
Synthesis and Antiinflammatory Activity of Schiff Bases of Mesalazine

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Schiff bases of mesalazine, (5 - Aminosalicyclic acid) with arylaldehydes were synthesised. They were screened for analgesic, antiinflammatory and antipyretic activities. All of them were found to have promising biological activities.

MESALAZINE is 5-aminosalicylic acid (5-ASA), the active moiety of sulphasalazine. It is used now-a-days to treat acute exacerbations of the inflammatory bowel disease and ulcerative colitis. The antiinflammatory action of 5-ASA in the gut is due to its powerful inhibition of synthesis of leukotrienes (via lipoxygenase pathway), highly potent inflammatory mediators playing a role in the pathogenesis of ulcerative colitis¹. *In vitro* studies showed that 5-ASA inhibits lymphocyte activation, killer cell activity and migration of macrophages into inflamed tissue. It is reported to be a weak inhibitor of cyclooxygenase. Orally ingested mesalazine is not effectively absorbed by gut and is even metabolised by intestinal epithelium. The major metabolites, Nacetyl-5-aminosalicylic acid, and 5-aminosalicylic acid appeared to have no therapeutic activity². When primary amino group is present along with carboxylic acid group in a molecule, it displays poor water solubility and liposolubility due to zwitter ion nature in the physiological pH range and thereby its bioavailability is restricted.

The present investigation is envisaged for further improving the pharmacological profile and bioavailability of 5-ASA. We decided to prepare bioprecursors (prodrugs) of 5-ASA by modifying the amino group into N-methylene derivatives³. Thus Schiff bases were synthesised by condensing biologically signif-

icant aldehydes like vanillin, anisaldehyde, salicylaldehyde, dimethylaminobenzaldehyde and furfural with 5-ASA. The antiinflammatory, analgesic and antipyretic activities of compounds synthesised were preformed and compared with 5-ASA.

MATERIALS AND METHODS

CHEMISTRY

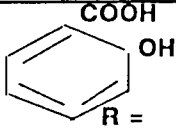
The Schiff bases were synthesised by condensing 5-ASA with respective aldehydes in ethanol⁴. They were recrystallised from ethanol and were characterized by TLC, MP, IR and NMR spectra. The structure, % yield and MP are given in the Table 1.

BIOLOGICAL ACTIVITY

The compounds synthesised are amorphous and insoluble in water and therefore all biological testings were carried out as their homogenised suspension in 0.3% w/v carboxymethylcellulose. Albino rats (wistar) and mice (swiss) were used for the experiments. Oral LD₅₀ was determined in albino mice in groups of 8 of either sex for each dose tested⁵. One tenth to one twelfth of oral LD₅₀ was selected for determination of biological activities. One m Mol/kg dose of each test compound was employed to compare the biological activity of the prodrugs which liberate 1 m Mol of 5-ASA in the system. One

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Table 1 : Schiff Bases of Mesalazine

Compound	RCH = H 	physical nature	% yield	mp * oC	Acute LD ₅₀ ** mg/kg p.o	m.Mol equivalent of test compounds
Ia	4 - Hydroxy-3-methoxyphenyl	yellow amorphous powder	92	241-42	3000	287
Ib	4 - Methoxyphenyl	pale yellow amorphous powder	90	216-17	3000	271
Ic	2 - Hydroxyphenyl	pale yellow amorphous powder	87	233-34	2750	257
Id	4 - N - Dimethylaminophenyl	pale pink amorphous powder	93	238-39	2500	284
Ie	2 - Furyl -	brown amorphous powder	85	220-21	3000	231

* melting points were taken in open capillary tube and are uncorrected

** LD₅₀ in albino mice (n = 8)

m Mol equivalence of the test compounds are given in the Table I.

ANTIINFLAMMATORY ACTIVITY

Test was performed by the technique of Winter et al⁶. The oedema was induced in albino rats in a group of 6 by injecting 0.1 ml carrageenin (1% w/v suspension in normal saline) into subplanter region of the left hind paw. The volume of paw was measured immediately using a plethysmometer after carrageenin injection and again 3 h later. The test compounds (1a to 1e) and 5-ASA were given orally 1 h prior to carrageenin injection. Indomethacin 5 mg/kg p.o. was used as standard drug. Mean increase in paw volume and standard error (S.E.) were calculated and the results were expressed as % inhibition of oedema as compared to the control. The compound Ic, which showed maximum activity was administered orally in graded dose (62.5 mg, 125 mg and 250 mg /kg) 1 h prior to introduction of inflammation in acute test. The statistical correlation of dose-response changes was determined by regression analysis.

ANALGESTIC ACTIVITY

The method was based on acetic acid-induced writhing syndrome in mice. The test compounds and aspirin in 0.5 m Mol/kg dose were used to antagonise the writhing produced by the injection of 0.6% acetic acid in albino mice. The analgestic activity is determined as % reduction of writhing in test animals in comparison with the control.

ANTIPYRETIC ACTIVITY

The method was based on Typhoid and Paratyphoid A & B, (TAB) vaccine-induced pyrexia in albino rats⁸. The animals were made pyretic by injecting TAB vaccine 0.1 ml/rat s.c. The compounds and paracetmol were administered orally (1 m Mol/kg) one hour after injection. The hourly rectal temperature was recorded upto 4 h using telethermometer.

Table II
BIOLOGICAL ACTIVITY STUDIES OF SCHIFF BASES OF MESALAZINE

Compound	Antiinflammatory Action *		Analgesic Action **		Antipyretic Action ***
	Mean redn of paw oedema \pm S.E.	% activity	No of writhing in 20 min. \pm S.E	% activity	Mean redn. in rectal Temp $^{\circ}$ C after 4 h.
CONTROL@	0.72 \pm 0.012	--	65.8 \pm 2.76	--	0.54 \pm 0.30
5-ASA	0.50 \pm 0.017	30.50	48.2 \pm 6.5	26.29	2.92 \pm 0.26
1a	0.28 \pm 0.069	61.11	35.6 \pm 1.14	45.80	3.18 \pm 0.24
1b	0.30 \pm 0.017	58.33	43.3 \pm 3.75	34.19	3.20 \pm 0.47
1c	0.18 \pm 0.010	75.00	27.1 \pm 2.24	60.33	4.40 \pm 0.47
1d	0.26 \pm 0.012	63.88	42.0 \pm 1.95	36.17	3.80 \pm 0.19
1c	0.34 \pm 0.022	52.91	34.4 \pm 3.80	47.72	4.04 \pm 0.32
Standard	0.21 \pm 0.013	70.8	26.7 \pm 2.28	59.42	4.10 \pm 0.31

* Percentage activity at 1 m mol/kg p.o dose by carrageenin- induced paw oedema in albino rats (n = 6). Standard used Indomethacin (5mg/kg, p.o.), P = < 0.01 (Student 't' test)

** Percentage activity at 0.5 m mol/kg. p.o. dose by acetic acid - induced writhing in albino mice (n + 6). Standard used Aspirin (0.5 m mol/kg. p.o.), P= <0.05 (Student 't' test)

*** Mean reduction of hyperpyrexia ($^{\circ}$ C) induced by TAb vaccine in albino rats. (n = 6). Standard used Paracetamol (1 m mol/kg. p.o.) P = <0.05 (Student5 't' test)

@ - vehicle 0.3% CMC in distilled water

RESULTS

The Schiff bases synthesised were characterised by their MP, TLC and by IR and NMR spectral data (Table 1). The compounds are amorphous in nature and practically insoluble in water but soluble in alcohol. The schiff bases of 5-ASA with aldehydes reported here were prepared easily in the experimental condition, with a % yield ranging from 85 to 93, but such condensation with benzaldehyde, cinnamaldehyde, citral etc. did not progress well. The compounds were less toxic on oral route as

shown in the actue LD₅₀ calculation. Compound 1d is slightly more toxic than others. Perusal of the Table II reveals that all the Schiff bases exhibited fairly good antiinflammatory activity at 1 m Mol/kg dose while comparing with 5-ASA. Among the Schiff bases, compound 1c exhibited the maximum activity 75%. The log dose- response of the compound 1c was found to be linear as shown in the graph (Fig.1). All compounds exhibited marked analgesic activity when given orally. The compound 1c showed maximum activity, comparable with that of aspirin (Fig.2).

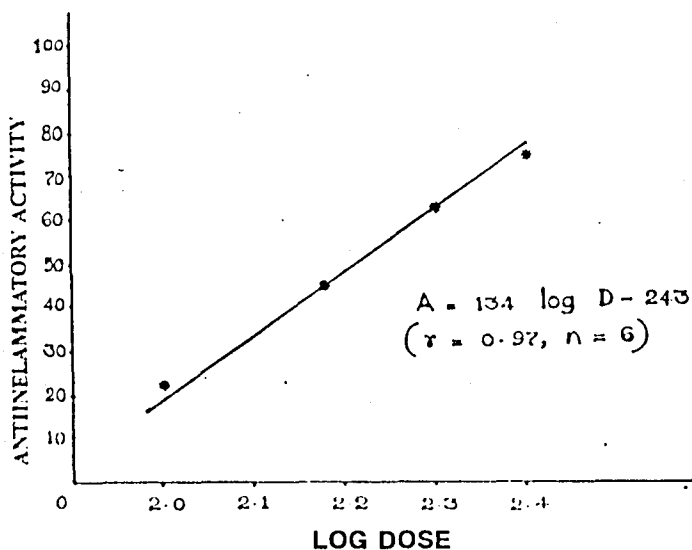


Fig. I : Log-dose response graph of Schiff base Ic. Antiinflammatory activity calculated as % inhibition of paw oedema in albino rats

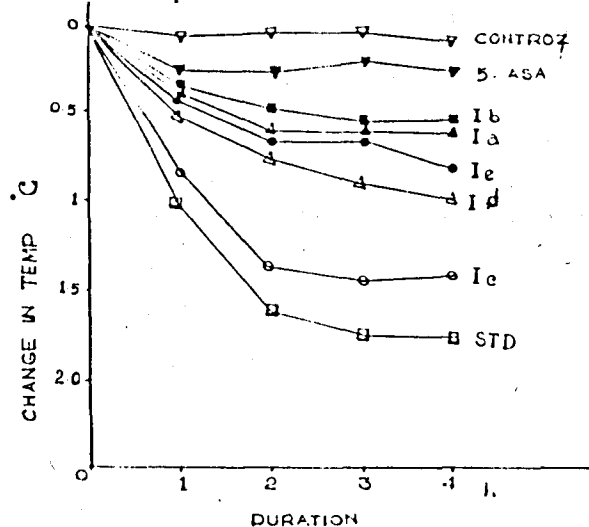


Fig. III : Mean reduction of hyperpyrexia ($^{\circ}\text{C}$) induced by TAB vaccine in albino rats. (n = 6) Standard used Paracetamol (1 m mol/Kg. p.o.) P = <0.05 (Student 't' test)

The antipyretic activity is calculated as mean change in rectal temperature ($^{\circ}\text{C}$) of TAB vaccine-induced hyperpyrexia in albino rats. All compounds showed fairly good activity than 5-ASA in 1 m Mol/kg p.o. The compound Ic had marked antipyretic activity (1 m Mol/kg p.o) even better than paracetamol in equimolar dose (Fig. 3).

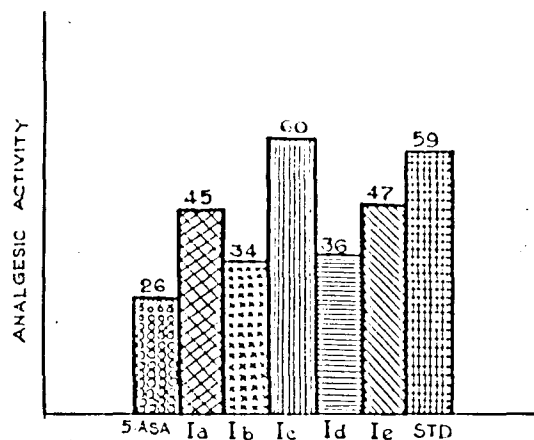


Fig. II Percentage activity at 0.5 m mol / Kg. p.o. dose by acetic acid - induced writhing in albino mice. (n = 6). Standard used Aspirin (0.5 m mol / Kg. p.o.), P = <0.05 (Student 't' test)

DISCUSSION

The condensation of 5-ASA with aldehydes depends on the nature of substitution in the benzene ring. The reported compounds are synthesised by simple condensation in ethanol by varying the duration and temperature of reaction. The aldehydes such as benzaldehyde, cinnamaldehyde and citral resists the condensation in the experimental condition. Verification of biological activity data reveals that all the Schiff bases have marked antiinflammatory, analgesic and antipyretic activities in comparison with 5-ASA. This indicates that absorption of these prodrugs is far better than the parent compound. It seems that blocking the amino group has helped the absorption and metabolism. Schiff base might have undergone oxidative cleavage and reduction mediated by cytochrome P 450 and other reductases to liberate bioactive moieties⁹. Compound Ic has exhibited better antiinflammatory and antipyretic activity (1 m Mole/kg P.O). Similarly it antagonised acetic acid-induced writhing syndrome in 0.5 m Mol/kg p.o. dose. These data clearly indicate that the schiff bases, unlike 5-ASA, exerts predominant inhibition of inflammatory mediators from phlogogenic stimuli. The better activity of compound

Ic over other schiff bases may be due to its *in-vivo* cleavage in two bioactive moieties namely 5-ASA and salicylic acid which are powerful inhibitors of lipoxygenase and cyclooxygenase respectively. We look forward to carry out the detailed toxicological studies, ulcerogenic property and other pharmacological parameters of the Salicylaldehyde schiff (Ic) as a promising antiinflammatory agent.

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