Synthesis and anti-tubercular and antimicrobial activities of some 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone derivatives

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Short communication

Synthesis and anti-tubercular and antimicrobial activities of some 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-one *N*-isonicotinoylhydrazone derivatives

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ABSTRACT

In this study, seven 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-one *N*-isonicotinoylhydrazones **8**–**14** were synthesized. The structure and stereochemistry of these compounds were established by IR and NMR spectral data. The purities were checked by elemental analysis. The synthesized compounds adopt twin-chair conformation with equatorial orientations of the aryl groups. The compounds were evaluated for their in vitro anti-tubercular and antimicrobial activities. The initial screen was conducted against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATTCC 27294) and *INH-TB* by luciferase reporter phage assay method. All the synthesized compounds showed very good activity against *MTB* and *INH-TB*. Though all the compounds showed good antimicrobial activity only **11** (Ar = *p*-chlorophenyl), **12** (Ar = *p*-fluorophenyl), **13** (Ar = *m*-chlorophenyl) and **14** (Ar = *m*-methoxyphenyl) exhibited activity against all the tested (bacterial and fungal) microorganisms. The results suggest that the formation of hydrogen bonds may play a significant role in drug action.

1. Introduction

Tuberculosis (TB) is one of the leading infectious disease among adults and youth, with one-third of the world's population infected with *Mycobacterium tuberculosis*. Two developments make the resurgence in TB especially alarming. The first is pathogenic synergy with HIV. The overall incidence of TB in HIV-positive patients is 50 times that of the rate for HIV-negative individuals [1]. The second is the emergence of drug-resistant and multi-drug resistant TB (MDR-TB). According to the World Health Organization (WHO), in 2006 there were 9.2 million new cases and 1.7 million deaths from TB around the world. The incidence of TB infection has steadily risen in the last decade [2]. Also WHO has reported that XDR TB, a virtually untreatable form of the respiratory disease is found in 45 countries [1,3]. The standard "short" course treatment for tuberculosis (TB) involves oral administration of isoniazid (INH), rifampicin, pyrazinamide and ethambutol for two months followed by INH and rifampicin alone for a further four months. For latent tuberculosis, the standard treatment involves oral administration of INH alone for six to nine months. MDR-TB is resistant to at least, INH and rifampicin, often taking a further two years to treat with second-line drugs [4,5].

Three reasons are usually given for needing new tuberculosis drugs: (i) to improve current treatment by shortening the total duration of treatment and/or by providing for more widely spaced intermittent treatment, (ii) to improve the treatment of MDR-TB, and (iii) to provide for more effective treatment of latent tuberculosis infection (LTBI) [6].

There are two basic approaches to develop a new drug for TB: (i) synthesis of analogues by modifications of existing compounds for shortening and improving TB treatment and, (ii) searching novel structures which the TB organism has never encountered with before, for the treatment MDR-TB [7].

To pursue this goal, our research efforts are directed to the modification of INH, which is a well known anti-tubercular agent bearing pyridine and hydrazine moieties. Modifying either of these moieties has been taken up by several research groups [8–12].

On the basis of these observations, we had the impetus to synthesize a number of 3-azabicyclo[3.3.1]nonan-9-one hydrazones and subsequently evaluate their in vitro antimicrobial activity. Molecules with the 3-azabicyclo[3.3.1]nonane nucleus are of great interest due to their presence in a wide variety of naturally occurring diterpenoid/norditerpenoid alkaloids and biological activities [13]. The chemistry of 3-azabicyclo[3.3.1]nonan-9-one has been reviewed

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[14]. The use of different substituents particularly aromatic substituents, with regard to biological activity, has been demonstrated [15].

In this paper, we report the synthesis of seven 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-one *N*-isonicotinoylhydrazones **8**–**14** and there in vitro anti-tubercular and antimicrobial activities.

2. Chemistry

The synthetic route of the compounds is outlined in Scheme 1. For the synthesis of the title compounds, 2*r*,4*c*-diaryl-3-azabicyclo [3.3.1]nonan-9-ones **1**–**7**, required as starting materials, were prepared by the reaction of cyclohexanone, aldehydes and ammonium acetate. The reaction of **1**–**7** with isonicotinoyl hydrazide, in the presence of acetic acid, resulted in the formation of the title compounds **8**–**14**. The structure and stereochemistry of compounds **8**–**14** have been established by IR and NMR spectral data. Their purities were checked by elemental analysis. For **8** all ^{1}H and ^{13}C signals have been assigned unambiguously using HOMOCOSY, HSQC and HMBC spectra.

3. Pharmacology

3.1. Anti-tubercular activity

Initial screen was conducted against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) and *INH-TB* by luciferase reporter phage assay method [16]. All the compounds showed very good anti-tubercular activity.

3.2. Antimicrobial activity

The in vitro activities of the compounds were tested in Nutrient broth (NB; Hi-media, Mumbai) for bacteria and Sabourauds dextrose broth (SDB; Hi-media, Mumbai) for fungi by the two-fold serial dilution method [17,18]. Some compounds showed significant antibacterial and antifungal activity.

4. Results and discussion

4.1. Designation of atoms

The atoms of the bicycle [3.3.1]nonane part are numbered as shown in Fig. 1. The *ipso* carbons of the aryl groups at C-2 and C-4 are designated as C-2′ and C-4′, respectively. The other carbons of the aryl group at C-2 are denoted as *o*, *m* and *p*- carbons and those of the aryl group at C-4 are denoted as *o*′, *m*′ and *p*′-carbons. In the *meta*-substituted compounds the *o*-carbon *ortho* to the substituent is denoted as *o*, *o*-C and that *para* to the substituent is denoted as *o*, *p*-C. The other aromatic carbons are designated in an analogous manner. The carbons of the pyridine ring are designated using Greek letters α, β and γ. The protons are numbered accordingly. For example, the benzylic proton at C-2 is denoted as H-2 that at C-α is denoted as H-α and so on. The methylene protons in the cyclohexane ring are denoted as axial and equatorial protons assuming chair conformation for the cyclohexane ring. Thus, the methylene protons at C-7 are denoted as H-7a and H-7e.

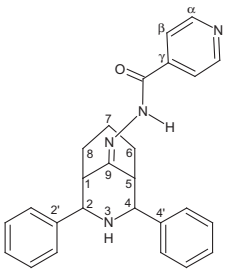


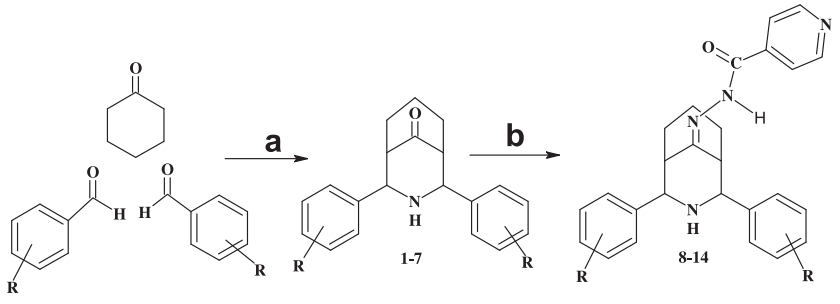
Fig. 1. Numbering of the atoms.

4.2. IR spectra

In all cases two separate bands were observed for the two different NH-stretching vibrations. The piperidine NH-stretching frequency was in the range 3310–3370 cm^{-1} and the amide NH-stretching frequency was in the range 3059–3183 cm^{-1}. The amide carbonyl stretching frequency was in the range 1635–1640 cm^{-1}. The C=N stretching frequency was in the range 1509–1524 cm^{-1}.

4.3. NMR spectra

For **8** the ^{1}H and ^{13}C signals were unambiguously assigned based on the observed correlations in the HOMOCOSY, HSQC and HMBC spectra. For all the other compounds the ^{1}H and ^{13}C signals were assigned by comparison with **8**. The signals for the aromatic protons and carbons in **9**–**14** were assigned based on known substituent effects [19,20]. In the case of **12** coupling with ^{19}F was



Entry		R
1	8	H
2	9	*p*-CH_3
3	10	*p*-OCH_3
4	11	*P*-Cl
5	12	*p*-F
6	13	*m*-OCH_3
7	14	*m*-Cl

Scheme 1. Synthesis of 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-one *N*-isonicotinoyl hydrazones, Reagents and conditions: (a) CH_3COONH_4, EtOH, warm; (b) Isoniazid, Methanol: Chloroform (1:1 v/v), AcOH, reflux.

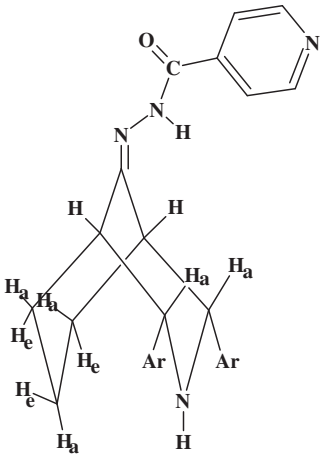


Fig. 2. Twin-chair (cc) conformation of compounds.

observed for the aromatic carbons except the *ipso* carbons C-2′ and C-4′ [20]. The signal for C-5 merged with the signal of DMSO-d_6 in all cases. This was conformed for **8** from its HSQC spectrum. For **8** the chemical shift of C-5 could be determined from its HSQC spectrum as 41.0 ppm approximately. The same value is assigned for all the other compounds.

In the HOMOCOSY spectrum of **8** the benzylic protons showed correlation with N(3)-H. Also H-2 showed correlation with H-1 and H-4 showed correlation with H-5. However, in all cases H-1, H-2, N (3)-H, H-4 and H-5 gave unresolved signals. The unresolved nature of the signals for H-1, H-2, H-4 and H-5 suggests that the vicinal couplings for these protons are very small. However, H-7a showed three large coupling (one geminal coupling with H-7e and two diaxial vicinal coupling with H-6a and H-8a) and two small couplings (one with H-6e and another with H-8e. All these observations are consistent with twin-chair (CC) conformation for these compounds (Fig. 2).

It is interesting to note that 2*r*,4*c*-diphenyl-9*t*-ethynyl-3-azabicyclo[3.3.1]nonan-9*t*-ol (**15)** has been to adopt twin-chair conformation, shown in Fig. 3 with equatorial orientations of the phenyl groups, by X-ray crystallographic study [21].

4.4. Anti-tubercular activity

The preliminary in vitro antimycobacterial activity of the synthesized compounds were evaluated by luciferase reporter phage assay method against *M. tuberculosis* $H_{37}Rv$ and *INH*-resistant *M. tuberculosis* at two different concentrations level of 1.00 and 2.00 μg/mL and the observed percentage inhibitions are tabulated in Table 1. A compound is considered to be an antimycobacterial agent if fifty percent reduction in the Relative Light Units (RLU) is observed when compared to the control using a luminometer. From Table 1 it is seen that, all the compounds show excellent in vitro activity against MTB with the percentage of reduction in RLU ranging 72–80%.

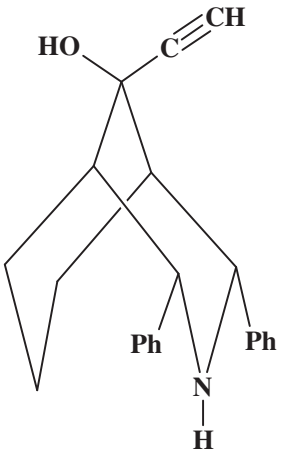


Fig. 3. Twin-chair (cc) conformation of compound **15**.

Table 1
The in vitro activity of compounds against *M. tuberculosis* $H_{37}Rv$ strain and *INH-MTB* (% reduction of RLU).

Compounds	*M. tuberculosis*		*INH-MTB*	
	1.00 μg/mL	2.00 μg/mL	1.00 μg/mL	2.00 μg/mL
8	77.76	87.90	69.60	77.52
9	72.03	82.04	62.30	71.07
10	73.07	83.02	67.07	74.19
11	75.61	85.22	63.61	73.28
12	76.63	86.12	67.63	75.08
13	77.18	87.06	69.18	78.33
14	75.42	85.34	65.42	75.83
INH	98.42	98.42	89.65	95.85

4.5. Antibacterial activity

The in vitro antibacterial activity of compounds **8**–**14** was examined against the bacterial strains *viz*., Gram positive [*Staphylococcus aureus* NCIM-2492 and *Bacillus subtilis* NCIM-2439] and Gram negative [*Escherichia coli* NCIM-2345, *Klebsiella pneumoniae* (derived from Medical College, Annamalai University) and *Pseudomonas aeruginosa* NCIM-2035] bacteria and are expressed as minimum inhibitory concentration (MIC, μg mL^{-1}) Streptomycin has been taken as the reference drug [22,23] and the observed minimum inhibitory concentration values are given in Table 2.

Compounds **11**, **13** and **14** are active against all the tested bacterial strains. Compound **10** is not active against *P. aeruginosa* and compound **12** is not active against *K. pneumoniae*. Compound **9** is active against three bacterial strains only, whereas **8** is only active against *S. aureus* and *P. aeruginosa*.

4.6. Antifungal activity

The in vitro antifungal activity of compounds **8**–**14** was examined against the fungal strains *viz*., *Candida albicans*-6 (NCIM-C27), *C. albicans* (NCIM-C27), *Aspergillus niger* (NCIM-590), *C. albicans*-51 (NCIM-C27) and *Aspergillus flavus* (NCIM-539). Amphotericin B was used as standard drug, the observed minimum inhibitory concentration values are given in Table 3.

Table 2
In vitro antibacterial activity of compounds **8**–**14**.

Compounds	Minimum Inhibitory Concentration (MIC) in μg/mL				
	Bacillus subtilis	*Klebsiella pneumoniae*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
8	–	–	–	100	200
9	200	–	–	200	100
10	200	200	100	100	–
11	100	100	50	50	100
12	50	–	100	50	100
13	50	100	200	200	200
14	100	50	50	100	100
Penicillin G	25	12.5	50	12.5	50
Streptomycin	12.5	50	12.5	50	25

'–' No inhibition even at a higher concentration of 200 μg/mL.

Table 3
In vitro antifungal activity of compounds **8**–**14**.

Compounds	Minimum Inhibitory Concentration (MIC) in μg/mL				
	Candida albicans-6	*C. albicans*	*Aspergillus niger*	*C. albicans*-51	*Aspergillus flavus*
8	–	–	200	100	–
9	200	100	100	100	–
10	100	200	200	100	200
11	100	100	100	50	200
12	50	50	100	50	100
13	200	100	100	200	100
14	50	200	100	200	200
Amphotericin B	25	25	50	25	50

'–' No inhibition even at a higher concentration of 200 μg/mL.

Compounds **10**–**14** are active against all the tested fungal strains. Compound **9** is not active against *A. flavus* whereas, **8** is active against only *C. albicans*-51 and *A. niger*.

4.7. Influence of aromatic substituents

The substituent in the aryl ring seems to have only little influence on the anti-tubercular activity. Compound **8** without a substituent in the aromatic ring and compound with an *m*-OCH_3 substituent in the aromatic ring show greater activities compared to the other compounds studied. Since the molecular mass of **8** is 60 units less than that of **13** by 60 units in 1 μg of 1**3** should contain less number of molecules than that 1 μg of **8**. Hence, **8** can be considered to be the most active compound studied.

However, the aromatic substituents substantially influence the antimicrobial activities. Thus, in the case of *S. aureus* the activity decreases in the order *p*-Cl > *p*-F > *m*-Cl >*p*-OCH_3 > *m*-OCH_3 > *p*-CH_3, if the MICs are expressed in μmol/mL. However, the same trend is not observed for the other bacterial strains. Thus, if MICs are expressed in μmol/mL the *m*-OCH_3 compound is the most active compound against *B. subtilis*. However, its activity against *S. aureus* is only slightly greater than that of the least active compound **9**.

On the same grounds the *m*-Cl compound **14** is the most active compound against *C. albicans*-6, whereas the *p*-F compound **12** is the most active compound against *C. albicans* and the *p*-Cl compound **11** is the most active compound against *C. albicans*-51. However, in the case of 4-aryl-5-isopropoxycarbonyl-6-methyl-3,4-dihydropyrimidinones [15], among the *p*-F, *p*-Cl, *p*-CH_3 and *p*-OCH_3 substituted compounds, the *p*-fluoro compound was found as the most active against all the tested organisms.

If the antimycobacterial and antimicrobial activities are due to the release of INH molecule by hydrolysis, the substituents in the aromatic ring cannot cause such a marked variation in the activities. The rate of hydrolysis cannot be influenced significantly by the aromatic substituent.

However, in all cases the most active compound contains a strong hydrogen-bonding atom O, F or Cl. Thus, it seems that formation of hydrogen bonds may play a significant role in drug action.

5. Conclusions

2*r*,4*c*-Diaryl-3-azabicyclo[3.3.1]-nonan-9-one *N*-isonicotinoylhydrazones **8**–**14** adopt twin-chair conformation with equatorial orientations of the aryl groups. All the newly synthesized compounds were screened for their preliminary antituberculosis, antibacterial and antifungal activities. All these compounds show good anti-tubercular activity against *M. tuberculosis* $H_{37}Rv$ and *INH-TB*. Most of the compounds show good antibacterial and antifungal activities.

The substituents in the aromatic ring do not have significant effect on the anti-tubercular activity. However, the antibacterial and antifungal activities are significantly influenced by the aromatic substituents. Analysis of these results and the observation earlier made on 4-aryl-5-isopropoxycarbonyl-6-methyl-3,4-dihydropyrimidinones suggest that the formation of hydrogen bond may play a significant role in drug action.

6. Experimental

6.1. Materials and methods

Isoniazid was purchased from Sigma–Aldrich and all other chemical were used as Analytical grade. Reactions were monitored by TLC. All the reported melting points were measured in open capillaries and are uncorrected. FT-IR spectra were recorded as potassium bromide pellets on AVATAR 330 FT-IR Thermo Nicolet Spectrometer. ^1H & ^{13}C NMR spectra were recorded at ambient temperature on a Bruker AMX 400 NMR spectrometer operating at 400.13 MHz for ^1H and 100.62 MHz for ^{13}C. Solutions for the measurement of spectra were prepared by dissolving 10 mg of the sample in 0.5 mL DMSO-d_6 and chemical shifts (δ) are expressed in ppm. Elemental analyses were carried out in Heraeus Carlo Erba 1108 CHN analyzer.

6.2. General procedure for synthesis of 2,4-Diaryl-3-azabicyclo [3.3.1]nonan-9-one N-isonicotinoylhydrazone (*8*–*14*)

2*r*,4*c*-Diaryl-3-azabicyclo[3.3.1]-nonan-9-ones **1**–**7** were prepared by the condensation of appropriate ketones, aldehydes and ammonium acetate in 1:2:1 ratio, using literature procedure [24]. A mixture of 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-one (**1**–**7**) (1 mmol), isoniazid (1.5 mmol) in methanol and chloroform (1:1 v/v), few drops of acetic acid was added and refluxed for 2–3 h. On the completion of reaction a solid mass was formed. After cooling to room temperature the precipitate was filtered off and washed with cold mixture of ethanol and water. The crude product was recrystallized from ethanol.

6.3. Analytical data for compounds (*8*–*14*)

6.3.1. 2r,4c-Diphenyl-3-azabicyclo[3.3.1]-nonan-9-one N-isonicotinoylhydrazone (*8*)

Yield: 85%, m.p. 216–218 °C. IR (KBr, ν_{max} cm^{-1}): 3312 (N–H stretching), 1637 (C=O stretching), 1524 (C=N stretching). ^1H NMR (δ, DMSO-d_6, ppm): 11.09 (s, 1H, amide NH), 8.75 (d, 2H, α-H), 7.77 (d, 2H, β-H), 7.63 (t, 2H, *o*-*H*), 7.62 (t, 2H, *o*′-*H*), 7.41 (m, 4H, *m*-*H*, *m*′-*H*), 7.28 (m, 2H, *p*-*H*, *p*′-*H*), 4.31(bs, 1H, H-2a,), 4.29 (bs, H, H-4a), 3.28 (s, 1H, H-5e), 2.74 (m, 1H, H-7a), 2.54 (s, 1H, H-1e), 1.63 (m, 1H, H-8e), 1.47 (m, 3H, H-6a, H-6e, H-8a), 1.26 (m, 1H, H-7e), 2.93 (s, 1H, ring NH). ^{13}C NMR (δ, DMSO-d_6, ppm): 64.2 (C-2), 62.2 (C-4), 45.9 (C-1), 41.0 (C-5), 28.2 (C-8), 26.9 (C-6), 20.8 (C-7), 170.7 (C-9), 162.5 (NHCO), 150.0 (C-α), 121.8 (C-β), 141.4 (C-γ), 142.8 (C-2′, C-4′), 127.0 (*o*-C, *o*′-C), 128.2 (*m*-C), 128.0 (*m*′-C), 126.8 (*p*-C, *p*′-C). Anal. Found (Cal.) for $C_{26}H_{26}N_4O$ (%): C, 75.89 (76.00); H, 6.23 (6.23); N, 13.42 (13.47).

6.3.2. 2r,4c-Bis(p-methylphenyl)-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone (*9*)

Yield: 80%, m.p. 210–212 °C. IR (KBr, ν_{max} cm^{-1}): 3303 (N–H stretching), 1654 (C=O stretching), 1512 (C=N stretching). ^1H NMR (δ, DMSO-d_6, ppm): 11.08 (s, 1H, amide NH), 8.75 (d, 2H, α-H), 7.75 (d, 2H, β-H), 7.49 (m, 4H, *o*-H, *o*′-H), 7.20 (t, 4H, *m*-H, *m*′-H), 4.25 (bs, 1H, H-2a), 4.33(bs, 1H, H-4a), 3.16 (s, 1H, H-5e), 2.72 (m, 1H, H-7a), 1.65 (m, 1H, H-8e), 1.44 (m, 3H, H-6a, H-6e, H-8a), 1.25 (m, 1H, H-7e), 2.77 (s, 1H, ring NH), 2.31 (d, 3H, *p*-CH_3), 2.29 (s, 3H, *p*-CH_3).

^{13}C NMR (δ, DMSO-d_6, ppm): 64.3 (C-2), 62.1 (C-4), 46.1(C-1), 41.0 (C-5), 28.2 (C-8), 26.9(C-6), 20.8(C-7), 20.7 (CH_3 at *p*), 170.9(C-9), 162.5(NHCO), 150.0(α-C), 121.8 (β-C), 141.4 (γ-C), 135.8 (C-2′, C-4′), 126.9 (*o*-C), 126.7(*o*′-C), 128.7 (*m*-C), 128.6 (*m*′-C), 139.7 (*p*-C, *p*′-C). Anal. Found (cal.) for $C_{28}H_{30}N_4O$ (%): C, 76.52 (76.61); H, 5.40 (5.47); N, 12.67 (12.77).

6.3.3. 2r,4c-Bis(p-methoxyphenyl)-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone (10)

Yield: 82%, m.p. 22–223 °C. IR (KBr, ν_{max} cm^{-1}): 3304 (N–H stretching), 1653 (C=O stretching), 1510 (C=N ring stretching), ^{1}H NMR (δ, DMSO-d_6, ppm): 11.06 (s, 1H, amide NH), 8,75 (d, 2H, α-H), 7.75 (d, 2H, β-H), 7.51 (q, 4H, *o*-H, *o*′-H), 6.96 (m, 4H, *m*-H, *m*′-H), 4.24 (bs, 1H, H-2a), 4.22 (bs, 1H, H-4a), 3.13 (bs, 1H, H-5e), 2.71 (m, 1H, H-7a), 1.67 (m, 1H, H-8e), 1.544 (m, 1H, H-6a), 1.44 (m, 2H, H-6e, H-8a), 1.24 (m, 1H, H-7e), 2.75 (s, 1H, ring NH),3.74 (s, 3H, *p*-OCH_3), 3.75 (d, 3H, *p*-OCH_3). ^{13}C NMR (δ, DMSO-d_6, ppm): 64.0 (C-2), 61.8 (C-4), 46.1(C-1), 41.0 (C-5), 28.2 (C-8), 26.9 (C-6), 20.8 (C-7), 55.0 (–OCH_3 at *p*), 170.9(C-9), 162.5 (NHCO), 150.0 (α-C), 121.8 (β-C), 141.4 (γ-C), 134.7 (C-2′, C-4′), 128.0 (*o*-C), 127.7 (*o*′-C), 113.6 (*m*-C), 113.4 (*m*′-C), 158.2 (*p*-C, *p*′-C). Anal. Found (cal.) for $C_{28}H_{30}N_4O_3$ (%): C, 71.35 (71.40); H, 6.28 (6.38); N, 11.82 (11.90).

6.3.4. 2r,4c-Bis(p-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone (**11**)

Yield: 87%, m.p. 230–232 °C. IR (KBr, ν_{max} cm^{-1}): 3301 (N–H stretching) 1652 (C=O stretching), 1489 (C=N stretching). ^{1}H NMR (δ, DMSO-d_6, ppm): 11.09 (s, 1H, amide NH), 8.75 (d, 2H, α-H), 7.76 (d, 2H, β-H), 7.67 (q, 4H, *o*-H, *o*′-H), 7.46 (m, 4H, *m*-H, *m*′-H), 4.28 (bs, 2H, H-2a, H-4a), 3.20 (s, 1H, H-5e), 2.65 (m, 1H, H-7a), 2.53 (s, 1H, H-1e), 1.60 (m, 1H, H-8e), 1.46 (m, 3H, H-6a, H-6e, H-8a), 1.24 (m, 1H, H-7e), 3.08 (s, 1H, ring NH). ^{13}C NMR (δ, DMSO-d_6, ppm): 63.6 (C-2), 61.4 (C-4), 45.6(C-1), 41.0 (C-5), 28.1(C-8), 26.8 (C-6), 20.7 (C-7), 169.7 (C-9), 162.6 (NHCO), 150.0 (α-C), 121.8 (β-C), 141.4 (γ-C), 141.6 (C-2′, C-4′), 128.1 (*o*-C), 127.9 (*o*′-C), 128.8 (*m*-C), 128.6 (*m*′-C), 131.3 (*p*-C, *p*′-C). Anal. Found (cal.) for $C_{26}H_{24}Cl_2N_4O$ (%): C, 65.13 (65.08); H, 5.22 (5.01); N, 11.75 (11.68).

6.3.5. 2r,4c-Bis(p-fluorophenyl)-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone (**12**)

Yield: 90%, m.p. 225–227 °C. IR (KBr, ν_{max} cm^{-1}): 3331 (N–H stretching), 1638 (C=O stretching). 1509 (C=N stretching). ^{1}H NMR (δ, DMSO-d_6, ppm): 11.08 (s, 1H, amide NH), 8.75 (d, 2H, α-H), 7.76 (d, 2H, β-H), 7.65 (m, 4H, *o*-H, *o*′-H), 7.22 (t, 4H, *m*-H, *m*′-H), 4.30 (bs, 1H, H-2a), 4.29 (bs, 1H, H-4a), 3.18 (s, 1H, H-5e), 2.67 (m, 1H, H-7a), 2.52 (s, 1H, H-1e), 1.61 (m, 1H, H-8e), 1.46 (m, 3H, H-6a, H-6e, H-8a), 1.26 (m, 1H, H-7e), 3.02 (s, 1H, ring NH). ^{13}C NMR (δ, DMSO-d_6, ppm): 63.6 (C-2), 61.4 (C-4), 45.7 (C-1), 41.0 (C-5), 28.1(C-8), 26.8 (C-6), 20.7 (C-7), 170.0 (C-9), 159.9 (NHCO), 150.0 (α-C), 121.8 (β-C), 141.4 (γ-C), 138.8 (C-2′, C-4′), 128.8 (*o*-C), 128.6 (*o*′-C), 114.9 (*m*-C), 114.7 (*m*′-C), 162.4 (*p*-C, *p*′-C). Anal. Found (cal.) for $C_{26}H_{24}F_2N_4O$ (%): C, 69.79 (69.89); H, 5.25 (5.37); N, 12.45 (12.54).

6.3.6. 2r,4c-Bis(m-methoxyphenyl)-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone (**13**)

Yield: 77%, m.p. 209–211 °C. IR (KBr, ν_{max} cm^{-1}): 3311 (N–H stretching), 1630 (C=O stretching), 1593 (C=N ring stretching). ^{1}H NMR (δ, DMSO-d_6, ppm): 11.07 (s, 1H, amide NH), 8,75 (d, 2H, α-H), 7.76 (d, 2H, β-H), 7.32 (m, 2H, m-H, m′-H), 7.20 (m, 2H, *o*,*p*-H, o,p′-H), 7.14 (m, 2H, *p*,*o*-H, *p*,*o*′-H), 6.86 (d, 2H, *o*,*o*-H, *o*,*o*′-H), 4.27 (bs, 1H, H-2a),4.26 (bs, 1H, H-4a), 3.19 (s, 1H, H-5e), 2.68 (m, 1H, H-7a), 2.56 (s, 1H, H-1e), 1.66 (m, 1H, H-8e), 1.54 (m, 1H, H-6a), 1.46 (m, 2H, H-6e, H-8a), 1.26 (m, 1H, H-7e), 2.88 (s, 1H, ring NH), 3.78 (s, 3H, *p*-OCH_3), 3.77 (s, 3H, *p*-OCH_3). ^{13}C NMR (δ, DMSO-d_6, ppm): 64.1 (C-2), 62.0 (C-4), 45.8 (C-1), 41.0 (C-5), 28.2 (C-8), 26.9 (C-6), 20.7 (C-7), 54.9 (OCH_3 at *m*), 170.5(C-9), 162.5 (NHCO), 149.9 (α-C), 121.7 (β-C), 141.3 (γ-C), 144.3 (C-2′, C-4'), 159.2 (*o*,*m*-C, *o*,*m*′-C),119.2(*o*, *p*-C), 118.9(*o*′,*p*-C), 113.2, 112.2 (*p*,*o*-C, *p*,*o*′-C), 112.0, 111.5 (*o*,*o*-C, *o*′,*o*-C).

6.3.7. 2r,4c-Bis(m-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-one N-isonicotinoylhydrazone (**14**)

Yield: 82%, m.p. 225–227 °C. IR (KBr, ν_{max} cm^{-1}): 3311 (N–H stretching, 1650 (C=O stretching), 1522 (C=N stretching). ^{1}H NMR (δ, DMSO-d_6, ppm): 11.09 (s, 1H, amide NH), 8,76 (d, 2H, α-H), 7.78 (d, 2H, β-H), 7.67(m, 2H, *o*,*o*-H, *o*′,*o*-H), 7.55 (d, 2H, *o*,*p*-H, *o*,*p*′-H)), 7.47 (t, 2H, *p*,*o*-H, *p*,*o*′-H),7.36 (m, 2H, *m*, *m*′), 4.30 (d, 2H, H-2a, H-4a), 3.27 (s, 1H, H-5e), 2.64 (m, 1H, H-7a), 1.61 (m, 1H, H-8e), 1.48 (m, 3H, H-6a, H-6e, H-8a), 1.27 (m, 1H, H-7e), 3.19 (s, 1H, ring NH). ^{13}C NMR (δ, DMSO-d_6, ppm): 63.6 (C-2), 61.4 (C-4), 45.5 (C-1), 41.0 (C-5), 28.2 (C-8), 26.9 (C-6), 20.7 (C-7), 168.7 (C-9), 162.7 (NHCO), 150.0(α-C), 121.9 (β-C), 141.4 (γ-C), 133.0 (m-C), 132.8 (m′-C), 130.1 (*m*,*m*-C), 130.0 (*m*,*m*′-C), 126.9 (*p*,*o*-C, *p*′,*o*-C) 126.8, 126.6 (*o*′,*p*-C, *o*,*p*-C), 125.8 (*o*, *o*-C), 125.6 (*o*′,*o*-C).

6.4. Pharmacology

6.4.1. Anti-tubercular activity

Primary in vitro antimycobacterial activities of the synthesized compounds were evaluated by luciferase reporter phage assay method [16] against *M. tuberculosis* H_{37}Rv and INH-resistant *M. tuberculosis* at two concentrations (1.00 and 2.00 μg/mL). Fifty-micro liter bacterial suspension equivalent to MacFarlands No.2 standard was added to 400 μL of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this setup was incubated for 72 h at 37 °C. After incubation 50 μL of the high titer Luciferase reporter phage (PhAE129) and 40 μL of 0.1 M $CaCl_2$ were added to all the vials and this setup was incubated at 37 °C for 4 h. After incubation 100 μL of the mixture was taken from each tube into a luminometer cuvette and equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10 s of integration in the Luminometer (Monolight 2010). Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1000.

6.4.2. Antimicrobial studies

The in vitro activities of the compounds were tested in Sabourauds dextrose broth (SDB; Hi-media, Mumbai) for fungi and Nutrient broth (NB; Hi-media, Mumbai) for bacteria by the two-fold serial dilution method [17,18]. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1 mg mL^{-1} stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB from 24-h-old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores from 24 h to 7-day-old Sabourauds agar slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4–10^5 cfu mL^{-1}. The final inoculums size was 10^5 cfu mL^{-1} for antibacterial assay and 1.1–1.5 × 10^2 cfu mL^{-1} for antifungal assay. Testing was performed at pH 7.4 ± 0.2. Exactly 0.2 mL of the solution of test compound was added to 1.8 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of the seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. The minimum inhibitory

concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Penicillin G, streptomycin and Amphotericin B were used as standards.

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