



**International Journal of Biological
&
Pharmaceutical Research**
Journal homepage: www.ijbpr.com

IJBPR

SYNTHESIS AND CHARACTERIZATION OF NOVEL AMINO ACID PRODRUG OF GLICLAZIDE

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ABSTRACT

Diabetes is a metabolic disorder which has emerged as a major healthcare threat throughout the world. Gliclazide is an extensively used sulphonyl urea compound in the treatment of diabetes. As per BCS classification gliclazide is categorized under class II drug which do have poor solubility. Limited aqueous solubility is a major pharmacokinetic barrier in development of new drug entity for discovery focused pharmaceutical companies. Prodrugs are recognized and well known concept to overcome pharmacokinetic barriers like poor solubility. Solubility enhancement is an important parameter for increasing the bioavailability. Hence the objective of the investigation was to improve the aqueous solubility and in turn bioavailability by synthesis of novel amino acid prodrug of Gliclazide. Characterization of prepared prodrug was done by IR, NMR, Mass and DSC. *In vitro* chemical hydrolysis profiles revealed that the synthesized amino acid derivatives of Gliclazide are chemically stable in Simulated Gastric fluid pH 1.2 and simulated Intestinal fluid pH 7.2. Decrease in Log P value, 0.41 of amino acid prodrug compared to 2.52 of Gliclazide indicates the increase in hydrophilic property of synthesized amino acid derivatives of Gliclazide. *In silico* analysis was done for synthesized prodrugs by ChemBio3D ultra 11.0 software.

Key Words: Synthesis, Characterization, Amino acid prodrug, Gliclazide.

INTRODUCTION

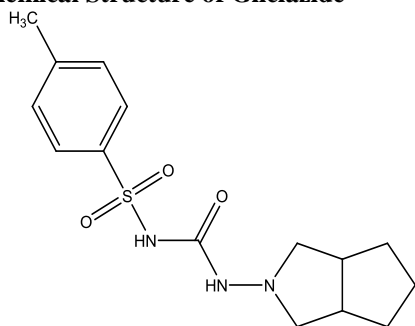
Diabetes mellitus (DM) is the most common endocrine disorder. It is estimated that more than 200 million people worldwide will have DM (Salim B, 2005). Gliclazide (GZ), chemically 1-[(4-methylbenzene) sulfonyl]-3-{octa-hydrocyclopenta [c] pyrrol-2-yl} urea (Fig-1) is a second generation sulphonyl urea which acts as a hypoglycemic agent (Pubchem compound, 2005). GZ belongs to Biopharmaceutical classification system (BCS) class -II drug which do have low aqueous solubility. Compounds with poor solubility influences both pharmacokinetic and pharmacodynamic properties of the

compound (Amidon GL *et al.*, 1995; Yu LX *et al.*, 1996). Although numerous strategies exist for enhancing the bioavailability of drugs with low aqueous solubility, the success of these approaches is not yet able to be guaranteed and is greatly dependent on the physical and chemical nature of the molecules being developed (Longqin Hu, 2004). 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 (Stella VJ, 2007; Kumpulainen H, 2007). Literature survey reveals that inclusion complex with β - cyclodextrin, solid dispersion techniques and pH change approach have been reported for enhancement of solubility of GZ (Roya T *et al.*, 2009; Ozkan Y, 2000; Shewale BD, 2008; Biswal S *et al.*, 2008). Oxime prodrug of gliclazide has been stated for

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Fig. 1. Chemical Structure of Gliclazide

increasing solubility and bioavailability of gliclazide (Vijayaraj S *et al.*, 2014). There are no reported methods for preparation of amino acid prodrug for solubility enhancement of GZ. The main purpose of present study is to increase aqueous solubility of GZ 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 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 desired concentration to the target site. The bioavailability of poorly water-soluble drug like GZ is a well-known difficulty to be coped with during drug delivery. The current study aims to resolve aforementioned issue by prodrug approach. Amino acid prodrug of GZ 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. Lipinski's rule of five, that is, molecular weight lower than 500 Da, number of donor hydrogen bonds less than 5, number of acceptor hydrogen bonds less than 10, and the lower than 5, has been adopted to check the suitability of synthesized drug for effective pharmacological activity.

MATERIALS AND METHODS

Instruments and Chemicals

Melting points were determined on a Differential Scanning Calorimeter (DSC) apparatus. The IR spectra were recorded in KBr discs on an FT-IR Bruker IFS 55 spectrophotometer and wave numbers are reported in cm^{-1} . The ^1H NMR spectra were obtained on a Bruker DRX-300 spectrometer (75 MHz) in CDCl_3 . Chemical shifts were recorded in ppm (d) relative to TMS as an internal standard. High resolution mass spectra were recorded on an Agilent 1100 series LC-MSD-TRAP-SL system using electro spray ionization technique. Aluminum sheets

coated with silica gel 60 F254 were used for column and TLC chromatography. All chemicals used were of analytical grade procured from SD fine, Himedia, and E. Merck while standard drug of Gliclazide was purchased from A to Z laboratories Limited Chennai. ChemBio 3D ultra 11.0 software was used to predict ADME characteristics of the drug.

Synthesis of Amino acid prodrug of Gliclazide

Water soluble amino acid prodrug of Gliclazide was synthesized by using polar amino acid tyrosine as promoiety. Microwave irradiation technique was preferred 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 (Nezhad A K *et al.*, 2003; Bobal P *et al.*, 2012).

Preparation and Purification

Accurately weighed quantity of 1 mM of tyrosine amino acid, 2 mM of Gliclazide drug and 1 mM of imidazole was taken and physically grinded 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 domestic microwave oven for 160 sec. Crude product was obtained. Fig 1. The Obtained crude product was then purified by running column chromatography using silica gel as stationary phase and ethyl acetate as eluent. The product was packed on top of the column and ethyl acetate was allowed run through the column. The product was separated and eluted out after 24hrs.

Recrystallisation

The product was placed the crystals in a clean round bottom flask and dissolved in 95% ethanol. To obtain a clear solution, the flask was heated until the crystals dissolve. Upon the addition of deionized water (15 mL water per gram of crude product recovered) the product was immediately crystallized. The mixture was cooled in an ice/water bath, and the recrystallized product was recovered via filtration using a Buchner funnel. The crystals were allowed to dry on filter paper. The recrystallised product was qualitatively analysed by HPLC using 70mL of Acetonitrile and 30mL of Water (HPLC grade) as mobile phase at the pH 3.0 and detection wavelength of 250nm. TLC analysis was performed to ensure purification fig. 4.

Chemical Scheme

Microscopical Characterisation

Morphology of the gliclazide and prepared amino acid prodrug were studied by optical microscopy at magnification of 45X.

Spectral and thermal characterization

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

Partition Coefficient

The partition coefficient of 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 each of n-octanol and aqueous phase. The mixture was shaken using mechanical shaker for 24 hrs until equilibrium was reached. Phases were separated by separating funnel and aqueous phase was analyzed for amount of product after appropriate dilution. Procedure was performed in triplicate (James S, 1989).

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

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 (Nezhad AK *et al.*, 2003). The aqueous solubility of compound was determined by adding excess amount of 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 hrs (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 273 nm, which was the absorption maxima and drug concentrations were calculated.

Chemical Hydrolysis Study

The rate of chemical hydrolysis of the prodrug was determined in Simulated Gastric Fluid (pH 1.2) and Simulated intestinal fluid (pH 7.4) at 37 °C. Solution of 10 mg of prodrug was prepared in 90 ml of SGF and 90ml of SIF individually. An aliquot of 15 ml of this solution was withdrawn repeatedly and kept in test tubes maintained at $37 \pm 0.5^\circ\text{C}$. At a definite interval of time (0.5, 1, 2 up to 8 h), an aliquot was withdrawn from 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 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 3000 rpm 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 273 nm (Mantyla A *et al.*, 2004; Bauer LA, 2008).

RESULTS AND DISCUSSION

Microscopical characterization shown irregular crystals of amino acid prodrug of gliclazide whereas GZ shown rectangular crystals (Figure 3). The FTIR spectrum of amino acid prodrug of Gliclazide ((Figure 3)) represents characteristic peaks at 1716.42 cm^{-1} & 1519.2 cm^{-1} (C=O ring stretch of NH bending), 3360.14 cm^{-1} (NH stretch), 3259.35 cm^{-1} (O-H stretch) (Figure 4).

The ^1H NMR of amino acid prodrug of GZ (DMSO) δ in ppm: 1.57 (m, 2H, Pyrrolidine protons), 2.42 (m, 4H, Pyrrolidine protons), 5.30 (s, 2H, -OH), 3.4 (s, 1H, -NOH), 6.8 (d, 1H, aromatic protons), 7.1 (d, 2H, aromatic protons) and 7.8 (d, 2H, aromatic protons) ((Figure 5).

The Mass spectrum of Gliclazide represents characteristic lines at $m/z = 339$ which is parent ion and also the base peak of gliclazide. The Mass spectrum of amino acid prodrug of Gliclazide represents characteristic lines at m/z value of 469 (M^+ peak). In mass spectra of G2 characteristic parent peak was at m/z value of 469 (M^+ peak). From the amino acid prodrug p- cresol group was cleaved leaving 3-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-1-tosylimidazo lidine-2, 4-dione of $m/z = 363$. Further 3-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-1-tosylimidazolidine-2, 4-dione was fragmented to 1-hydrosulfonyl-4-methylbenzene of $m/z = 156$, octahydrocyclopenta[c]pyrrole of $m/z = 111$ and 1-methylurea of $m/z = 74$ which showed the base peak of amino acid prodrug of GZ (Figure 6).

DSC experiments were carried out to study the thermal behavior of the synthesized prodrug in relation to the individual drug. DSC study of Gliclazide shows endothermic peak at 176.28°C , ((Figure 7) while DSC study of amino acid prodrug at 190.04°C ((Figure 8). The sharp endothermic values of synthesised prodrug and the individual drug agreed with the measured melting range in the melting point determination. The thermal profiles of synthesized prodrugs were distinct, with a different melting transition from that of individual drug. This indicates the formation of novel prodrug.

Log P value was calculated from Partition coefficient studies and was found to be 2.52 and 0.4024 for drug and prodrug respectively (Table 1). Aqueous solubility of GZ and amino acid prodrug was calculated in mg/ml and was found to be 1.012 and 154.8 for GZ and amino acid prodrug of GZ respectively ((Figure 9).

In Chemical hydrolysis study, rate of hydrolysis of amino acid prodrug in SGF and SIF was found to be 91.8% and 99.11% respectively at 37 °C in 240 min Table 2. Predicted physicochemical parameters of the synthesized amino acid prodrug of GZ falls within the acceptable values suggested in Lipinski's rule Table 3.

Table 1. Kinetic data for the chemical hydrolysis of G2 in SGF and SIF

Amino acid Prodrug	pH	Percent of Chemical hydrolysis								K_{obs} (min^{-1})	$t_{1/2}$ (min)
		30 min	60 min	90	120	150 min	180 min	210 min	240 min		
SGF	1.2	68.0	71.4	76.1	78.9	82.3	85.7	89.1	91.8	0.015	46.21
SIF	7.4	80.24	86.48	90.02	92.80	94.40	94.94	95.5	99.11	0.1038	6.6

Table 2. ADME predictions of synthesised amino acid prodrug of gliclazide

Sl. No	Parameters	Acceptable value	Predicted value for G2
1	Molar refractivity	40 to 130	102.51
2	Partition coefficient	-0.4 to +5.6	+ 3.92
3	Molecular weight	180 to 500	469.55
4	Number of atoms	20 to 70	60
5	Polar surface area	$\leq 140 \text{ \AA}^2$	98.23 \AA^2

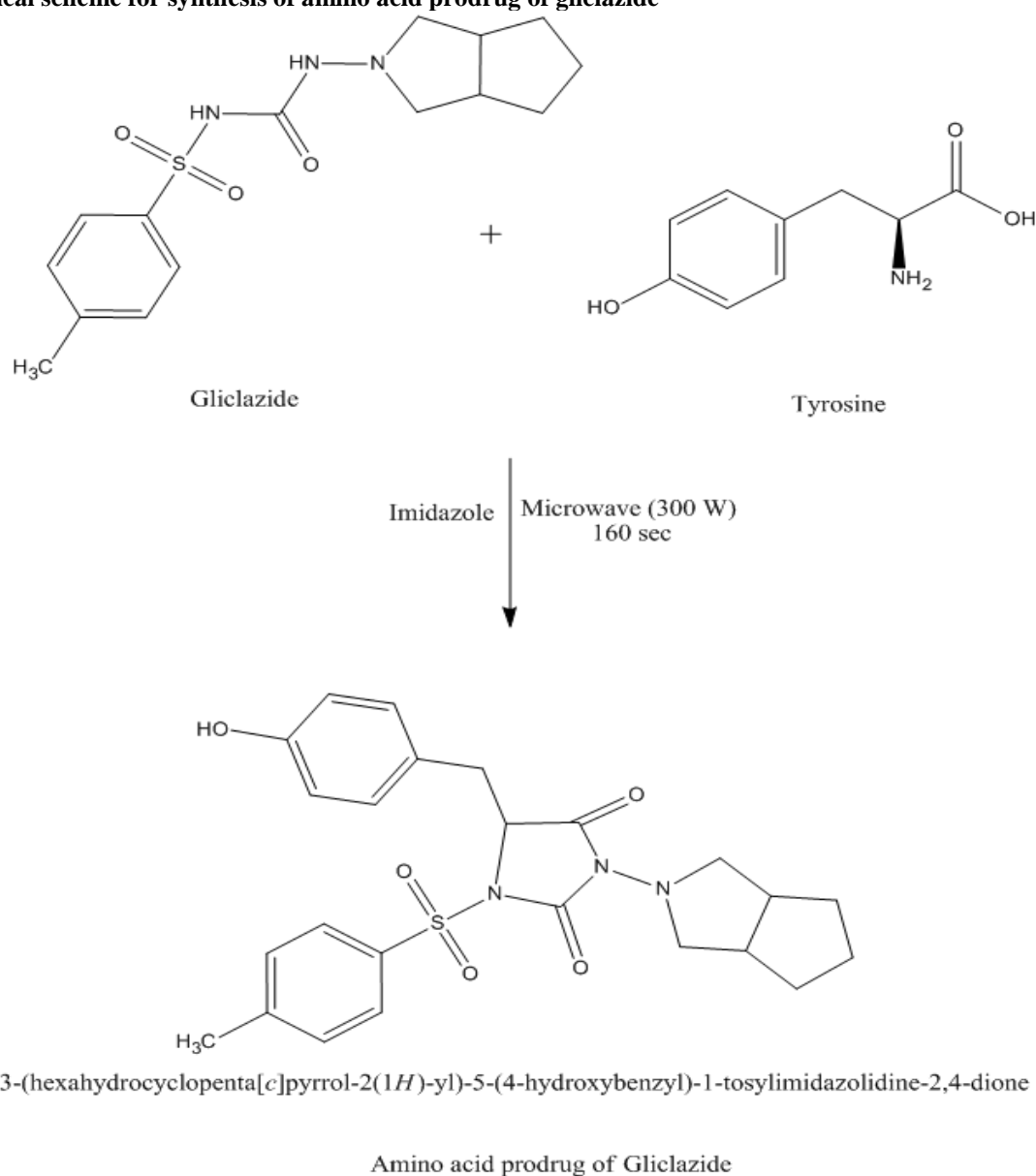
Fig. 2. Chemical scheme for synthesis of amino acid prodrug of gliclazide

Fig. 3. Optical Microscopy image of (I) Gliclazide (GZ) (II) Amino acid prodrug of gliclazide (Irregular crystals) at magnification of 45X

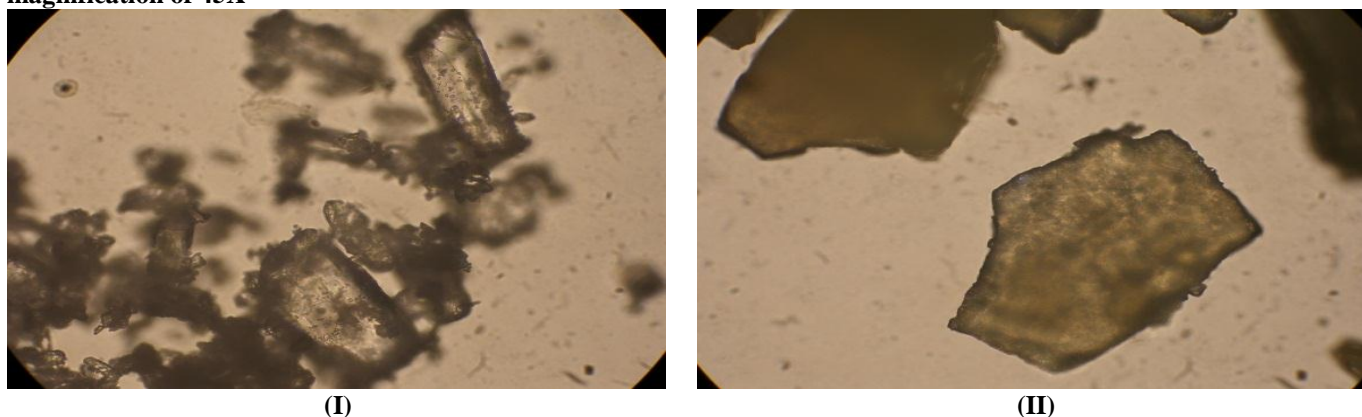


Fig. 4. FTIR spectrum of Gliclazide

Agilent Resolutions Pro

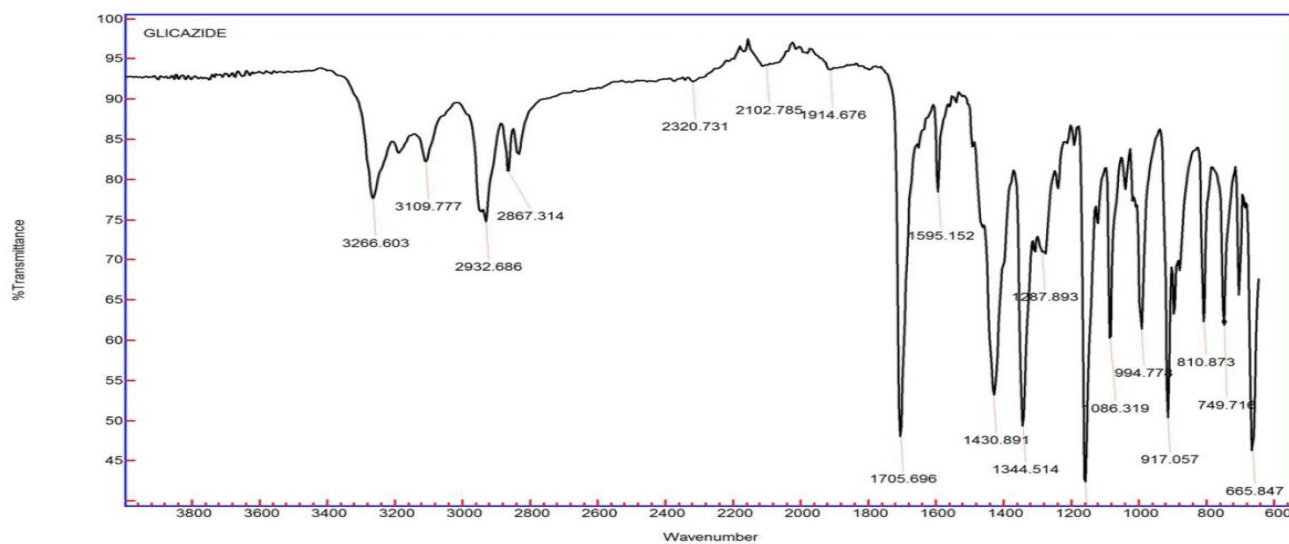


Fig. 5. FT-IR spectrum of synthesized Amino acid prodrug of Gliclazide (G2)

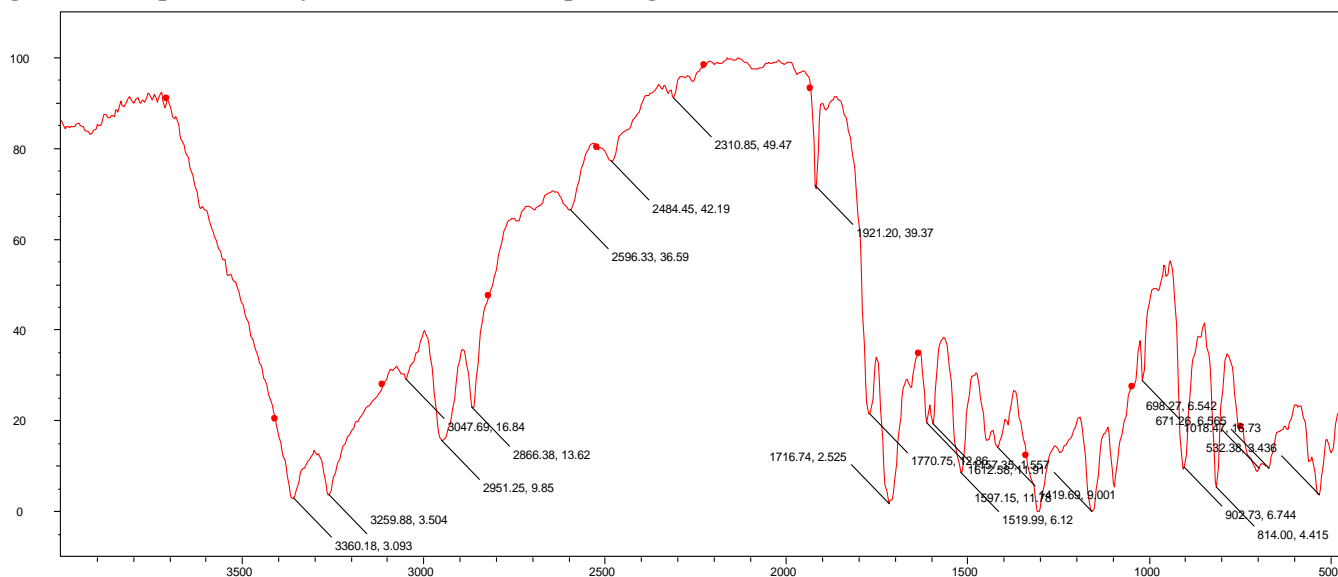


Fig. 6. NMR spectrum of synthesized amino acid prodrug (G2)

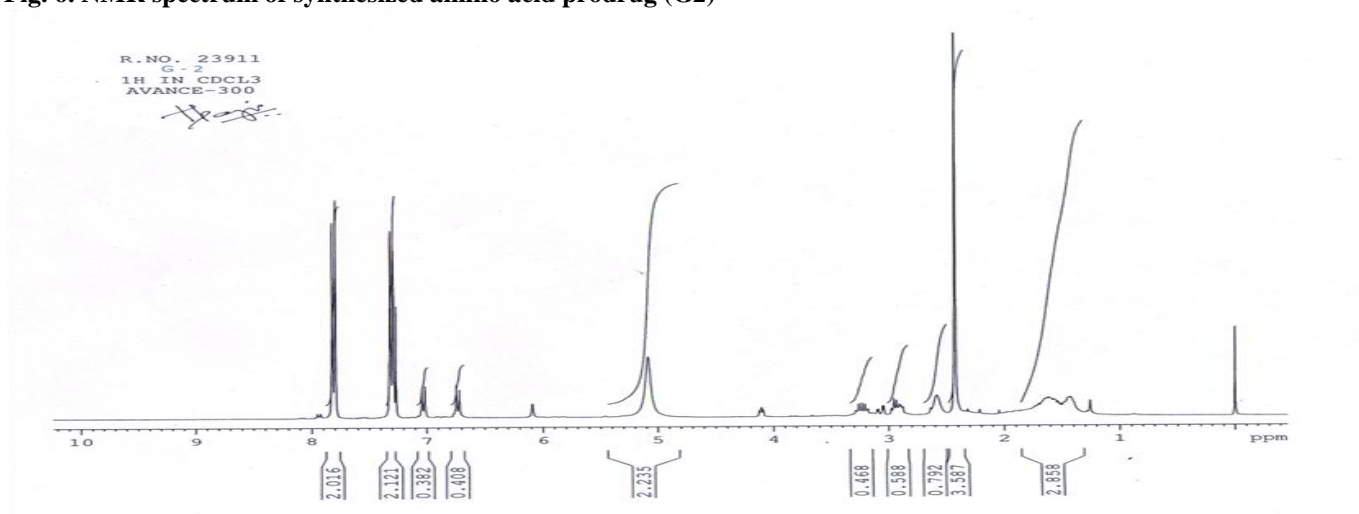


Fig. 7. Mass spectrum of Gliclazide

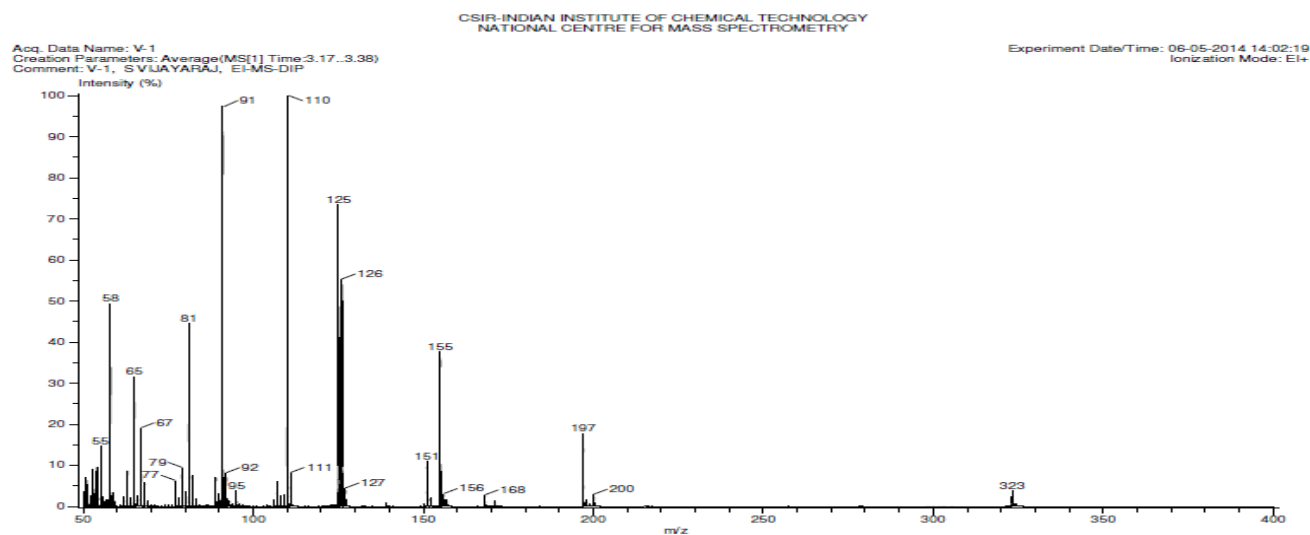


Fig. 8. Mass spectrum of synthesized Amino acid prodrug of Gliclazide

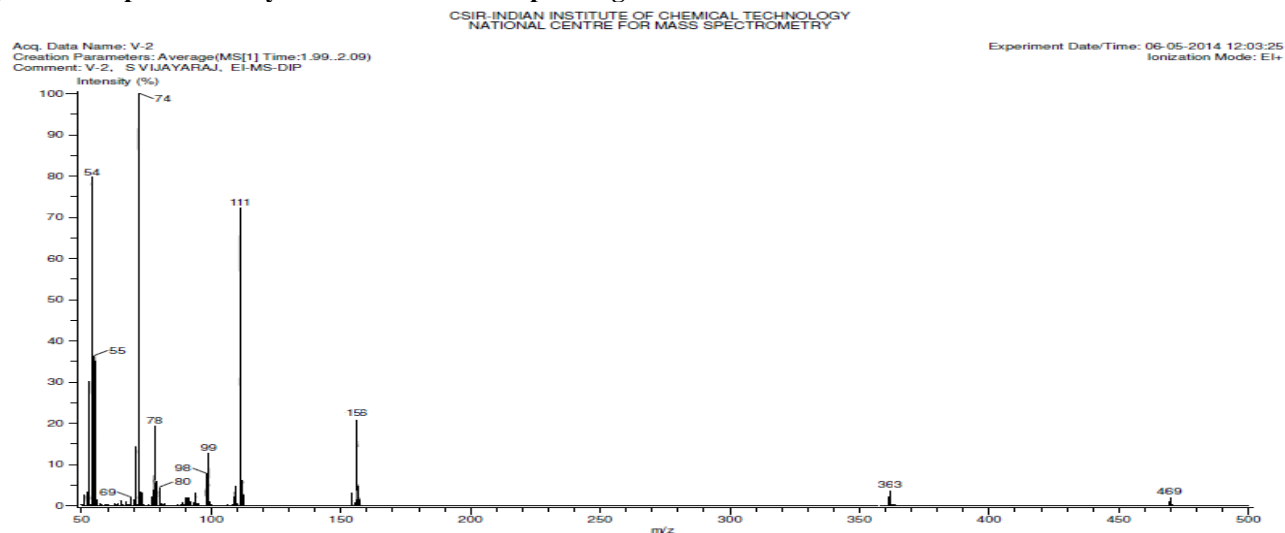
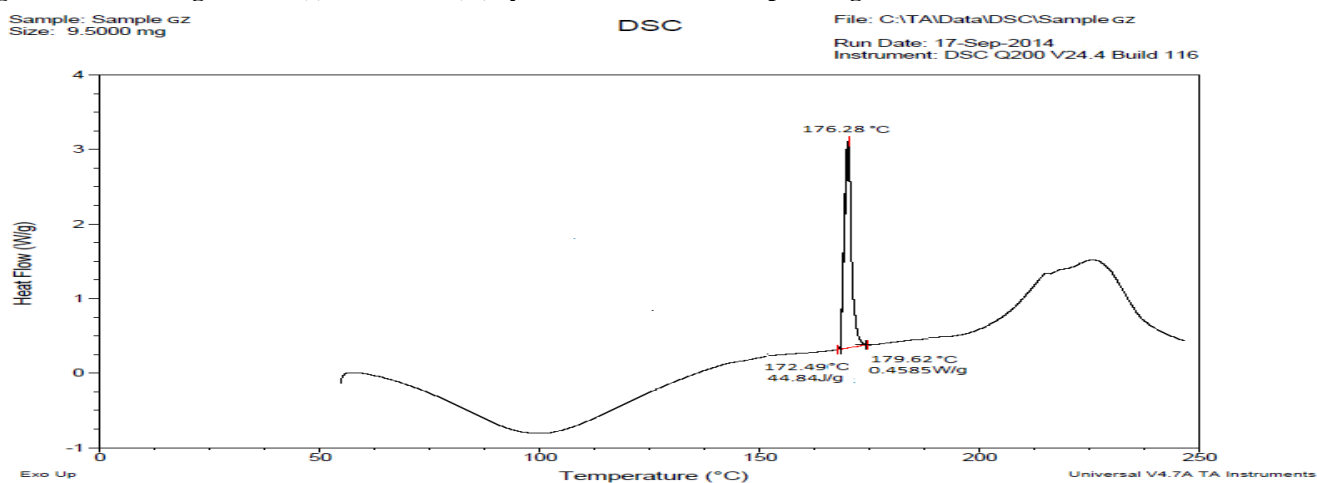
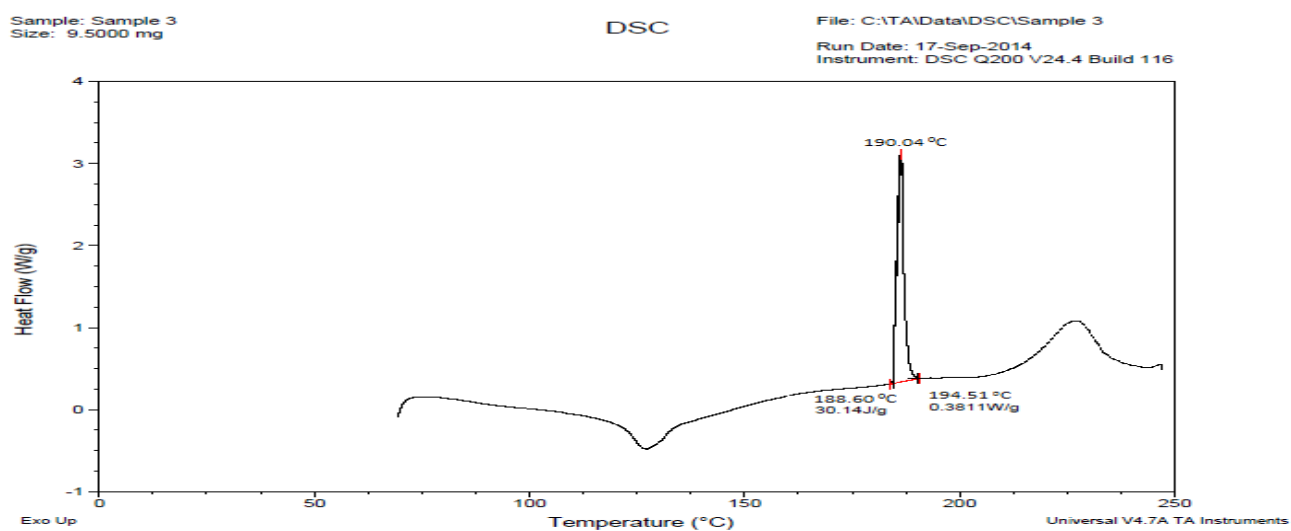


Fig. 9. DSC thermograms of (I) Gliclazide (II) synthesized amino acid prodrug

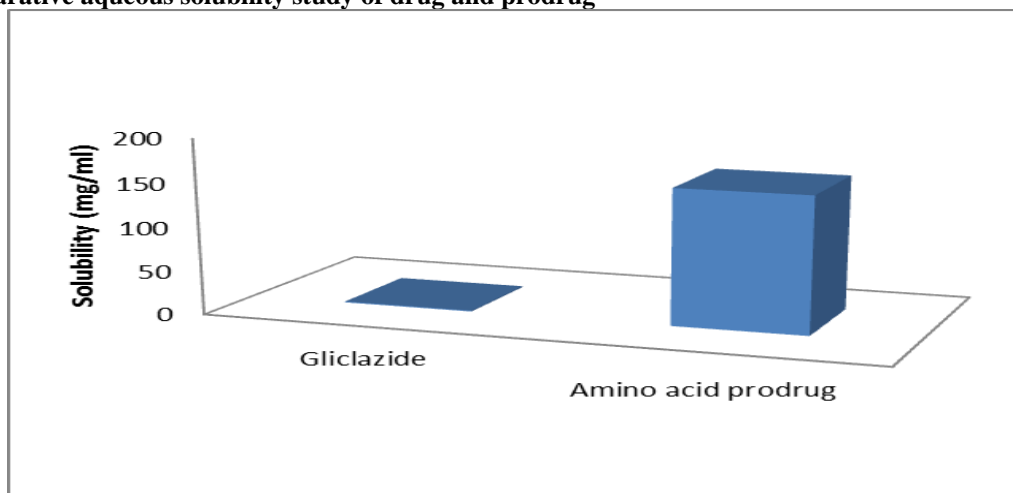


(I)



(II)

Fig. 10. Comparative aqueous solubility study of drug and prodrug



CONCLUSION

Novel amino acid prodrug of Gliclazide was successfully synthesized by using microwave irradiation technique with imidazole as base which suits green chemistry. The prepared prodrug exhibits good solubility, reasonable *invitro* chemical stability in acidic and alkaline medium. Partition coefficient studies ensure the increase in

hydrophilicity of the synthesized prodrug. ADME prediction studies shows synthesized amino acid prodrug may be formulated as an orally active compound with better bioavailability. These properties make the novel amino acid prodrug of gliclazide effective in treating diabetes.

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