

# The Reaction of Cyanoacetylhydrazine with Chloroacetone: Synthesis of 1,2,4-Triazine, 1,3,4-Oxadiazine and Their Fused Derivatives with Antitumor Activities

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## ABSTRACT

The reaction of cyanoacetylhydrazine with chloroacetone gave the N-(1-chloropropan-2-ylidene)-2-cyanoacetylhydrazide. This compound reacted with either hydrazine hydrate or phenylhydrazine to give the corresponding 1,2,4-triazine derivatives. On the other hand, its reaction with either benzenediazonium chloride or benzaldehyde gave in each case the 1,3,4-oxadiazine derivatives. Moreover, the reaction of the cyanoacetylhydrazine with 2-bromocyclohexanone gave the corresponding hydrazine-hydrazone derivative. The antitumor evaluation of the newly synthesized products against three cancer cell lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) was recorded. Some of the tested compounds showed activities which was higher than the reference doxorubicin.

**Keywords:** 1,2,4-Triazine; 1,3,4-Oxadiazine; Pyrazole; Antitumor

## 1. Introduction

It is well known that the hydrazone group plays an important role for the antimicrobial activity. Furthermore, a number of hydrazone-hydrazone claimed to possess interesting antibacterial antifungal [1-3] anticonvulsant [4-6] anti-inflammatory [7-9] antimalarial [10] and anti-tuberculosis activities [11-15]. With the aim of obtaining new hydrazone-hydrazone with such wide spectrum of pharmaceutical applications, we report here the synthesis of a series of hydrazone-hydrazone via the reaction of cyanoacetylhydrazine (**1**) with  $\alpha$ -halo ketones [16,17]. All synthesized compounds have been screened for anti-tumor activity against breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268).

## 2. Results and Discussions

The reaction of **1** with chloroacetone (**2**) in 1,4-dioxane gave the hydrazone-hydrazone derivative **3**. The structure of compound **3** was based on the analytical and spectral data. Thus, the <sup>1</sup>H NMR spectrum of the reaction product showed the presence of a singlet at  $\delta$  2.36 ppm corresponding to CH<sub>3</sub> group, two singlets at  $\delta$  3.37, 4.02 ppm

corresponding to two CH<sub>2</sub> groups and a singlet at  $\delta$  8.09 ppm for the NH group. Further confirmation for the structure of compound **3** was obtained through studying its reactivity towards some chemical reagents. Thus, the reaction of **3** with either hydrazine hydrate (**4a**) or phenylhydrazine (**4b**) gave the 1,2,4 triazine derivatives **6a** and **6b**, respectively. Formation of the latter products is explained through the intermediate formation of **5a,b** followed by water elimination.

The reaction of **6b** with benzenediazonium chloride (**7**) gave the phenyl hydrazone derivative **9** (Scheme 1). The reaction took place through the first coupling of **6** with **7** to give the phenylhydrazone derivative **8** followed by Michael addition of the N-NH group present in the triazine ring to the cyano group to give the pyrazolo[5-1-c][1,2,4]triazine derivative **9**. On the other hand, the reaction of **6b** with either malononitrile (**10a**) or ethyl cyanoacetate (**10b**) gave the tricyclic products **11a** and **11b**, respectively. Analytical and spectral data of **11a,b** are consistent with the proposed structures (see experimental section). The reaction of compound **3** with either benzenediazonium chloride **7** or p-chlorobenzenediazonium chloride **12** gave 1,3,4-oxadiazine derivatives **13a** and **13b** respectively. On the other hand, the reaction of compound **3** with potassium cyanide (**14**) gave the 3-amino

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or p-chlorobenzenediazonium chloride (**12**) gave the  $\alpha$ -phenyl hydrazone acetonitrilo-tetrahydrobenzo[e]-1,3,4 oxadiazine derivatives **25a,b**. The analytical and spectral data the latter products are in agreement with the proposed structure (**Scheme 3**).

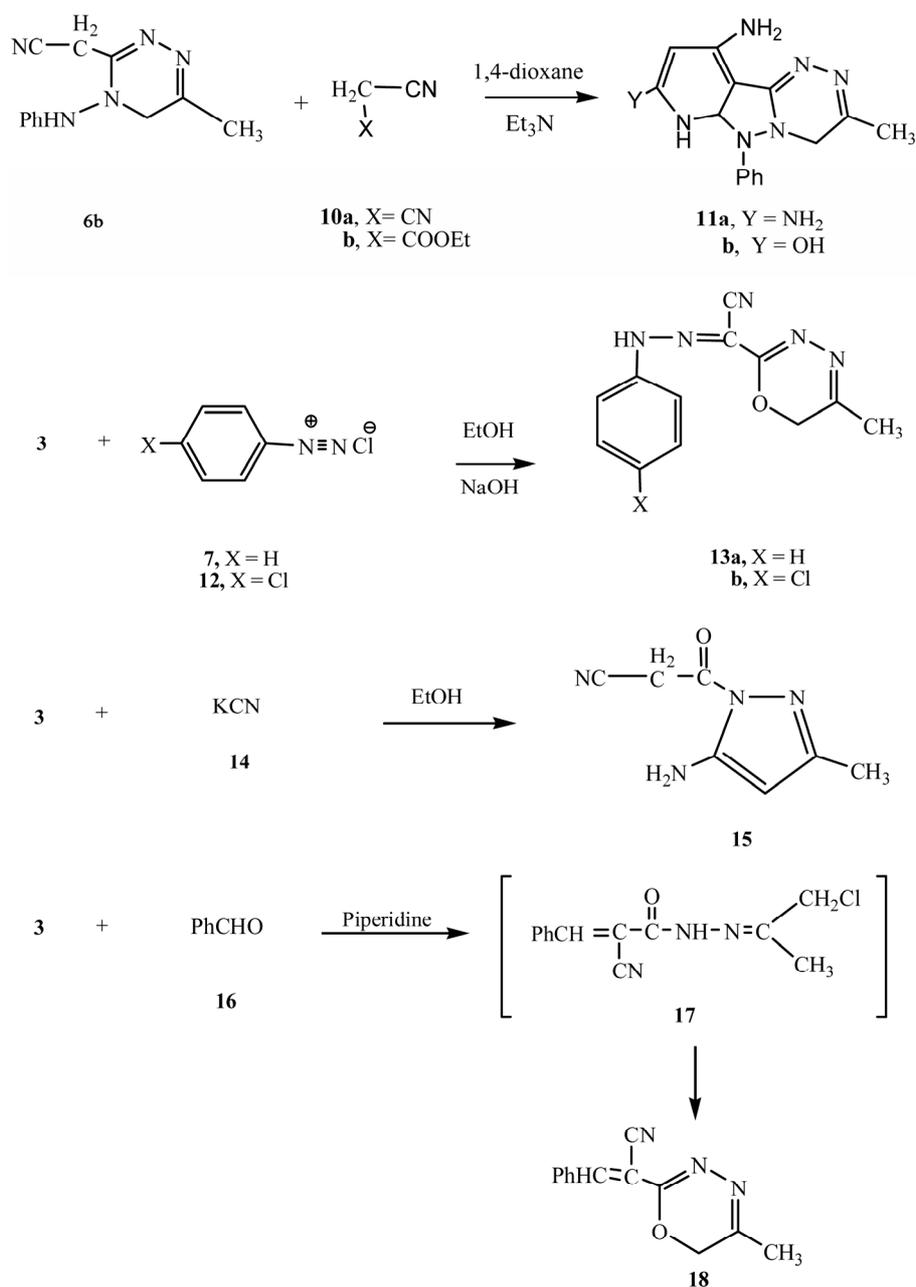
### 3. Antitumor Activity

#### 3.1. Material and Methods & Reagents

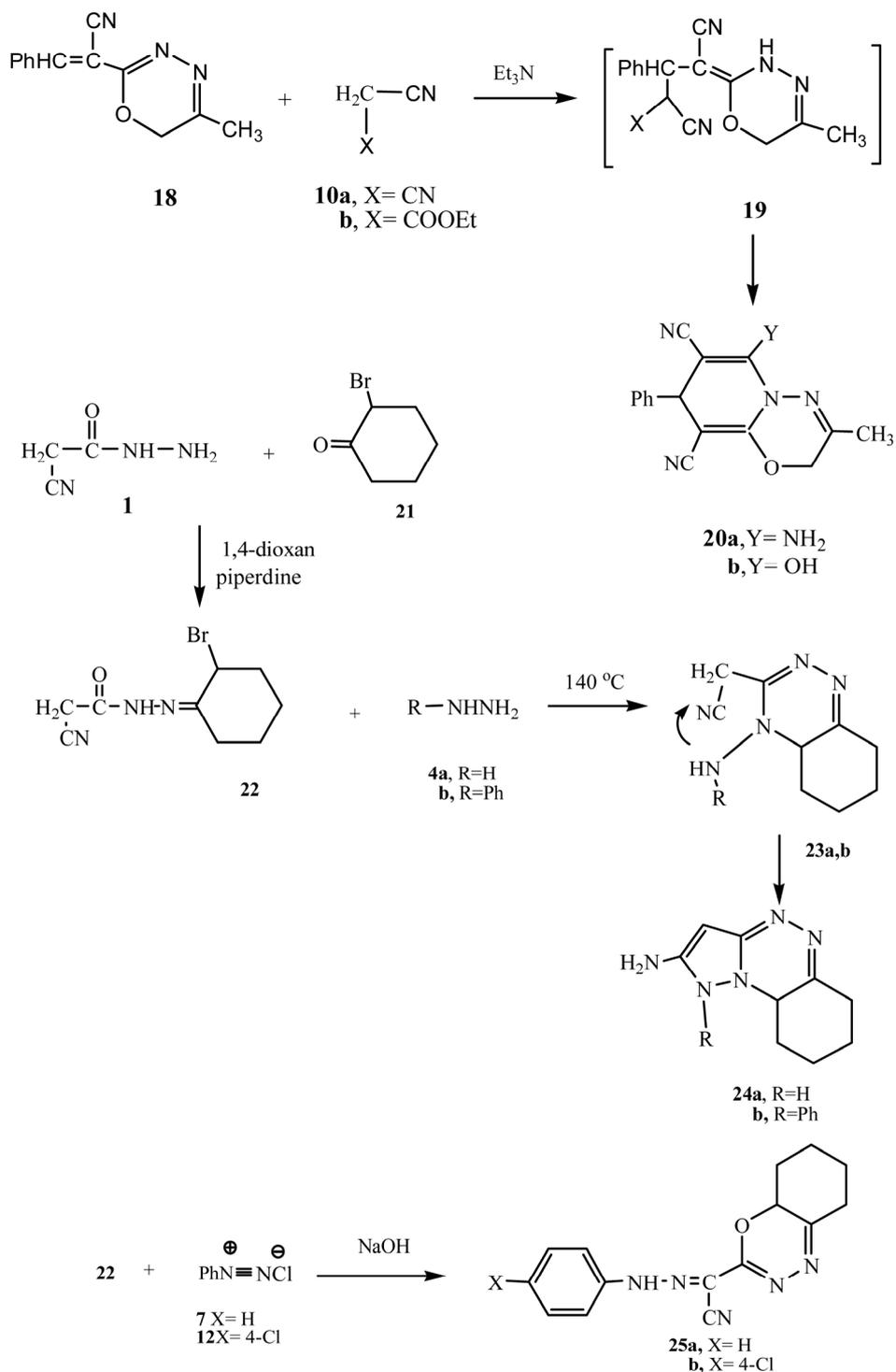
Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl

sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA). Samples: Stock solutions of new compounds through **3 - 25b** were prepared in DMSO and kept at  $-20^{\circ}\text{C}$ . Appropriate dilutions of the compounds were freshly prepared just prior the assays. Final concentrations of DMSO did not interfere with the cell growth.

*Cell cultures.* Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were



**Scheme 2.** Synthesis of compounds **11a,b-18**.



Scheme 3. Synthesis of compounds 20a,b - 25a,b.

kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 g/mL), at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

Exponentially growing cells were obtained by plating  $1.5 \times 10^5$  cells/mL for MCF-7 and SF-268 and  $0.75 \times 10^4$  cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum con-

centration (0.5%) of DMSO used in each assay.

### 3.2. Effect on the Growth of Human Tumor Cell Lines

The effect of compounds **3** - **25b** was evaluated on the *in vitro* growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268), after a continuous exposure of 48 h. The results are summarized in **Table 1**. All the compounds were able to inhibit the growth of the human tumor cell lines in a dose dependent manner (data not shown). Compound **11a** (7-methyl-9-phenyl-8,9-dihydro-1,5,6,8a,9-pentaaza-fluorene-2,4-diamine) and **20b** (2,8-dihydro-6-hydroxy-3-methyl-8-phenylpyrido[2,1-b][1,3,4]oxadiazine-7,9-dicarbonitrile) showed the best results, exhibiting an equivalent potency in all the three tumor cell lines; these values are much lower than that of the gram positive control doxorubicin. Compounds **20b** and **24b** are the following in relation to the anticancer activity. On the other hand, compounds **3**, **6a**, **9**, **11a**, **18**, **20a**, **22**, **24a** and **25b** showed moderated growth inhibitory effect which showed  $GI_{50} < 30 \mu\text{mol}\cdot\text{L}^{-1}$ .

Comparing the activities of **20a** and **20b** it is observed that the 2-amino group in **20a** showed a stronger growth inhibitory effect than the 2-hydroxy substituent in **20b** although the results in NCI-H460 cell line are comparable. Also comparing compounds **24a** and **24b**, it is obvious

**Table 1.** Effect of compounds **3** - **25b** on the growth of three human tumor cell lines.

Compound No.	$GI_{50}$ ( $\mu\text{mol}\cdot\text{L}^{-1}$ )		
	MCF-7	NCI-H460	SF-268
<b>3</b>	$8.0 \pm 0.3$	$12.6 \pm 1.8$	$12.3 \pm 1.0$
<b>6a</b>	$12.0 \pm 2.2$	$22.3 \pm 3.6$	$33 \pm 0.4$
<b>6b</b>	$66.2 \pm 12.7$	$30.9 \pm 8.2$	$42.8 \pm 8.3$
<b>9</b>	$10.2 \pm 0.8$	$8.6 \pm 1.4$	$6.2 \pm 1.6$
<b>11a</b>	$0.02 \pm 0.002$	$0.03 \pm 0.01$	$0.01 \pm 0.002$
<b>11b</b>	$30.5 \pm 12.0$	$20.1 \pm 12.8$	$20.0 \pm 4.31$
<b>13a</b>	$30.2 \pm 2.2$	$22.2 \pm 6.2$	$12.6 \pm 2.6$
<b>13b</b>	$16.2 \pm 2.2$	$20.6 \pm 1.6$	$33.2 \pm 3.7$
<b>15</b>	$30.0 \pm 2.5$	$22.0 \pm 4.6$	$20.5 \pm 2.8$
<b>18</b>	$14.0 \pm 0.4$	$12.3 \pm 0.6$	$26.5 \pm 4.3$
<b>20a</b>	$8.4 \pm 2.8$	$14.1 \pm 0.6$	$20.3 \pm 0.5$
<b>20b</b>	$0.02 \pm 0.002$	$0.01 \pm 0.008$	$0.02 \pm 0.008$
<b>22</b>	$22.8 \pm 10.0$	$30.2 \pm 6.4$	$18.2 \pm 10.6$
<b>24a</b>	$20.2 \pm 8.2$	$16.0 \pm 2.6$	$12.2 \pm 4.8$
<b>24b</b>	$0.4 \pm 0.08$	$0.2 \pm 0.04$	$0.1 \pm 0.01$
<b>25a</b>	$40.6 \pm 10.0$	$32.6 \pm 8.0$	$62.4 \pm 14.8$
<b>25b</b>	$12.2 \pm 4.0$	$16.0 \pm 2.8$	$14.2 \pm 4.6$
Doxorubicin	$0.04 \pm 0.008$	$0.09 \pm 0.008$	$0.09 \pm 0.007$

Results are given in concentrations that were able to cause 50% of cell growth inhibition ( $GI_{50}$ ) after a continuous exposure of 48 h and show means  $\pm$  SEM of three-independent experiments performed in duplicate.

that compound **24b** which is the (1-phenyl-1,6,7,8,9a-hexahydro-benzo[e]pyrazolo[5,1-c][1,2,4]triazin-2-ylamine) with its N-H group is much higher than **24b** with its N-Ph group. It is obvious that compounds **6b**, **11b**, **13a** and **25a** showed the lowest inhibitory effect towards the three cell line. The relative inhibitory effect of the newly synthesized and the reference doxorubicin, towards the three cancer cell lines, is indicated through **Figure 1**.

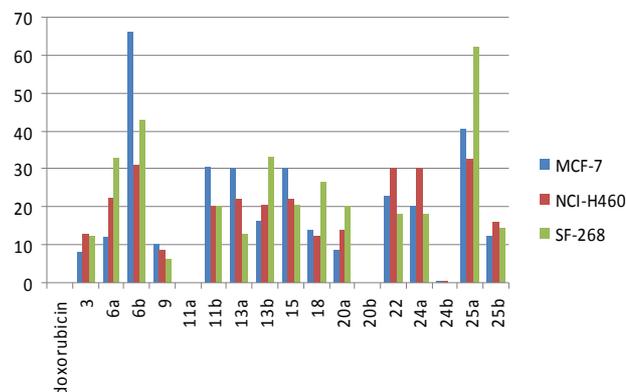
## 4. Experimental

### Materials

The requisite starting materials such as chloroacetone, hydrazine hydrate, phenyl hydrazine, sodium nitrite, malononitrile, ethyl cyanoacetate, aniline, p-chloro aniline, potassium cyanide, benzaldehyde, bromocyclohexanone were produced from Aldrich Company and used without any further purification. All the solvents were purified and dried by standard method. All melting points were determined in open capillaries and are uncorrected. IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer.  $^1\text{H}$ NMR spectra was measured on a varian EM390-200 MHz instrument in DMSO as solvent using tetramethylsilane (TMS) as internal reference standard. The chemical shifts are quoted as  $\delta$  ppm.

### N-(1-Chloropropan-2-ylidene)-2-cyanoacetohydrazide (**3**).

Equimolecular amounts of **1** (0.99 g, 0.01 mol) and chloroacetone (**2**) (0.92 g, 0.01 mol) in 1,4-dioxane (25 mL) and drops of dimethylformamide (5 mL) were stirred at room temperature for 1 h. The formed solid product was collected by filtration. White crystals from ethanol in a yield 88% (1.52) g and with m.p.  $160^\circ\text{C}$ ; Calculated for  $\text{C}_6\text{H}_8\text{N}_3\text{OCl}$  (173.59): C, 41.51; H, 4.64; N, 24.20. Found: C, 40.89; H, 4.10; N, 23.75. IR ( $\nu/\text{cm}^{-1}$ ) = 3450 - 3329 (NH), 2892, 2877 ( $\text{CH}_3$ ,  $\text{CH}_2$ ), 2260 (CN), 1688 (C=O).  $^1\text{H}$ NMR ( $\delta$  ppm): 2.36 (s, 3H,  $\text{CH}_3$ ), 3.37,



**Figure 1.** Inhibition of cell growth of the newly synthesized products towards the three cancer cell lines.

4.02 (2s, 4H, 2CH<sub>2</sub>), 8.09 (s, 1H, NH); MS: *m/z* 173 (M<sup>+</sup>, 10%).

**3**[(4-Amino-6-methyl-4,5-dihydro-[1,2,4]triazin-3-yl)]-acetonitrile (**6a**) and [6-Methyl-4-(N<sup>1</sup>-phenyl-hydrazino)-4,5-dihydro-[1,2,4]triazin-3-yl]-acetonitrile (**6b**).

*General procedure:* To a solution of **3** (1.73 g, 0.01 mol) in 1,4-dioxane (30 mL), and either hydrazine hydrate (**4a**) (0.50 g, 0.01 mol) or phenyl hydrazine (**4b**) (1.08 g, 0.01 mol) were added. The reaction mixture was kept on ice bath for 10 min. The solid product formed upon dilution with ice/water containing hydrochloric acid (till pH<sub>6</sub>), was collected by filtration.

**Compound 6a:** Brown crystals from ethanol, yield, 60% (0.9 g, m.p. >300°C. *Calculated for* C<sub>6</sub>H<sub>9</sub>N<sub>5</sub> (151.16): C, 47.67; H, 6.00; N, 46.33. Found: C, 47.00; H, 5.55; N, 45.86. IR (ν/cm<sup>-1</sup>) = 3466 - 3328 (NH<sub>2</sub>, NH), 3061 (CH aromatic), 2988 (CH<sub>3</sub>), 2226 (CN), 1658 (C=N), 1643 (C=C). <sup>1</sup>HNMR (δ ppm): 2.86 (s, 3H, CH<sub>3</sub>), 4.23 (s, 2H, CH<sub>2</sub>), 5.08 (s, 2H, NH<sub>2</sub>), 5.21 (s, 2H, triazine CH<sub>2</sub>); MS: *m/z* 151 (M<sup>+</sup>, 60%).

**Compound 6b:** Buff crystals from 1,4-dioxane, yield, 72% (1.63 g, m.p. 180°C. *Calculated for* C<sub>12</sub>H<sub>13</sub>N<sub>5</sub> (227.26): C, 63.42; H, 5.70; N, 30.80. Found: C, 62.89; H, 4.88; N, 29.99. IR (ν/cm<sup>-1</sup>) = 3476 - 3331 (NH), 3055 (CH aromatic), 2982 (CH<sub>3</sub>), 2261 (CN), 1655 (C=N), 1648 (C=C). <sup>1</sup>HNMR (δ ppm): 2.83 (s, 3H, CH<sub>3</sub>), 4.19 (s, 2H, CH<sub>2</sub>), 5.28 (s, 2H, triazine CH<sub>2</sub>), 7.28 - 7.39 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 8.78 (s, 1H, NH); MS: *m/z* 227 (M<sup>+</sup>, 40%).

**8-(2-Phenylhydrazono)-3-methyl-6-phenylpyrazolof 5,1-c][1,2,4]triazin-7(4H,6H,8H)-imine (9).**

To a cold solution (0°C - 5°C) of **6b** (2.27 g, 0.01 mol) in ethanol (30 mL) containing sodium hydroxide (10%, 6 mL) benzenediazonium chloride (**7**) (0.01 mol) [obtained via the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution (0°C - 5°C) of aniline (0.93 g, 0.01 mol) in hydrochloric acid (8 mL) was added with continuous stirring ] was added with continuous stirring. The reaction mixture was stirred for an additional 2 h then the formed solid product was collected by filtration. Orange crystals from ethanol in a yield, 80%, (2.65) g, with m.p. 236°C - 240°C. *Calculated for* C<sub>18</sub>H<sub>17</sub>N<sub>7</sub> (331.15): C, 65.22; H, 5.13; N, 29.59. Found: C, 64.66; H, 4.76; N, 28.91. IR (ν/cm<sup>-1</sup>) = 3455 - 3323 (2NH), 3052 (CH aromatic), 2980 (CH<sub>3</sub>), 1655 (C=N), 1648 (C=C). <sup>1</sup>HNMR (δ ppm): 2.83 (s, 3H, CH<sub>3</sub>), 4.65 (s, 1H, D<sub>2</sub>Oexchangeable, NH), 5.26 (s, 2H, triazine CH<sub>2</sub>), 7.29 - 7.37 (m, 10H, 2C<sub>6</sub>H<sub>5</sub>), 8.82 (s, 1H, D<sub>2</sub>Oexchangeable, NH); MS: *m/z* 331 (M<sup>+</sup>, 100%).

**7-Methyl-9-phenyl-8,9-dihydro-1,5,6,8a,9-pentaazafluorene-2,4-diamine (11a) and 4-Amino-7-methyl-9-phenyl-8,9-dihydro-1,5,6,8a,9-pentaazafluorene-2-ol (11b).**

*General procedure:* Equimolecular amounts of **6b** (2.27 g, 0.01 mol) and either malononitrile (**10a**) (0.66 g,

0.01 mol) or ethyl cyanoacetate (**10b**) (1.13 g, 0.01 mol) were added in 1,4-dioxane (25 mL) containing triethylamine (1.00 mL) were heated under reflux for 4 h. The formed solid product upon dilution with ice/water containing hydrochloric acid (till pH<sub>6</sub>), was collected by filtration.

**Compound (11a):** Red crystals from ethanol, yield 76% (2.22) g, m.p. 160°C. *Calculated for* C<sub>15</sub>H<sub>17</sub>N<sub>7</sub> (293.33) C, 61.00; H, 5.80; N, 33.20. Found: C, 61.27; H, 5.53; N, 33.44. IR (ν/cm<sup>-1</sup>) = 3473 - 3344 (2NH<sub>2</sub>), 3057 (CH aromatic), 2980 (CH<sub>3</sub>), 1662 (C=N), 1640 (C=C). <sup>1</sup>HNMR (δ ppm): 2.79 (s, 3H, CH<sub>3</sub>), 4.43, 5.18 (2s, 4H, 2NH<sub>2</sub>), 5.20 (s, 2H, triazine CH<sub>2</sub>), 7.23 - 7.36 (m, 8H, C<sub>6</sub>H<sub>5</sub>, pyridine 3H); MS: *m/z* 295 (M<sup>+</sup>, 65%).

**Compound (11b):** Red crystals from ethanol, yield 77% (2.26) g, m.p. 140°C. *Calculated for* C<sub>15</sub>H<sub>15</sub>N<sub>6</sub>O (296.33): C, 61.80; H, 5.44; N, 28.36. Found: C, 60.82; H, 5.64; N, 28.49. IR (ν/cm<sup>-1</sup>) = 3563 - 3324 (OH, NH<sub>2</sub>), 3053 (CH aromatic), 2980 (CH<sub>3</sub>), 1657 (C=N), 1637 (C=C). <sup>1</sup>HNMR (δ ppm): 2.82 (s, 3H, CH<sub>3</sub>), 4.32 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 5.18 (s, 2H, triazine CH<sub>2</sub>), 7.16 - 7.39 (m, 8H, C<sub>6</sub>H<sub>5</sub>, pyridine 3 h), 10.21 (s, 1H, OH); MS: *m/z* 296 (M<sup>+</sup>, 30%).

**2-(2-Phenylhydrazono)-2-(5-methyl-6H-1,3,4-oxadiazin-2-yl)acetonitrile (13a) and 2-(2-p-chlorolhydrazono)-2-(5-methyl-6H-1,3,4-oxadiazin-2-yl)acetonitrile (13b).**

*General procedure:* To a cold solution (0°C - 5°C) of **3** (1.73 g, 0.01 mol) in ethanol (30 mL) containing sodium hydroxide (10%, 6 mL) either benzenediazonium chloride (**7**) (0.01 mol) or P-chloro benzenediazonium chloride (**12**) obtained via the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution (0°C - 5°C) of aniline or p-chloro aniline (1.27 g, 0.01 mol) (0.01 mol) (0.93 g, 0.01 mol) in hydrochloric acid (8 mL) was added with continuous stirring was added with continuous stirring. The reaction mixture was stirred for an additional 2 h then the formed solid product was collected by filtration.

**Compound 13a:** Red crystals from ethanol, yield, 71% (1.71) g, m.p. 236°C - 240°C. *Calculated for* C<sub>12</sub>H<sub>11</sub>N<sub>5</sub>O (241.23): C, 59.74 ; H, 4.59; N, 29.03. Found: C, 60.01; H, 4.93; N, 28.7 0. IR (ν/cm<sup>-1</sup>) = 3467 - 3322 (NH), 3055 (CH aromatic), 2974 (CH<sub>3</sub>), 2227 (CN), 1656 (C=N), 1637 (C=C). <sup>1</sup>HNMR (δ ppm): 2.66 (s, 3H, CH<sub>3</sub>), 5.26 (s, 2H, triazine CH<sub>2</sub>), 7.23 - 7.36 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 8.26 (s, 1H, NH); MS: *m/z* 241 (M<sup>+</sup>, 70%).

**Compound 13b:** Red crystals from ethanol, yield, 70% (1.92 g), m.p. 100°C. *Calculated for* C<sub>12</sub>H<sub>10</sub>N<sub>5</sub>OCl (275.68): Calcd: C, 52.28; H, 3. 66; N, 25. 40. Found: C, 51.97; H, 3.45; N, 25.23. IR (ν/cm<sup>-1</sup>) = 3573 - 3312 (NH), 3053 (CH aromatic), 2983 (CH<sub>3</sub>), 1650 (C=N), 1634 (C=C). <sup>1</sup>HNMR (δ ppm): 2.80 (s, 3H, CH<sub>3</sub>), 5.16 (s, 2H, triazine CH<sub>2</sub>), 7.28 - 7.37 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 8.24 (s, 1H,

NH); MS:  $m/z$  275 ( $M^+$ , 80%).

**3-(5-Amino-3-methyl-1H-pyrazol-1-yl)-3-oxo-propenenitrile (15).**

To a solution compound **3** (1.73 g, 0.01 mol) in ethanol (25 mL) in a water bath at 60°C potassium cyanide (**14**) (0.65 g, 0.01 mol) was added with continues stirring. The reaction mixture was left in water bath for 30 min at 60°C then poured onto a beaker containing ice/water mixture and drops of hydrochloric acid. The formed solid product was collected by filtration and dried. Orange crystals from ethanol in a yield 59% (0.96 g), with m.p. 148°C - 150°C. *Calculated for* C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O (164.15): C, 51.21; H, 4.90; N, 34.12. Found : C, 51.41; H, 4.82; N, 34.10. IR ( $\nu/cm^{-1}$ ) = 3468 - 3348 (NH<sub>2</sub>), 3055 (CH aromatic), 2984 (CH<sub>3</sub>), 2227 (CN), 1644 (C= N), 1633 (C=C). <sup>1</sup>HNMR ( $\delta$  ppm): 2.74 (s, 3H, CH<sub>3</sub>), 4.11 (s, 2H, CH<sub>2</sub>), 4.84 (s, 2H, NH<sub>2</sub>), 5.53 (s, 1H, pyrazole); MS:  $m/z$  164 ( $M^+$ , 33%).

**2-(5-Methyl-6H-[1,3,4]oxadiazin-2-yl)-3-phenylacrylonitrile (18)**

Equimolecular amounts of **3** (1.73 g, 0.01 mol) and benzaldehyde (**16**) (1.06 g, 0.01 mol) in 1,4-dioxane (30 mL) containing piperidine (1.00 mL) were heated under reflux for 3 h. The solid product formed upon dilution with ice/water containing hydrochloric acid (till pH<sub>6</sub>), was collected by filtration. Red crystals from ethanol in a yield 76% (1.71 g) with m.p. 100°C. *Calculated for* C<sub>13</sub>H<sub>11</sub>N<sub>3</sub>O (225.24): C, 69.32; H, 4.92; N,18.66. Found: C, 68.84; H, 5.23; N,18.55. IR ( $\nu/cm^{-1}$ ) = 3057 (CH aromatic), 2985 (CH<sub>3</sub>), 2234 (CN), 1641(C=N), 1633 (C=C). <sup>1</sup>HNMR ( $\delta$ ppm): 2.68 (s, 3H, CH<sub>3</sub>), 5.26 (s, 2H, oxadiazine-CH<sub>2</sub>), 6.05 (s, 1H, CH=C), 7.22 - 7.35 (m, 5H, C<sub>6</sub>H<sub>5</sub>); MS:  $m/z$  255 ( $M^+$ , 80%).

**6-Amino-2,8-dihydro-3-methyl-8-phenylpyrido[2,1-b][1,3,4]oxadiazine-7,9-dicarbonitrile (20a) and 2,8-Di-hydro-6-hydroxy-3-methyl-8-phenylpyrido[2,1-b][1,3,4]oxadiazine-7,9-dicarbonitrile (20b).**

*General procedure:* Equimolecular amounts of **18** (2.25 g, 0.01 mol) either malononitrile (**10a**) (0.66 g, 0.01 mol) or ethyl cyanoactate (**10b**) were added(1.13 g, 0.01 mol) in 1,4-dioxane (25 mL) containing triethylamine (1.00 mL) were heated under reflux for 4 h. The solid product formed upon dilution with ice/water containing hydrochloric acid (till pH<sub>6</sub>), was collected by filtration.

**Compound (20a):** Brown crystals from ethanol, yield 71 % (2.06 g), m.p. 110°C. *Calculated for* C<sub>16</sub>H<sub>13</sub>N<sub>5</sub>O (291.26): C, 65.97; H, 4.50; N, 24.04. Found: C, 65.79; H, 4.36; N, 23.98. IR ( $\nu/cm^{-1}$ ) = 3473 - 3344 (NH<sub>2</sub>), 3057 (CH aromatic), 2980 (CH<sub>3</sub>), 2222, 2220 (2CN), 1662 (C=N), 1640 (C=C). <sup>1</sup>HNMR ( $\delta$  ppm): 2.79 (s, 3H, CH<sub>3</sub>), 4.43 (1s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 5.20 (s, 2H, Oxadiazine CH<sub>2</sub>), 5.52 (s, 1H, pyridine H-4), 7.23 - 7.36 (m, 5H, C<sub>6</sub>H<sub>5</sub>); MS:  $m/z$  291 ( $M^+$ , 40%).

**Compound (20b):** Orange crystals from 1,4-dioxane,

yield 69%, (2 g), m.p. 80°C. *Calculated for* C<sub>16</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub> (292.25): C, 65.75; H, 4.13; N,19.16. Found: C, 66.10; H, 4.43; N,19.58. IR ( $\nu/cm^{-1}$ ) = 3852 - 3480 (OH), 2980 (CH<sub>3</sub>), 2228, 2218 (2 CN), 1644 (C=N), 1636 (C=C). <sup>1</sup>HNMR ( $\delta$  ppm): 2.78 (s, 3H, CH<sub>3</sub>), 5.21 (s, 2H, oxadiazine-CH<sub>2</sub>), 5.51 (s, 1H, pyridine H-4), 7.21 - 7.33 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 10.22 (s, 1H, D<sub>2</sub>O exchangeable, OH); MS:  $m/z$  292 ( $M^+$ , 32%).

**N<sup>2</sup>-(2-bromocyclohexylidene)-2-cyanoacetylhydrazide (22).**

To a hot solution of cyanoacetylhydrazine (**1**) (0.99 g, 0.01mol), in 1,4-dioxane (40 mL),  $\alpha$ -bromocyclohexanone (**21**) (1.76 g, 0.01 mol) was added and drops of piperidine (1 mL). The reaction mixture was kept at room temperature with stirring for 1 h and the formed solid product was filtrated off. Orange crystals from 1,4-dioxane in a yield 88% (2.27 g) with m.p. 137°C - 140°C. *Calculated for* C<sub>9</sub>H<sub>12</sub>BrN<sub>3</sub>O (258.12): C, 41.88; H, 4.69; Br, 30.96; N, 16.27. Found: C, 41.67; H, 4.33; Br, 31.05; N, 16.34. IR ( $\nu/cm^{-1}$ ) = 3466 - 3329 (NH), 3055 (CH aromatic), 2260 (CN), 1640 (C=N), 1683 (C=O), 1630 (C=C). <sup>1</sup>HNMR ( $\delta$  ppm): 2.21 - 2.27 (m, 8H, 4CH<sub>2</sub>), 3.88 (s, 2H, CH<sub>2</sub>), 4.22 (s, 1H, CH), 8.30 (s, 1H, D<sub>2</sub>O exchangeable, NH); MS:  $m/z$  258 ( $M^+$ , 90%).

**1-amino-5,6,7,8-tetrahydro-4aHbenzo[e]pyrazolo[5,1-c][1,2,4]triazine (24a) and 1-Phenyl-5,6,7,8-tetrahydro-4aHbenzo [e]pyrazolo[5,1-c][1,2,4]triazine (24b).**

A mixture of **22** (2.58 g, 0.01 mol) and hydrazine hydrate (**4a**) (0.99 g, 0.01 mol) was heated in an oil bath 140°C for 1 h, then left to cool. The remaining product was heated in ethanol then poured into ice/water mixture and the formed solid product was collected by filtration.

**Compound (24a):** Brown crystals from 1,4-dioxane, yield 74% (1.41 g), m.p. > 300°C. *Calculated for* C<sub>9</sub>H<sub>13</sub>N<sub>5</sub> (191.23): C, 56.52; H, 6.85; N, 36.62. Found: C, 56.85; H, 6.60; N, 36.98. IR ( $\nu/cm^{-1}$ ) = 3478 - 3321 (NH<sub>2</sub>, NH), 3058 (CH aromatic), 1643 (C=N), 1636 (C=C). <sup>1</sup>HNMR ( $\delta$  ppm): 2.20 - 2.25 (m, 4H, 2CH<sub>2</sub>), 2.33 - 2.41 (m, 5H, 2CH<sub>2</sub>, CH), 4.82 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 5.32 (s, 1H, pyrazole H-4), 8.25 (s, 1H, D<sub>2</sub>O exchangeable, NH); MS:  $m/z$  191 ( $M^+$ , 100%).

**Compound (24b):** Equimolecular amounts of **22** (2.58 g, 0.01 mol) and phenylhydrazine (**4b**) (1.08 g, 0.01 mol) in 1,4-dioxane (40 mL) were heated under reflux for 4 h. The solid product formed upon dilution with ice/water containing hydrochloric acid (till pH<sub>6</sub>), was collected by filtration.

**Compound (24b):** Brown crystals from 1,4- dioxane, yield 78% (2.08 g), m.p. 217°C - 220°C. *Calculated for* C<sub>15</sub>H<sub>17</sub>N<sub>5</sub> (267.31): C, 67.39; H, 6.41; N, 26.19. Found: C, 67.69; H,6.32; N, 26.74. IR ( $\nu/cm^{-1}$ ) = 3465 - 3330 (NH<sub>2</sub>), 3056 (CH aromatic), 1640 (C=N), 1634 (C=C). <sup>1</sup>H NMR ( $\delta$  ppm): 2.23 - 2.28 (m, 4H, 2CH<sub>2</sub>), 2.32 - 2.39 (m, 5H, 2CH<sub>2</sub>, CH), 4.80 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 5.38(s,1H, pyrazole H-4), 7.28 - 7.36 (m, 5H,

C<sub>6</sub>H<sub>5</sub>); MS: *m/z* 267 (M<sup>+</sup>, 100%).

**2-(2-Phenylhydrazono)-2-(5,6,7,8-tetrahydro-4aH-benzo[e][1,3,4]oxadiazin-3-yl)acetonitrile (25a) and 2-(2-4-chlorophenylhydrazono)-2-(5,6,7,8-tetrahydro-4aH-benzo[e][1,3,4]oxadiazin-3-yl)acetonitrile (25b).**

*General procedure:* To a cold solution (0°C - 5°C) of **22** (2.58 g, 0.01 mol) in ethanol (35 mL) containing sodium hydroxide (6 mL, 10%) and a solution of benzenediazonium chloride (0.01 mol) or p-chlorobenzediazonium chloride (0.01 mol) obtained via the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution (0°C - 5°C) of either aniline (0.93 g, 0.01 mol) or p-chloroaniline (1.27 g, 0.01 mol) containing the appropriate amounts of hydrochloric acid (10 mL) was added with continuous stirring] was added with continuous stirring. The reaction mixture was stirred for an additional 2 h then the formed solid product was collected by filtration.

**Compound (25a):** Red crystals from ethanol, yield, 82% (2.31 g), m.p. 110°C. *Calculated for* C<sub>15</sub>H<sub>15</sub>N<sub>5</sub>O (281.30): C, 64.04; H, 5.37; N, 24.89. Found: C, 64.44; H, 5.00; N, 24.66. IR (ν/cm<sup>-1</sup>) = 3466 - 3316 (NH), 3053 (CH aromatic), 2890 (CH<sub>2</sub>), 2246 (CN), 1646 (C=N), 1632 (C=C). <sup>1</sup>HNMR (δ ppm): 2.22 - 2.27 (m, 4H, 2CH<sub>2</sub>), 2.30 - 2.43 (m, 5H, 2CH<sub>2</sub>, CH), 7.26 - 7.38 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 8.28 (s, 1H, NH); MS: *m/z* 281 (M<sup>+</sup>, 20%).

**Compound (25b):** Orange crystals from ethanol, yield 85% (2.72 g), m.p. 120°C. *Calculated for* C<sub>15</sub>H<sub>14</sub>N<sub>5</sub>OCl (315.74): C, 57.06; H, 4.47; Cl, 11.23; N, 22.18. Found: C, 57.45; H, 4.52; Cl, 11.09; N, 21.98. IR (ν/cm<sup>-1</sup>) = 3475 - 3322 (NH), 3059 (CH aromatic), 2251 (CN), 1649 (C=N), 1630 (C=C). <sup>1</sup>HNMR (δ ppm): 2.25 - 2.29 (m, 4H, 2CH<sub>2</sub>), 2.34 - 2.40 (m, 5H, 2CH<sub>2</sub>, CH), 7.29 - 7.42 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 8.24 (s, 1H, NH); MS: *m/z* 315 (M<sup>+</sup>, 70%).

## 5. Conclusion

The present work describes the synthesis of the new hydrazide-hydrazone derivative **3**. The latter was used for the synthesis of a series of heterocyclic products with antitumor activities. Compounds **11a** and **20b** showed the highest inhibitor effect towards the tested three cancer cells. The work opened the door towards pharmaceutical uses of other hydrazide-hydrazones that could be obtained by the sequence described in this article.

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