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4-HYDROXYPHENOXYMETHYLENE BISPHOSPHONIC ACID DERIVATIVES: POTENT, NON-HYDROLYSABLE INHIBITORS OF *MYO*-INOSITOL MONOPHOSPHATASE

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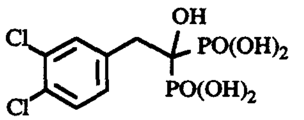
Abstract: From a series of 4-hydroxyphenoxymethylene bisphosphonic acid derivatives, 1-(4-Hydroxyphenoxy)-1-(methyl)methylenebisphosphonic acid has been identified as a structurally simple, competitive, inhibitor of *myo*-inositol monophosphatase (IC_{50}, 0.33 μM). Replacement of the 1-methyl group by a 3-(3,4-dichlorobenzamido)benzyl substituent affords the most potent inhibitor of the series (IC_{50}, 0.08 μM).

myo-Inositol monophosphatase is a key enzyme in the phosphoinositide cell signalling system[1]. It hydrolyses inositol 1-phosphate, inositol 3-phosphate and inositol 4-phosphate to provide the inositol required for the resynthesis of phosphatidylinositol and polyphosphoinositides. Blockade of inositol monophosphate hydrolysis and consequent depletion of inositol available for PI synthesis may underlie the anti-manic and anti-depressant activity of lithium[2]. Early papers from this laboratory described the preparation of phosphate containing competitive, reversible inhibitors of *myo*-inositol monophosphatase[3,4]. Recently, we have reported on the discovery of aromatic hydroxymethylene bisphosphonic acid derivatives as non-hydrolysable inhibitors of *myo*-inositol monophosphatase[5]. We have also shown that methylene bisphosphonic acid is an effective replacement for phosphate in phosphate containing inhibitors of the enzyme[6]. In this publication we wish to describe the optimisation of the *in vitro* potency of methylene bisphosphonic acid derivatives. This has led to the discovery of potent, non-hydrolysable inhibitors of *myo*-inositol monophosphatase[7] which may be used to compare the effects of competitive inhibitors of *myo*-inositol monophosphatase with the uncompetitive actions of lithium.

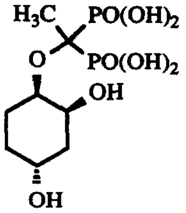
In studying hydroxybisphosphonates we found that lipophilic, aromatic substituents provide effective inhibitors[5]. Thus, 1-(2,4-dichlorobenzyl)-1-hydroxymethylene-1,1-bisphosphonic acid (1) is a moderately potent enzyme inhibitor (IC_{50} of 23 μM). On the other hand studies with methylene bisphosphonic acid derivatives showed an essential requirement for H-bonding groups attached to the cyclohexane ring[6] (Figure). In striving for optimum substitution of methylene bisphosphonic derivatives, inhibitors were designed incorporating both these features.

Figure



(1)

IC_{50}, 23 μM



(2)

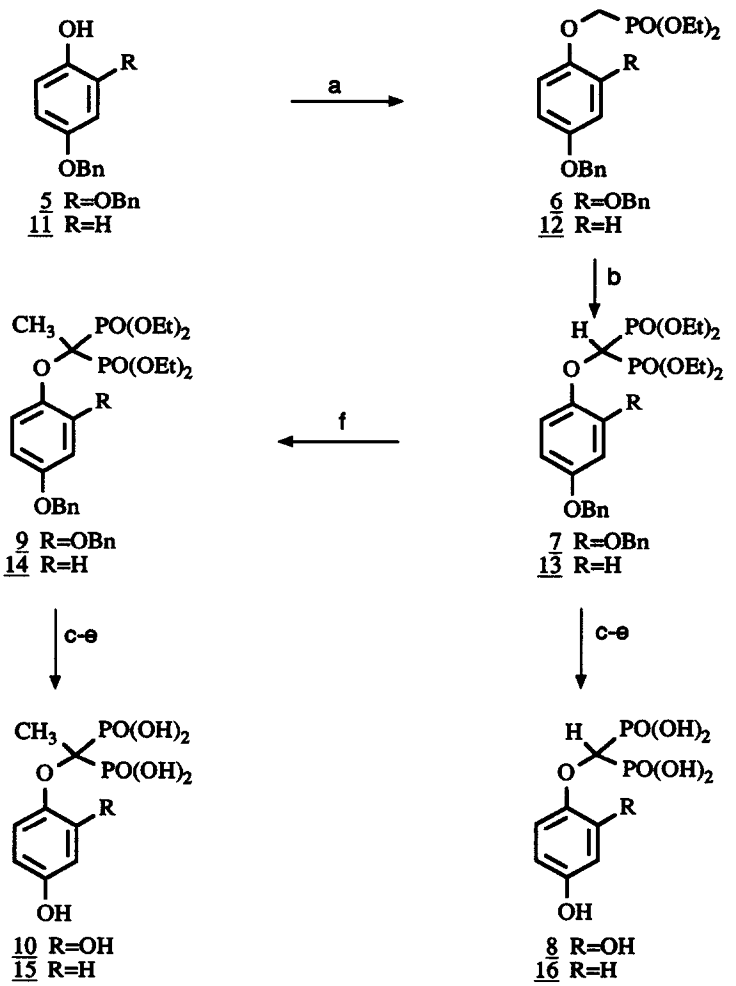
IC_{50}, 4 μM

Addition of lipophilic groups at the *alpha*-position of (2) using previously described methodology[6] failed to increase inhibitory potency (Table). Incorporation of a benzyl group (3) led to loss of inhibitory potency presumably due to an unfavourable steric interaction associated with the 2-hydroxy group. The introduction of spacer groups as exemplified by the phenylbutyl derivative (4), also failed to significantly increase inhibitor potency beyond that achieved with the simple methylated derivative (2). A search was thus undertaken for alternative H-bonding groups to which lipophilic substituents could be attached.

It was discovered that the cyclohexanediol moiety of (2) could be replaced with phenolic groups to provide improved enzyme inhibitors. The derivatives were prepared as outlined in Scheme 1. Alkylation of 2,4-dibenzyloxyphenol (5)[8] with diethyl trifluoromethylsulphonyloxymethylphosphonate[9] afforded monophosphonate (6) (Scheme 1). This was phosphorylated with diethyl chlorophosphate using lithium diisopropylamide as base to afford bisphosphonate ester (7). The esters were cleaved by treatment with trimethylsilyl bromide followed by hydrolysis of the resulting silyl esters. Catalytic hydrogenolysis of the benzyl ethers gave the phenolic bisphosphonic acid (8) which was characterised as the *tris*-cyclohexylamine salt[10]. The IC_{50} of 40.8 μM displayed by the unalkylated aromatic bisphosphonate (8) was encouraging since the introduction of a methyl group at the *alpha*-position in the acyclic series gave a 3-4 fold increase in inhibitory potency[6]. Methylation of (7) was carried out using methyl iodide with sodium hydride as base in DMF. The resulting compound was deprotected as above to afford (10) which had enhanced inhibitory potency comparable with that of the cyclohexanediol derivative (2) (Table).

In establishing the SAR of the phenolic derivatives it was discovered that in contrast with the cycloalkyl series, deletion of the 2-hydroxyl group leads to an increase in inhibitory potency. Thus, the 4-hydroxy derivative (15)[10] was prepared from 4-benzyloxyphenol (Scheme 1) and found to be 10-fold more potent than the 2,4-dihydroxy derivative (10) (Table). This may be due to alleviating an unfavourable steric interaction between the *alpha*-methyl and the 2-hydroxyl group attached to the phenyl ring of (10). To ascertain the contribution to binding made by the *alpha*-methyl group in (15) the *des*-methyl derivative (16) was prepared. This results in a 200-fold loss in inhibitory potency (Table). The enhanced binding is considered to arise from steric compression by the methyl group giving improved alignment of the phosphonate groups relative to the phenolic group when binding at the enzyme active site.

Scheme 1

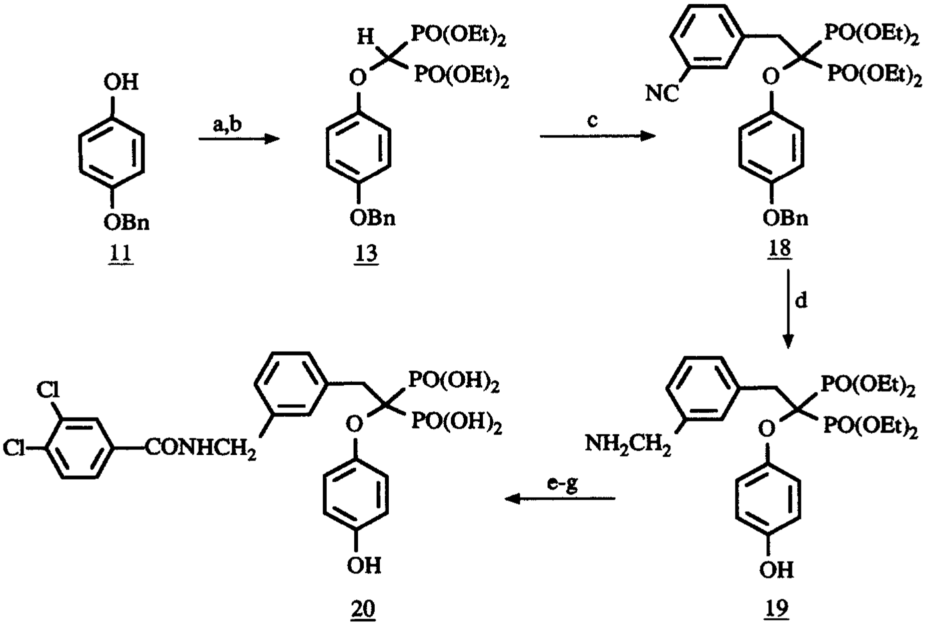


REAGENTS: a, NaH, $TfOCH_2PO(OEt)_2$, THF; b, LDA, $ClPO(OEt)_2$; c, TMS bromide; d, H_2O; e, H_2, Pd/C; f, NaH, MeI, DMF.

Methylenebisphosphonate derivative (15) was thus identified as a structurally simple, potent, competitive inhibitor[11] of *myo*-inositol monophosphatase (IC_{50}, 0.33 μM) which demonstrates the high affinity of the enzyme active site for the phenolic substituent (Table).

With (15) in hand attention was again directed towards the introduction of lipophilic groups at the *alpha*-position. Benzylation gave no significant increase in inhibitory potency (Table, Entry 17), but appending lipophilic groups at the 3- and 4-position did give rise to modest increases in inhibitory potency. Through this strategy the 3-(3,4-dichlorobenzamido)benzyl derivative (20) was prepared (Scheme 2). This compound (IC_{50}, 0.08 μM) is the most potent, non-hydrolysable inhibitor of *myo*-inositol monophosphatase reported to date.

Scheme 2

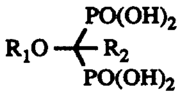


REAGENTS: a, NaH, $TfOCH_2PO(OEt)_2$, THF; b, LDA, $ClPO(OEt)_2$; c, NaH, *m*-Cyanobenzyl bromide, DMF; d, $Pd(OH)_2/C$, 55psi H_2; e, 3,4-Dichlorobenzoyl chloride, Et_3N; f, TMS bromide; g, H_2O

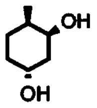
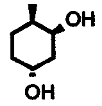
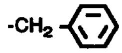
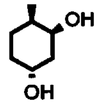
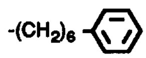
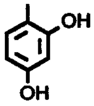
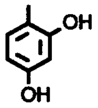
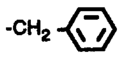
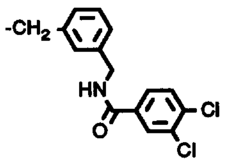
Development of potent, non-hydrolysable inhibitors has enabled studies to be carried out comparing the effects on the phosphoinositide (PI) cycle of competitive inhibitors of *myo*-inositol monophosphatase and the uncompetitive inhibitor, lithium. The results of these studies will be reported elsewhere.

Table

Inhibition Data For Methylene Bisphosphonate Derivatives



$$R_1O-C(PO(OH)_2)_2-R_2$$

No	R_1	R_2	IC_{50}* (μM)
2		$-CH_3$	4
3			19.2
4			2.5
8		-H	40.8
10		$-CH_3$	4.4
15		$-CH_3$	0.33
16		-H	27
17			0.5
20			0.08

* See Reference 7 for assay conditions

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