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DESIGN AND SYNTHESIS OF SOME NEW 1*H*-1,2,4-TRIAZOLES OF POTENTIAL ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES*

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مشتقات ١،٣،٥-ثلاثية الاستبدال-(ايد)-١،٢،٤-ترايازول التى تم نحضيرها واثبات تركيبها البنائى تعتبر نوعا مستحدثا من المركبات الحلقية المتوقع أن يكون لها فعالية كمثبطات للالتهابات وكمسكنات وقد انصب اهتمامنا بهذه المركبات لانها تحتوى على بعض التغيرات عن معظم مثبطات الالتهابات الغير استرويدية المعروفة. تمت دراسة قدرة المركبات لتثبيط الالتهابات بطريقتين: طريقة صبغة التريبان الزرقاء وطريقة التورم الناشئ بالكاولين على فئران التجارب. كما تمت دراسة قدرة المركبات كمسكنات بطريقتين أيضا هما: طريقة استخدام السطح وطريقة رد الفعل الناشئ عن البارابنزوكينون.

تمت دراسة علاقات التركيب البنائى للمركبات بالتأثير الفارماكولوجى لها وكذا علاقتهما بمكافئ التوزيع المحسوب للمركبات.

The synthesized 1H-1,2,4-triazoles (7a-c, 8a-c and 9a-c), a novel class of heterocyclic compounds of potential anti-inflammatory and analgesic activities, are of interest, since they present some differences compared to the classical non-steroidal anti-inflammatory agents. The anti-inflammatory activity of all derivatives was studied in albino rats using trypan-blue and kaolin-induced edema methods. The compounds were also tested in albino mice for their analgesic activity using the hot plate and p-benzoquinone-induced writhing methods. A correlation between the structures, pharmacological actions and the calculated partition coefficients of the products was studied.

INTRODUCTION

Beside the diverse biological activities that have been reviewed for 1*H*-1,2,4-triazoles[1], especially as antimycotic and as antifungal agents[2], the synthesis and anti-inflammatory activity of a number of 1-substituted-1*H*-1,2,4-triazoles have been reported recently[3,4]. In this work, we extended our study with the synthesis of some new 5-α-pyridyltriazoles, in order to investigate the substituent effect on some physico-chemical properties, such as partition coefficient[5], and on the biological activities of this class of compounds.

The title 5-α-pyridyltriazoles, a novel class of anti-inflammatory and analgesic structures, present some differences compared to the classical anti-inflammatory agents, since most of the latter are acidic in nature[6]. The non-acidic non-steroidal anti-inflammatories are gaining

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much interest, for their improved biological and pharmacokinetic properties[7] In this concern, we prepared a range of derivatives embodying different substituents in the para position of the 1-phenyltriazole, along with three selected moieties (carboxylic ester, carboxylic hydrazide and carboxyl) at the C-3 of the triazole system.

RESULTS AND DISCUSSION

Chemistry

The alkylation is the most frequently used method for the synthesis of N-substituted 1,2,4-triazoles, however, it does not only leads to N-1 substituted products, but more or less also to N-4 alkylated compounds[1,8-12].

A useful synthetic pathway to N-1 substituted-1,2,4-triazoles having carboxylic derivatives at C-3 comes through amidrazones **3a-c**[3].

Diazotization of *p*-substituted anilines, **1a-c** (Scheme **1**) followed by reaction with the active methylene compound ethyl acetoacetate had been described to provide **2a-c**, from which the key intermediates **3a-c** were obtained by the reaction of **2a-c** with bromine, and thence with ammonia[3]. On the other hand, the intermediate 2-methyl-3-nitropyridine-6-carbaldehyde **4** was prepared by nitration of 2,6-lutidine[13,14], followed by oxidation of the produced 3-nitro-2,6-lutidine with SeO_2 in dioxan[15]. The utilized oxidation process providing selectively the C-6 formyl isomer[16,17].

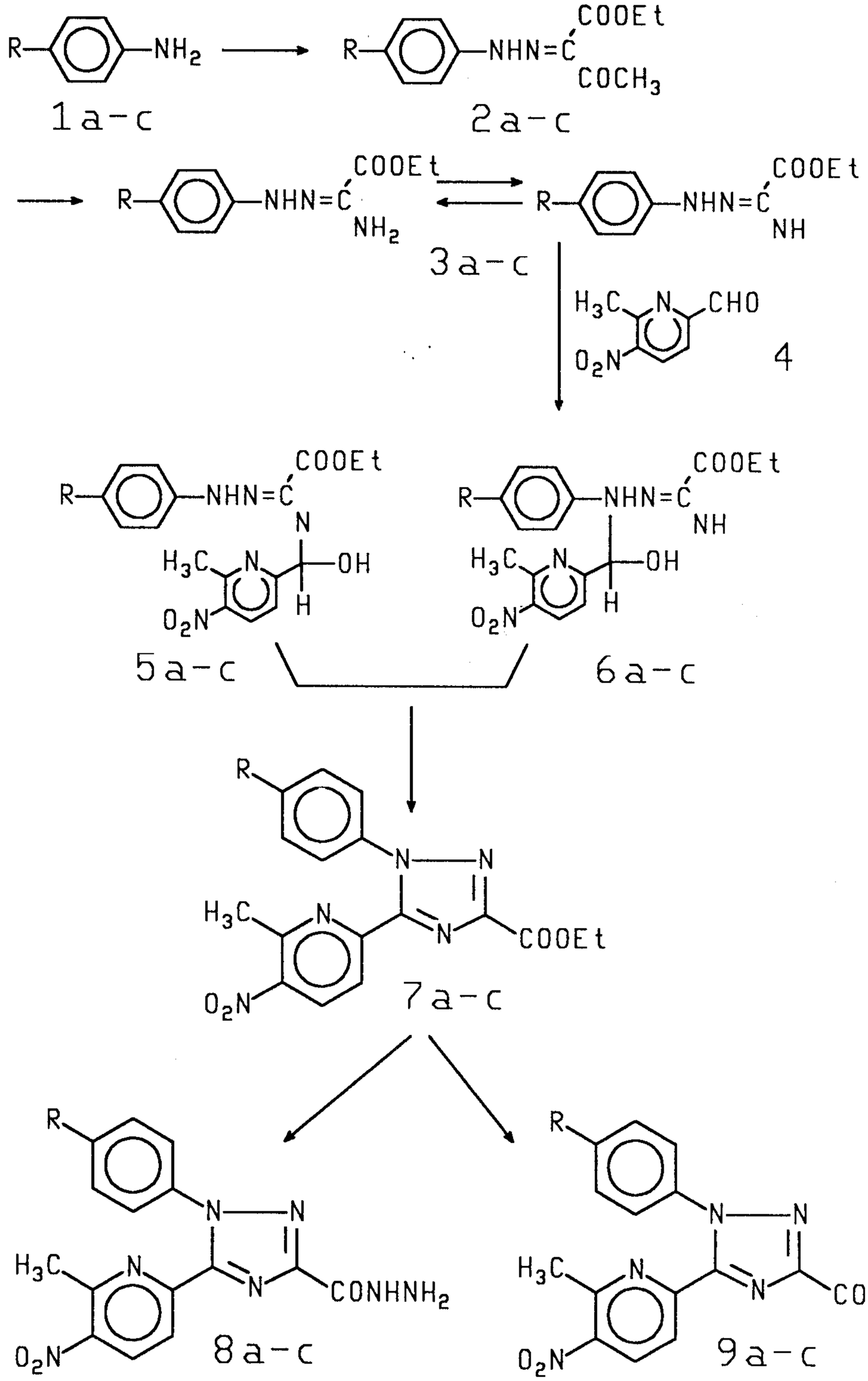
Condensation of the carbaldehyde **4** with the appropriate amidrazone **3a-c** in acetic acid containing acetic anhydride afforded the triazoles **7a-c** (Scheme **1**, Table **I**). The formation of **7a-c** is expected to proceed through either the intermediates **5a-c** or **6a-c**. The reaction of **7a-c** with hydrazine hydrate afforded the corresponding hydrazides **8a-c**. Furthermore, hydrolysis of **7a-c** with alkali followed by acidification afforded the free carboxylic acids **9a-c**. The structures of the prepared compounds were established by elemental microanalyses, IR, ^{1}H-NMR and MS spectral data.

The possibility that the reaction products, from **3a-c** with **4**, can be the triazolines was eliminated on spectral and chemical grounds. The IR spectra revealed the absence of an NH stretching or bending bands and the mass spectra produced molecular ion peaks corresponding to the triazoles **7a-c**. ^{1}H-NMR spectra showed, as a further evidence, the absence of both the C-5 proton and that at N-4(2). In addition, no oxidation of **7a-c** with MnO_2 in CH_2Cl_2 occurred and the separated compounds have the same R_f by TLC as well as other spectral data. This can be attributed to the stability of aromatic triazoles.

Pharmacology

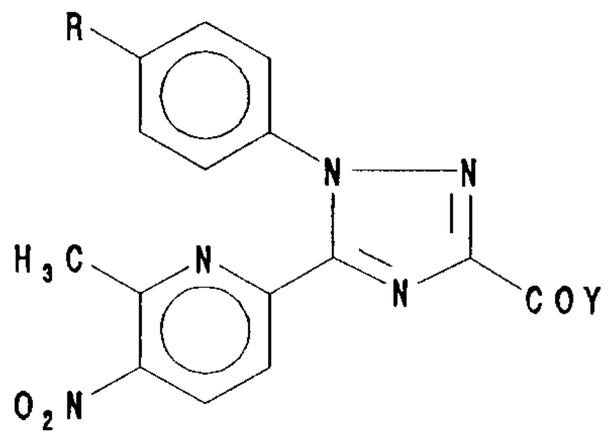
Anti-inflammatory Activity

Figure **1** presents the anti-inflammatory activity of the triazole derivatives against inflammation induced by histamine. All derivatives and the reference standard drug were given intramuscularly at two dose levels of 5 and 10 mg/kg into rats. It is evident from the figure that all tested compounds, except **8a, 9a, 7c** and indomethacin, produced in-significant effects when they were injected at a dose level of 5 mg/kg. Doubling the injected dose of the triazole derivatives was found to improve the anti-inflammatory activity. All the tested compounds when injected at a dose level of 10 mg/kg produced a significant anti-inflammatory action against histamine-induced inflammation. Compounds **8a** and **9a** of the series showed the highest activity. The ability of the triazole derivatives to protect against kaolin-induced rat paw edema was compared to that of indomethacin. The median effective dose of tested compounds and indomethacin are given in Table **II**. None of the triazole derivatives is different significantly in potency from indomethacin. However, the median effective dose (ED_{50}) of **8a** and **9a** were found to be less than that for indomethacin and compound **7c** was found to be almost equipotent to that of indomethacin. On the other hand, the median effective doses of the rest of compounds were found to be higher than that of indomethacin. As evident, compounds **8a** and **9a** showed the highest potency, while compound **7a** showed the least potency against kaolin-induced edema.


R
NH2
1a-c
COOEt
NHN=C
COCH3
2a-c
NH2
3a-c
NH
H3C
N
CHO
O2N
4
OH
H
5a-c
6a-c
CONHNH2
7a-c
8a-c
COOH
9a-c
a; (R = Br), b; (R = Cl), c; (R = NO2)

Scheme 1

Table 1: Physical and chemical properties of the synthesized 1,3,5-trisubstituted-1*H*-1,2,4-triazoles (**7a-c, 8a-c** and **9a-c**).



Comp. No.	R	Y	Yield (%)	m.p. °C (Reaction Time h')	Mol. Form. (Mol. Wt.)	Analysis % El.	Cal.	Found
7a	Br	OEt	78	156 (8)	$C_{17}H_{14}BrN_5O_4$ (432.26)	C H N	47.00 3.27 16.19	46.72 3.29 15.95
7b	Cl	OEt	84	139-140 (10)	$C_{17}H_{14}ClN_5O_4$ (387.82)	C H N	52.65 3.64 18.05	53.00 3.44 17.75
7c	NO_2	OEt	80	161-162 (14)	$C_{17}H_{14}N_6O_6$ (398.28)	C H N	51.26 3.54 21.09	49.81 3.27 21.70
8a	Br	$NHNH_2$	95	208-210 (3)	$C_{15}H_{12}BrN_7O_3$ (418.23)	C H N	43.07 2.89 23.43	42.85 2.92 23.22
8b	Cl	$NHNH_2$	94	220-221 (3)	$C_{15}H_{12}ClN_7O_3$ (373.79)	C H N	48.20 3.24 26.22	47.66 3.20 25.70
8c	NO_2	$NHNH_2$	95	249-251 (3)	$C_{15}H_{12}N_8O_5$ (384.25)	C H N	46.88 3.13 29.17	46.38 3.10 28.60
9a	Br	OH	87	204-205 (3)	$C_{15}H_{10}BrN_5O_6$ (404.14)	C H N	44.58 2.49 17.61	44.35 2.80 17.22
9b	Cl	OH	92	178-180 (3)	$C_{15}H_{10}ClN_5O_6$ (359.68)	C H N	50.09 2.80 19.47	49.76 3.31 18.92
9c	NO_2	OH	78	226-228 (3)	$C_{15}H_{10}N_6O_6$ (370.23)	C H N	48.66 2.72 22.69	48.51 3.13 22.24

Table II **a:** Comparison of the anti-inflammatory activity of the triazole derivatives against kaolin-induced rat paw edema.

Compd. No.	Ed_{50} (mg/kg) and its 95% confidence limits	P.R.[a]
Indomethacin	7.92 (4.74 - 13.22)	-
7a	11.41 (7.09 - 18.37)	2.01
7b	10.94 (6.75 - 17.72)	2.02
7c	7.97 (5.14 - 12.35)	1.96
8a	7.20 (4.92 - 10.50)	1.88
8b	13.13 (7.96 - 21.66)	2.05
8c	11.25 (6.62 - 19.13)	2.09
9a	7.50 (4.75 - 11.85)	1.99
9b	11.41 (6.79 - 19.17)	2.07
9c	11.25 (6.54 - 19.35)	2.20

b: Comparison of the analgesic activity of triazole derivatives and indomethacin against p-benzoquinone-induced writhing in mice.

Indomethacin	7.65 (4.75 - 12.32)	-
7a	9.06 (5.59 - 14.68)	1.96
7b	8.91 (5.30 - 14.97)	2.03
7c	7.81 (4.65 - 13.12)	2.03
8a	6.56 (4.79 - 8.99)	1.77
8b	11.88 (6.97 - 20.20)	2.04
8c	9.22 (5.59 - 15.21)	1.98
9a	6.88 (4.81 - 9.84)	1.82
9b	9.14 (5.86 - 14.26)	1.92
9c	9.06 (5.43 - 15.13)	2.02

a) P.R. = factor for potency ratio.
For the test compound to be different significantly in potency from indomethacin the value of potency ratio must exceed the value of P.R.

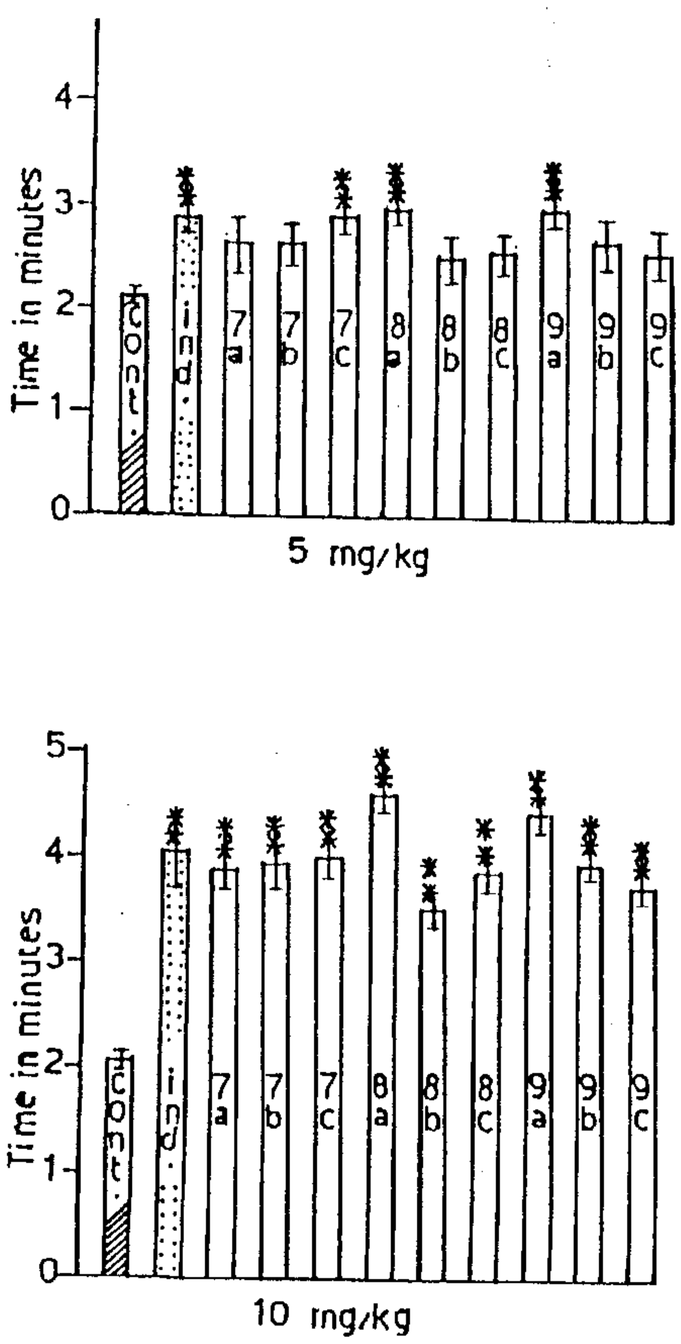


Fig. 1 Anti-inflammatory activity of the triazole derivatives against inflammation induced by histamine in rats. Cont. = control Ind. = indomethacin, the reference standard drug. Values are mean of 6 experiments ± standard error. ** Significant difference at $p < 0.01$. A = for dose 5 mg/kg B= 10 mg/kg.

Analgesic Activity

The study of the analgesic activity of the triazole derivatives revealed that all tested compounds have the ability to protect mice from thermal pain (Table **III**). The hot plate latencies were found to be significantly increased when these derivatives and indomethacin were given i.m. at a dose level of 5 and 10 mg/kg into albino mice. Again, compounds **8a** and **9a** of the series showed the highest activity.

The prepared compounds were also found to have the ability to protect mice from chemical pain induced by p-benzoquinone. The median effective doses of the tested compounds and indomethacin are given in Table **II**. None of the triazole derivatives is different significantly in potency from indomethacin. However, the median effective doses of compounds **8a** and **9a** were found to be less than indomethacin and compound **7c** exhibited an analgesic potency almost comparable to that of indomethacin. On the other hand, the median effective doses of the rest of compounds were found to be higher than that of indomethacin. As evident from Table **II**, compounds **8a** and **9a** showed the highest potency, while, compound **7a** showed the least potency against writhing induced by p-benzoquinone.

Gum acacia 5% suspension used as vehicle for the test compounds produced no anti-inflammatory or analgesic activities.

Conclusion

All of the tested compounds proved active as anti-inflammatory and analgesic agents. Upon comparison with indomethacin, compounds **8a** and **9a** are more potent, **7c** is equipotent, and the rest of compounds having activities 80-100% of it in different tests.

The overall high anti-inflammatory and analgesic activities obtained by the investigated compounds could point to the importance of the partition coefficients of the derivatives and the electronic parameters of the substituents. Otherwise, it might be indicative of the requirement for balanced hydrophobic-hydrophilic interaction at the receptor surface. The calculated partition coefficients, expressed as calculated log **p** (Table **IV**), were determined according to equation **1**[18].

$$\log \mathbf{p} = 1.244\,(CX)^{0.6} - 1.017\,(NO)^{0.9} + 0.406\,PRX - 0.145\,(UB)^{0.8} + 0.511\,HB + 0.264\,POL - 2.215\,AMP + 0.912\,ALK - 0.392\,RNG - 3.684\,QN + 0.474\,NO2 + 1.582\,NCS + 0.773\,BLM - 1.041 \quad \text{.....(Equation 1)}$$

Where CX; Summation of numbers of carbon and halogen atoms weighted by C: 1, Cl: 1, Br: 1.5. NO; Total numbers of N and O atoms. PRX; Proximity effect of N/O. UB; Total numbers of unsaturated bonds. HB; Dummy variable for the presence of hydrogen

Table III: Analgesic activity of the triazole derivatives as measured by hot plate method in mice.

Compd. No.	Dose (mg/kg)	Reaction time (sec.)
Control	-	6.17 + 0.30
Indo-methacin	5	10.50** + 0.43
	10	14.67** + 0.50
7a	5	9.67** + 0.21
	10	13.50** + 0.43
7b	5	9.83** + 0.40
	10	13.83** + 0.48
7c	5	10.33** + 0.40
	10	14.33** + 0.42
8a	5	11.33** + 0.56
	10	15.67** + 0.57
8b	5	8.17** + 0.31
	10	12.17** + 0.31
8c	5	9.83** + 0.31
	10	13.17** + 0.48
9a	5	10.67** + 0.42
	10	15.17** + 0.48
9b	5	10.17** + 0.37
	10	13.17** + 0.48
9c	5	9.67** + 0.21
	10	13.33** + 0.42

Values are mean of 6 experiments ± standard error.
** Significant difference at $p < 0.01$.

Table IV: The data of the calculated log P from equation 1 for the synthesized compounds.

Compd.	$(CX)^{0.6}$	PRX	HB	POL	ALK	NO_2	NCS	BLM	$(NO)^{0.9}$	$(UB)^{0.8}$	AMP	RNG	QN	Cal. log P
7a	5.76	4	1	5	1	1	-	-	7.19	5.76	-	2	-	2.05
7b	5.65	4	1	5	1	1	-	-	7.19	5.76	-	2	-	1.92
7c	5.47	4	1	5	1	2	-	-	9.36	5.76	-	2	-	-0.03
8a	5.37	3	1	5	1	1	-	-	7.94	5.76	-	2	-	0.40
8b	5.28	3	1	5	1	1	-	-	7.94	5.76	-	2	-	0.29
8c	5.07	3	1	5	1	2	-	-	10.05	5.76	-	2	-	-1.65
9a	5.37	4	2	5	1	1	-	-	8.57	5.76	1	2	-	-1.12
9b	5.28	4	2	5	1	1	-	-	8.57	5.76	1	2	-	-0.61
9c	5.07	4	2	5	1	2	-	-	10.30	5.76	1	2	-	-2.16

bonding. PO; Number of aromatic polar substituents. AMP; Amphoteric property. ALK; Dummy variable for alkane, alkene, alkyne or cycloalkene. RNG; Dummy variable for the presence of ring structures other than benzene. QN; Quaternary nitrogens. NO2; Number of nitro groups. NCS; Number of isothiocyanate groups. BLM; Dummy variable for the presence of ß-lactam.

Calculated log **p** data showed very weak lipid solubility for the esters **(7a-c)**, but, the corresponding hydrazides **(8a-c)** and free acids **(9a-c)** are calculated hydrophilic. Obviously upon comparison with indomethacin, **8a** and **9a** (calc. log p = 0.40, -1.12, respectively) are more potent, and **7c** (calc. log p = -0.03) is equipotent. Deviation from the range of calculated log p from (0.40) to (-1.12) resulted in a slight decrease in the activity.

The relation of the calculated log p using equation **1** and the pharmacological results showed a good fit for this type of molecules. Upon studying the substituent effect on activity it has been found that in case of N1-p-bromophenyl-substitution, the hydrazide **8a** is slightly more active than the acid **9a** which is more active than the ester **7a**. For the p-chlorophenyl- and the p-nitrophenyl derivatives, the esters **7b** and **7c** are slightly more potent than the corresponding hydrazides and carboxylic acids.

EXPERIMENTAL

Chemistry

Precoated silica gel 60 F-254 plates (Merck) were used for thin layer chromatography; spots were detected by ultraviolet light and/or iodine vapor staining. Melting points are determined in open capillary tubes using Electrothermal AI-9100 melting point apparatus and are uncorrected. IR spectra were recorded (KBr discs) on a Shimadzu 408 spectrometer. ^{1}H-NMR spectra were measured (DMSO-d_6 unless otherwise stated) on JEOL JNM-GSX-500 (500 MHz) and JEOL GX-270 (270 MHz) spectrometers. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Mass spectra were performed with MS Shimadzu QP 1000 EX, at 70 eV. Elemental analyses (C, H, N) were done Perkin-Elmer 240 analyzer, at the Faculty of Science, Assiut University. The 4-Br-, 4-Cl-, and 4-O_2N-anilines are commercially available. The 2-methyl-3-nitro-6-pyridinecarbaldehyde **4** was obtained by the conventional nitration of 2,6-lutidine followed by oxidation with SeO_2 of the resulting 3-nitro-2,6-lutidine[14-16]. Compounds **2a-c** and **3a-c** were obtained following the reported procedures[3].

Ethyl 5-(2-methyl-3-nitropyridine-6-yl)-1-(4-substituted phenyl)-1*H*-1,2,4 triazole-3-carboxylate 7a-c

General procedure: A solution of the appropriate **3a-c** (0.01 mol) and 2-methyl-3-nitro-6-pyridinecarbaldehyde **4** (0.01 mol) in a mixture of acetic anhydride (0.01 mol) and acetic acid (20 ml) was refluxed for 8-14 hr. The reaction mixture was cooled and poured onto ice-water (150 ml). The precipitated solid was filtered and air dried. Crystallization from ethanol (95%) afforded **7a-c** (Table **I**).

Ethyl 1-(4-bromophenyl)-5-(2-methyl-3-nitropyridine-6-yl)-1*H*-1,2,4-triazole-3-carboxylate 7a (Table I)

Measured in DMSO-d_6, compound **7a** revealed: 8.58 d, 1H (H-4", J= 8.60); 8.21 d, 1H (H-5", J= 8.56); 7.74 d, 2H (H-3' and H-5', J= 8.10); 7.51 d, 2H (H-2' and H-6', J= 8.20); 4.40 q, 2H ($COO\underline{CH_2}CH_3$, J= 6.10) 2.39 s, 3H (Ar-CH_3); 1.34 t, 3H ($COOCH_2\underline{CH_3}$, J= 7.3). In $CDCl_3$ it revealed: 8.32 d, 2H (H-4" and H-5", J= 3.20); 7.74 d, 2H (H-3' and H-5', J= 8.50); 7.25 d, 2H (H-2' and H-6', J= 8.50) 4.43 q, 2H ($COO\underline{CH_2}CH_3$, J= 7.00); 2.50 s, 3H (Ar-CH_3); 1.48 t, 3H ($COOCH_2\underline{CH_3}$, J= 7.25).

IR (KBr) spectrum: 740, 830, 900, 1570 (Aromatic), 1360, 1525 (NO_2), 1200 (Ar-Br), 1600 (C=N), 1745 (Ester C=O), 2850, 2920, 3080 (Aliphatic-H and Aromatic-H). MS (70 eV) spectrum: 433 (M^++2 22.5), 432 (M^++1 68.5), 431 (M^+ 26.1), 430 (66.7), 402 (32.6), 386 (47.8) 384 (39.9), 171 (70.3), 169 (73.3), 90 (100.0), 75 (23.9), 63 (51.1).

Ethyl 1-(4-chlorophenyl)-5-(2-methyl-3-nitropyridine-6-yl)-1*H*-1,2,4-triazole-3-carboxylate 7b (Table I)

Compound **7b** in DMSO-d_6 showed: 8.60 d, 1H (H-4", J= 8.60), 8.23 d, 1H (H-5", J= 8.50); 7.61 s, 4H (H-2', H-3', H-5' and H-6', changed to a doublet of J= 3.30 Hz with D_2O); 4.42 q 2H ($COO\underline{CH_2}CH_3$, J= 6.10); 2.40 s, 3H ($Ar\text{-}CH_3$); 1.35 t, 3H ($COOCH_2\underline{CH_3}$, J= 7.30).

IR (KBr) spectrum: 740, 830, 905, 1570 (Aromatic), 1185 (Ar-Cl), 1355, 1520 (NO_2), 1600 (C=N), 1740 (Ester C=O), 2850, 2940, 3070 (Aliphatic-H and Aromatic-H).

Ethyl 5-(2-methyl-3-nitropyridine-6-yl)-1-(4-nitrophenyl)-1*H*-1,2,4-triazole-3-carboxylate 7c (Table I)

Compound **7c** in DMSO-d_6 revealed 8.61 d, 1H (H-4", J= 8.50); 8.40 d, 2H (H-3' and H-5', J= 8.10); 8.27 d, 1H (H-5", J= 8.50); 4.43 q, 2H ($COO\underline{CH_2}CH_3$, J= 6.50); 2.37 s, 3H ($Ar\text{-}CH_3$); 1.36 t, 3H ($COOCH_2\underline{CH_3}$, J= 7.30).

IR (KBr) spectrum: 735, 830, 895, 1570 (Aromatic), 1350, 1360, 1520 (NO_2), 1600 (C=N), 1735 (Ester C=O), 2860, 2950, 3010, 3090 (Aliphatic-H and Aromatic-H).

5-(2-Methyl-3-nitropyridine-6-yl)-1-(4-substituted phenyl)-1*H*-1,2,4-triazole-3-carbohydrazide 8a-c (Table I)

General procedure: A solution **7a-c** (0.01 mol) in ethanol (30 ml) was added portionwise with stirring at room temperature to hydrazine hydrate (90%, 0.012 mol) in ethanol (10 ml) over a period of 30 min. The reaction mixture was refluxed for three hours and then left to crystallize out, filtered and washed with aqueous ethanol (50%). The solid obtained was recrystallized from ethanol to afford **8a-c**.

1-(4-Bromophenyl)-5-(2-methyl-3-nitropyridine-6-yl)-1*H*-1,2,4-triazole-3-carbohydrazide 8a (Table I)

Measured in DMSO-d_6, compound **8a** revealed: 10.01 s, 1H ($CONH\underline{NH_2}$, exchangeable with D_2O); 8.62 d, 1H (H-4", J= 8.00 Hz); 8.20 d, 1H (H-5", J= 8.07) 7.64 d, 2H (H-3' and H-5', J= 7.60); 7.52 d, 2H (H-2' and H-6', J= 7.55); 4.65 br s, 2H ($CONHN\underline{H_2}$, exchangeable with D_2O); 2.43 s, 3H ($Ar\text{-}CH_3$).

IR (KBr) spectrum: 750, 830, 1535 (Aromatic), 1190 (Ar-Cl), 1350, 1520 (NO_2), 1595 (C=N), 1690 (Hydrazide C=O), 2950, 3060 (Ar-H), 3100-3300 (N-H)

1-(4-Chlorophenyl)-5-(2-methyl-3-nitropyridine-6-yl)-1*H*-1,2,4-triazole-3-carbohydrazide 8b (Table I)

Compound **8b** in DMSO-d_6 showed: 10.04 s, 1H ($CONH\underline{NH_2}$, exchangeable with D_2O); 8.63 d, 1H (H-4", J= 8.20); 8.21 d, 1H (H-5", J= 8.30); 7.60 d, 4H (H-4', H-3', H-5' and H-6', J= 1.80 Hz, changed to doublet of J= 4.20 with D_2O); 4.64 br s, 2H ((CONHN$\underline{H_2}$, exchangeable with D_2O); 2.42 s, 3H ($Ar\text{-}CH_3$).

IR (KBr) spectrum; 755, 830, 1560 (Aromatic), 1350, 1530 (NO_2), 1600 (C=N), 1695 (Hydrazide C=O), 2950, 3070 (Aliphatic-H and Aromatic-H), 3100-3300 (N-H). MS (70 eV) spectrum: 375 (M^++2 14.0), 374 (M^++1 8.6), 373 (M^+ 39.4), 372 (61.1), 339 (26.7), 311 (19.1), 134 (44.2), 90 (100), 74 (17.8).

5-(2-Methyl-3-nitropyridine-6-yl)-1-(4-nitrophenyl)-1*H*-1,2,4-triazole-3-carbohydrazide 8c (Table I)

Compound **8c** in DMSO-d_6 revealed: 10.09 s, 1H ((CONH$\underline{H}NH_2$, exchangeable with D_2O); 8.64 d, 1H (H-4", J= 8.60); 8.38 d, 2H (H-3' and H-5', J= 8.00); 8.23 d, 1H (H-5", J= 8.61); 7.85 d, 2H (H-2' and H-6', J= 8.18) 4.64 br s, 2H ((CONHN$\underline{H_2}$, exchangeable with D_2O); 2.42 s, 3H ($Ar\text{-}CH_3$).

IR (KBr) spectrum: 760, 830, 900, 1580 (Aromatic), 1355, 1525 (NO_2), 1600 (C=N), 1700 (Hydrazide C=O), 2940, 3070 (Aliphatic-H and Aromatic-H), 3150-3400 (N-H).

5-(2-Methyl-3-nitropyridine-6-yl)-1-(4-substituted phenyl)-1*H*-1,2,4-triazole-3-carboxylic acids 9a-c (Table I)

General procedure: To a solution of the appropriate **7a-c** (0.01 mol) was added with stirring potassium hydroxide solution (2N, 10 ml). The reaction mixture was refluxed for 3 hours, cooled and neutralized with hydrochloric

acid (2N, 12 ml). The precipitated solid was filtered, washed with water, dried and crystallized from ethanol to afford **9a-c**.

1-(bromophenyl)-5-(2-methyl-3-nitropyridine-6-yl)-1*H*-1,2,4-triazole-3-carboxylic acid 9a (Table I)

IR (KBr) spectrum: 725, 820, 895, 1005 (Aromatic), 1070, 1220 (Ar-Br), 1475, 1580, 1335, 1515 (C=N and NO_2), 1720 (Carboxylic C=O), 2500-2460 (Combination overtones due to hydrogen bonding and carboxylate anion) 2700-3300 (Ar-H, N-H and O-H stretching).

1-(Chlorophenyl)-5-(2-methyl-3-nitropyridine-6-yl)-1*H*-1,2,4-triazole-3-carboxylic acid 9b (Table I)

IR (KBr) spectrum: 730, 830, 860, 1010, 1090, 1200, 1400, 1570 (Aromatic), 1315, 1500 (NO_2), 1590 (C=N), 1715 (C=O), 2500-3450 (Combination overtones due to hydrogen bonding and carboxylate anion embodying NH, OH, CH stretching).

5-(2-Methyl-3-nitropyridine-6-yl)-1-(4-nitrophenyl)-1*H*-1,2,4-triazole-3-carboxylic acid 9c (Table I)

IR (KBr) spectrum: 730, 855, 1200, 1570 (Aromatic), 1335, 1505 (NO_2), 1600 (C=N), 1720 (C=O), 2500-3480 (Combination overtones due to carboxylate anion and hydrogen bonding including the OH, NH_2 and CH stretching). MS (70 eV) spectrum: 372 (M^++2 0.8), 371 (M^++ 3.1), 370 (M^+ 9.9), 353 (18.3), 326 (37.8), 91 (100), 90 (87.1).

Pharmacology

Evaluation of the anti-inflammatory activity

The anti-inflammatory activity of the compounds under investigation was studied in rats using two different methods:

Trypan-blue method

This method was described by Golikov[19] and depends on the quantitative determination of the effect of drugs under investigation on the rate of capillary permeability disturbance induced by intradermal injection of a phlogogenic substance such as histamine. The rate of capillary permeability was calculated as the time taken for the appearance of a blue color around the site of the intradermal injection of histamine phosphate (0.02 ml, 0.1% solution) following an intravenous injection of 2 mg/kg of a solution of the trypan-blue dye. Suspensions of the tested compounds and indomethacin, the reference standard drug, in 5% gum acacia were intramuscularly injected into adult albino rats (200-260 g) in two dose levels (5 and 10 mg/kg) and their anti-inflammatory activity (Figure 1) was estimated 1 hour after injection. Control animals were treated similarly after injection of 5% suspension of gum acacia.

Kaolin edema method

The anti-inflammatory action was also determined by kaolin edema method as described by Vinegar[20]. Adult albino rats (200-260 g) was used. Kaolin as a 10% suspension in pyrogen-free saline, continuously stirred by magnetic stirrer, was injected into the hind paw of rats. The thickness of rat paw was measured by a Vernier caliper (SMIEC) before and 7 hours after kaolin injection to detect the inflammation induced by kaolin. Suspensions of the tested compounds and indomethacin were intramuscularly injected into rats 4 hours after the subplantar injection of kaolin and the thickness of the paw was measured 7 hours after kaolin injection. Control animals were treated with 5% suspension of gum acacia. Groups of 6 animals were used for each dose level. The percentage of anti-inflammatory activity was determined. The median effective dose (ED_{50}) and its confidence limits (Table II) were calculated for each compound by the method of Litchfield and Wilcoxon[21].

Evaluation of the analgesic activity

The analgesic activity of the compounds under investigation was studied in mice using two different methods.

Hot plate method

In this method, the time taken by the mouse to lick its feed to jump within a plexiglass cylinder placed on a hot plate surface (55°C) was determined. This reaction time was taken as the end point (Eddy and Leimbach[22]) and the increase in hot plate latency was taken as a

measure of the analgesic activity. Adult albino mice (20-28 g), grouped in 6 for each dose, were intramuscularly injected with test compounds and indomethacin, the reference standard drug, as suspensions in 5% gum acacia. The analgesic activity was evaluated 1 hour after injection (Table II). Control animals were treated with 5% suspension of gum acacia.

Writhing method

The analgesic activity of the investigated compounds was also studied by using p-benzoquinone writhing method in mice (Okun *et al.*[23]). Before carrying out the experiments, a sensitivity test was done to determine the sensitivity of mice to p-benzoquinone. In this test, animals were administered intraperitoneal injection of p-benzoquinone solution (0.25 ml, 0.02% in H_2O).

The animals were observed for writhing during a period of 1 hour. Only animals which responded to p-benzoquinone by writhing were used in the main experiment, not less than 48 hours later. Suspensions of test compounds and indomethacin, the reference standard drug, in 5% gum acacia were subcutaneously injected into the back of the neck of mice. Control animals were treated with 5% suspension of gum acacia. After 1 hour, the animals were intraperitonealy injected with 0.25 ml of 0.02% solution of p-benzoquinone H_2O. The animals were observed for writhing during a period of 1 hour. Groups of 10 adult albino mice (20-22 g) were used for each dose level. The percentage of protection against p-benzoquinone-induced writhing was determined. The median effective doses (ED_{50}) and its 95% confidence limits (Table II) were calculated for each compound by the method of Litchfield and Wilcoxon[21].

Chemicals

The following chemicals were used: Kaolin (Cid co.); Histamine phosphate (Merck Sharp Co.); Indomethacin (Cid. Co.); Trypan blue (Prolabo Co.) and p-Benzoquinone (Prolabo Co.)

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