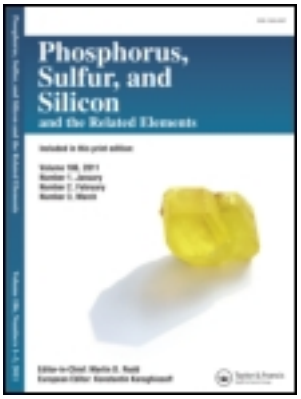

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An Efficient Synthesis of New Substituted Spiro Pyrazolethieno, Pyrimidinethieno, and Benzodiazepinethieno Pyridazine Derivatives with Biological Activities

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AN EFFICIENT SYNTHESIS OF NEW SUBSTITUTED SPIRO PYRAZOLETHIENO, PYRIMIDINETHIENO, AND BENZODIAZEPINETHIENO PYRIDAZINE DERIVATIVES WITH BIOLOGICAL ACTIVITIES

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3-Ethyl 2-amino-4-methyl-5-phenyl thiophene carboxylate 1 was used as a starting material to synthesize 2a,b via coupling with malononitrile or acetyl acetone, respectively. When heated, under reflux in sodium ethoxide solution, 2a,b give 3a,b. On the other hand, when compounds 3a,b were heated under reflux in ethanol with hydrazine hydrate, thiourea, or 1,1-phenylenediamine hydrochloride and a catalytic amount of piperidine 4a,b, 5a,b and 6a,b, were produced, respectively. The new compounds were tested for their antimicrobial activity. Compounds 2a–6b showed antibacterial activities, and 2a,2b and 4b showed antifungal activities.

Supplemental materials are available for this article. Go to the publisher's online edition of* Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.

Keywords Antibacterial; antifungal; benzodiazepinethieno-pyridazine; biological activity; pyrimidinethieno; spiro pyrazolethieno

INTRODUCTION

From a chemical structure point of view, various natural products or synthetic molecules containing the rigid conformations of spiro cyclic skeletons show a wide range of biological and pharmacological properties.[1–3] For example, spirohydantoins have been reported as glycogen and phosphorylase inhibitors, herbicides, and anti-inflammatory agents,[4] and spiro-isoxazolines and spiro-dioxazoles exhibit antitumor and anti-HIV activities.[5–8] In spite of the immense biological activities of many spiro heterocyclic compounds, no report is yet available on the synthesis of pyrazolothienopyridazine spiro derivatives. Prompted by this observation and in continuation of our earlier interest on chemical synthesis of biodynamic heterocyclic compounds,[9] we have made an attempt to synthesize novel tricyclic spiro derivatives.

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RESULTS AND DISCUSSION

Chemistry

For synthesis of novel spiro derivatives, at first, we attempted to obtain dinitrile or diacetyl derivatives by oxidative cyclization of diazo thieno derivatives. Thus, by using 3-ethyl 2-amino-4-methyl-5-phenylthiophene carboxylate[10] **1** as a starting material, it was coupled with some active methylene groups namely, malononitrile or acetylacetone, respectively, to obtain 3-Ethoxycarbonyl-4-methyl-5-phenyl(thien-2-ylazo)malononitrile **2a** or 3-ethoxycarbonyl-4-methyl-5-phenyl(thien-2-ylazo)acetylacetone **2b**. Elemental analyses as well as spectral data of compounds **2a**,**b** are in agreement with the proposed structures (see the Experimental section).

Heating **2a**,**b** under reflux with ethanolic sodium ethoxide solution for 3 h yielded 3,3-disubstituted thienopyridazine derivatives **3a**,**b**. Compounds **3a**,**b** give expected values in elemental analyses and spectral data. The IR spectrum (KBr) cm^{-1} of **3b**, for example, showed absorption bands at: 1719 (2CO), 1673 (CO); ^1H NMR (DMSO-d_6) δ ppm showed signals at: 2.31 (s, 3H, CH_3), 3.80 (s, 6H, $2CH_3$), 7.43–7.46 (m, 5H, aromatic protons); MS (m/z): 326 (M^+) 80%.

Compounds **3a**,**b** underwent oxidative cyclization upon heating under reflux in ethanol with hydrazine hydrate in the presence of a catalytic amount of piperidine to produce 3,5-diamino(diacetyl)-5′-methyl-6′-phenyl-4′H-spiro-(pyrazole-4,3′-thieno[2,3-c]pyridazine)-4′-one **4a**,**b** with new ring systems (Scheme 1). Compounds **4a**,**b** gave an expected elemental analyses (see the Experimental section). The IR spectrum (KBr) cm^{-1} of **4a**, for example, showed: 3313, 3180(NH_2), and 1660(CO); ^1H NMR (DMSO-d_6) δ ppm: 2.22 (s, 3H, CH_3), 3.81 (br., 2H, NH_2, D_2O exchangeable), 7.40–7.57 (m, 5H, aromatic protons), and 9.44 (br., 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) of **4a** for example showed signals at δ189 (S) ppm, 160 (S, 2C), 145 (S), 132 (S), 130 (S, 3C), 129 (d, 2C), 127 (d, 2C), 126 (d), 79 (S), 5 (q).

On the other hand, compounds **3a**,**b** also reacted with thiourea in boiling ethanol containing a catalytic amount of piperidine to produce other novel spiro- compounds 4,6-diamino(diacetyl)-5′-methyl-6′-phenyl-2-thioxo-2H,4′H-spiro-[pyrimidine-5,3′-thieno[2,3-c]pyridazin]-4′-one **5a**,**b**, which showed expected spectral data and elemental analyses (see the Experimental section and Scheme 1).

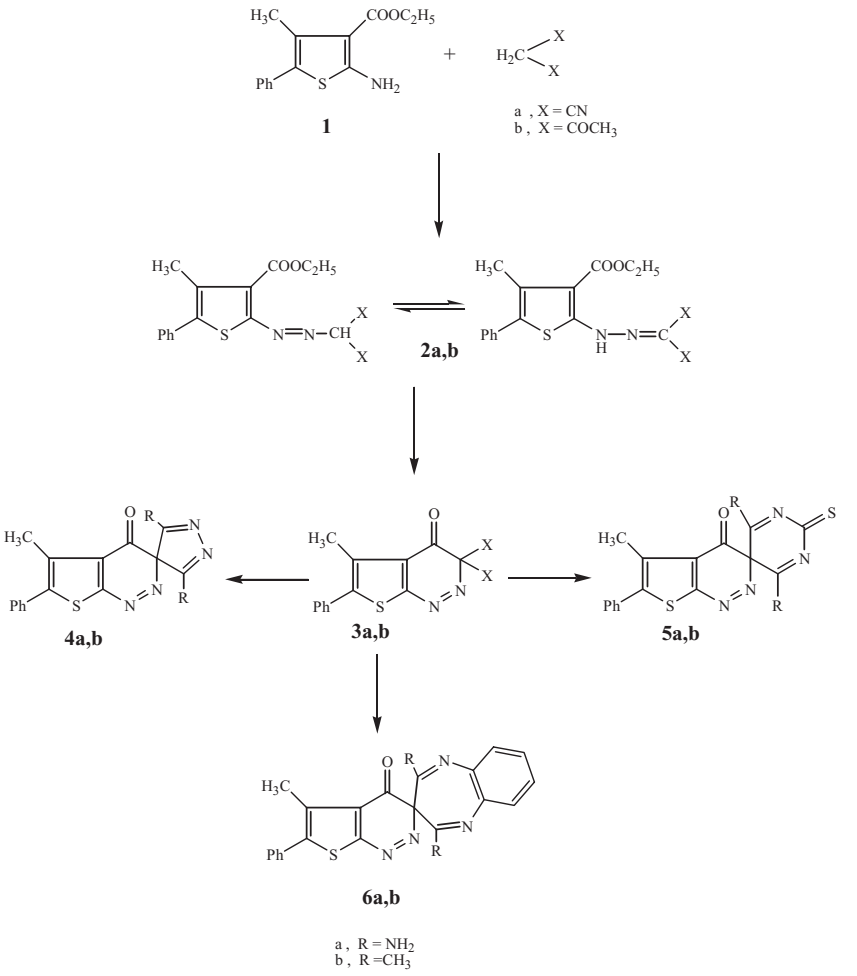
Also, heating **3a**,**b** under reflux with o-phenylenediamine in boiling ethanol containing a catalytic amount of piperidine produced other novel spiro compounds 2,4-diamino(diacetyl)-5′-methyl-6′-phenyl-4′H-spiro[1,5-benzodiazepine-3,3′-thieno[2,3-c]pyridazin]-4′-one **6a**,**b**. Besides the expected values of elemental analyses, the IR, ^1H NMR, and ^{13}C NMR spectra of **6a**,**b** are in agreement with the assigned structure (see the Experimental section and Scheme 1).

Biology

All new compounds were evaluated for their antimicrobial properties. Compound **2a** showed inhibition zones and therefore antibacterial activity against *staphylococcus aureus* (G^+) more than the reference compound tetracycline antibacterial agent. (See the Supplemental Materials available online, Table S1.)

CONCLUSION

This work is concerned with the synthesis and reactions of 3-ethyl-2-amino-4-methyl-5-phenyl thiophene carboxylate with functional and bifunctional groups leading to spiro



Scheme 1

pyrazolethieno, pyrimidinethieno and benzodiazepinethieno pyridazin derivatives. Nine of the synthesized compounds were found to be highly active against Gram positive bacteria (*Staphylococcus aureus* G^+), Gram negative bacteria (*Pseudomon asaeruginosa* G^-), and fungi (*Aspergillus flavus* and *Candida albicans*).

EXPERIMENTAL

All melting points are uncorrected and were taken in open capillaries on a Gallen Kamp Apparatus. Microanalyses were carried out at the Microanalytical Unit, National Research Center and Faculty of Science, Cairo University. IR spectra were recorded on a FT/IR-300 E Jasco using KBr discs. ^{1}H NMR spectra were measured in DMSO or $CDCl_3$, using a JEOL-JNM-Ex270 NMR spectrometer. Signals were measured with reference to TMS as an internal standard. The mass spectra were recorded on Finnigan SSQ 7000 spectrometer. Microbiological analyses were carried out by the Microanalytical Center,

Table I Physical data for the products **2a–6b**

Compound No.	Mp °C	Yield %	Molecular formala (M. wt.)	Elemental analyses (calcd./found) %C	%H	%N
2a	151–153	80	$C_{17}H_{14}N_4O_2S$	60.34	4.17	16.55
			338.39	60.30	4.00	16.50
2b	170–172	75	$C_{19}H_{20}N_2O_4S$	61.27	5.41	7.52
			372.44	61.22	5.40	7.50
3a	212–214	80	$C_{15}H_8N_4OS$	61.63	2.75	19.16
			292.32	61.60	2.74	19.12
3b	180–182	75	$C_{17}H_{14}N_2O_3S$	62.56	4.32	8.58
			326.37	62.54	4.30	8.55
4a	190–192	73	$C_{15}H_{12}N_6OS$	55.54	3.72	25.90
			324.36	55.50	3.70	25.90
4b	200–202	70	$C_{17}H_{14}N_4OS$	63.33	4.37	17.37
			322.39	63.30	4.35	17.35
5a	195–197	75	$C_{16}H_{12}N_6OS_2$	52.15	3.28	22.80
			368.44	52.10	3.25	22.80
5b	205–207	65	$C_{18}H_{14}N_4OS_2$	58.99	3.85	15.28
			366.47	58.97	3.85	15.26
6a	185–187	75	$C_{21}H_{16}N_6OS$	62.98	4.02	20.98
			400.46	62.97	4.00	20.97
6b	202–204	60	$C_{23}H_{18}N_4OS$	69.32	4.55	14.06
			398.49	69.32	4.55	14.00

Faculty of Science, Cairo University, Giza, Egypt. All solid compounds were recrystallized to produce constant melting points. See Table I.

3-Ethyl-2-amino-4-methyl-5-phenyl thiophene carboxylate (1)

Compound **1** was prepared according to the Gewald method.[10]

General Method for Preparation of 2a,b

To an ice-cold solution of the appropriate amine **1** (0.01 mol), glacial acetic acid (30 mL), and phosphoric acid (10 mL), a solution of sodium nitrite (1.03 g, 0.01 mol) dissolved in the minimum amount of water was added dropwise, in an ice bath at -5°C. This previously prepared diazonium salt was added dropwise to a mixture of active methylene malononitrile or acetyl acetone (0.01 mol) and anhydrous sodium acetate in ethanol. The reaction mixture was allowed to stand overnight at room temperature, then it was poured into water. The formed solid was filtered off, washed with water, dried, and recrystallized from the appropriate solvent to produce **2a**,**b**.

3-Ethoxycarbonyl-4-methyl-5-phenyl(thien-2-ylazo)malononitrile (2a). Compound **2a** was obtained by the reaction mixture of **1** (2.60 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol). The compound was recrystallized from ethanol to produce **2a** as deep orange crystals; IR spectrum (KBr) cm^{-1}: 3318 (br, NH), 2232, 2211 (2CN), and 1668 (CO); ^{1}H NMR (DMSO-d_6) δ ppm (J, Hz): 1.29 (t, 3H, CH_3, J = 4.2), 2.24(s, 3H,

CH_3), 3.12 (s, 1H, NH, D_2O exchangeable), 4.26 (q, 2H, CH_2, J = 4.2) and 7.34–7.47 (m, 5H, aromatic protons); MS (m/z): 338 (M^+) 80%.

3-Ethoxycarbonyl-4-methyl-5-phenyl(thien-2-ylazo)acetylacetone (2b). Compound **2b** was obtained by the reaction mixture of **1** (2.60 g, 0.01 mol) and acetyl acetone (1.00 g, 0.01 mol). The compound was recrystallized from ethanol to produce **2b** as orange crystals; IR spectrum (KBr) cm^{-1}: 3324(br, NH), 1671, 1645 (3CO); ^1H NMR (DMSO-d_6) δ ppm (J, Hz): 1.31 (t, 3H,CH_3, J = 4.7), 2.21 (s, 3H, CH_3), 4.00 (s, 6H, $2CH_3$), 4.10 (s, 1H,NH, D_2O exchangeable), 4.40 (q, 2H, CH_2, J = 4.7) and 7.34–7.45 (m, 5H, aromatic protons); MS(m/z): 372(M^+) 100%.

3,3-Dicyano(diacetyl)-5-methyl-6-phenylthieno[2,3-c]pyridazine-4-one (3a,b)

Compound **2a**,**b** (0.01 mol) was refluxed in sodium ethoxide solution [(prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (30 mL)] was heated under reflux for 5 h. The reaction mixture was allowed to cool to room temperature, poured into water, and neutralized by dilute acetic acid solution. The solid product that precipitated was filtered off, dried, and recrystallized from the proper solvent to produce **3a**,**b**.

3,3-Dicyano -5-methyl-6-phenylthieno[2,3-c]pyridazine-4-one (3a). Compound **3a** was obtained by refluxing **2a** (3.38 g, 0.01 mol) in sodium ethoxide solution. The solid product was recrystallized from dioxane to produce **3a** as deep orange crystals; IR spectrum (KBr) cm^{-1}: 2219 (2CN) and 1708 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.33 (s, 3H, CH_3), 7.34–7.47 (m, 5H, aromatic protons); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190 (s) ppm, 148 (s), 135 (s), 134 (s, 3C), 129 (d, 3C), 128 (d, 2C), 117(s, 2C), 58 (s), 5 (q); MS (m/z): 292(M^+) 70%.

3,3-Diacetyl-5-methyl-6-phenylthieno[2,3-c]pyridazin-4-one (3b). Compound **3b** was obtained by refluxing **2b** (3.72 g, 0.01 mol) in sodium ethoxide solution. The solid product was recrystallized from dioxane to produce **3b** as orange crystals; IR spectrum (KBr) cm^{-1}: 1719 (2CO), 1673 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.31(s, 3H, CH_3), 3.80 (s, 6H, $2CH_3$), 7.43–7.46 (m, 5H, aromatic protons); ^{13}C NMR (75 MHz, $CDCl_3$): δ 204 (s, 2C) ppm, 192 (s), 147 (s), 135 (s), 133 (s, 3C), 129 (d, 3C), 128 (d, 2C), 103 (s), 24 (q, 2C), 5 (q); MS(m/z): 326 (M^+) 80%.

3,5-Diamino(diacetyl)-5′-methyl-6′-phenyl-4′H-spiro[pyrazole-4,3′-thieno[2,3-c]pyridazin]-4′-one (4a,b)

A mixture of **3a**,**b** (0.01 mol) and hydrazine hydrate (99–100%) (7 mL, 0.03 mol) was refluxed in ethanol (30mol) containing a catalytic amount of piperidine for 5 h. The reaction mixture was cooled, filtered off, and recrystallized from the proper solvent to produce **4a**,**b**.

3,5-Diamino-5′-methyl-6′-phenyl-4′H-spiro[pyrazole-4,3′-thieno[2,3-c]-pyridazin]-4′-one (4a). Compound **4a** was obtained by refluxing **3a** (2.92 g, 0.01 mol) and hydrazine hydrate in ethanol containing a catalytic amount of piperidine. The solid product recrystallized from dioxane to produce **4a** as deep red crystals; IR spectrum (KBr) cm^{-1}: 3313, 3180 (NH_2) and 1660 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.22 (s, 3H, CH_3), 3.81 (br.,2H, NH_2, D_2O exchangeable), 7.40–7.57 (m, 5H, aromatic protons) and 9.44 (br.,2H, NH_2, D_2O exchangeable); MS (m/z): 324 (M^+) 14.80%.

3,5-Diacetyl-5′-methyl-6′-phenyl-4′H-spiro[pyrazole-4,3′-thieno[2,3-c]pyridazin]-4′-one (4b). Compound **4b** was obtained by refluxing **3b** (3.26 g, 0.01 mol) and hydrazine hydrate in ethanol containing a catalytic amount of piperidine. The solid product recrystallized from dioxane to produce **4b** as deep red crystals; IR spectrum (KBr) cm^{-1}: 1670 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.23 (s, 6H, $2CH_3$), 3.72 (s, 3H, CH_3) and 7.32–7.44 (m, 5H, aromatic protons); ^{13}C NMR (75 MHz, $CDCl_3$): δ190 (s) ppm, 165 (s, 2C), 147 (s), 136 (s), 134 (s, 3C), 129 (d, 3C), 128(d, 2C), 80 (s), 19 (q, 2C), 5 (s); MS (m/z): 322 (M^+) 65%.

4,6-Diamino(diacetyl)-5′-methyl-6′-phenyl-2-thioxo-2H-4′H-spiro-[pyrimidine-5,3′-thieno[2,3-c]pyridazin]-4′-one (5a,b)

A mixture of **3a**,**b** (0.01 mol) and thiourea (0.01, mol) was refluxed in ethanol containing a catalytic amount of piperidine for 5 h. The reaction mixture was cooled and poured into water. The solid product that precipitated was filtered off, dried, and recrystallized from the proper solvent to produce **5a**,**b**.

4,6-Diamino-5′-methyl-6′-phenyl-2-thioxo-2H-4′H-spiro[pyrimidine-5,3′-thieno[2,3-c]pyridazin]-4′-one (5a). Compound **5a** was obtained by refluxing of **3a** (2.92 g, 0.01 mol) and thiourea (0.76 g, 0.015 mol). The solid product recrystallized from dioxane to produce **5a** as yellow crystals; IR spectrum (KBr) cm^{-1}: 3310, 3150 (NH_2) and 1654 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.33 (s, 3H, CH_3), 3.88 (br., 2H, NH_2, D_2O exchangeable), 7.22–7.47 (m, 5H, aromatic protons) and 9.44 (br., 2H, NH_2, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$): δ229(s) ppm, 191 (s), 165 (s, 2C), 147 (s), 135 (s), 134 (s, 3C), 129 (d, 3C), 128 (d, 2C), 72 (s), 5 (q); MS (m/z): 368(M^+) 96%.

4,6-Diacetyl-5′-methyl-6′-phenyl-2-thioxo-2H-4′H-spiro[pyrimidine-5,3′-thieno[2,3-c]pyridazin]-4′-one (5b). Compound **5b** was obtained by refluxing **3b** (3.26g, 0.01 mol) and thiourea (0.76 g, 0.015 mol). The solid product recrystallized from dioxane to produce **5b** as orange crystals; IR spectrum (KBr) cm^{-1}: 1673 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.21 (s, 3H, CH_3), 3.20 (s, 6H, $2CH_3$) and 7.22–7.48 (m, 5H, aromatic protons); ^{13}C NMR (75 MHz, $CDCl_3$): δ 229(s) ppm, 192 (s), 165 (s, 2C), 147 (s), 135 (s), 134 (s, 3C), 129 (d, 3C), 128 (d, 2C), 72 (s), 15 (q, 2c), 5 (q); MS (m/z): 366(M^+) 70%.

2,4-Diamino(diacetyl)-5′-methyl-6′-phenyl-4′H-spiro[1,5-benzodiazepine-3,3′-thieno[2,3-c]pyridazin]-4′-one (6a,b)

A mixture of **6a**,**b** (0.01 mol) and *o*-phenylenediamine hydrochloride (0.01 mol) was refluxed in boiling ethanol containing a catalytic amount of piperidine for 5 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried, and recrystallized from the appropriate solvent to produce **6a**,**b**.

2,4-Diamino-5′-methyl-6′-phenyl-4′H-spiro(1,5-benzodiazepine-3,3′-thieno[2,3-c]pyridazin)-4′-one (6a). Compound **6a** was obtained by refluxing **3a** (2.92 g, 0.01 mol) and o-phenylenediamine hydrochloride. The solid product recrystallized from dilute dimethyl formamide (80%) to produce **6a** as yellow crystals; IR spectrum (KBr) cm^{-1}: 3430–3360 (br, $2NH_2$), 1680 (CO); ^1H NMR (DMSO-d_6) δ ppm: 2.13 (s, 3H, CH_3), 2.74 (br, 4H, $2NH_2$, D_2O exchangeable), 7.01–7.41 (m, 9H, aromatic protons). ^{13}C NMR (75 MHz, $CDCl_3$): δ190 (s) ppm, 160 (s, 2C), 145 (s), 140 (s, 2C), 136 (s), 134 (s,2C),

132 (s), 130 (d, 2C), 129 (d), 128 (d, 2C), 127 (d, 2C), 122 (d, 2C), 75 (s), 50 (q). MS (m/z): 400 (M^+) 70%.

2,4-Diacetyl-5′-methyl-6′-phenyl-4′H-spiro(1,5-benzodiazepine-3,3′-thieno [2,3-c]pyridazin)-4′-one (6b). Compound **6b** was obtained by refluxing **3b** (3.26g, 0.01 mol) and o-phenylenediamine. The solid product recrystallized from dilute dimethyl formamide (80%) to produce **6b**; 1710 (2CO), 1670 (CO); ^{1}H NMR (DMSO-d_6) δ ppm: 2.13 (s, 3H, CH_3), 2.24 (s, 6H, $2CH_3$), 7.01–7.41 (m, 9H, aromatic protons); MS (m/z): 398 (M-86) 20%.

Biology

Measurement of Antimicrobial Activity Using Diffusion Disc Method. The prepared compounds were tested against one strain of Gram positive bacteria (*Staphylococcus aureus* G^+), Gram-negative bacteria (*Esherichia coli* G^-), and fungi (*Aspergillus flavus* and *Candida albicans*).[11,12]

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