J Mol Model (2009) 15:665–672
DOI 10.1007/s00894-008-0421-x

ORIGINAL PAPER

Search for inhibitors of AminoAcyl-tRNA synthases by virtual click chemistry

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Received: 22 August 2008 / Accepted: 7 November 2008 / Published online: 2 December 2008
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Abstract The increase of multidrug-resistant strains of bacteria to known classes of antibiotics present a severe challenge for modern medicine. The most promising strategy to combat pathogenic bacteria is to discover new drug targets. In this regard, aminoacyl-tRNA synthetases are particularly well suited to develop novel drugs that show no cross-resistance to other classical antibiotics. To date various chemical structures that inhibit AA-RS have been identified. In this report we present an interesting approach towards generating of Leu-RS inhibitors by virtual click chemistry. That is we identified key fragments for ligand binding within catalytic pocket of Leu-RS, generated the collection of similar fragments with the use of Ligand.Info, identified the fragments that are most strongly bound in different areas within the catalytic pocket, and finally with the use of virtual click chemistry we generated a set of molecules which are most likely to act as highly potent bacterial Leu-RS inhibitors.

Electronic supplementary material The online version of this article (doi:10.1007/s00894-008-0421-x) contains supplementary material, which is available to authorized users.

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Keywords Aminoacyl-tRNA synthetases · Drug design · Molecular docking · Virtual click chemistry · Virtual screening

Introduction

The increasing ratio of bacteria resistant to various known classes of antibiotics (sometimes virtually all prescribed antibiotics) presents a severe challenge for modern medicine. The most promising strategy to combat such pathogenic bacteria strains is to discover new drug targets, taking advantage of the information obtained from genomic and proteomic research. In this regard, aminoacyl-tRNA synthetases (AA-RS) are particularly well suited to develop novel drugs that show no cross-resistance to other classical antibiotics [1]. AA-RS are essential enzymes for protein biosynthesis. When one AA-RS is inhibited, the protein synthesis is also inhibited, which, in turn, causes cell growth arrest. Consequently, each compound that inhibits any of the AA-RS is a potential antibacterial agent (Fig. 1).

Moreover, there are significant structural differences between the eukaryotic (human) and prokaryotic (bacterial) enzymes that can be exploited in drug design. The clinical utility of AA-RS inhibitors is proven by the natural product Ile-RS inhibitor pseudomonic acid, which is currently marketed as an antibacterial agent for topical application [2].

To date various chemical structures that inhibit AA-RS have been identified. These inhibitors have either been isolated from natural sources or have been generated synthetically. The synthetic inhibitors are modifications of natural inhibitors, derivatives of the natural synthetase substrates and reaction intermediates, or have been identified by screening of compound libraries.

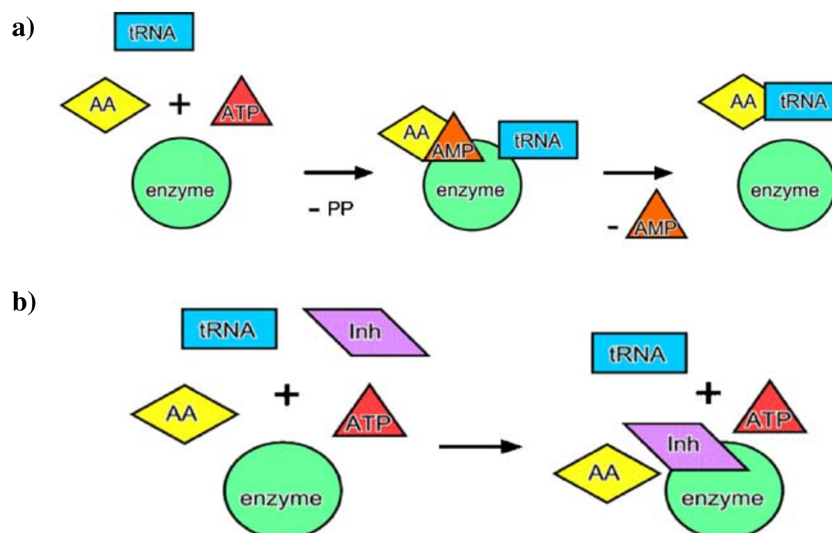

Fig. 1 Key mode of: **a)** action of aminoacyl-tRNA synthetases (in the first step enzyme catalyzes the reaction between a given aminoacid (AA) and ATP forming aminoacyl-AMP; then the aminoacyl moiety is transferred to tRNA; the final product, i.e. aminoacyl-tRNA is further utilized in protein synthesis), and **b)** the inhibition process (inhibitors binds inside the catalytic pocket of the enzyme and stops tRNA aminoacylation)

Computational methods

Previously, we have created freely available database of various AA-RS inhibitors [3]. Ligands were prepared in two conformations, A and B, the lowest energy in dreiding force field included in Marvin [4], and the most different from the first one (the biggest RMSD in a set of conformers of given molecule).

The only known structure of Leu-RS was derived from the protein from Thermus Thermophilus (Protein Data Bank [5]). Therefore, to study binding to Leu-RS from various organisms we have obtained sequences (see the Fig. S7 in supplementary materials for alignment) from UniProtKB/Swiss-Prot [6] and created 3D models of human (*H.c* – cytoplasmic, sequence Q9P2J5; *H.m* mitochondrial, sequence Q15031) and bacterial (*E.c* – *Escherichia coli*, sequence A7ZJ31; *H.p* – *Helicobacter pylori*, sequence P56457; *M.t* – *Mycobacterium tuberculosis*, sequence A5TYB2; *P.a* – *Pseudomonas aeruginosa*, sequence Q9HX33; *S.a* – *Staphylococcus aureus*, sequence Q5HF16) synthases using Metaserver [7] and Modeller [8] (we used 1GAX protein for creating model of human cytoplasmic Leu-RS and 1H3N protein for creating models of the rest of the proteins). As a result, we have obtained targets for docking different known and potential inhibitors.

Docking experiments were performed using AutoDock and MGLTools packages [9–11]. Bash shell script carried out following six steps for every receptor-ligand pair: preparing ligand (prepare_ligand4.py), preparing receptor (prepare_receptor4.py), preparing gpf file for AutoGrid (prepare_gpf4.py), preparing dpf file for AutoDock (prepare_dpf4.py), running AutoGrid (autogrid4), running AutoDock (autodock4).

We performed a blind docking with default parameters used in AutoDock 4 and flexible ligands approach. First of all, we checked how ‘flexible’ AutoDock calculations are? The AutoDock authors assert that during the docking the ligand is flexible [9–11]. We wanted to verify if the conformational space of a ligand is sufficiently probed during the docking, thus we used two different initial structures for every ligand. During AutoDock calculations ligands conformations are automatically modified (‘unbound extended state’ in AutoDock nomenclature) using Lamarckian genetic algorithm (LGA). Next the docking is performed, using LGA, to generate various poses of a ligand in the given protein-ligand complex. The AutoDock program calculates scores (protein-ligand binding free energies and K_i values) for each pose of a ligand and creates an ordered list of the poses in output dlg file. From the final output files we selected the docked structures of ligands with the lowest K_i value.

Results and discussion

Experimental data vs. *in silico* study

It is widely known, that the correlation between experimental values of inhibition constants and those gained using molecular docking software, contrary to the claims of software providers, is usually poor (40–60%). We docked

all the molecules stored in the IA database to the modelled targets (notice that they actually inhibit different AA-RS), and in Table 1 we compared experimental IC_{50} values with K_i values calculated using AutoDock only for Leu-RS inhibitors. Correlation is not too good (about 64%), but as good as could be expected in docking calculations. However, computed K_i values for known inhibitors seem to be sufficient to check if new potential inhibitors are better or worse than the known ones. Structures of the known inhibitors were presented in Fig. 2.

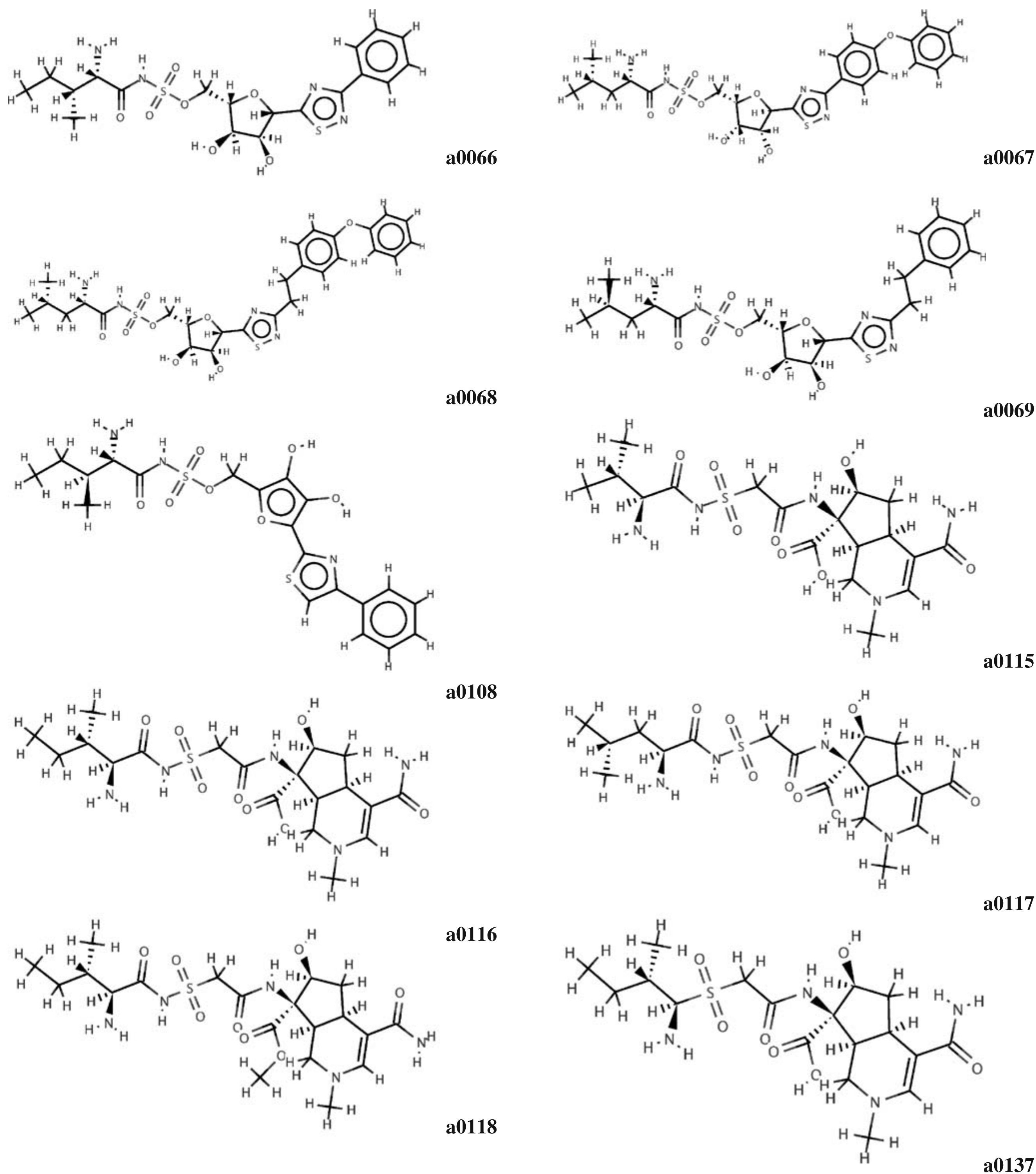

Fig. 2 Selected known inhibitors of Leu-RS [1, 2, 12, 13]

Table 1 Inhibition constant values (IC_{50}/μM) for known Leu-RS inhibitors compared with values (K_i/μM) calculated using AutoDock

Structure	Host	Experiment	*In silico* study
0066 [12]	*S. aureus*	>20	1.64
	E. coli	1.0	2.34
	Human	200	50.8 (*H.c*)
0067 [12]	*S. aureus*	0.054	0.047
	E. coli	0.0016	1.45
0068 [12]	*S. aureus*	0.1	0.15
	E. coli	0.006	0.92
	Human	1.15	3.31 (*H.c*)
0069 [12]	*S. aureus*	0.09	0.095
	E. coli	<0.002	3.64
	Human	0.73	0.47 (*H.m*)
0108 [1]	Human	0.6	4.09 (*H.m*)
0115 [13]	*S. aureus*	2.3	26.45
0116 [13]	*S. aureus*	1.55	1.41
0117 [13]	*S. aureus*	0.016	14.2
0118 [13]	*S. aureus*	186	37.95
0137 [2]	*S. aureus*	1.55	1.3

Strategy towards designing new inhibitors

It is possible to use known inhibitors to find new ones using Ligand.Info service [14]. Unfortunately, this strategy did not lead to better inhibitors than those known previously. As we observed, Ligand.Info service was unable to find similar molecules with aminoacid part. Ranges of inhibition constants were presented in Table 2.

Therefore, we applied another strategy-namely we divided the native reaction intermediate Leu-AMP into three fragments corresponding to the aminoacid, sugar, and nucleic base parts. Then we used Ligand.Info service to find compounds similar to these fragments. The comparison of the results from the docking experiment between the newly generated molecules and those obtained for the parental compounds (leucine, ribose, and adenine) allowed us to identify the molecules with stronger affinity towards prokaryotic enzymes than the parental molecules. We selected four compounds that are likely to bind stronger to the enzyme than leucine, three than ribose, and three than adenine (Table 3, Fig. 3).

Virtual click chemistry combined these fragments together and led to the generation of 36 new compounds. Results of the docking experiments are presented in Table 4. Molecules presented in Fig. 4 were identified as the most promising ones.

The results presented in Table 4 need comment due to sometimes huge differences in inhibition constants and only, at the first sight, few changes in the structures.

Firstly, the examined proteins' structures derived from different species differ from one another. The aligned sequences (Fig. S7 in supplementary materials) show differences, which due to protein structure building method can translate into even larger differences in the actual 3D structure of the catalytic pocket. Homology modelling may lead to proteins' models of different quality. It is virtually impossible to assess the model quality before the actual X-ray structure is recorded. However, the 3D-Jury score of our models (which to some degree describes the quality of the model) was high for all the structures - between 405 and 613. It is generally believed that the 3D-Jury score above 300 means that a protein model is of high quality [15].

Secondly, even small differences in the size of the molecules may translate into large differences of the K_i values. For example, for *P.aeruginosa* three similar molecules ade54leu3rib13, ade54leu3rib15, and ade54leu3rib16 (Fig. 4) have very different K_i values. The reason for such large differences in inhibition constants between ade54 leu3rib13 and ade54leu3rib15 is a linkage between sugar-like part and adenine-like part. As visible in the figures S1 and S2 (supplementary materials), ade54leu3rib15 is the

Table 2 Ranges of inhibition constant values (K_i/μM) for the known and potential Leu-RS inhibitors calculated using AutoDock

	Known inhibitors	Potential inhibitors
E.c	0.07564—7870	10.3–44400
H.p	0.00123–176.23	0.1–3120
H.c	61560–3.3	28560–11.9
H.m	715430–0.02	70270–0.2
M.t	0.04–33270	5.1–41120
P.a	0.006–2200	0.096–17670
S.a	0.003–6810	0.03–98760

The potential inhibitors were found via Ligand.Info service using set of the known inhibitors

Table 3 Inhibition constant values (K_i/μM) for adenine, leucine, ribose, and derivatives (generated via Ligand.Info), calculated using AutoDock for different hosts

	E.c	*H.p*	*H.c*	*H.m*	*M.t*	*P.a*	*S.a*
adenine	3780	1090	12580	114	7670	1310	1460
ade46	1120	213	1410	85	2080	49	184
ade47	521	123	2610	30	710	47	162
ade54	1580	73	5480	102	945	53	71
leucine	697	148	1250	220	2070	249	274
leu1	116	53	358	131	1460	39	15
leu3	358	10	132	5	504	30	36
leu5	530	30	651	38	602	12	3
leu15	702	27	1330	23	675	12	3
ribose	584	249	953	40	1320	320	349
rib13	1330	17	647	108	4550	56	15
rib15	224	26	424	66	797	41	14
rib16	477	44	244	86	2780	74	25

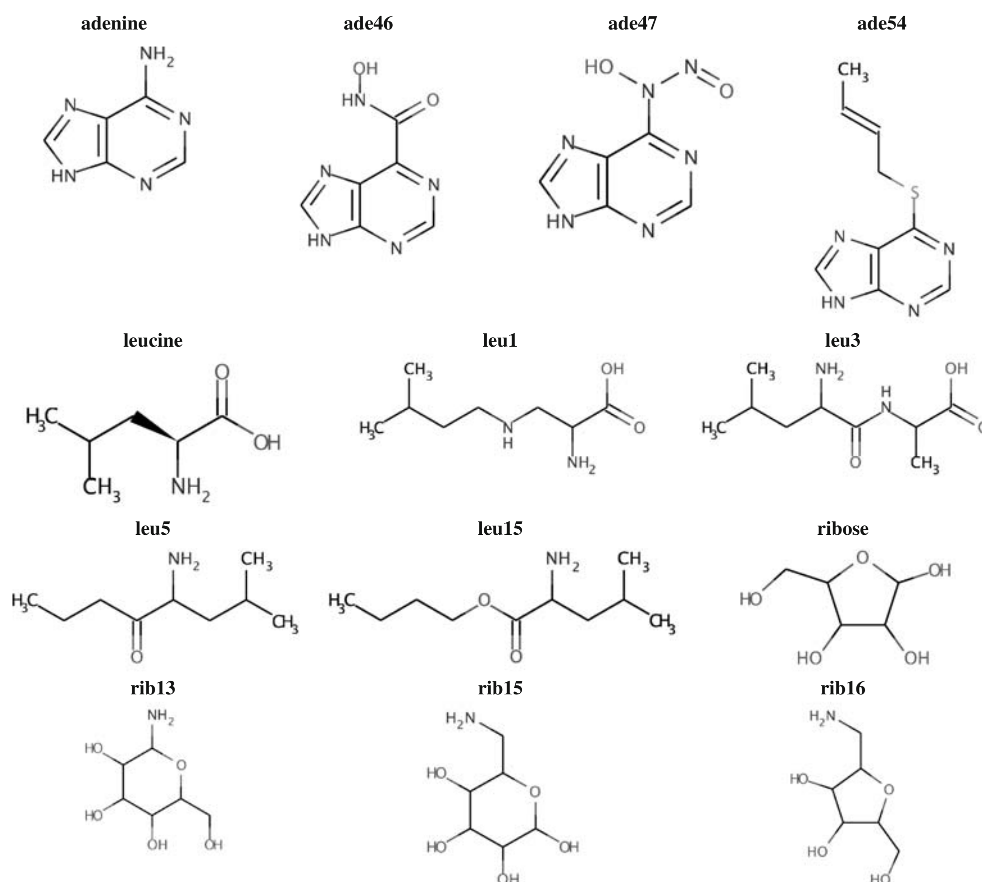

Fig. 3 Structures of fragments of Leu-AMP (adenine, leucine, and ribose) and their substitutes generated via Ligand.Info

worst ligand because it seems a little too big for the catalytic pocket - it has additional CH_2 linkage between the ribose-like and adenine-like parts. In the case of ade54leu3rib16 the ribose-like part is smaller than in ade54leu3rib15, thus this ligand can still find its place in the protein cavity.

Most promising inhibitors

In many cases, newly generated molecules are better inhibitors than the known Leu-RS inhibitors (compare inhibition constants given in Table 4 with those in the fourth column of Table 1). Interestingly, in the case of *M. tuberculosis*, the molecule **ade54leu3rib16** (K_i= 0.000314 μM) is a more potent inhibitor than all AA-RS inhibitors gathered in the IA database (the best one in IA database, acts according to literature on Ile-RS, CB286 from Ref. [16], has K_i=0.03998 μM). Although the calculated K_i values are likely to deviate strongly from the real values, they can be useful in planning experimental tests, and ade54leu3rib16 seems to be particularly interesting.

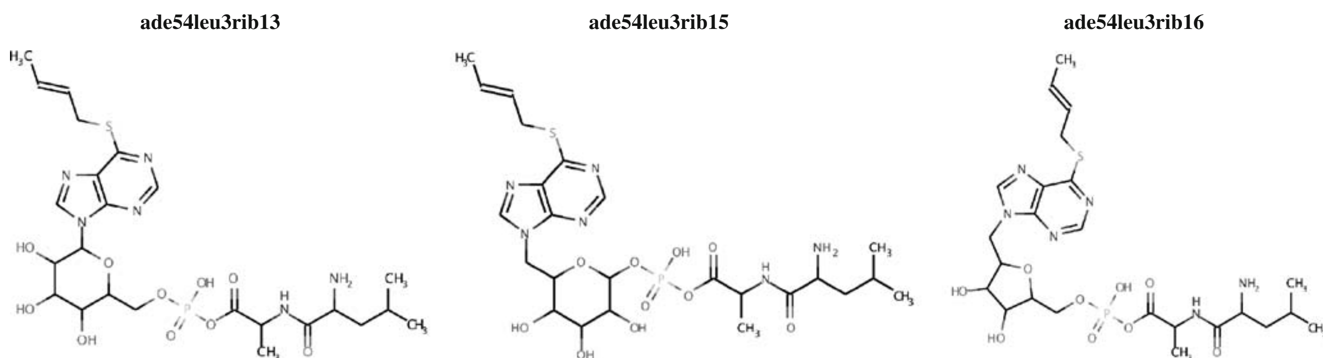

Fig. 4 The most promising potential antibacterial drugs

Table 4 Inhibition constant values (K_i/μM) for 36 new compounds acting on different hosts, calculated using AutoDock

	Bacterial					Human
	E.c	*H.p*	*M.t*	*P.a*	*S.a*	*H.c*
ade46leu15rib13	132	120	2110	8.6	14	1490
ade46leu15rib15	32	48	1040	80	58	124.89
ade46leu15rib16	86	53	2020	40	29	763.39
ade46leu1rib13	21	1.6	201	0.6	0.4	17.34
ade46leu1rib15	96	1.9	228	4	1.8	419.75
ade46leu1rib16	11	0.8	983	1.7	18	56.18
ade46leu3rib13	72	20	2520	8.4	36	258.56
ade46leu3rib15	295	0.3	65	5.8	20	493.06
ade46leu3rib16	265	9	4780	456	57	327.47
ade46leu5rib13	168	3.9	7930	59	34	336.21
ade46leu5rib15	315	32	3870	7.5	8.5	295.71
ade46leu5rib16	173	1	2820	81	37	949.53
ade47leu15rib13	466	2.4	399	65	16	1310
ade47leu15rib15	153	3.1	1400	33	213	1830
ade47leu15rib16	98	0.1	1150	11	47	693.71
ade47leu1rib13	122	19	8280	217	144	6670
ade47leu1rib15	31	3.2	283	4.6	3.4	174.52
ade47leu1rib16	5.4	3.7	26	7.4	2.9	373.2
ade47leu3rib13	0.3	0.7	91	1.2	5.7	4.59
ade47leu3rib15	20	6.5	585	33	53	365.97
ade47leu3rib16	26	0.8	397	30	25	399.32
ade47leu5rib13	9.8	5	87	0.8	13	87.69
ade47leu5rib15	91	1.3	3750	75	14	1490
ade47leu5rib16	27	3.9	162	3.3	7.2	537.24
ade54leu15rib13	80	11	2870	3.6	316	1950
ade54leu15rib15	732	1100	2650	375	97	2210
ade54leu15rib16	312	2	395	30	12	1070
ade54leu1rib13	182	21	0.8	22	0.7	11350
ade54leu1rib15	45	6	1970	86	16	283.09
ade54leu1rib16	42	30	1970	131	8.9	1010
ade54leu3rib13	1020	0.02568	1.6	**0.000000982**	**0.000332**	40350
ade54leu3rib15	**0.0532**	0.00871	21	83	0.9	21370
ade54leu3rib16	6.1	**0.00636**	**0.000314**	**0.00002062**	**0.00225**	5380
ade54leu5rib13	53	3.3	2.7	1110	95	15990
ade54leu5rib15	12	5.6	11390	457	352	1480
ade54leu5rib16	17	6.2	42	7.9	3.4	1040

The best results for each target are in bold

What is more, this molecule is amongst those which bind to *Human cytoplasmic* enzyme very poorly (K_i=5380 μM), suggesting that it may strongly inhibit protein synthesis in *M. tuberculosis* while it is not likely to interfere with Leu-tRNA synthesis in human.

Another promising compound, in the case of *E. coli*, is the molecule **ade54leu3rib15** (K_i=0.0532 μM), whereas the best docked known AA-RS inhibitor from the IA database (acts according to literature on Ile-RS, CB628 from Ref. [16]), has K_i=0.07564 μM. This molecule binds poorly to *Human cytoplasmic* Leu-RS (K_i=21370 μM).

In the case of *S. aureus*, two molecules (**ade54leu3rib13** with K_i=0.000332 μM, and **ade54leu3rib16** with K_i= 0.00225 μM) bind to the enzyme stronger than the best molecule in the IA database (acts according to literature on Met-RS, compound 25 from Ref. [17], K_i=0.00294 μM). **ade54leu3rib13** binds also poorly to *Human cytoplasmic* Leu-RS (K_i=40350 μM).

In the case of *P. aeruginosa*, we have also obtained interesting results: **ade54leu3rib13** with K_i=0.000000982 μM, and **ade54leu3rib16** with K_i=0.00002062 μM. For comparision, the best known inhibitor in IA database (acts according to literature on Met-RS, compound 7 from Ref. [18]), has K_i=0.00651 μM.

We also determined the closest interactions the docked ligand ands its receptor (our own script and also LIGPLOT [19] and HBPLUS [20] software) and listed them in Table 5.

Table 5 List of the closest interactions (distance between atoms under 3.5 Å) between the most promising new ligands and their receptors (LP - ligand part where interacting ligand atom is located, RR - receptor residue, D - distance, IT - interaction type, hph - hydrophobic interaction; parts: aa - aminoacid-like, su - sugar-like, ph - monophosphate, ad - adenine-like)

LP	RR	D/Å	IT	LP	RR	D/Å	IT
ade54leu3rib16 and *M. tuberculosis* **Leu-RS**							
aa	GLU253	2.92	CH…O	ph	ALA662	3.36	hph
ad	GLN299	2.63	CH…O	aa	VAL663	3.06	hph
ad	VAL300	2.81	hph	aa	LEU664	3.50	hph
ph	MET303	3.02	OH…S	ad	ASN693	2.82	CH…O
ph	ALA659	3.15	OH…O	ad	TYR696	2.68	N…HO
ph	GLU660	3.00	OH…O				
ade54leu3rib15 and *E. coli* **Leu-RS**							
ph	THR215	2.10	OH…O	ad	GLN566	2.95	CH…O
aa	MET219	3.22	hph	su	GLY567	3.28	hph
ph	ILE531	3.00	O…HC	ad	MET568	3.02	hph
aa	GLU532	3.27	CH…O	su	ASP653	2.86	CH…O
su	CYS565	3.30	OH…O	ad	THR655	3.44	CH…O
ade54leu3rib13 and *S. aureus* **Leu-RS**							
ad	SER214	3.44	CH…O	ad	GLN567	3.45	CH…O
ad	VAL532	3.14	hph	su	MET569	2.95	hph
aa	GLU533	2.60	NH…O	su	LYS577	2.86	O…HN
aa	HIS534	3.46	NH…N	ad	ASP611	3.22	CH…O
ad	ASN566	3.20	CH…O				
ade54leu3rib16 and *S. aureus* **Leu-RS**							
ad	ASP38	3.23	OH…S	aa	VAL532	2.73	hph
ad	MET39	2.76	hph	aa	GLU533	2.97	NH…N
ad	TYR42	3.35	hph	ad	HIS534	3.47	hph
ad	GLY51	3.23	CH…O	ad	GLN567	2.97	hph
ad	HIS52	3.29	CH…O	su	MET569	3.32	O…HC
ad	TYR56	3.12	CH…O	ph	LYS577	2.91	O…HC
ad	GLY531	3.30	hph				
ade54leu3rib13 and *P. aeruginosa* **Leu-RS**							
ad	HIS49	3.44	NH…π	ph	GLY579	2.70	CH…O
aa	GLN215	2.74	hph	su	MET580	2.93	hph
aa	ILE543	2.77	CH…O	ad	LYS632	3.49	CH…π
su	GLU544	2.86	OH…O	ad	MET633	3.00	C = O…π
ph	THR577	3.06	OH…O	aa	ASP666	3.26	CH…O
ph	GLN578	2.23	OH…O				
ade54leu3rib16 and *P. aeruginosa* **Leu-RS**							
ad	HIS49	3.07	CH…π	ad	GLU544	2.74	C = O…π
ad	GLY51	2.94	hph	aa	GLN578	2.90	CH…O
ad	HIS52	3.13	hph	aa	GLY579	3.46	O…HC
ad	ASN55	3.22	CH…O	ph	MET580	2.76	O…HC
aa	GLN215	3.04	CH…O	ad	MET633	3.24	C = O…π
aa	ILE543	2.30	hph				
ade54leu3ryb16 and *H. pylori* **Leu-RS**							
aa	MET36	3.38	hph	aa	ARG50	3.26	hph
ph	TYR39	2.82	CH…O	aa	GLU529	2.85	NH…O
su	HIS45	3.13	NH…N	ph	HIS530	3.47	NH…O
aa	GLY47	3.34	CH…O	aa	MET565	3.18	hph
su	HIS48	3.30	OH…π	ad	LYS572	3.46	NH…π

Conclusions

Even the most advanced computational methods predicting protein-ligand binding affinities are not capable of providing quantitative data. However, docking methods provide interesting information that can guide experimental test.

Even though it is hard to compare experimental inhibition constants with those from *in silico* studies, the

calculated K_i and experimental IC_{50} for Leu-RS do correlate, although, as expected, the correlation is not too good (about 64%).

The strategy to divide the native intermediate into fragments and generate new molecules from derivatives of these fragments can be successfully used in search towards new antibacterial drugs. Potential inhibitors bind stronger to selected bacterial Leu-RS than to human ones which is a very desired feature in rational drug design. Three particularly promising lead compounds (Fig. 4) have been identified for further experimental studies, which are expected to act as potent agents against *E. coli*, *H. pylori*, *M. tuberculosis*, *P. aeruginosa* and *S. aureus*.

Acknowledgments Financial support from European Committee grant no. LSHG-CT-2003-503265 and Polish Ministry for Science is gratefully acknowledged. Authors thank the Foundation for Polish Science for a FOCUS fellowship. Support from Academic Computer Center in Gdańsk (TASK) and Poznan Supercomputing and Networking Center (PCSS) is also gratefully acknowledged. We thank anonymous referees for their remarks.

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