ORIGINAL RESEARCH

Synthesis and chemotherapeutic activities of 5-chloro-1*H*-indole-2,3-dione 3-thiosemicarbazones

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ABSTRACT: A series of 5-chloro-1*H*-indole-2,3-dione 3-thiosemicarbazones 3a-g were synthesized to investigate the chemotherapeutic activities. The structures of 3a-g were confirmed by the spectral data (IR, ^{1}H NMR, ^{13}C NMR-APT, ^{13}C NMR-DEPT, HSQC, HMBC) and elemental analysis. Anticancer activities of the compounds were evaluated using cell kinetic parameters on HeLa cells derived from human cervix carcinoma. The preliminary screening results indicated that alkyl substituted compounds 3a, 3b and 3d were more effective in mitosis phase when compared to the synthesis phase. 3a-g were tested against some DNA and RNA viruses in CRFK, HeLa, HEL, MDCK and Vero cells. None of the compounds was active against any of the RNA or DNA viruses at 100 μM. All compounds were also evaluated for antimicrobial activity against selected strains. Methyl substituted 3a and allyl substituted 3c were found to be active against S. aureus and C. albicans.

KEY WORDS: Anticancer activity, antiviral activity, antimicrobial activity, 5-chloro-1*H*-indole-2,3-dione, thiosemicarbazone

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INTRODUCTION

Biological properties of thiosemicarbazone derivatives have been studied since 1946, when the activity of thiacetazone against *Mycobacterium tuberculosis* was reported. Since then, this and other biological properties of thiosemicarbazone derivatives, such as anticancer and antiviral activity have been described. Triapine is a ribonucleotide reductase inhibitor with promising anticancer activity against hematologic malignancies in clinical trials (1). Isatin-β-thiosemicarbazone (IBT) and N-methylisatin-β-thiosemicarbazone (M-IBT, methisazone) prevent the production of small-pox viruses. Methisazone has also been used in the clinical treatment of smallpox (2). N-methylisatin-β,4′,4′-diethylthiosemicarbazone (M-IBDET) specifically inhibits formation of Moloney leukemia virus structural proteins (3) (Figure 1).

Isatin (1*H*-indole-2,3-dione) is a synthetically versatile molecule which has led to an array of derivatives displaying a broad spectrum of biological properties (4,5). Investigation of the structure-activity relationships in 3-substituted 2-indolinone derivatives revealed that halogenation at the 5-position and 3-thiosemicarbazone formation were associated with increased activity against a range of human cancer cell lines, various bacteria and viruses (6-10). Selective activity toward multidrug resistant (MDR) cells of several thiosemicarbazone derivatives were recently tested. Pharmacophore analysis of active compounds revealed that isatin-3-thiosemicarbazone moiety was essential for the MDR1-selective activity (10,11).

In the light of these findings, 5-chloro-1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives were synthesized in order to obtain more potent and less toxic compounds. The structures of the

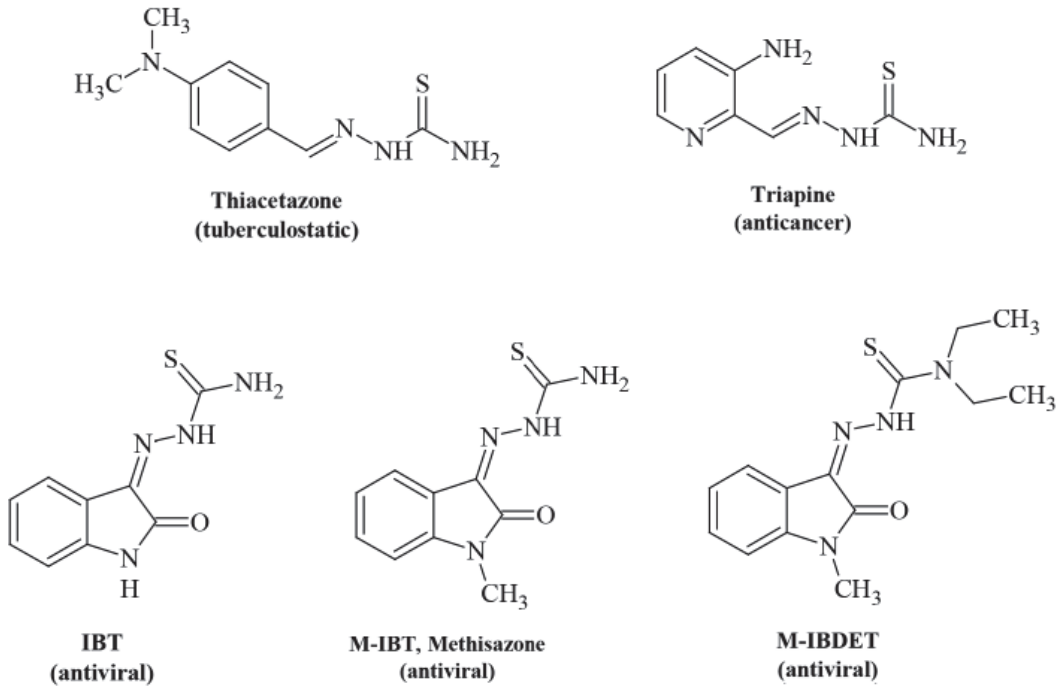


FIGURE 1. Representative bioactive thiosemicarbazone derivatives

synthesized compounds were determined by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR-APT, ^{13}C NMR-DEPT, HSQC-2D, HMBC-2D) methods. The compounds were tested to investigate their the chemotherapeutic activities.

MATERIALS AND METHODS

Chemistry

Chemicals and reagents used in the current study were of analytical grade. Melting points were estimated with a Buchi 540 melting point apparatus in open capillaries and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded on KBr discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer. ^{1}H NMR, ^{13}C NMR-APT, ^{13}C NMR-DEPT, HSQC-2D and HMBC-2D spectra were obtained on VarianUNITY INOVA (500 MHz) or Varian Mercury (300 MHz) spectrophotometers using DMSO-d_6.

The synthesis of 4-substituted thiosemicarbazides (1a-g)

To a solution of hydrazine hydrate (5 mmol) in ethanol (10 mL), a suspension of an appropriate isothiocyanate (5 mmol) in ethanol (10 mL) was added dropwise with vigorous stirring and cooling in an ice bath. The mixture was allowed to stand overnight. The crystals formed were recrystallized from ethanol.

The synthesis of 5-chloro-1H-indole-2,3-dione 3-thiosemicarbazones (3a-g)

A solution of 4-substituted thiosemicarbazides **1a-g** (3.5 mmol) in ethanol (10 mL) was added to a solution of 5-chloro-1*H*-indole-2,3-dione **2** (3.5 mmol) in ethanol (20 mL). After addition of a drop of concentrated sulfuric acid, the mixture was refluxed on a water bath for 5 h. The product formed after cooling was filtered and washed with ethanol or recrystallized from ethanol.

5-Chloro-1H-indole-2,3-dione 3-(4-methylthiosemicarbazone) **(3a)** (12)

Yield: 95%, mp: 254°C, IR (KBr) cm^{-1}: υ 3264 (NH), 1697 (C=O), 1142 (C=S); ^{1}H NMR (DMSO-d_6, 500 MHz) ppm: δ 3.07 (d, *J*= 4.40 Hz, 3H, CH_3), 6.91 (d, *J*= 8.30 Hz, 1H, indole C_7-H), 7.35 (dd, *J*= 8.29, 1.95 Hz, 1H, indole C_6-H), 7.67 (d, *J*= 1.95 Hz, 1H, indole C_4-H), 9.33 (q, *J*= 4.88 Hz, 1H, N_4-H), 11.26 (s, 1H, indole NH), 12.39 (s, 1H, N_2-H); HSQC-2D (DMSO-d_6, 125 MHz) ppm: δ 31.98 (CH_3), 113.23 (indole C_7), 120.98 (indole C_4), 122.62 (indole C_{3a}), 127.19 (indole C_5), 130.98 (indole C_6), 130.99 (indole C_{7a}), 141.55 (indole C_3), 163.07 (indole C=O), 178.37 (C=S). Anal. Cald for $C_{10}H_9ClN_4OS$ (268.72): C, 44.70; H, 3.38; N, 20.85%. Found: C, 44.70; H, 3.85; N, 20.95%.

5-Chloro-1H-indole-2,3-dione 3-(4-ethylthiosemicarbazone) **(3b)**

Yield: 94%, mp: 267°C, IR (KBr) cm^{-1}: υ 3365, 3255 (NH), 1689 (C=O), 1153 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz) ppm: δ 1.18 (t, *J*= 7.19 Hz, 3H, ethyl C_2-H), 3.62 (*quin.*, *J*= 6.82 Hz, 2H, ethyl C_1-H), 6.92 (d, *J*= 8.69 Hz, 1H, indole C_7-H), 7.36 (dd, *J*= 8.25, 2.25 Hz, 1H, indole C_6-H), 7.71 (d, *J*= 2.09 Hz, 1H, indole C_4-H), 9.38 (t, *J*= 5.85 Hz, 1H, N_4-H), 11.29 (s, 1H, indole NH), 12.36 (s, 1H, N_2-H). Anal. Cald for $C_{11}H_{11}ClN_4OS$ (282.75): C, 46.73; H, 3.92; N, 19.82%. Found: C, 46.41; H, 3.79; N, 19.62%.

5-Chloro-1H-indole-2,3-dione 3-(4-allylthiosemicarbazone) **(3c)**

Yield: 96%, mp: 215°C, IR (KBr) cm^{-1}: υ 3356, 3253 (NH), 1693 (C=O), 1170 (C=S); ^{1}H NMR (DMSO-d_6, 500 MHz) ppm: δ 4.25 (t, *J*= 4.87 Hz, 2H, allyl C_1-H), 5.14 (dd, *J*= 10.38, 1.52 Hz, 1H, allyl C_3-H $_{cis}$), 5.20 (dd, *J*= 17.22, 1.52 Hz, 1H, allyl C_3-H_{trans}), 5.87-5.95 (m, 1H, allyl C_2-H), 6.93 (d, *J*= 8.23 Hz, 1H, indole C_7-H), 7.36 (dd, *J*= 8.39, 2.29 Hz, indole C_6-H), 7.74 (d,

J= 2.44 Hz, 1H, indole C_4-H), 9.53 (t, *J*= 5.79 Hz, 1H, N_4-H), 11.27 (s, 1H, indole NH), 12.42 (s, 1H, N_2-H). Anal. Cald for $C_{12}H_{11}ClN_4OS.^1/_2H_2O$ (303.77): C, 47.44; H, 3.97; N, 18.44%. Found: C, 47.58; H, 3.96; N, 18.33%.

***5-Chloro-1H-indole-2,3-dione 3-(4-butylthiosemicarbazone)* (3d)**

Yield: 97%, mp: 224°C, IR (KBr) cm^{-1}: υ 3251 (NH), 1693 (C=O), 1169 (C=S). ^{1}H-NMR (DMSO-d_6, 300 MHz) ppm: δ 0.91 (t, *J*= 7.64 Hz, 3H, butyl C_4-H), 1.30-1.38 (m, 2H, butyl C_3-H), 1.60 (*quin.*, *J*= 7.49 Hz, 2H, butyl C_2-H), 3.59 (q, *J*= 7.49 Hz, 2H, butyl C_1-H), 6.93 (d, *J*= 8.39 Hz, 1H, indole C_7-H), 7.37 (dd, *J*= 8.25, 2.25 Hz, 1H, indole C_6-H), 7.73 (d, *J*= 2.1 Hz, 1H, indole C_4-H), 9.37 (t, *J*= 5.99 Hz, 1H, N_4-H), 11.29 (s, 1H, indole NH), 12.36 (s, 1H, N_2-H). Anal. Cald for $C_{13}H_{15}ClN_4OS$ (310.80): C, 50.24; H, 4.86; N, 18.03%. Found: C, 50.47; H, 4.98; N, 17.97%.

***5-Chloro-1H-indole-2,3-dione 3-(4-benzylthiosemicarbazone)* (3e)**

Yield: 93%, mp: 245°C, IR (KBr) cm^{-1}: υ 3373, 3159 (NH), 1691 (C=O), 1161 (C=S). ^{1}H-NMR (DMSO-d_6, 500 MHz) ppm: δ 4.87 (d, *J*= 6.1 Hz, 2H, benzyl CH_2), 6.92 (d, *J*= 8.24 Hz, 1H, indole C_7-H), 7.25-7.37 (m, 6H, indole C_6-H, C_6H_5), 7.71 (d, *J*= 2.13 Hz, 1H, indole C_4-H), 9.90 (t, *J*= 6.25 Hz, 1H, N_4-H), 11.29 (s, 1H, indole NH), 12.48 (s, 1H, N_2-H). ^{13}C NMR (APT, DMSO-d_6, 75 MHz) ppm: δ 47.9 (benzyl CH_2), 113.01 (indole C_7), 120.95 (indole C_4), 122.34 (indole C_{3a}), 126.95 (indole C_5), 127.53 (phenyl $C_{3,5}$), 127.74 (phenyl $C_{2,6}$), 128.78 (phenyl C_4), 130.87 (indole C_6), 131.29 (indole C_{7a}), 138.71 (phenyl C_1), 141.43 (indole C_3), 162.4 (indole C=O), 178.15 (C=S). Anal. Cald for $C_{16}H_{13}ClN_4OS.H_2O$ (362.84): C, 52.96; H, 4.16; N, 15.44%. Found: C, 53.13; 4.28; 15.58%.

***5-Chloro-1H-indole-2,3-dione 3-[4-(4-fluorophenyl)thiosemicarbazone]* (3f)** (13)

Yield: 97%, mp: 236°C, IR (KBr) cm^{-1}: υ 3326, 3215 (NH), 1690 (C=O), 1171 (C=S). ^{1}H-NMR (DMSO-d_6, 300 MHz) ppm: δ 6.92 (d, *J*= 8.40 Hz, 1H, indole C_7-H), 7.26 (t, *J*= 8.89 Hz, 2H, phenyl $C_{3,5}$-H), 7.35 (d, *J*= 2.10 Hz, 1H, indole C_6-H), 7.59 (dd, *J*= 8.99, 5.09 Hz, 2H, phenyl $C_{2,6}$-H), 7.82 (d, *J*= 1.80 Hz, indole C_4-H), 10.87 (s, 1H, N_4-H), 11.34 (s, 1H, indole NH), 12.60 (s, 1H, N_2-H). ^{13}C NMR DEPT, HMBC-2D (DMSO-d_6, 75 MHz) ppm: δ 113.01 (indole C_7), 115.65 (d, J= 22.47 Hz, phenyl $C_{3,5}$), 121.41 (indole C_4), 122.22 (indole C_{3a}), 127.03 (indole C_5), 128.43 (d, J= 8.67 Hz, phenyl $C_{2,6}$), 131.07 (indole C_6), 131.58 (indole C_{7a}), 135.08 (phenyl C_1), 141.59 (indole C_3), 158.91 (d, *J*= 242.99 Hz, phenyl C_4), 162.89 (indole C=O), 177.09 (C=S). Anal. Cald for $C_{15}H_{10}ClFN_4OS$ (348.78): C, 51.65; H, 2.89; N, 16.06%. Found: C, 51.79; H, 2.99; N, 16.06%.

***5-Chloro-1H-indole-2,3-dione 3-[4-(4-nitrophenyl)thiosemicarbazone]* (3g)**

Yield: 91%, mp: 258°C, IR (KBr) cm^{-1}: υ 3286, 3212 (NH), 1699 (C=O), 1179 (C=S). ^{1}H-NMR (DMSO-d_6, 300 MHz) ppm: δ 6.94 (d, *J*= 8.10 Hz, 1H, indole C_7-H), 7.40 (dd, *J*= 8.40, 2.10 Hz, 1H, indole C_6-H), 7.85 (d, *J*= 2.09 Hz, 1H, indole C_4-H), 8.06 (d, *J*=9.29 Hz, 2H, phenyl $C_{2,6}$-H), 8.29 (d, *J*= 9.79 Hz, 2H, phenyl $C_{3,5}$- H), 11.10 (s, 1H, N_4-H), 11.38 (s, 1H, indole NH), 12.79 (s, 1H, N_2-H). ^{13}C NMR (APT, DMSO-d_6, 75 MHz) ppm: δ 113.15 (indole C_7), 116.98 (indole C_4), 121.61 (indole C_{3a}), 122.0 (phenyl $C_{2,6}$), 124.48 (phenyl $C_{3,5}$), 127.07 (indole C_5), 131,45 (indole C_6), 132.49 (indole C_{7a}), 141.85 (indole C_3), 144.78 (phenyl C_1), 146.99 (phenyl C_4), 162.95 (indole C=O), 176.38 (C=S). Anal. Cald for $C_{15}H_{10}ClN_5O_3S$ (375.78): C, 47.94; H, 2.68; N, 18.64%. Found: C, 47.43; H, 2.81; N, 18.35%.

CYTOTOXICITY

Cell Culture

The HeLa cell line used in this experiment was obtained from European Cell Culture Collection (CCL). Cells were cultured in Medium-199 (M-199, Sigma, USA) containing 10% fetal bovine serum (FBS, Gibco Lab), 100 µg/mL streptomicin (Streptomicin sulphate, I. E. Ulugay), 100 IU/ml penicilin (Pronapen, Pfizer), Amphotericine B (Sigma, USA) and 2 mM glutamine at 37°C in humidified atmosphere of 5% CO_2 in air. The pH of the medium was adjusted to 7.4 with $NaHCO_3$.

Drug application

Drugs used in this study were dissolved immediately in DMSO before the preparation of the required concentrations. We used optimum doses of all drugs. Optimum doses were obtained by dilution of the stock solution. These doses were 1 mg/mL for **3a**, **3e** and **3g**; 10 mg/mL for **3b** and **3d**. The drugs were tested by using these doses and HeLa cells were treated with these doses in the time periods of 24, 48 and 72 h.

Mitotic index analysis

Mitotic index was studied by the methods of Feulgen. Before the cells were treated with Feulgen, they were treated with 1 N HCl at room temperature for 1 min and then hydrolized with 1 N HCl for 10.5 min at 60°C. After slides were treated with Feulgen, they were rinsed for a few minutes in distilled water and stained with 10% Giemsa stain solution (pH 6.8) for 3 min and washed twice in phosphate buffer. After staining, the slides were rinsed in distilled water. And then the slides were air dried. At last mitotic index was calculated by counting metaphases, anaphases and telophases for each tested drug concentration and control. At least three thousand cells were examined from each slide for mitotic index (14).

^{3}H-thymidine labelling index

At the end of drug administration, to investigate the parameter of labelling index, cells were treated with the medium containing 1 µCi/mL ^{3}H-thymidine for 20 min.

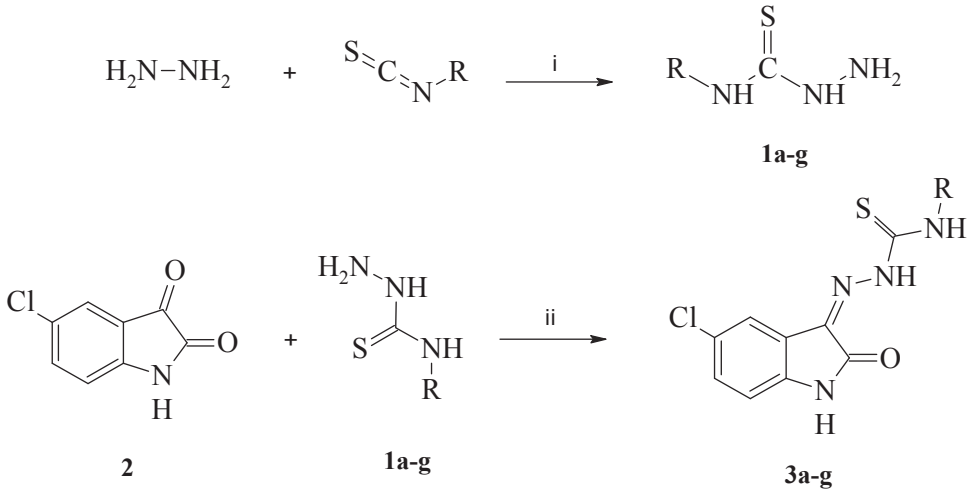
Autoradiography

After labelling, the cells were fixed with Carnoys fixative [ethanol: glacial acetic acid (3: 1)] and remaining radioactive materials were washed twice with 2% perchloric acid at 4°C for 30 mins. After preparing slides, they were coated with K.2 gel emulsion (Ilford, England) prepared with distilled water at 40°C to determine the thymidine labelling index. After 3 days of exposure at 4°C, autoradiograms were washed with D- 19 b developer (Kodak) and fixed with Fixaj B (Kodak). The slides were evaluated after being stained with Giemsa for 3 minutes. On each slide, with 100x12.5 magnification in 100 areas, the labelled cells were counted. The same person evaluated all the slides by counting at least 3000 cells from each slides. These data are typical results from a minimum of three independent experiments (15).

RESULTS AND DISCUSSION

Chemistry

In this study, hydrazine hydrate was reacted with an appropriate isothiocyanate in ethanol to give the corresponding



R= CH_3, C_2H_5, CH_2-CH=CH_2, n-C_4H_9, $CH_2C_6H_5$, 4-FC_6H_4, 4-$NO_2C_6H_4$

SCHEME. Synthesis of compounds **3a-g**. Reagents and conditions: i) EtOH, stirred, cooled ii) EtOH, H_2SO_4, reflux, 6 h.

4-substituted thiosemicarbazides **1a-g** (16-18). A series of 5-chloro-1*H*-indole-2,3-dione 3-thiosemicarbazones **3a-g** were synthesized by reacting 5-chloro-1*H*-indole-2,3-dione **2** with **1a-g** in ethanol containing a catalytic amount of sulphuric acid (7-9). The structures of **3a-g** were confirmed by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR-APT, ^{13}C NMR-DEPT, HSQC-2D, HMBC-2D) data (Scheme).

IR spectra of **3a-g** showed absorption bands in the 3373-3159 cm^{-1} region resulting from the NH stretchings of the lactam and thioamide functions. The lactam C=O and thioamide C=S stretchings were observed in the 1699-1689 and 1179-1142 cm^{-1} regions, respectively. The IR spectra provided evidence for the confirmation of the thiosemicarbazone structure. Amidic and ketonic C=O stretching bands of 5-chloro-1*H*-indole-2,3-dione **2** absorbing as two separate bands absorbed as a single amidic C=O stretching band and a ketonic C=O stretching band disappeared in the IR spectra of **3a-g** (19,20). ^{1}H NMR spectra of **3a-g** displayed the NH protons of the thiosemicarbazone moiety (δ 9.33-11.10 and 12.36-12.79 ppm) and the indole NH proton (δ 11.26-11.38 ppm) as three separate signals. HSQC spectra of **3a**, ^{13}C NMR-APT spectra of **3e** and **3g,** ^{13}C NMR-DEPT and HMBC spectra of **3f** supported the IR and ^{1}H-NMR findings, and displayed signals at δ 141.43-141.85, 162.4-163.07 and 176.38-178.37 ppm which were attributed to the quarternary indole C_3, indole C_2 and C=S atoms (7-9).

Chemotherapeutic activity

The anticancer activities of **3b**, **3d**, **3e** and **3g**, along with previously reported **3a** on HeLa cells derived from human cervix carcinoma were evaluated using cell kinetic parameters including mitotic index and labelling index. Mitotic index is the ratio of the number of cells undergoing mitosis (cell division) to the number of cells not undergoing mitosis in a population of cells. ^{3}H thymidine labelling index explains the ratio of cells with DNA synthesis to all the cells in growing cell population. Four different doses (D_1= 1 mg/mL, D_2= 5 mg/mL, D_3= 10 mg/mL and D_4= 50 mg/mL) were prepared for the compounds and the optimum dose was determined using mitotic index parameter for all of them. Optimum doses were determined as D_1 for **3a**, **3e** and **3g**, and D_3 for **3b** and **3d**. Then, they were applied to HeLa cells for 0-72 h. For both parameters all the differences between control and experimental groups were statistically significant ($p<0.01$). Sunitinib was used as the standard in the tests of the mitotic index (Table 1 and Figure 2) (21). The oxindole derived sunitinib was approved by the FDA in January 2006 for the treatment of gastroinestinal stromal cancers and renal cell carcinoma (22).

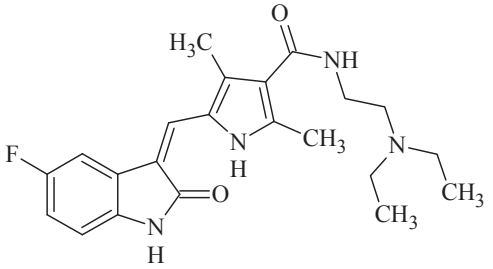


TABLE 1. Mitotic index (%) values of HeLa cells treated with 10μM of **Sunitinib**

Experiment Groups	Control	Sunitinib	% Inhibition
12 h	7.62 ± 0.24	5.00 ± 0.12	34.38
24 h	7.21 ± 0.51	3.44 ± 0.37	52.28
48 h	5.28 ± 0.22	2.42 ± 0.13	54.16
72 h	5.14 ± 0.21	1.10 ± 0.14	78.59

SD: Standard deviation
Significantly diffrent $p<0.01$

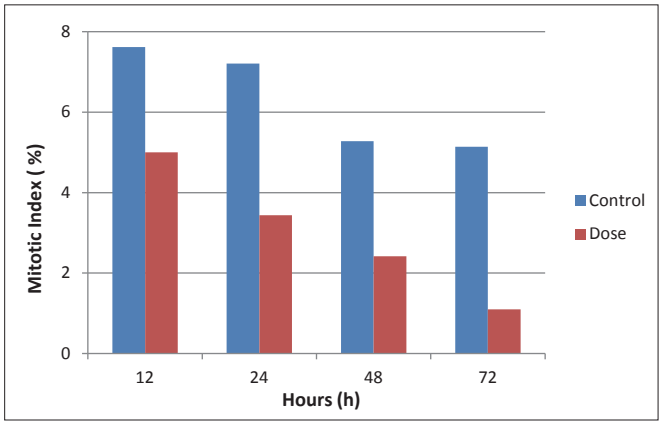


FIGURE 2. Mitotic index (%) values of HeLa cells treated with 10μM of **Sunitinib**

3a, **3b** and **3d** showed varying degrees of inhibition at optimum doses in the mitotic index tests as can be seen in Tables 2-4 and Figures 3-5. For ethyl substituted **3b**, an inhibition percentage of 50.41% was obtained. The inhibition percentages of methyl substituted **3a** and n-butyl substituted **3d** were 38.19% and 40.55%, respectively. In benzyl substituted **3e** and 4-nitrophenyl substituted **3g**, the activity significantly decreased when compared with the alkyl substituted derivatives. The inhibition percentages of the tested compounds were lower than the values observed for sunitinib.

TABLE 2. Mitotic index (%) values of HeLa cells treated with D1 (1 μg/mL) dose of **3a**

Experiment Groups	Control	3a	% Inhibition
24 h	4.34 ± 0.24	3.51 ± 0,23	19.12
48 h	3.65 ± 0.51	2.98 ± 0,26	18.35
72 h	2.54 ± 0.22	1.57 ± 0,28	38.19

SD: Standard deviation
Significantly diffrent $p<0.01$

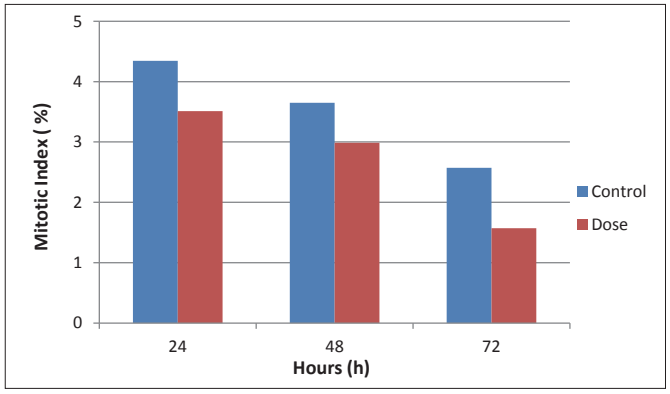


FIGURE 3. Mitotic index (%) values of HeLa cells treated with D1 (1 μg/mL) dose of **3a**

TABLE 3. Mitotic index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3b**

Experiment Groups	Control	3b	% Inhibition
24 h	4.34 ± 0.24	4.03 ± 0,31	7.14
48 h	3.65 ± 0.51	1.81 ± 0,22	50.41
72 h	2.54 ± 0.22	1.67 ± 0,28	34.25

SD: Standard deviation
Significantly diffrent $p<0.01$

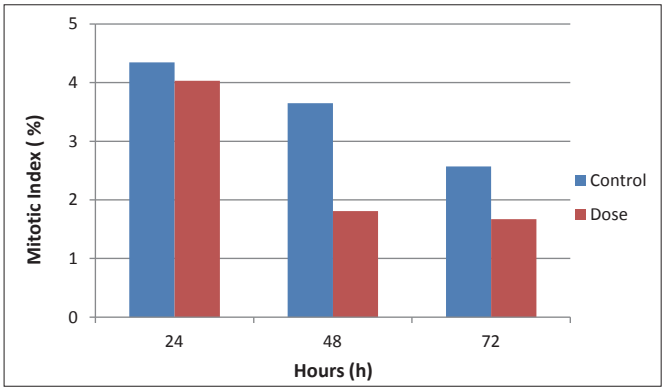


FIGURE 4. Mitotic index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3b**

TABLE 4. Mitotic index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3d**

Experiment Groups	Control	3d	% Inhibition
24 h	4.34 ± 0.24	3.29 ± 0.14	24.19
48 h	3.65 ± 0.51	2.54 ± 0.17	30.41
72 h	2.54 ± 0.22	1.51 ± 0.21	40.55

SD: Standard deviation
Significantly diffrent $p<0.01$

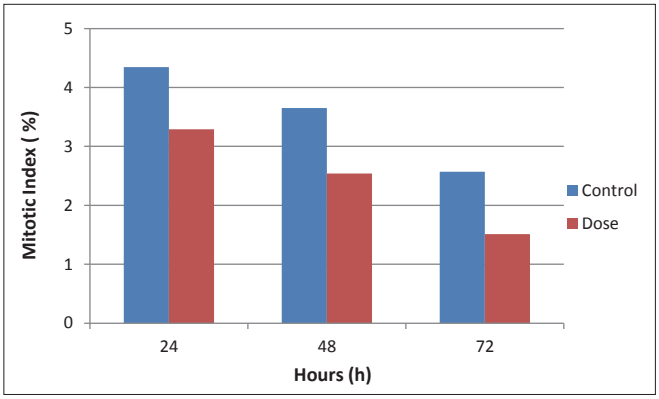


FIGURE 5. Mitotic index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3d**

In all experimental groups, labelling index values of 0-72 h were determined. The preliminary screening results indicated that all tested compounds were less effective in synthesis stage when compared to the mitosis phase (Tables 5-7 and Figures 6-8). n-Butyl substituted **3d** displayed the highest efficacy among the compounds and the inhibition percentage was 39.05% for **3d**.

TABLE 5. Labelling index (%) values of HeLa cells treated with D1 (1 μg/mL) dose of **3a**

Experiment Groups	Control	3a	% Inhibition
24 h	4.48 ± 0.24	4.42 ± 0.37	1.34
48 h	3.97 ± 0.31	3.78 ± 0.33	4.78
72 h	2.74 ± 0.27	2.44 ± 0.30	10.95

SD: Standard deviation
Significantly diffrent $p<0.01$

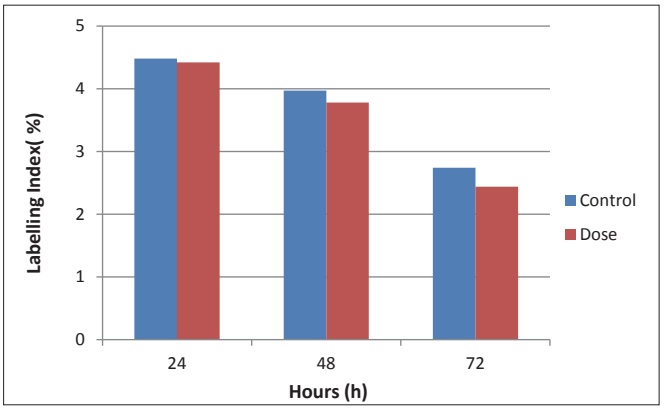


FIGURE 6. Labelling index (%) values of HeLa cells treated with D1 (1 μg/mL) of **3a**

TABLE 6. Labelling index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3b**

Experiment Groups	Control	3b	% Inhibition
24 h	4.48 ± 0.24	4.20 ± 0.22	6.25
48 h	3.97 ± 0.31	3.06 ± 0.31	22.92
72 h	2.74 ± 0.27	1.99 ± 0.19	27.37

SD: Standard deviation
Significantly diffrent $p<0.01$

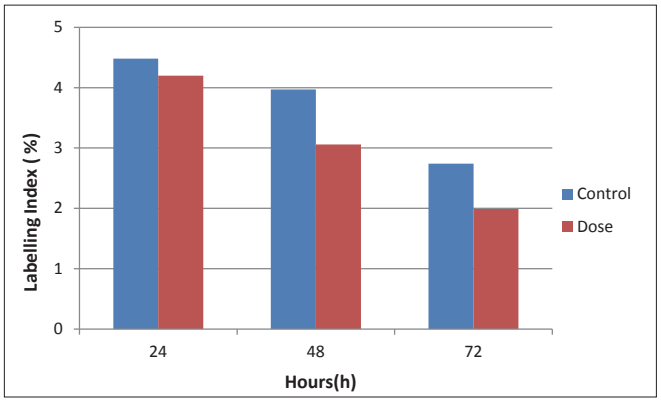


FIGURE 7. Labelling index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3b**

TABLE 7. Labelling index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3d**

Experiment Groups	Control	3d	% Inhibition
24 h	4.48 ± 0.24	4.14 ± 0.11	7.59
48 h	3.97 ± 0.31	3.22 ± 0.23	18.89
72 h	2.74 ± 0.27	1.67 ± 0.18	39.05

SD: Standard deviation
Significantly diffrent $p<0.01$

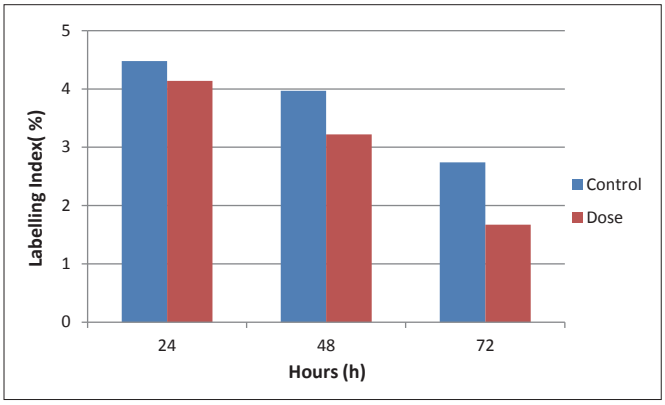


FIGURE 8. Labelling index (%) values of HeLa cells treated with D3 (10 μg/mL) dose of **3d**

3b-e and **3g**, along with previously reported **3a** and **3f** were evaluated against feline corona virus (FIPV), feline herpes virus (FHV) in Crandell-Rees feline kidney (CRFK), parainfluenza-3 virus, rheovirus-1, sindbis virus, coxsackie virus B4, punto toro virus in VERO, herpes simplex virus-1 (KOS)(HSV-1), herpes simplex virus-2 (G)(HSV-2), vaccinia virus, vesicular stomatitis virus (VSV), herpes simplex virus-1 TK KOS ACV in human embroyonic lung (HEL) and vesicular stomatitis virus, coxsackie virus B4 and respiratory syncytial virus (RSV) in Henrietta Lacks (HeLa) cell cultures (23). None of the test compounds was active against any of the RNA or DNA viruses, including influenza virus at 100 μM.

3b-e and **3g**, along with previously reported **3a** and **3f** were evaluated against *Staphylococcus aureus ATCC 6538*, *Staphylococcus epidermidis ATCC 12228*, *Escherichia coli ATCC 25922*, *Klebsiella pneumoniae ATCC 4352*, *Pseudomonas aeruginosa ATCC 27853*, *Proteus mirabilis ATCC 14153* ve *Candida albicans* ATCC 10231. The minimum inhibitory concentrations (MIC) of **3a** and **3c** were determined using a microdilution assay. Ciprofloxazin and clotrimazole were used as the standards in the tests (Table 12) (24,25). The antimicrobial activity results show that methyl substituted **3a** and allyl substituted **3c** have considerable antimicrobial effect on *S. aureus* and *C. albicans*.

TABLE 8. The MIC values of **3a** and **3c**

	Microorganisms	
Compounds	**S. aureus**	**C. albicans**
3a	9.8μg/ml	78μg/ml
3c	39μg/ml	78μg/ml
Ciprofloxazin	0.25 μg/ml	-
Clotrimazole	-	4.9μg/ml

5-Kloro-1H-İndol-2,3-Dion 3-tiyosemikarbazonların sentezi ve kemoterapötik aktiviteleri

ÖZET: Bir seri 5-kloro-1*H*-indol-2,3-dion 3-tiyosemikarbazon 3a-g, kemoterapötik etkilerini incelemek için sentezlenmiştir. 3a-g nin yapıları spektral bulgular (IR, ^{1}H NMR, ^{13}C NMR-APT, ^{13}C NMR-DEPT, HSQC, HMBC) ve elementel analiz ile kanıtlanmıştır. Bileşiklerin insan serviks kanserinden türetilmiş HeLa hücrelerine antikanser etkileri hücre kinetik parametreleri kullanılarak test edilmiştir. Ön inceleme sonuçları alkil sübstitüe 3a, 3b ve 3d' nin sentez evresine kıyasla mitoz evresinde daha etkili olduğunu göstermiştir. 3a-g CRFK, HeLa, HEL, MDCK ve Vero hücrelerinde bazı DNA ve RNA virüslerine karşı araştırılmıştır. Bileşiklerin hiçbiri DNA ve RNA virüslerine karşı etkili değildir. Tüm bileşiklerin seçilen suşlara karşı antimikrobial etkileri test edilmiştir. Metil sübstitüe 3a ve allil sübstitüe 3c' nin *S. aureus* and *C. albicans*'a karşı etkili olduğu bulunmuştur.

ANAHTAR KELİMELER: Antikanser aktivite, antiviral aktivite, antimikrobiyal aktivite, 5-kloro-1*H*-indol-2,3-dion, tiyosemikarbazon

CONCLUSION

A series of 5-chloro-1*H*-indole-2,3-dione 3-thiosemicarbazones were synthesized to investigate their chemotherapeutic activities. The compounds were evaluated for cytotoxic activities on HeLa cells derived from human cervix carcinoma. Replacement of the aralkyl or aryl at the R with alkyl have been found to yield more active compounds in mitosis phase, whereas none of the compounds was selective in the synthesis phase. The substitution of the methyl or allyl group at the R caused an increase in inhibitory activity against *S. aureus* and *C. albicans.* In conclusion, structural modification may lead to new derivatives with high selectivity for a range of human cancer cell lines, various bacteria and viruses.

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