Structure-based Virtual Screening and Molecular Dynamics Simulations For Detecting Novel Candidates as FGFR1 Inhibitors

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Figure S5. Atom names for Asp641 (FGFR1) and compound G9.

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Table S1. Docking scores and the suggested docking poses for compounds G1-G22 inside FGFR1 (Pdb id: 3rhx), using Goldscore.

| Compound  | FGFR1 | 3D Interaction of suggested binging poses of hit |
|-----------|-------|-----------------------------------------------|
| G1 (ID:316803) | 83.7928 | ![Image](image1) |
| G2 (ID:102217)  | 83.8963 | ![Image](image2) |
| G3 (ID:1572237) | 82.3749 | ![Image](image3) |
| ID   | Code | Score |
|------|------|-------|
| 1253838 | G4   | 83.0826 |
| 1084268 | G5   | 82.9231 |
| 335949  | G6   | 84.4309 |
| 230615  | G7   | 82.2494 |
G8 (ID:219085)  80.4187

G9 (ID:1088295)  82.8773

G10 (ID:151959)  82.0848

G11 (ID:218049)  81.3193
G20 (ID:392998)  81.9303

G21 (ID:359316)  80.6725

G22  (ID:1596766)  82.2491
Table S2. Energy decomposition analysis energies of compound G9 inside FGFR1 with standard errors of the mean.

| Residues | Van der Waals Energy (kcal/mol) | Electrostatic Energy (kcal/mol) | Polar solvation energy (kcal/mol) | Non-polar solvation energy (kcal/mol) | Total Energy (kcal/mol) |
|----------|---------------------------------|---------------------------------|----------------------------------|--------------------------------------|------------------------|
| Leu484   | -2.071 +/- 0.523                | 0.238 +/- 0.801                 | -0.433 +/- 0.531                 | -1.700 +/- 0.349                     | -3.966 +/- 0.904       |
| Val492   | -1.616 +/- 0.399                | 0.394 +/- 0.131                 | -0.753 +/- 0.156                 | -1.150 +/- 0.246                     | -3.125 +/- 0.665       |
| Lys514   | -2.158 +/- 0.572                | 19.081 +/- 2.680                | -25.715 +/- 1.595                | -2.078 +/- 0.143                     | -10.870 +/- 1.805      |
| Glu531   | -1.936 +/- 0.490                | -31.731 +/- 4.051               | 31.332 +/- 1.933                 | -1.247 +/- 0.162                     | -3.581 +/- 2.476       |
| Met535   | -1.677 +/- 0.329                | -1.340 +/- 0.706                | -0.056 +/- 0.404                 | -1.273 +/- 0.166                     | -4.347 +/- 0.651       |
| Ile545   | -0.748 +/- 0.146                | -1.470 +/- 0.308                | 1.413 +/- 0.297                  | -0.373 +/- 0.113                     | -1.177 +/- 0.268       |
| Val559   | -0.664 +/- 0.192                | -0.226 +/- 0.330                | 0.168 +/- 0.226                  | -0.587 +/- 0.075                     | -1.308 +/- 0.226       |
| Val561   | -1.346 +/- 0.291                | 0.740 +/- 0.141                 | -0.822 +/- 0.132                 | -1.229 +/- 0.111                     | -2.657 +/- 0.313       |
| Tyr563   | -0.830 +/- 0.322                | -0.388 +/- 0.247                | 0.196 +/- 0.212                  | -0.602 +/- 0.179                     | -1.625 +/- 0.505       |
| Ala564   | -0.661 +/- 0.293                | -0.330 +/- 0.446                | 0.231 +/- 0.253                  | -0.495 +/- 0.157                     | -1.256 +/- 0.523       |
| Gly567   | -0.617 +/- 0.225                | 0.348 +/- 0.263                 | -0.491 +/- 0.207                 | -0.530 +/- 0.157                     | -1.289 +/- 0.431       |
| Asn568   | -0.771 +/- 0.344                | 0.043 +/- 1.160                 | -0.877 +/- 0.373                 | -0.628 +/- 0.294                     | -2.233 +/- 1.467       |
| Arg627   | -0.519 +/- 0.133                | 11.900 +/- 0.743                | -12.192 +/- 0.738                | -0.322 +/- 0.088                     | -1.132 +/- 0.255       |
| Asn628   | -0.886 +/- 0.203                | 0.574 +/- 0.464                 | -0.347 +/- 0.426                 | -0.586 +/- 0.123                     | -1.245 +/- 0.280       |
| Leu630   | -1.814 +/- 0.331                | 0.061 +/- 0.138                 | -0.263 +/- 0.102                 | -1.413 +/- 0.176                     | -3.429 +/- 0.454       |
| Asp641   | -3.303 +/- 0.733                | -38.026 +/- 3.130               | 31.971 +/- 1.265                 | -2.283 +/- 0.159                     | -11.641 +/- 2.148      |
| Phe642   | -2.073 +/- 0.438                | -4.388 +/- 0.945                | 2.557 +/- 0.661                  | -1.459 +/- 0.225                     | -5.363 +/- 1.059       |
Table S3. Energy decomposition analysis energies of compound G10 inside FGFR1 with standard errors of the mean.

| Residues | Van der Waals Energy (kcal/mol) | Electrostatic Energy (kcal/mol) | Polar solvation energy (kcal/mol) | Non-polar solvation energy (kcal/mol) | Total Energy (kcal/mol) |
|----------|---------------------------------|---------------------------------|-----------------------------------|----------------------------------------|-------------------------|
| Leu484   | -2.358 +/- 0.396                | -0.670 +/- 0.324                | 0.174 +/- 0.206                   | -1.692 +/- 0.229                      | -4.546 +/- 0.664        |
| Phe489   | -2.149 +/- 0.419                | -0.331 +/- 0.203                | 0.352 +/- 0.166                   | -1.657 +/- 0.290                      | -3.785 +/- 0.712        |
| Val492   | -2.181 +/- 0.235                | -0.247 +/- 0.092                | -0.265 +/- 0.084                  | -1.425 +/- 0.153                      | -4.118 +/- 0.384        |
| Ala512   | -0.737 +/- 0.126                | -0.292 +/- 0.106                | 0.142 +/- 0.078                   | -0.452 +/- 0.084                      | -1.339 +/- 0.198        |
| Lys514   | -1.466 +/- 0.259                | 1.155 +/- 0.757                 | -2.035 +/- 0.473                  | -1.117 +/- 0.168                      | -3.462 +/- 0.777        |
| Glu531   | -1.120 +/- 0.327                | -2.344 +/- 0.709                | 2.343 +/- 0.496                   | -0.802 +/- 0.200                      | -1.923 +/- 0.552        |
| Met535   | -1.002 +/- 0.310                | -0.007 +/- 0.117                | -0.011 +/- 0.079                  | -1.027 +/- 0.174                      | -2.047 +/- 0.395        |
| Ile545   | -1.739 +/- 0.295                | -0.100 +/- 0.081                | -0.020 +/- 0.076                  | -1.414 +/- 0.170                      | -3.273 +/- 0.395        |
| Val561   | -0.931 +/- 0.189                | 0.468 +/- 0.087                 | -0.411 +/- 0.062                  | -0.819 +/- 0.162                      | -1.693 +/- 0.324        |
| Glu562   | -0.180 +/- 0.409                | -2.468 +/- 0.692                | 0.364 +/- 0.371                   | -0.235 +/- 0.026                      | -2.520 +/- 0.305        |
| Tyr563   | -1.236 +/- 0.238                | -2.450 +/- 0.329                | 0.455 +/- 0.158                   | -0.710 +/- 0.137                      | -3.941 +/- 0.460        |
| Ala564   | -0.991 +/- 0.475                | -2.092 +/- 0.574                | 0.049 +/- 0.168                   | -0.868 +/- 0.114                      | -3.903 +/- 0.537        |
| Gly567   | -0.774 +/- 0.277                | -0.458 +/- 0.291                | -0.205 +/- 0.165                  | -0.664 +/- 0.166                      | -2.101 +/- 0.575        |
| Asn568   | -0.962 +/- 0.377                | -0.670 +/- 1.133                | -0.436 +/- 0.324                  | -0.802 +/- 0.304                      | -2.869 +/- 1.460        |
| Arg627   | -0.264 +/- 0.545                | -1.124 +/- 1.647                | -0.066 +/- 0.708                  | -0.435 +/- 0.199                      | -1.889 +/- 0.999        |
| Asn628   | -0.627 +/- 0.344                | -0.949 +/- 0.971                | 0.341 +/- 0.446                   | -0.409 +/- 0.201                      | -1.643 +/- 0.872        |
| Leu630   | -2.580 +/- 0.419                | -0.374 +/- 0.104                | 0.063 +/- 0.093                   | -1.689 +/- 0.223                      | -4.580 +/- 0.608        |
| Ala640   | -1.720 +/- 0.294                | -1.167 +/- 0.249                | -0.493 +/- 0.145                  | -1.044 +/- 0.191                      | -4.424 +/- 0.727        |
| Asp641   | -2.178 +/- 0.421                | -3.111 +/- 0.877                | 1.898 +/- 0.765                   | -1.542 +/- 0.176                      | -4.934 +/- 0.665        |
| Phe642   | -1.581 +/- 0.389                | -0.075 +/- 0.103                | 0.216 +/- 0.077                   | -1.204 +/- 0.263                      | -2.645 +/- 0.588        |
Database Preparation and Docking Validation Protocol

The studied database was taken from ChEMBL KinaseSARfari database (ftp://ftp.ebi.ac.uk/pub/databases/chembl/KinaseSARfari/releases/5.01). The compounds, which have a molecular weight under 250 and over 650 and/or bearing the groups are able to form covalent bonding, were eliminated. The reason of a threshold value of molecular weight was selected between 250 and 650 is the FDA approved kinase inhibitors have molecular weight with a range of 306 (ruxolitinib) to 615 (trametinib).

Before the docking study of the studied compounds, in order to validate docking methods, erdafitinib as well-known FDA approved selective FGFR inhibitor, and the FDA approved kinase inhibitors as lenvatinib, and phase 3 stage compound as dovitinib, and selective FGFR inhibitor as AZD4547 were exposed to dock inside the ATP binding site of FGFR1 (PDB id: 3rhx). The best ranked docking poses of these inhibitors inside the prepared structure were superimposed with the crystal structures of inhibitor-FGFR1 complexes (PDB id: 5ew8, 5zv2, 5a46, 4v05). For instance, the superimpositions of the crystal structure of erdafinitinib-FGFR1 complex (PDB id: 5ew8.pdb) and AZD4547-FGFR1 complex (PDB id: 4v05.pdb) with the best ranked docking poses of erdafinitinib and AZD4547 inside the prepared structure of FGFR1 (3RHX) were figured in Figure S10 and Figure S11, respectively. The best ranked docking poses and the scoring values of these inhibitors inside prepared FGFR were figured and reported in Table S4. The calculated docking scores of FGFR inhibitors inside FGFR1 were observed between value as 63.97 and 85.75. Therefore, a threshold value of GoldScore fitness as 70 for virtual screening and 80 for docking study, respectively, were applied, the compounds which have GoldScore fitness value lower than these values, were gradually eliminated.

Determining MM-GBSA free binding energy threshold value

Regarding the chosen as a threshold value of MM-GBSA as -50 Kcal/mole, it was decided after the calculated MM-GBSA binding energy values for erdafitinib and AZD4547 inside FGFR1 from 10 ns MDS. First ranked docking poses of these inhibitors inside FGFR1 were used as initial structures of generated MDS. Free binding energies were calculated as -56.5209 Kcal/mole for erdafitinib in FGFR1 and -46.8109 Kcal/mole for AZD4547 in FGFR1. MM-GBSA calculation results for these inhibitors were given in Table S5. Therefore, the threshold value of MM-GBSA as -50 Kcal/mole was determined because of the fact that between these two calculated values.
Figure S10. Superimposition of the crystal structure of erdafitinib-FGFR1 complex (Pdb id: 5ew8.pdb) with the best ranked docking pose of erdafitinib inside the prepared structure of FGFR1 from 3rhx.pdb. Cyan and orange sticks represent erdafitinib in the crystal structure and the prepared structure of FGFR1, respectively. The active site residues are named using three letters code and represented as magenta sticks in the crystal structure of FGFR1 (Pdb id: 5ew8.pdb) and as green sticks in the prepared structure of FGFR1. The backbone structure of the crystal structure of FGFR1 (Pdb id: 5ew8.pdb) and the prepared structure of FGFR1 are represented as magenta and green ribbons, respectively. H bonds and CH–π interactions are represented by pale blue and pale green dashed lines, respectively. For clarity, all hydrogen atoms have been removed.
**Figure S11.** Superimposition of the crystal structure of AZD4547-FGFR1 complex (Pdb id: 4v05.pdb) with the best ranked docking pose of AZD4547 inside the prepared structure of FGFR1 from 3rhx.pdb. Yellow and cyan sticks represent AZD4547 in the crystal structure and the prepared structure of FGFR1, respectively. The active site residues are named using three letters code and represented as pink sticks in the crystal structure of FGFR1 (Pdb id: 4v05.pdb) and as green sticks in the prepared structure of FGFR1. The backbone structure of the crystal structure of FGFR1 (Pdb id: 4v05.pdb) and the prepared structure of FGFR1 are represented as pink and green ribbons, respectively. H bonds and CH–π interactions are represented by pale blue and pale green dashed lines, respectively. For clarity, all hydrogen atoms have been removed.
Table S4. Docking scores and first ranking poses for FGFR inhibitors inside FGFR1 (Pdb id: 3rhx), using Goldscore.

| Compounds   | GoldScore | 3D Interaction of first ranking poses of hit |
|-------------|-----------|--------------------------------------------|
| Erdafinitinib | 85.75 (1) | ![3D Interaction image](image)             |
| Lenvatinib   | 66.11 (1) | ![3D Interaction image](image)             |
| Dovitinib    | 63.97 (1) | ![3D Interaction image](image)             |
Table S5. MM-GBSA free binding energies (DELTA TOTAL) of the inhibitors in FGFR1.

| Compound          | Erdafitinib | AZD4547  |
|-------------------|-------------|----------|
| VDWAALS (kcal/mol)| -58.1595 ± 0.3354 | -53.7440 ± 0.3101 |
| EEL (kcal/mol)    | -104.4298 ± 1.3031 | 4.2426 ± 1.0395  |
| EGB (kcal/mol)    | 113.5529 ± 1.1392 | 9.1269 ± 1.0505  |
| ESURF (kcal/mol)  | -7.4845 ± 0.0283  | -6.4363 ± 0.0263 |
| DELTA G gas (kcal/mol) | -162.5893 ± 1.2278 | -49.5015 ± 1.1403 |
| DELTA G solv (kcal/mol) | 106.0684 ± 1.1370 | 2.6906 ± 1.0476  |
| DELTA TOTAL (kcal/mol) | -56.5209 ± 0.3255 | -46.8109 ± 0.4002 |
Table S6. Calculated molecular descriptors for compound G1-G22 using MOE 2016

| Compounds  | logP(o/w) | H Bond Acceptor | H Bond Donor | Molecular Weight |
|------------|-----------|-----------------|--------------|------------------|
| G1 (ID:316803) | 0.4481    | 4               | 3            | 547.449          |
| G2 (ID:102217) | 1.571     | 3               | 1            | 551.219          |
| G3 (ID:1572237) | 4.724     | 5               | 1            | 450.543          |
| G4 (ID:1253838) | 5.0108    | 4               | 2            | 505.884          |
| G5 (ID:1084268) | -0.0559   | 4               | 2            | 490.397          |
| G6 (ID:335949) | 3.239     | 6               | 2            | 553.643          |
| G7 (ID:230615) | 5.6755    | 6               | 1            | 482.632          |
| G8 (ID:219085) | 3.545     | 5               | 2            | 554.478          |
| G9 (ID:1088295) | 6.3828    | 4               | 1            | 529.566          |
| G10 (ID:151959) | 6.536     | 3               | 2            | 454.57           |
| G11 (ID:218049) | 4.3205    | 6               | 2            | 488.616          |
| G12 (ID:461758) | 3.711     | 5               | 1            | 514.614          |
| G13 (ID:150405) | 6.902     | 2               | 2            | 456.61           |
| G14 (ID:367127) | 4.724     | 9               | 7            | 569.552          |
| G15 (ID:518166) | 3.9551    | 5               | 1            | 510.538          |
| G16 (ID:593813) | 4.7201    | 7               | 6            | 529.004          |
| G17 (ID:605410) | 3.3441    | 8               | 6            | 519.5690         |
| G18 (ID:514509) | 6.528     | 4               | 2            | 508.581          |
| G19 (ID:358843) | 4.915     | 3               | 2            | 472.609          |
| G20 (ID:392998) | 4.0655    | 4               | 1            | 551.611          |
| G21 (ID:359316) | 5.922     | 3               | 2            | 440.543          |
| G22 (ID:1596766) | 3.882     | 5               | 2            | 434.52           |
Table S7. ATP binding site residues of FGFR1 (Pdb id:3rhx)

| ATP binding site residues                  |
|-------------------------------------------|
| LEU484, GLY485, PHE489, VAL492, ALA512, VAL513, LYS514, GLU531, MET535, ILE545, VAL559, ILE560, VAL561, GLU562, TYR563, ALA564, SER565, LYS566, GLY567, ASN568, LEU569, ARG570, GLU571, ARG627, ASN628, VAL629, LEU630, ILE639, ALA640, ASP641, PHE642, GLY643, LEU644 |