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A benzil and isoflavone derivatives from *Derris scandens* Benth.

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Abstract

A benzil derivative: scandione, 2′,2″-dihydroxy-4′-methoxy-4″,5″-methylenedioxybenzil and two isoflavones: scandenal, 3′-formyl-4′,5-dihydroxy-2″,2″-dimethylchromeno-[6,7:5″,6″]isoflavone and scanderone, 4′,5-dihydroxy-3′-prenyl-2″,2″-dimethylchromeno-[7,8:6″,5″]isoflavone together with fifteen known compounds were isolated from the stem of *D. scandens*. Their structures were determined by spectroscopic methods. Radical scavenging, antibacterial and hypertensive activities of some of the compounds were investigated.

Keywords: *Derris scandens*; Leguminosae; Radical scavenging; Antibacterial; Hypertension; Benzil; Isoflavones

1. Introduction

Derris scandens Benth. (Leguminosae), local Thai name, Tao-Wan-Priang, is well-known as an Asian medicinal plant. Its dried stem has been used as an expectorant, antitussive, diuretic, and antidysentery agent and for the treatment of muscle aches and pains (Chavalittumrong et al., 1999). A hydroalcoholic extract of the stem was reported to have both antimicrobial (Dhawan et al., 1977) and immunostimulating activities (Chuthaputti and Chavalittumrong, 1998). In a pharmacological study, the polar fraction when applied resulted in a marked decrease in blood pressure and heart rate (Jansakul et al., 1997). Coumarins, isoflavones and isoflavone glycosides have also been previously reported as chemical constituents of the stems of *D. scandens* (Chuankamnerdkarn et al., 2002; Rukachaisirikul et al., 2002; Sekine et al., 1999; Dianpeng et al., 1999; Rao et al., 1994; Falshaw et al., 1969; Johnson et al., 1966; Pelter and Stainton, 1966; Johnson and Pelter, 1966). The use of the stem of this plant as a folk medicine and the biological activity of the crude extract thus led us to examine the stem further for biologically active substances. We have isolated a new benzil (**1**), two new isoflavones (**2**, **3**) and 15 known compounds (**4**–**18**). Their structures were elucidated from analyses of 1D and 2D NMR spectroscopic data including ^{1}H, ^{13}C NMR, NOE, ^{1}H-^{1}H COSY, HMQC and HMBC. Radical scavenging, antibacterial and hypertensive activities of some of the compounds were investigated.

2. Results and discussion

D. scandens stems were sequentially extracted with acetone and methanol. The crude methanolic extract was further partitioned between ethyl acetate and water. The organic portion was fractionated into dichloromethane soluble- and insoluble fractions. Extensive chromatography of the dichloromethane-soluble fraction gave seven compounds: scandione, 2′,2″-dihydroxy-4′-methoxy-4″,5″-methylenedioxybenzil (**1**), scandenal, 3′-formyl-4′,5-dihydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone (**2**), scanderone, 4′,5-dihydroxy-3′-prenyl-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone (**3**), flemichapparin B (**4**) (Lin et al., 1991), lupalbigenin (**5**) (Sekine et al., 1999), flemichapparin C (**6**) (Aditachaudhury and Gupta, 1973), and isorobustone (**7**) (Garcia et al., 1986). Extensive chromatography of the dichloromethane-insoluble fraction gave nine compounds:

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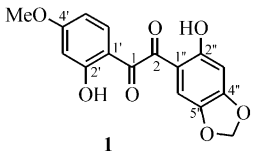

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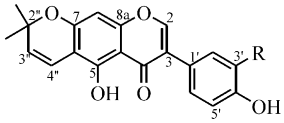
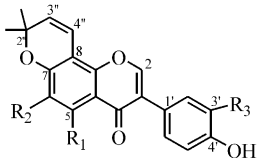
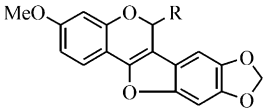


2 : R = CHO
10 : R = prenyl



	R	R_2	R_3	
3 :	OH	H	prenyl	:
13 :	OMe	prenyl	H	



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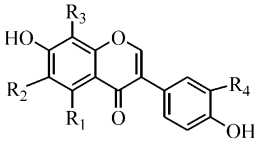
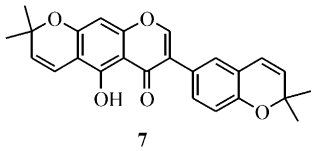
6 : R = O



	R_1	R_2	R_3	R_4
5 :	OH	prenyl	H	prenyl
14 :	OMe	prenyl	prenyl	H
19 :	OH	H	prenyl	H



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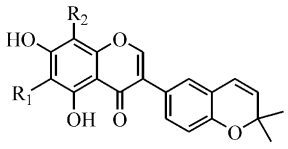
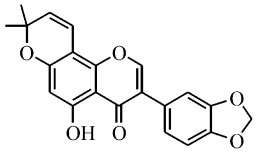


	R_1	R_2
9 :	H	prenyl
12 :	prenyl	H



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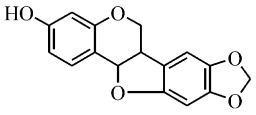

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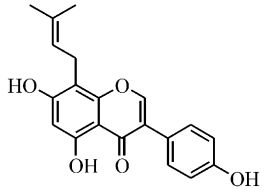

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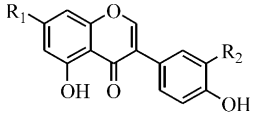
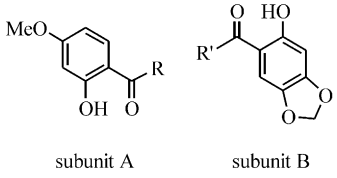


	R_1	R_2
15 :	OMe	OH
17 :	OH	OMe
18 :	OH	H



subunit A subunit B

5-hydroxy-2″,2″-dimethylchromeno-[6,7:5″,6″]-2‴,2‴-dimethylchromeno[3′,4′:5‴,6‴]isoflavone (**8**), ulexone A (**9**) (Maximo et al., 2000), chandalone (**10**) (Falshaw et al., 1969), maackiain (**11**) (Obara and Matsubara, 1981), isochandalone (**12**) (Tahara et al., 1989), scandinone (**13**) (Rao et al., 1994; Pelter and Stainton, 1966), derrisisoflavone A (**14**) (Sekine et al., 1999), santal (**15**) (Bohm and Choy, 1987) and lupiwighteone (**16**) (Hakamatsuka et al., 1991). Partitioning of the crude acetone extract with CH_2Cl_2 and 5% aqueous $Na_2B_4O_7$ followed by chromatography of material in the aqueous fraction gave 3′-methylorobol (**17**) (Kikuchi et al., 1989) and genistein (**18**) (Sang and Min, 2000).

Scandione (**1**) was a yellow solid, m.p. 132–133 °C, with the molecular formula $C_{16}H_{12}O_7$. The UV spectrum showed absorption maxima at 283, 259 and 212 nm. The IR spectrum exhibited O–H stretching at 3433 cm^{-1} and C=O stretching at 1624 cm^{-1}. The ^{13}C NMR spectrum showed that **1** contained two carbonyl carbons (δ 194.84 and 194.40). The ^1H NMR spectrum showed a singlet signal of a methoxy group at δ 3.87 (4′-OMe) and a singlet resonance of a methylenedioxy

group at δ 5.99. Two singlet signals of hydroxy protons hydrogen-bonded to carbonyl groups were displayed at δ 12.22 (2″-OH) and 11.81 (2′-OH). Three signals at δ 7.40 (*d*, H-6′), 6.51 (*d*, H-3′) and 6.45 (*dd*, H-5′) appearing as an ABX type suggested the presence of a 1,2,4-trisubstituted benzene ring. Two singlets of aromatic protons at δ 6.81 (H-6″) and 6.53 (H-3″) indicated that **1** also contained a 1,2,4,5-tetra-substituted aromatic nucleus. Consequently the OCH_3 was located at C-4′ and the methylenedioxy group was fused to an aromatic ring at C-4″ and C-5″; subunits A and B then were assigned. The HMQC and HMBC spectra suggested that the carbons of subunit A corresponded to 1-C=O, C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′ whereas the carbons of subunit B corresponded to 2-C=O, C-1″, C-2″, C-3″, C-4″, C-5″ and C-6″. In the MS the base peak at *m*/*z* 151 corresponded to the ion of subunit A and a strong peak at *m*/*z* 165 corresponded to the ion of subunit B, suggesting that the two subunits were joined together. Thus the structure, 2′,2″-dihydroxy-4′-methoxy-4″,5″-methylenedioxybenzil (**1**), was assigned for scandione and was confirmed by analysis of the complete HMBC data (Table 1).

Scandenal (**2**) was isolated as a yellow solid, m.p. 79–80 °C, with the molecular formula $C_{21}H_{16}O_6$. The UV spectrum showed absorption maxima at 279 and 231 nm. The IR spectrum showed a broad band of O–H stretching at 3450 cm^{-1} and a sharp band of C=O stretching at 1656 cm^{-1}. Two resonances of carbonyl carbons (δ 196.71 and 180.71) were present in the ^{13}C NMR spectrum. The ^1H NMR spectrum showed the signals of two hydrogen-bonded phenolic hydroxy groups at δ 12.98 (5-OH) and δ 11.11 (4′-OH), a formyl proton at δ 9.98 (CHO), a noncoupled olefinic proton at δ 7.89 (H-2) and an isolated aromatic proton at δ 6.36 (H-8). An ABX pattern from aromatic protons was present at δ 7.83 (*d*, H-2′), 7.09 (*d*, H-5′) and 7.68 (*dd*, H-6′). The signals of two methyl groups and vicinal olefinic protons associated with a chromene ring were present at δ 1.49 (*s*, 2×CH_3), 6.74 (*d*, H-4″) and 5.65 (*d*, H-3″). In a NOE experiment, irradiation of the formyl proton enhanced the signals of H-2′ and 4′-OH suggesting that the formyl group was at C-3. HMBC correlation of H-4″ to C-5, C-6, C-7, and H-3″ to C-6 indicated the chromene ring was fused to the parent structure at C-6 and C-7. The complete HMBC data (Table 2) confirmed the structure of scandenal as 3′-formyl-4′,5-dihydroxy-2″,2″-dimethylchromeno[6,7:5″,6″]isoflavone (**2**).

Scanderone (**3**) was obtained as a yellow solid, m.p. 115–116 °C, with the molecular formula $C_{25}H_{24}O_5$. The UV spectrum exhibited absorption maxima at 284 and 250 nm. Absorption bands of O–H stretching and C=O stretching were shown in the IR spectrum at 3450 and 1653 cm^{-1}, respectively. The ^1H NMR spectrum showed signals of a hydrogen-bonded hydroxy group at δ 13.18 (*s*, 5-OH), a free hydroxy group at δ 5.88 (*br s*, 4′-OH), a characteristic isoflavone proton at δ 7.80 (*s*, H-2), an isolated aromatic proton at δ 6.34 (*s*, H-6) and three aromatic protons which coupled as an ABX system at δ 7.22 (H-2′), 7.20 (H-6′) and 6.77 (H-5′). In addition, there were characteristic signals of a prenyl group at δ 3.37 (H_2-1‴), 5.34 (H-2‴) and 1.77 (H_3-4‴ and H_3-5‴). The remaining signals at δ 6.74 (*d*, 1H), δ 5.63 (*d*, 1H) and δ 1.48 (*s*, 6H) were due to olefinic protons H-4″, H-3″ and two methyl groups (2″-Me_2) of the chromene ring. The correlation of H-1‴ to C-2′, C-3′ and C-4′ in the HMBC spectrum indicated that the prenyl unit was at C-3′, whereas the correlation of H-4″ to C-7, C-8 and C-8a suggested that the chromene ring was connected to C-7 and C-8. The complete HMBC data (Table 2) confirmed the structure of scanderone as 4′,5-dihydroxy-3′-prenyl-2″,2″-dimethylchromeno[7,8:6″,5″]isoflavone (**3**).

Scandione (**1**) is a new benzil derivative. It is an addition to the small group of naturally occurring benzils (Ferrari et al., 1984; Miyase et al., 1999; Li et al., 1998) which presumably arise by oxidation of flavonoid precursors. Compounds **2** and **3** are new isoflavones. The formyl group in **2** possibly arises from oxidation of the prenyl chain in chandalone (**10**). Compounds **5**, **10**, **13**

Table 1
HMBC correlation data of compound **1**

H-position	**1**
3′	C-1′, C-2′, C-4′ C-5′
5′	C-1′, C-3′
6′	C-1, C-2′, C-4′
3″	C-1″, C-2″, C-4″, C-5″
6″	C-2, C-2″, C-4″, C-5″
2′-OH	C-2′, C-3′, C-1′
4′-OMe	C-4′
2″-OH	C-1″, C-2″, C-3″, C-4″
OCH_2O	C-4″, C-5″

Table 2
HMBC correlation of compounds **2** and **3**

H-position	**2**	**3**
2	C-3, C-8a, C-1′, C-4	C-3, C-4, C-8a, C-1′
6		C-4a, C-5, C-7, C-8
8	C-6, C-7, C-8a, C-4a	
2′	C-3, C-4′, C-6′, CHO	C-3, C-4′, C-6′, C-1‴
5′	C-1′, C-3′, C-4′	C-1′, C-3′, C-4′
6′	C-3, C-2′, C-4′	C-3, C-2′, C-4′
3″	C-6, C-2″, ($\underline{C}H_3$)$_2$-2″	C-8, C-2″, ($\underline{C}H_3$)$_2$-2″
4″	C-5, C-6, C-7, C-2″	C-4a, C-7, C-8, C-8a, C-2″
5-OH	C-5, C-6, C-7, C-4a	C-4a, C-5, C-7
CHO	C-2′, C-3′, C-4′, C-5′	
4′-OH	C-3′, C-4′, C-5′, C-6′	
2″-Me_2	C-2″, C-3″	C-2″, C-3″
1‴		C-2′, C-3′, C-4′, C-2‴, C-3‴
2‴		C-1‴, C-4‴
4‴		C-2‴, C-3‴, C-5‴
5‴		C-2‴, C-3‴, C-4‴

and **14** have been previously isolated from *D. scandens* but the isolation of compounds **4**, **6**, **7**, **8**, **9**, **11**, **12**, **15**, **16**, **17** and **18** is reported for the first time. Isoflavone **12**, like **10**, is a mono-cyclized product of **5** and isoflavone **8** is a bicyclic product; also, **3** and **9** represent two monocyclized forms of isolupalbigenin (**19**) (Tahara et al., 1994). Isoflavone **13** can arise from the cyclization of the C8-prenyl side chain in **14**. Genistein (**18**) has been found previously as a glycoside in this plant (Rukachaisirikul et al., 2002). The pterocarpan **4** can be derived from **11** by desaturation and methylation processes. Coumestan **6** is an oxidized form of pterocarpan **4**. This is the first report of coumestan and pterocarpan compounds in *D. scandens*.

The biological activities of some of the compounds are shown in Table 3. It was found that at a concentration of 10 μM, the compounds tested are able to scavenge the DPPH radical in a range of 5.3–86.8 percent. Compounds **13**, **14** and **15** exhibited the most potent radical scavenger effect. The activity of the flavonoid compounds, in general, arises from the donation of a hydrogen radical from a phenolic group to the free radical. The greater activity of **15** than the others may be because of the *ortho*-dihydroxy group in the B-ring, which confers higher stability to the radical form and participates in electron delocalization (Rice-Evans et al., 1996). Further evaluation indicated that compounds **13**, **14** and **15** acted as radical scavenger with IC_{50} of 8.75, 3.63 and 2.75, respectively. In addition, **14** and **15** were found to show greater antioxidative ability than butylated hydroxytoluene (BHT) (IC_{50} 6.88), a standard antioxidant. For antibacterial activity, compound **5** showed the strongest inhibitory activity against *Staphylococcus aureus*, both penicillin-sensitive strain ATCC 25923 and methicillin-resistant strain MRSA SK1, with MIC values of 4 and 2 μg/mL, respectively. Compounds **14** and **15** also exhibited strong antimicrobial activity against MRSA SK1 but weak activity against *S. aureus* ATCC 25923. It was also found that tested compounds caused moderate increases in mean arterial blood pressure in anesthetized rats ($n = 2$).

3. Experimental

3.1. General method

Melting points were measured on a digital Electrothermal 9100 Melting Point Apparatus and are uncorrected. Infrared spectra were recorded on an FTS 165 FT-IR spectrometer. Ultraviolet absorption spectra were recorded using a UV-160A spectrometer (SHIMADZU). ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were performed on a Varian UNITY INOVA 500 spectrometer in $CDCl_3$. The high resolution mass spectra were recorded on an MS25RFA spectrometer. Pre-coated TLC sheets (layer thickness 0.2 mm) and preparative TLC plate (layer thickness 1.25 mm) of silica gel 60 PF_{254} were used. Quick column and column chromatography were performed on silica gel 60H and silica gel 100 (Merck), respectively. Known compounds were identified by comparison of their spectroscopic data with published data.

3.2. Plant material

Derris scandens was collected from Phang-nga province in southern Thailand. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University, and a

Table 3
Radical scavenging activity, antibacterial activity and hypertensive activity (increase in mean arterial blood pressure, mmHg) of compounds from *D. scandens*

Compounds	Radical scavenging activity (% scavenging of DPPH)	Antibacterial activity (MIC, μg/ml)		Hypertensive activity increase in MAP (mmHg)	
		S. aureus ATCC 25923	MRSA SK1	0.4 mg/kg	4.0 mg/kg
2	–	–	–	11.67	–
3	–	–	–	–	20.0
4	5.26	>512	>512	8.9	–
5	26.32	2	4	–	–
6	21.05	–	–	–	–
7	13.16	>128	>128	–	–
10	18.42	128	16	–	11.67
11	–	>512	>512	8.3	–
12	10.53	>256	>256	–	–
13	63.16	>256	>256	–	9.17
14	81.58	16	4	–	7.5
15	86.84	128	2	–	21.7
16	15.79	–	–	–	–
BHT	71.05	–	–	–	–
Vancomycin	–	0.5	1	–	–

specimen deposited at Prince of Songkla University Herbarium. (Coll. No. 01, Herbarium No. 0012652).

3.3. Extraction and isolation

Chopped dry stems of *Derris scandens* (6.5 kg) were extracted at room temperature in turn with acetone (3 days) and methanol (5 days). Removal of the solvent from the methanolic extract yielded a brown gummy residue (363.5 g) which was subsequently separated into two fractions by partitioning between ethyl acetate and water. The ethyl acetate-soluble fraction was evaporated and was further fractionated by dissolving in dichloromethane to yield soluble (168.8 g) and insoluble (82.6 g) portions. The soluble portion was subjected to quick column chromatography on silica gel and eluted with hexane, hexane–C_6H_6, CH_2Cl_2, CH_2Cl_2–Me_2CO gradient solvent system. The eluted fractions were combined to ten sub-fractions (A1–A10) on the basis of TLC behaviour. A solid from fraction A1 was recrystallized from hexane to give flemichapparin B (**4**, 0.97 g). Fraction A3 was a mixture of a solid and a yellow gum. It was subjected to further CC; elution with hexane–dichloromethane (1:1) followed by recrystallization from dichloromethane–hexane afforded lupalbigenin (**5**, 0.32 g). Fraction A4 was subjected to preparative TLC using hexane–dichloromethane (1:1) as mobile phase (3 runs) to give two isolated bands. Scandione (**1**, 16.2 mg) was obtained from the first band after recrystallization from benzene as a yellow solid. Flemichapparin C (**6**, 14.5 mg) was obtained as a yellow solid from the second band. Fraction A6 was reapplied to a CC using dichloromethane as eluent to give scandenal (**2**, 40.0 mg). Fraction A8 was subjected to CC and eluted with hexane–dichloromethane, to yield subfractions isorobustone (**7**, 19.0 mg) and scanderone (**3**, 0.42 g). The insoluble portion was subjected to quick column chromatography and eluted with hexane, hexane–C_6H_6, CH_2Cl_2, CH_2Cl_2–Me_2CO gradient solvent system. The eluted fractions were combined on the basis of TLC behaviour to give eleven sub-fractions (B1–B11). Fraction B4 was further separated on CC and eluted with a gradient solvent system of benzene, dichloromethane and acetone. Repeated purification of subfractions from B4 by CC followed by recrystallization from dichloromethane-hexane gave 5-hydroxy-2″, 2″-dimethylchromeno[6,7: 5″,6″]-2‴,2‴-dimethylchromeno-[3′,4′:5‴,6‴]isoflavone (**8**, 0.08 g), ulexone A (**9**, 0.03 g), chandalone (**10**, 0.28 g) and maackiain (**11**, 0.16 g). Further chromatography of fraction B5, followed by preparative TLC using chloroform as the solvent system gave isochandalone (**12**, 0.03 g). A solid from fraction B6 B7 and B8 was recrystallized in benzene to give scandinone (**13**, 0.22 g), derrisisoflavone A (**14**, 0.96 g) and santal (**15**, 0.06 g), respectively. Lupiwighteone (**16**, 0.05 g) was obtained from further chromatography of Fraction B9.

The acetone extract was partitioned with dichloromethane and 5% $Na_2B_4O_7$. The aqueous layer was acidified and extracted with dichloromethane. The residue (4.2 g) after removal of the solvent was subjected to CC, using a CH_2Cl_2, Me_2CO gradient solvent system to give seven fractions (C1–C7). Fraction C3 was subjected to further CC and eluted with dichloromethane–chloroform (1:1) to give a yellow solid which was recrystallized from dichloromethane to give 3-methylorobol (**17**, 4.3 mg). Fraction C6 was further purified by CC and eluted with chloroform, followed by preparative TLC using 2% acetone-chloroform as a mobile phase (4 runs) to give genistein (**18**, 5.2 mg).

3.3.1. Scandione (*1*)

Yellow solid, m.p. 132–133 °C. HRESIMS *m/z* 339.0485 $[M+Na]^+$ (calcd for $C_{16}H_{12}NaO_7$, 339.0481). UV (CH_3OH) λ_{max} nm (log ϵ): 282.8 (3.99), 258.6 (3.62), 212.0 (4.13). IR (KBr) ν (cm^{-1}): 3433, 1624. ^1H NMR ($CDCl_3$) (δ ppm): 12.22 (1H, *s*, 2″-OH), 11.81 (1H, *s*, 2′-OH), 7.40 (1H, *d*, $J=8.4$ Hz, H-6′), 6.81 (1H, *s*, H-6″), 6.53 (1H, *s*, H-3″), 6.51 (1H, *d*, $J=2.2$ Hz, H-3′), 6.45 (1H, *dd*, $J=8.8$, 2.2 Hz, H-5′), 5.99 (2H, *s*, OCH_2O), 3.87 (3H, *s*, 4′-OMe) EIMS *m/z* (% relative intensity): 316 (15), 298 (16), 165 (55), 151 (100), 149 (9), 137 (5), 95 (10), 79 (5). ^{13}C NMR ($CDCl_3$) (δ ppm): 194.84 (1-C=O), 194.40 (2-C=O), 168.34 (C-4′), 167.40 (C-2′), 164.84 (C-2″), 156.86 (C-4″), 141.63 (C-5″), 134.32 (C-6′), 111.02 (C-1′), 109.58 (C-1″), 109.25 (C-5′), 108.02 (C-6″), 102.54 (OCH_2O), 101.31 (C-3′), 99.11 (C-3″), 55.72 (OCH_3).

3.3.2. Scandenal (*2*)

Yellow solid, m.p. 79–80 °C. HRESIMS *m/z* 387.0851 $[M+Na]^+$ (calcd for $C_{21}H_{16}NaO_6$, 387.0845). UV (CH_3OH) λ_{max} nm (log ϵ): 279.0 (4.42), 231.0 (4.22). IR (KBr) ν (cm^{-1}): 3450, 1656. ^1H NMR ($CDCl_3$) (δ ppm): 12.98 (1H, *s*, 5-OH), 11.11 (1H, *s*, 4′-OH), 9.98 (1H, *s*, CHO), 7.89 (1H, *s*, H-2), 7.83 (1H, *d*, $J=1.8$ Hz, H-2′), 7.68 (1H, *dd*, $J=9.1$, 1.8 Hz, H-6′), 7.09 (1H, *d*, $J=9.1$ Hz, H-5′), 6.74 (1H, *d*, $J=9.8$ Hz, H-4″), 6.36 (1H, *s*, H-8), 5.65 (1H, *d*, $J=9.8$ Hz, H-3″), 1.49 (6H, *s*, 2″-Me_2). EIMS *m/z* (% relative intensity): 364 (20), 349 (100), 300 (6), 271 (7), 255 (11), 213 (7), 174 (7), 135 (11), 97 (15), 83 (22), 69 (25), 55 (37). ^{13}C NMR ($CDCl_3$) (δ ppm): 196.71 (CHO), 180.71 (C=O), 161.90 (C-4′), 160.06 (C-7), 157.51 (C-8a), 157.08 (C-5), 152.89 (C-2), 137.45 (C-6′), 134.47 (C-2′), 128.60 (C-3″), 122.89 (C-1′), 122.49 (C-3), 120.82 (C-3′), 118.31 (C-5′), 115.59 (C-4″), 106.21 (C-6), 106.04 (C-4a), 95.26 (C-8), 78.45 (C-2″), 28.59 (2″-Me_2).

3.3.3. Scanderone (*3*)

Yellow solid, m.p. 115–116 °C. HRESIMS *m/z* 405.1707 $[M+H]^+$ (calcd for $C_{25}H_{25}O_5$, 405.1702). UV (CH_3OH) λ_{max} nm (log ϵ): 283.5 (4.65), 249.5 (4.00). IR

(KBr) ν (cm^{-1}): 3450, 1653. ^1H NMR ($CDCl_3$) (δ ppm): 13.18 (1H, *s*, 5-OH), 7.80 (1H, *s*, H-2), 7.22 (1H, *d*, $J=2.1$ Hz, H-2′), 7.20 (1H, *dd*, $J=7.7$, 2.1 Hz, H-6′), 6.77 (1H, *d*, $J=7.7$ Hz, H-5′), 6.74 (1H, *d*, $J=9.8$ Hz, H-4″), 6.34 (1H, *s*, H-6), 5.88 (1H, *br s*, 4′-OH), 5.63 (1H, *d*, $J=9.8$ Hz, H-3″), 5.34 (1H, *br t*, $J=7.4$ Hz, H-2‴), 3.37 (2H, *d*, $J=7.4$ Hz, H_2-1‴), 1.77 (6H, *s*, H_3-4‴, H_3-5‴), 1.48 (6H, *s*, 2″-Me_2). EIMS *m*/*z* (% relative intensity): 404 (23), 389 (100), 333 (14), 321 (14), 32 (7), 305 (5), 203 (9). ^{13}C NMR ($CDCl_3$) (δ ppm): 181.03 (C=O), 159.47 (C-5), 157.28 (C-8a), 156.81 (C-7), 154.65 (C-4′), 152.59 (C-2), 134.45 (C-3‴), 130.40 (C-2′), 128.10 (C-3″), 128.03 (C-6′), 127.42 (C-3′), 123.78 (C-3), 122.69 (C-1′), 121.69 (C-2‴), 115.84 (C-5′), 115.44 (C-4″), 106.07 (C-4a), 105.51 (C-8), 94.81 (C-6), 78.00 (C-2″), 29.45 (C-1‴), 28.25 (2″-Me_2), 25.72 (C-5‴), 17.83 (C-4‴).

3.4. Radical scavenging activity

An aliquot (50 μL) of the test sample in absolute ethanol was mixed with 3 mL of 0.05 mM DPPH ethanolic solution. The mixture was allowed to stand at 37 °C for 45 min. The absorbance was then measured at 517 nm against 0.05 mM DPPH. Butylated hydroxytoluene (BHT) was used for a positive control. Measurements were performed in triplicate.

3.5. Antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by the agar microdilution method (Lorian, 1996). Test samples were dissolved in dimethyl sulfoxide (DMSO, Merck). Serial 2-fold dilutions of the test samples were mixed with melted Mueller Hinton agar (Difco) in the ratio of 1:100 in microtiter plates (Nunc). Final concentration of the test samples in agar ranged from 512–0.25 μg/mL. *S. aureus* ATCC 25923 and MRSA SK1 isolated from clinical specimen were used as test strains. Ten μl of inoculum suspensions (10^4 cfu) were dropped on agar surface. The inoculated plates were incubated at 35 °C for 16–18 h. MICs were recorded by reading the lowest concentration that inhibited visible growth. The test was performed in triplicates. Vancomycin was used as a positive control drug. Growth controls were performed on agar containing DMSO.

3.6. Hypertensive activity

Male Wistar rats weighing 250–270 g were used in the study. The rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). A polyethylene catheter was cannulated through the right common carotid artery and connected to a pressure transducer and polygraph for monitoring blood pressure of the rats. Another polyethylene tube was cannulated through the left jugular vein for vehicle and drug injections. Bolus intravenous injection (0.1 mL) of vehicle or test compounds (0.4 or 4.0 mg/kg) dissolved in 20% DMSO in distilled water was performed after 1 h of animal equilibration, and blood pressure monitored. In blank tests, the vehicle showed no effect on blood pressure.

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