



## Synthesis and biological activity of ester derivatives of mycophenolic acid and acridines/acridones as potential immunosuppressive agents

Grzegorz Cholewinski, Dorota Iwaszkiewicz-Grzes, Piotr Trzonkowski & Krystyna Dzierzbicka

To cite this article: Grzegorz Cholewinski, Dorota Iwaszkiewicz-Grzes, Piotr Trzonkowski & Krystyna Dzierzbicka (2016) Synthesis and biological activity of ester derivatives of mycophenolic acid and acridines/acridones as potential immunosuppressive agents, Journal of Enzyme Inhibition and Medicinal Chemistry, 31:6, 974-982, DOI: [10.3109/14756366.2015.1077821](https://doi.org/10.3109/14756366.2015.1077821)

To link to this article: <https://doi.org/10.3109/14756366.2015.1077821>



View supplementary material [↗](#)



Published online: 24 Aug 2015.



Submit your article to this journal [↗](#)



Article views: 1180



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 1 View citing articles [↗](#)

RESEARCH ARTICLE

## Synthesis and biological activity of ester derivatives of mycophenolic acid and acridines/acridones as potential immunosuppressive agents

Grzegorz Cholewinski<sup>1</sup>, Dorota Iwaszkiewicz-Grzes<sup>1</sup>, Piotr Trzonkowski<sup>2</sup>, and Krystyna Dzierzbicka<sup>1</sup>

<sup>1</sup>Department of Organic Chemistry, Gdansk University of Technology, Gdansk, Poland and <sup>2</sup>Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Gdansk, Poland

### Abstract

Improved derivatives of mycophenolic acid (MPA) are necessary to reduce the frequency of adverse effects, this drug exerts in treated patients. In this study, MPA was coupled with *N*-( $\omega$ -hydroxyalkyl)-9-acridone-4-carboxamides or *N*-( $\omega$ -hydroxyalkyl)acridine-4-carboxamides to give respective ester conjugates upon Yamaguchi protocol. This esterification required protection of phenol group in MPA. Designed conjugates revealed higher potency *in vitro* than parent MPA. Acridine derivatives were more active than acridone analogs and length of the alkyl linker between MPA and heterocyclic units influenced the observed cytotoxicity. Derivatives **2b**, **2d**, **3a**, **3b** displayed the most promising immunosuppressive activity.

### Keywords

Acridines, acridones, esterification, IMPDH inhibitors, mycophenolic acid

### History

Received 14 April 2015  
Revised 10 June 2015  
Accepted 21 July 2015  
Published online 24 August 2015

### Introduction

Mycophenolic acid **1** (MPA) (Figure 1) is an uncompetitive inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH), the crucial enzyme in *de novo* purine nucleotide biosynthesis. This mechanism allows MPA **1** to decrease proliferation of lymphocytes. Hence, sodium mycophenolate [MPS, Myfortic (Novartis, Basel, Switzerland)] and MPA prodrug: mycophenolate mofetil [2-morpholinoethyl, MMF, CellCept (Roche, Basel, Switzerland)] are widely used in the clinic as immunosuppressants<sup>1–10</sup> in the prevention of allograft rejection and treatment of autoimmune diseases. However, adverse effects related to the treatment with MPA-based drugs, such as diarrhea, leukopenia, sepsis and vomiting, are the barrier to the administration of higher doses and more effective treatment. In order to solve this issue, many structural MPA modifications followed by the assessment of the antiproliferative activity were reported<sup>11–27</sup>.

Various types of compounds were considered as potent IMPDH inhibitors<sup>28,29</sup>. Among them acridines possess not only anticancer, antiviral and antibacterial<sup>30–36</sup>, but also antiproliferative and immunosuppressive features<sup>28,37</sup>. Additionally, activity can be improved by conjugate forming<sup>38–40</sup>. Recently, we reported potent IMPDH inhibitors bearing MPA covalently bonded to nitroacridine/nitroacridone derivatives via amide bond formation<sup>41</sup>. On the other hand, there are also described promising conjugates possessing ester linkages, i.e. derivatives of muramyldipeptide with acridine/acridone moieties<sup>35</sup> or analogs of MPA with quinic acid<sup>38</sup>. In current work, we elaborated synthesis of the ester conjugates of MPA and acridones/acridines **2a–e**, **3a–e**, in which

lack of nitro group may diminish toxicity and can help to optimize the biological activity when applied as a drug.

### Methods

#### Chemistry

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were measured in CDCl<sub>3</sub>, solutions with Varian Gemini 500 spectrometer (Varian Inc., Palo Alto, CA), with TMS as an internal reference. IR measurements were performed with Bruker IFS66 (Billerica, MA) and UV-VIS with PerkinElmer (Waltham, MA) UV-VIS LAMBDA 18. Mass spectra were recorded with MALDI-TOF spectrometer BRUKER BIFLEX III (DHB or CCA matrix) and HRMS ESI on MaldiSYNAPT G2-S HDMS (monoisotopic masses given). Column chromatography was performed using silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) and for thin-layer chromatography (TLC) silica gel 60 F254 was used. Solid-phase extraction (SPE) analyses were performed with CHROMABOND Macherey-Nagel columns (Düren, Germany).

*N*-( $\omega$ -hydroxyalkyl)-9-acridone-4-carboxamides **8a–e**, *N*-( $\omega$ -hydroxyalkyl)acridine-4-carboxamides **9a–e** were prepared according to the procedure reported in literature<sup>35</sup>.

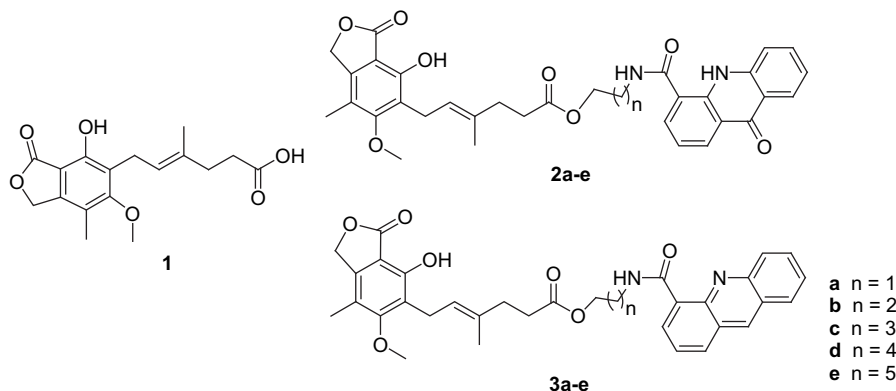
#### Synthesis of *tert*-butyldimethylsilyl ether of MPA **6**

MPA **1** (2 g, 6.2 mmol), *tert*-butyldimethylsilyl chloride (5.643 g, 37 mmol), imidazole (3.398 g, 50 mmol) were dissolved in dry DMF (10 mL), and the reaction mixture was stirred at room temperature. After 1 h no starting MPA **1** was observed (TLC). Subsequently, water (30 mL), diethyl ether (60 mL) were added and organic phase was separated, washed with five portions of water (20 mL each), and dried over anhydrous MgSO<sub>4</sub>. Solids were filtered off and solvent evaporated under reduced pressure.

The crude **4** was dissolved in THF (10 mL), water (10 mL), acetic acid (10 mL) and stirred at room temperature. The reaction

Address correspondence to Grzegorz Cholewinski, Department of Organic Chemistry, Gdansk University of Technology, ul. G. Narutowicza 11/12, 80-233 Gdansk, Poland. Tel: +48-58-347-23-00. Fax: +48-58-347-26-94. E-mail: grzchole@pg.gda.pl

Figure 1. Structures of MPA **1** and its conjugates with acridones **2a–e** and acridines **3a–e**.



mixture was monitored with TLC technique and after 1 h no substrate **4** was observed, and water (30 mL) and diethyl ether (60 mL) were added. Then, organic phase was separated, washed with five portions of water (20 mL each) and dried over anhydrous  $\text{MgSO}_4$ . Subsequently, solid were filtered off, solvent evaporated under reduced pressure. The residue was purified with column chromatography ( $\text{CH}_2\text{Cl}_2$ :MeOH 50:1 to 30:1 v/v) to give product **6** in yield 77% (2.075 g, 4.8 mmol).

MP 132–133 °C, white solid

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ [parts per million (ppm)] = 0.24 (s,  $\text{CH}_3$ , 6H), 1.03 (s,  $\text{CH}_3$ , 9H), 1.76 (s,  $\text{CH}_3$ , 3H), 2.15 (s,  $\text{CH}_3$ , 3H), 2.28–2.31 (m,  $\text{CH}_2$ , 2H), 2.40–2.43 (m,  $\text{CH}_2$ , 2H), 3.39 (d,  $J = 6.3$  Hz,  $\text{CH}_2$ , 2H), 3.74 (s,  $\text{OCH}_3$ , 3H), 5.07 (s,  $\text{CH}_2$ , 2H), 5.21 (t,  $J = 6.3$  Hz,  $\text{CH}_{\text{vinyl}}$ , 1H).

$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = –3.3, 11.6, 16.5, 19.0, 23.9, 26.3, 32.9, 34.3, 60.9, 67.9, 111.9, 118.2, 124.1, 127.8, 133.6, 146.3, 152.0, 163.4, 169.5, 179.4.

#### (Mycophenoyl-*N*-2-ethyl)-9-acridone-4-carboxamide ester **2a**

*N*-(2-hydroxyethyl)amide of 9-acrydone-4-carboxylic acid **8a** (56 mg, 0.2 mmol) was dissolved in dry chloroform (3 mL), then were added *tert*-butyldimethylsilyl ether of MPA **6** (87 mg, 0.2 mmol), 2,4,6-trichlorobenzoyl chloride (49 mg, 0.2 mmol), triethylamine (41 mg, 0.4 mmol), DMAP (5 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 24 h and water (5 mL), chloroform (5 mL) were added. Organic phase was separated, dried over anhydrous  $\text{MgSO}_4$ . Next, solids were filtered off and solvent evaporated under reduced pressure. The crude product was purified with column chromatography or SPE ( $\text{CH}_2\text{Cl}_2$ :MeOH 100:1 to 80:1, 0.1  $\text{Et}_3\text{N}$  v/v) to give *tert*-butyldimethylsilyl ether of (mycophenoyl-*N*-2-ethyl)-9-acridone-4-carboxamide ester **10a** in yield 58% (84 mg, 0.12 mmol).

MP 82–84 °C, yellow solid

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = 0.22 (s,  $\text{CH}_3$ , 6H), 1.01 (s,  $\text{CH}_3$ , 9H), 1.75 (s,  $\text{CH}_3$ , 3H), 2.10 (s,  $\text{CH}_3$ , 3H), 2.30–2.33 (m,  $\text{CH}_2$ , 2H), 2.44–2.47 (m,  $\text{CH}_2$ , 2H), 3.36 (d,  $J = 6.3$  Hz,  $\text{CH}_2$ , 2H), 3.68–3.74 (m,  $\text{NHCH}_2$ ,  $\text{OCH}_3$ , 5H), 4.31 (t,  $J = 5.4$  Hz,  $\text{OCH}_2$ , 2H), 5.03 (s,  $\text{OCH}_2$ , 2H), 5.18–5.20 (m,  $\text{CH}_{\text{vinyl}}$ , 1H), 7.17–7.22 (m,  $\text{NH}_{\text{amide}}$ ,  $\text{CH}_{\text{acr}}$ , 2H), 7.29 (dd,  $J = 8.3$  Hz,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 7.40 (d,  $J = 8.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 7.66–7.70 (m,  $\text{CH}_{\text{acr}}$ , 1H), 7.90 (d,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.45 (d,  $J = 8.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.63 (d,  $J = 8.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 12.3 (s,  $\text{NH}_{\text{acr}}$ , 1H).

Subsequently, compound **10a** was dissolved in dry THF (2 mL), treated with tetrabutylammonium fluoride (1 M solution in THF, 100  $\mu\text{L}$ ) and stirred at room temperature for 15 min. Then, the reaction mixture was partitioned between water (10 mL) and ethyl acetate (10 mL), and organic layer separated, dried over anhydrous  $\text{MgSO}_4$ . Next, solids were filtered off and solvent evaporated under reduced pressure. The crude product was purified with column

chromatography or SPE ( $\text{CH}_2\text{Cl}_2$ :MeOH 100:1 to 80:1, 0.1  $\text{Et}_3\text{N}$  v/v) to give 61 mg (0.104 mmol) of (mycophenoyl-*N*-2-ethyl)-9-acridone-4-carboxamide ester **2a** (52% according to **6**).

MP 84–86 °C, yellow solid

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = 1.77 (s,  $\text{CH}_3$ , 3H), 2.07 (s,  $\text{CH}_3$ , 3H), 2.32 (t,  $J = 7.3$  Hz,  $\text{CH}_2$ , 2H), 2.46–2.49 (m,  $\text{CH}_2$ , 2H), 3.32 (d,  $J = 6.8$  Hz,  $\text{CH}_2$ , 2H), 3.69 (s,  $\text{OCH}_3$ , 3H), 3.72–3.74 (m,  $\text{NHCH}_2$ , 2H), 4.33–4.35 (m,  $\text{OCH}_2$ , 2H), 5.13 (s,  $\text{OCH}_2$ , 2H), 5.20–5.23 (m,  $\text{CH}_{\text{vinyl}}$ , 1H), 7.15 (dd,  $J = 7.3$  Hz,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 7.26–7.31 (m,  $\text{NH}_{\text{amide}}$ ,  $\text{CH}_{\text{acr}}$ , 2H), 7.41 (d,  $J = 8.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 7.66–7.69 (m,  $\text{CH}_{\text{acr}}$ , 1H), 7.89 (d,  $J = 7.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.41 (d,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.57 (d,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 12.26 (s,  $\text{NH}_{\text{acr}}$ , 1H).

$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = 11.7, 16.4, 22.8, 33.1, 34.7, 40.0, 61.2, 63.1, 70.3, 106.6, 117.0, 117.5, 118.0, 120.0, 121.3, 122.1, 122.4, 122.6, 123.0, 127.0, 132.0, 132.1, 134.2, 140.4, 141.2, 144.4, 153.7, 163.8, 168.8, 173.1, 174.3, 178.1.

IR KBr  $\nu$ ( $\text{cm}^{-1}$ ): 3323, 2926, 1738, 1649, 1626, 1525, 1072, 1028

$\lambda_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ )/nm 256, 303, 409 ( $\epsilon/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$  29 580, 10 597, 7744)

MS ( $m/z$ ): for  $\text{C}_{33}\text{H}_{32}\text{N}_2\text{O}_8$  calculated: 584.6 found 585.2

HRMS ESI ( $m/z$ ): [ $\text{M}+\text{Na}$ ] for  $\text{C}_{33}\text{H}_{32}\text{N}_2\text{O}_8\text{Na}$  calculated: 607.2056 found 607.2048

Conjugates **2b–e** were obtained in the same way as **2a**. Intermediates **10b–e** were not isolated.

#### (Mycophenoyl-*N*-3-propyl)-9-acridone-4-carboxamide ester **2b**, 69 mg, 0.116 mmol, 58% according to **6**

MP 79–81.5 °C, yellow solid

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = 1.81 (s,  $\text{CH}_3$ , 3H), 1.96–1.98 (m,  $\text{CH}_2$ , 2H), 2.08 (s,  $\text{CH}_3$ , 3H), 2.34 (t,  $J = 7.3$  Hz,  $\text{CH}_2$ , 2H), 2.47 (t,  $J = 7.3$  Hz,  $\text{CH}_2$ , 2H), 3.37 (d,  $J = 6.4$  Hz,  $\text{CH}_2$ , 2H), 3.48–3.50 (m,  $\text{NHCH}_2$ , 2H), 3.73 (s,  $\text{OCH}_3$ , 3H), 4.24 (t,  $J = 5.9$  Hz,  $\text{OCH}_2$ , 2H), 5.15 (s,  $\text{OCH}_2$ , 2H), 5.26 (t,  $J = 6.4$  Hz,  $\text{CH}_{\text{vinyl}}$ , 1H), 7.16 (dd,  $J = 7.3$  Hz,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 7.26–7.31 (m,  $\text{NH}_{\text{amide}}$ ,  $\text{CH}_{\text{acr}}$ , 2H), 7.42 (d,  $J = 8.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 7.66–7.69 (m,  $\text{CH}_{\text{acr}}$ , 1H), 7.94 (d,  $J = 7.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.42 (d,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.58 (d,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 12.37 (s,  $\text{NH}_{\text{acr}}$ , 1H).

$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = 11.8, 16.4, 22.9, 28.9, 33.2, 34.8, 36.6, 61.2, 61.9, 70.3, 106.6, 117.0, 117.7, 118.1, 120.0, 121.4, 122.2, 122.4, 122.6, 123.0, 127.1, 131.8, 132.0, 134.2, 140.5, 141.2, 144.4, 153.8, 163.8, 168.6, 173.2, 174.3, 178.1.

IR KBr  $\nu$ ( $\text{cm}^{-1}$ ): 3304, 2924, 1735, 1649, 1619, 1524, 1073, 1030

$\lambda_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ )/nm 256, 304, 409 ( $\epsilon/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$  47 748, 17 266, 12 592)

MS ( $m/z$ ): for  $\text{C}_{34}\text{H}_{34}\text{N}_2\text{O}_8$  calculated: 598.6 found 599.2

HRMS ESI ( $m/z$ ): [M+Na] for  $C_{34}H_{34}N_2O_8Na$  calculated: 621.2213 found 621.2209.

(Mycophenoyl-*N*-4-butyl)-9-acridone-4-carboxamide ester **2c**,  
74 mg, 0.12 mmol, 60% according to **6**

MP 67–68 °C, yellow solid

$^1H$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 1.74 (m,  $CH_2$ , 4H), 1.78 (s,  $CH_3$ , 3H), 2.09 (s,  $CH_3$ , 3H), 2.28–2.31 (m,  $CH_2$ , 2H), 2.39–2.42 (m,  $CH_2$ , 2H), 3.34 (d,  $J$  = 6.8 Hz,  $CH_2$ , 2H), 3.54–3.55 (m,  $NHCH_2$ , 2H), 3.71 (s,  $OCH_3$ , 3H), 4.08 (m,  $OCH_2$ , 2H), 5.16 (s,  $OCH_2$ , 2H), 5.22 (t,  $J$  = 6.8 Hz,  $CH_{vinyl}$ , 1H), 7.12 (dd,  $J$  = 7.3 Hz,  $J$  = 7.3 Hz,  $CH_{acr}$ , 1H), 7.26–7.29 (m,  $NH_{amide}$ ,  $CH_{acr}$ , 2H), 7.41 (d,  $J$  = 8.3 Hz,  $CH_{acr}$ , 1H), 7.66 (dd,  $J$  = 7.3 Hz,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 7.99 (d,  $J$  = 7.3 Hz,  $CH_{acr}$ , 1H), 8.40 (d,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 8.53 (d,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 12.38 (s,  $NH_{acr}$ , 1H).

$^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 11.8, 16.4, 22.8, 26.2, 26.5, 33.2, 34.8, 39.9, 61.2, 64.0, 70.3, 106.5, 117.0, 117.9, 118.1, 120.0, 121.2, 122.3, 122.4, 122.9, 127.00, 131.7, 132.1, 134.2, 134.3, 140.4, 141.2, 144.3, 153.8, 163.8, 168.8, 173.2, 173.7, 178.0.

IR KBr  $\nu$ ( $cm^{-1}$ ): 3323, 2925, 1733, 1649, 1619, 1524, 1074, 1029

$\lambda_{max}$  ( $CH_2Cl_2$ )/nm 256, 304, 408 ( $\epsilon/dm^3 mol^{-1} cm^{-1}$  29 821, 10 892, 8019)

MS ( $m/z$ ): for  $C_{35}H_{36}N_2O_8$  calculated: 612.7 found 613.3

HRMS ESI ( $m/z$ ): [M+Na] for  $C_{35}H_{36}N_2O_8Na$  calculated: 635.2369 found 635.2366.

(Mycophenoyl-*N*-5-pentyl)-9-acridone-4-carboxamide ester **2d**,  
60 mg, 0.06 mmol, 48% according to **6**

MP 175–177 °C, yellow solid

$^1H$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 1.44–1.50 (m,  $CH_2$ , 2H), 1.68 (t,  $J$  = 7.3 Hz,  $CH_2$ , 2H), 1.71–1.74 (m,  $CH_2$ , 2H), 1.78 (s,  $CH_3$ , 3H), 2.19 (s,  $CH_3$ , 3H), 2.29 (t,  $J$  = 7.3 Hz,  $CH_2$ , 2H), 2.38–2.41 (m,  $CH_2$ , 2H), 3.36 (d,  $J$  = 6.8 Hz,  $CH_2$ , 2H), 3.51–3.55 (m,  $NHCH_2$ , 2H), 3.73 (s,  $OCH_3$ , 3H), 4.05 (d,  $J$  = 6.3 Hz,  $OCH_2$ , 2H), 5.18–5.21 (m,  $OCH_2$ ,  $CH_{vinyl}$ , 3H), 6.89 (s, OH, 1H), 7.21 (dd,  $J$  = 7.3 Hz,  $J$  = 7.3 Hz,  $CH_{acr}$ , 1H), 7.30 (dd,  $J$  = 7.3 Hz,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 7.43 (d,  $J$  = 8.3 Hz,  $CH_{acr}$ , 1H), 7.68–7.71 (m,  $NH_{amide}$ ,  $CH_{acr}$ , 2H), 7.99 (d,  $J$  = 7.3 Hz,  $CH_{acr}$ , 1H), 8.47 (d,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 8.63 (d,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 12.44 (s,  $NH_{acr}$ , 1H).

$^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 11.8, 16.3, 22.8, 23.6, 28.6, 29.2, 33.2, 34.8, 40.2, 61.2, 64.1, 70.3, 106.5, 117.0, 117.9, 118.00, 119.9, 121.4, 122.3, 122.4, 122.9, 127.1, 131.7, 132.0, 134.1, 134.3, 140.5, 141.3, 144.3, 153.9, 163.9, 168.8, 173.2, 173.7, 178.1.

IR KBr  $\nu$ ( $cm^{-1}$ ): 3330, 2925, 1733, 1650, 1523, 1070, 1027

$\lambda_{max}$  ( $CH_2Cl_2$ )/nm 256, 303, 409 ( $\epsilon/dm^3 mol^{-1} cm^{-1}$  35 225, 12 515, 9215)

MS ( $m/z$ ): for  $C_{36}H_{38}N_2O_8$  calculated: 626.7, found 627.3

HRMS ESI ( $m/z$ ): [M+Na] for  $C_{36}H_{38}N_2O_8Na$  calculated: 649.2526 found 649.2520.

(Mycophenoyl-*N*-6-hexyl)-9-acridone-4-carboxamide ester **2e**,  
69 mg, 0.108 mmol, 54%, according to **6**

MP 94–96 °C, yellow solid

$^1H$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 1.36–1.49 (m,  $CH_2$ , 4H), 1.59–1.65 (m,  $CH_2$ , 2H), 1.68–1.74 (m,  $CH_2$ , 2H), 1.79 (s,  $CH_3$ , 3H), 2.11 (s,  $CH_3$ , 3H), 2.30 (t,  $J$  = 7.3 Hz,  $CH_2$ , 2H), 2.39–2.42 (m,  $CH_2$ , 2H), 3.36 (d,  $J$  = 6.8 Hz,  $CH_2$ , 2H), 3.51–3.55 (m,  $NHCH_2$ , 2H), 3.73 (s,  $OCH_3$ , 3H), 4.03 (d,  $J$  = 6.8 Hz,  $OCH_2$ , 2H), 5.18 (s,  $OCH_2$ , 2H), 5.21–5.24 (m,  $CH_{vinyl}$ , 1H), 6.91 (s, OH, 1H), 7.22 (dd,  $J$  = 7.8 Hz,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 7.31 (dd,  $J$  = 7.3 Hz,

$J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 7.43 (d,  $J$  = 8.3 Hz,  $CH_{acr}$ , 1H), 7.68–7.72 (m,  $NH_{amide}$ ,  $CH_{acr}$ , 2H), 8.00 (d,  $J$  = 6.8 Hz,  $CH_{acr}$ , 1H), 8.48 (d,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 8.66 (d,  $J$  = 7.8 Hz,  $CH_{acr}$ , 1H), 12.48 (s,  $NH_{acr}$ , 1H).

$^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 11.8, 16.4, 22.8, 25.8, 26.8, 28.8, 29.6, 33.2, 34.8, 40.2, 61.2, 64.4, 70.4, 106.5, 117.0, 117.9, 118.1, 119.4, 120.0, 121.2, 122.4, 122.4, 122.9, 127.1, 131.9, 132.0, 134.3, 140.5, 141.2, 144.2, 153.8, 163.9, 168.7, 173.4, 173.8, 178.2.

IR KBr  $\nu$ ( $cm^{-1}$ ): 3336, 2936, 1734, 1645, 1619, 1596, 1524, 1077, 1030

$\lambda_{max}$  ( $CH_2Cl_2$ )/nm 256, 303, 408 ( $\epsilon/dm^3 mol^{-1} cm^{-1}$  38 230, 13 623, 9976)

MS ( $m/z$ ): for  $C_{37}H_{40}N_2O_8$  calculated: 640.7 found 641.3

HRMS ESI ( $m/z$ ): [M+Na] for  $C_{37}H_{40}N_2O_8Na$  calculated: 663.2682 found 663.2676.

### Synthesis of *tert*-butyldiphenylsilyl ether of MPA **7**

MPA **1** (2 g, 6.2 mmol), *tert*-butyldiphenylsilyl chloride (10.23 g, 37 mmol), imidazole (3.398 g, 50 mmol), DMAP (10 mg, 0.08 mmol) were dissolved in dry DMF (10 mL), and the reaction mixture was stirred at room temperature. After 24 h no starting MPA **1** was observed (TLC). Subsequently, water (30 mL) and diethyl ether (60 mL) were added and organic phase was separated, washed with 2 N HCl (20 mL) and dried over anhydrous  $MgSO_4$ . Solids were filtered off and solvent evaporated under reduced pressure.

The crude **5** was dissolved in THF (10 mL), acetic acid (10 mL), 1 N HCl (10 mL) and stirred at room temperature. The reaction mixture was monitored with TLC technique and after 12 h no substrate **5** was observed, and water (30 mL) and diethyl ether (60 mL) were added. Then, organic phase was separated, washed with five portions of water (20 mL each), and dried over anhydrous  $MgSO_4$ . Subsequently, solid were filtered off, solvent evaporated under reduced pressure. The residue was purified with column chromatography ( $CH_2Cl_2$ :MeOH 50:1 to 30:1 v/v, white foam during evaporation) to give product **7** in yield 89% (3.083 g, 5.52 mmol).

MP 61–64 °C, white foam

$^1H$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 1.10 (s,  $CH_3$ , 9H), 1.50 (s,  $CH_3$ , 3H), 2.12 (s,  $CH_3$ , 3H), 2.18–2.21 (m,  $CH_2$ , 2H), 2.32–2.35 (m,  $CH_2$ , 2H), 3.18 (d,  $J$  = 5.9 Hz,  $CH_2$ , 2H), 3.62 (s,  $OCH_3$ , 3H), 4.96–4.99 (m,  $CH_2$ ,  $CH_{vinyl}$ , 3H), 7.32 (dd,  $J$  = 6.8 Hz,  $J$  = 7.8 Hz,  $CH_{arom}$ , 4H), 7.38 (t,  $J$  = 7.3 Hz,  $CH_{arom}$ , 2H), 7.70 ( $J$  = 6.8 Hz,  $CH_{arom}$ , 4H).

$^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$ (ppm) = 11.6, 16.4, 20.6, 24.5, 26.8, 32.8, 34.2, 60.8, 67.7, 111.4, 118.1, 124.2, 127.4, 127.6, 129.7, 133.5, 133.8, 135.2, 146.2, 151.8, 163.4, 168.6, 179.4.

### (Mycophenoyl-*N*-2-ethyl)-acridine-4-carboxamide ester **3a**

*N*-(2-hydroxyethyl)acridine-4-carboxamide **9a** (53 mg, 0.2 mmol) was dissolved in dry chloroform (3 mL), then were added *tert*-butyldiphenylsilyl ether of MPA **7** (103 mg, 0.2 mmol), 2,4,6-trichlorobenzoyl chloride (49 mg, 0.2 mmol), triethylamine (41 mg, 0.4 mmol), DMAP (5 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 24 h and water (5 mL), chloroform (5 mL) were added. Organic phase was separated, dried over anhydrous  $MgSO_4$ . Next, solids were filtered off and solvent evaporated under reduced pressure. The crude product was purified with column chromatography or SPE ( $CH_2Cl_2$ :MeOH 100:1 to 80:1, 0.1  $Et_3N$  v/v) to give *tert*-butyldiphenylsilyl ether of (mycophenoyl-*N*-2-ethyl)-acridine-4-carboxamide ester **11a** in yield 61% (93 mg, 0.12 mmol).

MP 73–75 °C, yellow solid

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) = 1.06 (s, CH<sub>3</sub>, 9H), 1.42 (s, CH<sub>3</sub>, 3H), 2.08 (s, CH<sub>3</sub>, 3H), 2.21–2.24 (m, CH<sub>2</sub>, 2H), 2.40–2.43 (m, CH<sub>2</sub>, 2H), 3.09 (d, *J* = 5.9 Hz, CH<sub>2</sub>, 2H), 3.57 (s, OCH<sub>3</sub>, 3H), 3.916–3.925 (m, NHCH<sub>2</sub>, 2H), 4.41 (t, *J* = 5.4 Hz, OCH<sub>2</sub>, 2H), 4.91–4.94 (m, OCH<sub>2</sub>, CH<sub>vinyl</sub>, 3H), 7.25–7.28 (m, CH<sub>arom</sub>, 4H), 7.33 (t, *J* = 7.3 Hz, CH<sub>arom</sub>, 2H), 7.58 (dd, *J* = 7.3 Hz, *J* = 7.3 Hz, CH<sub>acr</sub>, 1H), 7.65 (d, *J* = 6.8 Hz, CH<sub>arom</sub>, 4H), 7.70–7.73 (m, CH<sub>acr</sub>, 1H), 7.76 (dd, *J* = 7.3 Hz, *J* = 7.8 Hz, CH<sub>acr</sub>, 1H), 8.05 (d, *J* = 8.3 Hz, CH<sub>acr</sub>, 1H), 8.14 (d, *J* = 8.8 Hz, CH<sub>acr</sub>, 1H), 8.17 (d, *J* = 8.3 Hz, CH<sub>acr</sub>, 1H), 8.93 (m, CH<sub>acr</sub>, 1H), 9.04 (s, NH<sub>amide</sub>, 1H), 12.06 (s, CH<sub>acr</sub>, 1H).

Subsequently, compound **11a** was dissolved in dry THF (2 mL), treated with tetrabutylammonium fluoride (1 M solution in THF, 100 μL), and stirred at room temperature for 15 min. Then the reaction mixture was partitioned between water (10 mL) and ethyl acetate (10 mL), and organic layer separated, dried over anhydrous MgSO<sub>4</sub>. Next, solids were filtered off and solvent evaporated under reduced pressure. The crude product was purified with column chromatography or SPE (CH<sub>2</sub>Cl<sub>2</sub>:MeOH 100:1 to 80:1, 0.1 Et<sub>3</sub>N v/v) to give 58 mg (0.102 mmol) of (mycophenoyl-*N*-2-ethyl)-acridine-4-carboxamide ester **3a** (51% according to **7**).

MP 136–138 °C, yellow solid

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) = 1.74 (s, CH<sub>3</sub>, 3H), 2.08 (s, CH<sub>3</sub>, 3H), 2.33–2.36 (m, CH<sub>2</sub>, 2H), 2.50–2.54 (m, CH<sub>2</sub>, 2H), 3.33 (d, *J* = 6.8 Hz, CH<sub>2</sub>, 2H), 3.70 (s, OCH<sub>3</sub>, 3H), 3.93–3.94 (m, NHCH<sub>2</sub>, 2H), 4.41 (t, *J* = 5.4 Hz, OCH<sub>2</sub>, 2H), 5.13 (s, OCH<sub>2</sub>, 2H), 5.20–5.22 (m, CH<sub>vinyl</sub>, 1H), 7.58–7.61 (m, OH, CH<sub>acr</sub>, 2H), 7.66 (dd, *J* = 7.3 Hz, *J* = 7.3 Hz, CH<sub>acr</sub>, 1H), 7.80 (dd, *J* = 7.3 Hz, *J* = 7.3 Hz, CH<sub>acr</sub>, 1H), 8.04 (d, *J* = 8.3 Hz, CH<sub>acr</sub>, 1H), 8.14–8.17 (m, CH<sub>acr</sub>, 2H), 8.90 (m, CH<sub>acr</sub>, 1H), 8.98 (s, NH<sub>amide</sub>, 1H), 12.07 (s, CH<sub>acr</sub>, 1H).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ(ppm) = 11.8, 16.4, 22.8, 33.3, 34.8, 39.0, 61.2, 63.8, 70.2, 106.5, 116.9, 122.3, 122.9, 125.6, 125.9, 126.2, 126.7, 128.5, 128.7, 128.8, 131.8, 132.8, 134.3, 135.9, 138.3, 144.2, 146.0, 147.2, 153.8, 163.9, 166.4, 173.1, 173.5.

$\lambda_{\max}$  (CH<sub>2</sub>Cl<sub>2</sub>)/nm 250, 305, 342, 359, 377 (ε/dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup> 92 249, 5376, 6653, 11 172, 5669).

IR KBr v(cm<sup>-1</sup>): 3405, 2922, 1736, 1655, 1625, 1565, 1552, 1524, 1164, 1138, 738.

MS (*m/z*): [M]<sup>+</sup> for C<sub>33</sub>H<sub>32</sub>N<sub>2</sub>O<sub>7</sub> calculated: 568.6, found: 569.0

HRMS ESI (*m/z*): [M+Na]<sup>+</sup> for C<sub>33</sub>H<sub>32</sub>N<sub>2</sub>O<sub>7</sub>Na calculated: 591.2107 found 591.2111.

Conjugates **3b–e** were obtained in the same way as **3a**. Intermediates **11b–e** were not isolated.

(Mycophenoyl-*N*-3-propyl)-acridine-4-carboxamide ester **3b**,

48 mg, 0.082 mmol, 41% according to **7**

MP 48–50 °C, yellow solid

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) = 1.66 (s, CH<sub>3</sub>, 3H), 1.99 (s, CH<sub>3</sub>, 3H), 1.93–2.18 (m, CH<sub>2</sub>, 4H), 2.29–2.36 (m, CH<sub>2</sub>, 2H), 3.24 (d, *J* = 6.4 Hz, CH<sub>2</sub>, 2H), 3.61 (s, OCH<sub>3</sub>, 3H), 3.70–3.94 (m, NHCH<sub>2</sub>, 2H), 4.17–4.23 (m, OCH<sub>2</sub>, 2H), 5.02–5.05 (m, CH<sub>vinyl</sub>, 1H), 5.19 (s, OCH<sub>2</sub>, 2H), 7.66–7.79 (m, OH, CH<sub>acr</sub>, 3H), 7.95 (dd, *J* = 6.8 Hz, *J* = 7.9 Hz, CH<sub>acr</sub>, 1H), 8.20–8.39 (m, CH<sub>acr</sub>, 3H), 8.72 (d, *J* = 6.8 Hz, CH<sub>acr</sub>, 1H), 9.32–9.36 (m, NH<sub>amide</sub>, 1H), 11.35 (s, CH<sub>acr</sub>, 1H).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ(ppm) = 11.2, 16.1, 22.6, 28.7, 32.5, 34.3, 36.4, 60.8, 62.3, 68.9, 107.2, 116.2, 122.5, 123.2, 125.6, 125.9, 126.7, 126.9, 128.4, 128.7, 128.8, 132.3, 133.2, 133.4, 134.8, 139.2, 145.6, 146.0, 147.2, 153.0, 162.8, 165.1, 170.4, 172.9.

IR KBr v(cm<sup>-1</sup>): 3421, 2928, 1731, 1654, 1623, 1562, 1522, 1162, 1132, 741.

$\lambda_{\max}$  (CH<sub>2</sub>Cl<sub>2</sub>)/nm 251, 305, 342, 359, 377 (ε/dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup> 85 875, 4692, 5749, 9995, 5096)

MS (*m/z*): [M]<sup>+</sup> for C<sub>34</sub>H<sub>34</sub>N<sub>2</sub>O<sub>7</sub>: calculated: 582.6, found: 583.1

HRMS ESI (*m/z*): [M]<sup>+</sup> for C<sub>34</sub>H<sub>35</sub>N<sub>2</sub>O<sub>7</sub> calculated: 583.2444 found 583.2445

(Mycophenoyl-*N*-4-butyl)-acridine-4-carboxamide ester **3c**,

72 mg, 0.12 mmol, 62% according to **7**

MP 51–53 °C, yellow solid

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) = 1.67 (s, CH<sub>3</sub>, 3H), 1.72–1.79 (m, CH<sub>2</sub>, 4H), 1.99 (s, CH<sub>3</sub>, 3H), 2.16 (t, *J* = 7.3 Hz, CH<sub>2</sub>, 2H), 2.34 (t, *J* = 7.3 Hz, CH<sub>2</sub>, 2H), 3.22 (d, *J* = 6.8 Hz, CH<sub>2</sub>, 2H), 3.52–3.55 (m, NHCH<sub>2</sub>, 2H), 3.60 (s, OCH<sub>3</sub>, 3H), 4.04–4.06 (m, OCH<sub>2</sub>, 2H), 5.07–5.10 (m, CH<sub>vinyl</sub>, 1H), 5.18 (s, OCH<sub>2</sub>, 2H), 7.66 (dd, *J* = 7.3 Hz, *J* = 7.8 Hz, CH<sub>acr</sub>, 1H), 7.72 (dd, *J* = 7.3 Hz, *J* = 7.8 Hz, CH<sub>acr</sub>, 1H), 7.89–7.93 (m, CH<sub>acr</sub>, 1H), 8.19–8.21 (m, OH, CH<sub>acr</sub>, 2H), 8.34 (d, *J* = 7.3 Hz, CH<sub>acr</sub>, 1H), 8.69–8.70 (m, CH<sub>acr</sub>, 1H), 9.27 (s, CH<sub>acr</sub>, 1H), 9.29 (s, NH<sub>amide</sub>, 1H), 11.36–11.38 (m, CH<sub>acr</sub>, 1H).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ(ppm) = 11.7, 16.5, 23.0, 26.4, 26.7, 33.1, 34.8, 39.4, 61.2, 64.2, 69.3, 107.6, 116.6, 122.9, 123.7, 126.0, 126.3, 127.2, 127.2, 129.1, 129.2, 129.2, 132.6, 133.5, 133.9, 135.0, 139.2, 146.0, 146.8, 147.3, 153.8, 163.8, 165.2, 170.2, 173.9.

IR KBr v(cm<sup>-1</sup>): 3418, 2923, 1732, 1652, 1624, 1562, 1522, 1161, 1132, 740.

$\lambda_{\max}$  (CH<sub>2</sub>Cl<sub>2</sub>)/nm 251, 305, 342, 359, 377 (ε/dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup> 58 658, 3378, 3897, 6723, 3371)

MS (*m/z*): [M]<sup>+</sup> for C<sub>35</sub>H<sub>36</sub>N<sub>2</sub>O<sub>7</sub>: calculated: 596.7 found: 597.0

HRMS ESI (*m/z*): [M+Na]<sup>+</sup> for C<sub>35</sub>H<sub>36</sub>N<sub>2</sub>O<sub>7</sub>Na calculated: 619.2420 found 619.2418.

(Mycophenoyl-*N*-5-pentyl)-acridine-4-carboxamide ester **3d**,

72 mg, 0.118 mmol, 59% according to **7**

MP 54–56 °C, yellow solid

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) = 1.50–1.72 (m, CH<sub>2</sub>, 6H), 1.60 (s, CH<sub>3</sub>, 3H), 2.01 (s, CH<sub>3</sub>, 3H), 2.04–2.07 (m, CH<sub>2</sub>, 2H), 2.19–2.22 (m, CH<sub>2</sub>, 2H), 3.21 (d, *J* = 6.8 Hz, CH<sub>2</sub>, 2H), 3.51–3.56 (m, NHCH<sub>2</sub>, 2H), 3.60 (s, OCH<sub>3</sub>, 3H), 3.96 (t, *J* = 6.3 Hz, OCH<sub>2</sub>, 2H), 5.00–5.02 (m, CH<sub>vinyl</sub>, 1H), 5.20 (s, OCH<sub>2</sub>, 2H), 7.67 (dd, *J* = 6.8 Hz, *J* = 7.8 Hz, CH<sub>acr</sub>, 1H), 7.73 (dd, *J* = 6.8 Hz, *J* = 7.8 Hz, CH<sub>acr</sub>, 1H), 7.91–7.94 (m, CH<sub>acr</sub>, 1H), 8.19–8.20 (m, OH, CH<sub>acr</sub>, 2H), 8.34 (d, *J* = 8.3 Hz, CH<sub>acr</sub>, 1H), 8.71 (d, *J* = 6.8 Hz, CH<sub>acr</sub>, 1H), 9.25–9.30 (m, CH<sub>acr</sub>, 1H), 9.33 (s, NH<sub>amide</sub>, 1H), 11.43 (s, CH<sub>acr</sub>, 1H).

<sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ(ppm) = 11.7, 16.5, 23.0, 24.0, 28.6, 29.4, 33.0, 34.7, 61.2, 64.4, 64.6, 69.3, 107.6, 116.6, 123.0, 123.6, 126.0, 126.3, 127.2, 127.2, 129.0, 129.1, 129.2, 132.7, 133.5, 133.8, 135.3, 139.4, 146.2, 146.4, 147.7, 153.4, 163.2, 165.3, 170.8, 173.1.

IR KBr v(cm<sup>-1</sup>): 3421, 2928, 1732, 1653, 1624, 1563, 1523, 1162, 1133, 741.

$\lambda_{\max}$  (CH<sub>2</sub>Cl<sub>2</sub>)/nm 251, 305, 342, 359, 377 (ε / dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup> 68 208, 4887, 5394, 9045, 4646)

MS (*m/z*): [M]<sup>+</sup> for C<sub>36</sub>H<sub>38</sub>N<sub>2</sub>O<sub>7</sub> calculated: 610.7, found: 611.1

HRMS ESI (*m/z*): [M]<sup>+</sup> for C<sub>36</sub>H<sub>39</sub>N<sub>2</sub>O<sub>7</sub> calculated: 611.2757 found 611.2750.

(Mycophenoyl-*N*-6-hexyl)-acridine-4-carboxamide ester **3e**,

73 mg, 0.118 mmol, 59%, according to **7**

MP 57–59 °C, yellow solid

<sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ(ppm) = 1.37–1.62 (m, CH<sub>2</sub>, 8H), 1.68 (s, CH<sub>3</sub>, 3H), 2.00 (s, CH<sub>3</sub>, 3H), 2.13 (t, *J* = 7.3 Hz, CH<sub>2</sub>, 2H), 2.29

(t,  $J = 7.3$  Hz,  $\text{CH}_2$ , 2H), 3.22 (d,  $J = 6.8$  Hz,  $\text{CH}_2$ , 2H), 3.52–3.54 (m,  $\text{NHCH}_2$ , 2H), 3.61 (s,  $\text{OCH}_3$ , 3H), 3.92 (t,  $J = 6.3$  Hz,  $\text{OCH}_2$ , 2H), 5.07–5.09 (m,  $\text{CH}_{\text{vinyl}}$ , 1H), 5.19 (s,  $\text{OCH}_2$ , 2H), 7.67–7.75 (m,  $\text{CH}_{\text{acr}}$ , 2H), 7.92–7.95 (m,  $\text{CH}_{\text{acr}}$ , 1H), 8.18–8.22 (m, OH,  $\text{CH}_{\text{acr}}$ , 2H), 8.35 (d,  $J = 7.8$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 8.71 (d,  $J = 7.3$  Hz,  $\text{CH}_{\text{acr}}$ , 1H), 9.302–9.307 (m,  $\text{CH}_{\text{acr}}$ ,  $\text{NH}_{\text{amide}}$ , 2H), 11.42 (s,  $\text{CH}_{\text{acr}}$ , 1H).

$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ (ppm) = 11.7, 16.5, 23.0, 25.9, 27.1, 29.7, 33.0, 34.8, 61.2, 64.4, 64.5, 69.3, 107.6, 116.6, 119.8, 123.0, 123.7, 126.0, 126.3, 127.2, 127.2, 129.0, 129.3, 132.7, 133.5, 133.9, 135.3, 139.4, 146.2, 146.4, 147.7, 153.4, 163.2, 165.3, 170.8, 173.1.

IR KBr  $\nu$ ( $\text{cm}^{-1}$ ): 3422, 2925, 1732, 1651, 1624, 1564, 1523, 1162, 1134, 741.

$\lambda_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ )/nm 251, 305, 342, 359, 377 ( $\epsilon/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ ) 10 3670, 6576, 7236, 12 002, 6230

MS ( $m/z$ ):  $[\text{M}]^+$  for  $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_7$  calculated: 624.7, found: 625.0

HRMS ESI ( $m/z$ ):  $[\text{M}]^+$  for  $\text{C}_{37}\text{H}_{41}\text{N}_2\text{O}_7$  calculated: 625.2914 found 625.2906.

### Biological activity evaluation

The following cells were used: Jurkat cell line (ATCC collection) or PBMC obtained from buffy coats of healthy volunteers by standard ficoll gradient centrifugation. The cells were disposed  $5 \times 10^4$  cells per well in RPMI medium containing 10% fetal calf serum in an atmosphere of 5%  $\text{CO}_2$  at 37 °C in triplicates with the following final concentration of the examined conjugates: 0 (control), 0.0001, 0.001, 0.01 and 0.1 mg/ml.

### Viability tests $\text{IC}_{50}$

The readout of the test was performed with colorimetric MTT assay. After 24 h of the incubation, (3–4.5 dimethylthiazol-2-yl)-2.5 diphenyl-tetrazolium bromide (MTT) was added to the wells in the final concentration of 1 mg/ml and the plates were incubated for additional 4 h. Then, the reaction was stopped by addition of 100  $\mu\text{l}$  of isopropanol. Optical density was read at 570 nm on the automated plate reader (Victor 4, PerkinElmer, Waltham, MA).

### Proliferation tests $\text{EC}_{50}$

The readout of the test was performed with tritium assay. The proliferative ability of the cells was measured in response to CD3/CD28 Dynabeads (Dyna, Dynabeads®, Life Technologies, Carlsbad, CA) in 1:1 ratio cultured for 4 days. Proliferation was measured after  $^3\text{H}$ -thymidine (0.5  $\mu\text{Ci}$ /well) incubation for the last 16 h [Liquid Scintillation Counter (LSC) reader, PerkinElmer, Waltham, MA] as counts per minute units.

The results from both tests were then adjusted with SigmaPlot Software (Systat Software Inc., San Jose, CA) in order to obtain  $\text{IC}_{50}$  or  $\text{EC}_{50}$  values for each compound.

## Results

### Chemistry

Direct condensation of MPA **1** with  $N$ -( $\omega$ -hydroxyalkyl)-9-acridone-4-carboxamides **8a–e** (35) or  $N$ -( $\omega$ -hydroxyalkyl)acridine-4-carboxamides **9a–e** (35) occurred to be laborious, probably due to participation of free phenolic group in undesired esterifications or macrolactonization. As a result, isolation of pure compounds **2a–e** and **3a–e** was difficult. Therefore, the preparation of MPA bearing blocked phenolic group was performed similarly to procedures reported in literature (14,26) (Scheme 1). Thus, MPA **1** was treated with excess of

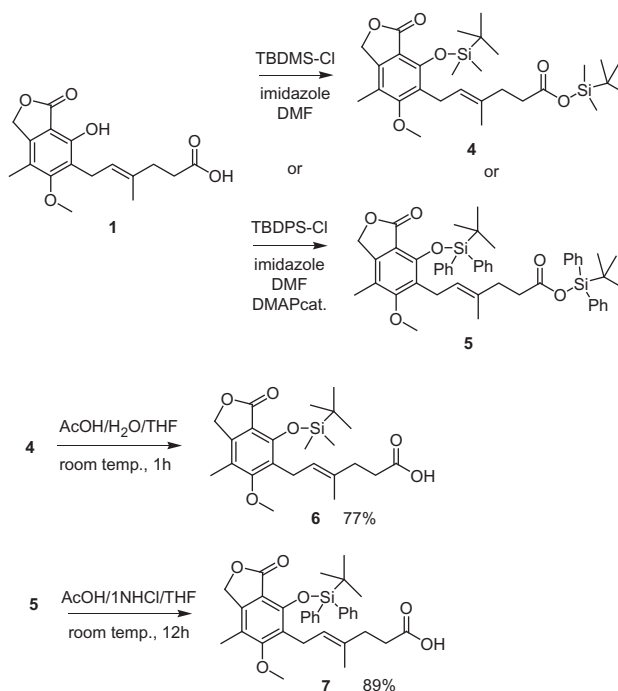
*tert*-butyldimethylsilyl chloride and both phenol and carboxylic groups underwent silylation to **4**. Subsequently, *tert*-butyldimethylsilyl ester was hydrolyzed in the presence of *tert*-butyldimethylsilyl ether to MPA derivative **6** bearing free carboxylic group, but protected phenolic group.

We tested several methods for esterification including commercially available coupling agents and the best results were achieved in case of Yamaguchi protocol<sup>42,43</sup> (Scheme 2). The reaction of blocked MPA **6** with  $N$ -( $\omega$ -hydroxyalkyl)-9-acridone-4-carboxamides **8a–e** led to esters **10a–e**, followed by deprotection of phenolic group by tetrabutylammonium fluoride treatment to conjugates **2a–e** in moderate yields (48–60%, according to **6**).

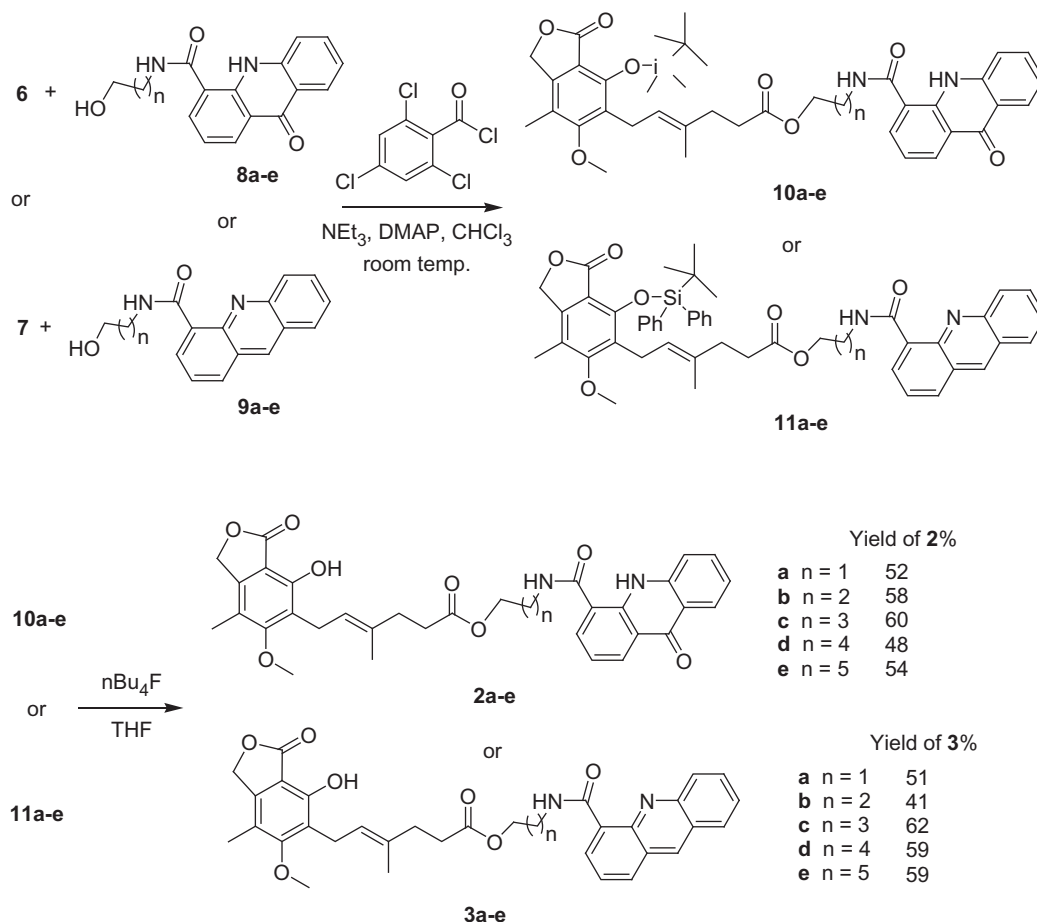
Similarly to this synthetic pathway, MPA derivative **6** was treated with  $N$ -( $\omega$ -hydroxyalkyl)acridine-4-carboxamides **9a–e** towards conjugates **3a–e**. However, we observed unexpected recovering of phenolic group during this esterification. The unprompted removal of *tert*-butyldimethylsilyl ether caused contamination of product **3**, which was difficult to purify. It suggested clearly, that nitrogen atom in acridine ring participated in this undesired deprotection.

It was widely reported, that stability of silyl ethers depends strongly on substituents at silicon atom<sup>44</sup>. Particularly, bulky alkyl or aryl groups increase resistance towards basic reagents and this correlation is a one possible basis in selective recovering of hydroxyl groups blocked with silyl ethers. For instance, *tert*-butyldimethylsilyl ether can be cleaved under basic conditions in the presence of *tert*-butyldiphenylsilyl ether, which is more stable due to steric reasons<sup>44</sup>.

Then we decided to obtain MPA bearing protected phenolic group as more stable *tert*-butyldiphenylsilyl ether **7** (Scheme 1). The synthetic pathway was similar to **6**, but silylation of MPA **1** to **5** with *tert*-butyldiphenylsilyl required catalytic amount of DMAP, and hydrolysis of *tert*-butyldiphenylsilyl ester **5** to **7** needed more acidic conditions. Noteworthy, we observed no interrupting deprotection in the course of this esterification and conjugates **3a–e** were obtained in moderate yields (41–62%, according to **7**).



Scheme 1. Synthesis of *tert*-butyldimethylsilyl ether of MPA **6** and *tert*-butyldiphenylsilyl ether of MPA **7**.

Scheme 2. Synthesis of conjugates of MPA with acridones **2a–e** and conjugates of MPA with acridones **3a–e**.Table 1. EC<sub>50</sub> [μM] values of conjugates **2a–e**, **3a–e**, *N*-(ω-hydroxyalkyl)-9-acridone-4-carboxamides **8a–e**, *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e**, **1** for cell line Jurkat and activated PBMC, obtained in antiproliferation test, *p* statistical significance.

Compound	EC <sub>50</sub> Jurkat	<i>p</i>	EC <sub>50</sub> PBMC	<i>p</i>
<b>2a</b>	0.86 ± 0.684	<0.05	0.001 ± 0.0004	<0.05
<b>2b</b>	0.33 ± 0.167	<0.05	2 × 10 <sup>-6</sup> ± 1 × 10 <sup>-6</sup>	<0.05
<b>2c</b>	0.98 ± 0.653	<0.05	0.01 ± 0.003	<0.05
<b>2d</b>	0.32 ± 0.159	<0.05	0.002 ± 0.001	<0.05
<b>2e</b>	0.48 ± 0.230	<0.05	0.002 ± 0.001	<0.05
<b>8a</b>	21.25 ± 4.605		6.38 ± 2.125	
<b>8b</b>	>33.75		5.74 ± 2.025	
<b>8c</b>	>32.22		2.90 ± 1.611	
<b>8d</b>	>30.29		1.23 ± 0.250	
<b>8e</b>	>29.55		1.48 ± 0.295	
<b>3a</b>	0.02 ± 0.006	<0.05	0.005 ± 0.001	<0.05
<b>3b</b>	0.01 ± 0.007	<0.05	0.003 ± 0.002	<0.05
<b>3c</b>	0.02 ± 0.009	<0.05	0.006 ± 0.004	<0.05
<b>3d</b>	0.02 ± 0.010	<0.05	0.01 ± 0.003	<0.05
<b>3e</b>	0.02 ± 0.008	<0.05	0.004 ± 0.003	<0.05
<b>9a</b>	0.098 ± 0.0049		1.13 ± 0.105	
<b>9b</b>	0.10 ± 0.005		0.71 ± 0.150	
<b>9c</b>	0.09 ± 0.004		0.34 ± 0.088	
<b>9d</b>	2.92 ± 0.324		0.32 ± 0.076	
<b>9e</b>	0.31 ± 0.161		0.31 ± 0.099	
MPA	0.07 ± 0.028	–	0.04 ± 0.004	–

### Biological results

Antiproliferative activity of the obtained compounds was measured with Jurkat cell line and PBMC (peripheral blood mononuclear cells from healthy donors). Data collected in Table 1

present EC<sub>50</sub> of conjugates **2a–e**, **3a–e**. These results were compared to starting acridones **8a–e**, acridines **9a–e** respectively, and MPA **1**. Acridine conjugates **3a–e** occurred to suppress proliferation more than acridone conjugates **2a–e**, starting

Table 2. IC<sub>50</sub> [μM] values of conjugates **2a–e**, **3, a–e**, *N*-(ω-hydroxyalkyl)-9-acridone-4-carboxamides **8a–e**, *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e**, **1** for cell line Jurkat and activated PBMC, obtained in MTT test, *p* statistical significance.

Compound	IC <sub>50</sub> Jurkat	<i>p</i>	IC <sub>50</sub> PBMC	<i>p</i>
<b>2a</b>	0.51 ± 0.171	<0.05	3.93 ± 1.882	<0.05
<b>2b</b>	2.34 ± 2.840	<0.05	>16.71	0.057
<b>2c</b>	1.31 ± 0.816	<0.05	>16.32	0.086
<b>2d</b>	3.99 ± 3.191	<0.05	>15.96	0.074
<b>2e</b>	1.56 ± 0.312	<0.05	>15.61	0.052
<b>8a</b>	15.59 ± 2.480		>35.42	
<b>8b</b>	9.79 ± 1.012		>33.75	
<b>8c</b>	10.31 ± 1.289		>32.22	
<b>8d</b>	2.16 ± 0.308		>30.83	
<b>8e</b>	7.68 ± 1.182		>29.55	
<b>3a</b>	0.35 ± 0.139	<0.05	>17.59	0.064
<b>3b</b>	0.86 ± 0.168	<0.05	4.98 ± 0.858	<0.05
<b>3c</b>	0.34 ± 0.121	<0.05	5.20 ± 3.687	<0.05
<b>3d</b>	0.49 ± 0.164	<0.05	2.62 ± 1.474	<0.05
<b>3e</b>	0.64 ± 0.160	<0.05	1.60 ± 0.960	<0.05
<b>9a</b>	8.26 ± 3.755		4.13 ± 1.878	
<b>9b</b>	9.99 ± 4.638		3.09 ± 2.141	
<b>9c</b>	9.17 ± 4.755		6.45 ± 1.698	
<b>9d</b>	11.67 ± 1.621		>32.42	
<b>9e</b>	10.55 ± 4.032		18.3 ± 4.03	
MPA	0.01 ± 0.059	–	>31.22	–

Table 3. Comparison of EC<sub>50</sub> [μM] values of conjugates **2a–e**, **3, a–e**, with mixtures of MPA and respective *N*-(ω-hydroxyalkyl)-9-acridone-4-carboxamides **8a–e**, *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e**, **1** for cell line Jurkat, *p* statistical significance.

Compound	EC <sub>50</sub> Jurkat	<i>p</i>	EC <sub>50</sub> Jurkat MPA + <b>8a–e</b> or <b>9a–e</b>	<i>p</i>
<b>2a</b>	0.86 ± 0.684	<0.05	0.33 ± 1.332 (MPA + <b>8a</b> )	<0.05
<b>2b</b>	0.33 ± 0.167	<0.05	1.30 ± 2.273 (MPA + <b>8b</b> )	<0.05
<b>2c</b>	0.98 ± 0.653	<0.05	0.63 ± 0.634 (MPA + <b>8c</b> )	<0.05
<b>2d</b>	0.32 ± 0.159	<0.05	1.86 ± 0.310 (MPA + <b>8d</b> )	<0.05
<b>2e</b>	0.48 ± 0.230	<0.05	1.52 ± 1.216 (MPA + <b>8e</b> )	<0.05
<b>3a</b>	0.02 ± 0.006	<0.05	0.69 ± 0.688 (MPA + <b>9a</b> )	<0.05
<b>3b</b>	0.01 ± 0.007	<0.05	2.68 ± 0.334 (MPA + <b>9b</b> )	<0.05
<b>3c</b>	0.02 ± 0.009	<0.05	0.65 ± 0.117 (MPA + <b>9c</b> )	<0.05
<b>3d</b>	0.02 ± 0.010	<0.05	0.64 ± 0.318 (MPA + <b>9d</b> )	<0.05
<b>3e</b>	0.02 ± 0.008	<0.05	1.56 ± 0.622 (MPA + <b>9e</b> )	<0.05
MPA	0.07 ± 0.028	–	–	–

Table 4. Selectivity index (IC<sub>50</sub>/EC<sub>50</sub>) calculated for conjugates **2a–e**, **3, a–e**.

Compound	SI Jurkat	SI PBMC
<b>2a</b>	0.6	3930
<b>2b</b>	7.1	>8355
<b>2c</b>	1.3	1632
<b>2d</b>	12	7980
<b>2e</b>	3.2	7805
<b>3a</b>	17	3518
<b>3b</b>	86	1660
<b>3c</b>	17	867
<b>3d</b>	24	262
<b>3e</b>	32	400
MPA	1.4	>780

acridines **9a–e**, and MPA **1** towards Jurkat cell line. In case of experiments with PBMC, both type of conjugates **2a–e** and **3a–e** revealed comparable suppressive activity, apart from **2b** which gave EC<sub>50</sub> better than **3b**.

Subsequently, toxicity measured as IC<sub>50</sub> of compounds **2a–e**, **3a–e** were investigated. These results are presented in Table 2 and

show, that acridine conjugates **3a–e** were more cytotoxic with Jurkat cell line than acridone conjugates **2a–e** and starting acridines **9a–e**, but less toxic if compared to MPA **1**. Conjugates **2a–e**, **3a–e** gave slightly higher cytotoxicity than MPA **1** towards PBMC, whereas acridine derivatives **3b–e** were mostly more toxic than acridone analogs **2b–e**, apart from compounds **2a** and **3a**.

## Discussion

The question arose on the influence of covalent bond between MPA and acridone/acridine moieties on observed antiproliferative activity.

Previously reported amide conjugates of MPA with nitroacridine/nitroacridone derivatives gave activities lower or comparable than their structural parts tested separately, but still better than parent MPA<sup>41</sup>. In other words, the observed effect was rather additive, not synergistic.

In contrast to that, EC<sub>50</sub> values of the most ester conjugates (**2b, d, e, 3a–e**) was higher than EC<sub>50</sub> of mixture of MPA **1** and relevant acridone **8b, d, e** or acridine **9a–e** (Table 3). These results strongly suggest, that covalent bond between both structural units is important for biological activity of conjugates **2a–e, 3a–e**. Noteworthy, mixtures of MPA with **8a–e, 9d** gave intermediate



EC<sub>50</sub> values, whereas mixtures of MPA with **9a–c, e** exhibited lower activity than both separate components (Table 1), which work with another mechanism of action and observed activities were not always additive.

In order to estimate efficacy of conjugates **2a–e, 3, a–e** as potential drugs, we calculated their selective index (SI). These values are provided in Table 4. MPA **1** gave SI value of 1.4 against Jurkat cell line and >780 against PBMC. The acridone derivatives **2b** ( $n=3$ ) and **2d** ( $n=5$ ) revealed the most interesting results from among conjugates **2a–e**. Compound **2b** exhibited SI value of 7.1 against Jurkat cell line and >8355 against PBMC. Similarly, analog **2d** displayed SI value of 12 against Jurkat cell line and 7980 against PBMC.

In the series of acridine conjugates **3a–e** a short linker occurred to be the most promising. Derivative **3a** ( $n=1$ ) provided SI value of 17 against Jurkat cell line and 3518 against PBMC. The highest value of SI against Jurkat cell line (86, Table 4) was observed in the case of compound **3b** ( $n=2$ ).

In the preliminary experiment we investigated mechanism of action of compounds **2a–e, 3a–e**. It was reported in literature, that the addition of guanosine monophosphate (GMP), which is the main product of IMPDH activity, reverses suppression of cell proliferation in the presence of this enzyme inhibitor<sup>1,12</sup>. In the similar test done in the presence of conjugates **2a–e, 3a–e** we observed, that the addition of 50 μM of GMP increased cells proliferation (results provided in Supplementary Figures 1 and 2). These results suggest that all compounds **2a–e, 3a–e** worked as IMPDH inhibitors.

## Conclusion

In summary, synthesis of novel MPA and acridone/acridine conjugates *via* Yamaguchi esterification was elaborated. For this purpose, synthesis of MPA bearing free carboxylic group and blocked phenol was optimized. The coupling of MPA with *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e** required conversion of phenol group in MPA to bulky *tert*-butyldiphenylsilyl ether. Obtained conjugates **2a–e, 3a–e** are IMPDH inhibitors and indicated interesting antiproliferative activity *in vitro* in comparison to parent MPA **1**. In most cases, acridine derivatives **3a–e** were more active than acridone analogs **2a–e**. Length of linker between MPA and heterocyclic units also influenced the observed activity. The most promising compounds **2b, 2d, 3a, 3b** are considered to farther examinations as potential immunosuppressive agents.

## Acknowledgements

Authors would like to thank Dr W.J. Watkins, Gilead Sciences Inc., Foster City, CA for MPA sample.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

This work has been supported by The National Centre for Research and Development (Poland, grant no. LIDER/07/58/L-2/10/NCBiR/2011) and Medical University of Gdansk (grant no. ST49).

## References

- Bentley R. Mycophenolic acid: a one hundred year odyssey from antibiotic to immunosuppressant. *Chem Rev* 2000;100:3801–25.
- Cholewinski G, Malachowska-Ugarte M, Dzierzbicka K. The chemistry of mycophenolic acid – synthesis and modifications towards desired biological activity. *Curr Med Chem* 2010;17:1926–41.
- Papageorgiou, C. Enterohepatic recirculation: a powerful incentive for drug discovery in the inosine monophosphate dehydrogenase field. *Mini-Rev Med Chem* 2001;1:71–7.
- Hedstrom L. IMP dehydrogenase: structure, mechanism and inhibition. *Chem Rev* 2009;109:2903–28.
- Sintchak MD, Nimmesgern E. The structure of inosine 5'-monophosphate dehydrogenase and the design of novel inhibitors. *Immunopharmacol* 2000;47:163–84.
- Kaufmann DB, Shapiro R, Lucey MR, et al. Immunosuppression: practice and trends. *Am J Transplant* 2004;4:38–53.
- Yang N, Wang J, Wang Z-W, et al. Computational insights into the inhibition of inosine 5'-monophosphate dehydrogenase by mycophenolic acid analogs: three dimensional quantitative structure-activity relationship and molecular docking studies. *Chem Biol Drug Des* 2012;79:1063–71.
- Kamar N, Oufroukhi L, Faure P, et al. Questionnaire-based evaluation of gastrointestinal disorders in de novo renal-transplant patients receiving either mycophenolate mofetil or enteric-coated mycophenolate sodium. *Nephrol Dial Transplant* 2005;20:2231–6.
- Aptaramanov B, Seyahi N, Alagoz S, et al. A comparison of mycophenolate mofetil with mycophenolate sodium in renal transplant recipients on tacrolimus-based treatment. *Transplant Proc* 2011;43:833–6.
- Silva Jr HT, Yang HC, Abouljoud M, et al. One-year results with extended-release tacrolimus/MMF, tacrolimus/MMF and cyclosporine/MMF in *de novo* kidney transplant recipients. *Am J Transplant* 2007;7:595–608.
- Felczak K, Chen L, Wilson D, et al. Cofactor-type inhibitors of inosine monophosphate dehydrogenase via modular approach: targeting the pyrophosphate binding sub-domain. *Bioorg Med Chem* 2011;19:1594–605.
- Mitsuhashi S, Takenaka J, Iwamori K, et al. Structure-activity relationships for inhibition of inosine monophosphate dehydrogenase and differentiation induction of K562 cells among the mycophenolic acid derivatives. *Bioorg Med Chem Lett* 2010;18:8106–11.
- Habib E, León F, Bauer JD, et al. Mycophenolic derivatives from *Eupenicillium parvum*. *J Nat Prod* 2008;71:1915–18.
- Nelson PH, Carr SF, Devens BH, et al. Structure-activity relationships for inhibition of inosine monophosphatedehydrogenase by nuclear variants of mycophenolic acid. *J Med Chem* 1996;39:4181–96.
- Chen Z, Zheng Z, Huang H, et al. Penicic acids A–C, three new mycophenolic acid derivatives and immunosuppressive activities from the marine-derived fungus *Penicillium* sp. SOF07. *Bioorg Med Chem Lett* 2012;22:3332–5.
- Iwaszkiewicz-Grzes D, Cholewinski G, Kot-Wasik A, et al. Synthesis and biological activity of mycophenolic acid-amino acid derivatives. *Eur J Med Chem* 2013;69:863–71.
- Malachowska-Ugarte M, Cholewinski G, Chojnacki J, Dzierzbicka K. 4-[(*tert*-Butyldimethylsilyloxy)-6-methoxy-7-methyl-5-(oxiran-2-ylmethyl)-2-benzofuran-3(1H)-one. *Acta Crystallogr E* 2011;67: o3393.
- Watkins WJ, Chen JM, Cho A, et al. Phosphonic acid-containing analogues of mycophenolic acid as inhibitors of IMPDH. *Bioorg Med Chem Lett* 2006;16:3479–83.
- Fardis M, Mertzman M, Thomas W, et al. Use of benzofuran for concomitant protection of aldehyde and phenol groups in the preparation of mycophenolic acid analogues. *J Org Chem* 2006;71:4835–9.
- Chen L, Wilson D, Jayaram HN, Pankiewicz KW. Dual inhibitors of IMP-dehydrogenase and histone deacetylases for cancer treatment. *J Med Chem* 2007;50:6685–91.
- Rohloff JC, Gardner JO, Towne RW. Mycophenolate dianions. *Tetrahedron Lett* 1995;43:7803–6.
- Smith DB, Waltos AM, Loughhead DG, et al. Asymmetric synthesis and stereochemical assignment of RS-97613, a potent immunosuppressive and antiinflammatory agent. *J Org Chem* 1996;61:2236–41.
- Jones DF, Moore RH, Crawley GC. Microbial modifications of mycophenolic acid. *J Chem Soc* 1970;1725–1737.
- Jones DF, Mills SD. Preparation and antitumor properties of analogs and derivatives of mycophenolic acid. *J Med Chem* 1971;14:305–11.
- Suzuki S, Takaku S, Mori T. Antitumor activity of derivatives of mycophenolic acid. *J Antibiotics* 1976;3:275–85.
- Sunohara K, Mitsuhashi S, Shigetomi K, Ubukata M. Discovery of *N*-(2,3,5-triazoyl)mycophenolic amide and mycophenolic epoxyketone as novel inhibitors of human IMPDH. *Bioorg Med Chem Lett* 2013;23:5140–4.

27. Felczak K, Vince R, Pankiewicz KW. NAD-based inhibitors with anticancer potential. *Bioorg Med Chem Lett* 2014;24:332–6.
28. Petrelli R, Vita P, Torquati I, et al. Novel inhibitors of inosine monophosphate dehydrogenase in patent literature of the last decade. *Recent Pat Anticancer Drug Discov* 2013;8:103–25.
29. Cholewinski G, Iwaszkiewicz Grzes D, Prejs M, et al. Synthesis of the inosine 5'-monophosphate dehydrogenase (IMPDH) inhibitors. *J Enzyme Inhib Med Chem* 2015;30:550–63.
30. Cholewinski G, Dzierzbicka K, Kolodziejczyk AM. Natural and synthetic acridines/acridones as antitumor agents: their biological activities and methods of synthesis. *Pharmacol Rep* 2011;63:305–36.
31. Denny WA. Acridine derivatives as chemotherapeutic agents. *Curr Med Chem* 2002;9:1655–65.
32. Dzierzbicka K, Gozdowska, M, Wysocka-Skrzela B, Kołodziejczyk AM. Synthesis and anticancer activities of new MDP conjugates with amino-acridine/acridone derivatives. *Drug Res* 1999;56:20–4.
33. Dzierzbicka K, Kołodziejczyk AM, Wysocka-Skrzela B, Kołodziejczyk AS. Synthesis of muramyl dipeptide conjugated with acridine derivatives, showing anti-HIV-1 and anticancer activity. *Polish J Chem* 1994;68:37–45.
34. Dzierzbicka K, Kołodziejczyk AM, Wysocka-Skrzela B, et al. Synthesis and antitumor activity of conjugates of muramyl dipeptides, normuramyl dipeptide, and desmuramyl peptides with acridine/acridone derivatives. *J Med Chem* 2001;44:3606–15.
35. Dzierzbicka K, Kołodziejczyk AM. Synthesis and antitumor activity of conjugates of muramyl dipeptide or normuramyl dipeptide with hydroxyacridine/acridone derivatives. *J Med Chem* 2003;46:183–9.
36. Kukowska-Kaszuba M, Dzierzbicka K, Serocki M, Skladanowski A. Solid phase synthesis and biological activity of tuftsin conjugates. *J Med Chem* 2011;54:2447–54.
37. Hess FK, Steward PB. Preparation of a new immunosuppressant 4,5-bis(aminomethyl)acridine. *J Med Chem* 1975;18:320–1.
38. Wu H, Pagadala J, Yates ChR, et al. Synthesis and characterization of an anti-apoptotic immunosuppressive compound for improving the outcome of islet transplantation. *Bioconjugate Chem* 2013;24:2036–44.
39. Dzierzbicka K, Kołodziejczyk AM. Adjuvants-essential components of new generation vaccines. *Post Bioch* 2006;52:204–11.
40. Wardowska A, Dzierzbicka K, Mysliwski A. Tuftsin-new analogues and properties. *Post Bioch* 2007;53:60–5.
41. Malachowska-Ugarte M, Cholewinski G, Dzierzbicka K, Trzonkowski P. Synthesis and biological activity of novel mycophenolic acid conjugates containing nitro-acridine/acridone derivatives. *Eur J Med Chem* 2012;54:197–201.
42. Inanaga J, Hirata K, Saeki H, et al. A rapid esterification by means of mixed anhydride and its application to large-ring lactonization. *Bull Chem Soc Jpn* 1979;52:1989–93.
43. Dhimitruka I, SantaLucia Jr J. Investigation of the Yamaguchi esterification mechanism. Synthesis of the Lux-S enzyme inhibitor using an improved esterification method. *Org Lett* 2006;8:47–50.
44. Crouch RD. Selective monodeprotection of bis-silyl ethers. *Tetrahedron* 2004;60:5833–71.

**Supplementary Material available online**  
Supplementary Figures 1 and 2

