SYNTHESIS AND BIOLOGICAL ACTIVITY OF MYCOPHENOLIC ACID-AMINO ACID DERIVATIVES

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Abstract

In search of new immunosuppressants we synthesized 11 amino acids derivatives of MPA as methyl esters **10a-k** using EDCI/DMAP and their corresponding amino acid derivatives in free acid form **11a-k** by hydrolysis of ester group with LiOH/MeOH. New analogs were evaluated as growth inhibitors of lymphoid cell line (Jurkat) and human peripheral blood mononuclear cells (PBMC) from healthy donors. According to obtained results recovering of free carboxylic group increased their activity. Additionally, the cytotoxic properties depends on the substituent and configuration at chiral center in amino acid unit. The compounds **10j, 11e** and **11h** exhibited higher potency than MPA **1** *in vitro*.

1. Introduction

Transplantation is the optimal treatment for selected patients with end-stages organ failure, increasing life expectancy and improving quality of life. Research in the field of immunosuppression has been continuous since 1954, when at Boston performed the first life-sustaining transplant [1,2]. The first successful chemical immunosuppressant was 6-mercaptopurine. Its derivative, azathioprine, is still used today [1]. Thereafter were discovered a number of new compounds including mycophenolic acid (MPA) **1** (Fig. 1) and its derivatives. Mycophenolate mofetil (MMF, CellCept) **2** (Fig. 1) and mycophenolate sodium (MPS, Myfortic) **3** (Fig. 1) are used clinically as immunosuppressants.
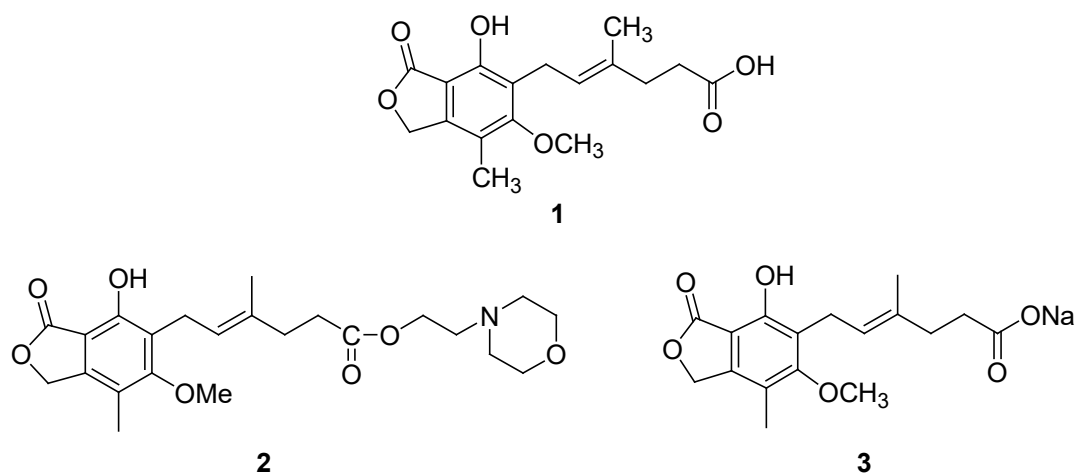

Figure 1. Structures of MPA **1**, MMF **2** and MPS **3**.

In modern transplantology inosine-5'-monophosphate dehydrogenase (IMPDH), is a major therapeutic target [3-9]. This enzyme is responsible for the catalysis of NAD-dependent oxidation of inosine monophosphate (IMP) to xanthosine 5′-monophosphate (XMP), which is used in the *de novo* biosynthesis of guanine.

Mycophenolic acid, (4*E*)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoic acid (MPA) **1** (Fig. 1), binds to the N subsite of IMPDH and is one of the most potent inhibitors of *h*IMPDH (human IMPDH) (K_i=7 nM) [5]. MPA **1** reduces the availability of guanine nucleotides, especially GTP. This causes a disturbance in DNA and RNA synthesis, while it induces apoptosis [9]. Reduction of GTP in lymphocytes and monocytes, causing cessation of proliferation, and inhibits glycosylation of membrane proteins [10,11]. MPA was first licensed for transplantation in 1995 and rapidly grew in popularity, becoming the second most widely prescribed immunosuppressant in the United States in 2004 [11]. MPA **1** was firstly isolated in 1896 by Gosio from *Pennicillium stoloniferum* and was probably first antibiotic [7, 12].

MMF **2** (Fig. 1) is the 2-morpholinoethyl ester prodrug of MPA and has been widely used as an immunosuppressant in kidney, heart, and liver transplantation procedures. The two most frequently observed adverse events with both drugs are leukopenia and gastrointestinal disorders, especially diarrhea [13, 14].

There were described many structural modifications of MPA [16-23], however only several ones displayed similar or better immunosuppressive activity. For example, it concerns β-aminophosphonic MPA derivatives **4** [24,25], hydroxamate **5** [26] or (S)-α-methylmycophenolic acid **6** [27], RS-97613 **7** [28] (Figure 2). These results are in good agreement with molecular modeling studies, that polar group at the and of the side chain interacts with Ser 276 of IMPDH [29]. In literature were also reported amide MPA analogs bearing glycine [30-32] and alanine [30] moieties **8** as potential anticancer agents. These compounds were obtained as metabolites of MPA by *Mucor rammamianus* or reaction of MPA with amino acid ester and DCC followed by alkaline hydrolysis.
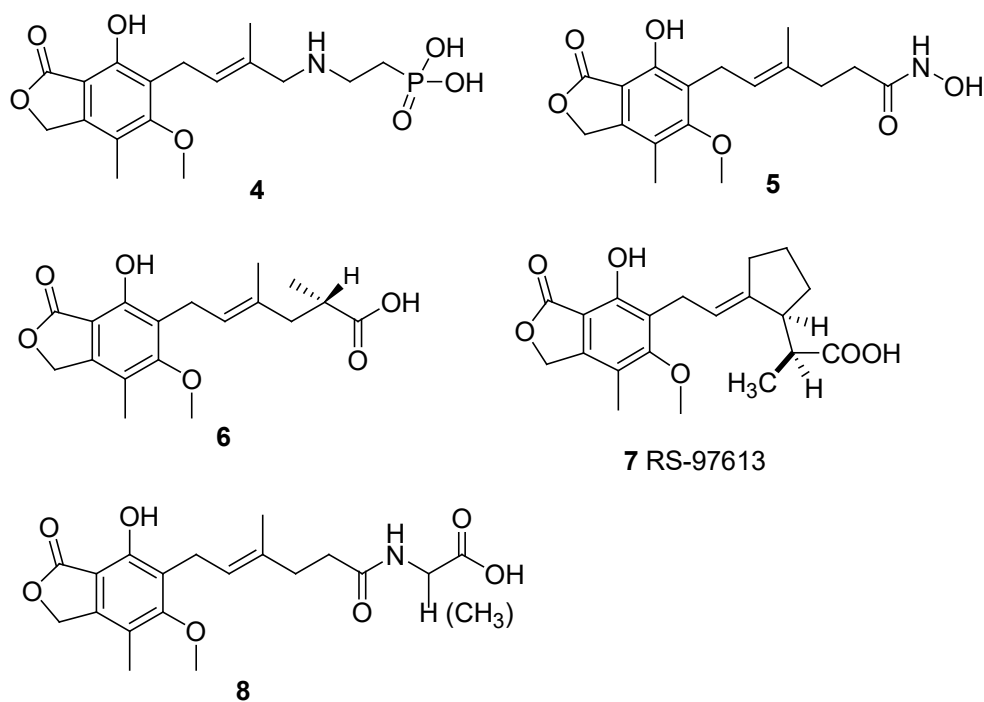

Figure 2. Structures of MPA analogs with modified side chain **4-8**.

In search of new immunosuppressants we decided to investigate the synthesis of MPA analogues **10, 11** (Scheme 1 and 2) possessing amino acids units which anti-proliferative and immunosuppressive activity were evaluated. The aim of our work is to examine the influence of the substituent R and configuration at chiral center in the amino acid units on the immunosuppressive activity.

2. Results and discussion

2.1. Chemistry

In order to form an amide bond between carboxyl group of **1** and amino acid **9** (Scheme 1) selectively, without protection of the phenol group in **1**, we tried several condensing agents: method of mixed anhydrides (isobutyl chloroformate), diphenyl phosphoroazidate (DPPA) with triethyloamine (TEA), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) with pyridine, *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) with *N*-methylomorpholine (NMM), *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) with 1-hydroxybenzotriazole (HOBt), (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) with HOBt, *N,N'*-dicyclohexylcarbodiimide (DCC) with NMM, *N*-Cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide methyl *p*-toluenesulfonate (CCMT) with 4-(dimethylamino)pyridine (DMAP) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI) with DMAP in the presence of HOBt. Unfortunately, none of the mentioned method occurred to be useful in synthesis of designed compounds **10**. The problem was low conversion of the substrates **1**, **9** or difficulties with purification of **10**, **11** as a material for biological activity examination.

There are widely reported additives applied in coupling, like HOBt or *N*-hydroxysuccinimide (HOSu), which reduce racemisation and increase yield of amide bond formation [33,34]. On the other hand, in the chemical literature are also described examples of using EDCI as a condensing reagent in the presence of DMAP without any racemisation suppressant in the synthesis of optically active amino acid derivatives, whereas amino group of α-amino acid is acylated [35-37].

The method of EDCI / DMAP without HOBt proved to be suitable for further synthesis of derivatives **10a-k** in moderate yields 54-86% (Scheme 1). Under these coupling conditions we observed the highest conversion of the substrates **1**, **9** and purity of the products **10a-k**. The best yield was achieved in case of glycine derivative **10c** probably due to steric reasons.
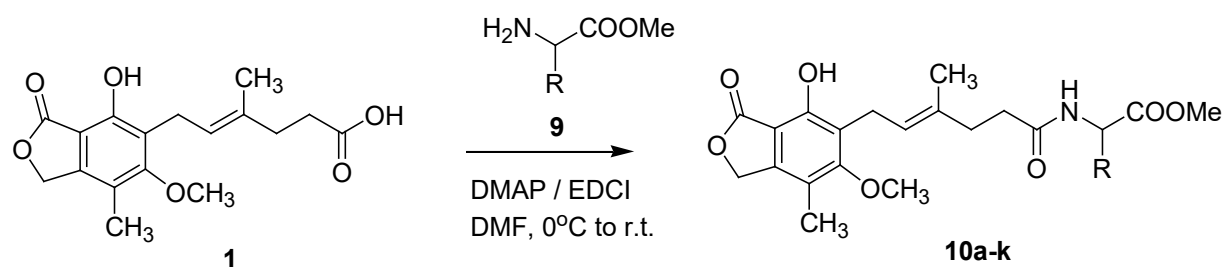
Scheme 1. Synthesis of amino acid derivative **10a-k**

For recovering free carboxylic group we performed hydrolysis methyl esters of derivatives **10** (Scheme 2) under mild conditions using LiOH [17] as a reagent. We obtained analogs **11a-k** in moderate yields 57-70% (Scheme 2).
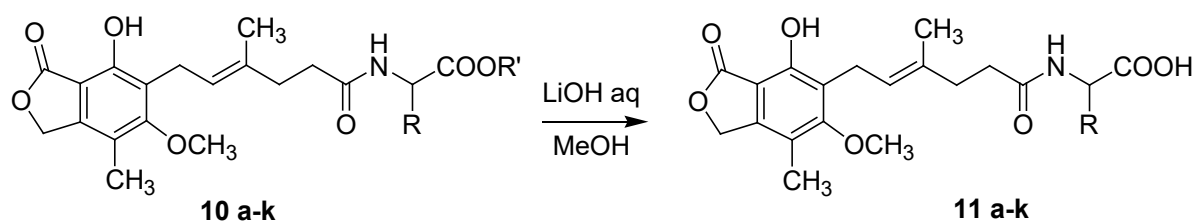
Scheme 2. Hydrolysis of amino acid methyl esters **10 a-k**.

2.2. Biological results

Cytotoxic activity of compounds **10a-k** and **11a-k** was specified against lymphoid cell line Jurkat and activated peripheral blood mononuclear cells (PBMC) as *in vitro* model of immunosuppression. Results (Tables 1 - 2) are expressed as micromolar IC_{50} concentrations. IC_{50} values were calculated with colorimetric MTT (λ=570nm) test and reported as the compound dose required to reduce the viability of the tested cells by 50% in regard to control sample.

According to data presented in Table 1, compounds **10a-k** exhibited lower or similar toxicity *in vitro* in comparison to compound **1**. Moreover, biological activity of esters **10** depends both on substituent and configuration in amino acid moiety. It can be clearly seen, that in case of alanine **10a,b**, valine **10f,g**, leucine **10h,i** D enantiomer is less toxic against Jurkat cells. In contrast to that, D-phenylalanine derivative **10k** occurred to be more toxic than its L enantiomer **10j**. Activities of D and L glutamic acid derivatives **10d,e** were comparable, and the best result in this series was achieved for glycine analog **10c**. Compound **10j** showed a little higher toxicity against Jurkat cells (IC_{50}=23.92μM, p=0.044610) but almost 250 times less cytotoxicity than MPA for activated PBMC (IC_{50}=12.06μM, p<0.05).

Compound	JURKAT			PBMC		
no	IC_{50}	p	F	IC_{50}	p	F
10a	65.70 ± 0,011	0.069	103.64	3.5 ± 0.0035	0.055	165.02
10b	23.47 ± 0.0059	0.051	194.48	<0.00025	0.394	2.71
10c	65.28 ± NAN	0.067	109.26	0.092±0.0008	<0.05	500.29
10d	20.33±0.0142	0.063	124.59	$0.05\pm2.12*10^{-5}$	0.184	14.27
10e	25.99±0.0036	<0.05	476.29	0.15±0.0002	0.060	136.14
10f	2.31 ± 0.0005	<0.05	1243.35	0.16 ± 0.00019	<0.05	761.10
10g	27.03 ± 0.018	<0.05	309.65	0.027 ± 0.0011	<0.05	689.42
10h	6.5±0.0009	<0.05	329.40	<0.0002	0.0863	67.62
10i	19.26±0.006	<0.05	365.16	0.67±0.0004	0.106	44.02
10j	23.92±0.0096	<0.05	250.75	12.06±0.0054	<0.05	2452.07
10k	6.45±0.0037	<0.05	3175.26	7.07±0.009	<0.05	1788.72
1	28.21±0.02536	-	-	$0.044\pm2.1*10^{-5}$	-	-

p- statistical significance, F-Fisher test, NAN-not a number

Table 1. IC_{50} [μM] values of **10a-k**, **1** for cell line Jurkat and activated PBMC obtained in MTT test.

Deprotection of methyl esters **10** changed activity considerably. Nevertheless influence of configuration at chiral centers remained similar, toxicity upon hydrolysis was increased (Table 2). Although toxicity of compound **11e** for Jurkat cells was higher than that of MPA, almost 2500-fold higher value than that for MPA could be seen for PBMC ($IC_{50}>111.5\mu M$, $p<0.05$). Also derivatives **11d**, **11g**, **11i** are promising in comparison with MPA, against Jurkat and PBMC as well.

Compound no	JURKAT			PBMC		
	IC_{50}	P	F	IC_{50}	p	F
11a	$9.73 \pm NAN$	0.113	38.43	$0.0033 \pm 3.6*10^{-5}$	0.074	90.97
11b	$1.85 \pm NAN$	<0.05	408.15	< 0.0003	<0.05	410.23
11c	$5.03 \pm NAN$	<0.05	202.62	$16.4 \pm NAN$	<0.05	703.29
11d	32.56 ± 0.0194	0.0548	173.48	$4.24 \pm NAN$	<0.05	801.51
11e	$21.18 \pm NAN$	<0.05	336.61	>111.5	0.078	80.55
11f	$0.19 \pm NAN$	<0.05	431.28	1.39 ± 0.0026	<0.05	1353.12
11g	38 ± 0.0406	<0.05	529.82	2.92 ± 0.0281	<0.05	1711.39
11h	8.78 ± 0.016	0.139	25.53	27.95 ± 0.0098	0.094	56.42
11i	41.8 ± 0.0146	<0.05	2010.23	27.26 ± 0.0082	0.074	89.73
11j	32.31 ± 0.025	<0.05	743.89	0.13 ± 0.018	0.051	657.78
11k	18.26 ± 0.056	<0.05	223.56	0.15 ± 0.0145	<0.05	485.57
1	28.21 ± 0.02536	-	-	$0.044 \pm 2,1*10^{-5}$	-	-

p- statistical significance, F-Fisher test, NAN-not a number

Table 2. IC_{50} [μM] values of **11a-m**, **1** for cell line Jurkat and activated PBMC obtained in MTT test.

Antiproliferation activity of compounds **10a-k** and **11a-k** was specified against lymphoid cell line Jurkat and activated peripheral blood mononuclear cells (PBMC) as *in vitro* model of immunosuppression. Results (Tables 3 - 4) are expressed as micromolar EC_{50} concentrations. EC_{50} values were calculated from incorporation of ^{3}H-TdR. EC_{50} was the concentration of a drug that gave half-maximal response during scintillation measurement (radiation β).

As we can see in Table 3 in case of PBMC all esters exhibited higher EC_{50} values than MPA **1**. In case of Jurkat cells five derivatives **10b, 10h, 10i, 10j, 10k** with lower EC_{50} values than that for MPA **1** were seen. All the differences are statistically significant ($p<0.05$). Enantiomers L were more active in case of alanine **10a,b** and valine **10f,g** derivatives against Jurkat, and the impact of chirality was diminished for glutamic acids **10d,e**, leucine **10h,i**, phenylalanine **10j,k** derivatives. Analogs with free carboxyl group (Table 4) **11a** ($EC_{50}=7.9\mu M$), **11c** ($EC_{50}=1.25\mu M$)**, 11e** ($EC_{50}=0.45\mu M$)**, 11h** ($EC_{50}=6.01\mu M$) gave lower EC_{50} values than MPA ($EC_{50}=9.45\mu M$) for Jurkat. Noteworthy, glycine **11c** and D-glutamic acid **11e** derivatives occurred to be the most active MPA analogs according to EC_{50} measurements. Compounds **11e** ($EC_{50}=0.0033\mu M$) and **11h** ($EC_{50}=0.0039\mu M$) also exhibited lower EC_{50} in comparison with others derivatives **11** for PBMC.

Compound	JURKAT			PBMC		
no	EC_{50}	p	F	EC_{50}	p	F
10a	30.134 ± 0.011	<0.05	285.37	0.0030 ± 0.0002	<0.05	69.08
10b	5.681 ± 0.0017	<0.05	29331.43	0.0017 ± 0.0001	<0.05	70.12
10c	24.58 ± 0.0028	<0.05	351.21	0.0069 ± 0.0007	<0.05	122.62
10d	26.2 ± 0.0174	<0.05	738.37	0.0021 ± 0.0002	<0.05	165.40
10e	24.73 ± 0.0272	<0.05	1594.73	0.0065 ± 0.0007	<0.05	194.19
10f	15.25 ± 0.0032	<0.05	6091.42	0.0005 ± 0.0001	<0.05	31.64
10g	36.04 ± 0.0236	0.059	141.23	0.0014 ± 0.0004	<0.05	56.03
10h	6.27 ± 0.0031	<0.05	204.07	$0.0016 \pm 6.9*10^{-5}$	<0.05	20.55
10i	8.29 ± 0.0036	<0.05	37153.29	0.0038 ± 0.0013	<0.05	100.81
10j	6.66 ± 0.0014	<0.05	5723.37	0.0042 ± 0.0004	<0.05	242.27
10k	7.9 ± 0.0304	<0.05	65934.89	0.0052 ± 0.0012	<0.05	220.02
1	9.45 ± 0.0789	-	-	$0.00006 \pm 4.6*10^{-6}$	-	-

p- statistical significance, F-Fisher test

Table 3. EC_{50} [μM] values of **10a-k**, **1** for cell line Jurkat and activated PBMC obtained in antiproliferation test.

Compound	JURKAT			PBMC		
no	EC_{50}	p	F	EC_{50}	p	F
11a	7.9 ± 0.0023	<0.05	2216.50	0.0056 ± 0.0003	<0.05	142.75
11b	13.3 ± 0.0028	<0.05	1250.55	0.0051 ± 0.0005	<0.05	61.84
11c	1.25 ± 0.008	<0.05	1719.10	0.0225 ± 0.0005	<0.05	316.24
11d	19.85 ± 0.0027	<0.05	1235.00	0.0065 ± 0.0007	<0.05	75.31
11e	$0.45 \pm 7.3*10^{-5}$	<0.05	7978.35	0.0033 ± 0.0003	<0.05	33.21
11f	21.51 ± 0.0015	<0.05	8855.69	0.0060 ± 0.0004	<0.05	67.84
11g	19.12 ± 0.0016	<0.05	17986	0.0062 ± 0.0002	<0.05	96.32
11h	6.01 ± 0.0016	<0.05	11663.82	0.0039 ± 0.0002	<0.05	32.79
11i	16.17 ± 0.0050	<0.05	2770.586	0.0155 ± 0.0012	<0.05	307.08
11j	19.49 ± 0.025	<0.05	659.89	0.016 ± 0.0003	<0.05	56.78
11k	21.20 ± 0.056	<0.05	157.54	0.01 ± 0.0013	<0.05	73.75
1	9.45 ± 0.0789	-	-	$0.00006 \pm 4.6*10^{-6}$	-	-

p- statistical significance, F-Fisher test

Table 4. EC_{50} [μM] values of **11a-k**, **1** for cell line Jurkat and activated PBMC obtained in antiproliferation test.

In order to identify analogs of MPA which showed the most favorable parameters we appointed selectivity index (SI) (Table 5). The selectivity index was calculated according to formula:

$$SI = \frac{IC_{50}}{EC_{50}}$$

where:

IC_{50} - half maximal inhibitory concentration obtained in MTT test [μM]

EC_{50} - half maximal effective concentration obtained in antiproliferation test [μM]

The most promising derivatives were **10j** (SI=2871.4)**, 11e** (SI=33787.9) and **11h** (SI=7166.7) in measurements for PBMC (Table 5). Even if EC_{50} values were higher than those for MPA, their cytotoxicity was much lower than for MPA. Particularly, D-glutamic acid **11e** derivative gave the best selective index against Jurkat cell line and activated PBMC

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as well. This result is consistent with reported molecular modeling studies, where polar group at the end of side chain is important for interaction with Ser 276 of IMPDH [29]. Although influence of chirality on observed activity is not unambiguous among derivatives **10** and **11**, in case of the most selective compound **11e** enantiomer D is preferred. One possible reason for increasing activity by replacement of L amino acid by D analog is enhanced resistance towards enzymatic hydrolysis [38]. Noteworthy, replacement of L-alanine by D enantiomer in case of muramyl dipeptide caused immunosuppressive activity [39].

Compound no	JURKAT SI	PBMC SI
10a	2.2	1166.7
10b	4.1	0.1
10c	2.7	13.3
10d	0.8	23.8
10e	1.05	23.1
10f	0.1	320.0
10g	0.75	19.3
10h	1.04	0.1
10i	2.3	176.3
10j	3.6	2871.4
10k	0.8	1359.6
11a	1.2	0.6
11b	0.1	0.1
11c	4.02	728.9
11d	1.6	652.3
11e	47.1	33787.9
11f	0.01	231.7
11g	2.0	471.0
11h	1.5	7166.7
11i	2.6	1758.7
11j	1.7	8.1
11k	0.9	15.1
1	2.99	7333

. **Table 5.** Selectivity index of **10a-k**, **11a-k**, **1** for cell line Jurkat and activated PBMC

Subsequently, we decided to establish whether obtained compounds **10**, **11** act as IMPDH inhibitors. In the literature was reported, that addition to the culture "guanylate pool" e.g. guanosine or guanosine monophosphate (GMP) suppress cell proliferation in the presence of IMPDH inhibitor [7,21,40]. We observed this effect both in case of MPA **1**, and investigated compounds **10**, **11** as well.

Figures 1 and 2 in Electronic Supplementary Information show data of inhibitory effect on Jurkat cell line proliferation of compounds **10a-k** and **11a-k**, respectively. The measurements were performed in absence or presence of GMP, at three concentration levels and compared to MPA **1**. The addition of 50 μM of GMP increased cells proliferation inhibition clearly. In case of derivatives **11a-k** observed effect was a little stronger than for compounds **10a-k**. These results prove that all compounds were IMPDH inhibitors.

3. Summary

As we expected, amino acid derivatives of mycophenolic acid showed similar antiproliferative activity to parent MPA **1** and acted as IMPDH inhibitors. However, their potency depends both on configuration and substituent R in amino acid moiety. Particularly, compounds **10j, 11e, 11h** exhibited higher activity *in vitro* than MPA **1** itself. Moreover, their toxicity was lower against Jurkat (**11e**, **11h**) and PBMC (**10j**). The best selectivity index was achieved in case of *N*-mycophenoyl-D-glutamic acid **11e**. In other words, derivatives **10j, 11e, 11h** revealed promising cytotoxic properties and have been selected as potential immunosuppressants to farther biological investigations including *in vivo* examinations.

4. Experimental Section

All reactions with DMF were performed without air with magnetic stirring. DMF was purified by distillation from benzene/water. Purification performed on ready plates coated with silica gel TLC [silica gel 60, Merck 1.05554.0001]. ^{1}H NMR and ^{13}C NMR spectra were taken on the camera Varian Unity 500 Plus in CDCl$_3$, acetone, DMSO or mixture of solvents. Mass spectra were performed at the Laboratory of Mass Spectrometry MALDI-TOF on the matrix DHB (BIFLEX III Bruker). Determination of optical rotation using a polarimeter Autopol ® II, Model: APII-6W-10 in the Department of Organic Chemistry at the Gdansk University of Technology. Designations HPLC-MS/MS were performed on an Agilent 1290 Infinity camera LC with an Agilent 6540 Accurate Mass Q-TOF LC / MS system in the Department of Analytical Chemistry at the Gdansk University of Technology.

Eluents for TLC chromatograpy:
- eluent A   10:1 CH$_2$Cl$_2$: MeOH
- eluent B   20 : 1 CH$_2$Cl$_2$: MeOH
- eluent C   15 : 1 : 0.1 CH$_2$Cl$_2$: MeOH : CH$_3$COOH
- eluent D   10 : 1 : 0.1 CH$_2$Cl$_2$: MeOH : CH$_3$COOH

*4.1.General procedure for the preparation amino acid derivatives of MPA **10,11***

Mycophenolic acid **1** (0.156 mmol), methyl ester amino acid hydrochloride **9** (0.178 mmol) and DMAP (0.178 mmol) was dissolved in dry DMF (3 mL). A reaction mixture was cooled to 0°C in an ice bath and with stirring was added EDCI (0.17053 mmol). The solution was stirred at 0°C for 2 h and after that time left at room temperature for 48 h. Progress of the reaction controlled by TLC plates. After completion of the reaction solution was poured into water (15 mL), extracted with ethyl acetate. The organic layer was separated, dried (MgSO$_4$), filtered and concentrated. The crude products was purified by chromatography (SiO$_2$). Structures of synthesized derivatives **10** were established by spectroscopic methods (^{1}H NMR, ^{13}C NMR, MS, HPLC-MS, optical rotation and melting point).

Ester hydrolysis: the methyl ester **10** was dissolved in MeOH (30mL/g), and a solution of LiOH·H$_2$O (3 mol equiv – 2-fold more for **10d** and **10e**) in an equal volume of water was added. When hydrolysis was complete (48 h), the solution was added to water and washed with ether. The aqueous phase was acidified with 2 N HCl and extracted with EtOAC. The extract was dried with MgSO$_4$ and evaporated, and the residue was recrystallized [17].

*4.1.1. Methyl ester N-mycophenoyl-D-alanine **10a*** elution with B (R$_f$=0.56) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.34 (d, 3H, J=7.3 Hz); 1.81 (s, 3H); 2.15 (s, 3H); 2.31 (s, 4H); 3.39 (d, 2H, *J*=7.3 Hz); 3.74 (s, 3H); 3.76 (s, 3H); 4.55-4.58 (m, 1H); 5.20 (s, 2H); 5.26 (t, 1H, *J*=6.8 Hz); 6.02 (s, 1H); 7.67 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.85; 173.15; 172,39; 163.90; 153.84; 144.26; 134.65; 123.10; 122.33; 116.98; 106.60; 70.27; 61.24; 52.68; 48.10; 35.27; 35.22; 22.83; 18.70; 16.39; 11.79. MS (DHB) *m/z* calcd for C$_{21}$H$_{27}$O$_7$N 405.4416 found 406.2 (M-H)[+]. HPLC-MS/MS found *m/z* 404,1726 (M-H)[+]. [α]$_D^{25}$= -4° (c=1, CHCl$_3$). mp. 135-137°C.

*4.1.2. Methyl ester N-mycophenoyl-L-alanine **10b*** elution with B (R$_f$=0,58) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.34 (d, 3H, J=7.3 Hz); 1.81 (s, 3H); 2.15 (s, 3H); 2.32 (s, 4H); 3.39 (d, 2H, *J*=6.8 Hz); 3.74 (s, 3H); 3.77 (s, 3H); 4.55-4.58 (m, 1H); 5.205 (s, 2H); 5.27 (t, 1H, *J*=6.8 Hz); 6.02 (s, 1H); 7.675 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.58; 172.88; 172.05; 163.66; 153.62; 143.99; 134.40; 122.86; 122.08; 116.71; 106.36; 70.01; 60.98; 52.40; 47.85; 35.40; 35.40; 22.59; 18.48; 16.15; 11.54. MS (DHB) *m/z* calcd for C$_{21}$H$_{27}$O$_7$N 405.4416 found 406.3 (M-H)[+]. HPLC-MS/MS found *m/z* 404.1724 (M-H)[+]. [α]$_D^{25}$=4° (c=1, CHCl$_3$). mp. 138-142°C.

*4.1.3. Methyl ester N-mycophenoylglycine **10c*** elution with A (R$_f$=0,6) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.82 (s, 3H); 2.15 (s, 3H); 2.34 (s, 4H); 3.40 (d, 2H, *J*=6.8 Hz); 3.75 (s, 3H); 3.77 (s, 3H); 3.98 (d, 2H, *J*=5.4 Hz); 5.205 (s, 2H); 5.27 (t, 1H, *J*=6.8 Hz); 5.98 (s, 1H); 7.68 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.26; 173.26; 170.94; 164.14; 154.09; 144.52; 134.87; 123.55; 122.57; 117.24; 106.87; 70.51; 61.48; 52.78; 41.61; 35.53; 35.28; 23.09; 16.58; 12.02. MS (DHB) *m/z* calcd for C$_{20}$H$_{25}$O$_7$N 391.4150 found 392.1 (M-H)[+]. HPLC-MS/MS found *m/z* 390.1585 (M-H)[+]. mp. 130-133°C.

*4.1.4. Dimethyl N-mycophenoyl-L-glutamate **10d*** elution with A (R$_f$=0.72) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.80 (s, 3H); 1.9-1.97 (m, 2H); 2.14 (s, 3H); 2.27-2.35 (m, 4H); 2.36-2.42 (m, 2H); 3.38-3.39 (d, 2H, *J*=6.8 Hz); 3.66 (s, 3H); 3.73 (s, 3H); 3.76 (s, 3H); 4.6-4.61 (m, 1H); 5.19 (s, 2H); 5.24-5.26 (t, 1H, *J*=6.3 Hz); 6.27 (d, 1H, *J*=7.3 Hz). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.54; 173.14; 173.02; 172.61; 163.88; 153.83; 144.30; 134.55; 123.16; 122.28; 116.99; 106.61; 70.28; 61.24; 52.77; 52.10; 51.74; 35.23; 35.13; 30.20; 27.55; 22.82; 16.37; 11.80. MS (DHB) *m/z* calcd for C$_{24}$H$_{31}$O$_9$N 477.5042, found 478.1 (M-H)[+]. HPLC-MS/MS found *m/z* 476.1956 (M-H)[+]. [α]$_D^{25}$=+12° (c=1, CHCl$_3$). mp. 76-79°C.

*4.1.5. Dimethyl N-mycophenoyl-D-glutamate **10e*** elution with A (R$_f$=0.7) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.81 (s, 3H); 1.9-1.98 (m, 2H); 2.15 (s, 3H); 2.27-2.35 (m, 4H); 2.36-2.42 (m, 2H); 3.38-3.395 (d, 2H, *J*=6.8 Hz); 3.67 (s, 3H); 3.73 (s, 3H); 3.76 (s, 3H); 4.6-4.60 (m, 1H); 5.20 (s, 2H); 5.25-5.27 (t, 1H, *J*=6.8 Hz); 6.27 (d, 1H, *J*=7.8 Hz). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.51; 173.13; 172.80; 172.63; 163.89; 153.85; 144.28; 134.59; 123.13; 122.29; 116.97; 106.62; 70.27; 61.24; 52.74; 52.07; 51.72; 35.23; 35.17; 30.21; 27.60; 22.83; 16.39; 11.79. MS (DHB) *m/z* calcd for C$_{24}$H$_{31}$O$_9$N 477.5042, found 477.9 (M-H)[+]. HPLC-MS/MS found *m/z* 476.1964 (M-H)[+]. [α]$_D^{25}$= -12° (c=1, CHCl$_3$). mp. 75 -78 °C.

*4.1.6. Methyl ester N-mycophenoyl-L-valine **10f*** elution with A (R$_f$=0.87) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 0.86 (dd, 6H, *J*=6.8 Hz); 1.82 (s, 3H); 2.08-2.12 (m, 1H); 2.15 (s, 3H); 2.30-2.36 (m, 4H); 3.39 (d, 2H, *J*=6.8 Hz); 3.72 (s, 3H); 3.76

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(s, 3H); 4.53-4.56 (m, 1H); 5.20 (s, 2H); 5.27 (t, 1H, J=6.8 Hz); 5.95 (d, 1H, J=8.8 Hz); 7.67 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 172.88; 172.59; 172.46; 163.68; 153.63; 144.00; 134.42; 122.77; 122.06; 116.70; 106.36; 70.00; 60.97; 56.83; 52.07; 35.06; 35.06; 31.27; 22.59; 18.84; 17.75; 16.14; 11.53. MS (DHB) m/z calcd for C$_{23}$H$_{31}$O$_7$N 433.4947, found 434.3 (M$^+$). HPLC-MS/MS found m/z 432.2038 (M-H)$^+$. [α]$_D^{25}$=+10° (c=1, CHCl$_3$). mp. 135-138°C.

4.1.7. Methyl ester N-mycophenoyl-D-valine 10g elution with A (R$_f$=0.86) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 0.86 (dd, 6H, J=6.8 Hz); 1.81 (s, 3H); 2.07-2.11 (m, 1H); 2.14 (s, 3H); 2.30-2.35 (m, 4H); 3.38 (d, 2H, J=6.8 Hz); 3.71 (s, 3H); 3.755 (s, 3H); 4.52-4.56 (m, 1H); 5.19 (s, 2H); 5.26 (t, 1H, J=6.8 Hz); 5.98 (d, 1H, J=8.7 Hz); 7.665 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 172.86; 172.58; 172.45; 163.62; 153.55; 143.97; 134.39; 122.71; 122.00; 116.69; 106.30; 70.00; 60.95; 56.77; 52.06; 35.06; 34.99; 31.23; 22.54; 18.82; 17.71; 16.11; 11.52. MS (DHB) m/z calcd for C$_{23}$H$_{31}$O$_7$N 433.4947, found 434.1 (M$^+$). HPLC-MS/MS found m/z 434.2154 (M+H)$^+$. [α]$_D^{25}$= -10° (c=1, CHCl$_3$). mp. 136-139°C.

4.1.8. Methyl ester N-mycophenoyl-L-leucine 10h elution with B (R$_f$=0.68) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 0.90 (dd, 6H, J=4.4 Hz); 1.44-1.48 (m, 2H); 1.56-1.62 (m, 1H), 1.80 (s, 3H); 2.14 (s, 3H); 2.31 (s, 3H); 3.38 (d, 2H, J=6.8 Hz); 3.71 (s, 3H); 3.76 (s, 3H); 4.59-4.63 (m, 1H); 5.19 (s, 2H); 5.25 (t, 1H, J=6.8 Hz); 5.89 (d, 1H, J=9.3 Hz); 7.67 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.89; 173.14; 172.66; 163.90; 153.85; 144.26; 134.74; 123.02; 122.30; 116.98; 106.61; 70.27; 61.25; 52.49; 50.74; 41.94; 35.28; 35.19; 25.07; 23.00; 22.84; 22.17; 16.37; 11.80. MS (DHB) m/z calcd for C$_{24}$H$_{33}$O$_7$N 447.5213, found 448.0 (M$^+$). HPLC-MS/MS found m/z 446.2193 (M-H)$^+$. [α]$_D^{25}$=+2° (c=1, CHCl$_3$). mp. 101-104°C.

4.1.9. Methyl ester N-mycophenoyl-D-leucine 10i elution with B (R$_f$=0.69) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 0.90 (dd, 6H, J=4.4 Hz); 1.44-1.50 (m, 2H); 1.56-1.63 (m, 1H), 1.80 (s, 3H); 2.14 (s, 3H); 2.31 (s, 3H); 3.38 (d, 2H, J=6.8 Hz); 3.71 (s, 3H); 3.76 (s, 3H); 4.59-4.63 (m, 1H); 5.19 (s, 2H); 5.25 (t, 1H, J=6.8 Hz); 5.89 (d, 1H, J=9.3 Hz); 7.67 (s, br s,1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.90; 173.14; 172.64; 163.90; 153.85; 144.26; 134.75; 123.02; 122.30; 116.98; 106.61; 70.27; 61.25; 52.49; 50.74; 41.94; 35.28; 35.20; 25.08; 23.00; 22.84; 22.17; 16.37; 11.80. MS (DHB) m/z calcd for C$_{24}$H$_{33}$O$_7$N 447.5213, found 448.0 (M$^+$). HPLC-MS/MS found m/z 446.2219 (M-H)$^+$. [α]$_D^{25}$= -2° (c=1, CHCl$_3$). mp. 100-104°C.

4.1.10. Methyl ester N-mycophenoyl-L-phenyloalanine 10j elution with B (R$_f$=0.82) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.79 (s, 3H); 2.13 (s, 3H); 2.28 (s, 4H); 2.98-3.07 (m, 2H); 3.37 (d, 2H, J=7.3 Hz); 3.69 (s, 3H); 3.75 (s, 3H); 4.82-4.86 (m, 1H); 5.14 (s, 2H); 5.24 (t, 1H, J=6.8 Hz); 5.94 (d, 1H, J=7.3 Hz); 7.04-7.23 (m, 5H, aromat); 7.67 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.14; 172.40; 172.30; 163.88, 153.83; 144.29; 136.11; 134.60; 129.43; 128.77; 127.33; 123.07; 122.27; 116.97; 106.61; 70.26; 61.24; 53.23; 52.52; 38.16; 35.23; 35.11; 22.83; 16.37; 11.80. MS (DHB) m/z calcd for C$_{27}$H$_{31}$O$_7$N 481.5375, found 482.2 (M$^+$). HPLC-MS/MS found m/z 480.2043 (M-H)$^+$. [α]$_D^{25}$=+20° (c=2, MeOH). mp. 87-91°C.

4.1.11. Methyl ester N-mycophenoyl-D-phenyloalanine 10k elution with B (R$_f$=0.80) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.79 (s, 3H); 2.13 (s, 3H); 2.28 (s,

4H); 2.98-3.07 (m, 2H); 3.37 (d, 2H, J=7.3 Hz); 3.69 (s, 3H); 3.75 (s, 3H); 4.82-4.86 (m, 1H); 5.14 (s, 2H); 5.24 (t, 1H, J=6.8 Hz); 5.94 (d, 1H, J=7.3 Hz); 7.04-7.28 (m, 5H, aromat); 7.67 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 173.14; 172.38; 172.29; 163.88; 153.83; 144.30; 136.12; 134.60; 129.43; 128.77; 127.32; 123.07; 122.27; 116.97; 106.61; 70.25; 61.24; 53.24; 52.52; 38.16; 35.23; 35.10; 22.83; 16.37; 11.79. MS (DHB) m/z calcd for C$_{27}$H$_{31}$O$_7$N 481.5375, found 482.2 (M$^+$). HPLC-MS/MS found m/z 480.2026 (M-H)$^+$. $[\alpha]_D^{25}$= -20° (c=2, MeOH). mp. 87-91°C.

*4.1.12. N-mycophenoyl-D-alanine **11a*** elution with D (R$_f$=0.73) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 1.31 (d, 3H, J=7.3 Hz); 1.81 (s, 3H); 2.05-2.07 (m, 3H); 2.26-2.32 (m, 2H); 3.39 (d, 2H, J=6.8 Hz); 3.795 (s, 3H); 4.38-4.41 (m, 1H); 5.27 (t, 1H, J=6.8 Hz); 5.32 (s, 2H); 7.28 (d, 1H, J=6.8 Hz). ^{13}C NMR (acetone-d$_6$, DMSO, 125 MHz) δ ppm: 174.13; 172.84; 172,68, 164.21; 153.86; 145.61; 135.02; 123.43; 122,04, 117.52; 106.83; 70.48; 61.30; 48.25; 35.87; 35.14; 23.10; 17.89; 16.32; 11.51. MS (DHB) m/z calcd for C$_{20}$H$_{25}$O$_7$N 391.4150, found 392.0 (M$^+$). HPLC-MS/MS found m/z 390.1617 (M-H)$^+$. $[\alpha]_D^{25}$=+2° (c=1, acetone). mp. 113-116°C.

*4.1.13. N-mycophenoyl-L-alanine **11b*** e elution with D (R$_f$=0.74) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 1.31 (d, 3H, J=7.3 Hz); 1.81 (s, 3H); 2.05-2.07 (m, 3H); 2.26-2.31 (m, 2H); 3.39 (d, 2H, J=6.8 Hz); 3.795 (s, 3H); 4.38-4.41 (m, 1H); 5.27 (t, 1H, J=6.8 Hz); 5.315 (s, 2H); 7.27 (d, 1H, J=6.8 Hz). ^{13}C NMR (acetone-d$_6$, 125 MHz) δ ppm: 173.62; 172.24; 172.18, 163.69; 153.34; 145.35; 134.61; 123.23; 122.17; 117.06; 106.58; 69.91; 60.74; 47.78; 35.34; 34.54; 22.56; 17.23; 15.65; 10.80. MS (DHB) m/z calcd for C$_{20}$H$_{25}$O$_7$N 391.4150, found 391.9 (M$^+$). HPLC-MS/MS found m/z 390.1558 (M-H)$^+$. $[\alpha]_D^{25}$= -2° (c=1, acetone). mp. 114-117°C.

*4.1.14. N-mycophenoyloglycine **11c*** elution with D (R$_f$=0.67) as a colorless solid. ^{1}H NMR (CDCl$_3$, 500 MHz) δ ppm: 1.83 (s, 3H); 2.15 (s, 1H); 2.28-2.34 (m, 3H); 3.31 (s, 3H); 3.39 (d, 2H, J=6.8 Hz); 3.795 (s, 3H); 5.25 (s, 2H); 5.27 (t, 1H, J=6.8 Hz). ^{13}C NMR (acetone-d$_6$, 125 MHz) δ ppm: 172.58; 172.20; 170.81; 163.67; 153.40; 145.37; 134.62; 122.98; 122.11; 117.05; 106.64; 69.88; 60.74; 40.66; 35.40; 34.60; 22.57; 15.62; 10.80. MS (DHB) m/z calcd for C$_{19}$H$_{23}$O$_7$N 377.3884, found 378.1 (M$^+$). HPLC-MS/MS found m/z 378.1511 (M+H)$^+$. mp. 114-118°C.

*4.1.15. N-mycophenoyl-L-glutamic acid **11d*** elution with D (R$_f$=0,68) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 1,82 (s, 3H); 1,89-1,95 (m, 2H); 1,97 (s, 1H); 2,06 (s, 1H); 2,17 (s, 3H); 2,19-2,46 (m, 6H, imposition of multiplets); 3,40 (d, 2H, J=6,8 Hz); 3,79 (s, 3H); 4,47-4,52 (m, 1H); 5,27-5,30 (t, 1H, J=6,3 Hz); 5,30 (s, 3H); 7,34 (d, 1H, J=7,3 Hz). ^{13}C NMR (acetone-d$_6$, 125 MHz) δ ppm: 173,420; 172,70; 172,61; 172,22; 163,66; 153,40; 145,36; 134,60; 122,91; 122,07; 117,06; 106,64; 69,89; 60,76; 51,54; 51,45; 35,35; 34,63; 27,17; 22,56; 15,68; 10,82. MS (DHB) m/z calcd for C$_{22}$H$_{27}$O$_9$N 449.4511 found 450,1 (M-H)$^+$. HPLC-MS/MS found m/z 448,1653 (M-H)$^+$. $[\alpha]_D^{25}$= -4° (c=1, acetone). mp. 109-113°C.

*4.1.16. N-mycophenoyl-D-glutamic acid **11e*** elution with D (R$_f$=0.66) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 1.82 (s, 3H); 1.89-1.95 (m, 2H); 1.97 (s, 1H); 2.06 (s, 1H); 2.17 (s, 3H); 2.19-2.46 (m, 6H, imposition of multiplets); 3.40 (d, 2H, J=6.8 Hz); 3.79 (s, 3H); 4.47-4.52 (m, 1H); 5.27-5.30 (t, 1H, J=6.3 Hz); 5.30 (s, 3H); 7.34 (d, 1H, J=7.3 Hz). ^{13}C NMR (acetone-d$_6$+DMSO, 125 MHz) δ ppm: 174.50; 173.76; 173.69; 173.38;

164.72; 154.41; 146.19; 135.55; 123.95; 123.05; 118.06; 70.98; 61.83; 52.57; 52.48; 36.37; 35.68; 28.15; 23.61; 20.99; 16.82; 11.98. MS (DHB) m/z calcd for $C_{22}H_{27}O_9N$ 449.4511, found 450.1 (M$^+$). HPLC-MS/MS found m/z 448.1659 (M-H)$^+$. $[\alpha]_D^{25}$=+4° (c=1, acetone). mp. 107-111°C.

*4.1.17. N-mycophenoyl-L-valine **11f*** elution with D (R$_f$=0.78) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 0.91 (dd, 6H, J=6.8 Hz); 1.81 (s, 3H); 2.15-2.30 (m, 1H); 2.15 (s, 3H); 2.32-2.38 (m, 4H); 3.39 (d, 2H, J=6.8 Hz); 3.76 (s, 3H); 4.52-4.54 (m, 1H); 5.20 (s, 3H); 5.27 (t, 1H, J=6.8 Hz); 6.23 (d, 1H, J=8.8 Hz); 7.27 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 175.51; 173.68; 173.22; 163.90; 153.86; 144.35; 134.47; 123.32; 122.29; 117.00; 106.62; 70.32; 61.25; 57.28; 35.29; 35.00; 31.15; 22.86; 19.17; 17.88; 16.33; 11.79. MS (DHB) m/z calcd for $C_{22}H_{29}O_7N$ 419.4682, found 420.1 (M$^+$). HPLC-MS/MS found m/z 418.1945 (M-H)$^+$. $[\alpha]_D^{25}$=+2° (c=1, acetone). mp. 130-133°C.

*4.1.18. N-mycophenoyl-D-valine **11g*** elution with D (R$_f$=0.76) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 0.91 (dd, 6H, J=6.8 Hz); 1.81 (s, 3H); 2.15-2.30 (m, 1H); 2.15 (s, 3H); 2.32-2.38 (m, 4H); 3.39 (d, 2H, J=6.8 Hz); 3.76 (s, 3H); 4.52-4.54 (m, 1H); 5.20 (s, 3H); 5.27 (t, 1H, J=6.8 Hz); 6.23 (d, 1H, J=8.8 Hz); 7.27 (s, 1H). ^{13}C NMR (CDCl$_3$, 125 MHz) δ ppm: 177.04; 172.60; 172.22; 163.71; 153.34; 145.34; 134.67; 122.78; 122.03; 117.06; 106.62; 69.92; 60.74; 57.02; 35.46; 34.53; 30.71; 22.56; 18.83; 17.49; 15.70; 10.81. MS (DHB) m/z calcd for $C_{22}H_{29}O_7N$ 419.4682, found 420.3 (M$^+$). HPLC-MS/MS found m/z 418.1941 (M-H)$^+$. $[\alpha]_D^{25}$= -2° (c=1, acetone). mp. 131-135°C.

*4.1.19. N-mycophenoyl-L-leucine **11h*** elution with D (R$_f$=0.68) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 0.91 (dd, 6H, J=6.8 Hz); 1.56-1.62 (m, 1H); 1.7-1.74 (m, 2H); 1.82 (s, 3H); 2.17 (s, 3H); 2.28-2.33 (m, 4H); 3.39 (d, 2H, J=6.8 Hz); 3.79 (s, 3H); 4.46-4.51 (m, 1H); 5.26-5.30 (t, 1H, J=6.8 Hz); 5.31 (s, 2H); 7.23 (d, 1H, J=6.8 Hz). ^{13}C NMR (acetone-d$_6$, 125 MHz) δ ppm: 174.29; 172.99; 172.84; 164.40; 154.04; 145.95; 135.36; 123.51; 122.70; 117.74; 107.26; 70.63; 61.46; 51.05; 41.64; 36.13; 35.36; 25.53; 23.36; 23.26; 21.91; 16.38; 11.55. MS (DHB) m/z calcd for $C_{23}H_{31}O_7N$ 433.4947, found 434.3 (M$^+$). HPLC-MS/MS found m/z 432.2094 (M-H)$^+$. $[\alpha]_D^{25}$=+8° (c=1, acetone). mp. 97-99°C.

*4.1.20. N-mycophenoyl-D-leucine **11i*** elution with D (R$_f$=0.70) as a colorless solid. ^{1}H NMR (acetone-d$_6$, 500 MHz) δ ppm: 0.91 (dd, 6H, J=6.8 Hz); 1.56-1.62 (m, 1H); 1.7-1.74 (m, 2H); 1.82 (s, 3H); 2.17 (s, 3H); 2.28-2.33 (m, 4H); 3.39 (d, 2H, J=6.8 Hz); 3.79 (s, 3H); 4.46-4.51 (m, 1H); 5.26-5.30 (t, 1H, J=6.8 Hz); 5.31 (s, 2H); 7.24 (d, 1H, J=6.8 Hz). ^{13}C NMR (acetone-d$_6$ + CDCl$_3$, 125 MHz) δ ppm: 174.17; 173.00; 172.90; 164.24; 153.90; 145.65; 135.14; 123.36; 122.54; 117.55; 107.07; 70.50; 61.33; 50.89; 41.49; 35.99; 35.24; 25.37; 23.29; 32.12; 21.85; 16.32; 11.51. MS (DHB) m/z calcd for $C_{23}H_{31}O_7N$ 433.4947, found 434.3 (M$^+$). HPLC-MS/MS found m/z 432.2094 (M-H)$^+$. $[\alpha]_D^{25}$= -8° (c=1, acetone). **mp.** 97-100°C.

*4.1.21. N-mycophenoyl-L-phenyloalanine **11j*** elution with D (R$_f$=0.70) as a colorless solid. ^{1}H NMR (CD$_3$OD, 500 MHz) δ ppm: 1.77 (s, 3H); 2.11 (s, 3H); 2.15-2.27 (dt, 4H); 2.78-3.09 (dq, 2H); 3.35 (d, 2H, J=7.3 Hz); 3.74 (s, 3H); 4.57-4.6 (m, 1H); 5.12 (s, 2H); 5.22 (t, 1H, J=6.8 Hz); 7.15-7.26 (m, 5H, aromat). ^{13}C NMR (CD$_3$OD, 125 MHz) δ ppm: 174.32; 173.45; 172.61; 163.64; 153.50; 145.45; 137.30; 133.95; 129.07; 128.21; 126.57; 123.23; 122.44; 116.64; 106.51; 69.57; 60.38; 53.78; 37.43; 35.32; 34.30; 22.42; 15.03; 10.21. MS

(DHB) *m/z* calcd for $C_{26}H_{29}O_7N$ 467.5110, found 468.3 (M^+). HPLC-MS/MS found *m/z* 468.2018 (M^+). $[\alpha]_D^{25}$=+2° (c=1, MeOH). mp. 61-65°C.

*4.1.22. N-mycophenoyl-D-phenyloalanine **11k*** elution with D (R_f=0.69) as a colorless solid. ^1H NMR (CD$_3$OD, 500 MHz) δ ppm: 1.77 (s, 3H); 2.12 (s, 3H); 2.16-2.27 (dt, 4H); 2.78-3.09 (dq, 2H); 3.36 (d, 2H, J=7.3 Hz); 3.75 (s, 3H); 4.57-4.6 (m, 1H); 5.13 (s, 2H); 5.22 (t, 1H, J=6.8 Hz); 7.15-7.26 (m, 5H, aromat). ^{13}C NMR (CD$_3$OD, 500 MHz) δ ppm: 174.32; 173.47; 172.62; 163.64, 153.50; 145.44; 137.31; 133.95; 129.07; 128.21; 126.56; 123.23; 122.45; 116.64; 106.51; 69.57; 60.38; 53.79; 37.43; 35.32; 34.30; 22.42; 15.02; 10.20. MS (DHB) *m/z* calcd for $C_{26}H_{29}O_7N$ 467.5110, found 468.2 (M^+). **HPLC-MS/MS** found *m/z* 468.2013 ($M+H$)$^+$. $[\alpha]_D^{25}$= -2° (c=1, MeOH). mp. 63-67°C.

4.2. Biological activity evaluation

Spectrophotometric measurements were performed using a spectrophotometer PerkinElmer VictorTMX4 2030Multilabel in the Department of Clinical Immunology and Transplantology, Medical University of Gdansk. Scintillation measurements were made by liquid phase scintillation reader LSC-Beckman in the Department of Biochemistry, Medical University of Gdansk during 2min/sample. EC_{50} and IC_{50} values were determined using SigmaPlot 11. F and p values were determined using the STATISTICA 10.0.

4.2.1. Preparing MPAs derivatives **10,11** concentrations
Compounds **10a-k**, **11a-k**, **1** were dissolved in DMSO (10mg/mL) and further dilutions were made with RPMI-1640 medium before being added to the 96-well microliter plates with cells.

4.2.2. RPMI-1640 medium
Medium consisting with RPMI-1640 (PAA) supplemented with 10% fetal bovine serum (Life Technologies), penicillin/streptomycin (Sigma-Aldrich).

4.2.3. Human peripheral blood mononuclear cells (PBMC)
PBMC were separated from heparinized whole blood by density-gradient centrifugation in Ficoll-paque (Gradisol L – Aqua-med). After washing with PBS (2 times), 10^5 cells/well were cultured in microtiter plates in 100 μL RPMI-1640 medium and activated by added antibodies anti-CD3/anti-CD28 (1μL/well, Dynal, Invitrogen, USA).

4.2.4. Colorimetric MTT test
Examined compounds **10a-k, 11a-k, 1** solved in RPMI-1640 medium were added to lymphoid cell line Jurkat or activated PBMC in an amount 10^5 cells/well. After 48h for Jurkat cells and 72h for PBMC 20μL of MTT was added (5mg/ml H_2O). After 3h of incubation the reaction was stopped 100mL of sour isopropanol (with 0,4N HCl). After 15 min spectrophotometric measurement was made. The results are shown in Tables 1-2.

4.2.5. Proliferation test with 3H-TdR
Examined compounds **10a-k, 11a-k, 1** solved in RPMI-1640 medium were added to lymphoid cell line Jurkat or activated PBMC in an amount 10^5 cells/well 0,5μCi/well [^3H]thymidine was added for the last 18h. Cells were collected on filters with an automatic harvester and radioactivity was measured by standard scintillation procedures. The results are shown in Tables 3-4.

Acknowledgements

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Figure 1. Structures of MPA **1**, MMF **2** and MPS **3**

Figure 2. Structures of MPA analogs with modified side chain **4-8**

Scheme 1. Synthesis of amino acid derivative **10a-k**

Scheme 2. Hydrolysis of amino acid methyl esters **10 a-k**