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Dual-Target Virtual Screening by Pharmacophore Elucidation and Molecular Shape Filtering

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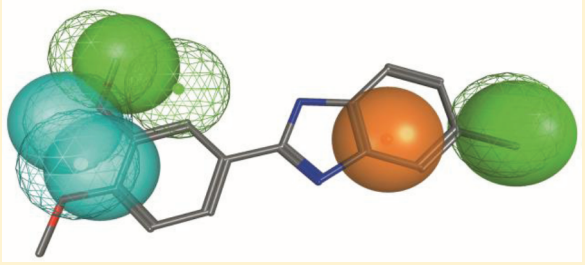
Supporting Information

ABSTRACT: Dual-target inhibitors gained increased attention in the past years. A novel in silico approach was employed for the discovery of dual 5-lipoxygenase/soluble epoxide hydrolase inhibitors. The ligand-based approach uses excessive pharmacophore elucidation and pharmacophore alignment in conjunction with shape-based scoring. The virtual screening results were verified in vitro, leading to nine novel inhibitors including a dual-target compound.



KEYWORDS: *computational chemistry, drug design, virtual screening, pharmaceutical chemistry*

Numerous approved drugs interact with macromolecules besides their main target,[1] which may contribute to overall efficacy or rather cause side effects (off-targets). Dual- or multitarget ligands have gained increased attention due to improved efficacy[2] and less side effects. Rational design of small molecules, which are able to interact with multiple targets involved in a disease pattern while leaving the off-targets untouched, is one of the challenging tasks.[3] Synthetic linking of two selective molecules has been an established approach to find novel dual ligands.[4] Although these hybrids yield high potency and can be used as tool compounds, they often exhibit increased molecular weight and leave little space for optimization of pharmacological and pharmacodynamic properties. A rational approach to find dual or multitarget leads has not been established yet, although the design of a common pharmacophore seems to be a straightforward way to deal with this task. A structure-based application by Wei et al.[5] demonstrates that a dual pharmacophore can be derived from two X-ray structures of the targets of interest. In this study, we present a generalized strategy for the generation of common pharmacophore models even in the absence of structural target information and an application for the design of dual ligands of 5-lipoxygenase (5-LO) and soluble epoxide hydrolase (sEH).

We started from the assumption that two targets share a common interaction pattern, although not necessarily at the same spatial distance. The latter circumstance makes the direct elucidation of the common pharmacophore from a combined set of active ligands of both targets unfeasible. Therefore, we developed a new approach for the in silico discovery of dual-target ligands using aligned pharmacophore models combined with shape-based scoring. The basic idea of this approach is the generation of a large number of "selective" pharmacophore models for each target and subsequent comparison of them (Figure 1). Two pharmacophore models are considered to be equal if they exhibit a similar interaction pattern but not necessarily at exactly the same distance. These two pharmacophore models are used for screening, and the shape of chemical compounds hitting both pharmacophore models is compared with the shape of active ligands to ensure that the screening hits are able to fit in the binding pocket.

Starting with two sets of known active compounds for each target, a number of different pharmacophore models are generated using the pharmacophore elucidator routine included in the MOE[6] software. The elucidator tries to enumerate all models that are matched by at least a given percentage of the molecules. Because the pharmacophore elucidation is very time-consuming, it may be necessary to apply a clustering algorithm in advance and to pick only the most active molecules of each cluster. Afterward, the pharmacophore models are subjected to pairwise alignment using a graph-based approach. First, an association graph is generated, followed by a clique detection[7] and their alignment using the Kabsch algorithm[8,9] (see the Supporting Information). Because a compound may be able to bind to different targets in different conformations, the algorithm aligns pairs of pharmacophore models sharing the same features, which are not necessarily at exactly the same spatial distance. Using the aligned models, a pharmacophore search (using MOE) on a multiconformation database is performed to find compounds matching both models. The potentially "dual" ligands are scored by a shape-based comparison with the known active molecules using

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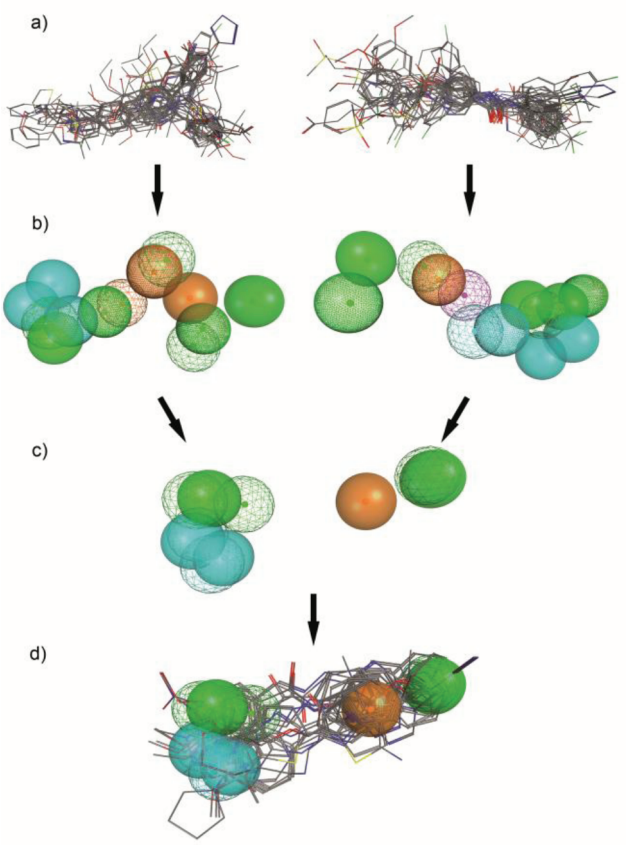

Figure 1. Virtual screening process. On the basis of multiple conformations of known ligands for both targets (a), a number of different pharmacophore models are generated (b). To find models sharing the same features at a similar spatial distance, pairwise alignments are computed (c). Using the aligned models, a pharmacophore search for molecules matching both models is performed (d). The potential "dual" compounds are scored by a shape-based comparison with the known active ligands (not shown). Different models are drawn in solid, as mesh, and as wireframe. The colors represent different pharmacophore features: green, hydrophobic; orange, aromatic; blue, H-bond acceptor; and purple, H-bond donor.

ShaEP.[10] ShaEP maximizes the volume overlap between two molecules, which is required to avoid steric clashes within the binding site.[11] Using this approach, we performed a prospective fragment-based virtual screening for dual 5-LO/sEH inhibitors. Both enzymes play an important role in the arachidonic acid cascade and are involved in inflammatory processes, pain, cardiovascular diseases, and allergic reactions.[12,13]

The sEH is a member of the cytochrome P450 branch of the arachidonic acid cascade and catalyzes the oxidation of epoxyeicosatrienoic acids (EETs) to the more soluble dihydroxyeicosatrienoic acids (DHETs). It has been shown that EETs are involved in a number of physiological processes,[14] and an increased EET level can mediate anti-inflammatory, antihypertensive, and vasodilatory effects.[13] Furthermore, a combined application of cyclooxygenase (COX), 5-LO activating protein (FLAP), and sEH inhibitors leads to a significantly increased anti-inflammatory efficacy as compared to the administration of a single compound.[15] However, inhibiting the sEH seems to induce a shift of the arachidonic acid cascade toward the 5-LO branch,[16] which caused albuminurea in the 5/6 nephrectomy model. Therefore, the simultaneous inhibition of both targets might lead to more effective anti-inflammatory compounds and also to safer antihypertensive drugs.

We used the ChEMBLdb database[17,18] (Version 9, 658075 molecules) as the source for the sets of known active compounds (Figure 2). For both targets, we retrieved all compounds with a reported $IC_{50} < 1\ \mu M$, which led to 677 sEH and 914 5-LO inhibitors. Because the whole ChEMBLdb was used, the IC_{50} values are not always comparable due to different assays or assay conditions. However, as our approach does not rely strongly on the exact activity data, they were suitable for our requirements.

The sets were both clustered using two different setups: one based on MACCS substructure keys[19] (as implemented in MOE) using the Jarvis–Patrick[20] clustering algorithm and the Tanimoto coefficient[21,22] as the distance metric, and the other one based on the CATS2D descriptor[23,24] using the *k*-means algorithm implemented in KNIME (Konstanz Information Miner)[25] with the Euclidean distance as the metric. The clustering parameters were adjusted to yield a total of 50 clusters whereof the most active compounds of each cluster were picked. Using the more diverse set of each target, we generated multiple conformations using the stochastic search in MOE with an output strain limit of 6 kcal/mol, which led to 2640 (sEH) and 532 (5-LO) structures. On the basis of these conformations, we used the pharmacophore elucidator of MOE to generate a multitude of pharmacophore models for both targets. The elucidation was realized with three different setups: The first setup did not emphasize any particular pharmacophore feature; the second and third setup emphasized aromatic and H-bond donor/acceptor features, respectively (see the Supporting Information). The three runs yielded 6764/1469 (sEH/5-LO), 33/118, and 6190/1362 different models, which led to 129, 31, and 92 aligned "dual" models.

For virtual screening, we used the "merged fragments" database provided by Asinex[26] (37429 molecules). Similar to the preparation of the known active compounds, we first generated multiple conformations (overall, 244423 conformations). Then, using the aligned models, we performed a pharmacophore search (yielding 645/200/2071 hits, respectively) followed by a scaffold analysis for further data reduction. By selecting the best-matching molecules of each scaffold class only, we obtained 360, 120, and 929 potential dual compounds. In the last step of the virtual screening workflow, these molecules were scored using ShaEP. As ShaEP returns two scores, shaep_best and shaep_average, we obtained two different rankings of molecules for each virtual screening setup. We considered the first elucidation setup to be less specific than the setups two and three as there was no particular emphasis on any feature leading to more general models. Therefore, we retrieved the top 20 from each ranking of the setups two and three for manual selection.

Out of these 80 molecules (69 unique compounds), we selected 36 molecules manually (see the Supporting Information) and ordered them for in vitro activity assessment against 5-LO and sEH. 5-LO inhibition was determined in a well-established, cell-free HPLC-based assay,[27] and sEH inhibition in a fluorescence-based assay[28] (see the Supporting Information). Nine out of the compounds exhibited functional IC_{50} values below 50 μM in either the 5-LO or the sEH assay.

None of the compounds has reported activities on these targets. The most potent 5-LO hits **3**, **4**, and **5** yielded functional IC_{50} values between 2.2 and 7.6 μM, whereas the

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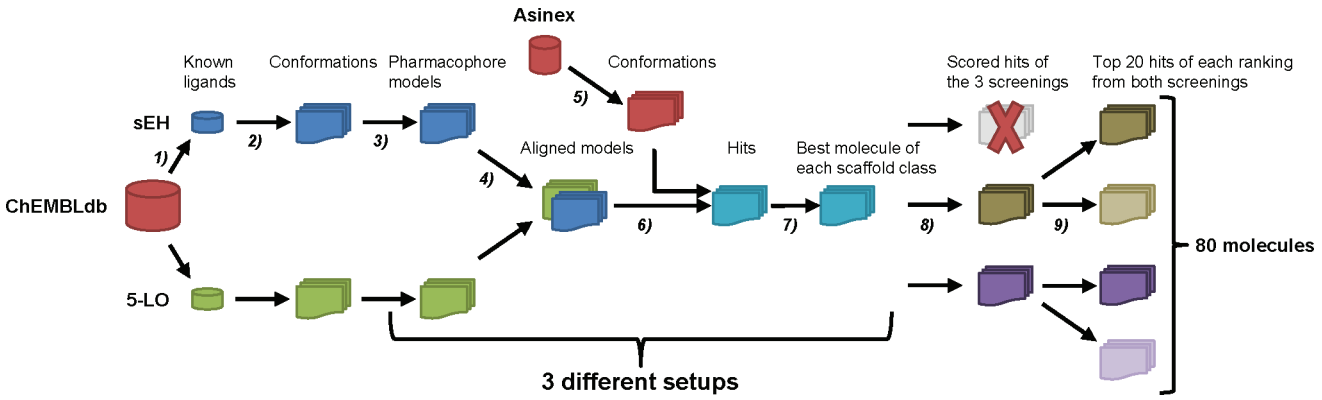

Figure 2. Overview of the virtual screening workflow. For a more detailed description, please see the main text. (1) First, all known ligands of sEH and 5-LO with an IC_{50} < 1 μM were retrieved from the ChEMBLdb. (2) The compounds were washed and clustered, and conformations were generated. (3) On the basis of these conformations, multiple pharmacophore models were generated using the pharmacophore elucidator routine of MOE with three different setups. (4) These models were subsequently aligned to yield "dual" models. (5) As the vendor database, the "merged fragments" database provided by Asinex was used. The compounds were washed, and conformations were generated. (6) With the aligned "dual" models, a pharmacophore search for molecules matching both models was performed on the Asinex database. (7) To further reduce the number of hits, a scaffold analysis was performed, and only the best matching molecule of each scaffold class was retained. (8) The resulting hits of the three screenings were scored using ShaEP. As mentioned in the main text, screening one was dropped. (9) As ShaEP returns two scores, we obtained two rankings for both remaining screenings. From each of these four lists, we picked the top 20, leading to a total of 80 molecules.

most potent sEH hits **6**, **7**, and **8** ranged between 0.47 and 3.5 μM (Figure 3). Besides these selective inhibitors, one compound, **6**, showed activity on both targets with IC_{50} values of 36 and 3.5 μM, respectively.

Regarding the structures of these hits, it seems obvious that an H-bond donor and acceptor feature is required to bind to the sEH. Although **5** and **6** are structurally similar, **5** shows no activity on sEH, which may be due to the missing H-bond donor feature at the benzoxazole ring. For the same reason, compound **4**, an N-substituted benzimidazole, shows no activity. Because urea-free, unsubstituted benzimidazoles have not yet been reported as sEH inhibitors, this hit could indicate a novel class of inhibitors. Remarkably, all 36 compounds were matched by only five different pharmacophore models, with one model accounting for 20 molecules. Although none of these models contained the mentioned H-bond donor/acceptor feature, the combination of pharmacophore search and shape-based comparison led to a number of hits for both targets, including a novel dual-target compound (**6**, Figure 4).

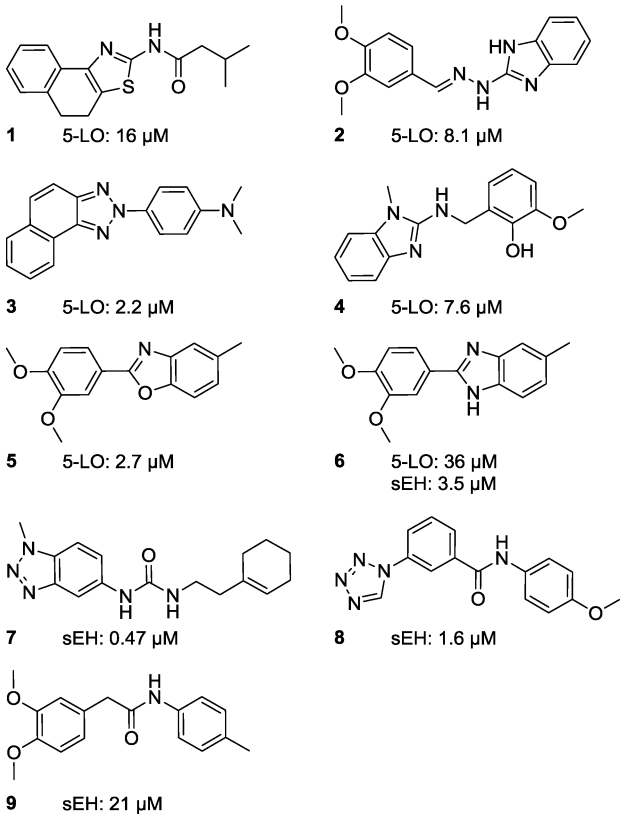

Figure 3. Virtual screening hits: IC_{50} values were determined only if a compound showed at least 50% inhibition at a concentration of 30 μM.

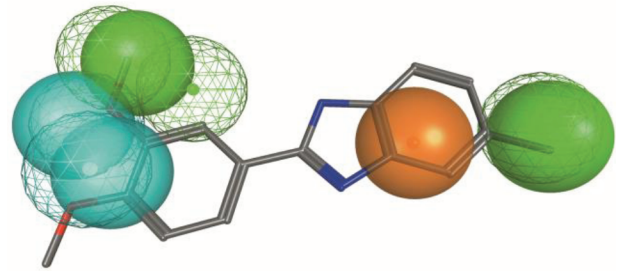

Figure 4. Compound **6** with the two matched pharmacophore models. Solid, 5-LO model; wireframe, sEH model.

In summary, we presented a virtual screening approach for the discovery of potential dual-target compounds. We derived a multitude of pharmacophore models from known ligands, computed pairwise alignments between models of both targets, and used these alignments for pharmacophore search. In conjunction with a shape-based similarity scoring, we were able to obtain a number of selective single-target ligands as well as a novel dual 5-LO/sEH inhibitor. These results indicate that the idea of aligned pharmacophore models can be successfully employed for the discovery of dual-target ligands. Nevertheless, pharmacophore elucidation is not only a crucial but also very time-consuming step; therefore, parametrization should be

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considered carefully since minor changes can have a major impact on the outcome of virtual screening.

■ ASSOCIATED CONTENT

Supporting Information

Detailed pharmacophore elucidation and alignment description, assay setups, and all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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