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INHIBITORS OF *MYO*-INOSITOL MONOPHOSPHATASE UNRELATED TO THE ENZYME SUBSTRATE

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Abstract: Hydroxymethylenebisphosphonate derivatives have been found to be competitive inhibitors of *myo*-inositol monophosphatase.

The enzyme *myo*-inositol monophosphatase plays a key role in controlling the phosphoinositide (PI) secondary messenger system[1]. Uncompetitive inhibition of this enzyme by lithium has been cited as a possible mode of action of lithium in the treatment of manic depression[2]. We wish to report the discovery of hydroxymethylenebisphosphonate derivatives as effective, competitive inhibitors of *myo*-inositol monophosphatase.

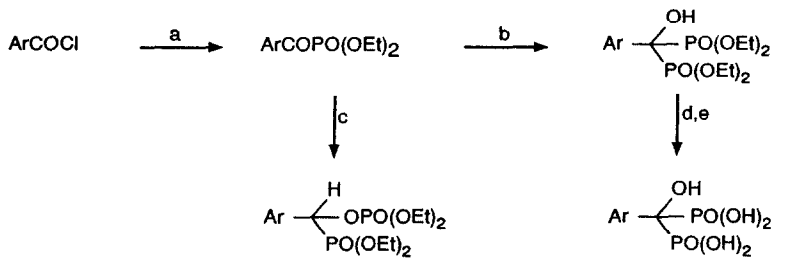
Moderately potent phosphate ester inhibitors have been previously reported[3] discovered by our approach based on hydroxyl deletion from the substrate, *myo*-inositol monophosphate. Subsequently, more potent phosphate inhibitors were prepared from considerations based on the observation that 2'-AMP is also a substrate of the enzyme[4]. This approach however is limited to the generation of substrate like inhibitors and the highly charged nature and metabolic instability of such compounds offers little potential for *in vivo* studies. Furthermore, simple phosphate isosteres such as phosphonate and thiophosphate derivatives have been found to lack inhibitory activity. A search for inhibitors unrelated to the enzyme substrate was thus initiated.

Selective screening of phosphonic acid derivatives led to the identification of 1-hydroxyethylidene-1,1-bisphosphonic acid (1) as a weak enzyme inhibitor, IC_{50}, 110 μM[5]. More detailed studies showed that the inhibition is competitive with respect to substrate[6] and the task of optimising this lead was undertaken

Since hydroxymethylenebisphosphonic acids have been extensively studied as metal chelators and certain derivatives are clinically effective as a treatment for osteoporosis[7] many derivatives are synthetically accessible. It has been shown that whilst treatment of arylketophosphonate esters[8] with phosphite and triethylamine forms phosphonophosphate esters

the use of di-n-butylamine generally yields hydroxybisphosphonate esters[9]. These are readily deesterified with TMS bromide followed by hydrolysis to give hydroxybisphosphonic acids (Scheme 1). A range of compounds were synthesised in this manner and used to establish SAR for the inhibition of *myo*-inositol monophosphatase (Table) [10]

Scheme 1



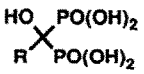
REAGENTS; a, $PO(OEt)_3$; b, $HPO(OEt)_2$, nBu_2NH, Et_2O: c, $HPO(OEt)_2$, Et_3N, Et_2O; d, TMS bromide; e, H_2O.

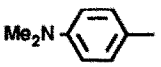
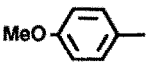
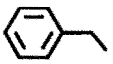
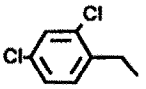
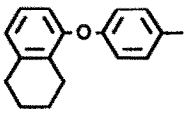
Replacing the methyl group of (1) with phenyl gives a 3-fold increase in inhibitory potency (2) IC_{50}, 29μM. Substitution of the phenyl ring of (2) revealed that chloro- and methoxy-groups are tolerated at the 4-position but such substitution does not lead to an improvement in inhibitory potency (Table). The unsubstituted benzyl derivative (6), (IC_{50}, 38 μM) was found to have similar potency to the phenyl analogue and the 2,4-dichlorobenzyl derivative (7) was identified as a readily accessible, moderately potent inhibitor of *myo*-inositol monophosphatase, IC_{50}, 23 μM. A more significant increase in potency was not found until the introduction of large lipophilic groups at the 4-position of the aromatic ring of 1-hydroxy-1-phenylmethylenebisphosphonic (2) was undertaken. These compounds were prepared via the SN_{Ar} displacement of fluoride from ethyl 4-fluorobenzoate followed by conversion to the hydroxybisphosphonate via the acid chloride (Scheme 2). This led to the identification of the tetralin derivative (8) as a highly potent, competitive enzyme inhibitor, IC_{50}, 0.61 μM.

In summary, a series of inhibitors of *myo*-inositol monophosphatase has been identified which are unrelated to the enzyme substrate. This offers the potential for carrying out *in vivo* studies on the effects of competitive inhibitors of *myo*-inositol monophosphatase on the secondary messenger system.

Table

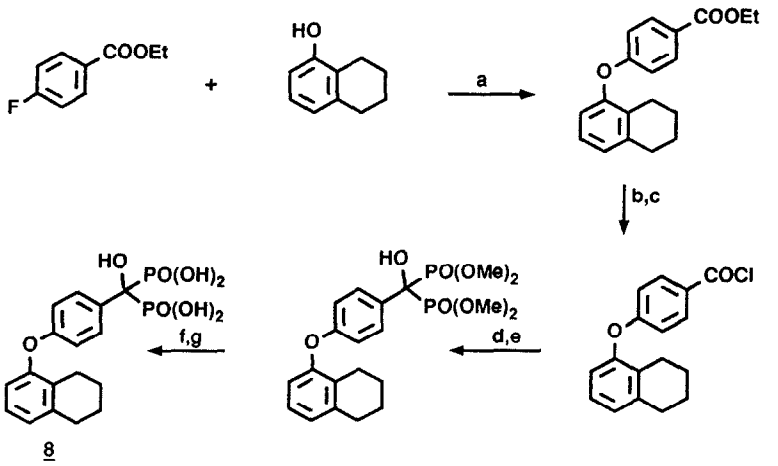
Inhibition Data for Hydroxymethylenebisphosphonic Acid Derivatives



No	R	IC_{50}(uM)*
1	CH_3-	110
2		29
3		140
4		40
5		36
6		38
7		23
8		0.61

*See Reference 5 for assay conditions

Scheme 2



REAGENTS: a, NaH, DMF, 120°, 2h; b, KOH/MeOH; c, $SOCl_2$; d, $P(OMe)_3$; e, $HPO(OMe)_2$, nBu_2NH, Et_2O; f, TMS bromide; g, H_2O.

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(4) Baker, R.; Carrick, C.; Leeson, P.D.; Lennon, I.C.; Liverton, N.J., J. Chem. Soc., Chem. Commun., **1991**, 298.

(5) IC_{50} determinations at 0.1 M *myo*-inositol-1-phosphate concentrations (n=3) were carried out using recombinant bovine *myo*-inositol monophosphatase. Diehl, R.E; Whiting, P; Potter, J; Gee, N; Ragan, C.I; Linemeyer, D; Schoepfer, R; Bennett, C, Dixon, R.A.F. J. Biol. Chem., **1990**, 5946.

(6) IC_{50} determinations at varying substrate concentrations showed the competitive nature of the inhibition with respect to *myo*-inositol-1-phosphate.

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(10) The hydoxybisphosphonate derivatives were prepared as *bis*-anisidine salts and satisfactory CHN data were obtained.