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RESEARCH ARTICLE

USE OF X-RAY CRYSTALLOGRAPHY TO DETERMINE THE CONFIGURATION OF SAQUINAVIR MYSELATE

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ABSTRACT

The absolute configuration of the Saquinavir myselate has been determined. The structural analysis confirms the absolute stereochemistry for (2S)-N-[(2S, 3R)-4-[(3S)-3-(tert-butylcarbamoyl)-decahydroisoquinolin-2-yl]-3-hydroxyl-1-phenylbutan-2-yl]-2-(quinolin-2ylformamido) butanedia midemyselate. Saquinqvir myselate exhibits monoclinic crystal system and P21 space group with unit cell dimensions of $\alpha=90^{0}$ $\beta= 114.848(2)^{0}$ and $\gamma=90^{0}$Stereochemistry observed using platon is N(1)-S, C(2)-S, C(7)-S, C(9)-S, C(16)-R, C(17)-S and C(26)-S.

INTRODUCTION

Single crystal X-ray crystallography is the most powerful structural method for the determination of the 3D structures of molecules. While the results of a routine diffraction experiment readily provide unambiguous determination of the relative configuration of all stereogenic centres in the molecule, determination of absolute configuration is more challenging. From single-crystal XRD data it is possible to solveandre fine the crystalline structure of a new material. It is an on-estructive analysis and the possibilities of success are very high and have radically increased over the last 15 years with the improvement of the experimental devices and the continuous progress in crystal-structure solution and refinement methodologies (**Clegg *et al.*, 2001; Müller *et al.*, 2005**). Knowledge of the crystal structure is of crucial relevance for aproper understanding of the material properties.

Perhaps the single most challenging task facing the research chemists today is the efficient, cost-effective synthesis and characterization of enantio-pure compounds. The paramount importance of chirality is based on the fact that the physiological and/or pharmacological activity of a compound is often determined by its interaction with chiral-based receptors in the body. It is now a standard requirement for all new drugs to be characterized as single enantiomers (**Food and Drug Administration, 1992; Gawley and Aubé, 1996**).

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Saquinavir, chemically (2S)-N-[(2S,3R)-4-[(3S)-3-(tert-butylcarbamoyl)-decahydroisoquinolin-2-yl]-3-hydroxyl-1-henylbutan-2-yl]-2-(quinolin-2ylformamido) but an ediamide, is a peptidomimetichydroxyl ethylamine highly specific HIV-1 and HIV-2protease enzyme inhibitor used in the management of human immunodeficiency virus, HIV (**Forestier *et al.*, 2001**) . It is used alone or in combination with other antiretroviral drugs. Combination of SQV with two nucleoside analogues has been regarded as standard therapy in HIV in fected patients (BHIVA Guidelines Co-ordinating Committee, 1997; Feinberg, 1997).

Different stereoisomers of chiral drug molecules possess different biological properties (**Wang *et al.*, 2010; Siccardi *et al.*, 2003**). Conjugates attached with different stereo isomeric promoieties may be featured with various physicochemical and biological properties. As concerns absolute-configuration determination using resonant- scattering effects, this is clearly impossible from a powder. The rings or lines in the powder pattern due to the *hkl*and *–h–k–l* reflections (Friedel opposites) necessarily over lap completely. It is thus only possible at best to obtain a value for the average intensity of each Friedel pair, their difference intensity engendered by resonant scattering being totally obscured by their mutual overlap.

MATERIALS AND METHODS

The saquinavir mesylate used for the analysis is synthesised and the identity and quality of the material is confirmed which includes purity and assay by HPLC, physical characteristics

such as differential scanning calorimetry and X-ray powder diffraction. The standard material mica NBS 675 was obtained from the national Institute of standards and technology. PXRD pattern of the sample were recorded at Room temperature on Bruker's D8 Advance diffract meter (Karlsruche, West Germany) Cu Kα radiation (1.54 ^{0}A), at 40Kv,35mA passing through Nickel filter with divergence slit 0.3^0,anti-scattering slit 0.3^{0}and receiving slit 1mm.The diffract meter was equipped with a 2θ compensating slit, and was calibrated for accuracy Korunprobe. Samples were subjected to X-ray powder diffraction analysis in continuous mode with a step size of 0.005^{0}and step speed of 1 Sec/Step over an angular range of 5-50^0 2θ.Five hundred milligrams powder mixture was loaded in a 25mm holder made of poly methyl methacry late and pressed by a clean glass slide to ensure co planarity of the powder surface with the surface of the holder. The sample holder was rotated in a plane parallel to its surface at 30rpm during the measurements. Obtained diffract grams were analyzed with DIFFRAC^{plus}EVA (ver.9.0) diffraction software.

Single crystal X-ray diffraction data were collected at Room temperature with a Bruker smart Apex CCD diffractometer. Program used of crystal resolution and refinement is SHELXTL-PLUS (**Sheldrick, 1994**).

RESULTS

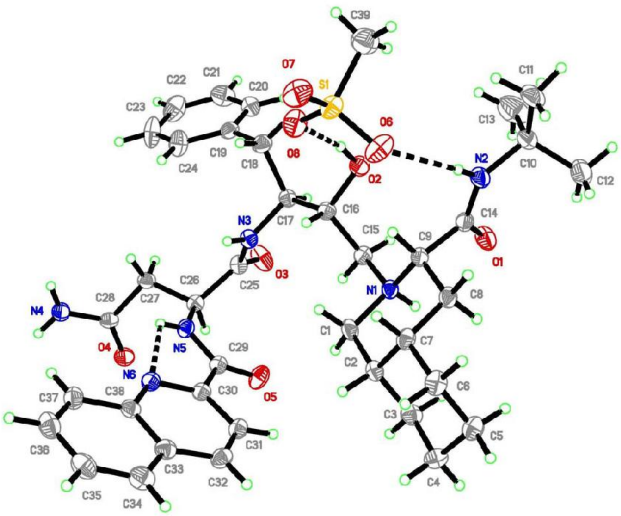

Fig.1. Crystal structure of saquinavir mesylate obtained by single crystal difractometer

Table 1. Selected crystal and Refinement details

1.	Crystal system	Monoclinic
2.	Unit cell dimensions	a= 14064^{0}A α=90^0 b= 9.3754^{0}A β=114.84^0 c=15.971^{0}A γ=90^0
3	Crystal size	0.18x 0.16x 0.08mm^3
4	Volume	1989.7(5)^{0}A^3
5	Z	2
6	Density	1.280Mg/m^3
7	Completeness to θ= 25.00^0	96.5%
8	Data/Resistants/ Parameters	5413/1/519
9	R indices	R1=0.0359, wR2=0.0937
10	Flack parameter	0.17

Table 2. Hydrogen bond data

D-H…A	d(D-H)	D(H-A)	D(D—A)	<(DHA)
N(1)-H(1N)...O(7)#1	0.83(3)	2.17(3)	2.859(3)	141(2)
N(2)-H(2N)...O(6)	0.84(3)	2.33(3)	3.160(3)	168(3)
N(3)-H(3N)...O(4)#2	0.83(3)	2.08(3)	2.891(3)	166(3)
N(4)-H(4N)...O(5)#2	0.85(3)	2.10(3)	2.935(3)	166(3)
N(4)-H(5N)...O(8)#3	0.86(3)	2.38(3)	3.222(3)	165(3)
N(5)-H(6N)...N(6)	0.77(3)	2.28(3)	2.673(3)	113(2)
O(2)-H(1O)...O(8)	0.93(4)	1.89(4)	2.801(3)	166(3)

Table 3. Displacement parameters data

	x	y	z	U(eq)
C(1)	9365(2)	6853(3)	6306(2)	40(1)
C(2)	10086(2)	6972(3)	5844(2)	38(1)
C(3)	10477(2)	8479(3)	5854(2)	44(1)
C(4)	11174(2)	8542(3)	5366(2)	47(1)
C(5)	12059(2)	7536(3)	5829(2)	45(1)
C(6)	11691(2)	6020(3)	5807(2)	44(1)
C(7)	10941(2)	5869(3)	6244(2)	37(1)
C(8)	11441(2)	6005(3)	7283(2)	38(1)
C(9)	10704(2)	5844(2)	7731(2)	34(1)
C(10)	12509(2)	4790(3)	10180(2)	44(1)
C(11)	12923(2)	3291(4)	10303(2)	64(1)
C(12)	13352(2)	5861(4)	10371(2)	70(1)
C(13)	11981(3)	5029(6)	10802(3)	92(1)
C(14)	11293(2)	6062(3)	8765(2)	37(1)
C(15)	9092(2)	6867(3)	7731(2)	36(1)
C(16)	8660(2)	5395(3)	7742(2)	35(1)
C(17)	7617(2)	5557(3)	7761(2)	34(1)
C(18)	7295(2)	4162(3)	8076(2)	44(1)
C(19)	6322(2)	4316(3)	8176(2)	40(1)
C(20)	6320(2)	4744(3)	9004(2)	49(1)
C(21)	5433(2)	4907(4)	9103(2)	62(1)
C(22)	4531(2)	4666(4)	8365(3)	71(1)
C(23)	4518(2)	4262(4)	7537(3)	76(1)
C(24)	5401(2)	4073(4)	7440(2)	59(1)
C(25)	6294(2)	7115(3)	6676(2)	36(1)
C(26)	5539(2)	7342(3)	5672(2)	34(1)
C(27)	4504(2)	6734(3)	5490(2)	37(1)
C(28)	3684(2)	7316(3)	4606(2)	36(1)
C(29)	6581(2)	7429(3)	4823(2)	34(1)
C(30)	6802(2)	6768(3)	4074(2)	33(1)
C(31)	7621(2)	7264(3)	3918(2)	37(1)
C(32)	7790(2)	6706(3)	3215(2)	41(1)
C(33)	7143(2)	5644(3)	2656(2)	39(1)
C(34)	7275(2)	4962(3)	1928(2)	50(1)
C(35)	6635(2)	3911(3)	1431(2)	52(1)
C(36)	5819(2)	3507(3)	1622(2)	52(1)
C(37)	5666(2)	4130(3)	2323(2)	43(1)
C(38)	6324(2)	5196(3)	2862(2)	35(1)
N(1)	9872(1)	6931(2)	7337(1)	33(1)
N(2)	11775(2)	4900(3)	9204(2)	42(1)
N(3)	6897(1)	5971(2)	6845(2)	35(1)
N(4)	2996(2)	6400(3)	4087(2)	49(1)
N(5)	5865(2)	6795(2)	4997(2)	35(1)
N(6)	6173(1)	5773(2)	3574(1)	36(1)
O(1)	11352(1)	7262(2)	9093(1)	53(1)
O(2)	9351(1)	4664(2)	8529(1)	46(1)
O(3)	6320(2)	7954(2)	7265(1)	60(1)
O(4)	3679(1)	8585(2)	4419(1)	52(1)
O(5)	7041(1)	8496(2)	5241(1)	49(1)
C(39)	10462(3)	670(4)	9104(2)	69(1)
O(6)	10765(2)	2240(2)	7936(2)	63(1)
O(7)	9917(2)	-23(2)	7417(2)	64(1)
O(8)	9047(1)	1938(2)	7740(2)	59(1)
S(1)	10011(1)	1250(1)	7955(1)	44(1)

Table 4. Bond lengths and bond angles

C(1)-N(1)	1.498(3)	C(6)-C(7)	1.537(3)
C(1)-C(2)	1.526(3)	C(6)-H(6A)	0.9700
C(1)-H(1A)	0.9700	C(6)-H(6B)	0.9700
C(1)-H(1B)	0.9700	C(7)-C(8)	1.512(4)
C(2)-C(3)	1.522(4)	C(7)-H(7)	0.9800
C(2)-C(7)	1.540(3)	C(8)-C(9)	1.532(3)
C(2)-H(2A)	0.9800	C(8)-H(8A)	0.9700
C(3)-C(4)	1.523(4)	C(8)-H(8B)	0.9700
C(3)-H(3A)	0.9700	C(9)-N(1)	1.508(3)
C(3)-H(3B)	0.9700	C(9)-C(14)	1.521(4)
C(4)-C(5)	1.519(4)	C(9)-H(9)	0.9800
C(4)-H(4A)	0.9700	C(10)-N(2)	1.479(3)
C(4)-H(4B)	0.9700	C(10)-C(13)	1.509(4)
C(5)-C(6)	1.515(4)	C(10)-C(11)	1.511(4)
C(5)-H(5A)	0.9700	C(10)-C(12)	1.519(4)
C(5)-H(5B)	0.9700	C(11)-H(11A)	0.9600
C(11)-H(11B)	0.9600	C(17)-H(17)	0.9800
C(11)-H(11C)	0.9600	C(18)-C(19)	1.506(3)
C(12)-H(12A)	0.9600	C(18)-H(18A)	0.9700
C(12)-H(12B)	0.9600	C(18)-H(18B)	0.9700
C(12)-H(12C)	0.9600	C(19)-C(20)	1.383(4)
C(13)-H(13A)	0.9600	C(19)-C(24)	1.386(4)
C(13)-H(13B)	0.9600	C(20)-C(21)	1.382(4)
C(13)-H(13C)	0.9600	C(20)-H(20)	0.9300
C(14)-O(1)	1.229(3)	C(21)-C(22)	1.370(5)
C(14)-N(2)	1.327(3)	C(21)-H(21)	0.9300
C(15)-N(1)	1.518(3)	C(22)-C(23)	1.369(5)
C(15)-C(16)	1.522(3)	C(22)-H(22)	0.9300
C(15)-H(15A)	0.9700	C(23)-C(24)	1.376(4)
C(15)-H(15B)	0.9700	C(23)-H(23)	0.9300
C(16)-O(2)	1.415(3)	C(24)-H(24)	0.9300
C(16)-C(17)	1.548(3)	C(25)-O(3)	1.214(3)
C(16)-H(16)	0.9800	C(25)-N(3)	1.343(3)
C(17)-N(3)	1.450(3)	C(25)-C(26)	1.532(3)
C(17)-C(18)	1.544(4)	C(26)-N(5)	1.444(3)
C(26)-C(27)	1.529(3)	C(34)-C(35)	1.362(4)
C(26)-H(26)	0.9800	C(34)-H(34)	0.9300
C(27)-C(28)	1.519(3)	C(35)-C(36)	1.403(4)
C(27)-H(27A)	0.9700	C(35)-H(35)	0.9300
C(27)-H(27B)	0.9700	C(36)-C(37)	1.363(4)
C(28)-O(4)	1.226(3)	C(36)-H(36)	0.9300
C(28)-N(4)	1.318(4)	C(37)-C(38)	1.405(4)
C(29)-O(5)	1.234(3)	C(37)-H(37)	0.9300
C(29)-N(5)	1.332(3)	C(38)-N(6)	1.359(3)
C(29)-C(30)	1.500(3)	N(1)-H(1N)	0.83(3)
C(30)-N(6)	1.319(3)	N(2)-H(2N)	0.84(3)
C(30)-C(31)	1.403(3)	N(3)-H(3N)	0.83(3)
C(31)-C(32)	1.352(4)	N(4)-H(4N)	0.85(3)
C(31)-H(31)	0.9300	N(4)-H(5N)	0.86(3)
C(32)-C(33)	1.405(4)	N(5)-H(6N)	0.77(3)
C(32)-H(32)	0.9300	O(2)-H(1O)	0.93(4)
C(33)-C(34)	1.409(4)	C(39)-S(1)	1.755(4)
C(33)-C(38)	1.433(3)	C(39)-H(39A)	0.9600
C(39)-H(39B)	0.9600	O(7)-S(1)	1.443(2)
C(39)-H(39C)	0.9600	O(8)-S(1)	1.4559(19)
O(6)-S(1)	1.453(2)		

DISCUSSION

Most biological molecule sarechiralin the sense that they can, in principle, exist as two identical structures that are non-super imposable mirror image so fone another. These two form sare referred to as enantiomersandare related to one another in the same senseasthelef than disrelated to the right hand. Although the molecules can exist in two forms, a ture has evolved such that only single. Enantiomeric forms of chiral molecules exist in living organisms. This is of enormouscon sequence in the field of pharmaceuticals because it means that the two enantiomers of a chiral pharmaceutical, although they may have the same nominal structure, they donotinteract with living organisms in the same manner. While one enantiomer of the drug may have the rapeutic properties, the other can betoxic. Heterogeneous catalytic synthesis offine chemical sand complex chiral molecules is in its infancy. The enantioselective synthesis of such molecules requires environments that are themselves chiraland of asingl ehandedness. This has generated a great deal of interest in the preparation of chiral materials and surfaces for use as hetero geneous catalysts. Them ostwidely pursued approach to the preparation of enantio selective heterogeneous catalysts has been the use of chiral organic templates which bind to the surface of acataly stand create achiral environment in which catalytic reaction scan occur (**Gellman *et al.*, 2001; Quiroga *et al.*, 2011**).

Polycrystalline crystals of Saquinavir mesylate are indexed with monoclinic $P2_1$ with unit cell dimensions of $\alpha=90^{0,}$ $\beta= 114.848\ (2)^0$ and $\gamma=90^0$. Form the single crystal X-ray data bonds, bond angles, iner molecular contacts, hydrogen bonds, displacement ellipsoid and their connectivity is studied. With all the data obtained and by using PLANTON data validation technique the stereo chemistry observed is N(1)-S; C(2)-S; C(7)-S, C(9)-S; C(16)-R; C(17)-S & C(26)-S (Spek, 2003).

Conclusion

From the above discussion it is concluded that Saquinqvir myselate exhibits monoclinic crystal system and P21 space group with unit cell dimensions of $\alpha=90^{0}$ $\beta= 114.848(2)^0$ and $\gamma=90^0$ Stereochemistry observed is N(1)-S, C(2)-S, C(7)-S, C(9)-S, C(16)-R, C(17)-S and C(26)-S.

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