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Synthesis and Bioactivity of Reduced Chalcones Containing Sulfonamide Side Chains

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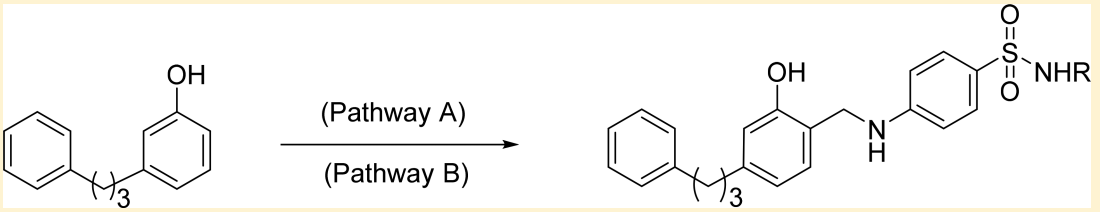
Supporting Information



ABSTRACT: The effect on the bioactivity of antibacterial sulfonamide drugs against malaria and tuberculosis via an increase of the lipid solubility groups by condensation with a reduced chalcone was investigated. Sulfonamide derivatives (**8a−8d**) were obtained via a 1,3-diarylpropane scaffold, prepared by reduction of the relevant chalcones, followed by the addition of a sulfonamide moiety via the Mannich and the Mannich exchange reactions. The ClogP values indicated that the lipophilicities of **8a−8d** and intermediate reduced chalcones and N-alkylated reduced chalcones (**5a−7a**) were much higher than those of the sulfonamides (**1a−1c**). The N-alkylated reduced chalcone derivatives **6** and **7** exhibited the highest antimalarial (*Plasmodium falciparum* (NF54 strain)) activity. Addition of the sulfonamide group weakened the activity, even though some ClogP values were higher, while **1a−1c** showed no activity. The reduced chalcones **5a** and **5** showed potent growth inhibition of *Mycobacterium tuberculosis* (H37Rv strain), but the sulfonamide derivatives **8a** and **8d** showed no or insignificant activity (0 and 14%, respectively) against *M. tuberculosis*, despite high ClogP values. Thus, the possible increase in bioactivity expected from an increase in ClogP values (lipophilicity) might be counteracted by the higher molecular weight of the studied analogues.

Malaria is a serious problem in the tropical and subtropical parts of Asia, Africa, Central and South America, and parts of the Middle East, where it affects millions of people.[1] The disease is caused by *Plasmodium*, a parasitic species carried by the female of the anopheles mosquito, and it is introduced into the bloodstream of humans by an infected mosquito. The costs involved in the treatment and management of malaria are exorbitant, taking into account not only the cost of medical treatment but also the cost of lost production.[2] One of the major obstacles to control malaria is the growing resistance of the malaria parasite to most of the commonly used antimalaria drugs.[3] Parallel to malaria, *Mycobacterium tuberculosis* remains a serious health problem in many regions of the world, especially in developing countries.[4] Tuberculosis is a transferable disease that can reach epidemic proportions. In South Africa the disease is exacerbated by the high incidence of HIV/AIDS infections that compromise the patients' immune systems. The added complication of the emergence of multidrug-resistant tuberculosis (MDR) and extensively drug resistant tuberculosis (XDR), as in the case of malaria, has complicated the treatment of TB considerably.[5] The world's future ability to treat these drug-resistant diseases depends on continued investigations into new, better performing drugs, including the exploration of the potential bioactivity of natural-product-derived compounds.

Chalcones are precursors in the biosynthesis of flavonoids and occur widely in medicinal plants. Chemically, their $C_6{\cdot}C_3{\cdot}C_6$ architecture comprised two aromatic rings linked via a three-carbon α,β-unsaturated carbonyl system.[6] A number of chalcones having hydroxy and alkoxy groups in different positions on the aromatic rings have been reported to possess antibacterial,[7] antifungal,[8] antioxidant,[9] and antimalarial[10] activities. Wilhelm et al. reported good antimalarial activity of N-alkylated 1,3-diarylpropanes, synthesized via reduction of chalcones with subsequent amino-alkylation via the Mannich reaction.[11] Mannich bases are furthermore known for their biological potential including anticonvulsant activity.[12,13]

Antibacterial sulfonamides such as sulfanilamide (**1a**) and derivatives **1b** and **1c** target a bacterial metabolic pathway as competitive inhibitors of the enzyme dihydropteroate synthetase (DHPS).[14] DHPS activity is vital in the synthesis of folate, needed by cells to synthesize nucleic acids such as DNA or RNA. Thus, inhibition of DNA replication and cell division leads to a bacteriostatic effect. The sulfonamide group is present in a number of biologically active molecules, e.g.,

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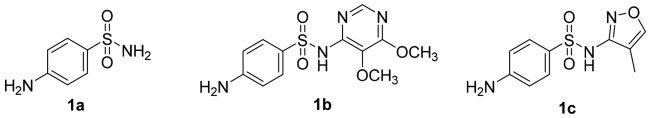
J. Nat. Prod. XXXX, XXX, XXX−XXX

antibacterial,[15,16] antifungal,[15−17] antimalarial,[16−18] anticancer,[18] and anti-inflammatory agents.[19]



Chemically modified sulfanilamide may lead to a wider spectrum of bioactivities with more prolonged action. Therefore, the aim of this study was to investigate if condensation of a sulfanilamide side chain with a reduced chalcone via the Mannich or Mannich exchange reaction, where a tertiary amine hydrochloride group is substituted with a primary amine,[20] could potentially increase the antimalaria and antituberculosis bioactivity by slightly increasing the lipophilicity.

RESULTS AND DISCUSSION

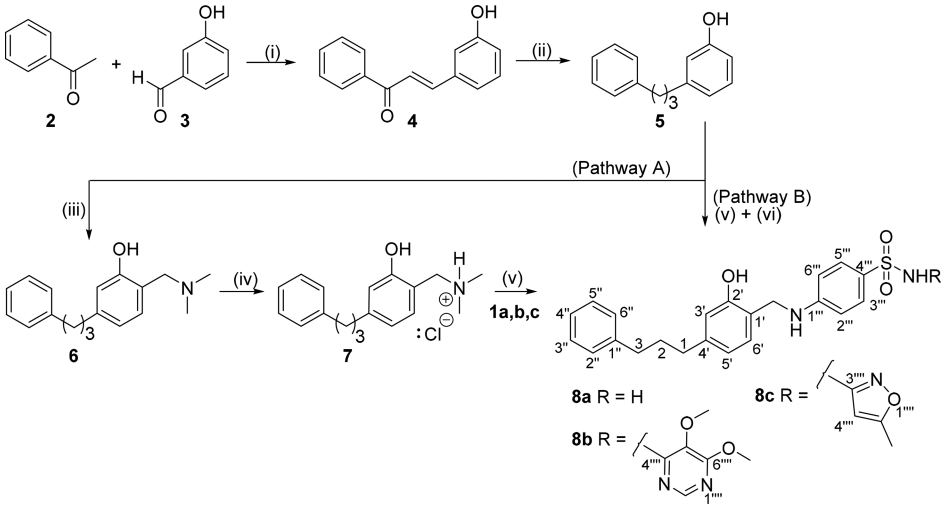
Membrane permeability is one of the key determinants in pharmacokinetic behavior and plays a role in absorption and distribution of drugs in the human body. The structure of cell membranes is a fluid-like bilayer arrangement of phospholipids, with the outer leaflet consisting of electrically neutral lipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), while the negatively charged lipids, such as phosphatidylserine, are located in the inner layer. Most drugs are delivered via passive diffusion across a biomembrane, and it may occur through its lipid structure.[21] It is generally accepted that more lipophilic molecules will interact more readily with the fatty acid tails of the lipid bilayer, thus allowing the molecule to cross cell membranes.[22] However, the optimal lipophilicity of a molecule needs to be determined, because if the solute is too lipophilic, it will remain trapped in the membrane. The best indication of lipophilicity is the logarithm of the octanol/water partition coefficient (ClogP). Compounds with a high ClogP (>6) display low absorption due to their poor aqueous solubility, whereas compounds with suboptimal lipophilicity (ClogP ← 3) are unable to penetrate membrane barriers.[23] The ClogP value forms part of the "rule of five" criteria for drug-likeness formulated by Lipinski in 1997,[24] i.e., a molecular mass smaller than 500, calculated octanol/water partition coefficient (CLogP) smaller than 5, number of hydrogen bond donors (HBD) smaller than 5, number of hydrogen bond acceptors (HBA) smaller than 10. The polar surface area (PSA) is a descriptor that shows good correlation with passive molecular membrane transport and thus is an indication of the transport properties of drugs. The PSA value should be no more than 140. However, the increased absorption of compounds with low molecular mass (M_r) and a suitable ClogP appear to be the two physicochemical parameters that best determine oral absorption potential.[11]

Chemistry. In this report, the influence on the antibacterial activity against *Plasmodium falciparum* and *Mycobacterium tuberculosis* when a sulfonamide group is added to an N-alkylated 1,3-diarylpropane scaffold was investigated.

The syntheses of 4-{[2-hydroxy-4-(3-phenylpropyl)benzyl]amino}benzenesulfonamide (**8a**), *N*-(5,6-dimethoxypyrimidin-4-yl)-4-{[2-hydroxy-4-(3-phenylpropyl)benzyl]amino}-benzenesulfonamide (**8b**), and 4-{[2-hydroxy-4-(3-phenylpropyl)benzyl]amino}-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide (**8c**) were achieved through the Mannich exchange reaction by replacement of the dimethylamine hydrochloride moiety of the reduced chalcone with different sulfonamide functionalities. The reaction is illustrated in Scheme 1. The amino functionality was introduced via the Mannich reaction into a series of chalcones available via the aldol reaction (Scheme 1). The Mannich reaction can be applied to aromatic systems provided that it possesses a vacant location ortho to the phenolic group (Scheme 2).

Structure Elucidation. The ^{1}H NMR spectra of the sulfonamides **8a**, **8b**, and **8c** displayed the following characteristics (ppm values given as a range to include values for **8a**, **8b**, and **8c**): a propyl group represented by two triplet resonances in the range δ_H 2.54−2.49 (t, J = 7.4−7.8 Hz) and δ_H 2.62−2.55 (t, J = 7.4−7.8 Hz) corresponding to H-1 and H-3, respectively, and a multiplet in the range δ_H 1.93−1.79 assigned to H-2, respectively; an aromatic ABX system comprising H-3′ (δ_H 6.65−6.60, d, J = 1.4−1.6 Hz), H-5′ (δ_H 6.60−6.56, dd, J = 1.4−1.6 Hz, 7.6−7.8 Hz), and H-6′ (δ_H 7.10−7.03, d, J = 7.6−7.8 Hz); an aromatic AA′BB′C system at δ_H 7.19−7.09 (H-2″/

Scheme 1. Synthesis of Sulfonamides 8a, 8b, and 8c via Pathways A and B[a]



[a](i) 50% KOH, EtOH, rt; (ii) H_2/Pd(OH)$_2$/C, EtOAc/H_2O; (iii) CH_2O/Me_2NH, CH_3CN, reflux; (iv) HCl (g)/*n*-hexane; (v) **1a** or **b** or **c**, EtOH, reflux; (vi) CH_2O.

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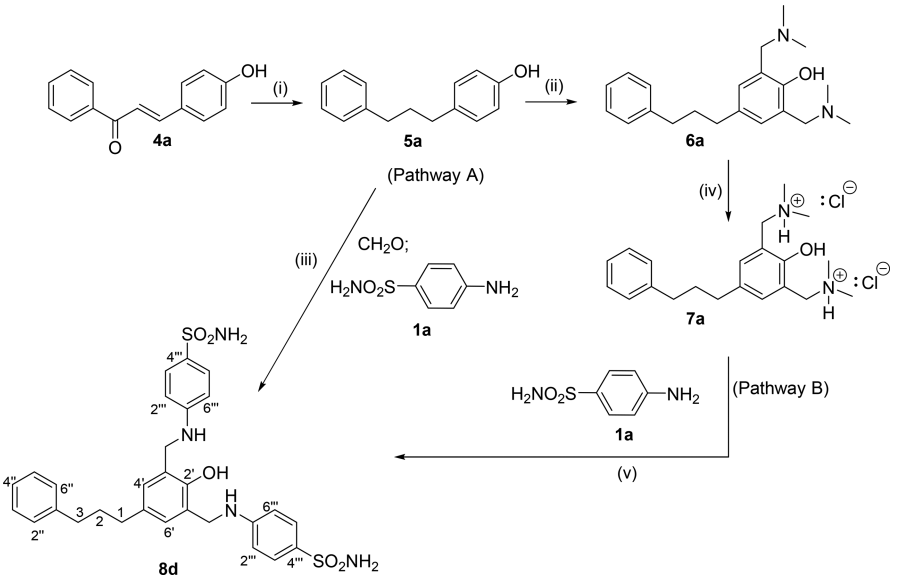
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of 8d via Pathway A (Direct Mannich Reaction) and Pathway B (Mannich Exchange Reaction)[a]



[a](i) H_2/Pd(OH)$_2$/C, EtOAc/H_2O; (ii) CH_2O/Me_2NH, CH_3CN, reflux; (iii) EtOH; (iv) HCl (g)/*n*-hexane; (v) EtOH/H_2O, reflux.

H-6″ and H-4″, overlapped) and δ_H 7.26–7.21 (H-3″/H-5″, t, J = 7.4–7.8 Hz); an aromatic AA′BB′ system representing H-2‴/H-6‴ (δ_H 7.73–7.52, d, J = 8.5–8.9 Hz) and H-3‴/H-5‴ (δ_H 6.67–6.58, d, J = 8.5–8.9 Hz); and the aminomethylene protons (−NH-CH_2) as broadened singlets in the range δ_H 4.32–4.25. Compound **8d** showed the same spin systems as **8a**, **8b**, and **8c**, except for the ABX system that is replaced by a two-proton singlet at δ_H 7.10 corresponding to H-4′/H-6′, indicating disubstitution during the Mannich reaction. This was confirmed by the observation that the H-2‴/H-6‴ and H-3‴/H-5‴ resonances integrated for four protons, respectively. Compounds **8b** and **8c** further displayed singlets at δ_H 8.01 and 5.99, respectively, corresponding to H-2⁗ and H-4⁗, respectively. The ^{13}C NMR spectra of compounds **8a**, **8b**, **8c**, and **8d** displayed the expected number of carbons, respectively, and all methine carbons were assigned via 2D HSQC experiments. Salient in the ^{13}C NMR spectra of **8a**, **8b**, **8c**, and **8d** are an oxygenated tertiary carbon at δ_C 156.3–151.7, a nitrogenated tertiary carbon at δ_C 154.1–151.7, and a sulfonylated tertiary carbon at δ_C 131.5–125.4. These tertiary carbon assignments were confirmed via long-range 2D HMBC correlations between the aminomethylene protons for **8a**, **8b**, and **8c** and C-2′ and C-1‴, respectively, and correlations between H-3‴, H-5‴, and the sulfonylated C-4‴ carbon (Figure 1). Assignments of three (four for **8d**) quaternary carbons at δ_C 125.2–122.1 (C-1′ for **8a**, **8b**, and **8c**, C-1′, C-3′ for **8d**, respectively), δ_C 143.9–142.3 (C-4′ for **8a**, **8b**, and **8c**), δ_C 143.6–142.2 for **8a**–**8d** (C-1″), and δ_C 133.5 for **8d** (C-5′) were confirmed via 2D HMBC correlations between H-1 and C-4′, H-3 and C-1″, and the aminomethylene protons and C-1′, respectively. Four (five for **8d**) aliphatic methylene carbons were assigned to the propyl groups (C-1, C-2, and C-3) and one resonance (two for **8d**) to the aminomethylene carbons. The long-range coupling data of compounds **8a**, **8b**, and **8c** observed between the aminomethylene protons and C-1′, C-2′, and C-6′ served as evidence for the presence of the Mannich functional group.

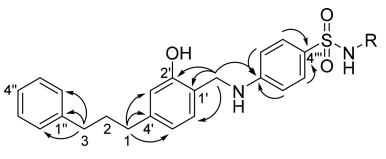


Figure 1. Long-Range HMBC correlations for compounds **8a**–**8c**.

Bioactivity. The results of the bioactivity tests are summarized in Tables 1 and 2. Testing against *M. tuberculosis* (**5**, **5a**, **6**, **6a**, **7**, **7a**, **1a**, **8a**, and **8d**) revealed that the reduced chalcones **5** and **5a** showed potent growth inhibition (94% and 96%, respectively), while the N-alkylated 1,3-diarylpropane **6** showed a moderate 74% inhibition. In the case of the sulfanilamide Mannich bases with substituted reduced chalcones, inhibition of *M. tuberculosis* of 14% (**8a**) compared to the zero activity of **1a** was observed (Table 1).

The synthesized compounds **5**, **6**, **7**, **8a**, **8b**, and **8c** were tested against *P. falciparum*, and the results are collated in Table 2. Compounds **6** and **7** showed moderate activity against *P. falciparum* of 58.3 and 117.7 nM, respectively. In the case of the sulfanilamide Mannich bases **8b** and **8c**, weak activity against *P. falciparum* of 497.5 and 324.5 nM was observed, respectively. Other compounds such as the reduced chalcone (**5**) and sulfonamide Mannich base (**8a**) showed no activity.

It is clear from the ClogP values that the lipophilicities of **8a**–**8c** and **5a**–**7a** are much higher than those of the sulfonamides **1a**–**1c**. The antimycobacterial tests showed a small increase in activity from **1a** (0%, ClogP 0.0035) to the derivative **8a** (14%, ClogP 3.888), supporting our hypothesis. However, the increased bulk of derivative **8d** based on the increase in molecular weight may account for the lack of activity. Compounds **5a** and **5** have relatively high ClogP values (4.450 for both), but much lower molecular weights and therefore show the highest inhibition. The same trends were observed in the antiplasmodium tests: the most potent compounds **6** and **7** have high lipophilicity and low molecular weights, sulfanilamide (**1a**) and derivatives **1b** and **1c** have very low lipophilicity and showed no activity, but the sulfonamide derivatives **8b** and **8c**, with the highest ClogP values of 5.691

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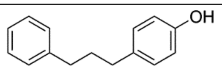
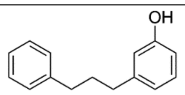
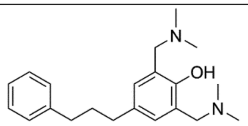
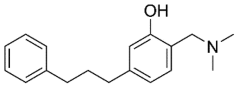
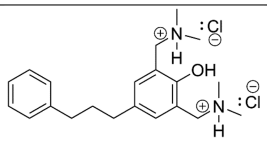
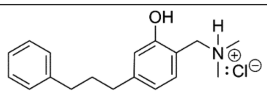
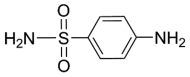
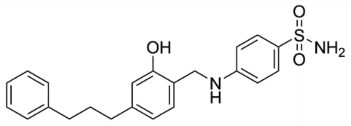
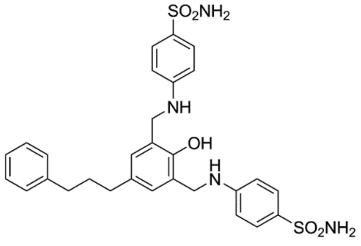
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. Bioactivity Screening against *Mycobacterium tuberculosis* (H37Rv Strain) (All Compounds Were Tested at 10 μM)

Compound	Structure	MIC99 (μM)	% Inhibition	ClogP	MW
5a		> 10	96	4.450	212.09
5		> 10	94	4.450	212.09
6a		> 10	42	4.018	326.48
6		> 10	74	4.234	269.39
7a		> 10	33	5.378	328.50
7		> 10	36	4.914	270.40
1a		> 10	0	-0.572	172.03
8a		> 10	14	3.888	396.51
8d		> 10	0	3.326	580.72

and 5.023, respectively, showed only slight activity, probably due to their high molecular weights.

In conclusion, it was established that reduced chalcones **5** and **5a** displayed potent inhibition against the growth of *M. tuberculosis* of 96% and 94%, respectively. However, derivatization of the reduced chalcones into sulfonamide Mannich bases reduced activity significantly, probably due to the increased molecular weight of the sulfonamide derivatives. Bioassays against *P. falciparum* revealed once more that activity is decreased when the reduced chalcone is derivatized to sulfanilamide Mannich bases, but **8b** and **8c** did indicate low antimalarial activity. Sulfanilamide and derivatives **1a**–**c** were inactive against *P. falciparum*. These results demonstrated that the lipophilicity of the tested compounds was not the sole parameter affecting antimycobacterial and antiplasmodium activity, but molecular weight and electrostatic effects caused by hydrogen bond donors and acceptors also play a role.

EXPERIMENTAL SECTION

General Experimental Procedures. A 600 MHz Bruker Avance spectrometer was used to record the ^{1}H NMR, COSY, HMBC, and HMQC (600 MHz) and ^{13}C, APT (150 MHz) experiments in either $CDCl_3$ (δ_H = 7.24; δ_C = 77.2), acetone-d_6 (δ_H = 2.04; δ_C = 29.8), or methanol-d_4 (δ_H = 4.87 and 3.31; δ_C = 49.2) with tetramethylsilane as internal standard. Chemical shifts were expressed as parts per million (ppm) on the delta (δ) scale, and coupling constants (J) are accurate to 0.01 Hz. Low-resolution mass spectrometry was performed on a Sciex instrument model API 4000 LC/MS/MS System (QTRAP triple quadrupole ion trap mass spectrometer). All samples were dissolved and diluted to ~2 ng/μL and infused without additives. Thin-layer chromatography (TLC) was performed on Merck aluminum sheets (silica gel 60 F_{254}, 0.25 mm). Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm). Thin-layer chromatograms were sprayed with a 2% (v/v) solution of formaldehyde (40% solution in H_2O) in concentrated H_2SO_4 and subsequently heated to 110 °C to effect maximum development of

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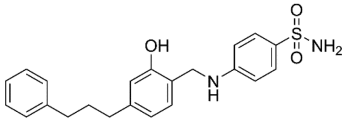
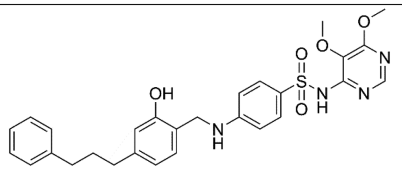
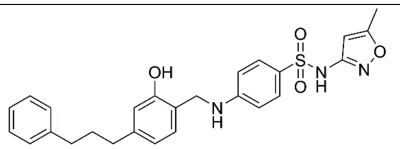
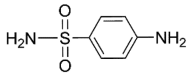
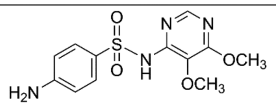
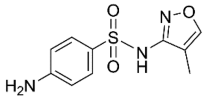
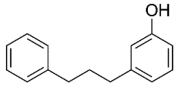
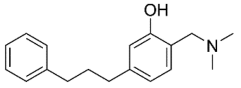
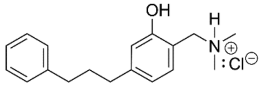
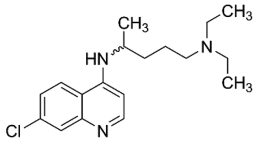
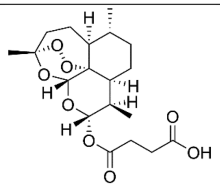
Table 2. Antiplasmodial Activity of Compounds against NF54 (Chloroquine-Sensitive *P. falciparum* Strain)

Compound	Structure	Antimalarial activity NF54 (nM)	ClogP	MW
8a		-	3.888	396.51
8b		497.5	5.691	534.63
8c		324.5	5.023	477.58
1a		-	-0.572	172.03
1b		-	1.231	310.33
1c		-	0.0035	253.28
5		-	4.450	212.09
6		58.3	4.234	269.39
7		117.7	4.914	270.40
Chloroquine (Control)		16.3		
Artesunate (Control)		<5.2		

color. Chemicals purchased from commercial vendors were used without purification.

General Procedure for the Synthesis of the Chalcones via the Aldol Condensation. A mixture of acetophenone (2.974 g; 24.7 mmol) and 3-hydroxybenzaldehyde (2.963 g; 24.3 mmol) was stirred in EtOH (50 mL) at room temperature. KOH solution (50%, 25 mL) was added after 10 min, which turned the reaction mixture bright yellow. The mixture was left to stir overnight, after which it was quenched with ice-cold 1 N HCl (100 mL) solution and extracted with EtOAc (2 × 50 mL). The organic layer was washed with H_2O (1 × 50 mL) and dried over Na_2SO_4, and the solvent evaporated under reduced pressure. Recrystallization from EtOH yielded the title compound. This is demonstrated for the synthesis of (*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one (**4**) using 3-hydroxybenzaldehyde (**3**).

(*E*)-3-(3-Hydroxyphenyl)-1-phenylprop-2-en-1-one (**4**) was obtained as yellow crystals[25] (1.260 g, 23%): ^{1}H NMR δ (600 MHz, acetone-d_6, Me_4Si) 8.60 (s, 1 × O*H*), 8.17−8.13 (2H, m, H-2″, H-6″),

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J. Nat. Prod. XXXX, XXX, XXX−XXX

7.81 (1H, d, J = 15.6 Hz, H-3), 7.73 (1H, d, J = 15.6 Hz, H-2), 7.66 (1H, tt, J = 6.9, 1.2 Hz, H-4″), 7.60−7.55 (2H, m, H-3″, H-5″), 7.33 (1H, dt, J = 7.6, 1.2 Hz, H-6′), 7.31 (1H, d, J = 7.6 Hz, H-5′), 7.29 (1H, t, J = 1.9 Hz, H-2′), 6.96 (1H, ddd, J = 7.6, 2.4, 1.3 Hz, H-4′); ^{13}C NMR δ (150 MHz, acetone-d_6, Me_4Si) 189.1 (C-1), 157.8 (C-3′), 144.1 (C-2), 138.2 (C-1″), 136.5 (C-1′), 132.8 (C-4″), 130.0 (C-5′), 128.7 (C-3″/ C-5″), 128.4 (C-2″/C-6″), 122.0 (C-3), 120.1 (C-6′), 117.6 (C-4′), 115.0 (C-2′).

General Procedure for the Synthesis of the Diarylpropanes. To a solution of the appropriate chalcone (1.000 g, 0.04 mol) in EtOAc (3 mL) and H_2O (9 mL) was added 20% $Pd(OH)_2/C$ (600 mg, 60 wt %). The flask was sealed, and hydrogen gas was passed through the reaction mixture. After stirring overnight at room temperature, the reaction mixture was filtered through silica gel. The filtrate was dissolved in EtOAc followed by extraction with EtOAc (2 × 50 mL). The organic layer was washed with H_2O (2 × 30 mL) and dried over anhydrous $MgSO_4$, and the solvent evaporated under reduced pressure. Column chromatography (*n*-hexane/EtOAc, 7:3) afforded the pure diarylpropanes. This is demonstrated for the synthesis of 3-(3-phenylpropyl)phenol (**5**) using (*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one.

3-(3-Phenylpropyl)phenol (**5**) was obtained as a brown oil (215 mg, 23%): ^{1}H NMR δ (600 MHz, $CDCl_3$, Me_4Si) 7.39 (2H, t, J = 7.6 Hz, H-2″, H-6″), 7.32−7.27 (3H, m, H-3″, H-4″, H-5″), 7.24 (1H, t, J = 6.6 Hz, H-5′), 6.86 (1H, d, J = 7.7 Hz, H-6′), 6.79−6.74 (2H, m, H-2′, H-4′), 2.74 (2H, m, H-3), 2.69 (2H, m, H-1), 2.07−1.99 (2H, m, H-2); ^{13}C NMR δ (150 MHz, $CDCl_3$, Me_4Si) 155.5 (C-3′), 144.4 (C-1′), 142.4 (C-1″), 129.6 (C-5′), 128.6 (C-2″/C-6″), 128.5 (C-3″/C-5″), 125.9 (C-5′), 121.1 (C-6′), 115.6 (C-2′), 112.9 (C-4′), 35.5 (C-3), 35.4 (C-1), 32.8 (C-2).

General Procedure for the Synthesis of Aminoalkylated Diarylpropanes. A mixture of the appropriate diarylpropane (0.212 g, 0.001 mol), paraformaldehyde (0.040 g, 0.01 mol), and Me_2NH (0.045 g, 1 mL, 0.01 mol) in dry MeCN (10 mL) was refluxed overnight. The mixture was purified by TLC chromatography (*n*-hexane/EtOAc, 7:3). This is demonstrated for the synthesis of 2-[(dimethylamino)methyl]-5-(3-phenylpropyl)phenol (**6**).

The title compound was obtained as a brown oil (0.242 g, 90%): LREIMS m/z 269.0 (calcd for $C_{18}H_{23}NO$, 269.4); ^{1}H NMR δ (600 MHz, $CDCl_3$, Me_4Si) 7.34 (2H, t, J = 7.6 Hz, H-2″, H-6″), 7.28−7.21 (3H, m, H-3″, H-4″, H-5″), 6.93 (1H, d, J = 7.6 Hz, H-5′), 6.77 (1H, d, J = 1.7 Hz, H-2′), 6.67 (1H, dd, J = 7.7, 1.7 Hz, H-6′), 3.66 (2H s, CH_2), 2.74−2.69 (2H, m, H-3), 2.68−2.63 (2H, m, H-1), 2.37 (6H, s, 2 × N-CH_3), 2.06−1.97 (2H, m, H-2).; ^{13}C NMR δ (150 MHz, $CDCl_3$, Me_4Si) 158.0 (C-3′), 143.3 (C-1″), 142.4 (C-1′), 128.5(C-2″/C-6″), 128.4 (C-3″/C-5″), 128.2 (C-5′), 125.8 (C-4″), 119.4 (C-4′), 119.2 (C-6′), 116.1 (C-2′), 62.7 (CH_2), 44.5 (2 × N-CH_3), 35.5 (C-3), 35.3 (C-1), 32.9 (C-2).

General Synthesis of HCl Salts of the Aminoalkylated Diarylpropanes 2-[(Dimethylamino)methyl]-5-(3-phenylpropyl)phenol Hydrochloride (7). The appropriate aminoalkylated diarylpropane (0.242 g, 0.9 mmol) was dissolved in *n*-hexane (5 mL) at 0 °C. HCl gas was bubbled through the mixture for 60 min to obtain the HCl salt as white crystals. This is demonstrated for the synthesis of 2-[(dimethylamino)methyl]-5-(3-phenylpropyl)phenol hydrochloride (**7**).

The title compound was obtained as an amorphous, white powder (0.30 g, 98%): LREIMS m/z 306.4 (calcd for $C_{18}H_{24}ClNO$, 305.2); ^{1}H NMR δ (600 MHz, $CDCl_3$, Me_4Si) 9.30 (1H, bs, OH), 7.24 (2H, d, J = 7.6 Hz, H-2″, H-6″), 7.18−7.08 (5H, m, H-2′, H-5′, H-3″, H-4″, H-5″), 6.65 (1H, dd, J = 7.7, 1.6 Hz, H-6′), 4.19 (2H, d, J = 3.3 Hz, CH_2), 2.72 (6H, d, J = 4.1 Hz, 2 × N-CH_3), 2.61−2.56 (2H, m, H-3), 2.55−2.48 (2H, m, H-1), 1.89−1.81 (2H, m, H-2); ^{13}C NMR δ (150 MHz, $CDCl_3$, Me_4Si) 156.8 (C-3′), 147.2 (C-1″), 142.2 (C-1′), 132.8 (C-5′), 128.6 (C-2″/C-6″), 128.5 (C-3″/C-5″), 126.0 (C-4″), 120.6 (C-6′), 117.5 (C-2′), 112.0 (C-4′), 55.6 (CH_2), 42.0 (2 × N-CH_3), 35.7 (C-3), 35.4 (C-1), 32.8 (C-2).

4-[(2-Hydroxy-4-(3-phenylpropyl)benzyl)amino]benzenesulfonamide (8a). *Pathway A.* 3-(3-Phenylpropyl)phenol (**5**) (0.212 g, 0.001 mol) and sulfanilamide (**1a**) (0.172 g, 0.001 mol) were dissolved in aqueous EtOH (2 mL), and paraformaldehyde solution (37% v/v, 0.25 mL) was added slowly with constant stirring. The pH of the mixture was adjusted to 3.5 by adding a 1 mol/L HCl solution (0.05 mL). The mixture was stirred on ice for 30 min followed by overnight reflux. The reaction mixture was purified by TLC chromatography (*n*-hexane/EtOAc/MeOH, 5:2:3). which yielded the title compound (**8a**) as pale yellow crystals, mp 108−110 °C (R_f 0.7, 0.039 g, 10%).

Pathway B. A mixture of 2-[(dimethylamino)methyl]-5-(3-phenylpropyl)phenol hydrochloride (**7**) (0.080 g, 262 mmol) and sulfanilamide (0.052 g, 291 mmol) in 50% aqueous EtOH (6 mL) was refluxed for 24 min. The solvent was removed at reduced pressure, and the mixture was separated by TLC chromatography (*n*-hexane/EtOAc/MeOH, 5:2:3) to afford the title compound (**8a**) as pale yellow crystals, mp 108−110 °C (R_f 0.7, 0.050 g, 50%).

LREIMS m/z 395.3 (calcd for $C_{22}H_{24}N_2O_3S$, 396.5); ^{1}H NMR δ (600 MHz, methanol-d_4, Me_4Si) 7.59 (2H, d, J = 8.9 Hz, H-2‴, H-6‴), 7.26 (2H, t, J = 7.2, H-3″, H-5″), 7.19−7.13 (3H, m, H-2″, H-4″, H-6″), 7.10 (1H, d, J = 7.7, H-6′), 6.67 (2H, d, J = 8.9 Hz, H-3‴, H-5‴), 6.65 (1H, d, J = 1.6 Hz, H-3′), 6.61 (1H, dd, J = 7.8, 1.6 Hz, H-5′), 4.32 (2H, s, N-CH_2), 2.62 (2H, t, J = 7.8 Hz, H-3), 2.54 (2H, t, J = 7.8 Hz, H-1), 1.93−1.86 (2H, m, H-2); ^{13}C NMR δ (150 MHz, methanol-d_4, Me_4Si) 154.9 (C-2′), 152.2 (C-1‴), 142.4 (C-4′), 142.2 (C-1″), 129.0 (C-4‴), 128.1 (C-6′), 128.0 (C-2″/C-6″), 127.9 (C-3″/C-5″), 127.4 (C-2‴/C-6‴), 125.3 (C-4″), 122.1 (C-1′), 119.2 (C-5′), 114.6 (C-3′), 111.2 (C-3‴/C-5‴), 41.3 (N-CH_2), 35.0 (C-3), 34.7 (C-1), 33.1 (C-2).

*N-(5,6-Dimethoxypyrimidin-4-yl)-4-{[2-hydroxy-4-(3-phenylpropyl)benzyl]amino}benzenesulfonamide (**8b**).* A mixture of 2-[(dimethylamino)methyl]-5-(3-phenylpropyl)phenol hydrochloride (**7**) (0.1 g, 0.47 mmol) and sulfadoxine (0.1 g, 0.32 mmol) in 50% aqueous EtOH (6 mL) was refluxed for 48 h. The solvent was removed under reduced pressure, and the mixture was purified by TLC chromatography (*n*-hexane/acetone, 6:4), to yield the title compound (**8b**) as a pale yellow oil (R_f 0.7, 0.04 g, 23%): LREIMS m/z 535.0 and [M + Na] 557.3 (calcd for $C_{28}H_{30}N_4O_5S$, 534.2); ^{1}H NMR δ (600 MHz, methanol-d_4, Me_4Si) 8.01 (1H, s, H-2⁗), 7.73 (2H, d, J = 8.5 Hz, H-2‴, H-6‴), 7.23 (2H, t, J = 7.5 Hz, H-3″, H-5″), 7.18−7.11 (3H, m, H-2″, H-4″, H-6″), 7.04 (1H, d, J = 7.7 Hz, H-6′), 6.64 (1H, d, J = 1.6 Hz, H-3′), 6.61 (2H, d, J = 8.6 Hz, H-3‴, H-5‴), 6.56 (1H, dd, J = 7.8, 1.7 Hz, H-5′), 4.28 (2H, s, N-CH_2), 3.91 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 2.59−2.55 (2H, m, H-3), 2.51 (2H, t, J = 7.7 Hz, H-1), 1.92−1.83 (2H, m, H-2); ^{13}C NMR δ (150 MHz, methanol-d_4, Me_4Si) 161.6 (C-6⁗), 154.9 (C-2′), 152.8 (C-1‴), 151.7 (C-4⁗), 150.6 (C-2⁗), 142.5 (C-4′), 142.2 (C-1″), 129.5 (C-2‴/C-6‴), 128.2 (C-6′), 128.1 (C-2″/C-6″), 128.0 (C-3″/C-5″), 127.9 (C-5⁗), 125.4 (C-4‴), 125.3 (C-4″), 122.0 (C-1′), 119.3 (C-5′), 114.7 (C-3′), 110.8 (C-3‴/C-5‴), 59.3 (OCH_3), 53.1 (OCH_3), 41.3 (N-CH_2), 35.0 (C-3), 34.7 (C-1), 33.0 (C-2).

*4-{[2-Hydroxy-4-(3-phenylpropyl)benzyl]amino}-N-(5-methylisoxazol-3-yl)benzenesulfonamide (**8c**).* A mixture of 2-[(dimethylamino)methyl]-5-(3-phenylpropyl)phenol hydrochloride (**7**) (101 mg, 332 mmol) and sulfamethoxazole (84 mg, 332 mmol) in 50% aqueous EtOH (12 mL) was refluxed for 48 h. The solvent was removed under reduced pressure, and the reaction mixture was purified by TLC chromatography (*n*-hexane/EtOAc/MeOH, 6:2:2), which yielded the title compound as an amorphous, white powder (R_f 0.7, 0.06 g, 40%). LREIMS m/z 476.2 (calcd for $C_{28}H_{30}N_4O_5S$, 477.2); ^{1}H NMR δ (600 MHz, methanol-d_4, Me_4Si) 7.52 (2H, d, J = 8.9 Hz, H-2‴, H-6‴), 7.21 (2H, t, J = 7.6 Hz, H-3″, H-5″), 7.14−7.09 (3H, m, H-2″, H-4″, H-6″), 7.03 (1H, d, J = 7.8 Hz, H-6′), 6.60 (1H, d, J = 1.4 Hz, H-3′), 6.58 (2H, d, J = 9.0 Hz, H-3‴, H-5‴), 6.56 (1H, dd, J = 7.7, 1.5 Hz, H-5′), 5.99 (1H, s, H-4⁗), 4.25 (2H, s, N-CH_2), 2.57 (2H, t, J = 7.4 Hz, H-3), 2.49 (2H, t, J = 7.4 Hz, H-1), 2.23 (3H, s, CH_3), 1.88−1.83 (2H, m, H-2); ^{13}C NMR δ (150 MHz, methanol-d_4, Me_4Si) 171.2 (C-5⁗), 161.0 (C-3⁗), 156.3 (C-2′), 154.1 (C-1‴), 143.9 (C-4′), 143.6 (C-1″), 129.9 (C-2‴/C-6‴), 129.6 (C-6′), 129.5 (C-2″/C-6″), 129.3 (C-3″/C-5″), 127.0 (C-4‴), 126.7 (C-4″), 123.4 (C-1′), 120.7 (C-5′), 116.0 (C-3′), 112.5 (C-3‴/C-5‴), 96.7 (C-4⁗), 42.7 (N-CH_2), 36.4 (C-3), 36.1 (C-1), 34.4 (C-2), 12.3 (CH_3).

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J. Nat. Prod. XXXX, XXX, XXX−XXX

*4,4′-{[(2-Hydroxy-5-(3-phenylpropyl)-1,3-phenylene)bis-(methylene)]bis(azanediyl)}dibenzenesulfonamide (**8d**).* A mixture of **7a** (100 mg, 327 mmol) and **1a** (130 mg, 582 mmol) in 50% aqueous EtOH (6 mL) was refluxed for 48 h. The solvent was removed under reduced pressure, and the mixture was separated by TCL chromatography (*n*-hexane/acetone, 5:5), which yielded the title compound (**8d**) as brown crystals: mp 125−128 °C (R_f 0.4, 0.054 g, 20%); LREIMS *m*/*z* 579.5 (calcd for $C_{29}H_{32}N_4O_5S_2$, 580.2); ^{1}H NMR δ (600 MHz, acetone-d_6, Me_4Si) 7.62 (4H, d, *J* = 8.8 Hz, H-2‴, H-6‴), 7.26 (2H, t, *J* = 7.6 Hz, H-3″, H-5″), 7.18−7.14 (3H, m, H-2″, H-4″, H-6″), 7.10 (2H, s, H-4′, H-6′), 6.79 (4H, d, *J* = 8.8 Hz, H-3‴, H-5‴), 6.19 (4H, s, 2 × $-SO_2NH_2$), 4.45 (4H, d, *J* = 5.4, 2 × $-NHCH_2-$), 2.58 (2H, t, *J* = 7.7 Hz, H-3), 2.52 (2H, t, *J* = 7.7 Hz, H-1), 1.89−1.81 (2H, m, H-2); ^{13}C NMR δ (150 MHz, acetone-d_6, Me_4Si) 151.7 (C-1‴), 151.5 (C-2′), 142.3 (C-1″), 133.6 (C-5′), 131.5 (C-4‴), 128.4 (C-3″/C-5″), 128.3 (C-2″/C-6″), 127.8 (C-4′/C-6′), 127.7 (C-2‴/C-6‴), 125.6 (C-4″), 125.2 (C-1′/C-3′) 112.0 (C-3‴/C-5‴), 43.1 (N-CH_2), 35.0 (C-3), 34.4 (C-1), 33.4 (C-2).

Synthesis of 4-{[2-hydroxy-4-(3-phenylpropyl)benzyl]amino}-benzenesulfonamide (**8a**) and 4,4′-{[(2-hydroxy-5-(3-phenylpropyl)-1,3-phenylene)bis(methylene)]bis(azanediyl)}dibenzenesulfonamide (**8d**) was achieved via two pathways (Schemes 1 and 2); first, via direct Mannich reaction by reacting a reduced chalcone with sulfanilamide and paraformaldehyde, and second via a Mannich exchange reaction by replacing the $Me_2NH.HCL$ moiety of a reduced chalcone with sulfonamides (Scheme 1).

Bioassays. *Antiplasmodial Assay.* Continuous in vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using the modified method of Trager and Jensen.[26] Quantitative assessment of antiplasmodial activity in vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[27] The test samples were tested in triplicate on one or two separate occasions. Compounds were initially screened for antiplasmodial activity against the chloroquine-sensitive (CQS) D10 or NF54 strains. The most promising compounds were subsequently tested against the chloroquine-resistant (CQR) strains, Dd2 and K1.

CQ was used as an internal standard to monitor the experimental conditions and showed IC_{50} values within an acceptable range of 0.018−0.060 μM for the CQS strains and 0.470−0.780 μM for the CQR strains. The 50% inhibitory concentration (IC_{50}) values were obtained from full dose−response curves (Supporting Information), using a nonlinear dose−response curve fitting analysis via GraphPad Prism v.4 software.

Anti-Tuberculosis Assay. BACTEC 460 TB system: The work was carried out in the BSL3 laboratory of the Division of Molecular Biology of the University of Stellenbosch. *M. tuberculosis* H37Rv reference strain (atcc 25618) was used to evaluate compounds against a well-characterized *M. tuberculosis* strain. Bacterial colonies were cultured and selected from Loewenstein-Jensen slant cultures followed by culture in 7H9 mycobacterial medium. Cultures were stained by acid-fast staining (ZN staining) to control for contamination. The H37Rv was subsequently cultured in 7H9 mycobacterial medium (Difco) enriched with ADC (Biolab art. C70) until it reached a density of approximately 0.16 at A600 nm (one McFarland), and then 0.1 mL was inoculated into a 4 mL Bactec vial (primary culture).[28]

Compounds were dissolved in H_2O or 100% DMSO. Subsequent dilutions were made in 100% DMSO. A final volume of 0.1 mL of DMSO was added to each 4 mL BACTEC vial, giving a final concentration of the required drug and also a final DMSO concentration of 2.5%. This final concentration did not have an effect on the growth rate of *M. tuberculosis* as compared to a non-DMSO-treated culture. All DMSO solvent and drug preparations were sterilized through a syringe filter with 0.22 μm pore size (Millex-LG, organic solvent resistant).

The BACTEC system was devised to monitor mycobacterial growth of the slow growing species. The bacteria were grown on a radioactive substrate, and the radioactive CO_2 produced was directly proportional to the mycobacterial growth rate.[28] Read-out values were expressed as growth index (GI). These primary cultures were incubated at 37 °C until a GI of 500 (± 50) was reached. A 0.1 mL amount of primary culture and 0.1 mL of drug compound were added to a BACTEC vial, the vials were incubated at 37 °C, and the growth (GI) was monitored every 24 h. Appropriate controls were included.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00570.

Experimental details and 1D and 2D NMR data (PDF)

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Notes

The authors declare no competing financial interest.

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