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Synthesis and characterization of new and potent α-lipoic acid derivatives

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Abstract—α-Lipoic acid [5-[1,2]-dithiolan-3-yl-pentanoic acid (LA)] is a natural antioxidant and cofactor of several enzymes. It increases the glucose transport activity in skeletal muscles and adipocytes in a non-insulin dependent manner. Therefore, LA is widely used in Type 2 diabetic patients as an oral auxiliary drug. However, large doses of LA (0.8–1.8 gr/day po) are required due to its unfavorable pharmacokinetic parameters. In order to improve these parameters, we synthesized ester and amide LA derivates. Two of these newly synthesized compounds, 5-[1,2]-dithiolan-3-yl-pentanoic acid 3-(5-[1,2]dithiolan-3yl-pentanoylamino)-propyl]-amide (AN-7) and 5-[1,2]-dithiolan-3-yl-pentanoic acid 3-(5-[1,2]-dithiolan-3yl-pentanoyloxy)-propyl ester (AN-8) augmented the rate glucose transport in myotubes in culture in the absence or presence of insulin. Their potency was 12-fold higher than that of the parent compound; their maximal stimulatory effect was 1.5-fold higher than that of LA. When tested in vivo in streptozotocin-diabetic C57/Black mice, AN-7 (10 mg/kg/day for 2 weeks, sc) reduced blood glucose level by 39% while a higher dose of LA (50 mg/kg/day for 2 weeks, sc) lowered it by 30%. These results indicate that AN-7 is more potent than LA in augmenting glucose transport in skeletal muscles and reducing blood glucose in diabetic animals.

1. Introduction

Lipoic acid (thiocitic acid) (LA) is a cofactor of mitochondrial decarboxylacion enzymatic reactions and is essential for an adequate ATP production from glucose via the citric acid cycle.[1] LA is also a multifunctional antioxidant.[2] Its antioxidant properties are divided into four categories: (I) scavenger of reactive oxygen species, (II) regeneration and de novo syntheses of endogenous antioxidants (i.e., glutathione and vitamin E), (III) metal ion chelating activity, and (IV) repairing oxidative damage in macromolecules.[3–5] Oxidative stress is associated with various diseases leading to multicellular and organ damages.[6] For instance, in diabetes mellitus oxidative stress is associated with an early onset of complications[7] and an impairment of peripheral insulin sensitivity.[8,9]

LA is an oral food additive and is sold over the counter.[10] Jacob et al. has reported that an acute iv infusion of LA improves insulin-stimulated metabolic clearance rate of glucose as well as insulin sensitivity in type 2 diabetes patients.[11] When tested in vitro, LA increases the rate of glucose transport in cultured rat L6 myotubes and 3T3-L1 adipocytes and in isolated rat diaphragm and cardiomyocytes.[12–14] This effect is blocked by wortmannin, an inhibitor of phosphoinositol-3-kinase (PI3-kinase), a key enzyme in the insulin transduction pathway. However, the concentrations of LA required to induce this direct stimulatory effects in vitro are considerably high (2.0–2.5 mM). It was calculated that a single oral dose of 600 mg in man elevates plasma LA concentration to only 10–25 μM without any considerable antihyperglycemic activity.[15] There are two possible reasons for the marginal effect of an oral dose LA

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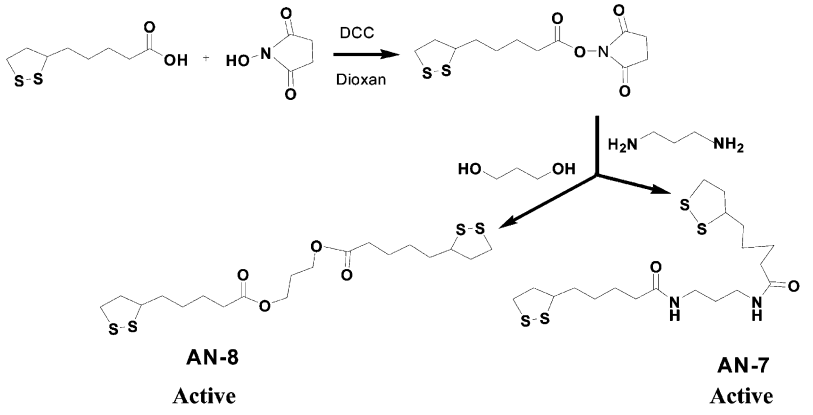
on blood glucose levels and peripheral insulin sensitivity: a short plasma half-life (30 min) and low bioavailability (30%).[15] Indeed, repeated oral administration of LA in man does not elevate its plasma level above 25 μM,[16] while an iv infusion of LA results in higher plasma concentration of the drug and improved effects.[2]

We have undertaken to synthesize more potent and effective LA derivatives. Several compounds were synthesized and tested both in vivo and in vitro. Among those 5-[1,2]-dithiolan-3-yl-pentanoic acid 3-(5-[1,2]dithiolan-3-yl-pentanoylamino)-propyl]-amide (AN-7) was found to be more potent than LA in vitro and in vivo.

2. Results and discussion

LA-N-hydroxy soccinimide (NHS) was synthesized according to Nefkens and Tesser[17] (Scheme 1). The bond between the alkyl chain and the LA was either an ester or amide. Amide derivatives (AN-11), (AN-5) and (AN-7) were synthesized by binding of LA-NHS to PEG-diamine, PEG-monoamine or propane-1,3-diamine, respectively[18] (Scheme 1). Ester derivatives were synthesized by coupling of LA-NHS to dihydroxy-PEG, propane-1,3-diol, hexane-1,6-diol, octane-1,8-diol and decane1,10-diol to produce (AN-6), (AN-8), (AN-30), (AN-31) and (AN-32), respectively[19] (Scheme 1).

None of the PEG-derivatives (AN-5, AN-6 and AN-11) or the ester derivatives of hexane (AN-30), octane (AN-31) and decane (AN-32) improved the rate of hexose transport in L6 myotubes (data not shown). However, AN-7 and AN-8 were active in vitro. These two compound as well as LA increased the rate of hexose transport in L6 myotubes in a dose- and a time-dependent manner, as shown in Figures 1–4. In these experiments the myotubes were pre-conditioned at 5.0 mM glucose or 23.0 mM glucose for 24 h. Pre-incubation of the myotubes at 23.0 mM glucose induced down-regulation of the hexose transport system, as was shown before.[20] Maximal stimulatory effects of LA (2.5 mM) on the rate of hexose transport were 39±9% (mean±SEM, $n=3$) and 51±6% in L6 myotubes maintained at 5.0 and 23.0 mM glucose, respectively (Fig. 1). Higher concentrations of LA were toxic to the myotubes. AN-7 and AN-8 increased the rate of hexose transport by 92±11% and 82±6.8% in L6 myotubes at 5.0 mM glucose and by 120±8.9% and 93±11.2% at 23 mM. These maximal effects were obtained with 200 μM of each compound (Fig. 2). Maximal stimulatory effect of LA on myotubes exposed to 5.0 and 23.0 mM glucose was observed 2 h after its addition and thereafter it rapidly declined (Fig. 3 and 4). In contrast, half maximal and maximal effects of AN-7 and AN-8 were observed at 27 and 36 h, respectively, after their addition to the myotubes cultures exposed to 5.0 mM (Fig. 3). The corre-



Non-active LA derivatives

(Y)LA-X-*R*-X-LA

Compounds	Y	X	*R*
AN-5	O-CH_3	NH	PEG
AN-6	-	O	PEG
AN-11	-	NH	PEG
AN-30	-	O	$(CH_2)_6$
AN-31	-	O	$(CH_2)_8$
AN-32	-	O	$(CH_2)_{10}$

Scheme 1.

sponding values in myotubes exposed to 23.0 mM glucose were 22 and 36 h (Fig. 4). These data suggest a slow release of active LA from the prodrug molecules following accumulation in myotubes. The combined stimulatory effects of insulin and LA, AN-7 or AN-8 were additive both in L6 myotubes under the normo- or hyperglycemic conditions (Fig. 5). AN-7 and AN-8 were not toxic to the myotubes cultures.

Table 1 shows the effects of a 2-week treatment with LA (50 mg/kg BW/day), AN-7 or AN-8 (10 mg/kg BW/day) on blood glucose levels in streptozotocin-diabetic male C57/Black mice. AN-7 and LA reduced blood glucose levels in mildly diabetic mice by 30.2±5 (mean±SEM, $n=8$) and 40±13%, respectively. Lower doses of AN-7 (1 or 5 mg/kg BW/day) had no noticeable effect on blood glucose level of mildly or severly diabetic mice. A higher dose of AN-7 (20 mg/kg BW/day) produced similar glucose lowering effect in mildly diabetic mice as 10 mg/kg/day (data not shown). AN-8 failed to change blood glucose levels in these mice. Neither LA nor AN-7 or AN-8 had a significant effect on blood glucose

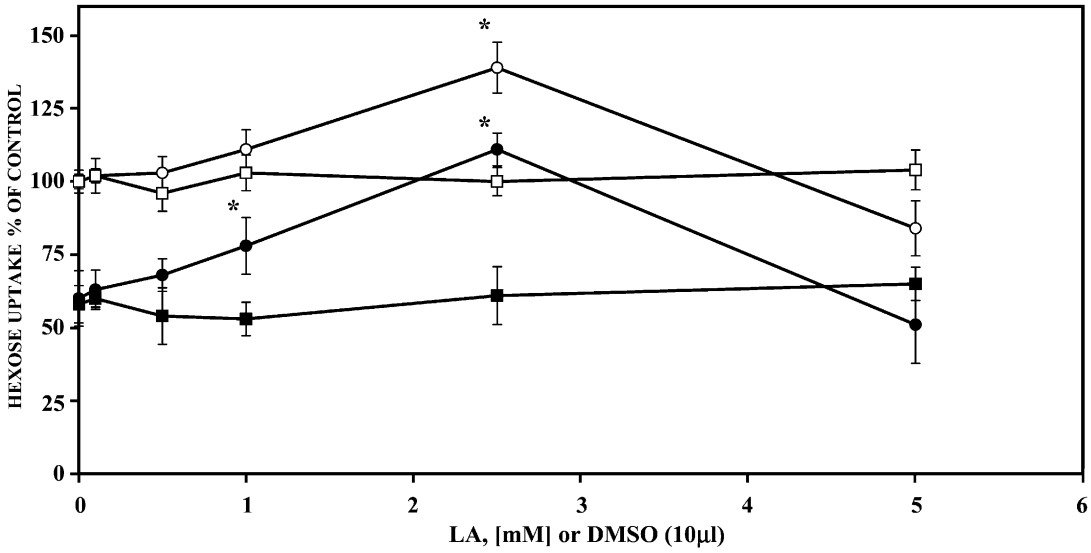


Figure 1. Dose–response of LA effect of on hexose transport in L6 myotubes. L6 myotubes were maintained for 24 h at 5.0 (open symbols) or 23.0 mM glucose (closed symbols). The cells then received increasing concentrations of LA (○) or 10 μL of the vehicle DMSO (□). The standard [^{3}H]dGlc uptake assay was performed 2 h after the addition of the LA to the myocyte cultures as described under. 100% value was taken for control cell maintained at 5.0 mM glucose (1.21±0.08 nmol dGlc/mg protein/min). Mean±SEM, are shown: $n=3$. *$p<0.05$, compared to the respective control.

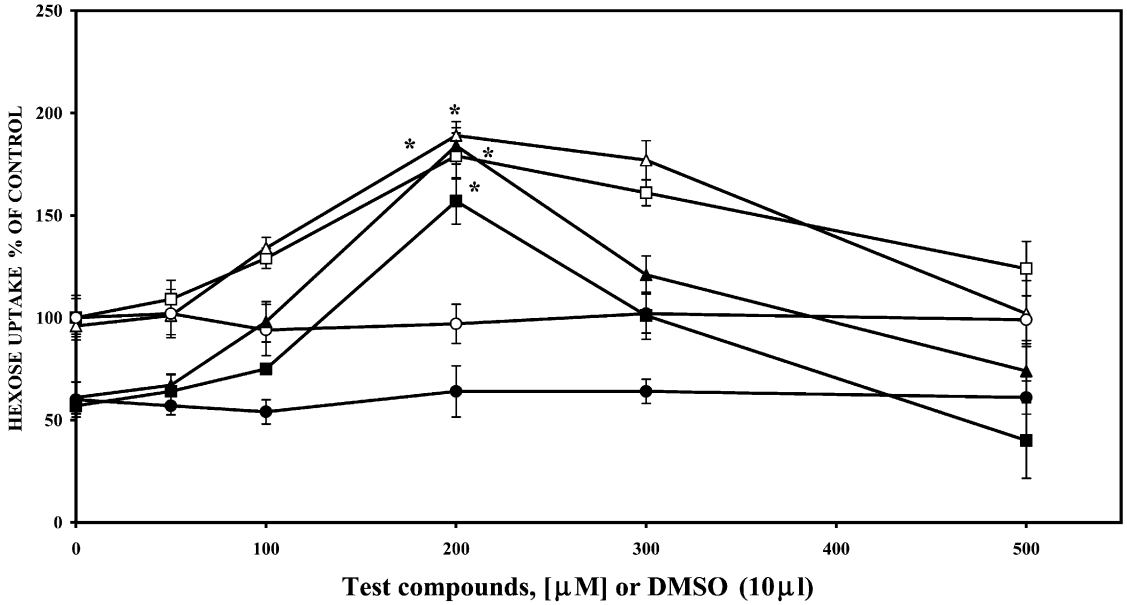


Figure 2. Dose–response of AN-7 and AN-8 effects on hexose transport in L6 myotubes. L6 myotubes were maintained for 24 h at 5.0 (open symbols) or 23.0 mM glucose (closed symbols). The cells then received increasing concentrations of △,▲: AN-7; □,■: AN-8; and ○,●: 10 μL of vehicle DMSO. After 36 h incubation, the myocyte cultures were taken for the standard [^{3}H]dGlc uptake assay as described. 100% value was taken for control cell maintained at 5.0 mM glucose (1.68±0.10 nmol dGlc/mg protein/min). Mean±SEM, are shown: $n=3$. *$p<0.05$, compared to the respective control.

levels in severely diabetic (Table 1) or in non-diabetic control mice (data not shown). One week after discontinuation of LA and AN-7 treatments, blood glucose levels returned to the same levels of control mice.

Clinical studies suggest that, due to the limited bioavailability of LA, large oral doses of the compound are required for an oral therapy.[21] Some studies[4] indicate that LA augments insulin sensitivity in Type 2 diabetics. However, this effect seems limited due to the reduced bioavailability of LA and its low plasma concentration in man.[21] Therefore, Evan and Goldfine[2] have suggested that effort should be made to maintain a therapeutically effective levels of LA in the plasma for an extended period of time that may result in an increased insulin sensitivity and eventually improvement of glycemic control in type 2 diabetic patients. To meet these requirements we have synthesized prodrugs with improved lipid solubility that can release LA intracellularly.[22,23]

Among all the LA derivatives we synthesized only the amidopropane derivative (AN-7) was active in both in

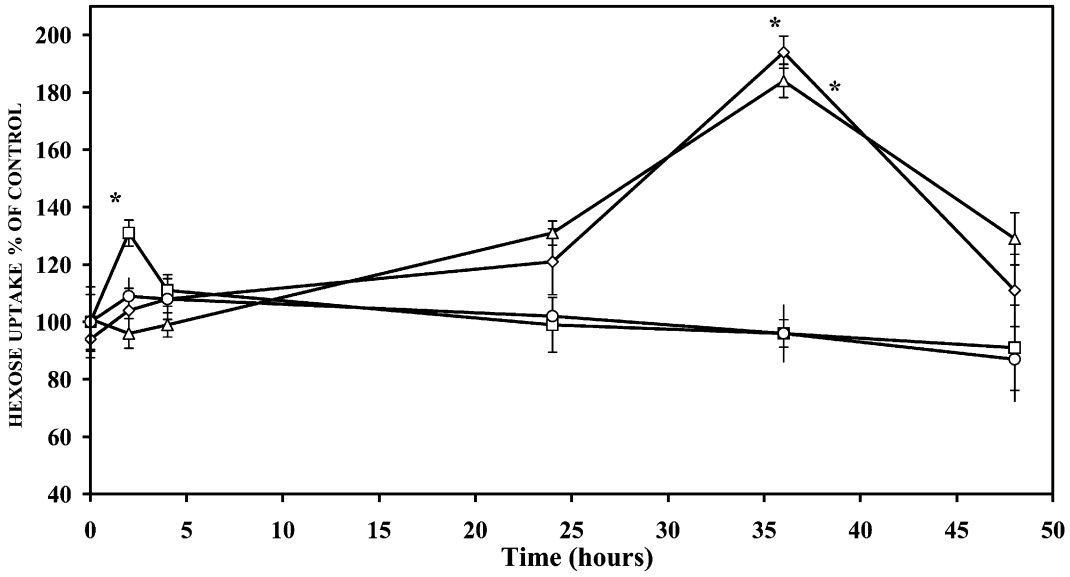


Figure 3. Time–course of α-Lipoic acid, AN-7 and AN-8-dependent stimulation of hexose transport in L6 myotubes at 5.0 mM glucose. L6 myotubes were preincubated at 5.0 mM glucose for 24 h, The culture medium was changed to fresh medium with the same glucose concentration in the presence 10 μL of vehicle DMSO (○), 2.5 mM LA (□), 200 μM AN-7 (◇), or 200 μM of AN-8 (△). Following incubations at the indicated times the myotubes were taken for the standard [^{3}H]dGlc uptake assay as described. 100% value was taken for control cell maintained at 5.0 mM glucose (1.11 ± 0.07 nmol dGlc/mg protein/min). Mean ± SEM, are shown: $n = 3$. *$p < 0.05$, compared to the respective control.

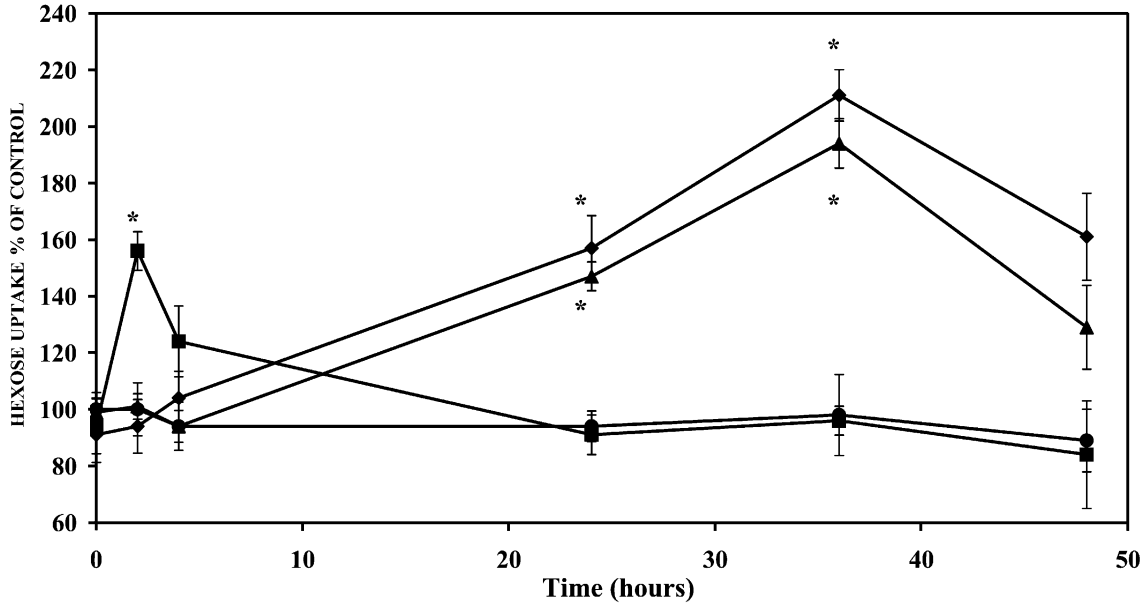


Figure 4. Time–course curves of α-lipoic acid, AN-7 and AN-8-dependent stimulation of hexose transport in L6 myotubes at 23.0 mM concentration glucose. L6 myotubes were preincubated at 23.0 mM glucose for 24 h. The culture medium was changed to fresh medium with the same glucose concentration in the presence DMSO (●), 2.5 mM LA (■), 200 μM AN-7 (◆), or 200 μM of AN-8 (▲). Following incubations at the indicated time the myotubes were taken for the standard [^{3}H]dGlc uptake assay as described. 100% value was taken for control cell maintained at 23.0 mM glucose. (0.69 ± 0.08 nmol dGlc/mg protein/min). Mean ± SEM, are shown: $n = 3$. *$p < 0.05$, compared to the respective control.

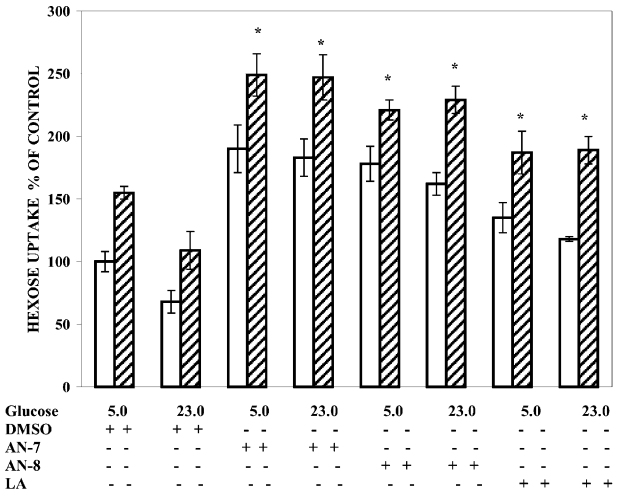


Figure 5. Effect of insulin α-lipoic acid, AN-7 and AN-8 on hexose transport in L6 myotubes. L6 myotubes were preincubated at 5.0 or at 23.0 mM glucose for 24 h before experiment. Medium was changed to fresh medium with the same glucose concentration in the presence of 10 μL of vehicle DMSO, 200 μM AN-7, or 200 μM of AN-8. Seven h before the uptake, assay medium was changed to a serum-free supplemented medium with the 0.5% (w/v) BSA and same additions. Insulin (200 nM) was added during the last 1 h of incubation. LA (2.5 mM) was added 2 h prior to the uptake assay. [^{3}H]dGlc uptake assay was performed 2 h after LA addition and 36 h after AN-7 and AN-8 additions. 100% value was taken for control cell maintained at 5.0 mM glucose (1.23±0.09 nmol dGlc/mg protein/min). Mean±SEM, are shown: $n=3$. *$p<0.05$, compared to the respective control.

vitro and in vivo experimental systems. This compound was 12-fold more potent in vitro than LA in increasing the rate of hexose transport in L6 myotubes with or without insulin. It was 5-fold more potent than LA in reducing blood glucose levels in mildly diabetic mice. Both AN-7 and AN-8 were ~1.5-fold more effective then LA in augmenting the rate of hexose transport in L6 myotubes. Another striking difference between LA and these compounds is the longer period required to exert their stimulatory effects. Yet, the effect of AN-7 in vivo was similar to that of LA, but a 5-fold lower doses. The lack of effect of both LA and AN-7 in severe diabetic mice is attributed to the impact of the disease on the entire glucose homeostatic system.

Table 1. Effects of LA and AN-7 on blood glucose level in diabetic mice

Test compound	Blood glucose (mg/dl)			
	Severe diabetes		Mild diabetes	
	Before treatment	After treatment	Before treatment	After treatment
DMSO	571±17	517±86	219±32	198±15
LA	514±38	547±62	235±34	163±33*
AN-7	556±23	502±71	246±33	148±29*
AN-8	582±20	530±79	194±21	209±69

Six-week old male C57 black mice (BW 27–32 g) were made diabetic by a single STZ injection as described above. Two weeks later they were divided into two groups (mild and severe diabetes) and received daily sc injections of DMSO (20 μL), LA (50 mg/kg BW, daily), AN-7 or AN-8, (10 mg/kg BW daily, for 2 weeks). Non-fasting blood glucose levels were determined at 9 AM by Glucometr Elite. MeanSD ($n=8$).

*$p<0.05$, significantly different from the respective to control, Mann–Whitney test.

The ester derivative AN-8, which was active in vitro, failed to decrease blood glucose level in diabetic mice. This is perhaps due to a rapid hydrolysis of the compound by non-specific esterases in the plasma,[24] which precludes a substantial accumulation of this molecule in cells. We suggest that the lack of in vitro effect of all the other ester and amide derivatives that we have synthesized may be due to steric hindrances related to the presence of PEG or an alkyl linker containing more than six methylene groups that produce compounds resistant to estrases or amidases.

3. Conclusions

1. AN-7 and AN-8 are more potent and effective than LA in augmenting the rate of hexose transport in L6 myotubes in culture. Their effects were additive to the stimulatory effect of insulin, indicating that they operate an intracellular mechanism distinguishable from that employed by insulin.
2. The longer time period required to achieve maximal effect of AN-7 and AN-8 in myotubes seems to depend on the release rate of LA from the produrg molecules intracellularly.
3. AN-7 is more potent in vivo then the parent compound LA; its blood glucose lowering effect was similar to that of LA, but was obtained with a 5-fold lower dose.
4. AN-7 and similar amide derivatives may serve as prototype drugs to develop potent LA prodrugs as an auxiliary drug therapy for the treatment of diabetes.

4. Experimental

1-(5-[1,2] Dithiolan-3-yl-pentanoyl)-pyrrolidine-2,5-dione (LA-NHS) and PEG diamine (H_2N-PEG-NH_2)[25–27] were synthesized and characterized in our laboratory. DL-α-lipoic acid (LA), *N*-*N*′-dicyclohexyl carbodiimide (DCC), *N*-hydroxy succinimide (NHS), PEG 2000, 1,3-propane diol, 1,3-propane diamine, 1,6-hexane diol, 1,8-ocatane diol, 1,10-decane diol, 2-deoxy-D-glucose (dGlc), streptozotocine, dimethyl sulfoxide (DMSO), sodium citrate, were dotained from Aldrich, Sigma Chemical Co (St. Louis, MO, USA) and used as received. α-Minimal essential medium (α-MEM), fetal calf serum (FCS), trypsin, glutamine and antibiotics were purchased from Biological Industries (Kibbutz Beth-Haemek, Israel). Insulin Actrapid® HM was from Novo Nordisk, Denmark. American Radiolabeled Chemicals (St. Louis, MO, USA) supplied 2-[1,2-^{3}H(N)] deoxy-D-glucose (60 Ci/mmol), D-glucose was purchased from Merck (Darmstadt, Germany). Bradford protein assay kit was from Bio-Rad Laboratories GmbH (Munich, Germany). Melting points were determined with a melting point apparatus (Melting Point SMP. Stuart Scientific). Infrared (IR) spectra were recorded an on VECTOR 22, Bruker spectrophotometer.

4.1. NMR

The proton NMR spectra were obtained on a Varian VXR-300 (300 MHz) spectrometer equipped with a 5-mm switchable probe, and data were processed using a VNMR software. Chloroform-*d* and dimethyl sulfoxide-d_6 were used as solvents; using tetramethylsilane Me_4Si (δ 0.00 ppm) as an internal standard. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, unresolved multiplet due to the field strength of the instrument; and dd, doublet of doublet.

4.2. Electrospray ionization mass spectrometry (ESI-MS)

Electrospray ionization mass spectrometry was measured on a Thermo Quest Finnigan LCQ-Duo in the positive ion mode. In most cases, elution was in 20:79:1 water/methanol/acetic acid at a flow rate of 15 μL/min. Data were processed using ThermoQuest Finnigan's Xcalibur™ Biomass Calculation and Deconvolution software.

4.3. Purification

Flash column chromatography on Merck silica gel 60 (particle size 230–400 mesh) was used for purification. CH_2Cl_2 and CH_3OH were used in different ratio as eluent. Purification on HPLC (Gilson) was performed on C18, preparative and semi preparative columns, using acetonitrile and double distilled water in different ratios as eluent solvent. Analytical TLC was performed on silica gel 60 F_{254}-precoated plates purchased from Merck (Darmstadt, Germany). The compounds were visualized by using UV light or I_2 vapor.

4.4. Cell cultures

A subclone of rat skeletal muscle L6 cells (curtsey of Dr. Nava Bashan, Ben-Gurion University, Beer-Sheva. Israel), selected for high fusion potential was used. Cells were grown in α-MEM supplemented with 10% (v/v) FCS, 100 U/mL penicillin G and 100 μg/mL streptomycin, in a 95%:5% air/CO_2 humidified incubator at 37 °C. Differentiation of myocytes into myotubes was induced with 2% (v/v) FCS, as described.[28] The culture medium was changed at least every 48 h.

4.5. Hexose uptake assay

The [^{3}H] dGlc uptake assay was performed as described.[29] Carrier-mediated dGlc uptake was calculated on the basis of protein quantity, determined by Bradford's method.[30] Test compounds were added to cell cultures from stocks solution in DMSO by a 1000-dilution (final concentration 1% v/v). DMSO reduced the rate of hexose transport by less than 5%.

4.6. Animals

All experiments were performed according to guidelines of the *Care and Use of Laboratory Animals* of the Hebrew University of Jerusalem. Male C57 black mice were purchased from Harlan Laboratories (Jerusalem, Israel) and housed (eight mice per cage) at the animal facility with a 12-h light–dark cycle at 23 °C. Diabetes was induced in 6-week-old mice by a single ip injection of streptozotocin (150 mg/kg body weight) as described.[31] The diabetic mice were divided into two groups: The mildly diabetic group that had non-fasting blood glucose less than 250 mg/dL and a severe diabetic group in which blood glucose ranged between 250 and 600 mg/dL. Two weeks following the induction of diabetes both diabetic and control animals received daily sc injections of LA (50 mg/kg body weight), AN-7 or AN-8 (10 mg/kg body weight) or the vehicle, DMSO, daily, for 2 weeks. The volume of each injection was 20 μL.

4.7. Glucose determination

Glucose concentration in culture medium samples and in blood (taken from the tip of tails) was determined with Glucometer Elite™ and blood glucose test strips (Bayer, Puteaux, France).

4.8. Statistical analysis

Statistical analysis was done using Mann–Whitney test.

4.9. Preparation of 1-(5-[1,2]dithiolan-3-yl-pentanoyl)-pyrrolidine-2,5-dione (LA-NHS)

NHS (3.1g, 27.0 mmol), and *N*,*N* dicyclohexyl-carbdiimide (DCC) (5.57 g, 27.0 mmol) were dissolved in dry dioxane (50 mL). A solution of LA (5 g, 26.0 mmol) in dry dioxane (20 mL) was added slowly and the stirred solution was left for 48 h at room temperature. The dicyclohexyl urea (DCU) precipitate was filtered and the solvent was evaporated. The powder was dissolved in 5 mL of 2-propanol and left overnight at 4 °C. The white precipitate formed was filtered and dried.

The yield was 57%. ^{1}H NMR (DMSO): δ 2.78 (t, 4H, [OC–$\underline{CH_2}$–$\underline{CH_2}$–CO]), 2.42–2,51 (m, 3H, [S–$\underline{CH_2}$–CH_2–$\underline{CH}$-S]), 2.29 (t, 2H, [$\underline{CH_2}$–CO–O–N]), 1.84 (m, 2H, [S–CH_2–$\underline{CH_2}$–CH–S]), 1.37–1.62 (m, 4H, [$\underline{CH_2}$–CH_2–$\underline{CH_2}$–CH_2–CO]), 1.25 (m, 2H, [CH_2–$\underline{CH_2}$–CH_2–CH_2–CO]), ^{13}C NMR (DMSO): δ 176.0, 166.4, 53.7, 42.9, 37.3, 36.8, 32.6, 25.9, 24.1, 21.3. Anal. calcd for $C_{12}H_{17}NO_4S_2$: C, 47.50; H, 5.65; N, 4.62; S, 21.14. Found: C, 47.48; H, 5.71; N 4.57; S, 21.10.

4.10. General procedure for the synthesis of (5-[1,2]dithiolan-3yl-pentanoylamino)-*O*-methoxy-PEG]-amide (AN-5) and di-(5-[1,2]dithiolan-3yl-pentanoylamino)-PEG]-amide (AN-11)

4.10.1. Preparation of di-(5-[1,2]dithiolan-3yl-pentanoylamino)-PEG]-amide, AN-11. PEG diamine $M_r = 2000$ g/mol (5 g, 2.5 mmol) was dissolved in 1:1 ratio of 15% $NaHCO_3$ in water and dioxane (50 mL). A solution of LA-NHS (3.03 g, 10 m mol) in dioxane (50 mL) was added and the stirred solution was left for 48 h at room

temperature. The solution was filtered and the solvent water and dioxane was removed. The powder left was dissolved in 30 mL of hot EtOH/CH_2Cl_2 and was filtered again and evaporated. The solid residue was dissolved in hot EtOH/ether 1:1 solution (10 mL), and left overnight at 4° C. The white precipitate formed was filtered and dried. The product was purified on silica-gel by flash chromatography.

The yield was 72%. IR V_{max} (KBr): 3447.13, 2887.35, 1653.27, 1280.22, 1113.27, 685.85 cm^{-1} ^1H NMR (DMSO): δ 3.70 (t, 4H, –NH–CH_2–CH_2–O), 3.62 (m, 4H, –NH–CH_2–CH_2–O, 1H, H–S–S–), 3.4–3.60 (m, 168H, –[CH_2–CH_2–O]n), 3.20 (m, 2H, –S–S–2H), 2.42 (m, 1H, CH_2–CH–S–), 2.38 (t, 2H, –CH_2–CO–), 1.90–1.45 (m, 7H, –(CH_2)3, CH_2–CH–S–). ^{13}C NMR (DMSO): δ 175.9, 75.4, 73.6, 60.0, 47.5, 42.6, 39.0, 38.0, 32.2, 28.9. Anal. calcd for $C_{105}H_{206}N_2O_{46}S_4$: C, 53.41; H, 8.79; N, 1.19; S, 5.43. Found: C, 54.68; H, 8.94; N 1.02; S, 5.00.

4.10.2. (5-[1,2]Dithiolan-3yl-pentanoylamino)-*O*-methoxy-PEG]-amide (AN-5). Yield 78%. IR V_{max} (KBr): 3447.69, 2886.80, 1653.99, 1280.35, 1113.75, 685.70 cm^{-1} ^1H NMR (DMSO): δ 3.70 (t, 2H, –NH–CH_2–CH_2–O), 3.62 (m, 4H, –NH–CH_2–CH_2–O, 1H, H–S–S–), 3.4–3.60 (m, 168H, –[CH_2–CH_2–O]n), 3.25 (s, 3H,OCH_3), 3.20 (m, 2H, –S–S–2H), 2.42 (m, 1H, CH_2–CH–S–), 2.38 (t, 2H, –CH_2–CO–), 1.90–1.45 (m, 7H, –(CH_2)3, CH_2–CH–S–). ^{13}C NMR (DMSO): δ 175.9, 75.2, 74.5, 73.7, 73.5, 72.1, 62.0, 60.1, 47.5, 42.6, 39.1, 38.0, 32.1, 29.0. Anal. calcd for $C_{97}H_{197}NO_{45}S_2$: C, 54.01; H, 9.02; N, 0.64; S, 2.91. Found: C, 53.31; H, 8.17 ; S, 2.97.

4.10.3. 3-(5-[1,2]dithiolan-3yl-pentanoylamino)-propyl]-amide (AN-7). 1,3 Diamino propane (0.25 mL, 3 mmol) was dissolved in a solution of 1:1 15% $NaHCO_3$ in water and dioxane. A solution of LA-NHS (3.63 g, 12 mmol) in dioxane was added and left stirred for 48 h at room temperature. The solution was then filtered, and the solvent was evaporated. The left residue was dissolved in 30 mL of a hot solution of EtOH/CH_2Cl_2 (1:1) and filtered again. After evaporation of the solvent the remaining solid was purified by silica-gel through flash chromatography and preparative HPLC and the pure product, AN-7, was collected.

The yield was: 75%; mp = 79–81 °C. IR V_{max} (KBr): 3303.18, 2930.69, 1732.75, 1205.83, 649.64 cm^{-1}. ^1H NMR (DMSO, δ) 3.62 (m, 1H, H–S–S–), 3.20–3.10 (dt, 4H, –NH–CH_2–), 3.07 (m,2H, –HN–CH_2–CH_2–) 3.05 (m, 2H, –S–S–2H), 2.4(m, 1H, CH_2–CH–S–), 2.03 (t, 2H, –CH_2–CO–NH), 1.9–1.45 (m, 7H, –(CH_2)$_3$, CH_2–CH–S–). ^{13}C NMR (DMSO): δ 175.8, 75.4, 73.6, 59.9, 44.0, 42.0, 39.2, 38.1,37.8, 31.6, 29.0. Anal. calcd for $C_{19}H_{34}N_2O_2S_4$: C, 50.63; H, 7.60; N, 6.21; S, 28.46. Found: C, 50.53; H, 7.59; S, 28.41. ESMS *m*/*z*: 451.3(MH^+).

4.10.4. Di-(5-[1,2]dithiolan-3yl-pentanoyloxy)-PEG ester (AN-6). HO-PEG-OH M_r = 2000 Da (5 g, 2.5 mmol) was dissolved in toluene (100 mL) and traces of water were removed by a Dean–Stark apparatus. The solution was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2. DCC (2.06 g, 10 mmol) and LA (2.06 g, 10 mmol) in CH_2Cl_2 was added and the mixture was stirred for 48 h at room temperature. Consequently, the precipitate was filtered and the solvent was evaporated to yield white solid. The solid was dissolved in a minimum volume of hot EtOH and the solution was left at 4° C for 24 h. The white precipitate was filtered, washed by ether and purified by silica-gel through flash chromatography technique.

The yield was: 85%. IR V_{max} (KBr): 2885.62, 1734.47, 1241.94, 1111.80, 685.38 cm^{-1}. ^1H NMR (DMSO, δ) 4.10 (t, 4H, 2* –(OC–O–CH_2–CH_2)), 3.70 (t, 1H, 1H, H–S–S–) 3.60 (m, 4H, –O–OC–CH_2–CH_2–O), 3.40–3.58 (m, 168H, –[CH_2–CH_2–O]n), 3.16 (m, 2H, –S–S–2H), 2.40 (m, 1H, CH_2–CH–S–), 2.30 (t, 2H, –CH_2–CO–), 1.9–1.45 (m, 7H, –[CH_2]$_3$, CH_2–CH–S–). ^{13}C NMR (DMSO): δ 176.7, 76.3, 73.8, 64.2, 60.0, 42.6, 42.1, 38.0, 37.2, 32.1, 28.2. Anal. calcd for $C_{106}H_{207}O_{48}S_4$: C, 53.37; H, 8.7; S, 5.43. Found: C, 54.21; H, 8.22; S, 4.62.

4.11. General procedure for the synthesis of 5-[1,2]dithiolan-3-yl-pentanoic acid 3-(5-[1,2]dithiolan-3yl-pentanoyloxy)-propyl ester (AN-8), 5-[1,2]dithiolan-3-yl-pentanoic acid 6-(5-[1,2]dithiolan-3yl-pentanoyloxy)-hexyl ester (AN-30); 5-[1,2]dithiolan-3-yl-pentanoic acid 8-(5-[1,2]dithiolan-3yl-pentanoyloxy)-octyl ester (AN-31); and 5-[1,2]dithiolan-3-yl-pentanoic acid 10-(5-[1,2]dithiolan-3yl-pentanoyloxy)-decyl ester (AN-32) 5-[1,2]dithiolan-3-yl-pentanoic acid 3-(5-[1,2]dithiolan-3yl-pentanoyloxy)-propyl ester (AN-8)

To propane-1,3-diol (0.25 mL, 3.45 mmol) in CH_2Cl_2 (100 mL), DCC (2.84 g, 13.8 mmol) and LA (2.8.4 g, 13.8 mmol) were dissolved in 100 mL CH_2Cl_2. The mixture was stirred for 48 h at room temperature. The DCU precipitate was filtered and the solvent was evaporated. The remaing powder was dissolved in a solution of 15% (w/v) $NaHCO_3$ in water, and the precipitate was, filtered and purified on a silica-gel column via flash chromatography, followed by preparative HPLC to obtain pure compound of AN-8.

The yield was: 80%, mp = 99–100 °C. IR V_{max} (KBr): 2930.15, 1709.33, 1234.59, 663.14 cm^{-1}. ^1H NMR (DMSO, δ) 4.10 (t, 1H, 1H, H-S-S-), 3.90 (t, 4H, 2* –(OC–O–CH_2–CH_2)), 3.60 (m, 2H, –OC–O–CH_2–CH_2–), 3.16 (m, 2H, –S–S–2H), 2.40 (m, 1H, CH_2–CH–S–), 2.30 (t, 2H, –CH_2–CO–), 1.9–1.45 (m, 7H, –[CH_2]$_3$, CH2–CH–S–). ^{13}C NMR (DMSO): δ 173.3, 64.5, 60.0, 42.0, 38.1, 38.0, 37.3, 32.1, 28.3. Anal. calcd for $C_{19}H_{32}O_4S_4$: C, 50.52; H, 7.36; N, 3.10; S, 28.39. Found: C, 50.50; H, 7.31; N, 2.98; S, 28.31. ESMS *m*/*z*: 452.4 (MH^+).

4.11.1. 5-[1,2]Dithiolan-3-yl-pentanoic acid 6-(5-[1,2]dithiolan-3yl-pentanoyloxy)-hexyl ester, (AN-30). Yield: 82%, mp = 205–207 °C. IR V_{max} (KBr): 2930.60, 1709.71, 1234.91, 664.06 cm^{-1}. ^1H NMR (DMSO, δ)

4.10 (t, 1H, 1H, H–S–S–), 3.90 (t, 4H, 2* –(OC–O–CH_2–CH_2)), 3.60 (m, 8H, –OC–O–CH_2–$(CH_2)_4$–), 3.16 (m, 2H, –S–S–2H), 2.40 (m, 1H, CH_2–CH–S–), 2.30 (t, 2H, –CH_2–CO–), 1.9–1.45 (m, 7H, –$[CH_2]_3$, CH2–CH–S–). ^{13}C NMR (DMSO): δ 174.3, 60.0, 56.5, 42.0, 38.1, 37.9, 35.7, 34.3, 32.2, 28.6, 28.4. Anal. calcd for $C_{22}H_{38}O_4S_4$: C, 53.40; H, 7.74; S, 25.92. Found: C, 53.31; H, 7.69; S, 26.10. ESMS *m*/*z*: 495.3(MH^+).

4.11.2. 5-[1,2]Dithiolan-3-yl-pentanoic acid 8-(5-[1,2]dithiolan-3yl-pentanoyloxy)-octyl ester, (AN-31). Yield: 79%, mp = 187–190 °C. IR V_{max} (KBr): 2929.81, 1709.33, 1234.85, 664.66 cm^{-1}. ^{1}H NMR (DMSO, δ) 4.10 (t, 1H, 1H, H–S–S–), 3.90 (t, 4H, 2* –(OC–O–CH_2–CH_2)), 3.60 (m, 12H, –OC–O–CH_2–$(CH_2)_6$–), 3.16 (m, 2H, –S–S–2H), 2.40 (m, 1H, CH_2–CH–S–), 2.30 (t, 2H, –CH_2–CO–), 1.9–1.45 (m, 7H, –$[CH_2]_3$, CH_2–CH–S–). ^{13}C NMR (DMSO): δ 173.4, 67.6, 59.9, 42.0, 38.0, 35.6, 34.3, 32.1, 32.0, 29.0, 28.6, 28.3 . Anal. calcd for $C_{24}H_{42}O_4S_4$: C, 55.13; H, 8.10; S, 24.53. Found: C, 55.12; H, 8.16; S, 24.63. ESMS *m*/*z*: 523.2 (MH^+).

4.11.3. 5-[1,2]Dithiolan-3-yl-pentanoic acid 10-(5-[1,2]dithiolan-3yl-pentanoyloxy)-decyl ester (AN-32). Yield: 87%, mp = 175–176 °C. IR V_{max} (KBr): 2918.94, 1709.11, 1234.53, 662.27 cm^{-1}. ^{1}H NMR (DMSO, δ) 4.10 (t, 1H, 1H, H–S–S–), 3.90 (t, 4H, 2* –(OC–OC−H_2–CH_2)), 3.60 (m, 12H, –OC–O–CH_2–$(CH_2)_6$–), 3.16 (m, 2H, –S–S–2H), 2.40 (m, 1H, CH_2–CH–S–), 2.30 (t, 2H, –CH_2–CO–), 1.9–1.45 (m, 7H, –$[CH_2]_3$, CH_2–CH–S–). ^{13}C NMR (DMSO): δ 173.3, 63.8, 59.9, 42.0, 37.9, 35.6, 34.3, 32.2, 32.0, 29.4, 28.9, 27.9 . Anal. calcd for $C_{26}H_{46}O_4S_4$: C, 56.68; H, 8.42; S, 23.48. Found: C, 56.64; H, 8.32; S, 23.39. ESMS *m*/*z*: 552.5 (MH^+).

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