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Synthesis and SAR of 2-arylbenzoxazoles, benzothiazoles and benzimidazoles as inhibitors of lysophosphatidic acid acyltransferase-β

Baoqing Gong, Feng Hong, Cory Kohm, Lynn Bonham and Peter Klein*

Cell Therapeutics, Inc., 201 Elliott Ave. W., Suite 400, Seattle, WA 98119, USA

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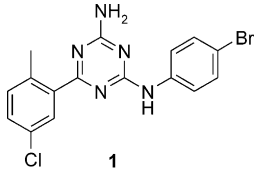
Abstract—2-Arylbenzoxazoles, benzothiazoles and benzimidazoles were identified as new classes of potent, isoform specific inhibitors of lysophosphatidic acid acyltransferase-β (LPAAT-β). Effects of selected inhibitors on proliferation of tumor cells in vitro were investigated.

LPAAT is an integral membrane protein that catalyzes the biosynthesis of phosphatidic acid (PA) from lysoPA (LPA). PA has been implicated in cell signaling events including Ras-associated factor (Raf) translocation to membranes, mammalian target of rapamycin (mTOR) activation, epidermal growth factor receptor (EGFR) internalization, and activation of protein kinase C-ζ (PKCζ).[1–4] LPAAT-α was shown to be uniformly expressed in all human tissues tested, while LPAAT-β displays distinct tissue distribution and is highly expressed in a wide variety of tumor cells and their surrounding stroma.[5–9] Ectopic over expression of LPAAT-β cooperates in activation of Ras/Raf/Erk pathway in *Xenopus* oocytes and contributes to transformation of human and rodent cells in vitro.[9,10] PA produced by LPAAT-β appears to play an important role in signaling pathways involved in tumor cell survival.[10] Knockdown of LPAAT-β expression by RNA interference (RNAi) blocked tumor cell proliferation.[9] Accordingly, LPAAT-β may provide a novel target for cancer therapy.

A group of aryl triazines have been reported as isoform specific inhibitors of LPAAT-β.[11] This group is exemplified by **1** (CT-32228, LPAAT-β $IC_{50} = 0.06$ μM), a highly effective antiproliferative agent with a broad range of efficacy toward a variety of tumor cells in vitro with IC_{50}'s ranging from 0.025 μM to 0.5 μM.[9,10]

We screened a structurally diverse library of compounds for their ability to inhibit the enzymatic activity of human LPAAT-β, which was over expressed in SF9 insect cell membranes, and found 2-arylbenzoxazole **2** to be a weak inhibitor ($IC_{50} = 1.7$ μM).[12] Representative of a new structural class of LPAAT-β inhibitors, **2** did not inhibit LPAAT-α up to at least 64 μM. In this report we summarize the synthesis and SAR of 2-arylbenzoxazole, 2-arylbenzothiazole and 2-arylbenzimidazole analogues of **2** as new classes of isoform specific LPAAT-β inhibitors. Selected inhibitors were tested for antiproliferative effects in tumor cell lines in vitro.



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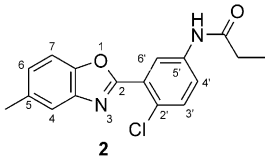

2

2-Arylbenzoxazole analogues of **2** were synthesized in 3 or 4 steps (Schemes 1). *N*-Acylation of substituted 2-aminophenol **3** with a 6-substituted 3-nitrobenzoyl chloride **4** yielded an anilide, which upon acid-promoted cyclization, provided the corresponding 2-(3-nitroaryl)-benzoxazole **6**.[13,14] For those 3-nitrobenzoyl chlorides that failed to produce **6** we employed the corresponding 6-substituted 3-nitrobenzoic acid **5** in a high temperature (150 °C) polyphosphoric acid facilitated condensation to generate **6**.[15] Reduction of **6** yielded aniline **7**, which upon *N*-acylation with an acid chloride or a

* Corresponding author. Fax: +1-206-284-6206; e-mail: jpeterklein@yahoo.com

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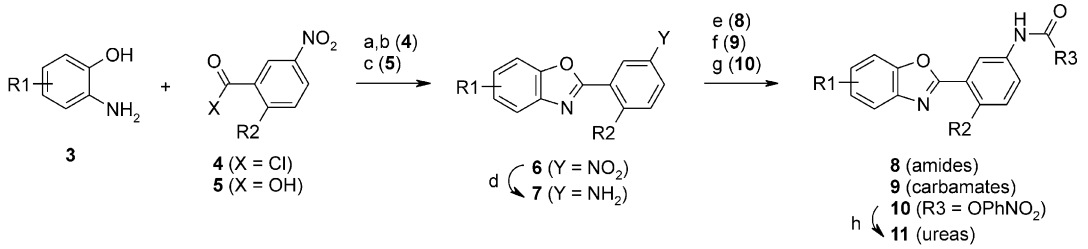


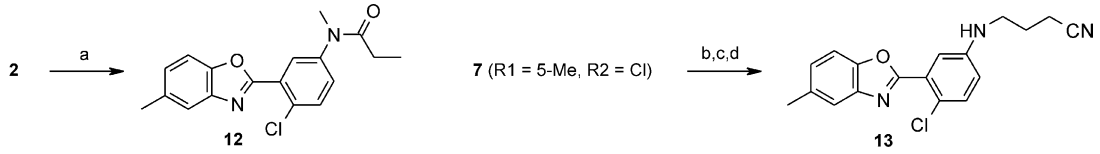
Scheme 1. Reagents and conditions: (a) TEA, THF; (b) *p*-TsOH, xylene, reflux; (c) PPA, 150 °C; (d) Fe powder, AcOH, MeOH, reflux; (e) an acid chloride (R3COCl), pyridine; (f) a chloroformate (R3COCl), pyridine; (g) 4-nitrophenyl chloroformate, TEA, THF; (h) an amine (R3).

chloroformate yielded amide **8** or carbamate **9**, respectively. Treatment of **7** with 4-nitrophenyl chloroformate yielded 4-nitrophenyl carbamate **10**, which, without isolation, was treated with a substituted amine to provide urea **11**. Structural diversity was afforded by substituents in the three reaction components; benzoic acid, 2-aminophenol and *N*-acylation reagent.

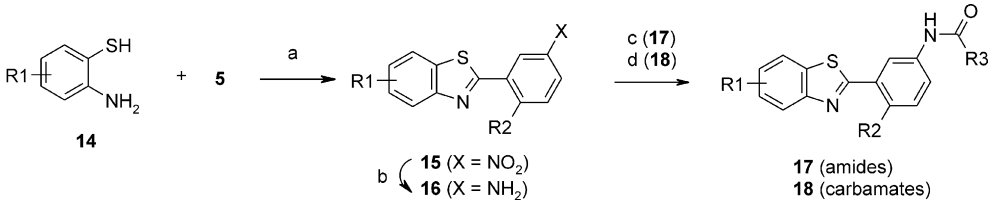
To evaluate structural requirements at the 5′-position of the 2-aryl substituent for LPAAT-β activity, we synthesized 2-arylbenzoxazole analogues of **2** with modifications at the 5′-position (Scheme 2). Methylation of the amide nitrogen in **2** using NaH-MeI provided **12**. Mono-*N*-alkylation of **7** involved *N*-formylation, followed by amide *N*-alkylation, and lastly deformylation to yield **13**, a compound lacking a carbonyl functionality in the 5′ substituent.

2-Arylbenzothiazole bioisoteres of active 2-arylbenzoxazoles were synthesized as outlined in Scheme 3. High temperature (150 °C) acid-promoted condensation of 2-aminothiophenol **14** and **6** yielded 2-(3-nitroaryl)-benzothiazole **15**.[15] Reduction to aniline **16** followed by *N*-acylation with an acid chloride or a chloroformate provided amide **17** or carbamate **18**, respectively.

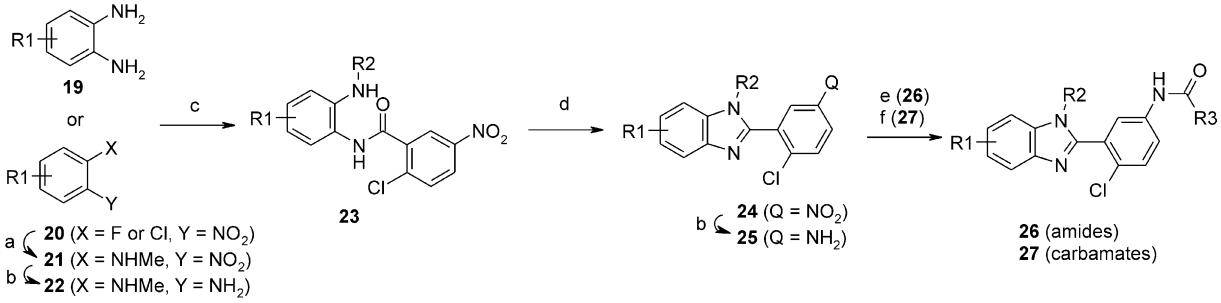
2-Arylbenzimidazole analogues of active 2-arylbenzoxazoles were synthesized as described in Scheme 4. Substitution on a benzimidazole nitrogen provided the opportunity for additional diversity. *N*-Unsubstituted benzimidiazole **24** (R2 = H) was synthesized from 1,2-phenylenediamine **19**. Synthesis of *N*-methyl benzimidazole **24** (R2 = Me) required synthesis of *N*-methyl 1,2-phenylenediamine **22**, accomplished by displacement of an *ortho*-nitro substituted aryl fluoride or chloride **20**



Scheme 2. Reagents and conditions: (a) NaH, MeI, THF; (b) 98% HCO_2H, EDC, $CHCl_3$; (c) NaH, $Br(CH_2)_3CN$, DMSO; (d) NH_2NH_2, H_2O, EtOH.



Scheme 3. Reagents and conditions: (a) PPA, 150 °C; (b) Fe powder, AcOH, MeOH, reflux; (c) an acid chloride (R3COCl), pyridine; (d) a chloroformate (R3COCl), pyridine.

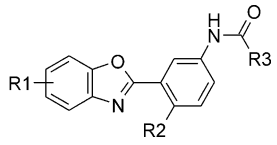


Scheme 4. Reagents and conditions: (a) NH_2Me, EtOH, reflux; (b) Fe powder, concd HCl, EtOH, water, reflux; (c) **4** (R2 = Cl), TEA, THF, 0 °C; (d) AcOH, reflux; (e) an acid chloride (R3COCl), pyridine; (f) a chloroformate (R3COCl), pyridine.

with methylamine to give **21** followed by reduction to nonsymmetrical 1,2-phenylendiamine **22**. Condensation of either **19** or **22** with **5** (R2 = Cl) yielded **23** which on acid-promoted cyclization gave benzimidazole **24**.[16] Reduction provided aniline **25** and *N*-acylation with an acid chloride or a chloroformate gave amide **26** or carbamate **27**, respectively.

The compounds listed in Tables 1–3 were tested for their ability to inhibit human LPAAT-β, which was over expressed in SF9 insect cell membranes.[12] None of the listed compounds inhibited similarly over expressed LPAAT-α up to at least 20 μM (data not shown).

Table 1. Inhibition of LPAAT-β by 2-arylbenzoxazoles



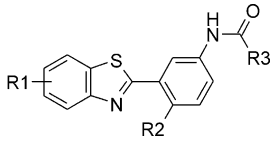
Compd	R1	R2	R3	LPAAT-β IC_{50} (μM)
2	5-Me	Cl	Et	1.5
8a	5-Me	Cl	H	> 5
8b	5-Me	Cl	Me	2.5
8c	5-Me	Cl	*n*-Pr	2.5
8d	5-Me	Cl	CH_2OH	31
8e	5-Me	Cl	C≡CMe	> 20
8f	5-Me	Cl	$(CH_2)_2C{\equiv}CH$	0.5
8g	5-Me	Cl	$CH_2C{\equiv}N$	0.1
8h	5-Me	Cl	CH_2N_3	0.6
8i	5-Me	Cl	Ph	40
8j	5-Cl	Cl	CH_2NHMe	> 5
8k	5-Cl	Cl	CH_2NMe_2	> 5
8l	5-Cl	Cl	C≡CMe	> 20
8m	5-Cl	Cl	$(CH_2)_2C{\equiv}CH$	0.1
8n	5-Cl	Cl	$CH_2C{\equiv}N$	0.027
8o	5-Cl	Cl	CH_2N_3	0.17
9a	5-Me	Cl	OMe	1
9b	5-Me	Cl	OEt	1.5
9c	5-Me	Cl	O(*n*-Pr)	12
9d	5-Me	Cl	O(*i*-Pr)	16
9e	5-Me	Cl	$OCH_2CH{=}CH_2$	1.4
9f	5-Me	Cl	$OCH_2C{\equiv}CH$	0.19
9g	5-Me	H	OMe	> 64
9h	5-Me	Me	OMe	1.5
9i	5-Me	OMe	OMe	64
9j	5-Me	F	OMe	> 64
9k	H	Cl	OMe	0.5
9l	4-Me	Cl	OMe	0.5
9m	6-Me	Cl	OMe	0.4
9n	5-CF_3	Cl	OMe	0.8
9o	5-Cl	Cl	OMe	0.3
9p	5-Ph	Cl	OMe	64
9q	5-CO_2H	Cl	OMe	> 20
9r	5-OH	Cl	OMe	> 20
9s	5-Cl	Me	OMe	0.55
9t	5-Cl	Cl	$OCH_2C{\equiv}CH$	0.035
9u	5-Cl	Cl	$O(CH_2)_2C{\equiv}CH$	0.075
9v	5-Cl	Cl	$OCH_2C{\equiv}CMe$	0.075
9w	H	Cl	$OCH_2C{\equiv}CH$	0.05
9x	4-Me	Cl	$OCH_2C{\equiv}CH$	0.16
9y	5-CF_3	Cl	$OCH_2C{\equiv}CH$	0.14
9z	5-OH	Cl	$OCH_2C{\equiv}CH$	2
9aa	5-Cl	Me	$OCH_2C{\equiv}CH$	0.1
11a	5-Cl	Cl	NHMe	0.95
11b	5-Cl	Cl	$NHCH_2C{\equiv}CH$	0.1

Analysis of the assay data for 2-arylbenzoxazoles (Table 1) indicated that small substituents (H, CH_3, Cl, CF_3) generally were well tolerated at positions 4, 5, or 6 (R1) of the benzoxazole, but comparable compounds with hydrogen bonding substituents in this ring (**9q**, **9r** and **9z**) were weak inhibitors.

Appropriate positional substitution on the 2-phenyl ring was essential for LPAAT-β inhibition. Compounds with the 2′,5′-disubstitution pattern in this ring were active inhibitors. Analogues with the 5′-substituent moved to either the 3′ or 4′ position were inactive (data not shown). Compound **9g**, unsubstituted at the 2′ position (R2 = H), was devoid of activity indicating a requirement for 2′ substitution. Compounds with a small substituent (R2 = Cl, Me) at the 2′ position were most active. However, highly electron withdrawing substituents as in **9i** (R2 = OMe) and **9j** (R2 = F) were not tolerated.

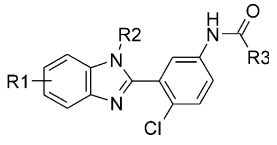
At the 5′ position, an acylamino substituent appears essential for LPAAT-β inhibition. Compounds with an amide, carbamate or urea as the 5′ substituent were highly active inhibitors. Weak inhibition by the *N*-methyl amide **12** (IC_{50} = 12 μM) and 5′-alkylamino compound **13** (IC_{50} > 5μM) indicated a preference for

Table 2. Inhibition of LPAAT-β by 2-arylbenzothiazoles



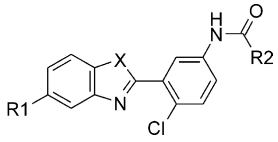
Compd	R1	R2	R3	LPAAT-β IC_{50} (μM)
17a	5-CF_3	Cl	$CH_2C{\equiv}N$	0.05
17b	5-Cl	Cl	$CH_2C{\equiv}N$	0.015
17c	H	Me	$CH_2C{\equiv}N$	0.07
18a	4-Me	Cl	OMe	9
18b	6-Me	Cl	OMe	> 64
18c	5-CF_3	Cl	OMe	0.2
18d	5-Cl	Cl	OMe	0.04
18e	5-CF_3	Cl	$OCH_2C{\equiv}CH$	0.015
18f	5-Cl	Cl	$OCH_2C{\equiv}CH$	0.006
18g	5-Cl	Me	$OCH_2C{\equiv}CH$	0.015

Table 3. Inhibition of LPAAT-β by 2-arylbenzimidazoles



Compd	R1	R2	R3	LPAAT-β IC_{50} (μM)
26	5-Cl	Me	$CH_2C{\equiv}N$	0.3
27a	5-Me	Me	OMe	11
27b	6-Me	Me	OMe	> 20
27c	5-CF_3	H	$OCH_2C{\equiv}CH$	5.2
27d	5-CF_3	Me	$OCH_2C{\equiv}CH$	0.12
27e	5-Cl	Me	$OCH_2C{\equiv}CH$	0.06
27f	5-Cl	Me	OMe	0.65

Table 4. Inhibition of growth of MCF-7 breast and DU145 prostate carcinoma cells by LPAAT-β inhibitors



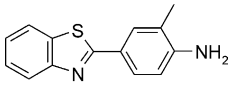
Compd	X	R1	R2	LPAAT-β IC_{50} (μM)	MCF-7 IC_{50} (μM)[a]	DU145 IC_{50} (μM)[a]
2	O	Me	Et	1.5	40	40
9t	O	Cl	$OCH_2C{\equiv}CH$	0.035	15	50
9w	O	H	$OCH_2C{\equiv}$ CH	0.05	20	50
18e	S	CF_3	$OCH_2C{\equiv}$ CH	0.015	30–40	20
18f	S	Cl	$OCH_2C{\equiv}$ CH	0.006	>50	>50

[a] IC_{50}'s were determined using a fluorescence-based indirect assay of proliferation; CyQuant (Molecular Probes, Eugene, OR).

–NHC(=O)– at the 5′-position. The most potent inhibitors in this series incorporated sp hybridization in the 5′-acylamino substituent. Analogues with an alkynyl or a cyano group in the 5′ substituent were particularly effective inhibitors. Comparing activities for an homologous series of 5′-substituted 3-carbon carbamates **9c**, **9e**, and **9f**, inhibition followed a trend with increased pi bonding: propyl <allyl <propargyl. Noteworthy exceptions to this enhanced activity with increased sp hybridization were observed with compounds **8e** and **8l** with the alkynyl group in conjugation with the 5′-acyl carbonyl group. These two agents were devoid of LPAAT-β inhibitory activity.

Analysis of the assay data for 2-arylbenzothiazoles (Table 2) indicated a trend toward increased LPAAT-β inhibition by replacement of the benzoxazole oxygen with sulfur. In fact, the most potent analogue of **2** tested was **18f**, a benzothiazole with $IC_{50}=0.006$ μM. However, SAR differences between benzoxazoles and benzothiazoles were observed that were contrary to this trend. For example, compounds **9l** with 4-methyl substitution and **9m** with 6-methyl substitution in the benzoxazole were effective inhibitors with $IC_{50}=0.5$ μM and 0.4 μM, respectively. In contrast, comparable compounds **18a** and **18b** in the benzothiazole series were significantly less active with $IC_{50}=9$ μM and >64 μM, respectively, indicative of the existence of more rigorous structural constraints in benzothiazole binding to LPAAT-β compared to the analogous benzoxazole.

Compound **28** is a member of a series 2-arylbenzothiazoles reported to have potent antitumor activity but its biomolecular target has not been identified.[17] Because of its structural similarity to our LPAAT-β inhibitors, we tested **28** for LPAAT-β inhibition and found it to be inactive ($IC_{50}>40$ μM).



28

Of the 2-arylbenzimidazoles listed in Table 3, compounds with R2 = Me were considerably more potent inhibitors than the compound with R2 = H. Within the group of 2-arylbenzimidazoles with R2 = Me, inhibitors were of comparable potency to 2-arylbenzoxazoles.

Selected LPAAT-β inhibitors were tested for their ability to inhibit proliferation of MCF-7 breast and DU145 prostate carcinoma cells in vitro (Table 4) but proved to be only weakly antiproliferative, suggestive of limited uptake by tumor cells.[18] While **9t** and **9w** display some increased antiproliferative effect on the mammary tumor cell line compared to the prostate tumor cell, the other compounds show a very similar level of antiproliferative effect on both lines. Although most of these agents were antiproliferative to both cell lines tested, the relatively high concentrations necessary to reach IC_{50}s (15–50 μM) would suggest that they might not prove to be particularly efficacious in vivo. However, with regard to their properties as new molecular classes that specifically inhibit the beta isoform of LPAAT these agents may prove useful as tools for the study of inhibition of cell signaling processes involving LPA and PA.

Acknowledgements

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References and notes

1. Ghosh, S.; Strum, J. C.; Sciorra, V. A.; Daniel, L.; Bell, R. M. *J. Biol. Chem.* **1996**, *271*, 8472.
2. Fang, Y.; Vilella-Bach, M.; Bachmann, R.; Flanigan, A.; Chen, J. *Science* **2001**, *294*, 1942.
3. Shen, Y.; Xu, L.; Foster, D. A. *Mol. Cell. Biol.* **2001**, *21*, 595.
4. Limatola, C.; Schaap, D.; Moolenaar, W. H.; van Blitterswijk, W. J. *Biochem. J.* **1994**, *3*, 1001.
5. West, J.; Tompkins, C. K.; Balantac, N.; Nudelman, E.; Meengs, B.; White, T.; Bursten, S.; Coleman, J.; Kumar, A.; Singer, J. W.; Leung, D. W. *DNA Cell Biol.* **1997**, *16*, 691.
6. Leung, D. W. *Frontiers Biosci.* **2001**, *6*, 944.
7. Kume, K.; Shimizu, T. *Biochem. Biophys. Res. Commum.* **1997**, *237*, 6632.
8. Eberhardt, C.; Gray, P. W.; Tjoelker, L. W. *J. Biol. Chem.* **1997**, *272*, 20299.

9. Bonham, L.; Leung, D.; White, T.; Hollenback, D.; Klein, P.; Tulinsky, J.; Coon, M.; de Vries, P.; Singer, J. W. *Exp. Opin. Ther. Targets* **2003**, *7*, 643.
10. Coon, M.; Ball, A.; Pound, J.; Ap, S.; Hollenback, D.; White, T.; Tulinsky, J.; Bonham, L.; Morrison, D. K.; Finney, R.; Singer, J. W. *Mol. Cancer Ther*. **2003**, *2*, 1067.
11. Bhatt, R.; Gong, B.; Hong, F.; Jenkins, S. A.; Klein, J. P.; Kumar, A. M.; Tulinsky, J. PCT Int. Appl. WO 03/037346, 2003.
12. LPAAT-α and LPAAT-β assays were conducted using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) as the colorimetric reagent according to the method described in ref 10.
13. Haugwitz, R. D.; Angel, R. G.; Jacobs, G. A.; Maurer, B. V.; Narayanan, V. L.; Cruthers, L. R.; Szanto, J. *J. Med. Chem*. **1982**, *25*, 969.
14. Suto, M. J.; Turner, W. R. *Tetrahedron Lett*. **1995**, *36*, 7213.
15. Richardson, T. O.; Shanbhag, V. P.; Adair, K.; Smith, S. *J. Heterocycl. Chem*. **1998**, *35*, 1301.
16. Nestor, J. J.; Horner, B. L.; Ho, T. L.; Jones, G. H.; McRae, G. I.; Vickery, B. H. *J. Med. Chem*. **1984**, *27*, 320.
17. Hutchinson, I.; Jennings, S. A.; Vishnuvajjala, B. R.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem*. **2002**, *45*, 744 and references cited therein.
18. Proliferation assays were conducted using the CyQuant Cell Proliferation Kit (Molecular Probes, Eugene, OR) as described in ref 10.