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Antiplasmodial flavanones and a stilbene from *Carpha glomerata*

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Abstract

Bioassay-guided fractionation of an extract of *Carpha glomerata* (Cyperaceae) led to the isolation of seven compounds. Compounds **1** (carphorin A), **3** (carphorin C), **4** (carphorin D), and **5** (carphabene) are new compounds, and compound **2** (8-(3''-hydroxyisoamyl)-naringenin) was isolated for the first time as a natural product. All structures were elucidated based on analyses of their HR-ESIMS and 1D and 2D NMR data. Compounds **1**, **2**, and **6**, which have prenyl or hydroxyprenyl side chains, exhibited antiplasmodial activities with IC₅₀ values of 5.2 ± 0.6, 3.4 ± 0.4, and 6.7 ± 0.8 μM against the drug-resistant Dd2 strain of *Plasmodium falciparum*. In addition the prenylated stilbene **5** also showed good activity, with IC₅₀ 5.8 ± 0.7 μM.

Graphical Abstract

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Conflict of Interest

The authors declare no competing financial interest.

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Supplementary Material

Supplementary material (experimental procedures, ¹H and ¹³C NMR, COSY, HSQC, and HMBC spectra, and dose-dependent test results) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2018.09.003>.

Keywords

Carpha glomerata; Cyperaceae; Bioassay-guided discovery; Antiplasmodial activity

Malaria is one of the world's most widespread diseases, with an estimated 216 million people being infected, primarily in Africa, and over 445,000 deaths in 2015, most of them in children.¹ It is transmitted by the bite of the female *Anopheles* mosquito, which infects the body with parasites of the *Plasmodium* genus,² and it causes symptoms such as yellow skin, seizures, and coma, leading to death in severe cases.¹ The major antimalarial drugs since 1945 are based on chloroquine and its analogs, such as sulphadoxine, pyrimethamine, and mefloquine, but resistance of the *Plasmodium* parasite to chloroquine developed in the 1950s, and is now widespread.^{3–4} Although the relatively new drug artemisinin and artemisinin-based combination therapies (ACT) are available, there is still an urgent need for new and more effective antimalarial medicines.⁵ In pursuit of this goal, we investigated an extract of *Carpha glomerata* (Cyperaceae) and found it to have moderate activity, with an IC₅₀ value of 5 µg/mL against the Dd2 drug-resistant strain of *P. falciparum*.

Carpha glomerata is a monotypic species in the family Cyperaceae from South Africa. Since no phytochemical studies of this plant have been carried out, we selected it for investigation of its bioactive constituents using bioassay-guided fractionation against the Dd2 strain of *P. falciparum*.

A CH₂Cl₂-soluble portion of a MeOH extract of *Carpha glomerata* had an IC₅₀ value of 5 µg/mL against the Dd2 drug-resistant strain of *P. falciparum*. Chromatography on a silica gel column followed by C-18 reverse-phase HPLC with a gradient of H₂O/MeOH gave compounds **3**, **4**, and **6**, and separation of the most active fractions by C-18 reverse-phase HPLC with elution by H₂O/CH₃CN gave compounds **1**, **2**, **5**, and **7**.

Compound **1** (Figure 1) was isolated as a yellow amorphous powder, and its molecular formula was determined to be C₂₁H₂₂O₇ by HRESIMS data (observed *m/z* 385.1307 [M – H][–], calculated for C₂₁H₂₁O₇[–], 385.1293). Its ¹H and ¹³C NMR spectra (Table 1) combined with its 2D NMR spectra indicated the presence of a flavanone moiety with a prenyl substituent. Its ¹H NMR spectrum displayed an oxymethine proton resonance (δ_H 5.33, 1H, dd, *J* = 11.9, 3.2 Hz, H-2) coupled to methylene protons (δ_H 3.02, 1H, dd, *J* = 17.1, 11.9 Hz, H-3ax; 2.71, 1H, dd, *J* = 17.1, 3.2 Hz, H-3eq), very similar to the corresponding signals of hesperetin,⁶ confirming that **1** is a flavanone derivative.

Comparison of the ¹H and ¹³C NMR signals of **1** with those of comophorin A (**1a**)⁷ indicated that both compounds had identical 3',5'-dihydroxy-4'-methoxyphenyl B-rings, and very similar 5,7-dihydroxy A-rings. The only difference between the two compounds was in the location of the prenyl group. The HMBC correlations from H-1'' (δ_H 3.09–3.11) to C-3'' (δ_C 130.6), C-2'' (δ_C 123.2), C-8 (δ_C 107.5), C-7 (δ_C 165.2), and C-9 (δ_C 159.8) determined the position of the prenylated group at C-8 of the A ring (Figure 2).

The specific rotation of compound **1**, as well as those of **2** – **4**, were all negative, which indicates that they have the *S* configuration at C-2.^{8–9} The structure of **1** was thus assigned

as (S)-2-(3,5-dihydroxy-4-methoxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-en-1-yl)chroman-4-one (carphorin A).

Compound **2** was obtained as a yellow amorphous powder, and its molecular formula was determined to be $C_{20}H_{22}O_6$ by HRESIMS data (observed m/z 359.1505 $[M + H]^+$, calculated for $C_{20}H_{23}O_6^+$, 359.1489). Its 1H and ^{13}C NMR spectra (Table 1) were similar to those of compound **1** except for signals for the B ring and the side chain attached in the A ring. Its ^{13}C NMR spectrum showed signals for two methylene carbons (δ_C 17.9 and 43.5), a quaternary hydroxylated carbon (δ_C 69.3), and two methyl carbons (δ_C 29.5) in a 3-hydroxy-3-methylbutyl side chain on the A ring. The HMBC cross-peak of H-2' (δ_H 7.31) to C-2 (δ_C 78.1), C-6' (δ_C 128.1), C-4' (δ_C 157.8), and C-3' (δ_C 115.5) allowed the assignment of the H-2', 3', 5', 6' spin system to ring B. Further HMBC correlations from H-2'' (δ_H 1.43-1.46) to C-1'' (δ_C 17.9), C-4'' (δ_C 29.5), C-8 (δ_C 108.9), and C-9 (δ_C 159.9) determined the position of the 3-hydroxy-3-methylbutyl side chain as C-8. In addition, the HMBC cross-peak from the chelated hydroxyl group (δ_H 12.13) to C-10 (δ_C 102.0), C-5 (δ_C 161.5), and C-6 (δ_C 95.9) as well as from H-6 (δ_H 5.88) to C-10 (δ_C 102.0), C-5 (δ_C 161.5), and C-8 (δ_C 108.9) also supported the determination of A ring. Although **2** was previously obtained as a by-product of demethylation of 4',7-dihydroxy-5-methoxy-6-(1''-geranyl) flavanone by Wilhelm et al.¹⁰ and as a by-product of hydrolysis of flavaprenin-7,4'-diglucoside by Gellert et al.,¹¹ it is described for the first time as a natural product in this report. Although it is possible that **2** is an artefact formed by hydration of its prenyl precursor during extraction or fractionation, this is considered unlikely since no corresponding derivative of **1** was isolated. Compound **2** is thus identified as (S)-5,7-dihydroxy-8-(3-hydroxy-3-methylbutyl)-2-(4-hydroxyphenyl)chroman-4-one, or 8-(3-hydroxy-3-methylbutyl)-naringenin (carphorin B).

Compound **3** was isolated as a white amorphous powder. It was assigned the molecular formula $C_{19}H_{20}O_7$ from HRESIMS (observed m/z 359.1155 $[M - H]^-$, calculated for $C_{19}H_{19}O_7^-$, 359.1136). Its 1H NMR spectrum (Table 1) was very similar to that of ophiopogon A, previously isolated from *Ophiopogon confertifolius*.¹² The only significant difference was that compound **3** had a signal for an additional OMe group. HMBC correlations from 8-CH₃ (δ_H 1.98) and H-2 (δ_H 5.43) to C-9 (δ_C 157.9), from 6-CH₃ (δ_H 1.96) and 5-OH (δ_H 12.13) to C-6 (δ_C 110.2), and from 5-OH (δ_H 12.13) and H-3 (δ_H 2.82) to C-10 (δ_C 105.0), as well as from 6-CH₃ (δ_H 1.96), 8-CH₃ (δ_H 1.98), and 7-OCH₃ (δ_H 60.3) to C-7 (δ_C 165.0) confirmed the nature of ring A and the attachments of methyl, methoxy, methyl, and hydroxy groups at the C-8, C-7, C-6 and C-5 positions respectively. The structure of **3** was thus assigned as (S)-2-(3,5-dihydroxy-4-methoxyphenyl)-5-hydroxy-7-methoxy-6,8-dimethylchroman-4-one (carphorin C).

Compound **4** was obtained as a white, amorphous powder and its molecular formula was determined to be $C_{18}H_{18}O_7$ by HRESIMS data (observed m/z 347.1159 $[M + H]^+$, calculated for $C_{18}H_{19}O_7$ 347.1125), 14 Daltons smaller than **3**. Its 1H and ^{13}C NMR spectra (Table 1) were similar to those of **3**, except for the presence of an aromatic hydrogen at δ_H 6.20 (1H, s, H-8) instead of the methyl group in **3**. HMBC correlations from H-8 (δ_H 6.20) to C-6 (δ_C 103.9), C-7 (δ_C 165.7), C-9 (δ_C 162.0), and C-10 (δ_C 102.8), from 7-OCH₃ (δ_H 3.84) to C-7 (δ_C 165.7), from 6-CH₃ (δ_H 1.91) to C-5 (δ_C 158.9), C-6 (δ_C 103.9), and C-7

(δ_C 165.7) as well as from 5-OH (δ_H 12.20) to C-10 (δ_C 102.8), C-5 (δ_C 158.9), and C-6 (δ_C 103.9) determined substitution of the A ring of the flavanone. The chemical structure of **4** was thus assigned as (*S*)-2-(3,5-dihydroxy-4-methoxyphenyl)-5-hydroxy-7-methoxy-6-methylchroman-4-one (carphorin D).

Compound **5** was isolated as a brownish amorphous powder and its molecular formula was established as $C_{20}H_{22}O_4$ by HRESIMS data (observed m/z 327.1565 $[M + H]^+$, calculated for $C_{20}H_{22}O_4^+$, 327.1591). Its 1H and ^{13}C NMR spectra (Table 2) combined with its 2D NMR spectra indicated the presence of a stilbene moiety with a prenyl substituent. A unique doublet at δ_H 6.70 (1H, d, J = 16.4 Hz, H- α) and δ_H 7.06 (1H, d, J = 16.4 Hz, H- β) indicated an *E*-stilbene configuration.¹³ The 1H NMR spectrum showed signals at δ_H 6.35 (2H, d, J = 2.2 Hz, H-2, H-6) and δ_H 6.11 (1H, t, J = 2.1 Hz, H-4) indicating a 1, 3, 5-trisubstituted aromatic ring in A ring. The 1H NMR spectrum combined with a 1H - 1H COSY spectrum displayed a pair of *ortho* aromatic protons at δ_H 7.24 (1H, d, J = 8.6 Hz, H-6') and δ_H 6.72 (1H, d, J = 8.6 Hz, H-5') of the ring. In its HMBC spectrum (Figure 3), the correlations from H- α (δ_H 6.70) to C-1 (δ_C 139.8), C-2 (δ_C 104.8), and C-1' (δ_C 127.9) and from H- β (δ_H 7.06) to C- α (δ_C 128.1), C-1 (δ_C 139.8), C-6' (δ_C 121.7), and C-2' (δ_C 133.5) confirmed the stilbene moiety. In addition, the coupling of the methoxy protons (δ_H 3.71) to C-3' (δ_C 145.8) indicated that the methoxy group is located on C-3'. Also, there were correlations from H-1'' (δ_H 3.40) to C-3' (δ_C 145.8), C-2' (δ_C 133.5) and C-1' (δ_C 127.9) indicating that the prenyl group is located on C-2', adjacent to the methoxy group. Further HMBC correlations from H-6' (δ_H 7.24) to C- β (δ_C 125.8), C-2' (δ_C 133.5), and C-4' (δ_C 150.2) and from H-5' (δ_H 6.72) to C-1' (δ_C 127.9), C-3' (δ_C 145.8), and C-4' (δ_C 150.2) supported the 1',2',3',4'-tetrasubstituted pattern of the B ring. Taking into account all of the above described spectroscopic data, compound **5** was assigned the structure (*E*)-5-(4-hydroxy-3-methoxy-2-(3-methylbut-2-en-1-yl)styryl)benzene-1,3-diol (carphabene).

Additionally, two known compounds were isolated and identified as 5,7,4'-trihydroxy-8-prenylflavanone (**6**)¹⁴ and ophiofolius A (**7**).¹⁵

The antiplasmodial activities of compounds **1** – **7** were determined against the chloroquine-resistant Dd2 strain of *P. falciparum* (Table 3 and Fig. S26). The compounds **1**, **2**, and **6** which have prenylated or hydroxyprenyl side chains showed the strongest inhibitory activity, with IC_{50} values of 5.2 ± 0.6 , 3.4 ± 0.4 , and 6.7 ± 0.8 μ M, respectively. In addition, the prenylated stilbene **5** also showed good activity, with an IC_{50} of 5.8 ± 0.7 .

In conclusion, the genus *Carpha* has yielded new and biologically active flavanones. Three new flavonoids along with one new stilbene were isolated and evaluated with *P. falciparum*. The good antiplasmodial activities of the compounds with prenyl or hydroxyprenyl side chains confirms previous studies that indicate that these substituents are important for antiplasmodial activity.¹⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- Three new flavanones
- One new stilbene derivative
- Good antiplasmodial activity

Figure 1.
Structures of compounds **1** – **7** from *Carpha glomerata*

Figure 2.
Selected ^1H - ^1H COSY and HMBC correlations for compounds **1** and **3**.

Figure 3.
Selected ^1H – ^1H COSY and HMBC correlations for compound 5

Table 1

¹H and ¹³C NMR Data of Compounds **1** – **4**.

Position	1^a		Posn	2^a		Position	3^b		Posn	4^a	
	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
2	78.3	5.33, dd (11.9, 3.2)	2	78.1	5.39, dd (12.5, 3.2)	2	78.2	5.43, dd (11.9, 3.2)	2	78.1	5.40, dd (11.6, 3.3)
3	42.5	ax, 3.02, dd (17.1, 11.9), eq, 2.71, dd (17.1, 3.2)	3	42.3	ax, 3.08, dd (16.9, 12.0), eq, 2.74, dd (16.9, 3.0)	3	42.7	ax, 3.14, dd (17.0, 11.8), eq, 2.82 (17.0, 3.2)	3	42.5	ax, 3.08, dd (17.1, 11.6), eq, 2.81, dd (17.1, 3.3)
4	196.6		4	196.5		4	198.5		4	197.4	
5	161.6		5	161.5		5	158.6		5	158.9	
6	95.8	5.93, s	6	95.9	5.88, s	6	110.2		6	103.9	
7	165.2		7	165.3		7	165.0		7	165.7	
8	107.5		8	108.9		8	109.2		8	92.5	6.20, s
9	159.8		9	159.9		9	157.9		9	162.0	
10	102.1		10	102.0		10	105.0		10	102.8	
1'	134.8		1'	129.9		1'	134.4		1'	134.6	
2'	105.9	6.42, s	2'	128.1	7.31, d (8.7)	2'	105.8	6.44, s	2'	105.7	6.47, s
3'	151.1		3'	115.5	6.77, d (8.7)	3'	151.2		3'	151.2	
4'	135.7		4'	157.8		4'	135.7		4'	135.7	
5'	151.1		5'	115.5	6.77, d (8.7)	5'	151.2		5'	151.2	
6'	105.9	6.42, s	6'	128.1	7.31, d (8.7)	6'	105.8	6.44, s	6'	105.7	6.47, s
1''	21.7	3.09-3.11, m	1''	17.9	2.42-2.45, m	6-CH ₃	8.2	1.96, s	6-CH ₃	8.1	1.91, s
2''	123.2	5.10, t (7.2)	2''	43.5	1.43-1.46, m	8-CH ₃	8.8	1.98, s			
3''	130.6		3''	69.3		7-OCH ₃	60.3	3.69, s	7-OCH ₃	56.6	3.84, s
4''	18.0	1.60, s	4''	29.5	1.05, s	4'-OCH ₃	60.1	3.67, s	4'-OCH ₃	60.1	3.67, s
5''	26.0	1.59, s	5''	29.5	1.05, s						
4'-OCH ₃	60.1	3.67, s									

^a₁H (500 MHz) and ¹³C (125 MHz) NMR data were obtained in DMSO-d₆

^b₁H (400 MHz) and ¹³C (100 MHz) NMR data were obtained in DMSO-d₆

Table 2¹H and ¹³C data of compound **5**^a

Position	5^a	
	δ_{C}	δ_{H} (J in Hz)
1	139.8	
2	104.8	6.35, d (2.2)
3	158.9	
4	102.3	6.11, t (2.1)
5	158.9	
6	104.8	6.35, d (2.2)
1'	127.9	
2'	133.5	
3'	145.8	
4'	150.2	
5'	115.2	6.72, d (8.6)
6'	121.7	7.24, d (8.6)
α	128.1	6.70, d (16.4)
β	125.8	7.06, d (16.4)
1''	25.3	3.40, bd (6.8)
2''	123.8	5.03, bt (6.8)
3''	131.0	
4''	25.9	1.63, s
5''	18.4	1.77, s
3'-OCH ₃	60.2	3.71, s

^a¹H (500MHz) and ¹³C (125MHz) NMR data were obtained in DMSO-*d*₆

Table 3.

Antiplasmodial activities of compounds 1 - 7

<i>P. falciparum</i> Dd2 IC ₅₀ (μM)	
carphorin A (1)	5.2 ± 0.6
carphorin B (2)	3.4 ± 0.4
carphorin C (3)	>10
carphorin D (4)	>10
carphabene (5)	5.8 ± 0.7
5,7,4'-trihydroxy-8-prenylflavanone (6)	6.7 ± 0.8
ophiofolius A (7)	>10
artemisinin ^a	0.012 ± 0.001

Each value represents the mean ± SEM of three experiments.

^a positive control