Bioorganic & Medicinal Chemistry Letters 23 (2013) 6794–6798

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The chemical synthesis and cytotoxicity of new sulfur analogues of 2-methoxy-lysophosphatidylcholine

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ARTICLE INFO

Article history:
Received 12 July 2013
Revised 3 October 2013
Accepted 8 October 2013
Available online 17 October 2013

Keywords:
Lysophosphatidylcholine analogues
2-Methoxylation
Phosphorothioates
Phosphorodithioates
Cytotoxicity

ABSTRACT

The chemical synthesis of phosphorothioate/phosphorodithioate analogues of 2-methoxy-lysophosphatidylcholine has been described. For the preparation of new sulfur derivatives of lysophosphatidylcholine both oxathiaphospholane and dithiaphospholane approaches have been employed. Each lysophospholipid analogue was synthesized as a series of five compounds, bearing different fatty acid residues both saturated (12:0, 14:0, 16:0, 18:0) and unsaturated (18:1). The methylation of glycerol 2-hydroxyl function was applied in order to increase the stability of prepared analogues by preventing 1→2 acyl migration. The cellular toxicity of newly synthesized 2-methoxy-lysophosphatidylcholine derivatives was measured using MTT viability assay and lactate dehydrogenase release method.

For many years lysophospholipids (LPL) have been considered mainly as 'structural and storage components without informational functions'. However, more recent studies have demonstrated that some lysophospholipids are intercellular lipid mediators acting as hormone-like signaling molecules. They can activate specific membrane receptors and/or nuclear receptors and in this way regulate many important physiological and pathophysiological processes.[1] The LPL have been shown to be involved in many cellular processes and diseases such as diabetes, obesity, atherosclerosis and cancer. Several lysophospholipids were identified under in vivo conditions including lysophosphatidic acid (LPA), cyclic phosphatidic acid (cPA), lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), lysophosphatidylethanolamine (LPE), lysophosphatidylserine (LPS) and lysophosphatidylthreonine (LPT).[1] There is a growing evidence that various lysophospholipids are ligands activating membrane receptors coupled to G proteins (GPCRs). However, the LPL receptors and corresponding signaling pathways have not been recognized so far. Lysophospholipids need to be further characterized with regard to diversity of their structures and specific biological activities. These studies will require the use of LPL analogs with well-defined fatty acid residues and various polar head groups.

One of the most important natural lysophospholipid is 1-*O*-acyl-*sn*-glycerol-3-*O*-phosphocholine (lysophosphatidylcholine, LPC, **1**).[2–5] LPC has a zwitterionic structure (see Fig. 1) and is the most abundant lysophospholipid in animals and plants.

Natural LPC exists in optically active form (R enantiomer), as a mixture of several compounds, possessing fatty acids of different length and saturation status (saturated, monounsaturated, polyunsaturated). Although LPC is only a minor phospholipid present in cell membranes (<3%), it is the most abundant LPL with relatively high (around 150 μM) concentration in human blood.[5] Most of the circulating LPC molecules are associated with albumin. LPC is also a major phospholipid component of oxidized low-density lipoproteins. LPC present in plasma is derived from phosphatidylcholine by lecithin–cholesterol acyltransferase that catalyzes the transacylation of the *sn*-2 fatty acid residue of lecithin to the free cholesterol, resulting in a formation of cholesterol ester and LPC formation.[6] LPC is generated by membrane phosphatidylcholine digestion with phospholipases A_2 (PLA_2) and phospholipases A_1 (PLA_1) that are able to cleave the *sn*-1 and *sn*-2 ester bond, respectively.[7,8] Appreciable amounts of LPC are also formed in plasma by the action of endothelial lipase.[5]

LPC is a proinflammatory, proatherogenic substance and plays an important role in the initiation, progression and destabilization of atherosclerotic plaques. It induces differentiation and morphological changes of various cells and plays important role in tumor progression. Recent reports have shown that LPC induces insulin secretion from pancreatic β-cells.[2] It has been also found that LPC activates glucose uptake and effectively lowers blood glucose levels in mouse models of type 1 and 2 diabetes.[3,4] However, to date the precise mechanism of this phenomenon is obscure. On the other hand, oleoyl-lysophosphatidylcholine (OLPC) was reported to bind to one of membrane receptors known as GPR119 that causes intracellular cAMP accumulation and an

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http://dx.doi.org/10.1016/j.bmcl.2013.10.020

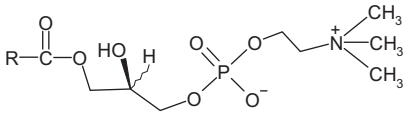


Figure 1. The structure of natural R-lysophosphatidylcholine (LPC, **1**). Substituents R–C(O)– refer to various fatty acid residues present in lysophospholipids.

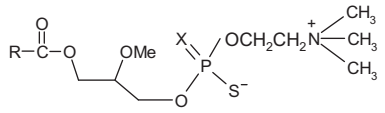
increase of glucose stimulated insulin secretion in NIT-1 insulinoma cells.[4] Nevertheless, the nature of the endogenous ligands of GPR119 and its physiological role in direct regulation of insulin secretion by the pancreatic β cells still have to be explained.[5]

In our previous publication we have described the chemical synthesis of sulfur analogues of LPA and cPA, in which either one or two nonbridging phosphate oxygen atoms are substituted by sulfur to give phosphorothioate or phosphorodithioate analogues, respectively.[9] Intriguing biological properties of natural LPC prompted us to undertake studies on the synthesis of its sulfur analogues modified in the same manner. On the basis of our earlier studies performed on the synthesis of phosphorothioate and phosphorodithioate derivatives of nucleotides and oligonucleotides[10] we expected that lysophospholipid sulfur analogues should have similar physicochemical properties as natural lysophospholipids, yet should be more resistant towards hydrolytic enzymes.[11] In order to prevent possible 1→2 acyl migration in LPC analogues, the oxygen atom in position 2 of glycerol was protected by methylation.[9,12–14] The conditions of chemical synthesis enabled us also to obtain LPC sulfur analogues as homogenic compounds, each containing only one fatty acid residue. Thus, LPC sulfur analogues, both phosphorothioates (**2a**–**e**) and phosphorodithioates (**3a**–**e**), were prepared as a series of five different compounds, bearing the residues of the following fatty acid: (a) lauric (12:0), (b) myristic (14:0), (c) palmitic (16:0), (d) stearic (18:0), (e) oleic (18:1) (see Fig. 2).

The diversity of biological functions and activity of lysophosphatidylcholine allowed us to assume that phosphorothioate/phosphorodithioate derivatives **2** and **3** may also have interesting biological or even therapeutic properties. For preliminary biological studies the phosphorothioates (**2a**–**e**) and phosphorodithioates (**3a**–**e**) were synthesized from racemic glycidol.

The chemical synthesis of 1-acyl-2-methoxyglycerols (**4a**–**e**), that were crucial building blocks for the preparation of **2a**–**e** and **3a**–**e**, was performed exactly as described in our previous paper (see Scheme 1).[9]

Thus, all five aforementioned fatty acids were reacted with commercially available racemic glycidol (**5**) in the presence of catalytic amounts of *n*-tributylamine into 1-acylglycerols (**6a**–**e**) according to the procedure described by Lok et al.[15] In the following step 1-acylglycerols were regioselectively silylated with *t*-butyldimethylsilyl chloride in the position 3 of glycerol (primary hydroxyl group).[16–18] The silyl ethers (**7a**–**e**) were then methylated at the central oxygen of glycerol with trimethylsilyldiazomethane in the presence of 40% fluoroboric acid.[19] The 2-methoxy ethers



LPC sulfur analogues **2,3**

2a-e X = O phosphorothioates **3a-e** X = S phosphorodithioates

a - lauroyl **b** - myristoyl **c** - palmitoyl **d** - stearoyl **e** - oleoyl

Figure 2. The structure of LPC 2-methoxy-sulfur analogues (phosphorothioates **2a**–**e** and phosphorodithioates **3a**-**e**).

(**8a**–**e**) were desilylated with tetra-*n*-butylammoniumfluoride in tetrahydrofuran, yielding desired glyceride building blocks **4a**–**e**.[16–18] All aforementioned products were isolated and purified by silica gel flash column chromatography and characterized by spectroscopic methods as described earlier.[9]

The introduction of one or two nonbridging sulfur atoms into lysophosphatitydylcholine molecule was performed using oxathiaphospholane or dithiaphospholane approach, respectively. These methods were successfully employed for the synthesis of phosphorothioate/phosphorodithioate nucleotide and oligonucleotide analogues of phosphodiester type (see Scheme 2 and the Supplementary data).[10]

The application of aforementioned approach to the synthesis of sulfur analogues of 2-methoxy-lysophosphatidylcholine **2** and **3** is shown on Scheme 3.

For the synthesis of phosphorothioate analogues racemic 1-acyl-2-methoxyglycerols **4a**–**e** were reacted in anh. dichloromethane solution with equimolar amount of 2-*N*,*N*-diisopropylamino-1,3,2-oxathiaphospholane in the presence of *S*-ethylthiotetrazole, and then with elemental sulfur, to give 3-*O*-(2-thio-1,3,2-oxathiaphospholane) lipid derivatives **9a**–**e** (X = O), each showing in ^{31}P NMR two signals at δ ca. 105 ppm. Such chemical shifts were earlier described as characteristic for various 2-alkoxy-2-thio-1,3,2-oxathiaphospholane derivatives.[10a] Compounds **9a**–**e** were isolated by silica gel flash column chromatography as pale-yellow oils in 54–78% yield, and characterized by spectroscopic methods. Due to appearance of two signals in ^{31}P NMR spectra it was concluded that each of **9a**–**e** is a mixture of two stereoisomers not separable by chromatography (TLC).

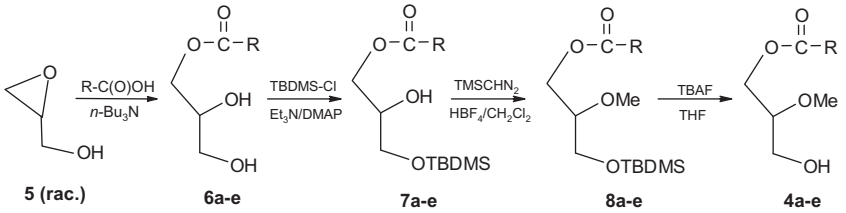
Each of oxathiaphospholane derivatives **9a**–**e** was then treated in anh. dichloromethane with 3 mol equiv of choline *p*-toluenesulfonate[20] and 2 mol equiv of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The ring opening condensation was accompanied by spontaneous elimination of episulfide.

Crude products were purified by silica gel flash column chromatography to give 2-methoxy-LPC phosphorothioates (**2a**–**e**) as white solids in 50–60% yield. The products were obtained as a mixture of stereoisomers (two signals in ^{31}P NMR at δ ca. 59 ppm), which could not be separated by chromatography (column or TLC). Such chemical shifts are characteristic for dialkyl phosphorothioates.[10a] The purified products **2a**–**e** were characterized by spectroscopic methods (^{1}H and ^{31}P NMR, MALDI TOF MS). They were soluble in chloroform, methanol and water, and did not decompose when stored at −20 °C. The details are given in Supplementary data.

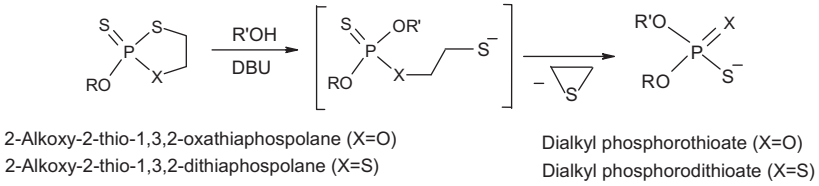
The presence of two signals in ^{31}P NMR spectra of each **2a**–**e** (and also **9a**–**e**) can be attributed to the presence of two centers of asymmetry (at C2 and at phosphorus), leading to the formation of two pairs of diastereoisomers. Unfortunately, unlike the previously phosphorothioate cPA analogues,[9] these stereoisomers could not be separated by either column chromatography or thin layer chromatography.

For the synthesis of phosphorothioate analogues racemic 1-acyl-2-methoxyglycerols **4a**–**e** containing one of the aforementioned fatty acid residues were reacted in anh. dichloromethane solution with 2-*N*,*N*-diisopropylamino-1,3,2-dithiaphospholane in the presence of *S*-ethylthiotetrazole, and then with elemental sulfur, to give 3-*O*-(2-thio-1,3,2- dithiaphospholane) lipid derivatives **10a**–**e** (X = S), showing in ^{31}P NMR single signals at δ ca. 123 ppm). Such chemical shifts were earlier described as characteristic for various 2-alkoxy-2-thio-1,3,2-dithiaphospholane derivatives.[10b,g] Pure compounds **10a**–**e** were isolated by silica gel flash column chromatography as pale-yellow oils in 39–50% yield, and characterized by spectroscopic methods.

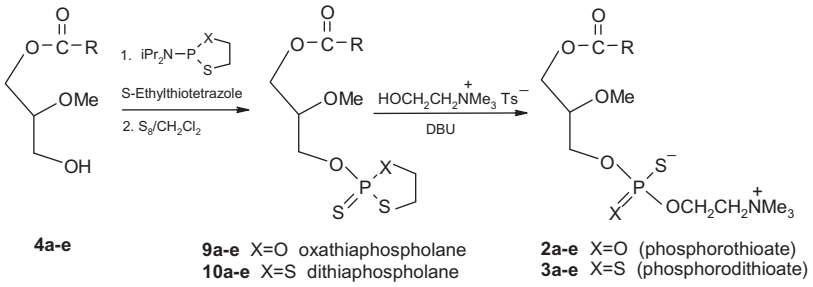
Each of dithiaphospholane derivatives **10a**–**e** was then treated in anh. dichloromethane with 3 mol equiv of choline



Scheme 1. The synthesis of racemic **4a**–**e**. The fatty acid residues correspond to those shown in Fig. 2.



Scheme 2. Oxathiaphospholane/dithiaphospholane methodology for the synthesis of phosphorothioate/phosphorodithioate diesters.



Scheme 3. The synthesis of 1-acyl-2-methoxy-phosphatidylcholine sulfur analogues **2a**–**e** and **3a**–**e**.

p-toluenesulfonate[20] and 2 mol equiv of DBU. Crude products were purified by silica gel flash column chromatography to give 2-methoxy-LPC phosphorodithioates (**3a**–**e**) as white solids in 18–46% yield. The products showed in ^{31}P NMR single signals at δ *ca* 116 ppm, characteristic for dialkyl phosphorodithioates.[10b,10g] The purified products **3a**–**e** were characterized by spectroscopic methods (^{1}H and ^{31}P NMR, MALDI TOF MS). They were soluble in chloroform, methanol and water, and were found to be stable on storage at −20 °C. The details are given in Supplementary data.

All newly synthesized compounds **2a**–**e** and **3a**–**e** were subjected to biological characterization for their in vitro cytotoxic activity. Commercially available unmodified LPC (12:0, 14:0, 16:0, 18:0, and 18:1) were used for comparison. The safety profile was tested on murine insulinoma βTC-3 cells. The choice of target cells was dictated by the fact that the aforementioned derivatives were to be used as possible inducers of insulin secretion. The cells (10,000 per well) were seeded onto 96-well plates and cultured for 24 and 48 h, followed by treatment with modified LPC derivatives or their natural counterparts. Cytotoxicity was evaluated by MTT colorimetric assay (see Table 1). To evaluate cell damage, lactate dehydrogenase (LDH) levels in the medium were also measured. In general, 2-methoxy-LPC phosphorothioates (**2a**–**e**) and 2-methoxy-LPC phosphorodithioates (**3a**–**e**) used at the concentration of 10 μM did not show any notable reduction in cell viability as determined by MTT assay after 24 and 48 h incubation. After 48 h of incubation, only 1-*O*-stearoyl-2-methoxy-lysophosphatidylcholine phosphorothioate (**2d**) showed significant inhibition of cellular metabolic activity. However, significant cytotoxic effect was observed for unmodified LPC, especially in the case of 14:0, 16:0, and 18:0 species, resulting in 26.3%, 29.7%, and 38.7% of cell viability, respectively, as compared to control. Only oleoyl LPC used at 10 μM concentration had no significant toxic effect on cell number. Cells exposed to 25 μM concentration of tested compounds showed greater reduction in their viability and, as expected, cytotoxic effect was stronger after 48 h. However, it is noteworthy to mention that only 1-*O*-oleoyl-2-methoxy-lysophosphatidylcholine phosphorothioate (**2e**) and 1-*O*-oleoyl-2-methoxy-lysophosphatidylcholine phosphorodithioate (**3e**) did not exhibit any cytotoxicity even at the 25 μM concentration. Other sulfur-containing compounds decreased the number of viable cells after 24 h from 58.7% in the case of 1-*O*-palmitoyl-2-methoxy-lysophosphatidylcholine phosphorodithioate (**3c**) to 90.7% for 1-*O*-lauroyl-2-methoxy-lysophosphatidylcholine phosphorothioate (**2a**). After 48 h of incubation with 25 μM concentration of LPC analogues, the most toxic appeared to be 1-*O*-myristoyl-2-methoxy-lysophosphatidylcholine phosphorothioate (**2b**), 1-*O*-palmitoyl-2-methoxy-lysophosphatidylcholine phosphorodithioate (**3c**) and 1-*O*-stearoyl-2-methoxy-lysophosphatidylcholine phosphorodithioate (**3d**), with 35.8%, 37.4%, and 38.9% of cell viability as compared to control, respectively. Among unmodified LPC tested, 14:0, 16:0, and 18:0 derivatives were found to be most toxic, as it could be concluded from a 24-h observation.

An easy insertion of cone-shaped lysophospholipids into the plasma membrane was demonstrated in several studies to result in disruption of membrane integrity.[21] Thus, we decided to evaluate if the cytotoxic effects observed with MTT assay may be caused by detergent properties of natural LPC and their modified derivatives. We measured LDH release from cells as a marker of cellular membrane damage. As shown in Fig. 3, all compounds tested at a concentration of 10 μM had only little effect on LDH release

Table 1
The cytotoxicity of unmodified lysophosphatidylcholines and 1-acyl-2-methoxy-phosphatidylcholine sulfur analogues **2a–e** and **3a–e** tested on βTC-3 insulinoma cells

Compound	Concentration (μM)	Cell viability (% of viability of untreated cells ± SD)	
		24 h	48 h
LPC 12:0	10	105.9 ± 21.6	86.1* ± 9.0
	25	83.0 ± 20.2	67.1* ± 4.1
LPC 14:0	10	75.5 ± 16.2	53.1* ± 6.0
	25	26.3* ± 7.6	7.5* ± 6.0
LPC 16:0	10	73.1 ± 21.2	59.8* ± 5.1
	25	29.7* ± 9.5	20.1* ± 6.3
LPC 18:0	10	88.8 ± 15.6	59.3* ± 11.8
	25	38.7* ± 5.1	29.5* ± 9.1
LPC 18:1	10	130.2 ± 28.7	93.4 ± 7.4
	25	81.1 ± 9.1	68.5* ± 7.3
2a	10	90.2 ± 8.3	106.0 ± 9.9
	25	90.7 ± 9.6	71.1* ± 10.8
2b	10	111.6 ± 4.8	100.8 ± 7.8
	25	73.7* ± 2.2	41.1* ± 10.5
2c	10	104.6 ± 12.2	87.7 ± 8.0
	25	60.4* ± 11.0	44.2* ± 6.2
2d	10	90.3 ± 6.8	61.4* ± 9.5
	25	82.9* ± 9.9	50.9* ± 10.3
2e	10	121.4* ± 2.6	92.4 ± 5.8
	25	144.5* ± 8.4	133.1 ± 14.1
3a	10	87.1 ± 12.0	98.2 ± 15.9
	25	83.7 ± 9.6	41.8* ± 6.1
3b	10	103.7 ± 11.4	87.9 ± 6.7
	25	64.7* ± 7.5	35.8* ± 4.4
3c	10	112.5 ± 6.2	100.5 ± 8.6
	25	58.7* ± 26.9	37.4* ± 14.6
3d	10	85.8* ± 7.7	96.8 ± 12.3
	25	63.1* ± 16.2	38.9* ± 7.8
3e	10	90.5 ± 13.1	95.9 ± 5.3
	25	111.2 ± 9.6	112.2* ± 6.8

Viability of the βTC-3 cells was investigated for 10 and 25 μM concentrations of tested compounds and for two times of incubation (24 and 48 h). Results are expressed as a mean of four independent experimental repeats. The data marked with * were found to be statistically significant ($p < 0.05$).

into the medium, suggesting that significant toxicity observed for unmodified 14:0, 16:0, and 18:0 LPC cannot be attributed to cell lysis. However, it was found that at the concentration of 25 μM the LDH release was considerably greater. The strongest effect was observed for 12:0 LPC (16.8%), as well as for 16:0 LPC and 18:1 LPC (both 9.04%). Among 2-methoxy-LPC phosphorothioates and 2-methoxy-LPC phosphorodithioates used at the concentration of 25 μM, compounds **1b**, **2b**, **2c**, and **3b** appeared to be most toxic. Negligible LDH release was seen only in the presence of LPC analogues **2d**, **2e**, **3d**, and **3e**. Thus, one can assume that 1-*O*-oleoyl-2-methoxy-lysophosphatidylcholine phosphorothioate (**2e**) and 1-*O*-oleoyl-2-methoxy-lysophosphatidylcholine phosphorodithioate (**3e**) did not exhibit cytotoxic properties because they did not disrupt plasma membrane integrity.

In summary, all tested compounds showed low toxicity against βTC-3 insulinoma cells when used at the 10 μM concentration. However, only 1-*O*-oleoyl-2-methoxy-lysophosphatidylcholine phosphorothioate (**2e**) and 1-*O*-oleoyl-2-methoxy-lysophosphatidylcholine phosphorodithioate (**3e**) exhibited good safety profile at 25 μM concentration. Based on these findings, due to toxicity of the sulfur LPC analogues for further cell culture studies their concentration should not exceed 10 μM.

The modification of lysophosphatidylcholine by introduction of one or two sulfur atoms into phosphate moiety may change its chemical and biological properties. We expect that phosphorothioate/phosphorodithioate analogues of LPC will be more stable toward phospholipid degrading enzymes. In our previous it was demonstrated that introduction of methyl group into *sn*-2 position of lysophosphatidic acid may also protect compounds against the action of lysophospholipid phosphatases in vivo.[12] On the other hand, introduction of sulfur into LPC structure will modify its biological activity The new LPC analogues can be valuable tools in the studies on the mechanism of binding of lysophosphatidylcholine to GPR119 receptors. Moreover, taking into account the involvement of LPC in insulin secretion, modified interactions of newly obtained LPC analogues with cell receptors may induce interesting pharmacological properties.

The new sulfur analogues of LPC were prepared employing oxathiaphospholane/dithiaphospholane chemistry. For each analogue a series of five compounds was synthesized, containing different fatty acid residues with both saturated (12:0, 14:0, 16:0, 18:0) and unsaturated (18:1) hydrocarbon chains. For preliminary biological studies, the LPC analogues were prepared from racemic glycidol. Thus, the phosphorodithioate products were also racemates, whereas phosphorothioates were obtained as a racemic mixture of diastereoisomers. The synthesis of most active compounds in optically pure form will be performed starting from enantiomeric precursors.

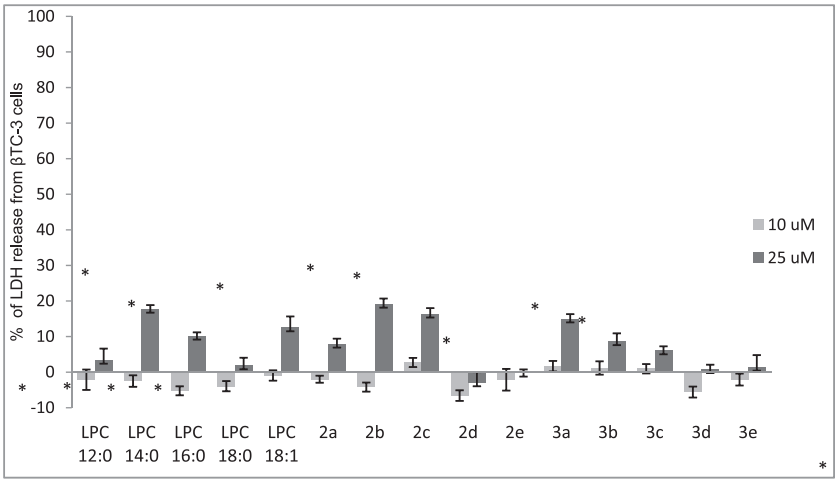


Figure 3. The influence of unmodified lysophosphatidylcholines and 1-acyl-2-methoxy-phosphatidylcholine sulfur analogues (**2a–e** and **3a–e**) on lactate dehydrogenase release from βTC-3 insulinoma cells measured after 24 h of incubation with tested compounds. Data points are expressed as a percentage of LDH release from the cells treated with tested compounds vs LDH release from cells treated with methanol alone. Each data point represents a mean ± SD ($n = 4$). The data marked with * were found to be statistically significant ($p < 0.05$).

Acknowledgment

This work was supported by a grant from the National Science Centre (grant no. 2011/01/B/ST5/06383).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.10.020.

References and notes

1. Makide, M.; Kitamura, H.; Sato, Y.; Okutani, M.; Aoki, J. *J. Prostaglandins Other Lipid Mediat.* **2009**, *89*, 135.
2. Soga, T.; Ohishi, T.; Matsui, T.; Saito, T.; Matsumoto, M.; Takasaki, J.; Matsumoto, S.; Kamohara, M.; Hiyama, H.; Yoshida, S.; Momose, K.; Ueda, Y.; Matsushime, H.; Kobori, M.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2005**, *326*, 744.
3. Yea, K.; Kim, J.; Yoon, J. H.; Kwon, T.; Kim, J. H.; Lee, B. D.; Lee, H. J.; Kim, J. K.; Lee, T. G.; Baek, M. C.; Park, H. S.; Park, K. S.; Ohba, M.; Sun, P. G.; Ryu, S. H. *J. Biol. Chem.* **2009**, *284*, 33833.
4. Overton, H. A.; Fyfe, M. C. T.; Reynet, C. *Br. J. Pharmacol.* **2008**, *153*, S76.
5. Schmitz, G.; Ruebsaamen, K. *Atherosclerosis* **2010**, *208*, 10.
6. 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.
7. Kitsiouli, E.; Nakos, G.; Lekka, M. E. *Biochim. Biophys. Acta* **2009**, *1792*, 941.
8. Yan, W.; Jenkins, C. M.; Han, X.; Mancuso, D. J.; Sims, H. F.; Yang, K.; Gross, R. W. *J. Biol. Chem.* **2005**, *280*, 26669.
9. Rytczak, P.; Koziołkiewicz, M.; Okruszek, A. *New J. Chem.* **2010**, *34*, 1008.
10. (a) Stec, W. J.; Grajkowski, A.; Koziołkiewicz, M.; Uznański, B. *Nucl. Acids Chem.* **1991**, *19*, 5883; (b) Okruszek, A.; Olesiak, M.; Balzarini, J. *J. Med. Chem.* **1994**, *37*, 3850; (c) Okruszek, A.; Sierzchała, A.; Fearon, K. L.; Stec, W. J. *J. Org. Chem.* **1995**, *60*, 6998; (d) Stec, W. J.; Grajkowski, A.; Kobylańska, A.; Koziołkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019; (e) Błaszczyk, J.; Wieczorek, M. W.; Okruszek, A.; Sierzchała, A.; Kobylańska, A.; Stec, W. J. *J. Chem. Cryst.* **1996**, *26*, 33; (f) Sierzchała, A.; Okruszek, A.; Stec, W. J. *J. Org. Chem.* **1996**, *61*, 6713; (g) Okruszek, A.; Olesiak, M.; Krajewska, D.; Stec, W. J. *J. Org. Chem.* **1997**, *62*, 2269; (h) Kobylańska, A.; Okruszek, A.; Stec, W. J. *Nucleosides & Nucleotides* **1998**, *17*, 1977; (i) Stec, W. J.; Karwowski, B.; Boczkowska, M.; Guga, P.; Koziołkiewicz, M.; Sochacki, M.; Wieczorek, M. W.; Błaszczyk, J. *J. Am. Chem. Soc.* **1998**, *120*, 7157; (j) Karwowski, B.; Okruszek, A.; Wengel, J.; Stec, W. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1001; (k) Olesiak, M.; Krajewska, D.; Wasilewska, E.; Korczyński, D.; Baraniak, J.; Okruszek, A.; Stec, W. J. *Synlett* **2002**, 967; (l) Guga, P.; Okruszek, A.; Stec, W. J. In *Topics in Current Chemistry*; Majoral, J. P., Ed.; Springer: Berlin-Heidelberg, 2002; p 169. Vol. 220.
11. Wójcik, M.; Cieślak, M.; Stec, W. J.; Goding, J. W.; Koziołkiewicz, M. *Oligonucleotides* **2007**, *17*, 134.
12. Gendaszewska-Darmach, E.; Laska, E.; Rytczak, P.; Okruszek, A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2698.
13. Qian, L.; Xu, Y.; Arai, H.; Aoki, J.; McIntyre, T. M.; Prestwich, G. D. *Org. Lett.* **2003**, *5*, 4685.
14. (a) Hasegawa, Y.; Erickson, J. R.; Goddard, G. J.; Yu, S.; Liu, S.; Cheng, K. W.; Aoki, J.; Jarosz, R.; Schrier, A. D.; Lynch, K. R.; Mills, G. B.; Fang, X. *J. Biol. Chem.* **2003**, *278*, 11962; (b) Qian, L.; Xu, Y.; Hasegawa, Y.; Aoki, J.; Mills, G. B.; Prestwich, G. D. *J. Med. Chem.* **2003**, *46*, 5575.
15. Lok, C. M.; Mank, A. P. J.; Ward, J. P. *Chem. Phys. Lipids* **1985**, *36*, 329.
16. Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
17. Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799.
18. Xu, Y.; Qian, L.; Prestwich, G. D. *Org. Lett.* **2003**, *5*, 2267.
19. Aoyama, T.; Shiori, T. *Tetrahedron Lett.* **1990**, *38*, 5507.
20. Brockenhoff, H.; Ayengar, N. K. N. *Lipids* **1979**, *14*, 88.
21. Grzelczyk, A.; Gendaszewska-Darmach, E. *Biochimie* **2003**, *21*, 667.