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Phosphonic acid-containing analogues of mycophenolic acid as inhibitors of IMPDH

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Abstract—The design, synthesis, and IMPDH inhibitory activity of a series of phosphonic acid-containing analogues of mycophenolic acid are described.

Mycophenolic acid (MPA, **1**) is a potent, uncompetitive inhibitor of inositol monophosphate dehydrogenase (IMPDH; EC 1.1.1.205), which catalyzes the rate-limiting step in the de novo biosynthesis of guanine nucleotides.[1] It binds to a covalent enzyme–substrate intermediate by occupying the recently vacated NAD binding site.[2] The compound exhibits ca. 4-fold greater potency against isoform II (upregulated in proliferating cells) than against isoform I (constitutively expressed). The consequent depletion of the guanosine pool results in inhibition of proliferation of a variety of cell types.[3]

MPA has seen widespread use in the prevention of rejection of solid organ transplants[4] in the form of its 2-morpholinoethyl ester prodrug, mycophenolate mofetil (MMF, **2**),[5] which is very efficiently cleaved by first-pass metabolism. The pharmacokinetic profile of the free acid is dominated by the presence of the phenol on the aromatic core, which is highly susceptible to glucuronidation. Following biliary excretion and enteric microbial hydrolysis of the biologically inactive glucuronide, the parent acid is reabsorbed; ca. 40% of the dose of MMF is subject to this enterohepatic recirculation process. Ultimately, >95% of the dose is excreted renally as the glucuronide.[6] Although an important component of immunosuppressive therapy, the drug has some liabilities as a result of this pharmacokinetic profile.[7] Doses of up to 1.5 g twice daily are required for optimal effect, and GI-related side effects (nausea, abdominal pain, and diarrhea) are common and dose-limiting in many patients (Fig. 1).

Recently, prodrugs of the antiviral nucleotide analogue tenofovir were described that are subject to preferential intracellular cleavage in lymphocytes.[8] The resulting free phosphonic acid (and its phosphorylated metabolites) are efficiently trapped inside cells, leading to prolonged inhibition of reverse transcriptase despite low plasma levels. We wished to explore the applicability of this concept in the search for an agent that might improve upon the therapeutic index of MMF. Herein we describe our initial work to identify potent, phosphonic acid-containing inhibitors of IMPDH.

Chemistry. All the compounds described herein were made by semi-synthesis from MPA itself. Phosphonic acid-containing analogues with alkyl- or ether-containing sidechains could be constructed without the need

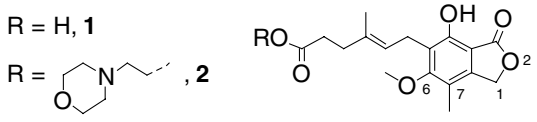

Figure 1. Mycophenolic acid (**1**) and mycophenolate mofetil (**2**).

Keywords: IMPDH; Immunosuppression; Transplant; Mycophenolic acid; Phosphonic acid; Enzyme inhibitor.
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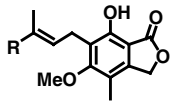
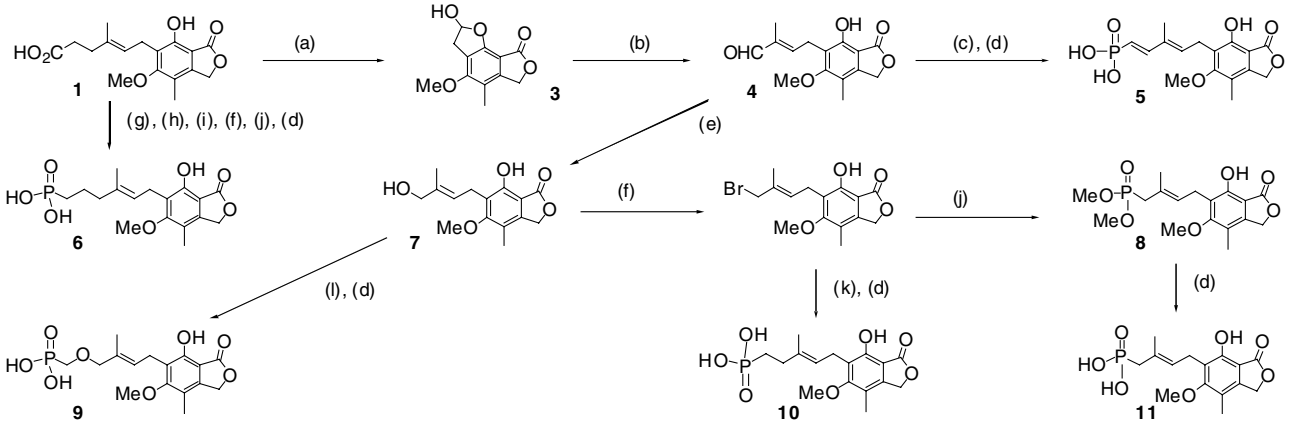
for protection of the phenol (Scheme 1). We found that cleavage of the olefin through ozonolysis to give the hemi-acetal **3**[9] was high-yielding and reproducible if MPA was pre-treated with an excess of TMSCl. Other oxidation methods that are more expedient for larger-scale work will be reported elsewhere.[10] For the synthesis of amine-containing analogues and derivatives thereof the (2-trimethylsilyl)ethyl-protected unsaturated aldehyde **12** (Scheme 2) served as a useful and flexible intermediate. We also found that treatment of the aldehyde **4** with di-*tert*-butyl dicarbonate in dichloromethane gave the corresponding Boc-protected material, which proved equally useful. The diethyl phosphonate **19** (Table 3) was generated from this intermediate by reductive amination followed by deprotection with TFA. Compound **20** (Table 1) was derived via the Boc-protected allylic bromide by cyanoborohydride-mediated reduction. Compound **21** was made through alkylation of the Boc-protected allylic alcohol with methyl iodide in the presence of lithium *tert*-butoxide. The synthesis of the *N*-methyl analogue **22** (Table 3) was achieved through cyanoborohydride-mediated reductive amination of the Boc-protected version of **14** (Scheme 2), followed by global deprotection with trimethylsilyl bromide buffered with 2,6-lutidine in acetonitrile. The sulfonyl urea **23** was made by reaction of the amine with 2-(trimethylsilyl)ethyl chlorosulfonyl carbamate (made in situ from 2-(trimethylsilyl)ethanol and chlorosulfonyl isocyanate) in the presence of triethylamine, followed by deprotection.

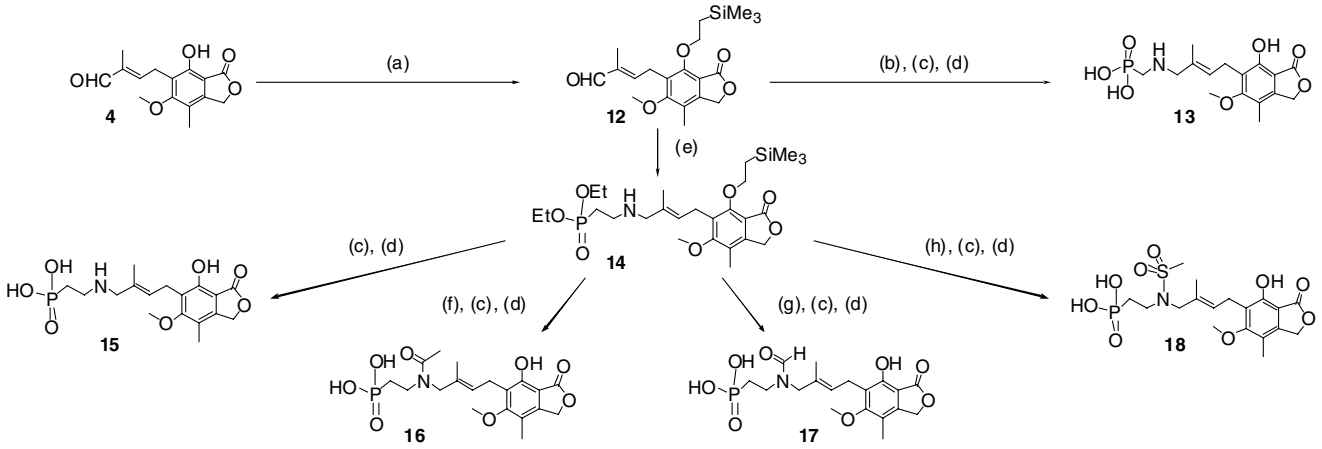
Table 1. Analogues of MPA lacking the carboxylic acid



Compound	R	IMPDH II IC_{50} (nM)
1	$CH_2CH_2CO_2H$	11
20	CH_3	254
21	CH_2OCH_3	273



Scheme 1. Reagents, conditions, and yields: (a) TMSCl, DABCO, CH_2Cl_2, 0 °C; O_3, −78 °C; Me_2S, −78 °C to rt, 73%; (b) $Ph_3P{=}C(Me)CHO$, C_6H_6, 77 °C, 77%; (c) $CH_2(P(O)(OMe)_2)_2$, NaHMDS, 71%; (d) TMSBr, 2,6-lutidine, CH_3CN; (e) $LiBH_4$, $CeCl_3$, THF/MeOH, 0 °C, 90%; (f) CBr_4, PS-PPh_3, CH_2Cl_2, 42%; (g) $ClCO_2CH_2CH(Me)_2$, TEA, THF, 0 °C; (h) $NaBH_4$, EtOH, 0 °C; (i) NaOH, then HCl, 84% from MPA; (j) $P(OMe)_3$, 100 °C, 93%; (k) $MeP(O)(OEt)_2$, *n*-BuLi, CuI, 50%; (l) LiO-*t*-Bu, $BrCH_2P(O)(O\text{-}i\text{-}Pr)_2$, DMF, 70 °C, 32%.



Scheme 2. Reagents, conditions, and yields: (a) $Me_3SiCH_2CH_2OH$, DIAD, PPh_3, Et_3N, THF, 45 °C, 70%; (b) $H_2NCH_2P(O)(OEt)_2$·oxalate, $NaBH(OAc)_3$, AcOH, DMF, 70%; (c) TMSBr, 2,6-lutidine, CH_3CN, 40–100%; (d) 20% TFA in CH_2Cl_2, 0 °C, 80–100%; (e) $H_2N(CH_2)_2P(O)(OEt)_2$·oxalate, $NaBH(OAc)_3$, AcOH, DMF, 96%; (f) AcOH, Ac_2O, EtOAc, 81%; (g) HCO_2H, Ac_2O, EtOAc, 85%; (h) MsCl, pyridine, CH_2Cl_2, 63%.

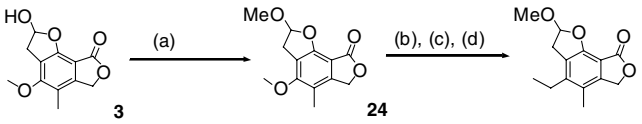
For manipulation of the C-6 substituent, it proved expedient to protect both the phenol and the sidechain simultaneously via the known methyl acetal **24** (Scheme 3).[11] Numerous methods were surveyed for demethylation of **24** without compromising the integrity of the acetal, and the best results were achieved by heating with DBN as solvent. The ethyl substituent could be introduced directly via Suzuki coupling, as shown, or by Pd-catalyzed reaction with tributylvinyl stannane, followed by hydrogenation.[12] Acidic hydrolysis, Wittig reaction, and Boc protection of the phenol gave an intermediate analogous to **12** (Scheme 2). A similar sequence of reactions to introduce the 6-ethyl substituent also proved feasible on the 2-(trimethylsilyl)ethyl-protected version of unsaturated alcohol **7** (Scheme 1). The 6-ethyl compounds **25**–**30** were then made by methods analogous to those described above.

Structure–activity relationships. Our chemistry strategy to achieve high and sustained lymphatic cellular concentrations of an MPA analogue was to incorporate a phosphonic acid, thereby generating a polyanionic entity whose permeability would be severely limited, and to mask the charges with a promoiety that is preferentially cleaved in lymph cells. In order to avoid the prospect of designing double prodrugs, we elected to explore analogues lacking the carboxylic acid moiety. This entity binds in the region of the NAD binding site that is occupied by the bridging phosphates of the cofactor,[2,13] and its removal leads to a 25-fold loss in potency (Table 1).

Initial attempts to regain potency through replacement of the carboxylate with phosphonates met with limited success (Table 2).

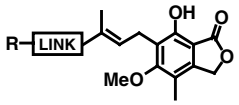
The analogue in which carboxylate is directly replaced by phosphonate (**10**) is 9-fold less active than MPA in the enzymatic assay. This is not surprising, given that in the conformation of MPA bound to IMPDH II, both oxygens of the carboxylate are involved in interactions with the protein: the carboxylate group is planar, unlike the spherical phosphonate, and the C–P and P–O bonds are ca. 20% longer than C–C and C–O. There may also be greater desolvation penalty upon binding for the phosphonate, due to the additional charge. Variation of the chain length by one atom gave analogues of similar potency (**6** and **11**). Rigidification of the chain, as in **5**, was detrimental. Overall, it appears that the phosphonic acid is contributing to binding, but to a lesser degree than the carboxylic acid of MPA.

To improve the potency, a wider selection of linkers was explored (Table 3). Side chains of the same length as that in **6**, but containing heteroatoms (**9**, **13**), are less



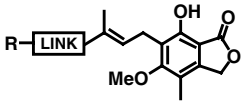
Scheme 3. Reagents and conditions: (a) *p*-TSA, MeOH, 50 °C; (b) DBN, 140 °C; (c) Tf_2O, pyridine; (d) $EtB(OH)_2$, $Pd(dppf)_2Cl_2$, Cs_2CO_3, THF, 70 °C.

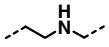
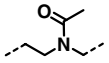
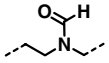
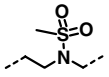
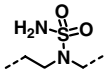
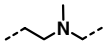
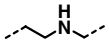
Table 2. Phosphonate replacements for carboxylate



Compound	Link	R	IMPDH II IC_{50} (nM)
1	CH_2CH_2	CO_2H	11
10	CH_2CH_2	$P(O)(OH)_2$	96
6	CH_2CH_2 CH_2	$P(O)(OH)_2$	86
11	CH_2	$P(O)(OH)_2$	168
5	CHCH(*E*)	$P(O)(OH)_2$	506
8	CH_2	$P(O)(OMe)_2$	289

Table 3. Linker variations



Compound	Link	R	IMPDH II IC_{50} (nM)
9		$P(O)(OH)_2$	246
13		$P(O)(OH)_2$	>1000
14		$P(O)(OH)_2$	499
16		$P(O)(OH)_2$	785
17		$P(O)(OH)_2$	749
18		$P(O)(OH)_2$	498
23		$P(O)(OH)_2$	93
22		$P(O)(OH)_2$	>1000
19		$P(O)(OEt)_2$	>1000

active. Furthermore, the activity of analogues with side chains containing a basic amine is quite sensitive to structural variation: the phosphonate ester **19** and the tertiary amine **22**, as well as **13**, prohibit binding of the molecule as a whole, but the β-aminophosphonate **14** retains some activity. While the majority of neutral derivatives of this amine retain similar potency, the sulfonyl urea **23** is ca. 5-fold more active—perhaps because of constructive H-bonds with the carboxylate binding site.

None of the analogues above displays potency equivalent to that of the parent MPA. In order to improve the activity of phosphonate-containing compounds, we resorted to modification of the phthalide core. In particular, it has previously been noted that variation of the C-6 methoxy substituent of MPA can improve potency.[12]

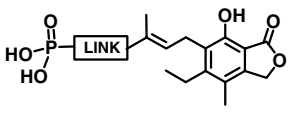
In this work, the most extensive variation of the C6-substituent was carried out with the allylic phosphonates (cf. **11**). We found that the SAR for this position is similar to that of MPA itself [12] and is very sensitive to small changes, particularly those that increase the polarity of the substituent (data not shown). The activity of the ethyl variant **25** (Table 4) is notable, however; although the corresponding analogue of MPA has been reported to be more active, the 8-fold increase in potency over **11** is striking. Replacing methoxy by ethyl as a tactic for improvement of potency is evident with the other linkers as well.

Finally, we measured the activity of phosphonate-containing analogues against the two different isoforms of IMPDH and found that they exhibited greater potency against IMPDH II (Table 5). The clinical relevance of this result is debatable (IMPDH functions as a tetrameric species that may be a mixture of isoforms in vivo),[14] but the similarity in isoform selectivity profile to that of MPA is consistent with a similar binding mode.

Replacement of the carboxylate of MPA by phosphonate gave analogues with slightly less potency for inhibition of IMPDH. However, it proved possible to incorporate a wide variety of different linker types and maintain activity. Through a conservative modification of the phthalide core several potent compounds were discovered, and in particular the amine-linked analogue **27** proved equi-potent to MPA itself. Like MPA, these compounds were somewhat selective for isoform II of the enzyme. Hence our initial objective of designing potent phosphonate-containing IMPDH inhibitors was achieved, and we were able to use these to explore the potential for intracellular delivery through the use of prodrugs.

Chemicals. All analogues were purified by reverse-phase HPLC using a C18 column with a gradient of 0.05% TFA–H_2O and 0.05% TFA–acetonitrile. The structural identity of each compound was confirmed by MS, ^1H NMR and ^{31}P NMR.

Table 4. C-6 ethyl analogues with various linkers



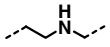
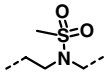
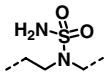
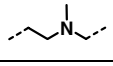
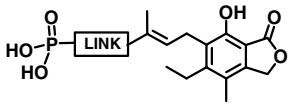
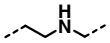
Compound	Link	IMPDH II IC_{50} (nM)
25	CH_2	20
26		23
27		13
28		68
29		24
30		132

Table 5. Inhibition of isoforms of IMPDH by key compounds



Compound	Link	IC_{50} (nM)		Ratio
		IMPDH I	IMPDH II	
1	–(MPA)	32	11	2.9
25	CH_2	71	20	3.6
26		38	23	1.7
27		37	13	2.8

Enzyme assays. Both IMPDH type I and type II genes were cloned from human fetal brain cDNA. The recombinant enzymes were expressed in BL21(DE3)pLysS (Invitrogen, Carlsbad, CA) with the plasmid pET15b (Novagen, Madison, WI) bearing the respective IMPDH open-reading frames. The assay for both isozymes was based on a previously reported method.[15] The reaction mixture contained 100 mM Tris–HCl, pH 8.0, 100 mM KCl, 3 mM EDTA, 1.1 mM DTT, 0.42 mM β-NAD, 0.2 mM IMP, and 3.4 nM IMPDH II (5.2 nM for IMPDH I). The total volume of each assay was 150 μL. The formation of NADH was monitored spectrophotometrically by measuring the difference in UV absorbance at 340 nm before and after incubation at 37 °C for 60 min. For IC_{50} determination, compounds were serially diluted in DMSO and added to the reaction mixture prior to the incubation; experiments were performed in duplicate. IC_{50} values were calculated from nonlinear curve fitting with GraphPad Prism software.

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