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Bis(sulfonamide) isosters of mycophenolic adenine dinucleotide analogues: Inhibition of inosine monophosphate dehydrogenase

Liqiang Chen, Riccardo Petrelli, Magdalena Olesiak †, Daniel J. Wilson, Nicholas P. Labello, Krzysztof W. Pankiewicz *

Center for Drug Design, Academic Health Center, University of Minnesota, 516 Delaware Street S.E., Minneapolis, MN 55455, USA

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ABSTRACT

Synthesis of novel inhibitors of human IMP dehydrogenase is described. These inhibitors are isosteric methylenebis(sulfonamide) analogues **5**–**8** of earlier reported mycophenolic adenine methylenebis(phosphonate)s **1**–**3**. The parent bis(phosphonate) **1** and its bis(sulfonamide) analogue **5** showed similar submicromolar inhibitory activity against IMPDH2 ($K_i \sim 0.2$ μM). However, the bis(sulfonamide) analogues **6** and **8** substituted at the position 2 of adenine were approximately 3- to 10-fold less potent inhibitors of IMPDH2 ($K_i = 0.3$–0.4 μM) than the corresponding parent bis(phosphonate)s **2** and **3** ($K_i = 0.04$–0.11 μM), respectively.

1. Introduction

In recent years, numerous studies of nicotinamide adenine dinucleotide (NAD)-dependent biological processes have demonstrated various significant roles of NAD in cell biology and medicine.[1] Consequently, molecules affecting interactions of NAD with NAD-dependent or NAD-utilizing enzymes are now of great therapeutic interest. For example, mycophenolic acid (MPA, Fig. 1), which targets the NAD-binding domain of IMP-dehydrogenase (IMPDH), has been approved by the FDA as an immunosuppressant.[2] Tiazofurin, an antileukemic C-nucleoside, is converted in the cell into tiazofurin adenine dinucleotide (TAD, Fig. 1), a mimic of NAD in which the nicotinamide riboside moiety is replaced by tiazofurin. TAD cannot participate in hydride transfer and selectively inhibits IMPDH.[3] Several other NAD-based potential therapeutics are now in Phase I/II clinical trials.[1]

NAD is usually bound at the characteristic Rossmann fold, a conserved structural motif that also binds nucleotides.[4–6] Large portions of the Rossmann folds of different NAD-dependent proteins superimpose well. Since this domain is conserved it was believed that it would be difficult if not impossible to design NAD-like molecules with good selectivity against a particular enzyme. However, dinucleotide-binding domains show very low overall sequence homology. Taken together with the enormous conformational flexibility of NAD the indication is that almost countless binding patterns between NAD and NAD-utilizing

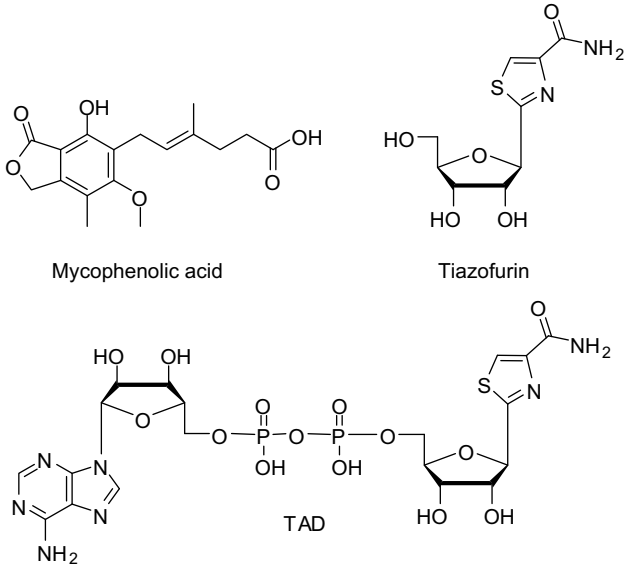

Figure 1. IMPDH inhibitors.

* Corresponding author. Tel.: +1 612 625 7968; fax: +1 612 624 825.
E-mail address: panki001@umn.edu (K.W. Pankiewicz).
† Department of Chemistry and Biochemistry, University of Colorado at Boulders, Boulder, CO 80309, USA.

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proteins are possible.[7] NAD has extraordinary hydrogen bonding potential. It contains 19 polar oxygen and nitrogen atoms and can form at least 16 hydrogen bonds with the NAD-binding proteins; some of these hydrogen bonds can be mediated by water molecules.[8] Thus, selective recognition of divergent three-dimensional arrangements of hydrogen bond acceptors and donors, hydrophobic groups as well as steric interactions should lead to selective or even specific inhibition of a single protein.

Indeed, NAD-dependent inosine monophosphate dehydrogenase (IMPDH) is a classic example of such selectively inhibited enzymes.[9] With NAD bound in an extended conformation[7] it is a major therapeutic target for the design of potential immuno-suppressants, anticancer, antiviral, and antimicrobial agents.[10,11] It catalyzes IMP to XMP conversion, the rate-limiting step in de novo synthesis of guanine nucleotides that are crucial for cell growth and proliferation. The mechanism of action of IMPDH has been recently reported.[12]

Crystal structures of complexes of human IMPDH with substrate and NAD analogues revealed that the dinucleotide-binding pocket can be divided into three regions: (1) the nicotinamide riboside sub-domain (N sub-site), (2) the adenosine sub-domain (A sub-site), and (3) the pyrophosphate linker sub-site (P sub-domain). The contribution of these regions to the potential binding of NAD-like inhibitors is not equal.

Mycophenolic acid (MPA) is a potent and specific inhibitor of the two known isoforms of human IMPDH with higher activity against IMPDH2 (K_i = 10 nM) than IMPDH1 (K_i = 40 nM). It binds at the N sub-domain of IMPDH with its six-member ring roughly mimicking the nicotinamide ring and its five-member ring extending to additional space in the IMPDH pocket (PDB 1JR1).[13,14] The side chain of MPA binds partially in the P sub-domain (groove) hydrogen bonding with one of the two serines (Ser 276) present at the floor of the groove. The A sub-site is empty.

Numerous inhibitors of IMPDH that take advantage of the strong-binding interactions at the N sub-domain have been designed by Vertex and then other major pharmaceutical companies.[14–17] Some of these compounds contained a phenyl-oxazole moiety [VX-497 (Merimepodib), VX-944 (now AVN-944), BMS-337197, Fig. 2] that binds at the N sub-domain, stacking under the covalently bound substrate hypoxanthine ring. AVN-944 is currently in Phase I/II clinical studies as an anticancer agent against blood malignancies. These inhibitors extend neither to the A sub-site nor to the P-groove.[9]

The active metabolite of Tiazofurin, TAD, that inhibits IMPDH (K_i = 100 nM) shows 3–4 orders of magnitude greater-binding affinity to IMPDH than to alcohol, glutamate or lactate dehydrogenases. A crystal structure of IMPDH2 in complex with SAD (PDB 1B30), a selenium analogue of TAD, showed that the selenazofurin moiety is bound at the N sub-domain.[18] The selenazole ring forms stacking interactions similar to that of MPA with the substrate analogue aromatic base and the carboxyamide group forming hydrogen bonds with the conserved Asn 303 residue. Only one phosphorous group of SAD interacts with both serines (275 and 276) of the P-groove. SAD-binding interactions are very similar to those of NAD with the adenine moiety located at the A sub-site. SAD is a slightly more potent inhibitor of IMPDH (K_i = 40 nM) than TAD.

We developed the mycophenolic adenine dinucleotide (MAD) analogue as a hybrid of C2-mycophenolic alcohol and adenosine linked through a metabolically stable bis(phosphonate) linker.[19,20] This compound, C2-MAD (**1**, Fig. 3), mimics the entire NAD in which nicotinamide riboside is replaced by a mycophenolic moiety. Good selectivity against IMPDH is preserved as no inhibition of cellular dehydrogenases (up to 50 μM) was determined. In a mouse model of chronic myelegenous leukemia C2-MAD showed lower toxicity and higher efficacy than tiazofurin.[21] Indeed, our crystal structure (PDB 1NF7) of the complex of IMPDH2 with C2-MAD (Fig. 4, coded by blue) revealed that the mycophenolic moiety is bound at the N sub-site of the cofactor-binding pocket whereas the adenine ring of C2-MAD is in exactly the same position at the A sub-domain as the adenine ring of NAD. It is sandwiched between the His 253 and Phe 282 and the N3 of the adenine ring is in close contact with Thr 45. This region around the adenine ring is not conserved between the human isoforms of IMPDH and can be

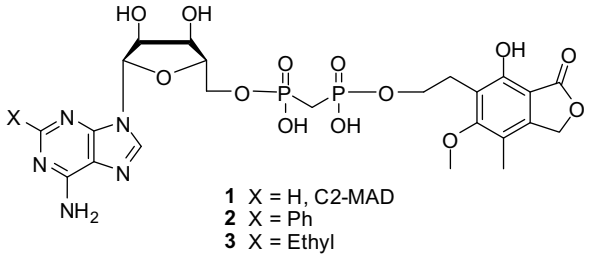


Figure 3. C2-MAD and its methylenebis(phosphonate) analogues.

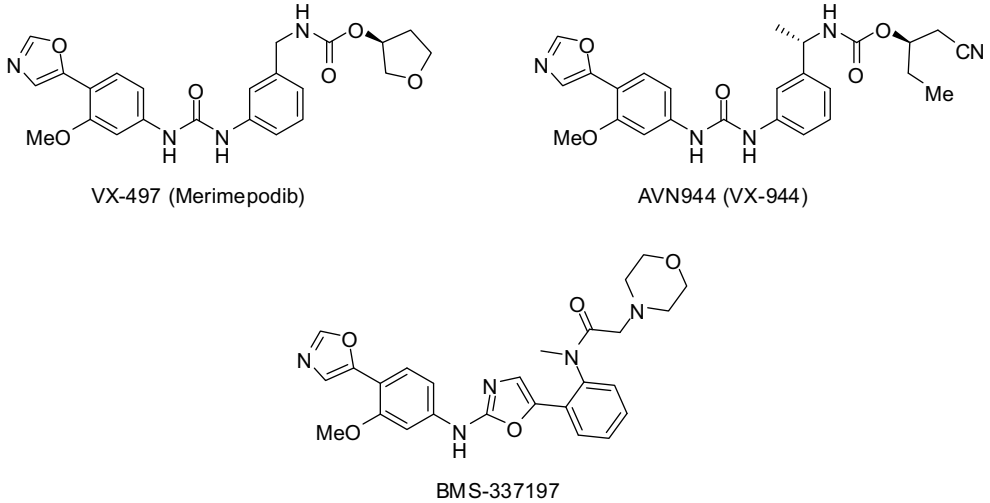


Figure 2. Inhibitors of IMPDH developed at Vertex and Bristol-Myers-Squibb.

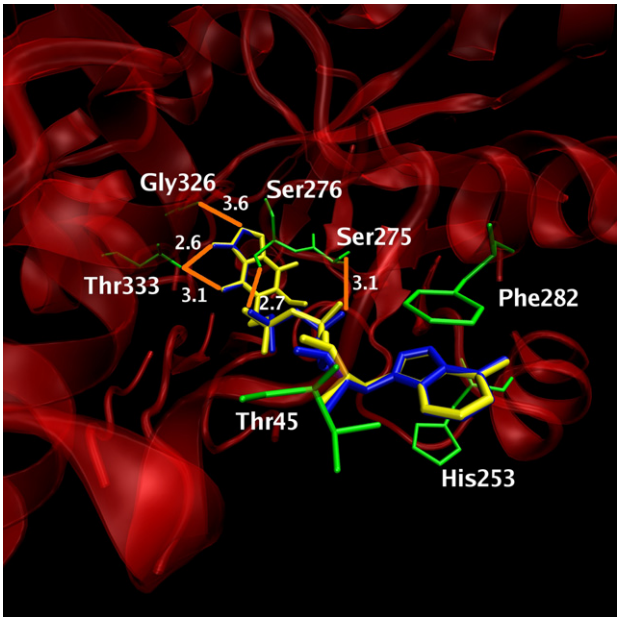


Figure 4. Bis(sulfonamide) 5 (coded in blue) modeled in IMPDH and superimposed with bis(phosphonate) 1 (C2-MAD, yellow). The starting conformation of each is taken from the ligand (C2-MAD) in crystal structure 1NF7. Both compounds were minimized. The protein is displayed in red ribbon while the ligands and several important residues (green) are presented in a tube representation. Key hydrogen bonds are shown with solid orange lines and distances measured for compound **5** are reported in angstroms.

exploited for the design of isoform selective inhibitors. Both adenosine hydroxyl groups interact with Gln 469. We found that even with the additional binding at the A sub-domain C2-MAD is less potent inhibitor of IMPDH (K_i = 250–330 nM) than MPA.

Recently, we found the C2-MAD analogue **2** (X = Ph, Fig. 3, Table 1) containing a phenyl group at the adenine 2 position is 5-fold more potent (K_i = 70 nM) against IMPDH1 than the parent analogue. Similarly, substitution with an ethyl group afforded analogue **3** (X = Et), which demonstrated a 16-fold improvement in potency (K_i = 20 nM) against IMPDH1.[22]

These studies indicate that binding interactions at the N sub-domain of IMPDH are crucial and sufficient for high affinity of inhibitors such as MPA or Vertex compounds. Additional MAD binding at the A sub-site is neither synergistic nor significant. It is likely that an entropy penalty for constructing the larger dinucleotide analogues negates any advantage of additional hydrogen bonding at the A sub-domain. The binding of the pyrophosphate

Table 1
Inhibition of human IMPDH type 1 and type 2

Compound	IMPDH1 K_i (μM)	IMPDH2 K_i (μM)
MPA	0.04	0.01
1 (C2-MAD)	0.33	0.25
2 (X = Ph)	0.07	0.11
3 (X = Et)	0.02	0.04
4	23.6[a]	18.8[a]
5 (MABS)	0.35 ± 0.03[b]	0.17 ± 0.02[b]
6 (X = Ph)	0.66 ± 0.11[b]	0.31 ± 0.14[b]
7 (X = C≡CH)	0.52 ± 0.06[b]	0.18 ± 0.03[b]
8 (X = Et)	0.82 ± 0.08[b]	0.44 ± 0.06[b]
14	18.2 ± 2.4[b]	4.0 ± 0.2[b]
15 (MP-alcohol)	15.4 ± 2.4[b]	3.0 ± 0.8[b]
18	>100	>100
Adenosine	>100	>100

[a] These values were reported as IC_{50}.
[b] The errors were given as 95% confidence intervals.

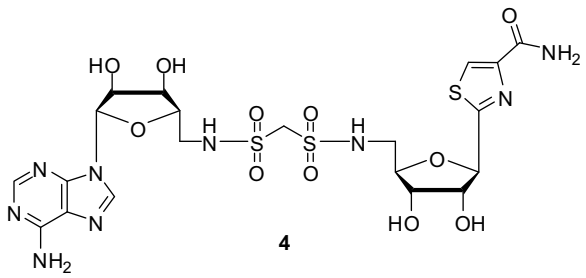


Figure 5. Tiazofurin adenine methylenebis(sulfonamide).

or bis(phosphonate) moieties in the P-groove seems to be even less important.

These results encouraged us to prepare NAD analogues with a longer linker than the typical pyrophosphate (–P–O–P–) or methylenebis(phosphonate) (–P–C–P–) group. Insertion of the oxygen atom between the phosphorus and carbon atom of C2-MAD afforded new inhibitors (–P–O–C–P– or P–C–O–P– linkers) that show 5- to 10-fold more potent inhibitory activity against the enzyme than the parent molecule (K_i's in the range of 20.0–87.0 nM).[23]

The relative flexibility of binding patterns at the P-groove inspired us to replace the phosphorous atoms of TAD with isosteric sulfur. In general, the similar geometry and shape of bis(sulfonamide) analogues of NAD may be well tolerated by NAD-dependent enzymes. However, we found that tiazofurin adenine methylenebis(sulfonamide) (**4**, Fig. 5) was about 200-fold less potent against IMPDH1 (IC_{50} = 23.6 μM) and IMPDH2 (IC_{50} = 18.8 μM, Table 1) than TAD (K_i = 0.1 μM) or its bis(phosphonate) analogue (K_i = 0.1 μM).[24]

2. Results and discussion

In our bis(phosphonate) series of mycophenolic adenine dinucleotides (MADs) the analogues **2** and **3** (Fig. 3) substituted at the 2 position of adenine were more potent than C2-MAD and TAD analogue (Table 1).[22] Therefore we expected that synthesis of novel mycophenolic adenine bis(sulfonamide) isosteres of C2-MAD (**5**, MABS, Fig. 6), and its substituted analogues, for example, MABS **6** (X = Ph), MABS **7** (X = C≡CH), and MABS **8** (X = Et), might afford more potent compounds than the parent molecules. With tetrahedral sulfur atoms, the proposed methylenebis(sulfonamide) linker should mimic the geometry of the methylenebis(phosphonate) group present in C2-MAD. Indeed, bis(sulfonamide) **5** (Fig. 4, coded in blue) modeled within the NAD-binding pocket of IMPDH2 showed very similar interactions (vide supra) as those observed in the crystal complex of C2-MAD/IMPDH2 (Fig. 4, coded in yellow). We expect that the weakly acidic bis(sulfonamide) functionality will not ionize under physiological conditions. At the same time, the partial negative charge delocalized onto the oxygen atoms would sufficiently mimic the negative charges present in

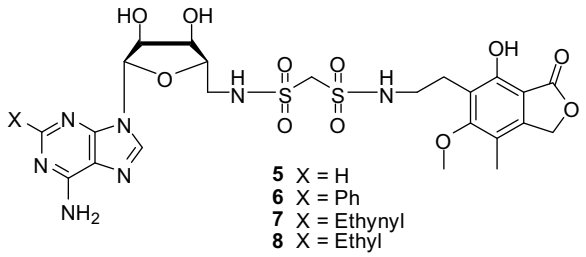
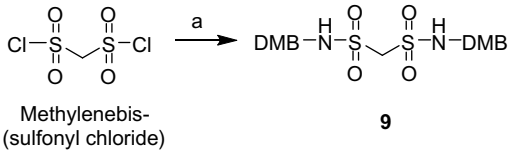


Figure 6. Mycophenolic adenine methylenebis(sulfonamide)s.



Scheme 1. Reagents and conditions: (a) 2,4-dimethoxybenzylamine, THF, 0 °C–rt.

the naturally occurring pyrophosphate linkage. Indeed, we found that new analogues **5**–**8** showed significantly more potent inhibitory activity against IMPDH than the corresponding bis(sulfonamide) analogue of TAD **4**.

2.1. Chemistry

We recently reported that bis(sulfonamide) analogues could be assembled by two sequential Mitsunobu reactions which linked protected mycophenolic and adenosine derivative through a properly protected methylenebis(sulfonamide) linker intermediate.[24] In our initial attempt on the synthesis of TAD analogue **4**, removal of the benzyl protective groups had been difficult under hydrogenolysis conditions.[24] To circumvent this problem, we decided to use 2,4-dimethoxybenzyl (DMB) protective groups, which could be cleaved under mild acidic conditions after the assembly of protected MABS analogues. To this end the key bis(sulfonamide) intermediate **9** (Scheme 1) was readily prepared by reaction of methylenebis(sulfonyl chloride)[25] with 2,4-dimethoxybenzylamine in high yield. For the protection of the 7-OH phenolic group 2-(trimethylsilyl)-ethoxymethyl (SEM) chloride was used based on the fact that SEM group could be removed under acidic conditions.

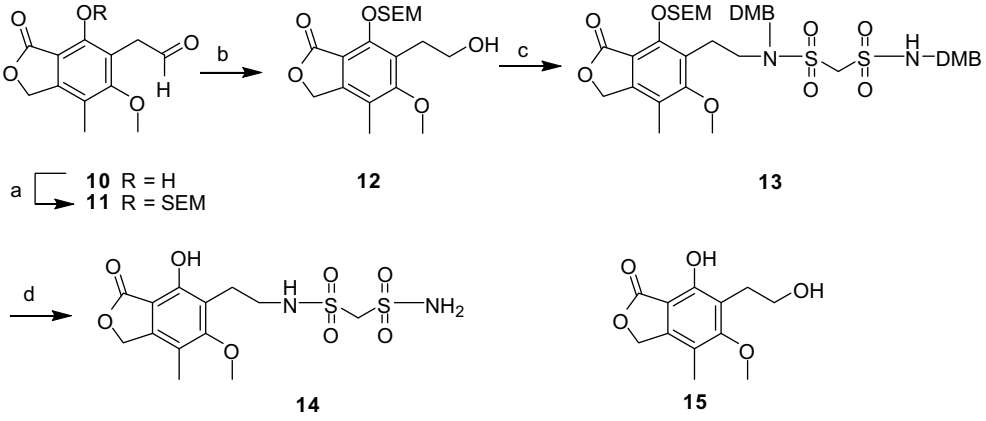
Alternatively, SEM group could also be cleaved with a fluoride source, such as tetrabutylammonium fluoride. Accordingly, the known aldehyde **10** (Scheme 2) was converted into aldehyde **11**, which upon reduction with sodium borohydride afforded protected alcohol **12**. It was subsequently used for the two sequential Mitsunobu reactions. The first Mitsunobu reaction between bis(sulfonamide) intermediate **9** and protected alcohol **12** produced a tri-substituted methylenebis(sulfonamide) **13**.

We were also interested in assessing the contribution of individual fragments, such as **14** and **15**, to the overall binding pattern. Thus, mildly acidic treatment of compound **13** led to the desired fragment **14** (Scheme 2). The Mitsunobu reaction between bis(sulfonamide) intermediate **9** and 2′,3′-isopropylidene-adenosine (**16**) gave tri-substituted methylenebis(sulfonamide) **17** (Scheme 3). A subsequent acidic treatment of this compound afforded the second desired fragment **18**, in which a bis(sulfonamide) group is attached to adenosine.

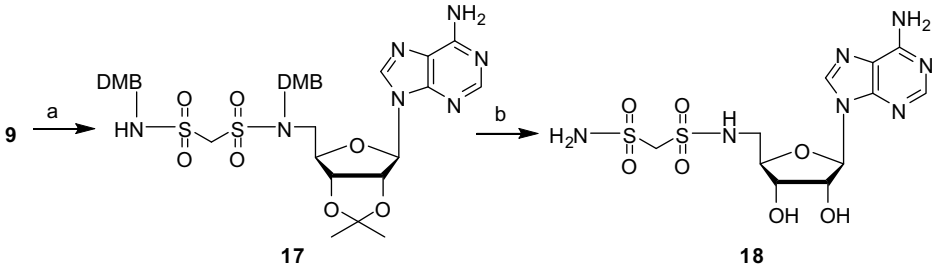
To prepare the fully assembled mycophenolic adenine bis(sulfonamide) analogue MABS **5**, tri-substituted methylenebis(sulfonamide) intermediate **13** was coupled with 2′,3′-isopropylidene-adenosine (**16**) via Mitsunobu reaction to give tetra-substituted sulfonamide **21**. Subsequent removal of SEM and DMB protective groups afforded MABS **5**. In a similar manner, the corresponding MABS analogues **6** (X = Ph) and **7** (X = C≡CH) were synthesized from 2′,3′-isopropylidene-2-phenyladenosine (**19**) and 2′,3′-isopropylidene-2-ethylnyladenosine (**20**), respectively. Finally, hydrogenation of compound **7** gave MABS analogue **8** (X = Et) (Scheme 4).

2.2. Biological evaluations

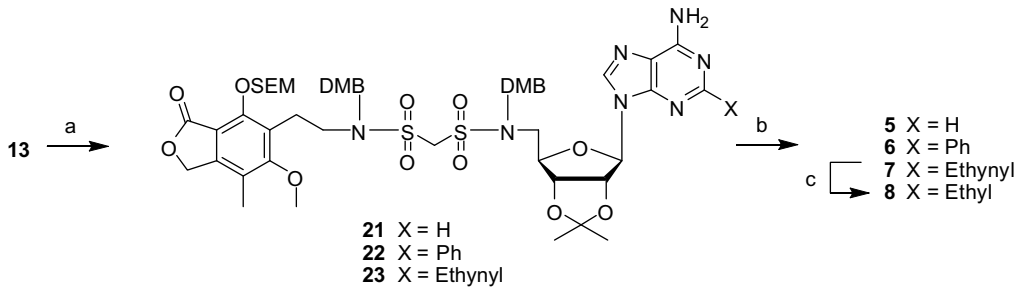
The final MABS analogues **5**–**8**, their fragments **14**, **15**, **18**, and adenosine were evaluated against human IMPDH1 and IMPDH2 (Table 1). Replacement of the bis(phosphonate) linker of C2-MAD (**1**) with the isosteric bis(sulfonamide) moiety afforded compound **5**, which showed as potent inhibitory activity against IMPDH1 (K_i = 0.35 μM) and IMPDH2 (K_i = 0.17 μM) as C2-MAD. The bis(sulfonamide) analogues **6**–**8** substituted at the 2-position showed



Scheme 2. Reagents and conditions: (a) SEMCl, Adogen 464, NaOH, CH_2Cl_2, H_2O; (b) $NaBH_4$, EtOH; (c) **9**, DIAD, PPh_3, THF, 0 °C–rt; (d) 40% (v/v) TFA, Et_3SiH, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) **16**, DIAD, PPh_3, THF, 0 °C–rt; (b) 40% (v/v) TFA, Et_3SiH, CH_2Cl_2; then 80% (v/v) TFA, H_2O.



Scheme 4. Reagents and conditions: (a) DIAD, PPh_3, THF, 0 °C–rt, **16**, **19** and **20** for compounds **21**, **22** and **23**, respectively; (b) 40% (v/v) TFA, Et_3SiH, CH_2Cl_2; then 80% (v/v) TFA, H_2O; (c) H_2, Pd/C, MeOH.

nanomolar inhibition in the range 0.18–0.84 μM however, they were less potent than the corresponding bis(phosphonate)s (0.02–0.11 μM).

Among fragments alcohol **15** (K_i = 15.4 μM, IMPDH1 and K_i = 3.0 μM, IMPDH2) retained a moderate inhibitory activity against the human enzyme. Replacement of the hydroxyl group with methylenebis(sulfonamide) as in compound **14** did not improve the potency. On the other hand, neither adenosine nor its methylenebis(sulfonamide) derivative **18** showed any activity against IMPDH.

This trend is not surprising, considering the different positions these fragments occupy in the NAD-binding site. Alcohol **15** presumably binds at the N sub-site, which has a great degree of rigidity. It is reasonable to expect that the functional groups on the phthalide core of alcohol **15** can engage in hydrogen bond interactions and aromatic stacking, as proposed for MPA. Therefore a portion of activity is retained for alcohol **15**. Nevertheless, the similar potency of bis(sulfonamide) **14** indicates that methylenebis(sulfonamide) contribution to binding is negligible and cannot compensate for strong binding of the crucial carboxylate group of MPA. It is likely that a bis(sulfonamide) group cannot form the desired hydrogen bonds needed for a high potency when adenosine is not attached. In contrast to fragments **14** and **15**, binding of adenosine and its bis(sulfonamide) derivative **18** to the A sub-site is weak if any as this region is exposed to solvent. Therefore it is energetically costly for adenosine or compound **18** to adopt a spatial configuration that enables them to favorably interact with the residues surrounding the A sub-site, resulting in no inhibition of IMPDH.

Even though fragment **14** exhibited only modest potency, the attachment of adenosine residue to compound **14** led to MABS analogue **5** with dramatically enhanced potency against both human IMPDH isoforms. When compared with fragment **14**, compound **5** displayed activity 52- and 24-fold more potent against IMPDH type 1 and type 2, respectively (Table 1). This dramatic gain of potency suggests that the methylenebis(sulfonamide) linker most likely facilitates the adenosine moiety to position itself so that it engages in favorable interactions with the residues surrounding the A sub-domain. Possibly, binding of the wholly assembled analogous such as MABS **5**–**8** is needed to trigger the conformational change of the enzyme (such as 'flap' closing) resulting in tight binding. This synergy also supports our current strategy for the design of IMPDH inhibitors based on linking two pharmacophores that interact with both N and A sub-sites.

However, modifications on the C2 position of the adenine ring resulted in bis(sulfonamide) analogues **6**–**8** with slightly reduced inhibitory activity. This finding is clearly in contrast with the trend we observed for the modifications made in the bis(phosphonate) C2-MAD series. For instance, introduction of an ethyl group at C2 position of the adenine ring led to bis(sulfonamide) derivative **8**, which displayed inhibitory activities approximately 2-fold lower than those of parent compound **5**. On the contrary, in the C2-MAD series attachment of an ethyl group at C2 position improved the potency by 21- and 6-fold for human IMPDH type 1 and type 2, respectively.[22]

2.3. Computational modeling

Bis(sulfonamide) **5** saw no loss in potency when compared to the analogous bis(phosphonate) suggesting that our hypothesis is correct and that a bis(sulfonamide) moiety can serve as an acceptable linker between the MPA and nucleoside pharmacophores. Interestingly, however, in the case of compound **4** identical substitution of the bis(phosphonate) results in a 230-fold loss of activity (IMPDH1).[24] Previously, on the basis of a structural analysis of ligands crystallized in IMPDH (particularly 1B3O), it was determined that at least one of the phosphate oxygen is protonated. In the case of tiazofurin methlenebis(phosphonate) the 3-hydroxy is donating a hydrogen bond paired with Asp 274 and accepting an internal hydrogen bond with the protonated adenosine phosphate. With an MPA head group, this internal hydrogen bond does not exist, and there is no penalty for substituting the bis(sulfonamide). With a tiazofurin head group, however, the internal hydrogen bond plays an important role in maintaining an optimal ligand conformation within the receptor. In the case of **4** the sulfonamide clearly cannot maintain this internal hydrogen bond resulting in the observed loss of potency when linking the tiazofurin and nucleoside groups with a bis(sulfonamide) linkage.

During study of the related NAD analogues with a di-phosphate linker it was noted that substitution of the adenine at the 2 position with ethyl and phenyl groups resulted in an increase in activity as much as 16-fold. Docking studies utilizing both molecular mechanics and QM/MM methods for geometry optimization are in agreement that while the positioning of the bis(sulfonamide) linker differs slightly from the (bis)phosphonate, displacement in the MPA head group and adenine moiety is much less. The RMSD of the heavy base atoms due to varying the linker is approximately 0.2 Å. Accordingly, a similar structure–activity relationship would be expected for substitution of the position 2. Interestingly, the relatively flat SAR suggests that no significant additional hydrophobic interactions occurred. Indeed, evaluation of the interaction energy for an ensemble of structures generated through molecular dynamics simulations of MABS **4**–**7** indicates that there is no energetic gain due to hydrophobic stacking for ligands in this series. Additionally the QM/MM interaction energy calculated for a minimum energy structure of each receptor–ligand complex indicates only very minor differences in agreement with the results of the enzyme assay.

3. Conclusions

It is well documented that the shape and physiochemical features around the three major sub-domains of NAD-dependent enzymes are significantly diversified. Numerous studies of the

Rossmann fold-NAD complexes showed that NAD-binding sites share a common mode of binding to the P and A sub-sites, whereas N sub-domain sites adopt a variety of conformations to fulfill different functions. The packing at the N sub-site is often strongly substrate-dependent and it enforces the proper chemical activity of NAD. It was believed that the substantial conservation of the A sub-domain indicates that the adenosine interactions are independent of an enzyme specificity. We found, however, that at least in the case of human IMPDH, the differences in the amino acids sequences at the A sub-site could be exploited toward design of inhibitors with some selectivity against the two isoforms of the human enzyme.[22]

The enzyme di-phosphate region (P-groove) appears to be highly promiscuous. It is not crucial for enforcing potent binding between ligands and their NAD-dependent enzymes. Therefore, we expected that NAD analogues containing structural changes at the pyrophosphate moiety would significantly affect neither binding affinity nor selectivity toward their corresponding proteins.

Herein, we replaced phosphorous atoms of methylenebis(phosphonate) analogues of MAD **1**–**3** with isosteric sulfur atoms that led to new sulfonamide analogues MPBS **5**–**8** with potent inhibitory activity against human IMPDH (K_is in the range of 0.17–0.82 μM). Our report indicates that further studies of structural diversity of the pyrophosphate moiety of NAD analogues on their binding ability at the P-groove of NAD-dependent enzymes are justified. Indeed, we found and will report shortly that connecting of pharmacophores binding at the N and A domains of IMPDH with a linker, which is neither isosteric nor isopolar, resulted in potent inhibition of the enzyme.

4. Experimental

4.1. General methods

All commercial reagents (Sigma–Aldrich, Acros) were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J.C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, while 2 packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Flash chromatography was performed with Ultra Pure silica gel (Silicycle) with the indicated solvent system. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz with Me_4Si or signals from residual solvent as the internal standard for ^1H or ^{13}C. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface.

4.2. Synthesis

4.2.1. Methylenebis(sulfonamide) (9)

To a solution of 2,4-dimethoxybenzylamine (33.0 mL, 220 mmol) in anhydrous THF (400 mL) at 0 °C was added dropwise a solution of methanedisulfonyl dichloride (10.7 g, 50.2 mmol) in anhydrous THF (100 mL). After stirring at 0 °C for 30 min, the reaction mixture was allowed to warm to rt and stir at rt for 5 h. After concentration, the residue was dissolved in EtOAc (600 mL) and washed with 0.1 N HCl (220 mL), water (100 mL), saturated $NaHCO_3$ (100 mL), and brine (2× 200 mL). The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated and then dried under high vacuum to give methylenebis(sulfonamide) **9** as a yellow solid (21.7 g, 91%). ^1H NMR (DMSO-d_6) δ 7.65 (br s, 2H), 7.20 (d, J = 8.4 Hz, 2H), 6.54 (s, 2H), 6.51 (d, J = 8.4 Hz, 2H), 4.75 (s, 2H), 4.10 (s, 4H), 3.78 (s, 6H), 3.74 (s, 6H). ^{13}C NMR (DMSO-d_6) δ 160.1, 157.6, 129.5, 117.7, 104.4, 98.2, 67.2, 55.4, 55.2, 41.0. $C_{19}H_{30}N_3O_8S_2$ 492.1468 $(M+NH_4)^+$, found 492.1512.

4.2.2. Protected aldehyde (11)

To a mixture of CH_2Cl_2 (24 mL) and 0.5 N aqueous NaOH solution (24 mL) were added Adogen 464 (3.60 mL, 2.42 mmol) and aldehyde **10** (2.82 g, 11.9 mmol). After stirring for 20 min, 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl, 2.60 mL, 14.7 mmol) was added dropwise. The resulting mixture was allowed to stir at rt for 1 h, and the organic layer was separated and then concentrated. The residue was purified by silica gel gravity column chromatography (10–30% EtOAc/hexanes) to give protected aldehyde **11** as a clear syrup (2.78 g, 64%). ^1H NMR ($CDCl_3$) δ 9.72 (s, 1H), 5.41 (s, 2H), 5.17 (s, 2H), 3.84 (s, 2H), 3.78 (t, J = 8.4 Hz, 2H), 3.75 (s, 3H), 2.21 (s, 3H), 0.94 (t, J = 8.4 Hz, 2H), 0.02 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 198.8, 168.8, 163.0, 154.3, 148.3, 121.3, 120.2, 112.3, 99.6, 68.3, 67.8, 60.8, 39.7, 18.1, 11.6, −1.5. $C_{18}H_{26}O_6S_iNa$ 389.1390 $(M+Na)^+$, found 389.1388.

4.2.3. Protected alcohol (12)

To a solution of protected aldehyde **11** (2.78, 7.58 mmol) in EtOH (80 mL) was added sodium borohydride (860 mg, 22.7 mmol) in small portions. The resulting mixture was allowed to stir for 30 min and then concentrated. The residue was re-dissolved in EtOAc (200 mL) and water (50 mL) and neutralized with 1 N HCl. The organic layer was separated, and washed with water (2× 50 mL) and brine (2× 75 mL). After drying over Na_2SO_4, the organic layer was filtered, and the filtrate was concentrated and dried in vacuo to give protected alcohol **12** as clear syrup (2.72 g, 97%). ^1H NMR ($CDCl_3$) δ 5.40 (s, 2H), 5.14 (s, 2H), 3.90–3.84 (m, 4H), 3.83 (s, 3H), 3.06 (t, J = 6.3 Hz, 2H), 2.30 (t, J = 5.7 Hz, 1H), 2.21 (s, 3H), 1.00 (t, J = 8.4 Hz, 2H), 0.03 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 169.1, 163.1, 154.6, 147.2, 126.8, 120.4, 112.2, 99.7, 68.2, 67.7, 62.5, 61.0, 27.8, 18.1, 11.7, −1.5. $C_{18}H_{28}O_6S_iNa$ 391.1547 $(M+Na)^+$, found 391.1535.

4.2.4. Protected mycophenolic bis(sulfonamide) (13)

To a solution of protected alcohol **12** (923 mg, 2.50 mmol), methylenebis(sulfonamide) **9** (3.56 g, 7.50 mmol), and triphenylphosphine (2.02 g, 7.70 mmol) in anhydrous THF (30 mL) at 0 °C was added via a syringe pump a solution of diisopropyl azodicarboxylate (DIAD, 1.50 mL, 7.74 mmol) in anhydrous THF (10 mL) over 5 h. The mixture was concentrated and the residue was dissolved in Et_2O (15 mL) and CH_2Cl_2 (5 mL). After an addition of hexanes (150 mL), a syrup formed on the wall of round-bottomed flask. The organic solvents were decanted and this cleaning process was then repeated for additional three times. The syrup obtained was purified by silica gel gravity column chromatography (0–3% MeOH/CH_2Cl_2) to give protected mycophenolic bis(sulfonamide) **13** as a white solid (1.24 g, 60%). ^1H NMR ($CDCl_3$) δ 7.22 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.48–6.40 (m, 3H), 6.38 (dd, J = 7.8, 2.4 Hz, 1H), 5.75 (t, J = 6.0 Hz, 1H), 5.39 (s, 2H), 5.12 (s, 2H), 4.35 (s, 2H), 4.23 (d, J = 6.0 Hz, 2H), 4.06 (s, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.78–3.73 (m, 5H), 3.72 (s, 3H), 3.38 (t, J = 7.2 Hz, 2H), 2.98 (t, J = 7.5 Hz, 2H), 2.16 (s, 3H), 0.90 (t, J = 8.4 Hz, 2H), −0.03 (s, 9H). $C_{37}H_{56}N_3O_{13}S_2Si$ 842.3018 $(M+NH_4)^+$, found 842.3030.

4.2.5. Mycophenolic bis(sulfonamide) (14)

To a solution of protected mycophenolic bis(sulfonamide) **13** (221 mg, 0.27 mmol) in dry CH_2Cl_2 (5.0 mL) and TFA (4.0 mL) was added dropwise triethylsilane (0.50 mL). During this period of time, the color of mixture changed from pink to light yellow. After stirring for 1 h at rt, the reaction mixture was concentrated

and the residue was triturated with hot CH_2Cl_2 (6 mL) to give bis(sulfonamide) **14** as pale solid (53.6 mg, 51%). ^{1}H NMR (DMSO-d_6) δ 9.51 (s, 1H), 7.58 (t, J = 5.7 Hz, 1H), 7.20 (s, 2H), 5.24 (s, 2H), 4.70 (s, 2H), 3.74 (s, 3H), 3.16-3.08 (m, 2H), 2.81 (t, J = 7.8 Hz, 2H), 2.08 (s, 3H). $C_{13}H_{18}N_2O_8S_2Na$ 417.0396 $(M+Na)^+$, found 417.0409.

4.2.6. Protected adenosine 5′-methylenebis(sulfonamide) (17)

In a manner similar to that described for the preparation of protected bis(sulfonamide) **13**, 2′,3′-*O*-isopropylideneadenosine (**16**, 986 mg, 3.21 mmol) was coupled with methylenebis(sulfonamide) **9** (2.53 g, 9.64 mmol) to give **17** as a pale solid (1.30 g, 53%). ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.84 (s, 1H), 7.11 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 6.45 (s, 1H), 6.42–6.36 (m, 2H), 6.29 (d, J = 8.4 Hz, 1H), 6.06 (s, 1H), 5.86 (s, 2H), 5.80 (t, J = 6.0 Hz, 1H), 5.40 (d, J = 6.0 Hz, 1H), 5.00–4.95 (m, 1H), 4.46–4.41 (m, 1H), 4.30 (d, J = 15.6 Hz, 1H), 4.22–4.16 (m, 3H), 4.11 (d, J = 15.0 Hz, 1H), 3.96 (d, J = 14.4 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 3.52 (dd, J = 15.6, 9.0 Hz, 1H), 3.39 (dd, J = 15.3, 3.3 Hz, 1H), 1.59 (s, 3H), 1.36 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 161.2, 161.0, 158.7, 158.5, 155.5, 153.0, 149.1, 140.1, 131.8, 130.6, 120.2, 116.3, 115.0, 114.5, 104.2, 103.9, 98.8, 98.4, 90.5, 86.1, 84.0, 82.3, 67.3, 55.3 (2C), 55.3, 55.1, 49.0, 46.5, 43.9, 27.1, 25.4. $C_{32}H_{42}N_7O_{11}S_2$ 764.2378 $(M+H)^+$, found 764.2391.

4.2.7. Adenosine 5′-methylenebis(sulfonamide) (18)

To a solution of protected adenosine 5′-methylenebis(sulfonamide) **17** (285 mg, 0.37 mmol) in dry CH_2Cl_2 (6.0 mL) and TFA (4.0 mL) was added dropwise triethylsilane (0.50 mL). The mixture was allowed to stir for 1.5 h and then concentrated. The residue was dissolved in TFA (8.0 mL) and water (2.0 mL) and the mixture was allowed to stir overnight. After concentration, the residue was co-evaporated with MeOH and purified by silica gel preparative TLC (1000 microns, 20% MeOH/CH_2Cl_2) to give **18** as a pale solid (58.5 mg, 37%). ^{1}H NMR (DMSO-d_6) δ 8.56 (t, J = 5.7 Hz, 1H), 8.32 (s, 1H), 8.19 (s, 1H), 7.46 (s, 2H), 7.24 (s, 2H), 5.85 (d, J = 6.6 Hz, 1H), 5.50 (br s, 1H), 5.30 (br s, 1H), 4.80 (s, 2H), 4.69 (t, J = 6.0 Hz, 1H), 4.12 (pseudo d, J = 2.4 Hz, 1H), 4.05 (pseudo d, J = 2.4 Hz, 1H), 3.37 (dt, J = 14.4, 4.2 Hz, 1H), 3.34-3.26 (m, 1H). HRMS calcd for $C_{11}H_{18}N_7O_7S_2$ 424.0703 $(M+H)^+$, found 424.0672.

4.3. Synthesis of protected mycophenolic adenine methylenebis(sulfonamide)s 21–23

4.3.1. Protected bis(sulfonamide) (21)

To a solution of protected mycophenolic bis(sulfonamide) **13** (995 mg, 1.21 mmol), 2′,3′-*O*-isopropylideneadenosine (**16**, 123 mg, 0.40 mmol), and triphenylphosphine (430 mg, 1.64 mmol) in anhydrous THF (10 mL) at 0 °C was added via a syringe pump a solution of DIAD (0.32 mL, 1.65 mmol) in anhydrous THF (5 mL) over 4.5 h. The mixture was allowed to slowly warm to rt and stir overnight. After concentration, the residue was purified by silica gel gravity column chromatography (0–6% MeOH/CH_2Cl_2) to give protected bis(sulfonamide) **21** as a white solid (152 mg, 34%). ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.83 (s, 1H), 7.25 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 9.0 Hz, 1H), 6.42 (d, J = 9.0 Hz, 1H), 6.40 (s, 1H), 6.36 (d, J = 1.8 Hz, 1H), 6.33 (dd, J = 8.7, 2.1 Hz, 1H), 6.04 (d, J = 1.8 Hz, 1H), 5.69 (br s, 2H), 5.41 (d, J = 6.6 Hz, 1H), 5.39 (d, J = 6.6 Hz, 1H), 5.36 (dd, J = 6.6, 1.2 Hz, 1H), 5.10 (s, 2H), 4.97 (dd, J = 6.6, 4.2 Hz, 1H), 4.50–4.46 (m, 1H), 4.46–4.40 (m, 3H), 4.36 (d, J = 15.0 Hz, 1H), 4.22 (d, J = 14.4 Hz, 1H), 4.10 (d, J = 14.4 Hz, 1H), 3.81–3.76 (m, 7H), 3.76–3.72 (m, 10H), 3.61 (dd, J = 15.3, 8.7 H, 1H), 3.51 (dd, J = 15.3, 4.5 Hz, 1H), 3.43–3.36 (m, 2H), 3.00 (t, J = 7.8 Hz, 2H), 2.14 (s, 3H), 1.58 (s, 3H), 1.35 (s, 3H), 0.89 (t, J = 8.4 Hz, 2H), −0.03 (s, 9H). HRMS calcd for $C_{50}H_{68}N_7O_{16}S_2Si$ 1114.3927 $(M+H)^+$, found 1114.3964.

4.3.2. Protected bis(sulfonamide) (22)

In a similar manner mycophenolic bis(sulfonamide) **13** (450 mg, 0.55 mmol) was coupled with 2′,3′-*O*-isopropylidene-2-phenyladenosine (**19**, 70 mg, 0.18 mmol) to give a pale solid (120 mg), which was used directly for the next reaction.

4.3.3. Protected bis(sulfonamide) (23)

In a similar manner mycophenolic bis(sulfonamide) **13** (1.64 g, 1.99 mmol) was coupled with 2′,3′-*O*-isopropylidene-2-ethynyladenosine (**20**, 223 mg, 0.67 mmol) to give **23** as a white solid (129 mg, 11%). ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1H), 7.26 (d, J = 7.8 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 6.42 (d, J = 8.4 Hz, 1H), 6.39 (s, 1H), 6.35 (s, 1H), 6.34 (d, J = 8.4 Hz, 1H), 6.04 (d, J = 1.2 Hz, 1H), 5.82 (br s, 2H), 5.40 (d, J = 6.6 Hz, 1H), 5.38 (d, J = 6.6 Hz, 1H), 5.29 (d, J = 6.6 Hz, 1H), 5.10 (s, 2H), 4.96 (dd, J = 6.6, 4.2 Hz, 1H), 4.48–4.40 (m, 4H), 4.35 (d, J = 15.0 Hz, 1H), 4.21 (d, J = 13.8 Hz, 1H), 4.13 (d, J = 14.4 Hz, 1H), 3.81–3.71 (m, 17H), 3.59 (dd, J = 15.6, 8.4 H, 1H), 3.54 (dd, J = 15.6, 4.8 Hz, 1H), 3.41 (t, J = 7.5 Hz, 2H), 2.99 (t, J = 7.8 Hz, 2H), 2.94 (s, 1H), 2.13 (s, 3H), 1.57 (s, 3H), 1.34 (s, 3H), 0.89 (t, J = 8.4 Hz, 2H), −0.03 (s, 9H). HRMS calcd for $C_{52}H_{68}N_7O_{16}S_2Si$ 1138.3927 $(M+H)^+$, found 1138.3942.

4.4. Synthesis of mycophenolic adenine methylenebis (sulfonamide)s (MABS) 5–7

4.4.1. MABS (5) (X = H)

To a solution of protected mycophenolic adenine methylenebis(sulfonamide) **21** (134 mg, 0.12 mmol) in dry CH_2Cl_2 (5.0 mL) and TFA (4.0 mL) was added dropwise triethylsilane (0.50 mL). The mixture was allowed to stir for 1.5 h and then concentrated. The residue was dissolved in TFA (4.0 mL) and water (1.0 mL) and the mixture was allowed to stir overnight. After concentration, the residue was co-evaporated with MeOH and purified by silica gel preparative TLC (1000 microns, 10% MeOH/CH_2Cl_2) to give 5 as a pale solid (23.6 mg, 30%). ^{1}H NMR (DMSO-d_6) (two signals overlap with the water signal) δ 9.53 (br s, 1H), 8.59 (s, 1H), 8.30 (s, 1H), 8.16 (s, 1H), 7.60 (t, J = 6.0 Hz, 1H), 7.43 (br s, 2H), 5.83 (d, J = 6.6 Hz, 1H), 5.49 (br s, 1H), 5.24 (s, 2H), 4.86 (s, 2H), 4.68 (t, J = 6.0 Hz, 1H), 4.11 (pseudo s, 1H), 4.05 (pseudo s, 1H), 3.72 (s, 3H), 3,12 (dd, J = 15.0, 6.0 Hz, 1H), 2.80 (t, J = 7.8 Hz, 2H), 2.07 (s, 3H). HRMS calcd for $C_{23}H_{30}N_7O_{11}S_2$ 644.1439 $(M+H)^+$, found 644.1415.

4.4.2. MABS (6) (X = Ph)

In similar manner compound **22** was deprotected to give mycophenolic 2-phenyladenine bis(sulfonamide) **6** as a pale solid (14.8 mg, 11% for two steps). ^{1}H NMR (DMSO-d_6) δ 9.51 (br s, 1H), 8.35 (s, 1H), 8.33 (d, J = 6.6 Hz, 2H), 7.74 (br s, 1H), 7.61 (br s, 1H), 7.48–7.38 (m, 3H), 7.32 (br s, 2H), 5.97 (d, J = 6.6 Hz, 1H), 5.54 (d, J = 5.4 Hz, 1H), 5.33 (br s, 1H), 5.23 (s, 2H), 4.83 (d, J = 14.4 Hz, 1H), 4.81-4.75 (m, 2H), 4.21 (pseudo s, 1H), 3.98 (dd, J = 9.3, 5.7 Hz, 1H), 3.70 (s, 3H), 3,43 (dd, J = 13.5, 5.1 Hz, 1H), 3.27 (dd, J = 14.4, 6.6 Hz, 1H), 3.09 (t, J = 7.8 Hz, 2H), 2.78 (t, J = 8.1 Hz, 2H), 2.05 (s, 3H). HRMS calcd for $C_{29}H_{34}N_7O_{11}S_2$ 720.1752 $(M+H)^+$, found 720.1728.

4.4.3. MABS (7) (X = C≡CH)

In similar manner compound **23** (115 mg, 0.10 mmol) was deprotected to give mycophenolic 2-ethynyladenine methylenebis(sulfonamide) (**7**) as a pale solid (56 mg, 83%). ^{1}H NMR (DMSO-d_6) (one signal overlaps with the water signal) δ 9.52 (s, 1H), 8.40 (s, 1H), 7.79 (t, J = 5.7 Hz, 1H), 7.61 (t, J = 5.7 Hz, 1H), 7.49 (br s, 2H), 5.85 (d, J = 5.4 Hz, 1H), 5.53 (d, J = 5.4 Hz, 1H), 5.30 (d, J = 5.4 Hz, 1H), 5.24 (s, 2H), 4.84 (d, J = 14.4 Hz, 1H), 4.81 (d, J = 14.4 Hz, 1H), 4.56 (pseudo d, J = 5.4 Hz, 1H), 4.10 (pseudo

d, J = 3.0 Hz, 1H), 4.00–3.93 (m, 2H), 3.72 (s, 3H), 3.18–3.08 (m, 4H), 2.80 (t, J = 7.5 Hz, 2H), 2.07 (s, 3H). HRMS calcd for $C_{25}H_{30}N_7O_{11}S_2$ 668.1439 $(M+H)^+$, found 668.1477.

4.4.4. MABS (8)

A mixture of MABS **7** (31 mg, 0.046 mmol) and 10% Pd/C (30 mg) in MeOH (10 mL) was hydrogenated (1 atm) overnight. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated. The resulting residue was purified by silica gel preparative TLC (1000 microns, 10% and then 15% MeOH/CH_2Cl_2) to give mycophenolic 2-ethyladenine methylenebis(sulfonamide) **7** as a pale solid (10.2 mg, 32%). ^{1}H NMR (DMSO-d_6) δ (one signal overlaps with the water signal) 8.22 (s, 1H), 7.80 (br s, 1H), 7.13 (br s, 2H), 5.76 (d, J = 6.6 Hz, 1H), 5.47 (d, J = 5.4 Hz, 1H), 5.27 (br s, 1H), 5.24 (s, 2H), 4.83 (d, J = 14.4 Hz, 1H), 4.79 (d, J = 14.4 Hz, 1H), 4.68 (pseudo d, J = 5.4 Hz, 1H), 4.15 (pseudo s, 1H), 3.96 (pseudo d, 2.4 Hz, 1H), 3.72 (s, 3H), 3.38 (dd, J = 13.8, 4.8 Hz, 1H), 3.11 (t, J = 7.5 Hz, 2H), 2.80 (t, J = 7.8 Hz, 2H), 2.66 (q, J = 7.2 Hz, 2H), 2.07 (s, 3H), 1.21 (t, J = 7.8 Hz, 3H). HRMS calcd for $C_{25}H_{34}N_7O_{11}S_2$ 672.1752 $(M+H)^+$, found 672.1767.

4.5. Computational chemistry

Model construction has been described elsewhere.[22] All calculations were completed within the Schrodinger suite of modeling programs, which includes MacroModel[26] for molecular mechanics minimization, conformational searching, and molecular dynamics and Qsite[27] for QM/MM calculations. The ligands were constructed by modifying the ligand present in 1NF7 and subsequent minimization. MD simulations were run for no less than 150 ps following minimization and equilibrium runs using the OPLS 2005[28] force field. QM/MM optimizations and interaction energy calculations were completed at the B3LYP[29,30]/6-31G* level of theory.[31,32]

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References and notes

1. Chen, L.; Petrelli, R.; Gao, G.; Bonnac, L.; Yu, J. S.; Bennett, E. M.; Pankiewicz, K. W. *Curr. Med. Chem.* **2008**, *15*, 650.
2. Bentley, R. *Chem. Rev.* **2000**, *100*, 3801.
3. Grifantini, M. *Curr. Opin. Invest. Drugs* **2000**, *1*, 257.
4. Rossmann, M. G.; Moras, D.; Olsen, K. W. *Nature* **1974**, *250*, 194.
5. Carugo, O.; Argos, P. *Proteins* **1997**, *28*, 10.
6. Carugo, O.; Argos, P. *Proteins* **1997**, *28*, 29.
7. Due to stacking abilities of its two aromatic moieties, adenine and nicotinamide, NAD is folded in water solution (with the distance between the bases of 4–5 Å). Several bacterial reductases bind NAD in such compact (folded) conformation (see PDB entries 1RZ1, 1ZPT, 2BKJ). Other enzymes, such as bacterial NAD kinases, including therapeutically important *Mycobacterium tuberculosis* enzyme, unfold NAD partially; NAD is bent with the distance around 12 Å between the bases. In majority of NAD-utilizing enzymes, including IMPDH, the distance is around 16–17 Å. This means that NAD must be unfolded into extended conformation to be able to bind.
8. Bottoms, C. A.; Smith, P. E.; Tanner, J. J. *Protein Sci.* **2002**, *11*, 2125.
9. *Inosine Monophosphate Dehydrogenase: A Major Therapeutic Target*; Pankiewicz, K. W., Goldstein, B. M., Eds.; Oxford University Press: Washington, 2003.
10. Chen, L.; Pankiewicz, K. W. *Curr. Opin. Drug Discov. Dev.* **2007**, *10*, 403.
11. Pankiewicz, K. W. *Expert Opin. Ther. Pat.* **2001**, *11*, 1161.
12. Hedstrom, L.; Gan, L. *Curr. Opin. Chem. Biol.* **2006**, *10*, 520.
13. Sintchak, M. D.; Fleming, M. A.; Futer, O.; Raybuck, S. A.; Chambers, S. P.; Caron, P. R.; Murcko, M. A.; Wilson, K. P. *Cell* **1996**, *85*, 921.
14. Sintchak, M. D.; Nimmesgern, E. *Immunopharmacology* **2000**, *47*, 163.
15. Iwanowicz, E. J.; Watterson, S. H.; Guo, J.; Pitts, W. J.; Murali Dhar, T. G.; Shen, Z.; Chen, P.; Gu, H. H.; Fleener, C. A.; Rouleau, K. A.; Cheney, D. L.; Townsend, R. M.; Hollenbaugh, D. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2059.
16. Watkins, W. J.; Chen, J. M.; Cho, A.; Chong, L.; Collins, N.; Fardis, M.; Huang, W.; Hung, M.; Kirschberg, T.; Lee, W. A.; Liu, X.; Thomas, W.; Xu, J.; Zeynalzadegan, A.; Zhang, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3479.
17. Birch, H. L.; Buckley, G. M.; Davies, N.; Dyke, H. J.; Frost, E. J.; Gilbert, P. J.; Hannah, D. R.; Haughan, A. F.; Madigan, M. J.; Morgan, T.; Pitt, W. R.; Ratcliffe, A. J.; Ray, N. C.; Richard, M. D.; Sharpe, A.; Taylor, A. J.; Whitworth, J. M.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5335.
18. Colby, T. D.; Vanderveen, K.; Strickler, M. D.; Markham, G. D.; Goldstein, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3531.
19. Lesiak, K.; Watanabe, K. A.; Majumdar, A.; Powell, J.; Seidman, M.; Vanderveen, K.; Goldstein, B. M.; Pankiewicz, K. W. *J. Med. Chem.* **1998**, *41*, 618.
20. Pankiewicz, K. W.; Lesiak-Watanabe, K. B.; Watanabe, K. A.; Patterson, S. E.; Jayaram, H. N.; Yalowitz, J. A.; Miller, M. D.; Seidman, M.; Majumdar, A.; Prehna, G.; Goldstein, B. M. *J. Med. Chem.* **2002**, *45*, 703.
21. Patterson, S. E.; Black, P. L.; Clark, J. L.; Risal, D.; Goldstein, B. M.; Jayaram, H. N.; Schinazi, R. F.; Pankiewicz, K. W. In *Developments in Nucleic Acids*; Schinazi, R. F. L. D., Ed.; IHL Press, 2004.
22. Chen, L.; Gao, G.; Felczak, K.; Bonnac, L.; Patterson, S. E.; Wilson, D.; Bennett, E. M.; Jayaram, H. N.; Hedstrom, L.; Pankiewicz, K. W. *J. Med. Chem.* **2007**, *50*, 5743.
23. Rejman, D.; Olesiak, M.; Chen, L.; Patterson, S. E.; Wilson, D.; Jayaram, H. N.; Hedstrom, L.; Pankiewicz, K. W. *J. Med. Chem.* **2006**, *49*, 5018.
24. Chen, L.; Gao, G.; Bonnac, L.; Wilson, D. J.; Bennett, E. M.; Jayaram, H. N.; Pankiewicz, K. W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3152.
25. Castro, A.; Erickson, S. K.; Shechter, I.; Spencer, T. A. *Bioorg. Chem.* **1996**, *24*, 242.
26. MacroModel. Schrodinger, LLC: New York, NY, 2006.
27. Qsite, version 4.5, Schrodinger, LLC.: New York, 2007.
28. Kaminski, G. A.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. *J. Phys. Chem. B* **2001**, *105*, 6474.
29. Kaminski, G. A.; Stern, H. A.; Berne, B. J.; Friesner, R. A.; Cao, Y. X.; Murphy, R. B.; Zhou, R.; Halgren, T. A. *J. Comput. Chem.* **2002**, *23*, 1515.
30. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
31. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
32. Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724.