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SYNTHESIS OF SOME PYRIMIDO-[4', 5' : 4,5]-PYRIMIDO- [1,6-a] INDOLE OF POTENTIAL ANTIINFLAMMATORY AND ANTIPYRETIC ACTIVITIES

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ABSTRACT

The synthesis of 12,12a-dihydropyrimido-[4',5' : 4,5]-pyrimido-[1,6-a]-indole-1,6 [2H, 5H]-dione is described. Treatment of enaminonitriles I with chloroacetyl chloride afforded the tetracyclic compounds II. In contrast compound I with fluorophenyl group at position 2 gave N-chloroacetylamino derivatives III which underwent cyclization either by acid or base. Preliminary Pharmacological screening of some compounds showed significant antiinflammatory and antipyretic activities.

INTRODUCTION

Gastric ulceration and haemorrhage are the major problems in therapy with antiinflammatory drugs [1]. Thereby nonsteroidal, nonacidic antiinflammatory (NSAI) agents are enjoying increasing favour due to the better gastrointestinal tolerability when compared with acidic agents [2]. As a part of our studies of nonacidic pyrimido-[1,6-a]-indole derivatives [3], it was promissing analgesic and antiinflammatory agents [4]. We report the synthesis of the nonacidic pyrimido-[4', 5': 4,5]-pyrimido-[1,6-a]-indole of general structure II-VI frequently bear substituents featured in known (NSAI) drugs [5].

Moreover, the titled compounds were designed to study the influence of added heterocyclic ring to the parent nucleus I and its relation to toxic and pharmacological effects.

The approach utilized in the synthesis of the designed compounds is given in scheme 1 and 2.

EXPERIMENTAL

All melting points are uncorrected and were determined by open capillary method. Microanalysis were performed by Microanalytical Center, University of Cairo. Ir spectra were determined on Perkin-Elmer PE-298 Spectrophotometer using KBr discs. ^{1}H-NMR were carried out in Faculty of Pharmacy, University of Cairo using JEOL FXQ 90 MHz Spectrometer.

1a- Preparation of 5-alkyl-3-chloro-methyl-12,12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole-1,6 [2H, 5H]-dione IIa-d :

A mixture of I (0.01 mole), chloroacetyl chloride (0.011 mole) and dry benzene (20 ml) was left overnight. The solvent was then alcoholic HCl for 12 hours. The product was crystallized from ethanol (Table 1).

1b- Compounds IIe-h were prepared from compounds IIIa-d by refluxing with alcoholic HCl for 12 hours. The product crystallized from ethanol (Table 1) :

1c- Preparation of 3-chloroacetamido-1-oxo-2-substituted-fluorophenyl-1,2,4a,5-tetrahydropyrimido [1,6-a] indole-4-carbonitrile IIIa-d :

Compounds IIIa-d were prepared from compound I, (R=o,m,p-FC_6H_4 and m-$CF_3C_6H_4$) respectively and applying the general method 1a (Table 2).

2a- 3-Alkylaminomethyl-5-substituted-fluorophenyl-12, 12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole-1,6 [2H, 5H]-dione IVa-d; IVe-h :

A mixture of III (0.01 mole), secondary amine (1.5 ml) and ethanol (30 ml) was refluxed for 9 hours. The solvent was evaporated under reduced pressure, the separated crystals washed with water and crystallized from ethano (Table 3).

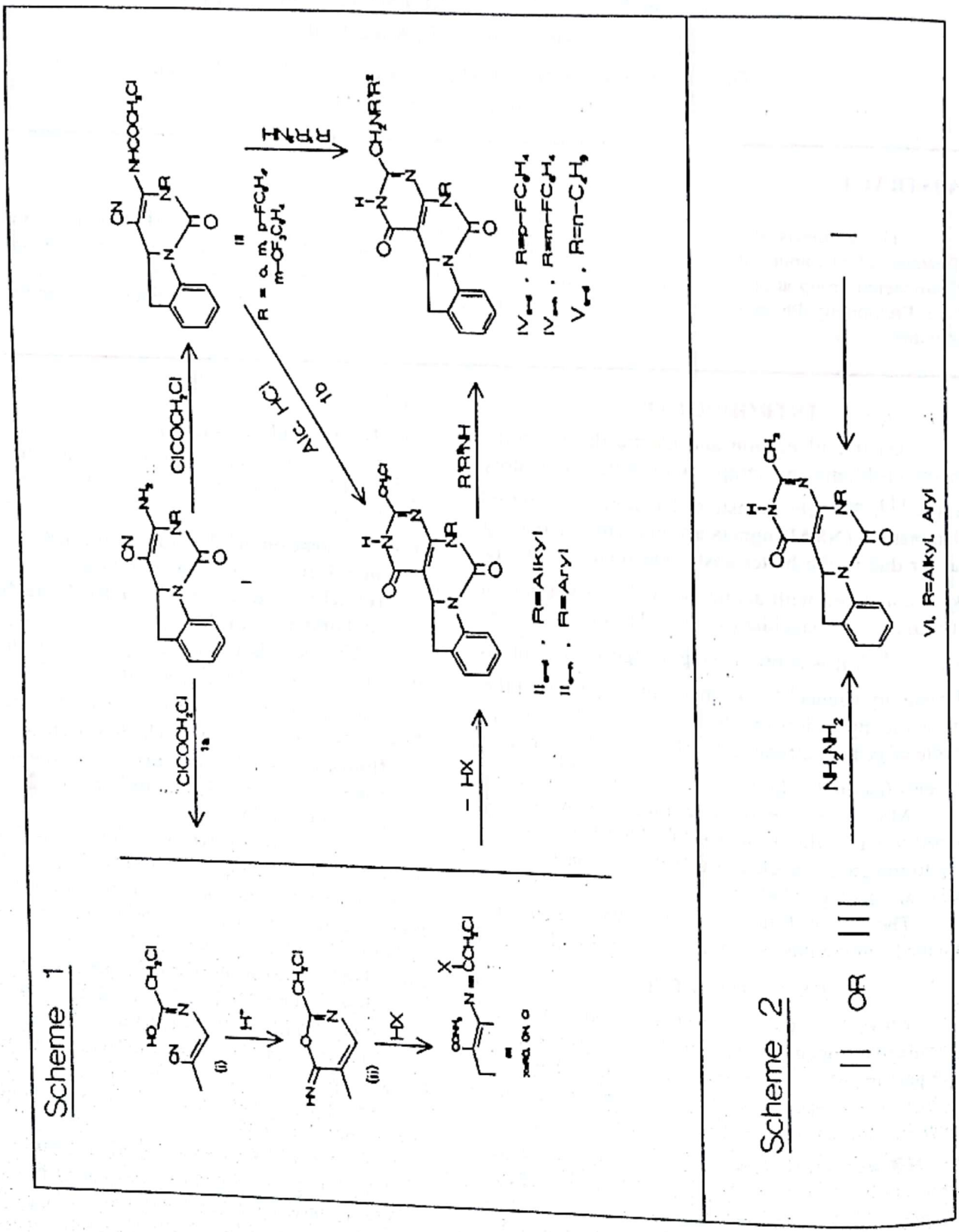
2b- 3-Alkylaminomethyl-5-n.butyl-12, 12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole-1,6 [2H, 5H] dione Va-d :

Compound Va-d were prepared from IIb and the corresponding amine by applying method 2 with 18 hours reflux. They were crystallized from aqueous ethanol (Table 3).

3- Preparation of 5-alkyl or aryl-3-methyl-12, 12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole-1,6 [2H, 5H] dione VIa-g

Method A : A mixture of II (0.01 mole), hydrazine hydrate (0.02 mole) and ethanol (20 ml) was refluxed for 10 hours. The product was crystallized from ethanol (Table 4).

Method B : A mixture of I (0.01 mole) and acetic anhydride (20 ml) was refluxed for 3 hours.


Scheme 1
ClCOCH2Cl
ClCOCH2Cl
AlCl3
RRNH
- HX
R = o, m, p-FC6H4, m-CF3C6H4
IV. R=p-FC6H4
IV. R=m-FC6H4
V. R=n-C3H9
II. R=Alkyl
II. R=Aryl
Scheme 2
N2H4
VI. R=Alkyl, Aryl

anhydride (20 ml) was refluxed for 3 hours. The solution was concentrated under reduced pressure and the residue was crystallized from ethanol (Table 4).

General IR spectra (cm^{-1}) : 3485 (NH),3050-3100 (CH-arom.), 2945 (CH-aliph.),2210 (CN), 1650-1680 (C=O), 1610 (NH), 750 (CH-arom.).

Special groups : 1075 (C-F), 750 (C-Cl), 1140 (C-F), 860 (C-Cl).

^{1}H-NMR (ppm) :

Compound # IIf : 1.9-2.1 (d, 2H, CH2 at position 12); 2.85-3.0 (t, 1H,CH at position 12a); 4.2 (s,2H,CH2Cl); 7.0-7.3 (m,8H,aromatic protons); 11.5 (br,s,1H,NH).

Compound # IIIb: 1.8-2.1 (d, 2H, CH2 at position 12);2.80-2.95 (t, 1H,CH at position 12a);4.15 (s,2H,CH2Cl); 6.95-7.2 (m,8H, aromatic protons); 8.7 (br,s,1H,NH).

Compound # IVh : 1.7-2.2 (d, 2H, CH2 at position 12); 2.8-3.1 (t, 1H,CH at position 12a); 2.6 (m,4H,-CH2N-CH2-); 3.4 (s,2H, >-CH2-); 3.75 (m,4H,-CH2-O-CH2-); 6.85-7.45 (m,8H,aromatic protons); 11.8 (br,s,1H,NH).

Compound # VIg : 1.7-2.0 (d,2H,CH2 at position 12); 2.7-2.95 (t,1H,CH at position 12a); 2.3 (s,3H,CH3); 7.0-7.2 (m,8H,aromatic protons); 11.9 (br,s,1H,NH).

Determination of LD_{50} :

LD_{50} of the tested compounds IIb and VIb was determined according to reported method.[12]

Compound	LD_{50} mg/100 gm
IIb	195
VIb	117

Comparing the LD_{50} of some reported pyrimido [1,6-a] indole [4] of general structure I with the present compounds, it can be stated that :

- The addition of further heterocyclic ring to I appears to highly reduce the toxicity.
- The 3-chloromethyl derivative IIb is less toxic than the 3-methyl analogue VIb.

Antiinflammatory effect :

Twenty rats of both sexes weighing 150-200 g were devided into four groups. The thickness of the left hand paw of each rat was measured using Vernier caliber. One of these groups was kept as control and injected with formalin whereas the other groups were subcutaneously injected with diclofenac sodium and the tested compounds in a dose of 250 mg/kg body weight. After 30 min of the drug administration the inflammation was induced by subcutaneous injection of 0.1 ml of 6% solution of formalin in normal saline [13] into the left hand paw of each rat. The paw thickness was then measured hourly for a period of 5 hours (Table 5).

Antipyretic effect :

Twenty rats of both sexes weighing 150-200g were devided into four groups. All rats were made hyperthermic by subcutaneous injection of Brewer's yeast [14] in physiological saline solution in a dose of 0.15/100 g body weight. After 17 hours the initial body temperature of each rat was measured rectally. One group was kept as a control while the other groups were subcutaneously injected with diclofenac sodium and the tested compounds in a dose of 250 mg/kg body weight. The temperature of each rat was then recorded at 30 min intervals after drug administration (Table 6).

RESULTS AND DISCUSSION

O-Acylaminonitriles are of considerable interest [6]. They are converted either by acid [7] or base [8] to condensed pyrimidines. Several reports have been considered for this conversion [9].

In the present work, chloroacetylation of enaminonitriles I, (R= alkyl, m-tolyl) directly afforded the tetracyclic compounds 5-alkyl-3-chloromethyl-12, 12a-dihydropyrimido-[4',5':4,5]pyrimido [1,6-a] indole-1,6-[2H, 5H]-dione IIa-d. The ir spectra of IIa-d have revealed the disappearance of the nitrile stretching band (2185 cm^{-1}) shown in I. The formation of the new compounds IIa-d presumed to have taken place through an acylaminonitrile intermediate (i) followed by an acid catalized intramolecular cyclization to a 4-imino-m-oxazine (ii). This underwent intramolecular rearrangement (during the work up) to the corresponding carboxamide (iii) which is known to undergo read intramolecular dehydration to a fused pyrimidone system [10]. Attempts for the isolation of the uncyclized intermediate (i) were unsuccessful. In contrast, chloroacetylation of I, (R=o,m,p-FC_6H_4; m- $CF_3C_6H_4$) gave the unciclized compounds : 3-chloroacetamido-1-oxo-2-substituted-fluorophenyl-1,2, 4a,5- tetra- hydropyrimido [1,6-a] indole-4-carbonitrile IIIa-d even at reflux.

The ir spectra of IIIa-d have revealed the presence of absorption band at 2210 cm^{-1} attributed to cyano group. Cyclization of IIIa-d was achieved by refluxing with alcoholic HCl to give 3-chloromethyl derivatives IIe-h. Also cyclization of III was accomplished by condensation with various amines to give the cyclized substituted derivatives : 3-alkylamino-methyl-1-oxo-5-substituted- fluorophenyl-12, 12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole-1,6 [2H, 5H] dione IVa-h. Moreover,

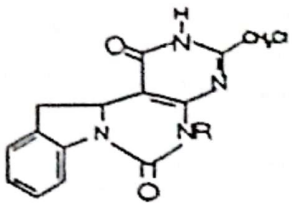


Table (1) :

No.	R	m.p.c	Yield %	Method	M.f & M.wt.	Microanalysis	Calcd	Found
IIa	iso-C_3H_7	205-6	83	1a	$C_{17}H_{17}ClN_4O_2$ (344.5)	C	59.21	59.4
						H	4.93	4.8
						N	16.25	16.4
IIb	n-C_4H_9	182-3	72	1a	$C_{18}H_{19}ClN_4O_2$ (358.5)	C	60.25	60.2
						H	5.29	5.4
						N	15.62	15.8
IIc	C_6H_{11}	210-1	74	1a	$C_{20}H_{21}ClN_4O_2$ (384.5)	C	62.41	62.5
						H	5.46	5.3
						N	14.56	14.6
IId	m-$CH_3C_6H_4$	233-4	84	1a	$C_{21}H_{17}ClN_4O_2$ (392.5)	C	64.20	64.4
						H	4.33	4.2
						N	14.26	14.4
IIe	o-FC_6H_4	245-6	86	1b	$C_{20}H_{14}ClFN_4O_2$ (396.5)	C	60.52	60.5
						H	3.53	3.5
						N	14.12	14.2
IIf	m-FC_6H_4	252-3	88	1b	$C_{20}H_{14}ClFN_4O_2$ (396.5)	C	60.52	60.6
						H	3.53	3.6
						N	14.12	14.3
IIg	p-FC_6H_4	265-6	89	1b	$C_{20}H_{14}ClFN_4O_2$ (396.5)	C	60.52	60.5
						H	3.53	3.7
						N	14.12	14.2
IIh	m-$CF_3C_6H_4$	207-8	88	1b	$C_{21}H_{14}ClF_3N_4O_2$ (446.5)	C	56.43	56.6
						H	3.13	3.1
						N	12.54	12.6

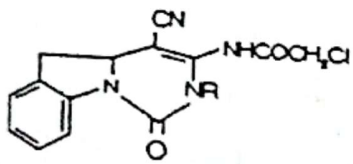


Table (2) :

No.	R	m.p.c	Yield %	Method	M.f & M.wt.	Microanalysis	Calcd	Found
IIIa	o-FC_6H_4	198-9	89	1c	$C_{20}H_{14}ClFN_4O_2$ (396.5)	C	60.52	60.7
						H	3.53	3.6
						N	14.12	14.3
IIIb	m-FC_6H_4	207-8	88	1c	$C_{20}H_{14}ClFN_4O_2$ (396.5)	C	60.52	60.6
						H	3.53	3.6
						N	14.12	14.4
IIIc	p-FC_6H_4	211-2	91	1c	$C_{20}H_{14}ClFN_4O_2$ (396.5)	C	60.52	60.7
						H	3.53	3.5
						N	14.12	14.3
IIId	m-$CF_3C_6H_4$	158-9	92	1c	$C_{21}H_{14}ClF_3N_4O_2$ (446.5)	C	56.43	56.5
						H	3.13	3.1
						N	12.54	12.7

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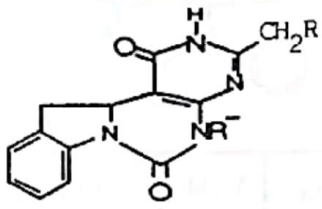
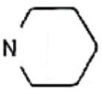
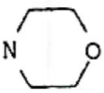
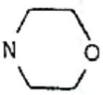
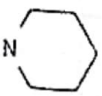
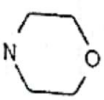


Table 3 :

No	R	R	m.p°C	Method	Yield%	M.f&M.wt.		Microanalysis Calcd	Found
IVa	$N(C_2H_5)_2$	p-FC_6H_4	255-6	2a	73	$C_{24}H_{24}FN_5O_2$ (433)	C	66.51	66.7
							H	5.54	5.4
							N	16.16	16.3
IVb		p-FC_6H_4	244-5	2a	65	$C_{24}H_{22}FN_5O_2$ (431)	C	66.82	66.9
							H	5.10	5.0
							N	16.24	16.4
IVc		p-FC_6H_4	235-6	2a	79	$C_{25}H_{24}FN_5O_2$ (445)	C	67.41	67.5
							H	3.39	3.3
							N	15.73	15.9
IVd		p-FC_6H_4	267-8	2a	79	$C_{24}H_{22}FN_5O_3$ (447)	C	64.42	64.6
							H	4.92	4.8
							N	15.65	15.8
IVe	$N(C_2H_5)_2$	m-FC_6H_4	262-3	2a	69	$C_{24}H_{24}FN_5O_2$ (433)	C	66.51	66.6
							H	5.54	5.5
							N	16.16	16.4
IVf		m-FC_6H_4	228-9	2a	54	$C_{24}H_{22}FN_5O_2$ (431)	C	66.82	66.9
							H	5.10	5.2
							N	16.24	16.5
IVg		m-FC_6H_4	224-5	2a	66	$C_{25}H_{24}FN_5O_2$ (445)	C	67.41	67.6
							H	3.39	3.4
							N	15.73	15.9
IV		m-FC_6H_4	228-9	2a	72	$C_{24}H_{22}FN_5O_3$ (447)	C	64.42	64.5
							H	4.92	4.9
							N	15.65	15.7
Va	$N(C_2H_5)_2$	n-C_4H_9	122-3	2b	52	$C_{22}H_{29}N_5O_2$ (395)	C	66.83	66.9
							H	7.34	7.2
							N	17.72	17.9
Vb		n-C_4H_9	112-3	2b	54	$C_{22}H_{27}N_5O_2$ (393)	C	67.17	67.3
							H	6.87	6.6
							N	17.81	17.7
Vc		n-C_4H_9	135-6	2b	53	$C_{23}H_{29}N_5O_2$ (407)	C	67.81	67.9
							H	7.12	7.0
							N	17.19	17.3
Vd		n-C_4H_9	155-6	2b	59	$C_{22}H_{27}N_5O_3$ (409)	C	64.54	64.7
							H	6.60	6.8
							N	17.11	17.3

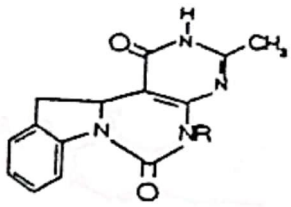


Table (4) :

No.	R	m.p.c	Yield %	M.f & M.wt.	Microanalysis	Calcd	Found
VIa	iso-C_3H_7	175-6	71	$C_{17}H_{18}N_4O_2$ (310)	C	65.80	65.9
					H	5.80	5.6
					N	18.06	18.2
VIb	n-C_4H_9	202-3	68	$C_{18}H_{20}N_4O_2$ (324)	C	66.66	66.8
					H	6.17	6.1
					N	17.28	17.3
VIc	C_6H_{11}	235-6	67	$C_{20}H_{22}N_4O_2$ (350)	C	68.57	68.7
					H	6.28	6.2
					N	16.00	16.2
VId	m-$CH_3C_6H_4$	216-7	72	$C_{21}H_{18}N_4O_2$ (358)	C	70.39	70.4
					H	5.02	5.00
					N	15.64	15.7
VIe	o-FC_6H_4	211-2	75	$C_{20}H_{15}FN_3O_2$ (362)	C	66.29	66.4
					H	4.14	4.00
					N	15.46	15.5
VIf	m-FC_6H_4	213-4	71	$C_{20}H_{15}FN_3O_2$ (362)	C	66.29	66.2
					H	4.14	4.2
					N	15.46	15.4
VIg	p-FC_6H_4	223-4	76	$C_{20}H_{15}FN_3O_2$ (362)	C	66.29	66.4
					H	4.14	4.1
					N	15.46	15.4

Table (5) : The antiinflammatory effect of 250 mg/kg body weight of diclofenac sodium and the tested compounds.

Compound	Thickness of Paw				
	1	2	3	4	5
Control	6.64 ± 0.37	6.60 ± 0.27	7.50 ± 0.25	7.70 ± 0.25	7.80 ± 0.10
IIb	7.70 ± 0.30	7.10 ± 0.27	7.00 ± 0.27	7.10 ± 0.26	7.30 ± 0.22
VIb	7.30 ± 0.17	6.80 ± 0.22	6.40±0.18**	5.90±0.04**	6.00±0.08**
Diclofenac	7.40 ± 0.17	6.50 ± 0.17	6.40±0.16**	6.40±0.22**	6.10±0.26**

Significant at $P < 0.01$

The antiinflammatory effect of compound VIb is more prominent than IIb.

Table (6) : The antipyretic effect of 250 mg/kg body weight of diclofenac sodium and the tested compounds.

Compound	Body temperature after drug administration					
	30 min	60 min	90 min	120 min	150 min	180 min
Control	38.64±0.40	37.42±0.03	38.04±0.04	37.40±0.04	37.88±0.71	37.94±0.07
IIb	36.66±0.07	36.52±0.12	36.60±0.06	36.68±0.04	36.72±0.07	36.60±0.09
VIb	36.72±0.13	36.63±0.15	36.74±0.10	36.30±0.07	36.24±0.05	36.22±0.04
Diclofenac sodium	37.06±0.08	37.02±0.06	36.96±0.12	36.32±0.09	36.40±0.04	36.32±0.07

Significant at $P < 0.01$

The two compounds show an interesting antipyretic activity level $P<0.01$ which appeared after 30 min. of adminstration.

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treatment of IIb with different amines gave Va-d.

On the other hand, refluxing II with equivalent amount of hydrazine hydrate in ethanol gave no successful results and in each case the starting material was separated. In contrary heating II with excess hydrazine hydrate in ethanol afforded compounds of definite melting points and give negative test for chlorine.

Elemental analysis and ir spectra of the products are not in accord with the expected for 3-hydrazino-methyl derivatives, but rather consistent with the structural formula of 5-alkyl-3-methyl-12, 12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole-1,6 [2H, 5H] dione Va-d. Treatment of IIIa-c with hydrazine hydrate under the same condition gave compounds of type VI, the formation of VI presumed to have taken place through the catalytic cyclization of IIIa-c followed by reductive dehalogenation.

The structure establishment of VI was confirmed by microanalysis and ^{1}H-NMR which showed a singlet peak at 2.3 integrated for three protons which is attributed to a methyl group at position # 3.

Our conclusion about the formation of VI was also supported by independent synthesis of VIa-g through unambiguous route [11] (scheme 2).

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تشييد بعض مشتقات البيريميدو (٤',٥' : ٤،٥) بيريميدو (١،٦-أ) اندول كمضادات للالتهابات وخافضات لدرجة الحرارة

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فى هذا البحث تم تحضير مشتقات (١٢ ، ١٢أ) ثنائى هيدروبيريميدو (٤،٥ : ٤ ، ٥) بيريميدو (١،٦-أ) اندول – {١،٦ (٢هـ ، ٥هـ)} دايون. وبمعالجة مركبات الامينونيتريل (I) بكلوروأسيتيل كلوريد ثم الحصول على مركبات رباعية الحلقات (II) وذلك على عكس تفاعل (I) مع مجموعة فلوروفينيل مما نتج عنه مشتقات ن – كلوروأسيتيل أمين (III) والتى تم تحويلها الى مركبات حلقية باضافة الاحماض او القلويات . ولقد اظهرت الدراسات الفارماكولوجية لبعض المركبات المحضرة أن لها نشاط كمضاد للالتهاب وخافض لدرجة الحرارة.