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Supplementary information

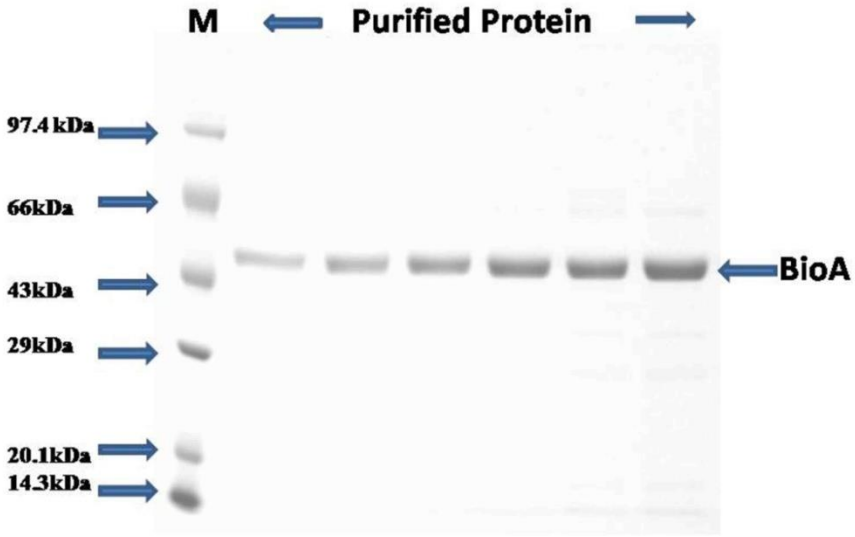
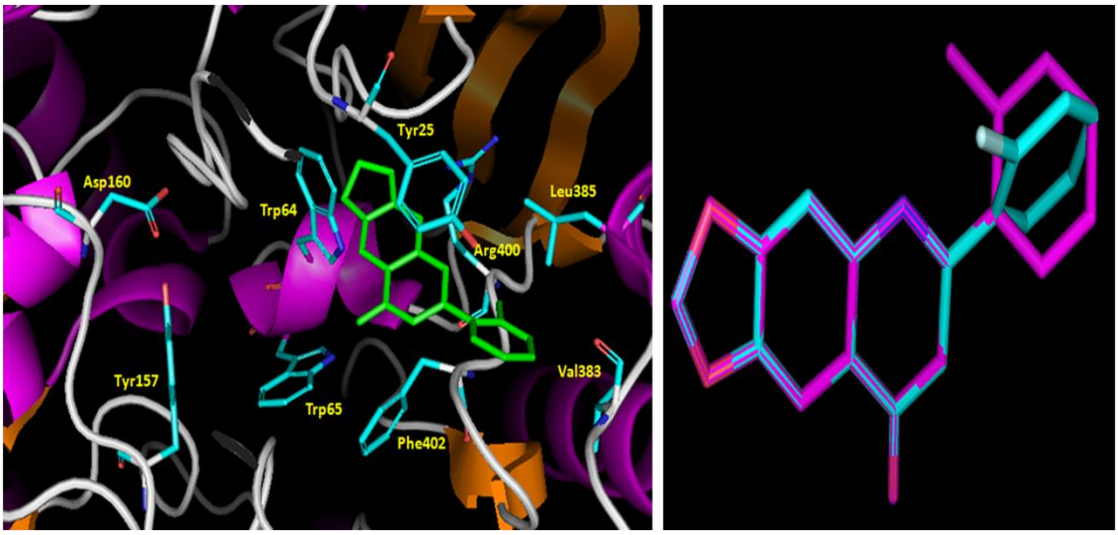


Figure S1: Purification of BioA protein. BioA was purified by using Ni-NTA affinity chromatography. BioA was eluted by using 250 mM imidazole and the purity of different eluted fractions was analyzed by SDS polyacryamide gel electrophoresis on 12.5% gel. BioA was observed at an estimated size of 48.6kDa.



(A) **(B)**

Figure S2: Docking of CHM-1 (the known inhibitor of BioA) at the active site of BioA. (A) CHM-1 was docked at the active site of BioA by Autodock4.2 by using the same grid co-ordinates as used in the docking studies. As expected, the docked complex of BioA-CHM-1 was found to be in the vicinity of the active site residues as reported earlier.[19] (B) Superimposition of docked and crystallographic mode of binding of CHM-1. Cyan colour represents the crystallographic mode of CHM-1 and magenta colour represents the docked conformation of CHM-1.

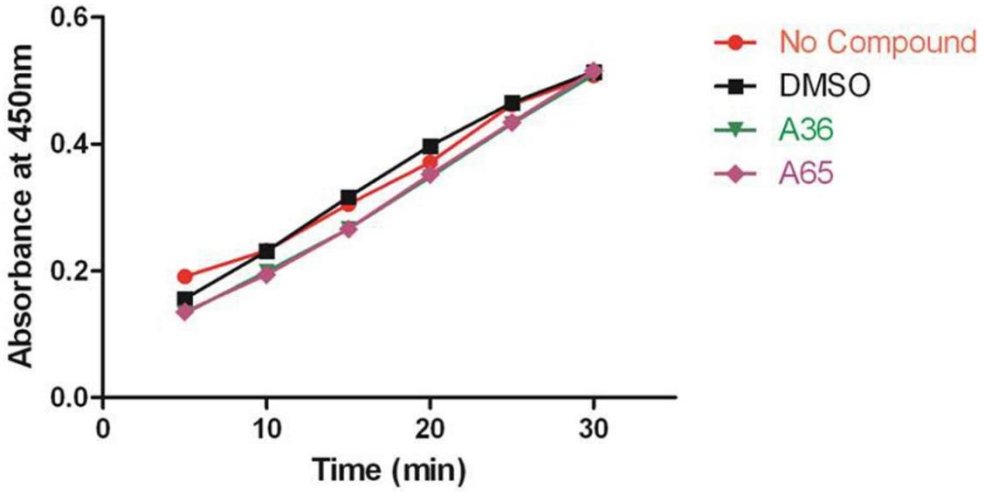


Figure S3: Evaluation of inhibitory potential of compounds against Aspartate transaminase (AST). Compounds exhibiting greater than 60 percent inhibition of BioA activity were evaluated for their potential to inhibit another PLP dependent enzyme i.e. AST. Reaction mix containing AST positive control and compound was incubated for 10 minutes at 37ºC followed by the addition of AST substrate, AST enzyme mix and AST developer and further incubated for 30 minutes at 37ºC. Formation of the product was detected by measuring the absorbance at 450nm. None of the compounds displayed any inhibition of AST activity and no reduction in the absorbance was observed at 450 nm. The figure depicts the results of two representative compounds.

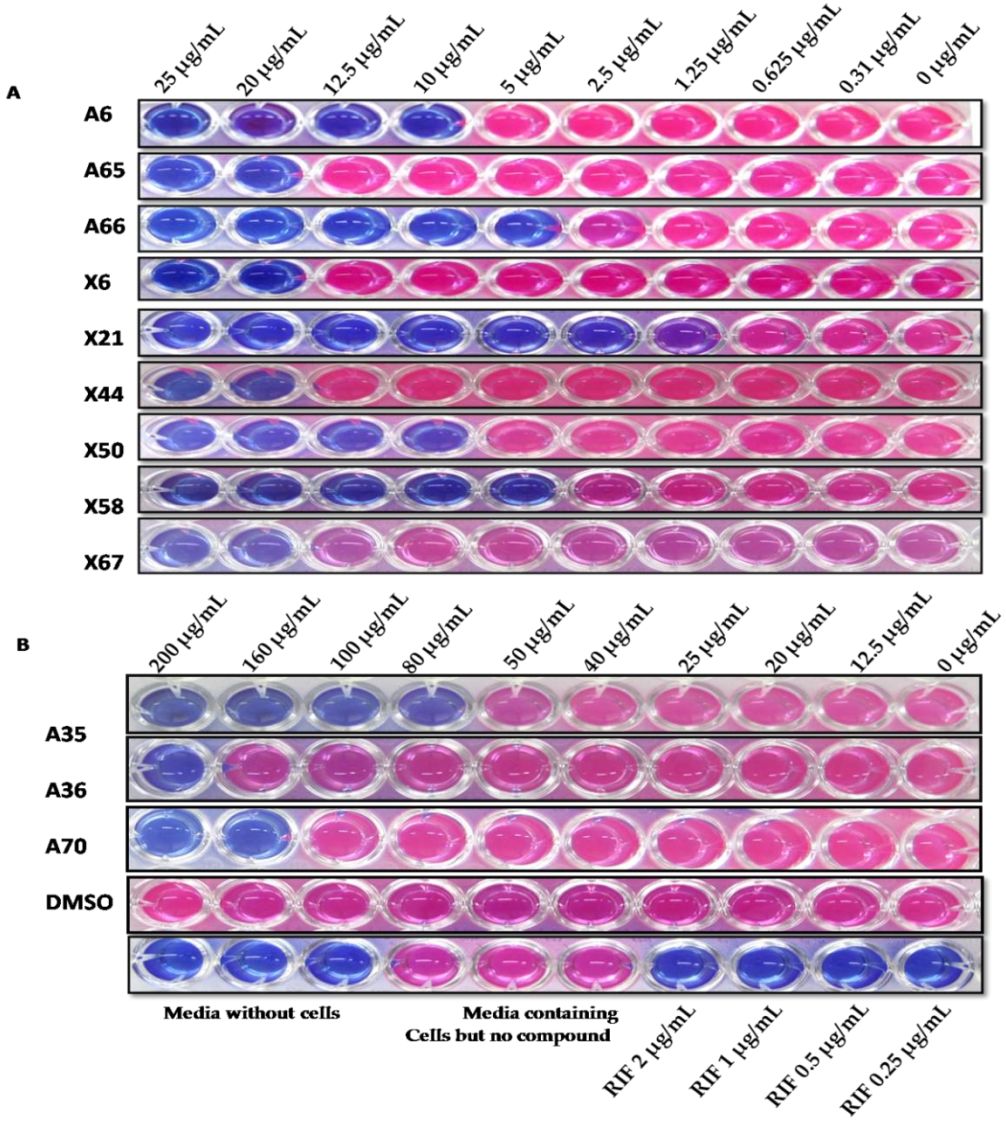


Figure S4: Evaluation of compounds for inhibition of *M. tuberculosis* growth. (A) Representative images after REMA assay for inhibition of *M. tuberculosis* growth by various compounds in the concentration range from 0.625 µg/mL to 25 µg/mL. (B) Compounds exhibiting greater than 60% inhibition of BioA enzyme activity were further tested for their potential to inhibit *M. tuberculosis* growth in the concentration from 12.5 µg/mL to 200 µg/mL. DMSO was used as a control whereas Rifampicin (RIF) was used as a positive control for the assay. DMSO did not display any inhibition of *M. tuberculosis* growth till the highest concentration employed.

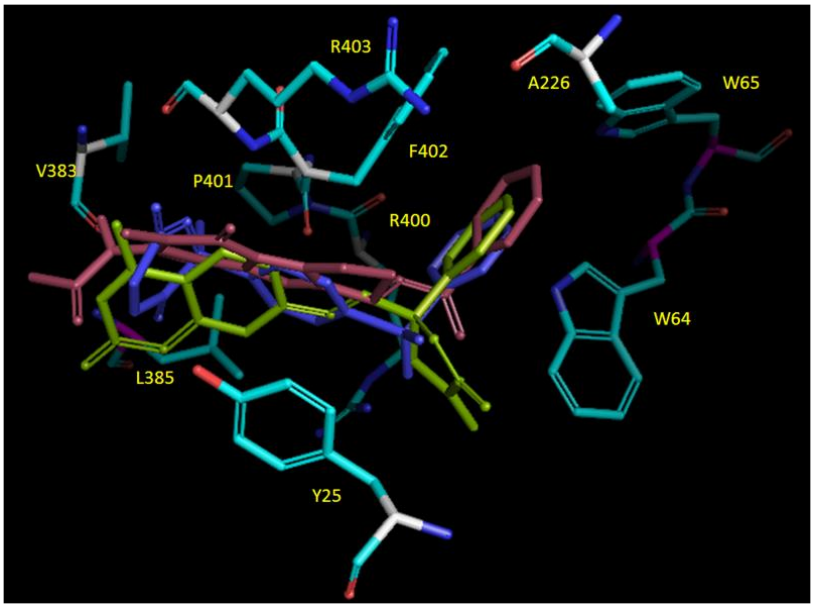


Figure S5: Superimposition of docked conformations of compound A35, compound A36 and compound 65 at the active site of BioA. The figure represents the compounds A35, A36 and A65 docked at the active of BioA. Surrounding residues were represented in cyan colour. Pink colour represents compound A35, green colour represents compound A36 and blue colour represents compound A65.

Figure S6: Multiple sequence alignment of *M. tuberculosis* BioA. Multiple Sequence alignment of *M. tuberculosis* BioA with the BioA sequences from the other species was performed by using ClustalW. Boxes represent the active site residues of BioA.

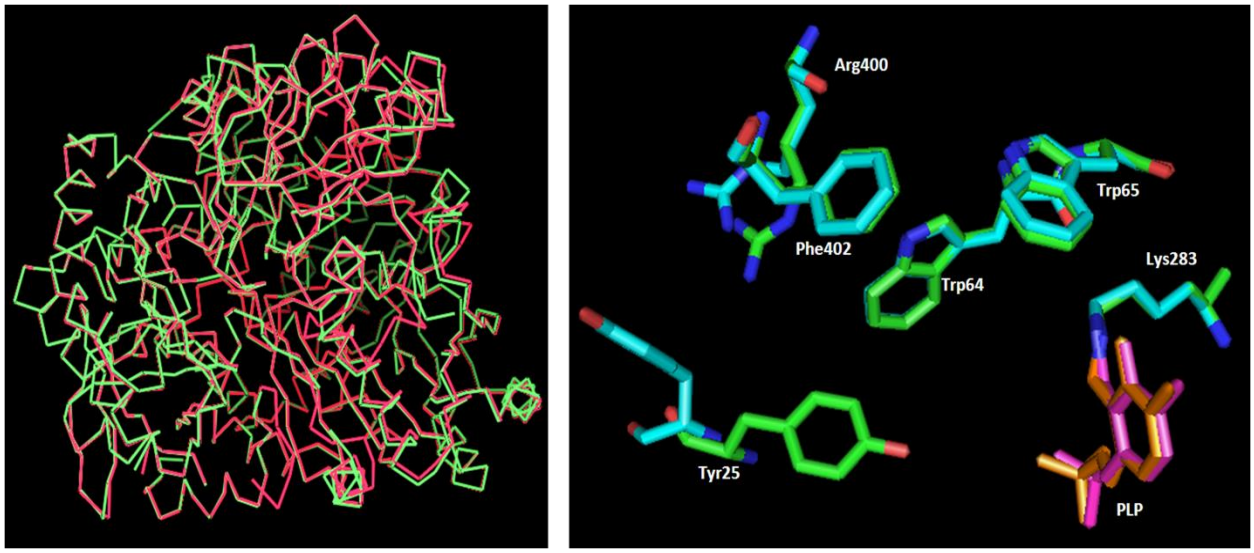


Figure S7: Comparison of structures of sinefungin bound BioA and KAPA bound BioA. (A) Alignment of crystal structures of BioA in complex with sinefungin (green) and BioA in complex with KAPA (red) which displayed the RMSD value of 0.205Å . (B) Alignment of active site residues of BioA in complex with sinefungin (cyan) and BioA in complex with KAPA (green) which displayed an RMSD value of 0.191Å. The PLP of BioA in complex with sinefungin and KAPA are represented in magenta and orange colour, respectively. The figure was generated by using Pymol.

Table S1: List of A series compounds that exhibited greater than 20% inhibition at 100 µg/mL

S.No.	Compound id	NSC id	Docking score	Percent inhibition at 100 µg/mL*
1	A1	1009	-10.59	33
2	A5	48602	-10.69	77
3	A6	56397	-10.58	32
4	A11	95154	-10.74	40
5	A12	108404	-10.60	56
6	A15	115767	-10.53	44
7	A18	163457	-11.30	31
8	A23	281774	-10.89	28
9	A24	298889	-10.74	45
10	A25	353454	-11.34	22
11	A26	373094	-10.26	65
12	A27	373236	-10.70	54
13	A28	373240	-11.24	21
14	A30	408904	-10.69	54
15	A31	609446	-10.60	22
16	A34	650017	-10.59	50
17	A35	658421	-10.85	85
18	A36	668266	-10.74	82
19	A39	690377	-10.58	30
20	A40	690380	-10.64	33
21	A42	16468	-10.46	26
22	A48	106111	-10.53	64
23	A49	107702	-10.33	25
24	A51	111575	-10.42	30
25	A55	127713	-10.36	27
26	A56	129519	-10.49	29
27	A57	152518	-10.32	43
28	A61	367105	-10.44	54
29	A62	373238	-10.65	47
30	A64	613565	-10.33	54
31	A65	615614	-10.47	84
32	A68	639914	-10.54	22
33	A70	652821	-10.35	74
34	A71	655494	-10.46	37
35	A72	671897	-10.36	34
36	A73	677026	-10.42	24
37	A79	687803	-10.43	21
38	A80	707084	-10.43	22
39	A81	710329	-10.38	20

* Data here depicts the values as the average of atleast two independent experiments.

Table S2: List of X series compounds that exhibited greater than 20% inhibition at 100 µg/mL

S.No.	Compound id	NSC id	Docking Score	Percent inhibition at 100 µg/mL*
1	X2	17490	7.30	38
2	X4	59310	7.27	27
3	X6	60622	7.31	28
4	X8	69356	7.38	20
5	X9	69360	7.27	38
6	X10	75500	7.50	47
7	X11	77534	7.33	32
8	X14	97308	7.41	45
9	X15	97752	7.39	34
10	X16	103111	7.32	36
11	X17	106062	7.39	33
12	X18	106728	7.35	30
13	X19	112336	7.30	26
14	X20	113521	7.47	26
15	X22	116631	7.31	58
16	X23	124222	7.55	34
17	X29	205574	7.50	53
18	X30	215214	7.28	53
19	X31	358779	7.33	27
20	X32	363071	7.29	44
21	X37	671451	7.26	26
22	X38	677807	7.34	53
23	X40	707079	7.47	51
24	X44	69576	7.19	29
25	X47	86505	7.20	21
26	X48	108405	7.21	25
27	X50	109838	7.25	53
28	X52	115981	7.18	32
29	X53	115984	7.23	21
30	X54	119447	7.20	23
31	X55	122433	7.26	20
32	X58	129077	7.22	23
33	X64	156171	7.19	21
34	X65	169450	7.18	56
35	X67	211542	7.20	55
36	X74	621516	7.18	26
37	X75	635330	7.18	46

* Data here depicts the values as the average of atleast two independent experiments.

Table S3: Assessment of the drug like properties of the potential inhibitors of BioA was calculated by AlogPs[31,32].

Compound Name	MW	logP^	logS$
A5	447.567	2.39	-4.86
A26	437	3.83	-4.97
A35	378.381	1.53	-3.21
A36	362.375	3.96	-5.11
A48	363	3.55	-4.88
A65	342.365	2.75	-4.80
A70	362.491	3.03	-4.41
CHM-1	283.258	2.92	-3.43

^ - logP represents partition coefficient of octanol and water

$-logS represents aqueous solubility of the compound.

Table S4: Evaluation of cytotoxicity of compounds in CHO cell line.

Compound	IC_{50} in CHO cell line (µg/mL)
A5	>200
A26	>200
A35	150
A48	>200
A65	50
A70	>200
RIF	>100