

Article

Semisynthesis and Biological Evaluation of Oleanolic Acid 3-*O*- β -D-Glucuronopyranoside Derivatives for Protecting H9c2 Cardiomyoblasts against H₂O₂-Induced Injury

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Abstract: A series of novel oleanolic acid 3-*O*- β -D-glucuronopyranoside derivatives have been designed and synthesized. Biological evaluation has indicated that some of the synthesized compounds exhibit moderate to good activity against H₂O₂-induced injury in rat myocardial cells (H9c2). Particularly, derivative 28-*N*-isobutyl ursolic amide 3-*O*- β -D-galactopyranoside (**8a**) exhibited a greater protective effect than the positive control oleanolic acid 3-*O*- β -D-glucuronopyranoside, indicating that it possesses a great potential for further development as a cardiovascular disease modulator by structural modification.

Keywords: oleanolic acid 3-*O*- β -D-glucuronopyranoside; semisynthesis; derivatives; H₂O₂-induced; protective effect

1. Introduction

Cardiovascular disease is among the leading causes of death worldwide. According to the World Health Organization, mortality from cardiovascular disease is expected to reach about 25 million by 2030 [1–3]. Despite the emergence of more and more listed drugs, the treatment of cardiovascular disease is still not optimistic [4–7]. Therefore, it is clinically necessary to research and develop more effective and safe agents for preventing and managing cardiovascular disease.

Aralia elata (Miq) Seem (AS), a kind of herbal medicine, has been used as a tonic, antiarrhythmic, anti-arthritic, antihypertensive, and anti-diabetic agent in traditional Chinese medicine [8,9]. The total saponins extracted from AS, which are found to be the main pharmacological active ingredients of AS, have been proven to exhibit anti-myocardial ischemic and anti-hypoxic activities, as well

as anti-oxidative, anti-inflammatory, and anti-apoptotic capacity [10–12]. Moreover, as the main components of *A. elata* Xinmaitong capsules (Clinical Trial Approval Number 2003L01111 by China Food and Drug Administration), AS was developed for the treatment of coronary heart disease and has successfully completed phase III clinical trials in China [13]. Oleanolic acid 3-*O*- β -D-glucuronopyranoside (**1**, Figure 1) is one of the major natural pentacyclic triterpenoid saponins isolated from AS [14,15]. We previously demonstrated that the triterpenoid saponins araloside C (**2**, Figure 1) and elatoside C (**3**, Figure 1) from AS can protect myocardial cells from ischemia/reperfusion (IR) injury and reduce I/R-induced oxidative stress and apoptosis in cardiomyoblasts [16–18]. We also reported that the proteomic profiling of oleanolic acid 3-*O*- β -D-glucuronopyranoside targets is associated with anti-apoptotic effects in endothelial cells by using biotinconjugated calenduloside E analogue (BCEA, **4**, Figure 1) [19]. However, there are no reports about the protective effects of oleanolic acid 3-*O*- β -D-glucuronopyranoside synthetic derivatives on cardiomyoblast damage.

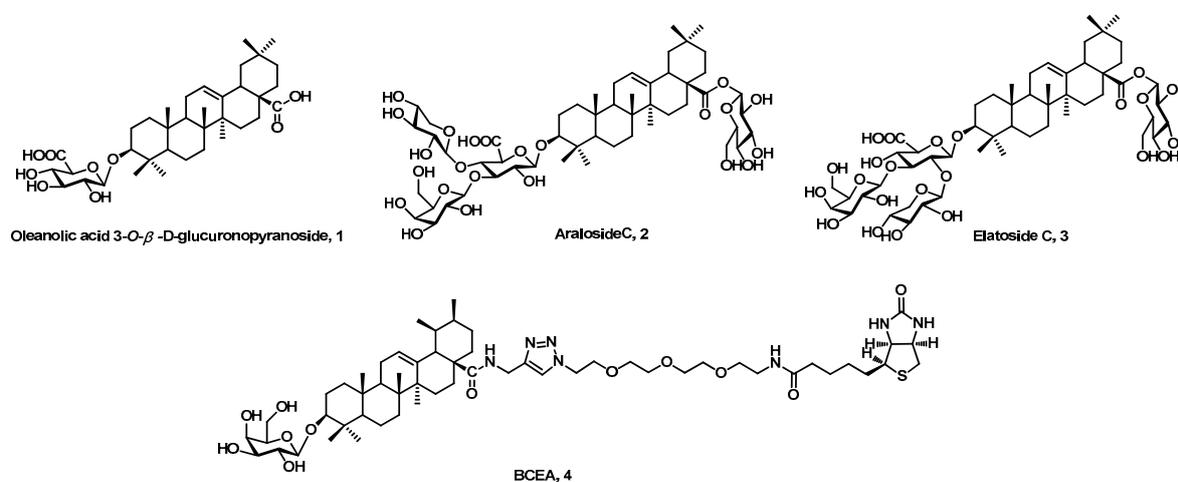


Figure 1. The structures of triterpenoid saponins from *Aralia elata* (Miq) Seem and the probe of oleanolic acid 3-*O*- β -D-glucuronopyranoside BCEA.

In this current study, we describe the preparation of a series of novel oleanolic acid 3-*O*- β -D-glucuronopyranoside derivatives with several amine moieties (isobutylamine, propynylamine, and 3,4-dimethoxybenzylamine) that are appended to the C-28 carboxyl group of the parental compound. Meanwhile, in order to extend the activity scaffold of oleanolic acid 3-*O*- β -D-glucuronopyranoside and improve its cardiovascular protective effects, we planned to change the aglycone moiety from oleanane to ursane, which is another natural pentacyclic triterpenoid that possesses biological activity. To further optimize the potency of oleanolic acid 3-*O*- β -D-glucuronopyranoside derivatives and to gain further insight into their structure–activity relationship, we also designed the analogues that derive from glycosylation at the C-3 position with D-glucose and D-galactose. For this purpose, herein we synthesized a series of novel oleanolic acid 3-*O*- β -D-glucuronopyranoside amide derivatives (**5a–c**, **6a–c**, **7a–c**, **8a–c**, Figure 2) and evaluated their protective activities as cardiovascular disease agents.

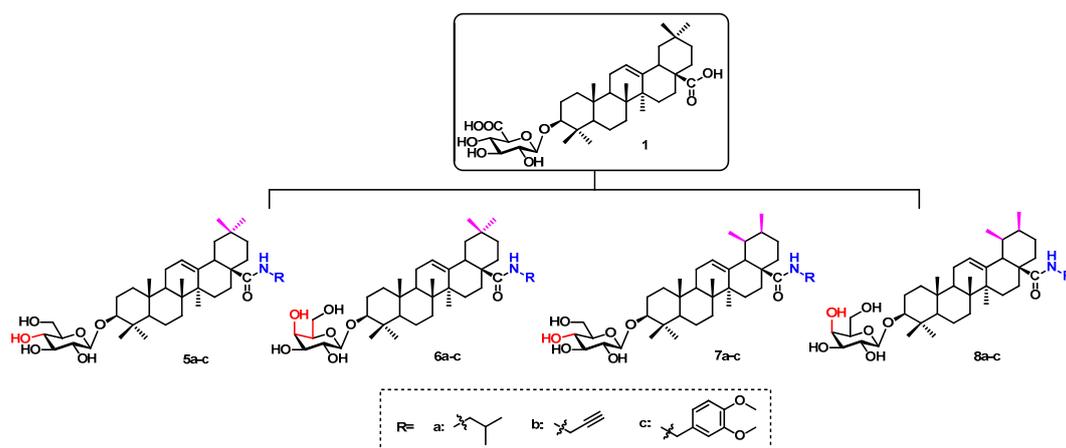
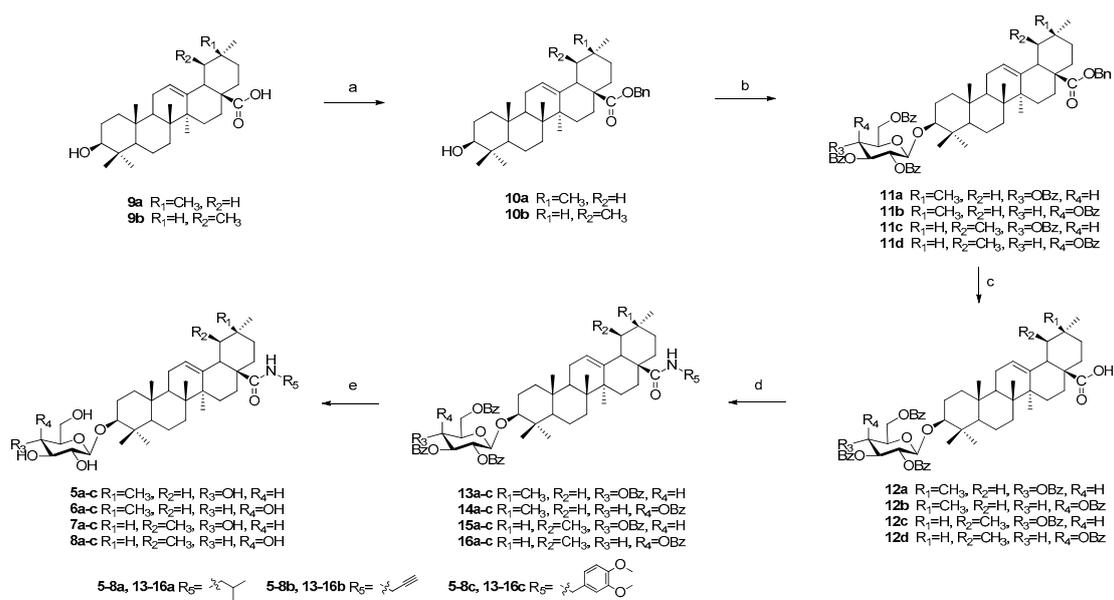


Figure 2. The structures of oleanolic acid 3-O- β -D-glucuronopyranoside derivatives.

2. Results and Discussion

2.1. Chemistry

The synthesis of derivatives **5a–c**, **6a–c**, **7a–c** and **8a–c** is outlined in Scheme 1. The naturally abundant oleanolic acid (**9a**) and ursolic acid (**9b**) were treated with benzyl bromide (BnBr), potassium carbonate solution (K_2CO_3), and tetrabutylammonium bromide (TBAB) in dry dichloromethane (DCM) to obtain **10a** and **10b**, respectively. Compounds **10a** and **10b** reacted with glucosyl donors and galactosyl donors in Lewis acid trimethylsilyl trifluoromethanesulfonate (TMSOTf) conditions to provide compounds **11a–d**, which were subjected to several hydrogenations to obtain compounds **12a–d** in the presence of catalytic amounts of 10% Pd–C at atmospheric pressure. Compounds **13a–c**, **14a–c**, **15a–c** and **16a–c** were attained via amidation with various amines of the C-28 carboxyl group of the saponin scaffold, and then followed by deprotection of the glycosyl groups in the presence of NaOMe/MeOH solution to gain compounds **5a–c**, **6a–c**, **7a–c** and **8a–c**. The above reaction conditions have also been described in our previous paper [19].



Scheme 1. The synthesis of target compounds. Reagents and conditions: (a) BnBr, K_2CO_3 , TBAB, DCM– H_2O , r.t., 18 h; (b) glycosyl donors, TMSOTf, 4 Å MS, DCM, r.t., 2–4 h; (c) H_2 , Pd–C (10%), EtOAc, reflux, 4–6 h; (d) HOBT, EDCl, R_5NH_2 , r.t., 4–16 h; (e) NaOMe, MeOH, r.t., 2–3 h.

2.2. Biological Results and Discussion

The protective effect of oleanolic acid 3-*O*- β -D-glucuronopyranoside and its derivatives against H₂O₂-induced cell injury was detected using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MTT results show that some compounds exhibited moderate to good protective effects against H₂O₂-induced injury in H9c2 cells. As shown in Figure 3, the preliminary test of compounds 5a–c, 6a–c, 7a–c and 8a–c at 0.1 μ g/mL revealed that 5a–b, 6a–b, 7a–b and 8a–b better increased the viability of H9c2 cells. Pretreatment of H9c2 cells with compounds 6–8a and 8b exhibited a better protective effect than oleanolic acid 3-*O*- β -D-glucuronopyranoside (OAGP). The survival rate increased from 49.69% (with H₂O₂ treatment alone) to 58.69%, 61.21%, 66.86% and 61.19% after pretreatment with 0.1 μ g/mL compounds 6–8a and 8b, respectively. Among them, analogue 8a exhibited a more potent cytoprotective effect than the others after pretreatment for 12 h, which suggests that 8a deserves further evaluation as a potential therapeutic agent for protection against H₂O₂-induced injury in H9c2 cells.

The preliminary structure activity relationships (SARs) suggest that amide derivatives of oleanolic acid 3-*O*- β -D-glucuronopyranoside containing ursane scaffolds were more potent than those containing oleanane scaffolds (e.g., compounds 7–8a vs. 5–6a). Beyond that, the compounds 5a–b, 6a–b, 7a–b and 8a–b with isobutylamine and propynylamine groups could increase cell viability after H₂O₂ treatment, compared to the control, indicating that the introduction of these aliphatic substituent groups could increase cell viability. However, compounds 5a–c, including the 3,4-dimethoxybenzylamine group, were inert, suggesting that the unsaturated aryl groups were the adverse substituent group in the derivatives. In addition, these above amide analogs possessed protective potency, indicating that the C-28 carboxyl group was not an essential group for protective activity, and the substituents on the amide nitrogen affected the cell viability obviously.

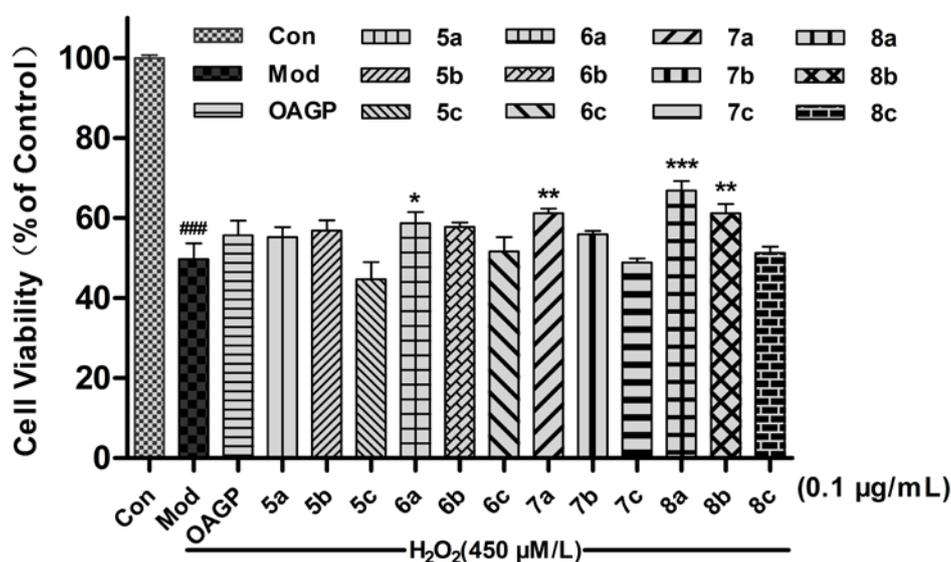


Figure 3. Effects of oleanolic acid 3-*O*- β -D-glucuronopyranoside derivatives on H₂O₂-induced cell damage in H9c2 cells. H9c2 cardiomyoblasts were pre-incubated with a 0.1 μ g/mL concentration of derivatives (5a–c, 6a–c, 7a–c and 8a–c) for 12 h and then exposed to H₂O₂ (450 μ mol/L) for 1 h. The data are expressed as the mean \pm S.D. from three independent experiments. ### p < 0.001 versus control group; * p < 0.05 versus H₂O₂-treated cells; ** p < 0.01 versus H₂O₂-treated cells; *** p < 0.001 versus H₂O₂-treated cells.

3. Experimental Section

3.1. General Information

All the reagents were used without further purification unless otherwise specified. Solvents were dried and redistilled in the usual manner prior to use. Analytical TLC was performed using silica gel HF254. Preparative column chromatography was performed with silica gel H. ^1H - and ^{13}C -NMR spectra were recorded on a Bruker Advance III 600 MHz spectrometer. HRMS were obtained on a Thermo Fisher LTQ-Orbitrap XL. Oleanolic acid 3-*O*- β -D-glucuronopyranoside was provided by the Institute of Medicinal Plant Development (Beijing, China) [20]. Cell culture products were purchased from Gibco BRL (Grand Island, NY, USA).

3.2. Chemistry

The synthesis of the glucosyl donor and galactosyl donor was described [20].

3.2.1. General Procedure for the Synthesis of Compounds 10a–10b

To a solution of oleanolic acid (**9a**) or ursolic acid (**9b**) (10.0 g, 21.8 mmol) in dry DCM (300 mL), TBAB (0.8 g, 2.5 mmol) and K_2CO_3 (7.4 g, 53.6 mmol) in water (50 mL) were added, and benzyl bromide (3.2 mL, 26.8 mmol) was dropped at 0 °C. Then, the reaction mixture was stirred at room temperature for 18 h. The reaction was monitored by TLC. The crude mixture was separated and the water layer was extracted with DCM (3 \times 100 mL). The combined organic layer was washed with 0.1 mol/L HCl aqueous solution, NaHCO_3 saturated aqueous solution, and NaCl saturated aqueous solution in sequence, and then dried over Na_2SO_4 and purified through column chromatography (eluent: PE-EtOAc, 8:1) to offer pure white solids **10a** (10.8 g, 91% yield) and **10b** (11.1 mg, 93% yield).

3.2.2. General Procedure for the Synthesis of Compounds 11a–11d

To a solution of compound **10a** or **10b** (3.3 g, 6.0 mmol) in dry DCM (50 mL), glucosyl donor or galactosyl donor (5.8 g, 7.9 mmol) and 4 Å molecular sieve 0.5 g were added and stirred at room temperature for 1 h under N_2 air. Then, Lewis acid TMSOTf (60 μg , 0.3 mmol) was dropped and reacted for 2–4 h. When complete, 1.0 mL triethylamine was added to quench the reaction. Then, the suspension was filtered and the filtrate was evaporated and the crude product was subjected to column chromatography (eluent: PE-EtOAc, 10:1) to gain the pure compounds **11a** (5.3 g, 79% yield), **11b** (5.1 g, 75% yield), **11c** (4.7 g, 69% yield), and **11d** (4.7 g, 70% yield) as white solids.

3.2.3. General Procedure for the Synthesis of Compounds 12a–12d

A mixture of **11a–11d** (3.0 g, 2.6 mmol) and 10% Pd/C (1.5 mg) was hydrogenated at 1 atm for 4–6 h in refluxing EtOAc (30 mL). The mixture was filtered and concentrated and the residue was purified by silica gel column chromatography (eluent: PE-EtOAc, 3:1) to get the pure compounds **12a** (2.5 g, 92% yield), **12b** (2.5 g, 93% yield), **12c** (2.4 g, 90% yield), and **12d** (2.4 g, 91% yield) as white solids.

3.2.4. General Procedure for the Synthesis of Compounds 13a–c, 14a–c, 15a–c, 16a–c and compounds 5a–c, 6a–c, 7a–c, 8a–c

To a solution of compounds **12a–12d** (1.0 g, 0.98 mmol) in dry DCM (15 mL), HOBt (0.2 g, 1.46 mmol) and EDCI (0.28 g, 1.46 mmol) were added and stirred at room temperature for 1 h. To this mixture, various amines (3.92 mmol) were added respectively at 0 °C and the reaction mixture was stirred 4–16 h until its completion. The solvent was washed with 0.1 mol/L HCl aqueous solution, NaHCO_3 saturated aqueous solution, and NaCl saturated aqueous solution in sequence, and then dried over Na_2SO_4 . The suspension was filtered and the filtrate was concentrated to give compounds **13a–c**, **14a–c**, **15a–c** and **16a–c**, which were used without further purification. To a solution of compounds

13a–c, 14a–c, 15a–c and 16a–c in MeOH/DCM (8 mL, 3:1) was added 1 mol/L NaOMe/NaOH solvent (1.6 mL). The reaction mixture was stirred for 2–3 h until its completion, after that Amberlite IR-120 was added to acidate pH 7. The suspension was filtered and the filtrate was evaporated and purified through column chromatography (eluent: DCM-CH₃OH, 10:1) to offer pure white solids **5a–c, 5a–c, 7a–c, and 8a–c**.

28-N-Isobutyl oleanolic amide 3-O-β-D-glucopyranoside (5a): White solid, 88% yield; $[\alpha]_{\text{D}}^{25}$: +56.25 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.25 (t, *J* = 5.8 Hz, 1H, N-H), 5.47 (t, *J* = 3.3 Hz, 1H, H-12), 4.96 (d, *J* = 7.8 Hz, 1H, H-1'), 4.61 (dd, *J* = 11.6 Hz, 2.1 Hz, 1H, Glu-H), 4.44 (dd, *J* = 11.6 Hz, 5.2 Hz, 1H, Glu-H), 4.29–4.23 (m, 2H, Glu-H), 4.08–4.03 (m, 2H, Glu-H), 3.46–3.41 (m, 2H, H-3, H-31-1), 3.12–3.07 (m, 2H, H-18, H-31-2), 1.35 (s, 3H, CH₃), 1.30 (s, 3H, CH₃), 1.04 (s, 3H, CH₃), 0.97 (s, 6H, 2 × CH₃), 0.95 (s, 3H, CH₃), 0.91 (d, *J* = 6.9 Hz, 6H, H-33, H-34), 0.90 (s, 3H, CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.5, 145.1, 122.8, 107.0, 88.9, 78.8, 78.4, 75.9, 71.9, 63.1, 55.8, 48.0, 47.3, 46.8, 46.6, 42.3, 42.1, 39.8, 39.6, 38.8, 37.0, 34.5, 33.9, 33.2 (C × 2), 30.9, 28.8, 28.3, 28.0, 26.6, 26.2, 23.9, 23.8 (C × 2), 20.5 (C × 2), 18.5, 17.5, 17.1, 15.5; HRMS (ESI): Calcd for [M + Na]⁺ C₄₀H₆₇NNaO₇: 696.4815, found 696.4811.

28-N-Propargyl oleanolic amide 3-O-β-D-glucopyranoside (5b): White solid, 75% yield; $[\alpha]_{\text{D}}^{25}$: +23.25 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 8.03 (t, *J* = 5.5 Hz, 1H, N-H), 5.43 (t, *J* = 3.5 Hz, 1H, H-12), 4.96 (d, *J* = 7.7 Hz, 1H, H-1'), 4.60 (dd, *J* = 11.6 Hz, 2.3 Hz, 1H, Glu-H), 4.44–4.38 (m, 2H, H-31-1, Glu-H), 4.33–4.23 (m, 3H, H-31-2, Glu-H), 4.07–4.01 (m, 2H, Glu-H), 3.41 (dd, *J* = 11.8 Hz, 4.4 Hz, 1H, H-3), 3.14 (dd, *J* = 13.6 Hz, 4.4 Hz, 1H, H-18), 3.09 (t, *J* = 2.5 Hz, 1H, H-33), 1.34 (s, 3H, CH₃), 1.29 (s, 3H, CH₃), 1.03 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 0.93 (s, 6H, 2 × CH₃), 0.88 (s, 3H, CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.3, 144.7, 122.9, 106.8, 88.8, 82.1, 78.7, 78.2, 75.7, 71.8, 71.7, 63.0, 55.7, 47.9, 46.7, 46.4, 42.1, 41.7, 39.8, 39.4, 38.7, 36.9, 34.3, 33.5, 33.1, 33.0, 30.8, 29.1, 28.2, 27.8, 26.5, 26.1, 23.7, 23.6 (C × 2), 18.4, 17.6, 17.0, 15.4; HRMS (ESI): Calcd for [M + H]⁺ C₃₉H₆₂NO₇: 656.4526, found 656.4514.

28-N-(3',4'-Dimethoxybenzyl) oleanolic amide 3-O-β-D-glucopyranoside (5c): White solid, 77% yield; $[\alpha]_{\text{D}}^{25}$: +21.00 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.90 (t, *J* = 5.7 Hz, 1H, N-H), 7.12 (d, *J* = 1.8 Hz, 1H, H-2''), 7.06 (dd, *J* = 8.1 Hz, 1.8 Hz, 1H, H-6''), 6.92 (d, *J* = 8.2 Hz, 1H, H-5''), 5.46 (t, *J* = 3.3 Hz, 1H, H-12), 4.97 (d, *J* = 7.8 Hz, 1H, H-1'), 4.79 (dd, *J* = 14.5 Hz, 5.9 Hz, 1H, H-31-1), 4.62–4.56 (m, 2H, H-31-2, Glu-H), 4.44 (dd, *J* = 11.6 Hz, 5.4 Hz, 1H, Glu-H), 4.30–4.24 (m, 2H, Glu-H), 4.08–4.02 (m, 2H, Glu-H), 3.77 (s, 3H, Ph-OMe), 3.74 (s, 3H, Ph-OMe), 3.42 (dd, *J* = 11.8 Hz, 4.3 Hz, 1H, H-3), 3.18 (dd, *J* = 13.1 Hz, 4.0 Hz, 1H, H-18), 1.35 (s, 3H, CH₃), 1.31 (s, 3H, CH₃), 1.03 (s, 3H, CH₃), 0.95 (s, 6H, 2 × CH₃), 0.91 (s, 3H, CH₃), 0.89 (s, 3H, CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.4, 149.1, 144.9, 133.3, 122.8, 120.5, 112.7, 106.8, 88.8, 78.7, 78.2, 75.8, 71.9, 63.1, 56.0, 55.8 (C × 2), 47.9, 46.8, 46.5, 43.4, 42.2, 42.0, 39.8, 39.5, 38.7, 37.0, 34.4, 33.8, 33.1, 33.0, 30.8, 28.2, 27.9, 26.5, 26.1, 23.8 (C × 2), 23.7, 18.4, 17.4, 17.0, 15.4; HRMS (ESI): Calcd for [M + Na]⁺ C₄₅H₆₉NNaO₉: 790.4870, found 790.4861.

28-N-Isobutyl oleanolic amide 3-O-β-D-galactopyranoside (6a): White solid, 87% yield; $[\alpha]_{\text{D}}^{25}$: +63.00 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.26 (t, *J* = 5.8 Hz, 1H, N-H), 5.47 (t, *J* = 3.3 Hz, 1H, H-12), 4.88 (d, *J* = 7.8 Hz, 1H, H-1'), 4.61–4.60 (m, 1H, Gal-H), 4.52–4.46 (3H, m, Gal-H), 4.19 (dd, *J* = 3.4 Hz, 9.5 Hz, 1H, Gal-H), 4.14 (t, *J* = 5.9 Hz, 1H, Gal-H), 3.46–3.40 (m, 2H, H-3, H-31-1), 3.12–3.06 (m, 2H, H-18, H-31-2), 1.34 (s, 3H, CH₃), 1.31 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 0.97 (s, 6H, 2 × CH₃), 0.95 (s, 3H, CH₃), 0.91 (s, 3H, CH₃), 0.91 (d, *J* = 6.7 Hz, 6H, H-33, H-34); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.4, 145.1, 122.7, 107.4, 88.7, 76.8, 75.4, 73.2, 70.3, 62.4, 55.8, 47.9, 47.3, 46.8, 46.5, 42.2, 42.1, 39.8, 39.5, 38.8, 37.0, 34.4, 33.8, 33.1 (C × 2), 30.8, 28.7, 28.2, 27.9, 26.6, 26.1, 23.8 (C × 2), 23.7, 20.4 (C × 2), 18.5, 17.5, 16.9, 15.5; HRMS (ESI): Calcd for [M + Na]⁺ C₄₀H₆₇NNaO₇: 696.4815, found 696.4811.

28-N-Propargyl oleanolic amide 3-O-β-D-galactopyranoside (6b): White solid, 75% yield; $[\alpha]_{\text{D}}^{25}$: +57.00 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 8.08–8.05 (m, 1H, N-H), 5.44 (t, *J* = 3.5 Hz, 1H, H-12), 4.88 (d, *J* = 7.7 Hz, 1H, H-1'), 4.61–4.60 (m, 1H, Gal-H), 4.52–4.46 (m, 3H, Gal-H), 4.43–4.39 (m, 1H, H-31-1), 4.33–4.29 (m, 1H, H-31-2), 4.19 (dd, *J* = 3.2 Hz, 9.3 Hz, 1H, Gal-H), 4.14 (t, *J* = 5.9 Hz, 1H, Gal-H), 3.41 (dd, *J* = 11.8 Hz, 4.4 Hz, 1H, H-3), 3.15 (dd, *J* = 13.6 Hz, 4.4 Hz, 1H, H-18),

3.10 (t, $J = 2.5$ Hz, 1H, H-33), 1.33 (s, 3H, CH₃), 1.30 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.93 (s, 6H, 2 × CH₃), 0.89 (s, 3H, CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.3, 144.7, 122.9, 107.4, 88.7, 82.1, 76.7, 75.4, 73.2, 71.7, 70.3, 62.4, 55.8, 47.9, 46.7, 46.4, 42.1, 41.7, 39.8, 39.5, 38.7, 37.0, 34.3, 33.5, 33.1, 33.0, 30.8, 29.2, 28.2, 27.8, 26.6, 26.1, 23.8, 23.7, 23.6, 18.4, 17.6, 16.9, 15.5; HRMS (ESI): Calcd for [M + H]⁺ C₃₉H₆₂NO₇: 656.4526, found 656.4516.

28-*N*-(3',4'-Dimethoxybenzyl) oleanolic amide 3-*O*- β -*D*-galactopyranoside (**6c**): White solid, 73% yield; $[\alpha]_{\text{D}}^{25}$: +20.25 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.88 (t, $J = 5.7$ Hz, 1H, N-H), 7.12 (d, $J = 1.8$ Hz, 1H, H-2''), 7.06 (dd, $J = 8.1$ Hz, 1.8 Hz, 1H, H-6''), 6.92 (d, $J = 8.2$ Hz, 1H, H-5''), 5.46 (t, $J = 3.3$ Hz, 1H, H-12), 4.88 (d, $J = 7.8$ Hz, 1H, H-1'), 4.79 (dd, $J = 14.5$ Hz, 5.9 Hz, 1H, H-31-1), 4.61–4.56 (m, 2H, H-31-2, Gal-H), 4.52–4.46 (3H, m, Gal-H), 4.19 (dd, $J = 3.5$ Hz, 9.4 Hz, 1H, Gal-H), 4.14 (t, $J = 6.1$ Hz, 1H, Gal-H), 3.77 (s, 3H, Ph-OMe), 3.74 (s, 3H, Ph-OMe), 3.41 (dd, $J = 11.7$ Hz, 4.3 Hz, 1H, H-3), 3.18 (dd, $J = 13.4$ Hz, 4.0 Hz, 1H, H-18), 1.33 (s, 3H, CH₃), 1.31 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 0.95 (s, 6H, 2 × CH₃), 0.91 (s, 3H, CH₃), 0.90 (s, 3H, CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.4, 149.1, 144.9, 133.3, 122.8, 120.5, 112.7, 107.5, 88.7, 76.8, 75.4, 73.2, 70.3, 62.5, 56.0, 55.8 (C × 2), 48.0, 46.8, 46.5, 43.4, 42.2, 42.0, 39.8, 39.5, 38.7, 37.0, 34.4, 33.8, 33.1, 33.0, 30.8, 28.2, 27.9, 26.6, 26.1, 23.8 (C × 2), 23.7, 18.4, 17.4, 17.0, 15.5; HRMS (ESI): Calcd for [M + Na]⁺ C₄₅H₆₉NNaO₉: 790.4870, found 790.4860.

28-*N*-Isobutyl ursolic amide 3-*O*- β -*D*-glucopyranoside (**7a**): White solid, 85% yield; $[\alpha]_{\text{D}}^{25}$: +27.00 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.18 (t, $J = 5.8$ Hz, 1H, N-H), 5.47 (t, $J = 3.4$ Hz, 1H, H-12), 4.97 (d, $J = 7.7$ Hz, 1H, H-1'), 4.62 (dd, $J = 11.7$ Hz, 2.4 Hz, 1H, Glu-H), 4.43 (dd, $J = 11.7$ Hz, 5.5 Hz, 1H, Glu-H), 4.29–4.22 (m, 2H, Glu-H), 4.08–4.02 (m, 2H, Glu-H), 3.44 (dd, $J = 11.9$ Hz, 4.5 Hz, 1H, H-3), 3.35–3.31 (m, 1H, H-31-1), 3.18–3.14 (m, 1H, H-31-2), 2.40 (d, $J = 10.6$ Hz, 1H, H-18), 1.35 (s, 3H, CH₃), 1.24 (s, 3H, CH₃), 1.04 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 0.97 (d, $J = 6.5$ Hz, 3H, CH₃), 0.95 (s, 3H, CH₃), 0.92–0.89 (m, 9H, 3 × CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.3, 139.8, 125.7, 106.9, 88.9, 78.7, 78.2, 75.8, 71.9, 63.1, 55.8, 53.7, 47.9 (C × 2), 47.4, 42.6, 40.0, 39.9, 39.5, 39.3, 38.9, 38.2, 36.9, 33.4, 31.2, 28.6, 28.3 (C × 2), 26.6, 24.9, 23.7, 23.6, 21.3, 20.5 (C × 2), 18.4, 17.4 (C × 2), 17.0, 15.6; HRMS (ESI): Calcd for [M + Na]⁺ C₄₀H₆₇NNaO₇: 696.4815, found 696.4807.

28-*N*-Propargyl ursolic amide 3-*O*- β -*D*-glucopyranoside (**7b**): White solid, 72% yield; $[\alpha]_{\text{D}}^{25}$: +24.00 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.85 (t, $J = 5.3$ Hz, 1H, N-H), 5.45 (t, $J = 3.3$ Hz, 1H, H-12), 4.97 (d, $J = 7.8$ Hz, 1H, H-1'), 4.62 (dd, $J = 11.5$ Hz, 1.9 Hz, 1H, Glu-H), 4.44–4.35 (m, 2H, H-31-1, Glu-H), 4.33–4.22 (m, 3H, H-31-2, Glu-H), 4.08–4.02 (m, 2H, Glu-H), 3.43 (dd, $J = 11.9$ Hz, 4.4 Hz, 1H, H-3), 3.10 (t, $J = 2.3$ Hz, 1H, H-33), 2.44 (d, $J = 10.8$ Hz, 1H, H-18), 1.35 (s, 3H, CH₃), 1.23 (s, 3H, CH₃), 1.03 (s, 3H, CH₃), 1.02 (s, 3H, CH₃), 0.97 (d, $J = 6.5$ Hz, 3H, CH₃), 0.94 (s, 3H, CH₃), 0.89 (s, 3H, CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.2, 139.4, 126.0, 106.9, 88.9, 81.9, 78.7, 78.3, 75.8, 71.9 (C × 2), 63.1, 55.8, 53.3, 47.9, 47.8, 42.5, 40.0, 39.7, 39.5, 39.3, 38.9, 37.8, 36.8, 33.3, 31.1, 29.2, 28.3, 26.6, 24.8, 23.8, 23.6, 21.3, 18.4, 17.6, 17.4, 17.0, 15.6; HRMS (ESI): Calcd for [M + H]⁺ C₃₉H₆₂NO₇: 656.4526, found 656.4520.

28-*N*-(3',4'-Dimethoxybenzyl) ursolic amide 3-*O*- β -*D*-glucopyranoside (**7c**): White solid, 74% yield; $[\alpha]_{\text{D}}^{25}$: +7.5 (c 0.13, MeOH); ¹H-NMR (600 MHz, pyridine-*d*₅) δ : 7.71 (t, $J = 5.6$ Hz, 1H, N-H), 7.11 (d, $J = 1.8$ Hz, 1H, H-2''), 7.05 (dd, $J = 8.1$ Hz, 1.9 Hz, 1H, H-6''), 6.94 (d, $J = 8.2$ Hz, 1H, H-5''), 5.45 (t, $J = 3.3$ Hz, 1H, H-12), 4.98 (d, $J = 7.7$ Hz, 1H, H-1'), 4.71 (dd, $J = 14.4$ Hz, 5.6 Hz, 1H, H-31-1), 4.64–4.60 (m, 2H, H-31-2, Glu-H), 4.44 (dd, $J = 11.8$ Hz, 5.6 Hz, 1H, Glu-H), 4.29–4.23 (m, 2H, Glu-H), 4.09–4.03 (m, 2H, Glu-H), 3.78 (s, 3H, Ph-OMe), 3.75 (s, 3H, Ph-OMe), 3.44 (dd, $J = 11.8$ Hz, 4.4 Hz, 1H, H-3), 2.45 (d, $J = 10.7$ Hz, 1H, H-18), 1.35 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 1.03 (s, 3H, CH₃), 0.97 (d, $J = 6.4$ Hz, 3H, CH₃), 0.95 (s, 3H, CH₃), 0.91 (s, 6H, 2 × CH₃); ¹³C-NMR (150 MHz, pyridine-*d*₅) δ : 177.2, 149.1, 139.7, 133.1, 125.8, 120.6, 112.8, 112.6, 106.9, 88.9, 78.7, 78.3, 75.8, 71.9, 63.1, 56.0, 55.9, 55.8, 53.6, 47.9, 47.8, 43.5, 42.5, 40.0, 39.8, 39.5, 39.3, 38.9, 38.1, 36.9, 33.4, 31.2, 28.4, 28.3, 26.6, 24.9, 23.8, 23.6, 21.3, 21.2, 18.4, 17.4, 17.0, 15.6; HRMS (ESI): Calcd for [M + Na]⁺ C₄₅H₆₉NNaO₉: 790.4870, found 790.4863.

28-N-Isobutyl ursolic amide 3-O- β -D-galactopyranoside (8a): White solid, 89% yield; $[\alpha]_D^{25}$: +41.25 (c 0.13, MeOH); $^1\text{H-NMR}$ (600 MHz, pyridine- d_5) δ : 7.19 (t, $J = 5.9$ Hz, 1H, N-H), 5.47 (t, $J = 3.4$ Hz, 1H, H-12), 4.89 (d, $J = 7.7$ Hz, 1H, H-1'), 4.60–4.59 (m, 1H, Gal-H), 4.52–4.46 (3H, m, Gal-H), 4.19 (dd, $J = 3.4$ Hz, 9.5 Hz, 1H, Gal-H), 4.14 (t, $J = 6.0$ Hz, 1H, Gal-H), 3.44 (dd, $J = 11.8$ Hz, 4.2 Hz, 1H, H-3), 3.35–3.31 (m, 1H, H-31-1), 3.17–3.13 (m, 1H, H-31-2), 2.41 (d, $J = 10.8$ Hz, 1H, H-18), 1.34 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 1.01 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 0.97 (d, $J = 6.6$ Hz, 3H, CH₃), 0.95 (s, 3H, CH₃), 0.92–0.89 (m, 9H, 3 \times CH₃); $^{13}\text{C-NMR}$ (150 MHz, pyridine- d_5) δ : 177.3, 139.8, 125.7, 107.5, 88.9, 76.8, 75.4, 73.1, 70.2, 62.4, 55.8, 53.6, 47.9 (C \times 2), 47.4, 42.6, 40.0, 39.9, 39.5, 39.3, 38.9, 38.2, 36.9, 33.4, 31.2, 28.6, 28.3, 28.2, 26.6, 24.9, 23.7, 23.6, 21.3, 20.5, 20.4, 18.4, 17.4 (C \times 2), 16.9, 15.6; HRMS (ESI): Calcd for $[\text{M} + \text{Na}]^+$ C₄₀H₆₇NNaO₇: 696.4815, found 696.4809.

28-N-Propargyl ursolic amide 3-O- β -D-galactopyranoside (8b): White solid, 74% yield; $[\alpha]_D^{25}$: +36.00 (c 0.13, MeOH); $^1\text{H-NMR}$ (600 MHz, pyridine- d_5) δ : 7.84 (t, $J = 5.4$ Hz, 1H, N-H), 5.45 (t, $J = 3.3$ Hz, 1H, H-12), 4.89 (d, $J = 7.7$ Hz, 1H, H-1'), 4.60–4.59 (m, 1H, Gal-H), 4.52–4.46 (m, 3H, Gal-H), 4.40–4.28 (m, 2H, H-31), 4.19 (dd, $J = 3.4$ Hz, 9.4 Hz, 1H, Gal-H), 4.13 (t, $J = 6.2$ Hz, 1H, Gal-H), 3.43 (dd, $J = 11.8$ Hz, 4.5 Hz, 1H, H-3), 3.10 (t, $J = 2.4$ Hz, 1H, H-33), 2.44 (d, $J = 10.8$ Hz, 1H, H-18), 1.34 (s, 3H, CH₃), 1.24 (s, 3H, CH₃), 1.02 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.97 (d, $J = 6.5$ Hz, 3H, CH₃), 0.94 (s, 3H, CH₃), 0.90 (s, 3H, CH₃); $^{13}\text{C-NMR}$ (150 MHz, pyridine- d_5) δ : 177.2, 139.4, 126.0, 107.5, 88.8, 81.9, 76.8, 75.4, 73.1, 71.9, 70.3, 62.5, 55.9, 53.3, 47.9, 47.8, 42.5, 40.0, 39.8, 39.5, 39.3, 38.9, 37.8, 36.8, 33.3, 31.1, 29.2, 28.3, 26.7, 24.8, 23.8, 23.6, 21.3, 18.4, 17.6, 17.4, 17.0, 15.6; HRMS (ESI): Calcd for $[\text{M} + \text{H}]^+$ C₃₉H₆₂NO₇: 656.4526, found 656.4516.

28-N-(3',4'-Dimethoxybenzyl) ursolic amide 3-O- β -D-galactopyranoside (8c): White solid, 69% yield; $[\alpha]_D^{25}$: +8.25 (c 0.13, MeOH); $^1\text{H-NMR}$ (600 MHz, pyridine- d_5) δ : 7.70 (t, $J = 5.8$ Hz, 1H, N-H), 7.11 (d, $J = 1.8$ Hz, 1H, H-2''), 7.05 (dd, $J = 8.2$ Hz, 2.0 Hz, 1H, H-6''), 6.94 (d, $J = 8.2$ Hz, 1H, H-5''), 5.45 (t, $J = 3.3$ Hz, 1H, H-12), 4.89 (d, $J = 7.7$ Hz, 1H, H-1'), 4.71 (dd, $J = 14.5$ Hz, 5.9 Hz, 1H, H-31-1), 4.62–4.59 (m, 2H, H-31-2, Gal-H), 4.53–4.46 (3H, m, Gal-H), 4.19 (dd, $J = 3.3$ Hz, 9.4 Hz, 1H, Gal-H), 4.14 (t, $J = 5.9$ Hz, 1H, Gal-H), 3.78 (s, 3H, Ph-OMe), 3.75 (s, 3H, Ph-OMe), 3.44 (dd, $J = 11.9$ Hz, 4.4 Hz, 1H, H-3), 2.45 (d, $J = 10.7$ Hz, 1H, H-18), 1.34 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 0.97 (d, $J = 6.5$ Hz, 3H, CH₃), 0.95 (s, 3H, CH₃), 0.93 (s, 3H, CH₃), 0.91 (s, 3H, CH₃); $^{13}\text{C-NMR}$ (150 MHz, pyridine- d_5) δ : 177.2, 149.1, 139.7, 133.1, 125.8, 120.6, 112.8, 112.6, 107.5, 88.8, 76.8, 75.4, 73.2, 70.3, 62.5, 56.0, 55.9, 53.6, 47.9, 47.8, 43.5, 42.5, 40.0, 39.8, 39.5, 39.3, 38.9, 38.1, 36.9, 33.4, 31.2, 28.4, 28.3, 26.7, 24.9, 23.8, 23.6, 21.3, 18.4, 17.5, 17.4, 17.0, 15.6; HRMS (ESI): Calcd for $[\text{M} + \text{Na}]^+$ C₄₅H₆₉NNaO₉: 790.4870, found 790.4860.

The spectrograms of the compounds **5a–c**, **6a–c**, **7a–c** and **8a–c** were shown in Electronic Supplementary Material (ESM).

3.3. Evaluation of the Biological Activity

3.3.1. Cell Culture and Treatment

The H9c2 rat myocardial cell line was obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China) and cultured as previously described [20]. Briefly, H9c2 cells were cultured in high glucose DMEM supplemented with 10% (*v/v*) fetal bovine serum, 1% penicillin/streptomycin (*v/v*), and 2 mM L-glutamine. The cells were maintained in a humidified incubator with 95% air and 5% CO₂ at 37 °C.

The cells were subcultured after reaching 70–80% confluence. Three sets of experiments were performed: (1) control cells; (2) cells treated with H₂O₂ (450 μM) for 1 h; (3) cells pretreated with oleanolic acid 3-O- β -D-glucuronopyranoside derivatives (0.1 $\mu\text{g}/\text{mL}$) for 12 h, then the medium was removed and the cells were treated with H₂O₂ (450 μM) for 1 h. For all of the experiments, the cells were plated at an appropriate density according to the experimental design and were grown for 36 h before experimentation.

3.3.2. Determination of Cell Viability

Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay as previously described [20]. Briefly, H9c2 cells were plated in 96-well plates at a density of 1×10^4 cells/well and incubated overnight. After the designated treatment, 20 μ L of MTT (5 mg/mL) was added to each well and cells were incubated at 37 °C for 4 h. Then, the culture medium with MTT was abandoned and the colored formazan crystals were dissolved in 100 μ L dimethyl sulfoxide (DMSO). The absorption values were measured at 570 nm using a microplate reader (TECAN Infinite M1000, Grödig, Austria). The viability of H9c2 cells in each well is presented as a percentage of control cells.

3.3.3. Statistic Analysis

The data are expressed as mean \pm SD. Comparisons were performed by Student's *t*-test or one-way ANOVA followed by Tukey's multiple comparison test with Prism 5.00 software. Statistical significance was set at $p < 0.05$. All data are the results of at least three independent experiments.

4. Conclusions

In summary, a new series of oleanolic acid 3-*O*- β -D-glucuronopyranoside derivatives were designed, synthesized, and evaluated for their cardiovascular protective effect. Some of the synthesized compounds showed potent protective activity against H₂O₂-induced injury in H9c2 cells. Particularly, compound **8a** exhibited a greater potential protective effect than the positive control oleanolic acid 3-*O*- β -D-glucuronopyranoside. Preliminary SAR analysis has shown that the isobutyl group of the amide derivatives had a good impact on the protective effect.

Supplementary Materials: Supplementary materials are available online, Figures S1–S24: ¹H-NMR and ¹³C-NMR of compound **5a–c**, **6a–c**, **7a–c** and **8a–c**.

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Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds 5–8a–c are available from the authors.



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