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ABSTRACT

Bioactivity guided isolation of an ethanol extract of the root of *Psoromanthus schottii* (Family Fabaceae) afforded a new prenylated isoflavone, named schottiin (**1**), together with four other isoflavones, including fremontone (**2**), 5,7,4´,5´-tetrahydroxy-2´-(3,3-dimethylallyl)-isoflavone (**3**), glycyrrhisoflavone (**4**) and fremontin (**5**), of which **3** and **4** identified as isomeric mixture. Structures of **1**-**5** were determined by full spectroscopic analyses. A comprehensive 2D NMR spectral data has allowed revising the structure of fremontone as **2** from previously reported **2A**. Compound **2** showed weak *in-vitro* antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). A combination study using a checkerboard assay between fremontone (**2**) and methicillin exhibited a synergistic activity with 8-fold decrease in MIC of methicillin, as well as an additive effect with vancomycin against MRSA ATCC 1708. Compounds **1** and **2** also showed moderate antiplasmodial activity against chloroquine-sensitive (D6) and -resistant (W2) strains of *Plasmodium falciparum* with no cytotoxicity to mammalian Vero cells.

METHODS

Root of *P. schotti*

Antimicrobial Assay

Antimicrobial assays were carried out using a modified version of the CLSI (formerly NCCLS) method.

Antimicrobial Combination Study by Checkerboard Method

The combination study of the compounds was carried out in MRSA using a standard Checkerboard method. The fractional inhibitory concentration (FIC) was calculated by using the following formula: FIC = [A*]/[A] + [B*]/[B], where [A*] is MIC of compound A in the presence of compound B, [A] is MIC of compound A alone, [B*] is MIC of compound B in the presence of compound A, and [B] is MIC of compound B alone. FIC: ≤0.5 = synergistic; 0.51-1.0 = additive; 1.1-2.0 = indifferent; >2.0 = antagonistic

Antiplasmodial and Cytotoxicity Assay

The antiplasmodial assay was performed against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *P. falciparum* using the *in-vitro* assay. The cytotoxicity of test samples was determined against a mammalian cell line (VERO) in order to calculate the selectivity index of antimalarial activity.

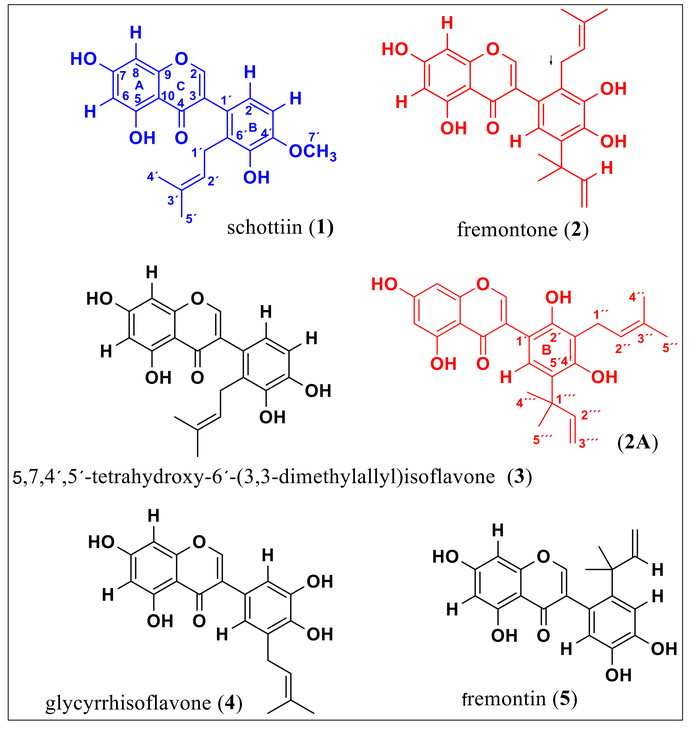

Figure 1. Structures of compounds isolated from *P. schottii*

DISCUSSION & CONCLUSIONS

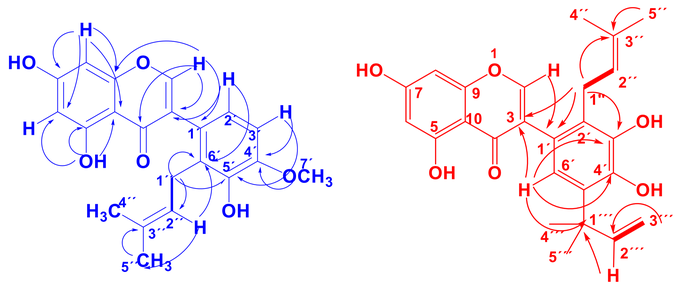

Figure 2: COSY and key HMBC correlations of **1** and **2**

Table 2. Combination study of compound **2** by checkerboard assay against MRSA

Compound	MRSA MIC (µg/mL)		
	MIC	Methicillin + 2	Vancomycin + 2
2	12.5	3.12 (4X)[a]	6.25 (2X)[d]
Methicillin	100	12.5 (8X)[b]	-
Vancomycin	0.5	-	0.25 (2X)[c]

[a)] MIC of **2** reduced from 12.5 to 3.12 µg/mL, [b)] MIC of methicillin reduced from >50 to 12.5 µg/mL, [c)] MIC of vancomycin reduced from 0.5 to 0.25 µg/mL, [d)] MIC of **2** reduced from 12.5 to 6.25 µg/mL. . In general, when the MIC of each compound-decreased 4X in the presence of the other, it is considered synergistic; reduction of MIC in parentheses. Antimicrobial assays were carried out in triplicate.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic data for compounds **1-2**

	1*		2**		2A**	
C #	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
2	154.0	7.69, s	155.1	7.74, s	154.8	7.75, s
3	123.9		120.4		120.3	
4	181.3		182.1		182.1	
5	162.5		161.8		161.7	
6	99.5	6.31,d (2.2)[c]	98.6	6.19, d (2.1)	98.4	6.23, d (2)
7	164.1		164.1		163.8	
8	93.3	6.39,d (2.2)	93.3	6.34, d, (2.1)	93.1	6.38, d (2)
9	158.4		157.5		157.3	
10	105.5		104.1		104.0	
1'	123.7		122.2		122.0	
2'	108.5		**128.8**		**140.9**	
3'	122.3		**141.1**		**129.0**	
4'	147.1		144.4		144.3	
5'	144.0		138.1		137.9	
6'	127.7		111.6	6.85, s	111.5	6.88, s
1''	26.3	3.32, bs, 1.45, m	26.8	2.75, 3.17, m	26.4	2.73, 3.24, m
2''	122.8	5.09, t (6.7)	123.3	4.88, t (6.1)	123.4	4.91, t (7)
3''	131.6		128.9		129.9	
4''	17.6	1.51, s	25.1	1.48, s	25.2	1.51, s
5''	25.6	1.61, s	16.9	1.21, s	16.7	1.27 s
1'''			41.1		40.9	
2'''			148.8	5.97, dd (10.6)	148.7	5.93, dd (10)
3'''			108.0	4.51, dd (1.3, 10.5)	108.0	4.54, dd (10, 17)
				4.65, dd (1.4, 17.5)		
4'''			29.2	1.24, s	29.3	1.23, s
5'''			29.2	1.19, s	29.4	1.21, s
7'	56.2	3.93, s				
5-OH		12.97, s		13.01, s		13.05, s

[a]Recorded at 100 mHz, [b]Recorded at 400 MHz. [c]Coupling constant (*J*) values in Hz. *in CDCl3/methanol-*d*4.**in DMSO-*d*6

A comprehensive 2D NMR spectral data has allowed revising the structure of fremontone as **2** from previously reported **2A (Table 1** & **Figures 1, 2)**. Among them, schottiin (**1**) showed weak antifungal activity (IC_{50} 19 µg/mL) against *C. neoformans*. Compound **2,** which contains both 1,1-dimethyl and 3.3-dimethylallyl prenylation groups in the ring B showed weak *in-vitro* antibacterial activity against MRSA, but a combination study, using a checkerboard method, of **2** and methicillin exhibited an 8-fold more potent activity (i.e., MIC 12.5 µg/mL), compared to methicillin (MIC 100 µg/mL), while vancomycin and **2** was found to be 2-fold more active (MIC 0.25 µg/mL) than vancomycin alone (MIC 0.5 µg/mL). On the other hand, the MIC value of **2** was reduced from 12.5 to 3.12 µg/mL, a 4-fold reduction due to synergism with methicillin, thus **2** displayed a potent synergistic effect (FIC = 0.375) with methicillin and an additive effect with vancomycin (FIC = 1.0) displaying a potentiation effect with both antibiotics (**Table 2**).

The antimicrobial activity of prenylated flavonoids and their potentiation effect with antibiotics against MRSA have been reported. Our study further substantiates that prenylated isoflavone (**2**) adjuvant therapy with methicillin, may have a potential value to address antibiotic-induce resistance by MRSA. However, further studies are needed to understand their exact role to combat antibacterial resistance. Compounds **1** and **2** also showed moderate anti-plasmodial activity against CQ-sensitive (D6) and -resistant (W2) strains of *P. falciparum* (IC_{50} 3.7, 3.5, and 1.2, 1.3 µg/mL, respectively, vs. IC_{50} of reference standards chloroquine and artemisinin 0.02, 0.16, and 0.005, 0.003 µg/mL, respectively), with no cytotoxicity to mammalian Vero cells (IC_{50} > 4.76 µg/mL).

REFERENCE & ACKNOWLEDGMENT

Kumarihamy et al. (2021): Schottiin, a new prenylated isoflavones from *Psorothamnus schottii* and antibacterial synergism studies between methicillin and fremontone against methicillin-resistant *Staphylococcus aureus* ATCC 1708, *Natural Product Research*, DOI: 10.1080/14786419.2021.1937157.

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