Synthesis of a Series of Promising Isobenzofuranones for the Treatment of Acute Mucositis Caused by Chemo- and Radiotherapy

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Abstract: A small series of 3,5-dihydroxy-7-methoxy-3,6-dimethyl-isobenzofuran-1(3*H*)-one analogues of the immunosuppressant, mycophenolate mofelite (MMF), has been identified during a screening campaign as possible mediators to prevent mucositis. Herein, we present a general seven-step approach for the preparation of small molecule mycophenolate mofelite analogues, which are readily available for further biological evaluation in our mucositis model.

Key words: isobenzofuranones, mycophenolic acid, Alder–Rickert reaction, acute mucositis

Chemotherapy agents continue to be the mainstay of cancer treatment, but unwanted deleterious effects on highly proliferative tissues remain a significant drawback. Furthermore, as radiation also affects rapidly dividing cells, patients receiving combined therapies are at even greater risk for treatment-related side-effects. Among these belongs the manifestation of mucositis, a general term that describes the damage of mucosal epithelial cells as a result of the cytotoxic effects of chemo- or radiation therapy.

The occurrence of oral mucositis constitutes a significant burden for patients undergoing chemo- or radiotherapy for solid tumors. Almost all patients treated with radiation therapy for head and neck cancer will develop some degree of oral mucositis, while patients treated with both radiation and chemotherapy have been found to present with more severe mucositis and increased morbidity.[1] Despite the prevalence of side-effects related to these treatments, clinically approved approaches to minimize epithelial damage caused by these invasive therapies are very restricted. Current treatments for mucositis are limited to palliative care, such as good oral care, or moderate therapeutic remediation.[2]

It has become clear that a mucositis-specific pain protocol addressing all elements of pain reduction including tissue damage, sensitization of pain receptors, and elaboration of inflammatory and pain mediators, has yet to be developed.[3] The identification of drugs further minimizing, or ideally preventing the development of mucositis would mark a major leap forward in anticancer therapy. To this end, we established a series of cellular assays[4] that are amenable for high-throughput screening in order to discover new classes of chemical modulators that could be used in the context of radio- and chemotherapy to prevent the development of severe oral mucositis, and thus reducing peritransplant morbidity.

Laure Bouchez was born in Orleans (France) in 1976. She graduated from the École Nationale Supérieure de Chimie Mulhouse in 2000. In November 2004, she received her PhD in Organic Chemistry from the EPFL (Lausanne, Switzerland) working with Prof. Pierre Vogel on sulfur dioxide mediated syntheses of polyene natural products. In February 2005, she joined the group of Prof. Alois Fürstner at the Max-Planck-Institüt für Kohlenforschung (Mülheim-an-der-Ruhr, Germany) where she was part of the team who accomplished the total synthesis of amphidinolides H, G and B. In April 2008, she worked in the group of Prof. Peter G. Schultz at the Scripps Research Institute (La Jolla, San Diego, USA) on the identification of chemical and biological modulators of human stem cell fates. Since November 2010, she is a Research Investigator at the Novartis Institute for BioMedical Research in Basel.

During our screening campaign, using the HEK293T cell line stably expressing a β-catenin/TCF luciferase reporter construct (STF),[4] a series of small molecule isobenzofuranones possessing a pentasubstituted aromatic ring were identified. All the compounds from this series yielded significant STF activity values [EC_{50} (compound **2**) = 800 nM and EC_{50} (compound **4**) = 2 μM].[5] Structurally, these isobenzofuranones resemble mycophenolate mofetil (MMF), a pro-drug of mycophenolic acid (MPA), approved by the Food and Drug Administration (FDA) (Figure 1),[6] suggesting a role for this recurring motif in the mechanism of mucositis alleviation.

To assist in future studies investigating the role of isobenzofuranone moieties as post-chemotherapeutic modulators of gastrointestinal side effects, the following

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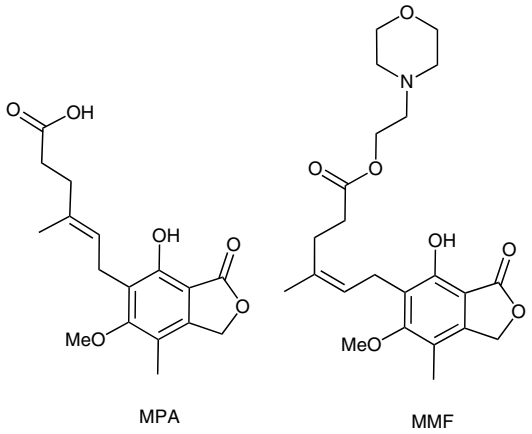


Figure 1 Structures of mycophenolic acid (MPA) and its pro-drug, mycophenolate mofetil (MMF)

communication reports a general strategy to access a small selection of highly substituted isobenzofuranones containing isoprenoid residues (Figure 2, analogues **1**–**4**).[7]

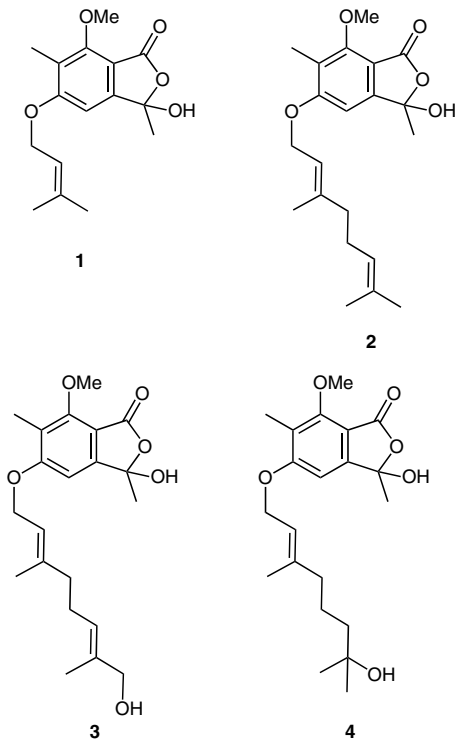
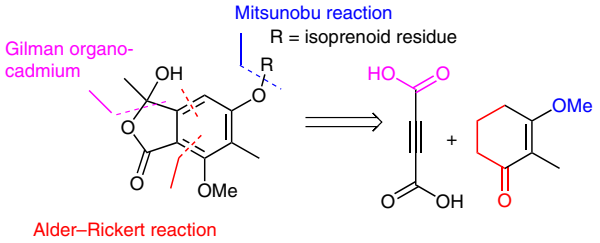


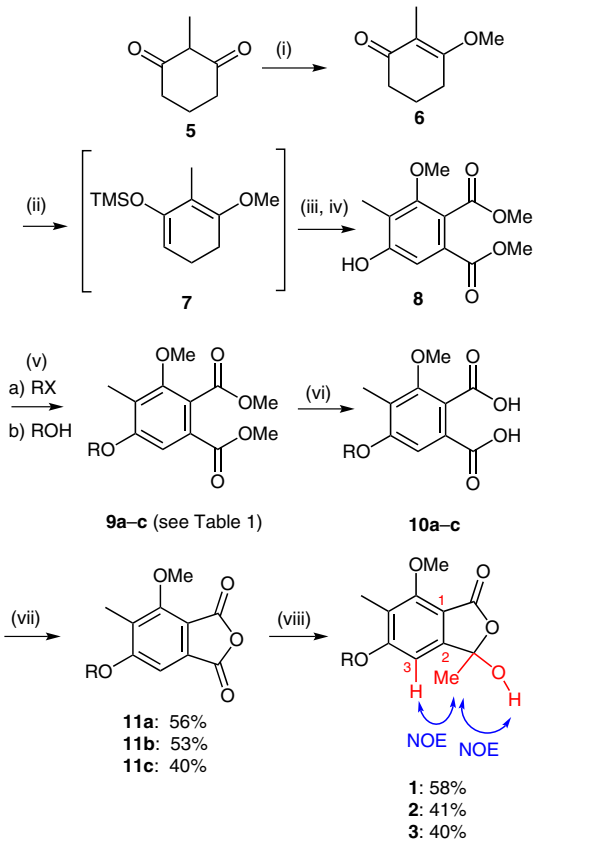
Figure 2 A small selection of 3,5-dihydroxy-7-methoxy-3,6-dimethylisobenzofuran-1(3*H*)-ones containing terpenoid residues

The proposed retrosynthesis of the corresponding mycophenolic acid analogues is depicted in Scheme 1. We envisioned a flexible approach based on the Alder–Rickert sequence, involving a suitably substituted cyclohexadiene (**7**) and dimethyl acetylenedicarboxylate (DMAD). A variety of terpenoid residues will be introduced by standard alkylation or under Mitsunobu reaction conditions, thus allowing for the completion of the synthesis of our small collection of isobenzofuranone derivatives.



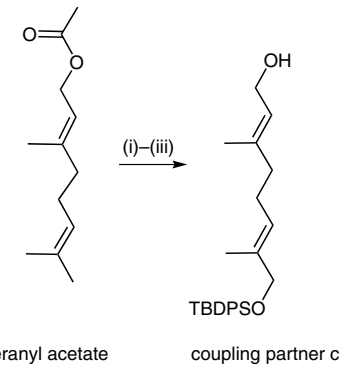
Scheme 1 Retrosynthetic approach for the synthesis of a small collection of isobenzofuranones

Inexpensive 2-methylcyclohexane-1,3-dione (**5**) served as the point of departure, which was advanced to the enol ether **6** by a previously reported procedure.[8] The intermediate enol silyl ether **7** underwent a [4+2] cycloaddition in presence of dimethyl acetylenedicarboxylate, at –10 °C, followed by a thermal elimination at 120 °C to give dimethyl 5-hydroxy-3-methoxy-4-methylphthalate (**8**)



Scheme 2 *Reagents and conditions*: (i) MeOH, PTSA, r.t., 16 h, 69%; (ii) LDA, TMSCl, THF, –78 °C, 2 h; (iii) DMAD, xylene, –10 °C, 2 h; (iv) 120 °C, 2 h, 36% over 3 steps; (v) see Table 1; alkylation under conditions (a) R–X, K_2CO_3, DMF, 85 °C, 3 h; under conditions (b) ROH, DIAD, PPh_3, THF, r.t., 16 h; (vi) KOH (15% in MeOH), reflux, 5 h, quant.; (vii) Ac_2O, 105 °C, 3 h; (viii) (a) MeMgBr (3 M in Et_2O), $CdCl_2$, Et_2O, 40 °C, reflux, 1 h; (b) **11**, Et_2O, 40 °C, reflux, 4 h; (c) (1) HCl (1 M), (2) TBAF (1 M in THF), r.t., 4 h in the case of **3**. LDA = lithium diisopropylamide, DIAD = diisopropyl azodicarboxylate.

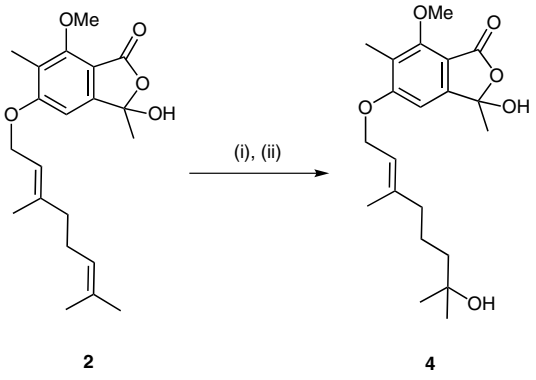
(Scheme 2).[9] Alkylation proceeded smoothly to enable the incorporation of the corresponding side chain[10] (Table 1, entries 1–3). The coupling partners **a** and **b** were commercially available, whereas **c** was prepared in a three-step operation (Scheme 3). Riley's oxidation of geranyl acetate,[11] followed by protection of the resulting alcohol to give the corresponding *tert*-butyldiphenylsilyl ether and saponification of the acetate moiety delivered coupling partner **c**, readily available for the synthesis of intermediate **9c**.



Scheme 3 *Reagents and conditions*: (i) geranyl acetate, salicylic acid, H_2SeO_3, *t*-BuOOH (70% in H_2O), CH_2Cl_2, 16 h, r.t., 73%; (ii) TBDPSCl, imidazole, 16 h, r.t., 94%; (iii) K_2CO_3, 4 h, r.t., 94%.

Subsequent hydrolysis by potassium hydroxide (KOH) (15% in MeOH) was slow but effective, and provided the required trisubstituted phthalic acid building blocks **10a**–**c** in essentially quantitative yields. These three intermediates were then subjected to a solution of refluxing acetic anhydride to yield the corresponding anhydrides **11a**–**c**. Gratifyingly, addition of the Gilman cadmium reagent, formed after addition of one equivalent of anhydrous cadmium chloride to a cold solution of two equivalents of methylmagnesium bromide[12,13] furnished the desired products **1**, **2** and **3** in moderate to good yields (Scheme 2, Table 1).[14]

For the preparation of compound **4**, we adapted a protocol from Piancatelli et al.[15] where a hypervalent iodine(III) reagent, [bis(acetoxy)iodo]benzene (BAIB or $PhI(OAc)_2$], was involved in a multicomponent reaction to facilitate the formation of the desired allylic alcohol. As predicted, the necessary tertiary alcohol motif was attained by oxidative removal of the phenylselenium group with aqueous sodium periodate in 1,4-dioxane (Scheme 4).[16,17]



Scheme 4 *Reagents and conditions*: (i) $(PhSe)_2$, BAIB, KSCN, MeCN, 5 min, r.t., then **2**, MeCN, 3 h, r.t., 34%; (ii) $NaHCO_3$, $NaIO_4$ in H_2O, 1,4-dioxane, 16 h, r.t., 55%.

In summary, we have successfully developed an efficient methodology for the synthesis of a small collection of isobenzofuranone compounds containing terpenoid motifs. These first lead compounds will be tested in our rodent models for radiotherapy-induced intestinal and oral mucositis. This experimental concept will be subject to further scrutiny using other disease models in order to allow potential therapeutic applications in the context of other gastrointestinal diseases. Biological data concerning the protective properties of our lead compounds against mucositis will be reported in due course.

Acknowledgment

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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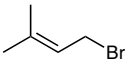
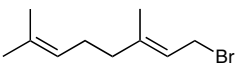
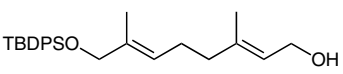
Table 1 Incorporation of Coupling Partners **9a**–**c**

Entry	Substrate	Coupling partner	Conditions	Time	Yield
1	**9a**		(v) a	8 h	51%
2	**9b**		(v) b	13 h	53%
3	**9c**		(v) b	16 h	87%

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(13) Reactions carried out without cadmium led to a mixture of compounds presenting the double addition of the methyl moiety and the expected mono-alkylated product **11**.

(14) The structures of the final compounds were fully characterized by NMR spectroscopy. They presented NOEs between H-3 and the methyl and between the methyl and the hydroxy function as depicted in the isobenzofuran-(3*H*)-one model (see Scheme 2).

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(16) See the Supporting Information for the preparation of compound **3.**

(17) **3-Hydroxy-5-{[(2*E*,5*E*)-7-hydroxy-3,7-dimethylocta-2,5-dien-1-yl]oxy}-7-methoxy-3,6-dimethylisobenzofuran-1(3*H*)-one (4)**

To a stirring suspension of KSCN (84 mg, 0.866 mmol) in MeCN (1 mL), $(PhSe)_2$ (108 mg, 0.346 mmol) followed by BAIB (139 mg, 0.433 mmol) were added. The cloudy yellow mixture was stirred at r.t. for 10 min before the addition of a solution of compound **2** (104 mg, 0.289 mmol) in MeCN (1 mL). The resulting mixture was stirred at r.t. for 16 h. After completion of the reaction, the mixture was washed with sat. $NaHCO_3$–10% $Na_2S_2O_3$ solution (5 mL) and extracted with EtOAc (2 × 50 mL). The organic layer was washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered and the volatiles evaporated under reduced pressure to afford the expected intermediate, (*E*)-3-hydroxy-5-{[7-isothiocyanato-3,7-dimethyl-6-(phenylselanyl)oct-2-en-1-yl]oxy}-7-methoxy-3,6-dimethylisobenzofuran-1(3*H*)-one as a yellow oil. Next, to a stirring mixture of $NaHCO_3$ (10.97 mg, 0.131 mmol) and $NaIO_4$ (55.8 mg, 0.261 mmol) in H_2O (4 mL) was added a solution of the phenylselenyl intermediate (50 mg, 0.087 mmol) in 1,4-dioxane (8 mL). The cloudy mixture was stirred overnight at r.t. Upon completion, the mixture was washed with sat. NH_4Cl solution (2 mL) and extracted with EtOAc (2 × 25 mL). The organic layer was washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified on silica gel (FC ISCO, gradient elution: 0–70% EtOAc in heptane) to afford compound **4** as a colorless oil (18 mg, 0.048 mmol, 55% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.57 (s, 1 H, exchangeable with D_2O), 7.02 (s, 1 H), 5.59 (d, *J* = 14.95 Hz, 1 H), 5.51–5.49 (m, 2 H), 4.73–4.70 (m, 2 H), 4.48 (s, 1 H, exchangeable with D_2O), 3.90 (s, 3 H), 2.72 (d, *J* = 6.35 Hz, 2 H), 2.05 (s, 3 H), 1.71 (s, 3 H), 1.70 (s, 3 H), 1.16 (s, 6 H, 2 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.4, 163.9, 157.7, 151.7, 141.1, 140.0, 124.6, 122.6, 120.4, 109.7, 104.2, 100.6, 71.4, 66.1, 62.5, 42.2, 30.4, 30.0, 26.6, 17.4, 9.3. LC–MS: *m*/*z* = 377 [M + H]$^+$; t_R = 1.03 min (>99% by LC–MS, UV). ROESY correlation data are reported in the Supporting Information.

