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Product-like inhibitors of inositol monophosphatase

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Abstract—A series of product-like inhibitors of inositol monophosphatase have been prepared and tested for activity in vitro as possible leads for treatment of bipolar disorders. Compounds possessing a 6-alkyloxy side chain were inhibitors but less efficacious than those possessing a 6-aminoalkyl side chain. These new structures show promise as inhibitors possessing the bioavailability and characteristics necessary for drug development.

Inositol monophosphatase (IMPase, EC 3.1.3.25) plays a crucial role in the recycling of metabolites of the important second-messenger molecule inositol-1,4,5-trisphosphate. The enzyme catalyses the cleavage of the phosphate group from various isomers of inositol monophosphate to generate *myo*-inositol. This is used to resynthesise phosphatidylinositol-4,5-bisphosphate, the membrane-bound lipid that is the direct precursor to the second messenger.[1–4] Inhibition of IMPase in vivo squeezes the supply of *myo*-inositol available for recycling, limiting the cellular production of inositol-1,4,5-trisphosphate and hence cell response to external stimuli. It is believed that this is the therapeutic mode of action of lithium ion in the treatment of bipolar disorders.[5,6] Lithium treatment unfortunately leads to many toxic side effects and so alternative inhibitors of IMPase are attractive therapeutic targets.[6]

Work performed both within our laboratories and elsewhere has led to the design and synthesis of numerous IMPase inhibitors, some with impressive potency yet to date, none have the required bioavailability of a drug-candidate.[7,8] Most members of the current range of inhibitors possess a phosphate monoester functionality and it seems that the negative charge on this group renders the molecules too hydrophilic to cross the lipophilic barrier between the blood and the brain where suppression of IMPase activity must occur.[7] It seemed to us, therefore that a new approach to this problem was needed.

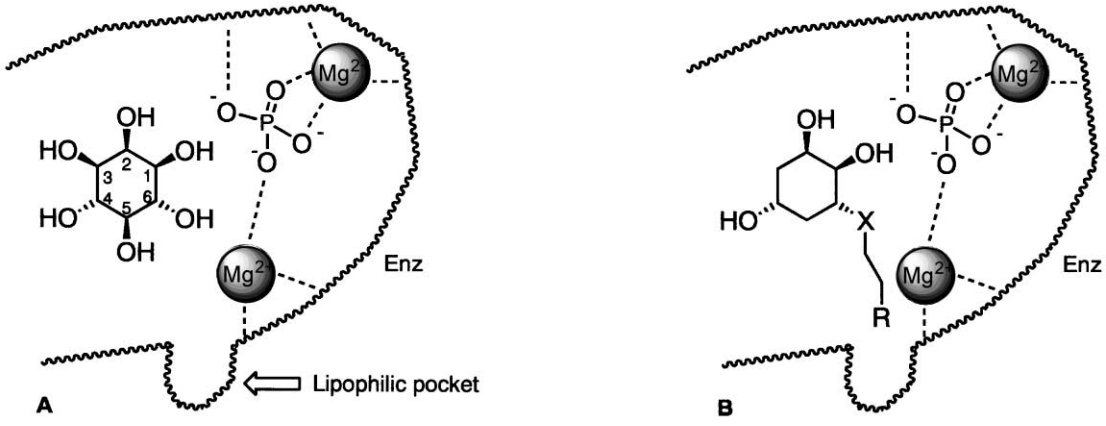

Figure 1. A Representation of the active site of IMPase after cleavage of the inositol–phosphate bond and before release of inositol. **B** Proposed product-like inhibitor bound to the enzyme–phosphate complex. X = O or NH, R = alkyl or aryl.

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Investigation of the kinetics of the reactions catalysed by IMPase has shown that after the enzyme has cleaved D-inositol-1-phosphate, the first molecule to leave the active site is *myo*-inositol followed by inorganic phosphate (Fig. 1) and it has been shown that P_i exhibits product inhibition ($K_i = 0.3$ mM at pH 8.0).[5] It has also been demonstrated that at therapeutic concentrations, lithium ion binds to the enzyme–P_i complex preventing the release of P_i and therefore the binding of another substrate molecule.[5] Moreover, it is estimated that the concentration of P_i in the brain is ~ 3 mM and so at any given time >90% of the enzyme must be P_i bound in vivo.[9] It was therefore reasoned that investigation of molecules that mimic the action of lithium by binding to the enzyme–P_i complex might lead to a new series of IMPase inhibitors. The proposed series of inhibitors were designed as mimics of the product *myo*-inositol (Fig. 1); such inhibitors would lack the usual phosphate group of IMPase inhibitors and so should be more lipophilic, hopefully improving the chances of transport across the blood-brain barrier. *myo*-Inositol itself displays poor product inhibition ($K_i = 400$ mM),[5] and so to improve binding to the enzyme–P_i complex structural features already known to enhance inhibitor binding to IMPase were included in the design.[8] The hydroxyl groups at positions 3 and 5 on the inositol ring would be deleted and on position 6 a lipophilic chain would replace the hydroxyl group of inositol. IMPase possesses a lipophilic pocket bounded by Val40 and Leu42 which this structural feature seeks to exploit.[10]

The synthesis of the first series of inhibitors of this type is illustrated in Figure 2. Epoxide **1** was used to prepare all of the required compounds.[11] Regioselective ring opening of **1** to the 6-alkyloxy derivatives **2a**–**c** was achieved in good to excellent yield by heating the epoxide with excess alcohol in 1,2-dichloroethane in the presence of catalytic ytterbium(III) triflate.[11,12] Similarly, amines **2d**–**h** were prepared in good yields by heating **1** with the parent primary amine and ytterbium-(III) triflate in a 3:1 mixture of toluene and THF. 1,2-Dichloroethane was not a suitable solvent in this instance as it was reactive towards the amines under the reaction conditions required to open the epoxide; it was also found necessary when opening the epoxide with amines to use 1 equivalent of ytterbium(III) triflate. To remove the benzyl protecting groups from compounds **2a**–**c** catalytic hydrogenolysis using hydrogen over 5% palladium on activated carbon was effective, **3a**–**c** being isolated in excellent yields. The benzyl groups proved resistant to hydrogenolysis in the presence of an amine side-chain but after some experimentation it was found that treatment of **2d**–**h** with trimethylsilyl bromide in chloroform at 50°C effected their cleavage in good yield.[13]

The results of initial testing of these compounds as inhibitors of IMPase are shown in Table 1. The assay procedure used was a modification of the procedure of Gee et al. using commercially available IMPase.[14] Assays (50 μl) were initiated by addition of enzyme solution (5 μl, 0.02 units) and contained 100 mM Tris–HCl, 50 mM KCl, 3 mM $MgCl_2$ and (2-^{3}H)-DL-*myo*-inositol-1-phosphate (500 dpm/nmol) plus inhibitor at pH 8.0. Assays were quenched after 20 min by addition of 1 M NaOH (5 μl). The solution was then passed down a small pre-equilibrated column of DOWEX 1X2-400 anion exchange resin (OH form, 200 mg) to remove unreacted substrate and the filtrate plus washings were emulsified with scintillation cocktail and then the radioactivity of each sample was determined by scintillation counting.

6-Propyloxy derivative **3a** was the first compound tested since its benzylated precursor had already been prepared within our laboratories.[11] It proved an ineffective inhibitor of IMPase, significantly retarding the turnover of inositol-1-phosphate only as it approached its saturating concentration in the assay buffer. Nevertheless, inhibition of IMPase with an IC_{50} of approximately 150 mM represented an improvement upon the efficacy of *myo*-inositol as a product inhibitor. The 6-hexyloxy derivative **3b** was a more effective inhibitor of IMPase but also was significantly more lipophilic

Table 1.

Compound	Side chain	IC_{50} (mM)
myo-Inositol	–	400 (K_i)
3a	OC_3H_7	≥150
3b	OC_6H_{13}	≥10
3c	OC_4H_8O-[(2-OH)C_6H_4]	4
3d	NHC_4H_9	0.5
3e	NHC_6H_{13}	0.5
3f	NHC_8H_{17}	4
3g	$NH(CH_2)_2C_6H_5$	6
3h	$NH(CH_2)_4C_6H_5$	10

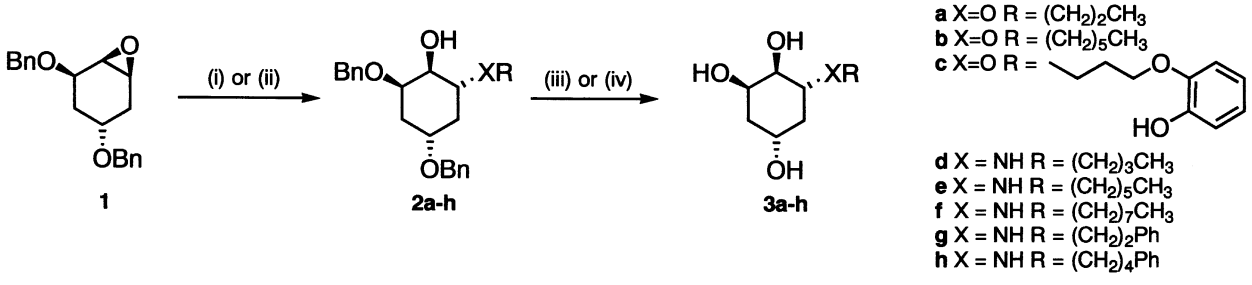

Figure 2. *Reagents and conditions*: (i) ROH, cat. $Yb(OTf)_3$, DCE, reflux, 72–99%; (ii) RNH_2, 3:1 toluene–THF, Tb(OTf), reflux, 76–96%; (iii) H_2, 5% Pd–C, MeOH, 91% quantitative; (iv) Me_3SiBr, $CHCl_3$, 50°C, 54–88%.

than **3a**. It appeared to have an IC_{50} value of around 10 mM but it was not possible to measure this precisely as above this concentration it too was insoluble in the assay buffer. Compound **3c** was expected to show much promise as a product-like inhibitor of IMPase. Phosphorylation of the hydroxyl group at position 1 on the inositol ring gives the most potent inhibitor of IMPase known ($IC_{50}=40$ nM),[7] and it was hoped that this compound would therefore bind effectively to the enzyme even in the absence of the phosphate group. In the event it proved the most effective of the 6-alkyloxy derivatives tested in this work but still gave a disappointing IC_{50} of only 4 mM. Removal of the phosphate group from position 1 of the inositol ring effectively retarded the binding to the enzyme by six orders of magnitude.

Amines **3d**–**h** proved more interesting when tested as inhibitors of IMPase. Of particular note is the fact that the structure–activity relationship with respect to the length of the side chain appears reversed when compared with the 6-alkyloxy derivatives. Long lipophilic side chains possessing aromatic functionality (compounds **3g** and **3h**) were the least effective of the 6-aminoalkyl derivatives and those possessing relatively short simple alkyl chains were the most effective. Indeed, the 6-butylamino derivative **3d** and the 6-hexyl-amino derivative **3e** were effective inhibitors at sub-millimolar concentrations and so represent an improvement of three orders of magnitude upon the product inhibition of *myo*-inositol itself.

The 6-aminoalkyl derivatives described above effectively validate our new approach to developing inhibitors of IMPase. Further analogues of this type are currently being investigated and a more detailed analysis of their mode of action will be published in due course.

Acknowledgements

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