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Hydrolyzable Tannins of Tamaricaceous Plants. V. Structures of Monomeric−Trimeric Tannins and Cytotoxicity of Macrocyclic-Type Tannins Isolated from *Tamarix nilotica*[1]

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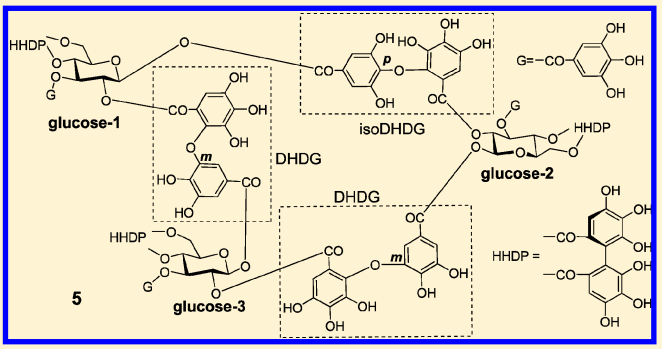
Supporting Information

ABSTRACT: Three new ellagitannin monomers, nilotinins M5−M7 (**1**−**3**), a dimer, nilotinin D10 (**4**), and a trimer, nilotinin T1 (**5**), together with three known dimers, hirtellin D (**7**) and tamarixinins B (**8**) and C (**9**), and a trimer, hirtellin T2 (**6**), were isolated from *Tamarix nilotica* dried leaves. The structures of the tannins were elucidated by intensive spectroscopic methods and chemical conversions into known tannins. The new trimer (**5**) is a unique macrocyclic type whose monomeric units are linked together by an isodehydrodigalloyl and two dehydrodigalloyl moieties. Additionally, dimeric and trimeric macrocyclic-type tannins isolated from *T. nilotica* in this study were assessed for possible cytotoxic activity against four human tumor cell lines. Tumor-selective cytotoxicities of the tested compounds were higher than those of synthetic and natural potent cytotoxic compounds, including polyphenols, and comparable with those of 5-fluorouracil and melphalan.



Ellagitannins are a unique group of polyphenols that are widely distributed in plant foods and beverages.[2] Ellagitannins from different plant sources have been demonstrated to have marked antiviral, antimicrobial, immunomodulatory, antitumor, and hepatoprotective activities.[2−8] Plants from the family Tamaricaceae have been shown to produce several types of tannins.[1,9−17] Ellagitannins from tamaricaceous plants were reported to exhibit significant host-mediated antitumor activities against sarcoma-180 in mice and strong cytotoxic effects with higher tumor specificity against four tumor cell lines.[11−13] Further investigation of the aqueous acetone extract of the leaves of *Tamarix nilotica* (Ehrenb.) Bunge (Tamaricaceae) has led to the isolation of several tannins. This paper describes the isolation and identification of three new ellagitannin monomers, nilotinins M5 (**1**), M6 (**2**), and M7 (**3**), a new dimer, nilotinin D10 (**4**), a new macrocyclic-type trimer, nilotinin T1 (**5**), and the known but first time isolated tannins hirtellins T2 (**6**) and D (**7**) and tamarixinins B (**8**) and C (**9**), as well as the purification of additional amounts of the known macrocyclic-type tannins nilotinin D9 (**10**) and hirtellins C (**11**) and F (**12**)[11] from *T. nilotica*. We also report the results of assessing the cytotoxic activities of the macrocyclic-type tannins **5**, **8**, and **10**−**12** against the human oral squamous cell carcinoma (HSC-2, HSC-3, and HSC-4) and human promyelocytic leukemia (HL-60) cell lines and human normal oral cells [gingival fibroblast (HGF), pulp cell (HPC), and periodontal ligament fibroblast (HPLF)] to determine the tumor specificity.

RESULTS AND DISCUSSION

An aqueous acetone extract of the dried leaves of *T. nilotica* was subjected to Diaion HP 20 column chromatography, and compounds were eluted with H_2O, MeOH/H_2O (40:60%, v/v), MeOH, and acetone, successively. The eluate with H_2O/MeOH (6:4, v/v) was submitted to Toyopearl HW-40, Sephadex LH-20, and MCI-gel CHP-20P gel chromatography, followed mainly by preparative HPLC purifications to furnish five new monomeric−trimeric tannins (**1**−**5**), four known oligomeric tannins, hirtellins T2 (**6**)[17] and D (**7**)[13] and tamarixinins B (**8**)[15] and C (**9**),[15] and additional amounts of the known macrocyclic-type tannins nilotinin D9 (**10**)[11] and hirtellins C (**11**)[11] and F (**12**).[11] Although tamarixinin C (**9**) was previously assigned to the structure unambiguously,[15] our results revealed the assignments of the protons and carbons of

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Table 1. ^{1}H NMR Data δ_H (J in Hz) for the Glucose Protons of 1−5 and 9 (600 MHz, acetone-d_6/D_2O, 9:1)

	1	2	3	9	4	5
glucose-1						
1	5.50, d (8.4)	6.02, d (7.8)	6.02, d (7.8)	6.21, d (8.4)	5.24, d (7.8)	6.16 br s
2	5.55, dd (8.4, 9.6)	5.65, dd (7.8, 9.6)	5.63, dd (7.8, 9.6)	5.63, dd (8.4, 9.6)	5.31, dd (7.8, 9.6)	5.56[a]
3	5.47, t (9.6)	5.72, t (9.6)	5.74, t (9.6)	5.81, t (9.6)	5.34, t (9.6)	5.70,[b] t (9.6)
4	5.11, t (9.6)	5.19, t (9.6)	5.19, t (9.6)	5.22, t (9.6)	3.85, t (9.6)	5.12,[c] t (9.6)
5	4.23, dd (6.6, 9.6)	4.43, dd (6.6, 9.6)	4.43, ddd (1.2, 6.6, 9.6)	4.54, dd (6.6, 9.6)	~3.60[d]	4.25,[e] dd (6.6, 9.6)
6	5.25, dd (6.6, 13.2)	5.28,dd (6.6, 13.2)	5.28,dd (6.6, 13.2)	5.33, dd (6.6, 12.6)	3.78, dd (6.6, 13.2)	5.26,[f] dd (6.6, 13.2)
	3.80, br d (13.2)	3.83, d (13.2)	3.83, dd (1.2, 13.2)	3.92, d (12.6)	3.93, dd (2.4, 13.2)	3.84,[g] d (13.2)
glucose-2						
1				6.03, d (8.4)	5.44, d (7.8)	5.76 br s
2				5.63, dd (8.4, 9.6)	5.51, dd (7.8, 9.6)	5.44, dd (7.8, 9.6)
3				5.76, t (9.6)	5.59, t (9.6)	5.80, t (9.6)
4				5.19, t (9.6)	5.10, t (9.6)	5.19, t (9.6)
5				4.45, dd (6.6, 9.6)	4.24, dd (6.6, 9.6)	4.34[e,h]
6				5.30, dd (6.6, 13.2)	5.26, dd (6.6, 13.2)	5.23,[f,i] dd (6.6, 13.2)
				3.85, d (13.2)	3.81, br d (13.2)	3.76,[g], d (13.2)
glucose-3						
1						5.24[i]
2						5.56[a]
3						5.56[a,b]
4						5.07,[c] t (10.2)
5						4.34[e,h]
6						5.36,[f] dd (6.6, 13.2)
						3.72,[g] d (13.2)

[a,h,i]Proton signals with these chemical shifts overlapped, and thus their coupling constants were not measured. [b,c,e−g]Interchangeable. [d]Overlapped by solvent signal.

the sugar cores in the NMR spectra should be corrected as shown in Tables 1 and 2.

Structural Elucidation of Monomeric Ellagitannins. Nilotinin M5 (**1**) was isolated as an off-white, amorphous powder. Its molecular formula was $C_{55}H_{38}O_{36}$ from the prominent ion peak ($[M + Na]^+$) at m/z 1297.10358 in the HRESIMS spectrum. The ^{1}H NMR spectrum of **1** displayed aromatic proton signals of two mutually coupled doublets [δ_H 7.21 and 6.46 (each 1H, d, J = 1.8 Hz)] and a one-proton singlet (δ_H 7.06), characteristic of the dehydrodigalloyl moiety (DHDG).[9−11,17] The spectrum also showed a two-proton singlet (δ_H 6.96) and one-proton singlet (δ_H 6.89), characteristic of an isodehydrodigalloyl moiety (isoDHDG),[9−11] and a second two-proton singlet (δ_H 6.93) and a pair of one-proton singlets (δ_H 6.58 and 6.51) ascribable to galloyl and hexahydroxydiphenoyl (HHDP) units, respectively.[18] The aliphatic region of the spectrum showed seven sets of well-resolved signals (δ_H 5.50−3.80) assignable to the protons of a fully *O*-acylated glucose core (Table 1).[9−11] The large coupling constants ($J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz) of these proton signals indicated the existence of the glucose core in the pyranose form with a $^4C_1$ conformation and a β-oriented acyl group at the anomeric carbon. The ^{13}C NMR spectrum of **1** showed the aliphatic, aromatic, and the carbonyl carbon peaks (Tables 2 and 3), which were assigned on the basis of correlations in the HSQC and HMBC spectra, characteristic of the structural moieties of **1**. A galloyl unit was placed at C-3 of the glucose core, as indicated from an HMBC correlation between a galloyl proton signal (δ_H 6.93, 2H, s) and a glucose H-3 signal (δ_H 5.47, 1H, t) through a common carbonyl peak (δ_C 166.6). The acylation of the glucose core C-4/C-6 by an HHDP unit was suggested by the large chemical shift difference ($\Delta\delta_H$ 1.45) between the geminal coupled C-6 methylene protons.[19] Bridging of the HHDP unit at C-4/C-6 of the glucose core was further confirmed by HMBC correlations among the HHDP proton signals (δ_H 6.58 and 6.51) and signals of H-6 (δ_H 5.25) and H-4 (δ_H 5.11) of the glucose core through the common carbonyl carbon peaks (δ_C 168.3 and 167.7), respectively. In the ^{13}C NMR spectrum of **1**, the carbon

Table 2. ^{13}C NMR Data for the Glucose Carbons of 1−5 and 9 (151 MHz, acetone-d_6/D_2O, 9:1)

	1	2	3	9	4	5
glucose-1						
1	93.8	93.7	93.5	93.7	93.3	93.3
2	71.4	71.9	72.0	71.7	71.5	71.6[a]
3	73.6	73.5	73.4	73.4	76.4	73.4[b]
4	70.5	70.6	70.6	70.65	68.7	70.8[c]
5	72.8	72.8	72.7	72.9	78.0	72.9[d]
6	62.9	63.0	63.0	63.0	61.4	63.4[e]
glucose-2						
1				93.5	93.6	93.3
2				71.9	71.6	72.5
3				73.3	73.5	73.4
4				70.58	70.6	70.5
5				72.8	72.8	72.7[d]
6				63.0	63.0	63.5[e]
glucose-3						
1						93.8
2						71.2[a]
3						74.4[b]
4						70.7[c]
5						73.0.[d]
6						63.1[e]

[a−e]Interchangeable.

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Table 3. ^{13}C NMR Data for the Aromatic Skeleton of the Tannins 1−5 (151 MHz, acetone-d_6/D_2O, 9:1)

	1	2	3	4	5[a]
DHDG					
1	121.8			119.9, 122.2	119.3, 119.6
2	106.7			106.6, 107.1	107.2, 107.8
3	147.6			147.4, 147.5	147.7 (2C)
4	138.8[b]			138.5,[c] 139.5[c]	139.43,[d] 139.7[d]
5	146.0			145.7, 145.8	145.6, 145.7
6	112.7			112.6, 112.8	112.2, 112.5
7	168.1			164.3, 168.9	163.4, 164.4
1′	113.4			113.1, 113.5	112.3, 112.5
2′	136.8			136.78, 136.84	135.8, 137.2
3′	140.4[b]			140.3,[c] 140.59[c]	139.9,[d] 140.2[d]
4′	140.8[b]			140.62,[c] 141.2[c]	140.1,[d] 140.9[d]
5′	143.4			143.2, 143.8	142.6, 143.4
6′	110.6			110.18, 110.23	109.6, 110.6
7′	164.7			164.8 (2C)	164.0, 164.1
isoDHDG					
1	124.6	124.9, 126.5	126.5		123.3
2/6	110.8 (2C)	110.5, 110.9 (2C each)	110.5		110.4 (2C)
3/5	150.3 (2C)	149.5, 150.7 (2C each)	149.4		148.1(2C)
4	139.3	138.4, 139.8	138.3		139.36[d]
7	164.3	164.8, 168.33	168.2		164.9
1′	114.4	113.5 (2C)	113.5		114.5
2′	139.5[b]	139.06, 139.14	139.2[e]		139.7[d]
3′	139.6[b]	139.6, 140.8	140.6[e]		139.65[d]
4′	139.9[b]	139.3, 140.1	140.1		139.9[d]
5′	142.2	142.0, 142.3	142.1		142.4
6′	108.3	108.2, 108.5	108.4		108.2
7′	170.3	166.6, 170.7	166.8		166.3
galloyl					
1	119.9	120.1	119.4, 120,0	119.1, 119.7, 120.5	120.4, 120.2, 120.0
2/6	110.16 (2C)	110.2 (2C)	110.18, 110.23	110.1, 110.18, 110.3 (2C each)	110.3 (2C), 110.4 (4C)
3/5	145.7 (2C)	145.7 (2C)	145.7, 145.9 (2C each)	145.8 (4C), 145.7 (2C)	145.7, 145.8, 145.9 (2C each)
4	139.0	139.3	139.3, 139.8	139.2, 139.3, 139.8	139.23, 139.26 139.6
7	166.6	166.8	165.2, 166.8	164.6, 166.8, 167.1	167.0 (2C), 167.4
HHDP					
1	115.7	115.6	115.6	115.7	115.6, 115.8 (2C)
1′	115.8	115.9	115.9	115.8	115.86, 115.93, 116.2
2, 2′	125.4, 126.0	125.5, 126.1	125.4, 126.1	125.4, 126.0	125.5, 125.6, 125.8, 126.1, 126.2 (2C)
3	107.9	108.0	107.9	107.84	108.1, 108.2 (2C)
3′	107.8	107.8	107.8	107.78	107.9 (3C)
4, 4′	145.1, 145.2	145.16, 145.23	145.15, 145.2	145.1, 145.2	145.2 (6C)
5	136.3	136.3	136.3	136.3	136.3, 136.4 (2C)
5′	136.5	136.6	136.6	136.5	136.5, 136.6, 136.8
6, 6′	144.3 (2C)	144.33, 144.36	144.3 (2C)	144.3 (2C)	144.27 (2C), 144.33 (4C)
7	168.3	168.3	168.3	168.3	168.3, 168.5 (2C)
7′	167.7	167.8	167.8	167.8	167.75, 167.87, 167.92

[a]The assignments of the carbons of the DHDG and isoDHDG moieties in **5** were achieved on the basis of HSQC and comparison with spectroscopic data of the monomeric tannins **1**−**3** and those of the previously isolated analogous macrocyclic-type tannins **10**−**12**.[11] [b−e]Interchangeable.

peak at δ_C 150.3 was assigned to C-3/C-5 of an isoDHDG moiety since it has been demonstrated that C-3/C-5 of the isoDHDG moiety resonate at lower field (δ_C ~150) than C-3/C-5 of the usual galloyl unit (δ_C ~145.7) (Table 3).[9,10] Assignment of a two-proton singlet (δ_H 6.96) to H-2/H-6 of an isoDHDG moiety was confirmed by HMBC connectivity with the characteristic carbon peak (δ_C 150.3) of C-3/C-5 of the same moiety.[9,10] The HMBC correlation of this two-proton singlet with the glucose H-1 signal (δ_H 5.50) through the ester carbonyl carbon (δ_C 164.3) indicated the location of the isoDHDG moiety at C-1 of the glucose core. A carboxylic carbon peak (δ_C 170.3) was assigned to C-7′ of an isoDHDG unit, as indicated from its HMBC correlation with the H-6′ signal (δ_H 6.89) of the same isoDHDG moiety. The absence of a similar correlation of any of the glucose proton signals with the latter carboxylic carbon peak satisfied orientation of the isoDHDG moiety as shown by the formula **1**. Similarly, placement of the DHDG moiety at C-2 of the glucose core was substantiated from connectivity in the HMBC spectrum between the H-6′ singlet signal (δ_H 7.06) of the DHDG

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moiety and the glucose H-2 signal (δ_H 5.55) through an ester carbonyl carbon peak (δ_C 164.7). The carboxylic carbon peak (δ_C 168.1) was assigned to C-7 of the DHDG moiety because of the HMBC correlation with the *meta*-coupled signals (δ_H 7.21 and 6.46) of the same ring. Lack of HMBC correlations of any of the glucose proton signals with this carboxylic carbon peak satisfied the attachment mode of the DHDG moiety as shown by formula **1** (Figure 1). The chiral HHDP unit in **1** was shown to have an *S* configuration, as deduced from a positive Cotton effect at 236 nm in the electronic circular dichroism (ECD) spectrum.[20] On the basis of these findings, nilotinin M5 was formulated as **1**. It is the first monomeric hydrolyzable tannin including both DHDG and isoDHDG moieties.

Nilotinin M6 (**2**) was isolated as an off-white, amorphous powder. Its molecular formula, $C_{55}H_{38}O_{36}$, the same as that of **1**, was determined by HRESIMS. The ^{1}H NMR spectrum displayed two two-proton singlets (δ_H 7.06 and 6.99) and a pair of one-proton singlets (δ_H 6.87 and 6.84), characteristic of two isoDHDG moieties.[9−11,15] Proton signals due to a galloyl (δ_H 6.97) and an HHDP (δ_H 6.60 and 6.49) unit were evident in the aromatic region.[18] The aliphatic proton signals comprised seven spin systems of well-resolved signals (δ_H 6.02−3.83) with large coupling constants (Table 1) assignable to protons of a fully *O*-acylated glucopyranose core with a $^4C_1$ conformation including a β-oriented acyl group at the anomeric center. The ^{13}C NMR spectrum also showed the aliphatic (Table 2), aromatic, and carbonyl carbon peaks (Table 3) consistent with the structural moieties of **2**. Acylation of the C-4/C-6 of the glucose core by an HHDP unit was suggested because of the large chemical shift difference ($\Delta\delta_H$ 1.45) between the *gem*-coupled proton signals (δ_H 5.28 and 3.83) of the C-6 methylene protons.[19] It was also evidenced by HMBC correlations between HHDP proton signals (δ_H 6.60 and 6.49) and signals of H-6 (δ_H 5.28) and H-4 (δ_H 5.19) of the glucose core through common carbonyl carbon peaks (δ_C 168.3 and 167.8), respectively (Figure 2). A galloyl unit was shown to be at C-3 of the glucose core by HMBC correlation between the galloyl proton signal (δ_H 6.97) and the glucose H-3 signal (δ_H 5.72) through a common carbonyl carbon peak (δ_C 166.8). Consequently, the isoDHDG moieties should be at C-1 and C-2 of the glucose core. A pair of two-proton singlets (δ_H 7.06 and 6.99) was assigned to H-2/H-6 of the two isoDHDG moieties, as indicated from two-bond HMBC connectivity with the characteristic carbon peaks (δ_C 150.7 and 149.5) of C-3/C-5 of the same moieties, respectivly.[9−11,16] The attachment mode of the isoDHDG moieties in **2** (Figure 1) was substantiated from HMBC correlations of H-2/H-6 [δ_H 7.06 (2H, s)] of an isoDHDG moiety and H-6′ [δ_H 6.84 (1H, s)] of the other isoDHDG moiety with the glucose H-1 signal [δ_H 6.02 (1H, d)] and the glucose H-2 signal [δ_H 5.65 (1H, dd)] via the carbonyl carbon peaks (δ_C 164.8 and 166.6), respectively. On the other hand, the remaining H-6′ signal [δ_H 6.87 (1H, s)] of the isoDHDG moiety at C-1 of glucose and the H-2/H-6 signal [δ_H 6.99 (2H, s) of the other one showed HMBC correlations with the carboxylic carbon peaks (δ_C 170.7 and 168.33), respectively. Lack of HMBC correlations from the aliphatic proton signals to these carboxylic carbon peaks satisfied orientations of the isoDHDG moieties as shown by formula **2** (Figure 1). A positive Cotton effect at 236 nm in the ECD spectrum indicated an *S* configuration of the chiral HHDP unit.[20] On the basis of this spectroscopic evidence, nilotinin M6 was assigned the structure **2** (Figure1), which is isomeric to nilotinin M5 (**1**), where the

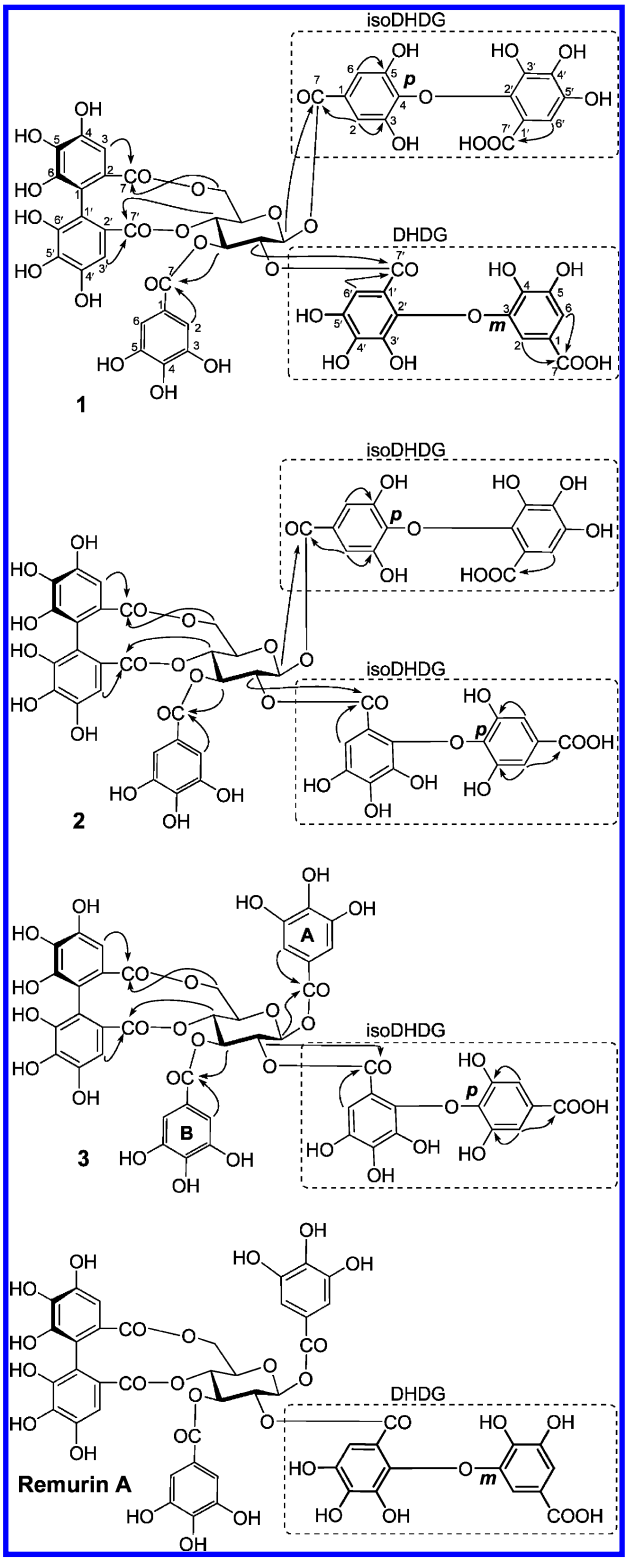

Figure 1. Structures of the new tannins **1**−**3** and the known one remurin A. The arrows (H→C) indicate important HMBC correlations.

DHDG moiety at C-2 of the glucose core in **1** was replaced with an isoDHDG moiety in **2**. It is the first monomeric hydrolyzable tannin including two isoDHDG moieties.

Nilotinin M7 (**3**) was isolated as an off-white, amorphous powder. Its molecular formula, $C_{48}H_{34}O_{31}$, the same as that of remurin A,[9] was determined from a prominent ion peak at *m*/*z*

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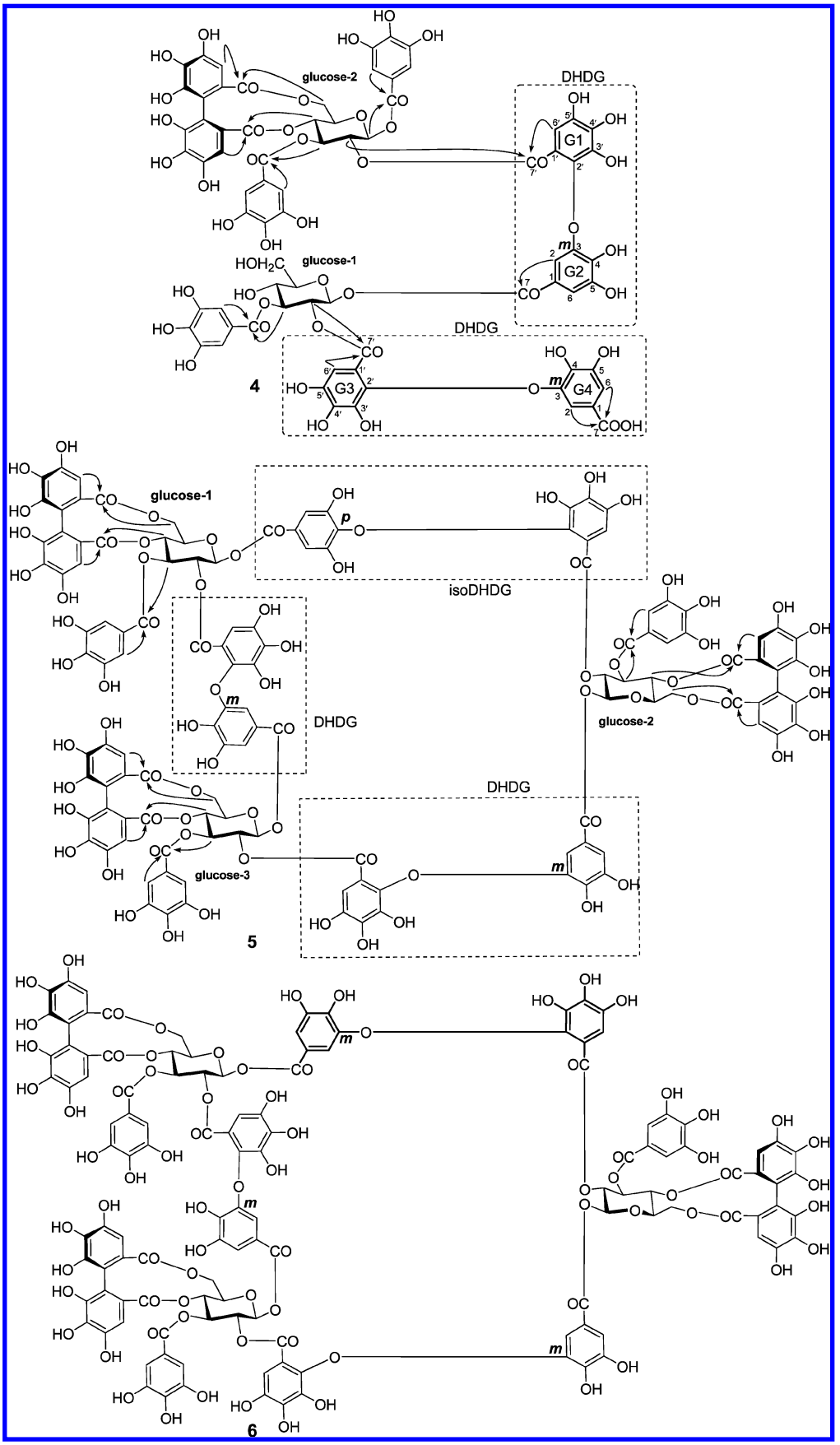

Figure 2. Structures of the new tannins **4** and **5** and the known tannin **6**. The arrows (H→C) indicate important HMBC correlations.

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1129.09566 ([M + Na]$^+$) in the HRESIMS spectrum. The ^{1}H NMR spectrum indicated the presence of two galloyl units [δ_H 7.07 and 6.965 (each 2H, s)], an HHDP unit [δ_H 6.60 and 6.48 (each 1H, s)], an isoDHDG moiety [δ_H 6.968 (2H, s) and 6.81 (1H, s)], and a fully *O*-acylated *β*-glucose core in the $^4C_1$ conformation (Table 1). The ^{13}C NMR spectrum showed carbon peaks (Tables 2 and 3) consistent in numbers and chemical shifts with the carbons in the structural components of **3**. Locations of the HHDP and galloyl units were the same as those of remurin A by close similarity of the respective glucose signals in the ^{1}H and ^{13}C NMR spectra and also HMBC correlations as shown in Figure 1. As mentioned for **2**, the two-proton singlet (δ_H 6.968) of H-2/H-6 of the isoDHDG moiety was differentiated from those of the galloyl (H-2/H-6) signals [δ_H 6.965 and 7.07 (each 2H, s)] on the basis of HMBC connectivity of the former proton signal with the carbon peak (δ_C 149.4) diagnostic of C-3/C-5 of the isoDHDG moiety.[9,10] One of the galloyl units was confirmed at C-1 of the glucose core by HMBC correlations between the galloyl-A proton signal [δ_H 7.07 (2H, s)] and the glucose H-1 signal [δ_H 6.02 (1H, d)] through the carbonyl carbon peak (δ_C 165.2). It was noteworthy that the H-6′ signal [δ_H 6.81 (1H, s)] of the isoDHDG and the H-2/H-6 signal [δ_H 6.965(2H, s)] of the galloyl-B moieties exhibited HMBC correlations with the glucose H-2 [δ_H 5.63 (1H, dd)] and H-3 [δ_H 5.74 (1H, t)] signals through two carbonyl carbons resonating at δ_C 166.8 (2C). This did not permit exact placement of these structural moieties on the glucose core on the basis of spectroscopic evidence. Alternatively, since isomerization of the isoDHDG moiety into DHDG through Smiles rearrangement in a weak alkaline solution (phosphate buffer, pH 7.4) was reported,[9,10,16] this reaction was applied here, and the rearranged product from **3** was identified as remurin A (Figure 1), to secure structure **3** including the *S* configuration of the HHDP unit for nilotinin M7.

Nilotinins M5−M7 (**1**−**3**) are further examples of the unique hydrolyzable tannins of *T. nilotica*. Among these tannins, the anomeric position, unless esterified with a galloyl unit, is always esterified by the galloyl part bearing two hydrogens of the DHDG or the isoDHDG moiety, while C-2 esterification of glucose was always by the galloyl part bearing an isolated hydrogen of the DHDG or the isoDHDG moiety. The upfield shift of H-1 (δ_H 5.50) of the glucose core in **1** relative to that (δ_H 6.02) of the glucose cores in **2** and **3** could be attributable to the anisotropic effect of the DHDG moiety at C-2 of the glucose core (Figure 1).[9,13]

Structural Elucidation of the Dimeric Ellagitannin. Nilotinin D10 (**4**) was isolated as an off-white, amorphous powder. Its HRESIMS exhibited a prominent ion peak ([M + Na]$^+$) at *m*/*z* 1763.17637, corresponding to the molecular formula $C_{75}H_{56}O_{49}$. The ^{1}H NMR spectrum exhibited 11 signals due to three galloyl units [δ_H 7.03, 6.948, 6.92 (each 2H, s)], an HHDP unit [δ_H 6.58 and 6.51 (each 1H, s)], and two DHDG moieties [δ_H 7.13, 6.95, 6.37, and 6.31 (each 1H, d, *J* = 1.8 Hz), DHDG (H-2 and H-6) × 2, δ_H 7.06, 7.02 (each 1H, s), DHDG H-6′ × 2]. In the upper-field region of the spectrum, two seven-spin aliphatic proton systems with large coupling constants (Table 1) were distinguished by the ^{1}H−^{1}H COSY experiment, indicating the presence of two *β*-glucopyranose cores in the $^4C_1$ conformation. The ^{13}C NMR spectrum exhibited carbon peaks (Tables 2 and 3) consistent with the structural units of **4** (Figure 2). An HHDP unit was located at C-4/C-6 of glucose-2, as indicated by the large chemical shift difference ($\Delta\delta_H$ 1.45) between the C-6 *gem*-proton signals (δ_H 5.26 and 3.81). Conversely, the C-4 and C-6 hydroxy groups of glucose-1 were unacylated because of the upfield shifts (δ_H 3.78−3.93) of the glucose-1 H-4 and H-6. The HMBC spectrum showed correlations among the galloyl proton signals [δ_H 7.03, 6.948, 6.92 (each 2H, s)] and signals of H-3 (δ_H 5.34) of glucose-2 and H-1 (δ_H 5.44) and H-3 (δ_H 5.59) of glucose-1 through the respective carbonyl carbon peaks (δ_C 167.1, 164.6 and 166.8). The remaining hydroxy groups of the glucose cores (at C-1 and C-2 of glucose-2 and C-1 of glucose-1) were also *O*-acylated because of the downfield shifts of the corresponding proton signals (Table 1). Consequently, three galloyl parts of the two DHDG moieties should be placed at these hydroxy groups. The HMBC correlations among two DHDG H-6′ signals [δ_H 7.05 and 7.02 (each 1H, s)] with the glucose-1 H-2 signal (δ_H 5.31) and the glucose-2 H-2 signal (δ_H 5.51) *via* two equivalent carbonyl carbon peaks (δ_C 164.8, 2C) substantiated attachment of the galloyl parts bearing an isolated hydrogen (G1 and G3) of the DHDG moieties to C-2 of both glucose cores. The orientation of the galloyl portion (G4) of the DHDG moiety was confirmed as shown in Figure 2 on the basis of the HMBC correlations of the *meta*-coupled proton signals due to the DHDG H-2 (δ_H 6.37) and H-6 (δ_H 7.13) with the carboxylic carbon peak (δ_C 168.9, DHDG C-7). In spite of the overlapping of the glucose-1 H-1 signal (δ_H 5.24) with the glucose-2 H-6 signal (δ_H 5.26) and that of the DHDG H-6 signal (δ_H 6.95) with a galloyl (H-2/H-6) signal (δ_H 6.948) in the ^{1}H NMR spectrum, and also the lack of HMBC cross-peaks for these proton signals, the galloyl part (G2) bearing two *meta*-coupled proton signals (δ_H 6.31 and 6.95) of the other DHDG moiety should necessarily be attached to C-1 of glucose-1. A positive Cotton effect at 238 nm in the ECD spectrum of **4** indicated an *S* configuration for the HHDP unit.[20] On the basis of the above spectroscopic findings, nilotinin D10 was assigned the structure **4** (Figure 2).

Structural Elucidation of the Trimeric Ellagitannin. Nilotinin T1 (**5**) was indicated to be a trimeric ellagitannin possessing the molecular formula $C_{123}H_{84}O_{78}$, as revealed by the ion peak ([M +Na]$^+$) at *m*/*z* 2831.24916 in the HRESIMS spectrum and the spectroscopic data shown below. The ^{1}H NMR spectrum showed proton signals [δ_H 6.97 (2H, br s) and 6.76 (1H, br s)] diagnostic for an isoDHDG moiety. Two pairs of mutually coupled broad signals [δ_H 6.81 and 6.44 (each 1H, br s), and δ_H 7.04 and 6.48 (each 1H, br s)] and a pair of one-proton singlets (δ_H 7.15 and 6.99) indicated the presence of two DHDG moieties.[9−11,16] Proton signals attributable to three HHDP units [δ_H 6.51, 6.52, 6.53, 6.58, 6.60, and 6.62 (each 1H, s)] and three galloyl units [δ_H 7.09, 6.972, and 6.968 (each 2H, s)] were also evident in the aromatic region of the spectrum. The ^{1}H NMR spectrum also showed three seven-spin systems assignable to protons of three glucose cores (Table 1). Among these signals, three broad signals (δ_H 6.16, 5.76, and 5.24) were assigned to anomeric protons of the glucose-1, 2, and 3 moieties on the basis of HSQC correlations with anomeric carbon peaks [δ_C 93.3 (2C) and 93.8], respectively. Despite the broadening of these anomeric proton signals, the $^4C_1$ conformations of the glucose cores were implied from the large coupling constants of the remaining proton signals (Table 1). Chemical shifts of the glucose proton signals (Table 1) indicated acylation of all of the hydroxy groups on the glucopyranose rings. The ^{13}C NMR spectrum of **5** showed carbon peaks (Tables 2 and 3) consistent with the presence of these acyl moieties and the glucose cores. The locations of the

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galloyl units were assigned to C-3 of each of the glucose cores, as determined from the HMBC correlations of the galloyl proton signals (δ_H 7.09, 6.972, and 6.968) with the H-3 signals (δ_H 5.80, 5.70, and 5.56) of the three glucose cores through common carbonyl carbon peaks [δ_C 167.4 and 167.0 (2C)]. Bridging of an HHDP unit at C-4/C-6 of each glucose core was evidenced from the large chemical shift difference ($\Delta\delta_H$ 1.42−1.64) between the C-6 methylene proton signals (δ_H 5.26/3.84, 5.23/3.76, and 5.36/3.72) of the glucosyl moieties. The location of the HHDP units was further confirmed by HMBC correlations among the HHDP proton signals (δ_H 6.51, 6.52, 6.53, 6.59, 6.60, and 6.63) and signals of H-4 (δ_H 5.07, 5.12, and 5.19) and H-6 (δ_H 5.23, 5.26, and 5.36) of the glucose cores through common ester carbonyl carbon peaks [δ_C 167.75, 167.87, 167.92, 168.3, and 168.5 (2C)]. Broadening of the ester carbonyl carbon peaks of the DHDG (δ_C 163.4, 164.0, 164.1, and 164.4) and the isoDHDG (δ_C 164.9 and 166.3) moieties suggested a macrocyclic structure for nilotinin T1, since analogous features were also observed in the ^{13}C NMR spectra of macrocyclic-type tannins with related structures such as nilotinin D9 (**10**) and hirtellins C (**11**) and F (**12**).[11] Consequently, the two DHDG and the isoDHDG moieties in **5** were presumed to be bridged between C-1 of a glucose core and the C-2 of another to form a macrocyclic structure. Linking modes of the DHDG and isoDHDG moieties to the glucose residues could not be established by HMBC due to the broadening of their proton signals. The structure of nilotinin T1 was subsequently formulated as **5** by its chemical conversion into hirtellin T2 (**6**) (Figure 2), a known tannin with the same molecular formula, through Smiles rearrangement of the less stable isoDHDG into the more stable DHDG unit under mild conditions. It is noteworthy that despite the lack of HMBC cross-peaks among the proton signals of the DHDG and isoDHDG moieties with their associated carbons, HSQC correlations of these proton signals with the corresponding carbons were clearly detected. Thus, assignments of the carbon resonances of **5** (Tables 2 and 3) were determined on the basis of HSQC data and comparison with the corresponding signals of the monomeric nilotinins M5−M7 and with signals of the analogous macrocyclic dimers nilotinin D9 (**10**) and hirtellins C (**11**) and F (**12**) (Figure 3).

Cytotoxic Activity of Tannins. Studies conducted with tumor cell lines have shown that several monomeric−oligomeric ellagitannins exhibit potent cytotoxicity against carcinoma cell lines and lower cytotoxicity to normal cells.[21,22] In our preceding paper,[11] we presented the effects of some monomeric and dimeric tannins on oral squamous cell carcinoma (HSC-2, HSC-3, and HSC-4) and promyelocytic leukemia (HL-60) cell lines compared with their effect on human oral normal cells (HGF, HPC, and HPLF). In our efforts to find candidates for developing into efficient antitumor agents, five macrocyclic-type tannins, nilotinin T1 (**5**, Figure 2), tamarixinin B (**8**), nilotinin D9 (**10**), and hirtellins C (**11**) and F (**12**) (Figure 3), were also investigated against these cell lines following the procedures shown in the Experimental Section.

Compounds examined in the present study showed significant cytotoxic effects with medium to high tumor-specificity (TS) indices against all of the tested tumor cell lines, as shown in Table 4. Although the effects of these macrocyclic-type tannins (**5**, **8**, and **10**−**12**) (TS = 2.2−2.9) are relatively lower than the linear and hellinoyl-type tannins isolated from the same plant (TS = 2.1−7.1),[11] their potencies are still higher than synthetic ketones (TS = 1.2−1.8), natural potent cytotoxic

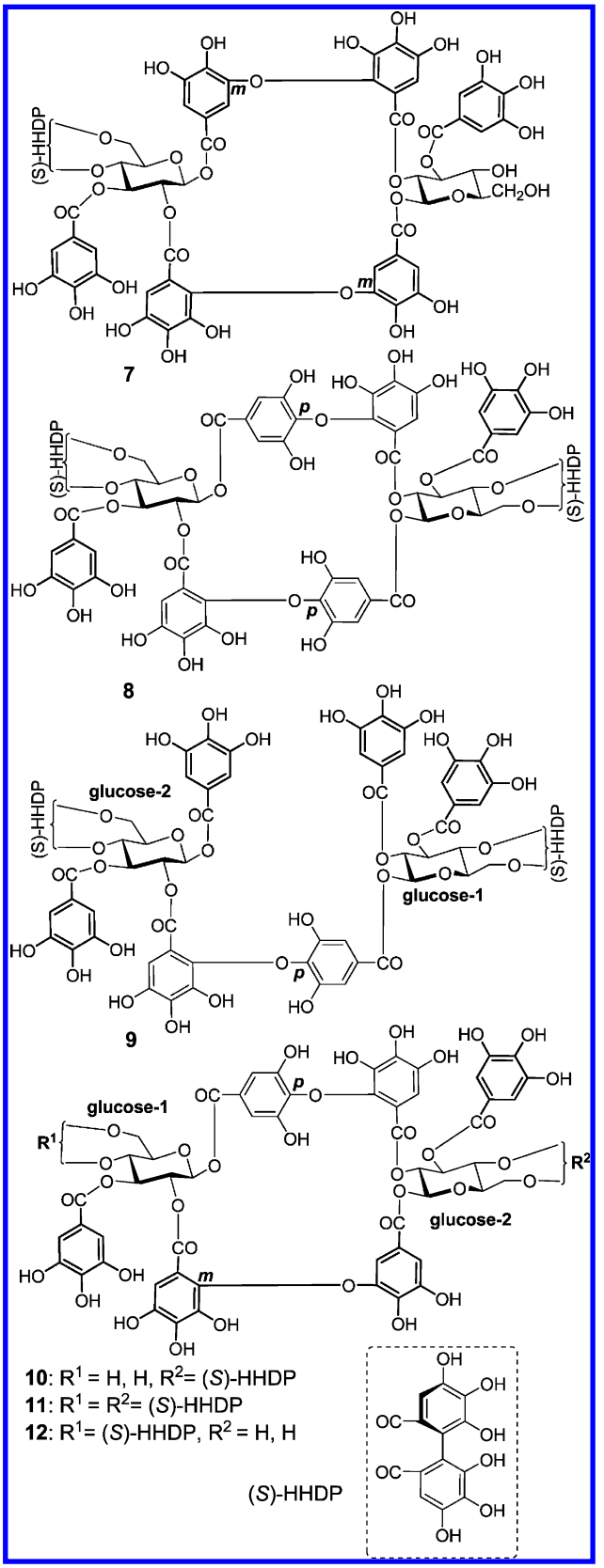

Figure 3. Structures of the known tannins **7**−**12**.

polyphenols (flavones, flavonols, and prenylated flavonoids) (TS = 1.2−2.3), terpenes (triterpene aglycones and triterpene glycosides) (TS = 1.2−1.6),[23] and coumarins (TS = 2.4) and

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Table 4. Cytotoxicity of Tannins 5, 8, and 10−12 against Human Normal and Tumor Cells

		CC_{50} (μM)[a]							
		human oral squamous cell carcinoma cells			promyelocytic leukemia cells	human normal oral cells			
tannin	MW	HSC-2	HSC-3	HSC-4	HL-60	HGF	HPC	HPLF	TS[b,c]
nilotinin T1 (**5**)	2808	22.3 ± 2.0	34.2 ± 1.1	42.9 ± 0.31	30.5 ± 0.23	96.6 ± 2.5	90.7 ± 2.1	>100	2.9
tamarixinin B (**8**)	1872	23.0 ± 1.8	47.8 ± 1.9	43.0 ± 0.78	55.9 ± 15.7	93.6 ± 3.2	88.3 ± 1.5	>100	>2.2
nilotinin D9 (**10**)	1570	18.5 ± 0.30	21.9 ± 0.98	24.5 ± 4.6	70.8 ± 17.0	>100	81.0 ± 1.7	>100	>2.8
hirtellin C (**11**)	1872	19.9 ± 1.2	24.3 ± 2.3	28.7 ± 2.6	54.2 ± 8.3	95.0 ± 1.6	84.3 ± 2.1	>100	>2.9
hirtellin F (**12**)	1570	20.2 ± 0.75	20.8 ± 0.90	26.6 ± 1.6	62.0 ± 0.48	>100	80.3 ± 2.1	>100	>2.9

[a]Each value represents the mean of at least three independent experiments. [b]TS, tumor specificity. [c]TS = {[CC_{50}(HGF) + CC_{50}(HPC) + CC_{50}(HPLF)]/[CC_{50}(HSC-2) + CC_{50}(HSC-3)] + CC_{50}(HSC-4) + CC_{50}(HL-60)]} × (4/3).

comparable with those of 5-fluorouracil (5-FU) (TS > 3.35) and melphalan (TS = 4.09).[24]

The ellagitannins, hirtellins A and B and tamarixinin A, from *Tamarix* plants exhibited strong antitumor activity upon single intraperitoneal injection to mice four days before intraperitoneal inoculation of the sarcoma-180 cells,[25] which agrees with our results of the cytotoxic activity study of the same and other tannins[11] and with the results of the cytotoxic investigation shown in this paper. These results support the possibility of developing antitumor ellagitannins with low toxicity from tamaricaceous plants.

T. nilotica has been known since pharaonic times and has been mentioned in medical papyri to relieve headache and as an antipyretic, an anti-inflammatory, and an aphrodisiac.[6] In our preceding paper on *T. nilotica* we reported several ellagitannins with strong cytotoxic effects.[11] We report here the isolation and structural elucidation of five new monomeric−trimeric ellagitannins, together with four known ones, from *T. nilotica*. Cytotoxic effects of macrocyclic-type tannins are also discussed. The mode of action of these compounds remains to be established.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 digital polarimeter. UV spectra were measured on a JASCO V-530 spectrophotometer, and ECD spectra on a JASCO J-720W spectrophotometer. ESIMS were performed using a Micromass Auto Spec OA-Tof spectrophotometer in positive ion mode. The solvent used was 50% MeOH + 0.1% NH_4OAc, and the flow rate was set at 20 μL/min. HRESIMS was performed using a micrOTOF-Q (Bruker Daltonics, USA) mass spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA AS 600 instrument (600 MHz for ^1H and 151 MHz for ^{13}C NMR). Chemical shifts are given in δ (ppm) values relative to those of the solvent signal [acetone-d_6 (δ_H 2.04; δ_C 29.8)] on the tetramethylsilane scale. The standard pulse sequences programmed into the instrument (Varian INOVA AS600) were used for each 2D measurement. The J_{CH} value was set at 5 Hz in the HMBC spectra. Normal-phase HPLC was conducted on a YMC-Pack SIL A-003 (YMC, Kyoto, Japan) column (4.6 i.d. × 250 mm) developed with *n*-hexane/MeOH/THF/formic acid (47:39:13:1) containing oxalic acid (450 mg/L) (flow rate, 1.5 mL/min; 280 nm UV detection) at room temperature. Analytical reversed-phase HPLC was performed on a YMC-Pack ODS-A A-303 column (4.6 i.d. × 250 mm) eluted with 0.01 M H_3PO_4/0.01 M KH_2PO_4/MeOH (2:2:1) (flow rate, 1 mL/min; 280 nm UV detection) at 40 °C. Preparative reversed-phase HPLC was performed at 40 °C on a YMC-Pack ODS-A A-324 column (10 i.d. × 300 mm) using 0.01 M H_3PO_4/0.01 M KH_2PO_4/MeOH [either 43:43:16 (solvent I), 40:40:20 (solvent II), 41:41:18 (solvent III), 41.5:41.5:17 (solvent IV), or 37.5:37.5:25 (solvent V), v/v], at a flow rate of 2 mL/min with detection at 280 nm UV. The tumor cell lines were obtained from Riken Bioresource Center, Tsukuba, Ibaraki, Japan. The normal cells were prepared from periodontal tissues, according to the guidelines of the intramural board of Meikai University Ethics Committee (no. A0808) after obtaining informed consent from the patients. Because HGF, HPC, and HPLF cells have a limited lifespan due to in vitro senescence, these cells were used at a population doubling level of 5−8.

Plant Material. Leaves of *T. nilotica* were collected at Al-Wadi Al-Assiuty, 20 km northeast of Assiut, Egypt, in October 2006, and identified by Prof. Mo'men Mostafa Zareh, Department of Botany, Faculty of Science, Assiut University. A voucher specimen (No. 1024) is deposited in the same department.

Extraction and Isolation. Air-dried, powdered leaves (1 kg) were homogenized in H_2O/acetone (30:70, v/v, 22 L) at room temperature. The filtered homogenate was concentrated in vacuo to 1.5 L at temperatures not exceeding 40 °C. The concentrated homogenate was applied to a Diaion HP-20 (5 i.d. × 50 cm) column and eluted with H_2O, MeOH/H_2O (40:60, v/v), MeOH, and acetone, successively, to give the corresponding H_2O (116 g), MeOH/H_2O (40:60) (52 g), MeOH (12.4 g), and acetone (0.629 g) fractions, respectively. The respective fractions were fractionated by monitoring normal- and reversed-phase HPLC. The MeOH/H_2O (40:60, v/v) fraction (52 g) was divided into two equal portions (26 g each). Each portion was separately subjected to a Toyopearl HW-40 (coarse) column (2.2 i.d. × 65 cm) and eluted with EtOH/H_2O (50:50 → 70:30, v/v), EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10 → 80:20 → 70:30 → 50:50, v/v), and acetone/H_2O (70:30, v/v), successively, collecting 500 mL fractions. The corresponding fractions from both Toyopearl columns were combined as seven Toyopearl (T1) fractions (T1−7).

The T1 fraction 3 (6.12 g), eluted with EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10, v/v), was subjected to the same Toyopearl (coarse) column and eluted with EtOH/H_2O (70:30, v/v), EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10), and acetone/H_2O (70:30, v/v), successively, collecting 900 drop fractions, to give 700 Toyopearl (T2) fractions. The T2 fractions 158−183 (322 mg), eluted with EtOH/H_2O (70:30, v/v), were subjected to an MCI-gel CHP-20P column (1.1 i.d. × 35 cm) with H_2O and H_2O/MeOH (85:15 → 80:20 → 75:25 → 70:30 and 0:100, v/v). The H_2O−MeOH (80:20, v/v) eluate (31 mg) was rechromatographed over an MCI-gel CHP-20P column (1.1 i.d. × 21 cm) with H_2O and H_2O/MeOH (85:15 → 80:20 and 0:100, v/v). A preparative HPLC purification with solvent I of the late eluate with H_2O/MeOH (85:15, v/v) (7 mg) yielded a pure sample of nilotinin M5 (**1**) (2.1 mg). Rechromatography of the H_2O/MeOH (80:20, v/v) eluate (63 mg) over the same MCI-gel CHP-20P column and by the same elution pattern afforded a H_2O/MeOH (75:25, v/v) eluate (19.8 mg), which upon preparative HPLC purification with solvent II gave nilotinin D10 (**4**) (4.5 mg). The T2 fractions 184−210 (297 mg), eluted with EtOH/H_2O (70:30, v/v), were subjected to an MCI-gel CHP-20P column (1.1 i.d. × 35 cm) with H_2O and H_2O/MeOH (85:15 → 80:20 → 75:25 → 70:30 and 0:100, v/v). The early eluate with H_2O/MeOH (80:20, v/v) (33.7 mg) was rechromatographed over an MCI-gel CHP-20P column (1.1 i.d. × 21 cm) isocratically with H_2O/MeOH (85:15, v/v), followed by preparative HPLC purification of the eluate (21 mg) with solvent II, affording an additional pure sample (8.2 mg) of nilotinin M5 (**1**). The

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late eluate (30 mg) with H_2O/MeOH (80:20, v/v) was also rechromatographed over an MCI-gel CHP-20P column (1.1 i.d. × 21 cm) with H_2O and H_2O/MeOH (90:10 → 85:15 → 80:20 → 75:25 and 0:100, v/v), and preparative HPLC purification of the H_2O/MeOH (75:25, v/v) eluate (55 mg) with solvent II yielded nilotinin M7 (**3**) (9.0 mg). The T2 fractions 211−264 (555 mg), eluted with EtOH/H_2O (70:30, v/v), were subjected to an MCI-gel CHP-20P column (1.1 i.d. × 37 cm) with H_2O and H_2O/MeOH (90:10 → 85:15 → 80:20 → 75:25 →70:30 and 0:100, v/v). The late eluate with H_2O/MeOH (80:20, v/v) (43 mg) with preparative HPLC purification with solvent II yielded nilotinin M6 (**2**) (7.2 mg). The T2 fractions 265−318 (535 mg), early eluted with EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10, v/v), were subjected to an MCI-gel CHP-20P column (1.1 i.d. × 37 cm) with H_2O and H_2O/MeOH (90:10 → 85:15 → 80:20 → 75:25 →70:30 and 0:100, v/v), and the late eluate with H_2O/MeOH (75:25, v/v) (59 mg) was rechromatographed over the same column with H_2O/MeOH (80:20 → 75:25 → 70:30 and 0:100, v/v). The eluate (42.0 mg) with H_2O/MeOH (75:25 and 70:30, v/v) was subjected to preparative HPLC purification with solvent III to yield nilotinin D9 (**10**) (5.4 mg) and hirtellin F (6.3 mg), respectively.

A part (3.6 g) of the T1 fraction 5 (4.6 g), eluted with EtOH−H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (70:30, v/v), was rechromatographed on a Toyopearl HW-40 (coarse) column (2.2 i.d. × 45 cm) with EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10 → 80:20 → 70:30, v/v), successively. The eluate (1.4g) with EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (80:20) was subjected to an MCI-gel CHP-20P column (1.1 i.d. × 36 cm) with H_2O and H_2O/MeOH (95:5 → 90:10 → 85:15 → 80:20 → 75:25 → 70:30 → 60:40 and 0:100, v/v), yielding 600 MCI fractions (MCIfrs, 600 drops each). The MCIfrs 11−60 (159 mg) and the MCIfrs 61−195 (235 mg), eluted with H_2O/MeOH (85:15, v/v), were separately rechromatographed over a Sephadex LH-20 column (2.2 i.d. × 21 cm) with EtOH/H_2O (70:30, v/v), EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10, v/v), and acetone/H_2O (70:30, v/v), and the crude tannins (69 mg) in the eluate with EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10, v/v) from these Sephadex columns yielded hirtellin T2 (**6**) (15.2 mg) upon preparative HPLC purification with solvents I and II, respectively. The MCIfrs 196−300 (268 mg), eluted with H_2O/MeOH (80:20, v/v), were subjected to an MCI-gel CHP-20P column (1.1 i.d. × 37 cm) with H_2O and H_2O/MeOH (95:5 → 90:10 → 85:15 → 80:20 → 75:25 → 70:30 → 60:40 and 0:100, v/v), and the early eluate (57 mg) with H_2O/MeOH (70:30, v/v) was purified by preparative HPLC with solvent IV to afford hirtellin T2 (**6**) (7.55 mg) and nilotinin T1 (**5**) (16.6 mg). The MCIfrs 301−471 (496 mg), eluted with H_2O/MeOH (75:25, v/v), were subjected to a Sephadex LH-20 column (1.1 i.d. × 37 cm) with EtOH/H_2O (70:30, v/v), EtOH−H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10 → 80:20, v/v), and acetone/H_2O (70:30, v/v), and the crude tannins (32 mg) in the EtOH/H_2O (70:30, v/v) eluate yielded hirtellin C (**11**) (7 mg) upon preparative HPLC purification with solvent II. The early eluate with EtOH/H_2O (70:30, v/v)/acetone/H_2O (70:30, v/v) (90:10, v/v) (88.7 mg) from the Sephadex column was dissolved in MeOH. Tamarixinin B (**8**) (29 mg) was obtained as an MeOH-insoluble fraction, whereas the MeOH-soluble fraction was purified by preparative HPLC with solvent IV and yielded additional crops of tamarixinin B (**8**) (10 mg) and nilotinin T1 (**5**) (9.3 mg). The MCIfrs 523−572 (72 mg), eluted with H_2O/MeOH (60:40, v/v), were rechromatographed over an MCI-gel CHP-20P column (1.1 i.d. × 21 cm) with H_2O and H_2O/MeOH (70:30 → 60:40 and 0:100, v/v). Preparative HPLC purification of the eluate with H_2O/MeOH (60:40, v/v) (17 mg) with solvent V afforded tamarixinin C (**9**) (5.7 mg).

*Nilotinin M5 (**1**):* off-white, amorphous powder; $[\alpha]^{26}_{D}$ +23.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218.5 (5.05), 270 (4.7); CD (MeOH) [θ] (nm) [θ] +0.75 × 10^5 (236), −1.8 × 10^4 (265), +1.3 × 10^4 (285); ^{1}H NMR (acetone-d_6/D_2O, 9:1, 600 MHz) δ_H 7.21 (1H, d, *J* = 1.8 Hz, DHDG H-6), 7.06 (1H, s, DHDG H-6′), 6.96 [2H, s, isoDHDG (H-2/H-6)], 6.93 [2H, s, galloyl (H-2/H-6)], 6.89 (1H, s, isoDHDG H-6′), 6.58 (1H, s, HHDP H-3), 6.51 (1H, s, HHDP H-3′), 6.46 (1H, d, *J* = 1.8 Hz, DHDG H-2), and glucose protons (Table 1); ^{13}C NMR assignments, see Tables 2 and 3; FABMS *m*/*z* 1297 [M + Na]$^+$; HRESIMS *m*/*z* 1297.10358 [M + Na]$^+$ (calcd for $C_{55}H_{38}O_{36}$ Na, 1297.10350).

*Nilotinin M6 (**2**):* off-white, amorphous powder; $[\alpha]^{18}_{D}$ +20.5 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218.5 (5.43), 264 (5.04); CD (MeOH) [θ] (nm) +1.5 × 10^5 (236), −7.6 × 10^4 (262), +4.8 × 10^4 (286); ^{1}H NMR (acetone-d_6/D_2O, 9:1) δ_H 7.06, 6.99 [each 2H, s, isoDHDG (H-2/H-6) × 2], 6.97 [2H, s, galloyl (H-2/H-6)], 6.87, 6.84 [each 1H, s, isoDHDG (H-6′) × 2], 6.60 (1H, s, HHDP H-3), 6.49 (1H, s, HHDP H-3′), and glucose protons (Table 1); ^{13}C NMR assignments, see Tables 2 and 3; HRESIMS *m*/*z* 1297.10145 [M + Na]$^+$ (calcd for $C_{55}H_{38}O_{36}$Na, 1297.10351).

*Nilotinin M7 (**3**):* off-white, amorphous powder; $[\alpha]^{25}_{D}$ +21.4 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 217.5 (5.11), 274 (4.74); CD (MeOH) [θ] (nm) +1.1 × 10^5 (237), −3.3 × 10^4 (262), +2.3 × 10^4 (283); ^{1}H NMR (acetone-d_6/D_2O, 9:1) δ_H 7.07 [2H, s, galloyl-A (H-2/H-6)], 6.968, [2H, s, isoDHDG (H-2/H-6)], 6.965 [2H, s, galloyl-B (H-2/H-6)], 6.81 (1H, s, isoDHDG H-6′), 6.60 (1H, s, HHDP H-3), 6.48 (1H, s, HHDP H-3′), and glucose protons (Table 1); ^{13}C NMR assignments, see Tables 2 and 3; FABMS *m*/*z* 1229 [M + Na]$^+$; HRESIMS *m*/*z* 1129.09566 [M + Na]$^+$ (calcd for $C_{48}H_{34}O_{31}$Na, 1129.09763).

*Nilotinin D10 (**4**):* off-white, amorphous powder; $[\alpha]^{27}_{D}$ +53.1 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218.5 (5.43), 277 (5.08); CD (MeOH) [θ] (nm) +0.9 × 10^5 (238), −1.6 × 10^4 (266), +2.6 × 10^4 (291); ^{1}H NMR (acetone-d_6/D_2O, 9:1) δ_H 7.13, 6.95 [each 1H, d, *J* = 1.8 Hz, (DHDG H-6) × 2], 7.06, 7.02 [each 1H, s, (DHDG H-6′) × 2], 7.03, 6.948, 6.92 [each 2H, s, galloyl (H-2/H-6) × 3], 6.58 (1H, s, HHDP H-3), 6.51 (1H, s, HHDP H-3′), 6.37, 6.31 [each 1H, d, *J* = 1.8 Hz, (DHDG H-2) × 2], and glucose protons (Table 1); ^{13}C NMR assignments, see Tables 2 and 3; FABMS *m*/*z* 1763 [M + Na]$^+$; HRESIMS *m*/*z* 1763.17637 [M + Na]$^+$ (calcd for $C_{75}H_{56}O_{49}$ Na, 1763.17824).

*Nilotinin T1 (**5**):* off-white, amorphous powder; $[\alpha]^{31}_{D}$ −5.8 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 218 (5.63), 274.5 (5.28); CD (MeOH) [θ] (nm) +4.2 × 10^5 (235), −1.9 × 10^5 (262), +1.2 × 10^5 (286); ^{1}H NMR (acetone-d_6/D_2O, 9:1) δ_H 7.15, 6.99 [each 1H, s, (DHDG H-6′) × 2], 7.04, 6.81 [each 1H, br s, (DHDG H-6) × 2], 6.48 and 6.44 [each 1H, br s, (DHDG H-2) × 2], 6.97 (2H, br s, isoDHDG H-2/H-6), 6.76 (1H, br s, isoDHDG H-6′), 7.09, 6.972, and 6.968 [each 2H, s, galloyl (H-2/H-6) × 3], 6.62, 6.60, and 6.58 [each 1H, s, HHDP (H-3′) × 3], 6.53, 6.52, 6.51 [each 1H, s, HHDP (H-3) × 3], and glucose protons (Table 1); ^{13}C NMR assignments, see Tables 2 and 3; HRESIMS *m*/*z* 2831.24916 [M + Na]$^+$ (calcd for $C_{123}H_{84}O_{78}$Na, 2831.24989).

*Chemical Conversion of Nilotinin M7 (**3**) into Remurin A.* A solution of **3** (2 mg) in 200 μL of phosphate buffer (pH 7.4) containing 1 drop of acetone was warmed at 37 °C for 1 h. The reaction mixture was acidified with 1 N HCl and poured into a Sep-Pak C_{18} cartridge. Inorganic solutes were washed out from the cartridge with H_2O, while adsorbed tannin was recovered (1.9 mg) with MeOH. Conversion of **3** into remurin A was monitored by co-chromatographic analyses with standard samples isolated from the plant on normal-phase (t_R 6.48 min, remurin A and **3**) and reversed-phase HPLC (t_R 7.02 min for remurin A and t_R 9.70 min for **3**) and was confirmed by the ^{1}H NMR data comparison.

*Chemical Conversion of Nilotinin T1 (**5**) into Hirtellin T2 (**6**).* A solution of **5** (2 mg) in 200 μL of phosphate buffer (pH 7.4) containing 1 drop of acetone was incubated at 37 °C for 2 h. The experiment was completed as mentioned above. A conversion of **5** into **6** was detected by co-chromatographic analyses with standard samples isolated from the plant on normal-phase (t_R 15.56 min, **5** and **6**) and reversed-phase HPLC (t_R 5.76 min for **1** and t_R 5.28 min for **6**) and the ^{1}H NMR data comparison.

Cytotoxic Activity Assay. HL-60 cells (Riken, Tsukuba, Japan) were cultured at 37 °C in RPMI1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma Chemical Corp., St. Louis, MO, USA), under a humidified 5% CO_2 atmosphere. Human oral squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4)

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(kindly provided by Professor Nagumo, Showa University, Japan) were cultured in Dulbecco's modified Eagle medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated FBS. Normal human oral cells, HGF, HPC, and HPLF were prepared from periodontal tissues, as previously reported,[24] and used at 8−15 population doubling levels. The cells (other than HL-60) were inoculated at 5×10^3 cells/well in 96-microwell plates (Becton Dickinson, Franklin Lakes, NJ, USA) unless otherwise stated. After 48 h, the medium was removed by suction with an aspirator and replaced with 0.1 mL of fresh medium containing different test compound concentrations. Each test compound was dissolved in DMSO at a concentration of 20 mM. The first well contained 100 μM of the test compound and was sequentially diluted 2-fold, with three replicate wells for each concentration. The cells were incubated for an additional 48 h, and the relative viable cell number was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. In brief, the cells were washed with phosphate-buffered saline without calcium and magnesium, which was replaced with fresh culture medium containing 0.2 mg/mL MTT, and the cells were incubated for another 4 h. The cells were lysed with 0.1 mL of DMSO, and the absorbance of the cell lysate at 540 nm (A_{540}) was determined using a microplate reader (Biochromatic Labsystem, Helsinki, Finland).[26] The A_{540} of the control cells was usually in the range from 0.40 to 0.90. The HL-60 cells were inoculated at 3.0×10^4 cells/0.1 mL in 96-microwell plates, and different concentrations of test compounds were added. After a 48 h incubation, the viable cell number was determined with a hemocytometer under a light microscope after trypan blue staining. The CC_{50} was determined from the dose−response curve.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for **1**−**5** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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