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Minor *C*-geranylated flavanones from *Paulownia tomentosa* fruits with MRSA antibacterial activity

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ABSTRACT

Exhaustive chromatographic separation of the chloroform portion of the ethanolic extract obtained from *Paulownia tomentosa* (Thunb). Steud. (Paulowniaceae) fruits has led to isolation of ten *C*-6 geranylated flavanones tomentodiplacone C–I and mimulone C–E, featured by 3′-methoxy and 4′-hydroxy or 4′-hydroxy substitution of the B-ring of the flavonoid, respectively. The structures of these compounds were determined by using mass spectrometry (including HRMS) and 1D and 2D NMR spectroscopy. The absolute configurations of the compounds at *C*-2 were determined using circular dichroism. The obtained compounds showed the presence of a geranyl moiety functionalized by a carbonyl, hydroxyl or methoxyl group, or by formation of tetrahydrofuran or fused-pyrane ring, respectively. All of the flavanones described were isolated for the first time from a natural source. The antibacterial activities of selected compounds isolated along with the previously isolated geranylated flavanones were evaluated against a common panel of microbes and MRSA strains. The selected isolated compounds were tested for their ability to affect eukaryotic translation initiation *via* dual-luciferase reporter assay (firefly and renilla).

1. Introduction

Prenylation plays an important role in the diversification of aromatic natural products, contributing to the description of more than 1000 prenylated polyphenols obtained from plants. The occurrence of prenylated flavonoids is rather limited in plant families such as *Asteraceae*, *Berberidaceae*, *Cannabaceae*, *Capparaceae*, *Euphorbiaceae*, *Fabaceae*, *Guttiferae*, *Moraceae*, *Myrsinaceae*, *Rutaceae* or *Umbellifereae* (Yazaki et al., 2009). The most frequent type of prenyl substitution of flavonoids is a 3,3-dimethylallyl side chain. Compounds with a C_5 (isopentenyl) or C_{10} (geranyl) side chain are quite abundant compared to those with a C_{15} (farnesyl) or with a further modified (e.g., by oxidation, reduction, dehydration, cyclization, or hydroxylation) prenyl moiety which are not common in nature (Barron and Ibrahim, 1996; Epifano et al., 2007; Yazaki et al., 2009). The addition of an isoprenoid chain renders the derivate molecule more effective than the parent compound from a pharmacological point of view, probably because a prenyl group increases the lipophilicity and confers on the molecule a strong affinity for biological membranes (Botta et al., 2005; Epifano et al., 2007).

Paulownia tomentosa (Thunb.) Steud. (Paulowniaceae) is a deciduous tree, about 10–20 m tall, native to China. The leaves are large and heart-shaped; the flowers are pale violet, blossoming before leaves appear. The fruits are dry, reddish-brown capsules approximately 3–4 cm long, containing numerous tiny winged

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seeds that are dispersed by wind and water (Erbar and Gülden, 2011) Previous publications have reported the content of phenolic glycosides, acylglycerols, furanquinones, naphthoquinones, iridoids, lignans, and phytosterols in MeOH and EtOH extracts obtained from *P. tomentosa* (Damtoft and Jensen, 1999; Franzyk et al., 1999; Babula et al., 2006; Kobayashi et al., 2008; Si et al., 2008; Asai et al., 2009). Furthermore, *P. tomentosa* (Thunb). Steud. (Paulowniaceae) is a rich source of prenylated flavonoids. More than 20, mostly novel compounds with a prenyl or geranyl side chain at C-6 of the flavonoid skeleton have been isolated from the flowers, fruits and leaves of *P. tomentosa* (Jiang et al., 2004; Šmejkal et al., 2007, 2008b; Asai et al., 2008; Kobayashi et al., 2008; Schneiderová et al., 2012). Only two compounds showed the presence of a five carbon side chain (Šmejkal et al., 2007; Asai et al., 2008); for the others a 10-carbon side chain was typical. Further, only a few compounds showed a geranyl moiety modified by hydroxylation at *C*-6″ or *C*-7″ (Šmejkal et al., 2007, 2008b; Asai et al., 2008; Schneiderová et al., 2012).

The antioxidant, antibacterial, antiphlogistic and cytotoxic activities of *P. tomentosa* geranyl flavonoids have been described recently (Šmejkal et al., 2007, 2008a,b, 2010; Asai et al., 2008; Hošek et al., 2010; Kollár et al., 2011). It has also been discovered that the glandular hairs on its young reproductive organs contain flavonoids at concentrations over 1000 times greater than those on the surfaces of its young leaves (Kobayashi et al., 2008). Some seasonal variations in the concentrations of *C*-geranyl flavonoids have also been described (Holubová and Šmejkal, 2011).

In this paper we report the isolation and structural elucidation of 10 new *C*-geranylated flavanones (**1**–**10**) (Fig. 1) substituted at position *C*-6 of the flavanone skeleton using 1D and 2D NMR experiments as well as MS, UV, IR, and CD. All of the isolated compounds showed a geranyl modified by formation of heterocyclic moiety (**6**, **8**, **9**), carbonyl (**1**–**3**, **7**, **10**), hydroxyl (**1**, **5**, **6**, **8**–**10**), or methoxyl (**1**, **2**, **4**, **5**, **10**) groups, respectively. We suppose that these compounds are not isolation artifacts.

The antimicrobial activity of some of the newly isolated compounds together with some previously obtained geranylated flavanones was tested against several Gram-negative and Gram-positive bacteria species, including methicillin-resistant *Staphylococcus aureus* strains, and showed varying degrees of activity.

Furthermore, some of the isolated compounds were tested to determine their ability to affect eukaryotic translation initiation *via* dual-luciferase reporter assay. The compounds tested showed little such ability.

2. Results and discussion

Compounds **1**–**10** were isolated as amorphous yellowish solids by the extensive chromatographic separation of the chloroform portion of an ethanolic extract of *P. tomentosa* fruits. The MeOH-soluble portion of the $CHCl_3$ extract was chromatographed on silica gel. On the basis of TLC and HPLC analysis, similar fractions were combined to make up 20 fractions marked alphabetically from A to T. Fractions C, D, and F were further separated using column chromatography, preparative RP-HPLC, prep. TLC or some combination of these techniques (Fig. 2).

The basic characteristics of the structures of compounds isolated were deduced by analyzing the UV and IR spectra. Compounds **1**–**7** and **10** showed similar maxima at ~230 (sh) nm, ~290 nm and ~340 (sh) nm; compounds **8** and **9** showed a different course of spectra with maxima at ~230 (sh) nm, ~275 (sh) nm, ~295 nm, ~315 (sh) nm and ~360 (sh) nm. Generally, the spectra corresponded to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions of the

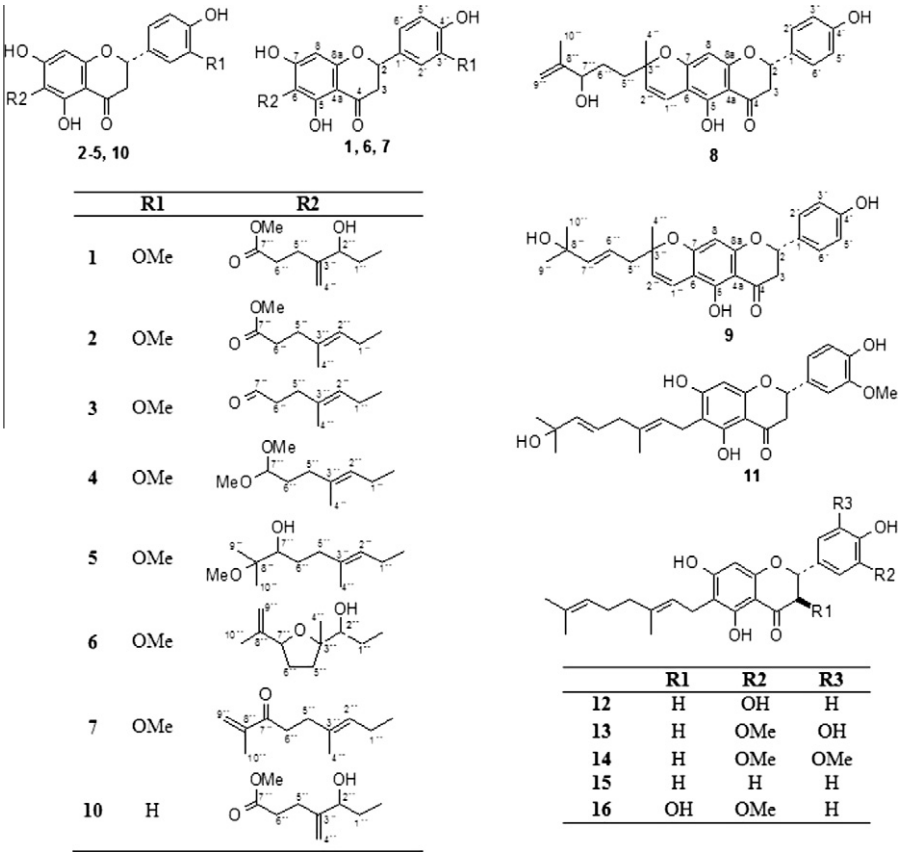

Fig. 1. Structures of compounds isolated.

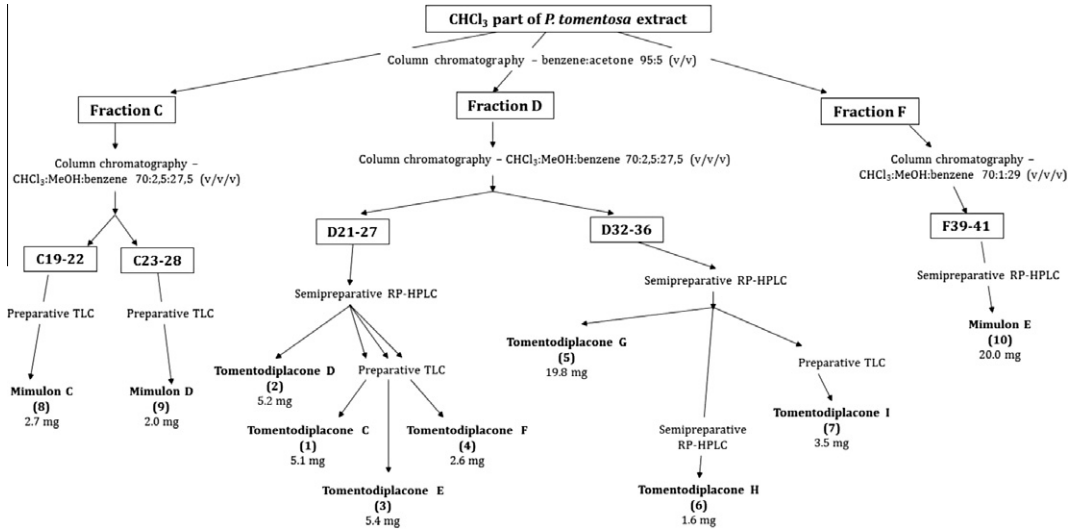

Fig. 2. Scheme of compounds **1**–**10** separation.

flavanone skeleton. The IR spectra clearly showed the presence of hydroxy groups (bands at 3500–3200 cm^{-1}, OH stretching vibrations), a large number of methyl or methylene groups (intensive absorption 3000–2850 cm^{-1}, CH stretching vibrations), 1640–1591 cm^{-1} corresponding to the C=O stretching vibrations of the carbonyl group, absorption bands typical for aromatic compounds (~1600–1450 cm^{-1}), an umbrella vibrations typical for methyl groups (~1375 cm^{-1}) and absorptions typical for aryl–alkyl ether bridges (~1265 cm^{-1}). Similar absorption maxima for *C*-geranyl flavanones had previously been observed (Šmejkal et al., 2008a,b).

Similar and characteristic signals for the ABX system of the flavanone nucleus were determined in the ^1H NMR spectra, 3′-methoxy and 4′-hydroxy (**1**–**7**, **10**) or 4′-hydroxy substitution only (**8** and **9**) at the B-ring of flavonoid skeleton. NMR signals which were not consistent with the published data (Šmejkal et al., 2007, 2008b; Asai et al., 2008) were attributed to modified geranylated side chains of flavonoids **1**–**10**.

The molecular formulas of **1** and **2** were determined on the basis of HRESIMS and the presence of pseudomolecular ions $[M+Na]^+$ at *m*/*z* 481.14682 (calcd. for $C_{24}H_{26}O_9Na^+$481.14745) and *m*/*z* 465.15182 (calcd. for $C_{24}H_{26}O_8Na^+$ 465.15254) to be $C_{24}H_{26}O_9$ and $C_{24}H_{26}O_8$, respectively. Their ^1H and ^{13}C NMR data (Table 1, Fig. S1–S9 of Supporting information) showed very similar signals which were assigned to the flavanone skeleton, a substituted phenyl group, and modified geranyl side chain. Differences in the spectroscopic analysis were observed for the signals of C-2″, C-3″, and C-4″. A carbon signal δ 75.2 confirmed hydroxy substitution at C-2″ for **1**, compared with carbon signals δ 123.6 and 133.5 in the spectrum of compound **2**, which indicated the presence of a double bond between C-2″ and C-3″. HMBC correlations supported the idea of a double bond outside of the prenyl side chain between C-3″ and C-4″ for **1**. Furthermore, analysis of the NMR data for compounds **1** and **2** revealed the unusual termination of the geranyl side chain with both a C-7″methoxycarbonyl group. Thus, in accordance with the chemical structures, compounds **1** and **2** were named tomentodiplacone C and D, respectively.

HRESIMS analysis of compound **3** revealed a molecular formula of $C_{23}H_{24}O_7$ based on the presence of a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 435.14135 (calcd. for $C_{23}H_{24}O_7Na^+$ 435.141975). A second pseudomolecular ion at *m*/*z* $[M+Na]^+$ 467.16762 (calcd. for $C_{24}H_{28}O_8Na^+$ 467.16891) was observed and its formula determined to be $C_{24}H_{28}O_8$. Because of the presence of methanol in the infusion sample solution, we suppose that this was an adduct formed by the addition of a methanol residue to a reactive formyl group of **3**, which was later confirmed by NMR analysis. ^1H and ^{13}C NMR of the derivative **3** (Table 1, Fig. S9–S12 of Supporting information) showed a set of resonances similar to those for **2** with the exception of the geranyl side chain termination (signals for the methoxy group are missing). A hydrogen signal at δ 9.62 and a corresponding carbon signal at δ 203.5 indicated the presence of an aldehyde group. Correlation of HMBC signals at δ 2.2 (H-5″) and 2.47 (H-6″) with δ 203.5 confirmed this atypical termination of the geranyl side chain at C-7″ (Table 1). Compound **3** was named tomentodiplacone E.

The molecular formula of **4** was determined to be $C_{25}H_{30}O_8$ based on HRESIMS analysis in the positive mode, which showed the presence of a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 481.18324 (calcd. for $C_{25}H_{30}O_8Na^+$ 481.18384). The ^1H and ^{13}CNMR data (Table 2, Fig. S13–S19 of Supporting information) indicated the structure of a flavanone skeleton with a substituted geranyl side chain that is the same as those of compounds **2** and **3** at the positions C-1″ through C-6″. The termination of side chain with two methoxy groups at C-7″ with identical chemical shifts (3.17 and 52.7 ppm) was confirmed by the presence of high carbon signal at δ 104.5 in the HSQC spectrum (Table 2). Structure **4** was named tomentodiplacone F.

The molecular formula of **5** was established to be $C_{27}H_{34}O_8$ by the presence of a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 509.21478 (calcd. For $C_{27}H_{34}O_8Na^+$ 509.21514). ^1H and ^{13}C data brought us general information about the presence of a C-6 geranyl-substituted flavanone (Table 2, Fig. S20–S23 of Supporting information). A carbon signal at δ 74.6 indicated hydroxy substitution of the side chain. HMBC correlations of the carbon signal at δ 76.9 with δ 3.37 (OH-7″) and δ 3.18 confirmed the presence and positions of a hydroxy group at C-7″ and a methoxy group at C-8″, respectively. Additionally, two methyl groups were found at C-8″ (Table 2, Fig. S20–S23 of Supporting information). Structure **5** was finally named tomentodiplacone G.

The molecular formula of **6** was determined to be $C_{26}H_{30}O_8$ by HRESIMS supported by the presence of a pseudomolecular ion $[M+Na]^+$ at *m*/*z* 493.18307 (calcd. for $C_{26}H_{30}O_8Na^+$ 493.18384). The carbon signals at δ 81.4 (C-7″) and 86.0 (C-3″) indicated an oxygen containing heterocycle because the presence of an open side chain with two hydroxy groups is usually characterized by chemical shifts with lower values of about 70–73. Further, the carbon signal at δ 76.3 ppm represented a hydroxyl group at C-2″ (Ta-

Table 1
^{1}H NMR and ^{13}C chemical shifts (δ in ppm) and spin–spin coupling constants (J in Hz) of compounds **1**–**3**.

1 (DMSO-d_6)				**2** (Methanol-d_4)			**3** (DMSO-d_6)		
Position	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC
2	78.6, CH	5.29 br, d (12.7)		79.2, CH	5.33, dd (2.6, 12.7)	1′, 2′, 6′	78.9, CH	5.39 dd (2.8, 13.0)	
3	43.2, CH_2	2.58, dd (3.0, 17.0) 3.16, dd (12.7, 17.0)	2, 4	42.6, CH_2	2.72, dd (2.6, 17.0) 3.15, dd (12.7, 17.0)	2, 4, 1′	42.4, CH_2	2.68, dd (2.8, 17.0) 3.28, dd (13.0, 17.0)	4
4	193.7, CO			196.0, CO			196.5, CO		
4a	100.0, C			101.0, C			101.2, C		
5	162.0, C			161.2, C			160.5, C		
6	106.7, C			108.0, C			108.3, C		
7	161.4, C			164.0, C			165.2, C		
8	96.6, CH	5.67, s	4a, 6, 7, 8a	94.3, CH	5.95, s	4, 4a, 6, 7, 8a	94.8, CH	5.97, s	4, 4a, 6, 7, 8a
8a	160.7, C			161.0, C			160.9, C		
1′	130.4, C			130.2, C			129.3, C		
2′	111.9, CH	7.07, d (1.9)	2, 3′, 4′, 6′	110.0, CH	7.08, d (1.8)	2, 3′, 4′, 6′	111.4, CH	7.08, d (2.0)	2, 3′, 4′, 6′
3′	147.9, C			147.6, C			148.1, C		
4′	147.0, C			146.5, C			147.1, C		
5′	116.0, CH	6.78, d (8.2)	1′, 3′	114.6, CH	6.82, d (8.0)	1′, 3′, 4′	115.7, CH	6.79, d (8.0)	1′, 3′, 4′
6′	120.4, CH	6.89, dd (1.9, 8.2)	2, 2′, 4′	119.4, CH	6.93, dd (1.8, 8.0)	2, 2′, 4′	120.1, CH	6.90, dd (2.0, 8.0)	2, 2′, 4′
1″	29.5, CH_2	2.56, m 2.68, m	5, 6, 7, 2″, 3″, 4″	20.1, CH_2	3.23, d (6.9)	5, 6, 7, 2″, 3″	20.3, CH_2	3.13, d (7.1)	5, 6, 7, 2″, 3″
2″	75.2, CH	4.11, m	6	123.6, CH	5.26, t (6.9)	1″, 4″, 5″	123.2, CH	5.16, t (7.1)	4″, 5″
3″	151.2, C			133.5, C			133.1, C		
4″	109.0, CH_2	4.61, s 4.81, s	2″, 5″	14.2, CH_3	1.78, s	2″, 3″, 5″	16.2, CH_3	1.70, s	2″, 3″, 5″
5″	25.9, CH_2	2.32, m 2.37, m	3″	34.4, CH_2	2.27, t (7.2)	2″, 3″, 4″, 6″, 7″	31.6, CH_2	2.20, t (7.6)	2″, 3″, 6″, 7″
6″	32.3, CH_2	2.47, m	3″, 5″, 7″	32.3, CH_2	2.43, t (7.2)	3″, 5″, 7″	41.6, CH_2	2.47, dt (1.8, 7.6)	3″, 5″, 7″
7″	173.6, CO			174.0, CO			203.5, CHO	9.62, t (1.8)	
OH-5		12.62, s	5					12.44, s	4a, 5, 6
OH-2″		4.10, s							
MeO-3′	55.9, CH_3	3.78, s	3′	55.0, CH_3	3.89, s	3′	54.9, CH_3	3.81, s	3′
MeO-7″	51.7, CH_3	3.59, s	7″	50.3, CH_3	3.59, s	7″			

n.d. – Not determined.

Table 2
^{1}H NMR and ^{13}C chemical shifts (δ in ppm) and spin–spin coupling constants (J in Hz) of compounds **4**–**6**.

4 (DMSO-d_6)				**5** (Methanol-d_4)			**6** (DMSO-d_6)		
Position	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC	δ_C, mult.	δ_H (J in Hz)	HMBC
2	78.9, CH	5.42, dd (3.0, 12.7)		78.2, CH	5.32 br, d (13.0)	2′, 6′	78.3, CH	5.26 br, d (12.3)	
3	42.6, CH_2	2.65, dd (3.0, 17.0)		43.2, CH_2	2.78, dd (2.6, 17.0)	2, 4	42.2, CH_2	2.57 br, d (17.0)	2, 4
		3.27, dd (12.7, 17.0)			3.12, dd (13.0, 17.0)			3.10 br, d (17.0)	
4	196.8, CO			197.0, CO			195.1, CO		
4a	101.4, C			101.0, C			100.7, C		
5	161.1, C			161.0, C			161.1, C		
6	108.0, C			106.0, C			108.2, C		
7	165.2, C			165.0, C			168.3, C		
8	95.4, CH	5.99, s	4, 4a, 6, 7	94.0, CH	5.96, s	4, 4a, 6, 7, 8a	96.7, CH	5.62, s	4a, 6, 8a
8a	n.d.			161.0, C			160.9,C		
1′	129.4, C			130.0, C			130.3, C		
2′	111.8, CH	7.12 br, s	2, 3′, 6′	109.8, CH	7.08 br, s	2, 3′, 4′, 6′	111.5, CH	7.06, d (2.0)	2, 4′, 6′
3′	147.9, C			147.8, C			147.7, C		
4′	147.4, C			146.8, C			147.0, C		
5′	116.0, CH	6.86, d (8.2)	1′, 3′	114.6, CH	6.83, d (8.4)	1′, 3′	115.5, CH	6.78, d (8.2)	1′, 4′
6′	120.2, CH	6.94 br, d (8.2)	2, 2′, 4′	119.4, CH	6.93 br, d (8.4)	1′, 2′, 4′, 5′	119.3, CH	6.88, dd (2.0, 8.2)	1′, 2′, 4′
1″	21.4, CH_2	3.13, d (7.1)	5, 6, 7, 2″, 3″	20.7, CH_2	3.26, d (7.3)	5, 6, 2″, 3″	24.7, CH_2	2.32, m	5, 6, 7, 5″
								2.90, m	
2″	123.3, CH	5.18, t (7.1)	1″, 5″	122.8, CH	5.29, t (7.3)	3″, 5″	76.3, CH	3.36 br, s[a]	
3″	133.0, C			133.3, C			86.0, C		
4″	16.2, CH_3	1.74, s	2″, 3″	14.8, CH_3	1.78, s	2″, 3″, 5″	21.6, CH_3	1.17, s	2″, 3″, 5″
5″	34.4, CH_2	1.96, t (7.4)	2″, 3″, 4″, 6″, 7″	35.9, CH_2	2.01, m	2″, 3″, 4″, 6″	35.5, CH_2	1.58, m	2″, 5″
					2.21, m			2.04, m	
6″	30.7, CH_2	1.58, m	3″, 5″, 7″	29.0, CH_2	1.32, m	4″, 5″	30.6, CH_2	1.58, m	7″, 8″, 9″
					1.65, m			1.96, m	
7″	104.5, CH	4.30, t (7.5)	5″, MeO-7″	74.6, CH	3.37, m	5″, 6″, 8″	81.4, CH	4.30, t (7.7)	6″, 9″
8″				76.9, C			147.0, C		
9″				19.3, CH_3	1.08, s	8″, 10″	110.2, CH_2	4.71, s	7″, 10″
								5.04, s	
10″				19.8, CH_3	1.12, s	8″, 9″	18.5, CH_3	1.68, s	7″, 8″, 9″
OH-5		12.50, s	4a, 5, 6					12.51, s	4a, 5, 6
OH-2″								3.36, s	
OH-7″					3.37, s				
MeO-3′	56.1, CH_3	3.83, s	3′	55.4, CH_3	3.89, s	3′	56.1, CH_3	3.79, s	3′
MeO-7″	52.7, CH_3	3.17, s	7″						
MeO-7″	52.7, CH_3	3.17, s	7″						
MeO-8″				48.1, CH_3	3.18, s	8″			

n.d. – Not determined.
[a] Overlapped with a signal of water.

ble 2). HMBC, COSY, and NOESY correlations supported the idea of a double bond between the C-8″ and C-9″ of the geranyl side chain (Fig. S24–S28 of Supporting information). The structure of **6** was concluded to be assigned as tomentodiplacone H.

The molecular formula of **7** was established to be $C_{26}H_{28}O_7$ by HRESIMS, supported by the presence of a pseudomolecular ion $[M+Na]^+$ at m/z 475.17263 (calcd. for $C_{26}H_{28}O_7Na^+$ 475.173275). NMR data analysis (Table 3, Fig. S29–S32 of Supporting information) showed a compound closely related to tomentodiplacone, which was reported in our previous work (Šmejkal et al., 2008b). According to the ^{1}H and ^{13}C NMR data, their structures differ in substitution at C-7″ of geranyl side chain only. The chemical shift at δ 202.2 indicated the presence of a carbonyl group. Unusually upfield hydrogen signal at δ 5.18 (H-8) was also observed. Compound **7** was named tomentodiplacone I.

The molecular formulas of **8** and **9** were determined to be $C_{25}H_{26}O_6$ for both compounds. The HRESIMS spectrum in the positive mode showed the presence of pseudomolecular ions $[M+Na]^+$ at m/z 445.16198 (**8**) and 445.16206 (**9**), respectively (calcd. for $C_{25}H_{26}O_6Na^+$ 445.16271). The ^{1}H and ^{13}C NMR data showed almost superimposable signals for flavanone skeletons each with 4′-hydroxyphenyl moiety. Furthermore, a geranyl side chain modified by the formation of a pyran ring and the presence of two double bonds and one hydroxyl group was discovered (Table 4, Fig. S33–S42 of Supporting information). Different carbon signals, HMBC, and COSY correlations between positions C-6″ and C-10″ of the prenyl side chain revealed different locations of the double bond between C-8″ and C-9″ in **8** and between C-6″ and C-7″ in **9**. The structures of **8** and **9** were closely related to cycloaltilisin 7, a compound previously isolated from *Artocarpus altilis* (Moraceae) (Patil et al., 2002). The structures differ only in the substitution patterns of the double bond at prenyl side chain and by the presence of additional hydroxy group, at positions C-7″ for **8** and C-8″ for **9**. Compounds **8** and **9** were named mimulone C and D, respectively.

The molecular formula of **10** was determined to be $C_{23}H_{24}O_8$ by HRESIMS and was supported by the presence of a pseudomolecular ion $[M-H]^-$ at m/z 427.1389 (calcd. for $C_{23}H_{23}O_8^-$ 427.13929). The NMR data were very similar to those for **1** (Table 4), the main difference being the presence of two doublets in the aromatic region at δ_H 7.33 and 6.84 ppm with a coupling constant of 8.5 Hz, corresponding to the four protons of the 4′-OH substituted ring B. In the geranyl chain, the carbon signals at δ_C 50.5 (MeO-7″), 74.4 (C-2″), 108.0 (C-4″), and 174.7 (C-7″), together with the HMBC and COSY data confirmed the same modifications of the geranyl chain as in the case of **1**. Compound **10** was named mimulone E.

The absolute configurations of the substances at stereogenic center C-2 were determined using analyses of their circular dichroism spectra. A positive Cotton effect for the $n \rightarrow \pi^*$ electronic transition at 320–360 nm and a negative Cotton effect for the $\pi \rightarrow \pi^*$ electronic transition at 280–310 nm were observed for compounds **2**–**5** and **8**–**10**. A 2*S*-configuration was assigned to these compounds by comparison of the CD and NMR data with those of previously reported flavanones (Šmejkal et al., 2008b). No Cotton effect was observed in the CD spectra of **1**, **6** and **7**, these

Table 3
^{1}H NMR and ^{13}C chemical shifts (δ in ppm) and spin–spin coupling constants (*J* in Hz) of compound **7.**

7 (DMSO-d_6)			
Position	δ_C, mult.	δ_H (*J* in Hz)	HMBC
2	77.7, CH	5.08, dd (3.0, 13.0)	
3	40.2, CH_2	2.38, dd (3.0, 17.0) 2.84, dd (13.0, 17.0)	2, 4, 1′, 6′
4	n.d.		
4a	96.8		
5	161.0, C		
6	109.5, C		
7	160.0, C		
8	98.1, CH	5.18, s	4, 4a, 6, 7
8a	160.6, C		
1′	131.6, C		
2′	111.2, CH	7.00, d (2.0)	2, 3′, 4′, 6′
3′	147.9, C		
4′	146.5, C		
5′	115.4, CH	6.75, d (8.0)	1′, 3′, 4′
6′	119.5, CH	6.84,dd (1.8, 8.1)	2′, 4′
1″	21.5, CH_2	2.93, d (6.8)	6, 2″, 3″
2″	125.6, CH	5.15, t (6.8)	6, 4″, 5″
3″	136.1, C		
4″	16.6, CH_3	1.67, s	2″, 3″, 5″
5″	34.2, CH_2	2.08, t (7.6)	2″, 4″, 6″, 7″
6″	36.3, CH_2	2.70, t (7.6)	5″, 7″, 8″
7″	201.2, CO		
8″	143.8, C		
9″	125.5, CH_2	5.82, s 6.03, s	7″, 10″
10″	17.4, CH_3	1.77, s	7″, 8″, 9″
OH-5		12.75, s	4a, 5, 6
MeO-3′	56.0, CH_3	3.77, s	3′

compounds therefore must be racemic mixtures of 2*R* and 2*S* enantiomers. Too little material was isolated to allow stereochemistry of any other stereogenic centers.

To prove that the compounds isolated are not artifacts formed during extraction and separation procedures, a test of stability was performed using mimulone (4′-hydroxy-6-geranyl-5,7-dihydroxyflavanone, **15**) as a reference compound (Šmejkal et al., 2007, 2008b). Mimulone (**15**) was subjected to conditions simulating the process of isolation (the influence of sunlight, temperature changes, and chemicals used in routine separation processes) to prove stability of compound. Results of this stability assay (HPLC analysis) confirmed that mimulone is a relatively stable compound (see Table 6). However, the long-termed exposition to a direct sun light and temperature of 50 °C led to significant decomposition of **15** both in all used solvents, and also as an amorphous solid. Because compounds isolated herein (**1**–**10**) have never been exposed to such conditions leading to decomposition of **15**, we conclude that they are not artifacts formed during separation process. Nevertheless, we cannot exclude that these compounds were formed during maturation of fruits from corresponding parent substances (mainly 3′-*O*-methyldiplacone), as also **15** in solid state decomposed under direct sunlight conditions and increased temperature. The similar functionalization of terpenoid chains has been described previously (Brown et al., 2003; Wang et al., 2007).

The results of antimicrobial activity assays are presented in Table 5. Following previously described testing procedures (Šmejkal et al., 2008b), minimum inhibitory concentrations (MICs) were determined for selected Gram-positive bacteria *S. aureus* and various methicillin resistant strains of *S. aureus*. Selected

Table 4
^{1}H NMR and ^{13}C chemical shifts (δ in ppm) and spin–spin coupling constants (*J* in Hz) of compounds **8**–**10**.

8 (DMSO-d_6)				**9** (DMSO-d_6)			**10** (Methanol-d_4)		
Position	δ_C, mult.	δ_H (*J* in Hz)	HMBC	δ_C, mult.	δ_H (*J* in Hz)	HMBC	δ_C, mult.	δ_H (*J* in Hz)	HMBC
2	78.7, CH	5.48 br, d (12.9)	4, 1′	78.9, CH	5.45 br, d (13.0)	4, 1′	78.6, CH	5.35, dd (2.6, 13.0)	4
3	40.3, CH_2	2.69 br, d (17.1) 3.29 br, d (17.1)	4	43.5, CH_2	2.60 br, d (17.1) 3.27 br, d (17.1)		42.5, CH_2	2.71, dd (2.6, 17.1) 3.12, dd (13.0, 17.1)	2, 4
4	197.4, CO			197.7, CO			196.1, CO		
4a	n.d.			n.d.			101.6, C		
5	n.d.			n.d.			161.8, C		
6	102.4, C			102.4, C			105.3, C		
7	162.3, C			163.9, C			165.2, C		
8	95.9, CH	5.92, s	6, 7	96.3, CH	5.91, s	6, 7	94.3, CH	5.95, s	4a, 6, 7, 8a
8a	n.d.			n.d.			161.6, C		
1′	128.7, C			129.0, C			129.4, C		
2′	128.8, CH	7.33, d (8.4)	1′, 4′, 6′	128.9, CH	7.32, d (8.2)	2, 1′, 4′, 6′	127.6, CH	7.23, d (8.5)	2, 4′, 6′
3′	115.6, CH	6.80, d (8.4)	1′, 4′, 5′	115.6, CH	6.83, d (8.2)	1′, 4′, 5′	114.7, CH	6.84, d (8.5)	1′, 4′, 5′
4′	158.3, C			158.0, C			157.5, C		
5′	115.6, CH	6.80, d (8.4)	1′, 3′, 4′	115.6, CH	6.83, d (8.2)	1′, 3′, 4′	114.7, CH	6.84, d (8.5)	1′, 3′, 5′
6′	128.8, CH	7.33, d (8.4)	1′, 2′, 4′	128.9, CH	7.32, d (8.2)	2, 1′, 2′, 4′	127.6, CH	7.23, d (8.5)	2, 2′, 4′
1″	115.3, CH	6.54, d (10.2)	6, 7	115.4, CH	6.52, d (10.1)	6, 7	28.2, CH_2	2.82, m 2.82, m	5, 6, 7, 2″, 3″
2″	126.4, CH	5.59, d (10.2)	6	126.2, CH	5.59, d (10.1)	6, 4″	74.4, CH	4.40, t (6.6)	6, 1″, 4″
3″	81.0, C			81.0, C			149.4, C		
4″	27.2, CH_3	1.38, s	2″, 3″, 5″	26.7, CH_3	1.39, s	2″, 3″, 5″	108.0, CH_2	4.74, s 4.91, s	2″, 5″
5″	37.5, CH_2	1.49, m 1.66, m	6″, 7″	43.8, CH_2	2.35, dd (2.0, 7.1)	2″, 3″, 4″, 6″, 7″	25.6, CH_2	2.52, m	4″, 6″
6″	29.4, CH_2	1.47, m 1.74, m	7″	120.0, CH	5.49, m		31.9, CH_2	2.53, m	5″, 7″
7″	73.9, CH	3.83, m		143.0, CH	5.60, m		174.7, C		
8″	148.0, C			74.2, C					
9″	110.7, CH_2	4.74, s 4.96, s	7″, 10″	30.1, CH_3	1.09, s	7″, 8″, 10″			
10″	18.3, CH_3	1.60, s	7″, 8″, 9″	30.1, CH_3	1.09, s	7″, 8″, 9″			
OH-5		12.48, s		12.47, s				n.d.	
OH-7″		3.83, s	7″						
OH-8″				3.82, s					
MeO-7″							50.5, CH_3	3.68, s	7″

n.d. – Not determined.

Table 5
Minimum inhibitory concentrations (μg/ml) of selected *C*-geranylated flavanones from *P. tomentosa* fruits.

Compound	Gram-positive bacteria					
	S. a.	MRSA 287	MRSA 4211	MRSA 6975	MRSA 630	MRSA 62059
1	>64	>64	>64	64	>64	>64
2	16	>64	>64	16	64	32
3	64	>64	>64	8	64	32
5	64	>64	>64	16	>64	>64
8	>64	>64	>64	8	64	64
9	>64	>64	>64	16	64	64
11	8	16	16	8	16	16
12	8	16	16	8	16	16
13	4	8	8	8	4	4
14	2	8	8	8	8	4
15	2	4	2	2	4	2
16	4	4	4	4	4	8
OXAC	0.125	0.5	0.25	>32	16	1
CIPR	0.5	0.5	0.5	>32	>32	1
VANC	1	1	1	1	1	1
CLIN	0.125	>16	0.125	>16	>16	0.25
ERYT	1	>32	0.5	>32	<0.007	>32
TETC	0.25	16	0.25	8	0.5	16
RIFP	<0.007	<0.007	<0.007	>16	<0.007	>16
DOXY	0.125	1	0.125	2	0.25	4

Oxacillin (OXAC), Ciprofloxacin (CIPR), Vancomycin (VANC), Clindamycin (CLIN), Erythromycin (ERYT), Tetracycline (TETC), Rifampicin (RIFP), and Doxycycline (DOXY) were used as positive controls for *S. a.*, *Staphylococcus aureus* ATCC 29213, MRSA (methicillin resistant *Staphylococcus aureus*): MRSA 287, MRSA 4211, MRSA 6975, MRSA 630, MRSA 62059. MIC breakpoints (*R*>) for *S. aureus* ATCC 29213 (μg/ml): OXAC $R > 2$ are mostly methicillin resistant due to the presence of the mecA gene, CIPR $R > 1$, VANC $R > 2$, CLIN $R > 0.5$, ERYT $R > 2$, TETC $R > 2$, RIFP $R > 0.5$, DOXY $R > 2$ (European Committee on Antimicrobial Susceptibility Testing, 2012).

Table 6
Results of stability assay of model *C*-6 geranyl flavanone **15.**

	Mimulone purity (% AUC)
Freezer	
DMSO	95.5
MeOH	98.3
Silica gel (MeOH)	98.3
0.2% HCOOH	98.2
Solid substance	96.6
Laboratory	
DMSO	93.3
MeOH	98.5
Silica gel (MeOH)	98.3
0.2% HCOOH	95.2
Solid substance	96.7
Sunlight	
DMSO	55.8
MeOH	85.9
Silica gel (MeOH)	74.3
0.2% HCOOH	36.7
Solid substance	39.4

compounds isolated (**1**–**3**, **5**, **8**, **9**) and some other C-6-geranylated flavanones previously isolated from *P. tomentosa* (Šmejkal et al., 2008a), namely tomentodiplacone B (**11**), diplacone (**12**), 3′-*O*-methyl-5′-hydroxydiplacone (**13**), 3′-*O*-methyl-5′-*O*-methyldiplacone (**14**), mimulone (**15**), and 3′-*O*-methyldiplacol (**16**) were tested. All of the compounds selected for testing exhibited some degree of activity in the range of the concentrations tested, however, with activity varying in dependence both on the structure and bacterial strains used in assay. Compounds **15** and **16** with MICs in range 2–4 μg/ml for all bacteria strains belonged among the most active anti-infectives analyzed in this assay, showing promising anti-MRSA activity (Ríos and Recio, 2005). Table 5 presents as the most active compound **15** showing antibacterial activity with MIC 2 μg/ml against MRSA 4211, against MRSA 6975 (better than all tested antibiotics with exception of vancomycin and doxycycline), and against MRSA 62059 (better activity than erythromycin, tetracycline, rifampicin and doxycycline). Slightly higher MICs for **15** (4 μg/ml) were obtained against MRSA 287 (better than clindamycin, erythromycin and tetracycline) and MRSA 630 (better than oxacillin, ciprofloxacin and clindamycin). The other substances with non-modified geranyl side chain showed better than intermediate antibacterial activity in range 4–16 μg/ml. The most active compound with modified geranyl side chain, **11**, inhibited the growth of bacteria with minimum inhibitory concentrations (MICs) in the range 8–16 μg/ml (against MRSA 287 better than clindamycin and erythromycin, comparable to tetracycline; against MRSA 630 better than ciprofloxacin and clindamycin, the same as oxacillin; and MRSA 62059 better than erythromycin and rifampicin, comparable to tetracycline). Compounds **2** and **3** showed much lower activities, inhibiting four bacteria strains with MICs in the range 16–64 μg/ml. Unfortunately, none of the compounds tested were able to inhibit the growth of Gram-negative bacteria (data not shown).

Several of the flavanones inhibited the growth of MRSA, suggesting that the MIC values indicate the relative influence of the different substituent groups. Hydroxylation at positions C-5, C-7, and C-4′ is a determining factor for the anti-MRSA activity of flavanones (Tsuchiya et al., 1996) and this corresponds with the presented results. The 3′-methoxy-4′-hydroxyphenyl ring B of **2**, **3**, and **11**, **13**, **14** and **16** probably increases the antibacterial activity because of the planar structure of the molecule; however, contrary to this assumption, compounds **1** and **5** had no significant antibacterial activity. This could be caused by the presence of changes at lipophilic aliphatic C-6 geranyl side chain, which Tsuchiya et al. (1996) described as the important structural factor for anti-MRSA activity. Homoeriodictyol (3′-methoxy-4′,5,7-trihydroxyflavanone) did not show an important antibacterial activity (Mori et al., 1987). Furthermore, as shown, the substitution of the geranyl side chain with carbonyl, hydroxyl and methoxyl groups decreases this activity similarly as the cyclization of the geranyl substituent attached to the C-6 (Kuete et al., 2009) with a ring closed *via* C-7 hydroxyl group (as observed in **8** and **9**).

The results of these assays on MRSA strains indicate that geranylated flavanones could be helpful in designing therapeutic strategies for the treatment of MRSA infections and also in the design and synthesis of different antibacterial agents.

The ability to affect eukaryotic translation initiation *via* dual-luciferase reporter assay (firefly and renilla) was tested for compounds **1**, **2**, **3**, **5**, and **8**, but only minor activity (for compound **1** 0.81 ± 0.07 and 0.64 ± 0.08, respectively) was observed in comparison to anisomycin, which was used as a positive control (0.01 ± 0.02 and 0.01 ± 0.14).

3. Concluding remarks

The data presented demonstrate that *P. tomentosa* is a rich source of multifarious geranylated flavonoids. Compounds **1**–**10** have been obtained from a natural source for the first time and their structures have been shown to possess unusually modified geranyl side chains at position *C*-6 of the flavanone nucleus. The antibacterial activity of compounds **1**–**3**, **5**, **8**, **9**, and **11**–**16** against different MRSA strains was tested, showing geranylated flavanones with unmodified side chain as compounds with promising activity against MRSA.

4. Experimental section

4.1. General experimental procedures

UV spectra were recorded in MeOH using a JASCO J-810 spectrometer (**1**–**3**, **5**, **8**, and **9**) and a UV/Vis Spectrometer Lambda 25 (PerkinElmer Instruments) (**4**, **6**, **7** and **10**). CD spectra were recorded on a JASCO J-810 spectrometer (MeOH; the molar elipticity Θ_λ values are presented). IR spectra were determined by the ATR method on a Nicolet Impact 400D FT-IR spectrophotometer. NMR spectra were recorded using a Bruker Avance 400 Ultrashield spectrometer operating at a frequency of 400 MHz (^{1}H). NMR spectra were acquired in methanol-d_4 at 298 K (**2**, **5**, **10**) and in DMSO-d_6 at 296 K (**1**, **3**, **4**, **6–9**) with TMS as an internal standard. The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signal of the solvent [3.30 ppm (^{1}H) and 49.9 ppm (^{13}C) for methanol-d_4, 2.49 ppm (^{1}H) and 53.6 ppm (^{13}C) for DMSO-d_6]. The 1D NMR experiment and 2D NMR experiments (COSY, HMBC, and HSQC for **1**–**10**, NOESY for **2**–**6**, **8**–**10**, and TOCSY for **4**) were used to assign the individual ^{1}H and ^{13}C resonances. NMR spectrometric processing was performed using the standard Bruker software (for detailed info please see Tables 1–4 and Supporting information Section S1–42). The elementary composition of compounds **1**–**9** were measured on an Orbitrap MS instrument (Thermo Scientific) using ESI in the positive mode. Samples were dissolved in MeOH and injected using a loop (2 μl) into the stream of the mobile phase (75% MeOH/25% H_2O; flowing at 40 μl/min). The mass resolution was 100,000, and the lock mass was 301.14 (dibuthylphthalate). Ions $[M+Na]^+$ were detected, and *m*/*z* MS/MS from parent ion $[M+Na]^+$ were measured. The elementary compositions of the compound **10** was measured using HRESITOFMS (PE Biosystems Mariner) in the negative mode. The compound was dissolved in 0.3% ammonia in MeOH and injected directly into the ESI source at a flow rate of 10 μl/min.

Column chromatography was carried out on Merck silica gel 60 (particle size 0.040–0.063 mm). TLC plates of Silicagel 60 F_{254} (Merck) (UV detection at 254 and 366 nm) were employed. TLC plates of Silicagel 60 F_{254} (500 μm, glass support) were used for prep. TLC. Analytical HPLC was performed on an Agilent 1100 apparatus equipped with a diode-array detector; Supelcosil™ ABZ+Plus column (150 mm × 4.6 mm i.d., particle size 3 μm) was used. YL9100 HPLC system (Young-Lin) was used for prep. RP-HPLC separation using a Supelcosil™ Ascentis RP-Amide (250 mm × 10 mm i.d., particle size 5 μm) column.

4.2. Plant material

P. tomentosa fruits were collected on the campus of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, during October 2004. The material was identified by Dr. Petr Babula (Department of Natural Drugs, UVPS Brno). A voucher specimen (PT-04O) has been deposited in the herbarium of the Department of Natural Drugs, Faculty of Pharmacy, UVPS Brno, Czech Republic.

4.3. Isolation method

The basic extraction and chromatographic sample preparation have been described previously (Šmejkal et al., 2008b). The $CHCl_3$ portion of the EtOH extract was separated by column chromatography using a mobile phase C_6H_6:Me_2CO (95:5, v/v) with an increasing proportion of Me_2CO. The collected fractions were combined according to the results of TLC and HPLC analyses to provide a final 20 fractions labeled A–T. Fractions C (0.8 g) and D (9.5 g) were subjected to further separation by column chromatography on silica gel using the mobile phase $CHCl_3$:MeOH:C_6H_6 (70:2.5:27.5, v/v/v). Compounds **8** (2.7 mg) and **9** (2.0 mg) were acquired from subfractions C19-22 and C23-28, respectively, by using of prep. TLC with a mobile phase of C_6H_6:EtOAc (1:1, v/v).

Subfractions of fraction D collected by column chromatography were combined on the basis of HPLC and TLC analyses. Subfractions D21-27 and D32-36 were further separated using prep. RP-HPLC and prep. TLC (Fig. 1). Subfraction D21-27 was subjected to separation by semiprep. RP-HPLC using a mobile phase of A (MeCN:MeOH:H_2O, 80:10:10, v/v/v) and B (0.2% HCOOH). The gradient elution started with a mobile phase composition of A:B (70:30, v/v), and reached the terminal composition of A:B (74:26, v/v) after 25 min at a flow rate of 5 ml/min. The injection volume was 20 μl. Using this method, pure compound **2** (5.2 mg) was acquired. The other subfractions collected based on UV detection at λ 280 nm were purified using prep. TLC with a mobile phase of C_6H_6:EtOAc (2:3, v/v) for compound **1** (5.1 mg) or $CHCl_3$:MeOH:C_6H_6 (6:1.25:2.75, v/v/v) for compounds **3** (5.4 mg) and **4** (2.6 mg). Subfraction D32-36 was separated repeatedly using prep. RP-HPLC, starting with a mobile phase composition of A (MeCN:MeOH:H_2O 80:10:10 v/v/v): B (0.2% HCOOH) of 72:28 (v/v), and finishing with a composition of A:B 75:25 (v/v) in the 20th min, at a flow rate of 5 ml/min. The injection volume was 20 μl. Pure compound **5** (19.8 mg) was obtained from subfraction D32-36 and the portion of subfraction D32-36/19 was further purified using preparative TLC with a mobile phase of $CHCl_3$:MeOH:C_6H_6 (6.25:1:2.75, v/v) to isolate compound **7** (3.5 mg). The portion of subfraction D32-36/12 was further purified repeatedly by prep. RP-HPLC with a mobile phase consisting of (A) MeOH and (B) 0.2% HCOOH. The gradient elution started with a mobile phase composition of A:B (70:30, v/v), and finished with a composition of A:B (73:27, v/v) in the 20th min. The flow rate was 5 ml/min, and the injection volume 20 μl. Pure compound **6** (1.6 mg) was obtained.

Fraction F (3.5 g) was separated on silica gel using column chromatography with a mobile phase of $CHCl_3$:MeOH:C_6H_6 (70:1:29, v/v/v). Compound **10** was obtained from subfraction F39-41 by using prep. RP-HPLC with a mobile phase of A (MeCN:MeOH:H_2O, 80:10:10, v/v/v) and B (0.2% HCOOH). The gradient elution started with a mobile phase composition of A:B (45:55, v/v), and reached a terminal composition of A:B (75:25, v/v) in the 30th min at a flow rate of 5 ml/min. The injection volume was 15 μl. Using this method, pure compound **10** (20 mg) was acquired.

Compounds **11**–**16** were obtained in a previously study (Šmejkal et al., 2008a).

*4.3.1. Tomentodiplacone C (**1**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 234 sh (4.37), 291 (4.25), 326 sh (3.97) nm; IR (ATR) ν_{max} cm^{-1}: 3971, 3724, 3510, 3390, 3325, 3253, 3212, 2903, 1714, 1633, 1551, 1516, 1454, 1343, 1266, 1152, 1089, 1028, 820, 772; HRESIMS $[M+Na]^+$ *m/z* 481.14682 (calcd. for $C_{24}H_{26}O_9Na^+$ 481.14745); for ^1H and ^{13}C NMR data, see Table 1.

*4.3.2. Tomentodiplacone D (**2**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 233 (sh) (4.54), 291 (4.42), 326 (sh) (3.71) nm; CD (MeOH): $\Theta_{335.5}$ +6184, Θ_{293} −21323, Θ_{219} +15287; IR (ATR) ν_{max} cm^{-1}:3725, 2925, 2900, 1723, 1627, 1516, 1439, 1342, 1304, 1274, 1234, 1207, 1154, 1082, 1032, 989, 817, 774; HRESIMS $[M+Na]^+$ *m/z* 465.15182 (calcd. for $C_{24}H_{26}O_8Na^+$ 465.15254); for ^1H and ^{13}C NMR data, see Table 1.

*4.3.3. Tomentodiplacone E (**3**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 234 (sh) (4.46) nm, 291.5 (4.34), 340 (sh) (3.59); CD (MeOH): $\Theta_{334.5}$ +5009, $\Theta_{293.5}$ −12147, $\Theta_{218.5}$ +10585; IR (ATR) ν_{max} cm^{-1}: 3853, 3651, 3369, 3304, 3117, 2924, 1712, 1630, 1597, 1515, 1487, 1441, 1334, 1294, 1272, 1186, 1152, 1077, 1028, 813, 770, 731; HRESIMS $[M+Na]^+$ *m/z* 467.16762 (calcd. for $C_{24}H_{28}O_8Na^+$ 467.16891), $[M+Na]^+$ *m/z* 435.14135 (calcd for $C_{23}H_{24}O_7Na^+$ 435.141975); for ^1H and ^{13}C NMR data, see Table 1.

*4.3.4. Tomentodiplacone F (**4**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 229 (sh) (4.81), 291 (4.25), 342 (sh) (3.56) nm; CD (MeOH):Θ_{344} +812, Θ_{294} −2 843; IR (ATR) ν_{max} cm^{-1} 3824, 3661, 3209, 2936, 2839, 2725, 2355, 1631, 1598, 1515, 1444, 1337, 1301, 1271, 1153, 1122, 1058, 1032, 817; HRESIMS $[M+Na]^+$ *m/z* 481.18324 (calcd. for $C_{25}H_{30}O_8Na^+$ 481.18384); for ^1H and ^{13}C NMR data, see Table 2.

*4.3.5. Tomentodiplacone G (**5**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 220 (sh) (4.43), 290.5 (4.25), 326 (sh) (3.61) nm; CD (MeOH): Θ_{343} +2520, Θ_{294} −11160, $\Theta_{219.5}$ +9624; IR (ATR) ν_{max} cm^{-1}: 3729, 3700, 3339, 3312, 3261, 3183, 2926, 1629, 1595, 1515, 1448, 1338, 1301, 1270, 1188, 1151, 1063, 1031, 816, 769; HRESIMS $[M+Na]^+$ *m/z* 509.21478 (calcd. for $C_{27}H_{34}O_8Na^+$ 509.21514); for ^1H and ^{13}C NMR data, see Table 2.

*4.3.6. Tomentodiplacone H (**6**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 231 (sh) (4.92), 290 (4.20), 338 (sh) (3.71) nm; IR (ATR) ν_{max} cm^{-1}: 3765, 3724, 3701, 3629, 3601, 3466, 3407, 3233, 3194, 3127, 3070, 2969, 2931, 2329, 1608, 1588, 1515, 1453, 1343, 1270, 1195, 1154, 1084, 1028, 900, 817, 769; HRESIMS $[M+Na]^+$ *m/z* 493.18307 (calcd. for $C_{26}H_{30}O_8Na^+$ 493.18384); for ^1H and ^{13}C NMR data, see Table 2.

*4.3.7. Tomentodiplacone I (**7**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 230 (sh) (4.80), 292 (4.11), 344 (sh) (3.86) nm; IR (ATR) ν_{max} cm^{-1}: 3449, 3268, 3239, 2913, 1636, 1598, 1553, 1516, 1453, 1377, 1348, 1308, 1263, 1181, 1152, 1123, 1078, 1030, 872, 818, 770; HRESIMS $[M+Na]^+$ *m/z* 475.17263 (calcd. for $C_{26}H_{28}O_7Na^+$ 475.173275); for ^1H and ^{13}C NMR data, see Table 3.

*4.3.8. Mimulone C (**8**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 227.5 (4.36), 274 (4.47), 296.5 (sh) (4.17), 352.5 (sh) (3.51) nm; CD (MeOH): Θ_{345} +2073, $\Theta_{292.5}$ −9200, $\Theta_{219.5}$ +6836; IR (ATR) ν_{max} cm^{-1}: 3294, 2948, 2921, 2854, 1632, 1514, 1446, 1371, 1338, 1259, 1153, 1089, 1022, 897, 798; HRESIMS $[M+Na]^+$ *m/z* 445.16198 (calcd. for $C_{25}H_{26}O_6Na^+$ 445.16271); for ^1H and ^{13}C NMR data, see Table 4.

*4.3.9. Mimulone D (**9**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 227.5 (4.33), 274.5 (4.44), 296 (sh) (4.14), 351 (sh) (3.48) nm; CD (MeOH): $\Theta_{344.5}$ +2069, $\Theta_{291.5}$ −3467, $\Theta_{219.5}$ +7089; IR (ATR) ν_{max} cm^{-1}: 2960, 2915, 1634, 1514, 1449, 1369, 1339, 1257, 1152, 1985, 1025, 798; HRESIMS $[M+Na]^+$ *m/z* 445.16206 (calcd. for $C_{25}H_{26}O_6Na^+$ 445.16271); for ^1H and ^{13}C NMR data, see Table 4.

*4.3.10. Mimulone E (**10**)*

Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 203 (4.58), 226 (sh) (4.50), 294 (4.30), 333 (sh) (3.67) nm; CD (MeOH): Θ_{331} +9693, Θ_{290} −45750; IR (ATR) ν_{max} cm^{-1}: 3246, 3081, 1584, 1519, 1441, 1336, 1296, 1268, 1155, 1078, 901, 832, 776; HRESIMS *m/z* $[M-H]^-$ 427.1389 (calcd. for $C_{23}H_{23}O_8^-$ 427.13929); for ^1H and ^{13}C NMR data, see Table 4.

4.4. Stability assay

1 mg of mimulone (**15**) was treated with DMSO, methanol, silica gel in MeOH, and 0.2% HCOOH (1 ml). All of these mixtures plus **15** in solid state were stored for 30 days in freezer (−20 °C, dark), under laboratory temperature (25 °C, dark), or under direct sunlight (50 °C, sunlight). The samples were analyzed using HPLC. The results were expressed as percentage of total area under curve of chromatogram, see Table 6. The initial purity of mimulone (**15**) used as tested substance was determined to be 97.26% (average value of five independent HPLC measurements).

4.5. Antimicrobial activity assay (Jorgensen et al., 1999; Schwalbe et al., 2007)

The compounds to be tested were dissolved in DMSO to a concentration of 128 μg/ml (stock solution) and subjected to an *in vitro* antimicrobial activity assay using the broth microdilution method. Nine successive 2-fold dilutions of each compound were mixed with the appropriate Mueller–Hinton broth to provide a concentration range from 64 down to 0.25 μg/ml. Placed in 96-well microtiter plates, these aliquots were then each inoculated with 3 μl of suspensions of standard bacteria strains of *K. p.* (*Klebsiella pneumonie* ATCC 700603), *P. a.* (*Pseudomonas aeruginosa* ATCC 27853), *E. f.* (*Enterococcus faecalis* ATCC 29212), *S. a.* (*Staphylococcus aureus* ATCC 29213) (deposited at the Department of Infectious Diseases and Microbiology, UVPS Brno) and several MRSA strains at a density of 0.5 McF. The resistant strains used were defined as MRSA based on their mecA gen. MRSA 287, MRSA 4211, MRSA 6975, MRSA 630 (Grundmann et al., 2010) are deposited at the National Reference Laboratory for Antibiotics, National Institute of Public Health, MRSA 62059 (clinical isolate) at the Department of Infectious Diseases and Microbiology, UVPS Brno. After inoculation, the plates were incubated at 37 °C for 24 h. The growth of microorganisms was observed using a UV–Vis spectrophotometer monitoring absorbance at 600 nm. The minimum inhibitory concentrations (MICs) were established based on the ratio of the absorbance of the growth of the negative control bacteria to the absorbance of the sample, and were calculated as the lowest concentration of the compound that resulted in an 80% reduction in the growth of the microorganism. The DMSO solvent solution

was assayed simultaneously as a negative control. All samples were tested in triplicate. The antibiotics oxacillin (OXAC), ciprofloxacin (CIPR), vancomycin (VANC), clindamycin (CLIN), erythromycin (ERYT), tetracycline (TETC), rifampicin (RIFP), and doxycycline (DOXY) were acquired from Sigma–Aldrich (UK) and used as positive controls. Due to the antibiotic resistance problems, breakpoints selected by European Committee on Antimicrobial Susceptibility Testing (2012) are presented in the Table 5. These MICs are commonly used for the interpretation of quantitative or qualitative susceptibility test values of antibacterial agents. MIC breakpoints basically divide bacteria into three categories of susceptibility: susceptible, intermediate, or resistant (Jorgensen, 2004). MRSA used were classified according to comparison between breakpoints and obtained MIC results of positive controls. MRSA 6975 and MRSA 630 have been considered as the most resistant bacteria to oxacillin, ciprofloxacin and clindamycin. MRSA 6975 and MRSA 62059 have shown resistance to erythromycin, tetracycline and rifampicin.

4.6. Dual-luciferase reporter assay

This assay was performed according to the previously published method (Novac et al., 2004). Samples were dissolved in DMSO to get a final concentration of 10 μM. The experiments were carried out in duplicate. The negative controls were water, and the DMSO that was used as a vehicle for the samples. Anisomycin (10 μM) was used as a positive control to inhibit the production of both the firefly (FF) and renilla (REN) luciferase. The FF and REN luciferase inhibitory activities were calculated from their relative light units compared with the DMSO control.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.01.002.

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