Current Literature Oct. 28, 2017

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Predicting a Drug's Membrane Permeability: A Computational Model Validated With in Vitro Permeability Assay Data

Bennion BJ, Be NA, McNerney MW, Lao V, Carlson EM, Valdez CA, Malfatti MA, Enright HA, Nguyen TH, Lightstone FC, Carpenter TS.

J. Phys. Chem. B 2017, 121, 5228-5237

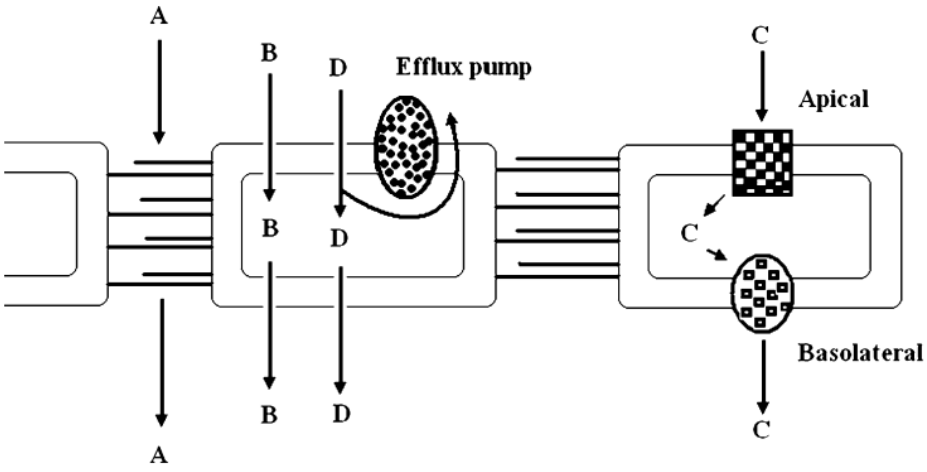
The transfer of drugs through cell membrane

- Passive diffusion

1. A process by which a compound moves down its concentration gradient without a membrane actively participating.
2. The rate of passive diffusion across of membrane is proportional to the partition coefficient of the compound, the diffusion coefficient through the membrane, and the compound's concentration gradient across the membrane.

- Active transport

1. A process by which a transport protein using energy (e.g. APT hydrolysis) to shuttle a molecule across the membrane against concentration gradient.
2. Some hydrophilic drugs could be transported through carrier-facilitated transport protein.
3. Efflux pumps (e.g. P-glycoprotein).



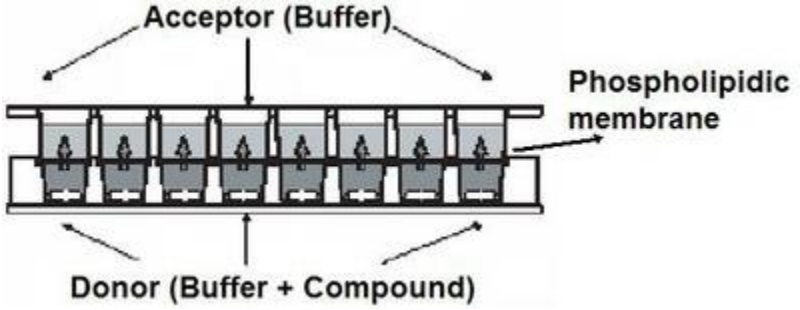
A: paracellular transport
B: transcellular transport
C: transporter-facilitated pathway
D: transport-restriced pathway

Pharm Res 2011 28, 962-977

in vitro models for predicting membrane permeability

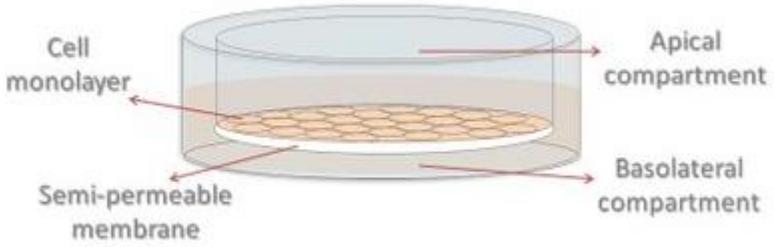
- PAMPA assay

1. Models transcellular (passive) absorption.
2. Two compartments are separated by one artificial membrane filter. 96-well plate permits for high-throughput compound screening.



- Caco-2 assay

1. Human colon carcinoma cell line spontaneously grows as a monolayer.
2. All mechanisms are modelled.



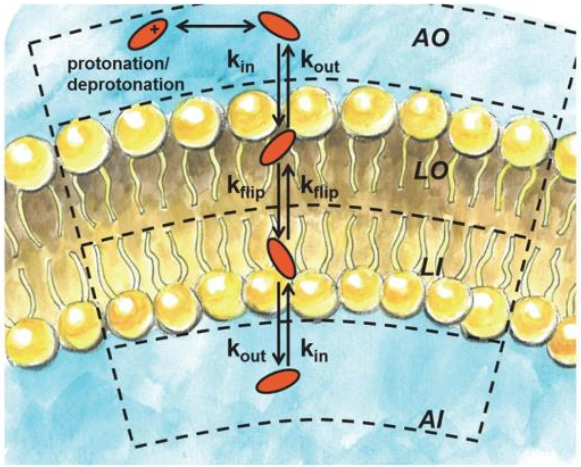
in silico models for predicting membrane permeability

- Knowledge-based QSPR model

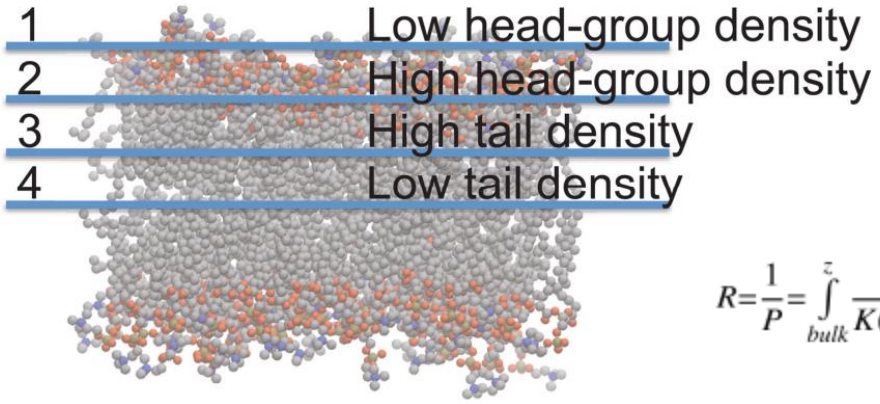
1. Mathematic description of the statistical relationship between experimental permeability measurements of training compounds and their chemical structure and physiochemical properties (descriptor).
2. The most critical parameter in QSPR model is Lipophilicity (LogP).

   $LogP_{oct}=5.83(\pm0.53)\cdot V/100-0.74(\pm0.31)\cdot\pi^*-3.51(\pm0.38)\cdot\beta-0.15(\pm0.23)\cdot\alpha-0.02(\pm0.34)$.
3. Success rate extremely depends on the compounds in the training set, thus transferability is limited.

- MD-based inhomogeneous solubility-diffusion model



Three-step process



$$R=\frac{1}{P}=\int_{bulk}^{z}\frac{dz'}{K(z')D(z')}$$

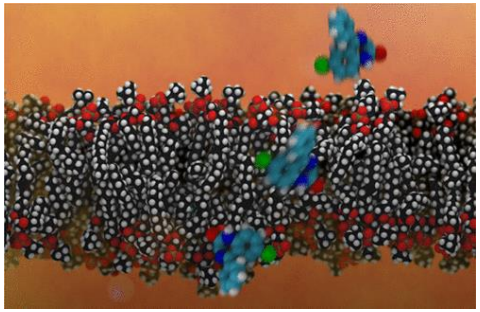
Anisotropic nature of membrane

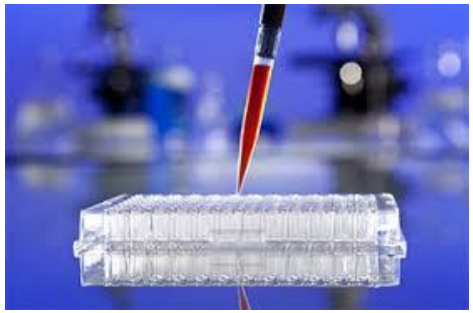
Pharm Res 2011,28, 962-977
Chem Bio Drug Des. 2013,81,61-71

Overview of this work

- Exploring the effectiveness of the combined use of umbrella sampling molecular dynamics simulation and PAMPA assay in predicting membrane permeability.

1. Calibration of MD model with PAMPA assay on training compounds.



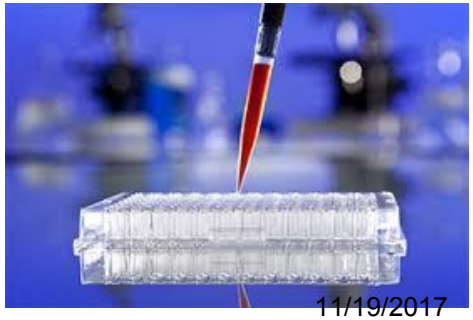


Permeability model

2. Assessing MD model against PAMPA assay on target compounds.

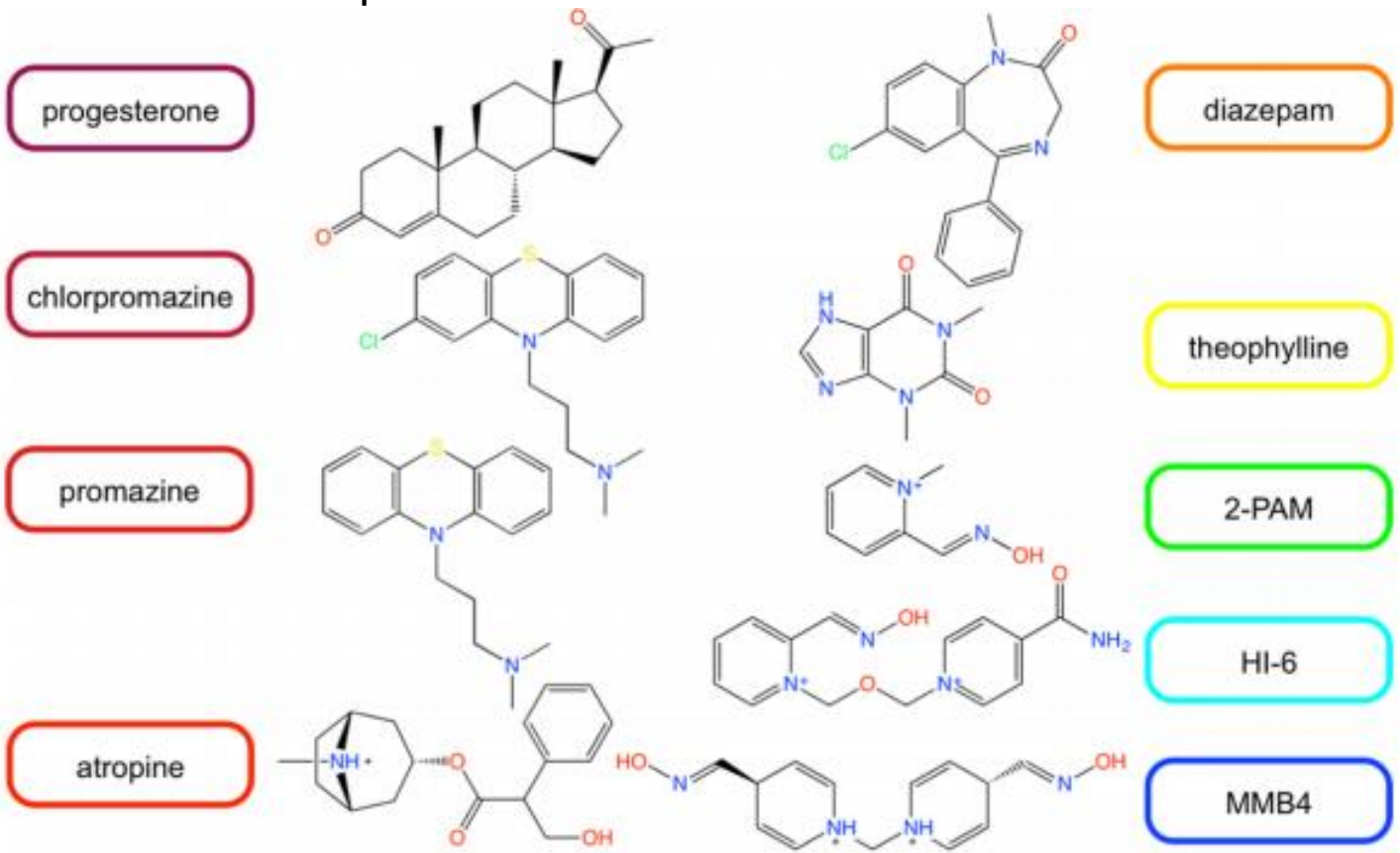
Permeability model

VS



Studied compounds

- Calibration compounds



- 18 structurally related testing compounds: LLNL1-LLNL18

Experimental procedures

- MD simulation

1. Each system contains 5124 water molecules and 72 DOPC molecules and a small compound.
2. Each system was coupled with 100 individual simulations, where compound was constrained at different z-axis position. Each simulation was run for ~50 ns.
3. The potential of mean force (PMF) profile and position-dependent diffusion of each compound was calculated using the last 30-ns MD trajectory.

potential of mean force (pmf) $w(z) = -\int_{-l}^{z} \langle F_z(z') \rangle_{z'} dz'$ position-dependent diffusion $D(\langle Z \rangle) = \frac{var(z)}{\int_0^\infty C_{ZZ}(t) dt}$

position-dependent resistance $R(z) = \frac{\exp(\beta \Delta G(z))}{D(z)}$ overall permeation coefficient $P_{eff} = \frac{1}{\int_{-z_b}^{z_b} R(z) dz}$

- PAMPA assay

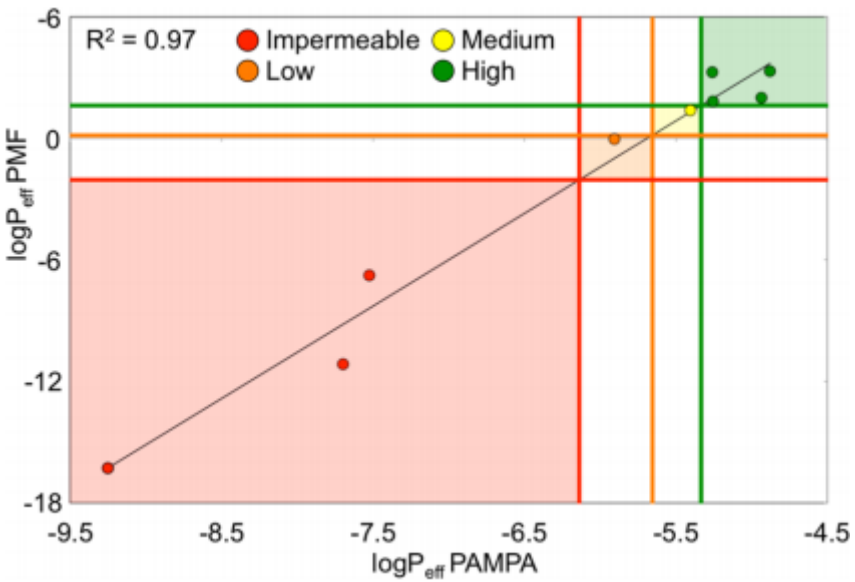
1. The Gentest Precoated PAMPA Plate System (Corning Discovery Labware) was applied.
2. Donor well and receiver well were separated by a filter plate precoated with phospholipid-oil-phospholipid trilayer consisting of DOPC phospholipids.
3. Compounds were incubated for 5 h at 25C° and then quantified using the Acquity ultra performance liquid chromatography (UPLC) system.

$$P_e = \frac{-\ln[1 - C_A(t)/C_{eq}]}{A \times (1/V_D + 1/V_A) \times t} \qquad C_{eq} = [C_D(t) \times V_D + C_A(t) \times V_A]/(V_D + V_A)$$

Results

- Assessment of MD-based prediction accuracy

Compound	$LogP_{eff}$ PAMPA	$LogP_{eff}$ PMF	Permeation category	
			From PAMPA	From PMF
MMB4	-9.25	-16.29	Impermeable	Impermeable
HI-6	-7.69	-11.16	Impermeable	Impermeable
2-PAM	-7.52	-6.77	Impermeable	Impermeable
Theophylline	-5.91	-0.02	Low	Low
Diazepam	-5.40	1.37	Medium	Medium
Chlorpromazine	-5.26	3.26	High	High
Atropine	-5.26	1.82	High	High
Progesterone	-4.94	1.99	High	High
Promazine	-4.88	3.31	High	High



Linear correlation between P_{eff}^{PMF} and P_{eff}^{PAMPA} is extremely good (R^2=0.97) among calibration set

In Vitro permeability cutoff:

$LogP_{eff}^{PAMPA} < -6.14$: impermeable

-6.14<$LogP_{eff}^{PAMPA}$<-5.66: low permeability

-5.66<$LogP_{eff}^{PAMPA}$<-5.33:medium permeability

-5.33<$LogP_{eff}^{PAMPA}$: high permeability

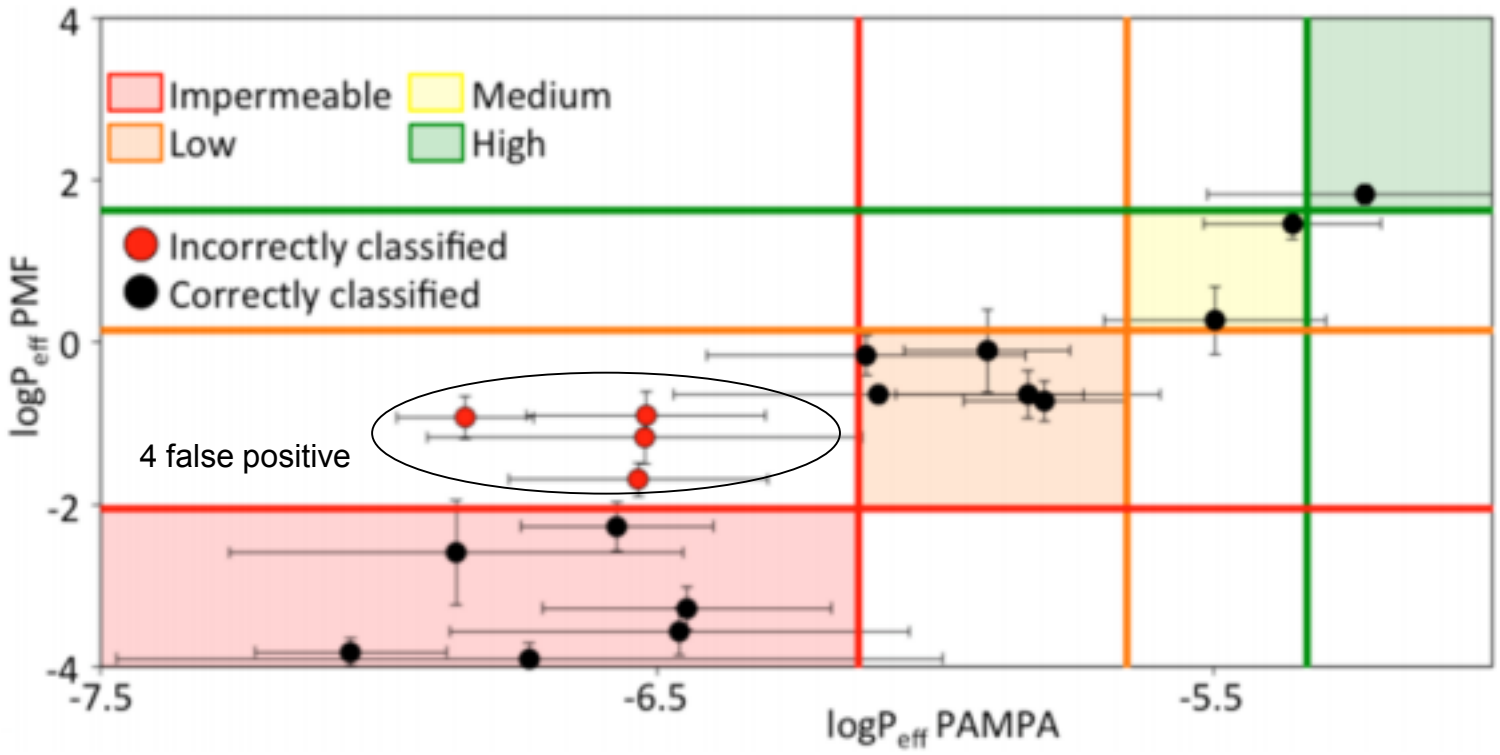
MD permeability cutoff:

$LogP_{eff}^{PMF} \leq -2.05$: impermeable

-2.05<$LogP_{eff}^{PAMPA}$<-0.15: low permeability

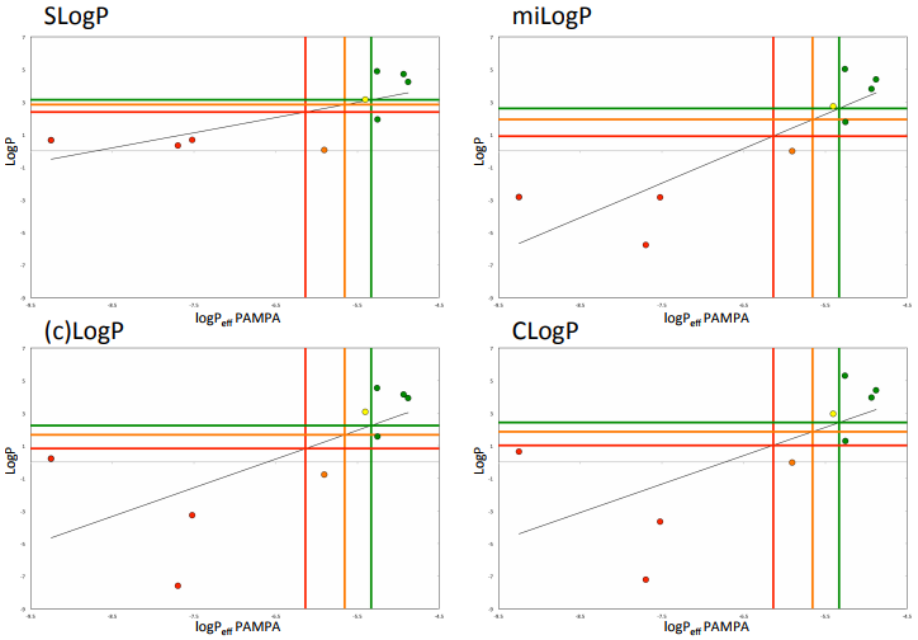
0.15<$LogP_{eff}^{PAMPA}$<1.62:medium permeability

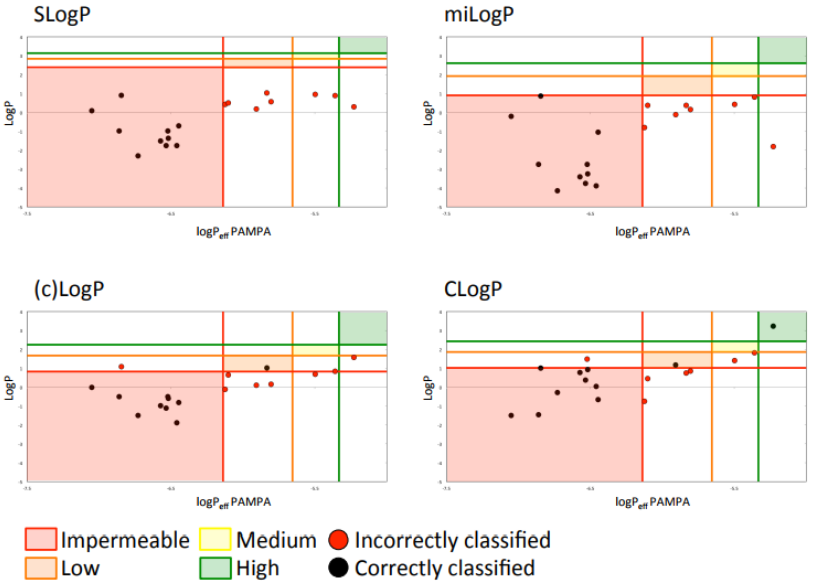
1.62<$LogP_{eff}^{PAMPA}$: high permeability



MD-based permeability prediction successful rate on testing set : 78% (14/18)

- Comparison with LogP prediction





method	calibration compound correlation (R^2)	LLNL1–LLNL18 compounds correct (%)	false positives	false negatives	"permeable" compounds correct
PMF	0.97	78	4	0	8/8
SLogP	0.53	56	0	8	0/8
miLogP	0.75	56	0	8	0/8
(c)LogP	0.45	56	1	7	1/8
CLogP	0.44	61	1	6	2/8

MD prediction outperforms LogP prediction

Conclusion

- MD-based computational model of membrane permeability can predict the PAMPA-defined permeability category of a compound with greater accuracy than LogP-based model.

- MD-based permeability prediction could be used as an evaluation tool to rule out impermeable drug candidates with a low false-negative rate.