Hindawi
Journal of Chemistry
Volume 2023, Article ID 4805466, 12 pages
https://doi.org/10.1155/2023/4805466

Research Article

Benzylated Sulfamethoxazole Derivatives with Improved Safety Profile as Potential Anti-*Mycobacterium tuberculosis* and Antibacterial Agents

Judas M. Maepa and Tlabo C. Leboho

Department of Chemistry, Faculty of Science and Agriculture, University of Limpopo, Private Bag X1106, Sovenga 0727, Polokwane, South Africa

Correspondence should be addressed to Tlabo C. Leboho; tlabo.leboho@ul.ac.za

Received 2 June 2023; Revised 26 July 2023; Accepted 25 August 2023; Published 12 September 2023

Academic Editor: Mozhgan Afshari

This study focussed on the synthesis of sulfamethoxazole derivatives and their biological evaluation. The sulfamethoxazole derivatives were successfully biologically evaluated against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and Chinese Hamster Ovarian (CHO) cells. The biological evaluation revealed compounds with improved antitubercular activity and antibacterial activity against *S. aureus* as well as safety profile when compared to the starting material, sulfamethoxazole. The most active compounds against *Mycobacterium tuberculosis* were**3q** with 92% inhibition followed by **3s** (90%), **3k** (88%), **3t** (85%), and **3o** (84%).

1. Introduction

Microbial infections pose a substantial health risk, especially to those people with a weakened immune system as a result of various factors such as other ailments, immunosuppressants, and cancer-treating strategies. As a result, the search for better acting, efficient, and safe antimicrobial agents remains of great interest [1]. Tuberculosis (TB), a communicable disease that is caused by bacillus *Mycobacterium tuberculosis* (Mtb), is one of the leading causes of death due to a single infectious agent worldwide even eclipsing HIV/AIDS. The World Health Organisation (WHO) has reported that the emergence of COVID-19 has negatively affected the whole process of TB management [2]. This resulted in an increase in the number of people who developed TB in 2020. In addition, all previously set goals for eradicating TB such as incidence rate, death rate, and cost reduction for the treatment of TB for the year 2020 were not met. To help WHO achieve its goals of eradicating TB by the year 2035, more intensified research and innovative methods are needed especially in identifying new drugs that can also treat drug-resistant TB strains such as multidrug and extended-drug TBs [3].

Sulfonamides are a class of synthetic antimicrobial compounds with broad pharmacological applications. An example of such sulphonamides includes sulfamethazine, sulfadiazine, sulfamethoxazole (SMZ) (**1**), sulfasalazine, sulfisoxazole, sulfamerazine, sulfadimethoxine, sulfafurazole, and sulphanilamide [4]. Sulfamethoxazole, an antibiotic that has caught the attention of a lot of researchers due to its wide biological activity, will be of focus in this research project. Sulfamethoxazole (**1**), which is usually used in combination with trimethoprim (Figure 1) (cotrimoxazole), has been extensively used for the treatment of microbial infections. For example, cotrimoxazole made it to the South Africa's list of essential medicines for use by people infected with HIV. This is because cotrimoxazole drastically prevented opportunistic infection by diseases such as malaria, pneumonia, and diarrhoea [5, 6]. In addition, cotrimoxazole was investigated for potential application against skin and soft tissue infections [7] and tuberculosis [8]. Sulfamethoxazole derivatives have been extensively investigated for

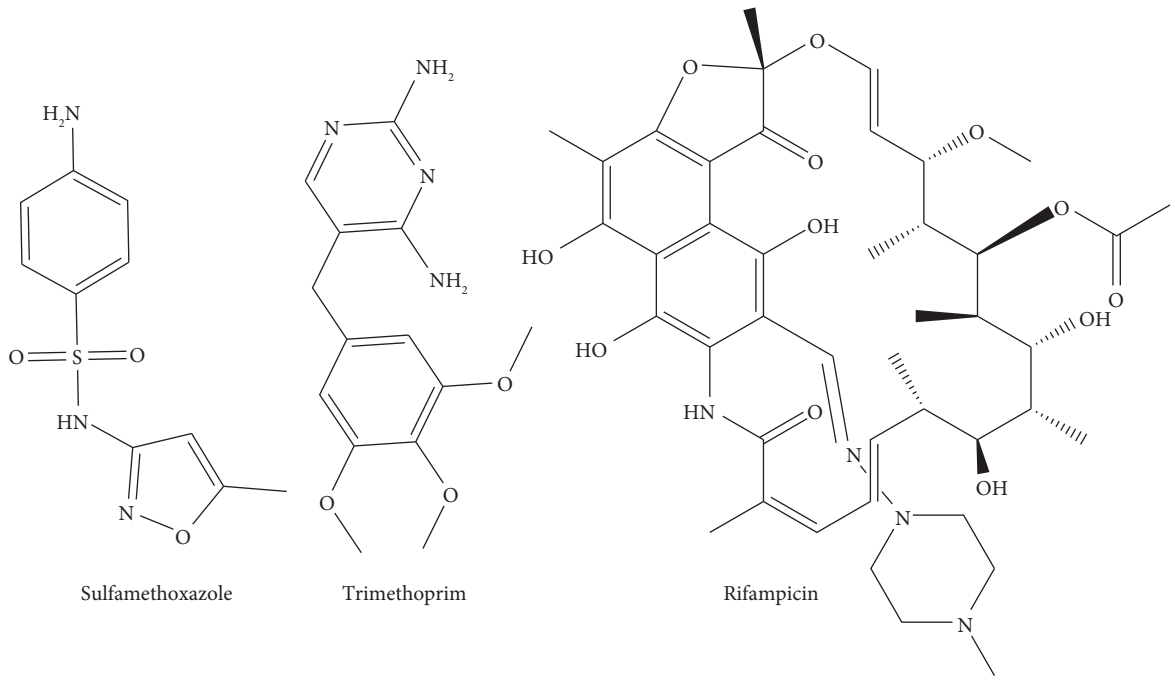


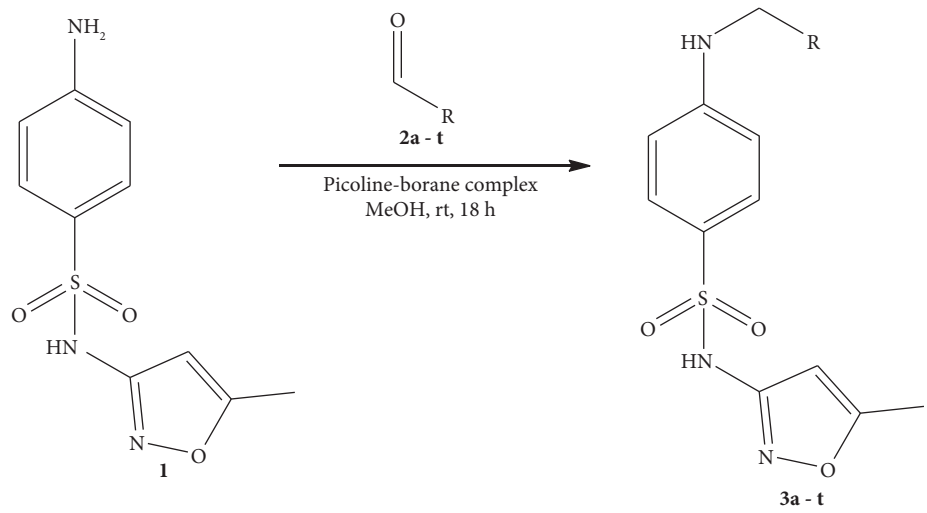
Figure 1: The structures of sulfamethoxazole, trimethoprim, and rifampicin.

their biological activity against various ailments. Rajamanickam et al. investigated sulfamethoxazole derivatives as potential antimicrobial agents [9], and Akili et al. used molecular modelling to design and synthesise sulfamethoxazole derivatives for the treatment of cancer [10, 11] while the mixture of sulfamethoxazole with rifampicin (Figure 1) showed improved anti-*Mycobacterium tuberculosis* activity [12]. Furthermore, sulfamethoxazole derivatives were evaluated as possible antimalaria agents [13] and were identified as potent inhibitors of *Trypanosoma brucei* and *Trypanosoma cruzi* phosphofructokinases. [14] Previously, we reported on benzylamine derivatives possessing sub 20 μM activity against *Mycobacterium tuberculosis* [15]. In our efforts to identify compounds with antibacterial activity, we report the synthesis and antibacterial evaluation of benzylated sulfamethoxazole derivatives as potential anti-*Mycobacterium tuberculosis*, their safety profile, and their activity against *Staphylococcus aureus* here.

2. Results and Discussions

2.1. Synthesis of Benzylated Sulfamethoxazole Derivatives. In order for us to gain access to the sulfamethoxazole derivatives with antibacterial activity, sulfamethoxazole (**1**) was treated with various salicylaldehyde derivatives under reductive amination reaction conditions. Reductive amination is a powerful synthetic method that is used to gain access to pharmaceutical drugs that are used in the treatment of various ailments such as cancer, diabetes, fungal infections, mental treatment, cardiovascular treatment, and many more [16]. Initially, sulfamethoxazole (**1**) was treated with various aromatic aldehydes under reductive amination reaction conditions using sodium borohydride in methanol to afford sulfamethoxazole derivatives. Unfortunately, the use of sodium borohydride returned sulfamethoxazole starting material and the reduced aldehyde product [17]. After the unsuccessful use of sodium borohydride, 2-methylpyridine borane complex was employed as the reducing reagent. Thus, sulfamethoxazole (**1**) was treated with various aromatic aldehydes under reductive amination reaction conditions using 2-methylpyridine borane complex in methanol to yield benzylated sulfamethoxazole derivatives as shown in Scheme 1 [18]. The successful synthesis of all compounds was confirmed by both ^{1}H and ^{13}C NMR, IR and mass spectroscopy.

The ^{1}H NMR spectra of the compounds showed the disappearance of the aldehyde functional group signal around 10 ppm and the appearance of the new methylene signals ranging from 4.21 to 4.32 ppm in addition to other proton signals from the aromatic aldehyde. To supplement the ^{1}H NMR spectroscopic data, the ^{13}C NMR spectral data were recorded. The ^{13}C NMR spectra of **3a**–**t** showed the disappearance of the aldehyde signal around 196 ppm and the appearance of the methylene signals in the region of 40.28–41.67 ppm. In addition, the disappearance of the NH_2 functional group that was observed around 6.06 ppm and the appearance of the new NH functional group signals (8.05–10.24 ppm) was observed on the ^{1}H NMR spectra of products **3a**–**t**. Furthermore, the analysis of the IR spectra of products **3a**–**t** supported their formation by revealing the presence of functional groups such as OH (~3500 cm^{-1}), NH



Scheme 1: Synthesis of sulfamethoxazole derivatives.

(~3300 cm^{-1}), CH stretch (~3000 cm^{-1}), C=C, C=N (~1600 cm^{-1}), S=O (~1400 cm^{-1}), N-O (~1300 cm^{-1}), C-O (~1000 cm^{-1}), and C-halide (>500 cm^{-1}).

3. Biological Assays of Benzylated Sulfamethoxazole Derivatives

3.1. Anti-Mycobacterium tuberculosis Activity. All synthesised compounds were evaluated for their biological activity against *Mycobacterium tuberculosis* (*Mtb*) $H_{37}R_V$ strain. The biological assays were performed following a broth dilution method in 7H9_ADC_GLU_TW (7 days), and rifampicin was used as a positive control [19]. In addition, sulfamethoxazole (SMZ) was also biologically evaluated to better understand the impact of the structural modifications. The results for the in vitro biological assays are displayed in Table 1. As observed in Table 1, SMZ achieved tuberculosis growth inhibition of 80% at the tested concentration of 20 μM. In our efforts to improve the activity of SMZ towards *Mycobacterium tuberculosis*, a series of SMZ derivatives based on salicylaldehydes were synthesised. This produced compounds with mixed activity against TB. For example, 5 compounds displayed improved antitubercular activity, 5 compounds displayed slightly reduced antitubercular activity, 4 compounds displayed significant loss of activity, while 7 compounds displayed total loss of antitubercular activity compared to sulfamethoxazole.

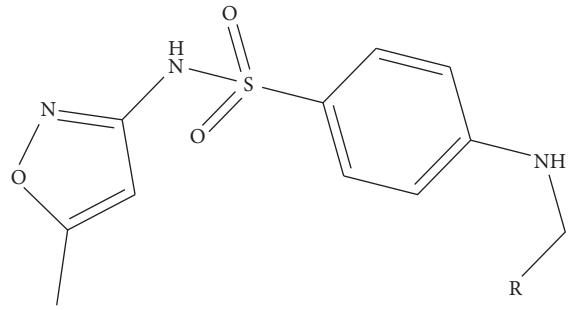
The most active compound in this series was **3q** with 92% inhibition, followed by **3s** (90%), **3k** (88%), **3t** (85%), and **3o** (84%). These compounds performed better than our starting material, SMZ (80%). However, most compounds displayed reduced activity to no activity against TB. For example, **3n** (78%), **3r** (77%), and **3l** (76%) displayed antitubercular activity closer to that of SMZ, while compounds such as **3c** (64%), **3b** (52%), and **3e** (36%) displayed significant loss of activity compared to SMZ. In addition, **3a**, **3d**, **3f**–**h**, **3j**, and **3m** all displayed complete loss of antitubercular activity (0%) in comparison to the activity of SMZ.

Interestingly, all compounds with a complete loss of activity possessed benzyl with substituents at the same positions (2, 3, and 5) with the exception of **3a** and **3d**. In general, trisubstituted benzyl substituents resulted in a complete loss of activity (e.g., **3d**) or reduced antitubercular activity (e.g. **3c**). Thus, we have synthesised SMZ derivatives, some with improved antitubercular activity, while others displayed reduced antitubercular activity when compared to SMZ.

3.2. Anti-Staphylococcus aureus (ATCC 25923) Activity. Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that is a member of the microbiota of the body, normally found in the upper respiratory tract and on the skin [20]. Although approximately 30% of the human population is designated permanent carriers of *S. aureus* where it is mostly found on the skin, nostrils, and women's lower reproductive tract, [21, 22] it remains the major cause of mild infections such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses to problematic infections such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis [23]. In addition, the emergence of antibiotic microbial resistant such as methicillin-resistant *S. aureus* (MRSA) has contributed to the increased economic burden of the world [24]. Thus, in our efforts of designing compounds with broad antibacterial properties, SMZ derivatives were evaluated for their potential activity against *S. aureus*. The single point growth inhibitory potential of the samples was determined using a 96-well plate-based assay following the Clinical and Laboratory Standards Institute guidelines [25].

All compounds were evaluated against *S. aureus* at a concentration of 32 μM. At this concentration, compounds were designated as either being active (100%) or inactive (0.00%). The activity of these compounds was compared to the activity of sulfamethoxazole in addition to the positive control, kanamycin. While SMZ showed activity against

Table 1: Summarised biological activity results of benzylated sulfamethoxazole derivatives.



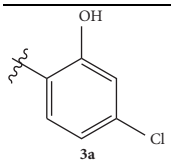
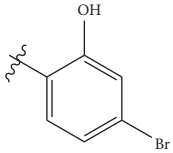
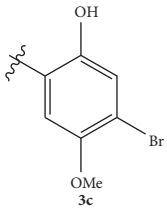
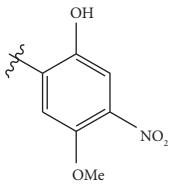
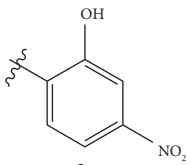
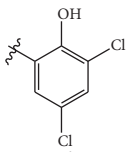
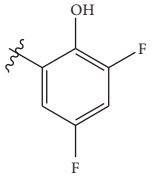
R =	Mtb % inhibition	*S. aureus*	CHO % survival
 **3a**	0	−	98.84
 **3b**	52.2	+	96.41
 **3c**	64.9	+	97.25
 **3d**	0	+	49.92
 **3e**	36.9	+	89.08
 **3f**	0	−	62.94
 **3g**	0	−	99.87

Table 1: Continued.

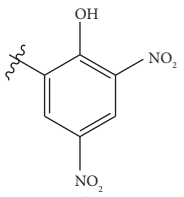
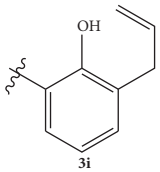
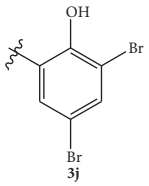
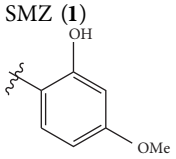
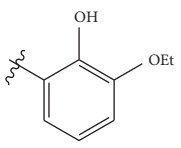
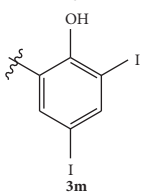
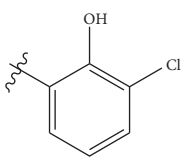
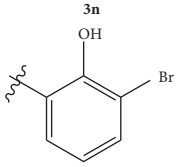
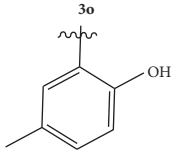
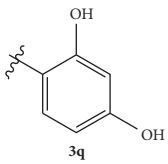
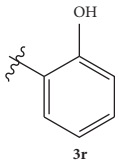
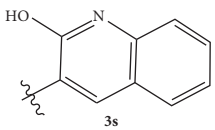
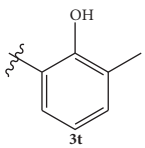
 **3h**	0	−	95.72
 **3i**	71.7	−	86.69
 **3j**	0	+	96.55
Rifampicin (0.01 μM)	+	ND	ND
Emitine (0.029 μM)	ND	ND	+
Kanamycin	ND	+	ND
SMZ (**1**)	80.8	−	67.6
 **3k**	88.6	−	82.91
 **3l**	76.6	−	103.5
 **3m**	0	+	97.31
 **3n**	78.4	+	85.63
 **3o**	84.7	+	84.61
 **3p**	54.3	−	90.24

Table 1: Continued.

 **3q**	92.4	+	94.10
 **3r**	77.9	−	87.26
 **3s**	90.0	+	83.45
 **3t**	85.9	−	86.54
Rifampicin (0.01 μM)	+	ND	ND
Emetine (0.029 μM)	ND	ND	+
Kanamycin	ND	+	ND
SMZ (**1**)	80.8	−	67.6

Active = +. Inactive = −. ND = not determined.

Mycobacterium tuberculosis, it was inactive against *S. aureus*. The modification of SMZ improved the activity of some derivatives, while others remained inactive like SMZ. For example, compounds **3b**–**e**, **3j**, **3m**–**o**, **3q**, and **3s** possessed antibacterial activity against *S. aureus* while the remainder of the compounds was inactive.

3.3. Further Anti-Bacterial Activity. To increase the scope and activity of the synthesized compounds, all benzylated sulfamethoxazole derivatives were also biologically evaluated against bacteria such as *Acinetobacter baumannii* (ATCC 19606), *Enterobacter cloacae* (ATCC 700323), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (BAA-1705), and *Pseudomonas aeruginosa* (ATCC 27853). These bacteria together with *Staphylococcus aureus* are responsible for the majority of nosocomial infections, and they have developed the ability to evade current antimicrobial agents [26, 27]. Unfortunately, all benzylated sulfamethoxazole derivatives were inactive against all five bacterial pathogens.

3.4. Cytotoxicity Activity Assays. In addition to the biological assays against *Mycobacterium tuberculosis* and *S. aureus*, cytotoxicity assays were also performed for all compounds against Chinese Hamster Ovarian (CHO) cells to determine the safety profile of these compounds at a specific concentration. Quantitative assessment of toxic activity *in vitro* was determined via the MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide] assay with emetine used as a standard. [28] The test compounds were mostly not toxic at the concentration 25 μM especially when compared to the starting material SMZ. Sulfamethoxazole showed a percentage survival of 67% at the same concentration of 25 μM. The most active SMZ derivative was **3d** with 49% cell survival followed by **3f** with 62%. These two derivatives showed improved toxicity to CHO cells compared to SMZ (67%). The remaining compounds possessed poor activity towards CHO cells. For example, SMZ derivative **3l** (103%) appeared to stimulate CHO cell growth, while other derivatives such as **3a** (98%), **3b** (96%), **3c** (97%), **3g** (99%), **3h** (95%), **3j** (96%), **3m** (97%), **3p** (90%), and **3q** (92%) possessed much improved safety profile compared to SMZ. In addition, the remainder of the compounds possessed cell survival of over 80%, which is still an improvement in comparison to SMZ (Figure 2). Thus, the modification of SMZ with salicylaldehyde produced compounds with improved safety profile (>80% cell survival) except for two compounds (<60%).

3.5. Structure-Activity Relationship. The effect of substituents on the antibacterial activity and cytotoxicity of the synthesised compounds was examined. The presence or absence of certain functional groups and their position can result in compounds with improved or decreased biological activity including their safety profile (cytotoxicity). For example, most SMZ derivatives with trisubstituted benzyl substituents were not active against Mtb (**3a**, **3d**, **3f**–**h**, **3j**, and **3m**). However, the same compounds showed excellent safety profile against CHO cells except for **3d** and **3f** which proved to be more toxic than SMZ. However, compounds

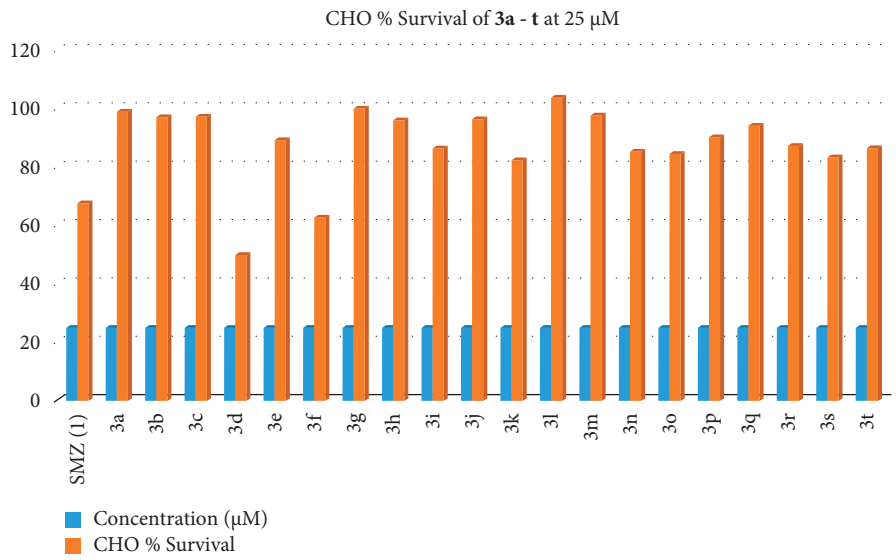


Figure 2: The plot of CHO % survival of sulfamethoxazole derivatives.

Table 2: ADMET predictions of the benzylated sulfamethoxazole derivatives.

Structure	MW	LogS	LogP	Predicted ADMET properties							Rules satisfied		
				Plasma protein binding (PPB) (%)	nHA	nHD	H-HT	Clearance	Half- life	TPSA	Lipinski	Pfizer	GSK
3a	393.06	−3.216	2.939	99.90	7	3	0.838	0.594	0.167	107.69	Yes	Yes	Yes
3b	437.00	−3.219	3.119	99.45	7	3	0.688	0.84	0.163	107.69	Yes	Yes	No
3c	467.02	−3.353	2.824	99.70	8	3	0.711	1.196	0.185	116.92	Yes	Yes	No
3d	434.09	−3.482	2.248	99.82	11	3	0.886	0.743	0.222	160.06	Yes	Yes	No
3e	404.08	−3.394	2.35	99.87	10	3	0.856	0.488	0.171	150.83	Yes	Yes	No
3f	427.02	−3.531	3.496	100.7	7	3	0.771	0.604	0.151	107.69	Yes	Yes	No
3g	395.08	−2.87	2.335	99.99	7	3	0.941	0.631	0.154	107.69	Yes	Yes	Yes
3h	449.06	−3.626	2.412	100.6	13	3	0.869	0.586	0.173	193.97	Yes	Yes	No
3i	399.13	−3.233	3.035	100.4	7	3	0.727	0.559	0.207	107.69	Yes	Yes	Yes
3j	514.92	−3.784	3.698	100.0	7	3	0.504	0.814	0.148	107.69	Yes	Yes	No
3k	389.1	−2.824	2.221	99.35	8	3	0.894	0.722	0.18	116.92	Yes	Yes	Yes
3l	403.12	−3.053	2.383	99.70	8	3	0.809	0.642	0.14	116.92	Yes	Yes	No
3m	610.89	−3.87	4.289	100.6	7	3	0.635	0.868	0.220	107.69	Yes	Yes	No
3n	393.06	−3.106	2.804	99.93	7	3	0.889	0.804	0.071	107.69	Yes	Yes	Yes
3o	437.0	−3.405	2.812	99.74	7	3	0.792	0.549	0.174	107.69	Yes	Yes	No
3p	373.11	−2.835	2.592	99.32	7	3	0.841	0.722	0.194	107.69	Yes	Yes	Yes
3q	375.09	−2.869	1.53	98.91	8	4	0.753	0.687	0.414	127.92	Yes	Yes	Yes
3r	359.09	−2.701	2.166	98.83	7	3	0.830	0.559	0.232	107.69	Yes	Yes	Yes
3s	410.1	−3.852	2.178	99.67	8	3	0.950	1.243	0.182	120.32	Yes	Yes	No
3t	373.11	−2.785	2.472	99.45	7	3	0.876	0.544	0.203	107.69	Yes	Yes	Yes
SMZ	253.05	−2.491	0.142	84.64	6	3	0.892	0.785	0.141	101.45	Yes	Yes	Yes

MW-molecular weight: 100–600 g/mol, LogS (predicted aqueous solubility): −4–0.5 log·mol/L, LogP (predicted octanol/water partition coefficient): 0–3, LogD (pH 7.4): 1–3, human hepatotoxicity (H H-T): 0 (nontoxic)– +1 (toxic), CL (clearance): 5–15 mL/min/kg (<5 (low), 5–15 (moderate), >15 (high), half-life ($T_{1/2}$): >3 hours, (short half-life <3 hours), protein plasma binding (PPB): <90%, number of hydrogen acceptors (nHA): 0–12, number of hydrogen donors (nHD): 0–7, GSK's rule: MW ≤ 400, LogP ≤4, Pfizer's rule: LogP ≤3, topological polar surface area (TPSA) >75, lipinski's rule: MW ≤ 500, LogP ≤5, number of hydrogen acceptors (nHA) ≤10, number of hydrogen donors (nHD) ≤5.

3d, **3j**, and **3m** displayed excellent antibacterial activity against *S. aureus* indicating their selectivity towards *S. aureus*. On the other hand, SMZ derivatives containing disubstituted benzyl substituents showed excellent Mtb activity. For example, **3q** (with 2,4-dihydroxy functional groups) was the most active, followed by **3k** (with 2-hydroxy-4-methoxy), **3t** (with 2-hydroxy-3-methyl), and **3o** (3-bromo-2-hyrodroxy), thus performing better than SMZ. Unfortunately, not all compounds with disubstituted benzyl substituents showed excellent activity against Mtb, indicating the important role played by different functional groups. For example, compounds **3b**, **3e**, **3i**, **3l**, **3n**, and **3p** all

possessed reduced antitubercular activity compared to SMZ, thus highlighting the role that was played by the functional groups including their position on the benzyl substituent.

3.6. Adsorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Predictions. ADMET properties of the synthesised compounds were analysed using the online prediction software ADMETlab 2.0 [29, 30]. These physiochemical predictions are very useful when designing a molecule for drug discovery. The ADMET predicted results are displayed in Table 2. The analysis of Table 2 results revealed the compounds with good predicted LogS values and mixed LogP values. For example, derivative **3q** possessed the best predicted LogP value of 1.53, derivatives **3b**, **3f**, **3i**, **3j**, and **3m** all gave poor predicted LogP values. In addition, all SMZ derivatives possessed poor predicted protein plasma binding properties (>90%) while the number of hydrogen acceptors and donors were within the required limits. Moreover, the predicted results for human hepatotoxicity (H-HT) were mixed, with some compounds predicted to be safer than SMZ, while others were predicted to be more toxic than SMZ. For example, derivatives **3b** (0.688), **3c** (0.711), **3i** (0.727), **3j** (0.504), and **3m** (0.635) were predicted to be safer than SMZ (0.892), while derivatives **3g** (0.941), **3k** (0.894), and **3s** (0.950) were predicted to be more toxic than SMZ (0.892). Thus, the safest compound was predicted to be **3j**, the most toxic compound was predicted to be **3s**, while the rest of the compounds possessed predicted values similar to the value of SMZ (0.892). The clearance and half-life of all compounds were also predicted. The derivatives with the best predicted clearance values were **3s** (1.243) and **3c** (1.196), while derivatives **3q** (0.414) and **3s** (0.232) displayed the best predicted half-life. Furthermore, all compounds possessed good predicted topological polar surface area (TPSA) with the highest value of 193.97 for derivative **3h** and the lowest value of 101.45 was predicted for SMZ. All compounds satisfied both Lipinski and Pfizer drug discovery rules, while some compounds failed GSK drug discovery rules.

4. Conclusion

The sulfamethoxazole benzylated derivatives were successfully synthesised and characterised using ^{1}H and ^{13}C NMR, IR, and mass spectroscopy. All characterised compounds were biologically evaluated against Mtb, *S. Aureus*, and CHO cells. Seven (7) compounds, mostly with similar structural properties, were not active when evaluated against Mtb, while the rest of the compounds showed weak (36%) to strong (92%) percentage inhibition of Mtb. In addition, ten (10) compounds were active when evaluated against *S.* Aureus, representing 50% of all compounds. Only two (2) compounds were active (toxic) against CHO cells, while the rest of the compounds performed better than SMZ (less toxic) against CHO cells. Thus, we have synthesised compounds with improved safety profile, better activity against *S. Aureus*, and improved antitubercular properties compared to SMZ.

5. Experimental Procedures

5.1. General Information. Commercially available reagents and solvents were purchased from Sigma Aldrich and Merck (South Africa). All chemicals were used as received, unless otherwise stated. The structural properties of the compounds were recorded and confirmed by: high-resolution mass spectra were recorded using Sciex X500R QTOF at the University of Limpopo Mass Spectrometry Facility; melting points were obtained using Lasec/SA-melting point apparatus from Lasec company, SA (Johannesburg, South Africa); IR spectra were recorded using Bruker technologies Alpha Platinum ATR FTIR spectrometer; and Nuclear Magnetic Resonance (NMR) (Bruker Ascend 400 MHz Topspin 3.2); ^{1}H NMR and ^{13}C NMR spectra were referenced internally using solvent signals, ^{1}H NMR: 7.250 ppm for $CDCl_3$, 2.500 ppm for DMSO-d_6; ^{13}C NMR: 77.00 ppm for $CDCl_3$, and 39.40 ppm for DMSO-d_6, respectively, which were used as the solvents at room temperature. Chemical shifts are expressed in δ-values parts per million (ppm) and the coupling constants (J) in Hertz (Hz). Multiplicity of the signals is given as follows: brs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, and m = multiplet.

5.2. General Synthetic Method for the Reductive-Amination of Sulfamethoxazole Derivatives [18]. Mixture of sulfamethoxazole **1** (0.0500 g, 0.197 mmol) and appropriate aldehyde **2** (0.207 mol, 1.05 eq.) in methanol (15 mL) was stirred for 14 hours before being reduced with 2-methylpyridine borane complex (0.02667 g, 0.257 mmol, 1.3 eq.) and stirred at room temperature for further 4 hours. Subsequently, the reaction mixture was quenched with a saturated aqueous solution of ammonium chloride and extracted with ethyl acetate (3×30 mL). The combined organic fractions were dried with anhydrous sodium sulphate, the solvent removed on a rotary evaporator, and the resulting product purified by flash silica gel column chromatography (5–30% ethyl acetate in hexane) to afford the appropriate products **3a**–**t** in good to excellent yields.

The exchangeable protons such as OH and NH may not display on the ^{1}H NMR spectra of compounds, thus affecting proton count of some of the compounds.

***N*-(4-Chloro-2-hydroxybenzyl)sulfamethoxazole (3a)** as a white solid, 0.06194 g, 80%, mp 100.9–101.8°C. ^{1}H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 9.99 (s, 1H), 7.51 (d, J = 9.0 Hz, 2H), 7.11 (d, J = 2.8 Hz, 1H), 7.10 (dd, J = 8.0, 2.8 Hz, 2H), 6.84 (d, J = 8.0 Hz, 1H), 6.62 (d, J = 9.0 Hz, 2H), 6.09 (d, J = 0.7 Hz, 1H), 4.21 (d, J = 5.8 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.38, 158.34, 154.42, 152.74, 129.18, 127.97, 127.42, 124.96, 122.83, 116.99, 95.72, 40.88, 12.50. V_{max} (FT-IR) 3480.70, 3449.13, 3250.08, 2250.87, 2125.01, 1657.70, 1598.51, 1160.16, 1052.56,

1023.41, 820.40, 757.81 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 394.2985; calculated mass for $C_{17}H_{16}ClN_3O_4S$ is 393.0550.

***N*-(4-Bromo-2-hydroxybenzyl)sulfamethoxazole (3b)** as a yellowish solid, 0.07430 g, 86%, mp 138.5–139.0°C. ^1H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 10.03 (s, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.23 (s, 1H), 7.22 (d, $J = 7.9$ Hz, 1H), 7.09 (t, $J = 5.9$ Hz, 1H), 6.81 (d, $J = 7.9$ Hz, 1H), 6.63 (d, $J = 8.8$ Hz, 2H), 6.09 (s, 1H), 4.21 (d, $J = 5.5$ Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.94, 157.93, 154.47, 152.33, 130.44, 128.76, 127.56, 117.18, 112.62, 110.07, 95.32, 40.43, 12.07.s V_{max} (FT-IR) 3466.19, 3427.55, 3249.63, 2249.60, 2124.58, 1617.23, 1597.83, 1328.04, 1272.70, 1160.58, 1052.83, 1023.85, 819.95, 757.29 cm^{-1}. HRMS (ESI) $[M + H]_+$:m/z 438.2715; Calculated mass for $C_{17}H_{16}BrN_3O_4S$ is 437.0045.

***N*-(4-Bromo-2-hydroxy-5-methoxybenzyl)sulfamethoxazole (3c)** as an orange solid, 0.08613 g, 96%, mp 186.4–186.9°C. ^1H NMR (400 MHz, DMSO) δ 10.94 (s, 1H), 9.17 (s, 1H), 7.51 (d, $J = 8.3$ Hz, 2H), 7.18–6.95 (m, 1H), 6.90 (d, $J = 8.3$ Hz, 2H), 6.69–6.48 (m, 1H), 6.10 (s, 1H), 4.24 (d, $J = 7.4$ Hz, 2H), 3.87 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.14, 158.03, 152.41, 148.57, 143.45, 128.99, 128.88, 127.43, 124.63, 113.53, 112.74, 109.98, 95.41, 56.33, 40.32, 12.20. V_{max} (FT-IR) 3500.69, 3461.80, 3258.61, 3072.42, 2250.05, 2124.86, 1657.85, 1598.61, 1160.18, 1052.25, 1023.33, 820.63, 758.06 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 468.3302; calculated mass for $C_{18}H_{18}BrN_3O_5S$ is 467.0151.

***N*-(2-Hydroxy-5-methoxy-4-nitrobenzyl)sulfamethoxazole (3d)** as a yellowish solid, 0.07451 g, 87%, mp 218.4–218.9°C. ^1H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 10.66 (s, 1H), 7.75 (s, $J = 2.4$ Hz, 1H), 7.69 (d, $J = 2.4$ Hz, 1H), 7.51 (d, $J = 8.8$ Hz, 2H), 7.25 (t, $J = 5.9$ Hz, 1H), 6.63 (d, $J = 8.8$ Hz, 2H), 6.08 (s, 1H), 4.32 (d, $J = 5.7$ Hz, 2H), 3.93 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.98, 157.90, 152.19, 150.99, 147.13, 139.19, 128.81, 125.77, 124.87, 116.50, 111.20, 105.70, 95.30, 56.45, 40.28, 12.08. V_{max} (FT-IR) 3489.51, 3453.91, 3253.91, 2250.01, 2124.41, 1654.02, 1507.54, 1335.44, 1160.32, 1052.54, 1023.48, 820.34, 757.70 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 435.0973; Calculated mass for$C_{18}H_{18}N_4O_7S$ is 434.0896.

***N*-(2-Hydroxy-4-nitrobenzyl)sulfamethoxazole (3e)** as a yellowish solid, 0.06266 g, 79%, mp 102.0–102.5°C. ^1H NMR (400 MHz, DMSO) δ 11.07 (s, 1H), 8.06 (d, $J = 2.6$ Hz, 1H), 8.03 (s, 1H), 7.52 (d, $J = 8.7$ Hz, 2H), 7.22 (t, $J = 5.8$ Hz, 1H), 7.01 (d, $J = 8.6$ Hz, 1H), 6.65 (d, $J = 8.7$ Hz, 2H), 6.58 (d, $J = 8.7$ Hz, 1H), 6.08 (s, 1H), 4.30 (d, $J = 5.6$ Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.01, 161.79, 157.94, 152.25, 139.60, 128.90, 128.87, 126.36, 124.90, 124.74, 123.89, 115.33, 95.33, 40.41, 12.11. V_{max} (FT-IR) 3523.64, 3417.50, 3256.84, 2250.26, 2125.31, 1598.04, 1498.17, 1398.63, 1335.70, 1291.82, 1160.65, 1052.92, 1023.82, 819.94, 757.1 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 405.0874; calculated mass for $C_{17}H_{16}N_4O_4S$ is 404.0791.

***N*-(3,5-Dichloro-2-hydroxybenzyl)sulfamethoxazole (3f)** as a light brown solid, 0.07901 g, 94%, mp 179.9–180.6°C. ^1H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 9.85 (s, 1H), 7.52 (d, $J = 8.9$ Hz, 2H), 7.40 (d, $J = 2.6$ Hz, 1H), 7.15 (t, $J = 6.0$ Hz, 1H), 7.10 (d, $J = 2.6$ Hz, 1H), 6.62 (d, $J = 8.9$ Hz, 2H), 6.09 (d, $J = 0.8$ Hz, 1H), 4.30 (d, $J = 5.8$ Hz, 2H), 2.29 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.01, 157.94, 152.14, 149.69, 130.04, 128.89, 128.83, 127.43, 126.45, 124.93, 123.38, 121.81, 112.63, 95.35, 41.17, 12.11. V_{max} (FT-IR) 3509.35, 3427.84, 3254.10, 2249.37, 2124.32, 1617.05, 1598.21, 1160.97, 1052.83, 1023.73, 819.99, 757.33 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 428.2865; calculated mass for $C_{17}H_{15}Cl_2N_3O_4S$ is 427.0160.

***N*-(3,5-Difluoro-2-hydroxybenzyl)sulfamethoxazole (3g)** as an orange solid, 0.06978 g, 90%, mp 158.9–159.3°C. ^1H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 9.75 (s, 1H), 7.51 (d, $J = 8.9$ Hz, 2H), 7.17–7.05 (m, 2H), 6.62 (d, $J = 8.9$ Hz, 2H), 6.09 (s, 1H), 4.28 (d, $J = 5.9$ Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.93, 157.89, 152.16, 138.81, 129.69, 128.72, 124.84, 112.58, 111.18, 109.74, 109.52, 102.70, 95.30, 40.53, 12.04. V_{max} (FT-IR) 3522.30, 3421.06, 3249.23, 2249.35, 2124.00, 1615.55, 1597.51, 1490.46, 1456.91, 1161.00, 1052.92, 1023.95, 819.97, 757.3 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 396.0837; calculated mass for $C_{17}H_{15}F_2N_3O_4S$ is 395.0751.

***N*-(2-Hydroxy-3,5-dinitrobenzyl)sulfamethoxazole (3h)** as an orange solid, 0.07019 g, 79%, mp 111.7–112.3°C. ^1H NMR (400 MHz, DMSO) δ 11.00 (s, 1H), 9.76 (s, 1H), 7.52 (d, $J = 8.7$ Hz, 2H), 7.12 (d, $J = 2.7$ Hz, 1H), 7.07 (d, $J = 2.7$ Hz, 1H), 6.63 (d, $J = 8.7$ Hz, 2H), 6.10 (s, 1H), 4.29 (d, $J = 5.6$ Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.03, 157.96, 152.22, 140.64, 128.80, 127.34, 124.85, 123.67, 123.41, 121.29, 112.64, 111.24, 95.35, 40.58, 12.10. V_{max} (FT-IR) 3520.64, 3414.50, 3250.84, 2255.26, 2126.31, 1598.04, 1493.17, 1388.63, 1335.70, 1281.82, 1157.65, 1050.92, 1022.82, 818.94, 754.1 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 450.0675; calculated mass for $C_{17}H_{15}N_5O_8S$ is 449.0641.

***N*-(3-Allyl-2-hydroxybenzyl)sulfamethoxazole (3i)** as a white solid, 0.05998 g, 76%, mp 99.2–99.9°C. ^1H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 8.55 (s, 1H), 7.50 (d, $J = 9.0$ Hz, 2H), 7.46 (d, $J = 8.8$ Hz, 1H), 6.75 (dd, $J = 14.4$, 7.2 Hz, 1H), 6.62 (d, $J = 8.9$ Hz, 2H), 6.58 (d, $J = 8.8$ Hz, 1H), 6.10 (s, 1H), 6.02–5.85 (m, 1H), 5.08–4.94 (m, 2H), 4.28 (d, $J = 5.5$ Hz, 2H), 3.38–3.30 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.95, 157.96, 152.16, 137.18, 137.08, 128.89, 128.66, 128.24, 126.43, 125.45, 119.26, 115.36, 112.61, 111.15, 95.31, 41.52, 33.74, 12.08. V_{max} (FT-IR) 3398.11, 3255.52, 3057.74, 2254.98, 2128.86, 1654.32, 1160.89, 1022.23, 996.26, 823.11, 760.70 cm^{-1}. HRMS (ESI) $[M + H]^+$: m/z 400.1328; calculated mass for $C_{20}H_{21}N_3O_4S$ is 399.1253.

***N*-(3,5-Dibromo-2-hydroxybenzyl)sulfamethoxazole (3j)** as an orange solid, 0.09398 g, 92%, mp 115.2–115.7°C. ^1H NMR (400 MHz, DMSO) δ 8.45 (s, 1H), 7.62 (s, 1H), 7.49 (d, $J = 8.8$ Hz, 2H), 7.26 (s, 1H), 7.11 (t, $J = 5.7.0$ Hz, 1H), 6.62 (s, $J = 8.8$ Hz, 2H), 6.07 (s, 1H), 4.30 (d, $J = 5.6$ Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.64, 158.48, 151.88, 151.28, 132.85, 130.54, 129.80, 128.69, 125.61, 112.56, 111.24, 111.08, 95.43, 41.43, 12.10. V_{max} (FT-IR) 3398.11, 3255.52, 3053.74, 2251.98, 2122.86, 1650.32, 1159.89, 1021.23, 996.26, 823.11, 754.70 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 515.9163; calculated mass for $C_{17}H_{15}Br_2N_3O_4S$ is 514.9130.

***N*-(2-Hydroxy-4-methoxybenzyl)sulfamethoxazole (3k)** as a yellow solid, 0.06810 g, 89%, mp 137.1–137.5°C. ^1H NMR (400 MHz, DMSO) δ 10.95 (s, 1H), 9.72 (s, 1H), 7.48 (d, $J = 9.1$ Hz, 2H), 7.06 (t, $J = 5.8$ Hz, 1H), 7.03 (d, $J = 8.4$ Hz, 1H), 6.61 (d, $J = 8.9$ Hz, 2H), 6.40 (d, $J = 2.5$ Hz, 1H), 6.33 (dd, $J = 8.4$, 2.4 Hz, 1H), 6.09 (s, 1H), 4.12 (d, $J = 5.6$ Hz, 2H), 3.65 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.91,

159.36, 157.99, 156.02, 152.55, 129.32, 128.63, 124.01, 116.83, 112.60, 110.99, 104.15, 101.20, 95.30, 54.89, 40.51, 12.07. V_{max} (FT-IR) 3521.42, 3407.11, 3266.62, 3249.79, 2250.10, 2124.04, 1658.00, 1618.25, 1597.15, 1160.15, 1053.00, 1023.81, 820.05, 757.41 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 390.2954; calculated mass for $C_{18}H_{19}N_3O_5S$ is 389.1045.

***N*-(3-Ethoxy-2-hydroxybenzyl)sulfamethoxazole (3l)** as a white solid, 0.06218 g, 78%, mp 199.1–199.8°C. ^{1}H NMR (400 MHz, DMSO) δ 10.96 (s, 1H), 8.60 (s, 1H), 7.48 (d, J = 8.9 Hz, 2H), 7.07 (t, J = 5.9 Hz, 1H), 6.83 (dd, J = 7.8, 1.5 Hz, 1H), 6.73 (d, J = 6.4 Hz, 1H), 6.68 (d, J = 7.7 Hz, 1H), 6.60 (d, J = 7.8 Hz, 2H), 6.09 (s, 1H), 4.23 (d, J = 5.8 Hz, 2H), 4.03 (q, J = 7.0 Hz, 2H), 2.28 (s, 3H), 1.34 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.96, 157.96, 152.59, 146.44, 144.17, 128.67, 125.14, 124.15, 120.11, 118.77, 111.70, 111.01, 95.31, 64.08, 40.76, 14.76, 12.10. V_{max} (FT-IR) 3527.72, 3405.57, 3265.70, 2237.38, 2166.20, 1615.36, 1593.99, 1520.16, 1272.10, 1229.64, 1091.79, 1074.45, 879.23, 746.4 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 404.1288; calculated mass for $C_{19}H_{21}N_3O_5S$ is 403.1202.

***N*-(2-Hydroxy-3,5-diiodobenzyl)sulfamethoxazole (3m)** as an orange solid, 0.1030 g, 85%, mp 180.0–180.9°C. ^{1}H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 9.56 (s, 1H), 7.88 (s, 1H), 7.52 (d, J = 7.2 Hz, 2H), 7.40 (s, 1H), 6.61 (d, J = 7.1 Hz, 2H), 6.09 (s, 1H), 4.27 (s, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.98, 157.91, 154.14, 152.10, 144.19, 136.51, 129.60, 128.86, 128.77, 124.89, 112.60, 111.31, 95.32, 90.30, 83.77, 41.57, 12.11. V_{max} (FT-IR) 3464.91, 3430.31, 3250.24, 2249.89, 2124.58, 1658.22, 1623.60, 1597.99, 1053.00, 1023.70, 820.16, 757.43 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 611.8964; calculated mass for $C_{17}H_{15}I_2N_3O_4S$ is 610.8873.

***N*-(3-Chloro-2-hydroxybenzyl)sulfamethoxazole (3n)** as a white solid, 0.06002 g, 77%, mp 148.8–149.5°C. ^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 9.46 (s, 1H), 7.51 (d, J = 9.0 Hz, 2H), 7.25 (dd, J = 8.0, 1.5 Hz, 1H), 7.11 (dd, J = 8.0, 7.8 Hz, 1H), 6.80 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 8.9 Hz, 2H), 6.10 (s, 1H), 4.30 (d, J = 5.5 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.00, 157.96, 152.41, 150.44, 128.75, 128.26, 128.04, 126.92, 124.55, 120.78, 120.45, 112.62, 95.34, 41.39, 12.11. V_{max} (FT-IR) 3480.70, 3449.13, 3250.08, 2250.87, 2125.01, 1657.70, 1598.51, 1160.16, 1052.56, 1023.41, 820.40, 757.81 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 394.0626; calculated mass for $C_{17}H_{16}ClN_3O_4S$ is 393.0550.

***N*-(3-Bromo-2-hydroxybenzyl)sulfamethoxazole (3o)** as a white solid, 0.07015 g, 81%, mp 110.2–111.0°C. ^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 9.32 (s, 1H), 7.51 (d, J = 8.9 Hz, 2H), 7.40 (dd, J = 8.0, 1.4 Hz, 1H), 7.12 (dd, J = 8.0, 1.4 Hz, 1H), 7.01 (t, J = 5.7 Hz, 1H), 6.76 (dd, J = 10.5, 5.0 Hz, 1H), 6.61 (d, J = 8.9 Hz, 2H), 6.10 (s, 1H), 4.30 (d, J = 5.6 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.02, 157.96, 152.39, 151.38, 131.40, 128.90, 128.76, 128.19, 127.56, 124.59, 121.25, 112.63, 111.42, 95.35, 41.67, 12.12. V_{max} (FT-IR) 3398.11, 3255.52, 3053.74, 2251.98, 2122.86, 1650.32, 1159.89, 1021.23, 996.26, 823.11, 754.70 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 438.0137; calculated mass for $C_{17}H_{16}BrN_3O_4S$ is 437.0045.

***N*-(2-Hydroxy-5-methylbenzyl)sulfamethoxazole (3p)** as a white solid, 0.06009 g, 82%, mp 102.9–103.8°C. ^{1}H NMR (400 MHz, DMSO) ^{1}H NMR (400 MHz, DMSO) δ 10.95 (s, 1H), 9.06 (s, 1H), 7.46 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 9.0 Hz, 1H), 6.57 (d, J = 9.0 Hz, 2H), 6.24 (d, J = 2.3 Hz, 1H), 6.18–6.13 (m, 1H), 6.09 (s, 1H), 4.34 (d, J = 5.1 Hz, 2H), 2.45 (s, 3H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.99, 169.98, 158.02, 157.99, 153.37, 152.81, 152.66, 140.65, 128.93, 128.91, 128.73, 128.37, 127.35, 127.28, 124.25, 124.12, 124.10, 114.96, 112.64, 95.34, 40.86, 20.33, 12.11. V_{max} (FT-IR) 3449.88, 3141.86, 2976.29, 2237.38, 2166.20, 1615.36, 1593.99, 1520.16, 1467.31, 1272.10, 1229.64, 1091.79, 1074.45, 825.48, 746.4 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 374.1196; calculated mass for $C_{18}H_{19}N_3O_4S$ is 373.1096.

***N*-(2,4-Dihydroxybenzyl)sulfamethoxazole (3q)** as a white solid, 0.05079 g, 78%, mp 106.2–106.9°C. ^{1}H NMR (400 MHz, DMSO) δ 10.95 (s, 1H), 9.16 (s, 1H), 9.06 (s, 1H), 7.46 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 9.0 Hz, 1H), 6.57 (d, J = 8.6 Hz, 2H), 6.30 (d, J = 2.3 Hz, 1H), 6.19–6.13 (m, 1H), 6.09 (s, 1H), 4.34 (d, J = 5.1 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.97, 158.00, 157.07, 153.36, 148.90, 136.44, 128.89, 124.09, 120.95, 112.62, 105.76, 102.12, 95.33, 40.68, 12.11. V_{max} (FT-IR) 3494.31, 3464.91, 3430.31, 3250.24, 2249.89, 2124.58, 1658.22, 1623.60, 1597.99, 1053.00, 1023.70, 820.16, 757.43 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 376.2020; calculated mass for $C_{17}H_{17}N_3O_5S$ is 375.0889.

***N*-(2-Hydroxybenzyl)sulfamethoxazole (3r)** as a white solid, 0.05317 g, 75%, mp 100.2–101.8°C. ^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 9.64 (s, 1H), 7.49 (d, J = 8.9 Hz, 2H), 7.11 (d, J = 7.5 Hz, 1H), 7.09–6.99 (m, 1H), 6.82 (d, J = 7.9 Hz, 1H), 6.78–6.69 (m, 1H), 6.61 (d, J = 7.3 Hz, 2H), 6.10 (s, 1H), 4.21 (d, J = 5.7 Hz, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.99, 157.98, 155.13, 152.63, 128.71, 128.00, 127.36, 124.51, 124.16, 118.92, 115.02, 112.63, 95.33, 40.88, 12.12. V_{max} (FT-IR) 3511.42, 3401.11, 3259.62, 3232.79, 2228.10, 2119.04, 1647.00, 1608.25, 1587.15, 1130.15, 1053.00, 1020.81, 825.05, 742.41 cm^{-1}. HRMS (ESI) $[M + H]^+$:m/z 360.1022; calculated mass for $C_{17}H_{17}N_3O_4S$ is 359.0940.

***N*-((2-Hydroxyquinolin-3-yl)methyl))sulfamethoxazole (3s)** as a cream white solid, 0.0.05183 g, 65%, mp 238.0–238.7°C. ^{1}H NMR (400 MHz, DMSO) δ 11.94 (s, 1H), 10.24 (s, 1H), 7.70 (s, 1H), 7.61 (d, J = 7.7 Hz, 1H), 7.52 (d, J = 8.8 Hz, 2H), 7.45 (m, 1H), 7.31 (d, J = 8.2 Hz, 1H), 7.13 (m, 1H), 6.66 (d, J = 8.8 Hz, 2H), 6.09 (s, 1H), 4.20 (d, J = 5.3 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 169.99, 161.62, 157.94, 152.40, 142.50, 137.95, 135.06, 130.99, 129.88, 128.80, 127.67, 124.72, 121.94, 119.03, 114.92, 95.33, 41.56, 12.09. V_{max} (FT-IR) 3457.05, 3445.66, 3261.99, 3164.90, 2239.79, 2121.48, 1615.57, 1590.90, 1505.72, 1464.07, 1263.12, 1082.93, 1026.46, 822.41, 746.89 cm^{-1}. HRMS (ESI) $[M + H^+]$:m/z 411.1082; calculated mass for $C_{20}H_{18}N_4O_4S$ is 410.1049.

***N*-(2-Hydroxy-3-methylbenzyl)sulfamethoxazole (3t)** as a white solid, 0.06352 g, 86%, mp 158.2–158.9°C. ^{1}H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 8.51 (s, 1H), 7.51 (d, J = 8.9 Hz, 2H), 7.03 (t, J = 5.6 Hz, 1H), 6.98 (d, J = 7.5 Hz, 1H), 6.69 (m, 1H), 6.62 (d, J = 8.9 Hz, 2H), 6.59 (d, J = 7.8 Hz, 1H), 6.11 (s, 1H), 4.27 (d, J = 5.5 Hz, 2H), 2.28 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 170.01, 158.01, 152.86, 152.66, 129.52, 128.92, 128.72, 125.99, 125.29, 124.74, 124.22, 119.41, 112.65, 111.15, 95.36, 41.56, 16.64, 12.12.

V_{max} (FT-IR) 3474.76, 3261.99, 3064.90, 2249.89, 2124.58, 1615.57, 1590.90, 1505.72, 1263.12, 1135.14, 1072.93, 1036.46, 891.55, 780.68 cm^{-1}. HRMS (ESI) $[M + H^+]$:m/z 374.1179; calculated mass for $C_{18}H_{19}N_3O_4S$ is 373.1096.

Data Availability

The data will be available on request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

JMM synthesised and characterised all compounds, while TCL conceptualised the research project and wrote the manuscript. In addition, JMM edited the manuscript.

Acknowledgments

The authors wish to thank University of Limpopo, Ms. Lerato Raphoko (University of Limpopo) for mass spectrometric data, and the Drug Discovery and Development Centre (H3D) at the University of Cape Town for biological assay data. The authors are thankful for financial support of this project from the University of Limpopo and the National Research Foundation (South Africa) through grant no. TTK180412320177.

References

[1] M. Mondelli, F. Pavan, P. C. de Souza et al., "Study of a series of cobalt(II) sulfonamide complexes: synthesis, spectroscopic characterization, and microbiological evaluation against M. Tuberculosis. Crystal structure of [Co(Sulfamethoxazole) 2(H 2O)2]·H2O," *Journal of Molecular Structure*, vol. 1036, pp. 180–187, 2013.

[2] Who, "Impact of the covid-19 pandemic on TB detection and mortality in 2020," *Journal of Chemical Information and Modeling*, vol. 53, no. 9, pp. 1689–1699, 2021.

[3] K. Dheda, M. Tomasicchio, A. Reuter et al., "Tuberculosis," *Encyclopedia of Respiratory Medicine*, pp. 75–98, 2022.

[4] A. Ovung and J. Bhattacharyya, "Sulfonamide drugs: structure, antibacterial property, toxicity, and biophysical interactions," *Biophysical reviews*, vol. 13, 2010.

[5] E. Bera and T. Leong, "South african national essential medicine list adult hospital level medication review process component: gynaecology," *Expert review of anti-infective therapy*, vol. 6, 2017.

[6] D. Lin, W. K. Li, and M. J. Rieder, "Cotrimoxazole for prophylaxis or treatment of opportunistic infections of HIV/AIDS in patients with previous history of hypersensitivity to cotrimoxazole," *Cochrane Database of Systematic Reviews*, vol. 2, Article ID CD005646, 2007.

[7] A. C. Bowen, J. R. Carapetis, B. J. Currie, V. Fowler, H. F. Chambers, and S. Y. C. Tong, "Sulfamethoxazole-trimethoprim (cotrimoxazole) for skin and soft tissue infections including impetigo, cellulitis, and abscess," *Open Forum Infectious Diseases*, vol. 4, no. 4, 2017.

[8] P. Forgacs, N. L. Wengenack, L. Hall, S. K. Zimmerman, M. L. Silverman, and G. D. Roberts, "Tuberculosis and trimethoprim-sulfamethoxazole," *Antimicrobial Agents and Chemotherapy*, vol. 53, no. 11, pp. 4789–4793, 2009.

[9] K. Rajamanickam, J. Yang, and M. K. Sakharkar, "A novel antimicrobial–phytochemical conjugate with antimicrobial activity against Streptococcus uberis, Enterococcus faecium, and Enterococcus faecalis," *Frontiers in Pharmacology*, vol. 10, pp. 1405-1406, 2019.

[10] S. Akili, D. ben Hadda, Y. Bitar, A. Najjar, and M. Fawaz Chehna, "Computer-aid design of novel sulfonamide derivatives as EGFR kinase inhibitors for cancer treatment," *International Journal of Organic Chemistry*, vol. 11, no. 4, pp. 171–186, 2021.

[11] S. Akili, D. ben Hadda, Y. Bitar, A. Balash, and M. Fawaz Chehna, "Design, synthesis and characterization of novel sulfonamides derivatives as anticancer agent targeting EGFR TK, and development of new methods of synthesis by microwave irradiation," *International Journal of Organic Chemistry*, vol. 11, no. 4, pp. 199–223, 2021.

[12] L. Macingwana, B. Baker, A. H. Ngwane et al., "Sulfamethoxazole enhances the antimycobacterial activity of rifampicin," *Journal of Antimicrobial Chemotherapy*, vol. 67, no. 12, pp. 2908–2911, 2012.

[13] A. E. M. Noreljaleel, A. Wilhelm, S. L. Bonnet, and J. H. van der Westhuizen, "Synthesis and bioactivity of reduced chalcones containing sulfonamide side chains," *Journal of Natural Products*, vol. 81, no. 1, pp. 41–48, 2018.

[14] K. R. Brimacombe, M. J. Walsh, L. Liu et al., "Identi fi cation of ML251, a potent inhibitor of T. Brucei and T," *Cruzi Phosphofructokinase*, vol. 8–13, 2014.

[15] M. R. Sedibana and T. C. Leboho, "Novel benzylamine derivatives: synthesis, anti-Mycobacterium tuberculosis evaluation and predicted ADMET properties," *The Open Medicinal Chemistry Journal*, vol. 17, no. 1, pp. 1–13, 2023.

[16] O. I. Afanasyev, E. Kuchuk, D. L. Usanov, and D. Chusov, "Reductive amination in the synthesis of pharmaceuticals," *Chemical Reviews*, vol. 11, pp. 11857–11911, 2019.

[17] A. V. Panfilov, Y. D. Markovich, I. P. Ivashev et al., "Sodium borohydride in reductive amination reactions," *Pharmaceutical Chemistry Journal*, vol. 34, no. 2, pp. 76–78, 2000.

[18] S. Sato, T. Sakamoto, E. Miyazawa, and Y. Kikugawa, "One-pot reductive amination of aldehydes and ketones with α-picoline-borane in methanol, in water, and in neat conditions," *Tetrahedron*, vol. 60, no. 36, pp. 7899–7906, 2004.

[19] C. Soares de Melo, T. S. Feng, R. van der Westhuyzen et al., "Aminopyrazolo[1,5-a]Pyrimidines as potential inhibitors of Mycobacterium tuberculosis: structure activity relationships and ADME characterization," *Bioorganic & Medicinal Chemistry*, vol. 23, no. 22, pp. 7240–7250, 2015.

[20] M. Masalha, I. Borovok, R. Schreiber, Y. Aharonowitz, and G. Cohen, "Analysis of transcription of the Staphylococcus aureus aerobic class ib and anaerobic class III ribonucleotide reductase genes in response to oxygen," *Journal of Bacteriology*, vol. 183, no. 24, pp. 7260–7272, 2001.

[21] J. Kluytmans, A. van Belkum, and H. Verbrugh, "Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks," *Clinical Microbiology Reviews*, vol. 10, no. 3, pp. 505–520, 1997.

[22] A. C. Senok, H. Verstraelen, M. Temmerman, and G. A. Botta, "Probiotics for the treatment of bacterial vaginosis," *Cochrane Database of Systematic Reviews*, vol. 4, Article ID CD006289, 2009.

[23] S. Y. C. Tong, J. S. Davis, E. Eichenberger, T. L. Holland, and V. G. Fowler, "Staphylococcus aureus infections epidemiology

pathophysiology clinical manifestations and management," *Clinical Microbiology Reviews*, vol. 623, 2015.

[24] X. Zhen, C. S. Lundborg, X. Sun, X. Hu, and H. Dong, "Economic burden of antibiotic resistance in ESKAPE organisms," *Systematic Reviews*, vol. 7, 2019.

[25] Clsi, *CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing*, Clsi, Wayne, PA, USA, 30th edition, 2020.

[26] S. Y. Park, J. S. You, S. Y. Moon, J. S. Oh, H. I. Choi, and G. W. Jung, "A literature review of infection with ESKAPE pathogens in oral and maxillofacial region," *Journal of Oral Medicine and Pain*, vol. 46, no. 3, pp. 75–83, 2021.

[27] M. S. Mulani, E. E. Kamble, S. N. Kumkar, M. S. Tawre, and K. R. Pardesi, "Emerging strategies to combat eskape pathogens in the era of antimicrobial resistance: a review," *Frontiers in Microbiology*, vol. 10, no. APR, p. 539, 2019.

[28] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, 1983.

[29] G. Xiong, Z. Wu, J. Yi et al., "ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties," *Nucleic Acids Research*, vol. 49, pp. W5–W14, 2021.

[30] Admet, "ADMETlab 2.0," 2020, https://admetmesh.scbdd.com/service/evaluation/cal.