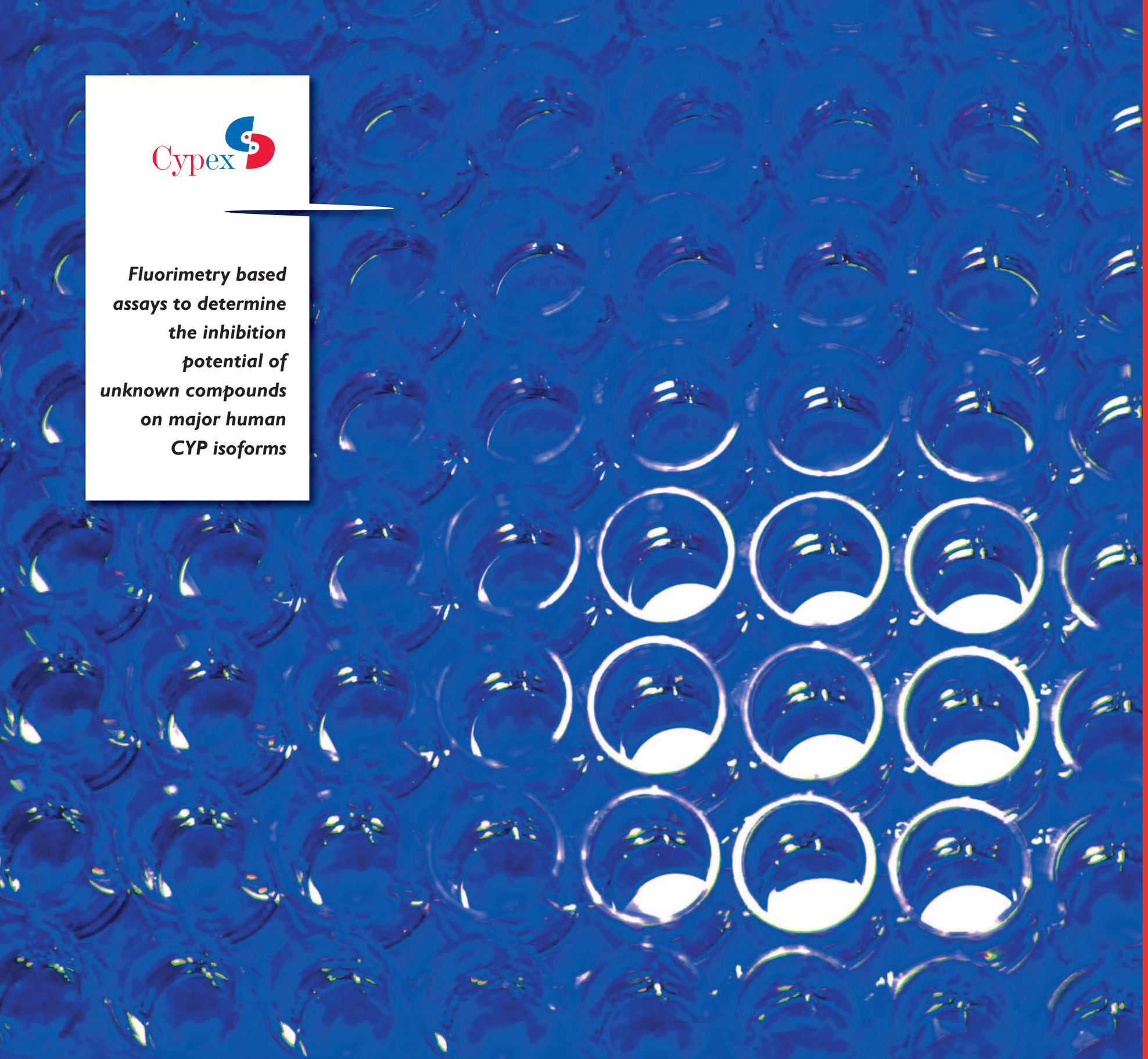

Cypex

Fluorimetry based assays to determine the inhibition potential of unknown compounds on major human CYP isoforms

Activity (compared to an uninhibited control) is followed for 30 mins at 7 different compound concentrations (100-0.1 µM) then fitted to a model using XLfit® software, before determining an IC_{50} value.

Shifts in IC_{50} value over time can indicate whether the unknown compound has the potential to be a time/metabolism-based inhibitor of each CYP isoform.

A positive control inhibitor is run in parallel on every plate, giving you confidence in the end result.

Suitable solvents are DMSO and Methanol up to 2.5% (v/v), assuming compound solubility.

Fast turnaround of data after receipt of compound, depending on number of compounds/isoforms to be assayed.

All work is carried out under Cypex's ISO9001:2008 accredited quality system.

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CYP inhibition screening using fluorometric assays

Figure 1 **Plate map for screening an indivdual CYP for inhibition by 10 compounds**

Column 1: Solvent controls for the plate
Column 2: Positive control (miconazole)
Columns 3-12: Test compounds

Row 8: Solvent controls for test compounds

The test and positive control compounds are serially diluted down the plate giving approximately 1000 fold concentration range from 0.14 µM - 100 µM.

The mean rates for the wells in column 1 are used as an internal QC for the plate:

Rates for individual test blank wells should fall within 20% of the mean rate in column 1. If rates in more than 33% of the test blank wells are outside the 20% limit then the plate must be repeated.

The mean rate in the wells in column 1 must be within two standard deviations of the mean rate derived from historical data.

The IC_{50} for the positive control inhibitor in column 2 (usually miconazole) must lie within two standard deviations of the mean IC_{50} derived from historical data.

Figure 2 **Typical raw data for two compounds**

The increase in fluorescence is plotted against time for 8 wells in a single column on the plate.

Each plate covers a full concentration range for a single compound.
Blank (yellow line) to 100 µM (red Line).

a Typical plot for a compound exhibiting direct inhibition

b Typical plot for a compound exhibiting direct inhibition in addition to a time dependent inhibition.

Figure 1

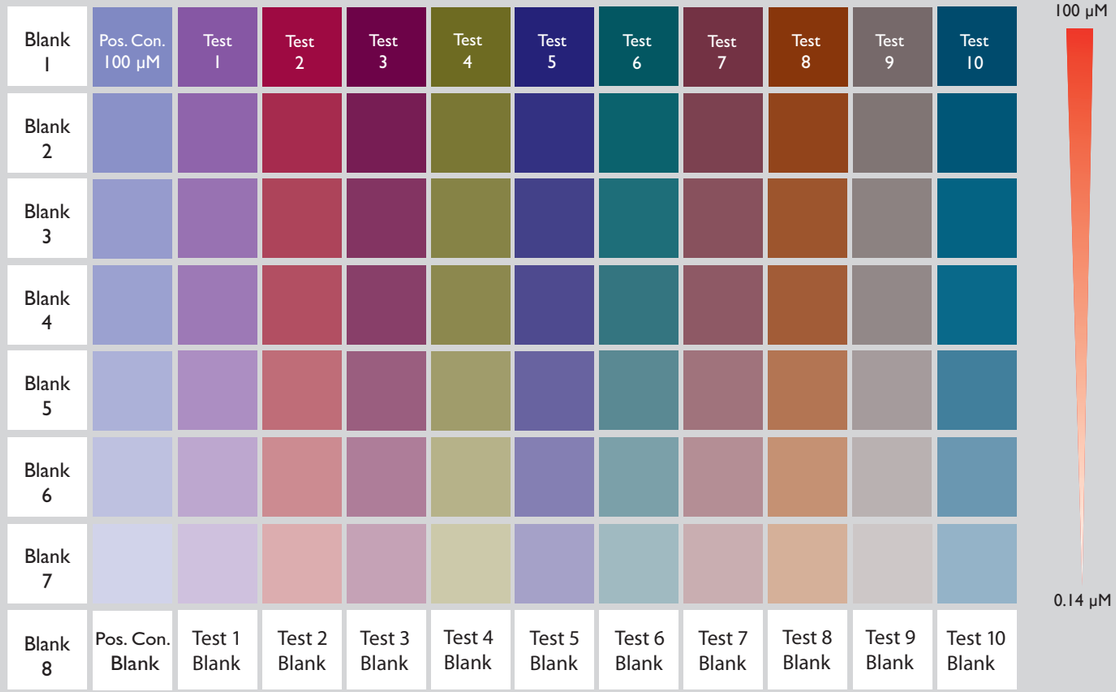


Figure 2a

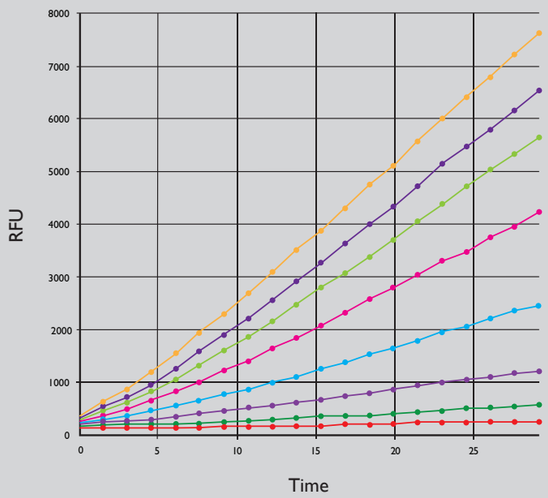


Figure 2b

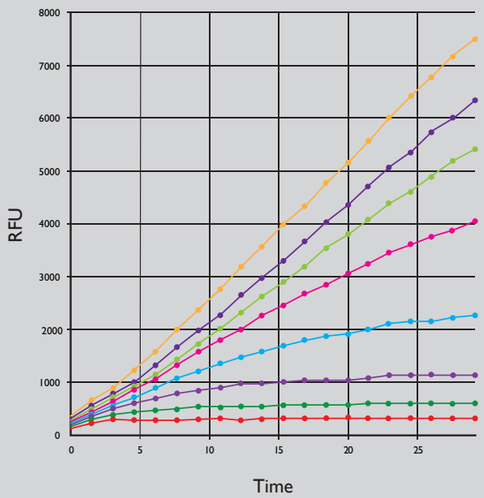
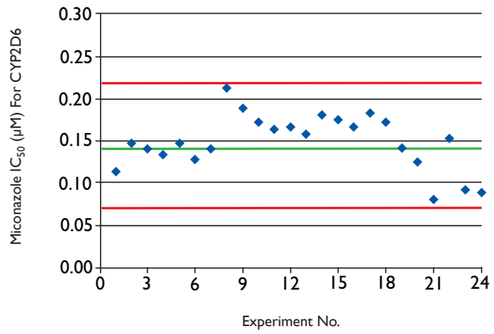


Figure 3



For each compound, the rates of increase in fluorescence at each concentration are expressed as a percentage of the control value and plotted in XLFit®. The IC_{50} is determined from the interpolated curve of best fit.

Typical positive control data over a number of experiments carried out with different batches of enzyme on different days are shown in **Fig. 3** demonstrating the reproducibility of the assay. The red lines mark ± 2 SD from the mean, the green line is the mean IC_{50}.

Because the increase in fluorescence is measured continuously over time it is possible to monitor changes in the IC_{50} of the compounds as the reaction progresses (**Fig. 4**).

For this analysis the rate of increase in fluorescence is calculated at each timepoint by taking the rate for 10.5 min spanning the nominal time (i.e. from 3 data points before to 3 data points after the nominal time). The IC_{50} is then plotted against time.

A decrease in the IC_{50} with time is a good indication that time/metabolism dependent inhibition (TDI/MDI) is occuring. Where a two fold reduction in the IC_{50} is observed over time then TDI/MDI is deemed to be present - **Fig 4c**.

Figure 4a No TDI/MDI

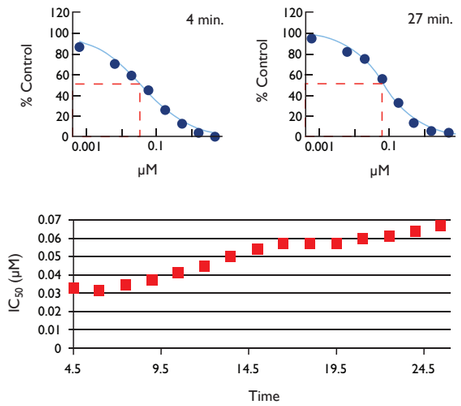


Figure 4b Possible TDI/MDI

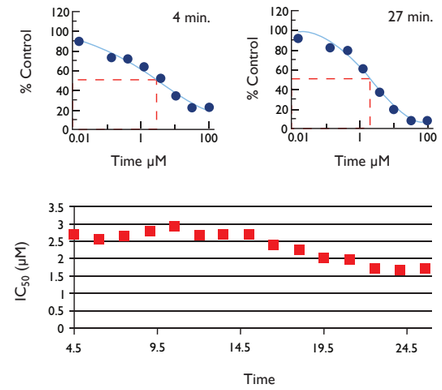
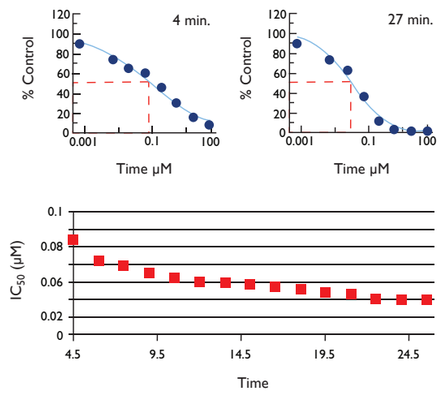


Figure 4c TDI/MDI



IC_{50} (µM) determined against each CYP isoform

Compound	Conc range tested (µM)	1A2	2C9	2C19	2D6	3A4
Furafylline	100-0.1	**76.6**	>100	**67.4**	**25.6**	**54.5**
Fluvoxamine	100-0.1	**0.08**	**1.2**	**<0.1**	**0.42**	**13.7**
Fluvoxamine	10-0.01	**0.07**	2.5	**0.07**	**0.41**	**>10**
Fluvoxamine	5-0.005	**0.11**	-	**0.05**	**0.52**	-
Fluvoxamine	2-0.002	-	-	**0.11**	-	-
Amiodarone	100-0.1	**59.1**	>100	**3.2**	**7.6**	**9.1**
Mibefradil	100-0.1	>100	**5.5**	**4.0**	**<0.1**	**0.15**
Mibefradil	10-0.01	-	-	-	-	**0.12**
Mibefradil	5-0.005	-	-	-	**0.08**	-
Isoniazid	100-0.1	>100	>100	**74.5**	**66.2**	**13.1**
Clopidogrel	100-0.1	**5.0**	**2.5**	**0.53**	**22.5**	**25.0**
Diltiazem	100-0.1	>100	**59.4**	>100	**73.4**	**29.3**
8-MOPS	100-0.1	**0.3**	**4.3**	**2.6**	**3.6**	**37.1**
8-MOPS	10-0.01	**0.5**	-	-	-	-
Ticlopidine	100-0.1	**15.2**	**8.4**	**0.21**	**0.43**	**22.3**
Montelukast	100-0.1	**28.8**	**0.56**	**2.2**	**4.3**	**2.3**
Montelukast	10-0.01	**>10**	-	-	-	-
Montelukast	5-0.005	-	**0.25**	-	-	-
Montelukast	2-0.002		**0.17**	-		-
Gemfibrozil	100-0.1	>100	**70.4**	**56.3**	>100	>100
Fluoxetine	100-0.1	>100	**4.0**	**<0.1**	**0.21**	**15.0**
Fluoxetine	10-0.01	>10	>10	**0.07**	**0.25**	-
Fluoxetine	5-0.005	-	-	**0.04**	**0.21**	-
Fluoxetine	2-0.002	-	-	**0.04**	-	-
Troglitazone	100-0.1	>100	>100*	**62.6**	>100	>100
Troglitazone	2.5-0.0025	-	**0.07**	-	-	-
Troglitazone	1-0.001	-	**0.04**	-	-	-
Sulphafenazole	100-0.1	>100	**0.40**	**62.0**	**92.9**	>100
Sulphafenazole	5-0.005	-	**0.23**	-	-	-
Sulphafenazole	2-0.002	-	**0.23**	-	-	-
Tienilic acid	100-0.1	>100	**0.23**	>100	>100	>100
Tienilic acid	5-0.005	-	**0.35**	-	-	-
Tienilic acid	2-0.002	-	**0.17**	-	-	-
Tranylcypromine	100-0.1	**46.9**	**3.4**	**1.1**	**10.1**	>100
Chloramphenicol	100-0.1	>100	>100	**2.0**	>100	>100
N-Bpb	100-0.1	>100	**7.3**	**0.13**	>100	>100
N-Bpb	10-0.01	-	-	**0.17**	-	-
Quinidine	100-0.1	>100	>100	**58.3**	**<0.1**	**17.9**
Quinidine	10-0.01	>10	>10	>10	**<0.01**	**>10**
Quinidine	2-0.002	-	-	-	**0.006**	-
Citalopram	100-0.1	>100	**76.6**	**55.9**	**2.2**	**58.9**
Paroxetine	100-0.1	**25.9**	**4.8**	**0.82**	**0.17**	**7.4**
Cinacalcet	100-0.1	**19.0**	**3.7**	**3.0**	**<0.1**	**5.4**
Cinacalcet	5-0.005	-	-	-	**0.009**	-
Ketoconazole	100-0.1	**45.3**	**0.71**	**1.4**	**11.9**	**<0.1**
Ketoconazole	5-0.005	-	-	-	-	**0.008**
Ketoconazole	2-0.002	-	-	-	-	**0.009**
TAO	100-0.1	>100	>100	>100	**86.6**	**8.4**
Erythromycin	100-0.1	>100	>100	>100	**90.5**	**19.6**
Verapamil	100-0.1	>100	**40.1**	>100	**19.5**	**9.2**
Fluconazole	100-0.1	>100	**4.8**	**1.3**	>100	**4.8**
Miconazole	10-0.01	**0.89**	**0.1**	**0.06**	**0.14**	**0.14**

- = Not Determined
***** = limited by solubility
8-MOPS = Methoxypsoralen
TAO = Troleandomycin
N-Bpb = R-(-)-N-Benzylphenobarbital