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New Derivatives of Lupeol and Their Biological Activity

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Abstract: The natural product lupeol (**1**) was isolated from *Bombax ceiba* leaves, which were used as starting material in the semisynthetic approach. Three new derivatives (**2a**, **2b**, and **3**) were synthesized using oxidation and aldolization. Their chemical structures were elucidated by spectroscopic analyses (HRESIMS and NMR). Compounds **3** showed significant α-glucosidase inhibition with an IC_{50} value of 202 µM, whereas **2a** and **2b** were inactive.

Keywords: lupeol derivative; benzylidene derivative; α-glucosidase inhibition; Oxone®

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1. Introduction

Diabetes mellitus (DM) causes high blood glucose after the consumption of a carbohydrate-enriched diet, leading to hyperglycemia. Uncontrolled diabetes is manifested by a very high rise in triglycerides and fatty acid levels [1]. Diverse antidiabetic drugs derived from synthetic compounds are of interest to chemists. However, these synthetic drugs come with several serious complications [1]. Due to the limitations associated with the use of existing synthetic antidiabetic drugs, the search for newer antidiabetic agents from natural sources continues. Lupeol is a pharmacologically active pentacyclic triterpenoid found in several medicinal plants worldwide [2]. It has several potential medicinal properties and is found in a variety of botanical sources [3]. Notably, lupeol has been reported to selectively target diseased and unhealthy human cells, while sparing normal and healthy cells [4]. Dozens of novel lupeol derivatives were synthesized and screened for their in vivo antihyperglycemic activity [5,6]. Most derivatives lowered the blood glucose levels, in a sucrose-challenged streptozotocin-induced diabetic rat (STZ-S) model [5]. To continue our ongoing search for highly efficient antidiabetic agents from derivatized lupeol [6,7], we herein describe the synthesis of lupeol derivatives **2**, **2a**, **2b**, and **3** (Figure 1). The structures of all the obtained compounds were characterized by ^{1}H, ^{13}C NMR, and HRESIMS. All derivatives were evaluated for α-glucosidase inhibition.

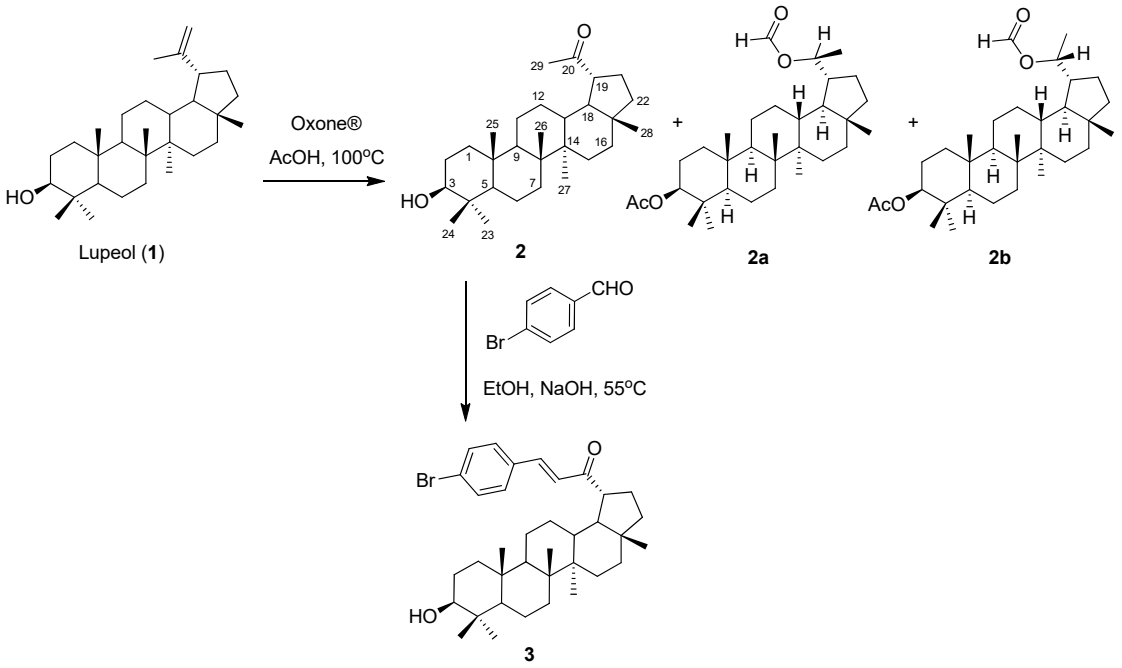

Figure 1. Synthesis of **2**, **2a, 2b**, and **3** from lupeol (**1**).

2. Results and Discussion

2.1. Synthesis

Lupeol was isolated from the Vietnamese plant *Bombax ceiba*, following our previously reported procedure [8]. Lupeol was transformed to products **2**, **2a**, and **2b** using oxidation with Oxone®, a potassium triple-salt ($KHSO_5 \cdot 1/2KHSO_4 \cdot 1/2K_2SO_4$) [6,9]. The conditions followed our previously reported method [6], with slight modifications. Both **2a** and **2b** had the same molecular formula as $C_{32}H_{52}O_4$. Comparison of NMR data of **2a**/**2b** and **1** indicated that oxidation occurred. The ^{1}H NMR spectrum of **2a**/**2b** showed differences with **1**: the downfield methine at δ_H 8.11, two oxymethines at δ_H 5.26 and 4.48, and a doublet methyl at δ_H 1.22. These signals indicated that the isopropenyl group of **1** was transformed to a 2-formylethyl group at C-19. Moreover, the downfield signal of H-3 (δ_H 4.48) indicated that 3-OH was esterified by acetic acid. The ^{13}C NMR spectrum of **2a**/**2b** showed one carbonyl ester at δ_C 171.1, one formyl group at δ_C 163.7 and two oxygenated carbons at δ_C 81.1 and 72.7, supporting the previous findings. Interestingly, **2a** and **2b** are C-20 epimers. Corbett and co-workers [10,11] indicated the method to define the absolute configuration of C-20 of lupane-type triterpenes. Particularly, the (20*S*) and (20*R*) isomers exhibited differences in the chemical shifts of C-19, C-20, C-29, and C-30, especially C-30. According to Corbett et al., **2a**, having C-30 at δ_C 20.1, would have a 20*R* configuration. On the other hand, **2b** would have the 20*S* configuration due to the lower chemical shift of C-30 at δ_C 14.2.

Compound **2** was further aldolized with 4-bromobenzaldehyde to afford compound **3**. Compound **3** had the same molecular formula as $C_{36}H_{51}BrO_2$, determined by a protonated ion peak at *m/z* 595.3188 in HRESIMS. Comparison of 1D NMR data of **2** and **3** indicated obvious differences. The first difference is the presence of a 1,4-disubstituted benzenoid characterized by two *ortho*-coupled protons at δ_H 7.51 and 7.42, and a *trans* double bond at δ_H 6.75 and 7.46. This was confirmed by the disappearance of a methyl ketone group at δ_H 2.15 (CH_3-29). This finding indicated that the aldolization occurred exclusively at C-29. The second difference was in the ^{13}C NMR spectrum. This spectrum showed the presence of seven aromatic carbons at δ_C 141.0 (C-1), 133.9 (C-5′), 132.3 (C-2′), 129.8 (C-3′,7′), and 126.9 (C-4′, 6′), supporting the reaction at C-29.

2.2. α-Glucosidase Inhibition of **2a**, **2b**, *and* **3**

Compounds **2a**, **2b**, and **3** were evaluated for α-glucosidase inhibition. Only compound **3** exhibited moderate α-glucosidase inhibition with an IC_{50} value of 202 μM, compared with an acarbose-positive control (IC_{50} 360 μM). Other compounds were inactive.

3. Materials and Methods

3.1. Materials

Reagents and solvents were obtained from commercial suppliers and were used without further purification. Column chromatography was carried out using Merck Kieselgel 60 silica gel (particle size: 32–63 Å). Analytical TLC was performed using Merck precoated silica gel 60 F-254 sheets.

NMR spectroscopic data were acquired on Bruker Avance III apparatus at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. HRESIMS spectra were recorded on a Bruker MICROTOF-Q 10187.

Extraction and Isolation. The air-dried *Bombax ceiba* leaves (4 kg) were ground into powder and exhaustively extracted at room temperature with MeOH (2 × 10 L). The filtered solution was evaporated under reduced pressure to afford a residue (473.4 g). This crude extract was subsequently partitioned using solvents of *n*-hexane and EtOAc to yield *n*-hexane (40 g) and EtOAc (88 g) extracts. The *n*-hexane extract was fractionated by silica gel column chromatography (CC), eluted with *n*-hexane–EtOAc (isocratic, 10:1, *v*/*v*), to produce five fractions (H1-H5). Fraction H2 (15 g) was rechromatographed by silica gel CC using *n*-hexane–$CHCl_3$ (isocratic, 12:1, *v*/*v*) as eluent to afford lupeol (**1**) (1.5 g).

3.2. Synthesis Procedure

Synthesis of **2**, **2a**, and **2b**: Lupeol (**1**, 200 mg, 0.469 mmol) was oxidized with Oxone® (951 mg, 1.548 mmol) in acetic acid (40 mL) at 100 °C for 3 h. The mixture was stirred and continuously monitored by TLC. The mixture was extracted with EtOAc–water (1:1) to gain the organic layer. This solution was evaporated to afford a residue. Then, the residue was purified by silica gel CC to give compounds **2**, **2a**, and **2b**.

Compound **2**. Isolated yield: 74.6 mg (37%), white solid. ^{1}H and ^{13}C NMR data were consistent with those reported previously [6].

(3a*R*,5a*R*,5b*R*,7a*R*,9*S*,11a*R*,11b*R*,13a*R*,13b*S*)-1-((*S*)-1-(formyloxy)ethyl)-3a,5a,5b,8,8,11a-hexamethylicosahydro-1*H*-cyclopenta[a]chrysen-9-yl acetate (2a). Isolated yield: 9.4 mg (4%), white solid. ^{1}H NMR (500 MHz, $CDCl_3$, *δ*, ppm): 2.05 (3H, *s*, CH_3-2′), 8.00 (1H, *s*, OCHO-29), 5.33 (1H, *m*, H-20), 4.48 (1H, *dd*, *J* = 10.5, 6.0 Hz, H-3), 2.13 (1H, *m*, H-3), 1.18 (3H, *d*, *J* = 6.5 Hz, CH_3-30), 1.03 (3H, *s*, CH_3-26), 0.90 (3H, *s*, CH_3-27), 0.87 (3H, *s*, CH_3-25), 0.85 (3H, *s*, CH_3-23), 0.84 (3H, *s*, CH_3-24), 0.79 (1H, *d*, *J* = 9.5 Hz, H-5), 0.76 (3H, *s*, CH_3-28). ^{13}C NMR (125 MHz, $CDCl_3$, *δ*, ppm): 171.2 (C-6′), 21.5 (C2′), 161.6 (C-29), 81.1 (C-3), 73.4 (C-20), 55.5 (C-5), 50.2 (C-9), 48.8 (C-18), 43.5 (C-17), 43.0 (C-14), 42.6 (C-19), 41.0 (C-8), 40.5 (C-22), 38.5 (C-4), 38.0 (C-1), 37.3 (C-13), 37.2 (C-10), 35.5 (C-16), 34.4 (C-7), 29.9 (C-21), 28.1 (C-23), 27.3 (C-15), 27.1 (C-2), 23.8 (C-12), 21.0 (C-11), 18.4 (C-6), 18.1 (C-28), 16.7 (C-25), 16.4 (C-26), 16.1 (C-24), 14.4 (C-27), 14.2 (C-30). HRESIMS calcd $C_{32}H_{52}NaO_4$ ($[M+Na]^+$): 523.3732, found: 523.3763.

(3a*R*,5a*R*,5b*R*,7a*R*,9*S*,11a*R*,11b*R*,13a*R*,13b*S*)-1-((*R*)-1-(formyloxy)ethyl)-3a,5a,5b,8,8,11a-hexamethylicosahydro-1*H*-cyclopenta[a]chrysen-9-yl acetate (2b). Yield: 9.4 mg (5%), white solid. ^{1}H NMR (500 MHz, $CDCl_3$, *δ*, ppm): 2.04 (3H, *s*, H-2′), 8.11 (1H, *s*, OCHO-29), 5.26 (1H, *m*, H-20), 4.48 (1H, *dd*, *J* = 11.5, 5.5 Hz, H-3), 2.31 (1H, *m*, H-19), 1.22 (3H, *d*, *J* = 6.5 Hz, CH_3-30), 1.03 (3H, *s*, CH_3-26), 0.86 (3H, *s*, CH_3-25), 0.85 (3H, *s*, CH_3-27), 0.85 (3H, *s*, CH_3-23), 0.84 (3H, *s*, CH_3-24), 0.77 (1H, *d*, *J* = 2.0 Hz, H-5), 0.75 (3H, *s*, CH_3-28). ^{13}C NMR (125 MHz, $CDCl_3$, *δ*, ppm): 171.1 (C-6′), 21.5 (C2′), 163.7 (C-29), 81.1 (C-3), 72.7 (C-20), 55.5 (C-5), 50.0 (C-9), 47.1 (C-18), 44.4 (C-19), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 40.1 (C-22), 38.5 (C-4), 38.0 (C-1), 37.5 (C-13), 37.3 (C-10), 35.3 (C-16), 34.4 (C-7), 29.9 (C-21), 28.1 (C-23), 27.4 (C-15), 26.9 (C-2), 23.9 (C-12), 21.0 (C-11), 20.1 (C-30), 18.4 (C-6), 18.1 (C-28), 16.7

(C-25), 16.3 (C-26), 16.1 (C-24), 14.4 (C-27). HRESIMS calcd $C_{32}H_{52}NaO_4$ ([M+Na+H_2O]$^+$): 541.3870, found: 541.3869.

Synthesis of **3**: Compound **2** (70 mg, 0.163 mmol) together with NaOH (35 mg, 0.875 mmol) in ethanol (7 mL) was stirred at 55 °C for 15 min. Then, 4–bromobenzaldehyde (64.35 mg, 0.35 mmol) was added to the mixture. The reaction was performed at 55 °C for 2 h. The mixture was extracted with EtOAc–water (1:1, *v*/*v*) to gain the organic layer. This solution was applied to silica gel CC using the gradient system of *n*-hexane–EtOAc (10:1, *v*/*v*) to obtain compound **3**. Isolated yield: 68 mg (48%), white solid.

(*E*)-3-(4-bromophenyl)-1-((1*R*,3a*R*,5a*R*,5b*R*,9S,11a*R*)-9-hydroxy-3a,5a,5b,8,8,11a-hexamethylicosahydro-1*H*-cyclopenta[a]chrysen-1-yl)prop-2-en-1-one (3): ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.51 (2H, *d*, *J* = 8.5 Hz, H-3′,7′), 7.46 (1H, *d*, *J* = 16.0 Hz, H-6′), 7.42 (2H, *d*, *J* = 8.5 Hz, H-4′,6′), 6.75 (1H, *d*, *J* = 16.0 Hz, H-29), 3.19 (1H, *dd*, *J* = 11.2, 4.8 Hz, H-3), 2.87 (1H, *td*, *J* = 11.5, 6.0 Hz, H-19), 1.02 (3H, *s*, CH_3-26), 0.98 (3H, *s*, CH_3-27), 0.96 (3H, *s*, CH_3-23), 0.84 (3H, *s*, CH_3-24), 0.80 (3H, *s*, CH_3-25), 0.75 (3H, *s*, CH_3-28). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 204.1 (C-20), 141.0 (C-6′), 133.9 (C-5′), 132.3 (C-3′,7′), 129.8 (C-4′,6 ‘), 126.9 (C-29), 124.7 (C-2′), 79.0 (C-3), 55.4 (C-5), 50.4 (C-9), 50.1 (C-18), 43.3 (C-17), 42.9 (C-14), 40.9 (C-8), 40.3 (C-22), 39.0 (C-4), 38.8 (C-1), 37.3 (C-10), 35.2 (C-16), 34.3 (C-7), 28.7 (C-15), 28.1 (C-23), 27.9 (C-2), 27.5 (C-12), 21.1 (C-11), 18.4 (C-6), 18.3 (C-28), 16.2 (C-26), 16.0 (C-25), 15.5 (C-24), 14.6 (C-27). HRESIMS calcd $C_{36}H_{52}BrO_2$ ([M−H]$^-$): 595.3151, found: 595.3188.

3.3. α-Glucosidase Inhibitory Assay

The *α*-glucosidase (0.2 U/mL) and substrate (5.0 mM *p*-nitrophenyl-*α*-D-glucopyranoside) were dissolved in 100 mM pH 6.9 sodium phosphate buffer [12]. The inhibitor (50 µL) was preincubated with *α*-glucosidase; then, the substrate (40 µL) was added to the reaction mixture. The enzymatic reaction was carried out at 37 °C for 20 min and stopped by the addition of 0.2 M Na_2CO_3 (130 µL). Enzymatic activity was quantified by measuring absorbance at 405 nm. All samples were analyzed in triplicate at five different concentrations around the IC_{50} values, and the mean values were retained. The inhibition percentage (%) was calculated as follows: Inhibition (%) = $[1 - (A_{sample}/A_{control})] \times 100$.

4. Conclusions

Three new derivatives, **2a**, **2b**, and **3**, from the natural product lupeol have been synthesized via oxidation and aldolization routes and evaluated for their *α*-glucosidase inhibition. Synthetic compound **3** showed much stronger *α*-glucosidase inhibitory activity (IC_{50} 202 µM) than acarbose (IC_{50} 360 µM). Synthetic products **2a** and **2b**, which lacked the 3-OH group, exhibited lower activity than **3** toward *α*-glucosidase. This result confirmed that this substituted group might be involved in *α*-glucosidase inhibition.

Supplementary Materials: The following are available online. Copies of HRESIMS and NMR spectra for compound **2a**, **2b**, and **3**.

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

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