Supporting Information

Fragments as Novel Starting Points for tRNA-Guanine Transglycosylase Inhibitors Found by Alternative Screening Strategies

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Table S1: Structures of fragments not included in SPR fragment screen.

| Jena Plate ID | Fragment | Jena Plate ID | Fragment |
|---------------|----------|---------------|----------|
| J1            | ![Image](image1.png) | J48           | ![Image](image2.png) |
| J11           | ![Image](image3.png) | J74           | ![Image](image4.png) |
| J16           | ![Image](image5.png) | J75           | ![Image](image6.png) |
| J21           | ![Image](image7.png) | J83           | ![Image](image8.png) |
| J26           | ![Image](image9.png) | J90           | ![Image](image10.png) |
| J33           | ![Image](image11.png) | J35           | ![Image](image12.png) |
| J53           | ![Image](image13.png) | J54           | ![Image](image14.png) |
| J57           | ![Image](image15.png) |               |          |
Table S2: Structures of fragments not included in NMR fragment screen.

| Jena Plate ID | Fragment | Jena Plate ID | Fragment |
|---------------|----------|---------------|----------|
| J2            | ![Image](image1.png) | J64           | ![Image](image2.png) |
| J4            | ![Image](image3.png) | J67           | ![Image](image4.png) |
| J6            | ![Image](image5.png) | J69           | ![Image](image6.png) |
| J13           | ![Image](image7.png) | J73           | ![Image](image8.png) |
| J14           | ![Image](image9.png) | J77           | ![Image](image10.png) |
| J17           | ![Image](image11.png) | J79           | ![Image](image12.png) |
| J59           | ![Image](image13.png) | J82           | ![Image](image14.png) |
| J35           | ![Image](image15.png) | J53           | ![Image](image16.png) |
| J54           | ![Image](image17.png) | J55           | ![Image](image18.png) |
| J57           | ![Image](image19.png) |               |          |
| PDB Code a | 6FSO | 5SW3 | 5N6F |
|------------|------|------|------|
| Fragment Jena ID | J14 | J41 | J64 |

**A) Data Collection and Processing**

| Wavelength | 0.9184 | 1.000 | 0.9184 |
| Beamline | BESSY BEAMLINE 14.1 | ELETTRA BEAMLINE 5.2R | BESSY BEAMLINE 14.1 |
| Detector | PILATUS 6M | DECTRIS PILATUS 2M | PILATUS 6M |
| Resolution range (Å) | 43.29 - 1.45 (1.54 - 1.45) | 44.46 - 1.38 (1.46 - 1.38) | 44.28 - 1.12 (1.19 - 1.12) |
| Space group | C 1 2 1 | C 1 2 1 | C 1 2 1 |
| Unit cell parameters (a,b,c Å) | 90.9 64.9 70.8 | 89.0 64.1 70.5 | 88.7 64.9 70.7 |
| Unit cell parameters (α, β, γ °) | 90.0 95.7 90.0 | 90.0 92.8 90.0 | 90.0 93.4 90.0 |
| Matthews coefficient b Å³/Da | 2.5 | 2.4 | 2.5 |
| Solvent content b (%) | 51.1 | 49.4 | 50.8 |
| Total reflections | 183142 (27068) | 303178 (47561) | 537838 (59077) |
| Unique reflections | 68249 (10718) | 81197 (12961) | 149008 (20548) |
| Completeness (%) | 94.8 (92.3) | 99.7 (98.9) | 97.1 (83.2) |
| Mean I/sigma(I) | 9.8 (2.2) | 12.4 (2.5) | 10.3 (2.0) |
| Wilson B-factor (Å²) | 11.5 | 13.6 | 10.9 |
| R-sym (% | 7.3 (48.6) | 6.0 (49.3) | 6.1 (48.7) |
| R-meas (%) | 9.0 (60.8) | 6.9 (57.7) | 7.1 (59.2) |
| CC1/2 | 99.6 (76.3) | 99.8 (79.7) | 99.7 (72.0) |

**B) Refinement**

| Resolution range (Å) | 43.29 - 1.45 | 44.46 - 1.38 | 44.28 - 1.12 |
| Total reflections used in refinement | 68178 | 81188 | 148997 |
| Reflections used for R-work | 64769 | 77129 | 141547 |
| Reflections used for R-free | 3409 | 4059 | 7450 |
| Final R value for R-work (%) | 16.6 | 13.3 | 13.6 |
| Final R value for R-free (%) | 18.6 | 16.2 | 15.4 |
| No. of Protein residues | 368 | 371 | 365 |
| No. of Water molecules | 252 | 223 | 280 |
| No. of Ligand molecules | 1 | 2 | 1 |
| No. of Other ligand molecules | 8 | 6 | 4 |
| RMSD bond lengths (Å) | 0.008 | 0.007 | 0.007 |
| RMSD bond angles (°) | 1.0 | 0.9 | 1.0 |
| Ramachandran plot d | | | |
| Residues in most favored regions (%) | 93.9 | 95 | 96.1 |
| Residues in additionally allowed regions (%) | 5.4 | 4.7 | 3.6 |
| Residues in generously allowed regions (%) | 0.3 | 0.3 | 0.3 |
| Residues in disallowed regions (%) | 0 | 0 | 0 |
| Average B-factor all atoms e (Å²) | 16.2 | 17.9 | 16.1 |
| Protein main chain | 13.4 | 15.7 | 13.7 |
| Protein side chain | 16.6 | 18.2 | 16.1 |
| Protein all atoms | 15.1 | 17.0 | 15.0 |
| Ligand | 21.6 | 30.1 | 12.2 |
| Water molecules | 25.5 | 26.4 | 27.4 |
| Other ligands | 36.3 | 36.3 | 33.5 |
| Number of TLS groups | 6 | N/A | N/A |

a) Statistics for the highest-resolution shell are shown in parenthesis, b) calculated with Matthews_coef program from CCP4 suite version 7.0.047, c) calculated by the equation: (SUM(ABS(I(h,i)-I(h))))/(SUM(I(h,i))) d) calculated with PROCHECK, e) calculated with MOLEMAN, f) other ligands include DMSO, PEG, Glycerol, 1PE, ACT, PG4, Zn.
**Table S4**: Data Collection and Refinement Statistics.

| PDB Code  | 5UTI | 5UTJ | SV3C |
|-----------|------|------|------|
| Fragment Jena ID | J72  | J79  | J86  |

**A) Data Collection and Processing**

| Wavelength | 1.000 | 0.9184 | 0.9184 |
| Beamline | ELETTRA BEAMLINE 5.2R | BESSY BEAMLINE 14.1 | BESSY BEAMLINE 14.1 |
| Detector | DECTRIS PILATUS 2M | PILATUS 6M | PILATUS 6M |
| Resolution range (Å) | 44.51 - 1.36 (1.44 - 1.36) | 44.50 - 1.55 (1.64 - 1.55) | 44.33 - 1.42 (1.51 - 1.42) |
| Space group | C 1 2 1 | C 1 2 1 | C 1 2 1 |
| Unit cell parameters (a,b,c Å) | 89.2 64.3 70.9 | 89.1 64.8 70.5 | 88.8 63.7 70.4 |
| Unit cell parameters (α, β, γ °) | 90.0 93.1 90.0 | 90.0 93.2 90.0 | 90.0 92.6 90.0 |
| Matthews coefficient b (Å³/Da) | 2.5 | 2.5 | 2.4 |
| Solvent Content b (%) | 50.1 | 51.0 | 48.8 |
| Total reflections | 239209 (38188) | 219498 (34906) | 276359 (43372) |
| Unique reflections | 81480 (12780) | 58079 (9314) | 73712 (11753) |
| Multiplicity | 2.9 | 3.8 | 3.8 |
| Completeness (%) | 95.0 (92.5) | 99.7 (99.4) | 99.4 (98.4) |
| Mean I/σ(I) | 11.6 (2.3) | 15.4 (2.0) | 15.6 (2.0) |
| Wilson B-factor (Å²) | 15.4 | 18.0 | 15.7 |
| R-sym c (%) | 5.0 (44.5) | 5.2 (55.6) | 4.6 (55.1) |
| R-meas (%) | 6.1 (54.2) | 6.0 (64.7) | 5.4 (64.3) |
| CC1/2 | 99.8 (81.6) | 99.9 (77.4) | 99.9 (74.1) |

**B) Refinement**

| Resolution range (Å) | 44.51 - 1.36 | 44.50 - 1.55 | 44.33 - 1.42 |
| Total reflections used in refinement | 81466 | 58069 | 73705 |
| Reflections used for R-work | 77393 | 55166 | 70220 |
| Reflections used for R-free | 4073 | 2903 | 3685 |
| Final R value for R-work (%) | 13.5 | 14.7 | 13.4 |
| Final R value for R-free (%) | 15.8 | 17.7 | 15.5 |
| No. of Protein residues | 371 | 364 | 373 |
| No. of Water molecules | 269 | 322 | 359 |
| No. of Ligand molecules | 1 | 1 | 1 |
| No. of Other ligand molecules | 4 | 4 | 6 |
| RMSD bond lengths (Å) | 0.007 | 0.008 | 0.007 |
| RMSD bond angles (°) | 0.9 | 0.9 | 0.9 |
| Ramachandran plot d | Residues in most favored regions (%) | 94.6 | 94.2 | 94 |
| Residues in additionally allowed regions (%) | 5 | 5.5 | 5.6 |
| Residues in generously allowed regions (%) | 0.3 | 0.3 | 0.3 |
| Residues in disallowed regions (%) | 0 | 0 | 0 |
| Average B-factor all atoms e (Å²) | 20.5 | 24.6 | 22.1 |
| Protein main chain | 17.9 | 22.1 | 19.7 |
| Protein side chain | 20.9 | 25.9 | 22.5 |
| Protein all atoms | 19.5 | 24.1 | 21.1 |
| Ligand | 27.1 | 57.5 | 26.8 |
| Water molecules | 29.8 | 30.4 | 31.2 |
| Other ligands | 44 | 36.4 | 46 |
| Number of TLS groups | N/A | N/A | N/A |

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a) Statistics for the highest-resolution shell are shown in parenthesis, b) calculated with Matthews_coef program from CCP4 suite version 7.0.047; c) calculated by the equation: (SUM(ABS((h,i) - (h))))/SUM((h,i)) d) calculated with PROCHECK; e) calculated with MOLEMAN; f) other ligands include DMSO, PEG, Glycerol, 1PE, ACT, PG4, Zn.
Table S5: Chemical Structures of the 96 fragments. Marvin was used for displaying chemical structures, Marvin 6.3.1, 2014, ChemAxon (http://www.chemaxon.com) and the web server chemicalize.com

| Jena Plate ID | Fragment | Jena Plate ID | Fragment | Jena Plate ID | Fragment |
|---------------|----------|---------------|----------|---------------|----------|
| 1             | ![Structure1](image1.png) | 8             | ![Structure8](image8.png) | 15           | ![Structure15](image15.png) |
| 2             | ![Structure2](image2.png) | 9             | ![Structure9](image9.png) | 16           | ![Structure16](image16.png) |
| 3             | ![Structure3](image3.png) | 10            | ![Structure10](image10.png) | 17           | ![Structure17](image17.png) |
| 4             | ![Structure4](image4.png) | 11            | ![Structure11](image11.png) | 18           | ![Structure18](image18.png) |
| 5             | ![Structure5](image5.png) | 12            | ![Structure12](image12.png) | 19           | ![Structure19](image19.png) |
| 6             | ![Structure6](image6.png) | 13            | ![Structure13](image13.png) | 20           | ![Structure20](image20.png) |
| 7             | ![Structure7](image7.png) | 14            | ![Structure14](image14.png) | 21           | ![Structure21](image21.png) |
| 22 | ![Molecule 22](image1.png) | 30 | ![Molecule 30](image2.png) | 38 | ![Molecule 38](image3.png) |
| 23 | ![Molecule 23](image4.png) | 31 | ![Molecule 31](image5.png) | 39 | ![Molecule 39](image6.png) |
| 24 | ![Molecule 24](image7.png) | 32 | ![Molecule 32](image8.png) | 40 | ![Molecule 40](image9.png) |
| 25 | ![Molecule 25](image10.png) | 33 | ![Molecule 33](image11.png) | 41 | ![Molecule 41](image12.png) |
| 26 | ![Molecule 26](image13.png) | 34 | ![Molecule 34](image14.png) | 42 | ![Molecule 42](image15.png) |
| 27 | ![Molecule 27](image16.png) | 35 | ![Molecule 35](image17.png) | 43 | ![Molecule 43](image18.png) |
| 28 | ![Molecule 28](image19.png) | 36 | ![Molecule 36](image20.png) | 44 | ![Molecule 44](image21.png) |
| 29 | ![Molecule 29](image22.png) | 37 | ![Molecule 37](image23.png) | 45 | ![Molecule 45](image24.png) |
|   | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
|---|---|---|---|---|
| 46 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 47 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 48 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 49 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 50 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 51 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 52 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
| 53 | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) | ![Image](https://via.placeholder.com/150) |
|    | Structure 70 | Structure 78 | Structure 86 | Structure 71 | Structure 79 | Structure 87 | Structure 72 | Structure 80 | Structure 88 | Structure 73 | Structure 81 | Structure 89 | Structure 74 | Structure 82 | Structure 90 | Structure 75 | Structure 83 | Structure 91 | Structure 76 | Structure 84 | Structure 92 | Structure 77 | Structure 85 | Structure 93 |
| 94 | ![Chemical Structure](image1) | 95 | ![Chemical Structure](image2) | 96 | ![Chemical Structure](image3) |
|----|-------------------------------|----|-------------------------------|----|-------------------------------|