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Synthesis of isonicotinic acid *N*′-arylidene-*N*-[2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl]-hydrazides as antituberculosis agents

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Abstract—A new series of antituberculosis agents **6**–**9** was designed, synthesized and evaluated for antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and clinical isolates in an agar dilution method. Compound **9h** showed comparable in vitro activity (MIC) to isoniazid against *M. tuberculosis* $H_{37}Rv$ and clinical isolates (sensitive strains) and superior activity against resistant strains of *M. tuberculosis*.

1. Introduction

Tuberculosis (TB), is a contagious disease caused by *Mycobacterium (M.) tuberculosis*, is one of the leading causes of death in the world. The World Health Organization (WHO) has reported that about 33% of the world's population is infected with TB, and has predicted that by year 2020 there will be one billion new active cases if new anti-TB drugs or treatments are not developed.[1] Treatment of TB infection that has been caused by multi drug resistant (MDR) *M. tuberculosis* has become a major concern worldwide. MDR TB and its synergy with HIV in immunocompromised patients have deteriorated the problem.[2–5] TB is therefore a leading cause of death among people who are HIV positive.[6] The increasing rate of MDR TB does not only create problems for treatment, but also costs are exploding. Since in the last 40 years no new drug has been added, there is an unmet medical need to discover newer synthetic molecules and drugs that shorten the duration of therapy and to combat MDR TB. We have earlier reported our research efforts towards the discovery of new chemical entities as potential antituberculosis agents.[7,8]

Several Schiff bases, hydrazones and hydrazides of isoniazid have shown good activity against tubercular, fungal and bacterial infections.[9] In the quest for biologically more potent antituberculosis compounds we envisioned to design and synthesize piperazine substituted hydrazone derivatives of isoniazid. In this short communication, we report the synthesis and in vitro antituberculosis activity of new series of isonicotinic acid *N*′-arylidene-*N*-[2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl]-hydrazide derivatives **6**–**9**.

2. Chemistry

The hydrazones **1**–**4** were prepared in one step by the condensation of isonicotinic hydrazide with the appropriate arylaldehydes according to the procedure described in literature.[9] The other intermediate 2-chloro-1-(4-aryl-piperazin-1-yl)-ethanone **5a**–**j** were synthesized in quantitative yields by the condensation of chloroacetyl chloride with various 1-aryl-piperazines according to literature method.[10] The final compounds **6**–**9** (Table 1) were prepared[11] in good yields by the condensation of *N*′-(arylmethylene)-isonicotinohydrazides (**1**–**4**) with

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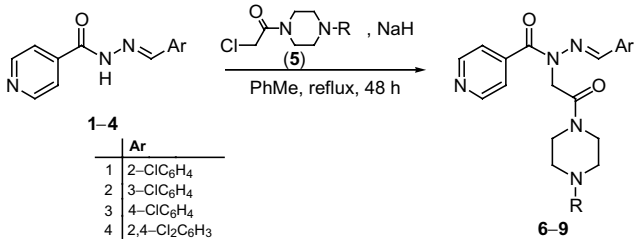
Table 1. Range of MIC values (μg/mL) of compounds **6**–**9** against $H_{37}Rv$ and clinical isolates of *Mycobacterium tuberculosis*

Compound	Ar	R	*M. tb.* $H_{37}Rv$ ATCC 27294	*M. tb.* clinical isolates	
				Sensitive ($N = 9$)[a]	Resistant ($N = 9$)[a]
6a	2-ClC_6H_4	4-FC_6H_4	>16	>16	>16
6b	2-ClC_6H_4	4-ClC_6H_4	>16	>16	>16
6c	2-ClC_6H_4	3-ClC_6H_4	>16	>16	>16
6d	2-ClC_6H_4	2-$MeOC_6H_4$	>16	>16	>16
6e	2-ClC_6H_4	3-MeC_6H_4	>16	>16	>16
6f	2-ClC_6H_4	4-$NO_2C_6H_4$	>16	>16	>16
6g	2-ClC_6H_4	C_6H_5	>16	8–>16	>16
6h	2-ClC_6H_4	1-Piperonyl	4	4–8	8–>16
6i	2-ClC_6H_4	3-$CF_3C_6H_4$	>16	>16	>16
6j	2-ClC_6H_4	2,3-$Cl_2C_6H_3$	>16	>16	>16
7a	3-ClC_6H_4	4-FC_6H_4	>16	>16	>16
7b	3-ClC_6H_4	4-ClC_6H_4	>16	>16	>16
7c	3-ClC_6H_4	3-ClC_6H_4	>16	>16	>16
7d	3-ClC_6H_4	2-$MeOC_6H_4$	>16	>16	>16
7e	3-ClC_6H_4	3-MeC_6H_4	>16	>16	>16
7f	3-ClC_6H_4	4-$NO_2C_6H_4$	>16	>16	>16
7g	3-ClC_6H_4	C_6H_5	>16	>16	>16
7h	3-ClC_6H_4	1-Piperonyl	4	2–4	4–8
8a	4-ClC_6H_4	4-FC_6H_4	>16	>16	>16
8b	4-ClC_6H_4	4-ClC_6H_4	>16	>16	>16
8d	4-ClC_6H_4	2-$MeOC_6H_4$	>16	>16	>16
8f	4-ClC_6H_4	4-$NO_2C_6H_4$	>16	>16	>16
8h	4-ClC_6H_4	1-Piperonyl	2	1–4	4–8
9a	2,4-$Cl_2C_6H_3$	4-FC_6H_4	>16	8–>16	>16
9b	2,4-$Cl_2C_6H_3$	4-ClC_6H_4	8	8	8–>16
9c	2,4-$Cl_2C_6H_3$	3-ClC_6H_4	>16	>16	>16
9d	2,4-$Cl_2C_6H_3$	2-$MeOC_6H_4$	>16	>16	>16
9e	2,4-$Cl_2C_6H_3$	3-MeC_6H_4	>16	>16	>16
9f	2,4-$Cl_2C_6H_3$	4-$NO_2C_6H_4$	>16	>16	>16
9g	2,4-$Cl_2C_6H_3$	C_6H_5	>16	>16	>16
9h	2,4-$Cl_2C_6H_3$	1-Piperonyl	1	0.25–0.5	2–4
9i	2,4-$Cl_2C_6H_3$	3-$CF_3C_6H_4$	>16	>16	>16
Isoniazid	—	—	0.25	0.12–0.25	8–>16
Rifampicin	—	—	0.125	0.03–0.5	2–>16

M. tb. = *Mycobacterium tuberculosis*.
[a] N = number of clinical isolates used per group.



Scheme 1. Synthesis of compounds **6**–**9**.

2-chloro-1-(4-aryl-piperazin-1-yl)-ethanone (**5**) in the presence of NaH in toluene at reflux temperature as shown in Scheme 1. All new compounds reported here were fully characterized on the basis of complementary spectroscopic (^{1}H NMR and MS) and analytical data.[12]

3. Results and discussion

The antimycobacterial activity of the compounds was determined with the objective to identify the compounds having inhibitory activity against susceptible (sensitive strains; inhibited by the two front line anti TB drugs viz. isoniazid, rifampicin) and resistant strains (not inhibited by either isoniazid or rifampicin or by both) of *M. tuberculosis* (causative agent of human tuberculosis).

The preliminary antimycobacterial activity of all the compounds was evaluated by the agar dilution assay[13] at three different concentrations (50, 25 and 12.5 μg/mL) against strains of mycobacterium that is *M. tuberculosis* $H_{37}Rv$ ATCC 27294. All compounds inhibited the mycobacterial species. The active compounds were then assayed for determination of minimum inhibitory concentration (MIC) against a panel of mycobacterial cultures consisting of appropriate reference strains of mycobacterial species, eighteen clinical isolates representing the sensitive and resistant (either isoniazid or rifampicin or by both) strains of *M. tuberculosis* were included in the study. Control drugs isoniazid and rifampicin were included in each batch of test.

The antituberculosis activity of all the screened compounds **6a**–**j**, **7a**–**h**, **8a**,**b**,**d**,**f**,**h** and **9a**–**i** is presented in Table 1. As evident from the in vitro antituberculosis

activity profile of all the tested compounds, **6h**, **7h**, **8h**, **9b** and **9h** were exhibited activity against *M. tuberculosis* $H_{37}Rv$ ATCC 27294 strain and clinical isolates (sensitive and resistant strains) of *M. tuberculosis* and in particular compound **9h** having 2-oxo-2-[4-(piperonyl)-piperazin-1-yl]-ethyl- residue at N_1 position of isonicotinic acid hydrazide was identified as the most active compound (Table 1). The in vitro antituberculosis activity of **9h** (MIC 0.25–1 μg/mL) against *M. tuberculosis* $H_{37}Rv$ ATCC 27294 strain and clinical isolates (sensitive strains) of *M. tuberculosis* was comparable to that of isoniazid. The compound **9h** showed activity against resistant strains of *M. tuberculosis* (MIC 2–4 μg/mL) also though isoniazid a well known drug for tuberculosis is not active against resistant strains of *M. tuberculosis*. Rest of the compounds had either little or no activity.

4. Conclusions

A new series of antituberculosis agents has been designed, synthesized and evaluated for antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ and clinical isolates. Some of these compounds showed good potency and their in vitro activity against sensitive and resistant strains of *M. tuberculosis* were found to be equivalent or better than isoniazid, a well-known drug for tuberculosis.

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11. *General procedure for the synthesis of N′-arylidene-N-[2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl]-hydrazides (**6**–**9**)*: To a stirred suspension of sodium hydride (0.13 g, 5.5 mmol) pre-washed with hexane, in toluene (25 mL) was added *N′*-(arylmethylene)-isonicotinohydrazides (**1**–**4**, 5.0 mmol) at 25–30 °C with stirring. The resulting reaction mixture was refluxed for 24 h. After cooling to 25–30 °C, 2-chloro-1-(4-aryl-piperazin-1-yl)-ethanone (**5**, 5.5 mmol) was added and the reaction mixture was refluxed for another 24 h. Toluene was evaporated under reduced pressure; the residue was suspended in the water (25 mL) and extracted with chloroform (2 × 50 mL). The combined organic layer was washed with water (2 × 25 mL), brine (1 × 25 mL), dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to obtain crude product. The crude product was purified by column chromatography over silica gel (100–200 mesh) using hexane–EtOAc (2:98) as eluent to give **6**–**9**.
12. *Analytical data for compounds **6**–**9***. Melting points were determined in open capillaries on a Büchi B-545 melting point apparatus. ^1H NMR spectra were recorded on Bruker Advance DRX 200 MHz instrument as solutions (in $CDCl_3$) using TMS as internal reference and chemical shifts values are expressed in δ units. Mass spectra were run on Applied Biosystems API 3000 instrument using direct inlet system under positive ion electrospray ionization source. Elemental analyses were carried out with a Perkin–Elmer 2400 analyzer and the values found were within ±0.4% of theoretical values. Physical data of some selected compounds. Compound **6a**: off white solid (44%). Mp 114–116 °C. ^1H NMR ($CDCl_3$) δ: 3.12–3.22 (m, 4H), 3.81–4.09 (m, 4H), 5.07 (s, 2H), 6.92–7.00 (m, 4H), 7.16–7.26 (m, 4H), 7.59 (d, J = 6.0 Hz, 2H), 8.04 (s, 1H), 8.78 (d, J = 6.0 Hz, 2H). MS: *m/z* 480 (M+1). Anal. Calcd for $C_{25}H_{23}ClFN_5O_2$ (479.93): C, 62.56; H, 4.83; N, 14.59. Found: C, 62.77; H, 5.07; N, 14.62. Compound **7d**: off white shining crystals (39%). Mp 77–79 °C. ^1H NMR ($CDCl_3$) δ: 3.09–3.17 (m, 4H), 3.82–3.84 (m, 4H), 3.89 (s, 3H), 5.03 (s, 2H), 6.88–7.02 (m, 4H), 7.26–7.32 (m, 4H), 7.60 (d, J = 6.0 Hz, 2H), 7.66 (s, 1H), 8.75 (d, J = 6.0 Hz, 2H). MS: *m/z* 492 (M+1). Anal. Calcd for $C_{26}H_{26}ClN_5O_3$ (491.97): C, 63.48; H, 5.33; N, 14.24. Found: C, 63.29; H, 5.11; N, 14.06%. Compound **8f**: yellow solid (36%). Mp 172–174 °C. ^1H NMR ($CDCl_3$) δ: 3.45–3.54 (m, 4H),

3.78–3.82 (m, 4H), 4.97 (s, 2H), 6.80 (d, J = 6.0 Hz, 2H), 7.20–7.31 (m, 4H), 7.53–7.65 (m, 3H), 8.10 (d, J = 6.0 Hz, 2H), 8.64 (d, J = 6.0 Hz, 2H). MS: *m/z* 507 (M+1). Anal. Calcd for $C_{25}H_{23}ClN_6O_4$ (506.94): C, 59.23; H, 4.57; N, 16.58. Found: C, 59.24; H, 4.71; N, 16.40. Compound **9h**: off white solid (44%). Mp 71–72 °C. ^{1}H NMR ($CDCl_3$) δ: 2.37–2.49 (m, 4H), 3.39 (s, 2H), 3.54–3.63 (m, 4H), 4.93 (s, 2H), 5.89 (s, 2H), 6.68 (s, 2H), 6.78 (s, 1H), 7.04–7.10 (m, 1H), 7.29–7.34 (m, 2H), 7.49 (d, J = 6.0 Hz, 2H), 7.84 (s, 1H), 8.63 (d, J = 6.0 Hz, 2H). MS: *m/z* 556 (M+1). Anal. Calcd for $C_{27}H_{25}Cl_2N_5O_4$ (554.42): C, 58.49; H, 4.54; N, 12.63. Found: C, 58.42; H, 4.61; N, 12.60.

13. *In vitro growth inhibition assay*. The ability of the compounds to inhibit the growth of mycobacterium species was determined by agar diffusion assay. Briefly, reference strains *M. tuberculosis* H_{37}Rv 27294, *M. avium* ATCC 49601 and *M. intracellulare* ATCC 13950 were grown in Middlebrook 7H9 broth containing 10% ADC supplement at 37 °C on a rotary shaker at 150 rpm for 7 days. The turbidity of the culture was adjusted to 0.5 McFarland. 0.50 mL of the individual cultures were then added to the molten Middlebrook 7H10 in 150 mm Petri plates. Uniform holes were then made in the media in which the three different concentration (50, 25 and 12.5 μg/mL) of individual compounds were added. The plates were then incubated at 37 °C for 21–28 days. Compounds showing zone of inhibition greater or equal to the control drugs were considered active. *In vitro Agar Dilution Assay*. MIC (in μg/mL) against *M. tuberculosis* strains in agar dilution assay as per the NCCLS-M24-T2 recommendations.[14] The compounds and control drugs were dissolved in DMSO and diluted twofold to obtain ten serial dilutions of each compound. Appropriate volumes of compounds were incorporated into duplicate plates of Middlebrook 7H10 agar medium supplemented with 10% Middlebrook supplement oleic acid–albumin–dextrose enrichment at concentration of 0.03–16 μg/mL. Test organisms (mycobacterium strains) were grown in Middlebrook 7H9 broth containing 0.05% Tween 80 and 10% OADC supplement. After 7 days of incubation at 37 °C the broths were adjusted to the turbidity of 1.0 McFarland standard; the organism were further diluted 10-fold in sterile water containing 0.10% Tween 80. The resulting mycobacterial suspensions were spotted (3–5 μL/spot) onto 7H10 media plates containing different dilution of compounds/control drugs. The plates were sealed and incubated at 37 °C for 3–4 weeks in upright position. The MIC was recorded as the lowest concentration/highest dilution of the compounds/control drugs that completely inhibited the growth of mycobacterial cultures.
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