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Suryanarayan Cherukuvada, and Ashwini Nangia

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Salts and Ionic Liquid of The Anti-Tuberculosis Drug *S,S*-Ethambutol

Suryanarayan Cherukuvada and Ashwini Nangia*
School of Chemistry, University of Hyderabad, Prof. C. R. Rao Road, Central University PO, Gachibowli, Hyderabad 500 046, India
E-mail: ashwini.nangia@gmail.com

Abstract

A salt screen of the anti-tuberculosis chiral basic drug Ethambutol with protic acids resulted in the formation of several salts and an ionic liquid. The protic salt/ionic liquid product was characterized by spectroscopic (ATR-IR and ss-NMR), thermal (DSC and TGA) and X-ray diffraction. Similar to the marketed Ethambutol dihydrochloride salt of the drug, all the new salts were found to be hygroscopic. Moisture-free conditions of desiccator and rotavapor gave non-hygroscopic materials in a few cases. X-ray crystal structures of two new salts were determined and that of Ethambutol base and Ethambutol dihydrochloride salt were redetermined in this work.

Introduction

Ethambutol (abbreviated as EMB; Figure 1) is the first-line anti-tuberculosis drug administered with Rifampicin, Isoniazid and Pyrazinamide in Fixed Dose Combinations (FDC).[1] It is a chiral basic drug (Figure 1) with the therapeutically active *S*,*S*-enantiomer whereas the *R*,*R*-form is inactive.[2] It is formulated as the dihydrochloride salt (*S*,*S*-EDH) which is synonymous with the drug.[1,3] Anti-TB FDC products are reported to be unstable due to drug–drug interactions between the component drugs.[3] The hygroscopicity of ethambutol dihydrochloride catalyzes the degradation of rifampicin and isoniazid in the FDC formulation, resulting in the loss of drug potency upon storage.[1a,3] As a consequence, individual drugs of the FDC formulation must be separately coated with polymers to avoid mutual interactions and water uptake, and then blended to make up the final product formulation.[4] Several salts of ethambutol base (dinitrate,[5] dihydrobromide,[6] oxalate pentahydrate,[7] and metal salts[8]) including a drug–drug methanesulfonic acid salt with isoniazid[9] are reported but without any discussion of their physico-chemical properties. The dihydrochloride salt of ethambutol is tetramorphic,[10] but neither the X-ray crystal structure nor any polymorphs of *S*,*S*-ethambutol base are reported.

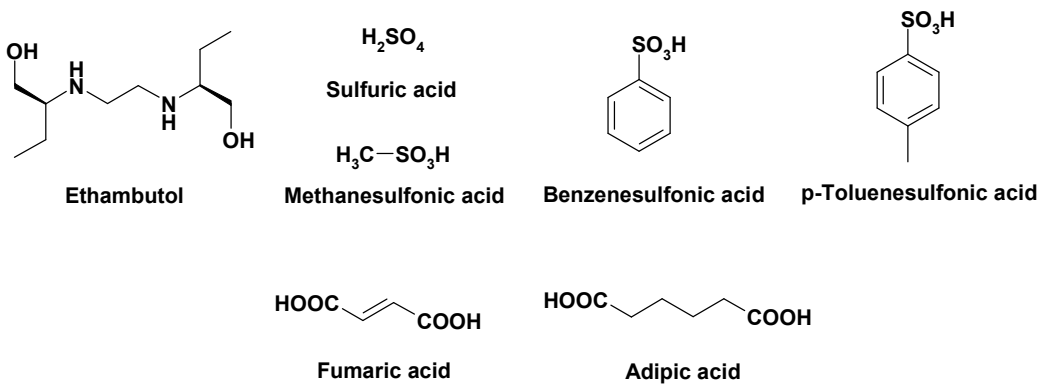


Figure 1 Molecular structure of ethambutol and protic acids used in this study.

In an attempt to solve the hygroscopicity problem of S,S-EDH, we performed a salt screen of EMB base with several salt-forming protic acids (Figure 1) to find new salts having lower hygroscopicity. We obtained five salts (sulfate, dimesylate, ditosylate, dibesylate, and fumarate) and two ionic liquids (dibenzoate[11] and adipate), but these new products were also found to be hygroscopic. Trimorphs of ethambutol dibenzoate ionic liquid were reported in a preliminary communication.[11] Ionic liquids (ILs) are salts with melting point below 100 °C[12] and if they are liquids at room temperature (m.p. ≤ 25 °C) then they are termed as 'room temperature ionic liquids' (RTILs).[13] The hygroscopic nature of the salts and ionic liquids was assessed by visual inspection and Karl Fischer (KF) titration. Out of the six salts, including the ionic liquid, of this report, two salts crystallized as diffraction quality single crystals (dimesylate and fumarate hydrate), and their X-ray crystal structures are reported.

Results and Discussion

The salts were prepared by different methods such as (i) direct reaction of the base (EMB) with an acid (the salt former) in a suitable solvent, (ii) co-grinding,[14] (iii) co-melting, (iv) solution crystallization, (v) crystallization in desiccator or rotavapor, etc. A summary of the experimental conditions is given in Table 1 and the methodology is detailed in the Experimental Section. All the product salts were obtained both in solid and liquid state (due to hygroscopic nature) in these experiments. The dimesylate, ditosylate, dibesylate and fumarate EMB salts crystallized as solids in a majority of experiments, whereas sulfate and adipate crystallized in fewer conditions (Table 1). Out of the six salts, five are high melting solids (sulfate, dimesylate, ditosylate, dibesylate and fumarate; m.p. > 100 °C) and one is an ionic liquid (adipate; m.p. 87 °C). The physical characterization of the protic salt/ionic liquid[15] was done by spectroscopic (ATR-IR and ss-NMR), thermal (DSC and TGA) and X-ray diffraction.

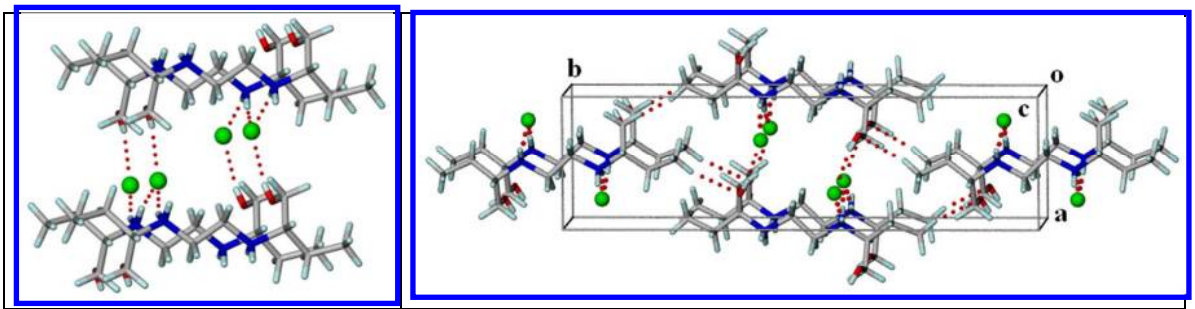
Table 1 Summary of salt screening experiments.

S. No.	Salt	Synthesis by direct reaction of base and acid	Solution crystallization in MeOH/ EtOH	Crystallization in desiccator	Crystallization in rotavapor	Co-grinding	Co-melting	Storage of physical mixture
1	Ethambutol sulfate	solid product[a]	viscous liquid	viscous liquid	semisolid	viscous liquid[b]	[c]	[c]
2	Ethambutol dimesylate	solid product[d]	solid[d]; single crystals at –20 °C	solid[d]	solid[d]	slight sticky solid[b]	-do-	-do-
3	Ethambutol dibesylate	-do-	solid[d]	-do-	liquid extract	viscous liquid[e]	semisolid	semisolid
4	Ethambutol ditosylate	-do-	-do-	-do-	solid[d]	-do-	-do-	-do-
5	Ethambutol fumarate (hydrate)	-do-	solid[d]	single crystals	semisolid	-do-	[f]	-do-
6	Ethambutol adipate	solid product[a]	viscous liquid	semisolid	-do-	-do-	semisolid	-do-

[a] Tends to liquefy during extraction upon concomitant exposure to atmospheric moisture, but crystalline solid by PXRD; [b] Acetone-assisted grinding; [c] Not feasible as the acid component is liquid; [d] Crystalline solid by PXRD; [e] Neat grinding and concomitant exposure to atmospheric moisture; [f] Not done since the acid component decomposes upon melting.

X-ray crystal structures

(+)-Ethambutol is a chiral molecule with the *S*,*S*-configuration (Figure 1). The reported crystal structures of EMB and its salts (dihydrochloride, dinitrate, dibromide, oxalate pentahydrate) in the Cambridge Structural Database[16] have no mention of the Flack parameter.[17] In this study, we inferred the absolute configuration of the drug by specific rotation measurement and the Flack parameter for the X-ray crystal structure of ethambutol dihydrochloride (EDH), salt which is the starting material in our experiments. Commercial EDH was neutralized to ethambutol base for making new salts (detailed in the Experimental Section). There is no information on the stereochemistry of the salt on the commercial package label. The chirality of the base and the dihydrochloride salt were found to be *S*,*S* by matching their specific rotation with the reported values ($[\alpha]_D^{25}$: ethambutol expt. +13.4° (+13.7°), ethambutol dihydrochloride expt. +7.8° (+7.6°).[18] A reliable Flack parameter to infer the absolute structure[17] was obtained for the dihydrochloride and dimesylate salts because they contain a heavy atom Cl and S (atomic number > Si). The Flack parameter from the X-ray crystal structure is 0.09(17) and –0.04(16), respectively, which confirms the assigned *S*,*S*-chirality for the salts. X-ray crystallographic parameters are listed in Table 2 and hydrogen bonds in Table 3. The space group and crystallographic features of the dihydrochloride salt (EDH) are the same as those for the reported structure[10,19] (CCDC Refcode CURJEE02, CURJEE)[16] (Figure 2). The Flack parameter of EMB base structure [–0.4(3)] is not reliable due to the absence of heavy atoms, but the absolute chirality is assumed to be the same as that in the salt. The crystal structure of EMB matches with that reported[20] for CCDC Refcode GEJHOT[16] (Figure 3).



(a) **(b)**

Figure 2 (a) N–H$^+$···Cl$^-$ and O–H···Cl$^-$ interactions connect translation related molecules along the *c*-axis in *S*,*S*-EDH. (b) Screw related molecules form zigzag tapes through C–H···O interactions along the *b*-axis which further extend into 2D sheets.

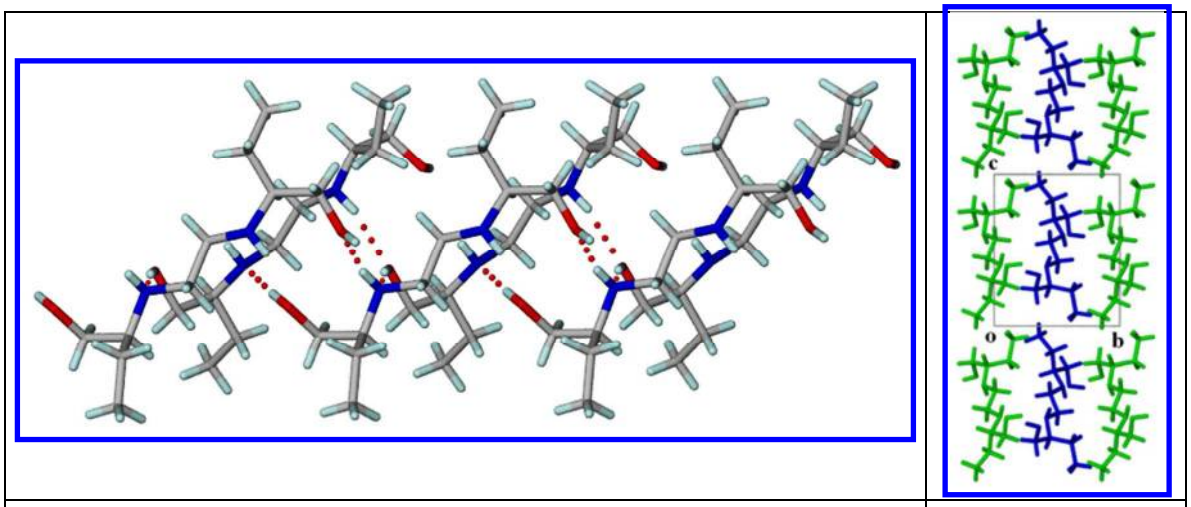


Figure 3 (a) Translation related molecules form a tape through N–H···O bonds in *S*,*S*-EMB. Screw related molecules through O–H···N bonds make parallel tapes which extend into (b) 2D sheets (shown in different color) along the *c*-axis through close pack.

[*S*,*S*]-Ethambutol dimesylate: A 1:2 salt of ethambutol and methanesulfonic acid was crystallized from methanol at –20 °C (Table 1). The X-ray crystal structure showed protonation of the secondary amine NHs of ethambutol by methanesulfonic acid. Linear tapes of ethambutol cations and mesylate anions are bonded via N^{+}–H···O^{-} and O–H···O hydrogen bonds (Figure 4a). These tapes make alternating sheets of drug and mesylate molecules (Figure 4b) such that there are strong heteromolecular interactions between the drug and mesylate ions.

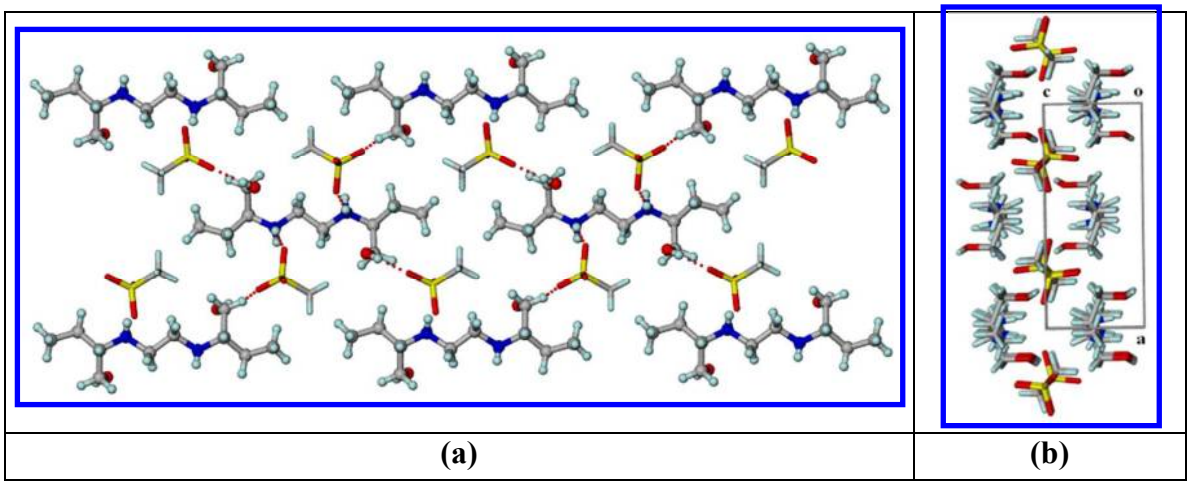


Figure 4 (a) Linear tapes of translation-related ethambutol molecules and screw-related mesylate ions are connected by N^{+}–H···O^{-} and O–H···O interactions. **(b)** Alternate sheets of ethambutol and mesylate molecules along the *a*-axis.

[*S*,*S*]-Ethambutol fumarate hydrate (2:2:1.145): A salt hydrate was obtained when ethambutol and fumaric acid were crystallized in 1:1 ratio from methanol in a desiccator. The same salt hydrate also crystallized at –20 °C (Table 1). The X-ray crystal structure shows two molecules of ethambutol and fumarate, one water molecule and a second water with 0.145 site occupancy of O atom (s.o.f. was derived from the electron density refinement). Each fumaric acid donates proton to ethambutol molecules lying above and below (Figure 5). The ethambutol molecules form parallel tapes along the *b*-axis through N^+–H···O^- and O–H···O bonds. Water molecules are connected through O–H···O^- and C–H···O bonds to fumarate and ethambutol to make channels along the *c*-axis.

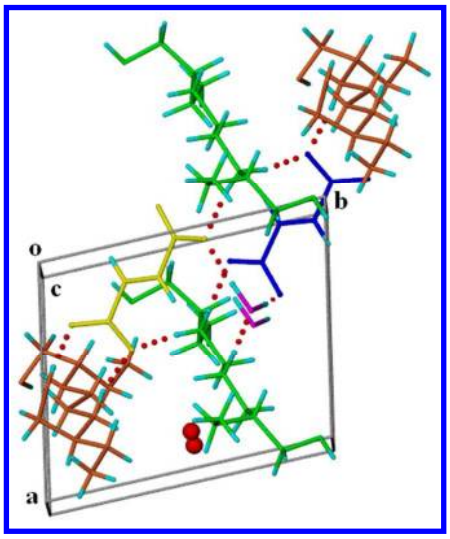


Figure 5 Linear tapes of ethambutol and fumarate crystallographic unique molecules (shown in different color) along the *b*-axis propagate through N^+–H···O^- and O–H···O interactions. Water molecules (magenta and red of 1.0 and 0.145 s.o.f.) form channels parallel to the *c*-axis.

Table 2 Crystallographic parameters.

Compound	Ethambutol dihydrochloride	Ethambutol	Ethambutol dimesylate	Ethambutol fumarate hydrate
empirical formula	$C_{10}H_{26}Cl_2N_2O_2$	$C_{10}H_{24}N_2O_2$	$C_{12}H_{32}N_2O_8S_2$	$C_{28}H_{58}N_4O_{13}$
formula weight	277.23	204.31	396.52	658.78
crystal system	orthorhombic	monoclinic	orthorhombic	triclinic
space group	$P2_12_12$	$P2_1$	$P2_12_12$	$P1$
Z*	6	2	6	5
T/K	100(2)	298(2)	100(2)	100(2)
a/Å	6.432(2)	7.1428(3)	12.239(3)	8.5813(6)
b/Å	22.966(9)	8.4149(3)	15.151(3)	10.5252(8)

c/Å	5.0934(19)	10.1973(4)	5.2980(11)	10.9210(8)
α/°	90	90	90	70.6260(10)
β/°	90	95.633(4)	90	68.8720(10)
γ/°	90	90	90	73.8670(10)
V/Å^3	752.4(5)	609.96(4)	982.4(3)	853.82(11)
D_{calc}/g cm^{-3}	1.224	1.112	1.340	1.281
μ/mm^{-1}	0.423	0.613	0.310	0.101
reflns. collected	2891	3541	10263	8775
unique reflns.	1445	2201	1951	6434
observed reflns.	1282	2157	1763	6382
$R_1[I > 2\sigma(I)]$	0.0588	0.0506	0.0646	0.0326
wR_2 [all]	0.1378	0.1438	0.1242	0.0859
goodness-of-fit	1.032	1.075	1.156	1.041
flack parameter	0.09(17)	–0.4(3)	–0.04(16)	0.1(5)
diffractometer	Bruker Smart-Apex	Oxford Xcalibur Gemini	Bruker Smart-Apex	Bruker Smart-Apex

* $Z = Z''$ (Number of crystallographically non-equivalent molecules of any type in the asymmetric unit)[21] × Number of independent general positions of the space group.

Table 3 Hydrogen bonds in the crystal structures.

Interaction	**H···A/Å**	**D···A/Å**	**∠D–H···A/°**	**Symmetry code**
Ethambutol dihydrochloride				
N1–H1A···Cl1	2.20(5)	3.046(4)	161(4)	*
N1–H1B···Cl1	2.35(5)	3.134(4)	159(4)	x, y, –1+z
O1–H1···Cl1	2.32(7)	3.055(3)	169(7)	–1+x, y, –1+z
C3–H3···O1	2.27(5)	3.234(5)	160(4)	x, y, 1+z
C1–H1D···O1	2.69	3.652(5)	165	–1/2+x, 1/2+y, –z
Ethambutol				
N2–H2···O1	2.22(2)	3.00(2)	156(2)	1+x, y, z
O1–H1D···N2	1.79(4)	2.763(2)	168(3)	1–x, 1/2+y, 1–z
O2–H2C···N1	1.98(3)	2.874(2)	176(3)	2–x, 1/2+y, 1–z
Ethambutol dimesylate				
N1–H1A···O2	1.92(4)	2.744(4)	159(3)	*
N1–H1B···O2	2.48(3)	3.060(4)	128(3)	x, y, –1+z
N1–H1B···O3	2.06(4)	2.864(4)	168(3)	x, y, –1+z
O1–H1···O4	1.98(5)	2.733(4)	176(4)	1/2+x, 1/2–y, 2–z
C5–H5A···O3	2.53	3.457(5)	155	1/2+x, 1/2–y, 2–z
C1–H1C···O3	2.64	3.621(6)	177	1/2–x, –1/2+y, 2–z
C6–H6B···O3	2.55	3.450(5)	152	1/2+x, 1/2–y, 1–z

C6–H6C···O1	2.69	3.543(5)	145	1–x, –y, z
Ethambutol fumarate hydrate				
N1–H1A···O3	1.87(3)	2.688(2)	158(2)	*
N1–H1B···O9	2.07(2)	2.881(2)	155(2)	*
N2–H2A···O11	1.80(3)	2.693(2)	167(2)	x, 1+y, z
N2–H2B···O5	1.90(3)	2.845(3)	166(3)	x, 1+y, z
N3–H3A···O9	1.82(3)	2.742(2)	166(2)	*
N3–H3B···O12	1.82(2)	2.701(2)	172(2)	1+x, y, z
N4–H4C···O4	1.84(2)	2.689(2)	168(2)	x, 1+y, z
N4–H4D···O5	1.77(3)	2.686(2)	164(2)	1+x, 1+y, z
O1–H1···O6	1.78(4)	2.692(2)	169(3)	x, 1+y, z
O2–H2···O10	1.82(2)	2.648(2)	175(2)	1+x, –1+y, z
O7–H7A···O10	1.88(3)	2.684(2)	173(3)	*
O8–H8···O7	1.90(3)	2.782(2)	177(2)	x, y, 1+z
O13–H13A···O11	1.82(3)	2.897(2)	169(2)	x, y, 1+z
O13–H13B···O4	1.59(4)	2.744(2)	173(4)	x, y, 1+z
C1–H1D···O13	2.63	3.136(3)	112	*
C5–H5A···O14	2.70	3.650(1)	159	x, y, 1+z
C6–H6A···O4	2.55	3.348(2)	138	*
C6–H6B···O9	2.42	3.225(2)	138	*
C9–H9C···O14	2.54	3.153(9)	121	x, y, –1+z
C18–H18B···O2	2.42	3.347(2)	156	x, 1+y, 2+z
C19–H19A···O13	2.46	3.362(2)	151	x, 1+y, 2+z
C23–H23A···O7	2.67	3.471(3)	139	x, y, 1+z
C26–H26···O6	2.59	3.251(2)	127	x, 1+y, z

* Molecules/ions in the same asymmetric unit.

Spectroscopic and Thermal analysis

A salt screen of EMB gave both solid and liquid products (Table 1) which were characterized as salts by ATR-IR spectroscopy. A shift in the vibrational frequency for the product compared to the starting materials indicates the formation of a multi-component adduct.[22] For example, the characteristic C=O absorption peak of COOH group at 1700 cm^{-1} disappeared and gave two peaks in the IR spectrum of the salt at 1550 and 1400 cm^{-1} for the asymmetric and symmetric stretch of COO^- group[22] for ethambutol adipate ionic liquid (Figure 6). Similarly, shifts in S=O vibrations of SO_3H group are indicative of sulfonate salts (e.g. ditosylate salt, Figure 7).

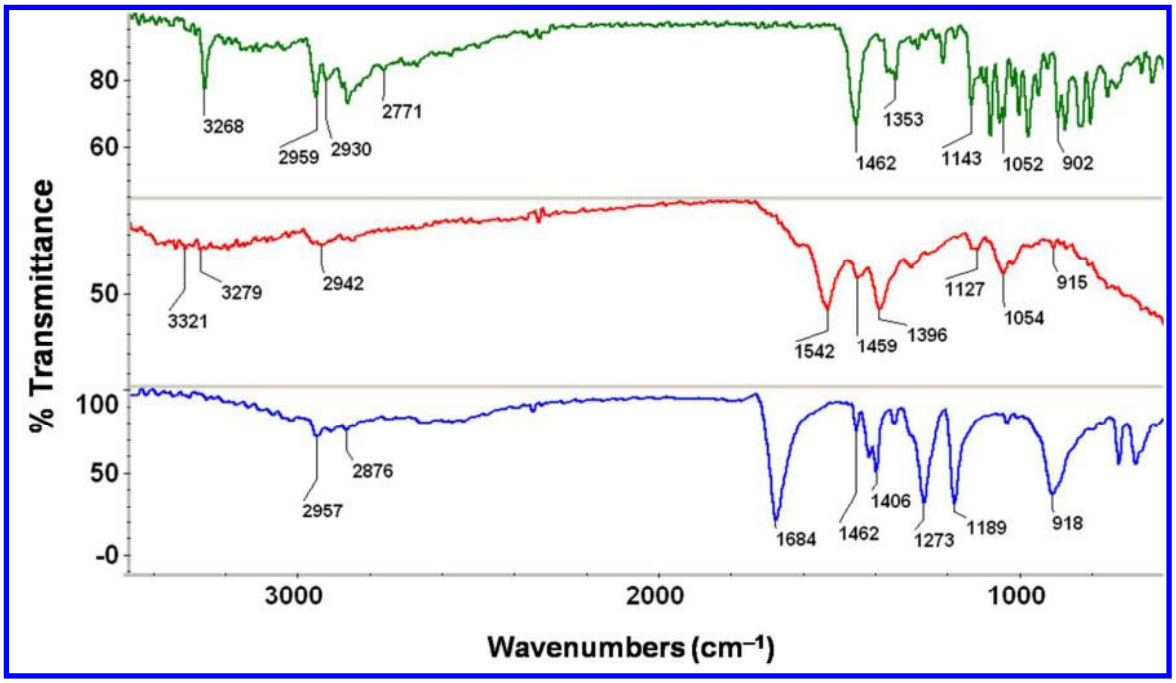


Figure 6 Ethambutol adipate liquid product (red) exhibits distinct ATR FT-IR spectrum compared to its parent compounds ethambutol (green) and adipic acid (blue) with respect to C=O, N–H, O–H and C–H bands.

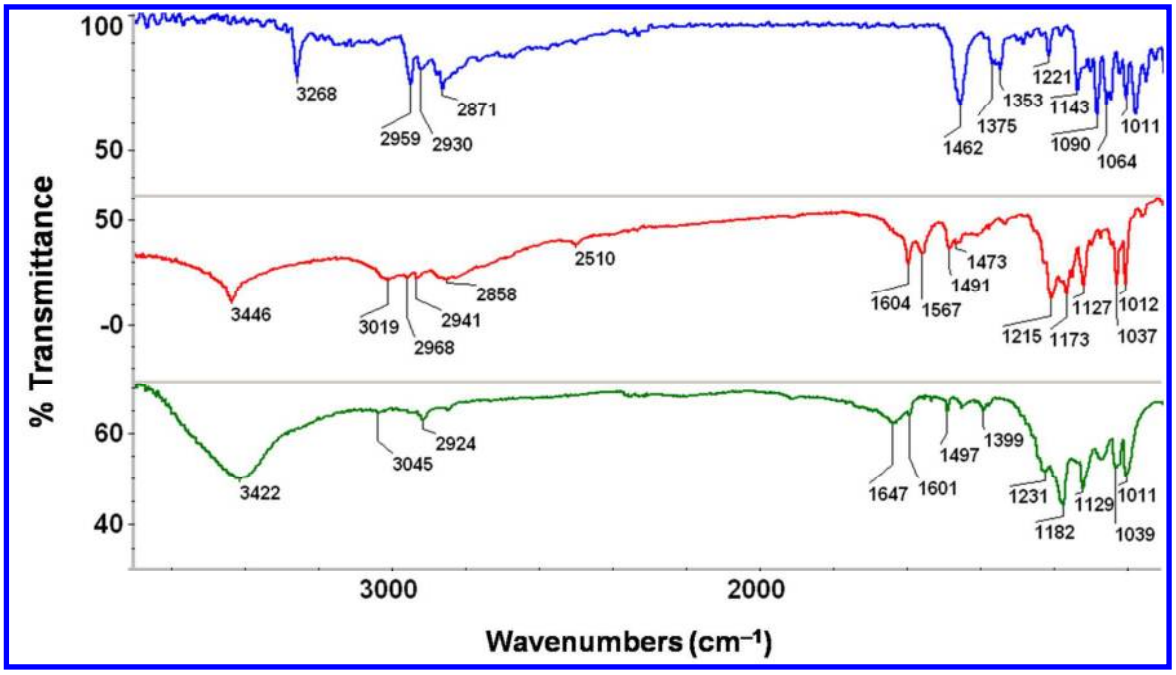
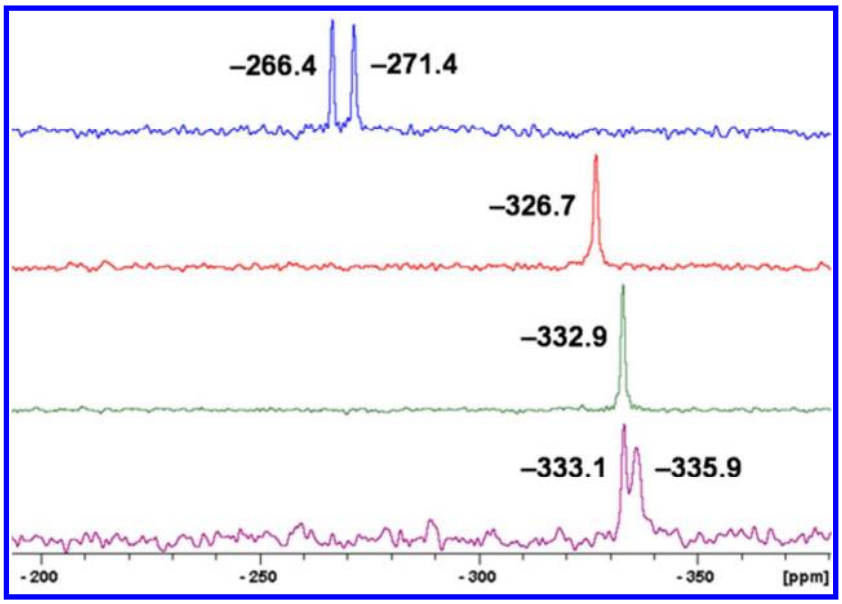
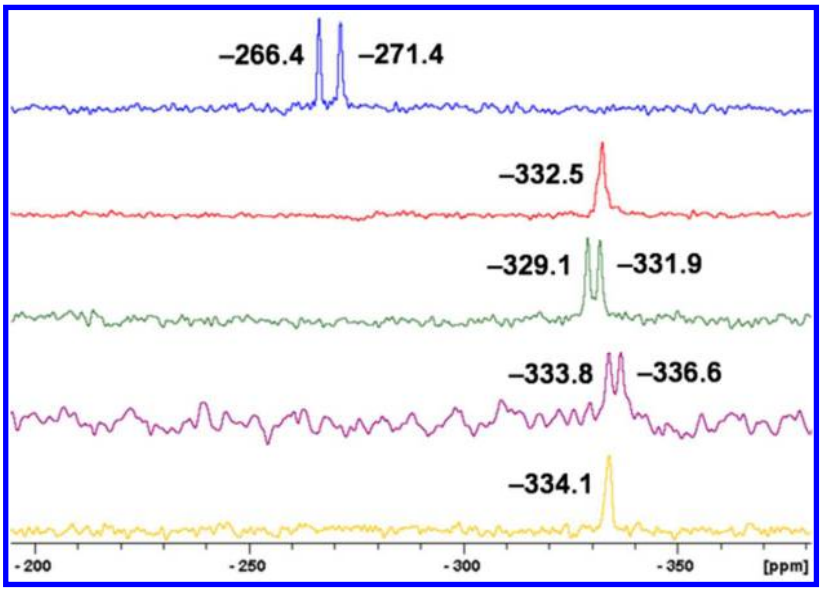


Figure 7 Ethambutol ditosylate salt (red) exhibits distinct ATR FT-IR spectrum compared to its parent compounds ethambutol (blue) and p-toluenesulfonic acid (green) with respect to S=O, O–H and N–H bands.

Solid-state NMR spectroscopy,[23] particularly ^{15}N nucleus chemical shifts, are useful to establish salt formation (protonation at basic N site). The conversion of the secondary amine of EMB base to NH_2^+ moves the nitrogen peak upfield.[11,23a] As such, an upfield shift of about 60 ppm was observed in the ^{15}N spectra of the salts (Figure 8). The secondary NHs of *S*,*S*-EMB base are non-equivalent in the crystal structure since one NH acts as a hydrogen bond donor and acceptor and the other is an acceptor only, and accordingly two ^{15}N peaks were observed. In case of *S*,*S*-ethambutol dihydrochloride and dimesylate salts, the protonated amines are equivalent in the crystal structure and therefore a single upfield signal was noted (Figure 8a). Similarly, the occurrence of an upfield shift in other cases confirms their salt nature (Figure 8b). PXRD of these salts established their crystalline nature (Figure 9). DSC showed distinct melting point endotherm and in two cases, sulfate and ditosylate salts, endothermic phase transitions were observed before melting (Figure 10). These transitions are attributed to polymorphic transformation of the salts at high temperature[24] since there is no weight loss in TGA in that temperature range (Figure 11).



(a)



(b)

Figure 8 ^{15}N ss-NMR spectra of ethambutol (blue) and its salts **(a)** ethambutol dihydrochloride (red), dimesylate (green) and fumarate hydrate (purple), and **(b)** sulfate (red), dibesylate (green), ditosylate (purple) and adipate (yellow) show characteristic upfield shift of secondary amine NH in the salts.

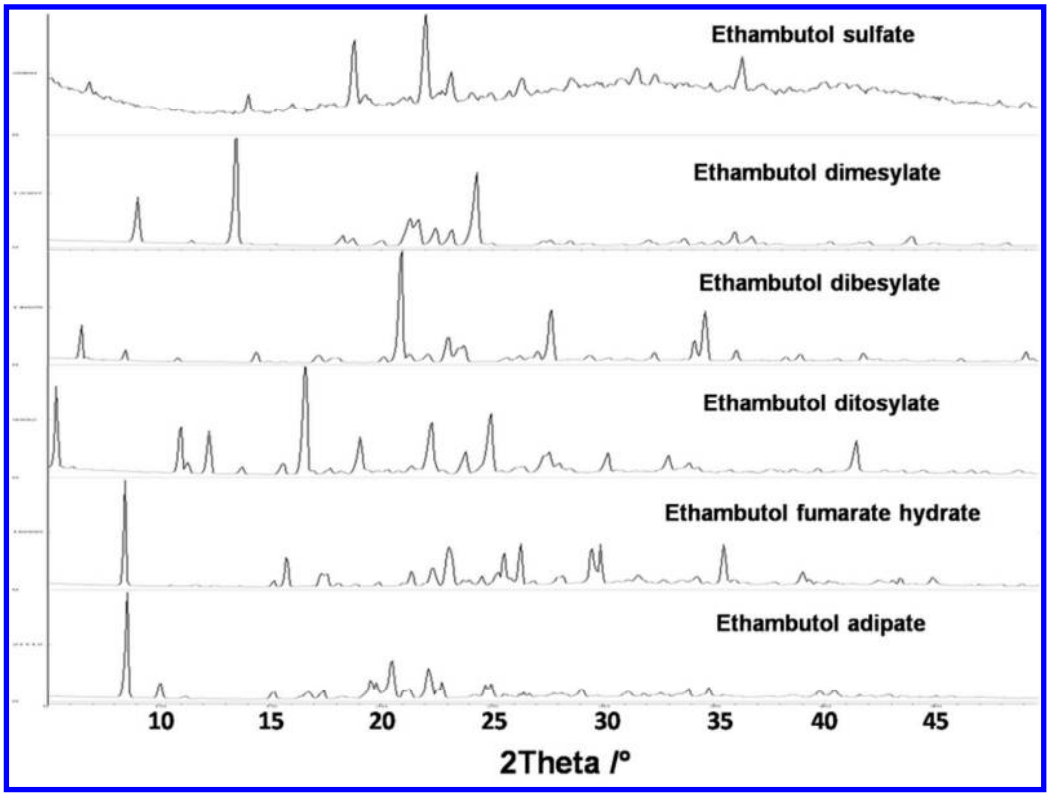


Figure 9 Experimental PXRD patterns of ethambutol salts.

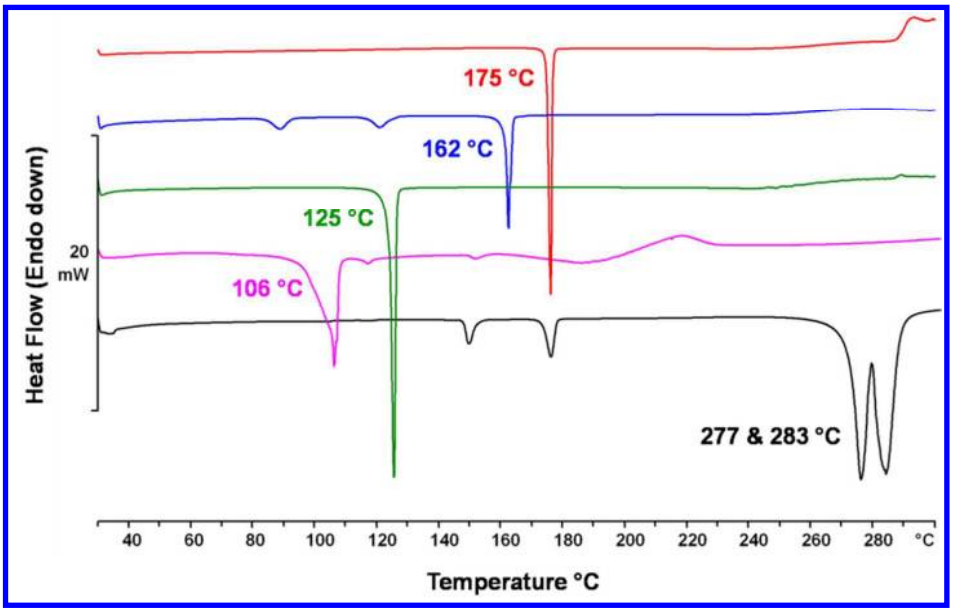
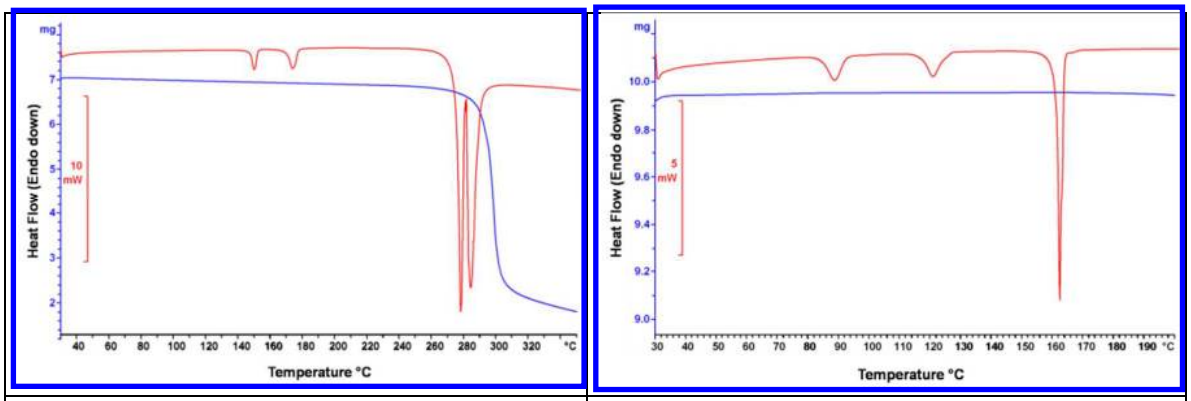


Figure 10 DSC of ethambutol sulfate (black), fumarate hydrate (magenta), dibesylate (green), ditosylate (blue) and dimesylate (red) salts.



(a) **(b)**

Figure 11 DSC (red) and TGA (blue) of **(a)** ethambutol sulfate and **(b)** ethambutol tosylate indicate polymorphic transformation of the salts at high temperature prior to melting as evident by no weight loss in the phase transition temperature range.

A hygroscopic salt can exist in the liquid state at room temperature due to deliquescence and the same is true for an ionic liquid, even though it is not an RTIL. If the melting point of a hygroscopic salt is less than 100 °C, then the solid residue upon water loss may not be visually observed if water loss and melting are concomitant (occur together) upon heating. Thus, a hygroscopic salt of m.p. <100 °C may be confused for an RTIL. A heat−cool−reheat DSC run will evolve any residual water and give T_g (glass transition temperature) or T_m (melting temperature) of the sample,[11,12b] thereby eliminating interference/ confusion from water escape for thermal phase transitions in

DSC. Thermal transitions of ethambutol adipate were observed in the DSC (Figure 12) for a sample containing 38.6% water content by KF titration. The thermal transitions suggested that (i) water is lost at the broad endotherm between 60-100 °C in the heat run, (ii) the anhydrous liquid solidified during the broad exotherm around –20 °C in the cooling cycle, and (iii) the solidified mass, which is an amorphous/glassy phase, transformed into a rubbery material after glass transition (T_g = –20 °C) and finally melted at 85 °C (Figure 12). The transitions of the hygroscopic sample were validated by DSC of the crystalline material which showed clean melting at 87 °C (Figure 12). Therefore, ethambutol adipate with T_m = 87 °C can be classified as an ionic liquid, more precisely as a protic ionic liquid,[15] evident from the proton transfer analyzed by ATR-IR and ^{15}N ss-NMR spectroscopy.

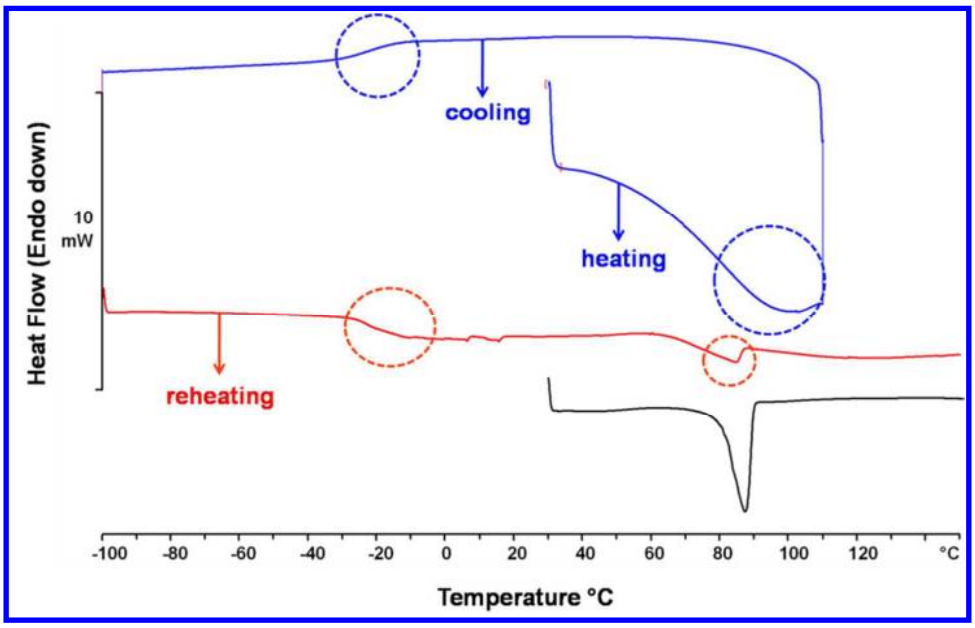


Figure 12 DSC of ethambutol adipate. (a) Heating and cooling of the hygroscopic liquid (blue trace) shows a broad endotherm at 100 °C indicative of water loss and a broad exotherm around –20 °C for solidification. Reheating (red trace) shows glass transition at –20 °C and melting at 85 °C. Crystalline sample (black trace) shows a single melting endotherm at 87 °C.

Conclusions

A salt screen of ethambutol base with several protic acids was carried out with the intent of solving the hygroscopicity problem of EDH. The tendency of ethambutol to form protic salts is so high that even a simple physical mixture of EMB and protic acid shows evidence of salt formation. Rotavaporization, a recent technique for new polymorphs[11,25] and cocrystals,[26] is successfully utilized to prepare salts in this study.

Polymorphism of ethambutol salts (EDH,[10] dibenzoate ionic liquid[11] and sulfate and tosylate salts of this study) warrants more studies in that ethambutol itself is not polymorphic to date. The salts of this study were found to be hygroscopic when exposed to ambient humidity, and therefore do not solve the original problem stated in the introduction of finding new non-hygroscopic salts of EMB. The common problem of hygroscopicity with salts/ILs[12,27] suggests that explorations of other solid forms such as cocrystals,[28] eutectics,[29] and solid dispersions[29c,30] are equally important in drug formulation. We are searching for improved stability and minimal hygroscopicity form of ethambutol dihydrochloride in solid screens.

Experimental Section

Materials and Methods

Ethambutol dihydrochloride (Lot#090M0189V) was purchased from Sigma-Aldrich (Hyderabad, India) and used without further purification. All other chemicals were of analytical or chromatographic grade. Water purified from a deionizer cum mixed bed purification system (AquaDM, Bhanu, Hyderabad, India) was used for experiments.

Preparation and Crystallization of Ethambutol Base

The free base of ethambutol was prepared as per the method reported by Bhutani et al.[1a] Ethambutol dihydrochloride (2 g) was added to 5N aqueous NaOH (20 mL). The mixture was stirred for 10-15 min and extracted with CH_2Cl_2 (25 mL). The organic extract was dried (Na_2SO_4) and left for evaporation at ambient temperature to afford block-shape crystals of ethambutol after one day. The product crystals were characterized by DSC (m.p. 88 °C), NMR and XRD. Recrystallization from acetone resulted in diffraction quality plate morphology crystals but they were very often twinned. Several crystals were checked for identical unit cell parameters during X-ray cell check and only that crystal which did not show a doubling of the unit cell was continued for X-ray data collection.

Ethambutol Dihydrochloride ($C_{10}H_{26}Cl_2N_2O_2$)

Colorless plate crystals of the salt, suitable for single crystal X-ray diffraction, were obtained from a methanolic solution, kept at –20 °C, after complete evaporation of the solvent.

^{1}H NMR (DMSO-d_6): δ 0.92 (3H, t, J 8), 1.68 (2H, m), 3.06 (1H, m), 3.71 (2H, m), 5.40 (1H, s), 9.29 (2H, d, J 80 (N–H coupling)). Protons of CH_2 group (attached to NH_2^+ group) merged with water peak of DMSO-d_6.

^{13}C NMR (DMSO-d_6): δ 10.24, 20.78, 41.27, 57.99, 60.74.

Ethambutol ($C_{10}H_{24}N_2O_2$)

^{1}H NMR (DMSO-d_6): δ 0.82 (3H, t, J 8), 1.31 (2H, m), 2.32 (1H, m), 2.55 (2H, s), 3.27 (2H, m). Exchange of NH and OH protons in the solvent.

^{13}C NMR (DMSO-d_6): δ 10.51, 24.22, 47.31, 60.72, 63.07.

Salt Synthesis

a. Synthesis by direct reaction of base and acid

Ethambutol and the salt-forming acid (in 1:1 molar ratio for diacids and 1:2 molar ratio for monoacids) were separately dissolved in acetone and kept at –20 °C. After incubation for 10 min, the two solutions were mixed and stirred for 5-10 min in ice cold bath. The product salt was obtained as a white precipitate and filtered on 2.5 μm Whatman filter paper and washed with acetone and dried.

b. Solution crystallization

Ethambutol and acid (in 1:1 molar ratio for diacids and 1:2 molar ratio for monoacids) were directly dissolved in methanol/ethanol. The solutions were kept for slow crystallization at (i) room temperature, in (ii) a –20 °C refrigerator and (iii) a desiccator separately. For fast crystallization of salts, the alcoholic solutions were subjected to rotavaporization.

c. Co-grinding

Ethambutol and acid (in 1:1 molar ratio for diacids and 1:2 molar ratio for monoacids) were subjected to grinding using a mortar-pestle for 1-2 min.

d. Co-melting

Ethambutol and acid (in 1:1 molar ratio for diacids and 1:2 molar ratio for monoacids) were taken together in a sublimation tube and heated in an oil/salt bath beyond the melting point of the higher melting compound. The tube was held until a uniform liquid with no trace of solid material is formed and then kept aside for ambient cooling.

e. Physical mixture

Ethambutol and acid (in 1:1 molar ratio for diacids and 1:2 molar ratio for monoacids) were gently mixed together with a spatula on a glass plate and the material was transferred to a vial for storage at ambient temperature and humidity.

Ethambutol Sulfate ($C_{10}H_{26}N_2O_6S$)

^{1}H NMR (D_2O): δ 0.94 (3H, t, J 8), 1.70 (2H, m), 3.25 (1H, m), 3.48 (2H, s), 3.73 (2H, m). Exchange of NH and OH protons in the solvent.

^{13}C NMR (D_2O): δ 9.00, 20.37, 40.82, 57.86, 61.40.

Ethambutol Dimesylate ($C_{12}H_{32}N_2O_8S_2$)

^{1}H NMR (DMSO-d_6): δ 0.90 (3H, t, J 8), 1.60 (2H, m), 2.39 (2H, s), 3.09 (1H, m), 3.42 (2H, m). Protons of CH_3 group (attached to SO_3^- group) merged with dissolved water peak of DMSO-d_6. Exchange of NH and OH protons in the solvent.

^{13}C NMR (DMSO-d_6): δ 10.14, 20.64, 39.10, 40.99, 57.99, 60.73.

Ethambutol Dibesylate ($C_{22}H_{36}N_2O_8S_2$)

^{1}H NMR (DMSO-d_6): δ 0.86 (3H, t, *J* 8), 1.57 (2H, m), 3.07 (1H, m), 3.30 (2H, s), 7.35 (2H, d, *J* 4), 7.64 (2H, d, *J* 4), 8.55 (1H, s). Protons of CH_2 group (attached to NH_2^+ group) merged with dissolved water peak of DMSO-d_6. Exchange of NH and OH protons in the solvent.

^{13}C NMR (DMSO-d_6): δ 10.07 (2C), 20.60 (2C), 41.02 (2C), 57.95 (2C), 60.79 (2C), 125.89 (4C), 128.43 (4C), 129.65 (2C), 147.32 (2C).

Ethambutol Ditosylate ($C_{24}H_{40}N_2O_8S_2$)

^{1}H NMR (DMSO-d_6): δ 0.86 (3H, t, *J* 8), 1.57 (2H, m), 2.27 (3H, s), 3.05 (1H, m), 3.29 (2H, s), 7.14 (2H, d, *J* 8), 7.51 (2H, d, *J* 8). Protons of CH_2 group (attached to NH_2^+ group) merged with dissolved water peak of DMSO-d_6. Exchange of NH and OH protons in the solvent.

^{13}C NMR (DMSO-d_6): δ 10.09 (2C), 20.63 (2C), 21.23 (2C), 41.06 (2C), 57.99 (2C), 60.77 (2C), 125.92 (4C), 128.82 (4C), 139.05 (2C), 144.73 (2C).

Ethambutol Fumarate ($C_{14}H_{28}N_2O_6$)

^{1}H NMR (DMSO-d_6): δ 0.86 (3H, t, *J* 8), 1.47 (2H, m), 2.69 (1H, m), 2.92 (2H, s), 3.39 (2H, m), 6.42 (1H, s). Exchange of NH and OH protons in the solvent.

^{13}C NMR (DMSO-d_6): δ 10.47, 22.30, 43.58, 60.39, 60.45, 135.88, 169.28.

Ethambutol Adipate ($C_{16}H_{34}N_2O_6$)

^{1}H NMR (DMSO-d_6): δ 0.83 (3H, t, *J* 8), 1.39 (2H, m), 1.43 (2H, d, *J* 4), 2.08 (2H, d, *J* 4), 2.73 (1H, m), 3.15 (2H, s), 3.36 (2H, m). Exchange of NH and OH protons in the solvent.

^{13}C NMR (DMSO-d_6): δ 10.51, 24.11, 35.29, 45.05, 49.05, 60.45, 61.55, 176.15.

X-ray Crystallography

X-ray reflections for ethambutol dihydrochloride, ethambutol dimesylate and ethambutol fumarate hydrate were collected at 100 K on a Bruker SMART-APEX CCD diffractometer equipped with a graphite monochromator and Mo-Kα fine-focus sealed tube (λ = 0.71073 Å). Data reduction was performed using Bruker SAINT software.[31] Intensities were corrected for absorption using SADABS.[32] Structures were solved and refined using SHELX-97.[33] X-ray reflections for ethambutol were collected at 298 K on an Oxford Xcalibur Gemini Eos CCD diffractometer using Cu-Kα radiation (λ = 1.5418 Å). Data reduction was performed using CrysAlisPro (version 1.171.33.55)[34] and OLEX2-1.0[35] was used to solve and refine the structure. All non-hydrogen atoms were refined anisotropically. In the crystal structure of ethambutol fumarate hydrate, site occupancy of 0.145 for O atom of the second water molecule was obtained by least squares refinement of electron density using FVAR command. Hydrogen atoms on heteroatoms were located from difference electron density maps and all C–H hydrogens were fixed geometrically. Flack parameter[17] was obtained upon structure solution and

refinement of X-ray data. The final CIF files and hydrogen bond geometries were validated in PLATON.[36] X-Seed[37] was used to prepare packing diagrams.

Powder X-ray Diffraction

PXRD were recorded on Bruker D8 Advance diffractometer using Cu-Kα X-radiation (λ = 1.5406 Å) at 40 kV and 30 mA. Diffraction patterns were collected over 2θ range of 5-50° at scan rate of 1° min^{-1}. Powder cell 2.4[38] was used to plot the diffraction patterns.

Spectroscopy

Nicolet 6700 FT-IR spectrometer equipped with a single bounce ATR accessory was used to record IR spectra. IR spectra were recorded on samples placed on Zn-Se crystal. Solution and solid state NMR spectra were recorded on a Bruker Avance spectrometer at 400 MHz. SS-NMR spectra were recorded on a Bruker 4 mm double resonance CP-MAS probe in zirconia rotors at 5.0 kHz with a cross-polarization contact time of 2.5 ms and a recycle delay of 8 s. ^{15}N CP-MAS spectra were recorded at 40 MHz and referenced to glycine N atom, and then the chemical shifts were recalibrated to nitromethane N ($\delta_{glycine}$ = –347.6 ppm). Additionally the identity and stoichiometry of the components of the salts were established through solution ^{1}H NMR integration and ^{13}C NMR spectra.

Thermal Analysis

DSC was performed on a Mettler Toledo DSC 1 module calibrated with indium (T_m = 156.60 °C; ΔH_f = 28.45 J g^{-1}) and zinc (T_m = 419.50 °C; ΔH_f = 107.50 J g^{-1}) as per the manufacturer's specifications. TGA was performed on a Mettler Toledo TGA/SDTA 851e module calibrated with indium (T_m = 156.60 °C) and aluminium (T_m = 660.30 °C). The temperature range used for solid samples in both DSC and TGA is 30–400 °C at 5 °C min^{-1}. For liquid samples, a heat-cool-reheat DSC cycle through liquid nitrogen cooling in the temperature range –100 to +150 °C at 5 °C min^{-1} was employed. The typical sample size is 5–10 mg for DSC and 10–15 mg for TGA. Samples were placed in crimped but vented aluminium pans for DSC and open alumina pans for TGA and were purged by a stream of dry nitrogen flowing at 50 mL min^{-1}.

Karl Fischer (KF) Titration

Water content of the samples was determined using a Spectralab volumetric MA 101 C Karl Fischer titrator with KF reagent (single solution) as the titrant and anhydrous methanol as the solvent.

Supporting Information Available

Crystallographic information (.cif files) are available free of charge via the Internet at http://pubs.acs.org.

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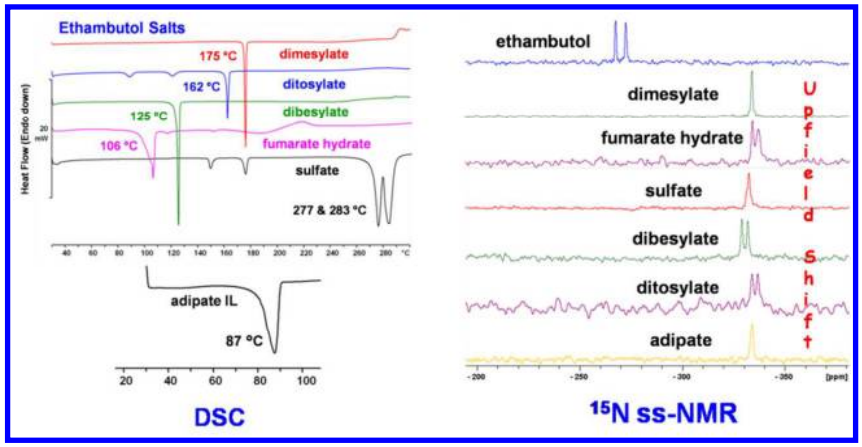
TOC and Synopsis



Salt screen of anti-tuberculosis chiral basic drug Ethambutol resulted in high melting salts (m.p. > 100 °C) and an ionic liquid (m.p. 87 °C). The upfield shift in the ^{15}N ss-NMR spectra established the products as protic salt/ionic liquid.