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Molecular and Crystal Structure of Efavirenz, a Potent and Specific Inhibitor of HIV-1 Reverse Transcriptase, and Its Monohydrate

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The crystal structures of efavirenz, systematic name: (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one, $C_{14}H_9ClF_3NO_2$, (I), a potent and specific inhibitor of HIV-1 reverse transcriptase, and its monohydrate, $C_{14}H_9ClF_3NO_2 \cdot H_2O$, (II), have been determined by single crystal X-ray diffraction (XRD) analysis. The molecular conformation in both structures has a crane bird like appearance. This structure analysis illustrates the influence of the self-complementary amide group in crystal packing through its involvement in forming helical hydrogen-bonding catemers in both structures. The water molecule in (II) has a cohesive function connecting the efavirenz molecules and, more significantly, forms one-dimensional water chains along the crystallographic a axis, thus providing a possible insight into the role of lattice water in drugs and its formulations.

Keywords: catemers; crane bird; helical chains; HIV-1 reverse transcriptase; molecular conformation

INTRODUCTION

Effective therapy for the treatment of HIV-1 infection and AIDS requires a combination of antiviral drugs. Currently, the standard of care for antiretroviral naïve patients is efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI), along with two other NNRTIs. Efavirenz [1], a HIV protease inhibitor, has demonstrated clinical efficacy in both antiretroviral naive and experienced patients. It is a synthetic purine derivative and similar to zidovudine, zalcitabine, and stavudine. Efavirenz is sold commercially as Sustiva by Bristor Myers Squibb. It was combined with the popular HIV medication

This paper is dedicated to Professor S. S. Rajan on the occasion of his 60th Birthday.

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Truvada, which consists of tenofovir and emtricitabine, all of which are reverse transcriptase inhibitors. This combination of three medications, approved by the USFDA in July 2006 under the brand name Atripla, provides highly active antiretroviral therapy (HAART) in a single tablet taken once a day.

The present study is a continuation of our investigation on the structural characterization of pharmaceutical compounds [2,3]. During the crystallization of efavirenz (I) for polymorphs screening, monohydrate (II) crystals were obtained. The fact that the pharmaceuticals can exist as various polymorphs and/or solvates can be problematic in terms of stability, processing, and solubility. Driven by this additional aspect, we have determined the solid-state structures of (I) and (II) and report them here.

EXPERIMENTAL

Crystal Growth

Efavirenz (100 mg, Emcure Pharmaceuticals Ltd., Pune, India) was dissolved in a boiling mixture of hexane (5 ml) and ethyl acetate (10 ml) in a stirred condition. The hot solution was filtered on a beaker and allowed to evaporate slowly at room temperature to give needles of (I). For the preparation of the monohydrate, 100 mg of efavirenz was dissolved in 2 ml of methonal and 8 ml of ethylacetate in a stirred condition at room temperature. Two days later crystals of (II) were obtained.

Structure Determination

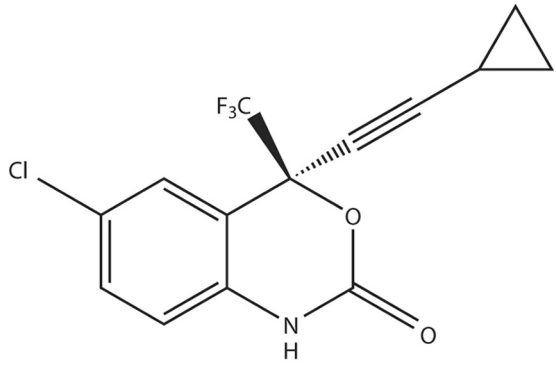
X-ray data for the compounds (I) and (II) were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoKα radiation ($\lambda = 0.71073$ Å) with ω-scan method [4]. Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined from the setting angles of 3461 reflections in the range of $2.37 < \theta < 21.55^\circ$ for (I) and 3751 reflections in the range of $2.31 < \theta < 24.12^\circ$.

Integration and scaling of intensity data were accomplished using SAINT program [4]. The structure was solved by direct methods using SHELXS97 [5] and refinement was carried out by full-matrix least-squares technique using SHELXL97 [5]. Anisotropic displacement parameters were included for all non-hydrogen atoms. All N-bound H atoms of both (I) and (II) were located in a difference density map and refined isotropically. All other H atoms were positioned geometrically and were treated as riding on their parent C atoms, with C-H distances of 0.93–0.98 Å, and with U_{iso}(H) values of 1.2 Ueq(C) for

other H atoms. Following refinement, the anisotropic displacement parameters of atoms C12/C13/C14 of (I) were restrained to be similar (SIMU instruction), and the direction of motion along the axis between these atoms was also restrained (DELU instruction) [6]. The geometries of the three-membered ring was restrained, where distances C12-C13, C13-C14, and C14-C12 were set to a target value 1.49 Å. H atoms attached to the water molecule were refined using geometrical restraints [O-H = 0.89(1) Å and $U_{iso}(H) = 1.5U_{eq}(O)$ for H_2O]. The absolute configuration of the procured material was known in advance and was confirmed by unambiguous refinement of the absolute structure parameter [7]. The crystal and refinement data are given in Table 1.

TABLE 1 Crystal Data and Structure Refinement for (I) and (II)

Compound CCDC No	(I) 728655	(II) 728656
Empirical formula	$C_{14}H_9ClF_3NO_2$	$C_{14}H_9ClF_3NO_2 \cdot H_2O$
Formula weight	315.67	333.69
Temperature	294(2) K	294(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions	a = 5.2111(7) Å b = 15.754(2) Å c = 17.182(2) Å	a = 5.2183(6) Å b = 8.000(1) Å c = 35.201(4) Å
Volume	1410.5(3) $Å^3$	1469.5(3) $Å^3$
Z	4	4
Calculated density	1.486 Mg/m^3	1.508 Mg/m^3
Absorption coefficient	0.307 mm^{-1}	0.304 mm^{-1}
F(000)	640	680
Crystal size	0.20 × 0.12 × 0.09 mm	0.17 × 0.16 × 0.10
Theta range	1.75 to 25.00°	1.16 to 25.00°
Index ranges	$-6 \le h \le 6$ $-18 \le k \le 18$ $-20 \le l \le 20$	$-6 \le h \le 6$ $-9 \le k \le 9$ $-41 \le l \le 41$
Reflections collected/ unique	13254/2490 [R(int) = 0.0563]	14226/2594 [R(int) = 0.0348]
Completeness	100.0%	100.0%
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2490/37/194	2594/2/209
Observed reflections [$I > 2\sigma(I)$]	2185	2374
Absolute structure parameter	0.10(16)	0.02(11)
Goodness-of-fit on F2	1.156	1.137
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0646, wR2 = 0.1486	R1 = 0.0436, wR2 = 0.1117
R indices (all data)	R1 = 0.0734, wR2 = 0.1542	R1 = 0.0500, wR2 = 0.1235
Largest diff. peak and hole	0.457 and −0.189 $eÅ^{-3}$	0.325 and −0.332 $eÅ^{-3}$



(I), (II) .H_2O

FIGURE 1 Schematic diagram of the compound (I) and (II).

Molecular graphics were computed using DIAMOND [8] and Mercury [9] programs. The schematic diagram of the title compound (I) and (II) is shown in Fig. 1.

RESULTS AND DISCUSSION

In both structures (I) and (II), the molecules crystallize in space group $P2_12_12_1$ with same handedness (the asymmetric center C8 has S configuration). The molecular structure observed in (I) (Fig. 2) and (II) (Fig. 3) is composed of a central bicyclic benzoxazin-2-one ring, substituted at C8 by cyclopropylethynyl and trifluoromethyl groups and at C5 by a chlorine atom. There are no major discrepancies in bond lengths and angles in the two structures (Table 2). The efavirenz molecule in both structures displays an overall appearance of a "crane bird" (Fig. 4). In both (I) and (II), the orientation of the cyclopropylethynyl and trifluoromethyl groups about the hetero ring is equatorial and axial, respectively. It is noteworthy to mention that in the crystal structure of the complex Efavirenz-HIV protease reverse transcriptase [10] (Protein Data Bank entry Ifk9), the extracted ligand structure (Fig. 4) shows the cyclopropylethynyl and trifluoromethyl groups having a similar orientation. Incidentally, in both structures, the cyclopropyl and trifluoromethyl groups face the same side, while in the complex efavirenz-HIV protease reverse transcriptase structure (Ifk9), they face opposite ways (Fig. 4). The conformation of the benzoxazin-2-one rings in both the structures are distorted half-chairs with O1 and C1 deviating from the mean plane described by the remaining four atoms.

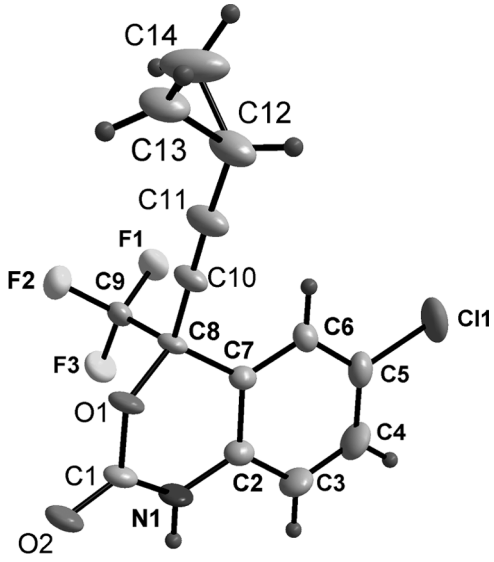


FIGURE 2 A perspective drawing showing the atom-numbering scheme of (I). Displacement ellipsoids are drawn at the 30% probability level, and H atoms are shown as small spheres of arbitrary radii.

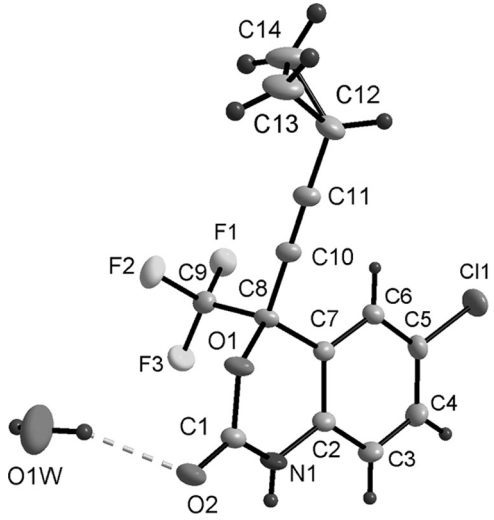


FIGURE 3 A perspective drawing showing the asymmetric unit of (II), with atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level, and H atoms are shown as small spheres of arbitrary radii. The intramolecular hydrogen bond is marked as dashed lines.

TABLE 2 Selected Geometric Parameters (Å and °) of (I) and (II)

Compounds	(I)	(II)
C1-N1	1.327(5)	1.327(4)
C1-O1	1.367(4)	1.364(3)
C2-N1	1.385(5)	1.409(4)
C8-O1	1.450(4)	1.460(3)
N1-C1-O1	116.8(3)	117.3(2)
O1-C8-C10	105.1(3)	105.1(2)
C1-O1-C8	120.5(3)	120.6(2)
N1-C2-C7-C8	−4.9(5)	−4.0(4)
C2-C7-C8-O1	28.7(4)	28.0(3)
O1-C1-N1-C2	5.8(6)	6.4(4)
C7-C2-N1-C1	−14.3(6)	−15.2(4)
N1-C1-O1-C8	22.6(5)	22.2(4)
C7-C8-O1-C1	−38.7(4)	−38.3(3)
C10-C8-O1-C1	−161.7(3)	−161.7(2)
C9-C8-O1-C1	82.9(4)	83.3(3)

Fig. 5 illustrates the packing for (I), which involves helical hydrogen bonding catemers proceeding from the amide NH of each molecule to amide C=O of a neighbor, screw related in b; the hetro ring oxygen is not involved in hydrogen bonding (Table 3). Screw related pairs of parallel single strand helices pass counterdirectionally along the bc faces of each chosen cell at 1/2 and 1 or 0 in c. Monohydrate (II) (Fig. 6), although having similar packing features as described for (I) above, has additionally a water molecule which plays a cohesive role by connecting the efavirenz molecules. Recently, the study of lattice water molecules has attracted much attention due to their

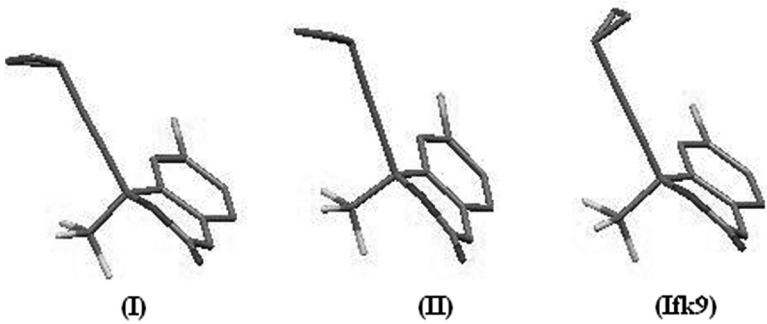


FIGURE 4 Overall molecular conformation of Efavirenz viewed along the aromatic ring (C2–C7) plane displaying the "crane bird"–like appearance. H atoms have been omitted for clarity.

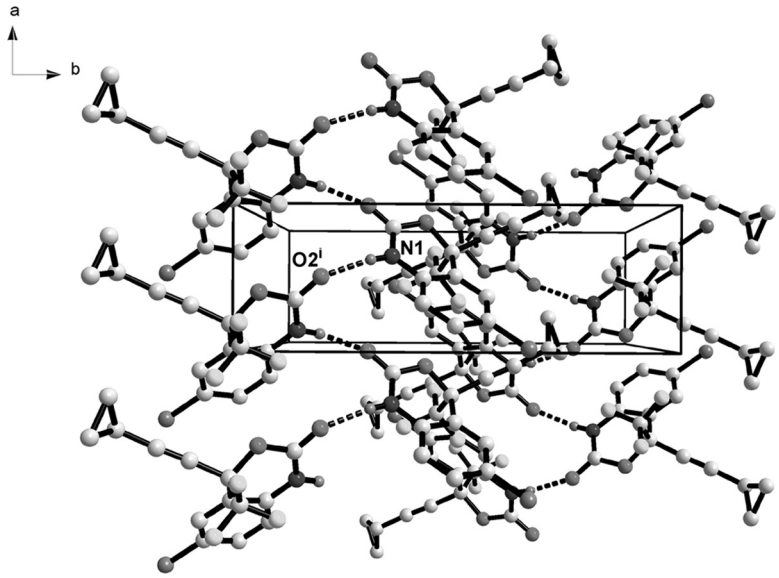


FIGURE 5 A partial packing diagram for (I) viewed down the c axis, depicting the catemers created by hydrogen-bonding proceeding from the NH of each molecule to the amide C=O of a screw-related neighbor. The parallel, screw related chains pass counterdirectionally through the cell in the b direction. H atoms not involved in hydrogen bonds have been omitted for clarity. [Symmetry code: (i) $x-1/2, -y+1/2, -z$].

fundamental importance in chemical and biological processes [11,12]. The water molecule acts as both donor and acceptor (Table 3). It connects the efavirenz molecules as a donor to the O2 atom of the carbonyl group of an efavirenz residue at (x,y,z) and to itself (screw related) to form one-dimensional infinite water chains (Fig. 7). As a result, the

TABLE 3 Hydrogen Bonding Geometry (Å and °) for (I) and (II)

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
(I)				
N(1)-H(1N)...O(2)#1	0.883(10)	1.977(14)	2.849(4)	169(5)
(II)				
N(1)-H(1N)...O(2)#2	0.77(3)	2.11(4)	2.873(3)	168(4)
O(1W)-H(1W)...O(1W)#3	0.91(4)	2.09(3)	2.861(3)	142(5)
O(1W)-H(2W)...O(2)	0.895(10)	2.063(19)	2.924(4)	161(5)

Where: #1 $x-1/2, -y+1/2, -z$ #2 $x-1/2, -y+3/2, -z$ #3 $x-1/2, -y+1/2, -z$.

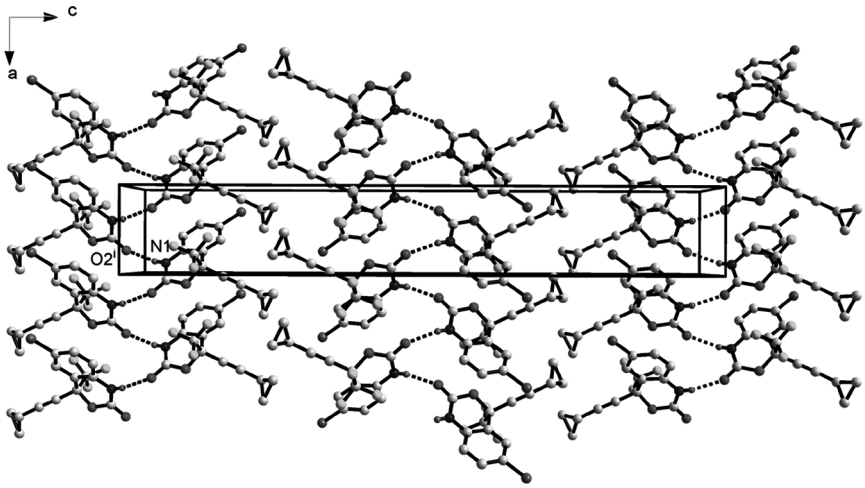


FIGURE 6 A partial packing diagram for (II) viewed down the b axis, showing the catemers created by hydrogen-bonding proceeding from the NH of each molecule to the amide C=O of a screw-related neighbor. The parallel, screw related chains pass counterdirectionally through the cell in the c direction. Water molecule and H atoms not involved in hydrogen bonds have been omitted for clarity. [Symmetry code: (i) $x-1/2, -y+3/2, -z$].

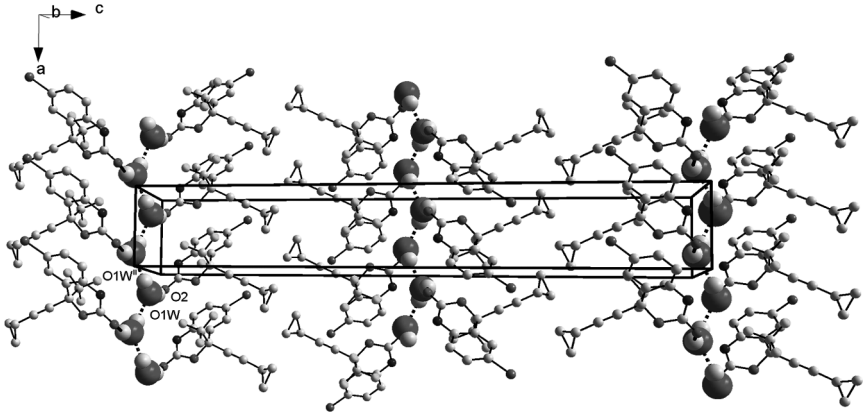


FIGURE 7 A packing view of (II) along the b axis, showing the infinite one-dimensional water chains. The water moelcules are shown in Corey–Pauling–Koltun (CPK); space-filling format. H atoms of efavirenz molecules have been omiited for clarity. [Symmetry code: (ii) $x-1/2, -y+1/2, -z$].

molecular framework in the crystal packing gets divided into hydrophobic and hydrophilic layers along the c axis, and this may provide some insight into the role of lattice water in drugs and its formulations. The water O atoms in the chain are coplanar.

In the context of the important chemical and geometric parameters required (pharmacophore) for biological activity [13], the distances of the two terminal atoms Cl and O2 from the centroid of the cyclopropyl plane are: 7.21 Å, 7.26 Å (I), 7.32 Å, 7.24 Å (II), and 6.56 Å, 7.25 Å (Ifk9).

To summarize, efavirenz and its monohydrate crystallize in the orthorhombic system. In both crystal structures, the efavirenz molecule adopts a similar conformation, although there is additionally a water molecule in (II). The water molecule plays an important role in the construction of the molecular frame work, particularly forming one dimensional water chains.

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