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Synthesis of galactopyranosyl amino alcohols as a new class of antitubercular and antifungal agents☆

Neetu Tewari,[a] V. K. Tiwari,[a] R. P. Tripathi,[a,*] V. Chaturvedi,[b] A. Srivastava,[b] R. Srivastava,[b] P. K. Shukla,[c] A. K. Chaturvedi,[c] A. Gaikwad,[d] S. Sinha[d] and B. S. Srivastava[b]

[a]*Division of Medicinal Chemistry, Central Drug Research Institute, Lucknow-226001, India*
[b]*Division of Microbiology, Central Drug Research Institute, Lucknow-226001, India*
[c]*Division of Medical Mycology, Central Drug Research Institute, Lucknow-226001, India*
[d]*Division of Biochemistry, Central Drug Research Institute, Lucknow-226001, India*

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Abstract—The galactopyranosyl amino alcohols (**3**–**16**) were synthesised by regioselective oxirane ring opening of compound **2** with variety of amines and screened for antitubercular and antifungal activities. One of the compounds (**16**) showed potent activity against *Mycobacterium tuberculosis* H37 Rv in vitro and also displayed activity in MDR TB. The compound (**16**) was found to be superior to ethambutol clinically used anti TB drug in in vitro screen.

1. Introduction

Tuberculosis (TB) an infectious disease caused by *Mycobacterium tuberculosis* affects about 8 million people worldwide and kills 2 million people annually. As per WHO estimates about one third of world population harbor latent infection of TB[1] and therefore it has been declared as global emergency.[2,3] Multiple drug resistant (MDR) tuberculosis and its synergy with HIV and mycotic infections particularly due to *Candida albicans*, *Candida* sp. and *Cryptococcus neoformans* in immunocompromised patients have worsened the problem.[4–7] Serious limitations of the existing drugs as well as resistance against them has led to an emergent need to develop new chemical entities (NCE) as antitubercular agents with novel mode of action. The additional chemotherapeutic potential of NCEs against opportunistic pathogens and immunopotentiation is also desired.

Cell wall of *M. tuberculosis* is an ideal and selective target in antitubercular drug development as it protects the bacterium and to a large extent responsible for drug resistance.[8–10] Galactose and arabinose are predominant in the cell wall mainly as arabino-D-galactan and lipoarabinomannans. Glycosyl transferases are intricately involved in the biosynthesis of these polymers. Ethambutol, an amino alcohol is well known anti TB drug presently in clinics as first line agent, acts through inhibition of arabinosyl transferases.[11,12] Unnatural arabinose based sugars have recently been shown to inhibit this enzyme and possess in vitro anti TB activity also.[13] We have also shown anti TB activity in unnatural sugars particularly glycosyl amino esters.[14,15] Galactopyranosyl amino sugars possess anti-infective[16] immunomodulatory, antiviral and antifungal activities.[17–20] In view of the above and in continuation of our ongoing programme to develop anti TB drugs from sugars it was thought to synthesise some β-hydroxy amino alkyl derivatives of galactopyranose which may inhibit glycosyl transferase and at the same time additional bioactivity in this class of molecule would lead to novel anti TB agents.

2. Results and discussion

2.1. Chemistry

The synthetic strategy as shown in Scheme 1 involves the reaction of diacetonide-α-D-galactose **1**[21] with (*R*/*S*) epi-

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* Corresponding author. Tel.: +91-522-212411-18x4382; fax: +91-522-223405/223938; e-mail: rpt_56@yahoo.com

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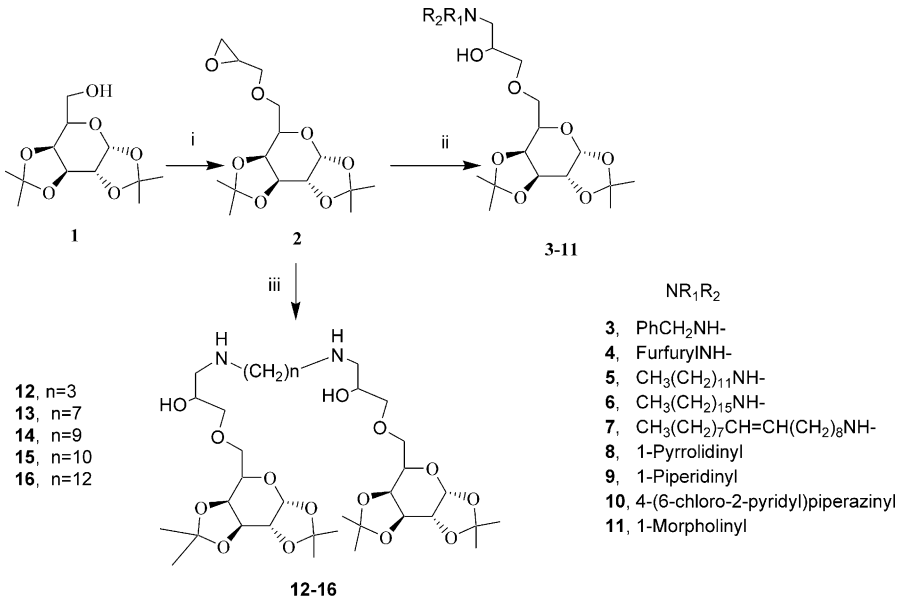

Scheme 1. Reagents and conditions: (i) epichlorohydrin, aq NaOH, THF, tetrabutyl ammonium bromide, 40 °C, 12 h; (ii) primary or secondary amines, ethanol, 30 °C, 12 to 18 h; (iii) diamines, ethanol, 30 °C, 12 to 18 h.

chlorohydrin in tetrahydrofuran. Ambient temperature reaction did not proceed and refluxing temperature led to only 30% yield of 6-*O*-(3′-epoxypropan-1′-yl] galactopyranosyl derivative **2**.[22] However the same reaction in presence of tetrabutyl ammonium bromide (TBAB) as phase transfer catalyst resulted in quantitative yield of compound **2** even at 25 °C in a shorter duration. Thus use of TBAB not only increases the yield of the product but also saves time and energy both. Nucleophilic opening of oxirane ring in compound **2** by selected primary and secondary amines in ethanol at room temperature was unsuccessful and at reflux temperature reaction never reached to completion. Therefore application of phase transfer catalyst (TBAB) was again sought in and to our pleasant surprise starting compound **2** was fully consumed within 6–12 h even at room temperature. Thus primary amines on regioselective oxirane ring opening resulted in galactopyranosyl amino alcohols (**3**–**7**)[23] in good yield. During these reactions, side products were also formed which could not be isolated in pure form. The secondary amines namely pyrrolidine, piperidine, 4-(6-chloro-2-pyridyl)-piperazine and morpholine on similar reaction with the above compound **2** gave corresponding galactopyranosylated amino alcohols (**8**–**11**)[23] in quantitative yields and no side products were observed. The reaction of 2 mol of the above 6-*O*-(3′-epoxypropan-1′-yl] galactopyranosyl derivative (**2**) with 1 mol of diamines resulted in the formation of N^1,N^n-digalactopyranosylated amino alcohols (**12**–**16**) under similar conditions in good yield.[23] The structures of all the compounds are based on their spectroscopic data. The ^{1}H NMR and ^{13}C NMR data of prototype compounds are given.

2.1.1. Antitubercular activity. The activity of the compounds against *M. tuberculosis* virulent strain H37Rv was determined in vitro. The minimum inhibitory concentration (MIC) of the test compounds that inhibit the colony forming ability of *M. tuberculosis* was determined by incorporating decreasing concentration of the test compound in Middle brook 7H10 agar medium supplemented with OADC. The activity is expressed in MIC as shown in Table 1. Compounds **5**–**7** inhibited growth at 12.5 μg/mL whereas compound **16** inhibited growth at 1.56 μg/mL concentrations. The MIC of these four compounds was confirmed in BACTEC.

As evident from the in vitro antitubercular activity profile of compounds (Table 1) antitubercular activities vary with the nature of hydroxy aminoalkyl chain as 1,2,3,4-bis-*O*-isopropylidene-α-D-galatopyranosyl moeity is common to all the compounds. Compounds with simple straight aminoalkyl chain offer better activity than furfuryl, benzyl or cyclic amine counterparts. Among the galactopyranosyl amino alcohols, compounds **5** and **16** with 12 carbon aminoalkyl chain showed better activity than other compounds. However, compound **16** having two galactopyranosyl units flanked by 18 carbon 1-[12-(2-hydroxy propyl amino)-dodecyl amino]-propan-2-ol moiety was more active

Table 1. In vitro antitubercular activities of compounds **3**–**16** on *M. tuberculosis*

Comp	MIC (μg/mL) against *M. tuberculosis* H37Rv
3	> 50
4	> 50
5	12.5
6	12.5
7	12.5
8	> 50
9	> 50
10	> 50
11	> 50
12	> 50
13	> 50
14	> 50
15	> 50
16	1.56
Ethambutol	3.25

than compound **5** (with only one galactopyranosyl residue) against *M. tuberculosis* H37Rv.

Since compound **16** was the most active compound against the virulent strain in vitro, it has also been screened against five clinical MDR strains of *M. tuberculosis* isolated from TB patients. The compound **16** was found effective against MDR strains in vitro at one concentration (50 μg/mL) while at the same concentration anti TB drugs were ineffective (Table 2).

2.2. In vivo activity

The activity of compound **16** was evaluated in vivo in experimental tuberculosis in mice as described previously.[24] The toxicity of the compound was determined in mice by giving single dose of different concentration, namely 100, 75, 50 and 25 mg/kg of the test compound. It was found that the compound was toxic to mice at 100, 75 and 50 mg/kg body weight whereas no toxicity was observed with even repeated doses of 25 mg/kg for 10 days. Hence, the efficacy of the compound **16** against challenge of *M. tuberculosis* H37Rv was tested at 25 mg/kg. Mice were infected intravenously via lateral veins with 10^7 CFU. Mice were divided into two groups of 10 mice each after 2 days of infection. One was of compound **16** treated by intraperitoneal (ip) route, whereas the other group served as untreated control. At 25 mg/kg dose, the compound gives a marginal protection (Fig. 1). The compound seems to protect mice at nontoxic concentration against *M. tuberculosis* infection. However, at higher doses it causes toxicity in mice. It will be intresting to prepare analogues of compound **16** which will be nontoxic to eukaryotes but are strongly antitubercular.

Table 2. In vitro activity of compound **16** (50 μg/mL) against MDR strains of *M. tuberculosis* H37 Rv isolated from TB patients

Compound or drug	Growth of MDR strains after 6 weeks				
	BC-248[a]	BC-243[a]	VA-101[b]	BC-426[c]	BC-437[c]
Compound **16**	−	−	+	−	−
Sparfloxicin	+	+ +	+ +	+ +	+ +
No drug control	+ +	+ +	+ +	+ +	+ +

−, no growth; +, 1–20 colonies; + +, heavy growth.
[a] Strains resistant to rifampicin, isoniazid, ofloxicin and ethambutol.
[b] Strains resistant to rifampicin, isoniazid and ethambutol.
[c] Strains resistant to rifampicin and isoniazid.

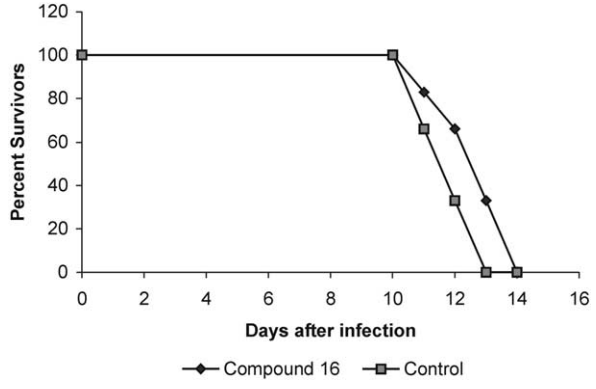

Figure 1. Comparative survival of untreated (control) and treated (compound **16**) Swiss mice infected with 10^7 CFU of *M. tuberculosis* H37Rv.

Table 3. Effect of compounds **3**–**16** on Fungi

Compd	Minimum inhibitory concn (MIC) in μg/mL against					
	1	2	3	4	5	6
3	> 50	25	> 50	50	> 50	> 50
4	> 50	25	> 50	50	> 50	> 50
5	12.5	1.56	6.25	6.25	6.25	25
6	25	6.25	25	12.5	25	25
7	50	6.25	50	25	50	> 50
8	> 50	25	> 50	50	> 50	> 50
9	> 50	25	> 50	50	> 50	> 50
10	> 50	12.5	> 50	25	> 50	> 50
11	> 50	25	> 50	50	> 50	> 50
12	> 50	25	> 50	50	> 50	> 50
13	> 50	50	> 50	50	> 50	> 50
14	> 50	25	> 50	50	> 50	> 50
15	> 50	12.5	50	50	> 50	50
16	> 50	12.5	50	50	> 50	> 50
Ketoconazole	0.001	0.12	0.50	0.25	0.25	0.12

1. *Candida albicans*; 2. *Cryptococcus neoformans*; 3. *Sporothrix schenckii*; 4. *Trichophyton mentagrophytes*; 5. *Aspergillus fumigatus*; 6. *Candida parapsilosis*.

2.2.1. In vitro antifungal activity. The in vitro antifungal activity of compounds was determined against *C. albicans, C. neoformans, Sporothrix schenckii, Trichophyton mentagrophytes, Aspergillus fumigatus* and *Candida parapsilosis* (ATCC-22019) by microbroth dilution technique as per guidelines of NCCLS.[25,26] The activity profile of the compounds is given in Table 3.

The in vitro screening against six fungal strains of all the compounds showed that only four compounds **5**, **6**, **7** and **16** were found to be active against almost all the fungi. Compound **16** the most potent antitubercular compound was found to be active against *C. neoformans,* one of the opportunistic pathogen during *M. tuberculosis* infection. Among the compounds **5**, **6** and **7**, only compound **5** was the best compound with MIC ranging from 1.56 to 25 μg/mL against different strains. It is interesting to note that all the compounds exhibited mild activity against *C. neoformans* and *T. mentagrophytes,* which are very important in clinical tuberculosis (Table 3).

3. Conclusion

In conclusion, we have synthesized galactopyranosyl amino alcohols having alkyl chain of varying length by a simple, practical and economical method. Compound **16** with an MIC value superior to clinically used drug ethambutol, active against MDR TB with antifungal activity offers a new lead to search better analogues which will be active in vivo too.

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22. *General procedure for the preparation of compound* **2**: A mixture of epichlorohydrin (60 mL), aq NaOH (50%, 100 mL) and tetra butyl ammonium bromide (6.0 g) was magnetically stirred at 0 °C for 30 min. To this stirring mixture diacetonide galactose (15 g, 57.6 mmol) was added slowly and stirring was continued at 0 °C for 3 h followed by 9 h stirring at 30 °C. The reaction mixture was poured over crushed ice. The reaction mixture was extracted with ethyl acetate (3×100 mL) and the organic layer was washed with aq NH_4Cl (10%, 2×50 mL), dried (Na_2SO_4) and evaporated under reduced pressure to get a crude product. The latter was purified by column chromatography using hexane:ethyl acetate (4:1) as eluent to give compound **2** as colourless oil. yield 90%,$[\alpha]_D$ −50.8 (*c*. 0.175, CH_3OH), FAB MS *m*/*z* 317 $[M+H]^+$ IR (film) ν_{max} 3399, 1630, 1459, 1380 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) δ 5.56 (d, *J* = 5.0 Hz, 1H, H-1), 4.62 (dd, *J* = 10.0 Hz and 2.8 Hz, 1H, H-3), 4.31 (m, 2H, H-2, H-4), 3.86 (m, 1H, H-5), 3.71 (m, 3H, H-6, H-1$_A$′), 3.59 (m, 1H, H-1′$_B$), 3.26 (m, 1H, H-2′), 2.89 (m, 1H, H-3′$_A$), 2.68 (m, 1H, H-3′$_B$), 1.53, 1.45 (each s, 6H, $C(CH_3)_2$), 1.33 (s, 6H, $C(CH_3)_2$). ^{13}C NMR (200 MHz, $CDCl_3$) δ 109.7, 108.9 ($\underline{C}(CH_3)_2$), 96.7 (C-1), 72.8, 72.6 (C-6), 72.2 (C-3), 71.5, 71.0 (C-2), 70.9, 70.6 (C-4), 70.4 (C-1′), 67.4, 67.1 (C-5), 51.1 (C-2′), 44.7 (C-3′), 26.4, 26.3 ($C(\underline{C}H_3)_2$), 25.3, 24.8 ($C(\underline{C}H_3)_2$).
23. *General procedure for the preparation of compound* **16**: A solution of compound **2** (1.5 g, 4.74 mmol) and 1,12- diaminododecane (0.47 gm, 2.37 mmol) in ethanol was magnetically stirred at 30 °C. Tetra butyl ammonium bromide (0.010 g) was added and the stirring continued for additional 12 h at the same temperature. The solvent was evaporated under reduced pressure and the residue obtained was purified by column chromatography using chloroform/methanol (98:2) as eluant to afford the desired compound as colourless oil. In a similar way, other compounds were prepared and purified. Physical data of some selected compounds **5**: Colourless oil, yield 80%, $[\alpha]_D$ −36.3 (*c*. 0.11, CH_3OH), FAB MS *m*/*z* 502 $[M+H]^+$ IR (film) ν_{max} 3394, 1628, 1457, 1379 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) δ 5.52 (d, *J* = 4.8 Hz, 1H, H-1), 4.57 (dd, *J* = 7.8 Hz and 2.9 Hz, 1H, H-3), 4.29 (m, 2H, H-2, H-4), 3.9 (m, 3H, H-5, H-6), 3.62 (m, 4H, H-1′ and H-3′), 2.60 (m, 3H, H-2′ and NCH_2), 1.53, 1.44 (s, 6H, $C(CH_3)_2$), 1.31 (s, 6H, $C(CH_3)_2$), 1.25 (s, 20H, CH_2's), 0.88 (t, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$) δ 109.7, 108.9 ($\underline{C}(CH_3)_2$), 96.7 (C-1), 74.3 (C-6), 71.5 (C-3), 71.0 (C-2), 70.9 (C-4), 70.3 (C-1′), 67.6, 67.2 (C-5), 58.3, 58.0 (C-3′), 56.1 (NCH_2), 32.2, 30.0, 29.7, 27.7, 27.0 (CH_2's), 26.3 ($C(\underline{C}H_3)_2$), 25.3, 24.8 ($C(\underline{C}H_3)_2$), 23.0 ($\underline{C}H_2CH_3$), 14.4 ($CH_2\underline{C}H_3$). **16**: colourless oil, yield 78%,$[\alpha]_D$ −46 (*c* 0.10, CH_3OH), FAB MS *m*/*z* 833 $[M+H]^+$ IR (film) ν_{max} 3394, 1628, 1457, 1379 cm^{-1}, ^1H NMR (200 MHz, $CDCl_3$) δ 5.53 (d, *J* = 5.0 Hz, 1H, H-1), 4.62 (dd, *J* = 7.8 Hz and 2.0 Hz, 1H, H-3), 4.31 (dd, *J* = 5.0 Hz and 2.0 Hz, 1H, H-2), 4.26 (d, *J* = 7.8 Hz, 1H, H-4), 3.98 (m, 1H, H-5), 3.68 (m, 1H, H-2′), 3.53 (m, 2H, H-6), 3.49 (m, 2H, H-1′), 2.71 (m, 2H, H-3′), 2.53 (m, 2H, NCH_2), 1.53, 1.44 (each s, each 3H, $C(CH_3)_2$), 1.33 (s, 6H, $C(CH_3)_2$), 1.25 (s, 10H, CH_2's). ^{13}C NMR (200 MHz, $CDCl_3$) δ 109.7, 109.6, 109.0, 108.9 ($\underline{C}(CH_3)_2$), 96.6 (C-1), 74.4, 74.2 (C-6), 71.5 (C-3), 71.0 (C-2), 70.9 (C-4), 70.7, 70.4 (C-1′), 68.2, 68.0 (C-5), 58.3, 57.9 (C-3′), 49.2 (NCH_2), 31.8, 30.5, 29.8, 27.7, 27.2, 29.7 (CH_2's), 26.4, 26.3 ($C(\underline{C}H_3)_2$), 25.3, 24.8 ($C(\underline{C}H_3)_2$).
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