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Efficient synthesis, spectral analysis and antimicrobial studies of nitrogen and sulfur containing spiro heterocycles from 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones

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ABSTRACT

Acetyl and propionyl group substituted thiadiazole derivatives (**4a**–**4h**, **5a**–**5h**, **6a**, **6b**, **7a** and **7b**) have been synthesized by the cyclization of 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one thiosemicarbazones (**2a**–**2h**, **3a** and **3b**) with acetic anhydride/propionic anhydride and were characterized by Elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectral analysis. Single crystal X-ray diffraction has also been recorded for compounds **4c** and **5a**. From the NMR and Single crystal X-ray diffraction analysis, compounds **4b**–**4d**, **4f**–**4h**, **5b**, **5c**, **5f**–**5h**, **6a**, **7a** and **7b** were found to adopt twin-chair conformations whereas compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b** adopt chair and boat conformation of cyclohexane and piperidine rings, respectively. Besides, the synthesized compounds were screened for antibacterial and antifungal activities using serial dilution method. The microbiological analysis showed that the electron withdrawing function substituted phenyl group at C-2 and C-4 of azabicyclononane based thiadiazoles **4c**/**4h** and **5c**/**5h** exposed significant antimicrobial activity against *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* at MIC of 6.25 μg/ml.

In the past years considerable evidences have been accumulated to demonstrate the efficacy of substituted 1,3,4-thiadiazoles in antibacterial, antifungal and HIV activities.[1–3] This analogue acts as 'hydrogen binding domain' and 'two-electron donor system' and naturally occur in four isomeric forms viz. 1,2,3-thiadiazole; 1,2,5-thiadiazole; 1,2,4-thiadiazole and 1,3,4-thiadiazole. A glance at literature studies show that more work has been carried out on the 1,3,4-thiadiazole than the other isomers. This five membered 1,3,4-thiadiazoles show diverse biological activities, probably by the virtue of =N–C–S moiety[4] and their toxicity. The toxicity of thiadiazole entity is related to substitutions like halogen, sulphur, oxygen and nitrile groups, for example, herbicides and insecticides. It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market as acetazolamide,[5] methazolamide,[6] sulfamethazole,[7] etc. The resistance towards available drugs is rapidly becoming a major worldwide problem. The necessity to design new compounds to overcome this resistance has become one of the most important areas of research today.

1,3,4-Thiadiazoles represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities such as antimicrobial,[8] antiviral,[9] anti-inflammatory,[10] herbicidal,[11] antidepressive,[12] antitumoral,[11] antitubercular,[13,14] anticancer,[15] anticonvulsant,[16] antioxidant/Radio protective activity,[17] antimycobacterial,[18,19] antimicotic,[20] antimalarial,[21] antileukemic,[22] antileishmanial[23,24] activities, pesticides[11] and cardiotonic[25] and their action is being noticeable.

Recently, we have reported several mono and bicyclic derivatives as antimicrobial agents.[26–28] Some of them show good growth inhibition at low concentration. Extending the research in this area, we decided to obtain the derivatives which contain the bicyclic based piperidine and 1,3,4-thiadiazole ring. The aim of this work is to investigate the antibacterial and antifungal activities of the target compounds by the modification of phenyl group substituents and the side chain (acetyl/propionyl group) in thiadiazole ring. The synthesized compounds (**4a**–**4h**, **5a**–**5h**, **6a**, **6b**, **7a** and **7b**) are characterized by analytical and spectral techniques. Conformational assignments of all the compounds were unambiguously achieved with the help of NMR and single crystal X-ray diffraction analysis.

The reaction pathways of different thiadiazole modifications are sketched in Scheme 1. The 2,4-diaryl-3-azbicyclo[3.3.1]nonan-9-ones were prepared according to the precedent literature[29] by the one pot multi-component condensation of ammonium acetate, benzaldehyde and cyclohexanone (1:2:1 ratio). This upon condensation with thiosemicarbazide under acidic medium afforded 2,4-diaryl-3-azbicyclo[3.3.1]nonan-9-one thiosemicarbazones along with aryl thiosemicarbazones.[27] In the present work, the key intermediate thiosemicarbazones react with acid anhydride (acetic

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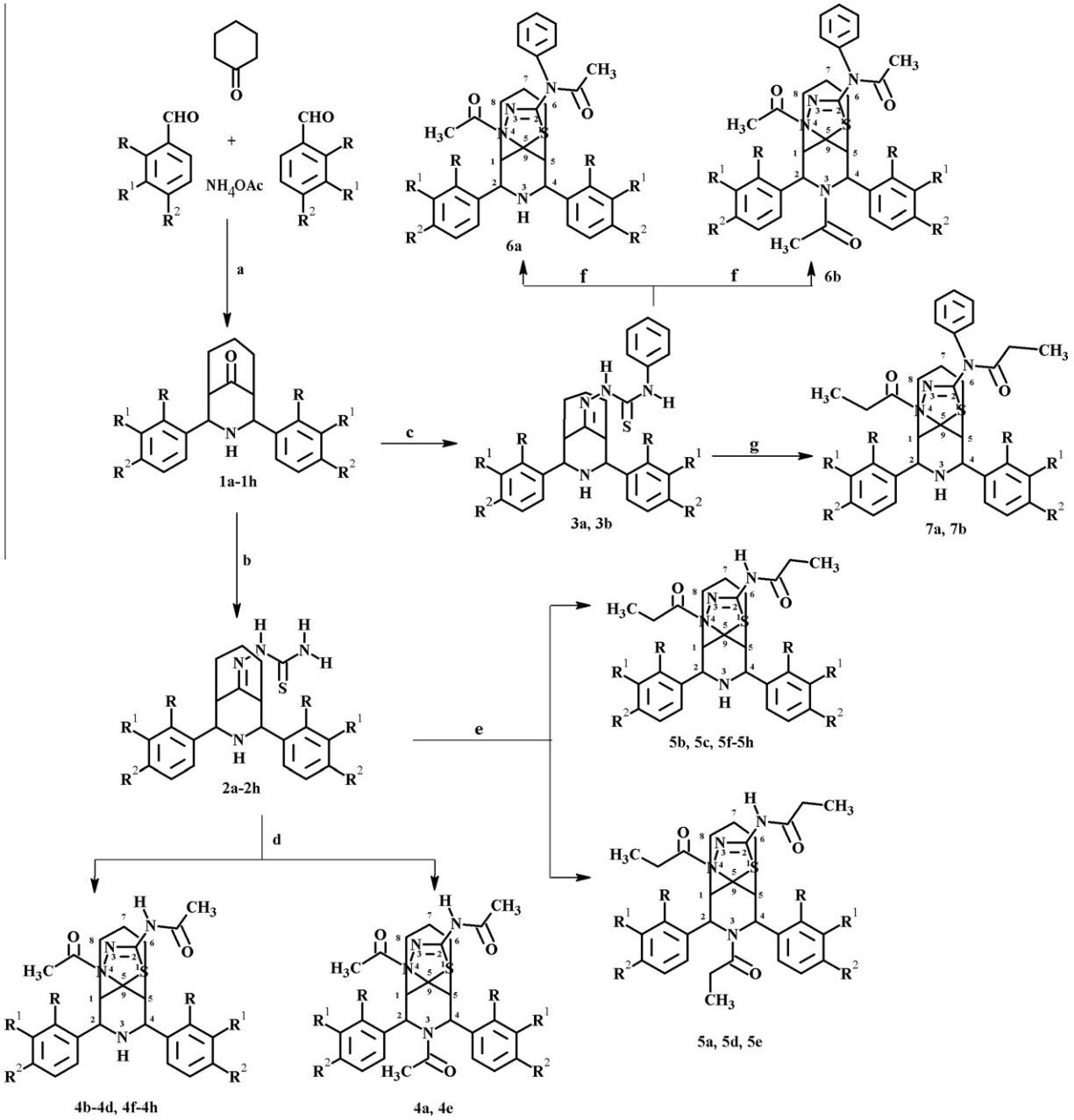


Scheme 1. Schematic diagram showing the synthesis of 1,3,4-thiadiazoles (**4a**–**7b**). Reagents and conditions: (a) EtOH, slight warm then rt, 24 h; (b) NH_2–NH–CS–NH_2·HCl, $CHCl_3$/EtOH (1:1), 4–5 h reflux; (c) PhNH–NH–CS–NH2·HCl, $CHCl_3$/EtOH (1:1), 6–7 h reflux; (d) acetic anhydride, 12–15 h reflux, 90 °C; (e) propionic anhydride, 5–8 h reflux, 90 °C; (f) acetic anhydride, 18–20 h reflux, 90 °C; (g) propionic anhdride, 7–10 h reflux, 90 °C.

anhydride/or propionic anhydride) under reflux condition (90 °C) afforded the corresponding cyclised thiadiazole derivatives. Then the synthesized compounds were purified by column chromatography using benzene–ethyl acetate (2:1) as eluent on neutral alumina.

According to Scheme 1, compounds **4b**–**4d**/**4f**–**4h**, **5b**–**5c**/**5f**–**5h**, **6a**, **7a** and **7b** were failed to undergo either acetylation or propionylation reaction at nitrogen site of piperidine ring due to steric hindrance caused by the presence of fluorine, chlorine and methyl moiety on the phenyl group at C-2 and C-4, whereas in compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b**, the formation of N-acetylation or N-propionylation reveals that the phenyl group did not cause any steric hindrance around the nitrogen site (piperidine ring). The target compounds were confirmed by elemental analysis and IR spectral analysis. The analytical data of the synthesized compounds were given in Table 1. Further, the structural assignments of the title compounds were made by using ^{1}H and ^{13}C NMR spectral analysis. Besides, single crystal X-ray diffraction has also been recorded for the representative compounds (**4c** and **5a**). A well numbered target compound structure was given in Figure 1 for structural and biological analysis.

IR spectra of 1,3,4-thiadiazoles (**4a**–**4h**, **5a**–**5h**, **6a**, **6b**, **7a** and **7b**) showed weak absorption band in the region of 3090–3000 cm^{-1} which is due to aliphatic and aromatic C–H stretching. A sharp and intense absorption band around 3200 and 3300 cm^{-1} is assigned to NH stretching vibration of side chain acetyl amino and piperidine analogue of compounds **4b**–**4d**/**4f**–**4h**, **5b**–**5c**/**5f**–**5h**, **6a**, **7a** and **7b** whereas in compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b**, the absence of stretching frequency around 3300 cm^{-1} suggests that the N-acetylation/propionylation occurred at nitrogen

Table 1
Analytical data for compounds **4a–7b**

Entry	Molecular formulae	Yield (%)	Mp (°C)	Elemental analysis (%)					
				Calculated			Found		
				C	H	N	C	H	N
4a	$C_{27}H_{30}N_4O_3S$	90	166	66.10	6.16	11.42	66.11	6.17	11.42
4b	$C_{25}H_{26}Cl_2N_4O_2S$	80	160	58.03	5.06	10.83	58.03	5.07	10.81
4c	$C_{25}H_{26}F_2N_4O_2S$	90	154	61.97	5.41	11.56	61.95	5.40	11.55
4d	$C_{27}H_{32}N_4O_4S$	75	152	63.76	6.34	11.02	63.76	6.35	11.00
4e	$C_{29}H_{34}N_4O_5S$	65	155	63.25	6.22	10.17	63.24	6.22	10.15
4f	$C_{27}H_{32}N_4O_2S$	60	164	68.04	6.77	11.75	68.03	6.75	11.73
4g	$C_{25}H_{26}Cl_2N_4O_2S$	80	168	58.03	5.06	10.83	58.01	5.07	10.83
4h	$C_{25}H_{26}F_2N_4O_2S$	75	158	61.97	5.41	11.56	61.95	5.43	11.58
5a	$C_{30}H_{36}N_4O_3S$	90	192	67.64	6.81	10.52	67.65	6.80	10.53
5b	$C_{27}H_{30}Cl_2N_4O_2S$	83	160	59.45	5.54	10.27	59.45	5.56	10.28
5c	$C_{27}H_{30}F_2N_4O_2S$	85	154	63.26	5.90	10.93	63.24	5.91	10.91
5d	$C_{32}H_{40}N_4O_5S$	65	168	64.84	6.80	9.45	64.84	6.81	9.47
5e	$C_{32}H_{40}N_4O_5S$	79	183	64.84	6.80	9.45	64.86	6.80	9.44
5f	$C_{29}H_{36}N_4O_2S$	85	171	69.02	7.19	11.10	69.03	7.17	11.10
5g	$C_{27}H_{30}Cl_2N_4O_2S$	81	181	59.45	5.54	10.27	59.47	5.51	10.25
5h	$C_{27}H_{30}F_2N_4O_2S$	79	174	63.26	5.90	10.93	63.25	5.91	10.94
6a	$C_{31}H_{30}Cl_2N_4O_2S$	84	210	62.73	5.09	9.44	62.71	5.08	9.44
6b	$C_{33}H_{32}Cl_2N_4O_3S$	88	216	62.36	5.07	8.81	62.36	5.05	8.80
7a	$C_{33}H_{34}Cl_2N_4O_2S$	81	214	63.76	5.51	9.01	63.74	5.50	9.02
7b	$C_{33}H_{34}Cl_2N_4O_2S$	75	185	63.76	5.51	9.01	63.77	5.49	9.01

site. Generally, the $\nu_{C=O}$ bands of amide carbonyl[30] group observed in the region 1690–1630 cm^{-1}. Likewise, the compounds in the present series also exhibit similar absorptions around 1690 cm^{-1} and 1595 cm^{-1} are characteristic for N–C=O and C=N bond stretching vibrations. In addition, the appearance of N–C–S bending vibration around 1375 cm^{-1} is the confirmatory evidence for five membered ring closure.

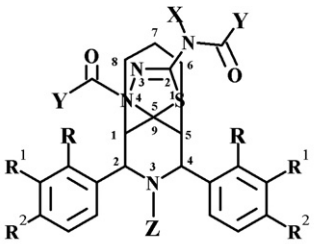
In order to assign the ring proton and carbon signals, compound **4c** has been chosen as representative compound. ^1H NMR spectrum of thiadiazole (**4c**) gives a singlet at 4.95 ppm with two protons integral which is assignable to benzylic (H-2a and H-4a) protons. Similarly, bridgehead protons (H-1e and H-5e) are resonated as a singlet at 2.73 ppm with two protons integral. Instead of doublet, a singlet was observed for benzylic and bridge head protons, therefore, the coupling constant values could not be extracted well, but its chemical shift values are similar to already reported twin-chair bicyclic compounds. So, we concluded that these compounds (**4b–4d**, **4f–4h**, **5b–5c**, **5f–5h**, **6a**, **7a** and **7b**) may also exist in twin-chair conformation with equatorial orientation of aryl substituents as shown in Figure 2. Single crystal XRD analysis was carried out for representative compound 2,4-[bis(*m*-fluorophenyl)-3-azabicyclo[3.3.1]nonan-9-yl]-5-spiro-4-acetyl-2-(acetylamino)-Δ^2-1,3,4-thiadiazoline **4c** and its ORTEP diagram is shown in Figure 3 with important bond length and bond angles. The XRD analysis also proved that both the piperidine and cyclohexane rings are exist in chair–chair conformation (Fig. 3). [Refer Supplementary data for detailed spectral assignments and the crystallographic data and refinement parameters of **4c** (Table 1)].

Interestingly, compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b** showed two benzylic proton signals in the deshielded region (around 6.0 ppm). This deshielding of chemical shift is due to the introduction of electron withdrawing acetyl or propionyl group at nitrogen site in piperidine ring. Due to the presence of *N*-acetyl/propionyl group, coplanarity was created between N–C=O bond to avoid $A^{1,3}$ allylic strain. Therefore, these compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b** should adopt boat–chair conformations of the piperidine and cyclohexane rings, respectively (Fig. 4) and the aryl groups occupy equatorial orientation. In order to confirm these assignments, single crystal XRD analysis was carried out for representative compound *N*-propionyl-2,4-[diphenyl-3-azabicyclo[3.3.1]nonan-9-yl]-5-spiro-4-propionyl-2-(propionylamino)-Δ^2-1,3,4-thiadiazoline **5a** and its ORTEP diagram is depicted in Figure 5 (with its important bond length and bond angles). The crystallographic data and structure refinement parameters of **5a** are given in Supplementary data (Table 2). According to XRD, the piperidine ring exists in half-boat conformation whereas the cyclohexane ring exists in chair conformation.

Among the cyclohexane ring proton (H-6, H-7 and H-8) signals, H-7a proton resonated in the deshielded region than its corresponding equatorial and other methylene (C-6 and C-8) protons. This is due to the deshielding effect exerted by the nitrogen lone pair and it creates steric interaction. Due to this, C–H(7a) bond becomes polarized and as a consequence, carbon and the attached proton acquire negative and positive charges, respectively. As a result, the H-7a proton signal is highly deshielded. Hence, a multiplet at 2.46 ppm is unambiguously assigned to H-7a proton for compounds **4b–4d**, **4f–4h**, **5b–5c**, **5f–5h**, **6a**, **7a** and **7b** and another multiplet at 1.28 ppm is assigned to H-7e proton. Conversely for compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b** the H-7a proton signal resonated in the shielded region (around 1.40 ppm). This is due to the absence of steric interaction between NH and H-7a proton (i.e., due to N-acylation, the free NH proton is absent). This clearly supports the N-acylation of these compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b**.

It is worthwhile mentioning that the equatorial proton in methylene carbon of cyclohexane ring is highly deshielded than the axial proton due to the anisotropy of the C–C single bonds. As a consequence, multiplets centered at 1.74 and 1.34 ppm, each two proton integral attributed to H-6e/H-8e and H-6a/H-8a protons, respectively. In addition, two singlets at 2.26 and 2.22 ppm with three protons integral confirms the methyl moieties of **4a–4h**, **6a** and **6b** series thiadiazoles but in the case of **5a–5h**, **7a** and **7b**, the side chain methyl and methylene protons observed as a triplet and a quartet around 1.50 and 2.50 ppm with three and two protons integration, respectively.

In compounds **4b–4d**, **4f–4h**, **5b**, **5c**, **5f–5h**, **6a**, **7a** and **7b**, the NH protons signal in piperidine and thiadiazole moiety resonated as singlets at 1.62 and 8.20 ppm, respectively, whereas in compounds **4a**, **4e**, **5a**, **5d**, **5e** and **6b** the expected NH proton signal in piperidine analogue was not observed. Instead, the acetyl and propionyl group protons signal appeared as a singlet, triplet and quartet around 2.20, 1.80 and 2.0–2.50 ppm with three and two protons integral, respectively. Therefore, the shielding of H-7a



Entry	R	R^1	R^2	X	Y	Z
4a	H	H	H	-	CH_3	$COCH_3$
4b	Cl	H	H	-	CH_3	H
4c	H	F	H	-	CH_3	H
4d	H	OCH_3	H	-	CH_3	H
4e	H	H	OCH_3	-	CH_3	$COCH_3$
4f	H	H	CH_3	-	CH_3	H
4g	H	H	Cl	-	CH_3	H
4h	H	H	F	-	CH_3	H
5a	H	H	H	-	CH_2CH_3	$COCH_2CH_3$
5b	Cl	H	H	-	CH_2CH_3	H
5c	H	F	H	-	CH_2CH_3	H
5d	H	OCH_3	H	-	CH_2CH_3	$COCH_2CH_3$
5e	H	H	OCH_3	-	CH_2CH_3	$COCH_2CH_3$
5f	H	H	CH_3	-	CH_2CH_3	H
5g	H	H	Cl	-	CH_2CH_3	H
5h	H	H	F	-	CH_2CH_3	H
6a	Cl	H	H	Ph	CH_3	H
6b	H	H	Cl	Ph	CH_3	$COCH_3$
7a	Cl	H	H	Ph	CH_2CH_3	H
7b	H	H	Cl	Ph	CH_2CH_3	H

Figure 1. A well numbered target molecules (**4a**–**7b**).

proton signal and the presence of methyl and methylene protons signal strongly confirms the N-acylation. The aryl protons appeared in the region of 6.97–7.32 ppm.

In ^{13}C NMR spectrum of target compounds (**4a**–**4h**, **5a**–**5h**, **6a**, **6b**, **7a** and **7b**), the disappearance of thiocarbonyl (C=S) signal around 180 ppm confirmed the cyclization. In addition, a new signal observed around 90 ppm is assigned to C-9 ipso (spiro) carbon. Similarly, the carbonyl, methyl and methylene moieties of the acetyl/propionyl groups signal further supports the formation of spiro thiadiazole analogue. Moreover, two sets of signals appeared in the downfield and upfield region at 172.6/167.8 and 25.7/23.6 ppm are due to carbonyl and methyl moieties of secondary and tertiary amide nitrogens, respectively, whereas the C=N signal is appeared around 156 ppm.

The benzylic (C-2/C-4) and bridgehead (C-1/C-5) carbon signals appeared at 60.2 and 41.9 ppm, respectively. Moreover, the signals which are appeared in the aliphatic region at 25.32 and 19.33 ppm are assigned to C-8/C-6 and C-7 carbon, respectively. A collection of signals resonated in the region of 129.87–113.40 ppm and a signal

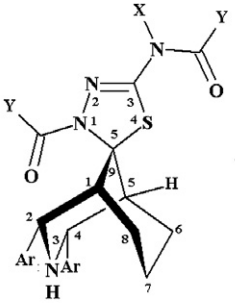


Figure 2

X = H,

Phenyl (6a, 7a & 7b)

Y = CH_3 / CH_2-CH_3

Entry	Ar
4b, 5b, 6a, 7a	*o*-cl-C_6H_4
4c, 5c	*m*-F-C_6H_4
4d	*m*-OCH_3-C_6H_4
4f, 5f	*p*-CH_3-C_6H_4
4g, 5g, 7b	*p*-cl-C_6H_4
4h, 5h	*p*-F-C_6H_4

Figure 2. Chair–chair conformation with equatorial orientation of the phenyl/substituted phenyl groups at C-2 and C-4.

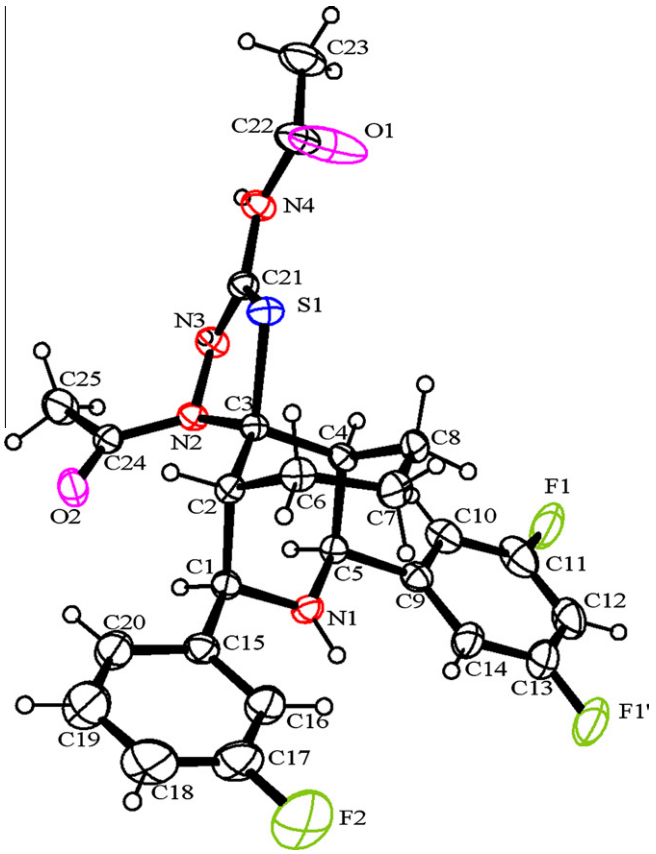


Figure 3. ORTEP diagram of compound **4c** with 50% probability. The important bond length (Å): C(3)–N(2) = 1.482 (4) ; C(3)–S(1) = 1.860 (3); N(2)–N(3) = 1.433 (4); C(21)–N(3) = 1.273 (4); C(21)–N(4) = 1.382 (4); C(21)–S(1) = 1.746 (3); C(22)–O(1) = 1.196 (5); C(22)–N(4) = 1.365 (5); C(24)–O(2) = 1.218 (4); C(24)–N(2) = 1.374 (4). The important bond angles (°): N(3)–C(21)–N(4) = 119.9 (3); N(3)–C(21)–S(1) = 118.3 (2); O(1)–C(22)–N(4) = 121.6 (4); N(4)–C(22)–C(23) = 115.6 (4); O(2)–C(24)–N(2) = 122.7 (3); N(2)–C(24)–C(25) = 116.8 (3); N(3)–N(2)–C(3) = 110.3 (2); N(2)–C(3)–S(1) = 100.68 (19).

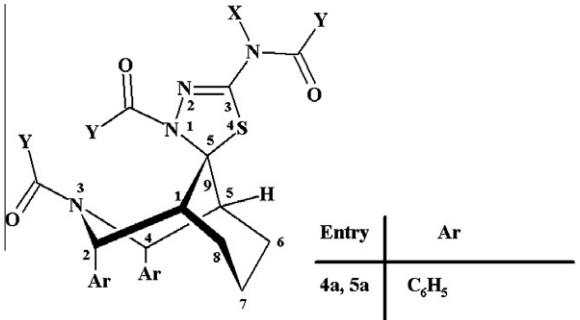


Figure 4

X = H,

Phenyl (6b)

Y = CH_3 / CH_2-CH_3

Entry	Ar
4a, 5a	C_6H_5
4e, 5e	*p*-OCH_3-C_6H_4
5d	*m*-OCH_3-C_6H_4
6b	*o*-cl-C_6H_4

Figure 4. Boat–chair conformation with equatorial orientation of the phenyl/substituted phenyl groups at C-2 and C-4.

at 164.21/161.77 ppm are assigned to aryl carbons and fluorine attached ipso carbons (C-2‴ and C-4‴), respectively.

All the newly synthesized compounds (**4a–4h**, **5a–5h**, **6a**, **6b**, **7a** and **7b**) were screened for in vitro antibacterial activity against *Staphylococcus aureus* (ATCC-25930), *Bacillus subtilis* (ATCC-530), *Salmonella typhi* (ATCC-25021), *Escherichia coli* (ATCC-26032) and *Klebsiella pneumoniae* (ATCC-16425) and antifungal activity against *Candida albicans* (ATCC-3430), *Cryptococcus neoformans* (ATCC-3235), *Rhizopus* species (ATCC-2842), *Aspergillus niger* (ATCC-635) and *Aspergillus flavus* (ATCC-525) and the MIC values were determined by serial dilution method.[31] Here, Streptomycin and Amphotericin B were used as standard drugs for bacterial and fungal studies, respectively. Tables 2 and 3 show the various bacterial and fungal growth inhibition level of compound **4a–4h**, **5a–5h**, **6a**, **6b**, **7a** and **7b** against different pathogenic strains.

All the compounds were shown their antibacterial activity ranging from 6.25 to 200 μg/ml. Table 2 revealed that the compounds **4a–4h**, **6a**, **6b** (cyclised compounds using acetic anhydride) were found to exhibit superior antibacterial activity against the tested bacterial strains than the compounds **5a–5h**, **7a**, **7b** (cyclised compounds using propionic anhydride). Among the different pathogenic strains, the compounds with unsubstituted phenyl groups at C-2 and C-4 of azabicyclononane based thiadiazoles (either acetylated or propionylated) showed better activity (12.5 μg/ml) against *S. aureus* and moderate activity (50–100 μg/ml) against rest of the strains used.

Conversely, the introduction of fluoro function at the *meta*/*para* position of **4a** (compounds **4c**, **5c** and **4h**, **5h**) showed a noticeable improvement in their activity even at very low concentration (6.25 μg/ml) against all the strains. Particularly, **4c** against *S. typhi*, *E. coli* and *K. pneumoniae*, **5c** against *B. subtilis*, *E. coli* and *S. typhi*, and **4h**/**5h** against *B. subtilis*, *S. typhi* exerted superior activity at MIC of 6.25 μg/ml. But, replacement of fluoro function by chloro function in **4h**/**5h** (compounds **4g**/**5g**) registered one fold diminished activity (12.5 μg/ml) against *B. subtilis* and *S. typhi*. Whereas the same compounds showed one fold increased activity against *S. aureus* and *K. pneumoniae* when phenyl group was substituted at amide nitrogen spiro (compounds **6a** and **7b**). In lieu of halogens, methyl substituted compounds **4f** and **5f** were more potent (12.5 μg/ml) against *B. subtilis* and *K. pneumoniae*, respectively. But the substitution of methoxy function in **4a**/**5a** (compound **4d**/**5d)** did not show any inhibition against *E. coli* and *S. typhi* even at maximum concentration (200 μg/ml), respectively.

The above discussion and the data of Table 2 disclosed that the activity is sensitive to position of the substituent in the aryl ring. The *para* methoxy substituted compounds (**4e**/**5e**) exhibited three fold increased activity (25 μg/ml) against *S. aureus*, *S. typhi*, *K. pneumoniae* than the *meta* methoxy substituted compounds (**4d**/**5d**), that is, MIC at 100 μg/ml. This suggests that besides electronic effects other factors also influence the activity.

A glance at the MIC_{50} value of Table 3 indicates that all thiadiazole compounds under this series exhibit their growth inhibition in the range of 6.25–200 μg/ml. However, among the thiadiazole derivatives **4a–4h**, **6a**, **6b** and **5a–5h**, **7a**, **7b**, unsubstituted phenyl bearing compound (**4a**) showed moderate activity (50–100 μg/ml). The modification of the acetyl group by propionyl group in **4a** (compound **5a**) does not show any change in activity against the tested organism. But the introduction of chloro function at *ortho*/*para* position of phenyl group in **4a** and **5a** (compound **4b**, **5b** and **4g**, **5g**) showed two fold increased growth inhibition (6.25–50 μg/ml). More precisely, **4b** and **5g** against *C. albicans* and *Rhizopus* sp. **5b** and **5g** against *A. niger* and **4b** against *A. flavus* were shown to possess significant antibacterial activity at 12.5 μg/ml. Replacement of chlorine from *ortho* to *para* in **4b** (compound **4g**) registered two fold enhanced activity (6.25 μg/ml) against *C. neoformans* and moderate activity against other strains but no significant change was noticed against the used strains when the amide group was modified (compound **6b**). Besides, Compound **4b** against *A. niger* is almost inactive up to 200 μg/ml, but introduction of phenyl group in the amide nitrogen in **4b** (compound **6a**)

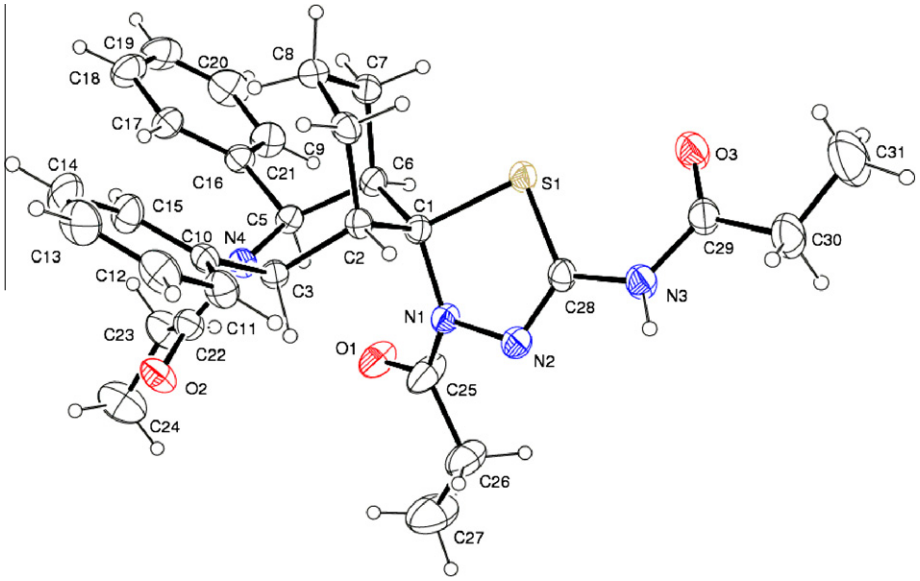


Figure 5. ORTEP diagram of compound **5a** with 50% probability. The important bond lengths (Å): C(1)–N(1) = 1.488 (3); C(1)–S(1) = 1.531 (3); N(1)–N(2) = 1.422 (3); C(28)–N(2) = 1.279 (3); C(28)–N(3) = 1.384 (3); C(28)–S(1) = 1.738 (2); C(29)–O(3) = 1.206 (3); C(29)–C(30) = 1.496 (4); C(30)–C(31) = 1.444 (5); C(25)–N(1) = 1.347 (4); C(3)–N(4) = 1.495 (3); N(4)–C(22) = 1.371 (3); N(4)–C(5) = 1.488 (3); C(22)–O(2) = 1.227 (3); C(22)–C(23) = 1.513 (4); C(23)–C(24) = 1.460 (4). The important bond angles (°): N(2)–N(1)–C(1) = 112.17 (18); N(1)–C(1)–S(1) = 100.46 (14); C(28)–S(1)–C(1) = 87.68 (11); N(2)–C(28)–N(3) = 120.0 (2); N(2)–C(28)–S(1) = 118.52 (18); N(3)–C(29)–C(30) = 114.8 (2); C(31)–C(30)–C(29) = 114.7 (3); C(27)–C(26)–C(25) = 113.5 (3); N(1)–C(25)–C(26) = 117.8 (2); N(4)–C(3)–C(10) = 113.88 (18); C(22)–N(4)–C(5) = 118.09 (19); C(22)–N(4)–C(3) = 112.79 (18).

Table 2
Antibacterial activity of compounds **4a**–**7b** against some bacterial strains (MIC in μg/ml)

Compounds	Minimum inhibitory concentration (MIC) in μg/ml				
	S. aureus	*B. Subtilis*	*S. typhi*	*E. coli*	*K. pneumonia*
4a	12.5	100	25	50	100
4b	12.5	12.5	12.5	12.5	25
4c	50	25	6.25	6.25	6.25
4d	100	50	–	200	100
4e	25	50	50	50	25
4f	25	12.5	100	100	12.5
4g	25	–	25	12.5	50
4h	50	6.25	6.25	12.5	12.5
5a	12.5	50	25	50	50
5b	50	12.5	25	50	25
5c	100	6.25	6.25	6.25	50
5d	100	50	200	50	–
5e	25	–	25	50	25
5f	50	100	50	25	25
5g	200	12.5	12.5	25	100
5h	25	6.25	6.25	12.5	25
6a	25	12.5	100	50	100
6b	12.5	25	12.5	25	50
7a	6.25	50	25	50	25
7b	12.5	100	50	25	200
Streptomycin	50	12.5	50	12.5	25

–, no inhibition even at maximum concentration.

Table 3
Antifungal activity of compounds **4a**–**7b** against some fungal strains (MIC in μg/ml)

Compounds	Minimum inhibitory concentration (MIC) in μg/ml				
	C. neoformans	*C. albicans*	*Rhizopus* sp.	*A. niger*	*A. flavus*
4a	50	100	50	25	100
4b	50	12.5	12.5	–	12.5
4c	25	25	12.5	25	6.25
4d	50	25	50	100	200
4e	25	25	100	–	12.5
4f	50	25	25	50	12.5
4g	6.25	25	200	25	50
4h	12.5	6.25	50	6.25	6.25
5a	6.25	50	12.5	50	12.5
5b	50	12.5	100	12.5	50
5c	25	25	25	12.5	50
5d	200	25	50	–	25
5e	50	25	100	25	100
5f	25	25	50	100	200
5g	25	12.5	12.5	12.5	50
5h	25	6.25	6.25	12.5	6.25
6a	50	6.25	25	12.5	100
6b	50	100	12.5	–	200
7a	200	50	100	50	50
7b	–	25	200	50	50
Amphotericin B	25	25	25	50	50

–, no inhibition even at maximum concentration.

showed significant activity (12.5 μg/ml) against the same strain. But moderate activity (50–100 μg/ml) was observed against all the strains for **6b**, **7a** and **7b**.

In order to gain more structure activity relationship in thiadiazole derivatives, chlorine function is replaced by fluoro function in **4g** and **5g** (compounds **4h** and **5h**); **4h** showed one fold decreased activity against *C. neoformans* (12.5 μg/ml), two and three fold increased activity against *C. albicans*, *A. niger* and *A. flavus*, (6.25 μg/ml), respectively, when compared to **4g**. Compound **5h** registered the analogous MIC values against *C. albicans* and *A. flavus* when the acetyl analogue is replaced by propionyl moiety in **4h**. The displacement of fluoro function from *para* to *meta* position in **4h** and **5h** (compounds **4c** and **5c**) showed one fold decreased activity against all the fungal strain except *A. flavus* and *A. niger*, which exhibited the same activity (6.25/12.5 μg/ml).

Surprisingly, the substitution of methoxy/methyl in **4a** and **5a** afforded **4d**, **4e**, **4f** and **5d**, **5e**, **5f**; all of them (either acetylated spiro or propionylated spiro compounds) endowed moderate activity (25 μg/ml) against *C. albicans* but poor activity (100–200 μg/ml) was registered against rest of the strains for the aforementioned spiro compounds. Besides, **4f** (*p*-methyl substituted acetylated spiro compound) were found to be more potent against *A. flavus*

(12.5 μg/ml) whereas **5f** (*p*-methyl substituted propionylated spiro compound) did not show any inhibition (200 μg/ml) against the same strain. This diminished antifungal activity can probably be ascribed by the presence of propionyl instead of acetyl group in spiro thiadiazoles.

In conclusion, some biologically important bicyclic based acetylated and propionylated spiro thiadiazoles (**4a**–**4h**, **5a**–**5h**, **6a**, **6b**, **7a** and **7b**) were synthesized by the treatment of bicyclic thiosemicarbazones (**2a**–**2h**, **3a**, **3b**) with acetic anhydride/propionic anhydride as cyclising agents. Besides this cyclization, acetylation/propionylation were also occurred at the nitrogen site of the piperidine ring in certain compounds (**4a**, **4e**, **5a**, **5d**, **5e**, **6b**) revealed that compounds did not show steric hindrance around the aryl ring (phenyl and OMe group substituted aryl ring). Pursuing the biological activity of these different substituted spiro compounds, the acetylated spiro compounds (**4a**–**4h**) exhibited better activity than the propionylated compounds (**5a**–**5h**), phenyl substituted amide derivatives (**6a**, **6b**, **7a** and **7b**) and N-acetylated/propionylated compounds (**4a**, **4e**, **5a**, **5d**, **5e** and **6b**). Especially, the fluoro phenyl substituted both acetylated and propionylated spiro thiadiazoles (**4c**, **4h** and **5c**, **5h**) exhibited excellent activity (6.25–12.5 μg/ml). From the microbiological results, a general trend emerges and the order of activity being: F > Cl > methyl > methoxy. This can probably be ascribed to enhancement of the activity of the azabicyclononane based thiadiazole pharmacophore by the electronic effects exerted by the substituents.

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Supplementary data

Supplementary data (complete experimental details and spectral data (IR, ^{1}H NMR and ^{13}C NMR) for all the reported compounds and single crystal X-ray data for compounds **4c** and **5a** were given. The crystallographic data of **4c** and **5a** have been deposited at Cambridge Crystallography Data Center (CCDC Nos. 706266 and 778612, respectively)) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.021.

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