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Small-molecule inhibitors of the cancer target, isoprenylcysteine carboxyl methyltransferase, from *Hovea parvicalyx*

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Structure elucidation

ABSTRACT

Isoprenylcysteine carboxyl methyltransferase (Icmt) is enzyme target in anticancer drug discovery. An Icmt natural product high-throughput screening campaign was conducted and a hit extract from the roots of *Hovea parvicalyx* was identified. 2′-Methoxy-3′-prenyl-licodione and 2′-methoxy-3′,3″-diprenyl-licodione, two prenylated β-hydroxychalcone compounds, together with the known flavanone (*S*)-glabrol, were isolated and identified as bioactive constituents. Their structures were determined largely by 1D and 2D NMR spectroscopy.

1. Introduction

It is now well established that isoprenylcysteine carboxyl methyltransferase (Icmt) methylation of prenylated proteins is important for the correct localization and function of a number of proteins ending in CAAX (C = cysteine, A = aliphatic residue, X = cysteine, serine, methionine, alanine) (Winter-Vann and Casey, 2005; Winter-Vann et al., 2005). There are ongoing efforts to assess the potential of targeting Icmt in anticancer drug discovery (Baron et al., 2007). Recently, we have reported the isolation of Icmt inhibitors, spermatinamine (Buchanan et al., 2007) and aplysamine 6 (Buchanan et al., in press) both from *Pseudoceratina* sp. Until now, cysmethynil and its derivatives were the only compounds discovered which were selective inhibitors of Icmt (Baron et al., 2007; Leow et al., 2007). During our campaign to discover Icmt inhibitors an extract of *Hovea parvicalyx* I. Thomps. (Fabaceae) showed Icmt inhibitory activity and was selected for further purification. Bioassay guided purification of this extract afforded two new prenylated β-hydroxychalcone derivatives, 2′-methoxy-3′-prenyl-licodione (**1**) and 2′-methoxy-3′,3″-diprenyl-licodione (**2**), together with the known flavanone (*S*)-glabrol (**3**) (Mizuno et al., 1990). Chalcones are known to show antibacterial, antifungal, antitumor and antiinflammatory properties and are also intermediates in the biosynthesis of flavonoids (Aoki et al., 2004). The chemical constituents of *H. parvicalyx* have not been studied previously. This paper reports the isolation, structure elucidation and Icmt inhibitory activity of these small-molecule inhibitors.

2. Results and discussion

2′-Methoxy-3′-prenyl-licodione (**1**) was isolated as an optically inactive yellow amorphous solid. Its HRESIMS (*m/z* 355.153132 $[M+H]^+$) established the molecular formula to be $C_{21}H_{22}O_5$ and the UV spectrum (λ_{max} 365, 301, 236 sh, 197 nm) resembled that of β-hydroxychalcone derivatives (Fukai et al., 1994; Fukai and Nomura, 1995). The ^{1}H NMR spectrum in DMSO-d_6 (Table 1) appeared as an equilibrium mixture of tautomers. A *ca.* 9:1 mixture consisting of β-hydroxychalcone (**1**) and dibenzoylmethane (**1′**) moieties, respectively were present. ^{1}H and ^{13}C NMR data clearly indicated the β-hydroxychalcone (δ_C 94.6; δ 7.04, 17.42) and dibenzoylmethane (δ_C 51.4; δ 4.53) groups (Fukai et al., 1994; Fukai

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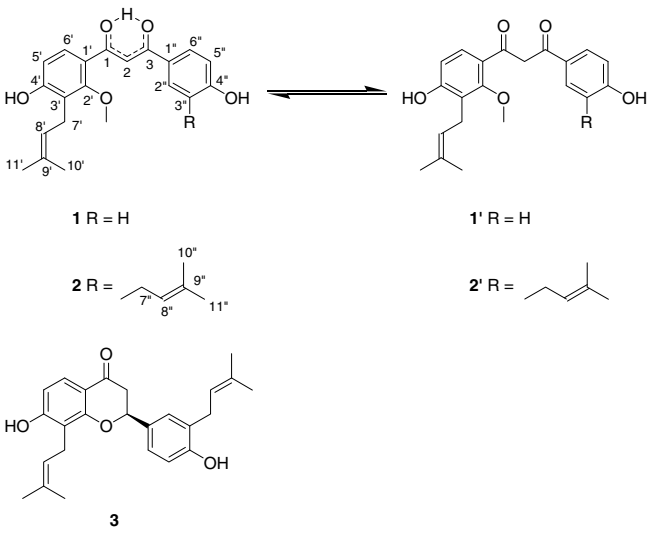
Table 1
^{1}H (600 MHz), ^{13}C (125 MHz), gCOSY and gHMBC NMR data for 2′-methoxy-3′-prenyl-licodione (**1**) in DMSO-d_6

Position	δ_C	δ_H enol/keto (mult., *J* Hz)	COSY (H no.)	$^{2,3}J_{CH}$ HMBC (C no.)
1	183.1 C			
2	94.6 CH_2	7.04/4.53 (*s*)		1, 3, 1′, 1″
3	185.0 C			
3-OH		17.42 (brs)		
1′	119.4 C			
2′	159.1 C			
3′	122.0 C			
4′	160.2 C			
5′	113.3 CH	6.73/6.67 (*d*, 8.5)	6′	1′, 3′, 4′
6′	128.4 CH	7.59/7.50 (*d*, 8.5)	5′	1, 2′, 4′
7′	22.4 CH_2	3.29/3.22 (brd, 7.2)	8′, 10′, 11′	2′, 3′, 4′, 8′, 9′
8′	122.8 CH	5.17/5.13 (tsep., 7.2, 1.4)	7′, 10′, 11′	
9′	130.5 C			
10′	17.7 CH_3	1.75/1.69 (brs)	7′, 8′	8′, 9′, 11′
11′	25.4 CH_3	1.65/1.62 (brs)	7′, 8′	8′, 9′, 10′
2′-OMe	61.6 CH_3	3.68/3.62 (*s*)		2′
4′-OH		10.30/10.30 (brs)		
1″	125.9 C			
2″	129.3 CH	7.85/7.81 (*d*, 8.5)	3″	3, 4″, 6″
3″	115.6 CH	6.89/6.84 (*d*, 8.5)	2″	1″, 4″, 5″
4″	161.8 C			
5″	115.6 CH	6.89/6.84 (*d*, 8.5)	6″	1″, 3″, 4″
6″	129.3 CH	7.85/7.81 (*d*, 8.5)	5″	3, 2″, 4″
4″-OH		10.30/10.30 (brs)		

and Nomura, 1995). The ^{1}H NMR data also revealed a 1,2,3,4-tetrasubstituted benzene ring {δ 6.73 (*d*, 8.5); 7.59 (*d*, 8.5)}, a 1,4-disubstituted benzene ring {δ 7.85 (*d*, 8.5, 2H); 6.89 (*d*, 8.5, 2H)}, a γ, γ-dimethylallyl (prenyl) group {δ 5.17 (tsep., 7.2, 1.4); 3.29 (brd, 7.2, 2H); 1.75 (brs, 3H); 1.65 (brs, 3H)}, a methoxyl (δ 3.68) and two phenolic hydroxyls {δ 10.30 (brs, 2H)}. Nineteen resonances corresponding to 21 carbon atoms were inferred from the ^{13}C NMR spectrum. Compound **1** therefore had a β-hydroxychalcone core containing 1,2,3,4-tetrasubstituted and 1,4-disubstituted benzene rings. What remained was to correctly assign the two hydroxyls, methoxyl and prenyl groups to the correct positions on the aromatic rings. This was achieved from analysis of the gHMBC spectrum. Thus, the aromatic proton at δ 7.59 (H-6′) showed gHMBC correlations to C-1 (δ_C 183.1), C-2′ (δ_C 159.1) and C-4′ (δ_C 160.2). The methoxyl at δ 3.68 (2′-OMe) also had a correlation to C-2′ (δ_C 159.1). Lastly the aromatic proton at δ 6.73 (H-5′) showed gHMBC correlations to C-1′ (δ_C 119.4), C-3′ (δ_C 122.0) and C-4′ (δ_C 160.2). This established that the 1,2,3,4-tetrasubstituted aromatic ring within the β-hydroxychalcone must be 2′-methoxy-3′-prenyl-4′-hydroxy and therefore the 1,4-disubstituted aromatic ring was substitiuted by a hydroxyl at C-4″. The ^{13}C NMR chemical shifts of C-1 (δ_C 183.1) and C-3 (δ_C 185.0) in the β-hydroxychalcone tautomer were very close in value indicating the presence of a delocalised six-membered ring as represented in **1**. All of the above evidence indicated compound **1** was 2′-methoxy-3′-prenyl-licodione.

The molecular formula of 2′-methoxy-3′,3″-diprenyl-licodione (**2**) was determined to be $C_{26}H_{30}O_5$ from HRESIMS (*m/z* 423.216746 $[M+H]^+$). The ^{1}H NMR data (Table 2) of **2** revealed that it had an additional prenyl group {δ 5.31 (tsep., 7.2, 1.4); 3.28 (brd, 7.2, 2H); 1.74 (brs, 3H); 1.69 (brs, 3H)}, replacing an aromatic proton, relative to **1**. Thus, compound **2** was 2′-methoxy-3′,3″-diprenyl-licodione.

2′-Methoxy-3′-prenyl-licodione (**1**), 2′-methoxy-3′,3″-diprenyl-licodione (**2**) and (*S*)-glabrol (**3**) showed inhibition of Icmt with IC_{50}′s of 30, 17 and 25 μM (assay performed in duplicate on two independent days), respectively. They showed no activity in the arterfact assay up to 100 μM, ruling out assay technology interference. *S*-Adenosylhomocysteine was used as the reference compound and had an IC_{50} of 1.2 μM. Compounds **1–3** add to the small list of inhibitors of the Icmt cancer target.



3. Experimental section

3.1. General experimental procedures

Water was Millipore Milli-Q PF filtered, while all other solvents used were Lab-Scan HPLC grade. A Betasil C_{18} 5 μm (21.2 mm × 150 mm i.d.) was used for semi-preparative HPLC. Waters 600 pump fitted with a 996 Photodiode Array Detector and 717 plus Autosampler was used for the semi-preparative separations. C_{18} was 04 K-4348 Sepra C_{18} end-capped silica. NMR spectra were recorded at 30 °C on Varian Inova 500 and 600 MHz NMR spectrometers. Samples were dissolved in DMSO-d_6 (residual ^{1}H δ 2.50 and ^{13}C δ 39.5 ppm). Multiplicity determined by DEPT (s = C, d = CH,

Table 2
^{1}H (600 MHz), ^{13}C (125 MHz), gCOSY and gHMBC NMR data for 2′-methoxy-3′,3″-diprenyl-licodione (**2**) in DMSO-d_6

Position	δC	δH enol/keto (mult., J Hz)	COSY (H no.)	^{2,3}JCH HMBC (C no.)
1	182.5 C			
2	94.6 CH2	7.05/4.51 (*s*)		1, 3, 1′, 1″
3	185.3 C			
3-OH		17.36 (brs)		
1′	119.3 C			
2′	159.1 C			
3′	122.0 C[a]			
4′	160.2 C			
5′	111.3 CH	6.74/6.68 (*d*, 8.5)	6′	1′, 4′
6′	128.4 CH[b]	7.60/7.49 (*d*, 8.5)	5′	1, 2′, 4′
7′	22.4 CH2	3.30/3.22 (brd, 7.2)[c]	8′, 10′, 11′	2′, 4′, 8′, 9′
8′	122.8 CH	5.18/5.13 (tsep., 7.2, 1.4)	7′, 10′, 11′	10′, 11′
9′	130.5 C			
10′	17.7 CH3	1.75 (brs)[d]	7′, 8′	8′, 9′
11′	25.4 CH3	1.65 (brs)[d]	7′, 8′	8′, 9′, 10′
2′-OMe	61.5 CH3	3.68/3.63 (*s*)		2′
4′-OH		10.31/10.34 (brs)		4′, 5′
1″	125.8 C			
2″	128.4 CH[b]	7.68 (brs)[d]		3, 4″, 6″, 7″
3″	127.9 C			
4″	159.6 C			
5″	115.0 CH	6.92/6.86 (*d*, 8.5)	6″	1″, 3″, 4″
6″	126.7 CH	7.69 (brd, 8.5)[d]	5″	3, 4″
7″	27.7 CH2	3.28 (brd, 7.2)[c,d]	8″, 10″, 11″	2″, 3″, 4″, 8″, 9″
8″	122.0 CH[a]	5.31/5.24 (tsep., 7.2, 1.4)	7″, 10″, 11″	10″, 11″
9″	132.2 C			
10″	17.6 CH3	1.69 (brs)[d]	7″, 8″	9″
11″	25.4 CH3	1.74 (brs)[d]	7″, 8″	9″, 10″
4″-OH		10.33/10.35 (brs)		3″, 4″, 5″

[a,b] Overlapping isochronous signals.
[c] Chemical shift determined from 2D experiments as ^{1}H signal obscured by H_2O.
[d] Cannot determine chemical shift of keto tautomer.

t = CH_2, q = CH_3). Standard parameters were used for the 2D experiments, which included gradient COSY, HSQC ($^{1}J_{CH}$ = 140 Hz) and HMBC ($^{n}J_{CH}$ = 8.3 Hz). HRESIMS were measured on a Bruker Daltonics Apex III 4.7e Fourier Transform Mass Spectrometer, fitted with an Apollo API source. FTIR and UV spectra were recorded on a Bruker Tensor 27 FTIR spectrophotometer and a Camspec M501 UV/vis spectrophotometer, respectively. Isoprenylcysteine carboxyl methyltransferase (Icmt) and biotin-*S*-farnesyl-L-cysteine (BFC) were provided by AstraZeneca (Boston, USA). *S*-Adenosylhomocysteine (SAH) was purchased from Fluka (Buchs Switzerland). *S*-Adenosylmethionine (SAM), magnesium chloride hexahydrate ($MgCl_2$), tartrazine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), dimethyl sulfoxide (DMSO) and dithiotreitol (DTT) were from Sigma (MO, USA). [3H] *S*-Adenosylmethionine, [3H] biotin and streptavidin PVT SPA Beads were purchased from Amersham (GE Healthcare, Cardiff UK). Assays were carried out in white clear flat-bottomed 384 well microtitre plates from BD Bioscience (CA, USA).

3.2. Plant material

The plant sample *Hovea parvicalyx* I. Thomps. (Fabaceae) was collected on the 12th May 1999 from Pannikin Springs Area, Blackdown Station, Queensland, Australia by Paul Forster. A voucher sample (PIF24387) is lodged at the Queensland Herbarium, Brisbane, Australia.

3.3. Extraction and isolation

The material was ground (6.0 g) and a CH_2Cl_2/MeOH extract prepared. The CH_2Cl_2/MeOH extract was further purified by being pre-adsorbed on C_{18} and loaded into a refillable preparative guard column (30 mm × 10 mm i.d.), in line with a semi-preparative C_{18} HPLC column. The following solvent conditions were used: H_2O/1% TFA isocratic for 1 min (flow 0 to 9 mL/min), then H_2O/1% TFA to MeOH/1% TFA in 35 min, and finally isocratic for 25 min (flow 9 mL/min), 60 fractions were collected. Bioactive fractions 36–39 were combined and further fractionated by semi-preparative C_{18} HPLC using the following conditions: H_2O/1% TFA to H_2O/1% TFA:MeOH/1% TFA (3:7) in 10 min, then to MeOH/1% TFA in 30 min, and finally isocratic for 20 min (flow 10 mL/min), 60 fractions were collected. 2′-Methoxy-3′-prenyl-licodione (**1**) (2.1 mg, 0.035% dry wt), 2′-methoxy-3′,3-diprenyl-licodione (**2**) (4.3 mg, 0.072% dry wt) and (*S*)-glabrol (**3**) (3.9 mg, 0.065% dry wt.) eluted with retention times of 31, 40 and 35 min, respectively.

3.3.1. 2′-Methoxy-3′-prenyl-licodione (**1**), 1-[4-hydroxy-2-methoxy-3-(3-methylbut-2-en-1-yl)phenyl]-3-(4-hydroxyphenyl)propane-1,3-dione

Isolated as a yellow amorphous solid; UV (MeOH) λ_{max} (log) 365 (3.72), 301 (3.34), 236 sh (3.42), 197 (3.98) nm; IR $_{max}$ (KBr film) 3424, 1597, 1171, 806, 462 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; positive-HRESIMS *m/z* 355.153132 $[C_{21}H_{22}O_5+H]^+$ (calcd 355.154004, Δ +2.5 ppm).

3.3.2. 2′-Methoxy-3′,3″-diprenyl-licodione (**2**), 1-[4-hydroxy-2-methoxy-3-(3-methylbut-2-en-1-yl)phenyl]-3-[4-hydroxy-3-(3-methylbut-2-en-1-yl)phenyl]propane-1,3-dione

Isolated as a yellow amorphous solid; UV (MeOH) λ_{max} (log) 370 (3.87), 307 (3.52), 239 sh (3.62), 200 (4.17) nm; IR $_{max}$ (KBr film) 3383, 1589, 1268, 808, 514 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 2; positive-HRESIMS *m/z* 423.216746 $[C_{26}H_{30}O_5+H]^+$ (calcd 423.216600, Δ +0.3 ppm).

3.4. Icmt assay

Test substances in a DMSO solution (final concentration 1%) were added to microplates. Then to each well was added 5 μL of Icmt enzyme and BFC (final concentrations of 20 μg/mL and 3 μM, respectively) in assay buffer (50 mM HEPES, 10 mM $MgCl_2$, 3 mM DTT, pH 7.5). The reaction was initiated with addition of 10 μL of [^{3}H] SAM (final concentration 6.2 μCi/mL) and unlabelled SAM (final concentration 0.6 μM). The plate was incubated at ambient temperature (~22 °C) for 60 min. The reaction was stopped with the addition of 20 μL of SAM (0.1 mM) and streptavidin SPA beads (12 mg/mL) added to capture the product BFC ^{3}H-methyl ester. Following overnight incubation at ambient temperature radioactivity was counted on a Microbeta Trilux™ (Perkin Elmer, Turku, Finland). The percentage inhibition relative to the reference compound SAH was then calculated.

3.5. Artefact assay

To determine if activity of test compounds was due to interference with the assay reagents or technology, an artefact assay in which [^{3}H] *S*-adenosylmethionine was replaced with [^{3}H] biotin was used. The assay measures the binding of ^{3}H-biotin to streptavidin on the SPA beads. The test substances were added to microtitre plates. The assay reagents, Icmt enzyme, BFC and [^{3}H] biotin (final concentration 1.25 μCi/mL) were dispensed into the 384 well plate. The plate was incubated for 60 min at room temperature. Steptavidin coated PVT SPA beads were added to each well to capture [^{3}H] biotin and the amount of radioactivity measured. The percentage inhibition relative to the reference compound tartrazine was then calculated. The artefact assay was performed using the Icmt assay method.

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