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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF PRENYLATED CHALCONE MANNICH BASE DERIVATIVES

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*Prenylated chalcones xanthohumol (**1**) and 2′-hydroxy-3,4,4′-trimethoxy-6′-*O*-prenyl chalcone (**2**) were synthesized through the Claisen–Schmidt condensation,* O*-prenylation, and Claisen rearrangement and deprotection respectively, using phloroglucinol and appropriate benzaldehydes as starting materials. Based on the Mannich reaction of prenylated chalcone **1** or **2** with various secondary amines and formaldehyde in acid alcohol solvent, 10 novel prenylated chalcone Mannich base derivatives **3a–3e** and **4a–4e** were synthesized. Furthermore, all synthetic compounds were evaluated for antiproliferative activities* in vitro *against four human cancer cell lines (Aspc-1, SUN-5, HepG-2, and HCT-116) by MTT assay. The results showed that most of them exhibit moderate to good antiproliferative activities against the four human cancer cells with IC_{50} values of 2.52 to 47.67 μM.*

Keywords: prenylated chalcones, xanthohumol, Mannich bases, synthesis, antiproliferative activity.

Chalcones are precursors of flavonoids in plants. Chemically, they consist of open-chain flavonoids, in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. Prenylated chalcones are a unique class of naturally occurring flavonoids characterized by the presence of a prenylated side chain in the chalcone skeleton [1, 2]. Owing to their unique structure and extensive bioactivities, prenylated chalcones have attracted a great deal of attention. Various prenylated chalcones have been investigated as antimalarial, antibacterial, and anticancer agents [3–6].

Xanthohumol is a representative prenylated chalcone extracted from the hop plant, *Humulus lupulus*, which has been characterized as having a broad spectrum of anticancer research [7]. Besides its remarkable antiproliferative activity against different cancer cell lines, xanthohumol also exhibited apoptotic activity and showed chemopreventive effects. Despite the well-documented anticancer activity of xanthohumol, its efficacy is moderate and its bioavailability is low due to poor solubility [8, 9]. Hence, it is of interest to improve its potency and increase its solubility in water.

The building block of Mannich base is an important model of drug biological activity. Studies demonstrated that compounds bearing phenolic Mannich base moieties exhibited good biological activities. Aminomethylation of drugs can increase the hydrophilic properties of drugs by introducing polar functional groups into the drug structure so as to improve the solubility of the drug in the human body. These results make Mannich bases promising for anticancer drug design [10, 11].

Due to their structural uniqueness and potent bioactivity, the synthesiss of prenylated chalcone products has attracted much attention in recent years. Based on our previous experience on flavonoid Mannich base derivatives as antitumor agents [12–14], we have synthesized two series of 10 prenylated chalcone Mannich base derivatives **3a–3e** and **4a–4e** and evaluated *in vitro* their antiproliferative activities against a panel of four human cancer cell lines including Aspc-1 (human pancreatic cancer), SUN-5 (human gastric cancer), HepG-2 (human hepatocellular carcinoma), and HCT-116 (human colon cancer), using the thiazolyl blue tetrazolium bromide (MTT) method with staurosporine as the positive control drug.

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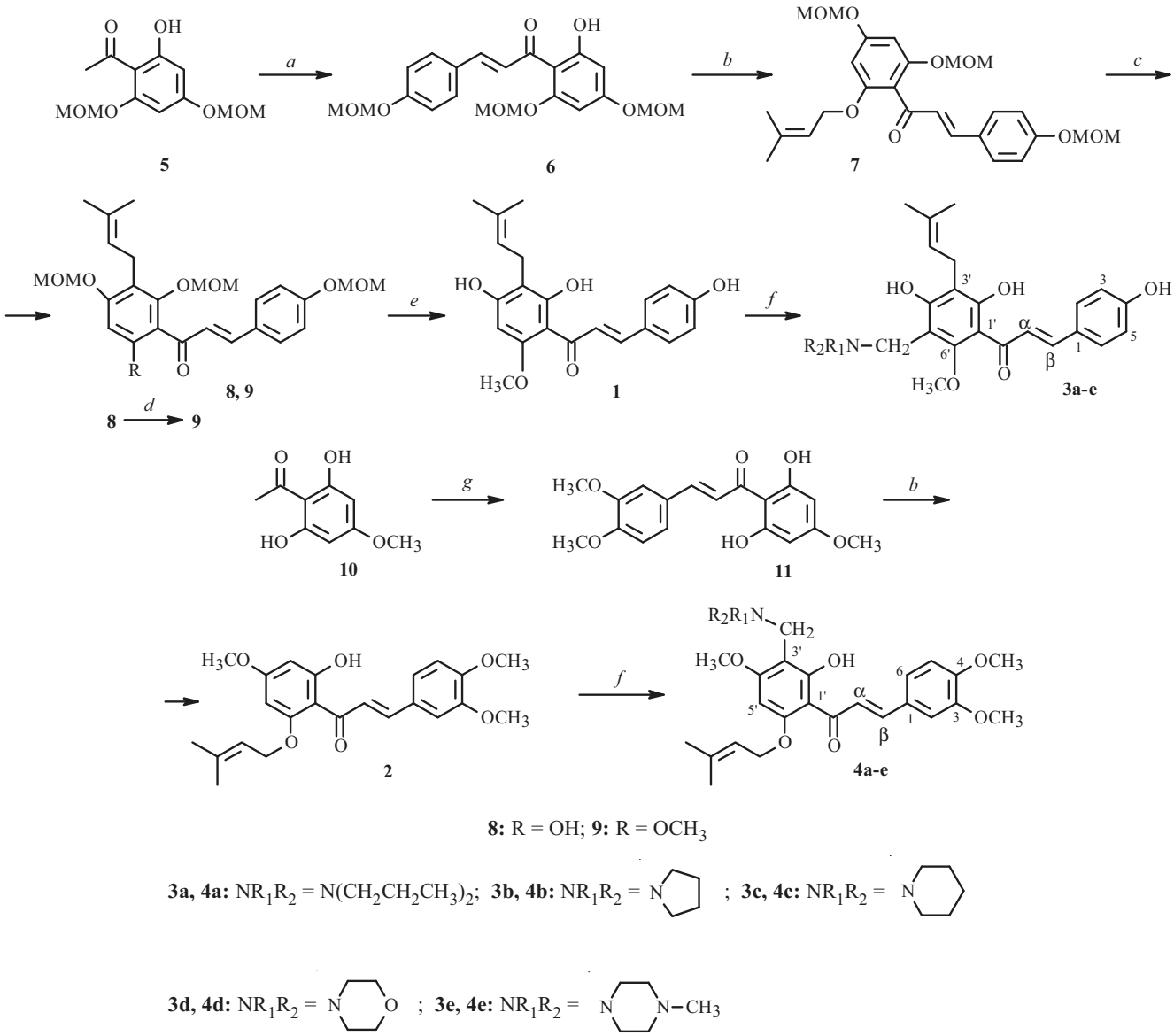

8: R = OH; **9:** R = OCH_3

3a, 4a: $NR_1R_2 = N(CH_2CH_2CH_3)_2$; **3b, 4b:** NR_1R_2 = ; **3c, 4c:** NR_1R_2 =

3d, 4d: NR_1R_2 = ; **3e, 4e:** NR_1R_2 =

a. 4-Methyloxymethylbenzaldehyde, KOH, EtOH; *b*. prenylbromide, K_2CO_3, acetone, 50°C; *c*. florisil, toluene, 100°C; *d*. $(CH_3)_2SO_4$, K_2CO_3, acetone, r.t.; *e*. 3M HCl (aq.); *f*. HCHO (aq.), CH_3OH, amines, HCl, 80°C; *g*. 3,4-dimethoxybenzaldehyde, KOH, EtOH

Scheme 1

The synthesis of naturally occurring *C*-prenylated chalcone xanthohumol (**1**), *O*-prenylated chalcone analogs (**2**), and their Mannich base derivatives **3a–3e** and **4a–4e** is outlined in Scheme 1. 2,4-Dimethoxymethoxy-6-hydroxyacetophenone (**5**) was prepared from commercially available phloroglucinol *via* Friedel–Crafts acetylation and selective *O*-methoxymethylation [13, 14]. Claisen–Schmidt aldol condensation of **5** or **10** with MOM-protected 4-hydroxybenzaldehydes or 3,4-dimethoxybenzaldehydes gave the corresponding chalcones **6** or **11**, which were refluxed with prenylbromide in K_2CO_3–acetone for 24 h to obtain *O*-prenylated chalcones **2** or **7** in high yield. Claisen rearrangement of *O*-prenylated chalcone **7** using Florisil as an effective and environmentally benign heterogeneous catalyst in toluene gave the MOM-protected *C*-prenylated chalcone **8** [15]. The *para*-rearranged product was obtained selectively in 85% yield and was the only product observed in the ^{1}H NMR spectrum of the product signals for the $–CH_2$-CH=C$(CH_3)_2$ in **1** at 3.16 ppm (2H, d). Methylation with dimethyl sulfate gave **9**. Deprotection with HCl (aq.) in MeOH (reflux) gave the prenylated chalcone xanthohumol (**1**). The spectroscopic data of **1** were identical to those reported in the literature [16]. *E*-Chalcone is characterized by a large $J_{\alpha,\beta}$ ^{1}H NMR coupling constant in the *trans* olefin bond region of 15–16 Hz.

TABLE 1. Half-Inhibitory Concentration of Compounds **1**, **2**, **3a–3e**, and **4a–4e** on Human Cancer Cell Lines (IC_{50}, μM)

Compound	Aspc-1	SUN-5	HepG-2	HCT-116
1	14.57 ± 1.21	16.20 ± 1.35	16.37 ± 1.46	9.32 ± 1.02
2	> 50	> 50	> 50	> 50
3a	28.5 ± 1.16	> 50	16.82 ± 1.45	6.05 ± 1.08
3b	11.23 ± 0.96	9.29 ± 0.68	13.37 ± 1.22	10.71 ± 0.98
3c	9.29 ± 1.06	> 50	> 50	> 50
3d	35.29 ± 1.64	34.66 ± 1.26	47.67 ± 1.68	15.06 ± 1.20
3e	> 50	36.51 ± 1.36	22.77 ± 1.64	19.81 ± 1.52
4a	> 50	> 50	> 50	6.98 ± 1.24
4b	6.0 ± 0.26	> 50	4.62 ± 1.06	2.52 ± 1.16
4c	5.37 ± 0.65	10.49 ± 1.02	4.83 ± 1.27	2.54 ± 1.04
4d	13.61 ± 0.86	12.96 ± 0.68	17.96 ± 0.88	8.47 ± 0.86
4e	14.49 ± 1.06	15.28 ± 0.55	5.97 ± 1.06	4.72 ± 0.96
Staurosporine[a]	0.052 ± 2.68	0.0208 ± 2.55	0.0575 ± 1.95	0.02 ± 1.46

[a] Staurosporine was employed as a positive control.

Further modifications of xanthohumol (**1**) and *O*-prenylated chalcone (**2**) were achieved through the introduction of an amino methyl functional group by means of the Mannich reaction. Compounds **1** or **2** reacted with various secondary amines and formaldehyde in acid alcohol solvent. Ten novel prenylated chalcone Mannich base derivatives **3a–3e** and **4a–4e** were synthesized. The Mannich reaction can be applied to aromatic rings as long as the hydroxyl group is available in the *ortho*-position of phenol. The synthesized compounds are classified as aminoalkylphenols. The exclusive formation of *ortho*-aminoalkylated products is attributed to the directing effect of the hydrogen bond between nitrogen and the *o*-hydroxyl group, which affords a stabilized six-membered ring. The electron density of the B ring is higher than that of the A ring due to the existence of multiple electron-donating groups in the B ring; the C-5′ or C-3′ position on the B-ring of chalcone is most nucleophilic and thus prone to electrophilic aromatic substitution. The difference in reactivity allows the regioselectivity for the Mannich reaction.

Substitution at the 5′-position for **1** and the 3′-position for **2** on the B-ring of chalcones was observed based upon the ^{1}H NMR. Compound **1** exhibited specific peaks at 6.10 ppm corresponding to 5′-positions, **2** exhibited specific peaks at 5.88 ppm corresponding to 3′-positions. All of the Mannich products exhibited methylene (CH_2) peaks around 3.60–3.85 ppm in the ^{1}H NMR, and a new sign appeared between 61.96–67.26 ppm in the ^{13}C NMR. With the notable absence of any peaks at 6.10 or 5.88 ppm, it is suggested that substitution occurred at the 5′-position for **1** and the 3′-position for **2** of the B-ring. It should be noted that in all cases the electrophilic substitution occurred solely in the B-ring, even at the large excess of the reagent and under more severe conditions.

The antiproliferative activity of all the synthesized compounds **1**, **2**, **3a–3e**, and **4a–4e** was investigated *in vitro* against a panel of four human cancer cell lines, Aspc-1, SUN-5, HepG-2, and HCT-116, by MTT cell proliferation assays. In these experiments, staurosporine, a known anticancer drug, was used as positive control. The antiproliferative activities of the tested compounds are described by the half maximal inhibitory concentrations (IC_{50}) value in Table 1. The results showed that most of them exhibited moderate to good antiproliferative activities against the four human cancer cells with IC_{50} values of 2.52 to 47.67 μM. As shown in Table 1, the *C*-prenylated chalcone xanthohumol **1** was more active than the *O*-prenylated chalcone **2**. The aminomethylene group substitution at the C-5′ position for **1** and the C-3′ position for **2** significantly enhanced the antiproliferative activity. For the Aspc-1 cell line, most of the compounds showed antiproliferative activity with IC_{50} from 5.37 to 35.29 μM. Compounds **4b** and **4c** showed the highest activity (IC_{50} 6.0 μM and 5.37 μM). For the SUN-5 cell line, compounds **3b**, **4c**, **4d**, and **4e** exhibited higher activity (IC_{50} 9.29–15.28 μM) compared to xanthohumol (IC_{50} 16.20 μM). For the HepG-2 cell line, most of the compounds showed antiproliferative activity with IC_{50} from 4.62 to 47.67 μM. Compounds **3b**, **4b**, **4c**, and **4e** exhibited higher activity (IC_{50} 4.62–13.37 μM) compared to xanthohumol (IC_{50} 16.37 μM). For HCT-116, most of the compounds showed antiproliferative activity with IC_{50} from 2.52 to 19.81 μM. Compounds **4b** and **4c** showed the highest activity (IC_{50} 2.52 and 2.54 μM, respectively). Compounds **3c** against Aspc-1 cell lines (IC_{50} 9.29 μM) and **4a** against HCT-116 (IC_{50} 6.98 μM) cell lines showed selective antiproliferative activity, respectively. Compounds **3b**, **3d**, **4c**, and **4e** showed antiproliferative activities with IC_{50} values ranging from 4.72 to 47.67 μM against all

cancer lines. Compound **4c,** having the piperidinomethyl group at the 3′-position, proved to be the most active of the synthesized prenylated chalcone Mannich base tested, with IC_{50} value ranging from 2.54 to 10.49 μM against all four cancer cell lines. The presence of a Mannich base group in natural products may increase the biological potency due to the greater number of molecular sites for electrophilic attack by cellular constituents, as well as due to the cascade effect of preferential chemosensitization.

In summary, we have synthesized prenylated chalcones xanthohumol (**1**) and 2′-hydroxy-3,4,4′-trimethoxy-6′-*O*-prenyl chalcone (**2**) and their 10 Mannich base derivatives. After antiproliferative activity screening of those compounds **4b**, **4c**, and **4e** against Aspc-1, HepG-2, and HCT-116 cells, and **3b** against SUN-5 cells, these compounds were identified as the most active with IC_{50} values ranging from 2.52 to 14.49 μM, and they showed more potency than the parent compounds prenylated chalcones **1** and **2**. Substitution of the aminomethylene group such as pyrrolidinomethyl, piperidinomethyl, and 4-methyl-piperazinomethyl at the C-5′ position for **1** and the C-3′ position for **2** significantly enhanced the antiproliferative activity.

EXPERIMENTAL

General Information Melting points were determined *via* an XRC-1 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker-AV 400 spectrometer at 400 and 100 MHz, respectively, with TMS as internal standard in $CDCl_3$ or DMSO-d_6. Mass spectra (MS) and high-resolution mass spectra (HR-MS) were determined on a VG Autospec-3000 or Mat 95 XP spectrometer by the EI or ESI method. Column chromatography was carried out on 200–300 mesh silica gel (Qingdao Ocean Chemical Products, China). Commercially available AR or chemically pure reagents were used, and anhydrous solvents were dehydrated and redistilled using standard experimental procedures.

Synthesis of 2′,4,4′-Trimethoxymethoxy-6′-hydroxychalcone (6). A 100 mL round-bottom flask was charged with 4-methoxymethoxybenzaldehyde (0.4 g, 2.4 mmol), 2,4-dimethoxymethoxy-6-hydroxyacetophenone (**5**, 0.5 g, 2.0 mmol), KOH (1.0 g, 0.018 mmol), and 20 mL of ethanol. After stirring at room temperature for 24 h, the reaction liquid changed from the original yellow to orange red. TLC analysis showed the complete consumption of the raw materials. The solvent was removed, 50 mL of water added, and the aqueous layers extracted with CH_2Cl_2 (15 mL × 3) and then washed with saturated brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (20:1) to give a yellow solid 0.7 g, yield 89%, mp 83–85°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.84 (1H, s, OH), 7.77 (1H, d, J = 15.5, H-*β*), 7.70 (1H, d, J = 15.5, H-*α*), 7.48 (2H, d, J = 8.1, H-2, 6), 7.00 (2H, d, J = 8.2, H-3, 5), 6.25 (1H, s, H-3′), 6.18 (1H, s, H-5′), 5.22 (2H, s, OCH_2), 5.15 (2H, s, OCH_2), 5.12 (2H, s, OCH_2), 3.47 (3H, s, OCH_3), 3.42 (6H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.83, 166.28, 162.31, 158.81, 157.95, 141.42, 128.96, 128.19, 124.42, 115.50, 106.53, 96.48, 94.12, 93.73, 93.10, 55.86, 55.45, 55.16. MS (EI, 70 eV) *m/z* 404.14 $[M]^+$.

Synthesis of 2′,4,4′-Trimethoxymethoxy-6′-*O*-prenylchalcone (7). A mixture of compound **6** (0.5 g, 1.2 mmol) and K_2CO_3 (1.0 g, 7.2 mmol) in acetone (30 mL) was stirred at room temperature for 0.5 h, then 3,3-dimethylallylbromide (0.3 mL, 2.5 mmol) was added. The reaction was carried out for 1 h after the temperature was raised to 50°C. The reaction solution changed from orange red to pale yellow; TLC analysis showed the complete consumption of the raw materials after 5 h. The mixture was filtered through a Celite pad, and the filtrate was concentrated to give the crude product. It was purified by neutral alumina column chromatography eluted with petroleum ether–ethyl acetate (30:1) to give 0.3 g of yellow solid substance, yield 62%, mp 76–78°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.32 (2H, d, J = 6.7, H-2, 6), 7.18 (1H, d, J = 16.2, 1H, H-*β*), 6.88 (2H, d, J = 6.6, H-3, 5), 6.74 (1H, d, J = 16.0, H-*α*), 6.38 (1H, s, H-3′), 6.27 (1H, s, H-5′), 5.20 (1H, t, J = 12.9, CH=), 5.03 (4H, s, OCH_2), 4.96 (2H, s, OCH_2), 4.36 (2H, d, J = 6.4, CH_2), 3.34 (3H, s, OCH_3), 3.29 (3H, s, OCH_3), 3.23 (3H, s, OCH_3), 1.54 (3H, s, CH_3), 1.51 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.22, 159.58, 158.98, 157.67, 155.77, 144.31, 137.59, 131.61, 129.86, 128.54, 128.27, 127.39, 119.58, 116.37, 116.06, 114.28, 95.90, 95.22, 94.43, 94.03, 65.54, 56.24, 25.60, 18.11. MS (EI, 70 eV) *m/z* 472.20 $[M]^+$.

Synthesis of 2′,4,4′-Trimethoxymethoxy-6′-hydroxy-3′-prenylchalcone (8). A round-bottom flask was charged with compound **7** (0.5 g, 1.1 mmol) and Florisil (2.0 g) in toluene (40 mL), and the reaction was carried out at 100°C for 48 h. The TLC was tracked and the material disappeared. The reaction solution was filtered to remove Florisil, 80 mL of water was added, and the mixture was extracted with CH_2Cl_2 (15 mL × 3); then the organic layer was washed with saturated brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (30:1) to give 0.4 g of yellow solid substance, yield 85%, mp 103–105°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.01 (1H, s, OH), 7.22 (2H, d, J = 8.0, H-2, 6), 6.94 (2H, d, J = 8.0, H-3, 5), 6.13 (1H, d, J = 15.6, H-*β*),

5.18 (1H, d, J = 15.6, Hα), 5.05 (6H, s, OCH_2), 3.34 (9H, s, OCH_3), 3.17 (2H, d, J = 7.0, CH_2), 2.95 (1H, s, H-5′), 2.61 (1H, t, J = 8.0, CH=), 1.66 (3H, s, CH_3), 1.54 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.28, 162.83, 161.04, 160.56, 157.60, 150.89, 142.95, 131.81, 131.40, 130.19, 127.64, 122.45, 116.46, 110.68, 103.44, 94.33, 93.84, 93.46, 78.88, 56.27, 55.96, 43.29, 29.59, 25.79, 21.30, 17.76. MS (EI, 70 eV) *m/z* 472.21 $[M]^+$.

Synthesis of 2′,4,4′-Trimethoxymethoxy-6′-methoxy-3′-prenylchalcone (9). A mixture of compound **8** (0.2 g, 0.4 mmol) and K_2CO_3 (0.2 g, 1.4 mmol) in acetone (20 mL) was stirred at room temperature for 20 min; then dimethyl sulfate (1 mL) was added. The TLC was traced at 2 h, and the starting material point disappeared. The reaction solution was filtered, and the filtrate was concentrated to give the crude product. It was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (20:1) to give 0.1 g of yellow solid material. Yield 52%, mp 108–110°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.40 (2H, d, J = 8.4, H-2, 6), 7.28 (1H, d, J = 16.0, H-β), 6.95 (2H, d, J = 8.4, H-3, 5), 6.85 (1H, d, J = 16.0, H-α), 6.48 (1H, s, H-5′), 5.16 (2H, s, OCH_2), 5.12 (4H, s, OCH_2), 5.11 (1H, t, J = 8.0, CH=), 3.68 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.39 (3H, s, OCH_3), 3.24 (2H, d, J = 6.6, CH_2), 1.69 (3H, s, CH_3), 1.60 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.60, 159.06, 157.54, 157.03, 156.26, 144.49, 131.14, 130.11, 128.64, 127.10, 123.28, 117.74, 116.86, 116.42, 94.56, 94.19, 62.84, 56.13, 56.03, 30.21, 29.70, 25.75, 22.68, 17.82. MS (EI, 70 eV) *m/z* 486.22 $[M]^+$.

Synthesis of Xanthohumol (1). Hydrochloric acid (3 mol/L, 3 drops) was added to a solution of compound **9** (0.1 g, 0.2 mmol) in anhydrous methanol (20 mL). The reaction was carried out at 40°C for 1 h. The solvent was evaporated, and water (80 mL) was added. The mixture was extracted with ethyl acetate (15 mL × 3); then the organic layer was washed with saturated brine, dried over Na_2SO_4, filtered, and the filtrate evaporated. The crude product was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (8:1) to give 40 mg of a yellow solid, yield 57%, mp 158–160°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.65 (1H, s, OH), 10.57 (1H, s, OH), 10.08 (1H, s, OH), 7.78 (1H, d, J = 15.5, H-β), 7.69 (1H, d, J = 15.5, H-α), 7.59 (2H, d, J = 8.0, H-2, 6), 6.86 (2H, d, J = 8.0, H-3, 5), 6.10 (1H, s, H-5′), 5.16 (1H, t, J = 8.0, CH=), 3.88 (3H, s, OCH_3), 3.16 (2H, d, J = 6.7, CH_2), 1.71 (3H, s, CH_3), 1.62 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.15, 165.10, 162.87, 161.00, 160.39, 143.00, 130.97, 130.39, 126.54, 124.26, 123.50, 116.46, 107.81, 105.06, 91.42, 56.22, 25.95, 21.52, 18.14. MS (EI, 70 eV) *m/z* 354.9 $[M]^+$.

Synthesis of Xanthohumol Mannich Base Derivatives 3a–3e. A round bottom flask was charged with secondary amines (3 mmol), methanol (10 mL), concentrated hydrochloric acid (3 drops), and 37% HCHO aqueous solution (1.0 mL). After stirring at room temperature for 20 min, compound **1** (100 mg, 0.28 mmol) and methanol (20 mL) were added to the reaction flask, and the whole heated to 80°C and reacted for 2 h. When TLC analysis showed the complete consumption of compound **1**, the mixture was concentrated, and then water (80 mL) was added. The mixture was extracted with dichloromethane (15 mL × 3), then the organic layer was washed with saturated brine, dried over Na_2SO_4, filtered, and the filtrate evaporated. The crude product was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (3:1) to give **3a–3e**.

5′-Dipropylaminomethylxanthohumol (3a). Light yellow solid 100 mg, yield 77%, mp 242–244°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.73 (1H, d, J = 15.6, H-β), 7.65 (1H, d, J = 15.6, H-α), 7.40 (2H, d, J = 8.0, H-2, 6), 6.78 (2H, d, J = 7.8, H-3, 5), 5.19 (1H, t, J = 6.8, CH=), 3.83 (2H, s, CH_2N), 3.52 (3H, s, OCH_3), 3.26 (2H, d, J = 6.7, CH_2), 2.66 (4H, q, J = 6.6, NCH_2), 1.71 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.09 (6H, t, J = 7.0, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.43, 163.86, 161.32, 158.89, 142.86, 131.80, 130.31, 128.74, 123.94, 122.66, 116.08, 115.49, 112.81, 106.22, 62.86, 50.03, 46.15, 31.43, 30.21, 29.71, 25.81, 21.70, 17.93, 10.68. MS (EI, 70 eV) *m/z* 467.1 $[M]^+$.

5′-Pyrrolidin-1-ylmethylxanthohumol (3b). Yellow solid 80 mg, yield 67%, mp 220–222°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.76 (1H, d, J = 16.2, H-β), 7.70 (1H, d, J = 15.8, H-α), 7.57 (2H, d, J = 7.9, H-2, 6), 6.86 (2H, d, J = 8.0, H-3, 5), 5.18 (1H, t, J = 6.5, CH=), 4.01 (3H, s, OCH_3), 3.60 (2H, s, CH_2N), 3.18 (2H, d, J = 6.9, CH_2), 2.83–2.85 (4H, m, NCH_2), 1.88–1.85 (4H, m, CH_2), 1.72 (3H, s, CH_3), 1.62 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 193.38, 164.30, 158.58, 143.56, 131.00, 128.71, 124.69, 122.97, 116.59, 113.31, 106.16, 67.26, 63.64, 55.08, 53.37, 30.32, 26.44, 22.30, 18.52. MS (EI, 70 eV) *m/z* 455.93 $[M + H_2O]^+$.

5′-Piperidin-1-ylmethylxanthohumol (3c). Yellow solid 90 mg, yield 71%, mp 248–250°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.69 (1H, d, J = 15.5, H-β), 7.64 (1H, d, J = 15.5, H-α), 7.40 (2H, d, J = 7.4, H-2, 6), 6.81 (2H, d, J = 7.4, H-3, 5), 5.18 (1H, t, J = 6.5, CH=), 3.80 (3H, s, OCH_3), 3.71 (2H, s, CH_2N), 3.54–3.52 (4H, m, CH_2), 3.28 (2H, d, J = 5.3, CH_2), 1.72 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.62–1.60 (4H, m, CH_2), 1.21–1.20 (2H, m, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.54, 163.07, 158.27, 157.88, 142.38, 131.44, 129.37, 126.39, 122.47, 121.26, 115.11, 111.59, 106.80, 104.34, 61.99, 54.12, 53.07, 52.48, 28.14, 24.79, 23.81, 22.26, 20.74, 16.90. ESI-MS *m/z* 452.07 $[M + H]^+$.

5′-Morpholine-1-ylmethylxanthohumol (3d). Pale yellow solid 90 mg, yield 71%, mp 230–232°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.76 (1H, d, J = 16.2, H-β), 7.67 (1H, d, J = 15.8, H-α), 7.46 (2H, d, J = 7.1, H-2, 6), 6.79

(2H, d, J = 6.8, H-3, 5), 5.19 (1H, t, J = 6.5, CH=), 3.85 (2H, s, CH_2N), 3.76–3.73 (4H, m, OCH_2), 3.54 (3H, s, OCH_3), 3.27 (2H, d, J = 5.5, CH_2), 2.58–2.55 (4H, m, NCH_2), 1.73 (3H, s, CH_3), 1.62 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.74, 162.66, 156.93, 141.92, 129.36, 127.06, 123.05, 121.33, 114.95, 111.67, 104.51, 65.62, 62.00, 53.44, 51.73, 29.19, 28.99, 28.68, 24.80, 20.65, 16.87. ESI-MS *m/z* 453.9 $[M + H]^+$.

5′-(4-Methyl)piperazin-1-ylmethylxanthohumol (3e). Light yellow solid 100 mg, yield 77%, mp 250–252°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.74 (1H, d, J = 15.6, H-*β*), 7.67 (1H, d, J = 15.5, H-*α*), 7.45 (2H, d, J = 7.9, H-2, 6), 6.78 (2H, d, J = 7.8, H-3, 5), 5.18 (1H, t, J = 6.8, CH=), 3.74 (2H, s, CH_2N), 3.51 (3H, s, OCH_3), 3.25 (2H, d, J = 6.5, CH_2), 2.88 (4H, t, J = 12.1, NCH_2), 2.60 (4H, t, J = 19.0, CH_2), 2.35 (3H, s, NCH_3), 1.73 (3H, s, CH_3), 1.62 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.74, 162.60, 162.26, 158.11, 157.82, 142.21, 130.86, 129.44, 126.33, 122.60, 121.41, 115.21, 111.57, 107.15, 104.72, 61.96, 53.45, 52.85, 50.55, 44.34, 24.80, 20.64, 16.88. ESI-MS *m/z* 467.13 $[M + H]^+$.

Synthesis of 2′-Hydroxy-3,4,4′-trimethoxy-6-*O*-prenylchalcone (2). A mixture of compound **11** (0.5 g, 1.5 mmol) and K_2CO_3 (1.0 g, 7.2 mmol) in acetone (30 mL) was stirred at 50°C for 1 h; then 3,3-dimethylallyl bromide (0.3 mL, 2.5 mmol) was added. The reaction was allowed to continue for 4 h. TLC analysis showed the complete consumption of compound **11,** and the reaction liquid changed from the original orange red to pale yellow. The mixture was filtered and the filtrate was concentrated. The crude product was chromatographed on a silica gel column eluted with petroleum ether–ethyl acetate (15:1) to give the a yellow solid 0.4 g, yield 67%, mp 82–84°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.45 (1H, s, OH), 7.83 (1H, d, J = 14.6, H-*β*), 7.69 (1H, d, J = 14.2, H-*α*), 7.12 (1H, d, J = 8.2, H-6), 7.01 (1H, s, H-2), 6.78 (1H, d, J = 8.3, H-5), 6.01 (1H, s, H-5′), 5.88 (1H, s, H-3′), 5.50 (1H, t, J = 5.7, CH=), 4.48 (2H, d, J = 6.6, CH_2), 3.87 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 1.72 (3H, s, CH_3), 1.66 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.59, 167.48, 164.98, 160.81, 149.94, 148.07, 141.36, 138.19, 127.71, 124.74, 121.20, 117.69, 110.12, 105.39, 92.73, 91.06, 86.68, 64.78, 57.54, 54.95, 54.52, 24.80, 17.30. ESI-MS *m/z* 439.9 $[M + H_2O + Na]^+$.

Synthesis of 2′-hydroxyl-3,4,4′-trimethoxy-6′-*O*-prenylchalcone Mannich base Derivatives 4a–4e. A solution of 37% HCHO aqueous solution (1 mL), amines (3.5 mmol), and concentrated hydrochloric acid (3 drops) in methanol (30 mL) was cooled to room temperature and reacted for 20 min; compound **2** (300 mg, 0.7 mmol) was added to the reaction flask, the whole warmed to 80°C, and the reaction continued for 3 h. When TLC analysis showed the complete consumption of compound **2**, the mixture was concentrated, and then water (80 mL) was added. The mixture was extracted with dichloromethane (15 mL × 3); then the organic layer was washed with saturated brine, dried over Na_2SO_4, filtered, and the filtrate evaporated. The crude product was purified by silica gel column chromatography eluted with petroleum ether–ethyl acetate (3:1) to give **4a–4e**.

2′-Hydroxy-3′-dipropylaminomethyl-3,4,4′-trimethoxy-6′-*O*-prenylchalcone (4a). Yellow solid 300 mg, yield 78%, mp 148–150°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.98 (1H, s, OH), 7.82 (1H, d, J = 15.6, H-*β*), 7.69 (1H, d, J = 15.3, H-*α*), 7.15 (1H, d, J = 8.0, H-6), 7.03 (1H, s, H-2), 6.81 (1H, d, J = 8.2, H-5), 6.00 (1H, s, H-5′), 5.52 (1H, t, J = 6.5, CH=), 4.60 (2H, d, J = 4.8, CH_2), 4.16 (2H, s, CH_2N), 4.01 (3H, s, OCH_3), 3.87 (6H, s, OCH_3), 3.06–2.91 (4H, m, NCH_2), 1.89–1.91 (4H, m, CH_2), 1.74 (3H, s, CH_3), 1.70 (3H, s, CH_3), 1.62–1.60 (6H, m, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.56, 150.42, 149.02, 142.07, 129.88, 127.77, 127.48, 121.56, 118.27, 111.34, 109.77, 86.76, 67.11, 64.96, 55.94, 54.32, 37.71, 29.34, 28.67, 27.90, 24.77, 22.72, 21.96, 21.66, 17.81, 17.31, 13.04, 10.79, 9.94. ESI-MS *m/z* 511.9 $[M + H]^+$.

2′-Hydroxy-3′-pyrrolidin-1-ylmethyl-3,4,4′-trimethoxy-6′-*O*-prenylchalcone (4b). Yellow solid 310 mg, yield 83%, mp 118–120°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.63 (1H, d, J = 15.6, H-*β*), 7.55 (1H, d, J = 15.6, H-*α*), 7.19 (1H, d, J = 8.2, H-6), 7.12 (1H, s, H-2), 6.88 (1H, d, J = 8.0, H-5), 6.04 (1H, s, H-5′), 5.53 (1H, t, J = 6.8, CH=), 4.61 (2H, d, J = 6.4, CH_2), 3.94 (6H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.79 (2H, s, CH_2N), 2.65–2.62 (4H, m, NCH_2), 1.79 (3H, s, CH_3), 1.74 (3H, s, CH_3), 1.78–1.76 (4H, m, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 193.45, 162.96, 162.19, 160.06, 150.87, 149.08, 142.57, 138.45, 128.66, 126.65, 122.40, 119.28, 111.08, 110.65, 108.59, 105.54, 87.65, 65.94, 55.95, 55.58, 53.77, 47.91, 25.80, 23.55, 18.34, 1.03. ESI-MS *m/z* 482.1 $[M + H]^+$.

2′-Hydroxy-3′-piperidin-1-ylmethyl-3,4,4′-trimethoxy-6′-*O*-prenylchalcone (4c). Yellow solid 300 mg, yield 81%, mp 108–110°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.45 (1H, d, J = 15.7, H-*β*), 7.19 (H, d, J = 15.7, H-1*α*), 7.07 (1H, d, J = 8.1, H-6), 7.02 (1H, s, H-2), 6.78 (1H, d, J = 8.1, H-5), 5.95 (1H, s, H-5′), 5.39 (1H, t, J = 6.8, CH=), 4.49 (2H, d, J = 6.4, CH_2), 3.84 (6H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.60 (2H, s, CH_2N), 2.45–2.42 (4H, m, CH_2), 1.66 (3H, s, CH_3), 1.63 (3H, s, CH_3), 1.53–1.52 (4H, m, CH_2), 1.37–1.36 (2H, m, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 180.44, 179.49, 166.62, 161.59, 149.82, 148.04, 141.94, 127.53, 125.93, 121.55, 109.98, 109.36, 103.53, 98.93, 86.82, 64.93, 54.94, 54.55, 52.93, 25.89, 24.75, 21.60, 17.28, 9.73. ESI-MS *m/z* 496.1 $[M + H]^+$.

2′-Hydroxy-3′-morpholin-1-ylmethyl-3,4,4′-trimethoxy-6′-*O*-prenylchalcone (4d). Pale yellow solid 330 mg, yield 82%, mp 144–146°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.65 (1H, d, J = 15.4, H-*β*), 7.59 (1H, d, J = 15.7, H-*α*), 7.12 (1H, d, J = 8.1, H-6), 7.02 (1H, s, H-2), 6.80 (1H, d, J = 8.0, H-5), 5.95 (1H, s, H-5′), 5.48 (1H, t, J = 6.8, CH=), 4.54

(2H, d, J = 6.4, CH_2), 3.85 (6H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.65–3.62 (4H, m, CH_2O), 3.61 (2H, s, CH_2N), 2.52–2.50 (4H, m, CH_2N), 1.71 (3H, s, CH_3), 1.67 (6H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.19, 163.63, 162.62, 160.21, 149.95, 148.08, 141.58, 138.05, 127.61, 125.18, 121.29, 117.86, 110.10, 109.87, 106.39, 86.53, 65.82, 64.91, 57.25, 54.95, 54.62, 52.15, 49.06, 24.81, 17.34. ESI-MS *m/z* 497.9 $[M + H]^+$.

2′-Hydroxy-3′-(4-methyl)piperazin-1-ylmethyl-3,4,4′-trimethoxy-6′-*O*-prenylchalcone (4e). Light yellow solid 300 mg, yield 79%, mp 136–138°C. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.54 (1H, d, J = 15.6, H-*β*), 7.48 (1H, d, J = 15.8, H-*α*), 7.10 (1H, d, J = 8.1, H-6), 7.02 (1H, s, H-2), 6.79 (1H, d, J = 8.1, H-5), 5.94 (1H, s, H-5′), 5.44 (1H, t, J = 6.8, CH=), 4.51 (2H, d, J = 6.4, CH_2), 3.85 (6H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.64 (2H, s, CH_2N), 2.55–2.52 (4H, m, NCH_2), 2.42–2.40 (4H, m, CH_2), 2.20 (3H, s, NCH_3), 1.69 (3H, s, CH_3), 1.65 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.36, 149.89, 148.07, 141.66, 137.63, 127.59, 125.48, 121.39, 118.13, 110.05, 109.66, 107.33, 86.67, 64.91, 54.93, 54.55, 53.85, 51.50, 49.33, 44.90, 33.64, 26.34, 25.89, 24.78, 24.25, 17.32. ESI-MS *m/z* 511.1 $[M + H]^+$.

Antiproliferative Activity Screening. Using MTT assay, all the cells were cultured in an RPMI 1640 medium containing 10%FBS and incubated at 37°C in a 5% CO_2 humidified incubator to keep the cells growing in the exponential phase. Briefly, MDA-MB-231 cells and K562 cells in a 100 μL culture medium were plated into a 96-well plate at 4000–5000 cells per well and subsequently cultured in the RPMI1640 medium containing 10% FBS, incubated at 37°C for 24 h prior to drug exposure. The selected compounds were weighed and dissolved in DMSO and then diluted with medium to the needed concentrations. Cells were treated to a final concentration of 100, 50, 25, 12.5, 6.25, and 3.125 μM of the tested compounds and incubated for 48 h; then 20 μL of 0.5% MTT solution was added to each well simultaneously and the whole incubated at 37°C for 4 h. The formed formazan crystals were dissolved by adding 150 μL of DMSO. The optical density at 570 nm was determined on a microliter-plate reader. According to the inhibition ratios, the IC_{50} value was obtained.

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