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SYNTHESIS OF NEW 6-[4-(2-FLUOROPHENYLPIPERAZINE-1-YL)]-3(*2H*)-PYRIDAZINONE-2-ACETHYL-2-(SUBSTITUTEDBENZAL)HYDRAZONE DERIVATIVES AND EVULATION OF THEIR CYTOTOXIC EFFECTS IN LIVER AND COLON CANCER CELL LINES

Zeynep Özdemir,[1,*] Neşe Başak-Türkmen,[2] İdris Ayhan,[3] Osman Çiftçi,[4] and Mehtap Uysal[5]

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In this study, seven new 3(2*H*)-pyridazinone derivatives expected to show cytotoxic activity in liver and colon cancer cell lines were synthesized. Their structures were confirmed by the IR, ^{1}H-NMR, ^{13}C-NMR spectra and elementary analyses. Compunds $\mathbf{V_1}$-$\mathbf{V_7}$ were tested on HEP3B (liver cancer) and HTC116 (colon cancer) cell lines for cytotoxicity by using MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] proliferation assay. Human fibroblast cells were used as safety control in these tests. 6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(2-chlorobenzal)hydrazone (compound $\mathbf{V_3}$) was the most active agent with respect to HEP3B and HTC116 cell lines.

Keywords: pyridazinone; cytotoxicity; liver cancer; colon cancer.

Abbreviations: HEP3B, liver cancer cell line; HTC116, colon cancer cell line; MTS, [3-(4,5-dimethylthi-azol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2H-tetrazolium]; IC50, half maximum inhibitory concentration; HF, human fibroblast cell line; SHSY5Y, neuroblastoma cell line; TLC, thin layer chromatography; ATR, attenuated total reflection; MM, molecular mass; MP, melting point.

1. INTRODUCTION

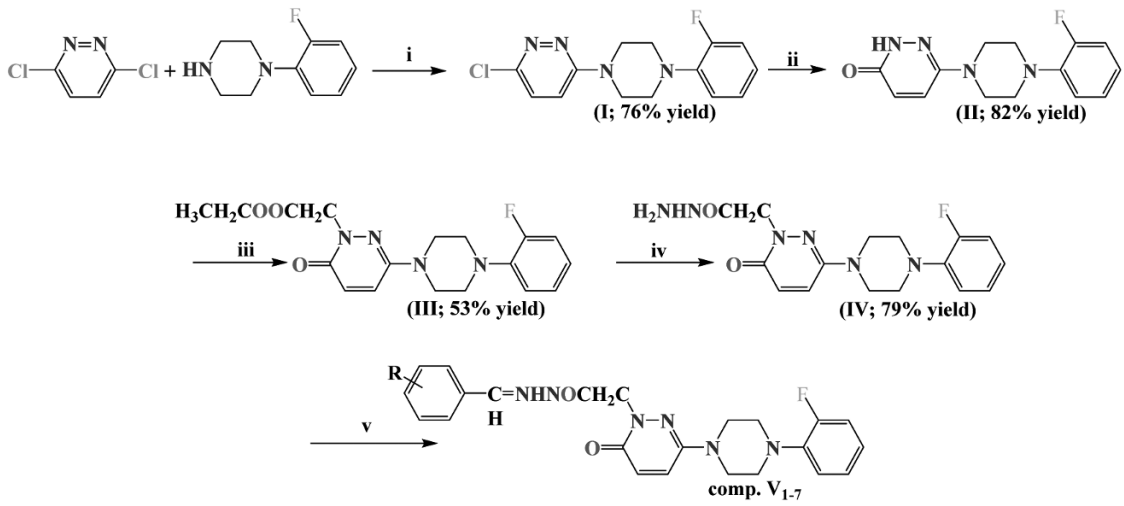
Cancer, one of the most important problems of today's medicine, is a disease with invasive features characterized by uncontrolled proliferation of cells. This disease that changes according to the tissue from which it was born is the second most common cause of death in the world today after cardiovascular disease [1 – 3]. It is also seen as an important social problem with the social and economic burden caused by the incidence of cancer [4 – 6].

Hepatocellular carcinoma, which is responsible for the death of more than 500,000 people per year and is associated with many etiological factors such as aflatoxin, hepatitis C and B viruses, is the most common primary carcinoma in the world. Colon tumors constitute 1% of all cancers and it is the third most common cause of cancer-related death among people aged 15-34 [7, 8].

3(2*H*)-Pyridazinone ring is a six-membered lactam ring system with $C_4H_4N_2O$ closed formula [9]. It is known that the tautomeric balance is due to the presence of free hydrogen in the nitrogen atom in the 3(2*H*)-pyridazinone derivatives having no substituent at second position [10]. Substituent-bearing derivatives of the pyridazinone ring, which is known to be aromatic in the ring nitrogen atom, are weakly acidic and they form salts with strong bases or with ammonia and amines [11].

In recent years, many pharmacological activity studies have been carried out with compounds bearing the 3(2*H*)-pyridazinone structure and showed that these compounds have analgesic, anti-inflammatory, antipyretic, antihypertensive, antiulcer, antioxidant, antiallergic, bronchospasmolytic, antibacterial, antifungal, and anthelmintic properties [12 – 20]. Some compounds were reported in literature to have anticancer activity due to 3(2*H*)-pyridazinone structure [18,

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Inonu University, 44280 Malatya, Turkey.
[2] Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Inonu University, 44280 Malatya, Turkey.
[3] Department of Medical Pharmacology, Faculty of Medicine, Yıldırım Beyazıt University, 06100 Ankara, Turkey.
[4] Department of Medical Pharmacology, Faculty of Medicine, Pamukkale University, 20160 Denizli, Turkey.
[5] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06100 Ankara, Turkey.
[*] e-mail: zeynep.bulut@inonu.edu.tr; zpozdmr@gmail.com



Scheme 1. Synthesis pathway of 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-acetyl-2-(substituted/nonsubstituted benzal)-hydrazone derivatives.

19, 21]. It was also reported that pyridazinones carrying substituents at fourth, fifth and sixth positions and containing phenyl groups attached to the ring nitrogen exhibit cytostatic activity [14, 16, 17]. These results suggest that pyridazinone compounds may be useful in cancer chemotherapy, depending on the type of cancer, and that derivatives bearing different substituents may exhibit varying degrees of cytotoxic effect.

This study was aimed to investigate the antiproliferative effects of seven newly synthesized 3(2*H*)-pyridazinone derivatives with respect to HEP3B, HTC116, and human fibroblast (HF) cell lines. The selective killing of cancer cells by test compounds without damaging healthy cells is very important in terms of chemotherapy. Therefore, cytotoxic effects of newly synthesized pyridazinone compounds on cancer cell lines were investigated in comparison to healthy HF cells.

2. RESULTS

2.1.Chemistry

Compounds $\mathbf{V_1}$-$\mathbf{V_7}$ were synthesized according to the literature methods as outlined in Scheme 1.

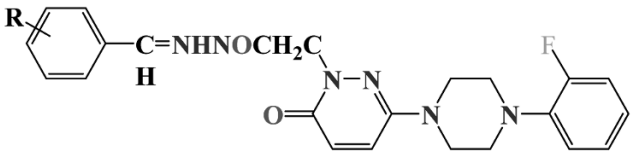
Target compounds have been synthesized starting from nucleophilic displacement reaction of commercial 3,6-dichloropyridazine with (2-fluorophenyl)piperazine in ethanol afforded 3-chloro-6-[4-(2-fluorophenyl)piperazine-1-yl]pyridazine. The physical and spectral properties of 3-chloro-6-[4-(2-fluorophenyl)piperazine-1-yl]pyridazine were in accordance with the literature [22]. Then, 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone was obtained as a result of hydrolysis of 3-chloro-6-substituted pyridazine by heating in glacial acedic acide [22]. The formation of these compounds was confirmed by the IR spectra with C=O signal at about 1660 cm^{-1}. Ethyl 6-(4-(2-fluorophenyl)piperazin-1-yl)-3(2*H*)-pyridazinone-2-ylacetate was obtained by the reaction of 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone with ethyl bromoacetate in the presence of K_2CO_3 in acetone [22]. The formation of these compounds was also confirmed by the IR spectra containing an ester C=O signal at about 1750 cm^{-1}. 6-(4-(2-Fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-ylacetohydrazide was synthesized by the condensation reaction of ethyl 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-ylacetate with hydrazine hydrate (99%) [22]. Eventually, the title compounds which have benzalhydrazone structure were obtained by the condensation reaction of 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(*2H*)-pyridazinone-2-ylacetohydrazide with substituted/nonsubstituted benzaldehydes. All these compounds are reported in this article for the first time. Molecular structures of new compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and LC/MS/MS spectral data and elementary analyses. Molecular structures, yields, melting points, molecular weights, and molecular formulas of compounds $\mathbf{V_1}$-$\mathbf{V_7}$ are given in Table I.

2.2.Pharmacological Activity

Cytotoxicty and cell viability

In this study, the cytotoxic effects of seven newly synthesized pyridazinone derivatives on HTC116 and HEP3B cancer cell lines were studied in comparison to the HF cell line. Six of these compounds did not show any toxic effect against HF cells as compared to cancer cells. Compound $\mathbf{V_3}$ is the most active one in HEP3B and HTC116 cell line, but it has not been considered as candidate anticancer drug since it also causes high mortality in HF cells. Data on the cell viability and IC_{50} of eight pyridazinone compound are presented in Fig. 1 and Table 2.

TABLE 1. Molecular structures, yields, melting points, molecular weights, and molecular formulas of compounds V_1-V_7



Compound	R	Yield (%)	MP	MM	Molecular formula
V_1	-H	79.90	226 – 228	434.48	$C_{23}H_{23}FN_6O_2$
V_2	-4CH_3	78.15	198 – 200	448.49	$C_{24}H_{25}FN_6O_2$
V_3	-2Cl	73.19	203 – 205	468.91	$C_{23}H_{22}ClFN_6O_2$
V_4	-4Cl	91.21	192 – 194	468.91	$C_{23}H_{23}ClFN_6O_2$
V_5	-4Br	85.69	209 – 211	513.36	$C_{23}H_{22}BrFN_6O_2$
V_6	-2CH_3O	77.45	239 – 241	464.49	$C_{24}H_{25}FN_6O_3$
V_7	-4$N(CH_3)_2$	68.77	237 – 239	477.53	$C_{25}H_{28}FN_7O_2$

3. DISCUSSION

Nowadays, investigation of potentially safer new anticancer agents is the focus of researchs in the area. Pyridazinone derivatives have been previously synthesized by our research group and the relationship between structure and anticancer activity has been investigated. Apoptosis and gene expression studies were carried out in order to elucidate the mechanism of action of the five most active compounds in these studies, in which a series of compounds with nonsubstitued phenyl and 4-chlorophenyl bound to the piperazine ring at sixths position of the pyridazinone ring were synthesized. According to the results of our previous study, the IC_{50} values of all compounds were lower than IC_{50} values for the HF cell line, and the substitution on the meta position of the benzalhydrazone phenyl ring at second position of 3(2*H*)-pyridazinone with strong electron-donating group such as -OCH_3 improved the cytotoxic effect in HEP3B cell line. In terms of IC_{50}, it was found that compound 4 was effective at lower concentrations for both SHSY5Y (24.3 μM) and HEP3B cells (23.2 μM) in our previous study [23].

In this study, which we have designed to develop more active and less toxic compounds, the structure-activity relationship has focused on the effect of substituent at the phenyl groups at second position of 3(2*H*)-pyridazinone ring. There were more halogen groups than previously, and these compounds caused higher cell death in cancer cell lines as well as higher damage to healthy cells. The compounds which have a halogen atom at the phenyl ring caused a further decrease of cell viability on L929 cell line as compared to other compounds. The pyridazinone derivative **V_2**, in which the methyl substituent was used, such as compound **4**, which was the most active compound in the previous study, did not cause a decrease in the number of cancerous cells but causes a decrease in the number of healthy cells. As a result of this study, it was found that the cell viability was decreased with **V_3**, **V_4** and **V_5** treatment in both HEP3B and HTC116 cells lines, when different concentrations were applied.

It is suggested that the synthesized pyridazinone derivatives may be used in the development of new chemotherapeutic agents, provided that the compounds are modified to increase their selectivity to cancer cells in order to reduce the toxicity.

4. EXPERIMENTAL

4.1.Chemicals and Methods

All chemicals used in this study were purchased from Aldrich, Fluka AG, and E. Merck. 3-Chloro-6-substituted pyridazines, 3-chloro-6-(4-(2-fluorophenyl)piperazine-1-yl)-pyridazine, 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone, ethyl 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-yl acetate and 6-(4-(2-fluorophenyl)-piperazine-1-yl)-3(2*H*)-pyridazinone-2-yl acetohydrazide

TABLE 2. The IC_{50} Values of Pyridazinone Dertvatives V_1-V_7

	IC_{50} (μM)						
Cell line	V_1	V_2	V_3	V_4	V_5	V_6	V_7
HEP3B	111,8	150,5	27,7	62,7	66,5	94,7	103,4
HTC116	110,8	110,8	32,7	65,8	62,2	110,6	105,9
HF	130,2	35,3	27,05	26,7	26,9	120,6	102,6

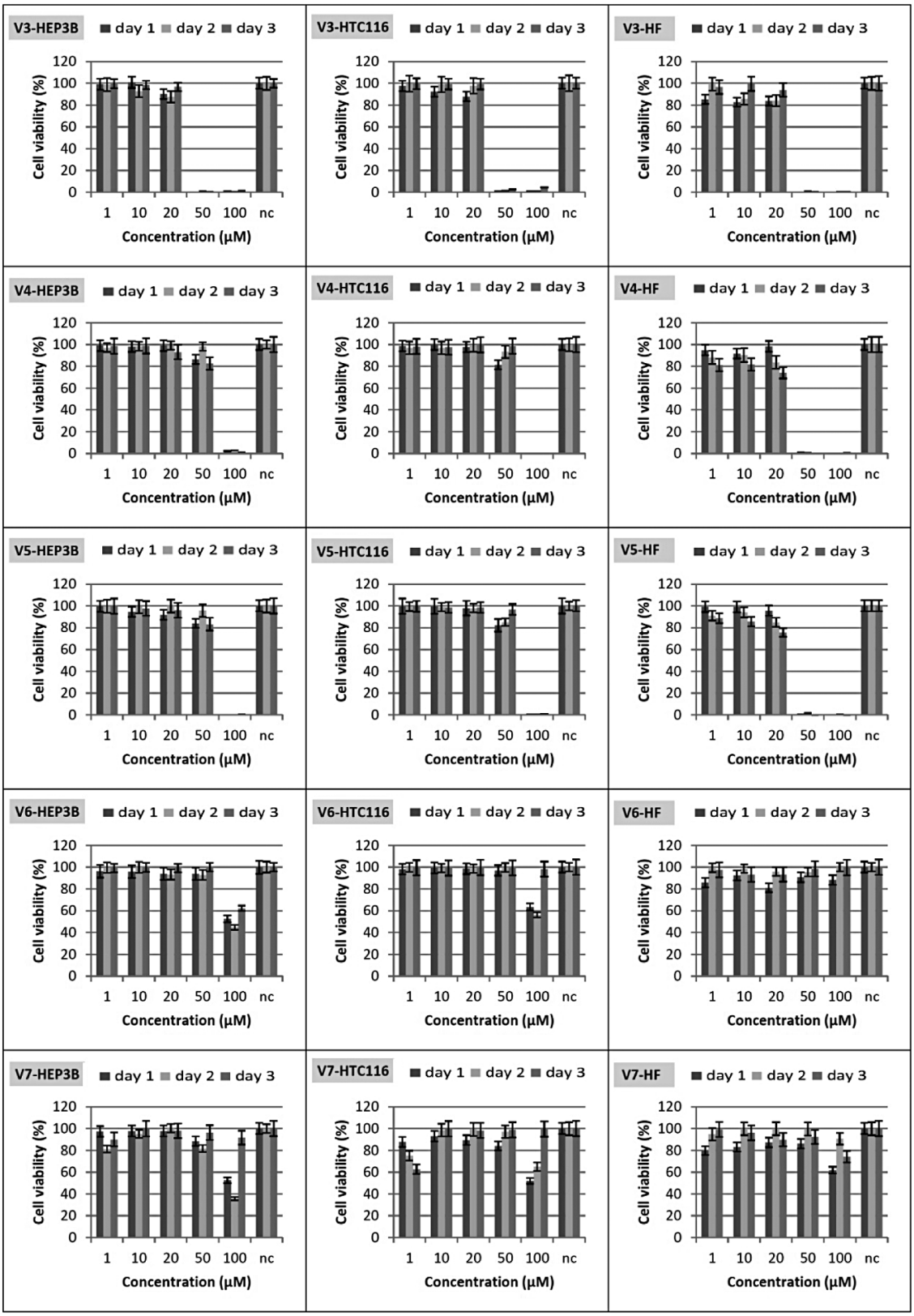


Fig. 1. The effect of compounds $\mathbf{V_3}$-$\mathbf{V_7}$ on cell viability: (1) cells were seeded in 96-well flat-bottomed tissue culture plates at a concentration of 5×10^3 cells/well; (2) cells were treated with DMEM medium containing various concentrations (25, 50, 100, 200, and 300 µM) of the test compounds for different incubation periods (24, 48 and 72 h); (3) fresh complete medium containing 10 µL of MTS solution was added at the end of the each time point, and incubated 2 h in incubator; (4) cell proliferation was assessed by measuring the absorbance with an ELISA microplate reader.

were synthesized according to literature methods [14, 17, 24, 25]. The purity of all compounds was checked by TLC with Merck Kieselgel F_{254} plates. Melting points were determined on Electrothermal 9200 melting points apparatus and the obtained values are uncorrected. IR spectra were recorded on a Perkin Elmer spectrometer by ATR technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance Ultrashield FT-NMR spectrometer in DMSO at 600 MHz and 75 MHz, respectively. Mass spectra (LC/MS-MS) were obtained using Agilent 6400 Series Triple Quadrupole B.08.00 (B8023.0). Elemental analyses were performed with Leco CHNS-932 instrument in the Inonu University (Malatya).

4.2.Synthesis of Intermediate Compounds

3-Chloro-6-[4-(2-fluorophenyl)piperazine-1-yl]pyridazine (I). 0.01 mol of 3,6-dichloropyridazine and 0.01 mol of (2-fluorophenyl)piperazine are stirred in 15 mL of ethanol under reflux with heating for 6 h according to the literature procedure. The reaction medium is poured into ice-cold water; the precipitate obtained by filtration is purified by crystallization from ethanol. The melting points of non-original compounds were in accordance with the literature data [22].

6-(4-(2-Fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone (II*).* A solution of 0.05 mol of 3-chloro-6-[4-(2-fluorophenyl)piperazine-1-yl]pyridazine in 30 mL glacial acetic acid was refluxed for 6 h. Acetic acid was removed under reduced pressure; the residue was dissolved in water and extracted with chloroform. The organic phase was dried over sodium sulphate and evaporated under reduced pressure. The residue was purified by recrystallization from ethanol. The melting points of non-original compounds were in accordance with the literature [22].

Ethyl 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-ylacetate (III). A mixture of 0.01 mol 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone, 0.02 mol ethyl bromoacetate, and 0.02 mol potassium carbonate in acetone (40 mL) was refluxed overnight. After the mixture was cooled, the organic salts were filtered off, the solvent evaporated, and the residue was purified by recrystallization from n-hexane to give the esters. The melting points of non-original compounds were in accordance with the literature [22].

6-(4-(2-Fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-ylacetohydrazide (IV). Hydrazine hydrate (99%, 3ml) was added to the solution of 0.01 mol ethyl 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-ylacetate in 25 mL methanol and stirred for 3 h at room temperature. The precipitate obtained was filtered off, washed with water, dried, and recrystallized from ethanol. The melting points of non-original compounds were in accordance with the literature [22].

4.3.General procedure for the synthesis of title compounds V_1-V_7

0.01 mol 6-(4-(2-fluorophenyl)piperazine-1-yl)-3(2*H*)-pyridazinone-2-ylacetohydrazide and 0.01 mol substituted/nonsubstituted benzaldehyde were stirred in ethanol (15 mL) and refluxed for 6 h. At the and of the reaction, the mixture was poured into ice water. The precipitate was filtered, dried and crystallized from methanol-water mixture.

All spectral data of the seven title compounds were in accordance with the assigned structures as shown below.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-benzalhydrazone (V_1). White crystals (methanol/water); yield 79.90%; m.p. 226 – 228°C; IR (ν, cm^{-1}, ATR): 3061 (C-H aromatic), 2853 (C-H aliphatic), 1693, 1651 (C=O), 1571 (C=N), 1239 (C-N), 1069 (C-O), 846 (C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 3.12 (4H; t; CH_2N; b+b′), 3.38 (4H; t; CH_2N; a+a′), 5.06 (2H; s; CH_2CO), 6.92 (1H; d; J = 4.15 Hz, pyridazinone H^5), 6.94 (1H; d; J = 4.16 Hz, pyridazinone H^4), 7.07 – 8.02 (9H; m; phenyl protons), 8.72 (1H; s; -N=CH-) and 11.65 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), δ 46.61 (2C, CH_2-N; b+b′), 50.13 (2C, CH_2-N; a+a′), 53.00 (1C; -N-$\underline{C}H_2$-C=O), 116.53 (1C; =CH), 119.89 (1C; pyridazinone C^5), 123.14 (1C; phenyl C^6), 125.35 (1C; phenyl C^2), 126.96 (1C; phenyl C^3), 127.36 (1C; phenyl C^5), 128.84 (1C; phenyl C^4), 129.30 (1C; pyridazinone C^4), 130.44 (1C; 2-fluorophenyl C^5), 131.01 (1C; 2-fluorophenyl C^4), 134.43 (1C; 2-fluorophenyl C^6), 140.04 (1C; 2-fluorophenyl C^5), 144.34 (1C; 2-fluorophenyl C^1), 147.44 (1C; phenyl C^1), 149.03 (1C; pyridazinone C^6), 154.63 (1C; 2-fluorophenyl C^2), 158.26 (1C; CH_2-N-$\underline{C}$=O) and 168.45 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 433.0 $[M]^+$; Anal. Calcd. for $C_{23}H_{23}FN_6O_2.1/2\ H_2O$: C, 62.29; H, 5.46; N, 18.95. Found: C, 62.46; H, 5.579; N, 18.69 %.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(4-methylbenzal)hydrazone (V_2). White crystals (methanol/water); yield 78.15%; m.p. 198 – 200°C; IR (ν, cm^{-1}, ATR): 3034 (C-H aromatic), 2851 (C-H aliphatic), 1651 (C=O), 1583 (C=N), 1240 (C-N), 1081 (C-O), 846 (C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 2.34 (3H; s; -CH_3), 3.11 (4H; t; CH_2N; b+b′), 3.38 (4H; t; CH_2N; a+a′), 5.05 (2H; s; CH_2CO), 6.99 (1H; d; J = 4.19 Hz, pyridazinone H^5), 7.01 (1H; d; J = 4.21 Hz, pyridazinone H^4), 7.06 – 7.98 (8H; m; phenyl protons), 8.67 (1H; s; -N=CH-) and 11.57 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), δ 21.48 (1C; -CH_3), 46.61 (2C; CH_2-N; b+b′), 50.11 (2C, CH_2-N; a+a′), 53.00 (1C; -N-$\underline{C}H_2$-C=O), 116.53 (1C; =CH), 119.89 (1C; pyridazinone C^5), 123.19 (1C; 4-methylphenyl C^6), 125.36 (1C; 4-methylphenyl C^2), 126.95 (1C; 4-methylphenyl C^3), 127.34 (1C; 4-methylphenyl C^5), 127.55 (1C; 4-methylphenyl C^4), 128.79 (1C; pyridazinone C^4), 130.00 (1C; 2-fluorophenyl C^3), 131.01 (1C; 2-fluorophenyl C^4), 131.74 (1C; 2-fluorophenyl C^5), 140.25 (1C; 4-methylphenyl C^1), 144.40 (1C; 2-fluorophenyl C^6), 149.01 (1C; 2-fluorophenyl C^1), 154.63 (1C; 2-fluorophenyl

C^2), 156.25 (1C; pyridazinone C^6) 158.25 (1C; CH_2-N-C=O) and 168.33 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 447.0 $[M]^+$; Anal. Calcd. for $C_{24}H_{25}FN_6O_2.1/3\ H_2O$: C, 63.42; H, 5.69; N, 18.49. Found: C, 63.66; H, 5.708; N, 18.26%.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(2-chlorobenzal)hydrazone (V_3). White crystals (methanol/water); yield 73.19%; m.p. 203 – 205°C; IR (ν, cm^{-1}, ATR): 3175 (C-H aromatic), 2851 (C-H aliphatic), 1690, 1656 (C=O), 1585 (C=N), 1242 (C-N), 881, 835 (C-Cl, C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 2.51 (4H; t; CH_2N; b+b′), 3.11 (4H; t; CH_2N; a+a′), 5.06 (2H; s; CH_2CO), 6.91 (1H; d; J = 4.15 Hz, H^5), 6.93 (1H; d; J = 4.16 Hz, H^4), 6.99 – 7.91 (8H; m; phenyl protons), 8.72 (1H; s; -N=CH-) and 11.71 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), δ 46.47 (2C, N-(CH_3)$_2$), 46.60 (2C, CH_2N; b+b′), 50.11 (2C, CH_2N; a+a′), 52.98 (1C; -N-CH_2-C=O), 116.39 (1C; =CH), 119.88 (1C; pyridazinone C^5), 123.14 (1C; 2-chlorophenyl C^6), 125.33 (1C; 2-chlorophenyl C^5), 126.97 (1C; 2-chlorophenylphenyl C^4), 129.03 (1C; 2-fluorophenyl C^5), 129.20 (1C; 2-fluorophenyl C^4), 129.57 (1C; pyridazinone C^4), 130.50 (1C; 2-fluorophenyl C^6), 131.05 (1C; 2-fluorophenyl C^3), 133.40 (1C; 2-chlorophenyl C^3), 140.09 (1C; 2-fluorophenyl C^1), 143.05 (1C; 2-chlorophenyl C^1), 146.14 (1C; 2-chlorophenyl C^2), 149.03 (1C; pyridazinone C^6), 156.24 (1C; 2-fluorophenyl C^2), 161.08 (1C; CH_2-N-C=O) and 168.53 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 467.0 $[M]^+$; 469.0 $[M+2]^+$; Anal. Calcd. for $C_{23}H_{22}ClFN_6O_2.2/3\ H_2O$: C, 57.44; H, 4.89; N, 17.47. Found: C, 57.48; H, 5.047; N, 17.06%.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(4-chlorobenzal)hydrazone (V_4). White crystals (methanol/water); yield 91.21%; m.p. 192 – 194°C; IR (ν, cm^{-1}, ATR): 3044 (C-H aromatic), 2852 (C-H aliphatic), 1689, 1656 (C=O), 1584(C=N), 1242(C-N), 834, 813 (C-Cl, C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 2.51 (4H; t; CH_2N; b+b′), 3.10 (4H; t; CH_2N; a+a′), 5.06 (2H; s; CH_2CO), 6.99 (1H; d; J = 4.19 Hz, pyridazinone H^5), 7.00 (1H; d; J = 4.20 Hz, pyridazinone H^4), 7.06 – 7.91 (8H; m; phenyl protons), 8.72 (1H; s; -N=CH-) and 11.71 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), δ 46.60 (2C, CH_2-N; b+b′), 50.11 (2C, CH_2-N; a+a′), 52.99 (1C; -N-CH_2-C=O), 116.39 (1C; =CH), 119.87 (1C; pyridazinone C^5), 123.19 (1C; 2-fluorophenyl C^4), 125.33 (1C; 4-chlorophenyl C^5), 127.96 (1C; 2-fluorophenyl C^5), 129.35 (1C; 4-chlorophenyl C^2), 131.00 (1C; 4-chlorophenyl C^6), 133.39 (1C; pyridazinone C^4), 134.85 (1C; 2-fluorophenyl C^6), 136.51 (1C; 4-chlorophenyl C^3), 140.03 (1C; 2-fluorophenyl C^3), 143.05 (1C; 4-chlorophenyl C^1), 149.03 (1C; 2-fluorophenyl C^1), 154.62 (1C; 4-chlorophenyl C^4), 156.24 (1C; pyridazinone C^6), 158.25 (1C; 2-fluorophenyl C^2), 163.87 (1C; CH_2-N-C=O), 168.53 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 466.9 $[M]^+$, 468.2 $[M+2]^+$; Anal. Calcd. for $C_{23}H_{22}ClFN_6O_2.1/3\ H_2O$: C, 58.17; H, 4.81; N, 17.70. Found: C, 58.36; H, 4.949; N, 17.82%.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(4-bromobenzal)hydrazon (V_5). White crystals (methanol/water); yield 85.69%; m.p. 209 – 211°C; IR (ν, cm^{-1}, ATR): 3023 (C-H aromatic), 2849 (C-H aliphatic), 1690, 1657 (C=O), 1584 (C=N), 1241 (C-N), 1068 (C-O), 834, 812 (C-Br, C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 2.51 (4H; t; CH_2N; b+b′), 3.10 (4H; t; CH_2N; a+a′), 5.06 (2H; s; CH_2CO), 6.91 (1H; d; J = 4.14 Hz, pyridazinone H^5), 6.94 (1H; d; J = 4.16 Hz, pyridazinone H^4), 6.99 – 7.99 (8H; m; phenyl protons), 8.70 (1H; s; -N=CH-) and 11.71 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), δ 46.60 (2C, CH_2-N; b+b′), 50.11 (2C, CH_2-N; a+a′), 52.99 (1C; -N-CH_2-C=O), 116.39 (1C; =CH), 119.87 (1C; pyridazinone C^5), 123.14 (1C; 2-fluorophenyl C^4), 123.63 (1C; 4-bromophenyl C^5), 125.35 (1C; 2-fluorophenyl C^5), 126.96 (1C; 4-bromophenyl C^2), 129.25 (1C; 4-bromophenyl C^6), 132.26 (1C; pyridazinone C^4), 133.73 (1C; 2-fluorophenyl C^6), 140.03 (1C; 4-bromophenyl C^3), 143.15 (1C; 2-fluorophenyl C^3), 146.23 (1C; 4-bromophenyl C^1), 149.03 (1C; 2-fluorophenyl C^1), 154.62 (1C; 4-bromophenyl C^4), 156.24 (1C; pyridazinone C^6), 158.25 (1C; 2-fluorophenyl C^2), 163.88 (1C; CH_2-N-C=O), 168.53 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 512.9 $[M]^+$, 514.9 $[M+2]^+$; Anal. Calcd. for $C_{23}H_{22}BrFN_6O_2.H_2O$: C, 51.99; H, 4.55; N, 15.82. Found: C, 52.23; H, 4.477; N, 15.47%.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(2-methoxybenzal)hydrazone (V_6). White crystals (methanol/water); yield 77.45%; m.p. 239 – 241°C; IR (ν, cm^{-1}, ATR): 3022 (C-H aromatic), 2840 (C-H aliphatic), 1683, 1652 (C=O), 1567 (C=N), 1240 (C-N), 1098 (C-O), 846 (C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 2.51 (4H; t; CH_2N; b+b′), 3.11 (4H; t; CH_2N; a+a′), 5.05 (2H; s; CH_2CO), 6.91 (1H; d; J = 4.15 Hz, pyridazinone H^5), 6.93 (1H; d; J = 4.16 Hz, pyridazinone H^4), 6.98 – 7.86 (8H; m; phenyl protons), 8.57 (1H; s; -N=CH-) and 11.60 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), 46.60 (2C, CH_2N; b+b′), 50.12 (2C, CH_2N; a+a′), 53.03 (1C; -N-CH_2-C=O), 56.17 (1C; -OCH_3), 112.27 (1C; =CH), 116.52 (1C; pyridazinone C^5), 121.20 (1C; 2-methoxyphenyl C^6), 122.43 (1C; 2-methoxyphenyl C^5), 123.18 (1C; 2-methoxyphenylphenyl C^4), 125.96 (1C; 2-fluorophenyl C^5), 127.01 (1C; 2-fluorophenyl C^4), 131.07 (1C; pyridazinone C^4), 132.10 (1C; 2-fluorophenyl C^6), 139.92 (1C; 2-fluorophenyl C^3), 142.95 (1C; 2-methoxyphenyl C^3), 149.00 (1C; 2-fluorophenyl C^1), 154.62 (1C; 2-methoxyphenyl C^1), 156.24 (1C; 2-methoxyphenyl C^2), 158.08 (1C; pyridazinone C^6), 159.22 (1C; 2-fluorophenyl C^2), 163.58 (1C; CH_2-N-C=O) and 168.32 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 463.0 $[M]^+$; Anal. Calcd. for $C_{24}H_{25}FN_6O_3.H_2O$: C, 60.88; H, 5.53; N, 17.75. Found: C, 60.74; H, 5.337; N, 17.57%.

6-[4-(2-Fluorophenyl)piperazine-1-yl]-3(2*H*)-pyridazinone-2-acetyl-2-(4-dimethylaminobenzal)hydrazone (V_7). Yellow crystals (methanol/water); yield 68.77%; m.p. 237 – 239°C; IR (ν, cm^{-1}, ATR): 3122 (C-H aromatic), 2857

(C-H aliphatic), 1648 (C=O), 1585 (C=N), 1241 (C-N), 1182 (C-O), 846 (C-F). ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 2.50 (6H; s; $N(CH_3)_2$), 2.97 (4H; t; CH_2N; b+b′), 3.38 (4H; t; CH_2N; a+a′), 5.01 (2H; s; CH_2CO), 6.73 (1H; d; J = 4.03 Hz, pyridazinone H^5), 6.75 (1H; d; J = 4.05 Hz, pyridazinone H^4), 6.98 – 7.88 (8H; m; phenyl protons), 8.06 (1H; s; -N=CH-) and 11.34 (1H; s; -NH-N). ^{13}C-NMR (DMSO-d_6, 600 MHz), δ 46.58 (2C, $N(CH_3)_2$), 46.62 (2C, CH_2-N; b+b′), 50.12 (2C, CH_2-N; a+a′), 52.98 (1C; -N-$\underline{C}H_2$-C=O), 112.25 (1C; =CH), 116.39 (1C; pyridazinone C^5), 119.88 (1C; 2-fluorophenyl C^4), 121.80 (1C; 4-dimethylaminophenyl C^5), 123.13 (1C; 2-fluorophenyl C^5), 125.35 (1C; 4-dimethylaminophenyl C^2), 126.89 (1C; 4-dimethylaminophenyl C^6), 128.63 (1C; pyridazinone C^4), 131.07 (1C; 2-fluorophenyl C^6), 140.10 (1C; 4-dimethylaminophenyl C^3), 145.11 (1C; 2-fluorophenyl C^3), 148.97 (1C; 4-dimethylaminophenyl C^1), 151.88 (1C; 2-fluorophenyl C^1), 154.63 (1C; 4-dimethylaminophenyl C^4), 156.24 (1C; pyridazinone C^6), 158.26 (1C; 2-fluorophenyl C^2), 163.11 (1C; CH_2-N-$\underline{C}$=O), 167.83 (1C; pyridazinone C^3); LC/MSMS (ESI-) *m/z* 476.1 $[M]^+$; Anal. Calcd. for $C_{25}H_{28}FN_7O_2.1/2H_2O$: C, 61.71; H, 6.01; N, 20.15. Found: C, 61.92; H, 6.097; N, 20.08%.

4.4. Activity Studies

4.4.1. Cell cultures and incubation. HTC116, HEP3B and HF (BJ, CRL-2522) cells were obtained from the American Type culture collection (ATCC). Cells were cultured in DMEM containing 10% fetal bovine serum and 1% antibiotics (100 µg/mL streptomycin and 10000 U /mL penicillin). Media were changed twice a week until cells reached 70 – 80% confluency.

4.4.2. Cell viability assay. The MTS [3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay was performed to determine viable cell ratio according to the manufacturer′s instructions (Promega). Briefly, cells were seeded in 96-well flat-bottomed tissue culture plates at a concentration of 5×10^{-3} cells/well. After 24 h, the cells were treated with DMEM medium containing different concentrations (25, 50, 100, 200, and 300 µM) of the test compounds for different incubation periods (24, 48, and 72 h). At the end of the each time point, fresh complete medium containing 10 µL of MTS solution was added and further incubated 2 h in incubator. Cell proliferation was assessed by measuring the absorbance with an ELISA microplate reader (Weida). Each experiment was performed quadruplet and results are expressed as the percentage growth inhibition with respect to the untreated cells.

Statistical deviations for the viability were calculated automatically by the Excel 2007 software (SE ? 5%), and for the $IC_{50,}$ values, by the ‘’Origin Pro 7.5” and “Origin 6.1” (for 7Crf) PC programs.

CONFLICT OF INTERESTS

No conflict of interests was reported by all authors.

REFERENCES

1. J. Ferlay, I. Soerjomataram, R. Dikshit, et al., *Int. J. Cancer,* **136**(5), 359–386 (2015).
2. N. Howlader, A. M. Noone, M. Krapcho, et al. (eds.), *SEER Cancer Statistics Review 1975 – 2014*, National Cancer Institute, Bethesda, MD (2017); https: // seer.cancer.gov / csr / 1975 2014 / .
3. R. Khoogar, B. C. Kim, J. Morris, and M. J. Wargovich, *Am. J. Physiol. Gastrointest. Liver Physiol*, **310**(9), 629 – 644 (2016).
4. S. M. S. Islam, T. D. Purnat, N. T. A. Phuong, et al., *Globalization and Public Health Symp.* (March 22, 2014, Munich, Germany) 10:81.
5. F. A. Sloan and H. Gelband, *Cancer Control Opportunities in Low- and Middle-Income Countries*, National Academies Press, Washington, DC (2007).
6. X. Ma and H. Yu, *Yale J. Biol. Med.,* **79**(3 – 4), 85–94 (2006).
7. A. G. Vaiopoulos, I. D. Kostakis, M. Koutsilieris, and A. G. Papavassiliou, *Stem Cells*, **30**(3), 63–371 (2012).
8. K. Bardhan and K. Liu, *Cancer*, **5**(2), 676–713 (2013).
9. R. N. Castle, A. Weissberger, and E. C. Taylor, *The Chemistry of Heterocyclic Compounds*, Vol. 28, John Wiley and Sons, New York (1973).
10. E. Matrai, *J. Mol. Struct.*, **408/409**, 467 – 472 (1997).
11. A. R. Katritzky and A. J. Boulton, *Advances in Heterocyclic Chemistry*, Vol. 9, Academic Press, New York – London (1968).
12. A. A. Siddiqui, R. Mishra, and M. Shaharyar, *Eur. J. Med. Chem.*, **45**, 2283 – 2290 (2010).
13. A. A. Siddiqui, R. Mishra, M. Shaharyar, et al*, Bioorg. Med. Chem. Lett.,* **21**, 1023 – 1026 (2011).
14. S. Utku, M. Gökçe, G. Aslan, et al., *Arzneim Forsch,* **61**, 1 – 7 (92011).
15. C. Yamali, G. H. Ozan, B. Kahya, et al., *Med. Chem. Res.*, **24**, 1210 – 1217 (2015).
16. P. Nagle, Y. Pawar, A. Sonawane, et al*., Med. Chem. Res.*, **23**, 918 – 926 (2014).
17. M. F. Şahin, B. Badıçoğlu, M. Gökçe, et al., *Arch. Pharm. Pharm. Med.,* **33**, 445 – 452 (2004).
18. I. G. Rathish, K. Javed, S. Ahmad, et al., *Eur. J. Med. Chem.*, **49**, 304 – 309 (2012).
19. N. F. Abd El-Ghaffar, M. K. Mohamed, M. S. Kadah, *J. Chem. Pharm. Res*., **3**, 248 – 259 (2011).
20. M. Asif, *Int. J. Adv. Chem.*, **2**(2), 148 – 161 (2014).
21. A. Bruel, C. Loge, M. L. Tauzia, et al., *Eur. J. Med. Chem*., **57**, 225 – 233 (2012).
22. Z. Özdemir, M. Gökçe, H. Uslu., et al., *Intern. Multidisciplinary Symp. on Drug Research and Development* (November 28 – 30, 2013, Antalya, Turkey).
23. O. Çiftçi, Z. Özdemir, C. Acar, et al., *Lett. Org. Chem.*, **15**(4), 323 – 331 (2017).
24. T. Önkol, M. Gökçe, İ. Orhan, and F. Kaynak, *Org. Commun.,* **6**, 55 – 67 (2013).
25. A. B. Özçelik, M. Gökçe, İ. Orhan, et al., *Arzneim Forsch*, **60**(7), 452 – 458 (2010).