Evaluation of *in vitro* and *in vivo* anti-*Toxoplasma gondii* activity of acridine and acridone derivatives

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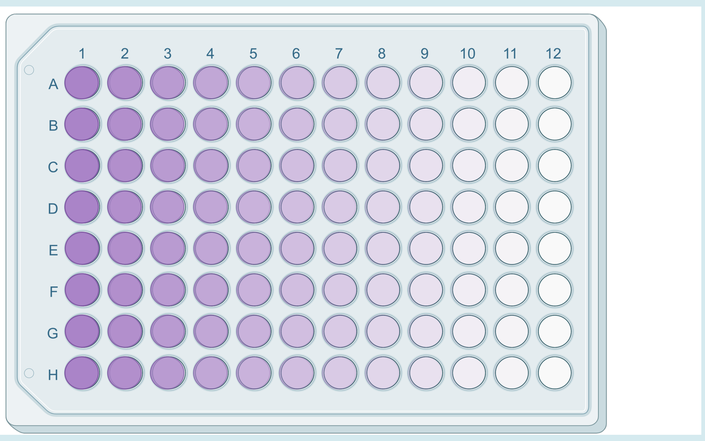
INTRODUCTION

Toxoplasma gondii is a ubiquitous protozoan parasite with a worldwide distribution that infects up to one third of the human population. Although usually asymptomatic in healthy individuals, toxoplasmosis, a disease caused by *T. gondii* poses a major health threat, causing life-threatening complications particularly in immunocompromised patients and fetuses. Considering the scarcity of the currently available treatment options for toxoplasmosis, especially those able to eradicate the parasite from the chronically infected hosts, the pursuit of novel drug candidates remains a top priority.

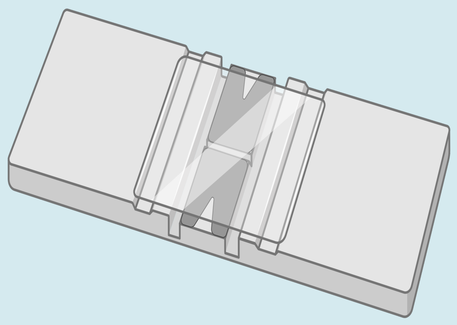
OBJECTIVE

The aim of this study was to evaluate selected acridine (AADs) and acridone (ACRs) derivatives, for both *in vitro* and *in vivo* anti-*T. gondii* activity.

METHODS

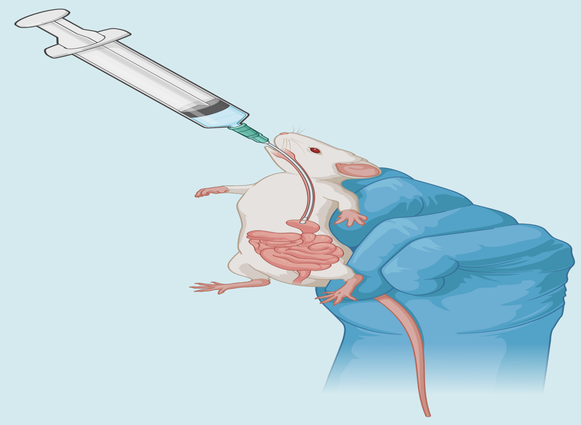


The MTT colorimetric assay, a widely used method for assessing the cytotoxicity of newly synthesized compounds, was used to evaluate the toxic effect of derivatives on non-infected Vero cells. All derivatives and positive control (combination of pyrimethamine and sulfadiazine, PYR+SDZ) were investigated in five different concentrations, using two-fold serial dillution method. CC50 values were obtained using GraphPad Prism. 10.4.1.



Anti-*T. gondii* activity screening on extracellular forms was evaluated after incubating *T. gondii* RH strain tachyzoites for 24h with the 25μM concentration of investigated AADs and ACRs and postive control. Reduction rates were calculated compared to untreated control.

To evaluate activity of the investigated AADs and ACRs on the intracellular forms of *T. gondii*, modified MTT assay was employed after confirming maximal cellular invasion of tachyzoites using light microscopy. EC50 values were obtained using GraphPad Prism 10.4.1.

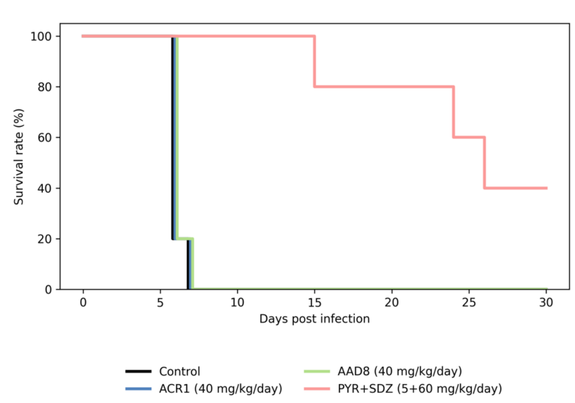


One acridine (AAD8) and one acridone derivative (ACR1), recognized as best overall candidates were evaluated in experimental models of toxoplasmosis using Swiss Webster mice, both acute and chronic, and compared to standard tratment option (PYR+SDZ).
In both models, investigated derivatives and standard treatment option were administered orally for five consecutive days, after establishing acute /chronic stage of infection.

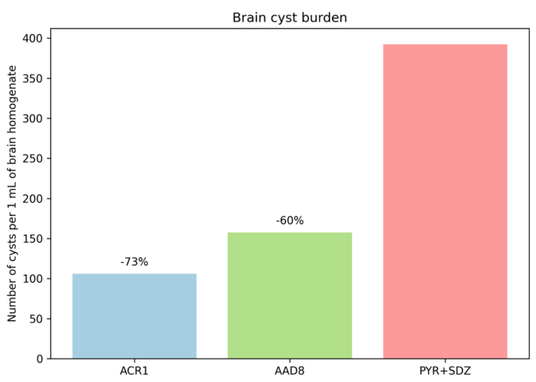
RESULTS

DERIVATIVE	CC50 value (μM)	Viability reduction of *T. gondii* tachyzoites (%)	EC50 value (μM)
AAD1	41.72	24.7	0.752
AAD2	60.22	25.8	0.727
AAD3	88.37	15.2	1.649
AAD4	133.70	4.5	49.68
AAD5	54.66	0	0
AAD6	154.10	22.0	0.867
AAD7	92.95	20.5	1.776
AAD8	102.00	33.3	0.477
AAD9	50.19	21.9	8.374
AAD10	176.80	21.9	0.879
AAD11	105.10	36.8	0.547
AAD12	121.50	36.6	0.575
AAD13	86.29	24.1	0.691
AAD14	90.38	28.1	0.644
AAD15	124.90	31.5	0.581
AAD16	148.80	34.7	0.587
ACR1	107.20	39.6	0.531
ACR2	112.20	41.1	0.646
ACR7	28.00	35.2	0.598
SDZ + PYR	2056 + 8.22	35.6	72.62 + 0.29

Acute toxoplasmosis - survival curves.



Chronic toxoplasmosis - brain cyst burden comparison between groups.



Investigated derivatives have displayed CC_{50} values ranging from 28.00 to 176.80 μM, which is in accordance with the cytotoxicity of the acridine derivatives in general. Four derivatives displayed superior activity on extracellular forms compared to standard treatment option, while all investigated derivatives were superior on intracellular forms of *T. gondii*, with EC_{50} values ranging from 0.477 μM (AAD8) to 49.68 μM (AAD4).

Derivatives AAD8 and ACR1 did not prolong survival of mice in model of acute toxoplasmosis. However, both derivatives provided significant reduction in mice brain cyst burden during chronic toxoplasmosis, i.e., 73% reduction for ACR1 and 60% for AAD8.

CONCLUSION

The obtained results indicate that the evaluated acridine and acridone derivatives possess significant anti-*T. gondii* activity, with favorable selectivity profiles. These findings highlight their potential as promising lead compounds for the development of new therapeutic agents against *T. gondii*, warranting further structural optimization and determination of mechanism of action.

ACKNOWLEDGMENT

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