Table S1. Data collection and refinement statistics of the ternary complexes of WT-PfDHFR-TS.

| PDB ID code | 148  | 263  | 820  |
|-------------|------|------|------|
| **Data collection** |      |      |      |
| Wavelength (Å) | 1.5418 | 1.5418 | 1.5418 |
| Space group | P2_12_1 | P2_12_1 | P2_12_1 |
| Unit-Cell Parameters |      |      |      |
| a, b, c (Å) | 56.638, 56.143, 56.185 | 155.639, 154.088, 154.730 | 164.614, 163.598, 163.816 |
| Resolution\(^a\) (Å) | Inf–2.65 (2.75–2.65) | Inf–2.80 (2.90–2.80) | Inf–2.65 (2.75–2.65) |
| Total reflections |      |      |      |
| Unique reflections |      |      |      |
| Completeness (%) | 97.8 (100.0) | 96.4 (87.5) | 97.8 (90.7) |
| \(<l/σ(l)\)> | 8.53 (2.10) | 12.68 (3.17) | 10.15 (2.03) |
| R\(_{merge}\)^b (%) | 15.76 (47.15) | 9.27 (18.76) | 13.95 (39.11) |
| **Refinement** |      |      |      |
| R\(_{work}\)/R\(_{free}\)(%)^c | 20.1/27.6 | 22.0/30.4 | 22.1/30.1 |
| No. of Atoms/Average B-factors (Å\(^2\)) |      |      |      |
| Protein | 17164/34.5 | 16744/29.5 | 17036/30.5 |
| Inhibitor | 38/34.4 | 58/35.3 | 30/46.9 |
| NDP | 138/44.4 | 67/19.4 | 138/52.4 |
| UMP | 60/29.4 | - | - |
| PO\(_4\) | - | 10/43.6 | 5/42.8 |
| Water | 58/15.1 | 16/6.5 | 65/10.5 |
| R.m.s. deviation |      |      |      |
| Bond lengths (Å) | 0.0102 | 0.0076 | 0.0078 |
| Bond angles (°) | 1.844 | 1.648 | 1.683 |
| Ramachadran Plot |      |      |      |
| favored (%) | 92.48 | 89.66 | 91.64 |
| allowed (%) | 5.90 | 6.93 | 6.63 |
| outlier (%) | 1.62 | 3.41 | 1.73 |

\(^a\)Values in parentheses are for the highest-resolution shell.

\(^b\)R\(_{merge}\) = \( \Sigma_{hkl} |I(hk\ell) - \langle I(hk\ell)\rangle|/\Sigma_{hkl}|I(hk\ell)|\), where \(I(hk\ell)\) is the intensity of an individual reflection and \(\langle I(hk\ell)\rangle\) is the mean intensity of symmetry-equivalent reflections.

\(^c\)R\(_{work}\) = \( \Sigma_{hkl} |F_{obs}| - |F_{calc}|/\Sigma_{hkl}|F_{obs}|\), where \(F_{obs}\) and \(F_{calc}\) are the observed and calculated structure-factor amplitudes, respectively. R\(_{free}\) was calculated in the same manner as R\(_{work}\) but using only a 5% unrefined subset of the reflection data.
**Figure S1.** Superimposition of crystal structure backbones for complexes with fragments 263 (red), 820 (blue) and 148 (green).

**Figure S2.** Superimposition of docking poses obtained for fragments 132 (green), 80 (cyan), 800 (orange), 240 (magenta), 1157 (pink) and 813 (yellow).
**Figure S3.** Examples of other piperidine and piperazine-based fragments from the BIONET Premium library and their inhibition of WT PfDHFR at 500 μM.

**Figure S4.** Structure of non-hit fragments analog to 148 from the BIONET Premium library and their inhibition of WT PfDHFR at 500 μM.