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Prediction of the permeability of drugs through study on quantitative structure–permeability relationship

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Abstract

This study is to research the quantitative structure–permeability relationship of 20 drugs having similar structure. Permeability was determined by using the Caco-2 cell in vitro model. The apparent permeability coefficient (P_{app}) of each drug both of apical to basolateral side and basolateral to apical side was measured at the concentration corresponding to 0.1 times the highest dose strength of 250 mL dissolved buffer. In order to test the permeability system suitability, we measured the P_{app} of 19 model drugs out of 20 which presented in 'Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms based on the Biopharmaceutics Classification System' of FDA guidance. Also, we demonstrated the functional expression of efflux systems (e.g., *p*-gp) by bi-directional transport studies with rhodamine 123. Also, as a result of the study on quantitative structure–permeability relationship by using the partial least square method, it was possible to predict the permeability of drugs from their 3D structure. The quantitative structure–permeability relationship provided a cross-validated $q^2 = 0.789$, a normal $r^2 = 0.998$. Considering all of above results, analysis on this quantitative structure–permeability relationship appears to be a very useful tool to predict the permeability.

Keywords: Biopharmaceutics classification system (BCS); Caco-2 cell; Permeability

1. Introduction

The estimation of human oral absorption of new drug candidates in the early stage of the drug discovery process is a useful tool in the lead-compound selection [1]. Several in vitro approaches, based on cell cultures, were used in this area over the last few years. Among the cell culture models, Caco-2 cell line is the most widely employed and it has been investigated like a potential in vitro model for drug absorption and metabolism studies. In the sense, considering the similarity of Caco-2 cell to small intestine enterocytes and their capacity to express carrier-mediated transport systems and typical small intestinal enzymes, the permeability coefficient across Caco-2 cell monolayer is increasingly used to estimate the oral absorption of new chemical entities. We used Caco-2 cell model to evaluate the cell permeability of 20 drugs having similar structures. Through this experience, we have come to have interest in applying the model to a broader exploration of structure–permeability relationships [2]. Quantitative structure–permeability relationships (QSPRs) of the intestinal permeability across Caco-2 cells monolayer have been studied by many researchers [1,3]. The theoretical approach appears to be good to predict the human absorption of new drug candidates obtained by combinatorial chemistry methodologies, avoiding significant failure in late stage of the drug-discovery process.

The objective of the present study is to predict in vivo pharmacokinetic performance of drug products from evaluation of computational approaches for estimation of passive intestinal absorption by applying them to Caco-2 cell permeability coefficient data for a series of structurally related 20 drugs [3]. This study describes how passive absorption can be predicted by using commercially available 3D-quantitative

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structure–activity relationships (QSAR) approaches, comparative molecular field analysis (CoMFA), to provide a readily interpretable in silico prediction of in vitro Caco-2 cell permeability. The CoMFA analysis has been used in a simple extension of the structure–activity relationship for predicting the physicochemical properties such as separation [4].

2. Materials and methods

2.1. Materials

We obtained 20 drugs from Dae-Woong Pharmaceutical (Seoul, Korea), etc. (Fig. 1) and obtained Caco-2 cells, originating from a human colorectal carcinoma, from American Tissue Culture Collection (Rockville, MD, USA). We purchased Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, penicillin and streptomycin from Gibco BRL (Grand Island, NY, USA); L-glutamine, sodium bicarbonate, nonessential amino acid, Hank's balanced salts solution (HBSS) and pyruvic acid from Sigma (Milwaukee, WI, USA) and Transwell polycarbonate filters from Costar Corning (NY, USA; diameter 12 mm, pore size 0.4 μm). Radiolabeled [^{14}C] mannitol (specific activity of 51.5 mCi/mmol) used in Caco-2 cell system suitability test was obtained from DuPont New England Nuclear (Boston, MA, USA) and measured with a β-counter (1450 microbeta, Wallac Counter, Turku, Finland). HPLC grade acetonitrile was purchased from J.T. Baker (Deventer, The Netherlands). Water was purified with an ultimate reverse osmosis system (Barnstead, Dubuque, USA). And all of the other reagents were of analytical or HPLC grade.

2.2. Caco-2 experiments

2.2.1. Caco-2 cell culture

Caco-2 cells purchased from American Type Culture Collection (ATCC) were grown in 75 cm^2 culture flasks at 37 °C in the atmosphere of 5% CO_2 and 90% relative humidity in DMEM growth medium supplemented with 10% (v/v) FBS, 1% (v/v) nonessential amino acids, penicillin (100 Unit/mL), and streptomycin (100 μg/mL). Confluent cell monolayers were subcultured every 7 days by treatment with 0.25% trypsin containing 1 mM EDTA. Caco-2 cells were seeded at the density of 80,000 cells/cm^2 in 12-well plates on Transwell polycarbonate filters. Cells were grown until fully differentiated after 21 days, and all experiments were conducted between 21 and 25 days. Cells of passage numbers 30–50 were used throughout, and Caco-2 cell monolayers with initial transepithelial electrical resistance (TEER) values measured with a Millicell-ERS Voltohmmeter (Millipore, Billerica, MA, USA) higher than 350 Ωcm^2 were used [5].

2.2.2. Caco-2 cell system suitability

The Caco-2 cell permeability model was used to determine the permeability class of the drugs used in this study. We had previously validated our permeability model using 19 model drugs. Our data demonstrated that our Caco-2 cell model was discriminating and that those compounds with permeability

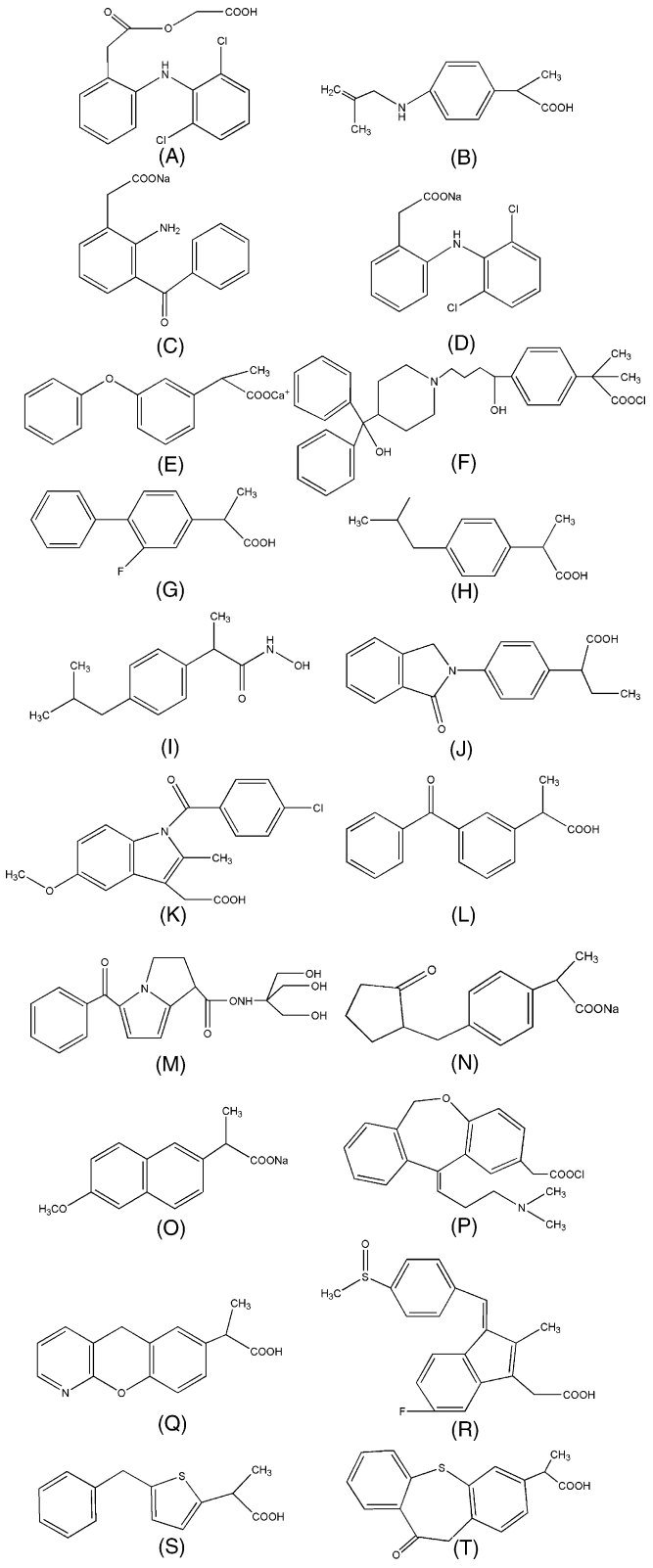


Fig. 1. Chemical structures of model drugs: (A) aceclofenac, (B) alminoprofen, (C) amfenac sodium, (D) diclofenac sodium, (E) fenoprofen calcium, (F) fexofenadine hydrochloride, (G) flurbiprofen, (H) ibuprofen, (I) ibuproxam, (J) indobufen, (K) indomethacin, (L) ketoprofen, (M) ketorolac tromethamine, (N) loxoprofen sodium, (O) naproxen sodium, (P) olopatadine hydrochloride (Q) pranoprofen, (R) sulindac, (S) tiaprofenic acid, and (T) zaltoprofen.

coefficients higher than $\sim 1 \times 10^{-6}$ cm/s had 90% of more of the administered dose absorbed and considered as having high permeability. The suitability of the Caco-2 cell method under this study was established by measuring the permeability of 19 model drugs selected from the list provided in the FDA Guidance. Thus, 10 highly permeable drugs (antipyrine, caffeine, carbamazepine, fluvastatin, ketoprofen, metoprolol, naproxen, propranolol, theophylline and verapamil) and nine low permeability drugs (amoxicillin, atenolol, furosemide, hydrochlorthiazide, mannitol, methydopa, PEG400, PEG4000 and ranitidine) were used as markers and also we demonstrated the functional expression of efflux systems, *p*-glycoprotein (*p*-gp), by transport studies with rhodamine123, fluorescent *p*-gp substrate. We found good correlation between high expression of the *p*-gp at the apical membrane and high rhodamine123 efflux into the apical extracellular space. Permeability values were determined from both apical to basolateral (AP → BL) and basolateral to apical (BL → AP) directions. We used [^{14}C] mannitol as a paracellular transport marker and measured with a β-counter. From the result of the [^{14}C] mannitol, we defined that [^{14}C] mannitol was zero-permeability marker. The AP → BL values for all highly permeable model drugs were higher than the permeability of verapamil, one of the highly permeable drugs and none appeared to have an affinity for cellular efflux pumps (Table 1). On the other hand, for the low permeable model drugs, the AP → BL values were less than hydrocholorothiazide, one of the low permeable drugs.

Table 1
Caco-2 cell permeability of model drugs for demonstration of system suitability of permeability test method ($P_{app} = \times 10^{-6}$ cm/s, $n = 6$)

Drug	Class	AP → BL
Antipyrine	High	40.9 ± 5.0
Caffeine	High	40.8 ± 15.8
Carbamazepine	High	35.8 ± 1.8
Fluvastatin	High	25.4 ± 1.3
Ketoprofen	High	16.9 ± 1.0
Metoprolol	High	23.6 ± 3.5
Naproxen	High	24.8 ± 6.3
Propranolol	High	14.2 ± 2.4
Theophylline	High	32.7 ± 1.3
Verapamil	High	11.7 ± 0.3
Amoxicillin	Low	1.8 ± 0.6
Atenolol	Low	0.2 ± 0.0
Furosemide	Low	0.2 ± 0.0
Hydrochlorthiazide	Low	5.0 ± 0.6
Mannitol	Low	0.5 ± 0.0
Methyldopa	Low	ND[a]
PEG400	Low	1.8 ± 0.9
PEG4000	Low	0.3 ± 0.1
Ranitidine	Low	0.3 ± 0.1

[a] ND, not detected.

2.2.3. Permeability studies

Caco-2 cell monolayers were preconditioned by incubating with HBSS (pH 7.4) consisting of 1.3 mM $CaCl_2$, 5.4 mM KCl, 0.44 mM KH_2PO_4, 0.49 mM $MgCl_2$, 0.41 mM $MgSO_4$, 137 mM NaCl, 0.34 mM Na_2HPO_4, 5.5 mM D-glucose, and 4.2 mM $NaHCO_3$, at 37 °C for 30 min. NSAIDs of the concentration corresponding to 0.1 times the highest dose strength dissolved in 250 mL of buffer were used in the permeability studies. For apical to basolateral (AP to BL) experiments, the solution was placed on the apical side of the cells, and samples were taken from the basolateral side. At many time

Table 2
HPLC analytical condition of drugs for permeability

Drug	Mobile phase	Detector/wavelength (λ)	Calibration range (μg/mL)
Antipyrine	50:50:1[a]	UV/243	0.1–200
Aceclofenac	50:50:1[a]	UV/275	0.2–400
Alminoprofen	50:50:1[a]	UV/255	0.1–300
Amfenac sodium	50:50:1[a]	UV/234	0.1–200
Diclofenac sodium	50:50:1[a]	UV/277	0.5–400
Fenoprofen calcium	50:50:1[a]	UV/229	2.8–277
Fexofenadine hydrochloride	65:35:0.3[b]	UV/210	0.2–360
Flurbiprofen	50:50:1[a]	UV/247	0.2–320
Ibuprofen	50:50:1[a]	UV/226	1.2–24
Ibuproxam	50:50:1[a]	UV/228	0.4–400
Indomethacin	50:50:1[a]	UV/318	0.5–100
Indobufen	50:50:1[a]	UV/281	0.1–400
Ketoprofen	50:50:1[a]	UV/255	0.2–200
Ketorolac tromethamine	50:50:1[a]	UV/313	0.1–80
Loxoprofen sodium	50:50:1[a]	UV/228	1.2–240
Naproxen sodium	50:50:1[a]	UV/232	0.1–44
Olopatadine hydrochloride	74:26[c]	UV/210	0.2–40
Pranoprofen	50:50:1[a]	UV/248	0.1–300
Sulindac	50:50:1[a]	UV/286	0.1–400
Tiaprofenic acid	50:50:1[a]	UV/305	0.1–300
Zaltoprofen	50:50:1[a]	UV/230	0.4–320

[a] Mobile phase composition—water:acetonitrile:glacial acetic acid.
[b] Mobile phase composition—0.1 M NaH_2PO_4 (pH 3):acetonitrile:triethylamine.
[c] Mobile phase composition—0.1 M KH_2PO_4 with 0.1% triethylamine (pH 3):acetonitrile.

points, samples were collected from the other side of the cell monolayers for quantification. All permeability studies were performed at 37 °C. The samples were analyzed by HPLC system (Waters, Milford, MA, USA) consisting of photo diode array detector (Waters 2996), fluorescence detector (Waters 2475), binary HPLC pump (Waters 1525) and autosampler (Waters 717 plus) to determine the drug concentration. A 150 mm × 4.6 mm, 5 μm Capcell pak C_{18} column (Shiseido, Tokyo, Japan) was used along with a mobile phase consisting of water–acetonitrile–glacial acetic acid (50:50:1, v/v/v), 0.1 M NaH_2PO_4 (pH 3)–acetonitrile–triethylamine or 0.1 M KH_2PO_4 with 0.1% triethylamine (pH 3)–acetonitrile with the flow rate of 1.5 mL/min (Table 2).

Permeability coefficient (P_{app}) was determined according to the following equation [6,7]:

$$P_{app} = \frac{J}{AC_i}$$

where J is the transport rate determined by plotting cumulative amount of drug permeated to the receiver chamber as a function of time, A the surface area of the filter, and C_i is the initial concentration of the solution in the donor chamber. To determine whether the drugs were substrate for the apically polarized efflux systems in Caco-2 cell monolayers, P_{app} of each drug from both AP to BL and BL to AP was measured. For cellular efflux pump substrates, P_{app} values from BL to AP are expected to be higher than from AP to BL. We used antipyrine (0.8 mg/mL) as internal standard of high permeability and calculated P_{app} ratio by P_{app} of each drug and P_{app} of antipyrine to compare with drug permeability. All experiments were performed in $n = 6$, and the data are expressed as mean ± standard deviation.

2.3. Quantitative structure–permeability relationships

2.3.1. Computational methods of quantitative structure–permeability relationships

All molecular modeling and statistical analyses were performed by using SYBYL 6.9 molecular modeling software from Tripos Inc. (Saintlouis, Missouri, USA) on Silicon Graphics Origin 300 (IRIX 6.5).

The 2D structure of each compound was built by using SYBYL Build program with the default SYBYL settings. The 2D structure was converted to a 3D structure by using Concord 4.0 program. The structural energy minimization was performed by using the SYBYL energy minimizer (Tripos Force Field) and Gasteiger–Huckel charge, with a 0.005 kcal/M energy gradient convergence criterion. Low energy conformation was searched by geometry optimization after rotating every 30° of single bond from 1° to 330° of tortional angle. All of the structures generated were aligned with the lattice box by fitting to β,γ-unsaturated ester as a common structure.

2.3.2. Calculation of CoMFA descriptors

Conventional CoMFA was performed with the quantitative structure activity relationship (QSAR) option of SYBYL. The steric and electrostatic field energies were calculated by using sp^3 carbon probe atoms with +1 charge. Maximum energy cutoff for steric and electrostatic energies was 30 kcal/M. The CoMFA grid spacing was 2.0 Å in all 3D within the defined region. The partial least squares (PLS) method was used for fitting the 3D structural features and their biological activities. The optimum number of components in the final PLS model was determined

Table 3
Caco-2 cell permeability of 20 selected drugs[a] ($P_{app} = \times 10^{-6}$ cm/s, $n = 6$)

Drug	Highest dose (mg)	Permeability class	AP → BL	IS[b] ratio
Diclofenac sodium	50	High	54.2 ± 3.1	1.72
Naproxen sodium	550	High	46.5 ± 1.2	1.42
Ibuprofen	600	High	46.3 ± 6.0	1.69
Alminoprofen	150	High	46.1 ± 1.9	1.43
Loxoprofen sodium	60	High	44.8 ± 5.0	1.32
Ketoprofen	50	High	44.6 ± 0.0	1.48
Indomethacin	25	High	42.9 ± 0.0	1.34
Fenoprofen calcium	692	High	41.4 ± 1.4	1.58
Indobufen	200	High	40.7 ± 1.0	1.38
Pranoprofen	75	High	40.5 ± 2.0	1.28
Zaltoprofen	80	High	39.9 ± 2.7	1.37
Tiaprofenic acid	300	High	39.3 ± 1.2	1.31
Aceclofenac	100	High	38.9 ± 1.7	1.20
Flurbiprofen	40	High	33.8 ± 2.0	0.92
Amfenac sodium	50	High	30.5 ± 3.0	0.98
Ibuproxam	400	High	23.2 ± 0.9	0.93
Sulindac	200	High	21.9 ± 0.0	0.76
Olopatadine hydrochloride	5	High	9.7 ± 4.2	0.30
Ketorolac tromethamine	10	High	8.3 ± 5.2	0.26
Fexofenadine hydrochloride	180	Low	2.0 ± 0.0	0.07

[a] Drugs concentrations corresponding to 0.1 times the highest dose strength dissolved in 250 mL of buffer.
[b] IS, internal standard (antipyrine).

by the q^2 value, obtained from the leave-one-out cross validation technique.

3. Results and discussion

3.1. Permeability coefficient evaluation

The Caco-2 cell permeability coefficient values of 20 drugs are presented in Table 3. Permeability values were determined from both apical to basolateral (AP → BL) and basolateral to apical (BL → AP) directions. Also we measured bi-directional P_{app} of each drug to determine whether the drugs were substrate for the efflux system (*p*-gp) in Caco-2 cell monolayers. As experimented data, the Caco-2 cell permeability coefficient values for all NSAIDs were higher than that of hydrochlorthiazide and were therefore classified as highly permeable. These NSAIDs are rapidly and completely absorbed with bioavailability values above 90%. Olopatadine hydrochloride of the two antihistamines was classified as having high permeable property, but the Caco-2 cell permeability coefficient value for fexofenadine hydrochloride was lower than that of hydrochlorthiazide, so was classified as low permeable drug. Our permeability data had similar results comparing with other studies. From the research of Wu and Benet [8] highly permeable drug (BCS classes 1 and 2) was highly metabolized, whereas BCS classes 3 and 4 drugs are primarily excreted unchanged via the biliary or renal routes. We expected that drug permeability study could be used in predicting the effects of efflux and absorptive transporters on drug absorption.

3.2. Quantitative structure–permeability relationships

The QSAR/CoMFA analysis developed by Cramer et al. [9,10] is a simple extension of the standard structure–activity relationship tables with ClogP and CoMFA. The statistical results of the CoMFA are summarized in Table 4. The CoMFA, molecular refractivity (CMR) and ClogP were used as descriptors, and the permeability (relative permeability to antipyrine) as a dependent column. The lipophilicity of model drugs was calculated by using CLOGP program. The statistical results of the CoMFA analyses, a cross-validated value q^2 was obtained as a result of PLS analysis served as a quantitative measure of the predictability of the CoMFA model. The cross-validated value q^2 appears to be a good indicator of the accuracy of actual predictions. In general, a comparative field analysis of any molecular property using PLS methodology that exhibits $q^2 \geq 0.5$ is indicative of the probability of a chance correlation between the molecular property and the CoMFA field examined to be ≤5%. Therefore, a model with $q^2 \geq 0.5$ is generally regarded as internally predictive.

Table 4
Statistical results

Number of component	5
q^2	0.789
r^2	0.998
Standard error of estimate	0.024
Contributions	
Steric	24.0%
Electrostatic	27.0%
CMR	24.8%
ClogP	24.1%

These cross-validated q^2 (0.789) and conventional r^2 (0.998) values proved the correlation between the descriptors and each of their activities, and gave reliability to the prediction of the permeability of the training set compounds. The relative contributions of steric, electrostatic field, CMR and ClogP were 24.0%, 27.0%, 24.8% and 24.1%, respectively as shown in Table 4. The contribution of electrostatic field was a little more important than others. Table 5 shows the comparison of the experimental and calculated values of permeability and the result is plotted in Fig. 3. The major steric and electrostatic features of the 3D QSAR derived from CoMFA study are illustrated in Fig. 2, as 3D transparent surfaces. Steric contours indicate the location of sterically less bulky group that enhances permeability in yellow color in this series of compounds. The green color region

Table 5
Actual and predicted permeability (IS ratio) of NSAIDs in training set

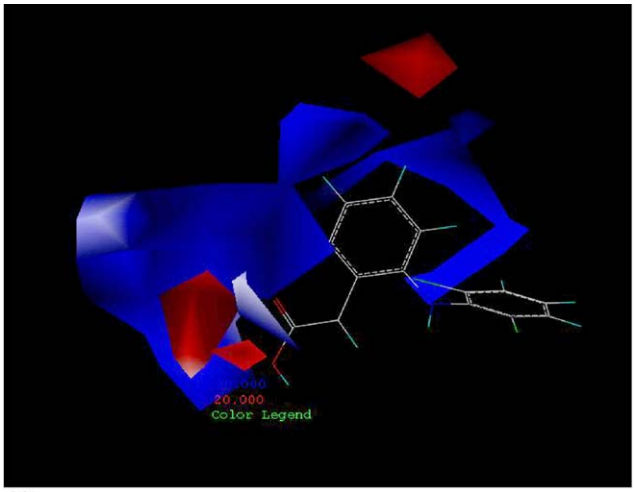
	Permeability	CoMFA	ClogP	CMR	Prediction	Residue
Aceclofenac	1.20	124	4.74	8.78	1.20	0.00
Amfenac sodium	0.98	102	2.12	7.10	1.00	−0.02
Diclofenac sodium	1.72	106	4.50	7.58	1.71	0.01
Fenoprofen Calcium	1.58	112	3.82	6.93	1.56	0.02
Fexofenadine Hydrochloride	0.07	226	1.96	14.89	0.08	−0.01
Ibuprofen	1.69	98	3.68	6.12	1.70	−0.01
Ibuproxam	0.93	106	2.61	6.49	0.96	−0.03
Indobufen	1.38	122	3.27	8.40	1.36	0.02
Indomethacin	1.34	140	4.18	9.51	1.34	0.00
Loxoprofen sodium	1.32	106	1.97	6.91	1.33	−0.01
Naproxen sodium	1.42	114	2.82	6.57	1.40	0.02
Olopatadine Hydrochloride	0.30	142	1.09	9.96	0.26	0.04
Pranoprofen	1.28	104	2.49	7.01	1.30	−0.02
Tiaprofenic acid	1.31	106	2.54	7.09	1.30	0.01
Zaltoprofen	1.37	116	3.50	8.37	1.40	−0.03

Table 6
Actual and predicted permeability (IS ratio) of NSAIDs in test set

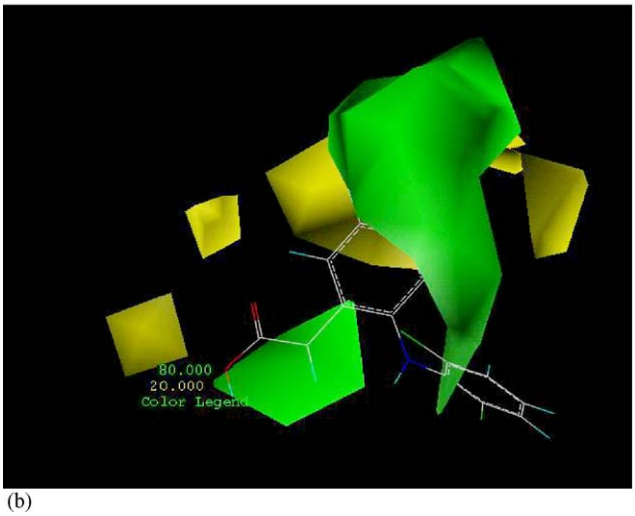
	Permeability	CoMFA	ClogP	CMR	Prediction	Residue
Alminoprofen	1.43	114	2.39	6.47	1.11	0.32
Flurbiprofen	0.92	98	3.75	6.8	1.65	−0.73
Ketorolac tromethamine	0.26	102	1.62	7.00	0.88	−0.62
Ketoprofen	1.48	112	2.76	7.28	1.24	0.24
Sulindac	0.76	138	3.02	9.71	1.02	−0.26

indicates the sterically bulky group enhanced the permeability. Electrostatic contours indicate the location of electropositive character on phenyl substituent in blue that enhances permeability. The red color in the region of amino group shows that the electronegative group enhanced the permeability.

The CoMFA analysis of the test set composed of five NSAIDs is reported in Table 6. Flurbiprofen gave a largest residue (−0.73), which might be resulted from little consideration of characteristic nature of fluorine in training set. Because the conformation of drug molecule plays an important role in CoMFA analysis, the differences present in the conformational factors between flurbiprofen and other drugs might have resulted in a fairly significant difference in their predicted permeability. Structurally, flurbiprofen has the moiety in which two aromatic rings are directly connected each other, whereas the corresponding part of the other analogous compounds possesses one atom linker between those two aromatic rings. The structural difference in this extent would certainly give caused enough conformational change. Still one has to admit that, in CoMFA analysis, less accurate predictability is obtained when conformation of one molecule is different from compounds in training set.



(a)



(b)

Fig. 2. CoMFA contour plots of NSAIDs (a) electrostatic model contours and (b) steric model contours.

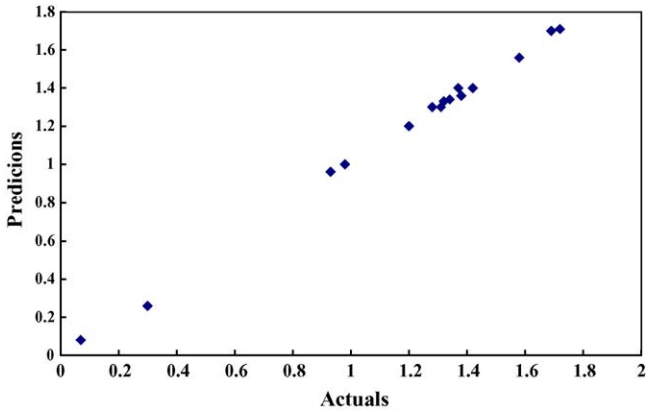


Fig. 3. Experimental vs. predicted permeability of training set.

4. Conclusion

The CoMFA method was successfully applied to NSAIDs, and prediction on the extent of permeability of drugs containing a carboxylic acid moiety would be possible from their 3D molecular structure. We expected that the computational approach using the CoMFA could be used in predicting in vivo the pharmacokinetic performance of the drugs.

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