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SYNTHESIS OF SOME PYRIMIDO-[1,6-a]- INDOLE OF POTENTIAL PHARMACOLOGICAL INTEREST

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ABSTRACT

The synthesis of some pyrimido-[1,6-a]- indole derivatives is described. Addition of dicyanomethylideneindoline (I) to various isocyanates gave 2-alkyl-3-imino-1-oxo-1,2,3,5-tetrahydropyrimido-[1,6-a]- indole-4-carbonitrile IIa-h. The corresponding enaminonitriles IIIa-h were obtained from II by reduction with sodium borohydride. Compounds IV-VII were also obtained. Preliminary pharmacological screening revealed antipyretic, antiinflammatory and analgesic activities.

INTRODUCTION

The interesting pharmacological features of nitrogen bridgehead compounds have been reviewed [1-9].

In previous work [10] we described the synthesis of pyrimido [1,6-a] indole. Careful study of potential analgesic, antipyretic, antiinflammatory, anticonvulsant and hypotensive activities of some examples led to the realization of synthesis of some pyrimido-[1,6-a]-indole that bear certain pharmacophores [11-14]. Obviously the inclusion of these groups seems to potentiate pharmacological activities among these compounds.

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

RESULTS AND DISCUSSION

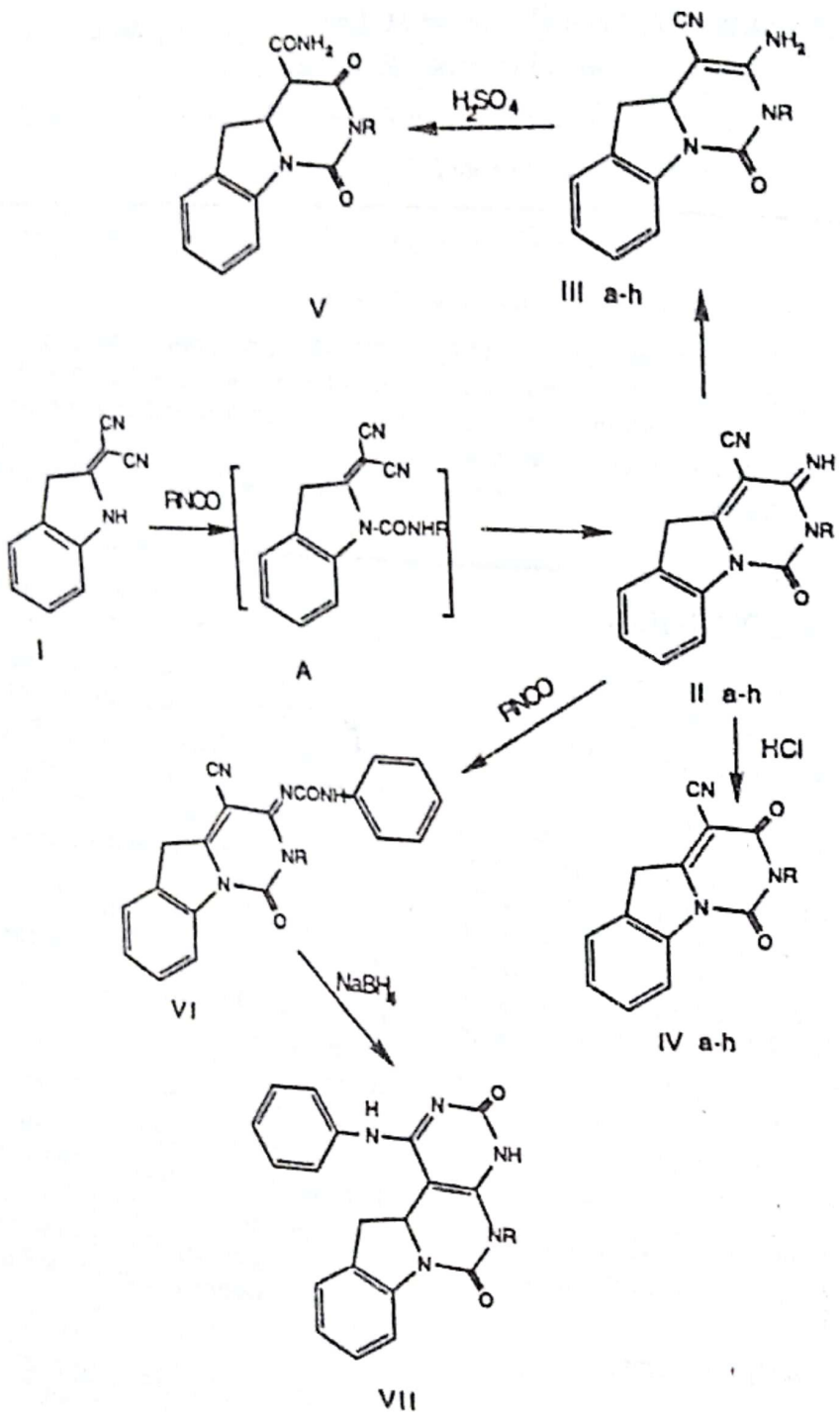
Reaction of 2-dicyanomethylideneindoline [10] with different isocyanates gave an intermediate (A) which underwent further nucleophilic addition affording enaminonitriles IIa-h. Reaction of II with sodium borohydride gave the enaminonitriles IIIa-h.

The IR spectra of II revealed the disappearance of the two absorption bands at 2225 and 2185 cm^{-1} corresponding to the geminal nitrile functions and instead two sharp bands at 2220 cm^{-1} corresponding to one cyano group and sharp stretching band appeared at 3320 cm^{-1} attributed to the 3-imino group.

The 1,4-addition [15] of two hydride ions was achieved by the reaction of sodium borohydride [16] with the 3-imino compounds IIa-h to give the 3-amino derivatives IIIa-h. The ir spectra of III showed two bands at 3200 and 3400 cm^{-1} attributed to an amino group. It is also worth mentioning that a lower absorption at 2180 cm^{-1} of the cyano group was observed, this lowering of the nitrile frequency is attributed to conjugation [17].

Acid hydrolysis of II gave the corresponding oxo derivatives IVa-h. Moreover, treatment of III with sulphuric acid yielded the carboxamide derivatives V.

The reaction of IIa with phenyl isocyanate afforded the 3-phenylureido derivative VI, which upon treatment with


CONH2
O
NR
N
O
V
H2SO4
CN
NH2
NR
N
O
III a-h
CN
CN
NH
RNCO
N-CONHR
I
A
CN
NH
NR
N
O
II a-h
RNCO
HCl
CN
NCONH
NR
N
O
VI
NaBH4
CN
O
NR
N
O
IV a-h
H
N
N
O
NH
NR
N
O
VII

Scheme 1

sodium borohydride underwent intramolecular addition to give the tetracyclic compound VII. The vanishing of the nitription band at 2220 cm^{-1} in the ir spectrum of VII, was taken as confirmation for the tetracyclic structure pyrimido [4', 5' : 4,5] pyrimido [1,6-a] indole which confirm Dimorth rearrangement [18].

(1) Determination of LD_{50} :

Kerber method (1941) [19] was used. The following table presents the LD_{50} of the studied compound IIf and A previously reported analogue [10] IIb in which R=C_2H_5.

Compound	R	LD_{50}
IIf	p.FC_6H_4	183
IIb	C_2H_5	62

It was observed that an aryl residue (p.FC_6H_4) at position 2 seems to lessen the toxicity.

(2) Pharmacological screening :

Compounds IIf, IIIf and IVf were evaluated for their analgesic and antiinflammatory activities.

a- Analgesic effect :

The analgesic activity was determined using the writhing test carried out according to Witkin et al. [20]. Mice weighing 20-30 g were assigned into four groups (9 mice each). The test compounds and antipyrine were given orally as suspension in 5% gum acacia in molar ratio doses (135, 136, 136, 80 mg/kg); respectively, after one and half hour, mice were intraperitoneally injected with p.Benzoquinone (0.2 ml of 0.02% in normal saline).

Mice of each group were placed in a separate glass cage for observation and number of the protected animals in each group was calculated (Table 1).

b- Antiinflammatory effect :

The antiinflammatory activity of the selected compounds was determined using the rat hand paw oedema method [21]. Male rats weighing 120-180 g were devided into five groups (six rats each). Three groups received the tested compounds IIf, IIIf and IVf intraperitoneally in propylene glycol in molar ratio doses (93, 94 and 93 mg/kg); respectively. The remaining two groups, one received propylene glycol only and the other received antipyrine (55 mg/kg).

One and half hour after injection of the tested compounds, rats were subcuta-

Table (1) : The absolute and relative analgesic activity of the tested compounds to antipyrine.

Compound	Absolute % of protection of writhing	Relative % of protection of writhing	Relative % of potancy to antipyrine
Antipyrine	89	100	1.0
IIf	78	87.6	0.9
IIIf	78	87.6	0.9
IVf	67	75.3	0.8

Table (2) : The absolute and relative antiinflammatory activities of the tested compounds to antipyrine.

Compound	Volume of oedema mm.Hg	% volume of oedema	Absolute % inhibition of oedema	Relative % inhibition of oedema	Relative % potancy to antipyrine
Control	± 3.7	100	0.00	0.00	0.00
Antipyrine	± 2.9	78.3	21.7	100	1.0
IIf	± 2.8	75.7	24.3	112	1.12
IIIf	± 2.9	78.3	21.7	100	1.0
IVf	± 2.6	70.3	29.7	137	1.37

neously injected with 100 µl of forma line (3.5%) in the subplanter region of the right hand paw of each animal. A 100 µl saline solution was similarly injected in the left hand paw of the same animal. The volume of the right and left hand paw was measured using plethysmograph immediately and four and half hours after injection of formaline. The difference in the volume of the paw before and after injection of formaline was considered the volume of oedema and the volume of inflammation. The volume of oedema in the control group was considered as 100%.

Conclusion :

From the previously mentioned preliminary pharmacological data it could be concluded that the tested compounds possess moderate analgesic activity, while compounds IIf and IVf demonstrate a marked antiinflammatory activity more than antipyrine.

EXPERIMENTAL

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

(1) General method of preparation of 2-alkyl-3-imino-1-oxo-1,2,3,5, tetra-hydropyrimido [1,6-a] indole-4-cabonitrile IIa-h. :

A mixture of I (0.02 mole) and appropriate isocyanate (0.025 mole) in methylene chloride (20 ml) was treated with few drops of triethylamine. The mixture was stirred with cooling for 45 min. and left overnight. The solvent was removed under reduced pressure and the residue was crystallized from ethanol (Table 3).

(2) General method of preparation of 2-alkyl-3-amino-1-oxo-1,2,4a,5-tetrahydropyrimido [1,6-a] indole-4-carbonitrile IIIa-h :

To compound II (0.1 mole) suspended in ethanol (20 ml), sodium borohydride (0.06 mole) was added in portions with stirring and the mixture was left overnight. Few drops of water were added and the separated crystals recrystallized from ethanol (Table 4).

(3) Preparation of 2-alkyl-1,3-dioxo-1,2,3,5-tetrahydro-pyrimido [1,6-a] indole-4-carbonitrile IVa-h :

Compounds II (0.03 mole) was dissolved in 18% hydrochloric acid (20 ml) and the mixture was refluxed for 3 hr. The mixture was concentrated under reduced pressure, cooled, the separated crystals were filtered and crystallized from ethanol (Table 5).

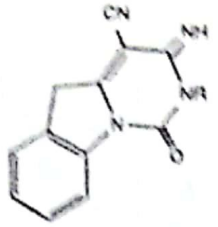


Table (3) :

No.	R	m.p°C	Yield %	Mol. Form. (Mol.wt.)		Microanalysis	
						Calcd.	Found
IIa*	iso-C_3H_7	162-3	81	$C_{15}H_{14}N_4O$ (266)	C H N	67.66 5.26 21.05	67.5 5.2 21.2
IIb	n-C_4H_9	123-4	73	$C_{16}H_{16}N_4O$ (280)	C H N	68.57 5.71 20.0	68.6 5.8 20.1
IIc**	C_6H_{11}	155-6	75	$C_{18}H_{18}N_4O$ (306)	C H N	70.58 5.88 18.30	70.7 5.8 18.4
IId	o.FC_6H_4	196-7	83	$C_{18}H_{11}FN_4O$ (318)	C H N	67.92 3.45 17.61	68.0 3.4 17.7
IIe	m.FC_6H_4	183-4	82	$C_{18}H_{11}FN_4O$ (318)	C H N	67.92 3.45 17.61	67.9 3.5 17.7
IIf ■	p.FC_6H_4	211-2	87	$C_{18}H_{11}FN_4O$ (318)	C H N	67.92 3.45 17.61	68.0 3.3 17.6
IIg	m.$CF_3C_6H_4$	143-4	88	$C_{19}H_{11}F_3N_4O$ (368)	C H N	61.95 2.98 15.21	62.0 2.9 15.3
IIh □	m.$CH_3C_6H_4$	193-4	92	$C_{19}H_{14}N_4O$ (314)	C H N,	72.61 4.45 17.83	72.7 4.4 17.9

* ^{1}H-NMR (ppm) : 1.42 (d,6H,2xCH3); 5.22-5.43 (m, 1H, isopropyl proton); 3.2(s,2H,CH2); 7.4(s,br,1H,NH); 8.8(m,4H,aromatic protons).

** ^{1}H-NMR (ppm) : 1.3-1.8 (m,11H,C6H11); 3.21 (s,2H,CH2); 7.4 (s,br,1H,NH); 8.6 (m,4H, aromatic protons).

■ ^{1}H-NMR (ppm) : 3.11 (s,2H,CH2); 6.3 (s,br,1H,NH); 7.3 (d,br,4H, aromatic protons); 8.8 (m,4H, aromatic protons).

□ ^{1}H-NMR (ppm) : 2.5 (s, 3H, tolyl CH3); 3.23 (s,2H,CH2); 7.2-7.6 (m,5H, 4 aromatic and NH proton); 8.7 (m, 4H, aromatic protons).

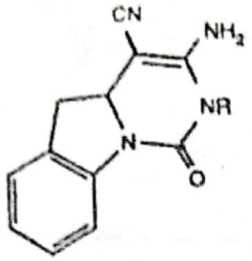


Table (4) :

No.	R	m.p°C	Yield %	Mol. Form. (Mol.wt.)		Microanalysis	
						Calcd.	Found
IIIa	iso-C_3H_7	111-2	73	$C_{15}H_{16}N_4O$ (268)	C	67.16	67.3
					H	5.97	5.9
					N	20.89	21.0
IIIb	n-C_4H_9	105-6	59	$C_{16}H_{18}N_4O$ (282)	C	68.08	68.2
					H	6.38	6.3
					N	19.85	19.9
IIIc	C_6H_{11}	117-8	75	$C_{18}H_{20}N_4O$ (308)	C	70.12	70.3
					H	6.49	6.6
					N	18.18	18.2
IIId	o.FC_6H_4	143-4	83	$C_{18}H_{13}FN_4O$ (320)	C	67.50	67.5
					H	4.06	4.1
					N	17.50	17.6
IIIe	m.FC_6H_4	203-4	78	$C_{18}H_{13}FN_4O$ (320)	C	67.50	67.6
					H	4.06	4.0
					N	17.50	17.5
IIIf	p.FC_6H_4	206-7	83	$C_{18}H_{13}FN_4O$ (320)	C	67.50	67.6
					H	4.06	4.1
					N	17.50	17.7
IIIg	m.$CF_3C_6H_4$	113-4	89	$C_{19}H_{13}F_3N_4O$ (370)	C	61.62	61.8
					H	3.51	3.6
					N	15.13	15.3
IIIh	m.$CH_3C_6H_4$	208-9	81	$C_{19}H_{16}N_4O$ (316)	C	72.15	72.1
					H	5.06	5.2
					N	17.72	17.8

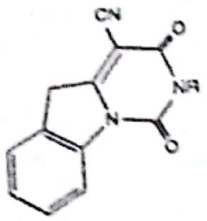


Table (5) :

No.	R	m.p°C	Yield %	Mol. Form. (Mol.wt.)		Microanalysis	
						Calcd.	Found
IVa	iso-C_3H_7	143-4	59	$C_{15}H_{13}N_3O_2$ (267)	C H N	67.41 4.86 15.73	67.5 4.7 15.9
IVb	n-C_4H_9	102-3	53	$C_{16}H_{15}N_3O_2$ (261)	C H N	68.32 5.33 14.94	68.5 5.3 14.9
IVc	C_6H_{11}	210-1	61	$C_{18}H_{17}N_3O_2$ (307)	C H N	70.33 5.53 13.65	70.5 5.5 13.8
IVd	o.FC_6H_4	253-4	63	$C_{18}H_{10}FN_3O_2$ (319)	C H N	67.71 3.13 13.16	67.8 3.0 13.3
IVe	m.FC_6H_4	241-2	64	$C_{18}H_{10}FN_3O_2$ (319)	C H N	67.71 3.13 13.16	67.7 3.1 13.2
IVf	p.FC_6H_4	263-4	65	$C_{18}H_{10}FN_3O_2$ (319)	C H N	67.71 3.13 13.16	67.8 3.1 13.3
IVg	m.$CF_3C_6H_4$	164-5	63	$C_{19}H_{10}F_3N_3O_2$ (369)	C H N	61.78 2.71 11.38	61.9 2.6 11.5
IVh	m.$CH_3C_6H_4$	223-4	59	$C_{19}H_{13}N_3O_2$ (315)	C H N	72.38 4.12 13.33	72.5 4.0 13.5

(4) Preparation of 2-n.butyl-1,3-dioxo-1,2,3,5-tetrahydro-pyrimido [1,6-a] indole-4-carbonitrile V :

A mixture of IIIb (0.01 mole), sulphuric acid (15 ml) was stirred untill complete dissolution, left over night and then poured onto ice-cooled ammonia solution. The separated crystals were filtered, washed with water and recrystallized from ethanol.

m.p 124-5 yield 62%

Microanalysis $C_{16}H_{19}N_3O_3$ (301)

	C%	H%	N%
Calcd.	63.78	6.31	13.95
Found	63.9	6.2	14.1

(5) Preparation of 2-isopropyl-1-oxo-3-phenylureido-1,2,3,5-tetrahydro-pyrimido [1,6-a] indole-4-carbonitrile VI :

To a mixture of IIa (0.01 mole) and methylene chloride (25 ml), phenyl isocyanate (0.011 mole) was added, followed by triethylamine (0.5 ml). The mixture was refluxed for two hours, the separated crystals were filtered, washed with water and recrystallized from ethanol.

m.p 189-90 yield 83%

Microanalysis $C_{22}H_{19}N_5O_2$ (385)

Calcd.	68.57	4.93	18.18
Found	68.6	4.8	18.3

(6) Preparation of 5-isopropyl-3,6-dioxo-1-phenylamino-12, 12a-dihydropyrimido [4', 5' : 4,5] pyrimido [1,6-a] 2H, 5H-indole VII :

A mixture of VI (0.01 mole) and sodium borohydride (0.005 mole) in ethanol (20 ml) was left over night. The separated crystals were filtered, washed with water and recrystallized from ethanol.

m.p 208-9 yield 83%

Microanalysis $C_{22}H_{21}N_5O_2$ (387)

Calcd.	68.21	5.42	18.08
Found	68.4	5.4	18.2

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تشييد بعض مشتقات البيريميدو (١,٦-أ) إندول ذات الاهمية الفارماكولوجية

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فى هذا البحث تم وصف طريقة تشييد لبعض مشتقات البيريميدو (١ . ٦-أ) اندول ، وذلك باضافة ثنائى سيانوميثيليدين اندولين الى مركبات الايزوسيانات المختلفة للحصول على ٢-الكيل ٣-ايمينو -١-اوكسو ١ . ٢ . ٣ . ٥ - رباعى هيدروبيريميدو (١ . ٦-أ) اندول -٤-كاربونيتريل.

ولقد تم تحضير مركبات الايناميننونيتريل المقابلة ، وذلك بطريقة الاختزال باستخدام بوروهيدريد الصوديوم. وبالدراسة الفارماكولوجية الأولية لبعض المركبات المحضرة وجد أن لها تأثير فارماكولوجى خافض للحرارة ومضاد للالتهابات ومسكنة للألم.