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Syntheses and Antimycobacterial Activities of [(2*S*,3*R*)-2-(Amino)-4-(Arenesulfonamido)-3-Hydroxy-1-Phenylbutane Derivatives

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Abstract: The syntheses of hydroxyethylsulfonamides, (2*S*,3*R*)-*tert*-butyl *N*-[4-(*N*-benzyl-4-R-phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl]carbamates and (**5**) (2*S*,3*R*)-2-amino-4-[*N*-benzyl-4-R-benzenesulfonamido]-3-hydroxy-1-phenylbutane hydrochlorides (**6**), derived from (2*S*,3*S*)-*Boc*-phenylalanine epoxide, are reported. None of the compounds, containing the *Boc* group, showed activity against *M. tuberculosis* ATTC 27294, while compounds **6** did, with the most active compounds having R = *p*-Cl, *p*-Br and *p*-Me. Results indicate that the presence of a free amino group at C2 and the sulphonamide moiety are important for biological activity. The antimycobacterial activity of compounds **6** correlated well with the calculated lipophilicities, but not with the electronic effects of the substituents, R. All compounds **6** were highly cytotoxic against the hepatoma cell lineage Hep G2 A16. The X-ray crystal structure of compound [(**6**: R = Me).H_2O] is also reported. In the propeller-like conformation adopted by the cation, the amino and hydroxy groups have a *cis* arrangement, and thus are suitably placed to form 5- membered chelates.

Keywords: Antimycobacterial activity, cytotoxicity, hydroxyethylamine derivatives, sulfonamide derivatives, x-ray crystallography.

1. INTRODUCTION

Tuberculosis (TB), an infectious bacterial disease caused by *Mycobacterium tuberculosis*, is a current worldwide problem mainly due to the increasing number of people infected with the HIV virus [1, 2]. In 2011, there were 1.1 million new cases of TB among people living with HIV. In the same year, some 430,000 people with HIV died due to TB [3]. Simultaneous tuberculosis and anti-retroviral treatment is complicated due to overlapping side effects of anti-tuberculosis drugs and anti-retroviral drugs, and the drug-drug interactions [4]. Thus, there is an urgent need to develop new drugs to combat dual TB and HIV infections.

Etambutol, see (Fig. **1**), is an amino-alcohol used in the first line treatment of (TB) [5]. Aminoalcohol derivatives are present in widely-used HIV protease inhibitors [6-10] providing further indications of their potential as anti-TB-HIV co-infection agents. *β*-Aminoalcohol derivatives thus appear a promising class of compounds to investigate as anti-TB-HIV agents and consequently we have studied the anti-tubercular activities of a number of 2-hydroxyethylamine derivatives [12-18].

Sulfonamides are widely used in the treatment of various bacterial infections. The sulfonamide moiety remains an important function in the development of novel antibacterial prototypes [19, 20]. Recently, cobalt (II) sulfonamide complexes were evaluated against *M. tuberculosis* by Mondelli *et al* but, in this case, the substances displayed low antimycobaterial activities. According to the authors, this was due to the low lipophilicity of the synthesized complexes, which would make penetration into the hydrophobic mycobacterium cell wall more difficult [21].

Following on from these studies, we now wish to report the synthesis and *in vitro* activity against *Mycobacterium tuberculosis* of series of sulfonamides derived from (2*S*,3*S*)-*Boc*-phenylalanine epoxide (Schemes **1** and **2**).

2. RESULTS AND DISCUSSION

2.1. Synthesis

Pure stereoisomer (*S,S*)-**1** is commercially available, however it can be prepared according to the method of Beau-

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1573-4064/13 $58.00+.00

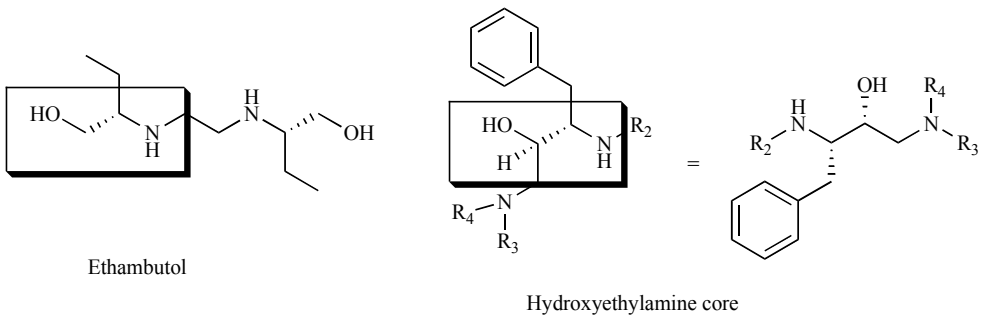
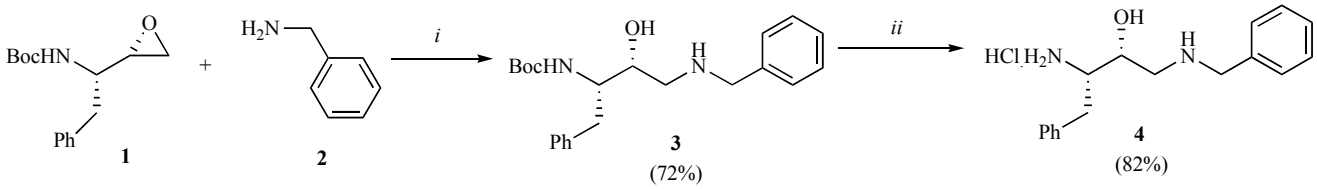
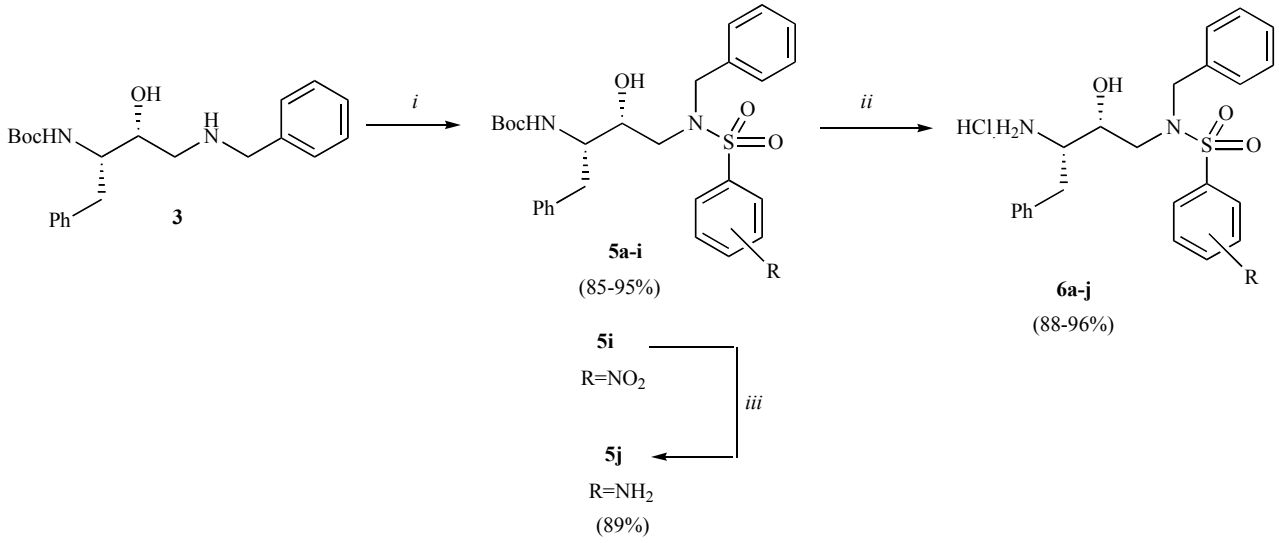


Fig. (1). Etambutol and hydroxyethylamine-based structures.



Scheme 1. Reaction and conditions: *i*: isopropanol, reflux, 16 h; *ii*: HCl gas, EtOH, r.t., 4h.



Scheme 2. Reaction and conditions: *i*: Et_3N, DMF, $RC_6H_4SO_2Cl$, CH_2Cl_2, r.t., 4 h; *ii*: HCl gas, EtOH, r.t., 4h, *iii*: H_2, Pd/C 10%, EtOH, r.t., 16 h.

lieu [22]. Compounds, **3**, **4**, **5a-j** and **6a-j**, were prepared as outlined in (Schemes **1** and **2**). The selective ring-opening of the (2*S*,3*S*)-*Boc*-phenylalanine epoxide **1** with benzylamine **2** in refluxing isopropanol afforded the chiral hydroxyethylamine intermediate **3**, which on treatment with gaseous hydrogen chloride in ethanol produced the hydrochloride **4** in quantitative yield (Scheme **1**). The intermediate **3** on reaction with arenesulfonyl chlorides in CH_2Cl_2 solution, containing excess triethylamine and a catalytic amount of DMF, at room temperature produced the hydroxyethylsulfonamides **5a-i** in good yields. Compound **5j** was prepared by reduction of the nitro derivative **5i** using H_2 and Pd/C (10%) in EtOH. Finally, compounds **5a-j** on reaction with gaseous hydrogen chloride in ethanol afforded the hydroxyethylamine hydrochlorides **6a-j** (Scheme **2**).

All the compounds were characterized by ^{1}H NMR and ^{13}C NMR spectral data, see (Table **2**), and specifically for **6e** by X-ray crystallography. Elucidation of the NMR spectra was aided by the use of HMBQ, HMQC and COSY techniques. Generally in the ^{1}H NMR spectra, the H1 protons were more shielded than the H4 protons, all appearing as double-doublets, while the two multiplets in the range of 3.59 - 3.37 ppm were assigned to H2 and H3 protons. The ^{13}C NMR spectra exhibited CH signals at 72 ppm for C3 (C-OH) and at 56-60 ppm for C2 (C-NH). The CH_2 signals for C4 and C1 appeared in the ranges 49-55 ppm and 35-38 ppm, respectively.

2.2. Antimycobacterial Activity

The antimycobacterial activities of compounds **3**, **4**, **5a-j** and **6a-j** were assessed against *M. tuberculosis* ATTC 27294, using the micro plate Alamar Blue assay (MABA) [23]. Compounds **3-5** were inactive at the tested concentrations. Compounds **6** exhibited moderate to good activities (Table **1**). Results suggest that both the presence of a free amino group at C2 and the sulfonamide moiety are important

Table 1. The *in vitro* activity of compounds 6a-h against the *Mycobacterium tuberculosis* H37Rv strain. Minimal Inhibitory Concentrations (MIC) and Minimal Lethal Doses (MLD_{50}).

Compound	R	MIC (μM)	MLD_{50} (μM)	TI*	miLogP
6a	4 -H	**111.86**	**237.14 ± 145.42**	2.12	**2.67**
6b	4-Cl	**51.93**	**< 33.23**	< 1	**3.35**
6c	4-Br	**47.54**	**41.83 ± 16.16**	< 1	**3.48**
6d	4-F	**107.53**	**45.16 ± 9.03**	< 1	**2.83**
6e	4-CH_3	**54.23**	**46.63 ± 19.96**	< 1	**3.12**
6f	4-OCH_3	**104.82**	**64.99 ± 20.75**	< 1	**2.72**
6g	4-NO_2	**101.63**	**< 32.52**	< 1	**2.63**
6h	4-NH_2	**216.45**	**309.52 ± 121.21**	1.43	**1.74**
6i	2-Br	**47.54**	**< 31.6**	< 1	**3.48**
6j	3-Br	**47.54**	**-**	-	**3.48**
ethambutol	-	77.82	> 48945.23	> 600	0.35

*TI, therapeutic index based on MLD_{50}/MIC ratios.

Table 2. Hydrogen-bond Parameters (Å, º)

a. Intra-cation hydrogen-bonds.

D—H···A	D—H	H···A	D···A	D—H···A
C(1)—H(1A)··O(3)	0.97	2.50	2.841(9)	101
C(4)—H(4A)··O(2)	0.97	2.31	2.820(9)	112
C(23)—H(23)··O(1)	0.93	2.55	2.903(9)	103

b. Inter-species hydrogen-bonds.

N(2)—HN(1)··Cl(1)^{i}	0.96(7)	2.30(7)	3.240(7)	166(6)
N(2)—HN(2)··O(3)	0.94(8)	2.51(8)	2.825(9)	100(5)
N(2)—HN(2)··Cl(1)^{ii}	0.94(8)	2.35(7)	3.192(7)	148(6)
O(3)—H(3)··Cl(1)	0.82	2.23	3.048(6)	173
N(2)—HN(3)··OW^{iii}	0.84(8)	2.03(8)	2.796(9)	153(7)
OW—HW(1)··Cl(1)	0.93(10)	2.20(10)	3.111(7)	170(9)
O(W)—H(W2)·· ?	1.06(11)			

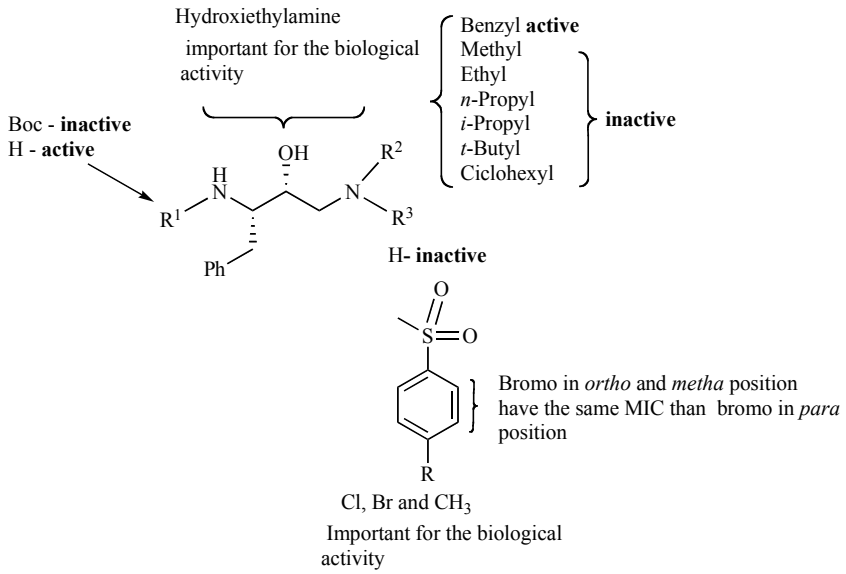
i = x,-1+y,z ; ii = 2-x,-1/2+y,2-z; iii = 2-x,-3/2+y,2-z.

C—H···Cg	H···Cg	H_{erp}	γ	C—H···Cg	C···Cg	C-H, π
C(8)-H(8)···Cg(1^{i})	2.74	2.72	7.14	141	3.511(9)	55
C(14)-H(14)···Cg(2^{ii})	2.68	2.66	6.59	136	3.416(8)	51
C(20)-H(20)···Cg(3^{iii})	2.89	2.86	7.08	130	3.553(8)	47

Cg(1) - Cg(3) are centoids of rings defined by [C6-C11], [C12-C17] and [C18-C23], respectively.
Symmetry operations: i = 2-x,1/2+y,1-z; ; ii = 1-x,-1/2+y,2-z ; iii = 1-x,1/2+y,1-z.

for the biological activity. Compounds **6b** (MIC = 51.93 μM), **6c, 61** and **6j** (all having MIC = 47.54 μM) as well as **6e** (MIC = 51.93 μM) displayed better *in vitro* activities then did the standard drug etambutol (MIC = 77.82 μM). The best results were obtained for compounds having bromo substituents. The same activity of the three compounds, **6c, 61** and **6j,** indicated the importance of the substituent rather than its position in the benzenesulfonamide group.

There is no correlation between the activities and electronic effects of the substituents in **6.** However, there does appear to be a correlation between the antimycobacterial activity and the lipophilicity of the compounds, with the bet-



Scheme 3. The SAR of hydroxyethylamine.

ter activity resulting from substances having larger log p values [24]. This result can be explained by the cell wall of the *M. tuberculosis* being rich in lipids. The SAR of this class of compound is summarized in (Scheme **3**).

2.3. Cytotoxicity

Unfortunately, the cytotoxicities determined using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and the hepatoma cell lineage Hep G2 A16 [25], indicated that compounds **6** were not suitable drug candidates, see (Table **1**). Cytotoxicity was evaluated from MTT-derived colorimetric values used to generate dose–response curves. The minimum lethal dose (MLD_{50}) based upon cell growth in drug-free controls was estimated by curve fitting using Microcal Origin Software (Northampton, MA, USA) [26]. The graphs for etambutol and compound **6h** are shown in (Fig. **2**). The therapeutic index (TI), calculated based on MLD_{50}/MIC ratios, see (Table **1**), clearly showed that the control drug, etambutol, exhibited a much higher TI value than did any of the compounds **6**.

2.4. Crystal Structure of [(6e).(H_2O)]

Recrystallization of **6e** from moist ethanol solution led to the isolation of good crystals of **[(6e).(H_2O)]**. (Fig. **3**) shows the atom arrangements and numbering scheme used for the hydrated salt. The asymmetric unit comprises a single cation, [(2*S*,3*R*)-2-(ammonio)-[4-(*N*-benzyl-4-arenesulfonamido)-3-hydroxy-1-phenylbutane]$^{+}$, a chloride ion and a water molecule. The stereochemistry of the cation was (*2S*,*3R*) as expected from the nucleophilic ring opening of the initial reagent, (2*S*,3*S*)-*Boc*-phenylalanine epoxide. All bond lengths and angles are in the expected regions and are not discussed further.

In the conformation determined for the cation in the solid state, the phenyl ring, C6-C11, is practically orthogonal to the C18-C23 phenyl ring and is also near orthogonal to the C12-C17 ring as shown by the angles between the two best planes of 89.4(3) and 80.4(2) ^{o}, respectively. One strong, N(2)-HN(2)---O(3), and three weak C-H---O intramolecular hydrogen bonds are present in the cation, see (Table **3**). It is apparent that the amino and hydroxyl groups have a *cis* arrangement, with a torsional angle, N(2)-C(2)-C(3)-O(3), of -66.8(7)^{o}, and thus are suitably sited to form chelates with appropriate centres. If this feature is maintained in solution, it could be of importance for biological activity. In the non-active compound, (*2S*,*3R*)-*tert*-butyl *N*-[4-(*N*-benzyl-4-fluoro-benzenesulfonamido)-3-hydroxy-1-phenylbutan-2-yl]carbamate, **5f**, the corresponding N-C-C-O dihedral angle was calculated to be 170.47(18) ^{0}, and hence a *trans* arrangement is present [27]. A comparison of the conformations adopted by the cation of [(**6e**).H_2O] and **5f** is shown in (Fig. **4**).

The cations are directly linked by weak C-H---π interactions, parameters of which are listed in (Table **2**), and indirectly via hydrogen bonds involving both water and chloride ions, see (Table **2**) for details. Each chloride ion is linked to three cations [*via* hydrogen bonds to H_3N centers in two cations and *via* OH in another] and to a water molecule. For each water molecule, there are strong (cation)N-H---OW and OW- H(W1)----Cl hydrogen bonds. The closest contacts of the other bond, OW-H(W2), are to a chloride ion, with a HW2---Cl separation of 2.910 Å and a OW- H(W2)----π type interaction with the C12-C17 phenyl ring, with a HW2---Cg separation of 3.484 Å and a OW-HW2---Cg angle of 67.65 ^{o} Such values suggest that these are weak interactions at best.

Overall, a three dimensional array is generated from the components of **[(6e).(H_2O)]**.

3. EXPERIMENTAL

3.1. Materials and Methods

Unless otherwise indicated, reagents and solvents were used as obtained from commercial suppliers without further purification. All melting points were determined on a Buchi Melting Point B-545 and are uncorrected. ^{1}H and ^{13}C NMR

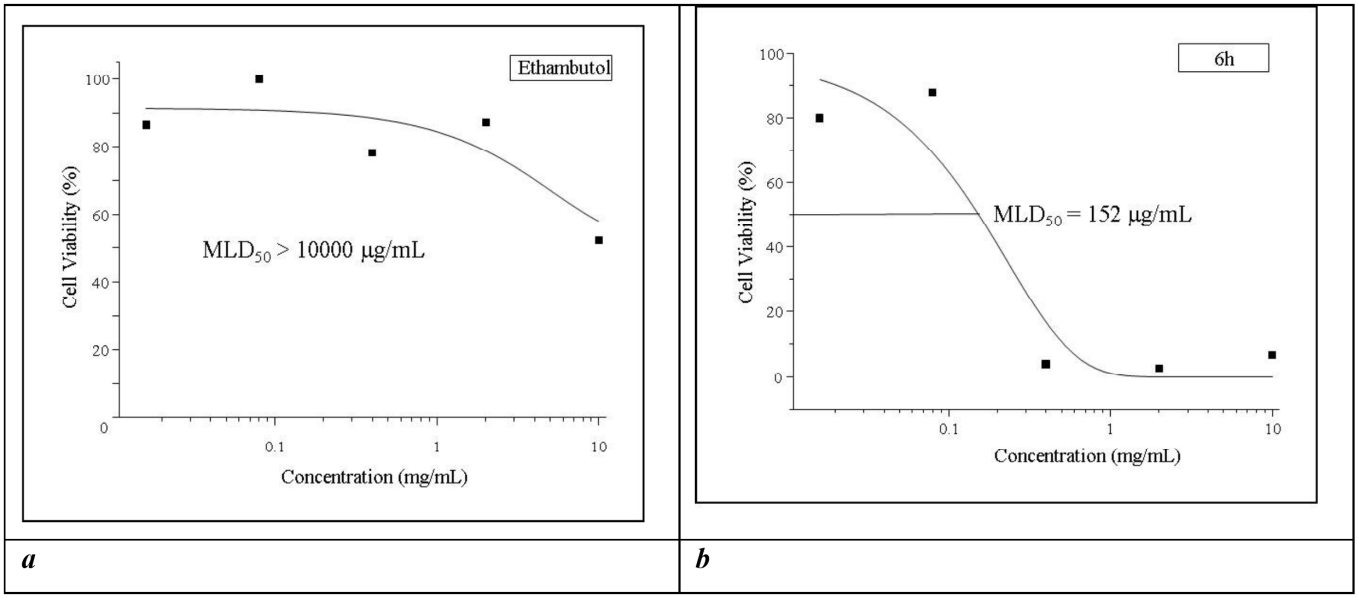


Fig. (2). Minimal lethal doses (MLD_{50}) of (***a***) etambutol and (***b***) compound **6h** on hepatoma HepG2 cells. Curves were obtained using Microcal Origin Software.

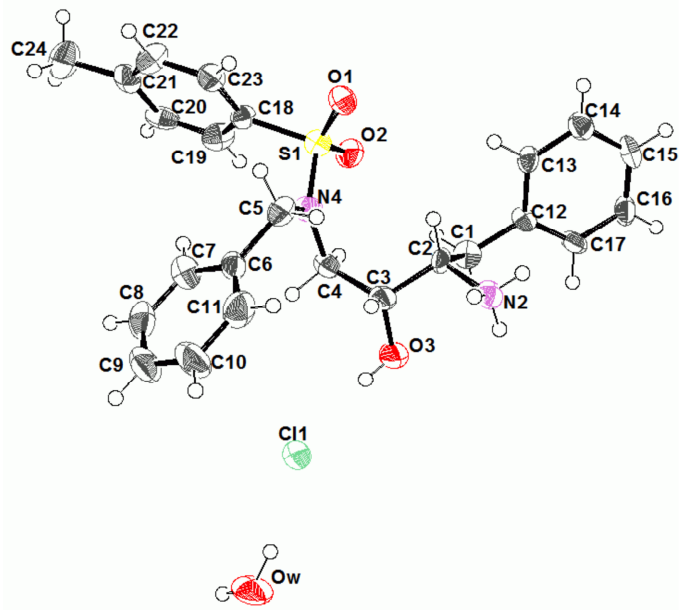


Fig. (3). Atom arrangements and numbering scheme [**6e**).(H_2O)]. Probability ellipsoids are drawn at the 50% level.

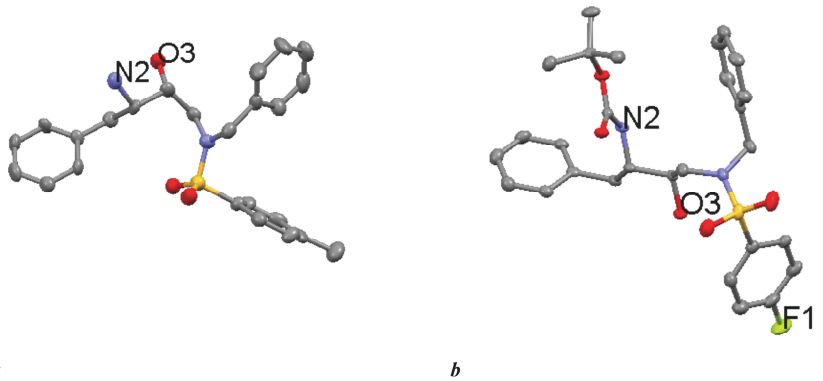
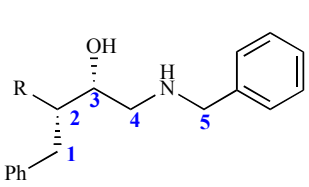
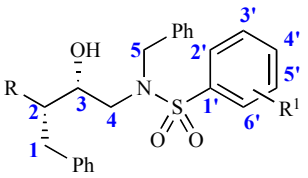


Fig. (4). Conformations adopted in the solid state by (a) the cation of active [(**6**: X = Me).H_2O], and (b) by inactive compound **5f** [21]. Hydrogen atoms have been omitted for clarity.



3: R = NHBoc
4: R= NH_2 . HCl



5: R = NHBoc
6: R= NH_2 . HCl

Fig. (5). Synthesized compounds.

Table 3. Crystal Data and Structure Refinement Details for [(6e).H_2O].

Identification code	
Empirical formula	$C_{24}H_{31}ClN_2O_4S$
Formula weight	479.02
Temperature, K	120(2)
Wavelength, Å	0.71073
Crystal system, space group	P21
Unit cell dimensions *a*, Å *b*, Å *c*, Å *β*, °	 13.855(2) 5.8473(10) 15.323(3) 91.856(8)
Volume, $Å^3$	1240.84)
Z	2
Density (calculated), Mg/m^3	1.282
Absorption coefficient, mm^-	0.270
F(000)	508
Crystal size, mm	0.4 x 0.03 x 0. 02
Theta range for data collection, °	2.94 to 25.00
Index ranges	-16 ≤ h ≤ 16 -6 ≤ k ≤ 6 -18 ≤ l ≤18
Reflections collected	7924
Independent reflections	4038 [R(int) = 0.0734]
Reflections observed (>2sigma)	2815
Data Completeness	0.985
Max. and min. transmission	0.669152 and 0.669152
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4038 / 1 / 306
Goodness-of-fit on F@2	1.174
Final R indices [I>2sigma(I)]	R_1= 0.086; wR_2 = 0.143
R indices (all data)	R_1= 0.140; wR_2 = 0.165
Largest diff. peak and hole, e. $Å^{-3}$	0.335 and -0.372

spectra were recorded using a Bruker DRX 400 spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) in DMSO-d_6 or in MeOD or in $CDCl_3$ with TMS as the internal standard. The low resolution Mass Spectrometry analyses were performed on an LC/MS micromass ZMD, using electrospray ionization in positive ion mode. Samples were introduced by the standard direct insertion probe method. The NMR numbering scheme is given in (Fig. **5**).

3.2. Preparation of *Tert*-butyl (2*S*,3*R*)-4-(Benzylamino)-3-hydroxy-1-phenylbutan-2-ylcarbamate (3)

Epoxide **1** (1.6 mmol) and benzylamine **2** (1.5 mmol) were dissolved in isopropanol (10 mL) and stirred under reflux for 16 h. The reaction mixture was rotary evaporated and the crude product was purified by recrystallization in methanol/water (7:3).

White solid, yield 72%; mp 130−132 °C.

^{1}H NMR (DMSO-d_6; 400 MHz): δ: 7.37-7.12 (m, 10H, Ph); 6.66 (d, 1H, $J_{H,H}$ = 7.2, NH); 6.58 (d, 1H, $J_{H,H}$ = 7.2, NH); 4.80-4.76 (m, 1H, H3); 3.75-3.69 (m, 1H, H2); 2.97 (dd, 1H, $J_{H,H}$ = 11.0; ^2J = 2.8, H4a); 2.63-2.46 (m, 1H, H4b); 2.59 (dd, 1H, $J_{H,H}$ = 9.6, $^2J_{H,H}$ = 2.4, H1a); 2.41-2.35 (m, 1H, H1b), 1.23 (s, 9H, $C(CH_3)_3$)

^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.2; 140.9; 139.8; 139.7; 129.3; 129.2; 129.1; 128.1; 127.9; 126.5; 125.6 (Ph); 77.3 ($C(CH_3)_3$); 71.9 (C3); 55.1 (C2); 53.1 (C4); 51.9 (C5); 36.1 (C1); 28.2 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3356 (OH); 1681 (C=O); 1523(NH carbamate); 1249 (C-N amine); 1172 (C-O alcohol)

MS: 307.8 [M^+], 100%.

3.3. Preparation of (2*S*,3*R*)-2-ammonio-4-benzylamino-3-hydroxy-1-phenylbutane Hydrochloride (4)

A solution of **3** (3.0mmol) in ethanol was treated with gaseous hydrogen chloride. Volatiles were removed by evaporation and the crude product was crystallized with diethyl ether.

Hygroscopic solid, yield 82%.

^{1}H NMR (DMSO-d_6; 400 MHz): δ: 8.31 (s, 2H, NH_2); 7.56-7.50 (m, 2H, Ph); 7.42-7.40 (m, 3H, Ph); 7.32-7.23 (m, 5H, Ph); 6.32 (d, 1H, $J_{H,H}$ = 4.8, NH); 4.22 (br, 1H, H3); 4.12 (s, 2H, H5); 3.53 (br, 1H, H2); 3.07 (d, 1H, J = 12.0, H4a), 2.91-2.82 (m, 3H, H4b, H1a and H1b).

^{13}C NMR (DMSO-d_6, 100 MHz) δ 136.3; 131.5; 130.3; 129.4; 128.9; 128.6; 126.8 (Ph); 65.9 (C3); 54.9 (C2); 50.1 (C4); 48.1 (C5); 33.1 (C1).

IR (KBr, ν = cm^{-1}): 3263 (OH).

MS: 299.3 [M^+], 100%.

3.4. General Procedure for the Preparation of (2*S*,3*R*)-*tert*-butyl *N*-[4-(*N*-benzyl-4-R-phenylsulfonamido)-3-hydroxy-1-phenylbutan-2-yl]carbamates (5)

To a solution of **3** (1.5 mmol) in CH_2Cl_2 (10 mL) was successively added triethylamine (2.2 mmol) and DMF (0.2 mmol). The mixture was stirred for 30 min under nitrogen before the portion-wise addition of an arenesulfonyl chloride (2.0 mmol). The reaction mixture was stirred for 8 h, and then treated successively with 5% aqueous hydrochloric acid, water and brine, before being dried over $MgSO_4$. The solvent was removed under vacuum and the products **5a-i** were recrystallized from hexane.

Compound (5a: R = H): yield, 89%; m.p. 124-126 °C.

^{1}H NMR (DMSO-d_6; 400 MHz): δ: 7.65 (s, 4H, Ph); 7.26-7.20 (m, 9H, Ph); 7.11 (d, 1H, $J_{H,H}$ = 6.8, Ph); 4.45 (d, 1H, $J_{H,H}$ = 14.4, H5a); 4.25 (d, 1H, $J_{H,H}$ = 14.4, H5b); 4.20 (d, 1H, $J_{H,H}$ = 7.6, NH); 3.63-3.59 (m, 2H, H3 and H2); 3.38 (br, 1H, OH); 3.22-3.17 (m, 1H, H4a); 3.14 (dd, 1H, $J_{H,H}$ = 14.8, $^2J_{H,H}$ = 8.4, H4b); 2.79 (dd, 1H, $J_{H,H}$ = 13.8; $^2J_{H,H}$ = 5.2, H1a); 2.76-2.72 (m, 1H, H1b); 1.34 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 140.0; 139.4; 136.7; 132.6; 129.2; 129.1; 128.2; 128.0; 127.8; 127.2; 126.9 (Ph); 77.4 ($C(CH_3)_3$); 72.0 (C3); 54.9 (C2); 51.4 (C5); 50.5 (C4); 35.2 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3556 and 3389 (OH); 1681 (C=O); 1327 and 1151 (S=O).

MS: 553.4 $[M + Na]^+$, 100%.

Compound (5b: R = Cl): yield, 93%; m.p. 144-146 °C.

^{1}H NMR (DMSO-d_6; 400 MHz): δ: 7.73 (d, 2H, $J_{H,H}$ = 8.5, Ph); 7.48 (d, 2H, $J_{H,H}$ = 8.5, Ph); 7.27-7.20 (m, 8H, Ph); 7.11 (d, 2H, $J_{H,H}$ = 7.5, Ph); 4.45 (d, 1H, $J_{H,H}$ = 14.5, H5a); 4.26 (d, 1H, $J_{H,H}$ = 14.5, H5b); 4.21 (d, 1H, $J_{H,H}$ = 7.0, NH); 3.62 (br, 1H, H3); 3.57 (br, 1H, H2); 3.38 (br,1H,OH); 3.21-3.10 (m, 2H, H4a and H4b); 2.81 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 5.0, H1a); 2.77-2.73 (m, 1H, $J_{H,H}$ = H1b); 1.34 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 139.4; 139.1; 137.7; 136.5; 129.2; 129.1; 128.9; 128.3; 128.0; 127.8; 127.3; 125.6; 77.4 ($C(CH_3)_3$); 71.5 (C3); 54.9 (C2); 51.1 (C5); 50.4 (C4); 35.2 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3560 and 3388 (OH); 1681 (C=O); 1333 and 1153 (S=O); 825 (C-Cl).

MS: 567.3 $[M + Na]^+$, 100% and 569.4, 42%.

Compound (5c: R = Br): yield, 90%; m.p. 161-163 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 7.77 (s, 4H, Ph'); 7.29-7.12 (m, 10H, Ph); 6.60 (d, 1H, $J_{H,H}$ = 8.8, NH); 4.98 (d, 1H, $J_{H,H}$ = 5.8, OH); 4.58 (d, 1H, $J_{H,H}$ = 15.6, H5a); 4.39 (d, 1H, $J_{H,H}$ = 15.6, H5b); 3.49-3.39 (m, 3H, H3, H2 and H4a); 3.06-2.86 (m, 2H, H4b and H1a); 2.49-2.38 (m, 1H, H1b); 1.21 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 139.5 (C4'); 139.4; 132.2; 129.1; 129.0; 128.3; 128.0; 127.8; 127.3 (Ph); 126.3 (C4'); 125.6 (Ph); 77.4 ($C(CH_3)_3$); 71.5 (C3); 54.9 (C2); 51.1 (C5); 50.4 (C4); 35.3 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3560 and 3383 (OH); 1681 (C=O); 1333 and 1151 (S=O); 723 (C-Br).

MS: 611.2 $[M + Na]^+$, 96% and 6.13,2, 100%.

Compound (5d: R = F): yield, 88%; m.p. 145-147 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 7.92-7.88 (m, 2H, Ph); 7.43-7.38 (m, 2H, Ph); 7.32-7.25 (m, 5H, Ph); 7.23-7.19 (m, 2H, Ph); 7.15-7.11 (m, 3H, Ph); 6.60 (d, 1H, $J_{H,H}$ = 8.8, NH); 4.97 (d, 1H, $J_{H,H}$ = 6.0, OH); 4.57 (d, 1H, $J_{H,H}$ = 15.6, H5a); 4.41 (d, 1H, $J_{H,H}$ = 15.6, H5b); 3.51-3.45 (m, 2H, H3 and H2); 3.38-3.32 (m, 1H, H4a); 3.00 (dd, 1H, $J_{H,H}$ = 14.8, $^2J_{H,H}$ = 8.8, H4b); 2.90 (dd, 1H, $J_{H,H}$ = 14.4, $^2J_{H,H}$ = 2.8, H1a); 2.45 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 10.4, H1b); 1.21 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 164.2 (d, $J_{C,F}$ = 254.6, C4'); 155.2 (C=O); 139.4; 136.6 (d, $^4J_{C,F}$ = 2.6, C1'); 136.5; 129.9 (d, $^3J_{C,F}$ = 9.4, C2' and C6'); 129.0; 128.2; 128.0; 127.8; 127.2; 125.6; 116.2 (d, $^2J_{C,F}$ = 22.4, C3' and C5'); 77.4 ($C(CH_3)_3$); 71.7 (C3); 54.9 (C2); 51.2 (C5); 50.4 (C4); 35.2 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3560 and 3383 (OH); 1681 (C=O); 1332 and 1147 (S=O); 833 (C-F).

MS: 551.0 $[M + Na]^+$, 100%.

Compound (5e: R = Me): yield, 95%; m.p. 155-157 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 7.70 (d, 2H, $J_{H,H}$ = 8.3, H2' and H6'); 7.31 (d, 2H, $J_{H,H}$ = 8.3, H3' and H5'); 7.26-7.18 (m, 8H, Ph); 7.11 (d, 2H, $J_{H,H}$ = 7.0, Ph); 4.45 (d, 1H, $J_{H,H}$ = 14.0, H5a); 4.18 (d, 1H, $J_{H,H}$ = 15.5, H5b); 4.14 (br, 1H, NH); 3.60-3.56 (m, H2 and H3); 3.32 (br, 1H, OH); 3.15-3.12 (m, H4a and H4b); 2.81 (dd, 1H, $J_{H,H}$ = 13.7, $^2J_{H,H}$ = 5.0, H1a); 2.77-2.73 (m, 1H, H1b); 2.44 (s, 3H, CH_3); 1.33 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 142.9 (C4'); 139.4; 137.1; 136.9; 129.7; 129.1; 128.2; 128.0; 127.8; 127.2; 127.0; 125.6 (Ph); 77.4 ($C(CH_3)_3$); 72.0 (C3); 54.9 (C2); 51.5 (C5); 50.7 (C4); 35.1 (C1); 28.9 ($C(CH_3)_3$); 20.9 (CH_3).

IR (KBr, ν = cm^{-1}): 3556 and 3387 (OH); 1681 (C=O); 1328 and 1147 (S=O).

MS: 547.3 $[M+Na]^+$, 42%.

Compound (5f: R = MeO): yield, 85%; m.p. 158-160 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 7.78 (d, 2H, $J_{H,H}$ = 8.5, H2' and H6'); 7.32-7.20 (m, 8H, Ph); 7.16-7.08 (m, 4H, Ph); 6.60 (d, 2H, $J_{H,H}$ = 8.8, NH); 4.94 (d, 1H, $J_{H,H}$ = 6.0, OH); 4.50 (d, 1H, $J_{H,H}$ = 15.6, H5a); 4.37 (d, 1H, $J_{H,H}$ = 15.6, H5b); 3.85 (s, 3H, OCH_3); 3.50-3.47 (m, 2H, H3 and H2); 3.39-3.34 (m, 1H, H4a); 2.94-2.89 (m, 2H, H4b and H1a); 2.45 (dd, 1H, $J_{H,H}$ = 13.8, $^2J_{H,H}$ = 10.4, H1b); 1.22 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 162.3 (C4'); 155.2 (C=O); 139.5; 136.9; 131.6 (C1'); 129.2; 129.1; 128.2; 128.0; 127.8; 127.2; 125.6; 114.3 (C3' and C5'); 77.4

($C(CH_3)_3$); 72.0 (C3); 55.6 (OCH_3); 54.8 (C2); 51.4 (C5); 50.7 (C4); 35.1 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3560 and 3389 (OH); 1680 (C=O); 1327 and 1147 (S=O).

MS: 563.3 $[M + Na]^+$, 100%.

Compound (5g: R = 2-Br): yield, 86%; Hygroscopic.

^{1}H NMR ($CDCl_3$, 500 MHz): δ: 8.12 (d, 1H, $J_{H,H}$ = 6.5, H6'); 7.75 (d, 1H, $J_{H,H}$ = 7.0, H3'); 7.44 (t, 1H, $J_{H,H}$ = 7.5, H5'); 7.41-7.38 (m, 1H, H4'); 7.26-7.24 (m, 8H, Ph); 7.09 (d, 2H, $J_{H,H}$ = 7.0, Ph); 4.66 (d, 1H, $J_{H,H}$ = 15.0, H5a); 4.57 (d, 1H, $J_{H,H}$ = 15.0, H5b); 4.25 (d, 1H, $J_{H,H}$ = 7.0, NH); 3.63 (br, 1H, H3); 3.58 (br, 1H, H2); 3.52 (s, 1H, OH); 3.41-3.37 (m,1H, H4a); 3.28 (dd, 1H, $J_{H,H}$ = 15.0, ^2J = 8.5, H4b); 2.75 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 4.5, H1a); 2.72-2.67 (m, 1H, H1b); 1.33 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.1 (C=O); 139.7; 139.3; 136.2; 135.4; 135.4; 133.9; 131.2; 128.9; 128.0; 127.9; 127.7; 127.4; 125.5 (Ph); 77.3 ($C(CH_3)_3$); 71.9 (C3); 54.9 (C2); 51.0 (C5); 49.5 (C4); 35.1 (C1); 28.0 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3396(OH), 1681 (C=O), 1334 and 1157 (S=O).

MS: 613.2 $[M + Na]^+$, 100% and 611.2, 98%.

Compound (5h: R = 3-Br): yield, 90%; m.p. 113-115 °C.

^{1}H NMR ($CDCl_3$, 500 MHz): δ: 7.93 (s, 1H, H2'); 7.73-7.71 (m, 2H, H4'and H6'); 7.38 (t, 1H, $J_{H,H}$ = 8.0H, H5'); 7.12 (d, 2H, $J_{H,H}$ = 7.0, Ph); 7.28-7.20 (m, 8H, Ph); 4.47 (d, 1H, $J_{H,H}$ = 14.5, H5a); 4.30 (d, 1H, $J_{H,H}$ = 14.5, H5b); 4.22 (d, 1H, $J_{H,H}$ = 8.0, NH); 3.62 (br, 2H, H3 and H2); 3.40 (br,1H, OH); 3.25-3.10 (m, 2H, H4a and H4b); 2.82 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 5.0, H1a); 2.78-2.74 (m, 1H, $J_{H,H}$ = H1b); 1.34 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 142.4 (C1'); 139.4; 136.3; 135.4; 131.3; 129.2; 129.1; 128.3; 128.1; 127.8; 127.4; 126.0; 125.6 (Ph); 77.4 ($C(CH_3)_3$); 71.7 (C3); 55.0 (C2); 51.1 (C5); 50.5 (C4); 35.4 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3560 and 3381(OH), 1681 (C=O), 1336 and 1155 (S=O).

MS: 613.3 $[M + 23]^+$, 100 and 611.3, 98%.

Compound (5i: R = NO_2): yield, 94%; m.p. 163-165 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 8.37 (d, 2H, $J_{H,H}$ = 9.0, H3' and H5'); 8.10 (d, 2H, $J_{H,H}$ = 9.0, H2' and H6'); 7.35-7.29 (m, 5H, Ph); 7.22-7.19 (m, 2H, Ph); 7.14-7.12 (m, 3H, Ph); 6.63 (d, 1H, $J_{H,H}$ = 9.0, NH); 5.00 (d, 1H, $J_{H,H}$ = 6.5, OH); 4.68 (d, 1H, $J_{H,H}$ = 15.5, H5a); 4.44 (d, 1H, $J_{H,H}$ = 15.5, H5b); 3.48-3.41 (m, 2H, H3 and H2); 3.36-3.34 (m, 1H, H4a); 3.10 (dd, 1H, $J_{H,H}$ = 15.0, $^2J_{H,H}$ = 9.0, H4b); 2.89 (dd, 1H, $J_{H,H}$ = 13.7, $^2J_{H,H}$ = 3.0, H1a); 2.42 (dd, 1H, $J_{H,H}$ = 13.5, $^2J_{H,H}$ = 11.0 H1b); 1.20 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 149.5 (C4'); 146.1 (C1'); 139.3; 136.1; 129.0; 128.5; 128.4; 128.1; 127.8; 127.5; 125.6; 124.3 (Ph); 77.5 ($C(CH_3)_3$); 71.2 (C3); 54.9 (C2); 50.9 (C5); 50.2 (C4); 35.2 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3574 and 3373 (OH); 1680 (C=O); 1336 and 1153 (S=O); 852 (C-NO_2)

MS 578.0 $[M + Na]^+$, 100%.

3.5. Preparation of Compound (5j: R = NH_2)

To a stirred solution of the nitro compound **5i** (0.57 mmol) in ethanol (15 mL) under nitrogen was added 10% palladium on activated charcoal (5 mg). The reaction mixture was evacuated, placed under a hydrogen atmosphere and stirred for 16 h. The reaction mixture was filtered through Celite and rotary evaporated. The brown oily residue was purified by chromatography on silica, eluting with 3:1 hexane/ethyl acetate.

Compound (5j: R = NH_2): yield, 89%; m.p. 93-95 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 7.58 (d, 2H, $J_{H,H}$ = 8.5, Ph); 7.26-7.18 (m, 8H, Ph), 7.11 (d, 2H, $J_{H,H}$ = 7.0, Ph); 6.67 (d, 2H, $J_{H,H}$ = 8.5, Ph); 4.41 (d, 1H, $J_{H,H}$ = 14.0, H5a); 4.21-4.11 (m, 2H, H2 and H3); 4.13 (d, 1H, $J_{H,H}$ = 14.0, H5b); 3.60 (br, 2H, H4a and H4b); 3.30 (br, 1H, OH); 3.12 (s, 2H, NH_2); 2.82 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 4.5, H1a); 2.76-2.72 (m, 1H, $J_{H,H}$ = H1b); 1.33 (s, 9H, $C(CH_3)_3$).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 155.2 (C=O); 152.8 (C4'); 139.6; 137.4; 129.3; 129.2; 129.0; 128.1; 128.0; 127.8; 125.6; 124.4; 112.7; 77.4 ($C(CH_3)_3$); 72.1 (C3); 54.8 (C2); 51.8 (C5); 50.9 (C4); 35.0 (C1); 28.1 ($C(CH_3)_3$).

IR (KBr, ν = cm^{-1}): 3558 and 3398 (OH); 1689 (C=O); 1367 and 1139 (S=O); 1137 (C-N amine).

MS: 548.4 $[M + Na]^+$, 100%.

3.6. General Procedure for the Preparation of (2*S*,3*R*)-2-amino-4-[*N*-benzyl-4-R-benzenesulfonamido]-3-hydroxy-1-phenylbutane Hydrochlorides (6)

A solution of **5** (3.0mmol) in ethanol was treated with gaseous hydrogen chloride. Volatiles were removed by evaporation and the crude product was crystallized from diethyl ether.

Compound (6a: R = H): yield, 92%; m.p. 113-115 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 8.03 (s, 2H, NH_2); 7.79 (d, 2H, $J_{H,H}$ = 7.2, Ph); 7.71-7.67 (m, 1H, Ph); 7.60-7.56 (m, 2H, Ph); 7.33-7.17 (m, 10H, Ph); 5.64 (d, 1H, $J_{H,H}$ = 5.2, OH); 4.46 (d, 1H, $J_{H,H}$ = 16.0, H5a); 4.41 (d, 1H, $J_{H,H}$ = 16.0, H5b); 3.98 (br, 1H, H3); 3.36-3.29 (m, 2H, H2 and H4a); 3.02 (dd, 1H, $J_{H,H}$ = 14.4, $^2J_{H,H}$ = 8.0, H4b); 2.88 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 6.0, H1a); 2.78 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 7.6, H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 139.5; 136.6; 136.3; 132.8; 129.3; 128.5; 128.3; 128.1; 127.3; 127.0; 126.7; 68.3 (C3); 55.4 (C2); 51.6 (C5); 49.3 (C4); 32.7 (C1).

IR (KBr, ν = cm^{-1}): 3600-3200 (OH); 1333 and 1157 (S=O).

MS: 411.4 $[M + H]^+$, 100%.

Found: C, 62.02; H, 6.03; N, 6.37. $[C_{23}H_{27}N_2O_3S]^+$ Cl^- requires C, 61.92; H, 6.10; N, 6.28%.

Compound (6b: R = Cl): yield, 96%; m.p. 164-166 °C.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 8.06 (br, 2H, NH_2); 7.80 (d, 2H, $J_{H,H}$ = 8.6, H3' e H5'); 7.63 (d, 2H, $J_{H,H}$ = 8.6, H2' and H6'); 7.34-7.21 (m, 10H, Ph); 5.65 (d, 1H, $J_{H,H}$ = 5.6, OH); 4.45 (s, 2H, H5); 3.95 (br, 1H, H3); 3.36 (br, 1H, H4a); 3.07 (dd, 1H, $J_{H,H}$ = 14.8, $^2J_{H,H}$ = 8.4, H4b); 2.87 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 8.0, H1a); 2.79 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 8.0, H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 138.4; 137.7; 136.5; 136.2; 129.3; 129.2; 129.0; 128.6; 128.4; 128.1; 127.5; 126.8 (Ph); 68.0 (C3); 55.2 (C2); 51.6 (C5); 49.3 (C4); 32.7 (C1).

IR (KBr, ν = cm^{-1}): 3600-3200 (OH); 1133 and 1155 (S=O).

MS: 445.3 $[M + H]^+$, 100% and 446.3, 41%.

Found: C, 57.29; H, 5.48; N, 5.92. $[C_{23}H_{26}ClN_2O_3S]^+$ Cl^- requires C, 57.48; H, 5.45; N, 5.83%.

Compound (6c: R = Br): yield, 90%; m.p. 184-186 °C.

^{1}H NMR (MeOD, 400 MHz): δ: 7.75-7.71 (m, 4H, Ph); 7.35-7.32 (m, 2H, Ph); 7.30-7.24 (m, 8H, Ph); 4.48 (d, 1H, $J_{H,H}$ = 15.0, H5a); 4.43 (d, 1H, $J_{H,H}$ = 15.0, H5b); 4.01-3.98 (m, 1H, H3); 3.51-3.48 (m, 1H, H2); 3.47 (dd, 1H, $J_{H,H}$ = 15.0, $^2J_{H,H}$ = 6.0, H4a); 3.12 (dd, 1H, $J_{H,H}$ = 14.7, $^2J_{H,H}$ = 7.5, H4b); 3.01 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 5.5, H1a); 2.75 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 9.5, H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 138.8 (C4'); 136.5; 136.2; 132.3; 129.3; 129.0; 128.6; 128.3; 128.0; 127.4; 126.8; 126.7 (Ph); 68.0 (C3); 55.2 (C2); 51.6 (C5); 49.3 (C4); 32.7 (C1).

IR (KBr, ν = cm^{-1}):3305 (OH); 1342 and 1151 (S=O).

MS: 488.9 $[M]^+$, 97% and 490.9, 100%.

Found: C, 52.65; H, 4.89; N, 5.47. $[C_{23}H_{26}BrN_2O_3S]^+$ Cl^- requires C, 52.82; H, 5.01; N, 5.35%.

Compound (6d: R = F): yield, 95%; m.p. 87-89 °C.

^{1}H NMR (MeOD, 400 MHz): δ: 7.90-7.87 (m, 2H, Ph); 7.36-7.25 (m, 12H, Ph); 4.48 (d, 1H, $J_{H,H}$ = 15.0, H5a); 4.43 (d, 1H, $J_{H,H}$ = 15.0, H5b); 4.03-4.01 (m, 1H, H3); 3.52-3.46 (m, 1H, H2); 3.48 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 6.0, H4a); 3,31 (br, 2H, NH_2); 3.13 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 7.5, H4b); 3.03 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 5.0, H1a); 2.77 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 9.5, H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 164.3 (d, $J_{C,F}$ = 250.0, C4'); 136.5; 136.2; 135.9 (d, $^4J_{C,F}$ = 2.8, C1'); 130.1 (d, $^3J_{C,F}$ = 9.4, C2' and C6'); 129.3; 128.5; 128.3; 128.0; 127.4; 126.8; 116.3 (d, $^2J_{C,F}$ = 22.4) (Ph); 68.1 (C3); 55.2 (C2); 51.6 (C5); 49.2 (C4); 32.6 (C1).

IR (KBr, ν = cm^{-1}): 3500-3200 (OH); 1333 and 1149 (S=O).

MS: 429.4 $[M]^+$,100%.

Found: C, 59.29; H, 5.75; N, 5.87. $[C_{23}H_{26}FN_2O_3S]^+$ Cl^- requires C, 59.41; H, 5.63; N, 6.02%.

Compound (6e: R = Me): yield, 95%; m.p. 121-123 °C.

^{1}H NMR (MeOD, 400 MHz): δ: 7.73 (d, 2H, $J_{H,H}$ = 8.0, H2' and H6'); 7.41 (d, 2H, $J_{H,H}$ = 8.0, H3' and H5'); 7.36-7.33 (m, 2H, Ph); 7.30-7.24 (m, 8H, Ph); 4.42 (d, 1H, $J_{H,H}$ = 15.0, H5a); 4.39 (d, 1H, $J_{H,H}$ = 15.0, H5b); 4.00 (dt, 1H, $J_{H,H}$ = 6.7, $^2J_{H,H}$ = 2.5, H3); 3.52-3.44 (m, 1H, H2); 3.46 (dd, 1H, $J_{H,H}$ = 14.7, $^2J_{H,H}$ = 6.5, H4a); 3.06 (dd, 1H, $J_{H,H}$ = 14.7, $^2J_{H,H}$ = 7.0, H4b); 3.02 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 5.0, H1a), 2.73 (dd, 1H, $J_{H,H}$ = 14.3, $^2J_{H,H}$ = 10.0; H1b) 2.45 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 143.2 (C4'); 136.5; 136.4; 136.3;129.8; 129.3; 128.6; 128.3; 128.1; 127.4; 127.1; 126.8 (Ph); 68.3 (C3); 55.1 (C2); 51.8 (C5); 49.5 (C4); 32.6 (C1); 21.0 (CH_3).

IR (KBr, ν = cm^{-1}): 3700-3200 (OH); 1334 and 1157 (S=O).

MS: 425.1 $[M]^+$, 100%.

Found: C, 62.41; H, 6.51; N, 5.97. $[C_{24}H_{29}N_2O_3S]^+$ Cl^- requires C, 62.52; H, 6.34; N, 6.07 %.

Compound (6f: R = MeO): yield, 88%; m.p. 131-133 °C.

^{1}H NMR (MeOD, 400 MHz): δ: 7.79 (d, 2H, $J_{H,H}$ = 9.0, Ph); 7.35-7.24 (m, 10H, Ph); 7.10 (d, 2H, $J_{H,H}$ = 8.5, Ph); 4.41 (d, 1H, $J_{H,H}$ =15.0, H5a); 4.37 (d, 1H, $J_{H,H}$ = 15.0; H5b); 4.00 (dt, 1H, $J_{H,H}$ = 6.5, $^2J_{H,H}$ = 2.0, H3); 3.89 (s, 3H, OCH_3); 3.50 (br, 1H, H2); 3.45 (dd, 1H, $J_{H,H}$ = 14.7, $^2J_{H,H}$ = 7.0, H4a); 3.05 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 7.0, H4b); 3.01 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 5.0, H1a); 2.73 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 9.5, H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 162.5 (C4'); 136.5; 130.8; 129.3; 128.5; 128.3; 128.0; 127.3; 126.7; 114.4 (Ph); 68.3 (C3); 56.0 (OCH_3); 55.7 (C2); 51.8 (C5); 49.5 (C4); 32.5 (C1).

IR (KBr, ν = cm^{-1}): 3315 (OH); 1328 and 1149 (S=O).

MS: 441.1 $[M]^+$, 100%.

Found: C, 60.32; H, 6.29; N, 5.73. $[C_{24}H_{29}N_2O_4S]^+$ Cl^- requires C, 60.43; H, 6.13; N, 5.87%.

Compound (6g: R = 2-Br): yield, 92%; Hygroscopic.

^{1}H NMR (DMSO-d_6, 400 MHz): δ: 8.05-8.03 (m, 3H, NH_2 and H6'); 7.89-7.84 (m, 1H, H3'); 7.58-7.54 (m, 2H, Ph, H5' and H4'); 7.31-7.20 (m, 8H, Ph); 7.11-7.08 (m, 2H, Ph); 5.74 (br, 1H, OH); 4.61 (d, 1H, $J_{H,H}$ = 16.0, H5a); 4.55 (d, 1H, $J_{H,H}$ = 16.0, H5b); 4.07 (br, 1H, H3); 3.37-3.33 (m, 1H, H2); 3.32 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 6.8, H4a); 3.13 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 8.0, H4b); 2.83 (dd, 1H, $J_{H,H}$ = 14.4, $^2J_{H,H}$ = 6.4, H1a); 2.75 (dd, 1H, $J_{H,H}$ = 14.4, $^2J_{H,H}$ = 8.0, H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 139.2; 136.5, 135.6, 135.5; 134.4; 131.6; 129.3; 128.6; 128.4; 128.3; 127.7; 126.8 (Ph); 68.1 (C3); 55.3 (C2); 50.7 (C5); 48.4 (C4); 32.5 (C1).

MS: 491.3 $[M + 1]^+$, 100% and 489.3, 98%.

Compound (6h: R = 3-Br): yield, 89%; Hygroscopic.

^{1}H NMR ($CDCl_3$, 500 MHz): δ: 7.99 (br, 2H, NH_2); 7.79 (s, 1H, H2'); 7.70 (d, 1H, $J_{H,H}$ = 8.0, H4'); 7,62 (d, 1H, $J_{H,H}$ = 8.0, H6'); 7.30 (t, 1H, $J_{H,H}$ = 8.0, H5'); 7.16-7.15 (m, 8H, Ph); 7.00 (d, 2H, $J_{H,H}$ = 6.5, Ph); 5.02 (s, 1H, NH); 4.40 (d, 1H, $J_{H,H}$ = 15.0, H5a); 4,34 (sl, 1H, H3); 4.30 (d, 1H, $J_{H,H}$ = 15.0, H5b); 3.77 (br, 1H, H3); 3.41 (br, 1H, H2); 3.18 (dd, 1H, $J_{H,H}$ = 14.0, $^2J_{H,H}$ = 4.5, H4a); 3.06 (dd, 1H, $J_{H,H}$ = 14.0,

$^2J_{H,H}$ = 8.0, H4b), 2.93 (dd, 1H, $J_{H,H}$ = 14.5, $^2J_{H,H}$ = 7.0, H1a); 2.19 (br, 3H, NH_2 and H1b).

^{13}C NMR (DMSO-d_6, 100 MHz): δ: 141.7 (C1'); 136.6; 136.0; 135.6; 131,4; 129.3; 129.2; 128.6; 128.3; 128.2; 127.6; 126.8; 126.1 (Ph); 68.1 (C3); 55.3 (C2); 51.5 (C5); 49.3 (C4); 32.8 (C1).

IR (KBr, ν = cm^{-1}): 3600-3400 (OH), 1333 and 1168 (S=O).

MS: 491.1 $[M + 1]^+$, 100% and 489.1, 98%.

Compound (6i: R = NO_2): yield, 94%; m.p. 200-201 °C.

^{1}H NMR ($CDCl_3$, 400 MHz): δ: 8.34 (d, 2H, $J_{H,H}$ = 8.6, H3' and H5'); 8.11 (br, 2H, NH_2); 8.06 (d, 2H, $J_{H,H}$ = 8.6, H2' and H6'); 7.31-7.24 (m, 10H, Ph); 5.67 (d, 1H, $J_{H,H}$ = 5.6, OH); 4.56 (d, 1H, $J_{H,H}$ = 16.0, H5a); 4.49 (d, 1H, $J_{H,H}$ = 16.0, H5b); 3.96 (br, 1H, H3); 3.41-3.36 (m, 2H, H2 and H4a); 3.16 (dd, 1H, $J_{H,H}$ = 14.8, $^2J_{H,H}$ = 8.8, H4b); 2.87 (dd, 1H, $J_{H,H}$ = 14.4, $^2J_{H,H}$ = 7.2, H1a); 2.82 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 7.6, H1b).

^{13}C NMR (DMSO-d6, 100 MHz): δ: 149.6 (C4'); 145.3 (C1'); 136.4; 135.9; 129.3; 128.6; 128.4; 128.1; 127.6; 126.8; 124.4 (Ph); 67.8 (C3); 55.2 (C2); 51.5 (C5); 49.1 (C4); 32.8 (C1).

MS: 456.0 $[M]^+$, 100%.

IR (KBr, ν = cm^{-1}): 3500-3200 (OH); 1348 and 1157 (S=O).

Found: C, 56.03; H, 5.45; N, 8.42. $[C_{23}H_{26}ClN_3O_5S]^+$ Cl^- requires C, 56.21; H, 5.33; N, 8.55 %.

Compound (6j: R = NH_2): yield, 92%; m.p. 167-169 °C.

^{1}H NMR (MeOD, 400 MHz): δ: 7.88 (d, 2H, $J_{H,H}$ = 8.4, Ph); 7.40 (d, 2H, $J_{H,H}$ = 8.4, Ph); 7.36-7.26 (m, 10H, Ph); 4.48 (d, 1H, $J_{H,H}$ = 15.2, H5a); 4.43 (d, 1H, $J_{H,H}$ = 15.2, H5b); 4.47-4.42 (m, 1H, H3); 3.54-3.49 (m, 1H, H2); 3.48 (dd, 1H, $J_{H,H}$ = 14.4, $^2J_{H,H}$ = 6.0; H4a); 3.15 (dd, 1H, $J_{H,H}$ = 14.8, $^2J_{H,H}$ = 7.6, H4b); 3.04 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 5.2, H1a), 2.77 (dd, 1H, $J_{H,H}$ = 14.2, $^2J_{H,H}$ = 9.6, H1b).

^{13}C NMR (DMSO-d6, 100 MHz): δ: 136.8; 136.6; 129.4; 129.0; 128.6; 128.3; 128.1; 127.3; 126.8; 115.4 (Ph); 68.4 (C3); 55.2 (C2); 51.9 (C5); 49.6 (C4); 32.5 (C1).

IR (KBr, ν = cm^{-1}): 3400 (OH); 1342 and 1159 (S=O).

MS: 426.2 $[M + H]^+$, 100%.

Found: C, 60.37; H, 6.04; N, 9.21. $[C_{23}H_{28}N_3O_3S]^+$ Cl^- requires C, 60.24; H, 6.11; N, 9.09%.

3.7. Antimycobacterial Study

The antimycobacterial activities of compounds **3**, **4**, **5a-j** and **6a-j** were assessed against *M. tuberculosis* ATTC 27294 using the micro plate Alamar Blue assay (MABA) [23] (Table **2**). This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods [28, 29].

Method: Sterile deionized water (200 µL) was added to all the outer-perimeter wells of the 96 sterile well plate system (Falcon, 3072: Becton Dickinson, Lincoln Park, NJ) to minimize evaporation of the medium in the test wells during incubation. Each of the 96 plates received 100 µL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) to which were added known concentrations of the compound under test. The final drug concentrations tested were 0.01 to 20.0 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. A freshly prepared 1:1 mixture of Alamar Blue (Accumed International, WestlakeOhio) reagent and 10% tween 80 (25 µL in all) was then added to the plate and incubated for 24 h. A blue color was interpreted as no bacterial growth, and a pink color was scored as growth. The minimal inhibition concentration (MIC) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

3.8. Cytotoxicity Assays

Cytotoxicity was determined using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and the hepatoma cell lineage Hep G2 A16 [25]. Cells were kept in RPMI medium supplemented with 10% FBS and confluent monolayers were trypsinized, washed in RPMI and applied in 96-well microtiter plates (4 X 10^4 cells/well). Dilution of compounds, which exhibited activity and Etambutol (control), were incubated with the cells (37 °C, 5% CO_2, 24h). Colorimetric reaction was developed after incubation with MTT (37 °C, 4 h) followed by addition of acidified isopropanol as previously described [29]. The reaction was read spectrophotometrically with a 570-nm filter and a background of 630 nm. Incubations were tested in triplicate in two independent experiments.

3.9. X-Ray Crystallography

Data for **[(6e).(H_2O)]** were obtained at 120 (2) K with Mo-Kα radiation by means of the Bruker-Nonius 95mm CCD camera on kappa-goniostat of the EPSRC crystallographic service, based at the University of Southampton. Data collection was carried out under the control of the program COLLECT [30] and data reduction and unit cell refinement were achieved with the COLLECT and DENZO programs [31]. Correction for absorption was achieved by a semi-empirical method based upon the variation of equivalent reflections using the program SADABS [32]. The programs ORTEP-3 for Windows [33] and MERCURY [34] were used in the preparation of the Figures. SHELXL97 [35] and PLATON [36] were used in the calculation of molecular geometry. The structures were solved by direct methods using SHELXS-97 [34] and fully refined by means of the program SHELXL-97 [33]. All hydrogen atoms were placed in calculated positions. Crystal data and structure refinement details are listed in (Table **3**).

4. CONCLUSION

Twenty two hydroxyethylamine derivatives, **3**, **4**, **5a-j** and **6a-j,** have been synthesized in good yields. All compounds were tested against *M. tuberculosis* with **6b, 6c, 6e, 6i** and **6j** showed the best activities with MIC values of 51.93 µM (**6b**), 47.54 µM (**6c, 6i** and **6j**) and 51.93 µM (**6e**). Both the presence of the sulfonamide moiety and a free amino group at C2, as in series **6**, are necessary for activity. Position of the substituent on the benzenesulfonamide does not seem to affect biological activity. Lipophilicity of the

compounds seems to play a very important role in the biological activity. X-Ray crystallography study indicated a *cis*-arrangement of the H_3N and OH groups in active **6e**, in contrast to the reported *trans* arrangement of Bu^t-OCONH and OH groups in non-active **5f** [21]. High cytotoxicity values indicate that further chemical modifications are needed in order to find useful lead compounds.

SUPPLEMENTARY MATERIAL

Full details of the crystal structure determinations in CIF format have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 886728. Copies of these can be obtained free of charge on written application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: 44 1223 336033); on request by e-mail to deposit@ccdc.cam.ac.uk or by access to http://www.ccdc.cam.ac.uk.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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