

Novel Derivative Of Arylideneimino-1,3-Pyrimidines (V-253-Met) As Potential Drug Candidates For The Treatment Of Pneumocystis Pneumonia and Other Fungal Infections



TGV
THERAPEUTICS



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Abstract

Pneumocystis pneumonia is a life-threatening lung disease caused by fungus *Pneumocystis* spp. Pneumocystis pneumonia (PCP) is still a frequent and severe opportunistic infection in immunocompromised patients.

Treatment of PCP remains a challenge due to high mutation rates of *Pneumocystis* spp. to standard compounds, and treatment-associated toxicity. Current therapeutic options do not address PCP with high breakthrough rates.

The goal of this study was to examine *in vitro* MICs and LD50 of the novel antifungal "V-253-Met", against *P. carinii* and *P. murina*.

Methods

P. carinii and *P. murina*, were distributed into triplicate wells of 48-well plates with final concentration of 7.5 log₁₀ nuclei/ml Pc and 6.5 log₁₀ Pm. Control and compound dilutions were added and incubated at 36°C, 5%CO₂. At 24-48-72h 10% of the well volume was removed and the ATP content was measured using luciferin-luciferase assay. IC₅₀ was calculated using INSTAT linear regression program. Triplicate IC₅₀ determinations were averaged. Oral LD50 of V-253-Met in both the mice and rats (10 in each group) was determined. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory. LD50 was calculated according to the method described by Litchfield and Wilcoxon. The minimal fungicidal concentration for *Candida glabrata*, *Cryptococcus neoformans* and *Histoplasma capsulatum* were determined by using microtiter assays in accordance with the CLSI guidelines.

Drugs:

- Pentamidine
- **V-253-Met** (SME, first-in-class antifungal, derivative of arylideneimino-1,3-pyrimidines)

Testing was performed by the NIH/NIAID

In vitro toxicity of V-253-Met

A549 %reduction in ATP/media control 24

V-253-met	24h	48h	72h
50µg/ml	0	21.50	2.38
10µg/ml	0	19.97	7.79
1µg/ml	0	24.59	5.30
IC50	-	-	>100µg/ml

V-253-Met had no toxic effect on cultured A549 cells after 72 hours exposure.

In vivo toxicity of V-Met-253

LD50 values

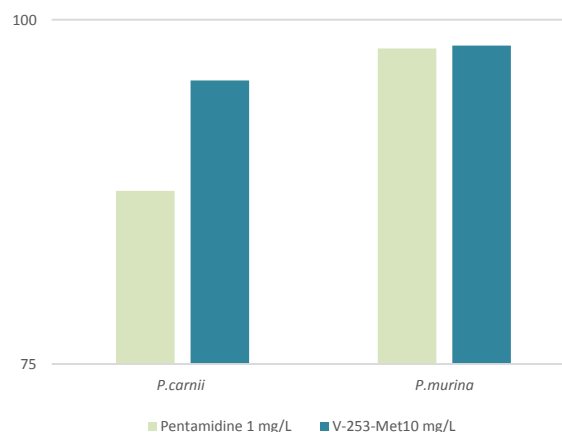
Mouse	Rat
7980 ± 750 mg/kg	13880 ± 920 mg/kg

Results

Average V-253-Met IC50 Determinations

Fungal strain	Hour (mg/L)		
	24 h	48 h	72 h
<i>P. carinii</i>	3.90	2.56	1.61
<i>P. murina</i>	3.30	1.50	0.165

Percent reduction in ATP/media control



V-253-Met demonstrated moderate activity in *P. carinii* and marked activity in *P. murina* when tested in a cell-free, suspension culture *in vitro* system. Reduction in ATP occurred in a time and dose dependent manner over the 72-hour testing period.

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Minimal Fungicidal Concentration

Species	Isolate No	Met-253 mg/L	Fluconazole mg/L
<i>C. glabrata</i>	CG1	1	16
<i>C. glabrata</i>	CG2	1	64
<i>C. glabrata</i>	CG3	1	32
<i>C. neoformans</i>	CN2	2	64
<i>H. capsulatum</i>	HC2	0.5	N/A

Conclusion

The toxicity of V-253-met was >100µg/ml, no toxicity for A549 cultured cell line. The drug candidate is of very low acute toxicity in the rat and mouse models.

A compound has shown marked effectiveness against *Pneumocystis in vitro* and no toxicity is considered for the following for PK/PD analysis and testing in the *in vivo P. murina* model.

Additional testing revealed marked activity against poorly curable strains of *C. glabrata* and *C. neoformans* that are the causative agents of a variety of currently poorly curable diseases including those associated with a high mortality rate.

Finally, our study (some data were generated by NIH/NIAID core) demonstrated that novel antifungal V-253-Met possesses high antifungal activity and low toxicity.

Further development of V-253-Met is suggested.

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