ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2014, *59*(9), 733-738

POTENTIAL INHIBITORS FOR BACTERIAL DIHYDROPTEROATE SYNTHASE. THE RESULTS OF A COMPREHENSIVE SCREENING BASED ON STRUCTURAL SIMILARITY WITH *p*-AMINO-BENZOIC ACID AND DOCKING SIMULATION ON THE SURFACE OF ENZYME

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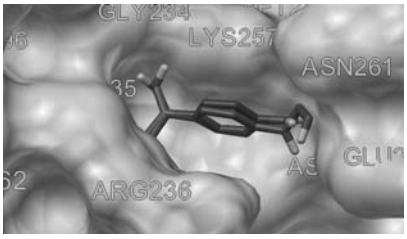
Received April 30, 2014

Sulfonamides are structural analogs and competitive antagonists of *para*-aminobenzoic acid and thus prevent normal bacterial utilization of this, for the synthesis of folic acid. Allergies to sulfonamide are common, hence medications containing sulfonamides are prescribed carefully. Because of this, it may be useful to found similar compounds in order to enlarge the spectrum of dihydropteroate synthase inhibitors used today in therapeutics. We used the Similar Compounds search type of the Chemical Structure Search of the PubChem Compound Database, to locate records that are similar to the chemical structure of *para*-aminobenzoic acid, using pre-specified similarity thresholds. Using the threshold ≥ than 95% for the similar structures criteria, we found 15 compounds that meet this criteria. In accordance with our calculations and molecular docking simulations, these compounds have a better binding affinity to the enzyme than *para*-amino benzoic acid acid, consequently they may act similar as sulfonamides, possibly lacking their adverse effects.



INTRODUCTION

Sulfanilamide (4-amino-benzenesulfonamide) was synthesized in 1908 and researchers proceeded to synthesize over 4500 sulfonamides by 1948. They are still an attractive group of drugs since they are very cheap. When they were first discovered, their mechanism of action was unknown. Observations that they structurally resemble *para*-amino benzoic acid (PABA, 4-aminobenzoic acid) prompted some scientists to suggest a connection. But it wasn't till the role of PABA in living cells was fully elucidated that the mechanism of action of sulfonamides was fully understood.

Sulfonamides are structural analogs and competitive antagonists of *para*-aminobenzoic acid and thus prevent normal bacterial utilization of PABA for the synthesis of folic acid. More specifically, sulfonamides are competitive inhibitors of dihydropteroate synthase[1] (DHPS, EC:2.5.1.15), the bacterial enzyme responsible for the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid. Folic acid is important as a one carbon source in many essential biochemical pathways. Sensitive microorganisms are those that must synthesize their own folic acid; bacteria that can utilize preformed folate are not affected. Bacteriostasis induced by sulfonamides is counteracted by PABA

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competitively. Sulfonamides do not affect mammalian cells by this mechanism, since they require preformed folic acid and cannot synthesize it. They are, therefore, comparable to sulfonamide-insensitive bacteria that utilize preformed folate.

Sulfonamides are bacteriostatic and they have a broad spectrum, but show poor activity against pseudomonas, enterococci and anaerobes. They are slow to act, since several generations are needed before appreciable depletion of folate pool and inhibition of growth. They are considered antimetabolites. Allergies to sulfonamide are common,[2] hence medications containing sulfonamides are prescribed carefully.

Because of this, it may be interesting to found similar compounds in order to enlarge the spectrum of DHPS inhibitors used today in therapeutics. We used the Similar Compounds search type of the Chemical Structure Search of the PubChem Compound Database, to locate records that are similar to the chemical structure of PABA, using pre-specified similarity thresholds. Using the threshold ≥ than 95% for the similar structures criteria, we found 15 compounds that meets this criteria. In accordance with our calculations and molecular docking simulations, these compounds have a better binding affinity to DHPS enzyme than PABA, consequently they may act similar as sulfonamides, probable lacking their adverse effects.

METHOD

Hardware: Asus X401A PC, CPU Dual Core Intel 820, 1.7GHz, 4 GB RAM.

Software: OS Windows 7 - 64 bit, Chem 3D Ultra 10.0, ChemDraw Pro 10.0, AutoDock Tools 1.5.6 Molecular Graphics Laboratory The Scripps Research Institute,[3] AutoDock Vina by Sargis Dallakyan, The Scripps Research Institute[4], Open Babel 2.3.2.,[5] PyRx 0.8, PubChem Compound Database, Firefox 28.0.

The Similar Compounds search type of the Chemical Structure Search of the PubChem Compound Database[6] allows to locate records that are similar to a chemical structure query using pre-specified similarity thresholds. Similarity is measured using the Tanimoto equation and the PubChem dictionary-based binary fingerprint. This fingerprint consists of series of chemical substructure "keys". Each key denotes the presence or absence of a particular substructure in a molecule. The fingerprint does not consider variation in stereo chemical or isotopic information. Collectively, these binary keys provide a "fingerprint" of a particular chemical structure valence-bond form.

The degree of similarity is dictated by the Threshold parameter. A threshold of "100%" effectively acts as an "exact match" to the provided chemical structure query (ignoring stereo or isotopic information), while a threshold of "0%" would return all chemical structures in the PubChem Compound database. Various predefined thresholds between 99-60% are allowed.

Searching the databases (with **over 30 million entries**) is possible for a broad range of properties including chemical structure, name fragments, chemical formula, molecular weight, XLogP, and hydrogen bond donor and acceptor count. PubChem can be accessed for free through a web user interface.

AutoDock Vina significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4. For its input and output, Vina uses the same PDBQT molecular structure file format used by AutoDock. PDBQT files can be generated (interactively or in batch mode) and viewed using MGLTools. Other files, such as the AutoDock and AutoGrid parameter files (GPF, DPF) and grid map files are not needed. The docking calculation consists of a number of independent runs, starting from random conformations. Each of these runs consists of a number of sequential steps. Each step involves a random perturbation of the conformation followed by a local optimization (using the Broyden-Fletcher-Goldfarb-Shanno algorithm) and a selection in which the step is either accepted or not. Each local optimization involves many evaluations of the scoring function as well as its derivatives in the position-orientation-torsions coordinates. The number of evaluations in a local optimization is guided by convergence and other criteria. The number of steps in a run is determined heuristically, depending on the size and flexibility of the ligand and the flexible side chains. However, the number of runs is set by the exhaustiveness parameter. Since the individual runs are executed in parallel, where appropriate, exhaustiveness also limits the parallelism. Unlike in AutoDock 4, in AutoDock Vina, each run can produce several results: promising intermediate results are remembered. These are merged, refined, clustered and sorted automatically to produce the final result.[7-11]

Vina creates *_out.pdbqt files where it stores all docked poses and scores.[4]

The predicted binding affinity of bound structures is given in kcal/mol. To compare the accuracy of the predictions of the experimental structure, AutoDock Vina use a measure of distance between the experimental and predicted structures, RMSD, root-mean-square deviation.

RMSD values are calculated relative to the best mode and using only movable heavy atoms. For scoring, AutoDock Vina uses a united-atom function, which involves only the heavy atoms.

Two variants of RMSD metrics are provided by the software, rmsd/lb (RMSD lower bound) and rmsd/ub (RMSD upper bound), differing in how the atoms are matched in the distance calculation:

- *rmsd/ub* matches each atom in one conformation with itself in the other conformation, ignoring any symmetry;
- *rmsd'* matches each atom in one conformation with the closest atom of the same element type in the other conformation (rmsd' can not be used directly, because it is not symmetric);
- *rmsd/lb* is defined as follows: $\text{rmsd/lb}(c_1, c_2) = \max((\text{rmsd}'(c_1, c_2), \text{rmsd}'(c_2, c_1))$.

RESULTS AND DISCUSSION

Using the Chemical Structure Search of the PubChem Compound Database and a threshold ≥ than 95% for the similar structures criteria, we detected 15 compounds 95% similar with PABA. We calculated the binding affinities for the ligands (including for *p*-aminobenzoic acid) to the surface of dihydropteroate synthase. Fifteen substances (with PubChem Compound ID = 22143252, 69285308, 2762775, 21063639, 22143251, 14332036, 302680, 45084823, 21446355, 11159424, 54746218, 95888, 69030033, 4465519, 154880) are better ligands for DHPS than PABA. These ligands are shown in Table 1 and below, together with PABA (CID 978). Except of CID 22143252 (with 6 H-bond donors, instead of 5), all substances accomplish the Lipinski Rule of Five,[12] also known as the Pfizer's rule of five or simply the Rule of five (RO5), which is a rule of thumb to evaluate druglikeness or determine, if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans: (i) no more than five hydrogen bond donors; (ii) no more than ten hydrogen bond acceptors; (iii) a molecular mass under 500 daltons; (iv) an octanol-water partition coefficient, LogP value under five.

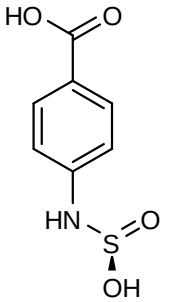
Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market.

In Table 2 are presented the calculated binding affinities in descending order for the ligands and the enzyme DHPS (with PDB code 4HB7[1]).

Table 1

The 15 ligands with a similarity threshold ≥ 95%, and PABA (CID 978)

PubChem CID	IUPAC Name	Molecular Formula	MW [g/mol]	H-bond Donor	H-Bond Acceptor	XLogP3-AA
22143252	4-(sulfinoamino)benzoic acid	$C_7H_7NO_4S$	201.19978	6	3	1
69285308	4-amino-2,3-diiodobenzoic acid	$C_7H_5I_2NO_2$	388.92904	2	3	1
2762775	4-amino-2,3-difluorobenzoic acid	$C_7H_5F_2NO_2$	173.116906	2	5	1
21063639	4-(hydroperoxyamino)benzoic acid	$C_7H_7NO_4$	169.13478	3	5	1
22143251	1-carboxy-4-(sulfinatoamino)benzene	$C_7H_6NO_4S^-$	200.19184	2	6	0.4
14332036	4-amino-2-iodobenzoic acid	$C_7H_6INO_2$	263.03251	2	3	1.4
302680	4-amino-2-fluorobenzoic acid	$C_7H_6FNO_2$	155.126443	2	4	1.2
45084823	4-(fluoroamino)benzoic acid	$C_7H_6FNO_2$	155.126443	2	4	2
21446355	4-(chloroamino)benzoic acid	$C_7H_6ClNO_2$	171.58104	2	3	2.3
11159424	4-aminobenzenecarboperoxoic acid	$C_7H_7NO_3$	153.13538	2	4	0.9
54746218	4-(oxidoamino)benzoic acid	$C_7H_6NO_3^-$	152.12744	2	4	1.6
95888	4-(hydroxyamino)benzoic acid	$C_7H_7NO_3$	153.13538	3	4	1.7
69030033	4-(phosphanylamino)benzoic acid	$C_7H_8NO_2P$	169.117682	2	3	2
4465519	(4-carboxyphenyl)azanium	$C_7H_8NO_2^+$	138.14392	2	2	0.8
154880	4-carboxybenzenediazonium	$C_7H_5N_2O_2^+$	149.1268	3	1	2.8
978	4-aminobenzoic acid	$C_7H_7NO_2$	137.13598	2	3	0.8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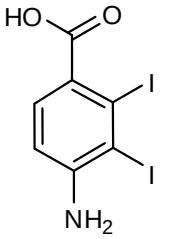
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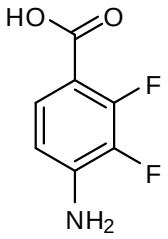
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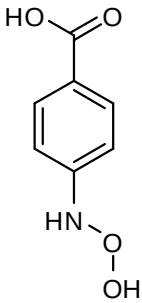
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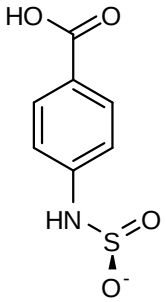
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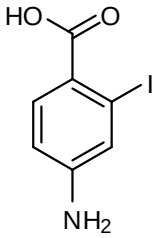
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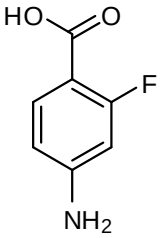
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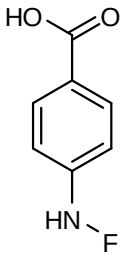
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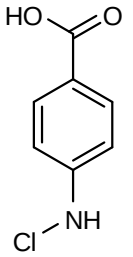
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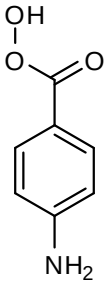
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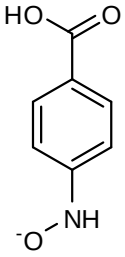
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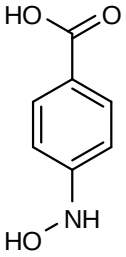
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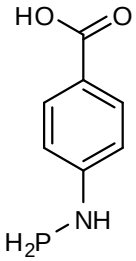
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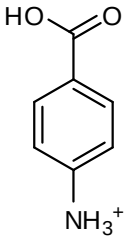
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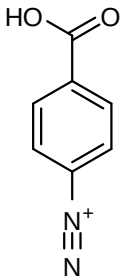
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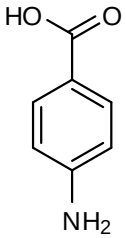
978

When using a flexible docking engine, then minimizing the input conformation of the ligands can reduce problems that are known to occur in conformer generation inside the docking engine, that arise if the input 3D conformation is not relaxed into good bond lengths and angles. For small molecules a good choice is to use some of molecular mechanics to optimize the structure down to a local energy minima, like UFF or mm2. The assignment of Universal Force Field (UFF) atom types and the calculation of the molecular connectivity (identifying bonds, angular, torsional and inversion terms) has been performed using the routines available in the Open Babel package.[4,5] OpenBabel can be used for refining initial geometries with UFF molecular-mechanics optimizations, adding or removing hydrogens to PDB protein files, and many other utility tasks that often arise in molecular modeling projects.

Open Babel supports a number of force fields which can be used for energy evaluation as well as energy minimization. We used the following

energy minimization parameters: Conjugate Gradients optimization algorithm, 200 total number of steps, stop if energy difference is less than 0.1 kcal/mol.

The virtual screening results show that the 15 compounds (Table 2) are strong inhibitors of DHPS (4HB7). The structure of enzyme was retrieved with Chem 3D's Online Find Structure from PDB ID option, and transformed to PDBQT wit AutoDock.

The RMSD cutoff of 2Å is usually used as criteria of the correct bound structure prediction.[13] Using the same cutoff value, the two metrics used for RMSD (summarized in Table 2) indicate that **15 compounds are better ligands** of DHPS **than PABA** (CID 978), because they require lesser energy for binding. This suggest, that these substances will successfully substitute PABA and will act similarily with sulfonamides.

Table 2

The calculated binding affinities greater than for PABA, in descending order for the enzyme dihydropteroate synthase (4HB7)

Enzyme-Ligand	Binding Affinity [kcal/mol]	rmsd/ub [Å]	rmsd/lb [Å]
4HB7_22143252_uff_E=105.40	-5.7	0	0
4HB7_69285308_uff_E=115.18	-5.6	0	0
4HB7_2762775_uff_E=71.95	-5.6	0	0
4HB7_21063639_uff_E=94.73	-5.6	0	0
4HB7_22143251_uff_E=101.25	-5.5	0	0
4HB7_14332036_uff_E=85.98	-5.5	0	0
4HB7_302680_uff_E=69.37	-5.5	0	0
4HB7_45084823_uff_E=66.69	-5.5	0	0
4HB7_21446355_uff_E=72.58	-5.5	0	0
4HB7_21063639_uff_E=94.73	-5.4	5.361	1.86
4HB7_11159424_uff_E=70.37	-5.4	0	0
4HB7_54746218_uff_E=68.72	-5.4	0	0
4HB7_95888_uff_E=81.41	-5.4	1.463	0.047
4HB7_95888_uff_E=81.41	-5.4	0	0
4HB7_21063639_uff_E=94.73	-5.3	29.271	28.19
4HB7_69030033_uff_E=-90.91	-5.3	0	0
4HB7_54746218_uff_E=68.72	-5.3	4.711	2.141
4HB7_4465519_uff_E=256.10	-5.3	0	0
4HB7_154880_uff_E=251.98	-5.2	26.716	25.983
4HB7_154880_uff_E=251.98	-5.2	0	0
4HB7_22143252_uff_E=105.40	-5.2	30.549	29.962
4HB7_22143252_uff_E=105.40	-5.2	4.151	3.367
4HB7_22143251_uff_E=101.25	-5.2	10.546	9.762
4HB7_54746218_uff_E=68.72	-5.2	4.933	2.162
4HB7_95888_uff_E=81.41	-5.2	4.705	2.176
4HB7_21446355_uff_E=72.58	-5.2	26.593	25.956
4HB7_978_uff_E=63.75	-5.2	0	0

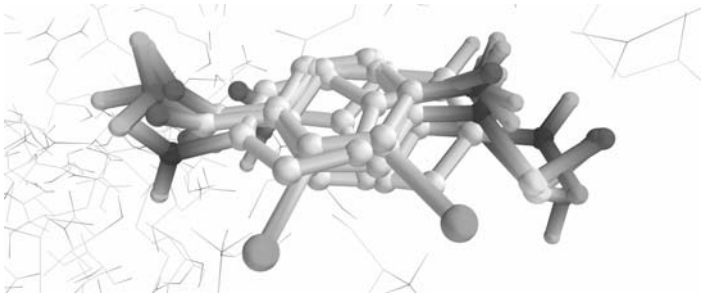

Fig. 1 – Molecular docking of PABA and other 6 other ligands (CID 22143251, 22143252, 4876, 9971, 16461, 69285308) in protein target (Mayavi).

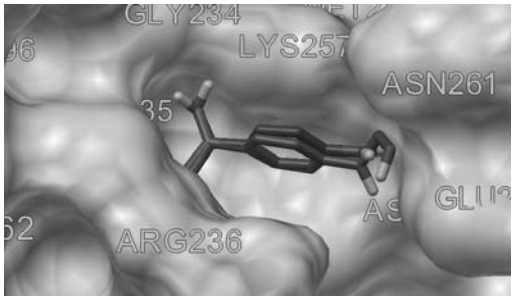

Fig. 2 – Ligand CID 21063639 and PABA on the binding site of DHPS (Autodock).

We used the default docking parameters:

- number of binding modes: 9,
- exhaustiveness (thoroughness of search): 8.

Larger values increase the probability of finding the global minimum, but also extend the computational time. Increasing the exhaustiveness value increases the time linearly and decreases the probability of not finding the minimum exponentially. Apart from exhaustiveness influenced by users, Vina has an internal heuristic algorithm to extend the search in accordance with an increasing number of atoms and rotatable bonds.[14]

With reference to (solid) diazonium salts (like CID 154880), especially diazonium chlorides, they are often dangerously explosive, but diazonium salts with weakly coordinating anions are quite stable. For example, tetrafluoroborates can be stored almost indefinitely at room temperature and decompose gently when heated.

CONCLUSIONS

Compounds with PubChem ID: 22143252, 69285308, 2762775, 21063639, 22143251, 14332036, 302680, 45084823, 21446355, 11159424, 54746218, 95888, 69030033, 4465519 and 154880 have better binding affinity to dihydropteroate synthase enzyme than PABA, they present the correct bound structure prediction, so they seem to act alike, and to be good substitutes for sulfonamides. Further investigations are needed to establish their pharmacodynamic properties and toxicity.

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