wwPDB X-ray Structure Validation Summary Report (i)

Apr 21, 2024 – 03:26 am BST

PDB ID	:	4ANU
Title	:	Complexes of PI3Kgamma with isoform selective inhibitors.
Authors	:	Foster, P.G.; Lougheed, J.C.
Deposited on	:	2012-03-22
Resolution	:	2.81 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:

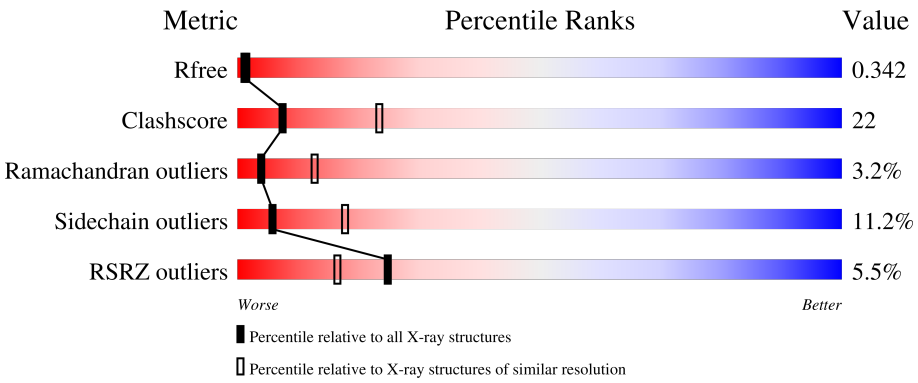
MolProbity	:	4.02b-467
Mogul	:	1.8.4, CSD as541be (2020)
Xtriage (Phenix)	:	1.13
EDS	:	2.36.2
buster-report	:	1.1.7 (2018)
Percentile statistics	:	20191225.v01 (using entries in the PDB archive December 25th 2019)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
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-RAY DIFFRACTION

The reported resolution of this entry is 2.81 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	3617 (2.84-2.80)
Clashscore	141614	4060 (2.84-2.80)
Ramachandran outliers	138981	3978 (2.84-2.80)
Sidechain outliers	138945	3980 (2.84-2.80)
RSRZ outliers	127900	3552 (2.84-2.80)

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	A	980	5% 46% 33% 6% 15%

2 Entry composition (i)

There are 4 unique types of molecules in this entry. The entry contains 6810 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.

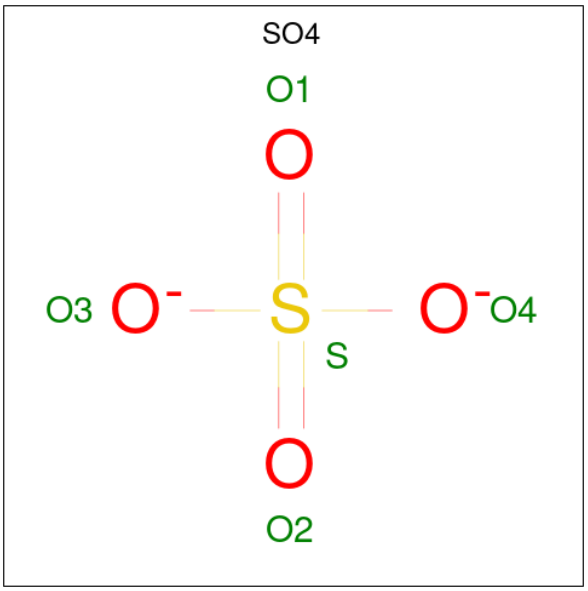
- Molecule 1 is a protein called PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE 3-KINASE CATALYTIC SUBUNIT GAMMA ISOFORM.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	833	6757	4334	1153	1235	35	0	0	0

There are 21 discrepancies between the modelled and reference sequences:

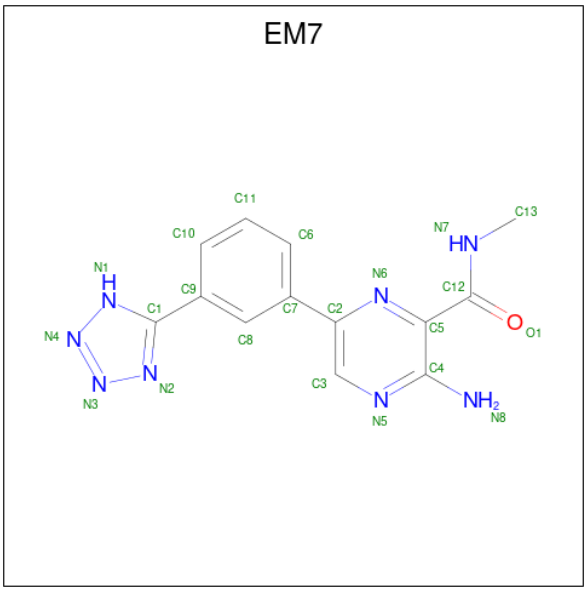
Chain	Residue	Modelled	Actual	Comment	Reference
A	139	MET	-	expression tag	UNP P48736
A	140	LEU	-	expression tag	UNP P48736
A	141	LEU	-	expression tag	UNP P48736
A	142	GLY	-	expression tag	UNP P48736
A	143	SER	-	expression tag	UNP P48736
A	1103	GLU	-	expression tag	UNP P48736
A	1104	PHE	-	expression tag	UNP P48736
A	1105	GLY	-	expression tag	UNP P48736
A	1106	LEU	-	expression tag	UNP P48736
A	1107	VAL	-	expression tag	UNP P48736
A	1108	PRO	-	expression tag	UNP P48736
A	1109	ARG	-	expression tag	UNP P48736
A	1110	GLY	-	expression tag	UNP P48736
A	1111	SER	-	expression tag	UNP P48736
A	1112	GLY	-	expression tag	UNP P48736
A	1113	HIS	-	expression tag	UNP P48736
A	1114	HIS	-	expression tag	UNP P48736
A	1115	HIS	-	expression tag	UNP P48736
A	1116	HIS	-	expression tag	UNP P48736
A	1117	HIS	-	expression tag	UNP P48736
A	1118	HIS	-	expression tag	UNP P48736

- Molecule 2 is SULFATE ION (three-letter code: SO4) (formula: O_4S).



Mol	Chain	Residues	Atoms			ZeroOcc	AltConf
			Total	O	S		
2	A	1	5	4	1	0	0

- Molecule 3 is 3-AMINO-N-METHYL-6-[3-(1H-TETRAZOL-5-YL)PHENYL]PYRAZINE-2-CARBOXAMIDE (three-letter code: EM7) (formula: $C_{13}H_{12}N_8O$).



Mol	Chain	Residues	Atoms				ZeroOcc	AltConf
			Total	C	N	O		
3	A	1	22	13	8	1	0	0

- Molecule 4 is water.

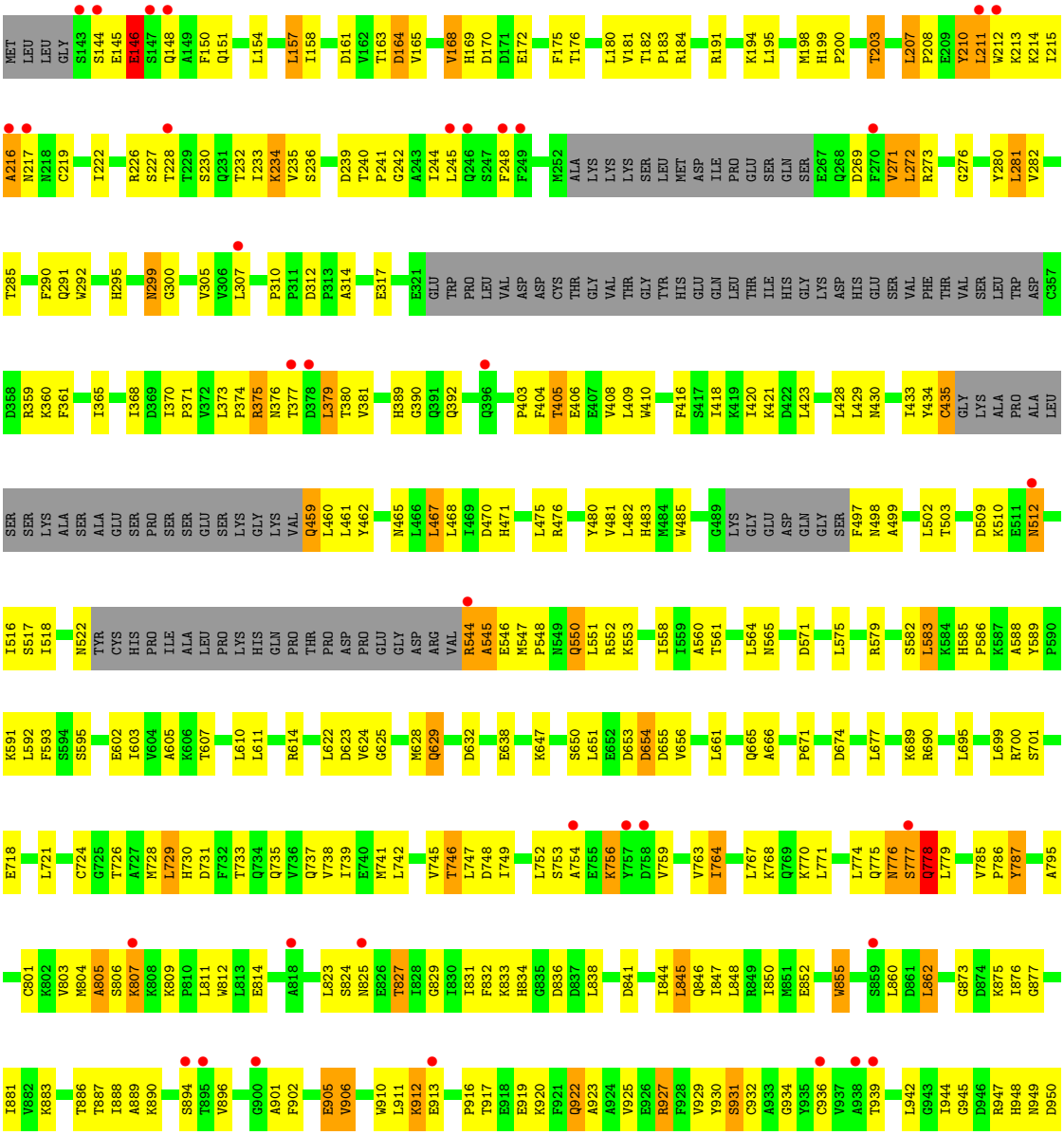
Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
4	A	26	26	26	0	0

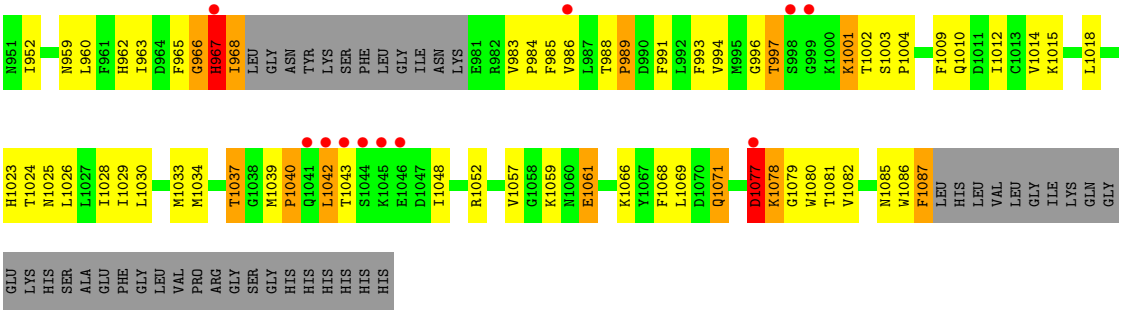
3 Residue-property plots (i)

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: PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE 3-KINASE CATALYTIC SUBUNIT GAMMA ISOFORM

Chain A: 5% 46% 33% 6% 15%




N951
I952
N959
L960
F961
H962
I963
D964
F965
G966
H967
I968
LEU
GLY
ASN
TYR
LYS
SER
PHE
LEU
GLY
ILE
ASN
LYS
E981
R982
V983
P984
F985
V986
L987
T988
P989
D990
F991
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F993
V994
H995
G996
T997
S998
G999
K1000
K1001
T1002
S1003
P1004
F1009
Q1010
D1011
I1012
C1013
V1014
K1015
L1018
H1023
T1024
N1025
L1026
L1027
I1028
I1029
L1030
M1033
M1034
T1037
G1038
M1039
P1040
Q1041
L1042
T1043
S1044
K1045
E1046
D1047
I1048
R1052
V1057
G1058
K1059
N1060
E1061
K1066
Y1067
F1068
L1069
D1070
Q1071
D1077
K1078
G1079
W1080
T1081
V1082
N1085
W1086
F1087
LEU
HIS
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4 Data and refinement statistics (i)

Property	Value	Source
Space group	C 1 2 1	Depositor
Cell constants a, b, c, α, β, γ	145.25Å 68.53Å 106.86Å 90.00° 95.29° 90.00°	Depositor
Resolution (Å)	28.70 – 2.81 28.70 – 2.81	Depositor EDS
% Data completeness (in resolution range)	93.9 (28.70-2.81) 93.9 (28.70-2.81)	Depositor EDS
R_{merge}	0.11	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.27 (at 2.80Å)	Xtriage
Refinement program	REFMAC 5.2.0019	Depositor
R, R_{free}	0.254 , 0.338 0.260 , 0.342	Depositor DCC
R_{free} test set	1242 reflections (5.14%)	wwPDB-VP
Wilson B-factor (Å^2)	78.9	Xtriage
Anisotropy	0.120	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.31 , 48.4	EDS
L-test for twinning[2]	$< \|L\| > = 0.51$, $< L^2 > = 0.34$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.89	EDS
Total number of atoms	6810	wwPDB-VP
Average B, all atoms (Å^2)	74.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 4.41% 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: SO4, EM7

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	0.87	1/6901 (0.0%)	0.89	6/9334 (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	A	0	2

All (1) bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
1	A	967	HIS	C-N	-48.27	0.23	1.34

The worst 5 of 6 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
1	A	967	HIS	CA-C-N	-27.87	55.88	117.20
1	A	967	HIS	O-C-N	26.25	164.70	122.70
1	A	967	HIS	C-N-CA	-20.88	69.49	121.70
1	A	210	TYR	N-CA-C	5.64	126.22	111.00
1	A	210	TYR	C-N-CA	5.27	134.88	121.70

There are no chirality outliers.

All (2) planarity outliers are listed below:

Mol	Chain	Res	Type	Group
1	A	777	SER	Peptide
1	A	967	HIS	Mainchain

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	6757	0	6788	302	0
2	A	5	0	0	0	0
3	A	22	0	12	0	0
4	A	26	0	0	4	0
All	All	6810	0	6800	302	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 22.

The worst 5 of 302 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:A:210:TYR:CE1	1:A:211:LEU:HD22	1.86	1.10
1:A:814:GLU:OE1	1:A:827:THR:HG21	1.59	1.03
1:A:628:MET:CE	1:A:1030:LEU:HD21	1.89	1.01
1:A:564:LEU:HD11	1:A:1048:ILE:HG22	1.38	1.01
1:A:628:MET:HE2	1:A:1030:LEU:HD21	1.42	1.00

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	819/980 (84%)	688 (84%)	105 (13%)	26 (3%)	4 13

5 of 26 Ramachandran outliers are listed below:

Mol	Chain	Res	Type
1	A	146	GLU
1	A	211	LEU
1	A	216	ALA
1	A	374	PRO
1	A	778	GLN

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	749/874 (86%)	665 (89%)	84 (11%)	6 18

5 of 84 residues with a non-rotameric sidechain are listed below:

Mol	Chain	Res	Type
1	A	807	LYS
1	A	959	ASN
1	A	823	LEU
1	A	912	LYS
1	A	1002	THR

Sometimes sidechains can be flipped to improve hydrogen bonding and reduce clashes. 5 of 26 such sidechains are listed below:

Mol	Chain	Res	Type
1	A	634	ASN
1	A	737	GLN
1	A	1071	GLN
1	A	711	GLN
1	A	743	GLN

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	Res	Link	Bond lengths			Bond angles		
					Counts	RMSZ	$\#\|Z\| > 2$	Counts	RMSZ	$\#\|Z\| > 2$
2	SO4	A	2088	-	4,4,4	0.14	0	6,6,6	0.25	0
3	EM7	A	2089	-	24,24,24	2.83	6 (25%)	30,33,33	1.93	7 (23%)

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
3	EM7	A	2089	-	-	4/14/14/14	0/3/3/3

The worst 5 of 6 bond length outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(Å)	Ideal(Å)
3	A	2089	EM7	N1-N4	-7.59	1.23	1.34
3	A	2089	EM7	N2-N3	-7.20	1.24	1.34
3	A	2089	EM7	C7-C2	-5.76	1.39	1.48
3	A	2089	EM7	N4-N3	-3.96	1.24	1.32
3	A	2089	EM7	C5-C12	3.58	1.56	1.50

The worst 5 of 7 bond angle outliers are listed below:

Mol	Chain	Res	Type	Atoms	Z	Observed(^{o})	Ideal(^{o})
3	A	2089	EM7	N1-C1-N2	-6.23	104.43	111.39
3	A	2089	EM7	C1-N1-N4	3.72	108.18	104.87
3	A	2089	EM7	N2-N3-N4	2.85	111.40	109.53
3	A	2089	EM7	C13-N7-C12	-2.76	118.76	121.89
3	A	2089	EM7	C9-C1-N2	2.74	128.76	124.12

There are no chirality outliers.

All (4) torsion outliers are listed below:

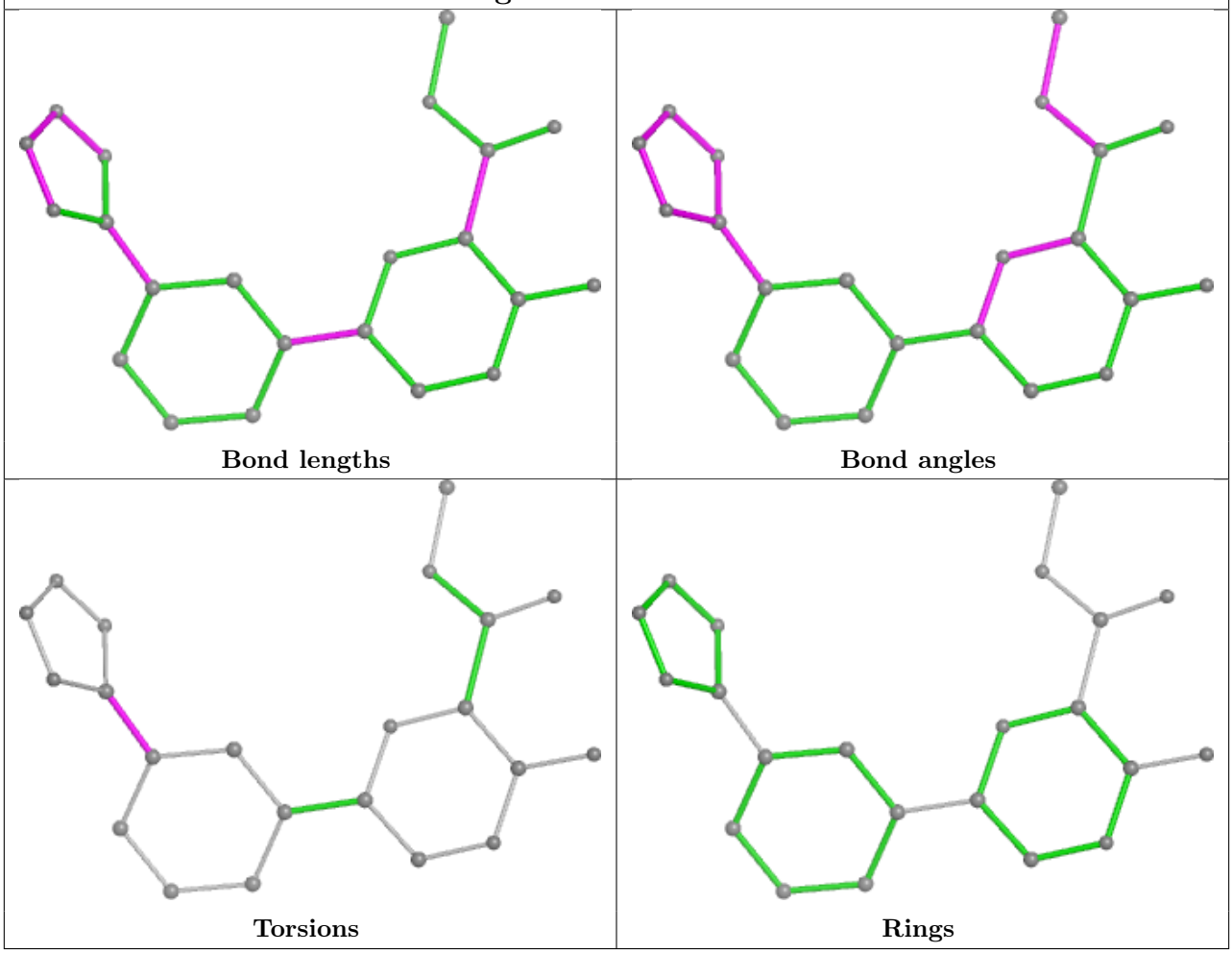
Mol	Chain	Res	Type	Atoms
3	A	2089	EM7	N1-C1-C9-C8
3	A	2089	EM7	N2-C1-C9-C10
3	A	2089	EM7	N2-C1-C9-C8
3	A	2089	EM7	N1-C1-C9-C10

There are no ring outliers.

No monomer is involved in short contacts.

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 EM7 A 2089



5.7 Other polymers (i)

There are no such residues in this entry.

5.8 Polymer linkage issues (i)

The following chains have linkage breaks:

Mol	Chain	Number of breaks
1	A	1

All chain breaks are listed below:

Model	Chain	Residue-1	Atom-1	Residue-2	Atom-2	Distance (Å)
1	A	967:HIS	C	968:ILE	N	0.23

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, 95th 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	A	833/980 (85%)	0.30	46 (5%)	25	16	45, 73, 99, 110	0

The worst 5 of 46 RSRZ outliers are listed below:

Mol	Chain	Res	Type	RSRZ
1	A	1044	SER	7.2
1	A	895	THR	7.1
1	A	143	SER	5.4
1	A	228	THR	5.0
1	A	754	ALA	4.8

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)

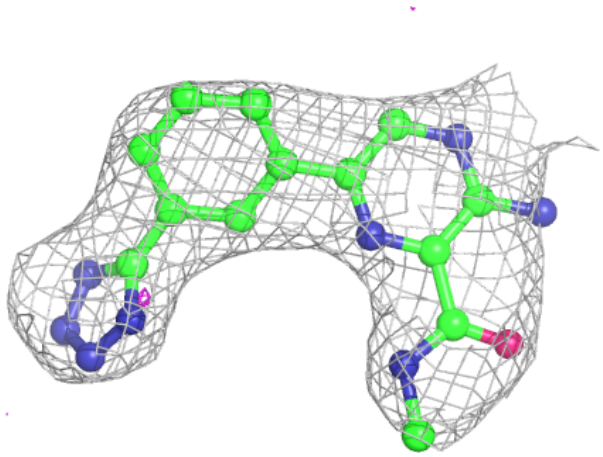
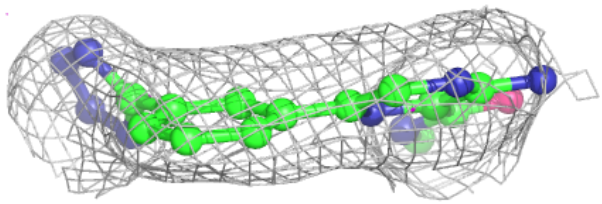
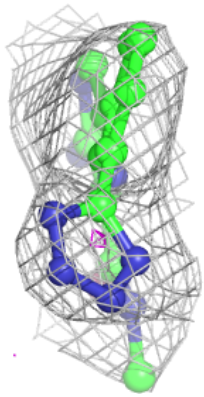
There are no monosaccharides in this entry.

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, 95th 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	EM7	A	2089	22/22	0.92	0.19	70,72,79,80	0
2	SO4	A	2088	5/5	0.94	0.13	97,98,98,99	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 EM7 A 2089: $2mF_o$-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.