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Similarity in the Amino Acid Sequences of *Mycobacterium tuberculosis* Protein Targets Involved in Binding Sites of Docking with Thiacetazone

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Abstract

Although according to WHO document, between 1990 and 2015, both TB mortality and its incidence has been fallen over 47% worldwide, the spread of multidrug-resistant strains of M*ycobacterium tuberculosis* reveals clearly that the efforts to find new drugs should not be stopped and the pathogenic microorganisms develop resistance. More extended knowledge about existing drugs is critical to design new and more effective medicines. In this study, we report the amino acid sequences involved in binding sites of 70 M. tuberculosis protein targets' docked with Thiacetazone (TAC), one of the extensively used antitubercular drug that is used in combination with other antitubercular agents to break multi-drug resistant TB. Categorization of protein targets was performed on the basis of the free energy of binding for the docked compounds. Comparison of the binding sites with the aim of ClustalW application indicated huge similarities in their amino acid sequences among target complexes.

Keywords Thiacetazone; Docking; Amino acid sequence; Binding site similarities; Mycobacterium; ClustalW; Arguslab

Introduction

Tuberculosis (TB) is a fatal contagious disease that can affect almost any part of the body especially the lungs. It is one of the top 10 causes of death worldwide. In 2015, 10.4 million people fell ill with TB and 1.8 million died from the disease [1]. Roughly one-third of the world's population has been infected with M*ycobacterium* tuberculosis, and new infections occur at a rate of one per second [2]. In 2015, the largest number of new TB cases occurred in Asia, with 61% of new cases, followed by Africa, with 26%. It is noted that 87% of new TB cases occurred in the 30 high TB burden countries. Six countries accounted for 60% of the new TB cases: India, Indonesia, China, Nigeria, Pakistan, and South Africa. Global progress depends on advances in TB prevention and care in these countries [3]. Annual average incidence rate of tuberculosis in Iran is 17.9 in 100,000 patients [4]. Tuberculosis incidence is higher in Balochistan, Khorasan, Golestan, Gilan, Kurdistan, Western Azerbaijan, Khuzestan, and southern coasts of Iran [5]. Multi-drug resistant (MDR) strains [6], extensively drug-resistant (XDR) strains [7] and XXDR strains as GB Migliori et al., mentioned in Italy in 2007 [8], drug-resistant tuberculosis (TDR) that has been identified in three countries; India, Iran and Italy and in all of them resistance to ordinary TB drugs is the common problem, make an important issue in TB treatment [9-11]. In 2015, an estimated 480,000 people worldwide developed MDR-TB, and an additional 100,000 people with rifampicin-resistant TB were also eligible for MDR-TB treatment. India, China, and the Russian Federation accounted for 45% of the 580,000 cases. It is estimated that about 9.5% of these cases were XDR-TB [12]. There are many efforts to combat with these drug resistant strains.

New drug synthesis and improve the properties of the old ones are included. Bedaquiline with a new mechanism of action (inhibits mycobacterial ATP synthetase and depletes cellular energy stores) is one of them. Unfortunately in one of the phase 2 studies, there were more deaths among patients who had bedaquiline added to an antimycobacterial drug regimen than among those who had placebo added to the same regimen. Therefore, FDA allows the approval of drug, only for serious or life-threatening conditions that provide meaningful therapeutic benefit over existing therapies [13]. For instance thiacetazone (Figure 1) that belong to World Health Organization group 5 drugs for the treatment of tuberculosis [14] despite cheapness and extreme usage has some serious side effect like Steven Johnson syndrome and cutaneous hypersensitivity reactions especially among patients with human immunodeficiency virus infection [15,16]. Hence many efforts are underway to synthesize its new and superior analogue with better properties [17,18]. Drugs and their targets are like lock and key, for making good key it is important to recognize and know the lock in advance and because of economical aspects, beginning such research with virtual screening is a better manner. For instance, Kandasamy et al., [19] looked at the pathogenesis of TB in order to find newer drugs they performed molecular docking studies with a library of kinase inhibitors. As a result T95 was found, which is a potent inhibitor for PknI, and Lys 41 along with Asp90, Val92 and Asp96 were identified as functionally important residues they suggested that docking studies helped in identifying ligand inhibitor specific to PknI which was confirmed by laboratory experimentation. Homology modelling, docking, pharmacophore and site directed mutagenesis analysis to identify the critical amino acid residue of PknI from *M. tuberculosis* [19].

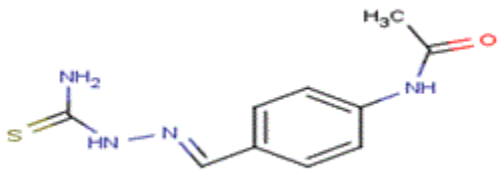

Figure 1: Thiacetazone.

In our study, a rapid and cheap method was used to study the amino acid sequences involved in the binding sites. This method leads to exploration of huge similarities that was not considered before.

Methods

At first the medical literature was retrospectively reviewed and well known *M. tuberculosis* protein target was chosen (Table 1) then they were downloaded in pdb format from protein Data Bank [20]. They were docked via Arguslab software version 4.0.1. Mark Thompson and Planaria Software LLC [21] to obtain free energy of binding measures between the thiacetazone and them. They were categorized on the basis of free energy of binding and then all amino acids involving in binding sites were recognized, briefly after docking all amino acids existed in the amino acid folder were selected, then at the binding site, it was chosen "hide" and then "show" option and after deselecting them it was chosen the "delete" option. Arguslab software deleted all amino acids except those involved in binding site. All binding site's amino acids' sequences belong to 70 *M. tuberculosis* target involved in docking with thiacetazone were compared via ClustalW application then it was performed a categorization on the basis of similar sequences that usually belong to same classes(Table 1) [22].

No	Category	Name	Classification	Free Energy of Binding (kcal/mol)	Sequence of amino acids in Binding Site
1		1GSI	Transferase	-9.55	RRNDFFPSYYY
2		1MRN	Transferase	-9.35	ARRRNDDDEEEHLFFPSYYYY
3		1G3U	Transferase	-9.28	RRNDFFPSYYY
4		1W2G	Transferase	-8.97	RRNFFPSYYY
5		1N5J	Transferase	-8.96	RRNDEFFPSYYY
6		1MRS	Transferase	-8.94	RRNDLFPSYYY
7		1N5I	Transferase	-8.78	RRNDDLFPSYYY
8	A	1W2H	Transferase	-8.59	RRNDLFFPSYY
9		3FNF	Oxidoreductase	-8.91	AADDGGGIIIIIILKMFFPSSTV
10		2PR2	Oxidoreductase	-8.69	ADDGGGIIIIILKMMFFPSSTWYV
11		2IEB	Oxidoreductase	-8.6	AADDGGGIIIIIILKMMMMFFPSTWYV
12		1ZID	Oxidoreductase	-8.58	ADDGGGIIIIIILKMMMFFFPSSTWYV
13		3FNH	Oxidoreductase	-8.47	AADDGGGIIIIIILLKMMFFFPSSTV
14		3FNG	Oxidoreductase	-8.45	AADDGGGIIIIIILLKMFFPSSYV
15		2IDZ	Oxidoreductase	-8.41	ADDGGGIIIIIILKMMMFFPSSTWYV
16		2IED	Oxidoreductase	-8.34	ADDGGGIIIIILKMMFFPSSTWTVV
17		1P44	Oxidoreductase	-8.32	ADDGGGIIIIIILKMMFFPSSTV
18		2B35	Oxidoreductase	-8.29	ADDGGGIIIIIILKMMFFPSSTV
19		2B36	Oxidoreductase	-8.19	ADDGGIIIIIILKMMMFFPSSTV
20	B	2NSD	Oxidoreductase	-8.15	ADDGGGIIIIIILKMMFFPSSTV
21		2AQI	Oxidoreductase	-8.12	ADDGGGIIIIIILKMMFFPSSTV
22		2H7N	Oxidoreductase	-8.09	ADDGGGIIIIIILKMMFFPSSTV
23		2AQ8	Oxidoreductase	-8.8	ADDGGGIIIIIILKKMMFFPSSTV
24		2NV6	Oxidoreductase	-8.03	AADDGGGIIIIIILKMMFFPSTWYV

25		2H9I	Oxidoreductase	-7.98	ADDGGGIIIIIILLKMMMFFPSSTWTV
26		3OEY	Oxidoreductase	-7.91	ADDGGGIIIIIILKMMFFPSSTV
27		2B37	Oxidoreductase	-7.89	ADDGGGIIIIIILKMMFFPSSTTV
28		1P45	Oxidoreductase	-7.77	AADDGGGIIIIIILKMMFFPSSTV
29		1ENZ	Oxidoreductase	-7.76	AADDGGGIIIIILKMFFFPSTV
30		3OEW	Oxidoreductase	-7.72	ADDGGGIIIIIILKMFFPSSTV
31		2AQK	Oxidoreductase	-7.64	AADDGGGIIIIIILKMFFPSTV
32		3OF2	Oxidoreductase	-7.58	ADDGGGIIIIIILKMFFPSSTV
33	B	2AQH	Oxidoreductase	-7.57	ADDGGGIIIIILKMFFPSSTVV
34		1ENY	Oxidoreductase	-7.57	ADDGGGIIIIIILKMFFPSSTV
35		2H7P	Oxidoreductase	-7.32	ADDGGGIIIIIILKMFFPSSTV
36		2X22	Oxidoreductase	-7.27	AADDGGGIIIIIILLKMMFFPSSTV
37		2H7L	Oxidoreductase	-7.25	ADDGGGIIIIIILKMMFFPSSTV
38		2H7M	Oxidoreductase	-6.97	ADDGGGIIIIIILMMFFPSSTV
39	C1	3F69	Transferase	-7.93	AANDEGGLLKMFTW
40		3F61	Transferase	-7.85	AANDEGGLKKMMMFSTVVV
41	C2	1DF7	Oxidoreductase	-7.5	AARRRDQQGGGGGGGIIILLSTWYV
42		1DG5	Oxidoreductase	-7.4	AARRRRDQQGGGGGGGIIILLSSTWYV
43	C3	3HEM	Transferase	-8.4	CEGGHILLFFYYYY
44		1KPI		-8.39	CEGGIILLLFFTWYYYY
45	C4	3HA5	Transferase	-7.85	AQEEGGGHIILLFSTTWY
46		2FK8		-7.69	ACQQEEGGGHIILLFSSTTWY
47	C5	2WGE	Transferase	-7.84	ACGGHHFFFPTTV
48		2AQB		-7.41	ADCGGHHMFFFPTTV
49	C6	1L1E	Transferase	-7.23	ARCQEEGGGGHILLFSSTTFT
50		3HA3		-7.21	AQEEGGGHIILFSTTWY
51	C7	2Q1Y		-7.04	AAARNNEEGGGGGGGGLFPTT
52		1RLU	Cell Cycle Signaling Protein	-6.93	AAARNNDEEGGGGGGGGLFPTT
53	C8	1RQ7		-6.93	ARNNDEEGGGGGGGLFPT
54		1QPN	Transferase	-6.96	AARRDGGHHLKST
55		1QPQ		-6.65	RRHLLKKST
56	C9	3PYF	Transferase	-6.72	AANDDGGGGLLLMPV
57		3PTY		-5.47	AANDDGGGGLLLMPV
58		2A8X	Oxidoreductase	-8.83	AAADCCNEGGGGGGGGGHILLKFFPPTYYY VVV
59		1KPG	Transferase	-8.39	AACQQGGGGHILLFSSTTWYYV
60		1M4I	Transferase	-7.94	ADDDDDEGFFSSTW

61		1X8A	Theoretical Models	-7.77	ADCHIFTYYYV
62		1EYE	Transferase	-7.75	RNNDDDGLKMFSVVVH
63		2HW2	Transferase	-7.72	ANGGGLLLLKKMFFSTWVV
64		2WGG	Transferase	-7.56	AAAEGILPV
65		1N4G	Oxidoreductase	-7.44	AFTWVV
66		3PYE	Transferase	-7.2	DGHLSTYY
67	M	2WGF	Transferase	-6.629	ELLV
68		3GWC	Transferase	-6.24	RRRHHHHM
69		3OXH	Hydrolase inhibitor	-6.73	ANHHIMY
70		1NKT	Protein Transport	-8.57	RRNDDQQEGGGLFPTTW

Table 1: Mycobacterium targets that docked with thiacetazone.

Results

There were some repeated patterns in amino acid sequences involved in binding site of thiacetazone and protein targets, among those targets. It was found out category A, B and C. Category A and B belong to the transferase and oxidoreductase classes, respectively. Category C consist of various target's classes such as oxidoreductase, transferase and the cell cycle signaling protein classes (Table 1). Figure 2 represents the frequency of amino acids involved in binding sites of our 70 Mycobacterium protein targets and thiacetazone.

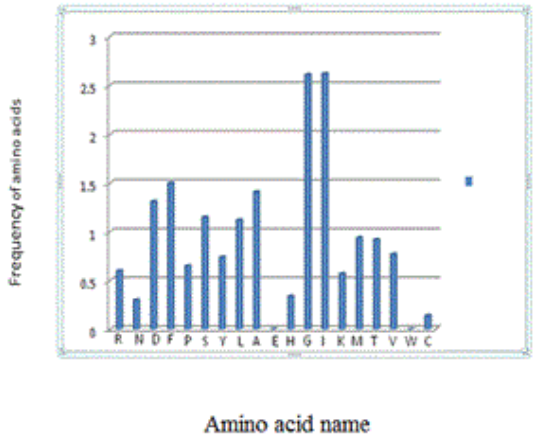

Figure 2: Frequency of amino acids in binding sites of Mycobacterium protein targets and thiacetazone.

Category A

This category consists of 8 targets: 1GSI, 1MRN, 1G3U, 1W2G, 1N5J, 1MRS, 1N5I, 1W2H (Figure 3). All of them belong to transferase class and form about 11% of these Mycobacterium targets. Free energy of binding in this Category is the least (-8.59_-9.55 kcal/mol). Amino acids sequences of their binding sites and the rate of similarity represent in Table 1.

Figure 4 shows a diagram of frequency belong to amino acids involved in binding sites of thiacetazone- target in group A. Table 1 also shows some diversity, in the case of 1MRN and though 1W2G, 1N5J, 1MRS, 1N5I and 1W2H have the same sequences but there is also a bit different. Generally, except 1MRN their binding sites begin with 2 arginine molecules and end with 2 to 4 tyrosine molecules. The number of amino acids in binding site of this target are 21 whereas others have only 10-12 (Figure 3).

```
1GSI     --RRNDE------FFPSYYY-        12
1W2H     --RRNDL------FFPSYY--        11
1MRN   ARRRNDDDEEEHLFFPSYYYY         21
1N5I     --RRNDD------ LFPSYYY-       12
1GSI     --RRND------- FFPSYYY-       11
1G3U     --RRND-------FF PSYYY-       11
1MRS     --RRND------- L FPSYYY-      11
1W2G     --RRN-------- F FPSYYY-      10
              ***          -*****
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Figure 3: Multiple sequence alignments of amino acids in binding site involved in docking between thiacetazone and protein targets belong to category A.

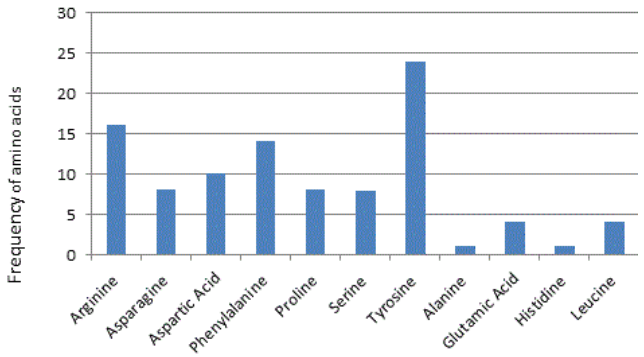

Figure 4: Frequency of amino acids involved in thiacetazone target binding site in group A.

Category B

This category consist of 30 targets: 3FNF, 2PR2, 2IEB, 1ZID, 3FNH, 3FNG, 2 IDZ, 2 IED, 1p44, 2B35, 2B36, 2NSD, 2AQI, 2AQ8, 2H7N, 2NV6, 2H9I, 3OEY, 2B37, 1P45, 1ENZ, 3OEW, 2AQK, 3OF2, 2AQH, 1ENY, 2H7P, 2X22, 2H7L , 2H7M (Figure 5). This group with one hydrophobic amino acid, alanine beginning and another hydrophobic amino acid, valine in the end form about 43% of these Mycobacterium targets (Figure 5) [15,16]. All of them belong to the oxidoreductase class and free energy of binding in this category is between-8.91_-6.97 kcal/mol (Table 1).

Category C

There are 9 small groups that consist of only two or three members and form about 27% of these targets. They are also very similar in Sequence of amino acids in binding site (Table 1).

The rest of 13 targets have no similarity in binding site amino acid sequences they are indicated as category M. Table 1 *Mycobacterium* targets that docked with thiacetazone.

Figure 6 shows a diagram of frequency belong to amino acids involved in binding sites of thiacetazone - target in group B.

Discussion

Results of multiple sequence alignment of protein targets belong to category A and B via ClustalW server represent a huge degree of similarity among the amino acids sequences involved in binding site in docking between thiacetazone and protein targets (Figures 2). Figure 3 and 4 represent frequency of amino acids involved in thiacetazone – target binding site in group A and B respectively. We found out high frequency of arginine and tyrosine in group A binding sites which meets the results have represented in prior studies [23, 24]. Protein targets belong to category A have the least free energy of binding (-8.59 _ -9.55 kcal/mol) among these 70 protein targets.

1P45	AADDGGG–IIIIIL - - KMMFFPSS- -T V-	24
1P44	- A DDGGG –IIIIIL - - KMMFFPSS- -T V-	23
3FNH	AADDGGG IIIIIL K – MMFFFPSS - -T V-	26
2B36	- ADDGG - - IIIIIL K – MMMFFPSS - -T V -	23
2H9I	- A DDGGGIIIIIIILL K - MMMFFPSSTWTV-	27
3FNG	AADDGGG – IIIIIL - - LKMFFPSS - -YV-	24
2AQH	- ADDGGG – IIII - - - - LKMFFPSS- -T VV	22
2B37	- ADDGGG - IIIIIL - - K MMFFPSS- -T TV	24
2H7M	- ADDGGG - IIIIIL - - - MMFFPSS- -T V -	22
3OEY	- ADDGGG - IIIIIL - - K MMFFPSS- -T V-	23
2AQI	- ADDGGG - IIIIIL - - K MMFFPSS- -T V-	23
2B35	- ADDGGG - IIIIIL - - K MMFFPSS- -T V-	23
2NSD	- ADDGGG - IIIIIL - - K MMFFPSS- -T V-	23
2H7N	- ADDGGG - IIIIIL - - K MMFFPSS- -T V-	23
2H7L	- ADDGGG - IIIIIL - - K MMFFPSS- -T V-	23

Figure 5: Multiple sequence alignment of amino acids sequences involved in binding site in docking between thiacetazone and protein targets belong to category B.

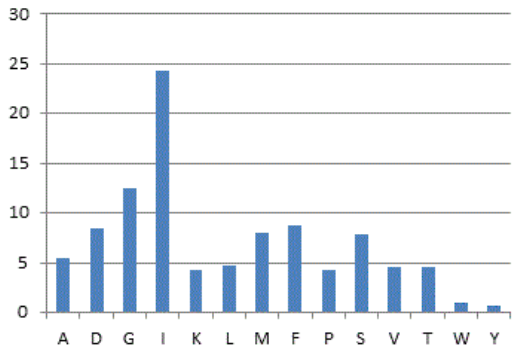

Figure 6: Frequency of amino acids involved in thiacetazone–target's binding site in group B.

The amino acids sequences of their binding sites begin with 2 arginine molecules which on the basis of its geometry, charge distribution and its ability to form multiple H-bonds are ideal for binding negatively charged groups such as thiacetazone with six potential negatively charged atoms (one oxygen, four nitrogen and one sulphur). On the other, hand these binding sites end with 2 to 4 tyrosine molecules which because their ability to make π interactions and their hydrophobic surface area are generally most abundant

residues in all binding sites and it is not surprisingly that the free energy of binding in this category is maximum [25-27] 1MRN begin with one alanine molecule which is second in rate of occurrence, accounting in a sample of 1150 protein [28] and then 3 arginine molecules. The arginine positive charge plus alanine hydrophobicity make it the second less free energy of binding in docking with in category A (Table 1). As Dennis A. Dougherty opinion that many drug–receptor interactions involve cation–π interactions, ammonium group belongs to thiacetazone in one side and aromatic ring available in arginine or tyrosine in binding site of group A and B respectively on the other side may involve in drug–receptor interactions [29].

As Pearson mentioned significant similarity can be to be homologous [24] and as Gary D. Stormo declared homologous sequences usually have the same, or very similar, functions [26] although there are some evidences to promote this idea that imidazo [1,2-c] pyrimidin-4-ol derivatives as antitubercular agents. One of their compound showed the highest docking score and H-bond interaction with Arg140 and Gly19 that was also confirmed by single crystal X-ray analysis. The in silico results are also validated with in vitro antitubercular activity of compound 7t. Compound 7b exhibited in vitro antitubercular activity [30].

Surekha et al., also used ClustalW application to Sequence alignment, found out the amino acid residues (Met1, Asp2, Glu43, Ala44, Glu47, Lys51, Ala157 and Leu158). We also found frequently repeated amino acid sequences of *M. tuberculosis* protein targets involved in binding sites of docking with thiacetazone [31].

Pulaganti.et al., (2014) performed a systematic study was conducted to get an insight about Mtb-OSBS enzyme and the corresponding inhibitors using in silico methods. The active site amino acids have been identified by comparing the template sequence with the Mtb-OSBS sequence. They identified that Lys (108), Asn (140), Asp (138), Lys (110), Glu (189), Ser(236), Asp (188), Arg (27), Tyr (52), and Ser (237) are highly conserved, and these may play a vital role as active residues, similar to that in template protein.

Molecular modeling and docking studies of O-succinyl benzoate synthase of M. tuberculosis—a potential target for antituberculosis drug design [32]. Surekha.et al., (2016) in the study of OPRTase as an anti-pathogenic target, a homology model of OPRTase was constructed using 2P1Z as a template. About 100 ns molecular dynamics simulation was performed to investigate the conformational stability and dynamic patterns of the protein. The aminoacid residues (Met1, Asp2, Glu43, Ala44, Glu47, Lys51, Ala157 and Leu158) lining in the binding site were predicted using Site Map. The amino acid residues (Met1, Asp2, Glu43, Ala44, Glu47, Lys51, Ala157 and Leu158) lining in the binding site were predicted using Site Map, a study that may provide better insight for designing potent anti-pathogenic agent [31].

Investigation of vital pathogenic target orotate phosphoribosyl transferases (OPRTase) from Thermus thermophilus HB8: Phylogenetic and molecular modeling approach [28].

Conclusion

Although many countries in sub-Saharan Africa still use extremely cheap thiocetazone, but severe (sometimes fatal) skin reactions in HIV positive patients due toit, lead to decline its usage and promote researches for synthesis of its other analogues to find out an new alternative has been performed concomitantly. Awareness about targets, rate of their maximum free energy of binding and their sequence of amino acids in binding site might be necessary for designing the new drugs that meet primary criteria. The implications of this study may be important for the design of those analogues. Although there are some evidences to promote this idea that the case of "function" is more complicated as the same enzyme have "different" roles in two tissues because of different circumstance but this method can be a good route for predicting of binding strength. Such methods may be present a good route for prediction hence as Stormo [26] mentioned we have expected that new agent with similar amino acids sequences involved in binding site in docking with protein targets has the same, or very similar, functions. Admittedly the second but more important step should be finding the function of these similar sequences.

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