Epunctanone, a New Benzophenone, and Further Secondary Metabolites from *Garcinia epunctata* STAPF (Guttiferae)

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A new polyprenylated benzophenone, named epunctanone (**1**), was isolated from the stem bark of *Garcinia epunctata* STAPF, together with eight known compounds, 7-epiisogarcinol (**2**), 2,6-dimethoxy-*p*-benzoquinone (**3**), friedelin (**4**), lupeol (**5**), 16β-hydroxylupeol (**6**), betulin (**7**), stigmasterol (**8**), and rheediaxanthone A (**9**). The structure of epunctanone (**1**) was established by detailed analysis of its spectroscopic data, especially 1D- and 2D-NMR, and HR-MS data. All these compounds were evaluated for their antimicrobial and anti-protozoan activities. They were also assayed to determine if any of the compounds were nonpeptide agonist ligands for nematodal G-protein-coupled receptors, which would be an indication of potential antinematodal activity. Among the isolated compounds, 7-epiisogarcinol (**2**) was the most active against *Candida albicans*.

Introduction. – *Garcinia epunctata* STAPF (syn. *Garcinia preussii* ENGL.) is a medicinal plant used in Cameroonian folk medicine for the treatment of stomach aches [1]. The genus *Garcinia* (Guttiferae) is known to produce a variety of biologically active secondary metabolites. Many biflavonoids with leishmanicidal, antiproteolytic, antioxidant, and antibacterial properties have been isolated from this genus [2][3], Polycyclic polyprenylated acylphloroglucinols (PPAPs) and xanthones with antimalarial, antibacterial, anticancer, cytotoxic, gastroprotective, or antioxidant activities were also isolated from different *Garcinia* species such as *Garcinia polyantha* [4], *G. Cowa* [5][6], *G. dulcis* [7], *G. hanburyi* [8], *G. indica* [9], *G. brasiliensis* [10], and *G. achachairu* [11]. Few triterpenes, lanosterol [4][12][13], and quinines [14] have been isolated so far from *Garcinia* species. As part of our continuous work on the plants with antimicrobial activities [15–18], we investigated the stem bark of *G. epunctata*. To the best of our knowledge, only one compound has so far been reported from this species [19].

Results and Discussion. – The air-dried and powdered stem bark of *Garcinia epunctata* (1,300 g) was sequentially extracted with CH_2Cl_2/MeOH 1:1 and MeOH to give 104.2 and 30.4 g of extracts, respectively, which were further combined on the basis of their TLC profile, then partitioned between petroleum ether (PE), $CHCl_3$, AcOEt, and BuOH. Extensive column chromatography of petroleum ether, $CHCl_3$, and AcOEt extracts yielded a new benzophenone, epunctanone (**1**), and eight known compounds, 7-epiisogarcinol (**2**) [20], 2,6-dimethoxy-*p*-benzoquinone (**3**) [21], friedelin (**4**) [22], lupeol (**5**) [13], 16β-hydroxylupeol (**6**) [23], betulin (**7**) [24], stigmasterol (**8**) [22], and rheediaxanthone A (**9**) [25] (*Fig. 1*). Herein, we describe the isolation and structure elucidation of the new compound, as well as the evaluation of the antimicrobial, antiprotozoa, and antinematodal activities of extracts and some isolated pure compounds.

Epunctanone (**1**) was isolated as brown powder. The molecular formula $C_{38}H_{52}O_6$ was deduced from HR-EI-MS (molecular-ion peak at *m*/*z* 604.3619). The UV spectrum showed absorption bands at 240, 276, and 440 nm consistent with aromatic rings and

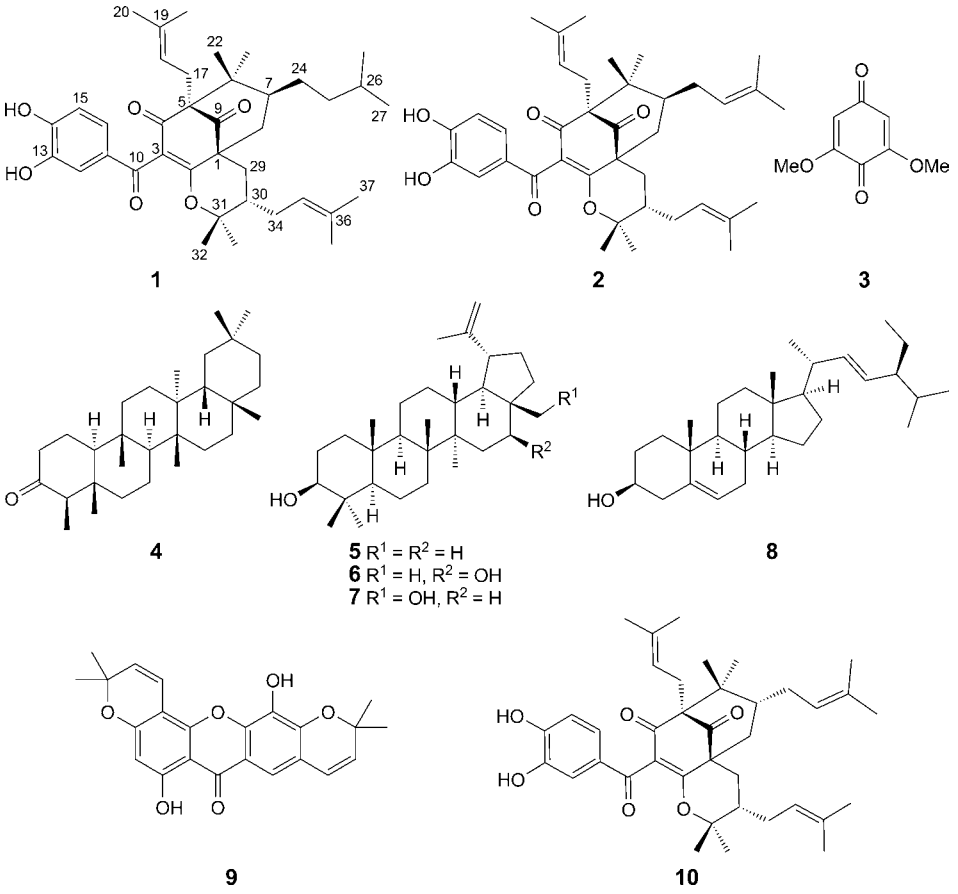

Fig. 1. *Structures of compounds* **1**–**10**

conjugated C=O groups [5][6]. The IR spectrum exhibited bands for OH (3350 cm^{-1}), α,β-unsaturated C=O (1650, 1670 cm^{-1}), and keto (1725 cm^{-1}) groups, and aromatic rings (1590 cm^{-1}). This information was confirmed by ^{13}C-NMR spectrum of **1** which showed the signals of two α,β-unsaturated C=O groups and a keto C=O group at δ(C) 195.3 and 192.2, and 208.5, respectively. These data coupled with two quaternary C-atom signals at δ(C) 69.4 (C(5)) and 46.9 (C(6)) were consistent with a polycyclic polyprenylated acylphloroglucinol (PPAP) structure [20][26]. The ^{1}H-NMR spectra revealed the presence of a 1,3,4-trisubstituted benzene ring with three aromatic H-atom signals appearing as an *ABC* system at δ(H) 7.38 (*d*, $J = 1.8$, H–C(12)), 6.83 (*d*, $J = 8.1$, H–C(15)), and 7.10 (*dd*, $J = 8.1$, 1.8, H–C(16)) and ten Me signals at δ(H) 1.02 (*s*, Me(22)), 1.15 (*s*, Me(23)), 0.86 (*s*, Me(32)), 0.93 (*s*, Me(33)), 1.70 (*s*, Me(37)), 1.72 (*s*, Me(38)), 1.58 (*s*, Me(20)), 1.63 (*s*, Me(21)), and 0.95 (*s*, Me(27,28)). The ^{13}C-NMR and DEPT spectra of epunctanone exhibited 38 signals (*Table 1*), including those of 14 quaternary C-atoms (including two α,β-unsaturated C=O signals at δ(C) 195.3 and 192.2, and one of the isolated C=O group at δ(C) 208.5), eight CH, six CH_2, and ten Me C-atoms. The spectroscopic data of epunctanone (**1**) were closely similar to those of 7-epiisogarcinol (**2**) and isogarcinol (**10**), previously isolated from the root barks of *Symphonia globulifera* [26] and from the trunk latex of *Moronobea coccinea* [20], respectively. However, the molecular formula of epunctanone ($C_{38}H_{52}O_6$) compared to those of isogarcinol or 7-epiisogarcinol ($C_{38}H_{50}O_6$) suggested that one of the three

Table 1. *^{1}H- and ^{13}C-NMR Data* (300 and 75 MHz, resp.) *of* **1** in (D_6)acetone[a]). δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	–	48.5 (*s*)	20	1.63 (*s*)	26.4 (*q*)
2	–	171.5 (*s*)	21	1.58 (*s*)	18.3 (*q*)
3	–	128.5 (*s*)	22	1.02 (*s*)	26.8 (*q*)
4	–	195.3 (*s*)	23	1.15 (*s*)	22.4 (*q*)
5	–	69.4 (*s*)	24	1.25–1.35 (*m*),	26.7 (*t*)
6	–	46.9 (*s*)		1.40–1.50 (*m*)	
7	1.58–1.62 (*m*)	37.6 (*d*)	25	1.15–1.23 (*m*)	38.4 (*t*)
8	2.00–2.12 (*m*),	42.4 (*t*)	26	1.65–1.76 (*m*)	31.1 (*d*)
	2.61–2.72 (*m*)		27	0.95 (*d*, $J = 10.1$)	27.8 (*q*)
9	–	208.5 (*s*)	28	0.95 (*d*, $J = 10.1$)	27.8 (*q*)
10	–	192.2 (*s*)	29	2.20 (*dd*, $J = 15.3$, 13.9),	30.2 (*t*)
11	–	131.1 (*s*)		3.00 (br. *s*)	
12	7.38 (*d*, $J = 1.8$)	115.9 (*d*)	30	1.55–1.63 (*m*)	47.0 (*d*)
13	–	145.8 (*s*)	31	–	84.3 (*s*)
14	–	151.2 (*s*)	32	0.93 (*s*)	29.1 (*q*)
15	6.83 (*d*, $J = 8.1$)	115.4 (*d*)	33	0.86 (*s*)	32.4 (*q*)
16	7.10 (*d*, $J = 8.1$, 1.8)	123.9 (*d*)	34	1.42 (*s*)	30.0 (*t*)
17	2.48 (*dd*, $J = 13.5$, 8.1),	26.3 (*t*)	35	5.03 (br. *t*, $J = 8.7$)	126.1 (*d*)
	2.65 (*dd*, $J = 13.5$, 8.4)		36	–	133.3 (*s*)
18	4.98 (br. *t*, $J = 8.7$)	121.3 (*d*)	37	1.70 (*s*)	26.1 (*q*)
19	–	134.5 (*s*)	38	1.72 (*s*)	18.6 (*q*)

[a]) All assignments are based on the ^{1}H,^{1}H-COSY, HMQC, and HMBC data.

prenyl group of isogarcinol or 7-epiisogarcinol should be hydrogenated. This information was confirmed by the ^{1}H-NMR spectra which showed only two vinylic H-atom signals at δ(H) 4.98 (br. *t*, *J* = 7.0, H–C(18)) and 5.03 (br. *t*, *J* = 7.0, H–C(35)). The location of the first prenyl side chain at C(5) was established by correlations between the CH_2 H-atom signals at δ(H) 2.48, 2.65 (CH_2(17)) and the C-atom signals at δ(C) 195.3 (C(4)), 69.4 (C(5)), 46.9 (C(6)), and 121.3 (C(18)) in the HMBC spectrum. The second prenyl was fixed at C(30) of the pyran ring according to the HMBCs between the CH_2 H-atom signals at δ(H) 1.42 (CH_2(34)), and the C-atom signals at δ(C) 30.2 (C(29)), 47.0 (C(30)), 84.3 (C(31)), and 126.1 (C(35)). The dihydroprenyl group (δ(H)/δ(C) 1.25 – 1.35 and 1.40 – 1.50/26.7 (CH_2(24)); 1.15 – 1.23/38.4 (CH_2(25)); 1.65 – 1.76/31.1 (H–C(26)), and 0.95/27.8 (Me(27,28))) was then located at C(7) and confirmed by the HMBC between the diastereotopic H-atom signals at δ(H) 1.25 – 1.35 and 1.40 – 1.50 (CH_2(24)) with the C-atom signals at δ(C) 46.9 (C(6)), 37.6 (C(7)), and 42.4 (C(8)). According to *Ciochina* and *Grossman*, and other authors [27 – 29], the orientation of the substituent at C(7) (axial or equatorial) can be deduced from the ^{1}H- and ^{13}C-NMR data based on the chemical shifts of Me(22) and Me(23). If the substituent at C(7) is axial, the ranges will be δ(C) 26 – 28 for Me_{ax}(22), and δ(C) 22 – 25 for Me_{eq}(23), while, if the substituent at C(7) is equatorial, the ranges will be δ(C) 15 – 17 for Me_{ax}(22) and δ(C) 22 – 24 for Me_{eq}(23). In epunctanone (**1**), the chemical shift of Me(22) (δ(C) 26.8) and Me(23) (δ(C) 22.4) evidenced the axial orientation of the dihydroprenyl group at C(7). This information was confirmed by the NOESY spectrum where correlations were observed between H_{ax}–C(8), H–C(7), and Me_{ax}(22) (δ(H) 2.00 – 2.12, 1.58 – 1.62, and 1.02, resp.). Taking into account the optical rotation ($[\alpha]_D = +24.9$) and published data for related compounds [30], structure **1**, depitced in *Fig. 1*, was proposed for epunctanone. To the best of our knowledge, **1** is a new natural compound. Key HMBC and NOESY correlations of epunctanone (**1**) are shown in *Fig. 2*.

To gain information on potentially interesting bioactivities of compounds **1** – **9**, an initial qualitative bioactivity profile was generated characterizing antimicrobial properties of the compounds (*Table 2*). Anti-protozoan (using the kinetoplatides model organism *Bodo caudatus*) activities of compounds serve as indicators for potential use as drug candidates for leishmaniasis, human African trypanosomiasis (HAT), and American trypanosomiasis or *Chagas* disease [31]. Yeast infections, such as those due *Candida albicans*, constitute one of the major opportunistic infections in immune-compromised patients. Up to 90% of HIV-infected individuals suffer from at least one episode of candidiasis, which is most commonly characterized by oral thrush

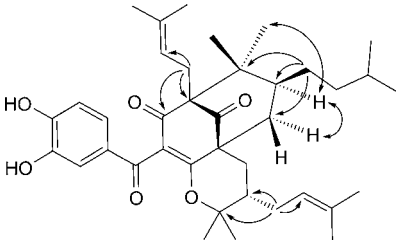

Fig. 2. *Key HMBCs* (H → C) *and NOE* (H ↔ H) *correlations of compound* **1**

Table 2. *Qualitative Bioactivity Screening of the Isolated Compounds.* +++ : active; ++ : moderately active; + : weakly active; − : not active.

Compound	Anti-yeast	Anti-protozoa	Anti-mycobacterial	Anti-gonococcal
	Candida albicans	*Bodo caudatus*	*Mycobacterium aurum*	*Neisseria gonorrhoeae*
1	−	−	−	++
2	+++	−	−	++
3	+	−	−	−
4	−	−	−	−
5	++	−	+	+
6	+	−	−	−
7	+	−	−	−
8	−	−	−	−
9	−	−	−	−

[32]. *Mycobacterium aurum* is a fast-growing, nonpathogenic mycobacterium, which has a very similar drug susceptibility characteristics as *Mycobacterium tuberculosis*, the causative agent of tuberculosis, and has, therefore, been recommended as the most suitable model organism to identify new potential therapeutics [33][34]. Additionally, compounds were assessed for their capacity to act as nematode-specific G-protein-coupled receptor (GPCRs) agonists.

None of the isolated compounds were active against the protozoa *Bodo caudatus.* They did also not turn out as potential activators of nematode-specific GPCR. However, epunctanone (**1**), 7-epiisogarcinol (**2**), and lupeol (**5**) showed weak-to-moderate activities against *Mycobacterium aurum*, *Neisseria gonorrhoeae*, and *Candida albicans* in the qualitative screening as compiled in *Table 2*. 7-Epiisogarcinol (**2**), which displayed inhibitory activity against *C. albicans* in this preliminary screening, was tested further to determine its minimum inhibitory concentration (*MIC*) and minimal mycocidal concentration. The *MIC* value of 2 mM was rather weak, and it was found that the antimicrobial property of **2** was mycostatic, but not mycocidal. Epunctanone (**1**) showed no activity, indicating that the C=C bond between C(25) and C(26) might play an essential role in the anti-yeast activity of these benzophenones.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 0.04–0.063 mm) (*Merck*) or *Sephadex LH-20* (*Sigma*). TLC: Precoated alumina sheets of SiO_2 F_{254} and $PF_{254+366}$ (*Merck*); detection under UV light and spraying with vanillin H_2SO_4. M.p.: *Stuart Scientific* (*SMP1*) melting-point apparatus. Optical rotations: *PerkinElmer 241* polarimeter at 25°. UV Spectra: *Shimadzu UV-210* PC UV/VIS spectrometer in acetone soln.; λ_{max} (log ε) in nm. IR Spectra: *Shimadzu FT-IR-8700 Fourier* transform infrared spectrometer as KBr discs; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker DMX Avance* spectrometers

operating at 300 and 75 MHz, and 600 and 150 MHz, resp.; in $CDCl_3$, (D_6)acetone, or (D_6)DMSO, with the residual solvent peak as internal references, δ in ppm, J in Hz. HR-EI-MS: *Waters GCT-Premier* spectrometer. EI-TOF-MS (pos.): *Finnigan SSQ-7000* spectrometer by direct inlet (70 eV); *m/z*.

Plant Material. The stem barks of *G. epunctata* were collected in July 2011 at Eloumden-Yaoundé in central Cameroon and identified by Mr. *Victor Nana* at the National Herbarium where a voucher specimen (19534/SRFCam) was deposited.

Extraction and Isolation. The air-dried and powdered stem bark of *G. epunctata* (1300 g) was macerated in CH_2Cl_2/MeOH 1:1 for 48 h. The solvent was evaporated under reduced pressure to give 104.2 g of crude extract. The residue was extracted with 2 l of MeOH at r.t. for 24 h to give 30.4 g of crude extract. The two extracts were mixed on the basis of their TLC profile to give a total of 134.6 g of crude extract. This extract was dissolved in MeOH/H_2O 2:1 and partitioned using (3×800 ml) each of petroleum ether (PE), $CHCl_3$, AcOEt, and BuOH to give 28.3, 6.6, 8.1, and 91.6 g of extracts, resp. The PE and $CHCl_3$ extracts were combined on the basis of their TLC profiles, and 30 g were subjected to CC (SiO_2; hexane/AcOEt gradients, 2 ml/min). Ninety-five fractions of 150 ml each were collected and combined on the basis of their TLC profile to seven subfractions, *Frs. 1–7* as follows *Fr. 1* (1–6), *2* (7–14), *3* (15–33), *4* (34–50), *5* (51–62), *6* (63–80), and *7* (81–95). *Frs. 1–4* were further purified by CC (SiO_2; hexane/AcOEt gradients) and prep. TLC. From *Fr. 1*, *friedelin* (**4**; 67.2 mg) and *lupeol* (**5**; 18.1 mg) were isolated. *Fr. 2* afforded *stigmasterol* (**8**; 20.1 mg), while *rheediaxanthone A* (**9**; 10.5 mg) was isolated from *Fr. 3*. From *Fr. 4*, *16β-hydroxylupeol* (**6**; 171.8 mg) and *betulin* (**7**; 7.5 mg) were isolated. *Frs. 6* and *7*, resp., were dissolved in MeOH, and subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 7:3). *Fr. 6* afforded *epunctanone* (**1**; 81.7 mg), while *7-epiisogarcinol* (**2**, 42.7 mg) was isolated from *Fr. 7*. The AcOEt extract was also investigated by CC (SiO_2; hexane/AcOEt gradients, 2 ml/min). *2,6-Dimethoxy*-p-*benzoquinone* (**3**; 2.1 mg) and *16β-hydroxylupeol* (**6**; 23.9 mg) were isolated.

Epunctanone (=*(3*S*,4a*S*,6*S*)-10-(3,4-Dihydroxybenzoyl)-3,4,5,6,7,8-hexahydro-2,2,7,7-tetramethyl-3,8-bis(3-methylbut-2-en-1-yl)-6-(3-methylbutyl)-2*H*,9*H*-4a,8-methanocycloocta[*b*]pyran-9,11-dione*; **1**). Brown crystals. M.p. 255°. $[\alpha]_D^{25} = +24.9$ ($c = 0.00985$, acetone). UV (acetone): 440 (0.015), 276 (9.99), 240 (9.90). IR (KBr): 3280, 2910, 2840, 1725, 1670, 1590, 1525, 1440, 1360, 1300, 1160. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 604.3499 (M^+, $C_{38}H_{52}O_6^+$; calc. 604.3764).

Biological Screening. Compounds were assayed at a concentration of 2 mM unless otherwise stated.

Antimicrobial Assays. Neisseria gonorrhoeae (ATCC 49226, *MicroBioLogics*, Minnesota, USA), *Mycobacterium aurum* A+ (kindly donated by Prof. *Pete Smith*, Department of Pharmacology, University of Cape Town, Medical School, Cape Town, South Africa), *Escherichia coli* (a kind gift from Mrs. *O. Tagwa*, Department of Biological Sciences, University of Botswana), and *Candida albicans* (kindly provided by Mr. *M. Morobe*, Department of Biological Sciences, University of Botswana) were used as test organisms. *Bodo caudatus* (kindly donated by the Global Institute for Bioexploration (GIBEX), Rutgers University, New Brunswick, NJ, USA) was also used. *E. coli* and *N. gonorrhoeae* were grown in nutrient broth (*HiMedia*, South Africa) at 37° for 24 and 48 h, resp. *M. aurum* was cultured in *Middlebrook 7H9* broth (*Difco Laboratories*, Detroit, USA), supplemented with 10% OADC (oleic acid/albumin/dextrose/catalase; *Difco Laboratories*), at 32° for 48 h. *C. albicans* was grown in *Sabouraud* dextrose broth (*HiMedia*, South Africa) at 32° for 48 h. Bacteria were maintained on agar plates at 4° or stored as glycerol stocks at −80°. Sterile molten agar medium (400 µl) was pipetted into each well of a sterile 24-well culture plate (*Corning*) and left to solidify. Bacteria or yeast (*Candida*) were grown in their respective broth media to a density of 1×10^6 cfu/ml. 10 µl of culture were pipetted onto each well of the agar plates. EtOH (60% aq. soln.; *B&M*, *Scientific*, Cape Town, South Africa) was the negative control across all antimicrobial assays and the anti-protozoan assay. For *N. gonorrhea*, kanamycin sulfate (100 µg/ml; *Sigma*) served as the positive control. For *M. aurum* and *C. albicans*, the positive controls were rifampicin (2 mg/ml; *Sigma*) and penicillin/streptomycin/fungizone (PSF, 100x; *Highveld Biological*, South Africa), resp. 10 µl of EtOH, antibiotic, or compound were pipetted onto the surface of the agar in duplicate wells. The plates were later inverted and incubated in a moist chamber. Inhibition was indicated by a clear area on the agar's surface, while visible growth of colonies indicated no/poor inhibition.

Compounds showing strong inhibitory activities in this preliminary screen were tested further to determine their minimum inhibitory and mycocidal concentrations (*MIC*s and *MMC*s, resp.). *MIC*

Values were determined by the microplate serial dilution method [35][36]. After incubation, *MIC* values were determined as the lowest concentrations at which growth inhibition occurred. To distinguish mycostatic from mycocidal effects of the compounds, 50 µl aliquots were taken from each well and spread evenly onto respective agar plates. The concentration at which a reduction of $\geq 99\%$ colony growth of microorganisms occurred was determined as minimal mycocidal concentration (*MMC*). Penicillin/streptomycin/fungizone (*PSF*, 100x) was used as positive control.

B. caudatus was grown to a density of $10 \cdot 10^4$ cells/ml in cereal grass medium (*Hay* medium *Ward*'s # *88v5205*) inoculated with *E. coli.*, kept in 250-ml tissue culture flasks at 25°, and a fresh culture was incubated for 72 h before being used for the assay. 100 µl of the culture were added to each well of a round-bottomed 96-well plate, and 10 µl of a mixture of 5,000 units/ml of penicillin, streptomycin, and 100 µg/ml of kanamycin were also added. 10 µl of each compound were added to each test well, and $CuSO_4$ served as a positive control at 0.6 mg/ml. The plates were incubated for 4 h after which 5 µl of 5 mg/ml of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) was added to each well. After addition of MTT, the plate was incubated for a further 12 h. Inhibition of growth was indicated by a yellow color, and growth of the protozoa resulted in a purple colorm and a collection of protozoan cells (seen as a purple spot) at the bottom of the well.

Antinematodal Assay. A set of four yeast strains of *Saccharomyces cerevisae* (obtained from Prof. *T. Geary*, Institute of Parasitology, McGill University, Canada), expressing three nematodal (*C. elegans*, Ce4; *C. elegans 19*, Ce19; *C elegans 50*, Ce50) and one insect (*Drosophila melanogaster*, DM4) G-protein-coupled receptors serving as control, were used for the antinematodal assay. Each yeast strain contained only one of the four receptors. The growth of the yeast strains was coupled to the activation of the G-protein coupled receptor such that the yeast cannot grow without the activation of the receptor or addition of histidine to the media. An overnight culture of each yeast strain was grown in media containing Yeast Nitrogen Base (YNB; *Sigma*, *Y-0626*), Yeast Synthetic Drop-Out Medium without amino acids (*Sigma*, *Y-2001*), dextrose (*Fischer Scientific*), histidine (*Sigma*, *H6034*), leucine (*Sigma*, *L8000*), tryptophan (*Sigma*, *T0254*), uracil (*Sigma*, *U1128*), and MOPS (*Fischer Scientific*, *BP308*). The culture was then diluted to $15 \cdot 10^4$ cells/ml in growth media without histidine. 100 µl of yeast culture were pipetted into individual wells of a 96-well plate. 2 µl of each compound were added to each test well and incubated for 48 h, followed by addition of 20 µl of the growth indicator dye alamar blue. A change in color from blue to pink indicated growth of yeast showing that the compound had activated the receptor enabling yeast proliferation. A negative result showing no growth of the yeast is indicated by a blue color. The positive control was the ligand flp18 for Ce4, Ce19, and Ce50, and the ligand allatostatin for DM4, the negative control was 2 µl of the solvent DMSO used to dissolve the compounds.

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