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3'-Tetrahydrofuranylglycine as a Novel, Unnatural Amino Acid Surrogate for Asparagine in the Design of Inhibitors of the HIV Protease

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Abstract

The blockade of the HIV protease has become a major target in the search for an effective therapy for AIDS.¹ While many reports of potent HIV-1 inhibitors have appeared recently, the compound Ro 31–8959 remains the least selective for the HIV-1 and HIV-2 proteases.² This property may result in reduced susceptibility to resistance since these represent the genetically most divergent strains of HIV presently known to exist.

Graphical Abstract

Ro 31-8959, R = CH₂CONH₂

12, R = 3(R)-tetrahydrofuranyl

13, R = 3(S)-tetrahydrofuranyl

Supplementary Material Available: Listings of experimental details and spectroscopic characterization for compounds 2–11 and analytical data and stereoview of the optimized bound conformations of Ro 31–8959 and inhibitor 12 superimposed in the inhibited HIV-1 protease active site (8 pages). Ordering information is given on any current masthead page.

Interestingly, no improvement over asparagine at the P₂ subsite has been reported. Computer-assisted modeling starting with the X-ray crystal structure of the L-689,502-HIV-1 protease complex³ and replacing the L-689,502 inhibitor with Ro 31–8959 suggested that one feasible explanation for this preference might be hydrogen bonding of the asparagine side chain to the Asp 29 and Asp 30 NH present in the HIV-1 P₂ subsite.⁴ Were this model correct, a rigid, cyclically constrained oxygen might be an effective replacement for the primary amide carbonyl oxygen atom. We considered employing the inherent propensity of the tetrahydro-furan (THF) oxygen atom to donate its lone pairs to protons and metal ions as a potential asparagine replacement for the protease enzyme. To test this concept, the unknown (2*S*,3'*R*)- and (2*S*,3'*S*)-tetrahydrofuranylglycines were prepared by chiral synthesis from D- or L-malic acid, respectively, in the following manner.

Enantiomerically pure (*S*)-(+)- and (*R*)-(–)-3-hydroxytetra- hydrofurans (**1a** and **1b**) were prepared by acid-catalyzed cy-clodehydration of 1,2,4-butanetriol as described by Tandon, van Leuson, and Wynberg.⁵ The corresponding mesylates were converted stereospecifically with inversion of configuration into diethyl (*R*)- and (*S*)-3-tetrahydrofuranylmalonates **2a** and **2b** by displacement with the sodium salt of diethyl malonate in DMF at 100 °C.⁶ Saponification followed by copper ion promoted decarboxylation⁷ afforded the optically pure (*R*)- and (*S*)-3- tetrahydrofuranylacetic acids **3a** and **3b** in excellent yields. The asymmetric azidation methodology of Evans⁸ was employed in excellent overall yields for the stereoselective introduction of the α-amino functionality as shown in Scheme I. The hydroxy- ethylamine isostere **11** was conveniently prepared from the readily available azido diol **7**^{9,10} by conversion into the (2*R*,3*S*)-azido epoxide **8**^{11,12} followed by reaction with the *tert*-butyl amide of (3*S*,4*aR*,8*aS*)-decahydroisoquinoline-3-carboxylic acid **9**^{13,14} in hot isopropyl alcohol. While the (2*S*,3'*R*)- and (2*S*,3'*S*)-tetra-hydrofuranylglycines could be obtained quantitatively from the (2*S*,3'*R*)-and (2*S*,3'*S*)-2-azido-3'-tetrahydrofuranylacetic acids **6a** and **6b** by hydrogenation over a palladium catalyst, we found the α-azido acids to be convenient, chirally stable intermediates for introduction of the 3'- tetrahydrofuranylglycines into the inhibitors (Scheme II).

A comparison of the in vitro potencies of Ro 31–8959 with the tetrahydrofuran-modified inhibitors **12** and **13** was rewarding. The inhibitor derived from (2*S*,3'*R*)- tetrahydrofuranylglycine (3'*R*-Thfg) **12**, IC₅₀ = 0.054 ± 0.027 nM (*n* = 4), was reproducibly 4-fold more potent as a competitive enzyme inhibitor than Ro 31–8959, IC₅₀ = 0.23 ± 0.10 nM (*n* = 3), containing asparagine in the P₂ position. In contrast, the (2*S*,3'*S*)- tetrahydrofuranyl- glycine (3'*S*-Thfg) **13**, IC₅₀ = 5.4 nM (*n* = 1), was 100-fold less effective than **12**.¹⁵ This strong stereochemical preference supports a hydrogen-bonding phenomenon as an important factor for ligand binding in the P₂ pocket. While the chirality of the HIV-1 protease active site requires a 3' *R*-Thfg, the 3' *S*-Thfg should prove a useful asparagine surrogate for other binding domains in nature.

The activities of the 3-Thfg-derived inhibitors **12** and **13** in the blockade of HIV-1 viral spread in cell culture using MT4 human T-lymphoid cells infected with the IIIb isolate¹⁵ paralleled the inhibitory potencies against the enzyme. In head to head comparison, the 3' *R*-Thfg-derived **12**, CIC₉₅ = 8 ± 4 nM (*n* = 8), was consistently at least 3-fold more potent than

the Asn-derived Ro 31–8959, $\text{IC}_{50} = 22 \pm 7 \text{ nM}$ ($n = 10$), or 3'-Thfg-derived **13**, $\text{IC}_{50} > 100 \text{ nM}$.

Finally, the inhibition of HIV-2 protease^{16,17} by the 3'-Thfg diastereomer **12** was measured and determined to be 2-fold more potent ($\text{IC}_{50} = 0.24 \text{ nM}$) than Ro 31–8959 ($\text{IC}_{50} = 0.5 \text{ nM}$). Further characterization and development of this novel, unnatural amino acid as a protease inhibitor building block are under investigation.¹⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- (18). Proton NMR and infrared spectra are consistent with assigned structures. Satisfactory ($\pm 0.4\%$) elemental analyses were obtained for new compounds, and all melting points were determined on a Thomas-Hoover capillary melting apparatus and are uncorrected.

Scheme 1^a

^aReagents and conditions: (a) M_5Cl , Et_3N , 0°C , CH_2Cl_2 ; (b) 5 equiv of $(\text{EtO}_2\text{C})_2\text{CH}_2$, NaH , DMF , 100°C , 12 h; (c) 1 N NaOH , EtOH , 48 h then HCl ; (d) CH_3CN , 0.05 equiv of Cu_2O , 80°C , 6 h; (e) Me_3COCl , Et_3N , THF , -78°C then *N*-lithio-(*S*)-(-)-4-benzyloxazolidinone; (f) KNTMS_2 , THF , -78°C , 30 min then trisyl azide, -78°C , 2 min, then AcOH , 30°C , 30 min; (g) LiOH , $\text{THF-H}_2\text{O}$, 45 min.

Scheme II^a

^aReagents and conditions: (a) AcOCMe₂COCl, 5 h, 25 °C then NaOMe, THF, 3 h, 25 °C; (b) 1.2 equiv of 9, 80 °C, *i*PrOH, 12 h; (c) H₂ (1 atm), 5% Pd/C, THF, MeOH, AcOH; (d) EDC, HOBT, DMF, *N*-methylmorpholine; (e) quinaldic acid, Ph₂POCl, Et₃N, THF, -10 °C, 1 h, then 12 h, 25 °C.