Communications to the Editor

Keto/Enol Epoxy Steroids: A New Structural Class of HIV-1 Tat Inhibitors

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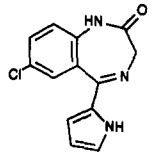
Received June 16, 1993

Introduction. The magnitude of the AIDS pandemic and the need for more effective therapy have focused much attention on the molecular biology of HIV-1. The result has been the identification of several viral-specific molecular targets for drug intervention.[1] Inhibitors of reverse transcriptase and protease have been found[2] which can be related structurally in each case to the natural substrates of the target molecules. The clinical utility of such compounds continues to be assessed.[3] Targeting the inhibition of the HIV-1 nuclear regulatory protein Tat could potentially yield useful drugs since this protein functions as an essential activator of transcription.[4,5] Tat has no known cellular counterpart, and deletions in its gene destroy the ability of HIV-1 to replicate.[6,7] One promising structural class of Tat inhibitors (benzodiazepines) has been reported. Ro 5-3335 (1) was discovered by screening,[8] and Ro 24-7429 (2) resulted from an analog program;[9] the latter compound has entered clinical trials.[10] We wish to report another structurally unique class of Tat inhibitors which resulted from the screening of our compound library.



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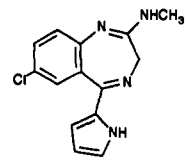

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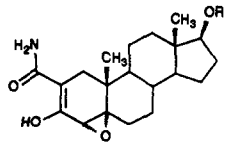
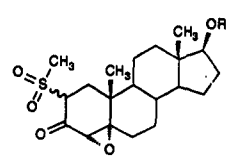


3a, R=H
3b, R=Ac



4a, R=H
4b, R=Ac

Experimental Summary. Chemistry. Compounds were screened as found in our chemical library. Active compounds were assayed for purity (TLC or HPLC) and purified as necessary. Structures were confirmed by ^1H and ^{13}C NMR, infrared, and mass spectrometries. Newly prepared compounds **3a** and **4b** gave acceptable spectral and elemental analyses. Compound 1 was prepared as described.[11]

Primary Assay. To assay for Tat inhibition, a plasmid[12] containing the chloramphenicol acetyl transferase (CAT) gene under the control of the HIV-1 long terminal repeat was transfected into SW480 cells together with a plasmid[13] containing the gene for Tat under the control of the simian virus 40 (SV40) promoter. Cells were incubated in the presence of test compounds for 48 h. The level of Tat-induced expression was determined by monitoring the level of CAT enzyme activity.[14] Compounds were dose-ranged, and percent inhibition dose–response curves were generated. The data from multiple runs (3–5) was used to calculate the IC_{50} (50% inhibitory concentration) and 95% confidence intervals for each compound. In order to determine that inhibition was due to a specific effect on Tat-induced expression rather than to nonspecific effects on transcription, compounds were also tested against a plasmid containing the CAT gene under the control of the Tat-independent SV40 promoter.[14] None of the compounds tested had any effect on Tat-independent transcription at concentrations ranging from 4- to 20-fold higher than their IC_{50} concentration for Tat inhibition.

Whole-Cell Assay. To assay for inhibition of HIV-1 replication,[15] H9 cells were infected with three different concentrations of the HIV IIIB strain. The infected cells were then exposed to the compounds (at 3–5 concentrations) for 5 days with replacement of fresh media and compound on day 3. On the last day of incubation, the amount of HIV replication was determined by assay of the tissue culture media for p24 antigen using the Coulter p24 Elisa kit. The average OD reading from the Elisa (n = 4 per dose) was calculated and the IC_{50} for at least two of the viral doses was determined for 3–5 individual assays. To measure cytotoxicity of these compounds, uninfected cells were incubated for 5 days with the same concentrations of compounds, and the number of viable cells was determined using trypan blue exclusion. The ratio of IC_{50} for cell cytotoxicity to IC_{50} for inhibition of HIV replication defined the therapeutic index; a lower limit of 3 was required for a compound to be considered positive.

Results and Discussion. Active compounds which resulted from screening (e.g., **3b** and **4a**) were all 3-keto-4,5-epoxy steroids. In order to have comparable compounds **3b** was hydrolyzed to give **3a**, and **4a** was acetylated to give **4b**. The biological data for these four compounds is shown in Table I along with comparison data for compound 1. Compound **3b** is about as potent as 1 in the primary assay but the activity observed in the whole cell assay may be due to cytotoxicity as indicated by the low therapeutic index (*vide supra*). Removal of the 17-acetate group to give **3a** resulted in decreased potency in the primary assay while improving the therapeutic index in

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0022-2623/93/1836-2701$04.00/0

Table I. Activities of Enol-Epoxy Steroids in the HIV Tat and Whole-Cell Assays

compd no.	R	Tat assay: IC_{50},[a] μM	whole-cell assay: IC_{50},[b] μM	whole-cell assay: therapeutic index[c]
3a	H	14.3 (10.3–22.1)	1.2 (3)	6.5
3b	Ac	2.6 (2.0–3.1)	2.4 (4)[d]	1.6
4a	H	8.4 (5.8–12.0)	1.4 (4)	3.9
4b	Ac	2.6 (2.0–7.4)	0.3 (4)	4.3
1		4.0 (2.0–7.4)	0.6 (3)	5.9

[a] Confidence limits (95%) are in parentheses. [b] Mean of (*n*) determinations. [c] Whole-cell assay. [d] Apparent activity may be due to cytotoxicity; note low therapeutic index. See text.

the whole-cell assay. By contrast, acetylation of **4a** to give **4b** resulted in increased potency in both assays. Compound **4b** compares favorably with 1 in the primary assay and is about twice as potent in the whole cell assay.

NMR data indicates that compounds **3a** and **3b** exist totally in the enolic form in $CDCl_3$. The C-2 amide group serves to stabilize this tautomer through hydrogen bonding to the enolic proton. In their ^1H spectra both compounds exhibit resonances near 14 ppm, indicative of protons involved in hydrogen bonding. In the ^{13}C spectra of these molecules there are no resonances assignable to ketone carbonyl carbons. There are, however, resonances at 167 and 91 ppm which are assignable as enolic systems.

By contrast, in $CDCl_3$ **4b** exists in the ketone form (50:1) as a 3:2 mixture of equatorial/axial methyl sulfone. The equatorial C-2 proton is completely exchanged, 30 min after addition of a small amount of CD_3OD, as opposed to only 15% of the axial proton. After 12 h in this protic medium the system had reached an equilibrium value of 8:1 in favor of the equatorial sulfone. Compound **4a** in $CDCl_3$ exists almost totally as a single configuration of the methyl sulfone. The hydroxyl group at C-17 apparently serves as a proton source for equilibration, as the CD_3OD did in the case of **4b**. The C-2/H resonance is overlapped with the C-17/H so that coupling constant information is not available. However, it seems reasonable to assume that this molecule would favor the equatorial placement of the methyl sulfone as does **4b**. Finally, ^{13}C NMR data supports the conclusion that **4a** and **4b** exist as the ketone forms. Both compounds have carbonyl resonances near 200 ppm and each lacks signal in the olefinic region which would arise from the enolic tautomer.

The fact that **3a** and **3b** are enolic while **4a** and **4b** are ketonic raises the question of the biologically active form of these molecules. The ketone forms of **3a** and **3b** have not been observed, and it is clear that **4a** and **4b** pass through the enol forms in protic media. It is thus tempting to speculate that it is the enol form, perhaps stabilized by interaction with a target macromolecule, which is responsible for the Tat inhibitory effect. We have carried out a synthetic program around these compounds using the primary assay to develop structure/activity relationships and to localize the pharmacophore. Our results indicate that the keto/enol–epoxide functionality is important to the observed activity and, therefore, suggest that the molecular interactions which result in Tat inhibition by the structurally dissimilar benzodiazepines and steroids may be different. Details of this work will be described in a full paper.

Acknowledgment. The original samples of **3b** and **4a** were prepared by R. G. Christiansen in 1981. We thank S. D. Kingsley, H. S. Putz, L. J. Shaw, and K. A. Ryan for their capable technical assistance, and G. D. Diana for insightful discussions of the work.

Supplementary Material Available: Detailed synthetic procedures and analytical data for **3a**, **3b**, **4a** and **4b** (8 pages). Ordering information is given on any current masthead page.

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