Asian Journal of Chemistry; Vol. 26, No. 20 (2014), 6989-6992

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17642

Synthesis and Characterization of Novel Oxime Prodrug of Gliclizide

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Received: 21 March 2014; *Accepted*: 31 May 2014; *Published online*: 25 September 2014; AJC-16052

Diabetes has emerged as a major healthcare problem throughout the world. Gliclizide is a widely used sulphonyl urea compound to treat diabetes. Gliclizide is categorized under biopharmaceutical classification system class II drug which do have poor solubility. Drug molecules with limited aqueous solubility are becoming increasingly prevalent in the research and development portfolios of discovery focused pharmaceutical companies. Prodrugs are an established concept to overcome barriers like poor solubility. Aqueous solubility is an important parameter to enhance the bioavailability of drug. Hence the present study aims to enhance aqueous solubility and in turn bioavailability by synthesis of novel oxime prodrug of gliclizide. The prepared prodrug was characterized by IR, NMR, Mass and DSC. *in vitro* chemical hydrolysis profiles revealed that the synthesized oxime derivatives of gliclizide are chemically stable in simulated gastric fluid pH 1.2. Decrease in log P value indicates the increase in hydrophilic property of synthesized oxime derivatives of gliclizide.

Keywords: Synthesis, Characterization, Oxime Prodrug, Gliclizide.

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder. It is estimated that more than 200 million people worldwide have diabetes mellitus and by 2025 the amount may rise to 300 million[1]. Gliclizide (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[2]. Gliclizide 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. 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[3]. 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[4]. Literature survey reveals that pH change approach, inclusion complex with β-cyclodextrin, solid dispersion techniques and have been reported for enhancement of solubility of gliclizide[5-8]. There are no reported method for preparation of prodrug in solubility enhancement of gliclizide. A huge sum of money has been invested in new drug research and it will take around 15 years to develop a new chemical entity whereas prodrug strategy is aimed to enhance the effectiveness of existing drug hence it is economic and time saving approach. 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[9].The bioavailability of poorly water-soluble drug like Gliclizide is a well-known difficulty to be coped with during drug delivery. The current research aims to resolve aforementioned issue by prodrug approach. Oxime prodrug of gliclizide was prepared and the synthesized prodrug was investigated by FT-IR, $1H^1$ NMR, Mass and DSC studies. Aqueous solubility studies were performed to ensure the enhancement in solubility.

EXPERIMENTAL

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 wavenumbers are reported in cm^{-1}. The $1H^1$ NMR spectra were obtained on a Bruker DRX-300 spectrometer (75 MHz) in $CDCl_3$. Chemical shifts were recorded in ppm (δ) relative

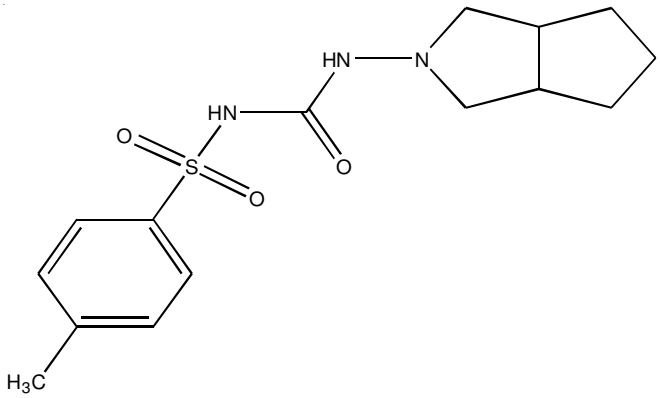

Fig. 1. Structure of gliclizide (GZ)

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 $60F_{254}$ were used for TLC chromatography. All chemicals used were of analytical grade procured from SD fine, Himedia and E. Merck while standard drug of Gliclizide was purchased from Yarrow Chem products Limited, Mumbai.

Synthesis of oxime prodrugs[10]

Preparation of N-[N-(hexahydrocyclopenta[c]pyrrol-2(1*H*)-yl)-N′-hydroxycarbamimidoyl]-4-methylbenzene sulfonamide: Accurately weighed quantity of 1.5 g of gliclizide was taken in a round bottom flask and is diluted with 8 mL of 95 % ethanol. To the above solution 0.6 g of hydroxylamine hydrochloride (97 % pure), along with 25 mL of deionized water was added. At the end 15 mL of a 10 % by weight aqueous solution of sodium hydroxide was added. The solution was refluxed at 100 °C. Completion of the reaction is marked by the formation of large crystals that float on the surface of the reaction mixture. The mixture was cooled in an ice/water bath, collected and then the recrystallised by using 95 % ethanol. Prepared prodrugs were physically chara-cterised by TLC method by using mixture of toluene: ethyl acetate in the ratio of 1:1 (v/v) as the mobile phase (Fig. 2).

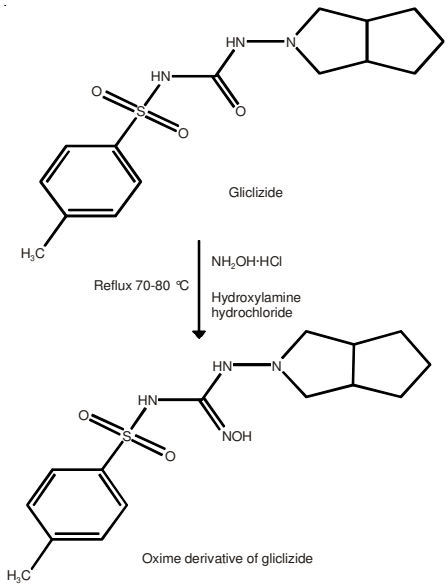

Fig. 2. Chemical scheme for synthesis of oxime prodrug of gliclizide

Microscopical characterization: Morphology of the gliclizide and prepared oxime 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 analyzed 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[11]: 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 h 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[13].

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

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[12]: Equilibrium solubility was determined by a "shake-flask" method[13]. 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 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 227 nm, which was the absorption maxima and drug concentrations were calculated.

Chemical hydrolysis study[13]: The rate of chemical hydrolysis of the prodrug was determined in Simulated Gastric Fluid (pH 1.2) at 37 °C. Solution of 10 mg of prodrug was prepared in 90 mL of SGF. An aliquot of 15 mL of this solution was withdrawn repeatedly and kept in test tubes maintained at 37 ± 0.5 ºC. 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 at 227 nm.

RESULTS AND DISCUSSION

TLC analysis reveals R_f values of gliclizide and its oxime prodrug to be 0.52 and 0.21 cm, respectively.

Microscopical characterization shown morphological difference between gliclizide and oxime prodrug of gliclizide (Fig. 3).

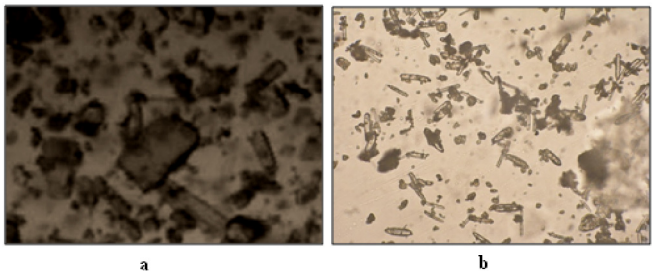

Fig. 3. Photomicroscopic Image of a) gliclizide b) oxime prodrug of gliclizide

The FTIR spectrum of oxime prodrug of gliclizide (Fig. 4) represents characteristic peaks at 1601.4 cm^{-1} and 1479.1 (C=N ring stretch of C=NOH), 1091.3 cm^{-1} (N-O stretch of NOH), 3661.0 cm^{-1} (O-H stretch) (Fig. 4).

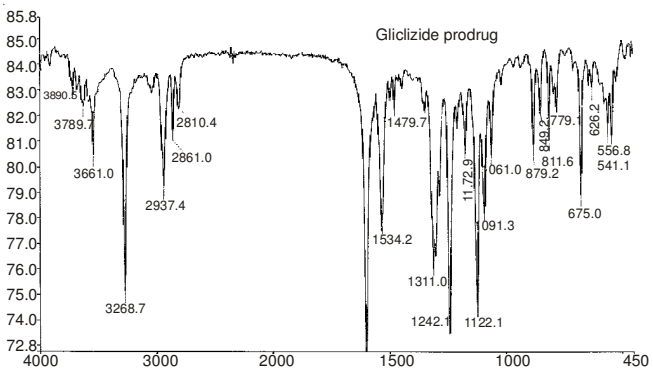

Fig. 4. FT-IR spectrum of oxime prodrug of Gliclizide

The ^1H NMR of oxime prodrug of gliclizide (DMSO) δ in ppm: 1.5(m, 3H, cyclopentane), 2.4 (m, 4H, Pyrrolidine protons), 2.90 (s, 3H, -CH_3), 3.4 (s, 1H, - NOH), 7.18 (d, 1H, aromatic protons), 7.65 (d, 1H, aromatic protons) (Fig. 5).

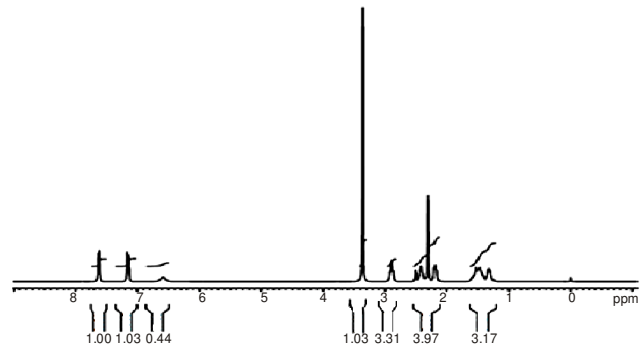

Fig. 5. ^1H NMR Spectra of oxime prodrug Gliclizide

The mass spectrum of Gliclizide represents characteristic lines at *m/z* 339 ($C_{15}H_{21}N_3O_3S$) which is parent ion and also the base peak of gliclizide. Mass spectrum of oxime prodrug of gliclizide represents characteristic lines at *m/z* 337 (M-2 parent ion peak, $C_{15}H_{19}N_3O_3S$). It has shown three fragmentation patterns. In the first fragmentation pattern, from oxime of gliclizide *m/z* 337, ($C_{15}H_{19}N_3O_3S$) sulphonyl group (SO_2) is fragmented apart giving the characteristic line at *m/z* 271 ($C_{15}H_{19}N_3O_2$). Two separate ring systems are formed namely methyl benzene of *m/z* 91 (C_7H_7) and azabicyclo carbamidoxime of *m/z* 181 ($C_8H_{15}N_4O$). From this azabicyclo carbamidoxime, the carbamidoxime gets fragmented and gives the characteristic line at *m/z* 73 (CH_3N_3O), which undergoes further fragmentation to give the characteristic line at *m/z* 60 ($C_9H_{19}N_2$, base peak). In the second fragmentation pattern, azabicyclo carbamidoxime fragmented in to azabicyclic amine ring of *m/z* 128 ($C_9H_{19}N_2$). Azabicyclic amine ring undergoes further fragmentation by the elimination of NH followed by C_2H_5N to form stable species of *m/z* 68 (C_5H_8). In the third fragmentation pattern, from methylbenzene sulphonyl compound *m/z* 155, ($C_7H_7SO_2^+$), (SO_2) is fragmented apart giving the characteristic line at *m/z* 91 ($C_7H_7^+$) of methyl benzene. Methyl benzene (parent tropylium ion) undergoes further fragmentation by eliminating C_2H_2 to produce stable simple tropylium ion of *m/z* 65 (C_5H_5) (Fig. 6).

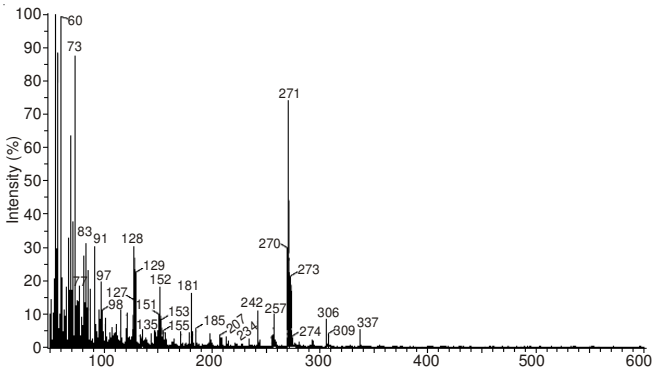

Fig. 6. Mass spectra of oxime prodrug of gliclizide

DSC experiments were carried out to study the thermal behaviour of the synthesised prodrug in relation to the individual drug. DSC study of gliclizide shows endothermic peak at 176.28 °C, (Fig. 7) while DSC study of oxime prodrug shows sharp endothermic peaks at 266.41 °C (Fig. 8), respectively. The sharp endothermic values of synthesized prodrug and the

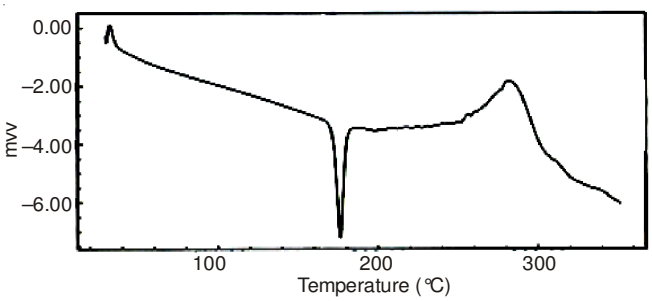

Fig. 7. DSC spectra of gliclizide

TABLE-1
PARTITION COEFFICIENT VALUES OF DRUG AND PRODRUGS

Compound	Concentration in octanol (a)	Concentration in water (b)	K (a/b)	Log P
Gliclizide	9.97	0.03	332.3	2.52
Oxime prodrug	9.74	0.26	37.4	1.57

TABLE-2
CHEMICAL HYDROLYSIS STUDY

Compound	pH	Hydrolysis (%)						K_{obs} (min^{-1})	$t_{1/2}$ (min)
		15 min	30 min	45 min	60 min	90 min	120 min		
Oxime prodrug	1.2	78.65	79.19	80.81	81.53	82.43	83.60	$8.52*10^{-3}$	81.33

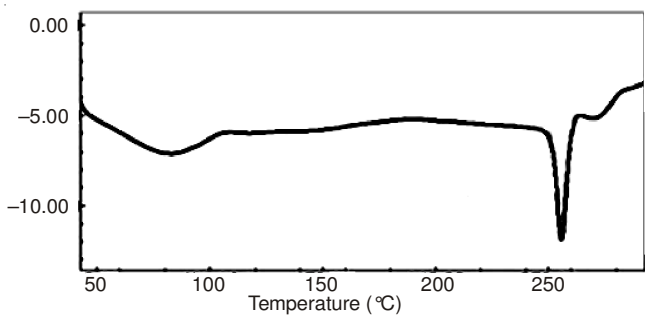

Fig. 8. DSC spectra of oxime prodrug of gliclizide

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 1.57 for drug and prodrug, respectively. Table-1

Aqueous solubility of gliclizide and oxime prodrug was calculated in mg/mL and was found to be 1.012 and 148.7 for gliclizide and prodrug of gliclizide, respectively (Fig. 9).

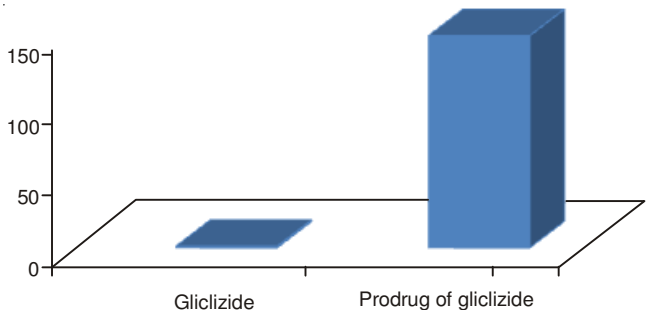

Fig. 9. Solubility of gliclizide and oxime prodrug of gliclizide

In chemical hydrolysis study, rate of hydrolysis of oxime prodrug in buffer solutions at 37 °C was found to be 83.6 % at 120 min (Table-2).

ACKNOWLEDGEMENTS

The authors are thankful to IICT, Hyderabad for their financial assistance in spectral studies. Thanks are also due to the Management of Sree Vidyanikethan College of Pharmacy, Tirupathi for providing necessary laboratory facilities to carry out the work.

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