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Physicochemical properties of a nonpeptide cyclic urea HIV protease inhibitor (DMP 323)[1]

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Abstract

DMP 323 ([4R-(4α, 5α, 6β, 7β)]-hexahydro-5,6-bis(hydroxy)-1,3-bis ([(4-hydroxymethyl)phenyl]methyl)-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one), a potent inhibitor of HIV protease and HIV replication, is a white irregular-shaped nonhygroscopic crystalline material. Differential scanning calorimetry revealed a single melt peak at 195.7°C. DMP 323 was practically insoluble in water at 10 μg/ml (pH 8.1) at 25°C. The aqueous solubility was unaffected by changes in pH. The logarithm of the solubility of DMP 323 is a linear function of the percentage of water miscible cosolvents. The highest solubility values of DMP 323 were 272 mg/ml in 95% (v/v) ethanol in water, 160 mg/ml in propylene glycol, 144 mg/ml in polyethylene glycol 400, 65.5 mg/ml in 70% (w/w) polyethylene glycol 1450 in water, 64.2 mg/ml in polyoxyethylene sorbitan monooleate, and 1.61 mg/ml in glycerin. DMP 323 was stable as a function of pH with no loss observed at pH 3, 5, 7, 9 and 12.7 in aqueous buffers containing 5% (v/v) methanol after eight weeks at 40°C. In polyethylene glycol 400 solutions, the degradation of DMP 323 was approximated with apparent first order kinetics at elevated temperatures. Butylated hydroxytoluene and butylated hydroxyanisole were effective antioxidants in reducing the degradation in polyethylene glycol 400 solutions while citric acid afforded no protection from the degradation. Argon flushing was effective at reducing the oxidative degradation in polyethylene glycol 400 solutions stored at room temperature. Consistent with the oxidative degradation, DMP 323 degraded to the mono- and dibenzaldehyde, the monobenzoic acid, and the monobenzaldehyde monobenzoic acid derivatives.

Keywords: DMP 323; Physicochemical properties; HIV protease inhibitor; Solution stability; Solubility behavior

1. Introduction

DMP 323 ([4R-(4α, 5α, 6β, 7β)]-hexahydro-5,6-bis(hydroxy)-1,3-bis ([(4-hydroxymethyl)phenyl]-methyl)-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-

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2-one) is a potent, oral, nonpeptide cyclic urea that inhibits HIV protease (Lam et al., 1994). By inhibiting HIV protease, the cleavage of the HIV gag polypeptide into ultimately active mature forms is inhibited. Loss of HIV protease function has been shown to block the production of infectious virus. DMP 323 was designed based on the three dimensional structure of the protease and inhibitor complex.

DMP 323 possesses several unique structural features that enable specific and potent binding to the symmetrical HIV protease dimer (Fig. 1). The structural features include a cyclic urea that acts as a mimic for the structural water of the enzyme permitting hydrogen-bonding to the isoleucine residues (Grzesiek et al., 1994), a diol that hydrogen bonds to the aspartic acid residues, and a stereochemistry that optimizes drug protein interactions (Lam et al., 1994).

As part of the development of an oral solution dosage form, the thermal properties, hygroscopicity, solubility behavior, and solution stability of DMP 323 were investigated. The objectives of this study were to determine the thermal behavior and water uptake pattern as a function of controlled humidity, to investigate the solubility profile in a variety of solvent systems, and to evaluate the aqueous and cosolvent solution stability of DMP 323.

2. Materials and methods

2.1. Materials

DMP 323 (lot XM323-12, empirical formula $C_{35}H_{38}N_2O_5$, molecular weight (MW) of 566.7) was used as received and prepared by Viral Diseases Research at the Experimental Station, Wilmington, Delaware. Ethanol (Quantum Chemical), polyoxyethylene cholesterol (Amerchol Corporation) and hydroxypropyl-β-cyclodextrin (Aldrich Chemical) were used as received. Polyethylene glycol 1450, propylene glycol, polyethylene glycol 400, polyoxyethylene sorbitan monooleate, polyethylenepolypropylene glycol and citric acid were obtained from Spectrum Chemical and were used as received. Glycerin and sodium lauryl sulfate were obtained from J.T. Baker and were used as received. Hexadecyltrimethylammonium bromide, dioctylsulfosuccinate, nicotinamide, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, butylated hydroxytoluene and butylated hydroxyanisole were obtained from Sigma Chemical and were used as received. The water was house-deionized water that was passed through a Nanopure II ion-exchange cartridge system resulting in a specific resistance of greater than 17 MΩ cm. All solvents were HPLC grade. All other reagents were of analytical grade.

2.2. Thermal analysis

The thermal properties of DMP 323 were characterized with hot stage microscopy (Hot Stage FP82 and Central Processor FP80, Mettler), differential scanning calorimetry (DSC 910, TA Instruments) and thermogravimetric analysis (TGA 2950, TA Instruments) with data analysis via a thermal analyzer (Analyzer 2100, TA Instruments). Heating rates of 5 or 10°C/min were employed over a temperature range of 25–220°C for hot stage microscopy and DSC and 25–160°C for TGA.

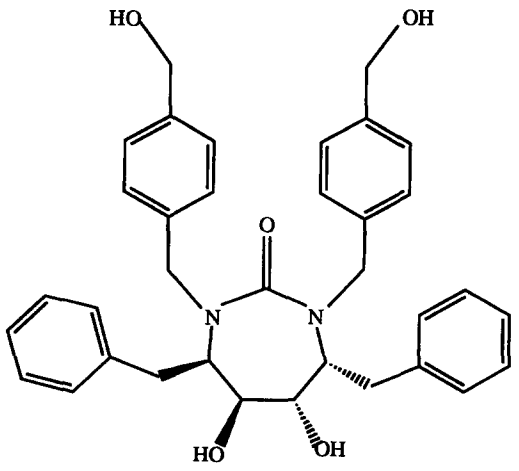

Fig. 1. The chemical structure of DMP 323.

2.3. Hygroscopicity

The water uptake of DMP 323 was followed with coulometric analysis (Coulometer 684 KF, Metrohm) of the drug substance after incubation at 0, 58, 85 and 100% relative humidity (RH). The humidity was maintained in sealed chambers (Dry Keeper, Samplatec Corporation) with a layer of a saturated aqueous salt solution in contact with excess NaBr or KCl for 58 or 85% RH, respectively. For 0 and 100% RH, lab desiccators were employed with the bases filled with Drierite® or water, respectively.

2.4. Solubility determination

Solubility studies were carried out by placing excess DMP 323 into a suitable container with the appropriate solvent. To examine the effect of pH on the aqueous solubility, varying amounts of either hydrochloric acid or sodium hydroxide were added to deionized water to adjust the pH. The suspensions were rotated end-to-end for at least 48 h at room temperature ($\approx$ 24°C). Preliminary experiments indicated that 3 h provided sufficient time to reach equilibrium. The suspension was passed through a 0.45 μm filter (Acrodisc® LC13 PVDF, Gelman Sciences) with the first portion discarded to ensure saturation of the filter. An aliquot of the filtrate was diluted and analyzed by an high performance liquid chromatography (HPLC) assay and the remainder of the filtrate was employed for pH determination.

2.5. Solution kinetics

DMP 323 was weighed into a suitable container and sufficient cosolvent was added to result in a 1 mg/ml concentration. To examine the effect of pH on the stability of DMP 323, buffers were employed that contained a minimal amount of methanol, 5% (v/v), such that facile solubilization was permitted at 10 μg/ml and detection of DMP 323 was feasible as degradation proceeded. The buffer or cosolvent was typically added at room temperature since the samples attained the desired temperature after placement into the stability oven (Model LDX1-42, Despatch Industries, Inc.). All solutions were prepared in triplicate. The solution was pipetted into 2 ml glass vials (Serum vials 621130-2, Kimble Glass), sealed with teflon-faced stoppers (4416-50, West Company), and placed upright into cardboard storage boxes to protect the compound from light. At appropriate intervals, samples were removed from the oven, cooled to room temperature, and diluted with mobile phase. In addition, the effect of argon head space flushing and various antioxidants was examined. The percent remaining from the initial values was determined at various time intervals by HPLC assay.

2.6. Chromatographic method

The concentration of DMP 323 was measured with an isocratic HPLC method. Separation was performed on a 4.6 mm × 25 cm reversed phase Zorbax® C_8 column (Mac Mod Chromatography) with the temperature maintained at 45°C (Column Heater Module and Temperature Control Module, Waters Chromatography). The mobile phase was composed of methanol:water (65:35) with 0.01 M acetic acid. A flow rate of 1.0 ml/min was employed (Controller, Model 680 and 2 HPLC Pumps, Model 510, Waters Chromatography). Ultraviolet detection was employed at 220 nm (HP 1050 Series Variable Wavelength Detector, Hewlett-Packard). Data acquisition was completed with a VAX-based program that calculated the sample concentrations from a standard curve using peak area (Multichrom® software, VG Instruments). The standards were prepared freshly before each analysis.

2.7. Liquid chromatography mass spectrometry

The mass of DMP 323 and its degradation products was measured with atmospheric pressure chemical ionization mass spectrometry (APCI-MS). Separation was performed isocratically on a 4.6 mm × 25 cm reversed phase Zorbax® R_x C_8 column (Mac Mod Chromatography) that was maintained at 45°C (HP 1090, Hewlett-Packard). The mobile phase was composed of methanol:water (65:35) adjusted to pH 3.4 with trifluoroacetic acid. A flow rate of 1.0 ml/min was

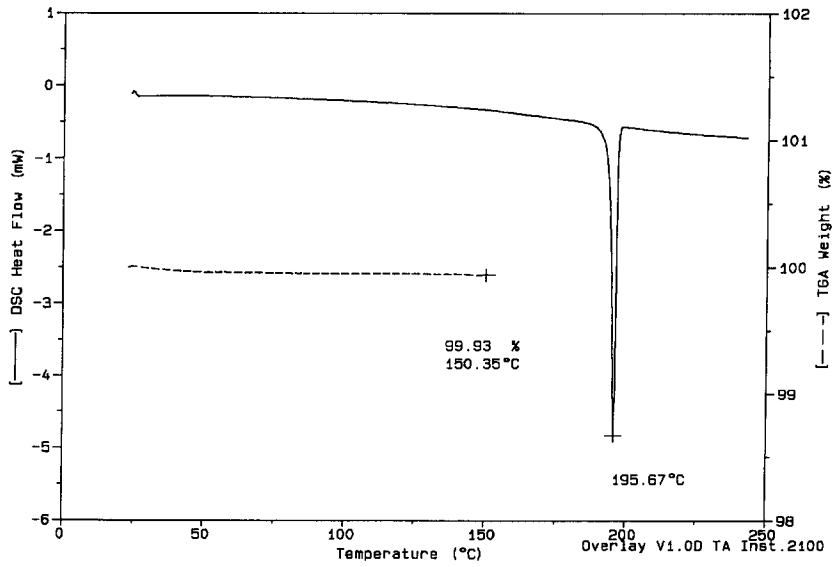

Fig. 2. DSC thermogram (solid line) with a single melt peak at 195.7°C and TGA profile with a weight loss of 0.07% at 150°C (dashed line) of DMP 323 (XM323-12) at 10°C/min.

employed (HP 1090, Hewlett-Packard). Ultraviolet detection was employed at 220 nm (HP 1090, Hewlett-Packard). Mass detection utilized a quadrupole mass spectrometer (TRIO-2000, Fisons Instruments).

3. Results and discussion

DMP 323 drug substance is a white irregular-shaped crystalline material with melting occurring at 197°C by hot stage microscopy. DSC thermogram in a sealed pan at 10°C/min resulted in a single melt peak at 195.7°C indicating the absence of solvates, hydrates, or polymorphs. A typical DSC thermogram is presented in Fig. 2. The compound exhibits a weight loss of 0.07% by TGA up to 150°C (Fig. 2).

DMP 323 was nonhygroscopic. The drug substance had an initial water content of 0.17% ± 0.02% ($n = 3$) by Karl Fischer Analysis. After three weeks at 0, 58 and 85% RHs, the water content remained virtually unchanged at 0.19 ± 0.02, 0.23 ± 0.05 and 0.21 ± 0.04%, respectively. The water content increased slightly to 0.27 ± 0.02% and equilibrated at that value within three weeks at 100% RH.

DMP 323 was practically insoluble in water at 10.0 ± 0.3 μg/ml (mean ± S.D., $n = 3$) pH 8.1 at 25°C. The solubility was characterized as a function of pH. The solubility remained virtually unchanged, 9.2 ± 0.6 μg/ml (mean ± S.D., $n = 19$), over the pH range of 1–13 consistent with the lack of an ionizable moiety on the molecule.

Three potential solubilization approaches were investigated, cosolvents, surfactants, and com-

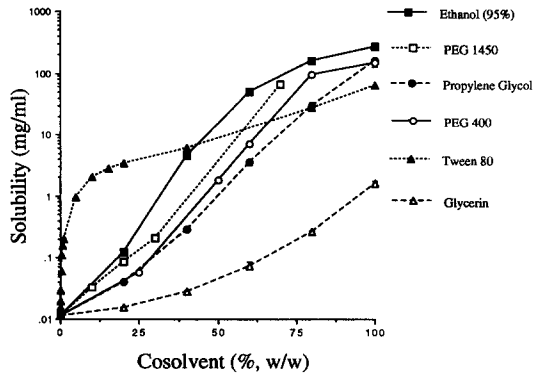

Fig. 3. DMP 323 solubility as a function of ethanol 95% (v/v) in water, polyethylene glycol 1450 (PEG 1450), propylene glycol, polyethylene glycol 400 (PEG 400), tween 80, and glycerin percentage (w/w) in water. The data points and error bars represent the mean ± S.D. of three replicates.

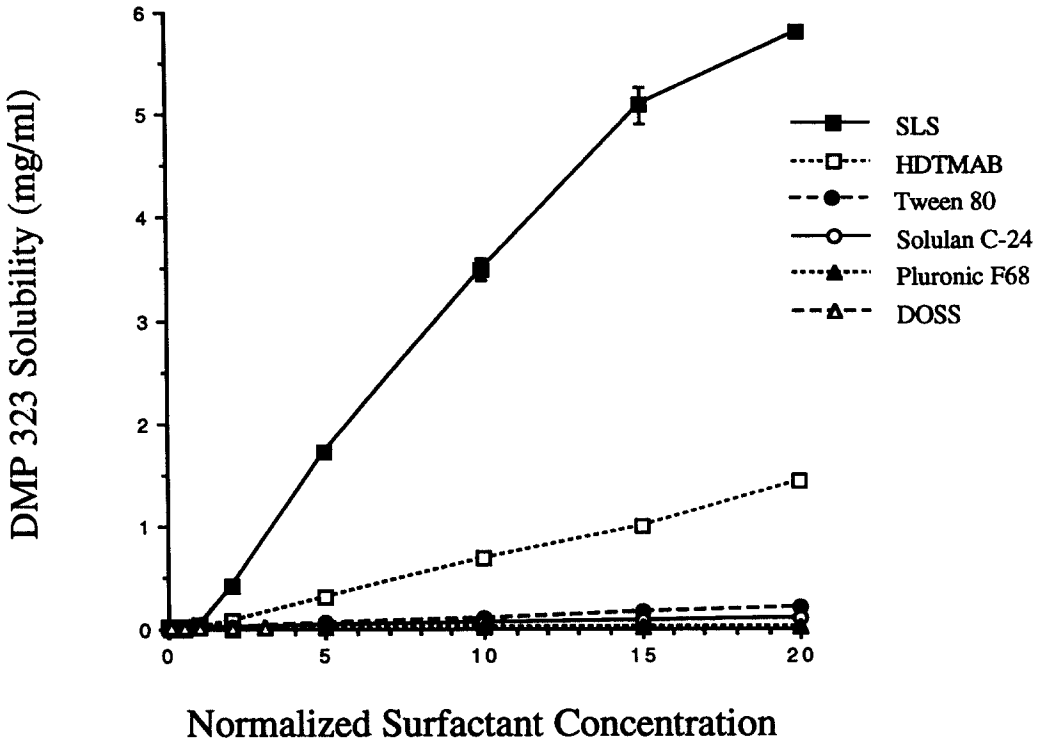

Fig. 4. DMP 323 solubility as a function of normalized surfactant concentration of sodium lauryl sulfate (SLS), hexadecyltrimethylammonium bromide (HDTMAB), Tween 80, Solulan C-24, Pluronic F68, and dioctylsulfosuccinate (DOSS) in water. The data points and error bars represent the mean ± S.D. of three replicates.

plexation (Yalkowsky, 1981). In the case of cosolvents, the solubility of DMP 323 was determined in the water miscible cosolvents, ethanol, polyethylene glycol 1450 (PEG 1450), propylene glycol, polyethylene glycol 400 (PEG 400), polyoxyethylene sorbitan monooleate (Tween 80), and glycerin. The logarithm of the solubility of DMP 323 increased as the percentage of water miscible cosolvent increased (Fig. 3). The highest solubility values of DMP 323 were 272 mg/ml in 95% (v/v) ethanol in water, 65.5 mg/ml in 70% (w/w) polyethylene glycol 1450 (PEG 1450) in water, 160 mg/ml in propylene glycol, 144 mg/ml in polyethylene glycol 400 (PEG 400), 64.2 mg/ml in Tween 80, and 1.61 mg/ml in glycerin. The positive deviation from a typical log–linear profile exhibited with low levels of Tween 80 was consistent with micellar solubilization. Surfactants formed a micellar environment that provided a hydrophobic core for solubilization and a hydrophilic surface for favorable interactions with the aqueous environment.

The solubility of DMP 323 was determined in solutions of the surfactants: Tween 80, sodium lauryl sulfate (SLS), hexadecyltrimethylammonium bromide (HDTMAB), polyoxyethylene cholesterol (Solulan C-24), polyethylenepolypropylene glycol (Pluronic F68), and dioctylsulfosuccinate (DOSS). The surfactant concentrations were normalized by dividing the concentration employed with their critical micelle concentrations (CMC), such that a normalized concentration of 1 was by definition the CMC for the surfactant. In solutions of Pluronic F68 and DOSS, no significant enhancement of solubility was observed. In solutions of Tween 80 and Solulan C-24, the solubility of DMP 323 increased slightly as the surfactant concentration approached their respective critical micelle concentrations (CMC) and then increased slightly

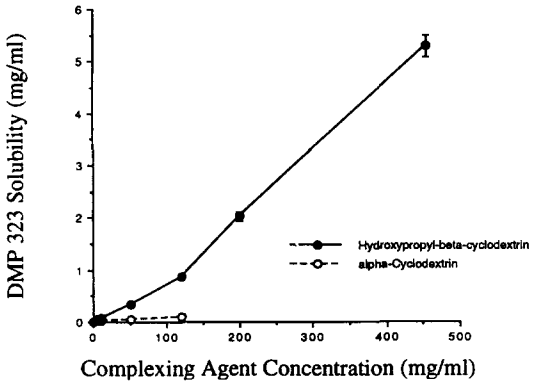

Fig. 5. DMP 323 solubility as a function of α-cyclodextrin and hydroxypropyl-β-cyclodextrin concentration in water. The data points and error bars represent the mean ± S.D. of three replicates.

(10–20-fold) at surfactant concentrations above their CMC (Fig. 4). The solubility of DMP 323 at a normalized concentration of 20 (20-fold the CMC) was improved dramatically in SLS and HDTMAB was 5.8 and 1.4 mg/ml, respectively. The greatest solubilizing capacity was observed with the linear charged surfactants SLS and HDTMAB. The increased solubilization by SLS and HDTMAB suggested that the charge of the surfactant afforded more favorable interaction and hence a greater solubility. The interaction may be potentially through an ion-hydrogen bond interaction with the protons of the benzyl alcohols or the ring diols and/or an ion-dipole interaction with the dipole of the cyclic urea.

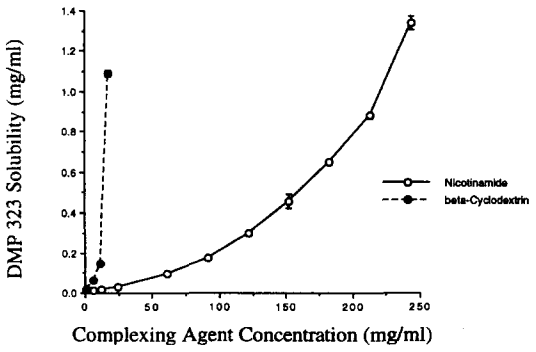

Fig. 6. DMP 323 solubility as a function of nicotinamide and β-cyclodextrin concentration in water. The data points and error bars represent the mean ± S.D. of three replicates.

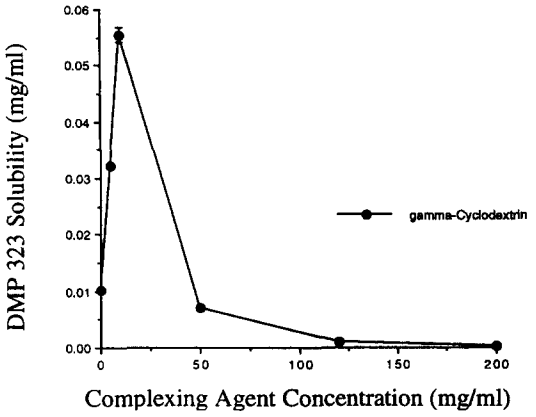

Fig. 7. DMP 323 solubility as a function of γ-cyclodextrin concentration in water. The data points and error bars represent the mean ± S.D. of three replicates.

Complexation was evaluated with nicotinamide, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, and hydroxypropyl-β-cyclodextrin. Three types of complexation behavior were observed. In the case of α-cyclodextrin and hydroxypropyl-β-cyclodextrin, the solubility of DMP 323 increased in a linear fashion with respect to the cyclodextrin concentration, consistent with the formation of 1:1 complexes (Fig. 5). In the case of nicotinamide and β-cyclodextrin, the solubility of DMP 323 increased with a positive deviation from linearity with respect to the concentration of the complexation ligand, consistent with the formation of higher order complexes (Fig. 6). In the case of γ-cyclodextrin, the solubility of DMP 323 increased initially in a linear fashion with respect to the cyclodextrin concentration and then dropped precipitously at γ-cyclodextrin concentrations above 10 mg/ml, consistent with the formation of 1:1 complexes with subsequent precipitation of the complex itself (Fig. 7). Although complexation provided an enhancement in solubility, the amount of the complexing agent that was required to provide the enhancement was relatively large especially in light of the high projected clinical dose.

DMP 323 was resistent to pH-catalyzed degradation with no loss observed in methanol 5% (v/v) aqueous buffers over pH range 3–13 after 8 weeks at 40°C. However, in PEG 400 solutions, the limited degradation (< 30% loss) of DMP 323

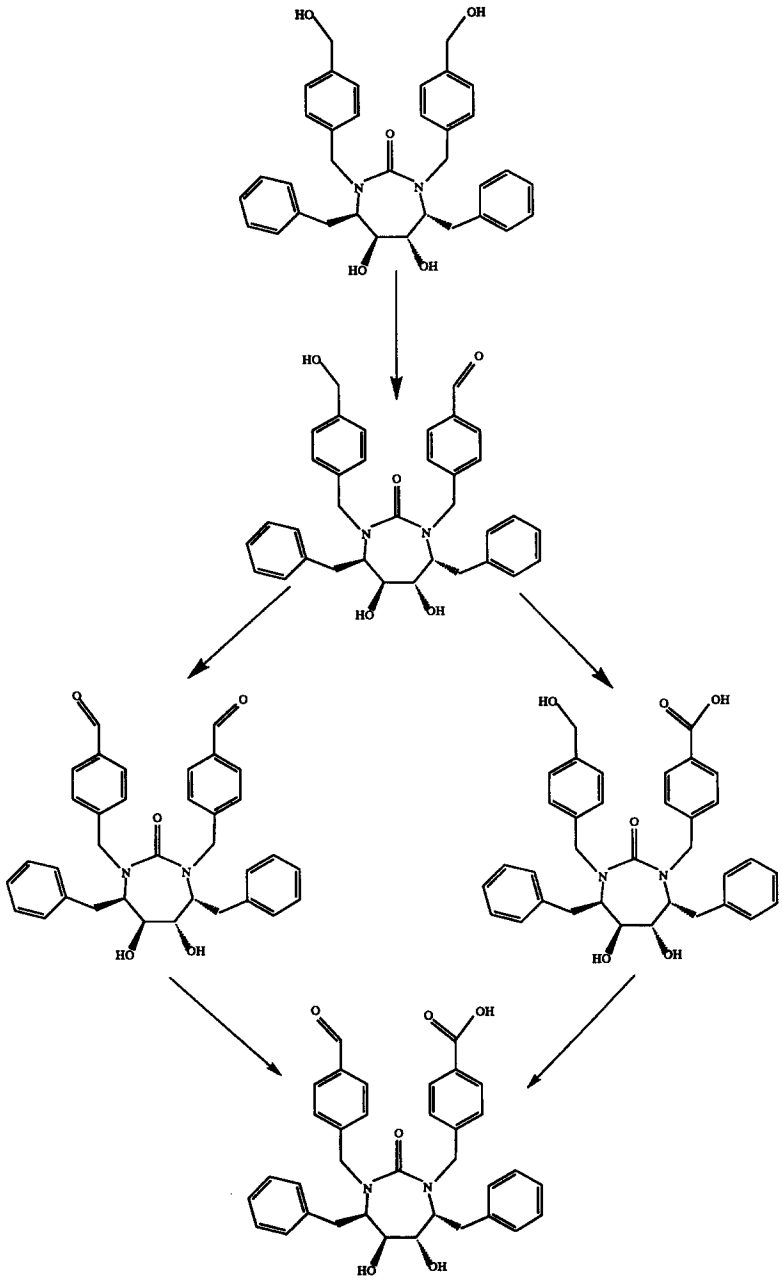

Fig. 8. Proposed degradation pathway for DMP 323 in polyethylene glycol 400 solutions.

Table 1
Percentage of DMP 323 remaining after 12 weeks at 50°C in polyethylene glycol 400 solutions with various additives

Additive	DMP 323 Remaining (%)[a]
Neat PEG 400 solution	78.6 ± 3.2
Citric acid 1 mg/ml	67.3 ± 3.7
Citric acid 0.3 mg/ml	76.4 ± 3.2
Citric acid 0.1 mg/ml	75.4 ± 2.9
BHT 1 mg/ml[b]	96.4 ± 1.9
BHT 0.3 mg/ml	98.2 ± 0.4
BHT 0.1 mg/ml	91.5 ± 2.3
BHA 1 mg/ml[c]	99.4 ± 1.6
BHA 0.3 mg/ml	102 ± 3.8
BHA 0.1 mg/ml	98.1 ± 0.9

[a] Mean ± S.D., $n = 3$.
[b] Butylatedhydroxytoluene.
[c] Butylatedhydroxyanisole.

was approximated with apparent first order kinetics at elevated temperatures (Table 1). Degradation was not detected in propylene glycol solutions after 12 weeks at 50°C. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were effective antioxidants in reducing the degradation in PEG 400 solutions while citric acid afforded no protection from the degradation. Argon flushing was effective at reducing the oxidative degradation in PEG 400 solutions stored at room temperature increasing the percent remaining from 84.6 to 90.2% after 12 weeks. The degradation was unaffected by initial concentration of DMP 323 with concentrations of 1, 10 and 100 mg/ml in PEG 400. The degradation was accelerated on exposure to intense light (600 foot candles) at 25°C for 6 weeks in PEG 400 with the percent remaining decreasing to 69.0 versus 84.6% for a light-protected solution. The protection afforded by BHA, BHT and argon headspace flushing and the light catalysis suggested an oxidative degradation mechanism.

A sample from the light-catalyzed degradation study was utilized to examine the predominant degradation products with HPLC-APCI/MS. The most labile oxidative site on DMP 323 was the benzylic hydroxy groups. The additional peaks on the chromatograms produced molecular ions consistent with MWs of 564, 562, 578 and 580 which were consistent with the MW of DMP 323 −2, DMP 323 −4, DMP 323 +12 and DMP 323 +14, respectively. Changes in MW of −2, −4, 12 and +14 were consistent with oxidation to the monobenzaldehyde, dibenzaldehyde, monobenzaldehyde monobenzoic acid and monobenzoic acid, respectively, (Fig. 8). Authentic samples of the monobenzaldehyde, dibenzaldehyde, monobenzoic acid and the monobenzaldehyde monobenzoic acid were found to have identical retention times to their corresponding peaks from the mass spectrometry characterization of the degraded solution of DMP 323 in PEG 400 and on co-injection with the degraded mixture the peak for the appropriate degradation product increased correspondingly. The predominant degradant was the monobenzaldehyde.

Based on the mass spectrometry characterization of the degraded solution and the chromatographic behavior that was consistent with authentic standards, the degradation scheme is proposed in Fig. 8. The degradation occurred via oxidation of one of the benzyl hydroxy groups to the corresponding aldehyde followed by oxidation of the monoaldehyde to either the dibenzaldehyde or the monobenzoic acid with subsequent convergence to the monobenzaldehyde monobenzoic acid.

References

Lam, P.Y.S., Jadhav, P.K., Eyermann, C.J., Hodge, C.N., Ru, Y., Bacheler, L.T., Meek, J.L., Otto, M.J., Rayner, M.M., Wong, Y.N., Chang, C.-H., Weber, P.C., Jackson, D.A., Sharpe, T.R. and Erickson-Viitanen, S., Rational design of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. *Science*, 263 (1994) 380–384.

Grzesiek, S., Bax, A., Nicholson, L.K., Yamazaki, T., Wingfield, P., Stahl, S.J., Eyermann, C.J., Torchia, D.A., Hodge, C.N., Lam, P.Y.S., Jadhav, P.K. and Chang, C.-H., NMR evidence for the displacement of a conserved interior water molecule in HIV protease by a non-peptide cyclic urea-based inhibitor. *J. Am. Chem. Soc.*, 116 (1994) 1581–1582.

Yalkowsky, S.H., Techniques of solubilization of drugs, *Drugs and the Pharmaceutical Sciences*, Vol. 12, Marcel Dekker, New York, 1981, pp. 159–182.