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Synthesis of 5-Phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic Acid Derivatives of Potential Anti-inflammatory Activity

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A series of 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid derivatives **4**–**10** were synthesized by rearrangement of 4-(3-pyridyl)-hydrazono-2-phenyl-2-oxazolin-5-one **3** in the presence of different nucleophiles to afford derivatives **4**, **7**, and **8**, while hydroxamic acid derivative **6** was prepared from reaction of methyl ester **4** with hydroxylamine hydrochloride. Semicarbazide **9** and thiosemicarbazide **10**, derivatives of the 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid, were synthesized via hydrazide **8** with potassium cyanate and appropriate isothiocyanate, respectively. The structures of the synthesized compounds were confirmed by elemental analyses, IR, ^{1}H-NMR, and mass spectra. The results of the anti-inflammatory activity of the synthesized derivatives showed that most of the tested compounds **4**–**10** showed significant inhibition against carrageenan-induced rat paw edema in albino rats. Derivatives **4** and **8** showed promising results and were found to be equipotent or more potent than Indomethacin and Celecoxib as reference drugs at two dose levels, 5 and 10 mg/kg, and they have no ulcerogenic activity.

Keywords: Triazole carboxylic acid / Carboxamides / Thiosemicarbazides / Anti-inflammatory / Ulcerogenic

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Introduction

Various derivatives of 1,2,4-triazole have been reported to possess interesting biological activities such as hypoglycemic [1], analgesic [2], anti-inflammatory [2–11], antibacterial [12–15], antifungal [16–18], anticancer [19, 20], antiviral [21], and antidepressant activities [22]. 3(5)-Substituted 1,2,4-triazole-5(3)-carboxylic acid, iminosemicarbazides, semicarbazides, and thiosemicarbazides showed different significant hypoglycemic activity [1].

Several studies of the anti-inflammatory activity of 1,5-diaryl-1*H*-1,2,4-triazole-3-carboxylic acid derivatives demonstrated that a halogen substituent in para position on the phenyl rings is necessary for potent anti-inflammatory activity [5]. 1,5-Diaryl-3-alkylthio-1*H*-1,2,4-triazoles and corresponding sulfoxides and sulfones, were prepared and tested for anti-arthritic potency. It was found that sulfones and sulfides derivatives were more potent as compared to sulfoxides [6]. 5-(2-Naphthyloxymethyl)-4-substituted-1,2,4-triazole-3-thione derivatives have been prepared and evaluated as orally active anti-inflammatory agents with reduced side effects [10].

New *S*-alkylated 5-(2,3- and 4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol and 5-(2,3-and 4-methoxyphenyl)-phenyl-4*H*-1,2,4-triazole-3-thiol have been synthesized and exhibited anti-inflammatory activity [11].

Various oxadiazole, triazole, thiadiazole, and triazine drivatives of Indomethacin have been synthesized and tested for anti-inflammatory activity. The test compounds inhibited the induction of gastric mucosal lesions and their protective effects may be related to inhibition of lipid peroxidation in gastric mucosa [9]. 1-(4-Substituted phenyl)-5-(6-methyl-5-nitropyridin-2-yl)-1*H*-1,2,4-triazole-3-carboxylic acid ester and hydrazide exhibited enhanced anti-inflammatory activity more than Indomethacin [2].

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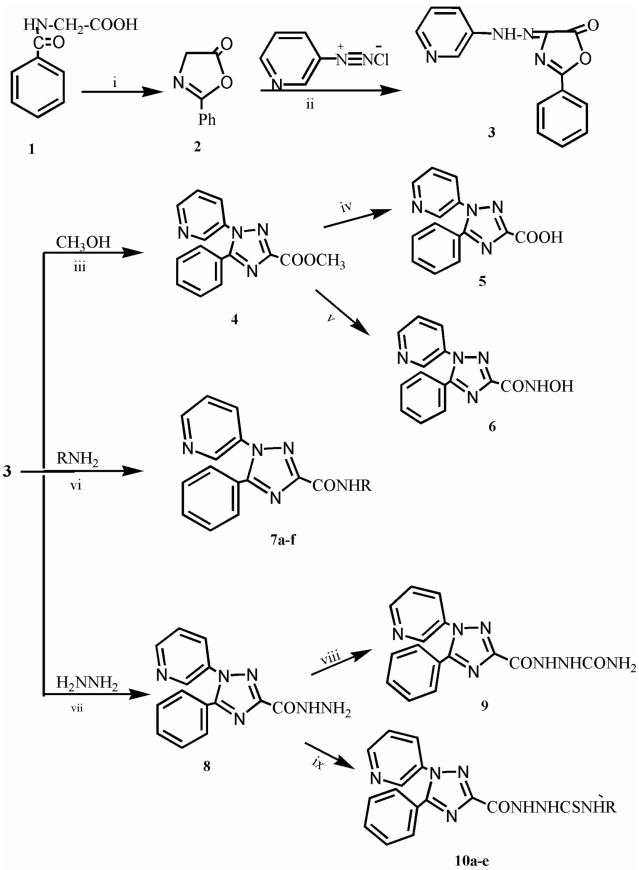
Promoted by these findings, it seemed of interest to synthesize 5-(3-pyridyl)-1-phenyl-1*H*-1,2,4-triazole-3-carboxylic acid derivatives, and investigate their anti-inflammatory activity to study the effect of positional substitution on the biological activity.

Results and discussion

The target compounds were synthesized according to the Scheme 1. Diazotization of 3-aminopyridine followed by reaction with the active methylene compound (2-phenyl-2-oxazolin-5-one) **2** had been described to provide the key intermediate 4-(3-pyridyl)-hydrazono-2-phenyl-2-oxazolin-5-one **3** [23]. IR spectra of **3** showed a weak and broad band at 3615–3300 cm^{-1}. The broadening of the NH stretching band indicates the effect of possible intramolecular hydrogen bonding with nitrogen atom of oxazolinone [24]. Strong stretching bands occurred at 1794 (C=O) of the lactone, bands at 1626 (C=N), 1229 (C–O–C), and 1600–1585 (C=C) cm^{-1} [25].



i) Ac_2O/Δ, (ii) NaOAc, (iii) KOH 5%, (iv) KOH 20%/HCl, (v) NH_2OH, (vi) CH_3OH or AcOH, (vii) CH_3OH, (viii) KOCN/AcOH, (ix) R-NCS/C_2H_5OH.
R: a = H; b = CH_3; c = C_6H_5; d = C_6H_4-4-CH_3; e = C_6H_4-4-Cl; f = C_6H_4-4-Br
R′: a = CH_3; b = C_2H_5; c = C_6H_{11}; d = C_6H_5; e = C_6H_4-4-CH_3; f = C_6H_4-4-Br

Scheme 1. Synthesis of 1,2,4-triazole-3-carboxylic acid derivates **4–10**.

Rearrangement of **3** upon treatment with methanolic potassium hydroxide afforded the triazole methyl ester **4**. Hydrolysis of compound **4** in aqueous ethanolic potassium hydroxide afforded the corresponding carboxylic acid 5. ^{1}H-NMR (DMSO-d_6) showed the appearance of broad singlet signal integrated for one proton of carboxylic group exchangeable on addition of D_2O.

Addition of carboxylic acid ester **4** to hydroxylamine hydrochloride at room temperature gave hydroxamic acid **6** in good yield.

Rearrangement of compound **3** with methanolic ammonia or methanolic methyl amine gave amides **7a** and **7b** respectively. While treatment of compound 3 with primary aromatic amines in acidic medium gave the target anilides **7c–f**. Physical and spectral data are listed in Tables 1 and 2.

Reaction of compound **3** with hydrazine hydrate in methanol afforded hydrazide **8**. While semicarbazide derivative **9** was synthesized via hydrazide **8** with potassium cyanate in glacial acetic acid. The preparation of N^4-substituted-5-phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid thiosemicarbazides **10a–f** involved the reaction of the hydrazide **8** with equimolar amounts of alkyl or aryl isothiocyanates in presence of fused sodium acetate, using ethanol as the solvent. Physical and spectral data are listed in Tables 3 and 4.

Anti-inflammatory activity

The anti-inflammatory activities of the tested compounds were evaluated by carrageenan-induced paw edema by the method of Hernandez-Perez et al [26]. The compounds were tested at 5 and 10 mg/kg oral dose and were compared with Indomethacin and Celecoxib as reference drugs. The results are listed in Table 5. The histograms, Figures 1, 2, 3, 4, 5, and 6 showed the per cent inhibition of edema induced by the reference drugs and tested compounds, respectively. Results showed that most of the tested compounds from **4–10** showed significant ($P < 0.05$) inhibition against carrageenan-induced edema in rats.

The anti-inflammatory activities of the tested compounds ranged from 45.6–94.5%, whereas standard drug Indomethacin showed an activity of 78.4% after 3 h. The anti-inflammatory activity of amide derivatives **7a–d** and **7f** revealed that the unsubstituted amide **7a** was found to be of lower activity (46.9%) than substituted derivatives **7b–d** and **7f** (48.0–78.4%).

Table 1. Physical constants of 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid derivatives **7a–f**

Compound	R	Mp. [°C] Cryst. Solvent	Yield [%]	Mol. Formula	Microanalyses Calcd./Found		
				Mol. Wt.	% C	% H	% N
7a	–H	204–206	71	$C_{14}N_{11}N_5$	63.39	4.18	26.40
		ethanol		(265.27)	62.99	4.17	26.43
7b	$-CH_3$	183–185	76	$C_{15}H_{13}N_5O$	64.51	4.69	25.08
		ethanol		(279.3)	64.78	5.24	25.16
7c	$-C_6H_5$	218–220	60	$C_{20}H_{15}N_5O$	70.37	4.43	20.52
		ethanol		(341.37)	69.82	4.53	20.48
7d	$-C_6H_4-4-CH_3$	234–236	52	$C_{21}H_{17}N_5O$	70.79	4.82	19.71
		ethanol		(355.39)	70.50	5.13	19.73
7e	$-C_6H_4-4-Cl$	237–239	44	$C_{20}H_{14}ClN_5O$	63.92	3.75	18.64
		ethanol		(375.81)	63.66	4.11	18.68
7f	$-C_6H_4-4-Br$	223–225	41	$C_{20}H_{14}BrN_5O$	57.16	3.36	16.66
		ethanol		(420.26)	57.26	3.37	16.68

Table 2. Spectral data of 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid derivatives **7a–f**.

Compound	R	IR [cm^{-1}]			^{1}H-NMR (DMSO-d_6, d ppm)
		NH	C=O	C=N	
7a	–H	3315	1668	1595	9.1–8.8 (2H, m, pyr. $H_{2,6}$); 8.2–7.9 (2H, m, pyr. $H_{4,5}$); 7.8–
		3430		1559	7.3 (5H, m, Ar-H); 6.8 (2H, br. s, $CONH_2$)[a]
7b	$-CH_3$	3375	1668	1570	9–8.7 (3H, m, CONH, pyr. $H_{2,6}$)[a]; 8.3–8 (2H, m, pyr. $H_{4,5}$);
				1562	7.9–7.6 (5H, m, Ar-H); 2.9 (3H, d, $NHCH_3$, became singlet after addition of D_2O)
7c	$-C_6H_5$	3415	1674	1591	10.6 (1H, br. s, CONH)[a]; 9–8.7 (2H, m, pyr. $H_{2,6}$); 8.1–7.9
				1553	(2H, m, pyr. $H_{4,5}$); 7.7–7.1 (10H, m, Ar-H)
7d	$-C_6H_4-4-CH_3$	3360	1669	1579	10.7 (1H, br. s, CONH)[a]; 9 (2H, m, pyr. $H_{2,6}$); 8.4–8.1 (2H,
				1559	m, pyr. $H_{4,5}$); 8.1–7.8 (2H, d, tolyl $H_{2,6}$); 7.8–7.5 (5H, m, Ar-H); 7.5–7.2 (2H, d, tolyl $H_{3,5}$); 2.4 (3H, s, CH_3)
7e	$-C_6H_4-4-Cl$	3200	1676	1590	10.9 (1H, br. s, CONH)[a]; 9–8.8 (2H, m, pyr. $H_{2,6}$); 8.2–7.9
				1530	(2H, m, pyr. $H_{4,5}$); 7.8–7.3 (9H, m, Ar-H)
7f	$-C_6H_4-4-Br$	3423	1668	1592	10.8 (1H, br. s, CONH)[a]; 8.8–8.6 (2H, m, pyr. $H_{2,6}$); 8.2–
				1565	7.8 (2H, m, pyr. $H_{4,5}$); 7.7–7.2 (9H, m, Ar-H)

[a] Exchangeable with D_2O

The substitution on the amide nitrogen by alkyl group **7b** lead to a slight increase of the anti-inflammatory activity (48.0%). While replacement of the alkyl group by a phenyl group or *p*-methyl-phenyl group **7c**, **7d** resulted in a dramatic increase of activity (62.3– 63.4%). The substitution by *p*-bromo-phenyl **7f** showed activity equal to Indomethacin (78.4%). On the other hand, replacement of the methyl group at N^4 of thiosemicarbazide **10a** by an ethyl group or cyclohexyl group **10b**, **10c** lead to a slight increase in activity (51.8–58.3%), while replacement by a phenyl or *p*-methyl-phenyl **10d**, **10e** lead to an increase in activity (68.6–69.0%). Higher activity was obtained by *p*-bromo-phenyl **10f** (82.6%). This means that the aryl substitution on amide nitrogen or N^4 of thiosemicarbazide is very important for the activity. Derivatives **4** and **8** showed maximum inhibition of inflammation ranging from (87.8–94.5%) and were found to be equipotent or more potent than Indomethacin and Celecoxib at two dose levels, 5 and 10 mg/kg. This was in agreement with reported data in reference [2].

Ulcerogenic activity

The compounds **4**, **7f**, **8**, and **10f** were screened for their ulcerogenic activity at dose level 10, 50, 100 mg/kg (Table 6) [27]. The tested compounds **4** and **8** have no ulcerogenic toxicity. While **7f** showed mild ulceration at dose 50 and 100 mg/kg (1.40 ± 0.06) and (1.68 ± 0.11) compound **10f** exhibited lower ulcerogenic activity at

Table 3. Physical constants of 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid derivatives **10a – f**.

Compound	R	Mp. [°C] Cryst. Solvent	Yield [%]	Mol. Formula	Microanalyses Calc./Found		
				Mol.Wt.	% C	% H	% N
10a	$-CSNHCH_3$	174 – 176	88	$C_{16}H_{15}N_7OS$	53.69	4.36	27.39
		aq. ethanol		(357.90) · $^1/_4H_2O$	53.47	4.43	27.36
10b	$-CSNHC_2H_5$	188 – 190	93	$C_{17}H_{17}N_7OS$	54.89	4.74	26.36
		aq. ethanol		(371.93) · $^1/_4H_2O$	54.92	4.44	26.65
10c	$-CSNHC_6H_{11}$	218 – 220	87	$C_{21}H_{23}N_7OS$	59.21	5.56	23.01
		aq. ethanol		(426.03) · $^1/_4H_2O$	59.49	6.00	23.30
10d	$-CSNHC_6H_5$	213 – 215	85	$C_{21}H_{17}N_7OS$	60.71	4.12	23.60
		ethanol		(415.47)	60.36	4.01	23.58
10e	$-CSNHC_6H_4-4-CH_3$	207 – 209	86	$C_{22}H_{19}N_7OS$	61.52	4.46	22.83
		ethanol		(429.50)	61.23	4.68	22.87
10f	$-CSNHC_6H_4-4-Br$	213 – 215	82	$C_{21}H_{16}BrN_7OS$	51.02	3.26	19.83
		aq. ethanol		(494.37)	50.56	2.93	19.70

Table 4. Spectral data of 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid derivatives **10a – f**.

Compound	R	IR [cm^{-1}]			^{1}H-NMR [DMSO-d_6 δ ppm]
		NH, NH_2	C=O	C=N	
10a	$-CSNHCH_3$	3315 3285 3190	1684	1605 1554	10.9 (1H, br. s, CO*NH*)[a]; 9.6 (1H, br. s, *NH*CS)[a]; 9 – 8.8 (2H, m, pyr. $H_{2,6}$); 8.3 – 7.9 (3H, m, *NH*CH_3, pyr. $H_{4,5}$)[a]; 7.8 – 7.5 (5H, m, Ar-H); 3.1 – 2.9 (3H, d, NH*CH_3*, became singlet after addition of D_2O)
10b	$-CSNHCH_2CH_3$	3310 3290 3200	1686	1611 1551	10.9 (1H, br. s, CO*NH*)[a]; 9.6 (1H, br. s, *NH*CS)[a]; 9 – 8.8 (2H, m, pyr. $H_{2,6}$); 8.3 – 7.9 (3H, m, *NH*CH_2, pyr. $H_{4,5}$)[a]; 7.8 – 7.5 (5H, m, Ar-H) 3.8 – 3.3 (2H, m, *CH_2*CH_3); 1.4 – 0.9 (3H, 2t, CH_2*CH_3*)
10c	$-CSNHC_6H_{11}$	3295 3200	1663	1573 1535	10.9 (1H, br. s, CO*NH*)[a]; 9.6 (1H, br. s, *NH*CS)[a]; 8.9 – 8.6 (2H, m, pyr. $H_{2,6}$); 8.2 – 7.9 (2H, m, pyr. $H_{4,5}$); 7.8 – 7.3 (5H, m, Ar-H); 4.2 (1H, br. s, *NH* C_6H_{11})[a]; 2.1 – 1 (11H, m, C_6H_{11})
10d	$-CSNHC_6H_5$	3300 3290 3170	1700	1590 1530	11(1H, br. s, CO*NH*)[a]; 10 (1H, br. s, *NH*CS)[a]; 8.9 – 8.6 (2H, m, pyr. $H_{2,6}$); 8.2 – 7.9 (3H, m, pyr. $H_{4,5}$, *NH*C_6H_4)[a]; 7.8 – 7.1 (10H, m, Ar-H)
10e	$-CSNHC_6H_4$-4-CH_3	3310 3280 3200	1665	1610 1550	11 (1H, br. s, CO*NH*)[a]; 10 (1H, br. s, *NH*CS)[a]; 9.1 – 8.8 (2H, m, pyr. $H_{2,6}$); 8.3 – 8 (2H, m, pyr. $H_{4,5}$); 7.9 – 7.1 (10H, m, N*H*C_6H_4, Ar-H)[a]; 2.3 (3H, s, CH_3)
10f	$-CSNHC_6H_4$-4-Br	3310 3385 3200	1670	1605 1550	11 (1H, br. s, CO*NH*)[a]; 10.1 (1H, br. s, *NH*CS)[a]; 8.9 – 8.6 (2H, m, pyr. $H_{2,6}$); 8.3 – 8 (2H, m, pyr. $H_{4,5}$); 7.9 – 7.5 (10H, m, *NH*C_6H_4, Ar-H)

[a] Exchangeable with D_2O.

100 mg/kg (0.75 ± 0.06), compared with Indomethacin which showed ulcerogenic activity from (1.35 ± 0.18 to 2.1 ± 0.17).

Acute toxicity (LD_{50})

LD_{50} of the most active compounds **4** and **8** was determined using graphical method [28]. LD_{50} of compounds **4** and **8** were found to be 150 mg/kg and 165 mg/kg (i.p.) while LD_{50} of Indomethacin is 50 mg/kg (i.p.).

In conclusion, 5-phenyl-1-(3-pyridyl)-1*H*-1,2,4-triazole-3-carboxylic acid derivatives were prepared with the objective of developing better anti-inflammatory molecules. From these studies compounds **4** and **8** showed significant anti-inflammatory activity without ulcerogenic toxicity. The effect of alkyl or aryl groups substituted on amides, or thiosemicarbazides at position 3 of triazole were also studied.

Experimental

General

Melting points were determined on Electrothermal Melting Point Apparatus (Stuart Scientific, UK) and are uncorrected. Ele-

Table 5. Anti-inflammatory activity of **4–10**

Compd. No.	Anti-inflammatory activity (5 mg/kg, p.o) (Inhibition ± S. E. M) [%]			Anti-inflammatory activity (10 mg/kg, p.o) (Inhibition ± S. E. M) [%]		
	1 h	2 h	3 h	1 h	2 h	3 h
Control	0	0	0	0	0	0
Ind.	36.8 ± 5.74[b)]	49.7 ± 6.32[b)]	56.0 ± 5.25[b)]	47.4 ± 5.56[b)]	57.9 ± 5.22[b)]	78.4 ± 5.88[b)]
Cel.	54.2 ± 1.56[b)]	68.6 ± 1.43[b)]	76.4 ± 1.18[b)]	65.4 ± 2.12[b)]	75.4 ± 2.56[b)]	91.7 ± 2.88[b)]
4	53.2 ± 1.37[b)]	66.2 ± 1.83[b)]	77.5 ± 1.56[b)]	65.4 ± 0.08[b)]	77.1 ± 1.05[b)]	87.8 ± 1.12[b)]
5	26.3 ± 2.25[a)]	30.0 ± 2.36[a)]	32.1 ± 1.18	34.1 ± 3.04[a)]	41.2 ± 3.56[a)]	45.6 ± 3.04[a)]
6	36.8 ± 4.83[b)]	49.6 ± 4.75[b)]	62.3 ± 3.39[b)]	45.6 ± 2.56[b)]	57.3 ± 2.44[b)]	77.7 ± 3.08[b)]
7a	21.1 ± 5.63[a)]	32.2 ± 5.84[b)]	37.6 ± 5.63[b)]	35.8 ± 4.75[a)]	41.2 ± 4.36[b)]	46.9 ± 4.75[b)]
7b	23.5 ± 4.96[a)]	38.4 ± 3.86[b)]	40.1 ± 4.88[b)]	37.9 ± 2.88[b)]	43.6 ± 2.12[b)]	48.0 ± 2.44[b)]
7c	36.9 ± 2.21[b)]	41.2 ± 2.21[b)]	53.2 ± 2.54[b)]	44.6 ± 3.55[b)]	55.3 ± 3.18[b)]	62.3 ± 3.12[b)]
7d	38.0 ± 2.21[b)]	42.4 ± 2.54[b)]	54.1 ± 2.21[b)]	44.9 ± 3.02[b)]	56.2 ± 3.39[b)]	63.4 ± 3.88[b)]
7f	44.7 ± 1.88[b)]	52.3 ± 1.56t	60.1 ± 1.88[b)]	52.6 ± 1.56[b)]	62.3 ± 2.02[b)]	78.4 ± 1.88[b)]
8	60.4 ± 1.42[b)]	72.0 ± 1.56[b)]	79.1 ± 1.88t	73.7 ± 5.08[b)]	80.3 ± 4.56[b)]	94.5 ± 5.18[b)]
9	23.6 ± 7.08[a)]	30.0 ± 6.38t	36.9 ± 7.08t	34.1 ± 3.12[a)]	44.1 ± 3.56[b)]	48.0 ± 3.18[b)]
10a	25.3 ± 5.56[a)]	30.8 ± 4.98[b)]	38.5 ± 5.56[b)]	40.1 ± 1.75[b)]	46.4 ± 1.88[b)]	51.8 ± 1.18[b)]
10b	28.8 ± 6.08[b)]	31.3 ± 6.38[b)]	38.0 ± 5.56[b)]	39.4 ± 2.25[b)]	48.0 ± 2.35[b)]	52.1 ± 2.88[b)]
10c	31.1 ± 2.25[b)]	34.2 ± 1.83[b)]	39.1 ± 1.56[b)]	42.6 ± 1.35[b)]	52.2 ± 1.44[b)]	58.3 ± 1.88[b)]
10d	40.8 ± 2.21[b)]	46.9 ± 2.54[b)]	54.4 ± 2.08[b)]	51.6 ± 3.04[b)]	62.2 ± 2.56[b)]	68.6 ± 2.88[b)]
10e	41.3 ± 1.88[b)]	47.2 ± 2.08[b)]	55.9 ± 1.56[b)]	51.9 ± 4.18[b)]	63.9 ± 4.55[b)]	69.0 ± 4.18[b)]
10f	49.9 ± 2.25[b)]	61.5 ± 2.54[b)]	65.3 ± 1.88[b)]	61.2 ± 0.08[b)]	70.3 ± 1.12[b)]	82.6 ± 1.44[b)]

[a)] Significant difference at $P < 0.05$.
[b)] Significant difference at $P < 0.001$
Ind. Indomethacin, Cel. Celecoxib

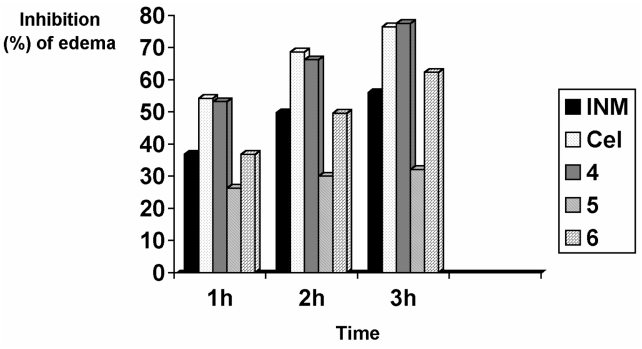


Figure 1. Inhibition (%) of edema induced by carrageenan in Indomethacin (**IND**), Celecoxib (**Cel**), and tested compounds **4–6** in treated groups of rats, at 5 mg/kg.

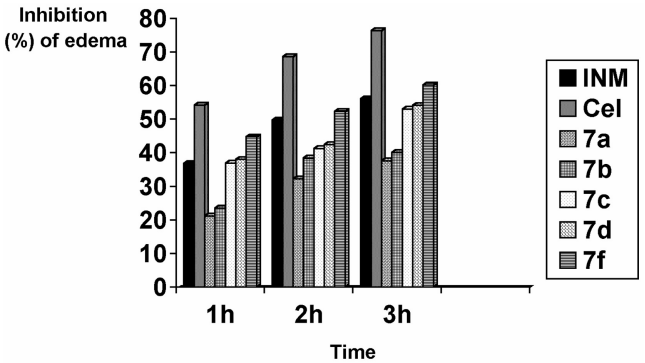


Figure 2. Inhibition (%) of edema induced by carrageenan in Indomethacin (**IND**), Celecoxib (**Cel**), and tested compounds **7a–d**, **f** in treated groups of rats, at 5 mg/kg.

mental microanalyses were performed on Perkin-Elmer, 240 Elemental Analyzer (Perkin-Elmer, Hitachi, Tokyo, Japan), at the central laboratory, Assiut University and Perkin-Elmer, 2400 CHN Elemental Analyzer at Micro-Analytical Center, Faculty of Science, Cairo University. TLC was carried out using silica gel 60 F_{254} precoated sheets 20 × 20 cm, layer thickness 0.2 mm (E. Merck, Darmstadt, Germany) and were visualized using UV lamp at 254 nm.

Column chromatography were carried out using silica gel 60 (0.063–0.200 mm; Merck). Dry-column flash chromatography were carried out using silica gel 60 GF_{254} (0.005–0.04 mm; Merck). IR (KBr) were recorded on a Shimadzu IR 200-91527 Spectrophotometer (Shimadzu Corp., Kyoto, Japan)at the Faculty of Pharmacy, Assiut University. ^{1}H-NMR spectra were carried out using Varian Em-360L NMR Spectrophotometer (60 MHz) (Varian, Palo Alto, CA, USA) at Faculty of Pharmacy, Assiut University using TMS as internal standard, DMSO-d_6 as solvent and the (chemical shifts in d ppm.). Mass spectra were performed on JEOL JMS600 (JEOL, Tokyo, Japan)at Assiut University Central Laboratory.

Chemistry

4-(3-Pyridyl)-hydrazono-2-phenyl-2-oxazolin-5-one ***3***

Hippuric acid **1** (23 g, 0.13 mol) in acetic anhydride (75 mL) was heated until a clear solution **2** was obtained, this solution was cooled to room temperature (solution A). To a cold solution of 3-aminopyridine (9.4 g, 0.1 mol) in 5N HCl (35 mL) in an ice-salt

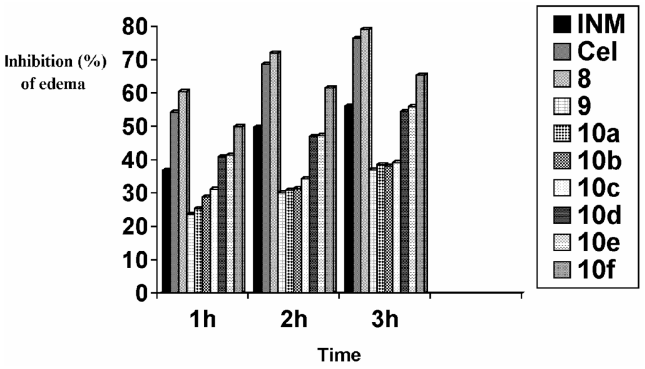


Figure 3. Inhibition (%) of edema induced by carrageenan in Indomethacin (**IND**), Celecoxib (**Cel**), and tested compounds **8–10** in treated groups of rats, at 5 mg/kg.

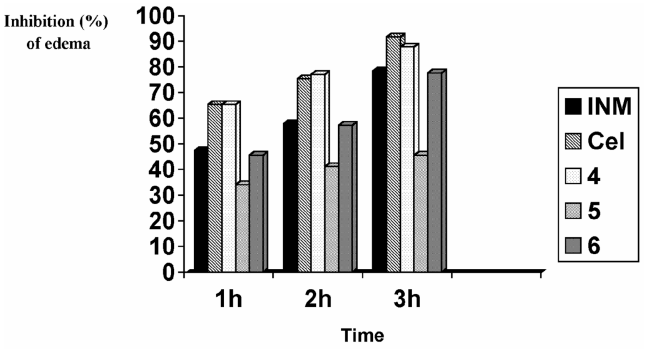


Figure 4. Inhibition (%) of edema induced by carrageenan in Indomethacin (**IND**), Celecoxib (**Cel**), and tested compounds **4–6** in treated groups of rats, at 10 mg/kg.

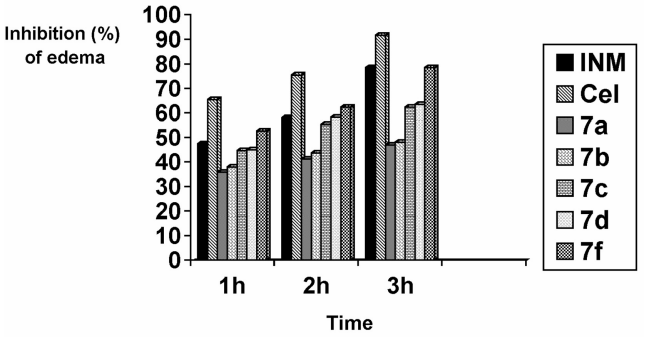


Figure 5. Inhibition (%) of edema induced by carrageenan in Indomethacin (**IND**), Celecoxib (**Cel**), and tested compounds **7a–d**, **f** in treated groups of rats, at 10 mg/kg.

bath −5–0 °C, a solution of sodium nitrite (8.97 g, 0.13 mol) in water (15 mL) was added dropwise. The reaction mixture was left for 10 min (solution B). Solution A was added to solution B in presence of anhydrous sodium acetate (15 g, 0.18 mol). The reaction mixture was stirred at 0–10°C for 2 h, the precipitate was filtered and dried (yield 70%).

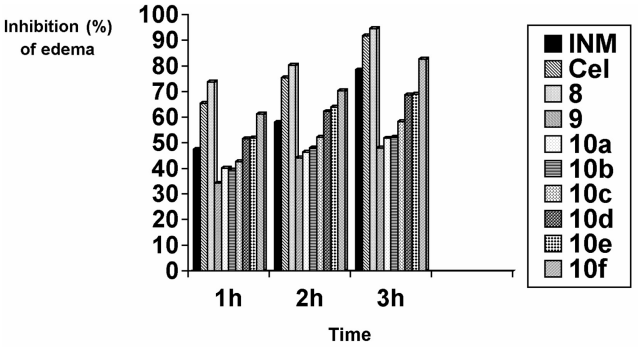


Figure 6. Inhibition (%) of edema induced by carrageenan in Indomethacin (**IND**), Celecoxib (**Cel**), and tested compounds **8–10** in treated groups of rats, at 10 mg/kg.

*Methyl-5-phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylate **4***

To a stirred suspension of **3** (4 g, 0.015 mol) in methanol (40 mL), potassium hydroxide (5%, 5 mL) was added. Stirring was continued at room temperature for 1 h. The solvent was removed under vacuum, the formed precipitate was purified by column chromatography using chloroform : methanol (9 : 1) as eluent. The product. was crystallized from ethanol, (2.2 g, 52%), mp. 140–142°C. IR (KBr cm^{-1}): 1735 (C=O), 1581, 1527 (C=N). ^{1}H-NMR (DMSO-d_6, d): 9.00–8.70 (2H, m, pyr. $H_{2,6}$), 8.10–7.80 (2H, m, pyr. $H_{4,5}$), 7.70–7.40 (5H, m, Ar-H), 4.10 (3H, s, $COOCH_3$). Analysis: $C_{15}H_{12}N_4O_2$ (280.28), Calcd: C: 64.28, H: 4.32, N: 19.99, Found: C: 64.09, H: 4.43, N: 19.88.

*5-Phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid **5***

A mixture of **4** (7 g, 0.025 mol) in ethanol (25 mL) and potassium hydroxide (20%, 25 mL) was refluxed for 1 h, cooled and neutralized with dilute hydrochloric acid. The precipitated product was filtered, washed with water, and crystallized from aqueous ethanol (4.17 g, 74%), mp. 202–204°C. IR (KBr cm^{-1}): 3210 (O–H), 1708 (C=O), 1586, 1565 (C=N). ^{1}H-NMR (DMSO-d_6, d): 10.20 (1H, br. s, COOH), 9.20–8.80 (2H, m, pyr. $H_{2,6}$), 8.40–8.00 (2H, m, pyr.$H_{4,5}$), 7.90–7.50 (5H, m, Ar-H). Analysis $C_{14}H_{10}N_4O_2$ (226.08), Calcd: C: 63.15, H: 3.79, N: 21.04, Found: C: 62.86, H: 3.51, N: 20.98.

*5-Phenyl-1-(3-pyridyl)-1,2,4-triazole-3-hydroxamic acid **6***

To a freshly prepared solution of hydroxylamine (prepared from a solution of hydroxylamine hydrochloride (7 g, 0.1 mol) in methanol (30 mL) by addition to a solution of potassium hydroxide (7 g, 0.125 mol) in methanol (30 mL), while cooling), a solution of **4** (1.4 g, 0.005 mol) in methanol (20 mL) was added portion-wise under stirring at room temperature. After complete addition, the mixture was left at room temperature overnight. The formed precipitate was filtered off and dissolved in water (15 mL); diluted acetic acid (5 mL) was added and the mixture was allowed to stand for 3 h at room temperature. The separated solid was filtered, dried, and crystallized from methanol; (0.89 g, 63%), m.p. 206–208°C. IR (KBr cm^{-1}): 3200 (O–H), 3145 (N–H), 1647 (C=O), 1578, 1555 (C=N). ^{1}H-NMR (DMSO-d_6, d): 11.90 (1H, br. s, CONH), 9.80 (1H, brs, OH), 9.00 (2H, m, pyr.$H_{2,6}$), 8.40–8.10 (2H, m, pyr.$H_{4,5}$), 7.90–7.60 (5H, m, Ar-H). MS m/z (%): 281[M^+] (65%), 280 (100%), 249 (88%),181 (77%), 78 (68%). Analysis

Table 6. Gastric ulceration in rats[a].

Compound	Dose [mg/kg]		
	10	50	100
Indomethacin	3/6 (1.35 ± 0.18)[b]	5/6 (1.88 ± 0.15)[b]	6/6 (2.1 ± 0.17)[b]
4	0/6 (0.00)	0/6 (0.00)	0/6 (0.00)
7f	0/6 (0.00)	2/6 (1.40 ± 0.06)[c]	4/6 (1.68 ± 0.11)[b]
8	0/6 (0.00)	0/6 (0.00)	0/6 (0.00)
10f	0/6 (0.00)	0/6 (0.00)	2/6 (0.75 ± 0.06)[c]

[a] Number of rats with lesions more than 0.5 mm in length per total number of rats. The number in parentheses is the mean ulcer index [mm] with S. E. M.
[b] Significant difference at $P < 0.001$.
[c] Significant difference at $P < 0.05$

$C_{14}H_{11}N_5O_2$ (281.27), Calcd: C: 59.78, H: 3.94, N: 24.90, Found: C: 59.99, H: 3.94, N: 24.70.

*5-Phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxamides **7a–f***

*Method A (**7a, b**)*

A mixture of **3** (2.66 g, 0.01 mol) in methanol (30 mL) and ammonium hydroxide (25%, 50 mL) or methyl amine solution 41% (0.62 g, 0.02 mol) was refluxed for 30 min. Then the solvent was evaporated *in vacuo*. The precipitated solid was crystallized from ethanol.

*Method B (**7c–f**)*

A mixture of **3** (2.66 g, 0.01 mol), appropriate primary aromatic amine (0.01 mol) in acetic acid (50 mL), and anhydrous sodium acetate (1.5 g, 0.018 mol) was refluxed for 2 h. The mixture was cooled, poured in ice cold water (50 mL). The formed precipitate was filtered, dried, and crystallized from ethanol. Physical data are listed in Table 1. IR and ^{1}H-NMR (DMSO-d_6) are listed in Table 3. MS m/z (%) of **7e**: 375 (100%), 249 (56%), 181 (91%), 78 (32%).

*5-Phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid hydrazid **8***

Hydrazine hydrate 85% (1.5 g, 0.03 mol) was added to a stirred suspension of **3** (8 g, 0.03 mol) in methanol (100 mL) and stirring was continued at room temperature for 30 min and left overnight. The solvent was removed under vacuum, the residue was purified by dry flash column chromatography using chloroform : methanol (9.5 : 0.5). The solvent was evaporated under vacuum and the residue was crystallized from ethanol. (2.05 g, 73%), mp. 180 – 182°C. IR (KBr, cm^{-1}): 3380, 3210, 3150 (N-H, NH_2), 1661(C=O), 1590, 1558 (C=N). ^{1}H-NMR (DMSO-d_6, d): 10.30 (1H, br.s, CONH), 9.20-8.90 (2H, m, pyr.$H_{2,6}$), 8.40 – 8.10 (m, 2H, pyr.$H_{4,5}$), 7.90 – 7.60 (5H, m, Ar-H), 4.80 (2H, br.s, $CONHNH_2$). Analysis $C_{14}H_{12}N_6O$ (280.28), Calcd: C: 59.99, H: 4.32, N: 29.98, Found: C: 59.60, H: 4.75, N: 29.77.

*5-Phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid semicarbazide **9***

A mixture of **8** (2.8 g, 0.01 mol) in glacial acetic acid (20 mL) and potassium cyanate (1.6 g, 0.02 mol) was refluxed for 2 h. The solvent was evaporated under vacuum and the product was crystallized from aqueous ethanol. (61%), mp. 210 – 212°C. IR (KBr cm^{-1}): 3400, 3310, 3200, 3150 (N – H, NH_2), 1700, 1670 (C=O), 1590, 1520 (C=N). ^{1}H-NMR (DMSO-d_6, d): 10.40 (1H, brs, CONH), 9.00 – 8.80 (2H, m, pyr. $H_{2,6}$), 8.30 – 7.90 (3H, m, CONHNH, pyr. $H_{4,5}$), 7.80 – 7.50 (5H, m, Ar-H), 6.00 (2H, br.s, $CONH_2$). Analysis: $C_{15}H_{13}N_7O_2$ (327.81, $^1/_4$ H_2O), Calcd: C: 54.99, H: 4.15, N: 29.92, Found: C: 55.08, H: 3.99, N: 29.86.

*N^4-Substituted 5-phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid thiosemicarbazides **10a–e***

To a suspension of **8** (2.8 g, 0.01 mol) in ethanol (40 mL); a solution of the appropriate isothiocyanate (0.01 mol) in ethanol (10 mL) was added. The mixture was refluxed for 2 h. The reaction mixture was cooled and the separated solid was filtered, washed with ethanol and crystallized from appropriate solvent. Physical data are listed in Table 2. IR and ^{1}H-NMR (DMSO-d_6) are listed in (Table 4).

Pharmacology

Chemicals and instruments

The following chemicals were used: Indomethacin was purchased from Nile Co. (Nile Co., for Drugs and Chem. Industries, Cairo, Egypt). Celecoxib was provided from EIPICO, Egyptian International Pharmaceutical Industries Co, Egypt), Carrageenan, used to induce edema, was purchased from Sigma Chemicals Co., St. Louis, MO, USA. Digital plethysmometer LE7500 (Panlab S.L., Cornella, Barcelona, Spain) was used to measure the volume of paw edema. Computer program Prism was used for carrying statistical analyses. Values of $P < 0.05$ (significant difference) or $P < 0.001$ (highly significant difference) were used as the limit for statistical significance [27].

Biological evaluation

The experiments were performed on adult male albino rats of Wistar strain, weighing (100 – -120 g) and male albino mice, weighing (25 – 30 g). All animals were obtained from the animal house, Faculty of Medicine, Assiut University, Assiut, Egypt. The animals were housed in stainless steel cages, maintained at 25 ± 2°C, 50 ± 5% relative humidity, 12 h light/dark cycle. Food and water (laboratory chow) *ad libitum* were freely available upto the time of experiments. The research was conducted in accordance

with the internationally accepted principles for laboratory animal use and care as found in the European community guidelines. The test compounds were dissolved in 0.5% w/v carboxy methyl cellulose (CMC) in water.

Anti-inflammatory activity

Anti-inflammatory activity of the compounds under investigation was studied in rats using carrageenan. A suspension of the tested compounds and reference drugs Indomethacin and Celecoxib in carboxy methyl cellulose (CMC) solution (0.5% w/v in water) was administrated orally to rats in two dose levels (5, 10 mg/kg). Control animals were similarly treated with CMC (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 according to the method of Hernandez-Perez et al. [26]. The right paw volume was measured by Digital plethysmometer LE7500 (Panlab S.L., Cornella, Barcelona, Spain). directly before and at 1, 2, 3 h intervals after administration of the tested compounds.

The anti-inflammatory activity of the tested compound and reference drugs was determined with the following formula [29]:

$$\% \text{ Anti-inflammatory activity} = (Vc - Vt/Vc) \cdot 100$$

where Vc represents the mean increase in paw volume in the control group of rats. Vt represents the mean increase in paw volume in rats treated with test compounds and data are expressed as mean ± S.E.M., the Students *t*-test was applied to determine the significance of the difference between the control group and rats treated with the test compounds.

Ulcerogenic activity [27]

Albino rats have been divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after *p.o.* administration of the tested compounds or Indomethacin at doses of 10, 50, and 100 mg/kg, control rats received *p.o.* administration of vehicle (suspension of 0.5% w/v CMC). Food but not water was removed 24 h before administration of the tested compounds. After 6 h, the rats were sacrified and the stomach was removed, and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosed damage for each stomach was examined using a Stereoscopic microscope (Nikon SMZ 1B stereoscopic microscope, Montana, USA), the mucosal damage was compared with Indomethacin. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage. Data are expresses as mean ± S.E.M., the Students *t*-test was applied to determine the significance of the difference between the standard group and rats treated with the test compounds.

Acute toxicity (LD_{50}) [28]

The median lethal doses (LD_{50}) of the most active compounds **4** and **8** were determined in mice. Groups of male adult albino mice, each of six animals, were injected *i.p.* with graded doses of each of the test compounds. The percentage of mortality in each group of animals was determined 24 h after injection. Computation of LD_{50} was processed by a graphical method.

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