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Preliminary communication

Synthesis and antitubercular screening of imidazole derivatives☆

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ABSTRACT

A series of imidazole based compounds were synthesized by reacting simple imidazoles with alkyl halides or alkyl halocarboxylate in presence of tetrabutylammonium bromide (TBAB). The compounds bearing carbethoxy group undergo amidation with different amines in the presence of DBU to give respective carboxamides. The synthesized compounds were screened against *Mycobacterium tuberculosis* where compound **17** exhibited very good in vitro antitubercular activity and may serve as a lead for further optimization.

1. Introduction

An increase in the global burden of tuberculosis with the worldwide mortality rate of 23% is a major concern in the socio-economic and health sectors [1–5]. The synergy of this disease with HIV infection and the emergence of multi drug resistance and extensively drug resistance tuberculosis (MDRTB and XDRTB) pose a threatening global challenge [6–8]. Although a number of lead molecules exist today to develop new drugs, no new chemical entity has emerged for clinical use for over the last 45 years in the treatment of this disease [9,10]. Therefore, there is an urgent need to develop new drugs, acting through a novel mechanism of action for the chemotherapy of tuberculosis.

Recently certain imidazole based compounds were reported to possess antimicrobial activities [11]. It is believed that aryl–azolyl–ethane moiety, present in many azole antifungal drugs serve as pharmacophore in compounds having *Mycobacterium* killing activity [12,13]. Many azole derivatives have also displayed interesting antimycobacterial activity in addition to antifungal activity [14–16]. It is established that these compounds target the sterol demethylase, a mixed-function oxidase involved in sterol synthesis in eukaryotic organisms [17]. The unraveling of *Mycobacterium* genome sequence has revealed that a protein having homology to one of the above mixed oxidase function is present in *Mycobacterium tuberculosis* [18]. Nitroimidazole derivative such as nitroimidazopyran is in advanced stage of clinical trial for the treatment of tuberculosis and it has been speculated that this compound is active against both the replicating and the latent *Mycobacterium* [19]. Keeping in mind the above facts, we were interested to see the antitubercular potential in simple imidazole derivatives.

2. Results and discussion

Compounds **3**–**5** and **15**–**18** were synthesized starting from simple imidazoles by reacting them with different alkyl halides (viz. 3,4-dichlorobenzyl bromide, ethyl bromoacetate, ethyl bromopropionate, 1,3-dibrompropane and 1,5-dibromopentane) in the presence of NaH/TBAB (tetrabutylammonium bromide) in anhydrous DMF or THF (Table 1). Although few such alkylation methods for the synthesis of 1-alkyl-, aralkyl imidazoles and bis-imidazolyl alkanes were reported earlier [32–35], however our method of alkylation of imidazole and 2-propylimidazole in the presence of TBAB offers advantages over previous ones in terms of mild reaction conditions along with shorter reaction time and better yield of the products.

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Table 1
Synthesis of substituted imidazoles and benzimidazoles (**3**–**18**).

Compound No.	Physical state	M.p. (°C)	Known m.p. (°C) [Ref]	% Yield
3	Yellow semi solid	–	–	65
4	White solid	120–124	124 [32]	70
5	Light brown solid	165–168	–	70
6	Viscous mass	–	–	78
7	Yellow semi solid	–	[33]	78
8	Yellow solid	74–76	–	80
9	Pale yellow semi solid	–	–	78
10	Colorless solid	37–39	36–40 [34]	46
11	Viscous mass	–	–	80
13	Pale yellow solid	116–118	–	70
14	Yellow solid	138–140	–	75
15	Yellow oil	–	[35]	68
16	Viscous mass	–	–	70
17	Colorless oil	–	–	55
18	Colorless oil	–	–	45

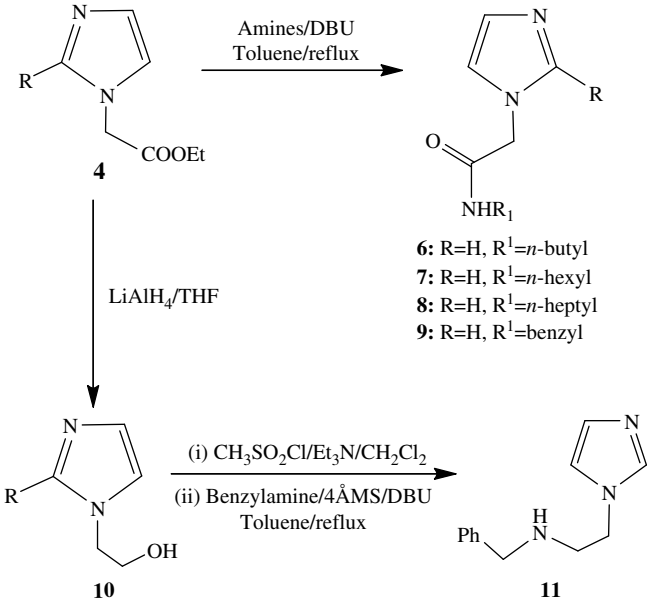
Thus, reaction of imidazole (**1**) with 3,4-dichlorobenzyl bromide, ethyl bromoacetate and ethyl bromopropionate separately in THF in the presence of NaH/TBAB gave 1-(3,4-dichlorobenzyl)-1*H*-imidazole (**3**), imidazol-1-yl-acetic acid ethyl ester (**4**) and 3-imidazol-1-yl-propionic acid ethyl ester (**5**) respectively in quantitative yield (Scheme 1).

Compounds (**6**–**9**) were prepared by reaction of compound **4** with different amines viz. *n*-butyl, *n*-hexyl, *n*-heptylamine, and benzylamine under refluxing condition. $LiAlH_4$ reduction of the above compound **4** gave respective 1-(2-hydroxy ethyl)-1*H*-imidazole (**10**) in good yield. The latter, on mesylation with methanesulphonyl chloride followed by reaction with benzyl amine in presence of DBU and 4 Å molecular sieve gave 1-(2-benzyl amino ethyl)-1*H*-imidazole (**11**) in quantitative yield (Scheme 2).

The structures of all the synthesized compounds were established on the basis of spectroscopic data and analysis. The IR data for compound **3** exhibited C=N and C=C stretching frequency at υ_{max} 1730 and 1642 cm^{-1}, respectively. In the ^1H NMR spectrum of compound **3**, appearance of a multiplet in the range of δ 7.56–6.89 corresponds to three imidazole and three phenyl protons and a singlet at δ 5.09 corresponds to methylene protons. In the ^{13}C NMR spectrum, peaks at δ 142.7, 137.6, 136.8, 136.4, 134.5, 134.1, 132.2, 128.2 and 124.6 showed the presence of imidazole carbons and aromatic carbons whereas peak at δ 54.3 showed the presence of methylene carbon. Finally, molecular ion peak at *m*/*z* 228 $(M+H)^+$ in MS spectrum confirms the structure of compound **3**.

Similarly, compounds **13** and **14** were prepared by benzylation of benzimidazole (**12**) with benzyl bromide and 3,4-dichlorbenzyl bromide respectively (Scheme 3) and the structures were established on the basis of spectroscopic data and analysis.

Compounds **15**–**18** were prepared by the reaction of imidazole (**1** or **2**) with dibromoalkanes in presence of NaH and TBAB in THF (Scheme 4). The reaction of 2 eq. of imidazole with 1 eq. of 1,3-dibromopropane and 1,5-1,5-dibromopentane separately led to

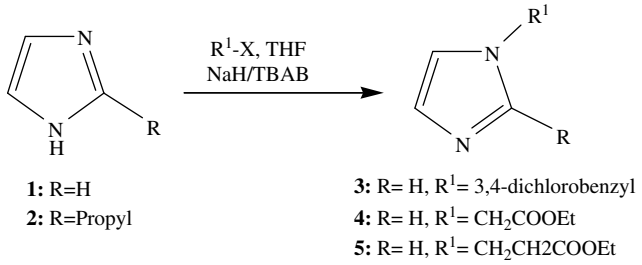


Scheme 2. Synthesis of imidazole derivatives.

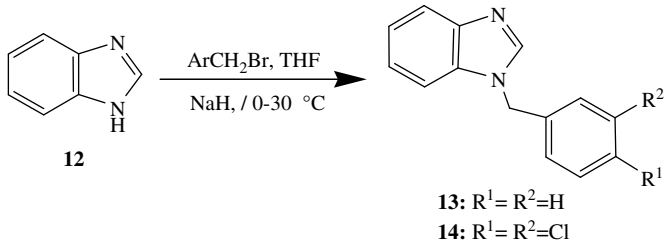
the formation of compounds **15** and **16** respectively in good yields. However, reacting 2 eq. of 2-propylimidazole with 1 eq. of 1,3-dibromopropane gave the expected 1,3-bis-(2-propylimidazol-1-yl)-propane (**17**) as major product along with another unusual minor product, 1-(4-allyl-2-propylimidazol-1-yl)-3-(2-propylimidazol-1-yl)-propane (**18**) (Scheme 4). Formation of compound **18** is speculated via electrophilic attack of carbocation generated from allyl bromide which in turn formed via rearrangement of 1,3-dibromopropane. However, exact mechanism is yet to be established.

Since glycosyl amino ester derivatives and other glycoconjugates bearing alkyl substitutents at the nitrogen atom [20–24] have been found to possess antitubercular activity, we were prompted to see the effect of imidazole ring at the C-5 of sugar moiety on antitubercular activity profiles. Synthesis of imidazolyl glycosyl uronoates (**19**–**22**, Fig. 1) in moderate yields was carried out by us as reported earlier [25].

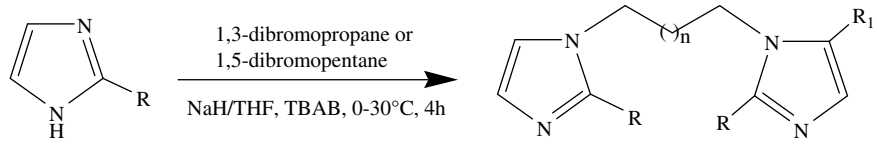
The imidazole derivatives (**3**–**22**) were screened for their antitubercular efficacy against *M. tuberculosis* using different test models [26–29] and the results are shown in Table 2. The antitubercular efficacy of these compounds were tested against avirulent strain *M. tuberculosis* $H_{37}Ra$ and virulent strain *M. tuberculosis* H37Rv at different concentrations ranging from 50 μg/ml to 3.25 μg/ml. As evident from Table 2 that most of the compounds displayed antitubercular activity with MIC ranging from 25 to >12.5 μg/ml against either the avirulent strain *M. tuberculosis* H37Ra or the virulent strain *M. tuberculosis* H37Rv. The only compound **17** showed MIC 6.25 μg/ml against virulent strain



Scheme 1. Synthesis of *N*-alkyl(aralkyl) imidazoles.



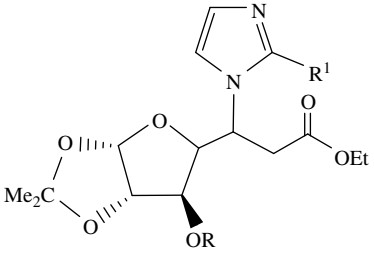
Scheme 3. Synthesis of benzimidazole derivatives.



1: R=H
2: R=C_3H_7

15: n=1, R=R^1=H
16: n=3, R=R^1=H
17: n=1, R=Propyl, R^1=H
18: n=1, R=Propyl,R^1=Allyl

Scheme 4. Synthesis of bis-imidazolyl derivatives.



19: R=CH_3; R^1=H, **20:** R=CH_2Ph; R^1=H, **21:** R=CH_3; R^1=Propyl, **22:** R=CH_2Ph; R^1=Propyl

Fig. 1.

Table 2
Antitubercular activities of synthesized imidazole derivatives.

Compound no.	MIC (μg/ml) using MABA method	MIC (μg/ml) using Agar microdilution method
3	>12.5	nd
4	>12.5	>6.25
5	nd	>12.5
6	>12.5	>12.5
7	25	>12.5
8	>12.5	>12.5
9	>12.5	>12.5
10	>12.5	>12.5
11	12.5	12.5
13	>12.5	>12.5
14	>12.5	>12.5
15	>12.5	>12.5
16	>12.5	>12.5
17	>12.5	6.25$^+$
18	>12.5	25
19	25	25
20	50	50
21	25	25
22	50	50

MIC, minimum inhibitory concentration; nd, not determined; MIC of the compounds used as control: EMB 1.5–5.0 μg/ml, INH 0.65 μg/ml; $^+$MIC confirmed by BACTEC method.

M. tuberculosis H37Rv. The MIC for this compound was also confirmed by using BACTEC assay and thus offers a prototype lead for further optimization and development.

3. Conclusion

In conclusion, we have synthesized simple imidazole derivatives either by simple alkylation of imidazoles followed by further manipulations or by conjugate addition of imidazoles to different glycosyl olefinic esters. An unusual observation of introducing an allyl moiety in 2-propylimidazole is also revealed. The compounds were screened against avirulent and virulent strains of *M. tuberculosis*. One of the compounds offers potential for further optimization and development to new antituberculars.

4. Experimental

4.1. Chemistry

Commercially available reagent grade chemicals were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light and/or spraying a 20% $KMnO_4$ aq. soln. Column chromatography was performed on silica gel (60–120 mesh, E. Merck). IR spectra were recorded as thin films or in chloroform soln. with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300 in $CDCl_3$. Chemical shift values are reported in ppm relative to $SiMe_4$ as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), m (multiplet); J in hertz. FAB mass spectra were performed using a mass Spectrometer Jeol SX-102 and ESI mass spectra with Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

4.2. General procedure for the synthesis of N-substituted imidazole or benzimidazoles (*3–5*, *13–14* and *15–18*)

To the stirred slurry of NaH (0.21 g, 14.7 mmol) in dry THF (5 ml) at 0 °C, a solution of imidazole (1.0 g, 14.7 mmol) in THF was added dropwise and the reaction mixture was stirred for half an hour. The corresponding alkyl halide or alkyl haloacetate (14.7 mmol) was added dropwise followed by addition of tetrabutylammonium bromide and the reaction mixture was further stirred at 30 °C till the disappearance of starting material (TLC). The reaction mixture was filtered through celite pad and filtrate thus obtained was evaporated under reduced pressure to give a residual mass. The latter was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to give a crude product, which was purified by column chromatography (SiO_2) using methanol:chloroform (1:5) as eluent.

4.2.1. 1-(3,4-Dichloro-benzyl)-1H-imidazole (*3*)

The reaction of **1** and 3,4-dichlorobenzyl bromide (2.1 ml, 14.1 mmol) as described above gave **3**. FT-IR (KBr, cm^{-1}): 3424, 2968, 1730, 1642; ESMS: 228 $(M+H)^+$. ^1H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.56–6.89 (m, 6H, Im–H and Ar–H), 5.09 (s, 2H, $-CH_2$). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 142.7, 137.6, 136.8, 136.4, 134.5, 134.1, 132.2, 128.2, 124.6, 54.3. Anal. Calcd for $C_{10}H_8N_2Cl_2$: C, 52.89; H, 3.55; N, 12.34; Found: C, 52.86; H, 3.57; N, 12.33.

4.2.2. Imidazol-1-yl-acetic acid ethyl ester (*4*) [32]

The reaction of **1** and ethyl bromo acetate (1.63 ml, 14.7 mmol) as described above gave **4**. FT-IR (KBr, cm^{-1}): 3436, 2989, 1743,

1637, 1514; ESMS: 155 $(M+H)^+$. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.60–6.92 (m, 3H, Im–H), 4.24 (m, 2H, –N–CH_2), 4.16 (dd, J = 7.1 Hz and 7.3 Hz, 2H, –OCH_2), 1.27 (m, 3H, –CH_3). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 167.6, 138.1, 129.9, 120.2, 62.2, 48.3, 14.4. Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.54; N, 18.17; Found: C, 54.52; H, 6.53; N, 18.15%.

*4.2.3. 3-Imidazol-1-yl-propionic acid ethyl ester (**5**)*

The reaction of **1** and ethyl bromo propionate (1.8 ml, 14.7 mmol) as described above gave **5**. FT-IR (KBr, cm^{-1}): 3418, 2986, 1726, 1513; ESMS: 169 (M + H). ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.52 (s, 1H, Im–H), 7.04 (s, 1H, Im–H), 6.93 (s, 1H, Im–H), 4.27 (t, J = 6.5 Hz, 2H, –N–CH_2), 4.15 (dd, J = 7.1 Hz and 7.1 Hz, 2H, –OCH_2), 2.77 (t, J = 6.5 Hz, 2H, –CH_2), 1.26 (m, 3H, –CH_3); ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 177.5, 142.3, 133.6, 124.5, 65.8, 47.8, 40.9, 19.3. Anal. Calcd for $C_8H_{12}N_2O_2$: C, 57.13; H, 7.19; N, 16.66; Found: C, 57.11; H, 7.20; N, 16.64%.

*4.2.4. 1,3-Bis-(imidazol-1-yl)-propane (**15**) [35]*

The reaction of **1** and 1,3- dibromo propane (1.53 ml, 14.6 mmol) as described above gave **15**. FT-IR (KBr, cm^{-1}): 3381, 2965, 1733, 1458; ESMS (M + H) = 177. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.64–6.69 (m, 6H, Im–H), 3.94–3.87 (m, 4H, 2 × –N–CH_2), 2.32–2.19 (m, 2H, –CH_2). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 137.3, 128.3, 122.1, 119.8, 43.8, 32.2. Anal. Calcd for $C_9H_{12}N_4$: C, 61.34; H, 6.86; N, 31.79; Found: C, 61.32; H, 6.88; N, 31.76%.

*4.2.5. 1,5-Bis-(imidazol-1-yl)-pentane (**16**)*

The reaction of **1** and 1,3-dibromo pentane (2.0 ml, 14.7 mmol) as described above gave **16**. FT-IR (KBr, cm^{-1}): 3435, 2989, 1743, 1636, 1513; ESMS: 205 (M + H). ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.50–6.84 (m, 6H, Im–H), 3.91 (m, 4H, 2 × –N–CH_2), 1.78 (m, 4H, 2 × –CH_2), 1.27 (m, 2H, –CH_2). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 137.2, 129.9, 118.9, 47.0, 30.9, 24.0. Anal. Calcd for $C_{11}H_{16}N_4$: C, 64.68; H, 7.89; N, 27.43; Found: C, 64.68; H, 7.89; N, 27.42%.

*4.2.6. 1,3-Bis-(2-propyl-imidazol-1-yl)-propane (**17**) and 1,3-(4-allyl-2-propyl-imidazol-1-yl)-(2-propyl-imidazol-1-yl)-propane (**18**)*

The reaction of 2-propylimidazole (**2**, 1.0 g, 8.26 mmol) and 1,3-dibromopropane (0.86 ml, 8.26 mmol) in the slurry of NaH (0.3 g, 12.5 mmol) as described above gave the title compounds **17** and **18** in quantitative yield as in the ratio of 55:45 respectively.

*4.2.6.1. 1,3-Bis-(2-propyl-imidazol-1-yl)-propane (**17**).* FT-IR (KBr, cm^{-1}): 3381, 2965, 1733, 1458; ESMS: 261 (M + H). ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.64 (s, 1H, Im–H), 7.54 (s, 1H, Im–H), 6.95–6.75 (m, 2H, Im–H), 3.97 (t, J = 6.5 Hz, 2H, –N–CH_2), 3.83 (t, J = 7.0 Hz, 2H, –N–CH_2), 2.70–2.48 (m, 4H, 2 × –CH_2), 2.25 (t, J = 6.9 Hz, 2H, –CH_2), 1.72 (m, 4H, 2 × –CH_2), 0.97 (m, 6H, 2 × –CH_3). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 147.6, 126.5, 120.2, 117.6, 41.5, 30.9, 29.5, 27.6, 21.0, 20.4, 18.1, 13.0. Anal. Calcd for $C_{15}H_{24}N_4$: C, 69.19; H, 9.29; N, 21.52; Found: C, 69.17; H, 9.30; N, 21.54%.

*4.2.6.2. 1,3-(4-Allyl-2-propyl-imidazol-1-yl)-(2-propyl-imidazol-1-yl)-propane (**18**).* FT-IR (KBr, cm^{-1}): 3381, 2965, 1733, 1458; ESMS: 302 $(M+H)^+$. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 8.48 (s, 1H, Im–H), 7.48 (s, 1H, Im–H), 7.31 (s, 1H, Im–H), 5.98–5.82 (m, 1H, –CH=CH_2), 5.24 and 5.05 (two d, J = 10.2 and 16.5 Hz, 2H, –CH_A and –CH_B), 4.48–4.45 (d, J = 3.8 Hz, 2H, –CH=CH_2), 2.98 (s, 2H, –N–CH_2), 2.87 (s, 2H, –CH_2), 2.60 (t, J = 7.4 Hz, 2H, –CH_2), 1.82–1.67 (m, 2H, –CH_2), 1.05–0.92 (m, 3H, –CH_3). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 162.8, 148.6, 133.4, 127.5, 119.5, 117.7, 66.6, 48.4, 36.7, 31.7, 28.9, 21.6, 14.2. Anal. Calcd for $C_{18}H_{28}N_4$: C, 71.96; H, 9.39; N, 18.65; Found: C, 71.94; H, 9.41; N, 18.64%.

*4.2.7. 1-Benzyl-1H-benzimidazole (**13**)*

The reaction of benzimidazole (**12**, 1.0 g, 8.47 mmol) and benzyl bromide (1.0 ml, 8.47 mmol) in the slurry of NaH (0.3 g, 12.5 mmol) as described above gave **13**. FT-IR (KBr, cm^{-1}): 3286, 3018, 1672, 1510, 1405; ESMS: 209 $(M+H)^+$. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.87–7.11 (m, 10H, Im–H and Ar–H), 5.32 (s, 2H, CH_2); ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 144.4, 143.2, 135.9, 134.2, 129.3, 128.5, 127.3, 123.3, 122.5, 120.9, 110.1, 49.07. Anal. Calcd for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81; N, 13.45; Found: C, 80.72; H, 5.83; N, 13.44%.

*4.2.8. 1-(3,4-Dichloro-benzyl)-1H-benzimidazole (**14**)*

The reaction of **12** and 3,4-dichloro benzyl bromide (1.26 ml, 8.47 mmol) as described above gave **14**. FT-IR (KBr, cm^{-1}): 3386, 3020, 1672, 1610, 1495; ESMS: 278 $(M+H)^+$. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.94–6.93 (m, 8H, Im–H and Ar–H), 5.30 (s, 2H, –CH_2). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 144.3, 143.4, 136.1, 133.9, 132.9, 131.4, 130.3, 129.3, 126.5, 123.8, 121.0, 112.41, 110.1, 48.0. Anal. Calcd for $C_{14}H_{10}N_2Cl_2$: C, 60.67; H, 3.64; Cl, 25.58; N, 10.11; Found: C, 60.65; H, 3.62; Cl, 25.56; N, 10.09%.

*4.2.9. Synthesis of 1-(2-hydroxy ethyl)-1H-imidazole (**10**) [34]*

To the stirred slurry of $LiAlH_4$ (0.34 g, 8.92 mmol) in dry THF under nitrogen atmosphere, compound **4** (1.0 g, 8.92 mmol) was added slowly at 0 °C and the reaction mixture was further stirred for 2 h at room temperature. The excess of the reducing agent was quenched with saturated NH_4Cl, filtered the reaction mixture on celite pad, the filtrate was evaporated. Water was added to the residue and the solution was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using methanol:chloroform (1:5) as eluent gave compound **10**. FT-IR (KBr, cm^{-1}): 3447, 2925, 1713, 1594; ESMS: 113 $(M+H)^+$. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.31 (s, 1H, Im–H), 6.89 (s, 1H, Im–H), 6.82 (s, 1H, Im–H), 3.98 (m, 2H, CH_2), 3.78 (m, 2H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 137.5, 128.5, 119.8, 61.5, 50.3. Anal. Calcd for $C_5H_8N_2O$: C, 53.56; H, 7.19; N, 24.98; Found: C, 53.54; H, 7.20; N, 24.99%.

*4.2.10. 1-(2-Benzyl amino ethyl)-1H-imidazole (**11**)*

A solution of the above compound **10** in anhydrous dichloromethane (20 ml) and triethyl amine was treated with methanesulphonyl chloride and reaction mixture was stirred at room temperature for 4 h. After the completion of the reaction, the reaction mixture was poured over a mixture of crushed ice and $NaHCO_3$ and extracted with dichloromethane. Dichloromethane layer was dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give a crude methanesulphonyloxy derivative which was used as such in the next step. The latter was refluxed with benzyl amine (0.7 ml, 6.41 mmol) in toluene in presence of DBU (5 mol%) and 4 Å molecular sieve for 4 h. The reaction mixture was extracted with chloroform and washed with aqueous $NaHCO_3$ followed by water (2 × 25 ml), organic layer was dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to give compound **11**. FT-IR (KBr, cm^{-1}): 3235, 3021, 1603, 1457; ESMS: 202 $(M+H)^+$. ^{1}H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.33–7.25 (m, 8H, Im–H and Ar–H), 4.86 (bs, 1H, –NH), 4.31–4.24 (m, 2H, $PhCH_2$), 2.82–2.70 (m, 4H, 2 × –CH_2). Anal. Calcd for $C_{12}H_{15}N_3$: C, 71.61; H, 7.51; N, 20.88; Found: C, 71.59; H, 7.60; N, 20.86%.

*4.3. General procedure for the synthesis of imidazol-1-yl-acetamides (**6**–**9**)*

A mixture of imidazol-1-yl-acetic acid ethyl ester (**4**, 1.0 g, 6.4 mmol), DBU (5 mol%) and appropriate amine (6.4 mmol) in the presence of 4 Å molecular sieve (1.0 g) in dry toluene (10 ml) was

stirred at room temperature for 10 min. The corresponding amine was added and the reaction mixture was heated to 80 °C for 10–18 h. After the completion of the reaction, it was cooled to ambient temperature, followed by extraction with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using methanol:chloroform (1:5) as eluent.

4.3.1. N-*(*n*-Butyl)-2-imidazol-1-yl-acetamide (**6**)*

The reaction of **4** and *n*-butylamine (0.64 ml, 6.45 mmol) as described above gave **6**. FT-IR (KBr, cm^{-1}): 3288, 2960, 1668, 1564; ESMS: 182 $(M+H)^+$. ^1H NMR (200 MHz, $CDCl_3$–CCl_4): $\delta = 7.50$–6.97 (m, 3H, Im–H), 5.96 (bs, 1H, –NH), 4.64 (s, 2H, $-COCH_2$), 3.28–3.19 (m, 2H, $-NHCH_2$), 1.50–1.18 (m, 4H, $2\times -CH_2$), 0.89 (m, 3H, $-CH_3$). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 167.1, 138.3, 130.3, 120.0, 50.4, 39.8, 31.6, 20.3, 14.0. Anal. Calcd for $C_9H_{15}N_3O$: C, 59.64; H, 8.34; N, 23.19; Found: C, 59.62; H, 8.35; N, 23.18%.

4.3.2. N-*(*n*-Hexyl)-2-imidazol-1-yl-acetamide (**7**) [33]*

The reaction of **4** and *n*-hexylamine (0.85 ml, 6.49 mmol) as described above gave **7**. FT-IR (KBr, cm^{-1}): 3307, 2933, 2361, 1669, 1570. ESMS: 210 $(M+H)^+$. ^1H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.27–6.83 (m, 3H, Im–H), 5.40 (bs, 1H, –NH), 4.55 (s, 2H, $-COCH_2$), 3.23 (m, 2H, $-NHCH_2$), 2.61 (m, 2H, $-CH_2$), 1.77 (m, 2H, $-CH_2$), 1.46–0.99 (m, 7H, $2\times -CH_2$ and $-CH_3$); ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 167.2, 149.3, 128.9, 119.9, 49.5, 39.9, 31.7, 29.6, 28.8, 26.7, 22.8, 21.4, 14.3. Anal. Calcd for $C_{11}H_{19}N_3O$: C, 63.13; H, 9.15; N, 20.08; Found: C, 63.12; H, 9.17; N, 20.10%.

4.3.3. N-*(*n*-Heptyl)-2-imidazol-1-yl-acetamide (**8**)*

The reaction of **4** and *n*-heptylamine (0.97 ml, 6.52 mmol) as described above gave **8**. FT-IR (KBr, cm^{-1}): 3300, 2933, 2360, 1669, 1569; ESMS: 224 $(M+H)^+$. ^1H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.51–6.93 (m, 3H, Im–H), 5.82 (bs, 1H, –NH), 4.64 (s, 2H, $-COCH_2$), 3.24 (m, 2H, $-NHCH_2$), 1.43 (m, 2H, $-CH_2$), 1.24 (s, 8H, $4\times -CH_2$), 0.87 (m, 3H, $-CH_3$). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 167.0, 138.4, 130.9, 120.1, 50.5, 40.1, 32.0, 29.6, 29.2, 27.1, 22.9, 14.9. Anal. Calcd for $C_{12}H_{21}N_3O$: C, 64.54; H, 9.48; N, 18.82; Found: C, 64.52; H, 9.50; N, 18.84%.

4.3.4. N-*Benzyl-2-imidazol-1-yl-acetamide (**9**)*

The reaction of **4** and benzylamine (0.7 ml, 6.41 mmol) as described above gave **9**. FT-IR (KBr, cm^{-1}): 3212, 2940, 1683, 1597; ESMS: 216 $(M+H)^+$. ^1H NMR (200 MHz, $CDCl_3$–CCl_4): δ 7.46–6.94 (m, 8H, Im–H and Ar–H), 6.32 (bs, 1H, –NH), 4.66 (s, 2H, $-COCH_2$), 4.41 (m, 2H, $-CH_2$). ^{13}C NMR (50 MHz, $CDCl_3$–CCl_4): δ 167.1, 138.3, 130.1, 129.1, 128.0, 120.3, 50.3, 43.9. Anal. Calcd for $C_{12}H_{13}N_3O$: C, 66.96; H, 6.09; N, 19.52; Found: C, 66.94; H, 6.10; N, 19.50%.

4.4. Biology

4.4.1. Activity against M. tuberculosis H_{37}Ra strain (microplate alamar blue assay – MABA method)

The compounds were evaluated against *M. tuberculosis* H_{37}Ra at concentration ranging from 50 μg/ml to 3.12 μg/ml using twofold dilutions in the initial screen. Log phase culture of *M. tuberculosis* H_{37}Ra was diluted so as to give final OD_{550nm} of 0.05 in Sauton's medium. In 96 well white plates 190 μl of culture was dispensed in each well. A dimethyl sulfoxide solution of test compounds was dispensed to 96 well plates so as to make final test concentration 25 μg/ml (5 μg test compound was dispensed in 10 μl of DMSO). Then the plate was incubated at 37 °C/5% CO_2 for 5 days. On 5th day 15 μl alamar blue solution was added to the each well of plate. The plate was again incubated overnight at 37 °C/5% CO_2 incubator. The fluorescence was read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found active (>90% inhibition as compared with control) at this concentration were further tested at 6 serial dilutions starting from 50 to 3.12 μg/ml.

4.4.2. Activity against M. tuberculosis H_{37}Rv strain (Agar dilution method)

Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis* H_{37}Rv was performed by agar microdilution method [23,24] where twofold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H_{37}Rv growing on L–J medium was harvested in 0.85% saline with 0.05% Tween-80. A solution of 1 μg/ml concentration of compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentrations of compound keeping the volume constant i.e. 0.1 ml. Medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H_{37}Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H_{37}Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

4.4.3. BACTEC method

Stock solution of the test compounds prepared in DMSO at 1 mg/ml was sterilized by passage through 0.22 μm filters. 50 μl were added to 4 ml radiometric 7H12Broth (BACTEC 12B; Becton Dickinson Diagnostic Instrument System US) to achieve final concentrations. Controls received 50 μl DMSO. Ofloxacin, streptomycin and rifampicin (Sigma Chemical Co. St. Louis, MO) were included as positive drug control. In BACTEC method, *M. tuberculosis* H_{37} Rv was scraped from fresh Lowenstein–Jensen slants resuspended in 3 ml diluting fluid and homogenized with glass beads (2 mm). Homogenous supernatant was taken; turbidity was adjusted to mc Farland 1 with diluting fluid and 0.1 ml injected into a BACTEC 12B vial which was used as a primary inoculum after growth index (GI) of 0 reached 500–700. 0.1 ml of this suspension was used to inoculate 4 ml fresh BACTEC 12B broth containing the test compounds. An additional control vial was included which received a further 1:100 inoculum. Cultures were incubated at 37 °C and the GI determined daily. When the GI of 1:100 control vials reached 30, the test was read for an additional day and then terminated. If the drug difference in the GI values from the previous day (called ΔGI) in case of drug containing vials was less than ΔGI of the 1:100 control, then the bacteria was defined as 1 − (GI of the test sample/GI of control) × 100. Assays were completed in 5–8 days and were carried out according to procedure reported earlier [29–31].

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