Synthesis of Some New Pyrazoline-Based Thiazole Derivatives and Evaluation of Their Antimicrobial, Antifungal, and Anticancer Activities

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Abstract—3-(2-Thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines were reacted with chloroacetone derivatives and hydrazonyl chloride derivatives in ethanol to afford the corresponding thiazolylpyrazoline derivatives and thiophenylpyrazolyl-5-substituted aryl-diazenylthiazole derivatives, respectively. The structures of the newly synthesized compounds were elucidated by different elemental and spectral analyses (IR, mass, ¹H and ¹³C NMR). The antimicrobial and antifungal activities of the newly synthesized compounds were evaluated against four bacterial species and five fungal strains. In addition, the antitumor activities of two of the newly synthesized compounds 1-(2-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-4-methyl thiazol-5-yl)ethan-1-one and 2-(5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-methyl-5-(phenyl-diazenyl)thiazole against HEPG-2, HCT-116, MCF-7, BHK, and CACO-2 were evaluated. From the obtained results, we found that these two compounds were the most potent candidates towards all gram-positive and gram-negative bacteria, as well as the fungi studied. Also, the same two compounds showed strong antitumor activities against two of the tumor cell lines (HCT-116 and CACO-2).

Keywords: thiophene, thiazole, pyrazoline, antimicrobial, antifungal, anticancer activity **DOI:** 10.1134/S1068162020030061

INTRODUCTION

The presence of pyrazole nucleus in different structures leads to diversified applications in different areas, such as technology, medicine, and agriculture. In particular, they are described as inhibitors of protein glycation, antibacterial, antifungal, anti-tuberculosis, antioxidant, and antiviral agents [1, 2]. Compounds including a pyrazole nucleus exhibit a wide spectrum of biological activities, such as antimicrobial, anti-inflammatory, antidepressant, and anticancer effects. Among the reported activities, it is important to note that pyrazolines are not only useful in treatment of various cancer types, including brain, bone, mouth, esophagus, stomach, liver, bladder, pancreas, cervix, lung, breast, colon, rectum, and prostate cancers, but also some of them act as cancer chemopreventive agents [3–21]. Also, pyrazoline derivatives were reported as epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors [17], aurora kinase inhibitors [18], COX-2/B-Raf inhibitors [19], telomerase inhibitors [20], and tubulin assembling inhibitors [21]. On the other hand, thiazole has been recognized as a promising scaffold for the design and development for central nervous system (CNS) active agents. There are thiazole-based CNS drugs currently used as therapeutic agents for the treatment of various CNS disorders and a number of thiazole derivatives are in clinical trials [22]. Diverse modifications of the thiazole ring at various positions have led to a variety of thiazole-based CNS agents as AChE and BuChE inhibitors, secretase inhibitors, monoamine oxidase (MAO) inhibitors, neuronal nitric oxide synthase inhibitors, Ach receptor ligands, adenosine receptor ligands, dopamine receptor ligands, serotonin receptor ligands, glutamate receptor ligands. γ -aminobutyric acid receptor ligands, opioid receptor ligands, neuroprotective, and anticonvulsant agents [22–25]. Acotiamide has been reported to be a prom-

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ising thiazole-based agent for the treatment of functional dyspepsia in clinical trials. Acotiamide enhances ACh release in the enteric nervous system through AChE inhibition and M1/M2 muscarinic receptor antagonism [26]. Many studies were carried out on heterocyclic systems bearing thiazole and pyrazoline [27–32] and pyrazolidinone [33] groups as pharmacophores. Sulfur and/or nitrogen heterocycles that possess pharmaceutical activities widely occur in nature in the form of alkaloids, vitamins, pigments, and as constituents of plant and animal cells. Thiazolyl-pyrazoline derivatives have been used as cholinesterase inhibitors [34]. From the above facts and our interest in designing new heterocyclic compounds that have the pharmaceutical properties [35-39], we synthesized a new system that combines these two bio-labile components, pyrazolines and thiazoles, together in order to give a compact structure like the titled compounds. Evaluation of the antimicrobial and antifungal activities of the newly synthesized compounds was investigated against four bacterial species and/or five fungal strains. In addition, the antitumor activities of two synthesized compounds against HEPG-2, HCT-116, MCF-7, BHK, and CACO-2 were also evaluated.

RESULTS AND DISCUSSION

Chemistry

3-(2-Thienyl)-5-aryl-1-thiocarbamoyl-2-pyrazolines (IIIa-c) ($\mathbf{R} = \mathbf{a}$, H; **b**, Cl; **c**, OMe) were prepared by the reaction of 1-(2-thienyl)-3-aryl-2propen-1-ones (Ia-c) (R = a, H; b, Cl; c, OMe) with thiosemicarbazide and sodium hydroxide in ethanol according to a reported procedure [40]. The reaction mechanism involves the formation of hydrazone followed by subsequent addition of N-H on the olefinic bond of the propenone moiety [41]. Reaction of the thioamide derivative (IIIa) (R = H)with chloroacetone derivatives (IVa-c) ($\mathbf{R}_1 = \mathbf{a}$; Me, b; OEt and c; NHPh) vielded thiophenyl-pyrazolyl-thiazoles (VIIa-c), respectively, as shown in Scheme 1. The reactions were performed in refluxing ethanol. Reaction of compound (IIIb) (R = CI)with compounds (IVa-c) under the same conditions afforded compounds (VIId-f), respectively. Finely, reaction of (IIIc) (R = OMe) with compounds (IVa-c) at the same conditions afforded compounds (VIIg-i), respectively. The mechanisms of all reactions passed through two intermediates (Va-i) and (VIa-i), respectively. The structures of compounds (VIIa-i) were confirmed by elemental and spectral analyses using methods of GC-MS, IR, ¹H, and ¹³C NMR. IR spectra of compounds (VIIa-i) showed stretching absorption

bands of C=O, C=N, and C=C groups at their expected regions. ¹H NMR spectra of the same compounds are consistent with their assigned structures. The ¹H NMR spectra of the compounds (VIIa-i) showed CH₂ signals of the pyrazoline ring resonating as a pair of doublets of doublets at δ 3.15-3.70 (H_a) and 3.98-4.16 ppm (H_b). The (H_c) proton appeared as a doublet of doublets at δ 5.59– 5.90 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring $(J_{a-b} =$ 18.50–23.10 Hz, $J_{a-c} = 6.0-6.80$ Hz, $J_{b-c} = 11.70-$ 12.10 Hz). The other aromatic and aliphatic protons were found at their expected regions. Also, ¹³C NMR spectral data of the same compounds gave another evidence for the assigned structures (Experimental part and Scheme 1).

On the other hand, reaction of 3-(2-thienyl)-5aryl-1-thiocarbamoyl-2-pyrazoline derivative (IIIa) (R = H) with hydrazonyl chloride derivatives (VIIIa-c) $(\mathbf{R}_2 = \mathbf{a}; \mathbf{H}, \mathbf{b}; 4$ -Me and $\mathbf{c}; 4$ -Cl) afforded 3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-diazenylthiazol derivatives (XIa-c), respectively. Also, reaction of compound (IIIb) (R = Cl) with compounds (VIIIa-c) afforded compounds (XId-f), respectively. Finely, reaction of compound (IIIc) (R = OMe) with hydrazonyl chloride derivatives (VIIIa-c) afforded compounds (XIg-i), respectively. All the reactions were performed in absolute ethanol under reflux temperature. The reaction mechanism involves two intermediates (IXa-i) and (Xa-i) that played important roles in thiazole formation step. The structures of compounds (XIa-i) were confirmed by elemental and spectral analyses, including GC-MS, IR, ¹H, and ¹³C NMR. Their ¹H NMR spectra showed the appearance of the characteristic signals due to the three pyrazoline ring protons in the regions of δ 3.33–3.37, 3.90–4.15, and 5.73–5.97 ppm, respectively, due to vicinal coupling with the two magnetically non-equivalent protons of the methylene group at position 4 of the pyrazoline ring $(J_{a-b} = 18.50 - 23.10 \text{ Hz}, J_{a-c} = 6.0 - 6.80 \text{ Hz}, J_{b-c} =$ 11.70-12.10 Hz). All other aromatic and aliphatic protons were found at their expected regions. The IR spectra of compounds (XIa-i) revealed absorption bands at about 1600–1605 cm^{-1} due to the C=N groups. ¹³C NMR spectral data of the compounds give us another proof for the same assigned structures (see the Experimental section and Scheme 2).











(VIIa-i)

(VIa-i)

(Va-i)

(V), (VI), (VII)	R	R ₁	Yield, %	
a	Н	Me	56	
b	Н	OEt	76	
с	Н	NHPh	67	
d	Cl	Me	77	
e	Cl	OEt	56	
f	Cl	NHPh	68	
g	OMe	Me	65	
h	OMe	OEt	78	
i	OMe	NHPh	72	

Scheme 1. Synthesis of pyrazoline-based thiazoles (VIIa-i).



Scheme 2. Synthesis of pyrazoline-based thiazoles (XIa–i).

Antimicrobial Activity

The antimicrobial activity of the newly synthesized compounds was evaluated against two gram-positive bacteria, *Staphylococcus aureus* (S. a.) and *Bacillus cereus* (B. c.), and two gram-negative bacteria, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, together with to five different fungus strains, namely, *Candida albicans*, *Syncephalastrum racemosum*, *Aspergillus fumigatus*, *Penicillium expansum*, and *Aspergillus flavus*.

The results of the antimicrobial screening of the newly synthesized pyrazole derivatives (VIIa-i) and (XIa-i) are shown in Table 1. The most important

part of the results was those that were obtained from antifungal activity screening. Most of the compounds were effective against *C. albicans* and *A. fumigatus* when compared with griseofulvin, especially compounds (VIIb) and (XId), which showed strong activities. Meanwhile, compounds (VIIe), (VIIa), (VIId), (VIIg), (VIIf), (Xia), (XId), and (VIIc) showed moderate activities against the same strains compared with griseofulvin. In the case of thiazole derivatives, potent antifungal activities were observed comparable with a commercially available drug, in particular against *C. albicans* and *A. fumigatus*. Antibacterial activity assessment revealed that the compounds had moderate or slight activities.

	Inhibition zone, mm									
Comp. (5 mg/mL)	Antibacterial activity			Antifungal activity						
	S. aureus	B. subtilis	K. pneumonia	P. aeruginosa	A. fumigatus	A. flavus	S. racemosum	P. expansum	C. albicans	
(VIIb)	2.2	1	0.9	_	20	0.5	1.8	1.2	0.9	
(VIIe)	1.1	0.5	_	_	—	0.5	_	0.9	1	
(VIIh)	_	_	_	_	—	_	—	_	_	
(VIIa)	_	_	_	_	—	0.5	—	_	0.8	
(VIId)	1.5	0.8	0.5	—	—	1.1	—	0.5	—	
(VIIg)	_	0.5	-	—	1	_	_	—	1.2	
(VIIc)	_	—	—	—	—	_	—	-	—	
(VIIf)	1.5	1.2	0.8	1	1.4	_	—	—	_	
(VIIi)	_	_	_	_	_	_	—	—	—	
(Xia)	_	1	0.2	—	—	0.5	0.8	—	0.5	
(XId)	1.8	1	1.1	0.9	1.9	2	1.2	1.5	2.2	
(XIg)	_	_	_	—	—		—	_	—	
(XIb)	1.5	0.8	0.9	1	0.6	1	0.6	—	1.1	
(XIe)	_	_	_	—	—	_	—	_	—	
Std. ^a	2.3	3.5	2.4	2.2	3.3	2.4	2.1	2.8	3	

Table 1. Antibacterial and antifungal activities of the synthesized compounds

^a Antibacterial reference drug is amoxicilline and antifungal reference drug is griseofulvin.

Minimum inhibitory concentration (MIC). By applying ethyl acetate solvent of the synthesized compounds started with a maximum concentration of 500 mg/mL and then reduced it by successive two fold dilutions of the stock solution using a calibrated micropipette. MIC of the sample determination was carried out by inoculation of their serial dilutions with test organisms and measurement of inhibition zones using diffusion agar technique. MIC was expressed as the lowest concentration inhibiting test. The results of minimum inhibitory concentration showed that, pyrazole derivatives (**VIIb**) and (**XId**) (MIC 1.25 mg/mL) showed better results than the reference drug (MIC 2.5 mg/mL).

Antitumor Activity

The antitumor activity of the two synthesized compounds (**VIIb**) and (**XId**) was evaluated against five tumor cell lines (HEPG-2 liver carcinoma cell line, MCF-7 breast carcinoma cell line, HCT-116 colon carcinoma cell line, CACO-2 colon carcinoma cell line, and BHK normal fibroblast cell line). The results of the antitumor activity of the newly synthesized compounds against the five tumor cell lines (survival, %) are summarized in Table 2, which shows the IC₅₀ values, i.e. the doses that reduces survival to 50%. From the results we conclude that compounds (**VIIb**) and (**XId**) have strong activities against the HCT-116 and CACO-2 lines while only compound (**XId**) shows high cytotoxic affect against the BHK normal fibroblast cell line. Compound (**VIIb**) did not show any cytotoxic effect on the BHK normal fibroblast cell line, so compound (**VIIb**) may be considered as a better candidate antitumor agent than compound (**XId**).

Structure–Activity Relationship (SAR)

From the results of the antibacterial and antifungal activities we concluded that compounds (VIIb) and (XId) were the most potent towards almost all the gram-positive and gram-negative bacteria, as well as all fungi, studied. Also, the two compounds, (VIIb) and (XId), showed strong antitumor activities against two tumor cell lines (HCT-116 and CACO-2). This may be due to the C=S, C=N, C=O, and N=N groups found in the heterocyclic rings, which gave good binding energies between the compounds and the active sites found in the proteins, in addition to the aromatic rings and the ester group found in compound (VIIb) and the halide atom (-Cl) found in compound (XId), which increased the activity of such compounds. The other compounds showed moderate effects toward the studied bacteria and fungus.

CONCLUSION

Thiazolyl-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives (**VIIa**-i) and (**XIa**-i) were synthesized and evaluated for their antibacterial, antifungal, and antitumor activities. A significant level of activity was illustrated. Compounds (**VIIb**) and (**XId**) were the most potent

Cell survival, %								
Conc	centration, µg/mL	(VIIb)	(XId)	Concentration, µg/mL		(VIIb)	(XId)	
HEPG2	0.0	100	100		0.0	100	100	
	1.0	70	96	CAC02	1.0	86	80	
	2.5	55	92		2.5	77	71	
	5.0	43	87		5.0	62	47	
	10.0	30	83		10.0	48	34	
	IC ₅₀ , μg/mL	100	—	IC ₅₀ , μg/mL 8.9		4.0		
MCF7	0.0	100	100		0.0	100	100	
	1.0	100	98	BHK	1.0	95	87	
	2.5	95	94		2.5	83	63	
	5.0	84	86		5.0	70	58	
	10.0	70	77		10.0	59	38	
	IC ₅₀ , μg/mL	_	—	IC ₅₀ , μg/mL		_	5.9	
	0.0	100	100		· · · · · ·			
HCT116	1.0	89	90					
	2.5	75	85					
	5.0	58	58					
	10.0	40	44]				
IC ₅₀ , μg/mL		7.5	6.0					

Table 2. Antitumor activities of compounds (VIIb) and (XId) against five tumor cell lines (survival, %, and IC₅₀, μ g/mL)

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towards almost all the gram-positive and gram-negative bacteria, as well as all fungi, studied. Also, the two compounds (VIIb) and (XId) showed a strong antitumor activity against two tumor cell lines (HCT-116 and CACO-2). Only compound (XId) showed high cytotoxic affect against the BHK cells. Compound (VIIb) did not show any cytotoxic effect on the BHK normal fibroblast cell line, so we concluded that compound (VIIb) may be considered as a better candidate antitumor drug than compound (XId).

EXPERIMENTAL

Chemistry

Melting points were measured with a Gallenkamp melting point apparatus and were uncorrected. Infrared (IR) spectra (v, cm⁻¹), were recorded as KBr disks using the Perkin Elmer FT-IR Spectrum BX apparatus. Mass spectral data are given as m/z (intensity, %). NMR spectra were recorded on a Bruker NMR spectrometer. ¹H NMR spectra were recorded at 400 MHz and ¹³C spectra, at 100 MHz, in deuterated dimethyl-sulfoxide (DMSO- d_6). Chemical shifts are expressed in δ values (ppm) using the solvent peak as internal standard. All coupling constants (J) values are given in hertz. The abbreviations used are as follows: s, singlet;

d, doublet; q, quartet; m, multiplet. The mass spectra were recorded on a Shimadzu GC-MS QP-1000 EX mass spectrometer operating at 70 eV. The progress of all reactions was monitored by thin layer chromatography using Merck Kiesel gel 60-F254 aluminum backed plates. Unless otherwise noted, all solvents and reagents were commercially available and used without further purification. Compounds (**IIIa–c**) were synthesized according to a reported procedure [40]. For compound (**IIIa**), mp 198–200°; (**IIIb**), mp 198– 200°C; (**IIIc**), mp 144–146°C. Hydrazonoyl halides were prepared as reported in the literature [45].

General Procedure for Synthesis of Compounds (VIIa-i) and (XIa-i)

To a suspension of compounds (IIIa–c) (10 mmol; (IIIa), 2.87 g; (IIIb), 3.21 g; (IIIc), 3.17 g) in absolute ethanol (30 mL), compounds (IVa–c) or (VIIIa–c) (10 mmol; α -chloro acetyl acetone (IVa), 1.29 g; α chloroethyl acetoacetate (IVb), 1.64 g; α -chloroacetoacetanilide (IVc), 2.11 g; α -chloroaceto phenylhydrazono, (*Z*)-1-chloro-1-(2-phenylhydrazono)propan-2-one (VIIIa), 1.96 g, (*Z*)-1-chloro-1-(2-p-tolylhydrazono)propan-2-one (VIIIb), 2.10 g; and (*Z*)-1chloro-1-(2-(4-chlorophenyl) hydrazono)propan-2one (VIIIc), 2.30 g) were added and heated at reflux temperature for 8 h. After cooling, the precipitates were collected. The products were crystallized from ethanol to afford the titled compounds (**VIIa**–i) and (**XIa**–i), respectively.

1-(4-Methyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5dihydropyrazol-1-yl)thiazol-5-yl)ethanone (VIIa). Yield 2.05 g (56%); dark green crystals; mp 202-204°C; IR: 3084 (Ar-CH), 2929 (aliphatic CH), 1625 (C=O); ¹H NMR: 2.38 (s, 3H, CH₃), 2.52 (s, 3H, $COCH_3$), 3.33 (dd, 1H, J = 11.6, J = 5.2 Hz, pyrazoline H_a), 4.04–4.12 (dd, 1H, J = 18.4, J = 11.6 Hz, pyrazoline H_b), 5.64–5.70 (dd, 1H, J = 9.6, J =5.2 Hz, pyrazol H_c), 7.16–7.30 (m, 3H, Ar-H), 7.34– 7.38 (m, 3H, thiophene- H_4 , Ar-H), 7.49–7.50 (d, 1H, J = 2.8 Hz, thiophene-H₅), 7.78–7.79 (d, 1H, J = 0.8Hz, thiophene-H₃); 13 C NMR: 16.0, 26.4 (2CH₃), 44.66 (CH₂), 63.73 (CH), 124.4, 128.10, 128.98, 129.28, 130.36, 130.98, 134.07, 136.0, 138.3 139.55, 141.66, 150.79, 155.27, 160.87 (Ar-C, thiophene-C, thiazole-C, pyrazole-C), 196.35 (CO); MS (m/z): 369 $([M^+ + 2], 5), 367 ([M^+], 12);$ analysis calcd. for C₁₉H₁₇N₃OS₂: C, 62.10; H, 4.66; N, 11.43; found: C, 62.21; H, 4.11; N, 10.50%.

Ethyl-4-methyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5dihydropyrazol-1-yl)thiazole-5-carboxylate (VIIb). Yield 3.02 g (76%); bright green crystals; mp 144– 145°C; IR: 1735 (C=O), 1618 (C=N), 1595 (C=C); ¹H NMR: 1.28 (t, 3H, J=7.2 Hz, OCH₂CH₃), 2.35 (s, 3H, CH₃), 3.39 (m, 1H, pyrazoline H_a), 4.04–4.12 (dd, J = 18.4, J =12 Hz, 1H, pyrazoline H_b), 4.16– 4.42 (q, J = 7.2 Hz, 2H, OCH₂CH₃), 5.72–5.77 (dd, J = 13.2, J = 6 Hz, 1H, pyrazol H_c), 7.16–7.38 (m, 6H, H₄ thiophene, Ar-H),7.49–7.50 (d, J = 3.6 Hz, 1H, H₅ thiophene), 7.78–7.79 (d, J=2.8 Hz, 1H, H₃-thiophene); MS (m/z): 397 ([M⁺], 5); analysis calcd. for C₂₀H₁₉N₃O₂S₂: C, 60.43; H, 4.82; N, 10.57; found: C, 60.11; H, 4.77; N, 10.40.

4-Methyl-N-phenyl-2-(5-phenyl-3-(thiophen-2-yl)-4.5-dihydropyrazol-1-yl)thiazole-5-carboxamide (VIIc). Yield 2.98 (67%); pale yellow crystals; mp 232-233°C; IR: 1570–1595 (C=C); ¹H NMR: 2.37 (s, 3H, <u>CH₃</u>), 3.34–3.39 (m, 1H, pyrazoline H_a), 4.05–4.13 $(dd, J = 19.2, J = 9.6 Hz, 1H, pyrazoline H_b), 5.74-$ 5.78 (dd, J = 12.4, J = 7.6 Hz, 1H, pyrazol H_c), 7.05-7.48 (m, 11H, thiophene H₄, Ar-H), 7.76–7.78 (d, J =4.8 Hz, 1H, thiophene H₅), 7.63–7.65 (d, J = 2.8 Hz, 1H, thiophene H₃), 9.67 (s, 1H, NH exchangeable); ¹³C NMR: 18.14 (CH₃), 44.66 (CH₂), 63.73 (CH), 115.55, 120.83, 121.0, 123.90, 124.4, 125.0, 126.28, 128.10, 128.98, 129.28, 130.36, 130.98, 134.07, 136.0, 138.3 139.55, 141.66, 150.79, 155.27, 160.87 (Ar-C, thiophene-C, thiazole-C, pyrazole-C), 163.35 (CO); MS (m/z): 444 $([M^+], 4)$; analysis calcd. for C₂₄H₂₀N₄OS₂: C, 64.84; H, 4.53; N, 12.60; found: C, 64.61; H, 4.73; N, 12.90%.

1-(2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5dihydropyrazol-1-yl)-4-methylthiazol-5-yl)ethan-1-one (VIId). Yield 3.09 g (77%); dark green crystals; mp 219–220°C; IR: 1660 (C=O), 1606 (C=N), 1575 (C=C); ¹H NMR: 2.37 (s, 3H, CH₃), 2.39 (s, 3H, COCH₃), 3.35 (m, 1H, pyrazoline H_a), 4.05 (dd, J =12.4, J = 11.2 Hz, 1H, pyrazoline H_b), 5.77 (dd, J =12.4, J = 5.2 Hz, 1H, pyrazole H_c), 7.16–7.49 (m, 4H, thiophene H₄, H₅, Ar-H),7.49–7.50 (d, J = 1.2 Hz, 1H, thiophene H₃),7.79–7.80 (m, 2H, Ar-H); MS (*m*/*z*): 402 ([M^+ + 1], 5); analysis calcd. for C₁₉H₁₆ClN₃OS₂: C, 56.78; H, 4.01; N, 10.45; found: C, 56.63; H, 4.22; N, 10.34%.

Ethvl 2-(5-(4-chlorophenvl)-3-(thiophen-2-vl)-4,5-dihydropyrazol-1-yl)-4-methylthiazole-5-carboxylate (VIIe). Yield 2.41 g (56%); pale green crystals; mp 160–162°C; IR: 1678 (C=O), 1579 (C=N); ¹H NMR: 1.26 (t, J = 6, 3H, OCH₂CH₂), 3.35 (s, 3H, CH_3 , 3.34 (m, 1H, pyrazoline H_3), 4.03–4.11 (dd, J= $17.6, J = 10.4 \text{ Hz}, 1\text{H}, \text{ pyrazoline } H_{\text{b}}), 4.16-4.22 \text{ (q,}$ J = 8.4 Hz, 2H, O<u>CH</u>₂CH₃), 5.73–5.77 (dd, J = 12, J = 6.4 Hz, 1H, pyrazol H_c), 7.17–7.31 (m, 3H, thiophene H_4 , Ar-H), 7.40–7.49 (m, 3H, thiophene H_5 , Ar-H), 7.79-7.80 (d, J = 3.2 Hz, 1H, thiophene H₃); ¹³C NMR: 14.73, 17.88 (2CH₃), 44.49, 60.69 (2CH₂), 63,07 (CH), 111.03, 125.6, 126.0, 127.6, 128.69, 129.28, 130.74, 131.35, 132.69, 133.74, 140.40, 151.66, 159.57, (Ar-C, thiophene-C, thiazole-C, pyrazole-C). 162.23 (CO).164.92 (CN): MS (m/z): 432 $(M^+ + M^-)$ 1], 6); analysis calcd. for $C_{20}H_{18}CIN_3O_2S_2$: C, 55.61; H, 4.20; N, 9.73; found: C, 55.41; H, 4.33; N, 10.0%.

2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5dihydropyrazol-1-yl)-4-methyl-*N***-phenyl-thiazole-5carboxamide (VIIf).** Yield 3.25 g (68%); bright green powder; mp 218–220°C; IR: 1641 (C=O), 1596 (C=N); ¹H NMR: 2.34 (s, 3H, CH₃), 3.37 (m, 1H, pyrazoline H_a), 4.04–4.12 (dd, J = 18.8, J = 14 Hz, 1H, pyrazoline H_b), 5.73–5.78 (dd, J = 14.8, J =5.6 Hz, 1H, pyrazol H_c), 7.05–7.32 (m, 8H, thiophene H₄, Ar-H),7.43–7.48 (m, 2H, Ar-H), 7.63– 7.65 (d, J = 6.8 Hz, 1H, thiophene H₅), 7.76–7.78 (d, J = 5.2 Hz, 1H, thiophene H₃), 9.66 (s, 1H, NH exchangeable); MS (m/z): 480 ([M^+ + 1], 11); analysis calcd. for C₂₄H₁₉ClN₄OS₂: C, 60.18; H, 4.00; N, 11.70; found: C, 60.11; H, 4.07; N, 11.77%.

1-(2-(5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5dihydropyrazol-1-yl)-4-methylthiazol-5-yl)ethanone (VIIg). Yield 2.58 g (65%); greenish silver crystals; mp 198– 200°C; IR: 1640 (C=O), 1579 (C=C); ¹H NMR: 2.38 (s, 3H, thiazole CH₃), 2.50 (s, 3H, COCH₃), 3.40 (m, 1H, pyrazoline H_a), 3.72 (s, 3H, OCH₃), 4.02 (dd, J =21, J = 12.4 Hz, 1H, pyrazoline H_b), 5.69 (dd, J = 11.6, J = 4.8 Hz, 1H, pyrazole H_c), 6.87–6.92 (d, J = 8 Hz, 2H, Ar-H), 7.17–7.19 (m, 3H, thiophene H₄, Ar-H), 7.30 (d, J = 5.6 Hz, 1H, thiophene H₅), 7.77 (d, J = 6 Hz, 1H, thiophene H₃); ¹³C NMR: 19.07, 30.08 (2CH₃), 40.40 (CH₂), 55.5 (CH₃), 63.2 (CH), 114.5, 115.55, 128.10, 128.98, 129.28, 130.98, 134.07, 136.0, 138.3 139.55, 141.66, 150.79, 155.27, 160.87 (Ar-C, thiophene-C, thiazole-C, pyrazole-C), 189.4 (CO); MS (m/z): 497 ($[M^+]$, 15); analysis calcd. for C₂₀H₁₉N₃O₂S₂: C, 60.43; H, 4.82; N, 10.57; found: C, 60.11; H, 4.75; N, 10.90%.

Ethyl 2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydropyrazol-1-yl)-4-methylthiazole-5-carboxylate (VIIh). Yield 3.33 g (78%); pale yellow crystals; mp 152–153°C; IR: 1735 (C=O), 1578 (C=C); ¹H NMR: 1.26 (t, J = 6.4 Hz, 3H, OCH₂<u>CH₃</u>), 2.35 (s, 3H, thiazole CH₃), 3.29–3.30 (m, 1H, pyrazoline H_a), 3.72 (s, 3H, OCH₃), 4.02 (dd, J = 19.6, J =12.8 Hz, 1H, pyrazoline H_b), 4.19 (q, J = 9.6 Hz, 2H, O<u>CH₂</u>CH₃), 5.66 (dd, J = 13.2, J = 6.0 Hz, 1H, pyrazole H_c), 6.90 (m, 2H, Ar-H), 7.12–7.19 (m, 3H, thiophene H₄, Ar-H), 7.48 (d, J = 2.8 Hz, 1H, thiophene H₅), 7.78 (d, J = 6.8 Hz, 1H, thiophene H₃); MS (m/z): 427 ([M^+], 22); analysis calcd. for C₂₁H₂₁N₃O₃S₂: C, 59.00; H, 4.95; N, 9.83; found C, 58.95; H, 4.90; N, 9.80%.

2-(5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5dihydropyrazol-1-yl)-4-methyl-*N***-phenyl-thiazole-5carboxamide (VIIi).** Yield 3.42 g (72%); pale yellow crystals; mp 224–225°C; IR: 1634 (C=O), 1615 (C=N), 1593 (C=C); ¹H NMR: 2.36 (s, 3H, thiazole CH₃), 3.77 (m, 1H, pyrazoline H_a), 3.72 (s, 3H, OCH₃), 4.06 (dd, J = 23.6, J = 14 Hz, 1H, pyrazoline H_b), 5.68 (dd, J = 13.2, J = 6 Hz, 1H, pyrazole H_c), 6.91 (d, J = 8 Hz, 2H, Ar-H), 7.05–7.50 (m, 8H, Ar-H, thiophene H4), 7.63–7.65 (d, J = 6.8 Hz, 1H, thiophene H₃), 7.76–7.74 (d, J = 6.8 Hz, 1H, thiophene H₅), 9.6 (s, 1H, NH exchangeable); MS (*m*/*z*): 476 ([M^+ + 2], 8); analysis calcd. for C₂₅H₂₂N₄O₂S₂: C, 63.27; H, 4.67; N, 11.81; found: C, 63.41; H, 4.53; N, 11.90%.

4-Methyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-(phenyldiazenyl)-thiazole (XIa). Yield 2.40 g (66%); orange crystals; mp $160-162^{\circ}$ C; IR: 1572 (C=N); ¹H NMR: 2.51 (s, 3H, <u>CH₃</u>), 3.91-3.97 (dd, J = 16.4, J = 10.8 Hz 1H, pyrazoline H_a), 4.01-4.17 (dd, J = 16, J = 10 Hz, 1H, pyrazoline H_b), $5.86-5.91 (dd, J = 14.8, J = 9.6 Hz, 1H, pyrazol H_c),$ 7.16-7.58 (m, 2H, H₄, thiophene H₅), 7.68-7.84 (m, 11H, Ar-H, thiophene H₃); ¹³C NMR: 10.07 (CH₃), 40.40 (CH₂), 60.2 (CH), 124.60, 125.0, 126.28, 128.10, 128.98, 129.28, 130.36, 134.07, 135.2, 136.0, 138.3 139.55, 141.66, 147, 150.79, 155.27, 160.87, 163.35 164.2, 165.8 (Ar-C, thiophene-C, thiazole-C, pyrazole-C); MS (m/z): 429 ([M^+], 10); analysis calcd. for C₂₃H₁₉N₅S₂: C, 64.31; H, 4.46; N, 16.30; found: C, 64.11; H, 4.55; N, 16.50%.

4-Methyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H***-pyrazol-1-yl)-5-(***p***-tolyldiazenyl)-thiazole** (**XIb**). Yield 2.927 g (66%); scarlet red crystals; mp 194– 196°C; IR: 1572 (C=N), 1595 (C=C); ¹H NMR: 2.36 (s, 3H, <u>Ar-CH₃</u>), 2.48 (s, 3H, thiazole C<u>H₃</u>), 3.91– 3.98 (dd, J = 18.8, J = 13.2 Hz, 1H, pyrazoline H_a), 4.08–4.13 (dd, J = 21.2, J = 12.8 Hz, 1H, pyrazoline H_b), 5.85–5.89 (dd, J = 13.6, J = 9.2 Hz, 1H, pyrazoline H_b), 5.85–5.89 (dd, J = 13.6, J = 9.2 Hz, 1H, pyrazoline H_b), 7.14–7.16 (m, 6H, Ar-H, thiophene H₄, H₅), 7.14–7.83 (m, 6H Ar-H, thiophene H₃); MS (*m*/*z*): 443 ([*M*⁺], 12); analysis calcd. for C₂₄H₂₁N₅S₂: C, 64.98; H, 4.77; N, 15.79; found: C, 64.81; H, 4.67; N, 15.90%.

5-((4-Chlorophenyl)diazenyl)-4-methyl-2-(5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (XIc). Yield 3.99 g (86%); reddish brown crystals; mp 190–192°C; IR: 1572 (C=N); ¹H NMR: 2.48 (s, 3H, thiazole <u>CH₃</u>), 3.38–3.44 (m, 1H, pyrazoline H_a), 3.89–3.98 (dd, J = 21.2, J = 12.8 Hz, 1H, pyrazoline H_b), 5.87–5.91 (dd, J = 12.8, J = 2.8 Hz, 1H, pyrazole H_c),7.16–7.85 (m, 12H, Ar-H, thiophene H₃, H₄, H₅); MS (*m*/*z*): 465 ([*M*⁺ + 1], 22); analysis calcd. for C₂₃H₁₈ClN₅S₂: C, 59.54; H, 3.91; N, 15.09; found: C, 59.85; H, 3.55; N, 15.10%.

2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H***-pyrazol-1-yl)-4-methyl-5-(phenyldiazenyl)thiazole (XId).** Yield 3.526 g (76%); orange crystals; mp 170– 172°C; IR: 1595 (C=N); ¹H NMR: 2.51 (s, 3H, thiazole CH₃), 3.13–3.18 (dd, 1H, J = 20, J = 4.0 Hz, pyrazoline H_a), 3.91–3.98 (dd, J = 20, J = 12.0 Hz, 1H, pyrazoline H_b), 5.93–5.97 (dd, J = 12, J = 4 Hz, 1H, pyrazole H_c), 7.16–7.55 (m, 7H, Ar-H, thiophene H₄, H₅), 7.69–7.71 (m, 5H, thiophene H₃, Ar-H); MS (*m/z*): 465 ([*M*⁺ + 1], 27); analysis calcd. for C₂₃H₁₈₋ ClN₅S₂: C, 59.54; H, 3.91; N, 15.09; found: C, 59.61; H, 4.01; N, 15.0%.

2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-methyl-5-(p-tolyldiazenyl)thiazole (XIe). Yield 4.30 g (90%); scarlet red crystals; mp 148–150°C; IR: 1618 (CN), 1595 (C=C); ¹H NMR: 2.27, 2.49 (2s, 6H, 2CH₃), 3.14-3.29 (dd, 1H, J = 16, J = 12 Hz, pyrazoline H_a), 3.91-3.98 (dd, J = 16, J = 12. Hz, 1H, pyrazoline H_b), 5.94–5.97 (dd, J = $11.6, J = 4.8 \text{ Hz}, 1\text{H}, \text{ pyrazole H}_{c}), 7.15-7.17 \text{ (m, 6H, }$ Ar-H, H4, thiophene H_5) 7.34–7.5 (m, 5H, thiophene H₃, Ar-H); 13 C NMR: 10.07, 21.0 (2CH₃), 40.40 (CH₂), 60.2 (CH), 115.55, 120.83, 121.0, 123.90, 124.4, 125.0, 126.28, 128.10, 128.98, 129.28, 130.36, 134.07, 136.0, 138.3 139.55, 141.66, 150.79, 155.27, 160.87, 163.35 (Ar-C, thiophene-C, thiazole-C, pyrazole-C), MS (m/z): 479 $([M^+ + 1], 23)$; analysis calcd. for C₂₄H₂₀ClN₅S₂: C, 60.30; H, 4.22; N, 14.65; found: C, 60.11; H, 4.33; N, 14.50%.

2-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-((4-chlorophenyl)diazenyl)-4**methylthiazole (XIf).** Yield 4.486 g (90%); brownish red crystals; mp 260–262°C; IR: 1625–1685 (C=N), 1570–1595 (C=C); ¹H NMR: 2.5 (s, 3H, thiazole CH₃), 3.13–3.19 (dd, 1H, J = 16, J = 5.2 Hz, pyrazoline H_a), 3.90–3.98 (dd, J = 17.6, J = 10.8Hz, 1H, pyrazoline H_b), 5.92–5.96 (dd, J = 16.8, J = 5.2 Hz, 1H, pyrazole H_c), 7.15–7.61 (m, 10H, Ar-H, thiophene H4, H5), 7.78–7.79 (d, J = 4.4 Hz, 1H, thiophene H₃); MS (m/z): 499 ([M^+ + 1], 12); analysis calcd. for C₂₃H₁₇Cl₂N₅S₂: C, 55.42; H, 3.44; N, 14.05; found: C, 55.61; H, 3.77; N, 14.10%.

2-(5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5dihydro-1*H***-pyrazol-1-yl)-4-methyl-5-(phenyldiazenyl)thiazole (XIg). Yield 3.033 g (66%); reddish brown crystals; mp 90–92°C; IR: 1605 (C=N),1578 (C=C); ¹H NMR: 2.5 (s, 3H, thiazole CH₃), 3.74 (s, 3H,OCH₃), 3.34–3.72 (m, 1H, pyrazoline H_a), 4.05– 4.12 (dd, J = 16, J = 8 Hz, 1H, pyrazoline H_b), 5.78– 5.87 (dd, J = 13.6, J = 11.6 Hz, 1H, pyrazole H_c), 6.87–7.84 (m, 12H, Ar-H, thiophene H₃, H₄, H₅); MS (***m***/***z***): 459 ([***M***⁺], 32); analysis calcd. for C₂₄H₂₁N₅OS₂: C, 62.72; H, 4.61; N, 15.24; found: C, 62.51; H, 4.77; N, 15.40%.**

2-(5-(4-Methoxyphenyl)-3-(thiophen-2-yl)-4,5dihydro-1*H*-pyrazol-1-yl)-4-methyl-5-(*p*-tolyldiazenyl)thiazole (XIh). Yield 4.262 g (90%); brown crystals; mp 200–202°C; IR: 1612 (C=N), 1580 (C=C); ¹H NMR: 2.36, 2.49 (2s, 6H, 2CH₃), 3.35 (m, 1H, pyrazoline H_a), 3.73 (s, 3H, OCH₃), 4.04–4.11 (dd, J = 19.2, J = 10.8 Hz, 1H, pyrazoline H_b), 5.79–5.83 (dd, J = 11.6, J = 3.6 Hz, 1H, pyrazole H_c), 6.92–6.94 (m, 2H, Ar-H), 7.18–7.58 (m, 8H, thiophene H₄, H₅, Ar-H), 7.83–7.82 (d, J = 5.6 Hz, 1H, thiophene H₃); MS (*m*/*z*): 474 ([*M*⁺ + 1], 42); analysis calcd. for C₂₅H₂₃N₅OS₂: C, 63.40; H, 4.90; N, 14.79; found: C, 63.61; H, 4.65; N, 14.90%.

5-((4-Chlorophenyl)diazenyl)-2-(5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1yl)-4-methylthiazole (XIi). Yield 3.26 g (66%); reddish brown crystals; mp 193–195°C; IR: 1610 (C=N), 1588 (C=C); ¹H NMR: 2.51 (s, 3H, CH₃), 3.74 (s, 3H, OCH₃), 3.68–3.81 (m, 1H, pyrazoline H_a), 4.22–4.32 (m, 1H, pyrazoline H_b), 5.40–5.50 (m, 1H, pyrazol H_c), 6.91–7.77 (m, 11H, Ar-H, thiophene H-3,4,5); ¹³C NMR: 10.07 (CH₃), 40.40 (CH₂), 55.0 (OCH₃), 60.2 (CH), 115.55, 120.83, 121.0, 123.90, 124.4, 125.0, 126.28, 128.10, 128.98, 129.28, 130.98, 134.07, 136.0, 138.3 139.55, 141.66, 150.79, 155.27, 160.87, 163.35 (Ar-C, thiophene-C, thiazole-C, pyrazole-C); MS (m/z): 494 ($[M^+]$, 34); analysis calcd. for C₂₄H₂₀₋ ClN₅OS₂: C, 58.35; H, 4.08; N, 14.18; found: C, 58.21; H, 4.07; N, 14.20%.

Antimicrobial Activities

Microorganisms. Nine clinical strains employed for this investigation include four filamentous fungi (Aspergillus fumigatus, Aspergillus flavus, Syncephlastarum racemosum, and Penicillium expansum), one yeast (Candida albicans), two gram-positive (Staphylococcus aureus and Bacillus subtilis) and two gram-negative (Klebsilla pnumonia and Pseudomonas aeruginosa) bacteria. All strains were provided from culture collection of Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Antimicrobial assays. By diffusion agar technique, the antifungal and antibacterial potentialities against the tested species were expressed as the measurement of diameter of their inhibition zone. Hole-plate diffusion method was used; six equidistant (1 cm diameter) holes were made using sterile cork borer in malt extract agar and nutrient agar sterile plates (10×10 cm), which had previously been seeded with tested fungal and bacterial isolates, respectively. Holes were filled with 100 mL of three concentrations 5, 2.5, and 1 mg/mL of each of the synthesized compounds after completely dissolving in ethyl acetate. Control holes were filled with ethyl acetate solvent.

Plates were left in a cooled incubator at $4 \pm 2^{\circ}C$ for 1 h, then incubated at $37 \pm 2^{\circ}C$ for bacterial isolates and incubated at $28 \pm 2^{\circ}C$ for fungal isolates. Inhibition zones developed due to active ingredients were measured after 24–48 h of incubation time. Griseofulvin was used as a standard antifungal agent while, amoxicilline was used as a standard antibacterial agent [42].

Minimum inhibitory concentration (MIC) assays. Determination of MIC was performed by a serial dilution technique [43]. Appling ethyl acetate solvent of the synthesized compounds starting with the maximum concentration of 500 mg/mL, which then was reduced by successive two-fold dilutions of the stock solution using a calibrated micropipette. MIC of the sample was determined by inoculation of the serial dilutions with tested organisms and measurement of inhibition zones using diffusion agar technique. MIC was expressed as the lowest concentration inhibiting test organism's growth [44].

Antitumor Activity

Five tumor cell lines (the HEPG2 liver carcinoma cell line, MCF7 breast carcinoma cell line; HCT116 colon carcinoma cell line, and CACO colon carcinoma cell line) and one normal cell line (BHK normal fibroblast cell line) were provided from the National Cancer Institute (NCI), Cairo University.

Cytotoxicity by SRB assay. Potential antitumor activity and cytotoxicity of the compounds were tested using the SRB technique. Tumor cells were plated in 96-well plate (10^4 cells/well) for 24 h before treatment with compounds to allow attachment of cell to the wall of the plate. Then, different concentrations of each

compound (0, 1, 2.5, 5, and 10 μ g/mL) were added to the cell monolayer triplicate wells. Monolayer cells were incubated with the compound for 48 h at 37°C and in atmosphere of 5% CO₂. After 48 h, cells were fixed, washed, and stained with sulfo-rhodamine B stain (SRB). Excess of stain was washed with acetic acid and attached stain was recovered with Tris– EDTA buffer. The color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line.

COMPLIANCE WITH ETHICAL STANDARDS

The work has no studies involving humans or animals as subjects of the study.

Conflict of Interests

Authors declare they have no conflicts of interest.

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