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**Original Article**

# Synthesis and anti-tubercular activity of a series of *N'*-substituted isonicotinohydrazide derivatives

Mahesh T. Chhabria*, Sweta Patel[1]

*Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Navrangpura, Ahmedabad, Gujarat, India.
[1]L. J. Institute of Pharmacy and Research Centre, Ahmedabad, Gujarat, India.

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## ABSTRACT

Tuberculosis is a leading cause of death worldwide and especially in developing countries due to the recent emergence and spread of multi-drug resistant tuberculosis (MDR TB), extensive drug resistant tuberculosis (XDR TB), total drug resistant tuberculosis (TDR TB) and HIV/AIDS pandemic. Discovery of target based newer anti-tubercular agent is area of interest for chemists. We have designed a series of *N'*-substituted isonicotinohydrazide derivatives based on pharmacophore modeling. Designed molecules were synthesized and evaluated for their anti-tubercular activity. Among synthesized compounds, *N'*-(2-(2-fluorophenyl) acetyl) isonicotinohydrazide **(4m)** was found to be the most active compound with MIC value of 6.25 µg/ml against *Mycobacterium tuberculosis* H37Rv strain using Almar Blue method. Compound **(4m)** was found active against MDR-TB and XDR-TB strain at 62.5 and 250 µg/mL.

**Keywords:** Pharmacophore modeling, Antitubercular activity, *Mycobacterium tuberculosis*, Isonicotinohydrazide derivatives.

## INTRODUCTION

Tuberculosis (TB) poses a global healthcare emergency with an estimated 1.5 million deaths (1.1 million HIV-negative and 0.4 million HIV-positive) from TB in 2014. Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014 which include 5.4 million men, 3.2 million women and 1.0 million children. Globally, 12% of the 9.6 million new TB cases in 2014 were HIV-positive. [1]. The length and complexity of current TB treatment regimens results into poor patient compliance, a major contributing factor in the emergence of multi-drug-resistant TB (MDR-TB), extensively drug-resistant (XDR-TB) and total drug resistant tuberculosis (TDR-TB). It was estimated that as much as one third of the world's population is currently infected with TB bacilli [2]. The WHO estimates that 36 million people will die of tuberculosis by 2020 if it is not controlled. TB is capable of establishing persistent infection in the host by using a complex interplay between the host immune system and bacterial survival mechanisms. Despite extensive research in the last 40 years, the treatment of TB is limited to a cocktail of drugs, including isoniazid, ethambutol, pyrazinamide and rifampicin, which target cell-wall biosynthesis and RNA synthesis. This need is driving force to identify and prioritize new potent molecules which can be effective against mycobacterial resistant strains.

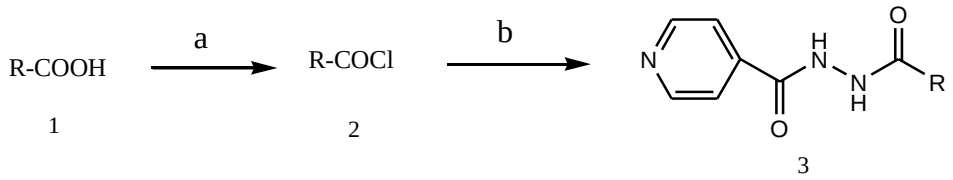
Isoniazid is most effective antitubercular drug of current tuberculosis chemotherapy. Isonicotinohydrazide is very important scaffold in medicinal chemistry and it is found to be associated with various biological activities like anticancer [3, 4], anti-malarial [5, 6], antifungal activity [7], antimicrobial activity [8, 9] and antitubercular agent [10-13]. This prompted us to design *N'*-substituted isonicotinohydrazide derivatives using pharmacophore modeling study. We had used different anti-tubercular agents like 3-nitropropionamides [14], Salicylanilide derivatives [15], 2-methoxybenzanilides and their thioxo analogues [16] and 2-Methoxy-2'-hydroxy benzanilides, their thioxo analogues and benzoxazoles for common feature pharmacophore generation using Hip Hop method in discovery studio (DS ver. 2.1; Accelrys Inc. USA). It gave ten

***Corresponding Author Address: Mahesh T. Chhabria,** Department of Pharmaceutical Chemistry, L. M. College of Pharmacy, Navrangpura, Ahmedabad, Gujarat 380 009, India; Tel: +91-79-26302746; E-mail: mahesh.chhabria@rediffmail.com*

pharmacophores, out of which best one was selected to obtain different lead molecules. Out of which we had selected lead molecule **(3)** and different derivatives were designed for further studies. Designed derivatives were synthesized and evaluated for their antitubercular activity.

## CHEMISTRY

General synthetic protocol of *N'*-substituted isonicotinohydrazide derivatives (**3**) is shown in Scheme **1**. The title compounds were prepared by reaction of different substituted aliphatic and aromatic carboxylic acids (**1**) with thionyl chloride in toluene which was followed by reaction of acid chloride (**2**) with isoniazid. Various derivatives with different substitution at *N'*- position were synthesized in good yield and purity.



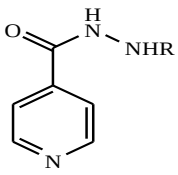

**Scheme 1** Synthetic protocol for *N'*-substituted isonicotinohydrazides. Reagent and conditions: (a) Thionyl chloride, Toluene, Reflux, 2 h; (b) Isoniazid, DMF, Reflux, 1 h.

## EXPERIMENTAL

The purity of compounds was checked by TLC. All the melting points of synthesized compounds were determined using open capillary method and are uncorrected. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer using DMSO-$d_6$ as solvent; the chemical shifts (δ) are reported in ppm. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet) and m (multiplet). IR spectra of all compounds were recorded on a Shimadzu FTIR-8400S with ATR-MIRACLE 10 spectrophotometer. Mass spectra were taken on an Advion Express CMS (Compact Mass Spectrometer) using ESI as ionization source.

***General procedure for the Synthesis of N'-substituted isonicotinohydrazides (4a to 4y)***

Aliphatic or aromatic carboxylic acid (10 mmol) was dissolved in toluene (15 mL) and thionyl chloride (15 mmol) was added and reaction mixture was refluxed for 2 h. After completion of reaction, toluene and excess of thionyl chloride were distilled off. To above reaction mixture, 10 mmol isoniazid dissolved in DMF (20 mL) was added and refluxed for 1h. Progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured in crushed ice-water mixture. Separated solid product was filtered and recrystallized from ethanol to obtain *N'*-substituted isonicotinohydrazide derivatives in 17-80 % yield.




**(4)**

***N'-tosylisonicotinohydrazide (4a):*** Yield: 42%; mp: 110-114°C; $^{1}$H NMR (δ, ppm): 2.50 (3H, s, -C<u>H</u>$_3$), 7.33-7.35 (2H, d, Ar-<u>H</u>), 7.57-7.59 (2H, d, Ar-<u>H</u>), 7.73 (2H, d, -C<u>H</u>-, Pyridine) , 8.71 (2H, d, -C<u>H</u>-, Pyridine), 10.11 (1H, s, -N<u>H</u>-) , 10.97 (1H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3248 (NH), 1674 (CO), 1550 (C=N), 1411 (C=C), 1334 ($SO_2$); Mass: *m/z* ($M^+$): 291.

***N'-(3-phenylacrylolyl)isonicotinohydrazide (4b)*** Yield: 17%; mp: > 340°C; $^{1}$H NMR (δ, ppm): 6.90 (1H, d, -C<u>H</u>-), 7.32 (1H, d, -C<u>H</u>-), 7.50 (5H, m, Ar-<u>H</u>)), 7.86 (2H, d, -C<u>H</u>-, Pyridine) , 8.52 (2H, d, -C<u>H</u>-, Pyridine), 10.30 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3309 (NH), 1504 (C=N); Mass: *m/z* $(M+H)^+$: 268.

***N'-stearoylisonicotinohydrazide (4c):*** Yield: 42%; mp: 80-85°C; $^{1}$H NMR (δ, ppm): 0.86 (3H, t, -C<u>H</u>$_3$) , 1.22 (32H, m, -C<u>H</u>$_2$-), 7.77 (2H, d, -C<u>H</u>-, Pyridine), 8.76 (2H, d, -C<u>H</u>-, Pyridine), 9.96 (1H, s, -N<u>H</u>-) , 10.62 (1H, s, -N<u>H</u>-); $^{13}$C NMR (δ, ppm): 171.46 (1C, <u>C</u>O), 163.86 (1C, <u>C</u>O), 150.36 (2C, -<u>C</u>H-, Pyridine), 139.47 (C, Pyridine), 121.26 (2C, -<u>C</u>H-, Pyridine), 33.21 (-<u>C</u>H$_2$-) , 31.27-22.07 (15C, -<u>C</u>H$_2$- aliphatic side chain), 13.94 (-<u>C</u>H$_3$, aliphatic side chain); IR (cm$^{-1}$): 3217 (NH); 1597 (CO); 1465 (C=C); Mass: *m/z* $(M+H)^+$: 404.

***N'-(3-(furan-2-yl)acryloyl)isonicotinohydrazide (4d):*** Yield: 21%; mp: 208-210°C; $^{1}$H NMR (δ, ppm): 6.59 (1H, t, -C<u>H</u>-, furan ring), 6.63 (1H, d, -C<u>H</u>-, furan), 6.88 (1H, d, -C<u>H</u>-, furan), 7.37-7.41 (1H, d, -C<u>H</u>-), 7.73-7.80 (2H, d, -C<u>H</u>-, Pyridine), 7.84 (1H, d, -C<u>H</u>-), 8.73-8.78 (2H, d, -C<u>H</u>-, Pyridine), 10.54-10.69 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3309 (NH), 1650 (C=O); 1550 (C=N), 1450 (C=C Ar); Mass: *m/z* $(M+H)^+$: 258.

***N'-(3-(4-methoxyphenyl)acrylolyl) isonicotinohydrazide (4e):*** Yield: 55%; mp: >

345°C; $^1$H NMR (δ, ppm): 3.78 (3H, s, -CH$_3$), 6.95 (1H, d, -CH-), 7.31 (1H, -CH-), 7.27-7.55 (4H, m, Ar-H), 7.82 (2H, d, -CH-, Pyridine) 8.47 (2H, d, -CH-, Pyridine), 10.24 (2H, s, -NH-); IR (cm$^{-1}$): 3093 (NH), 1604 (CO), 1512 (C=C), 1172 (C-O); Mass: *m/z* (M+H)$^+$: 298.

***N'-(2-nitrophenyl)isonicotinohydrazide (4f):*** Yield: 30%; mp: 280-282°C; $^1$H NMR (δ, ppm): 7.76-7.79 (2H, t, Ar-H), 7.85 (1H, d, Ar-H), 7.90 (1H, d, Ar-H), 8.13 (2H, d, -CH-, Pyridine), 8.80 (2H, d, -CH-, Pyridine), 10.84 (1H, s, -NH-), 11.08 (1H, s, -NH-); IR (cm$^{-1}$): 3201 (NH), 1643 (C=O); 1519 (C=N), 1350 (N=O); Mass: *m/z* (M+H)$^+$: 287.

***N'-(furan-2-carbonyl)isonicotinohydrazide (4g):*** Yield: 57%; mp: 210-212°C; $^1$H NMR (δ, ppm): 6.71 (1H, -CH-, t, furan), 7.29 (1H, -CH-, d, furan), 7.95 (1H, d, -CH-, furan), 7.82 (2H, d, -CH-, Pyridine), 8.80 (2H, d, -CH-, Pyridine), 10.5-10.8 (2H, s, -NH-); IR (cm$^{-1}$): 1635 (CO), 1519 (C=N), 1465 (C=C), 1010 (C-O); Mass: *m/z* (M+H)$^+$: 232.

***N'-(3,4-dichlorophenyl)isonicotinohydrazide (4h):*** Yield: 48%; mp: 220-225°C; $^1$H NMR (δ, ppm): 7.45-7.79 (3H, m, Ar-H), 7.82-7.84 (2H, d, -CH-, Pyridine), 8.79 (2H, d, -CH-, Pyridine), 10.67-11.02 (2H, s, -NH-); IR (cm$^{-1}$): 3178 (NH), 1612 (CO), 1519 (C=N), 1465 (C=C); Mass: *m/z* (M+H)$^+$: 311.

***N'-(3-methylphenyl)isonicotinohydrazide (4i):*** Yield: 34%; mp: 175-177°C; $^1$H NMR (δ, ppm): 2.47-2.50 (3H, s, -CH$_3$), 7.50-7.85 (4H, m, Ar-H), 7.86-7.89 (2H, d, -CH-, Pyridine), 8.80-8.83 (2H, d, -CH-, Pyridine), 10.30 (1H, s, -NH-), 10.90 (1H, s, -NH-); IR (cm$^{-1}$): 3008 (C=C-H), 1635 (CO), 1527 (C=N), 1481 (C=C); Mass: *m/z* (M+H)$^+$: 256

***N'-(2-methylphenyl)isonicotinohydrazide (4j)*** Yield: 23%; mp: 108-110°C; $^1$H NMR (δ, ppm): 2.43-2.51 (3H, s, -CH$_3$), 7.23-7.80 (4H, m, Ar-H), 7.83-7.85 (2H, d, -CH-, Pyridine), 8.79-8.81 (2H, d, -CH-, Pyridine), 10.33 (1H, s, -NH-), 10.86 (1H, s, -NH-); $^{13}$C NMR (δ, ppm): 168.37 (1C, CO), 164.12 (1C, CO), 150.46 (2C, -CH-, Pyridine), 139.39 (1C, Pyridine), 135.91, 134.63, 130.59, 129.99, 127.34, 125.80 (6C, Ar-C), 121.31 (2C, -CH- Pyridine), 19.29 (-CH$_3$); IR (cm$^{-1}$): 3008 (C=C-H), 1635 (CO), 1527 (C=N), 1481 (C=C); Mass: *m/z* (M+H)$^+$: 256.

***N'-(2-(2-bromophenyl)acetyl) isonicotinohydrazide (4k):*** Yield: 49%; mp: 162-164°C; $^1$H NMR (δ, ppm): 3.88 (2H, s, -CH$_2$-), 7.21-7.77 (4H, m, Ar-H), 8.59 (2H, d, -CH-, Pyridine), 8.73 (2H, d, -CH-, Pyridine), 10.53 (2H, s, -NH-); $^{13}$C NMR (δ, ppm):168.33(C, CO), 163.93 (C, CO), 150.39 (2C, -CH-, Pyridine), 139.36 (C, Pyridine), 133.51- 127.04 (6C, Ar-C), 121.29 (2C, -CH-, Pyridine), 37.69 (-CH$_2$-); IR (cm$^{-1}$): 3248 (NH), 3016 (C=C-H), 1658 (CO), 1527 (C=N), 1411 (C=C), 555 (C-Br); Mass: *m/z* (M+2): 336, (M+1): 335, M$^+$: 334, (M-1): 333.

***N'-(2-(2-chlorophenyl)acetyl) isonicotinohydrazide (4l):*** Yield: 29%; mp: 220-222°C; $^1$H NMR (δ, ppm): 3.73 (2H, s, -CH$_2$-), 7.33-7.50 (4H, m, Ar-H), 7.80 (2H, d, -CH-, Pyridine), 8.77 (2H, d, -CH-, Pyridine), 10.35 (2H, s, -NH-, D$_2$O exchangeable), 10.77 (2H, s, -NH-, D$_2$O exchangeable); IR (cm$^{-1}$): 3240 (NH), 3016 (C=C-H), 1658 (CO), 1527 (C=N), 1411 (C=C), 555 (C-Cl); Mass: *m/z* (M+2): 291, (M+1): 290, M$^+$: 289.

***N'-(2-(2-fluorophenyl)acetyl)isonicotinohydrazide (4m):*** Yield: 19%; mp: 236-240°C; $^1$H NMR (δ, ppm): 3.80 (2H, s, -CH$_2$-), 7.50-7.52 (4H, m, Ar-H), 7.83-7.88 (2H, d, -CH-, Pyridine), 8.80-8.82 (2H, d, -CH-, Pyridine), 10.80 (2H, s, -NH-); IR (cm$^{-1}$): 3232 (NH), 1658 (CO), 1411 (C=C), 1226 (C-F); Mass: *m/z* (M+H)$^+$: 274, (M$^+$): 273.

***N'-acryloyisonicotinohydrazide (4n):*** Yield: 31%; mp: 242-244°C; $^1$H NMR (δ, ppm): 3.36 (2H, d, -CH$_2$), 4.22 (1H, t, -CH-), 7.85-7.88 (2H, d, -CH-, Pyridine), 8.82-8.85 (2H, d, -CH-, Pyridine), 10.65 (2H, s, -NH-); IR (cm$^{-1}$): 3170 (NH), 1643 (CO), 1535 (C=N), 1404 (C=C); Mass: *m/z* (M+H)$^+$: 292.

***N'-isonicotinoylisonicotinohydrazide (4o):*** Yield: 53%; mp: 320-325°C; $^1$H NMR (δ, ppm): 7.75 (4H, d, -CH-, Pyridine), 8.59 (4H, d, -CH-, Pyridine), 10.65 (2H, s, -NH-); IR (cm$^{-1}$): 3317 (NH), 1728 (CO), 1512 (C=N); Mass: *m/z* (M+2): 291, (M+1): 290, M$^+$: 289.

***N'-(4-chlorophenyl)isonicotinohydrazide (4p):*** Yield: 43%; mp: 306-310°C; $^1$H NMR (δ, ppm): 7.52 (2H, d, Ar-H), 7.79 (2H, d, Ar-H), 7.85-7.87 (2H, d, -CH-, Pyridine), 8.60-8.62 (2H, d, -CH-, Pyridine), 10.70 (2H, -NH-); $^{13}$C NMR (δ, ppm):162.01 (1C, CO), 161.13 (1C, CO), 149.56 (2C, -CH-, Pyridine), 143.80 (1C, Pyridine), 135.18, 133.69, 130.84, 130.84, 128.65, 128.65 (Ar-C), 121.23 (2C, -CH-, Pyridine); IR (cm$^{-1}$): 3332 (NH), 1651 (CO), 1573 (C=N), 1481 (C=C); Mass: *m/z* (M+2): 277, (M+1): 276, M$^+$: 275.

***N'-(3,4-dimethoxyphenyl)isonicotinohydrazide (4q):*** Yield: 44%; mp: > 360°C; $^1$H NMR (δ, ppm): 3.81 (6H, s, -CH$_3$), 7.10-7.30 (3H, m, Ar-H), 7.78 (2H, d, -CH-, Pyridine), 8.45 (2H, d, -CH-, Pyridine), 10.36 (2H, -NH-); IR (cm$^{-1}$): 3456 (NH), 1689 (CO), 1442 (C=C); Mass: *m/z* (M+1): 302, M$^+$: 301.

***N'-(2-(3-bromophenyl)acetyl) isonicotinohydrazide (4r):*** Yield: 37%; mp: 158-160°C; $^1$H NMR (δ, ppm): 3.34 (2H, s, -C<u>H</u>$_2$-), 7.34-7.36 (2H, d, Ar-<u>H</u>), 7.46-7.48 (1H, t, Ar-<u>H</u>), 7.57 (1H, s, Ar-<u>H</u>), 7.78 (2H, d, -C<u>H</u>-, Pyridine), 8.76 (2H, d, -C<u>H</u>-, Pyridine), 10.37 (1H, s, -N<u>H</u>-), 10.74 (1H, s, -N<u>H</u>-); $^{13}$C NMR (δ, ppm): 168.85 (1C, CO), 163.92 (1C, <u>C</u>O), 150.41 (2C, -<u>C</u>H-, Pyridine), 139.27 (C, Pyridine), 138.26-121.47 (6C, Ar-<u>C</u>), 121.26 (2C, -<u>C</u>H-, Pyridine), 40.09 (-<u>C</u>H$_2$-); IR (cm$^{-1}$): 3186 (NH), 1743 (CO), 1550 (C=N), 1411 (C=C); Mass: *m/z* (M+2): 336, (M+1): 335 , (M-1): 333.

***N'-(3-(4-chlorophenyl)acryloyl) isonicotinohydrazide (4s):*** Yield: 21%; mp: 160-164°C; $^1$H NMR (δ, ppm): 6.90 (1H, d, -CH-), 7.32 (1H, d, -CH-), 7.50-7.60 (4H, m, Ar-<u>H</u>), 7.83 (2H, d, -C<u>H</u>-, Pyridine), 8.50 (2H, d, -C<u>H</u>-, Pyridine), 10.30 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3217 (NH), 1620 (CO), 1527 (C=N); Mass: *m/z* (M+2): 303, (M+1): 302 , (M$^+$): 301.

***N'-benzenesulfonylisonicotinohydrazide (4t):*** Yield: 51%; mp: 275-280°C; $^1$H NMR (δ, ppm): 7.60-7.65 (5H, m, Ar-<u>H</u>), 7.72 (2H, d, -C<u>H</u>-, Pyridine), 8.70 (2H, d, -C<u>H</u>-, pyridine), 10.90 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3240 (NH), 1689 (CO), 1527 (C=N), 1381 (-SO$_2$-); Mass: *m/z* (M$^+$): 277, (M-1): 276 .

***N'-(2-bromopropanoyl)isonicotinohydrazide (4u):*** Yield: 39%; mp: 166-170°C; $^1$H NMR (δ, ppm): 2.12 (3H, d, -CH$_3$), 4.25 (1H, m, -CH-), 7.90-7.92 (2H, d, -C<u>H</u>-, Pyridine), 8.85-8.87 (2H, d, -C<u>H</u>-, Pyridine), 10.50-10.52 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3101 (NH), 1658 (CO), 1550 (C=N), 1411 (C=C); Mass: *m/z* (M+2): 274, (M+1): 273.

***N'-(6-bromopiperidine-3-carbonyl)isonicotinohydrazide (4v):*** Yield: 44%; mp: 170-174°C; $^1$H NMR (δ, ppm): 1.80 (2H, m, -C<u>H</u>$_2$-, piperidine), 2.30 (2H, m, -C<u>H</u>$_2$- of piperidine), 2.43 (1H, m, -CH-, piperidine), 2.81 (2H, m, -C<u>H</u>$_2$-, piperidine), 4.10 (1H, m, -C<u>H</u>Br, piperidine), 4.22 (1H, s, -NH-, piperidine), 7.76-7.80 (2H, d, -C<u>H</u>-, Pyridine), 8.79-8.81 (2H, d, -C<u>H</u>-, Pyridine), 11.15 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3417 (NH), 1627 (CO), 1543 (C=N), 1411 (C=C); Mass: *m/z* (M+2): 328, (M+1): 327.

***N'-(2-phenoxyacetyl)isonicotinohydrazide (4w):*** Yield: 80%; mp: 148-152°C; $^1$H NMR (δ, ppm): 7.2-7.3 (5H, t, Ar-<u>H</u>), 8.13 (2H, d, -C<u>H</u>-, Pyridine), 8.97 (2H, d, -C<u>H</u>-, Pyridine), 10.54 (1H, s, -N<u>H</u>-), 11.11 (1H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3417 (NH), 1650 (CO), 1535 (C=N), 1415 (C=C), 1234(C-O); Mass: *m/z* (M+H)$^+$: 272.

***N'-(2, 5-dihydroxyphenyl) isonicotinohydrazide (4x):*** Yield: 60%; mp: 240-244°C; $^1$H NMR (δ, ppm): 6.46 (2H, d, Ar-<u>H</u>), 7.27 (1H, s, Ar-<u>H</u>), 7.89-7.91 (2H, d, -C<u>H</u>-, Pyridine), 8.84 (2H, d, -C<u>H</u>-, Pyridine), 11.07 (1H, s, -OH), 11.29 (1H, s, -OH), 12.36 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3441 (NH$_2$), 3348 (NH), 1728 (CO); Mass: *m/z* (M+H)$^+$: 274, (M-1): 272.

***N'-(5-amino-2-hydroxyphenyl)isonicotinohydrazide (4y):*** Yield: 49%; mp: 250-254°C; $^1$H NMR (δ, ppm): 5.70 (1H, s, -NH$_2$), 6.68 (1H, s, -OH), 7.35-7.50 (3H, m, Ar-<u>H</u>), 7.90 (2H, d, -C<u>H</u>-, Pyridine), 8.83 (2H, d, -C<u>H</u>-, Pyridine), 11.20 (2H, s, -N<u>H</u>-); IR (cm$^{-1}$): 3302 (NH), 3032 (OH), 1650 (CO), 1597 (C=N); Mass: *m/z* (M+H)$^+$: 274.

## RESULTS AND DISCUSSION

All the synthesized *N'*-substituted isonicotinohydrazide derivatives were characterized by the various spectroscopy (IR, Mass, $^1$H NMR and $^{13}$C NMR). In general, IR spectra of all derivatives (**4a-4y**) show the C=O peak at 1604–1743 cm$^{-1}$ and NH stretching vibrations at 3008–3348 cm$^{-1}$. The nuclear magnetic resonance spectra ($^1$H NMR) show hydrazide (NH) proton as a singlet at  9.96 - 11.11 ppm and imine proton (N=C-H) gives peak at 8.47- 8.97 ppm. The $^{13}$C NMR spectrum showed C=O signals at 161.13-171.46 ppm and C=N signals at 149.56-150.46 ppm.

***Antitubercular activity:*** In the present study, all synthesized derivatives were evaluated for their antimycobacterial activity against *M. tuberculosis* H37Rv strain using micro plate Alamar Blue assay [17]. The minimum inhibitory concentration (MIC) was determined by the serial dilution technique and Pyrazinamide, Streptomycin and Ciprofloxacin were used as standard drugs. Compound (**4m**) exhibited potent activity against *M. tuberculosis* H37Rv strain as indicated by its MIC value of 6.25 µg/mL which was further screened against MDR and XDR TB strain by method described by Rattan [18] using Lowenstein-Jensen medium. MIC observed was of 62.5 and 250 µg/mL respectively. The anti-tubercular activity of designed derivatives (**4a-4y**) are summarized in Table **I.**

Antitubercular activity of compounds with presence of unsaturation in side chain has found to be good **(4b)** and if electron releasing groups present on aromatic ring attached with unsaturated bond then it increased the potency **(4d & 4e).** Sulfonyl (-SO$_2$-) in side chain also showed better activity **(4a & 4t)**. Furan and other hetero aromatic rings are also important to improve the potency

**(4g)**. Electron withdrawing groups showed better activity as compared to electron releasing groups if present at 3rd and 4th position on aromatic ring in side chain **(4h & 4q)**. 2-substituted phenyl acetic acid substitution at *N'*-position of isonicotinohydrazide was resulted into good activity. Out of which 2-fluoro phenyl acetic acid derivative **(4m)** has most potent activity compared to other synthesized derivatives. All above results suggest that one to two carbon distance between aromatic ring and carbonyl group in side chain is optimum for better antitubercular activity of *N'*-substituted isonicotinohydrazide derivatives.

**Conclusion:** A series of *N'*-substituted isonicotinohydrazide derivatives was designed using pharmacophore modeling study and designed derivatives were synthesized and screened for antimycobacterial activity. Compounds (4d, 4e, 4g, 4h, 4i, 4m, 4o and 4s) showed good activity compared to other designed derivatives. Compound (**4m**) was found to be the most potent against *M. tuberculosis* H37Rv strain with MIC value of 6.25 µg/mL. Compound 4m was also screened against MDR and XDR TB, which has exhibited MIC values of 62.5 and 250 µg/mL respectively. The synthesized analogs have provided valuable information regarding further structural modification required for *N'*-substituted isonicotinohydrazide scaffold to exhibit better anti-tubercular activity. Thus, the synthesized compounds can be considered as promising novel lead with antitubercular potential.

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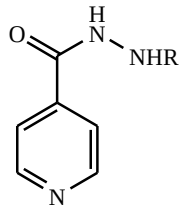
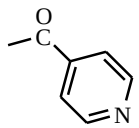
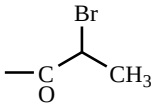
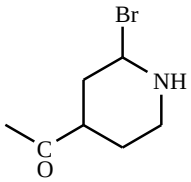
**Table I** Antiubercular activity of *N*'-substituted isonicotinohydrazides




| Sr. No. | Compound No. | R | MIC (µg/mL) |
|---|---|---|---|
| 1 | 4a | 4-$CH_3$-$C_6H_4$-$SO_2$- | 25 |
| 2 | 4b | $C_6H_5$-CH=CH-CO- | 50 |
| 3 | 4c | n-$C_{17}H_{35}$-CO- | 50 |
| 4 | 4d | 2-furyl-CH=CH-CO- | 12.5 |
| 5 | 4e | 4-$OCH_3$-$C_6H_4$-CH=CH-CO- | 12.5 |
| 6 | 4f | 2-$NO_2$-$C_6H_4$-CO- | 25 |
| 7 | 4g | 2-furyl-CO- | 12.5 |
| 8 | 4h | 3,4-Di-Cl-$C_6H_3$-CO- | 12.5 |
| 9 | 4i | 3-$CH_3$-$C_6H_4$-CO- | 12.5 |
| 10 | 4j | 2-$CH_3$-$C_6H_4$-CO- | 25 |
| 11 | 4k | 2-Br-$C_6H_4$-$CH_2$-CO- | 25 |
| 12 | 4l | 2-Cl-$C_6H_4$-$CH_2$-CO- | 25 |
| 13 | 4m | 2-F-$C_6H_4$-$CH_2$-CO- | 6.25 |
| 14 | 4n | $CH_2$=CH-CO- | 50 |
| 15 | 4o |  | 12.5 |
| 16 | 4p | 4-Cl-$C_6H_4$-CO- | 25 |
| 17 | 4q | 3,4-Di-$OCH_3$-$C_6H_3$-CO- | 50 |
| 18 | 4r | 3-Br-$C_6H_4$-$CH_2$-CO- | 25 |
| 19 | 4s | 4-Cl-$C_6H_4$-CH=CH-CO- | 12.5 |
| 20 | 4t | $C_6H_5$-$SO_2$- | 50 |

**(Table I) contd....**

| Sr. No. | Compound No. | R | MIC (µg/mL) |
|---|---|---|---|
| 21 | 4u |  | 50 |
| 22 | 4v |  | 25 |
| 23 | 4w | $C_6H_5$-O-$CH_2$-CO- | 25 |
| 24 | 4x | 2,5-Di-OH-$C_6H_3$-CO- | 50 |
| *25* | *4y* | *2-OH-5-$NH_2$-$C_6H_3$-CO-* | *50* |
| 26 | Pyrazinamide | - | 3.125 |
| 27 | Streptomycin | - | 6.25 |
| 28 | Ciprofloxacin | - | 3.125 |

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