Substrate specificity of the ileal and the hepatic Na^+/bile acid cotransporters of the rabbit. II. A reliable 3D QSAR pharmacophore model for the ileal Na^+/bile acid cotransporter

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Abstract To design a reliable 3D QSAR model of the intestinal Na^+/bile acid cotransporter, we have used a training set of 17 inhibitors of the rabbit ileal Na^+/bile acid cotransporter. The IC_{50} values of the training set of compounds covered a range of four orders of magnitude for inhibition of [^{3}H]cholyltaurine uptake by CHO cells expressing the rabbit ileal Na^+/bile acid cotransporter allowing the generation of a pharmacophore using the CATALYST algorithm. After thorough conformational analysis of each molecule, CATALYST generated a pharmacophore model characterized by five chemical features: one hydrogen bond donor, one hydrogen bond acceptor, and three hydrophobic features. The 3D pharmacophore was enantiospecific and correctly estimated the activities of the members of the training set. The predicted interactions of natural bile acids with the pharmacophore model of the ileal Na^+/bile acid cotransporter explain exactly the experimentally found structure–activity relationships for the interaction of bile acids with the ileal Na^+/bile acid cotransporter (Kramer et al. **1999.** *J. Lipid. Res.* 40: **1604–1617).** The natural bile acid analogues cholyltaurine, chenodeoxycholyltaurine, or deoxycholyltaurine were able to map four of the five features of the pharmacophore model: *a*) the five-membered ring D and the methyl group at position 18 map one hydrophobic site and the 21-methyl group of the side chain maps a second hydrophobic site; *b*) one of the α-oriented hydroxyl groups at position 7 or 12 fits the hydrogen bond donor feature; *c*) the negatively charged side chain acts as hydrogen bond acceptor; and *d*) the hydroxy group at position 3 does not specifically map any of the five binding features of the pharmacophore model. The 3-hydroxy group of natural bile acids is not essential for interactions with ileal or hepatic Na^+/bile acid cotransporters. A modification of the 3-position of a natural bile acid molecule is therefore the preferred position for drug targeting strategies using bile acid transport pathways.**—Baringhaus, K-H., H. Matter, S. Stengelin, and W. Kramer. **Substrate specificity of the ileal and the hepatic Na^+/bile acid cotransporters of the rabbit. II. A reliable 3D QSAR pharmacophore model for the ileal Na^+/bile acid cotransporter.** ***J. Lipid Res.*** **1999.** 40: **2158–2168.**

Supplementary key words bile acids • 3D QSAR • pharmacophore model • structure–activity relationships • ileum • liver

The enterohepatic circulation of bile acids is an efficacious biological recycling system established by active Na^+-dependent bile acid transport systems in the terminal ileum and the liver as well as the proximal tubule cells of the kidney (1, 2). These Na^+/bile acid cotransporters belong to the family of 7-transmembrane-domain solute transporters, showing 35–37% identity and 46–48% similarity between the ileal and the hepatic transporter (3–6). Based on the everted sac model and in vivo absorption measurements, Lack and coworkers (7) were able to suggest a hypothetical model for the interaction of bile acids with the intestinal Na^+/bile acid cotransporter with the following characteristics: *1*) a negatively charged side-chain of the bile acid molecule for a coulombic interaction with a positively charged group of the carrier protein; *2*) at least one axial hydroxyl group at the steroid nucleus at position 3, 7, or 12; *3*) a *cis* configuration of cyclohexyl rings A and B of the steroid nucleus for hydrophobic interaction with the carrier protein; and *4*) a negatively charged amino acid in the carrier protein for interaction with Na^+-ions.

This hypothetical model has meanwhile been strengthened by chemical modification techniques. Our laboratory was able to identify the positively charged group in the carrier protein as an ε-amino group of lysine and the negatively charged group of the transporter as vicinal cysteine residues (8).

The ileal Na^+/bile acid cotransport system regulates the activity of hepatic cholesterol-7α-hydroxylase as the rate-limiting step for the conversion of cholesterol into bile acids (1, 2) via the flux of bile salts recirculating back to the liver with portal blood. Thus, the ileal Na^+/bile acid cotransporter is a promising target for new nonsystemically acting hypolipidemic drugs (9–12). For the de-

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sign of optimized high affinity inhibitors, a fundamental understanding of the interaction of ligands and inhibitors with the ileal Na^+/bile acid cotransport system is necessary.

In the present study we describe the generation of a reliable 3D QSAR model of ileal Na^+/bile acid reabsorption inhibitors, which maps the variation of biological activity of our compounds as a function of their 3-dimensional structure. Thereby, molecular structure–activity relationships of the interaction of bile acid analogues with the ileal Na^+/bile cotransporter protein could be deduced. Furthermore, this 3D QSAR model was able to explain the experimentally found structure–activity relationships for the interaction of bile acids with the ileal Na^+/bile acid cotransporter (5).

MATERIALS AND METHODS

Materials

[G-^{3}H]cholyltaurine (specific radioactivity 2.1 Ci/mmol) was obtained from DuPont-New England Nuclear (Dreieich, Germany). All compounds of the training set were synthesized at Hoechst Marion Roussel Deutschland GmbH, Frankfurt. The bile acid analogues used for the transport studies were synthesized as described previously (5). Cell culture medium was Minimal Essential Medium (MEM) with 1% (v/v) of MEM nonessential amino acids and 10% (v/v) fetal bovine serum. All three medium components and PBS were from GIBCO-BRL (Life Science Technologies GmbH, Eggenstein, Germany).

Plasmid and cell lines

All cDNAs encoding bile acid transporters from rabbit or human ileum were cloned by RT-PCR. Cloning of the human and rabbit ileal Na^+/bile acid cotransporter was based on published sequences (13) and as described previously (5, 6).

Bile acid transport assay

The Na^+-dependent uptake of [^{3}H]cholyltaurine into stably transfected CHO cells expressing the rabbit Na^+/bile acid cotransporter (pKIBAT8) was measured in the presence of inhibitors to determine IC_{50} values. Cells were seeded and grown on 96-well plates as described (5). For transport measurements, cells were incubated at 22°C for 30 min with the respective inhibitors at concentrations of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, or 10^{-3} m dissolved in PBS. Afterwards, 10 μm [^{3}H]cholyltaurine was added and incubation was continued for 60 min. Then, cells were washed with PBS five times and cells were lysed by incubation for 15 min with 1 ml of a solution of 0.1 m NaOH/0.1% (w/v) SDS. Lysed material was triturated, mixed with 10 ml of a scintillation cocktail, and radioactivity was measured by liquid scintillation counting. All measurements were performed in triplicate and the mean values were used to determine the IC_{50} values. For the generation of the pharmacophore, the inhibition data obtained with pKIBAT 8 cells expressing the rabbit ileal Na^+/bile acid cotransporter were used. With pHIBAT 8 cells expressing the human ileal Na^+/bile acid cotransporter, a nearly identical ranking of the inhibitory potency of the test compounds was obtained; generally, a 50% inhibition of [^{3}H]cholyltaurine uptake was achieved at 1.5- to 3-fold lower concentrations with the human ileal Na^+/bile acid cotransporter compared to the rabbit transporter.

Generation of the 3D QSAR model

The 3D QSAR study was performed with the CATALYST software (14). All 17 bile acid reabsorption inhibitors of our training set were edited within CATALYST and minimized to the closest local minimum using the generalized CHARMm-like force field implemented in the program. The CATALYST model treats molecular structures as templates consisting of chemical functions positioned in space that will bind effectively with complementary functions on the respective binding proteins. The most relevant biological features are extracted from a small set of compounds that cover a broad range of activity (15). Molecular flexibility is taken into account by considering each compound as an ensemble of conformers representing different accessible areas in 3D space. The "best searching procedure" was applied to select representative conformers within 20 kcal/mol from the global minimum (16). CATALYST hypotheses are described by a set of hydrophobic, hydrogen bond donor, hydrogen bond acceptor, and positively and negatively ionizable sites distributed within 3D space. The hydrogen bonding features are vectors, whereas all other functions are points. The hypotheses are generated using the CatHypo module of CATALYST (14). The statistical relevance of the obtained hypothesis was assessed on the basis of their cost relative to the null hypothesis and their correlation coefficient.

RESULTS AND DISCUSSION

Biological basis for the 3D QSAR model

A prerequisite for the development of a reliable 3D QSAR model for the ileal Na^+/bile acid cotransporter is the correlation of a characteristic and reproducible biological activity to structural information of the respective compound. As biological activity a variety of parameters such as IC_{50} values, EC_{50} values, inhibition constants, or uptake rates may be used. Because our interest was primarily the design of high-affinity nonabsorbable inhibitors of the ileal Na^+/bile acid cotransporters, we decided to use the IC_{50} values of compounds inhibiting the ileal Na^+/bile acid cotransporter as the biological parameter to develop a pharmacophore model. In order to obtain a reliable 3D QSAR model adequately describing the interaction of ligands with a high predictability, several aspects have to be considered.

A collection of 15–25 chemically diverse molecules is necessary, i.e., the inhibitors should belong to different chemical classes. Using only bile acid analogues would lead to a pharmacophore model with a good predictability for bile acid analogues only.

The biological activity of the training set of compounds should cover at least 4–5 orders of magnitude. Therefore, we decided to use the IC_{50} values obtained by inhibition of [^{3}H]cholyltaurine by transfected CHO cell lines expressing the rabbit ileal Na^+/bile acid cotransporter. As the IC_{50} values for transport inhibition obtained with ileal brush border membrane vesicles covered a smaller range of 2–3 orders of magnitude compared to transfected CHO cells, we used the data elaborated with the transfected CHO cells for the following reasons. *a*) A broader range of IC_{50} values could be obtained, and *b*) the transfected CHO cells do not possess an extended glycocalix which can be a penetration barrier for inhibitors to get access to the ileal bile acid transporter at the surface of the brush border membrane. Thus, inhibition data obtained with transfected CHO cells reflect the direct molecular in-

teraction of the inhibitors with the transporter protein, whereas the data obtained with brush border membrane vesicles are the net result of direct transporter interaction and diffusion effects of the inhibitors across the unstirred water layer of the brush border membrane.

Compounds of the "training set"

As mentioned above, the training set of compounds used to generate a pharmacophore should exhibit chemical diversity. We therefore selected a training set of 17 structurally diverse bile acid reabsorption inhibitors which

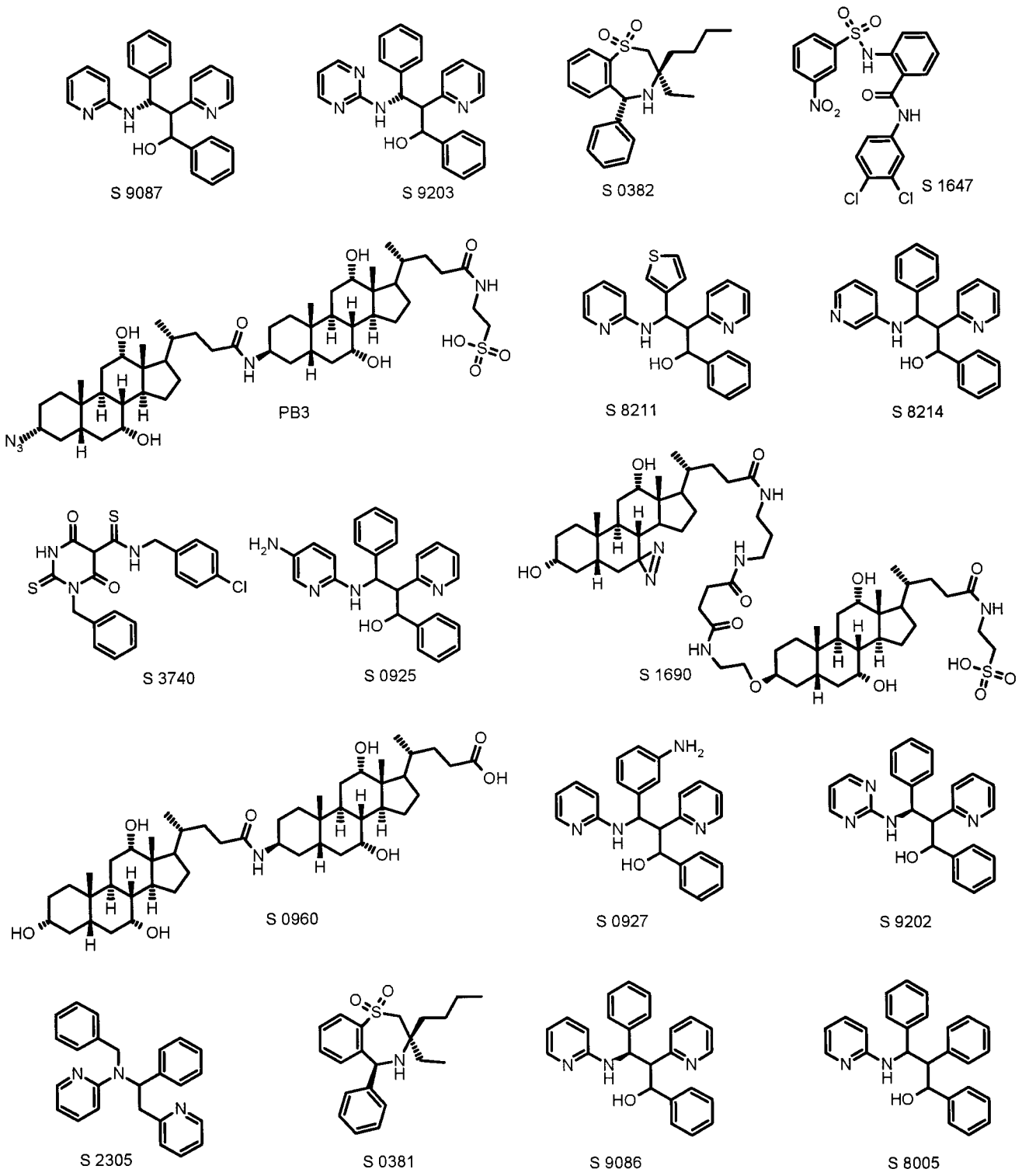

Fig. 1. Chemical structures of the molecules of the training set for generation of the 3D QSAR hypothesis.

TABLE 1. Estimated and experimentally measured activities (IC_{50} values) of the compounds of the training set

Compound	Estimated IC_{50} values	Measured IC_{50} values	Error
	μM	μM	
S 9087	0.2	0.3	−1.5
S 9203	2.8	1	+2.8
S 0382	8.9	3	+3
S 1647	33	4	+8.3
PB3	12	4	+3
S 8211	2	4	−2
S 8214	4.4	4	+1.1
S 3740	4.6	8	−1.7
S 0925	27	16	+1.7
S 1690	25	20	+1.3
S 0960	18	30	−1.6
S 0927	22	36	−1.7
S 9202	91	90	+1
S 2305	590	300	+2
S 0381	1100	700	+1.6
S 9086	890	1500	−1.7
S 8005	1600	3000	−1.9

The IC_{50} values were determined by measuring the uptake of 10 μm [^{3}H]cholyltaurine by pKIBAT 8 cells in the absence and presence of varying concentrations of the compounds in the concentration range 10^{-9} to 10^{-3} m as described in the Experimental Section. The values in the Error column represent the ratio of the estimated activity to the measured activity, or its negative inverse if this ratio is less than one.

were either identified by screening of chemical libraries or by rational design (the dimeric bile acid analogues S 0960, PB-3 and S 1690 (17)) as well as published bile acid reabsorption inhibitors, the benzothiazepines S 0382 and S 0381 (11, 18). The structures of the training set are shown in **Fig. 1**. The IC_{50} values of these compounds for inhibition of [^{3}H]cholyltaurine by pKIBAT 8 cells covered a range of 4 orders of magnitude between 300 nm and 3 mm (**Table 1**).

Generation of the pharmacophore

For the generation of the pharmacophore we used the CATALYST 2.3 software (Molecular Simulation Inc., Burlington, MA) to extract the information from the structure–activity data. Molecules were edited within CATALYST by assembling fragments. The resulting geometries were minimized to the closest local minimum using molecular mechanics (CHARMm force field). Conformational models were generated that emphasize representative coverage over a 20 kcal energy range above the computed global minimum. The conformational model of the training set was used for hypothesis (pharmacophore) generation within CATALYST, which aims to identify the best 3-dimensional arrangement of chemical functions explaining the activity variations among the training set. The chemical functions used in the hypothesis generation step include hydrogen bond acceptors, hydrogen bond donors, and hydrophobic interactions. The best hypothesis for the ileal Na^+/bile acid cotransporter proposed by CATALYST (default parameters for catHypo) is characterized by 1 hydrogen bond donor function, 1 hydrogen bond acceptor function, and three hydrophobic features (**Fig. 2**). Table 1 summarizes the estimated as well as the measured IC_{50} values for inhibition of [^{3}H]cholyltaurine uptake by pKIBAT 8 cells. Plotting of the experimentally found IC_{50} values versus the calculated affinities demonstrates a good correlation between the estimated and the experimentally found biological activities, indicating a good predictive power of this hypothesis (**Fig. 3**).

For example, the high affinity benzothiazepine inhibitor S 0382 (11) is able to map all five features of the hypothesis, explaining its high affinity for the ileal bile acid transporter (**Fig. 4**) (IC_{50} value: 3 μm, estimated activity 8.9 μm). The sulfonyl group acts as hydrogen bond acceptor and the secondary amino function as hydrogen bond donor. The hydrophobic interactions are occupied by the phenyl ring, the ethyl group, and the butyl group. The weakly active enantiomer S 0381 (IC_{50} value 700 μm, estimated activity 1100 μm) is able to map only the hydrogen bond acceptor site by the sulfonyl group and two of the three hydrophobic regions. Due to its shape, S 0381 cannot fit the hydrogen bond donor function and the nearby hydrophobic region as S 0382 does. This loss of two binding interactions explains the large difference in activity between the two enantiomers by a factor of 233 (Table 1).

Interaction of bile acids with the 3D QSAR hypothesis

The pharmacophore was generated with a training set of 17 chemically diverse compounds containing no bile acid monomer. For the reliability of the pharmacophore model to predict the activity of de novo designed compounds, it is of major importance whether the 3D QSAR hypothesis is able to explain the experimentally found structure–activity relationships for the interaction of the natural substrates, bile acids, with the ileal Na^+/bile acid cotransporter as described in a previous paper (5). **Figure 5** shows the mapping of the natural bile acid cholyltaurine to the pharmacophore. Cholyltaurine fits four of the five features of the pharmacophore: the five-membered ring D and the methyl group at position 18 map one hydrophobic site and the 21-methyl group of the side chain maps a second hydrophobic site. The hydroxy group at position 12 represents the hydrogen-bond donor function and the negatively charged sulfonyl group of the taurine-conjugated side chain at carbon C-17 functions as hydrogen-bond acceptor. Alternatively, the hydrogen bond donor function can also be mapped by the 7α-hydroxy group of a bile acid molecule explaining the nearly identical affinity of chenodeoxycholyl- and deoxycholyltaurine to the ileal Na^+/bile acid cotransporter (5, **Fig. 6**). By using the 7α-hydroxy group as the hydrogen-bond donor function also, only 4 out of 5 interactions can be mapped. The sulfonyl group fits with the hydrogen-bond acceptor function, ring D and methyl group 21 map one hydrophobic site and methyl group 18 fits with a second hydrophobic site leaving one hydrophobic feature unoccupied. The presence of one α-oriented hydroxy group either at position 7 or 12 allows an optimal interaction with the ligand binding site whereas the simultanous presence of two α-hydroxy groups at C-7 and C-12 affects optimal mapping. The simultaneous presence of an α-oriented hydroxy group at positions 7 and 12 may lead to intramolecular hydrogen

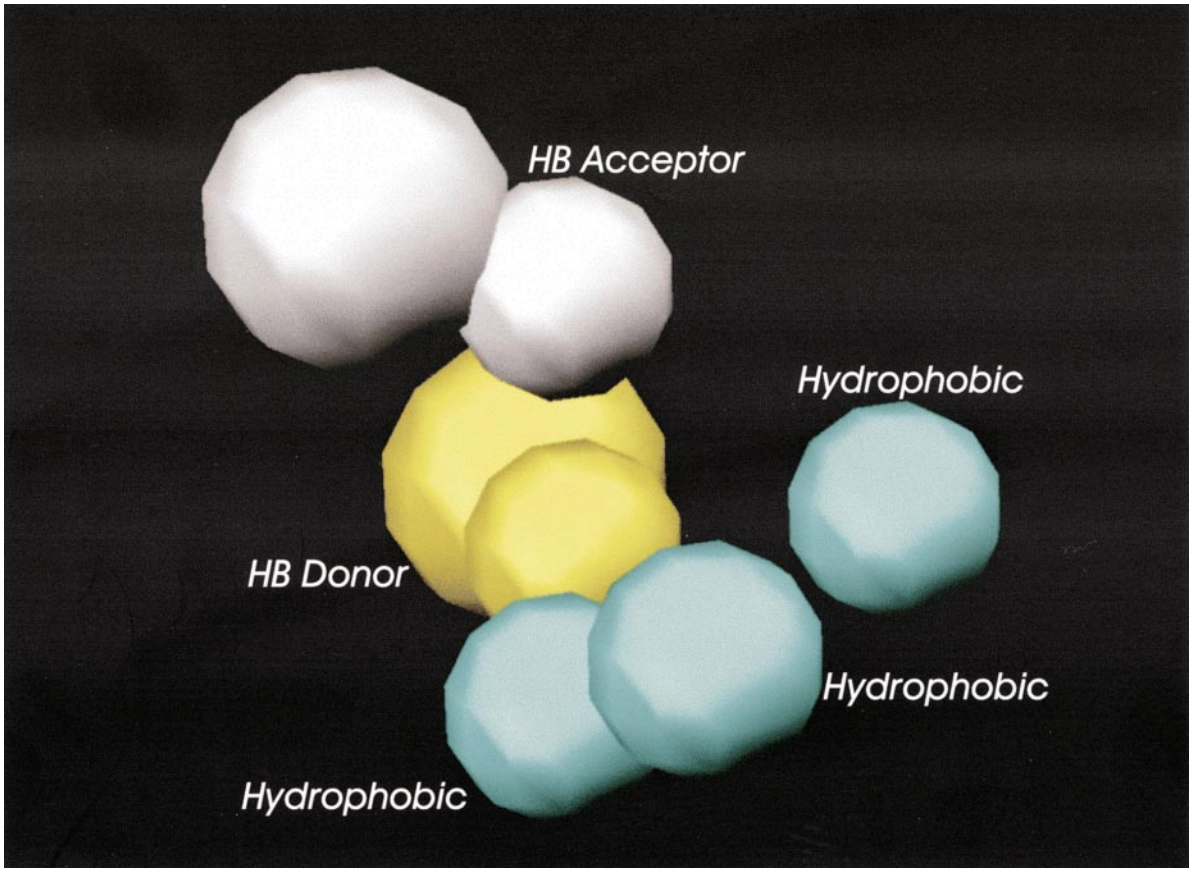

Fig. 2. Pharmacophore for the ileal Na^+/bile acid cotransporter.

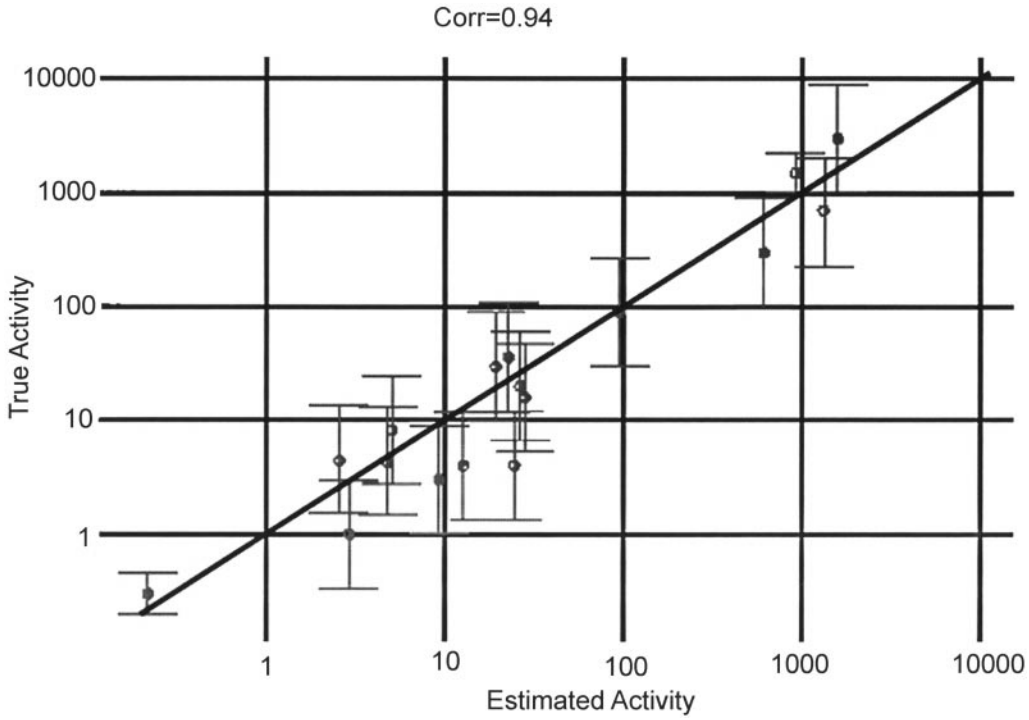

Fig. 3. Correlation line displaying the observed versus estimated IC_{50} values (μm) of the training set compounds by using the statistically most significant hypothesis.

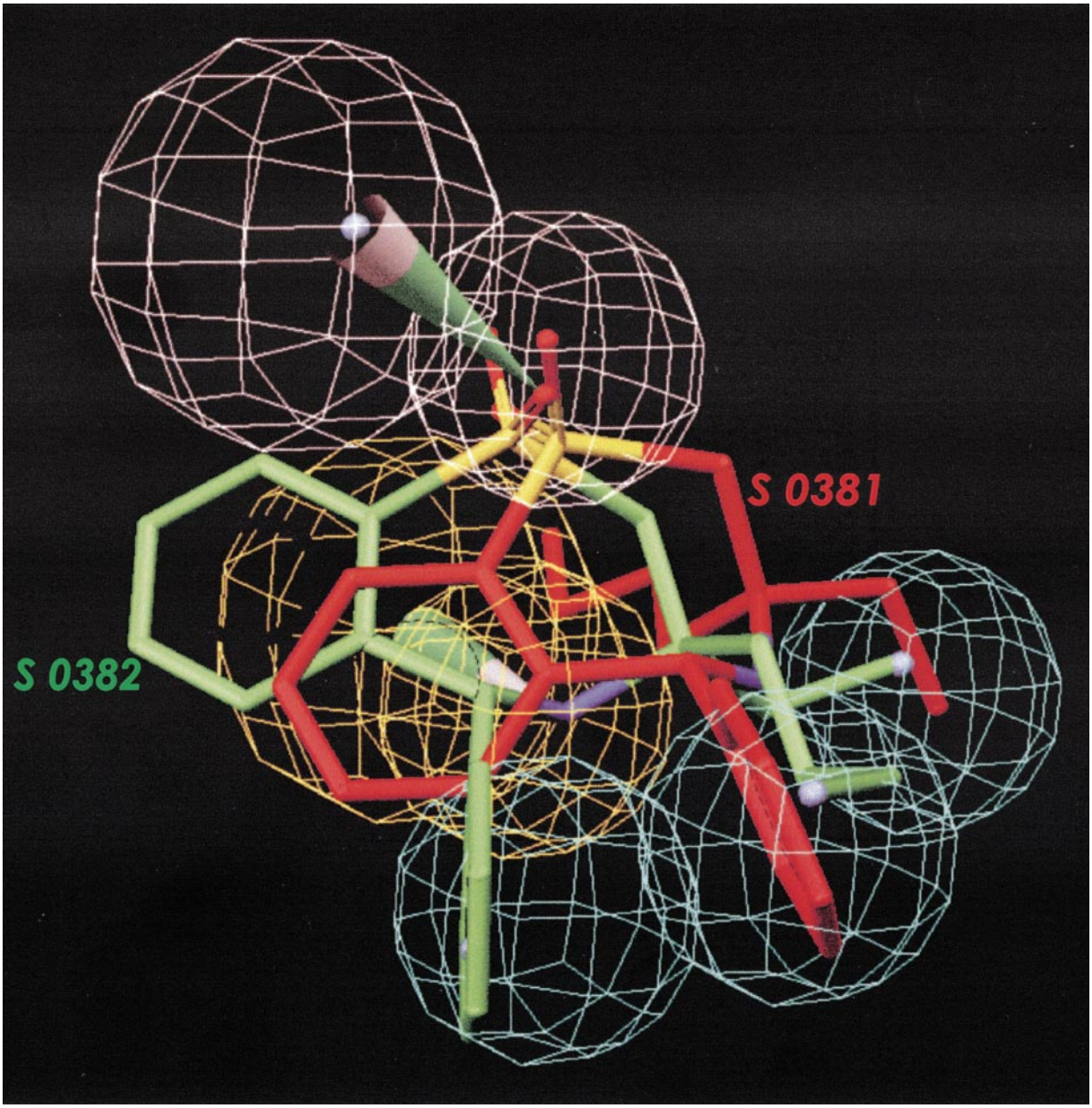

Fig. 4. Mapping of S 0382 (green) and S 0381 (red) onto the pharmacophore model.

bonding, weakening the hydrogen-bond donor function of either hydroxy group; such intramolecular hydroxy group interactions can explain the different chemical reactivity of the 7- and 12α-hydroxy group of bile acids during acetylation reactions (19). Interestingly, neither the 3α-hydroxy groups of the steroid nucleus nor the *cis*-oriented ring A maps one of the proposed structural features of our pharmacophore, explaining the specific character of drug bile acid conjugates as bile acids if the respective drugs are attached to the 3-position of the steroid nucleus (20–26).

In contrast, the dimeric bile acid-derived transport inhibitor S 0960 shows a different mode of interaction as compared with monomeric bile acids such as cholyltaurine. S 0960 maps four of the five features of the pharmacophore model (**Fig. 7**). The hydrogen-bond donor function is mapped by the 12α-hydroxy group of the cholyl moiety in S 0960 whereas the hydrogen-bond acceptor function is not mapped. Two of three hydrophobic features are mapped by ring D and the 21-methyl group of the cholyl moiety and, additionally, ring A of the 3β-amino-7α,12α-dihydroxy-cholanoate fragment occupies the third hydrophobic interaction (Fig. 7). Therefore, the affinity of S 0960 to the ileal Na^+/bile acid cotransporter mapping the hydrogen-bond donor function and the hydrophobic features is comparable to that of cholyltaurine mapping the hydrogen bond donor, the hydrogen-bond acceptor, and two of the hydrophobic features.

Recently, a structure-binding activity relationship model for the ileal bile acid transporter has been described using

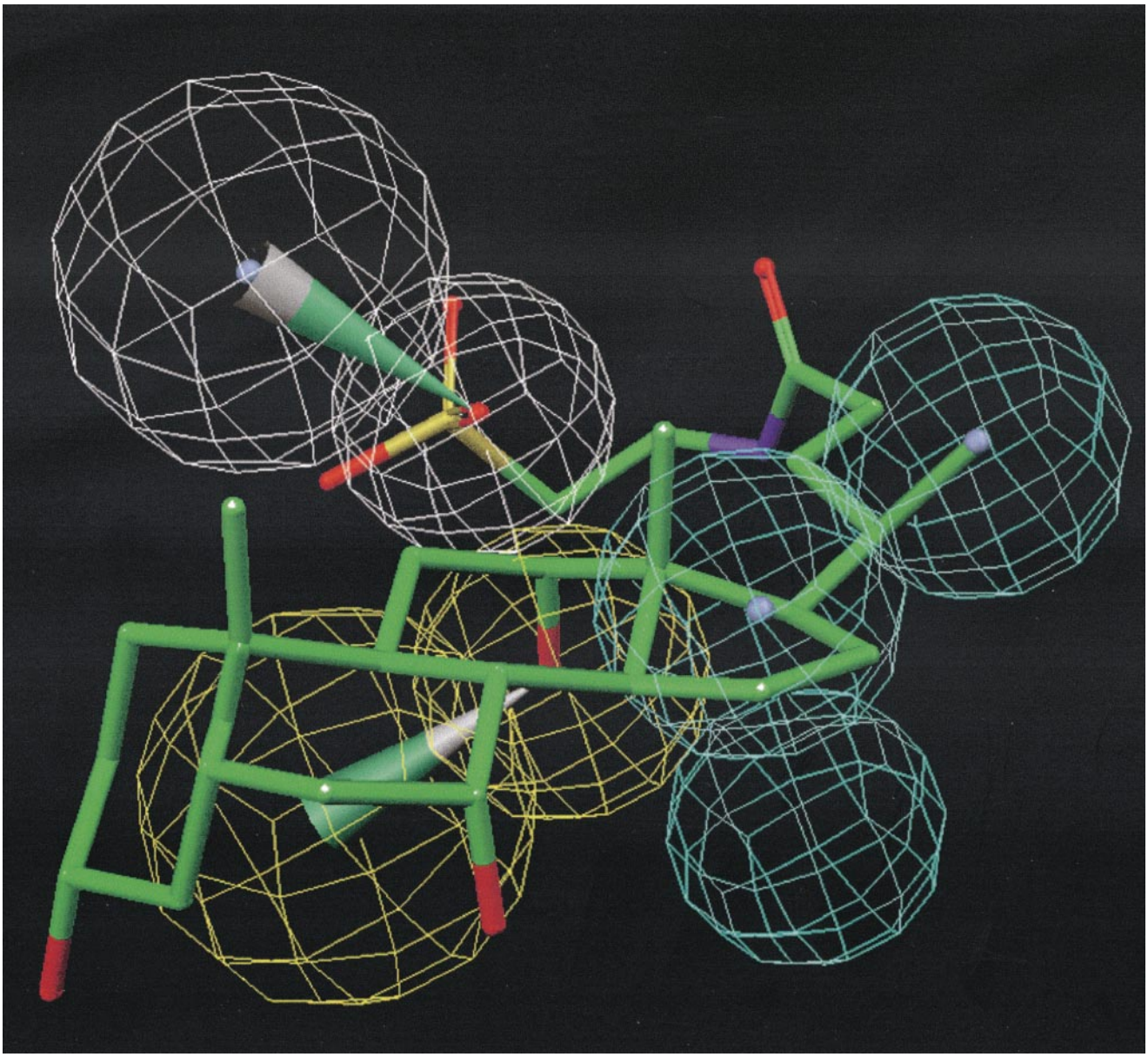

Fig. 5. Mapping of cholyltaurine onto the pharmacophore model by using 12-OH as hydrogen bond donor.

a comparative molecular field analysis (CoMFA) (27). A training set of 25 (peptide) bile acid analogues was used. Thereby, the old structure–activity relationships elaborated by Lack (7) was confirmed. As only chemically similar compounds were used for generation of the pharmacophore model, the predictability of this model is very limited in contrast to our 3D QSAR model which was able to explain and predict the affinities of several hundred diverse molecules for the ileal Na^+/bile acid cotransporter.

The computed interaction of cholyltaurine with the hypothesis shown in Fig. 5 predicts the following interactions of naturally occurring bile acids with four of the five interaction points.

1. The negatively charged side chain of the bile acid acts as hydrogen-bond acceptor. Substitution of the negatively charged side chain by uncharged polar or cationic groups leads to a strong drop in affinity to the ileal transporter as was previously shown (28–30).

2. The five-membered ring D of the steroid nucleus maps one of the three hydrophobic sites.

3. The methyl group of the bile acid side chain at position 21 maps the second hydrophobic site. The importance of the 21-methyl group for molecular recognition of bile acids by the ileal bile acid transporter was previously shown by us with bile acid–HMG-CoA reductase inhibitor hybrid molecules. Removal of the 21 methyl group increased affinity to HMG-CoA reductase but decreased the affinity to the ileal bile acid transport system by a factor of 3–4 (25, 26, 31).

4. The hydroxyl group in position 12α acts as hydrogen-bond donor. Oxidation of the 12α−hydroxy group to an oxo function significantly reduced the affinity and initial uptake rate of 3α,7α-dihydroxy-12-oxo-cholanoyltaurine

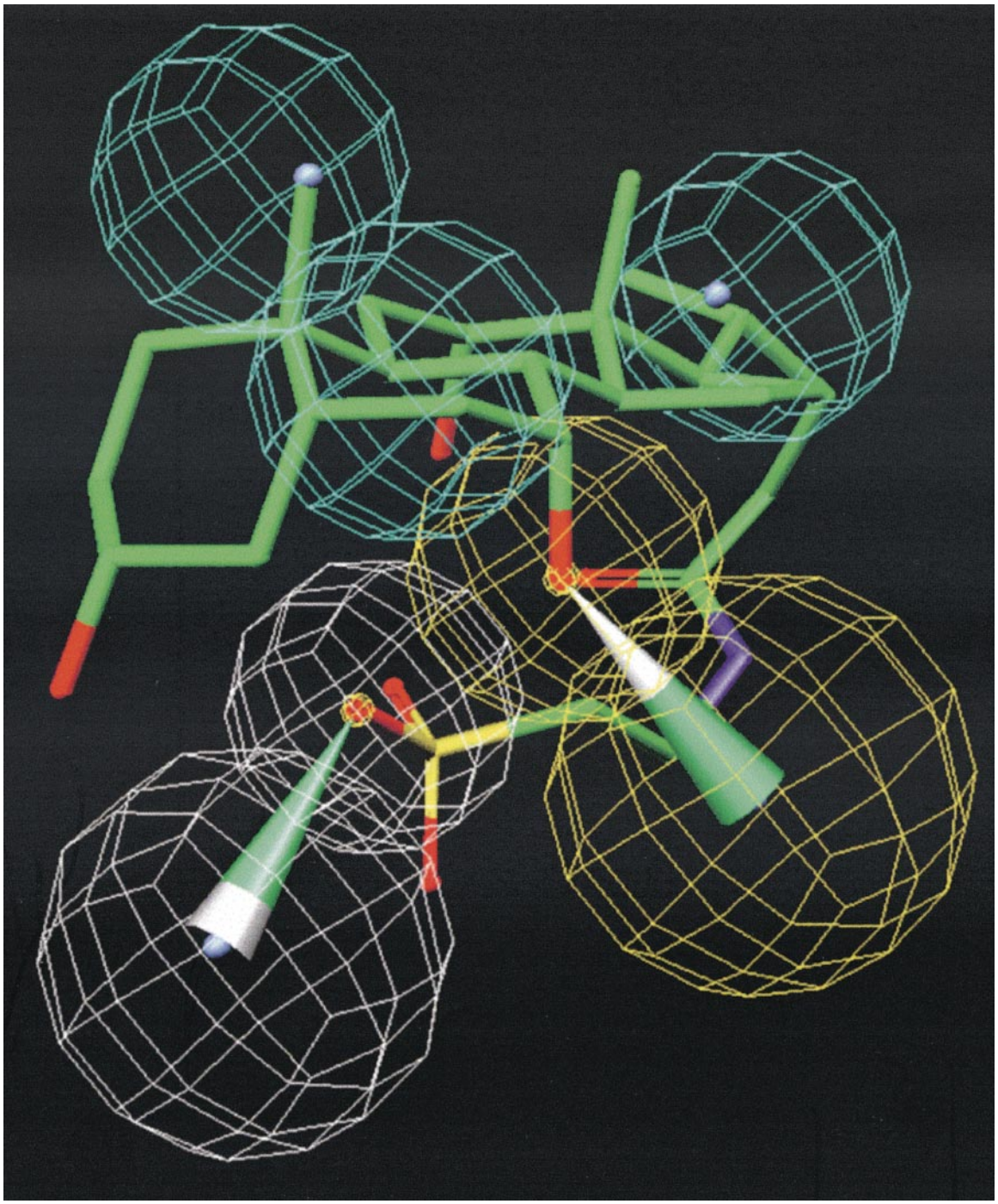

Fig. 6. Mapping of cholyltaurine onto the pharmacophore model by using 7-OH as hydrogen bond donor.

by a factor of 3 to 5 compared to cholyltaurine. In cholyltaurine, the 12α hydroxy group acts as a hydrogen-bond donor whereas the 12-oxo group as a hydrogen-bond acceptor thus disturbing this molecular interaction. Alternatively to the 12α-hydroxy group, the hydrogen-bond donor function can be mapped by the 7α-hydroxy group explaining the comparable high affinity and uptake rates of chenodeoxy- and deoxycholyltaurine. Removal of the unfavorable 12-oxo function as a hydrogen bond acceptor in 3α,7α-dihydroxy-12-oxo-cholanoyltaurine to yield chenodeoxycholyltaurine led to a 10- to 30-fold increase in affinity and a 4- to 7-fold higher initial uptake rate using brush border membrane vesicles or transfected CHO cells, respectively (5). Epimerization to the 7β-position, shift to 6α-position, or vicinal hydroxyl groups at positions 6 and 7 greatly reduced affinity and uptake rates. α-Oriented hydroxyl groups in bile acids are able to form intramolecular hydrogen bonds. Obviously the simultaneous presence of hydroxyl groups at positions 7 and 12 and especially positions 6 and 7 antagonizes a specific interaction of the

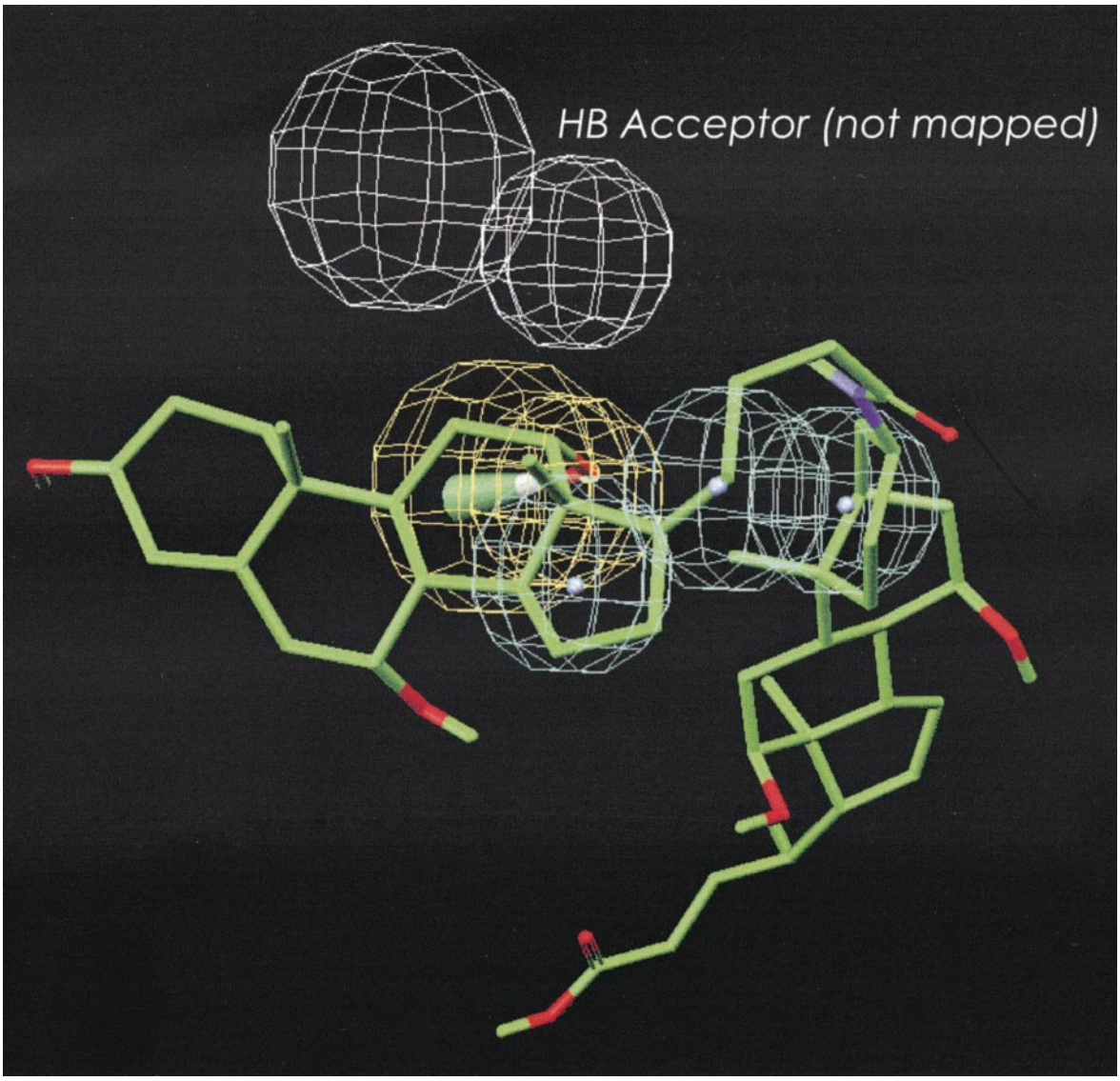

Fig. 7. Mapping of S 0960 onto the pharmacophore model.

7α-hydroxy group with the transporter protein, explaining the very low uptake rates of hyocholyltaurine or ω-muricholyltaurine.

5. The third hydrophobic interaction of the ileal Na^+/bile acid cotransporter is not mapped by a natural bile acid. This finding indicates that synthetic compounds as well as bile acid analogues being able to map all three hydrophobic interactions should show a significant higher affinity to the ileal bile acid transporter compared to hitherto known molecules.

A very important observation from the pharmacophore is the lack of any specific interaction of the 3α-hydroxy group in *cis*-oriented ring A of the steroid nucleus. A change of the *cis*-orientation of rings A and B of the steroid nucleus being present in all natural bile acids to the *trans*-orientation of the allo bile acids had only a moderate effect. The IC_{50} value for inhibition of [^{3}H]cholyltaurine uptake by ileal brush border membrane vesicles was 21 μm for allocholate compared to 67 μm for cholate, indicating a higher affinity of allocholate to the ileal Na^+/bile acid transporter. According to the pharmacophore model, steroid ring A does not map any of the five postulated binding regions. The missing interaction of the A ring with the five suggested binding regions also explains the behavior of bile acid analogues carrying no 3α-hydroxy group showing high affinity and uptake rate by the Na^+-dependent ileal and hepatic bile acid transporters (5). Hence, the 3α-hydroxy group being present in all natural bile acids is not essential for molecular recognition and transport of a bile acid by the ileal and the hepatic Na^+/bile acid cotransporters, which confirms our previous investigations with bile acids as a natural molecular drug delivery system (20–26, 31, 32). Bile acid conjugates carrying a drug or a peptide attached to the 3 position of the steroid nucleus behave in most aspects like bile acids. The pharmacophore clearly demonstrates that only ring A, preferably at position 3 of the bile acid molecule, allows attachment of compounds to achieve drug delivery by the natural bile acid

pathways. An attachment of a drug moiety to the side chain of a bile acid leads to a significant drop in the affinity to the ileal Na^+/bile acid cotransporter and a low to negligible intestinal absorption as was shown for fluorescent bile acid analogues such as (chenodeoxy)cholyl-N-rhodaminyl B lysine (33) or cholyl-N-fluoresceinyl-lysine (34). The intestinal absorption rates of the side-chain-modified bile acid analogues cholylglycylamidofluorescein (CGamF), cholylamidofluorescein (CamF), or ursodeoxycholylamidofluorescein (UDCAmF) in the rat were also negligible (35). In contrast, two bile acid analogues carrying the small fluorescent nitrobenzoxa-1,3-diazolyl group in the side chain, cholyl-(Nε-NBD)-lysine (C-L-NBD) and ursodeoxycholyl-(N-ε-NBD)-lysine (UDC-L-NBD) showed 28% and 14% relative ileal uptake compared to the natural bile acid cholyltaurine (35). Oligopeptides of 2 to 6 amino acids conjugated as amides to the C-24 carboxylic group of cholic acid consequently showed a negligible transport rate and a low affinity by the ileal Na^+/bile acid cotransport system (36, 37) in contrast to the attachment of oligopeptides to the 3 position of modified bile acid molecules (20–24, 32).

These results confirm our findings that attachment of a drug to the side chain of natural bile acids does not lead to high affinity substrates of the ileal Na^+/bile acid cotransport system.

The structure–activity relationships of bile acid derivatives to the hepatic Na^+/bile acid cotransporter can also be explained by the pharmacophore model for the ileal Na^+/bile acid cotransporter with respect to the following modifications of the bile acid molecule (5): modification of the side chain at C-24; modification of the 3-hydroxy group; *cis-trans*-isomerization of rings A and B; and removal of the 12α-hydroxy group and addition of 12-ethinyl groups.

The modifications at the side chain and the 3-position led to similar changes in affinity and transport rates as with the ileal transporter. A significant difference between the ileal and the hepatic Na^+/bile acid cotransporters for recognition of bile acids occurs upon modification of hydroxy groups at positions 7 and 6. A shift of the hydroxy function from positions 7 to 6 completely retained the affinity to the hepatic transporter in contrast to the ileal transporter. Vicinal hydroxyl groups at positions 6 and 7 only led to a small decrease in affinity and transport rates by the hepatic Na^+/bile acid cotransporter (5).

Twenty years after the first proposal for the interaction of a bile acid with the ileal Na^+/bile acid cotransporter (7) our investigations allow the definition of a new model for the interaction of bile acids with the ileal Na^+/bile acid cotransporter protein as follows.

An ε-amino group of a lysine residue interacts with the negatively charged bile acid side chain mapping the hydrogen bond acceptor function (38).

α-Oriented hydroxyl groups at positions 7 or 12 act as hydrogen-bond donors to an (hitherto unidentified) amino acid residue of the carrier protein.

Two of the three hydrophobic sites of the carrier protein are mapped by the 5-membered ring D of the steroid nucleus and the methyl group at position 21 of the side chain. A *cis*-configuration of rings A and B is not essential for high affinity of a bile acid to the ileal transporter as originally suggested.

Vicinal cysteine groups of the carrier protein function as negatively charged groups for interaction with Na^+ ions.

The 3α-hydroxy group present in all natural bile acids does not map any of the specific features of our model, indicating that the 3α-hydroxy group of bile acids does not significantly contribute to the affinity of a bile acid to the ileal as well as the hepatic Na^+/bile acid cotransporter.

The pharmacophore model described in this manuscript will be helpful for the design of optimized ligands and inhibitors for Na^+/bile acid cotransporters.

The authors are greatly indebted to Susanne Winkler and Meike Scharnagl for excellent secretarial assistance.

Manuscript received 13 May 1999 and in revised form 20 July 1999.

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