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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4703–4707

# Diamino-*C*,*N*-diarylpyridine positional isomers as inhibitors of lysophosphatidic acid acyltransferase-β

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Received 2 June 2005; revised 14 July 2005; accepted 27 July 2005
Available online 6 September 2005

**Abstract**—2,6-Diamino-4,*N*-diarylpyridines were identified as potent, isoform selective inhibitors of the enzymatic activity of lysophosphatidic acid acyltransferase-β (LPAAT-β).


Lysophosphatidic acid acyltransferase (LPAAT) activities, detected in bacteria, yeast, plant, and animal cells, catalyze the *sn*-2 acylation of lysophosphatidic acid (1-acyl-*sn*-glycerol-3-phosphate, LPA) to phosphatidic acid (1,2-diacyl-*sn*-glycerol-3-phosphate, PA). PA is a component of cell membranes and a key intermediate in the de novo synthesis of phosphoglycerides, which comprise the major components of cell membranes, and of triacylglycerol, the major form of energy storage in plants and animals. The majority of LPAAT activity in mammalian cells has been attributed to two membrane-associated isoforms, LPAAT-α and LPAAT-β. These isoforms share about 34% sequence identity, and contain putative transmembrane domains and two highly conserved motifs, NHQSXXD and EGTR, essential for the catalytic activity of a family of acyltransferases.[1,2] LPAAT-α and LPAAT-β also display similar substrate preferences.[3–7] While LPAAT-α is uniformly expressed in all human tissues tested, LPAAT-β appears to be expressed more prominently in liver, heart, and pancreatic tissues, as well as in a wide variety of tumor cells and their surrounding stroma.[1,3,4,8,9] Both isoforms are highly expressed in adipocytes.[10] Curiously, the LPAAT-β gene has been linked to a rare form of congenital, generalized lipodystrophy (CGL).[11] These individuals have non-functional or missing LPAAT-β genes and are characterized by a nearly complete absence of non-mechanical adipose tissue from birth. Patients also have high blood triacylglycerol levels and develop extreme insulin resistance along with its complications. The relationship between LPAAT-β and CGL has yet to be fully elucidated, particularly since patients presumably still have functional LPAAT-α activity.

PA has also been implicated as a lipid cofactor in cell signaling events including Raf translocation to membranes, mTOR activation, epidermal growth factor receptor (EGFR) internalization, and activation of PKCζ.[12–15] The production of PA associated with these cellular events is often attributed to the catalytic activity of phospholipase D. However, no small molecule inhibitor of this enzyme has been reported to help confirm this hypothesis. In contrast, ectopic overexpression of LPAAT-β has been shown to cooperate in activation of the Ras/Raf/Erk pathway in *Xenopus* oocytes and LPAAT-β appears to play a role in tumor cell survival.[16] RNAi knockdown of LPAAT-β blocks tumor cell proliferation.[9]

In spite of the ubiquity of LPAATs in all life forms and their potential importance in membrane homeostasis and possibly lipid signaling, little progress has been made to fully clarify their functions in cells. The synthesis of potent, isoform-specific, membrane permeable, small molecule inhibitors of LPAAT-β would greatly aid in elucidating the biological role of LPAAT-β.

Groups of isoform-specific inhibitors of LPAAT-β have been reported and include 2-arylbenzoxazoles **1**, 2-arylbenzothiazoles **2**, 2-arylbenzimidazoles **3**,[17] diamino-*C*,*N*-diaryltriazines **4**,[18] and diamino-*C*,*N*-diarylpyrimidine positional isomers **5**, **6**, and **7** (Fig. 1).[19] Triazine **4** and diamino-*C*,*N*-diarylpyrimidine positional isomer **5** emerged as potent LPAAT-β inhibitors.[18,19]

To clarify further the structural requirements for LPAAT-β inhibitor activity developed with the triazines

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doi:10.1016/j.bmcl.2005.07.055

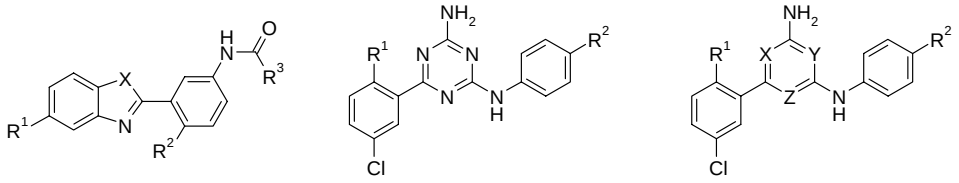


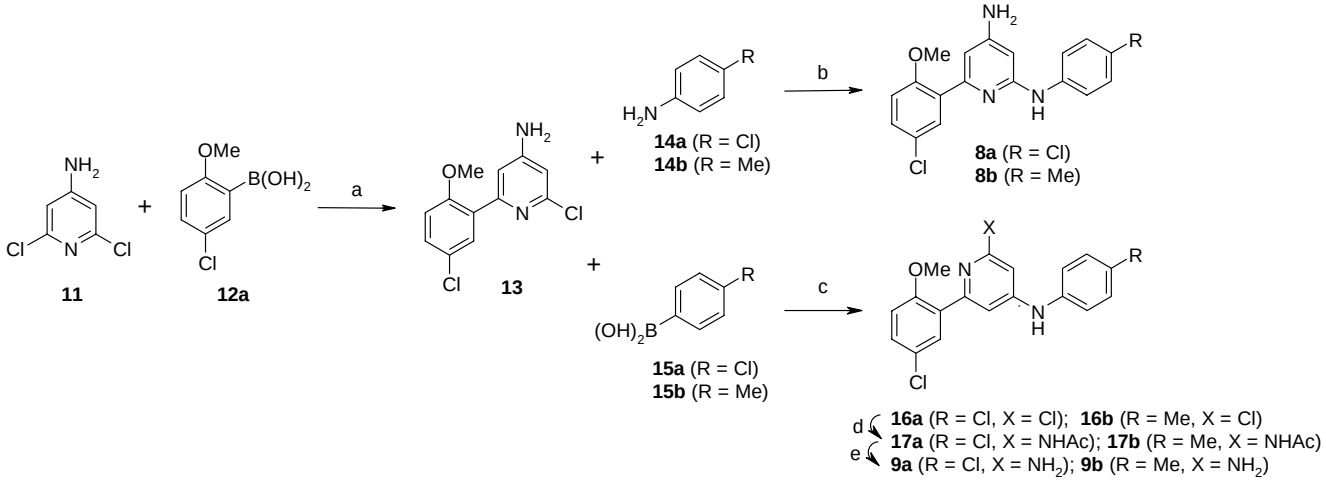

**Figure 1.**

and pyrimidines, we replaced these heterocyclic scaffolds with a pyridine ring. Although substitution on vicinal ring carbons is possible with pyridines, we chose to focus on non-vicinal positional isomers **8**, **9**, and **10** based on their structural similarities to the reported triazines and pyrimidine inhibitors. This report presents the syntheses of diamino-*C*,*N*-diarylpyridine positional isomers **8**, **9**, and **10**, and their ability to inhibit the enzymatic activity of LPAAT-β.

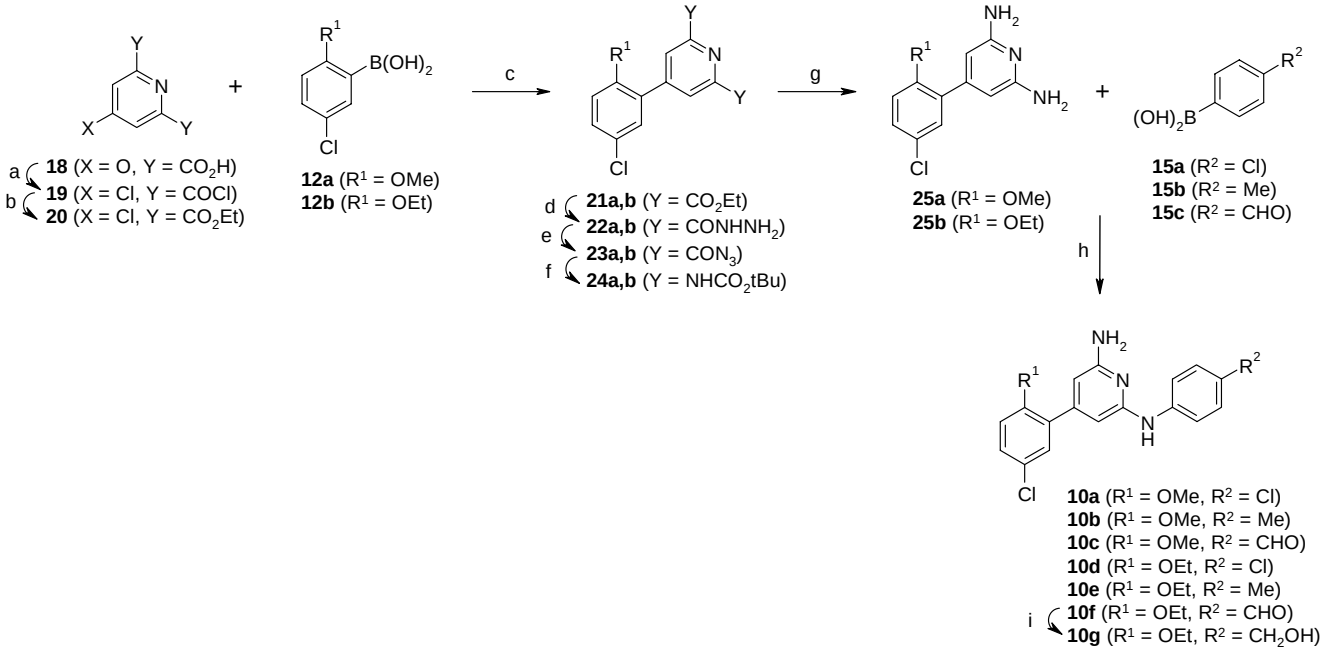
Approaches for the synthesis of 2,4,6-trisubstituted pyridines for use in drug discovery have been reported recently.[20–22] Our approach to synthesizing appropriately substituted pyridines as analogs of triazine **4** and pyrimidines **5**, **6**, and **7** utilized methods involving C-arylation of the pyridine ring and N-arylation of aminopyridines. The substitution patterns in the two aryl rings ($R^1$, $R^2$, and Cl), optimized for the triazine and pyrimidine series, were preserved in the pyridine series.[18,19]

Syntheses of 2,4-diamino-$N^2$,6-diarylpyridine **8** and 2,4-diamino-$N^4$,6-diarylpyridine **9** were carried out, as described in Scheme 1. Both **8** and **9** were prepared from 4-amino-2-aryl-6-chloropyridine **13**, which, in turn, was prepared via a palladium catalyzed Suzuki coupling of 4-amino-2,6-dichloropyridine (**11**) and aryl boronic acid **12**.[23] Displacement of the chloro group in **13** by heating with excess neat substituted aniline **14** at 180 °C yielded **8** after aqueous acid treatment to remove unreacted **14** and column chromatography. For the synthesis of **9**, Cu(II) promoted N-arylation of intermediate **13** with arylboronic acid **15** afforded 2-chloropyridine **16**.[24–26] Surprisingly, **16** proved exceptionally resistant to aminolysis procedures to produce **9**. This conversion was accomplished by a novel two-step approach. Heating a mixture of **16**, acetamide, and copper powder provided acetamide **17**. Amide hydrolysis of the acetamide group in **17** by refluxing with hydrazine and ethanol afforded **9**.

2,6-Diamino-$N^2$,4-diarylpyridine **10** was synthesized, as described in Scheme 2. Treatment of chelidamic acid (**18**) with oxalyl chloride yielded 4-chloropyridine dicarbonyl dichloride (**19**). Ethanolysis of **19** gave diester **20**. 4-Arylation of **20** by palladium catalyzed Suzuki coupling with arylboronic acid **12** afforded 4-arylpyridine diester **21**. Conversion of the two esters in **21** to amino groups utilized a 4-step sequence involving Curtius rearrangement. Heating **21** with hydrazine and ethanol yielded bis-acylhydrazide **22**, which upon treatment with nitrous acid gave bis-acylazide **23**. Curtius rearrangement proceeded upon heating **23** with *t*-butanol and toluene affording bis-*t*-butylcarbamate **24**, which was deprotected with TFA to give diaminopyridine **25**.




**Scheme 1.** Reagents and conditions: (a) $Pd(OAc)_2$, $PPh_3$, CsF, $H_2O$, DME, 80 °C (40% yield); (b) 180 °C (75–80% yield); (c) $Cu(OAc)_2$, $Et_3N$, 4 Å mol sieve, $CH_2Cl_2$ (68–70% yield); (d) acetamide, Cu powder, 180 °C (35–40% yield); (e) $NH_2NH_2$, EtOH, reflux (74–80% yield).



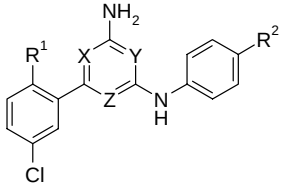

**Scheme 2.** Reagents and conditions: (a) $(COCl)_2$, DMF, $CH_2Cl_2$, reflux; (b) EtOH, pyridine (70% yield for 2 steps); (c) $Pd(OAc)_2$, $PPh_3$, $Na_2CO_3$, $H_2O$, DME (65–70% yield); (d) $NH_2NH_2$, EtOH, reflux; (e) $NaNO_2$, 0.5 M aq HCl (85–90% yield for 2 steps); (f) *t*-BuOH, toluene, reflux (35–40% yield); (g) $CF_3CO_2H$, $CH_2Cl_2$ (88–90% yield); (h) $Cu(OAc)_2$, $Et_3N$, 4 Å mol sieve, $CH_2Cl_2$ (20–35% yield); (i) $NaBH_4$, MeOH, THF (40% yield).

Cu(II) promoted N-arylation of **25** with **15** completed the synthesis of **10a**–**f**. Benzyl alcohol **10g** was synthesized by $NaBH_4$ reduction of aldehyde **10f**. Synthesized compounds were purified either by flash chromatography or preparative TLC to provide each compound to be screened for LPAAT activity as single spot by TLC. $^1H$ NMR and MS data of synthesized compounds are reported.[27]

Synthesized pyridines **8**, **9**, and **10** were tested for their ability to inhibit the enzymatic activity of human LPAAT-β and LPAAT-α, which were separately overexpressed in SF9 insect cell membranes.[28] Table 1 compares LPAAT-β inhibition by pyridines **8**, **9**, and **10**. Examples of positional isomer **10** were consistently more potent than examples of either **8** or **9**. For example, comparing inhibition by isomeric **8a**, **9a**, and **10a** it is evident that **10a** is at least 100-fold more potent than the other isomers. Of the pyridines listed in Table 1, **10a** with $IC_{50} = 0.04\ \mu M$ was the most potent. This potency preference for one of three positional isomers mirrors observations with pyrimidine positional isomers **5**, **6**, and **7**, where only isomer **5** displayed potent LPAAT-β inhibition. Comparing the potency preferences for pyridines, pyrimidines, and triazines, it is tempting to speculate that the presence of a nitrogen atom at the ring position labeled Y in Table 1 may be an important structural component influencing inhibitor potency. Contrary to the LPAAT-β data, none of the compounds measurably inhibited LPAAT-α activity up to a concentration of 40 μM.

In summary, we have synthesized pyridine ring analogs of potent triazine and pyrimidine LPAAT-β inhibitors. The three pyridine positional isomers **8**, **9**, and **10** were synthesized by novel approaches employing palladium promoted C-arylation and Cu(II) promoted N-arylation methodologies. These compounds potently inhibited LPAAT-β activity but not LPAAT-α activity, in spite

**Table 1.** LPAAT-β inhibition of triazines, diamino-*C*,*N*-diarylpyrimidine positional isomers, and diamino-*C*,*N*-diarylpyridine positional isomers



| Compound | X | Y | Z | $R^1$ | $R^2$ | LPAAT-β[a] ($IC_{50}$, μM) |
|---|---|---|---|---|---|---|
| **4a** | N | N | N | OMe | Cl | 0.07, 0.045 |
| **4b** | N | N | N | OEt | Cl | 0.14, 0.26 |
| **5a** | N | N | CH | OMe | Cl | 0.054 |
| **5b** | N | N | CH | OEt | Cl | 0.017 |
| **6a** | N | CH | N | OMe | Cl | 7 |
| **7a** | CH | N | N | OMe | Cl | 2.0, 2.05 |
| **8a** | CH | CH | N | OMe | Cl | 6.5 |
| **8b** | CH | CH | N | OMe | Me | 29 |
| **9a** | N | CH | CH | OMe | Cl | 18 |
| **9b** | N | CH | CH | OMe | Me | 35, > 40 |
| **10a** | CH | N | CH | OMe | Cl | 0.04 |
| **10b** | CH | N | CH | OMe | Me | 0.24 |
| **10c** | CH | N | CH | OMe | CHO | 0.26 |
| **10d** | CH | N | CH | OEt | Cl | 0.050 |
| **10e** | CH | N | CH | OEt | Me | 0.49 |
| **10f** | CH | N | CH | OEt | CHO | 0.050 |
| **10g** | CH | N | CH | OEt | $CH_2OH$ | 0.099 |

[a] LPAAT assay is described in Ref. 28.

of the two isoforms showing sequence similarities, conserved residues at their catalytic sites, and similar substrate preferences. New questions arise as to whether the dramatic selectivity of the reagents is due to binding to a unique site on LPAAT-β or whether the inhibitors exploit subtle structural differences at the catalytic site. These agents may prove useful as tools to study LPAAT-β functions in cells, including the de novo synthesis of phosphoglycerides and triacylglycerol, adipocyte differentiation and metabolism, as well as events involving PA signaling processes.

## Acknowledgments

We thank Lisa Romero for conducting LPAAT assays, and Man Shun Lai and Garland Bellamy for mass spectrometry analyses.

## References and notes

1. Leung, D. W. *Front. Biosci.* **2001**, *6*, 944.
2. Lewin, T. M.; Wang, P.; Coleman, R. A. *Biochemistry* **1999**, *38*, 5764.
3. 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.
4. Eberhardt, C.; Gray, P. W.; Tjoelker, L. W. *J. Biol. Chem.* **1997**, *272*, 20299.
5. Yamashita, A.; Kawagishi, N.; Miyashita, T.; Nagatsuka, T.; Sugiura, T.; Kume, K.; Shimizu, T.; Waku, K. *J. Biol. Chem.* **2001**, *276*, 26745.
6. Stamps, A. C.; Elmore, M. A.; Hill, M. E.; Kelly, K.; Makda, A. A.; Finnen, M. J. *Biochem. J.* **1997**, *326*, 455.
7. Aguado, B.; Campbell, R. D. *J. Biol. Chem.* **1998**, *273*, 4096.
8. Kume, K.; Shimizu, T. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 6632.
9. Bonham, L.; Leung, D.; White, T.; Hollenback, D.; Klein, P.; Tulinsky, J.; Coon, M.; de Vries, P.; Singer, J. W. *Expert Opin. Ther. Targets* **2003**, *7*, 643.
10. Agarwal, A. K.; Arioglu, E.; de Almeida, S.; Akkoc, N.; Taylor, S. I.; Bowcock, A. M.; Barnes, R. I.; Garg, A. *Nat. Genet.* **2002**, *31*, 21.
11. Garg, A. *Am. J. Med.* **2000**, *108*, 143.
12. Ghosh, S.; Strum, J. C.; Sciorra, V. A.; Daniel, L.; Bell, R. M. *J. Biol. Chem.* **1996**, *271*, 8472.
13. Fang, Y.; Vilella-Bach, M.; Bachmann, R.; Flanigan, A.; Chen, J. *Science* **2001**, *294*, 1942.
14. Shen, Y.; Xu, L.; Foster, D. A. *Mol. Cell. Biol.* **2001**, *21*, 595.
15. Limatola, C.; Schaap, D.; Moolenaar, W. H.; van Blitterswijk, W. J. *Biochem. J.* **1994**, *3*, 1001.
16. 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.
17. Gong, B.; Hong, F.; Kohm, C.; Bonham, L.; Klein, P. *Bioorg. Med. Chem. Lett* **2004**, *14*, 1455.
18. Bhatt, R.; Gong, B.; Hong, F.; Jenkins, S. A.; Klein, J. P.; Kumar, A. M.; Tulinsky, J. PCT Int. Appl. WO 03/037346, 2003; *Chem. Abstr.* **2003**, *138*, 354007.
19. Gong, B.; Hong, F.; Kohm, C.; Jenkins, S.; Tulinsky, J.; Bhatt, R.; de Vries, P.; Singer, J. W.; Klein, P. *Bioorg. Med. Chem. Lett* **2004**, *14*, 2303.
20. Zhao, L. X.; Moon, Y. S.; Basnet, A.; Kim, E. K.; Jahng, Y.; Park, J. G.; Jeong, T. C.; Cho, M. J.; Choi, S. U.; Lee, C. O.; Lee, S. Y.; Lee, C. S.; Lee, E. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1333.
21. Chiu, C.; Tang, Z.; Ellingboe, J. W. *J. Comb. Chem.* **1999**, *1*, 73.
22. Mello, J. V.; Finney, N. S. *Org. Lett.* **2001**, *3*, 4263.
23. Schomaker, J. M.; Delia, T. J. *J. Org. Chem.* **2001**, *66*, 7125.
24. Chan, D. M. T.; Monaco, K. L.; Wang, R.-P.; Winters, M. P. *Tetrahedron Lett.* **1998**, *39*, 2933.
25. Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. P.; Chan, D. M. T.; Combs, A. *Tetrahedron Lett.* **1998**, *39*, 2937.
26. Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Averill, K.; Chan, D. M. T.; Combs, A. P. *Synlett* **2000**, 674.
27. $^1$H NMR were recorded on a Bruker Avance 400 instrument and mass spectra were recorded on a Micromass Quatro II electrospray mass spectrometer. **8a**: (acetone-$d_6$) $\delta$ 3.90 (s, 3H, $CH_3$), 5.39 (s, 2H, $NH_2$), 6.10 (d, 1H, $J$ = 1.7 Hz, Ar), 6.90 (d, 1H, $J$ = 1.7 Hz, Ar), 7.12 (d, 1H, $J$ = 8.8 Hz, Ar), 7.24 (d, 2H, $J$ = 8.9 Hz, Ar), 7.34 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.8 Hz, Ar), 7.73 (d, 2H, $J$ = 8.9 Hz, Ar), 7.91 (d, 1H, $J$ = 2.8 Hz, Ar), 8.09 (s, 1H, NH); EIMS $m/z$ 360.0 $(M+H)^+$. **8b**: (acetone-$d_6$) $\delta$ 2.27 (s, 3H, $CH_3$), 3.89 (s, 3H, $CH_3$), 5.30 (d, 2H, $J$ = 6.4 Hz, $NH_2$), 6.11 (d, 1H, $J$ = 1.7 Hz, Ar), 6.89 (d, 1H, $J$ = 1.7 Hz, Ar), 7.07 (d, 2H, $J$ = 8.3 Hz, Ar), 7.10 (d, 1H, $J$ = 8.8 Hz, Ar), 7.32 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.8 Hz, Ar), 7.51–7.54 (m, 2H, Ar), 7.68 (s, 1H, NH), 7.99 (d, 1H, $J$ = 2.8 Hz, Ar); EIMS $m/z$ 339.9 $(M+H)^+$. **9a**: (acetone-$d_6$) $\delta$ 3.90 (s, 3H, $CH_3$), 5.27 (s, 2H, $NH_2$), 6.22 (t, 1H, $J$ = 1.9 Hz, Ar), 7.09–7.12 (m, 2H, Ar), 7.26–7.36 (m, 5H, Ar), 7.86 (s, 1H, NH), 7.96 (d, 1H, $J$ = 2.8 Hz, Ar); EIMS $m/z$ 360.2 $(M+H)^+$. **9b**: (acetone-$d_6$) $\delta$ 2.31 (s, 3H, $CH_3$), 3.90 (s, 3H, $CH_3$), 5.40 (s, 2H, $NH_2$), 6.16 (t, 1H, $J$ = 2.0 Hz, Ar), 7.04 (bs, 1H, Ar), 7.09 (t, 1H, $J$ = 8.8 Hz, Ar), 7.17 (bs, 4H, Ar), 7.32 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.8 Hz, Ar), 7.72 (s, 1H, NH), 7.93 (d, 1H, $J$ = 2.8 Hz, Ar); EIMS $m/z$ 340.2 $(M+H)^+$. **10a**: (acetone-$d_6$) $\delta$ 3.84 (s, 3H, $CH_3$), 5.33 (d, 2H, $J$ = 8.8 Hz, $NH_2$), 6.16 (d, 1H, $J$ = 1.1 Hz, Ar), 6.27 (t, 1H, $J$ = 1.1 Hz, Ar), 7.12 (d, 1H, $J$ = 8.8 Hz, Ar), 7.23 (d, 2H, $J$ = 8.9 Hz, Ar), 7.30 (d, 1H, $J$ = 2.7 Hz, Ar), 7.36 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.7 Hz, Ar), 7.76–7.79 (m, 2H, Ar), 8.05 (s, 1H, NH); EIMS $m/z$ 360.1 $(M+H)^+$. **10b**: (acetone-$d_6$) $\delta$ 2.26 (s, 3H, $CH_3$), 3.83 (s, 3H, $CH_3$), 5.24 (bs, 2H, $NH_2$), 6.10 (d, 1H, $J$ = 1.1 Hz, Ar), 6.26 (t, 1H, $J$ = 1.1 Hz, Ar), 7.06 (d, 2H, $J$ = 8.5 Hz, Ar), 7.08 (d, 1H, $J$ = 8.8 Hz, Ar), 7.29 (d, 1H, $J$ = 2.7 Hz, Ar), 7.35 (dd, 1H,$J$ = 9.0 Hz, $J$ = 3.3 Hz, Ar), 7.53–7.56 (m, 2H, Ar), 7.70 (s, 1H, NH); EIMS $m/z$ 340.1 $(M+H)^+$. **10c**: (acetone-$d_6$) $\delta$ 3.85 (s, $CH_3$), 5.51 (bs, 2H, $NH_2$), 6.27 (d, 1H, $J$ = 1.1 Hz, Ar), 6.38 (d, 1H, $J$ = 1.1 Hz, Ar), 7.14 (d, 1H, $J$ = 8.8 Hz, Ar), 7.32 (d, 1H, $J$ = 2.7 Hz, Ar), 7.38 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.7 Hz, Ar), 7.78 (d, 2H, $J$ = 8.7 Hz, Ar), 7.96 (d, 2H, $J$ = 8.7 Hz, Ar), 8.59 (s, 1H, NH), 9.84 (s, 1H, CH).**10d**: (acetone-$d_6$) $\delta$ 1.35 (t, 3H, $J$ = 7.0 Hz, $CH_3$), 4.08 (q, 2H, $J$ = 7.0 Hz, $CH_2$), 5.32 (bs, 2H, $NH_2$), 6.21 (d, 1H, $J$ = 1.2 Hz, Ar), 6.32 (t, 1H, $J$ = 1.1 Hz, Ar), 7.09 (d, 2H, $J$ = 8.6 Hz, Ar), 7.23 (d, 2H, $J$ = 8.9 Hz, Ar), 7.31–7.78 (m, 3H, Ar), 8.04 (s, 1H, NH); EIMS $m/z$ 374.1 $(M+H)^+$. **10e**: (acetone-$d_6$) $\delta$ 1.35 (t, 3H, $J$ = 7.0 Hz, $CH_3$), 2.27 (s, 3H, $CH_3$), 4.08 (q, 2H, $J$ = 7.0 Hz, $CH_2$), 5.24 (bs, 2H, $NH_2$), 6.14 (d, 1H, $J$ = 1.0 Hz, Ar), 6.32 (t, 1H, $J$ = 0.9 Hz, Ar), 7.06–7.10 (m, 3H, Ar), 7.29–7.34 (m, 2H, Ar), 7.51–7.54 (m, 2H, Ar), 7.67 (s, 1H, NH); EIMS $m/z$ 354.2 $(M+H)^+$. **10f**: (acetone-$d_6$) $\delta$ 1.36 (t, 3H, $J$ = 6.9 Hz, $CH_3$), 4.11 (q, 2H, $J$ = 7.0 Hz,

$CH_2$), 5.51 (bs, 2H, $NH_2$), 6.33 (d, 1H, $J$ = 1.1 Hz, Ar), 6.44 (d, 1H, $J$ = 1.0 Hz, Ar), 7.12 (d, 1H, $J$ = 8.5 Hz, Ar), 7.31–7.37 (m, 2H, Ar), 7.78 (d, 2H, $J$ = 8.8 Hz, Ar), 7.88–7.97 (m, 2H, Ar), 8.57 (s, 1H, NH), 9.84 (s, 1H, CH); EIMS $m/z$ 368.1 $(M+H)^+$. **10g**: (acetone-$d_6$) $\delta$ 1.36 (t, 3H, $J$ = 6.9 Hz, $CH_3$), 3.95 (bs, 1H, OH), 4.09 (q, 2H, $J$ = 7.0 Hz, $CH_2$), 4.56 (s, 2H, $CH_2$), 5.28 (bs, 2H, $NH_2$), 6.16 (d, 1H, $J$ = 1.1 Hz, Ar), 6.34 (d, 1H, $J$ = 1.1 Hz, Ar), 7.09 (d, 1H, $J$ = 8.5 Hz, Ar), 7.24 (d, 2H, $J$ = 8.8 Hz, Ar), 7.30–7.34 (m, 2H, Ar), 7.60–7.63 (m, 2H, Ar), 7.80 (s, 1H, NH); EIMS $m/z$ 370.1 $(M+H)^+$. **13**: (acetone-$d_6$) $\delta$ 3.91 (s, 3H, $CH_3$), 5.92 (s, 2H, $NH_2$), 6.60 (d, 1H, $J$ = 1.8 Hz, Ar), 7.14 (d, 1H, $J$ = 8.8 Hz, Ar), 7.33 (d, 1H, $J$ = 1.9 Hz, Ar), 7.37 (dd, 1H, $J$ = 8.8 Hz,$J$ = 2.8 Hz, Ar), 7.90 (d, 1H, $J$ = 2.8 Hz, Ar). **16a**: (acetone-$d_6$) $\delta$ 3.92 (s, 3H, $CH_3$), 6.86 (d, 1H, $J$ = 8.9 Hz, Ar), 6.90 (t, 1H, $J$ = 1.9 Hz, Ar), 7.18 (d, 1H, $J$ = 8.9 Hz, Ar), 7.20 (d, 1H, $J$ = 8.9 Hz, Ar), 7.36 (d, 2H, $J$ = 8.9 Hz, Ar), 7.41 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.8 Hz, Ar), 7.46 (d, 2H, $J$ = 8.9 Hz, Ar), 7.72 (t, 1H, $J$ = 1.9 Hz, Ar), 7.95 (d, 1H, $J$ = 2.8 Hz, Ar); EIMS $m/z$ 379.0 $(M+H)^+$. **16b**: (DMSO-$d_6$) $\delta$ 2.32 (s, 3H, $CH_3$), 3.91 (s, 3H, $CH_3$), 6.73 (d, 1H, $J$ = 1.9 Hz, Ar), 7.14 (d, 2H, $J$ = 8.4 Hz, Ar), 7.18 (d, 1H, $J$ = 8.9 Hz, Ar), 7.22 (d, 2H, $J$ = 8.2 Hz, Ar), 7.44–7.48 (m, 2H, Ar), 7.75 (d, 1H, $J$ = 2.8 Hz, Ar), 9.1 (s, 1H, NH). **17b**: (acetone-$d_6$) $\delta$ 2.19 (s, 3H, $CH_3$), 2.32 (s, 3H, $CH_3$), 3.91 (s, 3H, $CH_3$), 7.12 (d, 1H, $J$ = 8.8 Hz, Ar), 7.19–7.25 (m, 4H, Ar), 7.35 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.8 Hz, Ar), 7.47–7.48 (m, 1H, Ar), 7.86 (s, 1H, NH), 7.89 (d, 1H, $J$ = 2.8 Hz, Ar), 8.05 (s, 1H, NH); EIMS $m/z$ 382.2 $(M+H)^+$. **20**: (DMSO-$d_6$) $\delta$ 1.36 (t, 6H, $J$ = 7.1 Hz $CH_3$), 4.40 (q, 4H, $J$ = 7.1 Hz, $CH_2$), 8.31 (s, 2H, Ar). **21a**: (acetone-$d_6$) $\delta$ 1.42 (t, 6H, $J$ = 7.1 Hz, $CH_3$), 3.92 (s, 3H, $CH_3$), 4.45 (q, 4H, $J$ = 7.1 Hz, $CH_2$), 7.27 (d, 1H, $J$ = 8.9 Hz, Ar), 7.52 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.7 Hz, Ar), 7.56 (d, 1H, $J$ = 2.7 Hz, Ar), 8.43 (s, 2H, Ar). **22a**: (DMSO-$d_6$) $\delta$ 3.92 (s, 3H, $CH_3$), 4.68 (s, 4H, $NH_2$), 7.24 (d, 1H, $J$ = 8.6 Hz, Ar), 7.53–7.56 (m, 2H, Ar), 8.20 (s, 2H, Ar), 10.72 (s, 2H, NH). **23a**: (acetone-$d_6$) $\delta$ 3.94 (s, 3H, $CH_3$), 7.28 (d, 1H, $J$ = 8.9 Hz, Ar), 7.55 (dd, 1H, $J$ = 8.8 Hz, $J$ = 2.7 Hz, Ar), 7.62 (d, 1H, $J$ = 2.7 Hz, Ar), 8.54 (s, 2H, Ar). **24a**: (DMSO-$d_6$) $\delta$ 1.47 (s, 18H, $CH_3$), 3.79 (s, 3H, $CH_3$), 7.18 (d, 1H, $J$ = 8.9 Hz, Ar), 7.30 (d, 1H, $J$ = 2.6 Hz, Ar), 7.47–7.49 (m, 3H, Ar), 9.41 (s, 2H, NH).

28. LPAAT-$\alpha$ and LPAAT-$\beta$ assays were conducted using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) as the colorimetric reagent according to the method described in 16. $IC_{50}$ values were determined from experiments using 10 concentrations (0.001–40 μM) of each compound in duplicate and fit to a sigmoidal curve using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Duplicate values typically varied by less than 5% and the $R^2$ values for the goodness-of-fit were $\geqslant$0.95.