Bioorganic & Medicinal Chemistry Letters 23 (2013) 1187–1192

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of imidazole and triazole derivatives as Lp-PLA$_2$ inhibitors and the unexpected discovery of highly potent quaternary ammonium salts

Kai Wang, Wenwei Xu, Yang Liu, Wei Zhang, Wenyi Wang, Jianhua Shen *, Yiping Wang *

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 3 September 2012
Revised 7 November 2012
Accepted 8 January 2013
Available online 16 January 2013

Keywords:
Bioisosteric replacement
Imidazole
Triazole
Lp-PLA$_2$ inhibitor
Quaternary ammonium salt

ABSTRACT

New Lp-PLA$_2$ inhibitors were synthesized by the bioisosteric replacement of the amide group of Darapladib with an imidazole or a triazole. Unfortunately, the inhibitory activities of these derivatives were lower than that of Darapladib. But interestingly, a series of quaternary ammonium salts that were isolated as by-products during this synthetic work were found with high potency. Of these by-products, compound **22c** showed a similar profile to Darapladib both in vitro and in vivo.

With modern progress in understanding the role of inflammation in the pathogenesis of atherosclerosis,[1] researchers have focused significant research efforts on the development of drugs that target the inflammatory pathways. Of the large number of potential therapeutic targets, lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) has been regarded as a particularly promising one over the past decade.[2] The enzyme of Lp-PLA$_2$, also known as plasma platelet-activating factor acetylhydrolase (PAF-AH), is a 50 kDa, calcium-independent lipase that is secreted by inflammatory cells and binds mainly to low-density lipoprotein (LDL).[3] Lp-PLA$_2$ is responsible for the hydrolysis of oxidized phospholipids within LDL into two pro-inflammatory mediators, including lysophosphatidylcholine and oxidized non-esterified fatty acids.[4] Both products have been shown to elicit a series of inflammatory-immune responses within the arterial endarterium, ultimately leading to the initiation and progression of the atherosclerotic plaque.[5] The inhibition of Lp-PLA$_2$ activity is therefore expected to slow the hydrolysis process, reduce the content of the afore mentioned pro-inflammatory factors in circulation and stabilize the vulnerable plaque, as indicated by many animal studies.[6] Several epidemiological studies[7] have also presented a positive correlation between the elevated level of Lp-PLA$_2$ and the increased risk of adverse cardiovascular events. Lp-PLA$_2$ inhibitors as new therapeutic agents have great potential to further reduce the clinical event rates that cannot be entirely eliminated by current pharmaceuticals (e.g., lipid-lowering and antiplatelet agents).

Several different classes of selective and reversible Lp-PLA$_2$ inhibitors have been discovered in industrial and academic groups.[8] Some of these inhibitors have progressed into clinical studies, such as SB-435495,[8a] Rilapladib[8b] and Darapladib,[8c] which were all developed by GSK. Darapladib (Fig. 1) is now undergoing phase III trials to determine its clinical efficacy in reducing the incidence of death, myocardial infarction or ischemic stroke.

As the first drug in its class, Darapladib does not necessarily represent the perfect drug candidate in terms of its physiochemical properties, such as high log*P* value and low oral bioavailability. Further optimization of Darapladib would be desirable and several different research groups are already heavily involved in optimization programs. A survey of available patents and general literatures revealed that modifications to the pyrimidone moiety, the hydrophobic amino-chain and the biphenyl groups in Darapladib have been thoroughly investigated, whereas any exploration of the central amide has not yet been attempted. Bioisosteric replacement is a commonly used and effective strategy in drug design. We envisaged that the replacement of the amide group in Darapladib with a heterocyclic bioisostere would represent an interesting strategy for the development of new Darapladib analogues (Fig. 2). Imidazole and triazole were selected to evaluate this idea, mainly because they frequently appeared in approved drugs and have been used

* Corresponding authors. Tel.: +86 21 20231969; fax: +86 21 20231971 (J.S.); Tel.: +86 21 50806733 (Y.W.).
E-mail addresses: jhshen@mail.shcnc.ac.cn (J. Shen), ypwang@mail.shcnc.ac.cn (Y. Wang).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.029

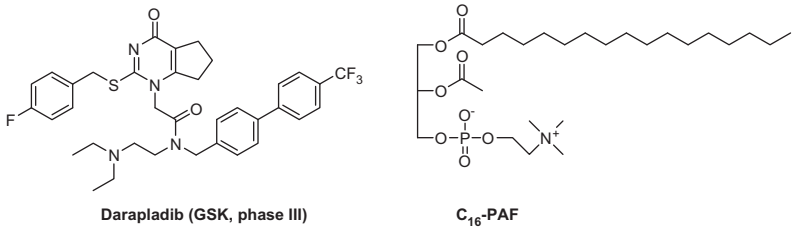


Figure 1. The structure of Darapladib and C_{16}-PAF (a substrate of Lp-PLA_2).

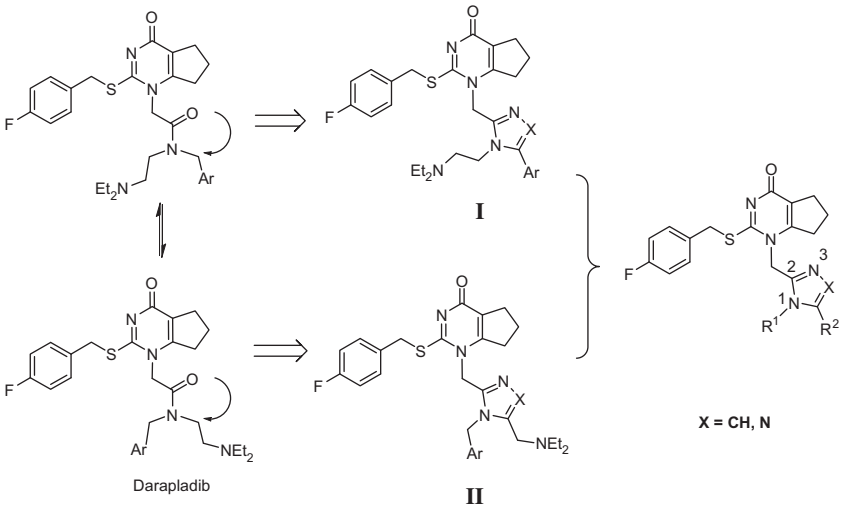
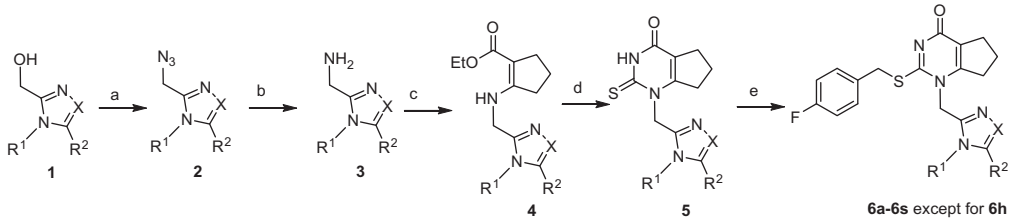


Figure 2. Design of imidazole/triazole derivatives as Lp-PLA_2 inhibitors.

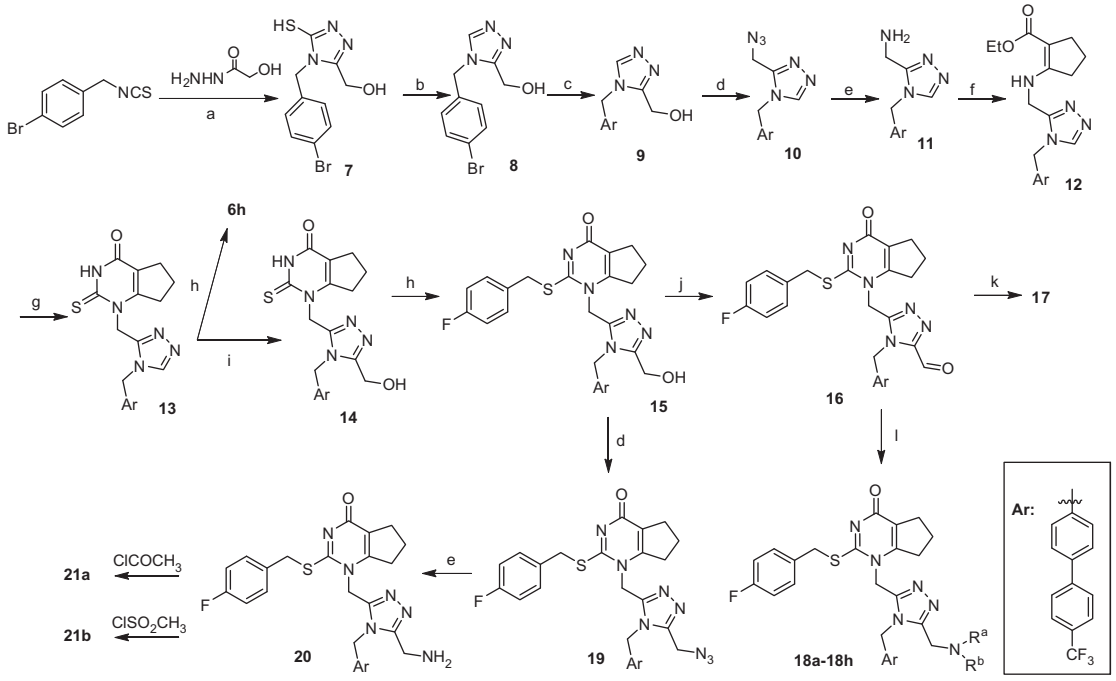
as amide bioisosteres to form H-bonds with proteins through their N-3 sites in the same way as the O-atom of the original amide. Herein, we describe our work on the preliminary structure–activity relationship (SAR) studies of the imidazole and triazole derivatives. Furthermore, we describe our discovery of a series of quaternary ammonium salts as highly potent Lp-PLA_2 inhibitors.

The synthesis of the imidazole derivatives together with part of the triazole derivatives is outlined in Scheme 1. Using the method initially reported by Thompson,[9] alcohol **1** was converted to the azide **2** by reaction with diphenylphosphoryl azide (DPPA). A subsequent Staudinger reaction provided the corresponding amine **3**, which underwent a condensation reaction with ethyl 2-oxocyclopentanecarboxylate in the presence of tetraethyl orthosilicate[10] to give **4**, which was then treated with trimethylsilyl isothiocyanate to produce **5**. The subsequent treatment of **5** with 4-fluorobenzyl bromide in boiling acetone gave rise to **6**. The intermediates represented by structure **1** were prepared according to procedures previously published in the literature and have therefore not been illustrated here.

Compounds **6h**, **15**, **17**, **18a–h** and **21a–b** were synthesized according to a well established procedure (Scheme 2). The starting material 1-bromo-4-isothiocyanoatomethylbenzene was converted to **7** via a condensation reaction with 2-hydroxyacetohydrazide, followed by oxidative desulfurization[11] and Suzuki coupling to form **9**. Azidation of **9** with DPPA, followed by catalytic hydrogenation of the resulting azide gave amine **11**, which was subsequently transformed into **13** under the same conditions as described for the conversion of intermediate **5** mentioned above. Using DBU as the base instead of K_2CO_3, compound **6h** was obtained in excellent yield via the reaction of **13** with 4-fluorobenzyl bromide in acetonitrile at ambient temperature. This process was also applied for the preparation of the key intermediate **15** from **14**, which was acquired itself from the hydroxymethylation of **13** in boiling formaldehyde solution. Compound **15** was then oxidized to **16** using MnO_2, followed by reaction with methylmagnesium bromide to afford compound **17**. The synthesis of the target compounds **18a–h** was completed by the reductive amination reactions of compound **16** with the corresponding amines HNR^aR^b. In a separate experiment, the azidation of **15** and subsequent catalytic hydrogenation led to the formation of **20**, which was ultimately converted to compounds **21a** and **21b** by treatment with the corresponding acylating agents. Compared with the synthetic

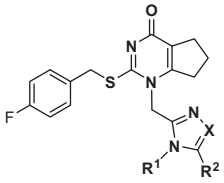


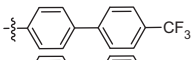
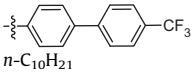
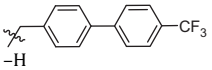
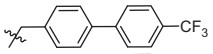
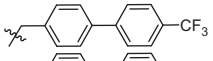
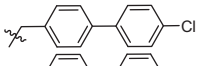
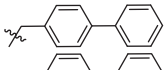
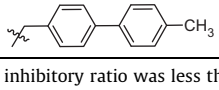
Scheme 1. Reagents and conditions: (a) DPPA, DBU, THF, reflux, 2–3 h; (b) Ph_3P, THF–H_2O, rt, 3 h; (c) ethyl 2-oxocyclopentanecarboxylate, $Si(OEt)_4$, EtOH, reflux, 3 h; (d) $(CH_3)_3SiNCS$, DMF, 140 °C , 3–4 h; (e) 4-fluorobenzyl bromide, K_2CO_3, acetone, reflux, 0.5–1 h.



Scheme 2. Reagents and conditions: (a) EtOH, reflux, 2 h then K_2CO_3, H_2O, reflux, 1 h; (b) H_2O_2 (30 wt % sol in water), AcOH, CH_2Cl_2, reflux, 1 h; (c) 4-trifluoromethylphenylboronic acid, Cs_2CO_3, $Pd(Ph_3P)_4$, dioxane, reflux, 18 h; (d) DPPA, DBU, THF, reflux, 3 h; (e) H_2, 10%Pd/C, 1 atm, EtOH, rt, 12 h; (f) ethyl 2-oxocyclopentanecarboxylate, $Si(OEt)_4$, EtOH, reflux, 5 h; (g) $(CH_3)_3SiNCS$, DMF, 140 °C , 4 h; (h) 4-fluorobenzyl bromide, DBU, KI (cat.), CH_3CN, rt, 5 h; (i) formaldehyde (37 wt% sol in water), reflux, 8 h; (j) MnO_2, dioxane, 70 °C , 3 h; (k) CH_3MgBr, THF, 0 °C , 2 h; (l) HNR^aR^b, $NaBH(OAc)_3$, CH_2Cl_2, rt, 2 h.

Table 1
Inhibitory activity of imidazole/triazole derivatives: study of the linkage site of the biphenyl group

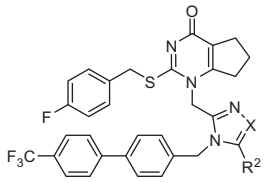


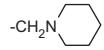
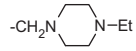
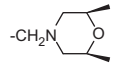
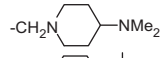
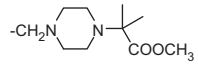
Compound	R^1	R^2	X	% Inhibition in rabbit plasma		
				10 μM	1 μM	100 nM
6a	$-CH_2CH_2NEt_2$		CH	20	16	NT[a]
6b	–Et		N	34	21	NT
6c	$-CH_2CH_2NEt_2$	n-$C_{10}H_{21}$	CH	6	NT	
6d	–Et	n-$C_{10}H_{21}$	N	27	NT	
6e	–Et		N	40	NT	
6f	n-$C_{12}H_{25}$	–H	CH	88	42	NT
6g		–H	CH	97	80	17
6h		–H	N	98	95	67
6i		–H	CH	50	2	
6j		–H	CH	3	NT	
6k		–H	CH	28	NT	

[a] Not tested. Usually, if inhibitory ratio was less than 50%, the compound would not be tested at lower concentration.

route shown in Scheme 1, this procedure was characterized by its high-efficiency in producing the derivatives that were commonly generated in the last step. Furthermore, over 9 steps in the preparation of the key intermediate **15**, only compound **10** required purification by column chromatography. All of the other intermediates could be purified either by recrystallization in moderate to high yields or used directly in the subsequent step without isolation. The synthetic route was therefore amenable to the prepara-

Table 2
Inhibitory activity of imidazole/triazole derivatives: modification at the R^2 position



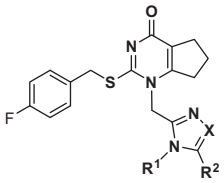
Compound	R^2	X	% Inhibition in rabbit plasma		% Inhibition in human plasma	
			100 nM	10 nM	10 nM	1 nM
6l	–Me	N	79	10	73	11
6m	*n*-Pr	N	88	25	88	65
6n	*i*-Pr	N	91	34	92	71
6o	*c*-Pr	N	87	34	93	68
6p	$-CH_2NMe_2$	CH	87	13	75	54
6q	$-CH_2NEt_2$	CH	88	10	75	52
6r		CH	79	10	67	21
6s		CH	86	11	69	58
15	$-CH_2OH$	N	73	23	65	58
17	$-CH(OH)CH_3$	N	90	29	89	68
18a	$-CH_2NMe_2$	N	88	21	85	62
18b	$-CH_2NEt_2$	N	85	21	78	60
18c	$-CH_2N(i\text{-}Pr)CH_3$	N	85	13	77	35
18d	$-CH_2N(c\text{-}Pr)CH_3$	N	86	16	77	38
18e		N	90	20	82	64
18f		N	76	16	69	23
18g		N	82	11	71	57
18h		N	67	15	51	45
21a	$-CH_2NHCOCH_3$	N	67	8	67	44
21b	$-CH_2NHSO_2Et$	N	75	7	68	49
Darapladib			99	82	95	80

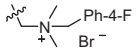
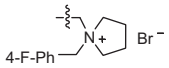
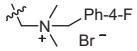
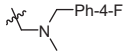
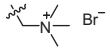
tion of selected compounds in multi-gram quantities, although 12 steps were usually required.

The assay for evaluating the potency of the compounds against the enzyme of Lp-PLA_2 was built in rabbit and human plasma according to the reference method.[12] The percentage inhibition of the enzyme activity at the tested concentration was specified as being indicative of the potency. Two kinds of scaffolds (represented by structures **I** and **II**) could be derived from Darapladib (Fig 2), which were distinguishable by the linking site of biphenyl group on the heterocycle (R^2 and R^1). In the first step, we established which scaffold represented the preferred conformation. For the biphenyl group at R^2 (**6a** and **6b** in Table 1), only limited inhibition (20–30%) of Lp-PLA_2 activity could be achieved at 10 μM concentration in rabbit plasma, regardless of whether the R^1 group was an alkyl or aminoalkyl substituent. The introduction of the more flexible and lipophilic decyl and biphenylmethyl groups gave no further increase in the activity (**6c–e**). In contrast, the introduction of a long aliphatic chain or biphenylmethyl group at the R^1 position (**6f–h**) provided significant improvements in the inhibitory ratio (98% of **6h** vs 34% of **6b** at 10 μM), even in the absence of any substitution at the R^2 position. On the basis of these results, scaffold **II** (i.e. with a biphenyl group at the R^1 position) was selected for further modification. Furthermore, the effect of substitution at the *para*-position of the biphenyl ring was also briefly evaluated (**6i–k**). The results indicated that trifluoromethyl was more effective than any of the other substituents tested in maintaining the potency, likely because of its strong electrophilic and high lipophilic properties.[13]

Then we proceeded to evaluate the potential for further replacements at the R^2 position. The results of this work have been summarized in Table 2. For synthetic convenience, we started with some of the alkyl substituted compounds **6l–o**. The increase in steric hindrance that occurred in the change from methyl to isopropyl was found to improve the activity, with a similar trend also being observed in the comparison of **15** with **17**. In spite of this trend, we decided not to explore the growth of the alky chain any further because the compounds invariably had high log *P* values and were not suitable for the formation of pharmaceutical salts. The inclusion of compounds with these physicochemical properties would very likely lead to poor oral absorbability. With a robust and well established synthetic route in hand, we proceeded to focus on the aminomethyl substituted derivatives (**6p–21b**). Amino groups of different shapes, sizes and electronic properties were therefore taken into consideration. We found that both the imidazole and triazole derivatives showed moderate potency in the rabbit plasma assay. The compounds behaved as potent inhibitors at 100 nM (80–90% inhibition) but suffered an abrupt drop in activity at 10 nM (10–20% inhibition). In the human plasma assay, the compounds performed much better. Several compounds, such as **18a** and **18b** showed significant levels of potency, with both inhibiting more than 60% of the enzyme activity at 1 nM. Unfortunately, this activity was still lower than the positive control Darapladib and the gap was even more pronounced in the rabbit plasma assay (only 21% inhibition at 10 nM vs 82% inhibition for Darapladib). The most potent compound in the series was **18e**, which was only poorly active in comparison to the *n*-propyl substituted compound

Table 3
Inhibitory activity of quaternary ammonium derivatives



Compound	R^2	X	% Inhibition in rabbit plasma		% Inhibition in human plasma	
			100 nM	10 nM	10 nM	1 nM
22a		CH	99	97	93	85
22b		CH	97	88	97	79
22c		N	97	91	99	95
22d		N	90	10	93	71
22e		N	93	91	97	77
Darapladib			99	82	95	80

6m. These results indicated that the amino N-atom did not effectively contribute to the potency of the compounds. Furthermore, when the N-atom was acylated (**21a** and **21b**) or hindered (**18c**, **d**, **f**, **h**), a reduction in the activity was observed. The significant loss in activity encountered following the replacement of the amide in Darapladib with a heterocycle might result from the associated reduction of the rotational degrees of freedom in the molecule. That was, the rotatable C–N bond in the amide was restricted in a cycle, preventing the molecule from reaching the optimal conformation necessary for a high level of affinity.

Although we were disappointed that the target compounds were not as potent as what we had expected, we were encouraged by the results achieved when we screened three quaternary ammonium byproducts **22a–c** (Table 3) that were generated from the over alkylation of intermediate **5** with an excess of 4-fluorobenzyl bromide during the preparation of **6p**, **6r**, and **18a**, respectively.[14] All of these compounds showed similar or higher levels of potency to Darapladib in both the rabbit and human plasma assays. At this stage we became interested in establishing whether the dramatically elevated potency observed in these compounds was derived from the introduction of the 4-flurophenyl group or the ammonium ion. To figure out this point, we synthesized compounds **22d** and **22e** and compared their activities with that of **22c**. Compound **22d** showed reduced activity, whereas **22e** maintained a similar level of potency to **22c**. These results confirmed that the ammonium ion was responsible for the significant improvement observed in the activity. Interestingly, both our most potent inhibitors and PAF (Fig. 1) contained quaternary amines and it was envisaged that these quaternary amines might bind to a similar position in the enzyme. But the accurate binding mode needs to be determined by the establishment of the crystal structure of Lp-PLA_2 with our compounds.

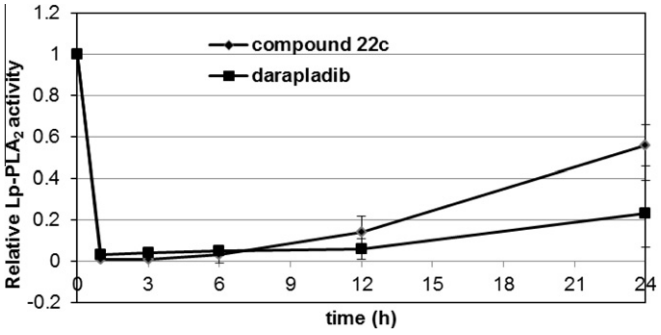


Figure 3. The relative plasma Lp-PLA_2 activity in the Apo-E mouse after a single dose ($n = 5$).

In view of their good in vitro activities, the quaternary ammonium compounds were selected for evaluation in the Apo-E mouse model. The mice were fasted for 16 h prior to being given a single dose of 50 mg/kg of the test compounds through an intraperitoneal injection and blood samples were then draw at different time points to measure the plasma Lp-PLA_2 activity.[15] Of the compounds tested, **22c** gave the best in vivo performance. As shown in Figure 3, compound **22c** effectively inhibited the plasma Lp-PLA_2 activity over a prolonged period of 24 h and showed a similar profile to that of Darapladib during the first 12 h after dosing.

In summary, we have designed and synthesized a series of imidazole and triazole derivatives as Lp-PLA_2 inhibitors using a bioisosteric replacement strategy. Unfortunately, these compounds provided only moderate levels of activity. Pleasingly, however, a series of quaternary ammonium salts that were obtained as byproducts showed comparable activity to Darapladib both in vitro and in vivo, as exemplified by compound **22c**. Compounds of this particular type could be valuable for researching the mechanism of drug action and help in the future design of Lp-PLA_2 inhibitors.

Acknowledgments

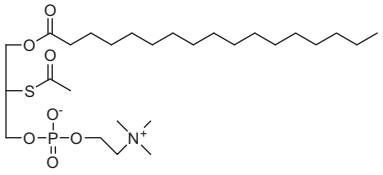
This work was financially supported by grants from the National Science & Technology Major Project 'Key New Drug Creation and Manufacturing Program' (Nos. 2012ZX09301001-001 and 2012ZX09103101-008).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.029.

References and notes

1. (a) Ross, R. *N. Engl. J. Med.* **1999**, *340*, 115; (b) Hansson, G. K. *N. Engl. J. Med.* **2005**, *352*, 1685.
2. Macphee, C. H.; Suckling, K. E. *Expert Opin. Ther. Targets* **2002**, *6*, 309.
3. Stafforini, D. M.; Tjoelker, L. W.; McCormick, S. P.; Vaitkus, D.; McIntyre, T. M.; Gray, P. W.; Young, S. G.; Prescott, S. M. *J. Biol. Chem.* **1999**, *274*, 7018.
4. Zalewski, A.; Macphee, C. H. *Arterioscler., Thromb., Vasc. Biol.* **2005**, *25*, 923.
5. Wilensky, R. L.; Macphee, C. H. *Curr. Opin. Lipidol.* **2009**, *20*, 415.
6. (a) Wilensky, R. L.; Shi, Y.; Mohler, E. R., 3rd; Hamamdzic, D.; Burgert, M. E.; Li, J.; Postle, A.; Fenning, R. S.; Bollinger, J. G.; Hoffman, B. E.; Pelchovitz, D. J.; Yang, J.; Mirabile, R. C.; Webb, C. L.; Zhang, L.; Zhang, P.; Gelb, M. H.; Walker, M. C.; Zalewski, A.; Macphee, C. H. *Nat. Med.* **2008**, *14*, 1059; (b) Wang, W. Y.; Zhang, J.; Wu, W. Y.; Li, J.; Ma, Y. L.; Chen, W. H.; Yan, H.; Wang, K.; Xu, W. W.; Shen, J. H.; Wang, Y. P. *PLoS One* **2011**, *6*, e23425.
7. (a) Ballantyne, C. M.; Hoogeveen, R. C.; Bang, H.; Coresh, J.; Folsom, A. R.; Heiss, G.; Sharrett, A. R. *Circulation* **2004**, *109*, 837; (b) Oei, H. H.; van der Meer, I. M.; Hofman, A.; Koudstaal, P. J.; Stijnen, T.; Breteler, M. M.; Witteman, J. C. *Circulation* **2005**, *111*, 570; (c) Brilakis, E. S.; McConnell, J. P.; Lennon, R. J.; Elesber, A. A.; Meyer, J. G.; Berger, P. B. *Eur. Heart J.* **2005**, *26*, 137; For a comprehensive review, please see: (d) Anderson, J. L. *Am. J. Cardiol.* **2008**, *101*, 23F.
8. (a) Blackie, J. A.; Bloomer, J. C.; Brown, M. J.; Cheng, H. Y.; Elliott, R. L.; Hammond, B.; Hickey, D. M.; Ife, R. J.; Leach, C. A.; Lewis, V. A.; Macphee, C. H.; Milliner, K. J.; Moores, K. E.; Pinto, I. L.; Smith, S. A.; Stansfield, I. G.; Stanway, S. J.; Taylor, M. A.; Theobald, C. J.; Whittaker, C. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2603; (b) Hickey, D. M. B.; Ife, I. G.; Leach, C. A.; Liddle, J.; Pinto, I. L.; Smith, S. A.; Stanway, S. J. Patent WO 0230,904, 2002.; (c) Blackie, J. A.; Bloomer, J. C.; Brown, M. J.; Cheng, H. Y.; Hammond, B.; Hickey, D. M.; Ife, R. J.; Leach, C. A.; Lewis, V. A.; Macphee, C. H.; Milliner, K. J.; Moores, K. E.; Pinto, I. L.; Stansfield, I. G.; Stanway, S. J.; Taylor, M. A.; Theobald, C. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1067; (d) Jeong, H. J.; Park, Y. D.; Park, H. Y.; Jeong, I. Y.; Jeong, T. S.; Lee, W. S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5576; (e) Wan, Z. H.; Zhang, X. M.; Tong, Z. L.; Long, K.; Dowdell, S. E.; Manas, E. S.; Goodman, E. S. Patent WO 2012,037,782, 2012.; (f) Jin, Y.; Wan, Z. H.; Zhang, Q. Patent WO 2012,076,435, 2012.
9. Tompson, A. S.; Humphrey, G. R.; DeMarco, A. M.; Mathre, D. J.; Grabowski, E. J. *J. Org. Chem.* **1993**, *58*, 5886.
10. Zhao, Y. H.; Zhao, J. F.; Zhou, Y. Y.; Lei, Z.; Li, L.; Zhang, H. B. *New J. Chem.* **2005**, *29*, 769.
11. Ivanova, N. V.; Sviridov, S. I.; Shorshnev, S. V.; Stepanov, A. E. *Synthesis* **2006**, *1*, 156.
12. *Assay:* DMSO solutions of the compounds were added to assay buffer containing 50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 150 μL dd H_2O and 10 μL plasma in a total volume of 200 μL. Following 5 min of preincubation at 37 °C, 10 μL [3H]PAF (1uCi) was added and incubated for 10 min at 37 °C. After incubation, 600 μL of $CHCl_3$/MeOH (2:1) was added and the resulting mixture was mixed thoroughly. After a period of centrifugation at 12000*g* at 4 °C for 15 min, 200 μL of supernatant was collected and mixed with 200 μL of $CHCl_3$. An 80 μL portion of the supernatant was then collected and its radioactivity was measured in a liquid scintillation counter. The inhibition rate was determined by the following equation:
Inhibitory rate (%) = $1-(CPM_{compound}-CPM_{blank})/(CPM_{positive}-CPM_{blank})^{*}100\%$
The blank sample contained no plasma or test compound in assay buffer and the positive sample contained no test compound.
13. Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006.
14. Prior to the development of the synthetic route depicted in Scheme 2, the synthesis of **18a** was performed according to the method shown in Scheme 1 via the reaction of the corresponding intermediate **5** with *p*-fluorobenzyl bromide. Compound **22c** was obtained in this procedure as the byproduct. For more synthetic details, please see the Supplementary data.
15. The serum Lp-PLA_2 activity of the Apo-E mouse was measured using 2-thio-PAF as the substrate. Briefly, 10 μL of the plasma were added to 0.1 mol/L Tris–HCl (pH 7.2) containing 1 mmol/L EGTA, 50 μmol/L 2-thio-PAF and 10 μL of 2 mmol/L 5,5′-dithiobis (2-nitrobenzoic acid) in a total volume of 200 μL. The assay was performed using a plate reader to obtain absorbance values at 414 nm every minute. The Lp-PLA_2 activity was calculated from the change in absorbance per minute.



2-thio PAF