Synthesis of 1-(N,N-disubstituted-aminomethyl)-2-oxo-3-(2,4-dinitrophenylacetyl hydrazono)-5-substituted-indoles as Anti-inflammatory agents

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Summary: Sixteen new 'Mannich Bases' were synthesised by the reaction of 2-oxo-3-(2,4-dinitrophenylacetyl hydrazono)-5-substituted-indoles with the appropriate secondary amines in the presence of formaldehyde solution. These indoles were prepared by the condensation of 2,4-dinitrophenylacetyl hydrazine with appropriate 2,3-dioxo-5-substituted indoles. Some of the title compounds have shown interesting anti-inflammatory activity against carrageenin-induced inflammation. An attempt has also been made to arrive at some structure activity relationship. In addition, the compounds were found to be Central Nervous System depressants and quite non-toxic.

Many indole derivatives play an important role in neuropharmacological systems. Serotonin, an indole derivative, is a chemical neurotransmitter[1], whereas oxindole and its derivatives have been found to be effective in CNS diseases, viz. convulsions[2] and depression[3]. Further different substituted indoles and oxoindoles are reported to be good anti-inflammatory agents[4,5]. On the other hand, different hydrazides are also reported to possess anti-inflammatory activity[6]. It is also recognised that different secondary amines and 'Mannich Bases'[7,8] have an effective therapeutic value against inflammation.

In view of these valid observations, we have synthesised a series of sixteen new title compounds having oxinodole, hydrazide and secondary amine moieties. The title compounds have been synthesised according to the Scheme-1 and their anti-inflammatory activity has been studied. The gross CNS activity of these compounds along with their toxicity test have also been observed.

The 'Mannich bases' (IV) have been synthesised by the reaction of secondary amines with 2-oxo-3-(2,4-dinitrophenylacetyl hydrazono)-5-substituted-indoles (III) in presence of aq. formaldehyde solution. The intermediate III in turn were obtained by the reaction of 2,4-dinitrophenylacetyl hydrazine (I) with respective 2,3-dioxo-5-substituted indoles (II). The structures of all the new compounds were assigned on the basis of elemental analysis I.R. and P.M.R. spectroscopy.

Pharmacological Studies

All the final compounds have been tested for their anti-inflammatory activity and gross Central Nervous System (CNS) activity on the brain of albino mice of either six. The ALD_{50} values were also determined for all the compounds.

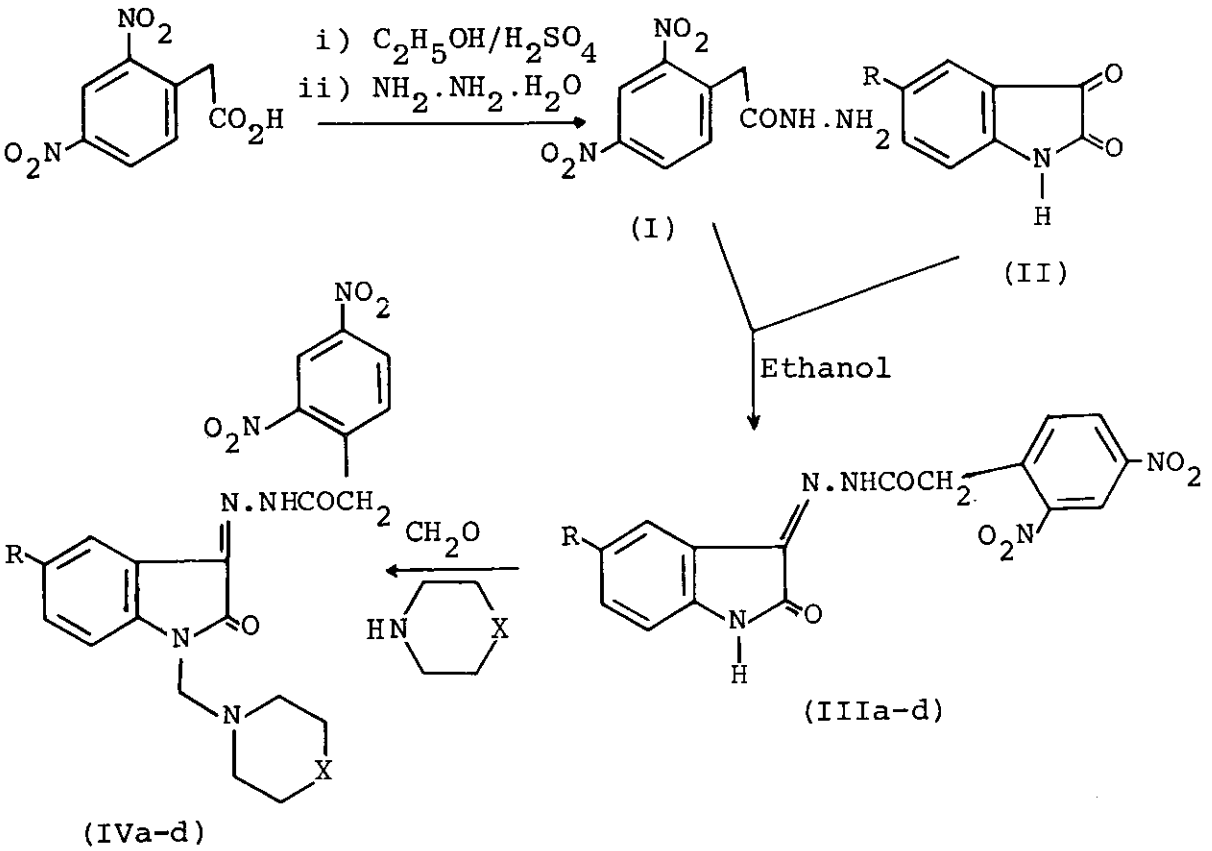
The term ALD_{50} defines the approximate lethal dose in 50% of animals tested. The method of Weil[9] was used to determine the lethal values. For the determination of ALD_{50}, the compounds were injected intraperitoneally at the doses of 464 and 1000 mg/kg weight of mice, as an aqueous gum acacia suspension. The general behavioural changes in the CNS were also observed at the same doses, after 1 hr of the compound's injection. To substantiate the gross CNS observations, decrease in mobility counts, rate of breathing and reactivities to sound and touch were also recorded.

The anti-inflammatory activity was done on the albino mice, adopting the method of Winter et al[10], by measuring the percentage protection against carrageenin induced inflammation. Indomethacin, (a patent drug) was used as the standard drug.

The compounds were found to be highly non-toxic. In gross CNS observations, all the compounds have been found to be CNS depressants, as they decreased the spontaneous motor acitivity (SMA) and reactivity to sound and touch at the doses of 464, 1000 and 1/5th of ALD_{50} mg/kg (mice).

Visualizing the data of anti-inflammatory activity (table 1) of these compounds against carrageenin-induced oedema in mice paw, the following inferences can be drawn:

(i) The N-methyl-piperazino group at position-1 of oxo-indole, confers the highest activity in



IIIa, IVa-d, R=H	IVa,e,i,& m; X=O
IIIb, IVe-h, R=CH_3	IVb,f,j&n; X=CH_2
IIIc, IVi-l; R=Cl	IVc,g,k&o, X=N-CH_3
IIId, IVm-p; R=Br	IVd,h,l&p; X=N-C_6H_4.CH_3(p)

its group of four compounds.

(ii) On the other hand, the introduction of morpholino group at the same position decreased the activity up to the extent of nil in three such compounds, whereas the fourth one (compound no. IVe) has negligible activity.

(iii) It is also noteworthy, that the compounds with methyl substitution at position-5 of oxindole have the highest activity in their group for secondary aminomethyl-substitution.

Experimental

M.ps. were taken in open capillaries, using AR H_2SO_4 bath, and are uncorrected. Ir spectra, in KBr phase, were recorded on the Perkin-Elmer 157 spectrophotometer (νmax in cm^{-1}) and PMR spectra in DMSO-D_6 or $CDCl_3$ on a varian A 60D instrument using TMS as internal standard (chemical shift in δ ppm). The purity of the compounds was checked by TLC using silica-gel coated plates (0.25 mm) and the solvent system: benzene-ethanol 90:10.

2,4-Dinitrophenylacetyl hydrazine (I)

It was prepared by the hydrazinolysis of the corresponding ethylester, according to the method of Bloom[11].

2,3-Dioxo-5-substituted-indoles (II)

These were synthesised via the Sandmeyer reaction, as per method of Marvel and Hiers[12]

Table 1. Gross CNS observations, ALD_{50} and Anit-inflammatory Activity of the compounds described in Table 3.

Compound	SMA & Reactivity*	ALD_{50} (mg/kg)	Anti-inflammatory activity (% protection)**
IVa	↓	> 1000	(–)
IVb	↓	> 1000	8.4
IVc	↓	> 1000	33.7
IVd	↓	> 1000	19.8
IVe	↓	> 1000	5.6
IVf	↓	> 1000	10.4
IVg	↓	> 1000	40.7
IVh	↓	> 1000	24.2
IVi	↓	> 1000	(–)
IVj	↓	> 1000	10.4
IVk	↓	> 1000	29.1
IVl	↓	> 1000	18.4
IVm	↓	> 1000	(–)
IVn	↓	> 1000	3.6
IVo	↓	> 1000	18.4
IVp	↓	> 1000	4.2
Indomethacin		–	39.00 at 10 mg/kg

↓= decreased; (–) = Not affected; – Not done

*= Doses: 464, 1000 and 1/5th of ALD_{50} mg/kg weight of mice.

**= Dose. 1/5th of ALD_{50}.

2-Oxo-3 (2,4-dinitropheynlacetyl hydrazono)-5-substituted indoles (IIIa-d)

To the solution of the appropriate 2,3-dioxo-5-subsitituted indole (0.01 mol) in ethanol (50 ml) containing 2,3 drops glacial AcOH, 0.01 mole of 2,4-dinitrophenyl-acetyl hydrazine was added. The resulting mixutre was refluxed on a steam bath for about 2 hours. A solid started to separate from the refluxing mixture nearby after half an hour but the refluxing was carried out for a further period of one and a half hour. The solid obtained on cooling the reaction mixture, was filtered after keeping aside for some time. It was washed with little ethanol (20 ml), dried well in air and finally recrystallised from glacial acetic acid. The compounds synthesised so far, are recorded in Table 2.

IR: 3400 and 3200-3240 (noncyclic and cyclic N-H respectively). 3010-3050 and 2900-2950 aromatic and aliphatic C-H), 1680-1700 & 1710-1730 (noncyclo and cyclo amidic C=O) and 1600-1620 cm^{-1} (C=N) etc. PMR (IIId); 11.30 (s. 1H, = N-NH), 9.74 (s, 1H, NH cyclic), 8.30 to 7.15 (m, 6H, Ar-H) and 4.22 (s, 2H,-CH_2-CO-) These peaks show the formation of the title compounds.

1-(N,N-Disubstituted aminomethyl)-2-oxo-3-(2,4-dinitrophenylacetyl hydrazono)-5-substituted-indoles (IVa-p)

2-Oxo-3-(2,4-dinitrophenylacetyl hydrazono)-5-substituted-indole (0.0025 mol) was suspended in ethanol (15 ml.). Formaldehyde solution (40%; 1 ml) was added to this suspension and the resulting mixture was heated till boiling on a steam bath. Thereafter, a secondary amine (0.0025 mol) was added to this boiling mixture, with constant vigorous stirring and again the solution was heated over a boiling water bath for about 10 mts. The reaction mixture was then left aside at the room temperature for 24 hrs. The solid obtained was filtered, washed several times with petroleum ether (60-80°C) and finally recrystallised from chloroform-petroleum ether (40-60°C). The physical data of the compounds are recorded in Table 3. IR: 3400-3440 (noncycloamidic N-H), 3010-3050 & 2910-2950 (C-H ar. & ali.), 1680-1700 & 1710-1730 (noncyclic & cylic C=O) and 1600-1610 cm^{-1} (C=N-) etc. PMR (IVn): 11.30 (s, 1H, = N-NH-), 8.30 to 7.15 (m 6H, Ar-H), 4.20 (s, 2H, - CH_2CO-) and 3.34-0.05 (m, 12H, 2H of N-CH_2-N and 10 of piperidine H). It is noteworthy that the peak at 3200-3240 cm^{-1} in the spectra of precursor compounds, referring to cycloamidic N-H, is lacking in the spectra of final compounds. This observation shows the utilisation of the same N-H group in the synthesis of the 'Mannich base'.

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Table 2. Physical data of 2-oxo-3-(2,4-dinitrophenylacetyl hydrazono)-5-substituted-indoles (III)

Compound	R	m.p. (°C)	Yield (%)	Mol. formula	Analysis N (%)* Calcd.	Found
a.	H	> 275	86	$C_{16}H_{11}N_5O_6$	18.97	19.01
b.	CH_3	272	80	$C_{17}H_{13}N_5O_6$	18.27	18.29
c.	Cl	> 275	90	$C_{16}H_{10}N_5O_6Cl$	17.34	17.35
d.	Br	> 275	95	$C_{16}H_{10}N_5O_6Br$	15.62	15.58

* All the compounds gave satisfactory analyses for C & H.

Table 3. Physical data of 1-(N,N-Disubstituted-aminomethyl)-2-oxo-3-(2,4-dintrophenylacetyl hydrazono)-5-substituted-indoles (IV)

Compound	X	m.p. (°C)	Yield (3)	Mol. formula	Analysis N (%)* Calcd.	Found
	R=H					
a.	O	181	69	$C_{21}H_{20}N_6O_7$	17.94	18.02
b.	CH_2	205	62	$C_{22}H_{22}N_6O_6$	18.02	18.19
c.	N-CH_3	120	70	$C_{22}H_{23}N_7O_6$	20.37	20.28
d.	N-$C_6H_4CH_3$ (p)	174	79	$C_{28}H_{27}N_7O_6$	17.59	17.56
	R=CH_3					
e.	O	200	50	$C_{22}H_{22}N_6O_7$	17.42	17.35
f.	CH_2	180	56	$C_{23}H_{24}N_6O_6$	17.50	17.47
g	N-CH_3	210	62	$C_{23}H_{25}N_7O_6$	19.79	19.64
h.	N-$C_6H_4CH_3$ (p)	170	65	$C_{29}H_{29}N_7O_6$	17.16	16.99
	R=Cl					
i.	O	259	79	$C_{21}H_{19}N_6O_7Cl$	16.71	16.73
j.	CH_2	250	80	$C_{22}H_{21}N_6O_6Cl$	16.78	16.81
k.	N-CH_3	285**	80	$C_{22}H_{22}N_7O_6Cl$	19.01	19.10
l.	N-$C_6H_4CH_3$(p)	245	89	$C_{28}H_{26}N_7O_6Cl$	16.56	16.50
	R=Br					
m.	O	165	90	$C_{21}H_{19}N_6O_7Br$	15.35	15.44
n.	CH_2	200	75	$C_{22}H_{21}N_6O_6Br$	15.41	15.62
o.	N-CH_3	215	80	$C_{22}H_{22}N_7O_6Br$	17.50	17.38
p.	N-$C_6H_4CH_3$(p)	242	86	$C_{28}H_{26}N_7O_6Br$	15.40	15.49

* Satisfactory analysis for C & H were also found for all the compounds.
** Mixed M.P. with the prefinal compound (C) is 255°C.

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