Bioorganic & Medicinal Chemistry Letters 20 (2010) 3078–3083

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and anticancer evaluation of α-lipoic acid derivatives

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ARTICLE INFO

Article history:
Received 26 January 2010
Accepted 27 March 2010
Available online 3 April 2010

Keywords:
Lipoic acid
Synthesis
Anticancer

ABSTRACT

α-Lipoic acid derivatives were synthesized and evaluated for their in vitro anticancer activities against NCI-460, HO-8910, KB, BEL-7402, and PC-3 cell lines. The results, for most compounds exhibited dose-dependent inhibitory property and several compounds had good inhibitions at the dose of 100 μg/mL. Compound **17m** was further selected for in vivo evaluation against S180 xenograft in ICR mice, which had 24.7% tumor-weight inhibition through intragastric administration of 200 mg/kg of body weight. Moreover, the LD_{50} in mice for **17m** through ig exceeded 1000 mg/kg of body weight.

α-Lipoic acid **1** is a naturally-occurring co-factor existed in a number of multi-enzyme complexes regulating metabolism and has arisen significant interest from its fascinating biological properties.[1] α-Lipoic acid **1** and its derivatives were proved to be effective against Type 2 diabetes,[2,3] atherosclerosis,[3] inflammatory skin diseases,[3] reperfusion arrhythmias,[4,5] and Parkinson's disease.[6] Moreover, lipoic acid **1** has been widely reported to induce apoptosis in various cancer cell lines. It induced apoptosis in human hepatoma cells SMMC-7721 of 65% after 96 h with 5 mM dose of lipoic acid **1**,[7] and human tumor cell lines FaDu and Jurkat were also initiated apoptosis following long-time exposure to lipoic acid in mM concentrations, indicating a time- and dose-dependent fashion.[8] Likewise, lipoic acid **1** was effective in suppressing growth on four different HNSCC cell lines (FaDu, SCC9, SCC25, and Detroit-562) at high concentrations up to 10 mM with more than 50% cell inhibition.[9] Lipoic acid **1** and dihydrolipoic acid **2** induced apoptosis in human colon cancer cells HT-29 with the corresponding EC_{50} values around 250 μM for both compounds.[10] Besides, there was an about 27% and 13% diminution of human lung epithelial cells H-460 growth after 24 h by 100 μM of lipoic acid **1** and dihydrolipoic acid **2**, respectively, and it seemed that the effect of lipoic acid **1** was more pronounced than dihydrolipoic acid **2**.[11] As expected, lipoic acid **1** was inactive at low μM concentrations,[12] and the concentrations required to induce effects were considerably high in mM ranges.[2] As an endogenous agent widely used as a dietary supplement, lipoic acid **1** is safe to normal cells without adverse side effects caused by many chemotherapeutic agents and the rat LD_{50} of lipoic acid **1** was assumed to exceed 2000 mg/kg of body weight.[1]

Some lipoic acid derivatives also possessed notable anticancer activities as illustrated in Figure 1. It was patented that bis acetyl lipoate **3**, bis benzoyl lipoate **4** and some other such sulfur-acylated derivatives (**6** and **7**) exhibited positive activities to kill a wide range of cancer cells at high concentrations between 60 μg/mL and 800 μg/mL; however, bis carbamoyl lipoate **5** seemed not active against these cell lines.[13,14] Compounds **8** and **9** were effective on inhibiting the proliferation of human breast cancer cells MCF-7 with IC_{50} at 15 μM and 25 μM, respectively; and of human colon cancer cells H-29 with IC_{50} at 10 μM and 8 μM.[3] And treatment of human skin cancer cells A-431 with quinazoline **10** related to PD168393, in which the acrylamide group at the 6-position was replaced by lipoic acid, induced a decrease of the cell proliferation with IC_{50} at 22 μM.[15] In addition, some thiolesters of lipoic acid derivatives (**11**, **12**, **13** and **14**) had an IC_{50} at 400, 240, 50, and 45 μM on a human epithelial prostate cancer cell line (DU-145), respectively.[16] All these modifications suggested that the variations on carboxyl group and disulfide bond of lipoic acid **1** with different substituents might enhance its anticancer efficacy.

With the knowledge of the anticancer activities of lipoic acid and its derivatives, we conceived that the skeleton of lipoic acid **1** could be modified, and such compounds may have potent anticancer activities with low toxicity. Therefore, in this Letter, we are endeavored to synthesize these α-lipoic acid derivatives and evaluate their anticancer activities.

Compound **3** was synthesized in three steps from **1** by sodium borohydride ($NaBH_4$) reduction to dihydrolipoic acid **2**, followed by treatment with acetyl chloride (3 equiv) in dichloromethane (DCM) to form anhydride **23** and selectively hydrolyzed in isopropanol/H_2O as illustrated in Scheme 1 and compound **4** was obtained by benzoyl chloride (3 equiv) to form anhydride **24** and selectively hydrolyzed in dioxane/H_2O.[13]

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doi:10.1016/j.bmcl.2010.03.112

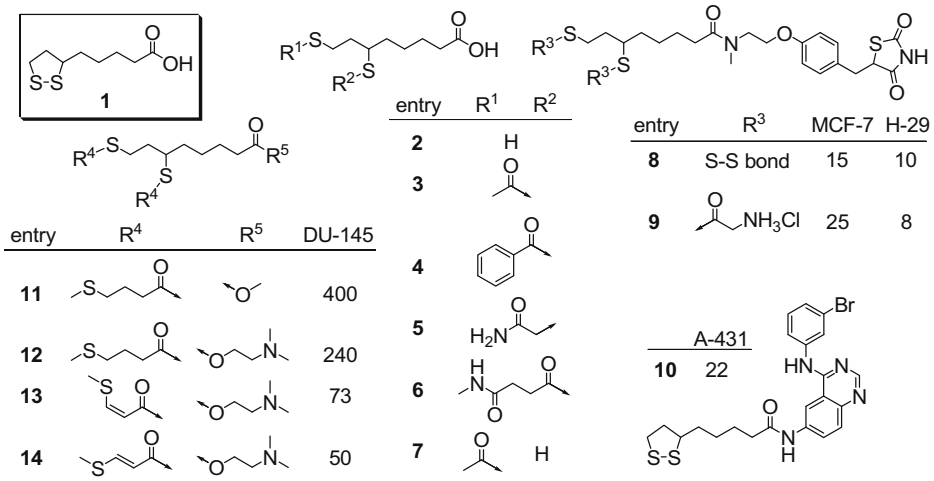
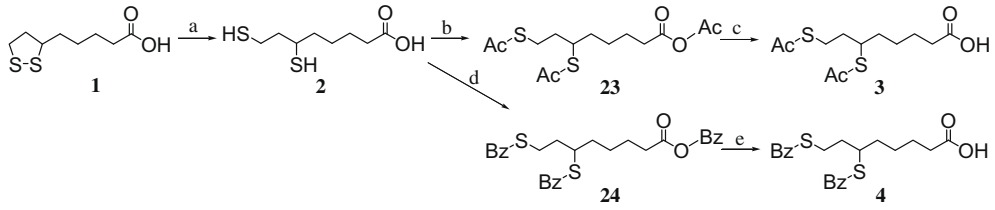
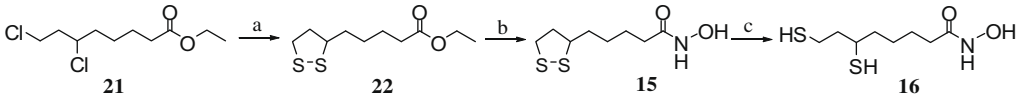


Figure 1. Structures of lipoic acid and related derivatives with anticancer property. Values refer to IC_{50} (μM).



Scheme 1. Reagents and conditions: (a) NaOH, $NaBH_4$, H_2O, 2 N HCl, rt, 2–3 h, 47%; (b) acetyl chloride, triethylamine, DCM, 0 °C to rt, 2 h; (c) isopropanol, H_2O, 40 °C, 4.5 h, 30% from **2**; (d) benzoyl chloride, triethylamine, DCM, 0 °C to rt, 9 h; (e) dioxane, H_2O, rt, 60 h, 24% from **2**.



Scheme 2. Reagents and conditions: (a) Na_2S, S, TBAB, toluene, reflux, 5 h; (b) hydroxylamine hydrochloride, KOH, methanol, rt, 4–6 h, 32%; (c) $NaBH_4$, THF, 0 °C, 3 h, 63%.

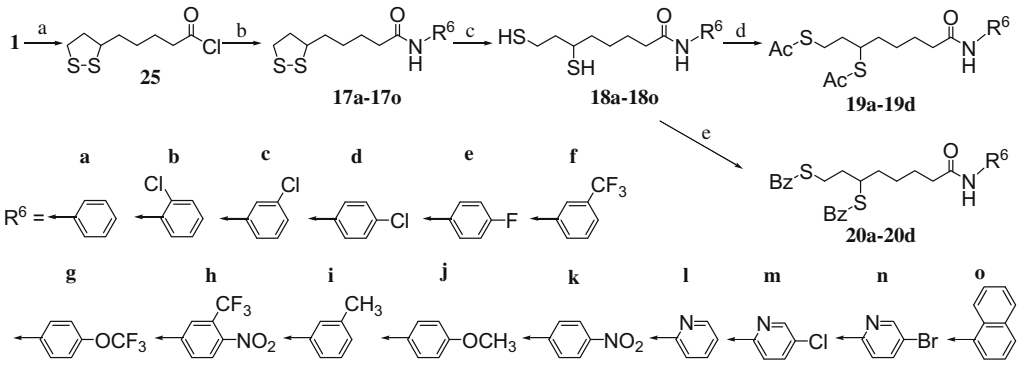
Lipoic acid-based hydroxamates **15** and **16** were obtained as outlined in Scheme 2. The esterification of **1** with ethanol under reflux was not feasible in a general way, as the procedure suffered from the formation of polymerized by-product. Therefore, to access ethyl lipoate **22**, ethyl 6,8-dichlorooctanoate **21**, a precursor in preparation of **1**, was used to react with Na_2S and elemental sulfur in presence of tetrabutylammonium bromide (TBAB) in toluene under reflux to form the heterocyclic disulfide circle with quantitative yield.[17] Hydroxamate **15** was obtained by treating with freshly prepared hydroxylamine and reduction of disulfide bond of **15** by $NaBH_4$ in tetrahydrofuran (THF) gave **16**.

The major series of lipoic acid derivatives as amides were prepared from **1** as shown in Scheme 3. Amidation of lipoyl chloride **25** with amines in presence of triethylamine as hydrogen chloride capture in DCM led to amides **17a**–**o**. Reduction of disulfide bond gave the corresponding dihydrolipoamides **18a**–**o**, several of which were followed by acylation with acetyl chloride and benzoyl chloride to afford **19a**–**d** and **20a**–**d**, respectively.

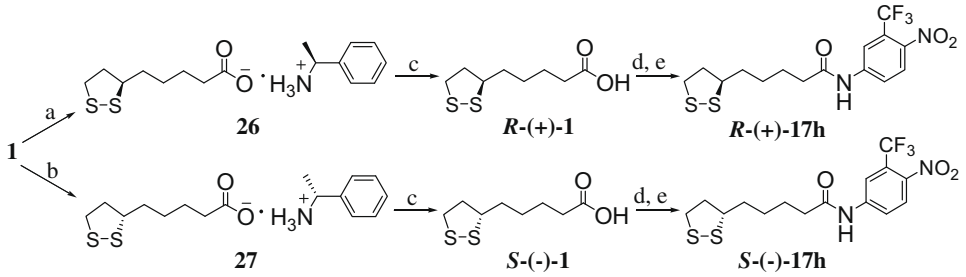
In order to probe the effect of chirality in the anticancer activities of lipoamides, we prepared enantiopure ***R*-(+)-1** ($[\alpha]_D^{25}$ +119.7 (*c* 1.0, EtOH), equals to ee >99%) according to a literature method.[18] Racemic lipoic acid **1** was treated with the optically active base *R*-(+)-α-methylbenzylamine (*R*-(+)-FEA) to obtain the diastereoisomeric salt, followed by repeatedly crystallization and finally scission of the salt with citric acid to get ***R*-(+)-1** and ***S*-(−)-1** ($[\alpha]_D^{25}$ −117.8 (*c* 1.0, EtOH)) was obtained with *S*-(−)-α-methylbenzylamine (*S*-(−)-FEA) under the same procedure. The obtained enantiomers were applied to synthesize amides with 4-nitro-3-(trifluoromethyl)aniline, respectively, as illustrated in Scheme 4.

In addition, some lipoamide analogues were synthesized without mercapto group at C_6 position as shown in Scheme 5. Compound **32** was prepared and compared with compound **18m** in anticancer activities. 8-Bromooctanoic acid **28** was converted to 8-bromooctanoyl chloride **29** and reacted with 5-chloropyridin-2-amine to get 8-bromo-*N*-(5-chloropyridin-2-yl)octanamide **30**, which was treated by a novel method we developed with potassium *O*-ethylxanthate in alkaline condition to have *S*-8-(5-chloropyridin-2-ylamino)-8-oxooctyl *O*-ethyl carbonodithioate **31** and acidified by hydrochloric acid to obtain *N*-(5-chloropyridin-2-yl)-8-mercaptooctanamide **32** one pot. Besides, *S*-8-oxo-8-(phenylamino)octyl ethanethioate **34** and 8-mercapto-*N*-phenyloctanamide **35** were synthesized to confirm the effect of C_6 position mercapto group compared with **18a** and **19a**.

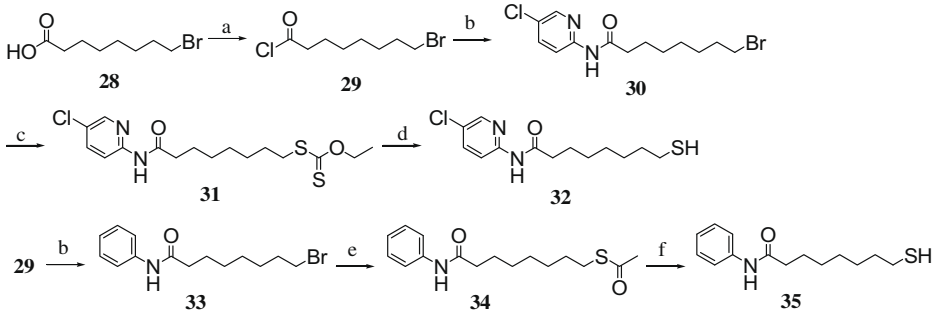
All the compounds were tested in vitro at different concentrations against human lung NCI-460, ovarian HO-8910, nasopharynx KB, hepatoma BEL-7402 cancer cell lines, in comparison with Vorinostat and Cisplatin. Some selected compounds were also tested against human prostate PC-3. By analysis of the data reported in Table 1, we can draw the following structure–activity relationships (SAR).



Scheme 3. Reagents and conditions: (a) thionyl chloride, benzene, 0 °C, 3–4 h; (b) R^6NH_2, triethylamine, DCM, 0 °C, 2–6 h, 19–76% from **1**; (c) $NaBH_4$, THF, 0 °C, 7 h, 22–87%; (d) acetyl chloride, triethylamine, DCM, 0 °C to rt, 2 h, 40–77%; (e) benzoyl chloride, triethylamine, DCM, 0 °C to rt, 9 h, 57–91%.



Scheme 4. Reagents and conditions: (a) *R*-(+)-FEA, toluene, 38–39 °C, recrystallization repeated 5 times; (b) *S*-(−)-FEA, toluene, 38–39 °C, recrystallization repeated 5 times; (c) citric acid, methanol, toluene, 1 h; (d) thionyl chloride, benzene, 0 °C, 3–4 h; (e) 4-nitro-3-(trifluoromethyl)aniline, triethylamine, DCM, 0 °C, 2–6 h, 70% for ***R*-(+)-17h** from ***R*-(+)-1**, 66% for ***S*-(−)-17h** from ***S*-(−)-1**.



Scheme 5. Reagents and conditions: (a) thionyl chloride, reflux, 1 h; (b) 5-chloropyridin-2-amine, triethylamine, DCM, 0 °C to rt, 3–4 h, 44% from **28**; (c) potassium *O*-ethylxanthate (freshly prepared from CS_2, EtOH, KOH in H_2O), NaOH, H_2O, overnight, without separation; (d) 1 N HCl, 80% from **30**; (e) AcSK, EtOH, rt, 20 h, 18%; (f) NaOH, H_2O, EtOH, rt, 48 h, 67%.

6,8-Bis(benzoylthio)octanoic acid **4** was most effective among compounds **1**, **2**, and **3** on the inhibition of NCI-460, HO-8910, KB, and BEL-7402 cancer cells. The results quite agreed with Zachar, Z.'s study that the threshold-killing concentration of **4** was 60 μg/mL while the concentration of **3** reached 600 μg/mL in killing a series of cancer cell lines.[13] However, comparison with **17a**–**20a**, **17b**–**20b**, **17c**–**20c**, and **17d**–**20d**, bis(benzoylthio) amides of lipoic acid (**20a**–**d**) retained little activities. In addition, it was difficult to draw a conclusion whether oxidized or reduced form of the corresponding lipoamides was preferable from the results of in vitro evaluation. It seemed that the amide group might play a more important role in anticancer efficacy. Comparing the compounds **17a**–**k**, it had the trends that the electron-withdrawing groups such as NO_2, CF_3, Cl on the aryl ring enhanced the inhibitory activities, and the electron-donating groups such as H, OCH_3 decreased the inhibitory activities. On the whole, these compounds exhibited dose-dependent anticancer property, most of which showed excellent inhibitory activities at the dose of 100 μg/mL.

Enantioselectivity was not a rare event in the screening of anticancer drugs. In our results, the anticancer activities of *S*-enantiomer of lipoamide (***S*-(−)-17h**) was about twofold stronger than the racemic **17h** against NCI-460, HO-8910 and KB cancer cell lines and fourfold stronger against BEL-7402 cells at the concentration of 100 μg/mL. The anticancer activities of its *R*-antipode (***R*-(+)-17h**) showed no improvement than racemic **17h**, but possessed twofold stronger inhibitory activity than the racemic **17h** against BEL-7402. These data underlined the different stereochemical preference in a series of cancer cell lines.

Figure 2 showed the in vitro anticancer activity of the representative compounds with or without the thiol side chain at C_6

Table 1
Anticancer activities against NCI-460, HO-8910, KB, BEL-7402, and PC-3 cell lines[a]

Compds	Molecular weight	Inhibition rate of NCI-460 (%) Concentration (μg/mL)				Inhibition rate of HO-8910 (%) Concentration (μg/mL)				Inhibition rate of KB (%) Concentration (μg/mL)				Inhibition rate of BEL-7402 (%) Concentration (μg/mL)				Inhibition rate of PC-3 (%) Concentration (μg/mL)			
		100	10	1	0.1	100	10	1	0.1	100	10	1	0.1	100	10	1	0.1	100	10	1	0.1
1	206.3	5.72	2.39	4.65	8.71	5.11	na	1.05	3.39	10.96	7.18	8.60	0.14	8.17	4.77	2.52	na	nt	nt	nt	nt
2	208.3	7.84	5.02	12.25	9.25	1.17	na	3.93	na	16.34	3.38	8.79	12.00	0.59	5.59	6.98	10.40	nt	nt	nt	nt
3	292.4	10.97	6.04	9.89	9.96	na	1.61	na	na	8.03	8.88	12.39	7.24	8.35	9.22	12.82	10.30	nt	nt	nt	nt
4	416.6	**72.80**	na	0.77	na	**86.71**	na	0.29	na	**89.81**	2.68	0.68	na	**80.97**	2.76	na	1.09	91.24	7.43	9.86	9.54
15	221.3	na	na	0.64	3.90	na	na	na	na	12.44	0.89	2.57	7.92	12.48	11.76	7.39	10.40	nt	nt	nt	nt
16	223.4	43.74	17.73	1.17	5.70	49.30	26.24	na	na	**58.00**	41.52	10.56	7.62	47.52	28.24	9.12	5.96	nt	nt	nt	nt
17a	281.4	47.59	0.75	5.86	9.48	57.76	4.71	10.95	11.16	**65.32**	10.24	6.06	10.92	**60.57**	2.27	3.28	3.22	59.24	12.98	13.62	5.50
18a	283.5	25.42	8.37	4.30	5.50	13.68	5.49	6.38	7.37	15.78	8.62	11.60	9.49	7.67	3.72	1.32	0.85	nt	nt	nt	nt
19a	367.5	44.77	14.24	13.07	15.39	22.27	3.20	10.63	8.33	22.95	2.50	1.68	7.24	14.13	na	2.84	na	nt	nt	nt	nt
20a	491.7	na	11.44	5.93	0.60	na	na	3.79	3.12	na	2.67	na	1.52	na	na	na	na	nt	nt	nt	nt
17b	315.9	3.38	14.64	10.14	8.00	na	4.66	7.74	8.17	0.72	na	na	5.11	4.74	na	5.33	6.83	nt	nt	nt	nt
18b	317.9	12.07	18.22	8.19	6.36	na	na	7.72	6.11	na	na	na	na	na	na	3.08	5.11	nt	nt	nt	nt
19b	402.0	15.24	11.43	13.19	16.58	na	na	na	2.48	6.93	6.55	8.21	7.05	na	3.09	5.01	6.61	nt	nt	nt	nt
20b	526.1	na	12.10	14.40	15.13	na	1.33	2.35	na	na	2.53	3.05	3.62	na	6.63	7.37	5.72	nt	nt	nt	nt
17c	315.9	**65.23**	10.14	8.85	6.08	**68.28**	na	na	na	**76.54**	7.88	na	2.85	**68.25**	4.15	1.11	0.33	79.46	13.85	8.44	17.06
18c	317.9	24.83	na	7.20	9.78	na	na	na	na	8.04	na	na	na	16.11	3.22	1.14	3.09	nt	nt	nt	nt
19c	402.0	42.81	2.09	0.31	na	38.15	na	na	0.27	48.79	na	0.04	5.72	20.76	na	0.56	3.72	nt	nt	nt	nt
20c	526.1	na	9.12	9.48	15.64	na	na	3.38	9.02	na	16.56	12.66	10.69	na	na	na	1.70	nt	nt	nt	nt
17d	315.9	**68.71**	18.32	10.90	12.97	**74.76**	0.75	na	na	**83.12**	21.72	18.64	16.36	**76.39**	7.01	0.64	3.37	41.17	14.67	13.66	18.89
18d	317.9	40.91	21.95	6.76	na	21.27	na	2.81	1.04	44.11	18.22	13.35	9.11	25.53	1.16	0.27	1.06	nt	nt	nt	nt
19d	402.0	47.34	14.85	15.40	9.89	58.76	1.47	0.35	1.81	**71.95**	18.08	14.79	11.44	45.50	5.78	3.07	9.96	nt	nt	nt	nt
20d	526.1	na	12.56	9.80	9.36	na	na	1.29	3.68	5.78	10.62	11.71	16.95	na	4.87	9.62	9.20	nt	nt	nt	nt
17e	299.4	**64.50**	18.79	11.44	15.64	**78.13**	3.89	5.82	6.59	**85.38**	4.44	4.05	11.27	**74.45**	1.92	1.79	na	80.79	13.85	14.49	15.64
18e	301.4	41.60	18.05	6.84	5.64	22.25	6.81	12.21	8.11	28.21	13.26	8.84	6.78	11.65	6.14	na	na	nt	nt	nt	nt
17f	349.4	**68.75**	6.77	4.39	na	**74.83**	5.99	4.76	5.00	**79.95**	10.86	9.50	0.33	**61.90**	4.43	na	na	88.74	38.50	8.44	6.27
18f	351.5	32.06	11.44	8.26	11.95	34.78	8.86	13.71	10.77	30.49	1.97	na	8.41	25.31	1.21	1.66	2.85	nt	nt	nt	nt
17g	365.4	**82.95**	20.32	12.16	7.05	**84.53**	8.57	10.02	5.53	**90.11**	8.49	1.54	1.97	**78.41**	1.24	0.29	na	88.55	38.17	10.84	6.69
18g	367.5	**65.14**	29.23	19.12.	17.25	**66.75**	6.11	0.56	na	**73.33**	14.99	6.00	7.27	49.69	14.16	7.32	na	75.45	42.46	12.58	14.94
17h	394.4	51.65	11.98	19.07	18.81	47.65	na	0.96	na	49.51	na	na	4.50	23.93	na	7.46	5.04	90.25	na	na	5.37
18h	396.4	**95.85**	0.97	6.29	3.20	**92.67**	na	1.17	1.95	**95.52**	4.04	10.19	na	**91.67**	na	0.68	6.54	79.88	45.52	1.08	8.06
17i	295.5	52.90	4.15	7.82	7.97	**62.72**	na	4.54	3.68	**67.95**	4.13	5.20	4.29	49.93	9.01	10.31	9.88	75.14	9.57	9.98	2.13
18i	297.5	23.07	7.67	9.43	9.34	na	na	2.76	4.92	17.49	7.23	3.31	5.75	7.08	14.07	11.13	10.05	nt	nt	nt	nt
17j	311.5	45.73	9.82	0.51	3.82	43.76	6.34	3.54	1.64	48.32	2.94	5.03	4.00	38.73	6.32	7.93	5.48	nt	nt	nt	nt
18j	313.5	23.49	1.43	na	5.69	12.55	4.14	1.79	na	22.21	8.93	2.49	na	22.50	10.43	3.96	5.35	nt	nt	nt	nt
17k	326.4	**56.67**	22.68	7.45	11.93	**64.52**	0.61	0.33	na	**71.31**	14.36	na	0.22	52.63	4.83	na	na	79.58	6.67	19.87	13.88
18k	328.5	39.11	13.38	4.64	5.69	51.21	8.96	0.76	na	41.06	13.68	0.93	na	33.35	14.44	1.73	1.88	nt	nt	nt	nt
17l	282.4	39.27	na	0.81	2.61	51.89	na	na	1.14	25.07	na	na	na	37.64	5.77	na	0.89	nt	nt	nt	nt
18l	284.4	11.71	na	2.31	5.01	14.17	na	na	na	18.53	0.28	na	na	3.62	na	na	na	nt	nt	nt	nt
17m	316.9	**58.55**	33.69	6.26	3.93	**68.55**	27.47	2.10	na	**74.45**	28.58	0.76	na	48.46	16.06	7.44	na	42.33	17.12	2.00	2.22
18m	318.9	**55.78**	27.38	na	na	**71.16**	21.90	na	2.65	**76.55**	21.59	na	na	50.67	17.82	2.34	na	32.37	10.76	0.89	na
17n	361.3	52.00	38.01	13.07	9.00	32.12	29.64	4.27	na	42.32	35.12	4.33	na	45.51	24.46	13.08	na	nt	nt	nt	nt
18n	363.3	**55.88**	39.25	10.69	6.40	56.84	29.42	2.51	na	**59.24**	34.06	2.51	na	41.15	23.17	4.71	na	nt	nt	nt	nt
17o	331.5	na	na	4.15	2.36	na	na	6.95	0.34	na	na	8.37	na	7.22	6.27	9.55	3.40	nt	nt	nt	nt
18o	333.5	2.18	na	na	na	na	na	na	0.76	na	na	na	na	na	na	0.69	na	nt	nt	nt	nt
***R*-(+)-17h**	394.4	35.44	na	na	na	39.24	na	na	na	49.15	na	na	na	43.35	na	na	na	59.00	na	na	na
***S*-(−)-17h**	394.4	**92.20**	na	na	na	**92.27**	na	na	1.06	**95.77**	na	na	na	**94.04**	na	na	na	79.83	na	na	na
Vorinostat	264.3	61.06	54.93	40.85	15.12	69.59	63.08	56.90	18.06	64.60	57.71	51.23	22.93	65.74	54.83	38.75	10.75	IC_{50} 6.5 μM[b]			
DDP	300.1	87.55	84.24	45.54	17.99	94.99	97.33	66.59	35.23	95.43	96.28	71.59	3.51	86.56	88.67	65.84	25.47	92.56	81.89	52.92	na

[a] Values presented are means of three experiments; values in bold are comparable to Vorinostat or DDP at the concentration; na = not active; nt = not tested.
[b] Data taken from the literature.[19]

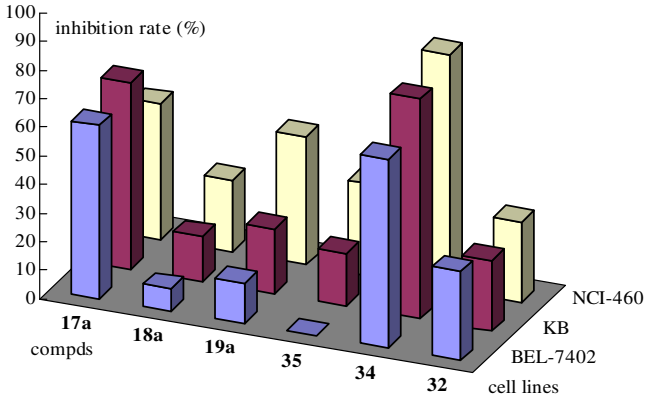


Figure 2. Comparative profiling of compounds against cancer cell lines at 100 μg/mL.

Table 2
Antitumor efficacy of selected compound **17m** against S180 xenograft in mice[a]

Groups	Dose (mg/kg)	AR[b]	No. animal[c]	TW[d] (g)	TWI[e] (%)
Control	/	Ig	8	1.53 ± 0.56	/
17m	100	Ig	6	1.53 ± 0.59	/
17m	200	Ig	6	1.15 ± 0.18	24.7
Vorinostat	100	Ig	6	1.61 ± 0.29	/
Vorinostat	200	Ig	6	1.53 ± 0.40	/
Cyclophosphamide	100	Ig	6	0.39 ± 0.15	74.5*

[a] Values presented are means ± SD. Statistical analysis: student *t*-test, $^{*}p$ <0.05 compared to control.
[b] Administration route.
[c] Number of mice tested in the group.
[d] Average tumor-weight after drug treatment.
[e] Percentage of tumor-weight inhibition versus control group.

Table 3
Anticancer activity against S180 cell lines[a]

Compds	Molecular weight	Inhibition rate of S180 (%) Concentration (μg/mL)			
		100	10	1	0.1
17m	316.9	74.28	24.51	13.03	5.92
Vorinostat	264.3	71.70	81.68	11.91	6.34
DDP	300.1	98.30	62.25	18.49	0.52

[a] Values presented are means of three experiments.

Table 4
Acute toxicity of selected compound **17m** on mice

Group	Dose	AR[a]	No. animal[b]	Sex	Gross pathological findings	Mortality
Experimental	1000 mg/kg	Ig	6	Female	Normal	0
Control	/	Ig	6	Female	Normal	0

[a] Administration route.
[b] Number of mice tested in the group.

position and the effect of sulfhydryl group protection. Compounds **18a**, **35** and **32** showed weak anticancer activities against BEL-7402, KB and NCI-460 at 100 μg/mL. This might due to their poor cell membrane permeability resulted from the highly polar character of the compounds with free thiols. Remarkably, masking the sulfhydryl group of compound **35** as thioester **34** enhanced its cancer cell growth inhibition rapidly. It stood to reason that the thioester **34** increased the possibility of permeating the membrane of cancer cells. However, bis acetyl compound **19a** failed to exhibit an admiring growth inhibitory effect as compound **34**, only similar to bis sulfhydryl compound **18a**. With the hypothesis that **19a** could get through the cell membrane as efficiently as **34**, the only explanation was that the thiol side chain at C_6 position impaired its anticancer activities. Hydrolysis of compound **34** under cellular physiological conditions could release free thiol compound **35**, which was considered as a good HDAC inhibitor with IC_{50} at 1.5 μM.[20,21] This might give explanation why compound **34** was of most active against these cancer cell lines. It could be inferred that the thiol side chain at C_6 position might prohibit compounds **19a** or **18a** from squeezing into the pocket-shaped HDAC tunnel.[22] It is worth noting that the anticancer activities of **17a** exhibited onefold higher against NCI-460, threefold higher against KB and sevenfold higher against BEL-7402 than **18a**, indicating lipoamides with intact dithiolane ring might function through other mechanisms rather than HDAC.[9] As far as we know, the exact mechanisms by which lipoic acid or its derivatives induce cancer cell inhibition are still unclear.[1,7,9,13,15,16,23]

We also evaluated the in vivo antitumor activity of lipoamide against mice S180 xenograft model. Compound **17m** was selected for the in vivo evaluation in comparison with cyclophosphamide and Vorinostat and 0.9% saline solution was used as blank control. All the drugs with different doses were administered once daily through intragastric (ig) administration route for consecutive seven days before animals were euthanized and the tumors were excised and weighted. The results presented in Table 2 showed that compound **17m** through ig administration of 200 mg/kg of body weight exhibited moderate experimental therapeutic efficacy in vivo against S180 xenograft in ICR mice by 24.7% compared with vehicle alone. This result was lower than that of cyclophosphamide at 74.5% but higher than Vorinostat, which showed no significant suppression of S180 tumor growth. In general, the in vitro evaluation against S180 revealed that the activity of **17m** was weaker than that of Vorinostat as shown in Table 3.

Compound **17m** was further profiled for its acute toxicity to mice as shown in Table 4. It should be noted that all the mice given a single injection of **17m** at the dose concentration of 1000 mg/kg of body weight through ig administration route showed no gross pathological changes and the mortality rate was zero, implying the normal growth of mice and the LD_{50} in mice was even higher than 1000 mg/kg. In preliminary studies, the maximum tolerated dose (MTD) of compound **17m** was determined following intraperitoneal (ip) dosed at 100 mg/kg per day for consecutive four days, as defined by no morality and body weight loss less than 10% on day eight. However, when the dose was increased to 200 mg/kg per day from day nine, none were alive on day 11.

In conclusion, these α-lipoic acid derivatives are quite promising for their low toxicity and in vivo efficacy and worth further studying as cancer preventive or therapeutic agents.

Acknowledgments

The authors thank Center of Analysis & Measurement of Zhejiang University and Zhejiang University of Technology for characterization of the compounds and partial financial support. We are also grateful to Zhejiang Provincial Top Key Discipline of Biochemical Engineering and Hangzhou Minsheng Pharmaceutical Group Co., Ltd for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.112.

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