Limiting Rheumatoid Arthritis through the designing PI3K protein inhibitors.

Kimberly Santaromita,[1] Earl Benjamin,[1] Ellis Benjamin*[1]
Department of Chemistry, The Richard Stockton College of New Jersey, Galloway NJ 08205-9441.

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease that often causes inflammation of the synovial joints resulting in severe pain, bone erosion, and joint deformity which affects more than 2 million Americans. Current treatments for RA are based on anti-inflammatory treatments including steroids, non-steroidial anti-inflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), immunosuppressants, and TNF-alpha inhibitors. Side effects of current treatments can lead to heart problems, liver and kidney damage, bone marrow suppression, and severe lung infections. Improved pharmaceutical targeting of inflammatory proteins such as phosphphatidylinositol 3-kinase (PI3K) should yield drugs with increased efficacy and decreased side effects.[1-9] This research sought to understand the pharmaceutical blockade of the PI3K kinase functionality to inhibit its function in the inflammatory pathway. 16 crystal structures of the tyrosine kinase of the PI3K protein were docked using IGEMDock to FDA approved pharmaceupticals, Alkaloids, Lactams, Lactones, Flavinoids, Sulfanilamide, Cyclic Imides, and NSAIDs drugs to determine structural correlation for the most effective binders. Structural similarities were determined with IGEMDock and partition coefficient was determined using DRAGON program. This data found a cluster of approximately 10 drugs to preferentially bind to the PI3K kinase for use as targeted anti-inflammatory treatments. This work will be used in the engineering of improved PI3K kinase inhibitors.

Introduction

This project was designed around structural understanding and pharmaceutical engineering of the PI3K inhibitors. Rheumatoid arthritis like many inflammatory diseases has the ability to be acute or chronic with few targeted treatments. Often treatments are based on wide spectrum anti-inflammatories, such as steroids, that show efficacy however do maintain some detrimental effect including heart problems and liver and kidney damage. A new understanding in pharmaceutical treatments is based on selective targeting of the immune pathways for decreased toxicity. This research sought to understand the PI3K for targeted treatment of Rheumatoid arthritis.

Specific and Overall Goal

The overall goal of this research is to investigate the interaction of multiple drug candidates to find the best drug candidates for targeted inhibition of the PI3K moiety. This research will first determine the binding and chemical properties of the PI3K 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

16 isoforms of PI3K that contained active site molecules were selected from the RCSB protein databank. The PI3K active site molecules were considered as controls versus drug candidates. 1172 drug candidates were chosen which included, 715 FDA approved, 197 Alkaloids, 73 Imides, 40 Lactams, 36 Lactones, 50 NSAIDs, 25 Sulfanilamide and 37 Flavonoids pharmaceuticals were selected and computationally bound to the PI3K kinase protein using IGEMDock. The 16 protein values were averaged for all 1172 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. Additionally grouping of the molecular functionalities was determined to find if any statistical difference in drug type were found. 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.

PI3K Crystal Structures from Protein Databank

#	PDB	Title
1	3T8M	Rational Design of PI3K-alpha Inhibitors that Exhibit Selectivity Over the PI3K-beta Isoform
2	3TJP	PI3K gamma with N6-(3,4-dimethoxyphenyl)-2-morpholino-[4,5'-bipyrimidine]-2',6-diamine
3	3TL5	Discovery of GDC-0980: a Potent, Selective… (PI3K)
4	3ZIM	Discovery of a potent and isoform-selective targeted covalent inhibitor of the PI3K alpha
5	3ZVV	FRAGMENT BOUND TO PI3KINASE GAMMA
6	4ANU	Complexes of PI3Kgamma with isoform selective inhibitors.
7	4ANW	Complexes of PI3Kgamma with isoform selective inhibitors.
8	4AOF	Selective small molecule inhibitor discovered by …reveals regulation by PI3Kgamma
9	4DK5	Crystal structure of human PI3K-gamma in complex with a pyridyl-triazine inhibitor
10	4F1S	Crystal structure of human PI3K-gamma in complex with a pyridyl-triazine-sulfonamide inhibitor
11	4FJY	Crystal structure of PI3K-gamma in complex with quinoline-indoline inhibitor 24f
12	4FJZ	Crystal structure of PI3K-gamma in complex with pyrrolo-pyridine inhibitor 63
13	4FLH	Crystal structure of human PI3K-gamma in complex with AMG511
14	4FUL	PI3 Kinase Gamma bound to a pyrmidine inhibitor
15	4G11	PI3K-gamma bound to a 4-(morpholin-4-yl)- (6-oxo-1,6-dihydropyrimidin-2-yl)amide inhibitor
16	4HLE	Compound 21 (1-alkyl-substituted 1,2,4-triazoles)

Summary of 1172 Drug Candidates vs Proteins

Groups	*Count*	*Sum*	*Average*	*Variance*
3T8M	2344	-152683	-65.138	6851.8
3TJP	2344	-154071	-65.73	5765.49
3TL5	2344	-157700	-67.278	4521.26
3ZIM	2344	-160872	-68.631	5425.93
3ZVV	2344	-150026	-64.004	6578.5
4ANU	2344	-153085	-65.309	5473.53
4ANW	2344	-152519	-65.068	7142.16
4AOF	2344	-155301	-66.255	5452.68
4DK5	2344	-154274	-65.817	5359.44
4F1S	2344	-151114	-64.469	4880.95
4FJY	2344	-157125	-67.033	4361.15
4FJZ	2344	-155003	-66.128	6069.49
4FLH	2344	-156284	-66.674	7179.44
4FUL	2344	-153956	-65.681	5696.61
4G11	2344	-157158	-67.047	5692.7
4HLE	2344	-155732	-66.439	6568.9

ANOVA of 1172 drug candidates

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	46980.73	15	3132.05	0.53873	0.92047	1.66665
Within Groups	2.18E+08	37488	5813.75			
Total	2.18E+08	37503				

Summary of control drugs (IGEMDock)

PI3K Control	
Low Value	-112.50144
High Value	-65.87927333
Average	-97.63339896
Standard Deviation	11.00546649

Breakdown of drug candidates by types

Types	Average	Standard Deviation
Alkaloids	-80.6951374	5.370858852
Flavonoids	-83.2733871	5.703398037
Imide	-79.4508933	4.602451471
Lactams	-77.4543402	5.081873303
Lactones	-75.793854	11.14768894
NSAIDS	-82.3829582	5.64418681
Sulfanilamide	-72.6882301	4.343798316

Drug Candidates Docking Energy

# of Drugs	Drug Title	Energy
1	FDA - 446-0	-146.374625
	FDA - 446-1	-143.449813
2	FDA - 570-1	-128.281563
	FDA - 570-0	-127.059688
3	FDA - 266-1	-121.817875
	FDA - 266-0.	-121.757125
4	FDA - 284-0	-115.846563
	FDA - 284-1	-114.707125
5	FDA - 503-0	-114.478381
	FDA - 503-1	-114.344825
6	Etoposide-1-1	-114.134113
	Etoposide-1-0	-114.0975
7	FDA - 533-1	-113.741063
	FDA - 533-0	-113.19425
8	FDA - 99-0	-130.736625
	FDA - 99-1	-124.42525
9	Paclitaxel-0-1	-112.402094
	Paclitaxel-0-0	-111.7048
10	Alkaloids - 504 -0-1	-127.742125
	Alkaloids - 504 -0-0	-118.756

Dragon Data of 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 - 99	504.72	2.029	4.117

Dragon Data of Control Molecules

NAME	MW	MLOGP	MLOGP2
4HLE_17V_A_1201	336.29	3.799	14.435
4G11_0W7_A_1201	296.2	2.637	6.953
4FUL_0VU_A_1201	414.34	2.762	7.63
4FLH_14K_A_1205	489.38	2.467	6.086
4FJZ_4FJ_A_1205	481.35	2.39	5.711
4FJY_FJY_A_1204	439.33	3.729	13.908
4F1S_F1S_A_1207	467.8	2.451	6.009
4DK5_0KO_A_1204	458.37	2.14	4.581
4AOF_7L0_A_2095	318.26	2.106	4.435
4ANW_O92_A_1189	439.75	2.32	5.385
4ANU_EM7_A_2089	284.21	2.119	4.492
3ZVV_XAZ_A_1500	152.12	1.622	2.631
3ZIM_KKR_A_2047	538.44	2.721	7.405
3TL5_980_A_1	468.38	1.483	2.199
3TJP_13K_A_1	386.27	1.474	2.173
3T8M_3T8_A_1	523.84	3.048	9.292

Discussion

Upon analysis of the data, 10 compounds were identified as effective based upon their interactions with each protein. Specifically an average energy of -121.453 was found for the drug candidates compared to -97.6334 for the control molecules. 10 drugs were chosen due to their low binding energies (for both binding interactions). An ANOVA determination of differences between the 16 proteins analyzed indicated no statistical differences were seen with an F value of 0.53873 compared to an F critical value of 1.66665. The data indicated that there were no statistical differences between all drug types with all averages with the standard deviations. 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.

Conclusion

By using the computational techniques we were able to identify several molecule that show improved binding efficacy over currently used PI3K inhibitors. These PI3K drug candidates indicated a diverse pool of PI3K binders with improved efficacy. This work can be used to engineer these motifs into novel PI3K inhibitors for improved drug efficacy.

Acknowledgments

This Project was supported by The College of Natural Science and Mathematics at The Richard Stockton College of New Jersey.

References

1. Banham-Hall, E.; Clatworthy, M. R.; Okkenhaug, K., The Therapeutic Potential for PI3K Inhibitors in Autoimmune Rheumatic Diseases. *Open Rheumatol J* 6, 245-58.
2. Ghigo, A.; Damilano, F.; Braccini, L.; Hirsch, E., PI3K inhibition in inflammation: Toward tailored therapies for specific diseases. *Bioessays* 32, (3), 185-96.
3. Han, W.; Xiong, Y.; Li, Y.; Fang, W.; Ma, Y.; Liu, L.; Li, F.; Zhu, X., Anti-arthritic effects of clematichinenoside (AR-6) on PI3K/Akt signaling pathway and TNF-alpha associated with collagen-induced arthritis. *Pharm Biol* 51, (1), 13-22.
4. Kelly, V.; Genovese, M., Novel small molecule therapeutics in rheumatoid arthritis. *Rheumatology (Oxford)* 52, (7), 1155-62.
5. Puri, K. D.; Gold, M. R., Selective inhibitors of phosphoinositide 3-kinase delta: modulators of B-cell function with potential for treating autoimmune inflammatory diseases and B-cell malignancies. *Front Immunol* 3, 256.
6. Safina, B. S.; Baker, S.; Baumgardner, M.; Blaney, P. M.; Chan, B. K.; Chen, Y. H.; Cartwright, M. W.; Castanedo, G.; Chabot, C.; Cheguillaume, A. J.; Goldsmith, P.; Goldstein, D. M.; Goyal, B.; Hancox, T.; Handa, R. K.; Iyer, P. S.; Kaur, J.; Kondru, R.; Kenny, J. R.; Krintel, S. L.; Li, J.; Lesnick, J.; Lucas, M. C.; Lewis, C.; Mukadam, S.; Murray, J.; Nadin, A. J.; Nonomiya, J.; Padilla, F.; Palmer, W. S.; Pang, J.; Pegg, N.; Price, S.; Reif, K.; Salphati, L.; Savy, P. A.; Seward, E. M.; Shuttleworth, S.; Sohal, S.; Sweeney, Z. K.; Tay, S.; Tivitmahaisoon, P.; Waszkowycz, B.; Wei, B.; Yue, Q.; Zhang, C.; Sutherlin, D. P., Discovery of novel PI3-kinase delta specific inhibitors for the treatment of rheumatoid arthritis: taming CYP3A4 time-dependent inhibition. *J Med Chem* 55, (12), 5887-900.
7. Tian, J.; Chen, J. W.; Gao, J. S.; Li, L.; Xie, X., Resveratrol inhibits TNF-alpha-induced IL-1beta, MMP-3 production in human rheumatoid arthritis fibroblast-like synoviocytes via modulation of PI3kinase/Akt pathway. *Rheumatol Int* 33, (7), 1829-35.
8. Wu, M. Y.; Yang, R. S.; Lin, T. H.; Tang, C. H.; Chiu, Y. C.; Liou, H. C.; Fu, W. M., Enhancement of PLGF production by 15-(S)-HETE via PI3K-Akt, NF-kappaB and COX-2 pathways in rheumatoid arthritis synovial fibroblast. *Eur J Pharmacol* 714, (1-3), 388-96.
9. Camps, M.; Ruckle, T.; Ji, H.; Ardissone, V.; Rintelen, F.; Shaw, J.; Ferrandi, C.; Chabert, C.; Gillieron, C.; Francon, B.; Martin, T.; Gretener, D.; Perrin, D.; Leroy, D.; Vitte, P. A.; Hirsch, E.; Wymann, M. P.; Cirillo, R.; Schwarz, M. K.; Rommel, C., Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* **2005,** 11, (9), 936-43.