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

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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.carnii* and *P. murina*.

Methods

P.carnii and *P.murina*, were distributed into triplicate wells of 48-well plates with final concentration of 7.5 log10 nuclei/ml Pc and 6.5log10 Pm. Control and compound dilutions were added and incubated at 36^{O}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 arylidenimino-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

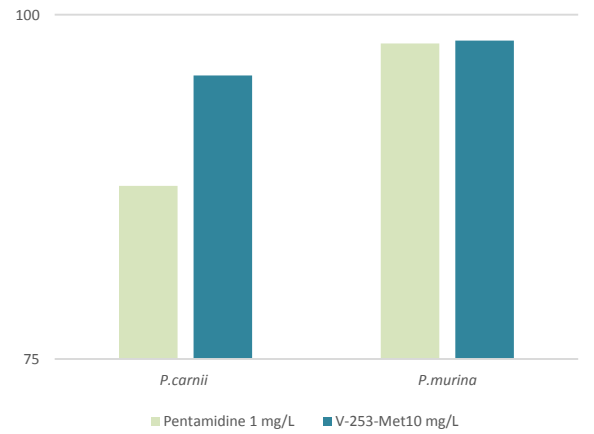
Mouse	Rat
7980 ± 750 mg/kg	13880 ± 920 mg/kg

Results

Average V-253-Met IC50 Determinations

Fungal strain	Hour (mg/L)		
	24 h	48 h	72 h
P.carnii	3.90	2.56	1.61
P.murina	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
C. glabrata	CG1	1	16
C. glabrata	CG2	1	64
C. glabrata	CG3	1	32
C. neoformans	CN2	2	64
H. capsulatum	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 anti-fungal activity and low toxicity.

Further development of V-253-Met is suggested.

References

•Tetz, G., Cynamon, M., Hendricks, G., Vikina, D., & Tetz, V. (2017). **In vitro activity of a novel compound, Mul-1867, against clinically significant fungi candida spp. and aspergillus spp.** *International Journal of Antimicrobial Agents.*

•Tetz G, Tetz V. 2016. **Study of Mul-1867 a Drug Candidate for Inhalation Therapy of Pulmonary Exacerbations in Patients With Cystic Fibrosis Against Mixed Infections Caused by Clinical Isolates of P.aeruginosa and A.fumigatus in Murine Lung Infection Model** *Pediatric Pulmonology,* The 30th Annual North American Cystic Fibrosis Conference October 27–29, 2016, Vol. 51,

•Tetz, G., & Tetz, V. (2016). **WS03. 6 Study of Mul-1867, a drug candidate for inhalation therapy clinical isolates of P. aeruginosa, S. aureus and C. albicans in murine lung infection model.** *Journal of Cystic Fibrosis*, *15*, S6.

•Tetz, G., Vikina, D., & Tetz, V. (2016). **Antimicrobial activity of mul-1867, a novel antimicrobial compound, against multidrug-resistant Pseudomonas aeruginosa**. *Annals of Clinical Microbiology and Antimicrobials*, *15*(1), 1.

•Tetz, G., & Tetz, V. (2015). **In vitro antimicrobial activity of a novel compound, Mul-1867, against clinically important bacteria Antimicrobial Resistance and Infection Control** 4:45.

•Clinical and Laboratory Standards Institute (2008). **M38-A2. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: second edition. Clinical and Laboratory Standards Institute**, *Wayne, PA 2008.*

•Rodriguez-Tudela JL, Arendrup MC, Cuenca-Estrella M, Donnelly JP, Lass-Flörl C. (2010) **EUCAST breakpoints for antifungals. Drug News Perspect, 23:93–97.**