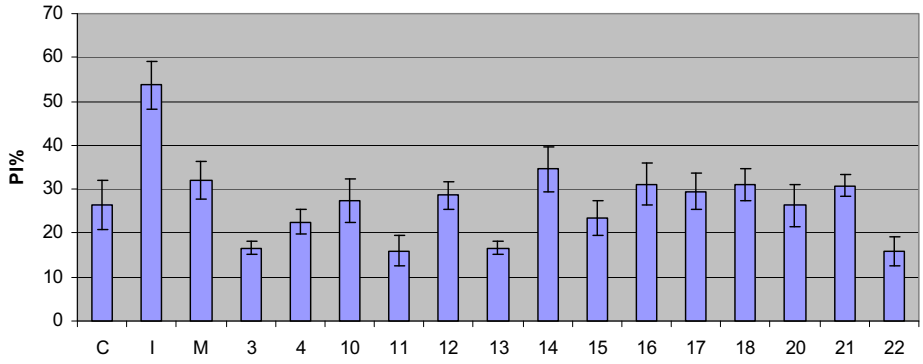



**Fig. 2.** The effect of the synthesized compounds on PI.

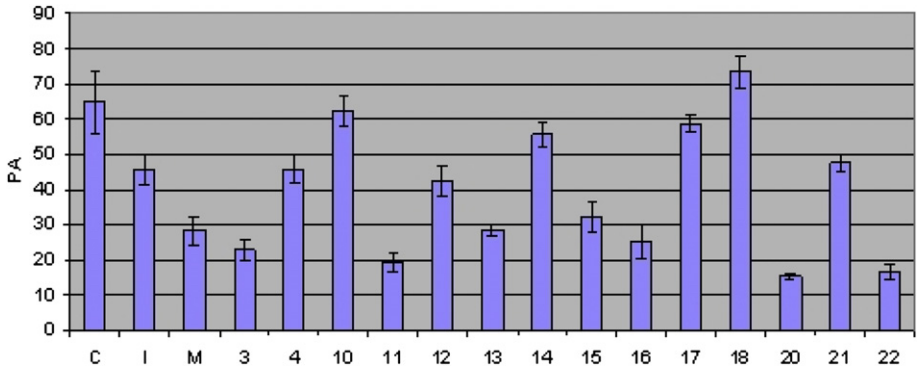



**Fig. 3.** The effect of the synthesized compounds on PA.

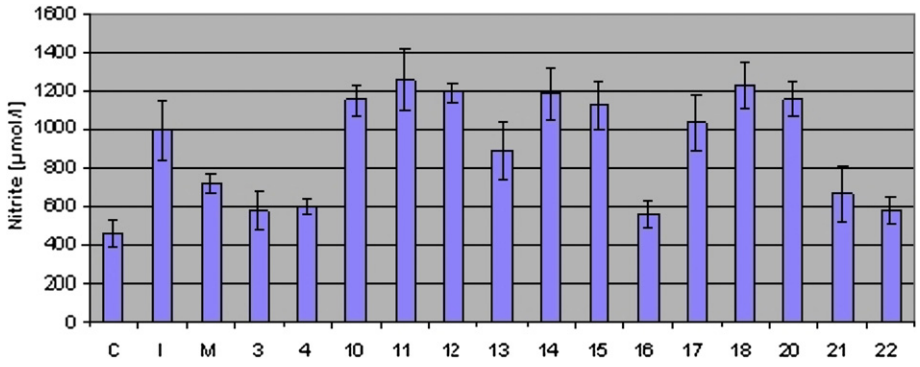



**Fig. 4.** The effect of the compounds on nitrates/nitrites amount.

compounds **10**–**12**, **14**, **18** and **20**, the results showed a significant increase ($p < 0.05$) of NO metabolites comparing with the Inflammation group, while compounds **3**, **4**, **16**, **21** and **22** reduced significantly ($p < 0.05$) the amount of nitrates/nitrites and exhibited a stronger inhibitory activity ($p < 0.01$) of NO synthesis than Meloxicam, excepting compound **21** ($p > 0.05$).

## 5. Conclusion

Various substituted 2-aryl-thiazole hydrazone derivatives were synthesized and screened for their anti-inflammatory potential. Compounds **10**, **15**, **17**, **18** showed to have a good inhibitory effect on the acute phase marrow response, by reducing the absolute leukocytes count due to the lower neutrophils percentage. Compound **10**, which has 2-phenyl-thiazole and [2-(4-methyl-phenyl)-4-methylen]-thiazole hydrazine moieties in its structure, proved to be a more active inhibitor of the marrow acute phase response than Meloxicam. From the results of *in vitro* phagocytosis test we could conclude that all the newly synthesized compounds reduced significantly PI, **3**, **4**, **11**, **13**, **15**, **20** and **22** being more potent inhibitors than Meloxicam. PA was significantly reduced by the compounds **3**, **11**, **13**, **15**, **16**, **20** and **22**, from which **3**, **11**, **20** and **22** were more potent inhibitors than Meloxicam. The NO synthesis was significantly reduced by **3**, **4**, **16**, **21** and **22** and they all, except of **21**, showed a stronger inhibitory activity than Meloxicam.

## 6. Experimental section

### 6.1. Chemistry

Solvents were obtained from commercial sources; the reagents were synthesized in our laboratory. Analytical thin layer chromatography was carried out on precoated Silica Gel $60F_{254}$ sheets using UV absorption for visualization. The melting points were taken with two melting point meters, Electrothermal and MPM-H1 Schorpp and are uncorrected. FT-IR spectra were recorded on a Nicolet 210 FT-IR spectrometer with a MCT detector and an Omnic 4.1b soft system, using potassium bromide. The $^1$H NMR and $^{13}$C NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 500 MHz and 125 MHz, respectively and were in accord with the assigned structures. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The samples were prepared by dissolving the synthesized powder of the compounds in DMSO-*d*6 ($\delta_H = 2.51$ ppm, $\delta_C = 40.02$ ppm) as solvent and the spectra were recorded using a single excitation pulse of 10.1 μs ($^1$H NMR) and 8 μs ($^{13}$C NMR), respectively. The two-dimensional NMR spectrum at 500 MHz was obtained on standard Bruker software. The conditions for ROESY phase-sensitive spectra via time proportional phase incrementation (TPPI) were: spectral widths of 6.0 ppm in both dimensions with a resolution of 0.7 and 1.4 Hz/point in $f_2$ and $f_1$ respectively and a mixing time of 450 ms. The experiment was performed using 4096 data points in $f_2$ and 2048 in f1 increments with 16 scans per $t_1$ value and a relaxation period of 2 s. A sine function (SSB = 2) was applied in $f_1$ and $f_2$ before Fourier transformation. GC–MS analyses were realized with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was registered with a Vario El CHNS instrument.

#### 6.1.1. General procedure for the synthesis of acyl-hydrazones ***3***, ***4***, ***10*–*18***, ***20*–*22***

4-[2-(4-Methyl-2-phenyl-thiazol-5-yl)-2-oxo-ethoxy]-benzaldehyde **1** or 2, 3 or 4-(2-Aryl-thiazol-4-ylmethoxy)-benzaldehydes **5**–**9** (1 mmol) was dissolved in 10 ml of acetic acid 50%. Separately, a suspension of acyl-hydrazide (1 mmol) in 10 ml acetic acid 50%

was prepared. This suspension of hydrazide was added under stirring to the solution of benzaldehyde. The reaction mixture was stirred for 1.5 h. For improving the solubility of products in acetic acid and the reaction, a small quantity of ethanol was added. After 1.5 h of refluxing, the reaction mixture was cooled off. The crude product was filtered under reduced pressure, washed with water on the filter and purified by crystallization from ethanol.

*6.1.1.1. (2-p-Tolyl-thiazol-4-yl)-acetic acid {4-[2-(4-methyl-2-phenyl-thiazol-5-yl)-2-oxo-ethoxy]-benzylidene}-hydrazide (**3**).* Yield 53%. Yellow powder, mp: 147–50 °C. IR (KBr, $cm^{-1}$): 3219 (NH), 3064 ($CH_{arom}$), 2921 and 2850 ($CH_{aliph}$), 1674 (C=O), 1652 (C=O Hydrazide), 1599 (C=C), 1554 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): δ 11.95 (s, 1H, NH); 8.2 (s, 1H, −CH=); 7.96 (dd, 2H, Ar-H); 7.824–7.8 (m, 3H, Ar-H); 7.66 (dd, 2H, Ar-H); 7.511 (d, 2H, Ar-H); 7.448 (s, 1H, H-Thiazole); 7.302 (dd, 2H, Ar-H); 7.148 (dd, 2H, Ar-H); 5.292 (s, 2H, $-CH_2-$); 4.183 (s, 2H, $-CH_2-$); 2.785 (s, 3H, $-CH_3$), 2.35 (s, 3H, Ar-$CH_3$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): δ 188.61 (C=O, ketone); 171.27 (C=O, hydrazide); 170.12 (C-2 Thiazole); 165.51 (C-4 Thiazole); 160.48 (C-2 Thiazole); 159.89 (=C−O, Ar); 153.39 (C-4 Thiazole); 151.61 (−CH=N); 143.21 (C, Ar); 140.01 (C, Ar); 134.19 (C, Ar); 132.27 (C, Ar); 131.04 (C-5 Thiazole); 130.21 (2C, CH, Ar); 128.84 (2C, CH, Ar); 128.54 (2C, CH, Ar); 128.02 (2C, CH, Ar); 127.11 (2C, CH, Ar); 122.31 (CH, C-5 Thiazole); 118.78 (CH, Ar); 115.58 (2C, CH, Ar); 72.05 ($CH_2$); 37.78 ($CH_2$); 21.39 ($C_{ar}-CH_3$); 19.01 ($C_{4-thiazole}-CH_3$). Anal. Calcd. (%) for $C_{31}H_{26}N_4O_3S_2$ (566.69): C, 65.70; H, 4.62; N, 9.89; S, 11.31. Found: C, 65.49; H, 4.61; N, 9.88; S, 11.28. MS (EI, 70 eV): *m*/*z* 567 (M+), 352 [M- $C_{12}H_{10}NSO$], 217 [$C_{12}H_{10}NSO$].

*6.1.1.2. [2-(3-Trifluoromethyl-phenyl)-thiazol-4-yl]-acetic acid {4-[2-(4-methyl-2-phenyl-thiazol-5-yl)-2-oxo-ethoxy]-benzylidene}-hydrazide (**4**).* Yield 80.64%. Yellow powder, mp: 167–9 °C. IR (KBr, $cm^{-1}$): 3161 (NH), 3060 ($CH_{arom}$), 2919 and 2847 ($CH_{aliph}$), 1670 (C=O), 1658 (C=O Hydrazide), 1587 (C=C), 1552 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): δ 11.559 (s, 1H, NH); 8.26 (s, 1H, −CH=); 8.18 (d, 2H, Ar-H); 7.994–7.952 (m, 3H, Ar-H); 7.81 (d, 2H, Ar-H); 7.686–7.66 (m, 2H, Ar-H); 7.52 (s, 1H, H-Thiazole); 7.508 (d, 2H, Ar-H); 7.149 (dd, 2H, Ar-H); 5.286 (s, 2H, $-CH_2-$); 4.22 (s, 2H, $-CH_2-$); 2.49 (s, 3H, $-CH_3$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): δ 188.65 (C=O, ketone); 171.19 (C=O, hydrazide); 170.2 (C-2 Thiazole); 165.04 (C-4 Thiazole); 160.43 (C-2 Thiazole); 159.5 (=C−O, Ar); 152.16 (C-4 Thiazole); 146.92 (−CH=N); 143.2 (C, Ar); 134.38 (C, Ar); 132.49 (C, Ar); 132.13 (CH, Ar); 131.04 (CH, C-5 Thiazole); 130.53 (CH, Ar); 129.89 (2C, CH, Ar); 128.77 (C, Ar); 128.4 (CH, Ar); 128.01 (2C, CH, Ar); 127.14 (2C, CH, Ar); 125.41 ($CF_3$); 123.24 (CH, Ar); 122.36 (CH, C-5 Thiazole); 118.66 (CH, Ar); 115.58 (2C, CH, Ar); 72.05 ($CH_2$); 37.7 ($CH_2$); 19.02 ($C_{4-thiazole}-CH_3$). Anal. Calcd. (%) for $C_{31}H_{23}F_3N_4O_3S_2$ (620.66): C, 59.99; H, 3.74; N, 9.03; S, 10.33. Found: C, 59.81; H, 3.74; N, 9.01; S, 10.29. MS (EI, 70 eV): *m*/*z* 621 (M+), 352 [M- $C_{12}H_7SOF_3$], 335 [M- $C_{12}H_8NSOF_3$], 217 [$C_{12}H_{10}NSO$].

*6.1.1.3. (2-p-Tolyl-thiazol-4-yl)-acetic acid [4-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (**10**).* Yield 82%. White powder, mp: 170–3 °C. IR (KBr, $cm^{-1}$): 3253 (NH), 3094 ($CH_{arom}$), 2920 and 2849 ($CH_{aliph}$), 1671 (C=O Hydrazide), 1615 (C=C), 1540 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): δ 11.35 (s, 1H, NH); 8.198 (s, 1H, −CH=); 7.972–7.953 (dd, 2H, Ar-H); 7.823–7.807 (m, 3H, Ar-H); 7.68–7.656 (dd, 2H, Ar-H); 7.511 (d, 2H, Ar-H); 7.485 (s, 1H, H-Thiazole); 7.448 (s, 1H, H-Thiazole); 7.315–7.290 (dd, 2H, Ar-H); 7.170–7.126 (dd, 2H, Ar-H); 5.286 (s, 2H, $-CH_2-$); 4.194 (s, 2H, $-CH_2-$); 2.37 (s, 3H, $-CH_3$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): δ 171.19 (C-2 Thiazole); 168.97 (C=O, hydrazide); 166.04 (C-2 Thiazole); 164.02 (C, Ar); 159.58 (C-4 Thiazole); 156.79 (C-4 Thiazole); 146.89 (−CH=N); 144.29 (C, Ar); 143.32 (C, Ar); 134.67 (C, Ar); 131.86 (2C, CH, Ar); 131.01 (C, Ar); 131.09 (2C, CH, Ar); 128.47 (2C, CH, Ar); 126.96 (2C, CH, Ar); 126.04 (CH, C-5 Thiazole); 122.43 (2C, CH, Ar); 119.29 (CH, C-5 Thiazole); 118.75 (CH, Ar); 115.59 (2C, CH, Ar); 65.90 ($CH_2$); 37.79 ($CH_2$); 21.41 ($CH_3$). Anal. Calcd. (%) for $C_{29}H_{24}N_4O_2S_2$ (524.65): C, 66.39; H, 4.61; N, 10.68; S, 12.22. Found: C, 66.15; H, 4.6; N, 10.64; S, 12.19. MS (EI, 70 eV): *m*/*z* 524 [M], 174 [$C_{10}H_8NS$], 104 [$C_7H_5O$].

*6.1.1.4. [2-(3-Trifluoromethyl-phenyl)-thiazol-4-yl]-acetic acid [4-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (**11**).* Yield 79%. White powder, mp: 175–6 °C. IR (KBr, $cm^{-1}$): 3442 (NH), 3063 ($CH_{arom}$), 2922 and 2850 ($CH_{aliph}$), 1672 (C=O Hydrazide), 1599 (C=C), 1524 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): δ 11.38 (s, 1H, NH); 8.205 (s, 1H, −CH=); 8.194 (s, 1H, Ar-H); 7.991–7.954 (m, 4H, Ar-H); 7.809 (d, 2H, Ar-H); 7.682–7.655 (m, 2H, Ar-H); 7.622 (s, 1H, H-Thiazole); 7.521 (s, 1H, H-Thiazole); 7.507 (d, 2H, Ar-H); 7.169–7.123 (dd, 2H, Ar-H); 5.28 (s, 2H, $-CH_2-$); 4.24 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): δ 171.19 (C-2 Thiazole); 168.06 (C-2 Thiazole); 165.44 (C=O, hydrazide); 165.11 (=C−O, Ar); 164.65 (C-4 Thiazole); 160.03 (C-4 Thiazole); 153.15 (−CH=N); 152.20 (C, Ar); 146.98 (C, Ar); 143.30 (C, Ar); 134.39 (CH, Ar); 133.34 (CH, Ar); 131.08 (2C, CH, Ar); 130.84 (C, Ar); 130.57 (2C, CH, Ar); 129.74 (CH, Ar); 128.98 (2C, CH, Ar); 127.69 ($CF_3$); 126.61 (CH, C-5 Thiazole); 122.38 (CH, Ar); 119.2 (CH, C-5 Thiazole); 118.69 (CH, Ar); 115.58 (2C, CH, Ar); 65.94 ($CH_2$); 37.7 ($CH_2$). Anal. Calcd. (%) for $C_{29}H_{21}F_3N_4O_2S_2$ (578.62): C, 60.20; H, 3.66; N, 9.68; S, 11.08. Found: C, 60.11; H, 3.65; N, 9.65; S, 11.11. MS (EI, 70 eV): *m*/*z* 578 [M], 243 [$C_{11}H_7NSCF_3$], 174 [$C_{10}H_8NS$], 104 [$C_7H_5O$], 71 [$C_2HNS$].

*6.1.1.5. (2-p-Tolyl-thiazol-4-yl)-acetic acid [3-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (**12**).* Yield 80%. White powder, mp: 173 °C. IR (KBr, $cm^{-1}$): 3253 (NH), 3094 ($CH_{arom}$), 2920 and 2849 ($CH_{aliph}$), 1671 (C=O Hydrazide), 1615 (C=C), 1540 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): δ 11.34 (s, 1H, NH); 8.2 (s, 1H, −CH=); 7.913–7.886 (m, 3H, Ar-H); 7.821–7.808 (m, 3H, Ar-H); 7.678–7.657 (dd, 2H, Ar-H); 7.482 (s, 1H, H-Thiazole); 7.442 (s, 1H, H-Thiazole); 7.311–7.286 (dd, 2H, Ar-H); 7.22 (m, 1H, Ar-H); 7.171–7.128 (dd, 2H, Ar-H); 5.28 (s, 2H, $-CH_2-$); 4.19 (s, 2H, $-CH_2-$); 2.39 (s, 3H, $-CH_3$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): δ 171.52 (C-2 Thiazole); 165.66 (C=O, hydrazide); 165.43 (C-2 Thiazole); 165.01 (=C−O, Ar); 164.87 (C-4 Thiazole); 158.99 (C-4 Thiazole); 151.90 (−CH=N); 146.69 (C, Ar); 143.26 (C, Ar); 134. 58 (C, Ar); 133.36 (C, Ar); 131.78 (2C, CH, Ar); 130.62 (CH, Ar); 130.43 (2C, CH, Ar); 128.71 (2C, CH, Ar); 122.37 (CH, C-5 Thiazole); 119.01 (CH, Ar); 118.85 (CH, C-5 Thiazole); 126.62 (2C, CH, Ar); 118.67 (CH, Ar); 117.19 (CH, Ar); 112.60 (CH, Ar); 65.94 ($CH_2$); 37.71 ($CH_2$); 21.40 ($CH_3$). Anal. Calcd. (%) for $C_{29}H_{24}N_4O_2S_2$ (524.65): C, 66.39; H, 4.61; N, 10.68; S, 12.22. Found: C, 66.63; H, 4.6; N, 10.64; S, 12.19. MS (EI, 70 eV): *m*/*z* 524 [M], 247 [$C_6H_5N_3O$], 232 [$C_{12}H_{11}N_2OS$], 215 [$C_{12}H_{10}NOS$], 189 [$C_{11}H_{10}NS$], 174 [$C_{10}H_8NS$], 104 [$C_7H_5O$], 71 [$C_2HNS$].

*6.1.1.6. [2-(3-Trifluoromethyl-phenyl)-thiazol-4-yl]-acetic acid [3-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (**13**).* Yield 72%. White powder, mp: 187–9 °C. IR (KBr, $cm^{-1}$): 3445 (NH), 3065 ($CH_{arom}$), 2920 and 2852 ($CH_{aliph}$), 1675 (C=O Hydrazide), 1600 (C=C), 1521 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): δ 11.32 (s, 1H, NH); 8.2 (s, 1H, −CH=); 8.178 (s, 1H, Ar-H); 7.989–7.948 (m, 4H, Ar-H); 7.779–7.769 (m, 3H, Ar-H); 7.68 (d, 2H, Ar-H); 7.62 (s, 1H, H-Thiazole); 7.523 (s, 1H, H-Thiazole); 7.51–7.432 (m, 2H, Ar-H); 7.165–7.151 (m, 1H, Ar-H); 5.22 (s, 2H, $-CH_2-$); 4.2 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): δ 171.48 (C-2 Thiazole); 167.88 (C-2 Thiazole); 165.68 (C=O, hydrazide); 165.07 (=C−O, Ar); 164.67 (C-4 Thiazole); 158.92 (C-4 Thiazole); 151.95 (−CH=N);

147.01 (C, Ar); 143.28 (C, Ar); 134.39 (C, Ar); 133.36 (C, Ar); 131.12 (CH, Ar); 130.83 (CH, Ar); 130.60 (CH, Ar); 130.45 (2C, CH, Ar); 129.72 (CH, Ar); 126.60 (2C, CH, Ar); 122.38 (CH, C-5 Thiazole); 120.50 (CH, Ar); 119 (CH, Ar); 118.86 (CH, C-5 Thiazole); 118.65 (CH, Ar); 117.15 (CH, Ar); 113.30 ($CF_3$); 112.62 (CH, Ar); 65.94 ($CH_2$); 37.71 ($CH_2$). Anal. Calcd. (%) for $C_{29}H_{21}F_3N_4O_2S_2$ (578.62): C, 60.20; H, 3.66; N, 9.68; S, 11.08. Found: C, 60.40; H, 3.65; N, 9.66; S, 11.03. MS (EI, 70 eV): *m/z* 578 [M], 336 [M- $C_{11}H_7F_3NS$], 270 [$C_{12}H_7F_3NOS$], 243 [$C_{11}H_7F_3NS$], 174 [$C_{10}H_8NS$], 104 [$C_7H_5O$].

*6.1.1.7. (2-p-Tolyl-thiazol-4-yl)-acetic acid {2-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (**14**).* Yield 68%. White powder, mp: 221–3 °C. IR (KBr, $cm^{-1}$): 3445 (NH), 3087 ($CH_{arom}$), 2921 and 2839 ($CH_{aliph}$), 1675 (C=O Hydrazide), 1621 (C=C), 1555 (C=N), 1072 (C–Br). $^1H$ NMR (500 MHz, DMSO-*d*6, ppm): $\delta$ 11.95 (s, 1H, NH); 8.3 (s, 1H, –CH=); 7.683–7.675 (dd, 2H, Ar-H); 7.52 (s, 1H, H-Thiazole); 7.517–7.502 (m, 1H, Ar-H); 7.478–7.454 (dd, 2H, Ar-H); 7.42 (s, 1H, H-Thiazole); 7.378–7.356 (m, 3H, Ar-H); 7.24–7.22 (dd, 2H, Ar-H); 7.154–7.126 (dd, 2H, Ar-H); 5.27 (s, 2H, $-CH_2-$); 4.2 (s, 2H, $-CH_2-$); 2.38 (s, 3H, $-CH_3$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 171.27 (C-2 Thiazole); 170.65 (C=O, hydrazide); 165.53 (C-2 Thiazole); 154.69 (C-4 Thiazole); 151.76 (C-4 Thiazole); 150.98 (C, Ar); 150.37 (–CH=N); 143.02 (C, Ar); 140.19 (C, Ar); 132.87 (C, Ar); 131.04 (2C, CH, Ar); 130.89 (CH, Ar); 130.18 (2C, CH, Ar); 128.91 (2C, CH, Ar); 127.24 (CH, Ar); 126.48 (2C, CH, Ar); 124.15 ($C_{ar}$–Br); 119.96 (CH, C-5 Thiazole); 119.82 (C, Ar); 117.01 (CH, C-5 Thiazole); 114.92 (CH, Ar); 110.87 (CH, Ar); 65.90 ($CH_2$); 38.85 ($CH_2$); 21.40 ($CH_3$). Anal. Calcd. (%) for $C_{29}H_{23}BrN_4O_2S_2$ (603.55): C, 57.71; H, 3.84; N, 9.28; S, 10.62. Found: C, 57.51; H, 3.83; N, 9.26; S, 10.59. MS (EI, 70 eV): *m/z* 604 [M+1], 373 [M- $C_6H_5N_2O$], 254 [$BrC_{10}H_7NS$], 189 [$C_{11}H_{10}NS$].

*6.1.1.8. [2-(3-Trifluoromethyl-phenyl)-thiazol-4-yl]-acetic acid {2-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (**15**).* Yield 64%. White powder, mp: 218–9 °C. IR (KBr, $cm^{-1}$): 3440 (NH), 3079 ($CH_{arom}$), 2919 and 2847 ($CH_{aliph}$), 1674 (C=O Hydrazide), 1615 (C=C), 1550 (C=N), 1070 (C–Br). $^1H$ NMR (500 MHz, DMSO-*d*6, ppm): $\delta$ 11.97 (s, 1H, NH); 8.3 (s, 1H, –CH=); 7.986–7.812 (m, 2H, Ar-H); 7.688–7.663 (m, 3H, Ar-H); 7.526 (s, 1H, H-Thiazole); 7.498–7.485 (dd, 1H, Ar-H); 7.479–7.461 (m, 3H, Ar-H); 7.44 (s, 1H, H-Thiazole); 7.184–7.163 (dd, 1H, Ar-H); 7.148–7.132 (dd, 2H, Ar-H); 5.3 (s, 2H,–$CH_2$); 4.23 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 171.20 (C-2 Thiazole); 170.74 (C=O, hydrazide); 165.62 (C-2 Thiazole); 155.43 (C-4 Thiazole); 153.12 (C-4 Thiazole); 152.21 (C, Ar); 152.01 (-CH=N); 143.20 (C, Ar); 135.37 (C, Ar); 133.36 (C, Ar); 131.64 (2C, CH, Ar); 130.89 (CH, Ar); 130.26 (CH, Ar); 129.27 (CH, Ar); 127.69 ($CF_3$); 127.24 (CH, Ar); 127.18 (CH, Ar); 126.50 (2C, CH, Ar); 124.82 ($C_{ar}$–Br); 120.45 (CH, C-5 Thiazole); 120.36 (C, Ar); 119.76 (CH, Ar); 117.88 (CH, C-5 Thiazole); 114.90 (CH, Ar); 110.89 (CH, Ar); 65.90 ($CH_2$); 39.11 ($CH_2$). Anal. Calcd. (%) for $C_{29}H_{20}BrF3N_4O_2S_2$ (657.52): C 52.97, H 3.07, N 8.52, S 9.75. Found: C 53.10, H 3.08, N 8.532, S 9.73. MS (EI, 70 eV): *m/z* 658 [M+1], 404 [$BrC_{10}H_7NS$], 373 [$BrC_{17}H_{12}N_2S$], 358 [$BrC_{17}H_{12}NS$], 269 [$BrC_{10}H_7NSO$], 254 [$BrC_{10}H_7NS$].

*6.1.1.9. [2-(3-Trifluoromethyl-phenyl)-thiazol-4-yl]-acetic acid {3-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (**16**).* Yield 73%. White powder, mp: 192–7 °C. IR (KBr, $cm^{-1}$): 3445 (NH), 3075 ($CH_{arom}$), 2921 and 2857 ($CH_{aliph}$), 1675 (C=O Hydrazide), 1620 (C=C), 1508 (C=N), 1071 (C–Br). $^1H$ NMR (500 MHz, DMSO-*d*6, ppm): $\delta$ 11.99 (s, 1H, NH); 8.2 (s, 1H, –CH=); 7.998–7.968 (m, 2H, Ar-H); 7.784–7.727 (m, 2H, Ar-H); 7.687–7.665 (dd, 2H, Ar-H); 7.526 (s, 1H, H-Thiazole); 7.498–7.488 (m, 1H, Ar-H); 7.478–7.465 (m, 2H, Ar-H); 7.44 (s, 1H, H-Thiazole); 7.139–7.122 (m, 3H, Ar-H); 5.28 (s, 2H, $-CH_2-$); 4.17 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 171.25 (C-2 Thiazole); 167.12 (C-2 Thiazole); 166.67 (C=O, hydrazide); 165.53 (=C–O, Ar); 160.28 (C-4 Thiazole); 153.32 (C-4 Thiazole); 151.65 (–CH=N); 146.91 (C, Ar); 143.22 (C, Ar); 143.45 (C, Ar); 140.34 (2C, CH, Ar); 133.97 (C, Ar); 131.12 (CH, Ar); 130.83 (CH, Ar); 130.60 (CH, Ar); 129.61 (CH, Ar); 126.53 (2C, CH, Ar); 124.35 ($C_{ar}$–Br); 122.57 (CH, C-5 Thiazole); 120.52 (CH, Ar); 119.22 (CH, Ar); 118.88 (CH, C-5 Thiazole); 118.68 (CH, Ar); 113.28 ($CF_3$); 112.58 (CH, Ar); 65.87 ($CH_2$); 37.70 ($CH_2$). Anal. Calcd. (%) for $C_{29}H_{20}BrF3N_4O_2S_2$ (657.52): C, 52.97; H, 3.07; N, 8.52; S, 9.75. Found: C, 53.99; H, 3.08; N, 8.53; S, 9.74. MS (EI, 70 eV): *m/z* 657 [M], 417 [M-$C_{11}H_7NSF_3$], 404 [M-$C_{10}H_7NSBr$], 254 [$BrC_{10}H_7NS$], 71 [$C_2HNS$].

*6.1.1.10. (2-p-Tolyl-thiazol-4-yl)-acetic acid {4-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (**17**).* Yield 89.4%. Yellow powder, mp: 204–5 °C. IR (KBr, $cm^{-1}$): 3445 (NH), 3079 ($CH_{arom}$), 2921 and 2837 ($CH_{aliph}$), 1674 (C=O Hydrazide), 1621 (C=C), 1514 (C=N), 1070 (C–Br). $^1H$ NMR (500 MHz, DMSO-*d*6, ppm): $\delta$ 11 (s, 1H, NH); 8.78 (d, 2H, Ar-H); 8.41 (s, 1H, –CH=); 8.04 (dd, 2H, Ar-H); 7.82 (dd, 2H, Ar-H); 7.72 (dd, 2H, Ar-H); 7.54 (dd, 2H, Ar-H); 7.52 (s, 1H, H-Thiazole); 7.48 (s, 1H, H-Thiazole); 7.13 (dd, 2H, Ar-H); 5.32 (s, 2H, $-CH_2-$); 4.2 (s, 2H, $-CH_2-$); 2.38 (s, 3H, $-CH_3-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 171.20 (C-2 Thiazole); 168.88 (C=O, hydrazide); 165.53 (C-2 Thiazole); 159.99 (=C–O, Ar); 153.31 (C-4 Thiazole); 151.63 (C-4 Thiazole); 146.89 (–CH=N); 143.22 (C, Ar); 140.39 (C, Ar); 132.62 (C, Ar); 130.99 (2C, CH, Ar); 130.21 (2C, CH, Ar); 128.99 (2C, CH, Ar); 128.54 (C, Ar); 127.73 (2C, CH, Ar); 126.41 (2C, CH, Ar); 124.14 ($C_{ar}$–Br); 119.78 (CH, C-5 Thiazole); 116.81 (CH, C-5 Thiazole); 115.60 (2C, CH, Ar); 65.85 ($CH_2$); 37.83 ($CH_2$); 21.39 ($CH_3$). Anal. Calcd. (%) for $C_{29}H_{23}BrN_4O_2S_2$ (603.55): C, 57.71; H, 3.84; N, 9.28; S, 10.62. Found: C, 57.55; H, 3.83; N, 9.27; S, 10.59. MS (EI, 70 eV): *m/z* 604 [M+1], 373 [M- $C_6H_5N_2O$], 254 [$BrC_{10}H_7NS$], 189 [$C_{11}H_{10}NS$].

*6.1.1.11. [2-(3-Trifluoromethyl-phenyl)-thiazol-4-yl]-acetic acid {4-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide (**18**).* Yield 81%. White powder, mp: 195–6 °C. IR (KBr, $cm^{-1}$): 3442 (NH), 3077 ($CH_{arom}$), 2920 and 2839 ($CH_{aliph}$), 1675 (C=O Hydrazide), 1621 (C=C), 1520 (C=N), 1072 (C–Br). $^1H$ NMR (500 MHz, DMSO-*d*6, ppm): $\delta$ 11.95 (s, 1H, NH); 8.4 (s, 1H, –CH=); 8.227–8.198 (m, 1H, Ar-H); 8.025–8.019 (m, 1H, Ar-H); 8.016 (dd, 2H, Ar-H); 7.864–7.847 (m, 2H, Ar-H); 7.718 (dd, 2H, Ar-H); 7.523 (dd, 2H, Ar-H); 7.516 (s, 1H, H-Thiazole); 7.447 (s, 1H, H-Thiazole); 7.121 (dd, 2H, Ar-H); 5.34 (s, 2H, $-CH_2$); 4.21 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 171.25 (C-2 Thiazole); 168.88 (C=O, hydrazide); 165.59 (C-2 Thiazole); 160.03 (=C–O, Ar); 153.61 (C-4 Thiazole); 151.72 (C-4 Thiazole); 146.76 (–CH=N); 143.12 (C, Ar); 140.41 (C, Ar); 133.09 (2C, CH, Ar); 132.67 (C, Ar); 130.21 (CH, Ar); 129.81 (CH, Ar); 128.48 (C, Ar); 127.67 (2C, CH, Ar); 127.29 (CH, Ar); 126.47 (2C, CH, Ar); 124.11 ($C_{ar}$–Br); 119.78 (CH, C-5 Thiazole); 118.81 (CH, Ar); 116.84 (CH, C-5 Thiazole); 115.61 (2C, CH, Ar); 114.25 ($CF_3$); 65.89 ($CH_2$); 37.82 ($CH_2$). Anal. Calcd. (%) for $C_{29}H_{20}BrF3N_4O_2S_2$ (657.52): C, 52.97; H, 3.07; N, 8.52; S, 9.75. Found: C, 53.00; H, 3.08; N, 8.54; S, 9.73. MS (EI, 70 eV): *m/z* 657 [M], 417 [M-$C_{11}H_7NSF_3$], 404 [M-$C_{10}H_7NSBr$], 254 [$BrC_{10}H_7NS$], 71 [$C_2HNS$].

*6.1.1.12. (4-Oxo-2-phenyl-4H-chromen-7-yloxy)-acetic acid [4-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide (**20**).* Yield 80%. White powder, mp: 234–5 °C. IR (KBr, $cm^{-1}$): 3446 (NH), 3123 ($CH_{arom}$), 2926 ($CH_{aliph}$), 1682 (C=O Chromone), 1650 (C=O hydrazide), 1627 (C=C), 1522 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): $\delta$ 11.59 (s, 1H, NH); 8.3 (s, 1H, –CH=); 7.729 (d, 2H, Ar-H); 7.685 (d, 2H, Ar-H); 7.605–7.599 (d, 1H, Ar-H); 7.585 (t, 1H, Ar-H);

7.568 (d, 2H, Ar-H); 7.526 (s, 1H, H-Thiazole); 7.513 (d, 2H, Ar-H); 7.379 (d, 1H, Ar-H); 7.348 (d, 2H, Ar-H); 7.180–7.163 (m, 2H, Ar-H); 7.143–7.121 (dd, 2H, Ar-H); 6.976 (s, 1H, Ar-H); 5.29 (s, 2H, $-CH_2-$); 4.87 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 176.90 (C=O, chromone); 168.58 (C-2 Thiazole); 167.96 (C=O, hydrazide); 163.73 (C, Ar); 163.48 (C, Ar); 162.73 (C, Ar); 160.12 (C-4 Thiazole); 157.81 (C, Ar); 153.11 (−CH=N); 144.33 (C, Ar); 133.35 (C, Ar); 132.15 (CH, Ar); 131.65 (2C, CH, Ar); 130.89 (C, Ar); 129.78 (2C, CH, Ar); 129.57 (2C, CH, Ar); 129.29 (CH, Ar); 129.11 (2C, CH, Ar); 127.42 (2C, CH, Ar); 126.67 (CH, C-5 Thiazole); 119.31 (C, Ar); 117.83 (CH, Ar); 115.63 (2C, CH, Ar); 115.46 (CH, Ar); 107.23 (CH, Ar); 102.36 (CH, Ar); 65.95 ($CH_2$), 65.88 ($CH_2$). Anal. Calcd. (%) for $C_{34}H_{25}N_3O_5S$ (587.64): C, 69.49; H, 4.29; N, 7.15; S, 5.46. Found: C, 69.34; H, 4.28; N, 7.14; S, 5.44. MS (EI, 70 eV): *m*/*z* 587 [M], 310 [M- $C_{17}H_{13}NOS$], 295 [$C_{17}H_{13}N_2OS$], 278 [$C_{17}H_{13}NOS$], 174 [$C_{10}H_8NS$], 104 [$C_7H_5O$], 71 [$C_2HNS$].

*6.1.1.13. (4-Oxo-2-phenyl-4H-chromen-7-yloxy)-acetic acid [3-(2-phenyl-thiazol-4-ylmethoxy)-benzylidene]-hydrazide* (***21***). Yield 76%. White powder, mp: 202–4 °C. IR (KBr, $cm^{-1}$): 3446 (NH), 3123 ($CH_{arom}$), 2926 ($CH_{aliph}$), 1683 (C=O Chromone), 1649 (C=O Hydrazide), 1627 (C=C), 1522 (C=N). $^1H$ NMR (DMSO-*d*6, 500 MHz, ppm): $\delta$ 11.68 (s, 1H, NH); 8.42 (d, 1H, Ar-H); 8.24 (s, 1H, −CH=); 8.128–8.104 (d, 2H, Ar-H); 7.976–7.962 (m, 3H, Ar-H); 7.742–7.721 (m, 2H, Ar-H); 7.678–7.649 (m, 4H, Ar-H); 7.536 (d, 2H, Ar-H); 7.522 (s, 1H, H-Thiazole); 7.139–7.119 (m, 3H, Ar-H); 6.965 (s, 1H, Ar-H); 5.24 (s, 2H, $-CH_2-$); 4.81 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 176.92 (C=O, chromone); 168.59 (C-2 Thiazole); 167.91 (C=O, hydrazide); 163.82 (C, Ar); 163.71 (C, Ar); 163.28 (C, Ar); 160.72 (C-4 Thiazole); 157.82 (C, Ar); 152.86 (−CH=N); 147.24 (C, Ar); 144.28 (C, Ar); 133.30 (C, Ar); 132.20 (CH, Ar); 131.61 (2C, CH, Ar); 130.73 (CH, Ar); 129.69 (2C, CH, Ar); 129.21 (CH, Ar); 129.11 (2C, CH, Ar); 127.53 (2C, CH, Ar); 126.49 (CH, C-5 Thiazole); 123.47 (CH, Ar); 120.04 (CH, Ar); 119.36 (C, Ar); 117.88 (CH, Ar); 115.61 (CH, Ar); 115.39 (CH, Ar); 107.29 (CH, Ar); 102.38 (CH, Ar); 65.93 ($CH_2$); 65.87 ($CH_2$). Anal. Calcd. (%) for $C_{34}H_{25}N_3O_5S$ (587.64): C, 69.49; H, 4.29; N, 7.15; S, 5.46. Found: C, 69.23; H, 4.28; N, 7.13; S, 5.47. MS (EI, 70 eV): *m*/*z* 587 [M], 238 [$C_{15}H_9O_3$], 174 [$C_{10}H_8NS$], 104 [$C_7H_5O$].

*6.1.1.14. (4-Oxo-2-phenyl-4H-chromen-7-yloxy)-acetic acid {3-[2-(4-bromo-phenyl)-thiazol-4-ylmethoxy]-benzylidene}-hydrazide* (***22***). Yield 77%. White powder, mp: 216–8 °C. IR (KBr, $cm^{-1}$): 3445 (NH), 3075 ($CH_{arom}$), 2921 and 2857 ($CH_{aliph}$), 1685 (C=O Chromone), 1652 (C=O Hydrazide), 1620 (C=C), 1541 (C=N), 1071 (C–Br). $^1H$ NMR (500 MHz, DMSO-*d*6, ppm): $\delta$ 12.1 (s, 1H, NH); 8.43 (d, 1H, Ar-H); 8.2 (s, 1H, −CH=); 8.129–8.115 (dd, 2H, Ar-H); 7.994–7.978 (m, 3H, Ar-H); 7.763–7.745 (m, 2H, Ar-H); 7.685–7.66 (dd, 2H, Ar-H); 7.627–7.615 (dd, 1H, Ar-H); 7.422 (s, 1H, H-Thiazole); 7.257–7.244 (m, 3H, Ar-H); 7.238–7.22 (m, 2H, Ar-H); 7.12 (s, 1H, Ar-H); 5.4 (s, 2H, $-CH_2-$); 4.37 (s, 2H, $-CH_2-$). $^{13}C$ NMR (DMSO-*d*6, 125 MHz, ppm): $\delta$ 176.93 (C=O, chromone); 168.64 (C-2 Thiazole); 168.03 (C=O, hydrazide); 163.91 (C, Ar); 163.68 (C, Ar); 163.22 (C, Ar); 160.70 (C-4 Thiazole); 157.75 (C, Ar); 152.73 (-CH=N); 147.21 (C, Ar); 144.17 (C, Ar); 133.45 (2C, CH, Ar); 133.30 (C, Ar); 132.20 (CH, Ar); 131.61 (2C, CH, Ar); 130.73 (CH, Ar); 129.22 (CH, Ar); 129.19 (2C, CH, Ar); 127.50 (2C, CH, Ar); 127.03 ($C_{ar}$–Br); 126.42 (CH, C-5 Thiazole); 123.39 (CH, Ar); 120.11 (CH, Ar); 119.32 (C, Ar); 115.58 (CH, Ar); 115.27 (CH, Ar); 107.30 (CH, Ar); 102.40 (CH, Ar); 65.94 ($CH_2$); 65.86 ($CH_2$). Anal. Calcd. (%) for $C_{34}H_{24}BrN_3O_5S$ (666.54): C, 61.27; H, 3.63; N, 6.3; S, 4.81. Found: C, 61.32; H, 3.62; N, 6.28; S, 4.8. MS (EI, 70 eV): *m*/*z* 667 [M+1], 372 [$C_{17}H_{12}N_2SBr$], 295 [$C_{17}H_{12}NO_4$], 254 [$C_{10}H_7NSBr$], 238 [$C_{15}H_9O_3$], 182 [$C_7H_4NBr$], 71 [$C_2HNS$].

### 6.2. Pharmacology

#### 6.2.1. Animals

The experiments were performed on adult male Wistar-Bratislava albino rats, weighing 200–250 g. The animals were obtained from the Biobase of University of Medicine and Pharmacy Cluj-Napoca and housed at 25 ± 2 °C, 50 ± 5% relative humidity and 12 h light/dark cycle. They were distributed in groups of ten and had free access to water and food. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of University of Medicine and Pharmacy Cluj-Napoca. Experiments were performed in triplicate.

#### 6.2.2. Anti-inflammatory activity

For the group called Inflammation, each animal was injected i.m. with 0.6 mL/100 g (body weight) of turpentine oil, the pro-inflammatory substance. The same procedure and dose were used for the other groups, too. After that, a 3.2 mg/kg dose, equivalent to 0.0091168 mmol/kg of Meloxicam, the reference standard drug, was administered i.p. to the animals from the reference group. The test groups received the synthesized compounds in an equi-molar dose with Meloxicam, by the i.p. administration of its 1% carboxymethyl celullose suspension.

*6.2.2.1. In vitro phagocytosis test.* Investigation of phagocytic activity of PMN cell was performed as described in literature [31–40] with slight modifications. Blood samples from treated animals were incubated in siliconated tube with E. coli suspension at 37 °C for 30 min. Smears were prepared on slides for microscopy. Cells were fixed with methanol, stained with May-Grünwald Giemsa and examined microscopically. Phagocytic activity was assessed by calculating two parameters: the phagocytic index (PI) (PI% = phagocytes with at least one phagocyted germ from 200 leukocytes counted) and the phagocytic activity (PA) (PA = number of germs phagocyted by 100 leukocytes).

*6.2.2.2. Measurement of serum nitrate and nitrite.* Reduction of nitrate to nitrite occurs in acidic solution by adding $VCl_3$ (100 ml) to serum samples (100 ml), rapidly followed by addition of the Griess reagents, SULF (50 ml) and NEDD (50 ml). The absorbance at 540 nm was measured following incubation (usually 30–45 min).

#### 6.2.3. Statistical analysis

The values are expressed as mean ± S.D. for Inflammation group, Meloxicam group and the healthy population, separately. The comparisons of parameters were performed with Student's *t*-test and correlation analyses were performed using Spearman correlation test. A $p$-value $<0.05$ was accepted as significant. Data were analyzed using the SPSS for Windows computing program (Version 11.0).

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