wwPDB X-ray Structure Validation Summary Report (i)

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Title	:	Discovery of IRAK4 Inhibitors BAY1834845 and BAY1830839
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Deposited on	:	2022-11-22
Resolution	:	2.12 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 (1)) were used in the production of this report:

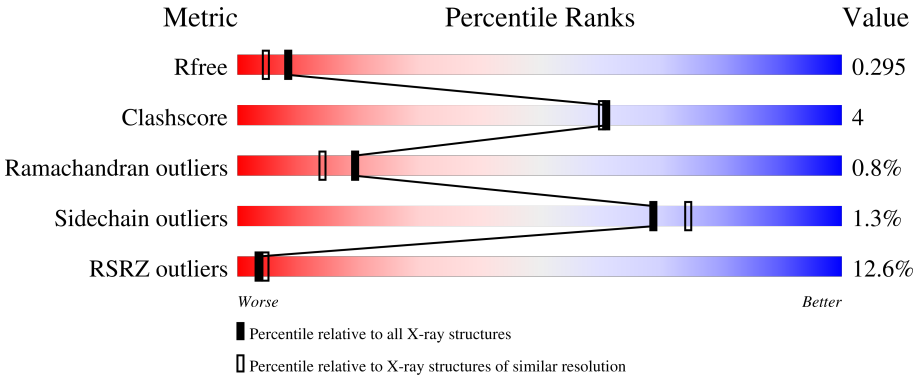
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
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 2.12 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	6241 (2.14-2.10)
Clashscore	141614	6778 (2.14-2.10)
Ramachandran outliers	138981	6705 (2.14-2.10)
Sidechain outliers	138945	6706 (2.14-2.10)
RSRZ outliers	127900	6112 (2.14-2.10)

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 chain
1	AAA	298	12% 84% 7% 8%
1	BBB	298	11% 79% 15% 7%

2 Entry composition (i)

There are 3 unique types of molecules in this entry. The entry contains 4461 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.

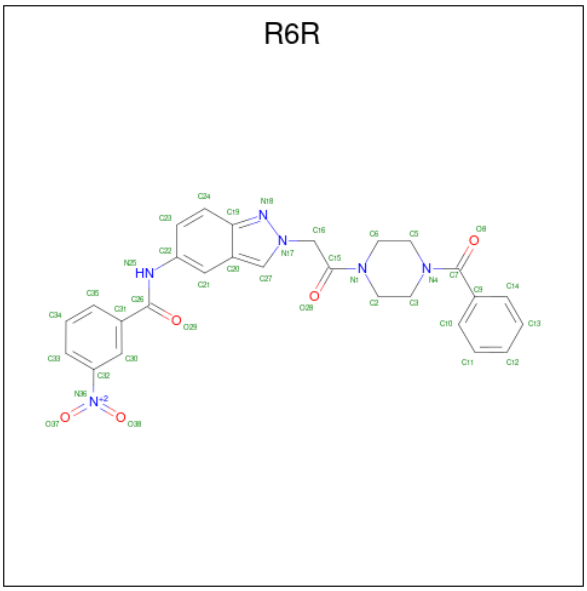
- Molecule 1 is a protein called Interleukin-1 receptor-associated kinase 4.

Mol	Chain	Residues	Atoms						ZeroOcc	AltConf	Trace
1	AAA	273	Total	C	N	O	P	S	0	0	0
			2161	1362	363	420	2	14			
1	BBB	278	Total	C	N	O	P	S	0	0	0
			2183	1371	368	428	2	14			

There are 10 discrepancies between the modelled and reference sequences:

Chain	Residue	Modelled	Actual	Comment	Reference
AAA	163	GLY	-	expression tag	UNP Q9NWZ3
AAA	164	SER	-	expression tag	UNP Q9NWZ3
AAA	400	ALA	LYS	engineered mutation	UNP Q9NWZ3
AAA	401	ALA	GLU	engineered mutation	UNP Q9NWZ3
AAA	402	ALA	GLU	engineered mutation	UNP Q9NWZ3
BBB	163	GLY	-	expression tag	UNP Q9NWZ3
BBB	164	SER	-	expression tag	UNP Q9NWZ3
BBB	400	ALA	LYS	engineered mutation	UNP Q9NWZ3
BBB	401	ALA	GLU	engineered mutation	UNP Q9NWZ3
BBB	402	ALA	GLU	engineered mutation	UNP Q9NWZ3

- Molecule 2 is 3-nitro- {N}-[2-[2-oxidanylidene-2-[4-(phenylcarbonyl)piperazin-1-yl]ethyl]indazol-5-yl]benzamide (three-letter code: R6R) (formula: $C_{27}H_{24}N_6O_5$) (labeled as "Ligand of Interest" by depositor).



Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
2	AAA	1	Total C N O 38 27 6 5	0	0
2	BBB	1	Total C N O 38 27 6 5	0	0

- Molecule 3 is water.

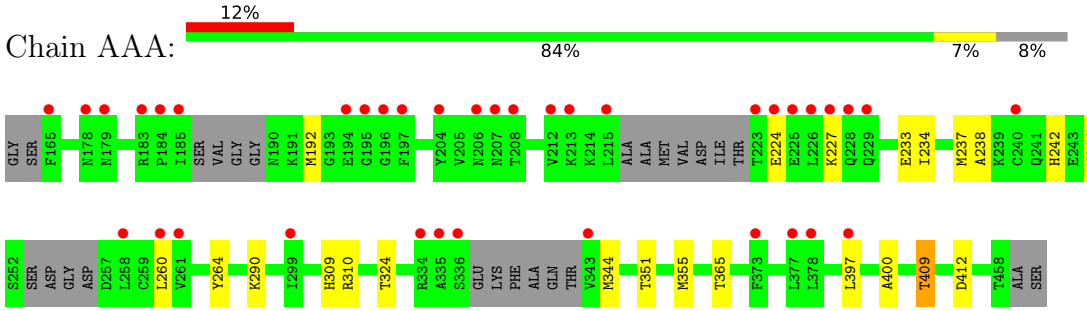
Mol	Chain	Residues	Atoms	ZeroOcc	AltConf
3	AAA	16	Total O 16 16	0	0
3	BBB	25	Total O 25 25	0	0

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.

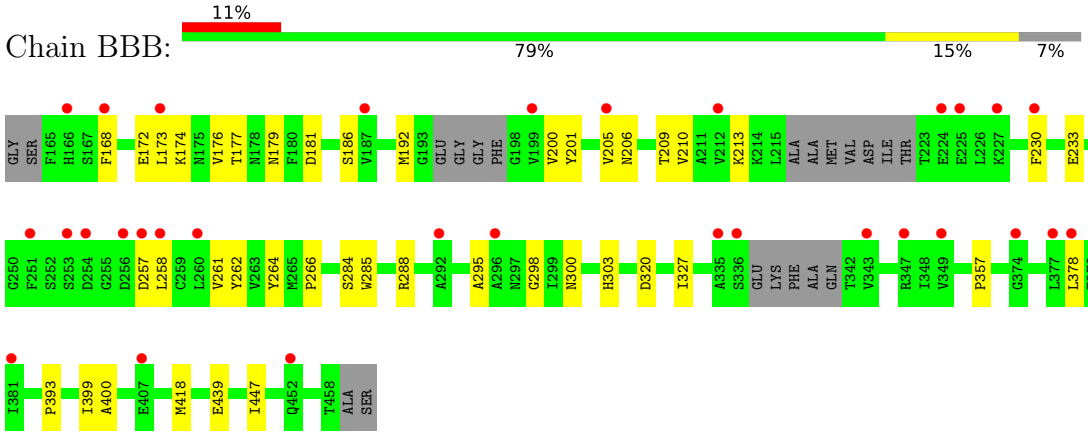
• Molecule 1: Interleukin-1 receptor-associated kinase 4

Chain AAA:



• Molecule 1: Interleukin-1 receptor-associated kinase 4

Chain BBB:



4 Data and refinement statistics (i)

Property	Value	Source
Space group	I 2 2 2	Depositor
Cell constants a, b, c, α, β, γ	87.26Å 117.97Å 139.86Å 90.00° 90.00° 90.00°	Depositor
Resolution (Å)	19.77 – 2.12 19.77 – 2.12	Depositor EDS
% Data completeness (in resolution range)	86.2 (19.77-2.12) 86.3 (19.77-2.12)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.05 (at 2.11Å)	Xtriage
Refinement program	REFMAC 5.8.0267	Depositor
R, R_{free}	0.239 , 0.288 0.250 , 0.295	Depositor DCC
R_{free} test set	1782 reflections (5.00%)	wwPDB-VP
Wilson B-factor (Å^2)	46.6	Xtriage
Anisotropy	0.187	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.34 , 44.2	EDS
L-test for twinning[2]	$< \|L\| > = 0.50$, $< L^2 > = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.94	EDS
Total number of atoms	4461	wwPDB-VP
Average B, all atoms (Å^2)	64.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 15.68% 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

5.1 Standard geometry

Bond lengths and bond angles in the following residue types are not validated in this section: R6R, SEP, TPO

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	AAA	0.64	0/2174	0.72	0/2927
1	BBB	0.66	0/2196	0.73	0/2960
All	All	0.65	0/4370	0.73	0/5887

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts

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	AAA	2161	0	2134	11	0
1	BBB	2183	0	2154	26	0
2	AAA	38	0	0	1	0
2	BBB	38	0	0	1	0
3	AAA	16	0	0	0	0
3	BBB	25	0	0	0	0
All	All	4461	0	4288	39	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 4.

The worst 5 of 39 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:BBB:176:VAL:HG11	1:BBB:205:VAL:HG12	1.73	0.70
1:BBB:177:THR:HG22	1:BBB:177:THR:O	1.94	0.67
1:BBB:357:PRO:HG2	1:BBB:439:GLU:HG2	1.79	0.65
1:BBB:172:GLU:O	1:BBB:176:VAL:HG13	1.99	0.63
1:BBB:173:LEU:HA	1:BBB:176:VAL:HG22	1.86	0.56

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	AAA	261/298 (88%)	252 (97%)	9 (3%)	0	100 100
1	BBB	268/298 (90%)	246 (92%)	18 (7%)	4 (2%)	10 5
All	All	529/596 (89%)	498 (94%)	27 (5%)	4 (1%)	19 14

All (4) Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	BBB	257	ASP
1	BBB	168	PHE
1	BBB	206	ASN
1	BBB	181	ASP

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	AAA	236/253 (93%)	233 (99%)	3 (1%)	69	74
1	BBB	239/253 (94%)	236 (99%)	3 (1%)	69	74
All	All	475/506 (94%)	469 (99%)	6 (1%)	69	74

5 of 6 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	BBB	186	SER
1	BBB	213	LYS
1	BBB	303	HIS
1	AAA	365	THR
1	AAA	344	MET

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

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	TPO	AAA	345	1	8,10,11	0.75	0	10,14,16	0.80	0
1	SEP	BBB	346	1	8,9,10	0.59	0	8,12,14	0.61	0
1	TPO	BBB	345	1	8,10,11	0.86	0	10,14,16	0.75	0
1	SEP	AAA	346	1	8,9,10	0.58	0	8,12,14	0.64	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	TPO	AAA	345	1	-	5/9/11/13	-
1	SEP	BBB	346	1	-	0/5/8/10	-
1	TPO	BBB	345	1	-	5/9/11/13	-
1	SEP	AAA	346	1	-	0/5/8/10	-

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

5 of 10 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
1	AAA	345	TPO	N-CA-CB-OG1
1	BBB	345	TPO	N-CA-CB-OG1
1	BBB	345	TPO	O-C-CA-CB
1	AAA	345	TPO	CA-CB-OG1-P
1	BBB	345	TPO	CA-CB-OG1-P

There are no ring outliers.

No monomer is involved in short contacts.

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	R6R	AAA	501	-	39,42,42	2.41	7 (17%)	53,59,59	1.30	6 (11%)
2	R6R	BBB	501	-	39,42,42	2.50	7 (17%)	53,59,59	1.68	5 (9%)

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	R6R	AAA	501	-	-	0/27/38/38	0/5/5/5
2	R6R	BBB	501	-	-	6/27/38/38	0/5/5/5

The worst 5 of 14 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
2	BBB	501	R6R	O37-N36	8.77	1.43	1.22
2	AAA	501	R6R	O38-N36	8.34	1.42	1.22
2	BBB	501	R6R	O38-N36	8.23	1.42	1.22
2	AAA	501	R6R	O37-N36	8.04	1.41	1.22
2	BBB	501	R6R	C32-N36	-5.76	1.32	1.47

The worst 5 of 11 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
2	BBB	501	R6R	C16-N17-C27	-7.75	119.31	129.19
2	BBB	501	R6R	C3-N4-C5	5.06	122.37	112.62
2	AAA	501	R6R	C3-N4-C5	3.98	120.28	112.62
2	AAA	501	R6R	C2-N1-C6	3.84	120.01	112.62
2	BBB	501	R6R	C2-N1-C6	3.77	119.89	112.62

There are no chirality outliers.

5 of 6 torsion outliers are listed below:

Mol	Chain	Res	Type	Atoms
2	BBB	501	R6R	N1-C15-C16-N17
2	BBB	501	R6R	C30-C32-N36-O37
2	BBB	501	R6R	C33-C32-N36-O37
2	BBB	501	R6R	C30-C32-N36-O38
2	BBB	501	R6R	C33-C32-N36-O38

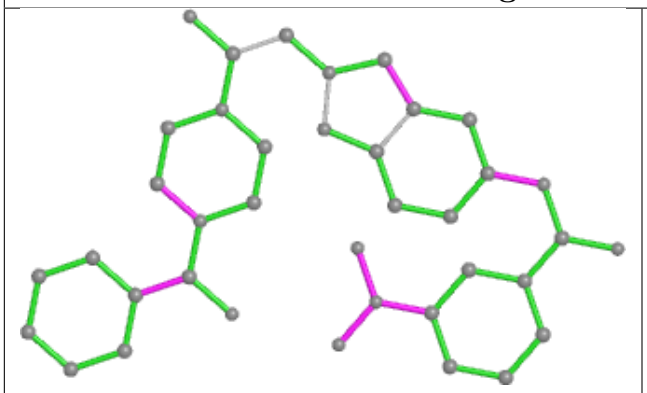
There are no ring outliers.

2 monomers are involved in 2 short contacts:

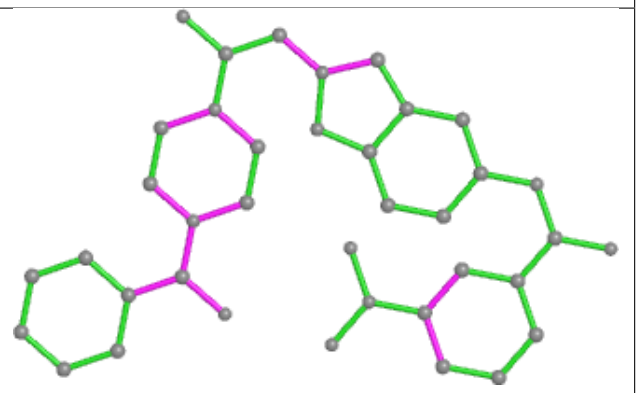
Mol	Chain	Res	Type	Clashes	Symm-Clashes
2	AAA	501	R6R	1	0
2	BBB	501	R6R	1	0

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.

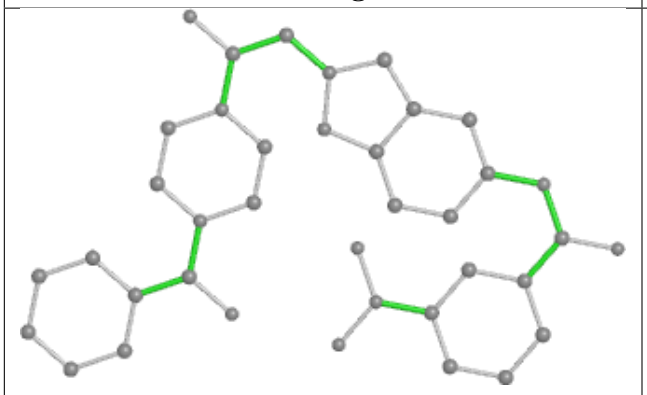
Ligand R6R AAA 501



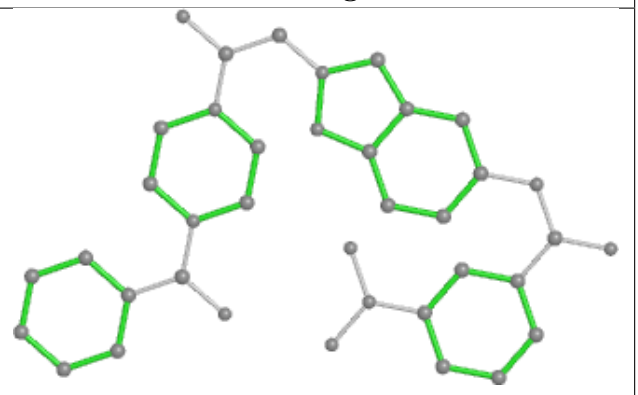
Bond lengths



Bond angles

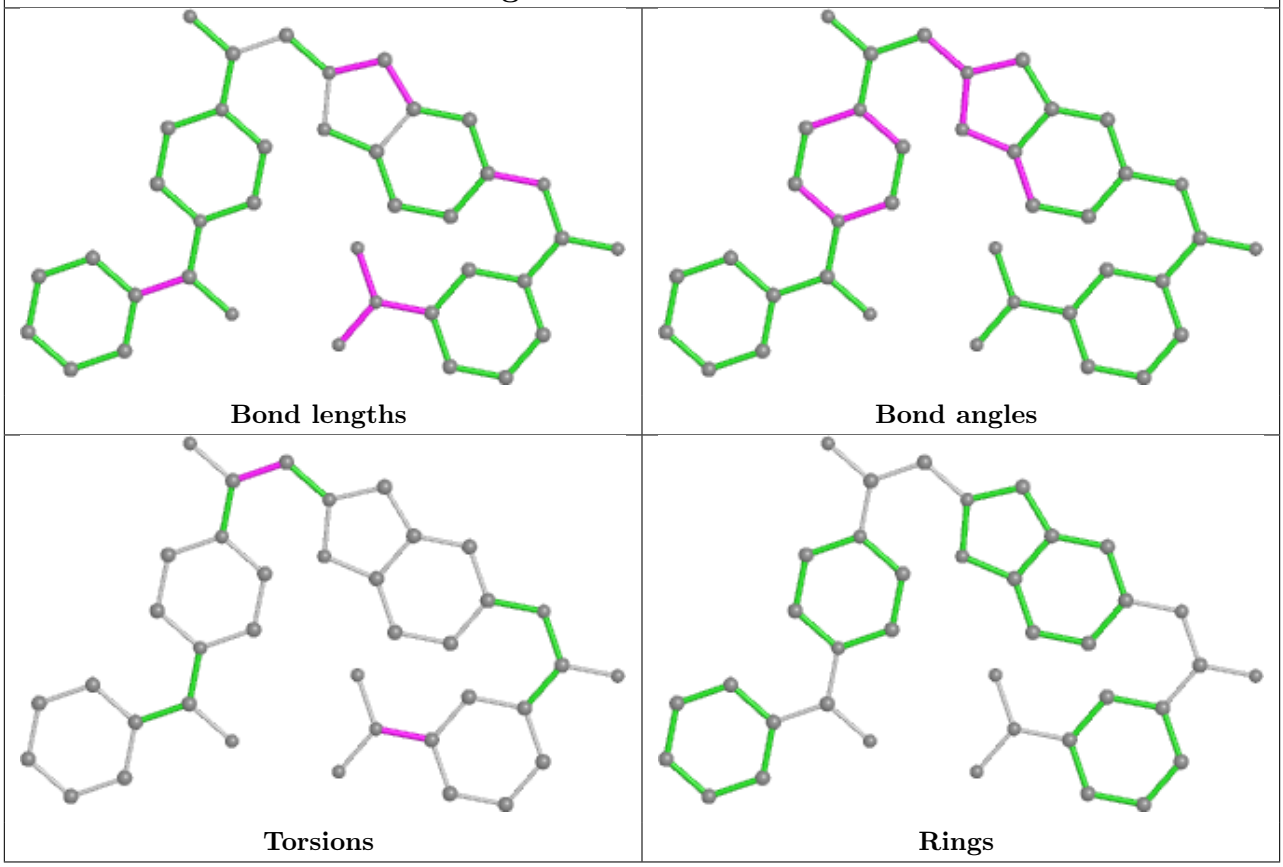


Torsions



Rings

Ligand R6R BBB 501



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

6.1 Protein, DNA and RNA chains

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	AAA	271/298 (90%)	0.69	37 (13%)	3	4	37, 57, 109, 135	0
1	BBB	276/298 (92%)	0.60	32 (11%)	4	6	36, 58, 95, 132	0
All	All	547/596 (91%)	0.64	69 (12%)	3	4	36, 58, 105, 135	0

The worst 5 of 69 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	AAA	226	LEU	8.7
1	BBB	256	ASP	5.0
1	AAA	334	ARG	4.7
1	AAA	204	TYR	4.7
1	BBB	225	GLU	4.3

6.2 Non-standard residues in protein, DNA, RNA chains

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
1	SEP	AAA	346	10/11	0.71	0.26	75,107,138,138	0
1	SEP	BBB	346	10/11	0.83	0.26	83,102,128,141	0
1	TPO	AAA	345	11/12	0.95	0.11	68,80,88,98	0
1	TPO	BBB	345	11/12	0.96	0.17	58,71,77,78	0

6.3 Carbohydrates

There are no monosaccharides in this entry.

6.4 Ligands (i)

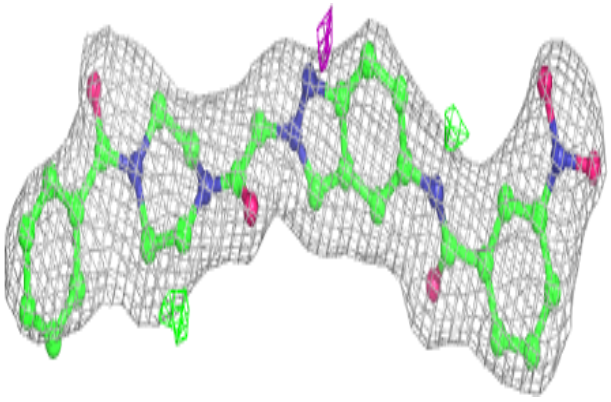
In the following table, the Atoms column lists the number of modelled atoms in the group and the number defined in the chemical component dictionary. The B-factors column lists the minimum, median, 95^{th} percentile and maximum values of B factors of atoms in the group. The column labelled 'Q< 0.9'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

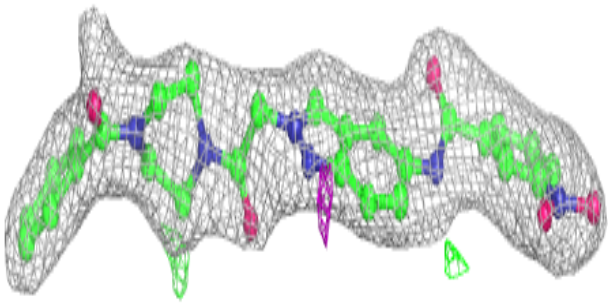
Mol	Type	Chain	Res	Atoms	RSCC	RSR	B-factors(Å^2)	Q<0.9
2	R6R	AAA	501	38/38	0.87	0.15	40,62,82,84	0
2	R6R	BBB	501	38/38	0.92	0.14	42,67,118,127	0

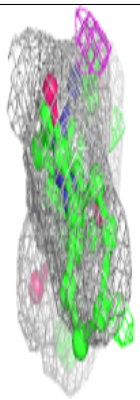
The following is a graphical depiction of the model fit to experimental electron density of all instances of the Ligand of Interest. In addition, ligands with molecular weight > 250 and outliers as shown on the geometry validation Tables will also be included. Each fit is shown from different orientation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

Electron density around R6R AAA 501:

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

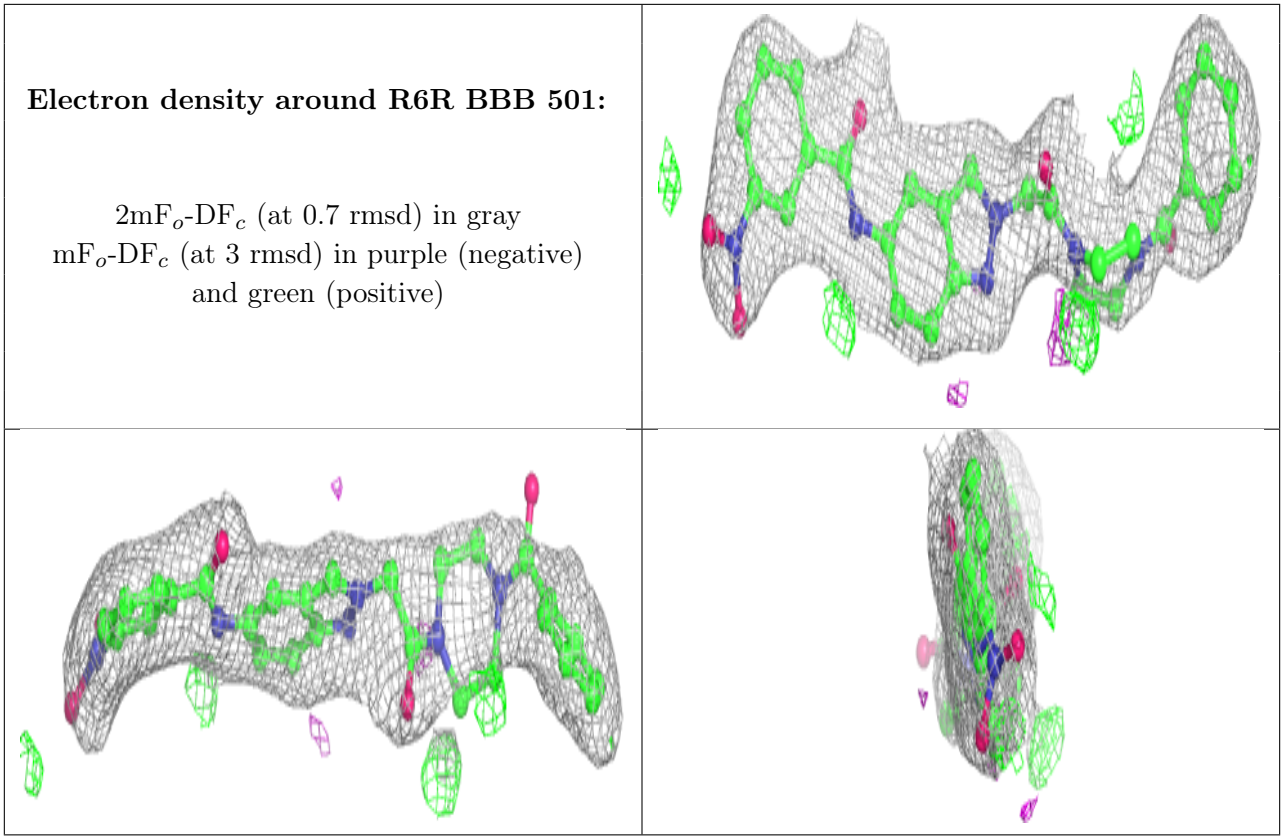






Electron density around R6R BBB 501:

$2mF_o$-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)

There are no such residues in this entry.