Med Chem Res (2012) 21:3312–3320
DOI 10.1007/s00044-011-9879-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antiproliferation, and docking studies of *N*-phenyl-lipoamide and 8-mercapto-*N*-phenyloctanamide derivatives: effects of C6 position thiol moiety

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Received: 21 July 2011 / Accepted: 4 November 2011 / Published online: 16 November 2011

Abstract Some *N*-phenyl lipoamide and 8-mercapto-*N*-phenyloctanamide derivatives were synthesized and their in vitro antiproliferative activity was evaluated. The experimental results indicated that 8-mercapto-*N*-phenyloctanamides might be good histone deacetylase inhibitors rather than *N*-phenyl lipoamides, who had thiol moiety at C6 position. To verify the antiproliferation data on structural basis, in silico docking studies of the representative compounds into the crystal structure of histone deacetylase-like protein using AutoDock 4.0 program were performed. Furthermore, sulfur acetylated 8-mercapto-*N*-phenyloctanamide improved its in vitro antiproliferative activity, probably due to the increasing of its cell membrane permeability. While the identification of enzymatic target of *N*-phenyl lipoamides with dithiolane is still ongoing.

Keywords Lipoic acid · 8-Mercapto-*N*-phenyloctanamide · Antiproliferation · HDAC · Molecular docking

Introduction

Vorinostat **1** (suberoylanilide hydroxamic acid, SAHA) has been approved for the clinical treatment of cutaneous T-cell lymphoma (CTCL) by the U.S. Food and Drug Administration (FDA) (Marks, 2007). As a promising lead compound, vorinostat **1** has shown good in vitro anticancer efficacy in a wide range of cancer cells (Curtin *et al*., 2002; Gediya *et al*., 2005; Hanessian *et al.*, 2007; Jones *et al.*, 2008). Crystallographic studies disclosed that the hydroxamic acid group of vorinostat **1** coordinated to the zinc ion in the active domain of *A. aeolicus* HDAC homolog HDLP in a bidentate fashion and also formed hydrogen bonds with Tyr 297, His 131, and His 132 residues (Finnin *et al.*, 1999). The complex thereby blocked substrate access, resulting in neoplastic cell cycle growth arrest, terminal cell differentiation, and cell death (Dokmanovic *et al.*, 2007). However, hydroxamic acid group was not the only coordinator that could bind to the zinc ion in enzymes (Suzuki and Miyata, 2005b), and some terminal sulfur-containing compounds were also found to bind tightly to zinc-dependent enzymes (Brown *et al.*, 2000; Opie and Kowolik, 1995). Researchers discovered that when the hydroxamic acid group of vorinostat **1** was replaced by proper sulfur-containing moieties, for instance, the corresponding α-mercaptoketone **2** and α-thioacetoxyketone **3** were observed to be even more potent HDAC inhibitors than that of vorinostat **1** (Gu *et al.*, 2006). The HDAC inhibition of thiol-containing analogs was distinctly dependent on chain length, such as ω-mercapto alkyl amide **5** with chain length of $n = 6$ resulting better potent inhibition than $n = 5$ (**4**) and $n = 7$ (**6**) (Suzuki *et al.*, 2005c). However, compounds with free thiol group might have low in vitro or in vivo anticancer efficacy because of its poor cell membrane permeability, and it would be enhanced by hiding the sulfhydryl group with a disulfide bond or by sulfur acylation (Gu *et al.*, 2006; Suzuki et al., 2005c). Sulfur-acylated natural product largazole **7**, isolated from Floridian marine cyanobacterium *Symploca* sp. (Taori *et al.*, 2008), had exceptionally potent inhibition in a

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number of cancer cell lines in vitro and by hydrolytic removal of the octanoyl group, its largazole thiol **8**, with exposure of the free sulfhydryl residue as a Zn^{2+}-binding arm, exhibited an extraordinarily potent HDAC inhibitory and antiproliferative activity (Bowers *et al.*, 2008; Seiser *et al.*, 2008). Many other modifications of hydroxamic acid group of HDAC inhibitors with sulfur-containing moieties brought out more or less exciting conclusions (Anandan *et al.*, 2005; Chen *et al.*, 2005; Dehmel *et al.*, 2007; Suzuki *et al.*, 2004, 2005a).

According to the SAR of the binding group of vorinostat **1**, we conceived that the skeleton of α-lipoic acid (LA) **9** could be modified as LA-based vorinostat analogs. In our previous article, we found that these analogs, mainly lipoamides exhibited dose-dependent inhibitory property and functioned at high concentration of 100 μg/ml (Zhang *et al.*, 2010). This was quite agreeable with the results made by Zachar's group, who found that the threshold-killing concentration of **12** was 60 μg/ml while the concentration of **11** even reached 600 μg/ml (Bingham and Zachar, 2001). In this article, we detailed the synthesis and antiproliferative activity of lipoamide analogs and compared with 8-mercapto-*N*-phenyloctanamide derivatives which have no thiol moiety at C6 position. In addition, molecular docking studies revealed the effect of C6 position thiol moiety on docking with HDAC.

Results and discussion

Chemistry

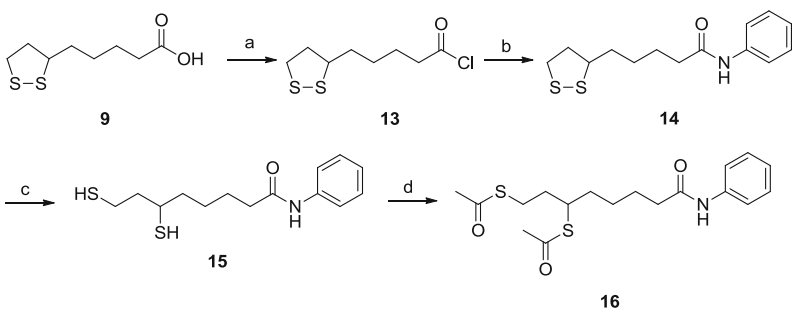
N-Phenyl-6,8-dithioctamide derivatives were prepared from **9** as shown in Scheme 1. Amidation of lipoyl chloride **13** and aniline in the presence of triethylamine as hydrogen chloride capture in dichloromethane led to lipoamide **14**. Reduction of disulfide bond by sodium borohydride gave the corresponding dihydro lipoamide **15**, which was followed by acylation with acetyl chloride to afford **16**.

The presence of thiol (S–H) absorption in an IR spectrum is diagnostic evidence for the sodium borohydride reduction of S–S bond that S–H stretching frequency can be distinguished at around 2,500–3,000 cm^{-1} with weak intensity. The free thiol group absorption of **15** is located at 2,557 cm^{-1}, which neither appears in the spectra of the corresponding disulfide **14** nor in bis acetyl lipoate **16**. The proton shifts on C6 (C–H) between lipoamide **14** and dihydro lipoamide **15** are not identical in ^1H NMR spectra and the protons come into resonance at different frequencies that in **14** it resonates about 0.6–0.7 ppm downfield ^1H chemical shift from that in **15**. Moreover, additional proton resonance signals in free thiols of **15** clearly show the difference.

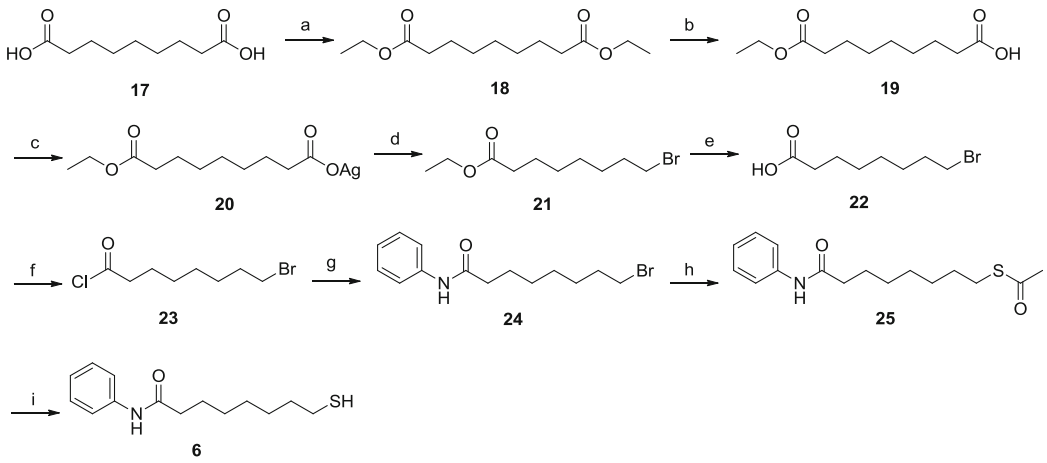
Given that these compounds may target the HDAC's active-site zinc ion, which was positioned near the bottom of the 11 Å deep tubular pocket of the enzyme, compounds with C6 position mercapto group may have difficulty in going through the pocket and binding the zinc ion with C8 position mercapto group. So compound **6** was synthesized as shown in Scheme 2 and compared with compound **15** in antiproliferative activity. Ethyl 8-bromooctanoate **21** was prepared through Hunsdriecke reaction from 9-ethoxy-9-oxononanoic acid **19**, which was esterified from azelaic acid **17** straightforward. Then compound **21** was converted to 8-bromooctanoyl chloride **23** and then reacted with aniline to get 8-bromo-*N*-phenyloctanamide **24**, which was treated with potassium thioacetate in ethanol to yield *S*-8-oxo-8-(phenylamino)octyl ethanethioate **25** and hydrolyzed by sodium hydroxide to obtain 8-mercapto-*N*- phenyloctanamide **6**.

Antiproliferation studies

Table 1 shows the in vitro antiproliferative activity of the representative compounds with or without the thiol side chain at C6 position and the effect of sulfhydryl group protection. In general, compounds **14**, **15**, **16**, **6**, **25** were more active than LA **9** and dihydro LA **10**, among which compounds **14** and **25** were of most active, comparable with or even better than vorinostat **1** at 100 μg/ml. It was reported that compound **6** with free thiol has good HDAC inhibitory activity with IC_{50} at 1.5 μM (Suzuki et al., 2005c), but its antiproliferative activity against BEL-7402, KB, NCI-460 shows weak at 100 μg/ml. This was due to its



Scheme 1 Reagents and conditions. *a* Thionyl chloride, benzene, 0°C, 3 h. *b* Aniline, triethylamine, dichloromethane, 0°C, 4 h. *c* Sodium borohydride, tetrahydrofuran, 0°C, 7 h. *d* Acetyl chloride, triethylamine, dichloromethane, 0°C to rt, 2 h



Scheme 2 Reagents and conditions. *a* Ethanol, *p*-methyl benzenesulfonic acid, toluene, reflux, 8 h. *b* Sodium hydroxide, ethanol, rt, 1 h, then 4 *N* hydrochloric acid. *c* Silver nitrate, potassium hydroxide, H_2O, rt, 15 min. *d* Bromine, carbon tetrachloride, 1.5 h. *e* Sodium hydroxide, H_2O, 45°C, 2 h, then 4 *N* hydrochloric acid. *f* Thionyl chloride, reflux, 1 h. *g* Aniline, triethylamine, dichloromethane, 0°C to rt, 4 h. *h* Potassium thioacetate, ethanol, rt, 20 h; *i* sodium hydroxide, H_2O, ethanol, rt, 48 h

Table 1 Antiproliferative activity of compounds against cancer cell lines at 100 μg/ml

Compound	BEL–7402	KB	NCI–460
14	60.57 ± 1.74	65.32 ± 2.30	47.59 ± 5.38
15	7.67 ± 4.96	15.78 ± 4.23	25.42 ± 2.59
16	14.13 ± 3.23	22.95 ± 1.54	44.77 ± 2.57
6	Na	18.59 ± 10.30	32.39 ± 7.35
25	66.31 ± 6.76	77.01 ± 2.75	82.26 ± 1.43
9	8.17 ± 2.14	10.96 ± 4.47	5.72 ± 6.86
10	0.59 ± 2.07	16.34 ± 0.34	7.84 ± 1.02
Vorinostat **1**	65.74 ± 2.28	64.60 ± 1.61	61.06 ± 2.89
Cisplatin	86.20 ± 0.79	88.83 ± 0.54	97.00 ± 0.46

Values presented are means ± S.D.

na not active

poor cell membrane permeability resulted from the highly polar character of the compound with free thiol. Remarkably, masking the sulfhydryl group of compound **6** as thioester enhanced its cancer cell growth inhibition rapidly. It stands to reason that the thioester **25** increased the possibility of permeating the membrane of cancer cells and then hydrolyzed under physiological conditions to release the free thiol compound **6** to target the active site of HDAC (Gu *et al.*, 2006). However, bis acetyl compound **16** failed to exhibit an admiring growth inhibitory effect as compound **25**, only relatively potent compared with bis sulfhydryl compound **15**. This result suggests that even though **16** can get through cell membrane as efficiently as **25** and convert to thiol **15** within the cell, hardly can it squeeze into pocket-shaped HDAC tunnel because of the block of the thiol side chain at C6 position. It is widely believed that in cellular environment, compounds with disulfide bond or sulfur-acylated can be reduced, releasing the free thiol analogs as the active species (Suzuki et al., 2005c), such as FK228, FR901375, Spiruchostatin (Bowers *et al.*, 2008), SCOP (Nishino *et al.*, 2008), largazole **7** (Bowers *et al.*, 2008), and **9** (Novotny *et al.*, 2008). Thus, it can be inferred that the antiproliferative activity of disulfide **14**, which can be reduced to bis sulfhydryl **15**, would be no better than that of **15** under the hypothesis of HDAC inhibitory action. In fact, the antiproliferative activity of **14** exhibits 1-fold higher against NCI-460, 3-fold higher against KB, and 7-fold higher against BEL-7402 than **15**, and the explanation might be that **14** is functioning through other mechanism such as mammalian pyruvate dehydrogenase complex (PDC) inhibition rather than HDAC inhibition.

In silico molecular docking

The docking studies of the compounds were performed with AutoDock 4.0 program, based on a Lamarckian genetic algorithm (LGA) method (Morris *et al.*, 1998; Huey *et al.*, 2007). Basically, this program calculates total interaction energies between random pairs of ligands and various selected portions of protein to determine docking poses. The AutoDock 4.0 program with the following parameters: AutoDockTools 1.4.5 (ADT) is prepared for ligands and receptor. The AutoDock search parameters select Genetic Algorithm. The number of GA-Run is 100, the population size 200, and the maximum number of Evals is 25,000,000 generations. Other parameters are default values. The generate files are calculated by AutoGrid 4.0 and AutoDock 4.0 (Figs. 1, 2).

As shown in Fig. 3a, b, compound **6** bound by inserting its unbranched long alkyl chain into the HDLP pocket snugly, making multiple contacts to the tube-like hydrophobic portion of the pocket. The thiol moiety at

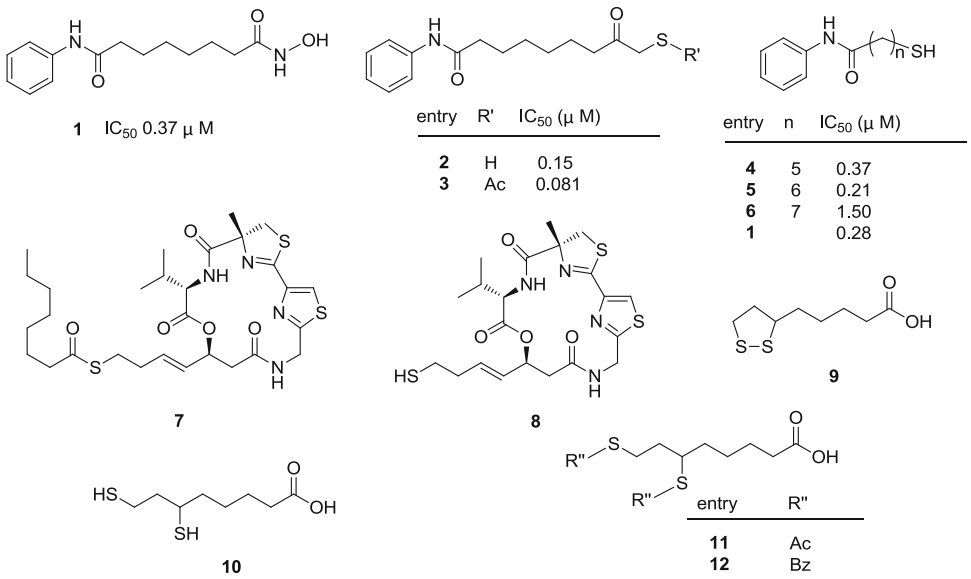


Fig. 1 Structures of known HDAC inhibitors and others related. IC_{50} refers to the inhibition of HDAC1, unless otherwise stated

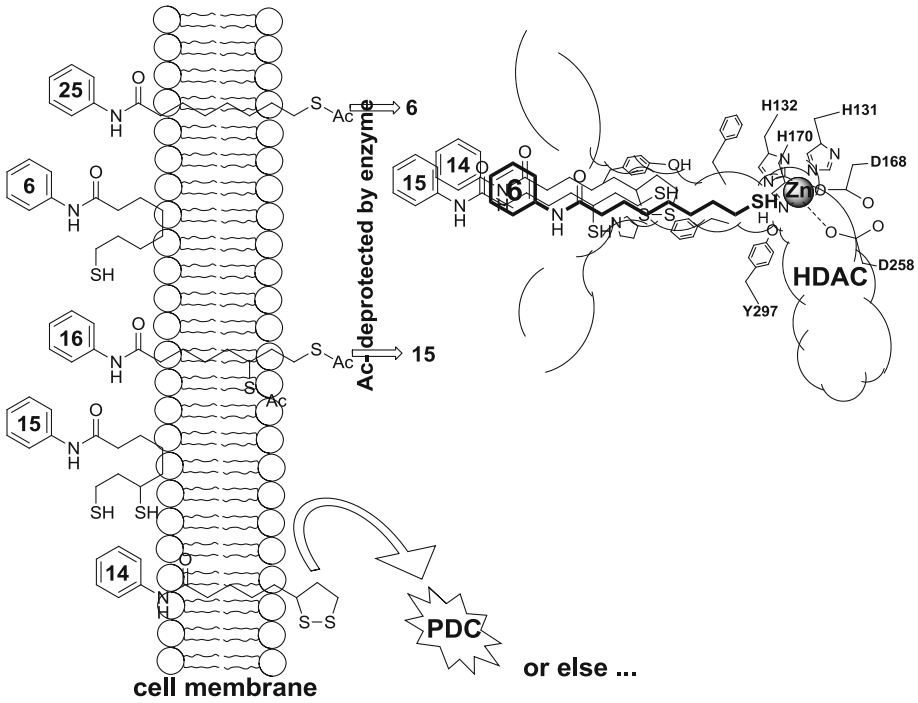


Fig. 2 Illustration of proposed pathway of compounds against cancer cell lines

one end of the chain reached the polar bottom of the pocket, where it coordinated the zinc ion and also contacted active-site residue Tyr 297 with a distance of 2.5 Å. While the phenyl-amino ketone group contacted residues at the rim of the pocket and in an adjacent surface groove formed by Pro 22, Tyr 91, and Phe 141. Due to the existence of C6 thiol moiety, compound **15** could not get into the pocket and was blocked at the tightest point of the pocket shaped by Phe 141 and Phe 198, where the distance of C8 thiol moiety to Tyr 297 was 2.7 Å and unbonded to zinc, as shown in Fig. 3c, d. For compound **14** in Fig. 3e, f, the dithiolane was almost sandwiched between the phenyl groups of Phe 141 and Phe 198 and could not reach the active site.

As shown in Table 2, compound **6** displayed free binding energy value of −7.36 kcal/mol, indicating that the affinity of compound **6** for HDLP was stronger than compounds **15** or **14**.

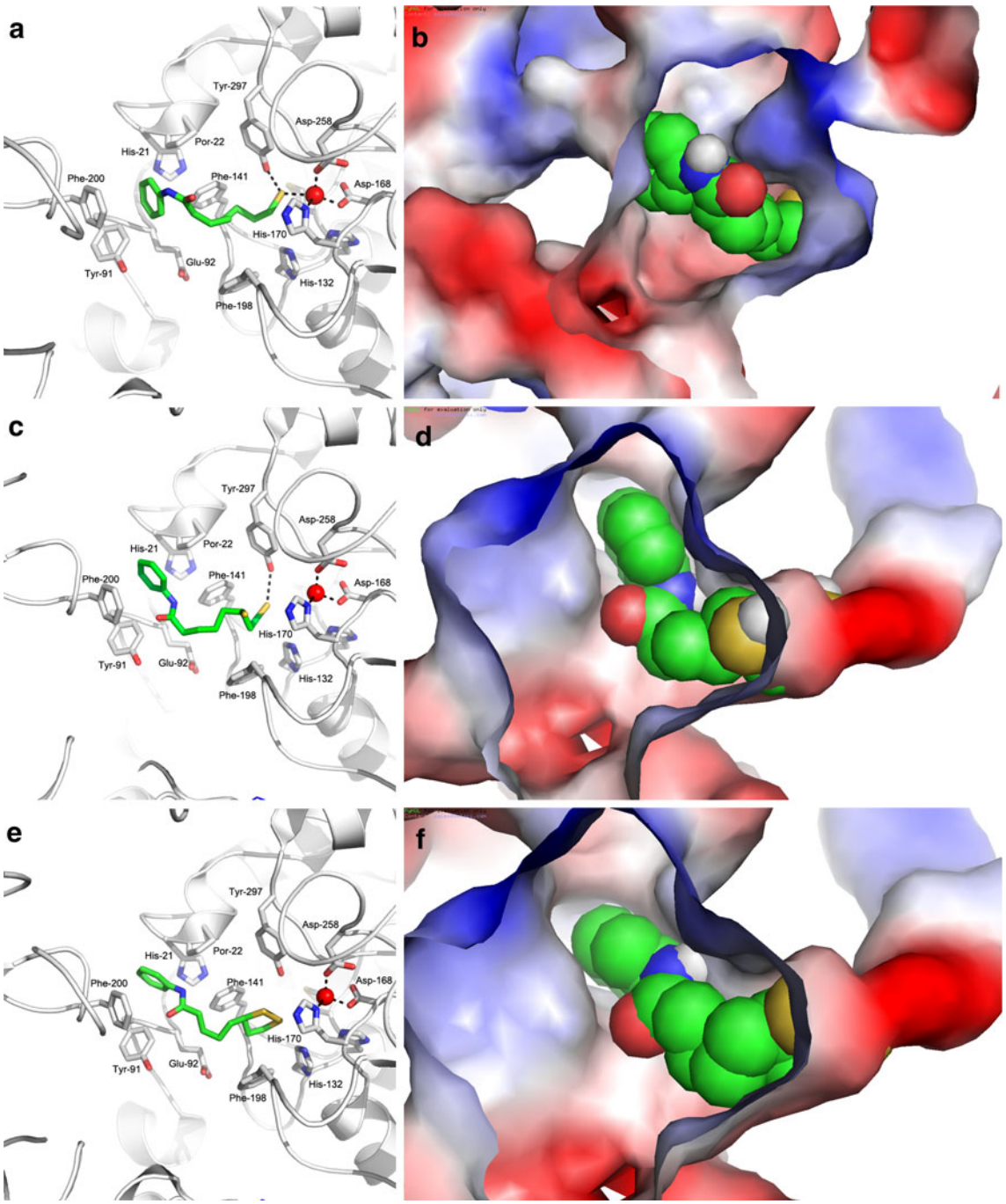


Fig. 3 **a**, **b** Docking interaction of compound **6** with the target HDLP. **c**, **d** Docking interaction of compound **15** with the target HDLP. **e**, **f** Docking pose of compound **14** with the target HDLP

Experimental

Chemistry-general aspects

Melting points were taken on an XRC-1 apparatus and are uncorrected. Infrared spectra (IR) were obtained on a Thermo Nicolet Avatar 370 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Brucker AC-80 spectrometer operating at 400 MHz using tetramethyl silane (TMS) as an internal standard in $CDCl_3$ and chemical shifts were expressed in δ (ppm) values downfield from TMS, and J values were in Hz (The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet and br, broad peak). Mass spectra (MS) were run on an HP 5989B instrument at an ionizing voltage of 70 eV. High resolution mass spectra (HRMS) were

Table 2 Binding energy data of compounds **6**, **15**, and **14**

Compound	Estimated free binding energy[a] (kcal/mol)
6	−7.36
15	−5.86
14	−6.01

[a] Free binding energy calculations were determined by docking studies using AutoDock 4.0 program

measured on Agilent 6210 TOF LC/MS instrument with ESI source. UV absorption spectra were recorded on UV-1800 UV–vis spectrophotometer from Shimadzu. Column chromatography was carried out on silica gel (200–300 mesh) eluting with solvents as indicated. Reaction progress was monitored by thin layer chromatography (TLC) on precoated silica gel GF254 plates with fluorescent indicator. All the chemicals and solvents were of analytical reagent and used as received, unless otherwise stated. Petroleum ether (PE) referred to a mixture of alkanes with the boiling range from 60 to 90°C. α-LA was racemic and used before recrystallization (cyclohexane:ethyl acetate (EtOAc) = 15:1).

*Lipoyl chloride (**13**) in dichloromethane*

To a stirred solution of thionyl chloride (0.89 g, 7.5 mmol) in benzene (10 ml, calcium chloride dried) at 0°C was added a solution of lipoic acid (1.03 g, 5 mmol) in dried benzene (20 ml) dropwise over 1 h. The mixture was allowed to stir for an additional 3 h at the same temperature. After completion of the reaction, the solvent was removed under vacuum to get lipoyl chloride **13** almost quantitatively. The residue without further purification was diluted in dichloromethane (20 ml) for the next step.

*5-(1,2-Dithiolan-3-yl)-N-phenylpentanamide (**14**)*

To an ice-cooled solution of aniline (0.56 g, 6 mmol) and triethylamine (0.61 g, 6 mmol) in dichloromethane (20 ml) was cautiously added the prepared solution of **13** in dichloromethane dropwise. The reaction mixture was stirred in an ice bath for 4 h to reach completion, monitored by TLC. The mixture was washed by saturated sodium bicarbonate (3 × 10 ml), 1 *N* hydrochloric acid (3 × 10 ml), brine (3 × 10 ml) and dried over anhydrous magnesium sulfate, and the solvent was then removed under reduced pressure. The crude product was purified with chromatography (PE:EtOAc = 1:1) to get **14** as yellow solid, 0.97 g (68.9%); mp: 67–69°C; IR ν_{max} (KBr)/cm^{-1}: 3296, 2920. 1658, 1599, 1537, 1498, 1444, 691, 502; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.52 (d, J = 8.0 Hz, 2H, Ar), 7.33 (t, J = 8.0 Hz, 2H, Ar), 7.16 (br, 1H, NH), 7.11 (t, J = 7.2 Hz, 1H, Ar), 3.59 (m, J = 6.9 Hz, 1H, –S–CH–), 3.22–3.09 (m, 2H, –S–CH_2–), 2.38 (t, J = 7.4 Hz, 2H, –CH_2–CO–), 1.97–1.49 (m, 8H, –CH_2–CH–CH_2–CH_2–CH_2–); EI-MS *m/z*(%): 281 (M^+, 97), 248 (16), 216 (17), 155 (30), 148 (27), 135 (51), 93 (100), 77 (18); UV (EtOH): λ_{max} (nm, log ε) = 202 (4.83), 243 (4.57).

*6,8-Dimercapto-N-phenyloctanamide (**15**)*

To a solution of **14** (0.84 g, 3 mmol) in tetrahydrofuran (30 ml) at 0°C was added a solution of sodium borohydride (0.23 g, 6 mmol in 5 ml H_2O) dropwise. The mixture was allowed to stir at the same temperature for an additional 7 h to reach completion. 1 *N* hydrochloric acid (10 ml) was added, and the solvent was removed under vacuum. The residue was extracted with dichloromethane (3 × 10 ml). The organic layer was washed with brine (3 × 10 ml), dried over anhydrous magnesium sulfate, and concentrated under vacuum to obtain crude product, which was chromatographed over silica gel (PE:EtOAc = 2:1) to yield **15** as white solid, 0.74 g (87.0%); mp: 43–44°C; IR ν_{max} (KBr)/cm^{-1}: 3310, 2929, 2557, 1653, 1599, 1534, 1498, 1442, 759, 692, 501; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.53 (d, J = 7.6 Hz, 3H, Ar + NH), 7.31 (t, J = 7.8 Hz, 2H, Ar), 7.11 (t, J = 7.4 Hz, 1H, Ar), 2.96–2.88 (m, 1H, –S–CH–), 2.78–2.62 (m, 2H, –S–CH_2–), 2.37 (t, J = 7.4 Hz, 2H, –CH_2–CO–), 1.94–1.45 (m, 8H, –CH_2–CH–CH_2–CH_2–CH_2–), 1.36 (t, J = 8.4 Hz, 1H, SH–CH_2–), 1.31 (d, J = 7.2 Hz, 1H, SH–CH); ESI-HRMS: $[M + H]^+$ 284.1157 for $C_{14}H_{21}NOS_2 + H$ (Calcd 284.1143), $[M + Na]^+$ 306.0976 for $C_{14}H_{21}NOS_2 + Na$ (Calcd 306.0962); UV (EtOH): λ_{max} (nm, log ε) = 202 (4.77), 243 (4.50).

*S,S′-(8-Anilino-8-oxo octane-1,3-diyl)diethanethioate (**16**)*

To a solution of **15** (0.28 g, 1 mmol) and triethylamine (0.25 g, 2.5 mmol) in dichloromethane (20 ml) at 0°C was cautiously added a solution of acetyl chloride (0.20 g, 2.5 mmol) in dichloromethane (5 ml). The mixture was stirred in an ice bath for additional 2 h to reach completion. Dichloromethane (15 ml) was added to dilute the mixture and washed with 1% citric acid (3 × 10 ml), brine (3 × 10 ml), and dried over anhydrous magnesium sulfate. The evaporation of solvent left crude product, which was purified with chromatography (PE: EtOAc = 2: 1) to obtain **16** as viscous liquid, 0.28 g (77.1%); IR ν_{max} (KBr)/cm^{-1}: 3311, 2933, 1690, 1600, 1541, 1499, 1442, 1134, 953, 757, 694, 630, 507; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.67 (br, 1H, NH), 7.54 (d, J = 7.6 Hz, 2H, Ar), 7.31 (t, J = 7.8 Hz, 2H, Ar), 7.09 (t, J = 7.2 Hz, 1H, Ar), 3.56

(m, $J = 6.8$ Hz, 1H, –S–CH–), 3.01–2.94 (m, 1H, –S–C*H*H–), 2.87–2.80 (m, 1H, –S–CH*H*–), 2.35 (t, $J = 7.6$ Hz, 2H, –CH_2–CO–), 2.32 (s, 6H, 2 × CH_3), 1.92–1.37 (m, 8H, –C*H*$_2$–CH–C*H*$_2$–C*H*$_2$–C*H*$_2$–); ESI-HRMS: $[M + H]^+$ 368.1381 for $C_{18}H_{25}NO_3S_2 + H$ (Calcd 368.1354), $[M + Na]^+$ 390.1202 for $C_{18}H_{25}NO_3S_2 + Na$ (Calcd 390.1174).

*S-8-Oxo-8-(phenylamino)octyl ethanethioate (**25**)*

A mixture of azelaic acid **17** (75.3 g, 400 mmol), excess ethanol (180 ml), toluene (100 ml), and catalytic amount of *p*-methyl benzenesulfonic acid (6.0 g, 35 mmol) was stirred at reflux for 8 h to get diethyl ester **18**, one ethyl ester moiety of which was hydrolyzed by sodium hydroxide (17.5 g, 440 mol) in ethanol (400 ml) for 1 h and then adjusted to pH 2 by 4 *N* hydrochloric acid. The aqueous phase was extracted by ethyl ether (3 × 100 ml) and dried over anhydrous magnesium sulfate to get azelaic acid monoethyl ester **19** as yellowish clear oil, 73.8 g (85.3%); IR ν_{max} (KBr)/cm^{-1}: 3446, 2934, 2858, 1736, 1710, 1384, 1182, 1096, 1035.

To a stirring solution of azelaic acid monoethyl ester **19** (21.6 g, 100 mmol) and potassium hydroxide (5.6 g, 100 mmol) in H_2O (240 ml) was added a solution of silver nitrate (17.0 g, 100 mmol) in H_2O (200 ml) in 5 min. The reaction continued for 15 min before filtration of the gray precipitation. The precipitated silver salt **20** was washed with water (60 ml) and methanol (60 ml) and then dried thoroughly under an oil pump at 110°C and powdered finely to get 19.4 g (60.1%).

To a mixture of silver salt **20** (19.4 g, 60 mmol) and carbon tetrachloride (70 ml, phosphorus pentoxide dried) was added bromine (8.8 g, 55 mmol, sulfuric acid dried) over 40 min in an ice bath with generation of carbon dioxide. After completion, the violet mixture was heated to reflux in a 110°C oil bath for 1.5 h. The mixture was filtered and the left silver bromide was washed with warm carbon tetrachloride. The filtrate was washed with 10% sodium carbonate (60 ml) and dried over anhydrous magnesium sulfate to obtain ethyl 8-bromooctanoate **21** as pale yellow oil, 8.9 g (64.4%); IR ν_{max} (KBr)/cm^{-1}: 2933, 2857, 1734, 1463, 1384, 1250, 1177, 1096, 1035, 782, 726, 644, 562; EI-MS *m/z*(%): 171 ([M-^{79}Br]$^+$, 100), 152 (32), 125 (17), 97 (50), 83 (28), 69 (31).

The mixture of ethyl 8-bromooctanoate **21** (3.8 g, 15 mmol) and sodium hydroxide (0.7 g, 16.5 mmol) in H_2O (60 ml) was heated to 45°C for 2 h and then more water (180 ml) was added and washed with carbon tetrachloride (3 × 30 ml). The aqueous phase was acidified by 4 *N* hydrochloric acid to pH 2. The solution was extracted by ethyl acetate (3 × 80 ml) and washed with brine and dried over anhydrous magnesium sulfate to get 8-bromooctanoic acid **22** as pale yellow crystal, 3.3 g (97.7%); low mp; IR ν_{max} (KBr)/cm^{-1}: 2933, 2852, 1701, 1467, 1410, 1252, 929, 726, 678.

The stirred mixture of 8-bromooctanoic acid **22** (3.2 g, 15 mmol) and thionyl chloride (20 ml) was refluxed for 1 h. Evaporation of excess thionyl chloride left 8-bromooctanoyl chloride **23** quantitatively.

8-Bromo-*N*-phenyloctanamide **24** was prepared from aniline (0.93 g, 9.9 mmol) and 8-bromooctanoyl chloride **23** using the procedure described for **14**: yellow solid, 1.1 g (44.6%); low mp; IR ν_{max} (KBr)/cm^{-1}: 3308, 2933, 2856, 1661, 1600, 1540, 1499, 1443, 1384, 1251, 1180, 757, 693.

To a suspension of 8-bromo-*N*-phenyloctanamide **24** (0.80 g, 2.68 mmol) obtained above in ethanol (10 ml) was added potassium thioacetate (1.1 g, 9.73 mmol), and the mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with ethyl acetate (50 ml) and tetrahydrofuran (50 ml), washed with H_2O (3 × 20 ml) and brine (3 × 20 ml), and dried over anhydrous sodium sulfate. Filtration and concentration in vacuo and purification by silica gel chromatography (PE:EtOAc = 1:1) gave **25** as brown oil, 142 mg (18.0%); IR ν_{max} (KBr)/cm^{-1}: 3312, 2929, 2856, 1690, 1667, 1600, 1543, 1499, 1443, 1384, 756, 693, 507; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.52 (d, $J = 7.6$ Hz, 2H, Ar), 7.32 (t, $J = 7.6$ Hz, 2H, Ar), 7.25 (brs, 1H, NH), 7.10 (t, $J = 7.4$ Hz, 1H, Ar), 2.86 (t, 2H, $J = 7.4$ Hz, –CH_2–S–), 2.35 (t, $J = 6.8$ Hz, 2H, –CH_2–CO–), 2.32 (s, 3H, CH_3–CO–), 1.77–1.72 (m, 2H, –C*H*$_2$–CH_2–S–), 1.60–1.53 (m, 2H, –C*H*$_2$–CH_2–CO–), 1.41–1.36 (m, 6H, –C*H*$_2$–C*H*$_2$–C*H*$_2$–CH_2–CH_2–S–); EI-MS *m/z*(%): 293 (M^+, 5), 253 (30), 218 (19), 204 (11), 135 (90), 120 (18), 93 (100), 77 (38).

*8-Mercapto-N-phenyloctanamide (**6**)*

To a solution of *S*-8-oxo-8-(phenylamino)octyl ethanethioate **25** (37.9 mg, 0.13 mmol) in ethanol (5 ml) was added sodium hydroxide (17.1 mg, 0.43 mmol) in H_2O (1 ml). The mixture was stirred at room temperature for 48 h before H_2O (10 ml) was added to dilute the mixture and neutralized with 2 *N* hydrochloric acid to pH 4–5 with cooling in an ice-water bath. The clear yellow solution was extracted with ethyl acetate (20 ml) and washed with H_2O (3 × 5 ml), brine (3 × 5 ml), and dried over anhydrous sodium sulfate. Filtration and concentration in vacuo gave **6** as light yellow solid, 21.8 mg (67.1%); low mp; IR ν_{max} (KBr)/cm^{-1}: 2961, 2926, 2855, 2549, 1663, 1600, 1545, 1499, 1443, 1384, 757, 693, 509; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.51 (d, $J = 8.0$ Hz, 2H, Ar), 7.31 (t, $J = 7.4$ Hz, 2H, Ar), 7.09 (t, $J = 7.0$ Hz, 1H, Ar), 2.73–2.65 (m, 2H, HS–C*H*$_2$–), 2.35 (t, $J = 7.6$ Hz, 2H, –CH_2–CO–), 1.81–1.43 (m, 10H, –C*H*$_2$–C*H*$_2$–C*H*$_2$–C*H*$_2$–C*H*$_2$–CH_2–HS), 1.36 (t, $J = 7.6$ Hz, 1H, S*H*–CH_2–); EI-MS

m/z(%): 219 ([M–SH + H]$^+$, 4), 205 (6), 191 (7), 179 (9), 163 (10), 149 (17), 137 (16), 123 (25), 93 (51).

Antiproliferation assay

The human cancer cell lines (BEL-7402, KB and NCI-460 derived from Shanghai Institutes for Biological Science, Chinese Academy of Sciences) were cultivated at 37°C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, purchased from Gibco) supplemented with 800 (U/v) penicillin, 0.1% (w/v) streptomycin, 10% (v/v) fetal bovine serum for 3–5 days. Human cancer cells, treated with Trypsin–EDTA solution, were seeded into 96-well flat bottom plates at 10^6 cells/well and incubated in a 5% CO_2 incubator at 37°C for 24 h. Cultures were treated with compounds prepared in 100 μg/ml. Mitochondrial metabolism was measured as a marker for cell growth by adding 10 μl/well MTT (5 mg/ml in medium, Sigma) with 3 h of incubation at 37°C. Crystals formed were dissolved in 150 μl of DMSO. The absorbance was determined using a microplate reader at 490 nm. The absorbance data were converted into a cell proliferation percentage, compared to DMSO treated cells, to determine growth inhibition. Each assay was performed in triplicate.

In silico molecular docking

The synthesized compounds with *R* conformation were taken for prediction of 3D structures and energy was minimized for flexible docking. Each molecule was added Gasteiger charges and nonpolar hydrogens were merged to carbon atoms by AutoDockTools (ADT 1.4.5). The 3D structure of HDLP (1C3R) was obtained from Protein Data Bank at the website: http://www.pdb.org/. HDLP is similar to human HDAC1 with 35.2% identity and specially, all of the polar residues in the active site and the hydrophobic residues that make up the walls of the pocket are identical (Finnin *et al.*, 1999). Trichostatin A (TSA) molecule and nonreceptor atoms such as water, ions were removed from 1C3R, and Kollmann charges were assigned and converted to pdbqt format as receptor file. The grid maps defining the search region and representing the protein in the docking process were calculated with AutoGrid and had dimensions of 40 × 60 × 40 Å centered by the predefined active site of the protein, with a spacing of 0.375 Å between the grid points. All ligands were transformed as pdbqt format for each docking run. The LGA parameters were accepted as number of GA runs 100, population size 200, maximum number of Evals 25,000,000 generations and others parameters were left as default values. The best affinity modes were screened by binding energy scores and analyzed by Pymol 0.99 program.

Conclusion

In summary, 8-mercapto-*N*-phenyloctanamide derivatives were successfully synthesized and their antiproliferative activity was evaluated and compared with *N*-phenyl-lipoamide analogs. The experimental results showed that in vitro antiproliferative activity of sulfur acetylated 8-mercapto-*N*-phenyloctanamide was much higher than that of 8-mercapto-*N*-phenyloctanamide, indicating the sulfur protected thioctamide increased its cell membrane permeability and would be deacetylated to become 8-mercapto-*N*-phenyloctanamide with free thiol moiety as HDAC inhibitor. In addition, docking studies of molecules with or without C6 thiol moiety were performed to determine their effectiveness as HDAC inhibitors. Based on their docking results and scoring, compounds with C6 thiol moiety or dithiolane were unable to reach the active site of HDLP, while 8-mercapto-*N*-phenyloctanamide without C6 thiol moiety successfully targeted HDAC with its C8 thiol moiety as zinc chelating group. *N*-Phenyl lipoamides with dithiolane also showed good antiproliferative activity, but it could not be docked with HDAC, the indentification of enzymatic target of which is still ongoing. In follow-up studies, these experimental and predictive results described may give rise to design new and more potent HDAC inhibitors with proper thiol moiety.

Acknowledgments The authors are thankful to Hangzhou Minsheng Pharmaceutical Group Co., Ltd. for antiproliferative evaluation. Partial financial support from Hangzhou Minsheng Pharmaceutical Group Co., Ltd. are also gratefully acknowledged.

Conflict of interest The authors declare that they have no conflict of interest regarding the work reported in this manuscript.

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