

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

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Abdelwahed R Sayed^{1,2}
Sobhi M Gomha^{3,4}
Eman A Taher^{5,6}
Zeinab A Muhammad⁵
Hesham R El-Seedi^{6,7}
Hatem M Gaber⁵
Mahgoub M Ahmed⁸

¹Department of Chemistry, Faculty of Science, KFU, Hofuf, Saudi Arabia;

²Department of Chemistry, Faculty of Science, Beni-suef University, Beni-suef, Egypt; ³Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt; ⁴Department of Chemistry, Faculty of Science, Islamic University in Almadinah Almonawara, Almadinah Almonawara 42351, Saudi Arabia; ⁵Department of Pharmaceutical Chemistry, National Organization for Drug Control and Research (NODCAR), Giza 12311, Egypt;

⁶Chemistry Department, Faculty of Science, El-Menoufia University, Shebin El-Kom 32512, Egypt; ⁷Division of Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, Biomedical Centre, Uppsala SE-75123, Sweden; ⁸Molecular Drug Evaluation Department, National Organization for Drug Control and Research (NODCAR), Giza 12311, Egypt

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, ¹H-NMR and ¹³C-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 IC₅₀ values of 3.80 ± 0.80, 3.65 ± 0.90 and 3.16 ± 0.90 μM, respectively, compared to harmine (IC₅₀ = 2.40 ± 0.12 μM) and cisplatin (IC₅₀ = 5.18 ± 0.94 μM) reference drugs. Also, compounds **8c**, **4d** and **4c** showed promising IC₅₀ 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 (IC₅₀ = 4.59 ± 0.67 μM) and cisplatin (IC₅₀ = 11.68 ± 1.54 μM). On the other hand, compounds **4d**, **4c**, **8c** and **11c** were the most active (IC₅₀ 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 (IC₅₀ = 2.54 ± 0.82 μM) and cisplatin (IC₅₀ = 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

Correspondence: Sobhi M Gomha
Tel +20 100-164-9576
Fax +20 25685799
Email s.m.gomha@gmail.com

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 Pye Unicam 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). ¹H spectra were recorded at 300 MHz and ¹³C 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 Elementar vario 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}; ¹H-NMR (DMSO-*d*₆): δ 1.91 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 6.97–8.10 (m, 10H, Ar-H), 8.69 (s, 1H, CH=N), 10.58 (brs, 1H, NH), 11.19 (brs, 1H, NH); ¹³C-NMR (DMSO-*d*₆): δ 8.89, 21.57, 23.56 (3CH₃), 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): ν 3428, 3210 (2NH), 3030, 2917 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.91 (s, 3H, CH₃), 2.26 (s, 3H, CH₃), 2.36 (s, 3H, CH₃), 2.51 (s, 3H, CH₃), 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 (4CH₃), 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): ν 3429, 3315 (2NH), 3020, 2922 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.91 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 2.42 (s, 3H, CH₃), 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): ν 3423, 3305 (2NH), 3040, 2916 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.91 (s, 3H, CH₃), 2.09 (s, 3H, CH₃), 2.40 (s, 3H, CH₃), 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): ν 3422, 3192 (2NH), 3062, 2916 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.91 (s, 3H, CH₃), 2.10 (s, 3H, CH₃), 2.57

(s, 3H, CH₃), 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 (CH₃), 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(5H)-One (8a)

Orange solid, 75% yield, m.p. 232–234 °C (DMF); IR (KBr): ν 3426, 3395, 3181 (3NH), 3050, 2924 (C–H), 1684 (C=O) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.82 (s, 3H, CH₃), 2.44 (s, 3H, CH₃), 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 (2CH₃), 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(5H)-One (8b)

Orange solid, 69% yield, m.p. 200–202 °C (EtOH); IR (KBr): ν 3421, 3280, 3183 (3NH), 3051, 2918 (C–H), 1685 (C=O) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 2.24 (s, 3H, CH₃), 2.45 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 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(5H)-One (8c)

Orange solid, 75% yield, m.p. 205–207 °C (DMF); IR (KBr): ν 3427, 3255, 3178 (3NH), 3060, 2926 (C–H), 1684 (C=O) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 2.44 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 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 (CH₃), 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(5H)-One (8d)

Orange solid, 70% yield, crystal (dioxane); m.p. 176–167 °C (EtOH); IR (KBr): ν 3429, 3210, 3180 (3NH), 3050, 2920 (C–H) 1701 (C=O) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.91 (s, 3H, CH₃), 2.08 (s, 3H, CH₃), 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 (2CH₃), 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): ν 3412, 3245, 3151 (2NH and NH₂), 3069, 2960 (C–a H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 2.34 (s, 3H, CH₃), 2.41 (s, 3H, CH₃), 7.12–8.14 (m, 7H, Ar–H and NH₂), 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-methylthiazol-5-yl)ethylidene)hydrazine-1-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): ν 3429, 3220 (2NH), 3063, 2920 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.91 (s, 3H, CH₃), 2.08 (s, 3H, CH₃), 2.42 (s, 3H, CH₃), 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 (3CH₃), 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): ν 3430, 3330 (2NH), 3110, 2938 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.92 (s, 3H, CH₃), 2.46 (s, 3H, CH₃), 3.76 (s, 3H, OCH₃), 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): ν 3426, 3260 (2NH), 3030, 2938 (C–H) cm^{-1} ; 1H -NMR (DMSO- d_6): δ 1.93 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 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): ν 3429, 3384 (2NH), 3072, 2918 (C–H) cm^{-1} ; 1H -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}C -NMR (DMSO- d_6): δ 13.7, 18.8 (2 CH_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): ν 3429, 3318 (2NH), 3115, 2920 (C–H) cm^{-1} ; 1H -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 (2 CH_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

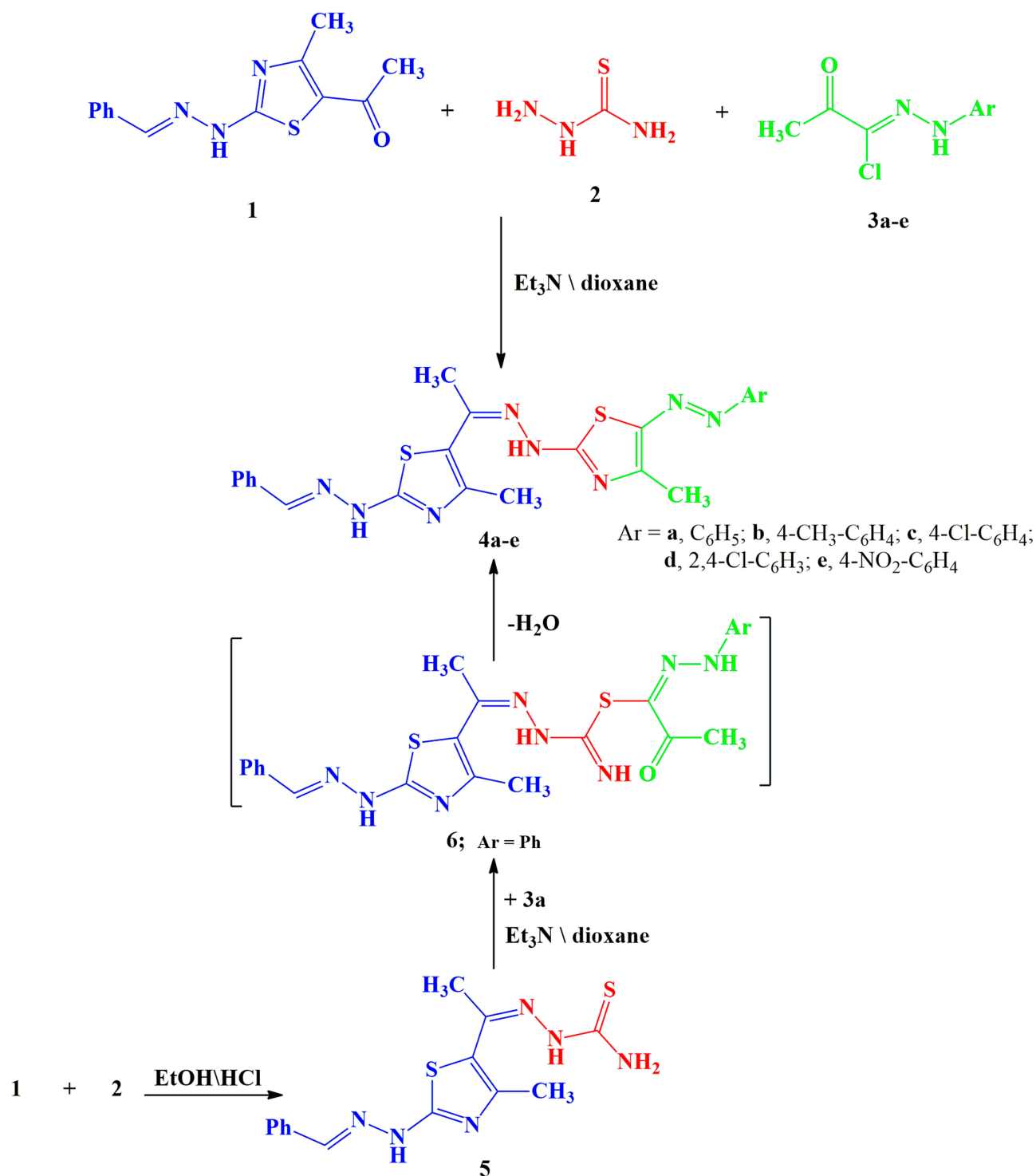
The analysis of the apoptotic markers Bax and caspase-3 levels as well as the anti-apoptotic marker Bcl-2 levels were assessed as reported earlier³⁵ for the most potent synthesized compounds on HCT-116 cells using ELISA colorimetric kits according to the manufacturer's instructions.

Results and Discussion Chemistry

2-(2-Benzylidenehydrazinyl)-4-methylthiazole (**1**),³⁶ thiosemicarbazide (**2**) and the appropriate *N*-aryl-2-oxopropenehydrazonoyl 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 1H -NMR spectra of **4a–e** showed generally three singlets at $\delta \sim 1.91$, 2.10 and 2.29 ppm due to the three CH_3 groups, a multiplet at $\delta \sim 6.97$ –8.10 ppm assignable to the aromatic protons, a singlet (1H) at $\delta \sim 8.69$ ppm assignable to the methine proton, beside two broad singlets (D_2O exchangeable) at $\delta \sim 10.58$ 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 $\nu \sim 3425$ and 3220 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

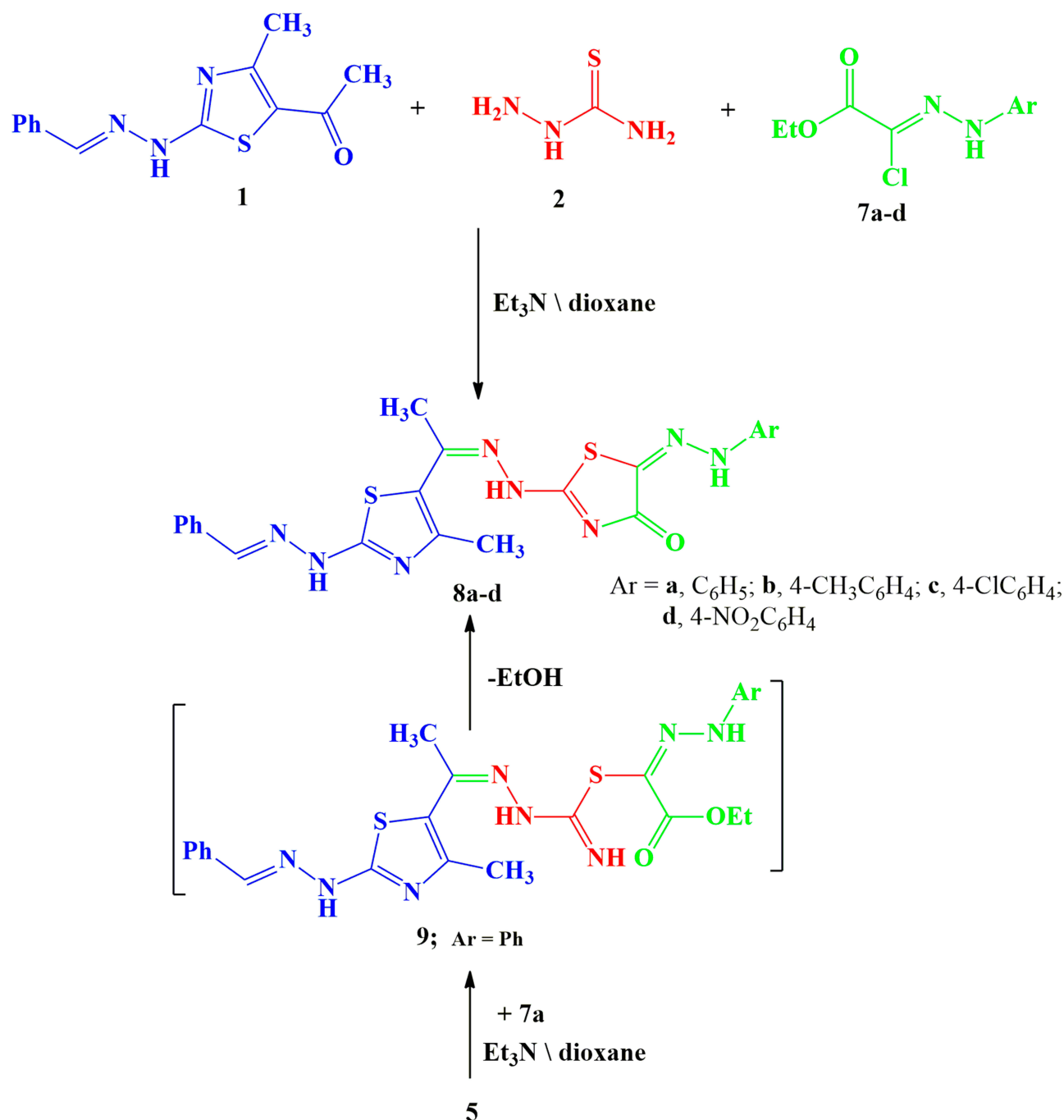


Scheme 1 Synthesis of arylazothiazoles **4a–e**.

8a–d were confirmed by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, 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 ν . 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 $^1\text{H-NMR}$ 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 $\nu = 3429$ and

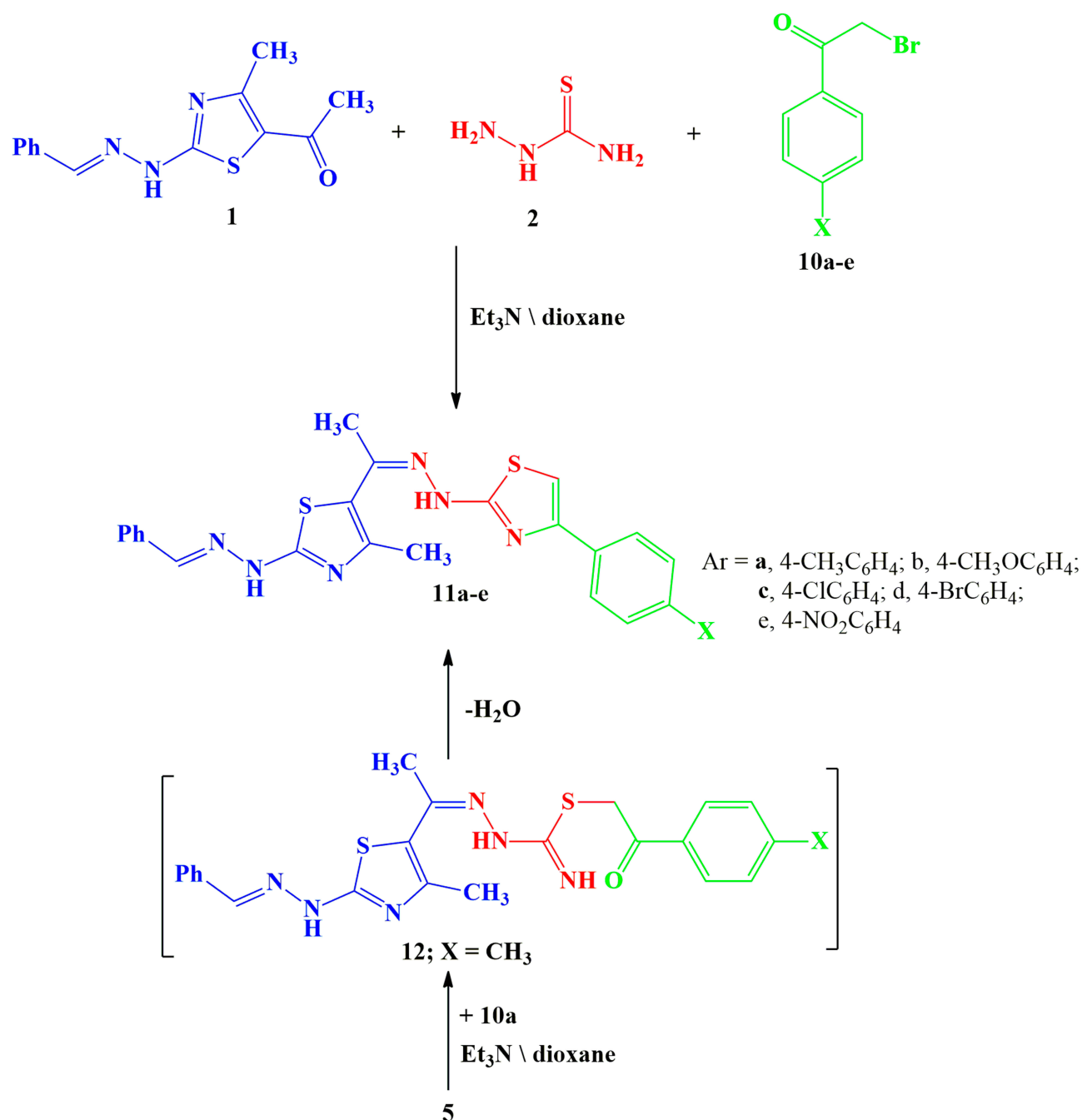
3220 cm^{-1} . The ^1H -NMR spectra of compounds **11a–d** revealed the characteristic three singlet signals for the 3CH_3 at δ 1.91, 2.08 and 2.42 ppm, a multiplet group at δ 7.22–8.05 ppm, a singlet signal at δ 8.27 ppm due to thiazole-H5, a singlet signal at δ 8.27 ppm due to $\text{CH}=\text{N}$ 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) \pm 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 $3.16 \pm 0.9 \mu M$, respectively) against the HCT-116 cell line compared with harmine reference drug with IC_{50} value of $2.40 \pm 0.12 \mu M$ as well as cisplatin ($IC_{50} = 5.18 \pm 0.94 \mu M$). Compounds **11c**, **11d**, **11b**, **11a**, **4b**, **4a**, **8a**, **8b** and **4e** have moderate inhibitory activity ($IC_{50} = 14.50$ – $31.50 \mu M$) while the other measured compounds **8d**, **11e** and **5** were inactive against HCT-116 (IC_{50} value > $48.20 \mu M$).

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 ($IC_{50} = 3.47 \pm 0.79 \mu M$) 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) \pm 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 IC₅₀ 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 IC₅₀ 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 CH₃ group, electron-donating group which decreases activity). For arylhydrazothiazolones

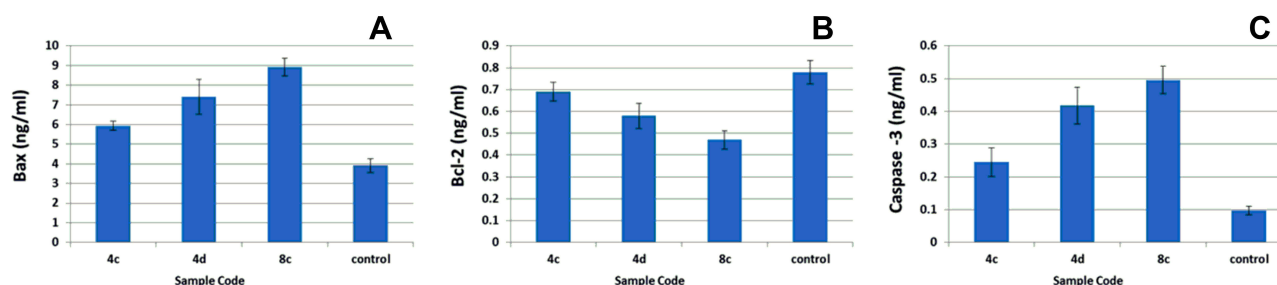
8a–d: thiazole **4c** (has Cl group, electron-withdrawing group which increases activity) > **4b** (has CH₃ group, electron-donating group that decreases activity). For arylthiazoles **11a–e**: thiazole **4c** and **4d** (have Cl or Br atoms, electron-withdrawing groups which increases activity) > **4b** and **4a** (have OCH₃ and CH₃ groups, electron-donating group decreases activity).

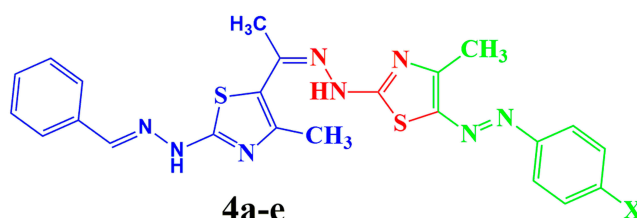
Moreover, chlorophenyl-hydrazothiazolone **8c** (IC₅₀ = 3.16 ± 0.90 μM) has greater activity than chlorophenyl-azothiazole **4c** (IC₅₀ = 3.80 ± 0.80 μM) and than chlorophenyl-thiazole **11c**.

Generally, in the three thiazole series **4a–e**, **8a–d** and **11a–e**, the Cl atom increases activity while the NO₂ group decreases the activity (Figure 2).

Conclusion

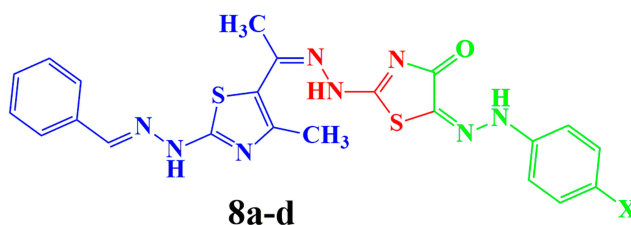
In our present work, we herein present an efficient synthesis of novel 1-(2-(2-benzylidenehydrazinyl)-4-methylthiazol-5-yl)ethanone. The latter compound was used as a building block for constructing novel three series of 5-(1-(2-(thiazol-2-yl)hydrazono)ethyl) thiazole derivatives in a one-pot three-component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



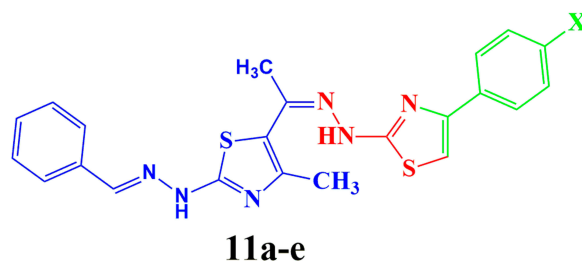
For HCT-116 and HT-29 cell lines: X = 2,4-diCl > Cl > Me > H >>> NO₂

For HepG2: X = 2,4-diCl > Cl >> Me > H > NO₂



For HCT-116 and HT-29 cell lines: X = Cl > H > Me >>> NO₂

For HepG2: X = Cl >> Me > H > NO₂



For HCT-116 and HT-29 cell lines: X = Cl > Br > Me > MeO >>> NO₂

For HepG2: X = Cl > MeO > Br > Me >> NO₂

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.

Acknowledgment

The financial support by the Deanship of Scientific Research (Project Number 1811008) King Faisal University, Saudi Arabia is gratefully acknowledged.

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.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65:87–108.
2. Coghlin C, Murray GI. Biomarkers of colorectal cancer: recent advances and future challenges. *Proteomics Clin Appl*. 2015;9:64–71. doi:10.1002/prca.201400082

3. Meier P, Finch A, Evan G. Apoptosis in development. *Nature*. 2000;407:796–801. doi:10.1038/35037734
4. Willis SN, Adams JM. Life in the balance: how BH₃-only proteins induce apoptosis. *Curr Opin Cell Biol*. 2005;17:617–625. doi:10.1016/j.ceb.2005.10.001
5. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol*. 2008;9(1):47–59. doi:10.1038/nrm2308
6. Dix MM, Simon GM, Cravatt BF. Global mapping of the topography and magnitude of proteolytic events in apoptosis. *Cell*. 2008;134(4):679–691. doi:10.1016/j.cell.2008.06.038
7. Kotschy A, Szlavik Z, Murray J, et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature*. 2016;538(7626):477–482. doi:10.1038/nature19830
8. da Silva EB, Silva DAO, Oliveira AR, et al. Design and synthesis of potent anti-*Trypanosoma cruzi* agents new thiazoles derivatives which induce apoptotic parasite death. *Eur J Med Chem*. 2017;130:39–50. doi:10.1016/j.ejmech.2017.02.026
9. Espindola JWP, de Oliveira Cardoso MV, de Oliveira Filho GB, et al. Synthesis and structure–activity relationship study of a new series of antiparasitic aryloxy thiosemicarbazones inhibiting *Trypanosoma Cruzi Cruza*. *Eur J Med Chem*. 2015;101:818–835. doi:10.1016/j.ejmech.2015.06.048
10. Netalkar PP, Netalkar SP, Revankar VK. Transition metal complexes of thiosemicarbazone: synthesis, structures and in vitro antimicrobial studies. *Polyhedron*. 2015;100:215–222. doi:10.1016/j.poly.2015.07.075
11. Hu WX, Zhou W, Xia CN, Wen X. Synthesis and anticancer activity of thiosemicarbazones. *Bioorg Med Chem Lett*. 2006;16:2213–2218. doi:10.1016/j.bmcl.2006.01.048
12. Wang Y, Wang Z, Kuang H, et al. Synthesis and antitumor activity of 2-isocamphanyl thiosemicarbazone derivatives via ROS-enhanced mitochondria damage. *Chem Biol Drug Des*. 2019. doi:10.1111/cbdd.13492
13. Sujatha K, Vedula RR. Novel one-pot expeditious synthesis of 2, 4-disubstituted thiazoles through a three-component reaction under solvent free conditions. *Synth Commun*. 2018;48:302–308. doi:10.1080/00397911.2017.1399422
14. Abdalla MA, Gomha SM, Abdelaziz M, Serag N. Synthesis and antiviral evaluation of some novel thiazoles and 1,3-thiazines substituted with pyrazole moiety against rabies virus. *Turk J Chem*. 2016;40:441–453. doi:10.3906/kim-1506-13
15. S M G, K D K. A convenient ultrasound-promoted synthesis and cytotoxic activity of some new thiazole derivatives bearing a coumarin nucleus. *Molecules*. 2012;17:9335–9347. doi:10.3390/molecules17089335
16. Nayak S, Gaonkar SL. A Review on recent synthetic strategies and pharmacological importance of 1, 3-thiazole derivatives. *Mini Rev Med Chem*. 2019;19:215–238. doi:10.2174/1389557518666180816112151
17. Kumar S, Aggarwal R. Thiazole: a privileged motif in marine natural products. *Mini Rev Org Chem*. 2019;16:26–34. doi:10.2174/1570193X15666180412152743
18. Gomha S, Edrees M, Altalbawy F. Synthesis and characterization of some new bis-pyrazolyl-thiazoles incorporating the thiophene moiety as potent anti-tumor agents. *Int J Mol Sci*. 2016;17:1499. doi:10.3390/ijms17091499
19. Gomha SM, Salah TA, Abdelhamid AO. Synthesis, characterization, and pharmacological evaluation of some novel thiadiazoles and thiazoles incorporating pyrazole moiety as anticancer agents. *Monatsh Chem*. 2015;146:149–158. doi:10.1007/s00706-014-1303-9
20. Dos Santos Silva TD, Bomfim LM, da Cruz Rodrigues ACB, et al. Anti-liver cancer activity in vitro and in vivo induced by 2-pyridyl 2, 3-thiazole derivatives. *Toxicol Appl Pharmacol*. 2017;329:212–223. doi:10.1016/j.taap.2017.06.003
21. Morigi R, Locatelli A, Leoni A, Rambaldi M. Recent patents on thiazole derivatives endowed with antitumor activity. *Recent Pat Anticancer Drug Discov*. 2015;10:280–297. doi:10.2174/1574892810666150708110432
22. Bremner WS, Organ MG. Multicomponent reactions to form heterocycles by microwave-assisted continuous flow organic synthesis. *J Comb Chem*. 2007;9:14–16. doi:10.1021/cc060130p
23. Murata T, Murai M, Ikeda Y, Miki K, Ohe K. Pd-and Cu-catalyzed one-pot multicomponent synthesis of hetero α , α' -dimers of heterocycles. *Org Lett*. 2012;14:2296–2299. doi:10.1021/ol300718x
24. Abdelrazek FM, Gomha SM, Shaaban ME, et al. One-pot three-component synthesis and molecular docking of some novel 2-thiazolyl pyridines as potent antimicrobial agents. *Mini Rev Med Chem*. 2019;19:527–538. doi:10.2174/1389557518666181019124104
25. Gomha SM, Muhammad ZA, Abdel-aziz MR, Abdel-aziz HM, Gaber HM, Elaasser MM. One-pot synthesis of new thiadiazolyl-pyridines as anticancer and antioxidant agents. *J Heterocycl Chem*. 2018;55:530–536. doi:10.1002/jhet.3088
26. Gomha SM, Edrees MM, Faty RA, Muhammad ZA, Mabkhot YN. Microwave-assisted one pot three-component synthesis of some novel pyrazole scaffolds as potent anticancer agents. *Chem Cent J*. 2017;11:37. doi:10.1186/s13065-017-0266-4
27. Gomha S, Kheder N, Abdelhamid A, Mabkhot Y. One pot single step synthesis and biological evaluation of some novel bis(1,3,4-thiadiazole) derivatives as potential cytotoxic agents. *Molecules*. 2016;21:1532. doi:10.3390/molecules21111532
28. Gomha SM. A facile one-pot synthesis of 6,7,8,9-tetrahydrobenzo [4,5]thieno[2,3-*d*]-1,2,4- triazolo[4,5-*a*]pyrimidin-5-ones. *Monatsh Chem*. 2009;140:213–220. doi:10.1007/s00706-008-0060-z
29. Gomha SM, Abdelrazek FM. A Facile three component-one-pot synthesis of some novel tri-cyclic hetero-ring systems. *J Heterocycl Chem*. 2016;53:1892–1896. doi:10.1002/jhet.2503
30. Gomha SM, Riyadh SM. Synthesis of triazolo[4,3-*b*][1,2,4,5]tetrazines and triazolo[3,4-*b*][1,3,4]thiadiazines using chitosan as eco-friendly catalyst under microwave irradiation. *Arkivoc*. 2009;xi:58–68.
31. Gomha SM, Abdalla MA, Abdelaziz M, Serag N. Eco-friendly one-pot synthesis and antiviral evaluation of pyrazolyl chalcones and pyrazolines of medicinal interest. *Turk J Chem*. 2016;40:484–498. doi:10.3906/kim-1510-25
32. Abbas IM, Riyadh SM, Abdallah MA, Gomha SM. A novel route to tetracyclic fused tetrazines and thiadiazines. *J Heterocycl Chem*. 2006;43:935–942. doi:10.1002/jhet.5570430419
33. Abbas IM, Gomha SM, Elaasser MM, Bauomi MA. Synthesis and biological evaluation of new pyridines containing imidazole moiety as antimicrobial and anticancer agents. *Turk J Chem*. 2015;39:334–346. doi:10.3906/kim-1410-25
34. Gomha SM, Riyadh SM, Mahmoud EA, Elaasser MM. Synthesis and anticancer activity of arylazothiazoles and 1,3,4-thiadiazoles using chitosan-grafted-poly(4-vinylpyridine) as a novel copolymer basic catalyst. *Chem Heterocycl Compd*. 2015;51:1030–1038. doi:10.1007/s10593-016-1815-9
35. Eldehna WM, Abo-Ashour MF, Nocentini A, et al. Novel 4/3--((4-oxo-5-(2-oxoindolin-3-ylidene) thiazolidin-2-ylidene) amino) benzene sulfonamides: synthesis, carbonic anhydrase inhibitory activity, anticancer activity and molecular modelling studies. *Eur J Med Chem*. 2017;139:250–262. doi:10.1016/j.ejmech.2017.07.073
36. Maccioni E, Cardia MC, Distinto S, Bonsignore L, De Logu A. An investigation of the biological effect of structural modifications of isothiosemicarbazones and their cyclic analogues. *Il Farmaco*. 2003;58:951–959. doi:10.1016/S0014-827X(03)00154-X
37. Shawali AS, Gomha SM. A new entry for short and regioselective synthesis of [1, 2, 4] triazolo [4, 3-*b*][1, 2, 4]-triazin-7(1*H*)-ones. *J Prakt Chemie Chem*. 2000;342:599–604. doi:10.1002/1521-3897-(200006)342:6<599::AID-PRAC599>3.0.CO;2-7
38. Tsujimoto Y. Cell death regulation by the Bcl-2 protein family in the mitochondria. *J Cell Physiol*. 2003;195:158–167. doi:10.1002/jcp.10254
39. Wu Z-R, Liu J, Li J-Y, et al. Synthesis and biological evaluation of hydroxycinnamic acid hydrazide derivatives as inducer of caspase-3. *Eur J Med Chem*. 2014;85:78–783. doi:10.1016/j.ejmech.2014.08.040

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>