Improving EGFR kinase inhibitor design for the targeted treatment of lung cancer.

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Abstract

With over 174,000 new cases of lung cancer being diagnosed in the United States each year novel chemotherapy treatments with efficacy towards both small-cell and non-small cell lung carcinoma is of interest to increase the survival rate of cancer patients.[1,2] Historically pharmaceutical treatments have been based on surgery, radiation therapy, and broad spectrum chemotherapies. New research is now focused on targeted approaches that seek to either inhibit specific proteins necessary for cellular proliferation or to initiate apoptosis for the removal of cancerous cells. The Epithelial Growth Factor (EGF) and its Receptor (EGFR) EGFR is protein that initiates cellular growth and has been found to be overexpressed in cancer cells which makes it an effective targeted approach to cancer treatment.[3-5] Specifically, this research determined structural blockade of the tyrosine kinase receptor of the EGFR as a way to inhibit cancer propagation with the use of FDA approved drugs. 22 crystal structures of the tyrosine kinase of the EGFR protein were docked using IGEMDock to 714 FDA drugs to determine structural correlation for the most effective binders. Structural similarities were determined wih IGEMDock and vROCS and partition coefficient was determined using DRAGON program. This data found a cluster of approximately 25 drugs to preferentially bind to the EGFR tyrosine kinase for use as targeted cancer treatments. This work will be used in the engineering of improved EGFR tyrosine kinase inhibitors.

Introduction

This project was designed around structural understanding and pharmaceutical engineering of the EGFR kinase. Epithelial Growth Factor (EGF) is a growth factor is that is expressed for the normal growth of epithelial cells as linings of cavities and surfaces inside the body. The EGFR is the receptor which is activated by the EGF to initiate epithelial propagation. Under normal circumstances the EGF – EGFR complex is initiated under growth conditions however overexpression or stimulation by cancer cells can cause or promote unchecked cellular proliferation (i.e. cancer). This research sought to understand the blockade of the EGFR to inhibit lung cancer formation.

Specific and Overall Goal

The overall goal is to investigate the interaction of multiple drug candidates to find the best structural motifs for targeted inhibition of the EGFR kinase moiety. This research will first determine the binding and chemical properties of the EGFR active site molecules as a control group. Secondly, a group of select drug candidates whose properties are more effective at binding to the active site versus the control molecules will be chosen. Drug classification analysis will indicate preferences to improved active site binding. Finally, quantitative structure and activity relationship (QSAR) analysis will be done on both the control and experimental molecules to identify similar trends and values.

Methods and Materials

- 22 isoforms of EGFR that contained active site molecules were selected from the RCSB protein databank. The EGFR active site molecules were considered as controls versus drug candidates. 715 FDA approved pharmaceuticals were selected and computationally bound to the PI3K kinase protein using IGEMDock. The 22 protein values were averaged for all 715 drug candidates and control molecules. IGEMDock used two independent docking with the average of both bindings factoring into binding selectivity. An ANOVA was done to determine if any discrepancies in binding were seen between proteins. The best 10 grouping based on binding energies was selected and structural data such as molecular weight and partition coefficient was collected using Dragon and compared to control molecules.

EGFR Crystal Structures from Protein Databank

Proteins #	PDB #	Structural Titles
1	1M17	EGFR tyrosine kinase domain with 4-anilinoquinazoline inhibitor erlotinib
2	1XKK	EGFR kinase domain complexed with a quinazoline inhibitor- GW572016
3	2ITN	COMPLEX WITH AMP-PNP
4	2ITO	EGFR KINASE DOMAIN G719S MUTATION IN COMPLEX WITH IRESSA
5	2ITP	EGFR KINASE DOMAIN G719S MUTATION IN COMPLEX WITH AEE788
6	2ITQ	EGFR kinase domain G719S mutation in complex with AFN941
7	2ITT	EGFR KINASE DOMAIN L858R MUTATION IN COMPLEX WITH AEE788
8	2ITX	EGFR KINASE DOMAIN IN COMPLEX WITH AMP-PNP
9	2J5F	EGFR KINASE DOMAIN WITH AN IRREVERSIBLE INHIBITOR 34-JAB
10	2J6M	EGFR KINASE DOMAIN IN COMPLEX WITH AEE788
11	2QLQ	SRC kinase domain with covalent inhibitor RL3
12	2QQ7	drug resistant SRC kinase domain with irreversible inhibitor
13	2RPG	EGFR in complex with hydrazone, a potent dual inhibitor
14	3BEL	EGFR in complex with oxime inhibitor
15	3IKA	EGFR 696-1022 T790M Mutant Covalently Binding to WZ4002
16	3LZB	EGFR kinase domain complexed with an imidazo[2,1-b]thiazole inhibitor
17	3POZ	EGFR Kinase domain complexed with tak-285
18	3VJN	Mutated EGFR kinase domain (G719S/T790M) in complex with AMPPNP.
19	3VJO	Wild-type EGFR kinase domain in complex with AMPPNP.
20	4G5J	EGFR kinase in complex with BIBW2992
21	4G5P	EGFR kinase T790M in complex with BIBW2992
22	4HJO	Inactive EGFR tyrosine kinase domain with erlotinib

EGFR Summary of Control Drugs

Control Molecules	
Low Value	-115.8353043
High Value	-82.42064783
Average	-96.66638533
Standard Deviation	8.06624206

Summary of 714 EGFR Drug Candidates vs Proteins

Groups	*Count*	*Sum*	*Average*	*Variance*
1M17	1296	-83144.7	-64.1548	3618.355
1XKK	1296	-89213.7	-68.8377	4889.467
2ITN	1296	-79023	-60.9746	4467.145
2ITO	1296	-81926.5	-63.2149	3613.618
2ITP	1296	-81046.1	-62.5356	3589.357
2ITQ	1296	-76326	-58.8935	4096.825
2ITT	1296	-78697	-60.723	5910.628
2ITX	1296	-78896.2	-60.8767	3624.527
2J5F	1296	-80263.4	-61.9316	3970.2
2J6M	1296	-79332.9	-61.2137	4038.261
2QLQ	1296	-82991.5	-64.0367	3088.686
2QQ7	1296	-82069	-63.3249	5354.947
2RPG	1296	-88939.2	-68.626	4576.86
3BEL	1296	-90249.5	-69.637	3865.085
3IKA	1296	-76066.7	-58.6935	5306.768
3LZB	1296	-92977.4	-71.7419	5193.512
3POZ	1296	-91255.4	-70.4131	4066.448
3VJN	1296	-71494.9	-55.1658	3991.113
3VJO	1296	-77837.7	-60.06	4108
4G5J	1296	-77021.4	-59.4301	5812.667
4G5P	1296	-85483.6	-65.9596	3660.031
4HJO	1296	-89332.5	-68.9294	3726.793

ANOVA of 714 EGFR Drug Candidates

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	539157	21	25674.1	5.97267	7.90E-17	1.55612
Within Groups	1.22E+08	28490	4298.6			
Total	1.23E+08	28511				

EGFR Drug Candidates Docking Energy

# of Drugs	Drug Title	Energy
1	FDA 2 - 446-1	-129.3
	FDA 2 - 446-0	-128.956
2	FDA 2 - 570-1	-124.991
	FDA 2 - 570-0	-124.859
3	FDA 2 - 266-0	-114.585
	FDA 2 - 266-1	-113.781
4	FDA 2 - 284-0	-113.107
	FDA 2 - 284-1	-112.753
5	FDA 2 - 503-0	-110.076
	FDA 2 - 503-1	-109.776
6	FDA 2 - 533-1	-108.068
	FDA 2 - 533-0	-107.705
7	FDA 2 - 525-1	-107.134
	FDA 2 - 525-0	-106.973
8	FDA 2 - 195-1	-105.761
	FDA 2 - 195-0	-104.738
9	FDA 2 - 150-1	-104.199
	FDA 2 - 150-0	-103.664
10	FDA 2 - 691-1	-100.742
	FDA 2 - 691-0	-100.093

Dragon Data of EGFR Drug Candidates

NAME	MW	MLOGP	MLOGP2
FDA 2 - 446	359.04	0.364	0.133
FDA 2 - 570	312.211	-0.009	0
FDA 2 - 266	254.15	0.372	0.139
FDA 2 - 284	281.13	2.402	5.771
FDA 2 - 503	872.96	0.319	0.102
FDA 2 - 533	265.13	3.174	10.076
FDA 2 - 525	586.75	2.813	7.914
FDA 2 - 195	543.57	-0.816	0.666
FDA 2 - 150	527.57	-0.099	0.01
FDA 2 - 691	168.11	0.315	0.099

Discussion

Upon analysis of the data, multiple compounds were identified as effective based upon their interactions with each protein. An ANOVA determination of differences between the 22 proteins analyzed indicated a statistical difference was seen. Specifically an average energy of -111.563 was found for the drug candidates compared to -96.6663for the control molecules. 10 drug candidates were chosen due to their low binding energies (for both binding interactions). An ANOVA determination of differences between the 22 proteins analyzed indicated a statistical difference was seen with an F value of 5.97267 compared to an F critical value of 1.55612. Structural analysis found that many of these molecules are relatively small with similar partition coefficient (-0.009 to 0.3.72) of the top binders when compared to control molecules.

Conclusion

By using the computational techniques we were able to identify several molecule that show improved binding efficacy over currently used EGFR inhibitors. These EGFR drug candidates indicated a diverse pool of EGFR binders with improved efficacy. This work can be used to engineer these motifs into novel EGFR inhibitors for improved drug efficacy.

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