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STUDIES ON *IN VITRO* ANTIMYCOBACTERIAL ACTIVITIES OF SOME 2-SUBSTITUTEDIMIDAZO[4,5-b] AND [4,5-c]PYRIDINE DERIVATIVES

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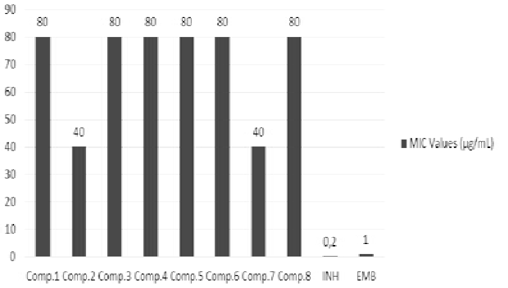
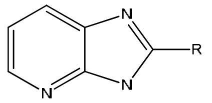
In this study, 2-substitutedimidazo[4,5-b]pyridine and 2-substitutedimidazo[4,5-c]pyridine which have -CH_2OH, -CH_2Cl, -CH_3 or -C_2H_5 groups in positions 2 have been synthesized and tested for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strains. The compounds were synthesized by previously reported methods. 2-Chloromethylimidazo[4,5-b]pyridine and 2-methylimidazo[4,5-c]pyridine were found to be more active than the other compounds tested.





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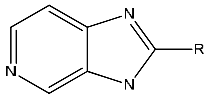

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INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis* is a leading cause of death worldwide.[1] Further contributing to the increased morbidity is the emergence of new strains of *Mycobacterium tuberculosis* resistant to some or all currently used antitubercular drugs.[2,3] Therefore, it is critical to develop novel drugs that are active against the drug-resistant strains, and shorten their treatment durations.[4]

Fused imidazole derivatives have occupred a prominent place in medicinal chemistry because of their important properties as therapeutics in clinical application.[5] In particular, compounds incorporating the imidazopyridine ring system have shown a broad range of biological and pharmacological activities.[6,7] Some 2-substitutedimidazo[4,5-b]pyridine derivatives have been reported for different biological activities including antituberculostatic,[8-17] antiviral,[18] inhibitors of Aurosa kinases,[19] inotropic,[20] antiproliferative,[21,22] nitric-oxide synthase inhibitors,[23] anticancer[24]and PAF (platelet activating factor) antagonist[25] activities. Additionally, some biological activity studies have been made for the [4,5-c] analog of 2-substitutedimidazopyridine derivatives. These activities include inotropic,[20] PAF antagonist,[25-28] antihistaminic,[29] antiviral,[30,31] pyrimidine-based Janus tyrosine kinase 3 inhibitors[32] and the Hsp90 Molecular Chaperone inhibitor activities.[33] It can be speculated that biological activity of this compunds may be related to their structural similarity to the natural purin bases.

Dimmling and Hein reported that 7-aminoimidazo [4,5-b] pyridine has (1-deazapurin) in vitro antibacterial effect.[34]

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Bukowski and coworkers reported that 2-cyanomethylimidazo[4,5-b]pyridine and its derivates have been found as antimycobacterial active. In the study, 2-(α-carbothioamide-β-arylvinyl)imidazo[4,5-b]pyridines have been found to be the most active compound among the tested compounds.[8] In an other study, QSAR (Analysis of Quantitative Structure-Activity Relationships) study of *in vitro* antimycobacterial activite 2-substitutedimidazo[4,5-b]pyridine.[10,11] The researchers investigated the importance of the hydrophobicity for antibacterial activity of the compounds. They have concluded that the antimycobacterial activity values of the imidazo[4,5-b] pyridine derivates tested increased by increasing the hydrophobic properties of the compounds. They have also reported that the steric and/or electronic effects caused by N1-methyl substitution oppose the effects resulting from increasing lipophilicty. [10,11] In an other study about the antimycobacterial activities of some imidazo[4,5-b]pyridine derivatives, the compounds having 2-(1-chloroethyl) substituent were found to be more active than the compound having 1-hydroxyethyl substituent on the same position.[13]

In this study, we synthesized four 2-substitutedimidazo[4,5-b]pyridine (Compound 1-4) and four 2-substitutedimidazo[4,5-c]pyridine (Compound 5-8) compounds to determine the in vitro antimycobacterial activities. The *in vitro* antimycobacterial activities of the compounds against *M. tuberculosis* H37Rv strains were performed by using the agar proportion method.

RESULTS

The melting points and spectral data of the compounds synthesized by us were in accordance with the literature.[35-40]

The compounds 1-4 and 5-8 which are imidazo[4,5-b] and [4,5-c]pyridine derivatives respectively were tested for their *in vitro* antimycobacterial activities against *M. tuberculosis* H37Rv, which is susceptible to INH (Isoniazid) and EMB (Ethambutol). The minimum inhibitory concentration (MIC) values were determined using the agar proportion method in Middlebrook 7H10 medium.

The MIC values of synthesized compounds 1-8 and reference compounds INH and EMB are given in Table 2. The activities of synthesized compounds 1-8 indicated that compounds 2 and 7 which are bearing chloromethyl substituent on position 2 of imidazo[4,5-b]pyridine and methyl substituent on position 2 of imidazo[4,5-c]pyridine rings respectively, were more potent than the other compounds synthesized, with MIC values 40 µg/mL The other compounds, exhibited inhibitory effects with MIC values 80 µg/mL The comparison of *in vitro* antimycobacterial activities of 2-substitutedimidazo[4,5-b]pyridine (Compound 1-4) and 2-substitutedimidazo[4,5-c]pyridine (Compound 5-8) derivatives showed that there were no significant differences between the activities of these two series (Fig. 1). All of the compunds we synthesized in this study may be considered as moderately *in vitro* antimycobacterial active compounds.

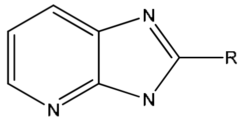
Table 1

Melting point and ^{1}H-NMR data of 2-substitutedimidazo[4,5-b] and [4,5-c] pyridine 1-8

Compounds	Melting Points	^{1}H-NMR (DMSO-d_6) ppm (δ)
1	214 °C (lit[38]: 219 °C)	4.75 (s,CH_2), 5.8 (b,1H), 7.2 (t, 1H), 7.9 (d, 1H), 8.2 (d,1H), 12.9 (s,1H)
2	261 °C (lit[38]: 255 °C)	5 (s,CH_2), 7.5 (t, 1H), 8.3 (d, 1H), 8.7 (d,1H)
3	188 °C (lit[37]: 188.5 °C)	2.64 (s, 3H), 7.24 (t, 1H), 8.01 (d, 1H), 8.33 (d,1H), 12.60 (s,1H)
4	153 °C (lit[37]: 152 °C)	1.54 (t, 3H), 3.10 (q, 2H), 7.23 (t, 1H), 8.02 (d, 1H), 8.32 (d,1H), 13.01 (s,1H)
5	197 °C (lit[34]: 198 °C)	4.9 (s,CH_2), 5.9 (b,1H), 7.2 (d, 1H), 8.3 (d, 1H), 8.8 (s,1H), 12.9 (s,1H)
6	Dec.≥ 300 °C (lit[36]: 345 °C)	5.1 (s,CH_2), 8.1 (d, 1H), 8.6 (d, 1H), 9.4 (s,1H)
7	168 °C (lit[34]: 170 °C)	2.65 (s, 3H), 7.3 (d, 1H), 8.4 (d, 1H), 8.9 (s,1H)
8	192 °C (lit[35]: 192 °C)	1.37 (t, 3H), 2.89 (q, 2H), 7.37 (d, 1H), 8.23 (d, 1H), 8.79 (s,1H), 12.55 (s,1H)

Table 2

In vitro antimycobacterial activity of 2-substitutedimidazo[4,5-b] and [4,5-c] pyridine 1-8



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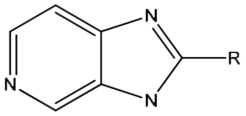

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Compounds	R	MIC(μg/mL)	Compounds	R	MIC(μg/mL)
1	CH_2OH	80	5	CH_2OH	80
2	$CH_2Cl.HCl$	40	6	CH_2Cl	80
3	CH_3	80	7	CH_3	40
4	C_2H_5	80	8	C_2H_5	80
INH		0.2	EMB		1

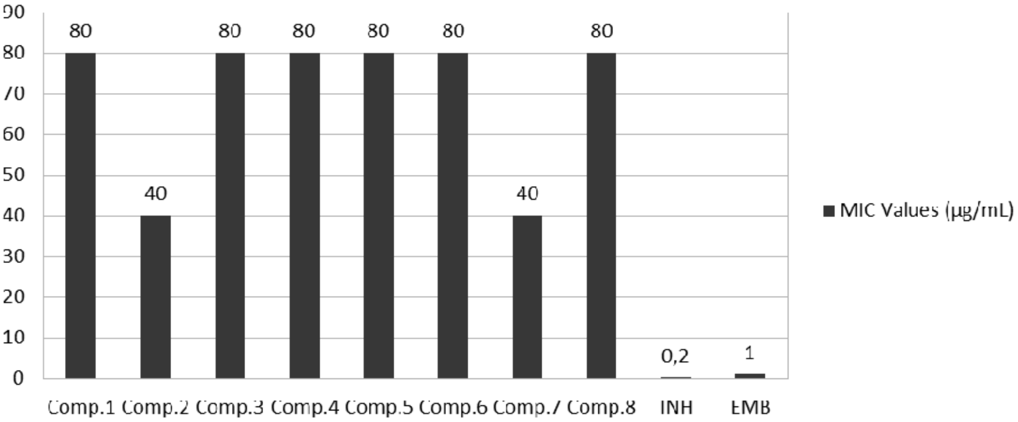

Fig. 1 – MIC values of 2-substitutedimidazo[4,5-b] and [4,5-c] pyridine 1-8.

EXPERIMENTAL

Synthesis

The compounds which were synthesized by the other researchers previously were synthesized by the methods as reported before.[35-40] Compounds (3,4,7,8) were synthesized by condensation of the corresponding carboxylic acid with 2,3-diaminopyridine or 3,4-diaminopyridine in the presence of PPA. The synthesis of compound 1 and 5 were performed by the heating of 2,3-or 3,4-diaminopyridine and glycolic acid at 160 ^{o}C. Compounds 2 and 6 were synthesized by the reaction of compound 1 or 5 and $SOCl_2$ (thionylchloride) in the medium of dry $CHCl_3$ (chloroform). [35,39]

Biological activity

Microorganism identification

M. tuberculosis H37Rv strain was provided from Refik Saydam National Public Health Agency, National Tuberculosis Reference Laboratory, Ankara, Turkey.

Agar proportion method

The minimum inhibitory concentration values of each synthesized compounds were obtained by agar dilution in duplicate as recommended by the Clinical Laboratory Standards Institute (CLSI).[41,42] Positive and negative growth controls were run in each assay. Isoniazid (Sigma I3377) and ethambutol (Sigma E4630) were used as control agents. H37Rv was used as the standard strain. Stock solutions of synthesized compounds and the reference compounds were prepared in dimethylsulfoxide (DMSO)/H_2O (50%) at a concentration of 1000 µg/mL. These solutions were then filtered through a 0.22 µm membrane filter (Millipore, USA). Middlebrook 7H10 agar medium (BBL, Becton Dickinson and Company, Sparks, MD, USA) was supplemented with oleic acid-albumin-dextrose-catalase (OADC, BBL, Becton Dickinson and Company, Sparks, MD, USA). Synthesized compounds and control agents were added to obtain an appropriate final concentration in the medium. The final concentrations of INH and EMB were 0.2-1 µg/mL and 1 µg/mL, respectively. The final concentrations of the synthesized compounds were of 5, 10, 20, 40 and 80 µg/mL. Agar without any references and synthesized compounds were used as a positive growth control, and 3 mL of prepared medium was dispensed into sterile tubes. The DMSO concentration in the final solutions was not above 1% for antimycobacterial activity.

Inoculum preparation

H37Rv was maintained in Lowenstein-Jensen medium. A culture suspension was prepared by subculturing in Middlebrook 7H9 broth (BBL, Becton Dickinson and Company, Sparks, MD, USA) supplemented with 10% OADC at 37°C for 7-10 days, until a density corresponding to 10^{-2} to 10^{-4} dilutions were obtained from McFarland standard No. 1. Then, 0.1 mL of the diluted suspension was inoculated onto the control and the other tubes with compounds in different concentrations. The tubes were incubated at 37°C in an atmosphere of 5% CO_2 for 3 weeks. The MIC values were defined as the lowest concentration that inhibited more than 90% of the bacterial growth, and the results of INH and EMB were interpreted according to the CLSI. The MIC was considered the lowest concentration that showed no visible colonies in all dilutions.

The biological activity studies of the compounds were made twice. The results of these two studies was almost same. The average MIC values were calculated as the activity values of the compounds. The results are given in Table 1 and Fig. 1.

CONCLUSIONS

2-substitutedimidazo[4,5-b]pyridine and 2-substitutedimidazo[4,5-c]pyridine which have $-CH_2OH$, $-CH_2Cl$, $-CH_3$ or $-C_2H_5$ groups in their positions 2 have been synthesized and tested for their *in vitro* antimycobacterial activities against *M. tuberculosis* H37Rv strains. This compounds showed moderate *in vitro* antimycobacterial activities. The structural modification on these compounds may be hopeful to improve the antimycobacterial activity of this series.

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