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Flavocillin: a potent TrxR and OATP inhibitor

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Abstract

Flavocillin, a newly discovered beta-lactam antibiotic, shows promise in its ability to be a TrxR and OATP inhibitor. Through our docking experiments, we identified key residues in the docking process, that proves the idea that this molecule can increase the susceptibility of various bacteria to beta-lactams since it has been proven that the combination of TrxR inhibitors, along with beta-lactams, can minimize the creation of MRSA. Also, Flavocillin shows an affinity for the metallo beta-lactamase, omitting the use of clavulanic acid. Possibly, the combination of Flavocillin with other beta-lactams, as a therapeutic approach, could minimize the chances of creating antibiotic-resistant strains.

Introduction

Beta lactams are a group of molecules that all share a common structure, that is the beta-lactam ring[1], with the help of which, the molecule manages to bind to transpeptidase, a known component of the cell wall of bacteria and thus, inhibit peptidoglycan, causing disruption of the wall and the dissolution of the cell due to extreme osmotic pressure from the environment.[2] Beta lactams have an indication for various bacteria that are either aerobic/anaerobic or gram-positive/gram-negative. Each bacteria can build up a tolerance to the beta-lactams, by producing beta-lactamases, enzymes that break up the beta-lactam ring, thus disallowing the interaction between the drug and the transpeptidase. [3] For this reason, scientists have developed beta-lactamase inhibitors, such as clavulanic acid and tazobactam. [4]

Another way via which the bacterium can withstand the effects of drugs is through the NADPH system and specifically, through its derivatives, the thioredoxin reductase (TrxR) and the glutaredoxin reductase (GrxR). These molecules exist both in bacteria and human tissues, with some minor differences in sequencing. Bacteria can have one, or none of them. [5] It has been proven that the inhibition of TrxR in E.coli tends to improve the efficacy profile of antibiotics![6] Most bacteria, such as E.coli, carry the TrxR, that is why our research focuses on that enzyme.

A recent discovery pointed out a new age in antibiotic production. This came with the discovery of Flavocillin. Flavocillin is essentially a beta-lactam that carries a flavonone in its group, as an R substitute and has shown great affinity, in vitro, for various strains of bacteria. Apart from the beta-lactam ring, the R group might be essentially an active component, offering to adjust properties, the same as other flavonoids. This should be tested in human cells and bacteria.

Methods: Through our research, we performed docking experiments for Flavocillin, with the help of the software mcule, against the TrxR of E.coli for the area (x, y,z)=(26.6282, 30.8226, -12.0442) and found an affinity of –10.1(high) that allowed us to assume that potentially, this antibiotic has some anti-inflammatory properties and could be used for far more conditions than merely an antibiotic. In more detail, the most prevalent binding site was for ALA295, GLN294, GLY41, GLY12, GLY36, GLY38, SER13, PRO15, ALA16, and SER298. The R group of flavocillin offers this affinity, proving that it is an active ingredient. (figure 1)

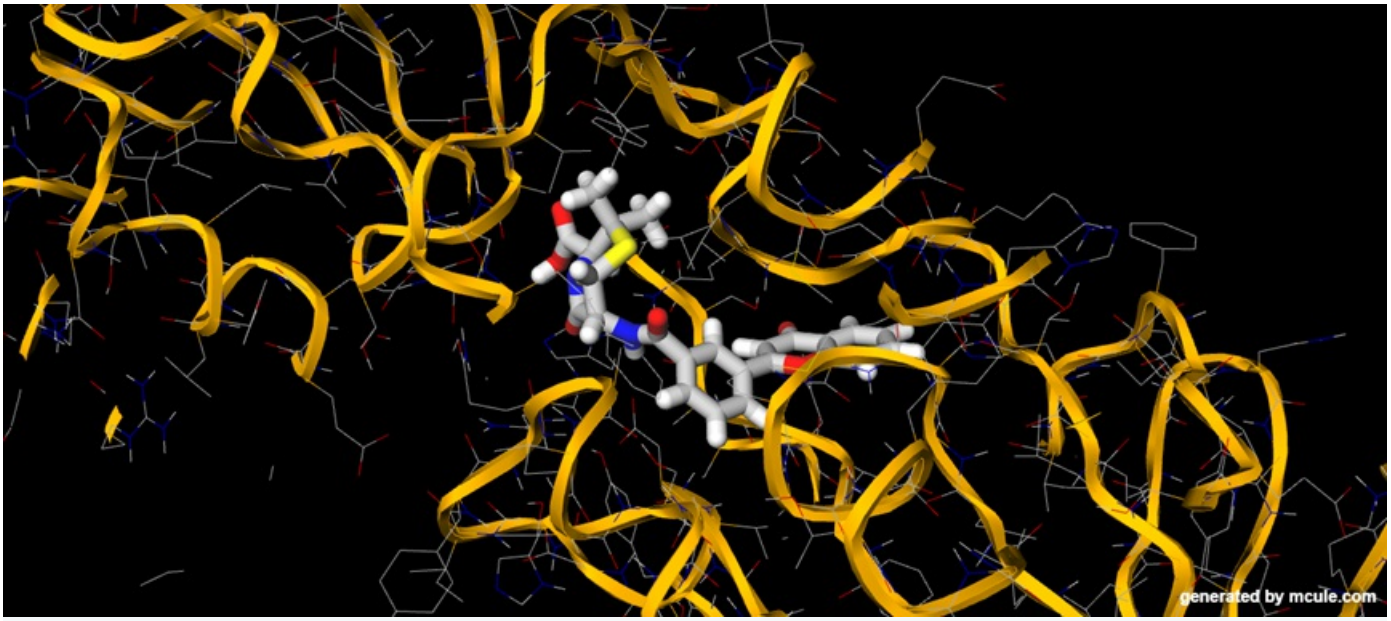


Figure 1. Docking results for Flavocillin

Results

In Silico docking, models were obtained for Flavocillin by using ADMETSar version 2 software.[7] According to these results, there were 0 violations of Lipinski's druggability rules [8]. The Human Intestinal absorption value for Flavocillin was 0.6236 which can be categorized as "middle'' intestinal absorption. [9] (figure 2). Since the prediction of oral bioavailability score was around 0.78, Flavocillin is considered to have good oral bioavailability. [10] In terms of drug safety profile prediction, Flavocillin is not an hERG inhibitor which would make it stronger as a new drug candidate because it will not enhance the risk of cardiovascular disease. [11] In addition, Flavocillin was OATP1B1 and OATP1B3 inhibitor according to the same modeling with high inhibitory scores of 0,88 and 0,92 respectively. This indicated that Flavocillin would additionally have a similar mechanism of action to other OATP inhibitors in the market which are already in clinical use against certain infectious diseases such as tuberculosis.

Property	Value
Molecular Weight	464.50
AlogP	2.71
H-Bond Acceptor	6
H-Bond Donor	2
Rotatable Bonds	4
Applicability Domain	In domain

ADMET predicted profile --- Classifications	ValueProbability	
Human Intestinal Absorption	-	0.6237
Caco-2	-	0.7788
Blood Brain Barrier	-	0.9750
Human oral bioavailability	-	0.7857
Subcellular localzation	Mitochondria	0.4607
OATP2B1 inhibitior	-	0.7264
OATP1B1 inhibitior	+	0.8809
OATP1B3 inhibitior	+	0.9296
MATE1 inhibitior	-	0.9600
OCT2 inhibitior	-	0.9425
BSEP inhibitior	+	0.8421
P-glycoprotein inhibitior	-	0.4719

P-glycoprotein substrate	+	0.5176
CYP3A4 substrate	+	0.6707
CYP2C9 substrate	-	1.0000
CYP2D6 substrate	-	0.8890
CYP3A4 inhibition	-	0.5894
CYP2C9 inhibition	-	0.7883
CYP2C19 inhibition	-	0.7715
CYP2D6 inhibition	-	0.8788
CYP1A2 inhibition	-	0.8052
CYP inhibitory promiscuity	-	0.8992
UGT catelyzed	-	0.0000
Carcinogenicity (binary)	-	0.7200
Carcinogenicity (trinary)	Non-required	0.6223
Eye corrosion	-	0.9891
Eye irritation	-	0.9600
Ames mutagenesis	-	0.6000
Human Ether-a-go-go-Related Gene inhibition	-	0.4702
Micronuclear	+	0.9100
Hepatotoxicity	+	0.6277
skin sensitisation	-	0.7909
Respiratory toxicity	+	0.9333

Reproductive toxicity	+	0.6444
Mitochondrial toxicity	+	0.9500
Nephrotoxicity	-	0.7207
Acute Oral Toxicity (c)	III	0.4790
Estrogen receptor binding	-	0.5384
Androgen receptor binding	+	0.5832
Thyroid receptor binding	-	0.5113
Glucocorticoid receptor binding	+	0.6058
Aromatase binding	-	0.5486
PPAR gamma	+	0.6936
Honey bee toxicity	-	0.7780
Biodegradation	-	0.9000
Crustacea aquatic toxicity	-	0.5400
Fish aquatic toxicity	+	0.9732

ADMET predicted profile --- Regressions	Value	Unit
Water solubility	-3.505	logS
Plasma protein binding	1.158	100%
Acute Oral Toxicity	1.375	log (1/(mol/kg))
Tetrahymena pyriformis	1.135	pIGC50 (ug/L)

Figure 2. ADMETSar version 2 based drug characteristics prediction In Silico for Flavocillin

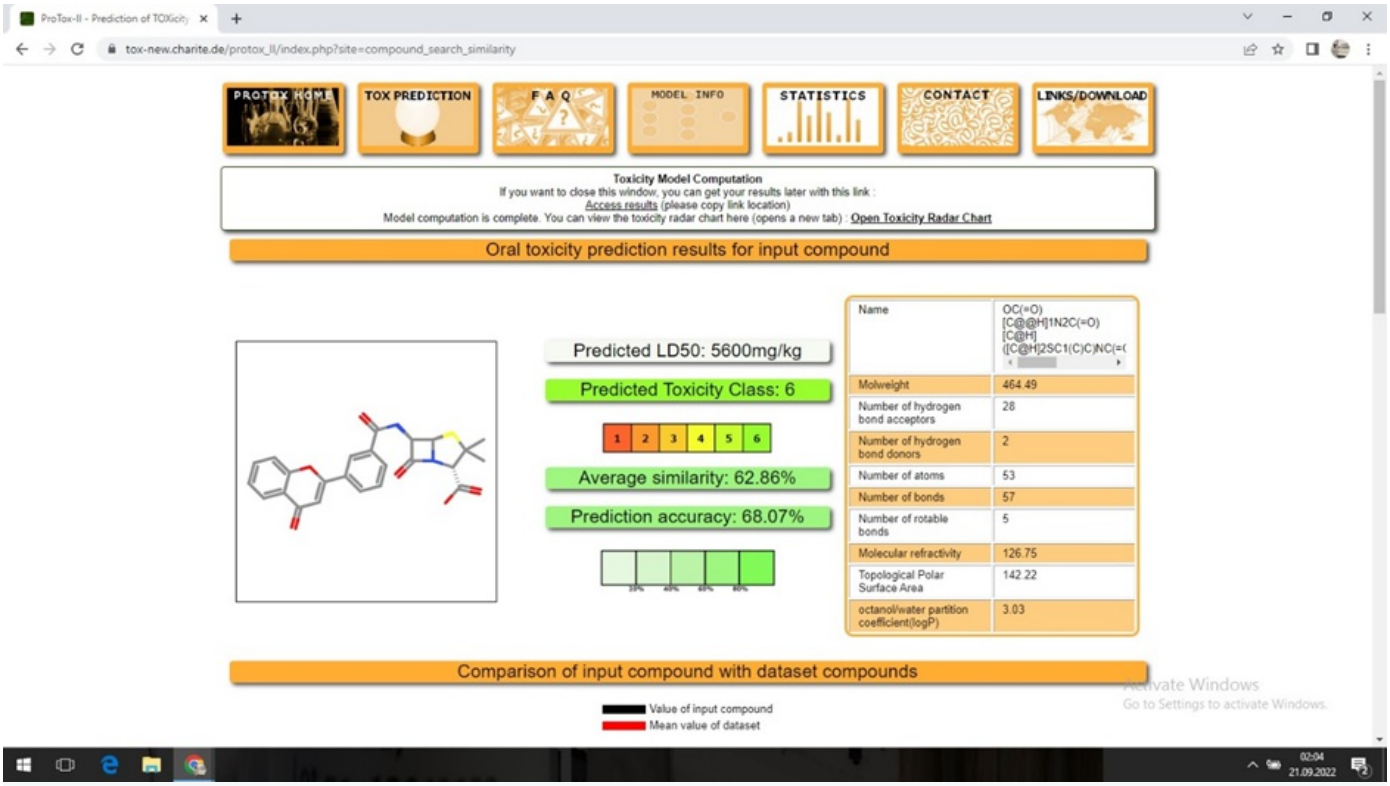
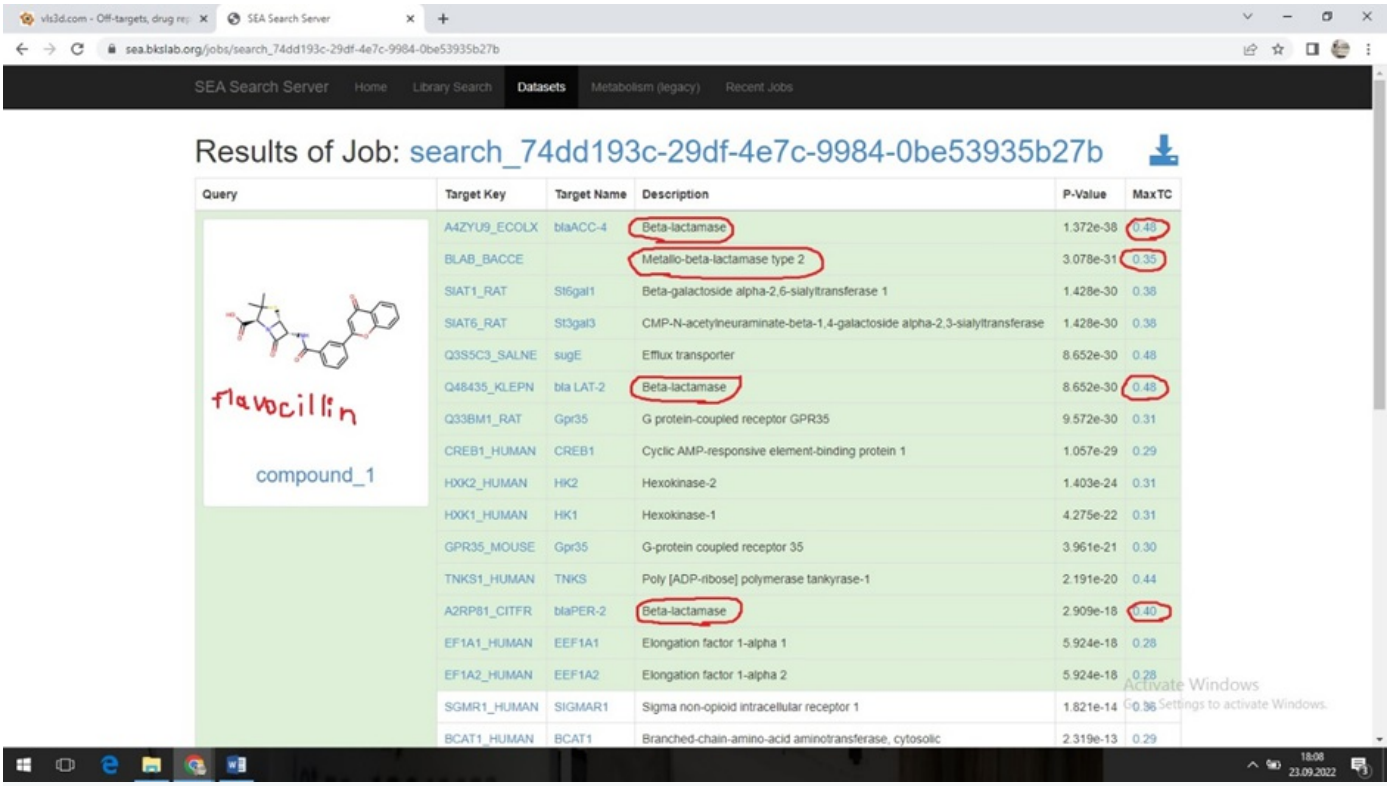


Figure 3. In Silico Predicted LD50 of Flavocillin



Query	Target Key	Target Name	Description	P-Value	MaxTC
	A4ZYU9_ECOLX	blaACC-4	Beta-lactamase	1.372e-38	0.48
	BLAB_BACCE		Metallo-beta-lactamase type 2	3.078e-31	0.35
	SIAT1_RAT	St6gal1	Beta-galactoside alpha-2,6-sialyltransferase 1	1.428e-30	0.38
	SIAT6_RAT	St3gal3	CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase	1.428e-30	0.38
	Q3S5C3_SALNE	sugE	Efflux transporter	8.652e-30	0.48
	Q48435_KLEPN	bla LAT-2	Beta-lactamase	8.652e-30	0.48
	Q33BM1_RAT	Gpr35	G protein-coupled receptor GPR35	9.572e-30	0.31
	CREB1_HUMAN	CREB1	Cyclic AMP-responsive element-binding protein 1	1.057e-29	0.29
	HXK2_HUMAN	HK2	Hexokinase-2	1.403e-24	0.31
	HXK1_HUMAN	HK1	Hexokinase-1	4.275e-22	0.31
	GPR35_MOUSE	Gpr35	G-protein coupled receptor 35	3.961e-21	0.30
	TNKS1_HUMAN	TNKS	Poly [ADP-ribose] polymerase tankyrase-1	2.191e-20	0.44
	A2RP81_CITFR	blaPER-2	Beta-lactamase	2.909e-18	0.40
	EF1A1_HUMAN	EEF1A1	Elongation factor 1-alpha 1	5.924e-18	0.28
	EF1A2_HUMAN	EEF1A2	Elongation factor 1-alpha 2	5.924e-18	0.28
	SGMR1_HUMAN	SIGMAR1	Sigma non-opioid intracellular receptor 1	1.821e-14	0.36
	BCAT1_HUMAN	BCAT1	Branched-chain-amino-acid aminotransferase, cytosolic	2.319e-13	0.29

Figure 4. SEA search server results for Flavocillin modelling

Because of the slightly larger structure of a flavonone group as a subgroup of Flavonoids on the variable R group, Flavocillin can enhance or cause the eradication of bacterial biofilm formation. This way, Flavocillin may target bacterial biofilms also. [12]

In Vitro, test results showed that Flavocillin targets mainly S. Aureus strains, MRSA, and S. mutans. M. Catarrhalis and was highly active on C. Stiratum when compared to Ampicillin and other antibiotics. [13]

Organic anion-transporting polypeptides (OATPs) are transporters that are useful targets against infectious diseases. [14] Flavocillin is one of the OATPB1/OATPB3 inhibitors. One of the uses of such inhibitors is against HIV, but research should not be limited only to the most notorious of infections, but also focus on other important diseases, such as tuberculosis.

The mechanism of action of OATPs is to carry xenobiotics, that has a molecular weight above 350 Da, such as natural products. It even extends to endogenous ligands, such as albumin. [15] Flavocillin shows a binding affinity for the OATPs that is measured to be 0,8809 for OATP1B1 and 0,9296 for OATP1B3. According to such findings, it is estimated that it would be a useful agent against Mycobacterium avium complex, leprosy, and, as mentioned, tuberculosis. The drug itself, being of 464.50 molecular weight, might also inhibit its metabolism, through the inhibition of OATPs and thus increase its levels in the bloodstream, bypassing first-pass metabolism, as shown in the case of simvastatin. However, there is a chance for increased drug-drug interactions, in terms of pharmacokinetics and this should be studied further. [16] Even more, the side effects would be potentially increased, causing a less tolerable safety profile.

Computational molecular modeling was performed for Flavocillin by using Protox and SEA search software. According to the obtained results, the drug safety profile of Flavocillin was high because the LD50 score was 5600 mg/kg and therefore high doses are required for Flavocillin to have toxic effects. On the other hand, SEA search results showed that Flavocillin is also a metallo beta-lactamase and beta-lactamase inhibitor. (figures 3 and 4)

For the tested Flavocillin Ammonium salt compound, in vitro test results concluded that Flavocillin antibiotics are effective on 10 types of bacteria including some of the antibiotic-resistant gram-positive and gram-negative bacteria and that they will be useful in the treatment of drug-resistant lung infections, pneumonia, hospital microbe, and septic bacteria. Among these, Flavocillin was the most effective against Corynebacterium Stiratum with a MIC value of 1, doing much better than Ampicillin and better than the combined effects of Amoxicillin and Clavulanic acid.

Flavocillin has a log P value of 2.7053 according to In Silico screening results. It obeys all 5 Lipinski rules such as a Molecular Weight of fewer than 500 daltons and no more than 5 proton donors and acceptors.

According to the results at Uppsala University, Department of Bacteriology, Flavocillin Ammonium salt is highly active against certain Staphylococcus Aureus strains and one strain of MRSA and cached some advantage in two strains over Ampicillin MIC values: EN1522 and EN1537.

According to the results at the Badebio lab of Eskişehir Anadolu University, Flavocillin Ammonium salts were highly active against S. mutans (bacteria that cause oral infections), and against C. Stiratum and M. catarrhalis (two drug-resistant dangerous bacterial species which causes upper and lower respiratory tract infections, pneumonia, and sepsis). In terms of the activity of Flavocillin on C. Stiratum, it had an advantage over Ampicillin as Ampicillin is not active on C. Stiratum whereas Flavocillin's MIC value against C. Stiratum is excellent. A combination of antibiotics was studied in comparison to Flavocillin, and in certain cases, Flavocillin MIC values are better when compared to these comparative In Vitro studies, as evident from the table on page 3 of the formal report at Badebio lab, which summarizes MIC values of Flavocillin Bioactivity over various bacteria. [13]

Discussion

A clinical study needs to be performed to evaluate the safety profile of the drug. Despite this, the multiple affinities for various substrates beget that flavocillin might not be a too strong inhibitor of OATPs in lower dosages and the toxicodynamic profile is dose-dependent.

In Silico testing for the activity was also carried out for Flavocillin Ammonium salt. The results showed that Flavocillin acid-free form had a binding affinity of 0,8624 for OATP1B1 and 0,9328 for OATP1B3. According to these results, Flavocillin acid-free form had higher binding activity on OATP1B1 whereas Flavocillin ammonium salt had higher binding affinity on OATP1B3. It is expected that clinical studies in the future will fully determine the exact characteristics and suitability of Flavocillin derivatives as new drug candidates.

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