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Characterization of Potential Drug Interactions and Off-Target Activities of VL-2397, A Novel Antifungal Agent against Invasive Aspergillosis

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Abstract

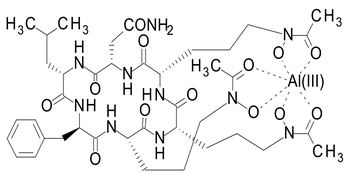
VL-2397, previously referred to as ASP2397, is a novel antifungal drug undergoing clinical development for the treatment of invasive aspergillosis (IA), a severe life-threatening fungal infection in immunocompromised individuals including high-risk cancer patients and allogeneic transplant recipients. VL-2397 is a cyclic metallohexapeptide that triggers a potent and rapid antifungal effect following active transport into fungal cells by siderophore iron transporter 1 (Sit1). Since mammalian cells lack Sit1, VL-2397 may be a much more specific antifungal agent compared with azoles which are notorious for drug-drug interactions (DDI). As part of a comprehensive nonclinical ADME characterization of VL-2397, studies were conducted to: 1) evaluate the inhibitory effects of VL-2397 on probe substrate metabolism by major cytochrome P450 isozymes, and 2) assess potential off-target activities in 54 ligand-binding assays using a variety of receptors, ion channels, and transporters as well as potential inhibitory activities in three enzyme assays.

The inhibitory activity of VL-2397 across a range of concentrations up to 500 µM was tested on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A using human liver microsomes. The IC_{50} values after 0 and 30 minutes pre-incubation were estimated to be > 500 µM for all CYP isozymes tested except CYP3A (midazolam), which was 206 µM and 160 µM, respectively. The selectivity of VL-2397 was calculated using inhibition ratios with each protein tested for off target binding activity. At 30 µg/mL, no inhibition > 30% was seen in any assay.

Collectively, the results suggest that VL-2397 has a low propensity for DDI because it does not tend to exert a time-dependent inhibitory effect on the CYP isozymes tested, and, at the drug exposure levels tested, appears to have a very low potential for off-target activity with a variety of cellular proteins tested. The favorable drug profiles presented here are consistent with the novel Sit1-dependent mechanism of action and suggest that VL-2397 may provide an effective therapeutic option for treating patients with IA infections.

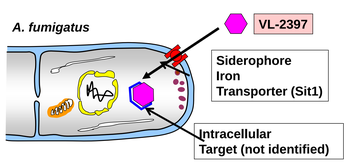
In the presence of VL-2397, the CYP isozyme-specific probe substrate was incubated with human liver microsomes. The formation of metabolite from probe substrate with CYP activity was detected using validated LC-MS/MS analytical methods, after which enzyme activity was calculated from the obtained metabolite concentration. In addition, the enzyme activity value was used to calculate the percent of control activity which was subsequently used for the calculation of IC_{50}. The inhibitory effects of VL-2397 on enzyme activities of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A were evaluated based on the percent of control activity and IC50 value as indicators. Typical inhibitors of CYP isozymes for direct and time-dependent inhibition were used as positive controls. For enzyme reactions, control, positive control and test samples were prepared with pre-incubation for 0 and 30 min.

VL-2397 Chemical Structure, Name, and Drug Product Formulation



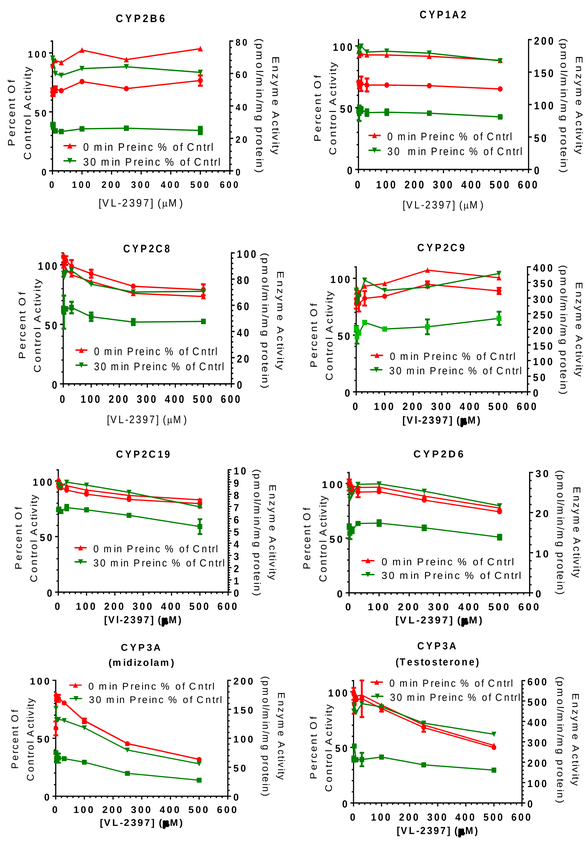
Cyclo{Asn-Leu-D-Phe-[(N^5-acetyl-N^5-hydroxy-Orn)-(N^5-acetyl-N^5-hydroxy-Orn)-(N^5-acetyl-N^5-hydroxy-Orn)]-} · Al(III) formulated in propylene glycol and ethanol at 150 mg/mL and diluted to 1.2 mg/mL in 5% Dextrose.

Putative Mechanism of Action



- Active transport of VL-2397 into *A.fumigatus* via Sit1 triggers antifungal effects
- An unidentified intracellular antifungal target for VL-2397 is presumed to exist
- Lack of Sit1 in mammalian cells may be responsible for higher selectivity

Impact of VL-2397 on Cytochrome P450 Isozyme Enzymatic Activity



Inhibitory effect of VL-2397 on enzyme activities of major cytochrome P450 isozymes CYP1A2 (phenacetin *O*-deethylation) CYP2B6 (bupropion hydroxyation), CYP2C8 (paclitaxel 6a-hydroxylation),CYP2D6 (dextromethrophan *O*-demethylation, CYP2C9 (diclofenac, 4'-hydroxylation), CYP2C19 ((S)-mephenytoin 4'-hydroxylation), CYP3A (midazolam1'-hydroxylation, and CYP3A (testosterone 6b-hydroxylation) were examined in human liver microsomes

VL-2397 IC_{50}Values (µmol/L) for CYP Isozyme Enzymatic Activities

CYP-mediated metabolism	0 min pre-incubation	30 min pre-incubation
CYP1A2 (phenacetin O-deethylation)	>500	>500
CYP2B6 (bupropion hydroxylation)	>500	>500
CYP2C8 (paclitaxel 6α-hydroxylation)	>500	>500
CYP2C9 (diclofenac 4'-hydroxylation)	>500	>500
CYP2C19 ((S)-mephenytoin 4'-hydroxylation)	>500	>500
CYP2D6 (dextromethorphan O-demethylation)	>500	>500
CYP3A (midazolam 1'-hydroxylation)	206	160
CYP3A (testosterone 6β-hydroxylation)	>500	>500

The IC_{50} values of VL-2397 after 0- and 30-min pre-incubation were 206 µM and 160 µM on CYP3A (midazolam), respectively. The IC_{50} values after 0- and 30-min preincubation were all estimated to be more than 500 µM on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A (testosterone).

Inhibitory Effect of Typical CYP Inhibitors: Enzyme Activity and Percent of Control Activity

CYP isozyme	0 min pre-incubation: Typical inhibitor	Enzyme activity (pmol/min/mg protein)	Percent of Control Activity	30 min pre-incubation: Typical inhibitor	Enzyme activity (pmol/min/mg protein)	Percent of Control Activity
CYP1A2	-	141 ± 3	100.0	-	92.2 ± 3.6	100.0
	Furafylline*	34.5 ± 3.0	24.5	Furafylline**	16.1 ± 1.2	17.5
	Furafylline**	94.2 ± 3.2	67.0			
CYP2B6	-	53.8 ± 1.0	100.0	-	29.9 ± 0.7	100.0
	Sertraline*	28.0 ± 0.7	52.0	Ticlopidine **	13.9 ± 0.6	46.6
	Ticlopidine **	48.6 ± 1.6	90.4			
CYP2C8	-	98.0 ± 6.4	100.0	-	61.2 ± 0.9	100.0
	Quercetin*	32.4 ± 1.6	33.1	Phenelzine**	20.1 ± 2.9	32.8
	Phenelzine**	50.8 ± 3.1	51.8			
CYP2C9	-	321 ± 2	100.0	-	226 ± 15	100.0
	Sulfaphenazole*	113 ± 4	35.1	Tienilic acid**	35.0 ± 1.1	15.5
	Tienilic acid**	194 ± 6	60.5			
CYP2C19	-	8.72 ± 0.21	100.0	-	7.00 ± 0.16	100.0
	Benzylnirvanol*	3.04 ± 0.18	34.8	Fluoxetine**	3.07 ± 0.02	43.9
	Fluoxetine**	6.13 ± 0.20	70.3			
CYP2D6	-	26.1 ± 0.4	100.0	-	17.4 ± 0.4	100.0
	Quinidine*	6.91 ± 0.42	26.5	Paroxetine**	5.20 ± 0.38	29.8
	Paroxetine**	21.1***	81.0			
CYP3A (midazolam)	-	199 ± 6	100.0	-	99.3 ± 3.9	100.0
	Ketoconazole*	26.5 ± 1.0	13.3	Verapamil**	32.9 ± 0.3	33.1
	Verapamil**	119 ± 4	59.5			
CYP3A (testosterone)	-	526 ± 12	100.0	-	261 ± 12	100.0
	Ketoconazole*	153 ± 10	29.1	Verapamil**	108 ± 4	41.3
	Verapamil**	395 ± 10	75.1			

Enzyme activity values represent the mean ± SD (n=3)
* Direct inhibitors
** Time-dependent inhibitors
*** Data is presented as the mean (n=2) for some operation error

Inhibitory Effect of Typical CYP Inhibitors: Enzyme Activity and Percent of Control Activity

Assay name	VL-2397 Inhibition (%)	Positive Substance Inhibition (%)
Adenosine A1 (Rat)	1.27	99.35 (DPCPX)
α1-Adrenergic receptor (Non-selective) (Rat)	2.09	100.00 (Prazosin)
α2-Adrenergic receptor (Non-selective) (Rat)	4.33	97.67 (Yohimbine)
β-Adrenergic receptor (Non-selective) (Rat)	3.64	100.00 ((±)-Propranolol)
Angiotensin AT1 (Human)	25.70	99.07 (Angiotensin II)
Angiotensin AT2 (Mouse)	5.80	99.07 (Angiotensin II)
Bradykinin B2 (Human)	0.00	99.11 (HOE140)
Ca Channel (Type L, Dihydropyridine) (Rat)	1.85	100.00 (Nitrendipine)
Ca Channel (Type N) (Rat)	2.44	100.00 (ω-Conotoxin GVIA)
CCK A (Human)	4.25	100.00 (CCK-8)
CCK B (Human)	0.00	99.94 (CCK-8)
CRF1 (Human)	0.00	100.00 (Urocortin human)
Dopamine D1 (Rat)	0.00	100.00 (R(+)-SCH-23390)
Dopamine D2 Short (Human)	0.00	100.00 ((+)-Butaclamol)
Dopamine Transporter (Human)	0.25	100.00 (GBR12909)
Estrogen (Rat)	0.59	99.79 (β-Estradiol)
Endothelin ETA (Human)	0.95	99.95 (Endothelin-1)
Endothelin ETB (Human)	2.67	98.59 (Endothelin-1)
GABA A (Agonist Site) (Rat)	11.35	99.79 (Muscimol)
GABA A (BZ Central) (Rat)	5.78	99.87 (Diazepam)
GABA B (Rat)	0.00	99.57 (GABA)
Glutamate (AMPA) (Rat)	0.00	99.51 ((S)-AMPA)
Glutamate (Kainate) (Rat)	0.00	100.00 (Kainic acid)
Glutamate (NMDA Agonist Site) (Rat)	7.67	100.00 (L-Glutamic acid)
Glutamate (NMDA Glycine Site) (Rat)	1.91	100.00 (MDL105,519)
Glycine (Strychnine Sensitive) (Rat)	1.53	96.14 (Strychnine)
Histamine H1 (Central) (Guinea pig)	0.33	100.00 (Pyrilamine)
Histamine H2 (Rat)	0.11	94.50 (Cimetidine)
Histamine H3 (Rat)	0.00	100.00 ((R)(−)-α-Methylhistamine)

Test substance concentration: 3.3 X 1X 10^{-5} µmol/mL positive substance concentration: 1×10^{-6} mol/L for HOE140, urocortin human and endothelin-1, or 1×10^{-5} mol/L for the others. Data expressed as the mean values of duplicate samples. The inhibition ratio was calculated from "100 – binding ratio".
Binding ratio: [(B – N) / (B_0 – N)] × 100 (%)
B: Bound radioactivity in the presence of test substance (individual value)
B_0: Total bound radioactivity in the absence of test substance (mean value)
N: Non-specific bound radioactivity (mean value)

Inhibition Effect of VL-2397 on Radioligand Binding to Various Receptors, Ion Channels and Transporters

Assay name	VL-2397 Inhibition (%)	Positive substance Inhibition (%)
K Channel KATP (Rat)	0.23	94.37 (Glybenclamide)
K Channel SkCa (Rat)	9.13	100.00 (Apamin)
Leukotriene B4 (Guinea pig)	1.25	97.56 (Leukotriene B_4)
Leukotriene D4 (Guinea pig)	1.32	100.00 (Leukotriene D_4)
Melatonin MT1 (Human)	3.46	100.00 (Melatonin)
Muscarinic (Non-selective) (Rat)	0.00	100.00 (Atropine)
Muscarinic M1 (Human)	0.00	100.00 (Atropine)
Muscarinic M2 (Human)	0.00	100.00 (Atropine)
Na Channel Site 2 (Rat)	1.85	100.00 (Dibucaine)
Neurokinin NK1 (Human)	0.00	99.30 (L-703,606)
Neurokinin NK2 (Human)	11.64	99.88 (Neurokinin A)
Neurokinin NK3 (Hu++++++man)	0.94	94.63 (Senktide)
Norepinephrine Transporter (Human)	17.56	99.31 (Desipramine)
Nicotinic (Neuronal) (Rat)	0.00	98.01 ((±)-Nicotine)
Opiate (Non-selective) (Rat)	0.00	99.84 (Naloxone)
Opiate µ (Human)	0.00	100.00 (DAMGO)
Oxytocin (Rat)	26.80	100.00 (Oxytocin)
PAF (Rabbit)	0.33	100.00 (PAF)
Serotonin 5HT1 (Non-selective) (Rat)	3.36	99.59 (Serotonin)
Serotonin 5HT2B (Human)	0.00	98.63 (Serotonin)
Serotonin Transporter (Human)	0.00	100.00 (Imipramine)
Sigma (Non-selective) (Guinea pig)	1.22	100.00 (Haloperidol)
Testosterone (Human)	0.93	99.14 (Testosterone)
Vasopressin V1 (Rat)	0.00	97.06 ([Arg^8]-Vasopressin)
VIP 1 (Human)	0.00	100.00 (VIP)

Test substance concentration: 3.3 x 10^{-5} mol/L, Positive substance concentration: 1×10^{-6} mol/L for leukotriene B_4, leukotriene D_4 and VIP, or 1×10^{-5} mol/L for the others. Data expressed as the mean values of duplicate samples. The inhibition ratio was calculated from "100 – binding ratio".
Binding ratio: [(B – N) / (B_0 – N)] × 100 (%)
B: Bound radioactivity in the presence of test substance (individual value)
B_0: Total bound radioactivity in the absence of test substance (mean value)
N: Non-specific bound radioactivity (mean value)

Inhibition Effect of VL-2397 on Various Enzymes

Assay name	VL-2397 Inhibition (%)	Positive substance Inhibition (%)
Acetylcholinesterase (Human)	3.06	99.32 (Eserine)
MAO-A (Rat)	0.00	97.11 (Clorgyline)
MAO-B (Rat)	0.04	94.23 (Ro 16-6491)

Test substance concentration: 3.3 X 10^{-5}mol/L, Positive substance concentration: 1×10^{-4} mol/L for Ro 16-6491, or 1×10^{-5} mol/L for the others. Data are expressed as the mean values of duplicate samples
The inhibition ratio was calculated from "100 – reaction ratio"
Reaction ratio: [(B – N)/(B_0 – N)] × 100 (%)
B: Radioactivity or fluorescence intensity in the tube or well for calculation of inhibition ratio (individual value)
B_0: Radioactivity or fluorescence intensity of the tube or well for calculation of total reaction (mean value)
N: Radioactivity or fluorescence intensity of the tube or well for calculation of non-specific reaction (mean value)

Conclusions

- The VL-2397 IC_{50} values after 0 and 30 min pre-incubations were estimated to be:
  - <500 mM for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYPC19, CYP2D6 and CYP3A (testosterone), and
  - 206 mM and 160 mM on CYP3A (midazolam)
- VL-2397 showed no significant inhibition of radioligand binding to any tested receptors, ion channels or transporters
- VL-2397 showed no significant inhibition of Acetylcholinesterase, or Monoamineoxidase A or B enzyme inhibition
- Based on lack of VL-2397 interaction with tested cytochrome P450s, receptors, ion channels, transporters and enzymes, drug-drug interactions with other therapeutics are unlikely