Identification of PTP1B and \( \alpha \)-Glucosidase Inhibitory Serrulatanes from *Eremophila* spp. by Combined use of Dual High-Resolution PTP1B and \( \alpha \)-Glucosidase Inhibition Profiling and HPLC-HRMS-SPE-NMR

Sileshi G. Wubshet,† Yousof Tahtah,† Allison M. Heskes,‡§ Kenneth T. Kongstad,† Irini Pateraki,‡§ Björn Hamberger,‡§ Birger L. Møller,‡§ and Dan Staerk*†

†Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark
‡Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark
§Center for Synthetic Biology “bioSYNergy”, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Supporting Information

ABSTRACT: According to the International Diabetes Federation, type 2 diabetes (T2D) has reached epidemic proportions, affecting more than 382 million people worldwide. Inhibition of protein tyrosine phosphatase-1B (PTP1B) and \( \alpha \)-glucosidase is a recognized therapeutic approach for management of T2D and its associated complications. The lack of clinical drugs targeting PTP1B and side effects of the existing \( \alpha \)-glucosidase drugs, emphasize the need for new drug leads for these T2D targets. In the present work, dual high-resolution PTP1B and \( \alpha \)-glucosidase inhibition profiles of *Eremophila gibbosa*, *E. glabra*, and *E. aff. drummondii* “Kalgoorlie” were used for pinpointing \( \alpha \)-glucosidase and/or PTP1B inhibitory constituents directly from the crude extracts. A subsequent targeted high-performance liquid chromatography—high-resolution mass spectrometry—solid-phase extraction—nuclear magnetic resonance spectroscopy (HPLC-HRMS-SPE-NMR) analysis and preparative-scale HPLC isolation led to identification of 21 metabolites from the three species, of which 16 were serrulatane-type diterpenoids (12 new) associated with either \( \alpha \)-glucosidase and/or PTP1B inhibition. This is the first report of serrulatane-type diterpenoids as potential \( \alpha \)-glucosidase and/or PTP1B inhibitors.

Diabetes mellitus caused 5.1 million deaths and constituted 11% (548 billion U.S. dollars) of the total health care expenditure worldwide in 2013. Type 2 diabetes (T2D), also known as noninsulin-dependent diabetes, accounts for 90% of the total 382 million diabetes cases. This alarming global burden emphasizes the need for new and effective therapeutic approaches. T2D is characterized by insulin resistance—possibly due to diminished postreceptor insulin signaling. This results in postprandial hyperglycemia (after-meal elevated blood glucose level), which is the major cause of the severe micro- and macro-vascular complications associated with T2D. One of the well-established therapeutic approaches for controlling postprandial hyperglycemia is inhibition of \( \alpha \)-glucosidase, an enzyme that catalyzes glucose release from the nonreducing end of dietary carbohydrates, thereby elevating the blood glucose level. The existing clinical drugs targeting this enzyme (i.e., acarbose, miglitol, and voglibose) are associated with gastrointestinal side effects, and new potent \( \alpha \)-glucosidase inhibitors are needed for management of blood glucose. Protein tyrosine phosphatase-1B (PTP1B) is responsible for catalyzing the dephosphorylation of the activated insulin receptor, which results in downregulation of the insulin-signaling pathway. Thus, PTP1B is an important drug target for the management of blood glucose in patients with T2D. Despite a number of drug lead candidates of both natural and synthetic origin, there are no clinically approved PTP1B inhibitors. This might be due to the fact that most of the discovered PTP1B inhibitors are highly charged molecules, which limits their potential as drug leads.

Several studies have shown that plants are important sources of new antidiabetic drug leads—often having multiple modes of action through interaction with different target enzymes related to diabetes. Within this realm, diterpenoids constitute an especially potent reservoir of drug leads. It is becoming increasingly clear that structurally unique diterpenoid ligands acting at the same receptor can preferentially activate different signaling pathways, which in turn may result in entirely different physiological responses.
different drug effects. This may partly explain why
diterpenoids are effective toward a multitude of diseases
ranging from cancers, diabetes, cardiovascular problems,
inflammation, stomach ulcers, and menopause-related condi-
tions. Diterpenoids are structurally complex molecules
produced in plants in complex mixtures from a single precursor
(geranylgeranyl diphosphate), typically in minute amounts,
thereby rendering isolation difficult, time-consuming, and
resource-demanding. Key classes of enzymes involved in
diterpenoid synthesis include terpenoid synthases and
cytochrome P450s. Elucidation of complete biosynthetic
pathways for structurally complex diterpenoids and biotechno-
logical approaches aimed at heterologous expression of spe-
cific diterpenoid pathways in yeast may facilitate access to otherwise
inaccessible diterpenoids and analogues of these.

In recent years, several advanced bioanalytical techniques
have been developed and utilized for screening plant extracts
against several therapeutic targets, including those associated
with T2D. Among such technological advances, high-resolution
bioassay-coupled HPLC-HRMS-SPE-NMR has proven to be an
effective technique for the targeted identification of bioactive
constituents directly from crude plant extracts. This platform
has been used for the identification of α-glucosidase inhibitors,
α-amylase inhibitors, aldose reductase inhibitors, monoamine oxidase-A inhibitors, antioxidants, and
PM H’-ATPase inhibitors from various natural product
extracts. In the current study, this platform is used for dual
high-resolution α-glucosidase/PTP1B inhibition profiling of
Eremophila gibbosa, E. glabra, and E. aff. drummondii
“Kalgoorlie”—plant species that are endemic to Australia and
known to contain an array of diterpenoids with a wide range of
structural features and pharmacological activities.

## RESULTS AND DISCUSSION

Crude ethyl acetate extracts of Eremophila gibbosa, E. glabra,
and E. aff. drummondii “Kalooorlie” were assessed for concen-
tration-dependent inhibition of PTP1B. The IC50 curves
are shown in Figure 1, and the resulting IC50 values were 0.10
± 0.03 mg/mL (E. gibbosa), 0.013 ± 0.004 mg/mL (E. glabra),
and 0.0057 ± 0.0175 mg/mL (E. aff. drummondii “Kalgoorlie”).
This was followed by optimization of a reversed-phase
analytical-scale HPLC method, microfractionation in two 96-
well microplates, α-glucosidase and PTP1B inhibition assaying
of the content in all wells, construction of dual high-resolution
PTP1B/α-glucosidase inhibition profiles, and targeted HPLC-
HRMS-SPE-NMR analysis of PTP1B and/or α-glucosidase
inhibitors.

### Dual High-Resolution PTP1B/α-Glucosidase Inhibition
Profiling.

For all three extracts, 176 fractions were collected in
the retention time range from 10 to 45 min, yielding a

![Figure 1. Protein-tyrosine phosphatase 1B (PTP1B) inhibition curves of crude extracts of E. gibbosa (A), E. glabra (B), and E. aff. drummondii “Kalgoorlie” (C).](image)

![Figure 2. UV chromatogram of E. gibbosa monitored at 254 nm (A), high-resolution α-glucosidase inhibition profile (B), and PTP1B inhibition profile (C).](image)

![Figure 3. UV chromatogram of E. glabra monitored at 254 nm (A), high-resolution α-glucosidase inhibition profile (B), and PTP1B inhibition profile (C).](image)

![Figure 4. UV chromatogram of E. drummondii monitored at 254 nm (A), high-resolution α-glucosidase inhibition profile (B), and PTP1B inhibition profile (C).](image)
Comparison of 1H NMR chemical shifts with their position marked with δ in Figures S1–S5. Additionally, 1D and 2D spectra of all new compounds are presented in Tables 1 and 2, and Table S1 (Supporting Information). Overlapping signals within the column.

Identification of α-Glucosidase Inhibitors. The material eluted as peak 3 from E. gibossa showed an [M + H]+ ion with m/z 287.0920, which suggested the molecular formula C_{16}H_{14}O_{5}. Comparison of 1H NMR chemical shifts with literature data resulted in identification of 3 as 4'-5-dihydroxy-7-methoxyflavanone. The structure shown in Figure 5 is in agreement with the 2S configuration observed for naturally occurring 4',5-dihydroxy-7-methoxyflavanone (sakuranetin), but based on the MS and NMR data obtained in the HPLC-HRMS-SPE-NMR mode, 3 could also be the 2R enantiomer. Sakuranetin has been shown previously to stimulate glucose uptake in differentiated 3T3-L1 adipocytes and has been recognized as a potential antidiabetic drug. The material eluted as peak 4 showed an [M + H]^+ ion with m/z 333.2062, which suggested the molecular formula C_{20}H_{26}O_{4}. Analysis of 2D COSY, NOESY, HSQC, and HMBC spectra allowed identification of 4 as 8,16-dihydroxyseruat-14-en-19-oic acid.

The relative configurations of C-1, C-4, and C-11 as well as the C-14—C-15 double bond were assigned based on the NOESY experiment. However, the absolute configuration of the stereogenic carbon atoms of 4 and the other isolated serrulatane-type diterpenoids, *vide infra*, could not be established based on the MS and NMR data obtained in the HPLC-HRMS-SPE-NMR mode. Therefore, the structures shown in Figure 5 represent only one of two possible enantiomers, but the configurations of C-1, C-4, and C-11 are in agreement with the 1R,4S,11S configuration previously established for dihydroxyseruat acid isolated from *Eremophila serrulata*. The material eluted as peak 6 showed an [M + H]^+ ion with m/z 331.1910, which suggested the molecular formula C_{20}H_{28}O_{4}. The 1H NMR spectrum of 6 showed resonances for the serrulatic acid core and a distinct formyl proton (δ 9.27, s). The position of the formyl group was not be established based on the MS and NMR data obtained in the HPLC-HRMS-SPE-NMR mode.

Table 1. NMR Spectroscopic Data (600 MHz) of 6, 7, 16, 18, and 24

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^aNMR spectra acquired in methanol-d₄ after HPLC-HRMS-SPE-NMR analysis. ^bNMR spectra acquired in CDCl₃ after preparative isolation. ^cN.d.: not detected. ^dOverlapping signals within the column.
Table 2. NMR Spectroscopic Data (600 MHz) of 11, 12, 21, 23, 25, and 27

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aNMR spectra acquired in methanol-\( d_4 \) after HPLC-HRMS-SPE-NMR analysis. bNMR spectra acquired in CDCl\( _3 \) after preparative isolation. cCompounds identified as a mixture, overlapping signals of two compounds across the row are highlighted in bold font. dN.d.: not detected; n.a.: not assigned. 99Chemically equivalent nuclei within the column. 77Overlapping signals within the column
established on the basis of $^1$J HMBC correlations from the olefinic H-14 ($\delta$ 6.47, t, 7.3 Hz) and the H-17 methyl group ($\delta$ 1.63, s) to the formyl C-16 ($\delta$ 197.4). After assigning all the $^1$H and $^{13}$C NMR resonances on the basis of analysis of homo- and heteronuclear 2D NMR experiments (COSY, NOESY, HSQC, and HMBC), 6 was identified as 8-hydroxy-16-oxoserrulat-14-en-19-oic acid. Compound 6 is a new compound, and $^1$H and $^{13}$C NMR data obtained in the HPLC-HRMS-SPE-NMR mode is given in Table 1, and selected NOE and HMBC correlations are shown in Figure 6. The amount of material eluted with peak 2 and 5 did not allow structural identification of these two minor metabolites. However, the material showed [M + H]$^+$ ions with $m/z$ 317.1027 and 335.2213, respectively, corresponding to molecular formulas C$_{17}$H$_{16}$O$_6$ (suggesting a dimethoxylated flavonoid for the material eluted with peak 2) and C$_{20}$H$_{30}$O$_4$ (suggesting a diterpenoid core for the material eluted with peak 5). The material eluted as peak 9 from E. glabra and peak 19 from E. aff. drummondii "Kalgoorlie" showed an [M + H]$^+$ ion with $m/z$ 335.2215 (corresponding to the molecular formula C$_{20}$H$_{30}$O$_4$), and comparison of $^1$H NMR chemical shifts with literature data, resulted in identification of 9/19 as 8,16-dihydroxyserrulat-19-oic acid. The material eluted with peak 20 and 21 from E. aff. drummondii "Kalgoorlie" are associated with $\alpha$-glucosidase as well as PTP1B inhibition and are therefore discussed in the next section.

**Identification of PTP1B Inhibitors.** The amount of material from the late eluting PTP1B inhibitory peaks 11, 12, and 13 from E. glabra afforded $^1$H NMR spectra in the HPLC-HRMS-SPE-NMR mode but was insufficient for high-quality 2D NMR data—even after multiple trappings on SPE cartridges. Similar challenges were encountered for HPLC-HRMS-SPE-NMR analysis of the late eluting peaks 21−27 from E. aff. drummondii "Kalgoorlie". The lipophilicity of such late eluting metabolites is expected to result in a stronger retention on the polydivinyl-benzene phase cartridges, thereby causing a lowered concentration in the final elution volume. Peaks 11−13 and 21−27 were therefore identified from material isolated using preparative-scale HPLC.

The material eluted as peak 12 from E. glabra showed an [M + H]$^+$ ion with $m/z$ 465.2614, which suggested the molecular

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**Figure 5.** Metabolites identified from crude EtOAc extracts of E. glabra, E. gibossa, and E. aff. drummondii "Kalgoorlie".
formula \( C_{29}H_{36}O_{5} \). The \(^1\)H NMR spectrum of 12 showed similar resonances as \( 8,16 \)-dihydroxyserrulat-19-oic acid (9) as well as additional resonances assigned to a cinnamoyl unit. \(^3\)HMBC correlations from the diastereotopic proton pair H-16a/H-16b to the C-1′ carbonyl group proved the cinnamoyl unit to be attached at C-16. The material eluted with peak 11 from \( E. \) aff. drummondii "Kalgoorlie" was a mixture showing \([M+H]^+\) ions with \( m/z \) 451.2673 and 487.2641, corresponding to the molecular formulae \( C_{26}H_{38}O_{5} \) and \( C_{29}H_{38}O_{5} \), respectively. Likewise, the \(^1\)H NMR spectrum showed two sets of signals both displaying similar resonances as \( 8,16 \)-dihydroxyserrulat-19-oic acid (9), and additional \( 4 \)-methylpent-3-enoyl (11a) and dihydrocinnamoyl (11b) signals. The esterification of the serrulatane core at C-16 was confirmed by \(^3\)HMBC correlations from the diastereotopic proton pair H-16a/H-16b to the C-1′ carbonyl group of the 4-methylpent-3-enoyl (11a) and dihydrocinnamoyl (11b), respectively. 2D homo- and heteronuclear experiments were used to assign all \(^1\)H and \(^13\)C NMR resonances of 11a, 11b, and 12 (Table 2), and selected NOE and HMBC correlations are shown in Figure 6. This led to identification of 11a as 8-hydroxy-16-\([4\text{-methylpent-3-enoyl}oxy]\)serrulat-19-oic acid, 11b as 8-hydroxy-16-dihydrocinnamoyloxyserrulat-19-oic acid, and 12 as 8-hydroxy-16-cinnamoyloxyserrulat-19-oic acid, which are all new compounds. These metabolites are the first O-methylpentenoyl, O-dihydrocinnamoyl, and O-cinnamoyl esters of serrulatane-type diterpenoids. The late eluting minor metabolite 21 was tentatively identified as 7,8-dihydroxy-16-cinnamoyloxyserrulat-19-oic acid (very low amount of 21 limited NMR analysis to a 1D \(^1\)H NMR experiment). Compound 21 is a new compound, and assignment of the \(^1\)H NMR collated in Table 2 was done.

Figure 6. Selected HMBC and NOE correlations used for structure elucidation of new compounds from \( E. \) glabra, \( E. \) gibboa, and \( E. \) aff. drummondii "Kalgoorlie".
by comparison of $^1$H NMR shifts with those unambiguously identified for 20.

The material eluted as peaks 23 and 24 showed $[M + H]^+$ ions with $m/z$ 527.2638 and 333.2058, which suggested the molecular formula $C_{20}H_{28}O_8$ and $C_{20}H_{28}O_9$. The $^1$H NMR spectrum of 23 showed all the characteristic resonances observed for 21 with the addition of a methoxy singlet at $\delta$ 3.92. Full assignment of all $^1$H and $^{13}$C NMR resonances from 2D homo- and heteronuclear NMR experiments allowed identification of compound 23 as 7,8-dihydroxy-16-feruloylserrulat-19-oic acid. The $^1$H NMR spectrum of compound 24 showed characteristic resonances of the serrulatane core structure with a single aromatic resonance ($\delta$ 7.28, br s) suggesting dihydroxylation of the aromatic ring. A detailed 2D NMR analysis confirmed the 7,8-dihydroxylation pattern of the serrulatane core, and compound 24 was identified as 7,8-dihydroxy-serrulat-14-en-19-oic acid. Both 23 and 24 are new compounds, and $^1$H and $^{13}$C NMR resonances are shown in Table 2 and selected NOE and HMBC correlations are shown Figure 6.

The material eluted as peaks 25 and 27 showed $[M + H]^+$ ions with $m/z$ 421.2586 and 435.2744, which suggested the molecular formula $C_{22}H_{30}O_7$ and $C_{22}H_{30}O_8$, respectively. Both compounds showed a singlet for H-5 in the $^1$H NMR spectrum, suggesting dihydroxylation of the aromatic ring. A detailed 2D NMR analysis resulted in identification of 25 as 7,8-dihydroxy-16-butanoxy-serrulat-19-oic acid and 27 as 7,8-dihydroxy-16-[(3-methylbutanoyl)-oxy]serrulat-19-oic acid (27). Both are new compounds, and $^1$H and $^{13}$C NMR resonances are shown in Table 2 and selected NOE and HMBC correlations are shown Figure 6. The material eluted as peak 26 showed an $[M + H]^+$ ion with $m/z$ 447.2745, which suggested the molecular formula $C_{22}H_{30}O_9$, but insufficient amounts of this metabolite prevented a complete structural elucidation.

**Identification of Additional Metabolites.** In addition to the metabolites correlated with $\alpha$-glucosidase and/or PTP1B inhibition, *vide supra*, other major metabolites from the three species were identified. Based on HRMS and NMR data, the material eluted with peak 1 from *E. gibosa* and peak 14 from *E. aff. drummondii* “Kalgoorlie” were both identified as dinatin (1/14), the material eluted with peaks 8 and 10 from *E. aff. drummondii* were identified as verbascoside (8) and 8-hydroxy-serrulat-14-en-19-oic acid (10), respectively. The material eluted with peak 15 from *E. aff. drummondii* “Kalgoorlie” was identified as jacobisin (15; a dimethoxylated flavonoid previously isolated from *Eremophila microtheca*), and the material eluted with peak 22 from *E. aff. drummondii* “Kalgoorlie” was identified as the only sesquiterpenoid, 7-hydroxy-acylamenene (22).

The material eluted as peak 7 from *E. aff. drummondii* “Kalgoorlie” showed a $[M + H]^+$ ion with $m/z$ 375.2155, which suggested the molecular formula $C_{22}H_{30}O_9$ and the $^1$H NMR spectrum showed resonances similar to those observed for dihydroxyserulateric acid (4). However, an additional methyl singlet ($\delta$ 2.02) and a downfield shift of the H-16 methylene resonance ($\delta$ 4.39) were consistent with acetylation at C-16. This was also in agreement with a $J_{\text{HMBC}}$ correlation from H-16 to the acetyl carbon (C 173.0), and 7 was thus identified as 8-hydroxy-16-acetoxy-serrulat-14-en-19-oic acid. Compound 7 is a new compound, and $^1$H and $^{13}$C NMR resonances are shown in Table 2, and selected NOE and HMBC correlations are shown in Figure 6.

The material eluted as peaks 16 and 18 from *E. aff. drummondii* “Kalgoorlie” showed $[M + H]^+$ ions with $m/z$ 363.1809 and 349.2014, which suggested the molecular formulae $C_{20}H_{26}O_6$ and $C_{20}H_{28}O_7$, respectively. The $^1$H NMR spectra of these metabolites showed a single aromatic resonance (unlike the serrulatanes 4, 6, 7, 11a, 11b, and 12, where two meta-coupled aromatic resonances were observed), and together with the $^{13}$C NMR chemical shift values of the aromatic carbons measured indirectly from HMBC experiments, this showed hydroxylation of the serrulatane core at C-7 and C-8. After establishing the necessary correlations using NOE and HMBC experiments (Figure 6), 16 was identified as 3,7,8-trihydroxy-16-oxoserrulat-14-en-19-oic acid and 18 as 3,7,8-trihydroxy-serrulat-14-en-19-oic acid. Compound 16 and 18 are new compounds, and $^1$H and $^{13}$C resonances are shown in Table 2. On the basis of HRMS data, peak 17 was assigned the molecular formula $C_{22}H_{30}O_7$, but the amount of material did not allow structural elucidation.

Preparative-scale isolation afforded material for determination of IC$_{50}$ values toward PTP1B for 12, 20, 25, and 27. IC$_{50}$ curves are shown in Figure S55 (Supporting Information) and resulted in the following IC$_{50}$ values: 12: 6.27 ± 1.31 μM; 20: 1260 ± 561 μM; 25: 7.67 ± 1.22 μM; and 27: 3.44 ± 0.88 μM. In comparison, the IC$_{50}$ value of the reference compound RK682 was 4.4 ± 0.3 μM. This shows that especially 12, 25, and 27, all serrulatanes substituted at C-16, are potent PTP1B inhibitors. With IC$_{50}$ values in the low μM range, their potency is comparable with the triterpenoids rhodendric acid A (6.3 μM) and 23-hydroxyursolic acid (7.4 μM) isolated from *Rhododendron brachycarpum*, the sesquiterpenoid dihydroav-losa C (1.5 μM) isolated from *Disidea villosa*, the diterpenoids lobophytin E (5.9 μM) and lobophytin C (7.8 μM) isolated from *Lobophytum cristatum*, the diterpenoid cyclonoside A (5.5 μM) isolated from *Cyclocarya paliurus*, and the benzophenanthro-thenenes oshiosin A, C, F, and G (3.5–7.6 μM) isolated from *Polystichastrum alpinum*.

In conclusion, the use of high-resolution $\alpha$-glucosidase and high-resolution PTP1B inhibition profiling combined with HPLC-HRMS-SPE-NMR resulted in identification of potential antidiabetic metabolites from three *Eremophila* species. A total of 21 compounds, including 12 new serrulatanes, were identified from the three species. Serrulatanes are the most common diterpenoids isolated from *Eremophila* species, and several studies have reported their potential antibacterial activity. However, the current work represents the first report of serrulatanes as $\alpha$-glucosidase and PTP1B inhibitors. These molecules may serve as new molecular templates for further development of potent $\alpha$-glucosidase and PTP1B inhibitors, based on either chemical synthesis of structurally simpler functional analogues or using combinatorial biochemistry.

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**EXPERIMENTAL SECTION**

**General Experimental Procedures.** $p$-Nitrophenyl phosphate (pNPP), $p$-nitrophenyl $\alpha$-n-glucopyranoside (pNPG), $\alpha$-glucosidase type 1 (EC 3.2.2.0, from *Saccharomyces cerevisiae*, lyophilized powder), DMSO, tris(hydroxymethyl)-aminomethane (Tris), bis(2-hydroxyethyl)-imin-tris(hydroxymethylmethane) (bis-Tris), dithiothreitol (DTT), N,N,N,N'-ethylenediaminetetraacetate (EDTA), and HPLC-grade MeCN were purchased from Sigma-Aldrich (St. Louis, MO). Formic acid was purchased from Merck (Darmstadt, Germany). Recombinant human Protein Tyrosine Phosphatase 1B (PTP1B) (BML-SE332–0050, EC 3.1.3.48) was purchased from Enzo Life
Plant Material and Extraction. Leaves of *E. gibossa* Chinnock, *E. glabra* (R.Br.) Ostenf., and *E. aff. drummondii* “Kalgoorlie” were collected from plants growing in a greenhouse at the University of Copenhagen (Frederiksberg, Denmark) under ambient photoperiod and day/night temperatures of 24/17 °C. *E. glabra* and *E. aff. drummondii* “Kalgoorlie” plants were established from bare-rooted specimens purchased from a nursery in Melbourne, Australia. Voucher specimens of *E. glabra* and *E. gibossa* Hans Griesser (University of South Australia). Voucher specimens of plants growing in South Australia and were provided by Professor P. L. O. Davies, School of Biological Sciences, University of Melbourne, Australia. The specimens of *E. gibossa* were established from cuttings taken from plants growing in South Australia and were provided by Professor Hans Griesser (University of South Australia). Voucher specimens of *E. gibossa* and *E. glabra* were established from cuttings taken from plants growing in South Australia and were provided by Professor Hans Griesser (University of South Australia).

**Determination of Inhibitory Concentrations.** Dilution series of the crude extracts and the isolated compounds were dissolved in MeOH. Thereafter, 180 μL aliquots of each concentration in the dilution series were added to 96-well microplates in triplicate, and after evaporation, the PTP1B assay was performed by the method described below. Dose–response curves and IC₅₀ values were obtained using GraphPad prism, version 6.02.833 (GraphPad Software, Inc., La Jolla, CA).

**High-Resolution α-Glucosidase and PTP1B Inhibition Assays.** HPLC separation of crude *E. glabra*, *E. gibossa*, and *E. aff. drummondii* “Kalgoorlie” extracts for the high-resolution PTP1B biochromatograms were performed with an Agilent 1200 system (Santa Clara, CA) consisting of a G1311A quaternary pump, a G1322A degasser, a G1316A thermostat column compartment, a G1315C photodiode-array detector, a G1367C high-performance autosampler, and a G1364C fraction collector, all controlled by Agilent ChemStation version B.03.02.2 software. Analyses were performed at 40 °C on a 150 x 4.6 mm i.d. Phenomenex Luna C₁₈(2) reversed-phase column (3 μm particle size, 100 Å pore size) with a flow rate of 0.5 mL/min. HPLC solvent A consisted of H₂O-MeCN 95:5 with 0.1% formic acid and solvent B consisted of MeCN-H₂O 95:5 with 0.1% formic acid. A single injection of 5 μL crude extract of *E. glabra* (containing the equivalent of 1 μg crude extract) was separated using the following gradient elution profile: 0 min, 30% B; 50 min, 100% B; 60 min, 100% B; 61 min, 30%. A single injection of 6 μL of crude extract of *E. glabra* (corresponding to 120 μg of crude extract) was separated using the following gradient elution profile: 0 min, 10% B; 10 min, 20% B; 25 min, 85% B; 35 min, 100% B; 45 min, 100% B; 46 min, 10% B. A single injection of 4 μL of crude extract of *E. aff. drummondii* “Kalgoorlie” (corresponding to 240 μg of crude extract) was separated using the following gradient elution profile: 0 min, 35% B; 25 min, 65% B; 27 min, 85% B; 38 min, 100% B; 45 min, 100% B; 46 min, 35% B. The eluate from 10 to 45 min for each HPLC run was fractionated into two 96-well microplates (omitting the 8 wells in the last column of each well for blank controls), leading to a resolution of 5.0 data points per min. The content in the microplates were subsequently evaporated using a SPD121P Savant SpeedVac concentrator (Thermo Scientific, Waltham, MA) equipped with an OFP400 oil Free Pump and a RVT400 Refrigerated Vapor Trap. After evaporation, the PTP1B and α-glucosidase assay were performed on the collected material.

The PTP1B inhibition assay was performed at 25 °C using a two-component buffer, consisting of 50 mM Tris and 50 mM bis-Tris containing 100 mM NaCl adjusted to pH 7.0 with citric acid, using a final reaction volume of 180 μL. Residues in each well were dissolved in DMSO (18 μL) followed by addition of 52 μL of buffer containing 3.46 mM EDTA (final well concentrations 10% DMSO and 1 mM EDTA), and 60 μL of 1.5 mM pNPP and 6 mM DTT (final well concentrations 0.5 mM pNPP and 2 mM DTT). After preincubation at 25 °C for 10 min, the reaction was started by adding 50 μL of 0.001 μg/μL PTP1B stock solution (final well concentration: 0.05 μg/well). The amount of p-nitrophenol produced was determined by measuring the absorbance at 405 nm every 30 s for 10 min to yield enzyme activity (cleavage rate) as ΔΔAΔ/s. Preincubation, incubation, and absorbance measurements were performed with a Multiscan FC microplate photometer with built-in incubator (Thermo Scientific, Waltham, MA) coupled to SkanIt version 2.5.1 software for data acquisition. Inhibition of PTP1B activity was plotted against chromatographic retention time to give PTP1B high-resolution biochromatograms. The inhibition of PTP1B was calculated using the below equation:

\[
\text{%inhibition} = \left(1 - \frac{\text{SLOPE}_{\text{sample}}}{\text{SLOPE}_{\text{blank}}}\right) \times 100\%
\]

The α-glucosidase inhibition assay was performed at 25 °C in 0.1 M phosphate buffer pH 6.9 and a final volume of 200 μL. Residues in each well were dissolved in 50 μL 25% DMSO prior to addition of 100 μL 1.0 U/mL α-glucosidase solution (final well concentrations 6.25% DMSO and 0.25 U/mL α-glucosidase). After preincubation at 25 °C for 10 min, the reaction was started by adding 50 μL of 5 mM pNPG (final well concentration 1.25 mM pNPG). The p-nitrophenol concentration was determined by measuring the absorbance at 405 nm every 30 s for 10 min to yield enzyme activity (cleavage rate) as ΔΔA/s. Inhibition of α-glucosidase activity was plotted against chromatographic retention time to give α-glucosidase high-resolution biochromatograms. The inhibition of α-glucosidase was calculated using the below equation:

\[
\text{%inhibition} = \left(1 - \frac{\text{SLOPE}_{\text{sample}}}{\text{SLOPE}_{\text{blank}}}\right) \times 100\%
\]

**HPLC-HRMS-SPE-NMR Analyses.** HPLC-HRMS-SPE-NMR analyses were performed on a platform consisting of an Agilent 1260 chromatograph (Santa Clara, CA), a Bruker microOTOF-Q II mass spectrometer (Bruker Daltonik, Bremen, Germany), a Knauer Smartline 120 pump (Knauer, Berlin, Germany), a Spark Holland Prospekt-2 SPE unit (Spark Holland, Emmen, The Netherlands), a Gilson 215 liquid handler, and a Bruker Avance III 600 MHz NMR spectrometer. The Agilent 1260 system consisted of a degasser, a quaternary pump, an autosampler, a column oven, and a diode array detector—all operated with similar chromatographic conditions (column, solvent composition, temperature, flow rate, and elution profile) as described above. The column eluate was connected to a T-piece splitter directing 1% of the flow to the mass spectrometer and 99% of the flow to SPE trapping after postcolumn dilution with 1 mL/min flow of H₂O delivered with the Knauer pump. The microOTOF-Q II mass spectrometer, equipped with an ESI source, was operated in positive-ion mode using drying temperature of 200 °C, capillary voltage of 4100 V, nebulizer pressure of 2.0 bar, and drying gas flow of 7 L/min. A solution of sodium formate clusters was automatically injected in the beginning of each run to enable internal mass calibration. For each of the three plants, peaks were trapped cumulatively on 10 x 2 mm i.d. Resin GP (general purpose, 5–15 μm, spherical shape, polydivinyl-benzene phase) SPE cartridges from Spark Holland (Emmen, The Netherlands) after 10 identical separations using UV absorption-thresholds to trigger trapping. The SPE cartridges were conditioned with 1000 μL MeOH (at 6 mL/min) and equilibrated with 500 μL H₂O (at 1 mL/min) prior to trapping. The loaded SPE cartridges were dried with pressurized N₂ gas for 45 min each and subsequently eluted with CD₃OD into 1.7 mm NMR tubes with a Gilson 215 liquid handler equipped with a 1 mm needle. Chromatographic separation, mass spectrometry, and analyte trapping on SPE cartridges were controlled using Hystar vers. 3.2 software (Bruker Daltonik, Bremen, Germany), whereas the elution process was mediated by Prep Gilson ST vers. 1.2 software (Bruker Biospin, Karlsruhe, Germany).
Preparative-Scale Isolation and Purification. Solutions of E. gibosa (232 mg/mL), E. glabra (108 mg/mL), and E. aff. drummondii “Kalgoorlie” (244 mg/mL) were prepared in MeOH. Injection volumes of 0.9 mL for all three species were used for preparative-scale isolation on an Agilent 1100 HPLC system equipped with two preparative-scale solvent delivery pumps, a multiple wavelength detector, and an autosampler. All separations were performed using a 250 mm × 21.2 mm i.d. Phenomenex Luna C18 (5 μm) column operated at room temperature. A gradient elution (similar to the one used for high-resolution screening) was used, and targeted peaks were collected manually. The collected peaks were concentrated under reduced pressure and lyophilized to a final purity.

NMR Experiments. The NMR experiments were recorded at 300 K either in methanol-d₄ (for material analyzed in the HPLC-HRMS-SPE-NMR mode) or in CDCl₃ (for material isolated on preparative scale). NMR experiments were acquired using either a Bruker Avance III HD spectrometer equipped with a cryogenically cooled 5 mm DCH probe optimized for 13C and 1H. All NMR experiments were acquired with 30°-pulses and 64k data points. Two dimensional homo- and heteronuclear experiments were acquired with 2048 data points in the direct dimension and 128 (HMBC) or 512 (DQF-COSY) in the indirect dimension, with spectral widths optimized from the corresponding 1H NMR spectra. The HMBC and HSQC experiments were optimized for 2J_HC = 8 Hz and 2J_HC = 145 Hz, respectively.

ASSOCIATED CONTENT

9 Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b01128.

Table S1 with retention time, HRMS and 1H NMR data of the material eluted with peaks 1–27, Figure S1–S54 with NMR spectra of new compounds identified in this work, and Figure S55 with IC₅₀ curves of 12, 20, 25, and 27 (PDF).

AUTHOR INFORMATION

Corresponding Author
E-mail: ds@sund.ku.dk. Phone: +45 3533 6177. Fax: +45 3533 6041.

Notes
The authors declare no competing financial interest.

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