Supplementary file 1

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Unravelling benzazepines and aminopyrimidine as multi-target therapeutic repurposing drugs for EGFR V774M mutation in neuroglioma patients

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Figure S1: Showing the cancer study origin and percentage of genetic alteration found in EGFR in neuroglioma.

Figure S2: Number of mutations types in EGFR gene in neuroglioma.

Figure S3: Overlapping of patients groups and samples in alter and unaltered from Of EGFR.
Fig. S4: (A) Spatio-temporal expression Heat Map of EGFR of neuroglioma in different part of brain. (**B)** Violin plot from GTEx portal expression levels of EGFR in different regions of the brain.

Fig. S5: Multiple sequence alignment of shortlisted PDB ids of wild type EGFR.

Fig. S6: 3D modeling of altered EGFR. **(A)** Overlapped of altered EGFR (Magenta color) and unaltered EGFR (Green color) 3D structure. **(B)** Conformational change was observed in altered EGFR (Magenta color). **(C)** Altered EGFR with changed amino acids in V774M positions.

Fig. S7: Validation of modelled 3D structure of altered EGFR, **(A)** Overall quality of 3D structure. **(B)** Verified 3D structure of mutant EGFR. **(C)** Ramachandran plot of modelled 3D structure of mutant EGFR. **(D)** Average Z score of modelled 3D structure of altered EGFR.

Fig. S8: Histograph showing the top drug lead compounds with least minimum free energy **(A)** For unaltered EGFR **(B)** for altered EGFR in comparision with standard drugs.

Fig. S9: Graph deficit the residues interaction fraction with and number of bonding interactions of **(A-D)** unaltered (normal) EGFR and **(E-H)** altered (Mutant) EGFR.

Fig. S10: Principal component analysis (PCA) of unaltered EGFR complexes **(A)** ZINC000006716957 **(B)** ZINC000011679756 **(C)** ZINC000068153186 and **(D)** ZINC000012503187.

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Fig. S11: Principal component analysis (PCA) of altered EGFR complexes **(A)** ZINC000003978005 **(B)** ZINC000006716957 **(C)** ZINC000012503187 and **(D)** ZINC000068153186.

Legends to supplementary Tables

Table S1: Number of shortlisted PDB IDs of EGFR from RCSB database.

Table S2: Predicted ligand binding sites of altered and unaltered EGFR.

Table S3: Pharmacokinetic (ADMET) analysis of top shortlisted compounds.

Table S4: FDA-approved shortlisted drugs currently used to treat other diseases.

Supp Figure 1:

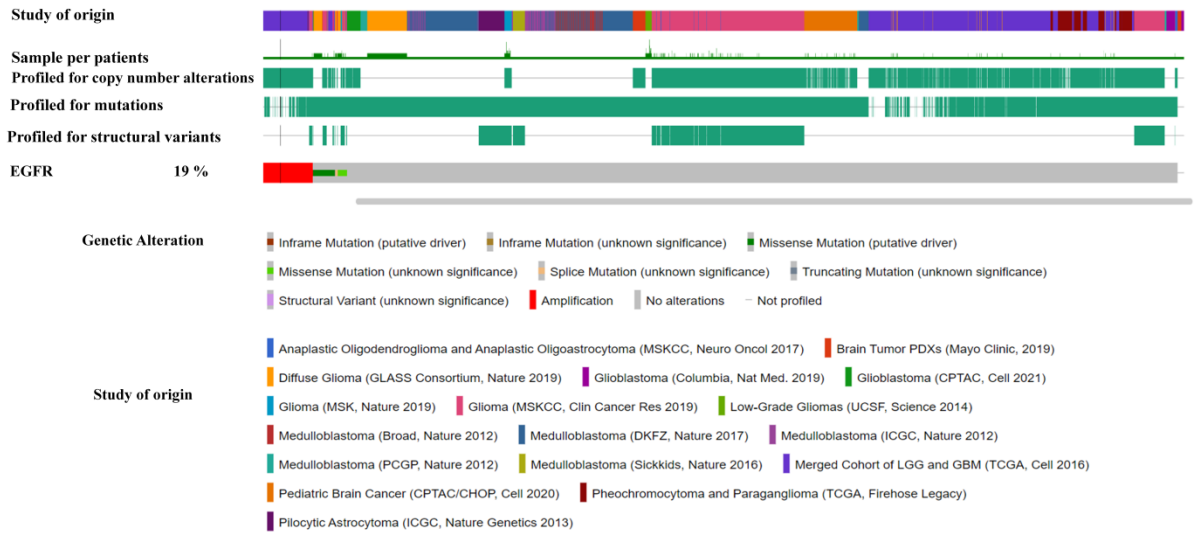

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Fig. S 2

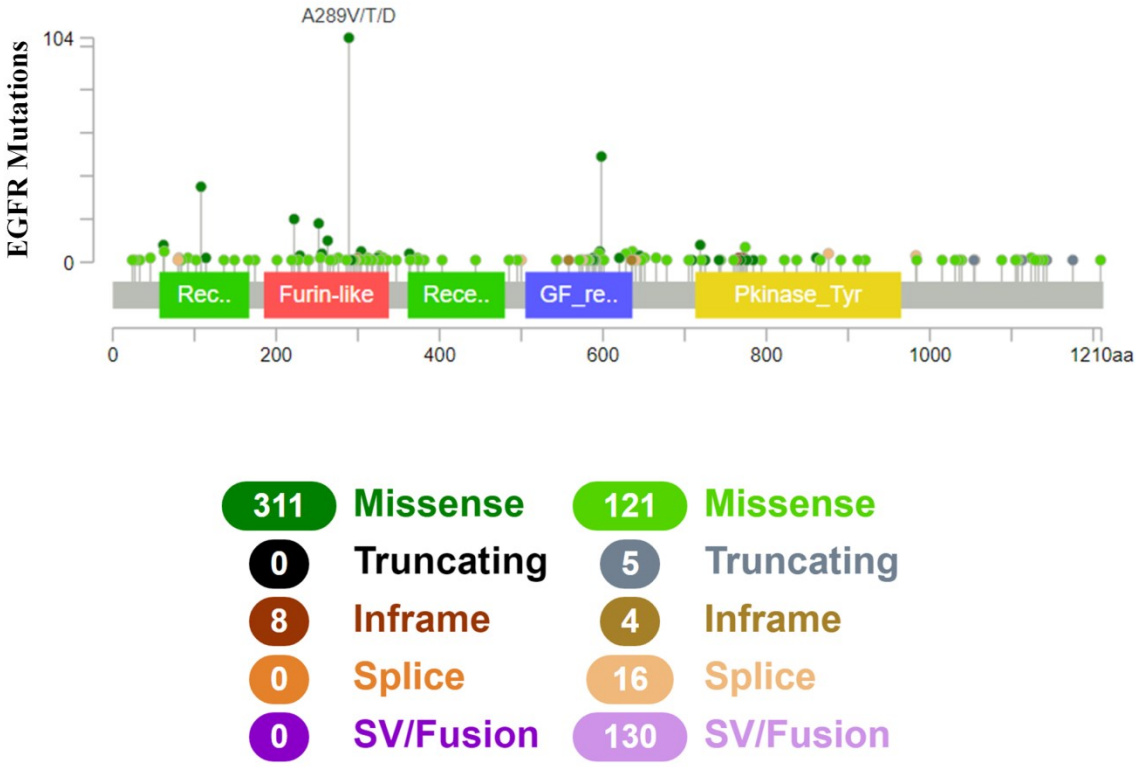

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Fig. S 3

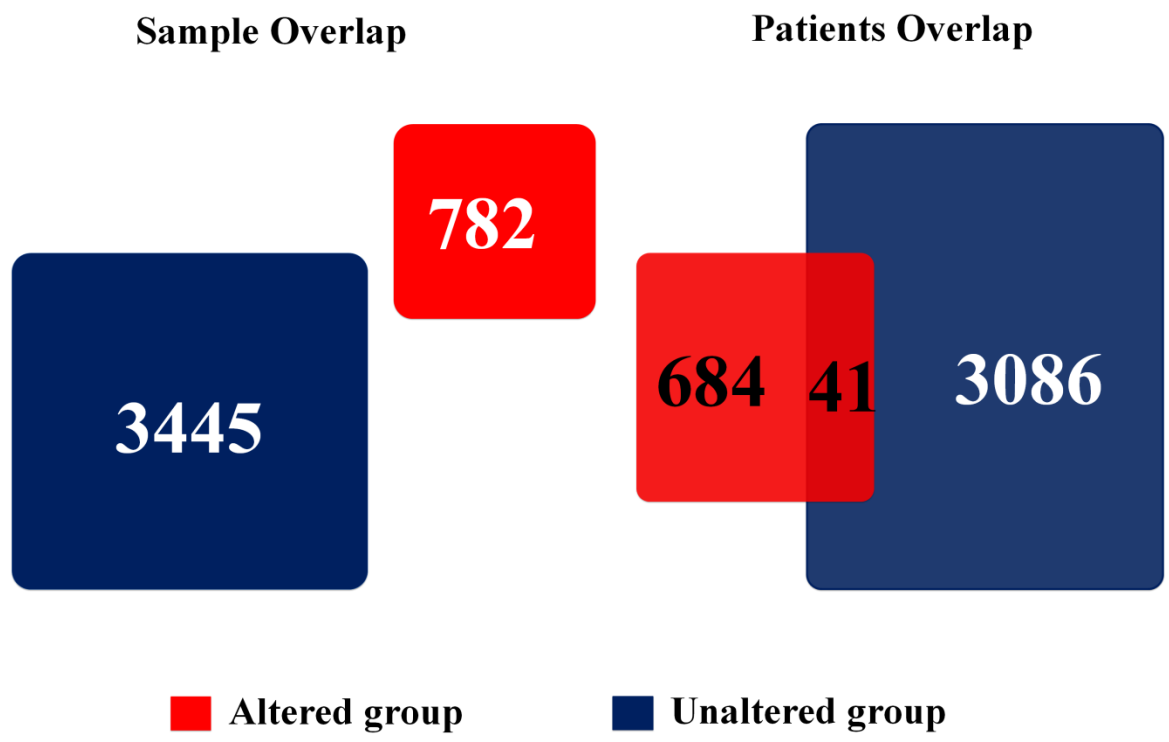

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Figure S4

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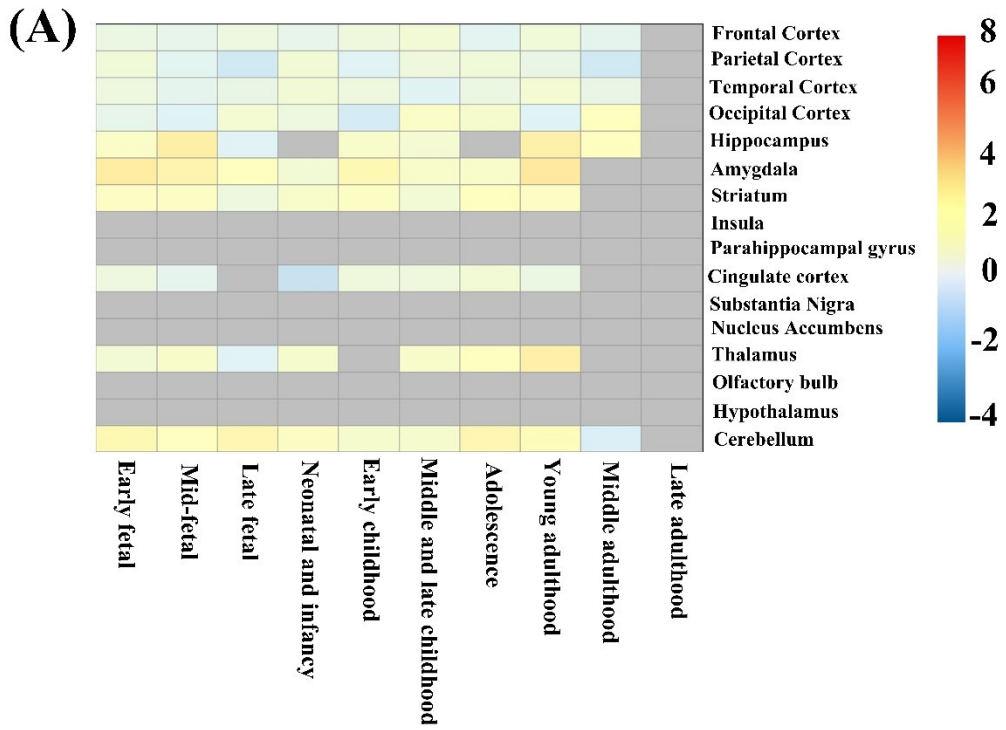

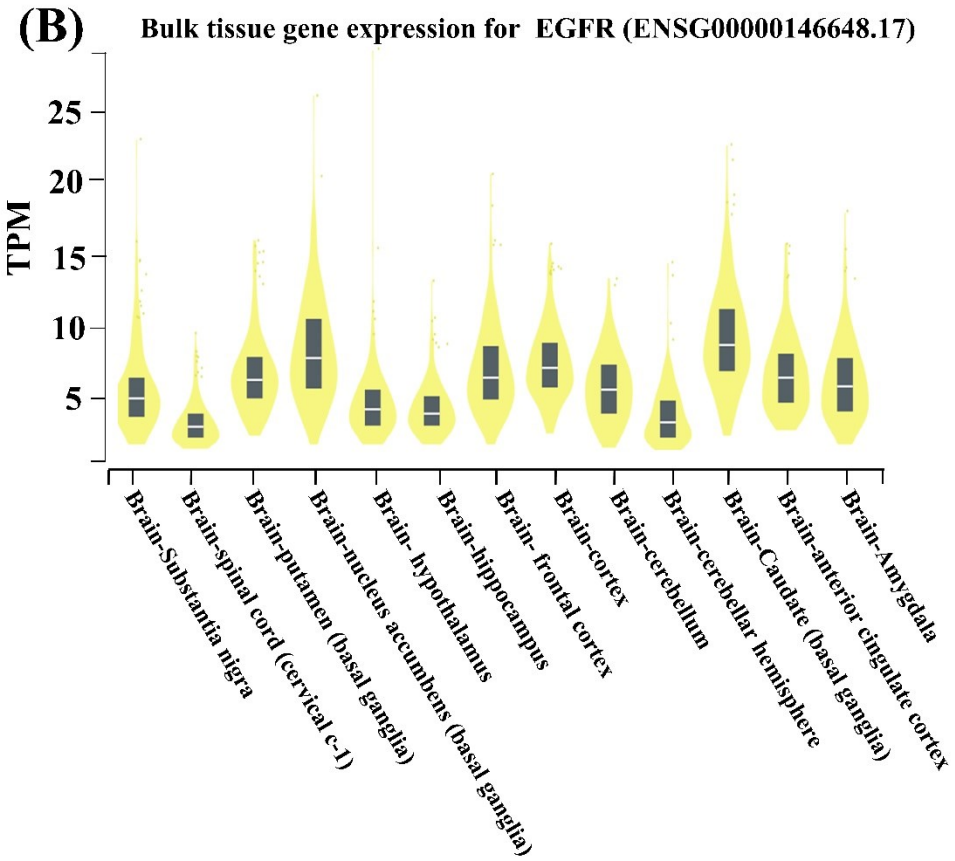

Fig. S5

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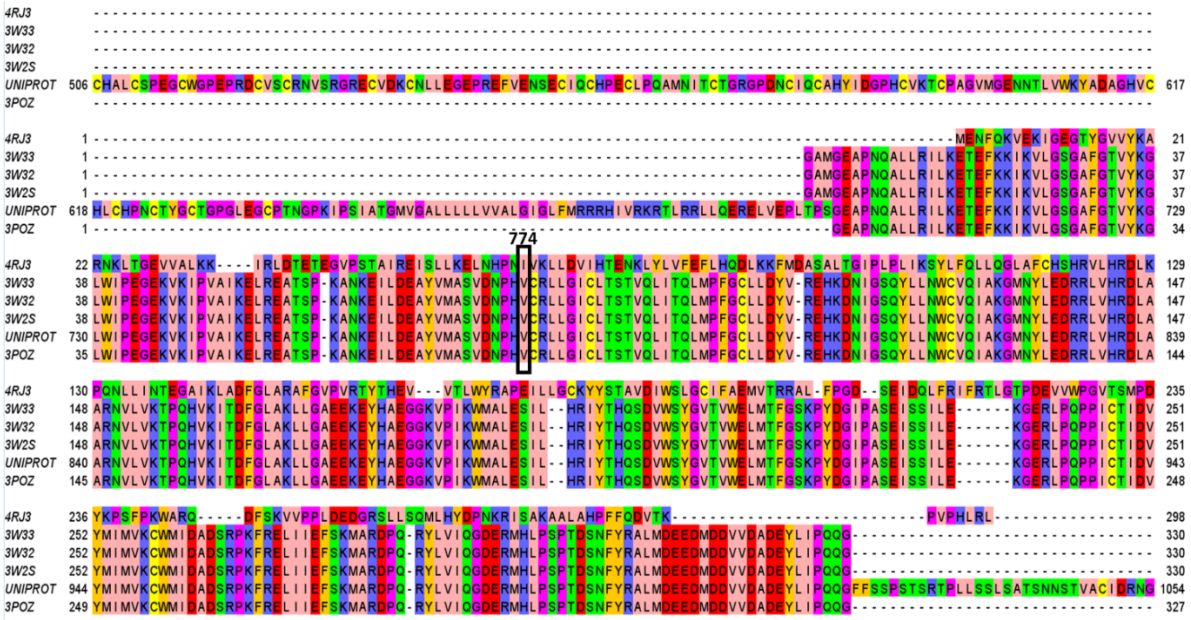

Figure S6

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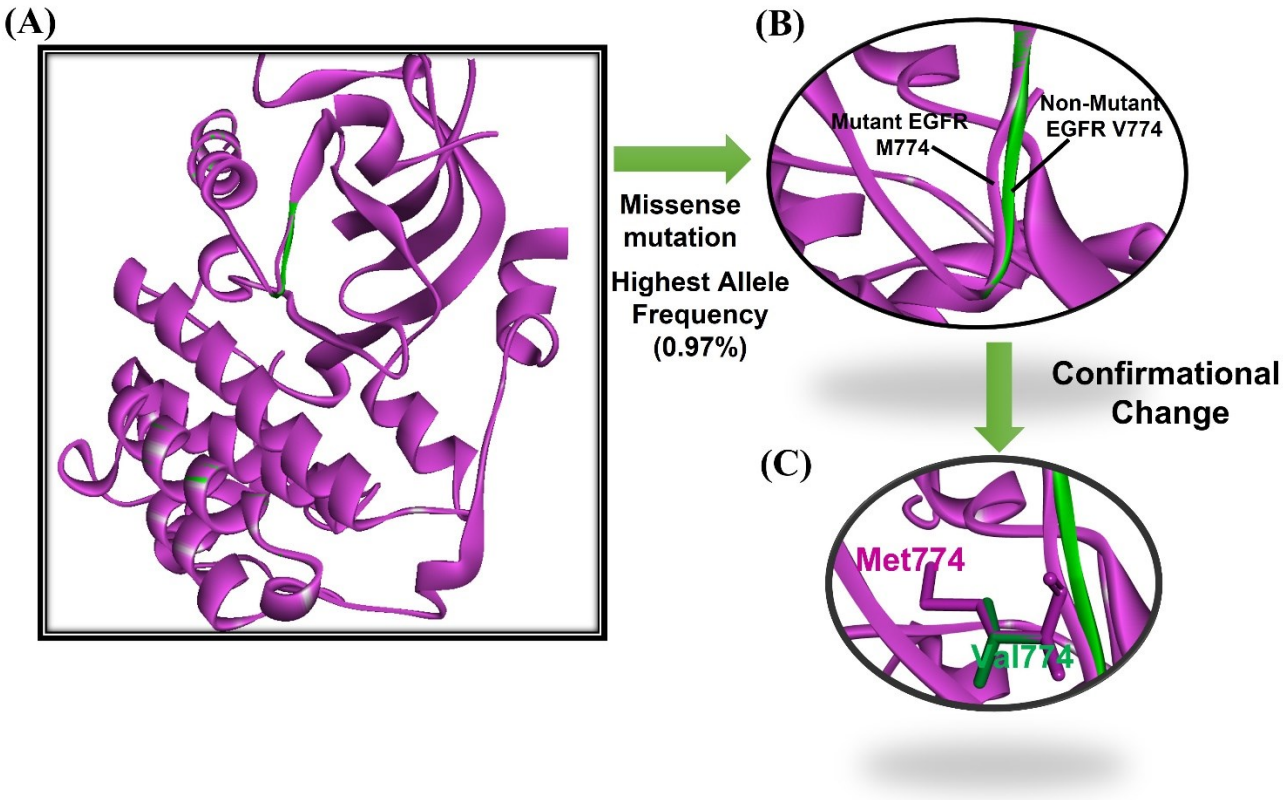

Figure S7

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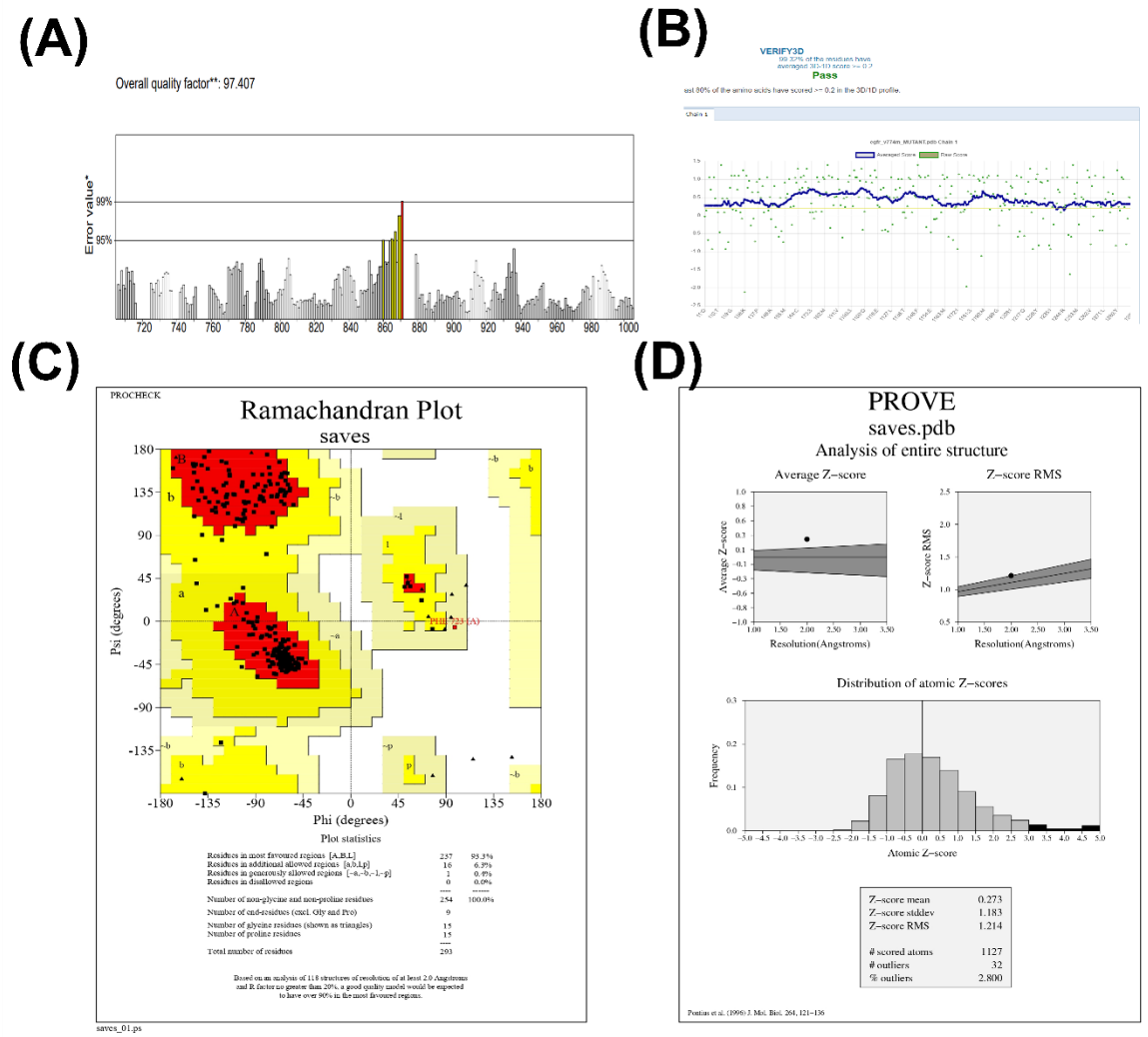

Figure S8

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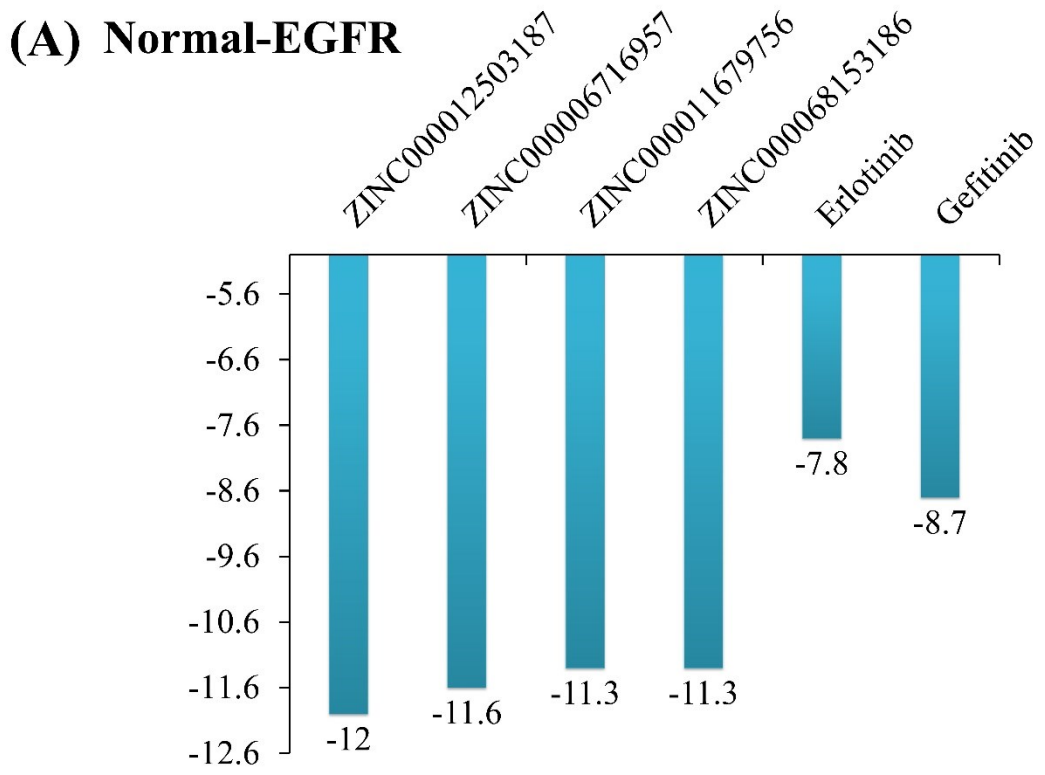

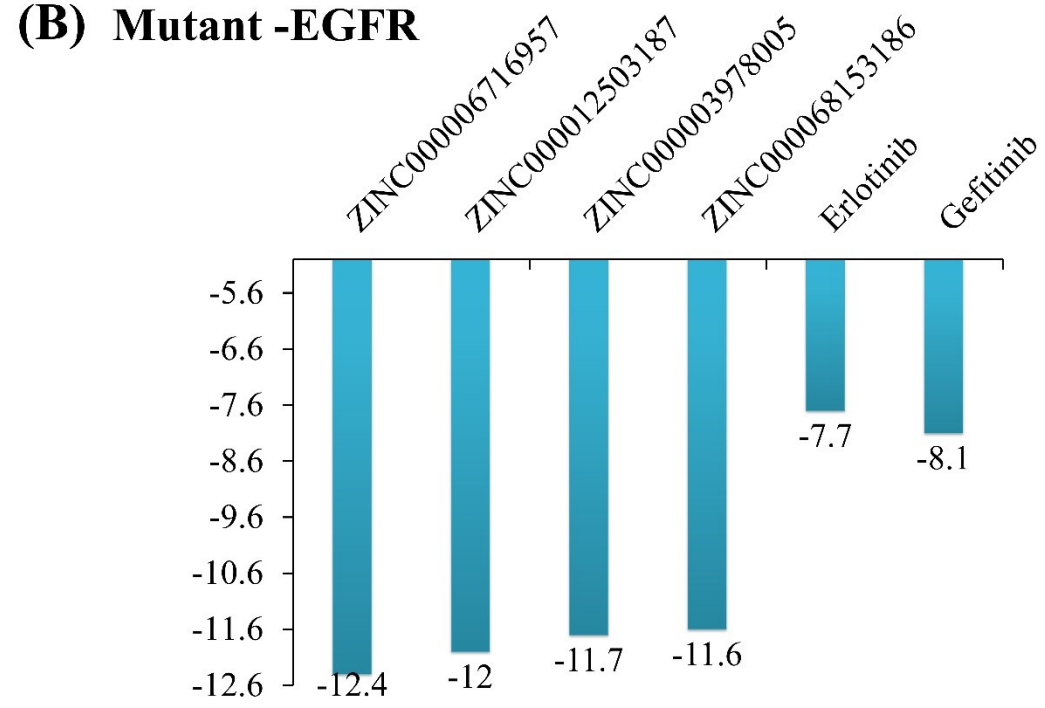

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Figure S9

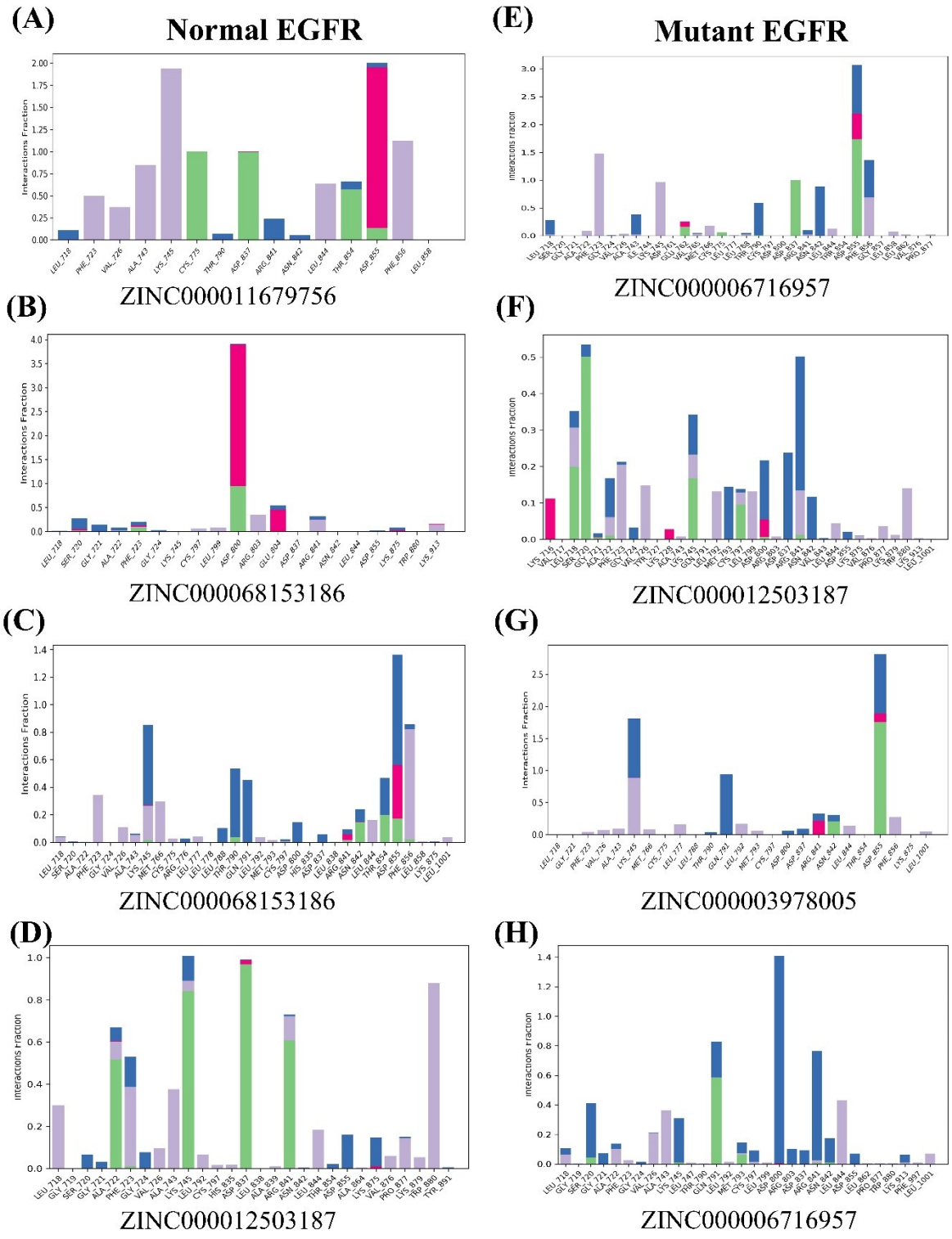

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Figure S10

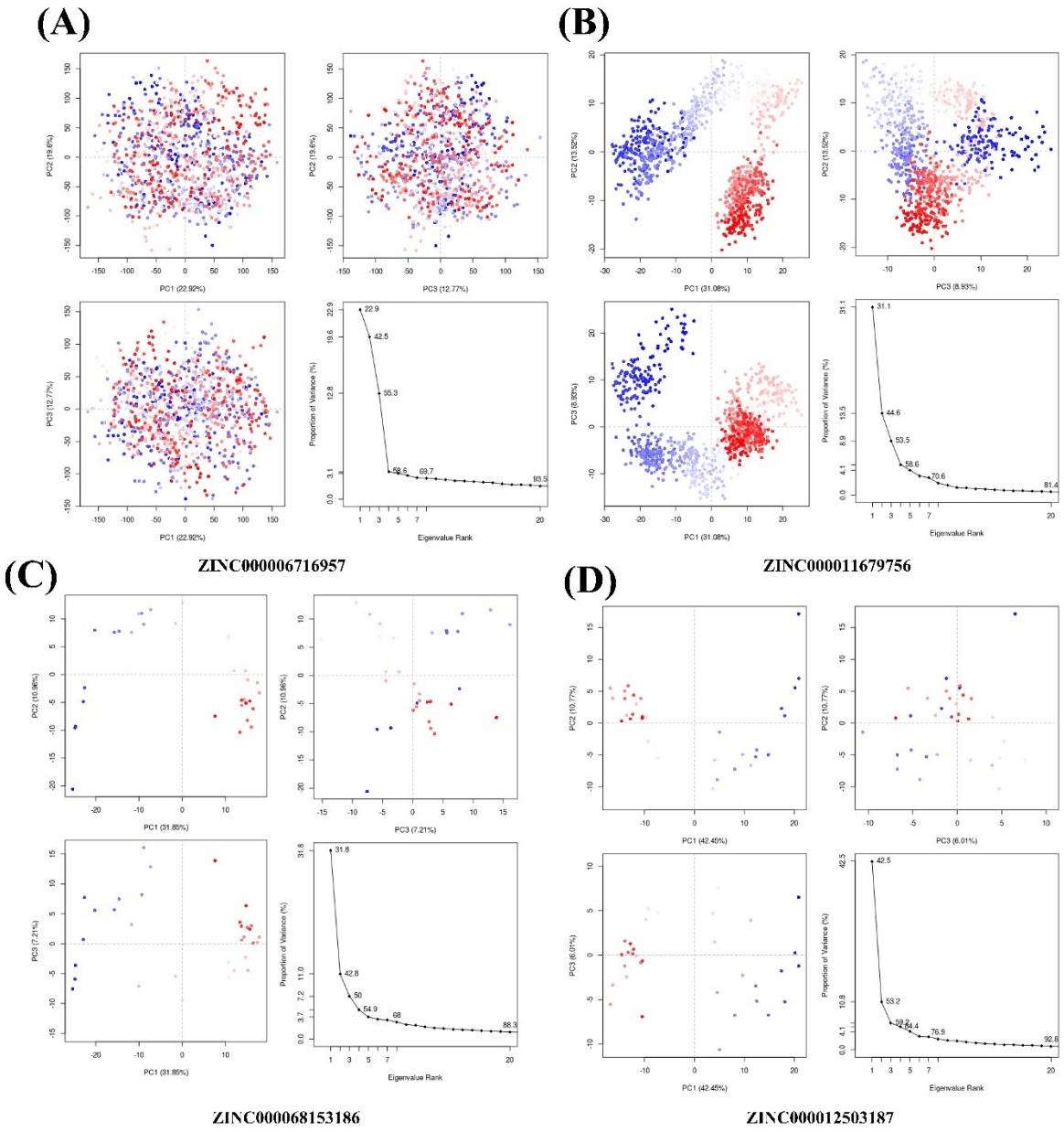

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Figure S11

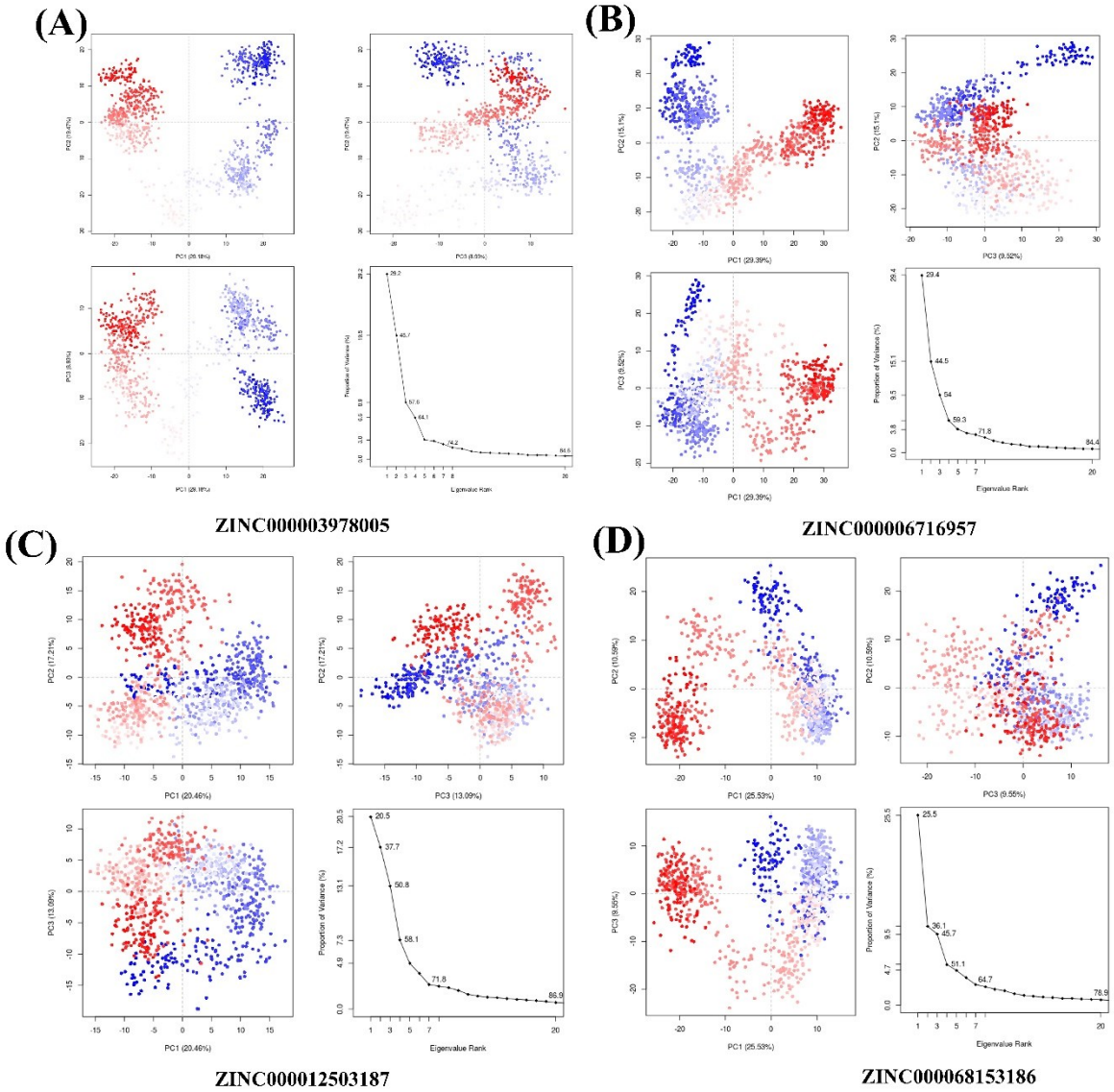

Table S1: Number of shortlisted PDB IDs of EGFR from RCSB database.

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Sr. no	PDB id	mutation	published	Subunit	Ligand	Resolution (A)	size
1	5GNK	Yes (99)	YES	monomer	80U.EDO.NO_3	1.80	297
2	4RJ3	NO	YES	Monomer (A)	3QS, ACT, ALY	1.63	298
3	5YU9	YES (99)	YES	Monomer (A)	1E8,CL	1.95	331
4	5HG7	YES (97,165,255)	YES	Monomer (A)	SO4,	1.85	329
5	5X2A	YES (99,257)	YES	Homo-2 mer	ANP.EDO.,7X0	1.85	331
6	3POZ	NO	YES	Monomer (A)	SO4,03P	1.50	327
7	7A2A	YES (101,259)	YES	Monomer (A)	7G9,57N,SO4	1.90	333
8	5UGA	Yes (97,165,255)	YES	Monomer (A)	8BM,SO4,GOL	1.82	329
9	5UGC	Yes (97,165,255)	YES	Monomer (A)	8BM,SO4,GOL	1.58	329
10	3W2S	NO	YES	Monomer (A)	W2R,SO4	1.90	330
11	6V66	YES (95,253)	YES	Homo-2mer	QP1, EDO, CL	1.79	327
12	5HG5	YES97,165, 255)	YES	Monomer (A)	GOL, SO4	1.52	329
13	6P8Q	YES (95,253)	YES	Homo-2mer	ANP,AMP	1.90	327
14	4I22	YES97,165, 255)	YES	Monomer (A)	IRE, SO4	1.71	329
15	4I24	YES (97)	YES	Monomer (A)	1C9,	1.80	329
16	3W32	NO	YES	Monomer (A)	W32, SO4	1.80	330
17	3W33	NO	YES	Monomer (A)	W19, SO4	1.70	330
18	3P0Y	NO	YES	Hetero-3 mer	-	1.80	214
19	5CNO	YES (256)	YES	Monomer A1	ANP,MG	1.55	330
20	5CNN	YES (14)	YES	Monomer (A)	ANP, MG	1.90	350
21	4ZSE	YES(99,257)	YES	Homo-2mer	EDO, ANP	1.97	331

Table S2: Predicted ligand binding sites of altered and unaltered EGFR.

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RSCB Database	
EGFR (PDB:3POZ)	Lys745, Val726, Asp837, Arg841, Asn842, Leu718, Leu844, Met793, Ala743, Gln791, Cys755, Leu788, Thr854, Arg776, Thr790, Phe856, Leu777, Met766, Asp855
Metapocket 2.0	
Non-Mutant (EGFR)	Cys775, Arg776, Leu777, Thr790, Phe856, Met766, Thr854, Ala743, Gln791, Leu792, Met793, Leu844, Gly796, Leu718, Pro794, Leu100, 1Phe997, Cys797, Val726, Phe795, Lys728, Asp855, Leu788, Lys745, Asp800, Gly719, Val717, Leu858, Arg841, Gly857, Asn842, Ser720, Phe723, Gly724, Thr725,Asp837, Leu799, Glu746, Gly721, Arg803, Ala722, Trp880, Glu906, Gly911, Ser912, Pro914, Lys913, Pro877, Arg836, Ala859, Ala864, Leu862, Lys875, Tyr891, Ala876, Gly863, Met881, Ser885, Ile878, Lys879, Glu866, Lys867, Asp916, Arg889, Tyr915, Glu865, Ile886, Ala920, Gly917, Ile918, Leu887, His888, Ser921, Leu861, Leu747, Ile759, Val786, Ala763, Leu782, Glu762, Thr785, Ser784
Mutant EGFR	Leu718, Gly719, Ser720, Gly721, Ala722, Phe723, Val726, Ala743, lys745, Met 766, Met774, Sys775, Arg776, Leu788, Thr790, Gln791, Met793, cys797, Asp837, Arg841, Asn842, Thr854, Asp855, Pro877
The consurf server	
Conserved residues	Gly721,Gly719,Gly724,Cys775,Glu762,Thr790,Gln791,Arg841,Thr790,Val726,Gln779,Ile759,Ala743,Gln791,Cyc797,Leu828,Gly857,Ala839,Val845,Ala859,Ala763,Val774,Glu884

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Table S3: Pharmacokinetic (ADMET) analysis of top shortlisted compounds.

Zinc id	Oral Toxicity class	Lipinski violation	BBB	Mutagenesis	Carcinogenicity
ZINC000012503187	III	0	+	No	Inactive
ZINC000006716957	III	1	+	No	Inactive
ZINC000003978005	III	1	-	No	Inactive
ZINC000011679756	III	0	+	No	Inactive
ZINC000068153186	III	1	+	No	Inactive

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Table S4: FDA-approved shortlisted drugs currently used to treat other diseases.

Zinc id	Drug Name	Role
ZINC000164760756	Olysio	For the treatment of chronic hepatitis-c
ZINC000012503187	Conivaptan	For the treatment of euvolemic or hypervolemia, hypernatremia, Stroke
ZINC000004099008	Vumon	Teniposide is used for the treatment of refractory acute lymphoblastic leukaemia
ZINC000006716957	Nilotinib	For the potential treatment of various leukaemia's, including chronic myeloid leukaemia (CML).
ZINC000003978005	Dihydroergotamine	For the acute treatment of migraine headaches with or without aura and the acute treatment of cluster headache episodes.
ZINC000052955754	Ergotamine	For use as therapy to abort or prevent vascular headache, e.g., migraine, migraine variants, or so called "histaminic cephalalgia".
ZINC000003932831	Avodart	For the treatment of symptomatic benign prostatic hyperplasia (BPH) in men with an enlarged prostate gland to improve symptoms, and reduce the risk of acute urinary retention.
ZINC000011679756	Eltrombopag	Thrombopoietin receptor agonists are pharmaceutical agents that stimulate platelet production in the bone marrow. In this, they differ from the previously discussed agents that act by attempting to curtail platelet destruction.
ZINC000068153186	Dabrafenib	For the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations,Skin
ZINC000242548690	Digoxin	For the treatment of mild to moderate heart failure in adult patients. To maintain control ventricular rate in adult patients diagnosed with chronic atrial fibrillation.