biochemical, immunological and functional characterization. *PhD Thesis-submitted to University of Hyderabad, India*. It has been reported that Mycobacterial protein, the polyketide Pks1 contributes to biofilm formation in *M. tuberculosis* (Pang *et al*, J. Bac. 2012, 194(3):715-21) but inhibitor against this protein is not known. It has also been reported that a chemical, TCA1 reduces the biofilm formation by *M. tuberculosis* (Wang *et al, PNAS,* USA, 2013; 110(27): E2510-7) but its target has not been studied.

[004] The most frequently used diagnostic methods for tuberculosis are the tuberculin skin test, acid-fast stain, and chest radiographs. Treatment of tuberculosis frequently involves multi drug therapy (MDT) treatment. This involves taking a combination of four drugs for a period of 6-9 months, often causing toxicity to the patients. The prolonged treatment is due to the ability of this slow growing bacterium to form the biofilm which makes the bacterium drug tolerant.

[005] The development of drug resistance in this bacterium has led to the development of *M. tuberculosis* variants which show multi-drug resistance (MDR). This is a major issue in the treatment of the disease and there is now a need to identify alternative drugs for the treatment of tuberculosis.

SUMMARY

[006] In an aspect of the present disclosure, there is provided a medicament comprising a drug selected from a group consisting of acarbose, cyclosporine A, its pharmaceutically acceptable salts thereof, and combinations thereof, for use in treatment of diseases caused by biofilm forming microorganisms.

[006a] In another aspect, the present disclosure refers to the use of acarbose and its pharmaceutically acceptable salts in the manufacture of a medicament for use in treatment of diseases caused by biofilm forming microorganisms.

[006b] In another aspect, the present disclosure refers to the useof acarbose, cyclosporine A, its pharmaceutically acceptable salts thereof, and combinations thereof

in the manufacture of a medicament for use in treatment of diseases caused by biofilm forming microorganisms.

[007] In an example of the present disclosure, there is provided a method of treatment of diseases caused by biofilm forming microorganisms, said method comprising:

(a) obtaining a drug selected from a group consisting of acarbose and its pharmaceutically acceptable salts; and

(b) administering a therapeutically relevant amount of acarbose.

[008] In an example of the present disclosure, there is provided a method of treatment of diseases caused by biofilm forming microorganisms, said method comprising:

(a) obtaining a drug selected from a group consisting of Cyclosporine A and its pharmaceutically acceptable salts; and

(b) administering a therapeutically relevant amount of Cyclosporine A.

[009] These and other features, aspects, and advantages of the present subject matter will be better understood with reference to the following description and appended claims. This summary is provided to introduce a selection of concepts in a simplified form. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

[0010] The following figures form part of the present specification and are included to further illustrate aspects of the present disclosure. The disclosure may be better understood by reference to the figures in combination with the detailed description of the specific embodiments presented herein.

[0011] Figure 1 depicts the enhanced biofilm and pellicle formation in recombinant *M. smegmatis* expressing *M. tuberculosis* PpiB protein, in accordance with an embodiment of the present disclosure.

[0012] Figure 2 depicts the effect of Cyclosporine A on the viability of *M. smegmatis* using the alamar blue assay, in accordance with an embodiment of the present disclosure.

[0013] Figure 3 depicts the effect of acarbose on the viability of *M. smegmatis* using the alamar blue assay, in accordance with an embodiment of the present disclosure.

[0014] Figure 4 depicts the effect of Cyclosporine A on biofilm formation in *M. smegmatis* expressing *M. tuberculosis* PpiB, using the crystal violet assay, in accordance with an embodiment of the present disclosure.

[0025] Figure 15A and 15B depicts multiple interactions between cyclosporine A and *M. tb* PpiB, in accordance with an embodiment of the present disclosure.

[0026] Figure 16 depicts protein homology of *M.tb* PpiB (Rv2582) with proteins from other biofilm forming bacteria, in accordance with an embodiment of the present disclosure.

[0026A] Figure 16A depicts protein homology of M.tb PpiB (Rv2582) with proteins from other biofilm forming bacteria, and the in silico interaction of acarbose with P162 and gallium with G203, in accordance with an embodiment of the present disclosure.

[0027] Figure 17 A depicts homology of *M. tb* PpiA or PpiB with *E. coli* Ppiase, and Figure 17 B depicts homology of *M. tb* Ppiase with trigger factor proteins (RopA) expressed in *S. mutans*, in accordance with an embodiment of the present disclosure.

[0028] Figure 18 A depicts SPR analysis of cyclosporine A interaction with *M. tb* PpiB, and Figure 18 B depicts SPR analysis of acarbose interaction with *M. tb* PpiB, in accordance with an embodiment of the present disclosure.

[0028A] Figure 19 depicts molecular docking study for interaction of dimeric gallium with G203 and nearby residue of T204/T247, in accordance with an embodiment of the present disclosure.

[0028B] Figure 20 depicts Surface plasma resonance (SPR) analysis of gallium nanoparticle (GaNP) interaction with M. tuberculosis PpiB protein, in accordance with an embodiment of the present disclosure.

[0028C] Figure 21 depicts dose dependent suppression in cell viability of M.smegmatis overexpressing M. tuberculosis PpiB in presence of GaNP treatment, in accordance with an embodiment of the present disclosure.

[0028D] Figure 22 depicts bacteriocidal activity of GaNP on M. smegmatis cells overexpressing M. tuberculosis PpiB, in accordance with an embodiment of the present disclosure3

[0028E] Figure 23 depicts inhibitory activity GaNP on Biofilm formation, in accordance with an embodiment of the present disclosure.

[0028F] Figure 24 depicts the synergistic effect of GaNP on Isoniazid in killing M. smegmatis expressing M. tuberculosis PpiB in accordance with an embodiment of the present disclosure. The reduction of MIC of Isoniazid by nearly two fold due to synergistic effect of GaNP is evident.

[0028G] Figure 25 depicts the synergistic effect of GaNP on Ethambutol in killing M. smegmatis expressing M. tuberculosis PpiB in accordance with an embodiment of the present disclosure. The reduction of MIC of Ethambutol by nearly two fold due to synergistic effect of GaNP is evident.

[0028H] Figure 26 depicts suppressive activity of GaNP on biofilm formation in Mycobacterium tuberculosis H37Rv strains, in accordance with an embodiment of the present disclosure.

[0028I] Figure 27 depicts the synergistic effect of GaNP with different concentration of cyclosporine A drug (CsA) or nano formulation of cyclosporine (Nano CsA) treatment on biofilm formation by H37 Rv cells during the course of biofilm formation, in accordance with an embodiment of the present disclosure.

[0028J] Figure 28 depicts the synergistic effect of GaNP with different concentration of cyclosporine A drug (CsA) or nano formulation of cyclosporine (Nano CsA) treatment post biofilm formation by H37 Rv cells, in accordance with an embodiment of the present disclosure.

[0028K] Figure 29 depicts the synergistic effect of GaNP with different concentration of cyclosporine A drug (CsA) or nano formulation of cyclosporine (Nano CsA) in presence of Isoniazid treatment (provided before biofilm formation) and the survival of H37 Rv cells thereof, in accordance with an embodiment of the present disclosure.

[0028L] Figure 30 depicts the synergistic effect of GaNP with different concentration of cyclosporine A drug (CsA) or nano formulation of cyclosporine (Nano CsA) in presence of Ethambutol treatment (provided before biofilm formation) and the survival of H37 Rv cells thereof, in accordance with an embodiment of the present disclosure.

[0028M] Figure 31 depicts the synergistic effect of GaNP with different concentration of cyclosporine A drug (CsA) or nano formulation of cyclosporine (Nano CsA) in presence of Isoniazid treatment (post biofilm formation) and the survival of H37Rv cells thereof, in accordance with an embodiment of the present disclosure.

[0028N] Figure 32 depicts the synergistic effect of GaNP with different concentration of cyclosporine A drug (CsA) or nano formulation of cyclosporine (Nano CsA) in presence of Ethambutol treatment (post biofilm formation) and the survival of H37Rv cells thereof, in accordance with an embodiment of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION:

[0029] Those skilled in the art will be aware that the present disclosure is subject to variations and modifications other than those specifically described. It is to be understood that the present disclosure includes all such variations and modifications. The disclosure also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any or all combinations of any or more of such steps or features.

Definitions:

[0030] For convenience, before further description of the present disclosure, certain terms employed in the specification, and examples are collected here. These definitions should be read in the light of the remainder of the disclosure and understood as by a person of skill in the art. The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

biofilm formation per se, that in turn could enhance penetrance of drugs across biofilms, thereby lowering the MIC values of drugs and also survival of the bacteria.

Experiment 9

Acarbose or Gallium binding sites in PpiB homologues in biofilm forming bacterial species have largely remained unaltered: Evidence of physical interaction

Results in Fig. 16A show the multiple sequence alignment of amino acid groups in the docking site of PpiB in biofilm forming bacteria that have remained conserved and could putatively interact with acarbose, or dimeric atomic gallium. Pro162 in PpiB are conserved for acarbose binding. Similarly for binding of dimer of atomic gallium, Gly203 in PpiB is conserved in all pathogens and the adjacent Thr204, present in the binding groove, is conserved in most of the pathogens. Presence of conserved amino acids at the acarbose and gallium binding site in PpiB homologues of several biofilm forming bacteria (Fig. 16A) indicate that these have also largely remained unaltered and hence could also prove to be an excellent putative target across bacterial species.

Molecular docking studies show that Thr204 interacts with dimeric atomic gallium (Fig. 19). Similarly, Gly in PpiB homologous proteins interacts with dimeric atomic gallium in most of the biofilm forming pathogens (Fig 16A). The interaction of gallium to PpiB is based on dimeric nature of bonding of gallium [Tonner R & Gaston N. (2014) *Phys Chem Chem Phys* 16: 24244-24249], however it remains to be shown whether the same will be true in a preparation of nanoparticles. Reports exist where atomic/molecule level docking involving specific amino acids in the target groove have been extrapolated to aggregation/complex/nanoparticle of the same [Asl BA et al. (2018) *Int J Biol Macromol.* 1(109); 639-650, Sekar G et al. (2017) *J Mol Liq* 241; 793-800]. These results, based on i*n-silico* docking and high binding capacity with

PpiB, point to the possibility of gallium in acting as inhibitor of *Mycobacterium tuberculosis (M.tb)* PpiB.

The actual physical interaction between purified recombinant *M.tb* PpiB and Gallium nanoparticle (GaNP) was tested using Surface plasma resonance (SPR) spectroscopy. SPR analyses show that GaNP (Fig. 20) interacts with *M.tb* PpiB in a dose dependent manner and bind with high affinity, suggesting that GaNP could modulate the activity of PpiB.

Experiment 10

GaNP suppresses the viability of *M. smegmatis*

The effect of GaNP on the viability of *M. smegmatis* cells expressing *M. tb* PpiB was examined colorimetrically using Alamar Blue assay. Control (Ms_VC) or recombinant (Ms_PpiB) strains were induced with anhydrotetracycline while Ms_VC or Ms_PpiB not induced with anhydrotetracycline were used as control. Results in Fig. 21 show that GaNP below 100 µg/ml had no significant effect on the number of viable *M. smegmatis* cells. Cell viability of the group, [VC (tet-/tet+) and PpiB (tet-/tet+)] treated with 100 µg/ml and above of GaNP, with the control group (no treatment of GaNP), showed that there is significant difference in viable cell number.

In order to assess whether GaNP has bacteriostatic or bactericidal effect on *M. smegmatis,* cells were cultured with GaNP (50, 100, 100 µg/ml) for upto 120 hours. A significant decrease in cell viability of the treatment group at 120 hours as compared to at 72 hours denotes bactericidal effect. Results in Fig. 22 show that GaNP exert bacteriostatic effect at 50 µg/ml (Fig. 22A). A very significant decline in growth at time 120 hours was evident (Fig. 22 panel B, C), pointing to bactericidal effect of GaNP at 100 µg/ml and above. These results indicate that GaNP at higher concentration is bactericidal.

Experiment 11

GaNP suppresses biofilm formation in *M.smegmatis* expressing *M.tb* PpiB

Given our earlier observation that *M.tb* PpiB activity is essential for biofilm formation, we speculated that modulation of PpiB activity upon binding with GaNP could affect biofilm formation. A threshold concentration of 50µg/ml of cyclosporine-A, acarbose and GaNP at which viability of PpiB expressing *M.smegmatis* was not significantly affected and did not show bactericidal effect was used for the assay. It was evident that in the absence of anhydrotetracycline induction, Ms_PpiB cells (PpiB tet-) do not develop significant levels of biofilm. Upon induction with anhydrotetracycline, Ms_PpiB cells (PpiB tet+) developed biofilm. Significant reduction in biofilm formation compared to control (no treatment) was observed in presence of GaNP at 50 µg/ml (Fig. 23). These *in vitro* biofilm inhibition results lend support to the earlier *in-silico* and SPR results, thereby demonstrating that GaNP physically interact with *M.tb* PpiB in a dose dependent manner and suppress the activity of PpiB protein resulting in inhibition of biofilm formation.

Experiment 12

GaNP co-treatment with anti-TB drugs increases susceptibility of mycobacteria to these drugs

The impact of reduced biofilm formation in terms of susceptibility to anti-TB drugs was examined. Isoniazid and ethambutol are front line antibiotics that are normally effective at a dosage of 16 µg/ml and 1 µg/ml, respectively. Since *M.tb* PpiB expression results in enhanced biofilm formation, it could abrogate drug sensitivity of *M.smegmatis* and hence the MIC of drugs is altered. In the absence of GaNP, PpiB tet+ induced cultures induced form biofilm, develop physical barrier over cells and prevent exposure to anti-TB drugs. PpiB tet- cultures, that were not induced to express PpiB proteins, are unaffected by the inhibitory action of cyclosporine-A or acarbose of GaNP on biofilms and hence are exposed directly to anti-TB drugs. Results in Fig. 24

show that in the presence of GaNP (50µg/ml), dosage of isoniazid was reduced from 64 µg/ml to 16 µg/ml, a four-fold decrease as compared to control.

Similar experiments were carried out for ethambutol. Results (Fig. 25) show that dosage of ethambutol in the presence of GaNP (50µg/ml), decreased from 16µg/ml to 1µg/ml, as compared to control. These results demonstrate that GaNP (50 µg/ml) inhibit the activity of PpiB protein which in turn negatively impact the ability of the bacterium to form biofilm efficiently, resulting in reduced percent viability thereby enabling greater access of anti-TB drugs to cells. The consequent reduced viability of *Mycobacterium* in the presence of GaNP points to its potential use as adjunct therapy.

Experiment 13

GaNP inhibit biofilm formation in pathogenic strains of *Mycobacterium tuberculosis* ($H_{37}Rv$)

While the experiments described so far were carried out on a non-pathogenic strain of *M.smegmatis*, we eventually tested the efficacy of GaNP on pathogenic strain of *Mycobacterium tuberculosis* ($H_{37}Rv$). Static culture of $H_{37}Rv$ cells were incubated in absence/presence of GaNP in a BSL-3 containment facility. Results in Fig. 26 show that while untreated control $H_{37}Rv$ cells formed pellicle, treatment with GaNP (50 µg/ml) resulted in reduction in biofilm formation. The role of GaNP in suppressing biofilm formation was further examined in two clinically relevant scenarios. $H_{37}Rv$ cells, pretreated for 6 hours and 24 hours with GaNP (25, 50 µg/ml), were incubated to allow biofilm formation. In another set of experiments, $H_{37}Rv$ cells were allowed to form biofilm and GaNP (25, 50 µg/ml) treatment was carried out post-biofilm formation. Pretreatment of $H_{37}Rv$ cells with GaNP resulted in dose dependent inhibition of biofilm formation that correlated with the duration for which the cells were pre-treated, the suppression in biofilm formation being enhanced when $H_{37}Rv$ cells were pre-treated with GaNP for 24 hours (Fig. 26C) as compared to 6 hours (Fig.

26B). Treatment with GaNP post-biofilm formation resulted in disintegration of pellicle at the liquid-air interface (Fig. 26 D).

Experiment 14

Efficacy of GaNP as adjunct with Cyclosporine A or nano formulation of cyclosporine

Our earlier results pointed to the efficacy of cyclosporine A in suppressing biofilm formation in $H_{37}Rv$ cells. However, the immunosuppressive activity of cyclosporine A made it mandatory for us to explore strategies to formulate alternative derivatives of cyclosporine A, which by virtue of their equivalent/better efficacy at lower doses, may be opted in place of cyclosporine A. Nano formulation of cyclosporine A, prepared by emulsifying cyclosporine A in presence of poly-ethylene glycol (80%) and Tween 80 (20%), was tested for their efficacy in inhibiting biofilm formation in $H_{37}Rv$ cells. $H_{37}Rv$ cells were initially treated with nano-cyclosporine (0, 5, 25, 50, 75, 100, 250, 500 μg/ml) or cyclosporine A (0, 5, 25, 50, 75, 100, 250, 500 μg/ml) and cultured in absence or presence of GaNP (25 μg/ml) as adjunct. Biofilm formation was recorded in static culture after four weeks. Results in Fig. 27 showed that $H_{37}Rv$ cells treated with nano-cylosporine A (50 μg/ml and above) showed significant reduction in biofilm formation as compared to cyclosporine A drug (control). A nearly four fold reduction in biofilm formation by $H_{37}Rv$ cells were observed when GaNP was used as an adjunct with either cyclosporine A drug or nano-cyclosporine. Treatment with GaNP significantly improves the efficacy of cyclosporine A drug or nano-cyclosporine in inhibiting biofilm formation, as is evident from the fact that both cyclosporine A drug or nano-cyclosporine can suppress biofilm formation even at lower dosage of 5 μg/ml.

In a separate study, $H_{37}Rv$ cells were cultured in static condition for four weeks to allow biofilm formation followed by treatment with combination of nano-cyclosporine (0, 5, 25, 50, 75, 100, 250, 500 μg/ml) and GaNP (25 μg/ml) or combination of cyclosporine A drug (0, 5, 25, 50, 75, 100, 250, 500 μg/ml) and GaNP (25 μg/ml), for

72 hours. Results in Fig. 28 showed that $H_{37}Rv$ biofilm culture treated with nano-cylosporine A (50 µg/ml and above) showed significant reduction in biofilm formation as compared to cyclosporine A drug (control). Treatment with GaNP, either in combination with cyclosporine A drug or nano-cyclosporine, reduced pellicle in $H_{37}Rv$ biofilm culture. However, treatment with cyclosporine A drug or nano-cyclosporine (in combination with GaNP) to cultures that had pre-formed biofilm had lesser effect in disrupting biofilm as compared to treatment of cyclosporine A drug or nano-cyclosporine (in combination with GaNP) during the period of biofilm formation in static culture. These results point that nano-cyclosporine is more efficacious in suppressing biofilm formation as compared to cyclosporine A drug. The efficacy of GaNP as adjunct treatment for inhibiting biofilm formation is also evident. A reduced dosage of nano-formulation of cyclosporine at which cyclosporine A drug is equivalently effective can help in reducing the deleterious effect of immunosuppressive activity of cyclosporine A drug. The immunosuppressive activity of nano-cyclosporine can be further reduced through the use of GaNP as adjunct with nano-cyclosporine.

Experiment 15

Nano-cyclosporine acts synergistically with GaNP in improving efficacy of anti-TB drugs

As showed previously, the efficacy of anti-TB drugs Isoniazid and Ethambutol, respectively, improved in presence of cyclosporine A drug. Cyclosporine A drug at 100 µg/ml reduced the MIC of Isoniazid and Ethambutol by two fold. However, given the fact that cyclosporine A drug could exert immunosuppressive activity, the efficacy of nano-cyclosporine in further improving the efficacy of isoniazid or ethambutol was examined. Our results in Fig. 27 and Fig. 28 highlighted the efficacy of GaNP as adjunct medication for inhibiting biofilm formation. The role of GaNP in reducing the MIC of Isoniazid (Fig. 24) or Ethambutol (Fig. 25) and in augmenting the inhibitory effect of nano-cyclosporine (Fig. 27 and Fig. 28) encouraged us to examine the

potential of combination of GaNP and nano-cyclosporine in improving the efficacy of anti-TB drugs. $H_{37}Rv$ cells were cultured in presence of combination of GaNP (25 µg/ml) and either cyclosporine A drug (25, 50 µg/ml) or nano-cyclosporine (25, 50 µg/ml) for 72 hours and the cell viability was assessed using Alamar Blue assay. Nano-cyclosporine exerted a dose dependent reduction in MIC of Isoniazid (Fig. 29A) or Ethambutol (Fig. 30A) which is more enhanced as compared to cyclosporine A drug at equivalent concentrations. Anti-TB drugs exhibited nearly two fold higher cell killing in presence of nano-cyclosporine as compared to cyclosporine A drug. Treatment with combination of 32 µg/ml of anti-TB drug and 50 µg/ml cyclosporine A drug had comparable killing as compared to treatment with combination of 8 µg/ml of anti-TB drug and 25 µg/ml nano-cyclosporine (Fig 29A, 30A). Nano-cyclosporine not only resulted in greater reduction in MIC of anti-TB drugs as compared to cyclosporine A drug, but also a lower dosage of nano-cyclosporine was required as compared to cyclosporine A drug for equivalent efficacy in cell killing. The adjunct activity of GaNP for improving the efficacy of cyclosporine A drug or nano-cyclosporine in reducing MIC of isoniazid and ethambutol are shown in Fig. 29B and Fig. 30B, respectively. Treatment with GaNP (25 µg/ml) reduced the concentration of anti-TB drugs by at least eight fold for equivalent efficacy in cell killing. While a dosage of 64 µg/ml of isoniazid or 16 µg/ml ethambutol resulted in 50% reduction of cell survival, isoniazid and ethambutol showed same efficacy in killing at 8 µg/ml (Fig. 29B) and 0.25 µg/ml (Fig. 30B) when treated in combination with GaNP. While a formulation of 25 µg/ml of either cyclosporine A drug or nano-cyclosporine in combination with anti-TB drugs (isoniazid or ethambutol) resulted in dose dependent killing of $H_{37}Rv$ cells, addition of GaNP to this formulation significantly increased killing of $H_{37}Rv$ cells. A formulation of GaNP in combination with isoniazid (8 µg/ml) or ethambutol (0.25µg/ml) showed 50% higher killing of $H_{37}Rv$ cells as compared to these anti-TB drugs alone, the efficacy of this formulation in killing $H_{37}Rv$ cells increased to 60%

when cyclosporine A drug was added and to 80% when nano-cyclosporine was added (Fig. 29B and 30B).

A clinically relevant case was also studied in which the efficacy of above formulations of combination of anti-TB drugs, cyclosporine A drug and nano-cyclosporine were examined on $H_{37}Rv$ biofilm culture. In this study, $H_{37}Rv$ cells were statically cultured for four weeks to allow biofilm formation. Post biofilm formation, anti-TB drugs alone or in combination with cyclosporine A drug or nano-cyclosporine and in presence or absence of GaNP as adjunct were added to $H_{37}Rv$ culture. Cell viability was assessed after 72 hours using Alamar Blue assay. Results obtained in Fig. 31 and Fig. 32 are similar to the above results show in Fig. 29 and Fig. 30 respectively, the efficacy of the above formulations provided post-biofilm formation being lower as compared to treatment of these formulations during the phase of biofilm formation. Interestingly, a dose dependent efficacy of anti-TB drugs in combination with cyclosporine A drug or nano-cyclosporine was observed when the formulations were given during biofilm formation phase. However, a dose dependent efficacy of anti-TB drugs in combination with cyclosporine A drug or nano-cyclosporine was not observed when the formulations were given after biofilm formation phase. The biofilm act as physical barrier and suppress penetration of drugs through matrix thereby preventing direct interaction of drugs with $H_{37}Rv$ cells and inhibitory activity of drugs. Addition of GaNP to the anti-TB formulations (cyclosporine A drug or nano-cyclosporine in combination with either isoniazid or ethambutol), provided post biofilm formation, significantly induced cell killing. GaNP can therefore act by disrupting biofilm that result in penetrance of anti-TB drugs and killing of $H_{37}Rv$ cells.

Our results provide evidence that nano-cyclosporine exhibit enhanced synergistically effect on anti-TB drugs as compared to cyclosporine A drug. GaNP acts as an adjuvant and reduces the MIC of anti-TB drugs treated in combination with cyclosporine A drug or nano-cyclosporine. Treatment with GaNP improves the efficacy of biofilm disruption and penetrance of anti-TB drugs.

Advantages of the present disclosure:

[00248] The present disclosure provides a medicament comprising a drug selected from a group consisting of acarboase, cyclosporine A, its pharmaceutically acceptable salts thereof, and combinations thereof, for use in treatment of diseases caused by biofilm forming microorganisms. The examples provided clearly depict that the composition of the present disclosure proves efficient enough to bind to PpiB and thus modulates its activity. Thus, the present disclosure provides an answer to problems posed by biofilm forming bacteria.

/We Claim:

1. A medicament comprising a drug selected from a group consisting of acarbose, cyclosporine A, its pharmaceutically acceptable salts thereof, and combinations thereof, for use in treatment of diseases caused by biofilm forming microorganisms.

2. The medicament as claimed in claim 1, wherein the drug is cyclosporine A for use in treatment of diseases caused by biofilm forming microorganisms.

3. The medicament as claimed in claim 1, wherein the drug is acarbose for use in treatment of diseases caused by biofilm forming microorganisms.

4. The medicament as claimed in any one of the claims 1-3, further comprising at least one compound for treatment of diseases caused by biofilm forming organisms.

5. The medicament as claimed in any one of the claims 1-4, wherein the biofilm forming microorganisms is selected from a group consisting of *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, multidrug resistant *M. tuberculosis*, extensively drug resistant *M. tuberculosis*, totally drug resistant variant of *M. tuberculosis*, *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus intermedius, Streptococcus mutans, Staphylococcus saprophyticus, Streptococcus constellatus, Pseudomonas aeruginosa,* and combinations thereof.

6. The medicament as claimed in any one of the claims 1-4, wherein said medicament is used in the treatment of diseases selected from a group consisting of tuberculosis, dental caries, and periodontitis.

7. The medicament as claimed in any one of the claims 1-4, wherein the drug is a pharmaceutically acceptable salt of acarbose.

8. The medicament as claimed in any of the claims 1-4, wherein the drug is a pharmaceutically acceptable salt of cyclosporine A.

9. The medicament as claimed in claim 4, wherein said compound is selected from a group consisting of isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, Fe_3O_4 nanocatalyst, clarithromycine, DNase, and pharmaceutically acceptable salts thereof.

10.. The medicament as claimed in claim 4, wherein said compound is to be administered, before, concurrently, or after administration of the drug selected from a group consisting of acarbose, cyclosporine A, and pharmaceutically acceptable salts thereof.

11. The medicament as claimed in claim 10, wherein said administration of said compound, and the drug selected from a group consisting of acarbose, and cyclosporine A, can be in the form of a single dose or multiple doses.

12. Use of acarbose and its pharmaceutically acceptable salts in the manufacture of a medicament for use in treatment of diseases caused by biofilm forming microorganisms

13. Use of acarbose, cyclosporine A, its pharmaceutically acceptable salts thereof, and combinations thereof in the manufacture of a medicament for use in treatment of diseases caused by biofilm forming microorganisms

14. The use as claimed in any one of the claims 12-13, wherein the medicament further comprises at least one compound for treatment of the disease caused by biofilm forming microorganisms.

15. The use as claimed in any one of the claims 12-14, wherein the biofilm forming microorganisms is selected from a group consisting of *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, multidrug resistant *M. tuberculosis*, extensively drug resistant *M. tuberculosis*, totally drug resistant variant of *M. tuberculosis*, *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus intermedius, Streptococcus mutans, Staphylococcus saprophyticus, Streptococcus constellatus, Pseudomonas aeruginosa,* and combinations thereof.

16. The use as claimed in any one of the claims 12-14, wherein said diseases are selected from a group consisting of tuberculosis, dental caries, and periodontitis.

17. The use as claimed in claim 14, wherein the at least one compound is selected from a group consisting of isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, Fe_3O_4 nanocatalyst, clarithromycine, DNase, and pharmaceutically acceptable salts.

18. The use as claimed in any one of the claims 14-17, wherein the at least one compound is to be administered before, concurrently or after administration of the drug selected from a group consisting of acarbose, and cyclosporine A.

19. The use as claimed in claim 18, wherein administration of the drug selected from a group consisting of acarbose, and cyclosporine A can be in the form of a single dose or multiple doses.