SYNTHESIS AND CHARACTERIZATION OF NOVEL AMINO ACID PRODRUG OF FAMOTIDINE

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ABSTRACT

Objective: Famotidine an H2 receptor antagonist is the drug of choice to treat ulcers in stomach (gastric and duodenal), erosive esophagitis (heartburn or acid indigestion) and gastroesophageal reflux disease (GERD). Drug molecules with limited aqueous solubility are becoming very common in the research and development portfolios of discovery focused pharmaceutical companies. Prodrugs are an established concept to overcome barriers like poor solubility to drug’s usefulness. Polar Amino acids which are biocompatible and easily ionisable were chosen as promoieties for the formation of prodrugs. Aqueous solubility is an important parameter to enhance the bioavailability of the drug. Hence the present study aims to enhance aqueous solubility and in turn bioavailability by prodrug approaches.

Methods: Synthesis of novel amino acid prodrug of famotidine was done by microwave irradiation technique. The synthesized amino acid prodrug was characterized by IR, NMR, Mass and DSC.

Results: In vitro chemical hydrolysis profiles revealed that the synthesized amino acid derivative of famotidine was chemically stable in Simulated Gastric fluid pH 1.2 and Simulated Intestinal Fluid pH 7.4. Decrease in Log P value, 0.39 of amino acid prodrug compared to -0.60 of famotidine was observed.

Conclusion: Hence a novel amino acid prodrug of famotidine with better solubility and bioavailability was synthesized and characterized.

Keywords: Famotidine, Amino acid prodrug, Characterization, Aqueous solubility.

INTRODUCTION

Famotidine (FM) chemically 3-[(2-{diaminomethylene amino) thiazol-4-yl} methyl-thio)-N' sulfamoyl propimidamide is a histamine H2 receptor blocker (fig 1).

Fig. 1: Chemical structure of famotidine

FM is used to treat and prevent ulcers in the stomach and intestine [1]. FM is a poorly water soluble drug and is poorly absorbed from the lower gastrointestinal tract. Dissolution is the rate limiting step in the process of drug absorption [2]. There are many stated techniques for solubility enhancement of FM like complexation, solid dispersion, micronization [3-5]. Prodrug is an efficient technique to enhance solubility of drugs. Prodrugs are defined as a biologically inactive derivative of a parent drug molecule that usually requires a chemical or enzymatic transformation within the body to release the active drug, and possess improved delivery properties over the parent molecule [6]. There are no reported methods for preparation of amino acid prodrug in solubility enhancement of FM. The main purpose of the present study is to increase aqueous solubility of famotidine by prodrug approach. Amino acids do have proven record of being successfully used as promoieties in synthesis of prodrugs. As amino acids are biocompatible and easily ionisable, synthesis of amino acid prodrug can be used for enhancing the solubility. New drug research consumes a lot of money and time, whereas prodrug strategy enhances the effectiveness of existing drug by overcoming its drawbacks. Therapy enhancement via successful delivery of a therapeutic agent is the principal goal of drug delivery research. Achieving therapeutic efficacy of any pharmaceutical dosage form mainly depends upon the availability of drug with the desired concentration to the target site [7]. The bioavailability of poorly water-soluble drug like FM is a well known difficulty to be coped with during drug delivery. The current research aims to resolve the aforementioned issue by prodrug approach. Amino acid prodrug of FM was synthesized and the synthesized prodrug was investigated by FT-IR, 1H NMR, mass and DSC studies. Aqueous solubility studies of prodrug were performed to ensure solubility enhancement.

MATERIALS AND METHODS

Instruments and chemicals

Melting points were determined on a Differential Scanning Calorimeter (DSC) apparatus. Aluminum sheets were coated with silica gel 60 F254 of Merck were used for TLC. Photographic images were taken using an Olympus research microscope. Elemental analysis was performed using Carlo - Erba model 1108. The IR spectra were recorded on Perkin Elmer spectrophotometer and wave numbers are reported in cm⁻¹. The 1H NMR spectra were obtained on a Bruker Avance-300 spectrometer (300 MHz) in deuterated methanol. Chemical shifts were recorded in ppm (δ) relative to TMS as an internal standard. High resolution mass spectra were recorded on an Agilent 5975 MSD series Direct Inlet Probe system using electro spray ionization technique. All chemicals used were of analytical grade procured from SD fine, Himedia, and E. Merck while standard drug of Famotidine was purchased from Yarrow Chem Products, Mumbai.

Synthesis of amino acid prodrug

Water soluble amino acid prodrug of Famotidine (FM1) was synthesized by using polar amino acid Glycine as promoety. Microwave irradiation technique was chosen, as the reaction time is short, less laborious, more yield and also it suits green chemistry. Imidazole was selected as the base due to its promotion ability, efficient microwave absorption and also it homogenizes the reaction mixture in dry medium [8].
Famotidine comprises of four reactive amine groups which may interfere in the prodrug synthesis. Hence except the intended amine group remaining amines will be protected for efficient synthesis of prodrugs. The tert-Butyloxycarbonyl (Boc) group is a commonly used protecting group for amines particularly in peptide synthesis. Formation of Boc amines (Boc protected amine groups) occurs in aqueous or anhydrous conditions on reaction of base and anhydride. After the reaction between Boc protected Famotidine and Glycine, the amino acid derivatives of Famotidine with Boc protection was deprotected completely within ten minutes using mineral acids like hydrochloric acid and dioxane as solvent.

**Boc protection of reactive amine groups in famotidine**

Accurately weighed amount of 2 mM of Famotidine was treated with 3.6 equivalents of di tertiary butyl di carbonate (Boc) group. The reaction mixture was dissolved in 100 ml of water and saturated solution of sodium bicarbonate was added. To the above solution 15 ml of tetrahydrofuran (THF) was added. The mixture was heated at 33°C for 45 min (fig. 2) and Boc protected compound was obtained [9].

**Reaction of amino acid with boc protected famotidine**

Accurately weighed quantity of 1 mM of Glycine amino acid, 2 mM of Boc protected Famotidine drug and 1 mM of imidazole was taken and physically ground by using mortar and pestle. Then the mixture was homogenized with ultra-homogenizer for 3 min and then the reaction mixture was exposed to microwave irradiation in a domestic microwave oven for 160 sec. Crude product was obtained [10] (fig. 3).

**Boc-deprotection**

The resultant Boc protected amino acid derivative of Famotidine was treated with 9 equivalents of 1M HCl and 5 ml of dioxane. The mixture was refluxed for 1 hr. The resulting product was filtered and the filtrate was washed several times with dioxane [11-13]. TLC analysis was performed to ensure the removal of Boc groups. The residue was dried weighed and the yield was calculated (fig. 4).

**Purification**

Synthesised product was purified by using column chromatography using silica gel as adsorbent and ethyl acetate/methanol/water (8:1.5:0.3 v/v/v) as mobile phase. The purified product was recrystallised by using methanol.

Further qualitative analysis of compound was performed by HPLC analysis using Acetonitrile: Water: Triethyl amine: Orthophosphoric acid (49:9.49:0.1: 0.1%v/v/v/v) at a detection wavelength of 280 nm. TLC analysis was performed to ensure purity of the compound (fig. 5).

**Spectral and thermal characterization**

Pressed pellet technique was adapted for FT-IR analysis. Drug admixed with KBr were made in to the disc and was analysed in the
spectral range of 4000 to 400 cm⁻¹ and IR spectrum was recorded. 1H, 13C NMR, Mass and DSC studies were carried out for the synthesized prodrug.

**Partition coefficient**

The partition coefficient of the product was determined in n-octanol/water system (10:10) by standard technique. Product (drug or prodrug) was accurately weighed (10 mg) and added to 10 ml of each n-octanol and aqueous phase. The mixture was shaken using a mechanical shaker for 24 h until equilibrium was reached. Phases were separated by separating funnel and aqueous phase was analyzed for the amount of product after appropriate dilution. Procedure was performed in triplicate [14].

\[
K_d = \frac{[\text{Solute}]_o}{[\text{Solute}]_{aq}} = \frac{C_o}{C_{aq}}
\]

Where \(K_d\) is partition coefficient

\(C_o\) = Concentration of solute distributed organic phase

\(C_{aq}\) = Concentration of solute distributed in aqueous phase

**Aqueous solubility**

Equilibrium solubility was determined by a “shake-flask” method [15]. The aqueous solubility of the compound was determined by adding an excess amount of a drug beyond its saturation limit in sealed conical flask containing 10 ml of water. This conical flask is placed in a mechanical shaker for 48 h (this duration was previously tested to be sufficient to reach equilibrium). The solvent was filtered through Whatmann filter paper No.42 and the portion of the filtrate was suitably diluted with water. Solutions were analyzed by using UV spectrophotometer at 281 nm, which was the absorption maxima and drug concentrations were calculated [16].

**Chemical hydrolysis study**

The rate of chemical hydrolysis of the prodrug was determined in Simulated Gastric Fluid (SGF, pH 1.2) and Simulated Intestinal Fluid (SIF, pH 7.4) at 37 °C. Solution of 10 mg of the synthesized prodrug was placed in dissolution basket containing 90 ml of SGF/SIF individually. An aliquot of 15 ml of this solution was withdrawn repeatedly and kept in test tubes maintained at 37±0.5 °C. At a definite interval of time (0.5, 1, 2 up to 8 h), an aliquot was withdrawn to different test tubes and was transferred to micro centrifuge tubes followed by addition of methanol to make up the volume. The tubes were placed in a freezing mixture in order to arrest further hydrolysis, followed by vortexing at high speed for 5 min. After vortexing, the tubes were centrifuged at high speed 3000rpm for 5 min. A 5 ml of clear supernatant obtained from each tube was measured on UV spectrophotometer for the amount of free drug released after the hydrolysis of prodrug in SGF and SIF at 281 nm [17].

**RESULTS AND DISCUSSION**

Amino acid prodrug of FM was synthesized by using microwave irradiation and its percentage yield was found to be 93.68%. TLC studies have shown the Rf values of FM and its amino acid prodrug to be 0.67 cm and 0.56 cm respectively, which ensures the formation of prodrug.

A photo microscopic image of FM and its amino acid prodrug was observed at 45X (fig. 6). Morphology of the synthesized amino acid prodrug was different from that of FM. Elemental analysis of synthesized prodrug shows C: 29.26, H: 4.42, N: 27.30, O: 15.59, S: 23.43.

The characteristic N-H stretch of amine in famotidine shifted in amino acid prodrug spectrum from 3401.8 cm⁻¹ to 3200.1 cm⁻¹. The carbonyl group formed in aminoacid prodrug and shown C=O stretch at 1581.9 cm⁻¹. Hence there is an interaction of N-H group of drug with C=O group of glycine resulting in formation of amide bond (-CONH) confirming the amino acid prodrug formation (fig.7 and fig. 8).

**Fig. 6: Microscopical characterization of compounds (a) FAM (b) FM1 amino acid prodrug of famotidine**

**Fig. 7: FT-IR spectra of famotidine**

**Fig. 8: FT-IR spectra of amino acid prodrug of famotidine**

1H-NMR of amino acid prodrug of FM (CD3OD) δ in ppm: 8.56 (s, 2H, NH₂ protons of sulfonamide), 7.34 (d, 1H, Heterocyclic protons), 4.45 (s, 2H, CH₂ protons), 3.92 (s, 2H, CH₂ protons), 3.62 (s, 2H, CH₃methylene), 2.61 (t, 2H, CH₂ protons), 1.89 (t, 2H, CH₃ protons), 1.53 (s, 2H, NH₂ protons). There is an increase in the number of
protons and signal for characteristic NH₂ group in FM1 compared to famotidine (fig. 9 and fig. 10). C[13]NMR of amino acid prodrug of FM (CD3OD) δ in ppm: 28.42, 31.93, 39.24, 50.11, 108.26, 139.14, 148.12, 152.44, 164.56, 170.11 (fig. 11).

From the IR, NMR & Mass studies, the molecular structure for synthesized amino acid prodrug was predicted and the proposed structure was confirmed to be an amino acid derivative of famotidine and the molecular formula was found to be C₁₀H₁₈N₈O₄S₃. DSC experiments were carried out to study the thermal behavior of the synthesized prodrug in relation to the individual drug. DSC study of FM shows an endothermic peak at 166.4 °C, while DSC study of amino acid prodrug shows sharp endothermic peaks at 148.47 °C respectively. Sharp endothermic values of synthesized prodrug and the individual drug agreed with the measured melting range in the melting point determination. The thermal profile of the synthesized prodrug was distinct, with a different melting transition from that of the individual drug. This indicates the formation of novel prodrug (fig. 14 and fig. 15).
Table 1: Kinetic data for the chemical hydrolysis of FM1 in SGF

<table>
<thead>
<tr>
<th>Amino acid Prodrug (FM1)</th>
<th>pH</th>
<th>Percent of Chemical hydrolysis</th>
<th>t₁/₂ (min)</th>
<th>Kobs (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>60 min</td>
<td>90 min</td>
<td>120 min</td>
</tr>
<tr>
<td>SGF</td>
<td>1.2</td>
<td>37.01</td>
<td>41.12</td>
<td>45.64</td>
</tr>
</tbody>
</table>

Table 2: Kinetic data for the chemical hydrolysis of FM1 in SIF

<table>
<thead>
<tr>
<th>Amino acid Prodrug (FM1)</th>
<th>pH</th>
<th>Percent of Chemical hydrolysis</th>
<th>t₁/₂ (min)</th>
<th>Kobs (min⁻¹)</th>
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<tr>
<td></td>
<td>1hr</td>
<td>2hr</td>
<td>3hr</td>
<td>4hr</td>
</tr>
<tr>
<td>SIF</td>
<td>7.4</td>
<td>6.11</td>
<td>28.45</td>
<td>32.33</td>
</tr>
</tbody>
</table>

Amino acid prodrug improves drug delivery of complex molecules [18]. Though earlier we reported synthesis of sulphoxide prodrug of famotidine [14], amino acid prodrug of famotidine was found to be better in terms of solubility enhancement as we observed 7.8 fold increment in solubility of famotidine whereas in sulphoxide prodrug it was found to be 6.7 folds. Moreover amino acids are biocompatible, easy to be metabolized in vivo compared to other organic groups used as promoieties. Microwave assisted synthesis is a vital technique in green chemistry. Green chemistry aims to remove hazards at the design stage. The practice of eliminating hazards from the beginning of the chemical design process has benefits for our health and the environment [19]. The synthesis adopted in the present study was microwave assisted irradiation technique which is an eco friendly method.

CONCLUSION

Novel amino acid prodrug of famotidine was successfully synthesized by using microwave irradiation technique with imidazole as base which suits green chemistry. Prepared prodrug exhibits good solubility, reasonable in vitro chemical stability in acidic and alkaline medium. Partition coefficient study ensures the increase in hydrophilicity of the synthesized prodrug. These properties make the novel amino acid prodrug of famotidine effective in treating gastro intestinal problems with enhanced bioavailability.

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CONFLICT OF INTERESTS

The authors have no conflict of interests to declare.

REFERENCES


