Regioselective Reactions, Spectroscopic Characterization, and Cytotoxic Evaluation of Spiro-pyrrolidine Thiophene

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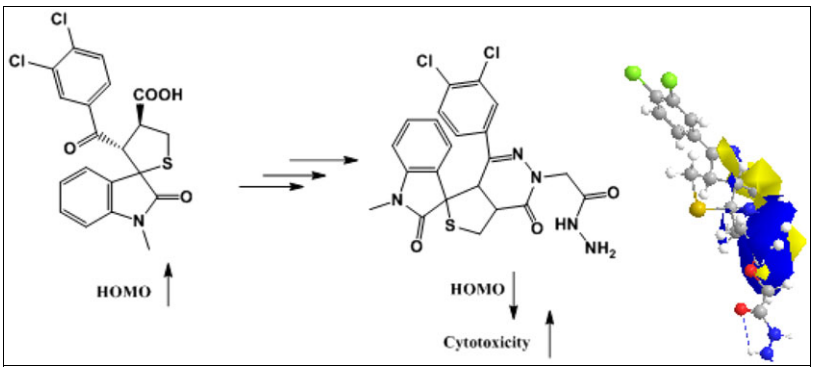

Spiro-oxo-indole/pyrrolidine-thiophene base possessed significant pharmacological activity. The [3 + 2] cycloaddition reactions of thia-methine ylide respected through multi-component reaction affording regioselective and stereoselective spiroindoline-3,2′-tetrahydrothiophene derivative **3**. Reaction of such compound with different electrophilic and nucleophilic reagents afforded bioactive heterocyclic compounds **4**–**16**. Biological evaluation showed that these synthesized spiro-pyrrolidine exhibited moderate to good cytotoxic activity. Among them, compounds **7** and **14** displayed the best cytotoxic activity against MCF-7 and Wl-38 cells with the IC_{50} values of 7.02 ± 0.6 and 8.97 ± 0.9 μm (very strong), respectively. Compounds **4**, **5**, and **12** exhibited strong cytotoxicity's with IC_{50} 16.28 ± 1.7, 11.16 ± 1.1, and 19.14 ± 1.7 μm, respectively, against MCF-7 mammary gland cell line. All compound structures were supported by spectroscopic data and elemental analysis.

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INTRODUCTION

Extreme potential for binding of spiro-cyclic compounds to biomolecules due to their non-planar rigid chiral structure. Their steric strain allied with the quaternary carbon in the structure owing structural rigidity that significantly influences the biological activities [1]. Spiro-oxindole system has a wide range of biological claim with hopeful antimicrobial and its cytotoxicity against MCF-7 mammary gland cell line and human lung fibroblast (WI-38) tumors [2,3]. Spiro-oxindoles annulated with heterocycles show the higher biological activities [3–5]. As smaller N-substituents in isatin such as a methyl group lead to higher reactivity, larger N-substituents resulted in decreased reactivity [6,7]. Oxindole derivatives are well known as powerful antitumor agent because of their kinase inhibitory properties, particularly, tyrosine kinase inhibitors [8,9]. In addition, thiophenes are unique sulfur-containing spiro-oxindole has attracted attention due to their structure-activity in natural products, pharmaceutical agents and possess a widespread in biological purposes (Fig. 1). For instance, essential coenzyme biotin with important biological functions [10], leukotriene antagonists [11], potential inhibitors of HIV [12], antitumor natural product [13] and human A3 adenosine receptor ligands [14]. The chiral thiophenes could attend building blocks in natural product synthesis [15,16]. Due to their important requests of chiral thiophenes in the aforementioned, the stereoselective synthesis of chiral tetrahydrothiophenes with high atomic efficiency and more significantly good feasibility to assemble various substitution patterns has become a very hot topic in synthetic organic and medicinal chemistry [17–19]. The multicomponent 1,3-dipolar cycloaddition of ylides, generated *in situ via* decarboxylative condensation of isatins, and amino acids or thioglycolic acid with olefinic dipolarophiles, represents main approach for the regioselective construction of spiro-oxindoles, have been described [20–30]. However, the construction of spiro-oxindoles is by far not tired with the aforementioned substances. Also, such compounds are encouraged by the search of new antidiabetic substances that strength inhibit DNA chain in metabolically relevant tissues as mammary

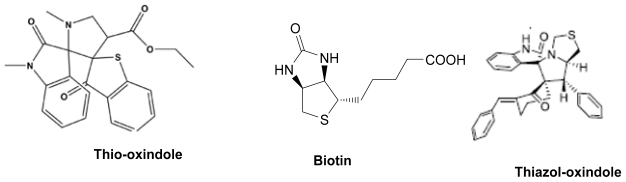


Figure 1. Some representative examples of biologically active compounds that contain a thiophene ring.

gland and human lung fibroblast. Recent studies have established that hydrazide of the spiro-cyclic system is molecular target for treating the antitumor *via* inhibiting the activity of DNA promising occasions for advance of therapeutic interferences [31–33]. Herein, we report the synthesis of spiro-oxindole-thiophene by utilizing a 1,3-dipolar cycloaddition of 4-aryl-4-oxo-but-2-enoic acid with thia-methine ylides, generated *in situ via* decarboxylative condensation of isatin and thioglycolic acid in a three-component fashion.

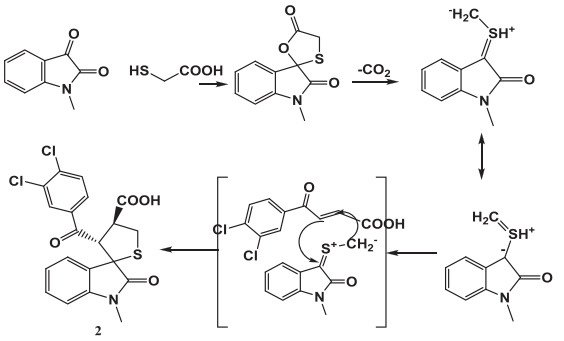
RESULTS AND DISCUSSION

Chemistry. Our present work current an atom-economical of spiro-annulated dihydro-thiophenes based on synthesis of enantioselective thia-[3 + 2]cyclization *via* addition of thia-ylide to 1,3-dicarbonyl, for example, 4-aryl-4-oxo-but-2-enoic acid **1**. During the investigation of compound **1** in organic synthesis that be highly reactive and convenience synthesize to various heterocycles [34–36]. Stimulated the successful aforementioned results, we considered that *S*-heterocycles (e.g., tetrahydrothiophenes) could be synthesized *via* smoothly reaction proceeded, and spiro compound **2** was formed in good yield with selective diastereomer.

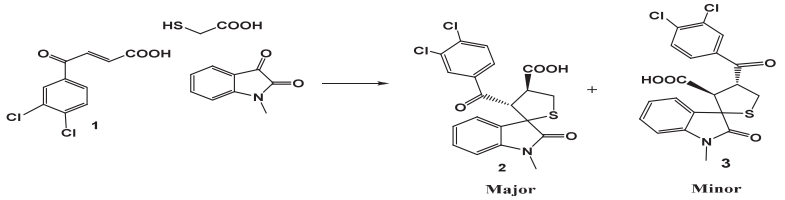
Three-component condensation of equimolar amounts of 4-(3,4-dichlorophenyl)-4-oxo-2-butenoic acid **1**, thioglycolic acid, and carbonyl compound, for example, isatin and/or hydroxy pyrrolidone in boiling aqueous methanol (1:3) afforded spiro-oxindole derivatives major product **2** and minor **3** (Scheme 1). The regiochemical product of the cycloaddition of such regioisomer confirmed by ^{1}H NMR, 4.43 (d, J = 11.4 Hz, 1H, CHCO), 3.69 (dd, J = 11.4, 8.4 Hz, 1H, CHCOO), 2.65–2.43 (d, J = 8.4 Hz, 2H, CH_2S) of hydro thiophene moiety shows signals to the neighboring methylene group that was indicative for the assigned virtual stereoisomer. Beside it shows a doublet at 4.43 ppm (J = 11.4 Hz) for CHCO and doublet at 3.69 ppm for CHCOO that was outlined the *trans*-configuration of the product.

The higher reactivity of 4-aryl-4-oxo-2-butenoic acids [37–43] induces remarkable rate acceleration and decreases the reaction time to only 10–15 min in a boiling mixture of methanol and water. The moderate yield of spiro compound **2** can be explained by formation of byproducts and supported by proposal mechanism (Scheme 2). To overcome these undesirable product, we carried out the reaction under stirring at room temperature. In this reaction, two regioisomers can be expected, but in experiment, the regioisomer **2** was isolated without detectable trace amounts of other isomers. Also, the chiral HPLC directly with chiral stationary phases have been used for enantiomeric separation successfully [34–36]. The intermolecular interactions between chiral target and its enantiomer can be calculated with quantum mechanics and quantitative structure enantioselectivity retention relationships [44]. S-Spiro-oxindole **2** (α = 1.12, high %) can be separated from its enantiomer R-isomer (α = 1.20, low %) (Fig. 2). The quantum chemical computation confirmed the stability of the S-spiro-oxindole is more than its enantiomer R-isomer according to dipole moment and energy gap ΔE = LUMO-HOMO (Supporting Information).

Scheme 2. Proposal mechanism of spiro-oxindole product **2**.



Scheme 1. Multicomponent reaction of aroyl acrylic acid, isatin, and thioglycolic acid.



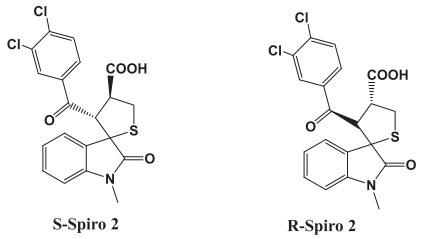
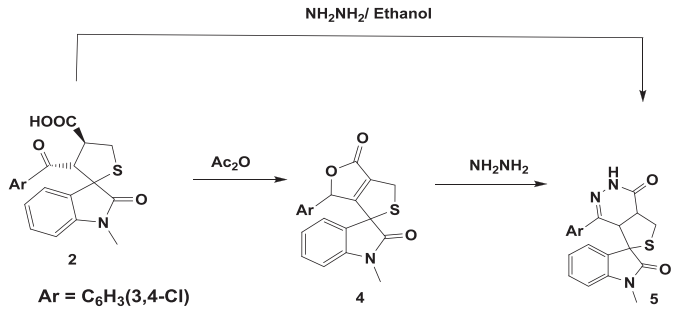


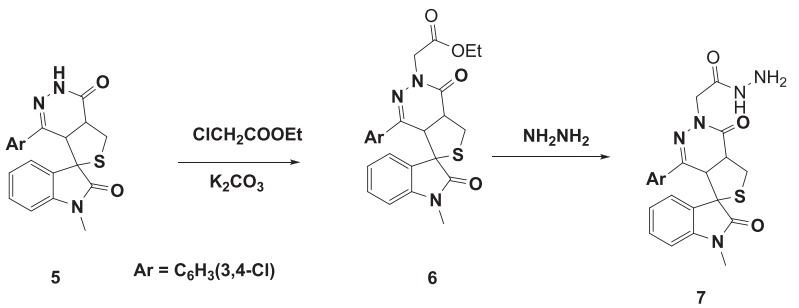
Figure 2. Outline the configuration of *S*-spiro-oxindole and its enantiomer R-isomer.

The cycloadduct spiro derivative **2** could react with acetic anhydride to afford the corresponding furanone **4**. Hydrazinolysis of the product **4** with hydrazine hydrate afforded the pyridazinone **5** (Scheme 3). The pyridazinone **5** can be obtained also via treatment of the Spiro **2** with hydrazine hydrate. Pyridazinone **5** was treated with ethylchloroacetate in the presence of anhydrous potassium carbonate in boiling acetone under SN^2 reaction afforded the corresponding ester **6**. Reaction of the ester **6** with hydrazine hydrate in boiling ethanol afforded the hydrazide **7** (Scheme 4). Reaction of the hydrazide **7** with different carbon electrophiles, for example, acetylacetone and acetic anhydride afforded the pyrazole and 1,3,4-oxadiazole derivatives **8** and **9**, respectively (Scheme 5). Moreover, by some previously manner, one pot reaction of thioglycolic acid, aroyl acrylic acid 2, and 1-(2-hydroxyethyl) pyrrolidin-2-one instead of isatin afforded spiro derivative **10** that cyclized with acetic anhydride to afford the corresponding

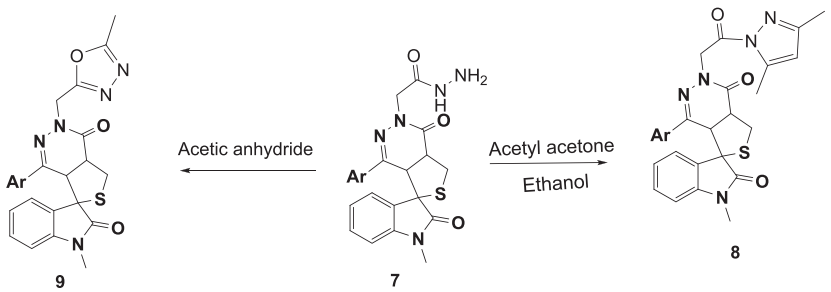
Scheme 3. Reaction of spiro aroyl carboxylic acid **2** with acetic anhydride and hydrazine hydrate to afford the corresponding furanone **4** and pyridazinone **5**, respectively.



Scheme 4. Reaction of the pyridazinone **5** with ethylchloroacetate afforded the ester **6** that reacted with hydrazine hydrate to give hydrazide **7**.



Scheme 5. Reaction of the hydrazide **7** with acetyl acetone and acetic anhydride afforded pyrazolo **8** and 1,3,4-oxadiazolo **9**, respectively.



furanone **11**. Hydrazinolysis of the compound **11** afforded the pyridazin-3-one **12**.

The power of the nucleophilicity functional group in the product **12** plays an important in orientation of the functionality building in the next products. So when the pyridazin-3-one **12** was allowed to react with ethyl chloroacetate in the presence of anhydrous potassium carbonate in boiling acetone afforded, the ester **13** *via* O-alkylation of the pyridazin-3-one **12** due to the nucleophilicity of aliphatic OH is more powerful than NH of the amide group.

Treatment of the ester **13** with hydrazine hydrate in boiling ethanol afforded the hydrazide **14** (Scheme 6). Reaction of the hydrazide **14** with aromatic aldehydes, for example, benzaldehyde and p-anisaldehyde afforded the corresponding arylidene derivatives **15** and **16**, respectively. All new cycloadducts obtained by the aforementioned method were characterized by spectral data and elemental analyses.

Pharmacology. ***Antitumor activity using* in vitro *Ehrlich ascites assay.*** We assessed the cytotoxic action of the compounds listed in Table 1 against two human tumor cell lines, namely, mammary gland breast MCF-7 and human lung fibroblast WI-38. In general, activity was observed by all of these molecules ranged from very strong to non-cytotoxic. The optimal results were observed for compounds **7** and **14** (very strong activity) with IC_{50} 8.9 ± 0.62 and 5.0 ± 0.41 μg/mL for MCF-7 cell and 7.8 ± 0.23 and 5.6 ± 0.22 μg/mL for WI cell, respectively.

The cytotoxicity effect or the inhibitory concentration (IC_{50}) of the synthesized compounds against cancer cell lines (MCF-7) and (WI-38) embraces at 50 μg/mL [45–47]. MTT assay was used to evaluate the effect of the newly synthesized compounds on the proliferation of (MCF-7) and (WI-38) cells. In our study, we describe the cytotoxicity of the new compounds against human breast cancer cell lines such as Michigan Cancer Foundation-7 (MCF-7) and human lung fibroblast (WI-38).

The dose reliant on cytotoxicity was detected in the newly compounds preserved (MCF-7) and (WI-38) cells. Fifty percent of the cell death, which regulates the inhibitory concentration IC_{50} value of newly synthesized compounds against (MCF-7) and (WI-38) cells, holds at 50 μg/mL that displayed a variation in inhibition of cell growth with changed concentration of compounds.

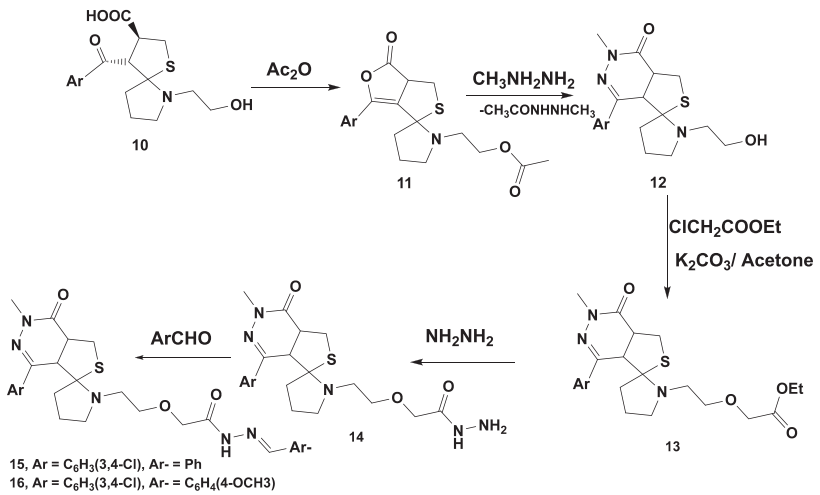
Table 1

Cytotoxic activity of some compounds against cell line

No.	Compounds	*In vitro* cytotoxicity IC_{50} (μM)[a]	
		MCF-7	WI-38
[b]	**DOX**	**4.17 ± 0.2**	**6.68 ± 0.5**
1	**2**	**36.28 ± 1.7**	**55.11 ± 1.9**
2	**4**	**23.79 ± 1.9**	**40.36 ± 4.0**
3	**5**	**22.37 ± 3.0**	**38.01 ± 3.2**
4	**6**	**20.83 ± 1.3**	**36.46 ± 2.5**
5	**7**	**8.16 ± 1.1**	**11.16 ± 4.7**
6	**8**	**29.14 ± 1.7**	**64.93 ± 4.2**
7	**9**	**38.91 ± 2.8**	**32.79 ± 2.3**
8	**10**	**31.85 ± 2.5**	**59.37 ± 3.9**
9	**11**	**27.02 ± 0.6**	**60.45 ± 2.9**
10	**12**	**18.97 ± 0.9**	**27.23 ± 1.6**
11	**13**	**25.04 ± 3.5**	**39.48 ± 2.2**
12	**14**	**7.72 ± 4.6**	**10.42 ± 1.8**
13	**15**	**19.83 ± 3.2**	**25.12 ± 2.7**
14	**16**	**22.12 ± 2.1**	**38.85 ± 3.6**

[a]IC_{50} (μM): 1–10 (very strong), 11–20 (strong). 21–50 (moderate), 51–100 (weak) and above 100 (non-cytotoxic);
[b]DOX: Doxorubicin.

Scheme 6. Reaction of spiro aroyl carboxylic acid **10** with acetic anhydride and methylhydrazine hydrate to afford the corresponding furanone **11** and pyridazinone **12** that with ethyl chloroacetate afforded the ester **13** that reacted with hydrazine hydrate to give hydrazide **14**. Reaction of hydrazide 14 with aromatic aldehyde afforded **15** and **16**.



Compounds **7** and **14** showed very strong; compounds **4**, **5**, and **13** showed strong cytotoxic activity against MCF-7 mammary gland cell line; and **2**, **6**, **10**, **11**, and **12** revealed moderate IC_{50} against MCF-7 cancer cell line compared with DOX that has very strong IC_{50} against MCF-7 cancer cell line.

Antibacterial activity. *Staphylococcus aureus* was significantly inhibited by compounds **7**, **13**, and **14** (Table 2). For Gram-negative clinical isolates, the compounds **7** and **14** significantly inhibited both *Escherichia coli* and *Pseudomonas aeruginosa*, and *Escherichia coli*. Both isolates were resistant to erythromycin. The mode of action of such compounds and inhibited the growth of Gram-positive clinical isolate and Gram-negative clinical isolates growth. Spiro thiophene hydrazides are competitive inhibitors as penicillin and are used as bacteriostatic and in the treatment of leprosy [48]. Many of aryl hydrazides are used as antifungal, antibacterial, or antitumor agents [49] and inhibitors for several [50] cyclic oxygenase-2 (COX-2). Compounds containing C═N may act as a Michael acceptor for nucleophiles containing NH_2 group of enzymes that converted to the inactive form. So the spiro thiophene hydrazide inhibited these enzymes are responsible for building cell walls of bacteria and/or fungi that explain the activities of compounds **15** and **16**.

Structure–activity relationship. DNA is made of chemical building blocks called nucleotides. The four types of nitrogen bases found in nucleotides are adenine (A), thymine (T), guanine (G), and cytosine (C). The base adenine always pairs with thymine, while guanine always pairs with cytosine through hydrogen bond. The cytotoxic activity toward different cell lines depends on two factors: (i) the formation of intermolecular hydrogen bond with DNA bases and (ii) the positive charge on the tested compounds attracted to the negative charge on the cell wall. By comparing the experimental cytotoxicity of the compounds reported in this study with their structures, the following structure–activity relationship were postulated.

Compounds **7** and **14** showed very strong activity, this is due to the presence of the hydrazide group ($CONHNH_2$) that may be added to any unsaturated moiety in DNA (oxa- or aza-Michael addition) or forming hydrogen bond with either one of the nucleobases of the DNA and causes it damage. Moreover, they have increased the acidic proton that facilitated the elimination reaction involving an oxygen of the phosphate group at the 3-position (Scheme 7) directed to single strand break of DNA molecule. It afforded lacking a hydroxyl and prevented its repair by ligation led to inhibition of the cell cycle. So the hydrazide **7** and **14** have controlled inadequate DNA such as incorporation of 2′-C-cyano-21-deoxy-1-D-arabinopento-furanosylcytosine triphosphate into DNA and extension during replication leads to single-strand breaks directly caused by elimination. These breaks, or the lesions that arise from further processing, cause cells to arrest in G2 [34,35,51–60]. Compounds **15** and **16** showed strong activity, this is due to the presence of NH group available to form hydrogen bond with either one of the nucleobases of the DNA and causes it damage. Incorporation of ─N═CHAr moiety has rendered compounds **15** and **16** with moderate to weak cytotoxicity activities against the two tumor cell types.

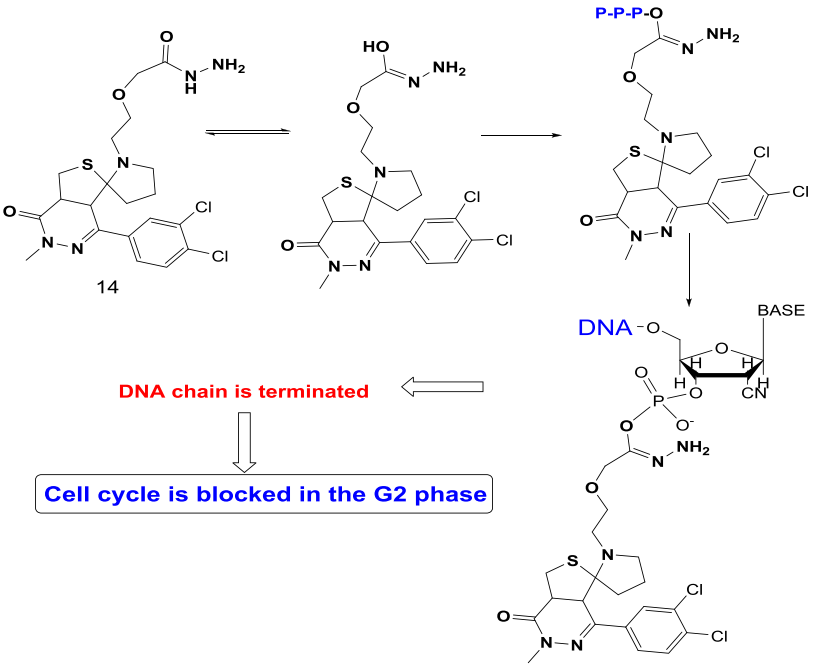
Table 2

Diameters (in mm) of inhibition zones and % activity of agar diffusion assays against a variety of fungi and bacteria (growth was quantified after 1 and 2 days)

		E. coli (mg/mL)		*S. aureus* (mg/mL)		*C. albicans* (mg/mL)	
No	Compound	(mm)	% Activity index	(mm)	% Activity index	(mm)	% Activity index
1	**2**	8	30.8	15	42.5	13	40.3
2	**4**	4	15.4	10	41.7	11	40.7
3	**5**	NA	—	2	8.3	6	22.2
4	**6**	NA	—	NA	—	4	14.8
5	**7**	24	65	20	92.5	23	91.1
6	**8**	NA	—	NA	—	NA	—
7	**9**	2	7.7	9	37.5	8	29.6
8	**10**	5	8.5	7	30.8	12	31.5
9	**11**	NA	—	NA	—	2	12.6
10	**12**	15	57.7	20	83.3	17	83.0
11	**13**	10	30.0	18	35.0	6	49.2
12	**14**	26	72	21	90	22	92
13	**15**	14	60.8	6	65.0	18	69.6
14	**16**	15	69.2	13	74.2	17	71.8
Ampicillin		26	100	24	100	NA	—
Clotrimazole		NA	—	NA	—	27	100

NA, no activity.

Scheme 7. Mechanism of the antitumor action of compound **14**. [Color figure can be viewed at wileyonlinelibrary.com]



CONCLUSION

The green synthesis using 1,3-dipolar cycloaddition of azomethine ylides (generated *in situ* from isatins and thioglycolic acid) to 4-aryl-4-oxo-but-2-enoic acid derivatives to afford the regioselective spiro oxindoles **2** in moderate to good yields. Compounds **7** and **14** exhibited very strong activity (IC_{50} 5.16 ± 0.22 μg/mL) approximately to the 5-fluorouracil (5-FU) as a standard (5.4 ± 0.21 μg/mL) for MCF-7. While compounds **4**, **5**, and **13** observed activity ranged from strong to moderate cytotoxic with IC_{50} 21.2 ± 2.96 to >100.

MATERIALS AND METHODS

Experimental. All melting points are corrected and were determined on a start electric melting point apparatus. Elemental analyses were carried out at the Micro-Analytical Center, National Research Center, Cairo, Egypt. By Elementary Viro El Microanalysis (Germany), the IR spectra (KBr pellets, ν_{max}/cm^{-1}) were recorded on infrared spectrometer FT-IR 400D using OMNIC program and reported frequency of absorption in terms of cm^{-1}, and ^{1}H-NMR spectra were recorded on a Bruker spectrophotometer (Germany) at 400 MHz using TMS as internal standard and with residual signals of the deuterated solvent δ = 7.26 ppm for $CDCl_3$ and δ = 2.51 ppm for DMSO-d_6. ^{13}C-NMR spectra were recorded on the same spectrometer at 125 MHz and referenced to solvent signals δ = 77 ppm for $CDCl_3$ and δ = 39.50 ppm for DMSO-d_6. The mass spectra were recorded on Shimadzu GCMS-QP 1000 EX mass spectrometer (Japan) at 70 eV using the electron ionization technique. Homogeneity of all compounds synthesized was checked by TLC.

General procedure for the synthesis of 4-(3,4-dichlorophenyl)-4-oxo-2-butenoic acid derivative. The 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) is equipped with a HSA quaternary pump, degasser, autosampler, thermostatic column compartment, a G1314A UV-detector, and data were processed using ChemStation program.

General procedure for the preparation of the spiro-oxindoline derivatives 2 and 10. Three-component reaction of 4-(3,4-dichlorophenyl)-4-oxo-2-butenoic acid **1** (0.01 mol), thioglycolic acid (0.9 g; 0.01 mol), and isatins or hydroxy pyrrolidone **2** (0.01 mol) in 10.0 mL aqueous methanol (1:3) was heated in an oil bath to reflux or stirred at room temperature [34]. The resulting precipitates were collected by filtration and washed with cold methanol to give the analytically pure products **2** and **10**, respectively. Enantio-separations of D/L-spiro-oxindole **2** on HSA columns were prepared by the SMCC method [36]. The HPLC conditions were as follows: sample concentration, 20 μM spiro-oxindole **2**; sample volume, 20 μL; mobile phase, pH 7.0, 0.067 M potassium phosphate buffer containing 5% 2-propanol and 1 mM octanoic acid; flow rate for the SMCC HSA column, 1.5 mL/min; column size, 5 cm *4.6 mm.

*3′-(3,4-Dichlorobenzoyl)-1-methyl-2-oxo-4′,5′-dihydro-3′H-spiro[indoline-3,2′-thiophene]-4′-carboxylic acid (**2**).* Colorless solid, 80%, mp: 252–254°C; IR (KBr) ν 3403 (OH), 3330, 3279 (NH), 3050 (CH_{Ar}), 1710, 1686, 1662 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H, 4′-COOH), 7.67 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.57 (d, 2H, 4-CH (indol)), 7.50 (s, 2H, 2-CH (indol)), 7.44 (s, 1H, 2-CH (Clbenz)), 7.36 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 4.43 (d, *J* = 11.4 Hz, 1H, CHCO), 3.69 (dd, *J* = 11.4, 8.4 Hz, 1H, CHCOO), 2.95 (s, 3H, ─NCH_3), 2.43 (d, *J* = 8.4 Hz, 2H, CH_2S);^{13}C NMR (125 MHz, DMSO-d_6) δ 195.09 (CO-benzoyl), 177.42 (CON), 173.16 (4′-COOH), 141.26 (C─N), 136.44 (C─CO), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl, 128.20 (CH Ist), 127.13 (CH Ist), 113.55 (CH Ist), 111.33 (CH Ist), 72.27 (C-spiro), 56.54 (CHCO), 54.66 (CHCOO), 42.96 (CH_2), 34.40 (CH_3). *Anal*. Calcd for $C_{20}H_{15}Cl_2N_2O_4S$ (436): C 55.06, H 3.47, N 3.21, S 7.35; found: C 54.80, H 3.25, N 3.07, S 7.12.

General procedure for the preparation of the compounds 4 and 11. A mixture of spiro compounds **2** and/or **10** (0.01 mol) and acetic anhydride (0.01 mol) was heated under reflux for 3 h. The reaction mixture was concentrated. The solid was separated out, filtered off, dried, and recrystallized from the proper solvent to afford furanone derivatives **4** and/or **11**, respectively.

*3′-(3,4-Dichlorophenyl)-1-methyl-3′,6′-dihydro-1′H-spiro[indoline-3,4′-thieno[3,4-c]furan]-1′,2-dione (**4**).* Colorless solid, 74%, mp: 260–262°C; IR (KBr) ν 3050, 2967 (CH), 1762, 1662 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.67 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.57 (d, 2H, 4-CH (indol)), 7.50 (s, 2H, 2-CH (indol)), 7.44 (s, 1H, 2-CH (Clbenz)), 7.36 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 6.96 (s, 1H, CH fur), 3.37 (s, 2H, SCH_2), 2.96 (s, 3H, NCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.32 (CO fur), 174.42 (CON), 169.30 (═C─O, fur), 160. 25 (C═), 147.4 (C─C-spiro), 141.26 (C─N, Ar), 136.44 (CH─C─O, fur), 132.08 (C─Cl), 131.65 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.42 (CH, 5-ArCl), 128.75 (CH, 6-ArCl), 128.20 (CH Ist), 127.13 (CH Ist), 113.55 (CH Ist), 111.33 (CH Ist), 76.42 (C-spiro), 53.16 (2CH), 41.26 (CH_2), 34.24 (CH_3). *Anal*. Calcd for $C_{20}H_{15}Cl_2NO_3S$ (418): C 57.43, H 3.13, N 3.35, S 7.66; found: C 57.21, H 2.94, N 3.17, S 7.43.

General procedure for the preparation of the compounds 5 and 12. A mixture of furanone derivatives **4** and/or **11** (0.01 mol) and hydrazine hydrate (0.5 mL, 0.01 mol) was heated under reflux in butanol (30 mL) for 3 h. The reaction mixture was concentrated, and the solid was separated out, filtered off, dried, and recrystallized from the proper solvent to afford the pyridazinone derivatives **5** and/or **12**, respectively.

*4′-(3,4-Dichlorophenyl)-1-methyl-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[indoline-3,5′-thieno[3,4-d]pyridazine]-1′,2-dione (**5**).* Colorless solid, 77%, mp: 276–278°C; IR (KBr) ν 3412, 3179 (NH), 3050 (CH_{Ar}), 1686, 1660 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H, 1-NH), 7.67 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.57 (d, 2H, 4-CH (indol)), 7.50 (s, 2H, 2-CH (indol)), 7.44 (s, 1H, 2-CH (Clbenz)), 7.36 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 4.66 (dd, *J* = 11.4, 8.4 Hz, 1H, CHCO), 3.69 (d, *J* = 8.4 Hz, 1H, CHC═N), 3.01 (s, 3H, CH_3), 2.34 (d, *J* = 8.4 Hz, 2H, CH_2S); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42 (2CON), 163.41 (C═N), 136.44 (C─CO), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 128.20 (CH Ist), 127.13 (CH Ist), 113.55 (CH Ist), 111.33 (CH Ist), 72.27 (C-spiro), 56.54 (CHCO), 54.66 (2CH), 42.96 (CH_2), 34.40 (CH_3). *Anal*. Calcd $C_{20}H_{15}Cl_2N_3O_2S$ (431): C 55.57, H 3.50, N 9.72, S 7.42; found: C 55.35, H 3.22, N 9.48, S 7.21.

General procedure for the preparation of the compounds 6 and 13. A mixture of pyridazinone derivatives **5** and/or **12** (0.01 mol) and ethyl chloroacetate (0.01 mol) in dry acetone (20 mL), in the presence of potassium carbonate (3 g), was refluxed for 24 h. The reaction mixture was concentrated, cooled, and poured into ice cold water. The participated solid was filtered off, washed, dried, and recrystallized from the proper solvent to afford spiro pyridazone ester **6** and/or **13**, respectively.

*Ethyl 2-(4′-(3,4-dichlorophenyl)-1-methyl-1′,2-dioxo-7′,7a′-dihydro-1′H-spiro[indoline-3,5′-thieno[3,4-d]pyridazin]-2′(4a′H)-yl)acetate (**6**).* Colorless solid, 80%, mp: 144–146°C; IR (KBr) ν 3400 (OH), 3050, 2960 (CH), 1740, 1686, 1662 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.63 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.57 (d, 2H, 4-CH (indol)), 7.50 (s, 2H, 2-CH (indol)), 7.44 (s, 1H, 2-CH (Clbenz)), 7.36 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 5.43 (s, 2H, NCH_2CO), 4.99 (q, *J* = 8.4 Hz, 1H, OCH_2CH_3), 3.93 (dd, *J* = 11.5, 8.4 Hz, 1H, COCH), 3.32–3.12 (d, *J* = 11.5 Hz, 1H, CHC═N), 3.02 (s, 3H, CH_3N), 2.62–2.60 (d, *J* = 8.4 Hz, 2H, SCH_2), 1.96 (t, *J* = 8.4 Hz, 1H, OCH_2CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 195.09 (CO-ester), 177.42 (2CON), 162.26 (C═N), 136.44 (C─C═N), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (2CH, 3,4-ArCl), 130.32 (CH), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 128.20 (CH Ist), 127.13 (CH Ist), 113.55 (CH Ist), 111.33 (CH Ist), 72.27 (C-spiro), 69.23 (NCH_2CO) 56.54 (CHCO), 54.66 (CH), 51.21 (OCH_2), 42.96 (CH_2), 34.40 (CH_3), 32.54 (CH_3). *Anal*. Calcd for $C_{24}H_{21}Cl_2N_3O_4S$ (517): C 55.61, H 4.08, N 8.11, S 6.18; found: C 55.42, H 3.86, N 7.92, S 5.92.

General procedure for the preparation of the compounds 7 and 14. A solution of ester **6** and/or **13** (0.01 mol) in

ethanol (40 mL) was treated with hydrazine hydrate 98% (1.5 mL; 0.04 mol) and then refluxed for 3 h. The solid that separated after concentration and cooling was recrystallized from the proper solvent to afford spiro pyridazone hydrazide **7** and/or **14**, respectively.

*2-(4′-(3,4-Dichlorophenyl)-1-methyl-1′,2-dioxo-7′,7a′-dihydro-1′H-spiro[indoline-3,5′-thieno[3,4-d]pyridazin]-2′(4a′H)-yl)acetohydrazide (**7**).* Colorless solid, 78%, mp: 268–270°C; IR (KBr) ν 3412, 3354, 3180 (NH), 3050 (CH_{Ar}), 1685, 1660 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H, CONH), 7.41 (d, 2H, 4-CH (indol)), 7.40 (s, 2H, 2-CH (indol)), 7.23 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.14 (s, 1H, 2-CH (Clbenz)), 7.06 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 5.44 (s, 2H, NH_2), 4.99 (s, 2H, NCH_2CO), 3.31 (s, 3H, NCH_3), 3.12–3.10 (dd, *J* = 13.4, 9.2 Hz, 1H, COCH), 3.02–3.00 (d, *J* = 13.4 Hz, 1H, CHC═N), 2.66–2.64 (d, *J* = 9.2 Hz, 2H, SCH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42–173.65 (3CON), 161.26 (C═N), 136.44 (C─C═N), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 128.20 (CH Ist), 127.13 (CH Ist), 113.55 (CH Ist), 111.33 (CH Ist), 72.27 (C-spiro), 56.54 (CHCO), 54.66 (CHCOO), 42.96 (CH_2), 34.40 (CH_3). *Anal*. Calcd for $C_{22}H_{19}Cl_2N_5O_3S$ (503): C 52.39, H 3.80, N 13.89, S 6.36; found: C 52.39, H 3.58, N 13.62, S 6.14.

General procedure for the preparation of the compound 8.
A solution of hydrazide **7** (3.4 g; 0.01 mol) and acetyl acetone (1.1 mL, 0.01 mol) in 30 mL ethanol, and four drops piper dine was refluxed for 4 h. The reaction mixture was allowed to cool, and the crude product was washed by petroleum ether (bp 40–60°C), filtered, dried, and then was crystallized from ethanol to afford pyrazolo spiro pyridazone derivative **8**.

*4′-(3,4-Dichlorophenyl)-2′-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-1-methyl-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[indoline-3,5′-thieno[3,4-d]pyridazine]-1′,2-dione (**8**).* Colorless solid, 76%, mp: 240–242°C; IR (KBr) ν 3400 (OH), 3050, 2955 (CH), 1711, 1688, 1664 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, 2H, 4-CH (indol)), 7.42 (s, 2H, 2-CH (indol)), 7.39 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.37 (s, 1H, 2-CH (Clbenz)), 7.29 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 6.44 (s, 1H, CHPyraz), 5.03 (s, 2H, NCH_2CO), 3.46–3.44 (dd, *J* = 12.6, 8.6 Hz, 1H, COCH), 3.31 (s, 3H, NCH_3), 3.11–3.10 (d, *J* = 12.6 Hz, 1H, CHC=N), 2.93 (s, 6H, $2CH_3$Pyraz), 2.75–2.72 (d, *J* = 8.6 Hz, 2H, SCH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42 (CON), 173.16 (2CON), 161.28 (C═N), 160.2 (2C═), 149.34 (CH═), 136.44 (C─C═N), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 128.20 (CH, Ist), 127.13 (CH, Ist), 113.55 (CH, Ist), 111.33 (CH, Ist), 72.27 (C-spiro), 28.47 (CH─C═O), 56.54 (CHC═N), 44.32 (CH_2), 42.96 (CH_2), 37.32 (CH_3), 34.40 (CH_3), 31.26 (CH_3). *Anal*. Calcd for $C_{27}H_{23}Cl_2N_5O_3S$ (507): C 57.05, H 4.08, N 12.32, S 5.64; found: C 56.93, H 3.90, N 12.03, S 5.42.

General procedure for the preparation of the compound 9.
A mixture of hydrazide **7** (3.4 g; 0.01 mol) and acetic anhydride (2 mL, 0.03 mol) in pyridine (30 mL) was refluxed for 3 h. The reaction mixture was left to cool then poured into cold ice/HCl. The participated solid was filtered off and was crystallized from ethanol to afford oxadiazolospiro pyridazone derivative **9**.

*4′-(3,4-Dichlorophenyl)-1-methyl-2′-((5-methyl-1,3,4-oxadiazol-2-yl)methyl)-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[indoline-3,5′-thieno[3,4-d]pyridazine]-1′,2-dione (**9**).* Colorless solid, 64%, mp: 232–234°C; IR (KBr) ν 3050 (CH_{Ar}), 1689, 1661 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.41 (d, 2H, 4-CH (indol)), 7.40 (s, 2H, 2-CH (indol)), 7.23 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.14 (s, 1H, 2-CH (Clbenz)), 7.06 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 3.32–3.22 (dd, *J* = 13.4, 9.2 Hz, 1H, COCH), 3.11 (s, 3H, NCH_3), 3.02–3.00 (d, *J* = 13.4 Hz, 1H, CHC═N), 2.66–2.64 (d, *J* = 9.2 Hz, 2H, SCH_2), 2.04 (s, 3H, CH_3);^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42 (CON), 175.16 (CON), 167.43 (3C═N), 162.2 (2C═), 149.34 (CH═), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 128.20 (CH, Ist), 127.13 (CH, Ist), 113.55 (CH, Ist), 111.33 (CH, Ist), 72.27 (C-spiro), 56.54 (CHC─N), 42.96 (CH_2), 37.32 (CH_3), 35.40 (CH_3), 33.26 (CH_3). *Anal*. Calcd for $C_{24}H_{19}Cl_2N_5O_3S$ (527): C 54.55, H 3.62, N 13.25; found: C 54.27, H 3.17, N 12.90.

*(3S,4S)-4-(3,4-Dichlorobenzoyl)-6-(2-hydroxyethyl)-1-thia-6-azaspiro[4.4]nonane-3-carboxylic acid (**10**).*
Colorless solid, 76%, mp: 188–190°C; IR (KBr) ν 3520, 3410 (OH), 3050, 2945 (CH), 1710, 1691,1664 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H, COOH), 7.23 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.14 (s, 1H, 2-CH (Clbenz)), 7.06 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 5.78 (s, 1H, OH), 3.64 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.32–3.22 (d, *J* = 9.2 Hz, 1H, CHCO), 3.02–3.00 (dd, *J* = 9.2, 7.6 Hz, 1H, CHCOO), 2.92 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz, 2H, NCH_2Pyrr), 2.04 (t, *J* = 7.7 Hz, 2H, CH_2Pyrr), 1.96 (m, 2H, CH_2Pyrr); ^{13}C NMR (125 MHz, DMSO-d_6) δ 195.09 (CO-benzoyl), 177.42 173.16 (4′-COOH), 141.26 (C─N), 136.44 (C─CO), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 72.27 (C-spiro), 68.20 (CH), 57.13 (CH_2), 55.35 (CH_2), 54.36

(CHCO), 52.66 (CHCOO), 44.40 (CH_2), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{17}H_{19}Cl_2NO_4S$ (403): C 50.50, H 4.74, N 3.46, S 7.93; found: C 50.24, H 4.49, N 3.16, S 7.68.

*2-(3′-(3,4-Dichlorophenyl)-1′-oxo-6′,6a′-dihydro-1′H-spiro[pyrrolidine-2,4′-thieno[3,4-c]furan]-1-yl)ethyl acetate (**11**).* Colorless solid, 72%, mp: 220–222°C; IR (KBr) ν 3052 (CH_{Ar}), 1782, 1767 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.62 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.52 (s, 1H, 2-CH (Clbenz)), 7.36 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 6.72 (s, 1H, CH fur), 3.67 (t, *J* = 8.4 Hz, 2H, CH_2O), 2.96 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.80–2.77 (d, *J* = 7.6 Hz, 2H, CH_2S), 2.62 (s, 3H, CH_3), 2.50 (t, *J* = 7.7 Hz, 2H, NCH_2Py), 2.11 (t, *J* = 7.7 Hz, 2H, CH_2Pyrr), 1.95 (m, 2H, CH_2Pyrr); ^{13}C NMR (125 MHz, DMSO-d_6) δ 175.59 (COO), 170.23 (O─C═), 161.36 (C═ fur), 141.26 (C─N), 136.44 (C─CO), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 72.27 (C-spiro), 68.20 (CH), 57.13 (CH_2), 55.35 (CH_2), 52.66 (CHCOO), 44.40 (CH_2), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{19}H_{19}Cl_2NO_{4S}$ (427): C 53.28, H 4.74, N 3.27, S 7.48; found: C 53.00, H 4.26, N 3.00, S 7.15.

*4′-(3,4-Dichlorophenyl)-1-(2-hydroxyethyl)-2′-methyl-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[pyrrolidine-2,5′-thieno[3,4-d]pyridazin]-1′-one (**12**).* Colorless solid, 74%, mp: 192–194°C; IR (KBr) ν 3356 (OH), 3050 (CH_{Ar}), 1689 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.33 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.24 (s, 1H, 2-CH (Clbenz)), 7.16 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 5.86 (s, 1H, OH), 3.67 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.22–3.16 (d, *J* = 9.2 Hz, 1H, CHCO), 3.14 (s, 3H, NCH_3), 3.08–3.04 (dd, *J* = 9.2, 7.6 Hz, 1H, CHPyrid), 2.92 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz, 2H, NCH_2Pyrr), 2.04 (t, *J* = 7.7 Hz, 2H, CH_2Pyrr), 1.96 (m, 2H, CH_2Pyrr); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42 (CON), 163.41 (C═N), 136.44 (C─CO), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 72.27 (C-spiro), 67.40 (2CH), 57.13 (CH_2), 55.35 (CH_2), 52.66 (CHCO), 44.40 (CH_2), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{18}H_{21}Cl_2N_3O_2S$ (413): C 52.18, H 5.11, N 10.14, S 7.74; found: C 51.95, H 4.86, N 9.94, N 7.50.

*Ethyl 2-(2-(4′-(3,4-dichlorophenyl)-2′-methyl-1′-oxo-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[pyrrolidine-2,5′-thieno[3,4-d]pyridazin]-1-yl)ethoxy)acetate (**13**).* Colorless solid, 65% (r.t.), mp: 202–204°C; IR (KBr) ν 3424 (OH), 3050 (CH_{Ar}), 1742, 1691 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.23 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.14 (s, 1H, 2-CH (Clbenz)), 7.06 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 4.95 (s, 2H, OCH_2CO), 4.02 (q, *J* = 7.8 Hz, 2H, OCH_2CH_3), 3.64 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, *J* = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, *J* = 9.2, 7.6 Hz, 1H, CHPyrid), 2.92 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz, 2H, NCH_2Pyrr), 2.04 (t, *J* = 7.7 Hz, 2H, CH_2Pyrr), 1.96 (m, 2H, CH_2Pyrr), 1.32 (t, *J* = 7.8 Hz, 3H, OCH_2CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 195.09 (CO-ester), 177.42 (CON), 162.26 (C═N), 136.44 (C─C═N), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (2CH, 3,4-ArCl), 130.32 (CH), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 72.27 (C-spiro), 69.23 (NCH_2CO) 56.54 (CHCO), 54.66 (CH), 68.20 (CH), 57.13 (CH_2), 55.35 (CH_2), 51.21 (OCH_2), 44.40 (CH_2), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{22}H_{27}Cl_2N_3O_4S$ (499): C 52.80, H 5.44, N 8.40, S 6.41; found: C 52.54, H 5.25, N 8.13, N 6.17.

*2-(2-(4′-(3,4-Dichlorophenyl)-2′-methyl-1′-oxo-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[pyrrolidine-2,5′-thieno[3,4-d]pyridazin]-1-yl)ethoxy)acetohydrazide (**14**).* Colorless solid, 72%, mp: 156–158°C; IR (KBr) ν 3341, 3186 (NH), 3050 (CH_{Ar}), 1690, 1665 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H, NH), 7.23 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.14 (s, 1H, 2-CH (Clbenz)), 7.06 (d, *J* = 8.4 Hz, 1H, 6-CH (Clbenz)), 5.38 (s, 2H, NH_2), 4.95 (s, 2H, OCH_2CO), 3.64 (t, *J* = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, *J* = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, *J* = 9.2, 7.6 Hz, 1H, CHPyrid), 2.92 (t, *J* = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, *J* = 7.6 Hz, 2H, CH_2S), 2.41 (t, *J* = 7.7 Hz, 2H, NCH_2Pyrr), 2.04 (t, *J* = 7.7 Hz, 2H, CH_2Py), 1.96 (m, 2H, CH_2Py); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42–173.65 (2CON), 161.26 (C═N), 136.44 (C─C═N), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 72.27 (C-spiro), 69.23 (NCH_2CO), 68.20 (CH), 56.54 (CHCO), 54.66 (CH), 54.35 (CH_2), 51.21 (OCH_2), 44.40 (CH_2), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{20}H_{25}Cl_2N_5O_3S$ (485): C 49.39, H 5.18, N 14.40, N 6.59; found: C 49.15, H 4.85, N 14.12, S 6.33.

General procedure for the preparation of the compounds 15 and 16. A mixture of compounds **14** (0.01 mol) and aromatic aldehyde, namely, benzaldehyde and/or *p*-methoxybenzaldehyde (0.01 mol) in ethanol (50 mL) was refluxed for 4 h. The solid that separated after cooling was filtered off, dried, and then crystallized from ethanol.

*N′-benzylidene-2-(2-(4′-(3,4-dichlorophenyl)-2′-methyl-1′-oxo-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[pyrrolidine-2,5′-thieno[3,4-d]pyridazin]-1-yl)ethoxy)acetohydrazide (**15**).* Colorless solid, 77%, mp: 174–176°C; IR (KBr) ν 3370 (NH), 3050 (CH_{Ar}), 1695, 1669 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H, CHAr), 9.32 (s, 1H, 1-NH), 7.43 (d, *J* = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.23–7.22 (d, *J* = 8.4 Hz, 2H, 2,6-CH (benz)), 7.31–7.30

(d, J = 8.4 Hz, 3H, 3,4,5-CH (Clbenz)), 7.16 (s, 1H, 2-CH (Clbenz)), 7.10–7.08 (d, J = 8.4 Hz, 1H, 6-CH (Clbenz)), 4.92 (s, 2H, OCH_2CO), 3.64 (t, J = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, J = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, J = 9.2, 7.6 Hz, 1H, CHPy), 2.92 (t, J = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, J = 7.6 Hz, 2H, CH_2S), 2.41 (t, J = 7.7 Hz, 2H, NCH_2Pyrr), 2.04 (t, J = 7.7 Hz, 2H, CH_2Py), 1.96 (m, 2H, CH_2Py); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42–173.65 (2CON), 162.26 (C═N), 141.65 (CH═), 136.44 (C─C═N), 133.43 (C, Ph), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl, 128.20 (CH, Ph), 127.13 (2CH, Ph), 113.55 (CH, Ph), 111.33 (CH, Ph), 72.27 (C-spiro), 68.20 (CH), 56.54 (CHCO), 54.66 (CH), 54.35 (CH_2), 51.21 (OCH_2), 44.40 ($2CH_2$), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{27}H_{29}Cl_2N_5O_3S$ (573): C 56.45, H 5.09, N 12.19, S 5.58; found: C 56.21, H 4.84, N 711.94, S 5.27.

2-(2-(4′-(3,4-Dichlorophenyl)-2′-methyl-1′-oxo-2′,4a′,7′,7a′-tetrahydro-1′H-spiro[pyrrolidine-2,5′-thieno[3,4-d]pyridazin]-1-yl)ethoxy)-N′-(4-methoxybenzylidene)acetohydrazide (***16***). Colorless solid, 70%, mp: 210–212°C; IR (KBr) ν 3330 (NH), 3050 (CH_{Ar}), 1691, 1667 (C═O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H, CH Ar), 9.28 (s, 1H, NH), 7.23 (d, J = 8.4 Hz, 1H, 5-CH (Clbenz)), 7.19 (s, 1H, 2-CH (Clbenz)), 7.13–7.12 (d, J = 8.4 Hz, 2H, 2,6-CH (anis)), 7.06 (d, J = 8.4 Hz, 1H, 6-CH (Clbenz)), 6.93–6.92 (d, J = 8.4 Hz, 2H, 3,5-CH (anis)), 4.91 (s, 2H, OCH_2CO), 3.64 (t, J = 8.4 Hz, 2H, CH_2O), 3.32–3.12 (d, J = 9.2 Hz, 1H, CHCO), 3.10 (s, 3H, NCH_3), 3.02–3.00 (dd, J = 9.2, 7.6 Hz, 1H, CHPyrid), 2.92 (t, J = 8.4 Hz, 2H, NCH_2), 2.82–2.78 (d, J = 7.6 Hz, 2H, CH_2S), 2.78 (s, 3H, OCH_3), 2.41 (t, J = 7.7 Hz, 2H, NCH_2Pyrr), 2.04 (t, J = 7.7 Hz, 2H, CH_2Pyrr), 1.96 (m, 2H, CH_2Pyrr); ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.42–173.65 (2CON), 162.26 (C═N), 141.65 (CH═), 138.20 (C, Ar), 136.44 (C─C═N), 133.43 (C, Ar), 132.08 (C─Cl), 131.70 (C─C spiro), 130.95 (CH, 3-ArCl), 130.32 (CH, 4-ArCl), 129.02 (CH, 5-ArCl), 128.55 (CH, 6-ArCl), 127.13 (2CH, Ar), 113.55 (CH, Ar), 111.33 (CH, Ar), 72.27 (C-spiro), 68.20 (CH), 57.45 (OCH_3), 56.54 (CHCO), 54.66 (CH), 54.35 (CH_2), 51.21 (OCH_2), 44.40 ($2CH_2$), 42.96 (CH_2), 39.33 (CH_2). *Anal*. Calcd for $C_{28}H_{31}Cl_2N_5O_4S$ (603): C 55.63, H 5.17, N 11.58, S 5.30; found: C 55.43, H 4.95, N 11.27, S 5.05.

In vitro cytotoxicity of the newly synthesized compounds. Anticancer activity screening of the newly synthesized compounds was measured *in vitro* utilizing two human tumor cell lines, namely, mammary gland (MCF-7) and human lung fibroblast (WI-38).

The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was used as a standard anticancer drug for comparison.

The reagents RPMI-1640 medium, MTT, and DMSO (Sigma co., St. Louis, USA), fetal bovine serum (GIBCO, UK).

The aforementioned cell lines were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT)-(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% CO_2 incubator. The cell lines were seeds in a 96-well plate at a density of 1.0 × 104 cells per well at 37°C for 48 h under 5% CO_2. After incubation, the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 μL of MTT solution at 5 mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 μL is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) × 100.

Antibacterial activity. The anti-bacterial activity of the synthesized compounds was tested against a panel of Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). The anti-fungal activities of the compounds were tested against (*Candida albicans*). Each of the compounds was dissolved in DMSO, and solution of the concentration 1 mg/mL were prepared separately; paper discs of Whatman filter paper were prepared with standard size (5 cm) and were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the Petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with *Staphylococcus aureus*, *E. coli*, and *Candida albicans*. The Petri dishes were incubated at 36°C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin and antifungal Clotrimazole was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as under:

$$\%\textbf{Activity Index} = \frac{\textbf{Zone of inhibition by test compound (diametre)}}{\textbf{Zone of inhibition by standard (diametre)}} \times 100$$

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