Bull. Pharm. Sci., Assiut University, Vol. 31, Part 2, 2008, pp. 197-214.

SYNTHESIS OF CERTAIN PYRAZOLO[3,4-d] PYRIMIDINE DERIVATIVES OF POTENTIAL ANTI-INFLAMMATORY ACTIVITY

Safinaz E. S. Abbas, Hanan H. Georgey, Shimaa M. Abdelrahman and Gamil M. El-Taliawi

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Egypt

يتناول هذا البحث تشييد تسعة وعشرين مركبا جديدا من مشتقات البيرازولوبيرميدين المستبدلة في الموقع ٤ للمركب ٤-كلورو-١-فينيل بيرازولو[٣،٤- د] بيرميدين بواسطة مجموعات مختلفة مثل ٤-أمينو-٣-ميثيل فينول، ٤-فينيلين داي أمين و ٤-أسيتاميدو فينول بغرض الحصول على مضادات للالتهاب غير حمضية. هذا وقد تضمن البحث الأسس التي اعتمد عليها في تصميم هذه المركبات وكذلك الخطط المستخدمة في بنائها. و قد تم التأكد من صحة المركبات الجديدة باستخدام مختلف الأساليب الطيفية. و تم عمل المسح البيولوجي في الفئران لثلاثة عشر مركب كمضادات للالتهاب بالمقارنة مع دواء الإيندوميثازين و قد وجد إن أثني عشرة منهم لهم فعالية كمضاد للالتهابات بينما مركب **10a** لم يظهر له أي فعالية كمضاد للالتهابات.

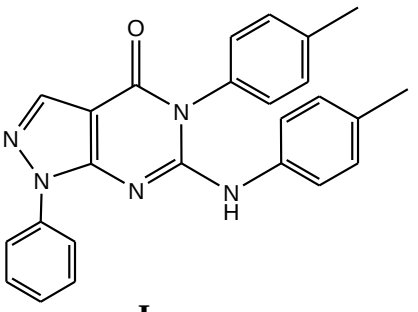
*The synthesis of twenty nine novel derivatives of pyrazolo[3,4-d]pyrimidines as non acidic nonsteroidal anti-inflammatory drugs has been achieved via reaction of 4-chloro-1-phenylpyrazolo[3,4-d]pyrimidine with different substituents including 4-amino-3-methylphenol, 4-phenylene diamine and 4-acetamidophenol.The anti-inflammatory activity of thirteen representative compounds have been screened compared to indomethacin as a reference drug. The results revealed that all the tested compounds showed anti-inflammatory activity with the exception of **10a**.*

INTRODUCTION

One of the most frequently encountered heterocycles in medicinal chemistry is pyrazolopyrimidine with antitumor[1-3], antihypertensive[4&5], against Alzheimer's disease[6], anti-allergic[7&8], antidiabetic[9], anti-viral[10&11], antibacterial[12&13] anti-fungal[14], and anti-inflammatory activities[15-17].

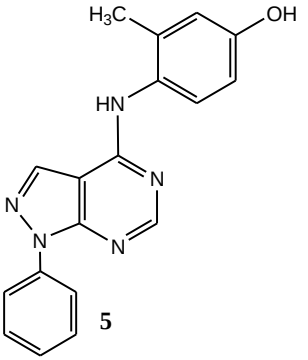
Received in 26/6/2008 Received in revised form in 29/9/2008 & Accepted in 4/10/2008

The common disadvantage of the conventional type of non-steroidal anti-inflammatory drugs (NSAID) are the induction of noxious ulcer[18&19] due to the non specific COX inhibition[20&21]. Furthermore, the erosion property of the carboxylic group usually present in the compounds leads to the primary insult effect. Therefore, non acidic NSAIDs show potent anti-inflammatory activity which is associated with remarkable systemic and gastric tolerance such as **I**[22]. Based on this consideration, it was suggested to synthesize novel substituted pyrazolo [3,4-d]pyrimidines aiming that the new compounds would find an acceptable value as anti-inflammatory with more pronounced action.

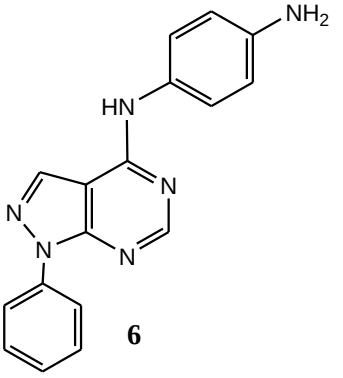


I

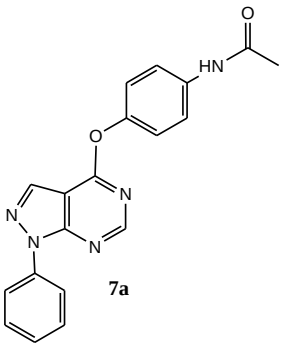
In the present investigation, motivated by the foregoing information, changing the substituent from position 5 and 6 in **I** into position 4 of the pyrazolo[3,4-d]pyrimidine ring was achieved in order to study the effect of different substituents on the anti-inflammatory activity. Therefore, compounds **5**, **6** and **7a** were synthesized using substituted anilino or substituted phenoxy moieties at position 4 and then anti-inflammatory screening was evaluated for them. The preliminary screening of these three derivatives revealed that, they showed anti-inflammatory activity. As a result, they were subjected to further derivatization to give **7b, 8a,b**, **9a-d**, **10a-e**, **11a,b**, **12**, **13a-d**, **14a-d**, **15** and **16a,b**.



5



6



7a

Chemistry

The present work involves the synthesis of certain pyrazolo[3,4-d]pyrimidine derivatives. Schemes 1, 2 and 3 summarize the steps involved in the synthesis of the key intermediates and the final compounds.

4-Chloro-2-phenyl-1*H*-pyrazalo [3,4-d]pyrimidine **4**; previously prepared according to Cheng and Robins[23]; was refluxed with 3-amino-3-methylphenol or 1,4-phenylene-diamine in absolute ethanol to yield **5** and **6**. Infrared spectra revealed the appearance of two absorption bands at 3393.1 and 3295.8 cm^{-1} attributed to the OH and NH respectively for **5** or at 3327.7 and 3211.0 cm^{-1} attributed to the NH_2 and NH for **6**. ^1H NMR of **5** showed the appearance of a singlet signal at 2.16 ppm integrated for three protons corresponding to the methyl protons, in addition to two singlet signals at 4.30 ppm and 9.80 ppm, each integrated for one proton that disappeared upon deuterium exchange assigned to OH and NH respectively, while that of **6** revealed the appearance of two singlet signals at 9.30 ppm and 9.90 ppm exchangeable with D_2O corresponding to NH_2 and NH protons respectively. Mass spectrum showed a molecular ion peak at m/z 317 as 1.19% and m/z 302 as 1.53% for **5** and **6** respectively (Scheme 1).

Furthermore, reaction of **4** with paracetamol or its substituted analogue afforded **7a,b**. Infrared spectra of **7a** and **7b** showed appearance of NH absorption band at 3112.6 and 3152.0 cm^{-1} respectively, CH aliphatic band at 2958.3 and 2952.0 cm^{-1} respectively and CO absorption band at 1730.8 and 1673.5 cm-1 respectively. ^1H NMR of compound **7a** showed the appearance of a singlet signal at 2.16 ppm corresponding to the three protons of the methyl group and a singlet signal at 8.30 ppm corresponding to NH proton. Mass spectrum of them showed a molecular ion peak at m/z 345 as 24.3% and M-2 at m/z 400 as 10.24% respectively (Scheme 1).

On the other hand, refluxing of **5** with alkyl/aralkyl halide in acetone in presence of potassium carbonate produced **8a,b** that were confirmed by microanalyses and spectral data. IR spectrum of **8a** revealed the disappearance of OH absorption band at 3393.1 cm-1. ^1H NMR data of it showed disappearance of OH signal and appearance of a singlet signal at δ 5.25 ppm equivalent to the two methylene protons. Also its mass spectrum showed a molecular ion peak at m/z 407(21.19%) (Scheme 2).

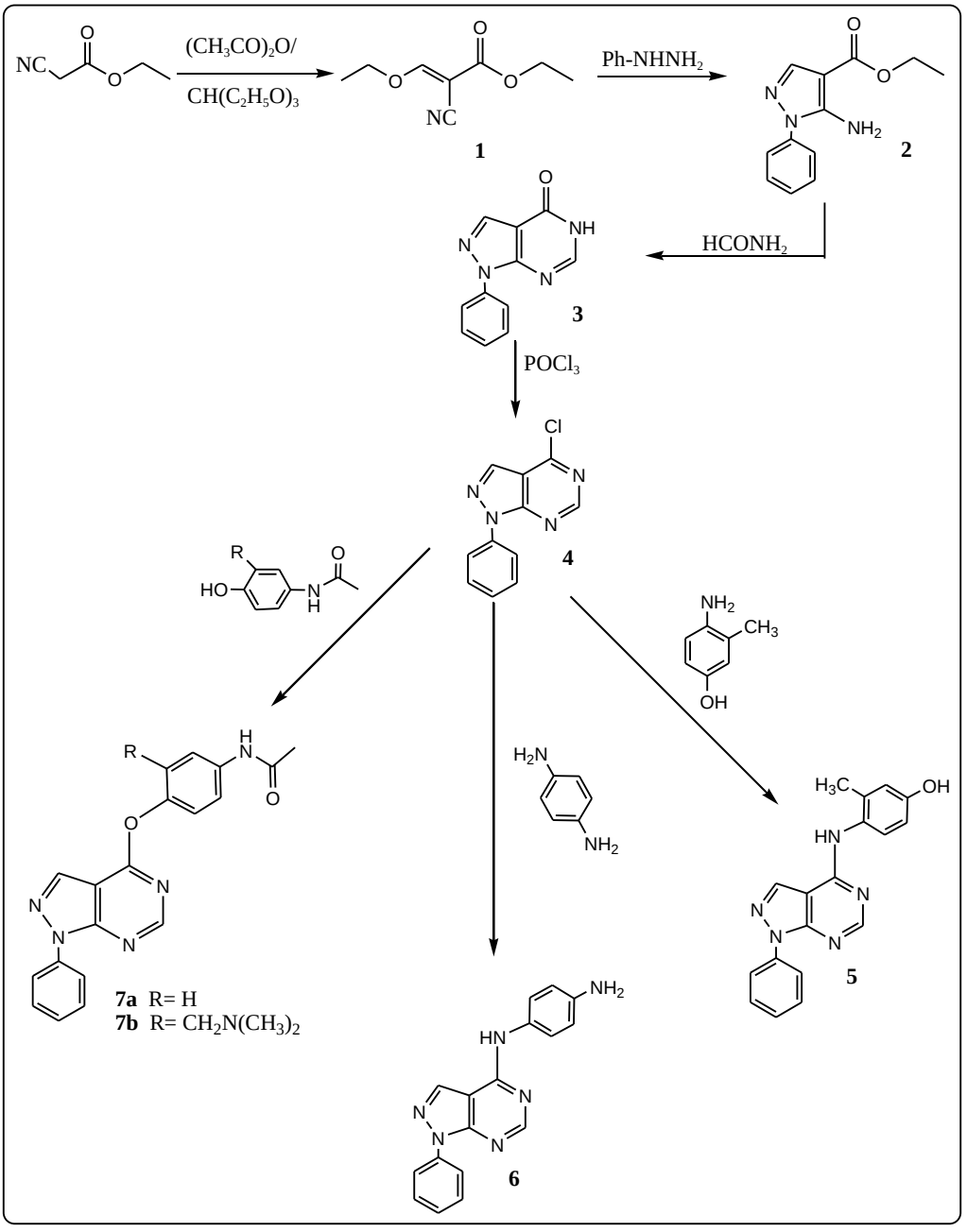
Further reaction of **5** with the appropriate isocyanate/ isothiocyanate

in methylene chloride in the presence of trietyl amine yielded **9a-d**. Infrared spectra of **9a,b** revealed the disappearance of OH absorption band and the appearance of CO absorption band at 1716.1 and 1700.0 cm^{-1} respectively or the appearance of CS absorption band at 1172.3 cm^{-1} for **9d**. ^1H NMR data of **9a** showed disappearance of OH signal and appearance of a singlet signal at 12.49 ppm exchangeable with D_2O corresponding to CO-NH proton. Mass spectrum of **9a** and **9b** showed a molecular ion peak at m/z 436 (4.9%) and M-3 at m/z 439 (0.76%) respectively (Scheme 2).

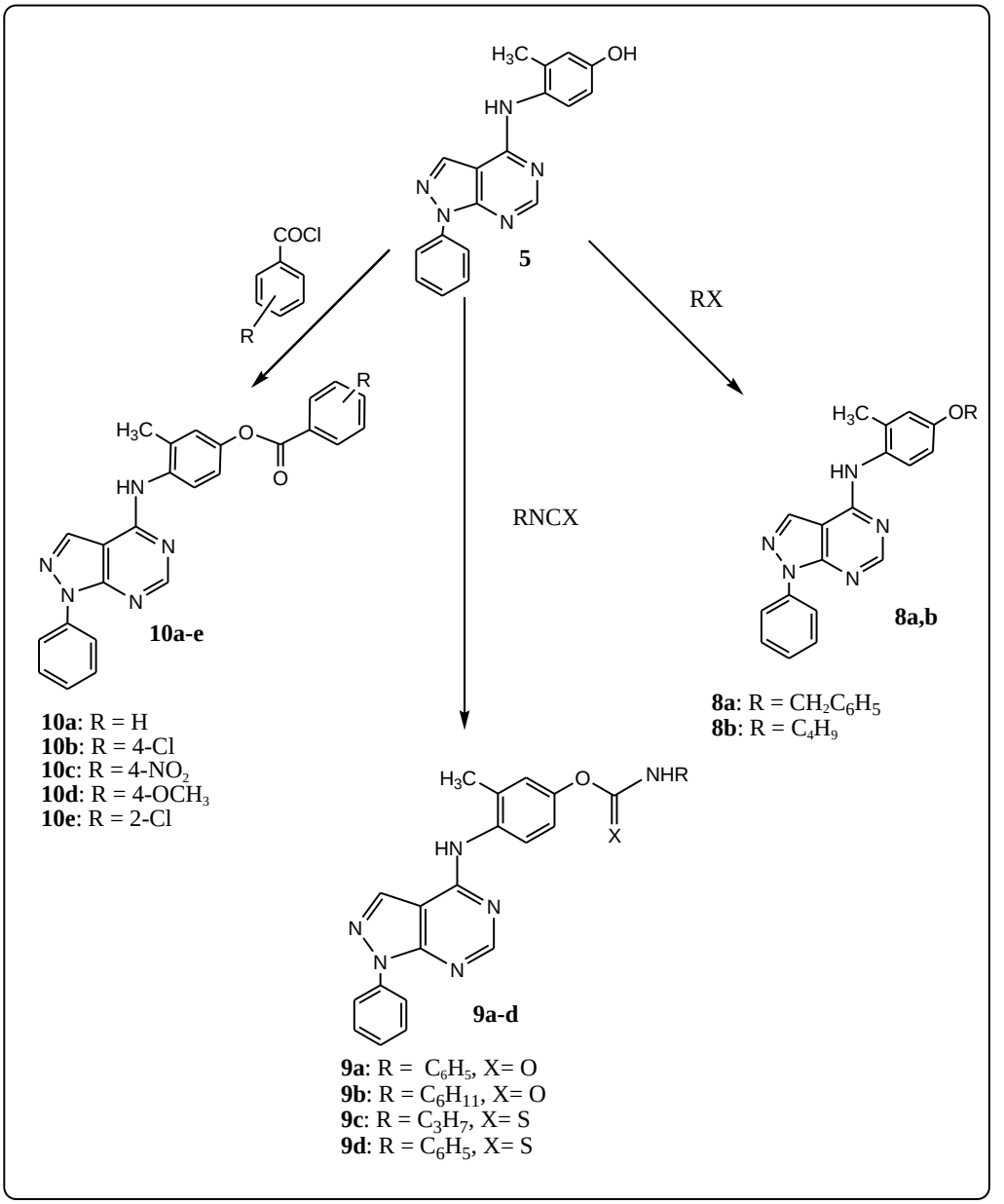
Moreover, refluxing of **5** with substituted benzoyl chloride afforded **10a-e**. IR spectra revealed the disappearance of OH absorption band at 3393.1 cm^{-1} and the appearance of CO absorption at 1685.0, 1741.4 and 1680.0 cm^{-1} for **10b**, **10c** and **10e** respectively. ^1H NMR spectrum of **10a** revealed the disappearance of OH signal. Mass spectrum of **10a** and **10c** showed a molecular ion peak at m/z 421 (0.03%) and 467 (0.44%) respectively (Scheme 2).

Besides, refluxing of the appropriate isocyanate with **6** formed urea derivatives **11a,b.** The trione derivative **12** was obtained by reaction of **11a** with oxalyl chloride. Infrared spectra of **11a** and **11b** showed the appearance of CO absorption band at 1733.7 and 1626.8 cm^{-1} respectively while **12** revealed the disappearance of two NH bands at 3324.7 and 3291.9 cm^{-1}.^1H NMR data of **11a** showed the appearance of a singlet signal at δ 12.38 ppm equivalent to CO-NH proton that was disappeared in **12**. Mass spectra of **11a** and **12** afforded M-2 at m/z 419 (0.7%) and 473 (3.3%) (Scheme 3).

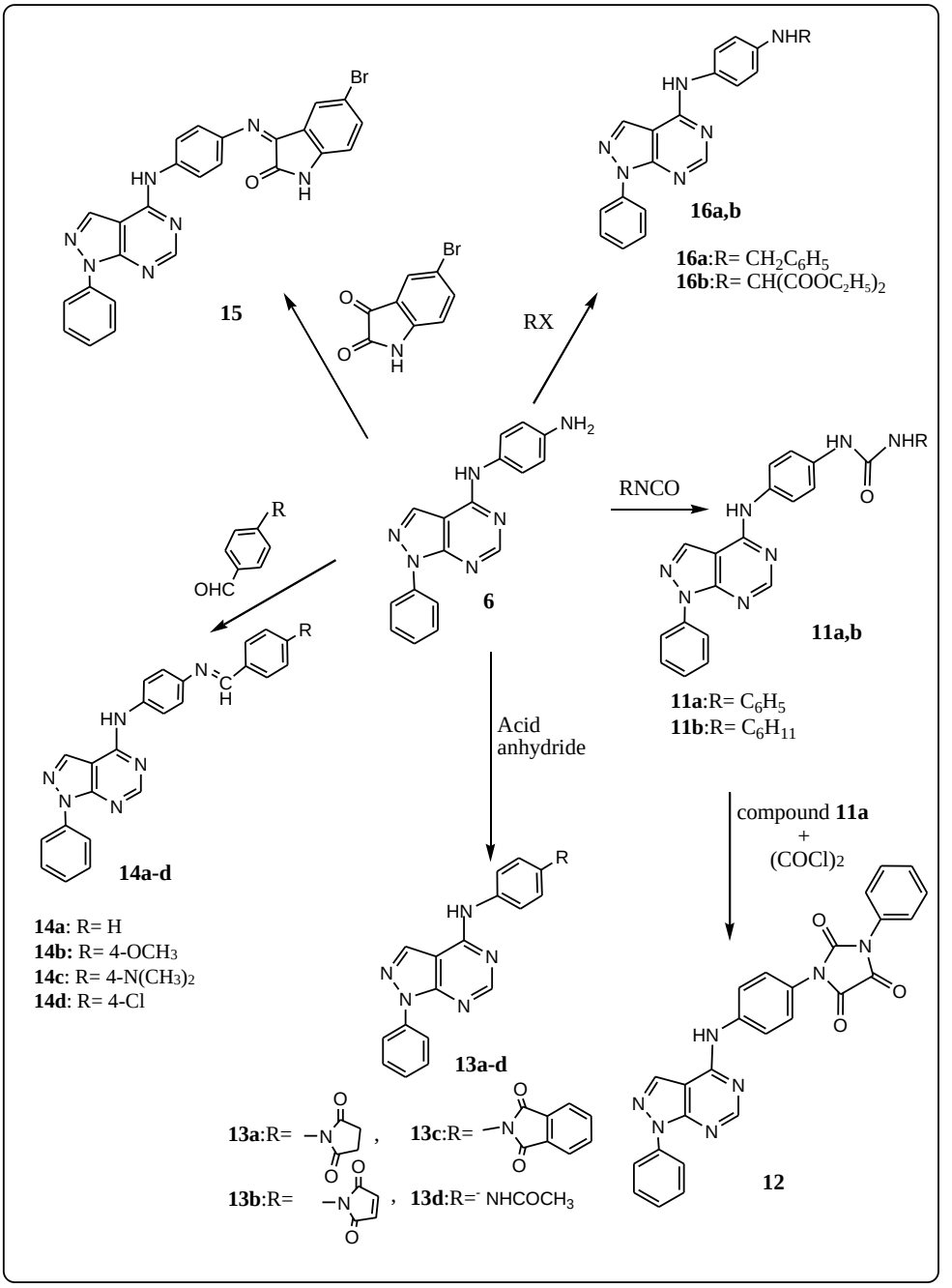
Additionally, the amino function of **6** is subjected to reaction with acid anhydrides, substituted aromatic aldehydes, 5-bromoisatin or alkyl halide to afford **13a-d**, the Schiff's bases **14a-d** and **15** or **16a-b** respectively. Infrared spectra of **13a** and **13d** revealed the disappearance of NH_2 absorption bands at 3327.7 and 3211.0 cm^{-1} and appearance of CO absorption band at 1750.0 – 1704.0 cm^{-1}. ^1H NMR spectra of **13a**, **13c** and **13d** showed the disappearance of NH_2 signal at 9.30 ppm and the appearance of a triplet signal at δ 1.91-2.06 equivalent to the four protons of the two methylene group of compound **13a** and a singlet signal at δ 2.02 equivalent to the three protons of CH_3 of compound **13d**. While ^1H NMR spectra of compounds **14c**, **15** and **16a** lacked the NH_2 signal and showed a singlet signal at δ 3.02 equivalent to six protons of $N(CH_3)_2$ together with a singlet signal at δ 9.64 equivalent to N=CH proton, a singlet signal at δ 11.2 exchangeable with D_2O correspond-ing to CO-NH proton and a singlet signal at δ 2.40 equivalent to the two methylene protons for **14c**, **15** and **16a** respectively. Mass spectra of **13a**, **13d**, **14b**, **14c**, **15**, **16a** and **16b** showed ion peak at m/z 384 (4.5%), 344 (100%), 420 (1.4%), 433 (6.37%), M+2 at m/z 512 (0.43%), 392 (1.28%) and 460 (33.30%) respectively (Scheme 3).


NC
O
O
(CH3CO)2O/
CH(C2H5O)3
NC
O
O
O
1
Ph-NHNH2
O
O
N
N
NH2
2
HCONH2
O
NH
N
N
3
POCl3
Cl
N
N
N
4
R
HO
N
H
O
NH2
CH3
OH
R
H
N
O
O
N
N
N
7a R= H
7b R= CH2N(CH3)2
H2N
NH2
H3C
OH
HN
N
N
N
5
NH2
HN
N
N
N
6

Scheme 1


5
RX
RNCX
COCl
R
10a-e
10a: R = H
10b: R = 4-Cl
10c: R = 4-NO2
10d: R = 4-OCH3
10e: R = 2-Cl
8a,b
8a: R = CH2C6H5
8b: R = C4H9
9a-d
9a: R = C6H5, X= O
9b: R = C6H11, X= O
9c: R = C3H7, X= S
9d: R = C6H5, X= S

Scheme 2


15
16a,b
16a:R= CH2C6H5
16b:R= CH(COOC2H5)2
RX
RNCO
6
11a,b
11a:R= C6H5
11b:R= C6H11
14a-d
14a: R= H
14b: R= 4-OCH3
14c: R= 4-N(CH3)2
14d: R= 4-Cl
Acid anhydride
13a-d
13a:R=
13b:R=
13c:R=
13d:R= NHCOCH3
compound 11a + (COCl)2
12

Scheme 3

EXPERIMENTAL

Chemistry

All melting points were determined by the open capillary tube method using GallenKamp digital melting point Griffin apparatus 1901 and are uncorrected. IR spectra were recorded on Bruker FT-IR spectrophotometer Vector 22, Schimadzu 435, Perkin-Elmer 457 and Jasco FT-IR plus 460 (Japan) Japan and expressed in wave number (cm^{-1}), using potassium bromide disc. ^1H NMR spectra were carried out using a Joel Ex 270 MHz spectrophotometer, Varian Gemini 200 MHz and Joel Fx 90Q, 90 MHz FT spectrophotometer.

The chemical shifts were expressed in δ ppm units using tetramethylsilane as the internal standard. Mass spectra were performed on Hewlett Packard 5988 at 70 eV. Elemental Microana-lysis were carried out using Heraew and Vario EL III (elemntar), CHNS analyzer (Germany) at the Microanalytical Center, Cairo University.

Compounds **1**[24], **2**[25], **3**[26], and **4**[23] were prepared according to the reported methods. Physical and microanalytical data of the newly synthesized compounds **7a,b, 8a,b**, **9a-d**, **10a-e**, **11a,b**, **13a-d, 14a-d** and **16a,b** are illustrated in Table 1.

Table 1: Physical and microanalytical data of the newly synthesized compounds.

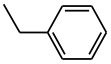
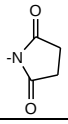
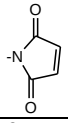
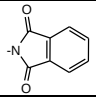
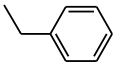
Compound No.	R	m.p. °C yield %	Mol. Formula Mol. Wt	Microanalysis	
				Calculated %	Found %
7a	H	290-293 40	$C_{19}H_{15}N_5O_2$ 345.36	C 66.08 H 4.38 N 20.28	66.00 4.50 20.25
7b	$-CH_2N(CH_3)_2$	300-3 55	$C_{22}H_{22}N_6O_2$ 402.46	C 65.66 H 5.51 N 20.88	65.39 5.22 20.85
8a		190-2 55	$C_{25}H_{21}N_5O$ 407.48	C 73.69 H 5.19 N 17.19	73.79 5.41 17.17
8b	$-CH_2CH_2CH_2CH_3$	147-50 50	$C_{22}H_{23}N_5O$ 373.46	C 70.76 H 6.21 N 18.75	70.83 5.48 19.01
9a	 (X= O)	160-2 45	$C_{25}H_{20}N_6O_2$ 436.47	C 68.80 H 4.62 N 19.25	68.90 4.90 19.57
9b	 (X= O)	120-2 42	$C_{25}H_{26}N_6O_2$ 442.52	C 67.86 H 5.92 N 18.99	67.59 5.77 19.13
9c	$-C_3H_7$ (X= S)	270-2 40	$C_{22}H_{22}N_6OS$ 418.52	C 63.14 H 5.30 N 20.08	62.89 5.59 19.92
9d	 (X= S)	260-2 42	$C_{25}H_{20}N_6OS$ 452.54	C 66.35 H 4.45 N 18.57	66.20 4.30 19.01
10a	H	187-90 55	$C_{25}H_{19}N_5O_2$ 421.46	C 71.25 H 4.54 N 16.62	71.20 4.50 16.33

Table 1: contiuned

Compound No.	R	m.p. °C yield %	Mol. Formula Mol. Wt	Microanalysis	
				Calculated %	Found %
10b	4-Cl	225-8 60	$C_{25}H_{18}ClN_5O_2$ 455.96	C 65.86 H 3.98 N 15.36	66.00 3.95 15.27
10c	4-NO_2	237-240 52	$C_{25}H_{18}N_6O_4$ 466.46	C 64.37 H 3.89 N 18.02	64.10 3.67 18.23
10d	4-OCH_3	220-2 57	$C_{26}H_{21}N_5O_3$ 451.49	C 69.17 H 4.69 N 15.51	68.70 4.50 15.32
10e	2-Cl	227-30 55	$C_{25}H_{18}ClN_5O_2$ 455.96	C 65.86 H 3.98 N 15.36	64.46 3.53 15.27
11a		250-2 60	$C_{24}H_{19}N_7O$ 421.46	C 68.40 H 4.54 N 23.26	68.52 4.71 23.26
11b		180-3 50	$C_{24}H_{25}N_7O$ 427.51	C 67.43 H 5.89 N 22.93	67.30 5.90 22.86
13a		170-3 53	$C_{21}H_{16}N_6O_2$ 384.40	C 65.62 H 4.20 N 21.86	65.41 4.23 21.78
13b		170-2 60	$C_{21}H_{14}N_6O_2$ 382.38	C 65.96 H 3.69 N 21.98	65.55 3.40 21.69
13c		190-2 65	$C_{25}H_{16}N_6O_2$ 432.44	C 69.43 H 3.73 N 19.43	68.57 3.73 19.63
13d	-$NHCOCH_3$	132-5 50	$C_{19}H_{16}N_6O$ 344.38	C 66.27 H 4.68 N 24.40	66.32 4.60 23.99
14a	H	145-7 60	$C_{24}H_{18}N_6$ 390.45	C 73.83 H 4.65 N 21.52	73.70 4.50 21.32
14b	4-OCH_3	230-2 55	$C_{25}H_{20}N_6O$ 420.48	C 71.41 H 4.79 N 19.99	71.62 4.94 19.95
14c	4-$N(CH_3)_2$	280-3 52	$C_{26}H_{23}N_7$ 433.52	C 72.04 H 5.35 N 22.62	71.86 5.20 22.40
14d	4-Cl	235-7 60	$C_{24}H_{17}ClN_6$ 424.94	C 67.84 H 4.03 N 19.78	67.90 4.30 19.49
16a		187-90 45	$C_{24}H_{20}N_6$ 392.46	C 73.45 H 5.14 N 21.41	73.10 5.14 21.62
16b	-$CH(COOC_2H_5)_2$	222-5 57	$C_{24}H_{24}N_6O_4$ 460.49	C 62.60 H 5.25 N 18.25	61.81 5.34 18.21

3-Methyl-4-(1-phenyl-1*H*-pyrazolo [3,4-d]pyrimidin-4-ylamino)phenol (5)

A mixture of compound **4** (2.3 g, 10 mmol) and 4-amino-3-methyl-phenol (1.35 g, 11 mmol) in absolute ethanol (20 ml) was heated under reflux for 5 hours. The solvent was evaporated under reduced pressure. The solid obtained was washed with water, filtered, dried and recrystallized from aqueous ethanol. M.p. 220-2°C, yield: 80%, M.wt: 317.35. Analysis of $C_{18}H_{15}N_5O$: Calculated C 68.13, H 4.76, N 22.07; Found C 68.00, H 4.80, N 22.17%. IR: 3393.1 (OH), 3295.8 (NH), 3035.4 (CH Ar), 2967.9 (CH aliphatic).—^{1}H NMR (DMSO-d_6): 2.16 (s, 3H, CH_3), 4.30 (s, 1H, OH (D_2O exchange)), 6.78-8.19 (m, 9H, Ar- Hs and pyrazole CH), 8.23 (s, 1H, pyrimidine CH), 9.80 (s, 1H, NH (D_2O exchange). Mass spectrum: m/z (relative abundance%) 317, M^+ (1.19), 212 (100).

***N*^{1}-(1-Phenyl-1*H*-pyrazolo[3,4-d] pyrimidin-4-yl)benzene-1,4-diamine (6)**

A mixture of compound **4** (2.30 g, 10 mmol) and 1,4-phenylenediamine (1 g, 11 mmol) in absolute ethanol (25 ml) was heated under reflux for 5 hours. The solvent was evaporated under reduced pressure, the solid obtained was washed with ethanol, filtered, dried and recrystallized from aqueous ethanol. M.p. 320-2°C, yield: 80%, M.wt: 302.34. Analysis of $C_{17}H_{14}N_6$: Calculated C 67.54, H 4.67, N 27.80; Found C 67.40, H 4.80, N 27.71%. IR: 3327.7, 3211.0 (NH_2, NH), 3040.2 (CH Ar), 1679.4 (NH bending). ^{1}H NMR (DMSO -d_6): 6.54-8.62 (m, 10H, Ar-Hs and pyrazole CH), 8.85 (s, 1H, pyrimidine CH), 9.30 (s, 2H, NH_2 (D_2O exchange)), 9.90 (s, 1H, NH (D_2O exchange)). Mass spectrum: m/z (relative abundance%) 302, M^+ (1.53), 212 (100).

***N*-(3-unsubstituted/substituted-4-(1-phenyl-1*H*-pyrazolo[3,4-d]-pyrimidin-4-yloxy)phenyl) acetamide (7a,b)**

A mixture of compound **4** (2.30 g, 10 mmol), paracetamol or N-(3-((dimethylamino)methyl)-4-hydroxy-phenyl)acetamide (11 mmol) in dry acetone (20 ml) and anhydrous potassium carbonate (0.36 g, 3 mmol) was heated under reflux for 24 hours. The reaction mixture was filtered while hot, then the solvent was evaporated under reduced pressure, the residual mass was triturated with ice water, and the resulting solid was filtered, washed with water, dried and recrystallized from acetone**.** IR of compound **7a**: 3112.6 (NH), 3037.3 (CH Ar), 2958.3 (CH aliphatic), 1730.8 (C=O). IR of **7b**: 3152.0 (NH), 3084.1 (CH Ar), 2952.0 (CH aliphatic), 1673.5 (C=O). ^{1}H NMR ($CDCl_3$) of **7a**: 2.16 (s, 3H, CH_3), 6.77-8.05 (m, 10H, Ar-Hs and pyrazole CH), 8.10 (s, 1H, pyrimidine CH), 8.30 (s, 1H, NH (D_2O exchange)). Mass spectrum of **7a**: m/z (%): 345, M^+ (24.3), 109 (100). Mass spectrum of **7b**: m/z (%): 400, M-2 (10.24), 57 (100).

_N_-(4-Aryloxy/Alkoxy)-2-methyl-phenyl)-1-phenyl-1_H_-pyrazolo[3,4-d]pyrimidin-4-amine (8a,b)

A mixture of compound **5** (3.17 g, 10 mmol) and the appropriate alkyl halide (11 mmol) in dry acetone (20 ml) and anhydrous potassium carbonate (0.36 g, 3 mmol) was refluxed for 18 hours. The reaction mixture was filtered while hot, then the solvent was evaporated under reduced pressure, the residue was poured onto crushed ice. The precipitated solid was filtered, washed, dried and recrystallized from acetone. IR of **8a**: 3168.5 (NH), 3058. 6 (CH Ar), 2917.8 (CH aliphatic). ^{1}H NMR (DMSO-d_6) of **8a**: 2.23 (s, 3H, CH_3), 5.25 (s, 2H, CH_2), 7.26-8.06 (m, 14H, Ar-Hs and pyrazole CH), 8.34 (s, 1H, pyrimidine CH), 8.68 (s, 1H, NH (D_2O exchange)). Mass spectrum of **8a**: m/z (%): 407, M^+ (21.19), 316 (100).

1-(3-Methyl-4-(1-phenyl-1_H_-pyrazolo[3,4-d]pyrimidin-4-yl-amino)phenyl)-3-aryl/alkyl urea/ thiourea (9a-d)

A mixture of compound **5** (3.17 g, 10 mmol) and the appropriate alkyl/aryl isocyanate/isothiocyanate (11 mmol) in methylene chloride (20 ml) and triethylamine (0.5 ml) was heated under reflux for 4 hours. The solid product separated on cooling was filtered, washed with water, dried and recrystallized from acetone. IR of **9a**: 3394.1, 3323.2 (NH), 3056.9 (CH Ar), 2923.5 (CH aliphatic), 1716.1 (C=O). IR of **9b**: 3412.3, 3332.4 (NH), 3064.0 (CH Ar), 2931.3 (CH aliphatic), 1700.0 (C=O). IR of **9d**: 3448.3, 3423.3 (NH), 3100.0 (CH Ar), 2940.3 (CH aliphatic), 1172.3 (C=S). ^{1}H NMR (DMSO-d_6) of **9a**: 2.11 (s, 3H, CH_3), 6.53-8.25 (m, 14H, Ar-Hs and pyrazole CH), 8.35 (s, 1H, pyrimidine CH), 8.73 (s, 1H, NH (D_2O exchange)), 12.49 (s, 1H, CO-NH (D_2O exchange)). Mass spectrum of **9a**: m/z (%): 436, M^+ (4.9), 57 (100). Mass spectrum of **9b**: m/z (%): 439, M-3 (0.76), 56 (100).

3-Methyl-4-(1-phenyl-1_H_-pyrazolo [3,4-d]pyrimidin-4-ylamino)phenyl unsubstituted/substituted benzoate (10a-e)

A mixture of Compound **5** (3.17 g, 10 mmol) and the appropriate substituted benzoyl chloride (11 mmol) in dry benzene (20 ml) and triethylamine (0.5 ml) was refluxed for 5 hour. The excess solvent was evaporated under reduced pressure, the residue was triturated with ice water, filtered, dried and recrystallized from acetone. IR of **10b**: 3448.0 (NH), 3092.5 (CH Ar), 2978.1 (CH aliphatic), 1685.0 (C=O), 760.8 (C-Cl). IR of **10c**: 3377.3 (NH), 3078.2 (CH Ar), 2919.9 (CH aliphatic), 1741.4 (C=O). IR of **10e**: 3440.0 (NH), 3010.0 (CH Ar), 2870.0 (CH aliphatic), 1680.0 (C=O), 780.1 (C-Cl). ^{1}H NMR (DMSO-d_6, δ ppm) of **10b**: 2.26 (s, 3H, CH_3), 7.54-8.15 (m, 14H, Ar-Hs and pyrazole <u>CH</u>), 8.16 (s, 1H, pyrimidine <u>CH</u>), 8.40 (s, 1H, NH (D_2O exchange)). Mass spectrum of **10a**: m/z (%): 421, M^+ (0.03), 86 (100). Mass spectrum of

10c: m/z (%): 467, M^+ (0.44), 212 (100).

1-Alkyl/Aryl-3-(4-(1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamino)phenyl) urea (11a,b)

A mixture of compound **6** (3.02 g, 10 mmol) and the appropriate alkyl/ aryl isocyanate (11 mmol) in methylene chloride (20 ml) and triethylamine (0.5 ml) was heated under reflux for 8 hours. The solid product separated on cooling was filtered, washed with water, dried and recrystallized from acetone. IR (cm^{-1}) of **11a**: 3324.7, 3291.9 (NH), 3093.3 (CH Ar), 1733.7 (C=O). IR (cm^{-1}) of **11b**: 3327.40 (NH), 3035.70 (CH Ar), 2927.80 (CH aliphatic), 1626.80 (C=O). ^{1}H NMR (DMSO-d_6) of **11a**: 6.91-8.31 (m, 15H, Ar-Hs and pyrazole CH), 8.61 (s, 1H, pyrimidine CH), 9.62 (s, 1H, NH (D_2O exchange)), 12.38 (s, 2H, CO-NH (D_2O exchange)). Mass spectrum of **11a**: m/z (%): 419, M-2 (0.7), 55 (100).

1-Phenyl-3-(4-(1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamino)phenyl) imidazoline-2,4,5(1*H*,3*H*)-trione (12)

A suspension of compound **11a** (4.2 g, 10 mmol) in dry benzene (25 mL) and triethylamine (0.5 mL) was heated with stirring at 60°C, then oxalyl chloride (1.4 g, 11 mmol) was added dropwise. The reaction mixture was then refluxed for 2 hours. The excess solvent was evaporated under reduced pressure, the residue was triturated with ice water, filtered, dried and recrystallized from ethanol. M. p. 218-20°C, yield: 62%, M. wt: 475.47. Analysis of $C_{26}H_{17}N_7O_3$: Calculated C 65.86, H 3.60, N 20.62; Found C 65.74, H 3.90, N 20.42%. IR: 3423.0 (NH), 3038.50 (CH Ar), 1704.30 (C=O). ^{1}H NMR (DMSO-d_6): 6.94-7.46 (m, 15H, Ar-Hs and pyrazole CH), 7.47 (s, 1H, pyrimidine CH), 8.62 (s, 1H, NH (D_2O exchange)). Mass spectrum: m/z (%): 473, M-2 (3.3), 103 (100).

4-(4-Substituted phenylamino)-1-Phenyl-1*H*-pyrazolo[3,4-d]pyrimidin (13a-d)

A mixture of compound **6** (3.02 g, 10 mmol) and the appropriate acid anhydride (11 mmol) in glacial acetic acid (10 ml) or acetic anhydride was heated under reflux for 10 hours (in case of **7a-c**) or 3hours (in case of **7d**). The excess solvent was distilled under reduced pressure. The residue was poured onto crushed ice and the precipitated solid was filtered, washed, dried and recrystallized from ethanol. IR (cm^{-1}) of **13a**: 3425.3 (NH), 3075.0 (Ar-CH), 2924.1 (CH aliphatic), 1704.0 (C=O). IR (cm^{-1}) of **13c**: 3369.7 (NH), 3056.4 (Ar-CH), 2923.3 (CH aliphatic), 1704.9 (C=O). IR (cm^{-1}) of **13d**: 3296.00 (NH), 3039.1 (Ar-CH), 2924.0 (CH aliphatic), 1708.20 (C=O). ^{1}H NMR (DMSO-d_6) of **13a**: 1.91-2.06 (t, 4H, $2CH_2$), 7.38-8.19 (m, 10H, Ar-Hs and pyrazole CH), 8.32 (s, 1H, pyrimidine CH), 9.82 (s, 1H, NH (D_2O exchange)). ^{1}H NMR (DMSO-d_6) of **13c**: 7.62-8.23 (m, 14H, Ar-Hs and pyrazole CH), 8.58 (s, 1H, pyrimidine

CH), 8.62 (s, 1H, NH (D_2O exchange)). ^{1}H NMR (DMSO-d_6) of **13d**: 2.02 (s, 3H, CH_3), 7.21-8.21 (m, 10H, Ar-CH, pyrazole CH), 8.35 (s, 1H, pyrimidine CH), 7.85 (s, 1H, NH (D_2O exchange)), 9.87 (s, 1H, CO-NH (D_2O exchange)). Mass spectrum of **13a**: m/z (%): 384 $(M)^+$ (4.5), 212 (100). Mass spectrum of **13d**: m/z (%): 344, M^+ (100).

N^1-Substituted benzylidene-N^4-(1-phenyl-1*H*-pyrazolo[3,4-d] pyrimidin-4-yl) benzene-1,4-diamine (14a-d)

A mixture of compound **6** (3.02 g, 10 mmol) and the appropriate aromatic aldehyde (11 mmol) in absolute ethanol (20 ml) containing 4 drops glacial acetic acid was heated under reflux for 8 hours. Ethanol was removed under reduced pressure and the residue was triturated with ice-water. The separated solid was filtered, washed, dried and re-crystallized from aqueous ethanol. IR of **14a**: 3380.0 (NH), 3030.0 (CH Ar), 2890.0 (CH aliphatic). IR of **14b**: 3420.0 (NH), 3055.0 (CH Ar), 2920.0 (CH aliphatic). IR of **14c**: 3108.0 (NH), 3036.0 (CH Ar), 2920.6(CH aliphatic). IR of **14d**: 3446.2 (NH), 3036.5 (CH Ar), 2923.1 (CH aliphatic). ^{1}H NMR (DMSO-d_6) of **14c**: 3.02 (s, 6H, $2CH_3$), 6.56-8.30 (m, 15H, Ar-Hs, pyrazole CH and NH (D_2O exchange), 8.45 (s, 1H, pyrimidine CH), 9.64 (s, 1H, N=CH). Mass spectrum of **14b**: m/z (%): 420, M^+ (1.4), 212 (100). Mass spectrum of **14c**: m/z (%): 433, M^+ (6.37), 368 (100).

5-Bromo-3-(4-(1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-yl-amino)phenylimino) indolin-2-one (15)

A mixture of compound **6** (3 g, 10 mmol) and 5-bromo isatin (5.1 g, 11 mmol) in glacial acetic acid (5 mL) was heated under reflux for 3 hours. The solvent was evaporated under reduced pressure, the residue was poured onto crushed ice, the precipitated solid was filtered, washed, dried and recrystallized from aqueous ethanol. M. p. 168-70°C, yield: 68%, M. wt: 510.35. Analysis of $C_{25}H_{16}BrN_7O$: Calculated C 58.84, H 3.16, N 19.21; Found C 59.21, H 3.11, N 19.32%. IR: 3296.6, 3172.2 (NH), 3076.1 (CH Ar), 2924.1 (CH aliphatic), 1750.0 (C=O). ^{1}H NMR (DMSO-d): 6.9-8.3 (m, 14H, Ar-CH, pyrazole CH), 8.4 (s, 1H, pyrimidine CH), 9.9 (s, 1H, NH (D_2O exchange)), 11.2 (s, 1H, CO-NH (D_2O exchange)). Mass spectrum: m/z (%): 512, M+2 (0.43), 108 (100).

4-(4-Substituted aminophenyl) amino-(1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine (16a,b)

A mixture of compound **6** (0.3 g, 1 mmol), benzyl choride or bromo diethylmalonate (11 mmol) and sodium ethoxde solution (0.25 g of sodium metal and 25 ml of absolute ethanol) was heated under reflux for 4 hours. The solvent was evaporated under reduced pressure. The residue was triturated with cold water and the solid obtained was filtered, washed, dried and recrystallized from ethanol (Table 2). IR of **16a**: 3403.2 (NH), 3032.8 (CH Ar), 2924.0 (CH

aliphatic). IR of **16b**: 3422.8 (NH), 3150.0 (CH Ar), 2989.0 (CH aliphatic), 1639.0 (C=O). ^{1}H-NMR (DMSO-d_6) of **16a**: 2.4 (s, 2H, CH_2), 7.2-8.4 (m, 17H, Ar-H, pyrazole-H and 2NH (D_2O exchange), 8.5 (s, 1H, pyrimidine CH). Mass spectrum of **16a**: m/z (%): 392, M^+ (1.28), 57 (100). Mass spectrum of **16b**: 460, M (33.30), 57 (100)

Biological Screening

Thirteen representative compounds **5**, **6**, **7a**, **7b**, **8a**, **9a**, **10a**, **11a**, **12**, **13a**, **14c**, **15** and **16b** were chosen and tested for their anti-inflammatory activity; it was done in the pharmacology department of National Research Center.

Method

The newly synthesized derivatives were dissolved in dimethylsulfoxide (DMSO). Rats used were wistar albino rat of both sex, ranged from 120-140 g, produced from National Research Center, Giza, Egypt and were housed under suitable laboratory conditions through the period of investigation. Animals were fed standard pellet chow (elnasr chemical company, Cairo, Egypt) and allowed free access of tap water. The anti-inflammatory activity was performed using carrageenan-induced paw edema according to Winter *et al*[27].

Rats used were divided into 15 groups, the first group (control group) received the vehicle only, the second group received indomethacin in a dose of 10 mg/kg body weight, and groups 3-15 received the tested compounds in a dose of 40 mg/kg body weight. The initial hind paw volume of rat was determined volumetrically. 0.1 ml of 1% (w/v) carragenan in saline was injected subcutaneously into the right hind paw one hour after the tested compounds had been administrated intraperitoneally. Paw volumes were measured up to 4 hours at the interval of 60 minutes using caliber post administration of the drug. The edema rate and inhibition rate of each group were calculated as follows:

$$\text{Edema rate (\%)} = (V_t - V_o) / V_o$$

$$\text{Inhibition rate (\%)} = (E_c - E_t) / E_c$$

Where: V_o is the volume before carrageenan injection (ml),V_t is the volume at t hour after carrageenin injection (ml), E_c is the edema rate of control group and E_t is the edema rate of treated group.

RESULTS AND DISCUSSION

Data are presented in Table 2 to reveal anti-inflammatory activity of indomethacin and the new synthesized compounds by carrageenan-induced Paw Edema in rats and illustrated by Figure 1.

Data revealed that, 3-methyl-4-(1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidin-4-ylamino)phenol **5** showed significant anti-inflammatory activity. Moreover, substitution on the phenolic group with benzyl moiety **8a** or phenyl amino carbonyl moiety **9a**

Table 2: Anti-inflammatory activity of Indomethacin and the new synthesized compounds by Carrageenan-Induced Paw Edema in rats.

Compound No	Dose mg/kg	1 h	2 h	3 h	4 h
		Edema rate % % Inhibition	Edema rate % % Inhibition	Edema rate % % Inhibition	Edema rate % % Inhibition
Control	0	68.12±9.09 0	111.41±10.20 0	121.07±8.58 0	120.60±8.32 0
Indomethacin	10	26.08±1.85* 61.71*	41.18±2.12* 63.04	47.79±2.31* 60.53*	53.35±2.55* 55.76*
5	40	37.54±2.87* 44.89*	55.40±4.11* 50.27	55.50±4.72* 54.16*	59.11±5.86* 50.99*
6	40	32.70±3.73* 52.00*	53.64±5.45* 51.80*	50.57±5.3* 58.23*	61.36±4.92* 49.13
7a	40	24.65±2.48* 63.81*	41.89±2.92* 62.40*	45.89±2.57* 62.1*	48.23±3.85* 60.00*
7b	40	21.95±2.04 67.78*	36.00±3.66* 67.69*	44.32±1.84* 63.39*	50.21±3.94* 58.37*
8a	40	2582±1.97* 62.10*	40.04±2.74* 64.06*	48.20±3.89* 60.19*	50.91±3.94* 57.79*
9a	40	22.6±2.15* 67.61*	34.94±3.79* 68.64*	43.24±4.42* 64.29*	57.18±4.36* 52.59*
10a	40	56.38±4.72 17.22	98.48±7.54 11.60	16.44±7.08 12.09	106.45±5.37 11.7
11a	40	55.14±4.21 19.05	89.07±4.96* 20.05*	79.64±5.59* 34.22*	80.10±5.92* 33.58*
12	40	28.58±1.25* 58.05*	38.02±2.74* 65.87*	43.97±3.51* 63.68*	45.73±4.07* 62.08*
13a	40	16.45±1.74* 75.85*	26.21±2.60* 76.45*	31.77±2.97* 73.76*	38.82±3.88* 67.81*
14c	40	55.57±0.89 18.42	84.89±4.46* 23.80*	75.87±5.29* 37.33*	88.78±7.26* 26.36*
15	40	20.12±1.22* 70.46*	40.70±3.92* 63.47*	41.99±3.54* 65.27*	47.12±3.57* 60.93*
16b	40	20.15±2.46* 70.41*	38.60±3.07* 65.35*	42.98±4.36* 64.50*	44.04±4.15* 63.48*

Values represent the mean ± S.E. of six animals for each group
P <0.05: Statistically significant from control, (Dunnett's test).

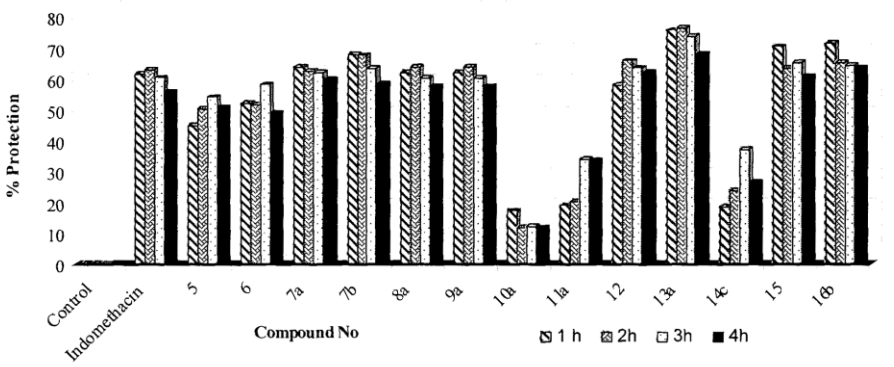


Fig. 1: Anti-inflammatory activity of the new synthesized compounds Carrageenan-Induced Paw Edema in rats.

showed increase in the activity while substitution with benzoyl moiety **10a** diminished the activity. On the other hand, substitution of the phenolic group by its bioisosteric amino group together with the removal of the methyl group to yield **6** led to increase in the anti-inflammatory activity. This latter, upon its substitution with 5-bromoindolin-2-one moiety or diethyl malonate moiety to yield **15** and **16b** respectively showed increase in the activity, while substitution with phenyl amino carbonyl moiety or 4-(dimethylamino)benzylidene to produce **11a** and **14c** showed decrease in the activity.

Moreover, conversion of the 4-amino group of **6** into a heterocyclic ring as imidazoline-2,4,5(1*H*,3*H*)-trione or pyrrolidine-2,5-dione to afford **12** and **13a** showed slight increase in the activity.

Finally, incorporation of 4-acetamidophenoxy group **7a** or substitution in position 2 by p-acetamidophenoxy moiety with (dimethylamino)methyl **7b** showed increase in the anti-inflammatory activity.

Conclusion

All of the tested compounds showed significant anti-inflammatory activity with the exception of **10a**. Although the phenyl amino carbonyl derivative of compound **5** showed increase in the anti-inflammatory activity, the reverse occurred with compound **6.** Additionally, incorporation of the 4-amino group into a heterocyclic ring increased the anti-inflammatory activity. Moreover, compound **13a** was the most potent one and compounds **7a**, **7b**, **9a**, **12**, **15** and **16b** were more potent than indomethacin at the given dose while the anti-inflammatory activity of the other tested compounds was arranged descendingly as follow: **8a** >**6 > 5 > 14c > 11a**.

REFERENCES

1- S. Schenone, O. Bruno, F. Bondavalli, A. Ranise, L. Mosti, G. Menozzi, P. Fossa, S. Donnini, A. Santoro, M. Ziche, F. Manetti and M. Botta, Eur. J. Med. Chem., 39, 939 (2004).

2- S. Schenone, O. Bruno, F. Bondavalli, A. Ranise, L. Mosti, G. Menozzi, P. Fossa, F. Manetti, L. Morbidelli, L. Trincavelli, C. Martini and A. Lucacchini, ibid., 39, 153-160 (2004).

3- F. Carraro, A. Naldini, A. Pucci, G. A. Locatelli, G. Maga, S. Schenone O. Bruno, A. Ranise,, F. Bondavalli, C. Brullo, P. Fossa, G. Menozzi, L. Mosti, M. Modugno, C. Tintori, F. Manetti and M. Botta, J. Med. Chem., 49, 1549 (2006).

4- B. Dumaitre and N. Dodic, ibid., 39, 1635 (1996).

5- A. S. Bell, D. G. Brown, D. N. A. Fox, I. R. Marsh, A. I. Morrel, M. J. Palmer and C. A. Winslow, PCT Int. Appl. WO 2004, 96, 810 (2004), through Chem. Abstr., 141, 410950c (2004).

6- F. Wunder, A. Tersteegen, A. Rebmann, C. Erb, T. Fahrig and M. Hendrix, Mol. Pharmacol., 68, 1775 (2005).

7- P. P. Gupta, R. C. Srimal, K. Avasthi, N. Garg, T. Chandra and D. S. Bhakuni, Indian J. Exp. Biol., 33, 38 (1995).

8- J. M. Quintela, C. Peinador, M. J. Moreira, A. Alfonso, L. M. Botana and R. Riguera, Eur. J. Med. Chem., 36, 321 (2001).

9- S. H. Dickerson, D. M. Garrido, W.Y. Mills, K. Kano, A. J. Peat, S. A. Thomson, J. L. R. Wilson and H. Zhouand, PCT Int. Appl. WO 2004, 9, 597 (2004), though Chem. Abstr., 140, 128435x (2004).

10- H. M. Vrupakshaiah, M. B. Talawar, U. V. Laddi, Y. S. Somannavar, V. V. Badiger and S. C Bennur., Indian J. Heterocycl. Chem., 6, 63 (1996).

11- V. A. Makarov, O. B. Riabova, B. Olga, V. G. Granik, H. M. Dahse, A. Stelzner, P. Wutzler and M. Schmidtke, Bioorg. Med. Chem., 15, 37 (2005).

12- M. M. Ghorab, Z. H. Ismail, S. M. Abdel-Gawad, and A. Abdel-Azeim, Heteroatom Chem., 15, 57 (2004).

13- Z. H. Ismail, S. M. Abdel-Gawad, A. Abdel-Azeim and M. M. Ghorab, Taylor and Francis Inc., 178, 1795 (2003), through Chem. Abstr. 140, 128362w (2004).

14- S. M. Abdel-Gawad, M. M. Ghorab, A. M. S. El-Sharief, F. A. El-Telbany and M. Abdel-Alla, Heteroatom Chem., 14, 530 (2003).

15- F. Bruni, A. Costanzo, S. Selleri, G. Guerrini, R. Fantozzi, R. Pirisino and S. Brunelleschi, J. Pharm. Sci., 82, 480 (1993).

16- H. Inoue, H. Murafuji and Y. Hayashi, PCT Int. Appl. WO 2004, 111, 054 (2004), through Chem. Abstr., 142, 74598y (2005).
17- M. C. Allison, A. G. Howatson, C. J. Torrance, F. D. Lee and R. I. G. Russel, N. Eng. J. Med., 327, 749 (1992).
18- R. T. Schon and R. J. Vender, Am. J. Med.**,** 86, 449 (1989).
19- G. Bold, J. Frei, M. Lang, P. Traxler and P. Furet, PCT Int. Appl. WO 9814, 451 (1998), Ann. Intern. Med., 115, 787 (1991).
20- S. E. Gabriel, L. Jaakkimainen and C. Bombardier; Ann. Intern. Med., 115, 787 (1991), through Chem. Abstr., 128, 257442y (1998).
21- H. E. Skipper, R. K. J. Robins and J. R. Thomson, Proc. Soc. Exper. Biol. Med., 89, 594 (1955).
22- C. J. Shishoo, U. S. Pathak, I. S. Rathod, K. S. Jain, L. G. Nargund, A. D. Taranalli, H. Patel, V. Kumar and V. S. Shirsath, Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem., 38B, 684 (1999).
23- C. C. Cheng and K. R. Robins, J. Org. Chem., 21, 1240 (1956).
24- De Bellemont, Bull. Soc. Chim. France, 25, 18 (1901).
25- N. P. Peet, N. L. Lentz, S. Sunder, M. W. Dudley and A. L. Ogden, J. Med. Chem., 35, 3263 (1992).
26- P. Schmidt and J. Druey, Helv. Chim. Acta, 39, 986 (1956).
27- C. A. Winter, E. A. Risley and G. W. Nuss, "Proceedings of the Society for Experimental Biology and Medicine", III, 544 (1962).