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Sesquiterpene Lactones from *Vernonia cinerascens* Sch. Bip. and Their in Vitro Antitrypanosomal Activity

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Abstract: In the endeavor to obtain new antitrypanosomal agents, particularly sesquiterpene lactones, from Kenyan plants of the family Asteraceae, *Vernonia cinerascens* Sch. Bip. was investigated. Bioactivity-guided fractionation and isolation in conjunction with LC/MS-based dereplication has led to the identification of vernodalol (**1**) and isolation of vernodalin (**2**), 11 β ,13-dihydrovernodalol (**3**), 11 β ,13-dihydrovernolide (**4**), vernolide (**5**), 11 β ,13-dihydrohydroxyvernolide (**6**), hydroxyvernolide (**7**), and a new germacrolide type sesquiterpene lactone vernocinerascoside (**8**) from the dichloromethane extract of *V. cinerascens* leaves. Compounds **3**–**8** were characterized by extensive analysis of their 1D and 2D NMR spectroscopic and HR/MS spectrometric data. All the compounds were evaluated for their in vitro biological activity against bloodstream forms of *Trypanosoma brucei rhodesiense* and for cytotoxicity against the mammalian cell line L6. Vernodalin (**2**) was the most active compound with an IC₅₀ value of 0.16 μ M and a selectivity index of 35. Its closely related congener 11 β ,13-dihydrovernodalol (**3**) registered an IC₅₀ value of 1.1 μ M and a selectivity index of 4.2.

Keywords: Asteraceae; sesquiterpene lactones; *Vernonia cinerascens*; *Trypanosoma brucei rhodesiense*; antitrypanosomal activity

1. Introduction

Human African trypanosomiasis (HAT or sleeping sickness), Chagas disease, and leishmaniases are some of the most neglected diseases among the eighteen neglected tropical diseases according to WHO [1,2]. The mentioned diseases are caused by protozoan parasites of the trypanosomatid family, order kinetoplastida, namely *Trypanosoma brucei* subsp., *Trypanosoma cruzi*, and *Leishmania* spp, respectively, which are transmitted by insect vectors [3–6]. Millions of people are affected so these diseases are a big public health concern, and more since the affected populations form a non-lucrative sector for drug development [5–8]. Moreover, the available drugs for the treatment of these infections, which were developed more than 50 years ago, are highly toxic, have a poor efficacy, and exhibit increasing drug resistance [4,5]. Therefore, new drugs are required; in particular, those that are appropriate for resource-limited rural health systems. Natural products have, in many instances, shown high activity against the mentioned parasites [9,10].

Plants of the family Asteraceae are a rich source of sesquiterpene lactones (STLs) [11–17], which have been reported to have a wide range of biological activity such as anti-inflammatory, cytotoxic, antiplasmodial, and antitrypanosomal, among others [18–23]. Previous work in our group has shown that STLs are good antiprotozoal agents, particularly against *T. brucei rhodesiense*, the causative agent

of the East African form of HAT, and therefore, could be of interest for drug discovery [17,24–26]. Very recently, we reported on antitrypanosomal STLs from *Vernonia lasiopis* with vernolepin as the most active compound from this plant [15]. In continuation of the study on STLs from this interesting plant genus with potential antitrypanosomal activity, we have now investigated *Vernonia cinerascens* Sch. Bip. (Asteraceae, subfamily Cichorioideae, tribe Vernonieae), which is an erect, highly branched annual herb that is 0.3–2 m tall [27,28]. It is native to Africa (Sudan, Ethiopia, Uganda, Kenya, Tanzania, Angola, Zimbabwe, Botswana, Namibia, South Africa) and Tropical Asia (India) [27]. It is used ethnomedicinally to treat urinary tract infections, male sterility, internal ulcers, constipation, navel aches, and gastritis [29,30]. Hirsutinolide type STLs have previously been reported from this plant [30,31], in addition to vanillic acid, isoferulic acid, caffeic acid, methyl gallate, uridine, 3'-methylquercetin, and quercetin [32]. We report herein on the antitrypanosomal activity of the dichloromethane leaf extract of this plant and its isolated constituents.

2. Results and Discussion

The dichloromethane (CH₂Cl₂) extract obtained from leaves of *V. cinerascens* was tested for in vitro antiprotozoal activity against *Trypanosoma brucei rhodesiense* (*Tbr*) (STIB 900 strain) trypomastigotes, *T. cruzi* (*Tcr*) (Tulahuen C4 strain) amastigotes, *Leishmania donovani* (*Ldon*) (MHOM-ET-67/L82 strain) axenic amastigotes, and *Plasmodium falciparum* (*Pf*) (NF54 strain) intra-erythrocytic forms. It was also evaluated for in vitro cytotoxicity against mammalian cells, specifically the rat-skeletal myoblast cell line L6. The extract was highly potent against *Tbr* with an IC₅₀ value of 0.24 µg/mL and moderately active against *Ldon* as well as *Pf* with IC₅₀ values of 4.0 and 5.0 µg/mL, respectively. The extract exhibited low activity against *Tcr* with an IC₅₀ value of 16.4 µg/mL, but was moderately cytotoxic with an IC₅₀ value of 6.1 µg/mL against the L6 cell line. Therefore, the CH₂Cl₂ extract was fractionated by silica gel column chromatography and representative fractions were subjected to bioactivity testing against *Tbr* (STIB 900 strain) trypomastigotes. Fractions F5, F6, F8, and F9 showed IC₅₀ values of 3.4, 1.4, 0.17, and 2.1 µg/mL, respectively.

The most active Fraction, F8, was analyzed by UHPLC/+ESIQTOFMS/MS and vernodalol (1), vernodalin (2), and 11β,13-dihydrovernodalol (3) were dereplicated (see Figure S1). These compounds had been isolated from *V. lasiopis* in our previous study and shown to have in vitro anti*Tbr* activity with IC₅₀ values of 0.26 µM and 0.07 µg/mL for compound 1 and a mixture of compounds 2 and 3 (in the ratio of 2:1), respectively, which could not be further separated due to the low amount obtained [17]. In the present study, vernodalin (2) and 11β,13-dihydrovernodalol (3) could be isolated in an attempt to determine their individual bioactivity. Through preparative purification of F6 and F9, compounds 4–8 were obtained. The structures of all isolated compounds were unambiguously confirmed from their NMR spectroscopic data and HR/MS data (obtained by UHPLC/+ESIQTOFMS/MS analysis). Except for the new compound 8, vernolide (4) [33], 11β,13-dihydrovernolide (5) [31,33], hydroxyvernolide (6) [31], and 11β,13-dihydrohydroxyvernolide (7) [31] were identified, and all analytical data obtained were in full agreement with those reported in literature.

Vernocinerascolide (8) was obtained as a colorless amorphous solid and determined to have the molecular formula C₁₉H₂₂O₈ by (+)-ESIQTOFMS/MS and was readily identified as a derivative of the germacrolide hydroxyvernolide 6 [28]. The ¹H and ¹³C NMR spectra of 8 were very similar to those of 6. However, in 8, there was a carbonyl group (δ_C 195.9/δ_H 9.52) characteristic of a formyl functional group. The C-15 (δ_C 195.5) formyl group was assigned based on its HMBC correlations with H-3 and H-5 (Table 1). Moreover, HMBC correlations of H-15 (δ_H 9.52) with C-3 and C-4 were observed. Additionally, in 8, there was an oxymethylene (δ_C 66.3/δ_H 4.07; 3.81) which replaced the doubly oxygenated C-14 methine in 6. This indicated that the C-14-O bond on the C-14-O-C-15 bridge was cleaved in 8. The β orientations of C-14, H-6, and H-8 were supported by NOESY correlations among H-14, H-6, and H-8. Similarly, the NOESY correlation between H-1 and H-7 affirmed the α orientation of H-1 and H-7. Thus, compound 8 was unambiguously assigned the depicted structure (Figure 1). We propose the generic name vernocinerascolide for this new germacrolide.

Table 1. NMR spectroscopic data of compound **8** (150 and 600 MHz, CDCl₃).

Position	δ_C	δ_H (multi., J in Hz)	HMBC
1	64.5, CH	2.75, dd (9.8, 4.9)	2, 3, 10
2	28.4, CH ₂	α 2.24, ddt [tt] (13.9, 4.8) β 1.58, m	3, 10
3	23.37, CH ₂	2.51, td (13.5, 4.8)	1, 2, 4, 5, 15
4	144.9, C		
5	149.3, CH	6.35, dd (10.6, 1.0)	3, 4, 7, 15
6	76.8, CH	6.10, dd (10.6, 1.5)	4, 7, 8, 11, 12
7	52.7, CH	3.01, ddd [dq] (9.9, 1.7)	5, 8, 9, 11, 12, 13
8	72.3, CH	5.58, ddd (11.5, 9.9, 3.4)	1'
9	47.2, CH ₂	β 2.83, m α 1.43, ddd (13.2, 11.5, 1.4)	1, 7, 8, 10, 14
10	60.0, C		
11	135.8, C		
12	171.2, C		
13	131.4, CH ₂	a 6.43, d (1.7) b 5.78, d (1.6)	7, 8, 11, 12
14	66.3, CH ₂	4.07, dd (11.0, 0.8) 3.81, dd (11.0, 1.4)	1, 9, 10
15	195.9, CH	9.52, d (1.0)	3, 4
1'	167.4, C		
2'	141.8, C		
3'	128.9, CH ₂	a 6.21, dt [q] (0.9) b 5.89, dt [q] (1.3)	1', 2', 4'
4'	64.9, CH ₂	4.29, dd [t] (1.1)	1', 2', 3'

All isolated STLs were tested in vitro for activity against *Tbr*. Vernodalin (**2**) was the most active with an IC₅₀ value of 0.16 μ M and a selectivity index (SI) of 35. The 11 β ,13-dihydro derivative of vernodaline, **3**, had a reduced inhibition activity with an IC₅₀ value of 1.1 μ M. The reduction in activity can be attributed to the loss of one of the Michael acceptor systems upon hydrogenation of the C-11-C-13 exomethylene group. The Michael acceptors, α,β -unsaturated carbonyl groups, have been shown to alkylate biological macromolecules, thus inhibiting their normal functions [20,24,25]. These groups are generally held responsible for the high and wide range of biological activities of most STLs and it has been shown for other examples that the presence of more than one such system in the structure can dramatically enhance the activity [20,24,25]. The contribution of these groups to the biological activity of STLs is further corroborated by a comparison of the potency of vernolide (**5**) and 11 β ,13-dihydrovernolide (**4**), which had IC₅₀ values of 0.50 and 17 μ M, respectively. Similarly, hydroxyvernolide (**7**) and its congener 11 β ,13-dihydrohydroxyvernolide (**6**) registered IC₅₀ values of 5.0 and 15 μ M, respectively. The new germacrolide vernocinerascolide (**8**) had a moderate IC₅₀ value of 4.8 μ M and an SI of 27 (Table 2).

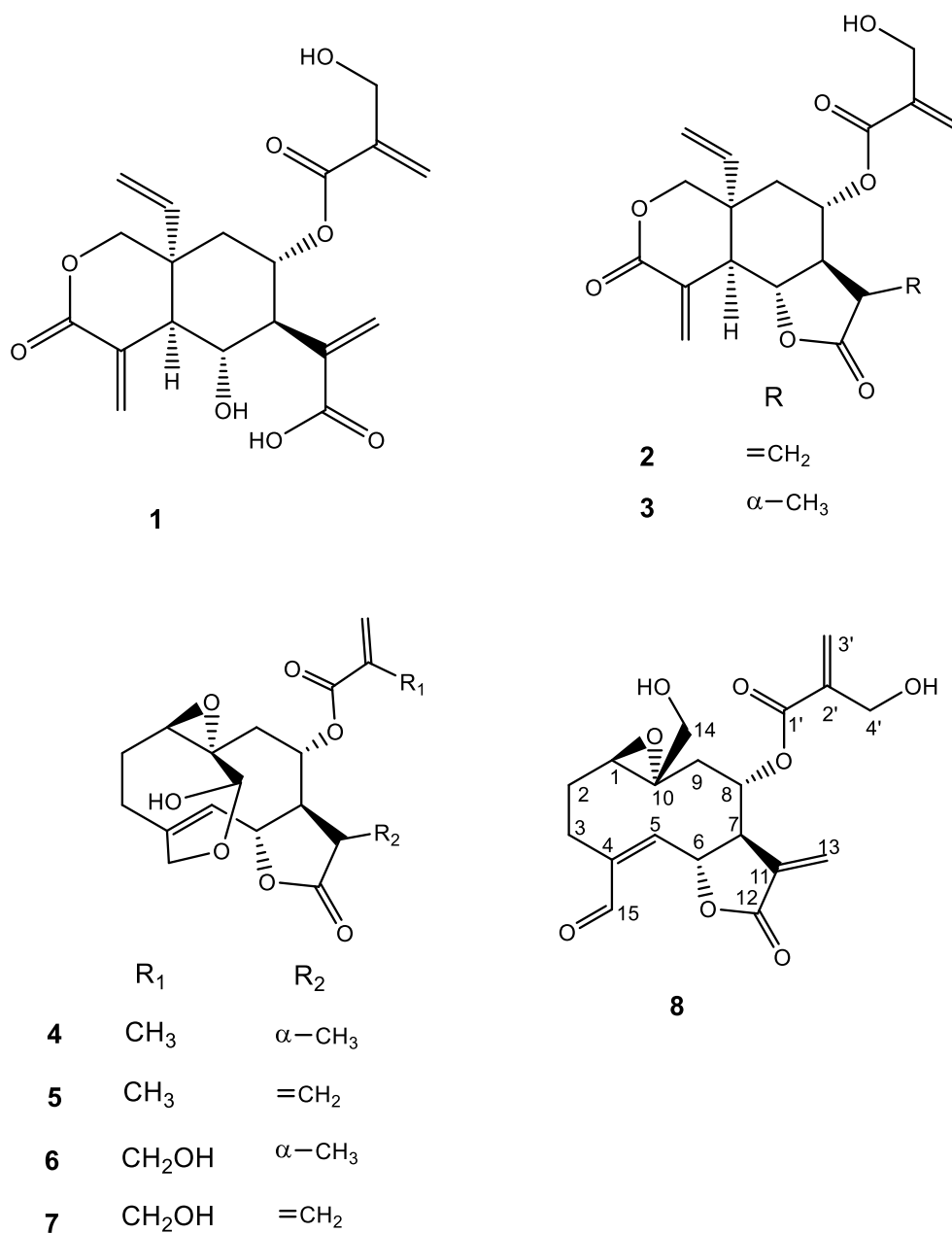


Figure 1. Sesquiterpene lactones isolated from *V. cinerascens*.

Table 2. In vitro antitrypanosomal and cytotoxic activity IC₅₀ values of the isolated compounds 2–8^a.

Compound	<i>Tbr</i> (μM)	Cytotoxicity (μM)	SI
2	0.16 ± 0.04	5.6 ± 0.0	35
3	1.1 ± 0.3	4.7 ± 0.4	4.2
4	17 ± 0	13 ± 2	0.8
5	0.50 ± 0.01	6.9 ± 0.0	13
6	15 ± 0	49 ± 1	3.2
7	5.0 ± 0.0	22 ± 1	4.3
8	4.8 ± 1.1	128 ± 1	27
PC ^b	0.003 ± 0.001	0.007 ± 0.001	

^a Data are means of two independent determinations ± absolute deviation; for activity of compound 1, see [15].

^b PC—Positive control: melarsoprol (*Tbr*), and podophyllotoxin (cytotox. L6).

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotation was measured on a JASCO P-2000 polarimeter (Groß-Umstadt, Germany). NMR spectra were recorded on a 600 MHz Agilent DD2 NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA) at 298 K in CDCl₃. The solvent signals (¹H: 7.260 ppm and ¹³C: 77.000 ppm) were used to reference the spectra. MestReNOVA v. 11 (Mestrelab Research, Chemistry Software Solutions, Santiago de Compostela, Spain) software was used to process and evaluate the spectra. HRESIMS spectra were obtained on a UHPLC/+ESIQTOFMS/MS instrument with a Bruker Daltonics micrOTOF-QII quadrupole/time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) with an Apollo electrospray ion source operated in positive ionization mode. A comprehensive description of the UHPLC/+ESIQTOFMS/MS instrument settings and elution systems has been given elsewhere [17]. TLC was performed on precoated silica gel plates 60 F₂₅₄ (Merck Chemicals GmbH, Darmstadt, Germany) with solvent systems consisting of hexane and ethyl acetate as the mobile phase. The plates were visualized under UV light at 254/360 nm and then sprayed with anisaldehyde/sulfuric acid reagent and heated on a hot plate. Column chromatography was performed on silica gel 60, 0.063–0.2 mm (Macherey-Nagel, Düren, Germany). Preparative HPLC isolations were carried out on a JASCO (Groß-Umstadt, Germany) preparative HPLC system (pump: PU-2087 plus; diode array detector MD 2018 plus; column thermostat CO 2060 plus; autosampler AS 2055 plus; LC Net II ADC Chromatography Data Solutions; sample injection loop: 2000 µL) on a preparative reversed-phase column Reprosil 100 C₁₈ (5 µm, 250 mm × 20 mm, Macherey-Nagel, Düren, Germany) with binary gradients of the mobile phase consisting of water and MeOH.

3.2. Plant Material

The plant material of *V. cinerascens* was collected and identified in April 2016 in Narok (1.0768° S, 35.9533° E), Kenya, by S.T. Kariuki, a taxonomist at the Biological Sciences Department, Egerton University, Kenya. A voucher specimen has been deposited at the Institute of Pharmaceutical Biology and Phytochemistry, University of Muenster, Germany (voucher number Kimani, 04). The sample was dried in the shade at ambient temperature to constant weight and then ground.

3.3. Extraction and Isolation

The powdered plant material (600 g) was exhaustively extracted with CH₂Cl₂ (4.5 L) in a soxhlet apparatus and evaporated under vacuum, yielding 20.34 g of crude extract. The extract was tested for in vitro activity against *Tbr*, *Tcr*, *Ldon*, and *Pf*. The extract was chromatographed on a silica gel column (800 g) and eluted with hexane (2 L) to afford hexane fraction. The column was further eluted with hexane-EtOAc (3:2, 1:1, 3:7, 1:2, 0:10, *v/v*) to yield nine fractions (F1–F9). The fractions were analyzed by UHPLC/+ESIQTOFMS/MS and representative fractions subjected to bioactivity testing. Fraction F6 (86.3 mg) was separated by preparative HPLC with an optimized mobile phase composed of water (A) and methanol (B) using the following binary gradient conditions: 50% of B (0–5 min), 50–55% of B (5–10 min), 55–60% of B (10–15 min), 60–70% of B (15–20 min), 70–80% of B (20–25 min), 80–100% of B (25–30 min) which was held for 5 min, 100–50% of B (45–40 min) which was held for a further 4 min, and a flow rate of 9 mL/min was maintained. This yielded compounds 4 (3.7 mg; *t*_R: 23.83–24.90 min) and 5 (6.4 mg; *t*_R: 25.23–25.8 min). Upon analysis of fraction F8 by UHPLC/+ESIQTOFMS/MS, compounds 1–3 were dereplicated. In order to isolate compounds 2 and 3, this fraction (99.2 mg) was separated using silica gel 60 column chromatography with hexane-EtOAc (3:7, *v/v*) as the mobile phase to yield two subfractions F8₁–F8₂. Subfraction F8₂ (33.1 mg) was then separated by preparative HPLC using an optimized binary gradient mobile phase composed of water (A) and methanol (B) as follows: 40% of B (0–10 min), 40–45% of B (10–15 min), 45–50% of B (15–20 min) which was held for a further 5 min, 50–100% of B (25–30 min) which was held for 5 min, and 100–40% of B (45–40 min) which was held for 4 min. The flow rate was maintained at 10 mL/min. This yielded compounds 2

(4.2 mg; t_R : 23.35–24.00 min) and **3** (3.7 mg; t_R : 25.00–26.30 min). Similarly, the separation of fraction F9 (22 mg) by preparative HPLC, using an optimized binary gradient mobile phase composed of water (A) and methanol (B), occurred as follows: 40% of B (0–10 min), 40–45% of B (10–15 min), 45–50% of B (15–20 min) which was held for a further 5 min, 50–100% of B (25–30 min) which was held for 5 min, and 100–40% of B (45–40 min) which was held for 4 min at a flow rate of 10 mL/min, gave compounds **6** (3.7 mg; t_R : 14.03–15.10 min), **7** (3.5 mg; t_R : 11.57–12.93 min), and **8** (2.8 mg; t_R : 9.13–9.84 min).

3.4. Analytical Data

Vernocinerascolide **8**: Colorless amorphous solid; $[\alpha]_D^{18} +28$ (c 0.1, MeOH); ^1H and ^{13}C NMR (CDCl_3), see Table 1; (+)HRESIMS m/z 379.3822 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{23}\text{O}_8$, 379.1393); 396.4218 $[\text{M} + \text{NH}_4]^+$ (calcd for $\text{C}_{19}\text{H}_{26}\text{NO}_8$, 396.1658); 401.3785 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{22}\text{O}_8\text{Na}$, 401.1212); UHPLC/+ESIQTOFMS t_R : 5.33 min.

3.5. In Vitro Bioassays

In vitro bioactivity tests were performed as previously described [24] against *Tbr* (bloodstream trypomastigotes, STIB 900 strain), *Tcr* (amastigotes, Tulahuen C4 strain), *Ldon* (axenic amastigotes, MHOM-ET-67/L82 strain), and *Pf* (intraerythrocytic forms, NF54 strain), and the cytotoxicity test was conducted against mammalian cells (L6 cell line from rat-skeletal myoblasts) at the Swiss Tropical and Public Health Institute (Swiss TPH, Basel, Switzerland).

4. Conclusions

In summary, the search for novel antiprotozoal agents from *V. cinerascens* is described in this work. The elemanolide type STLs vernodalol (**1**), vernodalin (**2**), and 11 β ,13-dihydrovernodalol (**3**) were dereplicated from the dichloromethane extract of *V. cinerascens* leaves. Moreover, isolation and the structure identification of a new germacrolide type STL vernocinerascolide (**8**), together with four known compounds (**4–7**) of a similar type, are described. These compounds were obtained from the leaves of *V. cinerascens* for the first time. In addition, compounds **2–8** displayed strong or moderate antitrypanosomal activity against *Tbr* blood stream forms and compound **2** was the most active with an IC_{50} value of 0.16 μM and an SI value of 35.2. Due to this considerably high activity, this compound warrants further investigations such as in vivo and mechanism of action studies.

Supplementary Materials: The UHPLC/+ESIQTOFMS/MS chromatograms and spectra of compounds **1–3** and **8**, and 1D and 2D NMR spectra of compound **8** as supplementary Figures S1–S8 available online.

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Author Contributions: N.M.K. isolated and characterized the compounds and wrote the manuscript. R.B and M.K. performed the biological activity tests. T.J.S. and J.C.M. conceived and initiated the study. T.J.S. finalized the manuscript and supervised the entire work.

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

References

1. World Health Organization (WHO) Neglected Tropical Diseases. Available online: http://www.who.int/neglected_diseases/diseases/summary/en/ (accessed on 13 November 2017).
2. World Health Organization Neglected Tropical Diseases. *Lowest Caseload Recorded as the World Prepares to Defeat Sleeping Sickness*. Available online: http://www.who.int/neglected_diseases/news/HAT_lowest_caseload_recorded/en/ (accessed on 13 November 2017).

3. Feasey, N.; Wansbrough-Jones, M.; Mabey, D.C.W.; Solomon, A.W. Neglected tropical diseases. *Br. Med. Bull.* **2010**, *93*, 179–200. [[CrossRef](#)] [[PubMed](#)]
4. Büscher, P.; Cecchi, G.; Jamonneau, V.; Priotto, G. Human African trypanosomiasis. *Lancet* **2017**, *390*, 2397–2409. [[CrossRef](#)]
5. Field, M.C.; Horn, D.; Fairlamb, A.H.; Ferguson, M.A.J.; Gray, D.W.; Read, K.D.; De Rycker, M.; Torrie, L.S.; Wyatt, P.G.; Wyllie, S.; et al. Anti-trypanosomatid drug discovery: An ongoing challenge and a continuing need. *Nat. Rev. Microbiol.* **2017**, *15*, 217–231. [[CrossRef](#)] [[PubMed](#)]
6. Cavalli, A.; Bolognesi, M.L. Multi-target-directed ligands in the search for novel lead candidates against *Trypanosoma* and *Leishmania*. *J. Med. Chem.* **2009**, *52*, 7339–7359. [[CrossRef](#)] [[PubMed](#)]
7. Sutherland, C.S.; Yukich, J.; Goeree, R.; Tediosi, F. A literature review of economic evaluations for a neglected tropical disease: Human African trypanosomiasis (“Sleeping Sickness”). *PLoS Negl. Trop. Dis.* **2015**, *9*, 1–22. [[CrossRef](#)] [[PubMed](#)]
8. Keating, J.; Yukich, J.O.; Sutherland, C.S.; Woods, G.; Tediosi, F. Human African trypanosomiasis prevention, treatment and control costs: A systematic review. *Acta Trop.* **2015**, *150*, 4–13. [[CrossRef](#)] [[PubMed](#)]
9. Schmidt, T.J.; Khalid, S.A.; Romanha, A.J.; Alves, T.M.; Biavatti, M.W.; Brun, R.; Da Costa, F.B.; de Castro, S.L.; Ferreira, V.F.; de Lacerda, M.V. The Potential of Secondary Metabolites from Plants as Drugs or Leads against Protozoan Neglected Diseases—Part I. *Curr. Med. Chem.* **2012**, *19*, 2128–2175. [[CrossRef](#)] [[PubMed](#)]
10. Schmidt, T.J.; Khalid, S.A.; Romanha, A.J.; Alves, T.M.; Biavatti, M.W.; Brun, R.; Da Costa, F.B.; de Castro, S.L.; Ferreira, V.F.; de Lacerda, M.V. The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases—Part II. *Curr. Med. Chem.* **2012**, *19*, 2176–2228. [[CrossRef](#)] [[PubMed](#)]
11. Liu, Y.; Nugroho, A.E.; Hirasawa, Y.; Nakata, A.; Kaneda, T.; Uchiyama, N.; Goda, Y.; Shirota, O.; Morita, H.; Aisa, H.A. Vernodalidimers A and B, novel orthoester elemanolide dimers from seeds of *Vernonia anthelmintica*. *Tetrahedron Lett.* **2010**, *51*, 6584–6587. [[CrossRef](#)]
12. Herz, W.; Govindan, S.V. Eucannabinolide and other constituents of *Schkuhria virgata*. *Phytochemistry* **1980**, *19*, 1234–1236. [[CrossRef](#)]
13. Romo de vivar, A.; Perez, A.L.C.; Leon, C.; Delgado, G. 11,13-Dehydroeriolin, schkuhrioidin and schkuhriolid, germacranolides from *Schkuhria* species. *Phytochemistry* **1982**, *21*, 2905–2908. [[CrossRef](#)]
14. Zhang, L.; Shao, Y.-L.; Hua, L.; Li, Y.; Hamid-Hussain, S.; Arfan, M.; Gao, K. Guaianolides and elemanolides from *Vernonia anthelmintica*. *Phytochem. Lett.* **2014**, *7*, 14–18. [[CrossRef](#)]
15. Zenebe, M.M.; Dessie, K.B.; Hana, G.M.; Werkneh, A.A. Isolation, structural elucidation, and bioactivity studies of leaf extract of *Vernonia Amygdalina*. *Am. J. Appl. Chem.* **2015**, *3*, 14–20. [[CrossRef](#)]
16. Buskuhl, H.; De Oliveira, F.L.; Blind, L.Z.; De Freitas, R.A.; Barison, A.; Campos, F.R.; Corilo, Y.E.; Eberlin, M.N.; Caramori, G.F.; Biavatti, M.W. Sesquiterpene lactones from *Vernonia scorpioides* and their in vitro cytotoxicity. *Phytochemistry* **2010**, *71*, 1539–1544. [[CrossRef](#)] [[PubMed](#)]
17. Kimani, N.M.; Matasyoh, J.C.; Kaiser, M.; Brun, R.; Schmidt, T.J. Anti-trypanosomatid elemanolide sesquiterpene lactones from *Vernonia lasiopus* O. Hoffm. *Molecules* **2017**, *22*, 597. [[CrossRef](#)] [[PubMed](#)]
18. Thao, N.P.; Luyen, B.T.T.; Brun, R.; Kaiser, M.; Van Kiem, P.; Van Minh, C.; Schmidt, T.J.; Kang, J.S.; Kim, Y.H. Anti-protozoal activities of cembrane-type diterpenes from Vietnamese soft corals. *Molecules* **2015**, *20*, 12459–12468. [[CrossRef](#)] [[PubMed](#)]
19. Schmidt, T.J. Structure-activity relationships of sesquiterpene lactones. In *Studies in Natural Products Chemistry*; ur Rahman, A., Ed.; Elsevier: Amsterdam, The Netherlands, 2006; Volume 33, pp. 309–392.
20. Schmidt, T.J. Helenanolide-type sesquiterpene lactones—III. Rates and stereochemistry in the reaction of helenalin and related helenanolides with sulfhydryl containing biomolecules. *Bioorg. Med. Chem.* **1997**, *5*, 645–653. [[CrossRef](#)]
21. Schmidt, T.J. Toxic activities of sesquiterpene lactones: Structural and biochemical aspects. *Curr. Org. Chem.* **1999**, *3*, 577–608.
22. Ghantous, A.; Gali-Muhtasib, H.; Vuorela, H.; Saliba, N.A.; Darwiche, N. What made sesquiterpene lactones reach cancer clinical trials? *Drug Discov. Today* **2010**, *15*, 668–678. [[CrossRef](#)] [[PubMed](#)]
23. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. Sesquiterpenoids lactones: Benefits to plants and people. *Int. J. Mol. Sci.* **2013**, *14*, 12780–12805. [[CrossRef](#)] [[PubMed](#)]
24. Schmidt, T.J.; Nour, A.M.M.; Khalid, S.A.; Kaiser, M.; Brun, R. Quantitative structure—Antiprotozoal activity relationships of sesquiterpene lactones. *Molecules* **2009**, *14*, 2062–2076. [[CrossRef](#)] [[PubMed](#)]

25. Schmidt, T.J.; Da Costa, F.B.; Lopes, N.P.; Kaiser, M.; Brun, R. In silico prediction and experimental evaluation of furanoheliangolide sesquiterpene lactones as potent agents against *Trypanosoma brucei rhodesiense*. *Antimicrob. Agents Chemother.* **2014**, *58*, 325–332.
26. Kimani, N.M.; Matasyoh, J.C.; Kaiser, M.; Brun, R.; Schmidt, T.J. Antiprotozoal sesquiterpene lactones and other constituents from *Tarchonanthus camphoratus* and *Schkuhria pinnata*. *J. Nat. Prod.* **2017**. [CrossRef]
27. *Vernonia cinerascens* in Global Plants on JSTOR. Available online: <http://plants.jstor.org/compilation/vernonia.cinerascens> (accessed on 3 December 2017).
28. Verma, S.C.; Sharma, N.K.; Sharma, J.L. Systematic survey of some angiosperms of family Asteraceae from Kota district of Rajasthan, India-II. *Int. J. Sci. Nat.* **2014**, *5*, 183–185.
29. Hussain, A.; Khan, M.N.; Iqbal, Z.; Sajid, M.S. An account of the botanical anthelmintics used in traditional veterinary practices in Sahiwal district of Punjab, Pakistan. *J. Ethnopharmacol.* **2008**, *119*, 185–190. [CrossRef] [PubMed]
30. Abdel-Sattar, E.; Mossa, J.S.; El-Askary, H.I. Hirsutinolides from *Vernonia cinerascens*. *Pharmazie* **2000**, *55*, 144–145. [PubMed]
31. Jakupovic, J.; Zdero, C.; Boeker, R.; Warning, U.; Bohlmann, F.; Jones, S.B. Vernocistifolide und andere Sesquiterpenlactone aus *Vernonia* und verwandten Arten. *Liebigs Ann. Chem.* **1987**, *1987*, 111–123.
32. Ahmad, I.; Chaudhary, B.A.; Ashraf, M.; Uzair, M.; Hussain Janbaz, K. Vernonione, a new urease inhibitory carvotacetone derivative from *Vernonia cinerascens*. *J. Chem. Soc. Pak.* **2012**, *34*, 639–642.
33. Rabe, T.; Mullholland, D.; Van Staden, J. Isolation and identification of antibacterial compounds from *Vernonia colorata* leaves. *J. Ethnopharmacol.* **2002**, *80*, 91–94. [CrossRef]

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