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Research Article

SYNTHESIS OF NOVEL SCHIFF BASES OF 5-AMINOSALICYLIC ACID BY GRINDING TECHNIQUE AND ITS EVALUATION FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES

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ABSTRACT
5-Aminosalicylic acid or Mesalamine are considered as amino derivative of salicylic acid with molecular formula $C_7H_7NO_3$. It is proved to a useful drug in an effective treatment of inflammatory bowel disease. It possess both anti-inflammatory and analgesic activity by targeting COX, Prostaglandins and lipoxygenase enzyme. Presence of primary amine group in the 5th position makes them an important substrate for Schiff base synthesis. Schiff base possess diverse biological activities and can be formed by various methods. In the present study grinding technique was used as a simple and effective way for synthesizing Schiff bases of 5-Aminosalicylic acid. A mixture of aromatic aldehyde and 5-Aminosalicylic acid were grinded to produce yellow coloured Schiff base and free amine group forms double bond with the carbon forming a condensation product. Further these schiff bases were evaluated for anti-inflammatory and analgesic activities. m-Chloro derivative was proved to be a potent analgesic and anti-inflammatory agent.

KEYWORDS: 5-Aminosalicylic acid, Anti-inflammatory, Analgesic, Prostaglandins.

INTRODUCTION

5-Aminosalicylic acid or Mesalamine belongs to the category of salicylates. This category is found to be active in inflammatory bowel disease. Sulphasalazine, Olsalazine and Balsalazide are considered as the prodrugs consisting of 5-Aminosalicyclic acid as active moiety[1]. It is very slightly soluble in dehydrated alcohol, acetone and metyl alcohol. Practically insoluble in chloroform, ether, n-hexane and ethyl acetate. It is prescribed in the treatment and management of ulcerative colitis. It basically provides relieve from pain to some extent in patients suffering from this disease[2-8]. Being salicylate derivative, it show its mechanism by inhibiting COX, level of prostaglandin and 5-lipoxygenase enzyme. It is also a radical scavenger, inhibits a link between formly-metionyl-lucyl-phenylalanin and the receptors located on the neutrophils[9-10]. Past studies suggest that 5-aminosalicylic acid is active in ulcerative colitis because of its multiple action on various functions of the immune system[11-12].

Schiff bases are widely applicated in biological systems, catalysis, dying processes and analytical field[13]. Schiff bases are associated with antibacterial, antifungal activities, analgesic, anti-inflammatory, cytotoxic, antitumor and antioxidant activities[14-16]. These are synthesized by the condensation of aromatic aldehyde and primary amine containing compounds with removal of a water molecule leading into formation of carbon nitrogen double bond. Various methods have been implicated for the synthesis of Schiff bases[17-19]. In the present study we have synthesized Schiff bases of 5-aminosalicylic acid by using grinding technique as a method employing green chemistry and evaluating them for anti-inflammatory and analgesic activities.

MATERIALS AND METHODS

Chemicals

5-Aminosalicylic acid and Carrageenan were obatined from HiMedia Labs, Mumbai. All other chemical reagents were used of analytical grade, which were procured from different companies (Loba Chem, Merck Limited and S D Fine). The progress of the reaction was monitored on readymade silica gel plates (Merck) using chloroform-methanol (6:4) as a solvent system. Iodine was used as a developing agent. Melting points were determined with a Buchi 530 melting point apparatus in open capillaries. IR spectra were recorded on KBr discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer. The proton magnetic resonance spectra (^{1}H-NMR) were recorded on Perkin Elmer Spectrophotometer-300MHz in DMSO-*d6* using TMS as an internal standard.

Animals

The wistar albino rats (150-200 g) of either sex were obtained from Zoin Co. Biologicals, Ambala. They were kept at standard laboratory diet, environmental temperature and humidity. A 12 hours light and dark cycle was maintained throughout the experimental protocol. The experimental protocol was duly approved by Committee for the Purpose of Control and Supervision of Experiments on Animals.

General Synthesis

A mixture of different aldehydes **(1)** (0.01 moL) and 5-aminosalicyclic acid **(2)** (0.01 moL) was grinded in a mortar with a pestle made of porcelain for 5 - 10 min. The mixture turned pasty after few minutes of grinding. It was grinded till yellow colour product appears. The mixture was left overnight. The resultant product **(3)** was recrystallized using ethanol. The reaction was monitored by TLC. Physiochemical and analytical data of Schiff bases of 5-Aminosalicylic acid is represented in Table 1 and 2 respectively and Figure 1.

5-{[(E)-(2-chlorophenyl)methylidene]amino}-2-hydroxybenzoic acid (3a)

A mixture of o-chlorobenzaldehyde (1.2 mL, 0.01 moL) and 5-aminosalicyclic acid (1.53 g, 0.01 moL) was grinded in a mortar with a pestle made of porcelain for 5-10 min. The mixture turned pasty after few minutes of grinding. It was grinded till yellow colour product appears. The mixture was left overnight. The resultant product was recrystallized using ethanol. The reaction was monitored by TLC.

5-{[(E)-(3-chlorophenyl)methylidene]amino}-2-hydroxybenzoic acid (3b)

A mixture of m-chlorobenzaldehyde (1.2 mL, 0.01 moL) and 5-aminosalicyclic acid (1.53 g, 0.01 moL) was grinded in a mortar with a pestle made of porcelain for 5-10 min. The mixture turned pasty after few minutes of grinding. It was grinded till yellow colour product appears. The mixture was left overnight. The resultant product was recrystallized using ethanol. The reaction was monitored by TLC.

5-{[(E)-(4-chlorophenyl)methylidene]amino}-2-hydroxybenzoic acid (3c)

A mixture of p-chlorobenzaldehyde (1.2 mL, 0.01 moL) and 5-aminosalicyclic acid (1.53 g, 0.01 moL) was grinded in a mortar with a pestle made of porcelain for 5-10 min. The mixture turned pasty after few minutes of grinding. It was grinded till yellow colour product appears. The mixture was left overnight. The resultant product was recrystallized using ethanol. The reaction was monitored by TLC.

Anti-inflammatory activity

Carrageenan-induced rat paw edema: The carrageenan-induced rat paw edema assay was carried out according to Winter et al., 1962. Wistar rats were divided into 8 groups each consisting of 6 animals[20-21].

Group I : (Disease Control) Carrageenan (1 %) was administered in the plantar surface of rat (p.o).

Group II : (Standard) Suspension of Mesalamine (200 mg/kg) in 1% Gum acacia (p.o) + Carrageenan.

Group III : (Test) Suspension of test compound **(3a)** (200 mg/kg) in 1 % Gum acacia (p.o) + Carrageenan.

Group IV : (Test) Suspension of test compound **(3b)** (200 mg/kg) in 1 % Gum acacia (p.o) + Carrageenan

Group V : (Test) Suspension of test compound **(3c)** (200 mg/kg) in 1 % Gum acacia (p.o) + Carrageenan

Edema was induced on the left hind paw of the rats by subplantar injection of 0.1 mL of a solution of 1 % (w/v) carrageenin in a 0.9 % NaCl (w/v). The paw volume was measured at intervals of 60, 120, 180 min by the mercury displacement method using a plethysmograph after administration of the suspension of test compounds in 1 % Gum acacia orally. The average paw edema volume of all the groups were calculated and compared with that of control. The percentage inhibition of paw edema in drug treated group was compared with the carrageenan control group and calculated according to the following Eq. 1.

$$\textbf{Percentage inhibition of drug} = \left(\frac{Vc - Vt}{Vc}\right) \times 100 \quad \text{Eq. 1}$$

where, Vc is the inflammatory increase in paw volume of control group of animals and Vt is the inflammatory increase in paw volume of drug-treated animals.

Analgesic activity

Swiss albino mice of either sex were divided into 8 groups each consisting of 6 animals[22-23].

Group I : (Control) 1 % Gum acacia (p.o).

Group II : (Standard) Suspension of Mesalamine (100 mg/kg) in 1% Gum acacia (p.o).

Group III : (Test) Suspension of test compound **(3a)** (200 mg/kg) in 1 % Gum acacia (p.o).

Group IV : (Test) Suspension of test compound **(3b)** (200 mg/kg) in 1 % Gum acacia (p.o).

Group V : (Test) Suspension of test compound **(3c)** (200 mg/kg) in 1 % Gum acacia (p.o).

Eddy's Hot plate method: The analgesic activity of the test compounds were measured by hot-plate method. The rats were placed on a hot plate maintained at 55±0.5 ^{o}C. The reaction time was taken as the interval from the instant animal reached the hot plate until the moment animal licked its feet or jumped out. The reaction time was recorded before and after 0, 30, 60 and 90 min following oral administration of tests compounds and standard drug in the form 1% Gum acacia suspension. Following groups were made and latency period in which rat responded to hot plate was calculated.

Statistical analysis: All the results were expressed as Standard Error of Means (SEM). The data was statistically analyzed by one way Analysis of Variance (ANOVA) followed by Tukey using GraphPad Prism 5 Software. The p-value<0.05 was considered to be statistically significant.

RESULTS

All the Schiff bases were obtained in good yield. Compound 3a showed maximum percentage yield of about 87 %.

Anti-inflammatory activity

The positive control, 5-Aminosalicylic acid and test compounds (3a-3c) significantly inhibited the paw edema response in comparison to control group. 5-Aminosalicylic acid showed an inhibition of 54.1 % after 3 hours. Compound 3b showed almost comparable activity with standard with an inhibition of 50.45 % and compound 3a showed minimum activity with an inhibition of 41.7 % after 3 hours as shown in Table 3.

Analgesic activity

All the test compounds (3a-3c) also showed good analgesic activity with compound 3b having maximum activity and compound 3c with minimum analgesic activity as compared to 5-Aminosalicylic acid (standard) as shown in Table 4.

DISCUSSION

Grinding technique was proved to be a better method for the synthesis of Schiff bases. Good yield was obtained and green chemistry was also followed. All the derivatives showed significant analgesic and anti-inflammatory activity. It may be concluded that all the derivatives may act through same mechanism as the parent drug.

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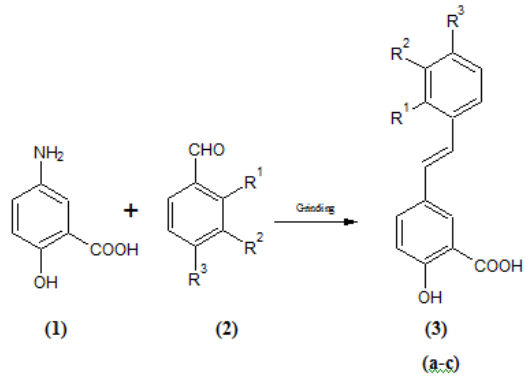
Figure 1: Synthesis of Novel 5-Aminosalicyclic acid Schiff bases



COMPOUND	R^1	R^2	R^3
3a	-Cl	H	H
3b	H	-Cl	H
3c	H	H	-Cl

Table 1: Physiochemical parameters of Schiff bases of 5-Aminosalicylic acid (3a-3c)

Compound	Ar	Molecular formula	MWt. g/moL	Yield [%]	M.pt [^{0}C]	R_f
3a	o-Chlorophenyl	$C_{14}H_{10}ClNO_3$	275.68	87	240-244	0.68
3b	m-Chlorophenyl	$C_{14}H_{10}ClNO_3$	275.68	83	237-241	0.67
3c	p-Chlorophenyl	$C_{14}H_{10}ClNO_3$	275.68	85	238-242	0.68

Table 2: Analytical data of Schiff bases of 5-Aminosalicylic acid (3a-3c)

Compound	FTIR (cm^{-1})					^{1}HNMR (δ ppm)
	-C=O	-C=N	-OH	-Cl	C-N	
3a	1689	1668	2914	743	1133.9	7.48-7.39 (4H, m, subst. Ar-H), 8.20 (1H, s, H_6), 7.8 (1H, d, H_8), 6.9 (1H, d, H_7), 10.42 (1H, s, H_9), 8.9 (1H, s, H_5), 5.06 (1H, s, H_{10}).
3b	1699	1623	2918	765	1133	7.3-7.49 (4H, m, H_1, H_2, H_3, H_4), 8.13 (1H, d, H_8), 6.9491 (1H, d, H_7), 11.4863 (1H, s, H_9), 5.42 (1H, s, H_{10}), 8.5919 (1H, s, H_5).
3c	1659	1606	3069	733	1129	7.30-7.87 (1H, d, H_8), 8.1743 (1H, s, H_6), 7.58 (1H, d, Ar-H), 6.963 (1H, d, H_7), 10.72 (1H, s, H_9), 4.33 (1H, s, H_{10}).

Table 3: Anti-inflammatory effect of Schiff bases of 5-Aminosalicylic acid (3a-3c) on carrageenan-induced paw edema

Treatment Groups	Dose (mg/kg) orally	Mean Paw Volume (mL)			% Inhibition
		60 min	120 min	180 min	
Control	-	0.62±0.014	0.66±0.034	0.72±0.012	-
Standard	200	0.43±0.011[a]	0.36±0.007[a]	0.30±0.01[a]	58.33
3a	200	0.50±0.038[ab]	0.46±0.017[ab]	0.42±0.02[ab]	41.7
3b	200	0.46±0.014[ab]	0.40±0.012[ab]	0.33±0.003[ab]	54.1
3c	200	0.48±0.023[ab]	0.45±0.008[ab]	0.41±0.01[ab]	43.0

Values are the average of triplicate experiments and represented as Mean±SEM. All values are significant, [a]p<0.05 compared to control, [b]p<0.05 compared to 5-Aminosalicyclic acid (tukey's test).

Table 4: Analgesic activity of Schiff bases of 5-Aminosalicylic acid (3a-3c)

Groups	Dose (mg/kg) orally	Lapse time (sec)			
		0 min	30 min	60 min	90 min
Control	-	3.30±0.083	3.32±0.19	3.40±0.062	3.35±0.053
Standard	200	3.24±0.050[a]	4.19±0.031[a]	5.12±0.003[a]	6.073±0.09[a]
3a	200	3.16±0.060[ab]	4.13±0.033[ab]	4.99±0.078[ab]	5.59±0.21[ab]
3b	200	3.20±0.051[ab]	4.25±0.05[ab]	5.19±0.098[ab]	6.19±0.076[ab]
3c	200	3.35±0.001[ab]	4.01±0.01[ab]	4.76±0.06[ab]	5.12±0.002[ab]

Values are the average of triplicate experiments and represented as Mean±SEM. All values are significant, [a]p<0.05 compared to control, [b]p<0.05 compared to 5-Aminosalicyclic acid (tukey's test).

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