Synthesis and antiproliferative activity of new mycophenolic acid conjugates with adenosine derivatives

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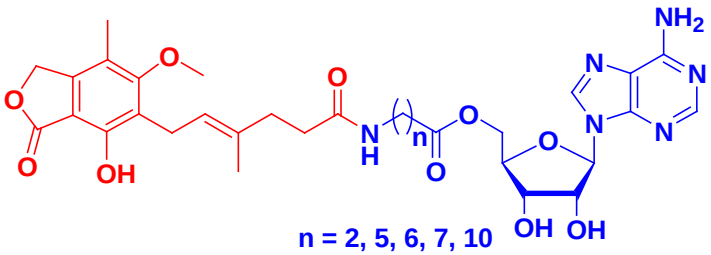
Abstract

New conjugates of mycophenolic acid (MPA) and adenosine derivatives were synthesized and assessed as potential immunosuppressants on Jurkat cell line and peripheral blood mononuclear cells (PBMC) from healthy donors. As compared to MPA, all compounds were found to be more active against Jurkat cell line. The antiproliferative activities were compared with MPA and adenosine, in both 2',3'-*O*-isopropylidene protected and free hydroxyl groups possessing forms. The obtained results were also discussed in terms of selectivity index, defined as $SI=IC_{50}/EC_{50}$.



mycophenolic acid (MPA) **5'-*O*-(ω-aminoalkylcarboxyl)adenosines**

Keywords: mycophenolic acid; adenosine derivatives; antiproliferative activity; esterification

1. Introduction

Mycophenolic acid (MPA) **1** (Figure 1) is a reversible, uncompetitive and potent inosine-5'-monophosphate dehydrogenase (IMPDH) inhibitor. This compound is currently used as immusuppressive drug. It is available as prodrugs: CellCept (mycophenolate mofetil (MMF), Roche AG) and Myfortic (mycophenolic acid sodium salt (MPS), Novartis Pharma AG) [1-5]. Despite high efficiency, MPA causes some severe side-effects within gastrointestinal tract, genitourinary system, circulatory system and nervous system. In addition, it undergoes glucuronidation *in vivo*, which limits its application in chemotherapy. Therefore, new MPA analogs are still desired.

On the other hand, adenosine **2** (Figure 1) exerts immunosuppressive effect *via* its receptors on immune cells. It interacts with the immune system in two ways, both as stimulant and immunosuppressant; however, its short half-time limits these effects *in vivo*. To improve pharmacokinetic properties of adenosine, many analogs of adenosine conjugates were synthesized and examined. Some of them were registered as drugs and others still are tested in clinical trials [6-10]. Hence, we decided to design novel MPA analogs possessing adenosine counterparts to improve pharmacological features of both compounds.

Numerous structural modifications of MPA have been reported. It was revealed that free phenol group and aromatic methyl substituent, presence of the lactone ring, *trans* configuration in the side chain (important for interactions between carboxylic group and IMPDH) are crucial for sustained biological activity. Various phenols, non-phenolic analogs or monocyclic amines were tested, however, pharmacological properties of the majority of them were worse than parent MPA. The modifications of carboxylic group in the side chain and phenol group in aromatic ring led to potent activity towards mouse leukemia and Ehrlich tumors. The immunosuppressive activities of some analogues occurred to be comparable with MPA [11-16]. Noteworthy, the conjugate of adenosine analog and fentanyl derivative (opioid receptor

antagonist) protected against decrease of blood pressure during septic shock [17], and the conjugates of adenosine and mycophenolic acid acted as inosine-5'-monophosphate dehydrogenase inhibitors [18].

In our current studies we attempted to modify MPA **1** towards novel adenosine derivatives with antiproliferative potential. According to the results published by Felczak [18], mycophenolic adenine dinucleotide (MAD) analogs bearing bis(phosponate) linker were resistant to hydrolysis and exhibited high inhibition towards IMPDH together with anticancer activity. Noteworthy, adenosin-5'-yl mycophenolate **3** despite containing ester bond, which should be susceptible to cellular esterases, also revealed significant activity. Moreover, D-adenosin-5'-yl mycophenolate occurred to be more active than its unnatural L-enantiomer, which might be caused by resistance of this type of compounds to glucuronidation. Recently, we reported conjugates of N^6-(ω-aminoalkyl)adenosines with MPA [19], where several obtained compounds gave similar or better antiproliferative activity *in vitro* than parent MPA **1**. These results encouraged us to synthesize adenosin-5'-yl esters of *N*-mycophenoylamino acid derivatives **4** (Figure 1) and investigate their antiproliferative activity.

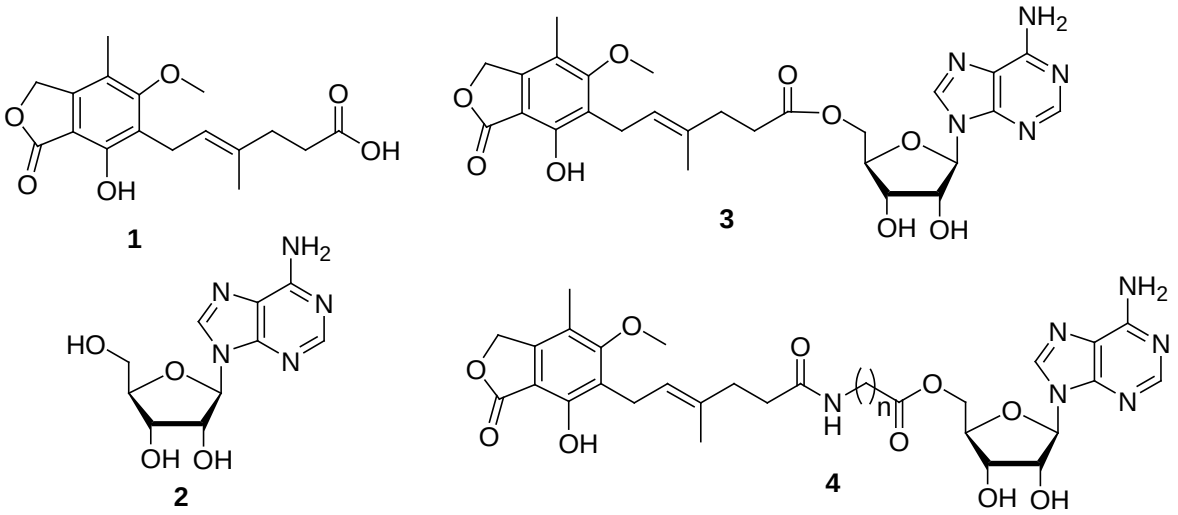
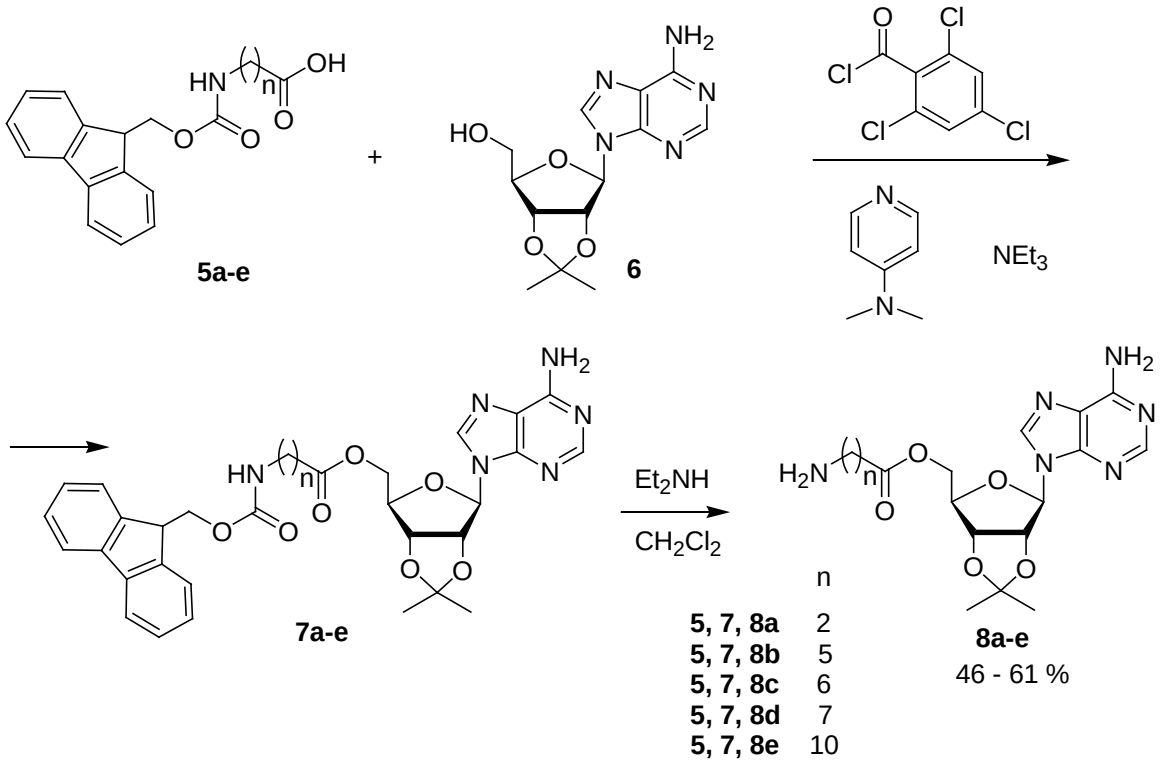


Figure 1. Mycophenolic acid (MPA) **1**, adenosine **2** and its adenosine derivatives **3**, **4**.

2. Results and discussion

2.1. Chemistry

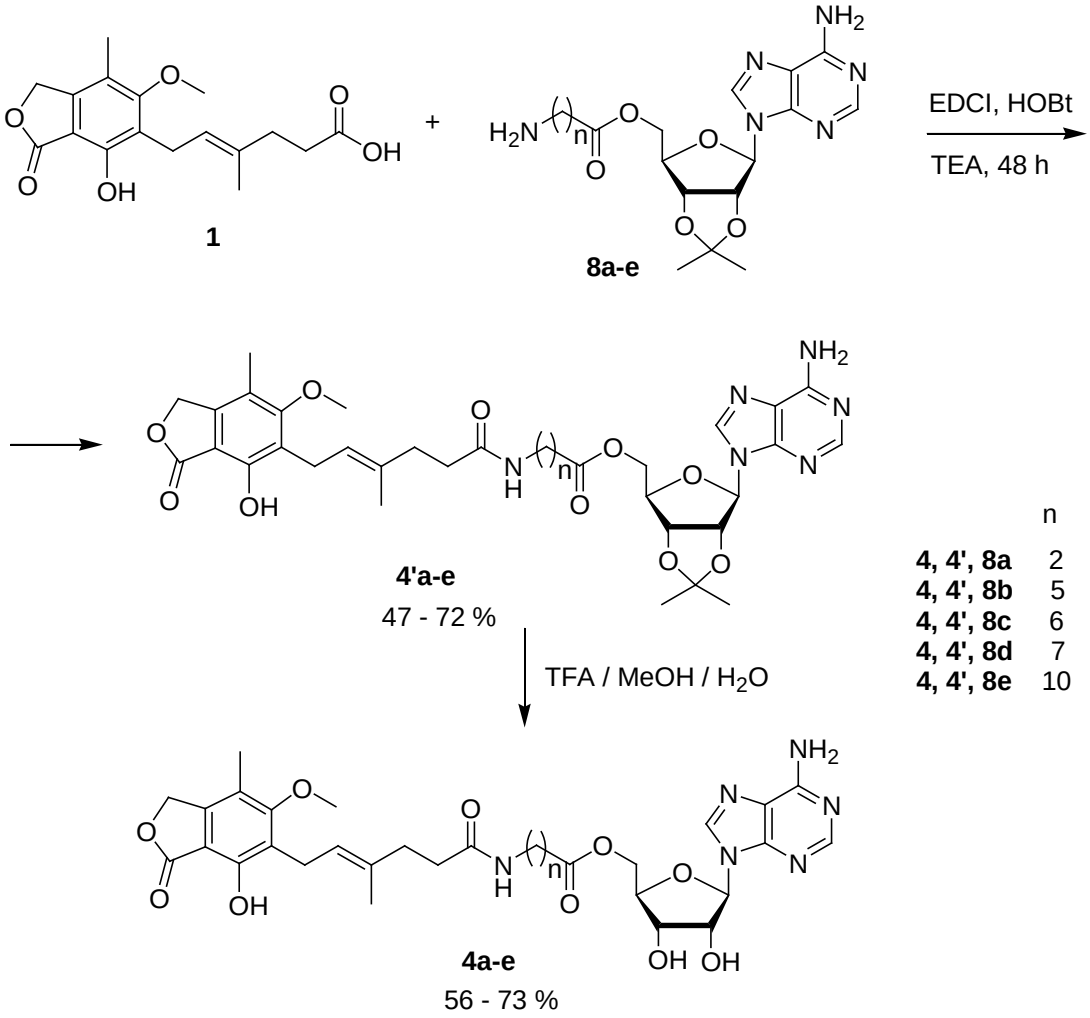
Synthetic pathway included preparation of 2',3'-*O*-isopropylideneadenosin-5'-yl esters of amino acids **8a-e** (Scheme 1) followed by coupling with MPA **1** (Scheme 2). First, *N*-(fluorenylmethyloxycarbonyl)amino acids **5a-e** were converted to 2',3'-*O*-isopropylideneadenosin-5'-yl esters of *N*-(fluorenylmethyloxycarbonyl)amino acids **7a-e** under Yamaguchi esterification with 2',3'-*O*-isopropylideneadenosine **6**. This method of ester bond formation under mild conditions was applied in case of multifunctional compounds and typically used 2,4,6-trichlorobenzoyl chloride to generate respective mixed anhydride and 4-dimethylaminopyridine DMAP as catalyst [20-21]. Then, esters **7a-e** were deprotected to amines **8a-e** in the reaction with diethylamine [22].



Scheme 1. Synthesis of 2',3'-*O*-isopropylideneadenosin-5'-yl esters of amino acids **8a-e**.

Subsequently, coupling of MPA **1** with amines **8a-e** was optimized with several condensing agents, and the highest yields and purities of amides **4'a-e** were achieved with 1-ethyl-3-(3'-

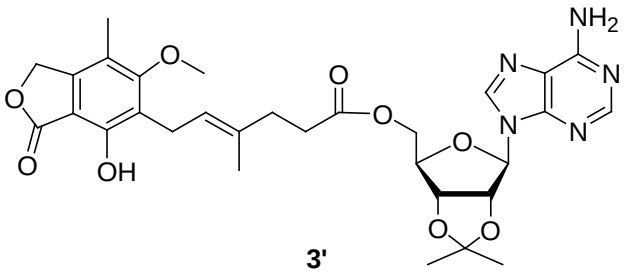
dimethylaminopropyl)carbodiimide (EDCI) / *N*-hydroksybenzotriazol (HOBt). Finally, 2',3'-*O*-isopropylideneadenosin-5'-yl esters of *N*-mycophenoylamino acids **4'a-e** were selectively hydrolyzed to adenosin-5'-yl esters of *N*-mycophenoylamino acids **4a-e** (Scheme 2). To avoid undesired hydrolysis of ester bond we tested several conditions to remove isopropylidene moiety from riboside unit and the best results were achieved with mixture of trifluoroacetic acid TFA and methanol 1:1 (v/v).



Scheme 2. Synthesis of 2',3'-*O*-isopropylideneadenosin-5'-yl esters of *N*-mycophenoylamino acids **4'a-e** and adenosin-5'-yl esters of *N*-mycophenoylamino acids **4a-e**

2.2. Biological evaluation

Since 2',3'-*O*-isopropylidene moiety did not exclude biological activities of adenosine [23], we considered for *in vitro* investigations also protected derivatives **4'a-e** together with those reported in literature [18] analog **3'** (Figure 2) without any amino acid linker as reference.



3'

Figure 2. 2',3'-*O*-isopropylideneadenosin-5'-yl mycophenolate **3'**.

Antiproliferative properties of compounds **3**, **3'**, **4'a-e**, **4a-e** were investigated on Jurkat cell line to research their activity against human lymphoid cell line (Table 1). Inhibition of proliferation was measured as EC_{50} from incorporation of ^{3}H-TdR, whereas tolerance of cells towards **3**, **3'**, **4'a-e**, **4a-e** was taken as IC_{50} in viability test with MTT (λ = 570 nm). These assessments were also performed against peripheral blood mononuclear cells PBMC from healthy donors as reference to select the most promising derivatives for further examinations as potential drug candidates. Results concerning cytotoxicity of investigated compounds are collected in Table 2.

Table 1. EC_{50} [μM] values of conjugates **3'**, **4'a-e**, **3**, **4a-e,** and MPA **1** for cell line Jurkat and activated PBMC, obtained in antiproliferation test.

Compounds	Jurkat			PBMC		
	EC_{50}	*p*	*F*	EC_{50}	*p*	*F*
3'	1.640±0.932	0.0019	13.025	0.984±0.492	<0.0001	82.8
4'a	1.322±0.108	0.0053	9.3904	0.147±0.0805	0.0081	122.14
4'b	1.384±0.393	0.0039	10.432	1.107±0.415	0.0001	28.164
4'c	0.814±0.543	0.0012	14.821	0.407±0.271	<0.0001	43.36
4'd	0.133±0.0266	0.0021	12.646	0.400±0.0354	<0.0001	57.475
4'e	1.2612±1.735	<0.0001	5.2767	39.737	0.252±0.0631	<0.0001
3	1.229±0.158	0.0014	14.165	0.351±0.0281	<0.0001	628.43
4a	4.995±0.562	0.0004	20.846	12.019±6.712	0.1113	48.47
4b	4.980±2.197	<0.0001	42.125	1.318±0.314	<0.0001	160.11
4c	1.435±0.431	<0.0001	43.393	1.292±0.0363	<0.0001	67.535
4d	1.407±0.240	<0.0001	36.048	0.422±0.0374	<0.0001	1319.9
4e	3.055±1.594	<0.0001	162.86	0.266±0.0664	0.0047	9.8017
1	30.592±12.306			0.0299±0.00403		

p-statistical significance, F-Fisher test

Derivatives bearing blocked hydroxyl groups with 2',3'-*O*-isopropylidene moiety **4'a** and **4'e** exhibited similar activity when compared with MPA **1** and analogs without amino acid linker **3**, **3'**. On the other hand, deprotected compounds **4a** and **4e** possessing free hydroxyl groups were less cytotoxic than MPA **1**, and **3**, **3'**. In general, removal of 2',3'-*O*-isopropylidene protection decreased cytotoxicity apart from **4d**. Noteworthy, derivative **4a** was found around 100 times less toxic against Jurkat cell lines and around 10 times less toxic against PBMC than MPA **1.** The cytotoxicity increased with the length of the amino acid linker, which could be due

to higher susceptibility to proteases and other cell enzymes. Lower stability probably resulted in faster increase in the concentration of toxic metabolites in the cell and caused higher toxicity, however this dependence did not match **4'e** and **4e**.

Table 2. IC_{50} [μM] values of conjugates **3'**, **4'a-e**, **3**, **4a-e,** and MPA **1** for cell line Jurkat and activated PBMC.

Compounds	Jurkat			PBMC		
	IC_{50} (MTT)	*p*	*F*	IC_{50} (MTT)	*p*	*F*
3'	0.164±0.06187	<0.0001	48.182	0.328±0.0328	0.1603	5.396
4'a	1.470±0.1866	<0.0001	28.358	0.881±0.0587	0.0009	16.586
4'b	0.830±0.0553	<0.0001	16.943	0.553±0.415	0.001	15.915
4'c	0.407±0.0271	<0.0001	26.805	0.136±0.0502	0.0052	9.4702
4'd	0.533±0.0266	<0.0001	25.694	0.133±0.0266	0.0005	19.277
4'e	1.328±0.332	0.0076	5.2767	0.378±NAN	0.0001	29.178
3	0.878±NAN	0.0238	3.9123	0.172±0.00351	0.0005	19.435
4a	35.745±6.447	<0.0001	40.264	15.453±1.897	0.0055	9.2732
4b	41.891±NAN	0.1109	2.2758	0.440±0.146	<0.0001	58.923
4c	1.292±0.175	<0.0001	19.707	0.574±0.316	0.0002	23.778
4d	0.2814±0.0844	0.0002	11.195	0.281±0.141	<0.0001	50.5559
4e	1.461±0.217	0.0127	0.0127	13.150±2.966	0.0052	9.4624
1	0.624±0.0937			2.185±1.561		

p-statistical significance, F-Fisher test

In the case of antiproliferative properties (Table 1), all EC_{50} values of **3**, **3'**, **4'a-e**, **4a-e** against Jurkat cell line were lower in comparison to MPA **1**. The protected analog **4'd** was the most active one (EC_{50} value of 0.133 μM). In contrary, none of tested compounds revealed higher

activity against PBMC than MPA **1**. Among linked derivatives **4'a-e**, **4a-e**, compound **4e** possessing 11 carbon atoms in amino acid chain revealed the lowest EC_{50} value (0.266 µM).

In most cases, 2',3'-*O*-isopropylidene protection increased both toxicity and antiproliferative activity of esters of *N*-mycophenoylamino acids **4'a-e**, **4a-e.** It could be due to diminished susceptibility towards adenosine deaminases [24]. This phenomena was especially evident for derivative **4b**, where IC_{50} against Jurkat cell line was 41.891 µM, and protected analog **4b'** gave IC_{50} value of 0.83 µM. Deprotection of **4b** to **4'b** decreased EC_{50} value from 4.980 µM to 1.384 µM. The stability of both analogs **4b**, **4'b** with Jurkat cell line was followed with high-performance liquid chromatography (HPLC) and compared with MPA **1** (see electronic supplementary information). The sample was taken each day and after 3 days MPA **1** was consumed in ca. 60 % (Fig. S-1), **4b** in ca. 70 % (Fig. S-2), and **4'b** in ca. 85 % (Fig. S-3). In other words, conversion of MPA **1** to conjugate **4b** accelerated metabolic activity, and protection with 2',3'-*O*-isopropylidene strengthened this effect additionally.

Subsequently, selectivity index SI (Table 3) was calculated for examined compounds to estimate their therapeutic use. The most promising result (SI = 49.500) gave analog **4e**. Although EC_{50} value was lower than in the case of MPA **1** (Table 1) against PBMC, derivative **4e** was less toxic against Jurkat and PBMC as well (Table 2).

Table 3. Selectivity index SI = IC_{50} / EC_{50} of conjugates **3'**, **4'a-e**, **3**, **4a-e,** and MPA **1**.

Compounds	SI	
	Jurkat	PBMC
3'	0.100	0.333
4a'	1.111	6.000
4b'	0.600	0.750
4c'	0.500	1.000
4d'	4.000	1.333
4e'	1.000	5.000
3	0.714	0.489
4a	7.156	1.286
4b	8.412	0.333
4c	0.900	0.444
4d	0.200	0.667
4e	0.478	49.500
1	0.020	73.069

3. Conclusions

To sum up, synthesis of 2',3'-*O*-isopropylideneadenosin-5'-yl esters of amino acids **8a-e** followed by their coupling with MPA **1** to 2',3'-*O*-isopropylideneadenosin-5'-yl esters of *N*-mycophenoylamino acids **4'a-e** and adenosin-5'-yl esters of *N*-mycophenoylamino acids **4a-e** was worked out. Both toxicity and antiproliferative properties of designed compounds **4'a-e**, **4a-e** were initially investigated to correlate length of the amino acid chain with potential use as immunosuppressive agents. According to the obtained results, compound **4e** gave the most promising activity and is considered for further studies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at….

Acknowledgements

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Conflict of interest

The authors declare no competing interest.

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