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Synthesis and Characterization of Some New *Bis*-Pyrazolyl-Thiazoles Incorporating the Thiophene Moiety as Potent Anti-Tumor Agents

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Abstract: A new series of 1,4-*bis*(1-(5-(aryldiazenyl)thiazol-2-yl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzenes **3a–i** were synthesized via reaction of 5,5′-(1,4-phenylene)*bis*(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) (**1**) with hydrazonoyl halides **2a–i**. In addition, reaction of **1** with ethyl chloroacetate afforded *bis*-thiazolone derivative **8** as the end product. Reaction of compound **8** with methyl glyoxalate gave *bis*-thiazolone derivative **10**. The structures of the newly synthesized compounds were established on the basis of spectroscopic evidences and their alternative syntheses. All the synthesized compounds were evaluated for their anti-tumor activities against hepatocellular carcinoma (HepG2) cell lines, and the results revealed promising activities of compounds **3g**, **5e**, **3e**, **10**, **5f**, **3i**, and **3f** with IC_{50} equal 1.37 ± 0.15, 1.41 ± 0.17, 1.62 ± 0.20, 1.86 ± 0.20, 1.93 ± 0.08, 2.03 ± 0.25, and 2.09 ± 0.19 μM, respectively.

Keywords: *bis*-heterocycles; *bis*-chalcones; hydrazonoyl halides; anticancer agents

1. Introduction

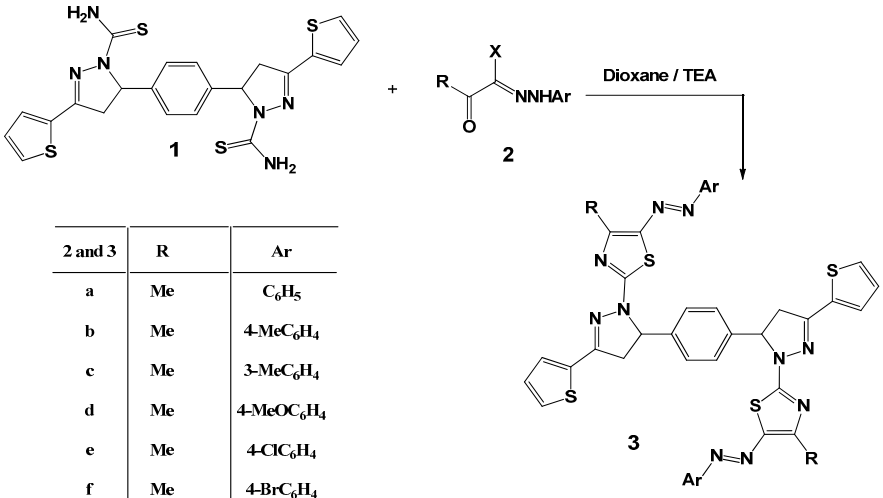
Thiophene and their *bis*-heterocycles have received considerable attention during the last two decades, as they are endowed with a wide range of therapeutic properties, such as analgesic, antibacterial, antioxidant, anti-inflammatory, antifungal, anticancer, and local anesthetic activities [1–6]. On the other hand, pyrazolines have been reported to possess a variety of significant and diverse pharmacological activities such as antibacterial [7], antifungal [8], anticonvulsant [9], DPPH radical scavenging and anti-diabetic [10], antitubercular [11], antidepressant [12], anti-inflammatory [13], antiamebic [14], analgesic [15], and anticancer [16] activities. Moreover, thiazoles are a familiar group of heterocyclic compounds possessing a wide variety of biological activities such as antimicrobial [17], antioxidant [18], antitubercular [19], anticonvulsant [20], anticancer [21–27], and anti-inflammatory [28] agents.

In view of these reports and in continuation of our previous works in synthesis of bioactive *bis*-heterocyclic compounds [29–31], we are herein interested in synthesis of *bis*-pyrazolyl-thiazoles incorporating the thiophene moiety using the hitherto unreported 5,5′-(1,4-phenylene)*bis*(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) as versatile building blocks for the synthesis of the title compounds. All the newly synthesized products were screened for their anti-tumor activities against hepatocellular carcinoma (HepG2) cell lines and showed activities with good IC_{50} for seven compounds.

2. Results

2.1. Synthesis

The reactivity of 5,5′-(1,4-phenylene)*bis*(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) (**1**) [32] was investigated towards hydrazonoyl halides aiming to synthesize new *bis*-heterocyclic compounds containing 1,3-thiazole ring. Thus, refluxing compound **1** with two moles of each of the hydrazonoyl halides **2a**–**i** in dioxane in the presence of triethylamine gave, in each case, a single product, as indicated by TLC analysis of the crude product, with 68%–76% yield. The structure of the product was identified as 1,4-*bis*(1-(5-(aryldiazenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)benzene **3** as outlined in Scheme 1 based on its elemental analysis and spectral (IR, ^{1}H NMR and MS) data [21,24,33]. For example, the ^{1}H NMR spectra of compound **3a**, taken as a typical example of the series, showed six signals at δ 2.59 (s, 6H, $2CH_3$), 3.15–3.19 (m, 2H, 2CH-pyrazoline), 3.77–3.82 (m, 2H, 2CH-pyrazoline), 5.92–5.96 (m, 2H, 2CH-pyrazoline), 7.02–7.82 (m, 16H, Ar-H and thiophene-H), and 7.99 (s, 4H, Ar-H). The mass spectrum of **3a** showed a peak corresponding to its molecular ion at *m*/*z* 780 (see Material and Methods).

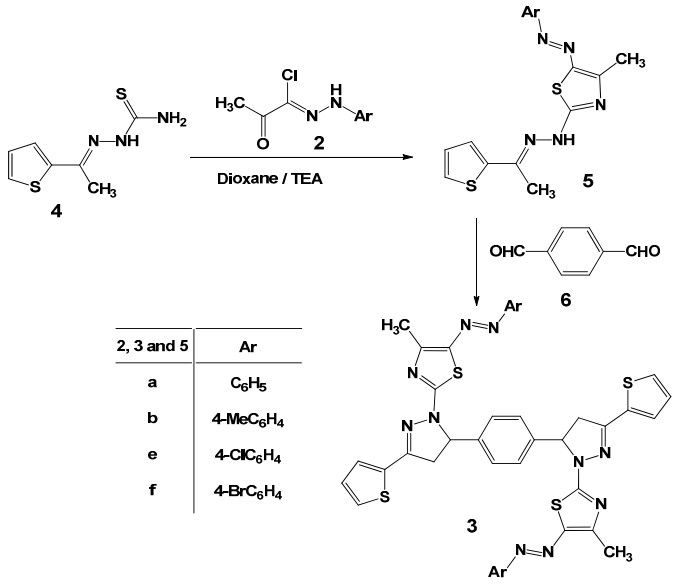


2 and 3	R	Ar
a	Me	C_6H_5
b	Me	4-MeC_6H_4
c	Me	3-MeC_6H_4
d	Me	4-MeOC_6H_4
e	Me	4-ClC_6H_4
f	Me	4-BrC_6H_4
g	Me	4-$NO_2C_6H_4$
h	C_6H_5	C_6H_5
i	C_4H_4S	C_6H_5

Scheme 1. Synthesis of *bis*-arylazothiazole derivatives **3a**–**i**.

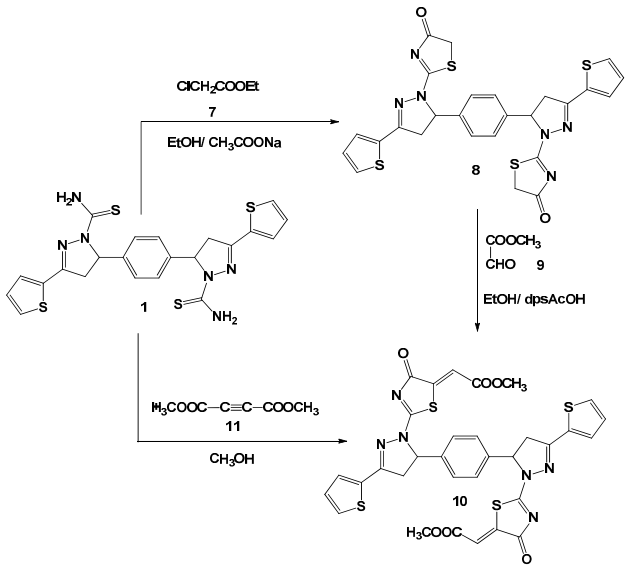
Next, the reaction of 2-(1-(thiophen-2-yl)ethylidene)hydrazinecarbothioamide (**4**) [34] with 2-oxo-*N*′-arypropanehydrazonoyl chlorides **2a**,**b**,**e**,**f** in dioxane under reflux in the presence of TEA as a basic catalyst afforded one isolable product (as evidenced by TLC analysis of the crude product), which were identified to be arylazothiazole derivatives **5a**,**b**,**e**,**f** (72%–76% yield) as outlined in Scheme 2. The structure of compounds **5a**,**b**,**e**,**f** was elucidated by elemental and spectral (IR, ^{1}H NMR, mass) data. ^{1}H NMR spectra of compounds **7** revealed in addition to the expected signals of the aromatic protons, and the protons of the two methyl groups, a singlet at δ 10.60–10.72 ppm, assigned to the –NH proton. The mass spectra of all products **5** exhibited, in each case, a molecular ion peak at the correct molecular weight for the respective compound (see Material and Methods).

Refluxing equimolar amounts of the appropriate arylazothiazole (**5**) and terephthalaldehyde (**6**) in EtOH containing catalytic amounts of glacial acetic acid gave the corresponding products, **3a**,**b**,**e**,**f**, which were identical in all respects (melting point (m.p.), mixed m.p. and IR spectra) with those obtained from reaction of compounds **1** with **2**.



Scheme 2. Alternate synthesis of *bis*-arylazothiazole derivatives **3a**,**b**,**e**,**f**.

Finally, compound **1** reacted with ethyl chloroacetate (**7**) in ethanol in the presence of fused sodium acetate as a basic catalyst to give the *bis*-thiazolone derivative **8** (77% yield) (Scheme 3). Condensation of the latter product **8** with methyl glyoxalate (**9**) in absolute ethanol containing catalytic amounts of glacial acetic acid afforded dimethyl (2*Z*,2′*Z*)-2,2′-(2,2′-(5,5′-(1,4-phenylene)*bis*(3-(thiophen-2yl)-4,5-dihydro-1*H*-pyrazole-3,1-diyl))*bis*(4-oxothiazole-2(4*H*)-yl-5(4*H*)-ylidene))diacetate (**10**) as the end product in 66% yield. Compound **10** was also prepared by a one-step reaction of compound **1** with dimethyl acetylenedicarboxylate (DMAD) (**11**) in refluxing methanol as outlined in Scheme 3. The structure of compounds **10** was confirmed by elemental analyses and spectral data. For example, the ^{1}H NMR spectrum of product **10** revealed the presence of a singlet at δ 6.68 ppm assigned to the olefinic CH proton of =CH–COOCH$_3$ group, in addition to the signals of the aromatic, methyl, and ester protons. Its mass spectrum revealed a peak corresponding to its molecular ion at *m*/*z* 716 (see Material and Methods). The stereochemistry of the methylidene proton of compound **10** is *Z*-configuration according to literature reports [35–37].



Scheme 3. Synthesis of *bis*-thiazolone derivative **10**.

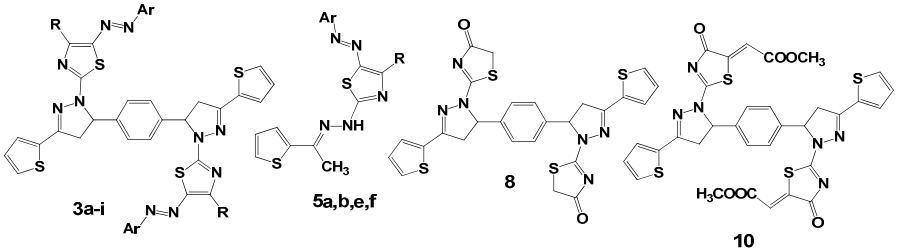
2.2. Anti-Cancer Activity

The anticancer activity of some newly synthesized compounds was determined against a liver carcinoma cell line HepG2, using doxorubicin as a reference drug. Data generated were used to plot a dose-response curve of which the concentration (μM) of test compounds required to kill 50% of cell population (IC_{50}) was determined. The cytotoxic activity was expressed as the mean IC_{50} of three independent experiments (Table 1), and the results revealed that all the tested compounds showed inhibitory activity to the tumor cell lines in a concentration dependent manner.

The results are represented in Table 1 showed the following:

1. The in vitro inhibitory activities of tested compounds against the human liver carcinoma (HepG2) have the descending order as follow: **3g** > **5e** > **3e** > **10** > **5f** > **3i** > **3f** > **3a** > **1** > **3a** > **3h** > **8** > **5b** > **3c** > **3b** > **3d**.
2. The thiazole derivatives **5a**,**b**,**e**,**f** have in vitro inhibitory activity greater than the *bis*-thiazole derivatives **3a**,**b**,**e**,**f** (**5a** > **3a**, **5b** > **3b**, **5e** > **3e**, and **5f** > **3f**).
3. The substituent at position 4 in the thiazole ring affect the in vitro inhibitory activity, while the thienyl ring has a greater effect than the methyl group, which has a greater effect than the phenyl group.
4. The introduction of electron-withdrawing group (nitro group > chlorine atom > bromine atom) at the fourth position of the phenyl group at position 5 in the thiazole ring enhances the antitumor activity. In contrast, the introduction of electron-donating group (methoxy group > methyl group) decreases the antitumor activity.

Table 1. The in vitro inhibitory activity of tested compounds against a liver carcinoma cell line HepG2 expressed as IC50 values (μM) ± standard deviation from six replicates.



Compound No.	R	Ar	IC_{50} (μM)
1	-	-	4.05 ± 0.17
3a	Me	C_6H_5	6.29 ± 0.22
3b	Me	4-MeC_6H_4	18.2 ± 0.14
3c	Me	3-MeC_6H_4	17.6 ± 0.09
3d	Me	4-MeOC_6H_4	52.4 ± 0.17
3e	Me	4-ClC_6H_4	1.62 ± 0.20
3f	Me	4-BrC_6H_4	2.09 ± 0.19
3g	Me	4-$NO_2C_6H_4$	1.37 ± 0.15
3h	C_6H_5	C_6H_5	10.49 ± 0.22
3i	C_4H_4S	C_6H_5	2.03 ± 0.25
5a	Me	C_6H_5	5.13 ± 0.09
5b	Me	4-MeC_6H_4	14.29 ± 0.22
5e	Me	4-ClC_6H_4	1.41 ± 0.17
5f	Me	4-BrC_6H_4	1.93 ± 0.08
8	-	-	12.37 ± 0.14
10	-	-	1.86 ± 0.20
Doxorubicin	-	-	0.72 ± 0.18

3. Materials and Methods

3.1. General Experimental Procedures

All melting points were determined on an electrothermal Gallenkamp apparatus (Bibby Sci. Lim. Stone, Staffordshire, UK). Solvents were distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument (Shimadzu, Tokyo, Japan) in potassium bromide discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury (Varian, Inc., Karlsruhe, Germany, 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) and the chemical shifts were related to that of the solvent DMSO-d_6. The mass spectra were recorded on GCMS-Q1000-EX Shimadzu (Tokyo, Japan), and the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl halides **2a–i** were prepared following a method from the literature [38].

3.2. Synthetic Procedures (See Supplementary Material Figures S1–S16)

3.2.1. General Method for the Synthsis of 1,4-*Bis*(1-(4-substituted-5-((*E*)-aryldiazenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)benzene **3a–i**

A mixture of *bis*-pyrazolylcarbothioamide **1** (0.336 g, 1 mmol) and the appropriate hydrazonoyl halides **2a–c** (2 mmol) in dioxane (20 mL) containing TEA (1 mL) was refluxed for 2–6 h (monitored by TLC) and allowed to cool, and the solid formed was filtered off, washed with EtOH, dried, and recrystallized from DMF to give **3a–i**. The products **3a–i** together with their physical constants is listed below.

1,4-Bis(1-(4-methyl-5-((E)-phenyldiazenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)-benzene (**3a**). Orange solid; yield (71%); m.p. 192–194 °C (from DMF); IR (KBr) ν_{max}: 3050, 2935 (CH), 1602 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.59 (s, 6H, 2CH$_3$), 3.15–3.19 (m, 2H, 2CH-pyrazoline), 3.77–3.82 (m, 2H, 2CH-pyrazoline), 5.92–5.96 (m, 2H, 2CH-pyrazoline), 7.02–7.82 (m, 16H, ArH and thiophene-H), 7.99 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 8.5 (CH$_3$), 43.3 (CH$_2$), 62.2 (CH), 121.2, 125.3, 125.8, 129.5, 130.4, 131.2, 131.7, 134.8, 136.2, 140.5, 141.8, 144.8 (Ar-C), 151.1, 158.3 (C=N); MS *m*/*z* (%): 780 (M$^+$, 9), 341 (13), 185 (51), 119 (66), 78 (42), 51 (100). Anal. Calcd. For $C_{40}H_{32}N_{10}S_4$ (780.17): C, 61.51; H, 4.13; N, 17.93; Found: C, 61.39; H, 4.08; N, 17.72%.

1,4-Bis(1-(4-methyl-5-((E)-p-tolyldiazenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3b**). Red solid; yield (74%); m.p. 180–182 °C (from DMF); IR (KBr) ν_{max}: 3036, 2931 (CH), 1600 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 6H, 2CH$_3$), 2.57 (s, 6H, 2CH$_3$), 3.04–3.09 (m, 2H, 2CH-pyrazoline), 3.56–3.60 (m, 2H, 2CH-pyrazoline), 5.92–5.94 (m, 2H, 2CH-pyrazoline), 7.15–7.86 (m, 14H, ArH and thiophene-H), 7.97 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 8.8, 20.4 (CH$_3$), 45.3 (CH$_2$), 67.2 (CH), 116.3, 125.6, 125.7, 129.0, 129.5, 129.6, 129.7, 131.6, 133.1, 133.4, 148.4, 149.9 (Ar-C), 160.5, 164.2 (C=N); MS *m*/*z* (%): 808 (M$^+$, 5), 522 (11), 185 (44), 106 (23), 78 (80), 51 (100); Anal. Calcd. For $C_{42}H_{36}N_{10}S_4$ (808.20): C, 62.35; H, 4.48; N, 17.31; Found: C, 62.27; H, 4.41; N, 17.24%.

1,4-Bis(1-(4-methyl-5-((E)-m-tolyldiazenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)-benzene (**3c**). Red solid; yield (68%); m.p. 164–166 °C (from DMF); IR (KBr) ν_{max}: 3052, 2939 (CH), 1600 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.32 (s, 6H, 2CH$_3$), 2.62 (s, 6H, 2CH$_3$), 3.03–3.07 (m, 2H, 2CH-pyrazoline), 3.53–3.57 (m, 2H, 2CH-pyrazoline), 5.90–5.94 (m, 2H, 2CH-pyrazoline), 7.06–7.82 (m, 14H, ArH and thiophene-H), 7.99 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 8.5, 20.0 (CH$_3$), 45.6 (CH$_2$), 66.3 (CH), 121.3, 125.7, 125.8, 127.7, 128.4, 128.6, 133.3, 138.5, 143.7, 144.5, 148.4, 148.8, 151.6 (Ar-C), 157.8, 163.6 (C=N); MS *m*/*z* (%): 808 (M$^+$, 9), 631 (6), 512 (30), 464 (35), 185 (32), 92 (80), 87 (100), 51 (50); Anal. Calcd. For $C_{42}H_{36}N_{10}S_4$ (808.20): C, 62.35; H, 4.48; N, 17.31; Found C, 62.26; H, 4.40; N, 17.22%.

1,4-Bis(1-(5-((E)-(4-methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3d**). Red solid; yield (70%); m.p. 190–192 °C (from DMF); IR (KBr) ν_{max}: 3052, 2936 (CH), 1601 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.59 (s, 6H, $2CH_3$), 3.04–3.07 (m, 2H, 2CH-pyrazoline), 3.79 (s, 6H, $2OCH_3$), 3.54–3.59 (m, 2H, 2CH-pyrazoline), 5.92–5.94 (m, 2H, 2CH-pyrazoline), 6.99–7.51 (m, 14H, ArH and thiophene-H), 7.90 (s, 4H, Ar-H); MS *m*/*z* (%): 840 (M^+, 13), 631 (4), 348 (10), 220 (36), 185 (25), 109 (37), 78 (86), 51 (100); Anal. Calcd. For $C_{42}H_{36}N_{10}O_2S_4$ (840.19): C, 59.98; H, 4.31; N, 16.65; Found C, 59.79; H, 4.26; N, 16.53%.

1,4-Bis(1-(5-((E)-(4-chlorophenyl)diazenyl)-4-methylthiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3e**). Red solid; yield (74%); m.p. 225–227 °C (from DMF); IR (KBr) ν_{max}: 3052, 2948 (CH), 1602 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.61 (s, 6H, $2CH_3$), 3.03–3.07 (m, 2H, 2CH-pyrazoline), 3.55–3.59 (m, 2H, 2CH-pyrazoline), 5.91–5.96 (m, 2H, 2CH-pyrazoline), 7.11–7.80 (m, 14H, ArH and thiophene-H), 7.98 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 8.4, 20.4 (CH_3), 45.5 (CH_2), 66.3 (CH), 115.5, 115.7, 125.2, 126.2, 126.3, 129.8, 131.9, 132.6, 135.2, 146.6, 155.7, 156.1 (ArC), 159.4, 162.6 (C=N); MS *m*/*z* (%): 850 (M^++2, 2), 848 (M^+, 7), 631 (13), 404 (22), 243 (15), 185 (67), 117 (26), 78 (80), 51 (100); Anal. Calcd. For $C_{40}H_{30}Cl_2N_{10}S_4$ (848.09): C, 56.53; H, 3.56; N, 16.48; Found: C, 56.44; H, 3.51; N, 16.36%.

1,4-Bis(1-(5-((E)-(4-bromophenyl)diazenyl)-4-methylthiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3f**). Red solid; yield (72%); m.p. 203–205 °C (from DMF); IR (KBr) ν_{max}: 3050, 2933 (CH), 1601 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.60 (s, 6H, $2CH_3$), 3.04–3.07 (m, 2H, 2CH-pyrazoline), 3.54–3.57 (m, 2H, 2CH-pyrazoline), 5.91–5.95 (m, 2H, 2CH-pyrazoline),7.30–7.86 (m, 14H, ArH and thiophene-H), 7.98 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 8.3 (CH_3), 45.2 (CH_2), 66.3 (CH), 116.1, 123.4, 124.2, 129.1, 130.9, 131.9, 132.2, 133.0, 141.3, 142.5, 142.8, 146.8, (Ar-C), 151.2, 162.1 (C=N); MS *m*/*z* (%): 937 (M^++2, 3), 935 (M^+, 3), 657 (83), 592 (86), 490 (75), 414 (99), 185 (91), 78 (72), 51 (100); Anal. Calcd. For $C_{40}H_{30}Br_2N_{10}S_4$ (935.99): C, 51.17; H, 3.22; N, 14.92; Found: C, 51.05; H, 3.13; N, 14.75%.

1,4-Bis(1-(5-((E)-(4-nitrophenyl)diazenyl)-4-methylthiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3g**). Red solid; yield (74%); m.p. 271–273 °C (from DMF); IR (KBr) ν_{max}: 3056, 2033 (CH), 1602 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 2.62 (s, 6H, $2CH_3$), 3.04–3.07 (m, 2H, 2CH-pyrazoline), 3.57–3.59 (m, 2H, 2CH-pyrazoline), 5.90–5.94 (m, 2H, 2CH-pyrazoline),7.08–7.79 (m, 14H, ArH and thiophene-H), 7.99 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 8.5 (CH_3), 45.4 (CH_2), 62.7 (CH), 114.8, 116.2, 123.5, 127.2, 128.6, 129.2, 139.4, 140.3, 141.0, 142.1, 142.8, 144.5, (ArC), 151.5, 162.1 (C=N); MS *m*/*z* (%): 870 (M^+, 8), 657 (64), 530 (77), 442 (40), 334 (57), 185 (64), 78 (50), 51 (100); Anal. Calcd. For $C_{40}H_{30}N_{12}O_4S_4$ (870.14): C, 55.16; H, 3.47; N, 19.30; Found: C, 55.07; H, 3.41; N, 19.21%.

1,4-Bis(1-(4-phenyl-5-((E)-phenyldiazenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3h**). Orange solid; yield (77%); m.p. 260–262 °C (from DMF); IR (KBr) ν_{max}: 3051, 2941 (CH), 1598 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.09–3.10 (m, 2H, 2CH-pyrazoline), 3.57–3.59 (m, 2H, 2CH-pyrazoline), 5.93–5.96 (m, 2H, 2CH-pyrazoline), 7.47–7.94 (m, 26H, ArH and thiophene-H), 8.27 (s, 4H, ArH); MS *m*/*z* (%): 904 (M^+, 3), 657 (64), 631 (7), 380 (12), 252 (37), 185 (42), 78 (77), 51 (100); Anal. Calcd. For $C_{50}H_{36}N_{10}S_4$ (904.20): C, 66.35; H, 4.01; N, 15.47; Found: C, 66.30; H, 4.13; N, 15.36%.

1,4-Bis(1-(5-((E)-phenyldiazenyl)-4-(thiophen-2-yl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)benzene (**3i**). Orange solid; yield (76%); m.p. 248–250 °C (from DMF); IR (KBr) ν_{max}: 3057, 2942 (CH), 1602 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.05–3.08 (m, 2H, 2CH-pyrazoline), 3.55–3.58 (m, 2H, 2CH-pyrazoline), 5.92–5.95 (m, 2H, 2CH-pyrazoline), 7.05–7.94 (m, 22H, ArH and thiophene-H), 8.10 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 43.6 (CH_2), 66.5 (CH), 119.0, 125.8, 126.0, 128.4, 128.5, 128.6, 128.9, 129.0, 129.4, 129.5, 130.8, 130.9, 131.1, 132.1, 132.3, 143.8 (ArC), 148.8, 162.8 (C=N); MS *m*/*z* (%): 916 (M^+, 13), 522 (20), 409 (48), 285 (40), 111 (100), 78 (65), 51 (84); Anal. Calcd. For $C_{46}H_{32}N_{10}S_6$ (916.11): C, 60.24; H, 3.52; N, 15.27; Found: C, 60.15; H, 3.40; N, 15.19%.

3.2.2. Synthesis of 4-Methyl-5-((*E*)-aryldiazenyl)-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl) Thiazole Derivatives **5a**,**b**,**e**,**f**

A mixture of thiosemicarbazone **4** (0.199 g, 1 mmol) and the appropriate hydrazonoyl halides **2** (1 mmol) in dioxane (20 mL) containing TEA (0.5 mL) was refluxed for 3–6 h and allowed to cool, and the solid formed was filtered off, washed with EtOH, dried, and recrystallized from DMF to give the corresponding arylazothiazoles **5a**,**b**,**e**,**f**. The products together with their physical constants are listed below.

4-Methyl-5-((E)-phenyldiazenyl)-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl)thiazole (**5a**). Red solid; yield (72%); m.p. 183–185 °C (from DMF); IR (KBr) ν_{max}: 3427 (NH), 3043 (=C–H), 2931 (–C–H), 1603 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 6.99–7.75 (m, 8H, ArH and thiophene-H), 10.67 (s, 1H, D_2O-exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 8.5, 15.5 (CH_3), 114.2, 122.1, 128.0, 128.3, 129.2, 130.2, 130.8, 137.9, 143.4 (ArC), 160.4, 170.6, 177.6 (C=N); MS *m*/*z* (%): 341 (M^+, 44), 238 (71), 106 (53), 78 (82), 51 (100); Anal. Calcd. for $C_{16}H_{15}N_5S_2$ (341.08): C, 56.28; H, 4.43; N, 20.51; Found: C, 56.22; H, 4.36; N, 20.40%.

4-Methyl-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl)-5-((E)-p-tolyldiazenyl)thiazole (**5b**). Red solid; yield (74%); m.p. 169–170 °C (from DMF); IR (KBr) ν_{max}: 3427 (NH), 3042 (=C–H), 2928 (–C–H), 1602 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 7.12–7.73 (m, 7H, ArH and thiophene-H), 10.60 (s, 1H, D_2O-exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 8.5, 15.5, 20.3 (CH_3), 114.2, 128.0, 129.6, 130.0, 130.7, 131.1, 137.3, 141.1, 142.7 (ArC), 160.1, 177.3, 170.8 (C=N); MS *m*/*z* (%): 355 (M^+, 20), 238 (53), 185 (77), 78 (69), 51 (100); Anal. Calcd. for $C_{17}H_{17}N_5S_2$ (355.09): C, 57.44; H, 4.82; N, 19.70; Found: C, 57.36; H, 4.77; N, 19.63%.

5-((E)-(4-Chlorophenyl)diazenyl)-4-methyl-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl) thiazole (**5e**). Red solid; yield (76%); m.p. 216–218 °C (from DMF); IR (KBr) ν_{max}: 3427 (NH), 3043 (=C–H), 2931 (–C–H), 1603 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.17–7.74 (m, 7H, ArH and thiophene-H), 10.71 (s, 1H, D_2O-exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 8.5, 15.4 (CH_3), 115.6, 125.6, 128.0, 128.3, 129.1, 129.3, 138.7, 142.4, 142.5 (ArC), 160.6, 170.4, 177.6 (C=N); MS *m*/*z* (%): 377 (M^++2, 32), 375 (M^+, 100), 222 (54), 186 (72), 78 (69), 51 (80); Anal. Calcd. for $C_{16}H_{14}ClN_5S_2$ (375.04): C, 51.12; H, 3.75; N, 18.63; Found: C, 51.03; H, 3.66; N, 18.51%.

5-((E)-(4-Bromophenyl)diazenyl)-4-methyl-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl) thiazole (**5f**). Red solid; yield (75%); m.p. 203–205 °C (from DMF); IR (KBr) ν_{max}: 3441 (NH), 3043 (=C–H), 2937 (–C–H), 1601 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.17–7.75 (m, 7H, ArH and thiophene-H), 10.72 (s, 1H, D_2O-exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 8.5, 15.5 (CH_3), 116.1, 128.0, 130.3, 130.9, 131.9, 132.1, 138.8, 142.5, 142.8 (ArC), 160.6, 170.4, 177.6 (C=N); MS *m*/*z* (%): 421 (M^+ + 2, 18), 419 (M^+, 19), 302 (100), 185 (83), 78 (82), 51 (74); Anal. Calcd. for $C_{16}H_{14}BrN_5S_2$ (418.99): C, 45.72; H, 3.36; N, 16.66; Found: C, 45.55; H, 3.30; N, 16.42%.

3.2.3. Alternate Synthesis of Compounds **3a**,**b**,**e**,**f**

A mixture of terephthalaldehyde (**6**) (0.134 g, 1 mmol) and the appropriate thiazole **5** (2 mmol) in EtOH (10 mL) containing 0.5 mL of glacial acetic acid was refluxed for 8 h and then cooled to room temperature. The solid precipitated was filtered off, washed with water, dried, and recrystallized from DMF to give the corresponding products, **3a**,**b**,**e**,**f**, which were identical in all respects (m.p., mixed m.p., and IR spectra) with those obtained from reaction of **1** with **2**.

3.2.4. Synthesis of 2,2′-(5,5′-(1,4-phenylene)*bis*(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-3,1-diyl))*bis*(thiazol-4(5*H*)-one) (**8**)

A mixture of *bis*-pyrazolylcarbothioamide **1** (0.336 g, 1 mmol) and ethyl chloroacetate (**7**) (0.244 g, 2 mmol) in EtOH (20 mL) containing fused sodium acetate (1 g) was refluxed for 8 h and cooled

to room temperature. The solid product was filtered off, washed with ethanol, and recrystallized from ethanol to afford the *bis*-thiazolone derivative **8** as yellow solid; yield (77%); m.p. 188–190 °C (from ethanol); IR (KBr) ν_{max}: 3066, 2951 (CH), 1682 (C=O), 1600 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.21–3.27 (m, 2H, 2CH-pyrazoline), 3.65–3.72 (m, 2H, 2CH-pyrazoline), 4.10 (s, 4H, $2CH_2$), 5.94–5.97 (m, 2H, 2CH-pyrazoline), 7.16–7.70 (m, 6H, thiophene-H), 7.97 (s, 4H, ArH); MS *m*/*z* (%): 576 (M^+, 15), 445 (39), 283 (31), 185 (64), 78 (79), 51 (100); Anal. Calcd. for $C_{26}H_{20}N_6O_2S_4$ (576.05): C, 54.15; H, 3.50; N, 14.57; Found: C, 54.15; H, 3.50; N, 14.57%.

3.2.5. Synthesis of (2*Z*,2′*Z*)-dimethyl 2,2′-(2,2′-(5,5′-(1,4-phenylene)*bis*(5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-3,1-diyl))*bis*(4-oxothiazole-2(4*H*)-yl-5(4*H*)-ylidene))diacetate (**10**)

A mixture of *bis*-thiazolone derivative **8** (0.580 g, 1 mmol), methyl glyoxalate (**9**) (0.176 g, 2 mmol), and glacial acetic acid (0.5 mL) in ethanol (20 mL) was refluxed for 6 h, left to cool, then poured gradually with stirring onto cold water. The solid formed was filtered off, washed with water, and crystallized from DMF to give compound **10** as canary yellow solid; yield (66%), m.p. 217–219 °C (from DMF); IR (KBr) ν_{max}: 3061, 2936 (CH), 1742, 1682 (C=O), 1601 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.54–3.57 (m, 2H, 2CH-pyrazoline), 3.71 (s, 6H, $2CH_3$), 3.84–3.87 (m, 2H, 2CH-pyrazoline), 5.97–6.01 (m, 2H, 2CH-pyrazoline), 6.68 (s, 2H, 2 =$CHCOOCH_3$), 7.23–7.72 (m, 6H, thiophene-H), 7.99 (s, 4H, ArH); ^{13}C NMR (DMSO-d_6) δ: 44.0 (CH_2), 52.4 (CH_3), 63.9 (CH), 114.2, 116.3, 126.5, 128.4, 131.9, 133.5, 142.4, 146.5 (ArC), 158.0, 165.8, (C=N), 171.5, 177.1 (C=O); MS *m*/*z* (%): 716 (M^+, 84), 631 (40), 314 (48), 185 (72), 116 (79), 51 (100). Anal. Calcd. For $C_{32}H_{24}N_6O_6S_4$ (716.06): C, 53.62; H, 3.37; N, 11.72; Found: C, 53.53; H, 3.31; N, 11.59%.

3.2.6. Alternate Synthesis of Compound **10**

To a solution of *bis*-pyrazolylcarbothioamide **1** (0.336 g, 1 mmol) in dry methanol (20 mL) was added dimethylacetylenedicarboxylate (**11**) (0.284 g, 2 mmol). The solution was refluxed for 4 h. The precipitated product after cooling was filtered, washed with methanol, and recrystallized from DMF to give product **10**, which was identical in all aspects (m.p., mixed m.p., and IR spectra) with that obtained from the reaction of **8** with **9**.

3.3. Anti-Tumor Activity

Human liver carcinoma (HepG2) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 μg/mL of gentamicin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were sub-cultured two to three times a week.

For antitumor assays, the tumor cell lines were suspended in medium at concentration 5×10^4 cell/well in corning® 96-well plates (six replicates) to achieve eight concentrations for each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96-well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media were removed from the 96-well plates and replaced with 100 μL of fresh culture RPMI 1640 medium without phenol red, followed by 10 μL of the 12 Mm MTT stock solutions (5 mg of MTT in 1 mL of PBS) to each well, including the untreated controls. The 96-well plates were then incubated at 37 °C and 5% CO_2 for 4 h. An 85-μL aliquot of the media was removed from the wells, and 50 μL of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, Männedorf, Switzerland) to determine the number of viable cells and the percentage of viability was calculated as $(1 - (ODt/ODc)) \times 100\%$, where ODt is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to obtain the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells,

was estimated from graphic plots of the dose-response curve for each concentration using Graphed Prism software (San Diego, CA, USA) [39–41].

4. Conclusions

In our present work, a new series of 2-ethylidenehydrazono-5-arylazothiazoles and 2-ethylidene-hydrazono-5-arylazothiazolones were synthesized from a reaction of ethylidene-thiosemicarbazide with various hydrazonoyl halides. The structures of the newly synthesized compounds were established on the basis of spectroscopic evidences and their synthesis by alternative methods. The in-vitro growth inhibitory activity of the synthesized compounds against hepatocellular carcinoma (HepG2) cell lines was investigated in comparison with doxorubicin as a standard drug using an MTT assay, and the results revealed promising activities of compounds **3g**, **5e**, **3e**, **10**, **5f**, **3i**, and **3f** with an IC_{50} equal to 1.37 ± 0.15, 1.41 ± 0.17, 1.62 ± 0.20, 1.86 ± 0.20, 1.93 ± 0.08, 2.03 ± 0.25, and 2.09 ± 0.19 μM, respectively.

Supplementary Materials: Supplementary materials can be found www.mdpi.com/1422-0067/17/9/1499/s1.

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