

# Full wwPDB X-ray Structure Validation Report (i)

#### Apr 21, 2024 - 08:41 am BST

PDB ID	:	4ALU
Title	:	Benzofuropyrimidinone Inhibitors of Pim-1
Authors	:	Stout, T.J.; Adams, L.
Deposited on		
Resolution	:	2.60  Å(reported)

This is a Full wwPDB X-ray Structure Validation Report for a publicly released PDB entry.

We welcome your comments at *validation@mail.wwpdb.org* A user guide is available at https://www.wwpdb.org/validation/2017/XrayValidationReportHelp with specific help available everywhere you see the (i) symbol.

The types of validation reports are described at http://www.wwpdb.org/validation/2017/FAQs#types.

The following versions of software and data (see references (1)) were used in the production of this report:

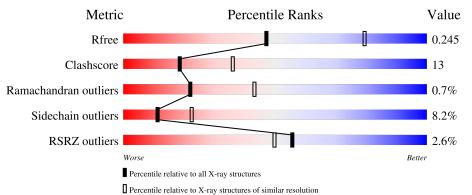
MolProbity		4.02b-467
5		
Mogul	:	1.8.4, CSD as $541be(2020)$
Xtriage (Phenix)	:	1.13
$\mathrm{EDS}$	:	2.36.2
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36.2

# 1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:  $X\text{-}RAY \, DIFFRACTION$ 

The reported resolution of this entry is 2.60 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	$\begin{array}{c} \textbf{Whole archive} \\ (\#\textbf{Entries}) \end{array}$	${f Similar\ resolution}\ (\#{ m Entries,\ resolution\ range}({ m \AA}))$
$R_{free}$	130704	3163 (2.60-2.60)
Clashscore	141614	3518 (2.60-2.60)
Ramachandran outliers	138981	3455 (2.60-2.60)
Sidechain outliers	138945	3455 (2.60-2.60)
RSRZ outliers	127900	3104 (2.60-2.60)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments of the lower bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of cha	in		
1	Δ	308	2%	2001		1.60/
1	A	328	61%	20%	•	16%



#### 4ALU

# 2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 2357 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

• Molecule 1 is a protein called SERINE/THREONINE-PROTEIN KINASE PIM-1.

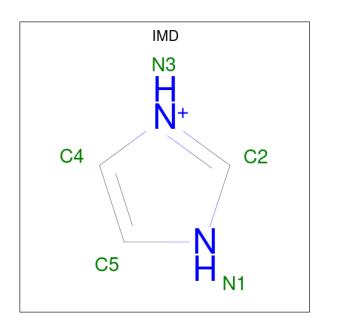
Mol	Chain	Residues	Atoms				ZeroOcc	AltConf	Trace	
1	А	274	Total 2239	C 1429	N 394	O 408	S 8	0	4	0

Chain	Residue	Modelled	Actual	Comment	Reference
А	-14	MET	-	expression tag	UNP P11309
А	-13	HIS	-	expression tag	UNP P11309
А	-12	HIS	-	expression tag	UNP P11309
А	-11	HIS	-	expression tag	UNP P11309
А	-10	HIS	-	expression tag	UNP P11309
А	-9	HIS	-	expression tag	UNP P11309
А	-8	HIS	-	expression tag	UNP P11309
А	-7	GLY	-	expression tag	UNP P11309
А	-6	GLU	-	expression tag	UNP P11309
А	-5	ASN	-	expression tag	UNP P11309
А	-4	LEU	-	expression tag	UNP P11309
A	-3	TYR	-	expression tag	UNP P11309
А	-2	PHE	-	expression tag	UNP P11309
A	-1	GLN	-	expression tag	UNP P11309
А	0	GLY	-	expression tag	UNP P11309
А	1	SER	-	expression tag	UNP P11309
А	49	SER	PHE	conflict	UNP P11309

There are 17 discrepancies between the modelled and reference sequences:

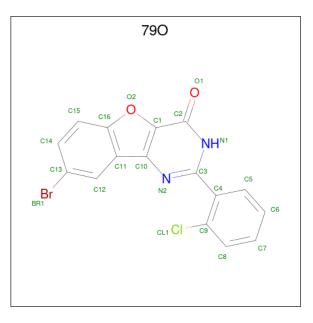
• Molecule 2 is IMIDAZOLE (three-letter code: IMD) (formula:  $C_3H_5N_2$ ).





Mol	Chain	Residues	Atoms		ZeroOcc	AltConf	
2	А	1	Total 5	${ m C} { m 3}$	N 2	0	0

• Molecule 3 is 8-bromo-2-(2-chlorophenyl)[1]benzofuro[3,2-d]pyrimidin-4(3H)-one (three-letter code: 79O) (formula:  $C_{16}H_8BrClN_2O_2$ ).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf		
3	А	1	Total 22	Br 1	C 16	Cl 1	N 2	O 2	0	0

• Molecule 4 is water.



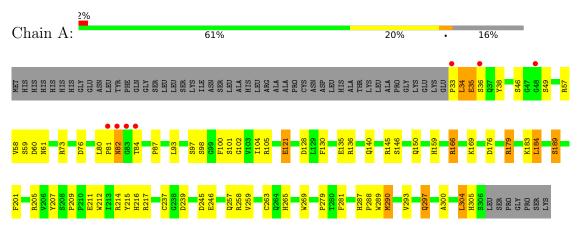
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
4	А	91	Total O 91 91	0	0



# 3 Residue-property plots (i)

These plots are drawn for all protein, RNA, DNA and oligosaccharide chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and electron density. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. A red dot above a residue indicates a poor fit to the electron density (RSRZ > 2). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

• Molecule 1: SERINE/THREONINE-PROTEIN KINASE PIM-1





# 4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 65	Depositor
Cell constants	97.16Å 97.16Å 81.05Å	Depositor
a, b, c, $\alpha$ , $\beta$ , $\gamma$	$90.00^{\circ}$ $90.00^{\circ}$ $120.00^{\circ}$	Depositor
Resolution (Å)	26.51 - 2.60	Depositor
Resolution (A)	26.51 - 2.60	EDS
% Data completeness	$99.8\ (26.51-2.60)$	Depositor
(in resolution range)	99.9(26.51-2.60)	EDS
R <sub>merge</sub>	0.10	Depositor
$R_{sym}$	(Not available)	Depositor
$< I/\sigma(I) > 1$	$2.24 (at 2.60 \text{\AA})$	Xtriage
Refinement program	REFMAC 5.2.0019	Depositor
$R, R_{free}$	0.181 , $0.248$	Depositor
II, II, <i>free</i>	0.181 , $0.245$	DCC
$R_{free}$ test set	679 reflections $(5.04%)$	wwPDB-VP
Wilson B-factor $(Å^2)$	29.2	Xtriage
Anisotropy	0.171	Xtriage
Bulk solvent $k_{sol}(e/Å^3), B_{sol}(Å^2)$	0.37, $44.1$	EDS
L-test for twinning <sup>2</sup>	$<  L  > = 0.46, < L^2 > = 0.29$	Xtriage
Estimated twinning fraction	0.076 for h,-h-k,-l	Xtriage
$F_o, F_c$ correlation	0.93	EDS
Total number of atoms	2357	wwPDB-VP
Average B, all atoms $(Å^2)$	28.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: The largest off-origin peak in the Patterson function is 3.49% of the height of the origin peak. No significant pseudotranslation is detected.

<sup>&</sup>lt;sup>2</sup>Theoretical values of  $\langle |L| \rangle$ ,  $\langle L^2 \rangle$  for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.



<sup>&</sup>lt;sup>1</sup>Intensities estimated from amplitudes.

# 5 Model quality (i)

# 5.1 Standard geometry (i)

Bond lengths and bond angles in the following residue types are not validated in this section: 79O, IMD

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with |Z| > 5 is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond	lengths	Bo	nd angles
	Chain	RMSZ	# Z  > 5	RMSZ	# Z  > 5
1	А	0.90	0/2318	0.88	3/3146~(0.1%)

Chiral center outliers are detected by calculating the chiral volume of a chiral center and verifying if the center is modelled as a planar moiety or with the opposite hand. A planarity outlier is detected by checking planarity of atoms in a peptide group, atoms in a mainchain group or atoms of a sidechain that are expected to be planar.

Mol	Chain	#Chirality outliers	#Planarity outliers
1	А	0	1

There are no bond length outliers.

All (3) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
1	А	184	LEU	CB-CG-CD2	-5.98	100.83	111.00
1	А	128	ASP	CB-CG-OD2	-5.89	113.00	118.30
1	А	128	ASP	CB-CG-OD1	5.87	123.58	118.30

There are no chirality outliers.

All (1) planarity outliers are listed below:

Mol	Chain	Res	Type	Group	
1	А	35	GLU	Peptide	

## 5.2 Too-close contacts (i)

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within



Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	А	2239	0	2194	58	0
2	А	5	0	5	1	0
3	А	22	0	8	1	0
4	А	91	0	0	9	0
All	All	2357	0	2207	59	0

the asymmetric unit, whereas Symm-Clashes lists symmetry-related clashes.

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 13.

All (59) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic	Clash
		distance (Å)	overlap (Å)
3:A:1308:79O:H12	4:A:2091:HOH:O	1.34	1.26
1:A:305:HIS:HB3	4:A:2090:HOH:O	1.44	1.17
1:A:179:ARG:HG2	1:A:179:ARG:HH11	1.25	1.00
1:A:265:HIS:HD2	1:A:287:HIS:HE1	1.11	0.97
1:A:287:HIS:CD2	1:A:289:TRP:H	1.84	0.95
1:A:287:HIS:HD2	1:A:289:TRP:H	0.97	0.93
1:A:265:HIS:CD2	1:A:287:HIS:HE1	1.92	0.87
1:A:36:SER:HA	4:A:2001:HOH:O	1.80	0.82
1:A:265:HIS:HD2	1:A:287:HIS:CE1	2.00	0.77
1:A:287:HIS:HD2	1:A:289:TRP:N	1.81	0.75
1:A:80:LEU:C	1:A:82:ASN:H	1.90	0.74
1:A:179:ARG:HG2	1:A:179:ARG:NH1	2.01	0.69
1:A:80:LEU:O	1:A:82:ASN:N	2.26	0.68
1:A:246:GLU:H	1:A:246:GLU:CD	1.97	0.68
1:A:104[B]:ILE:HG13	1:A:184:LEU:O	1.96	0.65
1:A:57:ARG:HD2	1:A:60:ASP:OD1	1.97	0.64
1:A:97:SER:HB2	4:A:2011:HOH:O	1.98	0.63
1:A:166:ARG:HD3	1:A:189:SER:O	2.00	0.61
1:A:35:GLU:OE1	1:A:35:GLU:N	2.34	0.60
1:A:33:PRO:O	1:A:34:LEU:HB2	2.02	0.60
1:A:140:GLN:HG3	4:A:2026:HOH:O	2.02	0.59
1:A:93:LEU:HD21	1:A:104[B]:ILE:CD1	2.33	0.58
1:A:259:VAL:HG12	1:A:263:CYS:HB3	1.86	0.58
1:A:80:LEU:C	1:A:82:ASN:N	2.57	0.54
1:A:300:ALA:HA	1:A:304:LEU:HB2	1.90	0.54
1:A:265:HIS:CD2	1:A:287:HIS:CE1	2.83	0.53
1:A:135:GLU:HG2	1:A:136:ARG:HG3	1.91	0.51
1:A:201:PHE:HZ	4:A:2043:HOH:O	1.93	0.51

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		Interatomic	Clash
Atom-1	Atom-2	distance $(\text{\AA})$	overlap (Å)
1:A:100:PHE:CE2	1:A:102:GLY:HA3	2.46	0.50
1:A:145:ARG:HG3	1:A:290:MET:HE3	1.93	0.50
1:A:104[A]:ILE:HG13	1:A:121:GLU:HG3	1.95	0.49
1:A:130:PHE:O	2:A:1307:IMD:H4	2.15	0.47
1:A:300:ALA:HA	1:A:304:LEU:HD22	1.96	0.47
1:A:38:TYR:CE2	1:A:57:ARG:HG3	2.51	0.46
1:A:258[B]:ARG:HD3	1:A:259:VAL:N	2.31	0.45
1:A:215:TYR:O	1:A:216:HIS:HB2	2.16	0.45
1:A:176:ASP:OD2	1:A:179:ARG:HD2	2.17	0.45
1:A:246:GLU:CD	1:A:246:GLU:N	2.67	0.45
1:A:145:ARG:HG2	1:A:293:VAL:HG23	1.98	0.44
1:A:146:SER:O	1:A:150:GLN:HG3	2.18	0.44
1:A:237:CYS:HA	1:A:257:GLN:HG3	1.99	0.44
1:A:269:TRP:CD1	1:A:279:PRO:HD3	2.52	0.44
1:A:159:HIS:CE1	1:A:281:PHE:HZ	2.36	0.44
1:A:201:PHE:HB3	1:A:212:TRP:CZ2	2.53	0.43
1:A:214:ARG:HG2	4:A:2061:HOH:O	2.17	0.43
1:A:305:HIS:CD2	1:A:305:HIS:O	2.71	0.43
1:A:101:SER:HB2	1:A:183:LYS:NZ	2.34	0.43
1:A:159:HIS:HD1	1:A:281:PHE:HE2	1.67	0.43
1:A:297:GLN:HB2	4:A:2089:HOH:O	2.19	0.43
1:A:98:SER:HB3	4:A:2012:HOH:O	2.19	0.42
1:A:205:ARG:NH1	1:A:245:ASP:OD1	2.53	0.42
1:A:93:LEU:HD21	1:A:104[B]:ILE:HD11	2.00	0.42
1:A:169:LYS:HB2	1:A:207:TYR:CZ	2.54	0.41
1:A:93:LEU:CD2	1:A:104[B]:ILE:CD1	2.99	0.41
1:A:57:ARG:O	1:A:61:ASN:N	2.49	0.41
1:A:209:PRO:HB2	1:A:211:GLU:CD	2.42	0.41
1:A:179:ARG:NH1	1:A:179:ARG:CG	2.77	0.40

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There are no symmetry-related clashes.

### 5.3 Torsion angles (i)

#### 5.3.1 Protein backbone (i)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the backbone conformation was analysed, and the total number of residues.



Mol	Chain	Analysed	Favoured	Allowed Outliers		Percentiles	
1	А	276/328~(84%)	257~(93%)	17~(6%)	2(1%)	22 43	

All (2) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	А	81	PRO
1	А	34	LEU

#### 5.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent side chain outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	А	249/290~(86%)	229~(92%)	20~(8%)	12 24	

All (20) residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	А	46	SER
1	А	49	SER
1	А	58	VAL
1	А	59	SER
1	А	73	ARG
1	А	76	ASP
1	А	82	ASN
1	А	84	THR
1	А	87	PRO
1	А	105	ARG
1	А	121	GLU
1	А	166	ARG
1	А	179	ARG
1	А	189	SER
1	А	217	ARG
1	А	239	ASP
1	А	288	PRO
1	А	290	MET
1	А	297	GLN

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Mol	Chain	Res	Type
1	А	304	LEU

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. All (5) such sidechains are listed below:

Mol	Chain	Res	Type
1	А	39	GLN
1	А	82	ASN
1	А	265	HIS
1	А	287	HIS
1	А	305	HIS

#### 5.3.3 RNA (i)

There are no RNA molecules in this entry.

### 5.4 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

### 5.5 Carbohydrates (i)

There are no monosaccharides in this entry.

### 5.6 Ligand geometry (i)

2 ligands are modelled in this entry.

In the following table, the Counts columns list the number of bonds (or angles) for which Mogul statistics could be retrieved, the number of bonds (or angles) that are observed in the model and the number of bonds (or angles) that are defined in the Chemical Component Dictionary. The Link column lists molecule types, if any, to which the group is linked. The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with |Z| > 2 is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol Type Chain		Chain Res Lii		Link Bond lengths		Bond angles		les		
	туре	Chain	nes		Counts	RMSZ	# Z >2	Counts	RMSZ	# Z  > 2
2	IMD	А	1307	-	$3,\!5,\!5$	0.47	0	$4,\!5,\!5$	0.38	0
3	790	А	1308	-	20,25,25	2.16	5 (25%)	24,37,37	2.78	9 (37%)



In the following table, the Chirals column lists the number of chiral outliers, the number of chiral centers analysed, the number of these observed in the model and the number defined in the Chemical Component Dictionary. Similar counts are reported in the Torsion and Rings columns. '-' means no outliers of that kind were identified.

Mol	Type	Chain	Res	Link	Chirals	Torsions	Rings
2	IMD	А	1307	-	-	-	0/1/1/1
3	790	А	1308	-	-	4/4/4/4	0/4/4/4

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	А	1308	790	C2-N1	6.24	1.47	1.37
3	А	1308	790	C1-C2	-3.99	1.38	1.47
3	А	1308	790	C3-N1	3.85	1.44	1.38
3	А	1308	790	C4-C3	-2.63	1.42	1.47
3	А	1308	790	C3-N2	2.06	1.37	1.31

All (5) bond length outliers are listed below:

All (9) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	$Observed(^{o})$	$Ideal(^{o})$
3	А	1308	790	C11-C10-N2	6.20	133.87	121.33
3	А	1308	790	N1-C3-N2	-6.16	116.91	123.10
3	А	1308	790	O1-C2-N1	5.02	126.57	120.65
3	А	1308	790	O1-C2-C1	-4.85	114.90	124.37
3	А	1308	790	C1-C10-C11	-4.63	103.76	109.56
3	А	1308	790	C10-C11-C16	3.30	108.92	106.17
3	А	1308	790	C4-C3-N2	2.70	121.99	118.93
3	А	1308	790	C1-C2-N1	2.59	118.52	113.95
3	А	1308	790	C4-C3-N1	2.05	121.10	118.19

There are no chirality outliers.

All (4) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
3	А	1308	790	N2-C3-C4-C9
3	А	1308	790	N1-C3-C4-C9
3	А	1308	790	N2-C3-C4-C5
3	А	1308	790	N1-C3-C4-C5

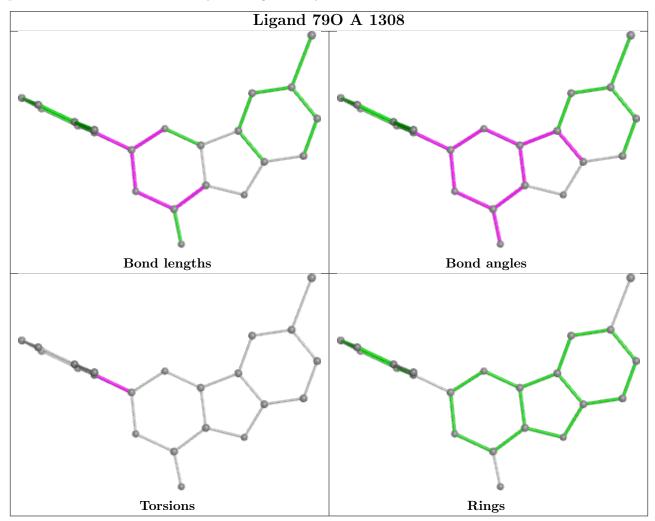
There are no ring outliers.

2 monomers are involved in 2 short contacts:



Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	А	1307	IMD	1	0
3	А	1308	790	1	0

The following is a two-dimensional graphical depiction of Mogul quality analysis of bond lengths, bond angles, torsion angles, and ring geometry for all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the validation Tables will also be included. For torsion angles, if less then 5% of the Mogul distribution of torsion angles is within 10 degrees of the torsion angle in question, then that torsion angle is considered an outlier. Any bond that is central to one or more torsion angles identified as an outlier by Mogul will be highlighted in the graph. For rings, the root-mean-square deviation (RMSD) between the ring in question and similar rings identified by Mogul is calculated over all ring torsion angles. If the average RMSD is greater than 60 degrees and the minimal RMSD between the ring in question and any Mogul-identified rings is also greater than 60 degrees, then that ring is considered an outlier. The outliers are highlighted in purple. The color gray indicates Mogul did not find sufficient equivalents in the CSD to analyse the geometry.





## 5.7 Other polymers (i)

There are no such residues in this entry.

## 5.8 Polymer linkage issues (i)

There are no chain breaks in this entry.



# 6 Fit of model and data (i)

## 6.1 Protein, DNA and RNA chains (i)

In the following table, the column labelled '#RSRZ> 2' contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median,  $95^{th}$  percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled 'Q< 0.9' lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ $>$	#RSRZ>2	$OWAB(Å^2)$	Q<0.9
1	А	274/328~(83%)	-0.47	7 (2%) 56 50	6, 24, 53, 71	0

All (7) RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	А	81	PRO	6.0
1	А	82	ASN	2.9
1	А	48	GLY	2.6
1	А	36	SER	2.6
1	А	83	GLY	2.6
1	А	33	PRO	2.5
1	А	84	THR	2.3

### 6.2 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

### 6.3 Carbohydrates (i)

There are no monosaccharides in this entry.

## 6.4 Ligands (i)

In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median,  $95^{th}$  percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9' lists the number of atoms with occupancy less than 0.9.

Mol	Type	Chain	Res	Atoms	RSCC	RSR	$B-factors(Å^2)$	Q<0.9
3	790	А	1308	22/22	0.92	0.24	47,49,49,52	6

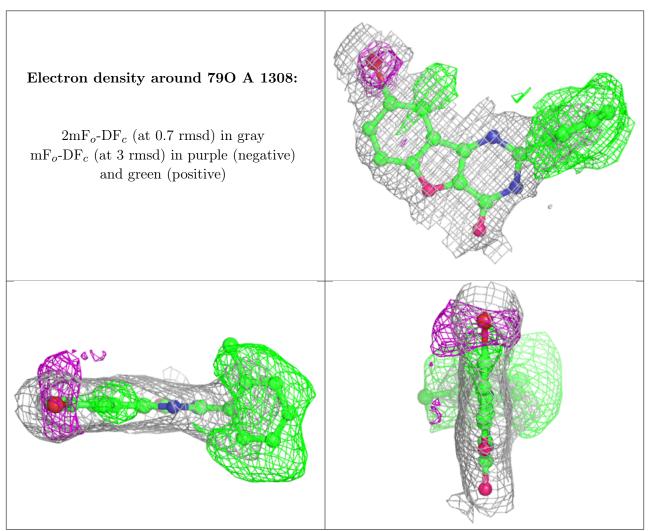
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Mol	Type	Chain	Res	Atoms	RSCC	RSR	$\mathbf{B} ext{-factors}(\mathrm{\AA}^2)$	Q<0.9
2	IMD	A	1307	5/5	0.97	0.12	$26,\!27,\!28,\!31$	0

The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation to approximate a three-dimensional view.



## 6.5 Other polymers (i)

There are no such residues in this entry.

