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Synthesis of Novel Metabolically Stable Analogues of D-myo-Inositol 1,4,5-Trisphosphate

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Abstract: Starting from L-quebrachitol, syntheses and biological activities of three novel analogues of the cellular second messenger D-*myo*-inositol 1,4,5-trisphosphate (IP₃), 3-deoxy-3-fluoro-D-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate (1a), 3-deoxy-3-fluoro-D-*myo*-inositol 1,5-bisphosphate 4-phosphorothioate (1b), and 3-deoxy-3-fluoro-D-*myo*-inositol 1-phosphate 4,5-bisphosphorothioate (1c) are described.

One of the major pathways of cellular signal transduction is a phospholipase C mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) in the membrane to generate two second messengers, myo-inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol.^{1a} The former binds to its receptor on the endoplasmic reticulum and mobilizes intracellular Ca^{2+} , which then elicits a host of important cellular responses. The IP₃ molecule is metabolized either by phosphorylation at the C-3 position by IP₃-3-kinase, or dephosphorylation at the C-5 position by an IP₃-5-phosphatase resulting in the termination of the signal.^{1b} A number of structural analogues of IP₃ have been synthesized² and pharmacologically evaluated to establish structure-activity relationships with regard to IP3 receptor affinity^{3a} and activity as a substrate for the metabolic enzymes.^{3b} Since the bioisosteric substitution of fluorine atoms for alcoholic oxygens,⁴ and of phosphorothioates² for phosphates have proved to be biologically useful, synthetic efforts have been mainly directed at substituting specific -OH groups by -F and phosphate groups by slow-hydrolyzing phosphorothioate groups.^{2,5} Some of these compounds, e.g., myoinositol 1,4,5-trisphosphorothioate,^{5a} the 5-phosphonate,^{5b} the 5-phosphorothioate,^{5c} and various C-3substituted IP₃ analogues,^{5d} have been reported to exhibit reasonable IP₃-receptor binding and [Ca²⁺]; releasing ability, albeit with less than desirable metabolic and/or chemical stability. We considered substituting simultaneously both the 3-kinase and 5-phosphatase sensitive target positions of the IP3 molecule with a C-F bond and a phosphorothioate moiety, respectively, to obtain stable, enzyme-resistant IP3 analogues while minimally sacrificing biological activity. Herein, we report the syntheses of 3-deoxy-3-fluoro-D-myo-inositol 1.4-bisphosphate 5-phosphorothioate (**1a**), 3-deoxy-3-fluoro-D-myo-inositol 4-1.5-bisphosphate phosphorothioate (1b), and 3-deoxy-3-fluoro-D-myo-inositol 1-phosphate 4,5-bisphosphorothioate (1c) along with their IP3 receptor binding and functional characteristics, as well as their inhibitory effects on the metabolic enzymes.6

The IP₃ analogues 1a, 1b, and 1c were synthesized starting from L-quebrachitol (2) (Scheme 1), a waste product of the rubber industry. Conversion of 2 to 3-deoxy-3-fluoro-D-*myo*-inositol (3) has been reported.⁷ The compound 3 was converted to a 2:1 mixture of regioisomeric hydroxydiacetonides 4a and 4b which could be readily interconverted under acid catalysis. After benzylation of the 6-OH of the diacetonide 4a, the *trans* acetonide group was selectively hydrolyzed with catalytic acetyl chloride in MeOH/CH₂Cl₂ to give diol 5 which served as the pivotal intermediate for the divergent syntheses of the IP₃ analogues 1a, 1b, and 1c.

The diol **5** was reacted with one equivalent of *p*-methoxybenzyl chloride in DMF to afford a 1:2 mixture of 4- and 5-hydroxyacetonides **6a** and **6b**.⁸ This simple approach was more convenient and higher yielding than an alternative scheme based on the acetal formation with *p*-methoxybenzaldehyde, followed by hydride cleavage. The acetonides **6a** and **6b** were carried forward in a parallel fashion for the syntheses of the IP₃ analogues **1a** and **1b**, respectively. The synthetic sequence for **1a** was concluded by: (i) benzoylation of **6a**, followed by acidic hydrolysis to yield the 1,2-*cis* diol **7**; (ii) selective benzoylation of the equatorial 1-OH with 1 equiv. of benzoyl chloride, followed by the protection of the axial 2-OH with a benzyloxymethyl (BOM) group to give a completely protected *myo*-inositol derivative **8**; (iii) saponification of the two 1,4-benzoate ester groups, and direct phosphorylation of the resulting diol by treatment with sodium hydride/tetrabenzyl pyrophosphate⁹ to provide a protected 1,4-bisphosphorylated *myo*-inositol derivative **9**; (iv) oxidative removal of the PMB group at C-5, followed by phosphitylation of the resulting alcohol with dibenzyl N,N-diisopropylphosphoramidite and Schönberg oxidative sulfurization¹⁰ with diphenyl disulfide to give **10**, and finally, (v) one-step deblocking of the fully-protected precursor **10** to furnish, after Sephadex A-25 chromatography, enantiopure **1a**.¹¹ Conceptually similar syntheses, beginning with the alcohol **6b** and the diol **5**, provide the optically pure IP₃ analogues **1b** and **1c**, respectively (Scheme 1).¹¹

The IP₃ analogues **1a**, **1b**, and **1c** were evaluated in the IP₃ receptor binding assay using pig cerebellar membranes, and in Ca²⁺ release assays using saponin permeabilized neuroblastoma SH-SY5Y cells (Table 1). The order of potency of the analogues for the binding and the $[Ca^{2+}]_i$ mobilization was **1b** > **1a** > **1c**. The binding of **1b** correlated well with the $[Ca^{2+}]_i$ release which was only 4 times less compared to IP₃. However, the IP₃ receptor binding observed for the analogues **1a** and **1c** lead to considerably diminished $[Ca^{2+}]_i$ release responses. The analogue **1a**, like **1b**, was found to be a full agonist, while **1c** was a partial agonist. These data indicate that the introduction of the 5-PS moiety is more perturbing for IP₃ receptor binding and function than is 4-PS, and that the 5-phosphate moity (vs the 4-phosphate) likely bears relatively higher structural importance vis-à-vis the 3-OH group in the IP₃ molecule. Also, preliminary data indicate that while the IP₃ analogues **1a** and **1c** were intrinsically resistant to IP₃-3-kinase and IP₃-5-phosphatase, **1b** and **1c** were potent inhibitors of these metabolic enzymes, the order of potency of inhibition being **1b** > **1c** > **1a**.

In conclusion, three novel, enzyme-resistant analogues of the second messenger molecule, IP₃, that exhibit a range of biological activities have been synthesized. These analogues provide further insight into the structure-activity relationships of the 4- and 5-phosphorylated functionalities and their inter-relationship with the 3-OH group in the IP₃ molecule. We believe these compounds help fill the need for tools in IP₃-receptor function studies targeted at, *inter alia*, deciphering the mechanisms of Ca²⁺ oscillations in relationship to the various modes of cellular Ca²⁺ entry. Further studies related to their metabolic stability will be reported elsewhere.

Scheme 1.^a Syntheses of Ins(1,4,5)P₃ analogues 1a, 1b, and 1c



^aReagents and conditions: (a) i) DAST, CH₂Cl₂, -40 °C to rt, 52%. ii) BBr₃, CH₂Cl₂, rt, overnight, 85%; (b) 2-methoxypropene, CSA, DMF, 65 °C, 5 h, 80%; (c) separate regioisomers **4a** and **4b** (ratio 1:2.5); (d) i) NaH, BnBr, DMF, 0 °C, 95%. ii) CH₂Cl₂/MeOH (2:1v/v), AcCl (cat), rt, 10 min, 80%; (e) NaH, PMB-Cl (1 eq), 0 °C, 5 h, then separate regioisomers, 60%, ratio **6a/6b** 1:2; (f) i) BzCl, Py, 0 °C, 12 h. ii) Conc. HCl (cat), MeOH, rt, 12 h, 82-88%; (g) i) BzCl (1.1 eq), Py, 0 °C, 82-90%. ii) BOM-Cl, DIPEA, THF , reflux, 72 h, 82-85%; (h) i) aq. NaOH, MeOH, rt, 80-95%. ii) NaH, tetrabenzyl pyrophosphate, DMF, 0 °C, 92-95%; (i) i) DDQ, H₂O, CH₂Cl₂, rt, 81-92%. ii) NaH, (BnO)₂P-N(^{*i*}Pr)₂, 1*H*-tetrazole, CH₂Cl₂, rt, 2 h, then (PhCH₂COS)₂, rt, 15 min, 85-87%; (j) Na, liq. NH₃, THF, -78 °C, 20 min, then Amberlite H⁺ form, and Sephadex A-25 chromatography, 62-71%; (k) i) NaH, PMB-Cl, DMF, rt, overnight, 90%. ii) Conc. HCl, MeOH, rt, 8 h, 90-95%.

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IP ₃ Analogue	^a IC ₅₀	^b EC ₅₀	IP ₃ Analogue	^a IC ₅₀	^b EC ₅₀
Ins(1,4,5)P ₃	14 nM	99.2 nM	1b	28 nM	424 nM
1a	80 nM	3579 nM	1c	109 nM	11345 nM ^c

^a Determined by displacement of [³H]Ins(1,4,5)P₃ binding from pig cerebellar membrane IP₃-receptors.

^b Determined by measuring [${}^{45}Ca^{2+}$]; released from saponin permeabilized SH-SY5Y cells. ^cThis EC₅₀ value was calculated from the maximally effective concentration (64.3%) of the partial agonist **1c**.

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- 11. Physical and spectral data for the triethylammonium salt of: a) (1a) $[\alpha]_D -1.05^\circ$ (c = 9.5 mg/mL, H₂O); ¹H NMR (D₂O) δ (ppm) 4.60 (ddd, J = 47.0, 9.5, 3.0 Hz, 1H), 4.56 (dd, J = 19.0, 9.5 Hz, 1H), 4.52-4.46 (m, 1H), 4.25 (dd, J = 22.5, 9.5 Hz, 1H), 4.30 (t, J = 9.5 Hz, 1H), 3.92 (t, J = 9.5 Hz, 1H); ³¹P NMR (D₂O, ¹H-decoupled) δ (ppm) 59.94, 3.04, 2.49; ¹⁹F NMR (D₂O, ¹H-decoupled) δ (ppm) -199.06; MS (ESI, negative ion mode) m/z 437 (M⁺-1). b) (1b) $[\alpha]_D$ -0.14° ($c = 3.0 \text{ mg/mL}, \text{H}_2\text{O}$); ¹H NMR (D₂O) δ (ppm) 4.90-4.70 (m, 1H), 4.60 (ddd, J = 47.0, 9.0, 2.5 Hz, 1H), 4.56-4.50 (m, 1H), 4.10-3.90 (m, 2H), 3.94 (t, J = 9.0 Hz, 1H); ³¹P NMR (D₂O, ¹H-decoupled) δ (ppm) 54.65, 3.96, 2.98: ¹⁹F NMR (D₂O, ¹H-decoupled) δ (ppm) -198.66; MS (ESI, negative ion mode) m/z 437 (M⁺-1). c) (1c) [α]_D -1.6° ($c = 12.0 \text{ mg/mL}, \text{H}_2\text{O}$); ¹H NMR (D₂O) δ (ppm) 4.80 (m, 1H), 4.55 (ddd, J = 47.0, 9.4, 3.0 Hz, 1H), 4.51-4.41 (m, 1H), 4.24 (dd, J = 19.0, 8.5 Hz, 1H), 4.02 (t, J = 8.5 Hz, 1H), 3.95 (t, J = 9.3 Hz, 1H); ³¹P NMR (D₂O, ¹H-decoupled) δ (ppm) 54.09, 52.34, 3.00; ¹⁹F NMR (D₂O, ¹Hdecoupled) δ (ppm) -197.86; MS (ESI, negative ion mode) m/z 453 (M⁺-1).

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