Supporting information

A structural and energetic model for the slow-onset inhibition of InhA, an enzyme drug target from *Mycobacterium tuberculosis*

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Supplemental Methods

Crystallization and structure determination of InhA ternary complexes with PT92, PT10, PT91 and PT155. Crystals of the ternary complex formed between InhA, NAD+ and PT92 were obtained by incubating 5 mg/mL of InhA with 2 mM NAD+ and 800 µM PT92 in 8% DMSO for 2 hr at RT before mixing with an equal volume of reservoir solution containing 100 mM Bis-tris pH 6.4, 200 mM NaCl, 14% PEG 3350 and 4% DMSO in a hanging drop. Before freezing, the crystals were cryo-protected in the solution containing 100 mM Bis-tris pH 6.4, 310mM NaCl, 16% PEG 3350, 25% DMSO, 2 mM NAD+ and 800 µM PT92. Crystals of the ternary complexes formed between InhA, NAD+ and PT10 or PT91 were both obtained by incubating 10 mg/mL of InhA with 2 mM NAD+ and 2 mM inhibitor in 4% DMSO for 2 hr at RT before mixing with an equal volume of reservoir solution containing 100 mM ADA pH 6.8, 200-250 mM ammonium acetate, 14-16% PEG 4000 and 6% DMSO in a hanging drop. Crystals of the ternary complex formed between InhA, NAD+ and PT155 were obtained similarly with a reservoir solution containing 100 mM HEPES pH 8.0 and 32% Jeffamine ED-2001 pH 7.0 in a hanging drop. Two crystal forms were taken from the same drop without further cryo protection and flash frozen in liquid nitrogen. Diffraction data were collected at beamlines X29, X25 and X12C at NSLS. The image frames were indexed, integrated and scaled using HKL2000(1) and structures solved using MolRep.(2) Structure refinement was performed in Phenix.(3) Data collection and refinement statistics are given in Table S1.

Computational Methods

Initial pose of inhibitors. The initial pose of PT70 was taken from the first monomer of the InhA:NAD+:PT70 complex (PDB ID: 2X23(4)). For the other inhibitors, the initial poses were generated by the DOCK 6.3(5) suite of docking software. The procedure for preparing the binding-site has been described previously.(6) Briefly, a molecular surface of InhA was computed using the program DMS.(7) The program SPHGEN in DOCK 6.3 was used to generate a set of spheres in regions where the inhibitor atoms could potentially interact favorably with the receptor, and 39 spheres were used to guide inhibitor placement during flexible docking. The grid file was computed with a 0.3 Å grid space using the program GRID in DOCK 6.3. Default parameters were then used in the flexible docking. We assumed that the other analogues adopted similar positions to that found for PT70. Thus, the results with the lowest RMSD value in the diphenyl ether moiety were chosen as the initial structures for MD runs.
Molecular dynamics (MD) simulations. All MD simulations were performed in a monomer system using the AMBER10 suite of molecular dynamics programs. The initial closed and open structures were taken from the first monomers of InhA:NAD⁺:PT70 complex (PDB ID: 2X23(4)) and the InhA:NAD⁺:C16-NAC complex (PDB ID: 1BVR(8)), respectively. AMBER ff99SB and GAFF force field parameters were assigned to the protein and inhibitor, respectively. The force field parameters of the cofactor NAD⁺ were taken from other studies.(11, 12) The partial atomic charges of the inhibitors were computed using Gaussian98(13) with the HF/6-31G* basis set, followed by a two-stage RESP fitting approach (Table S2). (14, 15) Each InhA:NAD⁺:inhibitor complex was solvated in a truncated octahedral TIP3P water box with a minimum distance of 8 Å between the water box edge and solute, resulting in ~ 23,000 atoms in total. SHAKE was used to constrain bonds to hydrogen.(17) The particle mesh Ewald method was used for calculating electrostatic energy with an 8Å nonbonded cutoff. Each end-point structure, including the open and closed conformations, was then equilibrated separately using the following procedures. The first equilibration step involved 10,000 steps of steepest descent minimization with 100 kcal mol⁻¹ Å⁻² restraints on all the atoms except the water molecules and hydrogen atoms. The second step involved heating the system from 100 to 300 K under constant volume conditions over 100 ps with 100 kcal mol⁻¹ Å⁻² restraints on non-water and non-hydrogen atoms, followed by 100 ps with the same restraints at a constant temperature of 300 K and at 1 atm of pressure. The third step was 250 ps MD with restraint weight of 10 kcal mol⁻¹ Å⁻² on the non-water and non-hydrogen atoms at constant temperature of 300 K and 1 atm pressure. The following steps only restrained the backbone atoms and gradually reduced the restraint weight from 10 to 0.1 kcal mol⁻¹ Å⁻² at constant temperature of 300 K and 1 atm of pressure. This was carried out by 100 ps with 10 kcal mol⁻¹ Å⁻² restraint, followed by 100 ps with 1 kcal mol⁻¹ Å⁻² restraint, and 100 ps with a 0.1 kcal mol⁻¹ Å⁻² restraint. The last step of the equilibration was 250 ps of unrestrained MD.

PNEB simulation. The protocol for generation of conformational change pathways and associated free energy profiles using PNEB and umbrella sampling was adapted from our recent study of DNA damage recognition pathways.(19) The partial nudged elastic band (PNEB)(20) variant of the nudged elastic band simulation approach was used in this study to generate the transition path between the open and closed conformations. The equilibrated open and closed structures were assigned as the two end-point structures and 30 windows (including end-points) were used in the simulation. The NVT ensemble was used with PNEB. A spring force was applied to the backbone atoms of α-helix-6 and α-helix-7 (residues 196 to 223)
in the subsequent steps. In the first 40 ps, the system was equilibrated at 300 K with a Langevin collision frequency of 50 ps\(^{-1}\) and spring force of 20 kcal mol\(^{-1}\) Å\(^{-2}\). The next step was 100 ps equilibration at 300 K with a 20 ps\(^{-1}\) Langevin collision frequency and a spring force of 75 kcal mol\(^{-1}\) Å\(^{-2}\). After conformations were generated along the open-to-closed path, simulated annealing was used to optimize the local energy minimized path.\(^{(20)}\) This was performed by heating the system from 300 to 375 K gradually over 175 ps, and subsequently cooling back to 300 K gradually over 175 ps with a 20 ps\(^{-1}\) Langevin collision frequency and a spring force of 75 kcal mol\(^{-1}\) Å\(^{-2}\). A 20 kcal mol\(^{-1}\) Å\(^{-2}\) Cartesian restraint was applied to the backbone atoms from residues 2 to 195 and 225 to 268 to prevent protein unfolding during the heating process. After this simulated annealing process, a 600 ps run at 300K was performed with the same Langevin collision frequency and PNEB spring forces, with a 10 kcal mol\(^{-1}\) Å\(^{-2}\) Cartesian restraint was applied to the backbone atoms from residues 2 to 195, and 225 to 268.

**Umbrella sampling.** Energy landscape plots were obtained using umbrella sampling (US). Two reaction coordinates (step and shear torsions) were used to describe the motion of α-helices 6 and 7 (Figure 5). In step torsion, point 1 is the center of mass (COM) of backbone atoms for residues 200–205. Point 2 is the backbone COM of residues 19–21 and 196. Point 3 is the backbone COM of residues 219–222. Point 4 is the backbone COM of residues 200–205. In shear torsion, points 1 and 4 are the Cβ of residue 203 and 215, respectively. Points 2 and 3 are the backbone COM of residues 98 and 158, respectively. A 2D grid along these 2 measures was constructed, using 3° increments in both step and shear torsions. The boundaries of the grid were determined from grid points sampled during the final PNEB run at 300K, with an extra 6° buffer region surrounding that sampled in PNEB, resulting in a total of 192 grid points. Initial structures for US windows were selected from the PNEB production trajectory snapshots with dihedral values closest to the respective window grid values. MD simulation in the NVT ensemble of 500 ps at 300 K using a Langevin thermostat with a collision frequency of 75.0 ps\(^{-1}\) was performed for each grid point. Structures were restrained to the two reaction coordinate values defining the grid point with a force constant of 1000 kcal mol\(^{-1}\) rad\(^{-2}\) s. The Weighted Histogram Analysis (WHAM)\(^{(22)}\) approach and analysis program\(^{(23)}\) were then used to generate the potential of mean force (PMF) from the umbrella sampling results. The convergence tolerance was set to zero during the WHAM calculation. Convergence of the free energy calculations was tested by extending the umbrella sampling of InhA:NAD\(^{+}\):PT70 complex for another 500 ps (Figure S10). There was no significant change in the free energy from 1ns run, thus 500ps umbrella sampling were run for other complexes.
Table S1. Data collection and refinement statistics for the structures of the InhA:NAD⁺:inhibitor ternary complexes.

| PDB ID  | InhA:NAD⁺:PT9 2 | InhA:NAD⁺:PT15 5 | InhA:NAD⁺:PT15 5 | InhA:NAD⁺:PT1 0 | InhA:NAD⁺:PT9 1 |
|---------|------------------|-------------------|-------------------|-----------------|-----------------|
| **Data Collection** |                  |                   |                   |                 |                 |
| Space group           | P2₁,2₁,2₁        | P2₁,2₁,2₁         | 2₁,2₁,2₁          | P2₁,2₁,2₁       | P2₁,2₁,2₁       |
| Unit cell dimensions  | 72.85, 90.49, 161.83 | 89.27, 7.44, 182.58 | 88.94, 97.15, 187.81 | 88.83, 91.08, 148.91 | 74.84, 90.65, 164.44 |
| a, b, c (Å)           |                  |                   |                   |                 |                 |
| Redundancy            | 7.1 (4.8)        | 6.6 (5.1)         | 7.4 (7.3)         | 7.2 (6.7)       | 4.2 (3.5)       |
| I/σI                 | 18.3 (4.0)       | 17.6 (2.4)        | 13.6 (2.8)        | 22.4 (4.6)      | 20.9 (3.2)      |
| R_merge (max)         | 0.084 (0.312)    | 0.110 (0.569)     | 0.167 (0.747)     | 0.080 (0.361)   | 0.062 (0.352)   |
| **Refinement**        |                  |                   |                   |                 |                 |
| Resolution (Å)        | 28.6-1.6         | 47.1-1.8          | 44.5-2.3          | 43.5-2.35       | 43.7-2.30       |
| No. unique reflections| 139747           | 136903            | 36121             | 50974           | 45401           |
| Completeness (%)      | 98.4             | 99.4              | 98.3              | 99.9            | 89.8            |
| R_work/R_free         | 0.171/0.188      | 0.158/0.177       | 0.172/0.205       | 0.166/0.220     | 0.194/0.244     |
| No. monomers in asu  | 4                | 4                 | 2                 | 4               | 4               |
| RMSD (max) from ideal values in |           |                   |                   |                 |                 |
| Bond length (Å)       | 0.006 (0.059)    | 0.007 (0.051)     | 0.008 (0.062)     | 0.007 (0.062)   | 0.007 (0.070)   |
| Bond angle (°)        | 1.2 (12.4)       | 1.2 (12.2)        | 1.1 (8.7)         | 1.1 (10.2)      | 1.1 (11.1)      |

Data collection numbers for the highest resolution shell are given in parentheses.
Table S2: Partial atomic charges of compounds

**PT3 partial atomic charges**

| Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge |
|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|
| C1 (ca)               | -0.223315      | C2 (ca)               | -0.007234      | C3 (ca)               | -0.229547      | C4 (ca)               | -0.211969      |
| C5 (ca)               | 0.148221       | C6 (ca)               | 0.285367       | C7 (ca)               | 0.222865       | C8 (ca)               | -0.135579      |
| C9 (ca)               | -0.197672      | C10 (ca)              | -0.123410      | C11 (ca)              | -0.197672      | C12 (ca)              | -0.135579      |
| C13 (c3)              | -0.056773      | C14 (c3)              | 0.002988       | C15 (c3)              | -0.002068      | C16 (c3)              | 0.026176       |
| H2 (ha)               | 0.155157       | H3 (ha)               | 0.183429       | H4 (ha)               | 0.137848       | H5 (ha)               | 0.161508       |
| H6 (ha)               | 0.136727       | H7 (ha)               | 0.161508       | H8 (ha)               | 0.137848       | H9 (ho)               | 0.452110       |
| H10 (hc)              | 0.041085       | H11 (hc)              | 0.041085       | H12 (hc)              | 0.010396       | H13 (hc)              | 0.010396       |
| H14 (hc)              | 0.009321       | H15 (hc)              | 0.009321       | H16 (hc)              | -0.008443      | H17 (hc)              | -0.008443      |
| H18 (hc)              | 0.006989       | H19 (hc)              | 0.006989       | H20 (hc)              | 0.006989       |

**PT70 partial atomic charges**

| Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge |
|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|
| C1 (ca)               | -0.305877      | C2 (ca)               | -0.005783      | C3 (ca)               | -0.207809      | C4 (ca)               | -0.182149      |
| C5 (ca)               | 0.112182       | C6 (ca)               | 0.269957       | C7 (ca)               | 0.123130       | C8 (ca)               | -0.141905      |
| C9 (ca)               | -0.194331      | C10 (ca)              | -0.164129      | C11 (ca)              | -0.234181      | C12 (ca)              | 0.111613       |
| C13 (c3)              | -0.192668      | C14 (c3)              | -0.013634      | C15 (c3)              | 0.024577       | C16 (c3)              | -0.010196      |
| C17 (c3)              | -0.005967      | C18 (c3)              | 0.065218       | C19 (c3)              | -0.045568      | O1 (os)               | -0.227720      |
| O2 (oh)               | -0.536475      | H1 (ha)               | 0.166880       | H2 (ha)               | 0.154695       | H3 (ha)               | 0.166490       |
| H4 (ha)               | 0.133435       | H5 (ha)               | 0.156808       | H6 (ha)               | 0.145586       | H7 (ha)               | 0.161364       |
| H8 (hc)               | 0.062701       | H9 (hc)               | 0.062701       | H10 (hc)              | 0.062701       | H11 (ho)              | 0.419315       |
| H12 (hc)              | 0.031061       | H13 (hc)              | 0.031061       | H14 (hc)              | 0.010100       | H15 (hc)              | 0.010100       |
| H16 (hc)              | -0.000394      | H17 (hc)              | -0.000394      | H18 (hc)              | -0.02013       | H19 (hc)              | -0.02013       |
| H20 (hc)              | -0.017212      | H21 (hc)              | -0.017212      | H22 (hc)              | 0.008652       | H23 (hc)              | 0.008652       |
| H24 (hc)              | 0.008652       |                       |                |                       |                |                       |                |
PT92 partial atomic charges

| Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge |
|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|
| C1 (ca)               | -0.273248      | C2 (ca)               | -0.021360      | C3 (ca)               | -0.191209      | C4 (ca)               | -0.179507      |
| C5 (ca)               | 0.121524       | C6 (ca)               | 0.239883       | C7 (ca)               | 0.272739       | C8 (ca)               | -0.159279      |
| C9 (ca)               | -0.184872      | C10 (ca)              | -0.155948      | C11 (ca)              | -0.072072      | C12 (ca)              | -0.107639      |
| C13 (c3)              | -0.036432      | C14 (c3)              | 0.028997       | C15 (c3)              | -0.004680      | C16 (c3)              | -0.010591      |
| C17 (c3)              | 0.056600       | C18 (c3)              | -0.043943      | O1 (os)               | -0.216836      | O2 (oh)               | -0.554134      |
| Br1 (br)              | -0.091016      | H1 (ha)               | 0.157112       | H2 (ha)               | 0.153929       | H3 (ha)               | 0.157876       |
| H4 (ha)               | 0.132010       | H5 (ha)               | 0.163768       | H6 (ha)               | 0.140505       | H7 (ha)               | 0.138119       |
| H8 (ho)               | 0.436221       | H9 (hc)               | 0.040829       | H10 (hc)              | 0.040829       | H11 (hc)              | 0.069003       |
| H12 (hc)              | 0.009003       | H13 (hc)              | -0.000839      | H14 (hc)              | -0.000839      | H15 (hc)              | 0.000284       |
| H16 (hc)              | 0.000284       | H17 (hc)              | -0.014184      | H18 (hc)              | -0.014184      | H19 (hc)              | 0.008780       |
| H20 (hc)              | 0.008780       |                      |                |                       |                |                       |                |

PT155 partial atomic charges

| Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge | Atom name (atom type) | Partial charge |
|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|
| C1 (ed)               | -0.438965      | C2 (cc)               | 0.053087       | C3 (cd)               | -0.207546      | C4 (cc)               | 0.026918       |
| C5 (c)                | 0.628641       | C6 (c3)               | -0.133660      | C7 (ca)               | 0.171512       | C8 (ca)               | -0.260329      |
| C9 (ca)               | -0.206477      | C10 (ca)              | 0.290422       | C11 (ca)              | -0.303745      | C12 (ca)              | 0.055573       |
| C13 (c3)              | -0.125502      | C14 (c3)              | -0.009664      | C15 (c3)              | -0.004296      | C16 (c3)              | -0.020813      |
| C17 (c)               | -0.002104      | C18 (c3)              | 0.032576       | C19 (c3)              | -0.136894      | N1 (na)               | 0.019303       |
| N2 (nh)               | -0.908259      | O1 (os)               | -0.284312      | O2 (o)                | -0.601764      | H1 (ha)               | 0.145029       |
| H2 (h4)               | 0.230152       | H3 (h1)               | 0.083804       | H4 (h1)               | 0.083804       | H5 (h1)               | 0.083804       |
| H6 (ha)               | 0.208299       | H7 (ha)               | 0.157432       | H8 (ha)               | 0.172183       | H9 (hc)               | 0.045560       |
| H10 (hc)              | 0.045560       | H11 (hc)              | 0.045560       | H12 (hn)              | 0.378067       | H13 (hn)              | 0.378067       |
| H14 (hc)              | 0.045818       | H15 (hc)              | 0.045818       | H16 (hc)              | 0.015768       | H17 (hc)              | 0.015768       |
| H18 (hc)              | 0.002572       | H19 (hc)              | 0.002572       | H20 (hc)              | 0.006981       | H21 (hc)              | 0.006981       |
| H22 (hc)              | 0.014048       | H23 (hc)              | 0.014048       | H24 (hc)              | 0.031192       | H25 (hc)              | 0.031192       |
| H26 (hc)              | 0.031192       |                      |                |                       |                |                       |                |
Figure S1. InhA in relevant liganded states (apo, binary and ternary (Fig. 3b) complexes) in the catalytic cycle that have been observed in the crystal structures.

(a)-(d) Chain A, B, C, D from PDB structure 2IED, apo InhA S94A.(24) (e) PDB structure 2AQ8, InhA binary complex in space group P6_22.(25) (f) InhA binary complex in space group C22_1, chain C. (g) InhA binary complex from PDB structure 1BVR,(8) chain D. (h) InhA binary complex from PDB structure 1BVR, chain E. Light pink, green and yellow show different subdomains in the same InhA subunit. Despite the structural variations in the vicinity of helix-6, I202, L207 or the substrate are positioned against the green loop, which keeps helix-6 away from strand-4 so that the ACP portal remains open.
Figure S2. Structures of the PT155 ternary complex. Unlike the ternary complex of slow-onset diphenyl ethers, the open structure becomes dominant in the ternary complex of PT155. Each panel shows one of the chains from the two structures of the complex. (a) chain B in the I2_12_1_2 crystal, (b) chain C in the P2_1_2_1 crystal (chain D is essentially identical), (c) chain A in the P2_1_2_1 crystal, (d) chain B in the P2_1_2_1 crystal, (e) chain A in the I2_12_1_2 crystal.
Figure S3. Simulated annealing Fo-Fc omit maps in chain C of PT155 complex structure from the P2₁2₁2₁ crystal
Figure S4: The substrate portal is blocked by helix-6 as a result of slow-onset inhibition. (a) InhA in complex with NAD* and the substrate analogue, C16-NAC (spheres) (PDB 1BVR, chain A). (b) InhA in complex with NAD* and PT70 (PDB 2X23, chain B) superimposed to the structure of enzyme-substrate complex. Only ligands from the enzyme-substrate complex are shown in (b), showing that C16-NAC is visible in (a) and obscured in (b). The opening between helix-6 (cyan), strand-4 (yellow) and the grey domain is the proposed space where ACP docks to InhA and delivers the fatty acyl substrate into the active site.
Figure S5. Inhibition of InhA by helix-6 induced by a slow-onset inhibitor. (a) Ordered buffer molecules (blue and pink stick) from PT155 ternary complex structure 12, chain B, superimposed on the substrate ternary complex structure, 1BVR. The bound substrate analogue from the structure is shown as black stick (b) The substrate analogue from 1BVR, and buffer components as well as the inhibitor (cyan) from the PT155 ternary complex structure in (a) are superimposed on the PT70 ternary complex structure where PT70 is not shown. Residues on helix-6 overlapping with the hypothetical natural substrate are shown in red.
Figure S6. Low occupancy of open and closed conformations in the PT03 ternary complex
Figure S7: Helix-6 and 7 conformations along the open to closed reaction coordinate. Structures are taken from (1) the binary complex (2AQ8, red),(25) (2) PT155 ternary complex (P2,2,2, chain C/D, green), (3) PT92 ternary complex (chain B, blue), (4) PT10 ternary complex (chain A, yellow), (5) PT70 ternary complex (2X23 chain B, purple). (4) The composite figure is shown in the main text (Figure 7).
Figure S8. Refolding of InhA by diphenyl ether inhibitors
(a) PDB structure 2AQ8 with re-modeled 2-methyl-2,4-pentanediol (MPD) in the active site; (b) the initial complex represented by chain C of the PT155 ternary complex structure; (c) the intermediate complex represented by chain B of the PT92 ternary complex structure; (d) the intermediate complex represented by chain A of the PT10 ternary complex structure; (e) the final complex represented by chain B of the PT70 ternary complex structure 2X23.(4)
Figure S9. Large-scale refolding of InhA caused by the effect of the diphenyl ether inhibitors on interactions of helix-6 and helix-7 with strand-4, strand-5 and neighboring subunits. Left panels show two sets of helix-6 and helix-7 and right panels zoom into one subunit. Left: (a) binary complex structure 2AQ8. (b) ternary complex of PT92, chain B in yellow. (c) ternary complex of PT70, chain B in yellow. The tetramer is colored by chain (by subunit). The SBL of the yellow and pink subunit is in red and green, respectively. Right: (a) binary complex PDB 2AQ8; (b) PT92 ternary complex chain B; (c) PT70 ternary complex PDB 2X23 chain B. SBL is in magenta ribbon, strand-4 in green, strand-5 in blue, and the C-terminus from the separate yellow subunit in the tetramer is in orange.
Figure S10. PMF of 1ns InhA:NAD⁺:PT70 complex. There is no significant change in the free energy from 1\textsuperscript{st} 500ps, 2\textsuperscript{nd} 500ps and 1ns runs.
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