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Review Article

INHIBITORS OF INOSITOL MONOPHOSPHATASE

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Inositol monophosphatase (IMPase) catalyses the hydrolysis of *myo*-inositol monophosphates to *myo*-inositol, which is required in the phosphatidyl inositol cell signalling pathway. Here the enzyme structure, mechanism and inhibition of IMPase are reviewed. Lithium, an effective therapy for manic depression, is an uncompetitive inhibitor. In the search for alternative inhibitors to lithium, substrate-based inhibitors, bisphosphonates, terpenoid and tropolone analogues are described.

Keywords: Inositol monophosphatase; *myo*-Inositol; Lithium; Inhibition; EC 3.1.3.25; Manic depression

INTRODUCTION

myo-Inositol and the Phosphatidyl Inositol Cell Signalling Pathway

The key step in the phosphatidyl inositol cell signalling pathway (Figure 1) is the formation of the second messengers, D-*myo*-inositol 1,4,5-trisphosphate (**2**) and diacylglycerol (**3**), from phosphatidyl inositol-4,5-bisphosphate (**1**).[1,2] The trisphosphate (**2**) is metabolised to *myo*-inositol (**4**) by three pathways as shown in Figure 1. To complete the cycle, *myo*-inositol is converted to (**1**) in three steps. Of most relevance to this review, the hydrolyses of D-*myo*-inositol 1-phosphate (**5**), D-*myo*-inositol 3-phosphate (**6**) and

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Abbreviations: IMPase, inositol monophosphatase; IC_{50}, 50% inhibitory concentration.

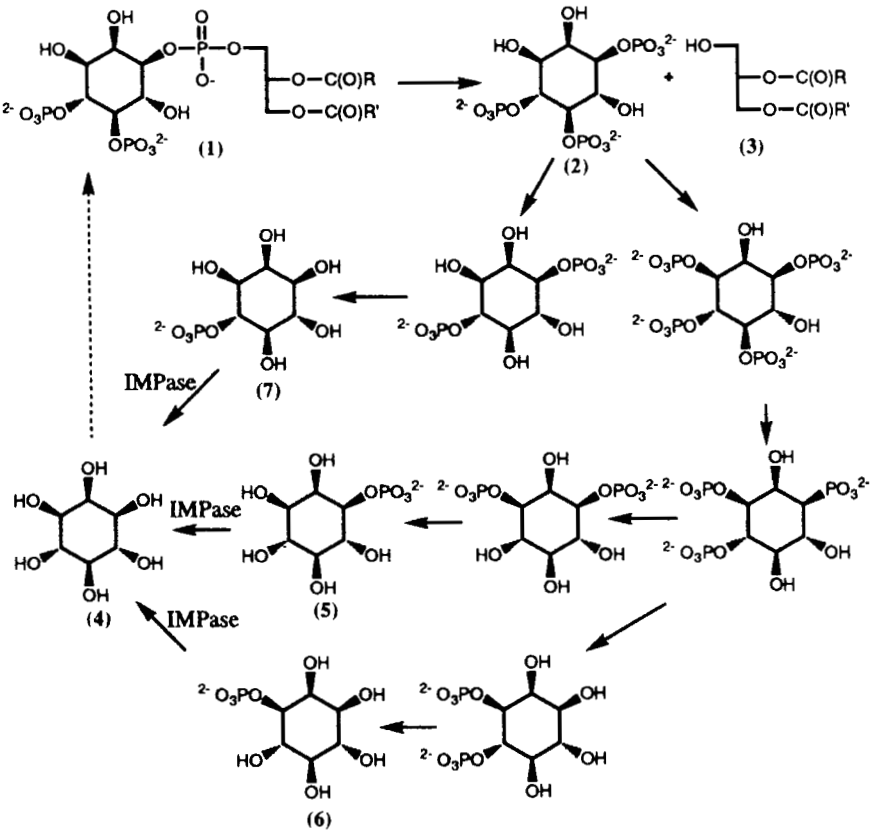


FIGURE 1 The phosphatidyl inositol cell signalling pathway, showing the hydrolysis of *myo*-inositol monophosphates (**5**), (**6**) and (**7**) to *myo*-inositol (**4**), catalysed by inositol monophosphatase (IMPase).

D-*myo*-inositol 4-phosphate (**7**), to *myo*-inositol and inorganic phosphate are all catalysed by *myo*-inositol monophosphatase (IMPase, E.C. 3.1.3.25).[1,2] *De novo* biosynthesis of *myo*-inositol can also occur,[3] the key step catalysed by D-*myo*-inositol 3-phosphate synthase, being the isomerisation of D-glucose 6-phosphate to D-*myo*-inositol 3-phosphate, with subsequent hydrolysis to *myo*-inositol also catalysed by IMPase. *myo*-Inositol is also available from the diet, however this source is not important for the brain, as *myo*-inositol does not cross the blood–brain barrier.[4] IMPase is therefore a crucial enzyme in the brain as it is responsible for maintaining the supply of *myo*-inositol for the phosphatidyl inositol cell signalling pathway.

IMPase

Structure

The structure of IMPase has been reviewed,[5] therefore only a brief outline is given here. IMPase has been cloned, expressed and purified from sources including bovine[6] and human brain.[7] The enzyme is a dimer (30 kD) and comprises of 277 residues per subunit. The amino acid sequences differ in only 32 residues between the bovine and human enzymes and these minor differences are thought not to perturb the substrate-binding conformation or affect the enzyme mechanism.[8] The X-ray crystal structure of human IMPase has been solved to a resolution of 2.1 Å with the lanthanide cation (Gd^{3+}) and sulphate, to 2.2–2.3 Å with Gd^{3+} and D- or L-*myo*-inositol 1-phosphate, and to 2.6 Å with Mn^{2+} and phosphate.[9–11] Each dimer is folded into a five-layered sandwich of three pairs of α-helices and two β-sheets. The active sites are located in large hydrophilic caverns at the base of the two central helices where several segments of secondary structure intersect.[9,10] Metal titration experiments have demonstrated the existence of two kinetically distinct metal-binding environments[12] suggesting that two magnesium ions are involved in the catalytic mechanism. One magnesium cation has octahedral coordination geometry and stabilises the development of negative charge on the nucleophilic water. The other magnesium cation has tetrahedral coordination geometry and stabilises the development of negative charge on the phosphate leaving group.

Mechanism

The mechanism of IMPase has been reviewed,[13] therefore only the major features will be discussed here. Although phosphatases typically proceed via a phospho-enzyme (P-E) intermediate, experiments with IMPase failed to detect one.[14] The X-ray crystallography of IMPase in the presence of D- or L-*myo*-inositol 1-phosphate also argues against the formation of a P-E intermediate, as there is no nucleophilic amino acid side chain close to the phosphoryl group.[11] For IMPase, kinetic studies show that phosphate ester hydrolysis is rate-limiting and that the substrate is attacked directly by water.[15] Two different mechanisms have been suggested depending on the location of water in the active site.[12,16,17] Pollack and coworkers[12] propose that the nucleophile attacks opposite the leaving group in the substrate with inversion of stereochemistry at phosphorus (Figure 2A). From structural and mutagenesis studies they implicate that the water nucleophile is

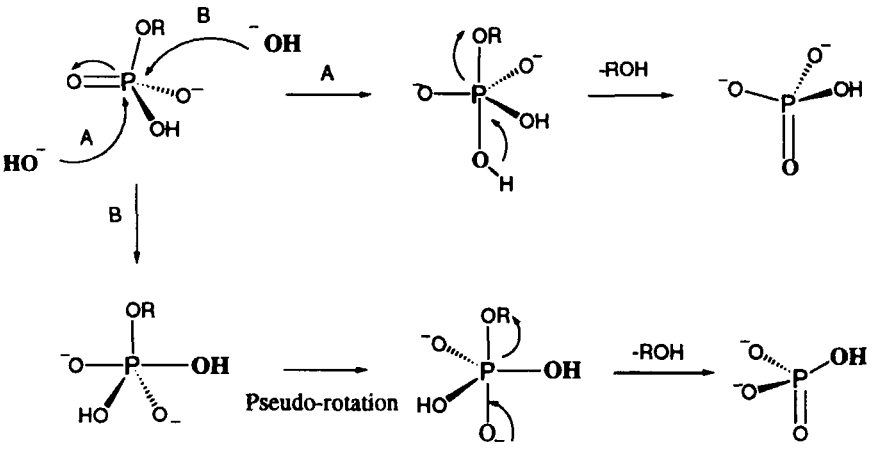


FIGURE 2 Mechanism for the hydrolysis of *myo*-inositol monophosphates catalysed by IMPase: Direct attack by water with inversion (A) or retention (B) of stereochemistry at phosphorus (R = *myo*-inositol).

activated by Glu-70, one Mg^{2+} ion, and possibly Thr-95. In contrast, Gani and coworkers[8,16] proposed a mechanism with retention of stereochemistry at phosphorus where the nucleophile attacks the phosphorus at the same face as the leaving group (Figure 2B). Pseudo-rotation is required to place the leaving group into an apical position. They suggest that Mg^{2+} is hexa-coordinated through the carboxylate groups of Asp-90, Asp-93 and Asp-220, to both terminal and bridging phosphate oxygen-atoms of the substrate and to one water molecule. IMPase shows significant structural similarity to fructose-1,6-bisphosphatase, an enzyme which also utilises two magnesium cations and is sensitive to lithium.[13] Therefore IMPase may proceed by the same mechanism as this enzyme, via a direct in-line displacement (Figure 2A),[18] however further experiments are required.

Substrate Specificity

IMPase shows a broad substrate specificity. This enzyme catalyses the hydrolyses of D-*myo*-inositol 1/3/4/5 or 6-monophosphates, however *myo*-inositol 2-phosphate, bearing an axial phosphate group, is not a substrate.[19] This enzyme can also catalyse the hydrolysis of other phosphate monoesters such as β-glycerophosphate,[15] adenosine-2′-monophosphate[14,20] and *p*-nitrophenyl phosphate (a very poor substrate).[6,15] The smallest molecule found to bind to the active site of IMPase and act as a substrate is ethane-1,2-diol phosphate.[20]

INHIBITORS OF IMPase

Manic depression may be caused by the over-activation of the phosphatidyl inositol cell signalling pathway.[21] Inhibition of IMPase will lower the concentration of *myo*-inositol and therefore should be useful in the treatment of this and related disorders.

Lithium

Lithium (Li^+) is the major drug therapy currently in use for the treatment of manic-depressive illness.[22] However, the mechanism by which Li^+ exerts its effects remains unclear.[23] The effects of Li^+ on the phosphatidyl inositol cell signalling pathway were first noted by Allison and Stewart who observed that following administration of Li^+, there was a decrease in *myo*-inositol levels in rat brain that was accompanied by an increase in *myo*-inositol 1-phosphate.[24] The biochemical basis of these effects remained vague until it was observed that Li^+ inhibits IMPase in an uncompetitive manner at low concentrations (K_i 0.8 mM) and non-competitive at high concentrations.[14,15,25] These data led Berridge and colleagues to propose that Li^+ might be exerting its therapeutic effects in manic depression through attenuation of phosphatidyl inositol cell signalling pathway caused by depletion of *myo*-inositol as a consequence of inhibition of IMPase.[26] The unusual uncompetitive nature of its inhibitory action implies that Li^+ will have little effect when the *myo*-inositol monophosphates are turning over slowly but will become increasingly effective as the level of signal generation increases.[27]

Li^+ is not an ideal therapy because serum levels of 1 mM are required, and severe toxicity is observed above 2 mM. Therefore lithium has a narrow therapeutic window which requires extensive monitoring of its plasma concentration. Therefore there is scope for the design of other inhibitors of IMPase, which may prove useful in the treatment of manic depression. Such an inhibitor should also confirm whether IMPase is the target for lithium therapy.

myo-Inositol Phosphate-based Inhibitors

Two reviews detailing inhibitor design based on structural modifications of *myo*-inositol phosphates have been published,[28,29] therefore only the most potent compounds and more recent results will be discussed here. Figure 3 summarises how the hydroxy and phosphate groups of *myo*-inositol 1-phosphate are thought to interact with the active site of IMPase. Only the 6-OH

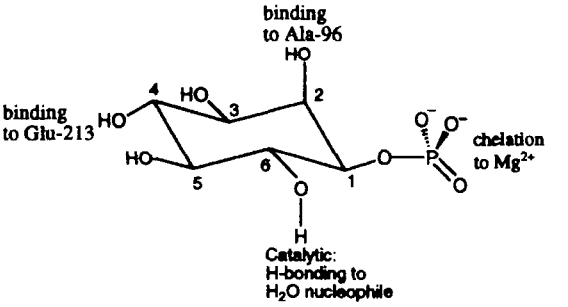


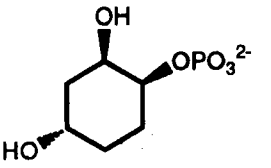
FIGURE 3 Proposed roles of the hydroxy and phosphate groups of D-*myo*-inositol 1-phosphate in its binding to the active site of IMPase.

group is implicated in catalysis, most likely through hydrogen-bonding to the water nucleophile,[12] which has been demonstrated by replacing 6-OH with -OMe or -H giving rise to tightly binding inhibitors.[28,29] Early substrate analogue studies involved systematic deletion of hydroxy groups from D-*myo*-inositol 1-phosphate, which led to the conclusion that the 3- and 5-hydroxy groups were not important for binding, and showed that (**8**) was a good enzyme inhibitor (IC_{50} 3 μM).[28,29] Comparison with other substrates demonstrated that a lipophilic group at the 6-position should be accommodated, which led to the design of (**9**), with an IC_{50} of 40 nM. In an attempt to develop compounds that would occupy the lipophilic cavity and chelate with the magnesium cations, (**10**) was designed and shown to have a K_i of 0.5 μM.[30]

However compounds of type (**9**) suffered from low bioavailability due to the presence of both a dianionic phosphate group (which also renders the compounds metabolically vulnerable to phosphatases) and the hydroxylated cyclohexane ring. van Steijn and coworkers[31] replaced the phosphate group of (**9**) with a wide range of less polar substituents including phosphate diester, phosphonate, sulphate, sulphonamide, carboxylate or carbamate, however in all cases they were significantly poorer inhibitors of IMPase, demonstrating that for this class of inhibitor, a phospho group, bearing two negative charges is required.

Bisphosphonates and their Prodrugs

Because of the low bioavailability and metabolic instability of cyclohexane phosphate compounds, a search for inhibitors not related in structure to *myo*-inositol monophosphates was initiated. Screening showed that



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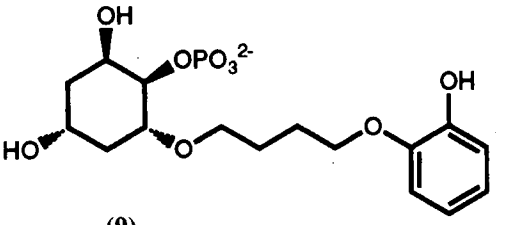

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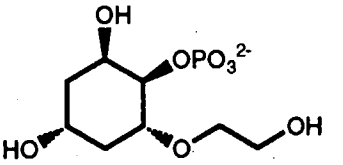

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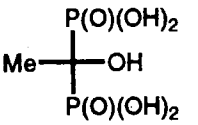

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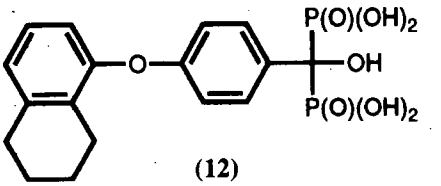

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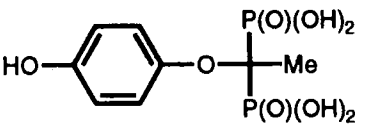

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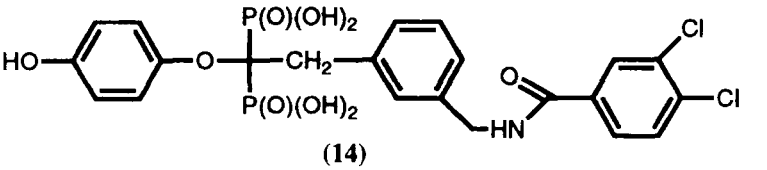
STRUCTURES (8)–(13)



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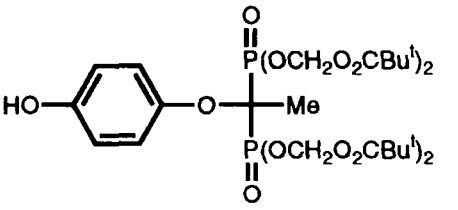


(15)

STRUCTURES (14) and (15)

1-hydroxyethylidene-1,1-bisphosphonic acid (**11**) was a weak inhibitor with an IC_{50} of 110 μM,[32] showing that the bisphosphonate grouping is an effective replacement for the phosphate group. This finding led to the synthesis of a range of phosphatase-stable hydroxymethylene bisphosphonic acid derivatives, in which the hydroxylated cyclohexane ring in the substrate was replaced with an aromatic ring. The most potent compound in this series was (**12**), with an IC_{50} of 0.61 μM.[32] Further optimisation recognised the requirement of hydrogen-bonding in the ring, which lead to the design of the competitive inhibitor (**13**) which had a K_i between 0.2 and 2 μM depending on the enzyme source,[33,34] being ~1000-fold more potent than lithium *in vitro*. An increase in the lipophilicity at the α-position improved inhibition, and (**14**) showed the best *in vitro* activity (K_i 0.07 μM) for this class of compound.

Although (**14**) was more potent than (**13**) *in vitro*, only (**13**) showed effects *in vivo*, presumably as a consequence of its better cell penetration.[34] However, at concentrations of 10 mM, lithium was 2.5-times more effective than (**13**) at the *in vivo* accumulation of *myo*-inositol monophosphates (a measure of IMPase inhibition) in muscarinic m1 receptor-transfected Chinese hamster ovary cells, suggesting that (**13**) only poorly crosses the cell membrane. By using high doses (0.8 mmol/kg) of (**13**) in mice whose phosphatidyl inositol cycle was stimulated with pilocarpine, increased concentrations of *myo*-inositol monophosphates were observed in the brain, kidney and liver. However, larger increases were observed with lithium. Thus despite promising *in vitro* activity, the *in vivo* results with (**13**) were disappointing,

and attributable to poor penetration through cell membranes (and specifically the blood–brain barrier).

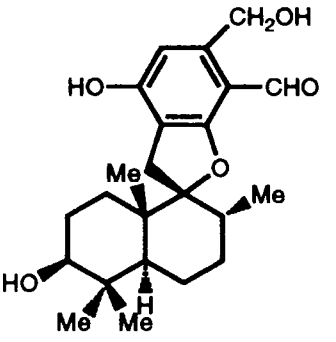
This result prompted the synthesis of a lipophilic prodrug of (**13**) in an attempt to improve bioavailability. The acyloxymethyl phosphonate esters [P–O–CH_2–O_2CR] were investigated as these groups were known to cleave to the chemically unstable hydroxymethyl phosphonate esters [P–O–CH_2–OH] in the presence of esterases.[35] The hydroxymethyl ester then cleaves to the phosphonic acid and formaldehyde. The pivaloyloxy ester was selected because of its good chemical stability, and the tetra(pivaloyloxymethyl) ester (**15**), as well as the mono-, di- and tri-esters were prepared for evaluation.[36] At a concentration of 10 μM, (**15**) gave a maximal increase in *myo*-inositol monophosphate concentration in rat cortical slices, whereas the tri-, di- and mono-esters showed decreasing activity, respectively, consistent with their increasing polarities and decreasing cell penetration. In a bovine brain homogenate (**15**) was shown to be converted to (**13**), showing that appropriate esterases are present in this tissue. The effects of the prodrug were similar to those observed with lithium, giving support to the theory that the therapeutic target of lithium is IMPase. Despite these encouraging results, when (**15**) was injected into animals, neither the prodrug nor phosphonic acid (**13**) could be detected in either the plasma or brain, suggesting that (**15**) is too insoluble to leave the site of injection, and is therefore unsuitable for further development.

Terpenoid Inhibitors

As part of a search for IMPase inhibitors from natural sources, a sesquiterpene was isolated from the hyphomycete, *Memnoniella echinata*. It was found to be a non-competitive inhibitor (with respect to both D-*myo*-inositol 3-phosphate and Mg^{2+}) with a K_i of 450 μM, and is therefore different to lithium in its inhibition profile.[37] In a rat parotid slice assay, the compound caused an accumulation of *myo*-inositol monophosphates, consistent with inhibition of IMPase. A structure of the sesquiterpene was proposed in the original paper, however subsequent studies using detailed NMR spectroscopy[38] and synthesis[39] revised this to (**16**).

Tropolone Inhibitors

Puberulonic acid (**17**), isolated from several *Penicillium* strains, was shown to be a competitive inhibitor of IMPase with an IC_{50} of 10 μM.[40] First it was proposed that the anhydride in (**17**) would hydrolyse to give a biscarboxylic



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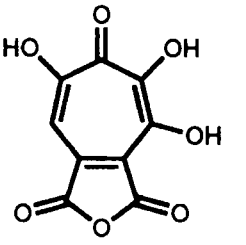

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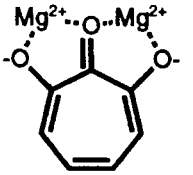

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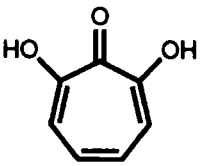

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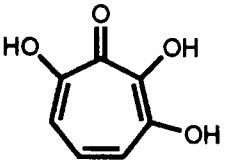

(20)

STRUCTURES (16)–(20)

acid, which would chelate the magnesium cations in the active site of IMPase. However, such biscarboxylic acids were shown to be inactive. Modelling studies supported this finding and led to the proposal that three adjacent unprotected oxygen atoms are required for chelation to the two magnesium cations as shown in model (**18**), indicating that the tropolone mimics the phosphate ester oxygens. To test this model, a series of tropolone analogues were synthesised and evaluated for inhibition of IMPase. 7-Hydroxytropolone (**19**) had an IC_{50} of 75 μM, whereas the addition of an extra hydroxy group in 3,7-dihydroxytropolone (**20**), gave an IC_{50} of 8 μM (K_i 5 μM), this inhibition being comparable to that observed with (**17**). Increased activity upon addition of the extra hydroxy group can be attributed to the formation of an additional hydrogen bond with the carbonyl group of leucine-42 in the active site. In support of model (**18**), methylated or acetylated analogues of (**20**) did not inhibit IMPase. In contrast to the micromolar inhibition observed with the seven-membered ring tropolones, pyrogallol (1,2,3-trihydroxybenzene) showed no inhibition of IMPase at 1 mM.

This was attributed to the tropolone system being more acidic (p*K*a 6.7) than pyrogallol (p*K*a 9.3), which is consistent with the increased ability of the anionic forms to chelate magnesium. In an attempt to increase potency, an extensive range of analogues of (**20**) were prepared in which aromatic substituents were incorporated into the 4- and/or 6-positions.[41] Although some of the analogues showed comparable activity to (**20**), no significant improvements were observed.

CONCLUSION

The therapeutic effect of lithium in the treatment of manic depression and related disorders is most likely attributable to its uncompetitive inhibition of IMPase. This serves to increase and decrease levels of *myo*-inositol monophosphates and *myo*-inositol, respectively, and therefore has an effect on the phosphatidyl inositol cell signalling pathway. However, lithium is difficult to administer as it has a narrow therapeutic window and is associated with a range of side-effects. Therefore, there has been substantial effort in the search for alternative inhibitors of IMPase. Phosphate analogues closely mimicking the natural substrate, with deletion or modification of the cyclohexane hydroxy groups, were inhibitors of IMPase, however they were unstable (hydrolysing with non-specific phosphatases) and showed poor bioavailability. Bisphosphonate (**13**) was a competitive inhibitor (K_i 0.2–2 μM) of IMPase *in vitro*. Although *in vivo* effects were observed with (**13**), high doses had to be employed, which was attributed to poor cell penetration properties. A lipophilic ester prodrug (**15**) was prepared, however experiments in animals were fraught with solubility problems. Terpenoid (**16**) and tropolone (**20**) were non-competitive and competitive inhibitors of IMPase, respectively. These compounds, not bearing a phospho group, may have improved bioavailabilities and may provide lead for the design of therapeutically acceptable inhibitors of IMPase for potential use in the treatment of manic depression.

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