ABSTRACT

Organic cation transporters play a critical role in the elimination of therapeutic compounds in the liver and the kidney. We used computational quantitative structure activity approaches to predict molecular features that influence interaction with the human ortholog of the organic cation transporter (hOCT1). [3H]tetraethylammonium uptake in HeLa cells stably expressing hOCT1 was inhibited to varying extents by a diverse set of 30 molecules. A subset of 22 of these was used to produce, using Catalyst, a pharmacophore that consisted of three hydrophobic features and a positive ionizable feature. The correlation coefficient of observed versus predicted IC50 was 0.86 for this training set, which was superior to calculated logP alone ($r = 0.73$) as a predictor of hOCT1 inhibition. A descriptor-based quantitative structure-activity relationship study using Cerius2 resulted in an equation relating five molecular descriptors to log IC50 with a correlation coefficient of 0.95. Furthermore, a group of phenylpyridinium and quinolinium compounds were used to investigate the spatial limitations of the hOCT1 binding site. The affinity for hOCT was higher for 4-phenylpyridiniums $>$ 3-phenylpyridiniums $>$ quinolinium, indicating that substrate affinity was influenced by the distribution of hydrophobic mass. In addition, supraplanar hydrophobic mass was found to increase the affinity for binding hOCT1. These results indicate how a combination of computational and in vitro approaches may yield insight into the binding affinity of transporters and may be applicable to predicting these properties for new therapeutics.

The transepithelial transport of organic cations (OCs) plays an important role in the excretion of xenobiotic compounds from the body by means of the liver and kidney, and from the cerebrospinal fluid via the choroid plexus (Pritchard and Miller, 1993). The proteins involved in the translocation of OCs transport a broad range of substrates. These substrates include naturally occurring plant alkaloids and synthetic drugs such as cimetidine and procainamide. These chemicals are not only therapeutically diverse but show remarkable structural diversity as well. A characterization of the structural parameters of substrates translocated by organic cation transporters may provide insight into the molecular determinants of substrate specificity (Ullrich, 1999). Because the elimination of many therapeutic drugs is significantly influenced by the interaction of these compounds with OC transporters, information pertinent to predicting the kinetics of such interactions may be useful in estimating the pharmacokinetics of a broad range of pharmaceuticals.

The organic cation transporter, OCT1, is likely to play a significant role in the elimination of a variety of therapeutic compounds. OCT1 is highly expressed in the sinusoidal membrane of liver cells (Meyer-Wentrup et al., 1998), where it is presumed to play a role in the hepatic metabolism and elimination of cationic drugs (Koopsell, 1998). In the rat, OCT1 is also expressed in the basolateral membrane of early (S1 segment) renal proximal tubule (Karbach et al., 2000), where it is presumed to play a role in the peritubular uptake step of OC secretion.

With the increasing number of discoveries of new molecules that interact with OC transporters (Zhang et al., 2000; Dresser et al., 2001), understanding the three-dimensional features fundamental to molecular interaction with the transporter is desirable. Hydrophobicity and basicity have been suggested to be the principal determinants of substrate interaction with OC transporters on both the apical and basolateral membranes of the rat renal proximal tubule (Ullrich et al., 1991). This observation was independently supported for apical and basolateral transporters in the rabbit (Groves et al., 1994; Wright et al., 1995; Wright and Wunz, 1999). However, the mechanisms of substrate interaction with proteins possessing multiple transmembrane domains are difficult to assess directly because of the absence of crys-
nal structures for these proteins. It is therefore desirable to take advantage of computational techniques such as three-dimensional quantitative structure-activity relationships (3D-QSAR) that may provide an effective means of assessing the basis of substrate-transporter interaction in the absence of knowledge of the 3D structure of the transport protein. Structure-activity relationships have been developed previously for substrates of the OC+/H+ exchanger (Wright and Wunz, 1999). Using this analysis, it was concluded that the binding site of the luminal transport step of rabbit renal proximal tubules, the OC+/H+ exchanger, includes a planar hydrophobic surface sufficiently accommodating that no steric exclusion is evident when a planar (9-×12-Å) hydrophobic mass is rotated about an N-pyridinium center. This work, although comparatively rudimentary, introduced the use of structure-activity relationship analysis as a way to understand how therapeutics may interact with OC transporters.

In the present report, multiple QSAR approaches were used to assess the influence of selected structural and physical factors on substrate interaction with the human organic cation transporter, hOCT1. The first technique involved development of a computationally derived pharmacophore, a cation transporter, hOCT1. The first technique involved derivation of a computationally derived pharmacophore, a cation transporter, hOCT1 (Wright and Wunz, 1999). Using this analysis, it was concluded that the binding site of the luminal transport step of rabbit renal proximal tubules, the OC+/H+ exchanger, includes a planar hydrophobic surface sufficiently accommodating that no steric exclusion is evident when a planar (9-×12-Å) hydrophobic mass is rotated about an N-pyridinium center. This work, although comparatively rudimentary, introduced the use of structure-activity relationship analysis as a way to understand how therapeutics may interact with OC transporters.

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Materials and Methods

Materials. [3H]Tetraethylammonium ([3H]TEA; 13.2 Ci/mmol) was a custom synthesis by Amersham Biosciences (Piscataway, NJ). [3H]1-Methyl-4-phenylpyridinium (80 Ci/mmol) was purchased from ARC, Inc. (St. Louis, MO). NBD-TMA was synthesized as described previously (Wright et al., 1995). Other chemicals were purchased from Sigma Chemical Corp. (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). Octanol/water partition coefficients (Log P) were calculated using CLOGP (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). Octanol/water partition coefficients (Log P) were calculated using CLOGP (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI). Octanol/water partition coefficients (Log P) were calculated using CLOGP (St. Louis, MO) or Aldrich Chemical Co. (Milwaukee, WI).

Generation, Transfection, and Culture of HeLa Cells Expressing hOCT1 and hOCT1-V5His cDNAs. The hOCT1 cDNA in the pEEXO vector was kindly provided by Dr. Kathleen M. Giacomini (University of California, San Francisco, CA). hOCT1 was removed from pEEXO using XhoI and ligated into pIZ-V5His (In Vitro-Gen, Carlshad, CA). hOCT1 was removed from pIZ-V5His at HindIII and Xhol and ligated into pcdNA3.1 to generate a full-length cDNA. hOCT1 in pcDNA3.1 (i.e., without addition of the C-terminal V5His sequence) was inserted into pIZ-V5His. This was accomplished using the following set of primers: the reverse primer containing the mutated sequence. The mutated fragment was cut and ligated back into the parent vector. Another mutation was performed on the pIZ-hOCT1-V5His sequence to insert an EcoRI cut site after the stop codon of the His-tag using the reverse primer 5′-GGTAATCGCTAGTCATATCAATGTTGTAAT-3′ and the forward primer 5′-GCCTCTAGATCCAGGGGCCTCGAGGGTC-3′ as the reverse primer containing the mutated sequence. The mutated PCR fragment was digested with HindIII and Xhol to generate a full-length cDNA. hOCT1 was removed from pIZ-V5His at HindIII and Xhol and ligated into pcdNA3.1 to generate a full-length cDNA. hOCT1 was removed from pIZ-V5His at HindIII and Xhol and ligated into pcdNA3.1 to generate a full-length cDNA.
of the maximal rate of TEA uptake and the ratio of the inhibitor IC50 and the Kt for TEA transport; [T] is the concentration of [3H]TEA; [I] is the inhibitor concentration, and C is a constant representing the nonsaturation component of [3H]TEA uptake.

Modeling with Catalyst and Cerius2. The computational molecular modeling studies were carried out using Silicon Graphics Octane workstations (SGI, Mountain View, CA). The 3D structures of substrates were built interactively using either Catalyst version 3.1 or 3.4. (Accelrys, San Diego, CA) and used to generate a 3D-QSAR model. The number of conformers generated for each substrate was limited to a maximum of 255 with an energy range of 20 kcal/mol. Ten hypotheses were generated using conformers for the 22 molecules in the training set and the IC50 values, after manual selection of the hydrogen bond donor, hydrogen bond acceptor, hydrophobic, and negative ionizable pharmacophoric features. After assessing all 10 hypotheses generated, the lowest energy cost hypothesis was considered the best. The goodness of the structure-activity correlation was estimated from calculated r values. Statistical significance of the retrieved hypothesis was verified by permuting the response variable; i.e., the activities of the training set compounds were mixed a number of times (so that each value was no longer assigned to the original molecule) and the Catalyst hypothesis generation procedure was repeated. Multiple conformations of the eight molecules in the test set were generated using the same method as the training set before fitting the conformers to the pharmacophore to generate a prediction.

Cerius2 (Accelrys) was used to generate more than 200 molecular descriptors for the 30 molecules used in the Catalyst studies. The forward stepwise regression method incorporated within Cerius2 was then used to relate the log IC50 to a selection of these descriptors, and hence result in a QSAR model. The model was validated for numerical stability and internal consistency using both the leave-one-out cross-validation method and by permuting, or randomizing, the response variable.

Results

hOCT1 Sequence Variation and Stable Cell Line Characteristics. The sequence of the hOCT1 cDNA provided by K. Giacomini differed by one base from the sequence reported by Zhang et al. (1997) (Genbank accession number U77086). That difference was a guanine, rather than an adenine, at nucleotide 1275. The resulting codon encoded a valine rather than a methionine at amino acid 408. The sequence of an hOCT1 fragment (from position 1197 to 1588), amplified using RT-PCR from human renal mRNA, confirmed the presence of G at position 1275 (i.e., valine at amino acid 408) in a sequence that otherwise matched that of Zhang et al. (1997). The full-length cDNA coding for hOCT1, as provided by Dr. Giacomini, was therefore used for the preparation of the hOCT1-V5His construct employed in the studies described below.

Figure 1A shows cells stably transfected with pcDNA3-hOCT1-V5His after a 10-min exposure to 20 μM NBD-TMA. Figure 1B is a fluorescence image of the same cells, showing that only a fraction of the transfected cells that were resistant to G418 actually transported NBD-TMA. Cells transfected with hOCT2, rbOCT1, and rbOCT2 also showed increased fluorescence after exposure to NBD-TMA (data not shown). In fact, only 5 to 10% of the ‘parent’ population of cells expressed transport activity. Cells positive for hOCT1-V5His-mediated transport of NBD-TMA were selected using flow cytometry, dilution-cloning, and cloning rings and discs. Figure 1C shows [3H]TEA uptake data from wild-type HeLa cells, the parent cell population, and cells selected from the parent cell line. The parent cell population showed about twice as much radiolabeled TEA accumulation as the wild-type HeLa cells. After selection of NBD-TMA-containing cells using flow cytometry, there was a 6-fold increase in accumulation of radiolabeled TEA. The remaining studies reported here used a clonal cell line selected from this population.

Figure 2A shows the time course of [3H]TEA uptake into HeLa cells stably expressing hOCT1-V5His. TEA uptake was approximately linear for 5 to 7 min, and a 5-min uptake provided a reasonable estimate of the initial rate of transport. Figure 2B shows the effect of increasing concentrations of unlabeled substrate on the rate of hOCT1-mediated transport of TEA from which the kinetics of transport of this substrate was estimated. In three separate experiments, the apparent Kt for TEA transport was 164 ± 17.9 μM, with a Jmax of 3.5 ± 0.33 nmol mg−1 min−1. These values are comparable with those reported by Zhang et al. (1998) in their study of hOCT1-mediated transport in transiently transfected HeLa cells. Consequently, we elected to use the transport of TEA, and the inhibition of TEA transport, in the remaining studies. We also measured the kinetic properties of TEA transport in cells stably expressing the wild-type hOCT1 (i.e., without the V5His tagged sequence; data not shown). In these experiments, the Kt for TEA transport in the wild-type clones was similar to that of the tagged protein, suggesting that the presence of the V5His sequence at the C terminus of hOCT1 had no significant effect on the interaction of substrate with the transport binding site. Hereafter, hOCT1-V5His is referred to simply as hOCT1.

To further assess the kinetic characteristics of our hOCT1 cell line, we examined the inhibition of hOCT1-mediated TEA transport produced by a series of tetraalkylammonium compounds (nTAAs: TEA, TPrA, TBA, and TPeA). The IC50 for each compound was determined by measuring the 5-min accumulation of [3H]TEA in the absence or presence of five incremental concentrations of inhibitor (Table 1). Figure 2C plots the relationship between the log of the measured IC50 values for the test agents and their calculated Log P values (CLog P), confirming the existence of a strong correlation between hydrophobicity and IC50 for the nTAAs commonly used in organic cation studies. Additionally, the IC50 values for these compounds compared favorably with those reported previously for nTAA inhibition of hOCT1 expressed in HeLa cells (Zhang et al., 1999) and Xenopus laevis oocytes (Dresser et al., 2000).

Pharmacophore 3D-QSAR Generation. We next used a computational approach to develop QSARs and a predictive model of inhibitor/hOCT1 interaction. The commercial software program Catalyst was used to model the structural features that facilitate interaction of inhibitors at the hOCT1 binding site. A set of chemically diverse molecules with IC50 values for hOCT1 spanning more than 3 orders of magnitude (Table 2) was used for pharmacophore construction. These 22 molecules identified a pharmacophore with four features of interaction with the transporter. The four features included a positive ionizable feature and three hydrophobic features at distances of 5.12, 4.19, and 5.32 Å from the center of the positive ionizable feature (Fig. 3A). Regression of the logs of experimentally determined and the estimated IC50 values for the compounds contributing to the model resulted in a correlation coefficient of 0.86 (Fig. 4, Table 2). Compounds with
the highest apparent affinity for the transporter (i.e., those with the lowest IC₅₀ values) generally possessed the greatest number of pharmacophore features, whereas compounds with structures that included fewer of these features generally showed a lower apparent affinity for the transporter. This can be seen in Fig. 3B, where the most potent inhibitor, clonidine (IC₅₀ = 0.71 µM) is characterized by all four pharmacophore features, but the least potent inhibitor, choline (IC₅₀ = 3.5 mM), is described by only one of the features, (i.e., the positive ionizable feature) (Fig. 3C). Additionally, choline has a hydrogen bond donor near two of the hydrophobes, perhaps contributing to its failure to inhibit TEA transport to a significant degree. Although many of the chemical features of the diverse group of molecules in the training set were in spatial agreement with the model pharmacophore, some molecular structures clearly lay outside the area described by the model. This can be readily seen for ranitidine (IC₅₀ = 21.7 µM; Fig. 3D). Furthermore, there was generally poor correlation (r = 0.54) between the Catalyst-produced estimates of IC₅₀ and the experimentally determined values for the pyridinium- and quinolinium-based set of substrates described in the following group of experiments (Fig. 4, C; Table 2). Nevertheless, the Catalyst-produced model was more accurate than the often-cited correlation between the hydrophobicity of substrates or inhibitors and the apparent affinity of OC transporters for these compounds. Figure 5 shows the relationship between the log of the IC₅₀ for each of the members of the training set and their CLog P values. The correlation (r = 0.73) was less than that of the Catalyst model (r = 0.86), which may reflect the substantial variation in the three dimensional structure of several inhibitors with similar CLog P values. Indeed, when a structurally homologous group of molecules is examined (e.g., the nTAA), the corre-

### Table 1

IC₅₀ values for selected nTAA compounds against [³H]TEA transport in hOCT1-expressing HeLa cells (mean ± S.E.M.; n = 3 to 4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>CLog P</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEA</td>
<td>-3.14</td>
<td>167.5 ± 25.9</td>
</tr>
<tr>
<td>TPrA</td>
<td>-1.02</td>
<td>22.0 ± 4.1</td>
</tr>
<tr>
<td>TBA</td>
<td>1.09</td>
<td>6.5 ± 1.5</td>
</tr>
<tr>
<td>TPeA</td>
<td>3.21</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

Fig. 1. A, differential interference contrast image (400×) of HeLa cells stably transfected with pcDNA3.1-hOCT1 after a 10 min exposure to 20 µM NBD-TMA⁺ (note the elongated cell for reference). B, fluorescence image of the same field of view showing NBD-TMA⁺ accumulation in a subpopulation of these cells. C, Accumulation of 0.04 µM [³H]TEA⁺ by untransfected wild-type HeLa cells (HeLa), HeLa cells stably transfected with pcDNA3-hOCT1-V5His (Parent), and cells sorted from the parent cell population by flow cytometry (Sorted). The height of each bar indicates the mean (± S.E.) of 60-min uptakes measured in three confluent wells (~1 x 10⁶ cells).

Fig. 2. Kinetic data acquired from HeLa cells stably transfected with pcDNA3-hOCT1-V5His. In each plot, the solid points represent the mean (± S.E.) of uptakes measured in three confluent wells of cells in a single, representative experiment. A, time-dependent accumulation of 0.04 µM [³H]TEA⁺ as shown by the profile in inhibition of 0.05 µM [³H]TEA⁺ uptake produced by increasing concentrations of unlabeled TEA⁺ (Malo and Berteloot, 1991). The curve was fit to the data using a nonlinear regression algorithm (SigmaPlot, v3.0). C, relationship between calculated hydrophobicity (Log P) of nTAA and the measured IC₅₀ each displayed against hOCT1-mediated TEA uptake. Each point represents the mean (± S.E.) of IC₅₀ values calculated in three separate experiments.
sectioned by the Cerius2 program. The following equation identifying molecular descriptors found to be correlated with inhibition of hOCT1 was built using the molecular descriptors generated by the Cerius program (Obach, 2000). Consequently, a descriptor-based QSAR model with parameters than a Catalyst-derived model (Ekins and Obach, 2000). The correlation of the experimentally observed and predicted IC50 values from computational models for a structurally diverse array of organic cations can be superior in its ability to predict activity of hOCT1 can be quite striking (Fig. 5). Kinetic parameters were measured against H2O transport in hOCT1-expressing HeLa cells (mean ± S.E.M.; n = 3 to 5).

### Descriptor-Based QSAR Generation

Previous work has shown that a descriptor-based QSAR model generated using Cerius can be superior in its ability to predict activity parameters than a Catalyst-derived model (Ekins and Obach, 2000). Consequently, a descriptor-based QSAR model for hOCT1 was built using the molecular descriptors generated by the Cerius program. The following equation identifying molecular descriptors found to be correlated with inhibition of hOCT1 activity was produced using forward stepwise regression:

\[
\text{Log IC}_{50} = 3.94081 - 0.212696 \times S_{\text{ssNH}} + 0.458416 \times \text{ADME solubility} - 0.0725515 \times Jurs-RNCS - 0.363722 \times \text{Shadow-ν} - 0.0899946 \times \text{Atype H}_{52}
\]

in which \(S_{\text{ssNH}}\) defines an E-state key for sum of nitrogens connected to a hydrogen and to two single bonds (Ghose et al., 1998); ADME solubility is a descriptor for prediction of aqueous solubility; Jurs-RNCS is related to the negative charge surface area mapped over the solvent-accessible surface area of individual atoms; Shadow-ν is a member of the shadow indices descriptors and is the ratio of largest to smallest dimension; and Atype H 52 is the number of times that each AlogP atom appears in the molecule and is part of the Thermodynamic family (AlogP atypes) of descriptors (Viswanadhan et al., 1989). The correlation of the experimentally determined (Log) IC50 values and those predicted for the descriptor-based model described in eq. 1 generated a correlation coefficient of 0.95 (Fig. 6). After permuting the log data with the descriptors and attempting to generate eqs. 9 times (to give 90% confidence in the models produced), the mean r value was 0.65 ± 0.14, which represented 2.1 S.D. from the r value described for the model in eq. 1

### Interaction of N-1-Substituted Pyridiniums and Quinoliniums with hOCT1

Of the five molecular descriptors presented in eq. 1, the relative importance of the Shadow-ν term (i.e., the ratio of the largest to the smallest dimension of the inhibitor) was particularly intriguing. In a previous study, we used a series of phenylpyridinium and quinolinium compounds to examine the influence of the spatial distribution of hydrophobic mass on the interaction of test agents with hOCT1. Consequently, we reasoned that the IC50 values of the test agents would vary inversely with the descriptors and attempting to generate eqs. 9 times (to give 90% confidence in the models produced), the mean r value was 0.65 ± 0.14, which represented 2.1 S.D. from the r value described for the model in eq. 1

### Test Set

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50</th>
<th>Catalyst Prediction</th>
<th>Cerius Prediction</th>
<th>CLog P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine</td>
<td>0.71 ± 0.08</td>
<td>3.1</td>
<td>1.8</td>
<td>3.08</td>
</tr>
<tr>
<td>TPEA</td>
<td>1.8 ± 0.2</td>
<td>1.0</td>
<td>2.2</td>
<td>3.21</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>4.3 ± 0.16</td>
<td>6.4</td>
<td>2.8</td>
<td>6.15</td>
</tr>
<tr>
<td>Quinidine</td>
<td>5.4 ± 0.22</td>
<td>4.5</td>
<td>7.1</td>
<td>3.24</td>
</tr>
<tr>
<td>TBA</td>
<td>6.5 ± 1.5</td>
<td>1.8</td>
<td>9.7</td>
<td>1.09</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>7.7 ± 1.94</td>
<td>9</td>
<td>3.1</td>
<td>6.25</td>
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<tr>
<td>Pindolol</td>
<td>9.7 ± 0.94</td>
<td>7.6</td>
<td>4.5</td>
<td>2.26</td>
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<tr>
<td>Procainamide</td>
<td>14.5 ± 0.83</td>
<td>8.4</td>
<td>18.9</td>
<td>0.90</td>
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<tr>
<td>1-Methyl-4-phenylpyridinium</td>
<td>15.7 ± 1.15</td>
<td>93</td>
<td>25.6</td>
<td>-2.31</td>
</tr>
<tr>
<td>Amantadine</td>
<td>18.4 ± 2.42</td>
<td>73</td>
<td>25.0</td>
<td>2.04</td>
</tr>
<tr>
<td>Ramitidine</td>
<td>21.7 ± 2.33</td>
<td>2.4</td>
<td>11.2</td>
<td>1.31</td>
</tr>
<tr>
<td>TPA</td>
<td>22 ± 4.1</td>
<td>7.4</td>
<td>45.1</td>
<td>-1.02</td>
</tr>
<tr>
<td>Nicotine</td>
<td>53.2 ± 9.07</td>
<td>71</td>
<td>81.7</td>
<td>1.97</td>
</tr>
<tr>
<td>1-(2-Hydroxyethyl)quinolinium</td>
<td>80.6 ± 3.2</td>
<td>73</td>
<td>67.6</td>
<td>-2.32</td>
</tr>
<tr>
<td>Tyraine</td>
<td>107 ± 6.7</td>
<td>71</td>
<td>187</td>
<td>0.81</td>
</tr>
<tr>
<td>TEA</td>
<td>168 ± 25.9</td>
<td>720</td>
<td>116</td>
<td>-3.14</td>
</tr>
<tr>
<td>Thiamine</td>
<td>434 ± 31.5</td>
<td>75</td>
<td>702</td>
<td>-5.04</td>
</tr>
<tr>
<td>Dopamine</td>
<td>487.2 ± 43.3</td>
<td>71</td>
<td>359</td>
<td>0.21</td>
</tr>
<tr>
<td>Cyclohexylamine</td>
<td>544 ± 19.0</td>
<td>76</td>
<td>187</td>
<td>1.41</td>
</tr>
<tr>
<td>N1-Methylnicotinamide</td>
<td>1035 ± 58.0</td>
<td>720</td>
<td>457</td>
<td>-5.21</td>
</tr>
<tr>
<td>Histamine</td>
<td>3007 ± 27.2</td>
<td>720</td>
<td>1771</td>
<td>-1.16</td>
</tr>
<tr>
<td>Choline</td>
<td>3540 ± 24.0</td>
<td>720</td>
<td>5409</td>
<td>-4.36</td>
</tr>
</tbody>
</table>

* The CLogP program was unable to calculate Log P for pindolol and histamine.
with hydrophobicity. This was generally the case for each 'genus' of chemical (i.e., 4-phenylpyridiniums, 3-phenylpyridiniums, and quinoliniums): for each 'species' within a given genus, increasingly hydrophobic residues at the N1 position were associated with a decrease in IC50 for hOCT1-mediated TEA transport (Fig. 8). However, there was systematic variation in the apparent affinity of hOCT1 for the different structural groups. The more elongated 4-phenylpyridiniums consistently had lower IC50 values for hOCT1 than did the

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**Fig. 3.** A, the structure of the Catalyst-produced pharmacophore illustrating three hydrophobic areas (cyan) and a positive ionizable feature (red). B, clonidine mapped to the pharmacophore model. C, choline mapped to the pharmacophore model. D, ranitidine mapped to the pharmacophore model.

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**Fig. 4.** The relationship between the Catalyst-predicted IC50 values and the measured IC50 values. The solid line describes the linear regression of the data representing the 22 molecules comprising the training set (\( r = 0.86 \)); the dotted lines illustrate the 95% confidence interval of the regression. ◦, the eight molecules from the test set (\( r = 0.54 \)).

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**Fig. 5.** Relationship between observed IC50 and calculated hydrophobicity (Log P) for the training set of compounds used to generate the Catalyst-produced pharmacophore. The solid line depicts the linear regression of these data (\( r = 0.73 \)), and the dotted lines show the 95% confidence interval for the regression. The dashed line depicts the linear regression for a subset of these data (i.e., the nTAAs) (\( r = 0.99 \)). Each point is the mean of measurements made in three or four separate experiments.
3-phenylpyridiniums, which, in turn, had lower IC\textsubscript{50} values than the least linear compounds, the quinoliniums (Fig. 8; Table 2). This observation suggests that, although hydrophobicity is clearly an important determinant of molecular interaction with hOCT1, changes in molecular dimension can also predictably influence apparent affinity of the transporter for a molecule.

In a previous study of the influence of planar hydrophobic mass on the binding of OCs to the luminal OC/H exchanger of rabbit renal BBMs (Wright and Wunz, 1999) we noted that, in general, planar substrates bind with greater affinity to the exchanger than nTAAs compounds of comparable hydrophobicity. Indeed, there is a marked discrepancy between hydrophobicity and binding efficacy between nTAAs and the selected pyridinium/quinolinium compounds at the luminal OC/H\textsuperscript{+} exchanger. This led to the hypothesis that the globular (rather than planar) nature of nTAAs places a substantial fraction of their hydrophobic mass away from the postulated planar binding surface of the OC/H\textsuperscript{+} exchanger, in contrast to the situation afforded by planar substrates. No such discrepancy was noted between the interactions of these two groups of compounds with hOCT1 (inset, Fig. 8). To extend the examination of the influence of globular, rather than planar, hydrophobic mass on interaction with hOCT1, the inhibitory efficacies of two compounds used in the development of the computational model were compared. Cyclohexylamine and amantadine are unsaturated hydrocarbons with a primary amine-containing side group. Importantly, the structure of cyclohexylamine is fully contained within amantadine, with the latter containing additional hydrophobic mass that is supraplanar to a postulated binding surface (refer to Fig. 7). Amantadine is known to be a substrate of basolateral OC transport in intact rat tubules (Wong et al., 1990), and it is likely that cyclohexylamine, which is a substructure of amantadine, is also a substrate. Interestingly, the presence of additional, nonplanar hydrophobic mass increased the inhibitory effectiveness of amantadine 25-fold over that observed for cyclohexylamine (IC\textsubscript{50} values of 19 and 540 \mu M, respectively; see Table 2).

Discussion

Although the interaction of substrate with OC transporters has been described for a variety of different compounds (e.g., Dresser et al., 2001), such descriptions have usually lacked structural/spatial information with regard to the substrates or transporters. Structure-related analysis of substrate interaction with cloned OC transporters has been performed using the nTAAs (Zhang et al., 1999; Dresser et al., 2000). These compounds, however, have limited structural diversity. In the present study, two groups of chemicals were employed, one with broad structural diversity and binding affinity for hOCT1, and a second of comparatively limited structural breadth that focused on the influence of a single structural parameter. Both groups were used to extend the

![Graph showing predicted vs measured IC\textsubscript{50} values for 30 molecules](image)

**Fig. 6.** The relationship between the Cerius 2 predicted IC\textsubscript{50} values and the measured IC\textsubscript{50} values. The solid line describes the linear regression of the data representing all 30 molecules used in the present study (\(r = 0.95\)); the dotted lines illustrate the 95\% confidence interval of the regression. \(\circ\), the eight molecules from the test set.

![Structural formulae of the molecules used to assess the influence of the Shadow-v dimensional descriptor on hOCT1-mediated TEA transport](image)

**Fig. 7.** Structural formulae of the molecules used to assess the influence of the Shadow-v dimensional descriptor on hOCT1-mediated TEA transport.
obtained by altering the structure of the test compounds to define the pharmacophore approach, the QSAR regression model was developed from this set of compounds. The Catalyst-based model had a very high correlation between measured and predicted IC50 values for all 30 molecules evaluated in this present study (Fig. 6; r = 0.95), and represented a marked improvement in predictability compared with the Catalyst, pharmacophore-based model, as well as with previous efforts to correlate OCT binding to the structural specificity. However, OC transporters do not display the narrow specificity of the typical receptor, which generally accepts a single, high-affinity interaction with a receptor that is expected to possess a marked degree of structural specificity. Therefore, one might predict a selective advantage arising from a process that can interact effectively with a diverse array of environmental chemicals, making it desirable for OC transporters to accept chemical structures that fit a generalized format, rather than one represented by a classic pharmacophore.

To achieve a computational model with greater predictability, a descriptor-based QSAR model was subsequently derived using Cerius2. In contrast to the structurally based pharmacophore approach, the QSAR regression model was based upon multiple regression of a number of quantitative molecular descriptors. The final model emphasized the importance of hydrophobic (ADME_solubility), hydrogen bond donor (S_sssNH), shape (Shadow-r), and charge (Jurs-RNCS) features. The Cerius2 model had a very high correlation between measured and predicted IC50 values for all 30 molecules evaluated in this present study (Fig. 6; r = 0.95), and represented a marked improvement in predictability compared with the Catalyst, pharmacophore-based model, as well as with previous efforts to correlate OCT binding to the single physical descriptor, CLogP.

Not surprisingly, greatest weight in the QSAR model was given to a molecular descriptor associated with hydrophobicity (ADME_solubility). Somewhat unexpected, however, was the next most heavily weighted term, Shadow-r (ratio of the longest to shortest molecular dimension). This dimensionality term, Shadow-r, describes a physical parameter not previously hypothesized to significantly contribute to molecular interaction with OC transporters. This term clearly distinguishes the phenylpyridinium and quinolinium compounds included in this study, and presumably explains the improved correlation of predicted and measured IC50 values for the test set compounds evident in the Cerius2 QSAR model.

Fig. 8. The relationship between the IC50 for inhibition of hOCT1-mediated [3H]TEA transport and the calculated hydrophobicity (Log P) of the members of the three sets of test compounds: 4-phenylpyridiniums (4P, •); 3-phenylpyridiniums (3P, □); and quinoliniums (Q, △). Each point is the mean (± S.E.) of IC50 values measured in three or four separate experiments. Inset, within each set of test agents, Log P was systematically increased by altering the N1-substituent (2-hydroxyethyl to -ethyl to -phenyl)methyl; see Fig. 7). The relationship between the IC50 for inhibition of hOCT1-mediated [3H]TEA transport and the calculated hydrophobicity (Log P) of the three sets of test compounds, and the nTAA compounds (○).
(Fig. 6), compared with the Catalyst pharmacophore model (Fig. 4).

Differences in relative geometric location of planar hydrophobic mass are intrinsic in the structures of the phenylpyridinium and quinolinium compounds used in this study. These changes in the geometric location of planar hydrophobic mass permitted a systematic assessment of the potential role that steric configuration can play in binding of substrate to an OC transporter. In fact, although for any chemical genus tested (i.e., 4-phenyl or 3-phenylpyridinium or quinolinium) increases in hydrophobicity (introduced through the R-group at the N1 position) were associated with decreases in IC₅₀ (Fig. 8), it was also evident that the location of planar hydrophobic mass had a clear and systematic effect on inhibitor interaction. For molecules of similar hydrophobicity, the rank order of apparent affinity for hOCT1 was 4-phenylpyridinium > 3-phenylpyridinium > quinolinium, which corresponded to the rank order of the mean Shadow-ᵣ values for each genus: 2.45 > 2.05 > 1.75, respectively. Although the basis for this effect is not clear, it may reflect a systematic misalignment of chemical features, such as the positive charge, with important structural elements of the binding site, resulting in decreased affinity for the molecule. Alternatively, the more elongated structure of the 4-phenylpyridiniums may permit association with a more stabilizing region of the binding site than the foreshortened quinoliniums. Additionally, it is possible that the hypothesized stabilizing feature of the protein is fundamental for π–π stacking type interactions with the phenyl group of the more elongated 4-phenylpyridiniums, and perhaps this stabilizing feature is misaligned or not adequately reached by the unsaturated rings within the other two groups of compounds.

Interestingly, the phenylpyridinium and quinolinium compounds used in the present study produced a very different inhibitor profile in a previous study on the molecular determinants of substrate interaction with the OC/H⁺ exchanger of renal brush border membranes (Wright and Wunz, 1999). Whereas for hOCT1, a change in molecular dimension (basis of renal brush border membranes (Wright and Wunz, 1999). OC/H effect on binding site interaction evident in Fig. 8, these using Cerius2 reiterated the importance of hydrophobicity features relative to one another. A QSAR model derived site for hOCT1 may be best described as a dimensionality parameter was validated by the empirical test of the effect of the placement of planar hydrophobic mass on binding to hOCT1. The developing view is of an hOCT1 binding site that is most conducive to interaction with comparatively elongated, cationic molecules capable of interacting with a supraplanar stabilizing structure within a hydrophobic pocket of the protein.

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References


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