Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Isoform selective PLD inhibition by novel, chiral 2,8-diazaspiro[4.5]decan-1-one derivatives

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ARTICLE INFO

Keywords:
PLD
Phospholipase D
Isoform
Inhibitors
Phosphodiesterase

ABSTRACT

This letter describes the on-going SAR efforts to develop PLD1, PLD2 and dual PLD1/2 inhibitors with improved physiochemical and disposition properties as well as securing intellectual property position. Previous PLD inhibitors, based on a triazaspiro[4.5]decanone core proved to be highly selective PLD2 inhibitors, but with low plasma free fraction (rat, human $f_u < 0.03$), high predicted hepatic clearance (rat $CL_{hep} > 65$ mL/min/kg) and very short half-lives *in vivo* ($t_{1/2} < 0.15$ h). Removal of a nitrogen atom from this core generated a 2,8-diazaspiro[4.5]decanone core, harboring a new chiral center, as well as increased sp^3 character. This new core demonstrated enantioselective inhibition of the individual PLD isoforms, enhanced free fraction (rat, human $f_u < 0.13$), engendered moderate predicted hepatic clearance (rat $CL_{hep} \sim 43$ mL/min/kg), improved half-lives *in vivo* ($t_{1/2} > 3$ h), and led to the first issued US patent claiming composition of matter for small molecule PLD inhibitors.

Phospholipase D (PLD) exists as two isoforms in mammals, coined PLD1 and PLD2, and is considered to be one of the major sources of the signal-activated second messenger phosphatidic acid (PtdOH). Recently, the development of isoform-selective PLD inhibitors, in combination with biochemical and molecular genetics studies, have suggested that PLD enzymes in mammalian cells and pathogenic organisms represent therapeutic targets for several human diseases.[1–5] Work from multiple labs have demonstrated that PLD dysfunction and/or overexpression can be modulated by small molecule, allosteric inhibitors **1–6** (Fig. 1) with efficacy in preclinical models relevant to oncology, viral infections and CNS disorders.[1–17] Despite the progress achieved in the past decade, small molecule tools with DMPK profiles suitable for robust *in vivo* proof of concept studies have remained elusive, and there have been no PLD inhibitor composition of matter patents (issued or published applications), further hindering translational potential for the target.[1–22] In this Letter, we describe the discovery and development of a new series of potent and selective PLD2 and dual PLD1/2 inhibitors from a series of 2,8-diazaspiro[4.5]decanones, that display enantioselective PLD isoform inhibition, provide improved DMPK profiles and enabled a robust intellectual property (IP) position.[23]

For several years, the triazaspiro[4.5]decanone core of PLD inhibitors **3–6** was the major focus of optimization efforts, as both PLD2-selective and dual PLD1/2 inhibitors could be realized within this core based on chiral α-methyl moieties along the ethyl linker chain (e.g., **5**).[7–14] Despite providing both peripherally restricted and highly CNS penetrant (brain-to-plasma K_ps 0.75–1.48) PLD inhibitors, this series uniformly afforded compounds displaying high plasma protein binding (rat and human $f_u < 0.03$), high predicted hepatic clearance (rat $CL_{hep} > 65$ mL/min/kg) and very short half-lives *in vivo* ($t_{1/2} < 0.15$ h).[7–14] Work within a well-known SERM chemotype afforded only weak PLD inhibitors, but they proved to be universal (mammalian and bacterial).[15] Thus, the ideal *in vivo* proof of concept tools were lacking, and the program also needed novel chemical matter to advance the target. Our strategy to potentially accomplish both goals focused on deletion of the *N*-aryl nitrogen atom in **7**, and replacement with a stereogenic carbon center, as in **8** (Fig. 2). We were also interested to see the impact of a chiral α-methyl moiety on the linker with regards to

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[f] This manuscript is in dedication to Prof. H. Alex Brown, on the anniversary of his death.

https://doi.org/10.1016/j.bmcl.2018.10.033
Received 31 July 2018; Received in revised form 18 October 2018; Accepted 20 October 2018

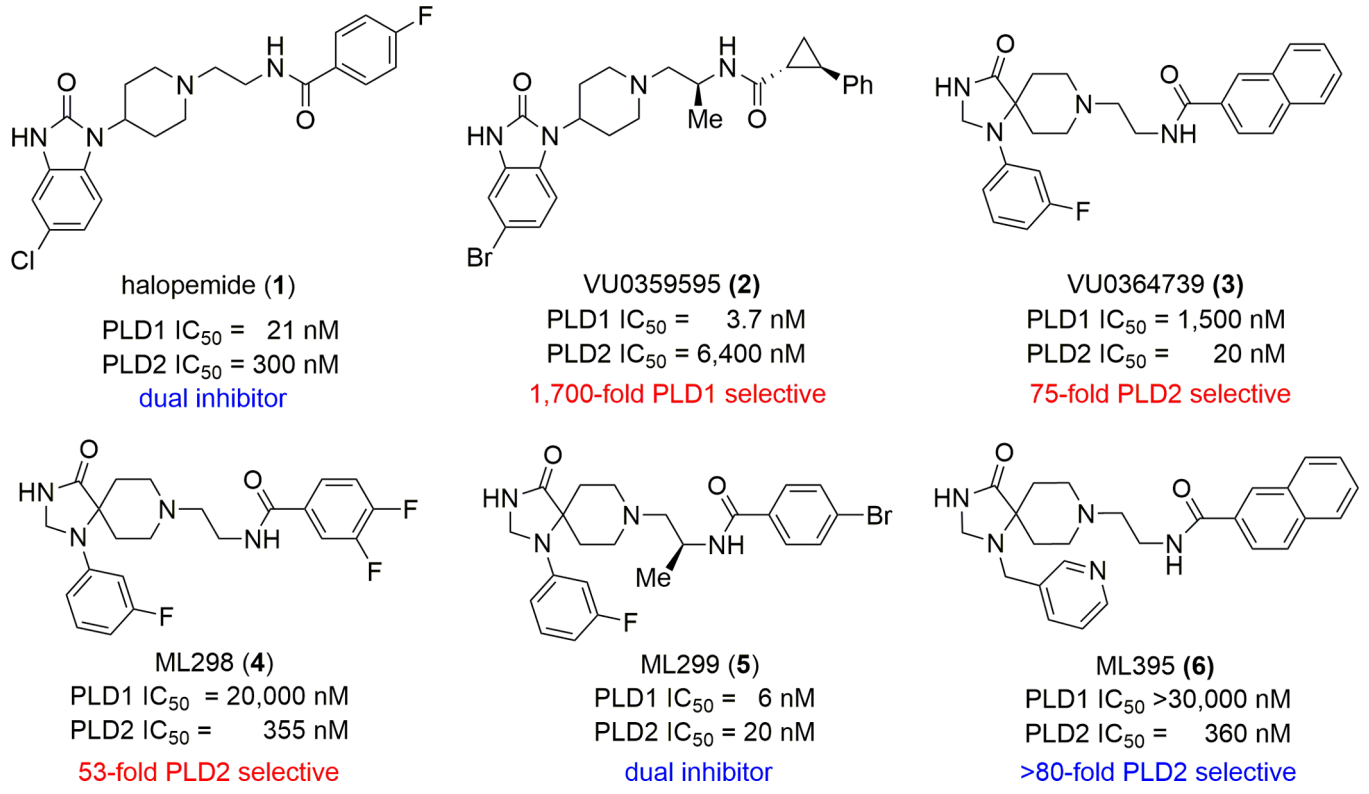


Fig. 1. Halopemide (1) was the progenitor of all PLD inhibitors, and a dual PLD1/2 inhibitor. Optimization efforts led to isoform-selective PLD inhibitors: **2** (1700-fold PLD1 selective), **3** (75-fold PLD2 selective), **4** (53-fold PLD2 selective), **5** (dual PLD1/2 inhibitor) and **6** (> 80-fold PLD2 selective).

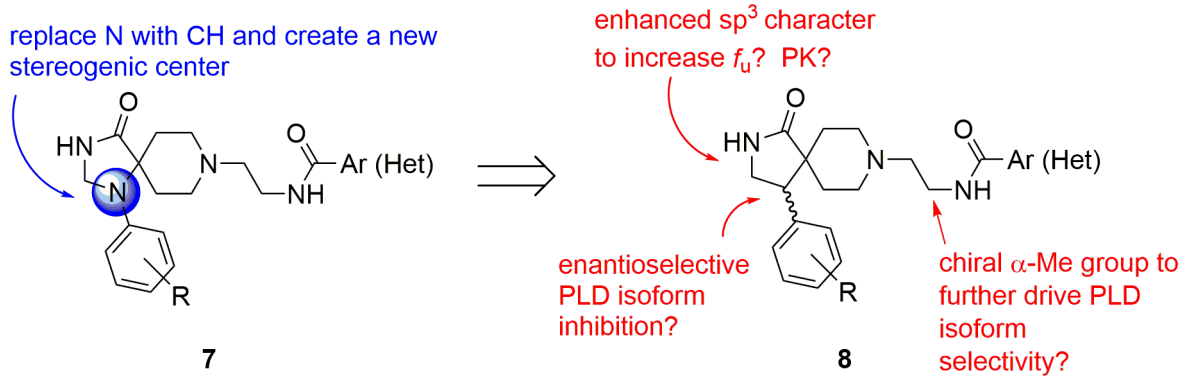


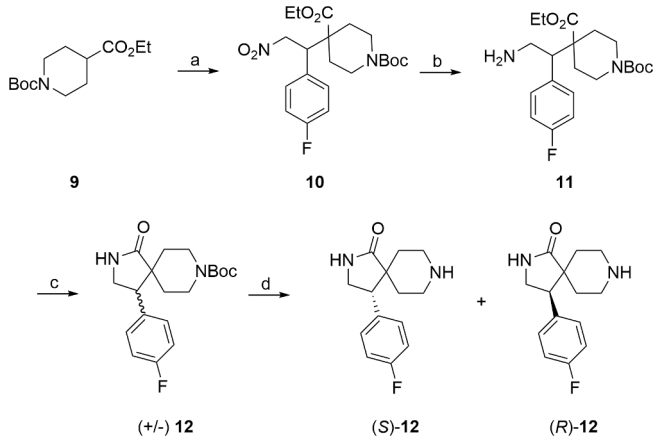
Fig. 2. Envisioned optimization strategy for **7** to improve disposition and structural novelty with analogs **8**.

PLD isoform inhibition (e.g., the 'magic methyl' effect)[24] in combination with the phenyl bearing stereocenter, and if previous SAR for **3–6** would be maintained.[7–14]

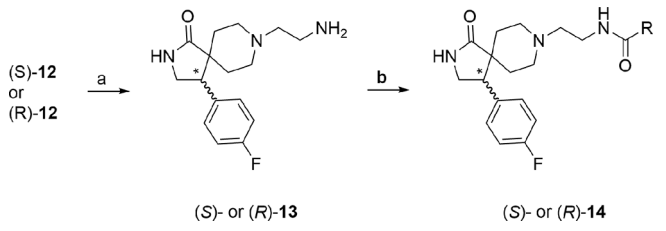
Fortunately, Thomas and co-workers form Roche had previously reported on a racemic synthesis of a 4-fluorophenyl 2,8-diazaspiro[4.5] decanone core,[25] and had provided details for the assignment of the individual enantiomers. Thus, we elected to follow their route and first evaluate both enantiomers of this core for PLD inhibition. As prescribed (Scheme 1), deprotonation of **9** with LDA followed by trapping with *trans*-1-fluoro-4-(2-nitrovinyl)benzene afforded the desired Michael adduct **10** in 90% yield. Reduction of the nitro moiety with Ra-Ni afforded primary amine **11** in 86% yield. Finally, closure to the racemic spiro-γ-lactam (±)-**12** was facilitated by refluxing **11** in toluene overnight; however, some deprotection of the Boc was noted, so the mixture was reprotected to afford Boc-protected (±)-**12** in 83% yield. Preparative chiral separation (2–3 g batches) was performed on a Gilson 215 Preparative HPLC with pumps capable of 200 mL/min using a Chiraltech OJ 20u, 50 × 250 mm column at a flow rate of 177 mL/min (95% hexanes and 5% isopropyl alcohol) to afford (*S*)-**12** and (*R*)-**12**, and in agreement with the work of Thomas.[25]

Once in hand, (*S*)-**12** and (*R*)-**12** were rapidly elaborated into putative PLD inhibitors (*S*)-**14** and (*R*)-**14** in a two-step sequence (Scheme 2).[7–14] Reductive amination with *tert*-butyl 2-oxoethylcarbamate and (*S*)-**12** and (*R*)-**12** provided (*S*)-**13** and (*R*)-**13**, respectively in 79–85% yield after Boc deprotection. Acylation of the primary amine with the requisite acid chloride delivered (*S*)-**14** and (*R*)-**14** in yields ranging from 58 to 95%. To assess the impact of the (*S*)-methyl group on the side chain, a similar two step route was employed (Scheme 3) wherein (*S*)-*tert*-butyl 1-oxo-2-propanylcarbamate is substituted for *tert*-butyl 2-oxoethylcarbamate with comparable yields at each step.

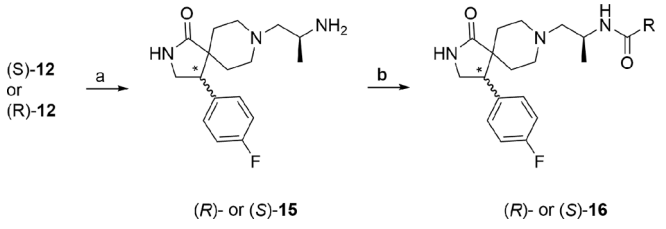
As shown in Table 1, clear enantioselective inhibition of the PLD isoforms was noted, with all (*R*)-**14** analogs typically > 10-fold weaker at inhibiting both PLD1 and PLD2 versus the (*S*)-**14** analogs. For example, (*S*)-**14a** was a 70 nM PLD 2 inhibitor with ~17-fold selectivity versus PLD1, while (*R*)-**14** was > 10-fold weaker at PLD2 (IC_{50} = 810 nM). This SAR trend was conserved across all enantiomeric



Scheme 1. Synthesis of 4-fluorophenyl 2,8-diazaspiro[4.5]decanone cores **12**.[a]
[a]Reagents and conditions. (a) *trans*-1-fluoro-4-(2-nitrovinyl)benzene, LDA, THF, −40 °C, 2 h, 90%; (b) H_2, Ra-Ni, EtOH, 50 °C, 20 h, 86%; (c) i) toluene, reflux; ii) Boc_2O, DIPEA, dioxane-H_2O, 0 °C, 2 h, 83%; d) i) Gilson 215 Preparative HPLC with pumps capable of 200 mL/min using a Chiraltech OJ 20u, 50 × 250 mm column at a flow rate of 177 mL/min (95% hexanes and 5% isopropyl alcohol. (*S*)-**12** is first eluting peak; ii) TFA, CH_2Cl_2, rt, 5 h, 95%.

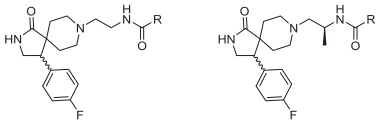


Scheme 2. Synthesis of 2,8-diazaspiro[4.5]decanone-based PLD inhibitors **14**.[a]
[a]Reagents and conditions: (a) i) *tert*-butyl 2-oxoethylcarbamate, $MPB(OAc)_3H$, DCM, rt; ii) 4.0 M HCl/dioxane, DCM, MeOH, rt, 79–85%; (b) RCOCl, DIEA, DMF, rt, 58–95%.

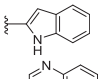
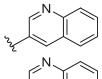
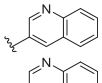
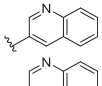
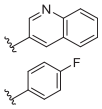
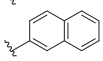


Scheme 3. Synthesis of 2,8-diazaspiro[4.5]decanone-based PLD inhibitors **16**.[a]
[a]Reagents and conditions: (a) i) (*S*)-*tert*-butyl 1-oxo-2-propanylcarbamate, $MPB(OAc)_3H$, DCM, rt; ii) 4.0 M HCl/dioxane, DCM, MeOH, rt, 70–82%; (b) RCOCl, DIEA, DMF, rt, 55–92%.

Table 1
Structures and PLD activities of (*S*)-and (*R*)-**14** and (*S*)-and (*R*)-**16**.



(*S*)- or (*R*)-**14** (*S*)- or (*R*)-**16**

Compound	R	PLD1 IC_{50} (nM)[a]	PLD2 IC_{50} (nM)[a]
(*S*)-**14a**		1200	70
(*R*)-**14a**		6400	810
(*S*)-**16a**		16	150
(*R*)-**16a**		320	4000
(*S*)-**14b**		21	2.5
(*R*)-**14b**		320	43
(*S*)-**16b**		1	12
(*R*)-**16b**		16	140
(*S*)-**14c**		1350	60
(*R*)-**14c**		12,000	450
(*S*)-**16c**		25	115
(*R*)-**16c**		200	700
(*S*)-**14d**		5500	220
(*R*)-**14d**		> 20,000	3175
(*S*)-**16d**		250	210
(*R*)-**16d**		1700	2800
(*S*)-**16e**		820	26

[b]PLD2 cellular assay in HEK293-gfp-PLD2 cells. IC_{50} values are the average of three independent measures (n = 3).
[a] Cellular PLD1 assay in Calu-1 cells.

pairs of (*S*)- and (*R*)-**14** derivatives. Thus, further optimization will maintain the (*S*)-configuration at the aryl bearing stereogenic center. The SAR trend within **3–6** by which a chiral (*S*)-methyl moiety on the linker not only improved PLD activity, but also enhanced PLD1 inhibitory activity was retained in the diazaspiro[4.5]decanone series.[7–14] For example, addition of the (*S*)-methyl moiety as in (*S*)-**16a** increased PLD1 activity ~9-fold over (*S*)-**14a**, providing an ~10-fold PLD1-prefering inhibitor. With the analogous (*R*)-**16a**, activity was increased at PLD1, but with a significant loss of inhibitory activity at PLD2. This enantiospecific modulation of PLD activity was also conserved within the entire series, with the (*S*)-methyl group engendering enhanced PLD1 inhibition with a concomitant loss of PLD2 activity. Notable examples include (*S*)-**16b**, a potent dual PLD1 and PLD2 inhibitor (IC_{50}s of 1 nM and 12 nM, respectively) and (*S*)-**14b**, a 25-fold PLD2 preferring inhibitor, which converts to a dual PLD1/PLD2 inhibitor (*S*)-**16d** upon incorporation of the (*S*)-methyl group. These data proved very exciting, confirming that a single chemotype can provide PLD1-preferring, PLD2-preferring and dual PLD1/PLD2 inhibitors (Fig. 3) based solely on the inclusion of either one or two easily established and constructed chiral centers. These data also supported the first, and only to date, issued U.S. composition of matter patent for PLD inhibitors.[23]

We next evaluated if these novel PLD inhibitors offer advantages in terms of disposition relative to the earlier generation **2–6**, which are plagued by high plasma protein binding (rat and human f_u < 0.03), high predicted hepatic clearance (rat CL_{hep} > 65 mL/min/kg) and very short half-lives *in vivo* ($t_{1/2}$ < 0.15 h). As shown in Table 2, analogs **14** and **16** uniformly showed improved free fraction (in both rat and human plasma, f_us > 0.10) and (*S*)-**14b** and (*S*)-**14d** displayed improved predicted hepatic clearance in rat (41.2 and 47.2 mL/min/kg, respectively) and human (10.7 and 10.1 mL/min/kg, respectively).

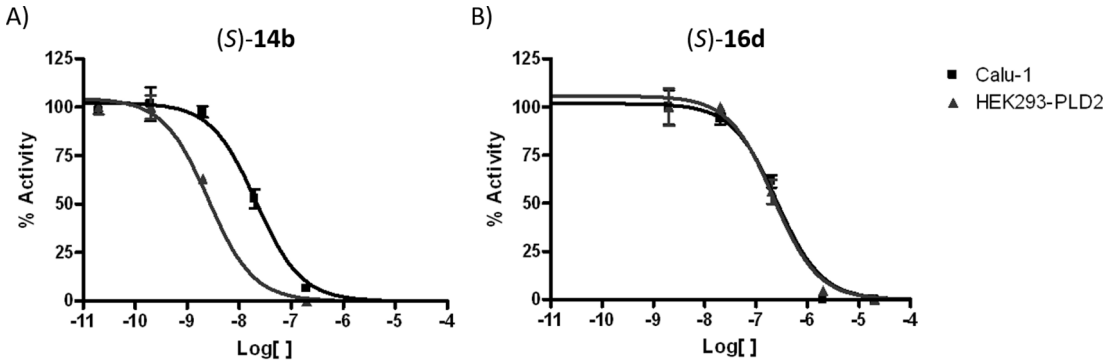


Fig. 3. Representative PLD1 (Calu-1) and PLD2 (HEK293-PLD2) concentration-response-curves for (*S*)-**14b**, a PLD2-preferring inhibitor and (*S*)-**16d**, a dual PLD1/PLD2 inhibitor.

Table 2
In vitro DMPK data for analogs (*S*)-**14** and (*S*)-**16**.

Property	(*S*)-14a	(*S*)-14b	(*S*)-14c	(*S*)-14d	(*S*)-16b	(*S*)-16e
MW (dal)	419.5	434.2	446.2	413.4	448.5	459.5
cLogP	2.87	2.29	2.68	2.84	2.73	3.31
TPSA (Å^2)	61.4	73.4	73.8	92.9	73.4	61.4
In vitro PK parameters						
Rat CL_{HEP} (mL/min/kg)	60.7	41.2	63.8	47.2	53.4	61.6
Human CL_{HEP} (mL/min/kg)	61.7	10.7	17.2	10.1	14.0	17.4
Rat f_u plasma	0.160	0.198	0.314	0.246	0.130	0.061
Hum f_u plasma	0.138	0.124	0.122	0.108	0.136	0.045
Rat f_u brain	0.013	0.004	0.026	0.027	0.004	0.003

Based on the improved predicted hepatic clearance of (*S*)-**14b** and (*S*)-**14d**, we evaluated both in a rat IV PK cassette study (0.25 mg/kg per compound in EtOH/PEG400/DMSO vehicle). Both compounds displayed suprahepatic clearance *in vivo* (CL_ps of 340 and 250 mL/min/kg for (*S*)-**14b** and (*S*)-**14d**, respectively), and thus a lack of an *in vitro*:*in vivo* correlation (IVIVC). However, both compounds showed improved half-lives ($t_{1/2}$s of 3.63 and 3.2 h, for (*S*)-**14b** and (*S*)-**14d**, respectively) driven by large volumes of distribution at steady state (V_{ss} of 80 and 56 L/kg for (*S*)-**14b** and (*S*)-**14d**, respectively). Thus, despite attractive cLogPs, these new inhibitors were clearly deporting in some tissue and being reabsorbed into plasma, providing a sustained half-life and mean residence time. Based on these data and associated concerns regarding accumulation with chronic dosing and the subsequent potential for tox findings, this series could not be further progressed. Still, this new series of 2,8-diazaspiro[4.5]decanones provided improvements in multiple domains over historical PLD inhibitors **2–6**.

In summary, we employed a scaffold-hopping strategy, moving from the classic triazaspiro[4.5]decanone-based PLD inhibitors to a novel 2,8-diazaspiro[4.5]decanone core, harboring a stereogenic center. This new core demonstrated enantioselective inhibition of the individual PLD isoforms (providing access to PLD1, PLD2 and dual PLD1/PLD2 inhibitors in the same scaffold, along with enhanced plasma free fraction (rat, human $f_u < 0.13$), engendered moderate predicted hepatic clearance (rat $CL_{hep} \sim 43$ mL/min/kg), and improved half-lives *in vivo* ($t_{1/2} > 3$ h). Further, this novel chemical matter led to the first issued US patent claiming composition of matter for small molecule PLD inhibitors. While this new series displayed robust and tractable SAR, the extremely high volume of distribution *in vivo* precluded further advancement. Follow-up efforts continue, and work is in progress to develop optimal *in vivo* tool compounds that selectively inhibit either PLD1 or PLD2 as well as clinical candidates.

Acknowledgments

Dr. Lindsley thanks William K. Warren, Jr. who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.) and the William K. Warren Foundation for support of our programs.

References

1. Brown HA, Thomas PG, Lindsley CW. *Nat Rev Drug Discov.* 2017;16:351–367.
2. Selvy PE, Lavieri R, Lindsley CW, et al. *Chem Rev.* 2011;111:6064–6119.
3. Brown HA, Henage LG, Preininger AM, et al. *Methods Enzymol.* 2007;434:49–87.
4. Bruntz RC, Lindsley CW, Brown HA. *Pharmacol Rev.* 2014;66:1033–1079.
5. Scott SA, Matthews TP, Ivanova PT, et al. *Biochim Biophys Mol Cell Biol Lipids.* 2014;1841:1060–1084.
6. Monovich L, Mugrage B, Quadros E, et al. *Bioorg Med Chem Lett.* 2007;17:2310–2311.
7. Scott SA, Selvy PE, Buck JR, et al. *Nat Chem Biol.* 2009;5:108–117.
8. Lewis JA, Scott SA, Lavieri R, et al. *Bioorg Med Chem Lett.* 2009;19:1916–1919.
9. Lavieri R, Lewis JA, Scott SA, et al. *Bioorg Med Chem Lett.* 2009;19:2240–2244.
10. Lavieri R, Scott SA, Selvy PE, et al. *J Med Chem.* 2010;53:6706–6719.
11. O'Reilly MC, Scott SA, Brown KA, et al. *J Med Chem.* 2013;56:2695–2699.
12. O'Reilly MC, Oguin III TH, Scott SA, et al. *ChemMedChem.* 2014;9:2633–2637.
13. Bruntz RC, Taylor HE, Lindsley CW, et al. *J Biol Chem.* 2014;289:600–616.
14. Oguin T, Sharma S, Stuart AD, et al. *J Biol Chem.* 2014;289:25405–25417.
15. Scott SA, Spencer CT, O'Reilly MC, et al. *ACS Chem Biol.* 2015;10:421–432.
16. Noble AR, Maitland NJ, Berney DM, et al. *Br J Cancer.* 2018;118:189–199.
17. Nelson RK, Ya-Ping J, Gadbery J, et al. *Sci Rep.* 2017;7:9112.
18. Stieglitz KA. *Curr Drug Discovery Technol.* 2018;15:81–93.
19. Song HI, Yoon MS. *Sci Rep.* 2016;22:36968.
20. Henkels KM, Muppani NR, Gomez-Cambronero J. *PLoS One.* 2016;7:e20166663.
21. Zhou G, Yu L, Yang W, et al. *Mediators Inflamm.* 2016:2543070.
22. Babenko NA, Kharchenko VS. *Int J Endocrinol.* 2015:7948838.
23. Brown HA, Lindsley CW, Waterson AG, et al. US 9,127,005, September 8; 2015.
24. Schonherr H, Cernak T. *Angew Chem Int Ed.* 2013;52:12256–12267.
25. Kraft EA, Kurt A, Maier A, et al. *Synthesis.* 2005;19:3245–3252.