Crystal Structure of Nevirapine, a Non-Nucleoside Inhibitor of HIV-1 Reverse Transcriptase, and Computational Alignment with a Structurally Diverse Inhibitor

Recently, a novel series of dipyridodiazepinones have been identified as potent inhibitors of HIV-1 reverse transcriptase (HIV-1 RT),[1,2] a key viral enzyme required for the replication of the virus.[3,4] A compound from this series, 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one (nevirapine, **1**, Figure 1), is presently undergoing clinical evaluation. Recent work has shown that nevirapine binds to a site on the RT enzyme that is distinct from the substrate binding sites.[5] In order to provide a better understanding of the structural requirements for binding to this allosteric site of the enzyme, a 3-dimensional structure of nevirapine was determined by single-crystal X-ray diffraction methods.[6]

Nevirapine adopts a "butterfly-like" conformation and is folded such that the angle that subtends the intersecting planes of the pyridine rings is 121°. Due to electron delocalization effects, the amide moiety in the 7-membered ring adopts a planar conformation (C4a–N5–C6–C6a torsion angle = −4°). The cyclopropyl substituent points up and away from the molecular framework of the tricyclic system, with the plane of the cyclopropyl ring being almost perpendicular to the pseudoplane of the boat conformation of the 7-membered ring. The hybridization at N11 exhibits

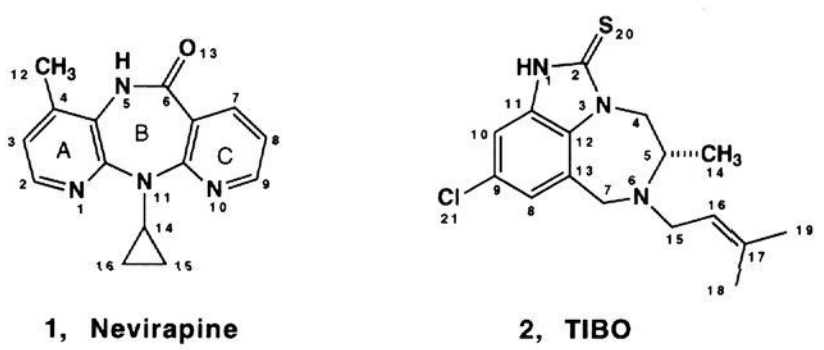

Figure 1. Chemical structures of nevirapine (**1**) and a TIBO derivative (**2**).

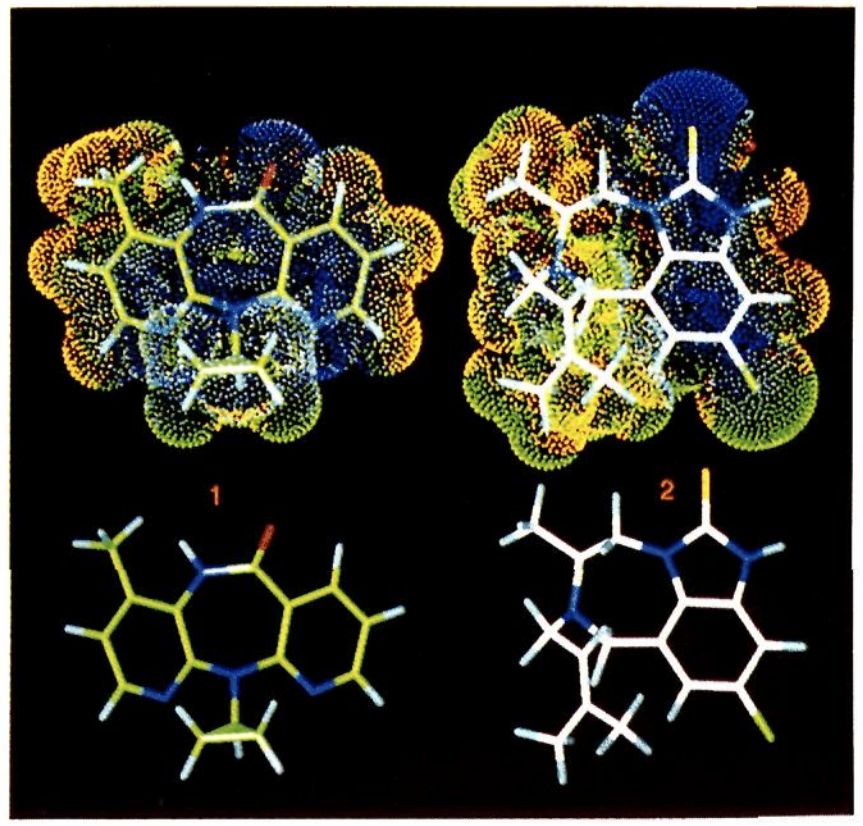

Figure 2. Optimized geometries of **1** and **2** showing electrostatic potential surfaces using Sybyl 5.41.[13] In this range yellow is positive, green is neutral, and blue is negative.

significant sp^3 character, as reflected by the N11–C10a and N11–C11a bond lengths of 1.418 (4) and 1.425 (4) Å, respectively, which compare to a mean C–N bond length of 1.47 Å and a C=N bond length of 1.28 Å.[7]

Semiempirical calculations[8] indicate that the low-energy conformation of nevirapine is in good agreement with the X-ray structure. The main differences obtained between the calculated and observed structures include smaller (by 3.5–4.2°) exocyclic valency angles at C10a, C11a, and C14 in the observed structure and small distortions of the 7-membered ring. For example, examination of the torsion angles indicate that in the crystal structure there is slight puckering in the 7-membered ring along the N5–C4a and C4a–C11a bonds (by 11.3° and 14.8°, respectively), and some flattening (by 14.6°) along the N11–C11a bond. It is felt that the calculated and observed structures are energetically similar since a least squares fit of the corresponding non-hydrogen atoms results in a RMS of 0.17 Å. In support of this claim, geometry optimization using the X-ray structure as starting input yielded back the calculated structure.

Recently, the structure of a tetrahydroimidazobenzodiazepinone (TIBO) (R82913, **2**) inhibitor of HIV-1 RT has

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(6) Single crystals of dimensions 0.15 × 0.35 × 0.70 mm obtained from methanol are: $C_{15}H_{14}N_4O$, M = 266.3, monoclinic, space group $P2_1/c - C^5_{2h}$ (No. 14), a = 7.009 (2) Å, b = 18.932 (6) Å, c = 9.647 (3) Å, β = 97.21 (2)°, V = 1269.9 (7) Å^3, Z = 4, d_{calcd} = 1.393 g cm^{-3}, μ_a(Cu Kα) = 0.70 mm^{-1}. A total of 1888 independent reflections having $2\theta < 120.0°$ were collected on a computer-controlled Nicolet autodiffractometer using θ–2θ scans and Nickel-filtered Cu Kα radiation (λ = 1.5419 Å). The structure was solved using Direct Methods techniques with the Siemens SHELXTL-PLUS software package as modified at Crystalytics Company, Lincoln, NE. The resulting structural parameters were refined to convergence {R_1 (unweighted, based on F) = 0.045 for 1237 independent reflections having $I > 3\sigma(I)$} using counter-weighted full-matrix least-squares techniques and a structural model which incorporated anisotropic thermal parameters for all non-hydrogen atoms and isotropic thermal parameters for all hydrogen atoms. Hydrogen atoms H_{5N}, H_{14}, H_{15a}, H_{15b}, H_{16a}, and H_{16b} were located from a difference Fourier map and refined as independent isotropic atoms. The methyl group (C_{12} and its hydrogens) was refined as a rigid rotor with sp^3-hybridized geometry and a C–H bond length of 0.96 Å. The refined positions for the rigid rotor methyl group gave C–C–H angles which ranged from 108° to 111°. The remaining hydrogen atoms were fixed at idealized sp^2- or sp^3-hybridized positions with a C–H bond length of 0.96 Å.

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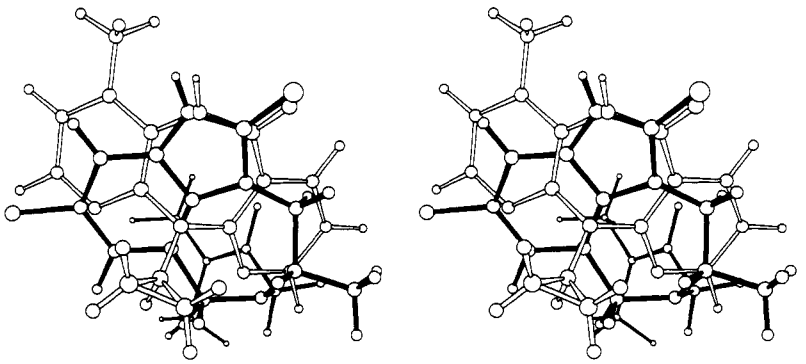

Figure 3. A structural alignment of **1** (open bonds) and **2** (filled bonds) giving the best score using the SEA program (E_{total} = -95.6 kcal/mol).

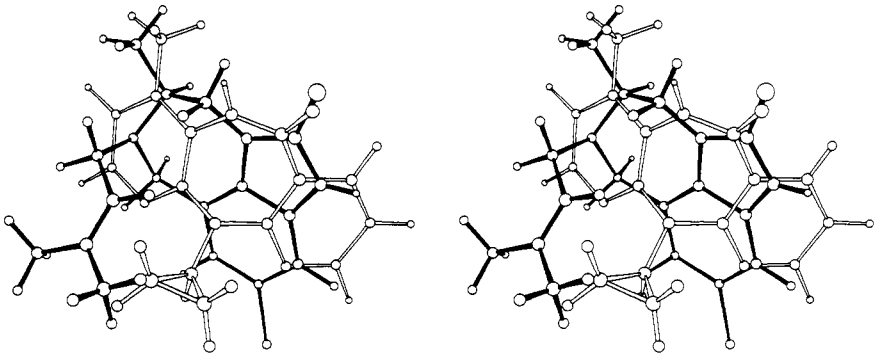

Figure 4. A structural alignment of **1** (open bonds) and **2** (filled bonds) giving the second best score using the SEA program (E_{total} = -93.3 kcal/mol).

been determined by X-ray crystallography.[9,10] Results from a photoaffinity labeling study[5] indicate that the two seemingly structurally distinct classes of inhibitors share a common allosteric binding site in the enzyme. In order to gain some molecular insight into the binding modes of these inhibitors, we have attempted to obtain the optimal alignment of the 3-dimensional structures of **1** and **2**, using both the electrostatic potential field and steric shape as guiding factors.

Using the SEA program,[11] 2000 overlays were generated. The top 20 overlays were ranked according to favorable energetic considerations and the best overlay structures were visually examined. The flexible side chain of **2** was adjusted to achieve a better visual fit,[12] a MOPAC calculation[8] was performed to optimize the geometry (see Figure 2), and the SEA program was rerun. The two best alignments (based on energy scoring) between the two molecules are shown in Figures 3 and 4. In Figure 3 (the alignment with the highest score) several features of the two molecules are well aligned. However, neither the C12-methyl and the C14-methyl of **1** and **2**, respectively, nor the cyclopropyl and 2-methylbutenyl side chains are proximal in conformational space. The better alignment between the two molecules is shown in Figure 4. In this overlay, there are several salient features which are compatible with the experimental findings.[2,9] First, the two methyl groups [at positions 4 (**1**) and 5 (**2**)] are proximal, thus accounting for the highly specific stereochemical requirement for the placement of a methyl group at position 5 in the TIBO series. Second, the carbonyl and thiocarbonyl groups are in relatively close proximity. Third, there is partial steric overlap between the methylbutenyl and the cyclopropyl side chains in the two inhibitors.[12] Both substitutions in the side chains of the respective molecules are known to be crucial elements for potent activity in the two series.[2,9] The proximity (<1 Å) of the methyl groups, the carbonyl and thiocarbonyl moieties, and the N-bonded lipophilic side chains in the overlay (Figure 2) suggests that they may be relevant pharmacophores. Further refinement and experimental verification of the validity of this alignment are in progress.

Acknowledgment. We are indebted to Dr. Cynthia Day of Crystalytics Co. for the crystallographic analysis of nevirapine.

Supplementary Material Available: Table listings of atomic coordinates, temperature factors, bond lengths, valency angles, and torsion angles (8 pages). Ordering information is given on any current masthead page.

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(12) The flexible side chain of **2** can adopt several low energy conformations without significant energetic penalties. In the computer model, the 2-methylbutenyl group[10] was manipulated in an effort to achieve a best fit to the cyclopropyl side chain in **1**.

(13) Tripos Associates, Inc., St. Louis, MO.

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