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RESEARCH ARTICLE

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

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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*-(ω-hydroxyalkyl)-9-acridone-4-carboxamides or *N*-(ω-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

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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 immunosuppresants[1–10] 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[11–27].

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

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Methods

Chemistry

^{1}H-NMR and ^{13}C-NMR spectra were measured in $CDCl_3$, 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 (Dűren, Germany).

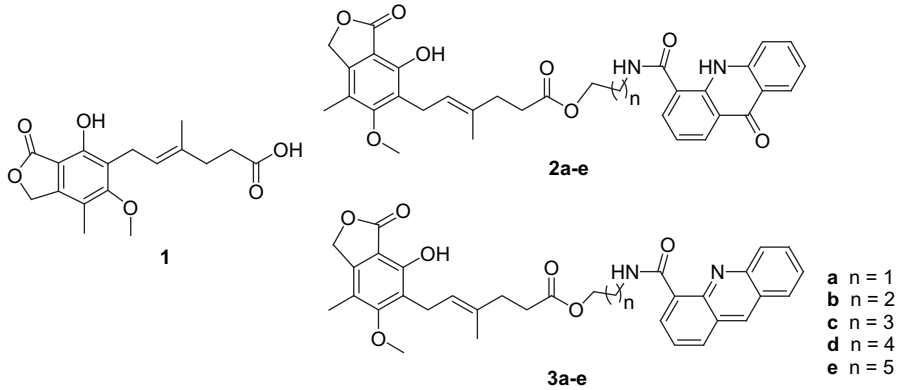
N-(ω-hydroxyalkyl)-9-acridone-4-carboxamides **8a–e**, *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e** were prepared according to the procedure reported in literature[35].

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 $MgSO_4$. 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

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 $MgSO_4$. 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) to give product **6** in yield 77% (2.075 g, 4.8 mmol).

MP 132–133 °C, white solid

^{1}H-NMR ($CDCl_3$): δ[parts per million (ppm)] = 0.24 (s, CH_3, 6H), 1.03 (s, CH_3, 9H), 1.76 (s, CH_3, 3H), 2.15 (s, CH_3, 3H), 2.28–2.31 (m, CH_2, 2H), 2.40–2.43 (m, CH_2, 2H), 3.39 (d, J = 6.3 Hz, CH_2, 2H), 3.74 (s, OCH_3, 3H), 5.07 (s, CH_2, 2H), 5.21 (t, J = 6.3 Hz, CH_{vinyl}, 1H).

^{13}C-NMR ($CDCl_3$): δ(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 $MgSO_4$. 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*-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}H-NMR ($CDCl_3$): δ(ppm) = 0.22 (s, CH_3, 6H), 1.01 (s, CH_3, 9H), 1.75 (s, CH_3, 3H), 2.10 (s, CH_3, 3H), 2.30–2.33 (m, CH_2, 2H), 2.44–2.47 (m, CH_2, 2H), 3.36 (d, J = 6.3 Hz, CH_2, 2H), 3.68–3.74 (m, $NHCH_2$, OCH_3, 5H), 4.31 (t, J = 5.4 Hz, OCH_2, 2H), 5.03 (s, OCH_2, 2H), 5.18–5.20 (m, CH_{vinyl}, 1H), 7.17–7.22 (m, NH_{amide}, CH_{acr}, 2H), 7.29 (dd, J = 8.3 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.40 (d, J = 8.3 Hz, CH_{acr}, 1H), 7.66–7.70 (m, CH_{acr}, 1H), 7.90 (d, J = 7.8 Hz, CH_{acr}, 1H), 8.45 (d, J = 8.3 Hz, CH_{acr}, 1H), 8.63 (d, J = 8.3 Hz, CH_{acr}, 1H), 12.3 (s, NH_{acr}, 1H).

Subsequently, compound **10a** 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 $MgSO_4$. 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 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}H-NMR ($CDCl_3$): δ(ppm) = 1.77 (s, CH_3, 3H), 2.07 (s, CH_3, 3H), 2.32 (t, J = 7.3 Hz, CH_2, 2H), 2.46–2.49 (m, CH_2, 2H), 3.32 (d, J = 6.8 Hz, CH_2, 2H), 3.69 (s, OCH_3, 3H), 3.72–3.74 (m, $NHCH_2$, 2H), 4.33–4.35 (m, OCH_2, 2H), 5.13 (s, OCH_2, 2H), 5.20–5.23 (m, CH_{vinyl}, 1H), 7.15 (dd, J = 7.3 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.26–7.31 (m, NH_{amide}, CH_{acr}, 2H), 7.41 (d, J = 8.3 Hz, CH_{acr}, 1H), 7.66–7.69 (m, CH_{acr}, 1H), 7.89 (d, J = 7.3 Hz, CH_{acr}, 1H), 8.41 (d, J = 7.8 Hz, CH_{acr}, 1H), 8.57 (d, J = 7.8 Hz, CH_{acr}, 1H), 12.26 (s, NH_{acr}, 1H).

^{13}C-NMR ($CDCl_3$): δ(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 ν(cm^{-1}): 3323, 2926, 1738, 1649, 1626, 1525, 1072, 1028

λ_{max} (CH_2Cl_2)/nm 256, 303, 409 (ε/$dm^3\,mol^{-1}\,cm^1$ 29 580, 10 597, 7744)

MS (*m/z*): for $C_{33}H_{32}N_2O_8$ calculated: 584.6 found 585.2

HRMS ESI (*m/z*): [M+Na] for $C_{33}H_{32}N_2O_8Na$ 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}H-NMR ($CDCl_3$): δ(ppm) = 1.81 (s, CH_3, 3H), 1.96–1.98 (m, CH_2, 2H), 2.08 (s, CH_3, 3H), 2.34 (t, J = 7.3 Hz, CH_2, 2H), 2.47 (t, J = 7.3 Hz, CH_2, 2H), 3.37 (d, J = 6.4 Hz, CH_2, 2H), 3.48–3.50 (m, $NHCH_2$, 2H), 3.73 (s, OCH_3, 3H), 4.24 (t, J = 5.9 Hz, OCH_2, 2H), 5.15 (s, OCH_2, 2H), 5.26 (t, J = 6.4 Hz, CH_{vinyl}, 1H), 7.16 (dd, J = 7.3 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.26–7.31 (m, NH_{amide}, CH_{acr}, 2H), 7.42 (d, J = 8.3 Hz, CH_{acr}, 1H), 7.66–7.69 (m, CH_{acr}, 1H), 7.94 (d, J = 7.3 Hz, CH_{acr}, 1H), 8.42 (d, J = 7.8 Hz, CH_{acr}, 1H), 8.58 (d, J = 7.8 Hz, CH_{acr}, 1H), 12.37 (s, NH_{acr}, 1H).

^{13}C-NMR ($CDCl_3$): δ(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 ν(cm^{-1}): 3304, 2924, 1735, 1649, 1619, 1524, 1073, 1030

λ_{max} (CH_2Cl_2)/nm 256, 304, 409 (ε/$dm^3\,mol^{-1}\,cm^1$ 47 748, 17 266, 12 592)

MS (*m/z*): for $C_{34}H_{34}N_2O_8$ 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

^{1}H-NMR ($CDCl_3$): δ(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, NHC$\underline{H}_2$, 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 ($CDCl_3$): δ(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 ν(cm^{-1}): 3323, 2925, 1733, 1649, 1619, 1524, 1074, 1029

λ_{max} (CH_2Cl_2)/nm 256, 304, 408 (ε/$dm^3\,mol^{-1}\,cm^1$ 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, 0.96 mmol, 48% according to **6***

MP 175–177 °C, yellow solid

H-NMR ($CDCl_3$): δ(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, NHC$\underline{H}_2$, 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 ($CDCl_3$): δ(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 ν(cm^{-1}): 3330, 2925, 1733, 1650, 1523, 1070, 1027

λ_{max} (CH_2Cl_2)/nm 256, 303, 409 (ε/$dm^3\,mol^{-1}\,cm^1$ 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

^{1}H-NMR ($CDCl_3$): δ(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, NHC$\underline{H}_2$, 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 ($CDCl_3$): δ(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 ν(cm^{-1}): 3336, 2936, 1734, 1645, 1619, 1596, 1524, 1077, 1030

λ_{max} (CH_2Cl_2)/nm 256, 303, 408 (ε/$dm^3\,mol^{-1}\,cm^{-1}$ 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

^{1}H-NMR ($CDCl_3$): δ(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 ($CDCl_3$): δ(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

^{1}H-NMR ($CDCl_3$): δ(ppm) = 1.06 (s, CH_3, 9H), 1.42 (s, CH_3, 3H), 2.08 (s, CH_3, 3H), 2.21–2.24 (m, CH_2, 2H), 2.40–2.43 (m, CH_2, 2H), 3.09 (d, J = 5.9 Hz, CH_2, 2H), 3.57 (s, OCH_3, 3H), 3.916–3.925 (m, $NHC\underline{H}_2$, 2H), 4.41 (t, J = 5.4 Hz, OCH_2, 2H), 4.91–4.94 (m, OCH_2, CH_{vinyl}, 3H), 7.25–7.28 (m, CH_{arom}, 4H), 7.33 (t, J = 7.3 Hz, CH_{arom}, 2H), 7.58 (dd, J = 7.3 Hz, J = 7.3 Hz, CH_{acr}, 1H), 7.65 (d, J = 6.8 Hz, CH_{arom}, 4H), 7.70–7.73 (m, CH_{acr}, 1H), 7.76 (dd, J = 7.3 Hz, J = 7.8 Hz, CH_{acr}, 1H), 8.05 (d, J = 8.3 Hz, CH_{acr}, 1H), 8.14 (d, J = 8.8 Hz, CH_{acr}, 1H), 8.17 (d, J = 8.3 Hz, CH_{acr}, 1H), 8.93 (m, CH_{acr}, 1H), 9.04 (s, NH_{amide}, 1H), 12.06 (s, CH_{acr}, 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 $MgSO_4$. 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 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

^{1}H-NMR ($CDCl_3$): δ(ppm) = 1.74 (s, CH_3, 3H), 2.08 (s, CH_3, 3H), 2.33–2.36 (m, CH_2, 2H), 2.50–2.54 (m, CH_2, 2H), 3.33 (d, J = 6.8 Hz, CH_2, 2H), 3.70 (s, OCH_3, 3H), 3.93–3.94 (m, $NHC\underline{H}_2$, 2H), 4.41 (t, J = 5.4 Hz, OCH_2, 2H), 5.13 (s, OCH_2, 2H), 5.20–5.22 (m, CH_{vinyl}, 1H), 7.58–7.61 (m, OH, CH_{acr}, 2H), 7.66 (dd, J = 7.3 Hz, J = 7.3 Hz, CH_{acr}, 1H), 7.80 (dd, J = 7.3 Hz, J = 7.3 Hz, CH_{acr}, 1H), 8.04 (d, J = 8.3 Hz, CH_{acr}, 1H), 8.14–8.17 (m, CH_{acr}, 2H), 8.90 (m, CH_{acr}, 1H), 8.98 (s, NH_{amide}, 1H), 12.07 (s, CH_{acr}, 1H).

^{13}C-NMR ($CDCl_3$): 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.

λ_{max} (CH_2Cl_2)/nm 250, 305, 342, 359, 377 (ε/dm^3 mol^{-1} cm^1 92 249, 5376, 6653, 11 172, 5669).

IR KBr ν(cm^{-1}): 3405, 2922, 1736, 1655, 1625, 1565, 1552, 1524, 1164, 1138, 738.

MS (*m/z*): $[M]^+$ for $C_{33}H_{32}N_2O_7$ calculated: 568.6, found: 569.0

HRMS ESI (*m/z*): $[M+Na]^+$ for $C_{33}H_{32}N_2O_7Na$ 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

^{1}H-NMR ($CDCl_3$): δ(ppm) = 1.66 (s, CH_3, 3H), 1.99 (s, CH_3, 3H), 1.93–2.18 (m, CH_2, 4H), 2.29–2.36 (m, CH_2, 2H), 3.24 (d, J = 6.4 Hz, CH_2, 2H), 3.61 (s, OCH_3, 3H), 3.70–3.94 (m, $NHC\underline{H}_2$, 2H), 4.17–4.23 (m, OCH_2, 2H), 5.02–5.05 (m, CH_{vinyl}, 1H), 5.19 (s, OCH_2, 2H), 7.66–7.79 (m, OH, CH_{acr}, 3H), 7.95 (dd, J = 6.8 Hz, J = 7.9 Hz, CH_{acr}, 1H), 8.20–8.39 (m, CH_{acr}, 3H), 8.72 (d, J = 6.8 Hz, CH_{acr}, 1H), 9.32–9.36 (m, NH_{amide}, 1H), 11.35 (s, CH_{acr}, 1H).

^{13}C NMR ($CDCl_3$): 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 ν(cm^{-1}): 3421, 2928, 1731, 1654, 1623, 1562, 1522, 1162, 1132, 741.

λ_{max} (CH_2Cl_2)/nm 251, 305, 342, 359, 377 (ε/dm^3 mol^{-1} cm^1 85 875, 4692, 5749, 9995, 5096)

MS (*m/z*): $[M]^+$ for $C_{34}H_{34}N_2O_7$: calculated: 582.6, found: 583.1

HRMS ESI (*m/z*): $[M]^+$ for $C_{34}H_{35}N_2O_7$ 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

^{1}H-NMR ($CDCl_3$): δ(ppm) = 1.67 (s, CH_3, 3H), 1.72–1.79 (m, CH_2, 4H), 1.99 (s, CH_3, 3H), 2.16 (t, J = 7.3 Hz, CH_2, 2H), 2.34 (t, J = 7.3 Hz, CH_2, 2H), 3.22 (d, J = 6.8 Hz, CH_2, 2H), 3.52–3.55 (m, $NHC\underline{H}_2$, 2H), 3.60 (s, OCH_3, 3H), 4.04–4.06 (m, OCH_2, 2H), 5.07–5.10 (m, CH_{vinyl}, 1H), 5.18 (s, OCH_2, 2H), 7.66 (dd, J = 7.3 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.72 (dd, J = 7.3 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.89–7.93 (m, CH_{acr}, 1H), 8.19–8.21 (m, OH, CH_{acr}, 2H), 8.34 (d, J = 7.3 Hz, CH_{acr}, 1H), 8.69–8.70 (m, CH_{acr}, 1H), 9.27 (s, CH_{acr}, 1H), 9.29 (s, NH_{amide}, 1H), 11.36–11.38 (m, CH_{acr}, 1H).

^{13}C NMR ($CDCl_3$): 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 ν(cm^{-1}): 3418, 2923, 1732, 1652, 1624, 1562, 1522, 1161, 1132, 740.

λ_{max} (CH_2Cl_2)/nm 251, 305, 342, 359, 377 (ε/dm^3 mol^{-1} cm^1 58 658, 3378, 3897, 6723, 3371)

MS (*m/z*): $[M]^+$ for $C_{35}H_{36}N_2O_7$: calculated: 596.7 found: 597.0

HRMS ESI (*m/z*): $[M+Na]^+$ for $C_{35}H_{36}N_2O_7Na$ 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

^{1}H-NMR ($CDCl_3$): δ(ppm) = 1.50–1.72 (m, CH_2, 6H), 1.60 (s, CH_3, 3H), 2.01 (s, CH_3, 3H), 2.04–2.07 (m, CH_2, 2H), 2.19–2.22 (m, CH_2, 2H), 3.21 (d, J = 6.8 Hz, CH_2, 2H), 3.51–3.56 (m, $NHC\underline{H}_2$, 2H), 3.60 (s, OCH_3, 3H), 3.96 (t, J = 6.3 Hz, OCH_2, 2H), 5.00–5.02 (m, CH_{vinyl}, 1H), 5.20 (s, OCH_2, 2H), 7.67 (dd, J = 6.8 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.73 (dd, J = 6.8 Hz, J = 7.8 Hz, CH_{acr}, 1H), 7.91–7.94 (m, CH_{acr}, 1H), 8.19–8.20 (m, OH, CH_{acr}, 2H), 8.34 (d, J = 8.3 Hz, CH_{acr}, 1H), 8.71 (d, J = 6.8 Hz, CH_{acr}, 1H), 9.25–9.30 (m, CH_{acr}, 1H), 9.33 (s, NH_{amide}, 1H), 11.43 (s, CH_{acr}, 1H).

^{13}C NMR ($CDCl_3$): 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 ν(cm^{-1}): 3421, 2928, 1732, 1653, 1624, 1563, 1523, 1162, 1133, 741.

λ_{max} (CH_2Cl_2)/nm 251, 305, 342, 359, 377 (ε / dm^3 mol^{-1} cm^1 68 208, 4887, 5394, 9045, 4646)

MS (*m/z*): $[M]^+$ for $C_{36}H_{38}N_2O_7$ calculated: 610.7, found: 611.1

HRMS ESI (*m/z*): $[M]^+$ for $C_{36}H_{39}N_2O_7$ 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

^{1}H-NMR ($CDCl_3$): δ(ppm) = 1.37–1.62 (m, CH_2, 8H), 1.68 (s, CH_3, 3H), 2.00 (s, CH_3, 3H), 2.13 (t, J = 7.3 Hz, CH_2, 2H), 2.29

(t, $J = 7.3$ Hz, CH_2, 2H), 3.22 (d, $J = 6.8$ Hz, CH_2, 2H), 3.52–3.54 (m, $NHC\underline{H}_2$, 2H), 3.61 (s, OCH_3, 3H), 3.92 (t, $J = 6.3$ Hz, OCH_2, 2H), 5.07–5.09 (m, CH_{vinyl}, 1H), 5.19 (s, OCH_2, 2H), 7.67–7.75 (m, CH_{acr}, 2H), 7.92–7.95 (m, CH_{acr}, 1H), 8.18–8.22 (m, OH, CH_{acr}, 2H), 8.35 (d, $J = 7.8$ Hz, CH_{acr}, 1H), 8.71 (d, $J = 7.3$ Hz, CH_{acr}, 1H), 9.302–9.307 (m, CH_{acr}, NH_{amide}, 2H), 11.42 (s, CH_{acr}, 1H).

^{13}C NMR ($CDCl_3$): δ(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 $\nu(cm^{-1})$: 3422, 2925, 1732, 1651, 1624, 1564, 1523, 1162, 1134, 741.

λ_{max} (CH_2Cl_2)/nm 251, 305, 342, 359, 377 (ε/$dm^3\,mol^{-1}\,cm^1$ 10 3670, 6576, 7236, 12 002, 6230)

MS (*m/z*): $[M]^+$ for $C_{37}H_{40}N_2O_7$ calculated: 624.7, found: 625.0

HRMS ESI (*m/z*): $[M]^+$ for $C_{37}H_{41}N_2O_7$ 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 5×10^4 cells per well in RPMI medium containing 10% fetal calf serum in an atmosphere of 5% 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 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 µl of isopropanol. Optical density was read at 570 nm on the automated plate reader (Victor 4, PerkinElmer, Waltham, MA).

Proliferation tests 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 (Dynal, Dynabeads®, Life Technologies, Carlsbad, CA) in 1:1 ratio cultured for 4 days. Proliferation was measured after ^3H-thymidine (0.5 µCi/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 IC_{50} or EC_{50} values for each compound.

Results

Chemistry

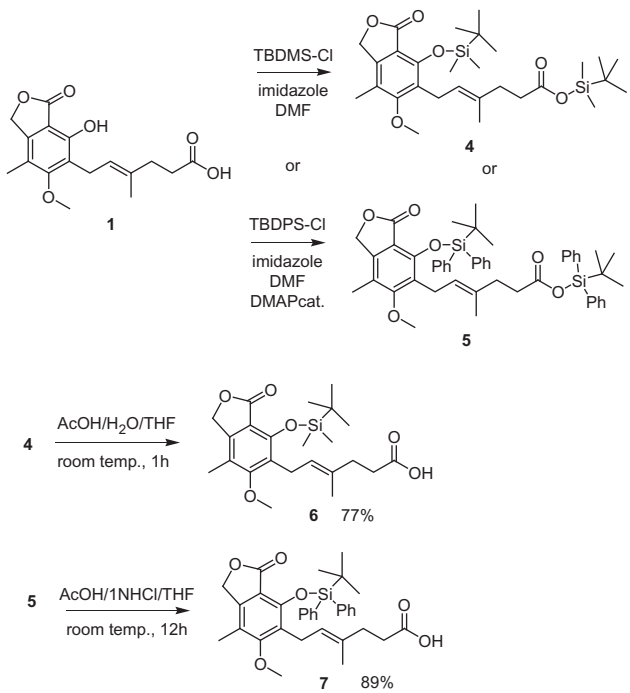
Direct condensation of MPA **1** with *N*-(ω-hydroxyalkyl)-9-acridone-4-carboxamides **8a–e** (35) or *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e** (35) occurred to be laborious, probably due to participation of free phenolic group in undesired esterifications or macrolactonization. As a result, 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[42,43] (Scheme 2). The reaction of blocked MPA **6** with *N*-(ω-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**).

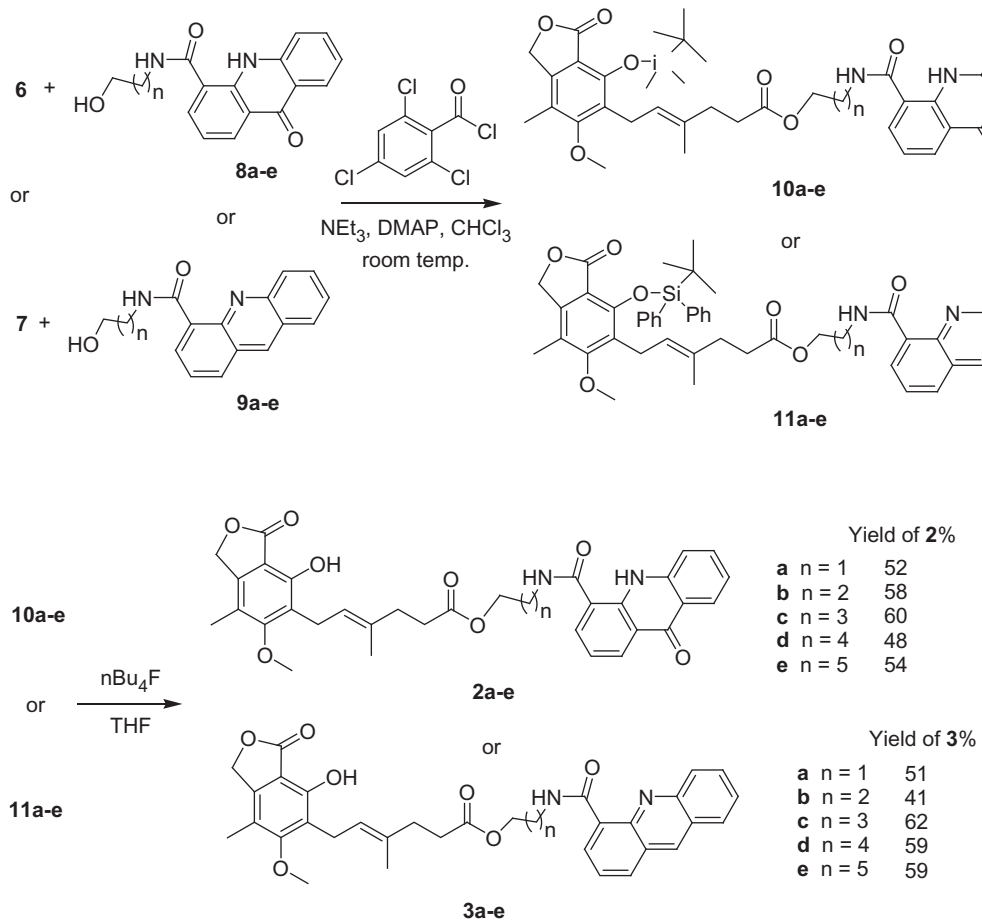
Similarly to this synthetic pathway, MPA derivative **6** was treated with *N*-(ω-hydroxyalkyl)acridine-4-carboxamides **9a–e** towards conjugates **3a–e**. However, we observed unexpected recovering of phenolic group during this esterification. The unprompted removal of *tert*-butyldimethylsilyl ether 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[44]. 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[44].

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*-butyldipfenylsilyl 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_{50} [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 antiproliferation test, *p* statistical significance.

Compound	EC_{50} Jurkat	*p*	EC_{50} PBMC	*p*
2a	0.86 ± 0.684	<0.05	0.001 ± 0.0004	<0.05
2b	0.33 ± 0.167	<0.05	$2 \times 10^{-6} \pm 1 \times 10^{-6}$	<0.05
2c	0.98 ± 0.653	<0.05	0.01 ± 0.003	<0.05
2d	0.32 ± 0.159	<0.05	0.002 ± 0.001	<0.05
2e	0.48 ± 0.230	<0.05	0.002 ± 0.001	<0.05
8a	21.25 ± 4.605		6.38 ± 2.125	
8b	>33.75		5.74 ± 2.025	
8c	>32.22		2.90 ± 1.611	
8d	>30.29		1.23 ± 0.250	
8e	>29.55		1.48 ± 0.295	
3a	0.02 ± 0.006	<0.05	0.005 ± 0.001	<0.05
3b	0.01 ± 0.007	<0.05	0.003 ± 0.002	<0.05
3c	0.02 ± 0.009	<0.05	0.006 ± 0.004	<0.05
3d	0.02 ± 0.010	<0.05	0.01 ± 0.003	<0.05
3e	0.02 ± 0.008	<0.05	0.004 ± 0.003	<0.05
9a	0.098 ± 0.0049		1.13 ± 0.105	
9b	0.10 ± 0.005		0.71 ± 0.150	
9c	0.09 ± 0.004		0.34 ± 0.088	
9d	2.92 ± 0.324		0.32 ± 0.076	
9e	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_{50} 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_{50} [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_{50} Jurkat	*p*	IC_{50} PBMC	*p*
2a	0.51 ± 0.171	<0.05	3.93 ± 1.882	<0.05
2b	2.34 ± 2.840	<0.05	>16.71	0.057
2c	1.31 ± 0.816	<0.05	>16.32	0.086
2d	3.99 ± 3.191	<0.05	>15.96	0.074
2e	1.56 ± 0.312	<0.05	>15.61	0.052
8a	15.59 ± 2.480		>35.42	
8b	9.79 ± 1.012		>33.75	
8c	10.31 ± 1.289		>32.22	
8d	2.16 ± 0.308		>30.83	
8e	7.68 ± 1.182		>29.55	
3a	0.35 ± 0.139	<0.05	>17.59	0.064
3b	0.86 ± 0.168	<0.05	4.98 ± 0.858	<0.05
3c	0.34 ± 0.121	<0.05	5.20 ± 3.687	<0.05
3d	0.49 ± 0.164	<0.05	2.62 ± 1.474	<0.05
3e	0.64 ± 0.160	<0.05	1.60 ± 0.960	<0.05
9a	8.26 ± 3.755		4.13 ± 1.878	
9b	9.99 ± 4.638		3.09 ± 2.141	
9c	9.17 ± 4.755		6.45 ± 1.698	
9d	11.67 ± 1.621		>32.42	
9e	10.55 ± 4.032		18.3 ± 4.03	
MPA	0.01 ± 0.059	–	>31.22	–

Table 3. Comparison of EC_{50} [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_{50} Jurkat	*p*	EC_{50} Jurkat MPA + **8a–e** or **9a–e**	*p*
2a	0.86 ± 0.684	<0.05	0.33 ± 1.332 (MPA + **8a**)	<0.05
2b	0.33 ± 0.167	<0.05	1.30 ± 2.273 (MPA + **8b**)	<0.05
2c	0.98 ± 0.653	<0.05	0.63 ± 0.634 (MPA + **8c**)	<0.05
2d	0.32 ± 0.159	<0.05	1.86 ± 0.310 (MPA + **8d**)	<0.05
2e	0.48 ± 0.230	<0.05	1.52 ± 1.216 (MPA + **8e**)	<0.05
3a	0.02 ± 0.006	<0.05	0.69 ± 0.688 (MPA + **9a**)	<0.05
3b	0.01 ± 0.007	<0.05	2.68 ± 0.334 (MPA + **9b**)	<0.05
3c	0.02 ± 0.009	<0.05	0.65 ± 0.117 (MPA + **9c**)	<0.05
3d	0.02 ± 0.010	<0.05	0.64 ± 0.318 (MPA + **9d**)	<0.05
3e	0.02 ± 0.008	<0.05	1.56 ± 0.622 (MPA + **9e**)	<0.05
MPA	0.07 ± 0.028	–	–	–

Table 4. Selectivity index (IC_{50}/EC_{50}) calculated for conjugates **2a–e**, **3**, **a–e**.

Compound	SI Jurkat	SI PBMC
2a	0.6	3930
2b	7.1	>8355
2c	1.3	1632
2d	12	7980
2e	3.2	7805
3a	17	3518
3b	86	1660
3c	17	867
3d	24	262
3e	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_{50} better than **3b**.

Subsequently, toxicity measured as IC_{50} 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[41]. In other words, the observed effect was rather additive, not synergistic.

In contrast to that, EC_{50} values of the most ester conjugates (**2b**, **d**, **e**, **3a–e**) was higher than EC_{50} 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_{50} 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[1,12]. 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.

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Declaration of interest

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

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Supplementary Material available online

Supplementary Figures 1 and 2