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Research Article

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Comparative study of microwave assisted and conventional synthesis, biological evaluation and molecular docking of some Heterobicyclic *N*-methylhydrazinecarbothioamide derivatives

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Abstract: A Rapid and efficient method for preparation of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazinecarbothioamides (**10-17**) were designed and synthesized from 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one (**1-8**) with 4-methyl-3-thiosemicarbazide under microwave irradiation. Newly synthesized compounds are screened for their *in vitro* antioxidant, antimicrobial studies and *in vivo* larvicidal activities. Among the tested compounds **12** and **17** showed potent antioxidants in comparison with standard ascorbic acid (Vit-C) while compounds **13** and **14** were emerged as outperformed antimicrobial activity than standard Ciprofloxacin and Ketoconazole respectively. The derivatives were further evaluated for *in vivo* larvicidal activities results revealed that compounds **11, 12** and **17** exhibited noticeable cent per cent mortality at ~100 ppm larvicidal activities against Aedes aegypti. Docking simulations demonstrated that compound **11** could bind tightly with the crystal structure of the NS2B/NS3 active site. Based on the results obtained, compound **11** may act as a better candidate for further development of direct NS2B/NS3 inhibitors. Thus, microwave heating technology in drug synthesis should be promoted in order to reducing the cost of basic drugs and chemicals.

Keywords: Antioxidant capacity, Antimicrobial, Mosquito larvicidal activity, DENs NS2B/NS3, Molecular docking.

1. INTRODUCTION

As an important heterocycle scaffold, 3-Azabicyclononones (3-ABN) hydrazine−hydrazone derivatives are attractive targets for organic synthesis and pharmaceutical because they characterize an important component of pharmacologically active compounds and are correlated with a wide spectrum of biological antimicrobial, antitubercular, and also anticonvulsant actions [1-4]. Molecules with the 3-azabicyclo[3.3.1]nonane nucleus are huge attention due to their occasion in a broad variety of naturally occurring alkaloids and biological activities [5]. Antioxidants inhibit the oxidation of other molecules from Reactive oxygen species (ROS) by being oxidized themselves. Synthetic compounds having groups like -S or -N, able to donating free electrons, whichever in the form of a negative charge or in the form of a lone pair of electrons, could be demonstrate higher antioxidant activity this is due to their redox property [6]. Consequently, thiosemicarbazones derivatives have demonstrated antitumor, antiviral,

antimalarial [7] anti-HIV [8] and antiamoebic studies [9-10]. It has also been shown that the active –NH– and C=S groups in the chitosan thiosemicarbazone derivatives strongly increase the ferrous ion-chelating effect and free radical-scavenging activity [11]. Every year, more than one billion people are infected and more than one million people die from vector-borne diseases, yellow fever and lymphatic filariasis [12,13]. The recent study is focused on the docking of the chemicals composition against NS2B-NS3 protease. The 184-residue NS3pro, with multiple enzyme activities that are essential for viral reproduction, is a trypsin-like protease through a serine protease catalytic triad (His51, Asp75, and Ser135) [14]. The proteolytic activity of NS3pro can be markedly enhanced when it is coupled to its cofactor, the central hydrophilic portion (residues 49−95) of NS2B [15,16]. The use of microwave irradiation in organic synthesis has become more and more popular within the pharmaceutical areas, as a new knowledge enabling drug discovery and development [17]. The use of microwave organic synthesis has been shown to spectacularly reduce processing times, increase product yields, and improve the purity of the product when compared to the conventionally processed experiments. Synthesis of bicyclic based piperidine and thiosemicarbazide derivatives are available in the articles [18,19] on the other hand, these methods undergo from disadvantages such as long reaction time, severe reaction conditions. Thus, it is plausible to develop a series of (3-ABN) hydrazine derivatives using microwave irradiation technique and also compare it with traditional method of conventional heating approach.

2. EXPERIMENTAL SECTION

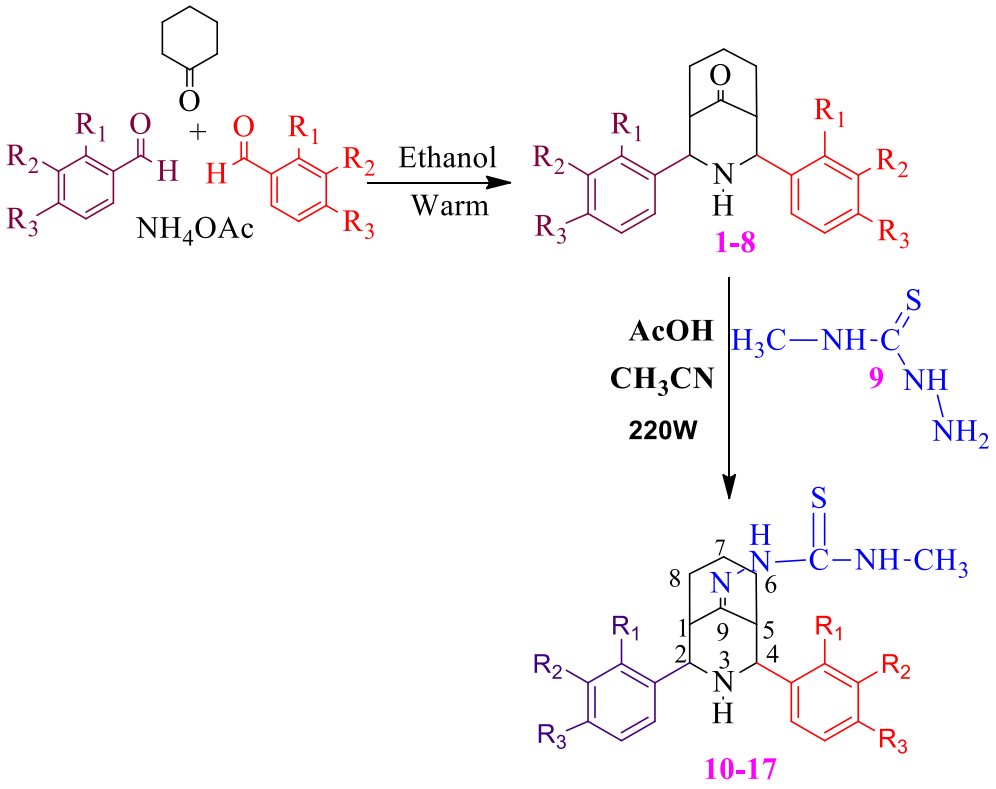
2.1 MATERIALS AND METHODS

All of the solvents were analytical reagent grade. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapour and UV light. The melting points of all the compounds were measured in open capillaries and were uncorrected. IR spectra were recorded on an AVATAR-330 FT-IR spectrometer (Thermo Nicolet) using KBr (pellet form). ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz Bruker instrument using $CDCl_3$ as solvent for all the compounds. Tetramethylsilane (TMS) was used as an internal reference for all NMR spectra with chemical shifts reported in δ units (parts per million) relative to the standard.

2.2 Molecular docking

Molecular docking experiment was carried out to study the exact binding location of ligand on protein. Molecular docking simulation was performed with the aid of Maestro 9.3.5 version. Three-dimensional (3D) structure of the Dengue virus NS2B/NS3 protease was retrieved from the Protein Data Bank (PDB) using PDB ID:2FOM [20] (http://www.rcsb.org/pdb) and was optimized by removing water molecules and hetero groups were deleted from receptor beyond the radius of 4Å of reference ligand (Flavin mononucleotide) and the resulting protein structure refined and minimized by protein preparation Wizard using OPLS-2005 force field. Receptor Grid Generation programs were used to prepare 2FOM Grid and all ligands were optimized by LigPrep program by using OPLS-2005 force field to generate lowest energy state of ligands. A binding sphere covering all the active site residues was generated using the Define and Edit Binding Site module, and docking was subsequently performed using the Flexible docking algorithm27 considering the default parameters. Of the total poses identified, the

best docked pose was selected on the basis of its scoring function (–CDOCKER energy), and processed further for the binding energy calculations.



Scheme 1

2.3 Synthesis

2.3.1 General procedure for the preparation of the title compounds (**10-17**)

Conventional method

The synthetic route for the 2,4-Diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazinecarbothioamide derivatives (**10-17**) was outlined in **Scheme 1**. The reported literature procedures [21] were used to synthesize the parent 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one (**1-8**). Compounds (**1-8)** react with 4-methyl-3-thiosemicarbazide **9** in the presence of acetic acid results the formation of the title compounds (**10-17**). In our study, 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one (1.0 mmol) and 4-methyl-3-thiosemicarbazide (1.1 mmol) were dissolved in a mixture of acetonitrile. Acetic acid (1 or 2 drops) was added as a catalyst and refluxed for 4-5 h at 40 °C afforded 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazinecarbothioamides were obtained. After the completion of reaction, the solvent mixture was removed under vacuum. The solid was separated and washed with cold water/ethanol mixture and recrystallized from ethanol.

Microwave method

To the mixture of compound (**1-8**) and 4-methyl-3-thiosemicarbazide in molar ratio of 1:1, 1-2 drops glacial acetic acid in acetonitrile (5 mL) was taken in round bottom flask placed in a microwave oven and irradiated (220 W, 80 °C) for 10-12 min, the reaction progress have been monitored by TLC using pet ether and ethylacetate 3:1 as a solvent. The reaction mixture was cooled and the formed precipitate was washed first with ethanol and then by ether to get pure compound. The products were obtained in good yield (77%–90%). The synthesized compounds **10**-**17** gave satisfactory analytical data and are given in (Table 1). The data obtained from ^{1}H NMR, ^{13}C NMR, and 2D spectra analysis confirmed the proposed structures (Spectral data results are provided in supporting information).

Table-1. Physical data of compounds **10-17**

Compd	R_1	R_2	R_3	M.P.(ºC)	Conventional %yield	t/hrs	Microwave %yield	t/min	p/watt
10	H	H	H	200-203	67	4	84	10	220
11	H	H	Me	195-198	54	5	80	12	220
12	H	H	OMe	190-193	52	5	79	12	220
13	H	H	F	209-212	67	4	90	10	220
14	H	H	Cl	207-210	55	4	88	10	220
15	H	Cl	H	203-206	62	4	84	10	220
16	H	H	Br	200-203	61	4	82	10	220
17	OMe	H	H	191-194	58	5	77	12	220

2.3.2 2-(2,4-diphneyl-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**10**)

White solid; yield 84**%**; mp 200-203 °C; IR (KBr, λmax cm^{-1}) 3194-3367 (N−H st), 2800-3086 (C−H st), 1650 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48 (s, 1H, H-1e), 4.35 (s, 1H, H-2a), 4.23 (s, 1H, H-4a), 2.84 (s, 2H, H-5e, H-7a), 1.83 (m, 3H, H-6e, H-8e, N−H), 1.49 (m, 3H, H-7e, H-8a, H-6a), 8.63 (s, 1H, ═N–NH), 7.67 (s, 1H, –C═S–NH), 3.28 (d, 3H, CH_3), 7.31−7.58 (m, 10H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.42 (C-1), 65.49 (C-2), 63.80 (C-4), 39.02 (C-5), 27.30 (C-6), 21.53 (C-7), 28.63 (C-8), 160.24 (C-9), 179.02 (C=S), 31.18 (CH_3), 126.89–142.03(Aromatic carbons and ipso carbons).

2.3.3 2-(2,4-bis(4-methylphenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**11**)

White solid; yield 80**%**; mp 195-198 °C; IR (KBr, λmax cm^{-1}) 3191-3374 (N−H st), 2801-2981 (C−H st), 1649 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44(s, 1H, H-1e), 4.30(s, 1H, H- 2a), 4.18 (s, 1H, H-4a), 2.82 (m, 2H, H-5e, H-7a), 1.83 (m, 3H, H-6e, H-8e, N−H), 1.43 (m, 3H, H-7e, H-8a, H-6a), 8.58 (s, 1H, ═N–NH), 7.66(s, 1H, –C═S–NH), 3.27 (d, 3H, CH_3), 2.37 (d, 6H, *p*-CH_3), 7.19−7.45 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.50 (C-1), 65.33 (C-2), 63.61 (C-4), 39.11 (C-5), 27.30 (C-6), 21.54 (C-7), 28.62(C-8), 160.58 (C-9), 178.98 (C=S), 31.17 (CH_3), 21.15 (CH_3 at *p*), 126.79–139.10(Aromatic carbons and ipso carbons).

2.3.4 2-(2,4-bis(4-methoxyphenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**12**)

White solid; yield 79%; mp 190-193 °C; IR (KBr, λmax cm^{-1}) 3212-3377 (N−H st), 2778-2985 (C−H st), 1649 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 1H, H-1e), 4.27(s, 1H, H- 2a), 4.16 (s, 1H, H-4a), 2.81(m, 2H, H-5e, H-7a), 1.83 (m, 3H, H-6e, H-8e, N−H), 1.46 (m, 3H, H-7e, H-8a, H-6a), 8.55(s, 1H, ═N–NH), 7.65 (s, 1H, –C═S–NH), 3.27 (d, 3H, CH_3), 3.83 (d, 6H, *p*-OCH_3), 6.92−7.48 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.56 (C-1), 65.05 (C-2), 63.35 (C-4), 39.20 (C-5), 27.27 (C-6), 21.58 (C-7), 28.59(C-8), 160.57 (C-9), 178.97 (C=S), 31.18 (CH_3), 55.33, 55.36 (OCH_3 at *p*), 113.84–159.13 (Aromatic carbons and ipso carbons).

2.3.5 2-(2,4-bis(4-fluorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**13**)

White solid; yield 90%; mp 209-212 °C; IR (KBr, λmax cm^{-1}) 3193-3379 (N−H st), 2778-3057 (C−H st), 1648 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 1H, H-1e), 4.32 (s, 1H, H-2a), 4.21 (s, 1H, H-4a), 2.83 (m, 2H, H-5e, H-7a), 1.81 (m, 3H, H-6e, H-8e, N−H), 1.47 (m, 3H, H-7e, H-8a, H-6a), 8.60 (s, 1H, ═N–NH), 7.65 (s, 1H, –C═S–NH), 3.27 (d, 3H, CH_3), 7.07−7.55 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.33 (C-1), 64.87 (C-2), 63.17 (C-4), 38.91 (C-5), 27.15 (C-6), 21.47 (C-7), 28.45 (C-8), 159.50 (C-9), 170.09 (C=S), 31.18 (CH_3), 115.31–137.53 (Aromatic carbons and ipso carbons).

2.3.6 2-(2,4-bis(4-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**14**)

White solid; yield 88%; mp 207-210 °C; IR (KBr, λmax cm^{-1}) 3258-3369 (N−H st), 2796-3060 (C−H st), 1659 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 1H, H-1e), 4.32(s, 1H, H- 2a), 4.21 (s, 1H, H-4a), 2.78 (m, 2H, H-5e, H-7a), 1.81 (m, 3H, H-6e, H-8e, N−H), 1.51 (m, 3H, H-7e, H-8a, H-6a), 8.58 (s, 1H, ═N–NH), 7.65 (s, 1H, –C═S–NH), 3.27 (d, 3H, CH_3), 7.07−7.55 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.12 (C-1), 64.87 (C-2), 63.17 (C-4), 38.90 (C-5), 27.15 (C-6), 21.48 (C-7), 28.46 (C-8), 159.49 (C-9), 179.05 (C=S), 31.20 (CH_3), 115.32–137.56(Aromatic carbons and ipso carbons).

2.3.7 2-(2,4-bis(3-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**15**)

White solid; yield 84%; mp 203-206 °C; IR (KBr, λmax cm^{-1}) 3187-3361 (N−H st), 2772-2970 (C−H st), 1652 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (s, 1H, H-1e), 4.32 (s, 1H, H- 2a), 4.21 (s, 1H, H-4a), 2.88 (s, 1H, H-5e), 2,72 (s, 1H, H-7a), 1.82 (m, 3H, H-6e, H-8e, N−H), 1.49 (m, 3H, H-7e, H-8a, H-6a), 8.63 (s, 1H, ═N–NH), 7.65 (s, 1H, –C═S–NH), 3.27 (d, 3H, CH_3), 7.28−7.57 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.12 (C-1), 64.97 (C-2), 63.26 (C-4), 38.72 (C-5), 27.20 (C-6), 21.48 (C-7), 28.48 (C-8), 158.91 (C-9), 179.09 (C=S), 31.18 (CH_3), 125.02–143.83(Aromatic carbons and ipso carbons).

2.3.8 2-(2,4-bis(4-bromophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**16**)

White solid; yield 82%; mp 200-203 °C; IR (KBr, λmax cm^{-1}) 3259-3345 (N−H st), 2814-3190 (C−H st), 1656 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 1H, H-1e), 4.30 (s, 1H, H- 2a), 4.19 (s, 1H, H-4a), 2.83 (s, 1H, H-5e), 2,72 (s, 1H, H-7a), 1.79 (m, 3H, H-6e, H-8e, N−H), 1.45 (m, 3H, H-7e, H-8a, H-6a), 8.59 (s, 1H, ═N–NH), 7.64 (s, 1H, –C═S–NH), 3.27 (d, 3H, CH_3), 7.42−7.54 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 46.11 (C-1), 64.87 (C-2), 63.16 (C-4), 38.65 (C-5), 27.14 (C-6), 21.43 (C-7), 29.73 (C-8), 158.99 (C-9), 179.11 (C=S), 31.20 (CH_3), 121.36–140.77(Aromatic carbons and ipso carbons).

2.3.9 2-(2,4-bis(2-methoxyphenyl)-3-azabicyclo[3.3.1]nonan-9-ylidine)-N-methylhydrazine carbothioamide (**17**)

White solid; yield 77**%**; mp 191-194 °C; IR (KBr, λmax cm^{-1}) 3159-3328 (N−H st), 2834-3041 (C−H st), 1611 (C═N st); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (s, 1H, H-1e), 3.76 (s, 1H, H- 2a), 3.65 (s, 1H, H-4a), 2.68 (dd, 1H, H-5e), 2,34 (dd, 1H, H-7a), 1.16 (m, 3H, H-6e, H-8e, N−H), 1.01 (m, 3H, H-7e, H-8a, H-6a), 8.49 (s, 1H, ═N–NH), 7.50 (m, 1H, –C═S–NH), 3.25 (d, 3H, CH_3), 3.81 (d, 6H, *o*-OCH_3), 6.83−7.41 (m, 8H aromatic hydrogens); ^{13}C (100 MHz, $CDCl_3$) δ 55.36 (C-1), 69.93 (C-2), 60.67 (C-4), 43.34 (C-5), 22.61 (C-6), 21.11 (C-7), 31.25 (C-8), 159.13 (C-9), 179.34 (C=S), 32.47 (CH_3), 55.27, 55.36 (OCH_3 at *o*), 113.11–157.87 (Aromatic carbons and ipso carbons).

2.4. Biological Screening

2.4.1 Free Radical Scavenging Assays

Free radical scavenging activity of compounds was measured by DPPH using the reported method [22]. The synthesized molecules (**10-17**) could quench $DPPH^{\bullet}$ free radicals (i.e., by providing hydrogen atoms or by electron donation, plausibly via a free-radical attack on the $DPPH^{\bullet}$ molecule) and convert them to a colourless/bleached product (i.e., 2,2-diphenyl-1-picryl-hydrazine, or a substituted analogous hydrazine), resulted in a decrease of absorbance at 517 nm. The total antioxidant potential was measured by 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) radical scavenging activity. Moreover this method measured the relative ability of antioxidants to scavenge, the $ABTS^{+\bullet}$ cation radical generated in the aqueous phase [23]. ABTS+$^{\bullet}$ radical scavenging assay is a facile and elegant method to exploit antioxidant activity of the array of newly synthesized compounds. The technique was based on direct production of the blue/green $ABTS^{+\bullet}$ chromophore through the reaction between $ABTS^{+\bullet}$ and potassium persulfate were determined spectrophotometrically at 734 nm. H_2O_2 radical scavenging was estimated based on the standard method [24]. A 40 mM solution of H_2O_2 was prepared in a phosphate buffer (pH 7.4). The concentration of H_2O_2 was determined spectrophotometrically at 230 nm. Different concentrations (1000, 500 and 250 µg/ml) of residue oil were added to 0.6 ml of H_2O_2 (40 mM) and absorbance of the solution was read at 230 nm after 10 min against a blank containing residue oil in phosphate buffer without H_2O_2.

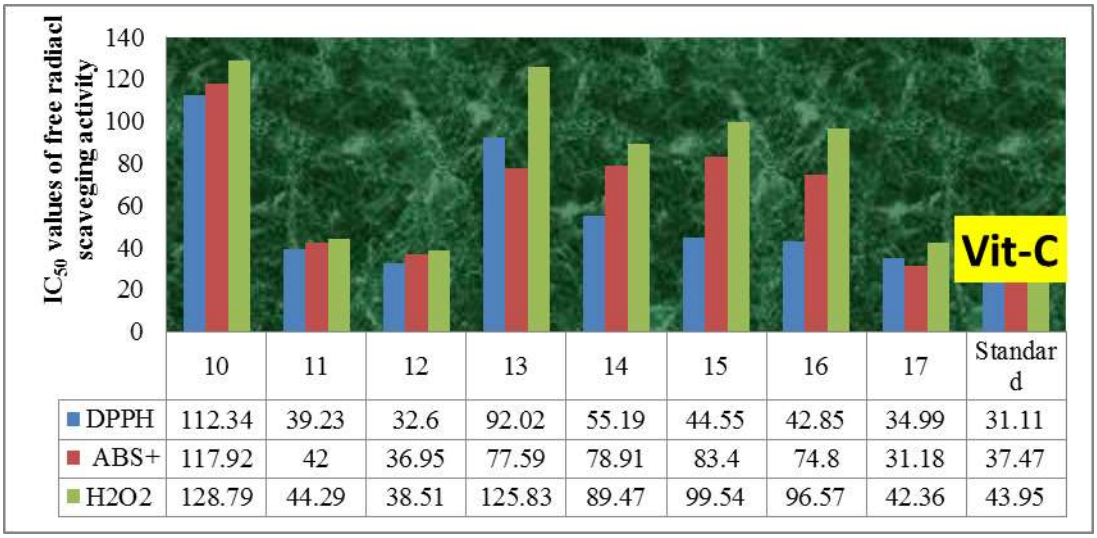
The percentage scavenging activity was calculated using the formula:

$$\% \text{ Radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.4.2 Mosquito Larvicidal Assay

The eggs of *A. aegypti* were received from the Field Station, Centre for Research in Medical Entomology, Indian council of medical research (ICMR)-Government of India, Virudhachalam. The larvae were fed with Brewer's yeast/dog biscuit (1:3). The larvicidal effect of the synthesized compounds against *A. aegypti* was studied with the standard procedures recommended by the World Health Organization (1981) [25]. The test compound was dissolved in 1ml of acetone and prepared into different concentrations *viz*. 12.5, 25, 50 and 100 ppm with distilled water. Twenty larvae (in a 100 ml beaker) of early fourth instars stage were used for Larvicidal assay and five replicates were maintained for each concentration. During this experiment, no food was offered to the larvae. The larval mortality was calculated after 12 and 24 hr of the exposure period. The lethal concentrations, LC_{50} and their 95 %

confidence limit of upper and lower confidence levels were calculated by probit analysis (SPSS, Version 11.0).



	10	11	12	13	14	15	16	17	Standard
DPPH	112.34	39.23	32.6	92.02	55.19	44.55	42.85	34.99	31.11
ABS+	117.92	42	36.95	77.59	78.91	83.4	74.8	31.18	37.47
H2O2	128.79	44.29	38.51	125.83	89.47	99.54	96.57	42.36	43.95

Figure 1. Antioxidant activity IC_{50} values of compounds **10-17.**

3. RESULTS AND DISCUSSION

3.1 Synthesis

The synthetic route for the 2,4-Diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazinecarbothioamide derivatives (**10-17**) was outlined in **Scheme 1**. The reported literature procedures [21] were used to synthesize the parent 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one (**1-8**). To the mixture of compound (**1-8**) and 4-methyl-3-thiosemicarbazide in molar ratio of 1:1, 1-2 drops glacial acetic acid in acetonitrile (5 mL) was taken in round bottom flask placed in a microwave oven and irradiated (220 W, 80 °C) for 10-12 min, the reaction progress have been monitored by TLC using pet ether and ethylacetate 3:1 as a solvent. The reaction mixture was cooled and the formed precipitate was washed first with ethanol and then by ether to get pure compound. The products were obtained in good yield (77%–90%). The synthesized compounds **10-17** gave satisfactory analytical data and are given in (**Table 1**).

3.2. Analytical Results

In the ^{1}H NMR spectrum of thiosemicarbazones two signals are observed due to NH−CS−NH and the corresponding protons (═N−NH−C═S−NH) are expected to appear in the most downfield region. However, in all thiosemicarbazones (**10-17**), the ═N−NH− proton signal is appeared at 8.63 ppm besides −C═S−NH proton doublet is appeared at 7.67 ppm. Methylene protons (H-6, H-7 and H-8) signals, H-7a proton resonated in the deshielded region than its corresponding equatorial and other methylene protons. The C-7 protons are magnetically non-equivalent and observed distinctly at 2.84 and 1.49 ppm, respectively. Moreover H-7e proton is observed at 1.49 ppm which is merged with H-6a and H-8a protons. Benzylic protons H-2a and H-4a are observed at 4.48 and 4.34 ppm respectively. In accumulation signals observed at 2.45 and 2.83 ppm should be due to bridgehead protons H-1e and H-5e respectively. While the methyl group of the thiosemicarbazide moiety was appeared at 3.28 ppm.

In ^{13}C NMR spectrum of thiosemicarbazones (**10**–**17**), the C═S and C═N carbon signals are appeared around 179.02 and 160.24 ppm respectively. The carbon resonances at 65.49 and 63.80 ppm are due to C-2 and C-4 carbons whereas the bridgehead carbons C-1 and C-5 were appeared around 46 and 39 ppm respectively. Further, the signal for methyl group of thiosemicarbazide moiety was observed at 31.18 ppm. This was confirmed from its HSQC spectrum. The ^{1}H and ^{13} C NMR spectra of the remaining compounds (**11-17**) are found to be like that of compound **10**.

3.3 In vitro antioxidant activity

The free radical scavenging activity of the synthesized compounds is determined by the $DPPH^{\bullet}$, $ABTS^{+\bullet}$ and $H_2O_2^{\bullet}$ radical scavengers. $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ are the main assays used for the evaluation of the ability of compounds to donate electrons or hydrogen atoms, respectively [26]. The results of antioxidant assays are carried out in triplicate for 3 separate experiments. Initially, 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazine carbothioamides compound **10** exhibited considerable activity. The bar graph representation of percentage of free radical scavenging activity is shows in **Fig 1**. The test results revealed that IC_{50} values of compounds **12(32.6, 36.95)** and **17(34.99, 31.18)** showed positive free radical scavenging $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ free radical compared to standard positive control ascorbic acid. Besides $H_2O_2^{\bullet}$ assays the abilities of the array of compounds **12** IC_{50} **38.5** and **17** IC_{50} **42.3** positive scavenge free radicals activity was further confirmed. Compounds **12** and **17** which are having electron donating substituent in the phenyl rings showed admirable radical scavenging activity. The findings results shows that the introduction of electron donating group in the different position of phenyl rings exhibited good scavenging ability compare with electron withdrawing substituent. The next lead antioxidants activity was showed by compounds **11.** The incorporation of $–CH_3$ groups on the phenyl ring at C-2 -C-4 position in the compounds **11** exhibited slightly lesser than compounds **12** and **17** antioxidant scavenging activity. Moreover, the electro donating substituents on a phenyl ring demonstrate a great influence on scavenging activity in a way that *p*-Methoxy substitution on the phenyl ring increases and *p*-Methyl substitution decreases towards entire radical scavenging activity of N-methylhydrazinecarbothioamide with a phenyl ring. Substitution on the phenyl ring such as *p* –Fluoro, *p* –Chloro, *p* –Bromo and *m* –Chloro are found to be enhancement antioxidant activity against $DPPH^{\bullet}$, $ABTS^{+\bullet}$ and $H_2O_2^{\bullet}$ scavenging radical activity [27]. When compared antioxidant assays performed in this work, compounds **12** and **17** exhibited superior antioxidant activity due to electron-donating group substitutents at C-2 and C-4 position of the phenyl rings, the presence of C=S function [28] and the presence of a labile hydrogen (–NH–N=) obtained from N-methylhydrazinecarbothioamide moiety.

3.4 Antimicrobial assay

MIC tests were carried out according to the method of Andrews [29] (Procedure method are provided in supporting information) using a microtitre plate (96 wells). The test compounds dissolved 1 mL chloroform were tested in the different concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.12 µg/Ml (2-fold serial dilution) with SDB and MHB. Compounds (**10-17**) were evaluated for their antibacterial activity screened against Gram positive strains *S. Aureus*, *B. Subtilis* and *K. pneumoniae* and Gram negative strains *E. Coli*, *P. Aeruginosa* by comparing their minimum inhibitory concentration values with Ciprofloxacin standard drug. All the MIC values obtained for compounds (**10-17**) are given in **Table 2.**

Table-2. *In vitro* antibacterial activity of compounds for compounds **10-17**

Compounds	Minimum inhibitory concentration (MIC) in µg/mL				
	S.Areus	*B.subtilis*	*P.aeruginosa*	*E coli*	*K.pneumoniae*
10	12.5	12.5	25	50	25
11	12.5	12.5	25	50	25
12	12.5	12.5	25	50	25
13	3.25	3.25	6.25	6.25	6.25
14	3.25	3.25	6.25	6.25	6.25
15	6.25	6.25	25	25	25
16	6.25	6.25	12.5	25	25
17	6.25	6.25	12.5	25	25
Ciprofloxacin	6.25	6.25	6.25	6.25	6.25

Table-3. *In vitro* antifungal activity of compounds for compounds **10-17**

Compounds	Minimum inhibitory concentration (MIC) in µg/mL				
	C.albicans	*C.tropicalis*	*C.galabarata*	*A.niger*	*A.flavus*
10	25	100	50	12.5	12.5
11	25	50	50	25	25
12	25	50	50	25	25
13	6.25	12.5	25	6.25	6.25
14	6.25	12.5	25	6.25	6.25
15	12.5	25	25	12.5	12.5
16	12.5	12.5	15	12.5	12.5
17	25	50	50	25	25
Ketaconazole	12.5	12.5	25	12.5	25

It is more attractive to speculate the observation that the result of the antimicrobial activities varied with the nature of substituents on the phenyl ring at C-2 and C-4 positions. The unsubstituent aryl rings in compound **10** showed good activity againt *S. Aureus, B. subtilis* at a MIC value of 12.5 µg/mL and *P. Aeruginosa K. pneumonia* at a MIC value of 25 µg/mL. However their *para* halo substituents compounds **13** and **14** possessing (electron-withdrawing groups fluoro and chloro) in the aryl rings in 3-ABN moiety account for the superior inhibitory effects against *B. subtilis* and *S. aureus* at MIC values of 3.25 µg/mL and against *E. Coli, P. Aeruginosa* and *K. pneumonia* at a MIC value of 6.25 µg/mL when compared to the standard antibiotic Ciprofloxacin. Compounds **15** and **16** possessing *m*-Cl and *p*-Br

substituents in the phenyl moiety displays moderate antibacterial activity against *B. subtilis* and *S. Aureus* (MIC = 6.25 μg/mL) expect *E. coli* (MIC = 12.5-25 μg/mL), *K. Pneumonia* and *P.aeruginosa* (MIC = 25μg/mL). Introduction of methoxy and methyl groups in compounds **11**, **12** and **17** were inadequate to show moderate antibacterial activity.The synthesized compounds **13**, **14**, **15** and **16** exhibited significant antibacterial activity against *B. Subtilis* and *S. Aureus* whereas the other strains shows moderate activity against *P. Aeruginosa*, *K. pneumonia* and *E. coli* than the standard Ciprofloxacin.

The antifungal screening revealed that tested compounds showed good inhibition against various tested fungal strains. The present study also afforded evidence for the antifungal effects of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one-N-methylhydrazinecarbothioamide derivatives (**10-17)** with MIC values ranging from 6.25 to 100 μg/mL. Compound **10**, which lacks any substituents at the aryl groups showed good antifungal activity against *A. flavus* (12.5 μg/mL), equivalent activity against *A. niger* (12.5 μg/mL) and moderate activity against *C. albicans* (25 μg/mL) and *C. tropicalis* (100 μg/mL) when compared to Ketoconazole, a known antifungal agent used as positive control. Among the tested compounds **13** and **14** has superior activity against C. *albicans*, *A. niger* and *A. Flavus* were compared with standard Ketoconazole. Compound **16** exerted 2-fold increase in antifungal activity against *A. flavus*. Besides, compounds **15** and **16** showed excellent antifungal activity against all the tested pathogens at MIC values of 12.5−25 μg/mL whereas the moderate antifungal activity has been observed for compounds **13**, **14** and **16** which may be due to the fluoro, chloro and bromo substituents at the *para* position of aryl groups. Introduction of methoxy and methyl groups at the *para* position of compounds **11**, **12** and **17** hinder the growth of all fungal strains at MIC values ranging from 25 to 50 μg/mL. Results of antifungal studies have been presented in **Table 3**.

The results suggest that the antibacterial and antifungal activities are markedly influenced by substituent in phenyl ring [18,19]. Compounds **13** and **14** exerted superior inhibitory effect against various tested microbes. The antifungal and antibacterial activities of compounds (**10−17)** generate promising leads for the development of potent antimicrobial agents.

3.5 Mosquito Larvicidal Assay

The larvicidal activities of *N*-methylhydrazinecarbothioamide derivatives (**10-17**) at different concentrations (12.5, 25, 50 and 100 ppm) were evaluated against the early 4th instar larvae of A. aegypti and the results are given in **Table 4**.

The number of conjugated double bonds appears to contribute to an increase in potency, since aromatic compounds such as thymol, carvacrol and eugenol exhibit higher larvicidal potencies than carvone [30]. The LC_{50} values of compounds **11**, **12** and **17** are 43.58, 45.43 and 45.28 respectively at 12 hr whereas the LC_{50} values of compounds **11**, **12** and **17** are 31.71, 37.77 and 37.14 respectively at 24 hr. Our findings revealed that the compound **11, 12** and **17** cent per cent mortality at ~100 ppm against A. aegypti. The LC_{50} values at 24 hrs of compound **11** were 43.58 besides 12 hr 31.71 ppm respectively against A. aegypti. At the lowest concentration (12.5

ppm) studied, the compound produced 17 ± 0.40% against A. aegypti at 12 hr while it produced 21± 0.28% ppm at 24 hr and the results were significant at $p < 0.05$.

Table 4. Mosquito Larvicidal Assay for compounds **10-17**

Compounds	Concentration (ppm)	Time interval		LC_{50}		$x^2(d=4)^b$	
		12 (hrs)	24(hrs)	12(hrs)	24(hrs)	12(hrs)	24(hrs)
	12.5	9±0.76	13±0.40				
10	25	15±0.50	25±0.50	80.90	69.50	0.84	7.20
	50	28±0.28	48±0.57				
	100	64±0.37	81±0.87				
	12.5	17±0.40	21±0.28				
11	25	33±0.28	43±0.31	43.58	31.71	0.718	1.31
	50	57±0.31	74±1.15				
	100	94±0.87	100±0.0				
	12.5	16±0.28	20±0.50				
12	25	30±0.50	38±1.12	45.43	37.77	1.04	1.17
	50	58±0.57	62±0.45				
	100	92±0.76	98±0.45				
	12.5	11±0.50	15±0.16				
13	25	20±0.37	28±0.50	68.88	54.86	0.86	3.00
	50	38±0.57	52±1.12				
	100	72±1.15	80±0.50				
	12.5	12±0.57	17±0.28				
14	25	23±0.34	28±0.50	69.88	55.93	2.31	4.47
	50	41±0.73	54±0.57				
	100	68±0.81	76±1.15				
	12.5	8±0.40	12±0.57				
15	25	15±0.87	21±0.45	85.55	64.76	1.57	0.52
	50	31±0.50	40±0.25				
	100	58±1.12	76±0.28				
	12.5	10±0.50	14±0.28				
16	25	21±0.25	26±0.45	75.43	59.11	2.83	6.44
	50	38±0.76	53±0.76				
	100	64±0.45	74±0.50				
	12.5	19±0.50	20±0.57				
17	25	36±0.40	37±0.76	45.28	37.14	1.17	3.44
	50	54±0.28	62 ±0.57				
	100	92 ±0.50	98±0.76				

Each value represents mean ± SD

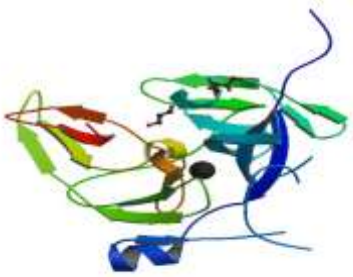


Figure 2. Crystal Structure of dengue virus NS2B/NS3 protease (2FOM)

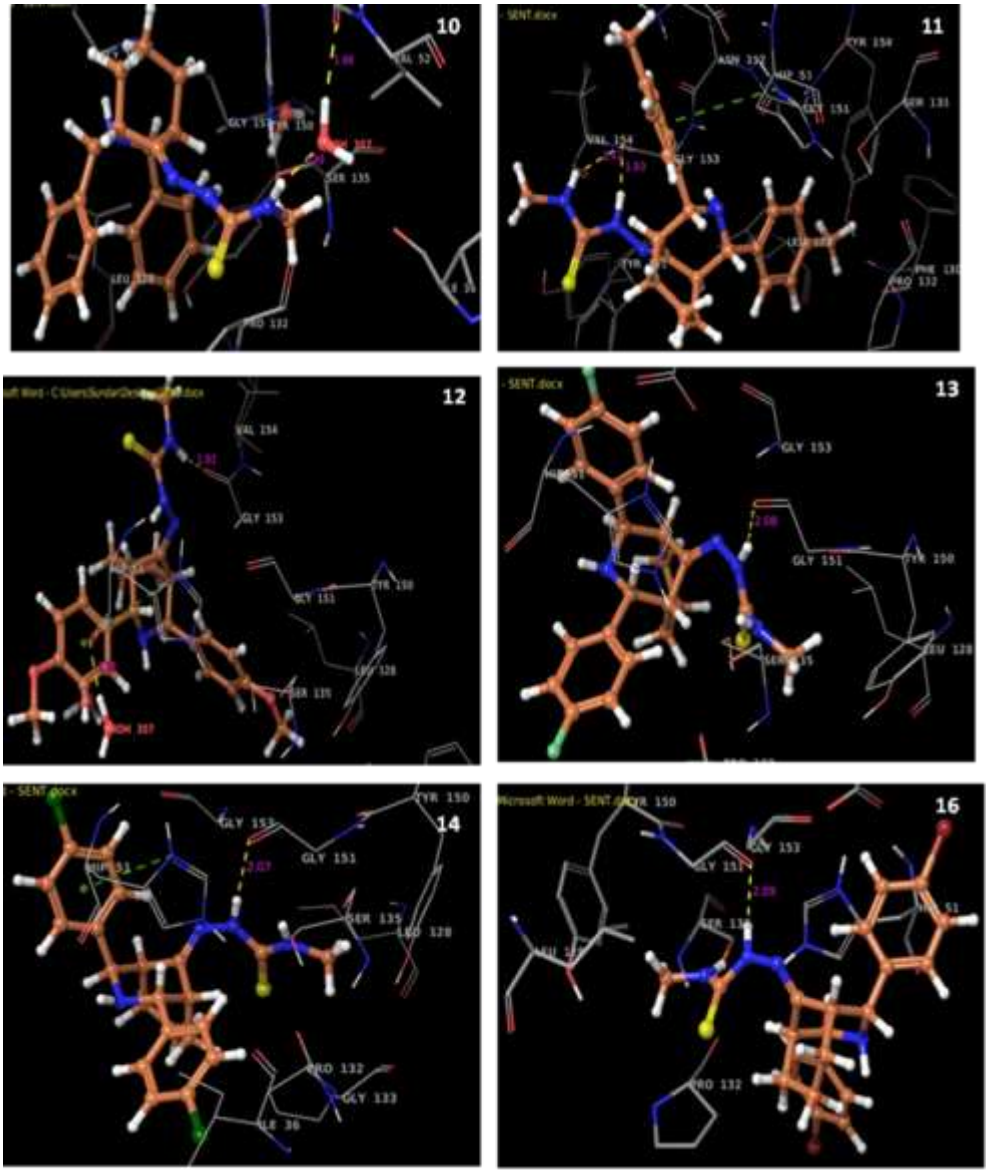


Figure 3. 3D model of the interaction between compound 10,11,12,13,14 and 16 into the active site of the NS2B/NS3 enzyme. For clarity, only the interacting residues are displayed. Hydrogen bonds are presented as yellow dotted lines, while the π- π* interactions are depicted in dotted green lines.

Table 5. Docking Results Of selected synthesized compounds

Compounds	H-interaction	Binding energy (kcal/mol)	Bond distance
10	——	-3.766	1.98 Å
11	GLY 153- NH of N-methylhydrazine carbothioamide group; HIP 51- Phenyl ring	−3.905	2.42 Å, 1.93 Å
12	GLY 153- NH of C=S–NH N-methylhydrazine carbothioamide group; HIP 51- Phenyl ring	−3.437	1.93 Å, 1.98 Å
13	GLY 151- NH of C=N–NH N-methylhydrazine carbothioamide group;	−3.377	2.08 Å,
14	GLY 151- NH of C=N–NH N-methylhydrazine carbothioamide group; HIP 51- Phenyl ring	−3.337	2.07 Å
15	——	——	——
16	GLY 151- NH of C=N–NH N-methylhydrazine carbothioamide group;	−3.675	2.09 Å
17	——	——	——

These results suggest that a compound having a conjugated double bonded system with the electron donating group substituents at C-2 and C-4 position of the phenyl rings has more larvicidal activity than the electron withdrawing substituted phenyl rings. Successive evaluation of the larvicidal activitiy of the synthesized compounds revealed that the compounds **12** and **17** exhibited good larvicidal activity against A. Aegypti. Compound **11** exhibited superior activity against A. Aegypti. The larvicidal study also inferred that more efficient larvicidal activity was achieved when electron donating groups substituted in the phenyl rings.

3.6 Molecular Docking

Docking of selected ligands, having anti-dengue activity with the active-site of protein 2FOM Fig 2 was complete to find the biochemical information. The synthesized compounds **10, 11, 12, 13, 14** and **16** were subsequently docked flexibly into the binding sites of the DENs NS2B/NS3 of using Maestro 9.3.5 software package to understand the binding interactions. The docking calculation and hydrogen bond interactions data obtained are presented in Table 5. The

interaction energy of the synthesized compounds and their larvicidal activity showed the corresponding results.

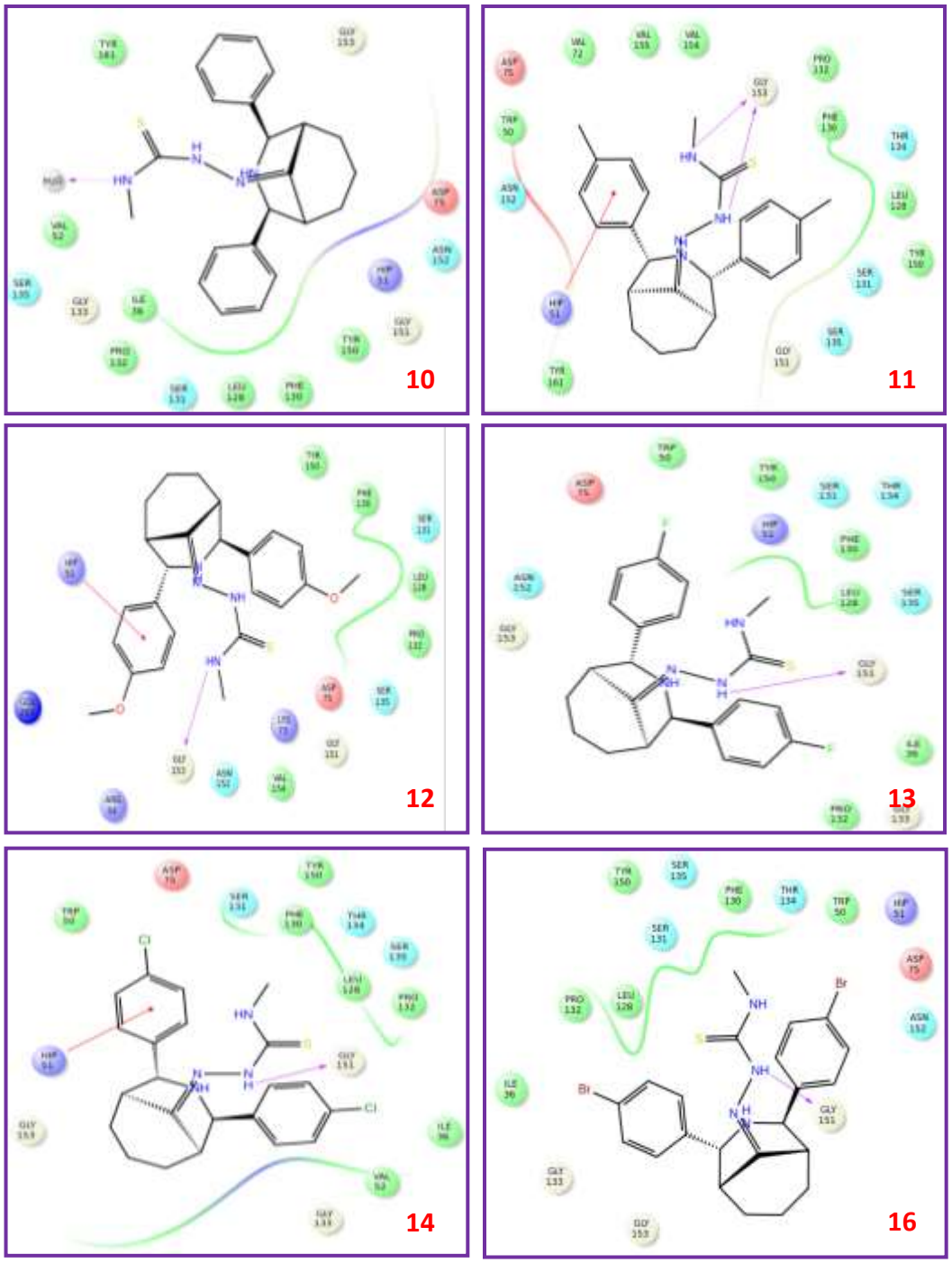


Figure 4. Interactions in ligand interaction viewer

The active compounds **11** showed lowest binding interaction energy, i.e −3.905 kcal/mol, respectively. The docking study also exposed that only N-methylhydrazinecarbothioamide group had formed various hydrogen bonding interactions with enzyme suggesting that N-methylhydrazinecarbothioamide group is more important for inhibiting the NS2B/NS3 when in combination with phenyl ring. The –NH-C=S and NH group of N-methylhydrazinecarbothioamide had formed the various hydrogen bonds with amino acids residue like GLY 153, HIP 51, and GLY 151. Thus, suggesting that –NH-C=S and NH group is important for larvicidal activity. The interactions of compound **11** and NS2B/NS3 are shown in **Fig.3**. In case of compound **11**, amino acids GLY 153, GLY 151 and HIP 51 had formed hydrogen bonds with –NH-C=S and NH and phenyl ring, respectively. On the basis of activity data and docking results, it was found that the compound **11** had potential to inhibit NS2B/NS3. Display of residual interactions and hydrogen bonding contacts between the ligand and the enzyme is shown in **Fig. 4**.

4. CONCLUSION

In conclusion, we have synthesized a series of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazinecarbothioamides (**10-17**) by a rapid, efficient, solvent-free, procedure with excellent yields under microwave irradiation. Antioxidant studies results revealed that the presence of electron donating groups at the C-2 and C-4 positions of phenyl rings, C=S and –NH–N=) function surprisingly enhanced their antioxidant activities. From the close assessment of the in vitro antimicrobial results revealed that the compounds with fluorine and chlorine substituents were found to be excellent in exhibiting antibacterial and antifungal activities against all the tested organisms. This is due to F and Cl compound is able interact with the microorganisms more effectively due to some intermolecular hydrogen bonding. The compound **11, 12** and **17** most potent *in vivo* larvicidal agents against Aedes aegypti among those synthesized compounds. Further, the docking studies of synthesized compounds with NS2B/NS3 modeled protein showed good binding interactions and formed various hydrophobic interactions with active site residues. As a result, suggesting that the compounds **11** most potent larvicidal activity. Toward our best knowledge, this is the first report about the syntheses and larvicidal activity of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-ylidine)-*N*-methylhydrazine carbothioamide derivatives.

It is evident that the presence of a phenyl rings and its substitution has a great impact on the activity. We expected that the present design strategy and the remarkable biological properties of this synthesized compound would help to extend its applications in pharmaceutical field. Promote studies will be needed on formulations against mosquitoes and their efficacy cost effectiveness.

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SUPPORTING INFORMATION

Spectral data (^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H-COSY, HSQC and HMBC) for the compound **10** and (^{1}H NMR, ^{13}C NMR) NMR data of compounds **11-17** can be found via the Supplementary Content section.

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The authors declare no conflict of interest

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