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Synthesis and antimicrobial activity of some novel 2-thienyl substituted heterocycles

Abstract: A series of 2-thienyl substituted derivatives of thiazoles, oxazoles and spiro(indole-azole) were synthesized. The structures of the synthesized compounds were confirmed by IR, NMR and mass spectral data.

Keywords: 2-acetylthiophene; antimicrobial activity; MIC; spiro; thiazolidines.

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Introduction

Thiophene derivatives are important compounds [1–8] with considerable biological activity, such as antimicrobial [9, 10], anti-inflammatory and analgesic activities [11–14]. Spiroindoles possessing a stereogenic center at C-3 [15, 16] and thiazolidine derivatives have also wide applications in synthetic and medicinal chemistry [17–21]. In view of the above mentioned findings and as continuation of our efforts in the synthesis of new biologically active heterocycles [22, 23]. We report herein the synthesis of thiazolidines and spiroindoles linked to thiophene and evaluation of their antimicrobial activities.

Results and discussion

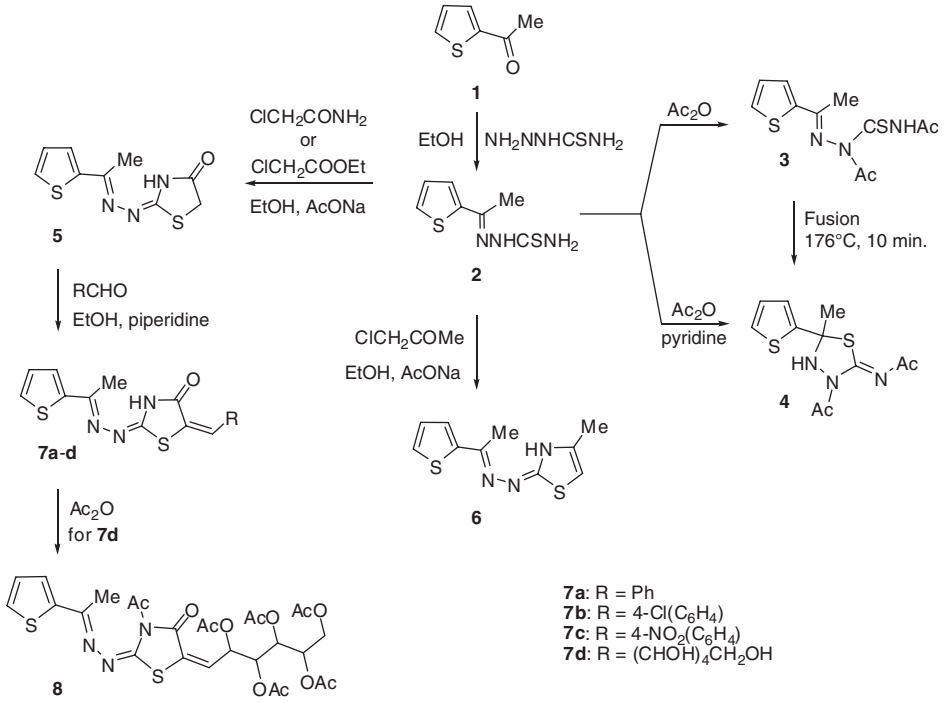
Chemistry

The reaction of 2-acetylthiophene (**1**) with thiosemicarbazide afforded 1-(thiophen-2-yl)ethanone thiosemicarbazone (**2**) according to a previously described method [24]. Treatment of the thiosemicarbazone **2** with freshly distilled acetic anhydride gave ethylidene thiosemicarbazide derivative **3**. Compound **3** was cyclized by heating to give 5-acetamidothiazolidine **4**. Compound **4** was also obtained by heating of **2** with acetic anhydride in pyridine. The structure of **4** was confirmed by IR, NMR and mass spectral analyses.

The reaction of thiosemicarbazone **2** with ethyl chloroacetate, chloroacetamide or chloroacetone afforded the thiazolidinones **5** and **6**. It is of interest to mention that the products of reacting **2** with ethyl chloroacetate or chloroacetamide were identical. The IR spectrum of **5** contains bands at ν 3120 cm^{-1} due to NH function and the C=O band of the thiazolidine ring at ν 1720 cm^{-1}. The ^1H NMR spectrum shows methylene protons signals of the thiazolidine function and NH signals at 3.82 and 11.93 ppm, respectively. The ^{13}C NMR spectrum of **5** is also consistent with the given structure. Compound **5** contains active methylene group. As such, it was condensed with aromatic aldehydes and *D*-glucose in the presence of piperidine to yield the arylidenes **7a**–**d**. Subjecting the prepared aldehydo-hexose chalcone **7d** to acetylation using a mixture of acetic anhydride and pyridine gave the crystalline product **8**. The ^1H NMR spectrum of **8** contains signals for five OAc groups in the range of δ 2.30–2.60 ppm. These acetyl groups show a characteristic absorption band around ν 1670 cm^{-1} in the IR spectrum (Scheme 1).

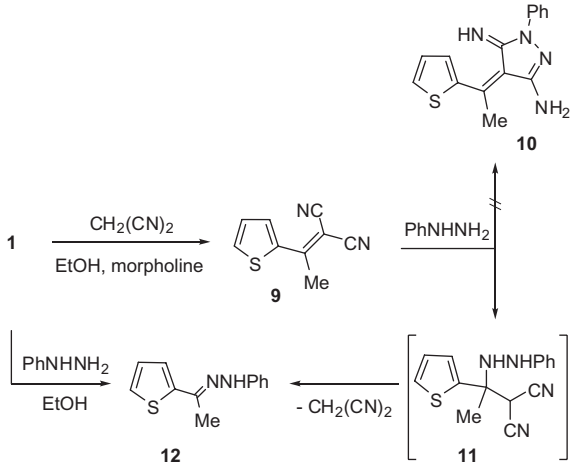
Condensation of **1** with malononitrile in the presence of morpholine led to the formation of dicyanoethylidene derivative **9**. The IR spectrum of **9** shows a strong band at ν 2200 cm^{-1} for two cyano groups. Treatment of **9** with phenylhydrazine in boiling ethanol furnished one isolable product for which structures **10**, **11** and **12** seemed possible (Scheme 2). The IR spectrum of the isolated product shows a lack of the CN absorption band and the presence of absorption band at 3385 cm^{-1} for an NH group. Furthermore, the ^1H NMR spectrum contains a broad singlet signal at 7.90 ppm (D_2O exchangeable) for the NH proton. These findings provide firm support for the structure **12** and exclude the pyrazoline derivative **10**. The structure of **12** was supported by its independent synthesis as shown in Scheme 2.

The chalcone **14** was prepared according to a previously described method [25]. Hydrazinolysis of **14** in ethanol yielded spiro[indole-pyrazole]-2(1*H*)-one **15**. Otherwise, the hydrazinolysis in acetic acid gave the



Scheme 1

acetyl derivative **16**. The presence of acetyl group in the product **16** was established using IR and ^{1}H NMR spectra. The reaction of **14** with hydroxylamine in ethanol containing AcONa produced spiro[indole-oxazole]-2(1*H*)-one **17**. Finally, phenyl-substituted spiro[indole-pyrazole]-2(1*H*)-one **18** was obtained by condensation of chalcone **14** with phenylhydrazine. Analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and EI-MS) confirmed the structures of the synthesized compounds **17** and **18** (Scheme 3).



Scheme 2

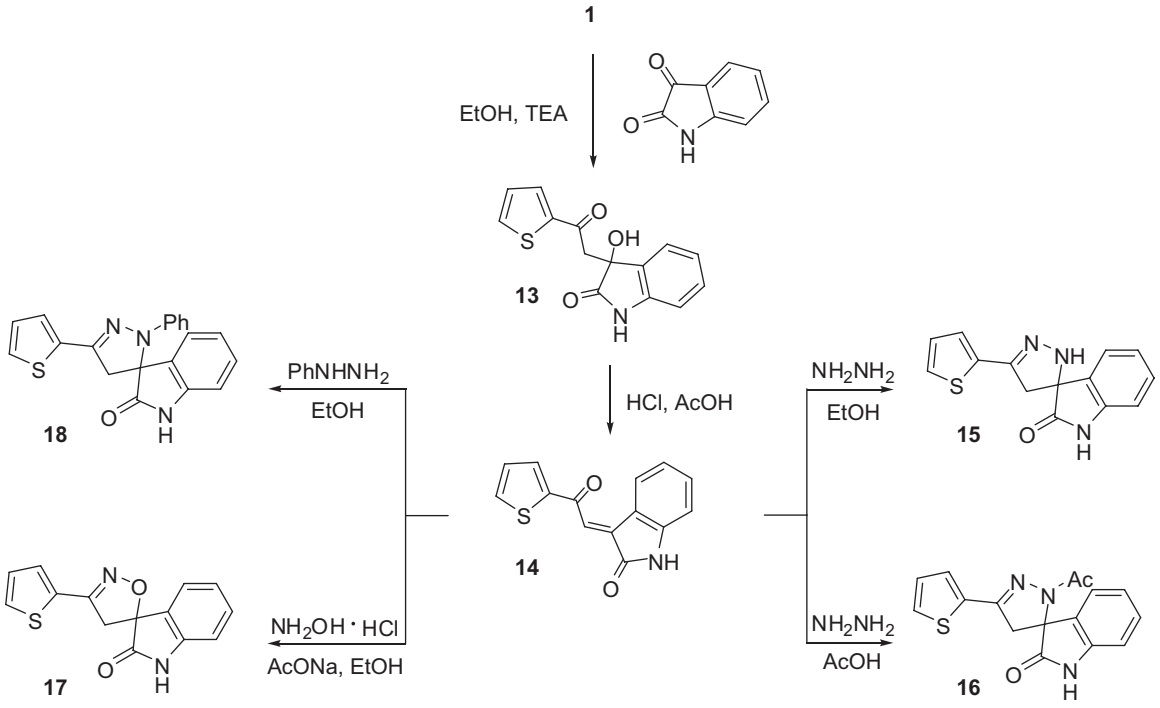
Antimicrobial evaluation

Antibacterial evaluation

All compounds were screened for their antibacterial activities against Gram-positive bacteria (*Staphylococcus aureus* AUMC B-54, *Micrococcus luteus* AUMC B-112, *Bacillus cereus* AUMC B-52, *Escherichia coli* AUMC B-53, *Pseudomonas aeruginosa* AUMC B-73, *Serratia marcescens* AUMC B-55). Chloramphenicol was used as the reference (Table 1). The minimum inhibitory concentrations (MICs) were recorded. All compounds exhibit significant antibacterial activities. Thiazolidinones **7b**–**d** and phenyl substituted spiro[indole-pyrazole]-2-one **18** are remarkably active against the tested antibacterial species at the MIC range of 5–41 μg/mL. Substitution of hydrogen atom at para position of the aromatic ring in compounds **7a–c** by a chlorine atom, or nitro group results in increasing of the antibacterial activity.

Antifungal evaluation

The compounds were also screened for their antifungal activities against six antifungal species, *Candida albicans* AUMC 1299, *Geotrichum candidum* AUMC 226, *Fusarium oxysporum* AUMC 5119, *Aspergillus flavus* AUMC 1276, *Trichophyton rubrum* AUMC 1804 and *Scopulariopsis*



Scheme 3

Table 1 The antibacterial activity of the synthesized compounds, MIC (μg/mL).

No.	*S. aureus* (Gram+)	*M. luteus* (Gram+)	*B. cereus* (Gram+)	*E. coli* (Gram-)	*P. aeruginosa* (Gram-)	*S. marcescens* (Gram-)
2	130	100	50	64	100	70
3	125	90	20	38.5	80	34.5
4	65	60	15	30	55	25
5	78	78	17	35	72	30
6	72	68	20	34	72	28
7a	49	60	15	25	59	15
7b	21.5	41	10	20	30	11
7c	10	30	10	15	20	10
7d	5	20	5	10	10	5
8	10	11	10	–	90	20
9	74	–	37	29	19	40
12	15	38	52	48	125	10
13	50	49	30	55	80	22
14	70	60	60	100	120	50
15	48	50	50	80	79	42
16	30	41	40	64	51	30
17	42	28	49	35	46	27
18	34	20	28	30	40	17
Ref.[a]	10	10	10	10	10	4.5

[a]Chloramphenicol was used as antibacterial standard. –, no activity.

brevicaulis AUMC 361. As shown in Table 2, most of the tested compounds are active. The spiro compounds **15–18** show strong antifungal activities in comparison to chalcone **14**.

Conclusion

A novel series of thiazolidines and spiro(indole-azole) derivatives was synthesized. The antimicrobial activity of

Table 2 The antifungal activity of the synthesized compounds, MIC (μg/mL).

No.	*C. albicans*	*G. candidum*	*T. rubrum*	*F. oxysporum*	*A. flavus*	*S. brevicaulis*
2	110	120	100	90	80	69
3	20	80	34	100	57	66
4	15	62	20	80	30	52
5	20	65	32	96	48	63
6	21	56	30	78	44	62
7a	12	46	20	58	34	36
7b	10	41	15	47	32	24
7c	8	25	10	25	20	20
7d	5	15	10	15	10	10
8	100	40	26	26	55	48
9	–	–	–	–	–	–
12	24	35	30	23	13	32
13	50	55	64	44	70	100
14	64	72	95	68	96	120
15	48	62	73	30	68	90
16	40	50	61	20	50	70
17	31	42	49	15	38	52
18	30	40	45	12	30	41
Ref.[a]	10	20	10	10	10	20

[a]Clotrimazole was used as antifungal standard. –, no activity.

these compounds was evaluated. Compounds **7a–d** and **15–18** display remarkable antimicrobial potency.

Experimental

Chemistry

Melting points were determined with an APP Digital ST 15 melting point apparatus. Infrared spectra were recorded in KBr pellets using a Pye-Unicam SP3–100 spectrophotometer. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were determined using a JNM-LA Series 400 spectrometer and DMSO-d_6. Mass spectra were measured using a JOEL JMS 600 spectrometer. Elemental analyses were carried out on a Vario EL C, H, N, S Analyzer.

1-[1-(2-Thienyl)ethylidene]-2,4-diacetylthiosemicarbazide (3)

A solution of thiosemicarbazone **2** (0.99 g, 0.005 mol) in acetic anhydride (20 mL) was heated under reflux for 2 h. After cooling, excess acetic anhydride was decomposed in an ice water mixture. The solid formed was filtered, dried and crystallized from ethanol to afford yellow powder; yield 65%; mp 173–175°C; ^{1}H NMR: δ 2.02 (s, 3H, CH_3), 2.31 (s, 3H, $COCH_3$), 2.49 (s, 3H, $NCOCH_3$), 7.04–7.46 (m, 3H, thiophene-H), 11.70 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 14.7, 22.2, 23.9, 122.3, 129.1, 134.7, 140.7, 155.9, 163.9, 171.0, 183.1; IR: ν 3200 (NH), 2920 (CH-aliph.), 1650 and 1670 for 2 (C=O), 1590 (C=N) cm^{-1}; MS: m/z 283.23 ([M]$^+$, 87%). Anal. Calcd for $C_{11}H_{13}N_3O_2S_2$ (283.37): C, 46.62; H, 4.62; N, 14.83; S, 22.63. Found: C, 46.65; H, 4.58; N, 14.80; S, 22.58.

N-(3-Acetyl-5-methyl-5-(thiophen-2-yl)-1,3,4-thiadiazolidin-2-ylidene)acetamide (4)

Method A A mixture of thiosemicarbazone **2** (0.99 g, 0.005 mol) and acetic anhydride (3 mL) in pyridine (25 mL) was heated under reflux for 3 h, then cooled and poured onto ice water. The solid formed was filtered, dried and crystallized from ethanol to afford red powder; yield 70%; mp 183–184°C; ^{1}H NMR: δ 2.02 (s, 3H, acetyl), 2.13 (s, 3H, acetyl), 2.31 (s, 3H, CH_3), 7.04–7.46 (m, 3H, thiophene-H), 11.70 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 22.5, 23.9, 27.6, 76.0, 120.9, 129.1, 134.7, 140.7, 148.7, 168.0, 169.5; IR: ν 3350 (NH), 2920 (CH-aliph.), 1720 and 1700 cm^{-1} (C=O); MS: m/z 283.28 ([M]$^+$, 91). Anal. Calcd for $C_{11}H_{13}N_3O_2S_2$ (283.37): C, 46.62; H, 4.62; N, 14.83; S, 22.63. Found: C, 46.58; H, 4.59; N, 14.85; S, 22.57.

Method B The thiosemicarbazide **3** was heated in an oil bath at 176°C for 10 min. The solid was triturated with petroleum ether (bp 60–80°C), filtered, dried and crystallized from ethanol. The product was identical to that produced by method A.

2-[(1-(2-Thienyl)ethylidene)hydrazono]thiazolidin-4-one (5)

Method A Thiosemicarbazone **2** (0.99 g, 0.005 mol) and ethyl chloroacetate (0.62 mL, 0.005 mol) were dissolved in hot absolute ethanol (20 mL) containing anhydrous sodium acetate (1 g). The reaction mixture was heated under reflux for 5 h, then cooled and poured onto crushed ice. The solid product was filtered, dried and crystallized from ethanol to afford pale yellow crystals; yield 74%; mp 278–279°C; ^{1}H NMR: δ 2.30 (s, 3H, CH_3), 3.82 (s, 2H, CH_2

thiazolidine), 7.00–7.57 (m, 3H, thiophene-H), 11.93 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 14.9, 32.9, 123.8, 129.1, 134.7, 141.7, 156.3, 163.6, 174.0; IR: ν 3120 (NH), 2825 (CH-aliph.), 1720 cm^{-1} (C=O thiazolidine); MS: m/z 239.24 ($[M]^+$, 82%). Anal. Calcd for $C_9H_9N_3OS_2$ (239.32): C, 45.17; H, 3.79; N, 17.56; S, 26.80. Found: C, 45.16; H, 3.81; N, 17.53; S, 26.78.

Method B A mixture of thiosemicarbazone **2** (0.99 g, 0.005 mol) and chloroacetamide (0.46 g, 0.005 mmol) in ethanol (20 mL) containing anhydrous sodium acetate (1 g) was heated under reflux for 5 h, then cooled and poured onto crushed ice. The solid product was filtered, dried and crystallized from ethanol to afford pale yellow crystals. The product was identical to compound **5** obtained by method A.

4-Methyl-2-[(1-(2-thienyl)ethylidene) hydrazono]-2,3-dihydrothiazole (6)

To a solution of thiosemicarbazone **2** (0.99 g, 0.005 mol) in absolute ethanol (20 mL) was added the equivalent amount of chloroacetone (0.46 mL, 0.005 mol) and anhydrous sodium acetate (1 g). The mixture was heated under reflux for 5 h, cooled and poured onto ice water. The solid product was collected by filtration, dried and crystallized from ethanol to afford yellow powder; yield 65%; mp 201–203°C; ^1H NMR: δ 1.80 (s 3H, CH_3), 2.30 (s, 3H, CH_3 thiazolidine), 6.20 (s, 1H, CH-thiazolidine), 6.40 (s, 1H, CH thiazolidine), 6.90–7.40 (m, 3H, thiophene-H), 9.30 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 14.9, 24.2, 98.4, 120.9, 129.1, 134.7, 140.9, 150.1, 155.3, 162.1; IR: ν 3250 (NH), 2978 cm^{-1} (CH-aliph.); MS: m/z 237.28 ($[M]^+$, 69). Anal. Calcd for $C_{10}H_{11}N_3S_2$ (237.34): C, 50.60; H, 4.67; N, 17.70; S, 27.02. Found: C, 50.62; H, 4.69; N, 17.68; S, 27.06.

General procedure for the synthesis of 7a–d

A mixture of **5** (1.20 g, 0.005 mol), aromatic aldehyde (0.005 mol) and piperidine (0.005 mol) in dioxane (20 mL) was heated under reflux for 3 h, then cooled and poured onto ice water. The solid product **7a–d** was collected by filtration, washed with water and crystallized from ethanol.

5-Benzylidene-2-[(1-(2-thienyl)ethylidene)hydrazono]thiazolidin-4-one (**7a**) A brown powder; yield 69%; mp 232–234°C; ^1H NMR: δ 2.20 (s, 3H, CH_3), 6.90–7.75 (m, 8H, aromatic-H + thiophene-H), 8.25 (s, 1H, =CH), 11.20 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 15.8, 115.8, 123.7, 129.2, 129.8, 131.2, 133.1, 134.8, 135.8, 137.2, 138.1, 140.3, 142.7, 163.3, 164.6, 173.0; IR: ν 3120 (NH), 3015 (CH arom.), 2920 (CH aliph.), 1690 cm^{-1} (C=O thiazolidine); MS: m/z 327.40 ($[M]^+$, 67%). Anal. Calcd for $C_{16}H_{13}N_3OS_2$ (327.42): C, 58.69; H, 4.00; N, 12.83; S, 19.59. Found: C, 58.65; H, 4.03; N, 12.80; S, 19.63.

5-*p*-Chlorobenzylidene-2-[(1-(2-thienyl)ethylidene)hydrazono] thiazolidin-4-one (**7b**) A red powder; yield 67%; mp 223–225°C; ^1H NMR: δ 2.15 (s, 3H, CH_3), 6.90–7.80 (m, 7H, aromatic-H + thiophene-H), 8.20 (s, 1H, =CH), 11.10 (s, 1H, NH, D_2O exchangeable); IR: ν 320 (NH), 3015 (CH arom.), 2960 and 2855 (CH aliph.), 1690 cm^{-1} (C=O thiazolidine); MS: m/z 361.81 ($[M]^+$, 67%), 363.64 ($[M+2]^+$, 23%). Anal. Calcd for $C_{16}H_{12}ClN_3OS_2$ (361.87): C, 53.11; H, 3.34; Cl, 9.80; N, 11.61; S, 17.72. Found: C, 53.09; H, 3.31; Cl, 9.77; N, 11.63; S, 17.74.

5-*p*-Nitrobenzylidene-2-[(1-(2-thienyl)ethylidene)hydrazono] thiazolidin-4-one (**7c**) Yellow crystals; yield 65%; mp 211–213°C; ^1H NMR: δ 2.15 (s, 3H, CH_3), 6.90–8.00 (m, 7H, aromatic-H + thiophene-H), 8.20 (s, 1H, =CH), 13.40 (s, 1H, NH, D_2O exchangeable); IR: ν 3150 (NH), 3020 (CH arom.), 2930 (CH aliph.), 1695 (C=O thiazolidine), 1570 cm^{-1} (NO_2); MS: m/z 372.39 ($[M]^+$, 67%). Anal. Calcd for $C_{16}H_{12}N_4O_3S_2$ (372.42): C, 51.60; H, 3.25; N, 15.04; S, 17.22. Found: C, 51.58; H, 3.21; N, 15.01; S, 17.25.

5-Glucosylidene-2-[(1-(thien-2-yl)ethylidene)hydrazono]thiazolidin-4-one (**7d**) White crystals; yield 61%; mp 201–203°C; ^1H NMR: δ 2.22 (s, 3H, CH_3), 2.90–3.02 (m, 2H, alditolyl 2H), 3.10–3.30 (m, 2H, alditolyl 2H), 4.10–4.30 (m, 2H, alditolyl 2H), 4.60 (s, 2H, 2 OH, D_2O exchangeable), 4.90–5.30 (2 m, 1H each, 2 OH, D_2O exchangeable), 5.70 (d, 1H, 1 OH, D_2O exchangeable), 6.10 (s, 1H each, CH=C), 6.49–7.98 (m, 3H, thiophene-H), 11.10 (s, 1H, NH, D_2O exchangeable); IR: ν 3150 (NH), 2950 (CH-aliph.), 1680 cm^{-1} (C=O thiazolidine); MS: m/z 401.83 ($[M]^+$, 70%). Anal. Calcd for $C_{15}H_{19}N_3O_6S_2$ (401.46): C, 44.88; H, 4.77; N, 10.47; S, 15.97. Found: C, 44.86; H, 4.74; N, 10.46; S, 15.94.

2,3,4,5,6-Penta-*O*-acetyl-aldehydo-*D*-glucose-3-acetyl-2-[(1-(2-thienyl)ethylidene) hydrazono]thiazolidin-4-one (8)

A solution of **7d** (2.0 g, 0.005 mol) in pyridine (15 mL) was treated with acetic anhydride (20 mL) and the mixture was stirred at ambient temperature for 24 h, then was poured onto ice water and extracted with $CHCl_3$ (3 × 20 mL). The organic layer was washed with 10% $NaHSO_4$ solution (2 × 20 mL), dried (Na_2SO_4), cooled and poured onto crushed ice. The precipitate was collected, dried and crystallized from ethanol to give white powder; yield 61%; mp 156–158°C; ^1H NMR: δ 2.10 (s, 3H, CH_3), 2.30–2.60 (m, 15H, 5 $COCH_3$), 3.20 (s, 3H, $NCOCH_3$), 4.30–5.50 (5 m, 6H, alditolyl H), 6.00–6.20 (m, 1H, C=CH), 6.90–7.45 (m, 3H, thiophene-H); ^{13}C NMR: δ 14.8, 19.0, 20.1, 21.2, 21.9, 22.5, 23.5, 60.6, 62.1, 63.9, 65.5, 68.7, 118.6, 123.7, 124.5, 135.6, 142.7, 145.1, 163.1, 165.0, 170.0, 175.0, 175.7, 175.2, 176.1, 176.9, 178.1; IR: ν 2920 (CH-aliph.), 1680 (C=O thiazolidine), 1670 (C=O) alditolyl, 1590 cm^{-1} (C=N); MS: m/z 653.60 ($[M]^+$, 91%). Anal. Calcd for $C_{27}H_{31}N_3O_{12}S_2$ (653.68): C, 49.61; H, 4.78; N, 6.43; S, 9.81. Found: C, 49.58; H, 4.82; N, 6.45; S, 9.78.

2-[1-(2-Thienyl)ethylidene]malononitrile (9)

To solution of **1** (0.63 mL, 0.005 mol) in absolute ethanol (20 mL) was added malononitrile (0.33 g, 0.005 mol) and a catalytic amount of morpholine. The mixture was heated under reflux for 3 h and then cooled. The solid product was collected, dried and crystallized from ethanol to give pale yellow crystals; yield 74%; mp 197–199°C; ^1H NMR: δ 2.40 (s, 3H, CH_3), 6.90–7.45 (m, 3H, thiophene-H); ^{13}C NMR: δ 30.2, 90.7, 118.1, 118.9, 122.7, 124.8, 135.7, 142.9, 161.5; IR: ν 2940 (CH-aliph.), 2200 cm^{-1} (CN); MS: m/z 174.20 ($[M]^+$, 64%). Anal. Calcd for $C_9H_6N_2S$ (174.22): C, 62.05; H, 3.47; N, 16.08; S, 18.40. Found: C, 62.08; H, 3.49; N, 16.11; S, 18.46.

N-[1-(2-Thienyl)ethylidene]-*N*′-phenylhydrazine (12)

Method A Phenylhydrazine (0.57 g, 0.005 mol) was added to a solution of **1** (0.63 mL, 0.005 mol) in ethanol (25 mL) and the mixture was heated under reflux for 1 h. Upon cooling, the precipitate was collected by filtration, dried and crystallized from ethanol to give white powder; yield 70%; mp 177–178°C; ^{1}H NMR: δ 2.40 (s, 3H, CH_3), 6.85–7.90 (m, 9H, Ar-H + thiophene-H + NH, D_2O exchangeable); ^{13}C NMR: δ 15.2, 120.4, 123.7, 126.2, 126.9, 135.8, 141.8, 147.3, 150.1, 160.5; IR: ν 3385 (NH), 3040, 3010 (CH-arom.), 2920 cm^{-1} (CH-aliph.); MS: m/z 216.22 ([M]$^+$, 61%). Anal. Calcd for $C_{12}H_{12}N_2S$ (216.30): C, 66.63; H, 5.59; N, 12.95; S, 14.82. Found: C, 66.61; H, 5.57; N, 12.92; S, 14.85.

Method B A mixture of **9** (0.87 g, 0.005 mol) and phenyl hydrazine (0.54 g, 0.005 mol) in ethanol (20 mL) was heated for 3 h. Upon cooling, the precipitate was filtered, dried and crystallized from ethanol to give white powder. The product was identical to that obtained by method A.

3-Hydroxy-3-(2-oxo-2-(2-thienyl)ethyl) indolin-2-one (13)

A mixture of **1** (0.63 g, 0.005 mol), isatin (0.73 g, 0.005 mol), 3–4 drops of diethylamine and ethanol (25 mL) was heated under reflux on the steam bath for 30 min. After standing for several days at room temperature, the product was collected by filtration, dried and crystallized from ethanol to afford white powder; mp 177–178°C [25].

3-(2-Oxo-2-(2-thienyl)ethylidene)indolin-2-one (14)

A mixture of **13** (1.36 g, 0.005 mmol), concentrated hydrochloric acid (0.25 mL) and glacial acetic acid (8 mL) was heated on the steam bath for 15–30 min. After addition of ethanol and standing at room temperature, the product was filtered, dried and crystallized from ethanol; mp 176–177°C [25].

5′-4-(2-Thienyl)-2′,4′-dihydrospiro[indole-3,3′-pyrazole]-2(1*H*)-one (15)

A mixture of chalcone **14** (0.26 g, 0.001 mol) and hydrazine hydrate (5 mL) in ethanol (20 mL) was heated under reflux for 6 h. On cooling, the solid formed was filtered and crystallized from ethanol to afford pale yellow crystals; yield 71%; mp 197–199°C; ^{1}H NMR: δ 2.20 (s, 2H, CH_2), 6.95–7.90 (m, 7H, A-H + thiophene-H), 8.50 (s, 1H, pyrazole-NH, D_2O exchangeable), 10.20 (s, 1H, NH, D_2O exchangeable); IR: ν 3250 and 3150 (NH), 2920 (CH-aliph.), 1690 cm^{-1} (C=O indole); MS: m/z 269.30 ([M]$^+$, 71%). Anal. Calcd for $C_{14}H_{11}N_3OS$ (269.32): C, 62.43; H, 4.12; N, 15.60; S, 11.91. Found: C, 62.41; H, 4.10; N, 15.63; S, 11.94.

2′-Acetyl-5′-(2-thienyl)-2′,4′-dihydrospiro[indole-3,3′-pyrazole]-2(1H)-one (16)

A mixture of chalcone derivative **14** (0.26 g, 0.001 mol) and hydrazine hydrate (5 mL) in acetic acid (20 mL) was heated under reflux for 5 h. On cooling, the solid formed was filtered and crystallized from dioxane; yield 69%; mp 201–203°C; ^{1}H NMR: δ 2.30 (s, 2H, CH_2), 2.60 (s, 3H, $COCH_3$), 6.90–8.10 (m, 7H, Ar-H + thiophene-H), 10.50 (s, 1H, NH, D_2O exchangeable); IR: ν 3210 (NH), 3010 and 3000 (CH-arom.), 2920 (CH-aliph.), 1690 (C=O indole), 1660 cm^{-1} (C=O); MS: m/z 311.32 ([M]$^+$, 71%). Anal. Calcd for $C_{16}H_{13}N_3O_2S$ (311.36): C, 61.72; H, 4.21; N, 13.50; S, 10.30. Found: C, 61.70; H, 4.18; N, 13.55; S, 10.24.

5′-(2-Thienyl)-1′,4′-dihydrospiro[indole-3,3′-oxazole]-2(1*H*)-one (17)

A solution of **14** (0.26 g, 0.001 mol) and hydroxylamine hydrochloride (0.07 g, 0.001 mol) in ethanol (20 mL) containing anhydrous sodium acetate (0.4 g) was heated under reflux for 5 h, then cooled and poured onto crushed ice. The solid product thus formed was collected by filtration, dried and crystallized from ethanol to give white powder; yield 69%; mp 193–195°C; ^{1}H NMR: δ 1.90 (s, 2H, CH_2), 6.90–8.00 (m, 7H, Ar-H + thiophene-H), 10.10 (s, 1H, NH, D_2O exchangeable); IR: ν 3310 (NH), 3010, 3000 (CH-arom.), 2960 (CH-aliph.), 1685 cm^{-1} (C=O indole); MS: m/z 270.28 ([M]$^+$, 64%). Anal. Calcd for $C_{14}H_{10}N_2O_2S$ (270.31): C, 62.21; H, 3.73; N, 10.36; S, 11.86. Found: C, 62.18; H, 3.75; N, 10.39; S, 11.89.

5′-(2-Thienyl)-2′-phenyl-2′,4′-dihydrospiro[indole-3,3′-pyrazole]-2(1*H*)-one (18)

A mixture of chalcone **14** (0.26 g, 0.001 mol) and phenylhydrazine (0.11 g, 0.001 mol) in ethanol (20 mL) was heated under reflux for 5 h and then cooled. The precipitate was filtered and crystallized from methanol to give white crystals; yield 60%; mp 183–185°C; ^{1}H NMR: δ 2.30 (s, 2H, CH_2), 6.70–8.15 (m, 12H, Ar-H + thiophene-H), 10.00 (s, 1H, NH, D_2O exchangeable); IR: ν 3300 (NH), 3010 and 3000 (CH-arom.), 2920 (CH-aliph.), 1690 cm^{-1} (C=O indole); MS: m/z 345.46 ([M]$^+$, 72%). Anal. Calcd for $C_{20}H_{15}N_3OS$ (345.42): C, 69.54; H, 4.38; N, 12.17; S, 9.28. Found: C, 69.52; H, 4.36; N, 12.19; S, 9.30.

Antimicrobial screening

The screened compounds were dissolved in DMSO to form a solution of 5% concentration. Filter paper discs (Whatman No. 3 and 5 mm diameter) were saturated with this solution. The discs were placed on the surface of solidified Nutrient agar dishes seeded by the tested bacteria or Czapek Dox agar dishes seeded by the tested fungi. The diameter of inhibition zones (mm) were measured at the end of the incubation period (24–48 h at 37°C for bacteria and for

4–7 days at 28°C for fungi) [26]. Discs saturated with DMSO were used as control. Chloramphenicol and clotrimazole were used as reference drugs. The biologically active compounds were then diluted with DMSO to prepare a series of concentrations in order to determine the MIC of each compound. The MIC values were calculated as μg/mL. The antibacterial and antifungal activities data are given in Tables 1 and 2.

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