Virtual screening cascade in search for novel purine-derivated MurD inhibitors as antibacterial agents

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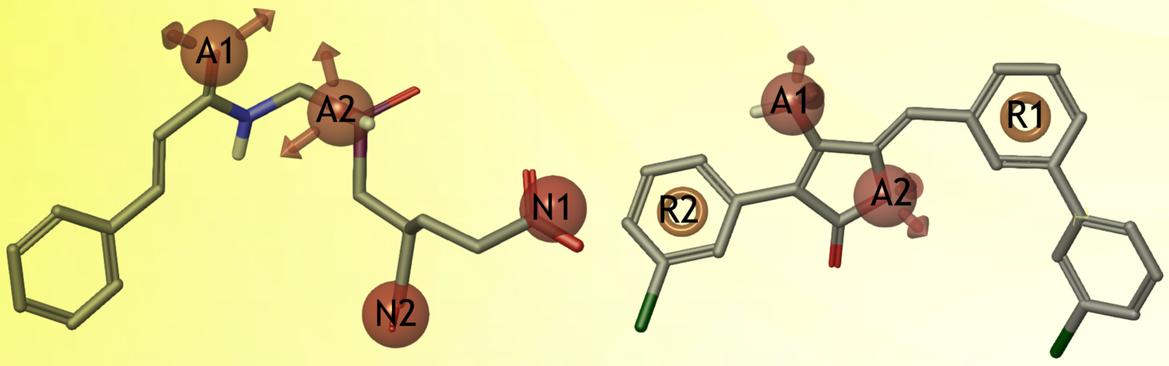
UDP-N-acetylmuramoylalanine glutamate ligase (MurD) is one of the emerging targets for the next-generation of anti-bacterial agents. Along with other members of the amide ligases (MurC-F) family, MurD inhibits the synthesis of peptidoglycan – a key bacterial metabolite, essential for bacterial growth. In addition, the anti-bacterial action of MurC-F is indeed very promiscuous, as this metabolic pathway is common for multiple bacterial strains and so the inhibitor of a single enzyme can be a multipotent antibacterial agent [1].
Herein, we developed a virtual screening protocol oriented at the identification of the new MurD inhibitors with purine scaffold. Organic synthesis schemes along with the databases of accessible building blocks were used for enumeration of combinatorial library of synthetically accessible compounds. This chemical subspace was used as an input for the multistep virtual screening (VS) protocol utilizing the previously developed methodology [2,3]. The VS cascade consisted of physicochemistry, ADME and pharmacophore filters, as well as the docking protocol. All stages were optimized to maximize the screening parameters in the retrospective experiments. Among the compounds that have passed through the entire cascade, the most promising will be selected by team members, synthetised and biologically evaluated.

PHARMACOPHORE MAPPING

All known MurD inhibitors were hierarchicaly clustered using Canvas with manual refinements to ensure proper chemotypes classification. Multiple hypotheses were developed for each cluster, employing the previously utilized approach [3]. After evaluation with DUD-like test set, one model per cluster (Figure 1) was selected to form the linear combination of pharmacophore models.



	A2	N1	N2
A1	3.38	8.87	7.66
A2		5.53	5.31
N1			4.88

	A2	R1	R2
A1	3.56	5.90	4.08
A2		4.11	5.12
R1			8.97

Figure 1. Exemplary pharmacophore models of MurD inhibitors along with a matrix of distances (in angstroms) between features. The feature abbreviations used are: hydrogen bond acceptor – A; negatively ionized group – N, aromatic ring – R.

DOCKING PROTOCOL

In silico models were generated using 18 crystal structures of MurD with resolution less than 2Å fetched from PDB. All models were evaluated on test set consisted of known MurD ligase inhibitors and DUD-decoys and only one model per crystal structure (in terms of MCC value) was selected. Then, models were combined into ensemble to maximize statistic parameters.

PHYSICOCHEMICAL FILTER

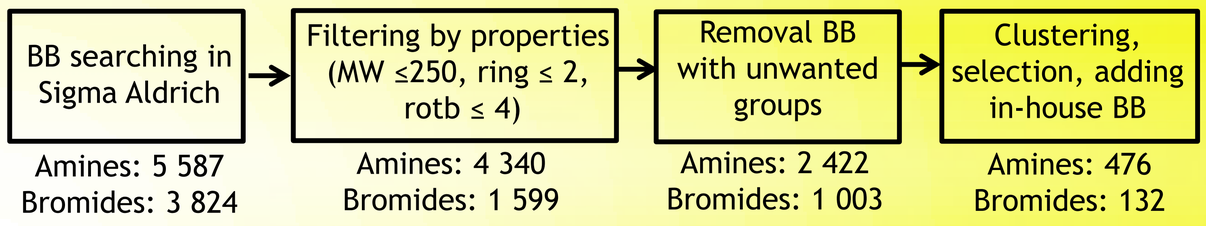
Due to the high acidicity of Mur D ligase inhibitors, physicochemical filter rejecting compounds with pKa > 6 was applied. 82 out of 87 Mur D ligases met this requirement and only 7.8% compounds from randomly selected set of 200K compounds from the ZINC database.

ADMETox FILTER

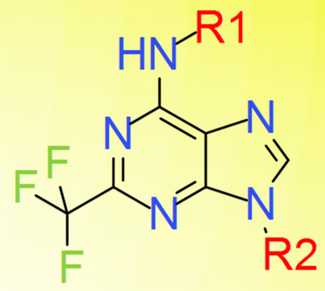
Among 51 ADMETox parameters available in Qikprop software, the four with the highest values of MI-DSE (Mutual Information Differential Shannon Enthropy – measurement of active/inactive discrimination potential) [4] were selected: total solvent accessible surface area (FISA); number of nitrogen and oxygen atoms (#NandO); predicted brain/blood partition coefficient (QLogBB); and predicted partition coefficient (QPlogPC16).

COMBINATORIAL LIBRARY

The library was enumerated by combining 2-(trifluoromethyl)-9H-purine-6-amine with two types of building blocks (BB): amines and bromides (Figure 2.). All BBs were fetched from Sigma Aldrich repository and after careful selection (removing BB with unwanted groups and properties, clustering, etc.) it allowed for generation of 63K virtual coumpounds.



Core:



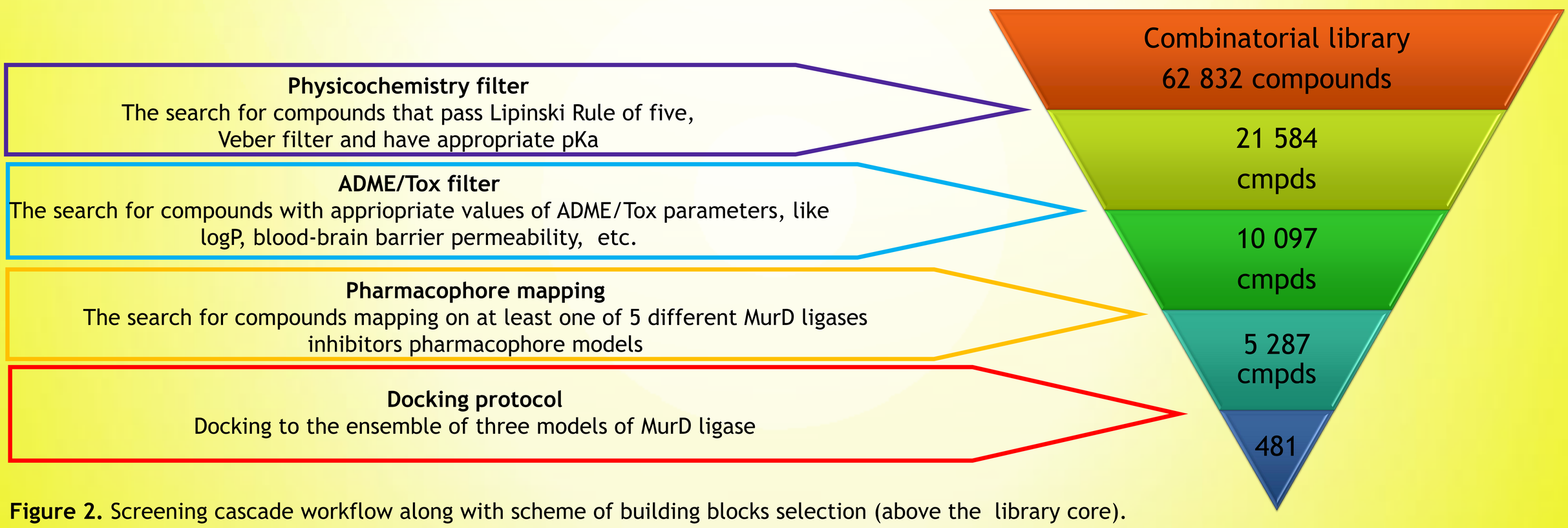


Figure 2. Screening cascade workflow along with scheme of building blocks selection (above the library core).

References

[1] Hrast et al, *Bioorg. Chem.***2014**, *55*, 2-15.
[2] Kurczab et al, *Bioorg. Med. Chem. Lett.* **2010**, *8*, 2465-2468.
[3] Warszycki et al. *PloS ONE*, **2013**, *8*, e84510.
[4] Wassermann et al. J. Chem. Inf. Model., 2010, 50,1935–1940.

Acknowledgements

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