Prediction of the Intestinal Absorption of Endothelin Receptor Antagonists Using Three Theoretical Methods of Increasing Complexity

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Purpose. Three new computational strategies have been evaluated for their ability to predict intestinal membrane permeability to a series of endothelin receptor antagonists.

Methods. The three methods were evaluated using a set of ten non-peptide endothelin receptor antagonists. The simplest method, "the rule of five", is based on 2D parameters such as the number of potential hydrogen bonds, molecular weight and calculated lipophilicity. A method based on molecular mechanics calculations is used to calculate 3D parameters such as polar and non-polar parts of the molecular surface area. The third method uses quantum mechanics to calculate molecular properties related to the valence region.

Results. Descriptors derived by the latter two methods correlated well with permeability coefficients of the endothelin receptor antagonists. On the other hand, the rule of five failed to discriminate between drugs with high and low permeability.

Conclusions. Molecular surface descriptors and descriptors derived from quantum mechanics are potentially useful for the virtual screening of the permeability of the intestinal membrane to endothelin receptor antagonists.

KEY WORDS: molecular surface area; hydrogen bonding; intestinal drug absorption; drug transport; rabbit colon.

INTRODUCTION

Lead compounds generated in high throughput drug discovery programmes are generally more lipophilic, less soluble and of higher molecular weight than conventional drugs (1). These characteristics often entail unfavorable biopharmaceutical properties resulting in a low success rate of such drug candidates in clinical development (2). The development of new methods to predict biopharmaceutical properties such as intestinal permeability is therefore desirable.

Indeed, several new *in vitro* methods have been developed to predict intestinal permeability (3,4). Of these, methods based on drug transport across intestinal epithelial cell monolayers or artificial model membranes have obtained much attention (5,6). In particular, Caco-2 cell monolayers have become well established as a model system for drug transport through the intestinal epithelial membrane (5) and attempts are currently being made to adopt this model into a high throughput screening (HTS) format, see for example (7,8). A drawback shared by all experimental methods is that they require synthesis of sufficient amounts of the compounds.

Therefore, simple theoretical molecular descriptors such as lipophilicity, hydrogen bonding potential and molecular size, are frequently used to predict membrane permeability (1,9–11). Recently, Lipinski and coworkers investigated the physicochemical properties of around 2,000 drug candidates that had entered clinical phase II trial (1). This investigation led to the formulation of simple rules aimed at increasing the possibility of discovering drug candidates with acceptable intestinal permeability and solubility. These rules, based on 2D descriptors of hydrogen bonding, size and lipophilicity were subsequently termed "The rule of five." Although the rule of five is much simpler than experimental methods and allows high throughput, it is based on highly generalized 2D descriptors, and gives relatively rough and sometimes false predictions.

We therefore developed an alternative theoretical method for the prediction of drug absorption. This method is based on the determination of a single molecular descriptor, the dynamic polar surface area (PSA_d) using an approach based on molecular mechanics calculations (12,13). Unlike the rule of five, this method takes the 3D shape and flexibility of the drug molecule into account. This allows the effects of internal hydrogen bonding and steric hindrance to be considered. PSA_d, which is related to the hydrogen bonding capacity of the drug molecule, showed strong relationships with the intestinal epithelial permeabilities to a homologous series of drugs measured *in vitro* (12,13). It also showed a strong relationship with the fraction absorbed after oral administration of a series of structurally diverse drugs to humans (14). A likely reason for this is that PSA_d provides a good description of the energetically costly process of transferring polar groups into the apolar regions of the cell membranes (15).

It is well established that non-polar substituents facilitate membrane transport, and compounds which are more lipophilic generally have higher membrane permeabilities than hydrophilic compounds with similar hydrogen bonding properties (16). We therefore suggested that not only PSA_d but also the dynamic non-polar surface area ($NPSA_d$) should be considered in the predictions (15). Indeed, the relationship between a combination of polar and non-polar surface areas and *in vitro* intestinal epithelial permeability to a series of oligopeptide derivatives was much stronger than that of single surface properties (15). Thus, theoretically derived molecular surface properties are strongly related to intestinal membrane permeability to both conventional drugs and peptide derivatives. The method based on molecular surface areas has not been adopted for drugs generated via high throughput screening.

A third theoretical method for the prediction of drug absorption is based on quantum mechanical calculations. In this more demanding approach, descriptors related to the valence

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regions of the molecules are generated and correlated to intestinal permeability by means of partial least squares projections to latent structures (17). Descriptors for conventional drugs that are well correlated to human intestinal absorption and permeability have been derived using such methods (17–19). The approach based on quantum mechanics has not yet been applied on the structurally different drugs generated in high throughput drug discovery programmes. Since both the method based on molecular mechanics calculations and that based on quantum mechanics have a potential application as virtual screening tools for the membrane permeability to drugs, it is important to establish whether these methods may be applied on modern drug candidates.

Endothelin receptor antagonists are examples of the new class of drugs generated via pharmacological screening (20,21). Therefore, a series of ten indane carboxylic acid endothelin receptor antagonists was selected for this study. As expected, these endothelin receptor antagonists are larger (average molecular weight of 527) and more lipophilic (the average octanol-water partition coefficient, logP, is 3.7) than conventional drugs (1). We have predicted the intestinal permeability to the endothelin receptor antagonists using the three approaches of increasing complexity presented above.

METHODS

Data Set

The colonic permeabilities to ten endothelin receptor antagonists in the indane carboxylic acid series have been determined previously (21). Briefly, segments of colon from male, New Zealand white rabbits were rinsed in bicarbonate-Ringer solution, stripped of underlying muscle tissue and mounted in diffusion chambers (1.78 cm^2). A bicarbonate-Ringer solution containing 200μM of the appropriate drug was added to the mucosal side of the diffusion chamber. Samples were withdrawn from the serosal side of the tissue at 60, 90, 120 and 150 minutes, replaced by an equal amount of fresh bicarbonate-Ringer solution and analyzed for drug content by reversed phase HPLC.

Rule of Five

The rule of five states that a compound is likely to have limited oral bioavailability due to low intestinal epithelial permeability or solubility if it generates two or more alerts (1). A rule of five alert is produced if the number of H-bond donors or acceptors in a compound exceeds 5 and 10, respectively, the molecular weight (M_w) exceeds 500 or the lipophilicity, expressed as the logarithm of the octanol-water partition coefficient (logP), exceeds 5. An H-bond acceptor is defined as any nitrogen or oxygen atom, and an H-bond donor is any hydrogen atom attached to an H-bond acceptor. The rule of five does not apply to compounds that are subject to active transport processes.

Conformational Analysis

A 10,000-(SB 202994) to 120,000-(SB 212314) step Monte Carlo conformational search (22) was performed using the MM2 force field in MacroModel v5.0 (23) on a Silicon Graphics O2 workstation. By using this procedure, we expected the entire conformational space of low energy conformations ($\Delta E_s \leq 2.5$ kcal/mol) to be covered. For all compounds but SB 202994, we searched the conformational space by two or more Monte Carlo simulations. The global minimum of each simulation was then used as the starting conformation for a subsequent conformational search. The resulting conformations from the searches were combined and duplicate conformations removed.

Surface Area Calculations

An in-house computer program, MAREA (24), was used to calculate the surface area of each conformer as described previously (14). The program calculates the free surface area of each atom as well as the molecular volume (V). The following atomic van der Waals radii were used: sp^2 carbon 1.94 Å, sp^3 carbon 1.90 Å, oxygen 1.74 Å, nitrogen 1.82 Å, sulfur 2.11 Å, electroneutral hydrogen 1.50 Å, hydrogen bonded to oxygen 1.10 Å and hydrogen bonded to nitrogen 1.125 Å (obtained from PCMODELv4.0 (25)).

The polar surface area (PSA) was defined as the area contributed by nitrogen and oxygen atoms, plus the area of the hydrogen atoms attached to these heteroatoms (14). The remaining surface area was defined as non-polar, denoted NPSA. The percentage of the total surface area contributed by polar atoms (%PSA) was also calculated. The dynamic surface area (SA_d) is a statistical average obtained by weighting the surface area of each low energy conformation ($\Delta E_s \leq 2.5$ kcal/mol) by its probability of existence according to a Boltzmann distribution (12,26). The dynamic polar surface area (PSA_d), dynamic non-polar surface area ($NPSA_d$), percentage of the total dynamic surface area that is polar ($\%PSA_d$) and dynamic volume (V_d) were determined in the same manner.

Semi-Empirical Calculations

The global minima from the conformational searches in MacroModel were used as starting geometries for semi-empirical geometry optimizations using the AM1 method in Spartan (27).

Ab Initio Calculations

An *ab initio* single point calculation using the 3-21G* basis set in Spartan was performed on each optimized geometry in order to obtain a wave function suitable for input to the MolSurf program.

MolSurf Calculations

The wave function of each compound was used as input for the MolSurf program (28). MolSurf calculates various molecular chemical and physico-chemical properties The molecular descriptors derived from MolSurf were for each compound: the strength of hydrogen bond donors of each compound, the number of hydrogen bond donors, the strength of hydrogen bond acceptor oxygens (HBAO), the number of hydrogen bond acceptor oxygens, the strength of hydrogen bond acceptor nitrogens (HBAN), the number of hydrogen bond acceptor nitrogens, the sum of HBAO and HBAN, the polarity of hydrogens, the number of polar hydrogens, the molecular electrostatic potential surface area, the octanol-water partition coefficient (logP), the

polarisability, the polarity, the Lewis acid strength, the Lewis base strength, the nucleophilicity, the electrophilicity and the acid dissociation constants (28).

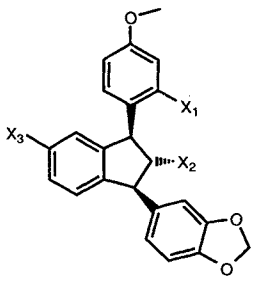
Data Analysis

The molecular descriptors from the MolSurf calculations were examined by principal component analysis (PCA) (29). Correlations between rabbit colonic permeability *in vitro* and calculated MolSurf descriptors were established by the method of partial least squares projections to latent structures (PLS) (30). The number of PLS components which was computed was determined by Q^2, the leave one out cross-validated R^2 (where R^2 is the fraction of the sum of squared permeability coefficients explained by the model). Only PLS components resulting in a positive Q^2 were computed, and the number of components were never allowed to exceed three. The model was further developed by a stepwise selection of variables. Initially, all descriptors were included in the PLS model. After the first round, the descriptor with least influence on the prediction was deleted and the PLS repeated. If exclusion of a descriptor resulted in a more predictive model (as assessed by Q^2), the descriptor was permanently left out of the model. The variable selection was repeated until no further improvement of Q^2 was achieved. PCA and PLS analyses were performed using Simca (31). The strength of linear relationships between single variables was assessed by the correlation coefficient, r. Unpaired two-tailed Student's t-test was used to test the significance of the difference between mean P_{app} values.

RESULTS AND DISCUSSION

In this study, we have used three computational strategies of different complexities to predict the intestinal permeability to a series of ten endothelin receptor antagonists generated via pharmacological screening. We found strong correlations between intestinal permeability to the drugs and molecular descriptors derived by molecular mechanics and quantum mechanics approaches, while the simpler rule of five failed to discriminate between high and low permeability drugs. These promising results indicate that computational approaches based on molecular mechanics and quantum mechanics should be further evaluated as virtual screening tools for the rapid prediction of passive drug transport across biological membranes.

Descriptors calculated by the three methods were correlated to permeability coefficients observed in excised tissue segments from rabbit colon. Colonic tissue was considered a better model for studies of passive transcellular drug transport than tissue from the small intestine since it is tighter (which gives a low paracellular permeability) and expresses less active transport mechanisms (32). The structural formulae of the endothelin receptor antagonists are shown in Fig. 1, and their colonic permeability coefficients (ranging from −0.76 to −2.62 log cm/h) are given in Table I. Earlier studies in rat report a bioavailability of 66% for SB 217242 and a limited bioavailability for SB 209670 (20,21). Based on these data, the endothelin receptor antagonists may roughly be divided into high and low permeability compounds with cut-offs in permeability coefficients around −1 and −2 log cm/h (Table I).



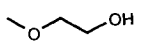
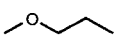
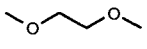
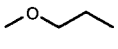
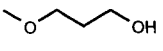
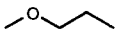
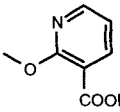
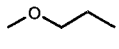
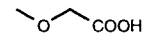
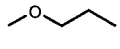
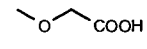
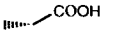
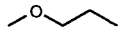
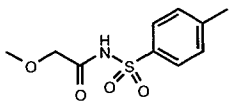
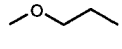
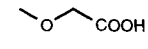
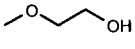
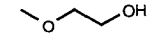
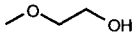
Compound	X_1	X_2	X_3
SB 202994	—H	COOH	—H
SB 217242		COOH	
SB 220501		COOH	
SB 208601		COOH	
SB 211835		COOH	
SB 209670		COOH	
SB 217585			
SB 212314		COOH	
SB 219730		COOH	
SB 219862		COOH	

Fig. 1. Structures of the endothelin receptor antagonists.

The endothelin receptor antagonists have high lipophilicities (Table I) and should experience high permeabilities (10). However, five out of the ten studied endothelin receptor antagonists have low permeability coefficients. Four of these have more than one negative charge at physiological pH (7.4), and this explains their low permeability coefficients. It is not clear why the permeability to SB 219862 is low, but it may be related to the increased hydrogen bonding capacity provided by the hydroxyl group on X_3 (Fig. 1). Thus, the compounds with low permeability have a higher negative charge than the high

Table I. Colonic Permeability, Surface Properties, Molecular Weight, Lipophilicity, and Acid Dissociation Constants of the Investigated Endothelin Receptor Antagonists

Compound	log P_{app}[a] (cm/h)	Permeability classification[b]	PSA_d[c] (Å^2)	$NPSA_d$[c] (Å^2)	M_w (g/mol)	logP[d]	pK_{a1}[d]	pK_{a2}[d]
SB 202994	−0.76	High	77.6	368.4	388	5.39	5.01	n/a
SB 217242	−1.02	High	103.8	474.1	506	4.22	5.36	n/a
SB 220501	−1.04	High	96.7	508.4	520	4.42	5.99	n/a
SB 208601	−1.54	Intermediate	100.4	495.8	520	4.29	4.78	n/a
SB 211835	−1.96	Low	129.3	495.4	583	3.25	3.90	5.51
SB 209670	−2.47	Low	126.8	452.4	520	3.56	4.33	4.60
SB 217585	−2.49	Low	124.8	468.4	534	3.68	2.99	6.41
SB 212314	−2.55	Low	145.3	542.1	673	2.63	5.80	6.95
SB 219730	−2.57	Low	137.2	402.6	522	2.51	4.41	5.20
SB 219862	−2.62	Low	125.6	434.4	508	3.36	5.17	n/a

[a] Data were obtained from reference (21).
[b] Based on bioavailability data (20), we divided the data set into high and low permeability compounds.
[c] Dynamic polar surface area (PSA_d) and non-polar surface area ($NPSA_d$) were determined using the molecular mechanics methodology described in the methods section.
[d] Lipophilicity was calculated as the octanol-water partition coefficient (logP). This partition coefficient and the acid dissociation constants (pK_a) were derived from quantum mechanics calculations described in the methods section.

permeability compounds and they may have an additional hydroxyl group.

We investigated whether the rule of five (1) could discriminate between the highly and poorly permeable compounds (Table II). All endothelin receptor antagonists but SB 202994 have a molecular weight over 500, which gives an alert, but only SB 212314 obtained an additional alert for having more than ten hydrogen bond acceptors (Table II). The rule of five requires at least two alerts to indicate limited permeability or solubility, and thus only one out of five poorly permeable endothelin receptor antagonists was identified. However, when the alert value for the number of hydrogen bond acceptors was decreased from ten to eight, the rule of five distinguished between the highly and poorly permeable endothelin receptor antagonists. We suggest that by optimizing the alert limits of the rule of five to the data set, this simple method may find a broader applicability in predictions of membrane permeability.

There was a good correlation between PSA_d and the log P_{app} values of the endothelin receptor antagonists (r^2 = 0.83; Fig. 2). More importantly, PSA_d discriminated between drugs with high and low permeability coefficients. For instance, the PSA_d of the low permeability compound, SB 209670, was 127 Å^2 as compared to 104 Å^2 for the high permeability compound SB 217242 (Fig. 2).

The most notable outlier in the correlation between PSA_d and colonic permeability is SB 208601. This compound differs from SB 217242 only by having an additional methylene group in the X_1 chain (Fig. 1), but has a significantly lower permeability coefficient (p = 0.001). We expected the addition of a

Table II. Rule of Five Alerts[a]

Compound	H-bond donors[b]	H-bond acceptors[b]	M_w	logP
SB 202994	−	−	−	+
SB 217242	−	−	+	−
SB 220501	−	−	+	−
SB 208601	−	−	+	−
SB 211835	−	−	+	−
SB 209670	−	−	+	−
SB 217585	−	−	+	−
SB 212314	−	+	+	−
SB 219730	−	−	+	−
SB 219862	−	−	+	−

[a] Lipinski *et al.* (1) proposed that a compound that obtained two or more alerts was likely to have limited permeability and/or solubility. A rule of five alert is produced if the number of H-bond donors or acceptors in a compound exceeds 5 and 10, respectively, the molecular weight (M_w) exceeds 500 or the lipophilicity, expressed as the logarithm of the octanol-water partition coefficient (logP), exceeds 5. In the table, an alert is indicated with a plus sign.
[b] Calculated as the sum of all hydrogen bond donor or acceptor atoms, respectively.

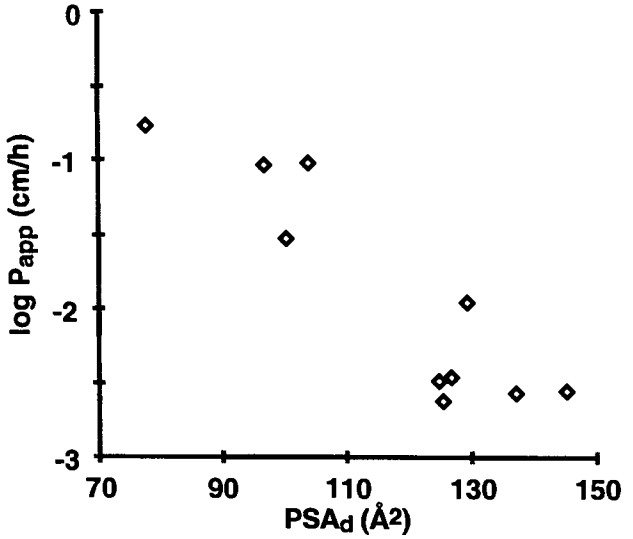


Fig. 2. Correlation between colonic permeability (P_{app}) to and dynamic polar surface area (PSA_d) of the series of ten indane carboxylic acid endothelin receptor antagonists (r^2 = 0.83). The relative standard error of the mean P_{app} values were 3–33% (21).

methylene group to increase rather than decrease the permeability, so we investigated whether the increased chain length induced a conformational change, which in turn decreased the intramolecular hydrogen bonding in SB 208601 compared to SB 217242. Increased intramolecular hydrogen bonding would lead to decreased intermolecular hydrogen bonding. The only possibility of forming intramolecular hydrogen bonds is by interaction between the hydroxyl and the carboxylic acid groups. Both compounds are deprived of the carboxylic acid proton at pH 7.4 and the increased chain length may unfavorably affect the possibility of forming an intramolecular hydrogen bond with the carbonyl oxygen in X_2. However, the molecular mechanics calculations did not support this conjecture, and SB 208601 was as prone as SB 217242 to form this particular hydrogen bond. The conformational searches were performed without solvent, which may promote the formation of intramolecular hydrogen bonds not seen in water. Therefore, we repeated the conformational search in simulated water for these two compounds. Neither of the compounds formed this internal hydrogen bond in water (results not shown). The two compounds were also studied by NMR-spectroscopy to determine their experimental hydrogen bonding patterns in solution. However, ^{1}H-NMR experiments performed in acetone, chloroform or methanol did not give conclusive results as the compounds appeared to be highly flexible under the experimental conditions (results not shown). However, when we performed calculations on the charged species in water, SB 217242 was stabilized by this internal hydrogen bond whereas SB 208601 was not. This indicates that the observed lower permeability coefficient for SB 208601 is the result of an exposure of the hydroxyl group.

A relationship has previously been established between PSA_d and the absorbed fraction after oral administration of conventional drugs to humans (14). Drugs with a $PSA_d < 60$ Å^2 are predicted to be completely (>90%) absorbed, whereas drugs with $PSA_d > 140$ Å^2 should be absorbed to less than 10%. When this relationship is applied to the endothelin receptor antagonists, an oral absorption of <20% is predicted for the low permeability endothelin receptor antagonists ($PSA_d > 120$ Å^2) while an oral absorption of 40–70% is predicted for the high permeability endothelin receptor antagonists (PSA_d 78–104 Å^2). This is consistent with earlier studies (20). We conclude that PSA_d successfully predicts intestinal permeability to these HTS compounds.

We recently showed that a combination of polar and non-polar surface areas ($PSA_d - 0.06 \times NPSA_d$) provided a much stronger relationship with in vitro intestinal epithelial permeability to a series of oligopeptide derivatives than any single surface property (15). In the present study, this particular combination of surface properties only slightly strengthened the relationship ($r^2 = 0.87$; Fig. 3). The limited improvement in r^2 is probably due to the fairly even distribution of $NPSA_d$ which is in contrast to the previous study (Table I). Further, the correlation between PSA_d and calculated logP values was strong ($r^2 = 0.95$), indicating that $NPSA_d$ is of minor importance for this particular series of lipophilic compounds. Although the combination of polar and non-polar surface areas only gave a minor improvement of the correlation with permeability, similar or larger improvements have been observed for other data sets (15). Thus, by accounting for the variability in hydrophobicity using a combination of polar and non-polar surface properties, a more general description of membrane permeability can be given.

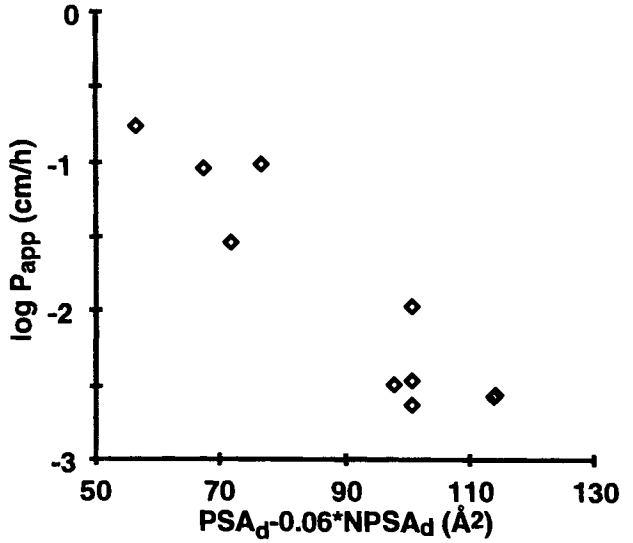


Fig. 3. Correlation between colonic permeability (P_{app}) to and a linear combination of polar and non-polar surface area ($PSA_d - 0.06*NPSA_d$) of the series of ten indane carboxylic acid endothelin receptor antagonists ($r^2 = 0.87$). The relative standard error of the mean P_{app} values were 3–33% (21).

Calculation of surface properties derived from molecular mechanics calculations using the analytical algorithm implemented in MAREA is a rapid process (24). For instance, to calculate the free surface area of each atom in SB 202994, a Silicon Graphics Indy workstation (R4400, 174 MHz) required 0.44 seconds of CPU time per conformation. However, since the calculation of surface properties by numerical methods (e.g., PCMODEL see (25)) is generally considered to be time consuming, we investigated whether colonic permeability to these compounds was correlated with the PSA calculated from single conformers only. The PSAs of the global minimum conformations generally differed from the corresponding PSA_ds by less than one Å^2 (SB 212314 differing by 4.6 Å^2 being an exception), and it was not surprising to obtain a similar correlation coefficient ($r^2 = 0.82$) for PSA as for PSA_d. However, dynamic surface properties take the shape of all low energy conformations into account and are less sensitive to the choice of force field and to the risk of not finding the global minimum in the conformational search. It proves especially important when investigating compounds stabilized by intramolecular hydrogen bonding, as described above for SB 208601 and SB 217242.

Molecular descriptors computed by quantum mechanics have previously been correlated with epithelial and endothelial permeabilities for structurally diverse sets of conventional drugs (17,33). We have used a method based on quantum mechanics to discover whether electronic effects not considered in molecular mechanics calculations provide a better model for the description of the colonic permeability to the endothelin receptor antagonists. Initially, a two component PLS model ($R^2 = 0.90$ and $Q^2 = 0.53$) was derived from the 20 calculated descriptors. Stepwise variable selection of this model resulted in a three component PLS model with $R^2 = 0.98$ and $Q^2 = 0.92$ (Fig. 4). The final model contained eight of the initial 20 descriptors: lipophilicity (logP), polarity of hydrogens, number of hydrogen

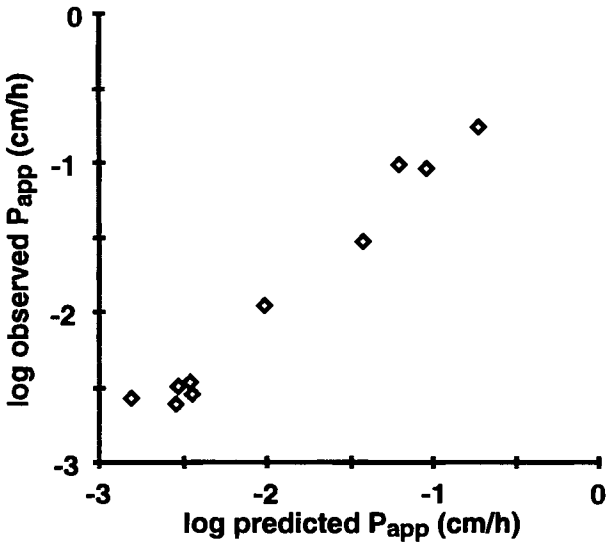


Fig. 4. Correlation between experimental colonic permeability (observed P_{app}) and permeability calculated by PLS (predicted P_{app}) from descriptors derived from quantum mechanics to the series of ten indane carboxylic acid endothelin receptor antagonists. The initial set of 20 descriptors was subjected to a stepwise variable selection procedure, yielding a model with eight descriptors.

bond donors, electrostatic potential surface area, pK_{a2}, strength of hydrogen bond accepting nitrogens, electrophilicity and nucleophilicity. We could not carry out an external validation of the model, since there are so few compounds in the data series. In particular, there are too few compounds with high permeability coefficients. An external test set would be required in order to draw any conclusions regarding the general applicability of the derived model. Still, the high Q^2 of the model indicates that the descriptors derived from quantum mechanics are predictive of intestinal permeability to these endothelin receptor antagonists. However, the optimization of geometry by quantum mechanical calculations requires considerably more computer time than molecular mechanics calculations. The generation of descriptors by MolSurf also takes longer than the calculation of surface properties by MAREA (hours versus seconds on a standard workstation). Further, the empirical nature of the model derived from MolSurf/PLS calculations makes it more difficult to explain structure-permeability relationships in terms of physico-chemical characteristics, especially when as many as eight descriptors are needed to give an optimal model.

The methods based on molecular mechanics and on quantum mechanics described in this paper have been successfully used to describe intestinal drug absorption in humans *in vivo* (14), and drug permeability in rat small intestine (12), in Caco-2 cell monolayers (12,13,15,17) and in rabbit colon (this study). We therefore speculate that, to a considerable extent, the same physico-chemical properties are important for passive transcellular membrane permeation in such diverse tissues.

In conclusion, the explosive development of computer technology makes it possible to adopt methods based on molecular mechanics and quantum mechanics as virtual screening tools. Using descriptors generated by these *in silico* methods we can construct highly predictive models of membrane permeability not only to conventional drugs and peptides, but also to non-peptide endothelin receptor antagonists.

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