Dual-target virtual screening by pharmacophore elucidation and molecular shape filtering

Daniel Moser, Joanna M. Wisniewska, Steffen Hahn, Janosch Achenbach, Estel·la Buscató, Franca-Maria Klingler, Bettina Hofmann, Dieter Steinhilber and Ewgenij Proschak

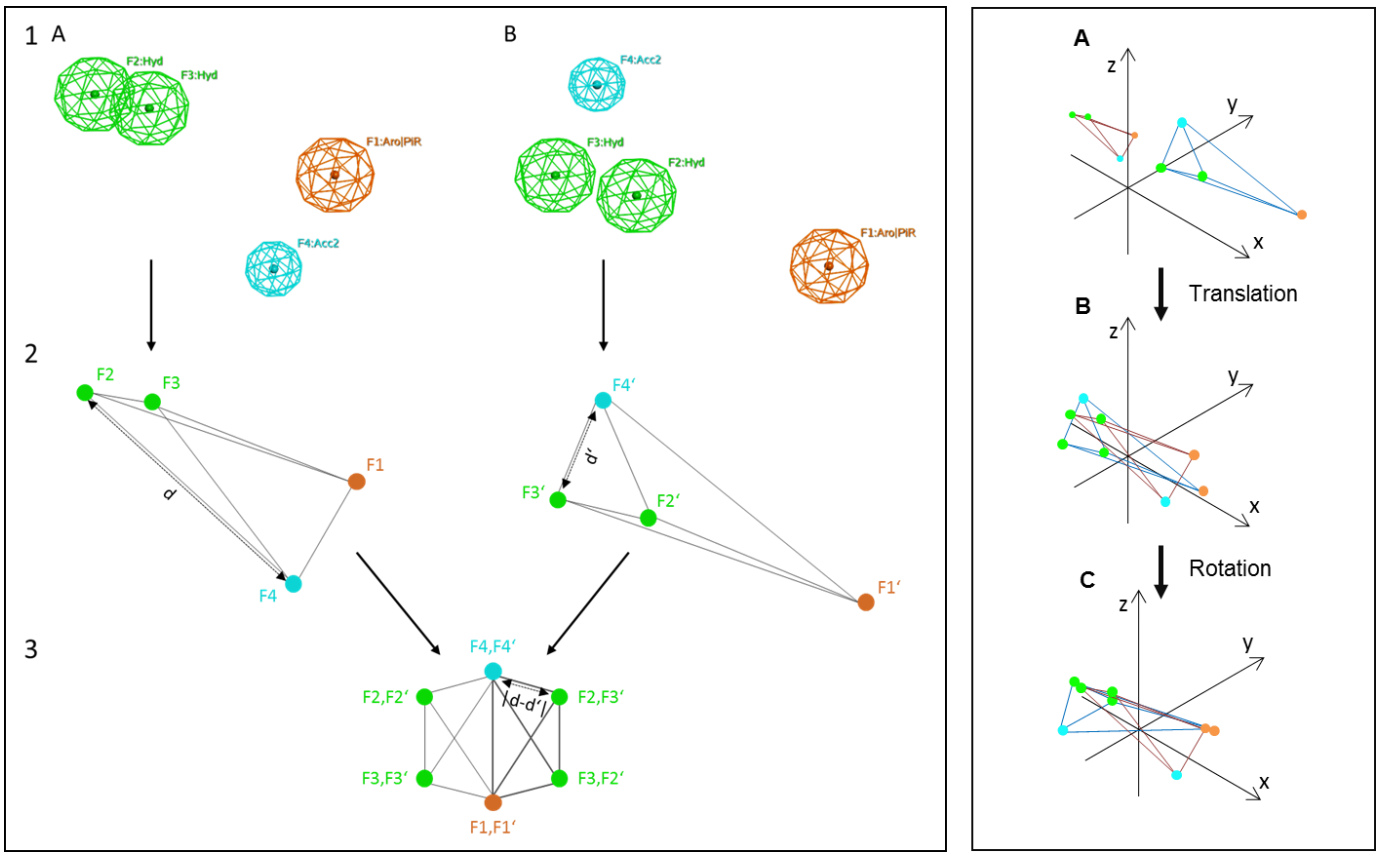
Computational Methods

Pharmacophore Elucidation

For the generation of pharmacophore models, the *pharmacophore elucidator* routine of the program MOE (Molecular Operating Environment (MOE), 2010.10, Chemical Computing Group Inc., Montreal, Canada **2010**) was employed. The elucidator tries to enumerate all models which are matched by at least a given percentage (active coverage) of the molecules. For all three virtual screening runs, the "Unified" annotation scheme was used with the following query expressions: "Aro|PiR, Hyd, Don2, Acc2, ML2, Cat, Ani, O2, CN2", "Aro|PiR, Hyd, Cat, Ani, O2, CN2" and "Aro|PiR, Hyd, Cat, Ani, O2, CN2, Don, Acc". The active coverage parameter was set to 0.2 (20 %), the query spacing to 1 and the query cluster to 1.25.

Pharmacophore Alignment

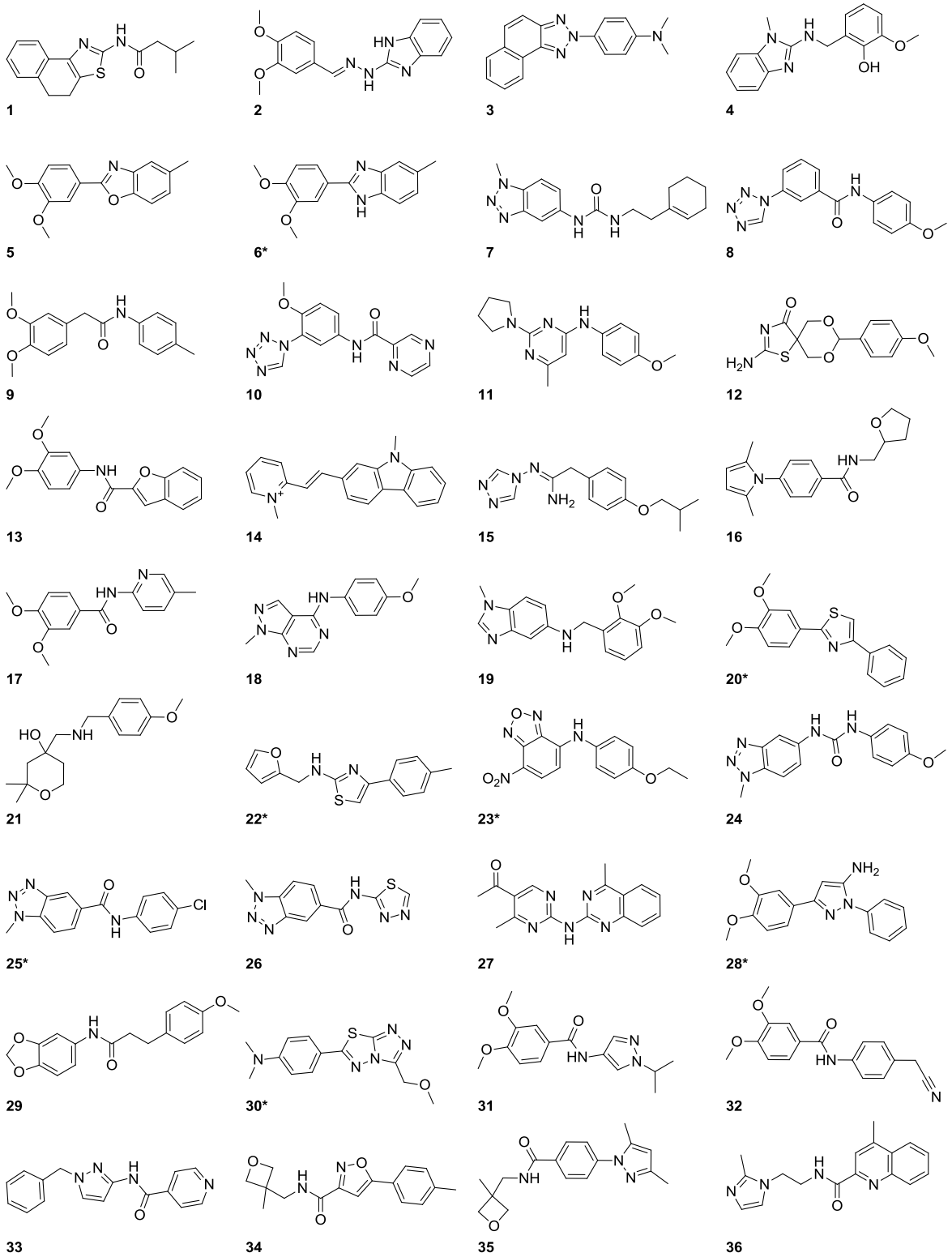
The alignment algorithm works basically as described by Proschak *et al.* (E. Proschak, M. Rupp, S. Derksen, G. Schneider, *J. Comput. Chem* **2008**, *29*, 108-114) and shown in Supplementary Figure 1. It was written in Java and implemented as a KNIME node.



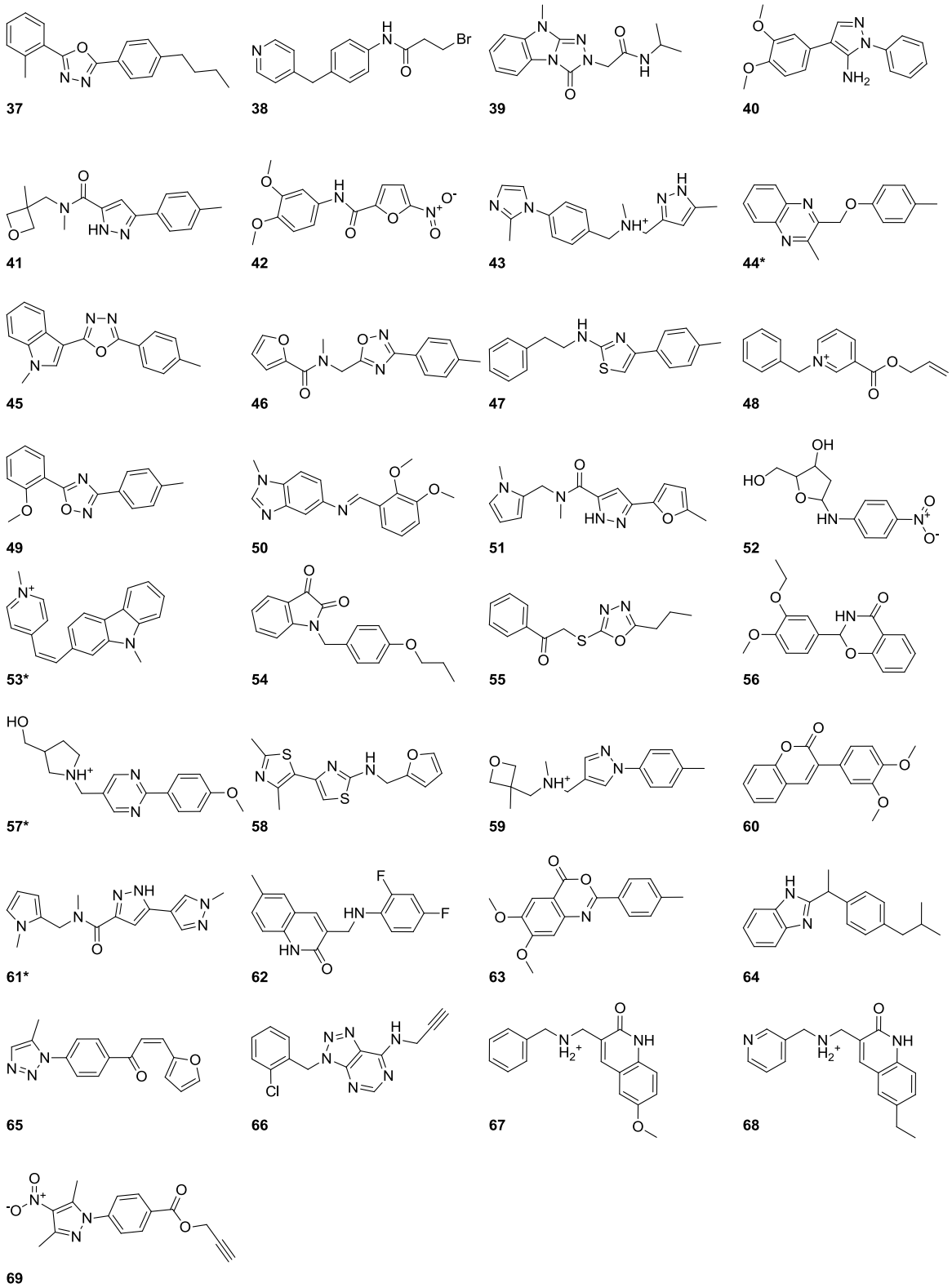
Supplementary Figure 1. Alignment of pharmacophore models. Left side: the models (1) are represented as fully connected, undirected graphs (2). The nodes are labeled with the feature, the edges with the *Euclidean distance* between two features. From these graphs, an association graph (3) is constructed, which represents corresponding nodes as well as their connectivity in both source graphs. For each pair of nodes from the source graphs, an association node is created, if these parent nodes have the same properties, i.e. pharmacophore features. Between two association nodes, an edge is inserted, if an edge between the corresponding parent nodes exists and the difference of the distances is below a user-defined threshold. Upon the association graph, a clique detection using Bron-Kerbosch Algorithm (C. Bron, J. Kerbosch, *Commun. ACM* **1973**, 16, 575-577) is performed to find the maximum common subgraph (highlighted bold in (3)) and therefore the greatest common subset of pharmacophore features between two models. Right side: Once the maximum cliques have been found, the corresponding nodes in the source graphs are aligned using the Kabsch Algorithm (W. Kabsch, *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1976**, 32, 922-923; W. Kabsch, *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1978**, 34, 827-828) which basically works in two steps: first, the graphs are translated to the origin of the coordinate system. Subsequently, an optimal rotation matrix is calculated and the graphs are rotated accordingly yielding the optimal alignment in terms of RMSD (root mean square deviation).

Selection of promising compounds

From the 80 compounds found by virtual screening (69 unique compounds), the 36 shown in Supplementary Figure 2 were chosen manually and or ordered for *in vitro* activity determination.



Supplementary Figure 2. The 36 compounds which were ordered for *in vitro* activity determination. Compounds marked with an asterisk were found in more than one ranking.



Supplementary Figure 3. The remaining 33 compounds which were not selected manually. Compounds marked with an asterisk were found in more than one ranking.

Assays

sEH

sEH assays were performed at room temperature (RT) in black polystyrol 96-well plates using PHOME (3-Phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester) as substrate (N. M. Wolf, C. Morisseau, P. D. Jones, B. Hock, B. D. Hammock, *Anal. Biochem* **2006**, *355*, 71-80). 10 µL of the respective test compound (dissolved in DMSO) at different concentrations in assay buffer (BisTris-HCl buffer (25 mM), 0.1 mg/mL BSA, 0.01 % Triton X-100, pH 7.0) were incubated with 100 µL of enzyme in assay buffer for 15 minutes at RT. The assay was performed in advance without inhibitors to determine the necessary amount of sEH to obtain about 1000 RFU (relative fluorescence units) after 15 minutes of measurement (*vide infra*). Subsequent to the incubation, 10 µL PHOME was added (final concentration 50 µM) and the increase in fluorescence was measured instantly over the next 15 minutes every minute. The increase of fluorescence during this time gives the activity of sEH in ΔRFU/min. The different activities of inhibited and not inhibited protein compared to the well without protein give the inhibition of the enzyme as follows (Eq. 1)

$$\text{Inhibition [\%]} = 100 \times \left[1 - \frac{V_I - V_B}{V_T - V_B}\right] \qquad \text{(Eq. 1)}$$

where V_I is the velocity obtained in the presence of an inhibitor (I); V_B is the velocity obtained for the blank (B) control; and V_T is the velocity obtained for the total activity (T) control (no inhibitor). The linearity of the reaction rate was checked.

5-LO

Recombinant 5 LO proteins were expressed at 22°C and purified from 1-liter cultures of *E. coli* BL21 transformed with the plasmid pT3-5-LO (YY. Zhang, O. Rådmark, B. Samuelsson, *Proc. Natl. Acad. Sci. U.S.A* **1992**, *89*, 485-489) according to the ATP affinity chromatography procedure as described previously (T. Hammarberg, YY. Zhang, B. Lind, O. Rådmark, B. Samuelsson, *FEBS J.* **1992**, *230*, 401-407; T. Hammarberg, P. Provost, B. Persson, O. Rådmark, *J. Biol. Chem.* **2000**, *275,* 38787-38793). For determination of 5-LO activity, 2 -3 µg protein was dissolved in 1 ml of reaction mix (PBS, pH 7.4, 1 mM EDTA, and 1 mM ATP). After pre-incubation with the test compounds or vehicle (DMSO) at the indicated concentrations for 15 min at 4°C, the samples containing purified 5-LO were pre-warmed for 30 sec at 37°C. The reaction was started after addition of 2 mM $CaCl_2$ and 20 µM AA. After 10 min at 37°C, the reaction was stopped with 1 ml of ice-cold methanol. 30 µl of 1 N HCl, 200 ng of prostaglandin B_1 as internal standard and 500 µl of PBS, pH 7.4 were added. 5-LO metabolites were extracted and analyzed by HPLC as described (M. Brungs, O. Rådmark, B. Samuelsson, D. Steinhilber, *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 107-111). 5 LO product formation was determined as ng of 5-LO products, which includes all-*trans* isomers of LTB_4, 5(*S*),12(*S*)-dihydroxy-6,10-*trans*-8,14-*cis*-eicosatetraenoic acid (5(S),12(S)-diHETE), and 5(*S*)-hydro(pero)xy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-H(p)ETE). Each experiment was performed at least three times. Data (mean ± S.E.) are expressed as percentage of control (DMSO).

IC_{50}-value estimation

IC_{50} values were estimated from the % of residual activity of the enzyme under influence of a compound compared to the average residual activity of the controls which were assigned 100 % residual activity. The values were plotted against the logarithmic inhibitor concentration (in molar) and fitted with Eq. 2 using GraphPad Prism 5 (log(inhibitor) vs. response (three parameters) equation with Least squares fit).

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{X - \log \text{IC}_{50}}} \qquad \text{(Eq. 2)}$$

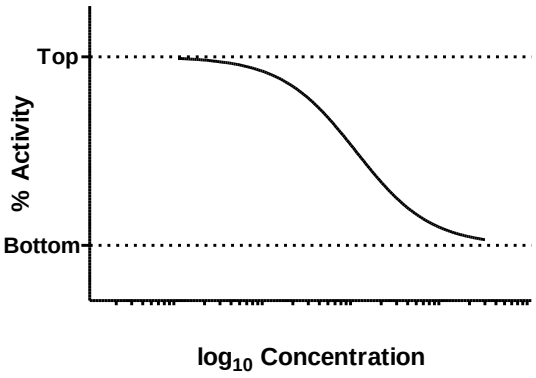
where

X: log of concentration

Y: Response, decreasing as X increases

Top and Bottom: Plateaus in same units as Y (Supplementary Figure 4)

$\log IC_{50}$: Same log units as X



Supplementary Figure 4. Top and bottom plateaus of an IC_{50} curve. The values are used to estimate the IC_{50} values using Eq. 3