Published in final edited form as:

Bioorg Med Chem Lett. 2014 December 15; 24(24): 5553–5557. doi:10.1016/j.bmcl.2014.11.017.

Further evaluation of novel structural modifications to scaffolds that engender PLD isoform selective inhibition

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Abstract

This letter describes the on-going SAR efforts based on two scaffolds, a PLD1-biased piperidinyl benzimidazolone and a PLD2-biased piperidinyl triazaspiro, with the goal of enhancing PLD inhibitory potency and isoform selectivity. Here, we found that addition of an α -methyl moiety within the PLD2-biased piperidinyl triazaspiro scaffold abolished PLD2 preference, while the incorporation of substituents onto the piperidine moiety of the PLD1-biased piperidinyl benzimidazolone, or replacement with a bioisosteric [3.3.0] core, generally retained PLD1 preference, but at diminished significance. The SAR uncovered within these two allosteric PLD inhibitor series further highlights the inherent challenges of developing isoform selective PLD inhibitors.

Keywords

PLD; Phospholipase D; isoform; inhibitors; phosphodiesterase

There are two isoforms of the phosphodiesterase phospholipase D (PLD) in mammals, termed PLD1 and PLD2, that catalyze the production of the lipid second messenger phosphatidic acid.¹⁻⁴ From studies in our labs as well as others, we have demonstrated that PLD dysfunction and/or overexpression can be modulated by small molecule, allosteric inhibitors **2-6** (Fig. 1) with therapeutic benefit in oncology, viral infections and CNS

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disorders.¹⁻¹¹ While significant progress has been made since the report of halopemide **1**,¹² an atypical antipsychotic, as a dual PLD1/2 inhibitor, the ideal, isoform selective PLD1 and PLD2 inhibitors with profiles suitable as *in vivo* proof of concept tools remain elusive.¹⁻¹² Here, we report the further chemical optimization and evaluation of novel modifications to the scaffolds represented by **2** and **3**, and the unexpected PLD pharmacological findings that resulted.

Within the triazaspirone-based series, represented by **3-6**, we previously reported that incorporation of an (*S*)- β -methyl moiety (as in ML299, **5**), dramatically enhanced PLD1 inhibitory activity converting a highly PLD2 selective inhibitor series into a dual PLD1/2 inhibitor series.^{7,8} This was a general finding within this series, and the magnitude of the impact of PLD1 activity exceeded 230-fold (**Fig. 2**) within the ML298 (**4**) molecule, (*S*)-**5**. The analogous (*R*)-enantiomer (*R*)-**5** was similar but of lesser magnitude.⁸ However, we had not yet surveyed the impact of addition of a methyl group in the α -position to the piperidine on PLD1 and PLD2 activity. The synthetic route to access these α -methyl analogs proved to be straightforward (**Scheme 1**).

Starting with commercial piperidine triazaspirone **6**, a reductive amination with *tert*-butyl-(2-oxopropyl)carbamate provided the α -methyl material **7**. Deprotection and standard amide coupling, with six optimal acids, delivered key racemic α -methyl congeners **8**. As shown in Table 1, five of the six analogs, while displaying greater inhibitory activity at PLD2, were essentially non-selective versus PLD1 (only 2- to 5-fold selectivity). Only the 3,4-difluorophenyl amide, **8f**, possessed potent PLD2 inhibitory activity (PLD2 IC₅₀ = 17 nM) and 63-fold selectivity versus PLD1 (PLD1 IC₅₀ = 1,080 nM). Despite this selectivity, the absolute potency at PLD1 (~1 μ M), rendered **8f** not useful as an *in vitro/in vivo* tool,⁸ as PLD1 would also be inhibited at standard testing concentrations. Therefore, we did not attempt to resolve the α -methyl enantiomers, and efforts focused on other domains of the PLD2-preferring core. The 'magic methyl' effect¹³ is very pronounced within this series and has profound impact on PLD1 and PLD2 activity.

In parallel, efforts were being directed at the PLD1-biased piperidinyl benzimidazolone scaffold represented by **2**.^{1,2,5,6} This series was plagued with ancillary pharmacology, due to the GPCR privileged structure, and poor metabolic stability (MET ID indicated oxidative metabolism on the central piperidine ring).^{5,6,9} In an attempt to address these issues, we elected to install a methyl group α to the piperidine nitrogen to block oxidative metabolism (as in **9** and **10**), as well as a β -fluorine atom (as in **11**) to modulate pK_a and potentially improve ancillary pharmacology at biogenic amine targets. The requisite functionalized piperidine benzimidazolones were prepared as previously described following literature routes^{5,6,14} and then elaborated via a reported variation on Scheme 1.^{5,6} While we were excited to note that these modifications to the piperidine core retained PLD inhibitory activity, the compounds were less potent and possessed diminished PLD1 selectivity as compared to the unsubstituted congeners (**Fig. 4**); thus, they did not represent a path forward towards improved *in vivo* tools. However, a brief metabolic stability assessment showed that **9** was more stable in rat microsomes than the corresponding unsubstituted derivative (Cl_{hep} = 70 mL/min/kg versus Cl_{hep} = 36 mL/min/kg), and that **11** was inactive at D₂ (IC₅₀ >10 μ M), whereas the des-fluorocongener possessed a D₂ IC₅₀ of 22 nM.

Finally, we decided to survey the replacement of the piperidine ring with a bioisosteric substitute, namely a [3.3.0] ring system, or octahydrocyclopenta[*c*]pyrrole.¹⁵ Commercial 2-fluoronitrobenzene **12** was heated under microwave irradiation with *tert*-butyl-5-aminohexahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate to deliver the S_NAr product **13** in 75% yield.^{5,14} A zinc mediated nitro reduction afforded **14** which was then treated with triphosgene to provide the benzimidazolone **15**. Acid deprotection and standard elaboration based on Scheme 1 yielded seven novel [3.3.0] analogs **16**.^{5,14}

As shown in Table 2, this effort, with a well documented piperidine biosistere, led to a significant loss in PLD1 potency and greatly diminished PLD2 selectivity (only 3- to 24-fold). Moreover, **16g** was 9.4-fold PLD2 preferring, whereas in the piperidine-based series, the *trans*-cyclopropylphenyl amide was ~111-fold PLD1 preferring. This is the first example in a benzimidazolone core that preferential PLD2 inhibition has been observed. Once again, metabolic stability in rat microsomes was slightly improved relative to the piperidine-based series, but neither PLD potency or isoform selectivity was acceptable. So, while the rationale was sound for the synthesis and evaluation of novel analogs **9-11** and **16**, the unpredictable nature of allosteric SAR once again thwarted our efforts, producing weak, dual PLD1/2 inhibitors.

In summary, we explored and evaluated novel SAR within two distinct chemotypes that afford isoform selective PLD inhibition. While the medicinal chemistry drivers proved to be correct to address ancillary pharmacology and metabolic stability, the inherent steep SAR of allosteric ligands led to a loss in PLD activity and/or PLD isoform selectivity, precluding the development of improved *in vivo* tools from this campaign. Interestingly, a pronounced 'magic methyl' effect was discovered. Efforts continue, and work is in progress to develop optimal *in vivo* tool compounds that selectively inhibit either PLD1 or PLD2.

Acknowledgments

Vanderbilt is a member of the MLPCN and houses the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development. This work was supported by the NIH/MLPCN grant U54 MH084659 (C.W.L.), the Vanderbilt Department of Pharmacology, Voices Against Brain Cancer and William K. Warren, Jr. who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.).

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Figure 1.

Halopemide **1**, and our recently reported isoform-selective PLD inhibitors: **2**, VU0359595 (1,700-fold PLD1 selective), **3**, VU0364739 (75-fold PLD2 selective), **4**, ML298 (53-fold PLD2 selective), **5**, ML299 (dual PLD1/2 inhibitor) and **6**, ML395 (>80-fold PLD2 selective).

Figure 2. Pharmacological impact of incorporation of an (*R*)- or (*S*)- β -methyl group into ML298 (**4**) affording (*R*)-**5** and (*S*)-**5**. Both enhance PLD1 activity, but the (*R*)-enantiomer dramatically decreases PLD2 activity (10-fold) as well.

Scheme 1.

a) *tert*-butyl-(2-oxopropyl)carbamate, MP-B(OAc)₃, DCE, rt, 16 h (94%); b) i) 4 N HCl/dioxane, MeOH (98%); ii) R₂COH, PS-DCC, HOBT, DCM, DIEA (76-92%).

Figure 3.
PLD1 (Calu-1) and PLD2 (293-PLD2) cell-based assay concentration-response curves (CRCs) for representative library members **8**. A) CRCs for **8e**; B) CRCs for **8f**; C) CRCs for **8c**.

Figure 4.

Pharmacological impact of incorporation of substituents on the central piperidine ring in the piperidine benzimidazooone series of PLD1 selective inhibitors.

Scheme 2.

Reagents: (a) *tert*-butyl-5-aminoheptahydrocyclopenta[*c*]pyrrole-2(1*H*)-carboxylate, Na₂CO₃, KI, cyclohexanol, μ w, 180°C, 10 min, 90%; (b) Zn, 1N HCl, MeOH; (c) i) triphosgene, Et₃N, THF, rt, 2 h; ii) 4 N HCl dioxane, rt, 82%.

Table 1

Structures and activities of analogs 8.

Cmpd	Ar	PLD1 IC ₅₀ (nM) ^a	PLD2 IC ₅₀ (nM) ^b	Fold PLD2-selective
8a		315	64	5
8b		1,490	290	5
8c		230	120	2
8d		35	18	2
8e		21	6	3
8f		1,080	17	63

^a Cellular PLD1 assay with Calu-1 Cells^b Cellular assay with HEK293-gfp PLD2 cells. Each IC₅₀ was determined in triplicate.

Table 2

Structures and activities of analogs 16.

Cmpd	Ar	PLD1 IC ₅₀ (nM) ^a	PLD2 IC ₅₀ (nM) ^b	Fold PLD1-selective
16a		244	1,790	7.3
16b		870	3,565	4
16c		468	2,590	5.5
16d		230	1,235	5.4
16e		72	220	3
16f		820	3,250	3.9
16g		1,080	115	-9.4

^a Cellular PLD1 assay with Calu-1 Cells^b Cellular assay with HEK293-gfp PLD2 cells. Each IC₅₀ was determined in triplicate.