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DHPMP: A NOVEL GROUP I SPECIFIC METABOTROPIC GLUTAMATE RECEPTOR AGONIST

E. Andrew Boyd,*[a] Stephen P.H. Alexander,[b] David A. Kendall[b] and Vincent M. Loh Jr.[a]

[a]*Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD U.K.*

[b]*Department of Physiology and Pharmacology, Medical School, Queens Medical Centre, Nottingham, NG7 2UH U.K.*

e-mail: *andy.boyd@nottingham.ac.uk*

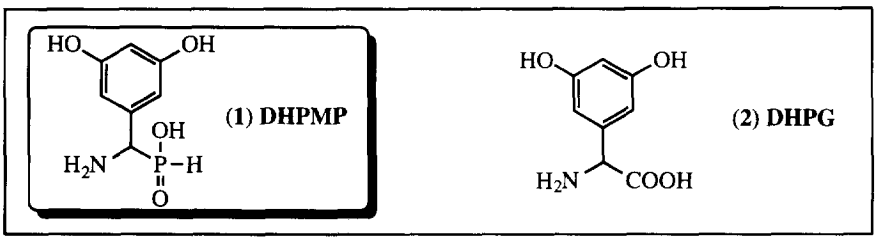
Abstract: *The synthesis and preliminary pharmacological evaluation of (RS)-amino(3,5-dihydroxyphenyl)methylphosphinic acid (DHPMP) is reported. DHPMP has been identified as possessing Group I specific metabotropic agonist activity.*

Recently we reported the multiple solid phase synthesis of 1-aminophosphinic acids[1] as part of a research programme directed towards the development of new methodologies for the construction of carbon-phosphorus bonds, in particular molecules containing the phosphinic acid motif.[2-5] As an integral component of this programme and to highlight our emerging synthetic methodology, the rational design and preparation of molecules which may have potential as biochemical tools was ensured. In this letter we report the synthesis of (*RS*)-amino(3,5-dihydroxyphenyl)methylphosphinic acid (DHPMP) **1** which we have identified to possess selective activity at Group I metabotropic glutamate receptors (mGluRs) in guinea-pig brain preparations.

Since the first report of mGluRs in 1985[6] much progress has been made in elucidating the pharmacology of this family of G-protein coupled receptors. The structure and function of the mGluRs has recently been reviewed[6] as have the pharmacological tools involved in its definition and modulation.[8] Recent perspectives on the mGluRs as novel targets for drug design have also been published[9,10] and the indispensable requirement for more potent and selective agents was identified as a major bottle-neck for further pharmacological development and eventual therapeutic exploitation.[9] To date eight sub-types of metabotropic receptor have been identified and on the basis of gene sequence homology, signal transduction mechanism and agonist pharmacology have been subdivided into three distinct groups.[7-10] We have previously identified three classes of mGluRs expressed in the guinea-pig brain.[11-13] Group I mGluRs are coupled to phosphoinositide hydrolysis whilst Group II and III are coupled to inhibition of cAMP generation. The preliminary pharmacological investigation detailed in this letter consists of evaluating phosphoinositide hydrolysis and the accumulation of cAMP assessed in guinea-pig cerebral cortex by methods which we have previously reported.[11-13]

Molecules incorporating phosphonic acid functionality have played a fundamental role in establishing and defining excitatory amino acid receptors.[14] However, surprisingly little has been published involving phosphinic acid containing entities, probably in part due to relatively limited general routes for their preparation. Monophosphinic acids as typified by DHPMP are isosterically and electronically more analogous to carboxylic acids than phosphonic acids. Due to the isoelectronic and isosteric analogy between carboxylic and phosphinic

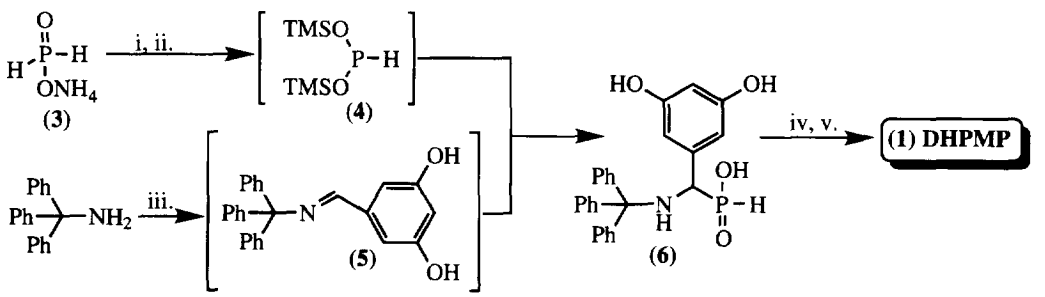
acid moieties coupled with the known selective action of 3,5-dihydroxyphenylglycine ((*R,S*)-3,5-DHPG) **2** at group I mGluR[8,15,16] various phenylglycine phosphinic acid containing isosteres were prepared and evaluated for potential metabotropic modulatory activity.



Synthesis of DHPMP

We have developed two complimentary synthetic approaches to DHPMP using either solution or solid phase based technologies. This synthetic strategy allows the convenient preparation of DHPMP on a small scale (5-15 mg) or on a multi-gram scale. The key synthetic step in both of these syntheses involve the formation of *bis*(trimethylsilyl) phosphonite[2] (BTSP) **4** and addition to a preformed imine. BTSP was conveniently generated *in situ* by the addition of hexamethyl disilazane (HMDS) to ammonium phosphinate **3**.[1-3]

The initial synthetic approach to DHPMP used solid phase based methodology[1] utilising Sieber resin[17] (xanthen-3-yloxymethyl polystyrene). The resin was transformed to the 9-(3,5-dihydroxyphenyl)imine by *in situ* condensation with 3,5-dihydroxybenzaldehyde and as the key step a solution of preformed BTSP was added to the imine resulting in formation of a new carbon-phosphorus bond. Due to the use of solid phase synthesis the resin bound DHPMP could be washed to efficiently remove impurities and after acidic cleavage from the resin resulted in DHPMP of excellent purity in a yield reproducibly greater than 90%. Using these solid phase conditions DHPMP was conveniently prepared on a small scale (5-15 mg).



(i) HMDS, 110 °C, Ar, 2 h. (ii) CH_2Cl_2 (iii) 3,5-dihydroxybenzaldehyde (iv) MeOH/HCl (2M), reflux (v) propylene oxide, EtOH

Scheme 1. Preparation of (*RS*)-amino(3,5-dihydroxyphenyl)methylphosphinic acid (**1**) DHPMP

As larger quantities of DHPMP were required we developed a solution synthesis which allows multigram preparation of **1**, Scheme 1. Previously 1-aminophosphinic acids have been prepared in solution by addition of phosphinic acid[18,19] or BTSP[20] to *N*-(diphenylmethyl)imines. However, problems have been encountered using this procedure to prepare primary aminophosphinic acids,[20] removal of the diphenylmethyl group to liberate the mono-phosphinic acids can also be problematic.[21] The use of triphenylmethyl 'protection' of amines has proved successful in the preparation of 1-aminophosphonic acids,[21] the triphenylmethyl group

being conveniently removed by dilute acid work-up. In analogy we found that BTSP generated *in-situ*[1-3] and subsequent addition to *N*-(triphenylmethyl)imine derivative **6**, followed by aqueous work-up resulted in DHPMP as its hydrochloride salt. The most convenient method for isolation and purification of free DHPMP was to dissolve the product in hot ethanol followed by addition of propylene oxide which resulted in precipitation of the product which was subsequently filtered.

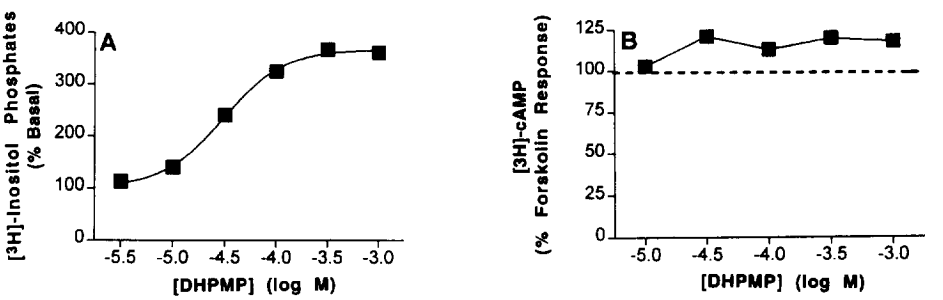
Pharmacological Evaluation of DHPMP

To assess the potential activity of agents at Group I mGluRs, concentration-responses to *(RS)*-DHPG and *(RS)*-DHPMP were examined for the generation of [^{3}H]-inositol phosphates using [^{3}H]-inositol pre-labelled guinea-pig cerebral cortex slices. In this tissue, the non-selective mGluR agonist 1-aminocyclopentane-1*S*,3*R*-dicarboxylic acid (1*S*,3*R*-ACPD) a conformationally restricted glutamate analogue elicits a phosphoinositide mobilisation with a pEC_{50} of 4.46 (pEC_{50} = -log concentration evoking half maximal response).[12] Both DHPMP and DHPG elicited significant accumulations of [^{3}H]-inositol phosphates with pEC_{50} values of 4.56 +/- 0.06 (Figure 1A), and 4.90 +/- 0.05 (data not shown. *cf.* reference 15 respectively).

To examine the potential activity of *(RS)*-DHPG and *(RS)*-DHPMP at Group II and Group III mGluRs, concentration-response curves for inhibition of forskolin-stimulated cAMP generation were examined (Figure 1B). (1*S*,3*R*-ACPD) inhibits forskolin-stimulated cAMP generation in this tissue with a pEC_{50} of 5.68.[12] However the putative Group I selective agonist *(RS)*-DHPG failed to elicit a significant inhibition of forskolin-stimulated cAMP generation at concentrations up to 1 mM (data not shown, *cf.* reference 15). DHPMP was similarly ineffective as an inhibitor of cAMP (Figure 1B). From this we may infer a lack of activity at Group II and Group III mGluRs at these concentrations of DHPG and *(RS)*-DHPMP.

In conclusion *(RS)*-DHPMP exhibits Group I mGluR selectivity in the guinea-pig brain which is of a similar magnitude to *(RS)*-DHPG. As this is the first example of a phosphinic acid-containing metabotropic active agent it could serve as a lead for exploitation of a novel class of ligands with Group I selectivity.

Figure 1. Preliminary pharmacological evaluation of *(RS)*-DHPMP: concentration-response curves for:
1A. Generation of [^{3}H]-inositol phosphates[a,b]
1B. Modulation of forskolin-stimulated [^{3}H]-cAMP[a,c]



Notes:
a The standard error of the means (SEM) are less than the size of the symbols used.
b Data shown are means of quadruplicate determinations from single experiments repeated with similar results on 4(A) on 2(B) further occasions.
c The dotted line in (B) shows a 100% forskolin response.

Further pharmacological evaluation of *(RS)*-DHPMP and other phenylglycine phosphinic acid containing isosteres is ongoing and the results will be published elsewhere. In addition we are currently developing methodology for the chiral synthesis of both *(R)*-DHPMP and *(S)*-DHPMP to evaluate the stereochemical implications (selectivity/potency) of these ligands at metabotropic receptors.

Synthetic procedures:

***N-(Triphenylmethyl)iminomethyl-3,5-dihydroxybenzene 5*:**

N-(Triphenylmethyl)amine (1.9 g, 7.4 mmol) and 3,5-dihydroxybenzaldehyde (1.0 g, 7.4 mmol) were refluxed in anhydrous ethanol (20 ml) for 12 h. The solution was stirred with sodium sulphate for 15 min, filtered and evaporated to yield **5** (2.8 g, 100%) which was used without further purification.

***(RS)-DHPMP 1*:** **CAUTION: BTSP is pyrophoric until it is dissolved in CH_2Cl_2.**

Ammonium phosphinate (2.5 g, 30.1 mmol) and HMDS (30.9 mmol) were heated at 115 °C for 2 h under Ar to generate BTSP **4**.[2] After cooling to room temperature the BTSP was dissolved in CH_2Cl_2 (30 ml) and a 7.5 ml (7.5 mmol) aliquot of BTSP solution was added to imine **5** (2.8 g, 7.4 mmol) in CH_2Cl_2 (20 ml) at 0 °C. After stirring under Ar for 12 h the reaction was filtered and concentrated *in vacuo* to yield **6**. *N*-(Triphenylmethyl)-aminophosphinic acid **6** was hydrolysed by solution in methanol containing hydrogen chloride (approximately 2M) (20 ml) followed by reflux for 15 min. Removal of the solvents *in vacuo* yielded the crude hydrochloride salt of DHPMP which was dissolved in water (20 ml). The aqueous DHPMP.HCl solution was washed with ethyl acetate (3 x 20 ml) and the water removed *in vacuo* to yield DHPMP.HCl as a beige solid (1.61 g, 91%). (This resulted in DHPMP.HCl of >98% purity as assessed by NMR, however DHPMP.HCl was further purified, and liberated from the hydrochloride before pharmacological investigation). To a filtered solution of DHPMP.HCl in hot ethanol (20 ml) was added propylene oxide until precipitation started to occur. The ethanolic solution of DHPMP was cooled to room temperature and refrigerated overnight. Filtration of the solution resulted in DHPMP which was washed with ethanol (0 °C) to yield the product as a white crystalline solid (0.80 g, 53%), m.p. 230-232 °C; ν_{max} cm^{-1} 2360, 1550, 1170, 1040; δ_H(250 MHz; D_2O) 4.05 (1 H, d, *J* 25 Hz, P-CH), 6.30-6.50 (3 H, m, Ar) and 6.85 (1 H, d, *J* 550 Hz, P-H); δ_P(101 MHz; D_2O) 19.6; *m/z* 203 (M^+, 100%).

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