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Antimicrobial Evaluation of a Set of Heterobicyclic Methylthiadiazole Hydrazones: Synthesis, Characterization, and SAR Studies

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Supporting Information

ABSTRACT: To exploit the potential antimicrobial activities of azabicyclic skeleton based compounds, a set of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one-4-methyl-1,2,3-thiadazole-5-carbonyl hydrazones were synthesized. Unambiguous structural elucidation has been carried out by investigating IR, H^1, C^{13} NMR, and elemental analysis. 2D NMR spectra ($^1H-^1H$ COSY, HSQC, HMBC, and NOESY) were recorded for a representative compound, **12**, to confirm the proposed structure for **9**−**15**. Antimicrobial activity assessment of synthesized hydrazones **9**−**15** has been evaluated by screening against selective strains. Both bacteria and fungi of various forms along with standard drug have been taken for the analysis. Difference in the potency of activity against the strains has been evaluated on the basis of SAR, and it has been revealed that substitution of electron-withdrawing halogens (chloro, fluoro, and bromo) at para positions of the phenyl (**10**, **12**, and **13**) enhanced the antifungal and antibacterial activities against tested organisms compared to other hydrazone derivatives.

KEYWORDS: *antibacterial, antifungal, hydrazone derivatives, hydrazides*

INTRODUCTION

3-Azabicyclonones (3-ABN) have attracted attention owing to their extensive scale of microbial potencies. Antifungal and antibacterial studies proved that the 3-ABN skeleton seems to be an effective pathogen killer.[1,2] Further reports revealed 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 are measured.[8] A wide variety of naturally abundant diterpenoid/norditerpinoid alkaloids have been found to possess various pharmacological actions due to the presence of the 3-azabicyclonone pharmocopore. Of late, hydrazide−hydrazone derivatives have received the attention of various medicinal chemists as a result of their effectual biological potencies, namely, antimicrobial, antitubercular, and also anticonvulsant actions.[9−11] Research in recent decades has provided evidence that compounds with the thiadiazole ring possess an essential structure with broad-spectrum biological activity. Hence, these compounds are of great interest for medicinal chemists and have been investigated for their insecticidal,[12] herbicidal,[13] antimicrobial,[14] antimycobacterial,[15] antimicotic,[16] antiviral,[17] antimalarial,[18] antidepressive,[19] anticonvulsant,[20] cardiotonic,[21] anti-inflammatory,[22] and antileukemic and anticancer activities.[23] The above observations provide impetus to synthesize a system that combines 3-azabicyclonone and thiadiazole moieties together to produce the corresponding hydrazones **9**−**15** with the anticipation of creating several promising antimicrobial agents.

EXPERIMENTAL METHODS

Chemicals and Instrumentation Techniques for Structural Characterization. 4-Methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide was purchased from Sigma-Aldrich. Other chemicals used for the synthesis of azabicyclic ketones were of analytical grade. Solvents were used after distillation. Reaction progress and purity were monitored by TLC. Melting points were determined in an Electrothermal 9100 instrument in open capillaries and are uncorrected. FT-IR analysis has been done in an AVATAR 330 FT-IR Thermo Nicolet spectrometer by making pellet of compound with KBr. Both one- and two-dimensional NMR spectra were recorded in a Bruker AMX 400 NMR spectrometer. Sample was prepared in a 5 mm diameter tube using DMSO-d_6 solvent (10 mg in 0.5 mL). ^1H NMR were data collected at 400.13 MHz operating frequency, and ^{13}C NMR was at 100.62 MHz. Chemical shifts (δ) are expressed in parts per million with respect to TMS. CHN analysis was provided from Heraeus Carlo Erba 1108.

Synthetic Procedure. *Typical Procedure for Synthesis of 2,4-Diaryl-3-azabicyclo[3.3.1]nonan-9-ones (**1**−**7**).* An often used typical procedure reported by Baliah and Jeyaraman[24] was adopted for the synthesis of 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones with convenient modification. A reaction mixture of cyclohexanone (1 equiv), respective aryl aldehyde (2 equiv), and ammonium acetate (1 equiv) dissolved in ethanol was kept in the water bath by maintaining the bath temperature at 60−75 °C with continuous stirring. The crude product thrown out from the solution was filtered off. It was washed through etheric ethanol solution. An ethanol/chloroform/acetone mixture was used for recrystallization of **1**−**7**.

*General Procedure for the Synthesis of 2r,4c-Diaryl-3-azabicyclo[3.3.1]nonan-9-one-4-methyl-1,2,3-thiadazole-5-carbonyl Hydrazones (**9**−**15**).* A mixture of 1 mmol of 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones (1 equiv) and 1.5 mmol of 4-methyl-

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1,2,3-thiadiazole-5-carboxylic acid hydrazide (1.5 equiv) was dissolved in the solvent mixture of chloroform and methanol (1:1 v/v). Acetic acid (1−2 mL) was added as catalyst. The reaction mixture was refluxed for about 3−4 h. After the completion of reaction, the solvent mixture was removed under vacuum. The separated solid was subjected to cold water/ethanol mixture washing. Compounds were recrystallized from ethanol.

Data for ***9:*** white solid; yield 58%; mp 211 °C; IR (KBr, ν_{max} cm^{-1}) 3330 (N−H st), 3058, 2920, 2844 (C−H st), 1624 (C═N st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.82 (m, 1H, H-1e), 4.47 (s, 1H, H-2a), 2.00 (s, 1H, N−H), 4.34 (s, 1H, H-4a), 3.29 (s, 1H, H-5e), 1.49 (m, 2H, H-6e, H-6a), 3.01 (m, 1H, H-7a), 1.27 (s, 1H, H-7e), 1.61 (m, 2H, H-8a, H-8e), 10.33 (s, 1H, amide NH), 2.92 (s, 3H, CH_3 at thiadiazole), 7.37−7.65 (m, 10H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 45.90 (C-1), 65.70 (C-2), 64.10 (C-4), 39.5 (C-5), 27.29 (C-6), 21.40 (C-7), 28.67 (C-8), 164.29 (C-9), 162.37 (NHCO), 15.3 (CH_3 at thiadiazole ring), 164.17 (C-4″), 135.31 (C-5″), 126.92−141.68 (aromatic carbons and ipso carbons).

Data for ***10:*** white solid; yield 54%; mp 204 °C; IR (KBr, ν_{max} cm^{-1}) 3054, 2926, 2850(C−H st), 1630, 1602 (C═C st), 3332 (N−H st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.69 (d, 1H, H-1e), 4.32 (s, 1H, H-2a), 1.91 (s, 1H, N−H), 4.23 (s, 1H, H-4a), 2.96 (s, 1H, H-5e), 1.51 (m, 1H, H-6a), 2.73 (m, 1H, H-7a), 1.25 (m, 1H, H-7e), 1.38 (m, 2H, H-8a, H-6e), 1.67 (m, 1H, H-8e), 10.78 (s, 1H, amide NH), 2.99 (s, 3H, CH_3 at thiadiazole), 7.31−7.70 (m, 8H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 45.00 (C-1), 64.53 (C-2), 62.35 (C-4), 39.43 (C-5), 27.99 (C-6), 20.63 (C-7), 26.83 (C-8), 164.02 (C-9), 162.90 (NHCO), 15.18 (CH_3 at thiadiazole ring), 161.24 (C-4″), 135.84 (C-5″), 126.83−142.48 (aromatic carbons and ipso carbons).

Data for ***11:*** white solid; yield 52%; mp 200 °C; IR (KBr, ν_{max} cm^{-1}) 3335 (N−H st), 3032 and 2925 (C−H st), 1631 (C═N st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.77 (d, 1H, H-1e), 4.38 (s, 1H, H-2a), 1.62 (s, 1H, N−H), 4.24 (s, 1H, H-4a), 3.26 (s, 1H, H-5e), 1.62 (m, 1H, H-6a), 1.80 (s, 1H, H-6e), 2.89 (m, 1H, H-7a), 1.85 (s, 1H, H-7e), 1.97 (m, 2H, H-8a, H-8e), 10.62 (s, 1H, amide NH), 2.77 (s, 3H, CH_3 at thiadiazole ring), 3.63 (s, 6H, methoxy hydrogen at aromatic ring), 7.20−7.76 (m, 8H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 46.00 (C-1), 65.01 (C-2), 63.60 (C-4), 39.80 (C-5), 27.40 (C-6), 21.52(C-7), 28.50 (C-8), 164.94 (C-9), 162.71 (NHCO), 15.40 (CH_3 at thiadiazole ring), 55.30 (OCH_3), 164.26 (C-4″), 135.35 (C-5″), 113.94−136.59 (aromatic carbons and ipso carbons).

Data for ***12:*** white solid; yield 67%; mp 204 °C; IR (KBr, ν_{max} cm^{-1}) 3030, 2944 and 2850 (C−H st), 1634 (C═N st), 1607 (C═C ring st), 3334 (N−H st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (d, 1H, H-1e), 4.30 (s, 1H, H-2a), 1.61 (s, 1H, N−H), 4.21 (s, H, H-4a), 3.08 (s, 1H, H-5e), 1.49 (m, 2H, H-6a, H-6e), 1.40 (m, 1H, H-7e), 2.67 (m, 1H, H-7a), 1.61 (m, 1H, H-8a), 1.63 (m, 1H, H-8e), 11.78 (s, 1H, amide NH), 3.09 (s, 3H, CH_3 at thiadiazole), 7.23−7.73 (m, 8H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 44.83 (C-1), 63.78 (C-2), 61.59 (C-4), 39.19 (C-5), 28.09 (C-6), 20.59 (C-7), 30.65 (C-8), 163.53 (C-9), 162.87 (NHCO), 15.18 (CH_3 at thiadiazole ring), 161.25 (C-4″), 135.85 (C-5″), 114.60−138.56 (aromatic carbons and ipso carbons).

Data for ***13:*** white solid; yield 61%; mp 201 °C; IR (KBr, ν_{max} cm^{-1}) 3339 (N−H st), 3054, 2920 and 2850 (C−H st), 1620 (C═N st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.52 (m, 1H, H-1e), 4.44 (d, 1H, H-2a), 1.86 (s, 1H, N−H), 4.24 (s, 1H, H-4a), 3.26 (s, 1H, H-5e), 1.80 (m, 2H, H-6a, H-6e), 1.12 (m, 1H, H-7e), 2.92 (m,1H, H-7a), 1.96 (m, 2H, H-8a, H-8e), 10.11 (s, 1H amide NH), 2.71 (s, 3H, CH_3 at thiadiazole ring), 6.88−7.76 (m, 8H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 46.00 (C-1), 65.29 (C-2), 63.67 (C-4), 40.01 (C-5), 26.70 (C-6), 20.81 (C-7), 28.50 (C-8), 164.90 (C-9), 164.20 (NHCO), 15.43 (CH_3 at thiadiazole ring), 162.71 (C-4″), 135.12 (C-5″), 128.02−136.59 (aromatic carbons and ipso carbons).

Data for ***14:*** white solid; yield 62%; mp 214 °C; IR (KBr, ν_{max} cm^{-1}) 3328 (N−H st), 3048, 2924 and 2845 (C−H st), 1624 (C═N st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.63 (d, 1H, H-1e), 4.15 (s, 1H, H-2a), 1.66 (s, 1H, N−H), 4.25 (d, 1H, H-4a), 2.98 (s, 1H, H-5e), 1.54 (m, 3H, H-6a, H-6e, H-8a), 2.84 (m, 1H, H-7a), 1.24 (m, 1H, H-7e), 1.62 (m, 1H, H-8e), 11.02 (s, 1H, amide NH), 2.32 (d, 3H, p-CH_3), 2.77 (s, 3H, CH_3 at thiadiazole ring), 7.12−8.24 (m, 8H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 45.80 (C-1), 64.20 (C-2), 62.00 (C-4), 40.20 (C-5), 28.00 (C-6), 20.70 (C-7), 28.00 (C-8), 164.20 (C-9), 162.20 (NHCO), 20.70 (CH_3 at phenyl ring), 15.20 (CH_3 at thiadiazole ring), 161.40 (C-4″), 135.94 (C-5″), 127.2−138.2 (aromatic carbons and ipso carbons).

Data for ***15:*** white solid; yield 55%; mp 196 °C; IR (KBr, ν_{max} cm^{-1}) 3342 (N−H st), 3050, 2920 and 2847 (C−H st), 1620 (C═N st); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.72 (m, 2H, H-1e, H-7a), 4.31 (d, 2H, H-2a, H-4a), 1.84 (s, 1H, N−H), 3.85 (s, 1H, H-5e), 1.44 (m, 1H, H-7e), 2.01 (m, 2H, H-6e, H-8e), 1.77 (m, 2H, H-6a,H-8a), 10.52 (s, 1H, amide NH), 2.78 (s, 1H, CH_3 at thiadiazole ring), 6.88−7.76 (m, 8H aromatic hydrogens); ^{13}C (400 MHz, DMSO-d_6) δ 46.00 (C-1), 63.60 (C-2), 61.19 (C-4), 39.70 (C-5), 27.40 (C-6), 21.50 (C-7), 28.50 (C-8), 164.26 (C-9), 162.71 (NHCO), 15.40 (CH_3 at thiadiazole ring), 164.11 (C-4″), 135.20 (C-5″), 128.02−136.59 (aromatic carbons and ipso carbons).

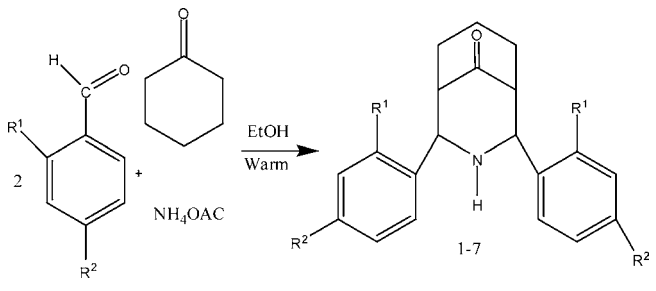
Biological Screening. Bacterial strains such as *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and fungal strains such as *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, and *Candida6* obtained from the Faculty of Medicine, Annamalai University, Annamalainagar, Tamil Nadu, India, were used to screen the antimicrobial activity of the newly synthesized compounds **9**−**15**. The bacterial and fungal strains were cultured in Sabourauds dextrose broth (SDB) at a pH 7.4 ± 0.2 (Hi-media, Mumbai, India) and nutrient broth (NB) (Hi-media) at pH 5.6, respectively.

In Vitro Antibacterial and Antifungal Activity by 2-Fold Serial Dilution Method. The in vitro potency of compounds **9**−**15** was examined by a 2-fold serial dilution method.[25] Stock solutions of **9**−**15** were made in DMSO (1 mg/mL). Compounds were tested in the concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.12 μg/mL (2-fold serial dilution) with SDB and NB. Then SDB and NB were suspended with 100 μL of bacterial spores from 24-h-old bacterial cultures on NB at 37 ± 1 °C and 100 μL fungal spores from 1−7-day-old SDB slant cultures at 28 ± 1 °C, respectively. Plating techniques were used to determine the colony-forming units (cfu) of the seeded broth in the adjusted range of 104−105 cfu/mL. Final inoculum sizes of 105 and (1.1−1.5) × 10^2 cfu/mL were used for antibacterial and antifungal assays, respectively. Microbial spore supplemented broth with DMSO at highest concentrations used in our experiments was used as the negative control. The growth of the microbes in the test medium was measured on the basis of the turbidity of the culture after 24 h of bacterial incubation and 72−96 h of fungal incubation. The lowest concentration of the test compound with the clear solution of test medium was considered as the minimum inhibitory concentration (MIC). Drug standards were streptomycin for antibacterial activity and fluconazole for fungal studies.

RESULTS AND DISCUSSION

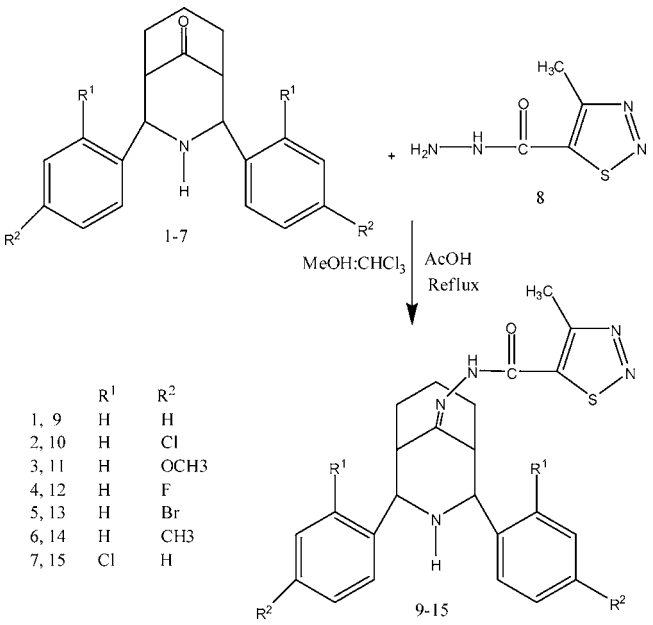
Chemistry. Synthesis of diversely substituted diaryl 3-azabicyclononanones (**1**−**7**)[24] and their methylthiadiazole hydrazones (**9**−**15**) was carried out according to the steps shown in Scheme 1. Compounds **9**−**15** were achieved by the

Scheme 1. Synthesis of 3-Azabicyclo[3.3.1]nonane and Hydrazones



reaction of the compounds **1**−**7** with 4-methyl-1,2,3-thiadiazole-5-carboxylic acid hydrazide (**8**) (Scheme 2). Acetic

Scheme 2



acid is preferred as suitable catalyst, and it was added in a small amount (∼1−2 mL). Reaction progress, completion, and purity were checked by TLC (chloroform/ethyl acetate/hexane mixture was used as eluent). Detailed investigations of IR, ^{1}H NMR, and C^{13} NMR spectral data with CHN analysis (Table 1) were made to identify and establish the newly synthesized compounds **9**−**15**. To substantiate the proposed structure, ^{1}H−^{1}H COSY, NOESY, HSQC, and HMBC were recorded for compound **12**. Figure 1 shows the numbering patterns of the compound. C-2′ and C-4′ are the ipso carbons of aryl groups at C-2 and C-4, respectively. Besides, the carbons of the thiadiazole part are designated using C-4″ and C-5″. Structural elucidation of **9** (aryl groups without substituent) has been described, and it was confirmed from the two-dimensional NMR reports of **12**.

Structural Elucidation of Compound 9. Hydrazone formation was supported by C═N stretching frequency at 1641 and 1646 cm^{-1} in the IR spectrum. We have observed the piperidine N−H stretching frequency around 3325−3375 cm^{-1}. Respective aliphatic and aryl C−H stretchings were found in the region of 3070−2800 cm^{-1}.

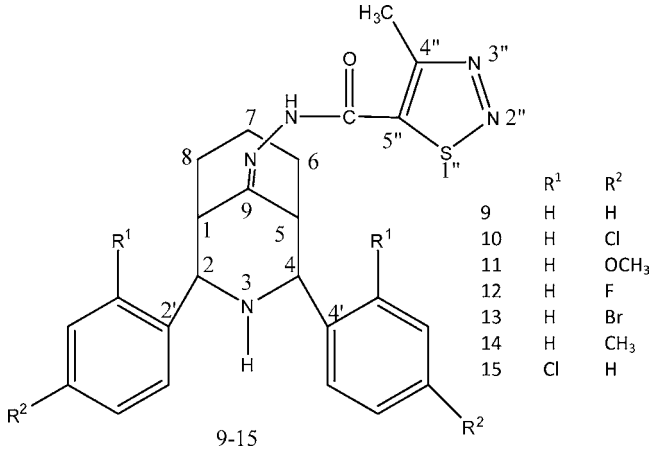


Figure 1. Numbering of the azabicyclo[3.3.1]nonane.

In ^{1}H NMR, characteristic hydrazide NH proton (═N−NH—CO−) is expected in the most downfield region. A broad peak resonating at 10.33 ppm was assigned for NH proton of the hydrazone part. Signal broadening is due to the faster exchange of NH proton with solvent moisture than the resonance time scale. The imine NH proton of the bicyclic ring was observed at 1.96 ppm. Methylene protons (both H_{ax} and H_{eq}) of C-6 and C-8 resonated as multiplets at 1.49 and 1.61 ppm, respectively. In contrast, C-7 protons are magnetically nonequivalent and observed distinctly at 2.94 and 1.27 ppm, respectively, for H-7a and H-7e. Benzylic protons H-2 and H-4 were observed at 4.48 and 4.34 ppm, respectively. In addition, signals appearing at 3.28 and 2.94 ppm were designated correspondingly for H-5 and H-1 bridgehead protons. The methyl substituent of the thiadiazole ring was observed at 2.92 ppm. Protons resonating with closer chemical shift values were distinct by two-dimensional NMR correlations.

In ^{13}C NMR of compound **9**, two downfield resonances at 164.29 and 162.37 ppm were consigned for C═N (C-9) and C═O (═N—NH—CO−) carbons, respectively. The carbon resonances at 65.70 and 64.10 ppm are respectively due to C-2 and C-4 carbons. Furthermore, the carbons involved in the fusion of bicyclic part C-5 and C-1 were consigned from the resonances at 39.50 and 45.90 ppm, respectively.

The assignment given for the respective carbons and protons of compound **9** is further confirmed from the 2D NMR spectra recorded for compound **12**. The benzylic protons showed correlation with N(3)−H in the HOMOCOSY spectrum. In addition, H-2 and H-4 were found to be positively correlated with H-1 and H-5, respectively. From this, benzylic protons and bridgehead proton assignment is confirmed. Methylene protons

Table 1. Analytical Data of Compounds 9−15[a]

			elemental analysis found (calculated) (%)		
compound	molecular formula	molecular weight	C	H	N
9	$C_{24}H_{25}N_5OS$	431.55	66.75 (66.80)	5.79 (5.84)	16.19 (16.23)
10	$C_{24}H_{23}Cl_2N_5OS$	500.44	57.52 (57.60)	4.57 (4.63)	13.94 (13.99)
11	$C_{26}H_{29}N_5O_3S$	491.61	63.49 (63.52)	5.85 (5.95)	14.16 (14.25)
12	$C_{24}H_{23}F_2N_5OS$	467.16	61.59 (61.65)	4.85 (4.96)	14.87 (14.98)
13	$C_{24}H_{23}Br_2N_5OS$	587.00	48.88 (48.91)	3.87 (3.93)	11.83 (11.88)
14	$C_{26}H_{29}N_5OS$	441.25	67.91 (67.94)	6.29 (6.36)	15.20 (15.24)
15	$C_{24}H_{23}Cl_2N_5OS$	500.44	57.57 (57.60)	4.57 (4.63)	13.89 (13.99)

[a]The observed microanalysis values for C, H, and N were within ±0.4% of the theoretical values.

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Table 2. In Vitro Antibacterial and Antifungal Activities (MIC[a], μg/mL) of Compounds 9−15 by 2-fold Serial Dilution Method

	bacterial strain					fungal strain			
compound	*B. subtilis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*S. aureus*	*A. flavus*	*A. niger*	*C. albicans*	*Candida6*
9	100	100	100	100	50	50	100	100	100
10	6.25	6.25	12.5	50	50	12.5	12.5	6.25	6.25
11	100	200	200	100	100	25	25	50	100
12	6.25	6.25	12.5	25	25	12.5	12.5	6.25	6.25
13	6.25	6.25	12.5	12.5	50	12.5	12.5	6.25	6.25
14	100	100	50	50	100	50	50	25	50
15	25	25	50	25	100	12.5	12.5	6.25	25
streptomycin	12.5	12.5	25	12.5	12.5				
fluconazole						25	25	12.5	25

[a]MIC, minimum inhibitory concentration.

at the 6-, 7-, and 8-positions of the bicyclic ring including the downfield H-7a proton were confirmed from their mutual correlations. Benzylic carbons, ipso carbons of aryl group, and methylene carbons (C-6, C-7, and C-8) are assigned with respect to their HSQC and HMBC correlations (refer to the Supporting Information). Assignments for the other compounds, **10**−**15**, were made by comparison with compound **9**. X-ray crystallographic study confirmed that 2r,4c-diphenyl-9t-ethynyl-3-azabicyclo[3.3.1]nonan-9t-ol exists in twin-chair conformation with equatorial orientations of the phenyl groups.[26] Taken together, all of the above observations substantiate the proposed structure and twin-chair (CC) conformation of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one-4-methyl-1,2,3-thiadazole-5-carbonyl hydrazones **9**−**15** (Table 2).

Biological Activity. *Antibacterial Activity.* Synthesized compounds **9**−**15** were examined for their antibacterial potencies. In vitro studies by 2-fold serial dilution method were adopted. Streptomycin was used as a positive control.[27] Table 1 shows the MICs of test compounds **9**−**15**. Analysis of in vitro antimicrobial effects of all the 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one-4-methyl-1,2,3-thiadazole-5-carbonyl hydrazones **9**−**15** revealed a diverse range (6.25−200 μg/mL) against the pathogens. The compounds deprived of any substituents at the aryl rings in **9** hinder the growth of *S. aureus* at a MIC value of 50 μg/mL and that of *B. subtilis*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* at a MIC value of 100 μg/mL. However, compounds **10**, **12**, and **13** possessing *para* halo (electron-withdrawing substituents chloro, fluoro, and bromo) substituted aryl groups in azabicyclononane moiety account for the enhanced inhibitory effects against *B. subtilis* and *K. pneumonia* at MIC values of 6.25 μg/mL and against *E. coli* at a MIC value of 12.5 μg/mL when compared to the standard antibiotic streptomycin. John Francis Xaiver et al. have also documented that electron-withdrawing group (fluoro, bromo, and chloro) substituted azabicyclononan-9-one derivatives exhibited outstanding antibacterial and antifungal activities.[28] Compound **15** with an ortho chloro substituent in the phenyl moiety displays good antibacterial activity against *B. subtilis*, *K. pneumoniae*, and *P. aeruginosa* (MIC = 25 μg/mL), *E. coli* (MIC = 50 μg/mL), and *S. aureus* (MIC = 100 μg/mL). Among the tested compounds **9**−**15**, compounds **10**, **12**, and **13** exhibit remarkable potencies against *B. subtilis*, *K. pneumonia*, and *E. coli*, whereas these compounds showed lesser activity against *P. aeruginosa* and *S. aureus* than standard streptomycin. Other compounds displayed reduced inhibitory effects against various bacterial strains compared to the standard streptomycin.

Antifungal Activity. The results of the present study also provide evidence for the antifungal effects of an array of 2r,4c-diaryl-3-azabicyclo[3.3.1]nonan-9-one-4-methyl-1,2,3-thiadazole-5-carbonyl hydrazones **9**−**15** with MIC values ranging from 6.25 to 200 μg/mL (Table 1). Compound **9**, which lacks any substituents at the aryl groups, showed mild and comparatively less antimicrobial activity against *A. flavus* (50 μg/mL), *A. niger* (100 μg/mL), *C. albicans* (100 μg/mL), and *Candida6* (100 μg/mL), compared to fluconazole, a known antifungal agent used as positive control. Compounds **10**, **12**, and **13** exerted 2-fold increased antifungal activity against *A. flavus*, *A. niger*, and *C. albicans* at MIC values of 6.25−12.5 μg/mL and show 4-fold increased activity against *Candida6* at a MIC value of 6.25 μg/mL when compared to the standard, fluconazole. In addition, compound **15** exerted 2-fold increases in antifungal activity against *A. flavus*, *A. niger*, *C. albicans*, and *Candida6* at MIC values of 6.25−25.0 μg/mL, whereas the enhanced antifungal activity of compounds of **10**, **12**, and **13** may be due to the chloro, fluoro, and bromo substituents at the para position of aryl groups. Furthermore, phenyl rings with electron-donating methoxy and methyl groups at the para position of compounds **11** and **14** hinder the growth of all fungal strains at MIC values ranging from 25 to 100 μg/mL.

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 test compounds and standard drug. Taken together, our findings provide evidence that the type of functional groups and the patterns 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 **9**−**15** create promising leads for the development of potent antimicrobial agents.

■ ASSOCIATED CONTENT

Supporting Information

Spectral data (^{1}H NMR, C^{13} NMR, H−H COSY, NOESY, HSQC, and HMBC) for compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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