Improving Drug Design for Resistances in Gram-Negative Bacteria.

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Abstract

Drug resistances in Gram-negative bacteria arise through several mechanisms including that of an over-expression of the multidrug transporter AcrB and its homologues. This research works sought to use computational methods to understand structure and functional relationship using the binding energies and structural similarity of the lowest binding molecules. A series of 479 molecules including alkaloids, flavinoids, cyclic imides, lactams, lactones, NSAIDS, sulfanilamides, and known pharmaceuticals were bound to 10 AcrB crystal structures (1IWG, 1OY6, 1OY8, 1OY9, 1OYD, 2W1B, 2RDD, 2J8S, 2HRT, 2DRD). Computational results matched found a group of current pharmaceuticals maintaining the lowest energy overall. Similarity searches of the lowest binding molecules were conducted to determine important structural motifs. This research allows a better understanding of drug interaction towards the blockade of Gram-negative resistances.

Introduction

AcrB is a multidrug exporter in E. coli that exports a wide variety of compounds from the cell, contributing to the bacterium's multidrug resistance.[1-3] In particular the AcrB works by proton-motive force which shows the widest substrate specificity among all known multidrug pumps, ranging from most of the antibiotics, disinfectants, dyes, and detergents to simple solvents.[4,5] Structurally, the AcrB protein is a homotrimer of 110kDa (per subunit) contains 12 transmembrane helices and two large periplasmic domains with a large 30A-wide central cavity that spans the cytoplasmic membrane and extends to the cytoplasm. The AcrB shows similarity to multiple microbial efflux pumps including the Mex protein involved in elevated intrinsic antibiotic resistance as well as in acquired multidrug resistance in Pseudomonas aeruginosa. Alignment of the (MexB, MexD, MexF, and MexY) amino acid sequences with those of the homologous AcrB

pump proteins of Escherichia coli showed conservation of five charged amino acid residues located in or next to transmembrane segments.[6] A better understanding of the AcrB protein would allow improved engineering of anti-microbials for both E. coli and other bacteria.

Experimental

iGEMDOCK by BioXGEM was used to determine protein –ligand docking interaction based on total binding energy, amino acid interaction energy, hydrogen bonding energy, and electrostatics. 8 categories of molecules were selected including Alkaloids (201 molecules), Flavonoids (37 molecules), Imides (73 molecules), Lactams (45 molecules), Lactones (36 molecules), NSAIDS (50 molecules), Sulfonamides (25 molecules), and known P- glycoprotein (Pgp) binders (12 molecules). The binding energy totals for all ten crystal structures (1IWG, 1OY6, 1OY8, 1OY9, 1OYD, 2W1B, 2RDD, 2J8S, 2HRT, 2DRD) were averaged and ANOVA calculations were done using Excel with the statistical Analysis ToolPak Addin. Additionally, Graphpad Prism was also used to determine non-parametric ANOVA with a Dunn's post-hoc calculations. Protein clustering of amino acid binding energies was conducted using iGEMDOCK. Additional structural clustering by shape and electrostatics was conducted using vROCS © (Open Eye Scientific).

Results

Computational results matched that of experimental result with a group of current Pgp binders which include Digoxin, Etoposide, Tacrolimus, and Paclitaxel maintaining the lowest energy (averaged over the ten proteins). This was confirmed using a one-way ANOVA when compared to the remaining 468 molecules. Additionally, a Dunn's post-hoc test found that known Pgp binders were significantly different (<0.001) than all all groups except Flavonids ($P<0.01$). 14 novel molecules were identified to bind comparable to known Pgp binders which included Alkaloids – 409 (-97.48614), Alkaloids – 442 (-90.61655), Alkaloids – 504 (-115.98222), Alkaloids – 619 -90.07489), Alkaloids – 633 (-90.18557), Alkaloids – 638 (-97.39622), Flavinoids – 17 (-91.27777), Imides – 4 (-96.78262), Imides – 6 (-93.63238), Lactones – 89 (-94.88379), NSAIDS – 4 (-91.06496), NSAIDS – 11 (-91.57011), NSAIDS – 18 (-94.75149) and NSAIDS – 20 (-

90.83211). This research allows a better understanding of drug interaction towards the blockade of the function of AcrB.

Conclusion

The computational calculation confirmed the experimental results with known Pgp binders statistically (P<0.001) being the best overall antagonist. Pgp binders should be tested to determine their anti-microbial characteristic. Additionally motifs of known Pgp binders should provide the best estimation of AcrB binders.

Overall Average and Standard Deviation					
Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Average	-71.8837994	-60.92417	-10.92945	-0.0301807	20.6792927
Standard Deviation	20.350326	19.596358	6.005277	0.1380734	4.93871253
Averages					
Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Alkaloids	-73.0292663	-62.9473	-10.05807	-0.023903	20.2972191
Flavinoids	-73.6019386	-62.86512	-10.6894	-0.0474138	21.2054475
Imides	-72.0103359	-60.77507	-11.19333	-0.0419372	22.7619015
Lactams	-63.2091656	-52.70895	-10.4769	-0.0233187	19.5942376
Lactones	-66.9353402	-53.69218	-13.22212	-0.021049	20.8679315
NSAIDS	-71.4096462	-61.80536	-9.554624	-0.049659	19.7355614
Sulfanamides	-69.8068156	-53.76267	-16.0296	-0.0145402	23.4959904
PGP binders	-102.918925	-86.67858	-16.24032	0	15.767675
Overall Standard Deviations					
Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Alkaloids	15.6393803	15.255212	5.461242	0.12264295	4.54747171
Flavinoids	14.5050899	13.821531	7.9344873	0.16397171	4.02367144
Imides	15.9213203	14.980601	5.2813063	0.0956885	5.09048230
Lactams	19.4848947	18.032534	6.2209276	0.15299825	5.80563022
Lactones	46.7097622	45.121244	4.898246	0.15222441	4.34747136
NSAIDS	20.2251514	19.58506	6.79894	0.20955531	6.10322500
Sulfanamides	16.7100293	16.642685	3.8879787	0.0865520	4.73345585
PGP binders	10.1212436	12.548317	2.4270733	0	2.53922045

ANOVA: Excel

Groups	*Count*	*Sum*	*Average*	*Variance*
Alkaloids	201	-14678.88253	-73.0292663	213.703044
Flavonoids	37	-2723.271728	-73.60193859	322.2903911
Imides	73	-5256.75452	-72.01033589	83.50207201
Lactams	45	-2862.50212	-63.61115822	643.8189889
Lactones	36	-2429.145258	-67.47625717	2126.732642
NSAIDS	50	-3570.48231	-71.4096462	415.524435
Sulfonamides	25	-1745.17039	-69.8068156	69.80287905
PGP binders	12	-1166.13108	-97.17759	77.50193749

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11949.519	7	1707.0742	4.3225810	0.0001	2.0290
Within Groups	186007.37	471	394.92012			
Total	197956.89	478				

Data for Pgp Binders

#Ligand	TotalEnergy	VDW	HBond	Elec	AverConPair
Cyclosporine	-108.9722	-95.92746	-13.044806	0	10.347066
Dexamethasone	-85.97912	-70.15382	-15.825249	0	18.19644
Digoxin	-106.39551	-85.91486	-20.480725	0	13.92222
Diltiazen	-87.56067	-78.89787	-8.662793	0	18.26552
Etoposide	-100.4417	-81.50776	-18.93403	0	15.88096
Hydrocortisone	-83.8703	-66.80757	-17.06273	0	19.51922
Nicardipine	-95.21412	-77.34742	-17.61633	-0.2504665	16.36858
Paclitaxel	-108.49361	-92.49126	-16.002396	0	12.238713
Tacrolimus	-103.44069	-90.06112	-13.379664	0	12.464918
Verapamil	-90.26009	-80.84795	-9.412135	0	17.53331
Vinblastine	-99.00943	-83.11372	-15.8957	0	12.252645
Vincristine	-96.49364	-81.37517	-15.11842	0	11.884486

ANOVA and Dunn's Post Hoc Test

Parameter	Value
Table Analyzed	
Data 1	
Kruskal-Wallis test	
P value	P<0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Do the medians vary signif. (P < 0.05)	Yes
Number of groups	8
Kruskal-Wallis statistic	46.46

Dunn's Multiple Comparison Test	Difference in rank sum	P value	Summary
Alkaloids vs Flavonoids	23.4	P > 0.05	ns
Alkaloids vs Imides	-39.1	P > 0.05	ns
Alkaloids vs Lactams	-55.66	P > 0.05	ns
Alkaloids vs Lactones	1.592	P > 0.05	ns
Alkaloids vs NSAIDS	2.283	P > 0.05	ns
Alkaloids vs Sulfonamides	-63.97	P > 0.05	ns
Alkaloids vs PGP binders	212.1	P < 0.001	***
Flavonoids vs Imides	-62.5	P > 0.05	ns
Flavonoids vs Lactams	-79.06	P > 0.05	ns
Flavonoids vs Lactones	-21.81	P > 0.05	ns
Flavonoids vs NSAIDS	-21.12	P > 0.05	ns
Flavonoids vs Sulfonamides	-87.37	P > 0.05	ns
Flavonoids vs PGP binders	188.7	P < 0.01	**
Imides vs Lactams	-16.56	P > 0.05	ns
Imides vs Lactones	40.69	P > 0.05	ns
Imides vs NSAIDS	41.38	P > 0.05	ns
Imides vs Sulfonamides	-24.87	P > 0.05	ns
Imides vs PGP binders	251.2	P < 0.001	***
Lactams vs Lactones	57.25	P > 0.05	ns
Lactams vs NSAIDS	57.94	P > 0.05	ns
Lactams vs Sulfonamides	-8.309	P > 0.05	ns
Lactams vs PGP binders	267.8	P < 0.001	***
Lactones vs NSAIDS	0.6911	P > 0.05	ns
Lactones vs Sulfonamides	-65.56	P > 0.05	ns
Lactones vs PGP binders	210.5	P < 0.001	***
NSAIDS vs Sulfonamides	-66.25	P > 0.05	ns
NSAIDS vs PGP binders	209.8	P < 0.001	***
Sulfonamides vs PGP binders	276.1	P < 0.001	***

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