Supplementary information

Homology modeling of DFG-in FMS-like tyrosine kinase 3 (FLT3) and structure-based virtual screening for inhibitor identification

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Figure S1. 3D structure alignment of the homology-modeled DFG-in FLT3 structure to the X-ray structure of phosphorylase B kinase (PDB ID: 2V7J) bound to the ligand sunitinib.
Figure S2. Assessment of the DFG-in FLT3-modeled structure by ERRAT server. The arrows represent the active site residues in the protein model. The overall quality factor of the modeled structure improved after MD simulation.

Figure S3. Assessment of the DFG-in FLT3-modeled structure by Profile-3D server. The arrowed regions represent the active site residues in the protein model. The Verify score improved after MD simulation of the modeled structure.
**Figure S4.** Ramachandran plot of the homology modeled DFG-in FLT3 structure after MD simulation.

Plot statistics:

- Residues in most favoured regions [A,B,L]: 235, 90.0%
- Residues in additional allowed regions [a,b,l,p]: 21, 8.0%
- Residues in generously allowed regions [−a,−b,−l,−p]: 3, 1.1%
- Residues in disallowed regions: 2, 0.8%
- Number of non-glycine and non-proline residues: 261, 100.0%
- Number of end-residues (excl. Gly and Pro): 2
- Number of glycine residues (shown as triangles): 20
- Number of proline residues: 10
- Total number of residues: 293

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.
Figure S5. FLT3 (DFG-in) ITD insert site and D835 mutation location. Tyrosine kinase domain-1 (TK1, blue color); juxtamembrane domain (JM, red color) and ligand binding site (blue transparent surface).
Figure S6. The IC_{50} determination dose-response curve of BPR056 and BPR080, along with sorafenib.

Figure S7. Molecular dynamics simulation (20 ns) of DFG-in FLT3 in complex with two hits identified from VS. (A) H-bond graph for BPR056 (blue) and BPR0080 (red). (B) Ligand root mean square deviation (RMSD) graph for BPR056 (blue) and BPR080 (red).
Figure S8. Aligned structures of the original ligand conformation (green) present in 2Y7J and the docked ligand conformation (gray) of sunitinib in 2Y7J crystal structure. RMSD = 1.50Å, calculation done using Discovery studio 2.1.
Table S1. The top 97 compounds ranked by DOCK6.0/LigFit score and their percent FLT3 inhibition at 10μM.

| Corporate ID          | MW    | Dock / Ligfit score | Inhibition % | Corporate ID          | MW    | Dock / Ligfit score | Inhibition % |
|-----------------------|-------|----------------------|--------------|-----------------------|-------|----------------------|--------------|
| BPR001(CSV0A031467)  | 500.559 | -372/110             | -0.90%       | BPR050(CSV0A062560)  | 500.471 | -356/104             | 0.40%        |
| BPR002(CSV0A011296)  | 485.46 | -369/107             | 1.70%        | BPR051(CSV0C001836)  | 521.368 | -356/96              | 0.90%        |
| BPR003(CSV0A042562)  | 501.584 | -369/101             | 0.50%        | BPR052(CSV0C008541)  | 523.596 | -356/109             | 6.00%        |
| BPR004(CSV0A048785)  | 533.501 | -369/108             | 0.80%        | BPR053(CSV0A008714)  | 532.745 | -355/108             | -2.20%       |
| BPR005(CSV0A013828)  | 523.431 | -368/108             | -2.50%       | BPR054(CSV0A013730)  | 541.58  | -355/105             | -0.20%       |
| BPR006(CSV0A014367)  | 481.637 | -368/100             | -2.70%       | BPR055(CSV0A031501)  | 462.574 | -355/100             | -1.00%       |
BPR007(CSV0A051509) 546.541 -368/108 2.80%  BPR056(CSV0A034160) 325.33 -355/101 82.10%
BPR008(CSV0A005771) 486.575 -367/98 0.10%  BPR057(CSV0A034206) 533.545 -355/106 0.90%
BPR009(CSV0A035490) 525.628 -367/102 6.60%  BPR058(CSV0A034595) 486.532 -355/103 2.60%
BPR010(CSV0A038176) 464.435 -367/101 2.50%  BPR059(CSV0A043513) 486.454 -355/104 0.80%
BPR011(CSV0A029882) 545.641 -366/107 7.60%  BPR060(CSV0A051510) 514.498 -355/107 0.90%
BPR012(CSV0A041534) 501.376 -366/104 2.10%  BPR061(CSV0A059600) 479.477 -355/102 4.90%
BPR013(CSV0A008382) 469.504 -365/103 0.00%  BPR062(CSV0A061707) 456.508 -355/108 0.80%
BPR021(CSV0A005350)  512.57 -362/100  13.20%  BPR070(CSV0A048895)  500.905 -353/103  2.30%

BPR022(CSV0A034868)  508.99 -362/98  -2.20%  BPR071(CSV0A061227)  522.013 -353/111  1.40%

BPR023(CSV0A019616)  538.564 -361/113  0.90%  BPR072(CSV0A004079)  479.883 -352/101  0.70%

BPR024(CSV0A024759)  535.567 -361/114  0.60%  BPR073(CSV0A026290)  479.883 -352/103  0.10%

BPR025(CSV0A032833)  476.545 -361/111  0.50%  BPR074(CSV0A030936)  500.474 -352/109  0.20%

BPR026(CSV0A034205)  519.518 -361/107  1.50%  BPR075(CSV0A045095)  540.624 -352/111  1.30%

BPR027(CSV0C001841)  504.473 -361/103  -0.20%  BPR076(CSV0A053019)  543.646 -352/118  1.10%
BPR028(CSV0A009104)  519.562 -360/109 -0.60%  BPR077(CSV0A055635)  468.56 -352/112  4.80%

BPR029(CSV0A043963)  508.516 -360/101 0.90%  BPR078(CSV0A043498)  538.421 -351/116  1.30%

BPR030(CSV0B003578)  494.551 -360/102 0.30%  BPR079(CSV0A015737)  524.375 -350/97  0.70%

BPR031(CSV0B028719)  436.446 -360/92  1.70%  BPR080(CSV0A027968)  479.512 -350/105  41.60%

BPR032(CSV0C002695)  531.619 -360/117 2.50%  BPR081(CSV0A034159)  390.4 -350/89  22.80%

BPR033(CSV0C002703)  544.409 -360/96  3.10%  BPR082(CSV0A034596)  516.558 -350/104  1.00%

BPR034(CSV0C012196)  506.604 -360/105 0.90%  BPR083(CSV0A034609)  482.587 -350/104  3.00%
| Code       | Compound  | 
|------------|-----------|
| BPR035(CSV0A038787) | ![Compound](image1.png) | 544.743 -359/114 1.50% BPR036(CSV0A053548) | ![Compound](image2.png) | 538.605 -359/114 4.20% BPR037(CSV0A062559) | ![Compound](image3.png) | 516.514 -359/114 4.90% BPR038(CSV0A029878) | ![Compound](image4.png) | 507.871 -358/98 1.70% BPR039(CSV0A043865) | ![Compound](image5.png) | 517.425 -358/99 1.70% BPR040(CSV0A059009) | ![Compound](image6.png) | 464.482 -358/102 2.40% BPR041(CSV0A022916) | ![Compound](image7.png) | 526.51 -357/104 0.70% BPR084(CSV0A043957) | ![Compound](image8.png) | 456.889 -350/96 2.90% BPR085(CSV0A056184) | ![Compound](image9.png) | 500.575 -350/106 1.00% BPR086(CSV0A058884) | ![Compound](image10.png) | 525.571 -350/108 0.90% BPR087(CSV0A000015) | ![Compound](image11.png) | 508.71 -349/101 -0.60% BPR088(CSV0A019152) | ![Compound](image12.png) | 544.664 -349/118 0.10% BPR089(CSV0A024460) | ![Compound](image13.png) | 515.533 -349/104 0.90% BPR090(CSV0A028568) | ![Compound](image14.png) | 515.598 -349/111 -0.20% |
| Assay control compound | IC₅₀ (µM) | 2 µM | 0.1 µM |
|------------------------|----------|------|-------|
| Sorafenib              | 0.102    | 82.10% | 45.20% |
### Table S2. Comparison of enrichment factor (EF) of our study with 5 recently reported virtual screening experiments

| Screen method | Our model | J Chem Inf Model. 2011, (51): 755. | J Chem Inf Model. 2013 (53),809 | ChemMedChem. 2014, (9): 953. | J Mol Graph Model. 2014, (53): 31 | Bioorg Med Chem Lett. 2014 (5): 1261. |
|---------------|-----------|-----------------------------------|---------------------------------|-------------------------------|-----------------------------------|-----------------------------------|
| Total number of active molecules identified (hits identified) | 2 | 3 | 1 | 7 | 5 | 2 |
| Total number of molecules selected (no. selected) | 97 | 25 | 24 | 151 | 357 | 50 |
| Total number of active molecules (hits total) | 2 | 3 | 1 | 7 | 5 | 2 |
| Total number of molecules in database (no. Total) | 125000 | 1125 | 77931 | 125000 | 200000 | 4000 |
| Hit IC$_{50}$ range | 2.3-10.7 $\mu$M | 2-58 $\mu$M | 14.4 $\mu$M | 1.29-11.71 $\mu$M | 0.83-1.12 $\mu$M | 4.05-5.54 $\mu$M |
| Hit rate$^a$ | 2.06 | 12 | 4.7 | 4.64 | 1.40 | 4 |
| Enrichment factor (EF)$^b$ | 1288.66 | 45.00 | 3247.13 | 827.81 | 560.22 | 80.00 |

$^a$Hit rate = (hits identified / no. selected); $^b$EF = (hits identified / no. selected) / (hits total / no. total)
| Docking parameters                                      | Value                          |
|--------------------------------------------------------|-------------------------------|
| calculate_rmsd                                         | yes                           |
| use_rmsd_reference_mol                                  | yes                           |
| orient_ligand                                          | yes                           |
| automated_matching                                     | yes                           |
| max_orientations                                       | 3333                          |
| critical_points                                        | yes                           |
| chemical_matching                                      | yes                           |
| use_ligand_spheres                                     | no                            |
| flexible_ligand                                        | yes                           |
| min_anchor_size                                        | 50                            |
| pruning_use_clustering                                  | yes                           |
| pruning_max_orients                                    | 10                            |
| pruning_clustering_cutoff                              | 10                            |
| use_internal_energy                                     | yes                           |
| internal_energy_att_exp                                | 6                             |
| internal_energy_rep_exp                                | 12                            |
| internal_energy_dielectric                             | 4                             |
| use_clash_overlap                                      | yes                           |
| clash_overlap                                          | 0.3                           |
| bump_filter                                            | yes                           |
| max_bumps_anchor                                       | 12                            |
| max_bumps_growth                                       | 8                             |
| score_molecules                                        | yes                           |
| contact_score_primary                                  | yes                           |
| contact_score_secondary                                | no                            |
| contact_score_cutoff_distance                          | 4.5                           |
| contact_score_clash_overlap                            | 0.5                           |
| contact_score_clash_penalty                            | 10                            |
| grid_score_secondary                                   | yes                           |
| grid_score_rep_rad_scale                               | 1                             |
| grid_score_vdw_scale                                   | 1                             |
| grid_score_es_scale                                    | 1                             |

