Current Pharma Research
ISSN: 2230-7842
CPR 1(2), 2011, 135-139.

Synthesis, antimicrobial and antimycobacterial activity of nicotinic acid hydrazide derivatives.

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Abstract
Pyridine 3-carboxillic acid **1** on treatment with phosphorous pentachloride and anhydrous carbon tetrachloride followed by reaction with hydrazine hydrate gave nicotinohydrazide **2**. And compound **2** on further reaction with acetyl acetone, ethyl acetoacetate, ethylcyanoacetate and different substituted aromatic acids yielded the corresponding (3,5-dimethyl-1H-pyrazol-1-yl)(pyridine-3-yl)methanone **3**, 3-methyl-1-nicotinoyl-1H-pyrazol-5(4H)-one **4**, 3-amino-1-nicotinoyl-1H-pyrazol-5(4H)-one **5** and 3-(5-substituted-1,3,4 oxadiazole) pyridine **6a-d**, respectively. All the synthesized compounds have been screened for their antimycobacterial activity.

Key Words
Pyridine, pyrazole, substituted oxadiazole, antimycobacterial activity.

Introduction
Heterocycles containing pyridine rings are associated with a wide range of biological properties such as anticonvulsant[1], antiparkinsonian[2], anti-inflammatory[2], antitumor[3,4], antimalarial[5], antimicrobial[5,6,7], antimycobacterial[8,9] activities due to toxophoric –N=C- group. Pyrazole represent one of the most active classes of compounds possessing a wide spectrum of biological activities, such as anti-inflammatory[10], antipyretic, analgesic and smooth muscle relaxant[11] activities. Many pyrazole derivatives are associated with antifungal, anti-diabetic[12] and bactericidal[13] activities. Large number of oxadiazole derivatives reported in the literature possesses a broad spectrum of pharmacological activity such as antimicrobial, antimalarial, anticonvulsant, anticancer, cyclooxygenase, anti HIV property[14] and anti-inflammatory[15] activities. In the present study we have aimed to achieve a better antimycobacterial profile at lower concentrations, by preparing nicotinic acid hydrazide derivatives. This report deals with the synthesis of nicotinic acid hydrazide derivatives and screening for their antimicrobial and antimycobacterial activities.

Materials and Methods
The starting material, nicotinic acid **1** was prepared in laboratory. Melting points were determined in open capillary tubes and are uncorrected.

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IR spectra were recorded in KBr discs (V_{max} in cm^{-1}) on Perkin-Elmer FT-IR (Spectrum ONE) spectrometer and ^{1}H NMR spectra on bruker AMX (400 MHz) spectrometer using DMSO-d_6 as solvent unless otherwise stated, using TMS as an internal standard (chemical shifts in δ,ppm) and mass spectra on a Jeol SX-102 (FAB) mass spectrometer.

Synthesis of nicotinohydrazide (2)
Compound **1** A mixture of nicotinic acid (0.03mole) and phosphorous pentachloride (0.07mole) in anhydrous carbon tetrachloride (20mL) were refluxed for 2 hrs at 100^{o}C. Solvent was distilled off and the solid acid chloride thus obtained was used for further reaction without any purification. To the nicotinoyl chloride (0.03mole) was added hydrazine hydrate (0.1mole) drop wise below 5^{o}C and the resultant mixture was stirred for 5 hrs at room temperature. A solid that separated out was washed with aqueous $NaHCO_3$ (10 %) and dried in vaccuo. It was recrystallized from methanol to obtain pure crystalline solid **2**,
(2): 76%, m.p.148^{o}C. ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 2H, NH_2), 8.0 (s, 1H, NH), 7.63-9.17 (m, 4H, Ar-H); IR (KBr): 1345 (C-N), 1628 (C=N), 1680 (C=O), 3335 (NH); *m/z* (%) 137 (7), 122 (38), 106 (100).

Synthesis of 3, 4 and 5
To a solution of **2** (0.001mole) in ethanol (10 mL), appropriate diketone ethylacetoacetate/ acetyl acetone/ ethylcynoacetate, 0.002 mol) was added and the reaction mixture was refluxed on a water-bath for 12 hrs in presence of catalytic amount of glacial

acetic acid (2-3 drops). The reaction contents were cooled to RT and the obtained product (**3,4,5**) was filtered, dried and purified by recrystallization from ethanol.

(3): (3, 5-dimethyl-1H-pyrazol-1-yl)(pyridine-3-yl)methanone, 71%, m.p. 155^{o}C. ^{1}H NMR (DMSO-d_6): δ 2.79 (s, 3H, CH_3), 5.9 (s, 1H, CH), 7.45-7.81 (m, 4H, Ar-H); IR (KBr): 1622 (C=N), 1350 (C-N), 1683 (C=O); *m/z* (%) 202(16), 200 (18), 186 (12), 175 (30), 106 (100).

(4): 3-methyl-1-nicotinoyl-1H-pyrazol-5(4H)-one, 74%,199^{o}C. ^{1}H NMR (DMSO-d_6): δ 0.9 (s, 3H, CH_3), 2.2 (s, 2H, CH_2), 7.44-7.95 (m, 5H, Ar-H); IR (KBr): 1615 (C=N), 1718, 1722 (C=O); *m/z* (%) 202 (19), 188 (31), 162 (43), 106 (100).

(5): 3-amino-1-nicotinoyl-1H-pyrazol-5(4H)-one, 68%, 204^{o}C. ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 2H, NH_2), 2.2 (S, 2H, CH_2), 7.44-7.95 (m, 4H, Ar-H); IR (KBr): 1320 (C-N), 1615 (C=N), 1696 (C=O), 3420 (NH); *m/z* (%) 202 (16), 188 (39), 162 (42), 106 (100).

Synthesis of 3-(5-substituted-1,3,4 oxadiazole) pyridine (6a-d)

A mixture of **2** (0.001mole), substituted aromatic acid(s) (0.002mole) and phosphorous oxichloride (15 mL) was refluxed on oil bath at 100-110^{o}C for 6 hrs. The excess of phosphorous oxichloride was distilled off and cooled residue was poured into icecold water. The content were neutralized with ammonia to offered crude product(s) **8a-d**, which were filtered, dried and purified by recrystallization from 1,4-dioxane.

(8a): 70%, m.p. 245^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.22-8.81 (m, 9H, Ar-H); IR (KBr): 1551, 1563 (C=N); *m/z* (%) 223 (25), 199 (40), 161 (54), 147 (48), 118 (100).

(8b): 76%, m.p. 240^{o}C. ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 2H, CH_2), 7.06-8.81 9m, 9H, Ar-H); IR (KBr): 1142 (C-O-C), 1578, 1610 (C=N); *m/z* (%) 237 (10), 227 (43), 189 (62), 147 (100).

(8c): 75%, m.p. 281^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.33-8.81 (m, 8H, Ar-H); IR (KBr): 1148 (C-O-C), 1686, 1599 (C=N), 712 (C-Cl); *m/z* (%) 259 (M+2, 15), 257 (19), 223 (31), 187 (42), 147 (53), 118 (100).

(8d): 73%, m.p. 223^{o}C. ^{1}H NMR (DMSO-d_6): δ 7.44-8.81 (m, 8H, Ar-H); IR (KBr): 1155 (C-O-C), 1620, 1642 (C=N), 1560 (C-NO_2); *m/z* (%) 268 (18), 227 (28), 161 (63), 118 (100).

Antimicrobial activity

The in-vitro biological screening of the synthesized compounds was undertaken against the bacteria species, namely, *Staphylococcus aureus*, *Escherichia coli,* and *Bacillus subtilus* and fungi species, namely, *Aspergillus niger* and *Candida albicans* by cup-plate method[6,7] using nutrient agar as a medium. The holes of 6mm diameter were punched carefully using a sterile cork borer and these were filled with test solutions (1000 µg/ml in DMF) and DMF was used as control. The plates were incubated at 37^{o}C for 24 hrs in case of antibacterial activity and antifungal activity, respectively. The diameter of the zone of inhibition for all the test compounds was measured and the results were compared with the standard drug gentamycin for antibacterial activity and Nystatin for antifungal activity (Table 1).

Antimycobacterial activity

Antitubercular activity was determined by the REMA plate method[15]. The Minimum Inhibitory Concentration (MIC) of the newly synthesized compound was tested against Mycobacterium tuberculosis $H_{37}R_V$. The REMA plate method was performed to determine the MICs of test compounds for all the mycobacterial isolates. The 100 ml volume of Middlebrook 7H9 broth (Difco, USA) was dispensed in each well of a 96-well cell culture plate (Nunc, Denmark). Test compound concentrations prepared directly in the medium were 1.25, 2.5, 3.75, 5.0, 6.25, 7.5, 8.75 and 10.0 mg/l. Perimeter wells of the plate were filled with sterile water to avoid dehydration of the medium during incubation. A standard bacterial suspension equivalent in turbidity to that of a no. 1 McFarland standard was prepared and diluted 1:20 in 7H9 broth; a 100 ml inoculum was used to inoculate each well of the plate. A growth control containing no test compound and a sterile control without inoculum were also included e. Plates were sealed and incubated at 37^{0}C for 1 week. 25µL of 0.02% resazurin (Sigma Chem. Co.) Solution was added to each well; plates were re-incubated for an additional 2 days. A change in colour from blue to pink indicated the reduction of resazurin and therefore bacterial growth, and the MIC was read as the minimum test compound concentration that

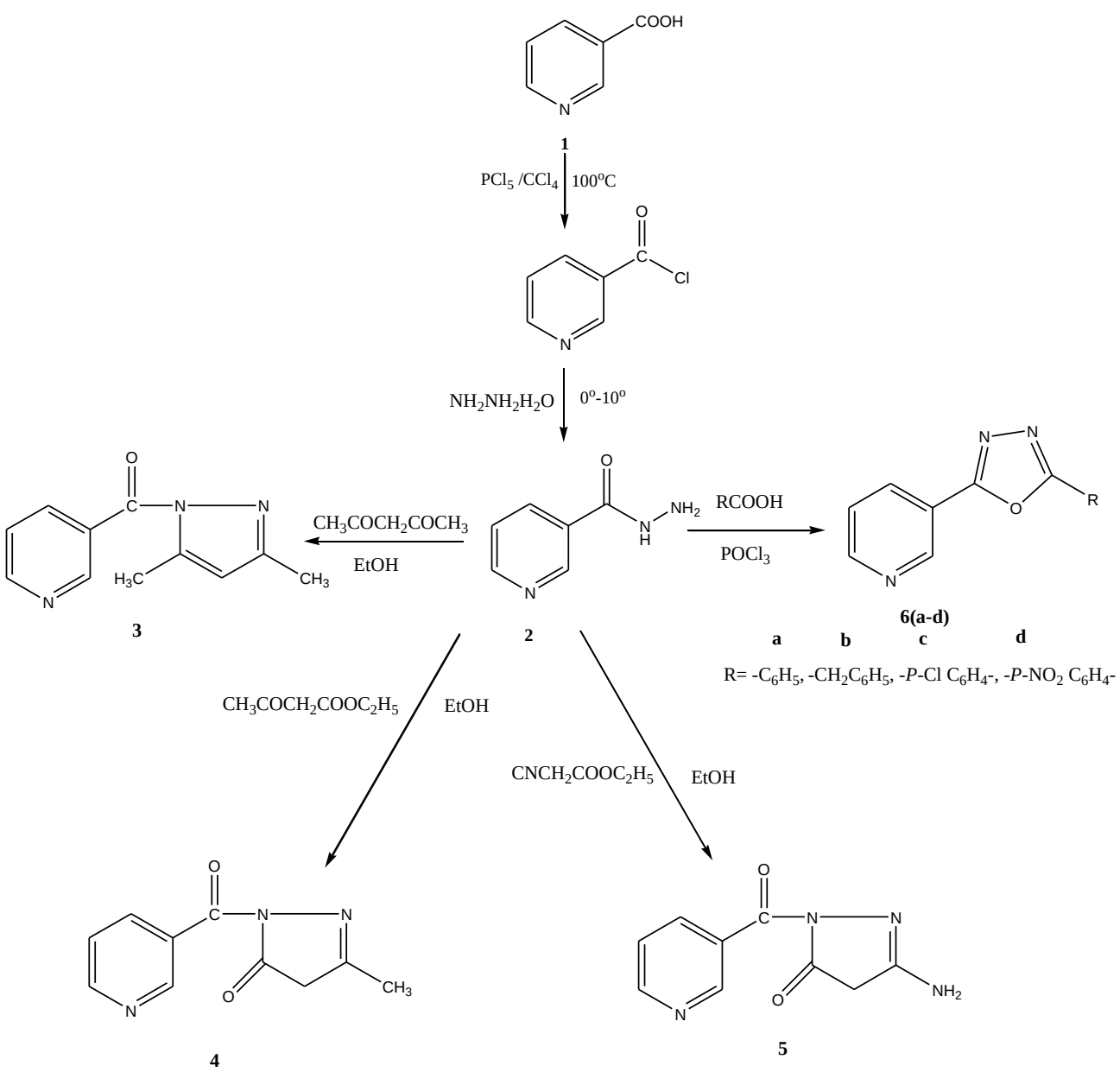
prevented the colour change in resazurin solution (Table 2).

Results and Discussion

The synthesized compounds were evaluated for their antimicrobial as well as antimycobacterial activities, in comparison with the standards, namely, Gentamycin, Nystatin and Isoniazid, respectively. In overall bioassay (Table 1) in general the compounds 4, 5, 6c and 6d exhibited good antimicrobial potency against both types of test species. In (Table 2) the compounds 4 and 6c exhibited good antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_V$

Conclusion

This study reports the successful synthesis of some new nicotinic acid hydrazide derivatives. The antimicrobial screening studies were also performed in the study. The substituted pyrazole and oxadiazole are the active components present in many standard drugs and it is known to increase the pharmacological activity of the molecules. The antimicrobial screening suggests that among the newly synthesized compounds, 4, 5, 6c and 6d exhibited good activity against all the tested microorganisms and the antimycobacterial screening suggest that 4 and 6c exhibited good antimycobacterial activity against tested microorganism.



Scheme 1

Table 1: Antimicrobial activity of the synthesized compounds.

Compound	Conc. (µg/0.1mL) in DMF	Zone of inhibition in mm*				
		Antibacterial activity			Antifungal activity	
		S. aureus	E. coli	B. subtilus	A. niger	C. albicans
2	100	09	11	12	11	12
3	100	14	12	11	12	14
4	100	19	17	18	17	18
5	100	16	16	19	14	15
6a	100	14	13	16	16	17
6b	100	15	13	15	08	09
6c	100	18	19	18	18	17
6d	100	17	15	18	17	18
Gentamycin	100	22	20	21	-	-
Nystatin	100	-	-	-	22	21
Control (DMF)	-	-	-	-	-	-
***Diameter of well (bore size) - 6 mm**						

Table 2: Antimycobacterial activity of the synthesized compounds.

Compound	MIC Concentration (µg/ml)
2	6.25
3	3.75
4	2.5
5	3.75
6a	5.0
6b	5.0
6c	2.5
6d	3.75
Isoniazid	1.25

Acknowledgement

The authors are thankful to the Directors, IIT, Chennai for spectral data. Authors are also grateful to HOD of Department of Chemistry, C.L.Baid Metha College of Pharmacy, Chennai, for providing laboratory facilities.

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