Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2535–2538
Bioorganic & Medicinal Chemistry Letters

Low molecular weight indole fragments as IMPDH inhibitors

Rebekah E. Beevers, George M. Buckley, Natasha Davies, Joanne L. Fraser, Francis C. Galvin, Duncan R. Hannah,* Alan F. Haughan, Kerry Jenkins, Stephen R. Mack, William R. Pitt, Andrew J. Ratcliffe, Marianna D. Richard, Verity Sabin, Andrew Sharpe and Sophie C. Williams

UCB Celltech, Granta Park, Great Abington, Cambridge CB1 6GS, UK

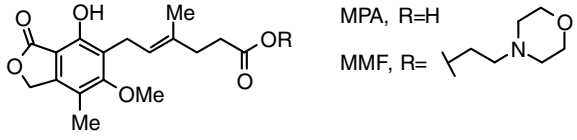
Received 21 November 2005; revised 19 January 2006; accepted 19 January 2006
Available online 17 February 2006

Abstract—The study of non-oxazole containing indole fragments as inhibitors of inosine monophosphate dehydrogenase (IMPDH) is described. The synthesis and in vitro inhibitory values for IMPDH II are discussed.

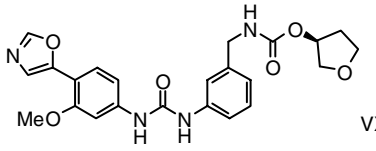
Proliferation of T and B lymphocytes is dependent on access to a large cellular pool of guanine nucleotides. Within the de novo purine biosynthetic pathway a key rate-limiting step is oxidation of inosine-5′-monophosphate to xanthosine-5′-monophosphate by the NAD-dependent enzyme inosine monophosphate dehydrogenase (IMPDH).[1] Two isoforms of the enzyme have been identified and designated type I and type II.[2] Of these it is IMPDH type II that is upregulated in actively proliferating cell types.[3,4] As a consequence inhibition of IMPDH II has become an attractive immunology target for the treatment of transplant rejection, psoriasis, systemic lupus erythematosus and rheumatoid arthritis.[5]

Mycophenolic acid (MPA) is a potent uncompetitive reversible inhibitor of both IMPDH I and II, which has been approved for clinical utility in transplant rejection in the form of Cellcept® or mycophenolate mofetil (MMF), an ester prodrug of MPA.[6]

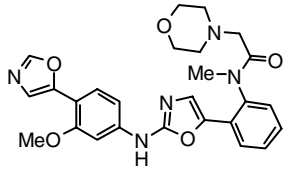
Despite clinical efficacy of Cellcept® its use in other disease end points is compromised by dose-limiting gastrointestinal (GI) side effects and efforts have focused on discovery of new IMPDH inhibitors with an improved therapeutic window.[7] Towards this end Vertex and BMS have reported *N*-[3-methoxy-4-(5-oxazolyl)phenyl-containing compounds, VX497[8] and BMS-337197[9] as potent IMPDH II inhibitors.



From protein/inhibitor crystallographic studies a binding model for VX497 complexed to IMPDH II has been reported.[8] Key interactions include formation of H-bonds between the NH of Gly 326 and the oxazole moiety, and carboxylate of Asp 274 with the urea NH's. In addition, favourable hydrophobic interactions result from binding of the methoxy in a pocket defined by the side chains of Asn 303 and Arg 332, and π-stacking of the phenyl oxazole with the bound nucleotide precursor. Based on this similar binding interactions have been suggested for BMS-337197 and used in the design of new IMPDH II inhibitors.[10]



VX-497

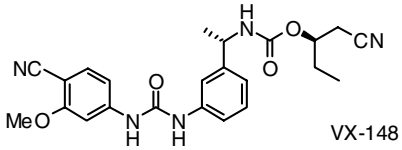


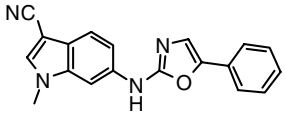
BMS-337197

Keywords: IMPDH; Inosine monophosphate dehydrogenase; Fragment.
* Corresponding author. Fax: +44 1223 896400; e-mail: duncan.hannah@ucb-group.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.089

We have disclosed two new classes of oxazole-containing IMPDH II inhibitors.[11,12] Further detailed investigation of these series revealed the oxazole moiety as a potential source of reactive metabolites. Recently, a number of non-oxazole nitrile containing inhibitors have been reported in VX148[13] and indole **1**.[14] This report describes our preliminary efforts to discover non-oxazole containing IMPDH II inhibitors using a fragment optimisation approach.





In a fragment study, commercially available 3-cyanoindole (**2**) has been reported to inhibit IMPDH II at approximately 30 μM.[15] GOLD[16] docking of **2** using an in-house crystal structure of IMPDH II was undertaken to define potential binding modes. Interestingly, the best fit predicts the indole NH forming a H-bond to Asp 274 with the nitrile potentially forming a hydrogen bond with Gly 326 (Fig. 1).

To further explore this concept with a view to improving the potency of **2** for IMPDH II, a series of indole fragments containing hydrogen bond acceptor groups at the 3-position were synthesised as described in Schemes 1–4 or obtained from commercial sources. A number of fragments substituted on the indole core were also prepared, to establish precise structural requirements and to assess potential sites for further elaboration.

3-Formylindole (**3**) was commercially available. The 3-carbamoyl derivative **4** was prepared directly from **2**, by reaction with hydrogen peroxide and sodium hydroxide in methanol. A series of heteroaromatic derivatives were also prepared. The 4-pyridyl indole **5** was synthesised by dehydrogenation of a commercially available precursor (Scheme 1). 1-Phenylsulfonylindol-3-yl boronic acid was reacted with aromatic bromides to prepare,

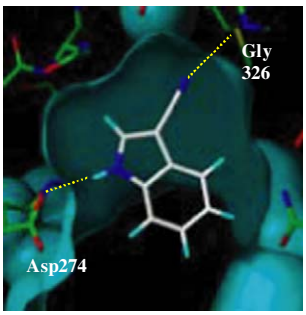
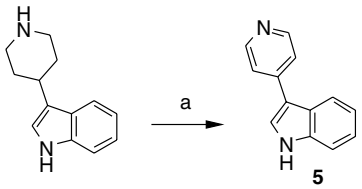
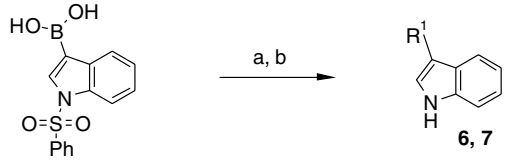


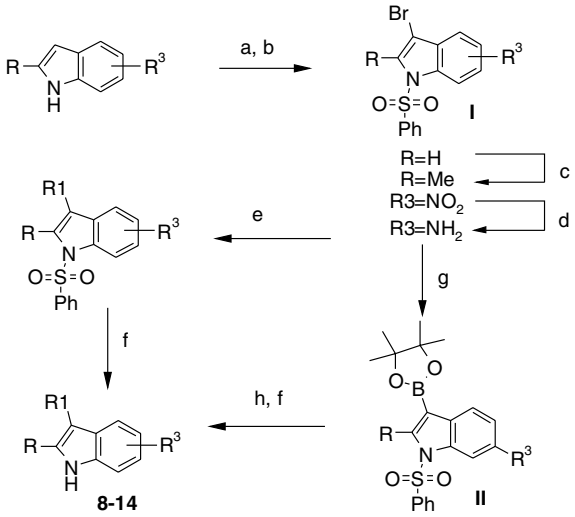
Figure 1. Indole **2** docked into IMPDH II, with potential H-bonds.



Scheme 1. Reagents and condition: (a) 1,1-Diphenylethylene, Pd/C, 250 °C (30%).

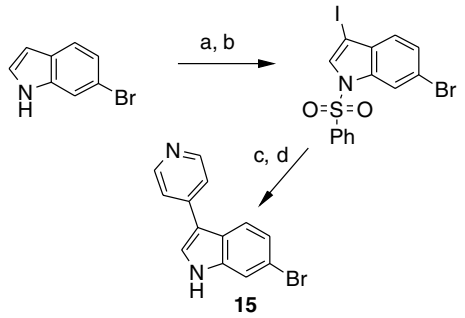


Scheme 2. Reagents and condition: (a) R^1-Br, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, microwave, 120–150 °C (74–81%); (b) NaOH, dioxane, reflux; or KOH, MeOH, reflux (86%).



Scheme 3. Reagents and conditions: (a) *N*-Bromosuccinimide, DCM, rt (77–93%); (b) $PhSO_2Cl$, TBAB, NaOH, toluene, H_2O; or NaH, THF; then $PhSO_2Cl$ (84–100%); (c) LDA, THF, −40 °C; then MeI, −40 °C to −20 °C; (d) $SnCl_2$, EtOH, H_2O, reflux (37–83%); (e) R^1-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, microwave, 120–150 °C (75–90%); (f) NaOH, dioxane, reflux; or, KOH, MeOH, reflux (12–86%); (g) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DME, 80 °C (or THF, microwave, 150 °C) (46%); (h) R^1-Br or R^1-Cl, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, microwave, 120–150 °C (51–90%).

after basic hydrolysis of the indole protecting group, indoles **6** and **7** (Scheme 2). Using similar chemistry, a set of related derivatives, additionally substituted on the indole core, were prepared starting from 6- and 7-nitroindole and 2-methylindole, (Scheme 3). The key step employed either Suzuki reaction of bromoindoles **I** with heteroaromatic boronic acids in the synthesis of **8**, **10**, **12** and **14**, or Suzuki reaction of the pinacolboronate ester **II** with heteroaromatic bromides (or chloride towards **11**) in the synthesis of **9**, **11** and **13**. The 2-methyl group of **14** was successfully introduced by deprotonation of the bromo nitroindole **I** followed by methyl iodide



Scheme 4. Reagents and conditions: (a) I_2, KOH, DMF, rt (quant.); (b) NaH, THF; then $PhSO_2Cl$ (85%); (c) pyridin-4-ylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, DME, H_2O, 80 °C (87%); (d) KOH, MeOH, reflux (74%).

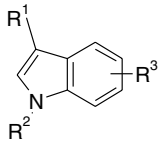
quench. In a variant of the above, 6-bromoindole was iodinated at the 3-position, allowing for the chemoselective Suzuki reaction towards pyridylindole **15** (Scheme 4). Indoles **2**, **7** and **15** were N-methylated by treatment with potassium hydroxide and methyl iodide in acetone at room temperature to provide indoles **16**, **19** and **20**, respectively. 3-Formyl-1-methylindole (**17**) was commercially available, and amide **18** was prepared from the nitrile **16** as in the synthesis of **4** above.

The in vitro potencies are shown in Table 1. The first observation is the sensitivity to variations of the cyano group for other small H-bond accepting groups, the aldehyde **3** conferring similar potency, while the primary amide **4** was inactive. A number of heteroaromatic replacements of the nitrile also showed activity, such as pyridine **5** and the five-membered furan **7**, thiophene **8** and thiazole **9**. With an IC_{50} of 1.15 μM, pyridine **5** demonstrated a significant improvement over **2**. The affinity was very sensitive to the position of heteroatom in the pendant heterocycles, for example, the pyrid-3-ylindole **6** was inactive, illustrating the importance of this interaction with the enzyme. In the case of the 3-(pyrid-4-yl)indoles, simple substitution was tolerated at the indole 6- and 7-position (see **10**, **12** and **15**), with a slight increase in affinity. Methylation at the 2-position in **13** also gave an improvement in activity (IC_{50} = 343 nM), however, these benefits were not additive (compare **14** with **10** and **13**). Substitution on the pyridine, however, did not appear to be tolerated (compare **11** and **10**).

Indole nitrogen methylation had a dramatic effect on the observed affinities of the fragments. Potency of the cyano and formylindoles was increased 3-fold on N-methylation (compare **16** and **17** with **2** and **3**, respectively). With five-membered heterocycles, illustrated with the furan **19**, potency was largely unaffected, but in the case of 4-pyridylindoles, N-methylation reduced activity at least 100-fold (compare **20** and **15**).

We were interested in establishing whether our small fragments were actually binding at the IMPDH active site, rather than exerting their effects through an allosteric interaction. Classical steady-state kinetic analysis with the most potent fragment, pyridylindole **13**, demonstrated uncompetitive inhibition with respect to NAD and IMP, analogous to the behaviour of MPA and VX497,[8] supporting binding at the active site at

Table 1. SAR of indoles



Compound	R^1	R^2	R^3	IMPDH II IC_{50}[17] (μM)
2	CN	H	H	20.9
3	CHO	H	H	22.7
4	$CONH_2$	H	H	IA[a]
5	Pyrid-4-yl	H	H	1.15
6	Pyrid-3-yl	H	H	IA
7	Furan-3-yl	H	H	5.84
8	Thiophen-3-yl	H	6-NH_2	2.32
9	Thiazol-5-yl	H	6-NH_2	27.9
10	Pyrid-4-yl	H	6-NH_2	0.637
11	2-Me-pyrid-4-yl	H	6-NH_2	IA[a]
12	Pyrid-4-yl	H	7-NH_2	0.524
13	Pyrid-4-yl	H	2-Me	0.343
14	Pyrid-4-yl	H	2-Me–6-NH_2	0.419
15	Pyrid-4-yl	H	6-Br	0.408
16	CN	Me	H	7.66
17	CHO	Me	H	6.84
18	$CONH_2$	Me	H	50% at 50 μM
19	Furan-3-yl	Me	H	6.21
20	Pyrid-4-yl	Me	6-Br	62% at 50 μM
21	—	—	—	8.20

[a] Inactive at 50 μM.

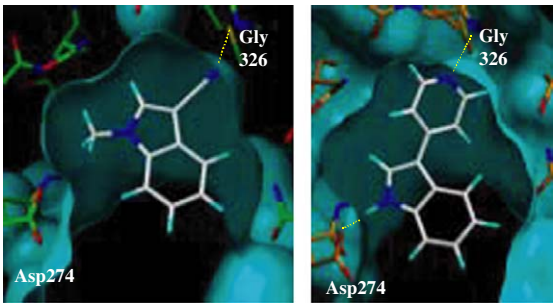


Figure 2. Docking of **16** (left) and **5** (right) in IMPDH II.

the same point in the enzymatic path as these known inhibitors.

Further docking studies were performed to understand the observed SAR, comparing the cyanoindoles **2** and **16** and looking at the pyridylindole **5**. The results from the docking of **2** and **16** suggest two different binding modes: although a H-bond between cyano and the NH of Gly 326 is a common feature, in the case of *N*-methylindole **16** the loss of a H-bond to Asp 274, as suggested for **2**, is compensated by placing the methyl group in the same hydrophobic region as the methoxy group of VX497 (Figs. 1 and 2).

In the case of pyridylindole **5**, H-bonds between both the indole NH to Asp 274 and pyridine N to Gly 326 can be realized and a good contact with the active site achieved, and it might be that this fragment can reach a more favourable geometry than **2**, resulting in the increased potency observed (Fig. 2). Docking also predicts the inactivity of an *N*-methyl-3-pyrid-4-ylindole, which would result in a steric clash in the active site. Docking studies also suggested the methyl group of 2-methylindole **13** may be able to access the hydrophobic region without disrupting the two H-bonds, which could account for the further increase in potency seen. Additionally, this methyl substitution would affect the torsion angle between pyridine and indole.

The most active fragments described here compare favourably with 3-methoxy-4-(oxazol-5-yl)aniline (**21**) from VX497 and BMS-337197 (IC_{50} = 8.2 μM). The implications of the proposed binding modes are that with the NH indoles, such as **2** and **5**, a greater variety of groups could be added to the carbocyclic ring without the need for a H-bond donor to interact with Asp 274, whereas with the *N*-methylindoles, such as **16**, this requirement still remains.

In conclusion, our fragment-based approach has delivered sub-micromolar, low molecular weight indole inhibitors of IMPDH II, with a platform to develop into new classes of IMPDH inhibitors. Our efforts to elaborate these hits further are described in the following paper.

Acknowledgment

The authors thank the Molecular and Cellular Systems department at Granta Park for in vitro measurements.

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. Jayaram, H. N.; Grusch, M.; Cooney, D. A.; Krupitza, G. *Curr. Med. Chem.* **1999**, *6*, 561.
4. Carr, S. F.; Papp, E.; Wu, J. C.; Natsumeda, Y. *J. Biol. Chem.* **1993**, *268*, 27286.
5. Dhar, T. G. M.; Shen, Z.; Gu, H. H.; Chen, P.; Norris, D.; Watterson, S. H.; Ballentine, S. K.; Fleener, C. A.; Rouleau, K. A.; Barrish, J. C.; Townsend, R.; Hollenbaugh, D. L.; Iwanowicz, E. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3557, and references cited therein.
6. Anderson, W. K.; Boehm, T. L.; Makara, G. M.; Swann, R. T. *J. Med. Chem.* **1996**, *39*, 46.
7. Sievers, T. M.; Rossi, S. J.; Ghobrial, R. M.; Arriola, E.; Nishimura, P.; Kawano, M.; Holt, C. D. *Pharmacotherapy* **1997**, *17*, 1178.
8. Sintchak, M. D.; Nimmesgern, E. *Immunopharmacology* **2000**, *47*, 163.
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. Dhar, T. G. M.; Shen, Z.; Fleener, C. A.; Rouleau, K. A.; Barrish, J. C.; Hollenbaugh, D. L.; Iwanowicz, E. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3305.
11. Buckley, G. M.; Davies, N.; Dyke, H. J.; Gilbert, P. J.; Hannah, D. R.; Haughan, A. F.; Hunt, C. A.; Pitt, W. R.; Profit, R. H.; Ray, N. C.; Richard, M. D.; Sharpe, A.; Taylor, A. J.; Whitworth, J. M.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 751.
12. 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.
13. Jain, J.; Almquist, S. J.; Heiser, A. D.; Shlyakhter, D.; Leon, E.; Memmott, C.; Moody, C. S.; Nimmesgern, E.; Decker, C. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 1272.
14. Dhar, T. G. M.; Shen, Z.; Gu, H. H.; Chen, P.; Norris, D.; Watterson, S. H.; Ballentine, S. K.; Fleener, C. A.; Rouleau, K. A.; Barrish, J. C.; Townsend, R.; Hollenbaugh, D. L.; Iwanoxicz, E. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3557.
15. Pickett, S. D.; Sherborne, B. S.; Wilkinson, T.; Bennett, J.; Borkakoti, N.; Broadhurst, M.; Hurst, D.; Kilford, I.; McKinnell, M.; Jones, P. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1691.
16. Jones, G.; Willett, P.; Glen, R. C. *J. Mol. Biol.* **1995**, *245*, 43–53.
17. For IMPDH II inhibition protocol, see Ref. 12 (Ref. 14).