Supplementary Information

Structure-Based in Silico Screening Identifies a Potent Ebolavirus Inhibitor from a Traditional Chinese Medicine Library

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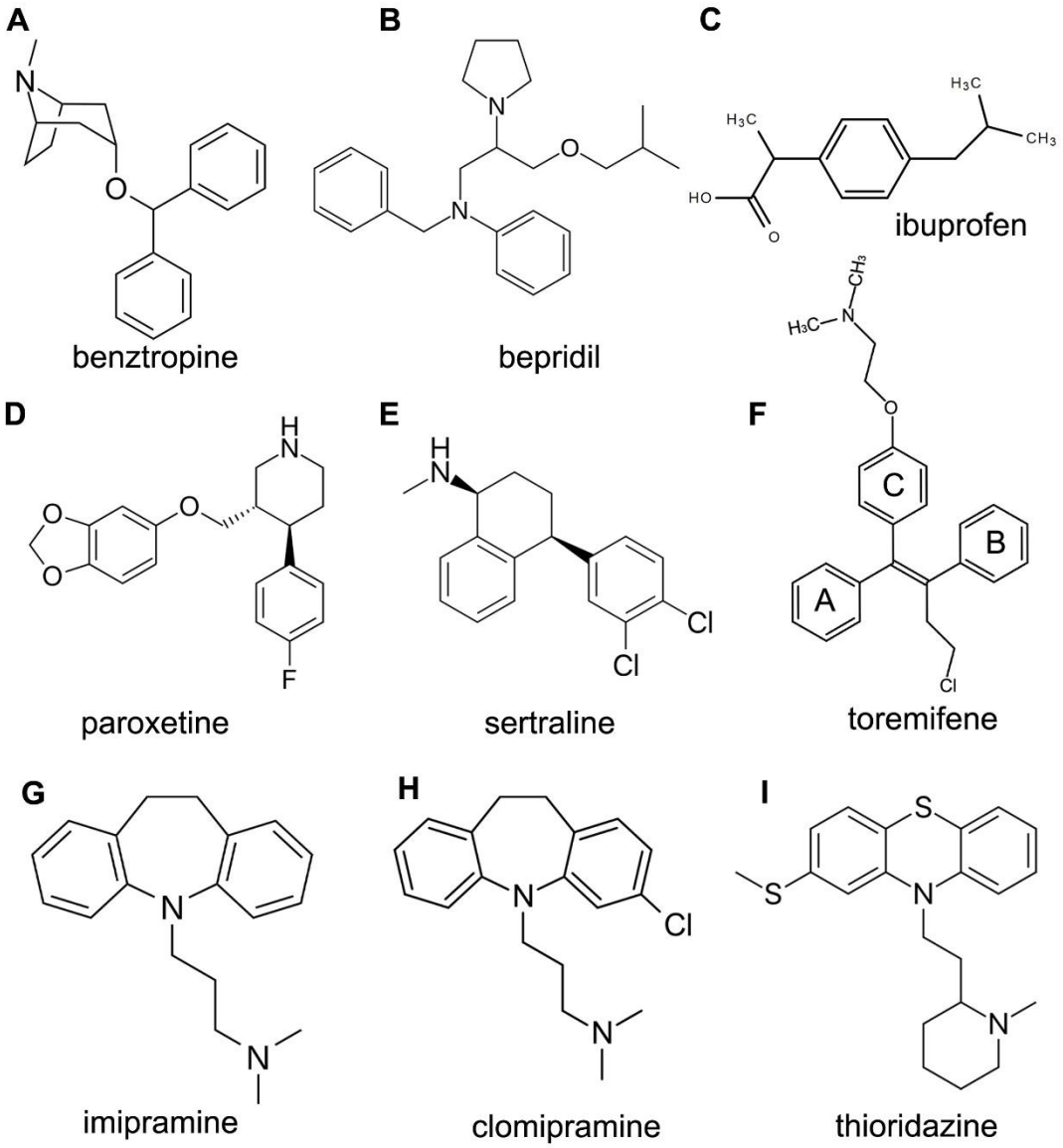

Figure S1. Chemical structures of approved drugs known to bind EBOV GP. Crystal structures of these nine drugs in complexes with EBOV GP have been previously reported.

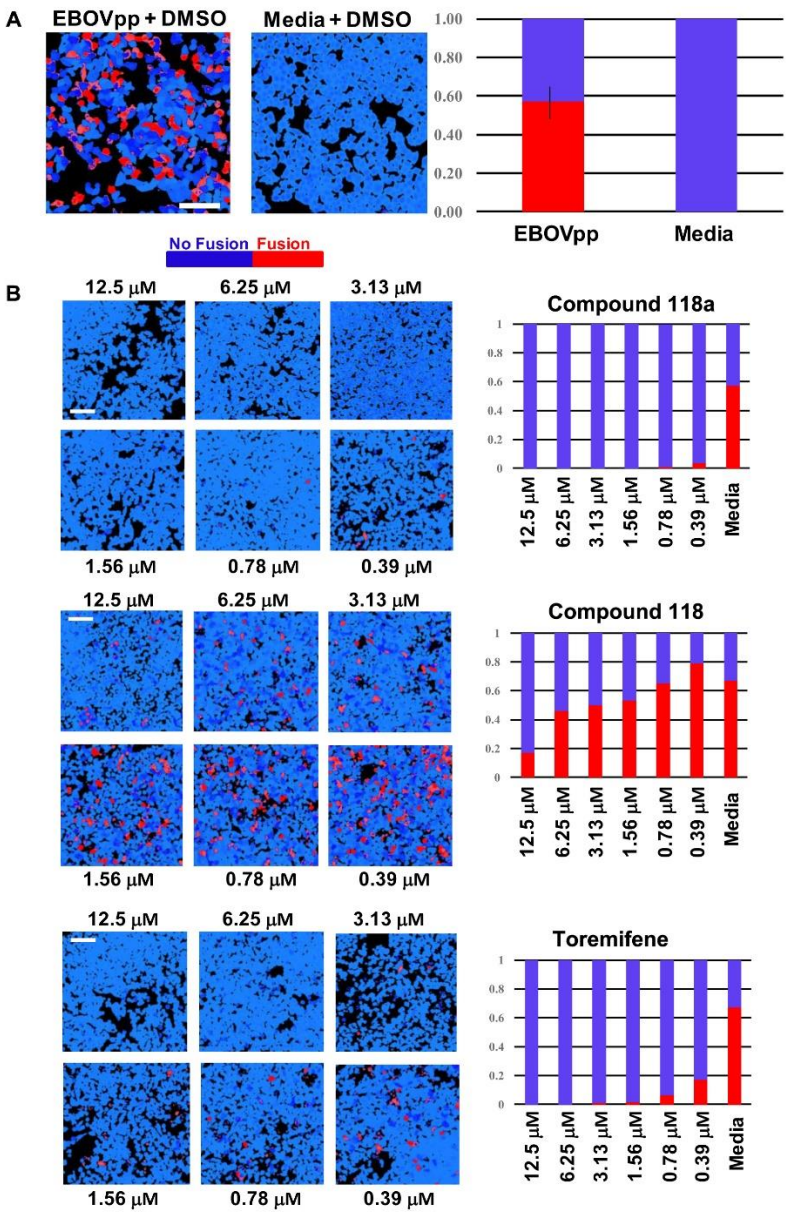

Figure S2. Compound **118a** is a potent and specific inhibitor of EBOVpp fusion in live cells. Fusion assays were carried out using a β-lactamase based reporter assay with different dilutions of **118a**. **118** and toremifene. (A) TZM-bl cells exposed to EBOVpp and media are shown, images were pseudocolored with red (fusion positive) and blue (fusion negative), error bar 10 µM. The bar diagram show the total proportion of fusion positive cells (57% +/-14) for TZM-bl cells exposed to EBOVpp (0.5 MOI) and Media (0%). (B) TZM-bl cells exposed to EBOVpp and **118a**, **118** and toremifene. Error bars showing the extent of fusion for each concentration of the corresponding compounds, error bar 20 µM.

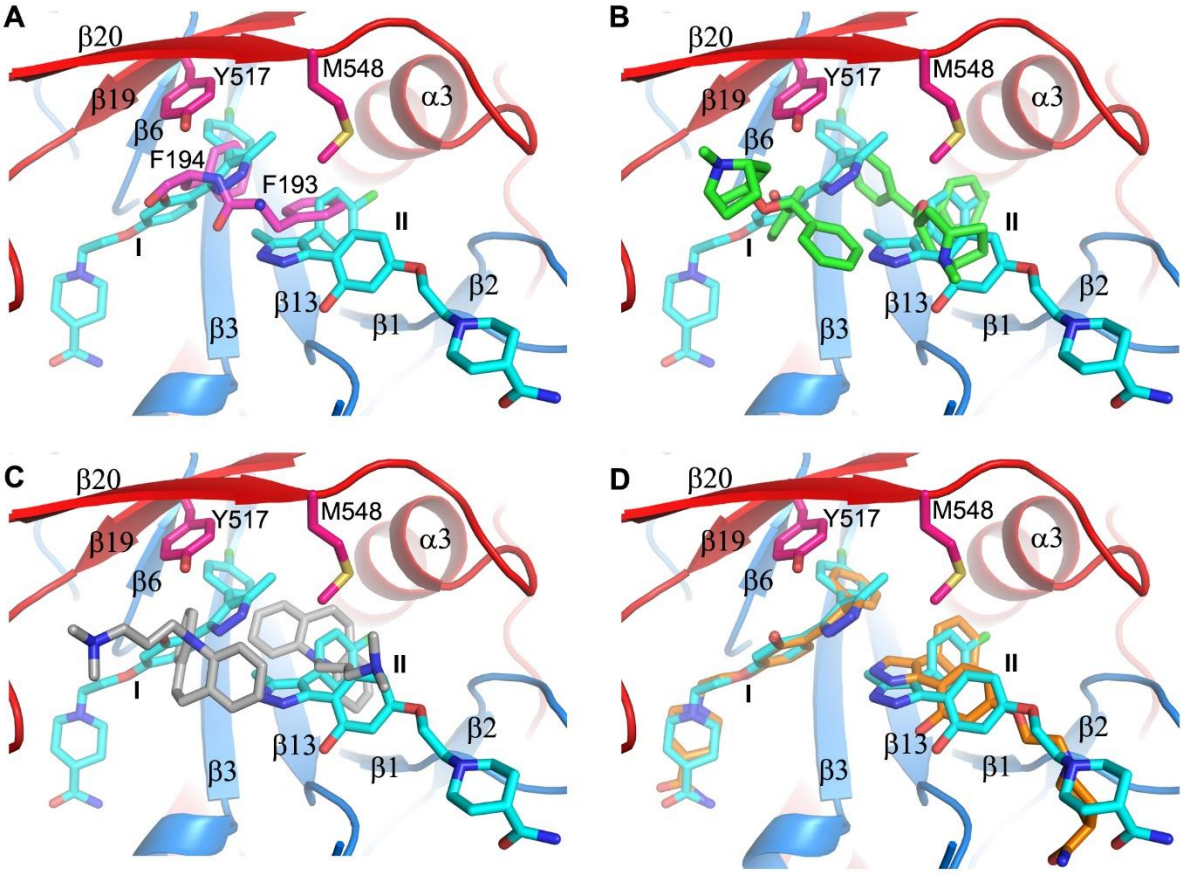

Figure S3. Comparison of binding modes of inhibitors that have two molecules bound in the GP cavity (these are labelled I and II, with I being the most internal). (A) The positions and orientations of the bound **118a** molecules are compared with F193 and F194 (magenta sticks) in the apo GP structure. (B to D) Overlays of bound benztropine (green sticks in (B)), imipramine (grey sticks in (C)) and compound **118** (orange sticks in (D)) molecules with **118a**. The superimpositions were done using protein Cα atoms only. In all panels protein structure shown is GP-**118a** with main-chain as ribbons (GP1 in blue and GP2 in red). For clarity, only side-chains of Y517 and M548 (red sticks) are shown to mark the relative position of the bound inhibitors.

Table S1. Glide and QPLD docking scores of known EBOV GP binders. pIC_{50} values are converted from the IC_{50}s obtained from virus infection assays in vero E6 cells (reference 10).

Drug	**Glide Emodel (kcal/mol)**	**QPLD Emodel (kcal/mol)**	**pIC_{50} ($-\log_{10}$ M)**
Toremifene	-67.733	-77.125	6.79
Bepridil	-50.37	-55.746	5.29
Benztropine	-47.379	-53.416	5.09
Paroxetine	-45.973	-51.792	5.13
Imipramine	-61.331	-44.892	4.86
Clomipramine	-44.13	-44.209	4.94
Thioridazine	-38.835	-44.026	5.20
Sertraline	-28.265	-31.91	5.50

Table S2. Glide XP and QPLD docking scores of top 16 natural compounds from *In silico* screening. About 2.5 million compounds in the ZINC natural compound library from the Traditional Chinese Medicine database@Taiwan have been screened (reference 27). All the top 16 hits have passed PAINS-Remover (reference 28). The docking results for toremifene are included for comparison. Compounds selected for *in vitro* experiment are marked with an asterisk.

Chemical ID	Glide XP Score (kcal/mol)	Glide XP Emodel (kcal/mol)	QPLD Score (kcal/mol)	QPLD Emodel (kcal/mol)	Ligand efficiency score (kcal/mol)
NP-005129	-12.582	-79.021	-12.53	-75.31	-0.307
NP-016821	-12.465	-77.247	-11.67	-83.967	-0.296
NP-019105	-11.006	-58.284	-9.407	-64.866	-0.297
***ZINC32540717 (118)**	-10.851	-70.036	-11.029	-73.801	-0.346
***ZINC00167626**	-10.894	-37.646	-9.143	-47.781	-0.545
ZINC 32540716	-10.781	-71.098	-10.645	-84.044	-0.333
***ZINC09410451 (118a)**	-9.210	-58.141	-9.210	-78.738	-0.288
NP-001471	-10.398	-54.738	-12.19	-70.721	-0.334
554127	-10.348	-37.327	-11.222	-59.189	-0.470
ZINC35464211	-10.127	-64.476	-9.887	-60.507	-0.316
***ZINC00407254**	-9.869	-44.638	-9.65	-57.035	-0.539
***ZINC12893941**	-9.791	-39.059	-9.187	-51.477	-0.484
***ZINC04772639**	-9.341	-49.893	-9.283	-55.833	-0.404
ZINC05071941	-9.275	-41.034	-9.323	-49.881	-0.459
***ZINC11865143**	-9.351	-40.886	-9.292	-53.402	-0.457
***ZINC00056827**	-8.166	-42.016	-9.694	-42.819	-0.606
Toremifene	-8.413	-67.733	-8.670	-77.125	-0.299

Table S3. Coordinates of compound **118** docked with the EBOV GP (PDB ID 5JQ7).

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HETATM    1  C   UNK   900     -47.581  11.604 -11.973  0.00  0.00           C
HETATM    2  C   UNK   900     -48.422  11.198 -10.946  0.00  0.00           C
HETATM    3  C   UNK   900     -48.109  11.514  -9.626  0.00  0.00           C
HETATM    4  C   UNK   900     -46.925  12.190  -9.316  0.00  0.00           C
HETATM    5  C   UNK   900     -46.088  12.603 -10.356  0.00  0.00           C
HETATM    6  C   UNK   900     -46.432  12.331 -11.680  0.00  0.00           C
HETATM    7  C   UNK   900     -46.599  12.453  -7.882  0.00  0.00           C
HETATM    8  C   UNK   900     -46.853  11.617  -6.802  0.00  0.00           C
HETATM    9  N   UNK   900     -46.388  12.274  -5.698  0.00  0.00           N
HETATM   10  H   UNK   900     -46.425  11.919  -4.752  0.00  0.00           H
HETATM   11  N   UNK   900     -45.860  13.464  -5.990  0.00  0.00           N
HETATM   12  C   UNK   900     -45.976  13.580  -7.297  0.00  0.00           C
HETATM   13  C   UNK   900     -45.498  14.811  -7.976  0.00  0.00           C
HETATM   14  C   UNK   900     -46.441  15.669  -8.542  0.00  0.00           C
HETATM   15  C   UNK   900     -46.024  16.798  -9.241  0.00  0.00           C
HETATM   16  C   UNK   900     -44.659  17.102  -9.352  0.00  0.00           C
HETATM   17  C   UNK   900     -43.725  16.241  -8.782  0.00  0.00           C
HETATM   18  C   UNK   900     -44.135  15.097  -8.106  0.00  0.00           C
HETATM   19  O   UNK   900     -43.182  14.258  -7.600  0.00  0.00           O
HETATM   20  O   UNK   900     -44.156  18.178 -10.051  0.00  0.00           O
HETATM   21  C   UNK   900     -44.909  19.385 -10.134  0.00  0.00           C
HETATM   22  C   UNK   900     -44.392  20.350  -9.065  0.00  0.00           C
HETATM   23  N   UNK   900     -45.309  21.593  -8.926  0.00  0.00           N1+
HETATM   24  C   UNK   900     -44.909  22.324  -7.626  0.00  0.00           C
HETATM   25  C   UNK   900     -45.863  23.502  -7.411  0.00  0.00           C
HETATM   26  C   UNK   900     -45.783  24.502  -8.585  0.00  0.00           C
HETATM   27  C   UNK   900     -46.079  23.784  -9.928  0.00  0.00           C
HETATM   28  C   UNK   900     -45.193  22.539 -10.150  0.00  0.00           C
HETATM   29  C   UNK   900     -46.719  25.713  -8.337  0.00  0.00           C
HETATM   30  O   UNK   900     -47.616  26.017  -9.117  0.00  0.00           O
HETATM   31  N   UNK   900     -46.509  26.453  -7.251  0.00  0.00           N
HETATM   32  H   UNK   900     -47.828  11.370 -12.999  0.00  0.00           H
HETATM   33  H   UNK   900     -49.309  10.629 -11.170  0.00  0.00           H
HETATM   34  H   UNK   900     -48.786  11.199  -8.851  0.00  0.00           H
HETATM   35  H   UNK   900     -45.171  13.134 -10.145  0.00  0.00           H
HETATM   36  H   UNK   900     -45.795  12.663 -12.487  0.00  0.00           H
HETATM   37  H   UNK   900     -47.309  10.640  -6.721  0.00  0.00           H
HETATM   38  H   UNK   900     -47.497  15.452  -8.457  0.00  0.00           H
HETATM   39  H   UNK   900     -46.783  17.408  -9.703  0.00  0.00           H
HETATM   40  H   UNK   900     -42.674  16.468  -8.887  0.00  0.00           H
HETATM   41  H   UNK   900     -42.295  14.518  -7.856  0.00  0.00           H
HETATM   42  H   UNK   900     -45.988  19.265 -10.061  0.00  0.00           H
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HETATM   45  H   UNK   900     -44.399  19.850  -8.096  0.00  0.00           H
HETATM   46  H   UNK   900     -43.869  22.643  -7.731  0.00  0.00           H
HETATM   47  H   UNK   900     -44.974  21.602  -6.814  0.00  0.00           H
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HETATM   57  H   UNK   900     -46.283  21.294  -8.851  0.00  0.00           H
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Table S4. Coordinates of compound **118a** docked with the EBOV GP (PDB ID 5JQ7).

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HETATM    1 Cl   UNK   900      -48.141  11.598 -12.609  0.00  0.00          Cl
HETATM    2  O   UNK   900      -43.308  18.223  -9.872  0.00  0.00           O
HETATM    3  O   UNK   900      -42.932  24.629  -9.642  0.00  0.00           O
HETATM    4  O   UNK   900      -42.364  15.448  -6.205  0.00  0.00           O
HETATM    5  N   UNK   900      -45.309  21.404  -9.352  0.00  0.00           N1+
HETATM    6  N   UNK   900      -43.309  25.525  -7.651  0.00  0.00           N
HETATM    7  N   UNK   900      -43.853  13.093  -5.902  0.00  0.00           N
HETATM    8  N   UNK   900      -44.757  12.194  -5.259  0.00  0.00           N
HETATM    9  C   UNK   900      -45.008  22.406 -10.487  0.00  0.00           C
HETATM   10  C   UNK   900      -44.712  21.872  -8.002  0.00  0.00           C
HETATM   11  C   UNK   900      -45.162  24.297  -8.714  0.00  0.00           C
HETATM   12  C   UNK   900      -45.612  23.778 -10.109  0.00  0.00           C
HETATM   13  C   UNK   900      -45.361  23.215  -7.623  0.00  0.00           C
HETATM   14  C   UNK   900      -44.909  19.962  -9.749  0.00  0.00           C
HETATM   15  C   UNK   900      -43.706  24.831  -8.712  0.00  0.00           C
HETATM   16  C   UNK   900      -43.470  19.547  -9.383  0.00  0.00           C
HETATM   17  C   UNK   900      -43.661  17.233  -9.020  0.00  0.00           C
HETATM   18  C   UNK   900      -42.806  16.811  -8.063  0.00  0.00           C
HETATM   19  C   UNK   900      -44.908  16.539  -9.251  0.00  0.00           C
HETATM   20  C   UNK   900      -44.256  14.816  -7.610  0.00  0.00           C
HETATM   21  C   UNK   900      -44.496  13.606  -7.023  0.00  0.00           C
HETATM   22  C   UNK   900      -45.478  12.555  -7.300  0.00  0.00           C
HETATM   23  C   UNK   900      -43.074  15.654  -7.188  0.00  0.00           C
HETATM   24  C   UNK   900      -45.174  15.397  -8.603  0.00  0.00           C
HETATM   25  C   UNK   900      -45.655  11.772  -6.218  0.00  0.00           C
HETATM   26  C   UNK   900      -46.167  12.339  -8.602  0.00  0.00           C
HETATM   27  C   UNK   900      -46.504  10.559  -5.918  0.00  0.00           C
HETATM   28  C   UNK   900      -47.468  11.831  -8.672  0.00  0.00           C
HETATM   29  C   UNK   900      -45.492  12.603  -9.799  0.00  0.00           C
HETATM   30  C   UNK   900      -48.078  11.597  -9.902  0.00  0.00           C
HETATM   31  C   UNK   900      -46.109  12.398 -11.029  0.00  0.00           C
HETATM   32  C   UNK   900      -47.394  11.884 -11.081  0.00  0.00           C
HETATM   33  H   UNK   900      -46.323  21.379  -9.251  0.00  0.00           H
HETATM   34  H   UNK   900      -45.467  22.017 -11.397  0.00  0.00           H
HETATM   35  H   UNK   900      -43.924  22.428 -10.612  0.00  0.00           H
HETATM   36  H   UNK   900      -44.909  21.104  -7.255  0.00  0.00           H
HETATM   37  H   UNK   900      -43.633  21.965  -8.137  0.00  0.00           H
HETATM   38  H   UNK   900      -45.751  25.179  -8.458  0.00  0.00           H
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HETATM   40  H   UNK   900      -46.701  23.690 -10.114  0.00  0.00           H
HETATM   41  H   UNK   900      -44.982  23.554  -6.657  0.00  0.00           H
HETATM   42  H   UNK   900      -46.431  23.061  -7.469  0.00  0.00           H
HETATM   43  H   UNK   900      -45.657  19.348  -9.252  0.00  0.00           H
HETATM   44  H   UNK   900      -45.090  19.851 -10.818  0.00  0.00           H
HETATM   45  H   UNK   900      -42.744  20.191  -9.886  0.00  0.00           H
HETATM   46  H   UNK   900      -43.250  19.626  -8.314  0.00  0.00           H
HETATM   47  H   UNK   900      -42.367  25.882  -7.646  0.00  0.00           H
HETATM   48  H   UNK   900      -43.891  25.599  -6.818  0.00  0.00           H
HETATM   49  H   UNK   900      -41.864  17.329  -7.962  0.00  0.00           H
HETATM   50  H   UNK   900      -45.640  16.965  -9.920  0.00  0.00           H
HETATM   51  H   UNK   900      -46.150  14.960  -8.765  0.00  0.00           H
HETATM   52  H   UNK   900      -43.507  13.726  -5.190  0.00  0.00           H
HETATM   53  H   UNK   900      -44.186  11.527  -4.758  0.00  0.00           H
HETATM   54  H   UNK   900      -46.556   9.900  -6.785  0.00  0.00           H
HETATM   55  H   UNK   900      -47.513  10.867  -5.645  0.00  0.00           H
HETATM   56  H   UNK   900      -46.099   9.999  -5.079  0.00  0.00           H
HETATM   57  H   UNK   900      -48.030  11.629  -7.775  0.00  0.00           H
HETATM   58  H   UNK   900      -44.477  12.973  -9.783  0.00  0.00           H
HETATM   59  H   UNK   900      -49.074  11.184  -9.941  0.00  0.00           H
HETATM   60  H   UNK   900      -45.594  12.645 -11.943  0.00  0.00           H
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