Efficient synthesis and antimicrobial evaluation of some Mannich bases from 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones

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Abstract

Background: Thiazolidinone, has been employed in the preparation of different important drugs required for treatment of inflammations, bacterial infections, and hypertension. Mannich bases have been shown to exhibit diverse biological activities, such as antibacterial, and antifungal activities. Spiroheterocycles including thiazolidine moiety have antimicrobial activity.

Results: In this study, a novel, rapid, and efficient protocol is developed for the synthesis of various 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones using sodium dodecylbenzene sulfonate (DBSNa) as an inexpensive and readily available reagent in acetic acid at room temperature. High yields, easy work-up, and short reaction times are advantages of this procedure. The synthesized arylidines were undergone Mannich reaction with formaldehyde and secondary amines in absolute ethanol at room temperature to afford the corresponding N-Mannich bases. All prepared Mannich bases were evaluated for their antimicrobial activity.

Conclusions: Good activity was noted for Mannich bases from 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones, with some members recorded higher antimicrobial activity.

Keywords: Spiro, 1-thia-4-azaspiro[4.5]decan-3-one, Sodium dodecylbenzene sulfonate, Antimicrobial activity, Mannich bases

Background

There are many bioactive molecules which possess various heteroatoms such as nitrogen, sulfur and oxygen, always taken the attention of chemists over the years mainly because of their biological significance. Thiazolidinones are thiazolidine derivatives which have a sulfur atom at position 1, a nitrogen atom at position 3 and a carbonyl group at position 2, 4, or 5 [1], is considered as an important biologically active scaffold that possesses almost all types of biological activities [2]. This heterocyclic system has been employed in the preparation of different important drugs required for treatment of inflammations [3], bacterial infections [4], and hypertension [5]. Some of the thiazole analogues are used as fungicides, inhibiting in vivo the growth of xanthomomas and as ingredients of herbicides, antischistosomidal, and anthelmintic drugs [6]. Mannich bases are reported to show a diversity of biological activities, such as antibacterial [7,8], antifungal [9,10] activities. Spiro derivatives have antibacterial, anticancer, and anticonvulsants activities. Spiro heterocycles were used as nitric oxide synthesis inhibitors [11] and potential topical agents for vaginal infection [12]. Spiro heterocyclic compounds including thiazolidine moiety have antimicrobial activity [13].

In this paper and as a consequence of our previous work on the synthesis of N-heterocyclic compounds [14–18], and bioactive heterocyclic agents [19–21], we reported herein an efficient protocol to the synthesis of N-Mannich bases (6a-r) from 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a-f). The anti-microbial activity of the prepared compounds (6a-r) was screened.
Results and discussion

Chemistry

First of all, 1-thia-4-azaspiro[4.5]decan-3-one (3) was prepared via the three component cyclocondensation reaction of cyclohexanone, thioglycolic acid, and ammonium carbonate according to the previously reported procedure [22] as shown in Scheme 1.

Synthesis of 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a-f)

In the first part of our research, we investigated a novel, rapid and efficient protocol that was developed for the synthesis of some 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a–f) by the condensation of 1-thia-4-azaspiro[4.5]decan-3-one (3) with aromatic aldehydes (4a–f) using sodium dodecylbenzene sulfonate (DBSNa) (20 mol %) in acetic acid at room temperature as shown in Scheme 2 and Table 1.

To find out the suitable conditions for the synthesis of 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones, a series of experiments were performed with the standard reaction of 1-thia-4-azaspiro[4.5]decan-3-one (3) and benzaldehyde (4a) as a model reaction (Scheme 3, Table 2).

Effect of the reaction conditions

In our initial study, we tried to optimize the model procedure mentioned above by detecting the efficiency of different reaction conditions, such as piperidine/EtOH, p-TSA/EtOH, AcOH/EtOH, AcONa/AcOH, H₂SO₄/AcOH, DBSNa/MeOH, DBSNa/EtOH, DBSNa/H₂O, DBSNa/AcOH (Table 2).

In each case, the reactants (10 mmol) were allowed together in 10 mL solvent at room temperature. In the case of piperidine/EtOH, p-TSA/EtOH, AcOH/EtOH, and AcONa/AcOH, the reaction proceeded with comparatively longer reaction time and poor reaction yield (Table 2, entries 1–4). Acetic acid acidified with a drop of H₂SO₄ can push the reaction towards the formation of product in yields of 61% (Table 2, entry 5).

In the presence of sodium dodecylbenzene sulfonate (DBSNa), the reaction was possible and the product (5a) was obtained in good yields.

Sodium dodecylbenzene sulfonate was used in different reaction media such as ethanol, methanol, water, and acetic acid (Table 2, entries 6–9). The best results were obtained when DBSNa was used as catalyst in acetic acid as reaction medium, which provided a yield of 94% (Table 2, entry 8).

Unfortunately, when the reaction was performed in water, the yield of the desired product was obtained in a trace amount (Table 2, entry 9).

Evaluation of catalytic activity of DBSNa To determine the appropriate concentration of the catalyst used, we investigated the model reaction at different concentrations of DBSNa (5, 10, 15, 20, and 25 mol %). It was found that when the amount of DBSNa was increased from 5 to 20 mol %, the yield increased from 68 to 94%, respectively. However, there was no significant change in reaction yield when the amount of catalyst was increased further, to 25 mol %. Thus, 20 mol % DBSNa in acetic acid is sufficient to push this reaction forward (Table 3).

Synthesis of Mannich bases of 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (6a-r)

The second part of the research includes the preparation of a series of Mannich bases (6a-r) in good yield (71-91%) by the reaction of 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a–f) with formaldehyde and secondary amines (piperidine, morpholine, and pyrrolidine) in absolute ethanol at room temperature for 1.5-4 h (Scheme 4 and Table 4).

The structures of the isolated new products (6a-r) were deduced by analyzing their physical and spectroscopic data, such as the data obtained using IR, ¹H NMR, and ¹³C NMR spectroscopy.

Taking (6b) as an example, the IR spectrum showed the lack of the absorption band corresponding to NH group. The ¹H NMR spectrum showed the presence of a singlet signal at δ = 4.15 ppm for the methylene protons and two triplet signals at 2.47, 3.59, ppm for morpholine ring protons.

Antimicrobial activity

In vitro antibacterial activity

The synthesized compounds (6a-r) were screened in vitro for their antibacterial at 50 mg/mL concentration against Staphylococcus aureus as Gram positive bacteria, Escherichia Coli, Pseudomonas aerogenosa as Gram negative bacteria using Ciprofloxacin as standard antibacterial reference.
Most of the tested compounds showed excellent antibacterial activities with respect to the reference drug.

The results obtained in Table 5 indicated that the type of substituents (Ar) and (R) are the controlling factors in developing the total antibacterial properties of the tested Mannich bases (6a-r).

Data in Table 5 revealed that compounds 6e, 6h, 6k, 6m, 6n, 6p and 6q, have superior significant antibacterial potency. Compounds 6n and 6q [Ar = 4-MeOC6H4 and 4-pyridyl; R = morpholin-1-yl] have excellent activities against Gram positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia Coli and Pseudomonas aeruginosa). Compound 6p [Ar = 4-pyridyl; R = piperidin-1-yl] has excellent potency against Staphylococcus aureus, moderate activity against Pseudomonas aeruginosa, and poor activity against Escherichia Coli. Structure-Activity relationships (SAR) based on the obtained results indicated that the best observed antibacterial activity is that which Ar is phenyl ring attached with electron donating function (MeO) as exhibit in compound 6n. However, substituting the phenyl ring with electron withdrawing function (Cl, NO2) the antibacterial behavior is decreased. When Ar is unsubstituted phenyl ring the antibacterial activity is not significant. It has also, been noticed that when Ar is pyridine ring exhibited in (6p) and (6q) increase in the observed antibacterial properties was noticed compared with the case of using phenyl ring.

In vitro antifungal activity

With respect to antifungal activity, the synthesized compounds were screened against three fungal strains; Aspergillus niger, Candida albicans, Fusarium oxysporium using Nystatin as standard antifungal reference (at 50 mg/mL concentration). Most of the tested compounds showed excellent antibacterial activities with respect to the reference drug.

As antibacterial activity, the obtained results indicated that the type of substituents (Ar) and (R) are the controlling factors in developing the antifungal properties of the tested compounds (6a-r). Results of antifungal activities were shown in Table 6. Data in Table 6 showed that compounds 6d, 6l, and 6p have remarkable antifungal potency. Compounds 6l [Ar = 4-pyridyl; R = piperidin-1-yl] exhibit excellent activities against Candida albicans and Fusarium oxysporium as well as good potency against Aspergillus niger. Compound 6p [Ar = 4-pyridyl; R = piperidin-1-yl] has excellent activity against Aspergillus niger as well as good potency against Candida albicans and Fusarium oxysporium. Compound 6d [Ar = 4-ClC6H4; R = piperidin-1-yl] exhibit good potency against Aspergillus niger, Candida albicans and Fusarium oxysporium. Structure-Activity relationships (SAR) based on the obtained results indicated that the best observed antifungal activity is that which Ar is phenyl ring attached with electron withdrawing function (NO2, Cl) as exhibit in compound 6l and 6d, respectively. However, substituting the phenyl ring with electron donating function (MeO) the antifungal behavior is decreased. When Ar is unsubstituted phenyl ring the antifungal activity is not remarkable. It has also, been noticed that when Ar is pyridine ring exhibited in (6p) increase in the observed potent antifungal properties was noticed compared with the case of using phenyl ring.

Conclusions

The authors have developed a novel, rapid and efficient protocol for the synthesis of various 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones using sodium dodecylbenzene sulfonate (DBSNa) in acetic acid at room temperature. The results clearly demonstrate that the using of the sodium dodecylbenzene sulfonate as an inexpensive and readily available reagent markedly enhances the efficiency of the chemical processes of interest. Mannich bases from the synthesized 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones were achieved and evaluated as antimicrobial agents and showed remarkable activities.

![Scheme 2 Synthesis of 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a-f).](image-url)
Experimental Chemistry

General methods The time required for completion of each reaction was monitored by TLC. All melting points are uncorrected and were measured on a Gallenkamp apparatus. The IR spectra were recorded on a Shimadzu 470 IR spectrometer (KBr) \( \nu_{\text{max}}, \text{cm}^{-1} \). The \(^1\)H and \(^{13}\)C NMR spectra were measured on a Varian EM-200 (\(^1\)H: 400 MHz, \(^{13}\)C: 100 MHz) spectrometer with TMS as internal standard. Mass spectra were determined on a JEOL JMS-600 spectrometer. Elemental analyses (C, H, N, and S) were performed on an elemental analysis system GmbH VarioEL V2.3.

General procedure for synthesis of 2-Arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a-f)

To a solution of (3) (1.71 g, 10 mmol) in acetic acid (10 mL), corresponding aromatic aldehyde (10 mmol) was added. Then DBS\(\text{Na} \) (20 mol%) was added and the reaction mixture was stirred at room temperature for the desired time as monitored by TLC (Table 1). After completion of the reaction, the solid product was filtered and washed with cold water, dried, and recrystallized from ethanol (95%).

| Table 2 The effect of reaction condition on the synthesis of (5a) under various conditions\(^a\) |
|---|---|---|---|
| Entry | Solvent\(^b\) | Catalyst\(^c\) | Time (min) | Yield\(^d\)(%) |
| 1 | EtOH | Piperidine | 180 | 37 |
| 2 | EtOH | AcOH | 180 | 43 |
| 3 | EtOH | p-TSA | 180 | 40 |
| 4 | AcOH | AcONa | 150 | 46 |
| 5 | AcOH | H\(_2\)SO\(_4\) | 120 | 61 |
| 6 | EtOH | DBS\(\text{Na} \) | 120 | 66 |
| 7 | MeOH | DBS\(\text{Na} \) | 120 | 64 |
| 8 | AcOH | DBS\(\text{Na} \) | 60 | 94 |
| 9 | H\(_2\)O | DBS\(\text{Na} \) | 180 | trace |

\(^a\) The reaction was carried out with 1-thia-4-azaspiro[4.5]decan-3-one (3) (10 mmol), benzaldehydes (4a) (10 mmol) at room temperature.

\(^b\) 10 mL solvent.

\(^c\) 20 mol%.

\(^d\) Isolated yields.

| 2-Benzylidene-1-thia-4-azaspiro[4.5]decan-3-one (5a) |
| Pale yellow crystals; mp 200–202°C; IR: 3200 (NH), 3020 (CH arom.), 1700 (C = O); \(^1\)H NMR (DMSO-d\(_6\)): 1.10–1.95 (m, 10H, 5 × CH\(_2\)), 7.50 (m, 5H, Ph-H), 7.85 (s, 1H, CH), 8.97 (s, 1H, NH, D\(_2\)O-exchangeable) ppm; \(^{13}\)C NMR: 21.7 (2 CH\(_2\)), 24.8 (CH\(_2\)), 25.6 (2CH\(_2\)), 47.9 (spiro C), 122.3 (CH), 124.8 (2CH), 126.1 (2CH), 129.4 (C), 131.1 (C), 136.7 (C), 170.2 (C = O) ppm; EI-MS, \( m/z \) (%): 260.10 (8) [M + 1], 259.01 (54) [M+], 216.05 (100.0). Calc. for C\(_{15}\)H\(_{17}\)NOS: C, 69.46; H, 6.61; N, 5.40; S, 12.36. Found: C, 69.28; H, 6.47; N, 5.24; S, 12.16.

| 2-(4-Chlorobenzylidene)-1-thia-4-azaspiro[4.5]decan-3-one (5b) |
| Pale yellow crystals; mp 210–212°C; IR: 3190 (NH), 3085 (CH arom.), 1695 (C = O), 695 (C-S); \(^{13}\)C NMR: 21.7 (2 CH\(_2\)), 24.8 (CH\(_2\)), 25.6 (2CH\(_2\)), 47.9 (spiro C), 122.3 (CH), 124.8 (2CH), 126.1 (2CH), 129.4 (C), 131.1 (C), 136.7 (C), 170.2 (C = O) ppm; EI-MS, \( m/z \) (%): 293.54 (44) [M+], 258.15 (30), 250.02 (100.0). Calc. for C\(_{15}\)H\(_{16}\)ClNOS: C, 61.32; H, 5.49; Cl, 12.07; N, 4.77; S, 10.91. Found: C, 61.12; H, 5.30; Cl, 12.07; N, 4.77; S, 10.78.

| 2-(4-Bromobenzylidene)-1-thia-4-azaspiro[4.5]decan-3-one (5c) |
| Pale yellow crystals; mp 221–222°C; IR: 3200 (NH), 3085 (CH arom.), 1695 (C = O), 695 (C-S); \(^{13}\)C NMR: 21.7 (2 CH\(_2\)), 24.8 (CH\(_2\)), 25.6 (2CH\(_2\)), 47.9 (spiro C), 122.3 (CH), 124.8 (2CH), 126.1 (2CH), 129.4 (C), 131.1 (C), 136.7 (C), 170.2 (C = O) ppm; EI-MS, \( m/z \) (%): 339.01 (20) [M+], 296.15 (100.0), 79 (15). Calc. for C\(_{15}\)H\(_{16}\)BrNOS: C, 63.22; H, 5.49; Cl, 12.07; N, 4.77; S, 10.78.

| 2-(4-Chlorobenzylidene)-1-thia-4-azaspiro[4.5]decan-3-one (5b) |
| Pale yellow crystals; mp 210–212°C; IR: 3190 (NH), 3085 (CH arom.), 1695 (C = O), 695 (C-S); \(^{13}\)C NMR: 21.7 (2 CH\(_2\)), 24.8 (CH\(_2\)), 25.6 (2CH\(_2\)), 47.9 (spiro C), 122.3 (CH), 124.8 (2CH), 126.1 (2CH), 129.4 (C), 131.1 (C), 136.7 (C), 170.2 (C = O) ppm; EI-MS, \( m/z \) (%): 293.54 (44) [M+], 258.15 (30), 250.02 (100.0). Calc. for C\(_{15}\)H\(_{16}\)ClNOS: C, 61.32; H, 5.49; Cl, 12.07; N, 4.77; S, 10.91. Found: C, 61.12; H, 5.30; Cl, 12.07; N, 4.59; S, 10.78.

Scheme 3 Synthesis of 2-benzylidene-1-thia-4-azaspiro[4.5]decan-3-ones (5a).

Table 3 Evaluation of catalytic activity of DBS\(\text{Na} \) in the synthesis of (5a)\(^a\)

| Entry | Amount of DBS\(\text{Na} \) (mol %) | Time (min) | Yield\(^b\)(%) |
|---|---|---|---|
| 1 | 5 | 90 | 68 |
| 2 | 10 | 70 | 81 |
| 3 | 15 | 60 | 87 |
| 4 | 20 | 60 | 94 |
| 5 | 25 | 60 | 94 |

\(^a\) The reaction was carried out with 1-thia-4-azaspiro[4.5]decan-3-one (3) (10 mmol), benzaldehydes (4a) (10 mmol) and DBS\(\text{Na} \) in 10 mL acetic acid at room temperature.

\(^b\) Isolated yields.
2-(4-Nitrobenzylidene)-1-thia-4-azaspiro[4.5]decan-3-one (5d)

Yellow crystals; mp 249–250°C; IR: 3220 (NH), 3090 (CH arom.), 1690 (C = O), 690 (C-S); 1H NMR (DMSO-d6): 1.20–2.10 (m, 10H, 5 × CH2), 8.10 (d, 2H, 2CH, J = 7.4), 8.50 (d, 2H, 2CH, J = 7.3), 8.70 (s, 1H, CH), 10.20 (s, 1H, NH) ppm; EI-MS, m/z (%): 304.61 (29) [M]+, 261.25 (100.0). Calc. for C15H16N2O3S: C, 59.19; H, 5.30; N, 9.20; S, 10.54. Found: C, 58.89; H, 5.43; N, 9.00; S, 10.30.

2-(4-Methylbenzylidene)-1-thia-4-azaspiro[4.5]decan-3-one (5e)

Yellow crystals; mp 230–231°C; IR: 3200 (NH), 3090 (CH arom.), 1695 (C = O), 690 (C-S); 1H NMR (DMSO-d6): 1.10–2.05 (m, 10H, 5 × CH2), 3.85 (s, 3H, CH3), 7.05 (d, 2H, 2CH, J = 7.3), 7.60 (d, 2H, 2CH, J = 7.3), 7.85 (s, 1H, CH), 8.90 (s, 1H, NH) ppm; 13C NMR: 22.2 (2 CH2), 24.7 (CH2), 26.1 (2CH2), 42.1 (CH3), 47.3 (spiro C), 121.9 (CH), 125.3 (2CH), 126.7 (2CH), 129.2 (C), 131.5 (C), 136.9 (C), 171.3 (C = O) ppm; EI-MS, m/z (%): 290.54 (12) [M]+, 289.02 (40) [M]+, 274.01 (17), 246.12 (100.0). Calc. for C16H19NO2S: C, 66.41; H, 6.62; N, 4.84; S, 11.08. Found: C, 66.09; H, 6.45; N, 4.68; S, 10.85.

2-(Pyridin-4-ylmethylene)-1-thia-4-azaspiro[4.5]decan-3-one (5f)

Pale yellow crystals; mp 225–227°C; IR: 3190 (NH), 3090 (CH arom.), 1695 (C = O), 690 (C-S); 1H NMR (DMSO-d6): 1.05–2.10 (m, 10H, 5CH2), 7.00 (d, 2H, 2CH, J = 6.8), 7.70 (d, 2H, 2CH, J = 6.8), 7.80 (s, 1H, CH), 8.10 (s, 1H, NH) ppm; EI-MS, m/z (%):260.15 (25) [M]+, 217.15 (100.0). Calc. for C14H16N2OSS: C, 64.58; H, 6.19; N, 10.76; S, 12.32. Found: C, 64.36; H, 6.00; N, 10.79; S, 12.20.

General procedure for synthesis of Mannich Bases (6a-r)

To a solution of 2-benzylidene-1-thia-4-azaspiro[4.5]decan-3-one (5a) (0.259 g, 1 mmol) in 5 mL of absolute ethanol was added a mixture of sec. amine (1.5 mmol) and aqueous formaldehyde 35% (0.2 mL, 1.5 mmol) also dissolved in 5 mL absolute ethanol. The reaction mixture was stirred for the desired time as monitored by TLC (Table 4), refrigerated for 24 h to form crystals. The crystalline product was separated by filtration, vacuum dried and recrystallized from ethanol.

2-Benzylidene-4-(piperidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6a)

Pale yellow crystals; mp 119–120°C; IR: 3030 (CH arom.), 2950 (CH aliph.), 1700 (C = O), 695 (C-S); 1H NMR (CDCl3): 1.38–1.51 (m, 12H, 6CH2), 2.10–2.37 (m, 4H, 2CH2), 2.43–2.48 (m, 4H, 2CH2), 4.08 (s, 2H, CH2),

| Mannich product | Ar            | R             | Time (h) | Yield (%) |
|-----------------|---------------|---------------|----------|-----------|
| 6a              | Ph            | piperidin-1-yl| 2.0      | 80        |
| 6b              | Ph            | morpholin-1-yl| 3.0      | 76        |
| 6c              | Ph            | pyrrolidin-1-yl| 2.5      | 79        |
| 6d              | 4-ClC6H4      | piperidin-1-yl| 1.5      | 91        |
| 6e              | 4-ClC6H4      | morpholin-1-yl| 2.5      | 88        |
| 6f              | 4-ClC6H4      | pyrrolidin-1-yl| 2.0      | 80        |
| 6g              | 4-BrC6H4      | piperidin-1-yl| 2.0      | 81        |
| 6h              | 4-BrC6H4      | morpholin-1-yl| 2.0      | 80        |
| 6i              | 4-BrC6H4      | pyrrolidin-1-yl| 3.0      | 80        |
| 6j              | 4-O2NC6H4     | piperidin-1-yl| 3.0      | 76        |
| 6k              | 4-O2NC6H4     | morpholin-1-yl| 4.0      | 71        |
| 6l              | 4-O2NC6H4     | pyrrolidin-1-yl| 3.5      | 73        |
| 6m              | 4-MeOC6H4     | piperidin-1-yl| 1.5      | 89        |
| 6n              | 4-MeOC6H4     | morpholin-1-yl| 2.5      | 81        |
| 6o              | 4-MeOC6H4     | pyrrolidin-1-yl| 2.0      | 83        |
| 6p              | 4-pyridyl     | piperidin-1-yl| 2.0      | 89        |
| 6q              | 4-pyridyl     | morpholin-1-yl| 3.0      | 79        |
| 6r              | 4-pyridyl     | pyrrolidin-1-yl| 2.0      | 82        |

a Reaction conditions: 2-arylidine-1-thia-4-azaspiro[4.5]decan-3-ones (5a-f) (10 mmol), formaldehyde (15 mmol), and secondary amine (15 mmol) in 10 mL absolute ethanol at room temperature.

b Isolated yields.
Table 5 Bactericidal activity of Mannich bases (6a-r) using Ciprofloxacin as standard antibacterial reference

| Compound | Staphylococcus aureus | Escherichia Coli | Pseudomonas aeroginosa |
|----------|-----------------------|------------------|------------------------|
| 6a       | –                     | +                | –                      |
| 6b       | ++                    | +                | ++                     |
| 6c       | –                     | –                | –                      |
| 6d       | ++                    | +                | –                      |
| 6e       | +++                   | +++              | +++                    |
| 6f       | –                     | +                | –                      |
| 6g       | +                     | ++               | –                      |
| 6h       | ++                    | ++               | +++                    |
| 6i       | –                     | –                | +                      |
| 6j       | ++                    | –                | –                      |
| 6k       | +                     | +++              | ++                     |
| 6l       | –                     | –                | –                      |
| 6m       | +++                   | +++              | ++                     |
| 6n       | +++++                 | +++              | +++                    |
| 6o       | +                     | +                | –                      |
| 6p       | +++++                 | +                | ++                     |
| 6q       | +++++                 | +++              | +++                    |
| 6r       | –                     | ++               | +                      |
| Ciprofloxacin | +++++             | +++              | +++                    |

*The activities are based on the diameter of zones of inhibition in mm. 50 μL of stock solution was applied in each hole of each paper disk. +: <15 mm; ++: 15–24 mm; +++: 25–34 mm; ++++: 35–44 mm.

Table 6 Fungicidal activity of Mannich bases (6a-r) using Nystatin as standard antifungal reference

| Compound | Aspergillus niger | Candida albicans | Fusarium oxysporium |
|----------|-------------------|------------------|---------------------|
| 6a       | +                 | +                | +                   |
| 6b       | +                 | –                | +                   |
| 6c       | –                 | –                | –                   |
| 6d       | +++               | +++              | +++                 |
| 6e       | ++                | +++              | +                   |
| 6f       | +                 | ++               | –                   |
| 6g       | ++                | ++               | ++                  |
| 6h       | +                 | +                | –                   |
| 6i       | –                 | ++               | –                   |
| 6j       | ++                | –                | +                   |
| 6k       | ++                | +                | +                   |
| 6l       | +++               | +++              | +++                 |
| 6m       | ++                | –                | –                   |
| 6n       | +                 | +                | ++                  |
| 6o       | +++               | +                | –                   |
| 6p       | +++               | +                | ++                  |
| 6q       | +++               | +                | +++                 |
| 6r       | +                 | +                | +                   |
| Nystatin | +++++             | +++              | +++                 |

*The activities are based on the diameter of zones of inhibition in mm. 50 μL of stock solution was applied in each hole of each paper disk. +: <15 mm; ++: 15–24 mm; +++: 25–34 mm; ++++: 35–44 mm.
2-Benzylidene-4-(morpholinomethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6b)

Pale yellow crystals; mp 135–137°C; IR: 3020 (CH arom.), 2955 (CH aliph.), 1700 (C = O), 695 (C-S); 1H NMR (DMSO-d$_6$): 1.42–1.51 (m, 6H, 3CH$_2$), 2.15-2.40 (m, 4H, 2CH$_2$), 2.47 (t, 4H, 2CH$_2$, J = 6.9), 3.59 (t, 4H, 2CH$_2$, J = 6.9), 4.15 (s, 2H, CH$_2$), 7.33-7.61 (m, 5H, Ph-H), 7.71 (s, 1H, CH) ppm; EI-MS, m/z (%): 359.01 (5) [M$^+$ + 1], 358.00 (32) [M$^+$], 258.09 (17), 216.05 (100.0). Calc. for C$_{25}$H$_{26}$N$_2$O$_2$: C, 70.71; H, 7.31; N, 7.76; S, 9.08. Found: C, 69.80; H, 7.43; N, 7.76; S, 9.08.

2-Benzylidene-4-(pyrrolidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6c)

Yellow crystals; mp 126–127°C; IR: 3020 (CH arom.), 2880 (CH aliph.), 1705 (C = O), 695 (C-S); 1H NMR (CDCl$_3$): 1.43-1.52 (m, 6H, 3CH$_2$), 1.62 (t, 4H, 2CH$_2$, J = 7.1), 2.17-2.40 (m, 4H, 2CH$_2$), 2.53 (t, 4H, 2CH$_2$, J = 7.1), 4.01 (s, 2H, CH$_2$), 7.30-7.58 (m, 5H, Ph-H), 7.60 (s, 1H, CH) ppm; EI-MS, m/z (%): 341.82 (15) [M$^+$], 258.09 (25), 215.85 (100.0). Calc. for C$_{25}$H$_{26}$N$_2$O$_2$: C, 70.14; H, 7.65; N, 8.18; S, 9.36. Found: C, 69.80; H, 7.43; N, 7.76; S, 9.08.

2-(4-Chlorobenzylidene)-4-(piperidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6d)

Yellow crystals; mp 126–127°C; IR: 3020 (CH arom.), 2950 (CH aliph.), 1705 (C = O), 695 (C-S); 1H NMR (CDCl$_3$): 1.44–1.61 (m, 12H, 6CH$_2$), 2.10-2.46 (m, 8H, 4CH$_2$), 4.25 (s, 2H, CH$_2$), 7.40 (d, 2H, 2CH, J = 7.6), 7.60 (s, 1H, CH), 7.72 (d, 2H, 2CH, J = 7.6) ppm; EI-MS, m/z (%): 390.65 (32) [M$^+$], 292.12 (16), 215.05 (100.0). Calc. for C$_{25}$H$_{26}$ClN$_2$O$_2$: C, 64.51; H, 6.96; Cl, 9.07; N, 7.17; S, 8.20. Found: C, 64.22; H, 6.66; Cl, 8.81; N, 7.00; S, 7.92.

2-(4-Chlorobenzylidene)-4-(piperidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6e)

Pale yellow crystals; mp 131–132°C; IR: 3020 (CH arom.), 2895 (CH aliph.), 1700 (C = O), 690 (C-S); 1H NMR (CDCl$_3$): 1.40–1.51 (m, 6H, 3CH$_2$), 2.10-2.40 (m, 4H, 2CH$_2$), 2.52 (t, 4H, 2CH$_2$, J = 6.9), 3.42 (t, 4H, 2CH$_2$, J = 6.3), 7.52 (d, 2H, 2CH, J = 6.3) ppm; EI-MS, m/z (%): 375.90 (19) [M$^+$], 291.89 (30), 215.07 (100.0). Calc. for C$_{25}$H$_{26}$ClN$_2$O$_2$: C, 63.73; H, 6.68; Cl, 9.41; N, 7.43; S, 8.51. Found: C, 63.45; H, 6.70; Cl, 9.19; N, 7.20; S, 8.27.
2-(4-Nitrobenzylidene)-4-(piperidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6j)

Yellow crystals; mp 142–144°C; IR: 3050 (CH arom.), 2900 (CH aliph.), 1700 (C = O), 690 (C-S); ¹H NMR (CDCl₃): 1.45–1.61 (m, 12H, 6CH₂), 2.17–2.49 (m, 8H, 4CH₂), 4.26 (s, 2H, CH₂), 7.92 (s, 1H, CH), 8.14 (d, 2H, 2CH, J = 6.8), 8.35 (d, 2H, 2CH, J = 6.8) ppm; EI-MS, m/z (%): 402.16 (15) [M⁺ + 1], 303.62 (22), 215.85 (100.0). Calc. for C₂₁H₂₈N₂O₅S: C, 62.82; H, 6.78; N, 10.47; S, 7.99. Found: C, 62.60; H, 6.57; N, 10.49; S, 7.76.

4-(Morpholinomethyl)-2-(4-nitrobenzylidene)-1-thia-4-azaspiro[4.5]decan-3-one (6k)

Yellow crystals; mp 158–160°C; IR: 3020 (CH arom.), 2900 (CH aliph.), 1700 (C = O), 695 (C-S); ¹H NMR (DMSO-d₆): 1.45–1.58 (m, 6H, 3CH₂), 2.20–2.52 (m, 4H, 2CH₂), 2.55 (t, 4H, 2CH₂, J = 7.5), 3.54 (t, 4H, 2CH₂, J = 7.5), 4.20 (s, 2H, CH₂), 7.71 (s, 1H, CH), 8.08 (d, 2H, 2CH, J = 6.8), 8.29 (d, 2H, 2CH, J = 6.8) ppm; EI-MS, m/z (%): 303.12 (41) [M⁺], 303.15 (35), 215.36 (100.0). Calc. for C₂₀H₂₇N₂O₅S: C, 59.53; H, 6.25; N, 10.41; S, 7.95. Found: C, 59.58; H, 6.01; N, 10.26; S, 7.71.

2-(4-Nitrobenzylidene)-4-(pyrrolidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6l)

Yellow crystals; mp 149–151°C; IR: 3025 (CH arom.), 2990 (CH aliph.), 1705 (C = O), 690 (C-S); ¹H NMR (CDCl₃): 1.43–1.52 (m, 6H, 3CH₂), 1.61 (t, 4H, 2CH₂, J = 6.9), 2.25–2.37 (m, 4H, 2CH₂), 2.58 (t, 4H, 2CH₂, J = 6.9), 4.20 (s, 2H, CH₂), 7.41 (s, 1H, CH), 7.93 (d, 2H, 2CH, J = 6.8), 8.21 (d, 2H, 2CH, J = 6.8) ppm; EI-MS, m/z (%): 387.20 (19) [M⁺], 303.10 (44), 215.87 (100.0). Calc. for C₂₀H₂₇N₂O₅S: C, 61.99; H, 6.50; N, 10.84; S, 8.27. Found: C, 62.05; H, 6.24; N, 10.70; S, 8.00.

2-(4-Methoxybenzylidene)-4-(piperidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6m)

Pale yellow crystals; mp 121–123°C; IR: 3200 (NH), 3090 (CH arom.), 1700 (C = O), 690 (C-S); ¹H NMR (CDCl₃): 1.42–1.53 (m, 12H, 6CH₂), 2.15–2.41 (m, 8H, 4CH₂), 3.50 (s, 3H, CH₃), 4.10 (s, 2H, CH₂), 6.92 (d, 2H, 2CH, J = 6.8), 7.45 (d, 2H, 2CH, J = 6.8), 7.56 (s, 1H, CH) ppm; EI-MS, m/z (%): 386.20 (15) [M⁺], 288.45 (28), 216.05 (100.0). Calc. for C₂₂H₂₃NO₅S: C, 68.36; H, 7.82; N, 7.25; S, 8.30. Found: C, 68.39; H, 7.70; N, 7.00; S, 8.02.

2-(4-Methoxybenzylidene)-4-(morpholinomethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6n)

Pale yellow crystals; mp 133–135°C; IR: 3050 (CH arom.), 2950 (CH aliph.), 1700 (C = O), 695 (C-S); ¹H NMR (CDCl₃): 1.41–1.52 (m, 6H, 3CH₂), 2.15–2.46 (m, 4H, 2CH₂), 2.51 (t, 4H, 2CH₂, J = 7.1), 3.50 (t, 4H, 2CH₂, J = 7.1), 4.08 (s, 2H, CH₂), 7.01 (d, 2H, 2CH, J = 6.9), 7.60 (d, 2H, 2CH, J = 6.9), 7.69 (s, 1H, CH) ppm; EI-MS, m/z (%): 388.19 (20) [M⁺], 288.75 (51), 215.06 (100.0). Calc. for C₂₁H₂₂N₂O₆S: C, 64.92; H, 7.26; N, 7.21; S, 8.25. Found: C, 64.70; H, 7.00; N, 7.30; S, 8.01.

2-(4-Methoxybenzylidene)-4-(pyrrolidin-1-ylmethyl)-1-thia-4-azaspiro[4.5]decan-3-one (6o)

Yellow crystals; mp 140–142°C; IR: 3050 (CH arom.), 2900 (CH aliph.), 1700 (C = O), 695 (C-S); ¹H NMR (CDCl₃): 1.40–1.49 (m, 6H, 3CH₂), 1.60 (t, 4H, 2CH₂), 2.20–2.32 (m, 4H, 2CH₂), 2.55 (t, 4H, 2CH₂, J = 7.3), 4.15 (s, 2H, CH₂, J = 7.3), 7.01 (d, 2H, 2CH, J = 6.8), 7.26 (d, 2H, 2CH, J = 6.8), 7.29 (s, 1H, CH) ppm; EI-MS, m/z (%): 372.19 (32) [M⁺], 289.02 (35), 215.97 (100.0). Calc. for C₂₁H₂₆N₂O₅S: C, 67.71; H, 7.58; N, 7.52; S, 8.61. Found: C, 67.58; H, 7.60; N, 7.40; S, 8.45.
Antimicrobial screening

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against bacterial isolate namely Staphylococcus aureus (ATCC 29213) as Gram positive bacteria, Escherichia Coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27953) as Gram negative bacteria by cup-plate method [23]. The sterilized nutrient agar medium was distributed 100 ml each in two 250 ml conical flasks and allowed to cool to room temperature. To these media, 18–24 h grown bacterial subcultures were added and shaken thoroughly to ensure uniform distribution of organism throughout the medium. Then, this agar medium was distributed in equal portions, in sterilized Petri dishes, ensuring that each Petri dish contains about 20 ml of the medium. The medium was then allowed for solidification. Then, cups were made with the help of a sterile cork borer (6 mm diameter) punching into the set of agar media.

The solutions of required concentration (50 μg/mL) of test compounds were prepared by dissolving the compounds in DMSO were filled into the cups with 1 ml of respective solution. Then, the Petri dishes were kept for incubation in an inverted position for 24–48 h at 37°C in an incubator. When growth inhibition zones were developed surrounding each cup, their diameter in cm was measured and compared with that of the Ciprofloxacin.

Antifungal activity

The newly synthesized compounds were screened for their antifungal activity against three fungal strains; Aspergillus niger, Candida albicans, Fusarium oxysporium as Gram positive candida albicans, Fusarium oxysporium–Clean Procedure for Three-component μcontributions′–Escherichia Coli–(2015) 9:25

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