SUPPLEMENTARY INFORMATION

SEARCH FOR NON-LACTAM INHIBITORS OF Mtb β-LACTAMASE LED TO ITS OPEN SHAPE IN APO STATE: NEW CONCEPT FOR ANTIBIOTIC DESIGN

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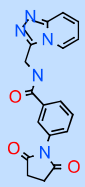
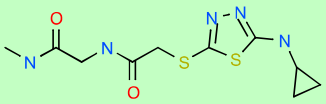
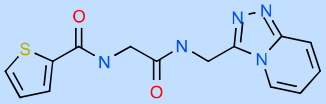
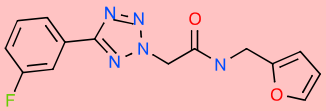
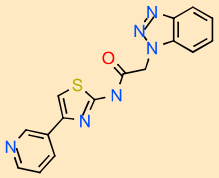
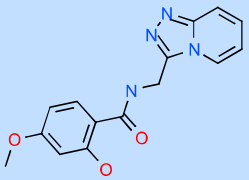
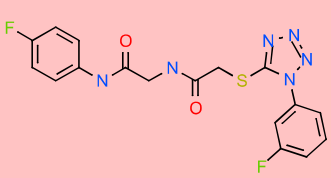
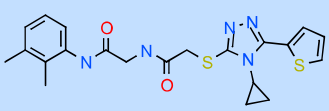
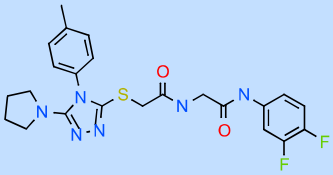
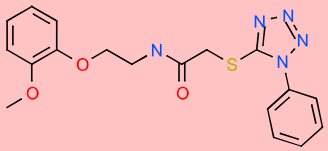
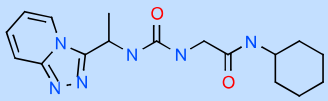
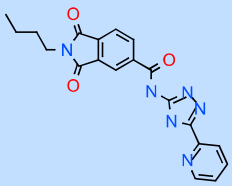
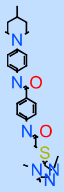
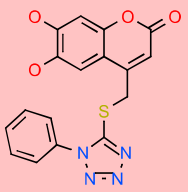
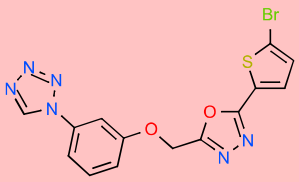
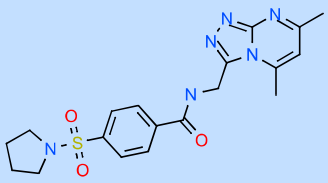
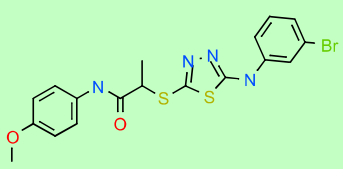
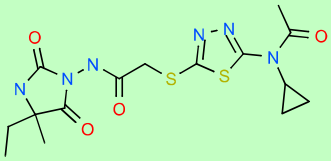
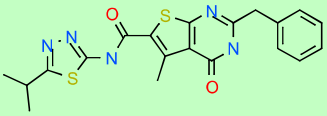
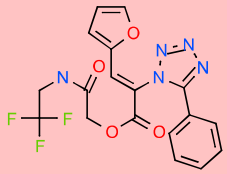
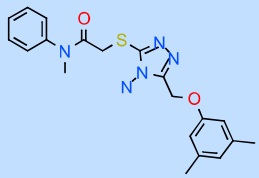
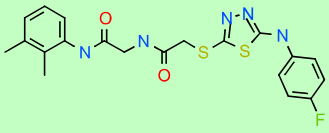
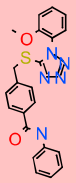
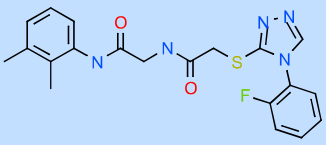
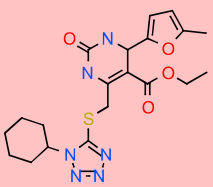
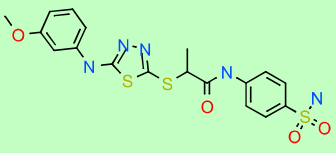
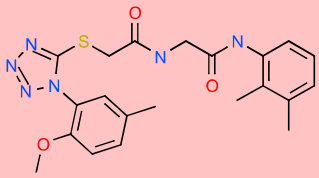
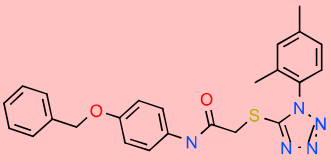
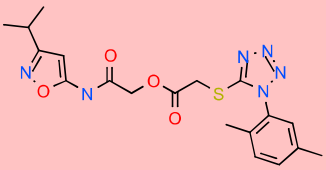
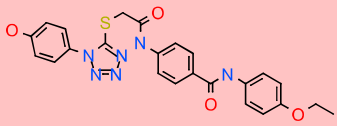
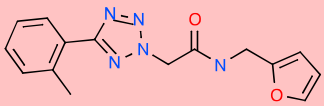
Supplementary Table S1

A table showing the compounds C1 through C32 which were purchased after virtual ligand screening and tested using the *In-vitro* β-lactamase activity assay. The 2D structures, IUPAC names and SMILES notations of the compounds have been tabulated with the rows colored according to the chemical moiety present in the compound. The color key is given at the bottom of the table.

	Molecule Name	Representation	IUPAC Name	SMILES
1	C1		1-(3-(((1,7,8-triaza-bicyclo[4.3.0]nona-2,4,6,8-tetraen-9-yl)-methylamino)-formyl)-phenyl)-pyrrolidine-2,5-dione	C1CC(N(C1=O)c1cccc(c1)C(NCc1nnc2ccccn12)=O)=O
2	C2		2-(2-(5-cyclopropylamino-1,3,4-thiadiazol-2-ylsulfanyl)-acetylamino)-1-methylamino-ethanone	CNC(CNC(CSc1nnc(NC2CC2)s1)=O)=O
3	C3		2-((thiophen-2-yl)-formylamino)-1-((1,7,8-triaza-bicyclo[4.3.0]nona-2,4,6,8-tetraen-9-yl)-methylamino)-ethanone	C(C(NCc1nnc2ccccn12)=O)NC(c1cccs1)=O
4	C4		2-(5-(3-fluoro-phenyl)-2H-1,2,3,4-tetraazol-2-yl)-1-((furan-2-yl)-methylamino)-ethanone	C(c1ccco1)NC(Cn1nc(c2cccc(c2)F)nn1)=O
5	C5		1-(4-(pyridin-3-yl)-thiazol-2-ylamino)-2-(7,8,9-triaza-bicyclo[4.3.0]nona-1(6),2,4,8-tetraen-7-yl)-ethanone	C(C(Nc1nc(cs1)c1cccnc1)=O)n1c2ccccc2nn1
6	C6		(2-hydroxy-4-methoxy-phenyl)-((1,7,8-triaza-bicyclo[4.3.0]nona-2,4,6,8-tetraen-9-yl)-methylamino)-methanone	COc1ccc(C(NCc2nnc3ccccn23)=O)c(c1)O
7	C7		1-(4-fluoro-phenylamino)-2-(2-(1-(3-fluoro-phenyl)-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-acetylamino)-ethanone	C(C(Nc1ccc(cc1)F)=O)NC(CSc1nnnn1c1cccc(c1)F)=O
8	C8		1-(3-((3-(1H-1,2,3,4-tetraazol-1-yl)-phenyl)-formylamino)-phenyl)-pyrrolidin-2-one	C1CC(N(C1)c1cccc(c1)NC(c1cccc(c1)n1cnnn1)=O)=O
9	C9		2-(2-(4-cyclopropyl-5-(thiophen-2-yl)-4H-1,2,4-triazol-3-ylsulfanyl)-acetylamino)-1-(2,3-dimethyl-phenylamino)-ethanone	Cc1cccc(c1C)NC(CNC(CSc1nnc(c2cccs2)n1C1CC1)=O)=O

	Molecule Name	Representation	IUPAC Name	SMILES
10	C10		1-(3,4-difluoro-phenylamino)-2-(2-(4-p -tolyl-5-(pyrrolidin-1-yl)-4H-1,2,4-triazol-3 -ylsulfanyl)-acetylamino)-ethanone	Cc1ccc(cc1)n1c(nnc1SCC(NCC(Nc1ccc(c(c1)F)F)=O)= O)N1CCCC1
11	C11		1-(2-(2-methoxy-phenoxy)-ethylamino)-2-(1 -phenyl-1H-1,2,3,4-tetraazol-5-ylsulfanyl) -ethanone	COc1ccccc1OCCNC(CSc1nnnn1c1ccccc1)=O
12	C12		1-cyclohexylamino-2-((1-(1,7,8-triaza -bicyclo[4.3.0]nona-2,4,6,8-tetraen-9-yl) -ethylamino)-formylamino)-ethanone	CC(c1nnc2ccccn12)NC(NCC(NC1CCCCC1)=O)=O
13	C13		8-butyl-3-((5-(pyridin-2-yl)-4H-1,2,4-triazol-3 -ylamino)-formyl)-8-aza-bicyclo[4.3.0]nona-1(6),2,4 -triene-7,9-dione	CCCCN1C(c2ccc(cc2C1=O)C(Nc1nnc(c2ccccn2)[nH]1)=O)=O
14	C14		1-(4-((4-(4-methyl-piperidin-1-yl)-phenylamino) -formyl)-phenylamino)-2-(4-methyl-4H-1,2,4 -triazol-3-ylsulfanyl)-ethanone	CC1CCN(CC1)c1ccc(cc1)NC(c1ccc(cc1)NC(CSc1nncn1 C)=O)=O
15	C15		8,9-dihydroxy-3-oxo-5-((1-phenyl-1H-1,2,3,4 -tetraazol-5-ylsulfanyl)-methyl)-2-oxa -bicyclo[4.4.0]deca-1(10),4,6,8-tetraene	C(C1=CC(=O)Oc2cc(c(cc12)O)O)Sc1nnnn1c1ccccc1
16	C16		1-(3-((5-(5-bromo-thiophen-2-yl)-1,3,4-oxadiazol-2 -yl)-methoxy)-phenyl)-1H-1,2,3,4-tetraazole	C(c1nnc(c2ccc(s2)[Br])o1)Oc1cccc(c1)n1cnnn1
17	C17		((2,4-dimethyl-1,5,7,8-tetraaza -bicyclo[4.3.0]nona-2,4,6,8-tetraen-9-yl) -methylamino)-(4-(pyrrolidin-1-ylsulfonyl)-phenyl) -methanone	Cc1cc(C)n2c(CNC(c3ccc(cc3)S(N3CCCC3)(=O)=O)=O)nnc2n1
18	C18		2-(5-(3-bromo-phenylamino)-1,3,4-thiadiazol-2 -ylsulfanyl)-1-(4-methoxy-phenylamino)-propan-1 -one	CC(C(Nc1ccc(cc1)OC)=O)Sc1nnc(Nc2cccc(c2)[Br])s1

	Molecule Name	Representation	IUPAC Name	SMILES
19	C19		3-(2-(5-(acetyl-cyclopropyl-amino)-1,3,4-thiadiazol-2-ylsulfanyl)-acetylamino)-5-ethyl-5-methyl-imidazolidine-2,4-dione	CCC1(C)C(N(C(N1)=O)NC(CSc1nnc(N(C2CC2)C(C)=O)s1)=O)=O
20	C20		3-benzyl-8-((5-isopropyl-1,3,4-thiadiazol-2-ylamino)-formyl)-7-methyl-9-thia-2,4-diaza-bicyclo[4.3.0]nona-1(6),2,7-trien-5-one	CC(C)c1nnc(NC(c2c(C)c3C(NC(Cc4ccccc4)=Nc3s2)=O)=O)s1
21	C21		2-oxo-2-(2,2,2-trifluoro-ethylamino)-ethyl 3-(furan-2-yl)-2-(5-phenyl-1H-1,2,3,4-tetraazol-1-yl)-prop-2-enoate	C(C(F)(F)F)NC(COC(C(=Cc1ccco1)n1c(c2ccccc2)nnn1)=O)=O
22	C22		2-(4-amino-5-((3,5-dimethyl-phenoxy)-methyl)-4H-1,2,4-triazol-3-ylsulfanyl)-1-(methyl-phenyl-amino)-ethanone	Cc1cc(C)cc(c1)OCc1nnc(n1N)SCC(N(C)c1ccccc1)=O
23	C23		1-(2,3-dimethyl-phenylamino)-2-(2-(5-(4-fluoro-phenylamino)-1,3,4-thiadiazol-2-ylsulfanyl)-acetylamino)-ethanone	Cc1cccc(c1C)NC(CNC(CSc1nnc(Nc2ccc(cc2)F)s1)=O)=O
24	C24		(4-((1-(2-methoxy-phenyl)-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-methyl)-phenyl)-phenylamino-methanone	COc1ccccc1n1c(nnn1)SCc1ccc(cc1)C(Nc1ccccc1)=O
25	C25		1-(2,3-dimethyl-phenylamino)-2-(2-(4-(2-fluoro-phenyl)-4H-1,2,4-triazol-3-ylsulfanyl)-acetylamino)-ethanone	Cc1cccc(c1C)NC(CNC(CSc1nncn1c1ccccc1F)=O)=O
26	C26		ethyl 6-((1-cyclohexyl-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-methyl)-4-(5-methyl-furan-2-yl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate	CCOC(C1C(c2ccc(C)o2)NC(NC=1CSc1nnnn1C1CCCCC1)=O)=O
27	C27		4-(2-(5-(3-methoxy-phenylamino)-1,3,4-thiadiazol-2-ylsulfanyl)-propionylamino)-benzenesulfonamide	CC(C(Nc1ccc(cc1)S(N)(=O)=O)=O)Sc1nnc(Nc2cccc(c2)OC)s1

	Molecule Name	Representation	IUPAC Name	SMILES
28	**C28**		**1-(2,3-dimethyl-phenylamino)-2-(2-(1-(2-methoxy-5-methyl-phenyl)-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-acetylamino)-ethanone**	**Cc1ccc(c(c1)n1c(nnn1)SCC(NCC(Nc1cccc(C)c1C)=O)=O)OC**
29	**C29**		**2-(1-(2,4-dimethyl-phenyl)-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-1-(4-(phenyl-methoxy)-phenylamino)-ethanone**	**Cc1ccc(c(C)c1)n1c(nnn1)SCC(Nc1ccc(cc1)OCc1ccccc1)=O**
30	**C30**		**2-(3-isopropyl-isoxazol-5-ylamino)-2-oxo-ethyl 2-(1-(2,5-dimethyl-phenyl)-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-ethanoate**	**CC(C)c1cc(NC(COC(CSc2nnnn2c2cc(C)ccc2C)=O)=O)on1**
31	**C31**		**1-(4-((4-ethoxy-phenylamino)-formyl)-phenylamino)-2-(1-(4-hydroxy-phenyl)-1H-1,2,3,4-tetraazol-5-ylsulfanyl)-ethanone**	**CCOc1ccc(cc1)NC(c1ccc(cc1)NC(CSc1nnnn1c1ccc(cc1)O)=O)=O**
32	**C32**		**1-((furan-2-yl)-methylamino)-2-(5-o-tolyl-2H-1,2,3,4-tetraazol-2-yl)-ethanone**	**Cc1ccccc1c1nnn(CC(NCc2ccco2)=O)n1**

Compounds having Tetrazole moiety

Compounds having 1,3,4 - Triazole moiety independently or as part of a larger ring

Compounds having Thiadiazole moiety independently or as part of a larger ring

Compounds having 1,2,3 - Triazole moiety independently or as part of a larger ring

Data-collection and scattering-derived parameters

Supplementary Table 2

Data-collection parameters	
Instrument /	SAXSpace (Anton Paar)
Beam geometry	10 mm slit
Wavelength (Å)	1.5418
Desmearing Software	Done using SAXSquant
q range (nm^{-1})	0.10–3.00
Temperature (K)	283
Molecular-mass determination†	
Partial specific volume ($cm^3\ g^{-1}$)	0.724
Calculated monomeric M_r from sequence	27.5
Dry volume calculated from sequence ($Å^3$)	34190
Software employed	
Primary data reduction	*SAXSquant*
Data processing	*PRIMUS QT*
Ab initio analysis	*GASBOR*
Validation and averaging	*DAMAVER*
Rigid-body modeling	N/A
Computation of model intensities	*CRYSOL*
Three-dimensional graphics representations	*PyMOL*

Table 2A

	Apo BlaC (Conc. Series)	**Apo BlaC (Conc. Series)**	**Apo BlaC (Conc. Series)**
Data-collection parameters			
Exposure time (min)	30	30	30
Concentration range (mg ml^{-1})	20	8	4
I(0)/C	886.1	880.0	888.3
Structural parameters†			
I(0) (Arbitrary Units) [from *P(r)*]	17820± 201	70±132	3485±104
R_g (nm) [from *P(r)*]	2.27± 0.04	2.30 ± 0.09	2.45 ± 0.16
I(0) (Arbitrary Units) (from Guinier)	17722± 284	7040±147	3553±110
R_g (nm) (from Guinier)	2.35 ± 0.012	2.38 ± 0.018	2.30 ± 0.025
D_{max} (nm)	6.7	6.8	6.9
Porod volume estimate ($Å^3$)	38503	38258	38365
Molecular-mass determination			
Molecular mass M_r [from V_c]	29.1	29.3	29.4

Table 2B

	Apo BlaC (Time Series)	BlaC+ CA (Time Series)	BlaC+ SB (Time Series)	BlaC+ TB (Time Series)	BlaC+ C5 (Time Series)	BlaC+ C13 (Time Series)	BlaC+ C16 (Time Series)	BlaC+ C28 (Time Series)
Data-collection parameters								
Exposure time (min)	5	5	5	5	5	5	5	5
Concentration range (mg ml^{-1})	20	20	20	20	20	20	20	20
Structural parameters†								
I(0) (from Guinier)	2968.24±135.43 2940.64±134.78 2911.41±139.99 2965.41±158.33 2934.97±136.12 2996.96±150.07 2966.75±161.12 2980.64±149.24	2958.99±151.07 2921.74±145.66 2969.78±150.96 2912.41±146.74 2955.25±145.32 2895.58±142.13 2958.35±148.42 2916.95±139.67	2914.08±142.78 2928.80±145.20 2953.57±150.79 2926.70±146.45 2937.87±150.68 2916.62±143.17 2941.73±151.52 2914.19±144.59	2940.78±143.75 2915.13±146.55 2996.80±147.41 2986.03±152.93 2980.35±149.17 2949.37±144.91 3024.01±156.85 2986.82±147.19	2932.99±145.29 2895.24±144.48 2954.58±146.53 2955.09±151.13 2930.92±143.42 2939.73±144.05 2973.23±146.34 2916.58±143.18	2951.25±150.28 2917.09±147.83 2997.49±146.42 2964.95±152.73 2926.70±147.20 2967.04±151.36 2931.05±145.34 2956.11±148.36	2940.90±147.00 2934.89±150.07 2956.45±154.29 2939.49±152.14 2951.54±146.20 2953.19±151.78 2969.84±147.45 2973.35±148.37	2908.22±145.29 2950.68±151.43 2962.11±146.44 3010.84±147.61 2922.80±149.83 2909.93±141.36 2975.66±143.54 2985.02±150.76
R_g(nm) (from Guinier)	2.35±0.048 2.32±0.053 2.33±0.058 2.31±0.047 2.34±0.060 2.32±0.042 2.36±0.031 2.34±0.040	2.01±0.041 1.98±0.048 1.97±0.05 2.01±0.038 2.04±0.042 2.03±0.036 2.04±0.033 2.04±0.04	1.97±0.035 2.04±0.031 2.15±0.039 2.28±0.041 2.34±0.042 2.36±0.034 2.32±0.036 2.34±0.041	1.98±0.029 2.09±0.038 2.13±0.044 2.16±0.031 2.34±0.027 2.32±0.033 2.31±0.028 2.30±0.030	1.98 ± 0.03 2.02 ± 0.039 2.04 ± 0.06 2.10 ± 0.046 2.05 ± 0.051 2.12 ± 0.041 2.13 ± 0.039 2.09 ± 0.034	2.04 ± 0.033 1.94 ± 0.047 2.09 ± 0.029 2.08 ± 0.066 2.05 ± 0.054 2.09 ± 0.032 2.11 ± 0.040 2.04 ± 0.038	1.98 ± 0.048 1.95 ± 0.041 2.06 ± 0.039 2.04 ± 0.052 2.10 ± 0.032 2.12 ± 0.037 2.07 ± 0.044 2.09 ± 0.030	2.11 ± 0.054 2.06 ± 0.032 2.13 ± 0.047 2.09 ± 0.039 2.13 ± 0.042 2.14 ± 0.034 2.08 ± 0.043 2.09 ± 0.033
Porod volume estimate ($Å^3$)	38091 37667 38358 39073 38012 38937 39154 38460	37840 39001 38137 38387 38346 37441 38540 38639	38912 38448 38827 38650 38182 38156 38568 37693	38119 38340 38912 38518 39001 38153 39085 38356	37947 38606 38189 38973 38523 38171 39235 38225	38325 38466 38346 38946 38093 38117 38199 38156	38429 39052 39079 39010 38485 38951 38620 38577	38925 38645 38320 39254 38454 38985 38092 38463
Molecular-mass determination								
Molecular mass M_r [from V_c]	28.9 28.8 29.5 28.7 28.6 29.2	29.2 28.7 29.1 29.0 28.9 29.0	29.2 29.4 29.4 28.8 29.9 29.2	29.0 29.1 28.9 28.9 29.5 28.9	29.3 29.1 29.0 28.6 28.8 29.5	29.3 28.9 29.3 29.2 28.9 29.4	29.1 28.6 29.3 29.0 28.5 28.8	29.1 28.9 28.8 28.9 29.2 28.7

Supplementary Figure Legends

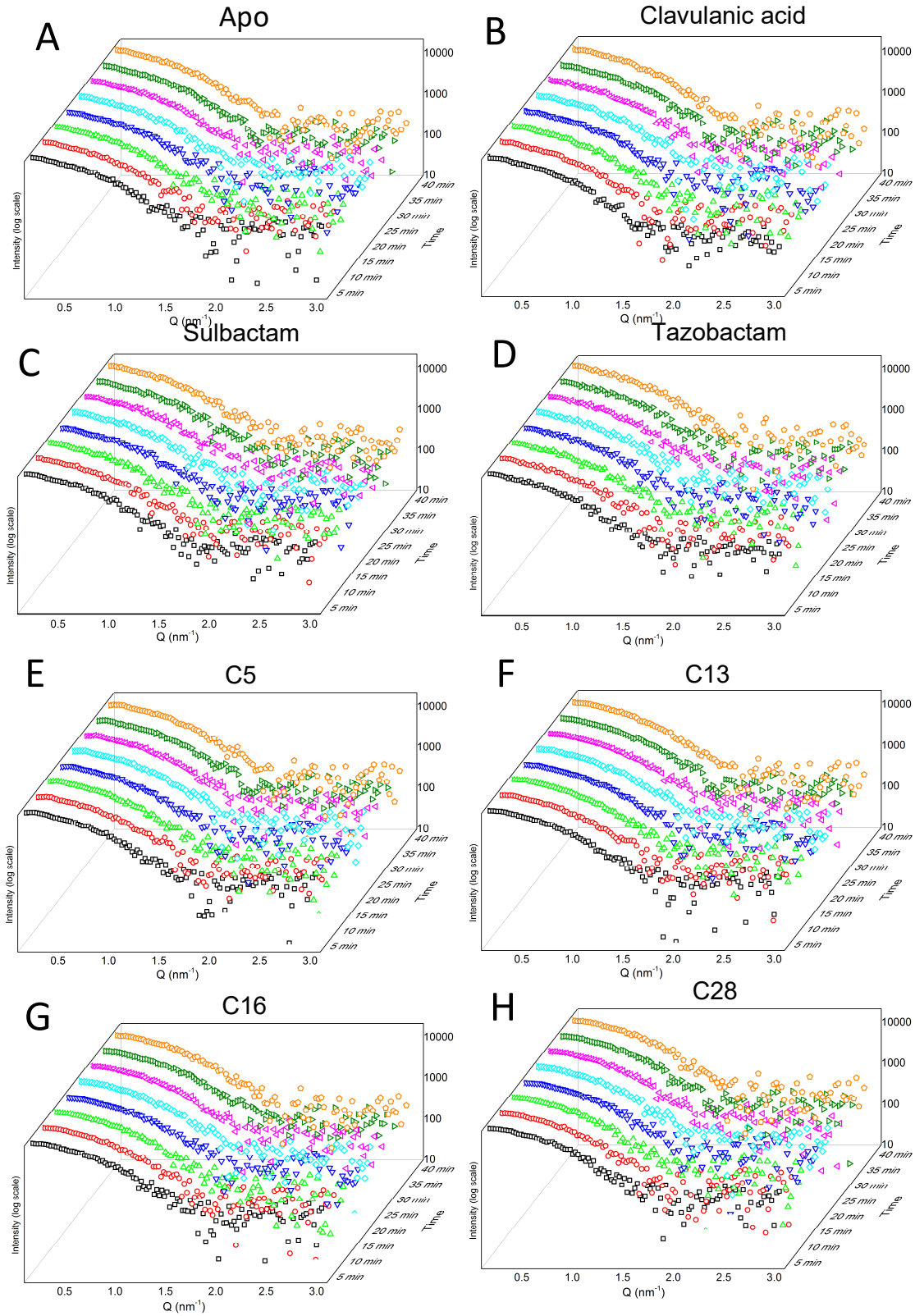
Figure S1 - The SAXS intensity profiles of BlaC in Apo state (A) and in presence of 100 fold molar excess of Clavulanic acid (B), Sulbactam (C), Tazobactam (D), C5 (E), C13 (F), C16 (G) and C28 (H) at various time points from 5 to 40 min.

Figure S2 - The Guinier Plots of BlaC in Apo state (A) and in presence of 100 fold molar excess of Clavulanic acid (B), Sulbactam (C), Tazobactam (D), C5 (E), C13 (F), C16 (G) and C28 (H) at various time points from 5 to 40 min. In the inset of each plot, the linear fits of the Guinier Plots at 5 and 40 min are presented close to each other to clearly show the difference in the slopes.

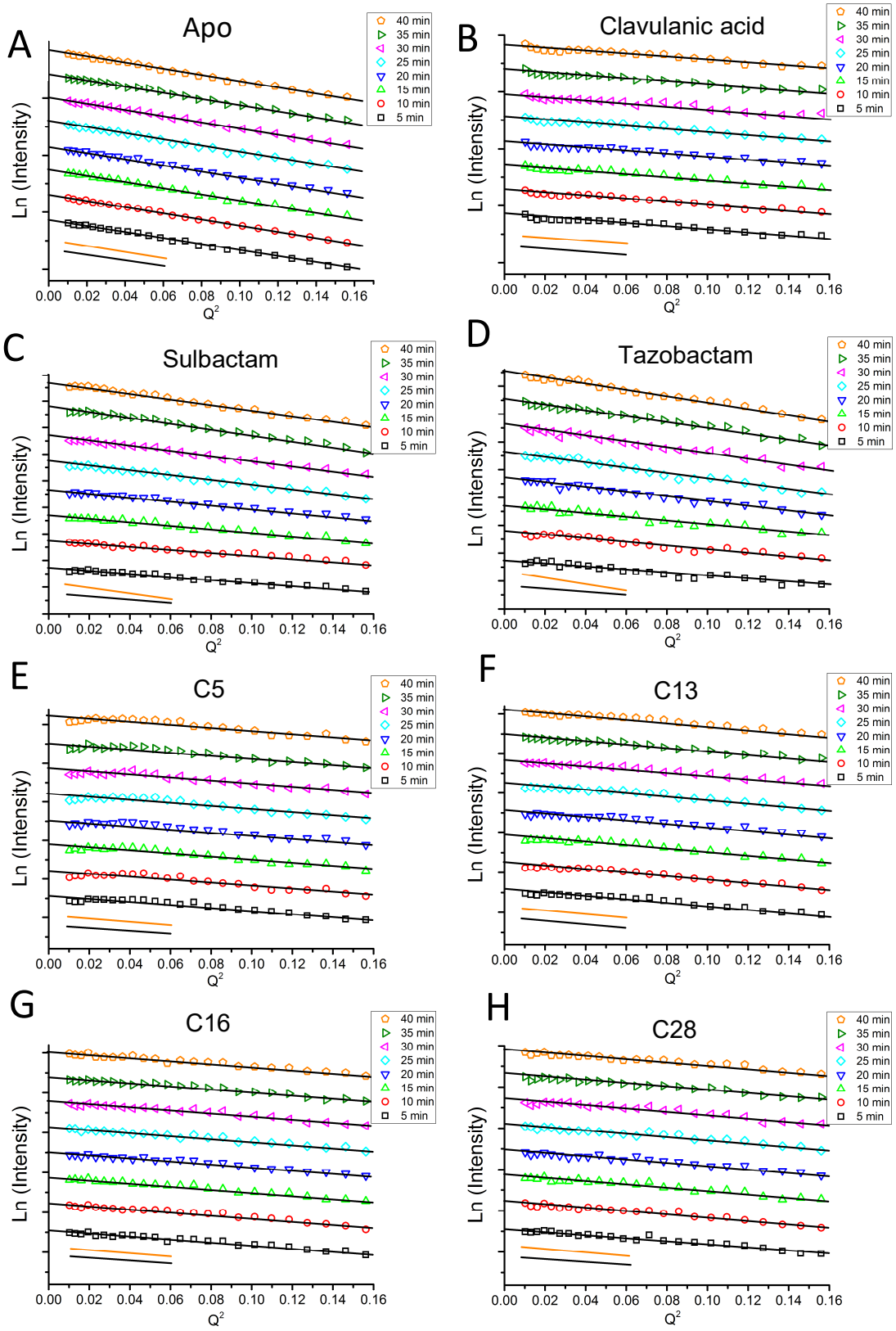
Figure S3 - The Porod-Debye Plots of BlaC in Apo state (A) and in presence of 100 fold molar excess of Clavulanic acid (B), Sulbactam (C), Tazobactam (D), C5 (E), C13 (F), C16 (G) and C28 (H) at various time points from 5 to 40 min. The gray histograms represent the value of Porod Exponent calculated from the SAXS profile.

Figure S4 - The Normalized Kratky Plots of BlaC in Apo state (A) and in presence of 100 fold molar excess of Clavulanic acid (B), Sulbactam (C), Tazobactam (D), C5 (E), C13 (F), C16 (G) and C28 (H) at various time points from 5 to 40 min.

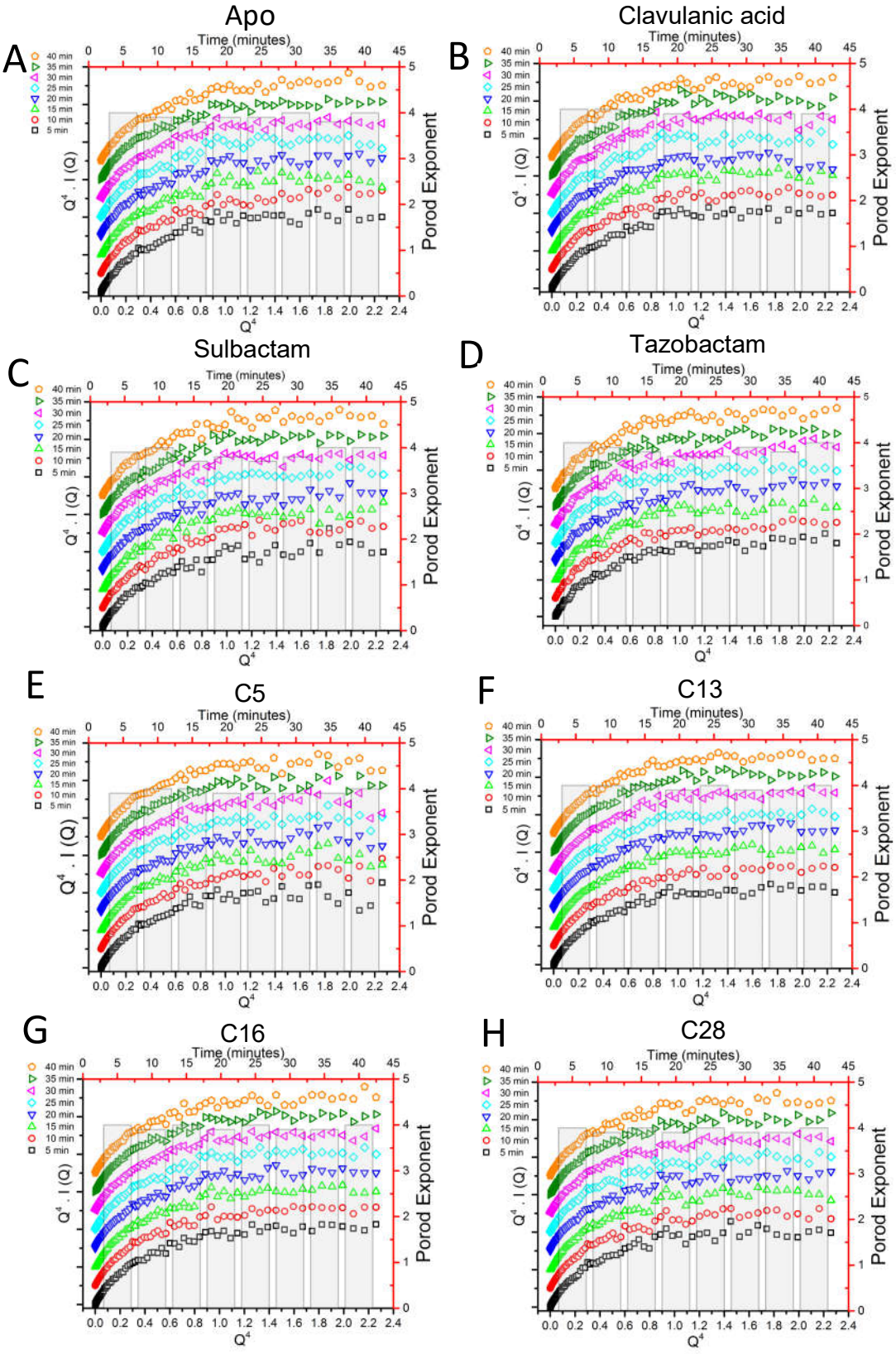
Supplementary figure S1



Supplementary figure S2



Supplementary figure S3



Supplementary figure S4