SUPPORTING INFORMATION

Exploring the activity profile of TbrPDEB1 and hPDE4 inhibitors using Free Energy Perturbation (FEP+)

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Materials and methods
This work aims to validate the use of FEP+ to assess the activity profiles of multiple series of TbrPDEB1 inhibitors against the off-target hPDE4. The flexibility of the specific P-pocket in TbrPDEB1 constituted the challenge for FEP+ calculations. We considered it essential to analyze the degree of induced fit of the different ligand classes upon binding to address this issue.

Ligand Preparation
Data sets were collected from an in-house database (SI Table 1, 2, and 3). All PDE inhibitors considered in this study were built using the Maestro 19 interface; tautomer enumeration and protonation state assignment at experimental pH of 7.0 +/- 2 was performed using LigPrep.

Protein Structure Preparation
All protein structures were obtained from the Protein Data Bank (PDB) (www.rcsb.org) and imported into Maestro. The tetrahydrophtalazinone series against TbrPDEB1 was investigated using the X-rays (PDB IDs: 5G57, 1.73 Å resolution, 5G2B, 1.83 Å resolution) in complex with NPD-038 and NPD-008, respectively. No ligands belonging to the tetrahydrophtalazinone series have nowadays been co-crystallized with any hPDE4. Therefore hPDE4B (PDB ID: 5LAQ, 2.40 Å resolution) and hPDE4D (PDB ID: 5LBO, 2.25 Å resolution) in complex with NPD-001 were investigated.

For the analysis of the alkynamide phthalazine series TbrPDEB1 (PDB ID: 6GXQ, 1.96 Å resolution) and hPDE4D (PDB ID: 6HWO, 1.99 Å resolution), in complex with NPD-1335, were selected.

The structures were prepared using the Protein Preparation Wizard tool in Maestro. Hydrogen atoms were added, missing side chains were filled, all crystal waters were retained.

The proper ionization state was assigned for both the amino acids and co-crystallized ligand at physiological pH.

The stability of the co-crystallized ligands in their corresponding binding site was assessed by running 100 ns MD Desmond simulations, (NPT ensemble (300 K, 1bar), SPC water, Langevin barostat), in each of the complexes under analysis (PDB IDs: 5G2B; 5G5V; 5L8Y; 5LAQ; 5LBO; 6HWO; 6GXQ; 6RFN; 6RFW).
**Ligand docking**
The ligands reported in SI Tables 1, 2, and 3 were docked into the TbrPDEB1 and hPDE4 structures using Glide\textsuperscript{8,9} core-constrained docking with default parameters, using the X-ray ligand as reference (with the standard precision scoring function).

We then refined the alignment into the binding site of both PDE models for the subsequent FEP+ calculation using the Flexible Ligand Alignment tool\textsuperscript{10} with the maximum common substructure algorithm.

**FEP+ calculation**
Free energy calculations were performed using the FEP+ method\textsuperscript{11} with Schrödinger suite in the versions 2019-4 to 2020-1. Maps were generated with default settings (optimal topology).

The OPLS3e\textsuperscript{12,13} force field with custom parameters was used to predict the relative energy of binding of the ligands. A detailed description of the implementation of the methodology can be found elsewhere\textsuperscript{14-16}. The missing torsions parameters of the ligands were computed using the Force Field Builder\textsuperscript{17} tool.

Maps were generated with default settings (optimal topology). FEP+ jobs were run for 10 ns sampling time per \( \lambda \)-window for a total of 12 \( \lambda \)-windows. The output was analyzed with the FEP+ panel in Maestro. Replica exchange with solute Tempering (REST) was used as sampling method as reported in prior publications\textsuperscript{16,18} (with the highest effective temperature of 1000 K for a typical perturbation with about 20 heavy atoms in the hot region)\textsuperscript{16} The Bennett acceptance ratio method was used to calculate the free energy difference between neighboring \( \lambda \) windows. The simulations were performed at NPT ensemble (300 K, 1bar), using SPC water model.

Calculations were run on the GPU compute nodes with Dual Intel Xeon CPU E5-2620 v4 @2.10GHz (16 core total), and NVIDIA GeForce GTX1080 GPUs. For this study, we specifically ran the FEP+ simulations across 4 GPUs. The FEP+ calculation with the highest number of ligands in the map from Table 3 took almost 27 h to complete. It must be noted that the duration of FEP+ calculations is also dependent by the hardware used, the size of the system, the number of ligands, the number of lambda windows (default for the FEP+ implementation are as follows: 12 for neutral perturbations, 16 for core-hopping perturbations, and 24 for charge-hopping perturbations), and the length of the simulation.
Figure S1: Sequence alignment of human PDE4D, PDE4B, and parasite TbrPDEB1, colored by identity.
Figure S2. Interaction fingerprints and 2D ligand graphs

Interaction graphs and fingerprints of NPD-1335 co-crystallized in PDB IDs: 6GXQ (TbrPDEB1) and 6HWO (hPDE4D); NPD-038 co-crystallized in PDB ID: 5G5V (TbrPDEB1); NPD-008 co-crystallized in PDB ID: 5G2B (TbrPDEB1) and NPD-001 co-crystallized in PDB ID: 5LAQ (hPDE4B).
5G5V (TbrPDEB1)
5G2B (TbrPDEB1)

| MB1 | MB2 | HC1 | Q1   | Q2   | HC  | Q2   | S    | Q2   | S    |
|-----|-----|-----|------|------|-----|------|------|------|------|
| Z1K | B19 | 22D | 852  | 23E  | 823 | 24S  | 824  | 23N  | 825  |
| 26V | 826 | 27S | 833  | 28R  | 834 | 28W  | 836  | 30A  | 837  |
| 31M | 838 | 32V | 840  | 33L  | 841 | 34E  | 843  | 35F  | 844  |
| 36Y | 845 | 37Q | 847  | 38L  | 859 | 39P  | 860  | 40M  | 861  |

| S   | Q2  | Q   | HC2 | HC1 | Q1  | HC2 | Q1   | Met1 | Met2 |
|-----|-----|-----|-----|-----|-----|-----|------|------|------|
| 41F | 862 | 42K | 866 | 43N | 867 | 44M | 868  | 45E  | 869  |
| 46L  | 870 | 47A  | 871 | 48K  | 872 | 49G  | 873  | 50Q  | 874  |
| 51G  | 876 | 52F  | 877 | 53I  | 878 | 54F  | 880  | 55V  | 881  |
| 56A  | 882 | 57W  | 911 | 58X  |     | 59X  |      |      |      |
5LAQ (hPDE4B)
Table S1. Chemical structures of tetrahydrophthalazinones ligands of validation set 1, Glide SP score, ΔG values experimentally determined and respective ΔG computed by FEP+.

| Ligand ID  | R₁   | R₂   | Glide SP score | Pred. ΔG | Exp. ΔG | Glide SP score | Pred. ΔG | Exp. ΔG |
|------------|------|------|----------------|----------|---------|----------------|----------|---------|
| NPD-008    | `NH` | `O`  | -11.75         | -10.60   | -10.23  | -8.31          | -7.67    | -8.46   |
| NPD-0734    | `NH` | `O`  | -7.13          | -8.01    | -9.41   | -8.09          | -7.93    | -8.32   |
| NPD-0800    | `NH` | `NH₂`| -11.09         | -9.13    | -9.00   | -8.43          | -8.51    | -8.59   |
| NPD-0936    | `NH` | `O`  | -11.41         | -8.97    | -8.73   | -8.04          | -8.24    | -7.64   |
| NPD-0935    | `NH` | `NH₂`| -10.45         | -9.96    | -8.73   | -6.28          | -7.83    | -7.64   |
| NPD-0937    | `NH` | `OH` | -11.94         | -8.71    | -8.73   | -7.96          | -7.61    | -7.50   |
| NPD-0878    | `NH` | `O`  | -11.98         | -9.27    | -8.59   | -8.43          | -7.71    | -7.37   |
| NPD-0801    | `NH` | `O`  | -11.00         | -8.29    | -8.59   | -8.53          | -7.85    | -7.64   |
| NPD-0942    | `NH` | `O`  | -10.87         | -7.94    | -7.78   | -8.34          | -8.07    | -7.09   |
Table S2. Chemical structures of tetrahydrophthalazinones ligands of validation set 2, Glide SP score, ΔG values experimentally determined and respective ΔG computed by FEP+.
Table S3. Chemical structures of alkylnamide phthalazinones ligands of validation set 3, Glide SP score, ΔG values experimentally determined and respective ΔG computed by FEP+.

| Ligand ID | R₁ | Glide SP score | Pred.ΔG | Exp.ΔG | Glide SP score | Pred.ΔG | Exp.ΔG |
|-----------|----|----------------|---------|--------|----------------|---------|--------|
| NPD-1335  | -  | -9.65          | -8.99   | -9.30  | -9.49          | -8.95   | -8.32  |
| NPD-0361  |    | -8.15          | -8.85   | -8.50  | -8.91          | -9.28   | -8.46  |
| NPD-1016  |    | -8.48          | -8.57   | -9.40  | -8.10          | -8.18   | -8.73  |
| NPD-1018  |    | -8.30          | -10.21  | -9.15  | -8.91          | -10.66  | -8.19  |
| NPD-1024  |    | -7.84          | -7.95   | -8.92  | -8.76          | -8.24   | -8.59  |
| NPD-1038  |    | -7.86          | -7.89   | -8.68  | -8.59          | -7.97   | -8.87  |
| NPD-1039  |    | -8.16          | -9.59   | -8.87  | -9.43          | -9.01   | -8.32  |
| NPD-1041  |  F | -7.28          | -7.42   | -8.40  | -5.19          | -8.16   | -8.73  |
| NPD-1042  |    | -8.09          | -8.42   | -8.91  | -8.80          | -8.54   | -8.32  |
| NPD-1168  |  S | -8.25          | -7.90   | -8.25  | -3.69          | -8.28   | -8.46  |
| Compound  | Structure | -8.60 | -8.75 | -9.52 | -9.33 | -8.44 | -8.59 |
|-----------|-----------|-------|-------|-------|-------|-------|-------|
| NPD-1169  | ![Structure](image) |       |       |       |       |       |       |
| NPD-1171  | ![Structure](image) | -8.58 | -9.38 | -8.92 | -9.39 | -8.90 | -8.19 |
| NPD-1174  | ![Structure](image) | -8.81 | -9.38 | -9.15 | -8.84 | -8.49 | -9.14 |
| NPD-1319  | ![Structure](image) | -8.85 | -9.80 | -8.96 | -8.85 | -8.02 | -8.73 |
| NPD-1320  | ![Structure](image) | -9.01 | -10.50 | -8.76 | -9.04 | -8.84 | -8.32 |
| NPD-1321  | ![Structure](image) | -8.29 | -8.96 | -9.39 | -9.21 | -8.48 | -8.46 |
| NPD-1322  | ![Structure](image) | -8.66 | -9.31 | -9.35 | -9.36 | -9.17 | -8.59 |
| NPD-1323  | ![Structure](image) | -7.94 | -8.66 | -8.72 | -9.20 | -7.92 | -8.46 |
| NPD-1334  | ![Structure](image) | -8.47 | -8.88 | -9.56 | -8.80 | -8.59 | -8.46 |
| NPD-3153  | ![Structure](image) | -8.66 | -9.83 | -9.13 | -8.22 | -9.31 | -9.00 |
| NPD-3155  | ![Structure](image) | -8.45 | -9.70 | -8.53 | -8.86 | -8.95 | -8.32 |
| NPD-1043a | ![Structure](image) | -7.94 | -8.21 | -8.69 | -8.77 | -7.07 | -8.73 |
| NPD-1043b | ![Structure](image) | -8.20 | -8.41 | -8.69 | -8.66 | -7.54 | -8.73 |
### Table S4. Statistics on Data Sets used for FEP+ calculations

| Protein system | PDB Ligand set | No. ligands | Expected Exp. R² | Expected FEP+ R² | Predicted R² | MUE (kcal/mol) | RMSE (kcal/mol) |
|----------------|----------------|-------------|------------------|------------------|--------------|----------------|----------------|
| **TbrPDEB1**   | 5G2B           | 1           | 0.74±0.1         | 0.48±0.2         | 0.63         | 1.47±0.3       | 2.05±0.4       |
|                | 5G2Ba          | 1           | 0.45±0.2         | 0.24±0.2         | 0.25         | 0.86±0.1       | 1.03±0.1       |
|                | 5G2B           | 2           | 0.49±0.2         | 0.28±0.2         | 0.37         | 1.70±0.2       | 1.86±0.3       |
|                | 5G5V           | 2           | 0.43±0.2         | 0.24±0.2         | 0.37         | 0.88±0.1       | 1.03±0.2       |
|                | 6GXQ           | 3           | 0.17±0.1         | 0.08±0.1         | 0.08         | 0.89±0.1       | 1.08±0.1       |
| **hPDE4**      | 5LAQ           | 1           | 0.49±0.2         | 0.26±0.2         | 0.45         | 0.69±0.1       | 0.90±0.2       |
|                | 5LAQ           | 2           | 0.49±0.2         | 0.28±0.2         | 0.15         | 0.76±0.2       | 0.98±0.3       |
|                | 6HWO           | 3           | 0.10±0.1         | 0.06±0.1         | 0.12         | 0.82±0.1       | 1.00±0.1       |

aThr841 included in the REST region

### Table S5. Flexibility and stability analysis of the X-ray structures in complex with PDEs inhibitors

| Ligand ID | Species | PDB ID | RMSD Ligand | RMSD FP | RMSD PP |
|-----------|---------|--------|-------------|---------|---------|
| NPD-008   | TbrPDEB1| 5G2B   | 1.8         | 0.34    | 3.53    |
| NPD-937   | TbrPDEB1| 5L8Y   | 3.5         | 0.50    | 1.49    |
| NPD-038   | TbrPDEB1| 5G5V   | 3.5         | 0.70    | 3.48    |
| NPD-001   | hPDE4B  | 5LAQ   | 1.8         | 0.62    | 1.08    |
|           | hPDE4D  | 5LBO   | 2.4         | 0.32    | 0.67    |
| NPD-1335  | hPDE4D  | 6HWO   | /           | 0.27    | 0.78    |

TbrPDEB1 6GXQ / 0.26 0.89

a RMSD of the ligands measured during the MD simulation. b RMSD of on the protein backbone between various apo-forms (PDB IDs: 4I15, 4WZI and 3SL3 for TbrPDEB1, hPDE4B and hPDE4D, respectively) and holo-forms (PDB IDs: 5G2B; 5G57; 6GXQ for TbrPDEB1; PDB IDs: 5LAQ for hPDE4B; PDB IDs: 5LBO; 6HWO for hPDE4D) measured on the full protein (FP) and c on the P-pocket region (PP).
Table S6. Convergence data from FEP+ calculation

Convergence criteria fully integrated in the graphical interface of the FEP+ implementation are programmatically assessed and sorted in 3 categories: Good convergence rate: < 0.3 kcal mol⁻¹ ns⁻¹; Fair convergence rate: 0.3 kcal mol⁻¹ ns⁻¹; Bad convergence rate: > 0.3 kcal mol⁻¹ ns⁻¹. Below are reported the number of edges and the associated convergence categories of FEP+ perturbations calculated for set 1, 2 and 3.

| Species | Lig. | Set  | PDB  | Total Edges | N. Edges with Good Convergence rate | N. Edges with Fair Convergence rate | N. Edges with Bad Convergence rate |
|---------|------|------|------|-------------|-------------------------------------|-------------------------------------|-----------------------------------|
| TbrPDEB1 | Set1 | 5G2B | 16   | 0           | 14                                  | 2                                   |
|         |      |      |      |             |                                     |                                     |                                   |
|         | Set1 | 5G2B (REST) | 17   | 17          | 0                                   | 0                                   |
|         |      |      |      |             |                                     |                                     |                                   |
|         | Set2 | 5G2B | 10   | 10          | 0                                   | 0                                   |
|         |      |      |      |             |                                     |                                     |                                   |
|         | Set2 | 5G5V | 9    | 8           | 1                                   | 0                                   |
|         |      |      |      |             |                                     |                                     |                                   |
|         | Set3 | 6GXQ | 34   | 30          | 4                                   | 0                                   |
| hPDE4   | Set1 | 5LAQ | 18   | 15          | 3                                   | 0                                   |
|         |      |      |      |             |                                     |                                     |                                   |
|         | Set2 | 5LAQ | 12   | 12          | 0                                   | 0                                   |
|         |      |      |      |             |                                     |                                     |                                   |
|         | Set3 | 6HWO | 35   | 33          | 2                                   | 0                                   |

*Thr841 included in the REST region.
Figure S3: Examples of Good, Fair, and Bad convergence plots and the associated maps.
References

1. Schrödinger Release 2021-1: Maestro, Schrödinger, LLC, New York, NY, 2021.
2. Schrödinger Release 2019-4: LigPrep.
3. Blaazer, A. R.; Singh, A. K.; de Heuvel, E.; Edink, E.; Orrling, K. M.; Veerman, J. J. N.; van den Bergh, T.; Jansen, C.; Balasubramaniam, E.; Mooij, W. J.; Custers, H.; Sijm, M.; Tagoe, D. N. A.; Kalejaüye, T. D.; Munday, J. C.; Tenor, H.; Matheeuissen, A.; Wijtmans, M.; Siderius, M.; de Graaf, C.; Maes, L.; de Koning, H. P.; Bailey, D. S.; Sterk, G. J.; de Esch, I. J. P.; Brown, D. G.; Leurs, R., Targeting a Subpocket in Trypanosoma brucei Phosphodiesterase B1 (TbrPDEB1) Enables the Structure-Based Discovery of Selective Inhibitors with Trypanocidal Activity. *J Med Chem* 2018, 61 (9), 3870-3888.
4. de Heuvel, E.; Singh, A. K.; Boronat, P.; Kooistra, A. J.; van der Meer, T.; Sadek, P.; Blaazer, A. R.; Shaner, N. C.; Bindels, D. S.; Caljon, G.; Maes, L.; Sterk, G. J.; Siderius, M.; Oberholzer, M.; de Esch, I. J. P.; Brown, D. G.; Leurs, R., Alkynamide phthalazinones as a new class of TbrPDEB1 inhibitors (Part 2). *Bioorg Med Chem* 2019, 27 (18), 4013-4029.
5. Sastry, G. M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W., Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des* 2013, 27 (3), 221-34.
6. Schrödinger Release 2021-1: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2021; Impact, Schrödinger, LLC, New York, NY; Prime, Schrödinger, LLC, New York, NY, 2021.
7. Schrödinger Release 2021-1: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2021. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY, 2021.
8. Schrödinger Release 2021-1: Glide, Schrödinger, LLC, New York, NY, 2021.
9. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T., Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem* 2006, 49 (21), 6177-96.
10. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S., Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem* 2004, 47 (7), 1739-49.
11. Schrödinger Release 2021-1: FEP+, Schrödinger, LLC, New York, NY, 2021.
12. Harder, E.; Damm, W.; Maple, J.; Wu, C.; Reboul, M.; Xiang, J. Y.; Wang, L.; Lupyan, D.; Dahlgren, M. K.; Knight, J. L.; Kaus, J. W.; Cerutti, D. S.; Krilov, G.; Jorgensen, W. L.; Abel, R.; Friesner, R. A., OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins. *J Chem Theory Comput* 2016, 12 (1), 281-96.
13. Shivakumar, D.; Harder, E.; Damm, W.; Friesner, R. A.; Sherman, W., Improving the Prediction of Absolute Solvation Free Energies Using the Next Generation OPLS Force Field. *J Chem Theory Comput* 2012, 8 (8), 2553-8.
14. Yu, H. S.; Deng, Y.; Wu, Y.; Sindhikara, D.; Rask, A. R.; Kimura, T.; Abel, R.; Wang, L., Accurate and Reliable Prediction of the Binding Affinities of Macrocycles to Their Protein Targets. *J Chem Theory Comput* 2017, 13 (12), 6290-6300.
15. Kuhn, B.; Tichy, M.; Wang, L.; Robinson, S.; Martin, R. E.; Kuglstatter, A.; Benz, J.; Giroud, M.; Schirmeister, T.; Abel, R.; Diederich, F.; Hert, J., Prospective Evaluation of Free Energy Calculations for the Prioritization of Cathepsin L Inhibitors. *J Med Chem* 2017, 60 (6), 2485-2497.
16. Abel, R.; Wang, L.; Harder, E. D.; Berne, B. J.; Friesner, R. A., Advancing Drug Discovery through Enhanced Free Energy Calculations. *Acc Chem Res* 2017, 50 (7), 1625-1632.
17. Shivakumar, D.; Williams, J.; Wu, Y.; Damm, W.; Shelley, J.; Sherman, W., Prediction of Absolute Solvation Free Energies using Molecular Dynamics Free Energy Perturbation and the OPLS Force Field. *J Chem Theory Comput* **2010**, *6* (5), 1509-19.

18. Wang, L.; Deng, Y.; Knight, J. L.; Wu, Y.; Kim, B.; Sherman, W.; Shelley, J. C.; Lin, T.; Abel, R., Modeling Local Structural Rearrangements Using FEP/REST: Application to Relative Binding Affinity Predictions of CDK2 Inhibitors. *J Chem Theory Comput* **2013**, *9* (2), 1282-93.