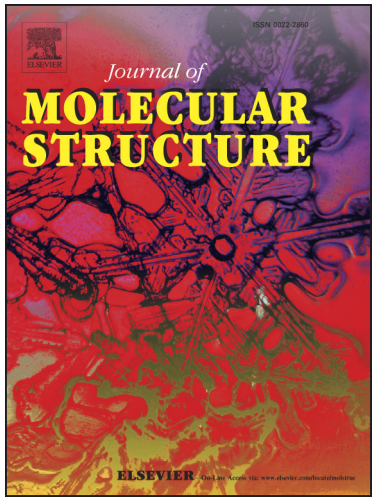

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A. Kamaraj, R. Rajkumar, K. Krishnasamy

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A. Kamaraj, R. Rajkumar and K. Krishnasamy*

Department of Chemistry, Annamalai University, Annamalainagar–608 002, Tamilnadu. India.

*Corresponding Author– K. Krishnasamy

K. Krishnasamy
Professor
Department of Chemistry
Annamalai University
Annamalainagar
Chidambaram
Mobile no.: 09942547856
Fax: 91 – 4144 – 238080
Email: krishnasamybala56@gmail.com

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A. Kamaraj, R. Rajkumar and K. Krishnasamy*

Department of Chemistry, Annamalai University, Annamalainagar–608 002, Tamilnadu. India.

ABSTRACT

Potential antimicrobial activities of azabicyclic skeleton based *N*–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene)hydrazinecarbothioamides (**6**–**10**) was synthesized. The newly synthesized compounds were characterized by FT–IR, ^{1}H NMR, ^{13}C NMR, HRMS and elemental analysis. The synthesized compound (**6**) was further compared by 2D NMR techniques (HOMOCOSY, NOESY and HSQC). The single crystal XRD study revealed that the compound **6** adopts a twin–chair conformation with equatorial orientation of the aryl groups. Antimicrobial activities have been carried out for compounds **6-10** against a panel of bacterial and fungal strains using Streptomycin and Amphotericin B as standards.

Keywords:
3–Azabicyclo[3.3.1]nonan-9–one, 2D NMR, Cyclohexylthiosemicarbazide, Antibacterial activity, Antifungal activity, Crystal structure

1. Introduction

3–Azabicyclo[3.3.1]nonanes (3–ABN) have attracted the attention of researchers owing to their extensive scale of microbial potencies. Antifungal and antibacterial studies proved that 3–ABN skeleton seems to be an effective pathogen killer [1, 2]. Further reports revealed that the

analgesic [3], anti–inflammatory [4], antipyretic [5] and antiphologistic [6] capabilities of azabicyclic ketones. Narcotic antagonism and antitussive and sedative properties of these heterobicycles were also described [7]. Calcium antagonist, hypotensive, and local anesthetic activities [8] are measured. A wide variety of naturally abundant diterpenoid/norditerpinoid alkaloids have been found to possess various pharmacological actions due to the presence of the 3–azabicyclo[3.3.1]nonane pharmocopore. The wide–spread nature and diverse biological actions of this heterocycle attracted more attention in the recent years and as a consequence, synthesis of large number of analogous molecules to improve their biological efficacy [9]. The chemistry of 3–azabicyclo[3.3.1]nonan–9–one has been reviewed [10]. The use of different substituents particularly aromatic substituents with regard to biological activity has been demonstrated [11]. The biological importance of bridged bicyclic compounds exhibit twin chair, chair-boat or twin boat conformations with interesting stereochemistry [12].

The correlation of reactivity and stability of heterocyclic compounds with their predominant conformation has provided a valuable method of assigning the stereochemical conformation of the isomers. The multicomponent reactions are importance now-a-days in synthetic organic chemistry, since they offer one pot combination of more than two components in one step allowing direct access to target molecule [13].

Thiosemicarbazones were reported to be associated with antimicrobial activities [14–16]. Several semicarbazones, as well as their sulfur analogues and derivatives, have proved their efficiency and efficacy in combating various diseases [17]. It is great interest because of their chemistry and beneficial biological activities such as antitumor, antibacterial, antiviral, antimalarial and antiprotozoal [18–21]. Semicarbazones have documented consistent advances in the design of novel anticonvulsant agents through the work of Dimmock and others [22–24].

By considering the synthetic and biological significance of 3–azabicyclo[3.3.1]nonan–9–one thiosemicarbazones, it was thought worthwhile to synthesize them and screen their biological activities. This study will be much useful for the development of new biologically active molecules.

A new series of *N*–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene) hydrazinecarbothioamides (**6**–**10**). The synthesized compounds (**6-10**) were confirmed by physical constants, elemental analysis and spectral techniques. Single crystal X–ray diffraction analysis has also been carried out for the representative compound **6**. The synthesized compounds were screened for their antibacterial and antifungal activities and their results were interpreted in terms of the SAR principles.

2. Experimental

2.1. General

All the chemicals used were of high grade and purchased from sigma Aldrich. Solvents used were of commercial grade and used after purification. The synthesized compounds melting points were taken in open capillaries and are uncorrected. FT–IR spectra were recorded in AVATAR 330 FT–IR Thermo Nicholet spectrophotometer (range 4000–400 cm^{-1}) as KBr pellets as reference standard. NMR spectra (^{1}H and ^{13}C) were recorded at ambient temperature on a BRUKER AMX 400 and 500 NMR spectrometer operating at 400 and 500 MHz for ^{1}H and 100 and 125 MHz for ^{13}C. Solutions for the measurement of spectra were prepared by dissolving 10 mg of the sample in 0.5 mL $CDCl_3$ and chemical shifts (δ) are expressed in ppm. High–resolution mass spectra (HRMS) were performed on VG 7070H mass spectrometer. Elemental analyses were performed on FINNAGAN EA1112 analyzer, their results were found to be in good agreement with the calculated values.

2.2. Synthesis of 2,4–diaryl– 3–azabicyclo[3.3.1]nonan–9–ones **(1–5)**

Substituted 3–azabicyclo[3.3.1]nonan–9–ones were prepared by following the procedure of Baliah and Jayaraman [25]. Dry ammonium acetate (100 mmol) was dissolved in ethanol and the solution was mixed with cyclohexanone (100 mmol) and substituted benzaldehyde (200 mmol). These reaction mixtures were very gently warmed and stirred until the completion of the reaction. The crude products formed were filtered and washed with an ethanol–ether (1:5) mixture to yield the compounds **1**–**5** and recrystallized from chloroform to obtain the pure compounds **1**–**5**.

*2.3. N–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene)hydrazinecarbothio amides **(6–10)***

Title compounds (**6**–**10**) were synthesized by stirring a mixture of respective 3–azabicyclo[3.3.1]nonan–9–ones (**1**–**5**) (25 mmol) and cyclohexylthiosemicarbazide (25 mmol) in ethanol (10 ml) containing and few drops of acetic acid at room temperature for 5 h. Then the separated solid was washed with ice–cold water [26]. The crude product was recrystallized from chloroform. A single-crystal suitable for X–ray structure analysis was obtained for compound **6** by slow evaporation of a solution in a mixture of chloroform and ethanol (1:1 *v*/*v*) at room temperature.

2.4. Analytical data for compounds (**6**-**10**)

*2.4.1. N–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene)hydrazine carbothioamide (**6**)*

White solid, mp=161–163 °C, FT–IR (KBr): 3331, 3303, 1528, 1204 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 8.51 (s, NH, 1H), 7.51 (d, NH, 1H), 7.29-7.58 (m, Ar–CH, 8H), 4.37

(s, H–2, 1H), 4.34 (m, H–1', 1H), 4.23 (s, H–4, 1H), 2.86 (s, H–5e, 1H), 2.82 (m, H–7a, 1H), 2.50 (s, H–1e, 1H), 2.17 (m, H–2'e/H-6'e, 2H), 1.76–1.83 (m, H–6e/H–8e/H–3'e/H–5'e/ NH, 5H), 1.68 (m, H–4'e, 1H), 1.25–1.54 (m, H–4'a/H–2'a/H–6'a/H–7e/H–3'a/H–5'a/H–6'a/H–6a/ H–8a, 9H), ^{13}C NMR (100 MHz, $CDCl_3$), 176.5 (C=S), 159.9 (C=N), 126.9–142.1 (Aromatic carbons), 65.3 (C–2), 63.7 (C–4), 53.2 (C–1'), 46.2 (C–1), 38.9 (C–5), 32.9 (C–2' and C–6'), 28.6 (C–8), 27.2 (C–6), 25.6 (C–4'), 24.9 (C–3' and C–5'), 21.5 (C–7), Anal. Found (Cal.) for $C_{27}H_{34}N_4S$ (%): C, 72.38 (72.60); H, 7.75 (7.67); N, 12.36 (12.54), HRMS (m/z) = 447.2581 $(M + H)^+$.

*2.4.2. 2–(2,4–bis(4–fluorophenyl)–3–azabicyclo[3.3.1]nonan–9–ylidene)–N–cyclohexyl hydrazinecarbothioamide (**7**)*

White solid, mp=196–198 °C, FT–IR (KBr): 3336, 3254, 1533, 1215 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$): 8.46 (s, NH, 1H), 7.50 (d, NH, 1H), 7.09–7.57 (m, Ar, 8H), 4.37 (s, H–2, 1H), 4.32 (m, H–1', 1H), 4.23 (s, H–4, 1H), 2.82 (s, H–5e, 1H), 2.76 (m, H–7a, 1H), 2.48 (s, H–1e, 1H), 2.18 (q, H-2'e/H-6'e, m, 2H), 1.72–1.84 (m, H–6e/H–8e/H–3'e/H–5'e/ NH, 5H), 1.63 (d, H–4'e, 1H), 1.25–1.49 (m, H–4'a/H–2'a/H–6'a/H–7e/H–3'a/H–5'a/H–6'a/H–6a/H–8a, 9H), ^{13}C NMR (100 MHz, $CDCl_3$), 176.5 (C=S), 159.2 (C=N), 115.2–163.2 (Aromatic carbons), 64.6 (C-2), 63.0 (C-4), 53.3 (C–1'), 46.1 (C–1), 38.8 (C–5), 32.9 (C–2' and C–6'), 28.4 (C–8), 27.1 (C–6), 25.5 (C–4'), 24.9 (C–3' and C–5'), 21.4 (C–7), Anal. Found (Cal.) for $C_{27}H_{32}F_2N_4S$ (%): C, 67.06 (67.19); H, 6.78 (6.68); N, 11.48 (11.61), HRMS (m/z) = 483.2396 $(M + H)^+$.

*2.4.3. 2–(2,4–bis(4–methoxyphenyl)–3–azabicyclo[3.3.1]nonan–9–ylidene)–N–cyclohexyl hydrazinecarbothioamide (**8**)*

White solid, mp=181–183 °C, FT–IR (KBr): 3325, 3265, 1533, 1248 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$): 8.46 (s, NH, 1H), 7.77 (d, NH, 1H), 6.94-7.52 (m, Ar–CH, 8H), 4.32–4.34 (d, H–2 and H–1',2H), 4.19 (s, H–4, 1H), 3.84–3.86 (d, *p*–OCH_3, 3H), 2.80 (s, H–5e, 1H), 2.83 (m, H–7a, 1H), 2.45 (s, H–1e, 1H), 2.18 (q, H–2'e/H–6'e, 2H), 1.79–1.87 (m, H–6e/ H–8e/H–3'e/H–5'e/ NH, 5H), 1.68 (d, H–4'e, 1H), 1.25–1.56 (m, H–4'a/H–2'a/H–6'a/H–7e/ H–3'a/H–5'a/H–6'a/H–6a/H–8a, 9H), ^{13}C NMR (100 MHz, $CDCl_3$), 176.4 (C=S), 160.2 (C=N), 113.7–159.0 (Aromatic carbons), 64.8 (C–2), 63.2 (C–4), 55.3 (*p*–OCH_3), 53.2 (C–1'), 46.3 (C–1), 39.1 (C–5), 32.9 (C–2' and C–6'), 28.5 (C–8), 27.2 (C–6), 25.5 (C–4'),

24.9 (C-3' and C–5'), 21.5 (C–7), Anal. Found (Cal.) for $C_{29}H_{38}N_4O_2S$ (%): C, 68.85 (68.74); H, 7.48 (7.56); N, 11.16 (11.06), HRMS (m/z) = 507.2794 $(M + H)^+$.

*2.4.4. 2– (2,4–bis(3–methoxyphenyl) –3–azabicyclo[3.3.1]nonan–9–ylidene)–N–cyclohexyl hydrazinecarbothioamide (**9**)*

White solid, mp=187–189 °C, FT–IR (KBr): 3353, 3216, 1534, 1265 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 8.45 (s, NH, 1H), 7.52 (d, NH, 1H), 6.85-7.53 (m, Ar–CH, 8H), 4.35 (s, H–2, 1H), 4.33 (m, H–1', 1H), 4.21 (s, H–4, 1H), 3.87 (*d*, *m*–OCH_3, 3H), 2.86 (s, H–5e, 1H), 2.81 (m, H–7a, 1H), 2.51 (s, H–1e, 1H), 2.19 (q, H–2'e/H–6'e, 2H), 1.78–1.88 (m, H–6e/H–8e/ H–3'e/H–5'e/NH, 5H), 1.70 (m, H–4'e, 1H), 1.23–1.56 (m, H–4'a/H–2'a/H–6'a/H–7e/H–3'a/ H–5'a/H–6'a/H–6a/H–8a, 9H), ^{13}C NMR (100 MHz, $CDCl_3$), 176.6 (C=S), 159.9 (C=N), 112.1–159.8 (Aromatic carbons), 65.1 (C–2), 63.5 (C–4), 55.3 (*m*–OCH_3), 53.2 (C–1'), 46.2 (C–1), 38.9 (C–5), 32.8 (C–2' and C–6'), 28.7 (C–8), 27.3 (C–6), 25.5 (C–4'), 24.9 (C–3' and C–5'), 21.4 (C–7), Anal. Found (Cal.) for $C_{29}H_{38}N_4O_2S$ (%): C, 68.85 (68.74); H, 7.48 (7.56); N, 11.21 (11.06), HRMS (m/z) = 507.2795 $(M + H)^+$.

*2.4.5. 2–(2,4–bis(4–chlorophenyl)–3–azabicyclo[3.3.1]nonan–9–ylidene)–N–cyclohexyl hydrazinecarbothioamide (**10**)*

White solid, mp=183–185 °C, FT–IR (KBr): 3347, 2931, 1523, 1210 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): 8.51 (s, NH, 1H), 7.52 (d, NH, 1H), 7.29-8.03 (*m*, Ar–CH, 8H), 4.77 (s, H–2, 1H), 4.35 (m, H–1', 1H), 4.66 (s, H–4, 1H), 3.21 (s, H–1e, 1H), 2.79 (*m*, H–7a, 1H), 2.86 (s, H–5e, 1H), 2.16 (q, H–2'e/H–6'e, 2H), 1.74–1.79 (m, H–6e/H–8e/H–3'e/H–5'e/ NH, 5H), 1.66 (m, H–4'e, 1H), 1.27–1.56 (m, H–4'a/H–2'a/H–6'a/H–7e/H–3'a/H–5'a/H–6'a/H–6a/H–8a, 9H), ^{13}C NMR (100 MHz, $CDCl_3$), 176.6 (C=S), 158.3 (C=N), 126.8–138.8 (Aromatic carbons), 62.0 (C–2), 60.3 (C–4), 53.2 (C–1'), 41.9 (C–1), 34.6 (C–5), 32.8 (C–2' and C–6'), 28.5 (C–8), 27.1 (C–6), 25.5(C–4'), 24.9 (C–3' and C–5'), 21.0 (C–7), Anal. Found (Cal.) for $C_{27}H_{32}Cl_2N_4S$ (%): C, 62.76 (62.90); H, 6.35 (6.26); N, 10.65 (10.87), HRMS (m/z) = 515.5487 $(M + H)^+$.

2.5. X–ray data collection and structure refinement

A colorless crystal of the compound **6** was mounted on a glass fiber with epoxy cement for the X–ray crystallographic study. The selected crystal data and data collection parameters of

the compound **6** were collected at 293 K. Bruker area data detector diffract meter was used for the measurement of data. The collected data were reduced using the SAINT program and structural refinement was carried out by Full–matrix least–squares on F^2 (SHELXL–97).

2.6. In vitro antibacterial and antifungal activity

The *in vitro* activities of the compounds were tested in Sabourauds dextrose broth (SDB; Hi–media, Mumbai) for bacteria by the 2–fold serial dilution method. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB (Nutrient Broth) from 24–h–old bacterial 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. The final inoculum size was 10^5 cfu/ml for antibacterial assay and 1.1–1.5 $x10^2$ cfu/ml for antifungal assay and testing was performed at pH 7.4 ± 0.2. Exactly 0.2 ml of the solution of each 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. Streptomycin and Amphotericin B were used as standards for antibacterial and antifungal activity evaluation respectively.

3. Results and discussion

3.1. Designation of atoms

The carbon atoms of the bicyclic[3.3.1]nonane part are numbered as shown in Fig. 1. The carbons of the aryl group at C–2 are denoted as 1", 2", 3", 4", 5" and 6"– carbons and those of the aryl group at C–4 are denoted as 1'", 2'", 3'", 4'", 5'"and 6'"–carbons. The carbons of the cyclohexyl ring at C–1, C–2, C–3, C–4, C–5 and C–6 are designated as C–1', C–2', C–3', C–4', C–5' and C–6', respectively and the protons are numbered accordingly. For example, the benzylic proton at C–2 is denoted as H–2 and so on. The methylene protons in the cyclohexane ring are denoted as axial and equatorial protons assuming chair conformation for the cyclohexane ring. The methylene protons at C–6, C–7 and C–8 are denoted as H–6a / H–6e, H–7a / H–7e and H–8a / H–8e.

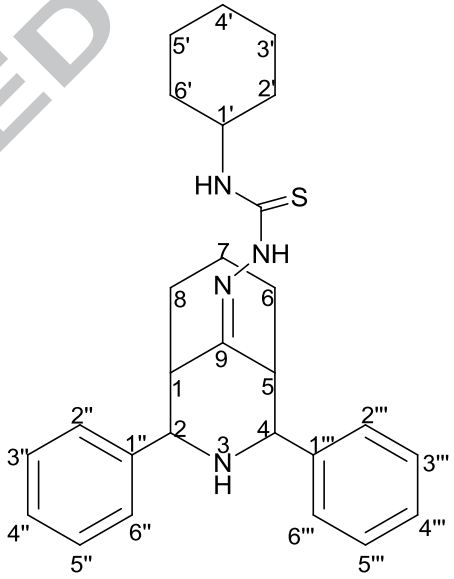

Fig. 1. Numbering of the atoms in the target compound

3.2. IR Spectral analysis of compounds **6–10**

The synthesized compounds, the NH stretching frequency was in the range of 3216–3353 cm^{-1}. The characteristic vibrational band in the region 1725-1675 cm^{-1} was observed for the carbonyl functionality [27]. For all the parent piperidones, the sharp band in the region 1720-1700 cm^{-1} of C=O group **1-5**. The synthesized compounds **6–10** showed the absence of C=O stretching frequency around 1720–1700 cm^{-1} and the presence of C=N and C=S stretching frequencies around 1528–1599 cm^{-1} and 1205–1248 cm^{-1} confirming the condensation of 2,4–diaryl–3–aza–bicyclo[3.3.1]nonan–9–ones with cyclohexylthiosemicarbazide. Literature report [28] shows that NH, C=N and C=S stretching frequency in the region of 3357-3171cm^{-1}, 1533-1516 and 1247-1205 cm^{-1}. The intense aliphatic and aromatic stretching frequencies appeared are 2969–2843 cm^{-1} due to the presence of the phenyl and cyclohexyl ring (Supplementary Material).

3.3. ^{1}H NMR spectral analysis of compound ***6***

The schematic diagram of compounds **6–10** is shown in Scheme 1. All the synthesized compounds were characterized by ^{1}H and ^{13}C NMR spectral studies. The signals were assigned with the help of earlier reports [28, 29]. The ^{1}H and ^{13}C NMR spectral analysis of compound **6** is discussed as follows. The observed ^{1}H and ^{13}C chemical shifts are given in Table 1 and 2 respectively.

We have chosen compound **6** as the representative system. ^{1}H NMR spectrum of compound **6** is given in Fig. 2. The ^{1}H NMR spectrum showed two broad signals in the downfield region at 8.51 ppm (singlet) with one proton integral, and a doublet at 7.51 ppm with one proton integral are due to NH protons of thiosemicarbazone moiety (=N-NH-CS and cyclohexyl-NH-CS). The benzylic protons (H–2 and H–4) appear separately at 4.37 and 4.23

ppm, respectively. Similarly, the bridgehead protons resonate at 2.50 ppm and 2.86 ppm (singlet) respectively. The shielded signal appeared at 2.50 ppm is attributed to H–1e proton whereas the deshielded signal observed at 2.86 ppm (singlet) is assigned to H–5e proton. The observed desheilding experienced by H–1e proton is attributed to the presence of electronegative nitrogen atom in the adjacent position.

The multiplet observed at 2.82 ppm is assigned for H-7a methylene proton and another multiplet observed at 4.34 ppm is assigned for H–1'a proton. Further, the multiplet observed at 2.17 ppm with two protons integral is assigned for H–2'e and H–6'e protons. The shielded region appeared at 1.68 ppm is attributed to the H–4'e proton. Furthermore, the multiplet observed in the region of 1.76–1.83 ppm is due to H–8e, H–6e, H–5'e and H–3'e and piperidone ring NH proton whereas another multiplet observed in the region of 1.25–1.54 ppm with eight protons integral is assigned for H–5'a, H–3'a, H–7e, H–8a, H–6a, H–2'a, H–6'a and H–4'a protons. The aromatic protons resonate in the region of 7.29–7.58 ppm due to anisotropic effect of the aromatic ring.

3.4. HOMOCOSY and NOESY spectral analysis

HOMOCOSY and NOESY spectrum of compound **6** shows Fig 3 and 4. The selected HOMOCOSY and NOESY correlations shown in Fig 5 and 6 whereas Table 3 and 4 represents the correlations chemical shifts values of compound **6**. The weak HOMOCOSY correlation between the signals at 2.86 and 2.50 ppm indicated that the correlation is due to the long-range coupling between the bridgehead protons (H-5e and H-1e) and this is possible only when both the protons are in equatorial orientation in chair conformation. This observation clearly confirms that the singlet at 2.86 ppm is due to H-5e proton. Another bridgehead proton (H-1e) appears at 2.50 ppm (singlet) has strong correlation with the benzylic proton at 4.23 ppm (singlet). Besides

H-1e proton also shows HOMOCOSY correlations with H-8a and H-8e protons. The strong correlation observed between the H-7a proton at 2.82 ppm and the H-7e proton at 1.39 ppm infers that both signals are due to the C-7 methylene protons (H-7a and H-7e). Furthermore, H-7a proton has correlations with H-6a, H-6e, H-8a and H-8e protons.

The signal due to cyclohexane ring proton (H-7a) appears in the higher frequency region than the equatorial proton (H-7e). The deshielding effect of H-7a proton signal is mainly due to the steric interaction between H-7a proton and the lone pair electrons on nitrogen atom. Due to this, C-H(7a) bond becomes polarized and as a consequence, carbon and its attached proton acquires negative and positive charges, respectively. Hence, the H-7a proton signal is highly deshielded than the H-7e. Further, H-6a and H-8a protons appear in the shielded region than the corresponding equatorial protons in accordance with C–C single bond anisotropy effect. In HOMOCOSY spectrum, the benzylic protons show correlation with that of N(3)-H proton. Based on the observed chemical shift values and the earlier report [30] it has been concluded that the compound **6** exists in the twin-chair (Fig**. 7**) conformation.

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 dispositions respectively. Besides, the nOe between the NH proton in hydrazone analogue and H-5e bridgehead proton suggests that the H-5e proton is *syn* to the hydrazone group.

The syn α–bridgehead proton (H-5e) is observed in the highly deshielded region than anti α–bridgehead proton (H-1e). This is due to the non–bonded (spatial) interaction between (H-5e) proton with nitrogen atom in the thiosemicarbazone moiety (Fig. 7). Owing to this interaction, syn α–carbon get partial negative charge and the attached proton acquires a slight positive charge

[30]. Based on this report, the shielded signal is assigned to (H-1e) proton whereas the deshielded signal is assigned to (H-5e) proton.

*3.5. ^{13}C NMR spectral analysis of compound **6***

In ^{13}C NMR spectrum of compound **6**, the thiosemicarbazone C=S and C=N carbons are resonated at 176.5 and 159.9 ppm respectively. In the aliphatic region the signals observed at 53.2, 25.6 and 32.8 are assigned to C–1' and C–4' carbons. This signal at 32.9 and 24.9 ppm are assigned to C–2'/C–6' and C–5'/C–3' carbons respectively. Further, two signals appeared at 65.3 and 63.7 ppm are conveniently assigned to C–2 and C–4 carbons whereas the bridged carbons C–1 and C–5 carbons resonated at 46.2 and 38.9 ppm respectively based on their HSQC correlations. The cyclohexyl ring methylene carbons C–6 and C–8 are appeared at 27.2 and 28.6 ppm whereas C–7 carbon is resonated at 21.5 ppm. The aromatic carbons are distinguished from other carbons by their characteristic absorption in the region of 126.9–128.6 ppm. *Ipso* carbons of phenyl groups resonated at 141.5–142.1 ppm are assigned based on their intension and chemical shift values.

*3.6. HSQC spectral analysis of compound **6***

HSQC spectrum of compound **6** is recorded to confirm the 1D NMR spectral assignments. In the HSQC NMR spectrum the benzylic protons observed at 4.37 and 4.23 ppm (H–2 and H–4) have cross peaks with the signals at 65.3 and 63.7 ppm, confirmed the C–2 and C–4 carbons. The bridgehead methine proton signals at 2.50 (H–1e) and 2.86 ppm (H–5e) have correlations with the carbon signals at 46.2 and 38.9 ppm. This inferred that the signals appeared at 46.2 and 38.9 ppm is due to C–1 and C–5 carbons. The HSQC spectrum of compound **6** shown in Fig 8 and the chemical shifts are reproduced in Table 5.

*3.7. Single crystal XRD study of compound **6***

Single crystal XRD analysis was carried out for the representative compound *N*–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene)hydrazinecarbothioamide **6** and its ORTEP was shown in Fig. 9 with selected bond length and bond angles. The bond lengths and bond angles are in good agreement with the literature values [28]. The thiosemicarbazone (**6**) crystallizes in triclinic system with P-1 space group as a chloroform solvate. The details of crystal data and refinement are given in Table 6.

From the single crystal XRD analysis, it is interesting to note that *N*–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene)hydrazinecarbothioamide (**6**) has adopted a twin–chair conformation with equatorial orientations of the phenyl groups shown in Fig. 10.

The condensation of ketone with thiosemicabazide resulted in a new C=N bond whose bond length is 1.265(7) Å [C3–N2]. The configuration about the C=N bond is E. The observed dihedral angle between S1 and N2 is 175.4(5)° [S1–C21–N3–N2]. The delocalization of π electron density over thiosemicarbazone moiety resulted in the variations of C=N, N-N and C=S bond distance from the normal value [31]. The torsion angles of phenyl rings C3–C4–C5–C15 and C3–C2–C1–C9 are –177.0 (6)° and –178.7(7)°, respectively. Thus, all parameters clearly indicate the twin–chair conformation [32] of the bicycle with an equatorial orientation of the phenyl groups (Fig 10). The cyclohexyl ring in the thiosemicarbazone fragment also adopts chair conformation as expected and all other bond parameters are normal. The packing of the molecule is given in Fig. 11.

3.8. Biological activity

The bacterial and fungal minimum inhibitory concentrations (MIC) values of the test compounds and the standard are furnished in Table 7.

All the compounds were screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi* and antifungal activity against, *Candida albicans, Aspergillus flavus, Rhizopus Mucor* and *Aspergillus niger*. The antimicrobial studies of compounds **6–10** with various substituents in the aromatic ring will be useful to understand the influence of steric and electronic effects on the biological activities. Also it reveals the effect of replacement of oxygen by thiosemicarbazone moiety on the biological activity.

*3.9. Antibacterial activity of compounds (**6-10**)*

For evaluating antibacterial activity, streptomycin was used as the standard drug. In general all the synthesized compounds exert a wide range of modest antibacterial activity *in vitro* against the tested organisms. Compound **6** without any substituent in the aryl moiety exhibited antibacterial activity *in vitro* at 100 μg ml^{-1} against *Escherichia coli* and *Pseudomonas aeruginosa* whereas it showed antibacterial activity for the other tested organisms only at 200 μg ml^{-1}.

Fluoro and chloro substituted compounds (**7** and **10**) demonstrated very good antibacterial activity against all the bacterial strains used in this study. Further when compared with standard drug streptomycin, compound **7** has two fold increased activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The antibacterial activity of compound **10** has been compared with the antibacterial activity of the standard drug streptomycin and it indicated that compound **10** has the same efficiency against *Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhi*. Further compounds **8** and **9** exhibited good antibacterial activity against all the bacterial strains at 50–100 μg ml^{-1}.

3.10. Antifungal activity *of compounds (**6-10**)*

For evaluating antifungal activity, Amphotericin B was used as the standard drug. The results of the present study also provide evidence for the antifungal effects of compounds **6**−**10** with MIC values ranging from 6.25 to 200 μg ml^{-1}. Compound **9**, without any substituents at the aryl groups, showed mild and comparatively less antimicrobial activity against *Candida albicans* (100 μg ml^{-1}), *Aspergillus flavus* (200 μg ml^{-1}), *Rhizopus* (100 μg ml^{-1}), *Mucor* (100 μg ml^{-1}) and *Aspergillus niger* (50 μg ml^{-1}) compared to Amphotericin B, a known antifungal agent used as positive control. Compound **7** exerted 3–fold increased antifungal activity against *C. albicans* and *A. niger* at MIC values of 6.25 μg ml^{-1} and 2–fold increased activity against *Aspergillus flavus*, *Rhizopus* and *Mucor* at a MIC value of 12. 5 μg ml^{-1} when compared to the standard. Furthermore, phenyl rings with electron–donating methoxy group at the *para* position of the phenyl rings (compounds **8** and **9**) hinder the growth of all fungal strains at MIC values ranging from 50 to 100 μg ml^{-1}. Compound **10** exhibited 3–fold increased antifungal activity against *Aspergillus flavus*, *Mucor* and *Aspergillus niger* and 2–fold enhanced antifungal activity against *Candida albicans* and *Rhizopus* when compared with Amphotericin B. The results of the present study demonstrate that electron withdrawing groups at the *para* position of the aromatic ring in the azabicyclononan–9–one moiety exert superior inhibitory effect against various tested microbes compared to the other tested compounds and standard drug.

The observed results indicated that the type of functional groups and the pattern of substitution on the azabicyclononan–9–one moiety could be responsible for the substantial influence on the antimicrobial activity of the hydrazone derivatives. The antifungal and antibacterial activities of compounds **6**−**10** create promising leads for the development of potent antimicrobial agents.

4. Conclusion

All the target compounds are synthesized successfully and were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, HRMS and elemental analysis. The piperidone ring adopts a twin–chair conformation with equatorial orientations of the aryl groups was confirmed by single crystal XRD (compound **6**). The cyclohexyl fragment present in thiosemicarbazone moiety also exhibits a normal chair conformation. From the close assessment of the *in vitro* antibacterial and antifungal results against a panel of microbial organisms revealed that the compounds with fluorine or chlorine substituents were found to be excellent antimicrobial activities against all the tested organisms.

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Figure captions

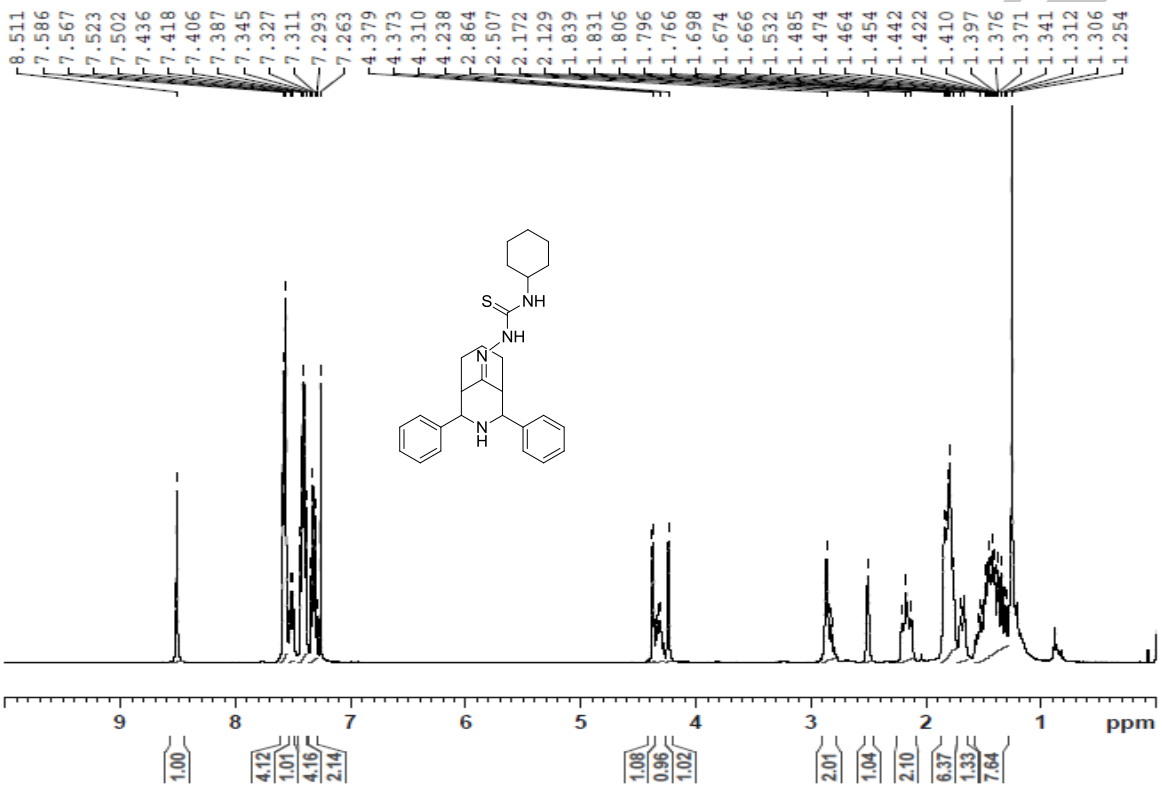


Fig. 2. ^{1}H NMR spectrum of compound **6**

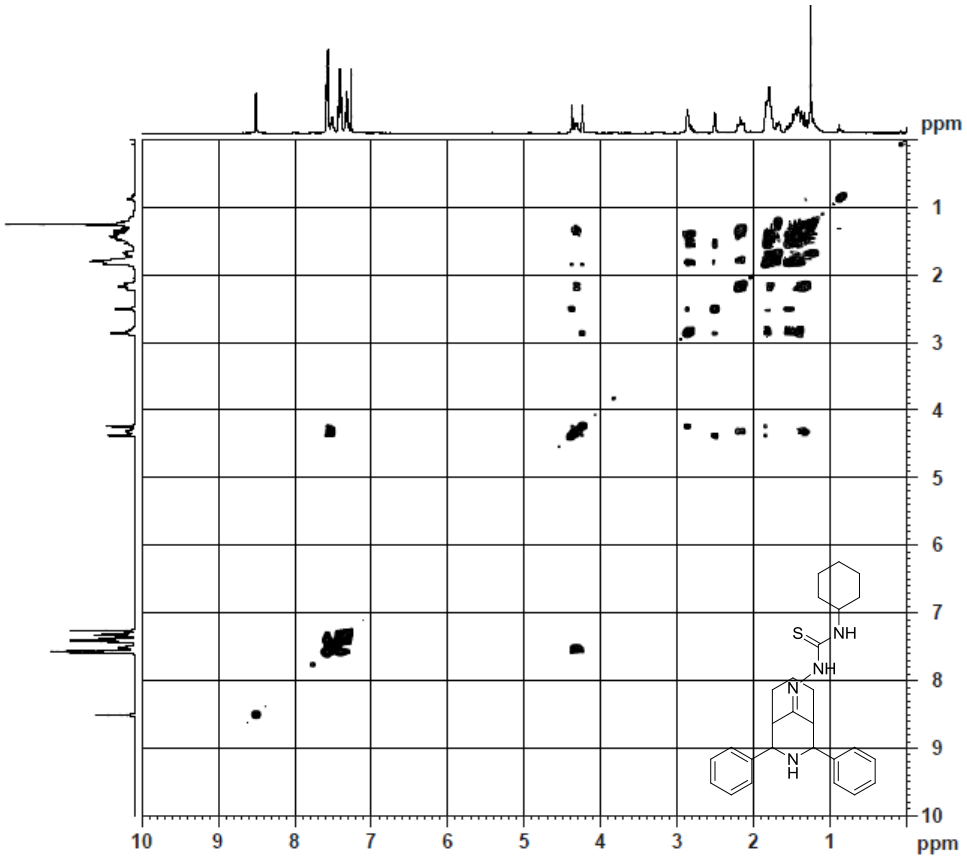


Fig. 3. HOMOCOSY spectrum of compound **6**

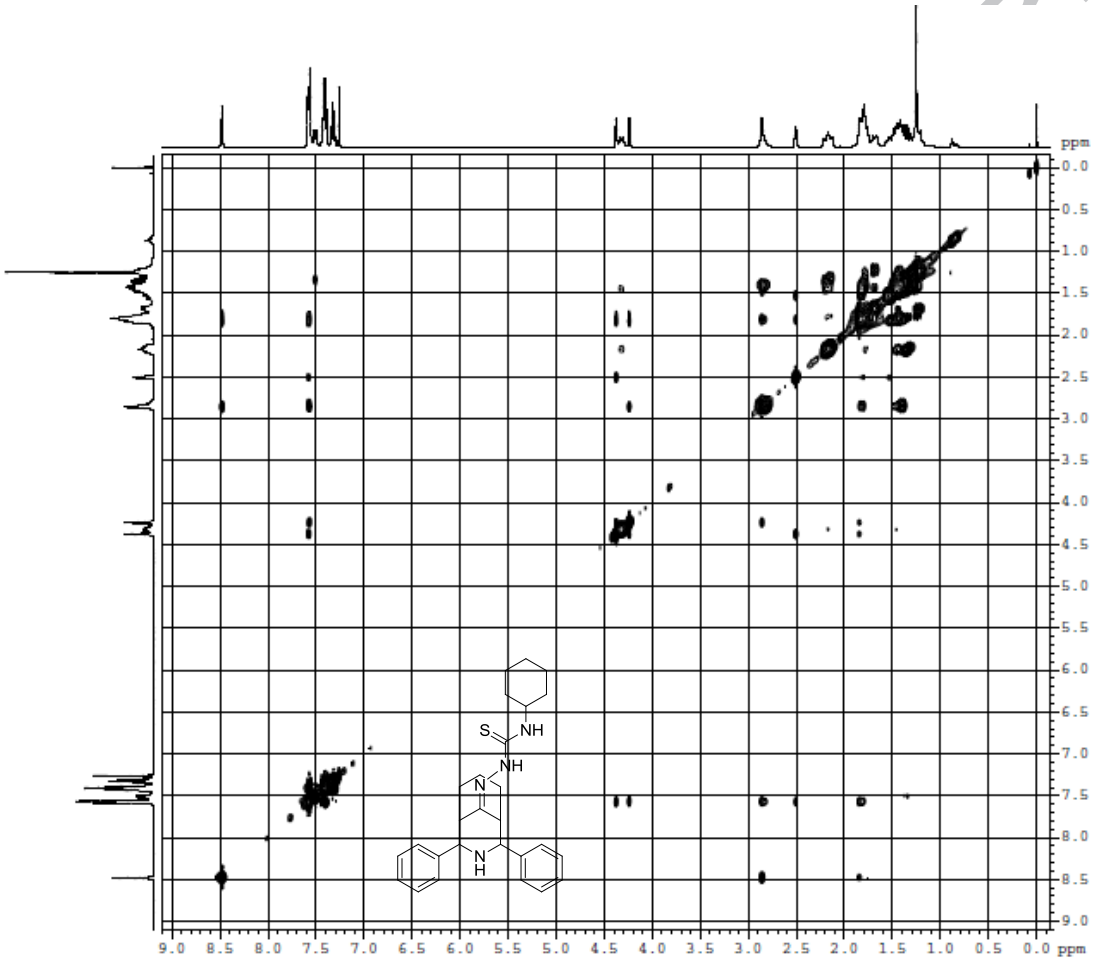


Fig. 4. NOESY spectrum of compound **6**

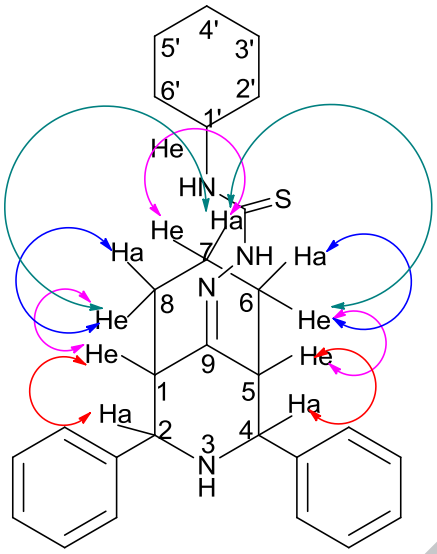


Fig. 5. HOMOCOSY- Selected correlations of compound **6**

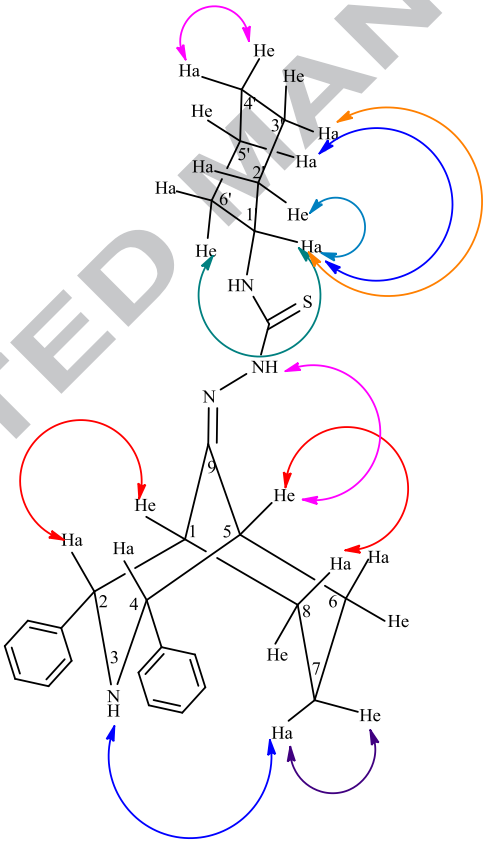


Fig.6. NOESY- Selected correlations of compound **6**

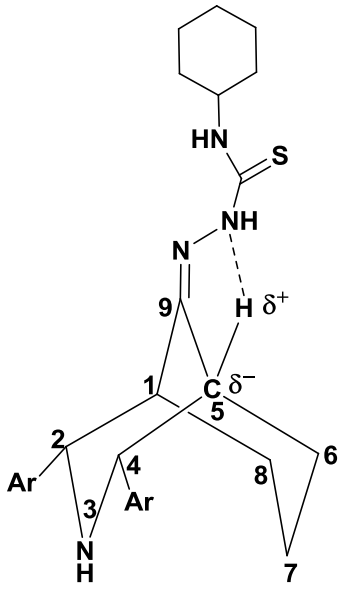


Fig. 7. Non-bonded interactions between CH(5e) and NH group.

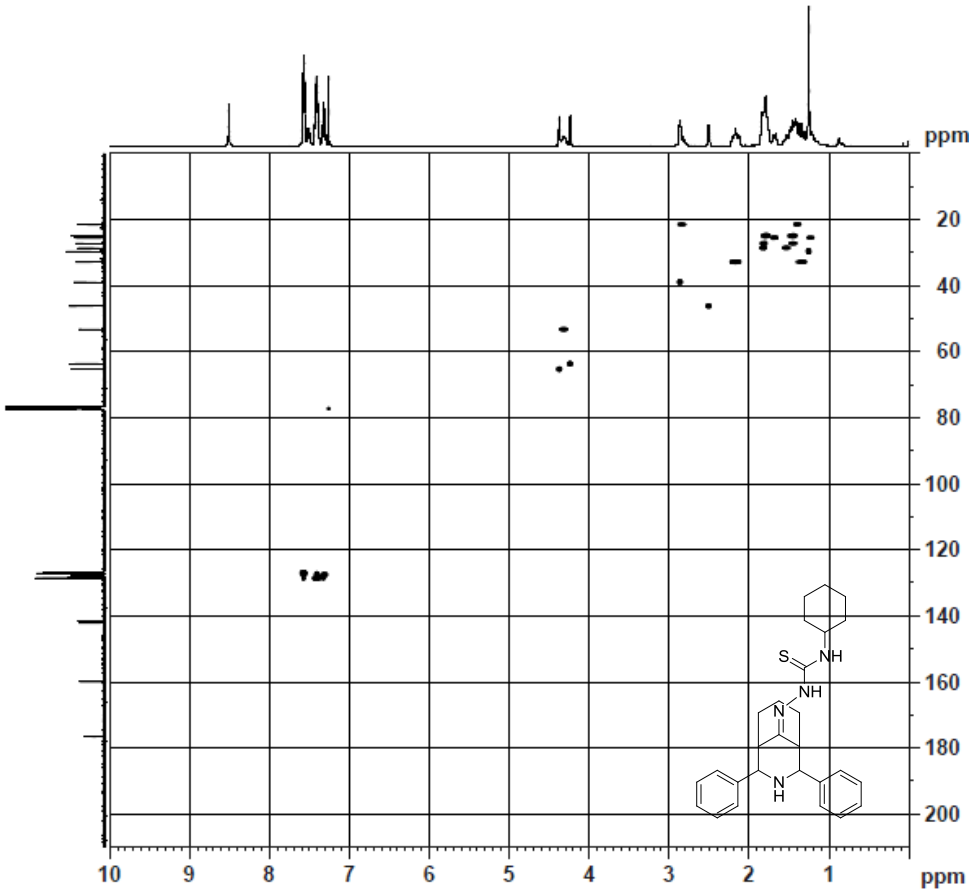


Fig. 8. HSQC spectrum of compound **6**

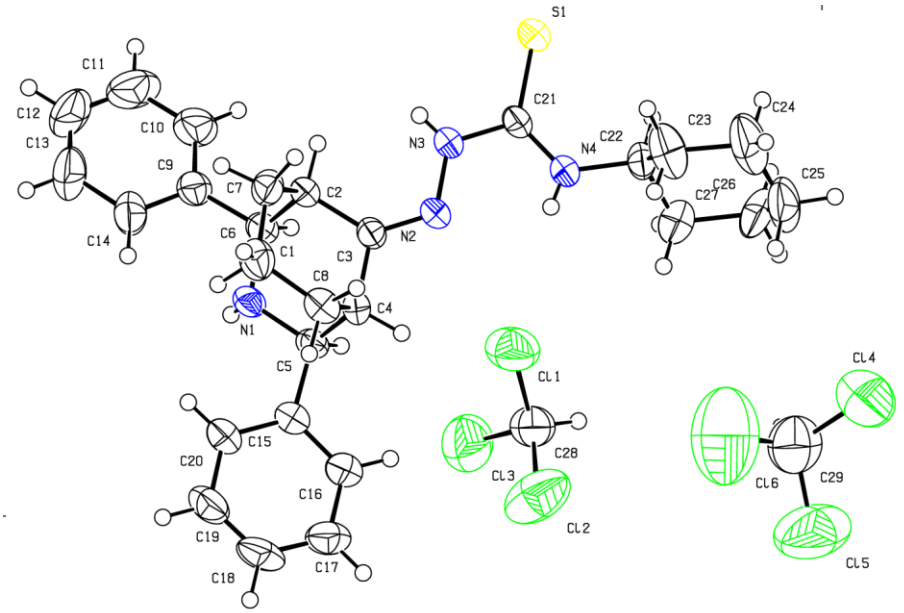


Fig. 9. ORTEP view of compound **6**

The important bond lengths (Å): N2-C3=1.265(7) ; N2-N3=1.38(1); N3-C21=1.350(7); N4-C21=1.29(1); C1-N1=1.44(1); N1-C5=1.45(1); C5-C4=1.52(1); C3-C8=2.429(9); C3-C6=2.42(1); C6-C7=1.53(1); C7-C8=1.51(1); C6-C2=1.51(1); C2-C3=1.48(1); C3-C4=1.49(1); C2-C1=1.53(1); C25-H26B=2.02(1); H24A-C23=2.058(8);C27-H22=2.0(1); H26B-C27=2.064(8); C27-C22=1.50(2); C26-C27=1.53(1).
The important bond angles (°): C3-N2-N3=118.1(7);C3-C2-C1=107.8(7);C3-C4-C5=109.3(7);C2-H6B-C7=77.0(3); C7-C8-C4=113.1(7); C21=117.5(7);N2-N3-C21=117.5(7); N3-C21-S1=117.9(6); N1-C5-C15=110.5(7); N1-C1-C9=111.9(7).

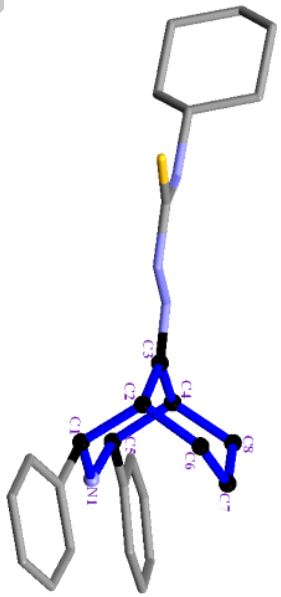


Fig. 10. Conformation of compound **6**

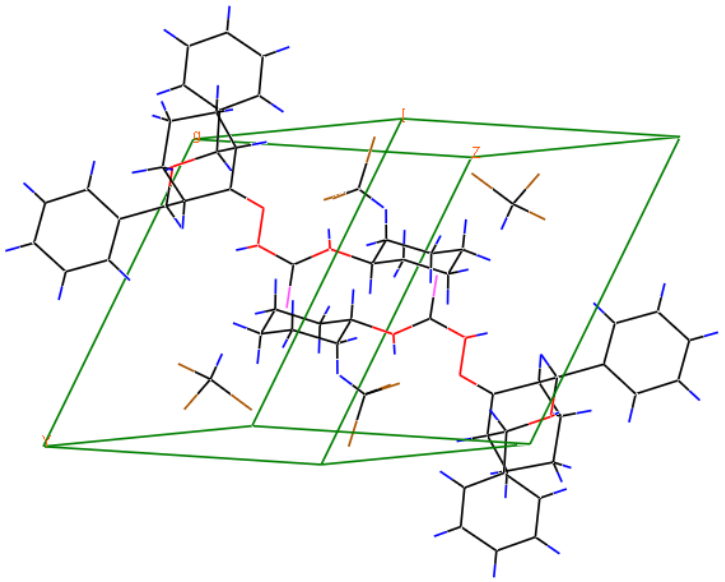
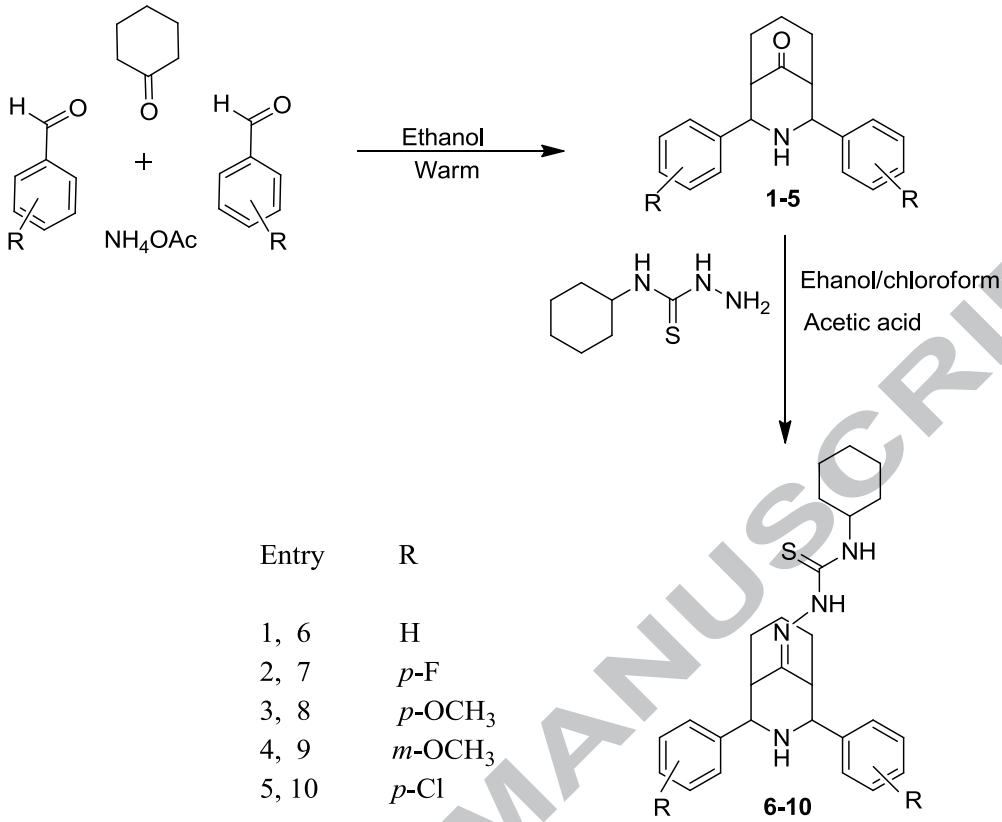


Fig. 11. Packing diagram of compound 6



Entry	R
1, 6	H
2, 7	*p*-F
3, 8	*p*-OCH_3
4, 9	*m*-OCH_3
5, 10	*p*-Cl

Scheme 1. Schematic diagram showing the synthesis of compounds **6**–**10**

Table 1

^{1}H Chemical shift (ppm) values of compounds **6-10**

Proton	Compound				
	6	**7**	**8**	**9**	**10**
NHCS	8.51(s)	8.46(s)	8.46(s)	8.45(s)	8.51(s)
CSNH-Cy	7.51(d)	7.50(d)	7.77(d)	7.52(d)	7.52(d)
H-2a	4.37(s)	4.37(s)	4.34(s)	4.35(s)	4.77(s)
H-4a	4.23(s)	4.23(s)	4.19(s)	4.21(s)	4.66(s)
H-1'a	4.34(m)	4.32(m)	4.36(m)	4.33 (m)	4.35(m)
H-5e	2.86(s)	2.82(s)	2.80(s)	2.86(s)	2.86(s)
H-7a	2.82(m)	2.76(m)	2.83(m)	2.81(m)	2.79(m)
H-1e	2.50(s)	2.48(s)	2.45(s)	2.51(s)	3.21(s)
H-4'e	1.68(m)	1.63(d)	1.68(q)	1.70(m)	1.66(m)
H-2'e, H-6'e	2.17(m)	2.18(q)	2.18(dd)	2.19(q)	2.16(q)
H-6e, H-8e, H-3'e, H-5'e, NH	1.76-1.83 (m)	1.72-1.84 (m)	1.79-1.87 (m)	1.78-1.88 (m)	1.74-1.79 (m)
H-3'a, H-5'a, H-7e, H-6a, H-8a, H-2'a, H-6'a, H-4'a	1.25-1.54 (m)	1.25-1.49 (m)	1.25-1.56 (m)	1.23-1.56 (m)	1.27-1.56 (m)
O(CH_3)	-	-	3.85(d)	3.87(d)	-
Aromatic protons	7.29-7.58 (m)	7.09-7.57 (m)	6.94-7.52 (m)	6.85-7.53 (m)	7.29-8.03 (m)

Table 2

^{13}C Chemical shift (ppm) values of compounds **6-10**

Carbon	Compound				
	6	**7**	**8**	**9**	**10**
C=S	176.5	176.5	176.4	176.6	176.6
C=N	159.9	159.2	160.2	159.9	158.3
C-2	65.3	64.6	64.8	65.1	62.0
C-4	63.7	63.0	63.2	63.5	60.3
C-1'	53.2	53.3	53.2	53.2	53.2
C-1	46.2	46.1	46.3	46.2	41.9
C-5	38.9	38.8	39.1	38.9	34.6
C-2', C-6'	32.9	32.9	32.9	32.8	32.8
C-8	28.6	28.4	28.5	28.7	28.5
C-6	27.2	27.1	27.2	27.3	27.1
C-4'	25.6	25.5	25.5	25.5	25.5
C-3', C-5'	24.9	24.9	24.9	24.9	24.9
C-7	21.5	21.4	21.5	21.0	21.0
O(CH_3)	-	-	55.3	53.3	-
Aromatic carbons	126.9-142.1	115.2-163.2	113.7-159.0	112.1-159.8	126.8-138.8

Table 3. Correlations in the HOMOCOSY spectrum of compound 6

^{1}H Chemical shifts	Correlations in the HOMOCOSY spectrum
2.50 (H-1e)	4.37
4.23 (H-4a)	2.86
2.82 (H-7a)	1.39
1.25 (H-4'a)	1.68
1.29-1.39 (H-2'a H-6'a)	2.17
1.41-1.49 (H-3'a, H-5'a)	1.76-1.83
4.34(H-1'a)	2.17

Table 4. Correlations in the NOESY spectrum of compound 6

^{1}H chemical shifts	Correlations in the NOESY spectrum
(H-1e) 2.50	4.37
(H-4a) 4.23	2.86
(H-6a/H-8a) 1.41-1.57	1.75-1.83
(H-7e) 1.39	2.82
(NH) 8.51	2.86
(H-4'a)1.25	1.68
(H-2'a H-6'a) 1.29-1.38	2.12-2.20
(H-3'a, H-5'a) 1.40-1.50	1.74-1.83

Table 5

Correlations in the HSQC spectrum of compound **6**

Signals (^{1}H Chemical shifts in ppm)	HSQC Correlations (^{13}C signals in ppm)
(H-4'a)1.21-1.26	25.6
(H-4'e) 1.66-1.68	25.6
(H-2'a/(H-6'a) 1.29-1.39	32.8
(H-2'a/(H-6'a) 2.12-2.20	32.8
(H-7e) 1.39	21.5
(H-7a) 2.82	21.5
(H-3'a/ H-5'a) 1.40-1.50	24.9
(H-3'e/ H-5'e) 1.74-1.83	24.9
(H-1'a) 4.34	53.2
(H-4a) 4.23	63.7
(H-2a) 4.37	65.3
(H-5e) 2.86	38.9
(H-1e) 2.17	46.2

Table 6

Crystal data and structure refinement parameters of compound **6**

Empirical formula	$C_{27}H_{34}N_4S$, 2($CHCl_3$)
Formula weight	685.39
Wavelength	0.71073Å
Temperature	293 K
Crystal system, space group	Triclinic, P-1
Unit Cell dimensions	a=10.6242(11) A alpha=105.013(11) deg.
	b=11.2159(18) A beta=101.195(8) deg.
	c=15.6817(16) A gamma=108.145(12) deg.
Z, Density (calculated)	2, 1.391 Mg/m3
Absorption coefficient	0.615 mm-1
Volume	1636.4(4)$Å^3$
F(000)	712
Crystal size	0.22 x 0.20 x 0.18 mm
Theta range for data collection	2.67 to 29.03 deg.
Goodness-of-fit on F^2	1.168
Largest diff. peak and hole	0.792 and -0.603 eA^{-3}
Data/restraints/parameters	7447 / 0 / 388
Refinement method	Full-matrix least-squares on F^2
CCDC No	1000456

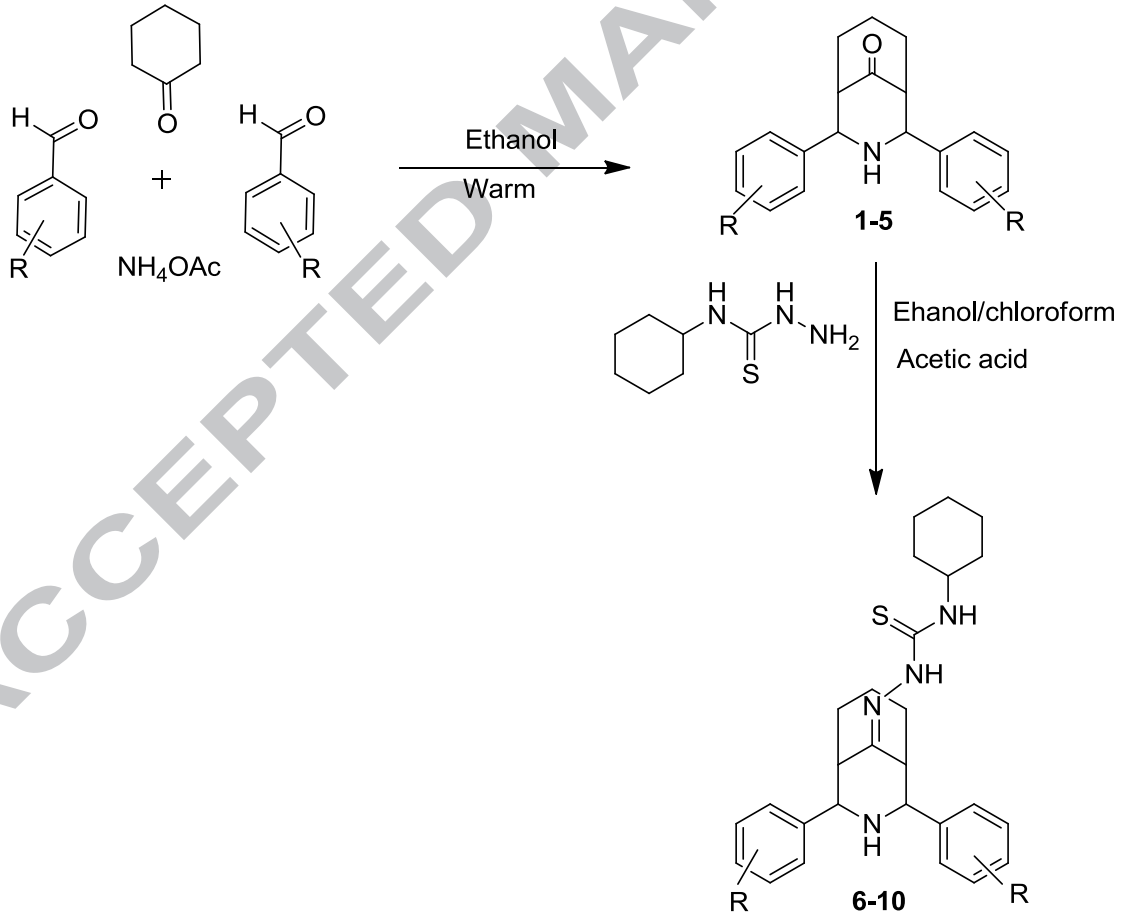
Table 7

Results of *in vitro* antibacterial and antifungal activity evaluation of compounds **6-10**

Compound	MIC in µg/ml									
	S. aureus	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. typhi*	*C. albicans*	*A. flavus*	*Rhizopus sp.*	*Mucor.*	*A.niger*
6	200	100	200	100	100	100	200	100	100	50
7	12.5	25	12.5	25	50	6.25	12.5	12.5	6.5	6.5
8	50	100	50	50	100	50	100	50	50	100
9	50	100	50	50	100	50	100	50	50	100
10	25	50	25	50	50	12.5	6.5	12.5	6.5	6.5
Streptomycin	25	25	25	12.5	50	-	-	-	-	-
Amphotericin **B**	-	-	-	-	-	25	25	25	12.5	25

GRAPHICAL ABSTRACT

Biologically active *N*–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1]nonan–9–ylidene)hydrazinecarbothioamide derivatives **(6–10)** were synthesized from substituted 2,4–diaryl–3–azabicyclo[3.3.1]nonan–9–ones **(1–5)** with thiosemicarbazone. The synthesized compounds subjected for spectral and antimicrobial studies and the results were suitably interpreted.



RESEARCH HIGHLIGHTS

- Biologically active novel *N*–cyclohexyl–2–(2,4–diphenyl–3–azabicyclo[3.3.1] nonan–9–ylidene)hydrazinecarbothioamide derivatives.
- Synthesized compounds are confirmed by FT-IR, ^{1}H and ^{13}C NMR spectral studies.
- The synthesized compounds adopted twin-chair conformation.
- All the synthesized compounds exhibited appreciable antimicrobial activities.