Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 23, pp. 2847-2852, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00528-8

EVALUATION OF FUROFURAN AS A P_2 LIGAND FOR SYMMETRY-BASED HIV PROTEASE INHIBITORS

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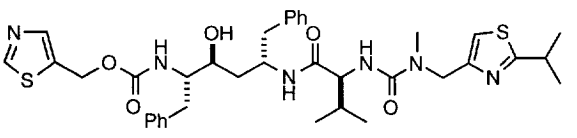
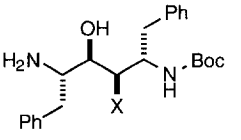
Abstract: The hexahydrofurofuranyloxy group was evaluated as a conformationally constrained P2 ligand for symmetry-based HIV protease inhibitors. A number of compounds showed nM level activity against HIV in MT4 cells and lower protein binding than the licensed protease inhibitor ritonavir. However, replacement of 5-thiazole of ritonavir with a furofuran caused a reduction of the bioavailability in vivo. Copyright © 1996 Elsevier Science Ltd

Inhibition of human immunodeficiency virus (HIV) protease is one of the most important and promising approaches for the therapeutic intervention of HIV infection.[1] This approach to anti-HIV therapy has been validated with the recent FDA approval of three HIV protease inhibitors-saquinavir, ritonavir, and indinavir.[2] A wide variety of classes of peptidomimetic inhibitors have been reported based upon HIV protease substrate sequences and on the three-dimensional structure of the C_2-symmetric, homodimeric enzyme active site.[3] There has also been rapid progress in the development of highly optimized P_2 ligands for hydroxyethylamine based HIV protease inhibitors.[4-10] In particular, the hexahydrofurofuranyloxy group as a conformationally constrained P_2 ligand discovered by Ghosh is of great interest.[11] Each of the two ether oxygen atoms of the furofuran hydrogen bonds to the NH groups of Asp_{29} and Asp_{30}, respectively, of the viral protease. These hydrogen bonding interactions were confirmed by X-ray studies.[11] Due to the dimeric nature of HIV protease, it is reasonable to speculate that this type of conformationally constrained ligand will also be well suited for the C_2-symmetry based HIV protease inhibitors core amines **1** and **2**.[12] As part of our continuing efforts, we prepared a number of HIV protease inhibitors containing furofuran as a P_2 ligand with the hope of improving the antiviral potency and reducing the high (99%) binding of human plasma proteins by ritonavir.[13] Attached to the symmetry-based core diamine **2**, the furofuran ligands provided inhibitors that displayed lower nM level activity against HIV in vitro.



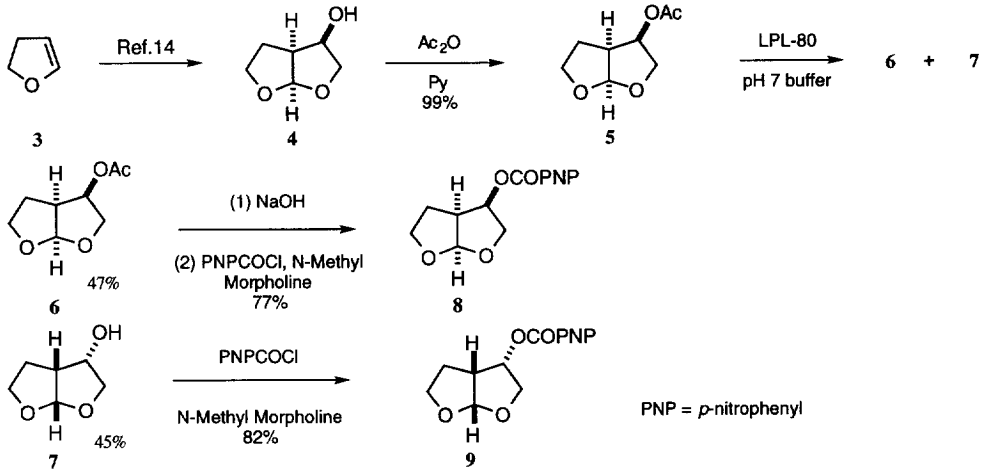
Ritonavir



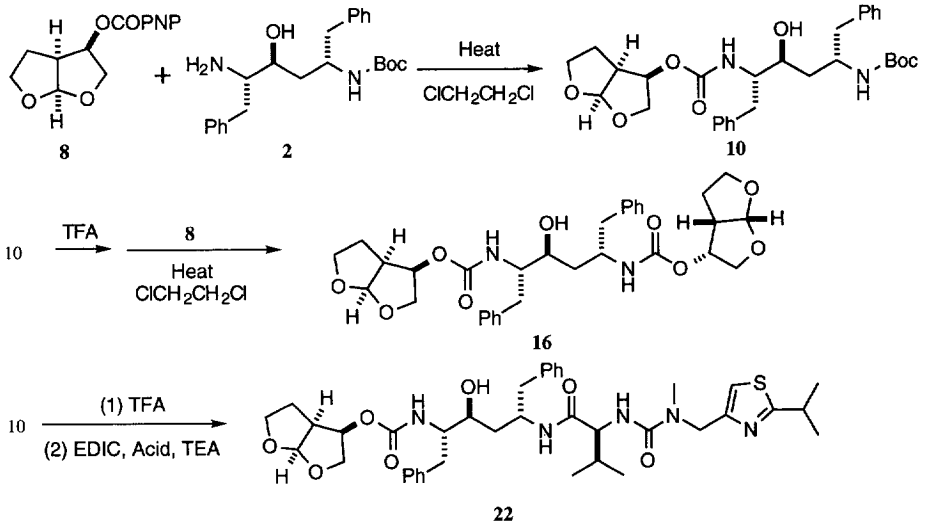
1, X = OH
2, X = H

Racemic 3-hydroxy-bistetrahydrofuran **4** was prepared from dihydrofuran through a four step reaction sequence as previously reported.[14] The two enantiomers of **4** were resolved by enzymatic hydrolysis of their acetates **5** by using LPL-80 (Amano). The enantiomeric purity of 3-hydroxy-(3R,3αS,6αR)-bis-tetrahydrofuran and 3-hydroxy-(3S,3αR,6αS)-bis-tetrahydrofuran **7** was (98% ee, $\alpha_D^{23°}$ -12.1°, MeOH) and (97% ee, $\alpha_D^{23°}$ +12.2°, MeOH), respectively. The enantiomeric bis-tetrahydrofuranes were converted to the corresponding activated carbonates **8** and **9** by reacting with *p*-nitrophenol chloroformate and N-methyl morpholine (Scheme I).

Scheme I. Synthesis of activated furofuran ligands



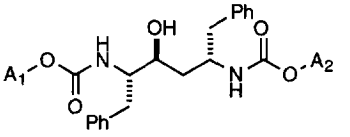
Scheme II. Synthesis furofuran containing HIV protease inhibitors.



The activated carbonates were heated with the Boc protected diamine core **2**[15] to furnish the key intermediate **10**. After removal of the Boc protecting group of **10** under acidic conditions, treatment with another equivalent of **8** or **9**, or standard peptide coupling to substituted valines provided the desired inhibitors (Scheme II). All final compounds showed satisfactory purity by ^{1}H NMR and mass spectral analysis. We first studied the stereochemical preference of the furofuran ligands in the context of C_2 symmetry based HIV protease inhibitors. The IC_{50} values for analogs A and B against HIV protease and the anti-HIV activity (EC_{50}) and cytotoxicity ($CCIC_{50}$) of each inhibitor in MT4 cells using a cytopathicity assay were measured according to reported

methods.[16] We also measured the EC_{50} in MT4 cells in the presence of 50% human serum as an empirical estimate of the effect of protein binding on the activity of the inhibitors.[17] The results are shown in Table I and II, respectively. As a result of unsymmetric binding of the core diamine **2** to the active site,[18] different binding affinities with HIV protease were observed depending upon the proximal or distal relationship of the furofuran to the hydroxyl group. In general, the 3R-furofuran was found to be a better P_2 ligand than the 3S-furofuran in this series. This was especially apparent when the furofuran occupied a position distal to the hydroxyl group. However, compounds with the 3S-furofuran in the position proximal to the hydroxyl group also displayed good

Table I Inhibition of HIV protease by furofuran containing analogs of **A**



A

No.	A1	A2	Inhib.[a] % (nM)	EC_{50} (μM)	EC_{50}[b] (μM)	$CCIC_{50}$ (μM)
Ritonavir			78(0.5)	0.07	0.81	56
11	R, S-FF	*t*-Bu	49(0.5)	0.2	0.2	56
10	R-FF	*t*-Bu	63(0.5)	0.18	nd	69
12	S-FF	*t*-Bu	45(0.5)	1.043	4.727	>100
13	R, S-FF	R,S-FF	53(0.5)	0.071	0.077	>100
14	S-FF	S-FF	50(4)	3.681	4.183	>100
15	S-FF	R-FF	73(0.5)	0.107	0.197	>100
16	R-FF	R-FF	64(0.5)	0.034	0.115	>100
17	R-FF	S-FF	39(0.5)	0.891	1.924	>100
18	R, S-FF	5-Thz	43(0.5)	0.502	1.204	>100
19	5-Thz	R, S-FF	52(0.5)	0.428	0.774	>100
20	R-FF	5-Thz	53(0.5)	0.140	0.290	>100

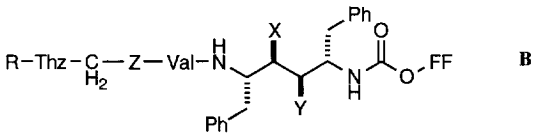
a: pencentage of inhibition of HIV protease was measured in the presence of inhibitor at indicated consentration.
b: antiviral activity was tested in the presence of 50% human serum.
nd = no data; FF = hexahydrofurofuranyl; Thz = Thiazolyl.

antiviral activities. These observations are consistent with the unsymmetric mode of binding previously observed in the crystal structure of a derivative of core diamine **2** bond to HIV protease.[18] Inhibitors with mixtures of 3R, 3S-furofuran retained most of the antiviral activities against HIV virus in MT4 cells in comparison to their corresponding 3R-furofuran stereoisomers.

Compounds **31** and **32** containing furofurans as P_2 ligands showed significantly higher binding affinities

to HIV protease than the simple tetrahydrofuran **29**, which suggests that both of the oxygen atoms of the furofuran are participating in the putative hydrogen bonds with Asp_{29} and Asp_{30} of the viral protein.[11] Replacement of L-valine with D-valine caused a slight lose of antiviral activity (**21** vs **22**; **23** vs **24** and **25** vs **26**). Compounds with larger P_3 ligands (**21-24** and **29-32**) showed improved antiviral activities over those with smaller groups. In parallel to the SAR studies of ritonavir development,[19] isopropyl substituted 4-thiazoles were superior to methyl substituted 4-thiazoles and 5-thiazoles. This observation is consistent with the previously

Table II Inhibition of HIV protease by furofuran containing analogs of **B**



B

No.	R	Thz	Z	Val	X	Y	FF	Inhib.%[a] (0.5nM)	EC_{50} (μM)	EC_{50}[b] (μM)	$CCIC_{50}$ (μM)
21	i-Pr	4	NMe	L	H	OH	R,S	52	0.009	0.14	56
22	i-Pr	4	NMe	D	H	OH	R,S	66	0.05	0.43	56
23	i-Pr	4	O	L	H	OH	R,S	83	0.01	0.137	>100
24	i-Pr	4	O	D	H	OH	R,S	66	0.03	0.33	66
25	H	5	O	L	H	OH	R	80	0.07	0.233	>100
26	H	5	O	D	H	OH	R	42	0.27	1.066	>100
27	H	5	NMe	L	H	OH	R	56	0.45	0.67	>100
28	H	5	NMe	D	H	OH	R	72	0.37	1.3	>100
29	i-Pr	4	NMe	L	H	OH	S-THF	68	0.03	0.435	56
30	i-Pr	4	NMe	L	OH	H	R	74	0.03	0.419	56
31	i-Pr	4	NMe	L	H	OH	R	83	0.01	0.116	>100
32	i-Pr	4	NMe	L	H	OH	S	80	0.01	0.239	47
33	Me	4	NMe	L	H	OH	R,S	69	0.07	0.166	>100
34	Me	4	NMe	L	H	OH	R	74	0.04	0.56	>100
35	Me	4	NMe	L	H	OH	S	82	0.06	0.216	>100

a: pencentage of inhibition of HIV protease was measured in the presence of inhibitor at indicated consentration.
b: antiviral activity was tested in the presence of 50% human serum.
FF = Furofuranyl; Thz = Thiazolyl; S-THF = (S)-3-Tetrahydrofuranyl; Val = Valinyl.

observed the hydrophobic interaction between the Val_{82} of HIV protease and the isopropyl substituent on the P_3 thiazole group.[13] The N-methyl urea linkage[12] between the P_2 and P_3 ligand produced slightly better antiviral activities than a carbamate linkage. However, this trend was not observed when D-valine is used as a P_2 ligand instead of L-valine (**22** vs **24** and **26** vs **28**).

Compound **13** displayed greater antiviral potency than ritonavir. However, the aqueous solubility of **13** was very low, and erratic results were observed when a suspension of **13** was dosed orally in rats. Compound **20** was twofold more active than ritonavir in MT4 cells in the presence of 50% of human serum, while it has a lower molecular weight than ritonavir. At a dose of 10 mg/kg, compound **20** exhibited 41% oral bioavailability in rat and a peak plasma level of 770 nM, in excess of its in vitro antiviral EC_{50} (290nM) in the presence of 50% human serum (Figure I). The small effect of human serum on these compounds is notable, and suggests that the incorporation of polar groups, in this case the furofuran and thiazole, significantly diminishes the protein binding.

Figure I Mean (±SEM) Plasma Concentration of **21** after a 5 (IV) or 10 (oral) mg/kg Dose in Rats

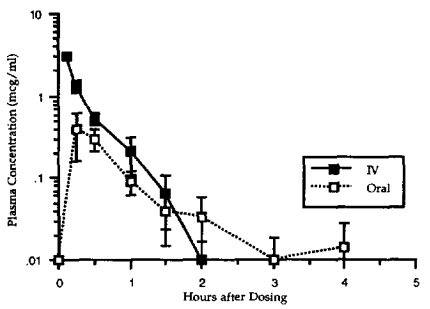
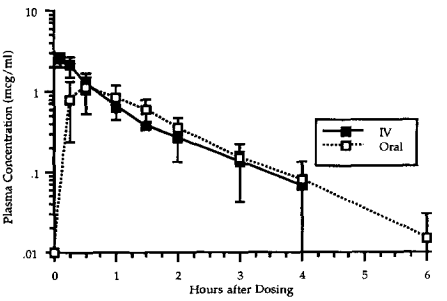


Figure II Mean (±SEM) Plasma Concentration of **20** after a 5 (IV) or 10 (oral) mg/kg Dose in Rats



Compound **21**: 5 mg/kg IV dose

Rat#	$t_{1/2}$(H)	Vc L/kg	AUC(0-8h) µg•h/mL
1	0.24	0.5	1.413
2	0.67	0.9	1.344
Mean	0.35	0.7	1.378

Compound **20**: 5 mg/kg IV dose

Rat#	$t_{1/2}$(H)	Vc L/kg	AUC(0-8h) mcg•h/mL
1	1.60	2.0	2.231
2	0.44	1.4	2.361
Mean	0.69	1.7	2.296

Compound **21**: 10 mg/kg Oral dose

Rat#	C_{max} µg/mL	T_{max} (H)	AUC(0-8h) µg•h/mL	F %
3	0.359	0.5	0.514	18.7
4	0.838	0.25	0.202	7.3
5	0.128	0.5	0.210	7.6
Mean	0.442	0.42	0.309	11.2

Compound **20**: 10 mg/kg Oral dose

Rat#	C_{max} µg/mL	T_{max} (H)	AUC(0-8h) µg•h/mL	F %
3	0.717	0.5	1.416	30.8
4	2.264	0.5	3.29	71.7
5	0.381	0.5	0.0951	20.7
Mean	1.121	0.5	1.886	41.1

However, further improvement in the potency and pharmacokinetic properties of these molecules is required to maintain suppression of HIV replication *in* vivo. Compound **21** demonstrated a five fold increase in antiviral activity in MT4 cells over ritonavir in the presence of 50% human serum. Unfortunately, a 10 mg/kg dose of compound **21** in rat achieved a calculated oral bioavailability of only 11.2%. Preliminary investigation of the hepatic metabolism of compound **21** in rat liver microsomes revealed that this compound is degraded an order of magnitude faster than ritonavir. Elucidation of the metabolic mode of the degradation of **21** will guide the future

direction of the structural modifications to this molecule.

In summary, we have incorporated conformationally constrained furofurans as a P_2 ligands in several novel C_2-symmetry based HIV protease inhibitors. Some of these compounds were potent HIV protease inhibitors and highly active in blocking the cytopathic affects of HIV in an MT4 cell culture assay and compound **20** showed 41% bioavailability in rats. Additional investigations will be required to further improve the pharmacokinetic profile of these compounds prior to clinical development.

ACKNOWLEDGMENT:

The authors thank Dr. John Dennison for the results of the *in* vitro hepatic metabolism studies in rat liver microcosms and Dr. Fariborz Mohamadi for helpful discussions.

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(*Received in USA* 23 *September* 1996; *accepted* 29 *October* 1996)