

## THE FLUROSOME<sup>®</sup> TECHNIQUE IN DRUG DISCOVERY

Winter 2012

Worcester, MA USA

### News Flashes:

*Publication “Determining P-glycoprotein-drug interactions: evaluation of reconstituted P-glycoprotein by a liposomal system and LLC-MDR1 polarized cell monolayers” by D.L. Melchior, F.J. Sharom, R. Evers, G.E. Wright, J.W.K. Chu, S.E. Wright, X. Chu and J. Yabut is in press in J. Pharm. Tox. Meth. (2012); doi: 10.1016/j.vascn.2012.02.002.*

*The Fluorosome Company offers inexpensive screening program for inhibition of pgp transport, p. 4.*

### BACKGROUND

Large numbers of compounds are generated by both traditional and combinatorial chemistry in the pharmaceutical industry, and various assays are used to select therapeutic hits. Methods to reduce the number of lead compounds based on these hits are in great demand. For example, many candidate drugs are ineffective because they cannot enter cells or cross the barriers that exist between various body compartments (e.g. intestine, blood-brain barrier, placenta). Many drugs are prevented from accessing organs and tissues, or are actually removed from them, by active transport proteins. In addition, the interaction of more than one drug with a transporter may change the effective concentration of either or both drugs, altering their disposition and leading to toxicity or reduced efficacy.

We have developed a sensitive *in vitro* spectroscopic approach, the Fluorosome<sup>®</sup> Technique, that can address these and related issues in drug discovery. This technique can measure both the rate of *passive diffusion* of a test compound through lipid bilayer membranes (permeability coefficient) and/or *active transport* of the compound by specific transport proteins. These properties may be correlated with both drug absorption and distribution, and drug interactions *in vivo*. The Fluorosome Technique is rapid, requires a minimal amount of the test compound, and is amenable to robotics for high throughput testing. Added advantages are that it uses no animals, cells, radiolabelled material or compound-specific detection methods such as LCMS. It is a valuable complement to existing tools used in the early screening of drugs.

### PASSIVE PERMEABILITY

**Cell-based methods.** Caco-2 cell lines, derived from human colon adenocarcinoma cells, exhibit some characteristics of the small intestinal epithelial layer, which presents a major barrier to gastrointestinal absorption. In this model system, membrane permeability coefficients are determined for test compounds by measuring transport of the test compound across a cell monolayer as a function of time. This model has advantages over animal testing – the cells are of human origin, results may provide good correlation to oral drug absorption in humans, and the method avoids the use of time-consuming, expensive, and controversial animal studies.

**In vitro methods.** Cell-free passive permeability methods include IAM chromatography which employs membrane lipid analogs immobilized on HPLC columns, and PAMPA (Parallel Artificial Membrane Permeation Assay), a lipid/organic solvent based method whose results are correlated with oral drug absorption. PAMPA is amenable to moderate throughput screening, and the assay can be conducted using commercial robotic equipment.

There are disadvantages to all these current methods. Cell-based assays are labor-intensive, require cell culture facilities and sterile conditions, and are limited to a narrow pH range. The *in vitro* methods described above do not utilize true bilayer membranes and lack flexibility. In all cases the analytical methods are compound-dependent, involving the use of radioactivity, suitable chromophores, or MS detection methods requiring expensive equipment. The assays are time consuming and not suitable for use in a high throughput screening program.

### The Fluorosome<sup>®</sup>-*trans* Technique

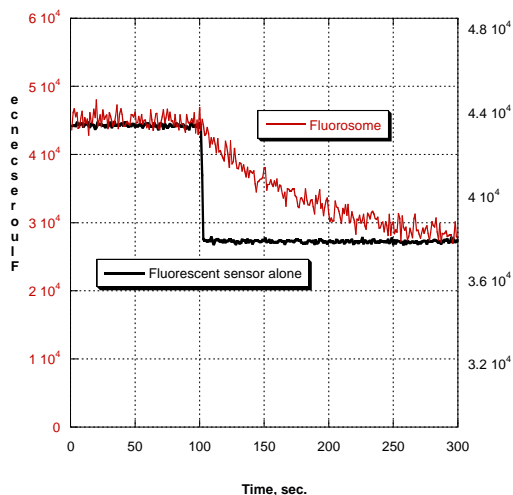
Fluorosome<sup>®</sup>-*trans* is a type of Fluorosome that is used for measuring passive diffusion of a drug across bilayer membranes. The Fluorosome<sup>®</sup>-*trans* technique is a rapid and universal *in vitro* method that is suitable for reliable measurement of drug permeability, and for high throughput screening of compound libraries. The technique is applicable to drugs in solution or drug delivery vehicles over a wide pH range, and can be modified for specialized membranes and active transport processes.

The technique is based on measurement of the *rate of diffusion* of a compound through the bilayer membrane of a liposome, which is detected via alteration of the fluorescence signal of special fluorescent probes trapped

within the liposome lumen. Thus, the assay can be run in any standard spectrofluorimeter. It is applicable to a wide range of drug molecules, and measures entry rates with half-lives from seconds to many minutes, corresponding to a wide range of permeability coefficients. It has the advantages of employing no animals or living cells, does not require sterile conditions, and uses no radiolabel or compound-specific detection method. Fluorosome<sup>®</sup>-*trans* are manufactured by proprietary methods, and qualified with respect to unit fluorescence, particle size and permeability to test compounds. The Fluorosomes can be pre-diluted and stored at room temperature for 3 months.

The basic experiment is illustrated in Figure 1. If a test compound is added to the fluorescent probe in solution (rather than trapped in liposomes) an instantaneous decrease in fluorescence emission is seen (Figure 1, black line). When the test compound is added to a Fluorosome<sup>®</sup>-*trans* suspension containing the same probe, the fluorescence decreases over time (Figure 1, red line). The time dependence of the fluorescence change is directly related to the rate of drug diffusion through the Fluorosome bilayer. The data are curve-fitted to give a first order rate constant, *k*, which is used in conjunction with the Fluorosome diameter to calculate the permeability coefficient, *P*, in cm/sec.

**Figure 1. Fluorescence quenching by a test compound injected at 100 sec of soluble fluorescent probe (black) and Fluorosome<sup>®</sup>-*trans* (red).**



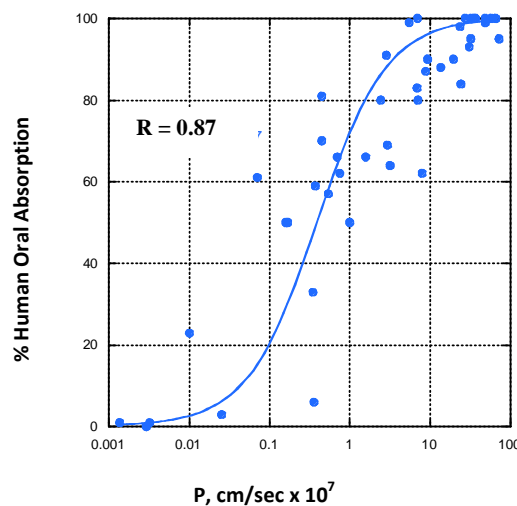
### Fluorosome-*trans* assay validation

The validity of the Fluorosome method was first demonstrated by permeability assays with 44 marketed drugs of documented low, medium and high human oral absorption. The drugs have diverse structures and properties, are from a variety of therapeutic classes, and are believed to be absorbed by passive diffusion. Membrane permeability coefficients of these drugs were measured and

compared with human oral absorption values (see the Appendix for data and literature references).

The permeability assay was performed at 25 °C by addition of a 20 µL aliquot of 50 mM drug solution in DMSO to 2 mL of diluted Fluorosome<sup>®</sup>-*trans* in a standard spectrofluorimeter. The time course of fluorescence change is converted to the rate constant for diffusion of drug into the vesicles, and then used to calculate the permeability coefficient, *P*. In all cases a clear, time-dependent change in fluorescence was observed. The magnitude of the change is different for each drug, but only the rate - not the extent - of the change, together with the particle diameter, are needed to calculate *P*. Values spanned a range of 10<sup>-6</sup> to 10<sup>-11</sup> cm/sec, and were highly correlated with human oral absorption for the 44 drugs analyzed (Figure 2). The correlation follows a sigmoidal relationship similar to that first reported by Artursson and Karlsson, who compared Caco-2 cell permeability with human oral absorption. The *R* value of 0.87 demonstrates the high correlation between *P* and oral absorption of the validation set of drugs.

**Figure 2. Correlation between permeability measured with Fluorosome<sup>®</sup>-*trans* and oral absorption for 44 drugs**



Published *P* values from Caco-2 and PAMPA assays for drugs in common with those of Figure 2 have been compared with the Fluorosome<sup>®</sup>-*trans* results (see Appendix I). Caco-2 results for 27 drugs had a similar correlation (*R* = 0.73 vs. 0.67 for Fluorosomes), suggesting the reliability of Fluorosomes in mimicking cell membranes; however, Caco-2 data showed more outliers than Fluorosomes. Published PAMPA permeabilities varied widely because different assay conditions were used. However, for a common subset of 22 drugs reported by *p*ION Inc., the PAMPA results had more scatter and were more poorly correlated than those obtained with Fluorosome<sup>®</sup>-*trans* (*R* = 0.46 vs. 0.72 for Fluorosomes).

Few published results for drugs with low oral absorption are available. As a consequence, PAMPA and Caco-2 P data are generally skewed to high absorption drugs (see Appendix I). The extension of Fluorosome permeability values to poorly permeable drugs is possible because of the convenient time course of the assays (seconds to minutes), and the wide dynamic range of permeability values (5 orders of magnitude) that can be measured using this technique.

## ACTIVE TRANSPORT

In contrast to *passive permeability*, many small hydrophobic molecules are actively transported (extruded, effluxed) from cells by membrane-bound protein pumps known as transporters. The most important mammalian drug transporter is P-glycoprotein (Pgp, ABCB1), originally described as MDR1 (multiple drug resistance 1 transpporter), an ABC superfamily efflux pump.

The three methods currently used to assess active transport of a compound are cell-based techniques, which estimate the ability of a compound to be transported or to compete for transport, leading to inhibition.

**Efflux in cell monolayers.** This assay measures the difference in flux of a compound across a monolayer of cultured cells overexpressing a transporter such as Pgp compared to cells that do not overexpress the transporter. The assay requires a radiolabelled drug or LCMS/MS for detection, and throughput is modest. Inhibition of transport of a radiolabelled substrate, e.g. [<sup>3</sup>H]digoxin, by a test compound is the most common application of this assay. *Advantage:* measures true drug efflux and/or its inhibition. *Disadvantages:* low throughput, labor intensive; requires sterile conditions and cell culture facilities, LCMS/MS instrumentation or radiolabelled drug. In addition, many cell lines contain a heterogeneous population of endogenous drug efflux pumps, and discrepancies may arise when comparing rates between different cell lines.

**Drug-stimulated ATPase activity in cells overexpressing Pgp.** This assay measures stimulation of the ATPase activity of cells overexpressing Pgp, or membrane vesicles isolated from them. The assay uses UV/visible absorption, and throughput of up to 150 compounds per week has been reported. *Advantages:* moderate throughput, simple instrumentation required. *Disadvantages:* the relationship between ATPase activity and transport is unclear (some Pgp substrates inhibit ATPase activity); cannot distinguish a transported drug (substrate) from a non-transported drug (inhibitor).

**Calcein AM inhibition assay.** This assay measures inhibition of Pgp-mediated calcein AM transport. When this non-fluorescent hydrophobic dye is added to cells, it crosses the membrane and is trapped intracellularly by conversion into fluorescent calcein. In cells containing

drug efflux pumps, calcein AM is extruded before its intracellular conversion to free calcein. If a competing drug inhibits extrusion, the fluorescent product accumulates intracellularly. *Advantage:* moderate throughput. *Disadvantages:* cannot distinguish a substrate from an inhibitor; requires cells expressing Pgp (although the assay is not specific for Pgp); results are variable depending on the cell line employed.

## Fluorosome<sup>®</sup>-trans-pgp

This is the first of a series of active transport Fluorosomes that The Fluorosome Company is developing. Fluorosome<sup>®</sup>-trans-pgp contains functional P-glycoprotein (Pgp) incorporated into the lipid bilayer. This system provides a rapid and sensitive screen of drug candidates for their potential to interact with Pgp (i.e. substrates/inhibitors). We have completed the development of assay procedures and tested a series of drugs known to interact with Pgp. Interim results were presented at the 2009 AAPS Workshop, Drug Transporters in ADME: From the Bench to the Bedside, Baltimore, MD, March 30-April 1, 2009.

A standard assay for inhibitors employs Fluorosome<sup>®</sup>-trans-pgp pre-equilibrated with the probe substrate “S-HR”, which is transported by both the H and R sites of Pgp. (We also have substrates “S-R” and “S-H” available to test for interaction with either site individually.) ATP is added to initiate Pgp-mediated active transport of probe substrate into the vesicle, leading to a change in the fluorescence emission over time (red line of Figure 3).

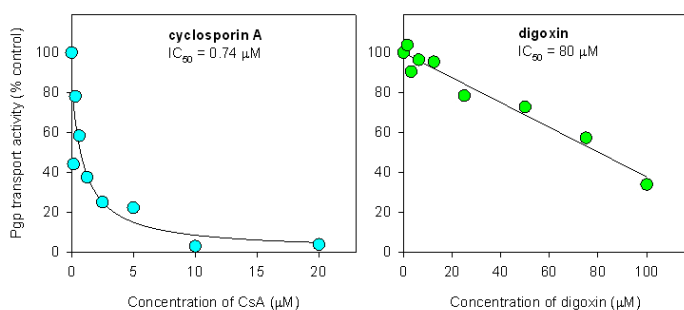
**Figure 3. Results of ATP-stimulated Fluorosome-trans-pgp. Control substrate transport vs. the effect of cyclosporin A at 2.5 and 20 μM.**



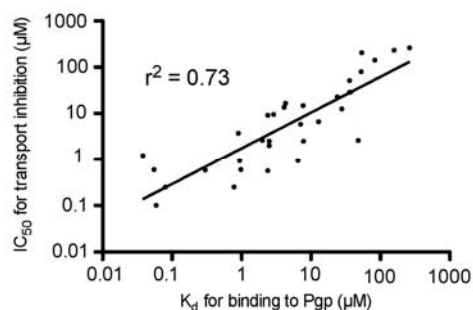
Addition of a test compound that interacts with Pgp, either as alternate substrate or as inhibitor, causes a reduction in S-HR transport to various extents, depending on the compound's potency and concentration. The magenta and blue lines of Figure 3, for example, illustrate the concentration-dependent reduction in slope of the substrate transport rate by cyclosporin A.

By comparing the effects of a test compound on substrate transport, i.e. reduction in slope of the substrate transport rate, over ca. 30 seconds after ATP addition, the % inhibition of Pgp-mediated transport can be calculated. IC<sub>50</sub> values can be obtained rapidly by repeating the assay at several concentrations of test compound. The results of 8-point IC<sub>50</sub> assays for test drugs cyclosporin A and digoxin together with controls, as illustrated in Figure 4, were completed in less than 10 minutes each.

**Figure 4. IC<sub>50</sub> determinations for two drugs that inhibit Pgp substrate transport using the Fluorosome<sup>®</sup>-*trans*-pgp assay system.**



The IC<sub>50</sub> values for 47 important drugs are listed in the Table of Appendix II. The values for 34 of these drugs were compared with their binding affinities (K<sub>d</sub>) to Pgp in detergent solution determined by fluorescence spectroscopy (see also Table in Appendix II). The plot of IC<sub>50</sub> vs. K<sub>d</sub> shown in Figure 5 illustrates the strong correlation (r<sup>2</sup>=0.73) between these two parameters over almost 4 orders of magnitude of IC<sub>50</sub> values, confirming the validity of the Fluorosome-*trans*-pgp results.



**Figure 5. Relation between IC<sub>50</sub>s for inhibition of drug transport and their K<sub>D</sub>s for binding to Pgp.**

Validation of the results of Fluorosome-*trans*-pgp inhibition assays was undertaken using the *in vivo* data for the effects of various co-administered second drugs ("inhibitors") on digoxin oral absorption (AUC) and plasma levels (C<sub>max,ss</sub>) in humans (Table, Appendix III). Plots using Fluorosome-*trans*-pgp IC<sub>50</sub> values illustrate the correlations (see Appendix III). In particular, the plot of AUC<sub>1</sub>/AUC vs. [I<sub>2</sub>]/IC<sub>50</sub> (Figure IIIb) resulted in only a single false negative, and the plot of C<sub>max,ss,1</sub>/C<sub>max,ss</sub> against [I<sub>2</sub>]/IC<sub>50</sub> (Figure IIIc) resulted in only two false negatives.

### Advantages of Fluorosome-*trans*-pgp

**Specificity:** Fluorosome<sup>®</sup>-*trans*-pgp contains **only** the Pgp transporter

**Low sample requirement:** Small amounts of test compounds are required. E.g. an assay at 10 μM requires 1 nanomole of compound

**Speed:** 1 minute or less per assay data point

**Convenience:** Real time measurement and full analysis in a fluorescence plate reader.

**Popular formats:** 96 half-well or 384 well microplates - one well per assay

**Dynamic range:** IC<sub>50</sub> determinations are possible over a concentration range of >1000

**Full coverage:** tests for drug interaction with both the H- and R-sites of Pgp

**Simple assay procedure:** amenable to robotics

### Service and sales

We offer both *service*, using our state of the art spectrofluorimeters and plate readers, and *sales* of reagents for customers' assays.

#### Fluorosome-*trans*

Service – \$200/test compound (minimum order \$1200)

Starter kit – \$1750 (consists of 1 mL Fluorosome-*trans*, sufficient for 50 assays in standard cuvettes, 100 mL buffer H, 100 μL test compounds A and B in DMSO, QC lot analysis, instructions) + shipping

Fluorosome-*trans*, 1 mL - \$1500 + shipping

#### Fluorosome-*trans*-pgp

Screening service - \$100/well (ca. 50 μL) (minimum order \$1000)

Standard compound testing protocols include:

- Assays at pre-selected concentrations
- 3-point range finding assays, e.g., 100, 10 and 1 μM
- Multi-point IC<sub>50</sub> determinations.

The Fluorosome Company, a division of GLSynthesis Inc., Spring 2011

Custom drug testing assays designed to meet your specific needs are also available.

Fluorosome-*trans*-pgp, 1 mL - \$1800 + \$65 packaging + express shipping

Volume discounts apply.

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Fluorosome® is a registered trademark of GLSynthesis Inc. U.S. patent 7,060,292 issued; international patents pending

**Appendix I. Reported human oral absorption data, permeability coefficients (P) measured with Fluorosome-*trans*,<sup>a</sup> and P values reported for Caco-2 and PAMPA assays.**

#	Drug	% human oral absorption (ref) <sup>b</sup>	Permeability coefficient, cm/sec x 10 <sup>7</sup>		
			Fluorosome- <i>trans</i>	Caco-2 <sup>c</sup>	PAMPA <sup>d</sup>
1	Caffeine	100 (1)	27.5	308	12.0
2	Diazepam	100 (1)	6.98	710	
3	Indomethacin	100 (1)	65.3	204	3.0
4	Salicylic acid	100 (1)	35.4	220	0.2
5	Verapamil	100 (1)	48.3	694	394
6	Phenobarbital	100 (2)	36.7		
7	Imipramine	100 (1)	57.6	141	140.4
8	Theophylline	100 (1)	32.3		0.4
9	Antipyrine	100 (1)	11.1		
10	Propranolol	99 (1)	22.3	218	143
11	Prednisolone	99 (1)	5.52		
12	Warfarin	98 (1)	23.6	211	15.8
13	Metoprolol	95 (1)	72.4	237	4.1
14	Timolol	95 (1)	31.9	128	6.1
15	Alprenolol	93 (1)	30.8	405	
16	Hydrocortisone	91 (1)	2.84	215	19.4
17	Barbital	90 (3)	-		
18	Sulindac	90 (1)	59.0		
19	Quinine	90 (2)	9.34		
20	Nicotinic acid	88 (1)	13.60		
21	Pindolol	87 (1)	8.81	167	1.2
22	Acetylsalicylic acid	84 (1)	24.2	90.9	1.1
23	Procainamide	83 (2)	6.91		3.1
24	Quinidine	81 (1)	0.45	204	45.6
25	Acebutolol	80 (1)	7.07	5.1	0.3
26	Dexamethasone	80 (1)	-	125	
27	Guanabenz	80 (1)	2.43	209	8.9
28	Carbamazepine	70 (2)	0.45	228	64
29	Ciprofloxacin	69 (1)	2.95		
30	Dipyridamole	66 (4)	0.70		
31	Enalapril	66 (1)	1.58	23.1	5.2
32	Ranitidine	64 (1)	3.17	4.9	0.1
33	Terbutaline	62 (1)	0.75	4.7	5.0
34	Ampicillin	62 (1)	7.94	2.27	
35	Furosemide	61 (1)	0.07	6.1	0.1
36	Sulfasalazine	59 (1)	0.37	3.0	2.3
37	Nadolol	57 (1)	0.54	38.8	2.8
38	Amiloride	50 (1)	0.17		2.7
39	Atenolol	50 (1)	0.16	5.3	0.6
40	Etoposide	50 (1)	1.0		
41	Sulpiride	44 (1)	-		0.3
42	Ribavirin	33 (1)	0.35		
43	Acyclovir	23 (1)	0.10	2.5	0.4
44	Oxybutynin	6 (2)	0.36		
45	Cidofovir	3 (1)	0.025		
46	Ceftriaxone	1 (1)	0.0014		1.7
47	Streptomycin	1 (1)	0.003		
48	Gentamycin	0 (1)	0.0029		

<sup>a</sup> At 25 °C. <sup>b</sup> (1) Zhao *et al.*, J. Pharm. Sci. 90:749-784, 2001. (2) Veber *et al.*, J. Med. Chem. 45:2615-2623, 2002. (3) AHFS Drug Information, American Society of Health System Pharmacists, Bethesda, MD, pp. 2102-2106, 2003. (4) Drug Information for the Health Care Professional, 23 Ed., Micromedex, Greenwood Village, CO, pp. 1103-1104, 2003. <sup>c</sup> Yazdani *et al.*, Pharm. Res. 15:1490-1494, 1998. Artursson and Karlsson, Biochem. Biophys. Res. Commun. 175:880-885, 1991. Mandagere *et al.*, J. Med. Chem. 45:304-311, 2002. Yee S. Pharm. Res. 14:763-766, 1997. Walter *et al.*, J. Pharm. Sci. 85:1070-1076, 1996. Zhu *et al.*, Eur. J. Med. Chem. 37:399-407, 2002. <sup>d</sup> www.pion-inc.com and Sugano *et al.*, J. Biomol. Screen. 6:189-196, 2001.

**Appendix II. IC<sub>50</sub>s for inhibition of substrate transport of important drugs by Fluorosome-*trans*-pgp, and binding affinities to Pgp.**

<b>Table. IC<sub>50</sub> values for drugs vs. Fluorosome-<i>trans</i>-pgp, and binding affinities of the drugs to Pgp.</b>					
Drug	Fl- <i>trans</i> -pgp IC <sub>50</sub> (μM)	Pgp binding K <sub>d</sub> (μM)	Drug	Fl- <i>trans</i> -pgp IC <sub>50</sub> (μM)	Pgp binding K <sub>d</sub> (μM)
Tariquidar	0.021		Mibefradil	7.1	
Elacridar	0.10	0.059 <sup>3</sup>	Ko143	7.5	
Telmisartan	0.12		Verapamil HCl	9.1	2.4 <sup>2</sup>
Ritonavir	0.25	0.78 <sup>3</sup>	Vinblastine	9.5	2.9 <sup>2</sup>
Valspodar	0.25	0.08 <sup>3</sup>	NAc-LLY-amide	12.5	28 <sup>2</sup>
MK571	0.56	2.4 <sup>2</sup>	Tamoxifen	13.5	4.1 <sup>2</sup>
Cyclosporin A	0.58	0.30 <sup>1</sup>	Trifluoperazine	14.8	7.7 <sup>2</sup>
Reserpine	0.31	0.73 <sup>2</sup>	Felodipine	16.5	4.3 <sup>3</sup>
Nelfinavir	0.59	0.98 <sup>3</sup>	Simvastatin acid	19.5	
Zosiquidar	0.59	0.055 <sup>2</sup>	Sufinpyrazone	22.8	24 <sup>2</sup>
Indinavir	0.93	0.94 <sup>3</sup>	Diltiazem	26.1	
Nicardipine	0.93	6.5 <sup>2</sup>	Pepstatin A	28.8	36 <sup>2</sup>
Paclitaxel	1.2	0.038 <sup>4</sup>	Sertraline	29	37 <sup>3</sup>
Ketoconazole	2.0	2.5 <sup>3</sup>	Benzbromarone	44	
Ivermetin	2.5	2.5 <sup>2</sup>	Indomethacin	51	36 <sup>2</sup>
Quinidine HCl	2.5	7.8 <sup>1</sup>	Ranolazine	64.1	
Nifedipine	2.6	48 <sup>3</sup>	Digoxin	80	53 <sup>3</sup>
Amiodarone	2.6	2.0 <sup>3</sup>	ALLN	142	138 <sup>4</sup>
Progesterone	3.7	0.9 <sup>2</sup>	Naloxone	206	54 <sup>3</sup>
Carvedilol	3.8		Colchicine	218	158 <sup>2</sup>
Troglitazone	5.2		Probenecid	235	
Nitrendipine	5.8	7.1 <sup>3</sup>	Captopril	429	
Simvastatin lactone	6.0		Digoxigenin	633	263 <sup>3</sup>
Isradipine	6.6	12.9 <sup>3</sup>			

<sup>1</sup> Liu, Siemiarczuk and Sharom, *Biochemistry* 39:14927-14938, 2000. <sup>2</sup> Sharom, F.J. (unpublished data). <sup>3</sup> Liu and Sharom, *Biochemistry* 35:11865-11873, 1996. <sup>4</sup> Sharom, Liu, Romsicki and Lu, *Biochim. Biophys. Acta* 1461:327-345, 1999. <sup>5</sup> Goard, Mather, Vinepal, et al. *Int. J. Cancer.* 127:2936-2948, 2010..

**Appendix III. Validation (*in vitro-in vivo* correlation) of Fluorosome-*trans*-pgp inhibition results.**

Validation of the Fluorosome-*trans*-pgp inhibition assay was done by correlating IC<sub>50</sub>s with *in vivo* data for the effects of various co-administered second drugs (“inhibitors”) on oral absorption (AUC) and plasma levels (C<sub>max,ss</sub>) of digoxin in human patients. Relevant data in the Table for digoxin disposition (AUC and AUC<sub>I</sub> and C<sub>max,ss</sub> and C<sub>max,ss,I</sub>) in the absence and presence of 16 drugs (inhibitors, I) were taken from Fenner *et al.* (Clin. Pharmacol. Ther. 85:173-181, 2009). [I] is the reported plasma concentration of drug (inhibitor) I, and [I<sub>2</sub>] is the estimated intestinal concentration of drug (inhibitor) I. The ratios AUC<sub>I</sub>/AUC and C<sub>max,ss,I</sub>/C<sub>max,ss</sub> represent the effect on absorption and plasma levels of digoxin by drug (inhibitor) I. IC<sub>50,F1</sub> values are half maximal pgp inhibitory concentrations of drugs from Fluorosome-*trans*-pgp assays.

Plots of AUC<sub>I</sub>/AUC and C<sub>max,ss,I</sub>/C<sub>max,ss</sub> against I/IC<sub>50</sub> and I<sub>2</sub>/IC<sub>50</sub> were made to evaluate the optimal correlation. It is assumed that a change in digoxin absorption or plasma concentration >25%, i.e. ratios >1.25, would represent a potentially toxic effect caused by the second drug (inhibitor).

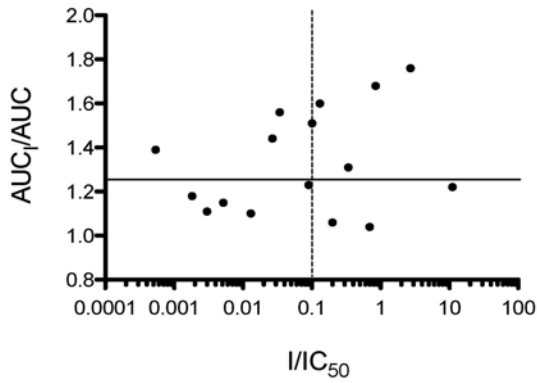
**Table. Data\* for correlations of Fluorosome-*trans*-pgp IC<sub>50</sub>s with effects on digoxin disposition.**

	Digoxin <i>in vivo</i>		“inhibitor” <i>in vivo</i>		Fl-t-pgp	[I]/IC <sub>50,F1</sub>	[I <sub>2</sub> ]/IC <sub>50,F1</sub>
	AUC <sub>I</sub> /AUC	C <sub>max,ss,I</sub> /C <sub>max,ss</sub>	[I]†	[I <sub>2</sub> ]‡	IC <sub>50,F1</sub> §		
Diltiazem	1.44	1.38	0.7	532	26.1	0.0268	20.383
Quinidine	1.76	1.75	3.54	3397	1.3	2.723	2613
Telmisartan	1.22	1.58	1.11	933	0.1	11.1	9330
Troglitazone	1.04	10.5	3.62	3264	5.2	0.696	627.7
Verapamil	1.51	1.44	1.2	652	11.9	0.101	54.79
Carvedilol	1.56	1.38	0.13	61.5	3.8	0.0342	65.3
Felodipine	1.18	1.34	0.03	104	16.5	0.00182	6.303
Mibefradil	1.31	1.41	2.42	1271	7.1	0.341	179.0
Nicardepine	1.06	1.06	0.18	248	0.9	0.2	275.6
Ranolazine	1.6	1.46	8.4	9356	64.1	0.131	145.96
Amiodarone	1.68	1.84	2.2	4693	2.6	0.846	180.5
Isradipine	1.11	1.26	0.02	161.5	6.6	0.003	24.47
Sertraline	1.1	1.05	0.39	2612	29	0.013	90.07
Captopril	1.39	1.59	0.23	230	ca. 425	0.00054	0.54
Nifedipine	1.23	1.06	0.23	115.5	2.55	0.09	45.29
Nitrendipine	1.15	1.57	0.03	222.0	5.8	0.0052	38.28

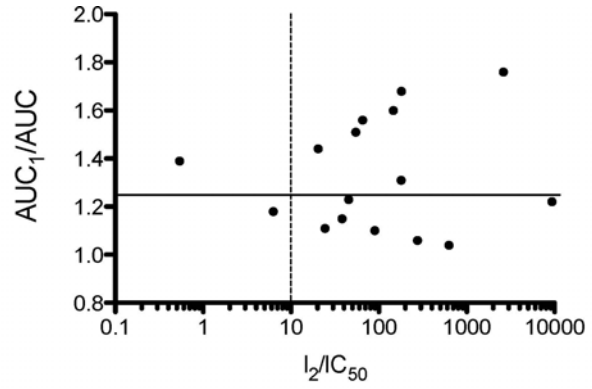
\*From Fenner *et al.*, 2009. †μM plasma conc. of drug (“inhibitor”) at steady state. ‡est. μM intestinal conc of drug (“inhibitor”) after oral dose. § μM conc. for half-maximal inhibition of drug (“inhibitor”) transport by Fluorosome-*trans*-pgp.

Using the 16 IC<sub>50</sub>s in Appendix II measured with Fluorosome-*trans*-pgp, the resulting plots (Figures 6a-d) are equivalent or superior to those based on net secretory flux results in Caco-2 cell monolayers reported by Fenner *et al.* In particular, the plot of AUC<sub>I</sub>/AUC vs. [I<sub>2</sub>]/IC<sub>50</sub> (Figure 6b) resulted in only a single false negative (captopril), and the plot of C<sub>max,ss,I</sub>/C<sub>max,ss</sub> against [I<sub>2</sub>]/IC<sub>50</sub> (Figure 6d) resulted in only two false negatives (captopril and felodipine).

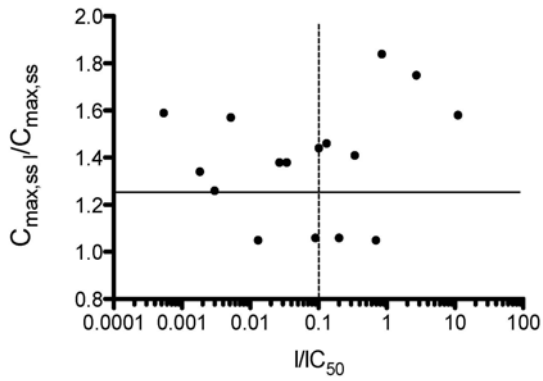
**Figure 6a. Digoxin abs. vs. plasma conc./IC<sub>50</sub>.**



**Figure 6b. Digoxin abs. vs. GI conc./IC<sub>50</sub>.**



**Figure 6c. Digoxin level vs plasma conc./IC<sub>50</sub>.**



**Figure 6d. Digoxin level vs GI conc./IC<sub>50</sub>.**

