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Synthesis and Antimicrobial Activity of Sulfenimines Based on Pinane Hydroxythiols

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Abstract: The widespread presence of multidrug-resistant pathogenic microorganisms challenges the development of novel chemotype antimicrobials, insensitive to microbial tools of resistance. To date, various monoterpenoids have been shown as potential antimicrobials. Among many classes of molecules with antimicrobial activity, terpenes and terpenoids are an attractive basis for the design of antimicrobials because of their low toxicity and availability for various modifications. In this work, we report on the synthesis of sulfenimines from chiral trifluoromethylated and non-fluorinated pinane-type thiols. Final compounds were obtained with yields of up to 81%. Among the 13 sulfenimines obtained, 3 compounds were able to repress the growth of both bacteria (*S. aureus*, both MSSA and MRSA; *P. aeruginosa*) and fungi (*C. albicans*) with an MIC of 8–32 μg/mL. Although compounds exhibited relatively high cytotoxicity (the therapeutic index of 3), their chemotype can be used as a starter point for the development of disinfectants and antiseptics for targeting multidrug-resistant pathogens.

Keywords: monoterpenoids; pinane; CF_3-containing hydroxythiol; sulfenimines; antibacterial; antifungal activity

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1. Introduction

The widespread presence of multidrug-resistant bacteria and fungi challenges the development of antimicrobials of a novel chemotype, as they are insensitive to microbial tools of resistance. The acquisition of genes encoding efflux systems and enzymes that hydrolyze antimicrobials and increase biofilm formation, as well as changes in target molecules and cell wall structure, reduces the effectiveness of conventional antibiotics [1].

Among various classes of molecules able to repress the growth of pathogenic bacteria and fungi, monoterpene derivatives have a wide spectrum of antimicrobial activity [2–4]. Thus, the repression of the growth of both various bacteria and fungi has been reported [5–10]. The combination of terpenes with conventional antimicrobials increases the activity of the latter [11,12]. Furthermore, the fusion in one molecule of a biologically active terpene fragment and sulfur-containing functional groups, which are part of many substances with bactericidal and fungicidal activity, leads to an increase in the efficiency of the resulting thioterpenoids [2,5,13,14]. The mechanism of these synergistic effects can be a consequence of targeting the membrane itself or membrane-related proteins with terpenes. Thus, the binding site for cyclic hydrocarbons, including terpene ones [15], has been reported to be located in the cell membrane of pathogenic microorganisms. The limonene, α- and β-pinenes, and γ-terpinene are able to inhibit respiration and other energy-dependent

processes localized in cell membranes of some fungi and bacteria [3,16–19]. Additionally, some derivatives of terpenes were shown to interact with membranes of eukaryotic cells [20,21].

Carane sulfenimines, sulfinimines, and *N*-substituted fluorine-containing sulfinamides, as well as pinane thiosulfonates obtained on the basis of monoterpene thiols, showed selective antimicrobial activity against yeasts *Candida albicans* and *Cryptococcus neoformans*, as well as the bacteria *Staphylococcus aureus* and *Acinetobacter baumannii* [22,23]. The introduction of a sulfenimine fragment in the structure of cephalosporin sulfoxides enhanced their inhibitory activity against cephalosporinase C. The activity was significantly affected by substituents in the sulfenimine moiety [24]. Substituted salicylic and nitrobenzylidene imines have been shown to be subjected as new chemotypes of antimicrobial drug candidates [25–29].

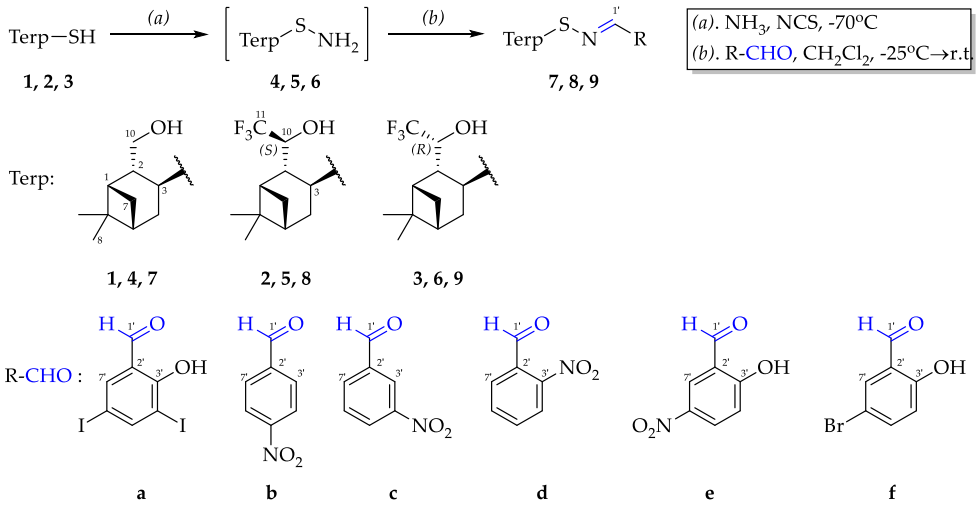
Nowadays, a third of the newly synthesized antimicrobials carry fluorine atoms [30], since the introduction of fluorine-containing groups enhances the membrane permeability and increases the resistance to biodegradation relative to their nonfluorinated analogs [31]. These modifications can lead to significant changes in interaction mechanisms of target with the drug and consequent shifts in biological activity of the latter, compared to hydrocarbon analogs [32,33]. Previously, CF_3-containing pinane-type monoterpene hydroxythiols for further functionalization were synthesized [34].

In this work, based on 10-hydroxyisopinocampheyl thiol **1** [35], of which its CF_3-containing analogs (10*S*)-**2** and (10*R*)-**3** were obtained earlier by our group [34], and nitrobenzaldehydes or substituted salicylic aldehydes, a series of sulfenimines were synthesized, and their antibacterial and antifungal activities, cytotoxicity, and mutagenicity were evaluated.

2. Results and Discussion

2.1. Synthesis of Sulfenimines from Pinane Hydroxythiols

Thiols **1**–**3** were treated with *N*-chlorosuccinimide (NCS) in liquid ammonia according to a known procedure [23,36–38] to form unstable sulfenamides **4**–**6**, which entered into a condensation reaction in situ with 3,5-diiodosalicylic aldehyde (**a**), 4-nitrobenzaldehyde (**b**), 3-nitrobenzaldehyde (**c**), 2-nitrobenzaldehyde (**d**), 5-nitrosalicylic aldehyde (**e**) and 5-bromosalicylic aldehyde (**f**) to provide the corresponding sulfenimines **7a**–**f**, **8a**–**f** and **9a** in 16–81% yields without isolation of sulfenamide intermediates (Scheme 1). Since the CF_3-containing thiol (10*R*)-**3** is synthetically less accessible than the thiol (10*S*)-**2** [34], only sulfenimine **9a** with the 3,5-diiodosalicylic moiety was synthesized on its basis.



Scheme 1. General scheme for the synthesis of sulfenimines from pinane hydroxythiols.

The structures and composition of sulfenimines have been proven by NMR and IR spectroscopy, and by elemental and X-ray diffraction analysis. The IR spectra of sulfenimines **7a–f**, **8a–f** and **9a** contain absorption bands characteristic of the C=N bond in the region of 1614–1574 cm^{-1}. The ^{1}H and ^{13}C NMR spectra contain signals from both terpene and aromatic fragments. In the ^{1}H NMR spectra of **7a–f**, **8a–f** and **9a**, in comparison with the starting thiols **1–3**, the proton signals of the SH groups disappear, whereas proton signals of the $C^{1\prime}H$=N group can be observed in the region of 8.34–8.95 ppm. In the ^{13}C NMR spectra, there are signals characteristic of the $C^{1\prime}$=N group in the downfield region (152.0–159.9 ppm). The ^{13}C NMR spectra of CF_3-containing sulfenimines **8a–f** and **9a** contain quartets of the $C^{11}F_3$ group in the range of 124.9–125.8 ppm (J_F = 283.0–284.2 Hz), as well as quartets of the C^{10} atom in the region of 71.1–72.6 ppm (J_F = 28.8–29.2 Hz); in contrast, in sulfenimines **7a–f**, the signals of the C^{10} atom are in the region of 65.9–66.4 ppm.

The structure and configuration of single-crystal **8b** were confirmed by X-ray diffraction analysis (Figure 1). This compound crystallizes in the chiral space group $P2_1$ of the monoclinic system. There are two independent molecules (A and B) of **8b** in the asymmetric unit cell. They have the same molecular structure. The root-mean-square deviation of the nonhydrogen atomic positions of the A and B molecules is 0.213 Å. The greatest difference is the slight rotation of the SNCHR group. The dihedral angle between the corresponding planes when the molecules are superimposed is 11.28°. All atoms of the NO_2 group, as well as the S(1), N(1) and C(12) atoms, lie in the plane of the C(13)–C(18) phenyl ring. The N-O distances in the NO_2 group are largely aligned. This is typical for NO_2 groups in similar compounds [23,39]. In general, the main geometric characteristics for compound **8b** are in good agreement with previously published related compounds [23,39].

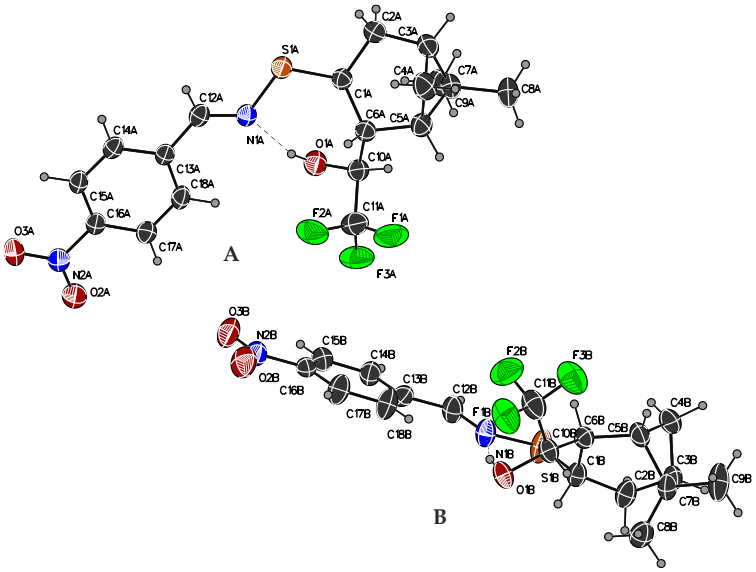


Figure 1. Molecular structure of two independent molecules (**A**,**B**) of compound **8b** with thermal ellipsoids drawn at the 30% probability level.

Compound **8b** has an intramolecular hydrogen bond O(1)-H(1) . . . N(1). The data set does not allow us to refine the position of the hydrogen atom without constraints. However, the distance between the oxygen and nitrogen atoms (2.830(5) and 2.852(5) Å in molecules A and B, respectively) indicates the implementation of the H...N interaction [40].

The resulting sulfenimines **7a–f**, **8a–f** and **9a** were further subjected to an antibacterial and antifungal activity test, and an assessment of mutagenicity and cytotoxicity.

2.2. Antimicrobial Activity

The activity of terpenes has been shown to vary against microorganisms with various cell wall structures [2–4,15,16,20]. Therefore, the antimicrobial activity of the newly

synthesized sulfenimine derivatives was assessed against Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 (MSSA) and a clinical isolate of *Staphylococcus aureus* resistant to methicillin (MRSA)), Gram-negative bacterium *P. aeruginosa* ATCC 27853, and a fluconazole-sensitive clinical isolate of *Candida albicans* 703. These microorganisms cause diseases of the skin, various mucosa and the respiratory tract and are characterized by a high frequency of occurrence of resistant isolates.

As could be seen from Table 1, compounds **7a, 8a**, **7c** and **8e** repressed the growth of all test microorganisms, although the activity was moderate and MIC values were generally significantly higher than those of reference antimicrobials. Notably, trifluoromethylated sulfenimines with salicylic moiety **9a** and **8f** were active only against methicillin-resistant *S. aureus* and *C. albicans*; while **9a** was even superior in activity to fluconazole.

Table 1. Antibacterial and antifungal activities, and cytotoxicity and mutagenicity of sulfenimines.

Compound	MIC, μg/mL				IC_{50} EBL, μg/mL	Mutagenicity in the Ames Test
	S. aureus ATCC 29213 (MSSA)	*S. aureus* Clinical Isolate (MRSA)	*P. aeruginosa* ATCC 27853	*C. albicans* 703 Clinical Isolate		
7a	8	8	8	64	21 ± 2.7	NF *
8a	16	16	32	32	23 ± 3.8	NF
9a	256	8	1024	8	14 ± 3.5	NF
7b	64	>1024	>1024	64	20 ± 2.8	NF
8b	>1024	>1024	>1024	>1024	ND	ND
7c	32	32	32	32	45 ± 10.1	NF
8c	512	>1024	512	>1024	ND	ND
7d	32	32	>1024	512	18 ± 4.5	TA 102
8d	>1024	>1024	>1024	1024	ND	ND
7e	32	64	32	>1024	14 ± 4.6	NF
8e	16	32	32	16	35 ± 9.1	NF
7f	32	32	1024	64	9 ± 1.9	TA 102
8f	256	64	1024	16	14 ± 3.1	NF
Amikacin	4	4	4	ND	ND	ND
Ampicillin	0.5	>1024	>1024	ND	ND	ND
Ciprofloxacin	2	4	4	ND	ND	ND
Fluconazole	ND *	ND	ND	16	ND	ND
Benzalkonium chloride	1	1	4	0.5	1 ± 0.3	ND

* ND—not determined; NF—not found.

Furthermore, **7d**, **7f** and, partially, **7b** were active only on *S. aureus* and *C. albicans*; **7d** and **7f** in the Ames test showed mutagenicity on the *Salmonella typhimurium* TA102 strain, causing point mutations and reversions [41]. Sulfenimines containing the CF_3 group did not show mutagenicity in this test. Compound **7e** was active only on bacteria. In general, a decrease in antibacterial properties upon the introduction of a CF_3 group in the terpene fragment of sulfenimines could be observed. Moreover, sulfenimines with a CF_3 group in the terpene moiety and a salicylaldehyde moiety, **8a**, **9a**, **8c** and **8f**, exhibit greater antifungal activity (MIC 8–32), in contrast to the nonfluorinated analogues **7a**, **7e** and **7f** (MIC $\geq$ 64). Compounds **8b**, **8c** and **8d** were inactive against all test strains, suggesting that the presence of nitrobenzylidene substituents abrogates their antimicrobial activity; neither cytotoxicity nor mutagenicity was tested.

In general, the synthesized sulfenimines exhibited high cytotoxicity on the embryonic bovine lung (EBL) cells: CC_{50} values were the least toxic, whereas active sulfenimines **7c** and **8e** exceeded only the corresponding MIC by 2–3-fold. This fact makes sulfenimines suitable only as antiseptics, which is similar to benzalkonium chloride due to a similar therapeutic index (CC_{50}/MIC).

3. Conclusions

Thus, new monoterpene sulfenimines based on monoterpene pinane thiols, including those containing a CF_3 group, have been synthesized for the first time. The described sulfenimines, generally, have moderate antibacterial and antifungal activity and high cytotoxicity in vitro, which limits their direct application. By contrast, the revealed effects of monoterpene and aromatic moieties on the antimicrobial activity of sulfenimines allows for further modeling of compounds with given selectivity for pathogenic microorganisms.

4. Materials and Methods

4.1. General Information

IR spectra were registered on a Shimadzu IR Prestige 21 infrared Fourier spectrometer in a thin layer or in KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 and 75 MHz) in $CDCl_3$ using the signal of the indicated solvent as an internal standard (See Supplementary Materials). ^{13}C NMR spectra were registered in the *J*-modulation mode. The complete assignment of ^{1}H and ^{13}C signals was performed using 2D homo- (^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY) and heteronuclear (^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC) experiments. ^{19}F NMR spectra were recorded on a Bruker Avance 300 spectrometer (282 MHz) and on a Spinsolve 60 HF Ultra spectrometer (58 MHz) in $CDCl_3$ using the signal of CF_3COOH as an external standard. For easier interpretation of the NMR spectra, the carbon atoms of structures **7a**–**f**, **8a**–**f** and **9a** were numbered, in some cases, contrary to the recommendations of IUPAC. Elemental analysis was carried out on an EA 1110 CHNS-O automatic analyzer. Melting points were determined on a Sanyo Gallenkamp MPD350BM3.5 instrument and were not corrected. Optical rotation was measured on an automated digital polarimeter, the Optical Activity PolAAr 3001 (UK). Sorbfil plates were used for thin-layer chromatography; the visualizing agent was a solution of phosphoromolybdic acid in ethanol. Alfa Aesar silica gel (0.06–0.2 mm) was used for column chromatography. The commercially available *N*-chlorosuccinimide, 98% (Alfa Aesar); 3,5-diiodosalicylaldehyde, 98+% (Alfa Aesar); 4-nitrobenzaldehyde, 99% (Alfa Aesar); 3-nitrobenzaldehyde, 99% (Alfa Aesar); 2-nitrobenzaldehyde, 98+% (Alfa Aesar); 5-nitrosalicylaldehyde, 98% (Alfa Aesar); and 5-bromosalicylaldehyde, 98% (Alfa Aesar) were used without additional purification.

The diffraction data for compound **8b** were collected on an Oxford Xcalibur Eos diffractometer (Mo-K_α radiation, ω-scan technique, λ = 0.71073 Å). The intensity data were integrated by the CrysAlisPro [42] program. The SCALE3 ABSPACK algorithm [42] was used to perform absorption corrections. The structure was solved by dual methods [43] and was refined on F^2_{hkl} using the SHELXTL package [44]. All nonhydrogen atoms were refined anisotropically. All H atoms, with the exception of hydrogens of the hydroxyl groups, were placed in calculated positions and were refined using a riding model (U_{iso}(H) = 1.5U_{eq}(C) for CH_3 groups and U_{iso}(H) = 1.2U_{eq}(C) for other groups). The H(1A) and H(1B) atoms in **8b** were located on the differential Fourier map and were refined isotropically with AFIX 147. The $PhNO_2$ fragment in molecule **B** was disordered over two positions. To refine the disordered atoms, the AFIX 66, SAME, SADI, FLAT and ISOR instructions were used. CCDC 2193190 contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via https://www.ccdc.cam.ac.uk/structures (accessed on 3 November 2022).

((1*S*,2*R*,3*S*,5*R*)-3-Mercapto-6,6-dimethylbicyclo[3.1.1]heptan-2-yl)methanol (**1**) [35], (*S*)-2,2,2-trifluoro-1-((1*S*,2*R*,3*S*,5*R*)-3-mercapto-6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethan-1-ol (**2**) and (*R*)-2,2,2-trifluoro-1-((1*S*,2*R*,3*S*,5*R*)-3-mercapto-6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethan-1-ol (**3**) [34] were synthesized according to known procedures.

4.2. General Procedure for the Synthesis of Sulfenimines

The procedure is based on methods for the synthesis of sulfenimines [23,36–38].

In a U-shaped tube, while cooling to −70 °C in an acetone bath, 7–10 mL of liquid NH_3 (dry) was condensed. While maintaining the bath temperature, NCS (143 mg, 1.069 mmol)

was carefully added. The mixture was stirred for 5 min, then thiol **1** (0.823 mmol, either **2** or **3**) dissolved in 2 mL of CH_2Cl_2 was introduced into the tube with a syringe. The reaction mixture was stirred for one hour, gradually increasing the temperature to −30 °C until unstable sulfenamide **2** was formed. After complete conversion of the thiol (monitored by TLC), the corresponding aldehyde (2.1 mmol, **a**–**f**) was added to the reaction mixture. As ammonia evaporated, CH_2Cl_2 was added. After complete evaporation of ammonia, the reaction mixture was heated to room temperature. After 12 h, the reaction mixture was filtered off on a Schott filter under reduced pressure. The organic phase was treated with a NaCl solution and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over Na_2SO_4. The solvent was then distilled off under reduced pressure. The resulting mixture was separated by silica gel column chromatography using the same eluent systems as for TLC.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-2-Hydroxy-3,5-diiodobenzylidene)-2-(hydroxymethyl)-6,6-dimethylbicyclo[3.1.1]heptane-3-sulfenamide (**7a**). Yield: 55.5%; yellow-orange powder; m.p.: 64.1 °C; $[\alpha]_D^{25}$ +56.6 (*c* 1.71, $CHCl_3$); R_f 0.43 (petr. ether:EtOAc, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.04 (3H, s, CH_3-8), 1.14 (1H, d, *J* = 9.8, H-7*α*), 1.27 (3H, s, CH_3-9), 2.04–2.11 (2H, m, H-5, C-10-OH), 2.18–2.29 (3H, m, H-1, H-4*α*, H-2), 2.50 (1H, dtd, *J* = 9.5, 6.6, 2.2, H-7*β*), 2.60–2.72 (1H, m, H-4*β*), 3.64 (1H, dt, *J* = 9.5, 6.6, H-3), 3.69–3.84 (2H, m, H-10), 7.43 (1H, d, *J* = 2.0, H-7′), 7.99 (1H, d, *J* = 2.0, H-5′), 8.35 (1H, s, H-1′), 12.45 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.5 (C-8), 27.5 (C-9), 32.4 (C-7), 35.5 (C-4), 38.8 (C-6), 41.7 (C-5), 42.5 (C-3), 42.7 (C-1), 49.7 (C-2), 66.0 (C-10), 80.7 (C-6′), 86.5 (C-4′), 121.7 (C-2′), 138.7 (C-7′), 147.7 (C-5′), 157.6 (C-3′), 158.8 (C-1′). IR spectrum (KBr, ν, cm^{-1}): 3404 (Ar-OH), 3383 (OH), 1574 (C=N). Elemental analysis calcd. (%) for $C_{17}H_{21}I_2NO_2S$: C 36.64, H 3.80, N 2.51; found: C 37.03, H 4.17, N 2.64.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-2-Hydroxy-3,5-diiodobenzylidene)-6,6-dimethyl-2-((*S*)-2,2,2-trifluoro-1-hydroxyethyl)bicyclo[3.1.1]heptane-3-sulfenamide (**8a**). Yield: 69%; yellow powder; m.p.: 98.5 °C; $[\alpha]_D^{25}$ +0.2 (*c* 0.85, $CHCl_3$); R_f 0.53 ($CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.06 (3H, s, CH_3-8), 1.25 (1H, d, *J* = 9.5, H-7*α*), 1.27 (3H, s, CH_3-9), 2.05–2.11 (1H, m, H-5), 2.20–2.21 (1H, m, H-1), 2.29–2.38 (2H, m, H-2, H-4*α*), 2.40–2.50 (1H, m, H-7*β*), 2.59–2.79 (2H, m, H-4*β*, C-10-OH), 4.05–4-21 (2H, H-3, H-10), 7.44 (1H, d, *J* = 2.0, H-7′), 8.00 (1H, d, *J* = 2.0, H-5′), 8.34 (1H, s, H-1′), 12.20 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.1 (C-8), 26.9 (C-9), 30.4 (C-7), 35.1 (C-4), 38.1 (C-6), 40.7 (C-5), 40.8 (C-3), 42.4 (C-1), 46.4 (C-2), 72.6 (q, J_F = 29.2, C-10), 80.7 (C-6′), 86.6 (C-4′), 121.6 (C-2′), 124.9 (q, J_F = 283.0, C-11), 138.8 (C-7′), 147.9 (C-5′), 157.4 (C-3′), 158.4 (C-1′). ^{19}F NMR (282 MHz, $CDCl_3$, δ, ppm): −76.20 (3F, s, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3460 (OH), 1576 (C=N), 1273, 1151, 1123 (CF_3). Elemental analysis calcd. (%) for $C_{18}H_{20}F_3I_2NO_2S$: C 34.58, H 3.22, N 2.24; found: C 34.95, H 3.60, N 2.08.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-2-Hydroxy-3,5-diiodobenzylidene)-6,6-dimethyl-2-((*R*)-2,2,2-trifluoro-1-hydroxyethyl)bicyclo[3.1.1]heptane-3-sulfenamide (**9a**). Yield: 16%; light-yellow gummy oil; $[\alpha]_D^{26}$ +30.8 (*c* 0.4, $CHCl_3$); R_f 0.37 ($CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.13 (3H, s, CH_3-8), 1.14 (1H, d, *J* = 9.5, H-7*α*), 1.30 (3H, s, CH_3-9), 2.05–2.13 (1H, m, H-5), 2.18 (1H, br.s, C-10-OH), 2.36–2.47 (3H, m, H-1, H-2, H-4*α*), 2.56–2.74 (2H, m, H-2, H-7*β*, H-4*β*), 3.87 (1H, td, *J* = 9.2, 6.6, H-3), 4.19–4-29 (1H, m, H-10), 7.45 (1H, d, *J* = 1.5, H-7′), 8.02 (1H, d, *J* = 1.5, H-5′), 8.40 (1H, s, H-1′), 12.32 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 24.3 (C-8), 27.9 (C-9), 33.8 (C-7), 35.6 (C-4), 38.4 (C-6), 41.7 (C-5), 42.0 (C-1), 43.4 (C-3), 46.3 (C-2), 71.2 (q, J_F = 29.9, C-10), 80.7 (C-6′), 88.5 (C-4′), 121.6 (C-2′), 125.1 (q, J_F = 283.1, C-11), 138.9 (C-7′), 148.0 (C-5′), 157.6 (C-3′), 159.4 (C-1′). ^{19}F NMR (58 MHz, $CDCl_3$, δ, ppm): −74.43 (3F, d, *J* = 7.4, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3466 (OH), 1576 (C=N), 1128, 1552, 1123 (CF_3). Elemental analysis calcd. (%) for $C_{18}H_{20}F_3I_2NO_2S$: C 34.58, H 3.22, N 2.24; found: C 34.58, H 3.14, N 2.63.

(1*S*,2*R*,3*S*,5*R*)-2-(Hydroxymethyl)-6,6-dimethyl-*N*-((*E*)-4-nitrobenzylidene)bicyclo-[3.1.1] heptane-3-sulfenamide (**7b**). Yield: 81%; yellowish oil; $[\alpha]_D^{26}$ −82.0 (*c* = 0.6, $CHCl_3$); R_f = 0.22 (petr. ether:EtOAc, 3:1). ^{1}H, ^{13}C NMR and IR spectral data correspond to those given in Ref. [37].

(1*S*,2*R*,3*S*,5*R*)-6,6-Dimethyl-*N*-((*E*)-4-nitrobenzylidene)-2-((*S*)-2,2,2-trifluoro-1-hydroxyethyl) bicyclo[3.1.1]heptane-3-sulfenamide (**8b**). Yield: 75%; light-yellow crystal; m.p.: 48.8 °C; $[\alpha]_D^{25}$ −1.5 (*c* 1.41, $CHCl_3$); R_f 0.28 (petr. ether:EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.09 (3H, s, CH_3-8), 1.28 (3H, s, CH_3-9), 1.28 (1H, d, *J* = 10.3, H-7α), 1.60–1.68 (1H, m, H-4α), 2.05–2.11 (1H, m, H-5), 2.28–2.35 (1H, m, H-1), 2.37–2.46 (1H, m, H-7β), 2.49–2.59 (1H, m, H-4β), 2.94 (1H, dt, *J* = 10.3, 3.3, H-2), 3.94–4.07 (1H, m, H-10), 4.37 (1H, dt, *J* = 10.8, 4.5, H-3), 5.58 (1H, br.d, *J* = 8.8, C-10-OH), 7.72 (2H, d, *J* = 8.8, H-3′, H-7′), 8.29 (2H, d, *J* = 8.8, H-4′, H-6′), 8.54 (1H, s, H-1′). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.0 (C-8), 26.9 (C-9), 29.7 (C-7), 29.9 (C-4), 38.1 (C-6), 38.7 (C-3), 40.1 (C-5), 43.3 (C-1), 51.5 (C-2), 71.1 (q, J_F = 28.8, C-10), 124.4 (C-4′, C-6′), 125.8 (q, J_F = 283.1, C-11), 127.8 (C-3′, C-7′), 140.1 (C-2′), 148.7 (C-5′), 155.3 (C-1′). ^{19}F NMR (282 MHz, $CDCl_3$, δ, ppm): −76.37 (3F, s, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3238 (OH), 1601 (C=N), 1522, 1344 (NO_2), 1267, 1169, 1125 (CF_3). Elemental analysis calcd. (%) for $C_{18}H_{21}F_3N_2O_3S$: C 53.72, H 5.26, N 6.96; found: C 53.33, H 5.21, N 7.04. A single crystal of **8b** was grown from the hexane-Et_2O system. A colorless prismatic crystal of the monoclinic system had a size of 0.77×0.39×0.13 mm, space group $P2_1$, *a* = 16.6479(3), *b* = 7.08470(10), *c* = 17.9640(4) Å, *β* = *111.720(2)*°, *V* = 1968.34(7) $Å^3$, Z = 4, μ = 0.212 mm^{-1}, d_{calc} = 1.358 g/cm^3 and F(000) = 840. A dataset of 42,548 reflections was collected at scattering angles of 2.101° < θ < 25.027°, of which 6946 were independent (R_{int} = 0.0357), including 5644 reflections with $I > 2\sigma(I)$. The final refinement parameters were R_1 = 0.0548, wR_2 = 0.1118 (all data), R_1 = 0.0406 and wR_2 = 0.1006 [$I > 2\sigma(I)$], with GooF = 0.945. $\Delta\rho_e$ = 0.266/−0.171 *e* $Å^{-3}$; Flack parameter = −0.03(2).

(1*S*,2*R*,3*S*,5*R*)-2-(Hydroxymethyl)-6,6-dimethyl-*N*-((*E*)-3-nitrobenzylidene)bicyclo-[3.1.1] heptane-3-sulfenamide (**7c**). Yield: 53%; light-yellow oil; $[\alpha]_D^{25}$ −40.4 (*c* 2.18, $CHCl_3$); R_f 0.30 (PhH:EtOAc, 10:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.04 (3H, s, CH_3-8), 1.26 (3H, s, CH_3-9), 1.27 (1H, d, *J* = 10.3, H-7α), 1.89 (1H, ddd, *J* = 14.1, 5.7, 2.9, H-4α), 2.02–2.15 (1H, m, H-5, H-1), 2.39–2.60 (1H, m, H-7β, H-4β), 2.67 (1H, dtd, *J* = 8.7, 5.9, 2.2, H-2), 3.37 (1H, br.s, C-10-OH), 3.64 (1H, dd, *J* = 10.3, 5.9, H-10α), 3.81–3.87 (2H, m, H-3, H-10), 7.58 (1H, t, *J* = 8.1, H-6′), 7.96 (1H, d, *J* = 8.1, H-7′), 8.22 (1H, d, *J* = 8.1, H-5′), 8.38 (1H, s, H-3′), 8.54 (1H, s, H-1′). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.5 (C-8), 27.3 (C-9), 31.5 (C-7), 32.4 (C-4), 38.6 (C-6), 41.2 (C-5), 42.8 (C-3), 44.1 (C-1), 53.1 (C-2), 66.4 (C-10), 122.1 (C-3′), 124.4 (C-5′), 129.9 (C-6′), 131.9 (C-7′), 137.4 (C-2′), 148.7 (C-4′), 154.0 (C-1′). IR spectrum (KBr, ν, cm^{-1}): 3402 (OH), 1614 (C=N), 1530, 1350 (NO_2). Elemental analysis calcd. (%) for $C_{17}H_{22}N_2O_3S$: C 61.05, H 6.63, N 8.38; found: C 61.38, H 6.37, N 7.91.

(1*S*,2*R*,3*S*,5*R*)-6,6-Dimethyl-*N*-((*E*)-3-nitrobenzylidene)-2-((*S*)-2,2,2-trifluoro-1-hydroxyethyl) bicyclo[3.1.1]heptane-3-sulfenamide (**8c**). Yield: 24%; white powder; m.p.: 122.7 °C; $[\alpha]_D^{27}$ −83.3 (*c* 0.6, $CHCl_3$); R_f 0.34 (petr. ether:EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.09 (3H, s, CH_3-8), 1.28 (3H, s, CH_3-9), 1.28 (1H, d, *J* = 10.3, H-7α), 1.64 (1H, dt, *J* = 14.3, 4.0, H-4α), 2.05–2.11 (1H, m, H-5), 2.28–2.35 (1H, m, H-1), 2.37–2.46 (1H, m, H-7β), 2.54 (1H, ddt, *J* = 13.9, 11.3, 2.5, H-4β), 2.96 (1H, dt, *J* = 10.3, 3.3, H-2), 3.94–4.07 (1H, m, H-10), 4.37 (1H, dt, *J* = 10.8, 4.5, H-3), 5.64 (1H, br.d, *J* = 8.1, C-10-OH), 7.63 (1H, t, *J* = 7.6, H-6′), 7.97 (1H, d, *J* = 7.6, H-7′), 8.27 (1H, d, *J* = 7.6, H-5′), 8.35 (1H, s, H-3′), 8.53 (1H, s, H-1′). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.0 (C-8), 26.9 (C-9), 29.7 (C-7), 29.9 (C-4), 38.1 (C-6), 38.5 (C-3), 40.1 (C-5), 43.2 (C-1), 51.4 (C-2), 71.1 (q, J_F = 28.8, C-10), 122.8 (C-3′), 125.1 (C-5′), 125.7 (q, J_F = 283.1, C-11), 130.3 (C-6′), 131.6 (C-7′), 136.6 (C-2′), 148.7 (C-4′), 155.2 (C-1′). ^{19}F NMR (282 MHz, $CDCl_3$, δ, ppm): −76.38 (3F, d, *J* = 7.0, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3379 (Ar-OH), 3264 (OH), 1595 (C=N), 1530, 1350 (NO_2), 1271, 1169, 1121 (CF_3). Elemental analysis calcd. (%) for $C_{18}H_{21}F_3N_2O_3S$: C 53.72, H 5.26, N 6.96; found: C 53.95, H 5.28, N 7.01.

(1*S*,2*R*,3*S*,5R)-2-(Hydroxymethyl)-6,6-dimethyl-*N*-((*E*)-2-nitrobenzylidene)bicyclo-[3.1.1] heptane-3-sulfenamide (**7d**). Yield: 81%; yellow oil; $[\alpha]_D^{25}$ −34.5 (*c* 1.4, $CHCl_3$); R_f 0.23 (PhH:EtOAc, 10:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.03 (3H, s, CH_3-8), 1.25 (3H, s, CH_3-9), 1.26 (1H, d, *J* = 9.8, H-7α), 1.86 (1H, ddd, *J* = 14.3, 5.5, 2.9, H-4α), 2.02–2.12 (2H, m, H-5, H-1), 2.39–2.59 (2H, m, H-7*β*, H-4*β*), 2.68 (1H, dtd, *J* = 8.7, 5.9, 2.2, H-2), 3.45 (1H, br.s, C-10-OH), 3.60 (1H, dd, *J* = 9.5, 6.6, H-10α), 3.78–3.90 (2H, m, H-10*β*, H-3), 7.52

(1H, td, *J* = 7.9, 1.7, H-5′), 7.66 (1H, t, *J* = 7.8, H-6′), 7.95 (1H, dd, *J* = 7.9, 1.7, H-7′), 8.00 (1H, d, *J* = 7.9, H-4′), 8.94 (1H, s, H-1′). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.5 (C-8), 27.2 (C-9), 31.4 (C-7), 32.0 (C-4), 38.6 (C-6), 41.2 (C-5), 42.6 (C-3), 44.2 (C-1), 53.2 (C-2), 66.4 (C-10), 124.6 (C-4′), 128.4 (C-7′), 130.3 (C-5′), 130.4 (C-2′), 133.6 (C-6′), 147.7 (C-3′), 152.0 (C-1′). IR spectrum (KBr, ν, cm^{-1}): 3395 (OH), 1605 (C=N). Elemental analysis calcd. (%) for $C_{17}H_{22}N_2O_3S$: C 61.05, H 6.63, N 8.38; found: C 61.29, H 7.04, N 8.19.

(1*S*,2*R*,3*S*,5*R*)-6,6-Dimethyl-*N*-((*E*)-2-nitrobenzylidene)-2-((*S*)-2,2,2-trifluoro-1-hydroxyethyl) bicyclo[3.1.1]heptane-3-sulfenamide (**8d**). Yield: 52%; yellow oil; $[\alpha]_D^{25}$ −58.8 (*c* 1.69, $CHCl_3$); R_f 0.35 (PhH). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.08 (3H, s, CH_3-8), 1.28 (3H, s, CH_3-9), 1.32 (1H, d, *J* = 10.6, H-7*α*), 1.60–1.68 (1H, m, H-4*α*), 2.05–2.11 (1H, m, H-5), 2.29–2.36 (1H, m, H-1), 2.37–2.46 (1H, m, H-7β), 2.49–2.59 (1H, m, H-4β), 2.97 (1H, dt, *J* = 10.3, 3.3, H-2), 3.93–4.06 (1H, m, H-10), 4.34 (1H, dt, *J* = 10.3, 4.4, H-3), 5.57 (1H, br.d, *J* = 8.1, C-10-OH), 7.58 (1H, t, *J* = 7.9, H-5′), 7.72 (1H, t, *J* = 7.9, H-6′), 7.85 (1H, d, *J* = 7.9, H-7′), 8.06 (1H, d, *J* = 7.9, H-4′), 8.95 (1H, s, H-1′). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.0 (C-8), 26.8 (C-9), 29.5 (C-7), 29.9 (C-4), 38.1 (C-6), 38.7 (C-3), 40.1 (C-5), 43.2 (C-1), 51.5 (C-2), 71.1 (q, J_F = 28.8, C-10), 124.8 (C-4′), 125.7 (q, J_F = 283.0, C-11), 128.3 (C-7′), 130.0 (C-2′), 130.9 (C-5′), 134.0 (C-6′), 147.7 (C-3′), 153.4 (C-1′). ^{19}F NMR (282 MHz, $CDCl_3$, δ, ppm): −76.12 (3F, d, *J* = 6.6, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3364 (OH), 1605 (C=N), 1528, 1344 (NO_2), 1269, 1169, 1123 (CF_3). Elemental analysis calcd. (%) for $C_{18}H_{21}F_3N_2O_3S$: C 53.72, H 5.26, N 6.96; found: C 53.98, H 5.31, N 6.60.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-2-Hydroxy-5-nitrobenzylidene)-2-(hydroxymethyl)-6,6-dimethylbicyclo [3.1.1]heptane-3-sulfenamide (**7e**). Yield: 66%; yellow powder; m.p.: 108.5 °C; $[\alpha]_D^{25}$ +65.4 (*c* 1.17, $CHCl_3$); R_f 0.29 (PhH:EtOAc, 10:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.05 (3H, s, CH_3-8), 1.15 (1H, d, *J* = 9.5, H-7*α*), 1.27 (3H, s, CH_3-9), 2.04–2.11 (2H, m, H-5, C-10-OH), 2.18–2.35 (3H, m, H-1, H-4*α*, H-2), 2.46 (1H, dtd, *J* = 9.5, 6.6, 2.2 H-7*β*), 2.58–2.68 (1H, m, H-4*β*), 4.36 (1H, dt, *J* = 10.3, 6.6, H-3), 3.73–3.86 (2H, m, H-10), 7.01 (1H, d, *J* = 8.8, H-4′), 8.13–8.18 (2H, m, H-5′, H-7′), 8.60 (1H, s, H-1′), 12.40 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.4 (C-8), 27.4 (C-9), 32.2 (C-7), 35.0 (C-4), 38.8 (C-6), 41.6 (C-5), 42.4 (C-3), 42.8 (C-1), 50.0 (C-2), 65.9 (C-10), 117.7 (C-4′), 119.4 (C-2′), 126.2 (C-7′), 126.9 (C-5′), 140.3 (C-6′), 158.8 (C-1′), 163.9 (C-3′). IR spectrum (KBr, ν, cm^{-1}): 3553 (Ar-OH), 3537 (OH), 1593 (C=N), 1520, 1339 (NO_2). Elemental analysis calcd. (%) for $C_{17}H_{22}N_2O_4S$: C 58.27, H 6.33, N 7.99; found: C 57.87, H 5.85, N 7.60.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-2-Hydroxy-5-nitrobenzylidene)-6,6-dimethyl-2-((*S*)-2,2,2-trifluoro-1-hydroxyethyl)bicyclo[3.1.1]heptane-3-sulfenamide (**8e**). Yield: 41%; yellow gummy oil; $[\alpha]_D^{25}$ +38.9 (*c* 1.14, $CHCl_3$); R_f 0.30 (petr. ether:EtOAc, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.07 (3H, s, CH_3-8), 1.27 (3H, s, CH_3-9), 1.30 (1H, d, *J* = 9.0, H-7*α*), 2.05–2.13 (1H, m, H-5), 2.20–2.29 (2H, m, H-1, H-4*α*), 2.40–2.51 (3H, m, H-2, H-7*β*, C-10-OH), 2.64–2.76 (1H, m, H-4*β*), 4.09–4.23 (2H, m, H-3, H-10), 7.02 (1H, d, *J* = 8.8, H-4′), 8.14–8.19 (2H, m, H-5′, H-7′), 8.59 (1H, s, H-1′), 12.10 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.1 (C-8), 26.8 (C-9), 30.0 (C-7), 34.3 (C-4), 38.0 (C-6), 40.3 (C-5), 40.6 (C-3), 42.4 (C-1), 46.7 (C-2), 72.4 (q, J_F = 29.2, C-10), 117.8 (C-4′), 119.4 (C-2′), 125.0 (q, J_F = 283.1, C-11), 126.4 (C-7′), 127.0 (C-5′), 140.3 (C-6′), 158.7 (C-1′), 163.9 (C-3′). ^{19}F NMR (282 MHz, $CDCl_3$, δ, ppm): −76.17 (1F, d, *J* = 6.5, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3509 (OH), 1593 (C=N), 1522, 1341 (NO_2), 1277, 1167, 1123 (CF_3). Elemental analysis calcd. (%) for $C_{18}H_{21}F_3N_2O_4S$: C 51.67, H 5.06, N 6.70; found: C 51.69, H 5.27, N 6.82.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-5-Bromo-2-hydroxybenzylidene)-2-(hydroxymethyl)-6,6-dimethylbicyclo [3.1.1]heptane-3-sulfenamide (**7f**). Yield: 61%; light-yellow gummy oil; $[\alpha]_D^{26}$ +55.9 (*c* 0.2, $CHCl_3$); R_f 0.36 (PhH:EtOAc, 5:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.04 (3H, s, CH_3-8), 1.14 (1H, d, *J* = 9.9, H-7*α*), 1.27 (3H, s, CH_3-9), 1.63 (1H, br.s, C-10-OH), 2.03–2.09 (1H, m, H-5), 2.18–2.34 (3H, m, H-1, H-4*α*, H-2), 2.44–2.53 (1H, m, H-7*β*), 2.56–2.66 (1H, m, H-4*β*), 3.59 (1H, dt, *J* = 9.9, 6.6, H-3), 3.69–3.85 (2H, m, H-10), 6.84 (1H, d, *J* = 8.8, H-4′), 7.27 (1H, d, *J* = 2.2, H-7′), 7.35 (1H, dd, *J* = 8.8, 2.2, H-5′), 8.48 (1H, s, H-1′), 11.51 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.4 (C-8), 27.5 (C-9), 32.4 (C-7), 35.1 (C-4), 38.8 (C-6), 41.7 (C-5), 42.4 (C-1), 42.8 (C-3), 50.1 (C-2), 66.1 (C-10), 110.8 (C-6′), 118.9 (C-4′),

121.5 (C-2′), 132.5 (C-7′), 134.3 (C-5′), 157.8 (C-3′), 159.7 (C-1′). IR spectrum (KBr, ν, cm^{-1}): 3341 (OH), 1587 (C=N). Elemental analysis calcd. (%) for $C_{17}H_{22}BrNO_2S$: C 53.13, H 5.77, N 3.64; found: C 53.10, H 5.71, N 3.55.

(1*S*,2*R*,3*S*,5*R*)-*N*-((*E*)-2-Hydroxy-5-bromobenzylidene)-6,6-dimethyl-2-((*S*)-2,2,2-trifluoro-1-hydroxyethyl)bicyclo[3.1.1]heptane-3-sulfenamide (**8f**). Yield: 38%; yellow oil; m.p.: 122.7 °C; $[\alpha]_D^{26}$ +29.0 (*c* 1.3, $CHCl_3$); R_f 0.34 (PhH:CH_2Cl_2, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.05 (3H, s, CH_3-8), 1.25 (1H, d, *J* = 9.9, H-7*α*), 1.25 (3H, s, CH_3-9), 2.02–2.09 (1H, m, H-5), 2.18–2.34 (2H, m, H-1, H-4*α*), 2.37–2.50 (2H, m, H-7*β*, H-2), 2.55–2.80 (2H, m, H-4*β*, C-10-OH), 4.00–4.20 (2H, m, H-3, H-10), 6.83 (1H, d, *J* = 8.8, H-4′), 7.27 (1H, s, H-7′), 7.34 (1H, d, *J* = 8.8, 2.2, H-5′), 8.45 (1H, s, H-1′), 11.25 (1H, br.s, C-3′-OH). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 23.0 (C-8), 26.9 (C-9), 30.3 (C-7), 34.7 (C-4), 38.1 (C-6), 40.5 (C-3), 40.8 (C-5), 42.5 (C-1), 46.6 (C-2), 72.6 (q, J_F = 29.8, C-10), 110.9 (C-6′), 118.9 (C-4′), 121.4 (C-2′), 125.0 (q, J_F = 284.2, C-11), 132.7 (C-7′), 134.5 (C-5′), 157.7 (C-3′), 159.9 (C-1′). ^{19}F NMR (58 MHz, $CDCl_3$, δ, ppm): −74.11 (3F, d, *J* = 6.1, CF_3-11). IR spectrum (KBr, ν, cm^{-1}): 3455 (OH), 1589 (C=N), 1271, 1171, 1123 (CF_3), 1076 (CBr). Elemental analysis calcd. (%) for $C_{18}H_{21}BrF_3NO_2S$: C 47.80, H 4.68, N 3.10; found: C 47.53, H 4.31, N 3.07.

4.3. Antibacterial Activity

Minimum inhibitory concentrations (MICs) of compounds were determined by the broth microdilution assay in 96-well plates (Eppendorf, Hamburg, Germany) according to the EUCAST rules for antimicrobial susceptibility testing [45] in full Mueller–Hinton broth (MH). Briefly, the bacterial suspension containing 10^8 CFUs/mL was subsequently diluted to 1:300 with MH broth in microwell plates to obtain a 10^6 cells/mL suspension, and then incubated at 37 °C for 24 h. The stock solutions of compounds to be tested were prepared in DMSO and added to the final concentrations of compounds to be tested, which ranged from 1 to 1048 μg/mL. The MIC was determined as the lowest concentration of an antibiotic for which no visible bacterial growth could be observed after 24 h of incubation. The assessment was performed five times and the typical (median) value was considered as the MIC.

4.4. Antifungal Activity

MICs on *C. albicans* were determined using the broth microdilution method in 96-well plates (Eppendorf) with MH broth, as recommended in the protocol CLSI M27-A3 [46]. *C. albicans* was grown overnight and diluted with MH broth until a density of 10^7 cells/mL was reached, obtaining the working solution. Then, 2-fold serial dilutions of compounds in concentrations from 1 to 1024 μg/mL were prepared in MH broth and seeded with fungi (1% *v*/*v* of working solution) with subsequent incubation at 37 °C for 24 h. The MIC was defined as the lowest concentration of the compound at which no visible growth could be seen. The assessment was performed five times and the typical (median) value was considered as the MIC.

4.5. Mutagenicity and Cytotoxicity

The mutagenicity of compounds was evaluated in the Ames test with *S. typhimurium* TA98, TA100 and TA102 strains, as described in [41]. The spot-test modification was applied to avoid false-negative results due to the antibacterial activity of compounds. The tested compound was considered to be mutagenic if the number of revertant colonies increased more than 2 times when close to the filter paper with the compound.

The cytotoxicity of compounds was determined using the microtetrazolium test (MTT) on BFL cells. The cells were cultured in DMEM—Dulbecco's Modified Eagle's Medium (Sigma Aldrich, St. Louis, MO, USA)—that was supplemented with 10% FBS, 2 mM of *L*-glutamine, 100 μg/mL of penicillin and 100 μg/mL of streptomycin. Cells were seeded in 96-well plates with a density of 3000 cells per well and left overnight to allow for the attachment. Next, cells were cultured at 37 °C and 5% CO_2 in the presence of compounds of interest at various concentrations from 1.25 to 160 μg/mL. After 24 h of cultivation, the

cells were subjected to the MTT assay. The formazan was solubilized by DMSO and the optical density was measured on the Tecan Infinite 200Pro at 570 nm. The concentration required to inhibit cellular dehydrogenase activity by 50% (CC_{50} value) was calculated by using the GraphPad Prism 6.0 software.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics11111548/s1. The ^{1}H NMR, ^{13}C NMR and IR spectra of novel compounds.

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