JOURNAL OF NATURAL PRODUCTS

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Article

Using a Bioactive *Eremophila*-Derived Serrulatane Scaffold to Generate a Unique Carbamate Library for Anti-infective Evaluations

Chen Zhang, Kah Yean Lum, Aya C. Taki, Robin B. Gasser, Joseph J. Byrne, Luis J. Montaner, Ian Tietjen, Vicky M. Avery, and Rohan A. Davis*

Cite This: https://doi.org/10.1021/acs.jnatprod.2c01041

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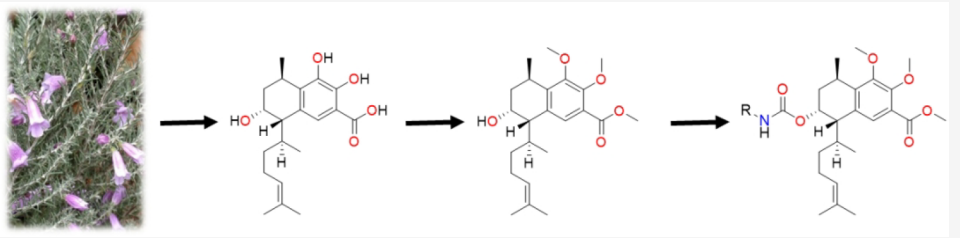

ABSTRACT: The known *Eremophila microtheca*-derived diterpenoid 3,7,8-trihydroxyserrulat-14-en-19-oic acid (**1**) was targeted for large-scale purification, as this bioactive plant compound has proven to be an attractive scaffold for semisynthetic studies and subsequent library generation. Compound **1** was converted to a selectively protected trimethyl derivative, 3-hydroxy-7,8-dimethoxyserrulat-14-en-19-oic acid methyl ester (**2**), using simple and rapid methylation conditions. The resulting scaffold **2** was reacted with a diverse series of commercially available isocyanates to generate an 11-membered carbamate-based library. The chemical structures of the 11 new semisynthetic analogues were fully characterized by spectroscopic and spectrometric analysis. All natural products and semisynthetic compounds were evaluated for their anthelmintic, antimalarial, and anti-HIV activities. Compound **3** was shown to elicit the greatest antiplasmodial activity of all compounds tested, with IC_{50} values of 4.6 and 11.6 μM against *Plasmodium falciparum* 3D7 and Dd2, respectively. Compound **11** showed the greatest inhibition of development to fourth-stage *Haemonchus contortus* larvae (L4) and induction of a skinny (*Ski*) phenotype (67.5% of nematodes) at 50 μM. Compound **7**, which inhibited 59.0% of HIV production at 100 μg/mL, was the carbamate analogue that displayed the best antiviral activity.

Over the millennia, natural products have been an important source of medicines for many diseases. During the past 40 years, approximately 40% of therapeutic drugs approved by the U.S. Food and Drug Administration (FDA) were either natural products, natural products derivatives, or natural products-related synthetic mimetics.[1] A strategy that has been used for some time now in natural products drug discovery is the use of a purified natural scaffold for medicinal chemistry and subsequent generation of unique screening libraries.[2] Scaffolds in this context are structural elements found in nature that contain mono- or multifunctional groups, which can be used as chemical handles for the semisynthesis of chemically diverse libraries. Numerous examples that use this approach exist in the literature and include libraries based on the natural products piperine,[3,4] nidulin,[5,6] psammaplysin F,[7,8] bilocularin A,[9,10] leubethanol,[11−13] and platensimycin.[14,15]

The endemic Australian plant genus *Eremophila* has been a rich source of new chemistry over the past four decades.[16−23] Numerous secondary metabolites reported from this source have been shown to exhibit novel and significant bioactivity.[24−27] Several recent studies have shown how unique and rare natural products from this plant genus can be used as scaffolds for semisynthetic studies, which result in the generation of novel screening libraries that have applications to not only drug discovery but also chemical biology.[7,9,28−30] For example, the serrulatane skeleton has been reported to exhibit various biological activities such as antibacterial,[20] anticancer,[27,31] and antihyperglycemic.[32]

3,7,8-Trihydroxyserrulat-14-ene-19-oic acid (**1**), previously isolated from *Eremophila microtheca* aerial parts, has been identified as an attractive scaffold for structural modification due to its natural abundance and array of functional groups that could potentially serve as chemical handles for medicinal chemistry efforts.[29] To date, two screening libraries have been generated based on this scaffold. The first library was synthesized via a one-pot mixed anhydride amidation method that generated 10 pivaloylated amides that displayed moderate to low activity against the *Plasmodium falciparum* 3D7 (half-

Special Issue: Special Issue in Honor of Mary J. Garson

Received: November 16, 2022

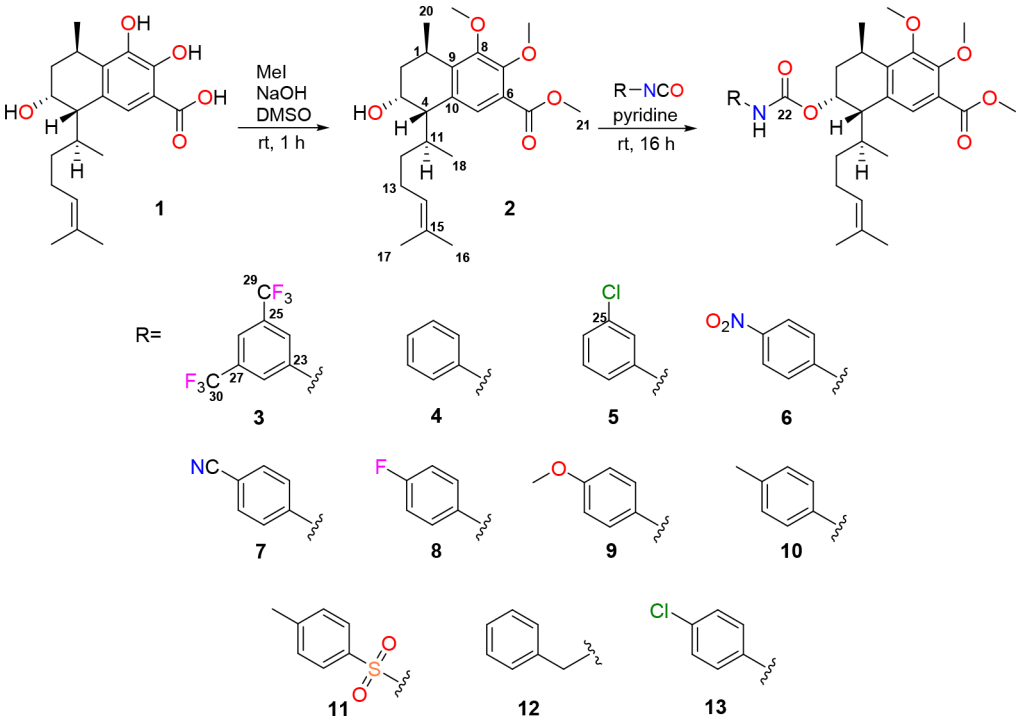

Figure 1. Reaction conditions used to generate the trimethyl-protected serrulatane scaffold (**2**) and the new serrulatane carbamate library (**3**−**13**).

maximal inhibitory concentration (IC_{50}) = 1.3−5.7 μM).[29] As these amides exhibited improved biological activity compared to the natural product scaffold **1**, a 12-membered amide library was then synthesized by converting natural product **1** to a trimethylated derivative **2**, which was subsequently reacted with a series of primary amines using a high-yielding aminolysis reaction. This library was tested for anthelmintic, antimicrobial, and antiviral activities. The trimethylated product **2** induced a skinny (*Ski*) phenotype in 78.4% of the fourth-stage larvae (L4s) of the parasitic nematode *Haemonchus contortus* at 100 μM (IC_{50} = 13.5 μM) and reduced the midbody width of L4 larvae to 8.8 ± 1.5 μm (untreated = 16.3 ± 1.5 μm).[30] Furthermore, testing of the library for activity against HIV latency reversal in inducible, chronically infected cells showed that **2** was the most active compound with a half-maximal effective concentration (EC_{50}) of 38 μM.[30]

Based on these encouraging data, we decided to synthesize a second-generation semisynthetic library using scaffold **2** to map structure activity relationships (SAR) around the secondary alcohol at C-3; none of the previous semisynthetic studies had involved analogue generation at the C-3 position. In recent years, carbamate derivatives have received considerable attention and played an important role in modern drug discovery and medicinal chemistry due to their chemical and proteolytic stability, ability to penetrate cell membranes, and similarity to peptide bond properties.[33] Carbamate stability originates from the resonance between the amide and carboxyl groups and has been investigated theoretically and experimentally by estimating the rotational barrier of the C−N bond.[33] Carbamates are found in many drugs or prodrugs approved by the FDA and the European Medicines Agency (EMA). Examples include cenobamate used for the treatment of partial-onset seizures in adults and mebendazole used to treat a broad range of parasitic infections, which were approved by the FDA in 2019 and 1974, respectively.[34,35] Additionally, carbamate groups also make up key structural motifs of many compounds that are in various stages of preclinical and clinical trials.[33] Due to our recent success with the semisynthesis of carbamate libraries with other natural product scaffolds,[9,28] we embarked on a project that aimed to generate a new carbamate library using the trimethylated serrulatane scaffold **2**.

All new semisynthetic compounds were fully characterized using spectroscopic and spectrometric techniques. The resulting carbamate library was evaluated for anthelmintic activity using the pathogenic ruminant parasite *Haemonchus contortus*, antiplasmodial activity against *Plasmodium falciparum*, anti-HIV activity using a cell-line model of inducible and chronic infection, and mammalian cell cytotoxicity.

RESULTS AND DISCUSSION

To obtain sufficient quantities of the desired serrulatane scaffold, large-scale extraction and isolation studies of kilogram quantities of the aerial parts of *E. microtheca* were undertaken as detailed by Zhang et al.[30] Briefly, following a CH_2Cl_2 extraction of the aerial parts, a C_{18}-flash column was employed ($MeOH/H_2O$/0.1% TFA). All resulting fractions were analyzed by ^1H NMR and LC-MS to identify the scaffold of interest, 3,7,8-trihydroxyserrulat-14-en-19-oic acid (**1**). The 80% MeOH (0.1% TFA)/20% H_2O (0.1% TFA) eluate contained the targeted serrulatane scaffold (**1**) in greatest abundance and was thus prioritized for further purification using reversed-phase semipreparative HPLC (H_2O/MeOH/0.1%TFA) that ultimately afforded >1 g of the previously reported compound 3,7,8-trihydroxyserrulat-14-en-19-oic acid (**1**, >95% purity). Comparison of NMR, $[\alpha]_D$, and MS data with literature values confirmed this known compound had been successfully purified.[20]

With large quantities of 3,7,8-trihydroxyserrulat-14-en-19-oic acid (**1**) in hand, the trimethylated serrulatane (**2**) was subsequently generated using a previously developed method for simple and rapid permethylation (MeI, NaOH, DMSO, rt, 1 h) of phenol and carboxylic acid functional groups (see

https://doi.org/10.1021/acs.jnatprod.2c01041
J. Nat. Prod. XXXX, XXX, XXX−XXX

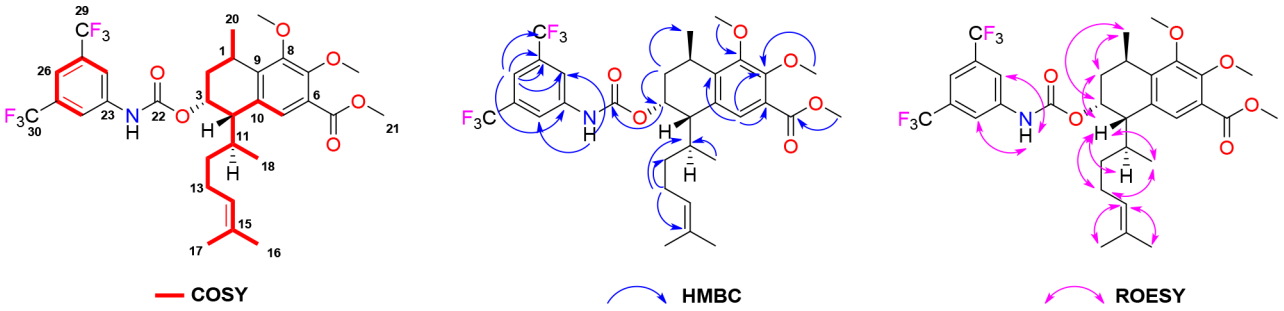

Figure 2. Key COSY, HMBC, and ROESY correlations of analogue **3**.

Figure 1).[30] The reaction afforded the desired partially protected serrulatane scaffold 3-hydroxy-7,8-dimethoxyserrulat-14-en-19-oic acid methyl ester (**2**, 98.8 mg, 22% yield, >95% purity), which was required for the planned C-3 focused carbamate library.

Scaffold **2** (20 mg) was subsequently reacted with a series of commercially available isocyanates (5−20 equiv) in dry pyridine at room temperature for 16 h. Following workup and HPLC purification, this resulted in a total of 11 new carbamate analogues (**3**−**13**) (see Figure 1) in yields ranging from 11% to 82% and purities of >95% as determined by UHPLC-MS analysis. Furthermore, all carbamate derivatives were fully characterized following 1D/2D NMR, UV, $[\alpha]_D$, ECD, LRESIMS, and HRESIMS data analysis.

For example, the (+)-HRESIMS spectrum of analogue **3** revealed an ion at *m*/*z* 668.2414 $[M + Na]^+$ that enabled a molecular formula of $C_{32}H_{37}F_6NNaO_6$ to be assigned to this carbamate analogue. The ^{1}H NMR spectrum of compound **3** in $CDCl_3$ indicated the presence of four aromatic protons [δ_H 7.91 (2H), 7.54, 7.28], one olefinic proton (δ_H 5.09), four methines (δ_H 5.40, 3.36, 3.13, 2.10), three methylenes (δ_H 2.25/1.75, 2.02, 1.50/1.30), and seven methyl signals (δ_H 3.90, 3.89, 3.88, 1.64, 1.54, 1.31, 0.63). The ^{13}C NMR and the edited HSQC spectrum of analogue **3** (Figures S2, S4) indicated a total of 29 unique carbons including seven methyls (δ_C 61.4, 60.8, 52.3, 25.7, 23.4, 19.4, 17.7), three methylenes (δ_C 37.4, 32.7, 26.2), four methines (δ_C 72.1, 45.8, 32.2, 28.8), two olefinic carbons (δ_C 131.9, 124.4), 10 aromatic carbons (δ_C 151.8, 151.5, 140.6, 139.7, 132.6, 132.5, 126.4, 123.3, 118.3, 116.7), two carbonyl carbon (δ_C 166.7, 152.7), and one CF_3 signal (δ_C 123.2, q, J = 273.3 Hz). The substitution of the hydroxyl group by a carbamate moiety was confirmed by a downfield shift of H-3 from δ_H 4.23 (observed in scaffold **2**) to δ_H 5.40 in **3**; a strong three-bond HMBC correlation from H-3 in compound **3** to a carbonyl at δ_C 152.7 (C-22) confirmed the substitution of the carbamate moiety at C-3 (Figure 2). The remaining NMR signals for carbamate **3** were assigned following comparison of the chemical shifts with the previously reported scaffold **2** and detailed analysis of the 2D NMR data of compound **3** (see Table 1 and Figure 2).

Based on some moderately active antimalarial amide analogues of serrulatane **1** (that we had previously reported during 2017[29]) and our interest in identifying other potential antimalarial compounds from natural sources, the natural product isolated from *E. microtheca* and the carbamate analogues generated in the current studies were screened for their antiplasmodial activities. All compounds (**1**−**13**) were tested against both *P. falciparum* 3D7 (chloroquine sensitive) and Dd2 (multidrug resistant) strains, and preliminary cytotoxicity data were acquired using the human embryonic kidney cell line HEK293 (Table 2). The parent molecule **1** demonstrated no activity at 10 μM, which was consistent with previous results,[29] while the trimethylated serrulatane **2** was also inactive. While the overall SAR data for the carbamate analogues are somewhat limited, moderate activity of eight analogues (**3**−**6**, **8**−**10**, **13**) was observed with IC_{50} values ranging from 4.6−12.2 μM and 9.2−13.2 μM against 3D7 and Dd2, respectively (see Table 2). The 3,5-bis(trifluoromethyl)-

Table 1. NMR Data for Compound 3 in $CDCl_3$[a]

position	δ_H (mult., J in Hz)	δ_C type
1	3.36 (ddq, 7.0, 2.2, 7.0)	28.8, CH
2a	2.25 (ddd, 13.0, 7.0, 5.0)	32.7, CH_2
2b	1.75 (ddd, 13.0, 3.8, 2.2)	
3	5.40 (ddd, 11.2, 5.0, 3.8)	72.1, CH
4	3.13 (dd, 4.1, 3.8)	45.8, CH
5	7.28 (s)	126.4, CH
6		123.3, C
7		151.5, C
7-OMe	3.88 (s)	61.4, CH_3
8		151.8, C
8-OMe	3.89 (s)	60.8, CH_3
9		140.6, C
10		132.5, C
11	2.10 (m)	32.2, CH
12a	1.50 (m)	37.4, CH_2
12b	1.30 (m)	
13	2.02 (ddd, 7.1, 7.1, 6.7)	26.2, CH_2
14	5.09 (br t, 7.1)	124.4, CH
15		131.9, C
16	1.64 (s)	25.7, CH_3
17	1.54 (s)	17.7, CH_3
18	0.63 (d, 7.0)	19.4, CH_3
19		166.7, C
20	1.31 (d, 7.0)	23.4, CH_3
21	3.90 (s)	52.3, CH_3
22-NH	7.19 (s)	
22		152.7, C
23		139.7, C
24	7.91 (s)	118.3[b], CH
25		132.6[c], C
26	7.54 (s)	116.7[d], CH
27		132.6[c], C
28	7.91 (s)	118.3[b], CH
29		123.2[e], C
30		123.2[e], C

[a]Spectra recorded at 25 °C (800 MHz for ^{1}H NMR and 200 MHz for ^{13}C NMR). [b]q, $^3J_{CF}$ = 3.8 Hz. [c]q, $^2J_{CF}$ = 33.7 Hz. [d]sept, $^3J_{CF}$ = 3.8 Hz. [e]q, $^1J_{CF}$ = 273.3 Hz.

https://doi.org/10.1021/acs.jnatprod.2c01041
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. Antimalarial Data for Compounds 1−13

compound	3D7[a] IC_{50} ± SD (μM)	Dd2[b] IC_{50} ± SD (μM)	Dd2/3D7 resistance index	HEK293[c] % inhibition at 80 μM	SI index HEK/3D7	SI index HEK/Dd2
1	[e]	[e]		8		
2	[d]	[d]		4		
3	4.6 ± 0.5	11.6 ± 4.8	2.5	34	17.5	6.9
4	11.6 ± 0.6	[d]	1.1	47	6.9	6.3
5	9.1 ± 2.4	9.2 ± 0.2	1.0	42	8.8	8.7
6	7.6 ± 2.1	10.6 ± 2.5	1.3	52	5.0	7.5
7	[e]	[e]		2		
8	8.7 ± 1.5	10.8 ± 1.7	1.2	25	9.2	7.4
9	9.3 ± 5.6	10.1 ± 3.6	1.1	26	8.6	7.9
10	12.2 ± 3.7	12.9 ± 4.5	1.1	12	6.6	6.2
11	[d]	[d]		11		
12	[d]	[d]		24		
13	11.7 ± 0.9	13.2 ± 0.7		3.1	6.9	6.1

compound	3D7[a] IC_{50}± SD (μM)	Dd2[b] IC_{50}± SD (μM)	Dd2/3D7 resistance index	HEK293 IC_{50} ± SD (μM)	SI index HEK/3D7	SI index HEK/Dd2
puromycin	0.0429 ± 0.0021	0.0814 ± 0.0071	1.9	0.6645 ± 0.029	15.5	8.2
dihydroartemisinin	0.0006 ± 0.0001	0.0013 ± 0.0005	2.2	>4	>6683	>3077
chloroquine	0.0219 ± 0.0052	0.5430 ± 0.0071	24.8	>10	>456	>18.4
pyrimethamine	0.0099 ± 0.0006	NT	>1010	>10	>1013	

[a]3D7 = *P. falciparum* (chloroquine sensitive strain). [b]Dd2 = *P. falciparum* (multidrug resistant strain). [c]HEK293 = human embryonic kidney cell line, approximate HEK293 IC_{50} used for selectivity. [d]>41% inhibition observed at the top dose of 80 μM. [e]<41% inhibition observed at the top dose of 80 μM. NT = not tested, SD = standard deviation, SI = parasite selectivity ratio (approximated HEK293 IC_{50}/parasite actual IC_{50}).

phenyl carbamate analogue (**3**) showed the strongest inhibition, with IC_{50} value of 4.6 μM against *P. falciparum* 3D7. Also, for this analogue, only 34% inhibition was observed against the HEK293 cell line at 80 μM. Hence, in this specific case, a natural product scaffold with no antimalarial activity was used to generate a series of new carbamate analogues, eight of which displayed moderate antiplasmodial activity and good selectivity.

As part of our ongoing research into the discovery of compounds or derivatives from nature that may hold promise as anthelmintic leads, we had previously identified that compound **2** induced a *Ski* phenotype in a portion (78.4%) of L4s after 7 days of treatment (IC_{50} = 14 μM),[30] but no significant inhibition of development of exsheathed third-stage larvae (xL3) of *H. contortus*. Thus, we decided to test the new semisynthetic analogues from this study for anthelmintic activity against *H. contortus*.[30,36] The screening of compounds **1**−**13** on xL3s of *H. contortus* indicated that almost all compounds reduced xL3 motility at 50 μM after 168 h, particularly compounds **3** and **12**, with IC_{50} values of 0.8 and 0.4 μM, respectively. Compound **11** showed the greatest inhibition of L4 development and was shown to induce a *Ski* phenotype in 67.5% of worms at 50 μM (see Table 3). Compounds **2** and **6** were also active, inducing a *Ski* phenotype in 59.1% and 60.0% of L4s, respectively.

Compounds were also assessed for their ability to suppress HIV induced from a latent cellular reservoir using the established J-Lat cell line model of HIV latency.[37] Briefly, J-Lat cells contain a latent, noninfectious HIV provirus with a green fluorescent protein (GFP) tag. Viral production is induced by treatment with proviral agents such as phorbol 12-myristate 13-acetate (PMA), which in turn can be inhibited by HIV suppressive agents and monitored in live cells by flow cytometry.[38] Using this assay, we previously observed that compound **2** could inhibit PMA (50 nM)-induced HIV production in J-Lat 10.6 cells with an EC_{50} of 38.3 μM.[30]

Table 3. Anthelmintic Data for Compounds 1−13[a]

compound	xL3 motility reduction, relative IC_{50} (μM)	xL3 motility reduction, at 50 μM (%)	L4 development inhibition, at 50 μM (%)	*Ski* phenotype induction, at 50 μM (%)
1	3.6	16.9	4.0	0
2	4.9	28.3	5.3	59.1
3	0.8	16.9	3.6	0
4	>50	9.6	4.0	0
5	1.6	39.1	7.1	0
6	6.4	30.4	4.8	60.0
7	9	6.8	12.0	1.7
8	31.6	36.2	3.2	0
9	>50	18.3	2.0	0
10	7.1	12.9	2.3	0
11	6.2	15.6	21.7	67.5
12	0.4	44.7	−4.4	0
13	1.8	26.4	2.5	0
monepantel	0.2	99.2	98.0	0
moxidectin	3	88.2	89.7	0

[a]The effect of *Eremophila*-derived compounds and two positive controls (monepantel and moxidectin) on exsheathed third-stage larvae (xL3s) of *Haemonchus contortus* on the larval motility, development to fourth-stage larvae (L4), and induction of a skinny (*Ski*) phenotype after 168 h of exposure.

Here, when J-Lat 10.6 cells were stimulated with 0.1 μg/mL PMA (~160 nM), we observed 33.6 ± 6.6% GFP-positive cells after 24 h of incubation (mean ± SEM). We further observed that co-incubation with test compounds at 100 μg/mL (corresponding to approximately 150−300 μM, depending on the compound) resulted in modest (<50%) inhibition of PMA-induced HIV production in most instances. The most active compounds were the positive control **2**, which inhibited 66.5 ± 14.8% (mean ± SEM) of HIV production at 100 μg/mL, and compound **7**, which inhibited 59.0 ± 14.6% of virus production. When these compounds were assessed in 2-fold

https://doi.org/10.1021/acs.jnatprod.2c01041
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serial dilutions, we determined EC_{50} values of 60.4 and 96.7 μg/mL, or 146.2 and 96.7 μM, for compounds **2** and **7**, respectively. The higher EC_{50} values observed here relative to previous results (i.e., 38.3 μM for compound **2**)[30] are consistent with competitive inhibition of PMA-induced provirus expression, as a roughly 3-fold higher concentration of PMA was used here for virus stimulation (i.e., 160 nM PMA used here vs 50 nM used previously).[30]

Table 4. Anti-HIV Data for Compounds 1−13

compound	average % block at 100 μg/mL (SEM)	EC_{50} (μM)
1	<10	
2	66.5 (14.8)	146.2
3	39.1 (11.3)	
4	29.3 (11.9)	
5	37.2 (6.6)	
6	43.5 (5.1)	
7	59.0 (14.6)	96.7
8	28.7 (25.7)	
9	22.9 (3.2)	
10	36.8 (10.7)	
11	20.7 (19.5)	
12	<10	
13	31.7 (3.1)	

In summary, the targeted diterpenoid scaffold **1** was isolated from the aerial parts of *E. microtheca*, converted to a trimethylated derivative **2**, then subsequently used to generate a new 11-membered carbamate library (**3**−**13**). The biological screening data of all 13 compounds identified that eight compounds displayed moderate antiplasmodial activity and good selectivity, especially the carbamate analogue **3**, which showed the strongest inhibition with IC_{50} values of 4.6 μM (3D7) and 11.6 μM (Dd2). For the anthelmintic study, almost all compounds reduced xL3 motility at 50 μM after 168 h. Compound **11** showed the greatest inhibition of development to L4s and was shown to induce a *Ski* phenotype in 67.5% of L4s at 50 μM. For the anti-HIV activity study, analogue **7**, which inhibited virus production with an EC_{50} of 96.7 μM, was determined to be the most active carbamate compared to the scaffold, which displayed an EC_{50} of 146.2 μM. These data identified scaffold **2** as a valuable starting point for the generation of unique carbamate analogues. These studies also further support the significance of the use of natural products scaffolds for screening library generation in drug discovery and chemical biology research.

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were determined on a JASCO P-2000 polarimeter. UV spectra were recorded using an Ocean Optics spectrometer. ECD spectra were obtained on a JASCO J-1500 spectropolarimeter and processed using SDAR software.[39] NMR spectra were recorded at 25 °C on a Bruker AVANCE III HD 500 or 800 MHz NMR spectrometer equipped with a cryoprobe. The ^{1}H and ^{13}C chemical shifts were referenced to solvent peaks for $CDCl_3$ at δ_H 7.26 and δ_C 77.16, respectively. LRESIMS data were recorded on an Ultimate 3000 RS UHPLC coupled to a Thermo Fisher Scientific MSQ Plus single quadruple ESI mass spectrometer. HRESIMS data were acquired on a Bruker maXis II ETD ESI-qTOF. GRACE Davisil (35−70 μm, 60 Å) C_{18} bonded silica was packed into an open glass column (65 × 40 mm) for C_{18} flash column chromatography. GRACE C_{18} bonded silica (35−70 μm, 60 Å) was used for preadsorption work before HPLC separations, and the preadsorbed sample was packed into a GRACE stainless steel guard cartridge (10 × 30 mm). A Thermo Fisher Scientific Dionex Ultimate 3000 UHPLC was used for semipreparative HPLC separations. A Thermo Betasil 5 μm C_{18} column (5 μm, 100 Å, 150 × 21.2 mm) was used for semipreparative HPLC separations. Merck silica gel 60 F_{254} precoated aluminum plates were used for thin layer chromatography (TLC) and analyzed under UV light at 254 and 365 nm. The air-dried aerial parts of *Eremophila microtheca* were ground using a Fritsch Universal Cutting Mill Pulverisette 19. The plant material was extracted at room temperature using an Edwards Instrument Company Bioline orbital shaker set to 200 rpm. All chemical reagents used for these studies were purchased from Sigma-Aldrich, and all solvents used for chromatography, UV, ECD, specific rotation, and MS measurements were Honeywell Burdick & Jackson or Lab-Scan HPLC grade. H_2O was filtered using a Sartorius Stedium Arium Pro VF ultrapure water system.

Plant Material. *Eremophila microtheca* (F. Muell. ex Benth.) F. Muell. aerial parts were collected on private property at Samford, Queensland, Australia, during March 2018. After air-drying for 1 month, the plant material was ground into a fine powder ready for extraction and isolation chemistry. A voucher specimen (RAD076) has been deposited at the Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia.

Extraction and Isolation. The air-dried and ground aerial parts of *E. microtheca* (1 kg) were extracted exhaustively with CH_2Cl_2 (6 × 1 L). These CH_2Cl_2 extracts were combined and dried under reduced pressure to yield a dark green amorphous powder (53 g). A portion of the CH_2Cl_2 extract (∼10 g) was preadsorbed to C_{18} bonded silica (∼10 g), then loaded onto a 10% MeOH (0.1% TFA)/90% H_2O (0.1% TFA) equilibrated C_{18}-bonded silica flash column (65 × 40 mm). The column was subsequently flushed with a 20% stepwise gradient from 10% MeOH (0.1% TFA)/90% H_2O (0.1% TFA) to 50% MeOH (0.1% TFA)/50% H_2O (0.1% TFA) (300 mL washes), followed by a 10% stepwise gradient to 100% MeOH (0.1% TFA) (300 mL washes), which resulted in eight fractions (F1−F8). LCMS and ^{1}H NMR experiments were used to analyze the eight fractions and revealed F6 contained the scaffold of interest. F6 (4.4 g) was further purified by a semipreparative C_{18} HPLC using a linear gradient from 40% MeOH (0.1% TFA)/60% H_2O (0.1% TFA) to 100% MeOH (0.1% TFA) over 60 min at a flow rate of 9 mL/min to give 3,7,8-trihydroxyserrulat-14-en-19-oic acid (**1**, 450 mg, t_R 24−30 min, 0.24% dry wt). This extraction and isolation procedure was repeated twice more during these studies to yield another portion (>1 g) of 3,7,8-trihydroxyserrulat-14-en-19-oic acid (**1**).

Methylation of 3,7,8-Trihydroxyserrulat-14-en-19-oic acid (1). 3,7,8-Trihydroxyserrulat-14-en-19-oic acid (**1**, 517 mg, 1.5 mmol) and NaOH (∼12 equiv, 720 mg, 18 mmol) were dissolved in DMSO (5 mL), followed by the slow addition of MeI (∼20 equiv, 1.8 mL, 30 mmol).[40] The reaction mixture was stirred for 1 h at room temperature and then transferred to a separatory funnel containing CH_2Cl_2 (30 mL) and H_2O (30 mL). The H_2O layer was washed with CH_2Cl_2 (2 × 30 mL), and the CH_2Cl_2-soluble material was dried under N_2. The crude product was preadsorbed to C_{18}-bonded silica (∼1.5 g) and subsequently subjected to HPLC using a semi-preparative C_{18} Betasil column at a flow rate of 9 mL/min with a linear gradient of 50% MeOH (0.1% TFA)/50% H_2O (0.1% TFA) to 100% MeOH (0.1% TFA) over 90 min. The fractions that eluted from 38−46 min yielded the trimethylated compound 3-hydroxy-7,8-dimethoxyserrulat-14-en-19-oic acid methyl ester (**2**, 98.8 mg, 22% yield, >95% purity). These methylation conditions were repeated several times in order to supply larger quantities of scaffold **2** for carbamate library generation.

General Preparation and Purification of the Carbamate Derivatives. The trimethylated scaffold (**2**, 20.8−25.6 mg, 0.053−0.066 mmol) was dissolved in dry pyridine (250 μL), and the relevant commercially available isocyanate (5−20 equiv, 38−225 μL, 41.0−295.7 mg) was slowly added at room temperature. The solution was stirred for 16 h before being quenched with MeOH, then dried under air. The crude reaction products were preadsorbed to C_{18}-bonded silica (∼1 g), packed into a guard cartridge, and subjected to

https://doi.org/10.1021/acs.jnatprod.2c01041
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semipreparative HPLC using a C_{18}-bonded silica column with a linear gradient from 60% MeOH (0.1% TFA)/40% H_2O (0.1% TFA) to 100% MeOH (0.1% TFA) over 60 min at a flow rate of 9 mL/min to give the desired carbamate products. Fractions containing UV-active material were analyzed by ^{1}H NMR and UHPLC-MS to determine purity. Yields ranged from 12% to 71%, and all compounds were obtained in purities of >95%.

Compound ***3****:* clear gum (20.8 mg, 71%); $[\alpha]_D^{24}$ +25.5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.17), 242 (4.24), 287 (3.35) nm; ECD λ_{ext} (MeOH) 212 ($\Delta\varepsilon$ +10.30), 231 ($\Delta\varepsilon$ +2.13), 244 ($\Delta\varepsilon$ +2.91) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.91 (2H, s, H-24, H-28), 7.54 (1H, s, H-26), 7.28 (1H, s, H-5), 7.19 (1H, s, NH-22), 5.40 (1H, ddd, *J* = 11.2, 5.0, 3.8 Hz, H-3), 5.09 (1H, br t, *J* = 7.1 Hz, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 3.36 (1H, ddq, *J* = 7.0, 2.2, 7.0 Hz, H-1), 3.13 (1H, dd, *J* = 4.1, 3.8 Hz, H-4), 2.25 (1H, ddd, *J* = 13.0, 7.0, 5.0 Hz, H-2a), 2.10 (1H, m, H-11), 2.02 (2H, ddd, *J* = 7.1, 7.1, 6.7 Hz, H-13), 1.75 (1H, ddd, *J* = 13.0, 3.8, 2.2 Hz, H-2b), 1.64 (3H, s, H-16), 1.54 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.31 (3H, d, *J* = 7.0 Hz, H-20), 1.30 (1H, m, H-12b), 0.63 (3H, d, *J* = 7.0 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.7 (C-19), 152.7 (C-22), 151.8 (C-8), 151.5 (C-7), 140.6 (C-9), 139.7 (C-23), 132.6 (q, $^2J_{CF}$ = 33.7 Hz, C-25, C-27), 132.5 (C-10), 131.9 (C-15), 126.4 (C-5), 124.4 (C-14), 123.3 (C-6), 123.2 (q, $^1J_{CF}$ = 273.3 Hz, C-29, C-30), 118.3 (q, $^3J_{CF}$ = 3.8 Hz, C-24, C-28), 116.7 (sept, $^3J_{CF}$ = 3.8 Hz, C-26), 72.1 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 45.8 (C-4), 37.4 (C-12), 32.7 (C-2), 32.2 (C-11), 28.8 (C-1), 26.2 (C-13), 25.7 (C-16), 23.4 (C-20), 19.4 (C-18), 17.7 (C-17); LRESIMS *m/z* 668 $[M + Na]^+$, 644 $[M - H]^-$; HRESIMS *m/z* 668.2414 $[M + Na]^+$ (calcd for $C_{32}H_{36}F_6NNaO_6$, 668.2417).

Compound ***4****:* clear gum (15.2 mg, 55%); $[\alpha]_D^{24}$ +46.5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.41), 234 (4.39), 281 (3.30) nm; ECD λ_{ext} (MeOH) 212 ($\Delta\varepsilon$ +14.21), 295 ($\Delta\varepsilon$ −0.78), 329 ($\Delta\varepsilon$ +0.30) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.30 (2H, s, H-25, H-27), 7.29 (2H, s, H-24, H-28), 7.28 (1H, s, H-5), 7.05 (1H, t, *J* = 7.3 Hz, H-26), 6.67 (1H, s, NH-22), 5.37 (1H, m, H-3), 5.10 (1H, br t, *J* = 6.7 Hz, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 3.35 (1H, ddq, *J* = 7.0, 2.5, 7.1 Hz, H-1), 3.13 (1H, br s, H-4), 2.23 (1H, ddd, *J* = 13.1, 7.0, 5.0 Hz, H-2a), 2.12 (1H, m, H-11), 2.02 (2H, ddd, *J* = 7.3, 7.1, 6.7 Hz, H-13), 1.74 (1H, ddd, *J* = 13.1, 3.6, 2.5 Hz, H-2b), 1.65 (3H, s, H-16), 1.55 (3H, s, H-17), 1.51 (1H, m, H-12a), 1.31 (3H, d, *J* = 7.1 Hz, H-20), 1.30 (1H, m, H-12b), 0.62 (3H, d, *J* = 6.7 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.7 (C-19), 153.1 (C-22), 151.8 (C-8), 151.3 (C-7), 140.5 (C-9), 138.0 (C-23), 132.1 (C-10), 131.7 (C-15), 129.1 (C-25, C-27), 126.4 (C-5), 124.5 (C-14), 123.5 (C-26), 123.1 (C-6), 118.9 (C-24, C-28), 71.0 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 46.0 (C-4), 37.6 (C-12), 32.6 (C-2), 32.1 (C-11), 28.9 (C-1), 26.2 (C-13), 25.8 (C-16), 23.4 (C-20), 19.3 (C-18), 17.8 (C-17); LRESIMS *m/z* 532 $[M + Na]^+$, 508 $[M - H]^-$; HRESIMS *m/z* 532.2668 $[M + Na]^+$ (calcd for $C_{30}H_{39}NNaO_6$, 532.2670).

Compound ***5****:* clear gum (14.3 mg, 49%); $[\alpha]_D^{24}$ +37.5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.43), 238 (4.37), 236 (3.47) nm; ECD λ_{ext} (MeOH) 213 ($\Delta\varepsilon$ +16.03), 295 ($\Delta\varepsilon$ −0.98), 329 ($\Delta\varepsilon$ +0.18) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.52 (H, s, H-28), 7.28 (1H, s, H-5), 7.20 (1H, m, H-27), 7.19 (1H, m, H-24), 7.02 (1H, dd, *J* = 6.8, 1.8 Hz, H-26), 6.62 (1H, s, NH-22), 5.37 (1H, ddd, *J* = 11.4, 5.3, 4.7 Hz, H-3), 5.10 (1H, br t, *J* = 7.0 Hz, H-14), 3.91 (3H, s, H-21), 3.90 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 3.35 (1H, ddq, *J* = 7.0, 2.4, 7.1 Hz, H-1), 3.12 (1H, dd, *J* = 4.1, 3.5 Hz, H-4), 2.23 (1H, ddd, *J* = 11.4, 7.0, 5.3 Hz, H-2a), 2.11 (1H, m, H-11), 2.02 (2H, ddd, *J* = 7.4, 7.1, 7.0 Hz, H-13), 1.74 (1H, m, H-2b), 1.66 (3H, s, H-16), 1.56 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.31 (3H, d, *J* = 7.1 Hz, H-20), 1.30 (1H, m, H-12b), 0.62 (3H, d, *J* = 7.0 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.7 (C-19), 152.8 (C-22), 151.8 (C-8), 151.3 (C-7), 140.6 (C-9), 139.0 (C-23), 134.9 (C-25), 132.8 (C-10), 131.8 (C-15), 130.1 (C-27), 126.4 (C-5), 124.5 (C-14), 123.5 (C-26), 123.2 (C-6), 118.8 (C-28), 116.5 (C-24), 71.5 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 45.9 (C-4), 37.5 (C-12), 32.7 (C-2), 32.1 (C-11), 28.9 (C-1), 26.2 (C-13), 25.8 (C-16), 23.4 (C-20), 19.4 (C-18), 17.8 (C-17); LRESIMS *m/z* 566 $[M + Na]^+$, 542 $[M - H]^-$; HRESIMS *m/z* 566.2276 $[M + Na]^+$ (calcd for $C_{30}H_{38}ClNNaO_6$, 566.2280).

Compound ***6****:* clear gum (20.0 mg, 68%); $[\alpha]_D^{24}$ +81 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.52), 242 (4.10), 316 (4.27) nm; ECD λ_{ext} (MeOH) 210 ($\Delta\varepsilon$ +15.84), 241 ($\Delta\varepsilon$ +2.03), 252 ($\Delta\varepsilon$ +3.00) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 8.17 (2H, d, *J* = 8.9 Hz, H-25, H-27), 7.55 (2H, br s, H-24, H-28), 7.27 (1H, s, H-5), 7.22 (1H, s, NH-22), 5.38 (1H, ddd, *J* = 11.2, 5.3, 3.5 Hz H-3), 5.06 (1H, br t, *J* = 7.1 Hz, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.87 (3H, s, OMe-7), 3.36 (1H, ddq, *J* = 7.0, 2.0, 7.2 Hz, H-1), 3.13 (1H, dd, *J* = 4.1, 3.5 Hz, H-4), 2.23 (1H, ddd, *J* = 13.1, 7.0, 5.3 Hz, H-2a), 2.09 (1H, m, H-11), 2.02 (2H, ddd, *J* = 7.3, 7.2, 7.1 Hz, H-13), 1.74 (1H, ddd, *J* = 13.1, 3.5, 2.0 Hz, H-2b), 1.62 (3H, s, H-16), 1.52 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.31 (3H, d, *J* = 7.2 Hz, H-20), 1.30 (1H, m, H-12b), 0.62 (3H, d, *J* = 6.8 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 152.4 (C-22), 151.8 (C-8), 151.4 (C-7), 144.2 (C-23), 143.0 (C-26), 140.5 (C-9), 132.5 (C-10), 131.9 (C-15), 126.3 (C-5), 125.3 (C-25, C-27), 124.3 (C-14), 123.3 (C-6), 117.9 (C-24, C-28), 72.2 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 45.8 (C-4), 37.4 (C-12), 32.6 (C-2), 32.0 (C-11), 28.8 (C-1), 26.1 (C-13), 25.8 (C-16), 23.4 (C-20), 19.4 (C-18), 17.7 (C-17); LRESIMS *m/z* 577 $[M + Na]^+$, 553 $[M - H]^-$; HRESIMS *m/z* 577.2516 $[M + Na]^+$ (calcd for $C_{30}H_{38}N_2NaO_8$, 577.2520).

Compound ***7****:* clear gum (3.5 mg, 12%); $[\alpha]_D^{24}$ +18 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.04), 260 (4.16), 303 (3.85) nm; ECD λ_{ext} (MeOH) 216 ($\Delta\varepsilon$ +4.73), 233 ($\Delta\varepsilon$ +0.37), 256 ($\Delta\varepsilon$ +1.86) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.60 (2H, d, *J* = 8.4 Hz, H-25, H-27), 7.49 (2H, s, H-24, H-28), 7.27 (1H, s, H-5), 6.78 (1H, s, NH-22), 5.38 (1H, ddd, *J* = 11.3, 4.4, 3.7 Hz,, H-3), 5.09 (1H, br t, *J* = 6.5 Hz, H-14), 3.91 (3H, s, H-21), 3.90 (3H, s, OMe-8), 3.89 (3H, s, OMe-7), 3.36 (1H, ddq, *J* = 7.1, 2.0, 7.2 Hz, H-1), 3.12 (1H, dd, *J* = 3.7, 3.5 Hz, H-4), 2.25 (1H, ddd, *J* = 13.1, 7.1, 4.4 Hz, H-2a), 2.10 (1H, m, H-11), 2.02 (2H, ddd, *J* = 7.4, 7.3, 7.1 Hz, H-13), 1.75 (1H, ddd, *J* = 13.1, 3.7, 2.0 Hz, H-2b), 1.65 (3H, s, H-16), 1.55 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.31 (3H, d, *J* = 7.1 Hz, H-20), 1.30 (1H, m, H-12b), 0.63 (3H, d, *J* = 6.8 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.7 (C-19), 152.1 (C-22), 151.8 (C-8), 151.5 (C-7), 142.2 (C-23), 140.5 (C-9), 133.5 (C-25, C-27), 132.6 (C-10), 131.9 (C-15), 126.3 (C-5), 124.4 (C-14), 123.4 (C-6), 119.0 (C-29), 118.4 (C-24, C-28), 106.5 (C-26), 72.0 (C-3), 61.4 (OMe-7), 61.0 (OMe-8), 52.4 (C-21), 45.9 (C-4), 37.4 (C-12), 32.7 (C-2), 32.1 (C-11), 28.8 (C-1), 26.2 (C-13), 25.9 (C-16), 23.5 (C-20), 19.4 (C-18), 17.8 (C-17); LRESIMS *m/z* 557 $[M + Na]^+$, 533 $[M - H]^-$; HRESIMS *m/z* 557.2615 $[M + Na]^+$ (calcd for $C_{31}H_{38}N_2NaO_6$, 557.2622).

Compound ***8****:* clear gum (17.8 mg, 63%); $[\alpha]_D^{24}$ +24 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.46), 234 (4.36), 286 (3.39) nm; ECD λ_{ext} (MeOH) 212 ($\Delta\varepsilon$ +17.74), 266 ($\Delta\varepsilon$ +4.30), 329 ($\Delta\varepsilon$ +2.42) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.34 (2H, br s, H-24, H-28), 7.27 (1H, s, H-5), 6.99 (2H, br s, H-25, H-27), 6.58 (1H, s, NH-22), 5.35 (1H, br m, H-3), 5.10 (1H, br s, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 3.35 (1H, ddq, *J* = 7.0, 2.5, 7.0 Hz, H-1), 3.12 (1H, br s, H-4), 2.22 (1H, m, H-2a), 2.10 (1H, br m, H-11), 2.02 (2H, br s, H-13), 1.74 (1H, m, H-2b), 1.66 (3H, s, H-16), 1.56 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.31 (3H, d, *J* = 7.0 Hz, H-20), 1.30 (1H, m, H-12b), 0.61 (3H, d, *J* = 6.7 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 159.1 (d, $^1J_{CF}$ = 243.4 Hz, C-26), 153.2 (C-22), 151.8 (C-8), 151.4 (C-7), 140.7 (C-9), 134.0 (C-23), 132.9 (C-10), 131.8 (C-15), 126.4 (C-5), 124.5 (C-14), 123.2 (C-6), 120.5 (d, $^3J_{CF}$ = 3.2 Hz, C-24, C-28), 115.8 (d, $^2J_{CF}$ = 23.0 Hz, C-25, C-27), 71.2 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 46.0 (C-4), 37.7 (C-12), 32.7 (C-2), 32.1 (C-11), 28.9 (C-1), 26.2 (C-13), 25.8 (C-16), 23.5 (C-20), 19.4 (C-18), 17.8 (C-17); LRESIMS *m/z* 550 $[M + Na]^+$, 526 $[M - H]^-$; HRESIMS *m/z* 550.2568 $[M + Na]^+$ (calcd for $C_{30}H_{38}FNNaO_6$, 550.2575).

Compound ***9****:* clear gum (23.1 mg, 70%); $[\alpha]_D^{24}$ +30 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.40), 241 (4.38), 291 (3.51) nm; ECD λ_{ext} (MeOH) 211 ($\Delta\varepsilon$ +14.84), 241 ($\Delta\varepsilon$ +5.72),

https://doi.org/10.1021/acs.jnatprod.2c01041
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322 (Δε +1.48) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.29 (2H, br s, H-24, H-28), 7.26 (1H, s, H-5), 6.83 (2H, br s, H-25, H-27), 6.55 (1H, s, NH-22), 5.34 (1H, br m, H-3), 5.11 (1H, br s, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 3.77 (3H, s, OMe-26), 3.35 (1H, ddq, J = 7.1, 2.5, 7.1 Hz, H-1), 3.12 (1H, br s, H-4), 2.26 (1H, m, H-2a), 2.10 (1H, m, H-11), 2.02 (2H, br s, H-13), 1.73 (1H, m, H-2b), 1.66 (3H, s, H-16), 1.56 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.30 (3H, d, J = 7.1 Hz, H-20), 1.29 (1H, m, H-12b), 0.60 (3H, d, J = 6.8 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 156.0 (C-26), 153.4 (C-22), 151.8 (C-8), 151.3 (C-7), 140.7 (C-9), 133.0 (C-10), 131.1 (C-23), 131.6 (C-15), 126.5 (C-5), 124.6 (C-14), 123.1 (C-6), 120.7 (C-24, C-28), 114.3 (C-25, C-27), 70.8 (C-3), 61.3 (OMe-7), 60.8 (OMe-8), 55.6 (OMe-26), 52.3 (C-21), 46.0 (C-4), 37.7 (C-12), 32.6 (C-2), 32.1 (C-11), 29.0 (C-1), 26.2 (C-13), 25.8 (C-16), 23.4 (C-20), 19.3 (C-18), 17.8 (C-17); LRESIMS m/z 562 $[M + Na]^+$, 538 $[M - H]^-$; HRESIMS m/z 562.2766 $[M + Na]^+$ (calcd for $C_{31}H_{41}NNaO_7$, 562.2775).

Compound ***10****:* clear gum (20.6 mg, 64%); $[\alpha]_D^{24}$ +57 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.18), 239 (4.15), 289 (3.09) nm; ECD λ_{ext} (MeOH) 209 (Δε +14.21), 260 (Δε −0.78), 334 (Δε +0.30) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.27 (1H, s, H-5), 7.26 (2H, br s, H-24, H-28), 7.09 (2H, br s, H-25, H-27), 6.52 (1H, s, NH-22), 5.35 (1H, br m, H-3), 5.11 (1H, br s, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 2.30 (3H, s, H-29), 3.35 (1H, ddq, J = 7.1, 2.6, 6.9 Hz, H-1), 3.12 (1H, br s, H-4), 2.22 (1H, m, H-2a), 2.12 (1H, m, H-11), 2.02 (2H, br s, H-13), 1.74 (1H, m, H-2b), 1.66 (3H, s, H-16), 1.56 (3H, s, H-17), 1.51 (1H, m, H-12a), 1.31 (3H, d, J = 7.1 Hz, H-20), 1.30 (1H, m, H-12b), 0.61 (3H, d, J = 7.0 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 153.1 (C-22), 151.8 (C-8), 151.3 (C-7), 140.7 (C-9), 135.4 (C-23), 133.1 (C-26), 133.0 (C-10), 131.7 (C-15), 129.6 (C-25, C-27), 126.5 (C-5), 124.6 (C-14), 123.1 (C-6), 118.8 (C-24, C-28), 70.9 (C-3), 61.3 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 46.0 (C-4), 37.7 (C-12), 32.7 (C-2), 32.1 (C-11), 29.0 (C-1), 26.2 (C-13), 25.9 (C-16), 23.5 (C-20), 20.9 (C-29), 19.4 (C-18), 17.8 (C-17); LRESIMS m/z 546 $[M + Na]^+$, 522 $[M - H]^-$; HRESIMS m/z 546.2819 $[M + Na]^+$ (calcd for $C_{31}H_{41}NNaO_6$, 546.2826).

Compound ***11****:* clear gum (29.5 mg, 82%); $[\alpha]_D^{24}$ +86 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.42), 250 (3.85), 293 (3.22) nm; ECD λ_{ext} (MeOH) 211 (Δε +14.08), 252 (Δε +3.84), 333 (Δε +0.20) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 8.05 (1H, s, NH-22), 7.78 (2H, d, J = 8.0 Hz, H-24, H-28), 7.28 (2H, d, J = 8.0 Hz, H-25, H-27), 7.18 (1H, s, H-5), 5.26 (1H, ddd, J = 11.1, 4.0, 3.8 Hz, H-3), 4.98 (1H, tqq, J = 7.1, 1.3, 1.4 Hz, H-14), 3.89 (3H, s, H-21), 3.88 (3H, s, OMe-8), 3.86 (3H, s, OMe-7), 3.35 (1H, ddq, J = 7.1, 2.7, 7.0 Hz, H-1), 2.98 (1H, br s, H-4), 2.41 (3H, s, H-29), 2.14 (1H, m, H-2a), 1.89 (1H, m, H-11), 1.83 (2H, m, H-13), 1.69 (3H, s, H-16), 1.60 (1H, m, H-2b), 1.57 (3H, s, H-17), 1.22 (3H, d, J = 7.1 Hz, H-20), 1.19 (1H, m, H-12a), 1.04 (1H, m, H-12b), 0.52 (3H, d, J = 6.9 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 151.8 (C-8), 151.4 (C-7), 150.2 (C-22), 145.0 (C-26), 140.5 (C-9), 135.7 (C-23), 132.1 (C-10), 131.7 (C-15), 129.7 (C-25, C-27), 128.3 (C-24, C-28), 126.0 (C-5), 124.4 (C-14), 123.3 (C-6), 73.6 (C-3), 61.3 (OMe-7), 60.7 (OMe-8), 52.3 (C-21), 45.5 (C-4), 36.6 (C-12), 32.5 (C-2), 31.8 (C-11), 28.7 (C-1), 26.0 (C-13), 25.8 (C-16), 23.3 (C-20), 20.9 (C-29), 19.1 (C-18), 17.8 (C-17); LRESIMS m/z 610 $[M + Na]^+$, 586 $[M - H]^-$; HRESIMS m/z 610.2434 $[M + Na]^+$ (calcd for $C_{31}H_{41}NNaO_8S$, 610.2445).

Compound ***12****:* clear gum (20.6 mg, 60%); $[\alpha]_D^{24}$ +15 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.23), 244 (3.81), 295 (3.12) nm; ECD λ_{ext} (MeOH) 210 (Δε +15.44), 252 (Δε +5.01), 319 (Δε +2.68) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.32 (2H, dd, J = 7.5, 7.5 Hz, H-26, H-28), 7.28 (2H, d, J = 7.5 Hz, H-25, H-29), 7.27 (1H, t, J = 7.5 Hz, H-27), 7.25 (1H, s, H-5), 5.37 (1H, m, H-3), 5.10 (1H, br t, J = 6.7 Hz, H-14), 4.96 (1H, t, J = 5.7 Hz, NH-22), 4.39 (1H, br dd, J = 14.7, 5.8 Hz, H-23a), 4.37 (1H, br dd, J = 14.7, 5.8 Hz, H-23b), 3.90 (3H, s, H-21), 3.88 (3H, s, OMe-8), 3.87 (3H, s, OMe-7), 3.35 (1H, ddq, J = 7.1, 1.7, 7.0 Hz, H-1), 3.09 (1H, br s, H-4), 2.16 (1H, ddd, J = 11.5, 7.0, 5.2 Hz, H-2a), 2.06 (1H, m, H-11), 2.01 (2H, m, H-13), 1.70 (1H, m, H-2b), 1.66 (3H, s, H-16), 1.58 (3H, s, H-17), 1.50 (1H, m, H-12a), 1.30 (3H, d, J = 7.1 Hz, H-20), 1.29 (1H, m, H-12b), 0.57 (3H, d, J = 7.1 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 156.1 (C-22), 151.8 (C-8), 151.3 (C-7), 140.8 (C-9), 138.6 (C-24), 133.2 (C-10), 131.5 (C-15), 127.7 (C-25, C-29), 127.6 (C-27), 126.6 (C-5), 124.7 (C-14), 123.1 (C-6), 128.8 (C-26, C-28), 70.7 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 45.2 (C-23), 46.1 (C-4), 37.8 (C-12), 32.6 (C-2), 32.2 (C-11), 29.0 (C-1), 26.3 (C-13), 25.8 (C-16), 23.4 (C-20), 19.3 (C-18), 17.8 (C-17); LRESIMS m/z 546 $[M + Na]^+$; HRESIMS m/z 546.2816 $[M + Na]^+$ (calcd for $C_{31}H_{41}NNaO_6$, 546.2826).

Compound ***13****:* clear gum (12.0 mg, 35%); $[\alpha]_D^{24}$ +82 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.48), 243 (4.54), 291 (3.45) nm; ECD λ_{ext} (MeOH) 212 (Δε +13.80), 243 (Δε +6.30), 334 (Δε +0.19) nm; ^{1}H NMR ($CDCl_3$, 800 MHz) δ_H 7.34 (2H, br m, H-24, H-28), 7.27 (1H, s, H-5), 7.24 (2H, br m, H-25, H-27), 6.63 (1H, br s, NH-22), 5.36 (1H, br m, H-3), 5.09 (1H, br s, H-14), 3.90 (3H, s, H-21), 3.89 (3H, s, OMe-8), 3.88 (3H, s, OMe-7), 3.35 (1H, ddq, J = 7.0, 2.5, 6.9 Hz, H-1), 3.11 (1H, br s, H-4), 2.22 (1H, m, H-2a), 2.10 (1H, br m, H-11), 2.01 (2H, m, H-13), 1.74 (1H, m, H-2b), 1.65 (3H, s, H-16), 1.55 (3H, s, H-17), 1.49 (1H, m, H-12a), 1.31 (3H, d, J = 7.0 Hz, H-20), 1.30 (1H, m, H-12b), 0.61 (3H, d, J = 6.6 Hz, H-18); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 166.6 (C-19), 152.9 (C-22), 151.8 (C-8), 151.4 (C-7), 140.6 (C-9), 136.6 (C-23), 132.8 (C-10), 131.8 (C-15), 129.2 (C-25, C-27), 128.5 (C-26), 126.4 (C-5), 124.5 (C-14), 123.2 (C-6), 119.9 (C-24, C-28), 71.4 (C-3), 61.4 (OMe-7), 60.8 (OMe-8), 52.3 (C-21), 46.0 (C-4), 37.6 (C-12), 32.7 (C-2), 32.1 (C-11), 28.9 (C-1), 26.2 (C-13), 25.9 (C-16), 23.5 (C-20), 19.4 (C-18), 17.8 (C-17); LRESIMS m/z 566 $[M + Na]^+$, 542 $[M - H]^-$; HRESIMS m/z 566.2271 $[M + Na]^+$ (calcd for $C_{30}H_{38}ClNNaO_6$, 566.2280).

In Vitro Antiplasmodial Image-Based Asexual Assay. *Plasmodium falciparum* 3D7 and Dd2 parasites were cultured in RPMI1640 (Life Technologies, Camarillo, CA, USA) supplemented with 2.5 mg/mL Albumax II, 5% AB human serum, 25 mM HEPES, and 0.37 mM hypoxanthine. Ring stage parasites were treated with compounds following two rounds of sorbitol synchronization as previously described.[41] Artesunate and pyrimethamine were incorporated as controls. Following incubation for 72 h at 37 °C, in 5% CO_2/5% O_2 in 384-well imaging plates, parasites were stained with 2-(4-amidinophenyl)-1*H*-indole-6-carboxamidine (DAPI) and imaged using an Opera Phenix high content screening system (PerkinElmer, Waltham, MA, USA). Images were analyzed using Harmony software (PerkinElmer, Waltham, MA, USA).[41]

In Vitro Cytotoxicity Assay. Human embryonic kidney cells (HEK293) were maintained in DMEM (Life Technologies, Camarillo, CA, USA) containing 10% fetal bovine serum (FBS) (Hyclone ThermoFisher, Melbourne, Australia). Cytotoxicity testing was undertaken as previously described.[42] In brief, test compound was added to wells of 384-well plates containing adherent HEK293 cells and incubated 72 h at 37 °C in 5% CO_2. After incubation, cells were incubated with Alamar Blue for 5−6 h and fluorescence was measured at 530 nm excitation and 595 nm emission. The % inhibition was calculated using 0.4% DMSO (no inhibition) and 5 μM puromycin (100% inhibition) data. IC_{50} values were obtained by plotting % inhibition against log dose using GraphPad Prism v.6 (San Diego, CA, USA), nonlinear regression with a variable slope plot.

Preparation of Nematode Larvae for Bioassays. *H. contortus* (Haecon-5 strain) L3s were produced and stored using an established protocol,[43] approved by the animal ethics committee of the University of Melbourne (permit no. 1714374). For use in the assay, L3s were exsheathed and sterilized by incubation in 0.15% (v/v) sodium hypochlorite (NaClO) at 38 °C for 20 min[44] and then washed five times in sterile saline by centrifugation at 500*g* (5 min) at room temperature (22−24 °C). After the last wash, exsheathed L3s (xL3s) were suspended in lysogeny broth (LB) containing 100 IU/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B, designated LB*. In vitro-raised L4s were produced by culturing xL3s for 168 h in LB* at 38 °C, 10% (v/v) CO_2, and a relative humidity of >90%.

https://doi.org/10.1021/acs.jnatprod.2c01041
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Anthelmintic Assay. All compounds (**1**−**13**) were individually tested for their anthelmintic effect on larvae (xL3s) of *H. contortus* using an established bioassay.[43] Each assay was performed in duplicate on different days. In brief, compounds were serially diluted in 50 μL of LB* (9-points, 2-fold dilution, 50 to 0.2 μM) and dispensed into the wells of sterile 96-well flat-bottom microtiter plates containing 300 xL3s, with six wells with no compound (LB* + 0.25% DMSO; negative control). A plate containing monepantel and/or moxidectin (positive control compounds) was serially diluted and prepared in the same manner. The motility of xL3s development to L4 and phenotypic alterations were assessed at 168 h. At 168 h, larvae in individual wells were fixed with 25 μL of 1% iodine and microscopically examined (using a DM1000 LED microscope, Leica) at 100-times magnification to assess their development (based on the presence/absence of a well-developed pharynx)[44] and morphology (phenotype).[43,45]

HIV-1 Latency Reversal Assay. J-Lat 10.6 cells were obtained from the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH [contributed by Dr. Eric Verdin].[37] Cells were cultured in RPMI 1640 medium with HEPES and L-glutamine, 10% fetal bovine serum, 100 U penicillin/mL, and 100 μg/mL streptomycin. Cells were seeded in 96-well plates at 2×10^5 cells/mL ± PMA and test agents and incubated at 37 °C in 5% CO_2 for 24 h. GFP expression was monitored using a BD FACSCelesta flow cytometer and analyzed as described previously.[38] Results presented here denote the mean ± SEM from at least three independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.2c01041.

Spectra of compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Rohan A. Davis − *Griffith Institute for Drug Discovery, School of Environment and Science, Griffith University, Brisbane, QLD 4111, Australia;* orcid.org/0000-0003-4291-7573; Phone: +61-7-3735-6043; Email: r.davis@griffith.edu.au; Fax: +61-7-3735-6001

Authors

Chen Zhang − *Griffith Institute for Drug Discovery, School of Environment and Science, Griffith University, Brisbane, QLD 4111, Australia*

Kah Yean Lum − *Griffith Institute for Drug Discovery, School of Environment and Science, Griffith University, Brisbane, QLD 4111, Australia*

Aya C. Taki − *Department of Veterinary Biosciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia;* orcid.org/0000-0003-3489-4367

Robin B. Gasser − *Department of Veterinary Biosciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia;* orcid.org/0000-0002-4423-1690

Joseph J. Byrne − *Department of Veterinary Biosciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia;* orcid.org/0000-0001-5235-8408

Luis J. Montaner − *The Wistar Institute, Philadelphia, Pennsylvania 19104, United States*

Ian Tietjen − *The Wistar Institute, Philadelphia, Pennsylvania 19104, United States*

Vicky M. Avery − *Griffith Institute for Drug Discovery, School of Environment and Science, Griffith University, Brisbane, QLD 4111, Australia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.2c01041

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the Australian Research Council (ARC) for support toward NMR and MS equipment (grants LE0668477, LE140100119, and LE0237908). We gratefully acknowledge NatureBank for supply of the plant sample investigated during these studies. C.Z. would like to thank Griffith University for Ph.D. scholarships (GUPRS and GUIPRS). The authors thank Dr. Sandra Duffy, Discovery Biology, for technical assistance. The authors also thank and acknowledge the Australian Red Cross Blood Bank for the provision of fresh red blood cells, critical for antiplasmodial research. Research at the University of Melbourne (Gasser Lab) was supported by the ARC, Yourgene Health and Melbourne Water Corporation. The HIV screening was supported by the Canadian Institutes for Health Research (CIHR PJT-153057) (I.T.). This work was also supported by the following grants to L.J.M.: The BEAT-HIV Delaney Collaboratory cofunded by NHLBI, NINDS, NIDDK, NIDA, and the National Institute of Allergy and Infectious Diseases (UM1 AI164570). It was also supported by the Robert I. Jacobs Fund of The Philadelphia Foundation and the Herbert Kean, M.D., Family Professorship.

■ DEDICATION

Dedicated to Dr. Mary J. Garson, The University of Queensland, for her pioneering work on bioactive natural products.

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https://doi.org/10.1021/acs.jnatprod.2c01041
J. Nat. Prod. XXXX, XXX, XXX−XXX