Synthesis, hypolipidemic and antifungal activity of tetrahydroberberrubine sulfonates*

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The paper describes the synthesis of new tetrahydroberberrubine derivatives containing polyfluorophenyl- or alkylsulfonate groups at the O(9) position as well as those containing or not containing a bromine atom at the C(12) position. In a model of acute Triton-induced hyperlipidemia, tetrahydroberberrubine 9-*O*-(heptafluoro-4′-toluene)sulfonate was shown to reduce the cholesterol level by 23.5%, which is comparable with the effect of Simvastatin. At a concentration of 32 μg mL^{-1}, tetrahydroberberrubine 9-*O*-pentafluorobenzenesulfonate inhibits the growth of the fungus *Cryptococcus neoformans* by 81.3±3.5%.

Key words: berberine, berberrubine, sulfonate, cholesterol, hyperlipidemia, antifungal activity, *Cryptococcus neoformans*, cytotoxicity.

The isoquinoline alkaloid berberine (**1**) is one of the most abundant members of the protoberberine alkaloid family.[1] Berberine has a broad spectrum of biological activity, with antibacterial, antimalarial, and antitumor activities studied in most detail.[2—4] In recent years, berberine was also extensively investigated as an agent capable of reducing the lipid level.[5] Berberine has a different mechanism of hypolipidemic effect compared to statins, which are most commonly used agents for the treatment of dyslipidemia. Statins are structurally similar to the substrate of HMG-CoA reductase, with the result that they block the biosynthetic pathway of farnesyl pyrophosphate, which is further converted not only into cholesterol but also into sterol, ubiquinone, and dolichol. Besides, statins have side effects, including increased concentrations of liver enzymes in the blood, myopathy and myalgia, rhabdomyolysis, and so on.[6] Another drawback of statins is that they induce an upregulation of the proprotein convertase subtilisin/kexin type 9 (PCSK9), which suppresses the expression of low-density lipoprotein receptors (LDLRs), thereby reducing the removal cholesterol-containing lipoproteins from the blood and offsetting beneficial effects of statins. Unlike statins, berberine does not inhibit cholesterol synthesis, but increases LDLR expression and was shown to inhibit PCSK9 expression, resulting in lowering of cholesterol levels in the blood and an increase in the efficacy of statins.[7]

It is known that modifications of the berberine molecule can give rise to compounds with a more pronounced hypolipidemic effect compared to the starting berberine. The modification of the berberine moiety at the O(9) position in order to prepare new biologically active compounds is a very popular line of research. We consider such compounds as derivatives of berberrubine (**2**), which is the demethylated analog of berberine. Thus, we showed that reduced berberine derivatives, such as 12-bromotetrahydroberberine (**3**) containing a bromine atom at C(12) and 9-*O*-tosyltetrahydroberberrubine (**4**) containing the sulfonate moiety at position 9, exhibit a pronounced hypocholesterolemic effect *in vivo*. In the experiment using Triton WR-1339-induced hyperlipidemic mice, compound **3** decreased the level of total cholesterol in the blood by 27%; compound **4**, by 33%.[8] In *in vitro* experiments, it was demonstrated that reduced berberine derivatives **5** and **6** containing the mono- and difluorinated phenylsulfonate group, respectively, increase the level of LDLR expression by a factor of 3.6—4.0.[9] An interesting

* Dedicated to Corresponding Member of the Russian Academy of Sciences A. V. Kutchin on the occasion of his 70th birthday.

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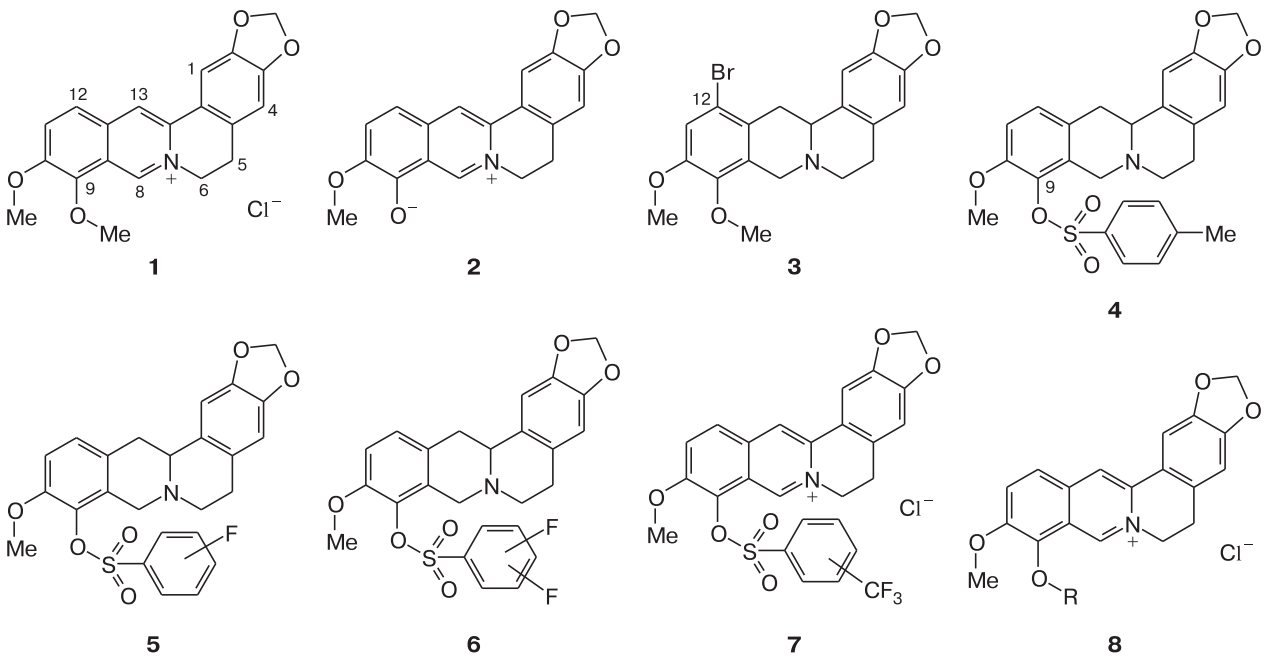


issue is the combined effect of the bromine atom and the sulfonate group, as pharmacophore moieties, on biological activity.

The modifications of berberine at the O(9) position giving compounds with different biological activities were described in the literature in detail. It was shown that compounds **7** containing the trifluoromethyl-substituted phenylsulfonate group have anti-inflammatory activity and suppress the expression (with an inhibitory rate of 85—87%) of nuclear factor-kappa B (NF-κB), which controls the expression of immune response genes, apoptosis, and cell cycle progression.[10] Some 9-*O*-alkyl- and acyl-substituted berberines **8** exhibit high activity against fungi (*Cryptococcus neoformans* ATCC 36556[11,12]) and bacteria (*Staphylococcus aureus*,[12] *Micrococcus luteus*[13]). These data suggest that 9-*O*-substituted berberine derivatives should be screened not only for hypolipidemic activity but also for other activities.

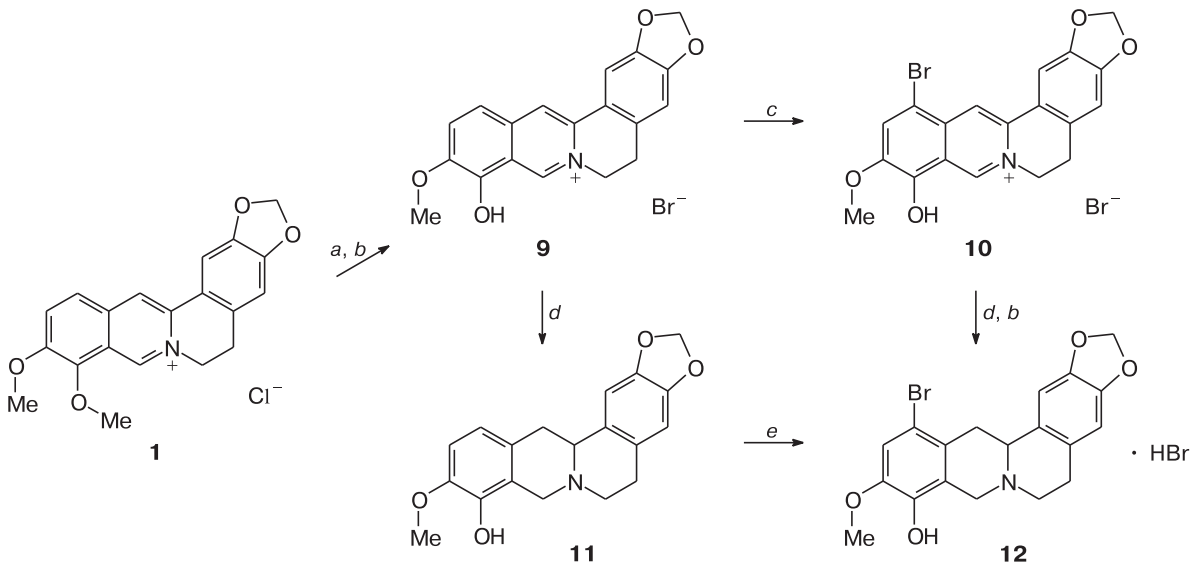
In the present work, we synthesized a series of berberrubine (berberine) derivatives, namely, tetrahydroberberrubine 9-*O*-sulfonates containing polyfluorophenyl- and alkylsulfonate groups. To test the hypothesis that the pharmacophore moieties have a combined effect,[8] we synthesized analogs of tetrahydroberberrubine sulfonates containing a bromine atom at the C(12) position. The synthesized berberrubine derivatives were examined for hypolipidemic activity *in vivo* using a model of Triton-induced hyperlipidemia. We also evaluated antibacterial and antifungal activity of these compounds and their cytotoxic effect.

Synthesis of berberrubine derivatives. The heating of berberine chloride (**1**) at 190 °C *in vacuo* (20 Torr)[14] leads to the elimination of the methyl group at the O(9) atom to form berberrubine (**2**). After the treatment with HBr, the reaction product was isolated in 89% yield as a salt — berberrubine hydrobromide (**9**) (Scheme 1). According to the published data, berberrubine **2** reacted with a solution of bromine in aqueous alkali to form 12-bromoberberrubine (**10**) (the yield was not reported).[15] We found that the reaction under these conditions gave compound **10** only in trace amounts. The use of a dioxane—water mixture as the solvent resulted in the bromination of compound **9** giving bromo derivative **10** in 53% yield.

Compound **9** was reduced with sodium borohydride in methanol according to a procedure described previously.[8] Tetrahydroberberrubine (**11**) was prepared in 78% yield. The bromination of compound **11** with a solution of bromine in dioxane afforded 12-bromotetrahydroberberrubine hydrobromide (**12**) in 52% yield. Compound **12** was also synthesized in 61% yield by the reduction of compound **10** with sodium borohydride in methanol.

The reaction of tetrahydro derivatives **11** and **12** with polyfluoroaryl and alkyl sulfonyl chlorides as well as with tosyl chloride in dichlorometane in the presence of triethylamine produced tetrahydroberberrubine 9-*O*-sulfonates **13a—g** (44—84% yields) and 12-bromotetrahydroberberrubine 9-*O*-sulfonates **14a—h** (49—93% yields) (Scheme 2). The structures of compounds **13** and **14** were established based on the analysis of ^{1}H, ^{13}C, and ^{19}F NMR spectra, elemental analysis data, high-resolution mass spectra, and IR and UV spectra. For compounds **13a** and **14h**, 2D correlation spectra (H—H, C—H, COLOC, NOESY) were recorded.

Scheme 1



Reagents and conditions: (*a*) 190 °C, 20 Torr, 30 min; (*b*) HBr; (*c*) Br_2/NaOH, dioxane—water; (*d*) $NaBH_4$, MeOH; (*e*) Br_2, dioxane.

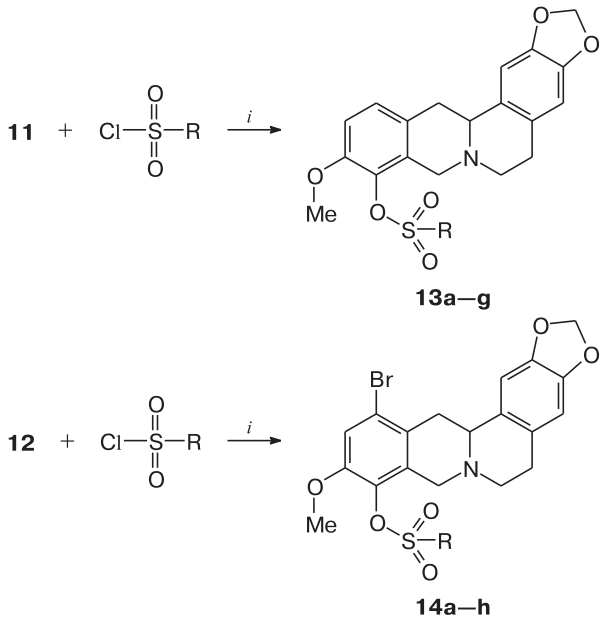
Evaluation of hypolipidemic activity. The ability of sulfonates **13a—g** and **14a—h** to reduce *in vivo* cholesterol and triglyceride levels in the blood was evaluated using a model of acute hyperlipidemia induced by the intraperitoneal administration of Tyloxapol (Triton WR1339). Triton WR1339 is a nonionic detergent that induces hyperlipidemia by inhibiting peripheral lipoprotein lipase enzymes, which are responsible for removal of lipid particles from the body, resulting in transient elevation of lipid levels.[16] The lipid level reaches a peak at 18—24 h after the administration of Triton WR1339 and then starts to decline.[17]

Compounds **13a—g** and **14a—h** were administered to mice once a day for four days at a dose of 100 mg kg^{-1}. Previously, it was shown that other berberine derivatives injected at this dose exhibit hypocholesterolemic effect.[8] After the last administration of compounds **13** and **14**, all animals, except for the control group, were injected with Tyloxapol. The animals were sacrificed 24 h after the injection, and the blood was collected for analysis. The drug Simvastatin at a dose of 40 mg kg^{-1} was used as the reference. Table 1 summarizes the cholesterol and triglyceride (TG) levels evaluated after the introduction of compounds **13a—g** and **14a—h.**

In the series of the compounds under study, only compound **13c** (R = *p*-$CF_3C_6F_4$) displays hypocholesterolemic effect. It causes a lowering of the cholesterol level by 23.5%. The efficacy of **13c** is comparable with that of Simvastatin (reduces the cholesterol level by 26.9%). These compounds had no effect on the triglyceride level.

As can be seen in Table 1, the introduction of a bromine atom at the C(12) position leads to a loss of hypocholesterolemic activity (*cf.* pairs of compounds **13c—14c** and **4**[8]**—14h)**. To evaluate this fact, further research on the mechanism of action of sulfonates and their possible binding site is needed.

Scheme 2



i. NEt_3, CH_2Cl_2.

13, **14**	R	**13**, **14**	R	**13**, **14**	R
a	C_6F_5	**d**	Me	**g**	Bu^n
b	*p*-HC_6F_4	**e**	Et	**h**	*p*-MeC_6H_4
c	*p*-$CF_3C_6F_4$	**f**	Pr^n		

Table 1. Hypolipidemic activity of tetrahydroberberrubine sulfonates in a model of Triton-induced hyperlipidemia at a dose of 100 mg kg^{-1}

Compound	Cholesterol	TG
	mmol L^{-1}	
Control	2.789±0.29	0.99±0.30
Triton WR1339	16.42±0.98	16.73±0.14
Simvastatin (40 mg kg^{-1})	12.00±0.87*	16.76±0.29
13a	16.16±0.35	17.59±0.34
13b	16.91±0.82	16.95±0.34
13c	12.56±1.11*	16.42±2.93
13d	16.71±1.21	16.59±0.09
13e	15.92±1.07	15.11±0.18
13f	15.35±2.09	15.70±2.09
13g	15.89±0.76	15.57±0.27
14a	16.03±0.77	16.64±0.20
14b	15.62±1.23	18.40±0.91
14c	16.34±1.73	15.16±0.34
14d	16.43±0.75	16.85±0.28
14e	15.57±2.09	16.54±2.07
14f	18.83±1.28	15.92±0.19
14g	17.87±1.29	15.84±0.25
14h	15.01±1.12	16.87±0.16

* The criterion of statistically significant differences between the results of evaluation compared to Triton WR1339 $p \le 0.05$.

Evaluation of antimicrobial activity. Antimicrobial activity of compounds **13a**,**d**—**e** and **14a**,**c**—**e**,**h** was kindly evaluated by The Community for Antimicrobial Drug Discovery (CO-ADD, Australia).[18] The compounds were tested for activity against Gram-negative (multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae* strains, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) and Gram-positive (methicillin-resistant *Staphylococcus aureus* (MRSA) strain) bacteria and against the fungi *Candida albicans* and *Cryptococcus neoformans*. The preliminary antimicrobial screening was performed using whole cell growth inhibition assays. Each compound was tested at a concentration of 32 μg mL^{-1}. The antibacterial activity was compared with the effect of the drugs Vancomycin and Amoxicillin; the antifungal activity, with the effect of the drug Fluconazole.

The tested compounds did not exhibit antibacterial activity against the Gram-positive and Gram-negative bacterial strains (the percentage of growth inhibition compared with the reference agent was less than 25%). At a concentration of 32 μg mL^{-1}, compound **13a** showed high activity against the fungus *Cryptococcus neoformans*. This fungus is an encapsulated organism, which usually grows in central nervous system tissues, although it can grow in other tissues of the body. This fungus causes the damage of the central nervous system and, in some cases, the lungs, mucosal and skin lesions.[19] Currently, there are no effective vaccines against cryptococcosis, and the available antifungal agents are inefficient, toxic, and expensive.[20] Compound **13a** showed 81.3±3.5% inhibition of *C. neoformans* (100% for the reference agent Fluconazole). The efficacy of compound **13a** can be enhanced by transforming it into hydrobromide. For compound **13a**·HBr, the inhibition of *C. neoformans* was 93.7±3.8% at a concentration of 32 μg mL^{-1}. Other analogs of compound **13a**, including those containing a bromine atom (**14a**,**c**,**h**), did not exhibit significant activity against *C. neoformans*; the minimal inhibitory concentration (MIC) of berberine is 64 μg mL^{-1} in the inhibition of *C. neoformans*.[21]

Evaluation of cytotoxic activity. Compounds **13a**—**g** and **14a**—**e**,**g**,**h** were tested for cytotoxic activity against the tumor cell lines MCF7 (ATCC HTB-22) and HepG2 (ATCC HB-8065) and immortalized human fibroblasts used as the normal cell line. Compounds **13b**,**e**—**g** and **14b**—**e**,**g**,**h** did not exhibit activity against all the cell lines under study (IC_{50} > 100 μmol L^{-1}). Table 2 summarizes the concentrations of the compounds that cause 50% cell growth inhibition (IC_{50}) after 72 h incubation. Four compounds (**13a**,**c**,**d** and **14a**) displayed moderate cytotoxicity against human fibroblasts.

In summary, we synthesized new tetrahydroberberine derivatives modified at one or two positions: at position 12 by introducing bromine and/or at position 9 by alkyl- and polyfluoroarylsulfonate moieties. Compound **13c** containing the perfluoro-*para*-toluenesulfonate substituent at position 9 and a bromine atom at position 12 displayed hypocholesterolemic effect in *in vivo* assays in mice comparable with that of Simvastatin. In the absence of a bromine substituent and in the presence of other substituents in the sulfonate group, the activity is lost. The screening of antibacterial activity revealed that at a concentration of 32 μg mL^{-1}, compound **13a** containing the perfluorophenylsulfonate and bromine substituents inhibits the growth of the fungus *C. neoformans*.

Experimental

Berberine chloride hydrate purchased from TCI (percent purity 81%) and a 48% aqueous HBr solution were used. Column chromatography was carried out on neutral alumina LL40/250;

Table 2. Cytotoxic activity of tetrahydroberberrubine sulfonates

Compound	IC_{50}±SEM/μmol L^{-1}		
	IFs*	MCF7	HepG2
Doxorubicin	4.2±0.7	3.17±0.41	11.23±2.38
13a	17.46±0.88	43.57±3.43	>100
13c	<10	>100	>100
13d	15.06±2.43	>100	>100
14a	20.92±2.3	78.93±2.95	48.56±5.7

* IFs are immortalized fibroblasts.

HPLC, on a Milichrom A-02 micro-column liquid chromatograph (EcoNova, Novosibirsk) equipped with standard chromatography columns packed with the reversed-phase sorbent ProntoSIL-120-5-C18 AQ (particle size 5 μm, 75×2 mm column) at 35 °C; the pressure was 30—36 atm; the flow rate was 150 μL min^{-1}. The injection volume was 2 μL. The linear gradient elution was peformed using the solvent system, from 100% A to 100% B for 25 min (solvent A, 0.1% aqueous trifluortoacetic acid solution; solvent B, methanol), with multi-wavelength detection at six wavelengths (220, 240, 260, 280, 320, 360 nm).

The spectroscopic and analytical measurements were carried out at the Multi-access Chemical Service Center of the Siberian Branch of the Russian Academy of Sciences. The melting points were measured on a Mettler Toledo thermosystem. The UV spectra were recorded on a HP 8453 UV-Vis spectrophotometer in EtOH ($c = 10^{-4}$ mol L^{-1}). The IR spectra were measured on a Vector 22 FTIR spectrometer in KBr pellets. High-resolution mass spectra were obtained on a DFS Thermo Scientific mass spectrometer; LC-MS, on a HPLC-MS system comprising an Agilent 1200 liquid chromatograph and a Bruker micrOTOF-Q hybrid quadrupole time-of-flight mass spectrometer. The mass detection parameters were as follows: the atmospheric pressure ionization electrospray (API-ES) mode, the positive ion scan in the range m/z = 100—3000. The nebulizer gas (nitrogen) flow rate was 4 L min^{-1}, the temperature was 220 °C, the pressure at the spraying nozzle was 1.0 atm. A solution of the compound (2 μL) at a concentration of 0.1 mg mL^{-1} in methanol was introduced into an electrospray ionization chamber of the mass spectrometer by injecting it into the solvent flow (MeOH, 0.1 mL min^{-1}) using an autosampler device. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer (400.13 and 100.61 MHz) for 5—10% solutions of the compounds in $CDCl_3$ or DMSO-d_6 using the signal of the solvent $CDCl_3$ as the standard (δ 7.24 for ^1H and δ 76.90 for ^{13}C). The ^{19}F NMR spectra were measured on a Bruker AV-300 spectrometer (282.36 MHz) using C_6F_6 (δ −162.9 with respect to the signal of $CFCl_3$) as the external standard. The assignments of the signals in the ^1H and ^{13}C NMR spectra marked with an asterisk and a double asterisk can be interchanged.

Berberrubine hydrobromide (9). Compound **1** (5 g, 0.011 mol) was heated in a 2 L flask on a sand bath at 180—200 °C using a water-jet vacuum (20—30 Torr) five times for 15 min. Between the heatings, the reaction mixture was stirred to homogeneity. The dark-maroon residue was dissolved in hot ethanol (200 mL) and then filtered. After cooling, concentrated HBr (3 mL) was added. The mixture was cooled and a precipitate of berberrubine hydrobromide dihydrate **9** was filtered off. The yield was 4.18 g (89%). The ^1H NMR spectroscopic data are consistent with those published in the literature.[22]

12-Bromoberberrubine hydrobromide (10). A solution of bromine (480 mg, 3.00 mmol) in a 6% aqueous NaOH solution (9 mL) was added dropwise to a magnetically stirred solution of berberrubine hydrobromide dihydrate **9** (595 mg, 1.36 mmol) in a mixture of dioxane (5 mL) and water (20 mL), during which a precipitate formed. Then the reaction mixture was stirred for 1 h at room temperature. The dark-cherry precipitate was filtered off, washed with a small amount of water, and dried in a desiccator. Then the precipitate was suspended in methanol (10 mL) and acidified with hydrobromic acid to acidic pH. The remaining precipitate was dissolved, after which a paler precipitate formed. The mixture was cooled, and the orange precipitate was filtered off and dried in a desiccator. Compound **10** was obtained in a yield of 347 mg (53%). The melting point was not determined (decomp.). Found (%): C, 46.73; H, 3.28; N, 2.43; Br, 32.91. $C_{19}H_{15}NO_4Br_2$. Calculated (%): C, 47.43; H, 3.14; N, 2.91; Br, 33.21. UV (EtOH), λ_{max}/nm: 239; 279; 356. IR (KBr), ν/cm^{-1}: 1315; 1497; 1591. MS (LC-MS): found: m/z 400.016 $[M - Br]^+$; $C_{19}H_{15}NO_4Br^+$; calculated: 400.018. ^1H NMR (400 MHz, DMSO-d_6), δ: 3.13—3.23 (m, 2 H, H(5)); 4.05 (s, 3 H, OCH_3); 4.86—4.97 (m, 2 H, H(6)); 6.17 (s, 2 H, OCH_2O); 7.06 (s, 1 H, H(4)); 7.82 (s, 1 H, H(1)); 8.31 (s, 1 H, H(11)*); 8.32 (s, 1 H, H(13)*); 9.97 (s, 1 H, H(8)); 11.67 (br.s, 1 H, OH). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 26.37 (C(5)), 54.97 (C(6)), 57.50 (OCH_3), 102.17 (OCH_2O), 105.80 (C(1)), 108.44 (C(4)), 108.64 (C(12)), 117.94 (C(11)), 118.06 (C(13b)), 120.24 (C(8a)), 128.46 (C(13)), 130.24 (C(12a)*), 131.12 (C(4a)*),138.06 (C(13a)), 144.44, 145.52 (C(2), C(3)), 147.80 (C(9)), 150.01 (C(10)).

Tetrahydroberberrubine (11). Sodium borohydride (1.35 g, 4 equiv.) was added portionwise to a magnetically stirred suspension of berberrubine hydrobromide dihydrate **9** (3.88 g, 8.86 mmol) in methanol (30 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2.5 h. The solvent was distilled off *in vacuo*, and the residue was dissolved in CH_2Cl_2 and passed through an alumina layer. The fractions of the eluate containing the product were combined, and the solvent was distilled off *in vacuo*. The residue was recrystallized from acetonitrile. Compound **11** was obtained in a yield of 2.14 g (74%). The spectroscopic data are consistent with those published in the literature.[22]

12-Bromotetrahydroberberrubine hydrobromide (12). ***A.*** A solution of bromine (3.53 g, 22.08 mmol) in dioxane (15 mL) was added dropwise to a mechanically stirred solution of tetrahydroberberrubine **11** (2.39 g, 7.36 mmol) in dioxane (50 mL) at room temperature, during which a brown-red precipitate formed. Then the reaction mixture was stirred for 3 h at room temperature. The precipitate was filtered off and successively washed with a saturated Na_2SO_3 solution (4×20 mL) and acetonitrile (3×10 mL). The residue was refluxed with methanol (80 mL) for 30 min and then cooled. The precipitate was filtered off and dried in a desiccator. Compound **12** was obtained in a yield of 1.87 g (52%).

B. Sodium borohydride (116 mg, 3.04 mmol) was added portionwise to a magnetically stirred suspension of aromatic hydrobromide **10** (244 mg, 0.51 mmol) in MeOH (5 mL) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then for 1.5 h at room temperature. The solvent was removed *in vacuo*, and the residue was separated by chromatography on silica gel (CH_2Cl_2—MeOH, 100 : 1). The recrystallization from acetonitrile gave compound **12** as a free base in a yield of 90 mg (44%). The mother liquor was acidified with HBr, and compound **12** as hydrobromide was additionally filtered off in a yield of 42 mg (17%). M.p. 289.9 °C (decomp.). Found (%): C, 42.74; H, 3.96; N, 2.61; Br, 30.11. $C_{19}H_{19}NO_4Br_2 \cdot 3\ H_2O$. Calculated (%): C, 42.32; H, 4.67; N, 2.60; Br, 29.64. UV (EtOH), λ_{max}/nm: 205; 290. IR (KBr), ν/cm^{-1}: 1296; 1498. MS (LC-MS): found: m/z 404.051 $[M - Br]^+$; $C_{19}H_{19}NO_4Br^+$; calculated: 404.049. ^1H NMR (400 MHz, DMSO-d_6), δ: 2.69—2.76 (m, 1 H, H(13)); 2.91—2.95 (m, 1 H, H(5)); 2.09—3.23 (m, 2 H, H(5), H(6)); 3.66 (m, 1 H, H(13)); 3.85 (s, 3 H, OCH_3); 3.95 (m, 1 H, H(6)); 4.32—4.35 (m, 1 H, H(8)); 4.57—4.65 (m, 2 H, H(8), H(13a)); 6.04 (d, 2 H, OCH_2O, J = 11.5 Hz); 6.84 (s, 1 H, H(4)); 7.16 (s, 1 H, H(1)); 7.29 (s, 1 H, H(11)); 9.71 (s, 1 H, OH); 10.44 (s, 1 H, NH+). ^{13}C NMR (125 MHz, DMSO-d_6), δ: 25.65 (C(5)),

34.29 (C(13)), 49.88 (C(6)), 51.06 (C(8)), 56.66 (OCH_3), 59.07 (C(13a)), 101.59 (OCH_2O), 105.82 (C(1)), 108.50 (C(4)), 111.95 (C(12)), 115.34 (C(11)), 118.30 (C(8a)*), 123.68 (C(12a)*), 124.98 (C(13b)*), 125.50 (C(4a)*), 142.30 (C(9)), 146.79, 147.02, 147.18 (C(2), C(3), C(10)).

Synthesis of sulfonates 13 and 14 (general procedure). Triethylamine (2.5 mmol, 0.35 mL; 3.5 mmol in the case of hydrobromide **12**) and sulfonyl chloride (1.5 mmol) were added dropwise to a solution of compound **11** or **12** (1 mmol) in CH_2Cl_2 (20 mL) at room temperature. The reaction mixture was stirred at room temperature for 5—8 h, the solvent was removed *in vacuo*, and the residue was separated by chromatography on silica gel ($CHCl_3$—MeOH, 100 : 0, 100 : 1, 100 : 2). After the recrystallization from MeCN, sulfonates **13** and **14** were obtained as crystalline compounds.

Tetrahydroberberrubine 9-*O*-pentafluorobenzenesulfonate (13a). Yield 44%. M.p. 213.8 °C (decomp.). Found (%): C, 49.32; H, 3.20; N, 2.44; S, 5.29; F, 15.73. $C_{25}H_{18}NO_6SF_5{+}3H_2O$. Calculated (%): C, 49.26; H, 3.97; N, 2.30; S, 5.26; F, 15.58. UV (EtOH), λ_{max}/nm: 202; 286. IR (KBr), ν/cm^{-1}: 1290; 1400; 1504. MS (HRMS): found: *m/z* 555.0778 $[M]^+$; $C_{25}H_{18}NO_6SF_5$; calculated: 555.0770. ^{1}H NMR (600 MHz, DMSO-d_6), δ: 2.80—2.95 (m, 1 H, H(5)); 3.03—3.19 (m, 1 H, H(13)); 3.30 (m, 1 H, H(5)); 3.45 (m, 1 H, H(6)); 3.56 (s, 3 H, OCH_3); 3.81—3.90 (m, 2 H, H(13), H(6)); 4.45—4.86 (m, 3 H, H(8), H(13a)); 6.03 (d, 2 H, OCH_2O, J = 3.0 Hz); 6.83 (s, 1 H, H(4)); 7.09 (s, 1 H, H(1)); 7.20 (d, 1 H, H(11), J = 8.7 Hz); 7.31 (d, 1 H, H(12), J = 8.4 Hz). ^{19}F NMR (282 MHz, DMSO-d_6), δ: 5.8 (m, 2 F, F(3′), F(5′)); 22.6 (t, 1 F, F(4′), J = 22.5 Hz); 28.5 (m, 2 F, F(2′), F(6′)). ^{13}C NMR (150 MHz, DMSO-d_6), δ: 25.41 (C(5)), 31.93 (C(13)), 49.76 (C(6)), 50.26 (C(8)), 56.04 (OCH_3), 58.66 (C(13a)), 101.28 (OCH_2O), 105.54 (C(1)), 108.26 (C(4)), 112.94 (C(1′)), 113.15 (C(11)), 124.06 (C(8a)), 124.96 (C(13b)), 125.48 (C(4a)), 125.86 (C(12a), 129.14 (C(12)), 134.22 (C(9)), 137.66 (d, (C(2′), C(6′), ^1J = 256 Hz), 144.40 (d, (C(3′), C(5′), ^1J = 260 Hz), 144.94 (d, (C(4′), ^1J = 258 Hz), 146.68, 146.87 (C(2), C(3)), 149.38 (C(10)).

Tetrahydroberberrubine 9-*O*-(2′,3′,5′,6′-tetrafluorobenzene)-sulfonate (13b). Yield 84%. M.p. 113 °C (decomp.). Found (%): C, 54.85; H, 2.41; N, 3.77; S, 6.35; F, 14.05. $C_{25}H_{19}NO_6SF_4{+}H_2O$. Calculated (%): C, 54.05; H, 2.52; N, 3.81; S, 5.77; F, 13.68. UV (EtOH), λ_{max}/nm: 205; 286. IR (KBr), ν/cm^{-1}: 1253; 1393; 1504. MS (HRMS): found: *m/z* 536.0782 $[M - H]^+$; $C_{25}H_{18}NO_6SF_4$; calculated: 536.0785. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 2.60—2.72 (m, 2 H, H(5), H(6)); 2.77—2.92 (m, 1 H, H(13)); 3.04—3.28 (m, 3 H, H(5), H(6), H(13)); 3.46 (s, 3 H, OCH_3); 3.58—3.66 (m, 1 H, H(13a)); 3.71 (d, 1 H, H(8), J = 16 Hz); 4.30 (d, 1 H, H(8), J = 16 Hz); 5.90 (s, 2 H, OCH_2O); 6.58 (s, 1 H, H(4)); 6.68 (s, 1 H, H(1)); 6.74 (d, 1 H, H(11), J = 8.4 Hz); 7.04 (d, 1 H, H(12), J = 8.4 Hz); 7.39 (tt, 1 H, H(4′), J = 9.0 Hz, J = 7.1 Hz). ^{19}F NMR (282 MHz, $CDCl_3$), δ: 25.7 (m, 2 F); 27.1 (m, 2 F). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 29.10 (C(5)), 35.68 (C(13)), 50.91 (C(6)), 53.47 (C(8)), 55.40 (OCH_3), 59.11 (C(13a)), 100.72 (OCH_2O), 105.26 (C(1)), 108.35 (C(4)), 110.65 (C(11)), 110.78 (C(4′)), 119.45 (C(1′)), 127.44 (C(8a*)), 128.24 (C(13b*)), 128.24 (C(12)), 129.87 (C(4a)*), C(12a)*), 135.37 (C(9)), 143.61 (d, C(2′), C(6′), ^1J = 262 Hz), 146.15 (d, C(3′), C(5′), ^1J = 251 Hz), 146.01, 146.12 (C(2), C(3)), 148.80 (C(10)).

Tetrahydroberberrubine 9-*O*-(heptafluoro-4′-toluene)sulfonate (13c). Yield 64%. M.p. 178.9 °C (decomp.). Found (%): C, 51.92; H, 3.07; N, 2.59; S, 5.42; F, 21.87. $C_{26}H_{18}NO_6SF_7$. Calculated (%): C, 51.58; H, 3.00; N, 2.31; S, 5.30; F, 21.96. UV (EtOH), λ_{max}/nm: 223; 228; 242; 289. IR (KBr), ν/cm^{-1}: 1159; 1327; 1400; 1410; 1502. MS (HRMS): found: *m/z* 605.0738 $[M]^+$; $C_{26}H_{18}NO_6SF_7$; calculated: 605.0741. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 2.60—2.72 (m, 2 H, H(5), H(6)); 2.77—2.93 (m, 1 H, H(13)); 3.02—3.29 (m, 3 H, H(5), H(6), H(13)); 3.47 (s, 3 H, OCH_3); 3.57—3.65 (m, 1 H, H(13a)); 3.71 (d, 1 H, H(8), J = 16 Hz); 4.28 (d, 1 H, H(8), J = 16 Hz); 5.90 (s, 2 H, OCH_2O); 6.58 (s, 1 H, H(4)); 6.68 (s, 1 H, H(1)); 6.76 (d, 1 H, H(11), J = 8.4 Hz); 7.07 (d, 1 H, H(12), J = 8.4 Hz). ^{19}F NMR (282 MHz, $CDCl_3$), δ: 24.2 (2 F, F(3′), F(5′)); 29.6 (m, 2 F, F(2′), F(6′)); 105.1 (t, 3 F, CF_3, J = 22.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 29.44 (C(5)), 36.02 (C(13)), 51.20 (C(6)), 53.70 (C(8)), 55.68 (OCH_3), 59.36 (C(13a)), 101.00 (OCH_2O), 105.52 (C(1)), 108.64 (C(4)), 113.15 (C(11)), 114.17 (qt, C(4′), ^2J = 35 Hz, ^2J = 12.5 Hz), 120.24 (q, CF_3, ^1J = 275 Hz), 122.66 (C(1′)), 127.73 (C(8a)*), 128.75 (C(13b*), 128.75 (C(12)), 130.17 (C(4a)*, C(12a)*), 135.58 (C(9)), 144.30, 144.52 (both d, C(2′), C(6′), C(3)′, C(5′), ^1J = 264 Hz), 146.30, 146.41 (C(2), C(3)), 148.75 (C(10)).

Tetrahydroberberrubine 9-*O*-methylsulfonate (13d). Yield 76%. M.p. 191.3 °C (decomp.). Found (%): C, 59.50; H, 5.06; N, 3.78; S, 7.85. $C_{20}H_{21}NO_6S$. Calculated (%): C, 59.54; H, 5.25; N, 3.47; S, 7.95. UV (EtOH), λ_{max}/nm: 201; 286. IR (KBr), ν/cm^{-1}: 1173; 1229; 1286; 1365; 1491. MS (HRMS): found: *m/z* 402.0998 $[M - H]^+$; $C_{20}H_{20}NO_6S$; calculated: 402.1006. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 2.56—2.68 (m, 2 H, H(5), H(6)); 2.80 (m, 1 H, H(13)); 2.99—3.27 (m, 3 H, H(5), H(6), H(13)); 3.32 (s, 3 H, CH_3SO_2O); 3.52—3.60 (m, 1 H, H(13a)); 3.65 (d, 1 H, H(8), J = 16 Hz); 3.85 (s, 3 H, OCH_3); 4.25 (d, 1 H, H(8), J = 16 Hz); 5.89 (s, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.68 (s, 1 H, H(1)); 6.82 (d, 1 H, H(11), J = 8.4 Hz); 7.03 (d, 1 H, H(12), J = 8.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 29.32 (C(5)), 35.96 (C(13)), 39.54 (CH_3SO_2O), 50.93 (C(6)), 53.79 (C(8)), 55.79 (OCH_3), 59.10 (C(13a)), 100.59 (OCH_2O), 105.25 (C(1)), 108.25 (C(4)), 110.60 (C(11)), 127.55 (C(12)), 127.60 (C(8a)*), 128.25 (C(13b*)), 130.32, 130.57 (C(4a)*, C(12a)*), 135.30 (C(9)), 145.77, 145.95 (C(2), C(3)), 149.20 (C(10)).

Tetrahydroberberrubine 9-*O*-ethylsulfonate (13e). Yield 58%. M.p. 186.5—187.0 °C. Found (%): C, 60.37; H, 5.34; N, 3.57; S, 7.68. $C_{21}H_{23}NO_6S$. Calculated (%): C, 60.42; H, 5.55; N, 3.36; S, 7.68. UV (EtOH), λ_{max}/nm: 201; 286. IR (KBr), ν/cm^{-1}: 1286; 1358; 1500. MS (HRMS): found: *m/z* 417.1239 $[M]^+$; $C_{21}H_{23}NO_6S$; calculated: 417.1241. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.57 (t, 3 H, $C\underline{H}_3CH_2$, J = 8 Hz); 2.58—2.67 (m, 2 H, H(5), H(6)); 2.75—2.85 (m, 1 H, H(13)); 3.00—3.25 (m, 3 H, H(5), H(6), H(13)); 3.39—3.54 (m, 2 H, CH_2SO_2O); 3.54—3.56 (m, 1 H, H(13a)); 3.68 (d, 1 H, H(8), J = 16 Hz); 3.83 (s, 3 H, OCH_3); 4.26 (d, 1 H, H(8), J = 16 Hz); 5.88—3.91 (m, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.68 (s, 1 H, H(1)); 6.81 (d, 1 H, H(11), J = 8.4 Hz); 7.02 (d, 1 H, H(12), J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 8.42 ($\underline{CH}_3CH_2$), 29.52 (C(5)), 36.16 (C(13)), 47.38 (CH_2SO_2O), 51.13 (C(6)), 54.06 (C(8)), 55.99 (OCH_3), 59.31 (C(13a)), 100.80 (OCH_2O), 105.47 (C(1)), 108.46 (C(4)), 110.78 (C(11)), 127.64 (C(12)), 127.80 (C(8a)*), 128.38 (C(13b*), 130.56, 130.91 (C(4a)*, C(12a)*), 135.44 (C(9)), 145.98, 146.16 (C(2), C(3)), 149.43 (C(10)).

Tetrahydroberberrubine 9-*O*-propylsulfonate (13f). Yield 74%. M.p. 179.9 °C (decomp.). Found (%): C, 61.08; H, 5.63; N, 3.31; S, 7.37. $C_{22}H_{25}NO_6S$. Calculated (%): C, 61.24; H, 5.84; N, 3.25; S, 7.43. UV (EtOH), λ_{max}/nm: 201; 286. IR (KBr), ν/cm^{-1}: 1284; 1365; 1487. MS (HRMS): found: *m/z* 431.1396 $[M]^+$; $C_{22}H_{25}NO_6S$; calculated: 431.1397. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.12 (t, 3 H,

$\underline{CH_3}CH_2$, J = 8 Hz); 2.06 (m, 2 H, $CH_3\underline{CH_2}$); 2.58—2.67 (m, 2 H, H(5), H(6)); 2.80 (m, 1 H, H(13)); 3.01—3.26 (m, 3 H, H(5), H(6), H(13)); 3.42 (m, 2 H, CH_2SO_2O); 3.56 (m, 1 H, H(13a)); 3.67 (d, 1 H, H(8), J = 16 Hz); 3.83 (s, 3 H, OCH_3); 4.26 (d, 1 H, H(8), J = 16 Hz); 5.89 (m, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.68 (s, 1 H, H(1)); 6.81 (d, 1 H, H(11), J = 8.4 Hz); 7.02 (d, 1 H, H(12), J = 8.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 14.42 ($\underline{CH_3}CH_2$), 18.90 ($CH_3\underline{CH_2}$), 30.90 (C(5)), 37.53 (C(13)), 52.50 (C(6)), 55.46 (C(8*)), 55.80 (CH_2SO_2O*), 57.41 (OCH_3), 60.70 (C(13a)), 102.17 (OCH_2O), 106.85 (C(1)), 109.84 (C(4)), 112.24 (C(11)), 128.98 (C(12)), 129.19 (C(8a)**), 129.80 (C(13b**), 131.97, 132.30 (C(4a)**, C(12a)**), 136.90 (C(9)), 147.39, 147.56 (C(2), C(3)), 150.86 (C(10)).

Tetrahydroberberrubine 9-*O*-butylsulfonate (13g). Yield 83%. M.p. 160.8 °C (decomp.). Found (%): C, 62.15; H, 5.95; N, 3.14; S, 7.12. $C_{23}H_{27}NO_6S$. Calculated (%): C, 62.00; H, 6.11; N, 3.14; S, 7.20. UV (EtOH), λ_{max}/nm: 201; 286. IR (KBr), ν/cm^{-1}: 1225; 1290; 1356; 1483. MS (HRMS): found: m/z 445.1550 $[M]^+$; $C_{23}H_{27}NO_6S$; calculated: 445.1554. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.99 (t, 3 H, $\underline{CH_3}CH_2$, J = 8 Hz); 1.47—1.56 (m, 3 H, $CH_3\underline{CH_2}$); 1.96—2.06 (m, 2 H, $CH_3CH_2\underline{CH_2}$); 2.58—2.68 (m, 2 H, H(5), H(6)); 2.75—2.85 (m, 1 H, H(13)); 3.00—3.26 (m, 3 H, H(5), H(6), H(13)); 3.37—3.51 (m, 2 H, CH_2SO_2O); 3.53—3.61 (m, 1 H, H(13a)); 3.67 (d, 1 H, H(8), J = 16 Hz); 3.83 (s, 3 H, OCH_3); 4.26 (d, 1 H, H(8), J = 16 Hz); 5.89 (m, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.68 (s, H(1) 1 H); 6.81 (d, 1 H, H(11), J = 8.4 Hz), 7.02 (d, 1 H, H(12), J = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 13.41 ($\underline{CH_3}CH_2$), 21.47 ($CH_3\underline{CH_2}$), 25.47 ($CH_3CH_2\underline{CH_2}$), 29.35 (C(5)), 35.99 (C(13)), 50.97 (C(6)), 52.37 (CH_2SO_2O*), 53.91 (C(8*)), 55.83 (OCH_3), 59.15 (C(13a)), 100.65 (OCH_2O), 105.31 (C(1)), 108.31 (C(4)), 110.64 (C(11)), 127.45 (C(12)), 127.63 (C(8a)**), 128.20 (C(13b**), 130.38, 130.72 (C(4a)**, C(12a)**), 135.31 (C(9)), 145.83, 146.00 (C(2), C(3)), 149.28 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-pentafluorobenzenesulfonate (14a). Yield 49%. M.p. was not determined (decomp.). Found (%): C, 47.63; H, 3.23; N, 2.99; S, 5.30; F, 14.30; Br, 12.63. $C_{25}H_{17}NO_6SF_5Br$. Calculated (%): C, 47.33; H, 2.70; N, 2.22; S, 5.05; F, 14.97; Br, 12.60. UV (EtOH), λ_{max}/nm: 204; 289. IR (KBr), ν/cm^{-1}: 1103; 1174; 1238; 1302; 1410; 1502. MS (HRMS): found: m/z 632.9881 $[M]^+$; $C_{25}H_{17}NO_6SF_5Br$; calculated: 632.9875. ^{1}H NMR (300 MHz, DMSO-d_6), δ: 2.80—2.97 (m, 2 H, H(5), H(13)); 3.16—3.28 (m, 1 H, H(5)), 3.45—3.56 (m, 1 H, H(6)); 3.62 (s, 3 H, OCH_3); 3.70—4.02 (m, 2 H, H(6), H(13)); 4.53—4.88 (m, 3 H, 2 H(8), H(13a)); 6.04 (d, 2 H, OCH_2O, J = 9.0 Hz); 6.84 (s, 1 H, H(4)); 7.15 (s, 1 H, H(1)); 7.58 (s, 1 H, H(11)); 11.51 (br.s, 1 H, NH^+). ^{19}F NMR (282 MHz, DMSO-d_6), δ: 4.0 (m, 2 F, F(3′), F(5′)), 21.1 (br.t, 1 F, F(4′), J = 21 Hz); 26.6 (m, 2 F, F(2′), F(6′)). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 25.44 (C(5)), 34.11 (C(13)), 49.47 (C(6)*), 50.02 (C(8)*), 56.65 (OCH_3), 58.55 (C(13a)), 101.35 (OCH_2O), 105.61 (C(1)), 108.33 (C(4)), 112.60 (C(1′)), 117.17 (C(11)), 123.57 (C(12)), 124.78, 125.09, 125.40 (C(8a)**, C(13b)**, C(4a)**), 126.15 (C(12a), 133.79 (C(9)), 137.75 (d, C(2′), C(6′), ^1J = 252 Hz), 144.36 (d, C(3′), C(5′), ^1J = 260 Hz), 144.99 (d, C(4′), ^1J = 258 Hz), 146.71, 147.01 (C(2), C(3)), 149.99 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-(2′,3′,5′,6′-tetrafluorobenzene)sulfonate hydrobromide (14b). Yield 56%. M.p. 243.4 °C (decomp.). Found (%): C, 43.80; H, 2.80; N, 2.15; S, 4.76; F, 11.44; Br, 23.50. $C_{25}H_{18}NO_6SF_4Br \cdot HBr$. Calculated (%): C, 43.06; H, 2.75; N, 2.01; S, 4.60; F, 10.90; Br, 22.92. UV (EtOH), λ_{max}/nm: 206; 288. IR (KBr), ν/cm^{-1}: 1172; 1261; 1402; 1504. MS (HRMS): found: m/z 614.9958 $[M]^+$; $C_{25}H_{18}NO_6SF_4Br$; calculated: 614.9969. ^{1}H NMR (300 MHz, DMSO-d_6), δ: 2.78—3.00 (m, 2 H, H(5), H(13)); 3.16—3.28 (m, 1 H, H(5)); 3.45 (m, 1 H, H(6)); 3.53 (s, 3 H, OCH_3); 3.72—4.06 (m, 2 H, H(6), H(13)); 4.56—4.90 (m, 3 H, 2 H(8), H(13a)); 6.03 (d, 2 H, OCH_2O, J = 8.0 Hz); 6.84 (s, 1 H, H(4)); 7.15 (s, 1 H, H(1)); 7.57 (s, 1 H, H(11)); 8.56 (tt, 1 H, H(4′), J = 10.3 Hz, J = 7.7 Hz); 11.47 (br.s, 1 H, NH^+). ^{19}F NMR (282 MHz, DMSO-d_6), δ: 26.3 (m, 2 F); 26.4 (m, 2 F). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 25.41 (C(5)), 34.11 (C(13)), 49.42 (C(6)), 50.04 (C(8)), 56.57 (OCH_3), 58.50 (C(13a)), 101.32 (OCH_2O), 105.53 (C(1)), 108.30 (C(4)), 113.96 (C(4′)), 116.98 (C(1′)), 117.11 (C(11)), 123.49 (C(12)), 124.63 (C(8a)*), 125.15, 125.35 (C(13b)*, C(4a)*), 126.29 (C(12a), 133.78 (C(9)), 143.60 (d, (C(2′), C(6′), ^1J = 254 Hz); 145.74 (d, (C(3)′, C(5′), ^1J = 253 Hz); 146,74, 146.98 (C(2), C(3)), 149.91 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-(heptafluoro-4′-toluene)sulfonate (14c). Yield 49%. M.p. was not determined (decomp.). Found (%): C, 45.38; H, 2.36; N, 2.24; S, 4.86; F, 19.31; Br, 12.72. $C_{26}H_{17}NO_6SF_7Br$. Calculated (%): C, 45.63; H, 2.50; N, 2.02; S, 4.69; F, 19.43; Br,11.68. UV (EtOH), λ_{max}/nm: 205; 289. IR (KBr), ν/cm^{-1}: 1161; 1331; 1504. MS (HRMS): found: m/z 682.9847 $[M]^+$; $C_{26}H_{17}NO_6SF_7Br$; calculated: 682.9843. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.57—2.72 (m, 3 H, H(5), H(6), H(13)); 3.00—3.31 (m, 3 H, H(5), H(6), H(13)); 3.50 (s, 3 H, OCH_3); 3.55—3.63 (m, 1 H, H(13a)); 3.68 (d, 1 H, H(8), J = 16 Hz); 4.25 (d, 1 H, H(8), J = 16 Hz); 5.92 (s, 2 H, OCH_2O); 6.59 (s, 1 H, H(4)); 6.75 (s, 1 H, H(1)); 7.03 (s, 1 H, H(11)). ^{19}F NMR (282 MHz, $CDCl_3$), δ: 24.5 (m, 2 F, F(3′), F(5′)); 29.7 (m, 2 F, F(2′), F(6′)); 105.1 (t, 3 F, CF_3, J = 22.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 29.19 (C(5)), 37.17 (C(13)), 50.55 (C(6)), 53.60 (C(8)), 55.72 (OCH_3), 58.99 (C(13a)), 100.77 (OCH_2O), 105.38 (C(1)), 108.36 (C(4)), 114.12 (qt, C(4′), ^2J = 35 Hz, ^2J = 12.5 Hz), 114.71 (C(11)), 119.92 (q, CF_3, ^1J = 275 Hz), 122.12 (C(1′)), 123.82 (C(12)), 127.44 (C(8a)*), 128.29 (C(13b*), 129.63 (C(4a)**), 131.98 (C(12a)**), 134.55 (C(9)), 144.05, 144.20 (both d,(C(2′), C(6′), C(3′), C(5′), ^1J = 267 Hz), 146.09, 146.15 (C(2), C(3)), 148.79 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-methylsulfonate (14d). Yield 93%. M.p. 234.2 °C (decomp.). Found (%): C, 50.50; H, 4.26; N, 2.84; S, 6.51; Br, 16.12. $C_{20}H_{20}NO_6SBr$. Calculated (%): C, 49.80; H, 4.18; N, 2.90; S, 6.65; Br, 16.57. UV (EtOH), λ_{max}/nm: 205; 288. IR (KBr), ν/cm^{-1}: 1165; 1222; 1361; 1483. MS (HRMS): found: m/z 481.0186 $[M]^+$; $C_{20}H_{20}NO_6SBr$; calculated: 481.0189. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 2.30—2.38 (m, 1 H, H(13)); 2.44—2.48 (m, 1 H, H(5)*); 2.58—2.66 (m, 1 H, H(6)*); 2.83—2.92 (m, 1 H, H(5)); 3.03—3.11 (m, 1 H, H(13)); 3.26—3.32 (m, 1 H, H(6)); 3.46—3.49 (m, 1 H, H(13a)); 3.50 (s, 3 H, CH_3SO_2O); 3.52 (d, 1 H, H(8), J = 16 Hz); 3.87 (s, 3 H, OCH_3); 4.06 (d, 1 H, H(8), J = 16 Hz); 5.96 (d, 2 H, OCH_2O, J = 9.6 Hz); 6.68 (s, 1 H, H(4)); 6.95 (s, 1 H, H(1)); 7.38 (s, 1 H, H(11)). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 28.92 (C(5)), 36.95 (C(13)), 39.82 (CH_3SO_2O), 49.86 (C(6)), 53.52 (C(8)), 56.51 (OCH_3), 58.54 (C(13a)), 100.67 (OCH_2O), 105.78 (C(1)), 108.20 (C(4)), 115.12 (C(11)), 122.23 (C(12)), 127.13 (C(8a)**), 127.40 (C(13b)**), 130.31 (C(4a)**), 132.17 (C(12a), 134.38 (C(9)), 145.66, 145.80 (C(2), C(3)), 150.04 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-ethylsulfonate (14e). Yield 54%. M.p. 195.5 °C (decomp.). Found (%): C, 50.70; H, 4.26; N, 2.84; S, 6.51; Br, 16.12. $C_{21}H_{22}NO_6SBr$. Calculated (%):

C, 50.81; H, 4.47; N, 2.82; S, 6.46; Br, 16.10. UV (EtOH), λ_{max}/nm: 204; 289. IR (KBr), ν/cm^{-1}: 1228; 1363; 1483. MS (HRMS): found: *m/z* 495.0337 $[M]^+$; $C_{21}H_{22}NO_6SBr$; calculated: 495.0346. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.56 (t, 3 H, CH_3, *J* = 8 Hz); 2.54—2.69 (m, 3 H, H(5), H(6), H(13)); 2.98—3.29 (m, 3 H, H(5), H(6), H(13)); 3.38—3.52 (m, 2 H, CH_2SO_2O); 3.53—3.58 (m, 1 H, H(13a)); 3.65 (d, 1 H, H(8), *J* = 16 Hz); 3.83 (s, 3 H, OCH_3); 4.24 (d, 1 H, H(8), *J* = 16 Hz); 5.91 (s, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.75 (s, 1 H, H(1)); 7.08 (s, 1 H, H(11)). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 8.23 (CH_3), 29.30 (C(5)), 37.31 (C(13)), 47.35 (C(6)), 50.60 (CH_2SO_2O*), 54.00 (C(8)*), 56.07 (OCH_3), 59.07 (C(13a)), 100.70 (OCH_2O), 105.41 (C(1)), 108.30 (C(4)), 114.64 (C(11)), 122.70 (C(12)), 127.52 (C(8a)**), 127.80 (C(13b**), 130.06 (C(4a)**), 132.76 (C(12a), 134.56 (C(9)), 145.94, 146.06 (C(2), C(3)), 149.67 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-propylsulfonate (14f). Yield 32%. M.p. 190.2 °C (decomp.). UV (EtOH), λ_{max}/nm: 205; 289. IR (KBr), ν/cm^{-1}: 1228; 1367; 1483. MS (HRMS): found: *m/z* 509.0510 $[M]^+$; $C_{22}H_{24}NO_6BrS$; calculated: 509.0502. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.02 (t, 3 H, $\underline{CH_3}CH_2$, *J* = 8 Hz); 2.99—2.11 (m, 3 H, $CH_3\underline{CH_2}$); 2.55—2.70 (m, 3 H, H(5), H(6), H(13)); 2.99—3.29 (m, 3 H, H(5), H(6), H(13)); 3.34—3.48 (m, 2 H, CH_2SO_2O); 3.52—3.60 (m, 1 H, H(13a)); 3.66 (d, 1 H, H(8), *J* = 16 Hz); 3.84 (s, 3 H, OCH_3); 4.24 (d, 1 H, H(8), *J* = 16 Hz); 5.91 (s, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.75 (s, 1 H, H(1)); 7.08 (s, 1 H, H(11)). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 12.89 ($\underline{CH_3}CH_2$), 17.34 ($CH_3\underline{CH_2}$), 29.24 (C(5)), 37.25 (C(13)), 50.60 (C(6)), 53.98 (C(8)*), 54.34 (CH_2SO_2O*), 56.10 (OCH_3), 59.08 (C(13a)), 100.72 (OCH_2O), 105.41 (C(1)), 108.31 (C(4)), 114.69 (C(11)), 122.69 (C(12)), 127.48 (C(8a)**), 127.74 (C(13b)**), 129.98 (C(4a)**), 132.65 (C(12a), 134.56 (C(9)), 145.97, 146.08 (C(2), C(3)), 149.70 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-butylsulfonate (14g). Yield 74%. M.p. 116.9 °C (decomp.). Found (%): C, 52.00; H, 4.73; N, 2.76; S, 6.66; Br, 16.04. $C_{23}H_{26}NO_6SBr$. Calculated (%): C, 52.68; H, 5.00; N, 2.67; S, 6.11; Br, 15.24. UV (EtOH), λ_{max}/nm: 205; 289. IR (KBr), ν/cm^{-1}: 1228; 1290; 1369; 1483. MS (HRMS): found: *m/z* 523.0652 $[M]^+$; $C_{23}H_{26}NO_6SBr$; calculated: 523.0659. ^{1}H NMR (400 MHz, $CDCl_3$). δ: 0.98 (t, 3 H, $\underline{CH_3}CH_2$, *J* = 8 Hz); 1.47—1.57 (m, 3 H, $CH_3\underline{CH_2}$); 1.94—2.06 (m, 2 H, $CH_3CH_2\underline{CH_2}$); 2.55—2.70 (m, 3 H, H(5), H(6), H(13)); 2.99—3.29 (m, 3 H, H(5), H(6), H(13)); 3.33—3.48 (m, 2 H, CH_2SO_2O); 3.53—3.60 (m, 1 H, H(13a)); 3.62—3.69 (d, 1 H, H(8), *J* = 16 Hz); 3.84 (s, 3 H, OCH_3); 4.24 (d, 1 H, H(8), *J* = 16 Hz); 5.91 (s, 2 H, OCH_2O); 6.57 (s, 1 H, H(4)); 6.75 (s, 1 H, H(1)); 7.08 (s, 1 H, H(11)). ^{13}C NMR (100 MHz, $CDCl_3$), δ: 13.39 ($\underline{CH_3}CH_2$), 21.44 ($CH_3\underline{CH_2}$), 25.43 (CH_3-$CH_2\underline{CH_2}$), 29.27 (C(5)), 37.28 (C(13)), 50.61 (C(6)), 52.48 (CH_2SO_2O*), 54.00 (C(8)*), 56.09 (OCH_3), 59.08 (C(13a)), 100.72 (OCH_2O), 105.42 (C(1)), 108.32 (C(4)), 114.69 (C(11)), 122.68 (C(12)), 127.51 (C(8a)**), 127.78 (C(13b)**), 130.02 (C(4a)**), 132.71 (C(12a), 134.60 (C(9)), 145.98, 146.08 (C(2), C(3)), 149.71 (C(10)).

12-Bromotetrahydroberberrubine 9-*O*-(4′-toluene)sulfonate (14h). Yield 67%. M.p. 170.1 °C (decomp.). Found (%): C, 55.59; H, 4.39; N, 2.81; S, 6.13; Br, 14.19. $C_{26}H_{24}NO_6SBr$. Calculated (%): C, 55.92; H, 4.33; N, 2.51; S, 5.74; Br, 14.31. UV (EtOH), λ_{max}/nm: 290. IR (KBr), ν/cm^{-1}: 1171; 1228; 1298; 1365; 1485. MS (HRMS): found: *m/z* 556.0427 $[M - H]^+$; $C_{26}H_{23}NO_6SBr$; calculated: 556.0424. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 2.29—2.36 (m, 1 H, H(6)*); 2.37—2.43 (m, 1 H, H(13)*); 2.45 (s, 3 H, Ar$\underline{CH_3}$); 2.58—2.62 (m, 1 H, H(5)); 2.81—2.90 (m, 1 H, H(5)); 2.94—2.98 (m, 1 H, H(6)); 3.26—3.31 (m, 1 H, H(13)); 3.34—3.37 (m, 1 H, H(8)); 3.42—3.44 (m, 1 H, H(13a)); 3.47 (s, 3 H, OCH_3); 3.94 (m, 1 H, H(8), *J* = 16 Hz); 5.94—5.97 (m, 2 H, OCH_2O); 6.68 (s, 1 H, H(4)); 6.94 (s, 1 H, H(1)); 7.26 (s, 1 H, H(11)); 7.51 (d, 2 H, H(3′), H(5′), *J* = 8 Hz); 7.82 (d, 2 H, H(2′), H(6′), *J* = 8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6), δ: 21.50 (CH_3), 29.15 (C(5)), 37.21 (C(13)), 50.10 (C(6)), 53.78 (C(8)), 56.24 (OCH_3), 58.87 (C(13a)), 100.97 OCH_2O), 106.02 (C(1)), 108.48 (C(4)), 115.29 (C(11)), 122.80 (C(12)), 127.32 (C(12a), 127.63 (C(4a)), 128.38 (C(2′), C(6′)), 130.20 (C(3)′, C(5′), 130.57 (C(8a)), 132.41 (C(13b)), 133.60 (C(1′)), 134.47 (C(9)), 145.90 (C(4′)), 145.95, 146.09 (C(2), C(3)), 150.42 (C(10)).

Biological assays were performed in CD-1 mice with a body weight of 25—35 g. The animals were obtained from the vivarium of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, where they were housed under standard vivarium conditions and had *ad libitum* access to water and standard granulated food. After the quarantine, the animals were randomized by weight into groups containing eight mice of the same sex per group. All manipulations on animals were carried out in strict compliance with the legislation of the Russian Federation (Order No. 708 N of the Ministry of Health of the Russian Federation, August 28, 2010, GOST 33044-2014 "Principles of Good Laboratory Practice") and in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Mouse model of acute experimental hyperlipidemia induced by the detergent Triton WR 1339. Sulfonates **13a—g** and **14a—h** or Simvastatin (as a positive control) were mixed with several drops of Tween-80 and then distilled water was added. The resulting suspension was orally administered to mice once a day for four days in a volume of 0.2 mL/10 g of body weight at a dose of 100 mg kg^{-1} for the sulfonates and at a dose of 40 mg kg^{-1} for Simvastatin.

One hour after the last (fourth) administration, all animals were intraperitoneally injected with a 7% Triton WR1339 (Tyloxapol, CAS 25301-02-4, Sigma) solution in a volume of 0.1 mL per 10 g of body weight. Then the mice were deprived of food and sacrificed 24 h after the administration of Triton. The blood was collected from neck vessels and centrifuged for 15 min at 3000 rpm to prepare the serum. The concentration of total cholesterol and triglycerides in the blood was determined using the standard diagnostic kit (Olvex-diagnosticum) on a StatFax 3300 spectrophotometer (USA).

Antibacterial and antifungal activity. The screening of antibacterial and antifungal activity was performed by the CO-ADD using whole cell growth inhibition assays. The tested compounds were predissolved in DMSO so that their concentration was 10 mg mL^{-1}. Then the solutions were diluted with water to the 32 μg mL^{-1} concentration of the tested compounds. Two portions of each sample with a volume of 5 μL were placed in a 384-well plate (Corning 3640) and then bacterial cells were added. The bacteria were cultured in CAMHB medium (Cation-adjusted Mueller Hinton broth) at 37 °C for 16 h, diluted in fresh CAMHB, and incubated at 37 °C. The bacterial cultures from the middle phase were diluted to the cell density of $5 \cdot 10^5$ CFU mL^{-1} (the absorbance was determined at a wavelength of 600 nm, OD600), and a portion (45 μL) of this solution was added to each well of

the plate. The plate was covered and incubated at 37 °C for 18 h without shaking. The bacterial growth inhibition was determined by measuring the absorbance at a wavelength of 600 nm using a Tecan M1000 Pro monochromator-based microplate reader. The percentage of growth inhibition was calculated for each well using the negative control (CAMHB medium) and the positive control (CAMHB medium supplemented with bacteria in the absence of inhibitors) as the reference in the same plate. All experiments were run in duplicate.

The strains of fungi were cultured for 3 days on the agar Yeast Extract-Peptone Dextrose (YPD) at 30 °C. Suspensions of the fungi were prepared from five colonies at concentrations from $1 \cdot 10^6$ to $5 \cdot 10^6$ cell mL^{-1} (OD530 was measured). Then these suspensions were diluted with Yeast NitrogenBase (YNB) medium to the concentration of $2.5 \cdot 10^3$ CFU mL^{-1}. The suspension of the fungi (45 μL) was added to each well of the plate containing the tested compound. The plate was covered and incubated at 35 °C for 24 h without shaking.

The growth inhibition of *C. albicans* was determined by measuring the absorbance at a wavelength of 630 nm (OD630). To determine the growth inhibition of *C. neoformans*, 0.001% resazurin was added, the incubation was performed for 2 h at 35 °C, and the difference between the absorbance at wavelengths of 600 and 570 nm (OD600-570) was measured on a Biotek Synergy HTX microplate reader. The percentage of growth inhibition was calculated for each well using the negative control (the medium without fungi) and the positive control (the medium containing fungi in the absence of inhibitors) as the rereference in the same plate. The significance of the results was estimated based on the Z-test values calculated from the mean and standard deviations of the samples in one plate. The samples with an inhibitory effect higher than 80% and the Z-score value higher than 2.5 for both repeated series of experiments were classified as active. All experiments were run in duplicate.

Cytotoxic activity. The cells were cultured in Dulbecco′s Modified Eagle′s medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, USA). The absorbance was measured on a Multiskan RC microplate photometer (LabSystems). The cytotoxicity was evaluated using the MTT assay, which was performed according to the protocol[23] using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, USA). The cell lines MCF7 and HepG2 were obtained from the American Type Culture Collection (ATCC, USA). Immortalized human fibroblasts were prepared and kindly provided by A. G. Shilov (Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk). All culture experiments were performed in triplicate and repeated three times with similar results. The final results are given as the average concentrations of the compounds that cause 50% cell growth inhibition (IC_{50}±SEM).

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References

1. L. Grycova, J. Dostal, R. Marek, *Phytochemistry,* 2007, **68**, 150.
2. I. V. Nechepurenko, N. F. Salakhutdinov, G. A. Tolstikov, *Chem. Sustain. Development,* 2010, **18**, 1.
3. P. R. Vuddanda, S. Chakraborty, S. Singh, *Expert Opin. Investig. Drugs*, 2010, **19**, 1297.
4. I. P. Singh, S. Mahajan, *Expert Opin. Ther. Pat.,* 2013, **23**, 215.
5. W. Kong, J. Wei, P. Abidi, M. Lin, S. Inaba, C. Li, Y. Wang, Z. Wang, S. Si, H. Pan, S. Wang, J. Wu, Y. Wang, Z. Li, J. Liu, J.-D. Jiang, *Nature Medicine,* 2004, **10**, 1344.
6. M. A. Silva, A. C. Swanson, P. J. Gandhi, G. R. Tataronis, *Clinical Therapeutics*, 2006, **28**, 26.
7. W.-J. Kong, J. Wei, Z.-Y. Zuo, Y.-M. Wang, D.-Q. Song, X.-F. You, L.-X. Zhao, H.-N. Pan, J.-D. Jiang, *Metabolism*, 2008, **57**, 1029.
8. I. V. Nechepurenko, U. A. Boyarskikh, M. V. Khvostov, D. S. Baev, N. I. Komarova, M. L. Filipenko, T. G. Tolstikova, N. F. Salakhutdinov, *Chem. Nat. Compd.*, 2015, **51**, 916.
9. H. Liu, J. Wang, R. Zhang, N. Cairns, J. Liu, *US Pat.* 200948246.
10. Y.-X. Wang, L. Liu, Q.-X. Zeng, T.-Y. Fan, J.-D. Jiang. H.-B. Deng, D.-Q. Song, *Molecules*, 2017, **22**, 1257.
11. S. H. Kim, S. J. Lee, J. H. Lee, W. S. Sun, J. H. Kim, *Planta Med.*, 2002, **68**, 277.
12. J.-H. Kim, S.-J. Lee, S.-H. Sung-Han, *Patent WO* 200351875.
13. S. W. Hong, S. H. Kim, J. A. Jeun, S. J. Lee, S. U. Kim, J. H. Kim, *Planta Med.*, 2000, **66**, 361.
14. K. Iwasa, M. Kamigauchi, *Phytochemistry*, 1996, **41**, 1511.
15. G. Frerichs, P. Stoepel, *Arch. Pharm.*, 1913, **251**, 321.
16. A. R. Patel, P. R. Vavia, *The AAPS Journal*, 2007, **9**, 344.
17. P. E. Schurr, J. R. Schultz, T. M. Parkinson, *Lipids*, 1972, **7**, 68.
18. M. A. Blaskovich, J. Zuegg, A. G. Elliott, M. A. Cooper, *ACS Infect. Dis.*, 2015, **1**, 285.
19. T. G. Mitchell, J. R. Perfect, *Clinical Microbiology Rev.*, 1995, **8**, 515.
20. A. Casadevall, *Clin. Infect. Dis.*, 2017, **64**, 823.
21. X. Bian, L. He, G. Yang, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1380.
22. I. V. Nechepurenko, N. I. Komarova, V. G. Vasilev, N. F. Salakhutdinov, *Chem. Nat. Compd.*, 2012, **48**, 1047.
23. T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.

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