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Original article

Facile synthesis of bis(4,5-dihydro-1*H*-pyrazole-1-carboxamides) and their thio-analogues of potential PGE_2 inhibitory properties

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ABSTRACT

A variety of bis(3-aryl-4,5-dihydro-1*H*-pyrazole-1-thiocarboxamides) **2a**–**h** were prepared via reaction of bis(2-propen-1-ones) **1a**–**h** with thiosemicarbazide in ethanolic KOH solution. Meanwhile, bis(3-aryl-4,5-dihydro-1*H*-pyrazole-1-carboxamides) **3a**–**d** were obtained through reaction of **1a**–**d** with semicarbazide hydrochloride in refluxing with acetic acid. Anti-inflammatory activity screening of the synthesized compounds **2a**–**f**,**h**; **3a**–**d** (at a dose of 50 mg/kg of body weight) utilizing in vivo acute carrageenan-induced paw oedema standard method in rats exhibited that many of the tested compounds reveal considerable anti-inflammatory properties, especially **2e** and **f** which reveal remarkable activities relative to indomethacin (which was used as a reference standard at a dose of 10 mg/kg of body weight). Ulcerogenic liability for the most promising prepared anti-inflammatory active agents (**2b**,**c**,**e** and **f**) (at a dose of 50 mg/kg of body weight) using indomethacin as a reference standard (at a dose of 10 mg/kg of body weight) indicated that compounds **2b** and **c** exhibit lower ulcer index values than the used reference standard itself. PGE_2 inhibitory properties of the highly promising synthesized anti-inflammatory active agents (**2b**,**c**,**e** and **f**) were determined by PGE_2 assay kit technique, which reveal remarkable activities coincide greatly with the observed anti-inflammatory properties.

1. Introduction

2-Pyrazolines represent an important class of heterocycles due to their highly pronounced biological and pharmacological activities. It has been reported that, 3,5-diaryl-4,5-dihydro-1-thiocarbamoyl-1*H*-pyrazole derivatives possess considerable anti-inflammatory activity with no ulcerogenic effects [1]. Additionally, many analogues were found to be highly inhibitory active agents against both monoamine oxidases A and B (MAO-A and MAO-B) isoforms, which indicate their efficiency in treating Alzheimer's disease [1]. A recent approach in the treatment of many serious diseases such as cancer, AIDS, cardiovascular diseases and Alzheimer's disease is the development of drugs with multiple actions [1,2]. In this regard, compounds such as pyrazolines, which possess different biochemical and pharmacological actions, may be helpful in this respect.

A variety of 1-thiocarbamoyl-2-pyrazoline derivatives and their palladium II complexes were reported to be antiamoebic active agents by screening their in vivo activity against *Entameoba histolytica* [3–7] where, the palladium complexes show better antiamoebic properties than their corresponding ligands [3–5]. Invasive amoebiasis caused by *E. histolytica* is one of the world's most prevalent and fatal infectious diseases in many Asian, South American and tropical parts of Africa [3,8]. Recently, 1-thiocarbamoyl-2-pyrazolines were exhibited anti-tubercular properties against *Mycobacterium tuberculosis* H37Rv [9], beside antidepressant and anticonvulsant activities [10–13].

In the present work, it is intended to investigate synthesis of bis(2-pyrazoline-1-carboxamide) containing compounds as well as their thio-analogues, i.e. construction of new heterocyclic derivatives possessing two pharmacologically active unites, utilizing easily accessible starting chemicals and facile synthetic approaches. The anti-inflammatory properties of the newly prepared heterocycles will be screened. In addition, the PGE_2 inhibitory properties for the most promising anti-inflammatory active agents will be determined.

2. Results and discussion

2.1. Chemistry

Reaction of bis(2-propen-1-ones) **1a**–**h** with thiosemicarbazide in refluxing ethanolic KOH solution afforded directly bis(3-aryl-4,5-dihydro-1*H*-pyrazole-1-thiocarboxamides) **2a**–**h** (Scheme 1). The

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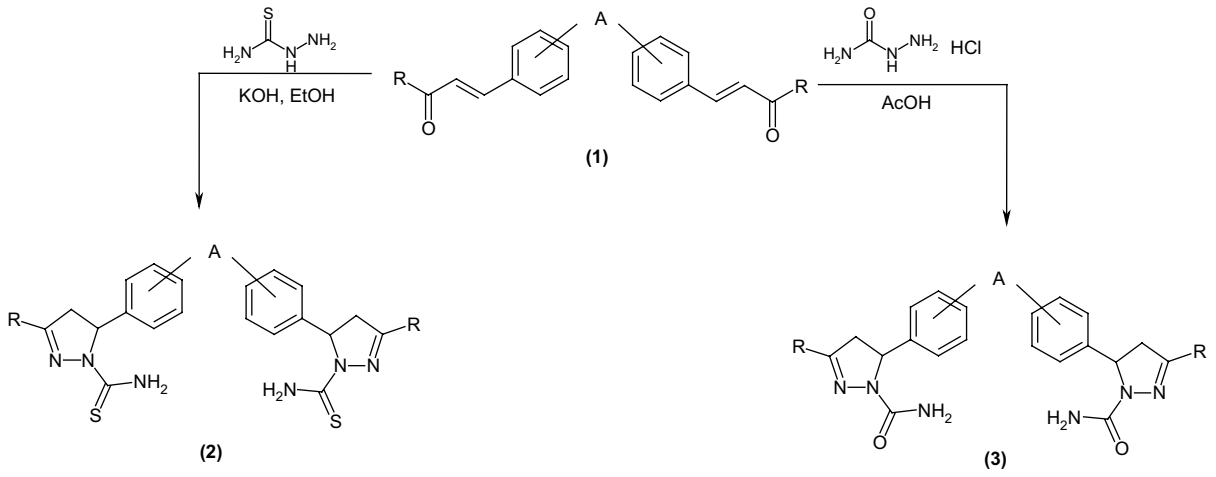

1a, 2a, 3a; A = 2-O$(CH_2)_2$O-2', R = Ph
1b, 2b, 3b; A = 2-O$(CH_2)_2$O-2', R = 4-ClC_6H_4
1c, 2c, 3c; A = 2-O$(CH_2)_2$O-2', R = 4-FC_6H_4
1d, 2d, 3d; A = 2-O$(CH_2)_2$O-2', R = 4-$H_3CC_6H_4$
1e, 2e; A = 2-O$(CH_2)_2$O-2', R = 2-thienyl
1f, 2f; A = 4-O$(CH_2)_2$O-4', R = Ph
1g, 2g; A = 4-O$(CH_2)_2$O-4', R = 4-ClC_6H_4
1h, 2h; A = 4-O$(CH_2)_2$O-4', R = 4-$H_3CC_6H_4$

Scheme 1.

structure of **2a–h** was established through spectroscopic (IR, ^{1}H NMR) as well as elemental analyses data. The IR spectra of **2a–h** reveal the presence of thiocarboxamide amino stretching vibration bands at $\nu = 3482–3123\ cm^{-1}$. ^{1}H NMR spectra of **2a–h** exhibit three signal sets each appears as a double doublets due to the presence of two symmetrical pyrazoline systems, assignable for nonmagnetically equivalent pyrazoline H_2C-4 (at $\delta = 3.00–3.20$, 3.80–3.89) coupled with each other and in turn with the vicinal methine proton *HC*-5 ($\delta = 5.98–6.10$, $J_{gem} = 17.7–18.0$ Hz, $J_{vic} = 3.3–3.6$, 11.1–11.7 Hz), in addition to the two methylene ether linkage which appears as a sharp singlet signal at $\delta = 4.26–4.38$.

Similarly, reaction of 3,3′-[1,2-ethanediylbis(oxy-2,1-phenylene)]bis(1-aryl-2-propen-1-ones) **1a–d** with semicarbazide hydrochloride in refluxing acetic acid gave directly bis(3-aryl-4, 5-dihydro-1*H*-pyrazole-1-carboxamides) **3a–d**. The IR spectra of **3a–d** exhibit the amidic carbonyl amino function (at $\nu = 3489–3132\ cm^{-1}$) beside the amidic carbonyl residue (at $\nu = 1683–1677\ cm^{-1}$). ^{1}H NMR spectral features of **3a–d** are closely similar to those of **2a–h** exhibiting the nonmagnetically equivalent methylene H_2C-4 as double doublet signals at $\delta = 2.91–2.95$, 3.63–3.70 beside the double doublet pyrazoline *HC*-5 at $\delta = 5.54–5.59$ ($J_{gem} = 17.7–18.0$ Hz, $J_{vic} = 5.1–5.4$, 12.0–12.6 Hz).

2.2. Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds **2a–f**,**h**; **3a–d** (at a dose of 50 mg/kg of body weight) was determined in vivo by the acute carrageenan-induced paw oedema standard method in rats [14–17]. The anti-inflammatory properties were recorded after 4 h of inflammation induction and compared with that of indomethacin (at a dose of 10 mg/kg of body weight) which was used as a reference standard. From the obtained results (Table 1, Figs. 1–3), it has been noticed that many of the tested compounds exhibit considerable anti-inflammatory properties, especially **2e** and **f** which reveal remarkable activities with potency (% oedema inhibition of the tested compounds relative to % oedema inhibition of indomethacin) 100.0 and 123.7, respectively.

Structure–activity relationships based on the observed results indicated that, the type of aryl group substitution attached to the 3-position of pyrazoline nucleus plays a controlling role for developing the exhibited pharmacological properties. It has been noticed that, attachment of the phenyl group oriented at the 3-position of pyrazoline heterocycle with a chlorine atom seems more favorable for constructing an anti-inflammatory active agent than the case of using a methyl residue as exhibited in pairs **2b**,**d** (potency, 81.8, 54.5, respectively) and **3b**, **3d** (potency, 67.1, 33.6, respectively). It is also obvious that, adopting a thienyl function (a bio-isostere of phenyl group) at the 3-position of pyrazoline heterocycle seems preferable for obtaining an effective pharmacological agent as exhibited in pairs **2a**, **2e** (potency, 36.3, 100.0, respectively). Alternatively, no precise rule could be attained through the observed results explaining the role of carbamoyl or thiocarbamoyl function in affecting the pharmacological properties.

Table 1
Anti-inflammatory activity of the tested compounds using acute carrageenan-induced paw oedema in rats at a dose of 50 mg/kg of body weight.

Compound	Mean swelling volume (ml)	% Inhibition of oedema	Potency[c]
Control	0.375 ± 0.063[b]	00.0	–
Indomethacin	0.122 ± 0.068[a]	67.5	100.0
2a	0.283 ± 0.063[b]	24.5	36.3
2b	0.168 ± 0.099[a]	55.2	81.8
2c	0.195 ± 0.115[a]	48.0	71.1
2d	0.237 ± 0.112[a]	36.8	54.5
2e	0.122 ± 0.086[a]	67.5	100.0
2f	0.062 ± 0.023[a]	83.5	123.7
2h	0.245 ± 0.166[a,b]	34.7	51.4
3a	0.213 ± 0.087[a]	43.2	64.0
3b	0.205 ± 0.093[a]	45.3	67.1
3c	0.297 ± 0.172[b]	20.8	30.8
3d	0.290 ± 0.086[b]	22.7	33.6

[a] Statistically significant from the control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.
[c] Potency was expressed as % oedema inhibition of the tested compounds relative to % oedema inhibition of indomethacin "reference standard".

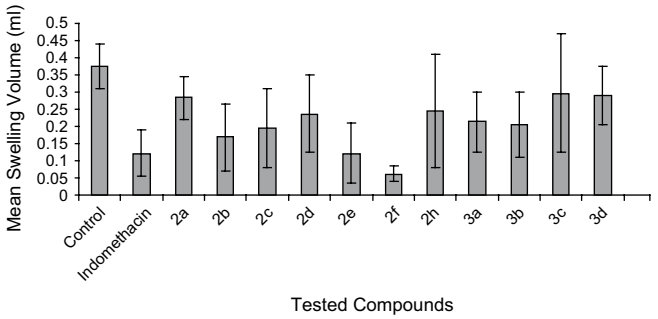

Fig. 1. Mean oedema volume (ml) of the tested compounds.

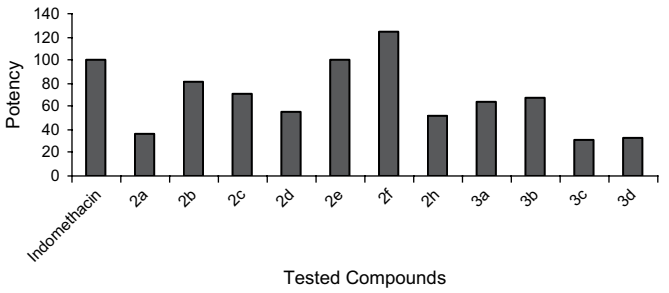

Fig. 3. Anti-inflammatory activity potency of the tested compounds relative to indomethacin which was used as a reference standard.

The most promising anti-inflammatory active agents (**2b**,**c**,**e** and **f**) were selected to be screened for their pharmacological properties at lower doses (20, 10 mg/kg of body weight). From the obtained results (Table 2), it has been noticed that, compound **2f** reveals highly distinguished activity at both 20 and 10 mg/kg of body weight (% inhibition of oedema 79.5, 74.4, respectively) comparable to indomethacin (% inhibition of oedema 67.3) which was used as a reference standard (at a dose of 10 mg/kg of body weight). Based on these observed pharmacological results, the ED_{50} values for the most promising prepared anti-inflammatory active agents (**2b**,**c**,**e** and **f**) were statistically calculated using the linear best fit technique giving values 34.59, 40.84, 28.82 and 23.05, respectively.

2.3. Ulcerogenic liability

Ulcerogenic liability of the most promising prepared anti-inflammatory active agents (**2b**,**c**,**e** and **f**) was determined using the standard method [18,19] at a dose of 50 mg/kg of body weight using indomethacin (at a dose of 10 mg/kg of body weight) as a reference standard. From the obtained data (Table 3, Fig. 4) it has been noticed that, compounds **2b** and **c** reveal the lowest ulcer index values (12.16, 11.51, respectively) and they are considered more safer than indomethacin (reference standard) itself which reveals ulcer index 13.49.

2.4. PGE_2 inhibitory properties

PGE_2 inhibitory properties of the most promising prepared anti-inflammatory active agents (**2b**,**c**,**e** and **f**) were determined using the previously described 6-day-old air pouch standard method in rats [20] via a commercial PGE_2 assay kit (R&D Systems, Inc., Minneapolis, USA). From the obtained results (Table 4, Fig. 5), it has been noticed that all the tested compounds effectively reduce PGE_2 level. It has also been noticed that the PGE_2 inhibitory properties of the tested compounds coincide greatly with our described anti-inflammatory properties. In other words, compound **2f**, which exhibits the highest promising anti-inflammatory properties also reveals the highest inhibitory level of PGE_2 (83.33 *pg*/ml) comparable with indomethacin (98.33 *pg*/ml) which was used as a reference standard in this study. Similarly, compounds **2b**,**c** and **e** reveal considerable PGE_2 inhibitory activities (110.83, 114.67, 96.50 *pg*/ml, respectively).

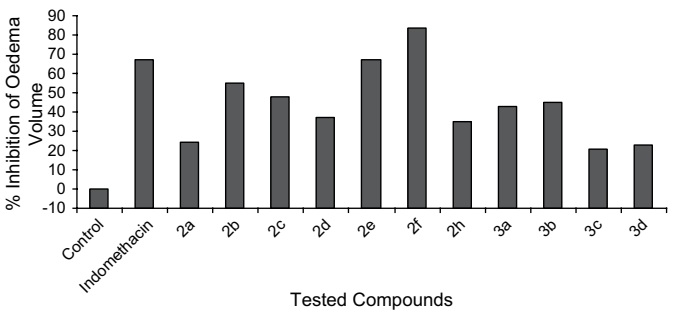

Fig. 2. % Inhibition of oedema for the tested compounds.

3. Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 digital melting point apparatus. IR spectra (KBr) were recorded on a Bruker Vector 22 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian MERCURY 300 (300 MHz) spectrometer. The starting compounds **1a**–**h** were prepared according to the previously reported procedures [18].

3.1. Synthesis of bis(3-aryl-4,5-dihydro-1H-pyrazole-1-thiocarboxamides) *2a*–*h* (general procedure)

A solution of **1a**–**h** (2.5 mmol) and thiosemicarbazide (5 mmol) in absolute ethanol (25 ml) containing KOH (6 mmol) was boiled under reflux for the appropriate time. The separated solid while refluxing was collected, washed with water and crystallized from a suitable solvent affording **2a**–**e**. In case of **1f**–**h**, the clear reaction mixture was stored at room temperature (25 °C) overnight so, the separated solid was collected, washed with water and crystallized from a suitable solvent affording **2f**–**h**.

3.1.1. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis(4,5-dihydro-3-phenyl-1H-pyrazole-1-thiocarboxamide) (*2a*)

Reaction time 1 h, pale yellow crystals from *N*,*N*-dimethylformamide – water as 1:1 v/v, mp 285–286 °C, yield 52%. IR: ν_{max}/cm^{-1} 3482, 3360 (NH_2), 1673, 1595, 1566, 1450 (C═N, C═C, C═S). ^{1}H NMR (DMSO-d_6): δ 3.03 (dd, 2H, upfield H's of pyr. H-4,

Table 2
Anti-inflammatory activity of the most promising prepared anti-inflammatory active agents using acute carrageenan-induced paw oedema in rats at doses of 20 and 10 mg/kg of body weight.

Compound		Mean swelling volume (ml)	% Inhibition of oedema
Control		0.352 ± 0.054[b]	00.0
Indomethacin		0.115 ± 0.038[a]	67.3
2b	20 mg/kg	0.163 ± 0.024[a]	53.7
	10 mg/kg	0.175 ± 0.025[a]	50.3
2c	20 mg/kg	0.200 ± 0.025[a]	43.2
	10 mg/kg	0.208 ± 0.038[a]	40.9
2e	20 mg/kg	0.135 ± 0.020[a]	61.6
	10 mg/kg	0.142 ± 0.018[a]	59.7
2f	20 mg/kg	0.072 ± 0.027[a]	79.5
	10 mg/kg	0.090 ± 0.014[a]	74.4

[a] Statistically significant from the control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.

Table 3
Ulcerogenic liability of the most promising prepared anti-inflammatory active agents

Compound	Number of animals with ulcer	% Incidence divided by 10	Average of ulcer number	Average severity	Ulcer index
Control	0/6	0.00	0.00	0.00	0.00
Indomethacin	5/6	8.33	3.83	1.33	13.49
2b	5/6	8.33	2.83	1.00	12.16
2c	4/6	6.67	3.67	1.17	11.51
2e	5/6	8.33	4.33	1.67	14.33
2f	6/6	10	7.17	1.33	18.50

H-4′, $J = 3.6$, 18.0 Hz), 3.87 (dd, 2H, downfield H's of pyr. H-4, H-4′, $J = 11.7$, 18.0 Hz), 4.36 (s, 4H, 2OCH_2), 6.09 (dd, 2H, pyr. H-5, H-5′, $J = 3.3$, 11.4 Hz), 6.87–7.95 (m, 22H, 18 arom. H + 2NH_2). Anal. Calcd. for $C_{34}H_{32}N_6O_2S_2$ (620.77): C, 65.78; H, 5.20; N, 13.54. Found: C, 65.99; H, 5.34; N, 13.65.

*3.1.2. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-thiocarboxamide] (**2b**)*

Reaction time 1.5 h, pale yellow crystals from *N*,*N*-dimethylformamide – water as 3:1 v/v, mp 267–269 °C, yield 41%. IR: ν_{max}/cm^{-1} 3443, 3231, 3123 (NH_2), 1671, 1581, 1462 (C═N, C═C, C═S). ^{1}H NMR (DMSO-d_6): δ 3.03 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.6$, 18.0 Hz), 3.81 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.4$, 18.0 Hz), 4.31 (s, 4H, 2OCH_2), 6.05 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.6$, 11.4 Hz), 6.85–7.81 (m, 16H, arom. H), 7.95 (br. s, 2H, NH_2), 8.00 (br. s, 2H, NH_2). Anal. Calcd. for $C_{34}H_{30}Cl_2N_6O_2S_2$ (689.66): C, 59.21; H, 4.38; N, 12.19. Found: C, 59.40; H, 4.44; N, 12.42.

*3.1.3. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[4,5-dihydro-3-(4-fluorophenyl)-1*H*-pyrazole-1-thiocarboxamide] (**2c**)*

Reaction time 1 h, colourless crystals from *N*,*N*-dimethylformamide – water as 2:1 v/v, mp 264–266 °C, yield 43%. IR: ν_{max}/cm^{-1} 3446, 3232, 3124 (NH_2), 1673, 1581, 1465 (C═N, C═C, C═S). ^{1}H NMR (DMSO-d_6): δ 3.04 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.3$, 18.0 Hz), 3.84 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.4$, 18.0 Hz), 4.34 (s, 4H, 2OCH_2), 6.07 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.3$, 11.1 Hz), 6.87–7.87 (m, 16H, arom. H), 7.91 (br. s, 2H, NH_2), 7.97 (br. s, 2H, NH_2). Anal. Calcd. for $C_{34}H_{30}F_2N_6O_2S_2$ (656.75): C, 62.18; H, 4.60; N, 12.80. Found: C, 62.08; H, 4.46; N, 12.97.

*3.1.4. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[4,5-dihydro-3-(4-methylphenyl)-1*H*-pyrazole-1-thiocarboxamide] (**2d**)*

Reaction time 1 h, colourless crystals from *N*,*N*-dimethylformamide – methanol as 1:3 v/v, mp 255–257 °C, yield 56%.

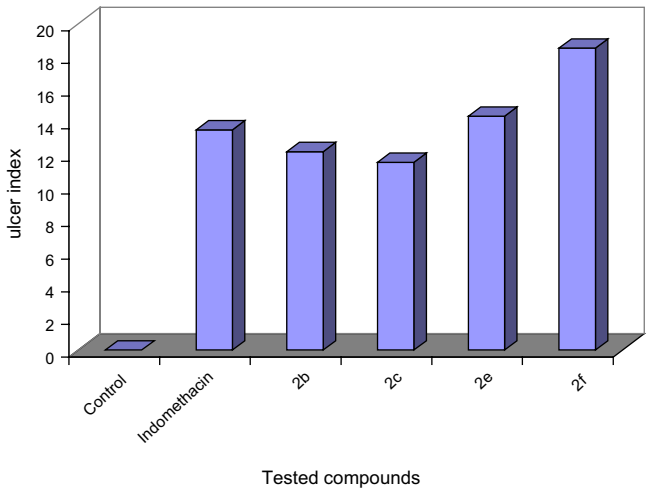

Fig. 4. Ulcer index for the most promising prepared anti-inflammatory active agents.

IR: ν_{max}/cm^{-1} 3438, 3239, 3130 (NH_2), 1659, 1583, 1470 (C═N, C═C, C═S). ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 6H, 2ArCH_3), 3.00 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.6$, 17.7 Hz), 3.80 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.7$, 18.0 Hz), 4.35 (s, 4H, 2OCH_2), 6.07 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.6$, 11.4 Hz), 6.87–7.68 (m, 16H, arom. H), 7.75 (br. s, 4H, 2NH_2). Anal. Calcd. for $C_{36}H_{36}N_6O_2S_2$ (648.82): C, 66.64; H, 5.59; N, 12.95. Found: C, 66.50; H, 5.51; N, 12.89.

*3.1.5. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[4,5-dihydro-3-(2-thienyl)-1*H*-pyrazole-1-thiocarboxamide] (**2e**)*

Reaction time 1 h, pale yellow crystals from *N*,*N*-dimethylformamide – water as 2:1 v/v, mp 269–270 °C, yield 44%. IR: ν_{max}/cm^{-1} 3443, 3230, 3125 (NH_2), 1660, 1580, 1472 (C═N, C═C, C═S). ^{1}H NMR (DMSO-d_6): δ 3.04 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.6$, 18.0 Hz), 3.89 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.4$, 17.7 Hz), 4.38 (s, 4H, 2OCH_2), 6.10 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.6$, 11.4 Hz), 6.87–7.72 (m, 14H, arom. H), 7.94 (br. s, 4H, 2 NH_2). Anal. Calcd. for $C_{30}H_{28}N_6O_2S_4$ (632.83): C, 56.93; H, 4.46; N, 13.28. Found: C, 56.71; H, 4.33; N, 13.37.

*3.1.6. 5,5′-[1,2-Ethanediylbis(oxy-4,1-phenylene)]bis(4,5-dihydro-3-phenyl-1*H*-pyrazole-1-thiocarboxamide] (**2f**)*

Reaction time 12 h, yellow crystals from ethanol, mp 145–147 °C, yield 45%. IR: ν_{max}/cm^{-1} 3434, 3263 (NH_2), 1566, 1509, 1466 (C═N, C═C, C═S). ^{1}H NMR ($CDCl_3$): δ 3.20 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.6$, 17.7 Hz), 3.82 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.4$, 17.7 Hz), 4.26 (s, 4H, 2OCH_2), 6.00 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.6$, 11.4 Hz), 6.87–7.76 (m, 22H, 18 arom. H + 2NH_2). Anal. Calcd. for $C_{34}H_{32}N_6O_2S_2$ (620.77): C, 65.78; H, 5.20; N, 13.54. Found: C, 65.62; H, 5.09; N, 13.46.

*3.1.7. 5,5′-[1,2-Ethanediylbis(oxy-4,1-phenylene)]bis[3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-thiocarboxamide] (**2g**)*

Reaction time 24 h, yellow crystals from ethanol, mp 142–144 °C, yield 29%. IR: ν_{max}/cm^{-1} 3429, 3260, 3139 (NH_2), 1576, 1509, 1460 (C═N, C═C, C═S). ^{1}H NMR ($CDCl_3$): δ 3.16 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.6$, 17.7 Hz), 3.80 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.4$, 17.7 Hz), 4.27 (s, 4H, 2OCH_2), 6.00 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.6$, 11.4 Hz), 6.87–7.68 (m, 20H, 16 arom. H + 2NH_2). Anal. Calcd. for $C_{34}H_{30}Cl_2N_6O_2S_2$ (689.66): C, 59.21; H, 4.38; N, 12.19. Found: C, 59.37; H, 4.49; N, 12.05.

*3.1.8. 5,5′-[1,2-Ethanediylbis(oxy-4,1-phenylene)]bis[4,5-dihydro-3-(4-methylphenyl)-1*H*-pyrazole-1-thiocarboxamide] (**2h**)*

Reaction time 24 h, yellow crystals from ethanol, mp 128–130 °C, yield 25%. IR: ν_{max}/cm^{-1} 3431, 3368, 3261, 3137 (NH_2), 1609, 1509, 1463 (C═N, C═C, C═S). ^{1}H NMR ($CDCl_3$): δ 2.41 (s, 6H, 2 ArCH_3), 3.18 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 3.6$, 17.7 Hz), 3.80 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 11.4$, 17.7 Hz), 4.26 (s, 4H, 2OCH_2), 5.98 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 3.6$, 11.4 Hz), 6.87–7.64 (m, 20H, 16 arom. H + 2NH_2). Anal. Calcd. for $C_{36}H_{36}N_6O_2S_2$

Table 4
PGE_2 inhibitory activity of the most promising prepared anti-inflammatory active agents.

Compound	Concentration of PGE_2 (pg/ml)
Control	533.33 ± 19.26[b]
Indomethacin	98.33 ± 3.33[a]
2b	110.83 ± 5.90[a]
2c	114.67 ± 1.84[a]
2e	96.50 ± 4.47[a]
2f	83.33 ± 4.84[a]

Results are means of 6 experiments ± standard error.
[a] Statistically significant from the control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.

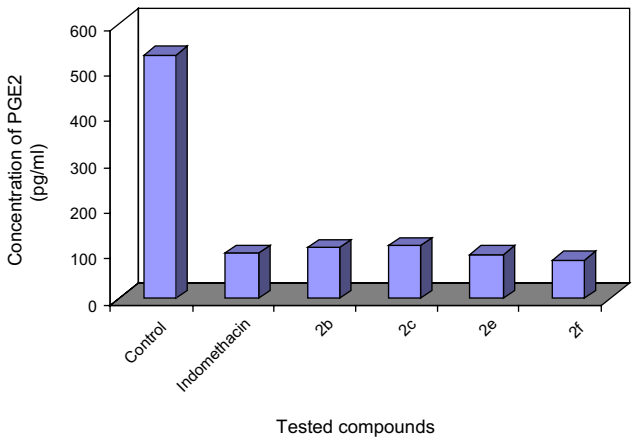

Fig. 5. PGE_2 inhibitory activity of the most promising prepared anti-inflammatory active agents.

(648.82): C, 66.64; H, 5.59; N, 12.95. Found: C, 66.86; H, 5.79; N, 12.87.

3.2. Synthesis of bis(3-aryl-4,5-dihydro-1H-pyrazole-1-carboxamides) **3a–d** (general procedure)

A solution of **1a–d** (2.5 mmol) and semicarbazide hydrochloride (5 mmol) in glacial acetic acid (25 ml) was boiled under reflux for the appropriate time. The separated solid upon storing the reaction mixture to cool at room temperature (25 °C) was collected, washed with water and crystallized from a suitable solvent affording **3a–d**.

3.2.1. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis(4,5-dihydro-3-phenyl-1H-pyrazole-1-carboxamide) (**3a**)

Reaction time 3 h, colourless crystals from *N*,*N*-dimethylformamide – methanol as 3:1 v/v, mp 282–284 °C, yield 48%. IR: ν_{max}/cm^{-1} 3481, 3263, 3177, 3135 (NH_2), 1677 (C═O), 1588, 1449 (C═N, C═C). ^1H NMR (DMSO-d_6): δ 2.94 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 5.1, 17.7$ Hz), 3.70 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 12.3, 17.7$ Hz), 4.33 (s, 4H, $2OCH_2$), 5.59 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 5.1, 12.3$ Hz), 6.45 (s, 4H, $2NH_2$), 6.86–7.70 (m, 18H, arom. H). Anal. Calcd. for $C_{34}H_{32}N_6O_4$ (588.65): C, 69.37; H, 5.48; N, 14.28. Found: C, 69.54; H, 5.61; N, 14.49.

3.2.2. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide] (**3b**)

Reaction time 4 h, colourless crystals from *N*,*N*-dimethylformamide, mp 291–293 °C, yield 43%. IR: ν_{max}/cm^{-1} 3487, 3266, 3184, 3132 (NH_2), 1678 (C═O), 1588, 1451 (C═N, C═C). ^1H NMR (DMSO-d_6): δ 2.95 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 5.4, 17.7$ Hz), 3.63 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 12.6, 18.0$ Hz), 4.27 (s, 4H, $2OCH_2$), 5.55 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 5.4, 12.3$ Hz), 6.48 (s, 4H, $2NH_2$), 6.86–7.69 (m, 16H, arom. H). Anal. Calcd. for $C_{34}H_{30}Cl_2N_6O_4$ (657.54): C, 62.10; H, 4.60; N, 12.78. Found: C, 61.86; H, 4.49; N, 12.94.

3.2.3. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[4,5-dihydro-3-(4-fluorophenyl)-1H-pyrazole-1-carboxamide] (**3c**)

Reaction time 4 h, colourless crystals from *N*,*N*-dimethylformamide – water as 4:1 v/v, mp 271–273 °C, yield 45%. IR: ν_{max}/cm^{-1} 3489, 3267, 3187, 3135 (NH_2), 1682 (C═O), 1584, 1449 (C═N, C═C). ^1H NMR (DMSO-d_6): δ 2.94 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 5.1, 17.7$ Hz), 3.65 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 12.0, 17.7$ Hz), 4.30 (s, 4H, $2OCH_2$), 5.56 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 5.1, 12.0$ Hz), 6.47 (s, 4H, $2NH_2$), 6.86–7.74 (m, 16H, arom. H). Anal. Calcd. for $C_{34}H_{30}F_2N_6O_4$ (624.63): C, 65.37; H, 4.84; N, 13.46. Found: C, 65.56; H, 4.92; N, 13.70.

3.2.4. 5,5′-[1,2-Ethanediylbis(oxy-2,1-phenylene)]bis[4,5-dihydro-3-(4-methylphenyl)-1H-pyrazole-1-carboxamide] (**3d**)

Reaction time 4 h, colourless crystals from *N*,*N*-dimethylformamide – methanol as 2:3 v/v, mp 278–280 °C, yield 52%. IR: ν_{max}/cm^{-1} 3480, 3269, 3196, 3140 (NH_2), 1683 (C═O), 1588, 1448 (C═N, C═C). ^1H NMR (DMSO-d_6): δ 2.28 (s, 6H, $2ArCH_3$), 2.91 (dd, 2H, upfield H's of pyr. *H*-4, *H*-4′, $J = 5.1, 17.7$ Hz), 3.64 (dd, 2H, downfield H's of pyr. *H*-4, *H*-4′, $J = 12.0, 18.0$ Hz), 4.31 (s, 4H, $2OCH_2$), 5.54 (dd, 2H, pyr. *H*-5, *H*-5′, $J = 5.1, 12.0$ Hz), 6.40 (s, 4H, $2NH_2$), 6.86–7.58 (m, 16H, arom. H). Anal. Calcd. for $C_{36}H_{36}N_6O_4$ (616.70): C, 70.12; H, 5.88; N, 13.63. Found: C, 70.20; H, 5.99; N, 13.82.

3.3. Anti-inflammatory activity screening

Anti-inflammatory activity screening for the prepared compounds was determined in vivo by the acute carrageenan-induced paw oedema standard method in rats [14–17]. Wister albino rats of either sex (pregnant female animals were excluded) weighing 160–190 g were divided into 13 groups of 6 animals each. Administration of indomethacin (reference standard at a dose of 10 mg/kg of body weight) and the tested compounds (**2a–f**,**h** and **3a–d**) dissolved in DMSO, at a dose of 50 mg/kg (body weight) was given intraperitoneally 1 h before induction of inflammation. The control group was given DMSO only. Carrageenan paw oedema was induced by subcutaneous injection of 1% solution of carrageenan in saline (0.1 ml/rat) into the right hind paw of rats. Paw volumes were measured volumetrically after 4 h of inflammation induction with plethysmometer 7140 (UGO BASILE, Italy) and compared with the initial hind paw volume of each rat for determining the oedema volume. Data were collected, checked, revised and analyzed. Quantitative variables from normal distribution were expressed as means ± SE "standard error". The significant difference between groups was tested by using one-way ANOVA followed by post hoc test and the chosen level of significance was $p < 0.05$.

The anti-inflammatory activity was expressed as percentage inhibition of oedema volume in treated animals in comparison with the control group (Table 1, Figs. 1–3):

$$\%\text{Inhibition of oedema} = \frac{V_c - V_t}{V_c} \times 100$$

where, V_c and V_t are the volumes of oedema for the control and drug-treated animal groups, respectively.

Potency of the tested compounds was calculated relative to indomethacin "reference standard" treated group according to the following equation.

$$\text{Potency} = (\%\text{Oedema inhibition of tested compound treated group})/(\%\text{Oedema inhibition of indomethacin treated group})$$

Additionally, the most promising prepared anti-inflammatory active agents (**2b**,**c**,**e** and **f**) were screened for their pharmacological properties at lower doses (20, 10 mg/kg body weight) using the same described experimental technique (Table 2).

3.4. Ulcerogenic liability

The ulcerogenic liability was determined in albino rats following the previously reported standard method [18,19]. Rats of either sex (pregnant female rats were excluded) weighing 130–150 g were divided into 6 groups of 6 animals each. The animals were fasted 18 h before drug administration. Indomethacin (reference standard

at a dose of 10 mg/kg of body weight) and the tested compounds (at a dose of 50 mg/kg of body weight), were suspended in saline solution by the aid of few drops of Tween 80 and were administered orally for three successive days to fasted rats. The control group animals were given saline with few drops of Tween 80. One hour following the last dose, the animals were sacrificed by cervical dislocation and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens (10×) for the presence of lesions and erosions. The ulcer index was calculated (Table 3, Fig. 4) and the degree of ulcerogenic effect was expressed in terms of:

1. percentage incidence of ulcer divided by 10;
2. average number of ulcers per stomach;
3. average severity of ulcers.

The ulcer index is the value that resulted from the sum of the above three values.

3.5. Measurement of PGE_2 level

Measurement of PGE_2 level was determined by the previously described 6-day-old air pouch standard method in rats [20]. Male albino rats weighing 200–250 g were divided into 6 groups of 6 animals each. The air pouch was induced as follows, on the first day of the experiment; 20 ml of air was injected subcutaneously in the back of each rat. Two days later, another 10 ml of air was injected at the same site. On the fifth day after the first injection, a further 10 ml of air was injected into the pouch. Then, 24 h later and before injecting the pouch with carrageenan (2 ml of 1% solution in saline), four groups of animals were treated orally with the tested compounds (**2b**,**c**,**e** and **f**) "at a dose of 50 mg/kg of body weight", one group with indomethacin (reference standard) "at a dose of 10 mg/kg of body weight" suspended in saline solution by the aid of few drops of Tween 80 and the last group with sterile saline (control group). All injections were conducted under light ether anaesthesia. Six hours after the carrageenan injection, animals were lightly anaesthetised with ether and the contents of the pouch were aspirated using a Pasteur pipette and transferred into graduated plastic tubes kept in ice. The bulk of the exudates was frozen and stored at −20 °C until required for PGE_2 assay. PGE_2 was measured by an ELISA (Beckman Biomek™ 1000 automated laboratory workstation apparatus) technique using PGE_2 assay kit supplied by R&D Systems Inc., Minneapolis, USA, according to the manufacturer's specifications.

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