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Short communication

Pterocarpans and isoflavones from the root bark of *Millettia micans* and of *Millettia dura*

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Dedicated to the late Professor Joseph Magadula.

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ABSTRACT

From the CH_2Cl_2/CH_3OH (1:1) extract of the root bark of *Millettia micans*, a new pterocarpan, (6a*R*,11a*R*)-3-hydroxy-7,8,9-trimethoxypterocarpan (**1**), named micanspterocarpan, was isolated. Similar investigation of the CH_2Cl_2/CH_3OH (1:1) extract of the root bark of *Millettia dura* gave a further new pterocarpan, (6a*R*,11a*R*)-8,9-methylenedioxy-3-prenyloxypterocarpan (**2**), named 3-*O*-prenylmaackiain, along with six known isoflavones (**3-8**) and a chalcone (**9**). All purified compounds were identified by NMR and MS, whereas the absolute configurations of the new pterocarpans were established by chriptical data analyses including quantum chemical ECD calculation. Among the isolated constituents, calopogonium isoflavone B (**3**) and isoerythrin A-4′-(3-methylbut-2-enyl) ether (**4**) showed marginal activities against the 3D7 and the Dd2 strains of *Plasmodium falciparum* (70–90% inhibition at 40 μM). Maximaisoflavone B (**5**) and 7,2′-dimethoxy-4′,5′-methylenedioxyisoflavone (**7**) were weakly cytotoxic (IC_{50} 153.5 and 174.1 μM, respectively) against the MDA-MB-231 human breast cancer cell line. None of the tested compounds showed *in-vitro* translation inhibitory activity or toxicity against the HEK-293 human embryonic kidney cell line at 40 μM.

1. Introduction

The genus *Millettia* (family Leguminosae, subfamily Papilionoideae) with approximately 260 species is widespread in Africa (139 species) and Asia (121 species) (Banzouzi et al., 2008). Out of the East African countries, the highest number of *Millettia* species, 25, is indigenous to Tanzania, followed by 6 species native to Kenya (Banzouzi et al., 2008). The genus is a rich source of secondary metabolites such as chalcones, isoflavones, rotenoids (Dagne et al., 1989; Yenesew et al., 2003), isoflavans (Khalid and Waterman, 1983), flavanones, coumarins (Baruah et al., 1984) and pterocarpans (Sritularak et al., 2002). Some of these metabolites inhibit nitric oxide formation or possess larvicidal, pesticidal, cytotoxic, anti-inflammatory, antimicrobial and cancer chemopreventive activities (Banzouzi et al., 2008).

Millettia dura (Dunn), growing in both Tanzania and Kenya, is used as food for livestock, as a source of firewood and charcoal, and as timber for construction since it is tough and resistant to termites (Orwa et al., 2009). Traditionally, in various parts of Africa, it is used to treat hernia, diarrhea, menstrual irregularities and for healing wounds (Banzouzi et al., 2008). Its seeds, seed pods, stem and root bark have been reported to contain rotenoids and isoflavones (Dagne et al., 1991; Derese et al., 2003; Ollis et al., 1967; Yenesew et al., 1996, 1997). In contrast, *Millettia micans* (Taub), a vulnerable small tree endemic to Tanzania, has no documented traditional uses and has not yet been phytochemically explored.

Here, we report the isolation and characterization of two new pterocarpans, named micanspterocarpan (**1**) and 3-*O*-prenylmaackiain (**2**), from the root bark of *Millettia micans* and that of *Millettia dura*, respectively. From the roots of *Millettia dura*, the known compounds (**3-9**) were also identified. The antiplasmodial and cytotoxicty profiles of

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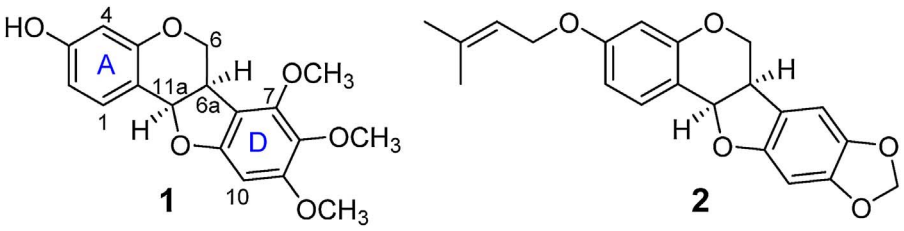

Fig. 1. Structures of micanspterocarpan (**1**) and 3-*O*-prenylmaackiain (**2**), the pterocarpans isolated from *Millettia micans* and *Millettia dura*, respectively.

Table 1
^{1}H and ^{13}C NMR data for **1** (CD_2Cl_2, 600.25 MHz for ^{1}H, 150.95 MHz for ^{13}C) and **2** ($CDCl_3$, 799.87 MHz for ^{1}H, 201.15 MHz for ^{13}C).

Micanspterocarpan (1)				3-*O*-Prenylmaackiain (2)		
Position	δ_H (*J* in Hz)	δ_C	HMBC (H → C)	δ_H (*J* in Hz)	δ_C	HMBC (H → C)
1	7.32 (*d*, 8.4)	132.4	3, 4a, 11a	7.39 (*d*, 8.8)	131.7	3, 4a, 11a
2	6.52 (*dd*, 8.4, 2.5)	109.7	4, 11b	6.64 (*dd*, 8.8, 2.4)	109.8	4, 11b
3		157.4			160.3	
4	6.39 (*d*, 2.5)	103.7	2, 11b	6.48 (*d*, 2.4)	102.4	2, 11b
4a		157.0			156.5	
6	eq: 4.29 (*ddd*, 10.6, 4.6, 0.4) ax: 3.63 (ψ-*t*, 10.6)	66.1	4a, 6a, 6b, 11a	eq: 4.23 (*dd*, 11.2, 4.8) ax: 3.66 (ψ-*t*, 11.2)	66.5	4a, 6a, 6b, 11a
6a	3.69 (*m*)	39.3	6, 6b, 10a	3.48 (*m*)	40.2	6, 6b, 10a
6b		110.0			117.9	
7		150.8		6.72 (*s*)	104.7	6a, 8, 9, 10a
8		135.7			141.7	
9		155.0			148.1	
10	6.21 (*s*)	90.8	8, 9, 6b, 10a	6.43 (*s*)	93.8	8, 9, 6b, 10a
10a		156.1			154.3	
11a	5.43 (*d*, 7.0)	78.4	1, 6, 4a, 11b	5.49 (*d*, 7.2)	78.6	1, 6, 4a, 11b
11b		113.1			112.3	
7-OCH_3	3.96 (*s*)	61.0	7			
8-OCH_3	3.73 (*s*)	61.1	8			
9-OCH_3	3.77 (*s*)	56.3	9			
8/9-OCH_2O				5.89 (*d*, 1.6) 5.92 (*d*, 1.6)	101.3	8, 9
1′				4.49 (*d*, 7.2)	64.9	3, 2′, 3′
2′				5.47 (*m*)	119.4	4′, 5′
3′					138.4	
4′				1.79 (*s*)	25.8	2′, 3′, 5′
5′				1.73 (*s*)	18.2	2′, 3′, 4′

some of the isolated compounds are also presented.

2. Results and discussion

Column chromatographic separation of the CH_2Cl_2/CH_3OH (1:1) extract of the root bark of *Millettia micans* resulted in the isolation of a new pterocarpan (**1**, Fig. 1). The HRMS (EI) spectrum indicated the molecular formula $C_{18}H_{18}O_6$ (*m/z* obs, 330.1115 $[M]^+$, calcd 330.1103), whereas the UV absorption maxima at 268, 269 and 281 nm, and the ^{1}H NMR (H-6*ax*, H-6*eq*, H-6a, H-11a) and ^{13}C NMR (C-6, C-6a, C-11a) spectral data (Table 1) are consistent with a pterocarpan skeleton (Tanaka et al., 1997; Yenesew et al., 2010). In the ^{1}H NMR spectrum (Table 1), the presence of signals for three methoxy groups (OCH_3-7, OCH_3-8 and OCH_3-9), three mutually coupled aromatic protons in an ABX spin system, and an aromatic singlet, suggested **1** to be a tetrasubstituted pterocarpan. The HMBC correlation of the methine proton at δ_H 5.43 (H-11a) with δ_C 132.4 (C-1) allowed the assignment of the ABX spin system to H-1, H-2 and H-4 of ring A. Whereas, the aromatic singlet at δ_H 6.21 was assigned to H-10 of ring D, which is otherwise fully substituted. The HMBC correlation of this proton (δ_H 6.21) to C-6b (δ_C 110.0), C-8 (δ_C 135.7), C-9 (δ_C 155.0) and C-10a (δ_C 156.1), and the absence of HMBC correlation of this proton with C-6a (δ_C 39.3), is consistent to it being assigned to H-10. The high chemical shift values of C-7, C-8 and C-9 of ring D reveal these carbon atoms to be oxygenated (Table 1). The deshielding of the ^{13}C NMR chemical shifts of two of the methoxy groups (δ_C 61.0 and 61.1) indicated that these are *di-ortho* substituted (Yenesew et al., 1998), and hence placed at C-7 and C-8, respectively. The connection of the third methoxy group (δ_H 3.77, δ_C 56.3) to C-9 (155.0) was revealed by their HMBC correlation, and the NOE interaction of OCH_3-9 and H-10 (δ_H 6.21). The $^{3}J_{H\text{-}6a,H\text{-}11a}$ = 7.0 Hz is typical of the energetically more favorable *cis* B/C ring junction (McKee et al., 1997). This relative configuration was further corroborated by the strong NOE observed between H-6a and H-11a that is in excellent agreement with the calculated (B3LYP/6-311G**) 2.4 Å distance of these protons in the lowest energy geometry of the *cis*-fused configurational isomers (Fig. 2).

A large and negative specific rotation ($[\alpha]_D$ − 134.3°), and a positive Cotton effect at 288 nm (Fig. 3) in the ECD spectrum, are consistent with the (6a*R*,11a*R*) absolute configuration at the B/C ring junction (Slade et al., 2005). This was confirmed by theoretical ECD calculations using the Time Dependent DFT (TDDFT) method (Autschbach et al., 2002; Bauernschmitt and Ahlrichs, 1996). It was further noted that different orientation of the methoxy and the hydroxy groups is

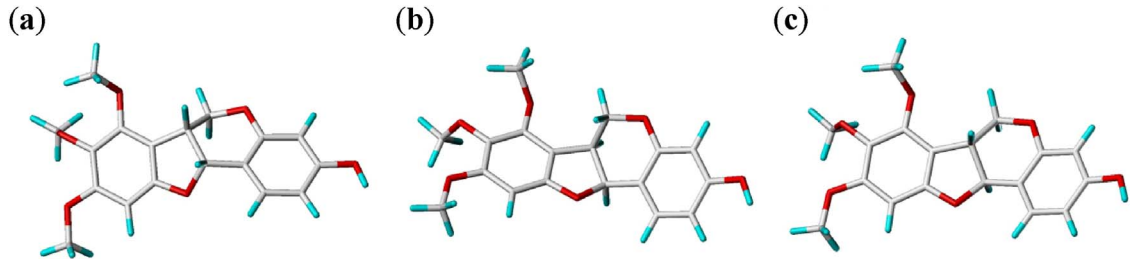

Fig. 2. Calculated global energy minimum geometries of (**a**) *cis*-fused (6a*R*,11a*R*)-**1**, (**b**) *cis*-fused (6a*S*,11a*S*)-**1**, and (**c**) *trans*-fused (6a*R*,11a*S*)-**1**.

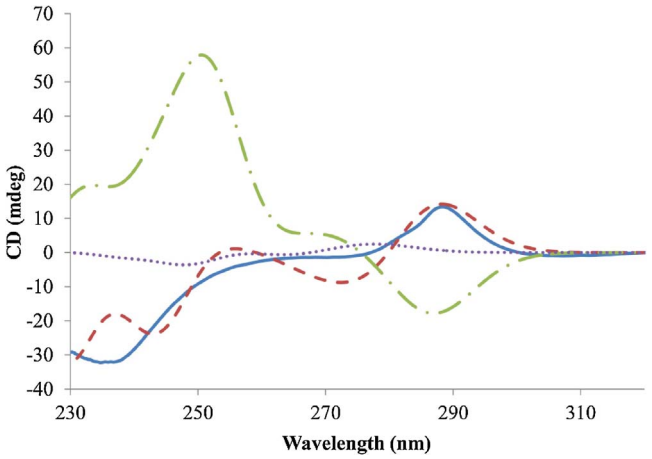

Fig. 3. ECD spectra: Experimental **1** (—, blue), computationally (DFT) predicted for the *cis*-fused (6a*R*,11a*R*)-**1** (——, red), *cis*-fused 6a*S*,11a*S*-**1** (— · —, green), *trans*-fused (6a*R*,11a*S*)-**1** (···, purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

predicted to lead to asymmetric behavior, concerning H-6a and H-11a, of the calculated ECD spectra of (6a*R*,11a*R*)-**1** (Fig. 2a) and (6a*S*,11a*S*)-**2** (Fig. 2b) enantiomers, the latter being 0.36 kcal/mol higher in energy. The orientation of these functionalities is expected to influence only the short-wave part of the spectrum, while retaining the symmetry of its long-wave part at 288 nm. Overall, based on the NMR, OR, ECD and DFT data, this compound was characterized as (6a*R*,11a*R*)-3-hydroxy-7,8,9-trimethoxypterocarpan (**1**), and was assigned the trivial name, micanspterocarpan.

Separation of the CH_2Cl_2/CH_3OH (1:1) extract of the root bark of *Millettia dura* led to the isolation of a new pterocarpan (**2**, Fig. 1) along with seven known compounds (Supplementary information, Fig. S1) that were identified as calopogonium isoflavone B (**3**) (Yankep et al., 1997), isoerythrin A-4′-(3-methylbut-2-enyl) ether (**4**) (Derese et al., 2003; Yenesew et al., 1996), maximaisoflavone B (**5**) (Dagne et al., 1991), durmillone (**6**) (Yankep et al., 1997; Yenesew et al., 1996), 7,2′-dimethoxy-4′,5′-methylenedioxyisoflavone (**7**) (Dagne et al., 1991; Yankep et al., 1997), 7-hydroxy-8,3′,4′-trimethoxyisoflavone (**8**) (Puebla et al., 2010) and butein (**9**) (Tian et al., 2011) upon comparison of their spectroscopic data (Supplementary information, Section S2) with literature.

Compound **2** was obtained as a colorless paste. Based on HRMS (ESI) analysis, the molecular composition of **2** was suggested to be $C_{21}H_{20}O_5$ ($[M+H]^+$, *m/z* obs 353.1386, calcd 353.1311). The NMR data (Table 1) showed high similarities to those of **1**, revealing that **2** also has a pterocarpan skeleton (Tanaka et al., 1997; Yenesew et al., 2010), with a prenyloxy (OCH_2-1′, CH-2′, CH_3-4′, CH_3-5′ and C-3′) and a methylenedioxy (8/9-OCH_2O) substituents. For ring A, similar to **1**, an ABX spin system was observed with the signal at δ_H 7.39 assigned to H-1 upon observation of its HMBC correlations to C-3 (δ_C 160.3), C-4a (δ_C 156.5), and C-11a (δ_C 78.6). Connection of the prenyloxy group to C-3 was indicated by the NOE of CH_2-1′ to H-2 (δ_H 6.64) and to H-4 (δ_H 6.48), and by the HMBC correlation of the CH_2-1′ protons to C-3 (δ_C 160.3). The presence of two singlet aromatic protons, H-7 (δ_H 6.72) and H-10 (δ_H 6.43), of ring D is consistent with the placement of the methylenedioxy group at C-8/9. Similar to compound **1**, a *cis* relative configuration at the B/C ring junction was proposed for **2** by the $^3J_{H\text{-}6a,H\text{-}11a}$ = 7.2 Hz, confirmed by the NOE correlation between H-6a and H-11a. Compound **2** also possessed a large and negative specific rotation ($[\alpha]_D$ −129.6°), which is consistent with (6a*R*,11a*R*) absolute configuration as in compound **1**. Based on the above information, this new compound was characterized as (6a*R*,11a*R*)-8,9-methylenedioxy-3-prenyloxypterocarpan (**2**), trivial name 3-*O*-prenylmaackiain was assigned.

Table 2
Cytotoxicity of the crude extract of root bark of *Millettia dura* and some of its isolated constituents against MDA-MB-231 breast cancer cells.

Sample	IC_{50}, μM
Crude extract of root bark of *M. dura*[a]	31.7
Calopogonium isoflavone B (**3**)	> 287.3
Isoerythrin A-4′-(3-methylbut-2-enyl) ether (**4**)	> 257.6
Maximaisoflavone B (**5**)	153.5
7,2′-Dimethoxy-4′,5′-dimethylenedioxy isoflavone (**7**)	174.1

[a] IC_{50} for the crude extract is given in μg/ml.

The crude extract of the root bark of *M. dura* and some of its constituents were tested for cytotoxicity against the MDA-MB-231 human breast cancer cell line (Table 2), the HEK-293 human embryonic kidney cell line and the chloroquine resistant (Dd2) and chloroquine sensitive (3D7) strains of *Plasmodium falciparum*. As part of an ongoing screening for small molecule inhibitors of eukaryotic protein synthesis, the isolated constituents were also tested in Krebs-2 *in vitro* translation extracts programmed with a bicistronic firefly-HCV IRES-Renilla luciferase mRNA construct, to simultaneously monitor cap-dependent and cap-independent translation (Novac et al., 2004). Minimal antiplasmodial activity was observed for compounds **3**-**5** and **7** against the chloroquine sensitive 3D7 and the chloroquine-resistant Dd2 *P. falciparum* strains; demonstrating activities between 70 and 90% inhibition at 40 μM. Among the compounds tested, **5** and **7** showed low toxicities against the Estrogen receptor (ER) negative MDA-MB-231 breast cancer cells. None of the compounds showed translation inhibitory activity *in vitro* or cytotoxicity against the HEK-293 human embryonic kidney cell line, up to 40 μM concentration.

3. Experimental section

3.1. General

Melting points were obtained on a Büchi Melting point B-545 instrument. NMR spectra were acquired on Varian MR-400, Bruker Avance III 600 and Bruker Avance III HD 800 MHz spectrometers. All spectra were processed using MestReNova 10.0 using the residual solvent peak as indirect chemical shift reference. HRMS (EI) spectra were obtained on a Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK), using direct inlet and 70 eV ionization voltage. LC–MS (ESI) chromatograms were acquired on a Perkin-Elmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source connected to a Gemini 5 mm RPC_{18} 110 Å column, applying a H_2O/MeCN 80:20-20:80 gradient with a separation time of 8 min. TLC was carried out on Merck pre-coated silica gel 60 F254 plates. Gel filtration was done on Sephadex LH-20. UV spectra were recorded using PerkinElmer Lambda 25, Serial No: 501S14030919 spectrophotometer. ECD spectra were recorded on a J-815 CD-spektrapolarimeter, serial No. A030261168. Optical rotations were measured on a PerkinElmer 341-LC Polarimeter. Preparative HPLC was carried out on a Waters 600E instrument using the Chromulan (Pikron Ltd) software and an RP C8 Kromasil® (250 mm × 55 mm) column with a H_2O/CH_3OH solvent system.

3.2. Plant materials

The root bark of *Millettia dura* was collected from Mulathankari, Meru County, Kenya in the year 2013. The plant material was identified by Mr. Patrick Chalo Mutiso of the Herbarium, School of Biological Sciences, University of Nairobi, Kenya, where a voucher specimen (number TD-2013/03) was deposited. The root bark of *Millettia micans* was collected from Kisarawe-Tanzania and was identified by Mr. F. Mbago of the Department of Botany, University of Dar es Salaam. A voucher specimen (FMM 3591/2013) was kept at the Institute of

Traditional Medicine, Muhimbili University of Health and Allied Sciences, Tanzania.

3.3. Extraction and isolation of compound 1 from the root bark of Millettia micans

The dried and ground root bark (1 kg) of *M. micans* was extracted with CH_2Cl_2/CH_3OH, (1:1) three times for 24 h each. The extract was filtered and concentrated using a rotary evaporator to give a brown crude extract (38 g). A 3.5 g portion of the crude extract was subjected to column chromatography (CC) on silica gel (150 g) and was eluted with mixture of *n*-hexane and EtOAc with increasing polarities. The fraction which was eluted with 6% EtOAc in *n*-hexane was purified by CC on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH; 1:1), and were subsequently recrystallized from *n*-hexane/CH_2Cl_2 to give micanspterocarpan (**1**, 40.4 mg).

3.4. Extraction and isolation of compounds from the root bark of Millettia dura

The air-dried and ground root bark (1.5 kg) of *M. dura* was extracted with CH_2Cl_2/CH_3OH (1:1), as described above, to give 100 g of a light brown crude extract. A portion of the crude extract (90 g) was subjected to column chromatography on silica gel eluting with *n*-hexane containing increasing amounts of EtOAc. The fraction eluted with 1% EtOAc in *n*-hexane was purified first by CC on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH; 1:1) and then by preparative HPLC (eluent: CH_3OH/H_2O; gradient) to give 3-*O*-prenylmaackiain (**2,** 1.5 mg). The fractions eluted with 2–4% EtOAc in *n*-hexane were combined and subjected to column chromatography on silica gel (eluent: *n*-hexane/EtOAc; 4:1) to give calopogonium isoflavone B (**3**, 140.9 mg), maximaisoflavone B (**5**, 23.6 mg) and impure isoerythrin A-4′-(3-methylbut-2-enyl) ether (**4**). The purification of **4** (6.8 mg) was achieved by reverse-phase preparative HPLC (eluent: CH_3OH/H_2O; gradient). Crystallization (from CH_3OH) of the 6% EtOAc in *n*-hexane elution gave durmillone (**6**, 7.4 mg). The fractions eluted with 8–10% EtOAc were combined and subjected to CC on silica gel (eluent: *n*-hexane/EtOAc; 4:1) to give 7,2′-dimethoxy-4′,5′-methylenedioxyisoflavone (**7**, 156.6 mg). Similar treatment of the fractions eluted with 12–15% EtOAc in *n*-hexane gave 7-hydroxy-8,3′,4′-trimethoxyisoflavone (**8**, 7.1 mg). The fractions eluted with 25–30% EtOAc in *n*-hexane were combined and applied on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH; 1:1) to give butein (**9**, 2.7 mg).

3.5. Physical and spectroscopic data of compounds 1 and 2

Micanspterocarpan (**1**). White amorphous powder. UV λ_{max} (CH_3OH) 268, 269 and 281 nm $[\alpha]_D$ − 134.3° (*c* 0.02, CH_2Cl_2). ECD (CH_3OH, Fig. 3); ^1H and ^{13}C NMR, see Table 1 and Supporting information; MS (EI, 70 ev) (rel. int.) *m/z* 330 (100, $[M]^+$), 315 (32, $[M\text{-}CH_3]^+$), 157 (25), 147 (31), 131 (41), 123 (41); HRMS (EI) *m/z* obs 330.1115 $[M]^+$, calcd 330.1103 ($C_{18}H_{18}O_6$).

3-O-Prenylmaackiain (**2**). Colorless paste. $[\alpha]_D$ − 129.6° (*c* 0.002, CH_2Cl_2); ^1H and ^{13}C NMR, see Table 1 and Supporting information; MS (ESI) *m/z* 353.6 $[M+H]^+$; HRMS (ESI) *m/z* obs 353.1386, calcd 353.1311 ($C_{21}H_{20}O_5$).

3.6. Theoretical calculations

Geometries were optimized at the B3LYP/6-311G** (Becke, 1993; Lee et al., 1988) level of theory without constraints. ECD spectra were computed using the Time Dependent DFT (TDDFT) (Autschbach et al., 2002; Bauernschmitt and Ahlrichs, 1996) algorithm encompassed into the program package GAUSSIAN 09 (Frisch et al., 2009) applying the 6-31G* basis set. For spectra prediction 15 singlet and 15 triplet states were solved using the keyword "TD" with Nstates = 15; 50-50. All GAUSSIAN results were analysed and the spectra were displayed using the SpecDis 1.62 software (Bruhn et al., 2014). Different conformations were weighted according to their Boltzmann distribution. Molecules were displayed using SYBYL (2013).

3.7. Cytotoxicity assays

MDA-MB-231 human breast cancer cells were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/mL streptomycin at 37 °C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density (10^4 cells per well) to ensure exponential growth for the duration of the assay. After a 24 h pre-incubation period, the medium was replaced with experimental medium containing the appropriate compound concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). After 72 h incubation, cell viability was measured using Alamar Blue (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± standard error for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least six times. Statistical analyses were performed using a two-tailed Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

To assess the cytotoxicity of compounds on HEK-293 cells in dose response, a resazurin-based viability assay was used. In brief, HEK-293 cells were grown in DMEM medium (Life Technologies), containing 10% fetal calf serum (FCS; Gibco), trypsinised, counted and seeded at 2000 cells per well in 45 µL media into TC-treated 384-well plates (Greiner) and left to adhere overnight at 37 °C, 5% CO_2 and 95% humidity. Test compounds were prepared by diluting compounds 1 in 25 in sterile water and then another 1 in 10 dilution, to give a top final test concentration of 40 µM, 0.4% DMSO. Plates were incubated for 72 h at 37 °C, 5% CO_2 and 95% humidity, and then the media was removed and replaced by 35 µL of 44 µM resazurin in DMEM without FCS. The plates were incubated for another 4–6 h at 37 °C, 5% CO_2 and 95% humidity, before reading on an EnVision® Plate Reader (PerkinElmer) using fluorescence excitation/emission settings of 530 nm/595 nm. The % growth was standardized to controls (40 µM puromycin as positive and 0.4% DMSO as negative control) using Microsoft® Excel 2013. A statistical analysis including IC_{50} determination and graphical output was performed in GraphPad Prism® 6 using nonlinear regression variable slope curve fitting.

3.8. Plasmodium falciparum culture

In vitro parasite culture of *P. falciparum* strains 3D7 and Dd2 were maintained in RPMI with 10 mM Hepes (Life Technologies), 50 µg/mL hypoxanthine (Sigma) and 5% human serum from male AB plasma and 2.5 mg/mL AlbuMAX II® (Life Technologies). Human 0+ erythrocytes were obtained from the Australian Red Cross Blood Service (Agreement No: 13-04QLD-09). The parasites were maintained at 2–8% parasitaemia (% P) at 5% haematocrit (% H), and incubated at 37 °C, 5% CO_2, 5% O_2, 90% N_2 and 95% humidity.

3.9. Plasmodium falciparum growth inhibition assay

A previously developed, well-established procedure (Duffy and Avery, 2012) of asexual *P. falciparum* imaging assay was used to determine parasite growth inhibition.

3.10. Translation inhibitory assay

A previously developed assay (Novac et al., 2004) was used to measure the translation inhibitory activity of the studied compounds. The compounds were suspended at a concentration of 10 mM in DMSO

and subsequently diluted to 200 μM in water. They were tested at a final concentration of 20 μM in Krebs-2 translation extracts programmed with a bicistronic Firefly-HCV IRES-Renilla luciferase mRNA construct. Translation reactions were incubated at 30 °C for 60 min at which point the luciferase activities were then measured. Compounds that inhibit only FF would be considered cap-dependent translation inhibitors, compounds that inhibit expression of Ren only would be inhibitors of HCV IRES translation while compounds that inhibit both FF and Ren would likely be translation elongation inhibitors. None of the compounds were observed to display significant inhibition of translation.

4. Conclusions

Two new pterocarpans were characterised from the CH_2Cl_2/CH_3OH (1:1) root bark extracts of *Millettia micans* and *Millettia dura*, along with seven known compounds from the latter extract. Marginal antiplasmodial activity was observed for calopogonium isoflavone B (**3**), isoerythrin A-4′-(3-methylbut-2-enyl) ether (**4**), maximaisoflavone B (**5**) and 7,2′-dimethoxy-4′,5′-methylenedioxyisoflavone (**7**) against the chloroquine-sensitive (3D7) and -resistant (Dd2) strains of *Plasmodium falciparum*. Whereas **5** and **7** showed some toxicity against the MDA-MB-231 human breast cancer cell line at high concentrations, **3** and **4** were inactive. None of the isolated compounds showed toxicity against the HEK-293 human embryonic kidney cell line, and they lacked translation inhibition potency.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2017.07.012.

References

Autschbach, J., Ziegler, T., van Giesbergen, S.J.A., Baerends, E.J., 2002. Chiroptical properties from time-dependent density functional theory. I. Circular dichroism spectra of organic molecules. J. Chem. Phys. 116, 6930–6940.
Banzouzi, J.T., Prost, A., Rajemiarimiraho, M., Ongoka, P., 2008. Traditional uses of the African *Millettia* species (Fabaceae). Int. J. Bot. 4, 406–420.
Baruah, P., Barua, N.C., Sharma, R.P., Baruah, J.N., Kulanthaivel, P., Herz, W., 1984. Flavonoids from *Millettia pulchra*. Phytochemistry 23, 443–447.
Bauernschmitt, R., Ahlrichs, R., 1996. Treatment of electronic excitations within adiabatic approximation of time dependent density functional theory. Chem. Phys. Lett. 256, 454–464.
Becke, A.D., 1993. Density-functional thermochemistry III. The role of exact exchange. J. Chem. Phys. 98, 1372–1377.
Bruhn, T., Schaumlöffel, A., Hemberger, Y., Bringmann, G., 2014. SpecDis, Version 1.62. University of Wuerzburg, Germany.
Dagne, E., Bekele, A., Waterman, P.G., 1989. The flavonoids of *Millettia ferruginea* subsp. *ferruginea* and subsp. *darassana* in Ethiopia. Phytochemistry 28, 1897–1900.
Dagne, E., Mammo, W., Bekele, A., 1991. Flavonoids of *Millettia dura*. Bull. Chem. Soc. Ethiop. 5, 81–86.
Derese, S., Yenesew, A., Midiwo, J.O., Heydenreich, M., Waterman, P.G., 2003. A new isoflavone from stem bark of *Millettia dura*. Bull. Chem. Soc. Ethiop. 17, 113–115.
Duffy, S., Avery, V.M., 2012. Development and optimization of a novel 384-well antimalarial imaging assay validated for high-throughput screening. Am. J. Trop. Med. Hyg. 86, 84–92.
Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, K., Toyota, R., Fukuda, J., Hasegawa, M., Ishida, T., Nakajima, Y., Honda, O., Kitao, H., Nakai, T., Vreven, J.A., Montgomery, J.R., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, O., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J., 2009. Gaussian 09, Revision A.02. Gaussian Inc, Wallingford, CT.
Khalid, S.A., Waterman, P.G., 1983. Thonningine-A and thonningine-B: two 3-phenylcoumarins from the seeds of *Millettia thonningii*. Phytochemistry 22, 1001–1003.
Lee, C., Yang, W., Parr, R.G., 1988. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys. Rev. B 37, 785–789.
McKee, T.C., Bokesch, H.R., McCormick, J.L., Rashid, M.A., Spielvogel, D., Gustafson, K.R., Alavanja, M.M., Boyd, M.R., 1997. Isolation and characterization of new anti-HIV and cytotoxic leads from plants, marine and microbial organisms. J. Nat. Prod. 60, 431–438.
Novac, O., Guenier, A.S., Pelletier, J., 2004. Inhibitors of protein synthesis identified by a high throughput multiplexed translation screen. Nucleic Acids Res. 32, 902–905.
Ollis, W.D., Rhodes, C.A., Sutherland, L.O., 1967. The extractives of *Millettia dura* (Dunn): the constitutions of durlettone, durmillone, milldurone, millettone and millettosin. Tetrahedron 23, 4741–4760.
Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Anthony, S., 2009. Agroforestree Database: A Tree Reference and Selection Guide Version 4.0. World Agroforestry Centre, Kenya.
Puebla, P., Oshima-Franco, Y., Franco, L.M., Santos, M.G.D., da Silva, R.V., Rubem-Mauro, L., Feliciano, A.S., 2010. Chemical constituents of the bark of *Dipteryx alata* Vogel, an active species against *Bothrops jararacussu* venom. Molecules 15, 8193–8204.
SYBYL-X 2.1.1. Tripos Inc, 2013. 1699 South Hanley Road. Tripos Inc., St. Louis, MO 63144.
Slade, D., Ferreira, D., Marais, J.P.J., 2005. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. Phytochemistry 66, 2177–2215.
Sritularak, B., Likhitwitayawuid, K., Conrard, J., Vogler, B., Reeb, S., Klaider, I., Kraus, W., 2002. New flavones from *Millettia erythrocalyx*. J. Nat. Prod. 65, 589–591.
Tanaka, H., Tanaka, T., Etoh, H., 1997. A pterocarpan from *Erythrina orientalis*. Phytochemistry 45, 205–207.
Tian, X., Zhou, S., Wei, H., Hu, N., Dai, Z., Liu, Z., Han, Z.-Z., Tu, P., 2011. Flavonoids from the herb of *Bidens pilosa* L. J. Chin. Pharm. Sci. 20, 518–522.
Yankep, E., Fomum, Z.T., Dagne, E., 1997. An *O*-geranylated isoflavone from *Millettia griffoniana*. Phytochemistry 46, 591–593.
Yenesew, A., Midiwo, J.O., Waterman, P.G., 1996. Four isoflavones from the seed pods of *Millettia dura*. Phytochemistry 41, 951–955.
Yenesew, A., Midiwo, J.O., Waterman, P.G., 1997. 6-Methoxycalopogoniumisoflavone A: a new isoflavone from seed pods of *Millettia dura*. J. Nat. Prod. 60, 806–807.
Yenesew, A., Midiwo, J.O., Miessner, M., Heydenreich, M., Peter, M.G., 1998. Two prenylated flavanones from stem bark of *Erythrina burttii*. Phytochemistry 48, 1439–1443.
Yenesew, A., Derese, S., Midiwo, J.O., Oketch-Rabah, H.A., Lisgarten, J., Palmer, R., Heydenreich, M., Waters, N.C., 2003. Antiplasmodial activities and X-ray crystal structures of rotenoids from *Millettia usaramensis* spp. *usaramensis*. Phytochemistry 64, 773–779.
Yenesew, A., Twinomuhwezi, H., Kabaru, J.M., Kiremire, B.T., Akala, H.M., Heydenreich, M., Peter, M.G., Eyase, F., Waters, N.C., Walsh, D., 2010. Antiplasmodial and larvicidal activities of flavonoids of *Derris trifoliata*. Bull. Chem. Soc. Ethiop. 23, 409–414.