JWH-018 CYP450 Inhibition

Fluorescent Cytochrome P450 Inhibition Experimental Conditions:

Test Compound	Test Conc.	Cyp Assays	Reference Compound	Analytical Method		
	100.0					
	33.3					
	11.1					
	3.7	Cyp1A2/CEC	α-naphthoflavone			
	1.2	Cyp2C9/DBF	sulphaphenazole	Fluorescent		
	0.4	Cyp2C19/DBF	tranylcypromine	Plate		
	0.14	Cyp2D6/AMMC	quinidine	Reader		
JWH-018	0.046	Cyp3A4/DBF	ketoconazole			

Fluorescent Cyp450 IC50 Summary

Test Compound	Cyp1A2 /CEC	Cyp2C9 /DBF	Cyp2C19 /DBF	Cyp2D6/AMMC	Cyp3A4/BFC	Cyp3A4/DBF
Controls	0.62 α-naphthoflavone	0.10 sulphaphenazole	10 tranylcypromine	0.15 quinindine	0.18 ketoconazole	0.050 ketoconazole
JWH-018	3.0	1.4	2.8	>100	>100	8.0

Standard Methods:

Fluorescent cytochrome P450 IC50 determination: Cytochrome P450 inhibition is measured using fluorogenic substrates. Test agents and nsubstrates are dissolved in acetonitrile for this assay, as DMSO significantly inhibits some cytochrome P450s. Assays were performed at 37 °C using commercially available recombinant human cytochrome P450 expressed in insect cells. Enzyme concentrations and reactions times are optimized for each batch of enzyme to ensure a linear production of product over the course of the reaction. Percent remaining activity is calculated by comparing product formation of wells treated with test agent against wells treated with vehicle, after subtraction of background fluorescence. Percent inhibition is 100% - percent remaining activity. IC50 is calculated using a four-point logistic curve model. The individual reaction conditions are summarized in the following Table.

Cytochrome	Substrate	Assay Buffer
1A2	5 µM CEC	Buffer B
2A6	3 µM coumarin	Buffer D
2B6	15 µM MFC	Buffer B
2C8	1 µM DBF	Buffer E
2C9	1 µM DBF	Buffer A
2C19	2 µM DBF	Buffer B
2D6	1.5 µM AMMC	Buffer C
2E1	100 µM MFC	Buffer B
3A4	50 µM BFC	Buffer B
3A4	1 µM DBF	Buffer B
3A5	50 µM BFC	Buffer B

Buffer A: 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 25 mM potassium phosphate, 3.3 mM $MgCl_2$, pH 7.4.

Buffer B: 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 100 mM potassium phosphate, 3.3 mM $MgCl_2$, pH 7.4.

Buffer C: 8.2 ⌈M NADP, 0.41 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 100 mM potassium phosphate, 0.41 mM $MgCl_2$, pH 7.4.

Buffer D: 0.066 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 100 mM Tris hydrochloride, 3.3 mM $MgCl_2$, pH 7.5.

Buffer E: 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 50 mM potassium phosphate, 3.3 mM $MgCl_2$, pH 7.4.

Abbreviations:
AMMC: 3-[2-(N,N-Diethyl-N-methylammonium)ethyl]-7-methoxy-4-methylcoumarin
BFC:7-Benzyloxy-4-(trifluoromethyl)couamrin
CEC: 3-Cyano-7-ethoxycoumarin; DBF: dibenzylfluorescein
MFC: 7-Methoxy-4-(trifluoromethyl)coumarin