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 belong 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-Aminosalicylic acid as active moiety. It is very slightly soluble in dehydrated alcohol, acetone and m ethyl 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. Being salicylate derivative, it show its mechanism by inhibiting COX, level of prostaglandin and lipoxygenase enzyme. It is also a radical scavenger, inhibits a link between formylmetionyl-lucyl-phenylalanin and the receptors located on the neutrophils. Past studies suggest that 5-aminosalicly acid is active in ulcerative colitis because of its multiple action on various functions of the immune system. Schiff bases are widely applied in biological systems, catalysis, dying processes and analytical field. Schiff bases are associated with antibacterial, antifungal activities, analgesic, anti-inflammatory, cytotoxic, antitumor and antioxidant activities. 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. 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 obtained 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 (1H-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-aminosalicylic 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 kept 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-aminosalicylic 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 kept overnight. The resultant product was recrystallized using ethanol. The reaction was monitored by TLC.
5-[(E)-3-chlorophenyl]methyldiene]amino)-2-hydroxybenzoic acid (3b)
A mixture of m-chlorobenzaldehyde (1.2 mL, 0.01 mol) and 5-aminosalicylic 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.

Group II

induced rat paw edema assay was carried out according to Carrageenan using mice. Anti-inflammatory activity

Group I

The anti-inflammatory activity of the test compounds were measured by hot-plate method. The rats were placed on a hot plate maintained at 55±0.5 °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.

\[ \text{Percentage inhibition of drug} = \frac{V_c - V_e}{V_c} \times 100 \]  

where, \( V_c \) is the inflammatory increase in paw volume of control group of animals and \( V_t \) 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. 2-3.

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 °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.

REFERENCES

2. Bird HA, Sulphasazin, Sulphapyridine or 5-Aminosalicylic acid Which is the Active Moiety in Rheumatoid Arthritis?, Br J Rheumatol 1995; 34: 16-9.
Figure 1: Synthesis of Novel 5-Aminosalicylic acid Schiff bases

![Schiff base reaction diagram]

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>Molecular formula</th>
<th>MWt. g/mol</th>
<th>Yield [%]</th>
<th>M.pt [°C]</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>o-Chlorophenyl</td>
<td>C₁₇H₁₆ClNO₂</td>
<td>275.68</td>
<td>87</td>
<td>240-244</td>
<td>0.68</td>
</tr>
<tr>
<td>3b</td>
<td>m-Chlorophenyl</td>
<td>C₁₇H₁₆ClNO₂</td>
<td>275.68</td>
<td>83</td>
<td>237-241</td>
<td>0.67</td>
</tr>
<tr>
<td>3c</td>
<td>p-Chlorophenyl</td>
<td>C₁₇H₁₆ClNO₂</td>
<td>275.68</td>
<td>85</td>
<td>238-242</td>
<td>0.68</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>FTIR (cm⁻¹)</th>
<th>¹H NMR (δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-C=O</td>
<td>-C=N</td>
</tr>
<tr>
<td>3a</td>
<td>1689</td>
<td>1668</td>
</tr>
<tr>
<td>3b</td>
<td>1699</td>
<td>1623</td>
</tr>
<tr>
<td>3c</td>
<td>1659</td>
<td>1606</td>
</tr>
</tbody>
</table>
### Table 3: Anti-inflammatory effect of Schiff bases of 5-Aminosalicylic acid (3a-3c) on carrageenan-induced paw edema

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dose (mg/kg) orally</th>
<th>Mean Paw Volume (mL)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
<td>120 min</td>
<td>180 min</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.62±0.014</td>
<td>0.66±0.034</td>
</tr>
<tr>
<td>Standard</td>
<td>200</td>
<td>0.43±0.011*</td>
<td>0.36±0.007*</td>
</tr>
<tr>
<td>3a</td>
<td>200</td>
<td>0.50±0.038**</td>
<td>0.46±0.017**</td>
</tr>
<tr>
<td>3b</td>
<td>200</td>
<td>0.46±0.014**</td>
<td>0.40±0.012**</td>
</tr>
<tr>
<td>3c</td>
<td>200</td>
<td>0.48±0.023**</td>
<td>0.45±0.008**</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean±SEM. All values are significant, *p<0.05 compared to control, **p<0.05 compared to 5-Aminosalicylic acid (tukey’s test).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg) orally</th>
<th>Lapse time (sec)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>3.30±0.083</td>
<td>3.32±0.19</td>
<td>3.40±0.062</td>
<td>3.35±0.053</td>
</tr>
<tr>
<td>Standard</td>
<td>200</td>
<td>3.24±0.050*</td>
<td>4.19±0.031*</td>
<td>5.12±0.003*</td>
<td>6.07±0.09*</td>
</tr>
<tr>
<td>3a</td>
<td>200</td>
<td>3.16±0.060**</td>
<td>4.13±0.033**</td>
<td>4.99±0.078**</td>
<td>5.59±0.21**</td>
</tr>
<tr>
<td>3b</td>
<td>200</td>
<td>3.20±0.051**</td>
<td>4.25±0.055**</td>
<td>5.19±0.098**</td>
<td>6.19±0.076**</td>
</tr>
<tr>
<td>3c</td>
<td>200</td>
<td>3.35±0.001**</td>
<td>4.01±0.011**</td>
<td>4.76±0.06**</td>
<td>5.12±0.002**</td>
</tr>
</tbody>
</table>

Values are the average of triplicate experiments and represented as Mean±SEM. All values are significant, *p<0.05 compared to control, **p<0.05 compared to 5-Aminosalicylic acid (tukey’s test).

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