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Preparation and antitubercular activities of alkylated amino alcohols and their glycosylated derivatives

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Abstract—A series of N- and C-alkylated amino alcohols and of their protected galactopyranosyl derivatives was synthesized and evaluated for antitubercular activity. Five of these compounds displayed good activity, with a MIC below 12.5 μg/mL. The presence of the carbohydrate slightly affected the antibacterial activity.

1. Introduction

Tuberculosis (TB), a contagious disease which spreads through air, is caused by *Mycobacterium tuberculosis*. World Health Organization reports that nearly 2 millions deaths result from tuberculosis every year, and that one-third of the world population is currently infected with the TB bacillus.[1] Tuberculosis is a leading cause of death among people who are HIV positive, forming with HIV a lethal combination, each one spreading the other's progress. Until 60 years ago there was no treatment for tuberculosis. Since then several drugs have been used, alone or in combination, and drug resistant strains of mycobacterium have emerged. This resistance is caused by partial treatment (patients stop taking the medicine), by prescription of the wrong treatment regimen, or by an unreliable drug supply. MDR-TB (multidrug resistant TB) is a particularly dangerous form of TB, being resistant to isoniazid and rifampicin, two of the most powerful anti-TB drugs.

During the last years, numerous new antitubercular drugs having different mechanisms of action have been synthesized.[2] Among the targets for the development of new anti-TB drugs, the mycobacterial cell membrane is of great interest, since it is responsible for the impermeability of the bacterial cell to numerous drugs.[3] Mycobacterial cell wall possesses in its structure complex polysaccharides, lipoarabinomannan and arabinogalactan, in which galactose and arabinose are predominant.[4–7] The inhibition of the glycosyltransferases evolved in their biosynthesis could result in a disturbance of the cell wall biosynthesis.[8–10] Good candidates for this type of drugs are sugar-based glycosylated amino alcohols and aminoesters.[11–16] We recently described the synthesis and anti-TB activity of some aminoacylated derivatives of galactopyranose.[17] In continuation to this work we report here the preparation and evaluation of anti-TB activity of C- and N-alkylated amino alcohols and of their D-galactose derivatives.

2. Results and discussion

For the obtention of N-alkylated compounds **3a**–**e**[18–20] and **7**[21] alcohols **1a**–**e** and **5** were first mesylated in pyridine at 0 °C, leading to compounds **2a**–**e** and **6** which were used without purification (90–96% yield, Scheme 1). Mesylated derivatives were then treated with ethanolamine (1.2 equiv/mol) in ethanol at 90 °C, furnishing the desired compounds **3a**–**e** and **7** in moderate yields

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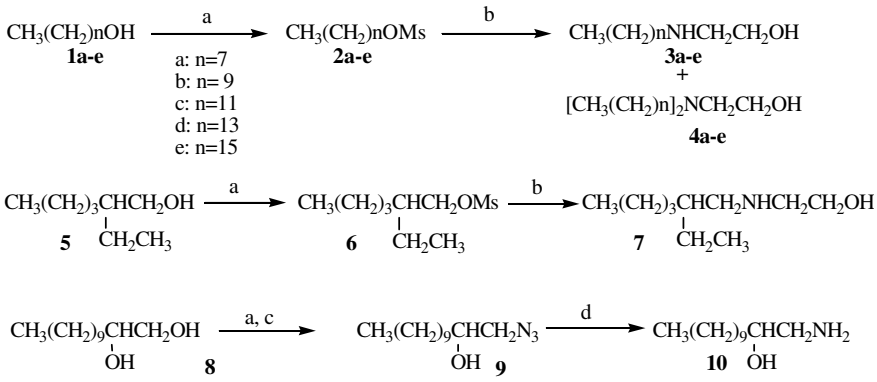


Scheme 1. Preparation of N- and C-alkylated amino alcohols. Reagents and conditions: (a) MsCl, pyridine, 0 °C; (b) $H_2N(CH_2)_2OH$, EtOH, 90 °C; (c) NaN_3, DMF, 120 °C; (d) H_2, Pd/C, EtOH.

(50–52%) along with N,N′-dialkylated compounds **4a**–**e** (8–10% yield). After purification by column chromatography amino alcohols **3a**–**e** and **7** were characterized by ^{1}H NMR and ^{13}C NMR spectroscopy. In the ^{1}H NMR spectra, the presence of signals in the regions of δ 3.6 and 2.7 ppm referring to the ethanolamine moiety, along with the disappearance of the singlet near 3.0 ppm corresponding to the methyl of the mesylate group, confirmed the conversion to amino alcohols. In the ^{13}C NMR spectra signals in the region of δ 60–61 and δ 49–53 ppm, corresponding to methylenic carbons CH_2N and CH_2O, were observed.

Primary hydroxyl group of diol **8** was first converted to mesylate and the resulting compound was treated with sodium azide in DMF at 120 °C, leading to azide **9** in quantitative yield. After a rapid purification, compound **9** was hydrogenated in the presence of palladium on charcoal 10% in ethanol, furnishing *C*-alkyl amino alcohol **10** in 70% yield[22] (Scheme 1).

No attempt was made to determine the stereochemistry of compounds **7** and **10** as they were prepared from the corresponding racemic alcohols (**5** and **8**, respectively).

D-Galactose **11** was converted into 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **12** according to the literature procedure[23] and then treated with amino alcohols **3a**–**e**, **7**, and **10** in DMSO at 90 °C (Scheme 2). All the compounds were purified by column chromatography (hexane:ethyl acetate) and were obtained in 40–56% yields. The structure was assigned by ^{1}H NMR, ^{13}C NMR, and COSY experiments. All ^{1}H NMR spectra showed signals referring to the carbohydrate moiety (3.8–5.6 ppm), to the alkyl chain (0.8–1.3 ppm), and, in the case of compounds **13a**–**e**, to the ethanolamine portion (2.7–3.6 ppm). ^{13}C NMR spectra showed characteristic signals near to δ 96 ppm (C_1) and between δ 66 and 73 ppm (C_2–C_5), corresponding to the carbohydrate portion of the molecule, and two signals corresponding to the isopropylidene groups were observed between δ 107 and 110 ppm. Three signals between 53 and 56 ppm (CH_2N) confirmed the presence of the N-alkylated amino alcohol (compounds **13a**–**e** and **14**).

3. Biological activity

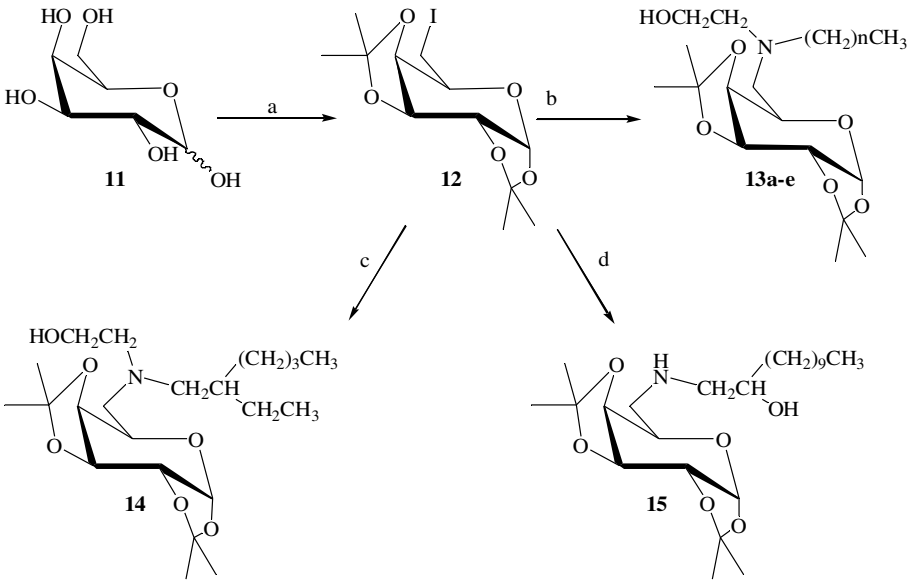
The activity of the compounds against *M. tuberculosis* virulent strain H37Rv was determined in vitro as previously described,[24] using rifampicin as a reference for activity. The minimum inhibitory concentration (MIC), concentration that inhibits the colony forming ability of *M. tuberculosis*, was determined by incorporating decreasing concentrations of the test compound in Middlebrook 7H9 agar medium. MIC values represent means of three separate experiments and are reported in Table 1.

It would appear that the antitubercular activity of the free amino alcohols depends on the length of the alkyl chain. The best results were obtained for the *N*-dodecyl-ethanolamine **3c** (MIC = 0.027 mM) and *C*-decyl-ethanolamine **10** (MIC = 0.015 mM). C-Alkylated compound **10** is almost twice more active than its N-alkylated analogue **3c**. Compounds **3d** and **3e** with longer alkyl chain were twice less active than **3c**, but still more active than shorter compounds. The same effect was observed for the glycosylated derivatives **13a**–**e**.

Among the glycosylated derivatives, compound **13c** was the most active (MIC = 0.006 mM), but this time the N-alkylated galactose derivative was four times more active than its C-alkylated analogue.

Glycosylation of N-alkylated amino alcohols increased their activity, suggesting that the carbohydrate moiety is important for the anti-TB activity of these compounds. The mechanisms of action of these two classes may be different. The glycosylated compounds could act as inhibitors of the cell wall biosynthesis. The anti-TB activity observed for compounds **3a**–**e** could be a result of their action on the cytoplasmic membrane, responsible for the antimicrobial action of some N-alkylated amino alcohols against several organisms.[25] *N*-Octyl-ethanolamine **3a** is also able to inhibit the growth of *Mycobacteria smegmatis* and *Mycobacteria marinum* in metalworking coolant fluid.[26]

Compound **7**, containing a branched alkyl chain, and its galactose derivative **14** did not exhibit antitubercular



Scheme 2. Preparation of D-galactose derivatives. Reagents and conditions: (a) i—$ZnCl_2$, H_2SO_4, acetone, t.a. (58%); ii—I_2, PPh_3, Imidazole, toluene, reflux (90%); (b) **3a**–**e**, DMSO, 90 °C, (40–56%); (c) **7**, DMSO, 90 °C (56%); (d) **10**, DMSO, 90 °C (41%).

Table 1. Antitubercular activity

Compound	MIC (μg/mL)	MIC (mM)
3a	100	0.578
3b	50	0.248
3c	6.25	0.027
3d	12.5	0.051
3e	12.5	0.046
7	>100	>0.578
10	3.12	0.015
13a	100	0.241
13b	100	0.225
13c	3.12	0.006
13d	6.25	0.012
13e	6.25	0.011
14	>100	>0.240
15	12.5	0.028
Isoniazid	0.2	—
Ethambutol	3.25	—

Rifampicin 1.0 μg/mL was used as internal control.

activity at the tested concentration, suggesting that the alkyl chain would have to be linear for biological activity.

Cell proliferation and cytotoxicity of the most active compounds **3c**, **10**, **13c**, **13d**, and **13e** were evaluated. Both activities were determined in parallel on lineage cell J744A.1 stimulated with interferon-gamma (IFN-γ) and evaluated by a colorimetric MTT assay. The determination was based on the viability of the cells in the presence of various concentrations of the tested compounds. Results are displayed in Table 2. Compounds **3c** and **13e** displayed low toxicity in all the tested concentrations. Compound **10** was cytotoxic at 0.5 μg/mL. Compounds **13c** and **13d** did not show any cytotoxicity in all tested concentrations. Despite its toxicity, compound **13e** showed a statistically significant increase of cell proliferation at 0.5 μg/mL and 0.05 μg/mL. These data could be explained by the fact that, due to cell proliferation, the higher number of cells inhibits the cellular survival conditions by the lack of nutrients. Compound **13d** stimulated cell proliferation at all the tested concentrations, and compound **10** displayed cell proliferation over 100% at 0.05 μg/mL and at 0.005 μg/mL.

4. Conclusion

A series of alkylated amino alcohols and their glycosylated derivatives were synthesized and evaluated for their in vitro antitubercular activity. Five of these compounds displayed good inhibition of *M. tuberculosis* growth, with MIC values below 12.5 μg/mL, and a low toxicity. This work may lead to further development of new efficient antitubercular compounds.

5. Experimental

5.1. General methods

Melting points were determined on a Microquímica MQAPF apparatus and are uncorrected. IR spectra were recorded using a BOMEM-FTIR MB102 spectrometer. Optical rotations were measured with a Perkin-Elmer 341 polarimeter, using a sodium lamp (λ 589 nm) at 20 °C. ^1H and ^{13}C NMR spectra were recorded on Bruker Advance DRX300 spectrometer. Elemental analyses were performed at the Central Analítica of Instituto de Química of the Universidade de São Paulo, Brazil. Thin-layer chromatography (TLC) was performed on glass plates and silica gel sheets (Silica Gel F254, Merck), visualized with iodine vapor, and/or revealed with ethanolic H_2SO_4 solution or with a 0.5% ninhydrin solution. Column chromatography was carried out on silica gel (E. Merck 230 and 400 mesh). Solvents were purchased from Vetec Química and were

Table 2. Cell proliferation and citotoxicity

Compound	Concentration					
	0.5 μg/mL		0.05 μg/mL		0.005 μg/mL	
	Proliferation (%)	Cytotoxicity (%)	Proliferation (%)	Cytotoxicity (%)	Proliferation (%)	Cytotoxicity (%)
3c	90.9	21.3	77.6	8.4	92.7	12.1
10	96.4	31.1	112	nc	110.2	nc
13c	96.6	nc	98.9	nc	98.3	nc
13d	117.7	nc	135.2*	nc	112.8	nc
13e	148.6*	17.4	110.4	16.3	104.3	10.6

nc, not cytotoxic.
* Statistically significant increase, $p < 0.05$ (ANOVA Bonferroni test).

distilled prior to use. Reagents were purchased from Aldrich and used without further purification.

5.2. General procedure for the preparation of N-alkylated amino alcohols 3a–e and 7

To a solution of the alcohol **1a**–**e**, **5** or **8** (50 mmol) in CH_2Cl_2 (25 mL) was added a solution of methanesulfonyl chloride (70 mmol) in CH_2Cl_2 (25 mL). The temperature of the reaction mixture was lowered to 0 °C and pyridine (5 mL) was added. The reaction mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was dissolved in hexane and washed three times with water. After concentration of the organic phase the crude mesylate was dissolved in ethanol (30 mL) and slowly added to a solution of 2-amino ethanol (100 mmol) in ethanol (30 mL). The reaction mixture was stirred for 24 h at 90 °C and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and water. After concentration of the organic phase the residue was purified by column chromatography (CH_2Cl_2/MeOH) to furnish the desired amino alcohols **3a**–**e** and **7**.

5.2.1. *N*-Octyl-amino ethanol (3a). Oil; yield, 50%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.83 (br s, 1H, NH); 3.60 (t, 2H, J = 5.1 Hz, CH_2OH); 3.20 (m, 1H, OH); 2.70 (t, 2H, J = 5.1 Hz, NHCH_2CH$_2$OH); 2.57 (t, 2H, J = 7.3 Hz, CH_2NHCH$_2$CH$_2$OH); 1.44 (m, 2H, CH_2); 1.22 (m, 10 H, CH_2); 0.82 (t, 3H, J = 6.7 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 60.6 (CH_2OH); 51.4 (NH*C*H$_2$CH$_2$OH); 49.8 (*C*H$_2$NHCH$_2$CH$_2$ OH); 31.9–22.7 (CH_2); 14.1 (CH_3).

5.2.2. *N*-Decyl-amino ethanol (3b). Oil; yield, 51%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.65 (t, 2H, J = 4.3 Hz, CH_2OH); 2.91 (m, 2H, NH and OH); 2.71 (t, 2H, J = 4.3 Hz, NHCH_2CH$_2$OH); 2.57 (t, 2H, J = 7.2 Hz, CH_2NHCH$_2$CH$_2$O H); 1.47 (m, 2H, CH_2); 1.27 (m, 14H, CH_2); 0.87 (t, 3H, J = 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 60.8 (CH_2OH); 51.4 (NH*C*H$_2$CH$_2$OH); 49.8 (*C*H$_2$NHCH$_2$CH$_2$ OH); 32.0–22.8 (CH_2); 14.2 (CH_3).

5.2.3. *N*-Dodecyl-amino ethanol (3c). Oil; yield, 51%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.58 (t, 2H, J = 5.2 Hz, CH_2OH); 3.48 (m, 2H, NH and OH); 2.66 (t, 2H, J = 5.2 Hz, NHCH_2CH$_2$OH); 2.54 (t, 2H, J = 7.3 Hz, CH_2NHCH$_2$CH$_2$OH); 1.41 (m, 2H, CH_2); 1.18 (m, 18H, CH_2); 0.82 (t, 3H, J = 6.8 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 60.4 (CH_2OH); 51.5 (NH*C*H$_2$CH$_2$OH); 49.8 (*C*H$_2$NHCH$_2$CH$_2$ OH); 31.9–22.7 (CH_2); 14.0 (CH_3).

5.2.4. *N*-Tetradecyl-amino ethanol (3d). White crystals; mp, 53.1–53.4 °C; yield, 52%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.66 (t, 2H, J = 5.0 Hz, CH_2OH); 3.57 (m, 2H, NH and OH); 2.76 (t, 2H, J = 5.0 Hz, NHCH_2CH$_2$OH); 2.62 (t, 2H, J = 7.3 Hz, CH_2NHCH$_2$CH$_2$O H); 1.48 (m, 2H, CH_2); 1.22 (m, 22H, CH_2); 0.85 (t, 3H, J = 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 60.4 (CH_2OH); 51.2 (NH*C*H$_2$CH$_2$OH); 49.5 (*C*H$_2$NHCH$_2$CH$_2$ OH); 32.0–23.0 (CH_2); 14.5 (CH_3).

5.2.5. *N*-Hexadecyl amino ethanol (3e). White crystals; mp 59.0–60.5 °C; yield, 50%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.71 (t, 2H, J = 5.0 Hz, CH_2OH); 2.80 (t, 2H, J = 5.0 Hz, NHCH_2CH$_2$OH); 2.66 (t, 2H, J = 7.3 Hz, CH_2NHCH$_2$CH$_2$O H); 1.55 (m, 2H, CH_2); 1.28 (m, 26H, CH_2); 0.89 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 60.7 (CH_2OH); 49.7 (NH*C*H$_2$CH$_2$OH); 49.5 (*C*H$_2$NHCH$_2$CH$_2$ OH); 32.1–22.9 (CH_2); 14.2 (CH_3).

5.2.6. *N*-(2-Ethyl-hexyl) amino ethanol (7). Oil, yield, 51%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.62 (t, 2H, J = 5.2 Hz, CH_2OH); 3.21 (m, 2H, NH and OH); 2.72 (t, 2H, J = 5.2 Hz, NHCH_2CH$_2$OH); 2.48 (d, 2H, J = 6.1 Hz, CH_2NHCH$_2$CH$_2$O H); 1.21 (m, 9H, CH_2aliph., CH_2ethyl, C*H*(Et)CH$_2$NH); 0.84 (t, 6H, J = 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 60.4 (CH_2OH); 52.8 (NH*C*H$_2$CH$_2$OH); 51.6 (CH$_3$CH$_2$CH*C*H$_2$ NH); 39.0 (*C*H(Et)CH$_2$NH); 31.2–23.1 (CH_2); 14.2 and 10.7 (CH_3).

5.3. Preparation of C-alkylated amino alcohol (10)

The crude monomesylated precursor (1.47 g, 5.22 mmol), obtained as described above, was dissolved in DMF (10 mL) and sodium azide (0.75 g, 10.45 mmol) was added. The reaction mixture was stirred at 120 °C for 24 h and concentrated under reduced pressure. The crude product was dissolved in hexane and washed 3 times with water. After evaporation of the organic phase, the residue was purified by column chromatography leading to 1-azidododecan-2-ol **9** as an oil. Yield, 94%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.77 (m, 1H,

CHOH); 3.36 (dd, 1H, J = 3.5 Hz, 12.5 Hz, CHH′N_3); 3.22 (dd, 1H, J = 7.2 Hz, 12.5 Hz, CHH′N_3); 2.90 (m, 1H, OH); 1.48 (m, 2H, CH_2CHOH); 1.28 (m, 16H, CH_2); 0.87 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 71.0 (CHOH); 57.2 (CH_2N_3); 34.5 (CH_2CHOH); 32.0–22.8 (CH_2); 14.5 (CH_3).

Compound **9** (0.681 g, 3 mmol) was dissolved in ethanol (10 mL) and Pd/C 10% (20 mg) was added. The mixture was stirred at room temperature for 48 h under an atmosphere of H_2. Catalyst was eliminated by filtration and the mixture was concentrated under reduced pressure. The crude residue was chromatographed on silica gel (methylene chloride/methanol), furnishing amino alcohol **10** as an oil. Yield, 70%; IR (ν, cm^{-1}, CsI): 3408, 2914; ^{1}H NMR (DMSO-d^6, 300 MHz): δ 3.30 (m, 1H, CHOH); 2.42 (dd, 1H, J = 3.5 Hz, 12.7 Hz, CHH′NH_2); 2.30 (dd, 1H,J = 7.3 Hz, 12.7 Hz, CHH′NH_2); 1.20 (m, 18H, CH_2); 0.81 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 72.2 (CHOH); 47.5 (CH_2NH_2); 35.0–22.9 (CH_2); 14.3 (CH_3); Anal. Calcd for $C_{12}H_{27}NO$: C, 71.58; H, 13.52; N, 6.96. Found C, 71.98; H, 13.64; N, 6.73.

5.4. General procedure for the preparation of the glycosylated derivatives 13a–e, 14 and 15

To a solution of **12** (1 mmol) in DMSO (5 mL) was added a solution of the amino alcohol (1.2 mmol) in DMSO (5 mL). The mixture was stirred at 90 °C for 48 h and the solution was concentrated under reduced pressure. The crude product was dissolved in methylene chloride and washed 3 times with water. After drying with sodium sulfate the organic phase was concentrated under reduced pressure. The residue was chromatographed on silica gel (methylene chloride/methanol) to furnish the desired compounds **13a**–**e**, **14**, and **15**.

5.4.1. 6-[(*N*-Octyl)-2-hydroxyethylamino]-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (13a). Oil; yield, 45%; $[\alpha]_D$: −2.3 (c 0.5, $CHCl_3$); IR (ν, cm^{-1}, CsI): 3488, 2928, 2856, 1070; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.53 (d, 1H, J = 5.0 Hz, H1); 4.60 (dd, 1H, J = 2.4, 7.9 Hz, H3); 4.30 (dd, 1H, J = 2.4, 5.0 Hz, H2); 4.25 (dd, 1H, J = 1.7, 7.9 Hz, H4); 3.90 (t, 1H, J = 6.6 Hz, H5); 3.58 (m, 2H, CH_2OH); 2.77 (m, 6H, CH_2N); 1.52; 1.44; 1.33; 1.32 (4s, CH_3iPr); 1.24 (m, 12H, CH_2aliph.); 0.89 (t, 3H, J = 6.7 Hz, CH_3aliph.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.3 and 108.6 (CiPr); 96.7 (C1); 72.1; 71.0; 70.7 (C2, C3, C4); 66.1 (C5); 59.3 (CH_2OH); 56.0; 55.5; 53.5 (CH_2N); 32.0–22.8 (CH_3iPr and CH_2aliph.); 14.2 (CH_3aliph.); Anal. Calcd for $C_{22}H_{41}NO_6$: C, 63.58; H, 9.94; N, 3.37. Found: C, 63.02; H, 9.91; N, 3.17.

5.4.2. 6-[(*N*-Decyl)-2-hydroxyethylamino]-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (13b). Oil; yield, 40%; $[\alpha]_D$: −76.2 (c 0.5, $CHCl_3$); IR (ν, cm^{-1}, CsI): 3480, 2914, 2849, 1070; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.52 (d, 1H, J = 5.0 Hz, H1); 4.61 (dd, 1H, J = 2.4, 7.9 Hz, H3); 4.30 (dd, 1H, J = 2.4, 5.0 Hz, H2); 4.25 (dd, 1H,J = 1.7, 7.9 Hz, H4); 3.88 (m, 1H, H5); 3.55 (m, 2H, CH_2OH); 2.76 (m, 6H, CH_2N); 1.51; 1.43; 1.33; 1.31 (4s, CH_3iPr); 1.24 (m, 16H, CH_2aliph.); 0.88 (t, 3H, J = 6.7 Hz, CH_3aliph.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.3 and 108.6 (CiPr); 96.1 (C1); 71.4; 71.0; 70.7 (C2, C3, C4); 66.1 (C5); 59.3 (CH_2OH); 56.9; 55.5; 53.6 (CH_2N); 32.0–22.8 (CH_3iPr and CH_2aliph.); 14.3 (CH_3aliph.); Anal. Calcd for $C_{24}H_{45}NO_6$: C, 64.98; H, 10.22; N, 3.16. Found: C, 65.50; H, 10.44; N. 2.71.

5.4.3. 6-[(*N*-Dodecyl)-2-hydroxyethylamino]-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (13c). Oil; yield, 53%; IR (ν, cm^{-1}, CsI): 3488, 2925, 2853, 1072; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.54 (d. 1H, J = 5.0 Hz, H1); 4.63 (dd, 1H, J = 2.0, 7.7 Hz, H3); 4.33 (dd, 1H, J = 2.0, 5.0 Hz, H2); 4.28 (dd, 1H, J = 1.7, 7.9 Hz, H4); 3.88 (m, 1H, H5); 3.61 (m, 2H, CH_2OH); 3.26 (m, 1H, OH); 2.82 (m, 6H, CH_2N); 1.54; 1.46; 1.35; 1.34 (4s, CH_3iPr); 1.26 (m, 20H, CH_2aliph.); 0.90 (t, 3H, J = 6.7 Hz, CH_3aliph.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.3 and 108.5 (CiPr); 96.6 (C1); 72.0; 70.8; 70.4 (C2, C3, C4); 66.0 (C5); 60.3 (CH_2OH); 56.1; 55.0 (CH_2N); 31.9–22.7 (CH_3iPr and CH_2aliph.); 14.2 (CH_3aliph.); Anal. Calcd for $C_{26}H_{49}NO_6$: C, 66.21; H, 10.47; N, 2.97. Found C, 65.82; H, 10.93; N, 2.92.

5.4.4. 6-[(*N*-Tetradecyl)-2-hydroxyethylamino]-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (13d). Oil; yield, 40%; $[\alpha]_D$: −21.9 (c 0.5, $CHCl_3$); IR (ν, cm^{-1}, CsI): 3488, 2928, 2856, 1070; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.54 (d, 1H, J = 5.0 Hz, H1); 4.62 (dd, 1H, J = 2.4, 7.9 Hz, H3); 4.32 (dd, 1H, J = 2.4, 5.0 Hz, H2); 4.27 (dd, 1H, J = 2.2, 7.9 Hz, H4); 3.90 (m, 1H, H5); 3.61 (m, 2H, CH_2OH); 2.64 (m, 6H, CH_2N); 1.53; 1.45; 1.34; 1.33 (4s, CH_3iPr); 1.26 (m, 24H, CH_2aliph.); 0.88 (t, 3H, J = 6.7 Hz, CH_3aliph.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.3 and 108.7 (CiPr); 96.8 (C1); 72.2; 70.9; 70.7 (C2, C3, C4); 66.1 (C5); 60.5 (CH_2OH); 56.2; 55.9; 55.5 (CH_2N); 32.0–22.8 (CH_3iPr and CH_2aliph.); 14.3 (CH_3aliph.); Anal. Calcd for $C_{28}H_{53}NO_6$: C, 67.30; H, 10.69; N, 2.80. Found: C, 66.93; H, 10.78; N, 2.63.

5.4.5. 6-[(*N*-Hexadecyl)-2-hydroxyethylamino]-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (13e). Oil; yield, 44%; $[\alpha]_D$: −133.5 (c 0.5, $CHCl_3$); IR (ν, cm^{-1}, CsI): 3494, 2928, 2856, 1071; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.55 (d, 1H, J = 4.8 Hz, H1); 4.63 (m, 1H, H3); 4.34 (dd, 1H, J = 2.4, 5.0 Hz, H2); 4.29 (m, 1H, H4); 3.93 (t, 1H, J = 6.2 Hz, H5); 3.65 (m, 2H, CH_2OH); 2.95 (m, 6H, CH_2N); 1.55; 1.47; 1.36; 1.34 (4s, CH_3iPr); 1.27 (m, 28H, CH_2aliph.); 0.89 (t, 3H, J = 6.7 Hz, CH_3aliph.); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.4 and 108.7 (CiPr); 96.8 (C1); 72.2; 71.0; 70.7 (C2, C3, C4); 66.2 (C5); 59.2 (CH_2OH); 56.9; 55.5; 53.6 (CH_2N); 32.1–22.9 (CH_3iPr and CH_2aliph.); 14.4 (CH_3aliph.); Anal. Calcd for $C_{30}H_{57}NO_6$:C, 68.27; H, 10.89; N, 2.65. Found: C, 59.90; H, 10.91; N, 2.63.

5.4.6. 6-{[*N*-(2-Ethyl-hexyl)]-2-hydroxyethylamino}-6-deoxy-1,2: 3,4-di-*O*-isopropylidene-α-D-galactopyranose (14). Oil; yield, 56%; $[\alpha]_D$: −35.1 (c 0.5, $CHCl_3$); IR (ν, cm^{-1}, CsI): 3494, 2928, 2858, 1071; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.47 (d, 1H, J = 5.0 Hz, H1); 4.54 (m,

1H, H3); 4.25 (dd, 1H, J = 2.4, 5.0 Hz, H3); 4.20 (m, 1H, H2); 3.84 (t, 1H, J = 5.8 Hz, H5); 3.52 (m, 2H, CH_2OH); 2.75 (m, 4H, $HOCH_2CH_2$ and CH_2N); 2.27 (m, 2H, $EtCHCH_2N$); 1.46; 1.38; 1.28; 1.26 (4s, CH_3iPr); 1.19 (m, 9H, $[(CH_2)_3CHCH_2CH_3]$; 0.84 (m, 6H, CH_3aliph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.4 and 108.8 (CiPr); 96.7 (C1); 72.2; 71.0; 70.6 (C2, C3, C4); 66.0 (C5); 60.4 (CH_2OH); 59.2; 57.5; 54.5 (CH_2N); 37.5 $[(CH_2)_3CHCH_2CH_3]$; 31.4–23.1 (CH_3iPr and CH_2aliph.); 14.2 and 11.0 (CH_3aliph.); Anal. Calcd for $C_{22}H_{41}NO_6$: C, 63.58; H, 9.94; N, 3.37. Found: C, 63.97; H, 9.83; N, 4.76.

5.4.7. 6-[*N*-2-Hydroxy-dodecylamino]-6-deoxy-1,2:34-di-*O*-isopropylidene-α-D-galactopyranose (15). Oil; yield, 41%; IR (ν, cm^{-1}, CsI): 3488, 2928, 2856, 1070; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.50 (d, 1H, J = 4.8 Hz, H1); 4.58 (dd, 1H, J = 2.4, 7.9 Hz, H3); 4.28 (dd, 1H, J = 2.4, 4.8 Hz, H2); 4.16 (dd, 1H, J = 2.4, 7.9 Hz, H4); 3.85 (m, 1H, H5); 2.94 (m, 1H, H6); 2.86 (m, 1H, CHOH); 2.73 (m, 6H, CH_2N); 1.49; 1.41; 1.29 (4s, CH_3iPr); 1.22 (m, 18H, CH_2aliph.); 0.84 (t, J = 6.6 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 109.3 and 108.6 (CiPr); 96.5 (C1); 72.0; 70.9; 70.7 (C2, C3, C4, CHOH); 66.8 (C5); 55.2 and 49.3 (CH_2N); 35.1–22.7 (CH_3iPr and CH_2aliph.); 14.2 (CH_3aliph.); Anal. Calcd for $C_{24}H_{44}NO_6$: C, 64.98; H, 10.22; N, 3.16. Found: C, 64.45; H, 10.61; N, 3.18.

5.5. Antitubercular activity

The antitubercular activity of the tested compounds was determined by incorporating decreasing concentrations to a culture of *M. tuberculosis* H37Rv (ATCC 27294) in Middlebrook 7H9 agar medium using the Microplate Alamar Blue Assay (MABA).[24] MIC values represent means of three separate experiments. Rifampicin was used as the reference compound.

5.6. MTT colorimetric assay

Cell proliferation was measured using the MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] test. Culture plates without supernatants were incubated with 100 μL of supplemented RPMI-medium and 10 μL of MTT (5 mg/mL) for 4 h at 37 °C in 5% CO_2. After this time, the reaction was stopped by adding to each weel 100 μL of an acidic isopropanol solution (100 mL of isopropyl alcohol/0.4 mL of HCl 10 N). Following 10 min incubation at room temperature, the optical density (OD) values at 570 nm were determined (Spectramax 190—Molecular Devices—US). For determination of cell proliferation and cytotoxicity the following formulae were used:

$$\text{Cell proliferation } (\%) = (\mathrm{OD}_{(J774A.1/IFN/\text{compound})}/\mathrm{OD}_{(J774A.1/IFN)}) \times 100$$

$$\text{Cytotoxicity } (\%) = [1 - (\mathrm{OD}_{(J774A.1/\text{compound})}/\mathrm{OD}_{(J774A.1)})] \times 100.$$

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