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Synthesis and biological evaluation of phosphonate derivatives as autotaxin (ATX) inhibitors

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Abstract—Autotaxin (ATX) is an autocrine motility factor that promotes cancer cell invasion, cell migration, and angiogenesis. ATX, originally discovered as a nucleotide phosphodiesterase, is known now to be responsible for the lysophospholipid-preferring phospholipase D activity in plasma. As such, it catalyzes the production of lysophosphatidic acid (LPA) from lysophophatidylcholine (LPC). ATX is thus an attractive drug target; small molecular inhibitors might be efficacious in slowing the spread of cancers. With this study we have generated a series of β-keto and β-hydroxy phosphonate derivatives of LPA, some of which are potent ATX inhibitors.

The autocrine motility factor autotaxin (ATX) was originally isolated from melanoma cell supernatants as a 125-kDa glycoprotein that stimulated tumor cell motility.[1] In vivo experiments documented that forced expression of ATX augments tumor cell invasion and metastasis.[2] Further, ATX promotes angiogenesis and may act in concert with other angiogenic factors to facilitate new blood vessel formation.[3] These biological properties require enzymatic activity.

ATX belongs to the nucleotide pyrophosphatase and phosphodiesterase (NPP) family of enzymes, which hydrolyze phosphodiester and diphosphate bonds, typically found in ATP and ADP.[4] Interest in ATX was stimulated by the identification of this enzyme as the long elusive plasma lysophospholipase D activity, which is responsible for the cleavage of choline group of lysophophatidylcholine (LPC) to form lysophosphatidic acid (LPA) (Fig. 1).[5,6] This is a major pathway of biosynthesis of LPA in plasma.[7,8] LPA is an intercellular lipid mediator that influences many biochemical processes including cell proliferation, smooth muscle contraction, platelet aggregation, and apoptosis.[9–11] For example, LPA is the "ovarian cancer activating factor" in ascitic fluid characteristic of ovarian cancer patients. Elevated levels of LPA are present both at early and late stages in ovarian cancer and may play a role in tumor cell proliferation and invasion.[12,13] LPA mediates its effects through the activation of G protein-coupled receptors (GPCR).[14] Thus, great efforts have been made on the study of LPA receptor antagonists and agonists due to their therapeutic potential.[15–21] In aggregate, these data suggest that ATX is an attractive pharmacological target; blockage of LPA production via ATX inhibition by small molecules could be a useful anticancer chemotherapy.[22,23]

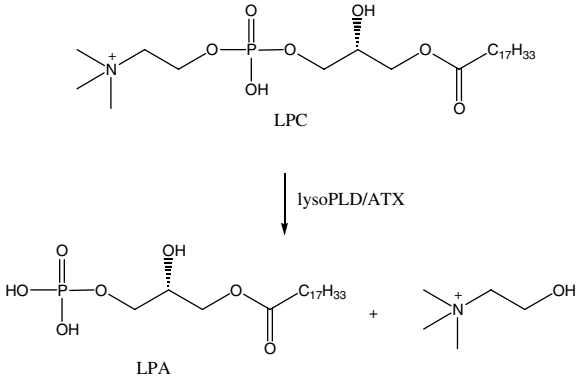

Figure 1. Hydrolysis of LPC by lysoPLD/ATX.

Keywords: Autotaxin; ATX; Phosphonate; Choline; LPA.
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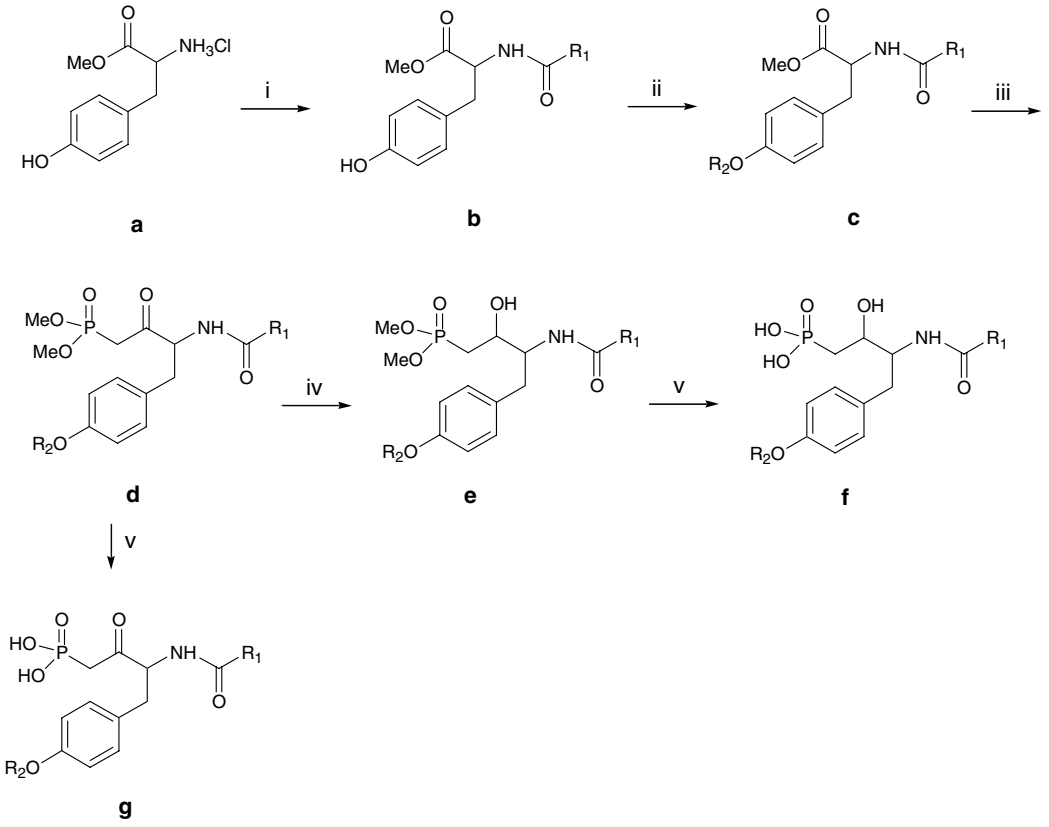
A lead towards developing ATX inhibitors was provided by the discovery that this enzyme undergoes end product inhibition by, for example, LPA.[24] Indeed a limited number of ATX inhibitors that are LPA analogs have been reported to date. Recently, a series of fatty alcohol phosphate analogs were identified as LPA receptor ligands.[20] Some of the analogs showed ATX inhibition activity. A series of phosphatidic acid derivatives were investigated and only two acyl thiophosphates showed autotaxin inhibition.[21] A group of Darmstoff analogs were reported as weak ATX inhibitors recently.[25] Most recently 3-carba analogs of cyclic phosphatidic acid were reported.[26] Although lacking significant activity at LPA receptors, they were potent inhibitors of ATX activity. In this report, we developed a series of β-hydroxy and β-keto phosphonate derivatives of LPA as ATX inhibitors.

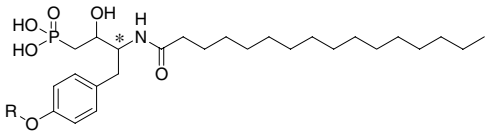
Synthesis of the phosphonate derivatives is described in Scheme 1. It began with the acylation of the ammonium hydrochloride salt of tyrosine *O*-methyl ester **a** with appropriate acyl chlorides followed by etherification of the free phenol with appropriate mesylates to afford the fully protected tyrosine **c**. Next, was the base mediated addition onto the methyl ester with the lithium anion of dimethyl methylphosphonate to achieve β-keto phosphonate dimethyl ester **d**. A bromotrimethylsaline mediated deprotection of the ester ensued to afford the β-keto phosphonate **g**.[27] Sodium borohydride reduction of **d** proceeded to give two possible diastereometic β-hydroxy phosphonate dimethyl esters which were separated by column chromatography. The stereochemistry determination is ongoing. The β-hydroxy phosphonate **f** was obtained by using the same deprotection method (for compounds **f**41 and **f**42, pyridine was used in the deprotection).

The phosphonate derivatives were tested in choline detection assay for ATX inhibition.[28] The ATX activity was measured in the presence of the compounds under different concentrations (100, 10, and 1 μM). The ATX activity without compounds was used as the standard (100% activity). Most β-hydroxy phosphonate derivatives inhibited ATX activity at only the highest concentration tested. However, **f**17 and **f**18 exhibited significant inhibition at 1μM (Table 1). These two compounds were synthesized from protected L-tyrosine and they are diastereomers in terms of the β-hydroxy groups. The less polar isomer, **f**17, (also known as VPC8a202) was able to inhibit 73% of ATX activity at 1 μM. Compounds **f**15 and **f**16, which were synthesized from D-tyrosine, did not potently inhibit ATX although they contained the same 4-methoxy-3,5-dimethyl-pyridyl structure moiety. Other groups including some alkyl chains and aromatic rings were also investigated. However, these compounds were not potent ATX inhibitors.



Scheme 1. Synthesis of compounds **f** and **g**. Reagents and conditions: (i) appropriate acyl chloride, Et_3N, CH_2Cl_2, 0 °C, 3 h, 70–80%; (ii) appropriate mesylate, K_2CO_3, 18-crown-6, acetone, reflux overnight, 90–95%; (iii) *n*-BuLi, dimethyl methylphosphonate, then add in ester **c**, −78 °C, 3 h, 50–60%; (iv) $NaBH_4$, THF, EtOH, 0 °C, 2 h, 70–80%; (v) bromotrimethylsaline, w/wo pyridine, CH_2Cl_2, rt, 4 h, then H_2O and MeOH, overnight, 90–95%.

Table 1. ATX inhibitory evaluation of compound **f**1–**f**34



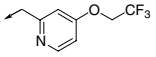
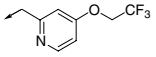
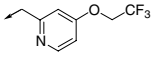
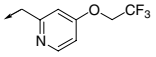
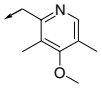
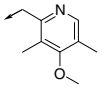
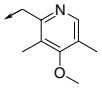
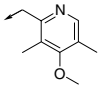
Compound	R	*S/R	ATX activity (%)		
			1 μM	10 μM	100 μM
f1		*R* (a[1])	92	84	55
f2		*R* (b[1])	N/D	84	64
f3		*S* (a)	92	80	32
f4		*S* (b)	103	83	56
f5		*R* (a)	83	82	83
f6		*R* (b)	80	81	83
f7		*S* (a)	97	77	68
f8		*S* (b)	76	83	78
f9		*R* (a)	103	76	60
f10		*R* (b)	92	78	69
f11		*S* (a)	104	81	52
f12		*S* (b)	N/D	74	65
f13		*R* (b)	84	80	67
f14		*S* (b)	80	80	80
f15		*R* (a)	108	95	63
f16		*R* (b)	69	64	47
f17		*S* (a)	27	13	6
f18		*S* (b)	63	21	8

Table 1 (*continued*)

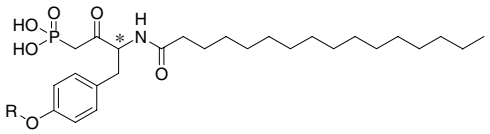
Compound	R	*S/R	ATX activity (%)		
			1 μM	10 μM	100 μM
f19	CH_3	*R* (a)	100	78	57
f20	CH_3	*R* (b)	95	84	82
f21	CH_3	*S* (a)	77	56	36
f22	CH_3	*S* (b)	84	80	69
f23	*n*-C_3H_7	*R* (a)	80	79	72
f24	*n*-C_3H_7	*R* (b)	108	108	90
f25	*n*-C_3H_7	*S* (a)	110	97	77
f26	*n*-C_3H_7	*S* (b)	100	100	83
f27	*n*-C_5H_{11}	*R* (a)	81	85	80
f28	*n*-C_5H_{11}	*R* (b)	86	82	70
f29	*n*-C_5H_{11}	*S* (a)	98	83	67
f30	*n*-C_5H_{11}	*S* (b)	94	90	70
f31		*R* (a)	103	104	72
f32		*R* (b)	N/D	104	94
f33		*S* (a)	104	104	82
f34		*S* (b)	106	101	91

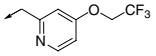
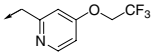
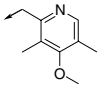
[1] a, refers to the diastereomer that elutes first; b, refers to the diastereomer that elutes second.

The corresponding β-keto phosphonate derivatives were also tested (Table 2). At the concentration of 100 μM, some compounds inhibited 50–70% of ATX activity. Further structure optimization was attempted based on the two lead compounds **f**17 and **f**18. We kept the 4-methoxy-3,5-dimethyl-pyridyl moiety and investigated a series of β-hydroxy phosphonate derivatives with a variety of lipophilic tails. These data are presented in Table 3. None of these compounds were as potent as **f**17 or **f**18. All of the compounds were tested as potential antagonists at the recombinant LPA_1, LPA_2, and LPA_3 receptors, but none showed significant blockade at concentrations up to 10 μM (data not shown).

A potential complication of our studies is the reported inhibition of ATX by its product, LPA,[24] which is generated in our assay using LPC as a substrate.[28] There, we measured the inhibition of ATX using the artificial

Table 2. ATX inhibitory evaluation of compound **g**1–**g**15

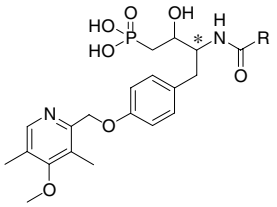


Compound	R	*S/R	ATX activity (%)		
			1 μM	10 μM	100 μM
g1		*R*	85	70	57
g2		*S*	79	68	44
g3		*R*	N/D	82	76
g4		*S*	N/D	71	61
g5		*R*	78	68	52
g6		*S*	102	72	50
g7		*S*	71	74	26
g8	CH_3	*R*	84	71	32
g9	CH_3	*S*	96	68	45
g10	*n*-C_3H_7	*R*	104	97	75
g11	*n*-C_3H_7	*S*	N/D	97	72
g12	*n*-C_5H_{11}	*R*	93	84	N/D
g13	*n*-C_5H_{11}	*S*	95	83	N/D
g14		*R*	97	101	76
g15		*S*	104	104	85

substrate, *para*-nitrophenol-thymidylic acid.[5,6] Using this substrate, we found the same rank order potency of our most potent inhibitors, including **f**17 and **f**18 (data not shown).

In summary, we developed a series of β-hydroxy and β-keto phosphonate derivatives. ATX inhibitory activity was determined for these compounds. Two β-hydroxy phosphonates, which originated from protected L-tyrosine, were identified as the lead compounds. The stereochemistry of original tyrosines and 4-methoxy-3,5-dimethyl-pyridyl moiety proved to be important to the activity. Further SAR studies are ongoing.

Table 3. ATX inhibitory evaluation of compound **f**35–**f**44



Compound	R	*S/R	ATX activity (%)		
			1 μM	10 μM	100 μM
f35	*n*-C_9H_{19}	*R* (a[1])	N/D	86	30
f36	*n*-C_9H_{19}	*R* (b[1])	N/D	N/D	N/D
f37	*n*-$C_{13}H_{27}$	*R* (a)	N/D	99	67
f38	*n*-$C_{13}H_{27}$	*R* (b)	N/D	N/D	35
f39	*n*-$C_{17}H_{35}$	*S* (a)	101	91	81
f40	*n*-$C_{17}H_{35}$	*S* (b)	N/D	90	62
f41	*n*-$C_{17}H_{33}$[2]	*S* (a)	N/D	45	15
f42	*n*-$C_{17}H_{33}$[2]	*S* (b)	77	68	58
f43	*n*-$C_{19}H_{39}$	*S* (a)	64	45	10
f44	*n*-$C_{19}H_{39}$	*S* (b)	N/D	N/D	N/D

[1] a, refers to the diastereomer that elutes first, b refers to the diastereomer that elutes second.
[2] *cis* double bond located between C9 and C10 from the carbonyl.

Acknowledgment

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References and notes

1. Stracke, M. L.; Clair, T.; Liotta, L. A. *Adv. Enzyme Regul.* **1997**, *37*, 135.
2. Nam, S. W.; Clair, T.; Campo, C. K.; Lee, H. Y.; Liotta, L. A.; Stracke, M. L. *Oncogene* **2000**, *19*, 241.
3. Nam, S. W.; Clair, T.; Kim, Y. S.; McMarlin, A.; Schiffmann, E.; Liotta, L. A.; Stracke, M. L. *Cancer Res.* **2001**, *61*, 6938.
4. Goding, J. W.; Terkeltaub, R.; Maurice, M.; Deterre, P.; Sali, A.; Bell, S. I. *Immunol. Rev.* **1998**, *161*, 11.
5. Tokumura, A.; Majima, E.; Kariya, Y.; Tominaga, K.; Kogure, K.; Yasuda, K.; Fukuzawa, K. *J. Biol. Chem.* **2002**, *277*, 39436.
6. Umezu-Goto, M.; Kishi, Y.; Taira, A.; Hama, K.; Dohmae, N.; Takio, K.; Yamori, T.; Mills, G. B.; Inoue, K.; Aoki, J.; Arai, H. *J. Cell Biol.* **2002**, *158*, 227.
7. Sano, T.; Baker, D.; Virag, T.; Wada, A.; Yatomi, Y.; Kobayashi, T.; Igarashi, Y.; Tigyi, G. *J. Biol. Chem.* **2002**, *277*, 21197.
8. Aoki, J.; Taira, A.; Takanezawa, Y.; Kishi, Y.; Hama, K.; Kishimoto, T.; Mizuno, K.; Saku, K.; Taguchi, R.; Arai, H. *J. Biol. Chem.* **2002**, *277*, 48737.
9. Tigyi, G.; Parrill, A. L. *Prog. Lipid Res.* **2003**, *42*, 498.
10. Mills, G. B.; Moolenaar, W. H. *Nat. Rev. Cancer* **2003**, *3*, 582.
11. Lynch, K. R.; Macdonald, T. L. *Prost. Lipid Med.* **2001**, *64*, 33.
12. Xu, Y.; Gaudette, D. C.; Boynton, J. D.; Frankel, A.; Fang, X.-J.; Sharma, A.; Hurteau, J.; Casey, G.; Goodbody, A.; Mellors, A.; Holub, B. J.; Mills, G. B. *Clin. Cancer Res.* **1995**, *1*, 1223.
13. Xu, Y.; Fang, X. J.; Casey, G.; Mills, G. B. *Biochem. J.* **1995**, *309*, 933.
14. Contos, J. J. A.; Ishii, I.; Chun, J. *Mol. Pharm.* **2000**, *58*, 1188.
15. Heasley, B. H.; Jarosz, R.; Carter, K. M.; Jenny Van, S.; Lynch, K. R.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4069.
16. Webster, L. S.; Heasley, B. H.; Jarosz, R.; Carter, K. M.; Lynch, K. R.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3473.
17. Heasley, B. H.; Jarosz, R.; Lynch, K. R.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2735.
18. Tamaruya, Y.; Suzuki, M.; Kamura, G.; Kanai, M.; Hama, K.; Shimizu, K.; Aoki, J.; Arai, H.; Shibasaki, M. *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 2834.
19. Qian, L.; Xu, Y.; Simper, T.; Jiang, G.; Aoki, J.; Umezu-Goto, M.; Arai, H.; Yu, S.; Mills, G. B.; Tsukahara, R.; Makarova, N.; Fujiwara, Y.; Tigyi, G.; Prestwich, G. D. *ChemMedChem.* **2006**, *1*, 376.
20. Durgam, G. G.; Virag, T.; Walker, M. D.; Tsukahara, R.; Yasuda, S.; Liliom, K.; van Meeteren, L. A.; Moolenaar, W. H.; Wilke, N.; Siess, W.; Tigyi, G.; Miller, D. D. *J. Med. Chem.* **2005**, *48*, 4919.
21. Durgam, G. G.; Tsukahara, R.; Makarova, N.; Walker, M. D.; Fujiwara, Y.; Pigg, K. R.; Baker, D. L.; Sardar, V. M.; Parrill, A. L.; Tigyi, G.; Miller, D. D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 633.
22. Mills, G. B.; Moolenaar, W. H. *Nat. Rev. Cancer* **2003**, *3*, 582.
23. Goto, M.; Tanyi, J.; Lahad, J.; Liu, S.; Lapushin, R.; Hasegawa, Y.; Lu, Y.; Trost, R.; Bevers, T.; Jonasch, E.; Aldape, K.; Liu, J.; James, R. D.; Ferguson, C. G.; Xu, Y.; Prestwich, G. D.; Mills, G. B. *J. Cell. Biochem.* **2004**, *92*, 1115.
24. van Meeteren, L. A.; Ruurs, P.; Christodoulou, E.; Goding, H.; Takakusa, K.; Kikuchi, K.; Perrakis, A.; Nagano, T.; Moolenaar, W. H. *J. Biol. Chem.* **2005**, *280*, 21155.
25. Gududuru, V.; Zeng, K.; Tsukahara, R.; Makarova, N.; Fujiwara, Y.; Pigg, K.; Baker, D.; Tigyi, G.; Miller, D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 451.
26. Baker, D. L.; Fujiwara, Y.; Pigg, K. R.; Tsukahara, R.; Kobayashi, S.; Murofushi, H.; Uchiyama, A.; Murakami-Murofushi, K.; Koh, E.; Bandle, R. W.; Byun, H.; Bittman, R.; Fan, D.; Murph, M.; Mills, G. B.; Tigyi, G. *J. Bio. Chem.* **2006**, *281*, 22786.

27. Rabinowitz, R. *J. Org. Chem.* **1963**, *28*, 2975.
28. ATX activity assay: in 0.1 ml, buffer: (in mM: Tris–HCl 100, pH9, NaCl 150, $MgCl_2$ 5, $CoSO_4$ 0.03, 0.05% Triton X-100), 1 mM 18:1 LPC, recombinant human ATX with or without inhibitors; incubation was for 16 h at 37 °C. Released choline was detected as follows: samples were mixed with 0.15 ml of 50 mM Tris–HCl containing 2.7 mM TOOS (*N*-ethyl-*N*-2-hydroxy-3-sulfopropyl)-*m*-toluidine,2.7 mM 4-AAP (4-aminoantipyrine), 47.7 U/ml horseradish peroxidase, 18 U/ml choline oxidase, and 5 mM $MgCl_2$. After 30 min incubation at 37 °C, light absorbance (550 nm) was determined, and the amount of choline release was calculated from a standard curve.