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# Dimeric chalcones derivatives from *Myracrodruon urundeuva* act as cathepsin V inhibitors

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## 1. Introduction

*Myracrodruon urundeuva* Allemão, a timber tree that is widely distributed in Brazil and commonly known as 'Aroeira do sertão', belongs to the family Anacardiaceae. Many studies have confirmed its effectiveness against fungal and bacterial infections (Sá et al., 2009a, 2009b), as well as its activity as a termite repellent (Napoleão et al., 2011), an insecticide against *Aedes aegypti* (Souza et al., 2011; Napoleão et al., 2012), and as a neuroprotective agent (Nobre-Júnior et al., 2009). Branch extracts are used in folk medicine to treat respiratory and urinary diseases, uterine haemorrhages, and diarrhoea (Braga, 1960).

Dimeric chalcones are a class of polyphenols found in some species of the Anacardiaceae family, especially in *M. urundeuva*, from where their identification was first reported. Urundeuvines are formed from two chalcone moieties with bonds from C-2 to C-7″ and C-8 to C-8″, resulting in a six-membered ring. In a previous study, the proposed structures of four dimeric chalcones isolated from *M. urundeuva* (called urundeuvines A, B, C, and matosine) were discussed (Bandeira et al., 2003).

Cysteine peptidases represent a large family of enzymes responsible for the cleavage of peptide bonds (Turk et al., 2012). These enzymes, also known as cathepsins, are involved in numerous physiological and pathological processes and have been studied for decades as potential drug targets to treat various diseases (Novinec et al., 2012; Severino et al., 2011). Cathepsin V is specifically expressed in the thymus, testis, and corneal epithelium and is associated with neurological diseases (Niwa et al., 2012). Under pathological conditions, the enzyme has been investigated as a potential diagnostic marker for colon tumours (Severino et al., 2011; Santamaria et al., 1998). Cathepsin V is involved in the control of human T cells (responsible for cell-mediated immunity) and shows the highest elastolytic activity among the proteolytic enzymes (Turk and Gunčar, 2003; Turk, 2006). This enzyme is an attractive molecular target for the treatment of atherosclerosis, but only a few inhibitors have been described (Turk, 2006; Puzer et al., 2008). Furthermore, cathepsin V is involved in antigen presentation in the thymus. Thus, it may be considered a drug target for the treatment of autoimmune diseases (Brömme and Kaleta, 2002).

Natural products represent a rich source of structural diversity and biological activities, providing many lead compounds for drug development as well as successful drugs for the treatment of human diseases

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(Newman and Cragg, 2016). Indeed, several natural products have been investigated for their inhibitory effects on the catalytic activity of cathepsins (Severino et al., 2011; Alvim et al., 2010; Vasiljeva et al., 2007; Lecaille at al., 2002; Brömme and Kaleta, 2002; Otto and Schirmeister, 1997).

As part of an ongoing project in the search for biologically active compounds from Anacardiaceae species, in this manuscript, we report the isolation, structural elucidation and the cathepsin V inhibitory activity of a flavonochalcone **1**, two dimeric chalcones, named urundeuvines D-F **2–3**, and a debenzoyl dimeric chalcone, named cajobin **4**. In addition, 11 known compounds were also isolated, including three dimeric chalcones, urundeuvines A and B, and matosine **5** (Bandeira et al., 1994, 2003). As stereochemistry plays a crucial role in biological activity (Lovering et al., 2009), the absolute configurations of compounds **2** and **3** were determined using electronic circular dichroism (ECD) and time-dependent functional theory calculations (TD-DFT). ECD spectroscopy, combined with quantum chemical calculations, has become popular in the stereochemical analysis of natural products, and good practices for the use of these techniques in the assignment of absolute configurations can be found in the literature (Pescitelli and Bruhn, 2016).

Finally, a molecular modelling study was conducted in order to elucidate the structural features underlying the biological activity of the most active compounds.

## 2. Results and discussion

From an EtOH extract of the inner bark of *M. urundeuva*, 11 known compounds were isolated, including three triterpenoids: cycloartenol, cycloeucananol (Anjaneyulu and Raju, 1987; Aplin and Hornby, 1966), and 30-norcyclopterospermone (Gan et al., 2009); two phenolic acids: 4-hydroxy-2-methoxy-3,6-dimethylbenzoic (Elix et al., 1990), and gallic acid; two gallates: methyl and ethyl gallate (Ceruks et al., 2007); three dimeric chalcones, urundeuvines A and B, and matosine (**5**) (Bandeira et al., 1994, 2003); and one phytosteroid: β-sitosterol. Additionally, it was possible to isolate four undescribed chalcone derivatives (**1–4**) that are described below.

Compound **1** was obtained as a pale yellow powder with $[\alpha]_D^{25}$ 0 (*c* 0.4, MeOH) and presented the molecular formula $C_{30}H_{20}O_9$ as determined by HRESIMS (*m/z* 523.1020 [M-H]-, calcd. for 523.1029) (Fig. S7, see Supporting Information). The IR spectrum revealed the presence of hydroxyl (3374-3336 $cm^{-1}$), carbonyl (1620-1614 $cm^{-1}$), and aromatic (1511-1409 $cm^{-1}$) groups and the UV-VIS spectrum showed absorption bands at 214, 232, 283, and 368 nm, which were representative of the absorption bands of aromatic rings (band I of B-rings and band II of A-rings of flavonoids). An examination of the 1H, 13C, and 2D NMR spectral data of **1** (Table 1, and Figs. S1–S5, see Supporting Information) showed the presence of four sets of substituted phenyl rings, a flavone, and a chalcone system. The 13C NMR data of **1** (Table 1) showed a total of 28 carbons, including two carbonyl carbons, eight aromatic oxygenated carbons, six quaternary carbons, and twelve tertiary $sp^2$ carbons. The 1H NMR data indicated the presence of two singlets, four *ortho*-coupled doublets, two *meta*-coupled doublets, two doublets of doublets indicating the presence of two trisubstituted aromatic systems, and two doublets characteristic of methine deshielded hydrogens.

Important HMBC correlations were observed between H-7″ ($\delta_H$ 4.28) and C-1′ ($\delta_C$ 129.0), C-2′ ($\delta_C$ 132.1), C-1″ ($\delta_C$ 134.2), C-2″ ($\delta_C$ 129.3), C-6″ ($\delta_C$ 129.3), and C-9″ ($\delta_C$ 202.3), as well as between H-8″ ($\delta_H$ 5.21) and C-2 ($\delta_C$ 161.0), C-4 ($\delta_C$ 177.0), C-1″ ($\delta_C$ 134.2), C-7″ ($\delta_C$ 46.5), and C-9″ ($\delta_C$ 202.3). Further HMBC correlations are presented in Table 1. These correlations showed the junction of two structural moieties: a 4′,5′,7 trihydroxy flavone and a 4″,2‴,4‴-trihydroxy-7″,8″-dihydrochalcone. The relative configuration of **1** was proposed based on the value of the coupling constant ($J$ = 3.2 Hz) corresponding to a vicinal spin-spin interaction [$^3J$(H,H)] between the H-8″ and H-7″. This is consistent with *pseudo* equatorial-equatorial coupling. This compound is optically inactive, as confirmed by its null optical rotation and ECD spectrum (Fig. S6, see Supporting Information), indicating the isolation of a racemic mixture.

**Table 1**
1H (400 MHz) and 13C (100 MHz) NMR spectroscopic data for **1** and **2** (in methanol-$d_4$).

| Position | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | HMBC | $\delta_C$ | $\delta_H$ ($J$ in Hz) | HMBC |
| 1 | – | | | 123.4 | | |
| 2 | 161.0 | | | 132.4 | | |
| 3 | 110.4 | | | 117.0 | 6.26, s | 1, 4, 5, 7″ |
| 4 | 177.0 | | | 148.0 | | |
| 4a | 116.4 | | | – | | |
| 5 | 127.6 | 7.86, d (8.8) | 4, 7, 8a | 145.7 | | |
| 6 | 113.0 | 6.86, dd (2.8, 8.8) | 4a | 117.5 | 6.88, s | 1, 4, 5, 7 |
| 7 | 157.7 | | | 79.2 | 5.25, s | 1, 2, 6, 8, 9, 8″ |
| 8 | 104.0 | 6.95, d (2.8) | 4a, 5, 8a | 82.2 | | |
| 8a | 164.4 | | | – | | |
| 9 | – | | | 190.1 | | |
| 1′ | 129.0 | | | 109.8 | | |
| 2′ | 132.1 | | | 174.6 | | |
| 3′ | 117.7 | 6.48, s | 2, 4′, 5′, 7″ | 104.4 | 6.20, d (2.2) | 1′, 5′ |
| 4′ | 149.7 | | | 164.1 | | |
| 5′ | 145.0 | | | 115.8 | 6.40, dd (2.2, 8.8) | 1′, 3′ |
| 6′ | 111.9 | 7.54, s | 2, 1′, 4′, 5′ | 130.5 | 7.50, d (8.8) | 1′, 4′, 9 |
| 1″ | 134.2 | | | 136.7 | | |
| 2″, 6″ | 129.3 | 6.98, d (8.4) | 1″, 3″ | 131.1 | 6.84, d (8.4) | 4″, 7″ |
| 3″, 5″ | 116.0 | 6.64, d (8.4) | 1″, 2″, 4″ | 116.3 | 6.65, d (8.4) | 1″, 4″ |
| 4″ | 157.0 | | | 157.0 | | |
| 7″ | 46.5 | 4.28, d (3.2) | 1′, 2′, 1″, 2″, 6″, 9″ | 47.3 | 4.00, d (8.8) | 1, 2, 1″, 2″, 8″, 9″ |
| 8″ | 48.5 | 5.21, d (3.2) | 2, 4, 1″, 7″, 9″ | 51.7 | 3.12, d (8.8) | 1″, 7″, 9″ |
| 9″ | 202.3 | | | 192.5 | | |
| 1‴ | 110.4 | | | 113.2 | | |
| 2‴ | 165.8 | | | 168.3 | | |
| 3‴ | 104.1 | 6.25, d (3.0) | 5‴ | 103.5 | 6.22, d (2.2) | 1‴, 5‴ |
| 4‴ | 166.6 | | | 165.0 | | |
| 5‴ | 109.9 | 6.45, dd (3.0, 9.2) | 1‴, 3‴ | 112.0 | 6.43, dd (2.2, 8.0) | 1‴, 3‴ |
| 6‴ | 133.8 | 7.99, d (9.2) | 2‴, 4‴, 9″ | 129.6 | 7.56, d (8.0) | 2‴, 4‴, 9″ |

$^{13}$C NMR values obtained from the projections of HSQC and HMBC data (100 MHz, methanol-$d_4$).

Compound **2** is similar to **5** (matosine; Bandeira et al., 2003) with an extra hydroxyl group at C-8 and was obtained as a pale yellow powder with $[\alpha]_D^{25}$ +52 (*c* 0.4, MeOH). Its molecular formula was determined as $C_{30}H_{24}O_{11}$ by HRESIMS (*m/z* 525.1193 [M+H] + -2H2O calcd. for 525.1185) (Fig. S13, see Supporting Information). The IR spectra revealed the presence of hydroxyl (3306 $cm^{-1}$), carbonyl (1614 $cm^{-1}$), and aromatic (1511-1446 $cm^{-1}$) groups, and the UV-VIS spectra showed absorption bands at 214, 233, 288, and 315 nm, which were representative of the absorption bands of aromatic rings (band I of B-rings and band II of A-rings of chalcones).

The relative configuration of **2** was proposed based on the coupling constant ($J$ = 8.8 Hz) corresponding to a vicinal spin-spin interaction [$^3J$(H,H)] between H-8″ ($\delta_H$ 3.12) and H-7″ ($\delta_H$ 4.00), which is consistent with *pseudo* axial-axial coupling and, consequently, with a *trans* relative configuration. In agreement with these spectral data, the NOE