

A Docking Study on Various Secondary Metabolites from *W. somnifera* on Tetrahydrodipicolinate N-Succinyltransferase Protein Involved in The Lysine Biosynthesis Pathway in *P. aeruginosa*

Nagwani AK* and Kashyap D

Department of Microbiology & Bioinformatics, University Teaching Department, Bilaspur University, Bilaspur, India

*Corresponding author: Nagwani AK, Department of Microbiology & Bioinformatics, University Teaching Department, Bilaspur University, Bilaspur, India, Tel: +91-9039950450; E-mail: amitkn52@gmail.com

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Abstract

A crucial survival factor for leading pathogen (carrying 40-60% mortality rate) is Tetrahydrodipicolinate N-Succinyltransferase (DAPD EC 2.3.1.117) involves in lysine biosynthesis pathway of *P. aeruginosa*. Targeting the lysine biosynthesis pathway is a opportunistic and logical site to inhibit the effect of this organism. *Withania somnifera*, also known as "Indian ginseng", a reputed herb in ayurvedic medicine, constituting various steroidal lactones (withanolides, withaferins) and saponins which shown various pharmacological activities. We described the docking of 11 secondary metabolites from *Withania somnifera* into the three dimensional structure of DapD protein of *P. aeruginosa* using swiss-dock server. Simultaneously, we have checked the ADME values and pharmacokinetics values of these secondary metabolites against the target protein. We have followed the "Lipinski rule of 5" principle and "Molinspiration" tool. Dihydrowithaferin, 4-B, Hydroxywithanolide, Withanoloide E, Withanoloide F, Withanoloide D, Withanoloide A) have potential to be used as medicine to treat *P.aeruginosa* infections. Several known antibiotics also studied along with this approach for comparison purpose.

Background/Objectives: *Withania somnifera* additionally referred to as "Ashwagandha" and "Indian ginseng" is a medicative plant constituting (isopelletierine, anaferine, cuseohygrine, and anahygrine, etc.), hormone lactones (withanolides, withaferins) and saponins., *P.aeruginosa* is a leading gram negative opportunist infectious agent, carrying a high 40-60% death rate and causes grievous infections in people with compromised immune systems. Tetrahydrodipicolinate N-Succinyltransferase (DAPD Europetwo.3.1.117) potential targets for brand spanking new antibacterial drug medication.

Methods/Statistical analysis: The tying up method involves the prediction of matter conformation and orientation (or posing) inside a targeted binding web site. Swiss Dock may be a tying up internet server. All calculations are performed on the server aspect, so for tying up run's don't need any machine power from the user. The ligands were neutralized and checked for his or her ADME properties victimization computer code Molinspiration obtainable at machine resources for Drug Discovery (CRDD). The secondary metabolites and antibiotics were conjointly subjected to Lipinski Rule of 5 value's on server The Supercomputing Facility for Bioinformatics & machine Biology (SCFBio), Indian Institute of Technology, N. Delhi.

Findings: Among the eleven secondary metabolites from *Withania somnifera* and seven antibiotics we elect for docking, Ciproflaxin, Cephalosporin, Tobramycin, and Azlocillin showing the higher result compare to alternative compounds. Results of ADME properties showed Compounds as 4-B hydroxywithanolide, 2-3 dihydowithaferin A, Withanoloide E, Withanoloide D, Withanoloide A, and Withanoloide F has higher ADME values then the unremarkably used antibiotics (Azlocillin, Ciproflaxin, Ticarcillin, and antibiotic). Results of Lipinski rule of five values are given below in one table. Secondary metabolites like 4-B hydroxywithanolide, 2-3 dihydowithaferin A, Withanoloide E, Withanoloide D, and Withanoloide F showed higher results for Lipinski rule of five compare to antibiotics like Alocillin, Mefoxin, Meropenem, and Tobramycin.

Improvements/Applications: Ashwagandha is employed from a few years for several treatments. This analysis additionally stresses the likelihood of this plant to use for cure of an added chronic infection. The experiment shows probabilities to develop a brand new drug.

Keywords: *P. aeruginosa*; *W. somnifera*; Tetrahydrodipicolinate N-Succinyltransferase; Swiss dock; Lysine biosynthesis pathway

Introduction

P. aeruginosa is associate expedient human infectious agent. It's an expedient as a result of it cause infection to healthy people. Instead, it typically affects immune-compromised patients, like those with monogenic disorder, cancer, or AIDS. *P. aeruginosa* may be a leading gram-negative expedient infectious agent and, carrying a 40-60% rate.

It complicates ninetieth of pancreatic fibrosis death, and lastly, it's invariably listed joined of the highest 3 most frequent gram-negative pathogens everywhere world [1]. *P. aeruginosa* conjointly carries a great deal of chromosome-mobilizing plasmids that a terribly vital to the organism's way as a microorganism [2].

P. aeruginosa attacks mucoviscidosis patients *via* airway and once it's in, it uses its flagellum for visit the hypoxic zone, associate degree oxygen depleted atmosphere. This can be the transition state for *P. aeruginosa* undergoes for associate degree aerobic to associate degree anaerobic microorganism and starts forming biofilms anaerobically. Once this can be fashioned, the *P. aeruginosa* will sense their population *via* gathering sensing, wherever they secrete low mass pheromones that change them to speak with one another at intervals a community [3,4]. This offers them the a resistance to several defenses, as well as anti-Pseudomonas antibiotics like ticarcillin, Mefoxin, tobramycin, and antibiotic, as a result of once the bacterium sense that their outer layer of biofilm is being destroyed, the inner layers can grow a lot of powerfully to alter the community [5].

Randomized transposon mutagenesis screens had indicated several genes from the diaminopimelate (DAP) biosynthetic pathway to lysine in *P. aeruginosa* as essential, suggesting that this gene products as potential targets for new antibacterial drugs. *P. aeruginosa* mutants where the dap A sequence had been deleted were viable and able to grow throughout a mouse internal organ infection model, suggesting that Dap A is not associate best target for drug development against this organism [6].

Plants are made during a wide selection of secondary metabolites like tannins, terpenoids, alkaloids, flavonoids etc. that are found to own antimicrobial properties. *Withania somnifera* (L) Dunal (Ashwagandha or Indian "Ginseng") is wide employed in ayurvedic medicines and is consumed as a dietary supplement round the world. The major bioactive compounds of Ashwagandha are steroidal lactones compounds referred to as withanolides. They conjointly contain many alkaloids, withanosides, reducing sugars, few flavonoids, tannins etc. [7].

There are several novel structural variants of withanolides with modifications either of the chemical element skeleton or the aspectchain and these have typically been delineate as changed withanolides or ergostan-type steroids associated with withanolides. Hence, the current study was aimed to spot the potential Dap D inhibitors from *W. somnifera* by molecular arrival studies exploitation Swiss Dock net server [8,9].

Materials and Methods

Selection of docking molecules

The SDF (SQL Server Compact info File) files of eleven varied chemicals (secondary metabolites) has been downloaded from Pubchem info of NCBI. For the docking studies. Equally the commercially offered medicine specifically antibiotics like Azlocillin, Ciproflaxin, Levofloxacin, Meropenem, Ticarcillin and antibiotic were taken from the literature.

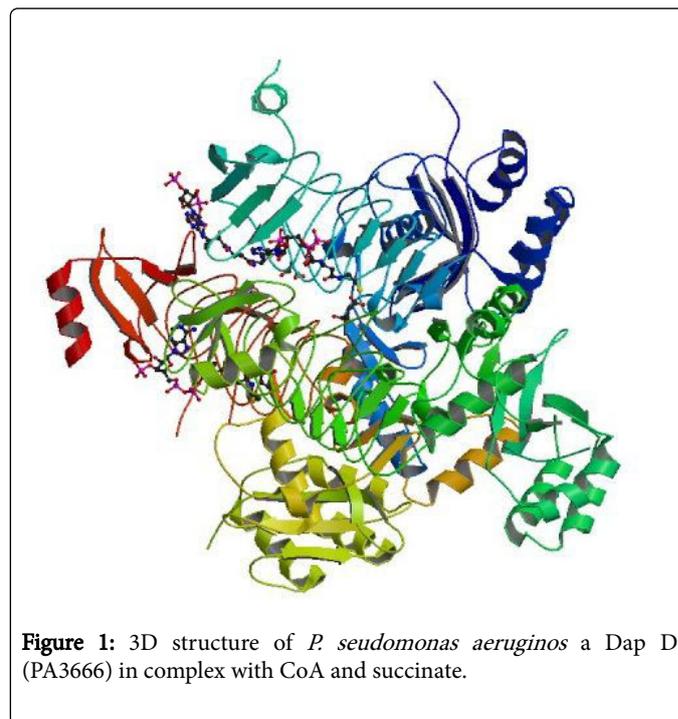
The ligands were neutralized and checked for their ADME properties mistreatment code Molinspiration on the market at machine resources for Drug Discovery (CRDD) (<http://www.molinspiration.com/cgi-bin/properties>). It helps in analyzing the

pharmacological medicine and pharmacodynamics of the matter by assessing the drug like properties.

The secondary metabolites and antibiotics were additionally subjected to Lipinski Rule of 5 values on server The Supercomputing Facility for Bioinformatics & machine Biology (SCFBio), Indian Institute of Technology, N. Delhi. Lipinski rule of five helps in characteristic between drug like and non-drug like molecules. It predicts high likelihood of success or failure because of drug likeness for molecules compliant with a pair of or a lot of rules [10,11].

Target protein

Tetrahydrodipicolinate N-Succinyltransferase (DAPD European Economic Community two.3.1.117) could be a succinyl-coenzyme A (SCoA) dependant accelerator. Dap D shows distinct options at the N and C terminal domains that area unit structurally completely different from those delineated for Dap D enzymes from gram-negative microorganism and shows additional similarity to Dap Ds from M. T.B. The sequence/structure of Dap D super molecule is downloaded from Protein data Bank (PDB ID-3R5A, B, C) As shown in Figure 1.



Docking

Docking was applied victimisation Swiss Dock internet server [12]. The structure of the target super molecule, similarly as that of the matter, is often mechanically ready for arrival. Additionally, the cumbersome syntax of the arrival engine is hidden behind a clean internet interface providing cheap various sets of parameters similarly as sample input files. All calculations area unit performed on the server aspect, so arrival runs don't need any machine power from the user. The interpretation of arrival results and their integration into existing analysis pipelines is greatly expedited by the seamless image of arrival predictions within the UCSF Chimera molecular viewer, which might be launched directly from the online browser. The sequence code 3R5A, B, C was used for arrival purpose.

Structure of 3R5A, B, C was submitted to Swiss Dock sever. The series (3R5A) has been used for Swiss Dock that is predicated on EADock DSS with anoloides and different compounds (from *W. somnifera*) were uploaded for docking.

The Predicted files from Swiss Dock server were analyzed exploitation UCSF Chimera. UCSF Chimera may be an extremely protractible program for interactive mental image and analysis of molecular structures [13]. Discovery Studio beholder version four, 2 were used for mental image of docked ligands. We have a tendency to were used PyMOL for analysis of docking results. The code free for academic and analysis purpose [14]. VEGA ZZ was used for format conversion and mental image of compounds and docking results [15-17] as shown in Figure 2.

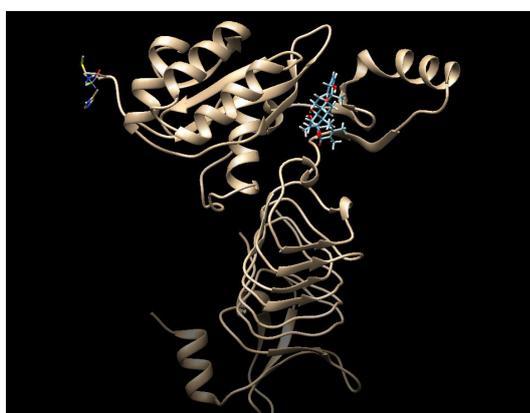


Figure 2a: With anolide E with Tetrahydrodipicolinate N-Succinyltransferase viewed in UCSF Chimer.



Figure 2b: With anolide F with Tetrahydrodipicolinate N-Succinyltransferase viewed in UCSF Chimera.



Figure 2c: 4-B, hydroxywithanoloide E with Tetrahydrodipicolinate N-Succinyltransferases viewed in UCSF Chimera.

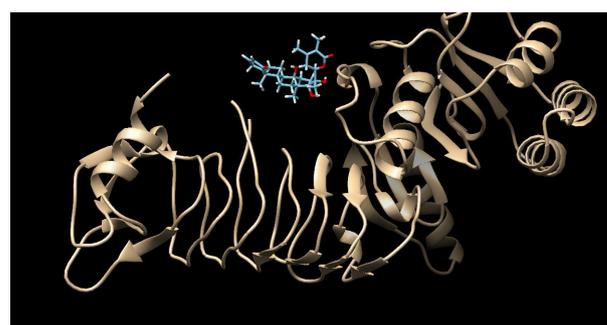


Figure 2d: 2-3dihydrowithaferin with Tetrahydrodipicolinate N-Succinyltransferase viewed in UCSF Chimer

Figures 2a, 2b, 2c and 2d show the best docking interaction of target protein and some secondary metabolites [18-21].

Results

We took eleven secondary metabolites from *Withania somnifera* and seven antibiotics for docking study. The docking confirmation with best docking Score had been analyzed. The results obtained from Swiss-Dock were analyzed mistreatment UCSF Chimera. Swiss-Dock results are given in terms of full fitness and ΔG Kcal/mol. The number of hydrogen bond found between the target super molecule and also the secondary metabolites ranged 1- 3.

Binding mode of available drug molecules

For comparison purpose, a bunch of antibiotics were extensively utilized for tying up with target macromolecule. square measure able to analyze that and therefore the results are, Ciproflaxin, antibiotic drug, Tobramycin, and Azlocillin showing sensible interaction in terms

of ΔG and nearly all are having sensible binding interactions as shown in Table 1.

S.no.	Antibiotics	Energy	Simple fitness	Full fitness	Cluster Rank	No. of Hydrogen Bond
1	Azlocillin	110.05	110.05	-1565	0	3
2	Cephalosporin	130.33	130.33	-1484.5	2	1
3	Ciproflaxcin	266.25	266.25	-1382.5	7	2
4	Levoflaxcin	62.47	62.47	-1565	7	1
5	Meropenem	107.12	107.12	-1548.7	5	2
6	Ticarcillin	96.91	96.91	-1564.2	7	2
7	Tobramycin	113.73	113.73	-1525.1	4	3

Table 1: Antibiotics with their docking results.

Binding mode of bioactive compounds from *W. somnifera*

After analysis of result, we will clearly observe that, after we have taken full fitness as criteria, we tend to area unit able to found Scopoletin, Cuscohygrine, Tropine, Cysteine showing high binding

affinity to the target macromolecule. Once the results of docking were analyzed using ΔG as criteria, 4-B hydroxywithanolide, 2-3 dihydowitaferin A, Withanoloide E, Withanoloide D, Withanoloide A, showed high affinity to focus on macromolecule as shown in Table 2.

S.no.	Compounds	Energy	Simple fitness	Full fitness	Cluster Rank	No. of Hydrogen Bond
1	Dihydowitaferin	274.27	274.27	-1334.1	7	1
2	4-B, Hydroxywithanolide	284.92	284.92	-1320.7	4	2
3	Withanoloide E	313.8	313.8	-1285.9	16	2
4	Withanoloide F	83.49	83.49	-1501.9	22	3
5	Withanoloide D	339.55	339.55	-1253.7	5	2
6	Withanoloide A	266.67	266.67	-1363.4	4	1
7	Choline	77.34	77.34	-1580.3	1	1
8	Tropine	34.67	34.67	-1597.7	7	2
9	Cuscohygrine	-5.71	-5.71	-1682.9	4	1
10	cysteine	1.45	1.45	-1626.8	3	3
11	scopoletin	1.66	1.66	-1618.7	38	2

Table 2: Compounds with their docking results.

Results of ADME properties

It showed Compounds as 4-B hydroxywithanolide, 2-3 dihydowitaferin A, Withanoloide E, Withanoloide D, Withanoloide A,

and Withanoloide F has higher ADME values then the normally used antibiotics (Azlocillin, Ciproflaxin, Ticarcillin, and Tobramycin) as shown in Table 3.

S.no.	Compounds	MiLogP	TPSA	MW	Antibiotics	MiLogP	TPSA	MW
1	Dihydowitaferin	3.88	96.36	472.62	Azlocillin	0.99	148.14	461.5
2	4-B, Hydroxywithanolide	2.26	136.82	502.6	Ticarcillin	0.44	124	384.44
3	Withanoloide E	3.18	116.59	486.61	Tobramycin	-5.7	268.19	467.52

4	Withanoloide F	3.75	104.06	470.61	Ciproflaxin	-0.7	74.57	331.35
5	Withanoloide D	4.15	96.36	470.61				
6	Withanoloide A	4.15	96.36	470.61				

Table 3: Comparison of ADME values.

Results of Lipinski rule of 5

Values given in Table no. 4. Secondary metabolites like 4-B hydroxywithanolide, 2-3 dihydowitaferin A, Withanoloide E,

Withanoloide D, and Withanoloide F showed higher results for Lipinski rule of five compare to antibiotics like Alocillin, Meropenem, and Antibiotic drug as shown in Table 4.

S.no.	Compounds	Molecular Weight	High Liphophicity	Molar Refractivity	Antibiotics	Molecular Weight	High Liphophicity	Molar Refractivity
		(130.0 to 725.0)	(-2 to 6.5)	(40-130)				
1	Dihydowitaferin	472	3.58	124.56	Azlocillin	460	3	110.85
2	4-B, Hydroxywithanolide	502	1.73	127.43	Cephalosporin	385	4	84.92
3	Withanoloide E	486	2.75	126.04	Meropenem	383	3	92.27
4	Withanoloide F	470	3.54	126.52	Tobramycin	472	3	101.87
5	Withanoloide D	470	3.5	124.51				
6	Withanoloide A	470	3.5					

Table 4: Comparison of Lipinski rule of 5 values.

Discussion

Interactions of secondary metabolites and antibiotics at the active site of Dap D. The two adjacent subunits that kind a lively website cleft however distinct from the binding sites for CoA and succinate. We found result in form of full fitness and ΔG Kcal/mol are reliable because it is additionally not showing results once there's no suitability of chemical with target super molecule for binding, whereas with Swiss dock, whenever results were obtained in terms of full fitness. There wasn't one case, after we didn't get results.

Conclusion

All the work done here recommend that the compounds beneath study (Dihydowitaferin, 4-B, Hydroxywithanolide, Withanoloide E, Withanoloide F, Withanoloide D, Withanoloide A) have potential to be used as drugs to treat *P. aeruginosa* infections in humans.

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References

1. Bhawsar NA, Singh M (2014) Isolation and characterization of *Pseudomonas aeruginosa* from waste soybean oil as biosurfactants which

- enhances biodegradation of industrial waste with special reference to kosmi dam, betul district, (M.P.). Int J Adv Res 2: 778-783.
2. Raj LM, Kalaigandhi V, Kanagaraj C (2015) The occurrence of (MDR/ MDS) *Pseudomonas aeruginosa* among nosocomial and community acquired infections in and around coimbatore, India. Int J Curr Microbiol App Sci 4:753-761.
3. Palamthodi SM, Gaikwad VJ, Ghasghase NV, Patil SS (2011) Antibacterial targets in *Pseudomonas aeruginosa*. Int J Appl Pharm Biol Res 2: 159-164.
4. Kitchen DB, Decornez H, Furr JR, Bajorath J (2004) Docking and scoring in virtual screening for drug discovery: Methods and application. Nat Rev Drug Discov 3: 935.
5. Smiley A, Hassett D (2010) *Pseudomonas aeruginosa* biofilm infections in cystic fibrosis, biofilms, infection, and antimicrobial therapy. Future Microbiol 5: 1663-1674.
6. Schnell R, Oehlmann W, Sandalova T, Braun Y, Huck C, et al. (2012) Tetrahydrodipicolinate N-Succinyltransferase and dihydrodipicolinate synthase from *Pseudomonas aeruginosa*: Structure analysis and gene deletion. PLoS One 7: e311.
7. Santhi N, Aishwarya S, (2010) Insights from the molecular docking of withanolide derivatives to the target protein PknG from *Mycobacterium tuberculosis*. Bioinformation 7: 1-4.
8. Meng XY, Zhang HX, Mezei M, Cui C (2011) Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7:146-157.
9. Elliotatn TSJ, Greenwood DD (1983) The response of *Pseudomonas aeruginosa* to azlocillin, ticarcillin and cefsulodin. J Med Microbiol 16: 351-362.
10. Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial- resistant *pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 22: 582-610.

11. Grosdidier A, Zoete V, Michielin O (2011) SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res 39.
12. SwissDock, a protein-small molecule docking web service based on EADock DSS.
13. UCSF Chimera: An extensible molecular modeling system
14. View 3D Molecular Structures. Pymol.
15. Molecular Modeling Toolkit, Drug Design Laboratory.
16. Oberhardt MA, Puchalka J, Fryer KE, Martins dos Santos VA, Papin JA (2008) Genome-Scale Metabolic Network Analysis of the Opportunistic Pathogen *Pseudomonas aeruginosa* PAO1. J Bacteriol 190: 2790-2803.
17. Hancock RE, Speert DP (2000) Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. Drug Resist Updat 3: 247-255.
18. Gfeller D, et al. (2014) SwissTarget Prediction: a web server for target prediction of bioactive small molecules. Nucleic Acids Res 42: 32-38.
19. Davis L, Kuttan G (2000) Immunomodulatory activity of *Withania somnifera*. J Ethnopharmacol 71: 193-200.
20. Velasco AM, Leguina JJ, Lazcano A (2002) Molecular Evolution of the Lysine Biosynthetic Pathways. J Mol Evol 55: 445-449.
21. Hutton CA, Perugini AM, Gerrard AJ (2007) Inhibition of lysine biosynthesis: an evolving antibiotic strategy. Mol BioSyst 3: 458-465.