Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 10, 2015

Original Article

SYNTHESIS AND CHARACTERIZATION OF NOVEL AMINO ACID PRODRUG OF FAMOTIDINE

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Received: 30 Jul 2015 Revised and Accepted: 02 Sep 2015

ABSTRACT

Objective: Famotidine an H_2 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 promoiety 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 H_2 receptor blocker (fig. 1).

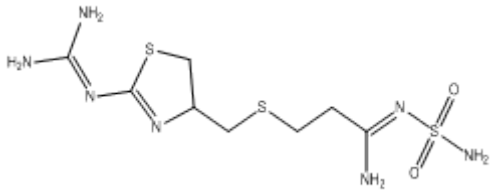

Fig. 1: Chemical structure of famotidine

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, $1H^1$ 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. Photo microscopic images were taken using an Olympus research microscope. Elemental analysis was performed using Carlo-Erba model 1108. The IR spectra were recorded in Agilent cary 630 FT-IR spectrophotometer and wave numbers are reported in cm^{-1}. The $1H^1$NMR spectra were obtained on a Bruker Avance-300 spectrometer (300 MHz) in deutrated methanol. Chemical shifts were recorded in ppm (d) 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 promoeity. 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].

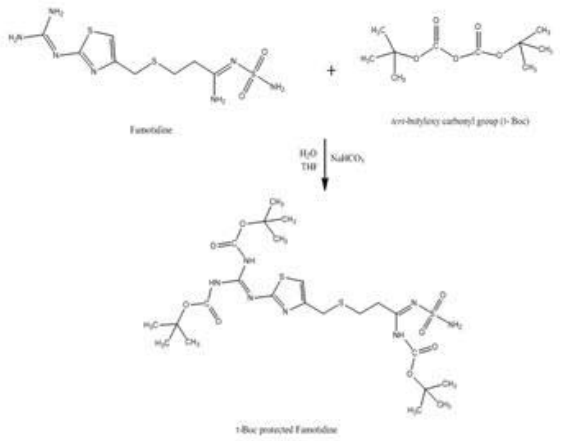

Fig. 2: t-boc protection of amine groups in famotidine

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).

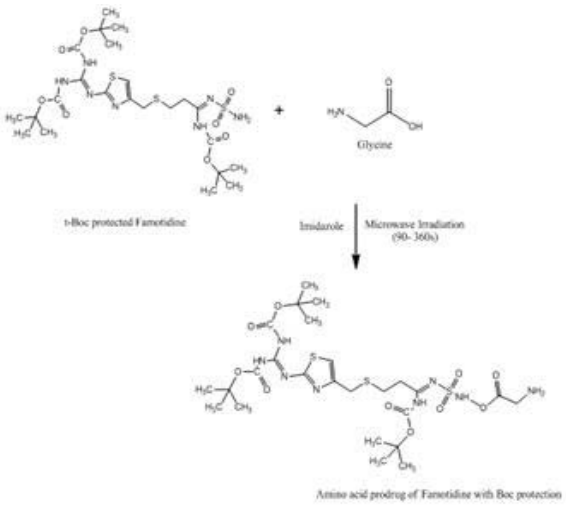

Fig. 3: Synthesis of amino acid prodrug of famotidine

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).

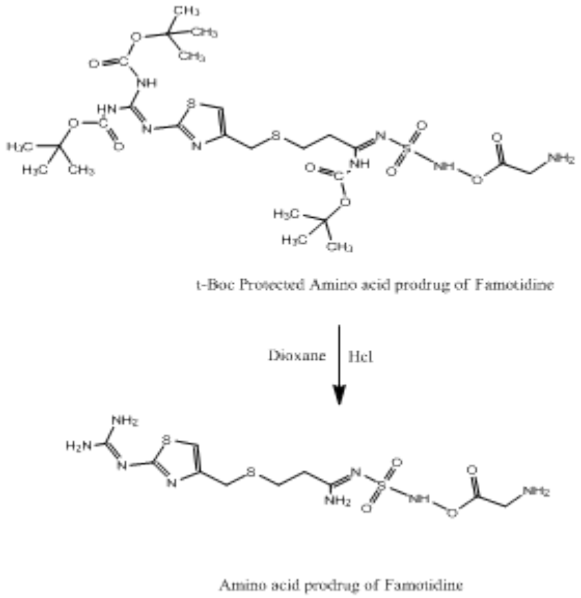

Fig. 4: t-boc deprotection

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: Orthophosporic acid (49.9: 49.9:0.1: 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).

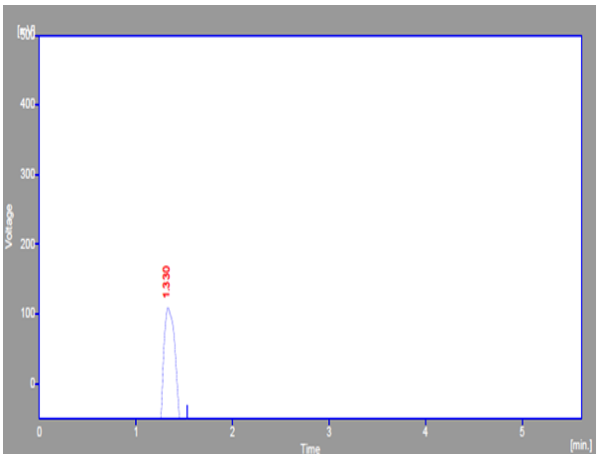

Fig. 5: HPLC spectrum of aminoacid prodrug FM1

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. $1H^1$, C[13] 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].

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

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 8h), 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.

(a) **(b)**

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

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).

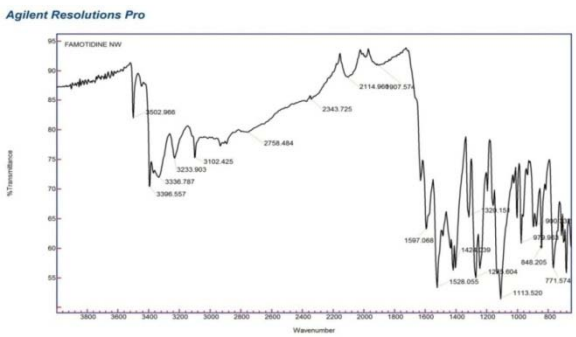

Fig. 7: FT-IR spectra of famotidine

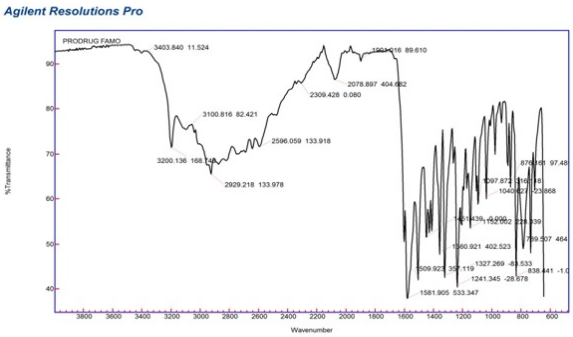

Fig. 8: FT-IR spectra of amino acid prodrug of famotidine

$1H^1$NMR of amino acid prodrug of FM (CD_3OD) δ in ppm: 8.56 (s, 2H, NH_2 protons of sulfonamide), 7.34 (d, 1H, Heterocyclic protons), 4.45 (s, 2H, CH_2 protons), 3.92 (s, 2H, CH_2 protons), 3.62 (s, 2H, CH_2methylene), 2.61 (t, 2H,-CH_2 protons), 1.89 (t, 2H,-CH_2protons), 1.53 (s, 2H, NH_2 protons). There is an increase in the number of

protons and signal for characteristic NH_2 group in FM1compared to famotidine (fig. 9 and fig. 10). C[13]NMR of aminoacid prodrug of FM (CD_3OD) 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).

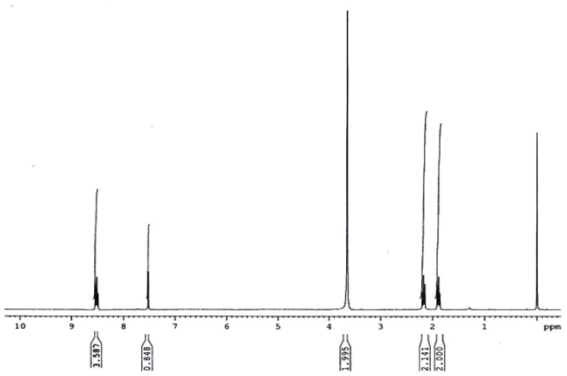

Fig. 9: NMR spectra of famotidine

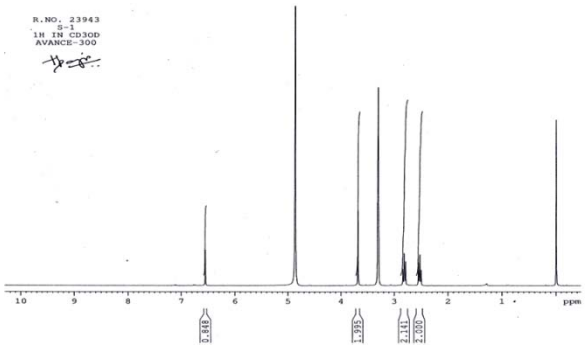

Fig. 10: NMR spectra of synthesized amino acid prodrug of Famotidine (FM1)

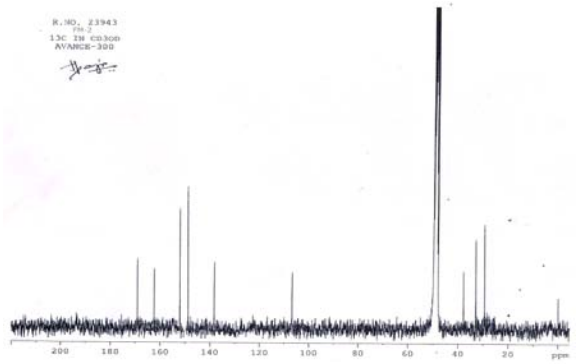

Fig. 11: ^{13}C NMR of synthesized amino acid prodrug of famotidine (FM 2)

Mass spectrum of FM represents characteristic parent ion peak at m/z = 336 (M-1 peak, $C_8H_{15}N_7O_2S_3$) and a base peak at m/z value = 80 (fig. 12). The mass spectrum of synthesized amino acid prodrug of FM exhibits parent ion peak at m/z = 410 (M^+peak, $C_{10}H_{18}N_8O_4S_3$), from parent peak, guanidine has been cleaved leaving N'-(N-(2-aminoacetoxy)sulfamoyl)-3-((2,3-dihydrothiazol-4-yl) methylthio) propanimidamide of m/z = 354, $C_9H_{15}N_5O_4S_3$. Further fragments like N-(2-aminoacetoxy) sulfonic amide (m/z = 155, $C_2H_6N_2O_4S$), (2,3-dihydrothiazol-4-yl) methanethiol (m/z = 131, $C_4H_7NS_2$), 2,3-dihydrothiazole (m/z = 87, C_3H_3NS) and guanidine (m/z = 60, CH_5N_3, base peak) were formed (fig. 13).

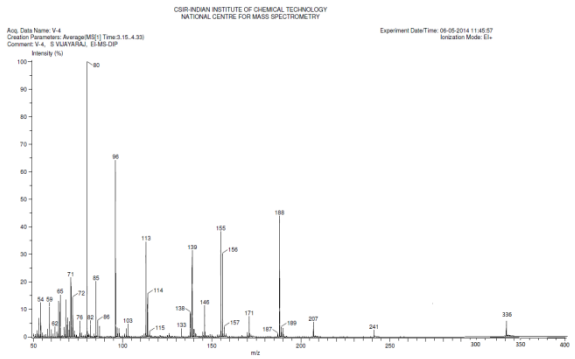

Fig. 12: Mass spectra of famotidine

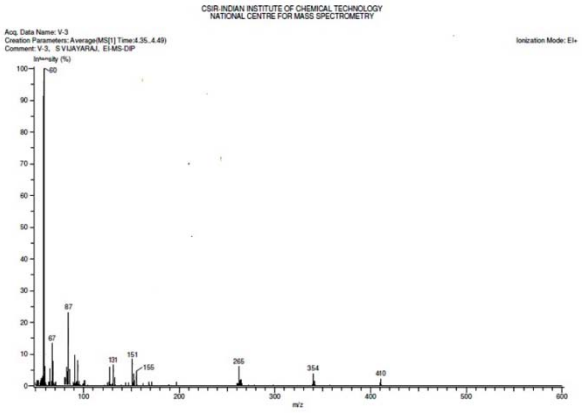

Fig. 13: Mass spectra of synthesized amino acid prodrug of famotidine (FM1)

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 $C_{10}H_{18}N_8O_4S_3$.

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).

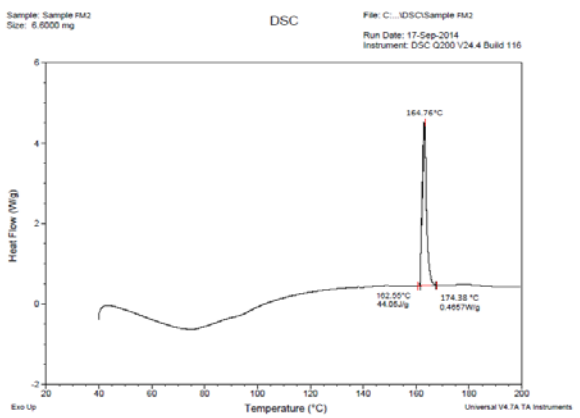

Fig. 14: DSC thermogram of famotidine

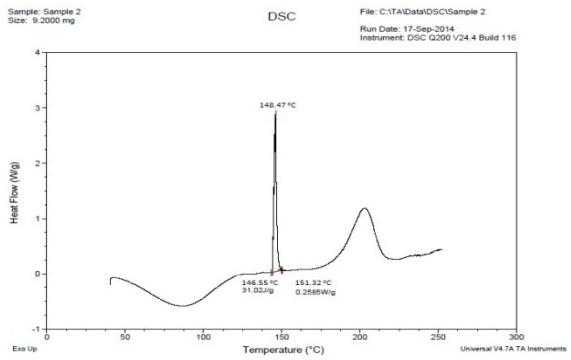

Fig. 15: DSC thermogram of amino acid prodrug of famotidine

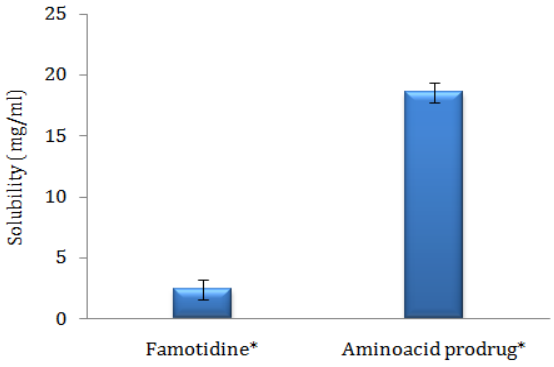

Fig. 16: Comparative aqueous solubility studies of drug and prodrug

Log P value was calculated from partition coefficient studies and was found to be-0.60 and-0.39 for drug and prodrug respectively. Decrease in log P values ensures increase in hydrophilic character of the synthesized prodrug.

Aqueous solubility of FM and amino acid prodrug was calculated in mg/ml and was found to be 2.386 and 18.565 for FM and prodrug of FM respectively. The solubility of prodrug FM1 enhanced by 7.8 folds when compared to pure drug (fig. 16)

*** Error bars represent standard deviation of the mean (n=3)**

Chemical hydrolysis study of amino acid prodrug in buffer solutions (Simulated Gastric Fluid, pH 1.2 and Simulated Intestinal Fluid, pH 7.4) at 37 °C was performed. The Rate of hydrolysis, Hydrolysis constant and $t_{½}$ of synthesized amino acid prodrug was found to be 92.45% and 98.52% at 300 min and 7hr, $6.81 \times 10^{-3}$and 3.28×10^{-3}, 102 and 204 min in SGF and SIF respectively (table 1 and table 2).

Table 1: Kinetic data for the chemical hydrolysis of FM1 in SGF

Amino acid Prodrug (FM1)	pH	Percent of Chemical hydrolysis							K_{obs} (min^{-1})	$t_{1/2}$ (min)
		30 min	60 min	90 min	120 min	150 min	210 min	300 min		
SGF	1.2	37.01	41.12	45.64	54.15	60.93	70.48	92.45	$6.81*10^{-3}$	102

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

Amino acid Prodrug (FM1)	pH	Percent of Chemical hydrolysis							K_{obs} (min^{-1})	$t_{1/2}$ (min)
		1hr	2hr	3hr	4hr	5hr	6hr	7hr		
SIF	7.4	6.11	28.45	32.33	49.15	60.23	91.41	98.52	$3.28*10^{-3}$	204

Amino acid prodrug improves drugability 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 promoeities. 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.

ACKNOWLEDGEMENT

The authors are thankful to Department of Science and Technology, India and IICT, Hyderabad for their financial assistance in spectral studies. They are Grateful to Dr. V. Murugesan for his assistance in spectral interpretation. They are also thankful to the management of Sree Vidyanikethan College of Pharmacy, Tirupathi for providing necessary laboratory facilities to carry out the work.

CONFLICT OF INTERESTS

The authors have no conflict of interests to declare

REFERENCES

1. Patel AR, Vavia PR. Preparation and evaluation of taste masked famotidine formulation using Drug/β-cyclodextrin/Polymer ternary complexation approach. AAPS Pharm Sci Tech 2008;9:544-50.
2. Hassan MA, Suleiman MS, Najib NM. Improvement of the *in vitro* dissolution characteristics of famotidine by inclusion in β-cyclodextrin. Int J Pharm 1990;58:19-24.
3. Dobetti L. Fast disintegrating tablets. US Patent No. B1 6,596,311, (Eurand International); 2003.
4. Brown D. Orally disintegrating tablets-taste over speed. Drug Delivery Technol 2003;3:58-61.
5. Seager HJ. Drug-delivery products and the Zydis fast-dissolving dosage form. Pharm Pharmacol 1998;50:375-82.
6. Bundgaard H. editor. Design of Prodrug. (Amsterdam): Elsevier; 1985.
7. Shargel L, Andrew Y, Susanna WP. Applied Biopharmaceutics and Pharmacokinetics. 6th ed. (USA): Mcgraw-Hill; 2012.
8. Balvinder SV, Kristiina MH, Krista L, Jarkko R. Amino acids as promoieties in prodrug design and development. Adv Drug Delivery Rev 2013;65:1370-85.
9. Zeng H, Li Y, Shao H. Simple and efficient method for *N*-Boc protection of amines using peg-400 as a reaction medium under mild conditions. Synth Commun 2012;42:25-32.

10. Khalafi NA, Mokhtari B, Rad MNS. Direct preparation of primary amides from carboxylic acids and urea using imidazole under microwave irradiation. Tetrahedron Lett 2003;44:7325-8.
11. Liu YS, Zhao C, Bergbreiter DE, Romo D. Simultaneous deprotection and purification of BOC amines based on ionic resin capture. J Org Chem 1998;63:3471-3.
12. Tang PW. Boric acid catalyzed amide formation from carboxylic acids and amines: N-Benzyl-4-Phenylbutyramide. Org Synth 2005;81:262.
13. Han G, Tamaki M, Hruby VJ. Fast, efficient and selective deprotection of the tert-butoxycarbonyl (Boc) group using HCl/dioxane (4 m). J Pept Res 2001;58:338-41.
14. Vijayaraj S, Omshanthi B, Anitha S, Sampathkumar KP**.** Synthesis and characterization of novel sulphoxide prodrug of famotidine**.** Indian J Pharm Educ 2014;48:35-44.
15. OECD. Guidelines for the Testing of Chemicals; 1981. p. 1-13.
16. Baka E, Comer JEA, Takacs NK. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochloro thiazide as model compound. J Pharm Biomed Anal 2008;46:335–41.
17. Mahdi MF, Alsaad HN. Design, Synthesis and hydrolytic behavior of mutual prodrugs of NSAIDs with Gabapentin using glycol spacers. Pharmaceuticals 2012;5:1080-91.
18. Balvinder V, Jarkko R. Amino acid prodrugs for oral delivery: challenges and opportunities. Ther Delivery 2011;2:959-62.
19. Anastas PT, Warner JC. Green Chemistry: Theory and Practice. New York: Oxford University Press; 1998.