wwPDB X-ray Structure Validation Summary Report (i)

Nov 7, 2023 – 01:14 AM EST

PDB ID	:	5TQ6
Title	:	Design and Synthesis of a pan-JAK Kinase Inhibitor Clinical Candidate (PF-06263276) Suitable for Inhaled and Topical Delivery for the Treatment of Inflammatory Diseases of the Lungs and Skin
Authors	:	Chrencik, J.; Jones, P.
Deposited on	:	2016-10-23
Resolution	:	2.06 Å(reported)

This is a wwPDB X-ray Structure Validation Summary 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 (i)) were used in the production of this report:

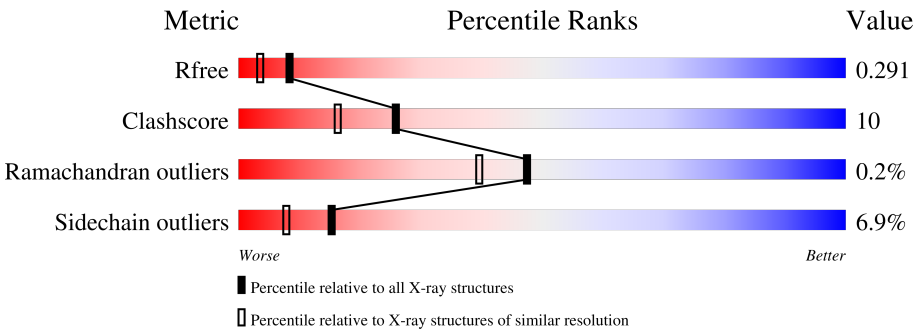
MolProbity	:	4.02b-467
Mogul	:	1.8.5 (274361), CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.36

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 2.06 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	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	130704	2684 (2.08-2.04)
Clashscore	141614	2801 (2.08-2.04)
Ramachandran outliers	138981	2768 (2.08-2.04)
Sidechain outliers	138945	2768 (2.08-2.04)

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%

Mol	Chain	Length	Quality of chain
1	A	304	59% 29% • 9%
1	B	304	67% 20% • 10%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 4846 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.

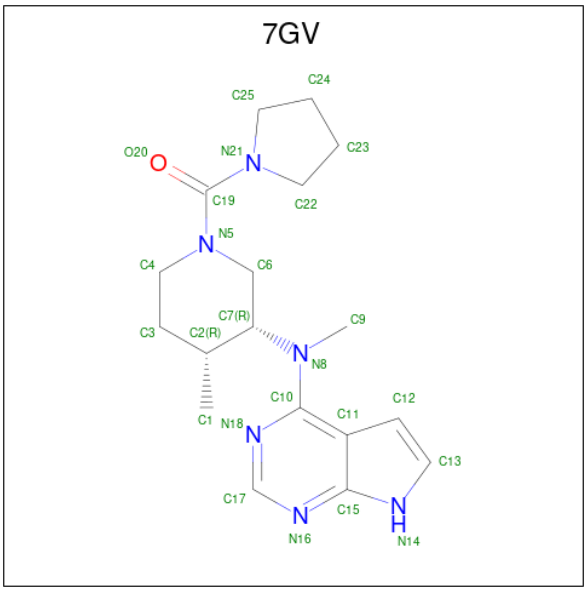
- Molecule 1 is a protein called Tyrosine-protein kinase JAK2.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
			Total	C	N	O	P	S			
1	A	276	2296	1459	394	428	2	13	0	0	0
1	B	273	2277	1449	391	422	2	13	0	0	0

There are 20 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
A	829	MET	-	initiating methionine	UNP O60674
A	830	GLY	-	expression tag	UNP O60674
A	831	HIS	-	expression tag	UNP O60674
A	832	HIS	-	expression tag	UNP O60674
A	833	HIS	-	expression tag	UNP O60674
A	834	HIS	-	expression tag	UNP O60674
A	835	HIS	-	expression tag	UNP O60674
A	836	HIS	-	expression tag	UNP O60674
A	1073	SER	MET	engineered mutation	UNP O60674
A	1076	THR	PHE	engineered mutation	UNP O60674
B	829	MET	-	initiating methionine	UNP O60674
B	830	GLY	-	expression tag	UNP O60674
B	831	HIS	-	expression tag	UNP O60674
B	832	HIS	-	expression tag	UNP O60674
B	833	HIS	-	expression tag	UNP O60674
B	834	HIS	-	expression tag	UNP O60674
B	835	HIS	-	expression tag	UNP O60674
B	836	HIS	-	expression tag	UNP O60674
B	1073	SER	MET	engineered mutation	UNP O60674
B	1076	THR	PHE	engineered mutation	UNP O60674

- Molecule 2 is {(3R,4R)-4-methyl-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl}(pyrrolidin-1-yl)methanone (three-letter code: 7GV) (formula: $C_{18}H_{26}N_6O$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	N	O		
2	A	1	25	18	6	1	0	0
			Total	C	N	O		
2	B	1	25	18	6	1	0	0

- Molecule 3 is water.

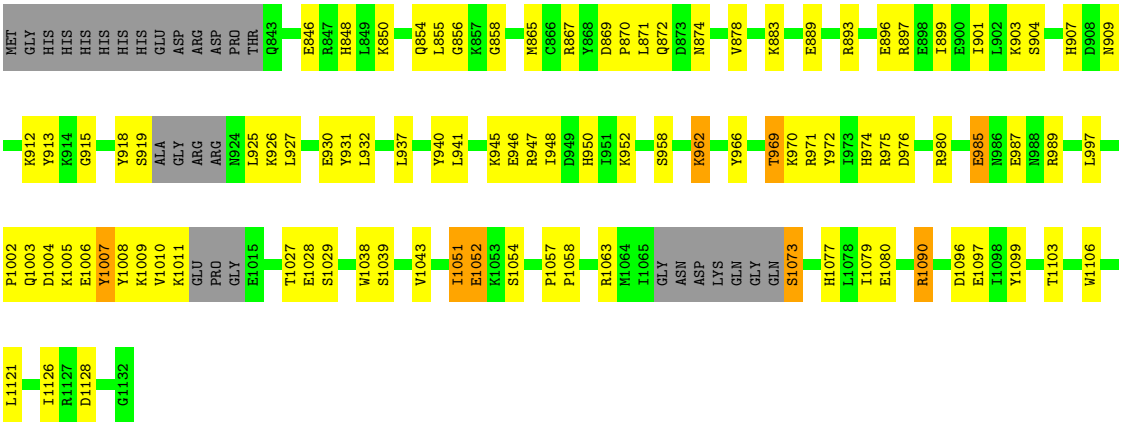
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
3	A	109	109	109	0	0
			Total	O		
3	B	114	114	114	0	0

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. 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. 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.

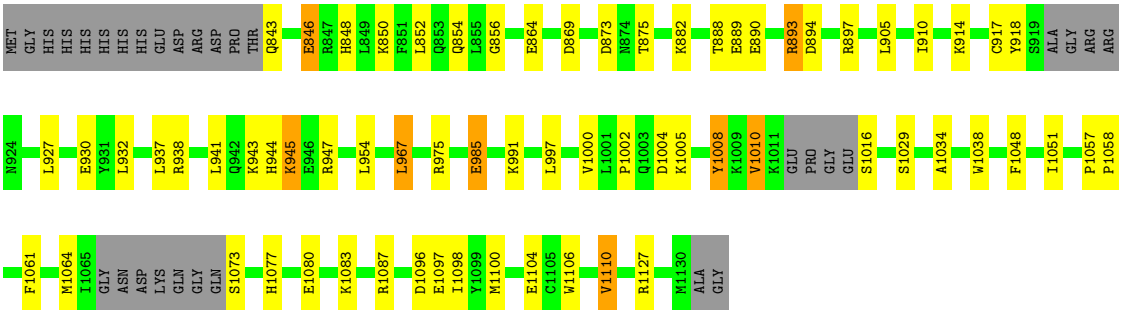
- Molecule 1: Tyrosine-protein kinase JAK2

Chain A:



- Molecule 1: Tyrosine-protein kinase JAK2

Chain B:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	36.98Å 183.40Å 52.58Å 90.00° 110.59° 90.00°	Depositor
Resolution (Å)	47.54 – 2.06 47.54 – 2.06	Depositor EDS
% Data completeness (in resolution range)	92.6 (47.54-2.06) 92.6 (47.54-2.06)	Depositor EDS
R_{merge}	0.07	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	4.54 (at 2.05Å)	Xtriage
Refinement program	REFMAC 5.8.0073	Depositor
R, R_{free}	0.257 , 0.289 0.259 , 0.291	Depositor DCC
R_{free} test set	1906 reflections (5.08%)	wwPDB-VP
Wilson B-factor (Å^2)	18.6	Xtriage
Anisotropy	0.103	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.36 , 29.0	EDS
L-test for twinning[2]	$< \|L\| > = 0.42$, $< L^2 > = 0.24$	Xtriage
Estimated twinning fraction	0.486 for h,-k,-h-l	Xtriage
F_o,F_c correlation	0.92	EDS
Total number of atoms	4846	wwPDB-VP
Average B, all atoms (Å^2)	20.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 14.64% of the height of the origin peak. No significant pseudotranslation is detected.*

[1]Intensities estimated from amplitudes.

[2]Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

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: PTR, 7GV

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		Bond angles	
		RMSZ	$\#\|Z\|>5$	RMSZ	$\#\|Z\|>5$
1	A	0.58	0/2308	0.72	1/3101 (0.0%)
1	B	0.57	0/2289	0.73	1/3077 (0.0%)
All	All	0.58	0/4597	0.73	2/6178 (0.0%)

There are no bond length outliers.

All (2) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	1051	ILE	CG1-CB-CG2	-5.24	99.88	111.40
1	B	967	LEU	CA-CB-CG	5.12	127.08	115.30

There are no chirality outliers.

There are no planarity outliers.

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

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	2296	0	2274	55	0
1	B	2277	0	2261	41	0
2	A	25	0	0	1	0
2	B	25	0	0	0	0
3	A	109	0	0	2	0
3	B	114	0	0	5	0
All	All	4846	0	4535	96	0

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 10.

The worst 5 of 96 close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:B:985:GLU:HG3	1:B:991:LYS:HE3	1.65	0.78
1:B:941:LEU:O	1:B:945:LYS:HB3	1.85	0.75
1:B:894:ASP:OD1	1:B:897:ARG:NH2	2.21	0.73
1:B:1096:ASP:O	1:B:1100:MET:HG3	1.88	0.73
1:A:1052:GLU:HG3	3:B:4104:HOH:O	1.89	0.73

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	A	266/304 (88%)	257 (97%)	9 (3%)	0	100	100
1	B	263/304 (86%)	250 (95%)	12 (5%)	1 (0%)	34	25
All	All	529/608 (87%)	507 (96%)	21 (4%)	1 (0%)	47	39

All (1) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	B	1002	PRO

5.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain 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	A	253/275 (92%)	234 (92%)	19 (8%)	13 6
1	B	252/275 (92%)	236 (94%)	16 (6%)	18 10
All	All	505/550 (92%)	470 (93%)	35 (7%)	15 8

5 of 35 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	B	985	GLU
1	B	1010	VAL
1	B	1087	ARG
1	A	1006	GLU
1	A	1003	GLN

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 14 such sidechains are listed below:

Mol	Chain	Res	Type
1	B	885	GLN
1	B	909	ASN
1	B	1129	ASN
1	B	955	GLN
1	B	1111	ASN

5.3.3 RNA (i)

There are no RNA molecules in this entry.

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

4 non-standard protein/DNA/RNA residues 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	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\#\|Z\| > 2$	Counts	RMSZ	$\#\|Z\| > 2$
1	PTR	B	1008	1	15,16,17	0.72	1 (6%)	19,22,24	0.84	0
1	PTR	A	1007	1	15,16,17	0.66	0	19,22,24	0.87	1 (5%)
1	PTR	B	1007	1	15,16,17	0.88	0	19,22,24	0.86	0
1	PTR	A	1008	1	15,16,17	0.72	0	19,22,24	0.82	0

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
1	PTR	B	1008	1	-	1/10/11/13	0/1/1/1
1	PTR	A	1007	1	-	2/10/11/13	0/1/1/1
1	PTR	B	1007	1	-	0/10/11/13	0/1/1/1
1	PTR	A	1008	1	-	1/10/11/13	0/1/1/1

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	B	1008	PTR	OH-CZ	-2.01	1.36	1.40

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	1007	PTR	OH-P-O1P	-2.03	101.64	109.31

There are no chirality outliers.

All (4) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	A	1007	PTR	CZ-OH-P-O1P
1	B	1008	PTR	CZ-OH-P-O1P
1	A	1008	PTR	CZ-OH-P-O1P
1	A	1007	PTR	CZ-OH-P-O2P

There are no ring outliers.

3 monomers are involved in 5 short contacts:

Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	B	1008	PTR	1	0

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Mol	Chain	Res	Type	Clashes	Symm-Clashes
1	A	1007	PTR	3	0
1	A	1008	PTR	1	0

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	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\#\|Z\| > 2$	Counts	RMSZ	$\#\|Z\| > 2$
2	7GV	A	4000	-	26,28,28	0.62	0	26,40,40	0.82	0
2	7GV	B	4001	-	26,28,28	0.75	0	26,40,40	1.10	1 (3%)

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	7GV	A	4000	-	-	1/16/36/36	0/4/4/4
2	7GV	B	4001	-	-	2/16/36/36	0/4/4/4

There are no bond length outliers.

All (1) bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	B	4001	7GV	N18-C10-N8	-3.27	113.55	117.06

There are no chirality outliers.

All (3) torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	B	4001	7GV	O20-C19-N21-C25
2	A	4000	7GV	O20-C19-N5-C4
2	B	4001	7GV	N5-C19-N21-C25

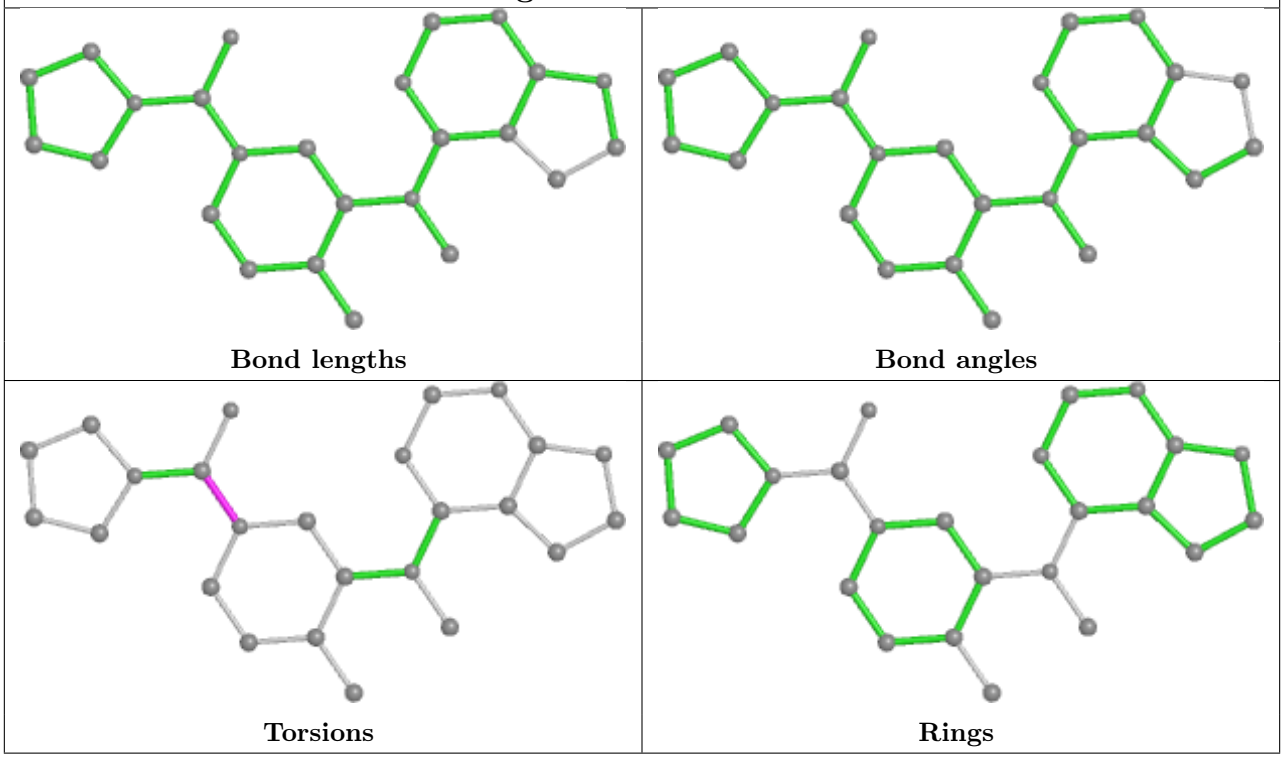
There are no ring outliers.

1 monomer is involved in 1 short contact:

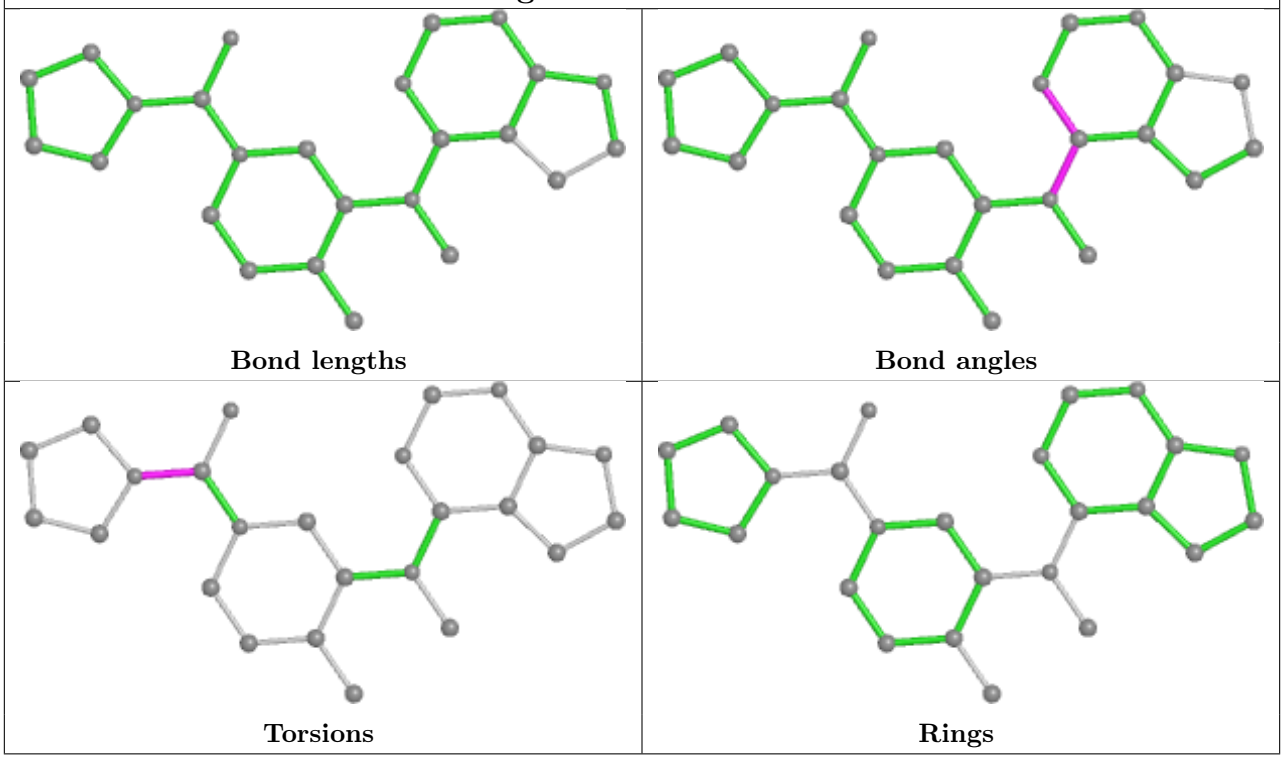
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	A	4000	7GV	1	0

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.

Ligand 7GV A 4000



Ligand 7GV B 4001



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)

Unable to reproduce the depositors R factor - this section is therefore empty.

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

Unable to reproduce the depositors R factor - this section is therefore empty.

6.3 Carbohydrates (i)

Unable to reproduce the depositors R factor - this section is therefore empty.

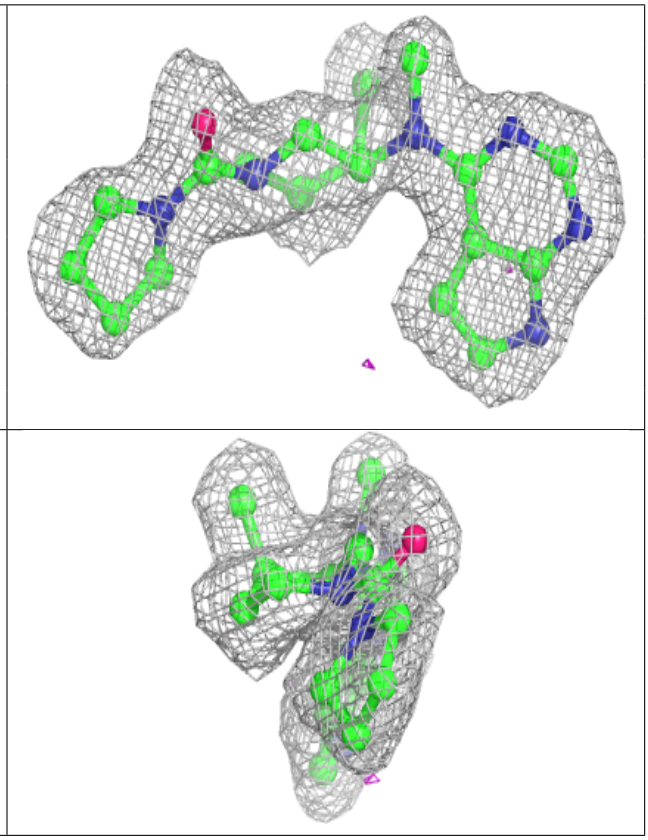
6.4 Ligands (i)

Unable to reproduce the depositors R factor - this section is therefore empty.

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.

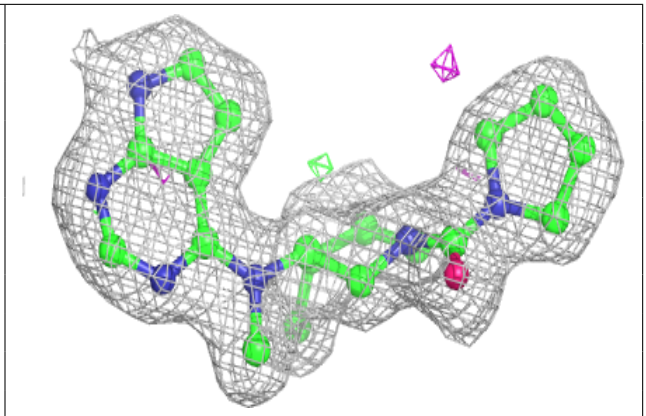
Electron density around 7GV A 4000:

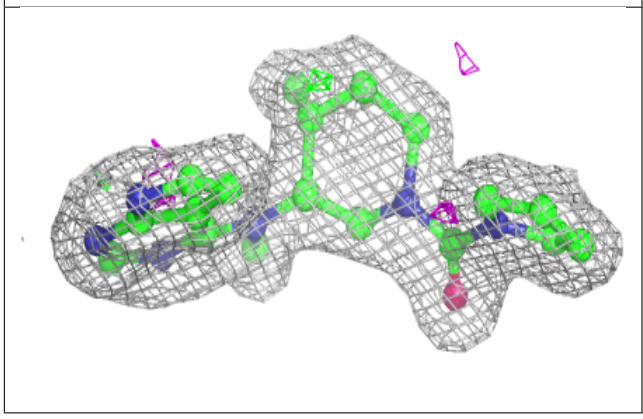
$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative) and green (positive)

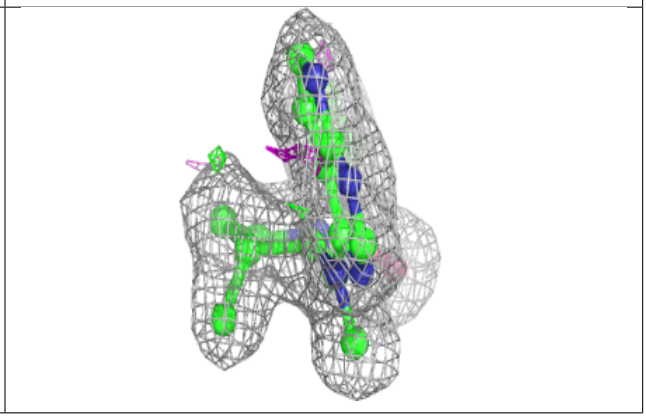


Electron density around 7GV B 4001:

$2mF_o$-DF_c (at 0.7 rmsd) in gray
mF_o-DF_c (at 3 rmsd) in purple (negative)
and green (positive)







6.5 Other polymers (i)

Unable to reproduce the depositors R factor - this section is therefore empty.