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Evaluation of inhibitory potencies for compounds inhibiting P-glycoprotein but without maximum effects: f2-values

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(JW and WEH)

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Running title: f2-values

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Abbreviations:
f1.5, f2, f3, and f4, concentration needed to increase baseline fluorescence by factor 1.5, 2, 3, and 4, respectively; FCS, fetal calf serum; DMSO, dimethylsulfoxide; LY335979, zosuquidar; GG918, elacridar; HBSS, Hanks' balanced salt solution; IC_{50}, concentration leading to 50% of I_{max}; I_{max}, maximum effect; I_{min}, minimum effect; pBCECs, porcine brain capillary endothelial cells; P-gp, P-glycoprotein; SDZ-PSC833, valspodar;

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Abstract

In cell culture systems with aqueous buffers concentration-response curves to lipophilic inhibitors are difficult to establish because plateau effects (I_{max}) are often not reached due to limited drug solubility. Consequently, the inhibitory potency of a compound will not be definable using IC_{50}-values (concentration exerting 50% of I_{max}). As alternative potency measures f2-values, the concentrations required to double baseline signals, have been proposed. Using both methods we reevaluated the concentration-response curves of calcein assays with 78 compounds in 3 different cell culture systems and found a close correlation between both methods (r_s = 0.93-0.99, $p \leq 0.0028$). These findings suggest that f2-values are a valuable alternative to define rank orders of highly lipophilic inhibitors as a basis for the prediction of pharmacological interaction properties in clinical settings. Although only tested for inhibition of P-glycoprotein it appears likely that this method may be transferred to other assays with other proteins.

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Introduction

The assessment of the concentration-response relationship is a key element in the pharmacologic characterization of inhibitors of important targets like the ATP-binding cassette (ABC)-transporter P-glycoprotein (P-gp, MDR1/ABCB1). The inhibitory potency of a compound is normally expressed as IC_{50} (concentration leading to half maximal inhibition) and typically derived from the Hill equation or its derivatives,

$$y = I_{min} + ((I_{max} - I_{min}) / (1 + (x/IC_{50})^{s})) \qquad \text{(equation 1)}$$

which describe concentration-response curves using four parameters: background (I_{min}), maximum effect (I_{max}), slope (s), and IC_{50}, the concentration leading to 50% of I_{max}.

Obviously, the accurate determination of IC_{50}-values depends on the reliable description of I_{min}, s, and I_{max} with the latter being the most challenging since maximum effects can often not be reached due to limited solubility or cytotoxic effects. We have previously shown that the poor solubility is frequently neglected and that numerous papers report to have tested inhibition at drug concentrations beyond the maximum solubility of the inhibitor in the respective buffers (Weiss et al., 2002).

In this study we have evaluated an alternative method to assess the inhibitory potency of compounds interacting with P-gp whose substrates are typically highly lipophilic and can typically not be tested up to maximal effects in vitro due to their low solubility in the aqueous solutions used in cell culture systems. P-gp inhibition was investigated with the well established and widely used calcein assay in three different cell systems expressing moderate to high amounts of this efflux transporter: primary porcine brain capillary endothelial cells (pBCECs), L-MDR1 cells with overexpression of human P-gp, and in the murine leukemic cell line P388/dx overexpressing murine P-gp.

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Materials and Methods

Materials

Culture media, fetal calf serum (FCS), medium supplements, antibiotics, and Hanks' balanced salt solution (HBSS) were purchased from Invitrogen (Karlsruhe, Germany), dimethylsulfoxide (DMSO) from Sigma-Aldrich (Taufkirchen, Germany), calcein-AM from MoBiTec (Göttingen, Germany), and 96-well microtiter plates were from Nunc (Wiesbaden, Germany). Drugs were obtained from Sigma-Aldrich (Taufkirchen, Germany) or from the corresponding manufacturer.

LLC-PK1 and L-MDR1 cells

As model for human P-gp we used L-MDR1 cells, a cell line generated by transfection of the porcine kidney epithelial cell line LLC-PK1 with the human MDR1 gene (Schinkel et al., 1996) and the parental cell line LLC-PK1 (available at ATCC, Manassas, U.S.A.) as a control. The L-MDR1 cell line was kindly provided by Dr. A. H. Schinkel (Amsterdam, the Netherlands). The cells were cultured and seeded as described previously (Weiss et al., 2003a).

P388 and P388/dx cells

As an alternative model for P-gp we used the murine monocytic leukaemia cell line P388 and the corresponding doxorubicin-resistant cell line P388/dx over-expressing *mdr1a/1b* (Boesch et al., 1991). Both cell lines were kindly provided by Dr. Dario Ballinari (Pharmacia & Upjohn, Milano, Italy). The cells were cultured and seeded as described previously (Fröhlich et al., 2004).

pBCECs

Isolation, culturing, and seeding of pBCECs expressing porcine pgp1A was essentially based on the method described by Audus and co-workers (Audus et al., 1996) with minor alterations Weiss et al., 2003a).

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Stock solutions

Stock solutions of test compounds were prepared strictly following the manufacturers instructions. Only very few compounds were soluble in aqua bidest. All others were dissolved in DMSO. The DMSO concentration in the assays never exceeded 1% (v/v), a concentration which was found not to influence the results of the assay.

Calcein uptake assay

The calcein assay was used to assess P-gp inhibition. Because the transport capacity of P-gp is inversely proportional to the accumulation of intracellular calcein fluorescence, inhibition of P-gp leads to an increase in intracellular calcein fluorescence. The assay was conducted and validated as described previously (Weiss et al., 2003a, Fröhlich et al., 2004). Each experiment was performed at least in duplicate (if no inhibition was observed) or in triplicate on different days.

Statistical analysis

For calculation of the inhibitor effects, a non-linear four parameter fit was used (Grafit, version 4, Erithacus Software, Middlesex, UK) according to the sigmoidal I_{max} model with the formula specified in equation 1 (Hill equation). The f1.5, f2, f3, and f4-values (concentration needed to increase baseline fluorescence by factor 1.5, 2, 3, and 4, respectively) were derived from the corresponding concentration-response curve (Fig. 1) as published previously for the f2-value (Weiss et al., 2003).

Correlations were assessed by Spearman rank correlation and characterized by the corresponding correlation coefficient r_S (GraphPad Prism, version 4.0, GraphPad Software, San Diego, USA). A p-value of ≤ 0.05 was considered significant.

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Results and Discussion

A total of 78 compounds was tested in the calcein assay in the three different cell systems (50 compounds in P388/dx cells, 67 in L-MDR1 cells, and 48 in pBCECs). Twenty-seven compounds were investigated in all three cell systems, 35 in two systems, and 16 in only one cell system. Among them were highly potent P-gp inhibitors like LY335979 (zosuquidar), SDZ-PSC833 (valspodar), and GG918 (elacridar), drugs used for HIV therapy, fungicides, newer antidepressants (Weiss et al., 2003a), progestins (Fröhlich et al., 2004), fibrates (Ehrhardt et al., 2004), amphetamines (Ketabi-Kiyanvash et al., 2003), antiepileptic drugs (Weiss et al., 2003b), kava-kava extracts and kavalactones (Weiss et al., 2005). Of all these compounds, 21 revealed no P-gp inhibition up to the highest soluble concentration (10 in P388/dx cells, 19 in L-MDR1 cells, and 11 in pBCECs). Plateau effects were only reached by 15 compounds in P388/dx cells, 6 compounds in L-MDR1 cells, and 9 compounds in pBCECs, a prerequisite for a valid calculation of IC_{50} (Tables 1-3). For all other compounds (44 in P388/dx cells, 42 in L-MDR1 cells, and 28 in pBCECs) with obvious P-gp inhibitory effects evaluation of the inhibitory potency based on IC_{50}-values would be inappropriate because they did not reach I_{max}. Indeed, the common practice to calculate IC_{50} values from truncated curves covering not the whole range of the dose-response relationship (e.g. Tiberghien and Loor, 1996; Ji and Morris, 2004; Zhou et al., 2005) bears a significant potential of over- or underestimation of a compound's potency. To illustrate this fact, we have calculated IC_{50} with truncated concentration-response curves (using only values below I_{max}) for the 15 compounds reaching plateau effects in P388/dx cells. Fig. 2 demonstrates that such calculations will result in IC_{50}-values of up to two orders of magnitude over the 'true' IC_{50} calculated with the complete concentration-response curves. Moreover, when IC_{50} were calculated from truncated concentration-response curves, variation coefficients of the test series were up to 171% illustrating the extent of inaccuracy by this mode of calculation. In

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addition, the ranking order of the compounds changed substantially from A-B-C-D-E-F-G-H-I-J-K-L-M-N-O to B-A-C-E-F-K-G-J-I-M-L-D-H-O-N.

Provided that the maximal intracellular calcein fluorescence were identical for all compounds, it would be possible to calculate IC_{50} by fixing I_{max} to a certain value. However, as Fig. 3 demonstrates I_{max}-values may differ between different inhibitors by half an order of magnitude and indeed when IC_{50}-values were calculated with I_{max}-values fixed to 35 they differed up to 5-fold (Fig. 2) and the potency ranking order changed to A-B-C-H-D-F-E-G-I-M-L-K-N-J-O. Moreover, when IC_{50}-values were calculated with fixed I_{max}, variation coefficients reached up to 157% emphasizing that this mode of calculation is also inadequate. Having faced this problem when P-gp inhibitory properties of antidepressants were assessed (Weiss et al., 2003a), we have established the f2 method. The f2-value corresponds to the concentration of the compound leading to a 2-fold increase in the baseline calcein fluorescence (Fig. 1). While f2 is obviously rather independent of I_{max} its values are only a useful alternative if they are closely correlated with IC_{50}-values. Table 4 demonstrates the excellent correlation between f2 and IC_{50} in P388/dx cells, L-MDR1 cells, and pBCECs for all compounds that reached plateau effects in the calcein assay and Fig. 4 exemplifies this correlation in P388/dx cells. In all cell lines, which differ in the expression level and the species origin of P-gp, the relationship was very close and highly significant. The same applies to the correlation between IC_{50} and f1.5 and f3 but not to f4, where the correlation is the weakest (Table 4). Fig. 5 demonstrates, that the ranking order between IC_{50}, f2, and f3 only differed slightly and switches mostly occurred between compounds with IC_{50} laying closely together. However, f2 was favoured over f1.5 or f3, because (a) in contrast to f1.5 with the common slopes observed f2 always lies in the log-linear steep part of the concentration-response curve (similar to IC_{50}) and (b) in contrast to f3 only for few of the compounds tested in the calcein assay the poor solubility prevented the quantification of the f2-value (Table 5). Only for very weak inhibitors, which do not lead to a 2-fold increase in

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basal fluorescence up to the highest soluble concentration, this method is not suitable. However, such minor effects appear negligible because these compounds do not bear a clinically meaningful interaction potential.

The following limitations merit discussion: First, differences in rank order between the two methods are more likely if compounds with substantially different I_{max} are compared. However, as illustrated in Figures 4 it is very unlikely that potent inhibitors are misclassified as weak and that their interaction potential remains undiscovered.
Second, in this paper we have only assessed a series of P-gp inhibitors and we have thus not proven universal validity of the proposed approach. It appears, however, rather likely that this method may also readily be transferred to other assays evaluating the inhibition of other transporters provided that the slopes of the concentration-response curves are not too variable. Finally f2-values may depend on assay conditions and the cell system used, and these values are thus not readily comparable with absolute f2-values generated in other test systems. This is, however, also true for full concentration-response curves and figures directly derived from it such as IC_{50}. Irrespective of the method used it is therefore critical to have appropriate controls to which the results may be related to. Moreover, the clinical relevance of the observed potencies in vitro always depends on the concentrations encountered in vivo at the site of the transporter and on other factors influencing the interaction with the transporter like the presence of inhibitors or inducers. Indeed, this represents a general problem of data assessed in vitro and is not restricted to f2- or IC_{50}-values.

In conclusion, in the frequent case when inhibitor potencies cannot be assessed with full concentration-response relationships because of restricted solubility of the (often lipophilic) test compounds calculation of IC_{50}-values as a gold standard is inaccurate and will cause misinterpretation of relative potencies. In these instances the computation of f2-values

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(concentration doubling baseline effects) is a robust and reliable alternative which even provides correct rank orders if I_{max}-values largely differ between different test compounds.

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References

Audus KL, Ng L, Wang W, and Borchardt RT (1996) Brain microvessel endothelial cell culture systems. *Pharm Biotechnol* **8**:239-258.

Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzane-Do A, Bollinger P, and Loor F (1991) In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res* **51**:4226-4233.

Ehrhardt M, Lindenmaier H, Burhenne J, Haefeli WE, and Weiss J (2004) Influence of lipid lowering fibrates on P-glycoprotein activity in vitro. *Biochem Pharmacol* **67**:285-292.

Fröhlich M, Albermann S, Sauer A, Walter-Sack I, Haefeli WE and Weiss J (2004) In vitro and ex vivo evidence for modulation of P-glycoprotein activity by progestins. *Biochem Pharmacol* **68**:2409-2416.

Ji Y and Morris ME (2004) Effect of organic isothiocyanates on breast cancer resistance protein (ABCG2)-mediated transport. *Pharm Res* **21**:2261-2269.

Ketabi-Kiyanvash N, Weiss J, Haefeli WE, and Mikus G (2003) P-glycoprotein modulation by the designer drugs methylendioxymethamphetamine, methylenedioxyethylamphetamine, and paramethoxyamphetamine. *Addict Biol* **8**:413-418.

Schinkel AH, Wagenaar E, Mol CA, and van Deemter L (1996) P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* **97**:2517-2524.

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DMD Fast Forward. Published on November 4, 2005 as DOI: 10.1124/dmd.105.007377
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Tiberghien F and Loor F (1996) Ranking of P-glycoprotein substrates and inhibitors by a calcein-AM fluorometry screening assay. *Anti-Cancer Drugs* **7**:568-578.

Weiss J, Burhenne J, Riedel K-D, and Haefeli WE (2002) Poor solubility limiting significance of in-vitro studies with HIV protease inhibitors. *AIDS* **16**:674-676.

Weiss J, Dormann S-MG, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash N and Haefeli WE (2003a) Inhibition of P-glycoprotein by newer antidepressants. *J Pharmacol Exp Ther* **305**:197-204.

Weiss J, Kerpen CJ, Lindenmaier H, Dormann S-MG, and Haefeli WE (2003b) Interaction of antiepileptic drugs with human P-glycoprotein in vitro. *J Pharmacol Exp Ther* **307**:262-267.

Weiss J, Sauer A, Frank A, and Unger M (2005) Extracts and kavalactones of Piper methysticum G. FORST (kava-kava) inhibit P-glycoprotein in vitro. *Drug Metab Dispos* **33**, Epub ahead of print, PMID:16051732.

Zhou X-F, Yang X, Wang Q, Coburn RA, and Morris ME (2005) Effects of dihydropyridines and pyridines on multidrug resistance mediated by breast cancer resistance protein: in vitro and in vivo studies. *Drug Metab Dispos* **33**:1220-1228.

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Figure legends:

Figure 1: Evaluation of the inhibitory effect of a compound by calculation of the concentration needed to increase baseline calcein fluorescence by factor 1.5, 2, or 3 and by calculation of the IC_{50} (example: loperamide in P388/dx cells; n= 8 wells).

Figure 2: Comparison of the ranking of the 15 compounds reaching plateau effects in P388/dx cells after different IC_{50} calculations. For the calculation of the truncated curves only values below I_{max} were used.

Figure 3: Calcein assay with (a) P388/dx cells (b) L-MDR1 cells, and (c) pBCECs with compounds revealing substantially differing plateau effects (I_{max}) (different intrinsic activity). Each curve depicts one representative experiment of a series of 3-4 with each concentration tested in octuplet. Data are expressed as mean ± S.E.M. for n = 8 wells.

Figure 4: Correlation of f2 and IC_{50}-values for the 15 compounds with plateau effects in the calcein assay in P388/dx cells. B represents a zoomed section of a (indicated by the square) with seven highly potent inhibitors revealing very low f2 and IC_{50}-values.

Figure 5: Comparison of the ranking order of the 15 compounds reaching plateau effects in P388/dx cells after calculation of IC_{50}, f2, and f3. f2, f3: concentrations needed to increase baseline calcein fluorescence by factor 2 and 3, respectively.

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Table 1: f2 and IC_{50}-values for compounds exerting maximum effects in the calcein assay in P388/dx cells

	P388/dx	
Compound	**f2 (µM)**	**IC_{50} (µM)**
LY335979	0.010 ± 0.006	0.025 ± 0.008
GG918	0.011 ± 0.004	0.021 ± 0.005
SDZ-PSC833	0.020 ± 0.004	0.052 ± 0.018
Ketoconazole	0.15 ± 0.02	0.74 ± 0.31
Itraconazole	0.25 ± 0.11	0.66 ± 0.04
Verapamil	0.55 ± 0.20	2.91 ± 0.80
Loperamide	0.62 ± 0.11	2.28 ± 0.95
Nelfinavir	1.11 ± 0.25	1.65 ± 0.68
Lopinavir	2.05 ± 0.17	2.27 ± 0.78
Quinidine	2.43 ± 0.91	3.90 ± 1.05
Chlormadinone acetate	2.87 ± 1.27	4.87 ± 1.30
Cyproterone acetate	2.92 ± 1.46	4.30 ± 1.06
Norgestimate	4.32 ± 0.60	8.27 ± 0.32
Delavirdine	4.97 ± 0.27	6.67 ± 1.22
Amprenavir	9.62 ± 1.12	24.12 ± 4.41

Values represent mean ± S.D. of 3-5 independent assays each performed in octuplet.

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Table 2: f2 and IC_{50}-values for compounds exerting maximum effects in the calcein assay in pBCECs

	pBCECs	
Compound	**f2 (µM)**	**IC_{50} (µM)**
GG918	0.001 ± 0.0003	0.002 ± 0.0006
LY335979	0.003 ± 0.0007	0.007 ± 0.001
SDZ-PSC833	0.006 ± 0.003	0.02 ± 0.01
Itraconazole	0.02 ± 0.01	0.05 ± 0.01
Loperamide	0.79 ± 0.11	1.86 ± 0.41
Desmethylloperamide	0.96 ± 0.29	2.35 ± 0.60
Verapamil	0.60 ± 0.20	3.22 ± 0.82
Quinidine	1.12 ± 0.42	7.19 ± 1.60
Amprenavir	8.61 ± 0.83	19.7 ± 6.78

Values represent mean ± S.D. of 3-5 independent assays each performed in octuplet.

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Table 3: f2 and IC_{50}-values for compounds exerting maximum effects in the calcein assay in L-MDR1 cells

	L-MDR1	
Compound	**f2 (µM)**	**IC_{50} (µM)**
GG918	0.006 ± 0.002	0.038 ± 0.004
LY335979	0.02 ± 0.01	0.14 ± 0.05
Itraconazole	0.14 ± 0.08	0.40 ± 0.07
Verapamil	4.66 ± 0.8	20.0 ± 3.1
Paroxetine	14.3 ± 3.5	29.8 ± 11.1
Fluoxetine	91.4 ± 7.7	115.5 ± 12.1

Values represent mean ± S.D. of 3-5 independent assays each performed in octuplet.

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Table 4: Correlation between f-values and IC_{50} in the different cell lines

Cell system	Analyzed compounds (n)	f1.5	f2	f3	f4
P388/dx	15	$r_S = 0.94$ $p < 0.0001$	$r_S = 0.95$ $p < 0.0001$	$r_S = 0.95$ $p < 0.0001$	$r_S = 0.73$ $p = 0.02$
L-MDR1	6	$r_S = 1.0$ $p = 0.0028$	$r_S = 0.99$ $p = 0.0028$	$r_S = 1.0$ $p = 0.0028$	n.d.
pBCECs	9	$r_S = 0.92$ $p = 0.0013$	$r_S = 0.93$ $p = 0.0007$	$r_S = 0.93$ $p = 0.0022$	n.d.

f1.5, f2, f3, f4: concentrations needed to increase baseline calcein fluorescence by factor 1.5, 2, 3, and 4, respectively; r_S = Spearman rank correlation coefficient; n.d. not definable (too few values for calculation).

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Table 5: Percentage of calculable f-values in the different cell lines

Cell system	**% of P-gp inhibiting compounds not reaching**		
	f2	**f3**	**f4**
P388/dx	7.5	22.5	50.0
L-MDR1	20.8	52.1	56.3
pBCECs	13.5	43.2	59.5

f2, f3, f4: concentrations needed to increase baseline calcein fluorescence by factor 2, 3, and 4, respectively.

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Figure 1

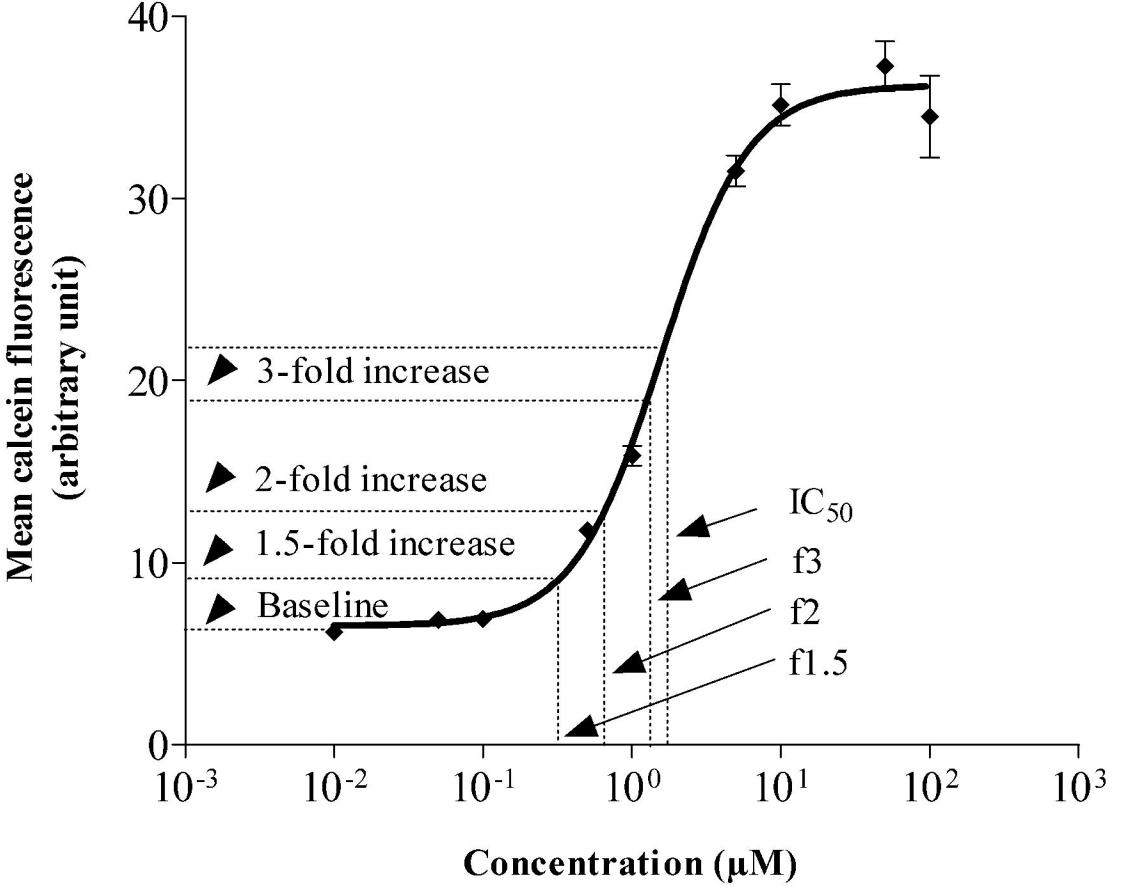

40
30
20
10
0
Mean calcein fluorescence (arbitrary unit)
3-fold increase
2-fold increase
1.5-fold increase
Baseline
IC50
f3
f2
f1.5
10-3
10-2
10-1
100
101
102
103
Concentration (µM)

Figure 2

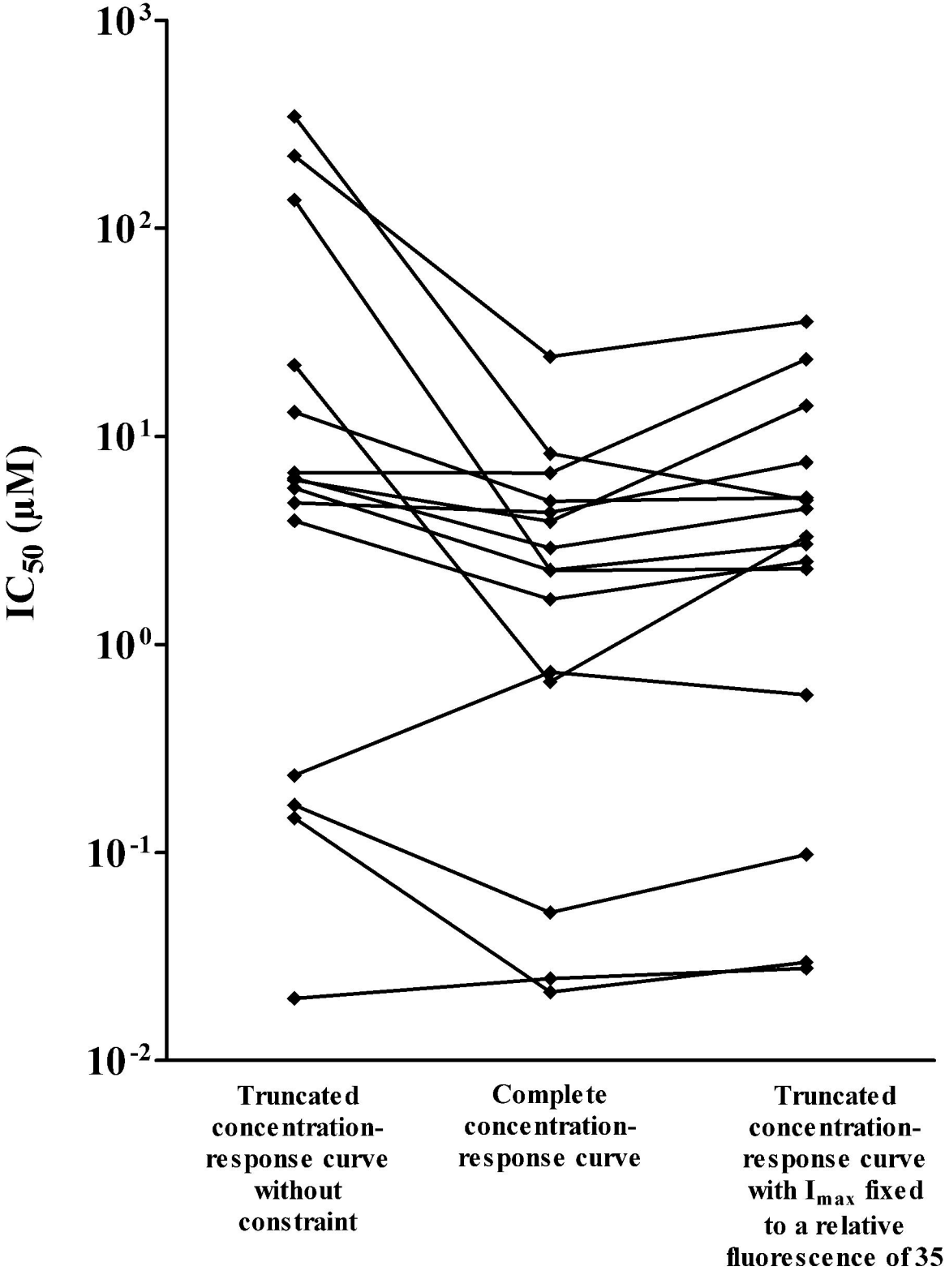

10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
IC_{50} (µM)
Truncated concentration-response curve without constraint
Complete concentration-response curve
Truncated concentration-response curve with I_{max} fixed to a relative fluorescence of 35

Figure 3a

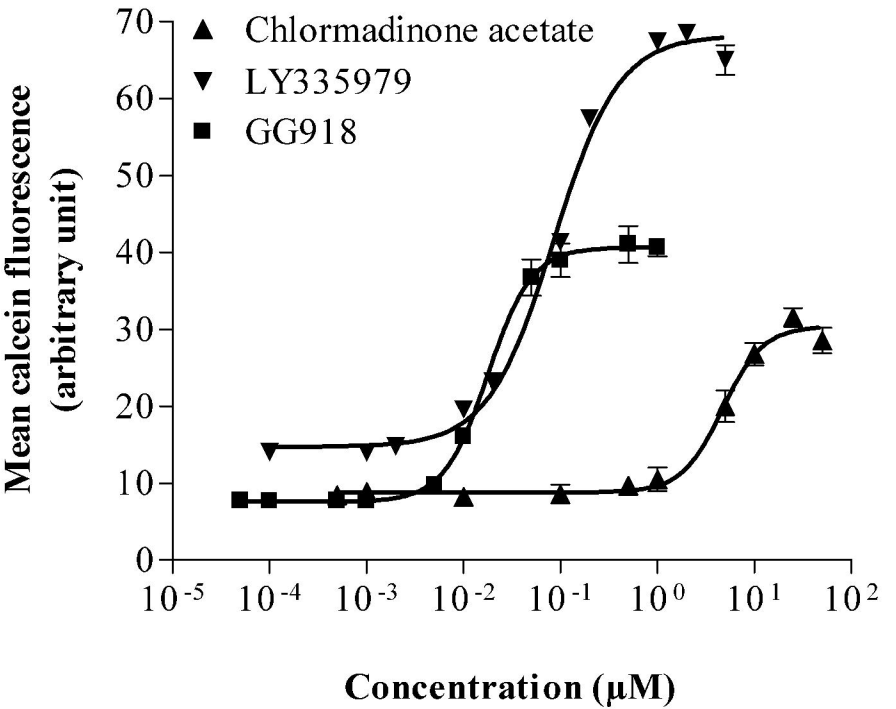

Chlormadinone acetate
LY335979
GG918
Mean calcein fluorescence (arbitrary unit)
70
60
50
40
30
20
10
0
10-5
10-4
10-3
10-2
10-1
100
101
102
Concentration (µM)

Figure 3b

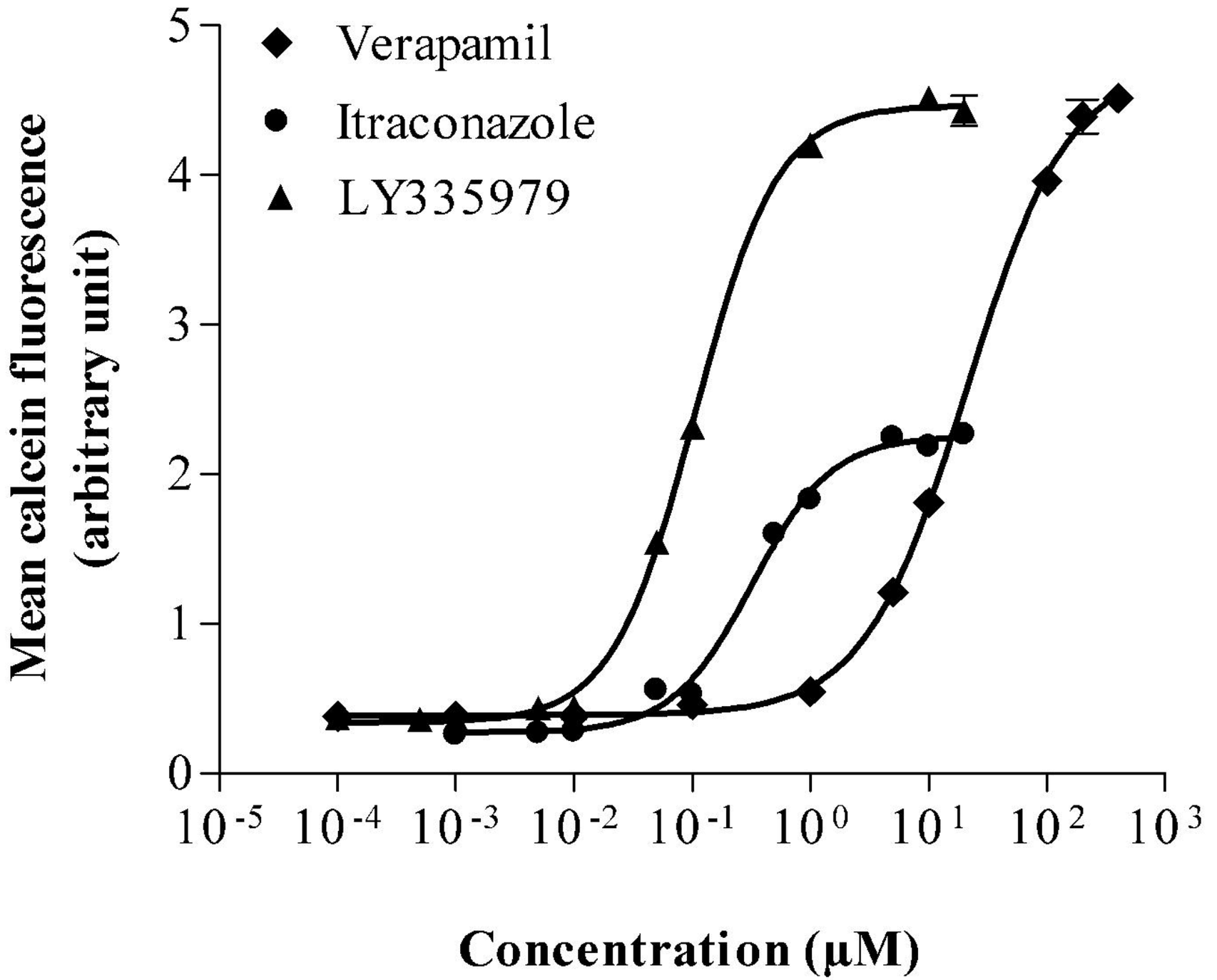

Verapamil
Itraconazole
LY335979
Mean calcein fluorescence (arbitrary unit)
0
1
2
3
4
5
10 -5
10 -4
10 -3
10 -2
10 -1
10 0
10 1
10 2
10 3
Concentration (μM)

Figure 3c

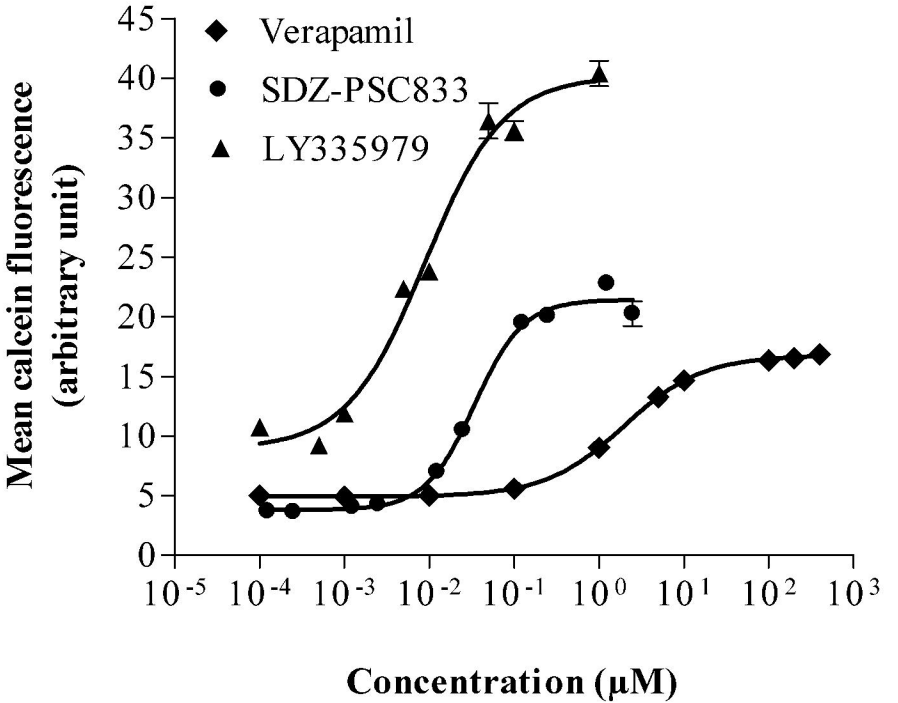

Verapamil
SDZ-PSC833
LY335979
Mean calcein fluorescence (arbitrary unit)
45
40
35
30
25
20
15
10
5
0
10-5 10-4 10-3 10-2 10-1 100 101 102 103
Concentration (μM)

Figure 4

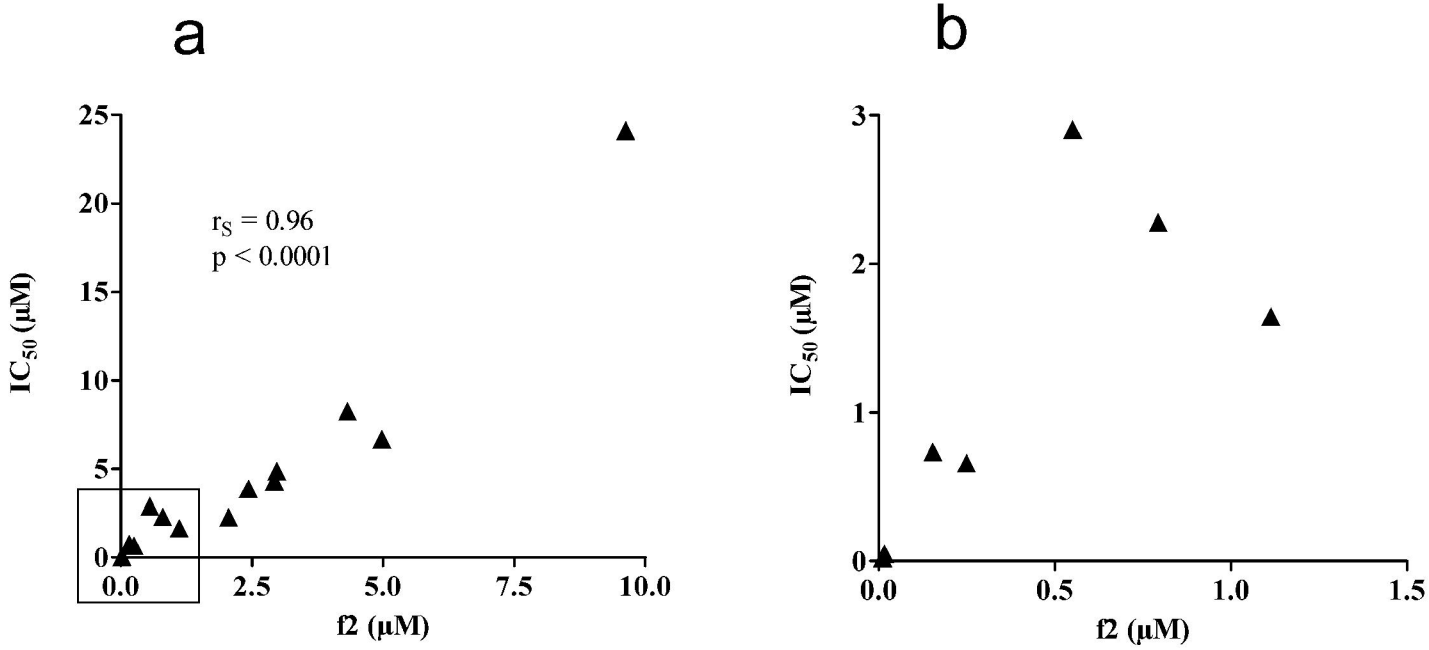
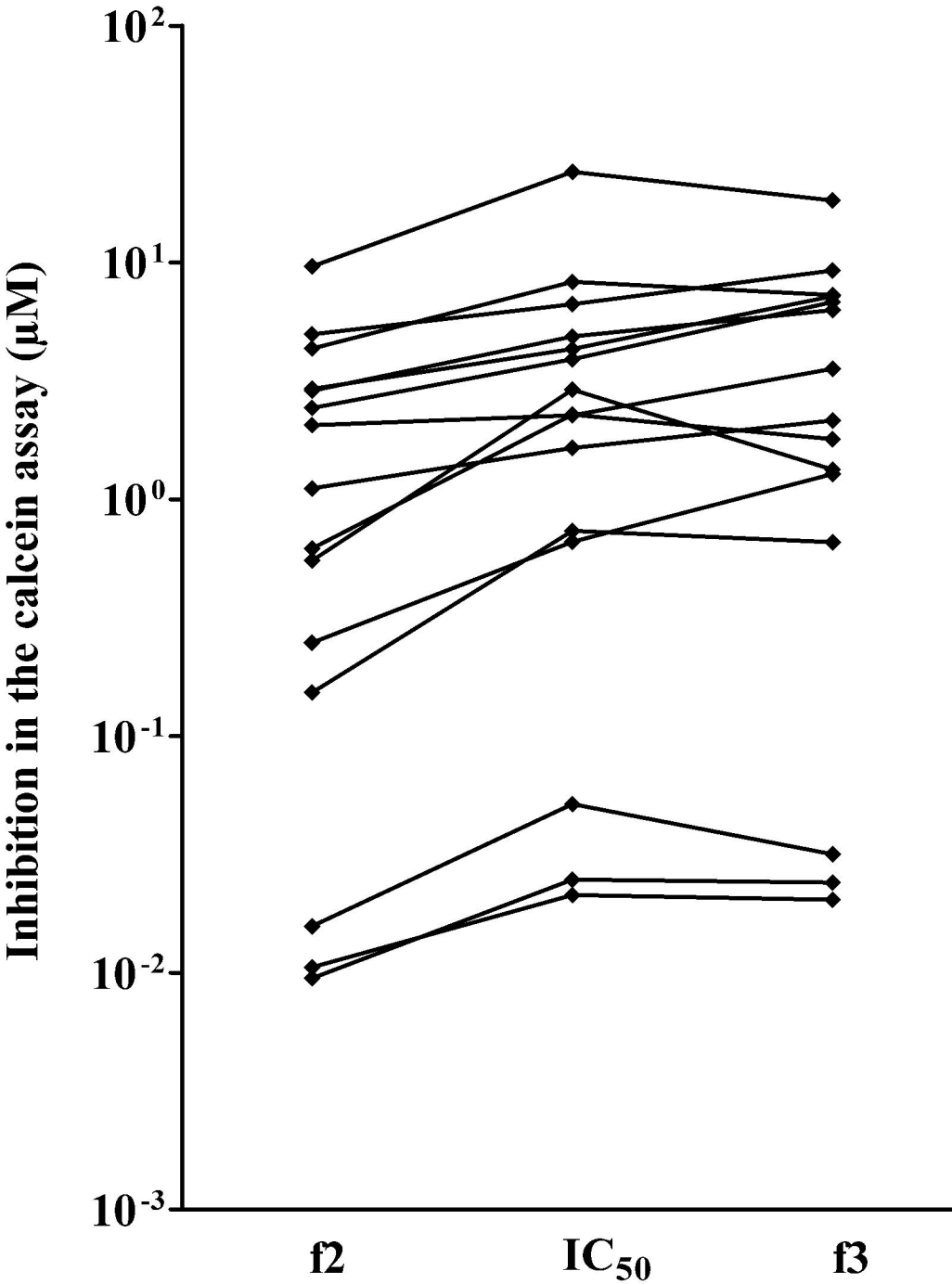

a
b
IC_{50} (µM)
f2 (µM)
r_S = 0.96
p < 0.0001
0
5
10
15
20
25
0.0
2.5
5.0
7.5
10.0
1
2
3
0.5
1.0
1.5

Figure 5


Inhibition in the calcein assay (µM)
10^2
10^1
10^0
10^-1
10^-2
10^-3
f2
IC50
f3