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A Simple Synthesis of Some Novel Bi-Thiazoles as Anti-Tumor Agents

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Abstract

A series of novel bi-thiazoles was synthesized by reaction of 2-(1-(2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)-4-methylthiazol-5-yl)ethylidene) hydrazine-1-carbothioamide **3** with a variety of hydrazonoyl chloride derivatives **4a-d**, and 7 a-d affording **6a-d** and **9a-d** respectively in dioxane in the presence of triethyl amine as a base. Also, hydrazine-1-carbothioamide **3** reacts with substituted phenacyl bromide derivatives **10 a-d** yielding **12a-d** in appropriate reaction conditions. The mechanisms of the titled reactions were discussed. Structural assignments were based on spectroscopic methods (NMR, FTIR, MS). The new compounds were tested in vitro for their were subjected to *in vitro* anticancer screening against human hepatocellular carcinoma (HCT-116), (HepG-2) and the results showed that compounds **6a, 6d, 9a, 9c** and **12a** have promising activities compared with cisplatin reference drug.

Key Words: heterocycles, hydrazonoyl halides, cyclization, hydrazones, bi-thiazoles.

1. Introduction

Human colon carcinoma (HCT) is the most leading cause of cancer-related death globally. Recently, Cancer Facts & Figures reported around 1,693,900 deaths in 2012 and diagnosed more than 1 million people.[1-4] HCT mainly occurs in Australia, Canada, the U.S., and parts of Europe for over 63% of all cancer cases. [5] In the U.S., the average incidence rates of HCT ranges were 40 per 100,000 people in 2007.[6] Surgical resection is the most principle treatment for HCT patients. However, 5-fluorouracil (5-FU), capecitabine (Xeloda), irinotecan (Camptosar), and oxaliplatin (Eloxatin) representing current drugs have proved as effective first-line drugs for HCT. These findings indicate that there is an urgent need for the exploring of new effective therapies.[7,8]

Thiosemicarbazones represented an important class of compounds with tremendous therapeutic value as antiparasitic and antimicrobial diseases [9,10] and microbial infections.[11] Recently, They also reported as one of the interesting antitumor inhibitors due to the induction of oxidative stress and ROS-mediated cell injury. [12,13] Thiazole are scaffolds of many natural and synthetic drugs with numerous exciting and significant pharmacological activities [14,18] (Figure 1 adapted from Sujatha and Vedula, 2019 [14]).

Thiazole derivatives showed significant anticancer activity with a mechanism of action related to inhibition of matrix metalloproteinases kinases and anti-apoptotic BCL2 family proteins.[19] Multi-component reactions (MCR) as one-pot processes that nowadays occupy great importance of the interesting sustainable synthetic tools for their high efficiency and atom-economy.[20,21]

Herein, this study explores a convenient and rapid method for the synthesis of thiazolyl-hydrazono-ethylthiazole derivatives by one-pot three-component reactions using 1-(4-fluorophenyl)ethanone (5-acetyl-4-methyl-1,3-thiazol-2-yl)hydrazine 1, thiosemicarbazide 2 and the appropriate hydrazonoyl chlorides 4a-d or 7a-d or phenacyl bromides 10a-d in the presence of a catalytic amount of TEA in dioxane.

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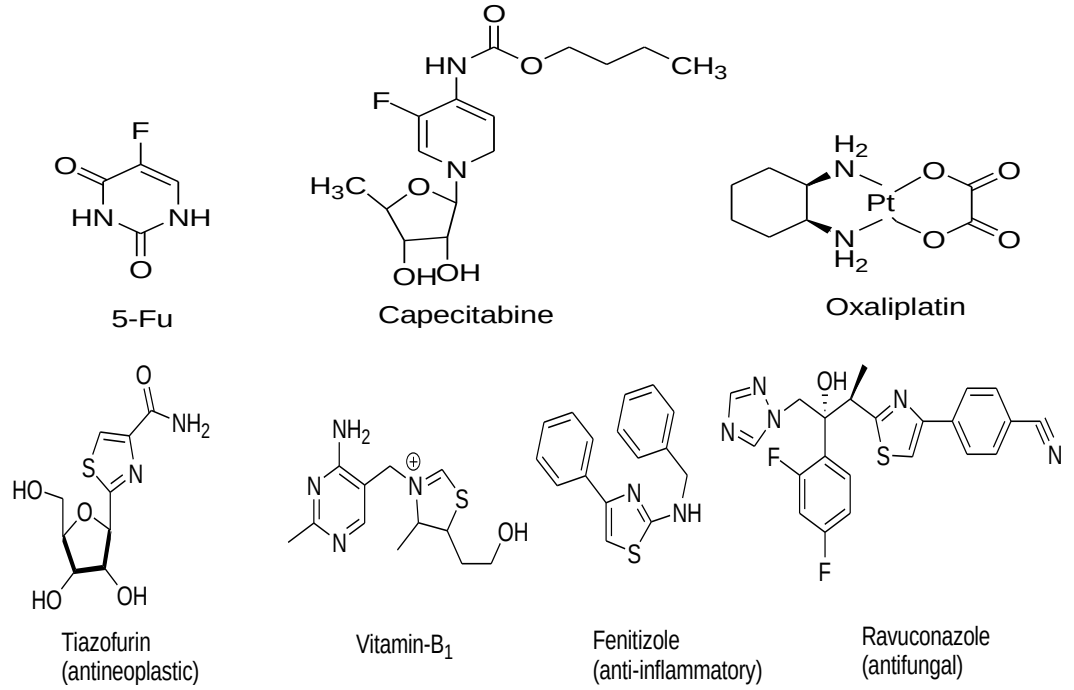
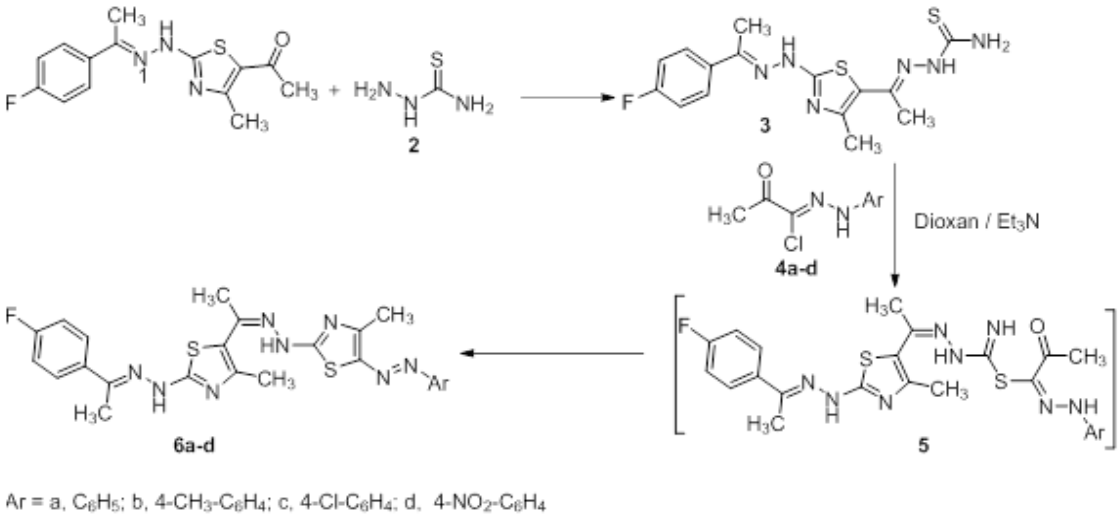


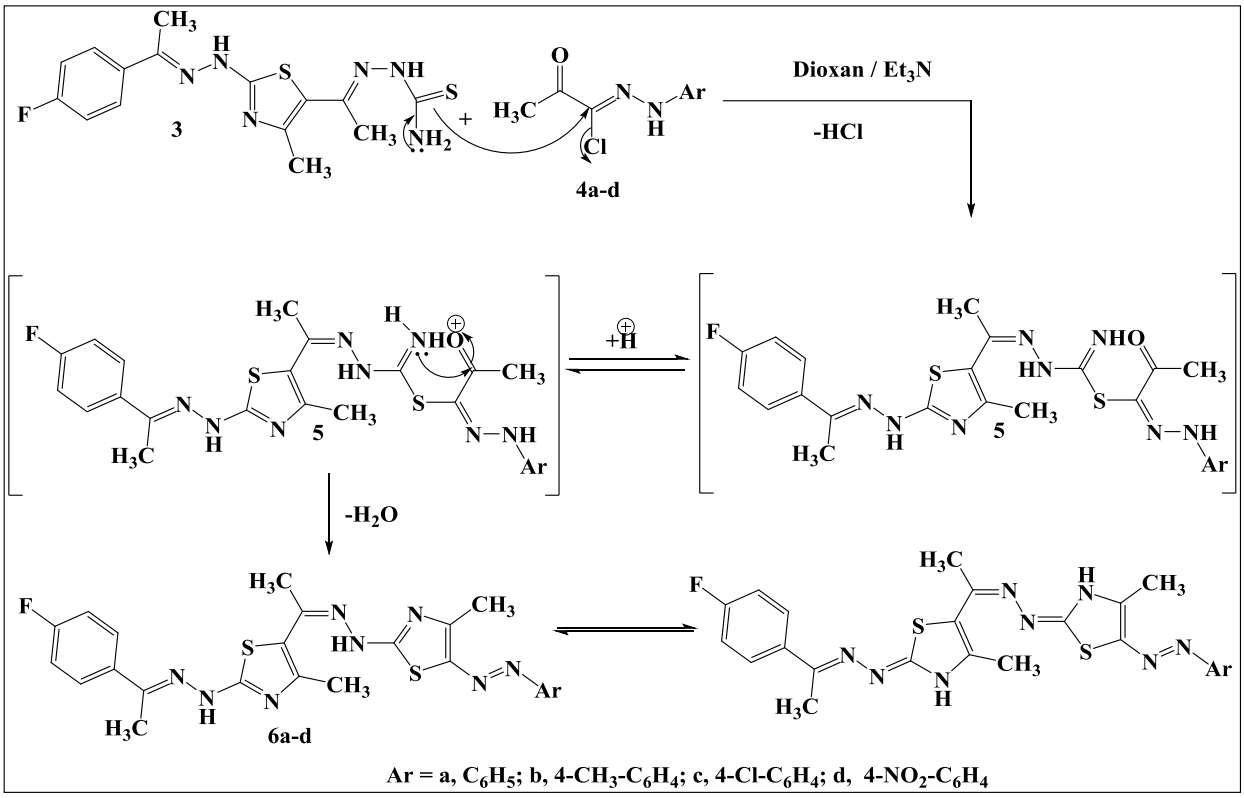
Results and discussion

Heating 1-(4-fluorophenyl)ethanone (5-acetyl-4-methyl-1,3-thiazol-2-yl)hydrazine 1 with thiosemicarbazied 2 in ethanol catalyzed by a catalytic amount of hydrochloric acid afforded 1-(2-{2-[1-(4-fluorophenyl)ethylidene]hydrazino}-4-methyl-1,3-thiazol-5-yl)ethan-1-one thiosemicarbazone 3 in moderate yield (Scheme 1). The structure of 3 was established on the basis of elemental analysis and its spectral data. The ^{1}HNMR showed three signals at δ 2.10, 2,31, 2.45 ppm corresponding to three CH_3 groups, a multiplet at δ 6.99-7.97 ppm assignable to aromatic protons, and a broad signals at δ 7.52 ppm corresponding to NH_2 , and two broad singlets at δ 11.32, 11.92 ppm corresponding to 2 NH, integrated for one proton for each. The combination of thiazolyl semicarbazone 3 with appropriate -aryl-2-oxopropane hydrazonoyl chlorides 4a-d takes place in dioxane and a catalytic amount of triethyl amine affording the expected product 1,3-thiazol-5-yl)hydrazino -2-oxo-*N*-arylpropanehydrazonothioate derivatives 5a-d or 1-(4-fluorophenyl)ethanone [4-methyl-5-(*N*-{4-methyl-5-[aryldiazenyl]-1,3-thiazol-2-yl}ethanehydrazonoyl) -1,3-thiazol-2-yl]hydrazine derivatives 6a-d or both depending on the reaction conditions used and mode of cyclization.



Scheme 1: Synthesis of bi-thiazole 6a-d.

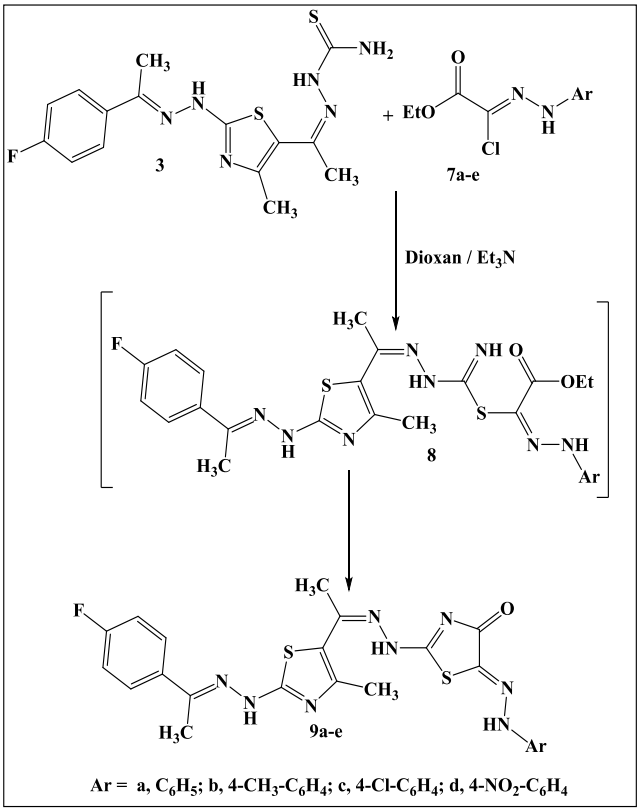
In the present work, the treatment of thiazolyl thiosemicarbazone **3** with appropriate hydrazonyl chloride **4a-d** occurred in the presence of triethylamine as a catalytic base might ease the removal of chlorine. The nucleophilic attack of sulfur on the generated carbocation resulting from chlorine elimination in **4** affording 1,3-thiazol-5-yl)hydrazino -2-oxo-N-arylpropanehydrazonothioate derivatives **5a-d** as an expected product. Spectroscopic methods confirmed the structure of 1,3-thiazol-2-yl}ethanehydrazonoyl)-1,3-thiazol-2-yl]hydrazine derivatives **6a**; its IR spectrum shows two weak bands of the two imino group at 3420, 3221 cm^{-1}. The two amino groups are confirmed by its ^1H NMR spectrum as two broad singlets at δ 10.65, 11.19 ppm, integrating for one proton of each, and revealed the characteristic four singlet signals at δ 1.94, 2.12, 2.45, 2.84 for the $4CH_3$ respectively. The formation of compound **6** can explained by Intramolecular cyclization of compound **5a-d** occurred in *situ* affording 1,3-thiazol-2-yl[(ethanehydrazonoyl)-1,3-thiazol-2-yl]hydrazine **6a-d** in good yield. We do believe that the concentration of hydrochloric acid formed in the reaction mixture as a factor-induced the intramolecular cyclization yielding **6a-d** (Scheme 2).



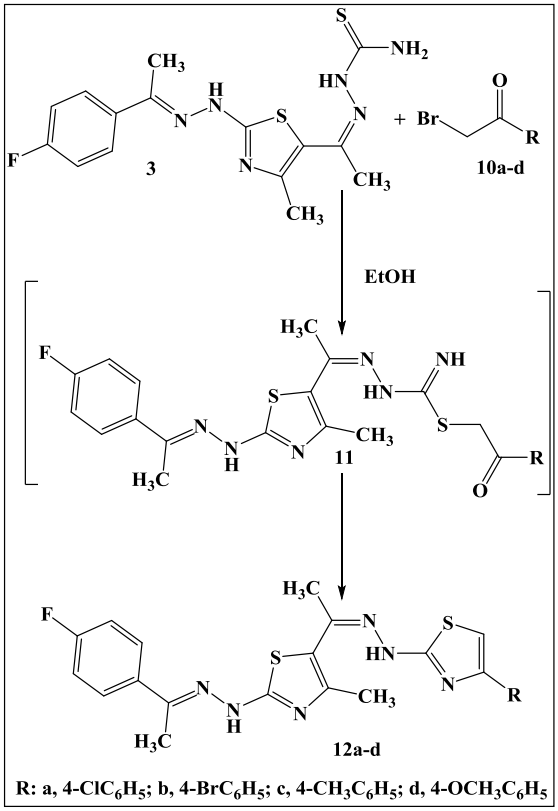
Scheme 2: Possible Mechanism of cyclization of bi-thiazole 6a-d.

Reaction of compound **3** with *N*-aryl-2-oxopropanehydrazonoyl chlorides **7a-d** in dioxane under reflux in the presence of trimethylamine led to formation of products **9a-d** (Scheme 3). The other possible products **8a-d** were excluded based on the spectral data (IR and ^1H NMR). The isolated product **9a** has confirmed spectroscopically, its IR spectra showed the presence of stretching bands for 3NH and C=O groups at ϒ 3426, 3395 , and 1684 cm^{-1}. The presence of the three imino groups is confirmed by its ^1H NMR spectrum as three broad singlets at δ 10.48, 10.51, 12.20 ppm, integrating for one proton of each, and at revealed the characteristic three singlet signals at δ 1.80, 2.18, 2.47 for the 3 CH_3 respectively.

Reaction of compound **3** with phenathyl bromide **10a-d** in dioxane under reflux in the presence of a catalytic amount of trimethylamine led to formation **12a-d** (Scheme 4). Spectroscopic methods confirmed the isolated product **12a-d** excluding the formation of **11** as a product. Its IR spectra of products **12c** showed at ϒ 3429, 3220 cmt^{-1} corresponding to 2NH, and its ^1H NMR spectrum revealed the characteristic three singlet signals for the $3CH_3$ at δ 1.40, 1.97, 2.10 ppm corresponding to three CH^3 also singlet signal at δ8.30 ppm revealed to CH-thiazole-H, in additional broad singlet signal at 11.55 due to 2NH ppm groups.



Scheme 3: Synthesis of bi-thiazolone 9a-e



Scheme 4: Synthesis of bi-thiazole 12a-d.

Antitumor Activity

The pharmacological activities of the synthesized products **3, 6a-d, 9a-d,** and **12a-d** were investigated in comparison with cisplatin reference drugs using 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 in Table 1 showed that:

1) HePG-2

- The anti-proliferative activities of the tested compounds depend on their structure.
- The activity of the measured compounds are varies with their concentration.
- The descending order of *in vitro* inhibitory activity of the tested compounds towards the HepG-2 was as follow: **6e > 12d > 6b > 9b > 9c >12a >3 > 12c > 6a > 9a > 6d > 12b > 9d.**
- Compounds **6e, 12d, 6b** and **9b** were the most active (IC_{50} value of 4.30 ± 1.80, 6.40 ± 2.10 and 8.90 ± 2.70, 9.15 ± 1.80 μM**,** respectively) against the HepG-2 cell line, compared with cisplatin reference drug with IC_{50} value of 2.80 ±1.10 μM.
- Compound **9c, 12a, 3** has moderate inhibitory activity, while the other measured compounds **6d, 12b** were weak activity
- Compound **9d** has inactive against HepG-2 (IC_{50} value > 50 μM).
- Compounds **6c** with chlorine atom (IC_{50} = 4.30 ± 1.80 μM) has greater activities than compound **12d** with methoxy group moeity (IC_{50} = 6.40 ± 2.10 μM).

2) HCT-116

- The descending order of *in vitro* inhibitory activity of the tested compounds towards the HCT-116 was as follow: **9c > 12d > 6c > 12a > 6b >3 >12c > 9b > 6a > 9a > 12b > 6d > 9d.**
- Compounds **9c, 12d** and **6c** were the most active (IC_{50} value of 3.16 ± 1.90, 9.25 ± 2.10 and 10.85 ± 2.80 μM**,** respectively) against the HCT-116 cell line, compared with cisplatin reference drug with IC_{50} value of 3.20 ±1.10 μM.
- Compound **12a, 6b, 3** has moderate inhibitory activity, while the other measured compounds 12c, **9b, 6a** were weak activity

- Compound **9d** has inactive against HCT-116 (IC_{50} value > 50 μM).
- Compounds **12d** with methoxy group moeity (IC_{50} = 9.25 ± 2.10 μM) has nearly equal to compound **6c** with one halogen group moeity (IC_{50} = 10.85 ± 1.80 μM).

For bi-thiazole **9c** and **6c** (has one atom of Cl, and the electron-withdrawing group that increases activity compared with **9d** with a nitro group). Compound **12d** has a methoxy group acting as a donating group to decrease the activity but the nearly equal Chlorine atom activity inside HCT-116. We do believe that the presence of hydrazine moiety attached to thiazole system play a role in increasing the activity of chlorine atom, but their absence, increase the activity of donating group as methoxy group in compound **12d**

Table 1: The anti-proliferative activity of compounds **3, 6a-d, 9a-d,** and **12a-d** against HepG-2 and HCT-116 cell lines expressed as IC_{50} values (Mm) ± standard deviation from three replicates

Tested compounds	IC_{50} (Mm) HepG-2	IC_{50} (Mm) HCT-116
3	16.50 ± 1.70	19.30 ± 1.80
6a	26.00 ± 1.10	38.35 ± 1.10
6b	8.90 ± 2.70	15.70 ± 2.70
6c	4.30 ± 1.80	10.85 ± 1.80
6d	113.60 ± 2.90	143.65 ± 2.90
9a	37.40 ± 2.10	116.20 ± 2.10
9b	9.15 ± 1.80	27.40 ± 1.80
9c	13.19 ± 1.90	3.16 ± 1.90
9d	348.44 ± 1.20	248.20 ± 1.20
12a	15.50 ± 1.80	12.50 ± 1.80
12b	116.35 ± 1.60	125.10 ± 1.80
12c	20.50 ± 1.20	19.50 ± 8.20
12d	6.40 ± 2.10	9.25 ± 2.10
Cisplatin	2.80 ± 1.12	3.20 ± 1.10

Experimental

Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded in potassium bromide using Pye-Unicam SP300 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in deuterated DMSO-d_6 using a Varian Gemini 300 NMR spectrometer (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR) and the chemical shifts were related to that of the solvent DMSO-d_6. Mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV.

Reaction of Acetyl derivatives 1 with Thiosemicarbazied 2.

General procedure: To a stirred solution of 1-(4-fluorophenyl)ethanone (5-acetyl-4-methyl-1,3-thiazol-2-yl)hydrazine **1** (1.0 g, 2.5 mmol) and the appropriate thiosemicarbazone derivative **3** (2.5 mmol) in ethanol (30 mL), was added HCl (0.35 mL) and the mixture was refluxed for 2 h. The filtrate was evaporated under reduced pressure. The residue was triturated with methanol. The solid product, so formed in each case, was collected by filtration, washed with water, dried, and crystallized from dioxane to afford the corresponding carbazone derivative **3**.

1-(2-{2-[1-(4-Fluorophenyl)ethylidene]hydrazino}-4-methyl-1,3-thiazol-5-yl)ethan-1-one thiosemicarbazone 3. Yellow solid, 82% yield; mp. 289-290 °C; IR (KBr): *ν* 3463, 3354 (2NH), 3254, 3190 (NH_2), 3073, 2998 (CH), 1663 (CS), 1594 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 2.10 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 7.52 (br. s, 2H, NH_2), 6.99-7.97 (m, 4H, Ar-H), 11.32 (s, 1H, NH), 11.92 (s, 1H, NH); MS m/z (%): 365 (M^+, 28).Anal. Calcd for $C_{15}H_{17}FN_6S_2$ (364.46): C, 49.43; H, 4.70; N, 23.06. Found: C, 49.70; H, 4.50; N, 23.25 %.

Reaction of Thiosemicarbazone derivatives 3 with Hydrazonoyl chlorides 4a-f and 7a-d and 10a-h.

General procedure: To a stirred solution of thiosemicarbazone derivative **3** (1.0 g, 2.5 mmol) and the appropriate hydrazonoyl chloride **4a-d** or **7a-d** and phenathylbromide **10a-d** (2.5 mmol) in dioxane (30 mL), was added trimethylamine (0.35 mL) and the mixture was refluxed for 5 h. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was evaporated under reduced pressure. The residue was triturated with methanol. The solid product, so formed in each case, was collected by filtration, washed with water, dried, and crystallized from dioxane to afford the corresponding thiazole derivatives **6a-d** or **9a-d**, respectively. The compounds **6a-d** and **9a-d** prepared are listed below together with their physical constants.

2-(2-(1-(4-Fluorophenyl)ethylidene)hydrazinyl)-4-methyl-5-(1-(2-(4-methyl-5-(phenyldiazenyl) thiazol-2-yl)hydrazono)ethyl)thiazole (6a).

Red solid, 70% yield; mp. 220-221 °C; IR (KBr): *ν* 3420, 3221 (2NH), 3034, 2932 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.94 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.83-7.86 (m, 9H, Ar-H), 10.65 (s, 1H, NH), 11.19 (s, 1H, NH); MS m/z (%): 507 (M^+, 34). Anal. Calcd for $C_{24}H_{23}FN_8S_2$ (506.62): C, 56.90; H, 4.58; N, 22.12. Found: C, 57.20; H, 4.40; N, 21.90%.

2-(2-(1-(4-Fluorophenyl)ethylidene)hydrazinyl)-4-methyl-5-(1-(2-(4-methyl-5-(p-tolyldiazenyl) thiazol-2-yl)hydrazono)ethyl)thiazole (6b).

Red solid, 73% yield; mp. 215-216 °C; IR (KBr): v 3431, 3310 (2NH), 3031, 2921 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.92 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 7.30-8.22 (m, 8H, Ar-H), 10.81 (s, 1H, NH), 11.38 (s, 1H, NH); MS m/z (%): 520 (M^+, 37). Anal. Calcd for $C_{25}H_{25}FN_8S_2$ (520.65): C, 57.67; H, 4.84; N, 21.52. Found: C, 60.28; H, 4.68; N, 4.55%.

5-((4-Chlorophenyl)diazenyl)-2-(2-(1-(2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)-4-methyl thiazol-5-yl)ethylidene)hydrazinyl)-4-methylthiazole (6c).

Red solid, 68% yield; mp. 241-242 °C; IR (KBr): *v* 3421, 3320 (2NH), 3020, 2922 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.95 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.12-7.80 (m, 8H, Ar-H), 10.62 (s, 1H, NH), 11.10 (s, 1H, NH); MS m/z (%): 541 (M^+, 49). Anal. Calcd for $C_{24}H_{22}ClFN_8S_2$ (541.06): C, 53.28; H, 4.10; N, 20.71. Found: C, 53.55; H, 3.90; N, 21.60 %.

2-(2-(1-(4-Fluorophenyl)ethylidene)hydrazinyl)-4-methyl-5-(1-(2-(4-methyl-5-((4-nitrophenyl) diazenyl)thiazol-2-yl)hydrazono)ethyl)thiazole (6d).

Red solid, 79% yield; mp. 253-255 °C; IR (KBr): v 3412, 3266 (2NH), 3060, 2910 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.92 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 7.10-8.20 (m, 8H, Ar-H), 11.10 (br s, 2H, 2NH); ^{13}C NMR (DMSO-d_6): δ 8.8, 16.9, 18.9 ($3CH_3$), 56.1, 66.0, 114.9, 126.3, 127.1, 129.0, 130.8, 134.2, 136.1, 141.1, 143.0, 149.3, 150.7, 154.9, 162.5, 168.1; MS m/z (%): 551 (M^+, 46). Anal. Calcd for $C_{24}H_{22}FN_9O_2S_2$ (551.62): C, 52.26; H, 4.02; N, 22.85. Found: C, 52.55; H, 3.75; N, 22.60 %.

2-(2-(1-(2-(2-(1-(4-Fluorophenyl)ethylidene) hydrazinyl)-4-methylthiazol-5-yl)ethylidene) hydrazinyl)-5-(2-phenylhydrazono)thiazol-4(5H)-one (9a).

Orange solid, 74% yield; mp. 233-234 °C; IR (KBr): *v* 3426, 3395, 3181 (3NH), 3050, 2924 (CH), 1684 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.80 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 6.98-8.52 (m, 9H, Ar-H), 10.48 (s, 1H, NH), 11.51 (s, 1H, NH), 12.20 (s, 1H, NH); MS m/z (%): 508 (M^+, 43). Anal. Calcd for $C_{23}H_{21}FN_8OS_2$ (508.59): C, 54.32; H, 4.16; N, 22.03. Found: C, 54.55; H, 3.98; N, 21.80 %.

2-(2-(1-(2-(2-(1-(4-Fluorophenyl)ethylidene) hydrazinyl)-4-methylthiazol-5-yl)ethylidene) hydrazinyl)-5-(2-(p-tolyl)hydrazono)thiazol-4(5H)-one (9b).

Orange solid, 64% yield; mp. 158-160 °C; IR (KBr): *v* 3430, 3274, 3191 (3NH), 3051, 2918 (CH), 1685 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.80 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.04-8.01 (m, 8H, Ar-H), 10.31 (s, 1H, NH), 10.80 (s, 1H, NH), 12.11 (s, 1H, NH); MS m/z (%): 522 (M^+, 37). Anal. Calcd for $C_{24}H_{23}FN_8OS_2$ (522.62): C, 55.16; H, 4.44; N, 21.44. Found: C, 55.35; H, 4.20; N, 21.15 %.

5-(2-(4-Chlorophenyl)hydrazono)-2-(2-(1-(2-(2-(1-(4-fluorophenyl)ethylidene) hydrazinyl)-4-methyl thiazol-5-yl)ethylidene)hydrazinyl)thiazol-4(5H)-one (9c).

Orange solid, 68% yield; mp. 198-199 °C; IR (KBr): *v* 3427, 3255, 3178 (3NH), 3060, 2926 (CH), 1684 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.86 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.05-50 (m, 8H, Ar-H), 10.55 (s, 1H, NH), 10.88 (s, 1H, NH), 12.10 (s, 1H, NH); MS m/z (%): 543 (M^+, 42). Anal. Calcd for $C_{23}H_{20}ClFN_8OS_2$ (543.04): C, 50.87; H, 3.71; N, 20.64. Found: C, 51.02; H, 3.50; N, 20.45 %.

2-(2-(1-(2-(2-(1-(4-Fluorophenyl)ethylidene)hydrazinyl)-4-methy lthiazol-5-yl)ethylidene)hydrazinyl)-5-(2-(4-nitrophenyl)hydrazono)thiazol-4(5H)-one (9d).

Orange solid, 77% yield; mp. 200-202 °C; IR (KBr): *v* 3429, 3210, 3180 (3NH), 3050, 2920 (C-H) 1701 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.92 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 7.38-8.45 (m, 8H, Ar-H), 10.24 (s, 1H, NH), 11.11 (s, 1H, NH), 12.34 (s, 1H, NH); MS m/z (%): 554 (M^+, 20). Anal. Calcd for $C_{23}H_{20}FN_9O_3S_2$ (553.59): C, 49.90; H, 3.64; N, 22.77. Found: C, 50.15; H, 3.40; N, 22.55 %.

5-(1-(2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazon o)ethyl)-2-(2-(1-(4-fluorophenyl) ethylidene)hydrazinyl)-4-methylthiazole (12a).

Green solid, 76% yield; mp. 196-197 °C; IR (KBr): *v* 3421, 3250 (2NH), 3030, 2938 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.95 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.39-7.86 (m, 8H, Ar-H), 7.33 (s, 1H, thiazole-H), 11.55 (br s, 2H, 2NH); MS m/z (%): 499 (M^+, 54). Anal. Calcd for $C_{23}H_{20}ClFN_6S_2$ (499.02): C, 55.36; H, 4.04; N, 16.84. Found: C, 55.55; H, 3.85; N, 16.65 %.

5-(1-(2-(4-(4-Bromophenyl)thiazol-2-yl)hydrazono)ethyl)-2-(2-(1-(4-fluorophenyl)ethylidene) hydrazinyl)-4-methylthiazole (12b).

Green solid, 83% yield; mp. 198-201 °C; IR (KBr): *v* 3490, 3385 (2NH), 3072, 2918 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): *δ* 1.82 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.38-8.35 (m, 8H, Ar-H), 7.80 (s, 1H, thiazole-H), 11.40 (s, 1H, NH), 12.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 8.9, 18.8, 20.2 ($3CH_3$), 56.5, 66.7, 115.8, 116.5, 125.5, 126.8, 127.7, 129.4, 129.1, 130.9, 134.8, 143.3, 153.3, 158.4, 163.3, 167.4 (Ar-H); MS m/z (%): 543 (M^+, 67). Anal. Calcd for

$C_{23}H_{20}BrFN_6S_2$ (543.48): C, 50.83; H, 3.71; N, 15.46. Found: C, 51.15; H, 3.50; N, 15.15 %.

2-(2-(1-(4-Fluorophenyl)ethylidene)hydrazinyl)-4-methyl-5-(1-(2-(4-(p-tolyl)thiazol-2-yl)hydrazono)ethyl)thiazole (12c).

Green solid, 69% yield; mp. 235-236 ºC; IR (KBr): *v* 3429, 3220 (2NH), 3063, 2920 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.82 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.27-8.13 (m, 8H, Ar-H), 8.30 (s, 1H, thiazole-H), 10.90 (br s, 2H, 2NH); MS m/z (%): 478 (M^+, 40). Anal. Calcd for $C_{24}H_{23}FN_6S_2$ (478.61): C, 60.23; H, 4.84; N, 17.56. Found: C, 60.44; H, 4.55; N, 17.25 %.

2-(2-(1-(4-Fluorophenyl)ethylidene)hydrazinyl)-5-(1-(2-(4-(4-methoxyphenyl) thiazol-2-yl)hydrazono)ethyl)-4-methylthiazole (12d).

Green solid, 71% yield; mp. 249-250 ºC; IR (KBr): *v* 3430, 3330 (2NH), 3110, 2938 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.47 (s, 3H, OCH_3), 7.45-8.36 (m, 8H, Ar-H), 7.72 (s, 1H, thiazole-H), 11.52 (br s, 2H, 2NH); MS m/z (%): 494 (M^+, 41). Anal. Calcd for $C_{24}H_{23}FN_6OS_2$ (494.61): C, 58.28; H, 4.69; N, 16.99. Found: C, 58.45; H, 4.55; N, 16.70 %.

Anticancer Activity

Evaluation of Cytotoxic Effects of certain Chemical compound

Mammalian cell line: HCT-116 cells (human Colon carcinoma) were obtained from VACSERA Tissue Culture Unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA).

Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH_2O and filtered through a Whatmann No.1 filter paper.

Cell line Propagation:

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were sub-cultured two times a week.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get 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 conc. using Graphpad Prism software (San Diego, CA. USA).

Conclusion

In our present work, we herein present an efficient synthesis of novel 1-(2-(2-benzylidenehydrazinyl)-4-methylthiazol-5-yl)ethenone **3**. The latter compound was used as a building block for constructing novel three series of 5-(1-(2-(thiazol-2-yl)hydrazono)ethyl) thiazole derivative. 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 tow

tumor cells (HCT-116 and HepG2) were investigated in comparison with cisplatin reference drugs and the results revealed promising activities of three compounds. The study also suggested that the mechanism of the anticancer action exerted by most active compounds (**9c, 12d** and **6c**) inside HCT-116 and (**6c, 12d, 6b** and **9b**) inside HepG-2 cells.

References

1. Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A., Global cancer statistics. *CA Cancer J. Clin.* ***65***, 87-108 (2015).
2. Bhandari A., Woodhouse M., Gupta S., Colorectal cancer is a leading cause of cancer incidence and mortality among adults younger than 50 years in the USA: A SEER-based analysis with comparison to other young-onset cancers. *J. Investig. Med*., **65**, 311-315 (2017).
3. American Cancer Society. Cancer Facts & Figures 2012. Atlanta: American Cancer Society; 2012.
4. Siegel R.L., Jemal A., Ward E.M., Increase in incidence of colorectal cancer among young men and women in the United States. *Cancer Epidemiol Biomarkers Prev.* **18**, 1695-1698 (2009).
5. Haggar F.A., Boushey R.P., Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. *Clin. Colon Rectal. Surg.* **22**, 191-197 (2009).
6. World Cancer Research Fund and American Institute for Cancer Research Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research; 2007.
7. Twelves C., Boyer M., Findlay M., Cassidy J., Weitzel C., Barker C., Osterwalder B., Jamieson C., Hieke K., Xeloda., Colorectal Cancer Study Group. Capecitabine (Xeloda™) improves medical resource use compared with 5-fluorouracil plus leucovorin in a phase III trial conducted in patients with advanced colorectal carcinoma. *Eur. J. Cancer*, **37**, 597-604 (2001).
8. Scheithauer W., McKendrick J., Begbie S., Borner M., Burns W.I., Burris H.A., et al. Oral capecitabine as an alternative to iv 5-fluorouracil-based adjuvant therapy for colon cancer: safety results of a randomized, phase III trial. *Ann. Oncol.*, **14**, 1735-1743 (2003).
9. da Silva E.B., Silva D.A.O., Oliveira A.R., da Silva Mendes C.H., dos Santos T.A. .R, da Silva A.C., et al. Desing and synthesis of potent anti-*Trypanosoma cruzi* agents new thiazoles derivatives which induce apoptotic parasite death. *Eur. J. Med. Chem.*, **130**, 39-50 (2017).
10. Espíndola J.W.P., de Oliveira Cardoso M.V., de Oliveira Filho G.B., e Silva D.A.O., Moreira D.R.M., Bastos T.M. et al., Synthesis and structure–activity relationship study of a new series of antiparasitic aryloxyl thiosemicarbazones inhibiting *Trypanosoma cruzi cruzain*. *Eur. J. Med. Chem.*, **101**, 818-835 (2015).
11. Netalkar P.P., Netalkar S.P., Revankar V.K., Transition metal complexes of thiosemicarbazone: Synthesis, structures and in vitro antimicrobial studies. *Polyhedron*, **100,** 215-222 (2015).
12. Hu W.X, Zhou W., Xia C.N., Wen X., Synthesis and anticancer activity of thiosemicarbazones. *Bioorg. Med. Chem. Lett.*, **16,** 2213-2218 (2006).
13. Wang Y., Wang Z., Kuang H., Zhang Y., Gu W., Zhu Y., Wang S., Synthesis and antitumor activity of 2-isocamphanyl thiosemicarbazone derivatives via ROS-enhanced mitochondria damage. *Chem. Biol. Drug Des*. 2019; https://doi.org/10.1111/cbdd.13492.
14. Sujatha K., Vedula R.R., Novel one-pot expeditious synthesis of 2, 4-disubstituted thiazoles through a three-component reaction under solvent free conditions. *Synth.Commun.*, **48**, 302-308 (2018).
15. Nayak S., Gaonkar S.L., A Review on recent synthetic strategies and pharmacological importance of 1, 3-thiazole derivatives. *Mini Rev. Med. Chem.*, **19**, 215-238 (2019).
16. Kumar S., Aggarwal R., Thiazole: A Privileged Motif in Marine Natural Products. *Mini Rev. Org. Chem.*, **16**, 26-34 (*2019)*.
17. dos Santos Silva T.D., Bomfim L.M., da Cruz Rodrigues A.C.B., Dias R.B., Sales C.B.S., Rocha C.A.G., et al. Anti-liver cancer activity in vitro and in vivo induced by 2-pyridyl 2, 3-thiazole derivatives. *Toxicol. Appl. Pharmacol*., **329**, 212-223 (2017).
18. Morigi R., Locatelli A., Leoni A., Rambaldi M., Recent patents on thiazole derivatives endowed with antitumor activity. *Recent Pat. Anticancer Drug Discov*., **10**, 280-297 (2015).
19. Bremner W.S., Organ M.G., Multicomponent reactions to form heterocycles by microwave-assisted continuous flow organic synthesis. *J. Comb. Chem.*, **9**, 14-16 (2007).
20. 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.*, **14**, 2296-2299 (2012).
21. De Logu A., An investigation of the biological effect of structural modifications of isothiosemicarbazones and their cyclic analogues. *Il Farmaco*., **58**, 951-959 (2003).