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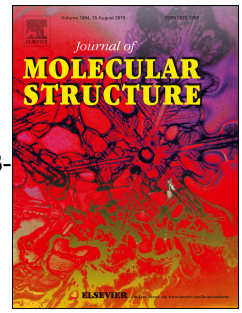

Synthesis, stereochemical, structural and biological studies of some N′-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide

M. Mangalam, C. Sebastian Antony Selvan, C. Sankar

PII: S0022-2860(16)30960-7

DOI: 10.1016/j.molstruc.2016.09.033

Reference: MOLSTR 22945

To appear in: *Journal of Molecular Structure*

Received Date: 21 April 2016

Revised Date: 20 August 2016

Accepted Date: 12 September 2016

Please cite this article as: M. Mangalam, C.S.A. Selvan, C. Sankar, Synthesis, stereochemical, structural and biological studies of some N′-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide, *Journal of Molecular Structure* (2016), doi: 10.1016/j.molstruc.2016.09.033.

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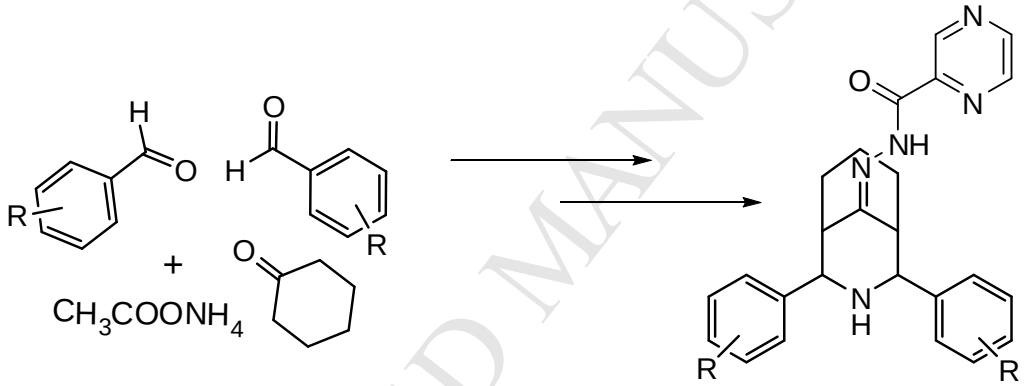
M. Mangalam[a], **C. Sebastian Antony Selvan**[b*] and **C. Sankar**[c]

[a]Department of Chemistry, Rajah serfoji Govt. College (auto), Thanjavur – 613005, Tamilnadu, India.
[b]Department of Chemistry, R.V. Govt. Arts College, Chengalpattu, Chennai, India
[c]Department of Chemistry, TRP Engineering College, Tiruchirappalli – 626 126, Tamil Nadu, India

A new series of N′-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazides (**8-14**) have been synthesized. All the compounds have been tested for their antibacterial activity.



* Corresponding author
E. mail address: sebantonyselvan@gmail.com
* Department of Chemistry, R.V. Govt. Arts College, Chengalpattu, Chennai, Tamil Nadu, India

Synthesis, Stereochemical, Structural and Biological studies of some *N'*-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide

M. Mangalam[a], C. Sebastian Antony Selvan[b*] and C. Sankar[c]

[a]Department of Chemistry, Rajah serfoji Govt. College (auto), Thanjavur– 613005, Tamilnadu,India.
[b]Department of Chemistry, R.V.Govt.Arts College, Chengalpattu, Chennai, India
[c]Department of Chemistry, TRP Engineering College, Tiruchirappalli – 626 126, Tamil Nadu, India

ABSTRACT

A new series of *N´*-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazides (**8-14**) were synthesized by corresponding 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ones (**1-7**) reaction with pyrazine-2-carbohydrazide. The stereochemistry of the newly synthesized compounds were unambiguously assigned by using FT-IR, ^{1}H, ^{13}C and 2D (COSY, HSQC, HMBC, ROESY) NMR spectral data. The chemical shifts suggest that in all these compounds adopts twin-chair conformation with equatorial orientation of aryl substitutions in solution. Hydrazones were screened for their *in vitro* antitubercular activity against *Mycobacterium Tuberculosis* $H_{37}Rv$ and antibacterial activity against a set of pathogenic bacteria's. Most of the halogenated compounds expressed promising antitubercular and antibacterial activities.

Keywords: Stereochemistry - Conformation - Anti-TB – Hydrazone - Azabicyclo[3.3.1]nonan-9-ones.

*Corresponding author,
E. mail address: sebantonyselvan@gmail.com
*Department of Chemistry, R.V.Govt. Arts College, Chengalpattu, Chennai, Tamil Nadu, India

Introduction

NMR techniques have been extensively applied in deriving stereo-dynamical information about a wide variety of organic systems. Conformation studies of heterocyclic compounds have helped in understanding the influence of electronic and conformation effects on chemical shifts, coupling constant and bioactivity [1-10]. Such, studies have furnished information about the dependence of NMR spectral parameters such as chemical shifts and coupling constants on electronegativities of heteroatoms. Proton chemical shifts are influenced by electronic and magnetic anisotropic effects, whereas carbon chemical shifts are largely influenced by electronic and steric effects only.

The heterocyclic system of 3-azabicyclononane are distributed in various alkaloids such as kobusine, hetisine, delcorine, deltaline, elatine, inulin, *etc*., They are having interesting chemical and biological actions such as antibacterial antimycobacterial, antiinflammatory, antiarrhythmic, antifungal, antiallergic, antiprotozoan, antioxidant, antitumor, anticonvulsant, antiviral, antimalarial and cytotoxic activity [11-17]. In addition a family of hydrazone / hydrazide derivatives are containing highly reactive azomethine group (CO-NH-N=CH) and thus useful in new drug development [18]. Also, these are found to possess antimicrobial [16,19], antimalarial [20], anticonvulsant [21], analgesic [22], antiinflammatory [23], antiplatelet [24], antitumoral [25] and antiviral [26] activities. The structural requirement for inhibition of antimycobacterial activity suggests the presence of a pyrazine ring with an acyl moiety [27].

The main reason for selection of pyrazine-2-carbohydrazide as a lead molecule in this study that are simplicity of synthesis methodology and search on new compounds related to pyrazine derivatives which are known for their antitubercular activity [28]**.** In the present study, 3-azabicyclo[3.3.1]nonan-9-one carbohydrazide which are active against MTB as attempt has been made to condense with pyrazine-2-carbohydrazide to explore the

possibilities of their conformation and the MTB activity. Based on the higher bioactivity of hydrazones, we have synthesized novel hydrazones (**8-14**) from piperazine-4-carbohydrazide coupled with 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ones (**1-7**). The assignment of all proton and carbon resonances was achieved by double resonance experiments. The antitubercular and antibacterial studies were effectively done for newly synthesized hydrazones with different concentrations.

Experimental

Purchasing materials and recording spectra

All the solvents and chemicals were purchased from Sigma–Aldrich and were used as received and the purity of the compounds was checked by TLC. The melting points were recorded in open capillaries and are uncorrected. FT-IR spectra were recorded in KBr (pellet forms) on AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and noteworthy absorption levels (reciprocal centimeters) alone are listed. ^{1}H and ^{13}C NMR spectra of **8-14** have been recorded on a BRUKER AMX 400 NMR spectrometer operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C, in DMSO-d_6. The following spectral parameters were used ^{1}H; acquisition time = around 3.0 s, number of scans = around 100 s, number of data points = 32 K and special with = 5000 Hz. ^{13}C; acquisition time = around 0.5 s, number of scans = around 1000, number of data points = 32 K and special with = 30,000 Hz. ^{1}H–^{1}H COSY, HSQC, HMBC and ROESY NMR spectra were recorded on a BRUKER DRX 500 MHz NMR spectrometer using standard parameters. All NMR measurements were made on 5 mm NMR tubes. The solutions were prepared by dissolving about 10 mg of the compound in 0.5 mL of DMSO-d_6.

Synthesis of 2,4-diaryl-3-azabicyclo [3.3.1]nonan-9-ones

The synthetic route of the target compounds **8-14** is illustrated in **Scheme 1**. The starting compounds 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ones **1-7**, were prepared by the mixture of cyclohexanone, substituted benzaldehyde and ammonium acetate in 1:2:1.5 ratio

in ethanol was gently warmed and allowed overnight [29]. The crude products formed were filtered and washed with an ethanol-ether (1:5) mixture. Then the bicyclic ketones were recrystallized from ethanol-chloroform to obtain the pure compounds.

General procedure for synthesis of N′-(2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide (**8-14**)

The quest for synthesis of *N′*-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide (**8-14**) was accomplished in two steps as outlined in **Scheme 1**. A mixture of 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-one (**1-7**) (1 mmol), commercially available pyrazine-2-carbohydrazide (PZH) (1.5 mmol) in methanol and chloroform mixture (1:1 v/v), catalytic amount of acetic acid (0.1 ml) 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 [16].

N′-(2r,4c-diphenyl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide (**8)**

M.P 220-221°C. Yield 95%. IR (KBr, ν_{max} cm^{-1}): 3322 (N–H stretching), 1650 (C=O stretching), 1546 (C=N stretching).^{1}H NMR (δ, DMSO-d_6-ppm): 11.08 (s, 1H, amide NH), 9.65 (s, 1H, H-3‴), 8.72 (d, 1H, H-5‴), 7.93 (d, 1H, H-6‴), 7.64 (t, 2H, *o-H*),7.63 (t, 2H, *o′-H*), 7.41 (m, 4H, *m-H, m′-H*), 7.28 (m, 2H, *p-H, p′-H*), 4.32 (bs, 1H, H-2a,), 4.20 (bs, H, H-4a), 3.28 (s, 1H, H-5e), 2.73 (m, 1H, H-7a), 2.54 (s, 1H, H-1e), 1.63 (m, 1H, H-8e), 1.46 (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): 64.4 (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), 149.3 (C-3‴), 121.8 (C-2‴), 141.4 (C-5‴), 150.37 (C-6‴) 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. Calculate (Found) for $C_{25}H_{25}N_5O$ (%): C, 72.90 (72.97); H, 6.12 (6.18); N, 17.02 (16.97).

N′-[2r,4c-bis(p-methylphenyl-3-azabicyclo[3.3.1]nonan-9-ylidene]pyrazine-2-carbohydrazide (**9**)

M.P 210-212°C. Yield 80%. IR (KBr, ν_{max} cm^{-1}): 3303 (N–H stretching), 1654 (C=O stretching), 1512 (C=N stretching).^{1}H NMR (δ, DMSO-d_6-ppm): 11.08 (s, 1H, amide NH), 9.65 (s, 1H, H-3‴), 8.73 (d, 1H, H-5‴), 7.94 (d, 1H, H-6‴), 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): 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), 149.3 (C-3‴), 121.8 (C-2‴), 141.4 (C-5‴), 150.37(C-6‴), 126.9 (*o*-C), 126.7(*o′*- C), 128.7 (*m*-C), 128.6 (*m′*-C), 139.7 (*p*-C, *p′*-C). Anal. Calculate (Found) for $C_{27}H_{29}N_5O$ (%): C, 73.78 (73.7); H, 6.65 (6.59); N, 15.93 (15.97).

N′-[2r, 4c-bis(p-chlorophenyl-3-azabicyclo[3.3.1]nonan-9-ylidene]pyrazine-2-carbohydrazide (***10***)

M.P 245-246°C. Yield 88%. 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), 9.65 (s, 1H, H-3‴), 8.75 (d, 1H, H-5‴), 7.78 (d, 1H, H-6‴), 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): 63.6 (C-2), 61.4 (C4), 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), 149.3 (C-3‴), 121.8 (C-2‴), 141.4 (C-5‴), 150.37(C-6‴), 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. Calculate (Found) for $C_{25}H_{23}Cl_2N_5O$ (%): C, 62.51 (62.47); H, 4.83 (4.89); N, 14.58 (14.65).

N′-[2r, 4c-bis(p-fluorophenyl-3-azabicyclo[3.3.1]nonan-9-ylidene]pyrazine-2-carbohydrazide ***(11)***

M.P 235-236 °C. Yield 90%. IR (KBr, $\nu_{max}cm^{-1}$): 3330 (N–H stretching), 1638 (C=O stretching). 1510 (C=N stretching). ^{1}H NMR (δ, DMSO-d_6-ppm): 11.08 (s, 1H, amide NH),9.68 (s, 1H, H-3‴), 8.75 (d, 1H, H-5‴), 7.76 (d, 1H, H-6‴), 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): 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), 149.3 (C-3‴), 121.8 (C-2‴), 141.4 (C-5‴), 150.37(C-6‴), 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. Calculate (Found) for $C_{25}H_3F_2N_5O$ (%): C, 67.10 (67.15); H, 5.18 (5.13); N, 15.36 (15.40).

N′-[2r, 4c-bis(p-methoxyphenyl-3-azabicyclo[3.3.1]nonan-9-ylidene]pyrazine-2-carbohydrazide ***(12)***

M.P 221-223°C. Yield 89%. IR (KBr, ν_{max} cm^{-1}): 3324 (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), 9.65 (s, 1H, H-3‴), 8.75 (d, 1H, H-5‴), 7.75 (d, 1H, H-6‴), 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): 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), 149.3 (C-3‴), 121.8 (C-2‴), 141.4 (C-5‴), 150.37(C-6‴), 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. Calculate (Found) for $C_{27}H_{29}N_5O_3$ (%): C, 68.77 (68.70); H, 6.20 (6.28); N, 14.85 (14.90).

N′-[2r, 4c-bis(m-chlorophenyl-3-azabicyclo[3.3.1]nonan-9-ylidene]pyrazine-2-carbohydrazide (**13)**

M.P 239-240°C. Yield 86%. IR (KBr, $\nu_{max}cm^{-1}$): 3321 (N–H stretching, 1653 (C=O stretching), 1532 (C=N stretching). ^{1}H NMR (δ, DMSO-d_6-ppm): 11.09 (s, 1H, amide NH), 9.67 (s, 1H, H-3‴), 8.76 (d, 1H, H-5‴), 7.78 (d, 1H, H-6‴), 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): 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 (C-3‴), 121.9 (C-2‴), 141.4 (C-5‴), 150.37(C-6‴), 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). Anal. Calculate (Found) for $C_{25}H_{23}Cl_2N_5O$ (%): C, 62.51 (62.56); H, 4.83 (4.82); N, 14.58 (14.53).

N′-[2r, 4c-bis(m-methoxyphenyl-3-azabicyclo[3.3.1]nonan-9-ylidene]pyrazine-2-carbohydrazide (**14)**

M.P 210-211°C. Yield 73%. IR (KBr, $\nu_{max}cm^{-1}$): 3325 (N–H stretching), 1644 (C=O stretching), 1593 (C=N ring stretching). ^{1}H NMR (δ, DMSO-d_6-ppm): 11.07 (s, 1H, amide NH), 9.64 (s, 1H, H-3‴), 8.75 (d, 1H, H-5‴), 7.76 (d, 1H, H-6‴), 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): 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 (C-3‴), 121.7 (C-2‴), 141.4 (C-5‴), 150.37(C-6‴), 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). Anal. Calculate (Found) for $C_{27}H_{29}N_5O_3$ (%): C, 68.77 (68.83); H, 6.20 (6.16); N, 14.85 (14.80).

Evaluation of antitubercular activity

The *in vitro* antitubercular activity of the synthesized compounds was evaluated by the help of Microplate Alamar Blue Assay method (MABA). The resazurin colorimetric MIC assay method was used to test the MIC of compounds [30]. A color change from blue to pink was observed when growth occurs. Compounds were initially tested at a single point concentration of 10 µg/mL against Mycobacterium Tuberculosis ($H_{37}Rv$), obtained from Colorado State University. Test compounds were logged into the inventory spreadsheet and placed in a -20ºC freezer.

$H_{37}Rv$ is grown in Middle brook 7H9 broth medium (7H9 medium) supplemented with 0.2% (v/v) glycerol, 10% (v/v) ADC (albumin, dextrose, catalase), and 0.05% (v/v) Tween 80. The bacteria were inoculated in 100 ml of 7H9 medium in 1 liter roller bottles that were placed on a roller bottle apparatus in an ambient 37^{o}C incubator. When the cells reach an OD600 of 0.150 (equivalent to ~1.5 x 107 CFU/ml), they were diluted 200-fold in 7H9 medium. The procedure was the same as that used for the MIC procedure described, but only the first 2 fold dilution was made that reduces the stock solution to 1.6 mg/ml. An additional 1:10 dilution was made in water, which reduces the stock solution further to 0.16 mg/ml. Addition of 6.25 µl of the 1:10 dilution to the wells in a final volume of 100 µl will give rise to a concentration equivalent to 10µg/ml.

Antibacterial studies

The *in vitro* antibacterial activity of the compounds was tested in Nutrient broth (NB; Hi-media, Mumbai) by two-fold serial dilution method [31]. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1mg 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. 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. 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. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h. Ciprofloxacin was used as standard drug.

Results and Discussion

Chemistry

In a series of recent reports, methods of synthesis and reactivity of new hydrazone derivatives bearing pyrazines function were described [21, 26]. *N´*-(2*r*,4*c*-diaryl-3-azabicyclo [3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide (**8-14**) can be smoothly obtained by the condensation of corresponding 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ones (**1-7**) with pyrazine-2-carbohydrazide in presence of glacial acetic acid. In all cases expected hydrazones **8-14** were formed as single compound, and isolated in good to excellent yield.

Numbering and designation of atoms

The numbering of the carbons of the piperidine and cyclohexane rings are shown in **Fig. 1**. The *ipso* carbons of the aryl groups at C-2 and C-4 are designated as C-2′ and C-6′. 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. The carbons of the pyrazine ring are designated using 2″, 3″, 5″ and 6″. The protons are denoted accordingly. For example, the benzylic proton at C-2 is denoted as H-2 and that at C-2″is denoted as H-2″ and so on.

The methylene protons in the cyclohexane ring at C-6, C-7 and C-8 are denoted as axial and equatorial protons assuming chair conformation for the cyclohexane ring. Thus, the methylene protons at C-8 are denoted as H-8a and H-8e.

IR spectral studies

In IR spectra, the presence of C=N stretching frequency around 1540 cm^{-1} confirm the hydrazone formation. In all cases two separate N-H stretching bands were observed for two different NH stretching vibrations. In the range 3160–3220 cm^{-1} are due to N-H of hydrazone analogues and the piperidine NH stretching frequency are in the range 3230–3310 cm^{-1}. The absorption band in the region 3070–2800 cm^{-1} are ascribed to aromatic and aliphatic C-H stretching frequencies. The band observed in the region 1645–1660 cm^{-1} are due to C=O stretching frequency of amide carbonyl group. The important FT-IR stretching frequencies of **8-14** are given in experimental section.

NMR Spectroscopy

Proton NMR spectral analysis of compound (**8**)

In order to analysis the spectral assignments of synthesized compounds **8-14,** we have chosen compound **8** as the representative compound. The ^{1}H and ^{13}C signals for the remaining compounds were assigned by comparison with **8** and using known effects [32] of the Cl, CH_3 and OCH_3 substituents in the phenyl rings. In compound **11** coupling with ^{19}F was observed for the aromatic carbons except the *ipso* carbons C-2′ and C-4′ [33]. ^{1}H NMR spectrum of **8** there is a sharp singlet at 11.08 ppm, corresponding to one proton. This should be due to the amide NH proton. There are two doublets at 8.72 and 7.93 ppm, each corresponding to two protons and a sharp singlet at 9.65 ppm corresponding to one proton. These signals must be, respectively, due to the H-5‴, H-6‴and H-3‴ protons of pyrazine ring. There are two doublets at 7.64 and 7.63 ppm, each corresponding to two protons. These are due to the *ortho* protons (*o*-H and *o*'-H). There is one quartet at 7.41 ppm corresponding to

four protons. This signal is an overlap of two triplets and it should be due to the *meta* protons (*m-H* and *m'*-H). There is one quartet at 7.28 ppm corresponding to two protons. This signal should be due to the *mata* protons of the phenyl groups. The *ortho* protons appear at a higher frequency than the other aromatic protons due to the deshielding by the nitrogen lone pair.

There are two singlets at 4.32 and 4.29 ppm each corresponding to one proton. These signals are due to the benzylic protons H-2a and H-4a. There are two broad singlets at 2.54 and 3.28 ppm each corresponding to one proton. These must be due to bridgehead protons H-1e and H-5e. Since H-5e should be deshielded by steric interaction with NHCOPZH moiety the signal at 3.28 ppm should be due to H-5e. It has been shown [16] that in 2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ones proton H-7a appears as a multiplet around 3.0 ppm. Hence, the well resolved multiplet at 2.73 ppm, corresponding to one proton, can be assigned to H-7a. Proton H-7e appears as a quintet at 1.26 ppm.

There is a broad singlet at 2.93 ppm corresponding to one proton. This signal is due to the NH proton of the piperidine ring. There are two multiplets at 1.63 and 1.47 ppm. The multiplet at 1.63 ppm corresponds to one proton. This signal is due to H-8e. The multiplet at 1.47 ppm corresponds to three protons, ascribed to H-6a, H-6e and H-8a.

In order to confirm the above assignment ^{1}H-^{1}H COSY and ROESY spectra have been recorded. The observed correlations of **8** are given in **Table 1** and Shown in **Fig. 2.** In the ^{1}H-^{1}H COSY spectrum the bridgehead proton H-1e has weak correlation with the benzylic proton at 4.32 ppm and has strong correlation with the three protons signal at 1.46 ppm. The signal at 2.73 ppm (H-7a) also has strong correlation with multiplet centered at 1.46 ppm. These observations suggest that the signal at 1.46 ppm can be unambiguously assigned to H-6a, H-8a and H-6e. Furthermore, H-7a has strong correlation with the signals at 1.63 and 1.26 ppm. Hence, the signal at 1.63 ppm is due to H-8e and that at 1.26 ppm is due to H-7e. The two benzylic protons at 4.32 and 4.20 ppm have strong correlations with the

signal at 2.93 ppm. These correlations suggest that the signal 2.93 ppm is due to piperidine NH proton.

In the ROESY spectrum the doublet at 7.64 ppm has strong nOes with H-4a at 4.29 ppm and H-7a at 2.73 ppm. Hence, the doublet at 7.64 ppm must be due to the *ortho* protons (*o*'-H) of the phenyl ring at C-4. Likewise, the doublet at 7.63 ppm has strong nOes with H-2a at 4.32 ppm and H-1e at 2.54 ppm. Hence, the doublet at 7.63 ppm must be due to the ortho protons (*o*-H) of the phenyl ring at C-2.

The strong nOes between H-2a and H-1e and also between H-4a and H-5e clearly confirm that the benzylic and bridgehead protons are occupying axial and equatorial positions, respectively. There is significant nOe between the hydrazone NH proton at 11.08 ppm and bridgehead proton at 3.28 ppm. This shows that the signal at 3.28 ppm is due to H-5e. All these observations are consistent with the assignments of ^{1}H signals and conformation **8CC** assigned to **8** (**Fig. 3)**.

In addition, the NOE between NH proton in hydrazone analogue and H-5e bridgehead proton clearly reveals that the H-5e proton is *syn* to the hydrazone analogue.

In **9-14** individual assignments of the protons were made based on their positions, multiplicities, integral values of the signals and by comparison with **8**. Normally, the *ortho, meta* and *para* protons of piperidine ring give separate signals. Ortho protons are deshielded by the lone pair of electron on the nitrogen while the *meta* and *para* protons are shielded and resonate in the upfield region.

Analysis of ^{13}C NMR spectra

In order to assign the ^{13}C signals unambiguously HSQC and HMBC spectra have been recorded. The observed correlations in the HSQC and HMBC spectra are given in **Table 2**. There is a weak signal at 170.7 ppm. This signal has no correlation in the HSQC spectrum. In the HMBC spectrum, this signal shows correlation with NH proton at 11.08 ppm. Hence,

this signal must be due to C-9. There are four other weak signals at 162.5, 142.8 and 141.4 ppm. These signals have no correlation in the HSQC spectrum. The signal at 162.5 ppm shows correlation with amide NH proton at 11.08 ppm in the HMBC spectrum. Hence, this signal should be due to the carbonyl carbon (C=O). The signals at 142.8 ppm show correlation with the *meta* protons at 7.41 ppm, can be assigned to the *ipso*-carbons C-2' and C-4'.

In the HMBC (**Fig. 4**) spectrum the signal at 141.4 ppm has correlation with H-5‴ at 8.72 ppm. Hence, this signal should be due to C-5‴. The other signals are assigned based on the observed correlations in the HSQC spectrum. The ^{13}C chemical shifts are given experimental section.

The proton signal at 3.28 ppm shows correlation with the signal of DMSO-d_6. From this correlation, it is obvious that the signal for C-5 is mixed with the solvent signal. By careful inspection the chemical shift of C-5 was determined as 41.0 ppm.

Among the bridgehead carbons C-5 is shielded by about 5 ppm relative to C-1. This is due to the interaction between H-5e and nitrogen in hydrazone unit (**Fig. 5**). This interaction induces a polarity in the C-5 - H-5e bond so that the proton H-5e gets a partial positive charge and C-5 gets a partial negative charge. Consequently, the proton is deshielded and the carbon is shielded. Such an effect has been observed in related compounds and has been termed as γ-*syn* effect [8].

Interestingly, among the three methylene carbons of the cyclohexane ring, C-7 is shielded by about 7 ppm than C-6 and C-8. This is due to the steric interaction between H-7a and lone pair on the nitrogen atom of the piperidine ring. Owing to this interaction, C-7 - H7a bond is polarized, H-7a acquires a partial positive charge and C-7 acquires a partial negative charge. Therefore, C-7 is shielded and H-7a is deshielded. The partial negative charge on C-7 shields H-7e. Hence, H-7e appears at a considerably lower frequency (δ = 1.26 ppm).

Antitubercular evaluation

The primary antimycobacterial screening was performed in accordance with the protocol of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) Southern Research institute [30]. All compounds were screened for their *in vitro* antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ (ATCC2729) using isoniazid and rifampicin as the standards. Compounds demonstrating a percent inhibition of bacterial growth greater than or equal to 90% in the primary screening was re-tested against *M. tuberculosis* $H_{37}Rv$, to determine the actual minimum inhibition concentration (MIC) in the MABA. The MIC was defined as the minimum concentration of the compound required to inhibit 90% of the bacterial growth and reported in **Table 3** along with the standard drugs for comparison. This values was determined from the dose-response curve as the IC_{90} using a curve fitting program. Any IC_{90} value of ≤10 μg/mL was considered "Active" for the antitubercular activity. Compounds active in the initial screen were tested for cytotoxicity (IC_{50}) in VERO cells. Cytotoxicity was determined from the does-response curve as the IC_{50} using a curve fitting programs. Concurrent with the determination of MICs, compounds were tested for cytotoxicity in VERO cells at concentration 10×MIC for the *M. tuberculosis* $H_{37}Rv$ (**Table 3**). The selectivity index (SI) was defined as the ratio of the measured IC_{50} in VERO cells to the MIC.

The percentage inhibition was found to lie in a large range of 60-70%. The resazurin MIC assay is a colorimetric assay used to test compounds for antimycobacterial activity. A color change from blue to pink is observed when growth occurs. All of these compounds displayed good *in vitro* activity against *M .tuberculosis* $H_{37}Rv$ strain. The MIC values are within a range of 0.312 μg/mL to 0.625 μg/mL. By comparing their MIC values with INH, the compounds under study were good active against *M. tuberculosis* $H_{37}Rv$. Among the

newly synthesized compounds, two compounds **10** and **14** exhibited excellent antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ at MIC 0.312 μg/mL. The obtained results revealed that the nature of substituent's and substitution pattern on the aryl ring may have a considerable impact on the antimycobacterial activities of the target hydrazones. By closer look into structure–activity relationship of the compounds, it is evident that substitution in aromatic ring by any of the group like methyl, methoxy, fluoro and chloro affects the activity profile. Compounds with aromatic ring having electron donating and electron withdrawing groups are results in loss of activity with compare unsubstituent. Compounds with aromatic ring having fluoro group are more active than the compounds with chloro, methoxy and methyl groups. With respect to structure-MTB activity relationship, the results demonstrated that the antimycobacterial activity was in the order, 4-fluoro > 3-methoxy > 4-chloro >phenyl > 4-methyl as evident from the compounds **8 – 14**.

Beside on our data it is clear that the antimycobacterial activities of the all compounds are comparable to the standard drug isoniazid. Among the seven synthesized compounds two compounds (**11** and **14**) were found to be most active compounds with high percentage growth inhibition at 0.62 μg/mL was found. Compound with electron releasing *meta* substituted phenyl group showed more activity, when compared to methoxy substituent at *para* position. The mycobacterial activity profile of **8**, **11**, **12** and **14** compounds suggests that each compound has a bactericidal effect as there has been no growth in treated control.

Antibacterial evaluation

The newly synthesized compounds were assayed for their antibacterial activity against selected some Gram positive such as *Staphylococcus Aureus* and *Bacillus Subtilis* and Gram negative bacteria *Escheichia Coli*, *Enterobacter Aerogenes*. The result of antibacterial screening is shown in **Table 4**. In table 4 clearly shows that the compound **11** fluoro substituent in the phenyl ring exhibited good activity against all the bacterial strains.

Compounds **10** and **13** had an almost similar range of activity, they differ only by one or two fold dilution, in spite of having a chloro substituent at different positions. The replacement of the halogen substituent by a methoxy or methyl group in phenyl ring as in **9**, **12** and **14** respectively, caused a reduction of activity. From the antibacterial screening, it can be concluded that the compounds show better activity toward gram positive bacteria than gram negative bacteria. Compound **11** shows superior activity against *B. Subtilis.*

Conclusion

In summary seven *N´*-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide (**8-14**) were synthesized in good yield and characterized by FT-IR , ^{1}H, ^{13}C NMR spectral data and elemental analyses. Based on the observed chemical shifts and 2D correlations the hydrazones are twin-chair conformation with equatorial orientations of the aryl groups. All the newly synthesized compounds were screened for their anti-mycobacterium tuberculosis activity. Among the synthesized hydrazones, **10** and **14** showed excellent antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ at the concentration of 0.312 µg /mL. In antibacterial activity that the compound **11** exhibited good activity against all the bacterial strain.

Acknowledgements

The authors are thankful to SIF, Indian Institute of Science, Bangalore and to SAIF IIT-Madras for recording NMR spectra. We thank to Mr. Robert C. Goldman, Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research institute, USA.

Conflict of Interest

The authors have declared no conflict of interest.

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FIGURE CAPTIONS

Scheme 1. Schematic diagram showing the synthesis of title compounds **8-14.**

Figure 1. The numbering of the carbons of the piperidine and cyclohexane ring of compounds.

Figure 2. COSY and (dash line) **ROESY** correlations of compound **8.**

Figure 3. Twin chair conformation of compound **8.**

Figure 4. HMBC Correlation of compound **8.**

Figure 5. The non-bond interaction between H-5e and Nitrogen in hydrazone unit.

Table 1. Correlations in the COSY and ROESY spectra of compound **8.**

Table 2. Correlations in the HSQC and HMBC spectra of compound **8.**

Table 3. *In vitro* antimycobacterial screening data of the compounds **8-14.**

Table 4. *In vitro* antibacterial activity of the compounds **8-14.**

Table 1: Correlations in the ^{1}H-^{1}H COSY and ROESY spectra of 8

Protons	Correlations in the **COSY** spectrum	Correlations in the **ROESY** spectrum
11.09 (amide NH)	Nil	7.93 (H-6‴), 3.28 (H-5e)
8.72 (H-5‴)	7.93 (H-6‴)	-
7.93 (H-6‴)	8.72 (H-5‴)	11.09 (amide NH)
7.64 (*o′*-H)	7.41 (*m*-H, *m*'-H)	2.73 (H-7a), 2.93 (NH), 3.28 (H-5e), 4.29 (H-4a)
7.63 (*o*-H)	7.41(*m*-H *m*'-H)	2.54 (H-1e), 2.73 (H-7a), 2.93 (NH), 4.32 (H-2a)
7.41 (m-H, m'-H)	7.62 (*o*-H), 7.64 (*o′*-H)	7.63 (*o*-H), 7.64 (*o′*-H)
7.28 (*p*-H, *p*'-H)	7.41(*m*-H, *m*'-H)	7.63 (*o*-H), 7.64 (*o′*-H)
2.54 (H-1e)	1.47, 4.31(H-2a)	4.32 (H-2a) 7.64 (*o′*-H), 7.63 (*o*-H)
4.32 (H-2a)	2.54 (H-1e), 2.93 (NH)	2.54 (H-1e), 2.93 (NH), 7.63 (*o*-H)
4.29 (H-4a)	2.93 (NH), 3.28 (H-5e)	2.93 (NH), 3.28 (H-5e), 7.64 (*o*'-H)
3.28 (H-5e)	1.47, 4.29 (H-4a)	1.47, 4.29 (H-4a), 7.64 (*o*'-H)
1.47 (H-6a, H-6e & H-8a)	2.73 (H-7a)	1.63 (H-8e), 7.64 (*o*'-H)
2.73 (H-7a)	1.47 (H-6a, H-6e & H-8a)	1.26 (H-7e), 1.47, 7.63, 7.64
1.26 (H-7e)	1.47 (H-6a, H-6e & H-8a), 2.73 (H-7a)	2.73 (H-7a)
1.63 (H-8e)	1.47 (H-6a, H-6e & H-8a), 2.73 (H-7a)	2.54 (H-1e), 1.47 (H-6a, H-6e & H-8a)
2.93 (piperidine NH)	4.29 (H-4a), 4.32 (H-2a)	3.28 (H-5e), 4.29 (H-4a), 4.32 (H-2a), 7.63 (*o*-H), 7.64 (*o′*-H)

Table 2: Correlations in the HSQC and HMBC spectra of **8**

^{13}C Chemical shifts (δ, ppm)	Correlations in the **HSQC** spectrum	Correlations in the **HMBC** spectrum
170.7	-	11.09 (amide NH)
162.5	-	11.09, 9.65 (H-3‴)
150.0	7.93 (H-6‴)	8.72 (H-5‴)
121.8	-	9.65 (H-3‴)
142.8, 142.7	-	7.41(*m*-H, *m*'-H)
141.4	8.72 (H-5‴)	7.93 (H-6‴)
127.0	7.63 (o-H), 7.64 (o'-H)	4.32 (H-2a), 4.29 (H-4a)
128.2, 128.0	7.41 (*m*-H, *m*'-H)	-
126.8	7.28 (*p*-H, *p*'-H)	-
64.4	4.32 (H-2a)	7.63 (*o*-H)
62.2	4.29 (H-4a)	7.64 (*o*'-H)
45.9	2.54 (H-1e)	-
41.0	3.28 (H-5e)	-
28.2	1.63 (H-8e), 1.47 (H-8a)	-
26.9	1.47 (H-6a, H-6e)	-
20.8	2.73 (H-7a), 1.26 (H-7e)	-

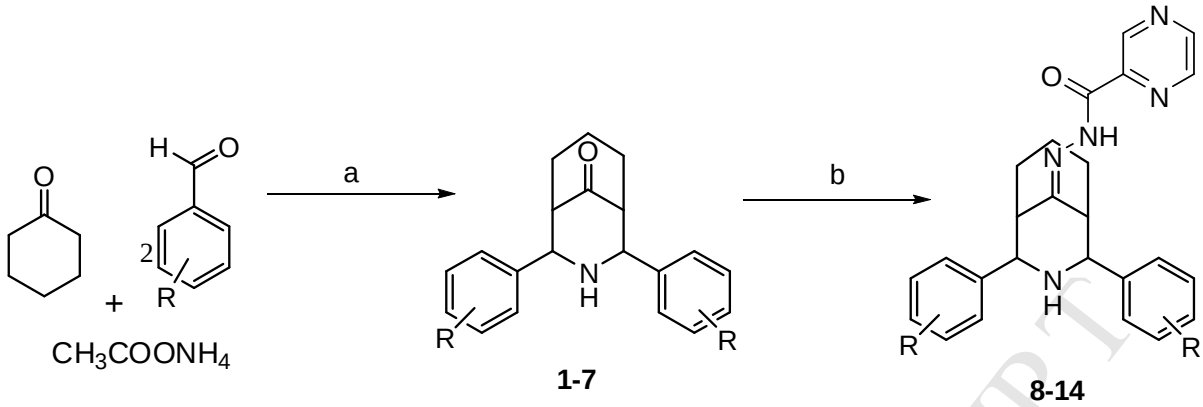
Table 3. *In vitro* antimycobacterial activity of the compounds **8-14**

Comp.	R	MIC (μg/mL)	IC_{50}	IC_{90}	Growth Inhibition (%)	log P[a]	SI
8	H	0.625	0.29	0.32	62.66	3.58	0.93
9	*p*-CH_3	0.625	0.57	0.64	62.30	4.50	0.91
10	*p*-Cl	0.625	0.57	0.64	63.61	4.77	0.91
11	*p*-F	0.312	0.4	0.59	67.63	3.68	0.64
12	*p*-OCH_3	0.625	0.39	0.63	67.07	3.41	062
13	*m*-Cl	0.625	0.57	0.65	65.42	4.77	0.91
14	*m*-OCH_3	0.312	0.29	0.32	59.18	3.41	0.93
INH	-	0.125 - 0.0625	-	-	-	-0.58	-
RIF	-	0.006 - 0.003	-	-	-	-2.38	-

Table 4. *In vitro* antibacterial activity of the compounds **8-14**

Compds.	R	Gram positive				Gram negative			
		S. Aureus		*B. Subtilis*		*E. Coli*		*E. Aerogenes*	
		ZI	AI	ZI	AI	ZI	AI	ZI	AI
8	H	-	-	8	0.27	-	-	-	
9	*p*-CH_3	8	0.25	-	-	-	-	9	0.29
10	*p*-Cl	18	0.56	20	0.67	12	0.43	18	0.64
11	*p*-F	20	0.63	25	0.83	15	0.54	22	0.79
12	*p*-OCH_3	12	0.38	14	0.47	10	0.36	11	0.39
13	*m*-Cl	15	0.47	17	0.57	8	0.29	13	0.46
14	*m*-OCH_3	10	0.31	7	0.23	-	-	8	0.29
Ciprofloxacin		32		30		28		28	

Z.I.–Zone of Growth Inhibition (mm); A.I.–Activity Index (Inhibition Zone of Compound / Inhibition Zone of the Standard Drug).



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

Scheme 1. Reagents and conditions: (a) EtOH, warm, allow overnight; (b) PZH, 0.2 mL AcOH, Methanol and Chloroform (1:1 v/v), reflux 2-3 h.

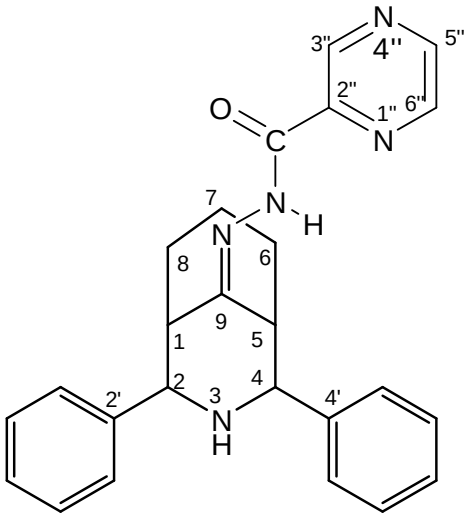


Figure 1. The numbering of the carbons of the piperidine and cyclohexane ring of compounds **8-14**

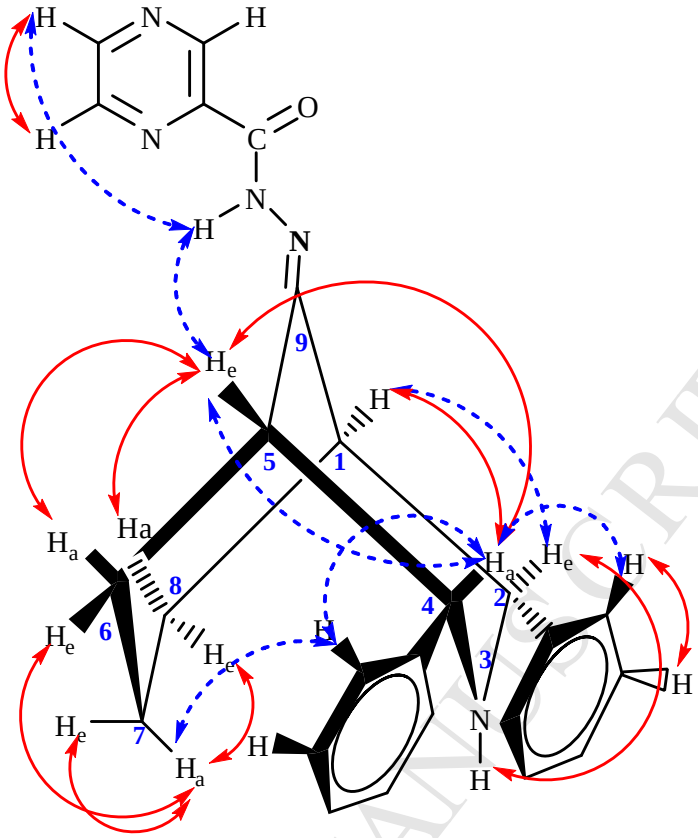
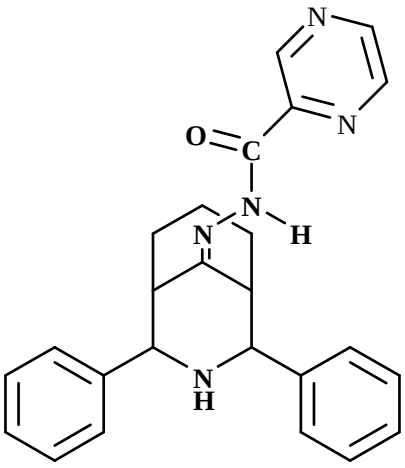
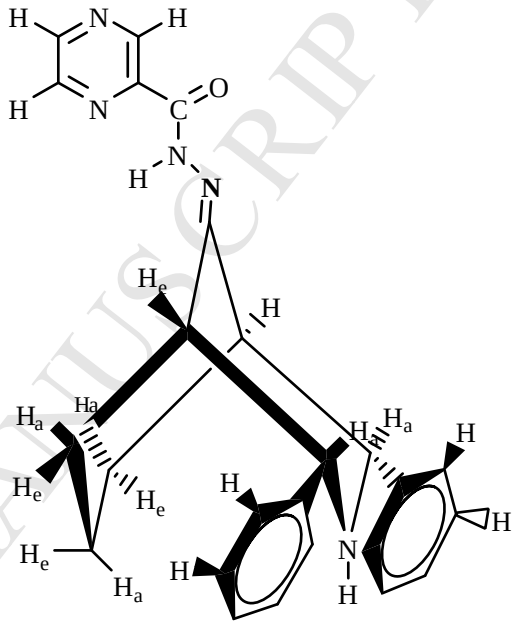


Figure 2. COSY and (dash line) ROESY correlations of compound **8.**



8



8CC

Figure 3. Twin chair conformation of compound **8**.

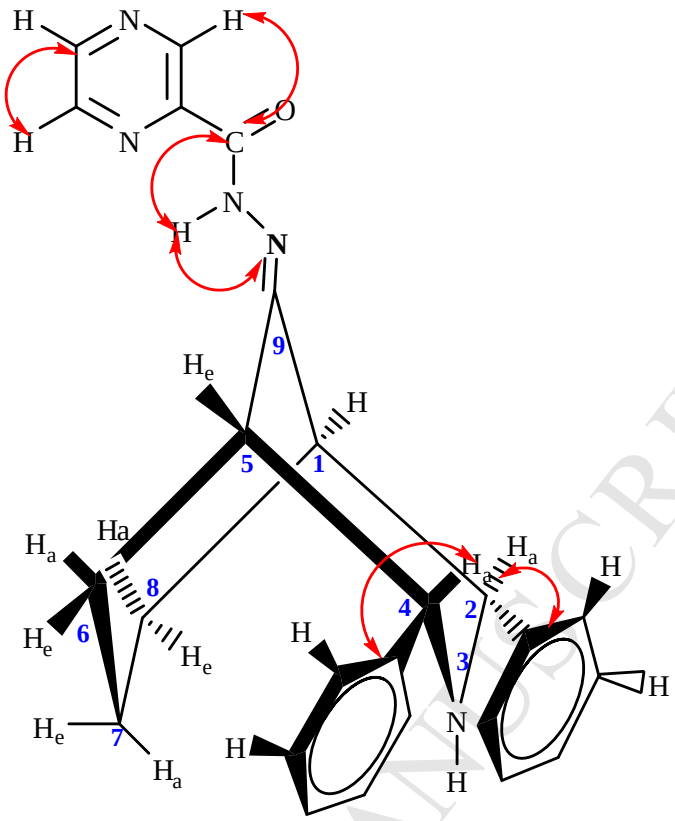


Figure 4. HMBC Correlation of compound **8**.

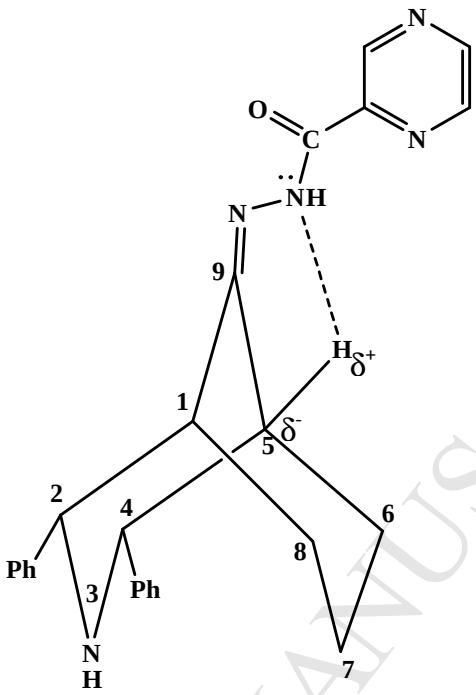


Figure 5.The non-bond interaction between H-5e and Nitrogen in hydrazone unit

H I G H L I G H T S

- *N′*-(2*r*,4*c*-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidene)pyrazine-2-carbohydrazide have been synthesized in good yield.
- All the compounds exists in twin chair conformations with equatorial orientation of the aryl groups.
- All the compounds showing very good antitubercular activity against *Mycobacterium Tuberculosis* H_{37}Rv.