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ORIGINAL RESEARCH

One-Pot Synthesis of Novel Thiazoles as Potential Anti-Cancer Agents

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Background: Thiazole and thiosemicarbazone derivatives are known to have potential anticancer activity with a mechanism of action related to inhibition of matrix metalloproteinases, kinases and anti-apoptotic BCL2 family proteins.

Materials and Methods: A novel three series of 5-(1-(2-(thiazol-2-yl)hydrazono)ethyl) thiazole derivatives were prepared in a one-pot three-component reaction using 2-(2-benzylidene hydrazinyl)-4-methylthiazole as a starting precursor. MS, IR, ^{1}H-NMR and ^{13}C-NMR were used to elucidate the structures of the synthesized compounds. Most of the synthesized products were evaluated for their in vitro anticancer screening against HCT-116, HT-29 and HepG2 using the MTT colorimetric assay.

Results: The results indicated that compounds **4c, 4d** and **8c** showed growth inhibition activity against HCT-116 with IC_{50} values of 3.80 ± 0.80, 3.65 ± 0.90 and 3.16 ± 0.90 µM, respectively, compared to harmine (IC_{50} = 2.40 ± 0.12 µM) and cisplatin (IC_{50} = 5.18 ± 0.94 µM) reference drugs. Also, compounds **8c, 4d** and **4c** showed promising IC_{50} values of 3.47 ± 0.79, 4.13 ± 0.51 and 7.24 ± 0.62 µM, respectively, against the more resistant human colorectal cancer (HT-29) cell line compared with harmine (IC_{50} = 4.59 ± 0.67 µM) and cisplatin (IC_{50} = 11.68 ± 1.54 µM). On the other hand, compounds **4d, 4c, 8c** and **11c** were the most active (IC_{50} values of 2.31± 0.43, 2.94 ± 0.62, 4.57 ± 0.85 and 9.86 ± 0.78 µM, respectively) against the hepatocellular carcinoma (HepG2) cell line compared with harmine (IC_{50} = 2.54 ± 0.82 µM) and cisplatin (IC_{50} = 41 ± 0.63 µM). The study also suggested that the mechanism of the anticancer action exerted by the most active compounds (**4c, 4d** and **8c**) inside HCT-116 cells was apoptosis through the Bcl-2 family.

Conclusion: Thiazole scaffolds **4c, 4d** and **8c** showed anticancer activities in the micromolar range and are appropriate as a candidate for cancer treatment.

Keywords: hydrazones, hydrazonoyl halides, cyclization, harmine, HCT-116, HepG2, HT-29

Introduction

Colorectal cancer (CRC) is considered one of the most common malignancies with a high incidence and mortality worldwide. It is estimated that 1.4 million individuals were newly diagnosed with CRC in 2012, which resulted in 693,900 mortalities.[1] Surgery, chemotherapy and radiation treatment are the three main standard therapies for CRC. Chemotherapy is an important treatment for cancer, particularly in tumors with a propensity to invade adjacent tissues and metastasize to other organs. Challenges remain that require the continued search for novel effective and less toxic chemotherapeutic agents for the treatment of colon cancer.[2] Apoptosis is a form of regulated cell death that is triggered in response to developmental cues or cellular stress. This

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selective cell suicide plays an essential role in numerous physiological and pathological processes including development, immunity and disease where the elimination of damaged or superfluous cells helps to ensure organismal health.[3] There are two apoptotic pathways – the extrinsic pathway and the intrinsic (mitochondrial) pathway. The intrinsic apoptotic pathway (the mitochondrion-mediated pathway) is initiated in response to a variety of stress signals,[4] and a complex interplay of Bcl-2 (B-cell lymphoma 2) proteins relays this signal to the mitochondrial outer membrane (OM) to initiate Bak and Bax activation, oligomerization and OM damage. Breaching the mitochondrial OM releases apoptogenic factors, including cytochrome c and Smac, which activate a group of aspartate-specific proteases (caspases).[5] Caspases, in turn, cleave several hundred cellular proteins to coordinate the destruction of the cell.[6] Recent pharmaceutical advances have allowed the specific targeting of protein–protein interactions in the BCL-2 family.[5,7]

Thiosemicarbazones are a large class of compounds, which represent great therapeutic value against parasitic diseases[8,9] and microbial diseases.[10] They were also identified to be among the most interesting antitumor inhibitors due to induction of oxidative stress and ROS-mediated cell injury.[11,12] Thiazole heterocycles, derivatives of thiosemicarbazone, are scaffolds of many natural, synthetic and semi-synthetic drugs which exhibit numerous remarkable pharmacological activities including antiparasitic, anti-inflammatory and antineoplastic activities.[13–17] Thiazole derivatives are also known to have potential anticancer activity with a mechanism of action related to inhibition of matrix metallo-proteinases, kinases and anti-apoptotic BCL2 family proteins.[18–21]

Multicomponent reactions (MCR) are one-pot processes which always occupy great importance in the repertoire of sustainable synthetic tools because of their high efficiency and atom economy.[22,23] As part of our ongoing studies on the synthesis of new heterocyclic compounds via one-pot, multicomponent reactions,[24–33] herein this study describes a convenient and rapid method for the synthesis of thiazolyl-hydrazono-ethylthiazole derivatives by one-pot three-component reactions using 2-(2-benzylidenehydrazinyl)-4-methylthiazole (**1**), thiosemicarbazide (**2**) and the appropriate hydrazonoyl chlorides (**3a–e** or **7a–d**) or phenacyl bromides (**10a–e**) in the presence of a catalytic amount of TEA in dioxane. The cytotoxic potential of the newly synthesized compounds was examined against HCT-116, HT-29 and HepG2 cells using the MTT assay to elucidate its underlying mechanisms of action related to their anti-apoptotic BCl2 family proteins.

Materials and Methods

Chemistry

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus (Bibby Sci. Lim., Stone, UK). IR spectra were recorded in potassium bromide discs on PyeUnicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers (Shimadzu, Tokyo, Japan). NMR spectra were measured on a Varian Mercury VX-300 NMR spectrometer (Varian, Inc., Karlsruhe, Germany). ^{1}H spectra were recorded at 300 MHz and ^{13}C spectra were recorded at 75.46 MHz in deuterated dimethyl sulfoxide (DMSO-*d*6). Mass spectra were run on a Shimadzu GCMS-QP1000 EX mass spectrometer (Tokyo, Japan) at 70 eV. Elemental analyses were measured using a German-made Elementarvario LIII CHNS analyzer. Biological activities of the synthesized compounds were carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

General Procedure for Synthesis of Thiazole Derivatives 4a–e and 8a–d

A mixture of 2-(2-benzylidenehydrazinyl)-4-methylthiazole (**1**) (0.259 g, 1 mmol), thiosemicarbazide (**2**) (0.091 g, 1 mmol) and the appropriate hydrazonoyl chlorides (**3a–e** or **7a–d**) (1 mmol) in dioxane (20 mL) containing catalytic amounts of TEA was refluxed for 2–4 h (monitored by TLC). The formed precipitate was isolated by filtration, washed with methanol, dried and recrystallized from appropriate solvent to give products **4a–e** or **8a–d**. The physical properties and spectral data of the obtained products **4a–e** and **8a–d** are listed below.

2-(2-(Benzylidene)Hydrazinyl)-4-Methyl-5-(1-(2-(4-Methyl-5-(Phenyldiazenyl)Thiazol-2-yl)Hydrazono)Ethyl)Thiazole (4a)

Red solid, 70% yield, m.p. 235–237 °C (DMF); IR (KBr): *ν* 3425, 3220 (2NH), 3035, 2914 (C–H) cm$^{-1;}$ ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 6.97–8.10 (m, 10H, Ar-H), 8.69 (s, 1H, CH=N), 10.58 (brs, 1H, NH), 11.19 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 8.89, 21.57, 23.56 ($3CH_3$), 45.86, 66.81, 114.62, 115.63, 117.86, 118.99, 121.70, 122.89, 125.60, 126.60, 127.66, 129.81, 137.19, 140.76, 161.91, 165.21 (Ar–C and C=N); MS *m/z* (%): 474 (M^+,

14). Anal. Calcd for $C_{23}H_{22}N_8S_2$ (474.14): C, 58.21; H, 4.67; N, 23.61. Found: C, 58.36; H, 4.51; N, 23.47%.

2-(2-(Benzylidene)Hydrazinyl)-4-Methyl-5-(1-(2-(4-Methyl-5-(*p*-Tolyldiazenyl)Thiazol-2-yl)Hydrazono)Ethyl)Thiazole (4b)

Red solid, 73% yield, m.p. 220–222 °C (DMF); IR (KBr): *v* 3428, 3210 (2NH), 3030, 2917 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.17–8.13 (m, 9H, Ar–H), 8.66 (s, 1H, CH=N), 10.48 (brs, 1H, NH), 11.23 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 9.21, 16.78, 18.90, 19.82 ($4CH_3$), 112.97, 114.15, 116.69, 120.79, 128.63, 129.94, 131.57, 132.35, 135.18, 137.05, 139.35, 143.49, 151.36, 156.16, 157.05, 159.27 (Ar–C and C=N); MS *m/z* (%): 488 (M^+, 17). Anal. Calcd for $C_{24}H_{24}N_8S_2$ (488.63): C, 58.99; H, 4.95; N, 22.93. Found: C, 59.08; H, 4.78; N, 22.79%.

2-(2-(Benzylidene)Hydrazinyl)-5-(1-(2-(5-((4-Chlorophenyl) Diazenyl)-4-Methylthiazol-2-yl)Hydrazono)Ethyl)-4-Methylthiazole (4c)

Red solid, 68% yield, m.p. 215–217 °C (DMF); IR (KBr): *v* 3429, 3315 (2NH), 3020, 2922 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.36–8.11 (m, 9H, Ar–H), 8.70 (s, 1H, CH=N), 10.65 (brs, 1H, NH), 11.40 (brs, 1H, NH); MS *m/z* (%): 511 (M^++2, 6), 509 (M^+, 19). Anal. Calcd for $C_{23}H_{21}ClN_8S_2$ (509.05): C, 54.27; H, 4.16; N, 22.01. Found: C, 54.14; H, 3.99; N, 21.90%.

2-(2-(Benzylidene)Hydrazinyl)-5-(1-(2-(5-((2,4-Dichlorophenyl)Diazenyl)-4-Methylthiazol-2-yl)Hydrazono) Ethyl)-4-Methylthiazole (4d)

Red solid, 78% yield, m.p. 188–190 °C (DMF); IR (KBr): *v* 3423, 3305 (2NH), 3040, 2916 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 7.39–8.10 (m, 8H, Ar–H), 8.22 (s, 1H, CH=N), 9.70 (s, 1H, NH), 10.71 (s, 1H, NH); MS *m/z* (%): 543 (M^+, 17). Anal. Calcd for $C_{23}H_{20}Cl_2N_8S_2$ (543.49): C, 50.83; H, 3.71; N, 20.62. Found: C, 50.62; H, 3.55; N, 20.46%.

2-(2-(Benzylidene)Hydrazinyl)-4-Methyl-5-(1-(2-(4-Methyl-5-((4-Nitrophenyl)Diazenyl) Thiazol-2-yl)Hydrazono)Ethyl) Thiazole (4e)

Red solid, 70% yield, m.p. 172–174 °C (EtOH); IR (KBr): *v* 3422, 3192 (2NH), 3062, 2916 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.43–8.18 (m, 9H, Ar–H), 8.61 (s, CH=N), 10.63 (brs, 1H, NH), 11.10 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 8.86, 16.91, 18.92 (CH_3), 114.09, 118.11, 120.55, 126.03, 127.11, 129.30, 130.08, 131.84, 134.62, 136.11, 141.11, 143.07, 149.73, 154.89, 162.35, 168.51 (Ar–C and C=N); MS *m/z* (%): 520 (M^+, 19). Anal. Calcd for $C_{23}H_{21}N_9O_2S_2$ (519.60): C, 53.17; H, 4.07; N, 24.26. Found: C, 53.45; H, 3.85; N, 24.00%.

2-(2-(1-(2-(2-(Benzylidene)Hydrazinyl)-4-Methylthiazol-5-yl)Ethylidene)Hydrazinyl)-5-(2-Phenylhydrazono)Thiazol-4(5*H*)-One (8a)

Orange solid, 75% yield, m.p. 232–234 °C (DMF); IR (KBr): *v* 3426, 3395, 3181 (3NH), 3050, 2924 (C–H), 1684 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.82 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 6.92–8.11 (m, 10H, Ar–H), 8.52 (s, 1H, CH=N), 10.45 (brs, 1H, NH), 10.80 (brs, 1H, NH), 12.47 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 12.89, 18.50 ($2CH_3$), 115.07, 119.85, 123.16, 126.11, 126.72, 128.98, 129.38, 129.57, 129.81, 131.31, 133.48, 143.10, 153.77, 157.93, 163.05 (Ar–C and C=N), 172.94 (C=O); MS *m/z* (%): 476 (M^+, 13). Anal. Calcd for $C_{22}H_{20}N_8OS_2$ (476.58): C, 55.45; H, 4.23; N, 23.51. Found: C, 55.29; H, 3.98; N, 23.36%.

2-(2-(1-(2-(2-(Benzylidene)Hydrazinyl)-4-Methylthiazol-5-yl)Ethylidene)Hydrazinyl)-5-(2-(p-Tolyl)Hydrazono) Thiazol-4(5*H*)-One (8b)

Orange solid, 69% yield, m.p. 200–202 °C (EtOH); IR (KBr): *v* 3421, 3280, 3183 (3NH), 3051, 2918 (C–H), 1685 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.04–8.09 (m, 9H, Ar–H), 8.11 (s, 1H, CH=N), 10.38 (brs, 1H, NH), 10.78 (brs, 1H, NH), 12.24 (brs, 1H, NH); MS *m/z* (%): 490 (M^+, 37). Anal. Calcd for $C_{23}H_{22}N_8OS_2$ (490.60): C, 56.31; H, 4.52; N, 22.84. Found: C, 56.20; H, 4.36; N, 22.65%.

2-(2-(1-(2-(2-(Benzylidene)Hydrazinyl)-4-Methylthiazol-5-yl)Ethylidene)Hydrazinyl)-5-(2-(4-Chlorophenyl) Hydrazono)Thiazol-4(5*H*)-One (8c)

Orange solid, 75% yield, m.p. 205–207 °C (DMF); IR (KBr): *v* 3427, 3255, 3178 (3NH), 3060, 2926 (C–H), 1684 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.11–8.11 (m, 9H, Ar–H), 8.53 (s, 1H, CH=N), 10.55 (s, 1H, NH), 10.88 (brs, 1H, NH), 12.21 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 8.95, 18.87 (CH_3), 115.84, 116.59, 125.58, 126.38, 127.07, 129.34,

129.51, 130.09, 134.68, 143.23, 147.14, 153.43, 158.64, 163.83, 167.54 (Ar–C and C=N), 173.93 (C=O); MS *m/z* (%): 513 (M^++2, 5), 511 (M^+, 17). Anal. Calcd for $C_{22}H_{19}ClN_8OS_2$ (511.02): C, 51.71; H, 3.75; N, 21.93. Found: C, 51.52; H, 3.64; N, 21.79%.

2-(2-(1-(2-(2-(Benzylidene)Hydrazinyl)-4-Methylthiazol-5-yl)Ethylidene)Hydrazinyl)-5-(2-(4-Nitrophenyl) Hydrazono)Thiazol-4(5*H*)-One (8d)

Orange solid, 70% yield, crystal (dioxane); m.p. 176–167 ° C (EtOH); IR (KBr): *v* 3429, 3210, 3180 (3NH), 3050, 2920 (C–H) 1701 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 7.38–8.43 (m, 9H, Ar–H), 8.54 (s, 1H, CH=N), 10.26 (brs, 1H, NH), 11.09 (brs, 1H, NH), 12.31 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 12.10, 19.45 ($2CH_3$), 113.52, 116.62, 118.33, 120.94, 123.75, 125.55, 128.23, 129.99, 130.86, 134.39, 134.96, 147.92, 154.70, 158.09, 162.10 (Ar–C and C=N), 172.59 (C=O); MS *m/z* (%): 521 (M^+, 20). Anal. Calcd for $C_{22}H_{19}N_9O_3S_2$ (521.57): C, 50.66; H, 3.67; N, 24.17. Found: C, 50.95; H, 3.40; N, 23.77%.

Alternative Synthesis for 4a and 8a

Synthesis of 2-(1-(2-(2-(Benzylidene)Hydrazinyl)-4-Methylthiazol-5-yl)Ethylidene)Hydrazine-1-Carbothioamide (5)

A mixture of 2-(2-benzylidenehydrazinyl)-4-methylthiazole (**1**) (2.59 g, 10 mmol) and thiosemicarbazide (**2**) (0.91 g, 10 mmol) in EtOH (20 mL) containing catalytic amounts of HCl was refluxed for 2 h (monitored by TLC). The formed precipitate was isolated by filtration, washed with methanol, dried and recrystallized from EtOH to give thiosemicarbazone derivative **5**. Yellow solid, 75% yield, m.p. 190–192 °C; IR (KBr): *v* 3412, 3245, 3151 (2NH and NH_2), 3069, 2960 (C–a H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.-12–8.14 (m, 7H, Ar–H and NH_2), 8.20 (s, 1H, CH=N), 10.36 (brs, 1H, NH), 11.41 (brs, 1H, NH); MS *m/z* (%): 332 (M^+, 17). Anal. Calcd for $C_{14}H_{16}N_6S_2$ (332.44): C, 50.58; H, 4.85; N, 25.28. Found: C, 50.37; H, 4.65; N, 25.07%.

Reaction of 5 with Hydrazonoyl Chlorides 3a and 7a

A mixture of 2-(1-(2-(2-benzylidenehydrazinyl)-4-methyl thiazol-5-yl)ethylidene)hydrazine- carbothioamide (**5**) (0.332 g, 1 mmol) and the appropriate 2-oxo-*N*'-phenylpropanehydrazonoyl chloride (**3a**) or ethyl 2-chloro-2-(2-phenylhydrazono)acetate (**7a**) (1 mmol) in dioxane (20 mL) containing a catalytic amount of TEA was refluxed for 4 h (monitored by TLC). The formed precipitate was isolated by filtration, washed with methanol, dried and recrystallized from DMF to give a product proved to be identical in all respects (m.p., mixed m.p. and IR spectra) with the products **4a** or **8a** obtained from reaction of **1** + **2** + **3a** or **1** +**2** + **7a**, respectively.

Synthesis of Thiazole Derivatives 11a–e

A mixture of 2-(2-benzylidenehydrazinyl)-4-methylthiazole (**1**) (0.259 g, 1 mmol), thiosemicarbazide (**2**) (0.091 g, 1 mmol) and the appropriate phenacyl bromides **10a–e** (1 mmol) in EtOH (20 mL) was refluxed for 3–5 h, allowed to cool and the solid formed was filtered off, washed with EtOH, dried and recrystallized from DMF to give the corresponding thiazoles **11a–e**. The products **11a–e** together with their physical constants are listed below.

2-(2-(Benzylidene)Hydrazinyl)-4-Methyl-5-(1-(2-(4-(p-Tolyl) Thiazol-2-yl)Hydrazono)ethyl) Thiazole (11a)

Green solid, 68% yield, m.p. 250–252 °C; IR (KBr): *v* 3429, 3220 (2NH), 3063, 2920 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.22–8.05 (m, 9H, Ar–H), 8.13 (s, 1H, thiazole-H5), 8.27 (s, 1H, CH=N), 10.11 (brs, 1H, NH), 10.90 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.45, 18.41, 24.09 ($3CH_3$), 115.75, 116.59, 120.79, 123.22, 125.03, 127.50, 129.36, 133.29, 134.48, 137.93, 142.15, 143.10, 152.10, 156.84, 160.09, 162.64 (Ar–C and C=N); MS *m/z* (%): 446 (M^+, 4). Anal. Calcd for $C_{23}H_{22}N_6S_2$ (446.59): C, 61.86; H, 4.97; N, 18.82. Found: C, 61.69; H, 4.75; N, 18.68%.

2-(2-(Benzylidene)Hydrazinyl)-5-(1-(2-(4-(4-Methoxyphenyl) Thiazol-2-yl)Hydrazono)Ethyl)-4-Methylthiazole (11b)

Green solid, 69% yield, m.p. 203–205 °C; IR (KBr): *v* 3430, 3330 (2NH), 3110, 2938 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.92 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 7.32–7.79 (m, 10H, Ar–H and thiazole-H5), 8.18 (s, 1H, CH=N), 10.14 (brs, 1H, NH), 11.00 (brs, 1H, NH); MS *m/z* (%): 462 (M^+, 11). Anal. Calcd for $C_{23}H_{22}N_6OS_2$ (462.59): C, 59.72; H, 4.79; N, 18.17. Found: C, 59.91; H, 4.58; N, 18.05%.

2-(2-(Benzylidene)Hydrazinyl)-5-(1-(2-(4-(4-Chlorophenyl) Thiazol-2-yl)Hydrazono)Ethyl)-4-Methylthiazole (11c)

Green solid, 73% yield, m.p. 240–242 °C; IR (KBr): *v* 3426, 3260 (2NH), 3030, 2938 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.93 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.-39–7.86 (m, 10H, Ar–H and thiazole-H5), 8.25 (s, 1H,

CH=N), 10.05 (brs, 1H, NH),10.91 (br s, 1H, NH); MS *m/z* (%): 467 (M^+, 14). Anal. Calcd for $C_{22}H_{19}ClN_6S_2$ (467.01): C, 56.58; H, 4.10; N, 18.00. Found: C, 56.88; H, 3.85; N, 17.70%.

2-(2-(Benzylidene)Hydrazinyl)-5-(1-(2-(4-(4-Bromophenyl) Thiazol-2-yl)Hydrazono)Ethyl)-4-Methylthiazole (11d)

Green solid, 71% yield, m.p. 257–259 °C; IR (KBr): *v* 3429, 3384 (2NH), 3072, 2918 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 7.-38–8.14 (m, 10H, Ar–-H and thiazole-H5), 8.35 (s, 1H, CH=N), 10.03 (brs, 1H, NH), 10.86 (brs, 1H, NH); ^{13}CNMR (DMSO-d_6): δ 13.7, 18.8 ($2CH_3$), 112.6, 119.6, 126.1, 127.8, 128.4, 129.2, 129.9, 130.6, 133.3, 134.8, 136.2, 138.9, 140.8, 142.0, 145.0, 151.4; MS *m/z* (%): 513 (M^++2, 5), 511 (M^+, 7). Anal. Calcd for $C_{22}H_{19}BrN_6S_2$ (511.46): C, 51.66; H, 3.74; N, 16.43. Found: C, 51.35; H, 3.59; N, 16.31%.

2-(2-(Benzylidene)Hydrazinyl)-4-Methyl-5-(1-(2-(4-(4-Nitrophenyl)Thiazol-2-yl)hydrazono)Ethyl)Thiazole (11e)

Green solid, 71% yield, m.p. 225–227 °C; IR (KBr): *v* 3429, 3318 (2NH), 3115, 2920 (C–H) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.-41–7.72 (m, 9H, Ar–H), 8.06 (s, 1H, thiazole-H5), 8.36 (s, 1H, CH=N), 10.21 (brs, 1H, NH), 11.09 (brs, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.06, 18.98 ($2CH_3$), 124.64, 126.80, 129.34, 132.16, 133.18, 134.78, 135.23, 137.82, 141.29, 143.89, 150.70, 151.50, 157.23, 158.76, 164.69, 166.94 (Ar–C and C=N); MS *m/z* (%): 477 (M^+, 10). Anal. Calcd for $C_{22}H_{19}N_7O_2S_2$ (477.56): C, 55.33; H, 4.01; N, 20.53. Found: C, 55.14; H, 3.94; N, 20.45%.

Alternative Synthesis for 11a

A mixture of thiosemicarbazone derivative **5** (0.332 g, 1 mmol) and 2-bromo-1-(p-tolyl)ethanone **(10a)** (0.213 g, 1 mmol) in EtOH (20 mL) was refluxed for 3 h. The formed precipitate was isolated by filtration, washed with MeOH, dried and recrystallized from DMF to give **11a**.

Anticancer Activity

The cytotoxic potential of the newly synthesized compounds was examined against HCT-116, HT-29 and HepG2 cells using the MTT assay after 24 h of incubation.[34] For more details, see the Supplementary Materials.

Mammalian cell line: HCT-116, HT-29 and HepG2 cells were obtained from VACSERA Tissue Culture Unit, Cairo, Egypt.

Mechanistic Study on the Antitumor Activity

The analysis of the apoptotic markers Bax and caspase-3 levels as well as the anti-apoptotic marker Bcl-2 levels were assessed as reported earlier[35] for the most potent synthesized compounds on HCT-116 cells using ELISA colorimetric kits according to the manufacturer's instructions.

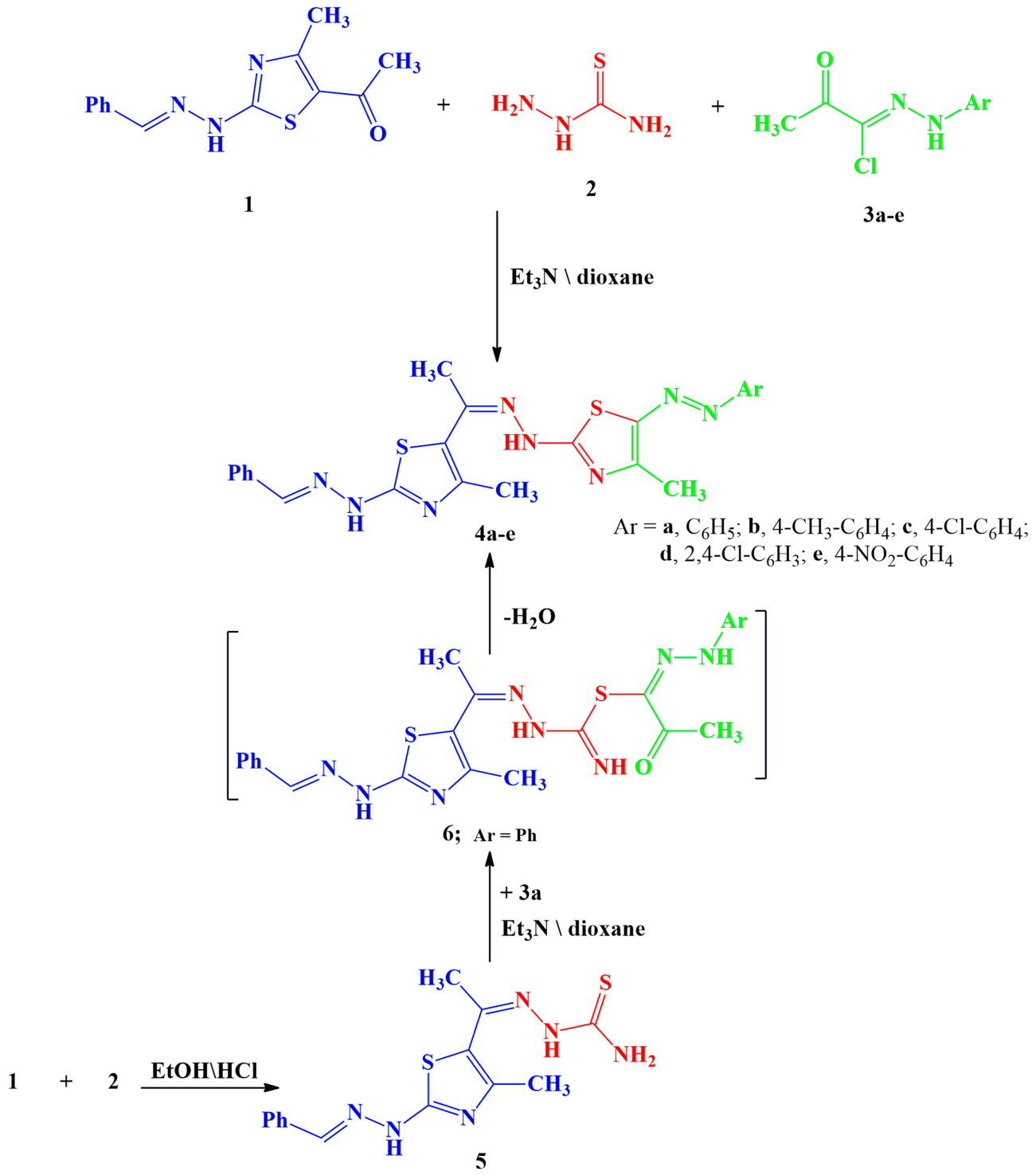
Results and Discussion

Chemistry

2-(2-Benzylidenehydrazinyl)-4-methylthiazole (**1**),[36] thiosemicarbazide (**2**) and the appropriate *N*-aryl-2-oxopropanehydrazonoyl chlorides (**3a**–**e**)[37] were allowed to react in a one-pot three-component reaction in refluxing dioxane containing a catalytic amount of TEA to afford the arylazothiazoles **4a**–**e**, respectively (Scheme 1). The structures of these products were established on the basis of elemental analyses as well as spectral data. The ^{1}H-NMR spectra of **4a**–**e** showed generally three singlets at δ ~ 1.91, 2.10 and 2.29 ppm due to the three CH_3 groups, a multiplet at δ ~ 6.97–8.10 ppm assignable to the aromatic protons, a singlet (1H) at δ ~ 8.69 ppm assignable to the methine proton, beside two broad singlets (D_2O exchangeable) at δ ~ 10.58 and 11.19 ppm due to the two NH protons. IR spectra of the compounds **3a**–**e** showed two absorption bands due to the two NH groups at *v* ~ 3425 and3220 cm^{-1}. Moreover, the mass spectrum of **4a** revealed a molecular ion peak at *m/z* = 474 which is consistent with its molecular weight.

Compound **4a** was alternatively synthesized by reacting 2-(1-(2-(2-benzylidenehydrazinyl)-4-methylthiazol-5-yl)ethylidene)hydrazine-carbothioamide (**5**) (prepared separately from reaction of acetylthiazole **1** and thiosemicarbazide **2** under reflux in ethanol in the presence of HCl drops for 1 h) with 2-oxo-*N*'-phenylpropanehydrazonoyl chloride (**3a**) in refluxing dioxane/TEA (Scheme 1). The obtained product was found to be identical with **4a** in all respects (TLC, m.p. and IR spectrum) which affords further evidence to all structures **4a**–**e**.

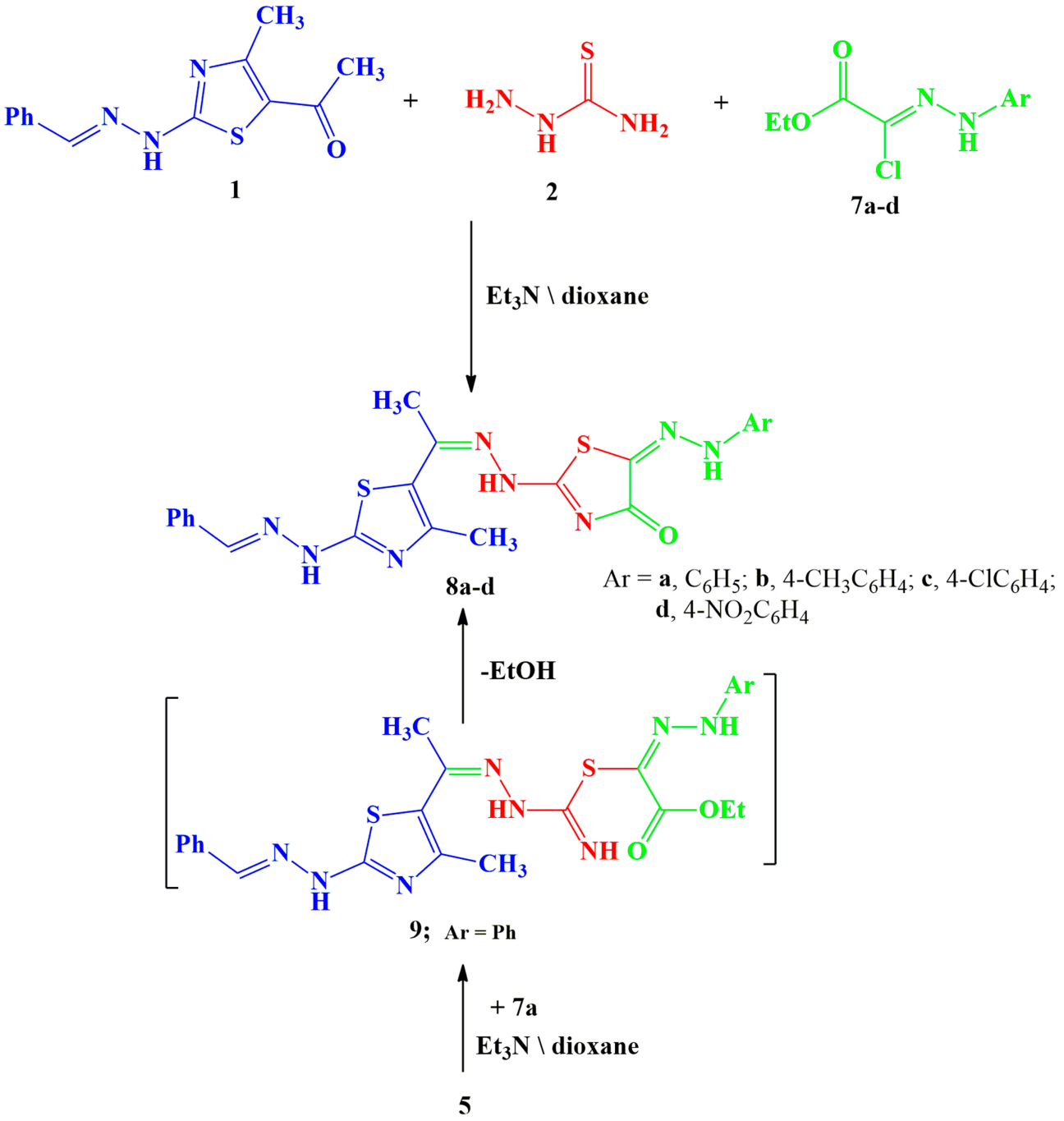
In a similar way, acetylthiazole **1** reacted with thiosemicarbazide **2** and the appropriate hydrazonoyl chlorides **7a**–**d**[34] in a one-pot three-component reaction in refluxing dioxane/TEA to afford the respective arylhydrazothiazolones **8a**–**d** (Scheme 2). The structures of compounds



Scheme 1 Synthesis of arylazothiazoles **4a–e.**

8a–d were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR and MS. For example, the IR spectra of product **7a** showed the exhibited presence of stretching bands for 3NH and C=O groups at the normal wave number *v*. The mass spectra of the products **8a–d** revealed in each case a molecular ion peak *m/z* at the expected molecular weight calculated for each compound (see Experimental).

In the ^{1}H-NMR spectrum of **7a**, six singlet signals were observed (δ 1.82, 2.44, 8.52, 10.45, 10.80, 12.47 ppm) for two CH_3 groups, CH=N proton and the three



Scheme 2 Synthesis of arylhydrazothiazoles **8a–d.**

NH groups, respectively, in addition to the expected aromatic protons.

To support this mechanism, compound **5** was allowed to react with ethyl 2-chloro-2-(2-phenylhydrazono)acetate (**7a**) in refluxing dioxane/TEA to afford a product identically similar to **8a** (Scheme 2).

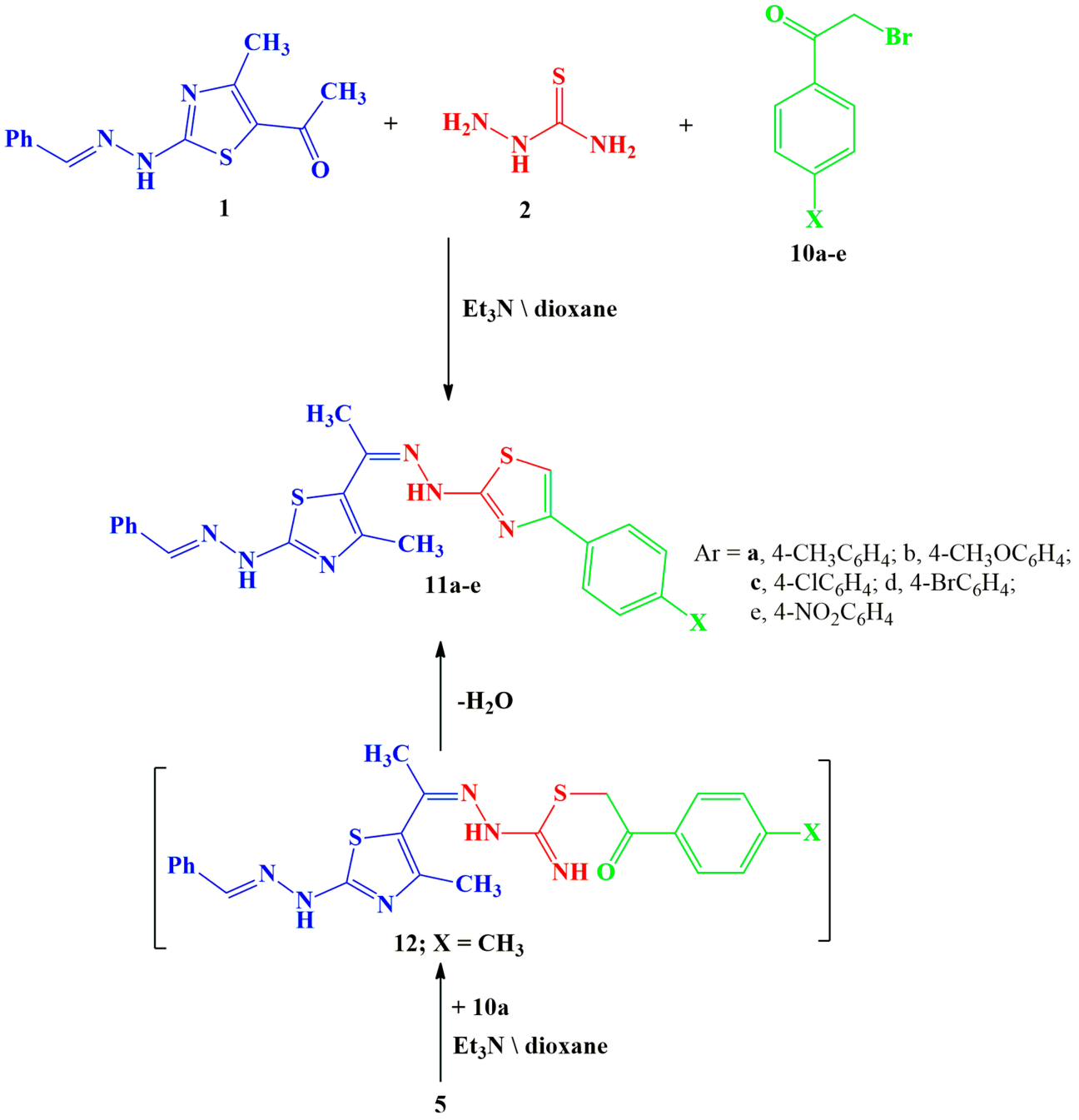
The chemical reactivity of acetylthiazole **1** toward thiosemicarbazide **2** and a variety of phenacyl bromides **10a–e** was also studied aiming to synthesize another series of novel thiazole derivatives. Thus, reaction of compound **1** with **2** and *p*-substituted phenacyl bromide derivatives **10a–e** in EtOH under reflux led to the formation of products **11a–e** (Scheme 3). Analytical and spectral data of these reaction products are in complete agreement with their proposed structures. The IR spectra of the products showed two NH absorption bands in **11a** at υ = 3429 and

3220 cm^{-1}. The ^{1}H-NMR spectra of compounds **11a–d** revealed the characteristic three singlet signals for the $3CH_3$ at δ 1.91, 2.08 and2.42 ppm, a multiplet group at δ 7.22–8.05 ppm, a singlet signal at δ 8.27 ppm due to thiazole-H5, a singlet signal at δ 8.27 ppm due to CH=N and also two broad singlet signals at δ 10.11 and 10.90 ppm due to 2NH groups.

The structure assigned for products **11** was further evidenced via the alternative method. Thus, reaction of **5** with 2-bromo-1-(p-tolyl)ethanone (**10a**) in EtOH afforded a product identical in all respects (m.p., mixed m.p. and IR spectra) with compound **11a** obtained from reaction of **1** + **2** + **10a** (Scheme 3).

Anticancer Activity

The pharmacological activities of the synthesized products **4a–e, 5, 8a–d** and **11a–e** were investigated for their human colon carcinoma cell line in comparison with harmine and



Scheme 3 Synthesis of thiazoles **11a–e.**

cisplatin as reference drugs using the colorimetric MTT assay. The relation between drug concentration and surviving cells is plotted to get the survival curve. The 50% inhibitory concentration (IC_{50}) was obtained and the anti-proliferative activity was expressed as the mean IC_{50} of 3 independent experiments (μM) ± standard deviation from three replicates.

The outline data presented in Tables 1–3 show that the anticancer activity of the tested compounds depends on their structures and the concentration. The descending order of in vitro inhibitory activity of the tested compounds toward the HCT-116 was as follows: **8c > 4d > 4c > 11c > 11d >11b >11a > 4b > 4a > 8a > 8b > 4e > 8d > 11e > 5**. Compounds **4c, 4d** and **8c** were the most active (IC_{50} value of 3.80 ± 0.8, 3.65 ± 0.9 and 3.16 ± 0.9 μM, respectively) against the HCT-116 cell line compared with harmine reference drug with IC_{50} value of 2.40 ± 0.12 μM as well as cisplatin (IC_{50} = 5.18± 0.94 μM). Compounds **11c, 11d, 11b, 11a, 4b, 4a, 8a, 8b** and **4e** have moderate inhibitory activity (IC_{50} = 14.50–31.50 μM) while the other measured compounds **8d, 11e** and **5** were inactive against HCT-116 (IC_{50} value > 48.20 μM).

Moreover, the activity of the most active compounds (**4c, 4d** and **8c**) was also performed on another human colorectal cancer cell line like HT-29, which is a more resistant cell line, to explore the efficiency of the synthesized compounds (Table 3). Interestingly, compound **8c** exerted higher potency (IC_{50} = 3.47± 0.79 μM) compared with harmine and cisplatin reference drugs with IC_{50} values of 4.59 and 11.68 μM, respectively. Also, compounds **4c** and **4d** showed promising IC_{50} values of 7.24 and 4.13 μM, respectively.

On the other hand, the order of inhibitory activity of the tested compounds toward the hepatocellular carcinoma (HepG2) cells was as follows: **4d > 4c > 8c > 11c > 11b > 11d > 8b > 4b >11a > 4a > 8a > 4e > 8d > 11e > 5** as indicated in Table 2. Compounds **4d, 4c, 8c** and **11c** were the most active (IC_{50} values of 2.31, 2.94, 4.57 and 9.86 μM, respectively) against the HepG2 cell line compared with harmine and cisplatin reference drugs with IC_{50} values of 2.54 and 9.41 μM.

To explore the mechanism of the anticancer action exerted by most active compounds (**4c, 4d** and **8c**) inside the HCT-116 cancer cell line, apoptotic cell marker analysis was also investigated in this study. The Bcl-2 family, the best-characterized protein family involved in the regulation of apoptosis, consists of anti-apoptotic and pro-apoptotic members that modulate this programmed process.[38] The anti-apoptotic members, such as Bcl-2, attenuate apoptosis either by preventing the release of mitochondrial apoptogenic factors like cytochrome C into the cytoplasm or by sequestering pro-forms of the caspases. On the other hand, pro-apoptotic members of the Bcl-2 family, such as Bax, trigger the release of caspases.[5] Caspase-3 is a cysteine-containing aspartic acid-specific protease that provides crucial roles in cell regulatory systems directing cell death pathways.[39]

When HCT-116 cells were treated with compounds **4c, 4d** and **8c** there was a significant increase in the levels of the pro-apoptotic molecule Bax by 1.5, 1.9 and 2.28 folds, respectively, compared to control (Figure 1A). In contrast, exposure of HCT-116 cells to compounds **4c, 4d** and **8c** resulted in a significant decrease in the protein expression levels of the anti-apoptotic protein Bcl-2 by about 11.5, 25.6 and 39.7%, respectively, compared to control (Figure 1B). Furthermore, the Bax/Bcl2 ratio was calculated to give more profound insight into the pro-apoptotic activity. Analyzing the results revealed that compounds **4c, 4d** and **8c** increased the Bax/Bcl-2 ratio about 2, 2.5 and 4 folds in comparison to the control. These results agreed with the previous reports where the bax/bcl-2 ratio is the major

Table 1 The Anticancer Activity of Compounds **4a–e, 5, 8a–d** and **11a–e** Against Colon Carcinoma (HCT-116) Cell Line Expressed as IC_{50} Values (μM) ± Standard Deviation from Three Replicates

Tested Compounds	X	IC_{50} (μM)	Tested Compounds	X	IC_{50} (μM)
4a	H	26.50 ± 1.10	8d	NO_2	48.20 ± 1.20
4b	Me	25.90 ± 0.70	11a	Me	19.50 ± 0.20
4c	Cl	3.80 ± 0.80	11b	OMe	19.20 ± 1.10
4d	2,4-diCl	3.65 ± 0.90	11c	Cl	14.50 ± 1.80
4e	NO_2	31.50 ± 1.90	11d	Br	16.10 ± 1.80
5	–	59.50 ± 1.80	11e	NO_2	50.20 ± 1.20
8a	H	27.20 ± 1.10	Harmine	–	2.40 ± 0.12
8b	Me	27.40 ± 1.80	Cisplatin	–	5.18± 0.94
8c	Cl	3.16 ± 0.90			

Table 2 The Anticancer Activity of the Synthesized Compounds Against HepG2 Cell Line Expressed as IC_{50} Values (μM) ± Standard Deviation from Three Replicates

Tested Compounds	X	IC_{50} (μM)	Tested Compounds	X	IC_{50} (μM)
4a	H	33.48 ± 1.64	8d	NO_2	61.74 ± 2.36
4b	Me	23.52 ± 1.12	11a	Me	24.56 ± 1.18
4c	Cl	2.94 ± 0.62	11b	OMe	14.39 ± 0.89
4d	2,4-diCl	2.31 ± 0.43	11c	Cl	9.86 ± 0.78
4e	NO_2	36.91 ± 2.34	11d	Br	21.32 ± 1.43
5	–	81.76 ± 3.88	11e	NO_2	76.93 ± 2.75
8a	H	34.63 ± 2.04	Harmine	–	2.54 ± 0.82
8b	Me	21.38 ± 1.26	Cisplatin	–	9.41± 0.63
8c	Cl	4.57 ± 0.85			

Table 3 The Anticancer Activity of Compounds **4c, 4d** and **8c** Against HT-29 Cell Line Expressed as IC_{50} Values (μM) ± Standard Deviation from Three Replicates

Tested Compounds	X	IC_{50} (μM)
4c	Cl	7.24 ± 0.62
4d	2,4-diCl	4.13 ± 0.51
8c	Cl	3.47 ± 0.79
Harmine	–	4.59 ± 0.67
Cisplatin	–	11.68 ± 1.54

influential value to determine cell susceptibility to apoptosis.[35]

However, treatment of HCT-116 cells by compounds **4c, 4d** and **8c** resulted in a significant elevation in the protein expression levels of active caspase-3 by about 2.59, 3.75 and 5 folds, respectively, compared to the non-treated HCT-116 control (Figure 1C).

The Anticancer Activity of Tested Compounds Against HCT-116 Cell Lines

For arylazothiazoles **4a–e**: thiazole **4d**(has two Cl atoms, electron-withdrawing group which increases activity) > **4c** (has one Cl atom) > **4b** (has CH_3 group, electron-donating group which decreases activity). For arylhydrazothiazolones **8a–d**: thiazole **4c**(has Cl group, electron-withdrawing group which increases activity) > **4b** (has CH_3 group, electron-donating group that decreases activity). For arylthiazoles **11a–e**: thiazole **4c** and **4d** (have Cl or Br atoms, electron-withdrawing groups which increases activity) > **4b** and **4a** (have OCH_3 and CH_3 groups, electron-donating group decreases activity).

Moreover, chlorophenyl-hydrazothiazolone **8c** (IC_{50} = 3.16 ± 0.90 μM) has greater activity than chlorophenyl-azothiazole **4c** (IC_{50} = 3.80 ± 0.80 μM) and than chlorophenyl-thiazole **11c**.

Generally, in the three thiazole series **4a–e, 8a–d** and **11a–e**, the Cl atom increases activity while the NO_2 group decreases the activity (Figure 2).

Conclusion

In our present work, we herein present an efficient synthesis of novel 1-(2-(2-benzylidenehydrazinyl)-4-methylthiazol-5-yl)ethanone. The latter compound was used as a building block for constructing novel three series of 5-(1-(2-(thiazol-2-yl)hydrazono)ethyl) thiazole derivatives in a one-pot three-component reaction. The structures of the newly synthesized compounds were established on the basis

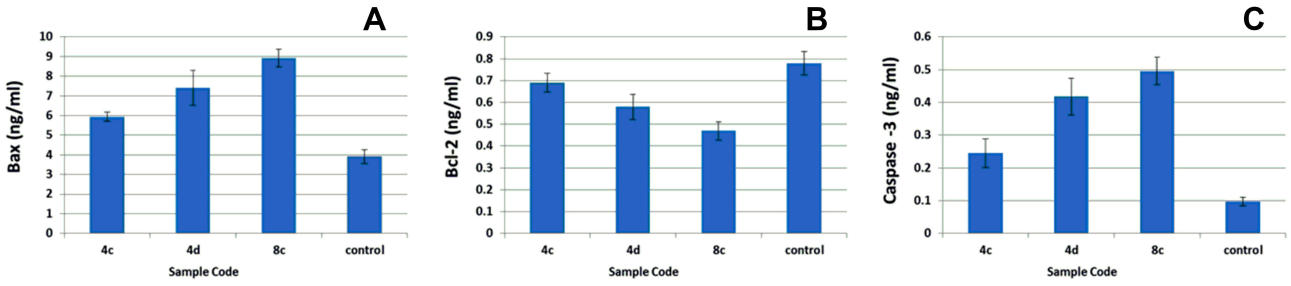
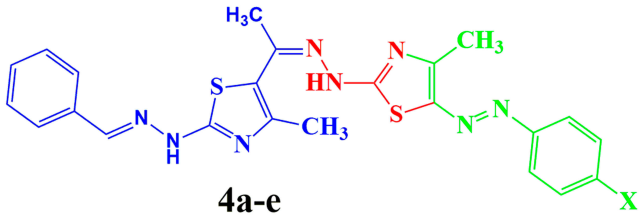
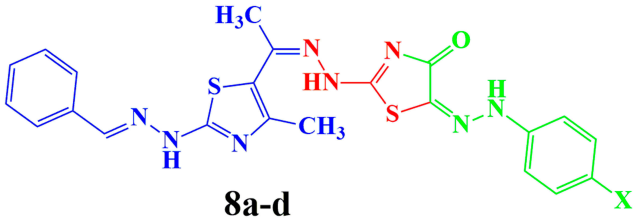


Figure 1 The apoptotic cell marker analysis exerted by the most active compounds (**4c, 4d** and **8c**) inside the HCT-116 cancer cell line suggested an apoptosis mechanism of the anticancer action; (**A**) Bax; (**B**) Bcl-2 and (**C**) caspase-3 levels compared with non-treated cell control.



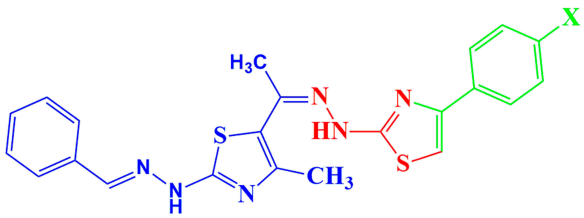
4a-e

For HCT-116 and HT-29 cell lines: X = 2,4-diCl > Cl > Me > H >>> NO_2
For HepG2: X = 2,4-diCl > Cl > > Me > H > NO_2



8a-d

For HCT-116 and HT-29 cell lines: X = Cl > H > Me >>> NO_2
For HepG2: X = Cl >> Me > H > NO_2



11a-e

For HCT-116 and HT-29 cell lines: X = Cl > Br > Me > MeO >>> NO_2
For HepG2: X = Cl > MeO > Br > Me >> NO_2

Figure 2 The anticancer activity of tested compounds (ordered) against HCT-116, HT-29 and HepG2 cell lines.

of spectroscopic evidence and their synthesis by alternative methods. The in vitro growth inhibitory activity of the synthesized compounds against three tumor cells (HCT-116, HT-29 and HepG2) was investigated in comparison with harmine and cisplatin reference drugs using an MTT assay and the results revealed promising activities of three compounds. The study also suggested that the mechanism of the anticancer action exerted by the most active compounds (**4c, 4d** and **8c**) inside HCT-116 cells was apoptosis through the Bcl-2 family.

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Author Contributions

All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest regarding this paper.

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