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A *BIS*-[N-3-(1-HYDROXY-1-METHYL-ETHYL)-BENZYL)-CYCLIC UREA AS A HIV PROTEASE INHIBITOR

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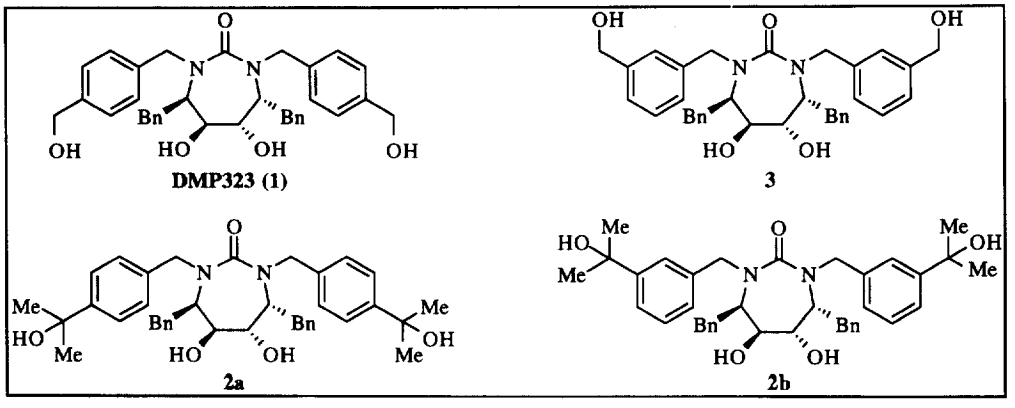
Abstract: Synthetic efforts to overcome the metabolic oxidative degradation of the HIV protease inhibitory cyclic urea **DMP323**, a benzyl alcohol, have led to the discovery of a tertiary carbinol with superior affinity for the viral protease and more potent inhibitory activity against viral replication. Synthetic approaches to this new carbinol and comparative analysis of its pharmacology and pharmacokinetics are presented.

Introduction

The virally encoded aspartyl protease of the human immunodeficiency virus (HIV) is responsible for the processing of the viral polyprotein precursors to the mature structural proteins and enzymes that comprise the virus particle. Since correct processing of the viral polypeptides is essential for the production of infectious virus, HIV protease represents a potential target for therapeutic agents that may prove beneficial in the treatment of AIDS. HIV-I protease is a member of the aspartyl protease family of enzymes, and through molecular modeling studies, we discovered that some seven-membered cyclic ureas were potent inhibitors of the protease. The first compound of this series to advance to clinical trial was [4R-(4b,5α,6β,7β)]-hexahydro-5,6-dihydroxy-1,3-bis[[4-(hydroxymethyl)phenyl]methyl]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (**DMP323**) (**1**) (Figure 1).[1-3] Unfortunately, **1** was found through pharmacokinetic studies to produce variable blood levels in man. Compound **1** is a *para*-benzyl alcohol (P2 = P2′ = CH_2-Ph-(4-CH_2OH), and one of the reasons identified as a cause of the low blood level was the facile metabolism of the benzyl alcohol moiety (-CH_2OH -> -CHO -> -CO_2H). Authentic samples of the proposed metabolites were synthesized and validated (data not included). The objective was to prepare analogues of **1** that would be resistant to the proposed oxidative metabolism of the benzyl alcohol group while maintaining the potency, selectivity and antiviral properties of the parent compound. Typically, this would involve the replacement of the benzilic protons, and to this effect, we set out to prepare the corresponding dimethyl carbinol where P2, P2′ = [Ar-C(Me)$_2$OH].

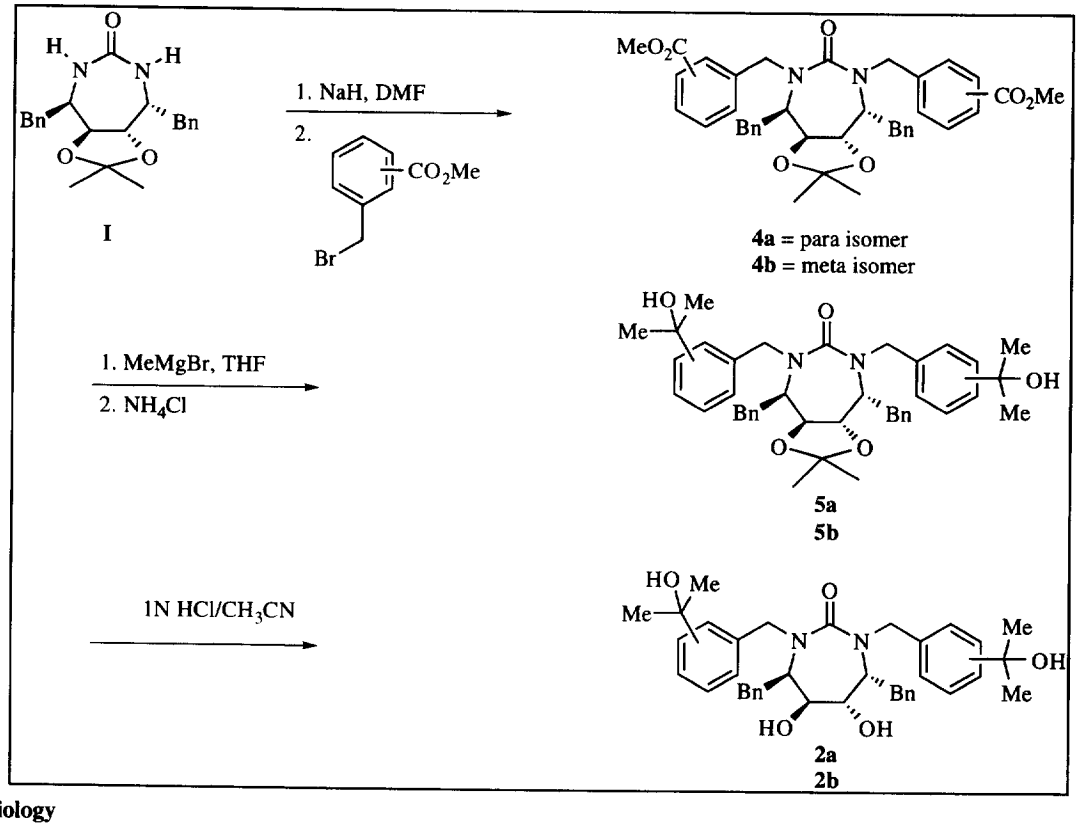
Figure 1. Structures of the cyclic urea HIV protease inhibitors.



Chemistry

The syntheses of **1** and **3** have previously been disclosed.[4] For this study, most of the compounds were prepared from a common intermediate (**I**).[4] The unsubstituted cyclic urea **I** was dialkylated with methyl 4-(bromomethyl)benzoate using NaH in DMF or KO^{t}Bu in THF to give the **4a.** The ester **4a** was reacted with excess MeMgBr to give the **5a** which was treated with 1N HCl in acetonitrile to give the **2a** (Scheme 1). By substituting methyl 3-(bromomethyl)benzoate in the synthesis of **2a**, the corresponding tetraol **2b** was obtained.

Scheme 1. Synthetic approach to the carbinols **2a** and **2b**.



Biology

The **K_i values** were determined using a fluorescent peptide substrate and recombinant single-chain dimeric HIV protease (see Erickson-Viitanen, et al.).[5] The use of single-chain dimeric protease allows enzyme concentrations as low as 0.0625 nM to be used. Reaction products were separated by HPLC with a Pharmacia Mono Q anion-exchange column, and the product was quantified by fluorescence. The ability of test compounds to block cleavage of the HIV-1 gag polyprotein was assessed with [^{35}S]methionine-labeled *in vitro* translation product corresponding to gag p17 plus the first 78 amino acids of gag p24 and recombinant HIV PR as described by Erickson-Viitanen, et al.[6] The K_i values were measured with 62.5 to 250 pM HIV PR dimer and 1 to 10 nM inhibitor.

HIV RNA Assay.[7] Antiviral assays were carried out in the human lymphocytoid cell lines MT-2 or MT-4 using the RF strain of HIV-1 or variant forms of HIV containing mutations within the gene encoding the HIV protease. Virus replication was measured after 3 days by either a microtiter well-based viral RNA hybridization assay, or by measurement of viral p24 antigen (Intercompany Consortium Assays[8]). For all determinations, the concentration that decreased by 90% the amount of virus present in the assay, relative to untreated, infected control cells was designated the IC_{90}. Because the level of virus replication depends on the integrity of the infected cell population compound cytotoxicity was also determined in companion, uninfected cell cultures using the metabolizable tetrazolium dye MTT. The concentration of compound reducing by 50% the viable cell population as based on MTT oxidation was designated the TC_{50}.

Pharmacokinetic studies (PK) were conducted in the male CD rat and the female Beagle dog. The oral study, at 10 mg/kg, was conducted in three dogs, and the IV study was done at 5 mg/kg. The vehicle used was PEG400, water, ethanol (60/30/10). In the current study, the concentration was 10 mg/mL and the dosing volume was 1 mL/kg. The IV formulation was the same as the oral, 10 mg/mL with dosing volume 0.5 mL/kg. The method was validated in dog plasma at a concentration range of 0.10 to 50 μg/mL. The same formulation and vehicle were used for the rat study.

Discussion

The data in Table 1 shows that the replacement of the benzilic protons of **1** to produce **2a** resulted in a loss of binding and the ability to inhibit viral replication. Because of these results, no additional pharmacological or pharmacokinetic studies were conducted on **2a.** A comparison between **1** and it's *meta*-derivative **3** show that there is no statistical difference in their K_i values (0.31 nM vs. 0.40 nM) while their ability to inhibit viral replication would appear to be different (IC_{90} = 176.5 nM vs. 38.8 nM, respectively). Since we had structure-activity relationship data that suggested that most of our 3-substituted benzyl P2 derivatives were more active than the corresponding 4-substituted derivatives, we decided to investigate the 3-carbinol derivative. Clearly **2b** (K_i = 0.067 nM, IC_{90} = 33.7 nM) was superior to **2a** (K_i = 2.20 nM, IC_{90} = 8044.2 nM) as an anti-HIV agent. More importantly, **2b** was superior to **1** (K_i = 0.31 nM, IC_{90} = 176.5 nM) and **3** (K_i = 0. 140 nM, IC_{90} = 38.8 nM). The structural differences between **2b** and **2a** would suggest that regiochemistry (*meta*- vs. *para*-substituent) was more important for activity than molecular lipophilicity and/or size since both compounds have the same ClogP (6.164) and CMR (18.387).[9] For **1** and **3,** regiochemistry would appear to be more important for IC_{90} than K_i. Similar to other cyclic ureas, compound **2b** shows high specificity for the viral aspartic acid protease, causing 0%, 0%, and 43% inhibition of pepsin, cathepsin D and renin, respectively at test concentrations of 12.5 μM or higher (data not shown). In addition to potency against wild type HIV-1, as measured by the IC_{90} for inhibition of replication of the laboratory isolate, HIV-1(RF) or the recombinant infectious clone HxB2, carbinol **2b** also inhibited mutant variants of HIV-1 carrying single mutations corresponding to amino acids 82 and 84 within the gene encoding the HIV protease. Mutations at these positions are known to cause a loss in inhibitory potency for cyclic ureas such as **1** (see Table 1). With the double mutant corresponding to amino acid changes at both positions 82 and 84, compound **2b** shows a significant loss of potency, although maintaining 4- to 5-fold improvement over its predecessor, compound

1. To the extent that such mutant viruses may reside within the infected cell population of patients (Ho, Wei, and Coffin refs. of *Science* and *Nature* 1995), a compound such as **2b** could be advantageous because of its attenuated resistance profile. In order for **2b** to be an effective anti-HIV drug useful for the treatment of AIDS, potency against wild type and mutant forms of the virus must be combined with adequate oral bioavailability, such that blood levels in excess of those required to inhibit virus replication are achieved. This study has resulted in the identification of a superior anti-HIV agent (**2b**) to **1**.

Table 1. Pharmacological Profile for **1**, **2a**, **3**, and **2b**.

					p24 Mutant Variants IC_{90} nM			
cmpd	Ki, nM	Gag IC_{50}, nM	HIV-1 RNA IC_{90}, nM	MMT TC50, μM	HxB2 wild type	V82F	184V	V82F/184V
1	0.310	35.7	176.5	67.5	95.6	675.0	988.9	22045.9
	(0.120)		(42.4)	(31.4)				
2a	2.200		8044.2	39.3				
3	0.400	7.9	38.8	54.7				
	(0.140)			(11.5)				
2b	0.067	15.0	33.7	33.4	22.2	84.3	72.2	5778.5
	(0.029)		(11.2)	(29.1)				

numbers in parentheses () = ± standard deviation

To achieve high plasma level with good duration of action, a compound should have low systemic clearance and excellent absorption after oral dosing. We determined the systemic clearance of a compound after an intravenous (iv) dose, and the bioavailability and maximum plasma concentration after oral administration in rats and dogs. Statistically, the systemic clearance in the dog for **2b** and **1** was not different (0.93 ± 0.36 vs. 1.48 ± 0.58 L/h/kg) and the difference in clearance for the rat (1.91 ± 0.34 vs. 7.12 ± 1.28 L/h/kg) was large and significant (see Table 2). This observation was consistent with the hypothesis that the carbinol group was less susceptible to metabolism than the benzilic alcohol. The volume of distribution at steady-state (Vss) is a parameter that describes the extent of distribution of a compound into tissues. Apparently, the *meta*-benzilic alcohol distributes more extensively than the *para*-benzilic alcohol in rats as Vss increased from 6.36 to 9.8 L/kg in rats. The carbinol, on the other hand, distributed less extensively than the benzilic alcohol in both rats and dogs. Upon oral dosing, the maximum plasma concentration of **2b** in rat and dog was 0.63 and 0.24 μg/mL, respectively. There was considerable variability in the plasma concentration data, therefore bioavailability of **2b** in rat could not be determined. The maximum concentration for **2b** was higher in the rat than **1** but lower than **1** in the dog. Typically, when a compound has low systemic clearance, one expects high blood levels. However, incomplete absorption is usually an important factor when both the systemic clearance and blood levels are low. Based on the limited *in vivo* data, it appeared that the introduction of the dimethyl substituent diminished metabolism at the P2 position, but poor solubility and incomplete absorption might be the reason for low bioavailability and plasma concentration of **2b.** Though more potent as an anti-HIV entity, **2b** did not result in an improved PK profile as compared to **1**. The PK data in both rat and dog would suggest that the problem may reside more in drug solubility and absorption than drug metabolism.

Table 2. Pharmacokinetics Profile for **1**, **3**, and **2b**.

	1[3]	1[3]	3	2b	2b
	Rat	**Dog**	**Rat**	**Rat**	**Dog**
iv Dose, mg/kg	5.0	5.0	5.0	5.0	5.0
CL, L/h/kg	7.12 (1.28)	1.48 (0.58)	6.2	1.91 (0.34)	0.93 (0.36)
Vss, L/kg	6.36 (1.84)	2.28 (0.91)	9.8	3.04 (0.40)	1.65 (0.66)
iv $t_{1/2}$, h	0.95 (0.10)	1.80 (0.54)	1.1	1.03	1.20 (0.36)
po Dose, mg/kg	10.0	10.0	10.0	10.0	10.0
C_{max}, μg/mL	0.45 (0.24)	1.48 (0.31)	0.47	0.63 (0.12)	0.24 (0.20)
po $t_{1/2}$, h	1.50 (0.80)	1.08 (0.52)		1.50 (0.80)	0.65 (0.26)
PO t_{max}, h	0.25 (0.00)	0.50 (0.29)			0.42 (0.14)
F, %	27.1 (7.0)	38.4 (10.0)			<4

numbers in parentheses () = ± standard deviation

Conclusion

Structural modification of **1** to eliminate some of its metabolic liabilities resulted in the discovery of **2b** that was superior to **1** in both protease and viral replication inhibitory properties. Unfortunately, the pharmacokinetic properties were not as satisfactory. Oral PK of a compound can be affected by two important processes: drug absorption and metabolism. The oxidative metabolic degradation of the benzilic alcohol of **1** was eliminated by converting the benzilic primary carbon into a tertiary center. Thus, the CH_2OH -> CHO -> CO_2H pathway was eliminated. However, in the process, a less soluble compound was produced which we believe retards gastrointestinal absorption. It is possible for this problem to be overcome through different formulations of the **2b.**

Experimental

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The NMR spectra were recorded with a Varian-300S spectrometer, IR spectra were recorded with a Perkin Elmer 1650 FTIR spectrophotometer, UV spectra were obtained with a Cary 2415 spectrophotometer, optical rotations (OR) were determined on a Perkin-Elmer 241 polarimeter, and mass spectra (MS) were obtained using the Hewlett Packard HP5988A GC-MS system. Thin layer chromatography (TLC) was performed on silica gel plates.

Synthesis

(4b,5α,6β,7β)-Hexahydro-5,6-dihydroxy-1,3-bis[[4-(1-hydroxy-1-methylethyl)phenyl]methyl]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (2a). A solution of the **I**[4] (3.66 g, 0.010 mol) in dry THF (50 mL) was treated with KO^{t}Bu (2.47 g, 0.022 mol) and staffed at room temperature for 30 min. The mixture was treated with methyl 4-(bromomethyl)benzoate (4.58 g, 0.020 mol) and stirred at room temperature for 72 h. The mixture was concentrated *in vacuo*, and the residue was partitioned between EtOAc (150 mL) and water (200 mL). The organic layer was washed with additional water and brine, dried over $MgSO_4$, filtered, and concentrated to give **4a** as a thick oil: IR (neat) 1723 (C=O) cm^{-1}; MS (NH_3-CDI) m/e 663(M+1).

The ester **4a** (1.0 g, 0.0015 mol) in dry THF (33 mL) was treated with excess 1.4 M MeMgBr (6.47 mL, 0.009 mol) and stirred at room temperature for 16 h. The mixture was treated with MeOH (10 mL), stirred for 30 min, and concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (100 mL) and 5% citric acid (100 mL), and the organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give

5a as a yellow gum (0.541 g). The protected diol **5a** was deprotected by dissolving in 30 mL MeCN, treating with 5 mL 1N HCl, stirring for 1 h, and diluting with 200 mL water. The crude product was isolated by decanting the aqueous phase, dissolving the residue in CH_2Cl_2, washing the organic phase with water and brine; drying the organic phase over $MgSO_4$; filtering; and concentrating to a foam. The foam was dissolved in Et_2O, and the Et_2O was allowed to evaporated to produce a saturated solution. The resulting white crystals were collected by filtration, washing with cold Et_2O and petroleum ether, and drying *in vacuo* to give **2a** in 77% (0.481 g) yield: m.p. 189-193°; ^{1}H NMR (300 MHz, DMSO-d_6 TMS) δ 1.38 (s, 12H, CH_3CCH_3), 2.82 (m, 4H, $PhCH_2$), 2.96 (d, J = 14.3 Hz, 2H, NCH), 3.3-3.55 (m, 4H, NCH, and OCH), 4.62 (d, J= 14.3 Hz, 2H, NCH), 4.97 (s, 2H, OH), 5.07 (s, 2H, OH), [6.97 (d, 4H), 7.07 (d, 4H), 7.28 (m, 6H), 7.41 (d, 4H, Ar); IR (nujol) 3355 (OH), 1608 cm^{-1}; MS (NH_3-DCI) m/e 623(M+I); Analysis calc'd for $C_{39}H_{46}N_2O_6$ MW 622.81: C, 75.21; H, 7.45; N, 4.50; found: C, 75.21; H, 7.61; N, 4.46.

(4b,5α,6β,7β)-Hexahydro-5,6-dihydroxy-1,3-bis[[3-(1-hydroxy-1-methylethyl)phenyl]methyl]-4,7-bis (phenylmethyl)-2*H*-1,3-diazepin-2-one (2b). By substituting methyl 3-(bromomethyl)benzoate in the method for **2a**, the desired product was obtained in 86% yield: m.p. 216-217°; ^{1}H NMR (300 MHz, DMSO-d_6 TMS) δ 1.36 (s, 12H, CH_3), 2.78-2.99 (m, 6H, Ar'CH_2 + NCH), 3.40 (s, 2H, OCH), 3.45 (d, J = 10.98 Hz, 2H, OCCH), 4.67 (d, J = 13.91 Hz, 2H, NCH), 4.97 (s, 2H, OH), 5.08 (s, 2H, OH), [6.88 (d, 2H), 7.01 (d, 4H), 7.25 (m, 10H), 7.3 (d, 2H), Ar]; IR (KBr) 3462(OH), 1592(C = O) cm^{-1}; MS (NH_3-DCI) m/e 640(M+NH_4); $[\alpha]_D^{20}$ +98.68° (c = 0.454, MeOH); Analysis calc'd for $C_{39}H_{46}N_2O_5$, MW 622.81: C, 75.21; H, 7.46; N, 4.41; found: C, 75.48; H, 7.55; N, 4.40.

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