Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3125–3128

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Survey of Cyclic Replacements for the Central Diamide Moiety of Inhibitors of Inosine Monophosphate Dehydrogenase

T. G. Murali Dhar,* Chunjian Liu, William J. Pitts, Junquing Guo, Scott H. Watterson, Henry Gu, Catherine A. Fleener, Katherine Rouleau, N. Z. Sherbina, Joel C. Barrish, Diane Hollenbaugh and Edwin J. Iwanowicz

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

Received 30 April 2002; accepted 30 July 2002

Abstract—A series of heterocyclic replacements for the central diamide moiety of **1**, a potent small molecule inhibitor of inosine monophosphate dehydrogenase (IMPDH) were explored The synthesis and the structure–activity relationships (SARs), derived from in vitro studies, for these new series of inhibitors is given.

Inosine monophosphate dehydrogenase (IMPDH), an enzyme in the de novo synthesis of guanosine nucleotides, catalyzes the irreversible NAD-dependent oxidation of inosine-5′-monophosphate (IMP) to xanthosine-5′-monophosphate (XMP).[1] Two distinct cDNA's encoding IMPDH have been identified and isolated. These transcripts labeled type I and type II are of identical size (514 amino acids).[2–4] IMPDH II activity is markedly upregulated in actively proliferating cell types including cancers and activated peripheral blood lymphocytes.[5]

CellCept® (mycophenolate mofetil, MMF), a prodrug of mycophenolic acid (MPA), has clinical use due to its inhibition of IMPDH, for the treatment of transplant rejection. Dose-limiting gastrointestinal (GI) toxicity exhibited from administration of either MMF or MPA limits this drugs potential for treatment of other autoimmune disorders, such as psoriasis and rheumatoid arthritis.[6] The formation of conjugates at either the carboxylic acid or phenolic residues has been postulated as a contributing factor in the poor therapeutic index observed for MMF.[7,8] Structurally related analogues, devoid of acid and phenolic functionalities, are reputed to have an improved therapeutic window with regard to dose limiting GI toxicity.[9,10]

Recently we have disclosed a new class of inhibitors of IMPDH, exemplified by diamide **1** (Fig. 1).[11] This report describes a series of structural modifications aimed at replacing the diamide linkage. An alternative approach to IMPDH inhibition has been described by Pankiewicz.[12]

Ligand-based modeling suggested that the diamide moiety might be replaced by either five– and six–membered ring systems. This strategy would maintain a hydrogen bond between the NH of the inhibitor and the carboxylate of Asp 274 in the IMPDH enzyme active site. This hydrogen

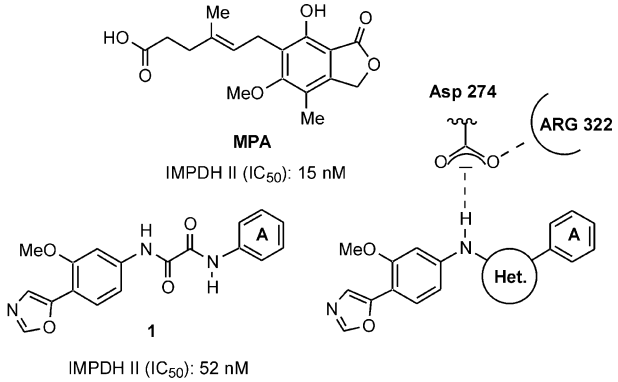


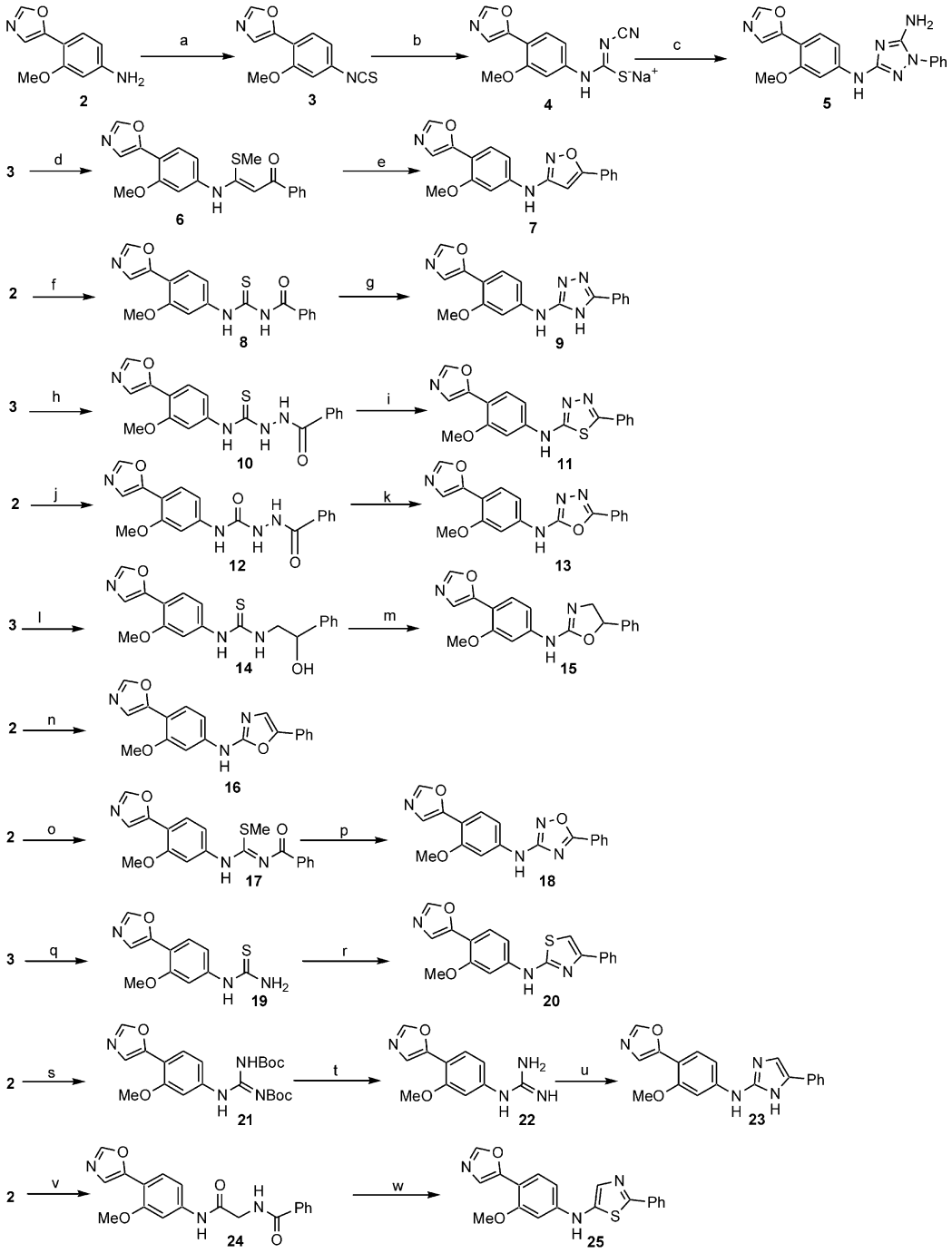
Figure 1. The chemical structures of MPA and diamide **1**. In addition, the schematic showing the key hydrogen bond between the NH and the carboxylate of Asp 274 and the site of heterocyclic insertion (Het.).

*Corresponding author. Tel.: +1-609-252-4158; fax: +1-609-252-6804; e-mail: dharm@bms.com

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00641-8

bond appears to be a critical interaction supported by protein/inhibitor crystallographic studies and SAR (Fig. 1).[13] Additionally, replacement of the diamide with five- and six- membered ring systems, permit the terminal phenyl group (A) to project into a similar region of space.

The synthesis of the various heterocycles is described in Schemes 1 and 2. 5-(4-amino-2-methoxyphenyl)-oxazole **2**[14] is used as a common intermediate in the synthesis of these heterocycles. The 2-(*N*-aryl)-1,3-oxazole **16** was synthesized employing the tandem iminophosphorane/heterocumulene mediated annulation.[15] The 1,1′-



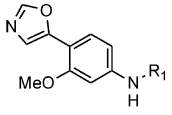
Scheme 1. Reagents and conditions: (a) 1,1′thiocarbonyldi-2(1H)-pyridone, CH_2Cl_2, rt (94%); (b) NaNHCN, EtOH, rt (67%); (c) phenylhydrazine, EDC, DMF, 50 °C (36%); (d) PhCOMe, NaH, DMF, MeI, rt (70%); (e) NH_2OH, EtOH, reflux (15%); (f) PhC(O)NCS, CH_2Cl, rt (94%); (g) hydrazine, THF/EtOH, reflux, (32%); (h) $PhC(O)NHNH_2$, EtOH, (94%); (i) CH_3SO_3H, toluene, reflux, (14%); (j) CDI, THF, $PhC(O)NHNH_2$, DMAP, EtOH, (27%); (k) $C_2Cl_4Br_2$, PPh_3 CH_3CN, Et_3N, rt (64%); (l) $NH_2CH_2CH(OH)Ph$, dioxane, reflux (75%); (m) 2-chloro-3-ethylbenzoxazolium tetrafluoroborate CH_3CN, Et_3N, rt, (70%); (n) $PhC(O)CH_2N_3$, PPh_3, CH_2Cl_2, rt, (80%); (o) PhCONCS, CH_2Cl_2, rt then 0.1 NaOH, MeI (98%); (p) NH_2OH, EtOH, reflux 98%); (q) NH_3, dioxane, rt, (90%); (r) $PhC(O)CH_2Br$, EtOH, reflux, (50%); (s) BocNHC(NBoc)SMe, DMF, rt (100%); (t) TFA, rt, then, AG1-X8 hydroxide form resin (81%); (u) $PhC(O)CH_2Br$, DMF (20%); (v) $PhC(O)NHCH_2CO_2H$, CDI, THF, rt (80%); (w) Lawesson's reagent, pyridine, 100 °C (60%).

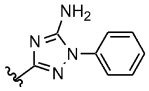
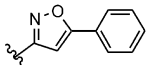
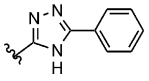
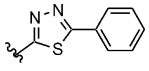
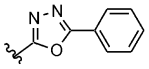
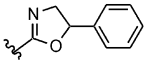
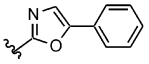
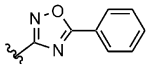
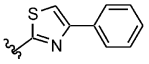
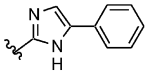
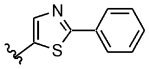
biphenylaniline **26** was synthesized employing the Buchwald protocol.[16] These compounds were tested for the in vitro inhibition of the IMPDH II enzyme[17] and the data is summarized in Tables 1 and 2.

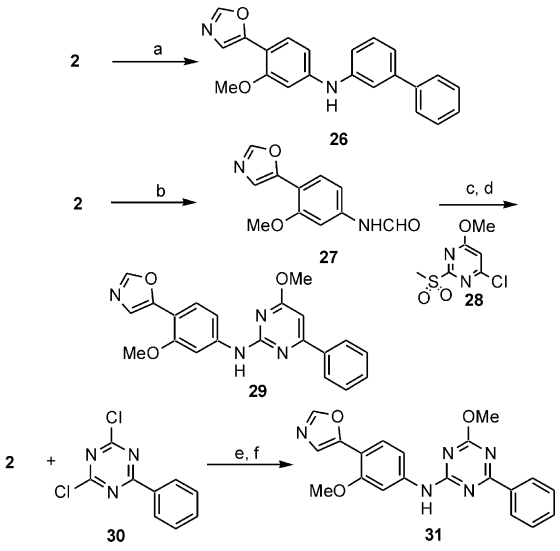
Table 1 reports the in vitro inhibitor potencies against IMPDH II for a series of inhibitors featuring a central five-membered heterocyclic residue. All heterocycles are commonly substituted with a phenyl and aniline moieties. Replacement of the diamide linkage of **1** with triazole **5** looked favorable by molecular modeling (Fig. 2) and was only a 3-fold less potent than **1**. This result suggested an in-depth examination of other heterocyclic cores was warranted. Thiadiazole **11** and related thiazole **20** displayed inhibitory activities of 300 nM or less. The oxazole, isoxazole and oxadiazole analogues were the more potent members of this series. The isoxazole **7**, was the least potent of this group whereas **16**, **13** and **18** displayed similar inhibitory activity relative to **2**. The non-aromatic heterocycle **15**, shows that an aromatic system is not a requirement for potency. The low potency seen with imidazole **23**, suggests that the IMPDH II enzyme does not tolerate basic residues in this region of the inhibitor. This might be explained by charge repulsion, since the negative charge of the carboxylate group of ASP 274 is satisfied by the close proximity of the guanidine group of ARG 322.

The IMPDH activity of 6-membered ring systems is shown in Table 2. The methoxytriazine **31** was of

Table 1. SAR of the five-membered heterocyclic inhibitors of IMPDH

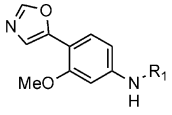


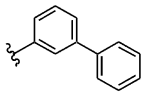
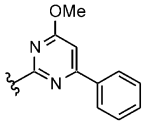
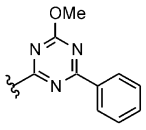
Compd	R_1	IMPDH II IC_{50}, μM
1	NA	0.052
5		0.15
7		0.12
9		0.19
11		0.33
13		0.044
15		0.11
16		0.020
18		0.059
20		0.30
23		7% @ 100
25		0.76



Scheme 2. Reagents and conditions: (a) 3-bromobiphenyl, $Pd_2(dba)_3$, BINAP, NaO*t*-Bu, toluene, 80 °C, (11%); (b) HCO_2H, 150 °C, (96%); (c) NaH, DMF, **28**,[18] then 4N HCl, 70 °C, (75%); (d) $PhB(OH)_2$, DME, K_2CO_3, $Pd(PPh_3)_4$, 90 °C (81%); (e) THF, rt (99%); (f) NaOMe, dioxane, 80 °C (75%).

Table 2. SAR of the six-membered heterocyclic inhibitors of IMPDH



Compd	R_1	IMPDH II IC_{50}, μM
26		30% @ 500
29		0.11
31		0.041

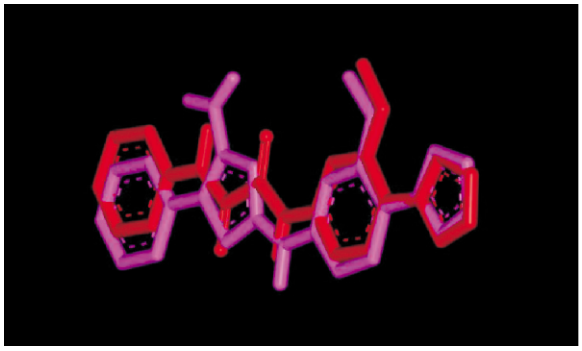


Figure 2. Overlap of minimized structures: diamide **1** (red) and triazole **5** (magenta).

comparable potency to the more potent analogues in the five-membered ring series. In contrast, replacement of the six-membered heterocyclic core with a phenyl residue, **26**, led to a significant loss in affinity. Biphenyl **26** can not assume the same dihedral angle of **29** and **31** because of unfavorable steric interactions. This may be responsible for the loss of activity observed with **26**, although a direct comparison is not possible due to the lack of a methoxy group in the appropriate position.

In summary a number of ring systems were examined as replacements for the diamide linkage in compound **1**. Several heteroaromatic systems (**13**, **16**, **18** and **31**) inhibited IMPDH as potently as diamide **1**. Compound **16** is similar in potency to MPA, and this series is the subject of further effort to enhance potency and in vivo efficacy in a T-cell mediated pharmacodynamic model.

References and Notes

1. Jackson, R. C.; Weber, G. *Nature* **1975**, *256*, 331.
2. Collart, F. R.; Huberman, E. *J. Biol. Chem.* **1988**, *263*, 15769.
3. Natsumeda, Y.; Ohno, S.; Kawasaki, H.; Konno, Y.; Weber, G.; Suzuki, K. *J. Biol. Chem.* **1990**, *265*, 5292.
4. Collart, F. R.; Huberman, E. US Patent 5,665,583, 1990. *Chem. Abstr.* **1990**, *113*, 186080.
5. Jayaram, H. N.; Grusch, M.; Cooney, D. A.; Krupitza, G. *Curr. Med. Chem.* **1999**, *6*, 561.
6. Sievers, T. M.; Rossi, S. J.; Ghobrial, R. M.; Arriola, E.; Nishimura, P.; Kawano, M. *Holt Pharmacotherapy* **1997**, *17*, 1178.
7. Papageorgiou, C. *Mini-Rev. Med. Chem.* **2001**, *1*, 71.
8. Shipkova, M.; Wieland, E.; Schutz, E.; Wiese, C.; Niedmann, P. D.; Oellerich, M.; Armstrong, V. W. *Transplant. Proc.* **2001**, *33*, 1080.
9. 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.
10. Jain, J.; Almquist, S. J.; Shlyakhter, D.; Harding, M. W. *J. Pharm. Sci.* **2001**, *90*, 625.
11. Gu, H. H.; Iwanowicz, E. J.; Guo, J.; Watterson, S. H.; Shen, Z.; Pitts, W. J.; Dhar, T. G. M.; Fleener, C. A.; Rouleau, K.; Sherbina, N. Z.; Witmer, M.; Tredup, J.; Hollenbaugh, D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1323.
12. Pankiewicz, K. W.; Lesiak-Watanabe, K. B.; Watanbe, 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.
13. Sintchak, M. D.; Nimmesgern, E. *Immunopharm.* **2000**, *47*, 163.
14. Armistead, D. M.; Badia, M. C.; Bemis, G. W.; Bethiel, R. S.; Frank, C. A.; Novak, P. M.; Ronkin, S. M.; Saunders, J. O. PCT Int. Appl. WO 9740028 1997. *Chem. Abstr.* **1997**, *128*, 717901.
15. Froyen, P. *Phosphorous, Sulfur Silcon* **1991**, *60*, 81.
16. Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 7215.
17. The enzymatic activity of human IMPDH II was quantitated as follows. The conversion of NAD^+ to NADH was followed spectrophotometrically at 340 nm. A reaction mixture containing 0.1 M Tris, 0.1 M KCl, 3 mM EDTA pH 8.0, 400 μM IMP, 2 mM DTT and 40 nM IMPDH II was added to the wells of flat bottom UV-transparent 96-well plates (Costar 3635). To test inhibitors, compounds resuspended in DMSO were diluted in the reaction to give a final DMSO concentration of 2.5%. IMPDH II used in these assays was purified from *Escherichia coli* expressing the gene for the human Type II enzyme. The reaction was initiated by addition of NAD to a final concentration of 400 μM. After a 2-h incubation at 25 °C, readings were taken at 340 nM. The concentrations of compound required to inhibit NADH accumulation by 50% (IC_{50}) were calculated using a four-parameter logistic plot.
18. Kanno, H.; Kubota, Y.; Sato, T.; Arahira, M. *EP 694538* **1996**; *Chem. Abstr.* **1996**, *124*, 289562.