Antibacterial Cannabinoids from *Cannabis sativa*: A Structure−Activity Study

Giovanni Appendino,*[,†,‡] Simon Gibbons,*[,⊥] Anna Giana,[†,‡] Alberto Pagani,[†,‡] Gianpaolo Grassi,[§] Michael Stavri,[⊥] Eileen Smith,[⊥] and M. Mukhlesur Rahman[⊥]

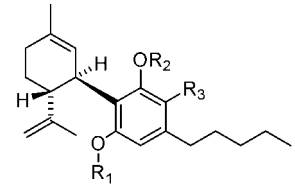
Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy, Consorzio per lo Studio dei Metaboliti Secondari (CSMS), Viale S. Ignazio 13, 09123 Cagliari, Italy, Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, U.K., and CRA-CIN Centro di Ricerca per le Colture Industriali, Sede distaccata di Rovigo, Via Amendola 82, 45100 Rovigo, Italy

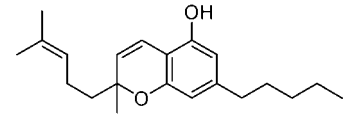
Received May 1, 2008

Marijuana (*Cannabis sativa*) has long been known to contain antibacterial cannabinoids, whose potential to address antibiotic resistance has not yet been investigated. All five major cannabinoids (cannabidiol (**1b**), cannabichromene (**2**), cannabigerol (**3b**), Δ^9-tetrahydrocannabinol (**4b**), and cannabinol (**5**)) showed potent activity against a variety of methicillin-resistant *Staphylococcus aureus* (MRSA) strains of current clinical relevance. Activity was remarkably tolerant to the nature of the prenyl moiety, to its relative position compared to the *n*-pentyl moiety (abnormal cannabinoids), and to carboxylation of the resorcinyl moiety (pre-cannabinoids). Conversely, methylation and acetylation of the phenolic hydroxyls, esterification of the carboxylic group of pre-cannabinoids, and introduction of a second prenyl moiety were all detrimental for antibacterial activity. Taken together, these observations suggest that the prenyl moiety of cannabinoids serves mainly as a modulator of lipid affinity for the olivetol core, a *per se* poorly active antibacterial pharmacophore, while their high potency definitely suggests a specific, but yet elusive, mechanism of activity.

Several studies have associated the abuse of marijuana (*Cannabis sativa* L. Cannabinaceae) with an increase in opportunistic infections,[1] and inhalation of marijuana has indeed been shown to interfere with the production of nitric oxide from pulmonary macrophages, impairing the respiratory defense mechanisms against pathogens and causing immunosuppression.[2] The association of *C. sativa* with a decreased protection against bacterial infections is paradoxical, since this plant has long been known to contain powerful antibacterial agents.[3] Thus, preparations from *C. sativa* were investigated extensively in the 1950s as highly active topical antiseptic agents for the oral cavity and the skin and as antitubercular agents.[3] Unfortunately, most of these investigations were done at a time when the phytochemistry of *Cannabis* was still in its infancy, and the remarkable antibacterial profile of the plant could not be related to any single, structurally defined and specific constituent. Evidence that pre-cannabidiol (**1a**) is a powerful plant antibiotic was, nevertheless, obtained,[4] and more recent investigations have demonstrated, to various degrees, antibacterial activity for the nonpsychotropic cannabinoids cannabichromene (CBC, **2**),[5] cannabigerol (CBG, **3b**),[6] and cannabidiol (**1b**),[7] as well as for the psychotropic agent Δ^9-tetrahydrocannabinol (THC, **4b**).[7] These observations, and the inactivity of several noncannabinoid constituents of *C. sativa* as antibacterial agents, suggest that cannabinoids and their precursors are the most likely antibacterial agents present in *C. sativa* preparations.[8] However, differences in bacterial strains and end-points make it difficult to compare the data reported in these scattered studies, and the overall value of *C. sativa* as an antibacterial agent is therefore not easy to assess.

There are currently considerable challenges with the treatment of infections caused by strains of clinically relevant bacteria that show multidrug-resistance (MDR), such as methicillin-resistant *Staphylococcus aureus* (MRSA) and the recently emerged and extremely drug-resistant *Mycobacterium tuberculosis* XDR-TB. New antibacterials are therefore urgently needed, but only one new class of antibacterial has been introduced in the last 30 years.[9] Despite the excellent antibacterial activity of many plant secondary metabolites[10] and the ability of some of them to modify the resistance associated with MDR strains[11] and efflux pumps,[12] plants are still a substantially untapped source of antimicrobial agents.





2

	R_1	R_2	R_3
1a	H	H	COOH
1b	H	H	H
1c	Ac	Ac	H
1d	Me	H	H
1e	Me	Me	H
1f	H	H	COOMe
1g	H	H	$COOCH_2CH_2Ph$

These considerations, as well as the observation that cross-resistance to microbial and plant antibacterial agents is rare,[10] make *C. sativa* a potential source of compounds to address antibiotic resistance, one of the most urgent issues in antimicrobial therapy. To obtain structure−activity data and define a possible microbiocidal cannabinoid pharmacophore, we investigated the antibacterial profile of the five major cannabinoids, of their alkylation and acylation products, and of a selection of their carboxylic precursors (pre-cannabinoids) and synthetic positional isomers (abnormal cannabinoids).

Results and Discussion

The antibacterial cannabinoid chemotype is poorly defined, as is the molecular mechanism of its activity. Since many simple phenols show antimicrobial properties, it does not seem unreasonable to assume that the resorcinol moiety of cannabinoids serves as the antibacterial pharmacophore, with the alkyl, terpenoid, and carboxylic appendices modulating its activity. To gain insight into

* To whom correspondence should be addressed. Tel: +39 0321 373744 (G.A.); +44 207 753 5913 (S.G.). Fax: +39 0321 375621 (G.A.); +44 207 753 5909 (S.G.). E-mail: appendino@pharm.unipmn.it (G.A.); simon.gibbons@pharmacy.ac.uk (S.G.).

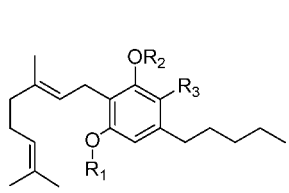
† Università del Piemonte Orientale.

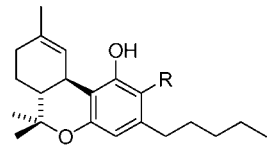
‡ Consorzio per lo Studio dei Metaboliti Secondari.

⊥ University of London.

§ Centro Ricerca Colture Industriali.

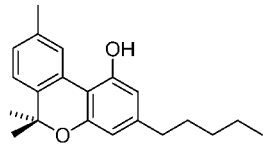
10.1021/np8002673 CCC: $40.75
Published on Web 08/06/2008



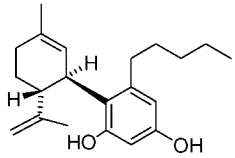


	R_1	R_2	R_3
3a	H	H	COOH
3b	H	H	H
3c	Ac	Ac	H
3d	Me	H	H
3e	Me	Me	H
3f	H	H	COOMe
3g	H	H	$COOCH_2CH_2Ph$

	R
4a	COOH
4b	H



5



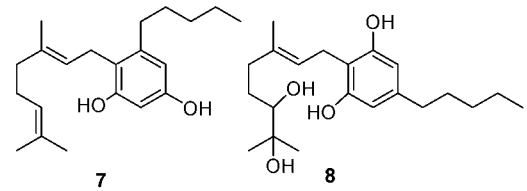
6

the microbiocidal cannabinoid pharmacophore, we have investigated how the nature of the terpenoid moiety, its relative position compared to the *n*-pentyl group, and the effect of carboxylation of the resorcinyl moiety are translated biologically, assaying the major cannabinoids and a selection of their precursors and regioisomeric analogues against drug-resistant bacteria of clinical relevance. Within these, we have selected a panel of clinically relevant *Staphylococcus aureus* strains that includes the (in)famous EMRSA-15, one of the main epidemic methicillin-resistant strains,[13] and SA-1199B, a multidrug-resistant strain that overexpresses the NorA efflux mechanism, the best characterized antibiotic efflux pump in this species.[14] SA-1199B also possesses a gyrase mutation that, in addition to NorA, confers a high level of resistance to certain fluoroquinolones. A macrolide-resistant strain (RN4220),[15] a tetracycline-resistant line overexpressing the TetK efflux pump (XU212),[16] and a standard laboratory strain (ATCC25923) completed the bacterial panel.

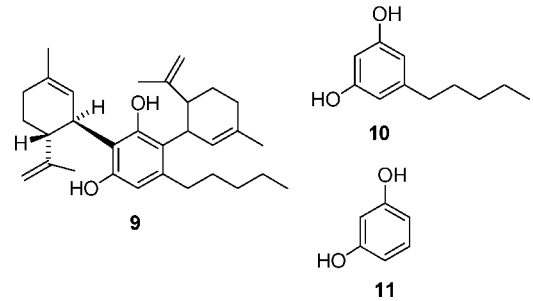
Δ^9-Tetrahydrocannabinol (THC, **4b**), cannabidiol (CBD, **1b**), cannabigerol (CBG, **3b**), cannabichromene (CBC, **2**), and cannabinol (CBN, **5**) are the five most common cannabinoids.[17] They could be obtained in high purity (>98%) by isolation from strains of *C. sativa* producing a single major cannabinoid (THC, CBD, CBG), by total synthesis (CBC),[6] or by semisynthesis (CBN).[18] Their antimicrobial properties are listed in Table 1. All compounds showed potent antibacterial activity, with MIC values in the 0.5−2 μg/mL range. Activity was exceptional against some of these strains, in particular the multidrug-resistant (MDR) SA-1199B, which has a high level of resistance to certain fluoroquinolones. Also noteworthy is the potent activity demonstrated against EMRSA-15 and EMRSA-16, the major epidemic methicillin-resistant *S. aureus* strains occurring in U.K. hospitals.[13,19] These activities compare highly favorably with the standard antibiotics for these strains. The potent activity against strains possessing the NorA and TetK efflux transporters suggests that cannabinoids are not substrates for the most common resistance mechanisms to current antibacterial agents, making them attractive antibacterial leads.

Given their nonpsychotropic profiles, CBD (**1b**) and CBG (**3b**) seemed especially promising, and were selected for further structure−activity studies. Thus, acetylation and methylation of their phenolic hydroxyls (compounds **1c**−**e** and **3c**−**e**, respectively) were both detrimental for activity (MIC >100 μg/mL), in accordance with the essential role of the phenolic hydroxyls in the antibacterial properties. However, in light of the potent activity of the monophenols CBC (**2**), THC (**4b**), and CBN (**5**), it was surprising that monomethylation of the diphenols CBD (**1b**) and CBG (**3b**) was so poorly tolerated in terms of antibacterial activity.

Cannabinoids are the products of thermal degradation of their corresponding carboxylic acids (pre-cannabinoids).[17] Investigation of the antibacterial profile of the carboxylated versions of CBD, CBG, and THC (compounds **1a**, **3a**, and **4a**, respectively) showed a substantial maintenance of activity. On the other hand, methylation of the carboxylic group (compounds **1f** and **3f**, respectively) caused a marked decrease of potency, as did esterification with phenethyl alcohol (compounds **1g** and **3g**, respectively). This operation is associated with a potentiation of the antibacterial properties of phenolic acids, as exemplified by phenethyl caffeate (CAPE), the major antibacterial from propolis, compared to caffeic acid.[20] Remarkably, the synthetic abnormal cannabinoids abn-CBD (**6**)[21] and abn-CBG (**7**)[22] showed antibacterial activity comparable to, although slightly less potent than, their corresponding natural products, while olivetol (**10**) showed modest activity against all six strains, with MICs of 64−128 μg/mL, and resorcinol (**11**) did not exhibit any activity even at 256 μg/mL. Thus, the pentyl chain and the monoterpene moiety greatly enhance the activity of resorcinol.



7 **8**



9 **10** **11**

Taken together, these observations show that the cannabinoid antibacterial chemotype is remarkably tolerant to structural modification of the terpenoid moiety and its positional relationship with the *n*-pentyl chain, suggesting that these residues serve mainly as modulators of lipid affinity, and therefore cellular bioavailability. This view was substantiated by the marked decrease of activity observed when the antibacterial activity of CBG (**3b**) was compared to that of its polar analogue carmagerol (**8**).[23] The results against the resistant strains confirm this suggestion, and it is likely that the increased hydrophilicity caused by the addition of two hydroxyls greatly reduces the cellular bioavailability by substantially reducing membrane permeability. Conversely, the addition of a further prenyl moiety, as in the bis-prenylated cannabinoid **9**,[21] while increasing membrane solubility, may result in poorer aqueous solubility and therefore a lower intracellular concentration, similarly leading to a substantial loss of activity. A single unfunctionalized terpenyl moiety seems therefore ideal in terms of lipophilicity balance for the antibacterial activity of olivetol derivatives. The great potency of cannabinoids suggests a specific interaction with a bacterial target, whose identity is, however, still elusive.

Given the availability of *C. sativa* strains producing high concentrations of nonpsychotropic cannabinoids, this plant represents an interesting source of antibacterial agents to address the problem of multidrug resistance in MRSA and other pathogenic bacteria. This issue has enormous clinical implications, since MRSA

Table 1. MIC (μg/mL) Values of Cannabinoids and Their Analogues toward Various Drug-Resistant Strains of *Staphylococcus aureus*[a,b]

compound	SA-1199B	RN-4220	XU212	ATCC25923	EMRSA-15	EMRSA-16
1a	2	2	2	2	2	2
1b	1	1	1	0.5	1	1
2	2	2	1	2	2	2
3a	4	2	4	4	2	4
3b	1	1	1	1	2	1
3f	64	[c]	64	[c]	[c]	[c]
4a	8	4	8	4	8	4
4b	2	1	1	1	2	0.5
5	1	1	1	1	1	[c]
6	1	1	1	1	1	1
7	2	1	0.5	1	2	[c]
8	32	32	16	16	16	32
10	64	64	64	128	64	64
norfloxacin	32	1	4	1	0.5	128
erythromycin	0.25	64	>128	0.25	>128	>128
tetracycline	0.25	0.25	128	0.25	0.125	0.125
oxacillin	0.25	0.25	128	0.125	32	>128

[a] Compounds **1c**−**g**, **3c**−**e**, **3g**, and **9** exhibited MIC values of >128 μg/mL for all organisms in which they were evaluated. [b] Compound **11** exhibited MIC values of >256 μg/mL for all organisms in which they were evaluated. [c] Not tested.

is spreading throughout the world and, in the United States, currently accounts for more deaths each year than AIDS.[24] Although the use of cannabinoids as systemic antibacterial agents awaits rigorous clinical trials and an assessment of the extent of their inactivation by serum,[25] their topical application to reduce skin colonization by MRSA seems promising, since MRSA resistant to mupirocin, the standard antibiotic for this indication, are being detected at a threatening rate.[26] Furthermore, since the cannabinoid anti-infective chemotype seems remarkably tolerant to modifications in the prenyl moiety, semipurified mixtures of cannabinoids could also be used as cheap and biodegradable antibacterial agents for cosmetics and toiletries, providing an alternative to the substantially much less potent synthetic preservatives, many of which are currently questioned for their suboptimal safety and environmental profile.[27]

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Shimadzu DR 8001 spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained at room temperature with a JEOL Eclipse spectrometer. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. Silica gel 60 (70−230 mesh) and Lichroprep RP-18 (25−40 mesh) were used for gravity column chromatography. Reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates and were visualized by UV inspection and/or staining with 5% H_2SO_4 in ethanol and heating. Organic phases were dried with Na_2SO_4 before evaporation. All known cannabinoids were identified according to their physical and spectroscopic data.[28] Semisynthetic cannabinoids **1c**−**f**, and **3c**−**f** were prepared and identified according to their corresponding literature references.[22,29,30] Synthetic [abnormal (**6**,[21] **7**[6]) and polyprenyl (**9**)[21]] cannabinoids were synthesized and characterized according to the literature.

Plant Material. The three strains of *Cannabis sativa* used for the isolation of THC, CBD, and CBG came from greenhouse cultivation at CRA-CIN, Rovigo (Italy), where voucher specimens are kept for each of them, and were collected in September 2006. The isolation and manipulation of all cannabinoids were done in accordance with their legal status (License SP/101 of the Ministero della Salute, Rome, Italy).

Isolation of Cannabinoids (1b, 3b, 4b). The powdered plant material (100 g) was distributed in a thin layer on cardboard and heated at 120 °C for 2 h in a ventilated oven to affect decarboxylation, then extracted with acetone (ratio solvent to plant material 3:1, ×3). The residue (6.5 g for the CBD chemotype, 4.1 g for the CBG chemotype, 7.4 g for the THC chemotype) was purified by gravity column chromatography on silica gel (ratio stationary phase to extract 6:1) using a petroleum ether−ether gradient. Fractions eluted with petroleum ether−ether (9:1) afforded **1b** (628 mg, 0.63%, from the CBD chemotype) and **3b** (561 mg, 0.56%, from the CBG chemotype), precipitated from hot hexane to obtain white powders. Crude THC (3.2 g, 3.2%, from the THC chemotype) was obtained as a greenish oil, part of which (400 mg) was further purified by RP-18 flash chromatography with methanol−water (1:1) as eluant, affording **4b** as a colorless oil (315 mg).

Isolation of Pre-cannabinoids (1a, 3a, 4a). The powdered plant material (100 g) was extracted with acetone (ratio solvent to plant material 5:1, ×3). After removal of the solvent, the residue (7.7 g for the CBD chemotype, 4.9 g for the CBG chemotype, 7.9 g for the THC chemotype) was fractionated by vacuum chromatography on RP-18 silica gel (ratio stationary phase to extract 5:1) using methanol−water (75:25) as eluant. Fractions of 100 mL were taken, and those containing pre-cannabinoids were pooled, concentrated to ca. half-volume at 30 °C, saturated with NaCl, and extracted with EtOAc. After removal of the solvent, the residue was further purified by gravity column chromatography on silica gel (ratio stationary phase to crude compound 5:1) using a petroleum ether−EtOAc gradient (from 8:2 to 5:5) to afford 1.59 g (1.6%) of **1a** from the CBD chemotype, 0.93 g (0.93%) of **3a** from the CBG chemotype, and 2.1 g (2.1%) of **4a** from the THC chemotype. All pre-cannabinoids were obtained as white foams that resisted crystallization.

Synthesis of CBC (2) and CBN (5). CBG (**2**) was synthesized from olivetol,[6] and CBN was prepared from THC (**6**) by aromatization with sulfur.[18]

Mitsunobu Esterification of Pre-cannabinoids (synthesis of 3g as an example). To a cooled (ice bath) solution of **3a** (360 mg, 1.1 mmol) in dry CH_2Cl_2 (4 mL) were added sequentially phenethyl alcohol (92 μL, 0.76 mmol, 0.75 molar equiv), triphenylphosphine (TPP) (220 mg, 0.84 mmol, 0.80 molar equiv), and diisopropyldiazodicarboxylate (DIAD) (228 μL, 1.1 mmol, 1 molar equiv). At the end of the addition, the cooling bath was removed, and the reaction was stirred at room temperature. After 16 h, the reaction was worked up by evaporation, and the residue was dissolved in toluene and cooled at 4 °C overnight to remove most of the TPPO-dihydroDIAD adduct. The filtrate was evaporated and purified by gravity column chromatography on silica gel (10 g, petroleum ether as eluant) to afford 126 mg (32%) of **3g**. Under the same reaction conditions, the yield of **1g** from **1a** was 26%.

Pre-cannabigerol Phenethyl Ester (3g): colorless foam; IR ν^{KBr}_{max} 3746, 3513, 3313, 1715, 1589, 1421, 1274, 1164, 980, 804, 690 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.08 (1H, s), 7.25 (5H, m), 6.02 (1H, s), 5.98 (1H, s), 5,25 (1H, br t, J = 7.0 Hz), 5.01 (1H, br t, J = 6.5 Hz), 4.56 (2H, t, J = 6.6 Hz), 3.40 (2H, d, J = 7.3 Hz), 3.1 (2H, t, J = 6.6 Hz), 2.7 (2H, t, J = 6.6 Hz), 2.05 (4H, m), 1.79 (3H, s), 1.65 (3H, s), 1.57 (3H, s), 1.24 (6H, m), 0.88 (3H, t, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1 (s), 162.7 (s), 159.5 (s), 148.8 (s), 139.1 (s), 137.4 (d), 132.1 (s), 128.8 (d), 126.8 (d), 125.9 (d), 121.5 (d), 111.5 (s), 110.8 (s), 65.8 (t), 39.8 (t), 36.6 (t), 35.0 (t), 32.0 (t), 31.5 (t), 26.5 (t), 25.8 (q), 22.2 (t), 17.8 (q), 16.3 (q), 14.2 (q); CIMS m/z [M + H] 465 [$C_{30}H_{40}O_4$ + H].

Pre-cannabidiol Phenethyl Ester (1g): colorless oil; IR (KBr) ν_{max} 3587, 3517, 3423, 3027, 1642, 1499, 1425, 1274, 1172, 1143, 980, 894 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 12.13 (1H, s), 6.23 (5H, m), 6.48 (1H, s), 6.19 (1H, s), 5,55 (1H, s), 4.52 (3H, m), 4.4 (1H, s), 4.08 (1H, br s), 3.08 (2H, t, $J = 7.0$ Hz), 2.7 (2H, m), 2.11 (1H, m), 1.78 (3H, s), 1.71 (3H, s), 1.5 (4H, m), 1.28 (6H, m), 0.88 (3H, t, $J = 6.9$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2 (s), 171.5 (s), 163.5 (s), 160.0 (s), 148.8 (s), 147.0 (s), 145.9 (s), 140.2 (s), 137.4 (s), 128.7 (d), 126.7 (d), 124.0 (d), 114.4 (t), 112.3 (d), 105.8 (s), 65.6 (t), 46.6 (d), 39.1 (t), 37.0 (d), 31.9 (d), 31.5 (t), 27.8 (t), 25.3 (q), 22.6 (t), 21.9 (t), 18.5 (q), 14.1 (q); CIMS *m/z* [M + H] 463 [$C_{30}H_{38}O_4$ + H].

Bacterial Strains and Chemicals. A standard *S. aureus* strain (ATCC 25923) and a clinical isolate (XU212), which possesses the TetK efflux pump and is also a MRSA strain, were obtained from E. Udo.[16] Strain RN4220, which has the MsrA macrolide efflux pump, was provided by J. Cove.[30] EMRSA-15[13] and EMRSA-16[19] were obtained from Paul Stapleton. Strain SA-1199B, which overexpresses the NorA MDR efflux pump, was the gift of Professor Glenn Kaatz.[14] Tetracycline, norfloxacin, erythromycin, and oxacillin were obtained from Sigma Chemical Co. Oxacillin was used in place of methicillin as recommended by the NCCLS. Mueller-Hinton broth (MHB; Oxoid) was adjusted to contain 20 mg/L Ca^{2+} and 10 mg/L Mg^{2+}.

Antibacterial Assays. Overnight cultures of each strain were made up in 0.9% saline to an inoculum density of 5×10^5 cfu by comparison with a MacFarland standard. Tetracycline and oxacillin were dissolved directly in MHB, whereas norfloxacin and erythromycin were dissolved in DMSO and then diluted in MHB to give a starting concentration of 512 μg/mL. Using Nunc 96-well microtiter plates, 125 μL of MHB was dispensed into wells 1−11. Then, 125 μL of the test compound or the appropriate antibiotic was dispensed into well 1 and serially diluted across the plate, leaving well 11 empty for the growth control. The final volume was dispensed into well 12, which being free of MHB or inoculum served as the sterile control. Finally, the bacterial inoculum (125 μL) was added to wells 1−11, and the plate was incubated at 37 °C for 18 h. A DMSO control (3.125%) was also included. All MICs were determined in duplicate. The MIC was determined as the lowest concentration at which no growth was observed. A methanolic solution (5 mg/mL) of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliium bromide (MTT; Lancaster) was used to detect bacterial growth by a color change from yellow to blue.

References and Notes

(1) Caiffa, W. T.; Vlahov, D.; Graham, N. M. H.; Astemborski, J.; Solomon, L.; Nelson, K. E.; Munoz, A. *Am. J. Resp. Crit. Care Med.* **1994**, *150*, 1593–1598.

(2) Roth, M. D.; Whittaker, K.; Salehi, K.; Tashkin, D. P.; Baldwin, G. C. *J. Neuroimmunol.* **2004**, *147*, 82–86.

(3) (a) Krjci, Z. *Lekarske Listy* **1952**, *7*, 500–503; *Chem. Abstr.* **1952**, *48*, 78326. (b) Ferenczy, L.; Gracza, L.; Jakobey, I. *Naturwissenschaften* **1958**, *45*, 188. (c) Krejci, Z. *Pharmazie* **1958**, *13*, 155–156. (d) Rabinovich, A. S.; Aizenman, B. L.; Zelepukha, S. I. *Mikrobiol. Zh* **1959**, *21*, 40–48. (PubMed ID 14435632). (e) Turner, C. E.; Elsohly, M. A.; Boeren, E. G. *J. Nat. Prod.* **1980**, *43*, 169–243.

(4) Schultz, O. E.; Haffner, G. A. *Z. Naturforsch.* **1959**, *14b*, 98–100.

(5) Turner, C. E.; Elsohly, M. A. *J. Clin. Pharmacol.* **1981**, *21*, 283S–291S.

(6) Elsohly, H. N.; Turner, C. E.; Clark, A. M.; Elsohly, M. A. *J. Pharm. Sci.* **1982**, *71*, 1319–1323.

(7) Van Klingeren, B.; Ten Ham, M. *Lab. Chemother. Natl. Inst. Public Health* **1976**, *42*, 9–12; *Chem. Abstr.* **1976**, *85*, 14622.

(8) Molnar, J.; Csiszar, K.; Nishioka, I.; Shoyama, Y. *Acta Microb. Hung.* **1986**, *33*, 221–231.

(9) Barrett, C. T.; Barrett, J. F. *Curr. Opin. Biotechnol.* **2003**, *14*, 621–626.

(10) Gibbons, S. *Nat. Prod. Rep.* **2004**, *21*, 263–277.

(11) Stavri, M.; Piddock, L. J. V.; Gibbons, S. *J. Antimicrob. Chemother.* **2007**, *59*, 1247–1260.

(12) Smith, E. C. J.; Kaatz, G. W.; Seo, S. M.; Wareham, N.; Williamson, E. M.; Gibbons, S. *Antimicrob. Agents Chemother.* **2007**, *51*, 4480–4483.

(13) Richardson, J. F.; Reith, S. J. *Hosp. Infect.* **1993**, *25*, 45–52.

(14) Kaatz, G. W.; Seo, S. M.; Ruble, C. A. *Antimicrob. Agents Chemother.* **1993**, *37*, 1086–1094.

(15) Ross, J. I.; Farrell, A. M.; Eady, E. A.; Cove, J. H.; Cunliffe, W. J. *J. Antimicrob. Chemother.* **1989**, *24*, 851–862.

(16) Gibbons, S.; Udo, E. E. *Phytother. Res.* **2000**, *14*, 139–140.

(17) (a) Mechoulam, R.; McCallum, N. K.; Burstein, S. *Chem. Rev.* **1976**, *76*, 75–112. (b) Elsohly, M. A.; Slade, D. *Life Sci.* **2005**, *78*, 539–548.

(18) (a) Ghosh, R.; Todd, A. R.; Wilkinson, S. *J. Chem. Soc.* **1940**, 1393–1396. (b) Bastola, K. P.; Hazekamp, A.; Verpoorte, R. *Planta Med.* **2007**, *73*, 273–275.

(19) Cox, R. A.; Conquest, C.; Mallaghan, C.; Maples, R. R. *J. Hosp. Infect.* **1995**, *29*, 87–106.

(20) Castaldo, S.; Capasso, F. *Fitoterapia* **2002**, *73*, S1−S6.

(21) Razdan, R. K.; Dalzell, H. C.; Handrick, G. R. *J. Am. Chem. Soc.* **1974**, *96*, 5860–5865.

(22) Baek, S. H.; Srebnik, M.; Mechoulam, R. *Tetrahedron Lett.* **1985**, *26*, 1083–1086.

(23) Appendino, G. Unpublished results.

(24) Bancroft, E. A. *J. Am. Med. Assoc.* **2007**, *298*, 1803.

(25) Mechoulam, R.; Gaoni, Y. *Tetrahedron* **1965**, *21*, 1223–1229.

(26) Simor, A. E.; Stuart, T. L.; Louie, L.; Watt, C.; Ofner-Agostini, M.; Gravel, D.; Mulvey, M.; Loeb, M.; McGeer, A.; Bryce, E.; Matlow, A. *Antimicrob. Agents Chemother.* **2007**, *51*, 3880–3886.

(27) McGrath, K. G. *Eur. J. Cancer Prev.* **2003**, *12*, 479–485.

(28) Hazekamp, A.; Peltenburg, A.; Verpoorte, R.; Giroud, C. *J. Liq. Chromatogr. Rel. Technol.* **2005**, *28*, 2361–2382.

(29) Vailancourt, V.; Albizati, K. F. *J. Org. Chem.* **1992**, *57*, 3627–3631.

(30) Mechoulam, R.; Gaoni, Y. *Tetrahedron* **1965**, *21*, 1223–1229.

NP8002673