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Exploring the Chemical Space of Multitarget Ligands Using Aligned Self-Organizing Maps

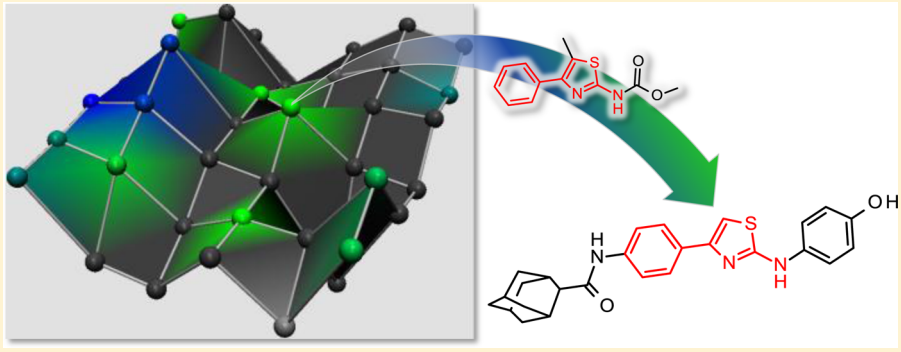
Janosch Achenbach,[†] Franca-Maria Klingler,[†] René Blöcher,[†] Daniel Moser,[†] Ann-Kathrin Häfner,[†] Carmen B. Rödl,[†] Simon Kretschmer,[†] Björn Krüger,[‡] Frank Löhr,[§] Holger Stark,[†] Bettina Hofmann,[†] Dieter Steinhilber,[†] and Ewgenij Proschak*[,†]

[†]Institute of Pharmaceutical Chemistry, ZAFES/OSF, Goethe University, Max-von-Laue-Strasse 9, D-60438 Frankfurt am Main, Germany

[‡]Chemical R&D—Drug Design, Merz Pharmaceuticals GmbH, Eckenheimer Landstrasse 100, D-60318 Frankfurt, Germany

[§]Institute of Biophysical Chemistry, Goethe University, Max-von-Laue Strasse 9, D-60438 Frankfurt am Main, Germany

S *Supporting Information*



ABSTRACT: Design of multitarget drugs and polypharmacological compounds has become popular during the past decade. However, the main approach to design such compounds is to link two selective ligands via a flexible linker. Although such chimeric ligands often have reasonable potency in vitro, the in vivo efficacy is low due to high molecular weight, low ligand efficiency, and poor pharmacokinetic profile. We developed an unprecedented in silico approach for fragment-based design of multitarget ligands. It relies on superposition of the chemical spaces related to the affinity on single targets represented by self-organizing maps. We used this approach for screening of molecular fragments, which bind to the enzymes 5-lipoxygenase (5-LO) and soluble epoxide hydrolase (sEH). Using STD-NMR and activity-based assays, we were able to identify fragments binding to both targets. Furthermore, we were able to expand one of the fragments to a potent dual inhibitor bearing a reasonable molecular weight (MW = 446) and high affinity to both targets (IC_{50} of 0.03 μM toward 5-LO and 0.17 μM toward sEH).

KEYWORDS: *Aligned self-organizing maps, 5-lipoxygenase, soluble epoxide hydrolase, structure−activity relationships*

The one drug−one target−one disease paradigm in drug discovery has been reconsidered during the last decade. This paradigm change was mainly caused by high attrition rates in drug approvals due to toxicity and lack of efficacy. On top of that, the results of post-genomic and network biology showed that putative drug targets rarely act within isolated systems but rather as a part of a highly connected network.[1] Furthermore, the efficacy of several approved drugs has been traced back to the interaction with multiple targets.[2] Inhibition of a single target in such a network might not lead to the desired therapeutic effect, which explains a large proportion of clinical study failures. A paradigm shift towards designed polypharmacology should overcome the lack of efficacy;[3] however, the design of selective multi-target drugs is still challenging. In particular, sufficient affinity to each target as well as an adequate pharmacokinetic profile has to be considered.[4] Early design strategies comprise linking the relevant pharmacophores of known selective ligands, but these methods often lead to compounds exhibiting high molecular weight and low ligand efficiency.[3,4] The application of computer-aided techniques could be beneficial for the development of multi-target drugs[5] as it was recently shown by Besnard et al.[6]

Fragment-based techniques have been broadly applied to design potent and selective drugs.[7,8] In this study, we extend this successful rational design strategy to multitarget ligands. We present an in silico approach based on alignment of self-organizing maps (SOM),[9,10] which we call multiSOM, for the identification of multitarget fragments with low molecular weight bearing space for further optimization. Our workflow

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comprises the search for common substructures of known ligands for each target followed by the identification of multitarget relevant substructures among the target-specific substructures. We used our multiSOM approach to retrieve molecular fragments, which target 5-lipoxygenase (5-LO)[11] and soluble epoxide hydrolase (sEH).[12,13] We validated these findings by saturation transfer difference (STD)-NMR[14,15] and functional in vitro assay systems. Both enzymes are part of the arachidonic acid cascade and involved in inflammatory processes, pain, and cardiovascular diseases.[16,17] The simultaneous inhibition of both enzymatic pathways was shown to have synergistic effects in treatment of inflammation in vivo.[18]

The initial step in our screening workflow was the identification of characteristical molecular substructures of known active compounds for each target (5-LO, sEH). For the identification of the relevant substructures for each target we created two compound sets derived from the ChEMBLdb (v. 12) with annotated affinity data for each target.[19] We considered only ligands with IC_{50} and K_i values equal or less than 10 μM. The DrugBank (v. 3.0) database served as a background distribution of the drug-like chemical space for the multiSOM training.[20] We generated a virtual fragment library as a subset of all compounds available from Specs (v. May 2011) by applying the "Astex Rule of 3" filter.[21] All computational procedures described were implemented as KNIME nodes or workflows.[22]

We used the Molecular Substructure Miner (MoSS) KNIME node,[23] a maximum common substructure based approach to search for frequent substructures in each active compound set. A substructure has been defined as frequent for one of the targets if it contains more than seven heavy atoms and occurs in at least 5% of the known active compounds of the corresponding target and only at most in 1% of the DrugBank compounds. We performed that kind of search for both known active sets and additionally (with swapped active/inactive definition) for the DrugBank compounds to generate a set of prevalent frequent substructures as background distribution. We could find 173 substructures that were characteristic for sEH inhibitors, 150 for 5-LO inhibitors, and 312 that were common for DrugBank compounds representing the drug-like chemical space. The subsequent step was the identification of potential dual-target substructures, i.e., the intersection of both sets of frequent substructures derived from sEH and 5-LO ligands that are most similar to each other. For the identification of this intersection, we used our multiSOM approach, which is based on the alignment of multiple self-organizing maps (Figure 1).

In this study we used the RDKit FeatMorgan fingerprint, a functional-class, generalized extended-connectivity fingerprint[24] to encode the identified frequent substructures. The multiSOM approach yielded three self-organized maps containing 42 neurons: two primary maps, each trained on one of both active sets and the inactive set, respectively, and additionally one aligned multiSOM (Figure 1b). High-lying neurons that contain similar substructures only from both active sets (colored) and not from the background set (gray) have been considered to be relevant for both targets. All substructures contained on those neurons were used for the following virtual screening procedure.

The resulting collection containing 229 dual relevant substructures was used to search for similar fragments in the prefiltered Specs database containing 8417 fragment-like small molecules. On the basis of the previously prepared active and

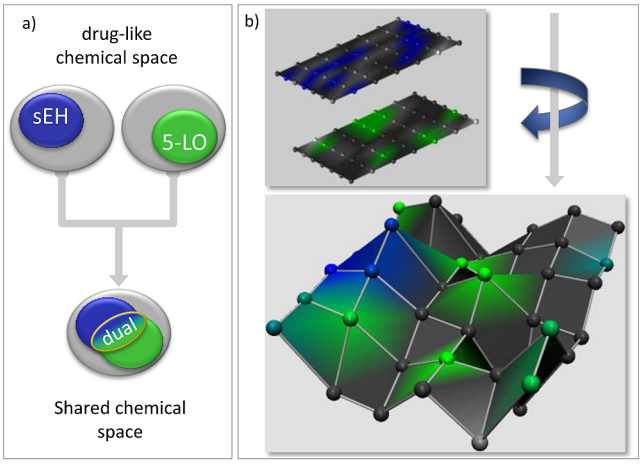

Figure 1. (a) Schematically depicted principle of the multiSOM approach. Two chemical subspaces described by the ligands of two distinct targets are aligned to determine the shared chemical space. (b) Primary maps of the multiSOM approach trained on the 5-LO/DrugBank and sEH/DrugBank frequent substructures (top). The resulting multiSOM map represents the similarity of the underlying, aligned neurons by its third dimension (bottom); a higher lying neuron means a higher similarity of the aligned, original neurons.

inactive substructure sets, we used the same multiSOM approach for the virtual screening. Because of the difference in size between the Specs fragment library and the frequent substructures collection, we decided to perform multiple multiSOM calculations in parallel. Each of these multiSOMs was trained on the dual substructures, the inactive DrugBank substructures and an equally sized subset of the Specs fragments library. Again, multiSOM neurons, which aggregated only dual relevant substructures and Specs fragments were further analyzed and served as a source for the final list of fragments purchased. We performed this procedure with three different fingerprint types, including the RDKit FeatMorgan, the RDKit layered fingerprint[25], and the 2D CATS descriptor,[26] to maximize the chemical diversity of the purchased fragments. All three screenings yielded a total of 274 fragments of which we purchased 24 compounds regarding manual inspection and availability.

We had to determine the activity of the purchased fragments on two separate targets, which led to the decision to use a combination of two complementary in vitro assay approaches consisting of STD-NMR as binding assay under the same conditions for both targets, followed by functional assays for 5-LO and sEH. STD-NMR allows the testing of multiple fragments in a single experiment. Because of this fact, we clustered all 24 compounds in five subsets leading to a total of ten STD-NMR experiments for both targets. The composition of each subset was aimed to maximize the difference between the chemical shifts of the included fragments to facilitate the discrimination of the subsequent STD-NMR results. All fragments were tested at a final concentration of 400 μM. Despite these preparations, we were not able to determine an utterly clear discrimination between all fragments on the STD-NMR spectra; thus, we had to classify the fragment in binders, nonbinders, and potential binders. We additionally determined the inhibitory potency of all fragments at a single concentration of 100 μM in the particular functional in vitro assays for both recombinant enzymes. In the case of the 5-LO, we used a HPLC-based assay to determine the remaining 5-LO product

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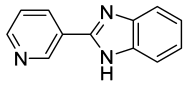
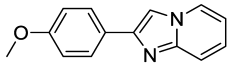
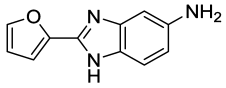
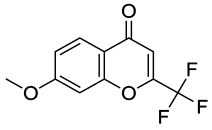
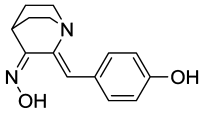
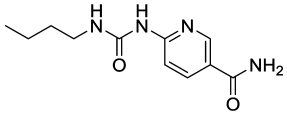
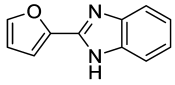
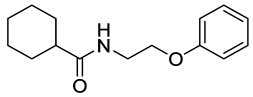
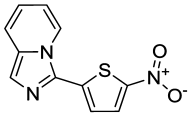
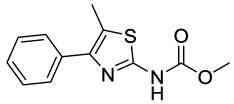
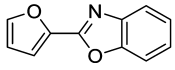
formation.[27] The sEH activity was determined in a fluorescence-based assay, which uses (3-phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester (PHOME) as substrate (for all STD-NMR data and inhibition at 100 μM, see Supporting Information)

Afterward, we determined an IC_{50} of all fragments, which showed a potential dual binding in the STD-NMR and an inhibition of at least 25% in the functional assays. The results of the 11 compounds matching these criteria are shown in Table 1. We were able to identify fragments with a functional IC_{50} value in the range of 3 to 379 μM for sEH and in the range of 7 to 237 μM for 5-LO. For both targets, we identified known, already described active scaffolds like benzimidazole, urea, and amide (**l**, **q**, **o**, and **r**) for the sEH or imidazo-[1,2-a]-pyridine, aminothiazole, and benzoxazole (**g**, **w**, and **x**) for the 5-LO as well as new, so far not described scaffolds like compounds **m** and **n**.

Furthermore, we were able to identify five fragments **i**, **m**, **n**, **w**, and **x**, which exhibit inhibitory activity on both targets. Especially compound **n** showed inhibitions in a low micromolar concentration range for both sEH and 5-LO. To investigate the optimization potential of at least one of the fragments, we searched our in-house library for compounds containing the dual hit fragments. We found a derivative of fragment **w**, an enlarged aminothiazole **1** (ST-1366, Figure 2).

Table 1. IC_{50} Values (μM) and STD-NMR Binding Data of the Most Promising Candidates

ID	Cpd.	sEH IC_{50}[a]	5-LO IC_{50}[a]	sEH STD[b]	5-LO STD[b]
f		91	-	×	✓
g		75	-	✓	✓
l		18	66	~	~
m		91	237	✓	~
n		95	27	✓	~
o		57	-	✓	✓
q		207	-	×	~
r		3	-	✓	~
v		-	8	n.s.	n.s.
w		379	89	n.s.	n.s.
x		133	7	n.s.	n.s.

[a]IC_{50} values in μM; each experiment was performed at least three times. [b]STD-NMR data: checkmark = binder; × = nonbinder; ~ = ambiguous; and n.s. = not soluble under STD conditions.

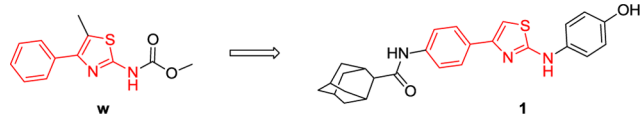


Figure 2. Substructure search in our in-house library based on compound **w** lead to compound **1**.

Compound **1** was subsequently tested in both assay systems yielding IC_{50} values of 0.03 μM at 5-LO and 0.17 μM at sEH (Figure 3). Thus, compound **1** is a good starting point for lead optimization.

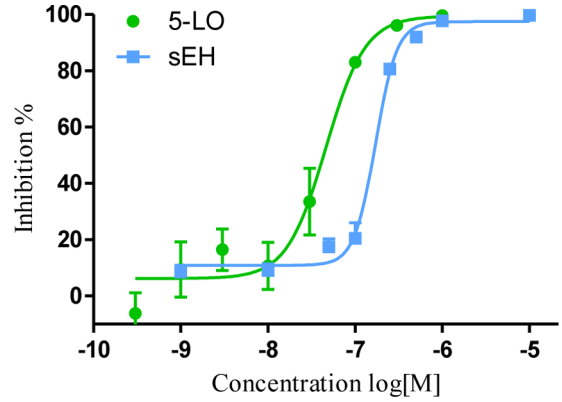


Figure 3. Dose–response curves for the IC_{50} determination of compound **1** on 5-LO (green) and sEH (blue).

In conclusion, our study presents a novel approach for the development of multitarget drugs. We show that fragment-based techniques are applicable to design multitarget ligands, as postulated in theoretical papers by Morphy and Rancovic[29,30] and Bottegoni et al.[5] We suggest an in silico technique for recognition of molecular fragments suitable for multitarget drug design, which led to enrichment of dual fragments targeting sEH and 5-LO in a prospective study. An exemplary testing of an enlarged fragment yields a potent lead structure for further optimization. Further studies following this multiSOM strategy are needed to demonstrate the broad applicability of diverse fragment-based design.

■ ASSOCIATED CONTENT

Supporting Information

More detailed description of the multiSOM approach, the identified relevant substructures for each target, all purchased compounds and assay setups, and synthesis of compound **1**.

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AUTHOR INFORMATION

Corresponding Author

*(E.P.) Tel: +49 69 798 29301. Fax: +49 69 798 29258. E-mail: proschak@pharmchem.uni-frankfurt.de.

Notes

The authors declare no competing financial interest.

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