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Prediction of Permeability Coefficients of Compounds Through Caco-2 Cell Monolayer Using Artificial Neural Network Analysis

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ABSTRACT Artificial neural network (ANN) analysis was used to predict the permeability of selected compounds through Caco-2 cell monolayers. Previously reported models, which were shown to be useful in the prediction of permeability values, use many structural parameters. More complex equations have also been proposed using both linear and non-linear relationships, including ANN analysis and various structural parameters. But proposed models still need to be developed using different neuron patterns for more precise predictions and a better understanding of which factors affect the permeation. To develop a simple and useful model or method for easy prediction is also a general need. Permeability coefficients (log k_p) were obtained from various literature sources. Some structural parameters were calculated using computer programs. Multiple linear regression analysis (MLRA) was used to predict Caco-2 cell permeability for the set of 50 compounds (r^2=0.403). A successful ANN model was developed, and the ANN produced log *kp* values that correlated well with the experimental ones (r^2=0.952). The permeability of a compound, famotidine, which has not previously been studied, through the Caco-2 cell monolayer was investigated, and its permeability coefficient determined. It was then possible to compare the experimental data with that predicted using the trained ANN with previously determined Caco-2 cell permeability values and structural parameters of compounds. The model was also tested using literature values. The developed and described ANN model in this publication does not require any experimental parameters; it could potentially provide useful and precise prediction of permeability for new drugs or other penetrants.

KEYWORDS Artificial neural network, Prediction of permeability, Caco-2 cell, Molecular modeling

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INTRODUCTION

Oral bioavailability of the molecule under investigation in drug discovery or development of the drug formulation is a highly desirable property. In the discovery or formulation development process, the second step is to achieve enough cellular permeability. Caco-2 cell experiments provide successful results, but to perform these kinds of experiments requires expensive equipment and adequate laboratory conditions. Computational modeling for prediction of drug permeability through intestinal cells provides an inexpensive and fast way to assess the potential for a molecule before another synthesis or further formulation developments, and enables a precise decision and prioritization of a molecule for further studies (Egan & Lauri, 2002). Therefore, prediction equations and computational analysis of passive drug absorption and intestinal permeability have recently attracted considerable attention (Clark, 2001; Clark & Pickett, 2000; Krämer, 1999; Stenberg et al., 2000; Waterbeemd, 2000; Waterbeemd et al., 2001). These attempts became more attractive, easy, and of lower cost following the development of faster and more effective computer hardware and software.

The best known model was proposed by Lipinski et al. (1997) for the prediction of passive intestinal absorption with five consecutive models in 1997. The molecular weight (MW) and the number of hydrogen bond donors and acceptors of the penetrant molecules were considered for the prediction in this model. A similar analysis was performed by Ghose et al. (1999) using a larger data base and log Koct, MW, and molar refractivity. Some recent models consider dynamic polar surface area of the penetrant (Palm et al., 1997), using it as a describing effect of hydrogen bonding on the permeability. A strong and inverse, sigmoidal relationship was found for 20 molecules (r^2=0.940). Lipophilicity was weakly correlated alone with fraction absorbed (r^2=0.340), but the dynamic polar surface area for six β-adrenoreceptor antagonists was found to be highly correlated with their Caco-2 cell permeabilities (Palm et al., 1996). When the dynamic surface area of the penetrant and the other molecular descriptors such as atom type counts were considered for 12 oligopeptide derivatives, moderate predictive ability for Caco-2 cell permeability was achieved (Stenberg et al., 2001). Waterbeemed et al. modeled the Caco-2 cell permeability for 17 compounds using a series of linear models containing hydrogen bonding, hydrogen bond acceptor, donor ability, and MW terms, and produced the best fit with an r^2 value of 0.883 (Waterbeemd et al., 1996). A neural network with generic algorithm descriptor selection was also used to predict human intestinal absorption for selected compounds (Wessel et al., 1998), but ANN approaches have not been applied to Caco-2 cell experiments with our proposed neuron topology and descriptors.

This publication builds on the use of dipole moments, polarizability log Koct, and MW data to predict Caco-2 cell monolayer permeability and uses these factors as inputs into an ANN propagated back towards the input of the network; results were compared with classical MLRA method. To train the ANN model, a series of compounds were selected and their structural parameters and permeabilities through Caco-2 cells were obtained from literature sources. To test the ANN model, famotidine was selected and the permeability value was determined experimentally, using Caco-2 diffusion cells. Seven compounds were also selected from the literature to test a new ANN model. Their permeability value was also predicted using the new ANN model. The predicted and experimental results and the models were then compared.

MATERIALS AND METHODS

Materials

Famotidine was kindly provided by İlsan-İltasi, Ltd., Ankara, Turkey. Transwell (24mm diameter, 0.4mm pore size polycarbonate membrane filter) was purchased from Corning and Costar, New York, USA. Caco-2 cells were obtained from American Type Culture Collection, Rockville, MD, USA. Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), and penicillin-streptomycin solution were purchased from Gibco Laboratory, Paisley, UK. Methanol and potassium dihydrogen phosphate (HPLC grade) were purchased from Sigma. All other chemicals and solvents were of analytical grade except for those used in the high performance liquid chromatography (HPLC) procedure, which were of HPLC grade.

Methods

Diffusion/Permeation Studies

Caco-2 cell monolayers were first prepared by cultivating Caco-2 cells on polycarbonate membrane

filter for three weeks. The pore size of the membrane was 0.4 μm and the diameter of the membrane was 24 mm. Radial diffusion cells were used for the determination of drug absorption. Caco-2 cell monolayer on the membrane filter was fixed between the donor and receptor compartment. Famotidine solution (5 mg/ml) or famotidine microspheres (20 mg) in DMEM were applied to the donor chamber (4 ml), and receptor compartment (4 ml) was also filled with DMEM solution. Transport studies across Caco-2 monolayers were performed in a carbogen atmosphere (5% CO_2 and 95% O_2) at 37°C. The samples were collected from the donor compartment during 30 hours and analyzed by HPLC. Permeability values were calculated by dividing the flux values (μg/h) with the concentrations of donor phases (μg/ml).

HPLC Analysis

HPLC analyses were conducted with a pump, injection port, and 20 mL Rheo-dyne, UV detector, integrator (Hewlett-Packard series 1050, Waldbronn, Germany).

Software

The ANN Program Pythia

The Neural Network Designer version 1.0 was used (Runtime Software LLC; Carson City, NV). Pythia is a computer program for the development and design of neural networks. Pythia uses back propagation networks to detect hidden relationships in a set of patterns. The network parameters (weights) are initially set to random values. During the training phase, the actual output of the network is compared with the desired output and the error propagated back towards the input of the network. A special feature of the program is the evolutionary optimizer. This module of the software automatically generates suitable networks for a given training data set. The best network model was developed using the optimizer and the ANN that achieved the lowest square deviations. Figure 1 shows the details of the developed ANN model.

A neural network has two phases, commonly referred to as the "training phase" and the "reproduction phase." During the training phase, sample data containing both (inputs and desired outputs) are processed to optimize the network's output, meaning to minimize the deviation (OutputData OutputNet).

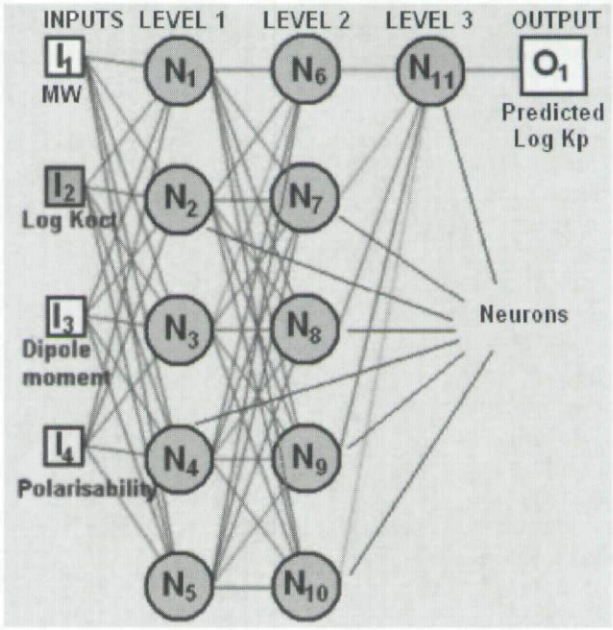

FIGURE 1 The Developed ANN Model for Predicting Caco-2 Cell Permeability.

OutputData is the output value in the training data; OutputNet is the output value provided by reproducing the input data with the network. During the "reproduction phase," the network's parameters are no longer changed and the network is used for the reproduction of input data in order to "predict" suitable output data.

The computer program trained itself (program parameters set as follows: trained until: repetition 1000,000, deviation^2 <0.00380, time passed 300; use learn rate 0.5, automatically adjust; finally: reproduce pattern set and show results in native form). Other parameters such as transfer function, etc. were selected as default. The program trained itself until the square deviations were less than 0.0038 (0.003753 is the lowest value that the program could achieve). Figure 1 shows a typical backpropagation network. In backpropagation networks, each neuron has one output and as many inputs as neurons in the previous level. Each network input is connected to every neuron in the first level. Each neuron output is connected to every neuron in the next level. The network's output is the output of the last level's neurons. The network is processed from the left to the right.

The ANN has one input layer, one or more hidden layers, and the output layer. Each layer is composed of a number of units. The units in neighboring layers are fully interconnected with links. The strengths of the

TABLE 1 Caco-2 Cell Permeabilities and Structural Descriptors of Compounds

Compound[Lit.]	MW	log Koct	Dipole moment (Debye)	Polarizability	log kp (cm/s)[a]
Acebutolol[h]	334,46	2,86	6,28	173,90	−6,29
Acyclovir[h]	224,22	−2,91	5,96	117,70	−6,6
Alprenolol[h]	249,35	2,88	1,99	131,10	−4,6
Aminopyrine[h]	231,30	0,76	4,38	133,60	−4,44
Artemisinin[e]	282,33	1,97	6,14	124,30	−4,52
Atenolol[a]	266,34	0,10	4,13	133,90	−6,7
Azithromycin[g]	748,99	2,37	4,86	330,00	−5,98
Benzyl penicillin[g]	334,39	1,67	4,51	157,20	−5,71
Betaxolol[d]	307,43	2,66	1,14	190,80	−4,31
Caffeine[g]	194,19	−0,08	3,78	100,70	−4,51
Chlorothiazide[g]	295,71	−0,63	4,71	140,50	−6,72
Chlorpromazine[h]	318,86	5,36	2,67	180,20	−4,7
Cimetidine[f]	252,34	0,36	9,74	141,30	−6,3
Corticosterone[h]	346,46	1,76	1,48	161,50	−4,67
Desipramine[g]	266,39	3,97	2,31	157,60	−4,67
Dexamethasone[h]	392,46	2,06	1,96	175,20	−4,91
Diazepam[h]	284,74	2,96	3,05	161,30	−4,48
Dopamine[h]	153,18	0,12	2,16	78,90	−5,03
Doxorubicin[g]	543,52	2,12	4,02	259,50	−6,8
Erythromycin[g]	733,93	2,49	9,47	314,30	−5,43
Flucanozole[g]	306,27	0,31	1,95	138,60	−4,53
Griseofulvin[h]	352,77	2,36	3,16	172,30	−4,44
Hydrochlorothiazide[h]	297,73	−0,07	9,15	136,80	−6,29
Hydrocortisone[h]	362,46	1,43	2,85	164,90	−4,85
Imipramine[g]	280,41	4,47	1,25	164,60	−4,85
Indomethacin[h]	357,79	3,10	1,45	196,00	−4,69
Labetalol[h]	328,41	2,87	5,03	176,00	−5,03
Mannitol[a]	182,17	−4,96	4,48	62,80	−6,75
Meloxicam[h]	351,39	0,98	4,58	199,40	−4,71
Methanol[c]	32,04	−0,72	1,59	10,90	−3,88
Naloxone[g]	327,38	2,23	4,78	162,70	−4,55
Nicotine[h]	162,23	0,72	3,02	86,00	−4,71
Phenytoin[h]	252,27	2,52	3,42	139,00	−4,57
Pindolol[h]	248,32	1,97	1,60	132,60	−4,78
Pirenzepine[h]	351,41	−0,08	5,62	195,10	−6,36
Piroxicam[h]	331,34	−0,02	4,03	190,00	−4,45
Practolol[b]	266,34	0,76	4,49	140,40	−6,05
Prazocin[g]	383,40	−2,06	3,36	226,90	−4,36
Progesterone[h]	314,47	4,04	2,67	148,20	−4,63
Quinidine[g]	324,42	3,44	1,96	180,60	−4,69
Salicylic acid[a]	138,12	2,06	1,17	67,50	−4,92
Scopolamine[h]	303,36	1,34	1,25	140,60	−4,93
Sucrose[h]	342,29	−3,65	3,80	136,00	−5,77
Sulfasalazine[a]	398,39	3,18	5,64	240,90	−6,89
Terbutaline[a]	225,29	0,48	3,96	109,90	−6,42
Testosterone[h]	228,43	3,48	4,14	134,30	−4,6
Timolol[h]	316,42	−0,15	1,53	153,40	−4,89
Urea[h]	60,06	−1,58	3,18	24,20	−5,34
Valproic acid[g]	144,21	2,72	1,80	76,20	−4,32
Warfarin[h]	308,33	3,47	1,87	167,90	−4,68

[a]Artursson 1990.
[b]Artursson and Karlsson 1991.
[c]Rubas et al. 1993.
[d]Hovgaard et al. 1995.
[e]Augustijns et al. 1996.
[f]Collett et al. 1996.
[g]Yee 1997.
[h]Yazdanian et al. 1998.

connections between two units are called "weights." In each hidden layer and output layer, the processing unit sums its input from the previous layer and then applies the function to compute its output according to the following Eqs. 1 and 2:

$$Y_i = \sum w_{ij} \cdot x_i \quad (1)$$

$$F_{(i)} = 1/[1 + \exp(\alpha - y_j)] \quad (2)$$

where w_{ij} is the weight of the connection between unit i in the current layer and j in the following layer, and x_i is the output value from the previous layer. The parameter relating to the shape of the function is α.

Calculation of Log Koct Values

Log Koct values were calculated using the computer program Medchem (Biobyte, Claremont, CA) and ACD-Log P (Advanced Chemistry Development Inc., Ontario, Canada).

Calculation of Dipole Moment and Polarizability Values

Molecular modeling for program NEMESIS V1.0 package (Oxford Molecular, Oxford, UK) was used to calculate dipole moment and polarizability values of compounds. Conformation analysis using a step size of 500 was used to find the approximate energy minimum conformation, followed by optimization to identify the minimum energy conformation. This two-step approach reduces the risk of finding a local minimum energy form. The program calculates dipole moment and polarizability of the molecule on the basis of inductive effects in saturated molecules and Huckel molecular orbital calculations in pi systems; values were also checked from literature sources. These calculations were performed using NEMESIS without altering any parameter of the program (default values were used) for all chemicals (for selected chemicals from literature and for famotidine).

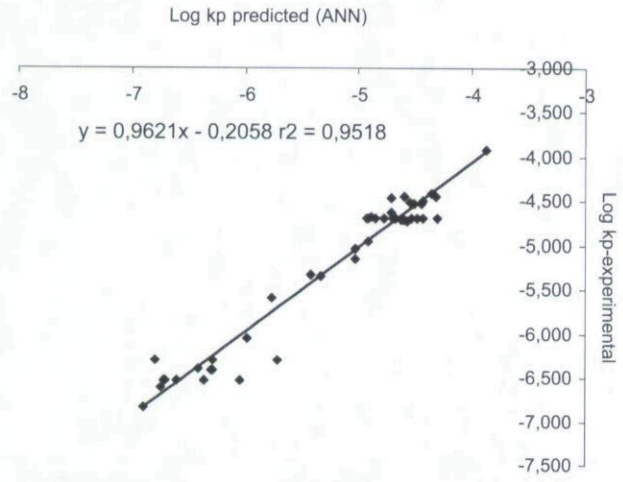

FIGURE 2 The Relationship Between the Theoretically Calculated Log kp Values Using the ANN Model and Experimental Results.

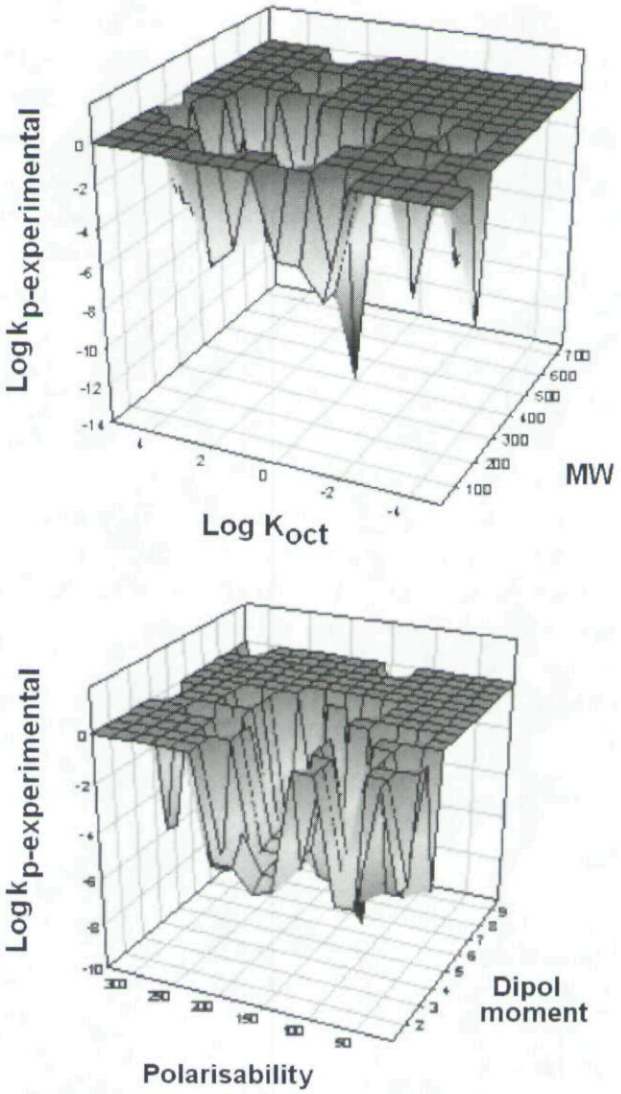

FIGURE 3 The Functional Dependence Surfaces of the Descriptors Used for ANN Analysis.

RESULTS AND DISCUSSION

The chosen data from literature sources are shown in Table 1. Pythia was used to construct an appropriate ANN. The optimizer function of the program was used; MW, log Koct, dipole moment, and polarizability values were used as inputs, and the literature log *kp* values as the output. The most

successful ANN created contained five neurons at levels 1 and 2, and one neuron at level 3 (model described as ANN-551). Other configurations were also studied but none gave superior results. The optimization of the model (number of hidden layers and hidden units) was performed automatically and the lowest value of square deviation was obtained with this model (for instance, the square deviation of model ANN-551 was 0.0038, compared to typical values of around 0.03 for the other tested models such as ANN-221, ANN-441, ANN-31, ANN-321, and ANN-51). Therefore, the model ANN-551 was used for further calculations.

It is also important to note that an ANN model was attempted using inputs of log Koct and MW, but it was not possible to obtain an adequate ANN from these two simple inputs for our data set.

The MLRA analysis has been used for prediction and comparison in many studies. In the MLRA analysis, the regression equation was obtained as follows:

$$Log\ k_p = -4.35 - 0.00091\ MW + 0.129 Log\ K_{OCT} - 0.185\ Dipole\ m - 0.00041\ Polarizability$$

The r^2 value was calculated as 0.403. This coefficient was highly improved (r^2=0.952) when ANN modeling was used.

Figure 2 shows the relationship between the theoretically calculated log *kp* values and experimental results using the ANN model.

The interpretation of effects of each descriptor was difficult because the model was multivariate and nonlinear. However, some insight into the degree of nonlinear behavior of descriptors has been assessed with

TABLE 2 The Statistical Test Results of the MLRA and ANN Models

MLRA

Regression statistics	
Multiple R	0,634627
R Square	0,402752
Adjusted R square	0,390309
Standard error	0,661176
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	14,15005	14,15005	32,36862	7,45039E-07
Residual	48	20,98335	0,437153		
Total	49	35,1334			

ANN-551

Regression statistics	
Multiple R	0,975623
R Square	0,951840
Adjusted R square	0,950837
Standard error	0,187751
Observations	50

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	33,44139	33,44139	948,6847	2,84E-33
Residual	48	1,692013	0,03525		
Total	49	35,1334			

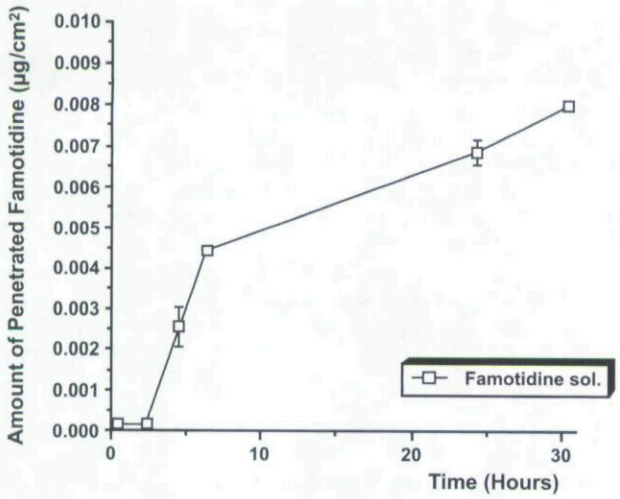

FIGURE 4 Permeability Properties of Famotidine Through Caco-2 Cell Monolayers.

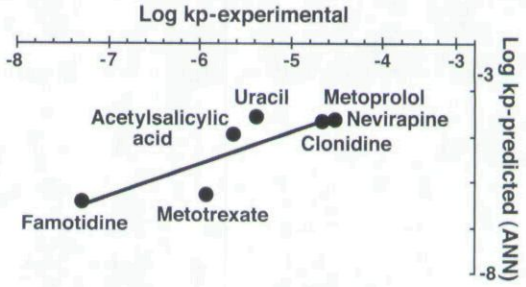

FIGURE 5 The Relationship Between Experimental and ANN-551 Produced Results.

a functional dependence to understand relationships. The value of input variables was varied through its range, whereas others were held constant. The network output was plotted against two input descriptors to generate a functional dependence surface. This gives an idea and indication of how the network output alters in response to two selected input variables. Figure 3 shows the functional dependence surfaces of these descriptors. Non-linearity of inputs is clearly evident, suggesting a very complex relationship.

The r^2 value may not be the most suitable parameter for the comparison because the calculation methods and equations are different. In fact, it could be useful to use another parameter for testing the model, but ANN modeling uses the previous data over and over to produce the final output value. The technique and the equations are complex; therefore, another parameter such as Akaike Information Criterion (AIC) (Yamokawa et al., 1978) could not be used for comparison. Since the final output values can be used for the comparison, the regression analysis and the analysis of variance (ANOVA) test were performed. Table 2 shows the statistical test results of ANN modeling. When all results were considered, the ANN seemed to be the most suitable model.

The next stage of the evaluation of the ANN was to test the trained model. The permeability coefficient of famotidine through Caco-2 cell monolayer was determined experimentally. Figure 4 shows the permeability properties of famotidine.

The log *kp* value of famotidine was then calculated. Some other compounds were also selected from the literature that have a range of physicochemical properties; their permeability coefficients were already determined using standard techniques (their permeability data of compounds were obtained from the literature to make a better comparison). The results are given in Table 3 [the calculated values of log *kp* (from the ANN) are also given in the Table] and Fig. 5. The quality of the data has a very important role in modeling; this was particularly important in neural computing (Murtoniemi et al., 1994).

Some outliers appear in all prediction equations, and this may be because of the unreliable log *kp*

TABLE 3 Experimental and Literature Values to Developed ANN Model

Compound	Source	MW	Log Koct	Dipole moment	Polarizability	Log kp-exp	Log kp-ANN	%Recovery
Acetyl-salicylic acid	Lit.	180,16	1,19	1,45	85,1	−5,62	−4,965	88,3451957
Clonidine	Lit	230,1	1,54	0,62	111,4	−4,66	−4,7	100,858369
Famotidine	Experimental	337,43	−1,2	9,22	109	−7,274	−6,424	88,314545
Metoprolol	Lit.	267,37	1,76	0,64	136,4	−4,63	−4,6899	101,293737
Metotrexate	Lit.	454,44	−0,09	5,35	264,7	−5,92	−6,3018	106,449324
Nevirapine	Lit.	266,3	−0,31	2,6	157,2	−4,52	−4,6748	103,424779
Uracil	Lit	112,09	−0,71	4,19	54,6	−5,37	−4,5975	85,6145251

$$\% \text{ Recovery} = \frac{\text{Log } kp_{ANN-551} * 100}{\text{Log}kp_{exp}}$$

values. Their log *kp* values may need to be redetermined, but within the range of the compounds tested, ANN modeling gave closer results to experimental results. This may indicate that the permeability of compounds through Caco-2 cell monolayer has a very complex mechanism and that the proposed model is easy to use and gives accurate results.

CONCLUSION

An ANN (551) model was used for predicting Caco-2 cell permeability for a data set of 50 selected compounds for training. The r^2 value was calculated as 0.952 when the ANN predicted and experimental log *kp* values from the literature sources were correlated. A complex relationship exists between structure of the penetrant and permeability. Finally, six chosen compound's descriptors from literature sources and experimentally determined values (for famotidine) were then used to test the developed ANN model. Unlike many previously determined models, the ANN model developed and described herein does not require any experimental attempt; it could potentially provide a useful and precise prediction of intestinal permeability for new chemical entities in terms of both therapy and toxicity. It could reduce the need for performing permeability experiments using expensive material and equipment or other model membranes. The results indicate that the simple predictors investigated to date can still be capable of predicting intestinal cell permeability.

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