Four Prenylflavone Derivatives with Antiplasmodial Activities from the Stem of Tephrosia purpurea subsp. leptostachya

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Abstract: Four new flavones with modified prenyl groups, namely (E)-5-hydroxytephrostachin (1), purleptone (2), (E)-5-hydroxanhydrotephrostachin (3), and terpurlepflavone (4), along with seven known compounds (5–11), were isolated from the CH2Cl2/MeOH (1:1) extract of the stem of Tephrosia purpurea subsp. leptostachya, a widely used medicinal plant. Their structures were elucidated on the basis of NMR spectroscopic and mass spectrometric evidence. Some of the isolated compounds showed antiplasmodial activity against the chloroquine-sensitive D6 strains of Plasmodium falciparum, with (E)-5-hydroxytephrostachin (1) being the most active, IC50 1.7 ± 0.1 μM, with relatively low cytotoxicity, IC50 > 21 μM, against four cell-lines.

Keywords: Tephrosia purpurea subsp. leptostachya; stem; flavone; antiplasmodial; cytotoxicity

1. Introduction

Tephrosia purpurea (family Leguminosae) is one of the most widely distributed Tephrosia species and is found in tropical, subtropical, and other arid parts of the world. It consists of the four subspecies purpurea, leptostachya, appolinea, and barbigera, and four varieties, namely under subsp. leptostachya var. leptostachya and var. pubescens, and under subsp. barbigera var. barbigera and var. rufescens [1–5]. In Africa, a decoction of roots, leaves, and fruits of Tephrosia purpurea is given as a diuretic, for blood purification, and for the treatment of a cough and cold [6]. Its macerated leaves are used for curing diarrhoea and whooping cough in children [6]. In East Africa, its roots are used against stomach pains, while its leaves are used to treat snake bites and headaches. A decoction of its leaves and roots is used as a purgative [7], whereas that of the roots of T. purpurea subsp. leptostachya is employed for the treatment of schistosomiasis [6].

Phytochemical studies on T. purpurea collected from different parts of the world have resulted in the isolation of a wide variety of flavonoids; flavones [8,9], rotenoids [10], chalcones [11], and flavanones [12]. The crude extracts and pure compounds obtained from T. purpurea have shown
a wide range of biological activities including antiplasmodial [12,13], anticancer [14], antacid [15], antidiabetic [16], analgesic and anti-inflammatory [17], and hepatoprotective [18] activities, and were also shown to be applicable to treat *Helicobacter pylori* infection [19]. Despite the presence of several subspecies and varieties of the taxa *T. purpurea*, the ethnobotanical, bioactivity, and phytochemical reports available so far have not been specific on the particular subspecies and variety. In order to better understand the relationship between *T. purpurea* and other species, the chemical variability among its subspecies and varieties has to be documented. With this in mind, the first phytochemical and biological report on *T. purpurea* subsp. leptostachya is reported here.

2. Results and Discussion

Extraction of the air dried stem of *T. purpurea* subsp. leptostachya with CH$_2$Cl$_2$/MeOH (1:1) at room temperature, followed by a combination of chromatographic separations, gave four new (1–4) and seven known (5–11) compounds (Figure 1).

![Figure 1. Structures of compounds isolated from *T. purpurea* subsp. leptostachya.](image-url)

Compound 1 was isolated as yellow crystals, and its molecular formula C$_{21}$H$_{20}$O$_5$ was established from HRMS ($m/z$ 352.1315) and $^1$H- and $^{13}$C-NMR data (Table 1, Figures S1–S6). The UV ($\lambda_{max}$ 230, 270 and 310 nm), $^1$H ($\delta$$_H$ 6.67 for H-3), and $^{13}$C ($\delta$$_C$ 164.2 for C-2, 105.5 for C-3, and 182.9...
for C-3) NMR spectral data suggested that this compound is a flavone derivative substituted with methoxy (δ_C 3.92; δ_H 56.1), hydrogen bonded hydroxyl (δ_H 13.08), and 2-methylbut-3-en-2-ol (Table 1, Tables S1–S6) substituents. The HMBC correlation of H-3 (δ_H 6.67) with C-2 (δ_C 164.2), C-4 (δ_C 182.9), and C-4a (δ_C 105.2) further supported the proposed flavone structure. Three sets of mutually coupled protons resonating at δ_H 7.91 (H-2'/6'), 7.52 (H-3'/5'), and 7.55 (H-4') with corresponding carbons at δ_C 126.5 (C-2'/6'), 129.1 (C-3'/5'), and 131.9 (C-4'), respectively, were assigned to ring-B, which is unsubstituted (Table 1). The 1H-NMR data (Table 1) of 1 possesses a singlet at δ_H 6.40 (δ_C 95.3) on ring-A, which is hence trisubstituted with a methoxy (at C-7), a hydrogen bonded hydroxy (at C-5), and a (E)-2-methylbut-3-en-2-ol group. The HMBC correlations of the singlet at δ_H 6.40 with C-4a (δ_C 105.2), C-5 (δ_C 161.3), C-7 (δ_C 163.1), and C-8 (δ_C 105.3) allowed its assignment to H-6. Based on HMBC correlations, the methoxy group (δ_H 3.92, δ_C 56.1) was placed at C-7 (δ_C 163.1) and the hydrogen bonded hydroxy group (δ_H 13.08) at C-5, and the 2-methylbut-3-en-2-ol group could only be placed at C-8. This regiochemistry was confirmed by the HMBC correlation of OH-5 (δ_H 13.08) to C-4a (δ_C 105.2), C-5 (δ_C 161.3), and C-6 (δ_C 95.3), and of the olefinic proton H-1" (δ_H 6.85) to C-7 (δ_C 163.1) and C-8a (δ_C 154.1). The J = 16.5 Hz coupling between H-1" (δ_H 6.85) and H-2" (δ_H 6.70) is consistent with the E-configuration of the double bond of the 2-methylbut-3-en-2-ol group [20]. Therefore, compound 1 was characterized as (E)-5-hydroxy-8-(3-hydroxy-3-methylbuta-1,3-dien-1-yl)-7-methoxy-2-phenyl-4H-chromen-4-one. It is a 5-hydroxy derivative of trans-tephrostachin [20] and hence was given the trivial name (E)-5-hydroxytephrostachin.

The molecular formula of compound 2 was established as C_{20}H_{15}O_{5} from HRMS (m/z 336.0980), and 1H- and 13C-NMR data (Table 1, Figures S9–S13). Its UV spectrum (λ_max 230, 290, and 330 nm), along with its NMR spectra (Table 1), suggested that 2 had a flavone skeleton. Its 1H- and 13C-NMR spectra (Table 1) showed high similarities to those of 1. Thus, ring-B of 2 is unsubstituted, while its ring-A is trisubstituted, with a hydroxy at C-5, a methoxy at C-7, and a modified prenyl group at C-8 (Table 1). The 1H-NMR spectral data further suggested the presence of trans-oriented and mutually coupled (J = 16.4 Hz) olefinic protons, which are deshielded (δ_H 8.06, H-1", and δ_H 7.18, H-2"), suggesting a different substituent at C-8 of 2 as compared to 1. Furthermore, a single, deshielded methyl signal (δ_H 2.41; δ_C 27.8) was observed, which along with an additional carbonyl signal (δ_C 199.1) showing HMBC correlations to H-1" (δ_H 8.06) and H-2" (δ_H 7.18), suggests that the C-8 substituent is the rare (E)-but-3-en-2-one group, similar to that reported for (2S)-5-hydroxy-7-methoxy-8-[(E)-3-oxo-1-butenyl]flavanone [21], and for erylivingstone F [22]. Based on the above spectroscopic data, compound 2 was characterized as (E)-5-hydroxy-7-methoxy-8-(3-oxobut-1-en-1-yl)-2-phenyl-4H-chromen-4-one and was given the trivial name purleptone.

Compound 3 ([M + 1]^+ m/z 335.1227, C_{21}H_{18}O_{4}) was also found to be a flavone derivative (λ_max 230, 280 and 310 nm), whose 1H- and 13C-NMR spectra (Table 1, Figures S16–S21) showed close similarities to those of 1 and 2. It was found to have an unsubstituted ring-B, and trisubstituted ring-A with hydroxy at C-5, methoxy at C-7, and a modified prenyl group at C-8. The structure of the latter substituent was established to be (E)-3-methylbuta-1,3-dien-1-yl from the 1H- and 13C-NMR spectral data (Table 1), and was confirmed by the HMBC correlations of CH_{2}-4" (δ_H 5.10) with C-2" (δ_C 135.4), C-3" (δ_C 142.9), and C-5" (δ_C 18.2). The placement of this group at C-8 was established from the HMBC correlations of H-2" (δ_H 6.29) to C-8 (δ_C 106.0), C-3" (δ_C 142.9), C-4" (δ_C 116.8), and C-5" (δ_C 18.2), and of H-5" (δ_H 2.06) with C-2" (δ_C 135.4), C-3" (δ_C 142.9), and C-4" (δ_C 116.8). In agreement with this, H-1" also showed HMBC correlation with C-7 (δ_C 163.2), C-8a (δ_C 154.2), C-2" (δ_C 135.4), and C-3" (δ_C 142.9). Compound 3 was therefore characterized as (E)-5-hydroxy-7-methoxy-8-(3- methylbuta-1,3-dien-1-yl)-2-phenyl-4H-chromen-4-one, and was given the trivial name (E)-5-hydroxyanhydrotephrostachin as it is structurally closely related to anhydrotephrostachin [20].
Table 1. $^1$H- (800 MHz) and $^{13}$C- (200 MHz) NMR data for compounds 1, 2, and 3 (in CDCl$_3$) at 25 °C.

| Position | δ$_C$ (ppm) | δ$_H$, m (J in Hz) | HMBC (H→C) | δ$_C$ | δ$_H$, m (J in Hz) | HMBC (H→C) | δ$_C$ | δ$_H$, m (J in Hz) | HMBC (H→C) |
|----------|--------------|---------------------|-------------|-------|---------------------|-------------|-------|---------------------|-------------|
| 2        | 164.2        |                     |             | 164.6 |                     |             | 164.2 |                     |             |
| 3        | 105.5        | 6.57 s              | C-2, C-4, C-4a, C-1’ | 106.2 | 6.74 s              | C-2, C-4, C-4a, C-1’ | 105.5 | 6.71 s              | C-2, C-4, C-4a, C-1’ |
| 4        | 182.9        |                     |             | 182.6 |                     |             | 183.0 |                     |             |
| 4a       | 105.2        |                     |             | 105.4 |                     |             | 105.3 |                     |             |
| 5        | 161.3        |                     |             | 164.2 |                     |             |       |                     |             |
| 5-OH     | 95.3         | 13.08 s             | C-4a, C-5, C-6 | 95.6  | 13.41 s             | C-4a, C-5, C-6 | 95.4  | 13.11 s             | C-4a, C-5, C-6 |
| 6        | 163.1        | 6.40 s              | C-4a, C-5, C-7, C-8 | 165.0 | 6.40 s              | C-4a, C-5, C-7, C-8 | 163.2 | 6.45 s              | C-4a, C-5, C-7, C-8 |
| 7        | 105.3        |                     |             | 103.4 |                     |             | 106.0 |                     |             |
| 8        | 154.1        |                     |             | 156.0 |                     |             | 154.2 |                     |             |
| 8a       | 131.5        |                     |             | 131.5 |                     |             | 131.5 |                     |             |
| 1’       | 126.5        | 7.91 m              | C-2, C-4’, C-2’, C-6’ | 126.5 | 7.92 m              | C-2, C-4’, C-2’, C-6’ | 126.4 | 7.93 m              | C-2, C-4’, C-2’, C-6’ |
| 2’,3’    | 129.1        | 7.52 m              | C-1’, C-3’, C-5’ | 129.4 | 7.59 m              | C-1’, C-3’, C-5’ | 129.2 | 7.54 m              | C-1’, C-3’, C-5’ |
| 4’       | 131.9        | 7.55 m              | C-2’, C-6’ | 132.2 | 7.59 m              | C-2’, C-6’ | 132.0 | 7.56 m              | C-2’, C-6’ |
| 5’       | 114.9        | 6.85, d (16.5)      | C-7, C-8a, C-2”, C-3” | 132.0 | 8.06, d (16.4)      | C-7, C-8a, C-2”, C-3” | 117.5 | 6.83, d (16.5)      | C-7, C-8a, C-2”, C-3” |
| 6’       | 141.3        | 6.70, d (16.5)      | C-8, C-3”, 3’-Me$_2$ | 128.8 | 7.18, d (16.4)      | C-8, C-3”, 3’-Me$_2$ | 135.4 | 6.29, d (16.5)      | C-8, C-3”, 3’-Me$_2$ |
| 7’       | 71.5         |                     |             | 199.1 |                     |             | 142.9 |                     |             |
| 3’-Me$_2$| 30.0         | 1.50 s              | C-2”, C-3”, 3’-Me$_2$ | 27.8  | 2.41 s              | C-2”, C-3” | 116.8 | 5.10 s              | C-2”, C-3”, 3’-Me$_2$ |
| 4’       | 5’           |                     |             |       |                     |             | 18.2  | 2.06 s              | C-2”, C-3”, 3’-Me$_2$ |
| 7(OMe)   | 56.1         | 3.92 s              | C-7        | 56.4  | 4.01 s              | C-7        | 56.2  | 3.97 s              | C-7        |
The structure of compound 4 ([M + 1]^+, m/z 423.1465, C_{24}H_{22}O_7), also a flavone, was established from \(^1\)H- and \(^{13}\)C-NMR data (Table 2, Figures S24–S29), as well as from its UV spectrum (\(\lambda_{\text{max}} 230, 260, \text{and } 310 \text{ nm}\)). Its NMR spectra (Table 2) revealed the presence of an unsubstituted ring-B (\(\delta_H 7.70, \delta_C 126.3 (\text{H-2}^′/6′), \delta_H 7.45, \delta_C 128.7 (\text{H-3}′/5′), \text{and } \delta_H 7.49, \delta_C 131.1 (\text{H-4}′ \text{ m})\)), a methoxy (\(\delta_H 3.96, \delta_C 56.7\)) at C-5, an acetate ([\(\delta_H 2.11, \delta_C 21.4 \text{ (Me)}\), \(\delta_C 170.0 \text{ (C=O)}\)] at C-7, and a modified prenyl group in the form of a tetrahydrofuran ring at C-8 (Table 2), similar to terpurinflavone [12] and tephroglabrin [23]. The presence of an additional carbonyl (\(\delta_C 206.1\)) and two geminal methyl groups (\(\delta_C 24.0 \text{ and } \delta_C 165.2, \delta_C 23.9\)), and three mutually coupled protons at \(\delta_H 4.95 \text{ (dd, } J = 6.1, 10.2 \text{ Hz})\), \(\delta_H 4.90 \text{ (dd, } J = 6.1, 8.8 \text{ Hz})\) indicated that the C-8 substituent was a 5,5-dimethyl-4-oxo-tetrahydrofuran-3-yl group. In agreement with this, H-4′ (\(\delta_H 4.95\)), H-5′ (\(\delta_H 4.90\)), and 2′-(Me) \(\delta_H 1.57 \text{ and } 1.65\) showed HMBC correlations to the carbonyl carbon C-3′ (\(\delta_C 206.1\)). The HMBC correlation of H-4′ (\(\delta_H 4.95\)) with C-7, H-6 (\(\delta_H 6.41\)) with C-4a (\(\delta_C 109.1\)), C-5 (\(\delta_C 162.9\)), C-7 (\(\delta_C 166.3\)), and C-8 (\(\delta_C 103.9\)); and the OMe (\(\delta_H 3.96\)) with C-5 (\(\delta_C 162.9\)) confirmed the substitution pattern of this ring. The coupling constant \(J = 10.2 \text{ Hz}\) of H-4′ and H-5′ indicated a 1,2-diaxial orientation of these protons [12]. Hence, compound 4 was characterized as 8-(5,5-dimethyl-4-oxotetrahydrofuran-3-yl)-5-methoxy-4-oxo-2-phenyl-4H-chromen-7-yl acetate and was given the trivial name terpurlepflavone.

Table 2. \(^1\)H-(800 MHz) and \(^{13}\)C-(200 MHz) spectroscopic data for compound 4 (CDCl\(_3\)) at 25 °C.

| Position | \(\delta_C\) | \(\delta_H, m (J \text{ in Hz})\) | HMBC (H→C) |
|----------|---------------|-----------------|--------------|
| 2        | 160.6         |                 |              |
| 3        | 110.1         | 6.55 s          | C-2, C-4, C-4a, C-1′ |
| 4        | 177.2         |                 |              |
| 4a       | 109.1         |                 |              |
| 5        | 162.9         |                 |              |
| 6        | 91.1          | 6.41 s          | C-4a, C-5, C-7, C-8 |
| 7        | 166.3         |                 |              |
| 8        | 103.9         |                 |              |
| 8a       | 154.9         |                 |              |
| 1′       | 131.7         |                 |              |
| 2′, 6′   | 126.3         | 7.70 m          | C-2, C-4′, C-2′, C-6′ |
| 3′, 5′   | 128.7         | 7.45 m          | C-1′, C-3′, C-5′ |
| 4′       | 131.1         | 7.49 m          | C-2′, C-6′ |
| 2′″      | 83.9          |                 |              |
| 3″       | 206.1         |                 |              |
| 4″       | 47.7          | 4.95 dd (10.2, 6.1) | C-7, C-8, C-8a, C-2″, C-3″, C-5″ |
| 5″       | 75.8          | 4.90 dd (10.2, 8.8) | C-7, C-8, C-3″, C-4″ |
| 2″-Me    | 24.0          | 1.57 s          | C-2″, C-3″, C-2″-Me |
| 2″-Me    | 23.9          | 1.65 s          | C-2″, C-3″, C-2″-Me |
| 5″-OMe   | 56.7          | 3.96 s          | C-5 |
| 7-COMe   | 170.0         |                 |              |
| 7-COMe   | 21.4          | 2.11 s          | 7-COMe |

The known compounds were identified as derrone (5) [24], glabranin (6) [25], obovatin methyl ether (7) [26], genistein (8) [27], tachrosin (9) [28], kaempferitrin (10) [29], and D-pinitol (11) [30] by a comparison of their spectroscopic data (Tables S1 to S7) with that available in the literature. The major flavones of this plant were tested for antimalarial activity against the D6 strain of Plasmodium falciparum (Table 3). Among these, (E)-5-hydroxytephrostachin (1) showed good activity, IC\(_{50}\) 1.7 \(\mu\)M, while terpurlepflavone (4) and tachrosin (9) showed low antimalarial activities. The compounds were also tested for cytotoxicity against two non-tumoral and two cancerous cell-lines (Table 3). Most of these did not show cytotoxicity (IC\(_{50}\) > 100 \(\mu\)M), while compound 1 showed IC\(_{50}\) between 21–100 \(\mu\)M, which is still significantly lower than its antimalarial activity with a selectivity
index > 12. The results observed here demonstrate the potential of flavones as antiplasmodial agents, parallel to the in vitro and in vivo antiplasmodial activities reported earlier for some flavones [12,31].

Table 3. In vitro antiplasmodial activity and cytotoxicity of compounds 1, 2, 4 and 9 (IC₅₀, µM).

| Samples                        | Antiplasmodial Activity against P. falciparum | Cytotoxicity |
|--------------------------------|---------------------------------------------|--------------|
|                                | D6                           | LO2 *        | BEAS *        | A549 **        | HepG2 **        |
| (E)-5-Hydroxytephrostachin (1) | 1.7 ± 0.1                    | 21.7 ± 4.8   | 24.5 ± 2.7    | 76.1 ± 2.9     | >100            |
| Purleptone (2)                 | NT                          | >100         | >100          | >100           | >100            |
| Terpurlepflavone (4)           | 14.8 ± 3.2                   | >100         | >100          | >100           | >100            |
| Tachrosin (9)                  | 27.1 ± 3.2                   | >100         | >100          | >100           | >100            |
| Chloroquine                    | 0.037 ± 0.003                |              |              |                |                 |
| Artesunate-Mefloquine          | 0.075 ± 0.006                |              |              |                |                 |

* Non-tumoral cell: LO2, Immortal human hepatic cell line; BEAS, Lung/bronchus cell line (epithelial virus transformed); ** Cancer cell: A549, adenocarcinomic human alveolar basal epithelial cells; HepG2, human liver cancer cell line; NT = Not Tested.

3. Materials and Methods

3.1. General Experimental Procedure

UV spectra were recorded on a Specord S600 (Analytik Jena AG, Jena, Germany) spectrophotometer. Melting points were obtained on a Büchi Melting point B-545 (Flawil, Switzerland) apparatus, and optical rotations were measured on Perkin Elmer 341-LC (Perkin Elmer, Wellesley, MA, USA), whereas CD experiments were run on a Jasco J-715 spectropolarimeter (Jasco, Corp., Tokyo, Japan). NMR spectra were acquired on a Bruker Avance III HD 800 spectrometer (Bruker BioSpin AG, Fallanden, Switzerland) equipped with a TXO cryogenic probe using the residual solvent peak as the reference. Analytical reversed phase liquid chromatography (RP-HPLC)—mass spectrometry (MS) was performed on a API SCIEX 150 EX Perkin Elmer (Perkin Elmer, Waltham, MA, USA) ESI-MS (30 eV) connected to a Perkin Elmer gradient pump system and a C8 column (120 Å, 4 µm, 4.6 mm × 50 mm) using gradients of acetonitrile/water (CH₃CN/H₂O) with 1% formic acid (HCOOH) as the mobile phase at a flow rate of 1 mL/min. TLC was carried out on Merck pre-coated silica gel 60 F254 plates (Merck, Darmstadt, Germany). Column chromatography was run on silica gel 60 (70–230 mesh). Gel filtration was done on Sephadex LH-20 (Fluka, Buchs, Switzerland). Preparative HPLC was carried out on a Waters 600E instrument using the Chromulan (Pikron Ltd., Praha, Czech Republic) software and a RP-C₈ Kromasil® (250 mm × 55 mm, Kromasil, Bohus, Sweden) column with an H₂O/MeOH solvent system for elution. HRESIMS were obtained with a Q-TOF-LC/MS spectrometer (Stenhagen Analyslab AB, Gothenburg, Sweden) using a 2.1 mm × 30 mm, 1.7 µm RPC18 column and a H₂O–CH₃CN gradient system (5:95–95:5 gradient and 0.2% formic acid).

3.2. Plant Material

The stems of Tephrosia purpurea subsp. leptostachya were collected in April 2015 from the Kilungu hills in Makueni County, Kenya. The plant specimen was identified by Mr. Patrick C. Mutiso of the Herbarium, School of Biological Sciences, University of Nairobi, where a voucher specimen (Mutiso-841/April 2015) was deposited.

3.3. Extraction and Isolation

The air dried and ground stems (2 kg) of T. purpurea subsp. leptostachya were extracted with CH₂Cl₂/MeOH (1:1) for seven days at 20–25 °C by percolation (3 × 2 L) to yield a dark yellow paste (80 g, 4%). Hence, it was soaked for 24 h with 2 L solvent, filtered, and concentrated using a rotatory evaporator. This procedure was then repeated three times. A portion of the extract (31 g) was subjected to column chromatography over silica gel (300 g) eluting with iso-hexane containing increasing amounts of EtOAc. The fraction that eluted with 3% EtOAc in iso-hexane was purified by
gel filtration on Sephadex LH-20 (eluent: CH₂Cl₂/MeOH; 1:1) to give 2 (16.2 mg, ≥97% purity) and 3 (23.4 mg, ≥97% purity). The eluent with 5% EtOAc in iso-hexane was first separated over Sephadex LH-20 (CH₂Cl₂/MeOH; 1:1) followed by preparative HPLC (20:80 MeOH/H₂O–100% MeOH gradient elution for 20 min with flow rate 8 mL/min) to give 5 (derrone, 28 mg, ≥98% purity) [24], 6 (glabranin, 52 mg, ≥98% purity) [25], 7 (obovatin methyl ether, 47 mg, ≥99% purity) [26], and 8 (genistein, 53 mg, ≥98% purity) [27]. Elution with 6% EtOAc in iso-hexane gave a yellow solid which was recrystallized from CH₂Cl₂/MeOH (1:1) to give 1 (550 mg, ≥99% purity). Further elution with 8% EtOAc in iso-hexane gave 4 (67.5 mg, ≥99% purity); the eluent with 9% EtOAc in iso-hexane gave 9 (tachrosin, 158 mg, >99% purity) [28], and the 10% EtOAc in iso-hexane eluent gave 10 (kaempferitin, 97 mg, >99% purity) [29]. Fraction elution with 15% EtOAc in iso-hexane gave 11 (d-pinitol, 650 mg, >99% purity) [30].

(E)-5-Hydroxytetraphostachin (1): Yellow crystals (CH₂Cl₂/MeOH; 1:1). mpt 160–162 °C. UV λmax (CH₂Cl₂): 230, 270 and 310 nm. ¹H- and ¹³C-NMR (Table 1). EIMS m/z (rel. int.) 353.6 [M]+ (100). HRMS [M]+ m/z 352.1315 C₂₁H₂₃O₅ (Calculated: 352.1311).

Purleptone (2): Colourless amorphous solid. UV λmax (CH₂Cl₂): 230, 290 and 330 nm. ¹H- and ¹³C-NMR (Table 1). EIMS m/z (rel. int.) 337 [M]+ (100). HRMS [M]+ m/z 336.0980 C₂₅H₁₆O₅ (Calculated: 336.0998).

(E)-5-Hydroxyanhydrotetraphostachin (3): Colourless amorphous solid. UV λmax (CH₂Cl₂): 230, 280 and 310 nm. ¹H- and ¹³C-NMR (Table 1). EIMS m/z (rel. int.) 336.1276 [M]+. HRMS [M + 1]+ m/z 335.1227 C₂₁H₁₈O₄ (Calculated: 335.1283).

Terpurlepflavone (4): White amorphous solid. m.p. 210–214 °C. UV λmax(CH₂Cl₂): 230, 260 and 310 nm. CD (MeOH) λ nm (Δε; M⁻¹cm⁻¹): (122.83)₂₁₂; (−58.17)₂₁₂. [α]D²⁰ +14.00° (c 0.001, MeOH). ¹H- and ¹³C-NMR (Table 2). EIMS m/z (rel. int.) 423 [M]+. HRMS [M + 1]+ m/z 423.1465 C₂₄H₂₂O₇ (Calculated: 423.1444). 3.4. In Vitro Antiplasmodial Activity

The pure compounds were assayed using a non-radioactive assay technique as described by Smilkstein et al., 2004 [32] with modifications given in the literature [12,33].

3.5. Cell Culture

A549, HepG2, and non-tumoral cells were all purchased from ATCC. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics penicillin (50 U/mL) and streptomycin (50 µg/mL; Invitrogen, Paisley, Scotland, UK). All cell cultures were incubated at 37 °C in a 5% humidified CO₂ incubator.

3.6. Cytotoxicity Assay

All tested compounds were dissolved in DMSO at a final concentration of 50 mmol/L and stored at −20 °C before use. Cytotoxicity was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5.0 mg/mL) assay as previously described [34]. Briefly, 4 × 10⁴ cells per well were seeded in 96-well plates before drug treatments. After overnight culture, the cells were then exposed to different concentrations of selected compounds (0.039–100 µmol/L) for 72 h. Cells without drug treatment were used as the control. Subsequently, MTT (10 µL) solution was added to each well and incubated at 37 °C for 4 h followed by the addition of 100 µL solubilization buffer (10% SDS in 0.01 mol/L HCl) and overnight incubation. A₅₇₀ nm was then determined in each well on the next day. The percentage of cell viability was calculated using the following formula: Cell viability (%) = A_treated/A_control × 100. Data were obtained from three independent experiments and the standard error was calculated.
4. Conclusions

Four new prenylflavones with seven known compounds were isolated from the stem of *Tephrosia purpurea* subsp. *leptostachya*. The isolated flavones were tested for antiplasmodial activity against the D6 strain of *Plasmodium falciparum*. Among these, (E)-5-hydroxytephrostachin (1) showed good activity (IC$_{50}$ 1.7 µM). The compounds were also tested for cytotoxicity against two non-tumoral and two cancerous cell-lines. Most of these did not show cytotoxicity (IC$_{50}$ > 100 µM), while compound 1 showed IC$_{50}$ between 21–100 µM.

**Supplementary Materials:** The Supplementary Materials are available online. NMR, UV and MS spectra for all new compounds and spectral data for the known compounds are available as Supporting Information.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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