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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of oleanolic acid/ursolic acid/glycyrrhetinic acid-hydrogen sulfide donor hybrids and their antitumor activity

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Abstract

A total of 25 targeted hydrogen sulfide donor–pentacyclic triterpene hybrids were designed, synthesized and evaluated on the basis of inspiring reports about hydrogen sulfide donor molecules and oleanolic acid derivatives on anti-tumor activity. The results revealed that only some hybrids (**10a**, **10b**, **12a**, **12b**, **13a**, and **15b**) showed moderate anti-proliferation activity against K562 cell line. It indicated that oleanane-type, (*R*)-lipoic acid and TBZ groups were much more favorable to the anti-proliferation activity. Furthermore, C-3 OH was more beneficial than C-28/29 COOH in the oleanane-type to the antitumor activity of the batch of (*R*)-lipoic acid derivatives. And among them, only compound **13a** exhibited moderate anti-proliferation activity against both K562 and K562/ADR cell lines, while it exhibited no anti-proliferation activity against BEL-7402 and L-O2 cell lines. Therefore, it suggested that it was not suitable for hybridization of hydrogen sulfide donors attached to oleanolic acid, ursolic acid and glycyrrhetinic acid in the field of anti-tumor.

Keywords Oleanolic acid · Ursolic acid · Glycyrrhetinic acid · Hydrogen sulfide donor · Antitumor activity

Introduction

Though hydrogen sulfide (H_2S) has been long considered to be a poison similar to cyanide metabolism (Imbrogno et al. 2018), in the latest researches (Olson et al. 2013; Olas 2015; Bazhanov et al. 2017; Powell et al. 2017), it has been demonstrated that it plays an important role in life and possesses an irreplaceable physiological regulation in life activities, controlling of a variety of signal transduction in cells and playing a positive role in regulating process. Studies (Caliendo et al. 2010; Chen et al. 2015; Ercole et al. 2017) on biological effects and mechanisms of signal pathways of H_2S revealed that, as a signaling molecule, H_2S involved in the cell signal transduction of cardiovascular system, nervous system, circulatory system, and many other organs, and possessed intermediary effect on multiple physiological processes in body. 5-(4-Hydroxyphenyl)-3H-1,2-dithiole-3-thione (ADT-OH, **1**, Fig. 1), (R)-lipoic acid (**2**, Fig. 1) and 4-hydroxy benzothioamide (TBZ, **3**, Fig. 1) were reported as excellent hydrogen sulfide donors with sustained release, high tissue specificity, long half-life, and low toxicity (Bharath et al. 2002; Jia et al. 2013; Hammers et al. 2016).

In retrospect, researchers (Kodela et al. 2012) synthesized a series of new hybrids of aspirin (ASA), bearing both nitric oxide (NO) and hydrogen sulfide-releasing moieties (ADT-OH, *R*-lipoic acid, and TBZ), and discovered these compounds were extremely effective in inhibiting the growth of human cancer cell lines by arresting cell proliferation, inducing apoptosis, and blocking cell cycle of G_0/G_1. These hybrids, which were covalently attached H_2S-releasing moiety (ADT-OH) to non-steroidal anti-inflammatory drugs (NSAIDs), could inhibit the growth of cancer cell lines with potencies of at least 28-fold greater than that of their traditional counterparts, and some of them even up to 3000-fold. Among these reported hybrids, the two components were individually less active than the hybrid itself (Chattopadhyay et al. 2012a, b, c). Novel H_2S-releasing hybrids have been

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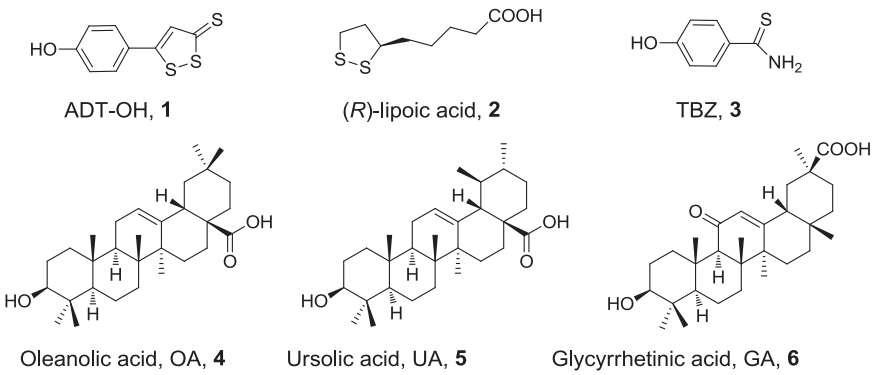
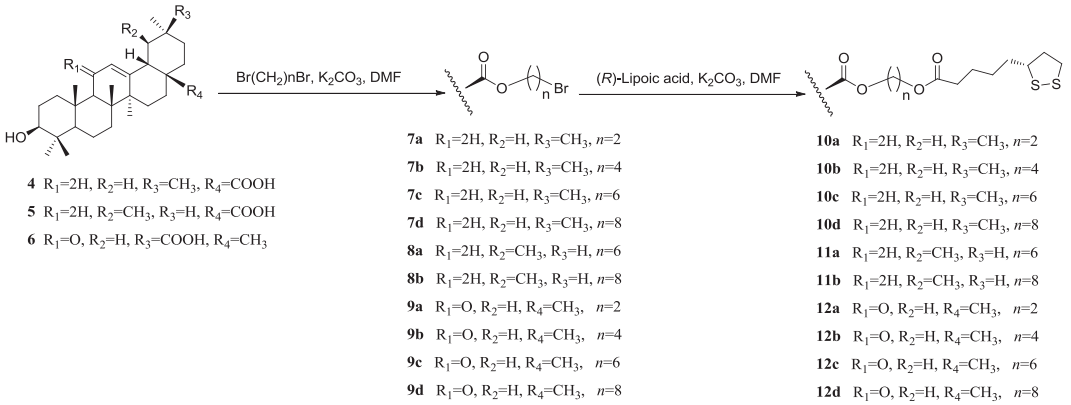


Fig. 1 Chemical structures of ADT-OH, (*R*)-lipoic acid, TBZ, OA, UA, and GA



Scheme 1 Synthesis of pentacyclic triterpenes-(*R*)-lipoic acid derivatives, where *n* is the number of methylene groups

developed in order to investigate some new chemical entities containing hydrogen sulfide-releasing moieties (Chattopadhyay et al. 2012a, b, c; Kaium et al. 2011; Ryazantseva et al. 2011; Frantzias et al. 2012; Lee et al. 2011). Furthermore, with the invention and application of new hydrogen sulfide release donors and the introduction of hydrogen sulfide metabolic mechanism in the treatment of abnormal diseases, the research on H_2S-donor is entering an era of exponential exploration.

Natural products are the major resources of bioactive agents and their derivatives also play as protagonists for discovering new entities (Shen et al. 2003). Oleanolic acid (OA, **4**, Fig. 1), ursolic acid (UA, **5**, Fig. 1) and glycyrrhetinic acid (GA, **6**, Fig. 1) are the representative compounds of pentacyclic triterpenes, which are the active ingredients of herbal medicines (Kandefer-Szerszen and Paduch 2014), and have been deeply researched as they possessing biodiversity activities (Bhragual et al. 2010; Shyu et al. 2010; Tsai and Yin 2010; Huang et al. 2014; Luzina and Salakhutdinov 2018). Our previous studies (Cheng et al. 2016a, b) exhibited that the derivatives of oleanolic acid possessed excellent anti-proliferation activity against tested tumor cell lines with some IC_{50} values under 0.1 μM.

Based on above inspiring reports about hydrogen sulfide donor molecules (Chattopadhyay et al. 2012a, b, c; Frantzias et al. 2012; Kodela et al. 2012) and oleanolic acid derivatives (Cheng et al. 2016a, b; Huang et al. 2018) on anti-tumor activity, in the present study, hydrogen sulfide donors (ADT-OH, (*R*)-lipoic acid and TBZ) were attached to OA, UA, and GA to afford series of pentacyclic triterpenes-H_2S donor hybrids (Schemes 1–3), and to evaluate the anti-proliferation activity against tested cell lines (BEL-7402, K562, K562/ADR, and L-O2) through the MTT assay (Van Meerloo et al. 2011) while 5-fluorouracil (5-FU) used as a positive control (Table 1).

The results revealed that most of these pentacyclic triterpenes-H_2S donor hybrids exhibited no anti-proliferation activity against tested cell lines (BEL-7402, K562, K562/ADR and L-O2). Only some compounds (**10a**, **10b**, **12a**, **12b**, **13a**, and **15b**) showed moderate anti-proliferation activity against K562 cell line. It indicated that oleanane-type, (*R*)-lipoic acid and TBZ groups were much more favorable to the anti-proliferation activity. Furthermore, in the oleanane-type, C-3 OH was more beneficial than C-28/29 COOH to the antitumor activity of the batch of (*R*)-lipoic acid derivatives. And among them, only compound **13a** exhibited moderate anti-proliferation

Table 1 Evaluation of compounds against cell viability of different cell lines

Compound	IC_{50} (μM)[a]			
	BEL-7402	K562	K562/ADR	L-O2
1	100.5	56.3	59.6	71.5
2	>200	>200	>200	>200
3	>200	>200	>200	>200
4	59.9	>200	>200	>200
5	64.6	41.0	38.1	58.4
6	>200	>200	86.5	>200
10a	>200	89.637	>200	>200
10b	>200	92.397	>200	>200
10c	>200	184.8	>200	>200
10d	>200	>200	>200	>200
11a	>200	>200	>200	>200
11b	>200	>200	>200	>200
12a	>200	64.3	>200	>200
12b	>200	68.7	>200	>200
12c	>200	126.2	>200	>200
12d	>200	135.7	>200	>200
13a	>200	55.2	74.6	>200
13b	>200	95.0	181.0	>200
14a	>200	99.3	>200	>200
14b	>200	>200	>200	>200
15a	171.2	97.5	>200	161.2
15b	93.5	47.8	150.8	>200
16a	>200	>200	>200	>200
16b	>200	>200	>200	>200
17a	>200	>200	>200	>200
17b	>200	>200	>200	>200
18a	>200	>200	>200	>200
18b	>200	183.9	>200	>200
19	134.8	190.9	>200	113.8
20	>200	>200	>200	76.94
21	>200	>200	>200	>200
5-FU	53.3	41.7	31.9	>200

[a]The IC_{50} values are the concentrations of the compounds which inhibit tumor cell growth by 50%, and are presented from three separated experiments

activity against both K562 and K562/ADR cell lines, while it exhibited no anti-proliferation activity against BEL-7402 and L-O2 cell lines. All these results revealed that, though the reports about hydrogen sulfide donor molecules (Chattopadhyay et al. 2012a, b, c; Frantzias et al. 2012; Kodela et al. 2012) and oleanolic acid derivatives (Cheng et al. 2016a, b; Huang et al. 2018) on anti-tumor activity were inspiring, it seemed that it was not suitable for hybridization of hydrogen sulfide donors attached to OA, UA, and GA in the field of anti-tumor.

Materials and methods

General

All the chemical reagents and solvents were used of analytical grade and used without further purification unless specified. All commercial reagents were purchased from Aladdin (Shanghai) Industrial Corporation. Melting points were measured on a RY-1 melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-500 or AV-400 spectrometer. Chemical shifts were reported as values from an internal tetramethylsilane standard. HR-MS were recorded on a Thermo Scientific Accela – Exactive High Resolution Accurate Mass spectrometer.

Synthesis

General procedure I for *O*-alkylation reaction from (*R*)-lipoic acid to afford compounds 10a–12d

To a solution of **7a**–**9d** (0.44 mmol, 1 equiv.) in DMF (5 mL) were added K_2CO_3 (182.16 mg, 1.32 mmol, 3 equiv.) and (*R*)-lipoic acid (90.64 mg, 0.44 mmol, 1 equiv.). After stirring at 50 °C for 24 h, the mixture was diluted with EtOAc (50 mL). The organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography.

[2-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)ethyl]-3*β*-hydroxyolean-12-en-28-oate (10a) Prepared from **7a** (Cheng et al. 2016a, b) (0.25 g, 0.44 mmol) and (*R*)-lipoic acid (90.64 mg, 0.44 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.227 g, 74.0%; faint yellow solid, m.p. 116–118 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.71, 0.76, 0.90, 0.97 and 1.11 (5 s, each 3H, 5 × CH_3), 0.88 (s, 6H, 2 × CH_3), 0.64–2.02 (m, 30H), 2.32 (t, J = 7.5 Hz, 2H, $COCH_2$), 2.45 (m, 1H, S-CH), 2.84 (dd, J = 4.2, 13.7 Hz, 1H, 18-H), 3.04–3.23 (m, 3H, OH and SCH_2), 3.55 (dd, J = 6.3, 8.4 Hz, 1H, 3-H), 4.24 (m, 4H, 2 × OCH_2), 5.26 (s, 1H, 12-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.0, 18.4, 23.1, 23.5, 23.7, 24.7, 26.0, 27.3, 27.8, 28.2, 28.9, 30.8, 32.5, 32.8, 33.2, 34.0, 34.7, 37.1, 38.6, 38.9, 39.4, 40.3, 41.4, 41.8, 45.9, 46.9, 47.7, 55.3, 56.4, 62.1, 79.1, 122.6, 143.7, 173.2, 177.6. HRMS (ESI) *m/z*: $[M+Cl]^+$ calcd for $C_{40}H_{64}ClO_5S_2$ 723.3884; found 723.3903.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)butyl]-3*β*-hydroxyolean-12-en-28-oate (10b) Prepared from **7b** (Cheng et al. 2016a, 2016b) (0.25 g, 0.42 mmol) and (*R*)-lipoic acid (90.64 mg, 0.42 mmol) according to general

procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.284 g, 94.0%; faint yellow solid, m.p. 80–82 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.70, 0.76, 0.90, 0.96 and 1.11 (5 s, each 3H, 5 × CH_3), 0.88 (s, 6H, 2 × CH_3), 0.64–2.07 (m, 34H), 2.30 (t, J = 7.5 Hz, 2H, $COCH_2$), 2.40–2.54 (m, 1H, S-CH), 2.84 (dd, J = 4.2, 13.8 Hz, 1H, 18-H), 3.01–3.20 (m, 3H, OH and SCH_2), 3.55 (dd, J = 6.2, 8.5 Hz, 1H, 3-H), 4.02 (t, J = 5.85 Hz, 2H, OCH_2), 4.07 (t, J = 6.18 Hz, 2H, OCH_2), 5.26 (d, J = 3.5 Hz, 1H, 12-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.1, 18.4, 23.1, 23.5, 23.7, 24.8, 25.4, 25.5, 26.0, 27.3, 27.7, 28.2, 28.9, 30.8, 32.6, 32.9, 33.2, 34.0, 34.2, 34.7, 37.1, 38.6, 38.8, 39.4, 40.3, 41.4, 41.8, 46.0, 46.8, 47.7, 55.3, 56.4, 63.7, 64.0, 79.1, 122.5, 143.9, 173.6, 177.8. HRMS (ESI) *m/z*: $[M+Cl]^+$ calcd for $C_{42}H_{68}ClO_5S_2$ 751.4197; found 751.4216.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)hexyl]-3β-hydroxyolean-12-en-28-oate (10c) Prepared from **7c** (Cheng et al. 2016a, 2016b) (0.50 g, 0.81 mmol) and (*R*)-lipoic acid (182.60 mg, 0.81 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.580 g, 96.0%; faint yellow solid, m.p. 45–47 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.73, 0.78, 0.89, 0.90, 0.92, 0.99 and 1.13 (7 s, each 3H, 7 × CH_3), 0.66–1.77 (m, 38H), 2.31 (t, J = 7.4 Hz, 2H, $COCH_2$), 2.44 (m, 1H, S-CH), 2.86 (dd, J = 4.1, 13.8 Hz, 1H, 18-H), 3.17 (m, 3H, OH and SCH_2), 3.57 (dd, J = 6.5, 8.0 Hz, 1H, 3-H), 4.01 (t, J = 6.48 Hz, 2H, OCH_2), 4.06 (t, J = 6.68 Hz, 2H, OCH_2), 5.27 (s, 1H, 12-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.5, 15.7, 17.2, 18.5, 23.2, 23.6, 23.8, 24.9, 25.8, 26.0, 27.4, 27.8, 28.3, 28.7, 28.9, 30.9, 32.6, 32.9, 33.3, 34.1, 34.2, 34.8, 37.2, 38.6, 38.9, 39.5, 40.4, 41.5, 41.9, 46.1, 46.8, 47.8, 55.4, 56.5, 64.2, 64.5, 79.2, 122.5, 144.0, 173.7, 177.9. HRMS (APCI) *m/z*: $[M+Cl]^+$ calcd for $C_{44}H_{72}O_5ClS_2$ 779.4510; found 779.4518.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)octyl]-3β-hydroxyolean-12-en-28-oate (10d) Prepared from **7d** (Cheng et al. 2016a, 2016b) (0.50 g, 0.77 mmol) and (*R*)-lipoic acid (158.87 mg, 0.77 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.467 g, 78.0%; faint yellow solid. m.p. 50–52 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.71, 0.75, 0.89, 0.96 and 1.10 (5 s, each 3H, 5 × CH_3), 0.88 (s, 6H, 2 × CH_3), 0.63–1.73 (m, 42H), 2.28 (t, J = 7.5 Hz, 2H, $COCH_2$), 2.43 (m, 1H, S-CH), 2.84 (dd, J = 3.8, 13.7 Hz, 1H, 18-H), 3.03–3.34 (m, 3H, OH and SCH_2), 3.54 (dd, J = 6.6, 7.8 Hz, 1H, 3-H), 3.98 (t, J = 6.27 Hz, 2H, OCH_2), 4.03 (t, J = 6.74 Hz, 2H, OCH_2), 5.26 (d, J = 11.9 Hz, 1H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.1, 18.4, 23.1, 23.5, 23.7, 24.8, 25.9, 26.1, 27.3, 27.7, 28.2, 28.7, 28.8, 29.2, 29.3, 30.8, 32.6, 32.8, 33.2, 34.1, 34.7, 37.1, 38.5, 38.8, 39.4, 40.3, 41.4, 41.8, 46.0, 46.7, 47.7, 55.3, 56.4, 64.3, 64.5, 79.0, 122.4, 143.9, 173.6, 177.8. HRMS (APCI) *m/z*: $[M+Cl]^+$ calcd for $C_{46}H_{76}O_5ClS_2$ 807.4823; found 807.4813.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)hexyl]-3β-hydroxyurs-12-en-28-oate (11a) Prepared from **8a** (Mallavadhani et al. 2013; Huang et al. 2018) (0.50 g, 0.81 mmol) and (*R*)-lipoic acid (182.60 mg, 0.81 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3: 1). Yield: 0.567 g, 94.0%; faint yellow solid, m.p. 62–64 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.73, 0.76, 0.89, 0.93, 0.97 and 1.06 (6 s, each 3H, 6 × CH_3), 0.85(d, J = 6.2 Hz, 3H, CH_3), 0.63–2.05 (m, 38H), 2.21 (d, J = 11.2 Hz, 1H, 18-H), 2.29 (t, J = 7.4 Hz, 2H, $COCH_2$), 2.44 (m, 1H, S-CH), 3.04–3.23 (m, 3H, OH and SCH_2), 3.55 (dd, J = 6.5, 8.0 Hz, 1H, 3-H), 3.98 (t, J = 6.78 Hz, 2H, OCH_2), 4.05 (t, J = 6.54 Hz, 2H, OCH_2), 5.21 (s, 1H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.5, 15.7, 17.1, 17.2, 18.4, 21.2, 23.3, 23.6, 24.3, 24.7, 25.7, 25.8, 27.3, 28.0, 28.2, 28.5, 28.6, 28.8, 30.7, 33.1, 34.1, 34.6, 36.8, 37.0, 38.5, 38.7, 38.8, 38.9, 39.1, 39.6, 40.3, 42.1, 47.6, 48.1, 52.9, 55.3, 56.4, 64.1, 64.3, 79.0, 125.6, 138.3, 173.6, 177.6. HRMS (APCI) *m/z*: $[M+H]^+$ calcd for $C_{44}H_{73}O_5S_2$ 745.4899; found 745.4876.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)octyl]-3β-hydroxyurs-12-en-28-oate (11b) Prepared from **8b** (Mallavadhani et al. 2013; Huang et al. 2018) (0.50 g, 0.77 mmol) and (*R*)-lipoic acid (158.87 mg, 0.77 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3: 1). Yield: 0.222 g, 37.0%; faint yellow solid, m.p. 49–51 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.75, 0.78, 0.91, 0.94, 0.99 and 1.08 (6 s, each 3H, 6 × CH_3), 0.85(d, J = 6.4 Hz, 3H, CH_3), 0.64–2.05 (m, 42H), 2.23 (d, J = 11.3 Hz, 1H, 18-H), 2.31 (t, J = 7.4 Hz, 2H, $COCH_2$), 2.46 (m, 1H, S-CH), 3.06–3.26 (m, 3H, OH and SCH_2), 3.56 (dd, J = 6.4, 8.1 Hz, 1H, 3-H), 3.97 (t, J = 6.56 Hz, 2H, OCH_2), 4.06 (t, J = 6.76 Hz, 2H, OCH_2), 5.23 (s, 1H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.5, 15.7, 17.0, 17.1, 18.3, 21.2, 23.3, 23.5, 24.2, 24.7, 25.9, 26.0, 27.2, 28.0, 28.2, 28.6, 28.8, 29.1, 30.7, 33.1, 34.1, 34.6, 36.7, 37.0, 38.5, 38.6, 38.7, 38.9, 39.1, 39.6, 40.2, 42.0, 47.5, 48.0, 52.9, 55.2, 56.3, 64.2, 64.4, 78.9, 125.5, 138.2, 173.5, 177.6. HRMS (APCI) *m/z*: $[M+H]^+$ calcd for $C_{46}H_{77}O_5S_2$ 773.5212; found 773.5183.

[2-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)ethyl]-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (12a) Prepared from **9a** (Yang et al., 2017) (0.25 g, 0.43 mmol) and (*R*)-lipoic acid (88.58 mg, 0.43 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 2:1). Yield: 0.231 g, 76.0%; faint yellow solid, m.p. 64–66 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.77, 0.78, 0.97, 1.09, 1.10, 1.13 and 1.34 (7 s, each 3H, 7 × CH_3), 0.60–2.14 (m, 27H), 2.31 (s, 1H, 9-H), 2.34 (t, J = 7.5 Hz, 2H, $COCH_2$), 2.44 (m, 1H, S-CH), 2.75 (m, 1H, 18-H), 2.98–3.25 (m, 3H, OH and SCH_2), 3.52 (m, 1H, 3-H), 4.18–4.41 (m, 4H, 2 × OCH_2), 5.62 (s, 1H, 12-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.3, 15.7, 16.4, 17.6, 18.8, 23.5, 24.6, 26.5, 27.3, 28.2, 28.3, 28.7, 28.8, 31.2, 31.9, 32.8, 34.0, 34.6, 37.2, 37.8, 38.6, 39.2, 40.3, 41.1, 43.3, 44.1, 45.5, 48.4, 55.0, 56.4, 61.9, 62.3, 78.8, 128.5, 169.2, 173.3, 176.2, 200.1. HRMS (ESI) *m/z*: $[M+Cl]^+$ calcd for $C_{40}H_{62}ClO_6S_2$ 737.3676; found 737.3696.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)butyl]-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (12b) Prepared from **9b** (Shen et al. 2008) (0.25 g, 0.41 mmol) and (*R*)-lipoic acid (88.12 mg, 0.41 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 2:1). Yield: 0.190 g, 63.0%; faint yellow solid, m.p. 60–62 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.78 (s, 6H, 2 × CH_3), 0.98, 1.09, 1.10, 1.13 and 1.34 (5 s, each 3H, 5 × CH_3), 0.61–2.15 (m, 29H), 2.30 (t, J = 7.0 Hz, 2H, $COCH_2$), 2.31 (s, 1H, 9-H), 2.45 (m, 1H, S-CH), 2.75 (dd, J = 3.3, 13.3 Hz, 1H, 18-H), 3.02–3.21 (m, 3H, OH and SCH_2), 3.54 (m, 1H, 3-H), 4.09 (m, 4H, 2 × OCH_2), 5.60 (s, 1H, 12-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.7, 16.5, 17.6, 18.8, 23.5, 24.7, 25.5, 25.6, 26.5, 26.6, 27.4, 28.2, 28.5, 28.7, 28.8, 31.2, 31.9, 32.8, 34.1, 34.7, 37.2, 37.8, 38.6, 39.2, 40.3, 41.1, 43.3, 44.1, 45.5, 48.5, 55.0, 56.4, 61.9, 63.9, 78.8, 128.6, 169.3, 173.6, 176.5, 200.2. HRMS (ESI) *m/z*: $[M+Cl]^+$ calcd for $C_{42}H_{66}ClO_6S_2$ 765.3989; found 765.4008.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)hexyl]-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (12c) Prepared from **9c** (Huang et al. 2018) (0.50 g, 0.79 mmol) and (*R*)-lipoic acid (163.0 mg, 0.79 mmol) according to general procedure I. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.402 g, 67.0%; faint yellow solid, m.p. 51–53 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.77 (s, 6H, 2 × CH_3), 0.97, 1.09, 1.10, 1.11 and 1.35 (5 s, each 3H, 5 × CH_3), 0.58–2.12 (m, 33H), 2.28 (t, J = 7.4 Hz, 2H, $COCH_2$), 2.31(s, 1H, 9-H), 2.42 (m, 1H, S-CH), 2.75 (m, 1H, 18-H), 3.03–3.25 (m, 3H, OH and SCH_2), 3.54 (m, 1H, 3-H), 4.05 (m, 4H, 2 × OCH_2), 5.60 (s, 1H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.7, 16.4, 17.6, 18.8, 23.5, 24.8, 25.6, 25.8, 26.5, 27.4, 28.2, 28.6, 31.2, 31.9, 32.8, 34.2, 34.7, 37.2, 37.8, 38.5, 39.2, 40.3, 41.2, 43.3, 44.1, 45.5, 48.5, 55.0, 61.9, 64.4, 78.8, 128.6, 169.3, 173.6, 176.5, 200.2. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{44}H_{71}O_6S_2$ 759.4692; found 759.4696.

[*n*-((5-((*R*)-1,2-Dithiolan-3-yl)pentanoyl)oxy)octyl]-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (12d) Prepared from **9d** (Huang et al. 2018) (0.50 g, 0.76 mmol) and (*R*)-lipoic acid (156.81 mg, 0.76 mmol) according to general procedure I. The residue wad purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.475 g, 80.0%; faint yellow solid, m.p. 52–54 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.77 (s, 6H, 2 × CH_3), 0.96, 1.08, 1.09, 1.10 and 1.38 (5 s, each 3H, 5 × CH_3), 0.60–2.10 (m, 37H), 2.27 (t, J = 7.4 Hz, $COCH_2$), 2.30 (s, 1H, 9-H), 2.42 (m, 1H, S-CH), 2.74 (m, 1H, 18-H), 3.27 (m, 3H, OH and SCH_2), 3.53 (m,1H, 3-H), 3.97–4.13 (m, 4H, 2 × OCH_2), 5.60 (s, 1H, H-12). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.7, 16.4, 17.5, 18.7, 23.5, 24.8, 25.9, 26.5, 27.4, 28.2, 28.5, 28.6, 28.7, 28.8, 29.1, 31.2, 31.9, 32.8, 34.1, 34.6, 37.1, 37.8, 38.5, 39.2, 40.2, 41.1, 43.3, 44.0, 45.4, 48.4, 55.0, 56.4, 61.9, 64.5, 78.7, 128.5, 169.3, 173.6, 176.5, 200.2. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{46}H_{75}O_6S_2$ 787.5005; found 787.5019.

General procedure II for *O*-alkylation reaction from TBZ to afford compounds 13a–15b

To a solution of **7c/7d/8a/8b/9c/9d** (0.40 mmol, 1 equiv.) in DMF (5 mL) were added K_2CO_3 (56 mg, 0.40 mmol, 1 equiv.) and TBZ (61.79 mg, 0.40 mmol, 1 equiv.). After stirring at 35 °C for 12 h, the mixture was diluted with EtOAc (50 mL). The organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography.

[*n*-(4-Carbamothioylphenoxy)hexyl]-3*β*-hydroxyolean-12-en-28-oate (13a) Prepared from **7c** (Cheng et al. 2016a, 2016b) (0.25 g, 0.40 mmol) and TBZ (61.79 mg, 0.40 mmol) according to general procedure II. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.100 g, 36.0%; faint yellow solid, m.p. 83–85 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.71, 0.75, 0.86, 0.88, 0.90, 0.96 and 1.11 (7 s, each 3H, 7 × CH_3), 0.67–2.05 (m, 30H), 2.85 (dd, J = 4.2, 13.8 Hz, 1H, 18-H), 3.19 (dd, J = 4.5, 11.3 Hz, 1H, 3-H), 4.00 (m, 4H, 2 × OCH_2), 5.25 (t, J = 3.4 Hz, 12-H), 6.85 (d, J = 8.9 Hz, 2H, Ar–H), 7.33 (s, 1H, NH), 7.75 (s, 1H, NH), 7.88 (d, J = 8.9 Hz, 2H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.2, 18.5, 23.2, 23.6, 23.8, 25.8, 26.0, 27.3, 27.8, 28.3, 28.7, 29.2, 30.8, 32.6, 32.9, 33.2, 34.0, 37.2, 38.6, 38.9, 39.5, 41.5, 41.9, 46.0, 46.8, 47.7, 55.3,

64.2, 68.3, 79.1, 114.2, 122.5, 129.2, 131.2, 144.0, 162.6, 178.0, 201.5. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{43}H_{65}NNaO_4S$ 714.4532; found 714.4538.

[*n*-(4-Carbamothioylphenoxy)octyl]-3*β*-hydroxyolean-12-en-28-oate (13b) Prepared from **7d** (Cheng et al. 2016a, 2016b) (0.25 g, 0.39 mmol) and TBZ (59.12 mg, 0.39 mmol) according to general procedure II. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.110 g, 40.0%; faint yellow solid, m.p. 84–86 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.70, 0.73, 0.85, 0.87, 0.89, 0.94 and 1.10 (7 s, each 3H, 7 × CH_3), 0.65–2.03 (m, 34H), 2.83 (dd, *J* = 4.2, 13.7 Hz, 1H, 18-H), 3.18 (dd, *J* = 4.5, 11.3 Hz, 1H, 3-H), 3.96 (m, 4H, 2 × OCH_2), 5.24 (t, *J* = 3.5 Hz, 12-H), 6.83 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.54 (s, 1H, NH), 7.87 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.89 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.1, 18.4, 23.1, 23.7, 25.9, 26.1, 27.2, 27.7, 28.2, 28.6, 29.1, 29.3, 30.7, 32.6, 32.8, 33.2, 33.9, 37.1, 38.8, 39.4, 41.4, 41.7,45.9, 46.8, 47.6, 55.2, 64.3, 68.3, 79.0, 114.0, 122.4, 129.3, 131.1, 143.9, 162.5, 178.0, 201.2. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{45}H_{68}NO_4S$ 718.4869; found 718.4880.

[*n*-(4-Carbamothioylphenoxy)hexyl]-3*β*-hydroxyurs-12-en-28-oate (14a) Prepared from **8a** (Mallavadhani et al. 2013; Huang et al. 2018) (0.25 g, 0.40 mmol) and TBZ (61.20 mg, 0.40 mmol) according to general procedure II. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.102 g, 37.0%; faint yellow solid, m.p. 79–81 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.72, 0.73, 0.86, 0.95 and 1.04 (5 s, each 3H, 5 × CH_3), 0.83 (d, *J* = 6.4 Hz, 3H, CH_3), 0.91 (d, *J* = 6.1 Hz, 3H, CH_3), 0.64–2.04 (m, 30H), 2.19 (d, *J* = 11.2 Hz, 1H, 18-H), 3.18 (dd, *J* = 4.3, 11.4 Hz, 1H, 3-H), 3.97 (m, 4H, 2 × OCH_2), 5.20 (t, *J* = 3.3 Hz, 1H, 12-H), 6.83 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.49 (s, 1H, NH), 7.86 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.87 (s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 14.2, 15.5, 15.8, 17.1, 17.2, 18.4, 21.2, 23.3, 23.6, 24.3, 25.7, 25.9, 27.2, 28.0, 28.2, 28.5, 29.3, 30.7, 33.1, 36.8, 37.0, 38.7, 38.8, 39.1, 39.6, 42.1, 47.5, 48.1, 52.9, 55.2, 60.5, 64.2, 68.2, 79.1, 114.0, 125.6, 129.3, 131.1, 138.3, 162.5, 177.8, 201.2. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{43}H_{64}NO_4S$ 690.4556; found 690.4571.

[*n*-(4-Carbamothioylphenoxy)octyl]-3*β*-hydroxyurs-12-en-28-oate (14b) Prepared from **8b** (Mallavadhani et al. 2013; Huang et al. 2018) (0.25 g, 0.38 mmol) and TBZ (58.14 mg, 0.38 mmol) according to general procedure II. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.069 g, 24.0%; faint yellow solid, m.p. 83–85 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.72, 0.74, 0.87, 0.96 and 1.05 (5 s, each 3H, 5 × CH_3), 0.84 (d, *J* = 6.4 Hz, 3H, CH_3), 0.92 (d, *J* = 6.2 Hz, 3H, CH_3), 0.65–2.04 (m, 34H), 2.20 (d, *J* = 11.2 Hz, 1H, 18-H), 3.03–3.20 (dd, *J* = 4,5, 11.2 Hz, 1H, 3-H), 3.82–4.10 (m, 4H, 2 × OCH_2), 5.21 (t, *J* = 3.4 Hz, 1H, 12-H), 6.85 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.45 (s, 1H, NH), 7.81 (s, 1H, NH), 7.88 (d, *J* = 8.9 Hz, 2H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.4, 15.6, 17.0, 17.1, 18.3, 21.1, 23.2, 23.5, 24.2, 25.8, 25.9, 27.1, 27.9, 28.1, 28.5, 29.00, 29.07, 29.2, 30.6, 33.0, 36.7, 36.9, 38.6, 38.8, 39.0, 39.5, 42.0, 47.5, 48.0, 52.8, 55.1, 64.1, 68.2, 79.0, 113.9, 125.4, 129.1, 131.0, 138.2, 162.4, 177.7, 201.2. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{45}H_{68}NO_4S$ 718.4869; found 718.4881.

[*n*-(4-Carbamothioylphenoxy)hexyl]-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (15a) Prepared from **9c** (Huang et al. 2018) (0.25 g, 0.39 mmol) and TBZ (60.35 mg, 0.39 mmol) according to general procedure II. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.091 g, 33.0%; faint yellow solid, m.p. 119–120 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 0.67 and 1.00 (2 s, each 6H, 4 × CH_3), 0.72, 0.89 and 1.08 (3 s, each 3H, 3 × CH_3), 0.63–2.18 (m, 27H), 2.30 (s, 1H, 9-H), 2.55 (m, 1H, 18-H), 2.98 (dd, *J* = 4.5, 11.5 Hz, 1H, 3-H), 3.96–4.02 (m, 4H, 2 × OCH_2), 5.40 (s, 1H, 12-H), 6.99 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.48 (d, *J* = 8.8 Hz, 2H, Ar–H), 8.23 (s, 1H, NH), 9.30 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 16.1, 16.2, 17.2, 18.4, 22.2, 23.0, 25.4, 25.6, 25.8, 26.1, 27.0, 27.8, 28.2, 28.3, 28.56, 28.59, 28.7, 29.1, 30.4, 31.6, 32.1, 36.7, 37.4, 42.9, 43.6, 44.9, 48.1, 54.1, 61.2, 63.9, 67.8, 76.6, 115.0, 120.9, 126.4, 134.2, 161.6, 169.5, 175.9, 191.7, 199.1. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{43}H_{64}NO_5S$ 706.4505; found 706.4500.

[*n*-(4-Carbamothioylphenoxy)octyl]-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (15b) Prepared from **9d** (Huang et al. 2018) (0.25 g, 0.38 mmol) and TBZ (57.80 mg, 0.38 mmol) according to general procedure II. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.107 g, 39.0%; faint yellow solid, m.p. 117–119 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 0.68 and 1.02 (2 s, each 6H, 4 × CH_3), 0.73, 0.90 and 1.09 (3 s, each 3H, 3 × CH_3), 0.64–2.15 (m, 31H), 2.31 (s, 1H, 9-H), 2.58 (dd, *J* = 3.3, 10.1 Hz, 1H, 18-H), 3.01 (dd, *J* = 4.4, 11.4 Hz, 1H, 3-H), 4.01 (m, 4H, 2 × OCH_2), 5.42 (s, 1H, 12-H), 6.91 (d, *J* = 8.9 Hz, 2H, Ar–H), 7.94 (d, *J* = 8.9 Hz, 2H, Ar–H), 9.31 (s, 1H, NH), 9.63 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 14.4, 16.0, 16.2, 18.4, 20.8, 23.0, 25.2, 25.4, 25.8, 26.1, 27.0, 27.8, 28.2, 28.5, 30.4, 31.5, 32.1, 36.7, 37.4, 42.9, 43.6, 44.9, 48.1, 54.1, 59.8, 61.2, 63.9, 67.7, 76.6, 113.4, 127.4, 129.5, 131.1, 161.4, 169.4, 175.8, 198.5, 199.0. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{45}H_{68}NO_5S$ 734.4818; found 734.4816.

General procedure III for *O*-alkylation reaction from ADT-OH to afford compounds 16a–18b

To a solution of **7c/7d/8a/8b/9c/9d** (0.81 mmol, 1 equiv.) in DMF (5 mL) were added K_2CO_3 (335.85 mg, 2.43 mmol, 1 equiv.), KI (13.28 mg, 0.08 mmol, 0.1 equiv.) and ADT-OH (182.60 mg, 0.81 mmol, 1 equiv.). After stirring at 65 °C for 24 h, the mixture was diluted with EtOAc (50 mL). The organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography.

[*n*-(4-(3-Thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)hexyl]-3*β*-hydroxyolean-12-en-28-oate (16a) Prepared from **7c** (Cheng et al. 2016, ab) (0.50 g, 0.81 mmol) and ADT-OH (182.60 mg, 0.81 mmol) according to general procedure III. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.231 g, 35.0%; orange solid, m.p. 81–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.73, 0.76, 0.88, 0.89, 0.92, 0.98 and 1.13 (7 s, each 3H, 7 × CH_3), 0.65–2.10 (m, 30H), 2.87 (dd, J = 4.0, 13.7 Hz, 1H, 18-H), 3.21 (dd, J = 4.6, 11.1 Hz, 1H, 3-H), 4.03 (dd, J = 6.2, 11.4 Hz, 4H, 2 × OCH_2), 5.28 (t, J = 4.3 Hz, 1H, 12-H), 6.95 (d, J = 8.8 Hz, 2H, Ar–H), 7.39 (s, 1H, CH), 7.60 (d, J = 8.8 Hz, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.2, 18.5, 21.2, 23.2, 23.7, 25.7, 26.0, 27.3, 27.8, 28.3, 28.7, 29.2, 30.8, 32.7, 32.9, 33.2, 34.0, 37.2, 38.9, 39.5, 41.9, 46.0, 46.8, 47.7, 55.3, 64.2, 68.4, 79.1, 115.6, 122.5, 124.2, 128.7, 134.7, 144.0, 162.6, 173.2, 177.9, 215.3. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{45}H_{63}O_4S_3$ 763.3889; found 763.3878.

[*n*-(4-(3-Thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)octyl]-3*β*-hydroxyolean-12-en-28-oate (16b) Prepared from **7d** (Cheng et al. 2016a, 2016b) (0.50 g, 0.77 mmol) and ADT-OH (174.29 mg, 0.77 mmol) according to general procedure III. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.247 g, 40.0%; orange solid, m.p. 68–70 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.74, 0.77, 0.92, 0.98 and 1.13 (5 s, each 3H, 5 × CH_3), 0.89 (s, 6H, 2 × CH_3), 0.71–2.00 (m, 34H), 2.87 (dd, J = 3.4, 11.0 Hz, 1H, 18-H), 3.21 (dd, J = 3.6, 9.0 Hz, 1H, 3-H), 4.01 (t, J = 6.3 Hz, 4H, 2 × OCH_2), 5.27 (s, 1H, 12-H), 6.96 (d, J = 8.7 Hz, 2H, Ar–H), 7.39 (s, 1H, CH), 7.60 (d, J = 8.7 Hz, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.4, 15.7, 17.2, 18.5, 23.1, 23.5, 23.7, 26.0, 27.3, 27.8, 28.2, 28.7, 29.2, 29.4, 30.8, 32.6, 32.9, 33.2, 34.0, 37.1, 38.6, 38.9, 39.5, 41.4, 41.8, 46.0, 46.8, 47.7, 55.3, 64.3, 68.5, 79.1, 115.5, 122.4, 124.1, 128.7, 134.6, 144.0, 162.7, 173.2, 177.9, 215.2. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{47}H_{67}O_4S_3$ 791.4202; found 791.4193.

[*n*-(4-(3-Thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)hexyl]-3*β*-hydroxyurs-12-en-28-oate (17a) Prepared from **8a** (Mallavadhani et al. 2013; Huang et al. 2018) (0.50 g, 0.81 mmol) and ADT-OH (182.60 mg, 0.81 mmol) according to general procedure III. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.226 g, 37.0%; orange solid, m.p. 83–85 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.75, 0.77, 0.91, 0.94, 0.98 and 1.07 (6 s, each 3H, 6 × CH_3), 0.85 (d, J = 8.1 Hz, CH_3), 0.65–2.12 (m, 30H), 2.22 (d, J = 11.3 Hz, 1H, 18-H), 3.21 (dd, J = 4.4, 11.1 Hz, 1H, 3-H), 4.02 (m, 4H, 2 × OCH_2), 5.23 (t, J = 3.4 Hz, 1H, 12-H), 6.96 (d, J = 8.9 Hz, 2H, Ar–H), 7.39 (s, 1H, CH), 7.60 (d, J = 8.9 Hz, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.5, 15.7, 17.1, 17.2, 18.4, 21.3, 23.4, 23.6, 24.3, 25.7, 25.9, 27.3, 28.1, 28.2, 29.1, 30.8, 33.2, 36.8, 37.0, 38.7, 38.8, 39.2, 39.6, 42.2, 47.6, 48.5, 53.0, 55.3, 64.1, 68.3, 79.0, 115.5, 124.1, 125.6, 128.7, 134.6, 138.3, 162.6, 173.2, 177.7, 215.1. HRMS (APCI) *m/z*: $[M+H]^+$ calcd for $C_{45}H_{65}O_4S_3$ 765.4045; found 765.4009.

[*n*-(4-(3-Thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)octyl]-3*β*-hydroxyurs-12-en-28-oate (17b) Prepared from **8b** (Mallavadhani et al. 2013; Huang et al. 2018) (0.50 g, 0.77 mmol) and ADT-OH (174.97 mg, 0.77 mmol) according to general procedure III. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.098 g, 16.0%; orange solid, m.p. 64–66 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.74, 0.75, 0.89, 0.92, 0.97 and 1.06 (6 s, each 3H, 6 × CH_3), 0.84 (d, J = 6.4 Hz, 3H, CH_3), 0.66–2.11 (m, 34H), 2.21 (d, J = 11.3 Hz, 1H, 18-H), 3.20 (dd, J = 4.6, 11.1 Hz, 1H, 3-H), 3.89–4.06 (m, 4H, 2 × OCH_2), 5.22 (t, J = 3.4 Hz, 1H, 12-H), 6.94 (d, J = 8.8 Hz, 2H, Ar–H), 7.38 (s, 1H, CH), 7.59 (d, J = 8.7 Hz, 2H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 15.6, 15.7, 17.1, 17.2, 18.4, 21.3, 23.4, 23.6, 24.3, 26.0, 26.1, 27.3, 28.1, 28.2, 28.6, 29.1, 29.2, 29.4, 30.8, 33.2, 36.8, 37.1, 38.7, 38.8, 39.0, 39.2, 39.7, 42.2, 47.6, 48.2, 53.0, 55.3, 64.3, 68.5, 79.1, 115.5, 124.0, 125.6, 128.7, 134.6, 138.3, 162.7, 173.2, 177.7, 215.1. HRMS (APCI) *m/z*: $[M+H]^+$ calcd for $C_{47}H_{69}O_4S_3$ 793.4358; found 793.4322.

[*n*-(4-(3-Thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)hexyl-3*β*-hydroxy-11-oxo-olean-12-en-29-oate (18a) Prepared from **9c** (Huang et al. 2018) (0.50 g, 0.79 mmol) and ADT-OH (178.57 mg, 0.79 mmol) according to general procedure III. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.126 g, 21.0%; orange solid, m.p. 92–94 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80, 0.81, 1.00, 1.12, 1.13 1.14 and 1.36 (7 s, each 3H, 7 × CH_3), 0.69–2.13 (m, 27H), 2.34 (s, 1H, 9-H), 2.79 (m, 1H, 18-H), 3.23 (dd, J = 5.3, 10.9 Hz, 1H, 3-H), 4.04 (t,

$J = 6.4$ Hz, 4H, 2 × OCH_2), 5.63 (s, 1H, 12-H), 6.96 (d, $J =$ 8.8 Hz, 2H, Ar–H), 7.39 (s, 1H, CH), 7.60 (d, $J = 8.8$ Hz, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.7, 16.5, 17.6, 18.8, 23.5, 25.7, 25.9, 26.51, 26.54, 27.4, 28.2, 28.5, 28.7, 28.8, 29.0, 31.2, 31.9, 32.9, 37.2, 37.8, 39.2, 41.2, 43.3, 44.1, 45.5, 48.6, 55.0, 61.9, 64.4, 68.3, 78.8, 115.6, 124.0, 128.6, 128.7, 134.6, 162.6, 169.5, 173.3, 176.6, 200.3, 215.1. HRMS (APCI) m/z: $[M+H]^+$ calcd for $C_{45}H_{63}O_5S_3$ 779.3838; found 779.3820.

[*n*-(4-(3-Thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)octyl]-3β-hydroxy-11-oxo-olean-12-en-29-oate (18b) Prepared from **9d** (Huang et al. 2018) (0.50 g, 0.76 mmol) and ADT-OH (169.74 mg, 0.76 mmol) according to general procedure III. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.118 g, 19.0%; orange solid, m.p. 87–89 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 6H, 2 × CH_3), 1.00, 1.12, 1.13, 1.14 and 1.37 (5 s, each 3H, 5 × CH_3), 0.64–2.17 (m, 31H), 2.34 (s, 1H, 9-H), 2.78 (m, 1H, 18-H), 3.22 (dd, $J = 5.3$, 10.7 Hz, 1H, 3-H), 3.98–4.14 (m, 4H, 2 × OCH_2), 5.64 (s, 1H, 12-H), 6.96 (d, $J = 8.8$ Hz, 2H, Ar–H), 7.39 (s, 1H, CH), 7.60 (d, $J = 8.8$ Hz, 2H, Ar–H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.7, 16.5, 17.6, 18.8, 23.5, 25.96, 26.03, 26.5, 26.6, 27.4, 28.2, 28.5, 28.7, 29.1, 29.2, 29.3, 31.3, 31.9, 32.9, 37.2, 37.9, 39.2, 41.2, 43.3, 44.1, 45.5, 48.5, 55.1, 61.9, 64.6, 68.5, 78.8, 115.6, 124.0, 128.6, 128.7, 134.6, 162.7, 169.4, 173.3, 176.6, 200.3, 215.2. HRMS (APCI) *m/z*: $[M+H]^+$ calcd for $C_{47}H_{67}O_5S_3$ 807.4151; found 807.4118.

General procedure IV for esterification from (*R*)-lipoic acid to afford com°pounds 19–21

To a solution of OA, UA or GA (1.09 mmol) in dry CH_2Cl_2 (5 mL) were added DCC (674.70 mg, 3.27 mmol), DMAP (66.58 mg, 0.55 mmol) and (*R*)-lipoic acid (224.90 mg, 1.09 mmol). After stirring at room temperature overnight, the mixture was diluted with EtOAc (50 mL) and washed by 1 N HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography.

3β-[(*R*)-5-(1,2-Dithiolan-3-yl)valeryl]oxyolean-12-en-28-oic acid (19) Prepared from OA (0.50 g, 1.09 mmol) and (*R*)-lipoic acid (224.90 mg, 1.09 mmol) according to general procedure IV. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.194 g, 27.0%; white solid, m.p. 138–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.75, 1.13 (2 s, each 3H, 2 × CH_3), 0.86 (s, 6H, 2 × CH_3), 0.92 (s, 9H, 3 × CH_3), 0.75–2.04 (m, 30-H), 2.32 (t, $J = 7.4$ Hz, 2H, $COCH_2$), 2.46 (m, 1H, S-CH), 2.82 (d, $J = 10.3$ Hz, 1H, 18-H), 3.14 (m, 2H, 3-H and S-CH), 3.56 (m, 1H, S-CH), 4.50 (t, $J =$ 7.9 Hz, 1H), 5.28 (t, $J = 6.4$ Hz, 1H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.5, 16.9, 17.3, 18.3, 23.1, 23.6, 23.8, 25.0, 26.0, 27.1, 27.8, 28.3, 29.0, 30.8, 32.6, 33.2, 34.0, 34.8, 37.1, 37.9, 38.2, 38.6, 39.4, 40.4, 41.1, 41.7, 41.9, 46.0, 46.7, 47.7, 48.5, 55.5, 56.5, 80.9, 122.7, 143.8, 173.4, 183.5. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{38}H_{59}O_4S_2$, 643.3855; found 643.3867.

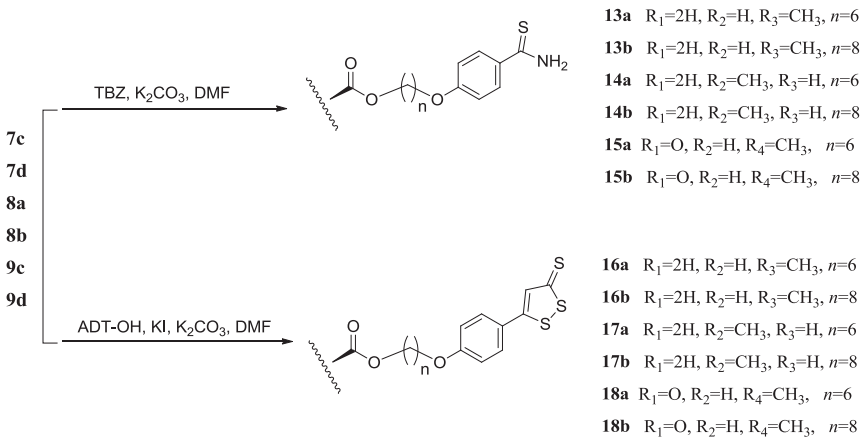
3β-[(*R*)-5-(1,2-Dithiolan-3-yl)valeryl]oxyurs-12-en-28-oic acid (20) Prepared from UA (0.50 g, 1.09 mmol) and (*R*)-lipoic acid (224.90 mg, 1.09 mmol) according to general procedure IV. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.196 g, 28.0%; white solid, mp 208–210 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.77, 0.86, 0.96, 1.08 and 1.43 (5 s, each 3H, 5 × CH_3), 0.85 (s, 6H, 2 × CH_3), 0.67–2.08 (m, 30H), 2.18 (d, $J = 11.1$ Hz, 1H, 18-H), 2.32 (t, $J =$ 7.4 Hz, 2H, $COCH_2$), 2.45 (m, 1H,), 3.15 (m, 2H), 3.57 (m, 1H, 3-H), 4.50 (t, $J = 7.9$ Hz, 1H), 5.24 (s, 1H, 12-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 15.7, 17.0, 17.3, 18.3, 21.3, 23.4, 23.7, 24.2, 25.0, 27.0, 28.3, 29.0, 30.8, 33.0, 34.8, 36.9, 37.1, 37.9, 38.4, 38.6, 39.0, 39.2, 39.7, 40.4, 42.1, 47.6, 48.1, 52.7, 55.5, 56.5, 81.0, 125.9, 138.1, 173.4, 183.2. HRMS (APCI) *m/z*: $[M-H]^+$ calcd for $C_{38}H_{59}O_4S_2$ 643.3855; found 643.3846.

3β-[(*R*)-5-(1,2-Dithiolan-3-yl)valeryl]oxy-11-oxo-olean-12-en-29-oic acid (21) Prepared from GA (0.50 g, 1.06 mmol) and (*R*)-lipoic acid (219.18 mg, 1.06 mmol) according to general procedure IV. The residue was purified by flash column chromatography (petroleum ether:EtOAc = 3:1). Yield: 0.118 g, 17.0%; white solid, mp 118–120 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.77, 0.84, 0.85, 1.09, 1.13, 1.25 and 1.32 (7 s, esch 3H, 7 × CH_3), 0.64–2.20(m, 27H), 2.29 (t, $J = 7.4$ Hz, 2H, $COCH_2$), 2.32 (s, 1H, 9-H), 2.43 (m, 1H), 2.76 (dd, $J = 3.4$, 10.2 Hz, 1H, 18-H), 3.12 (m, 2H), 3.58 (m, 1H, 3-H), 4.49 (dd, $J = 4.7$, 11.7 Hz, 1H), 5.73 (s, 1H, 12-H), 9.73 (s, 1H, 29-COOH). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 16.4, 16.8, 17.4, 18.7, 23.0, 23.6, 24.9, 26.2, 28.1, 28.7, 28.9, 30.9, 31.6, 32.1, 34.6, 36.9, 38.1, 38.5, 38.8, 40.2, 43.2, 43.9, 45.3, 47.0, 48.2, 50.3, 55.0, 55.7, 56.4, 61.6, 80.5, 128.6, 169.2, 173.3, 175.3, 199.9. HRMS (ESI) *m/z*: $[M-H]^+$ calcd for $C_{38}H_{57}O_5S_2$ 657.3647; found 657.3627.

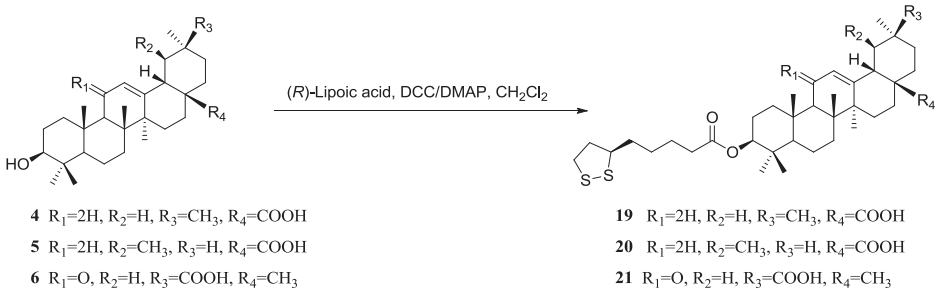
In vitro cytotoxicity

All tests were conducted at Nanjing Kaiji Biotechnology Development Co., Ltd. Using MTT assay (Van Meerloo et al. 2011): cells were seeded in 96-well plates and incubated in the CO_2 incubator at 37 °C. When the cells

Scheme 2 Synthesis of pentacyclic triterpenes-TBZ/ADT-OH derivatives, where *n* is the number of methylene groups



Scheme 3 Synthesis of derivatives of (*R*)-lipoic acid linked to pentacyclic triterpenes at 3-OH



adhered, compounds at different concentrations were added to every well. After incubation for another 72 h, 20 µL of MTT (5%) was added to each well and incubated for an additional 4 h. The viable cells were stained with MTT and scanned with an electrophotometer at 570 nm. Each concentration treatment was done in triplicate wells. The IC_{50} values were estimated by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation.

Results and discussion

Synthesis

As outlined in Schemes 1 and 2, ω-Bromine compounds **7a–9d** (Shen et al. 2008; Mallavadhani et al. 2013; Cheng et al. 2016a, b; Yang et al. 2017; Huang et al. 2018) were respectively treated with hydrogen sulfide donors (*R*-lipoic acid, TBZ or ADT-OH) under potassium carbonate to generate corresponding targeted compounds **10a–12d** (37.0–96.0%), **13a–15b** (24.0–40.0%) and **16a–18b** (16.0–40.0%). Compounds **19–21** were afforded from esterification of OA, UA and GA respectively with (*R*)-lipoic acid in the presence of DCC/DMAP at a very low yield (17.0–28.0%, Scheme 3). Targeted compounds have been fully characterized by ^{1}H- and ^{13}C-NMR spectroscopy and high resolution mass spectrometry.

Evaluation of antitumor activity

In vitro cytotoxicities of synthesized target compounds were evaluated by MTT assay (Van Meerloo et al. 2011) against human liver tumor cell line BEL-7402, chronic myelogenous leukemia cell line K562, K562 multidrug-resistant cell line K562/ADR and human normal liver cell line L-O2.

As shown in Table 1, the results revealed that, though the reported hydrogen sulfide donor molecules (Chattopadhyay et al. 2012a, b, c; Frantzias et al. 2012; Kodela et al. 2012) and oleanolic acid derivatives (Cheng et al. 2016, ab; Huang et al. 2018) on anti-tumor activity were inspiring, most of synthesized pentacyclic triterpenes-H_2S donor hybrids exhibited no anti-proliferation activity against tested cell lines (BEL-7402, K562, K562/ADR, and L-O2). Some compounds (**10a**, **10b**, **12a**, **12b**, **13a**, **13b**, **14a**, **15a**, and **15b**) showed moderate anti-proliferation activity against K562 cell line. Among them, only compound **14a** belongs to ursane-type pentacyclic triterpenes, it indicated that oleanane-type derivatives (**10a**, **10b**, **12a**, **12b**, **13a**, **13b**, **15a**, and **15b**) exhibited better anti-proliferation activity than ursane-type derivatives. And it also indicated that (*R*)-lipoic acid and TBZ groups were much more favorable than ADT-OH to the anti-proliferation activity (**10a**, **10b**, **12a**, **12b**, **13a**, **13b**, **15a**, and **15b**). Compared from compounds **10a–12d** and **19–21**, the results showed that C-3 OH was more favorable than C-28/29 COOH to the antitumor

activity of the batch of (*R*)-lipoic acid derivatives. Only 4 compounds (**12a**, **12b**, **13a**, and **15b**) exhibited IC_{50} about 55 μM. Furthermore, only compound **13a** exhibited moderate anti-proliferation activity against both K562 and K562/ADR cell lines, while it exhibited no anti-proliferation activity against BEL-7402 and L-O2 cell lines.

Conclusions

In this study, a total of 25 targeted hydrogen sulfide donor–pentacyclic triterpene hybrids were designed and synthesized on the basis of inspiring reports about hydrogen sulfide donor molecules and oleanolic acid derivatives on anti-tumor activity. The results revealed that only some hybrids (**10a**, **10b**, **12a**, **12b**, **13a**, and **15b**) showed moderate anti-proliferation activity against K562 cell line. It indicated that oleanane-type, (*R*)-lipoic acid and TBZ groups were much more favorable to the anti-proliferation activity. Furthermore, in the oleanane-type, C-3 OH was more beneficial than C-28/29 COOH to the antitumor activity of the batch of (*R*)-lipoic acid derivatives. And among them, only compound **13a** exhibited moderate anti-proliferation activity against both K562 and K562/ADR cell lines, while it exhibited no anti-proliferation activity against BEL-7402 and L-O2 cell lines. Therefore, in the present study, the hybrids didn't exhibit prospective anti-tumor activity, it could deny the study which built the base for the researches of hydrogen sulfide donors and pentacyclic triterpene hybrids. And this also suggested that it was not suitable for hybridization of hydrogen sulfide donors attached to OA, UA, and GA in the field of anti-tumor. It seems that there is a long way to go.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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