Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2059–2063

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Inhibitors of Inosine Monophosphate Dehydrogenase: SARs about the *N*-[3-Methoxy-4-(5-oxazolyl)phenyl Moiety

Edwin J. Iwanowicz,* Scott H. Watterson, Junqing Guo, William J. Pitts, T. G. Murali Dhar, Zhongqi Shen, Ping Chen, Henry H. Gu, Catherine A. Fleener, Katherine A. Rouleau, Daniel L. Cheney, Robert M. Townsend and Diane L. Hollenbaugh

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA

Received 25 October 2002; accepted 29 January 2003

Abstract—The first reported structure–activity relationships (SARs) about the *N*-[3-methoxy-4-(5-oxazolyl)phenyl moiety for a series of recently disclosed inosine monophosphate dehydrogenase (IMPDH) inhibitors are described. The syntheses and in vitro inhibitory values for IMPDH II, and T-cell proliferation (for select analogues) are given.

Despite CellCept®'s commercial success, dose-limiting toxicities restrict its use primarily to the treatment of transplant rejection. There is a continuing effort to identify and develop new inhibitors of inosine monophosphate dehydrogenase (IMPDH) differentiated from CellCept® (MMF) by an improved side-effect profile. A key objective of these efforts is to reduce the incidence of GI-related disorders, a common adverse event for patients administered CellCept®.[1] Two strategies have emerged in the literature, the first is to identify inhibitors which exclude the chemical moieties implicated in the mechanisms responsible for the overt GI toxicity.[2] Our group has focused on this approach and has identified a number of new classes of inhibitors.[3–13] The second approach is to appropriately formulate mycophenolic acid (MPA), the active species of MMF, to reduce upper GI toxicity and improve absorption.[14] With favorable clinical data,[15,16] Novartis is poised to launch an enteric-coated formulation of mycophenolate sodium (ERL-80, Myfortic).

Table 1 shows selected analogues from several recently disclosed chemical series. All of these compounds contain an oxazol-5-yl group adjacent to a methoxy residue. This communication will focus on alterations in this conserved molecular array.

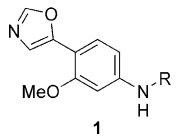
*Corresponding author. Tel.: +1-609-252-5416; e-mail: iwanowie@bms.com

Vertex has reported crystallographic studies of mycophenolic acid and VX-497 complexed with Chinese hamster IMPDH type II.[17] Consistent with the uncompetitive kinetics of inhibition for both compounds, each binds in the NAD pocket and prevents the hydrolysis of the hypoxanthine ring system (XMP*) and subsequent liberation of XMP. Shown in Figure 1 are the key binding interactions for the benzofuranone residue of MPA and the *N*-[3-methoxy-4-(5-oxazolyl)phenyl (MOA) fragment of VX-497. The benzofuranone ring of MPA binds in a spatially restricted region of the NAD binding site packing with the hypoxanthine ring of XMP*. The carbonyl oxygen and hydroxy residues are involved in a hydrogen bond array that involves Gly 326, Thr 333 and Gln 441. The appending methyl substituent of the benzofuranone system, resides in a small hydrophobic pocket defined by the planes of Asn 303 amide and Arg 322 guanidine. The phenyl oxazole moiety of VX-497 binds in the same region as the benzofuranone ring of MPA, forming hydrogen bonds between the oxazole nitrogen and oxygen with Gly 326 and possibly Thr 333, respectively. The methoxy residue binds in the same pocket as the benzofuranone methyl in MPA.

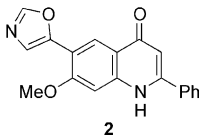
The first objective of this study was to prepare analogues with variations in the substitution of the phenyl residue while maintaining the 5-oxazolyl ring and its point of attachment. The anilines were generally pre-

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00258-0

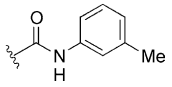
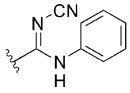
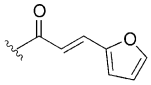
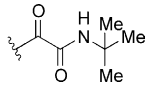
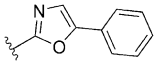
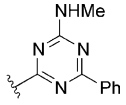
Table 1. Various reported inhibitors of IMPDH utilizing a 5-oxazolyl residue



1



2

Compd	R	IMPDH II IC_{50} (μM)	CEM IC_{50} (μM)
1a		0.019	2.3
1b		0.24	—
1c		0.028	6.6
1d		0.010	2.3
1e		0.020	>10
1f		0.076	3.7
2	NA	0.008	0.69

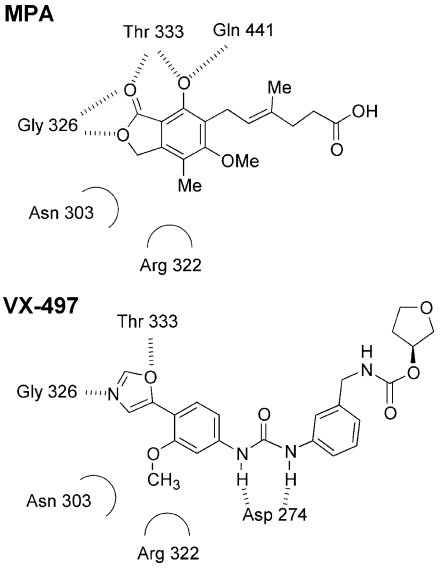
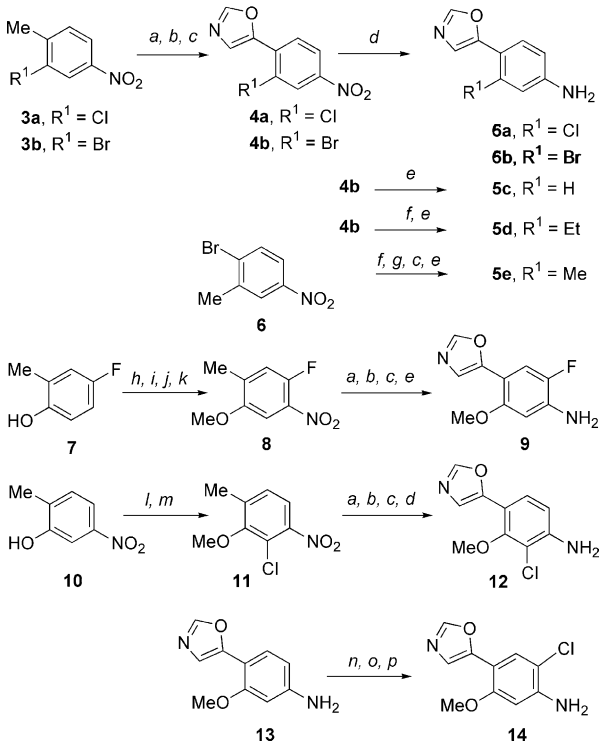


Figure 1. Key binding interactions for the benzofuranone moiety of MPA and the oxazol-5-yl and methoxy substituents of VX-497 with Chinese hamster IMPDH II. The hexenoic acid side chain of MPA is depicted in an extended conformation for clarity. Hydrogen bond interactions between the inhibitors and enzyme are shown as dashed lines and hydrophobic interactions are denoted with partial circles.

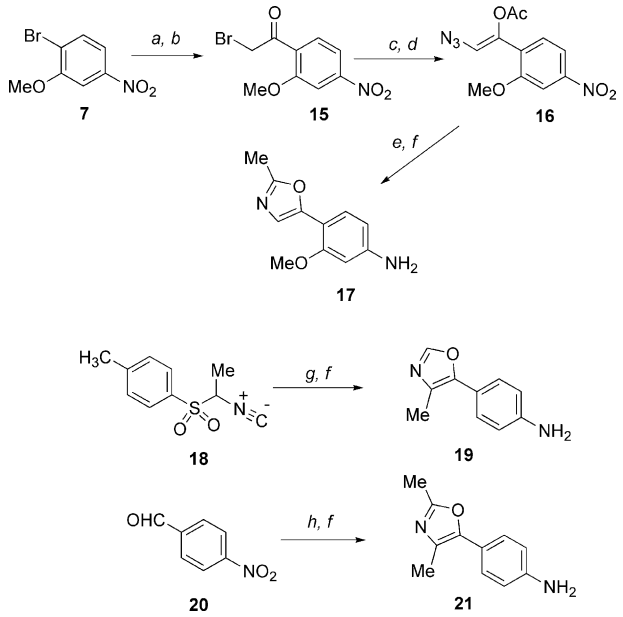
pared by condensation of appropriately substituted nitrobenzaldehydes with anion derived from tosylmethyl isocyanide (TosMIC) (Scheme 1). The various classes of final compounds were prepared, utilizing the anilines described herein, making use of the synthetic procedures described in the original disclosures.[3–13]

Our next objective was to place a methyl probe on the oxazol-5-yl system (Scheme 2) to examine the effect of increasing steric congestion in an area which appeared to make important interactions with the enzyme. The 2-methyloxazole intermediate **17** was prepared by intramolecular cyclodehydration. The 4-methyloxazole intermediate **19** was prepared utilizing a substituted TosMIC derivative. The 2,4-dimethyloxazole intermediate **21** was prepared by reaction of aldehyde **20** with 2-acetamidoacrylic acid in the presence of pyridine.

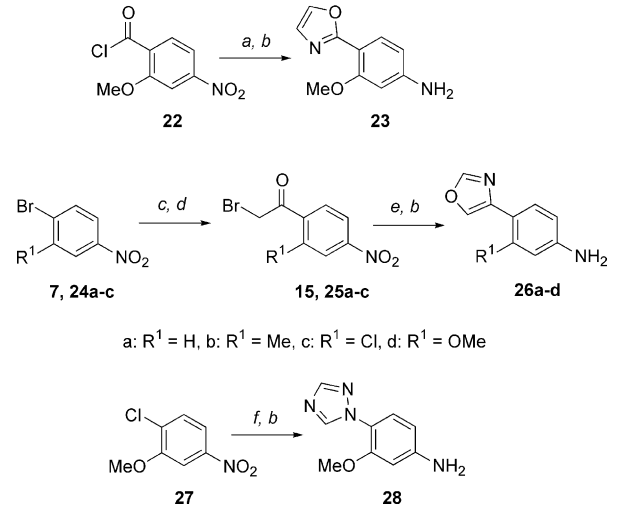
Finally, analogues were fashioned where the oxazole connectivity to the phenyl moiety was varied, and where the oxazole was replaced with a triazole. As shown in Scheme 3, the oxazol-2-yl derivative was prepared by reaction of the acid chloride **22** with 1,2,3-triazole. A series of oxazole-4-yl derivatives was prepared by a four-step procedure. This synthetic sequence features the formation of the corresponding bromoketones, **15**, **25a–c** and ring formation by reaction with formamide



Scheme 1. Reagents and conditions: (a) HOAc, Ac_2O, concd H_2SO_4, CrO_3, 0–10 °C, 35–55%; (b) concd HCl, dioxane, reflux, 90–95%; (c) TosMIC, K_2CO_3, MeOH, reflux, 80–85%; (d) $SnCl_2{\cdot}2(H_2O)$, EtOAc/ETOH(2:1), 70 °C, 95–98%; (e) 40 psi H_2, Pd/C, EtOH, 92–95%; (f) $CH_2{=}CHSnBu_3$, Pd_2dba_3, toluene, 110 °C, 82–96%; (g) O_3, CH_2Cl_2, −78 °C, then Me_2S, 82%; (h) $CH_3OC(O)Cl$, pyridine/dioxane, 0 °C – rt, 86%; (i) H_2SO_4/HNO_3, 0 °C, 30 min, 90%; (j) BBr_3, CH_2Cl_2, 0 °C – rt, 18 h, 85%; (k) K_2CO_3, MeI, acetone, reflux, 1 h, 90%; (l) $Cl_2/CHCl_3$, reflux 30 min, 96%; (m) MeI, NaH, DMF, 0 °C –rt, 90 min, 50%; (n) AcCl, Et_3N, CH_2Cl_2, 0 °C –rt, 2 h, 76%; (o) NCS, DMF, 1 h, 80 °C, 31%; (p) NaOH, H_2O, EtOH, reflux, 3 h, 95%.



Scheme 2. Reagents and conditions: (a) $EtOC(CH_2)SnBu_3$, $Pd(Ph_3)_2Cl_2$, dioxane, 90 °C, 2 h, 91%; (b) NBS, THF, H_2O, rt, 1 h, 90–95%; (c) NaN_3, acetone, H_2O, 50 °C, 1 h, 90%; (d) NaHMDS, Ac_2O, −60 °C –rt, 2 h, 72%; (e) $P(OEt)_3$, dioxane, rt, 30 min, warm to 90 °C, 18 h, 84%; (f) H_2 (45 psi), Pd/C, MeOH, rt, 2 h, 80%; (g) 4-nitrobenzaldehyde, MeOH, reflux, 96%; (h) $AcNHC(CH_2)CO_2H$, pyridine, reflux, 46%.

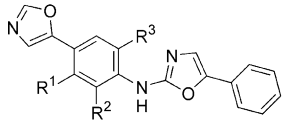


Scheme 3. Reagents and conditions: (a) 1,2,3-triazole, K_2CO_3, sulfolane 140 °C, 16 h, 90%; (b) H_2 (1 atm), Pd/C, MeOH, rt, 1–2 h, 90–95%; (c) $EtOC(CH_2)SnBu_3$, $Pd(Ph_3)_2Cl_2$, dioxane, 90 °C, 2 h, 89–95%; (d) NBS, THF, H_2O, rt, 1 h, 90–95%; (e) $HC(O)NH_2$, 130 °C, 2–18 h, 17–31%; (f) 1,3,4-triazole, KOH, DMSO, 110 °C, 24 h, 19%.

and reduction to provide **26a–d**. The triazole **28** was prepared by reaction of **27** with triazole, followed by reduction.

The structure-activity relationship (SAR) studies discussed in this paper involve the evaluation of various replacements for the conserved methoxy and oxazole-5-yl residues, a key structural feature for the various classes of inhibitors given in Table 1.[18] We report the SAR in several chemical series including **30** which represents a more soluble version of **1e**.

Table 2. Phenyl substitution analogues of **1e**

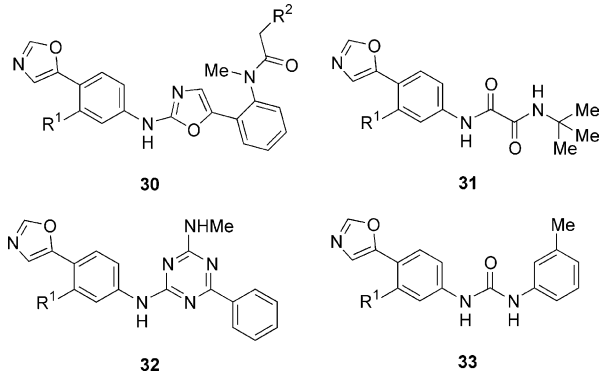


29

Compd	R^1	R^2	R^3	IMPDH II IC_{50} (μM)	CEM IC_{50} (μM)
29a	H	H	H	>5.0	—
29b[20]	OH	H	H	>1.6	—
29b[20]	OEt	H	H	>10	—
29d	Cl	H	H	0.088	>10
29e	OMe	H	F	>5.0	—
29f	OMe	Cl	H	>5.0	—
29g	OMe	H	Cl	>1.6	—

Examination of Table 2 demonstrates that substitution of the methyoxy group in **1e**, by hydrogen (**29a**), hydroxy (**29b**), or ethoxy (**29c**), resulted in a decrease in potency.[19] Only the chlorine analogue (**29d**) maintained similar activity to **1e**. These results suggested that a suitable replacement for the methoxy residue might be halo-

Table 3. Substitution about the phenyl moiety in several chemical series

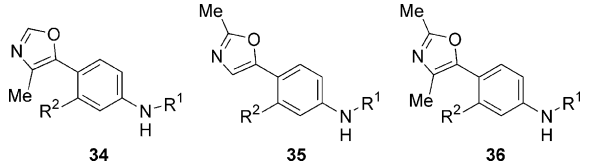


30 **31**

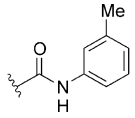
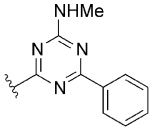
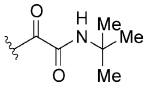
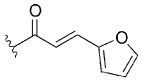
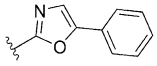
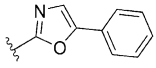
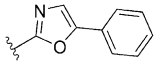
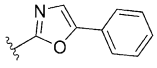
32 **33**

Compd	R^1	R^2	IMPDH II IC_{50} (μM)	CEM IC_{50} (μM)
30a	OMe	Morpholino	0.016	0.52
30b	OMe	OH	0.021	1.4
30c	Me	Morpholino	0.045	3.0
30d	Me	OH	0.10	4.5
30e	Cl	Morpholino	0.047	4.3
30f	Cl	OH	0.041	3.6
30g	Br	Morpholino	0.028	3.2
30h	Br	OH	0.021	2.4
30i	Et	Morpholino	0.31	>10
30j	Et	OH	0.10	4.5
31a	Cl	NA	0.055	>10
31b	Br	NA	0.050	5.5
31c	H	NA	>5.0	—
32a	Cl	NA	0.340	—
32b	H	NA	>5.0	—
33a	Cl	NA	0.043	>10
33b	H	NA	0.64	>10

Table 4. Methyl probes on the oxzaole ring



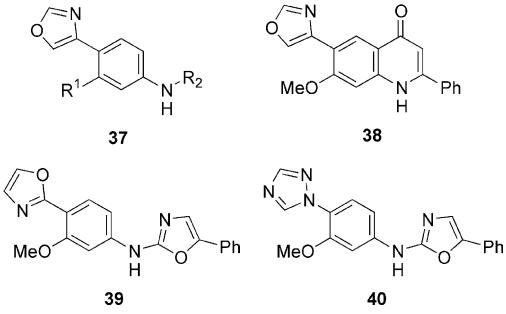
34 **35** **36**

Compd	R^1	R^2	IMPDH II IC_{50} (μM)
34a		H	0.50
34b		H	>5.0
34c		H	>5.0
34d		H	>2.0
34e		H	>5.0
34f		OMe	>5.0
35		OMe	>2.0
36		H	>2.0

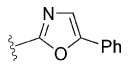
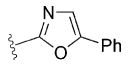
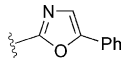
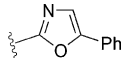
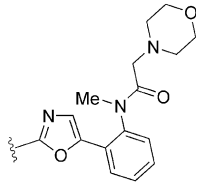
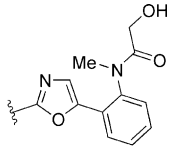
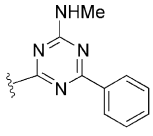
gen. Interestingly, addition of chlorine (**29f**, **29g**) or fluorine (**29e**) to the phenyl group which contained the optimal methoxy residue also decreased activity. Quantum mechanical calculations suggest that halogen substitution at R3 significantly perturbs the planarity of the *N*-phenyl-2amino-oxazole system presumably resulting in decrease binding affinity to this sterically narrow cavity.

The anti-arthritic potential of **30a** has been established in an adjuvant induced arthritis model.[3] This chemical series was central in our MOA SAR studies. Examination of Table 3 confirms the importance of the methoxy group since its replacement by hydrogen in analogues **31c**, **32b**, **33b** resulted in a significant (at least 10-fold) loss in potency in these chemical series. The suggestion that halogens could effectively replace the methoxy group was confirmed by chlorine analogues **30e**,**f**, **31a**, **32a**, and **33a** and bromine analogues **30g**,**h**, and **31b**. Likewise, methyl, **30c**,**d**, was also an effective replacement for methoxy, whereas the corresponding ethyl analogues, **30i**,**j** were less potent. In general, changes in R^2 functionality (morpholino, or hydroxy) did not significantly alter the potency.

Table 5. Oxazole connectivity



37 **38** **39** **40**

Compd	R^1	R^2	IMPDH II IC_{50} (μM)	CEM IC_{50} (μM)
37a	H		>5.0	—
37b	OMe		0.057	>10
37c	Me		>5.0	—
37d	Cl		>5.0	—
37e	OMe		0.091	9.9
37f	OMe		0.041	3.7
37g	OMe		1.6	—
38	NA	NA	0.21	3.5
39	NA	NA	0.19	—
40	NA	NA	0.41	8.3

The methoxy residue appears to have two major functions, the most important of which is to fill the small hydrophobic pocket formed by the side chains of Asn 303 and Arg 322, as described above. Quantum mechanical calculations also indicate that the methoxy residue, presumably through electrostatic repulsive interactions, renders the conformer most suitable for forming a hydrogen bond with Thr 333, of lowest energy.

Table 4 illustrates the SAR associated with a methyl probe placed on the oxazol-5-yl ring system. Due to the significant effects on the rotomeric conformations for the oxazole phenyl moiety, studies were conducted with

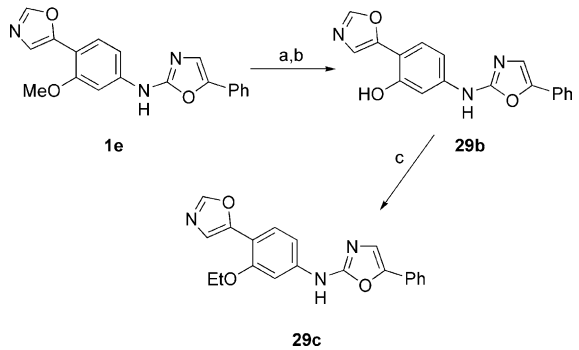
and without the appending methoxy moiety. Thus, the methoxy group on the phenyl ring was omitted in the 4-methyl oxazole containing analogues **34a**–**e**, and the 2,4-dimethyl analogue **36**. The methoxy group was retained in the 2-methyl analogue **35**. With the exception of **34a**, all of these compounds were significantly less potent than the corresponding analogues fashioned with MOA.

The oxazole-4-yl analogues of **1e** were generally slightly less potent than their corresponding oxazole-5yl analogues. For example, compare **1e** to **37b**, **30a** to **37e**, and **30b** to **37f** (Table 5). The difference in potency was more pronounced in other inhibitor series, as oxazol-4-yl analogues **37g**, and **38** were greater than 20-fold less potent than the corresponding analogues **1f** and **2**, respectively. It is interesting to note that the chloro analogue **37d** was significantly less potent when compared to the oxazole-5-yl analogue **29d**, which is in contrast to the results previously discussed for the oxazol-5-yl ring system. The triazole analogue **40** may enter into a similar hydrogen bond array upon binding as the oxazole-5-yl analogues however this analogue displays only moderate inhibitory potency. The level of binding affinity of **39** was 10-fold less than **1e**. Our binding hypothesis predicts that the oxazol-2-yl ring system would not be able to effectively hydrogen bond with Gly 326, in contrast to the oxazole-4-yl and oxazole-5-yl ring systems.

From an examination of the reported crystallographic data for MPA and VX-497 bound to IMPDH, it is clear that key hydrophobic and hydrogen bonding interactions involving the benzofuranone and oxazole phenyl moieties are critical for the low nM binding affinities observed for these analogues. Our studies have defined the sensitivity to chemical modification about the oxazole phenyl moiety and have established that formation of a hydrogen bond between the inhibitor, Gly 326 and Thr 333 as important for achieving high potency. Additional studies of structurally novel inhibitors will further our understanding of the requirements for achieving maximal binding affinity for MOA replacements. These studies will be the subject of future reports.

References and Notes

1. Sievers, T. M.; Rossi, S. J.; Ghobrial, R. M.; Arriola, E.; Nishimura, P.; Kawano, M.; Holt, C. D. *Pharmacotherapy* **1997**, *17*, 1178.
2. Jain, J.; Almquist, S. J.; Shlyakhter, D.; Harding, M. W. *J. Pharm. Sci.* **2001**, *90*, 625.
3. Dhar, T. G. M.; Shen, Z.; Guo, J.; Liu, C.; Watterson, S. H.; Gu, H. H.; Pitts, W. J.; Fleener, C. A.; Rouleau, K. A.; Sherbina, N. Z.; McIntyre, K. W.; Witmer, M. R.; Tredup, J. A.; Chen, B.-C.; Zhao, R.; Bednarz, M. S.; Cheney, D. L.; MacMaster, J. F.; Miller, L. M.; Berry, K. K.; Harper, T. W.; Barrish, J. C.; Hollenbaugh, D. L.; Iwanowicz, E. J. *J. Med. Chem.* **2002**, *45*, 2127.
4. Iwanowicz, E. J.; Watterson, S. H.; Lui, C.; Gu, H. H.; Mitt, T.; Leftheris, K.; Barrish, J. C.; Fleener, C. A.; Rouleau, K.; Sherbina, N. Z.; Hollenbaugh, D. *Bioorg. Med. Lett.* **2002**, *12*, 2931.
5. Watterson, S.; Liu, C.; Dhar, T. G. M.; Gu, H. H.; Pitts, W. J.; Barrish, J. C.; Fleener, C. F.; Rouleau, K.; Sherbina, N. Z.; Hollenbaugh, D. H.; Iwanowicz, E. J. *Bioorg. Med. Lett.* **2002**, *12*, 2879.
6. Gu, H. H.; Iwanowicz, E. J.; Guo, J.; Watterson, S. H.; Shen, Z.; Pitts, W. J.; Dhar, T. G. M.; Fleener, C. F.; Rouleau, K.; Sherbina, N. Z.; Witmer, M.; Tredrup, J.; Hollenbaugh, D. H. *Bioorg. Med. Lett.* **2002**, *9*, 1323.
7. Pitts, W. J.; Guo, J.; Dhar, T. G. M.; Shen, Z.; Gu, H. H.; Watterson, S. H.; Bednarz, M. S.; Chen, B.-C.; Barrish, J. C.; Bassolino, D.; Cheney, D.; Fleener, C. A.; Rouleau, K. A.; Hollenbaugh, D. L.; Iwanowicz, E. J. *Bioorg. Med. Lett.* **2002**, *12*, 2137.
8. Dhar, T. G. M.; Watterson, S. H.; Liu, C.; Barrish, J. C.; Fleener, C. A.; Rouleau, K.; Sherbina, N. Z.; Hollenbaugh, D.; Iwanowicz, E. J. *Bioorg. Med. Lett.* **2002**, *12*, 3153.
9. Dhar, T. G. M.; Watterson, S. H.; Liu, C.; Barrish, J. C.; Fleener, C. A.; Rouleau, K.; Sherbina, N. Z.; Hollenbaugh, D.; Iwanowicz, E. J. *Bioorg. Med. Lett.* **2002**, *12*, 3125.
10. Dhar, T. G. M.; Shen, Z.; Fleener, C. A.; Rouleau, K.; Barrish, J. C.; Hollenbaugh, D. L.; Iwanowicz, E. J. *Bioorg. Med. Lett.* **2002**, *12*, 3305.
11. Watterson, S. H.; Carlsen, M.; Dhar, T. G. M.; Shen, Z.; Pitts, W. J.; Guo, J.; Gu, H. H.; Norris, D.; Chorba, J.; Chen, P.; Cheney, D.; Fleener, C. A.; Rouleau, K.; Hollenbaugh, D. H.; Iwanowicz, E. J., *Bioorg. Med. Lett.* **2003**, *13*, 543.
12. Dhar, T. G. M.; Watterson, S. H.; Chen, P.; Shen, Z.; Gu, H. H.; Norris, D.; Carlsen, M.; Haslow, K. D.; Pitts, W. J.; Guo, J.; Chorba, J.; Fleener, C. A.; Rouleau, K. A.; Townsend, R. Hollenbaugh, D.; Iwanowicz, E. J. *Bioorg. Med. Lett.* **2003**, *13*, 547.
13. Chen, P.; Norris, D.; Halsow, K. D.; Dhar, T. G. M.; Pitts, W. J.; Watterson, S. H.; Cheney, D. L.; Fleener, C. A.; Rouleau, K. A.; Hollenbaugh, D. L.; Barrish, J. C.; Iwanowicz, E. J. **2003**, *13*, 1345.
14. Bjarnason, I. *Transplant. Proc.* **2001**, *33*, 3238.
15. Salvadori, M. *Transplant. Proc.* **2001**, *33*, 3245.
16. Granger, D. K. *Transplant. Proc.* **2001**, *33*, 3241.
17. 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.
18. Sintchak, M. D.; Nimmesgern, E. *Immunopharmacology* **2000**, *47*, 163.
19. The IMPDH II inhibition assay and the CEM proliferation protocol are outlined in ref 2.
20. Conversion of the methyoxy group to an ethoxy group was accomplished at a late stage for **26c** as depicted below:



Reagents and conditions: (a) $(Boc)_2O$, DMAP, Et_3N, DMSO 95%; (b) BBr_3, CH_2Cl_2, 93%; (c) EtI, K_2CO_3, DMF, reflux, 2 h, 20%.