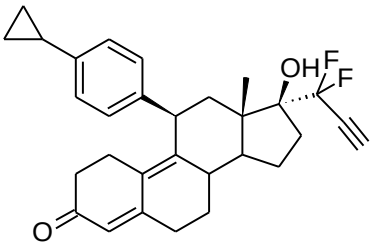
Supplementary Materials

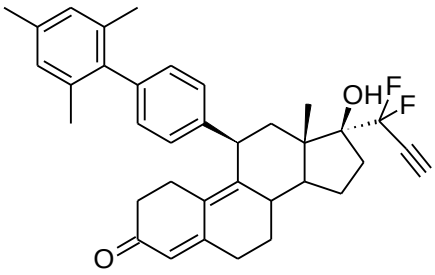
Synthesis and characterization of EC330 and EC359

Synthesis and characterization of EC330 and EC359 was done using the protocol described in the patent WO 2016/154203A1.



EC330

EC330 was isolated as a white crystalline powder with a melting point of 187-188ºC.
The spectroscopic data are given below.
UV(nm): 200, 230, 303.
FT IR (ATR, cm^{-1}): 3284, 3217, 2947, 2124, 1645, 1604.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.61 (s, 3H, H-18), 0.65 (m, 2H, cyclopropyl), 0.93(m, 2H, cyclopropyl), 2.90 (t, *J*= 5.4 Hz, 1H, acetylenic hydrogen), 4.3 (d, *J*= 7.2 Hz, 1H, H-11), 5.76 (s, 1 H, H-4), 6.9 (d, *J*= 8.4 Hz, 2H, H-Ar), 7.06 (d, *J*= 8.4 Hz, 2H, H-Ar).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.17 (cyclopropyl), 9.26 (cyclopropyl), 14.89 (C-18), 16.61 (cyclopropyl), 86.17 (t, *J*= 24 Hz, ($\underline{C}F_2CC$)), 123.01 (C-4), 125.76 (C-Ar), 126.67 (C-Ar), 129.56 (C-10), 141.18 (C-Ar), 141.44 (C-Ar), 145.05 (C-9), 156.34 (C-5), 199.46 (C-3).



EC359

EC359 was isolated as a white crystalline powder with a melting point of 255-256ºC.
The spectroscopic data are given below.
UV (nm): 300.
FT IR (ATR, cm^{-1}): 3305, 2947, 2873, 2130, 1638.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.65 (s, 3H, H-18), 1.96 (s, 6H, Ar-C$\underline{H}_3$), 2.32 (s, 3H, Ar-C$\underline{H}_3$), 2.9 (t, *J*= 5.4 Hz, 1H, acetylenic hydrogen), 4.52 (d, *J*= 6.9 Hz, 1H, H-11), 5.78 (s, 1H, H-4), 6.93 (s, 2H, H-Ar), 7.05 (d, *J*= 8.4 Hz, 2H, H-Ar), 7.23 (d, *J*= 8.1 Hz, 2H, H-Ar).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.40 (C-18), 20.61 (Ar-$\underline{C}H_3$), 25.84 (Ar-$\underline{C}H_3$), 27.69 (Ar-$\underline{C}H_3$), 85.92 (t, *J*= 23 Hz, ($\underline{C}F_2CC$)), 123.08 (C-4), 126.87 (C-Ar), 128.0 (C-Ar), 129.43 (C-Ar), 129.70 (C-10), 135.99 (C-Ar), 136.54 (C-Ar), 138.53 (C-Ar), 138.61 (C-Ar), 142.51 (C-Ar), 145.04 (C-9), 156.45 (C-5), 199.52 (C-3).

SPR studies

Confirmation of functionality of LIF/LIFR interaction (Supplementary Fig. S1A). 5 μg/mL LIF protein was immobilized on a COOHV sensor chip to 2,000 RU in PBST buffer (pH 7.4). LIFR was analyzed at serial doubling concentrations maximized at 2 μM. The ligand bound to the receptor in a dose dependent manner. The sensorgram was fit based on 2:2 Langmuir model simulation (orange color) and used to derive kinetic parameters of the bimolecular interactions using QDAT software. Confirmation of LIFR-inhibitor interaction (Supplementary Fig. S1B) . 5 μg/mL LIFR protein was immobilized on a COOHV sensor chip to saturation (1500 RU) in PBST buffer (pH 7.4). LIF inhibitor was analyzed at serial doubling concentrations maximized at 50 μM. The inhibitor bound to the receptor in a dose dependent (various colors) manner. The sensorgram was fit to a simple 2:2 Langmuir model using QDAT to obtain K_D value. The simulation fit is shown in orange color (right panel).

Structural and sequence comparison of human and mouse LIFR

The primary sequences of human (P42702) and mouse (P42703) LIFR were collected from SwissProt and subjected to BLAST against the Protein Data Bank to retrieve the corresponding three dimensional structures. The corresponding X-ray crystallographic structures of hLIFR (PDB ID 3E0G) and hLIF-mLIFR complex (PDB ID 2Q7N) were superimposed using SuperPose version 1.0. The average RMSD between the Cα atoms is 2.2 Å (Supplementary Fig. S2). For the better visibility, the glycosylations were removed from the structures. The sequence alignment was also performed to identify the similar regions in both of these LIFRs. Pairwise alignment was done between the sequences and revealed an alignment score of 75 %. The regions where the structural information is available for both of these sequences were highlighted in the Supplementary Fig. S3. After superimposition of hLIFR to the hLIF-mLIFR complex, the mLIFR was removed and hLIF-LIFR complex was created. The glycosylations were also removed from the hLIFR structure to avoid possible clashes during the superimposition. Furthermore, the hLIF-LIFR was energy minimized extensively to avoid the clashes between the residues especially at the interface.

Energy minimization of hLIFR

The hLIF-LIFR complex was energy minimized using ‘macromodel’ module implemented in Schrödinger with a force field, OPLS3. The default parameters were used for the minimization using a method PRCG with maximum iterations 2500. The final energy of the minimized model was analyzed for the short contacts especially at the hLIF-LIFR interface. The energy calculated by prime for the minimized model was -95317 kcal/mol.

Protein and ligand preparation

From the energy minimized structure, the hLIFR was separated and prepared for molecular docking studies. The ‘Protein preparation wizard’ (Schrödinger) was used for the receptor preparation. During the preparation, the bond orders were assigned and disulfide bonds were treated properly. Hydrogen atoms were also added to the polar groups and the structure was optimized and minimized with a RMSD cutoff of 0.30 Å. The ligand EC359 was drawn using 2D sketch option and prepared for the docking studies using ligprep module (Schrödinger). Multiple states of the ligand were generated at a pH level 7.0±2.0. All the generated states were used for the docking studies.

Molecular docking and MM-GBSA calculations

Since there was no information on the binding sites, the whole receptor was probed for the potential binding sites using the sitemap option of Schrödinger. The sitemap defined different set of site points using site-finding algorithm by placing a 1 Å grid of possible grid points around the entire protein. Further, the distance from each grid point to nearby protein atoms was measured and compared to the van der Waals radius of each protein atom. Those grid points which can create most number of van der Waals contact with the receptor were selected as potential ligand binding sites. Consequently, 5 potential sites were identified and shown in Supplementary Fig. S4. The grid with a diameter 20 Å was prepared on each binding sites and molecular docking was carried out. Standard precision (SP) docking was used initially to place and score the ligands on to the respective binding sites. Flexible ligand sampling was carried out with default parameters using OPLS3 force field. Multiple ligand poses were generated for each site during the docking step. After docking, the corresponding ligand poses were analyzed and superimposed on to the hLIF-LIFR complex to evaluate the possible clashes. None of the ligand poses apart from site-3 were making any contacts with LIF since they are far away from the LIF binding regions. The distance to site-2 (18 Å) and site-3 (15 Å) were measured from a randomly selected residue 'L104' of LIF and found that these sites are close to the LIF binding regions. It was observed that majority of the poses at site-3 are creating close contacts with LIF (Supplementary Fig. S5). Moreover, the docking scores at site-3 are more favorable compared to the other sites. The docking scores at site-3 range from -5.8 to -2.8 kcal/mol. One of the best scored poses was selected and further IFD calculations were performed. Flexibility was applied to the residues at 6 Å away from the centroid of the ligand. In the IFD protocol, as an initial step, glide docking of the ligand was carried out using a softened potential. By default, 20 poses per ligand was generated. Then side-chain prediction followed by minimization for each protein-ligand complex was carried out within a radius of 6 Å away from the ligand. Later, the Glide re-docking of each protein-ligand complex structure has been carried out with a default energy cutoff value. A total 28 poses were generated as a part of IFD calculations. Finally, the output poses were subjected to MM-GBSA calculations. All the MM-GBSA calculations were performed using VSGB solvation model with OPLS3 force field. The MM-GBSA scores of the 28 poses were shown in Supplementary Fig. S6. It was observed that a significant structural deviation has been observed in the chosen model due to the ligand binding (Supplementary Fig. S7).

Molecular dynamics simulation

In order to estimate the stability of the ligand at the binding site, molecular dynamics (MD) simulation was carried out using Desmond. The best binding pose obtained after IFD studies was utilized for MD simulation. Prior to this, the hLIFR-EC359 complex was prepared by soaking into an orthorhombic box (volume 1156533 $Å^3$) consisting 34898 TIP3P water molecules and 3 Na+ ions. The force field used for the simulation was OPLS2005. The total time of the MD simulation was 25 ns and the intermediate structures (snapshots) were saved at each 25 ps. Later, these snapshots were superimposed to the original structure (reference structure) in order to deduce the average changes in displacement of atoms with respect to a reference frame. The RMSD of ligands and proteins were deduced and finally plotted against time. The average RMSD for protein was found to be 4.6 ± 1.2 Å at the same time the average RMSD found for the ligand is 3.0 ± 1.5 Å (Supplementary Fig. S8A). The snapshots were analyzed to understand the structural deviations. It was found that a majority of the structural changes occurred to the loops connecting the different domains (D1-D5). Hence, flexibility in the domain has been observed. At the same time the ligand is found to be stable in the binding position. Even though the RMSD is 3.0 Å, the two key hydrogen

bonds with T308 and T16 was found to be conserved during the simulation (Supplementary Fig. S8 B). At the same the hydrogen bond between the hydroxyl group and side chain of E340 was found not conserved during the simulation.