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Research Article

NOVEL PRODRUG OF SIMVASTATIN AS CASCADE LATENTIATED INHIBITOR OF HMG-COA REDUCTASE

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ABSTRACT

Novel Oxime derivative of simvastatin as prodrug were designed, synthesized and evaluated as mutual prodrug with the aim to enhance aqueous solubility and thus bioavailability to improve therapeutic potency and retard the adverse effects. The synthesized mutual prodrug was confirmed by IR, ^{1}H NMR, mass spectroscopy (MS) and their purity was ascertained by TLC analyses. *In vitro* chemical hydrolysis profiles revealed that the synthesized oxime derivatives of simvastatin are chemically stable within a range of pH 3.0 to 7.4. Decrease in Log P value (1.196 of oxime prodrug as compared to 4.7 of simvastatin) indicates the increase in hydrophilic property of synthesized oxime derivatives of simvastatin.

Key Words: prodrugs, simvastatin, hypercholesterolemia, oxime.

INTRODUCTION

Simvastatin (SV) is a cholesterol-lowering agent derived synthetically as a fermentation product of *Aspergills terreus* and widely used as a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase to treat hypercholesterolemia[1, 2]. HMGCoA reductase catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol. Chemically SV is an inactive lactone, converted to 3', 5'-dihydrodiol SV in liver by cytochrome P-450 (CYP) 3A after oral administration[3]. However it is practically insoluble in water and poorly absorbed from the gastrointestinal (GI) tract[4]; it is very important to introduce effective methods to enhance the solubility, substantially enhancing its bioavailability[5-6]. Prodrugs are an established concept to overcome such barriers to drug's usefulness. The term prodrug was first introduced in 1958 by Adrien Albert to describe compounds that undergo biotransformation prior to eliciting their pharmacological effects[7-9]. According to his definition, prodrugs are "therapeutic agents which are inactive but are transformed into one or more active metabolites." A later definition by Bundgaard states that "By attachment of a pro-moiety to the active moiety, a prodrug is formed which is designed to overcome the barrier that hinders the optimal use of the active principle"[10-12]. Prodrugs can be classified according to two major criteria, namely, (a) chemical classes (carrier-linked prodrugs, bioprecursors (i.e., prodrugs lacking a promoiety), site-specific chemical delivery systems, macromolecular prodrugs, and drug-antibody conjugates) and (b) mechanism of activation (enzymatic versus nonenzymatic, activation by oxidation, reduction or hydrolysis, catabolic versus anabolic reactions)[13-16]. Such techniques has proven to be useful in defeating pharmaceutical and pharmacokinetic barriers in clinical drug application, including toxicity, chemical instability, low oral absorption, lack of site specificity, poor patient acceptance, low bioavailability, low aqueous solubility etc.[17-18].

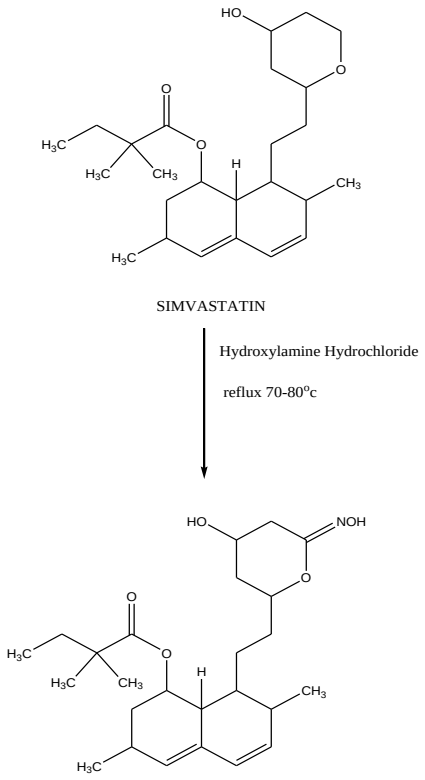
With respect to simvastatin, many efforts had been made to increase the aqueous solubility of the drug substantially leading to increase its bioavailability[19-20].

In present study, we synthesized and characterize oxime derivative of simvastatin as novel prodrug to increase aqueous solubility. C=NOH group in oxime compounds reported to increased water solubility. It was also reported that oxime compounds containing C=N-OH gr. gets oxidized by cytochrome P-450 with the formation of nitrogen oxides, corresponding C=O gr. containing parent compounds and NO which gets released from endothelial cells as a crucial regulator of arterial conductance[21-22].

MATERIALS AND METHODS

Melting points were determined by open capillary method on melting point apparatus and are uncorrected. Thin layer chromatography was carried out using silica gel-G on glass plate and spots were detected using iodine vapor and UV light. The progress of the reactions was monitored by TLC technique for formation of product. The intermediates and final compounds were purified through column chromatography using Silica gel 240-400 mesh as stationary phase. IR spectra were recorded as ATR technique on FTLA 2000 series FTIR (ABB USA) at SCOPE, Indore. ^{1}H NMR was recorded on Brucker (500 MHz) Advance NMR spectrophotometer using DMSO as solvent and chemical shifts were given in d ppm relative to tetra methyl silane (TMS). The mass spectra were recorded on Agilent 1100 series LC-MSD-TRAP-SL 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 Simvastatin was obtained from Ranbaxy laboratories Limited Dewas as gift sample.

Scheme I:



SIMVASTATIN OXIME

HPLC analysis was carried out to evaluate the *In vitro* chemical hydrolysis profiles of synthesized derivative using RP-HPLC system, consisting of a binary pump, a variable wavelength UV detector and a 20µL injection loop. The column used was a reversed phase C_{18} (250 X 4.6 mm; 5 µm particle) connected with a cartridge guard column. Quantification of the eluted compounds was obtained from the area under the peak measurements in relation to those of standards chromatographic conditions.

EXPERIMENTAL

Simvastatin (0.002 mol) was taken in round bottom flask containing 10 ml of ethanol. To this add a mixture Hydroxylamine Hydrochloride (0.002 mol) in water (2 ml). Mix the contents and add 0.002 mole of triethylamine with constant stirring (Scheme I). Reaction mixture was reflux for three days. Progress of the reaction mixture was checked through TLC. After completion of reaction, solvent was removed under reduce pressure and crude product was purified by silica gel column chromatography (petroleum ether/ ethyl acetate 3:1). The purity of the resulted compound was examined by TLC, Rf = 0.672; IR (FTIR) cm^{-1}: (C=C) 1456.5, (C=N) 1698.3, (C=O aromatic ester) 1714, (C-H stretch) 2928, (O-H stretch) 3648; ^{1}H NMR (DMSO): 6.87-7.38 δ (m, Ar-H), 2.093 δ (s, N-OH*-), 4.108 δ (-CH_2-CH_2*-) 1.45 δ (t-CH_3-CH_3*); MS, m/z: 434.9 (M^+H^+); Melting Point- 105- 107 ^{0} C; λ_{max}- 230.0 nm

Partition coefficient

The partition coefficient of product was determined in n-octanol/water system (10:10) by standard technique. Product 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.

Chemical Hydrolysis Study

The rates of chemical hydrolysis of the prodrugs were determined in 0.04M citric acid buffer (pH 3.0), 0.05M sodium acetate buffer (pH 5.0), and 0.185 M borate buffer (pH 7.4) at 37 °C. Solutions were prepared by dissolving an appropriate amount of prodrug (about 0.5 mg) in 5ml of preheated buffer, and then the solutions were filtered through a 0.45 µm Millipore and were placed in a water bath at 37 °C. At determined intervals, 200 µl of sample was withdrawn and 200 µl of acetonitrile was added to hinder hydrolysis during HPLC analysis. Pseudo-first-order half-life ($t_{1/2}$) for the hydrolysis of prodrug was calculated from the slope of the linear portion of the plotted logarithm of remaining prodrug versus time. Rates of hydrolysis of oxime prodrug in buffer solutions (pH 7.4, 5.0 and 3.0) at 37 °C (n = 1) is given in **Table** 1 and chromatogram of hydrolysis study in phosphate buffer pH 7.4 is given in **Figure** 1.

Table 1: Half-life of oxime prodrug in buffer solutions (pH 7.4, 5.0 and 3.0) at 37 °C (n = 1).

Compound	$t_{1/2}$		
	Phosphate buffer pH 7.4	Acetate buffer pH 5.0	Citric acid buffer pH 3.0
oxime prodrug	97 mins	158 mins	223 mins

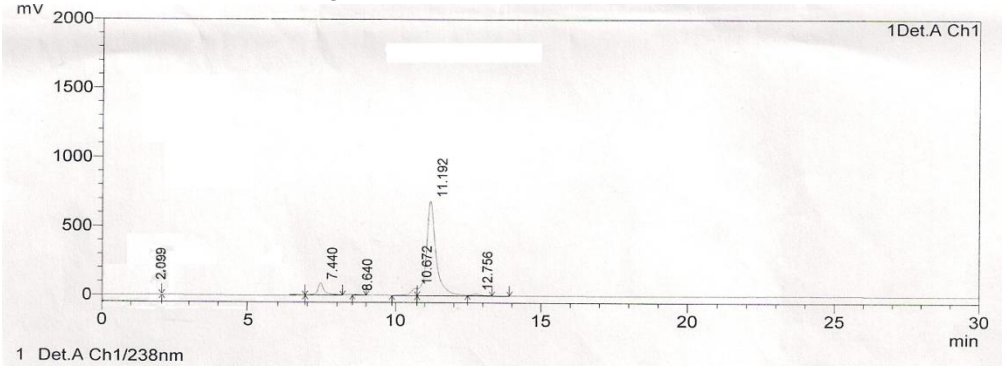


Figure 1: Chromatogram of hydrolysis study of simvastatin oxime prodrug at pH 7.4.

RESULT AND DISCUSSION

Modification of Simvastatin into prodrug (Oxime derivatives) may lead to enhance aqueous solubility and thus bioavailability. The formation of oxime derivatives was confirmed by means of a variety of techniques including FTIR, 1H NMR, mass analysis and TLC.

The TLC of oxime derivatives was performed in solvent system of petroleum ether: ethyl acetate mixture (3:2). TLC was visualized in iodine chamber. Retention factor (R_f) of oxime prodrug is found to be 0.672, which shows non polar nature in solvent system (Pet. Ether: Ethyl Acetate, 3:2) compared to that of simvastatin (R_f 0.41) in same solvent system.

Functional group modification was studied through IR spectra. Peak for 6-membered lactone ring is 1735-1770 cm^{-1} and carbonyl stretching in lactone ring is at 1740 cm^{-1}. In the FTIR spectrum the absorption band obtained at 1740 cm^{-1} as sharp and strong peak is attributed to the carbonyl groups of lactone ring of simvastatin. For oxime prodrug disappearance of carbonyl group peak at 1740 cm^{-1} indicates synthesis of derivative.

Mass spectrum of oxime prodrug exhibited [M/Z, $M+H]^+$ = 434.9. Calculated Molecular weight of compound is 433.58.

NMR spectroscopy was carried out on Bruker 500 MHz spectrometer. Data obtained in DMSO is as follows ^{1}H NMR (DMSO): 6.87-7.38 δ (m, Ar-H), 2.093 δ (s, N-OH*-), 4.108 δ (-CH_2-CH_2*-) 1.45 δ (t-CH_3-CH_3*).

Melting point was found to be in range of 105-107 ^{0}C which is decreased when compared with that of simvastatin (135- 138 ^{0}C). λ_{max} of compound is found to be 230 nm, which is also found to be decreased compared to that of simvastatin (238 nm). It can be due to increase in conjugation (C=N-OH). These characterization data suggest that compound was synthesized.

Partition coefficient is important parameter to assess lipophilicity and hydrophilicity of compounds. Log P value of synthesized compound was determined using n-Octanol and distilled water through standard procedure. Log P value of oxime prodrug was found to be 1.196, which is decreased from simvastatin having Log P value 4.7. This change in Log P value shows that synthesized compound has increased hydrophilicity over that of simvastatin.

Chemical Hydrolysis study of synthesized compound was done to determine conversion of prodrug into drug in buffer solution (phosphate buffer pH 7.4). After 120 mins 39.7% of drug was recovered from synthesized compound. This study revealed that prodrug is hydrolyzed and converted back into drug.

HMG CoA reductase inhibitors catalyses the conversion of HMG CoA to mevalonate, the rate limiting step in cholesterol synthesis. Competitive inhibition of this enzyme by statin's decrease hepatocyte cholesterol synthesis. All statin's are relatively

hepatoselective with respect to inhibition of HMG-CoA reductase because majority of cholesterol production occur in liver. The mechanism contributing this hepatoselective effect is governed by solubility profile of statin's. For lipophilic statin's, passive diffusion through hepatocyte cell membrane is responsible for uptake; while for hydrophilic statin's, uptake is through active carrier mediated process through organic anion transporter. Lipophilicity increases hepatic shunting but also results in ready passage of drugs into non hepatic cells and thus decreases bioavailability of the drug and increase toxicity (inhibition of smooth cell proliferation causing myopathy).

CONCLUSION

Novel water soluble Oxime prodrug of simvastatin was synthesized by using hydroxylamine hydrochloride by using triethylamine as base. Prodrug significantly increases the water solubility and release corresponding drug in aqueous buffer. Prodrug also had chemical stability over a pH range of 3.0- 7.4. Decrease in Log P value indicates that hydrophilicity has been increased. These properties make the novel oxime prodrug promising for treatment of hypercholesterolemia with greater efficacy and reduced toxicity. Moreover, the novel oxime promoiety is very useful for improving the aqueous solubility of other drugs that have ketone groups.

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