4-(2-Chloroethyl) semicarbazide의 히드라존 유도체 합성 : 새로운 종류의 세포독성요법제

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Synthesis of Hydrazone Derivatives of 4-(2-Chloroethyl) semicarbazide: A New Class of Cytotoxic Agents

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요 약. 새로운 종류의 히드라존 유도체는 4-(2-chloroethyl) semicarbazides로 부터 합성되었고, 인간 두뇌(U251)와 간(Hepg2)의 암세포에 대해 항증식성을 보였다. 히드라존 화합물은 벤즈알데하يد, 아세토페논, 3-formylindole 유도체이다. 아세토페논 유도체들에 3e (p-methoxy substituted)와 3f (p-nitro substituted)는 Hepg2 세포 (각각IC50 = 6,8 µg/mL)에 대해 가장 높은 세포독성이성을 보인다. 3-Formylindole 유도체들에 4a (hydrazone of 3-formylindole)은 U251 (IC50 = 21 µg/mL)와 Hepg2 (IC50 = 7 µg/mL)에 강한 세포독성이성을 보인다.

주제어: 히드라존, 2-(Chloroethyl)요소들, 4-(2-Chloroethyl) semicarbazides, 세포독성요법 활성

ABSTRACT. A new series of hydrazone derivatives were synthesized from 4-(2-chloroethyl)semicarbazide and their antiproliferative activity against human brain (U251) and liver (Hepg2) carcinoma cell lines were evaluated. The hydrazone compounds are benzaldehyde (2a-2g), acetophenone (3a-3f), and 3-formylindole derivatives (4a-4d). Among the acetoephene derivatives, 3e (p-methoxy substituted) and 3f (p-nitro substituted) showed the highest cytotoxic activity against Hepg2 cell line (IC50 = 6 and 8 µg/ml, respectively). Among the 3-formylindole derivatives, 4a (hydrazone of 3-formylindole itself) showed a pronounced cytotoxic activity against both U251 (IC50 = 21 µg/ml) and Hepg2 (IC50 = 7 µg/ml).

Keywords: Hydrazones, 2-(Chloroethyl)ureas, 4-(2-Chloroethyl) semicarbazides, Cytotoxic activity

INTRODUCTION

1-Aryl-3-(2-chloroethyl)ureas (CEUs) were found to exhibit better cytotoxicity1 than the parent drugs i.e. chlorambucil and carmustine.2-6 These derivatives as for examples 1-(4-tert-butylphenyl)-3-(2-chloroethyl) urea (A) and 1-(4-iodophenyl)-3-(2-chloroethyl) urea (B) were designed as hybrid based on the conjugation of aromatic ring of chlorambucil as the prosthetic group and the cytotoxic 2-chloroethylnitrosourea moiety of carmustine as the pharmacophore.7
The SAR of the CEU derivatives showed that the unniitrosated derivatives were the only active ones; meanwhile, the exocyclic urea function and the 2-chloroethyl group were essential for activity; moreover, the preferred aryl groups were 4-substituted phenyl with alkyl or an indanyl group. This class was found to irreversibly alkylate β-tubulin inside the cancer cell causing the cytotoxic effect. Unfortunately, these derivatives caused many side effects such as developing resistance through P-glycoprotein overexpression and modification of topoisomerase I.

On the other side, a series of synthetic indole derivatives such as compound D-64131(C) have proven to have significant antitumoral activity both in vitro and in vivo.

In our previous work, a series of benzamides (D) and 2-indolecarboxamides (E) derivatives of the 4-(2-chloroethyl)semicarbazide were prepared as a new class of cytotoxic agents. These derivatives were structurally related to 1-aryl-3-(2-chloroethyl) ureas derivatives with some structural variations whereas the aryl moiety whether p-substituted phenyl (D) or 5-substituted indolyl (E) was linked indirectly to (2-chloroethyl)urea function through carboxylylamino moiety. It was found that semicarbazides containing 2-indolyl carbonyl moiety (E) especially those having electron-donor substituents at position 5 of the indole ring like methoxy (IC50 = 8 µg/ml against U251) and benzylxoy (IC50 = 21 µg/ml against Hepg2) showed a higher cytotoxic activity than their corresponding benzoyl derivatives (D) against human brain (U251) and liver (Hepg2) carcinoma cell lines.

In continuation to our previous work, a new series of hydrazone derivatives belonging to p-substituted benzaldehyde or p-substituted acetophenone were synthesized from 4-(2-chloroethyl) semicarbazide to study, firstly, the effect of different substituents on the cytotoxic activity. Secondly, we studied also the effect of replacement of p-substituted phenyl belonging to benzaldehyde hydrazone of the 4-(2-chloroethyl)semicarbazide by 5-substituted indolyl moieties upon the resultant cytotoxic activity against human brain and liver carcinoma cell lines.

Discussions

A) Chemistry

To achieve the first aim, a new series of hydrazones derived from p-substituted benzaldehyde (2a-g) and p-substituted-acetophenone (3a-f) were prepared as shown in Scheme 1.

The novel and valuable key intermediate, 4-(2-chloroethyl) semicarbazide intermediate (1), was prepared for the first time by adding 2-chloroethyl isocyanate dropwise to excess hydrazine hydrate in ethanol. Both the sequence of addition and room temperature were very important in order to minimize the formation of the expected side products. The intermediate 1 should be used directly in the next step, because it is very hygroscopic. Its structure was confirmed using the elemental analyses and mass spectroscopy.

The novel hydrazone derivatives of the 4-(2-chloroethyl)semicarbazide 2a-g or 3a-f were pre-
pared by condensation of intermediate 1 with the corresponding \( p \)-substituted benzaldehyde or acetophenones in ethanol containing few drops of glacial acetic acid (Scheme 1).

To achieve the second aim, \( p \)-substituted phenyl of benzaldehyde hydrazone (2) was replaced by 5-substituted indolyl moiety at position 1 of the semicarbazide (4) as shown in Scheme 2.

Thus, the novel 1-[(5-substituted-1H-indol-3-yl)methylidene]-4-(2-chloro ethyl)semicarbazides (4a-d) were synthesized by stirring the intermediate 1 with the corresponding 5-substituted 1H-indole-3-carboxaldehyde in dioxane \(^{14}\) containing few drops of glacial acetic acid at room temperature for two hours.

The new compounds were characterized using melting points and thin layer chromatography techniques in different solvent systems. Moreover, the structures of the novel compounds were determined using elemental analyses and various spectral data. From the mass spectra, the absence of molecular ion peaks for compounds 2b, 4c, 4d and the appearance of \( M^+ - HCl \) \((M^+ - 36.5)\) peaks were observed, indicating the instability of molecular ion peak which undergoes rapid fragmentation and also the low relative intensity of such peak in some compounds such as 2a (3.8\%) and 4b (5.9\%).

The structure of some compounds as for example 4d which showed \( M^+ -36.5 \) peak in the mass spectra was established using \(^1\)H-NMR. It confirmed the presence of straight chain for chloroethyl semicarbazide moiety through appearance of two peaks at various chemical shift 3.52 and 3.69 ppm for the two methylene groups as well as the appearance of two peaks at 6.78 and 10.05 ppm for the \( NH \) groups in addition to singlet peak at 8.07 ppm due to azomethine proton.

**B) Cytotoxic evaluation**

The new compounds 2a-g, 3a-f and 4a-d were evaluated for their \( in \) \( vitro \) cytotoxic activity against human brain (U251) and liver carcinoma (Hepg2)
4-(2-Chloroethyl) semicarbazide and its hydrazone derivatives: synthesis and exploration of potential cytotoxic activity

Table 1. Cytotoxic evaluation for compounds 2a-g, 3a-f and 4a-d against U251 and Hepg2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>U251 (µg/ml)</th>
<th>Hepg2 (µg/ml)</th>
<th>Compound</th>
<th>U251 (µg/ml)</th>
<th>Hepg2 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>35</td>
<td>28</td>
<td>3c</td>
<td>39</td>
<td>95</td>
</tr>
<tr>
<td>2b</td>
<td>25</td>
<td>65</td>
<td>3d</td>
<td>73</td>
<td>NA</td>
</tr>
<tr>
<td>2c</td>
<td>35</td>
<td>26</td>
<td>3e</td>
<td>72</td>
<td>6</td>
</tr>
<tr>
<td>2d</td>
<td>38</td>
<td>NA</td>
<td>3f</td>
<td>NA</td>
<td>8</td>
</tr>
<tr>
<td>2e</td>
<td>40</td>
<td>80</td>
<td>4a</td>
<td>21</td>
<td>7</td>
</tr>
<tr>
<td>2f</td>
<td>30</td>
<td>40</td>
<td>4b</td>
<td>65</td>
<td>NA</td>
</tr>
<tr>
<td>2g</td>
<td>NA</td>
<td>32</td>
<td>4c</td>
<td>28</td>
<td>63</td>
</tr>
<tr>
<td>3a</td>
<td>87</td>
<td>52</td>
<td>4d</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>3b</td>
<td>42</td>
<td>95</td>
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<td></td>
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</tr>
</tbody>
</table>

*IC50: Dose which required to inhibit cell growth by 50%.

It was observed through analysis of Table 1 that variable results were obtained depending not only on the type of the used cell line but also on the type of substitution at position 1 of the semicarbazides as seen in comparison of hydrazone derived from benzaldehyde (2a-g) with that of acetophenone (3a-f).

Firstly, regarding the U251 cell line, it could be concluded that cytotoxic activity has been increased in the order 3 > 2 as seen in comparison of the unsubstituted or methyl or chloro derivatives belonging to the general formulas 3 with that of 2.

Secondly, for Hepg2 cell line, the cytotoxic activity has increased in the order 2 > 3 as observed from IC50 values for compounds 3e (6 µg/ml) and 3f (8 µg/ml) which showed the higher cytotoxic activity than their corresponding 2 derivatives.

Table 1 revealed that replacement of phenyl ring of the parent compound 2a, (IC50 = 35 µg/ml against U251 and 53 µg/ml against Hepg2) by indole moiety as seen in compound 4a, (IC50 = 21 µg/ml against U251 and 7 µg/ml against Hepg2) led to an increase in the cytotoxic activity against the assigned cell lines. The last phenomena was also observed upon comparing the IC50 values for compounds 2e, 2f which have an electron donating substituents with their corresponding indole derivatives 4c, 4d. Thus, hydrazones 4a, 4c, 4d containing an indole moiety were more active than their corresponding p-substituted phenyl derivatives.

It was also found that all chloro derivatives 2d, 3d and 4b have no cytotoxic activity upon Hepg2 which could arise from biochemical variations between different cancer cell phenotypes.

**CONCLUSIONS**

The main objective of this study was to synthesize new hydrazone derivatives of 4-(2-chloroethyl)semicarbazide and exploration of their potential cytotoxic activity.

SAR studies revealed that:
- Regarding human brain carcinoma cell line, the hydrazones of the 4-(2-chloroethyl)semicarbazide derived from p-substituted benzaldehyde especially derivative 2b exhibited the highest cytotoxic activity while in case of human liver carcinoma cell line, hydrazones 3e, 3f belonging to acetophenone gave the highest cytotoxic activity.
- It could be concluded that replacement of p-substituted phenyl group of benzaldehyde hydrazones (2a, 2e, 2f) by indole moiety (4a, 4c, 4d) led to enhancement of the cytotoxic activity against the assigned cell lines.
- It was also found that semicarbazide 4a bearing an indole moiety was the most active one against the two cell lines and recommended for further future optimization to be potential candidate for new anticancer agents.

**EXPERIMENTAL**

A) Chemistry

**Materials and Methods**

Melting points were determined with a Gallenkamp digital melting point apparatus in open
capillaries and are uncorrected. IR spectra (KBr, cm⁻¹) were recorded on Bruker or Testscan Shimadzu FT 8000 spectrometer. ¹H NMR spectra were determined using DMSO-d₆ as a solvent and TMS as an internal standard (chemical shifts in δ, ppm). Mass spectra were measured on a GCMS-QP1000EX-SHAMADZU with an ionization energy equals to 70 eV. Elemental analyses were determined using Heraew and Vario EL-III (Elementar), CHNS analyzer (Germany) at Microanalytical Center, (Faculty of Science, Cairo University) and National Researches Centre, Egypt. All the results of elemental analyses corresponded to the calculated values within experimental error. TLC was performed on silica gel G (Fluka), and spots were visualized by UV irradiation (254 nm). Starting materials were purchased from Lancaster Synthesis Corporation (U.K.).

1. 4-(2-chloroethyl) semicarbazide. A solution of 2-chloroethylisocyanate (10 mmol) in EtOH (10 ml) was added dropwise to a stirred solution of hydrazine hydrate (15 mmol) in EtOH (20 ml). The resulting solution was stirred at rt for 12 h. The separated precipitate was dissolved in CHCl₃ and then filtered over anhydrous Na₂SO₄. The dried chloroformic solution was added to equal volume of petroleum ether (60-80) which led to separation of white precipitate in the form of clusters. The intermediate 1 was filtered, air dried and then used directly for preparation of final products. Yield: 20%; m.p.: 62-64 °C; MS: m/z (rel. int.) = 138 (M+, 68.3), 102 (44.4), 63 (100). Anal. Calcd. for C₃H₈ClN₃O: C, 26.19; H, 5.86; N, 30.55. Found: C, 25.96; H, 6.05; N, 30.65.

General procedure for the preparation of 2a-g and 3a-f. To a solution of the appropriate carbonyl starting material (1 mmol) in EtOH (10 ml) and 10 drops glacial acetic acid was added equimolar amount of 1. The resulting mixture was stirred for 2 h then added to 50 mL ice-cold water. The resulting precipitate was filtered under vacuum, washed with water and finally crystallized from EtOH.

2a. 1-Benzylidene-4-(2-chloroethyl) semicarbazide. Yield: 53%; m.p.: 112-113 °C; IR: ν = 3410, 3189 (NH), 3093 (CH, aromatic), 2957(CH, aliphatic), 1685 (C=O), 1605(C=N), 1543 (C=C) cm⁻¹; MS: m/z (rel. int.) = 227 (M⁺=2, 2.0), 226(M⁺+1, 2.5), 225 (M⁺, 3.8), 188 (9.3), 147 (3.6), 119 (100), 91 (17). Anal. Calcd. for C₁₀H₁₂ClN₃O: C, 53.22; H, 5.36; N, 18.62. Found: C, 53.03; H, 5.66; N, 18.45.

2b. 1-(p-Methylbenzylidene)-4-(2-chloroethyl) semicarbazide. Yield: 79%; m.p.: 148-150 °C; IR: ν = 3414, 3174 (NH), 3090 (CH, aromatic), 2944 (CH, aliphatic), 1674 (C=O), 1613(C=N), 1537 (C=C) cm⁻¹; MS: m/z (rel. int.) = 202(M⁺=36.5, 13.8), 133 (87.9), 91 (64.9), 73 (100). Anal. Calcd. for C₁₁H₁₄ClN₃O: C, 55.12; H, 5.89; N, 17.53. Found: C, 55.24; H, 5.41; N, 17.3.

2c. 1-(p-Fluorobenzylidene)-4-(2-chloroethyl) semicarbazide. Yield: 80%; m.p.: 161-163 °C; IR: ν = 3431, 3182 (NH), 3090 (CH, aromatic), 2953 (CH, aliphatic), 1685 (C=O), 1610(C=N), 1540 (C=C), 1225 (C-F) cm⁻¹; MS: m/z (rel. int.) = 245 (M⁺=2, 6.6), 244(M⁺+1, 8.1), 243 (M⁺, 19), 208 (0.7), 165 (3.0), 137 (100), 109 (9.3), 95 (29.05), 63 (35.7). Anal. Calcd. for C₁₀H₁₁ClFN₃O: C, 49.29; H, 4.55; N, 17.25. Found: C, 49.24; H, 4.62; N, 17.20.

2d. 1-(p-Chlorobenzylidene)-4-(2-chloroethyl) semicarbazide. Yield: 73%; m.p.: 175-177 °C; IR: ν = 3408, 3184 (NH), 3091 (CH, aromatic), 2944 (CH, aliphatic), 1678 (C=O), 1607(C=N), 1532 (C=C) cm⁻¹; ¹H NMR (90 MHz, δ): 3.45-3.50 (m, 2H, CH₂), 3.70-3.80 (m, 2H, CH₂), 7.35 (t, 1H, NHCH₂), 7.40-7.45 (d, 2H, ArH), 7.75-7.80 (d, 2H, ArH), 7.85 (s, 1H, CH = N), 10.60 (s, 1H, C=NNH) ppm; MS: m/z (rel. int.) = 259 (M⁺, 6.6), 222 (5), 181 (2.7), 154 (61.3), 119 (25.6), 73 (100). Anal. Calcd. for C₁₀H₁₂Cl₂N₃O: C, 46.39; H, 4.35; N, 16.09.

2e. 1-(p-Methoxybenzylidene)-4-(2-chloroethyl) semicarbazide. Yield: 65%; m.p.: 167-168 °C; IR: ν = 3401, 3181 (NH), 3014 (CH, aromatic), 2948 (CH, aliphatic), 1694(C=O), 1605(C=N), 1534 (C=C) cm⁻¹; MS: m/z (rel. int.) = 255 (M⁺, 39.5), 220(10.4), 219 (11.7), 150 (6.1), 165 (56.5), 149 (60.2), 133(81.6), 63(100.0). Anal. Calcd. for C₁₁H₁₄ClN₃O₂: C, 51.67; H, 5.52; N, 16.43. Found:
4-(2-Chloroethyl) semicarbazide의 허드라존 유도체 합성: 새로운 종류의 세포독성요법체

2f. 1-(p-Benzoyloxybenzylidene)-4-(2-chloroethyl) semicarbazide. Yield: 60%; m.p.: 145-147 °C; IR: ν = 3403, 3186 (NH), 3092 (CH, aromatic), 2959 (CH, aliphatic), 1687 (C=O), 1606(C=N), 1550 (C=C) cm⁻¹; ¹H NMR (200 MHz, δ): 3.42-3.48 (m, 2H, CH₂), 3.66-3.68 (m, 2H, CH₂), 5.14 (s, 2H, OCH₂), 7.02-7.05 (d, 2H, ArH), 7.18-7.22 (t, 1H, N/H/CH₂, exch.), 7.33-7.47 (m, 5H, ArH), 7.64-7.67 (d,2H,ArH),7.79(s,1H,CH=N);10.29(s,1H,NNH, exch.)ppm; Anal. Calcd. For C₁₇H₁₈ClN₃O₂: C, 61.54; H, 5.47; N, 12.66. Found: C, 61.77; H, 5.68; N, 12.18.

2g. 1-(p-Nitrobenzylidene)-4-(2-chloroethyl) semicarbazide. Yield: 75%; m.p.: 190-192 ℃; IR: ν = 3420, 3200 (NH), 3082 (CH, aromatic), 2940 (CH, aliphatic), 1682(C=O), 1598(C=N), 1575(C=C), 1533(NO₂) cm⁻¹; MS: m/z (rel. int.) = 273(M++2, 6.0), 272(M++1, 3.8), 271 (M +, 13.4), 270 (3.4), 235 (3.7), 166 (6.1), 165 (51.4), 63 (100.0). Anal. Calcd. for C₁₀H₁₁ClN₄O₃: C, 44.37; H, 4.10; N, 20.70. Found: C, 44.56 ; H, 4.22 ; N, 20.60.

3a. 1-(1-Phenylethylidene)-4-(2-chloroethyl) semicarbazide. Yield: 37%; m.p.: 120-121 ℃; IR: ν = 3415, 3185 (NH), 3077 (CH, aromatic), 2939 (CH, aliphatic), 1677 (C=O), 1613 (C=N), 1533 (C=C) cm⁻¹; MS: m/z (rel. int.) = 240(M⁺+1, 1.8), 239 (M⁺, 15.3), 133 (62.23), 77 (100), 63 (64.0). Anal. Calcd. for C₁₁H₁₄ClN₃O: C, 55.12; H, 5.89; N, 17.53. Found: C, 55.02; H, 5.95; N, 17.36.

3b. 1-[1-(p-Methoxyphenyl)ethylidene]-4-(2-chloroethyl) semicarbazide. Yield: 55%; m.p.: 148-150 ℃; IR: ν = 3415, 3189 (NH), 3083 (CH, aromatic), 2946 (CH, aliphatic), 1671 (C=N), 1530 (C=C) cm⁻¹; ¹H NMR (90 MHz, δ): 2.16 (s, 3H, CH₃C=N), 3.47-3.52 (m, 2H, CH₂), 3.65-3.69 (m, 2H, CH₂), 7.17-7.72 (d, 2H, ArH), 8.10(br s, 1H, N/H/CH₂), 9.45 (s, 1H, C=NNH)ppm; MS: m/z (rel. int.) = 255(M⁺+2, 4.5),254(M⁺+1, 5.0), 253 (M⁺, 11.7), 218 (2.5), 175 (5.6), 147 (100), 117 (29.2), 91 (61.2). Anal. Calcd. for C₁₁H₁₃BrClN₄O : C, 56.80; H, 6.36; N, 16.56. Found: C, 56.42; H, 6.51; N, 16.65.

3c. 1-[1-(p-Bromophenyl)ethylidene]-4-(2-chloroethyl) semicarbazide. Yield: 40%; m.p.: 177-179 ℃; IR: ν = 3413, 3195 (NH), 3086 (CH, aromatic), 2957 (CH, aliphatic), 1676 (C=O), 1608 (C=N), 1523 (C=C) cm⁻¹; ¹H NMR (90 MHz, δ): 2.15 (s, 3H, CH₃C=N), 3.45-3.55 (m, 2H, CH₂), 3.65-3.75 (t, 2H, CH₂), 7.35 (t, 1H, N/H/CH₂), 7.55-7.60 (d, 2H, ArH), 7.80-7.85 (d, 2H, ArH), 9.65 (s, 1H, C=NNH)ppm; MS: m/z (rel. int.) = 320(M⁺+1, 3.2), 319 (M⁺, 9.1), 280 (11.3), 213 (49.7), 212 (49.0), 182 (24.6), 103 (66.7), 63 (100). Anal. Calcd. for C₁₁H₁₃BrClN₄O : C, 41.47; H, 4.11; N, 15.19. Found: C, 41.64; H, 4.02; N, 13.30.

3d. 1-[1-(p-Chlorophenyl)ethylidene]-4-(2-chloroethyl) semicarbazide. Yield: 32%; m.p.: 160-162 ℃; IR: ν = 3416, 3185 (NH), 3078 (CH, aromatic), 2940 (CH, aliphatic), 1676 (C=O), 1613 (C=N), 1533(C=C) cm⁻¹; MS: m/z (rel. int.) = 275(M⁺+2, 7.7),273 (M⁺, 14.2), 238 (10.2), 195 (14.8), 167 (73.3), 137 (17.4), 103 (53.6), 63 (100). Anal. Calcd. for C₁₁H₁₃Cl₂N₄O : C, 48.19; H, 4.78; N, 15.33. Found: C, 48.06; H, 4.63; N, 15.51.

3e. 1-[1-(p-Nitrophenyl)ethylidene]-4-(2-chloroethyl) semicarbazide. Yield: 39%; m.p.: 155-157 ℃; IR: ν = 3405, 3192 (NH), 3083 (CH, aromatic), 2947(CH, aliphatic), 1675 (C=O), 1608 (C=N), 1533(C=C) cm⁻¹; MS: m/z (rel. int.) = 269 (M⁺, 36.9), 233 (10.5), 191 (12.7), 163(100), 134 (43.6), 63 (67.0). Anal. Calcd. for C₁₁H₁₃ClN₃O₂ : C, 53.43; H, 5.98; N, 15.58. Found: C, 53.45; H, 5.94; N, 15.63.

3f. 1-[1-(p-Methoxyphenyl)ethylidene]-4-(2-chloroethyl) semicarbazide. Yield: 87%; m.p.: 251-253 ℃; IR: ν = 3436, 3182 (NH), 3092 (CH, aromatic), 2972 (CH, aliphatic), 1679 (C=O), 1613 (C=N), 1575(C=C) cm⁻¹; MS: m/z (rel. int.) = 284 (M⁺, 13.1), 249 (7.3), 179 (100), 149(4,2), 103 (14.1), 63 (32.88). Anal. Calcd. for C₁₁H₁₃ClN₄O₃ : C, 46.41; H, 4.60; N, 19.68. Found: C, 46.41; H, 4.57; N, 19.79.

General procedure for the preparation of 4a-d
To a solution of the appropriate 5-substituted-1H-indole-3-carboxaldehyde (1 mmol) in dioxane (10 ml), an equimolar amount of I and glacial acetic acid (10 drops) were added. The resulting mixture was stirred for 2 h and then added to 50
mL ice-cold water. The separated product was filtered under vacuum, washed with water, and finally crystallized from EtOH.

4a. 1-[(1H-indol-3-yl)methylidene]-4-(2-chloroethyl) semicarbazide. Yield: 54%; m.p.: 176-178 °C; IR: \(v = 3399-3210\) (NH), 3056 (CH, aromatic), 2979, (CH, aliphatic), 1663 (C=O), 1621 (C=N), 1544(C=C) cm\(^{-1}\); \(^1\)H NMR (90 MHz, \(\delta\)): 3.45-3.54 (m, 2H, CH\(_2\)), 3.65-3.75 (t, 2H, CH\(_2\)), 6.85 (t, 1H, N/CH\(_2\)), 7.10-8.20 (m, 5H, ArH + CH of fused pyrole), 8.05 (s, 1H, HC=N), 10.05 (s, 1H, C=NNH), 11.50 (s, 1H, NH of indole) ppm; MS: \(m/z\) (rel. int.) = 264 (M\(^+\), 21.5), 228 (19.5), 185 (12.2), 158 (22.3), 142 (100.0), 129 (75.6), 116 (62.3), 105 (59.0). Anal. Calcd. for C\(_{12}\)H\(_{13}\)ClN\(_4\)O: C, 54.45; H, 4.95; N, 21.17. Found: C, 54.54; H, 5.36; N, 20.79.

4b. 1-[(5-Chloro-1H-indol-3-yl)methylidene]-4-(2-chloroethyl) semicarbazide. Yield: 50%; m.p.: 219-221 °C; IR: \(v = 3389-3173\), (NH), 3091 (CH, aromatic), 2913 (CH, aliphatic), 1656 (C=O), 1610 (C=N), 1543 (C=C) cm\(^{-1}\); MS: \(m/z\) (rel. int.) = 300 (M\(^+\) 2, 4.3), 298 (M\(^+\), 5.9), 262 (28.5), 193 (14.3), 163 (100), 151 (12.1), 63 (59.0). Anal. Calcd. for C\(_{12}\)H\(_{12}\)Cl\(_2\)N\(_4\)O: C, 48.18; H, 4.04; N, 18.73. Found: C, 48.18; H, 4.37; N, 18.44.

4c. 1-[(5-Methoxy-1H-indol-3-yl)methylidene]-4-(2-chloroethyl) semicarbazide. Yield: 71%; m.p.: 189-190 °C; IR: \(v = 3418-3230\) (NH), 3081 (CH, aromatic), 2933, (CH, aliphatic), 1657 (C=O), 1619 (C=N), 1538 (C=C) cm\(^{-1}\); MS: \(m/z\) (rel. int.) = 259 (M\(^+\)-36.5, 17.4), 172 (69.6), 160 (23.2), 116(62.3), 87(100.0), 63(97.1). Anal. Calcd. for C\(_{13}\)H\(_{15}\)ClN\(_4\)O\(_2\): C, 52.98; H, 5.13; N, 19.01. Found: C, 52.95; H, 5.54; N, 18.82.

4d. 1-[(5-Benzoyloxy-1H-indol-3-yl)methylidene]-4-(chloroethyl) semicarbazide. Yield: 37%; m.p.: 162-163 °C; IR: \(v = 3420-3170\) (NH), 3094 (CH, aromatic), 2930 (CH, aliphatic), 1667 (C=O), 1621 (C=N), 1577 (C=C, aromatic) cm\(^{-1}\); \(^1\)H NMR (200 MHz, \(\delta\)): 3.52-3.56 (m, 2H, CH\(_2\)), 3.69-3.73 (t, 2H, CH\(_2\)), 5.16 (s, 2H, OCH\(_2\)), 6.78 (t, 1H, N/CH\(_2\)), 6.87-7.71 (m, 9H, ArH + CH of fused pyrole), 8.07 (s, 1H, HC=N), 10.05 (s, 1H, C=NNH, exch.), 11.34 (s, 1H, NH of indole, exch.) ppm; MS: \(m/z\) (rel. int.) = 334 (M\(^+\)-36.5, 30.1), 265 (19.4), 248 (11.7), 146(28.1), 91 (100.0), 63 (17.9). Anal. Calcd. for C\(_{19}\)H\(_{18}\)ClN\(_4\)O\(_2\): C, 61.54; H, 5.16; N, 15.11. Found: C, 61.20; H, 5.53; N, 14.87.

B) Cytotoxic activity evaluation. The Sulforhodamine B (SRB) assay of Skehan\(^{15}\) was used to evaluate the cytotoxic activity of the semicabazides 2a-g,3a-f and 4a-d against two cell lines, U251 and Hepg2. These assays were performed at the Cancer Biology Department, Pharmacology Unit, National Cancer Institute, Cairo, Egypt. Cells were plated in 96-multiwell plates (10\(^4\) cells/well) for 24 h before treatment with the compound to allow attachment of the cells to the wall of the plate. Using DMSO (0.02%) and saline solution as solvent system\(^{16,17}\), different concentrations of the compounds under test (10, 25, 50, 100 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each dose. Monolayer cells were incubated with the compound under test for 48 h at 37 °C and in atmosphere of 5% CO\(_2\). After 48 hours, cells were fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and then after attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. IC\(_{50}\) value was determined for each tumor cell line for the specified compound, calculated by an available computerized program, which was defined as the concentration of drug to produce a 50% reduction in the viability relative to the control and results were recorded in table 1.

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