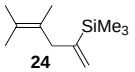
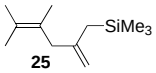
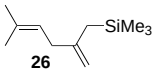
Table 3. Cobalt(I)-mediated hydrovinylation of silyl substituted alkenes with 1,3-dienes.

Entry	Substrates	Product	Yield [%]
1	 	 **24**	90
2	 	 **25**	92
3	 	 **26**	83

masked carbon nucleophiles is well described and very interesting transformations seem possible with these higher-substituted vinyl and allyl silanes.[7]

In conclusion, interesting linear and branched 1,4-dienes can be generated under very mild reaction conditions with good selectivities and in good to excellent isolated yields from the 1,4-hydrovinylation reactions. The investigation of reactions with higher-substituted functionalized alkenes and nonsymmetrical 1,3-dienes are currently underway.

Experimental Section

Representative procedure (synthesis of 4,5-dimethyl-2-methylene-4-hexenyloxybenzene (**12**)): A 50 mL flask was charged with [$CoBr_2$(dppe)](40 mg, 65 μmol, 1.8 mol%) and dry zinc iodide (100 mg, 313 μmol, 8.6 mol%) under nitrogen atmosphere and suspended in dry dichloromethane (2.0 mL). After the addition of 2,3-dimethyl-1,3-butadiene (0.5 mL, 363 mg, 4.42 mmol) and allyl phenyl ether (0.5 mL, 489 mg, 3.64 mmol), tetrabutylammonium borohydride (18 mg, 70 μmol, 1.9 mol%) was added, inducing a color change from green to brown. The mixture was stirred overnight at room temperature, then pentane (10 mL) was added and the solution filtered through silica gel with pentane/diethyl ether (10/1) as the eluent. The filtrate was reduced in volume and the product purified by column chromatography on silica gel with pentane/diethyl ether (50/1) as the eluent. The product **12** was obtained as a colorless liquid (772 mg, 3.57 mmol, 98% yield).

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Subnanomolar Inhibitors from Computer Screening: A Model Study Using Human Carbonic Anhydrase II

Sven Grüneberg, Bernd Wendt, and Gerhard Klebe*

The search for novel lead structures as potential drugs or herbicides is increasingly time consuming and costly. Modern methods of disease characterization at the molecular level, driven in particular by the Genome Project,[1] promise to deliver a plethora of potential therapeutic targets. How well are we prepared to transform this flood of information into lead structures? Enormous effort has been put into large-scale automation of experimental hi-tech high-throughput screening (HTS). Initial euphoria surrounding this technique as a universal lead generator has subsided as a result of the considerable costs involved, coupled with frequent inadequacies in quality and quantity of the available compounds for screening.[2] This raises the question: are computer methods sufficiently mature to complement the experimental screening process for new lead structures?

The requirements for computer screening[3] are opposite to those for HTS. The latter is technology-driven, delivering potentially structurally diverse hits by identifying an interaction with the target. No insights are obtained into why a particular hit interacts, however. In contrast, virtual computer screening is dependent upon prior information about factors necessary for binding to the target; thus, it is knowledge-driven. Computer screening should therefore provide the interactions responsible for binding. The rules governing protein–ligand interactions are, however, complex and are only in part understood.[4] We decided to investigate whether the rules incorporated in current programs are able to reliably predict novel ligands.

We chose human carbonic anhydrase II for our test study, as its structure has been solved to high resolution. We utilized existing computational tools to analyze the binding pocket in

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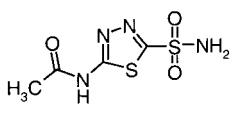
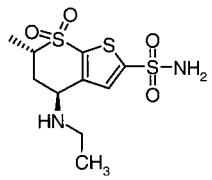
detail. To convert the binding pocket properties into a query that could be applied to a database of chemical structures it was necessary to develop a number of new strategies. A variety of (in part new) filters were applied to the hits obtained, reducing the data to an essential core by way of molecular similarity, ligand docking, and estimation of binding affinity.

Three-dimensional molecular candidates were generated by using the program CORINA[5] from about 90 000 entries of the Maybridge[6] and LeadQuest[7] databases. The latter database has been designed to contain molecules that are easily synthesized yet highly chemically diverse.[8] The 13 best hits from this search were tested for inhibition experimentally; of these, three could be shown to be subnanomolar, one nanomolar, while a further seven were micromolar inhibitors. The structures of two of the best inhibitors could be determined in complex with the human carbonic anhydrase II crystallographically, providing a "proof of concept".

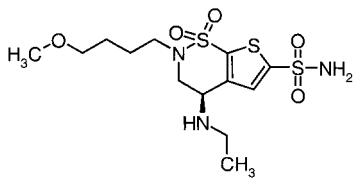
Carbonic anhydrase II (CAII; E.C. 4.2.1.1) is a metalloenzyme that catalyzes the reversible hydration of CO_2 to HCO_3^-.[9] The reactive zinc center is coordinated by three histidine groups at the base of a conical amphiphilic binding pocket. One isoform of CAII is concentrated in the ciliary body of the eye, where it is responsible for aqueous production. Glaucoma leads to reduced ocular water removal with subsequent increase in intraocular pressure (IOP) and thereby damage to the optic nerve. Inhibitors of CAII thus offer a possible route to reducing intraocular pressure. The binding modes of acetazolamide (**1**),[10] dorzolamide (**2**; Figure 1),[11] and brinzolamide (**3**)[12] have been determined by using crystallographic methods. Derivatives of both sulfonamide and hydroxamate[13] have been identified as inhibitors, although the latter exhibit much lower affinity for CAII. Initial analysis of the binding pocket entailed multiple superpositioning of the C_α atoms of 24 high-resolution complex crystal structures[14] by using our modified version of Relibase+.[15] The pocket is rather rigid, with only His64 exhibiting alternative conformations (Figure 2).[11]



1



2



3

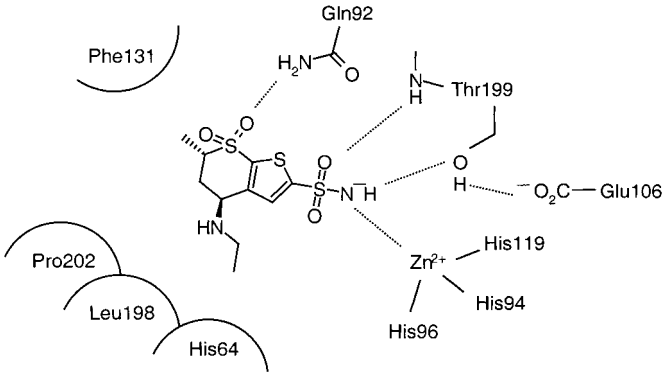


Figure 1. The binding of the the zinc ion to a sulfonamide group, which is most probably singly deprotonated, is common to all sulfonamide inhibitors, and results in a tetrahedral coordination geometry at the zinc center. The remaining sulfonamide proton forms a hydrogen bond to the hydroxy oxygen atom of the Thr199. A further hydrogen bond exists between the amide nitrogen atom of Thr199 and one of the two sulfonamide oxygen atoms; the remaining oxygen atom makes no contacts with the protein. For dorzolamide, an additional hydrogen bond is formed between one of the two oxygen atoms of the second sulfone moiety and the side chain nitrogen atom of Gln92.

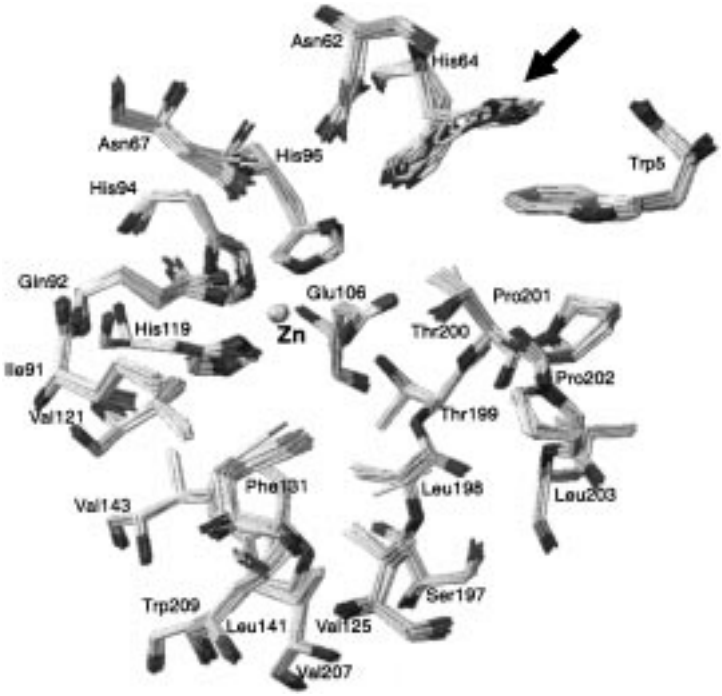


Figure 2. Superposition of 24 protein–ligand complexes of CAII (ligands not shown) calculated in Relibase+ based on C_α positions. With the exception of His64 (arrow), the binding pocket is rather rigid.

The pocket was then investigated by using various probes in GRID,[16] SuperStar,[17] LUDI,[18] and DrugScore.[19] The pocket was systematically searched for favorable interactions with possible functional groups of a ligand; the probes used were a C=O group (H-bond acceptor), an amide NH group (H-bond donor), and a CH_3 group (hydrophobic probe). Although each method highlighted qualitatively similar regions (hot spots), the maps differed quantitatively due to different relative weightings. Hot spots for the three probes were converted into a pharmacophore model to allow the

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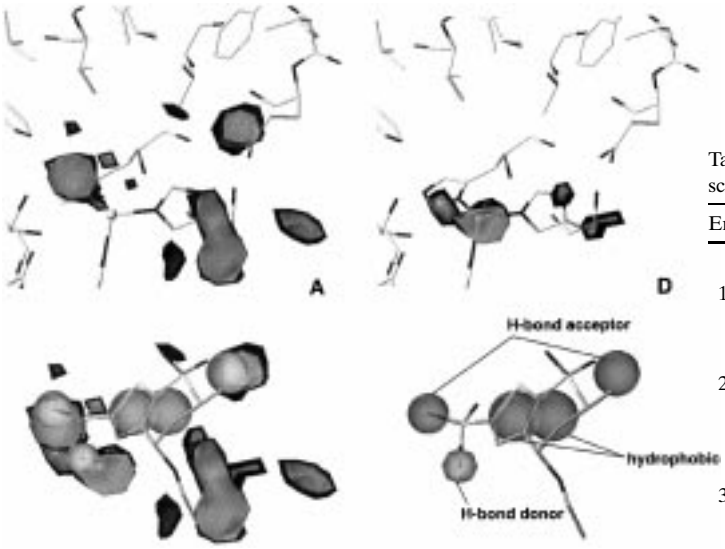
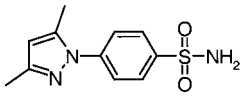
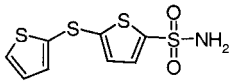
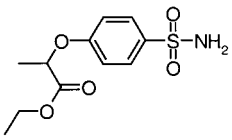
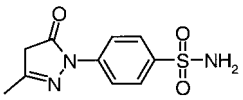
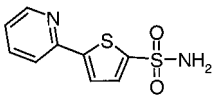
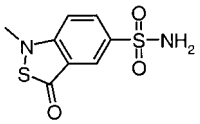
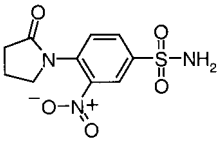
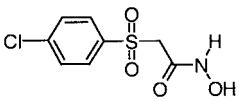
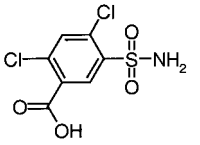
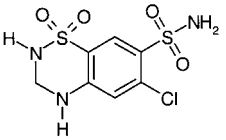
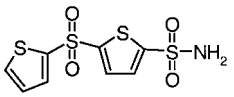
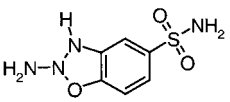
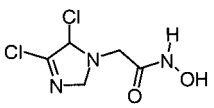


Figure 3. Analysis of preferred interactions for hydrogen-bond acceptors (A; top left) and hydrogen-bond donors (D; top right) within the binding pocket of CAII. These maps were calculated using SuperStar; analogous results were obtained with GRID and DrugScore, albeit with different relative weightings. LUDI generated interaction centers in similar positions for C=O, NH, or aliphatic carbon probe. This "hot spot" distribution was converted into a pharmacophore query for UNITY (bottom right), taking into account the hydrophobic excluded volume according to known inhibitor binding modes. SuperStar calculates probability densities based on contact geometries determined from low molecular weight crystal structures. GRID is based on a force-field approach, allowing calculation of interaction potentials for a large variety of different probes within the binding pocket. DrugScore uses the "inverse Boltzmann principle", evaluating the binding pocket according to frequency for which a particular atom type of a potential ligand is found in contact with a protein in known protein–ligand complexes. LUDI places potential interaction sites for hydrogen-bond donors, acceptors, and hydrophobic groups within the binding pocket according to a set of empirical rules derived from experimental data.

database search using UNITY.[20] In addition to matching the thus formatted query, the molecular structures extracted from Maybridge and LeadQuest had also to contain at least one zinc-binding head group.[21] A total of 35 known inhibitors of CAII were added to the database, whose appearance in the hit list served to calibrate and validate the method at each stage.[22]

The 2D and 3D searches with UNITY yielded 3314 molecules after taking flexibility into consideration. The resulting compounds were ordered according to their potential binding abilities using the program FlexS.[23] FlexS flexibly superimposes a test ligand on a reference molecule through optimizing the spatial distribution of various physicochemical parameters. The highly potent inhibitor dorzolamide, in the conformation known from the crystal structure, was used as reference. Scores for the additional test ligands indicated that a size normalization was important to obtain the correct order in the hit list. Use of such a similarity search has the advantage that information on the chemical structures of known inhibitors is implicitly taken into account, information that is ignored in a simple docking strategy. All CAII inhibitors reported to date, for example, possess a sulfonamide anchor as zinc-coordinating element; the superposition using FlexS ensures that this fact is utilized in the ranking of possible lead structures. Procedures for combining docking and similarity to ligands of known potency have recently been implemented in DOCK[24] and DrugScore.[25]

Table 1. Lead structures for CAII inhibitors generated by using the virtual screening procedures described here.

Entry	Compound	FlexX[a]	DrugScore[a]	IC_{50} [nM]	Databank
1		– 25.7	– 102.2	21	LeadQuest
2		– 15.0	– 107.1	0.9	LeadQuest
3		– 17.3	– 116.5	185	LeadQuest
4		– 23.4	– 100.6	488	LeadQuest
5		– 19.9	– 100.9	117	LeadQuest
6		– 21.6	– 112.4	248	LeadQuest
7		– 25.7	– 105.1	0.6	LeadQuest
8		–	–	$> 10^6$	LeadQuest
9		– 16.0	– 83.5	169	LeadQuest
10		– 23.4	– 88.6	5500	Maybridge
11		– 16.5	– 97.4	0.8	Maybridge
12		– 23.0	– 94.1	2300	Maybridge
13		–	–	$> 10^6$	Maybridge

[a] Large negative scores correspond to high predicted affinities. FlexX and DrugScore values cannot be compared directly with one another, relative comparisons can only be made within each of the methods. No score has been given for the two hydroxamate inhibitors (entries 8 and 13) as these cannot be compared directly due to the different zinc-coordinating groups.

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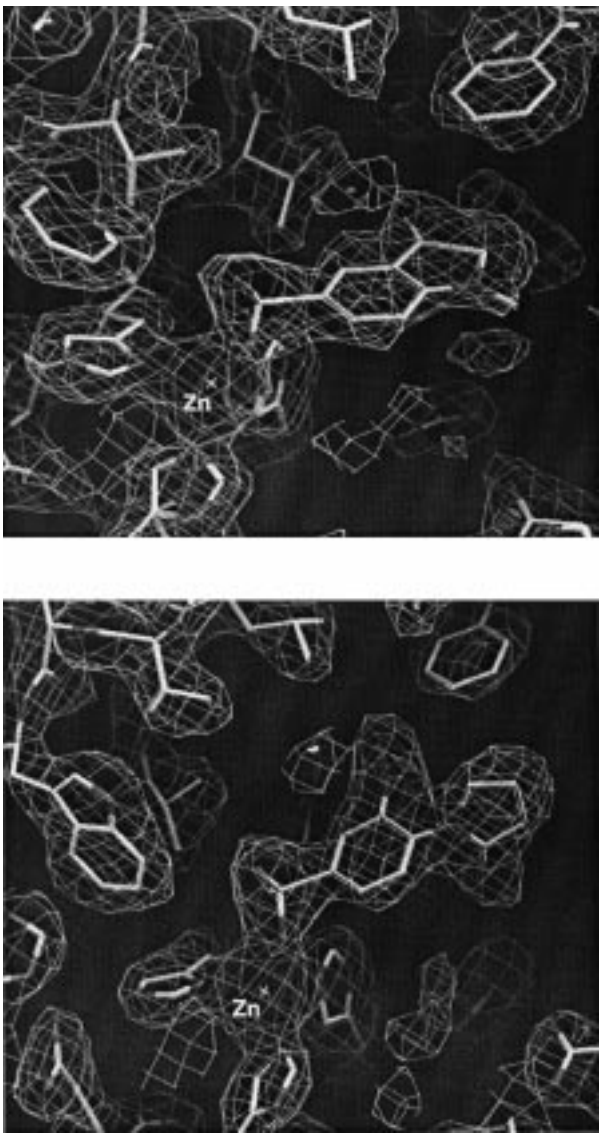

Figure 4. Experimental electron density for the inhibitors given in entries 6 (top) and 7 (bottom) in Table 1 in the binding pocket of CAII.

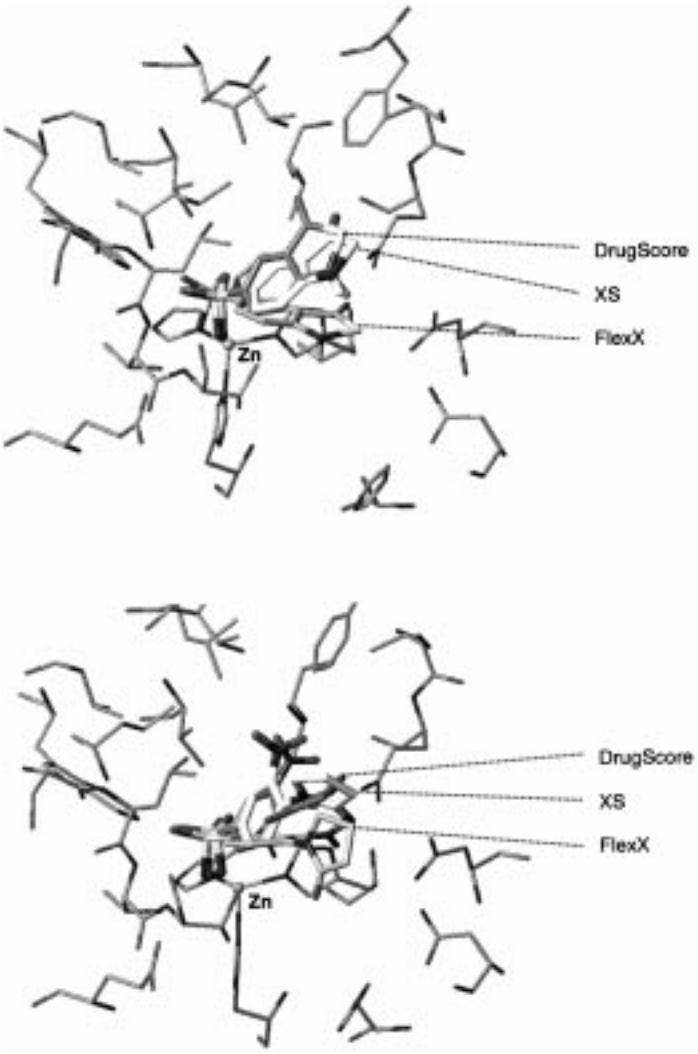

Figure 5. Superposition of the X-ray structure (XS) and docked models for the inhibitors given in entries 6 (top) and 7 (bottom) in Table 1 in CAII. In both cases, DrugScore predicts a binding geometry (generated using FlexX) that is very close to the experimentally observed geometry. The original scoring function in FlexX places a binding geometry as highest ranking that diverges significantly from the experimental one.

The best scoring hits derived from this procedure were then docked into the binding pocket using FlexX,[26] in which the partial hydration of the pocket proved to play an important role. Four conserved water molecules could be identified following superposition of all complex- and apo-structures in Relibase+, taking into account B-factors, local hydrophobicity, and neighboring hydrogen-bonding partners, and backed up by an analysis of the H-bond network using MAB/Moloc.[27] These solvent molecules added to the steric restriction of the binding pocket.

Following the docking procedure, binding affinity was estimated using FlexX and DrugScore, and the top ranking 13 hits were chosen for experimental testing. These 13 hits were also inspected visually for their FlexX-generated binding modes. The 13 compounds (with 10 from LeadQuest) are shown in Table 1, together with their computer scorings and experimental IC_{50} values.

Two of the resulting high affinity hits were investigated crystallographically (Figure 4).[29] In both cases the binding mode generated by FlexX and ranked as highest using DrugScore is in good agreement with the experimental structure (Figure 5).

This emphasizes the improvement afforded by the knowledge-based scoring function of DrugScore over the regression-based function implemented in FlexX, which placed these solutions at rank 51 and 61, respectively. Validation using the compounds of known structure also indicated that DrugScore showed a marked improvement in finding the correct binding mode.

The correlation between the IC_{50} values and the affinities predicted by DrugScore or FlexX is rather disappointing; at best, a correct order of magnitude could be found. Other studies have shown predicted affinities to be accurate to about 1.5 lg units.[28] Experimental measurements are often only correct to an order of magnitude, however; methods for affinity prediction from structures must be brought to within these limits. The development of a better method to define

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native-like binding geometries represents an important first step towards this goal.

Finally, we have shown that the LeadQuest Database provides an ideal source for the discovery of novel lead compounds,[31] in terms of both structural diversity[8] and chemical purity.[30]

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Thioformaldehyde *S*-sulfide (Thiosulfine)**

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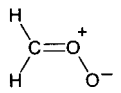
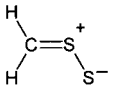
The elucidation of the course of events in the ozonolysis of olefins by Criegee[1] was a milestone in the effort to understand the mechanism of organic reactions. As early as in 1949 it was recognized that carbonyl oxides are the decisive intermediates in this process.[2] However, to date it has not been possible to observe these species directly during the transformation of "primary" into "secondary" ozonides. In contrast, an entry into carbonyl oxides exists in the trapping of carbenes with oxygen under matrix conditions;[3] however, the unsubstituted formaldehyde *O*-oxide **1** cannot be isolated even upon using this procedure. In the reaction of methylene with oxygen in an argon matrix only formic acid was detected.[4]



1



2

The idea that it might be possible to generate formaldehyde together with its oxide by cycloreversion of 1,2,4-trioxolane has not yet been realized, presumably due to the fact that the required ozonide of ethene is difficult to handle.[5] As is shown herein,[6] the situation is completely different in the sulfur series. 1,2,4-Trithiolane (**3**) is a stable, easily accessible

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