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Mycophenolic Acid and Its Derivatives as Potential Chemotherapeutic Agents Targeting Inosine Monophosphate Dehydrogenase in *Trypanosoma congolense*

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This study aimed to evaluate the trypanocidal activity of mycophenolic acid (MPA) and its derivatives for *Trypanosoma congolense*. The proliferation of *T. congolense* was completely inhibited by adding <1 μM MPA and its derivatives. In addition, the IMP dehydrogenase in *T. congolense* was molecularly characterized as the target of these compounds. The results suggest that MPA and its derivatives have the potential to be new candidates as novel trypanocidal drugs.

Trypanosoma congolense causes animal African trypanosomiasis (AAT) in livestock. The lack of effective vaccines makes the use of chemotherapeutic agents the most effective measure for controlling AAT. Limited numbers of commercial drugs have long been used to treat AAT. The emergence of drug-resistant trypanosomes and cases of drug-refractory trypanosomiasis have been reported (1–4), underscoring the need for development of new drugs.

A candidate target for drug development is IMP dehydrogenase (IMPDH). This enzyme is very important in the *Trypanosoma* spp. because it lacks a *de novo* purine synthesis pathway, which makes the purine nucleotide synthesis in these parasites solely dependent on a salvage pathway in the glycosomes (5–7). IMPDH converts IMP into XMP through this pathway, which is a rate-limiting step in the metabolism of guanine nucleotides (8). Mycophenolic acid (MPA), compound 1, is a well-known IMPDH inhibitor (Fig. 1). Its enzymatic activity has already been proven in many protozoan parasites (9–14). The antiprotozoan activities of MPA against *Babesia* spp. were reported in *in vivo* and *in vitro* studies (9, 15). Thus, the activity of MPA against IMPDH

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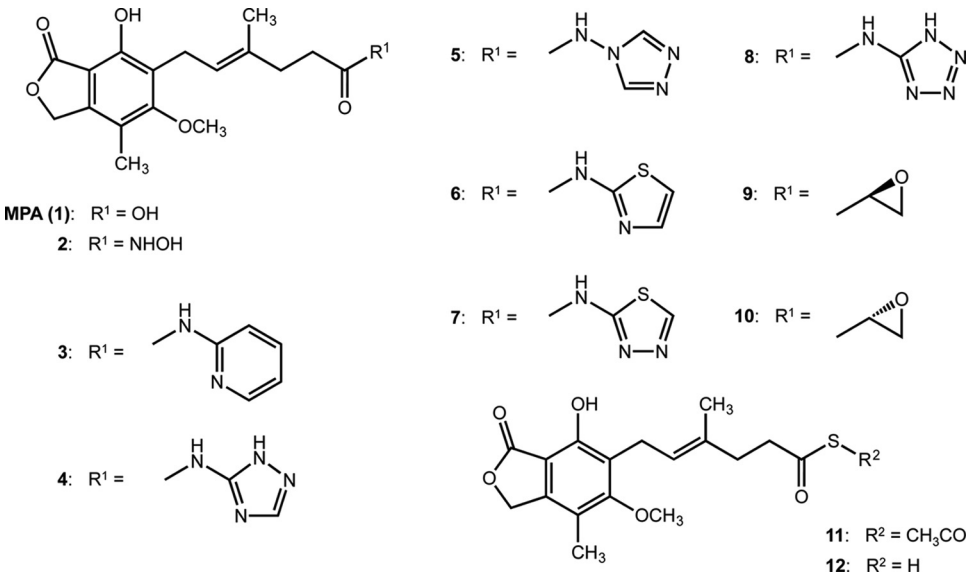

FIG 1 The structures of mycophenolic acid (MPA) and its derivatives.

TABLE 1 Trypanocidal activity of MPA and its derivatives

Compound	Inhibition rate (%)[a]		
	T. congolense	*T. b. brucei*	*T. evansi*
1 (MPA)	99.60 ± 0.38	82.99 ± 2.82	90.53 ± 1.22
2	94.46 ± 3.89	5.24 ± 13.12	14.21 ± 8.64
3	2.36 ± 8.64	7.83 ± 10.35	16.66 ± 5.55
4	98.87 ± 0.78	46.13 ± 5.21	42.79 ± 4.58
5	4.65 ± 15.29	14.29 ± 34.17	32.43 ± 4.88
6	1.45 ± 10.94	22.27 ± 4.81	17.11 ± 6.14
7	4.59 ± 15.12	14.50 ± 13.76	29.44 ± 10.03
8	3.59 ± 14.06	22.99 ± 12.90	19.94 ± 8.44
9	0.06 ± 8.66	9.28 ± 5.15	11.99 ± 1.59
10	3.15 ± 8.43	9.03 ± 7.91	9.49 ± 6.13
11	6.51 ± 14.38	16.47 ± 6.97	12.79 ± 4.49
12	3.03 ± 12.91	11.56 ± 4.17	13.61 ± 8.67
Pentamidine[b]	99.93 ± 0.07	99.96 ± 0.06	99.94 ± 0.07
Control[c]	0.00 ± 1.74	0.48 ± 1.58	−0.24 ± 2.25

[a] Trypanocidal activity of MPA (compound 1) and 11 MPA derivatives (see Fig. 1) at a concentration of 1 μM was evaluated for *T. congolense* IL3000 strain, *T. b. brucei* GUTat 3.1 strain, and *T. evansi* Tansui strain. The inhibition rate was calculated from 3 independent experiments and expressed as the mean inhibition rate ± SD.
[b] Pentamidine 500 ng/ml was used as a 100% inhibition control.
[c] HMI-9 medium with 0.25% dimethyl sulfoxide (DMSO) was used as a 0% inhibition control.

is expected to lead to a novel strategy for the development of trypanocides.

The novel *IMPDH* orthologue of *T. congolense* (*TcIMPDH*) (accession no. LC094350) was identified from *T. congolense* resequencing data (unpublished data). The recombinant *Tc*IMPDH showed IMPDH activity *in vitro* (see Fig. S1A and B in the supplemental material). The nanomolar levels of MPA clearly inhibited NADH production by *Tc*IMPDH in a dose-dependent manner (50% inhibitory concentration [IC_{50}] = 26.2 nM) (see Fig. S1C in the supplemental material). The expression profile and cellular localization of *Tc*IMPDH were analyzed by Western blotting and immunofluorescence microscopy. *Tc*IMPDH was expressed in glycosomes as granulated forms throughout the life cycle stages of *T. congolense* (see Fig. S2 in the supplemental material). *Tc*IMPDH was expressed at similar levels in bloodstream form (BSF), procyclic form (PCF), and epimastigote form (EMF). In contrast, *Tc*IMPDH expression in the metacyclic form (MCF) was significantly lower than in the other stages ($P < 0.05$, Tukey's multiple-comparison test). This result suggests that purine synthesis is highly important in the proliferative stages of the parasite but not in the nonproliferative MCF stage.

The aim of this study was to reveal the trypanocidal activities of MPA derivatives for developing effective trypanocidal drugs. Various inhibitory activities and the cell-differentiation activity of MPA derivatives against mammalian cells have been reported *in vitro*. Some MPA derivatives (compounds 2, 4, 9, and 10) have shown particularly significant inhibitory activities against human IMPDH and were observed to induce erythroid differentiation in K562 cells (16, 17). The earlier reports suggested that some MPA derivatives might be specific inhibitors for *Trypanosoma*. The chemical structures of the MPA derivatives in this study are shown in Fig. 1. We evaluated the trypanocidal activity against *T. congolense*, *T. b. brucei*, and *T. evansi* using an ATP-based luciferase viability system (18). To evaluate the trypanocidal activity of MPA (compound 1) and its derivatives *in vitro*, BSFs were cultivated with 1 μM of each compound. At 1 μM, nine derivatives showed <10% anti-*T. congolense* activity (Table 1). In contrast, only three compounds, 1, 2, and 4, inhibited *T. congolense* growth by 99.60 ± 0.38%, 94.46 ± 3.89%, and 98.87 ± 0.78% at 1 μM, respectively (Table 1). Although compound 1 showed high trypanocidal activity against *T. b. brucei* and *T. evansi*, compounds 2 and 4 showed lower inhibitory activities at 1 μM against *T. b. brucei* and *T. evansi* than against *T. congolense* (Table 1). The low plasma membrane permeability of compounds 3, 5, 6, 7, 8, 11, and 12 might account for their low trypanocidal activity, while the low trypanocidal activity of compounds 9 and 10 against all of the tested trypanosome species and of compound 2 against *T. b. brucei* and *T. evansi* suggests their low affinity with these trypanosome IMPDHs or the deactivation of these compounds by other species-specific enzymes in cytosol. The IC_{50}s of compounds 1, 2, and 4 to *T. congolense* were 0.10 ± 0.04, 0.56 ± 0.21, and 0.16 ± 0.04 μM, respectively (Table 2). The IC_{50}s of these three compounds to MDBK cells were 0.52 ± 0.12, 1.40 ± 0.18, and 0.84 ± 0.21 μM, respectively. The selectivity indices of MPA and the two derivatives in *T. congolense* were 5.14, 2.62, and 5.10, respectively (Table 2). However, the higher IC_{50}s and lower selectivity indices of these three compounds were shown in *T. b. brucei* and *T. evansi* (Table 2). The cytotoxicity of these compounds was higher than that of commercial drugs (19). However, the IC_{50}s of compounds 1 and 4 for *T. congolense* BSF were comparable to those of two commercially available trypanocides (pentamidine [0.17 μM] and diminazene [0.11 μM]) against *T. congolense* (18). These results suggest that compounds 1, 2, and 4 might be potential lead compounds in the development of trypanocides, especially against *T. congolense*.

To clarify the mode of action of compounds 1 and 4 in trypanosomes, the effects of guanosine and xanthine supplementation on the trypanocidal effects of these compounds were examined. The IC_{50}s of compounds 1 and 4 were increased by guanosine in a dose-dependent manner (Table 3), while xanthine supplementation did not alter the IC_{50}s of either compound 1 or compound 4 in *T. congolense* BSF (Table 3). These results suggest that guanosine was transported into the *T. congolense* BSF and converted into GMP as a purine nucleotide source, while no xanthine was transported or converted into XMP by hypoxanthine-guanine phosphoribosyltransferase in *T. congolense*. We therefore concluded that the proliferation inhibitory

TABLE 2 IC_{50} and selectivity index of MPA and MPA derivatives 2 and 4 against *T. b. brucei* and *T. evansi*

Compound	IC_{50} (μM)[a] for:				Selectivity index[a,b] for:		
	T. congolense	*T. b. brucei*	*T. evansi*	MDBK cell	*T. congolense*	*T. b. brucei*	*T. evansi*
1 (MPA)	0.10 ± 0.04	0.62 ± 0.05	0.61 ± 0.002	0.52 ± 0.12	5.14	0.84	0.85
2	0.56 ± 0.21	>2.5	>2.5	1.4 ± 0.18	2.62	ND	ND
4	0.16 ± 0.04	1.26 ± 0.009	1.38 ± 0.10	0.84 ± 0.21	5.10	0.67	0.61

[a] All values were calculated from 3 independent experiments and expressed as means ± SD.
[b] Mean IC_{50} of MDBK cells/mean IC_{50} of trypanosomes. ND, not determined.

TABLE 3 Effects of guanosine and xanthine on parasite proliferation under IMPDH inhibition by MPA and *N*-(2,3,5-triazolyl) mycophenolic amide (compound 4)

Guanosine or xanthine supplementation (μM)	IC_{50} (μM)[a] with:			
	Guanosine for compound:		Xanthine for compound:	
	1 (MPA)	4	1 (MPA)	4
250	>5.0	>5.0	0.09 ± 0.001	0.21 ± 0.01
50	0.29 ± 0.19	0.50 ± 0.31	0.09 ± 0.003	0.22 ± 0.01
0	0.07 ± 0.006	0.13 ± 0.02	0.09 ± 0.004	0.22 ± 0.02

[a] All values were calculated from 3 independent experiments and are shown as means ± SD.

effects of MPA against *T. congolense* BSF were caused by the inhibition of intracellular *Tc*IMPDH.

Hypoxanthine and inosine were predicted to be the main purine sources in *T. brucei* (20). Hypoxanthine and inosine have also been shown to be present in the blood at higher concentrations than other purines (21), suggesting their roles as the main purine sources in trypanosomes and that they are supplied via the salvage pathway. The concentration of purine bases and nucleosides in the extracellular environment is lower than that in the intracellular environment (21). *T. brucei* spp. proliferate in blood circulation and then invade the central nervous system through the blood-brain barrier (22, 23), while *T. congolense* only proliferates in blood circulation by adhesion to the vascular endothelium (24). In conclusion, MPA and its derivatives might therefore also inhibit trypanosome proliferation *in vivo*, particularly in *T. congolense*.

Nucleotide sequence accession number. The sequence for the novel *IMPDH* orthologue of *T. congolense* (*TcIMPDH*) can be found in the GenBank database under accession no. LC094350.

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