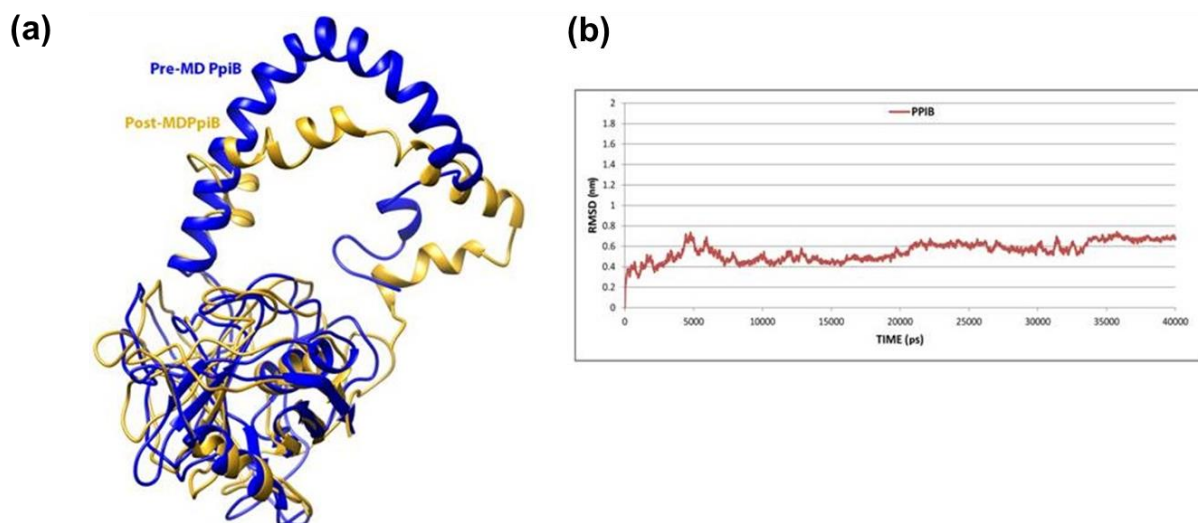


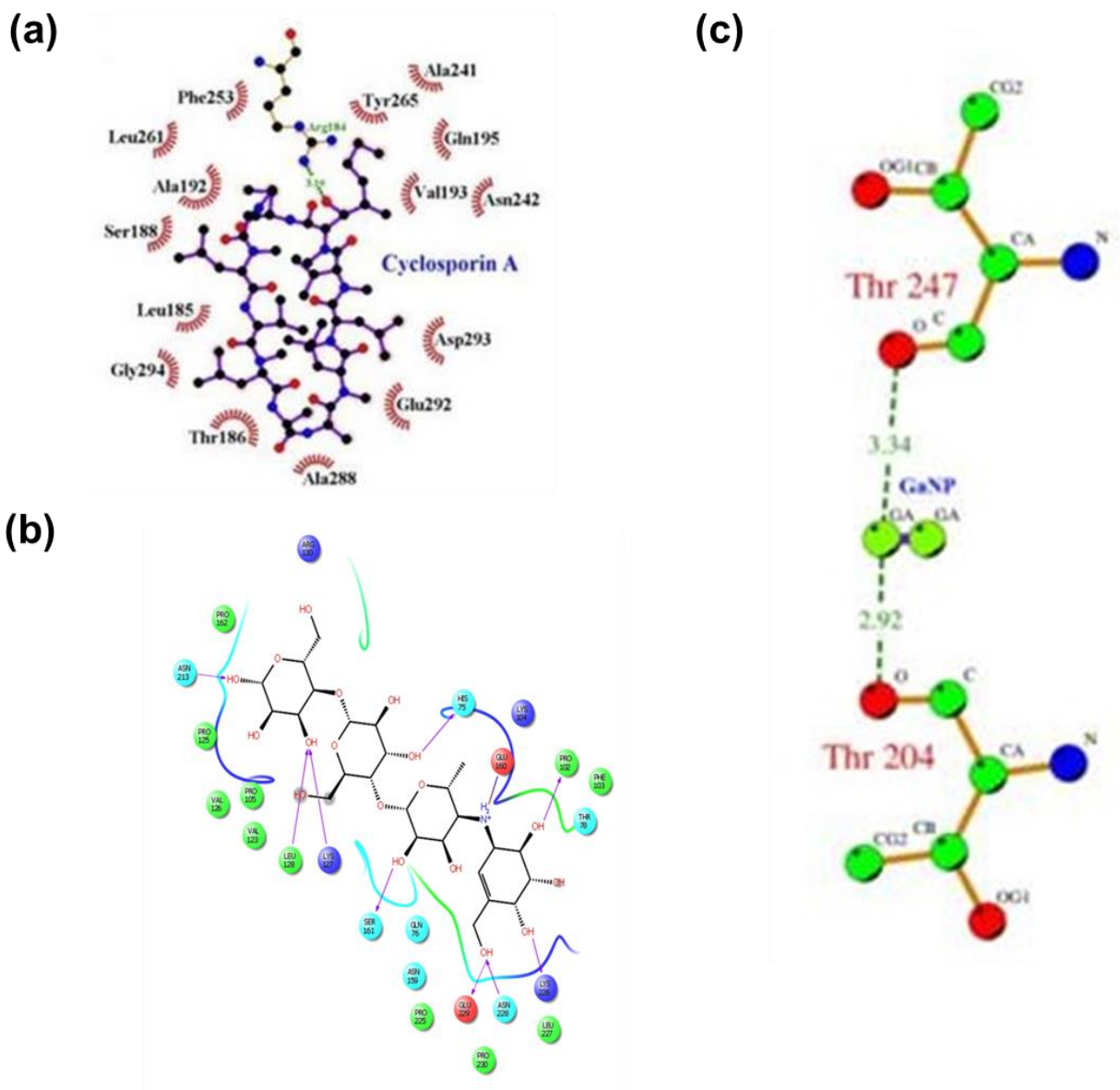
**Figure S1: Sequence alignment and comparison of different PPIase.** (Panel a): Multiple sequence alignments using BLASTp were performed to assess similarities between *M.tb* PpiB, *M.tb* PpiA and *E.coli* peptidyl-prolyl isomerase. (Panel b): Multiple sequence alignments of *M.tb* PpiB, *M.tb* PpiA and peptidyl-prolyl isomerase RopA (trigger factor) of *S.mutans* were tested using BLASTp. Highly conserved and less conserved amino acids are shown in red and blue, respectively.

*M.tb* PpiA or PpiB exhibit high degree of similarity in conserved amino acids found in *E.coli* PPIase. *M.tb* PpiB also possesses an extended sequence of 100 amino acids in the N-terminal end and is absent in either *M.tb* PpiA or *E.coli* PPIase. That *M.tb* PPIase exhibits high degree of sequence similarity with RopA proteins expressed in *S.mutans* is evident.



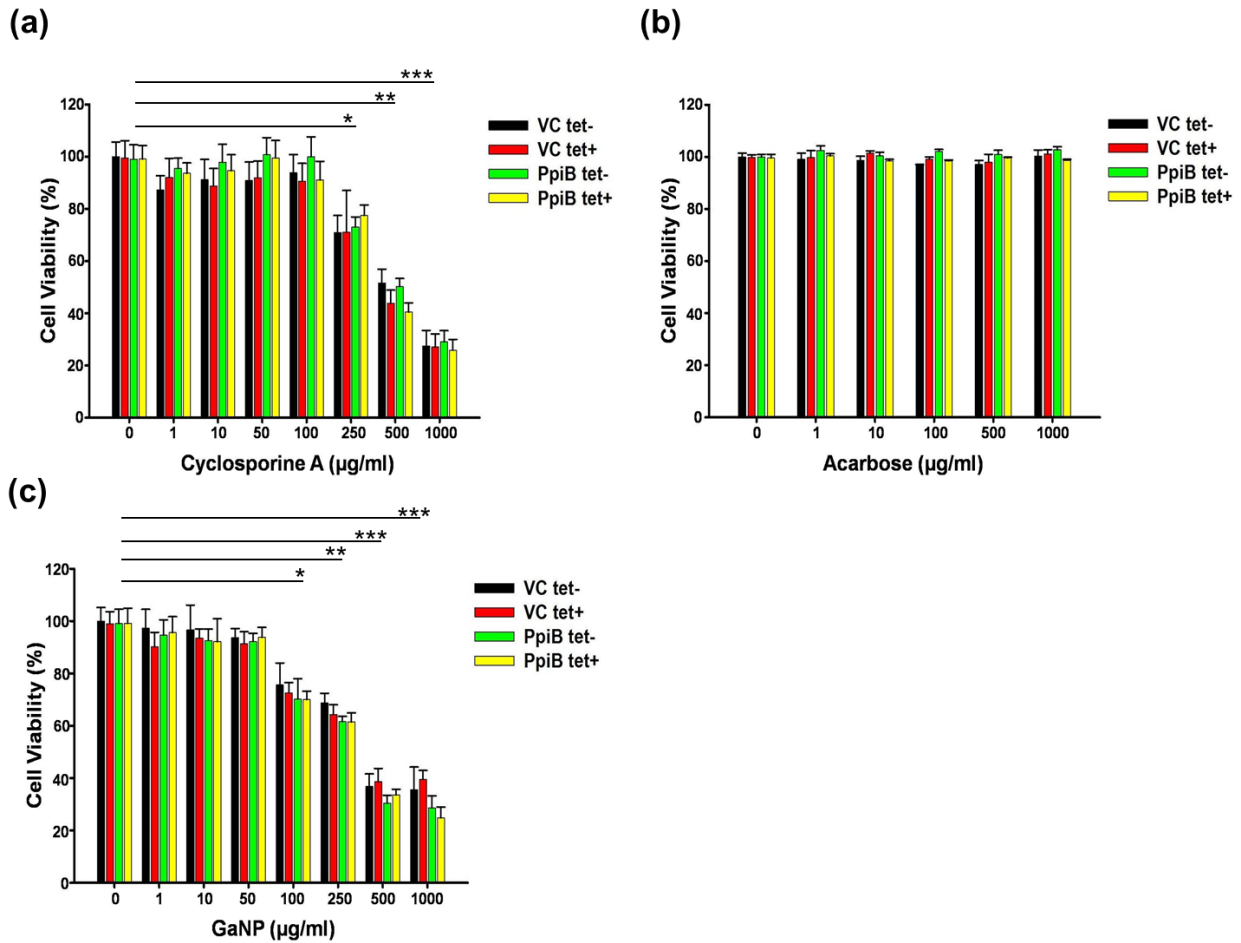
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 16 **Figure S2: Molecular Modelling of PpiB and *in-silico* docking of acarbose**  
 17 **with PpiB.** The modeled structure of PpiB obtained using Phyre2 or  
 18 MODELLER was found to have overall 98% residues in the allowed regions.  
 19 Our model scored -1.23 in the MolProbity Clashscore that was greater than the  
 20 recommended Global Z-score values of -3, suggestive of being an adequate  
 21 model. Verify3D also corroborated the reasonable quality of the model. (Panel  
 22 a): Ribbon representation showing superimposition of the Pre-MD PpiB (shown  
 23 in blue) and Post-MD PpiB structure obtained from the 40 ns molecular  
 24 dynamic simulations (shown in golden-yellow). (Panel b): RMSD plot of PpiB  
 25 apo structure molecular dynamics trajectory.

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 29 **Figure S3: Ligplot of interactions of PpiB with Cyclosporine-A, acarbose**  
 30 **and dimeric atomic gallium.** (Panel a): Ligplot of interacting amino acid  
 31 residues of PpiB interacting with cyclosporine-A. (Panel b): Ligplot of  
 32 interacting amino acid residues of PpiB interacting with acarbose. (Panel c):  
 33 Ligplot of interacting amino acid residues of PpiB interacting with dimeric  
 34 atomic gallium<sup>17</sup>.

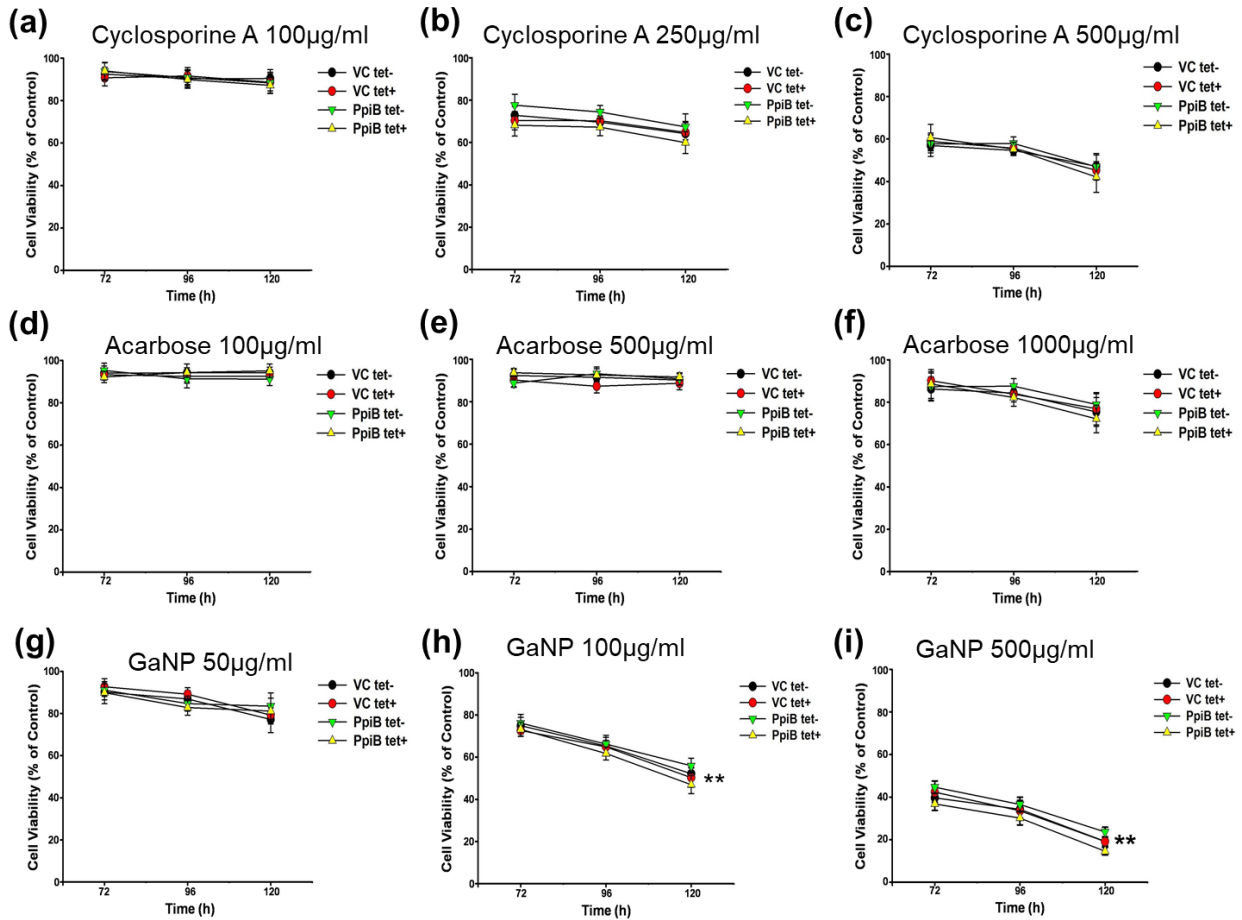
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39 **Figure S4: Cyclosporine-A, acarbose or GaNP impact the viability of**  
 40 ***M. smegmatis*.** Ms\_VC and Ms\_PpiB cells were induced with  
 41 anhydrotetracycline to express *ppiase* gene in absence and presence of  
 42 cyclosporine-A (0, 1, 10, 50, 100, 250, 500, 1000 µg/ml) (Panel a), acarbose (0,  
 43 1, 10, 100, 500, 1000 µg/ml) (Panel b) or GaNP (0, 1, 10, 50, 100, 250, 500,  
 44 1000 µg/ml) (Panel c), as described in methods. At the end of incubation  
 45 period, cell viability was assessed in a 4 h alamar blue redox assay. Values  
 46 shown from a representative experiment are means [±SEM] of percent cell  
 47 viability for *M. smegmatis* (VC and PpiB) cultured in absence [(■)VC tet-, (■) PpiB tet-]  
 48 or presence [(■) VC tet+, (■) PpiB tet+] of anhydrotetracycline.  
 49 Note the decline in cell viability evident at higher concentration of only GaNP  
 50 but not cyclosporine A or acarbose. \*p<0.05, \*\*p<0.01, \*\*\*p<0.005 (ANOVA).

51 Initially, the effect of acarbose, cyclosporine-A and GaNP on the viability  
52 of *M. smegmatis* cells expressing *M. tb* PpiB was examined colorimetrically  
53 using Alamar Blue redox dye assay, as described in methods. Control (Ms\_VC)  
54 and recombinant (Ms\_PpiB) strains were induced with anhydrotetracycline,  
55 hereafter referred as VC tet<sup>+</sup> and PpiB tet<sup>+</sup>, respectively. Ms\_VC and Ms\_PpiB  
56 not induced with anhydrotetracycline were used as control, hereafter referred  
57 as VC tet<sup>-</sup> and PpiB tet<sup>-</sup>, respectively. It can be seen (supplementary Fig. S4,  
58 Panel a) that cyclosporine-A below 250 µg/ml had insignificant effect on the  
59 number of viable *M. smegmatis* cells. However, a comparison of the cell  
60 viability of the group, [VC (tet<sup>-</sup>/tet<sup>+</sup>) and PpiB (tet<sup>-</sup>/tet<sup>+</sup>)], treated with 250  
61 µg/ml and above of cyclosporine-A with the control group (no treatment of  
62 cyclosporine-A), showed significant difference (P<0.05, P<0.01 and P<0.005) in  
63 viable cell numbers. Acarbose up to (1000 µg/ml) had no effect on the number  
64 of viable Ms\_VC or Ms\_PpiB cells (supplementary Fig. S4, Panel b). Even at 10  
65 mg/ml, acarbose showed no significant effect on cell viability (data not shown),  
66 implying that it did not exhibit inhibitory effect on survival of *M. smegmatis*.  
67 Results in supplementary Fig. S4 (Panel c) show that GaNP below 100 µg/ml  
68 had no significant effect on the number of viable *M. smegmatis* cells. Cell  
69 viability of the group, [VC (tet<sup>-</sup>/tet<sup>+</sup>) and PpiB (tet<sup>-</sup>/tet<sup>+</sup>)] treated with 100  
70 µg/ml and above of GaNP, with the control group (no treatment of GaNP),  
71 shows that there is significant difference (P<0.05 P<0.01 and P<0.005) in viable  
72 cell number. A threshold value of 100 µg/ml, 1000 µg/ml and 50 µg/ml of  
73 cyclosporine-A, acarbose and GaNP, respectively was selected for further  
74 experiments to examine the effect of these components on biofilm formation.  
75 These threshold values of cyclosporine-A, acarbose and GaNP at which viability  
76 of PpiB expressing *M. smegmatis* is not affected was crucial for validating the  
77 effect of these components on biofilm formation. A decrease in biofilm  
78 formation may be a result of decreased cell numbers *per-se*, so it was  
79 important to ascertain the threshold dose of cyclosporine-A, acarbose or GaNP  
80 that does not affect the overall growth of *M. smegmatis*.



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 82 **Figure S5: Bacteriostatic versus bactericidal effect of cyclosporine-A,**  
 83 **acarbose and GaNP.** Ms\_VC and Ms\_PpiB cells were induced with  
 84 anhydrotetracycline to express *ppiase* gene in absence and presence of  
 85 cyclosporine-A (100 µg/ml, Panel a; 250 µg/ml, Panel b; 500 µg/ml, Panel c ),  
 86 acarbose (100 µg/ml, Panel d; 500 µg/ml, Panel e; 1000 µg/ml, Panel f ) or  
 87 GaNP (50 µg/ml, Panel g; 100 µg/ml, Panel h; 500 µg/ml, Panel i), as described  
 88 in methods. At the end of incubation period (72 h, 96 h, 120 h), cell viability  
 89 was assessed in a 4 h alamar blue redox assay. Values shown from a  
 90 representative experiment are means [±SEM] of percent cell viability for  
 91 *M.smegmatis* (VC and PpiB) cultured in absence [(●)VC tet-, (▼) PpiB tet-] or  
 92 presence [(●) VC tet+, (▲) PpiB tet+] of anhydrotetracycline.  
 93 \*\*p<0.01(ANOVA). Note the bacteriostatic effect of cyclosporine-A, acarbose and  
 94 GaNP (panels a to g). In panels c, f and g the values at time 72 h and time 120  
 95 h are not significant. However, in panels (h) and (i), a very significant decline in

96 growth at time 120 h is evident when compared with 72 h, pointing to the  
97 bactericidal effect of GaNP.

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99 In order to assess whether cyclosporine-A, acarbose and GaNP has  
100 bacteriostatic or bactericidal effect on *M. smegmatis*, cells were cultured with  
101 these drugs for upto 120 h. A significant decrease in cell viability of the  
102 treatment group at 120 h as compared to at 72 h denoted bactericidal effect of  
103 the drug. Results in supplementary Fig. S5 show that at 100 µg/ml, both  
104 cyclosporine-A and acarbose exert bacteriostatic effect. It is also evident that  
105 cyclosporine-A, acarbose and GaNP have bacteriostatic effect upto 500 µg/ml,  
106 1000 µg/ml, 50 µg/ml, respectively (supplementary Fig. S5, panels a to g). The  
107 apparent decline in the values at time 120 h as compared to at 72 h  
108 (supplementary Fig. S5, panels c, f and g) were not significant based on ANOVA  
109 analysis. A very significant decline in growth at time 120 h was evident  
110 (supplementary Fig. S5, panels h, i), pointing to bactericidal effect of GaNP at  
111 100 µg/ml and above. These results indicate that cyclosporine-A and acarbose  
112 have bacteriostatic effect on cell viability whereas GaNP at higher concentration  
113 is bactericidal.

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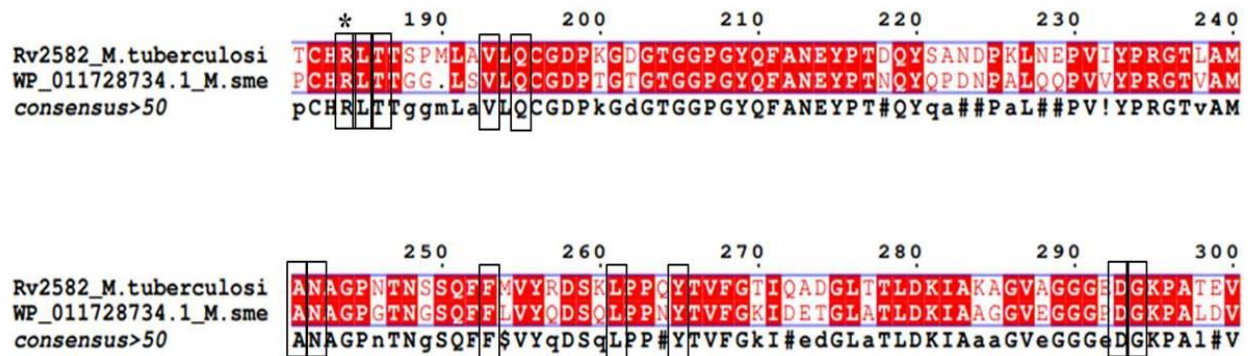
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126 **Figure S6: Sequence alignment of Rv2582 (PPIB protein) from *M.***  
 127 ***tuberculosis* and WP\_011728734 from *M.smegmatis*.** The binding pocket  
 128 residues present in Rv2582 are Ala241, Tyr264, Gln195, Val193, Asn242,  
 129 Asp293, Thr186, Gly294, Leu185 Ser188, Ala192, Leu261, Phe253, Arg184,  
 130 Glu292, Ala288, Ser188, Ala192. Sequence alignment showed that all the  
 131 above mention residues are also conserved in WP\_011728734 from  
 132 *M.smegmatis* except Glu292, Ala288, Ser188 and Ala192 and t6e conserved  
 133 residue Arg184 from PPIB protein which was used as catalytic centre for  
 134 docking with cyclosporine-A is also conserved in WP\_011728734 gene.

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**Supplementary Table 1: Docking results of PpiB with FDA Approved Drugs**

	<b>GENERIC NAME</b>	<b>PRODUCTS</b>	<b>DOCKING SCORE</b>	<b>FUNCTIONS</b>
1	Acarbose	Acarbose; Glucobay; Precose	-13.3	Reversible binding to pancreatic alpha-amylase & membrane-bound intestinal alpha-glucoside hydrolases. Used for treatment of diabetes type II.
2	Cyclosporine A <sup>#</sup>	Gengraf, Sandimmune	-5.2	It is an immunosuppressant used in patients undergoing organ transplantation. It is also used for treatment of psoriasis and severe rheumatoid arthritis.
2	Diosmin		-12.2	It is a semisynthetic drug used for the treatment of venous diseases.
3	Ouabain		-9.8	It inhibits Na-K-ATPase membrane pump and used in treatment of atrial fibrillation & heart failure.
4	Ticagrelor	Brilinta	-9.7	It blocks ADP receptors. Used for prevention of thrombotic events such as stroke & heart failure.
5	Flavin adenine dinucleotide		-9.5	It is used to treat eye diseases due to vitamin B2 deficiency.
6	Travoprost	Izba; Travatan Z	-9.4	It is a selective prostanoid receptor agonist that is used to reduce elevated intraocular pressure.
7	Cytarabine	Cytarabine;Cytosar	-8.9	It acts by direct DNA damage as well as incorporation into DNA. Used in treatment of different forms of leukaemia.

8	Lymecycline		-8.7	Inhibits cell growth via inhibition of translation and used for treatment of infections & acne.
9	Azacitidine	Azacitidine; Vidaza	-8.7	Inhibits the DNA methyltransferase at low doses while incorporates into DNA and RNA at high doses, resulting in cell death. Used for treatment of patients with French-American-British myelodysplastic syndrome subtypes.
10	Paromomycin		-8.5	Inhibits protein synthesis via 16S ribosomal RNA binding. Used for treatment of acute as well as chronic intestinalamebiasis.
11	Adenosine monophosphate (AMP)		-8.3	Dietary supplement to boost immune activity. Also used as substitute sweetener for low-calorie diet.
12	Daunorubicin	Daunorubicin Hydrochloride; Daunoxome	-8.3	It forms complexes with DNA thereby having cytotoxic and antimitotic activity. Used in the treatment of nonlymphocyticleukaemia.
13	Epirubicin	Ellence	-8.3	Inhibits nucleic acid and protein synthesis via different mechanisms. Used in adjuvant therapy for patients with axillary node tumor involvement.
14	Doxorubicin	Caelyx; Doxil; Myocet	-8.3	Intercalates between base pairs and inhibits topoisomerase II activity

				thereby having antimitotic and cytotoxic activities. Used in treatment of acute myeloblastic leukemia and acute lymphoblastic leukemia,
15	Valrubicin	Valstar; Valtaxin	-8.2	An anthracycline that affects nucleic acid metabolism and used in bladder cancer treatment.
16	Pentostatin	Nipent	-8.1	A transition state inhibitor of ADA(adenosine deaminase) and used in treatment of hairy cell leukaemia refractory to alpha interferon.
17	Sofosbuvir	Sovaldi	-8.1	A nucleotide analog inhibitor, that inhibits HCV NS5B polymerase and used in combination therapy for treatment of chronic hepatitis C virus.
18	Glyburide	Diabeta; Euglucon; Glyburide; Glynase	-8.1	It binds to ATP-sensitive potassium channels on pancreatic cell surface and used as an adjunct to diet for lowering blood glucose in patients with NIDDM.
19	Fludarabine	Fludara; Fludarabine; Fludarabine Phosphate; Oforta	-8.1	It gets converted to 2-fluoro-ara-ATP and this metabolite inhibits DNA synthesis. Used for treatment of B-cell chronic lymphocytic leukaemia
20	Pioglitazone	Actos; Pioglitazone; Hydrochloride	-8.1	Agonist of peroxisome proliferator activated receptors (PPAR) and used in treatment of Type II diabetes mellitus.

21	Idarubicin	Idarubicin; Idarubicin Hydrochloride	-8.1	Intercalates between base pairs and inhibits topoisomerase II activity thereby having antimitotic and cytotoxic activities.  Used for treatment of acute myeloid leukemia (AML
22	Canagliflozin	Invokana	-8.0	Inhibitor of (SGLT2) Sodium-glucose co-transporter 2 and an adjunct to diet for improving glycemic control in adult patients of type 2 diabetes mellitus.

149 # Inhibitor lead obtained from previously published reports<sup>18</sup>

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172 **Supplementary Table 2: Amino acid residues in active site of PpiB homologues, from**  
 173 **other biofilm forming bacteria, that interact with cyclosporine-A or acarbose or dimer of**  
 174 **atomic gallium.**

<b>Name of the organism</b>	<b>Role in biofilm</b>	<b>GenBank accession number</b>	<b>Homology with PpiB</b>	<b>E-value</b>	<b>Query coverage</b>	<b>Binding Pocket Residues of Cyclosporin</b>	<b>Binding Pocket Residues of Acarbose</b>	<b>Binding Pocket Residues of dimer of atomic gallium<sup>29</sup></b>
<i>Mycobacterium tuberculosis</i>	(Present study)	AL123456	NA	NA	NA	Arg184	Pro162	Gly203
<i>Staphylococcus aureus</i>	Cystic Fibrosis, Pacemakers, Prosthetic heart valves, Contact Lenses, Orthopaedic implants	<a href="#">WP_0617360_25.1</a>	35%	9e-20	54%	His186, His58, Pro184, Asp187, Lys183, Val160, Arg59, Leu185, Val178, Lys177	Ala2, Asn3, Tyr4, Pro5, Gln6, Leu7, Gly14, Glu15, Ile16, Pro33, Asn34, Pro37, Lys38, Glu41, Tyr82	Met76, Gly77, Gly78,
<i>Staphylococcus epidermidis</i>	Prosthetic heart valves, Wounds,	<a href="#">WP_0493741_78.1</a>	33%	8e-18	53%	Tyr186, Asp187, Val160, Pro184, His58, Val185, Arg59, Val178, Ile61, Lys177	Lys9, Asn14, Ile16, Lys17, Lys30, Phe32, Pro33, Asp34, Glu197	Gly75, Gln114
<i>Staphylococcus intermedius</i>	Wounds	<a href="#">WP_0191682_88.1</a>	31%	3e-19	54%	Asp187, Tyr186, Pro184, His58, Met185, Arg59,	Met1, Thr2, Tyr4, Gln6, Leu7, Lys9,	Gly75, Met76

						Val178, Val60, Lys177	Gln12, Glu13, Pro37, Lys39, Gln41, Tyr82, Glu87	
<i>Streptococcus mutans</i>	Dental biofilm, Orthopaedic implants, Wounds, Prosthetic heart valves, Pacemakers	<u>WP_0193205</u> <u>73.1</u>	33%	1e-22	56%	Gln238, Lys240, Lys236, Thr104, Ser234, His106, Gly235, Gln233, Asn232, Pro119, Lys120, Gly121, Gln171, Arg107	Leu5, Val8, Leu9, Phe12, Lys41, Lys43, Leu44, Lys45, Gln46, Leu47, Glu63, Ala64, Leu81, Lys82, Pro85, Val88, Glu89, Leu92, Asp251	Gly123
<i>Staphylococcus saprophyticus</i>	Contact lenses	<u>WP_0487926</u> <u>81.1</u>	31%	2e-18	53%	His58, His186, Asp187, Pro184, Leu185, Arg59, Val60, Val178, Lys177	Asn3, Tyr4, Pro5, Gln6, Leu7, Ile16, Lys17, Lys30, Leu31, Leu32, Pro33, Asp34, Val35, Glu93, Gln162, Asp197	Gly75, Gln114
<i>Streptococcus constellatus</i>	Dental biofilm	<u>WP_0062700</u> <u>79.1</u>	32%	1e-19	52%	Ala374, His424,	Leu140, Pro141,	Gln335

						Asn373, Gly343, Met344, His326, Met450, Asp451, Lys452, Thr342, Arg327	Val178, Arg179, Trp180, Glu218, Leu219, Gly235, Ile236, Ser237, His238, Lys239, Lys242	
<i>Pseudomonas aeruginosa</i>	Cystic Fibrosis, Wounds, Contact Lenses, Orthopaedic implants, Breast implants,	<u>CRO97127.1</u>	38%	1e-21	45%	Arg46, Gly141, Leu139, Asp142, His45, Phe44, Glu140, Val47, Gly131, Ile48, Asp132	Lys22, Ala23, Pro24, Leu25, Glu71, Asp72, Glu73, Lys74, Phe115	Thr63

175 NA: not applicable