Predicting Gastrointestinal Absorption of Prodrugs and their Drugs with the ANDROMEDA by Prosilico Software

Urban Fagerholm[1,*], Sven Hellberg[1], Jonathan Alvarsson[2] and Ola Spjuth[1,2]

[1]Prosilico AB, Lännavägen 7, SE-141 45 Huddinge, Sweden.

[2]Department of Pharmaceutical Biosciences and Science for Life Laboratory, Uppsala University, Box 591, SE-751 24 Uppsala, Sweden.

*Corresponding author: urban.fagerholm@prosilico.com

ABSTRACT

Introduction Some prodrugs are developed in order to improve gastrointestinal absorption properties such as permeability and solubility/dissolution. Prediction of the uptake of prodrugs and their drugs is challening for reasons including gastrointestinal hydrolysis and active transport.

Objective and Methodology The objective was to use the ANDROMEDA software by Prosilico to predict absorption characteristics - passive fraction absorbed ($f_{a,passive}$), dose-adjusted dissolution potential (f_{diss}) and total f_a (f_a) - of prodrugs and their drugs (including drugs and their active metabolites), and to evaluate how they differ between prodrugs and drugs and the predictive accuracy of the software.

Results 70 prodrug-drug pairs were found and selected for the study. The mean predicted $f_{a,passive}$ and f_{diss} for the prodrugs were 0.74 and 0.94, respectively. Corresponding estimates for the drugs were 0.72 and 0.98, respectively. For non-hydrolyzed prodrugs, the median relative and absolute prediction errors for f_a were 1.17-fold and 0.08, respectively. Corresponding values for drugs were 1.11-fold and 0.07, respectively. The correlation between predicted and observed f_a for non-hydrolyzed ester prodrugs and drugs combined (predictive accuracy) was 0.6.

Conclusion Prodrugs and drugs had similar average predicted $f_{a,passive}$ and f_{diss}, and most had or were predicted to have at least 50 % f_a. The f_a for about 1/3 of non-hydrolyzed prodrugs was higher than for corresponding drugs, showing successful prodrug design. Adequate prediction accuracy validates ANDROMEDA for prediction of prodrug and drug absorption in man.

INTRODUCTION

Prodrugs are developed in order to decrease local side effects and prolong patents of drugs and to improve their pharmacokinetic (PK) properties, for example, enhancing gastrointestinal absorption characteristics, such as permeability (P_e) and solubility/dissolution (and thereby, increasing the oral bioavailability), and prolonging the elimination half-life (Fagerholm and Björnsson 2005; Rautio et al. 2018).

It has been approximated that 10 % of all marketed drugs can be considered prodrugs (Rautio et al. 2018). Many of these are esters that undergo hydrolysis in the gastrointestinal tract, which makes predictions of human *in vivo* gastrointestinal uptake challenging/difficult. The absorption of prodrugs designed to utilize intestinal uptake transporters is also challenging to predict.

Prediction of the gastrointestinal uptake requires prediction of absorption parameters/determinants such as passive P_e-driven fraction absorbed ($f_{a,passive}$), active efflux by transporters such as MDR-1, BCRP and MRP2, active influx by tranporters such as PEPT1 (utilized by cephalosporins, penicillins, ACE-inhibitors, protease inhibitors, valacyclovir and levodopa) and *in vivo* dissolution potential (f_{diss}; the maximum fraction dissolved).

The ANDROMEDA software by Prosilico for prediction, simulation and optimization of human clinical PK (with a major domain for compounds with molecular weight (MW) from 100 to 700 g/mole) has been sucessfully applied and validated for prediction of absorption/biopharmaceutics of small compounds in man in many studies (Fagerholm et al. 2022a-g). The software predicts $f_{a,passive}$ (70 % predictions within ca ±7 %), f_{diss} (70 % predictions within ca ±12 %), efflux by MDR-1, BCRP and MRP2, and f_a.

The objective of this study was to use ANDROMEDA to predict absorption characteristics ($f_{a,passive}$, dose-adjusted f_{diss} and f_a) of prodrugs and their drugs (including drugs and their active metabolites), and to evaluate how they differ between prodrugs and drugs and the predictive accuracy of the software.

METHODOLOGY

ANDROMEDA by Prosilico was used to predict $f_{a,passive}$, dose-adjusted f_{diss} (50 mg oral dose is the default for predicted f_{diss}) and f_a for selected prodrugs and their drugs (or drugs and their active metabolites). Prodrug-drug pairs were searched for in the literature and the aim was to find at least 50 pairs of various kinds of prodrugs (including hydrolysis sensitive esters that are degraded to some extent before gastrointestinal uptake). No prodrug was actively excluded during this search process. Degradation in gastrointestinal fluids were not considered in the predictions. Observed/measured human *in vivo* f_a-data were taken from various sources (Pham-The et al. 2013; Varma et al. 2010; Skolnik et al. 2010; Lin et al. 2011; Thomas et al. 2005; Matsson et al. 2005, Sköld et al. 2006; and data proprietary of Prosilico). Normally, compounds and data from training sets are not included in validation of the software. In this study, however, training set compounds and data were allowed in order to get better estimations of differences in absorption for each prodrug-drug (or drug-active metabolite) pair.

RESULTS & DISCUSSION

70 prodrug-drug pairs (including many ester prodrugs and their drugs) were found and selected for the study (Table 1). Their MWs ranged from 130 to 744 g/mole. Data/compounds from training sets of prediction models were available in 32 cases (out of 140 cases), which is anticipated to have influenced the overall results to some minor extent.

The mean predicted $f_{a,passive}$ and f_{diss} for the prodrugs were 0.74 (range 0.02-1.00) and 0.94 (range 0.32-1.00), respectively (Figures 1 and 2). Corresponding estimates for the drugs were 0.72 (range 0.02-1.00) and 0.98 (range 0.65-1.00), respectively. 29 (41 %) and 7 (10 %) prodrugs had predicted $f_{a,passive} \geq 0.9$ and <0.25, respectively. For drugs, corresponding values were 31 (44 %) and 7 (10 %), respectively. 29 (41 %) and 7 (10 %) prodrugs had predicted $f_{a,passive} \geq 0.9$ and <0.25, respectively. For drugs, corresponding values were 31 (44 %) and 7 (10 %), respectively. 59 (84 %) and 6 (9 %) prodrugs had predicted $f_{diss} \geq 0.9$ and <0.7, respectively. For drugs, corresponding values were 66 (94 %) and 1 (1 %), respectively.

Thus, the majority of the selected prodrugs and drugs have good/adequate predicted passive P_e and f_{diss}.

For prodrugs, the median (mean) relative and absolute prediction errors for f_a were 1.17 (1.24)-fold and 0.08 (0.15) (n=17; non-hydrolyzed prodrugs with observed estimates only), respectively (Table 2). For 7 prodrugs with approximated f_a (for example, moderate, >0.90 and

>0.26-0.91), the median relative maximum prediction error for f_a was 1.69-fold. Only 1 to 4 (4 to 16 %) of the prodrugs had or were predicted to have a f_a of less than 50 %.

For drugs, the median (mean) relative and absolute prediction errors for f_a were 1.11 (1.63)-fold and 0.07 (0.11) (n=45; drugs (or active metabolites) with observed estimates only), respectively (Table 3). For 12 drugs with approximated f_a the median relative maximum prediction error for f_a was 1.49-fold. 6 to 8 (13 or 18 %) of the drugs had or were predicted to have f_a<50 %.

The correlation (Q^2; predictive accuracy) between predicted and observed f_a was 0.62 (observed f_a = 0.84 • predicted f_a + 0.15; n=50; data for both non-hydrolyzed ester prodrugs and drugs combined; Figure 3). Removal of the active uptake component for 7 compounds resulted in a higher intercept (0.33), but similar Q^2 (0.57).

Among 25 pairs with observed and/or predicted f_a for both the prodrug (excluding hydrolyzed esters) and the drug, 8 (32 %) showed apparently higher f_a for the prodrug (Table 4). These were adefovir dipivoxil (active uptake), enalapril (active uptake; bioavailability of enalaprilat increased from 3-12% to 60-70% on dosing enalapril), fenofibrate (higher $f_{a,passive}$ and possibly removed efflux), levodopa (active uptake), loratadine (higher $f_{a,passive}$ and f_{diss}), terfenadine (higher $f_{a,passive}$), valaciclovir (active uptake; 3-5 times higher oral bioavailability of acyclovir following valaciclovir dosing) and ximelagatran (higher $f_{a,passive}$).

Limitations with the study include the fact that all available prodrugs were not selected and uncertainties regarding active uptake and gastrointestinal hydrolysis. Inclusion of compensation for active uptake was done in retrospect (when active transport was known). There are also uncertainties/variability of collected *in vivo* f_a-data. Despite this, prediction results are encouraging.

In conclusion, prodrugs did not have larger or smaller average predicted $f_{a,passive}$ and f_{diss} than their drugs. However, the f_a for about 1/3 of non-hydrolyzed prodrugs was higher than for corresponding drugs, showing successful prodrug design. Most prodrugs and drugs had or were predicted to have at least 50 % f_a. The overall prediction accuracy was adequate, which validates ANDROMEDA for prediction of prodrug and drug absorption in man.

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Figure 1. Ranked predicted passive fraction absorbed ($f_{a,passive}$) for 70 prodrug-drug pairs. Ranking was based on data for drugs.

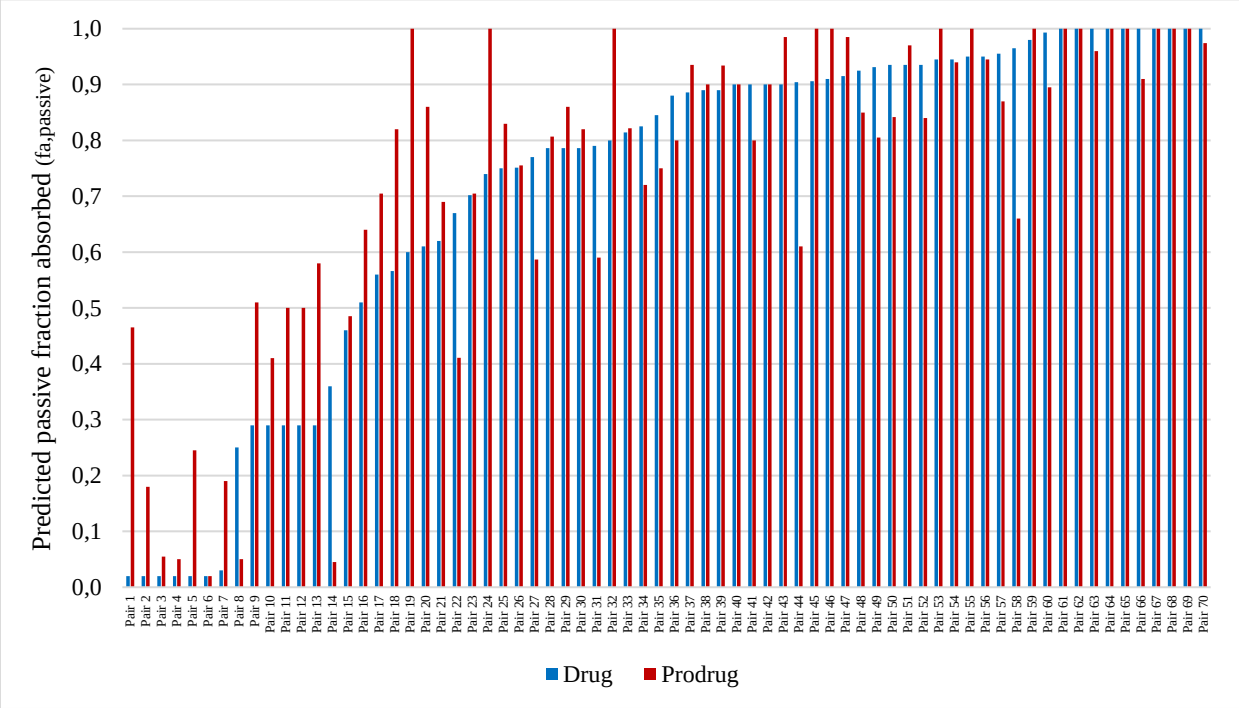

Figure 2. Ranked predicted maximum dissolution potential (f_{diss}) for 70 prodrug-drug pairs. Ranking was based on data for drugs.

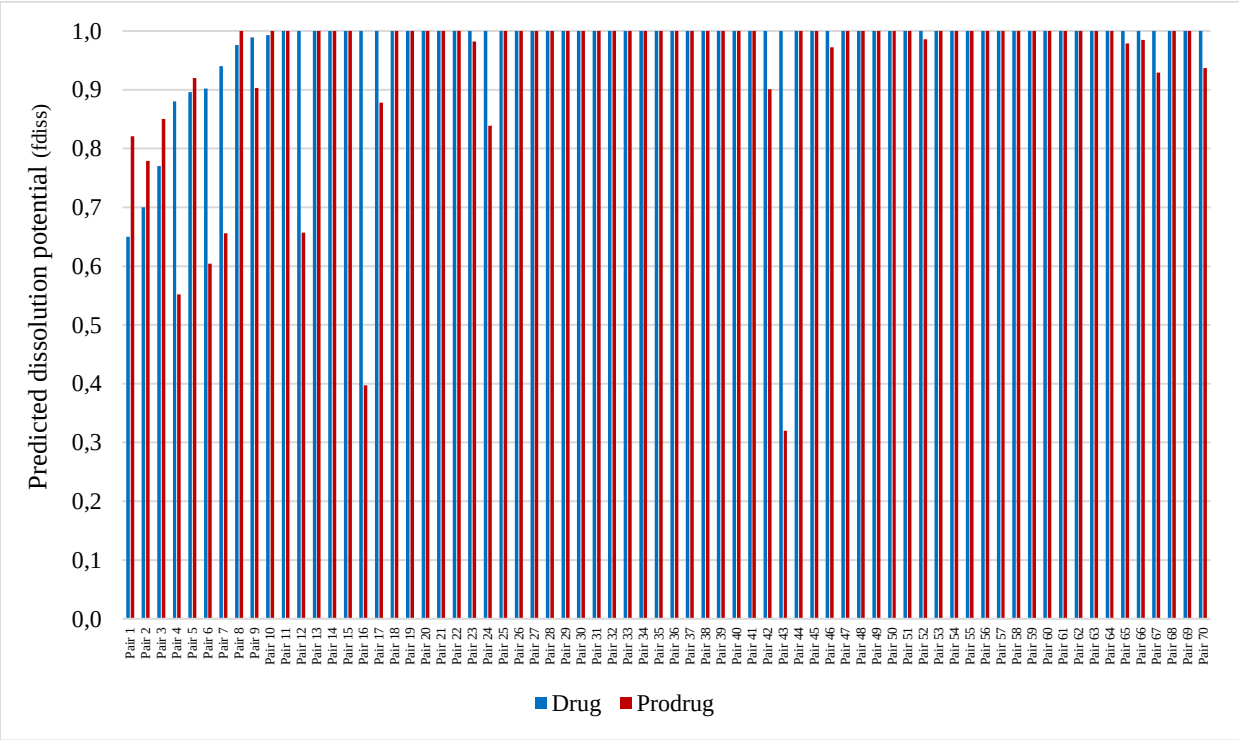

Figure 3. *In silico* predicted *vs* observed fraction absorbed (f_a) for 50 prodrugs and drugs.

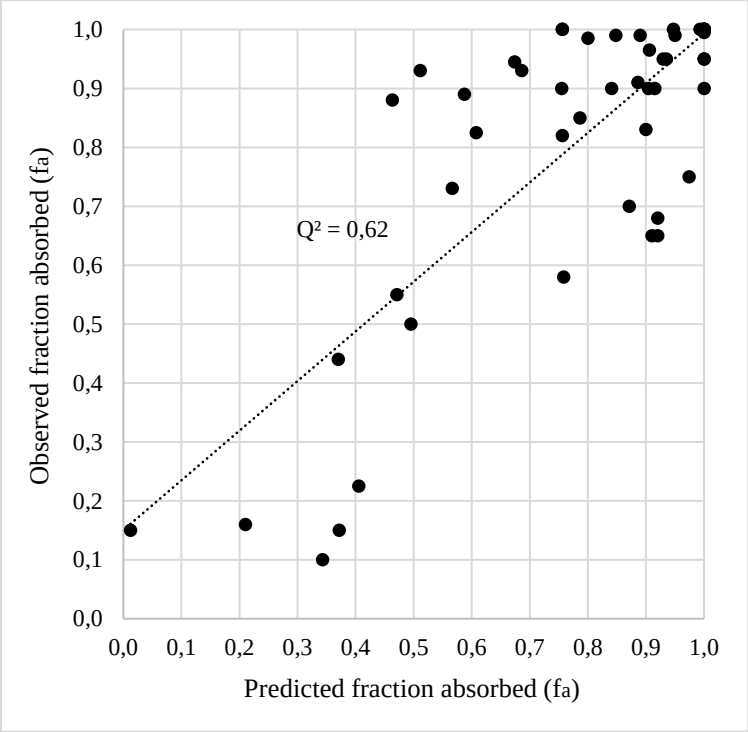

Table 1. The 70 prodrug-drug (alternatively drug-active metabolite) pairs selected for the study.

Prodrug	Drug
6-monoacetylmorphine	Morphine
Abiratarone acetate	Abiratarone
Adefovir dipivoxil	Adefovir
Amitriptyline	Nortriptyline
Ampiroxicam	Piroxicam
Aripiprazole lauroxil	Aripiprazole
Azathioprine	Mercaptopurine
AZD3582 (naproxinod)	Naproxen
Azilsartan medoximil	Azilsartan
Bambuterol	Terbutaline
Becampicillin	Ampicillin
Caffeine	Theophylline
Candesartan cilexitil	Candesartan
Capecitabine	5-FU
Carisoprodol	Meprobamate
Cefditoren pivoxil	Cefditoren
Cefotiam hexetil	Cefotiam
Cefpodoxime proxetil	Cefpodoxime
Ceftaroline fosamil	Caftaroline
Cefuroxime axetil	Cefuroxime
Chloramphenicol succinate	Chloramphenicol
Codeine	Morphine
Cortisone	Hydrocortisone
Cyclophosphamide	Aldophosphamide
Dabigatran etexilate	Dabigatran
Diazepam	Oxazepam
Enalapril	Enalaprilat
Eslicarbazepine acetate	Eslicarbazepine
Estramustine phosphate	Estramustine
Famciclovir	Penciclovir
Fenofibrate	Fenofibric acid
Fludarabine phosphate	Fludarabine
Fosamprenavir	Amprenavir
Fosaprepitant	Aprepitant
Fosfluconazole	Fluconazole
Fosphenytoin	Phenytoin
Fospropofol	Propofol
Gabapentin enacarbil	Gabapentin
Haloperidol decanoate	Haloperidol
Heroin	Morphine
Hetacillin	Ampicillin
Metoxibutropate	Ibuprofen
Imipramine	Desipramine
Irinotecan	SN-38
Lenampicillin	Ampicillin
Levodopa	Dopamine
Lisdexamphetamine	Dextroamphetamine
Loratadine	Desloratadine
Midodrine	Desglymidodrine
Mycophenolate mofetil	Mycophenolic acid
Olmesartran medoxomil	Olmesartan
Paliperidone	Risperidone
Parecoxib	Valdecoxib
Pivampicillin	Ampicillin
Prednisone	Prednisolone
Primidone	Phenobarbital

Psilocybin	Psilocin
Rolitetracycline	Tetracycline
Salicylic acid	Acetylsalicylic acid
Serdexmethylphenidate	Dexmethylphenidate
Sulfasalazine	5-aminosalicylic acid
Sulindac	Sulindac sulfide
Talampicillin	Ampicillin
Tedizolid phosphate	Tedizolid
Tenofovir disoproxil	Tenofovir
Terfenadine	Fexofenadine
Valaciclovir	Aciclovir
Valganciclovir	Ganciclovir
Valpromide	Valproic acid
Ximelagatran	Melagatran

Table 2. *In silico* prediction results for 25 non-hydrolyzed prodrugs.

Prodrug	Observed f_a	Predicted f_a	Error	Max error	Abs error
Adefovir dipivoxil	>0,6	1,00* (0,47)		1,67	
Amitriptyline	0,95	1,00	1,05		0,05
Azathioprine	0,88	0,46	1,90		0,42
Caffeine	1,00	0,76	1,32		0,24
Capecitabine	Mod/high	0,59		1,69	
Cefpodoxime proxetil	Poor	0,03			
Codeine	0,93	0,69	1,36		0,24
Cortisone	>0,26-0,91	0,94		3,48	
Cyclophosphamide	0,90	0,76	1,19		0,15
Diazepam	1,00	1,00	1,01		0,01
Enalapril	0,65	0,92* (0,15)	1,38		
Fenofibrate	>0,90	0,99		1,09	
Imipramine	1,00	1,00	1,00		0,00
Irinotecan	>0,09	0,46		4,60	
Levodopa**	1,00	1,00* (0,41)	1,00		
Loratadine	0,90	0,92	1,02		0,02
Midodrine	0,93	0,51	1,82		0,42
Paliperidone	>0,28	0,68		2,34	
Prednisone	0,95	0,93	1,02		0,02
Primidone	0,75	0,97	1,30		0,22
Psilocybin	>0,53	0,65		1,54	
Sulindac	0,90	0,84	1,07		0,06
Terfenadine	1,00	0,95	1,06		0,05
Valaciclovir	0,68	0,92* (0,64)	1,35		0,24
Ximelagatran	0,55	0,47	1,17		0,08
		Min	1,00	1,09	0,00
		Max	1,90	4,60	0,42
		Mean	1,24	2,34	0,15
		Median	1,17	1,69	0,08

*Considering active uptake (passive contribution within parentheses)
**Stabilized with the dopa decarboxylase inhibitor benserazide

Table 3. *In silico* prediction results for 45 drugs.

Drug	Observed f_a	Predicted f_a	Error	Max error	Abs error
5-aminosalicylic acid	High	0,61		1,64	
5-FU	High	0,57		1,76	
Acetylsalicylic acid	0,82	0,76	1,08		0,06
Aciclovir	0,23	0,41	1,80		0,18
Adefovir	0,16	0,21* (0,01)	1,31		0,05
Ampicillin	0,65	0,91* (0,18)	1,40		0,26
Amprenavir	0,70	0,87	1,24		0,17
Aprepitant	>0,60-0,65	0,58		1,72	
Aripiprazole	>0,87	0,88		1,13	
Cefuroxime	0,44	0,37* (0,02)	1,19		0,07
Chloramphenicol	0,89	0,59	1,52		0,30
Desglymidodrine	>0,5	0,64		1,56	
Desipramine	1,00	1,00	1,00		0,00
Dexmethylphenidate	0,90	0,90	1,00		0,00
Dextroamphetamine	>0,75	0,93		1,07	
Enalaprilat	0,15	0,01	12,50		0,14
Estramustine	0,83	0,61	1,36		0,22
Fenofibric acid	>0,81	0,78		1,28	
Fexofenadine	0,58	0,76	1,31		0,18
Fluconazole	0,95	0,67	1,40		0,27
Gabapentin	>0,65	0,70		1,42	
Ganciclovir	>0,06	0,30		3,29	
Haloperidol	0,95	1,00	1,05		0,05
Hydrocortisone	0,91	0,89	1,03		0,02
Ibuprofen	0,97	0,91	1,07		0,06
Melagatran	0,15	0,37	2,48		0,22
Mercaptopurine	0,50	0,50	1,01		0,01
Morphine	0,85	0,79	1,08		0,06
Mycophenolic acid	>0,4	0,39		2,59	
Naproxen	0,99	0,85	1,17		0,14
Nortriptyline	1,00	1,00	1,00		0,00
Olmesartan	>0,26	0,82		1,22	
Oxazepam	0,95	0,94	1,02		0,01
Penciclovir	0,10	0,34	3,43		0,24
Phenobarbital	1,00	1,00	1,00		0,00
Phenytoin	0,90	1,00	1,11		0,10
Piroxicam	1,00	1,00	1,00		0,00
Prednisolone	0,99	0,89	1,11		0,10
Propofol	1,00	0,99	1,01		0,01
Psilocin	>0,5	0,82		1,22	
Risperidone	0,99	0,80	1,23		0,19
Terbutaline	0,73	0,57	1,29		0,16
Tetracycline	0,83	0,90* (0,42)	1,08		0,07
Theophylline	1,00	0,76	1,32		0,24
Valproic acid	0,99	0,95	1,04		0,04
		Min	1,00	1,07	0,00
		Max	12,50	3,29	0,30
		Mean	1,63	1,66	0,11
		Median	1,11	1,49	0,07

*Considering active uptake (passive contribution within parentheses)

Table 4. *In silico* prediction results for 25 prodrug-drug pairs (excluding hydrolyzed esters).

Prodrug	Observed fa	Predicted fa	Drug	Observed fa	Predicted fa	Prodrug >Drug
Adefovir dipivoxil	>0,6	1,00* (0,47)	Adefovir	0,16	0,21* (0,01)	Yes
Amitriptyline	0,95	1,00	Nortriptyline	1,00	1,00	
Azathioprine	0,88	0,46	Mercaptopurine	0,50	0,50	
Caffeine	1,00	0,76	Theophylline	1,00	0,76	
Capecitabine	Mod/high	0,59	5-FU	High	0,57	
Cefpodoxime proxetil	Poor	0,03	Cefpodoxime	-	0,01	
Codeine	0,93	0,69	Morphine	0,85	0,79	
Cortisone	>0,26-0,91	0,94	Hydrocortisone	0,91	0,89	
Cyclophosphamide	0,90	0,76	Aldophosphamide	-	0,75	
Diazepam	1,00	1,00	Oxazepam	0,95	0,94	
Enalapril	0,65	0,92* (0,15)	Enalaprilat	0,15	0,01	Yes
Fenofibrate	>0,90	0,99	Fenofibric acid	>0,81	0,78	Yes
Imipramine	1,00	1,00	Desipramine	1,00	1,00	
Irinotecan	>0,09	0,46	SN-38	-	0,66	
Levodopa**	1,00	1.00* (0,41)	Dopamine	-	0,47	Yes
Loratadine	0,90	0,92	Desloratadine	-	0,69	Yes
Midodrine	0,93	0,51	Desglymidodrine	>0,5	0,64	
Paliperidone	>0,28	0,68	Risperidone	0,99	0,80	
Prednisone	0,95	0,93	Prednisolone	0,99	0,89	
Primidone	0,75	0,97	Phenobarbital	1,00	1,00	
Psilocybin	>0,53	0,65	Psilocin	>0,5	0,82	
Sulindac	0,90	0,84	Sulindac sulfide	-	0,83	
Terfenadine	1,00	0,95	Fexofenadine	0,58	0,76	Yes
Valaciclovir	0,68	0,92* (0,64)	Aciclovir	0,23	0,41	Yes
Ximelagatran	0,55	0,47	Melagatran	0,15	0,37	Yes

*Considering active uptake (passive contribution within parentheses)
**Stabilized with the dopa decarboxylase inhibitor benserazide