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Cytotoxic flavonoids from two *Lonchocarpus* species

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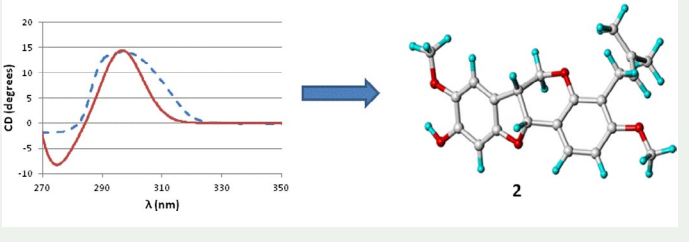
ABSTRACT

A new isoflavone, 4′-prenyloxyvigvexin A (**1**) and a new pterocarpan, (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**2**) were isolated from the leaves of *Lonchocarpus bussei* and the stem bark of *Lonchocarpus eriocalyx*, respectively. The extract of *L. bussei* also gave four known isoflavones, maximaisoflavone H, 7,2′-dimethoxy-3′,4′-methylenedioxyisoflavone, 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone, durmillone; a chalcone, 4-hydroxylonchocarpin; a geranylated phenylpropanol, colenemol; and two known pterocarpans, (6a*R*,11a*R*)-maackiain and (6a*R*,11a*R*)-edunol. (6a*R*,11a*R*)-Edunol was also isolated from the stem bark of *L. eriocalyx*. The structures of the isolated compounds were elucidated by spectroscopy. The cytotoxicity of the compounds was tested by resazurin assay using drug-sensitive and multidrug-resistant cancer cell lines. Significant antiproliferative effects with IC_{50} values below 10 µM were observed for the isoflavones 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone and durmillone against leukemia CCRF-CEM cells; for the chalcone, 4-hydroxylonchocarpin and durmillone against its resistant counterpart CEM/ADR5000 cells; as well as for durmillone against the resistant breast adenocarcinoma MDA-MB231/*BCRP* cells and resistant gliobastoma U87MG.Δ*EGFR* cells.



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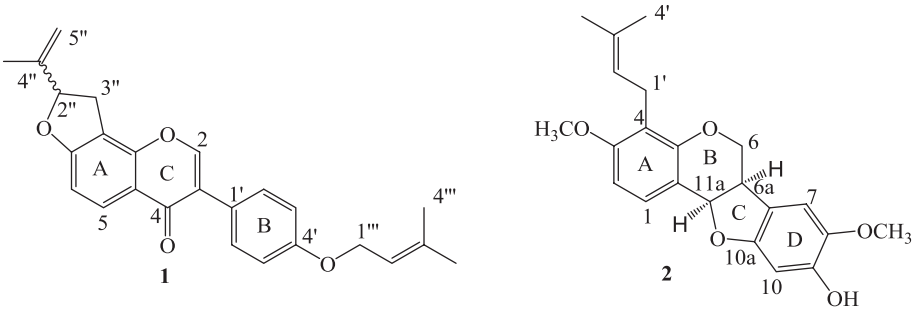

Figure 1. Structures of new compounds isolated from *Lonchcarpus* species.

1. Introduction

The genus *Lonchocarpus* (family Leguminosae, sub-family Papilionoideae, tribe Millettieae) comprises over 100 species dispersed in the tropical America, Africa and the Caribbean Islands (Magalhães et al. 1996). Some taxonomists consider the genus *Lonchocarpus* to be one of the genera within the complex tribe Millettieae, which also includes the genera *Derris* and *Millettia* (Polhill and Raven 1981; Lavin et al. 1998). Previous phytochemical studies on different *Lonchocarpus* species have resulted in the isolation of flavonoids, chalcones, pterocarpans, dibenzoylmethane derivatives and rotenoids (Magalhães et al. 1997, 2000; Borges-Argáez et al. 2002; Lawson et al. 2010). Some flavonoids isolated from the traditionally used Asian plants, *Epimedium koreaonum* and *Terminalia arjuna* have showed cytotoxicity against MCF-7 and Hep G2 cancer cell lines (Pettit et al. 1996; Wang et al. 2007).

In Kenya, *Lonchocarpus bussei*, *Lonchocarpus eriocalyx* and *Lonchocarpus kanuri* are the only species that are taxonomically recognized (Beentje 1994). The bark of *L. eriocalyx* is used in the treatment of blood pressure and to reduce sugar level by Embu and Mbeere communities in Kenya (Kareru et al. 2006). The roots and the stem bark of *L. bussei* are used in the management of fever and abdominal pain in Tanzania (Chhabra et al. 1990). Herein, we report the isolation, characterization and the cytotoxicity of a new isoflavone (**1**, Figure 1) and a new pterocarpan (**2**, Figure 1) from *L. bussei* and *L. eriocalyx,* respectively, along with eight known compounds (**3**–**10**, Figure S1).

2. Results and discussion

The air dried and powdered leaves of *L. bussei* were extracted with CH_2Cl_2/MeOH (1:1). The extract was separated by the use of a combination of chromatographic techniques which led to the isolation of a new isoflavone (**1**) along with nine known compounds. The known compounds were identified as maximaisoflavone H (**3**) (Dagne et al. 1990; Yenesew et al. 1996), 7,2′-dimethoxy-3′,4′-methylenedioxyisoflavone (**4**) (Veitch et al. 2003), 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone (**5**) (Campbell et al. 1969), durmillone (**6**) (Yenesew et al. 1996), 4-hydroxylonchocarpin (**7**) (Dagne et al. 1989) and colenemol (**8**) (Brader et al. 1997). Similar investigation of the roots of this plant afforded (6a*R*,11a*R*)-maackiain (**9**) (Abdel-Kader 2001) and (6a*R*,11a*R*)-edunol (**10**) (Reyes-Chilpa et al. 1994).

Compound **1** was obtained as a colourless paste; HRESIMS analysis showed a $[M + H]^+$ peak at *m/z* 389.1759, which together with NMR data (Section 3.4, Table S1, Figure S2–S7)

allowed the assignment of the molecular formula $C_{25}H_{24}O_4$. The UV (λ_{max} 250 and 312 nm), ^{1}H NMR (δ_H 7.91 for H-2) and ^{13}C NMR (δ_C 152.0 for C-2, 124.9 for C-3, 175.9 for C-4) spectral data were characteristic of an isoflavone moiety (Yenesew et al. 1998a). Two *ortho*-coupled proton signals at δ_H 8.09 (*d*, *J* = 8.6 Hz) and 6.92 (*d*, *J* = 8.6 Hz) were assigned to H-5 and H-6, respectively, of ring-A which is substituted at C-7 and C-8. In the HMBC spectrum, H-5 (δ_H 8.09) correlated to C-4 (δ_C 175.9), C-7 (δ_C 165.2) and C-8a (δ_C 153.9); H-6 to C-7 (δ_C 165.2), C-8 (113.5) and C-4a (δ_C 119.1), consistent with 7,8-disubstituted ring-A. This substituent was identified as 2-isopropenyl-2,3-dihydrofuran group as shown from the ^{1}H NMR spectrum [δ_H 3.22 (1H, *dd*, *J* = 15.8, 9.9 Hz, H-3″a), 3.58 (1H, *dd*, *J* = 15.8, 7.9 Hz, H-3″b), 5.43 (1H, *br t*, *J* = 8.9 Hz, H-2″), 4.98 (1H, *m*, H-5″a), 5.13 (1H, *m*, H-5″b) and 1.80 (3H, *s*, 6″-CH_3)]. The placement of the 2-isopropenyl-2,3-dihydrofuran ring at C-7/C-8 of ring-A was supported by HMBC correlation of CH_2-3″ to C-7 (165.2), C-8 (113.5) and C-8a (153.9); and H-2″ to C-7 (165.2) (Table S1).

In the ^{1}H NMR spectrum, an AA′XX′ spin system at δ_H 7.45 (*d*, *J* = 8.8 Hz) and 6.96 (*d*, *J* = 8.8 Hz) was assigned to H-2′/6′ and H-3′/5′, respectively, of 4′-oxygenated ring-B. In addition, the ^{1}H NMR spectrum revealed the presence of a prenyloxy group in ring-B based on the signals at δ_H 4.56 (2H, *d*, *J* = 6.7 Hz, CH_2-1‴), 5.49 (1H, *m*, H-2‴), 1.76 (3H, *s*, 4‴-CH_3) and 1.80 (3H, *s*, 5‴-CH_3) together with the corresponding ^{13}C signals at δ_C 65.2 (C-1‴), 120.0 (C-2‴), 138.2 (C-3‴) and two methyl carbon signals at δ_C 18.2 (4‴-CH_3) and 25.8 (5‴-CH_3). The attachment of the prenyloxy group to C-4′ was confirmed by the HMBC correlation (Table S1) of H-3′/5′ (δ_H 6.96) to C-4′ (δ_C 159.2). On the basis of the above data and comparison with related compounds (Leu et al. 2012; Derese et al. 2014), compound **1** was characterised as 7,8-(2″-isopropenyl-2″,3″-dihydrofuran)-4′-prenyloxyisoflavone, named 4′-prenyloxyvigvexin A. The absolute configuration at C-2″ has not been determined.

Investigation of the stem bark of *L. eriocalyx* afforded a new pterocarpan (**2**) along with the known pterocarpan (6a*R*,11a*R*)-edunol (**10**) (Reyes-Chilpa et al. 1994). HRESIMS of compound **2**, $[\alpha]_D^{22} - 114°$, showed a molecular ion peak at *m/z* 368.1626, which along with NMR data (Table S2, Figures S9–S14) suggested the molecular formula $C_{22}H_{24}O_5$. The UV (λ_{max} 288 and 304 nm), ^{1}H NMR spectrum [δ_H 3.60 and 4.34 (CH_2-6), 3.58 (H-6a), and 5.48 (H-11a)], with the corresponding carbon signals (from HSQC spectrum, Figure S13) appearing at δ_C 68.3 (C-6), 41.9 (C-6a) and 80.0 (C-11a), suggested that compound **2** has a pterocarpan skeleton. The presence of a prenyl, two methoxy and a hydroxy substituents was also evident from the NMR spectra (Table S2). In ring-A, the ^{1}H NMR spectrum showed two *ortho*-coupled aromatic protons at δ_H 6.71 (*d*, *J* = 8.5 Hz) and 7.31 (*d*, *J* = 8.5 Hz), the latter of which showed HMBC correlation with C-3 (δ_C 159.7), C-4a (δ_C 155.6) and C-11a (δ_C 80.0), allowing the assignment of this signal (δ_H 7.31) to H-1 and its coupling partner (δ_H 6.71) to H-2. One of the methoxy protons (δ_H 3.84) showed NOESY correlation (Figure S11) with H-2 (δ_H 6.71), and HMBC (Table S2, Figure S14) correlation with C-3 (δ_C 159.7) and hence placed at C-3. The methylene protons of the prenyl group (δ_H 3.30) showed HMBC correlation with C-3 (δ_C 159.7), C-4 (δ_C 118.9) and C-4a (δ_C 155.6), consistent with its placement at C-4.

In ring-D, two singlet aromatic protons resonating at δ_H 7.02 and 6.33 were assigned to H-7 and H-10, respectively, indicating that ring-D is 8,9-disubstituted with hydroxy and methoxy groups. This was supported by HMBC spectrum where both H-7 and H-10 correlated with C-8 (δ_C 143.4) and C-9 (δ_C 149.2) (Table S2). The methoxy group (δ_H 3.80) in this ring showed NOESY correlation with H-7 (δ_H 7.02) and HMBC correlation with C-8 (δ_C 143.4) and hence was placed at this carbon, then the hydroxy group should be located at C-9. Compound

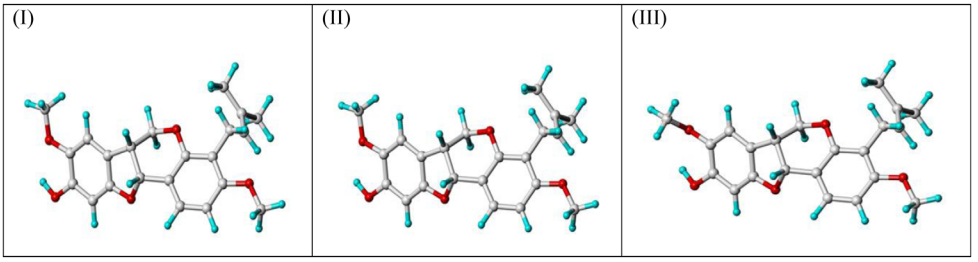

Figure 2. The calculated global energy minimum geometries of conformers of (6a*R*,11a*R*)-**2** (conformation I: global minimum, conformation II:+0.10 kcal/mole, confirmation III:+0.99 kcal/mole).

Table 1. Cytotoxicity of the studied compounds and doxorubicin towards leukemia cells as determined by resazurin assay.

	IC_{50} value in μM and degree of resistance		
Compound[a]	CCRF-CEM	CEM/ADR5000	Degree of resistance
1	18.92 ± 4.88	25.53 ± 9.05	1.34
2	31.82 ± 3.40	16.87 ± 1.94	0.53
3	>135.10	>135.10	N/A
4	49.49 ± 11.27	32.47 ± 5.53	0.65
5	6.27 ± 1.41[b]	29.51 ± 3.75	6.70
6	0.54 ± 0.17[b]	0.86 ± 0.02[b]	1.59
7	88.91 ± 1.18	3.43 ± 1.21[b]	0.03
8	37.74 ± 14.12	21.58 ± 0.84	0.57
9	>140.70	81.34 ± 4.10	>0.57
10	34.11 ± 0.80	33.22 ± 4.07	0.97
Doxorubicin	0.02 ± 0.00[b]	66.83 ± 2.20	3341

Note: N/A = Not Applicable.
[a]Refer to Figures 1 and S1 for structures of compounds.
[b]Values in bold: Significant activity.

2 was therefore characterised as 9-hydroxy-3,8-dimethoxy-4-prenylpterocarpan, named 3,8-dimethoxybitucarpin B.

The high negative specific rotation, $[\alpha]_D^{22}$ − 114°, and the experimental ECD spectrum (Figure S16) which showed a positive Cotton effect at 300 nm suggested (6a*R*,11a*R*) configuration (Yenesew et al. 1998b). In order to ascertain this, the energies of different conformers for (6a*R**,11a*R**)-**2** were calculated and the one with minimum energy and two immediately above it with Δ*E* < 0.99 kcal/mol were identified (Figure 2). Then the ECD spectra for these conformations were calculated separately and compared with experimental ECD of **2**. The weighed Boltzmann sum of calculated for three energy minimum conformations of (6a*R*,11a*R*)-**2** showed a positive Cotton effect at *ca*. 300 nm as in the experimental ECD spectrum (Figure S16), confirming this configuration. Therefore on the bases of the above evidence this new compound was characterised as (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B.

The cytotoxicity of compounds **1**–**10** as well as the standard doxorubicin was first determined against sensitive leukemia CCRF-CEM cells and the drug resistant subline CEM/ADR5000 cells. Apart from maximaisoflavone H (**3**) against CCRF-CEM and CEM/ADR5000 cells, and (6a*R*,11a*R*)-maackiain (**9**) against CCRF-CEM cells, all other compounds showed activities with IC_{50} values below 90 μM against the two cell lines (Table 1). Significant activity, with IC_{50} values less than 10 μM (Brahemi et al. 2010; Kuete and Efferth 2015), was observed

for 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone (**5**, IC_{50} 6.27 µM) and durmillone (**6**, IC_{50} 0.54 µM), against the CCRF-CEM cells. Similarly, 4′-hydroxylonchocarpin (**7**, IC_{50} 3.43 µM) and durmillone (**6**, IC_{50} 0.86 µM) showed moderate to good activities against the CEM/ADR5000 cells, respectively. It is worth noting that CEM/ADR5000 cells were highly resistant to the drug doxorubicin; on the other hand, all the tested compounds (except for compounds **3** and **9**) showed better activity than the standard against this cell-line. Hypersensitivity (degree of resistance below 0.90) (Mbaveng et al. 2017) of CEM/ADR5000 compared to its sensitive parental cell line CCRF-CEM was noted with (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**2**), 7,2′-dimethoxy-3′,4′-methylenedioxyisoflavone (**4**), 4-hydroxylonchocarpin (**7**) and colenemol (**8**), suggesting that they might have inhibitory effect on P-glycoprotein's expression (Mbaveng et al. 2017). With regards to their activity in leukemia cells, 4′-prenyloxyvigvexin A (**1**), 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone (**5**) and durmillone (**6**), displaying IC_{50} values below 20 µM towards CCRF- CEM cells, were selected and screened further against a panel of 7 carcinoma cells and normal AML12 hepatocytes (Table S3). Of the three compounds, the isoflavone durmillone (**6**) displayed IC_{50} values below 10 µM towards resistant MDA-MB231/*BCRP* cells (8.97 µM) and resistant glioblastoma U87MG.Δ*EGRF* cells (5.83 µM). Interestingly, hypersensitivity was obtained with the three selected compounds against U87MG.Δ*EGRF* cells as well as with 4′-prenyloxyvigvexin A (**1**) and durmillone (**6**) against MDA-MB231/*BCRP* cells compared to their respective sensitive counterparts U87MG cells and MDA-MB231 cells. It is also important to note that the selectivity indexes of 4′-prenyloxyvigvexin A (**1**), 6,7,3′-trimethoxyl-4′,5′-methylenedioxyisoflavone (**5**) and durmillone (**6**) for the normal AML12 hepatocytes versus hepatocarcinoma HepG2 cells are below 1, suggesting their poor selectivity to liver cancer cells. However, a closer look at the IC_{50} values of the compounds against AML12 cells versus other cell lines indicated that higher selectivity can be achieved with other cancer types. Overall the study has showed the good activity of durmillone (**6**), with IC_{50} below 1 µM towards the two leukemia cells, and IC_{50} below 10 µM against two carcinoma cells. This compound is a cytotoxic agent that can be explored further towards the development of an anticancer drug.

3. Experimental

3.1. General experimental procedure

NMR spectra were recorded on BRUKER AVANCE 500 MHz and 600 MHz instruments. HRESIMS were acquired on GC-TOF micromass (Waters Inc.). UV spectra were acquired on specord S 600. Melting points were determined on SMP 10 apparatus. CD spectra were measured using Jasco J-815. Optical rotations were read on AUTOPOL IV. IR spectra were measured on Perkin Elmer UATR Two instrument. Column chromatography on Merck silica gel 60 (70-230 mesh). Final purifications were conducted on Sephadex LH-20 and on PTLC silica gel 60 (20 cm x 20 cm) plates. In the cytotoxicity assay, fluorescence was measured on an Infinite 200 Pro TECAN plate reader.

3.2. Plant materials

L. bussei were collected from Muhaka-Kaya forest, Kwale County, Kenya in January, 2016. *L. eriocalyx* were collected from Mutheteni location, Machakos County, Kenya in January,

2016. The plants were authenticated by Mr. Patrick C. Mutiso of the University Herbarium, School of Biological Sciences, University of Nairobi, where a voucher specimen, PBC2016/005 for *L. bussei*, and PBC2016/006 for *L. eriocalyx*, were deposited.

3.3. Extraction and isolation

3.3.1. Isolation of compounds from the leaves of L. bussei

The air dried and powdered leaves of *L. bussei* (2 kg) were extracted by percolation with CH_2Cl_2/MeOH (1:1) (3 × 2L) to yield 100 g of crude extract. A portion of the extract (85 g) was subjected to column chromatography (CC) over silica gel (450 g, 80 × 4 cm) and eluted with *n*-hexane containing increasing amounts of EtOAc. A total of 75 fractions each *ca*. 500 mL were collected. The fraction eluted with 3% EtOAc in *n*-hexane was separated by CC over Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to give compounds **1** (15 mg) and **8** (17 mg). Compound **3** (25 mg) crystallized from the fraction eluted with 5% EtOAc in *n*-hexane. The mother liquor of this fraction was purified by CC on Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to give compound **7** (50 mg) and more amounts of compound **3** (3 mg). The fraction eluted with 10% EtOAc in *n*-hexane was separated by CC over Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) and crystallisation to give compounds **4** (10 mg) and **5** (12 mg). Similar treatment of the fraction eluted with 20% EtOAc in *n*-hexane afforded compound **6** (22 mg).

3.3.2. Isolation of compounds from the roots of L. bussei

The air dried and ground roots of *L. bussei* (2 kg) were extracted as described above to afford 84 g of crude extract. A portion of the extract (75 g) was subjected to column chromatography on silica gel (400 g, 80 × 4 cm) and eluted with *n*-hexane containing increasing amounts of EtOAc. A total of 50 fractions, each about 500 mL were collected. PTLC (eluent: *n*-hexane-CH_2Cl_2–EtOAc, 8:1:1) separation of the fraction eluted with 15% EtOAc in *n*-hexane afforded compounds **9** (5 mg) and **10** (4 mg).

3.3.3. Isolation of compounds from the stem bark of L. eriocalyx

Powdered stem bark of *L. eriocalyx* (2 kg) were extracted using CH_2Cl_2/MeOH (1:1). The solvent was removed under reduced pressure to yield 105 g of crude extract. A portion of the extract (85 g) was subjected to CC over silica gel (450 g, 80 x 4 cm) and a total of 100 fractions, each about 500 mL were collected. The fraction eluted with 5% EtOAc in *n*-hexane was purified by PTLC (eluent: *n*-hexane-CH_2Cl_2–EtOAc, 8:1:1) to give compound **2** (20 mg). The fraction eluted with 10% EtOAc in *n*-hexane was purified by CC over Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to afford compound **10** (3 mg).

3.4. Physical and spectroscopic data of compounds 1 and 2

4′-Prenyloxyvigvexin A (**1**): colourless paste; IR ν_{max}: 1704, 1625, 1605, 1510, 1449 and 1380 cm^{-1}; UV λ_{max} (CD_2Cl_2): 250 and 312 nm; ^1H NMR (500 MHz, CD_2Cl_2): δ_H 8.09 (*d*, *J* = 8.6, H-5), 7.91 (*s*, H-2), 7.45 (*d*, *J* = 8.8 Hz, H-2′/6′), 6.96 (*d*, *J* = 8.8 Hz, H-3′/5′), 6.92 (*d*, *J* = 8.6, H-6), 5.49 (*m*, H-2‴), 5.43 (*br t*, *J* = 8.9 Hz, H-2″), 5.13 (*m*, H-5″b), 4.98 (*m*, H-5″a), 4.56 (2H, *d*, *J* = 6.7 Hz, H-1‴), 3.58 (*dd*, *J* = 15.8, 9.9 Hz, H-3″b), 3.22 (*dd*, *J* = 15.8, 9.9 Hz, H-3″a), 1.80 (*s*, H-4″/H-5‴, 1.76 (*s*, H-4‴); ^{13}C NMR (125 MHz, CD_2Cl_2): δ_C 175.9 (C-4), 165.2 (C-7), 159.2 (C-4′), 153.9 (C-8a), 152.0 (C-2), 143.7 (C-3″), 130.5 (C-2′/6′), 128.0 (C-5), 124.9* (C-3), 124.7* (C-1′), 119.1 (C-4a),

114.8 (C-3′/5′),113.5 (C-8), 108.6 (C-6), 112.7 (C-5″), 88.2 (C-2″), 31.8 (C-1″α), 25.8 (C-5‴), 18.2 (C-4″/C-4‴); HR-HRESIMS: *m/z* 389.1759 $[M + H]^+$ (Calcd for $C_{25}H_{25}O_4$, 389.1747).

(6a*R*,11a*R*)-3,8-Dimethoxybitucarpin B (**2**): colourless paste; $[\alpha]_D^{22} - 114°$ (*c* 0.12, acetone); UV λ_{max} (CH_2Cl_2): 287, 304 nm; ^{1}H NMR (500 MHz, acetone-d_6): δ_H 7.31 (*d*, *J* = 8.5 Hz, H-1), 6.71 (*d*, *J* = 8.6 Hz, H-2), 3.60 (*m*, H-6ax), 4.34 (*m*, H-6 eq), 3.58 (*m*, H-6a), 7.02 (*s*, H-7), 6.33 (*s*, C-10), 5.48 (*d*, 6.6), 3.30 (*m*, H-1′), 5.15 (*m*, C-2′), 1.73 (*s*, H-4′), 1.60 (*s*, H-5′), 3.84 (*s*, 3-OCH_3), 3.80 (*s*, 8-OCH_3); ^{13}C NMR (125 MHz, acetone-d_6): δ_C 159.7 (C-3), 155.7* (C-10a), 155.6* (C-4a), 149.2 (C-9), 143.4 (C-8), 131.8 (C-3′), 130.7 (C-1), 124.4 (C-2′), 118.9 (C-4), 118.6 (C-6b), 115.4 (C-1a), 111.2 (C-7), 106.1 (C-2), 99.3 (C-10), 80.0 (C-11a), 68.3 (C-6), 58.2 (8-OCH3), 56.8 (3-OCH_3), 41.9 (C-6a), 26.3 (C-5′), 23.5 (C-1′), 18.5 (C-4′) EIMS *m/z* (rel int): 368 $[M]^+$, 312 (16), 297 (16); HRESIMS: *m/z* 368.1626 $[M]^+$, (Calcd for $C_{22}H_{24}O_{5,}$ 368.1624).

3.5. Cytotoxicity assays

The resazurin reduction assay (O'Brien et al. 2000) was performed to assess the cytotoxicity of compounds **1**–**10** and doxorubicin as control drug towards various sensitive and drug-resistant cancer cell lines, including the CCRF-CEM and CEM/ADR5000 leukemia, MDA-MB231 breast cancer cells and its resistant subline MDA-MB231/*BCRP*, HCT116*p53*$^{+/+}$ colon cancer cells and its resistant subline HCT116*p53*$^{-/-}$, U87MG glioblastoma cells and its resistant subline U87MG.Δ*EGFR* and HepG2 hepatocarcinoma cells and normal AML12 hepatocytes, the details of which have been described (Kuete et al. 2017). The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; CEM/ADR5000, MDA-MB-231-BCRP, HCT116 (p53−/−), U87MG.Δ*EGFR* and HepG2 were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-pcDNA, HCT116 (p53+/+), U87MG and AML12, respectively.

3.6. Theoretical calculation

Different conformations and configurations of compound **2** were optimized at the B3LYP/6-311G** (Lee et al. 1988; Becke 1993) level of theory without any restrictions. The ECD were computed using the Time Dependent DFT (TDDFT) (Bauernschmitt and Ahlrichs 1996; Autschbach et al. 2002) algorithm in the program package GAUSSIAN 09 (Frisch et al. 2009). The 6-31G* basis set was applied. 10 singlet and 10 triplet states were solved (keyword TD (NStates = 10, 50–50). All GAUSSIAN results were analysed and the spectra display using the SpecDis 1.62 (Bruhn et al. 2013). The molecules are displayed using SYBYL-X 2.1.1 (2013).

4. Conclusion

Phytochemical study of two *Lonchocarpus* species led to the isolation of a new isoflavone, 4′-prenyloxyvigvexin A (**1**), and a new pterocarpan, (6a*R*,11a*R*)-3,8-dimethoxybitucarpin B (**2**) along with eight known compounds, **3**–**10**. Investigation for cytotoxicity of the isolated compounds, based on the resazurine assay on drug sensitive and multidrug resistant cancer cell lines, showed significant antiproliferative effects with IC_{50} values below 10 µM for compounds 3′,6,7-trimethoxy-4′,5′-methylenedioxyisoflavone (**5**) and durmillone (**6**) against leukemia CCRF-CEM cells; for 4-hydroxylonchocarpin (**7**) and durmillone (**6**) against its

resistant counterpart CEM/ADR5000 cells; as well as for durmillone (**6**) against the resistant breast adenocarcinoma MDA-MB231/*BCRP* cells and resistant gliobastoma U87MG.Δ*EGFR* cells.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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