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Design, synthesis, and evaluation of novel ethambutol analogues

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Abstract—Ethambutol is one of the front-line agents recommended by the World Health Organization for the treatment of tuberculosis. In an effort to develop more potent therapies to treat tuberculosis, novel unsymmetrical ethambutol analogues were successfully synthesized by a new route utilizing novel building blocks synthesized using Ellman's sulfinyl chemistry. The resulting analogues were tested for anti-tuberculosis activity yielding compounds with comparable anti-tuberculosis activity to ethambutol and increased lipophilicity that may instill better tissue penetration and serum half-life.

Isoniazid, rifampin, pyrazinamide, and ethambutol are the front-line agents that are recommended by World Health Organization (WHO) for the treatment of tuberculosis (TB).[1] The problems with the current TB treatment regimens are complex and include: a prolonged standard course regimen of 6 months, which often results in patient non-compliance; the emergence of extensively drug-resistant tuberculosis strains[2] (XDRTB); and the lack of effective drugs against the latent state. One approach to decrease the treatment time is to improve the potency of currently used anti-tuberculosis drugs. Wilkinson and coworkers from Lederle laboratories first reported the synthesis and activity of ethambutol (EMB) (**1**) (Fig. 1) in 1961.[3,4] EMB is primarily a bacteriostatic anti-tuberculosis agent. EMB targets the arabinosyl transferases responsible for arabinogalactan biosynthesis, a key component of the unique mycobacterial cell wall.[5–7] Despite modest anti-tuberculosis activity, EMB is used in combination with other front-line anti-tuberculosis agents mainly owing to its synergy with the other drugs and low toxicity. EMB is a simple diamine molecule that was synthesized by reacting 1,2-dihaloethane with chirally pure (*S*)-2- amino 1-butanol.[3,4] Based on the early SAR study it appears that the distance between the two nitrogens, the presence of two hydroxyl groups, and small side chains are the key pharmacophoric elements.[8] The chirality of the molecule is also very crucial in determining the activity, as EMB, the (*S*,*S*) isomer is approximately 500 times more potent than the (*R*,*R*) isomer.[8] Recently, novel EMB analogues **2**, **3**, and **4** were found to be active against *Mycobacterium smegmatis* but the corresponding anti-tuberculosis activity was not reported for these compounds.[9] Lee et al. recently synthesized a large library of asymmetrical 1, 2 diamines using combinatorial chemistry to explore the SAR around the diamine pharmacophore. In this process SQ 109 (**5**) was identified as the most active compound possessing 35-fold improved activity when compared to EMB.[10] SQ109 has recently advanced into clinical trials for the treatment of tuberculosis, though it appears that SQ109 does not have the same target as EMB as originally intended.[11]

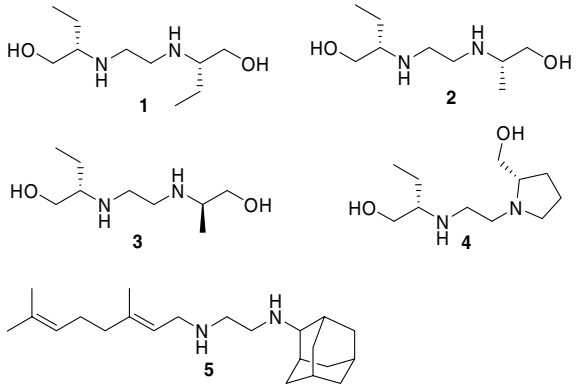

Figure 1. Structures of ethambutol and its analogues.

In this study we further expand the SAR of unsymmetrical EMB derivatives by taking advantage of new chemistries and building blocks that were not previously available to synthesize asymmetric and constrained

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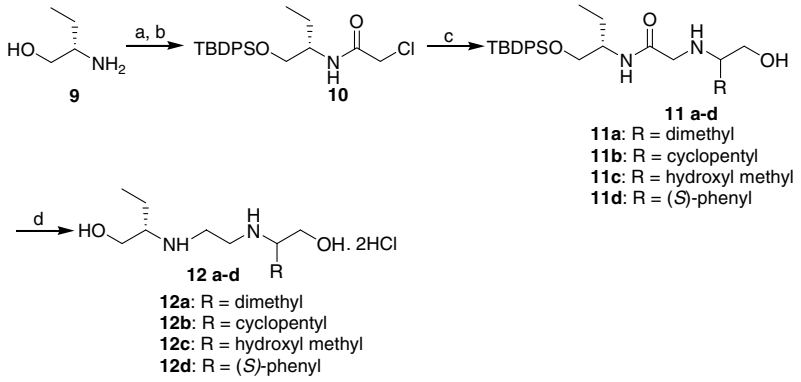
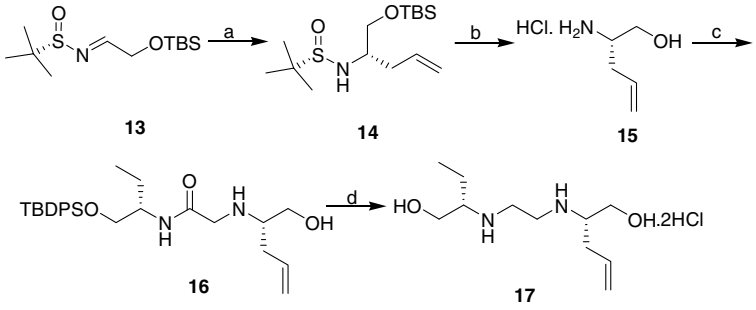
analogues. Accordingly, novel EMB analogues were synthesized and tested with an aim not only to improve the anti-tuberculosis activity but also with a view toward improving the pharmacokinetic profile of the emerging compounds. Initially, EMB hydrobromide salt **6**, and its symmetrical analogues **7**,[12,13] and **8**[14] were synthesized by reacting amino alcohols with 1, 2-dihaloethanes.[3] These compounds were synthesized as standards and the activities of these compounds were compared with the novel unsymmetrical derivatives described below.

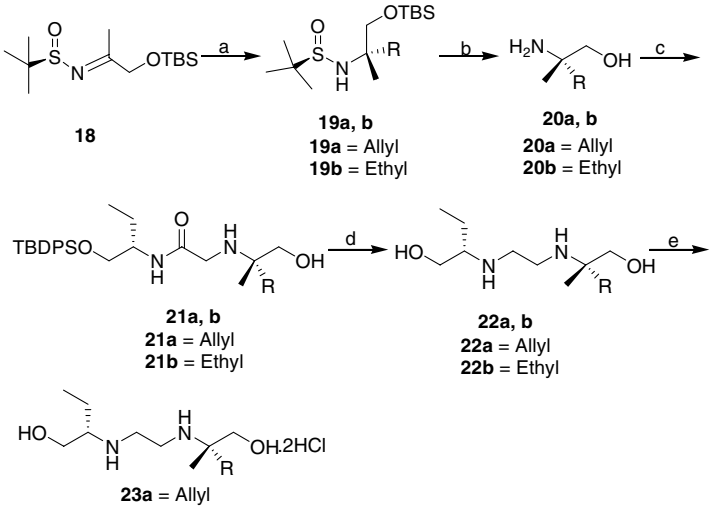
To evaluate the structural importance of the side chains and to explore non-commercially available amino alco-



Scheme 1. Reagents and conditions: (a) TBDPSiCl, imidazole, CH_2Cl_2, rt, 16 h, 97%; (b) chloroacetylchloride, DIPEA, CH_2Cl_2, rt, 16 h, 51%; (c) amino alcohols, DIPEA, DMF, 70 °C, 14 h, 63–90%; (d) i—$LiAlH_4$, THF, reflux, 16 h; ii—1.25 M Hydrogen chloride–methanol solution, rt, 1 h, 11–45% yields.



Scheme 2. Reagents and conditions: (a) Allyl magnesium bromide, toluene, −78 °C, 3 h, 67%; (b) 4 M hydrogen chloride dioxan solution, rt, 1 h, 91%; (c) **10**, DIPEA, DMF, 70 °C, 14 h, 87%; (d) i—$LiAlH_4$, THF, reflux, 16 h; ii—1.25 M hydrogen chloride–methanol solution, rt, 1 h, 15%.

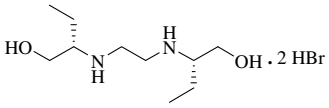
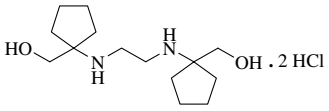
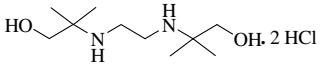
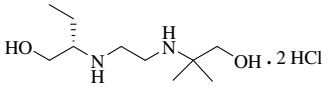
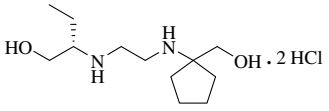
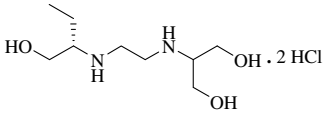
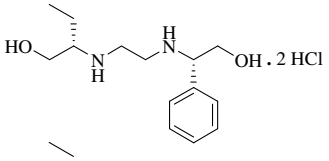
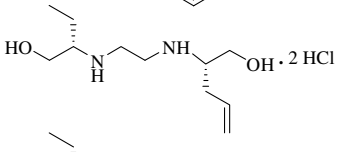
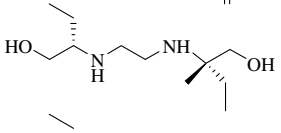
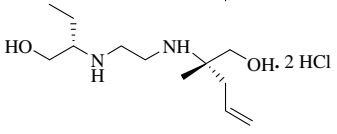


Scheme 3. Reagents and conditions: (a) AllylMgBr, or EtMgCl, toluene, −78 °C, 3 h, 46% (**19a**), 48% (**19b**); (b) i—4 M hydrogen. chloride dioxan solution, rt, 1 h; ii—Et_3N, rt, 1 h (c) **10**, DIPEA, DMF, 70 °C, 14 h, 45% (**21a**), 29% (**21b**); (d) $LiAlH_4$, THF, reflux, 16 h, 56% (**22b**); (e) 1.25 M hydrogen chloride–methanol solution, 19% yield from **21a** to **23a**.

hol building blocks, seven unsymmetrical EMB analogues **12a–d**, **17**, **22b**, and **23a** were synthesized. Synthesis of **12a–d** was achieved by a novel synthetic route as depicted in Scheme 1. In the first step alcohol **9** was protected using TBDPSiCl in the presence of imidazole in DCM to afford silyl-protected intermediate in 97% yield.[15] N-acylation of this intermediate was achieved using chloroacetylchloride in the presence of DIPEA in DCM to give α-halo amide **10** in 51% yield.[16] This was subsequently reacted with a variety of commercially available amino alcohols in the presence of DIPEA in DMF at 70 °C for 14 h to afford respective amides **11a–d** in 63–90% yields.[10] TBDPS deprotection and amide reduction were achieved in a single step using an excess of $LiAlH_4$ at reflux temperatures for 16 h to yield free amino alcohols, which were subsequently converted into hydrochloride salts **12a–d** in 11–45% yields.[20]

The synthesis of *S*-amino alcohol **17** was performed as described by Ellman and co-workers[17] as depicted in Scheme 2. (*S*)-*tert*-butanesulfinamide[18] was reacted with (*tert*-butyldimethylsilyloxy) acetaldehyde in the presence of $CuSO_4$ in DCM at room temperature for 24 h to yield aldimine **13**.[19] Aldimine **13** was reacted with 1.0 M allyl magnesium bromide diethyl ether solution in THF at −78 °C for 3 h to give intermediate **14** in 67% yield. N and O deprotection was achieved using HCl dioxan solution to afford 2(*S*)-2-amino-pent-4-en-1-ol hydrochloride **15** in 91% yield.[17] This was converted into **16** by reacting with intermediate **10** and subsequent reac-

Table 1. Structures of ethambutol analogues and their anti-tuberculosis activity

No.	Structures	*M. tb* H37Rv MIC_{90} μg/mL[21]	*C*Log *P*[a]
6 EMB		0.8	0.11
7		3.12	1.36
8		6.25	−0.14
12a		1.6	−0.01
12b		1.6	0.74
12c		3.12	−0.75
12d		50	0.84
17		25	0.16
22b		3.12	0.51
23a		6.25	0.56

[a] *C*Log *P* was calculated using the ChemDraw Ultra, version 7, software by Cambridge Soft.

tions were performed as described in Scheme 2 to afford compound **17**.

To evaluate the effect of β,β-disubstitution of the amino alcohol unit for anti-tuberculosis activity, compounds **23a** and **22b**[20] were synthesized by applying Ellman's sulfinyl chemistry as depicted in Scheme 3. (*R*)-*tert*-butanesulfinamide[18] was reacted with 1-{[*tert*-butyl(dimethyl)silyl] oxy}acetone in the presence of $Ti(OEt)_4$ in THF at 70 °C to give ketamine **18**,[19] which was subsequently treated with allyl magnesium bromide and ethyl magnesium chloride to give intermediate **19a** (46% yield) and **19b** (48% yield). N and O deprotection was achieved using HCl dioxan solution to yield **20a** and **20b**.[17] Final products **23a** and **22b** were obtained by reacting amino alcohols **20a** and **20b** with intermediate **10** and performing subsequent reactions as described in Scheme 3.

To establish the structure–activity relationship (SAR) of EMB, compounds in Table 1 were tested for their anti-tuberculosis MIC_{90} activity against *M. tuberculosis* $H_{37}Rv$.[21] Our resynthesized EMB standard (**6**) had an MIC of 0.8 μg/mL. Symmetrical compounds with cyclopentyl side chain (**7**) and dimethyl (**8**) side chain were less active than that of the EMB salt (**6**) which is consistent with previous reports.

Unsymmetrical compounds in which one-half of the ethyl side chain of EMB was replaced with smaller dimethyl (**12a**), cyclopentyl (**12b**), and hydroxy methyl **(12c)** side chains, resulted in MIC values in between EMB and the corresponding symmetrical derivatives (**8** and **7**). Replacement of the ethyl side chain with larger (*S*) allyl (**17**) and (*S*) phenyl (**12d**) side chains had a large detrimental effect on activity. Interestingly, the replacement of hydrogen at the chiral carbon of the amino alcohol section of (**17**) with a methyl group (**23a**) resulted in increased anti-tuberculosis activity. However, the analogous substitution to the chiral center of EMB (**6**) reduced anti-tuberculosis activity (**22b**). The tight SAR observed for the compounds in this study is consistent with previous reports for ethambutol analogues in the literature.[8,9] Therefore, careful attention must be paid when designing new potential EMB analogues. Currently we believe that modifications that also seek to improve the pharmacokinetic properties of EMB rather than simply the improve the anti-tuberculosis activity may be a more productive approach. The *C*Log *P* values for the compounds in this study were estimated using ChemDraw Ultra and are reported in Table 1. Most of the synthesized novel compounds had higher *C*Log *P* values than that of EMB indicating that they may have better pharmacokinetic profiles leading to an increase in cerebrospinal fluid (CSF) penetration, serum binding and serum half-life. EMB analogues with these properties may be more useful the treatment of CSF infections than EMB, which has limited application due to moderate penetration into the CSF.

In summary we have developed a new route for the synthesis of novel EMB analogues. In future, using this new synthetic route many more side chain modifications can be explored in detail. Even though none of the molecules that were synthesized in this study improved upon the activity of EMB, some of these molecules did have comparable in vitro activity. These compounds now require further in vivo testing. If these compounds retain their activity in vivo and have better pharmacokinetic properties such as better absorption and half-life than that of EMB then they can be considered as an alternative for replacement of EMB in anti-tuberculosis drug therapy.

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References and notes

1. World Health Organization. http://whqlibdoc.who.int/hq/2003/WHO_CDS_TB_2003.313_eng.pdf.
2. Shah, N. S.; Wright, A.; Bai, G.-H.; Barrera, L.; Boulahbal, F.; Martin-Casabona, N.; Drobniewski, F.; Gilpin, C.; Havelkova, M.; Lepe, R.; Lumb, R.; Metchock, B.; Portaels, F.; Rodrigues, M. F.; Rusch-Gerdes, S.; Van Deun, A.; Vincent, V.; Laserson, K.; Wells, C.; Cegielski, J. P. *Emerging Infect. Dis.* **2007**, *13*, 380.
3. Wilkinson, R. G.; Shepherd, R. G.; Thomas, J. P.; Baughn, C. *J. Am. Chem. Soc.* **1961**, *83*, 2212.
4. Thomas, J. P.; Baughn, C. O.; Wilkinson, R. G.; Shepherd, R. G. *Am. Rev. Respir. Dis.* **1961**, *83*, 891.
5. Lee, R. E.; Mikusova, K.; Brennan, P. J.; Besra, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 11829.
6. Zhang, J.; Khoo, K. H.; Wu, S. W.; Chatterjee, D. *J. Am. Chem. Soc.* **2007**, *129*, 9650.
7. Alderwick, L. J.; Seidel, M.; Sahm, H.; Besra, G. S.; Eggeling, L. *J. Biol. Chem.* **2006**, *281*, 15653.
8. Shepherd, R. G.; Baughn, C.; Cantrall, M. L.; Goodstein, B.; Thomas, J. P.; Wilkinson, R. G. *Ann. NY. Acad. Sci.* **1966**, *135*, 686.
9. Hausler, H.; Kawakami, R. P.; Mlaker, E.; Severn, W. B.; Stutz, A. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1679.
10. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E., III *J. Comb. Chem.* **2003**, *5*, 172.
11. Boshoff, H. I.; Myers, T. G.; Copp, B. R.; McNeil, M. R.; Wilson, M. A.; Barry, C. E., 3rd *J. Biol. Chem.* **2004**, *38*, 40174.
12. Cremieux, A.; Baghdadi, N.; Berthelot, P.; Debaert, M. *Ann. Microbiol. (Paris)*. **1983**, *134A*, 177.
13. Berthelot, P.; Debaert, M.; Cremieux, A.; Baghadi, N. *Farmaco, Edizione Scientifica.* **1983**, *38*, 73.
14. Wilkinson, R. G.; Cantrall, M. B.; Shepherd, R. G. *J. Med. Pharmaceut. Chem.* **1962**, *5*, 835.
15. Brickmann, K.; Yuan, Z.; Sethson, I.; Somfai, P.; Kihlberg, J. *Chem. Eur. J.* **1999**, *5*, 2241.
16. Kim, K. S.; Kimball, S. D.; Misra, R. N.; Rawlins, D. B.; Hunt, J. T.; Xiao, H.-Y.; Lu, S.; Qian, L.; Han, W.-C.; Shan, W.; Mitt, T.; Cai, Z.-W.; Poss, M. A.; Zhu, H.; Sack, J. S.; Tokarski, J. S.; Chang, C. Y.; Pavletich, N.; Kamath, A.; Humphreys, W. G.; Marathe, P.; Bursuker, I.; Kellar, O. K. A.; Roongta, U.; Batorsky, R.; Mulheron, J. G.; Bol, D.; Fairchild, C. R.; Lee, F. Y.; Webster, K. R. *J. Med. Chem.* **2002**, *45*, 3905.
17. Tang, T. P.; Volkman, S. K.; Ellman, J. A. *J. Org. Chem.* **2001**, *66*, 8772.

18. Liu, G.; Cogan, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1997**, *119*, 9913.
19. Liu, G.; Cogan, D. A.; Owens, T. D.; Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 1278.
20. Analytical data for a representative compounds Compound **12a**: ^{1}H NMR (500 MHz, D_2O): 0.87 (3H, t, J = 7.56 Hz), 1.22 (6H, s), 1.54–1.72 (2H, m), 3.17 (1H, sextet), 3.26–3.32 (2H, m), 3.34–3.42 (2H, m), 3.51 (2H, s), 3.66 (1H, dd, J = 5.12, 12.93 Hz), 3.78 (1H, dd, J = 3.17, 12.93 Hz); ESI-MS: 205.2 (M+1). $[\alpha]_D^{25.7}$ +5.7 (c = 1.25 %, MeOH). Anal. Calcd for $C_{10}H_{26}Cl_2N_2O_2$: C, 43.32; H, 9.45; N, 10.1. Found: C, 43.23; H, 9.35; N, 9.52. Compound: **12b**: ^{1}H NMR (500 MHz, D_2O): 0.87 (3H, t, J = 7.32 Hz), 1.54–1.82 (10H, m), 3.18 (1H, sextet), 3.28–3.34 (2H, m), 3.36–3.42 (2H, m), 3.55 (2H, s), 3.66 (1H, dd, J = 5.37, 13.18 Hz), 3.79 (1H, dd, J = 3.17, 12.93 Hz); ESI-MS: 231.2 (M+1). $[\alpha]_D^{26.4}$ +3.1 (c = 1 %, MeOH). Anal. Calcd for $C_{12}H_{28}Cl_2N_2O_2$: C, 47.52; H, 9.31; N, 9.24. Found: C, 47.51; H, 9.23; N, 9.1. Compound: **22b**: ^{1}H NMR (500 MHz, D_2O): 0.74 (3H, t, J = 7.56 Hz), 0.79 (3H, t, J = 7.56 Hz), 0.88 (3H, s), 1.36–1.44 (4H, m), 2.48 (1H, pentet), 2.52–2.68 (4H, m), 3.32–3.38 (2H, m), 3.41 (1H, dd, J = 5.85, 11.47 Hz), 3.51 (1H, dd, J = 4.63, 11.47 Hz); ESI-MS: 219.2 (M+1); $[\alpha]_D^{26.3}$=+10.0 (c = 1%, MeOH). Anal. Calcd for $C_{11}H_{26}N_2O_2$: C, 60.51; H, 12.0; N, 12.83. Found: C, 60.58; H, 12.1; N, 12.74. Compound: **23a**: ^{1}H NMR (500 MHz, D_2O): 0.87 (3H, t, J = 7.56 Hz), 1.21 (3H, s), 1.54–1.71 (2H, m), 2.37 (2H, d, J = 7.56 Hz), 3.17 (1H, sextet), 3.3-3.4 (4H, m), 3.5–3.62 (2H, m), 3.66 (1H, dd, J = 5.37, 13.18 Hz), 3.78 (1H, dd, J = 3.17, 12.93 Hz), 5.16-5.24 (2H, m), 5.68-5.78 (1H, m); ESI-MS: 217.2 (M+1).
21. MIC values were determined against *M. tuberculosis* H37Rv by the microbroth dilution method. A broth culture of *M. tuberculosis* was grown in Middlebrook 7H9 medium with 10% ADC supplement to an OD_{600} of 0.4–0.6. The culture was diluted with 7H9 medium to an OD_{600} of 0.01, and 100 μL was added to a microtiter plate containing twofold serial dilutions of the ethambutol analogues for a final volume of 200 μL. The plates were incubated at 37 °C for 7 days. The MIC_{90} was determined by visual inspection and defined as the concentration that inhibited 90% of growth.