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# The Uses of 2-Amino-4-Phenylthiazole in the Synthesis of Coumarin, Pyran, Pyridine and Thiazole Derivatives with Antitumor Activities

**Faten I. Hamed[1], Abeer A. Mohamed[2], Amr S. Abouzied[1,3]**

[1]National Organization for Drug Control & Research, Cairo, Egypt
[2]National Organization for Research and control of Biologicals, Giza, Egypt
[3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail, Kingdom of Saudi Arabia
Email: fatenismail@yahoo.com

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## Abstract

The thiazole derivative **3** was used for a series of heterocyclization reaction to produce pyran, pyridine and thiazole derivatives. The cytotoxicity of the newly synthesized compounds was studied against the six cancer cell lines namely NUGC, HR, DLD1, HA22T, HEPG2, MCF, HONE1 and normal fibroblast cells (WI38). The results showed that most of the synthesized compounds were of high potency. Among the tested compounds, 2-Amino-4-(4-chlorophenyl)-6-(4-phenylthiazol-2-yl)-4H-pyran-3,5-dicarbonitrile **17b** showed the highest potency among the tested compounds.

## Subject Areas

Organic Chemistry

## Keywords

Thiazole, Pyran, Pyridine, Antitumor

## 1. Introduction

Thiazole is a core structural motif present in a variety of natural products, such as vitamin B1 (thiamine) and penicillin. Thiazole derivatives also exhibit a broad spectrum of medicinal and biological properties, such as antibacterial, antifungal [1], anti-inflammatory [2], antiviral [3], antimalarial [4] and anti-HIV activities [5]. Thiazole analogs have also been reported as ligands at estrogen receptors [6], neuropeptide Y5 [7], adenosine receptors [8], and act as inhibitors of human platelet aggregation factor [9], urokinase [10] and poly (ADP-Ribose) polymerase-1 [11]. Selenazoles have been reported to possess antibacterial [12], and su-

peroxide anion scavenging activity [13], and exhibit cytotoxicity and DNA fragmentation effects in human HT-1080 fibrosarcoma cells [14]. The structures of sulfathiazole, meloxicam, and selenazofurin and their pharmacological activities are given in Figure 1.

## 2. Results and Discussion

The reaction of ω-bromoacetophenone (**1**) with thiourea (**2**) in ethanol gave the thiazole derivative (**3**) [15].

The latter compound underwent acetylation when reacted with acetic anhydride to give the N-acetyl derivative **5**. The structure of compound **5** was confirmed on the basis of analytical and spectral data. The reaction of compound **3** with phenylisothiocyanate gave the N-phenylthiourea derivative **7**. On the other hand, the reaction of compound **3** with ethyl cyanoacetate in dimethylformamide gave N-cyanoacetamide derivative **9**. The reaction of compound **9** with any of the aromatic aldehydes namely benzaldehyde (**10a**), 4-chlorobenzaldehyde (**10b**) or 4-methoxybenzaldehyde (**10c**) gave benzylidene derivatives **11a-c**, respectively. In addition, the reaction of compound **9** with salicylaldehyde (**12**) gave the coumarin derivative **13** (Figure 2).

The structure of compound **13** was established on the basis of analytical and spectral data. Thus, the $^1$H NMR spectrum showed $\delta$ = 6.13 (s, 1H, thiazole H-5), 6.29 (s, 1H, coumarin H-4), 7.21 - 7.43 (m, 9H, $C_6H_5$, $C_6H_4$), 8.30 (s, 1H, $D_2O$ exchangeable, NH). The reaction of compound **9** with any of the benzenediazonium chloride derivatives **14a-c** gave aryl hydrazone derivatives **15a-c**, respectively. Moreover, the multi-component reaction of compound **9** with any of the aromatic aldehydes **10a**, **10b** or **10c** and malononitrile (**16**) gave the pyran derivatives **17a-c**, respectively (Figure 3).

The analytical and spectral data of **17a-c** were the basis of their structural identification. Thus, the $^1$H NMR spectrum of compound **17a** (as an example) showed $\delta$ = 4.82 (s, 2H, $D_2O$ exchangeable, $NH_2$), 6.14 (s, 1H, thiazole H-5), 6.28 (s, 1H, $D_2O$ exchangeable, NH), 6.49 (s, 1H, pyran H-4), 7.28 - 7.42 (m, 10H, $2C_6H_5$). Similarly, the multi-component reaction of compound **9** with any of the aromatic aldehydes **10a**, **10b** or **10c** and ethyl cyanoacetate (**8**) gave the pyran derivatives **18a-c**, respectively (Figure 4).

The analytical and spectral data of **18a-c** were the basis of their structural identification. In addition, the multi-component reaction of compound **9** with any of the aromatic aldehydes **10a**, **10b** or **10c** and thiourea (**2**) gave the pyrimidine derivatives **19a-c**, respectively. The analytical and spectral data of **19a-c**

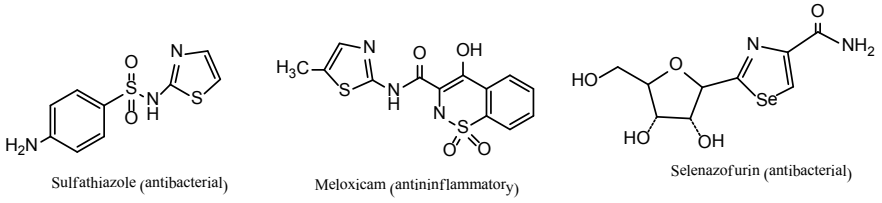


Figure 1. Biologically active thiazole and selenazole derivatives.

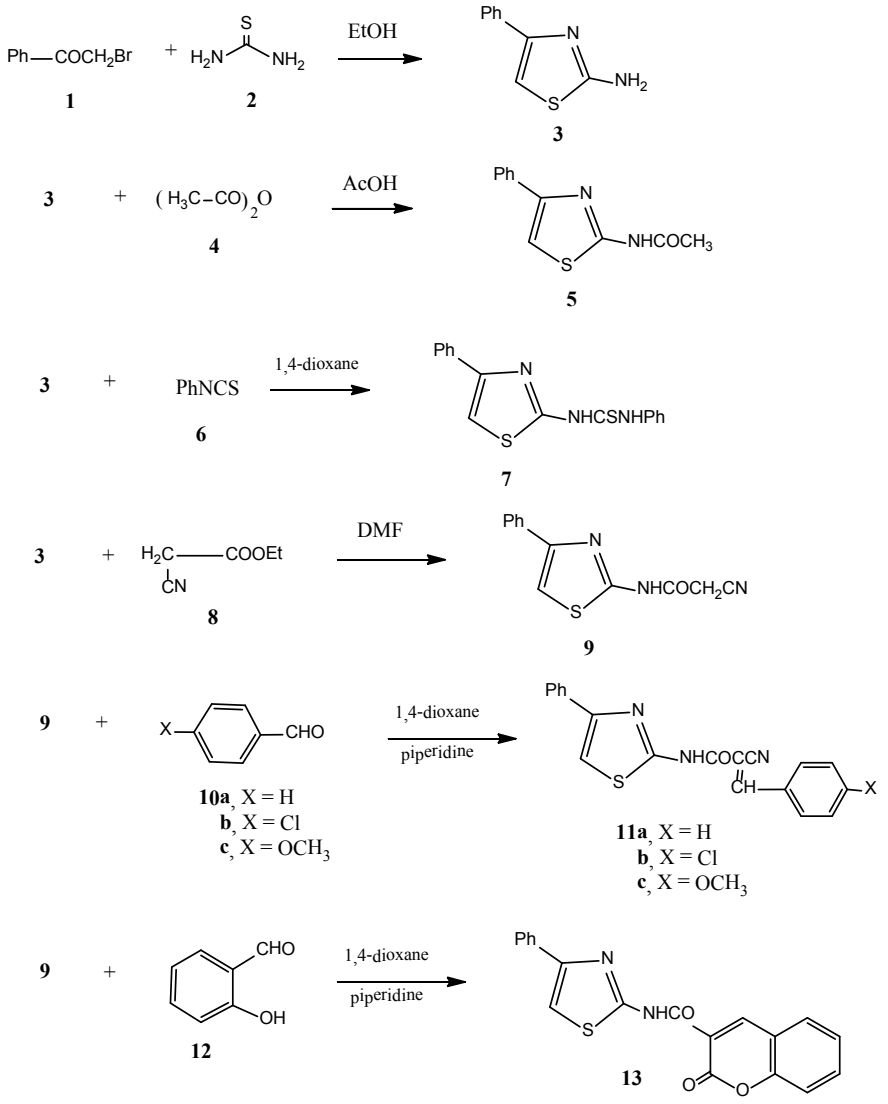


Figure 2. Compounds **3**, **5**, **7**, **9**,**11a-c**, **13**.

were the basis of their structural identification. Thus, the $^1$H NMR spectra of compound **19a** (as an example) showed $\delta$ = 6.18 (s, 1H, thiazole H-5), 6.28 (s, 1H, $D_2O$ exchangeable, NH), 7.29 - 7.36 (m, 10H, $2C_6H_5$), 8.24 (s, 1H, $D_2O$ exchangeable, NH). Compound **9** was capable for thiazole synthesis, thus the reaction of compound **9** with elemental sulfur and phenylisothiocyanate (**6**) gave the thiazole derivative **20**.

## 3. Experiment

### General

All melting points were determined on an electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer. $^1$H NMR spectra were recorded with Varian Gemini-200 (200 MHz) and Jeol AS 500 MHz instruments spectra were performed in DMSO-*d*6 as solvent using TMS as internal standard-

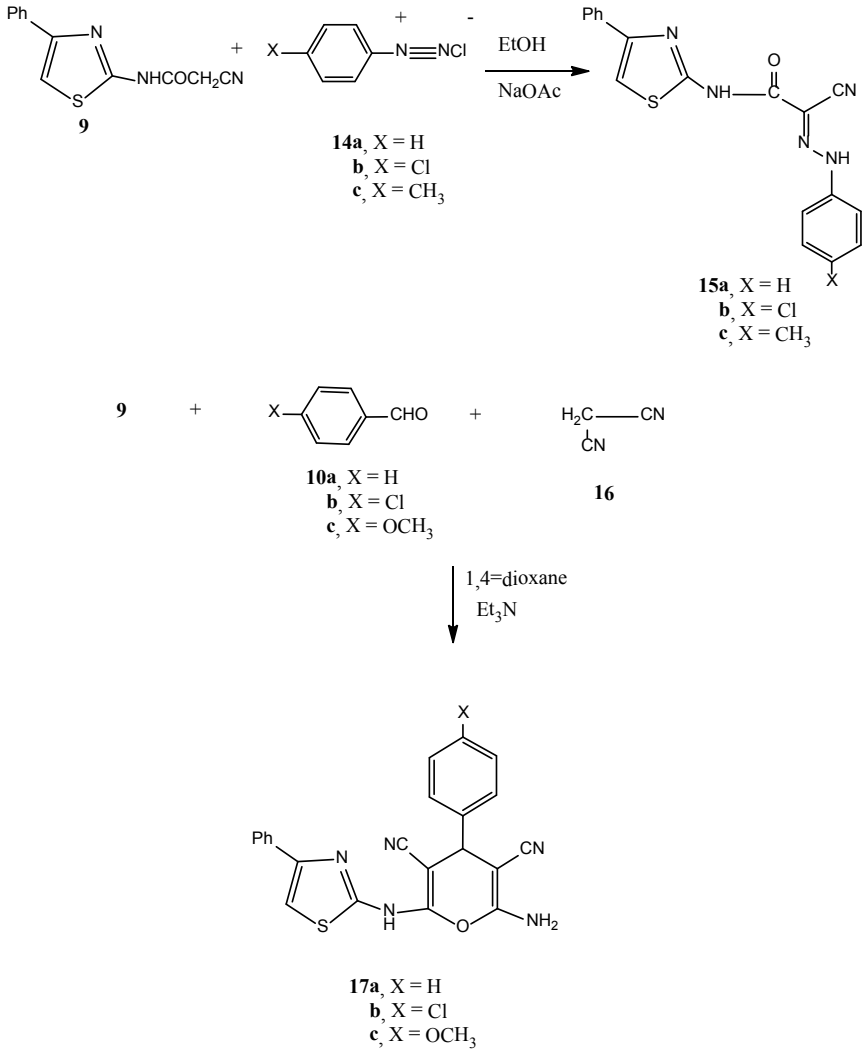


Figure 3. Compounds **15a-c**, **17a-c**.

and chemical shifts are expressed as $\delta$ ppm. MS (EI) spectra were recorded with Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Analytical data were obtained from the Micro-analytical Data Unit at Cairo University and were performed on Vario EL III Elemental analyzer. Compound **3** was synthesized according to method reported in literature [15]. All synthesized compounds are filtered using Whatman filter paper 42 Ashless.

1) *N*-(4-*phenylthiazol*-2-*yl*)*acetamide* **(5)**

To a solution of compound **3** (1.76 g, 0.01 mol) in acetic acid (40 mL) acetic anhydride (10 mL) was added. The reaction mixture was heated under reflux (118˚C) for 2 h then poured onto ice/water and left to room temperature for 4 h. The formed solid product was collected by filtration.

Yellow crystals from ethanol, yield 70% (1.52 g), m.p. 206˚C - 208˚C. *Anal.* Calculated for $C_{11}H_{10}N_2OS$ (218.27): C, 60.53; H, 4.62; N, 12.83; S, 14.69. Found: C, 60.74; H, 4.59; N, 12.93; S, 14.80. MS: m/e 218 ($M^+$, 28%). IR, υ: 3492 - 3330 (NH), 3056 (CH, aromatic), 2970 ($CH_3$), 1688 (CO), 1638 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 2.80 (s, 3H, $CH_3$), 6.12 (s, 1H, thiazole H-5), 7.26 -

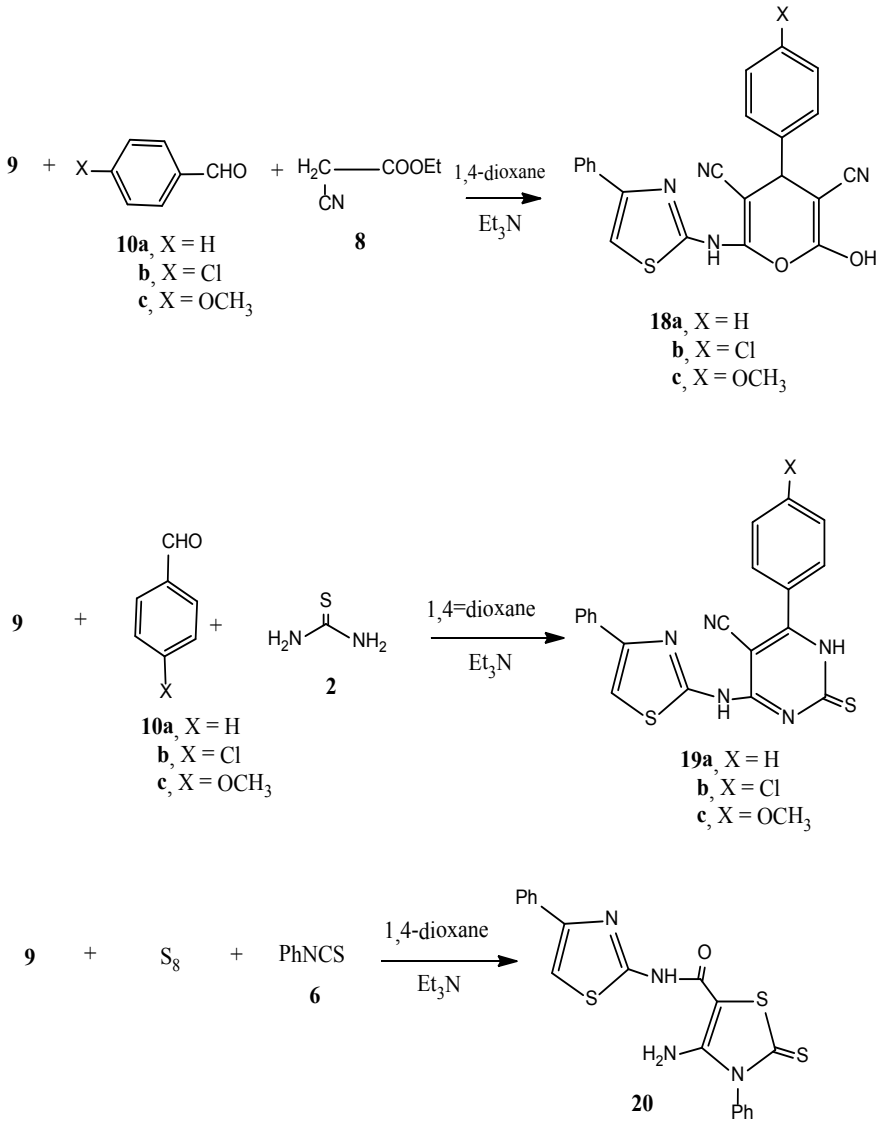


Figure 4. Compounds **18a-c**, **19a-c**, **20**.

7.39 (m, 5H, $C_6H_5$), 8.30 (s, 1H, $D_2O$ exchangeable, NH).

2) 1-*phenyl*-3-(4-*phenylthiazol*-2-*yl*)*thiourea* **(7)**

To a solution of compound **3** (1.76 g, 0.01 mol) in 1,4-dioxane (20 mL) phenylisothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was heated under reflux (101˚C) for 3 h then poured onto ice/water and the formed solid product was collected by filtration.

Orange crystals from ethanol, yield 78% (2.42 g), m.p. 130˚C - 132˚C. *Anal.* Calculated for $C_{16}H_{13}N_2S_2$ (311.42): C, 61.71; H, 4.21; N, 13.49; S, 20.59. Found: C, 61.95; H, 4.31; N, 14.22; S, 20.72. MS: m/e 311 ($M^+$, 22%). IR, υ: 3468 - 3324 (2NH), 3054 (CH, aromatic), 1638 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.14 (s, 1H, thiazole H-5), 7.23 - 7.42 (m, 10H, $2C_6H_5$), 8.26, 8.32 (2s, 2H, $D_2O$ exchangeable, 2NH).

3) 2-*Cyano*-*N*-(4-*phenylthiazol*-2-*yl*)*acetamide* **(9)**

To a solution of compound **3** (1.76 g, 0.01 mol) in dimethylformamide (20

mL) ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux (153˚C) for 2 h then poured onto ice/water and the formed solid product was collected by filtration.

Orange crystals from ethanol, yield 67% (1.63 g), m.p. 154˚C - 157˚C. *Anal.* Calculated for $C_{12}H_9N_3OS$ (243.28): C, 59.24; H, 3.73; N, 17.27; S, 13.18. Found: C, 59.36; H, 4.01; N, 16.96; S, 13.47. MS: m/e 243 ($M^+$, 36%). IR, υ: 3452-3328 (NH), 3057 (CH, aromatic), 2220 (CN), 1678 (CO), 1632 (C=C),. $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 3.84 (s, 2H, $CH_2$), 6.13 (s, 1H, thiazole H-5), 7.28 - 7.39 (m, 5H, $C_6H_5$), 8.30 (s, 1H, $D_2O$ exchangeable, NH).

4) *General procedure for the synthesis of the benzylidine derivatives* **11a-c**

To a solution of compound **9** (2.43 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL), any of benzaldehyde (1.08 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The reaction mixture was heated under reflux (101˚C) for 3 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product, formed in each case was collected by filtration.

5) 2-*Cyano*-3-*phenyl*-*N*-(4-*phenylthiazol*-2-*yl*)*acrylamide* **(11a)**

Pale brown crystals from ethanol, yield 70% (2.32 g), m.p. 139˚C - 141˚C. *Anal.* Calculated for $C_{19}H_{13}N_3OS$ (331.39): C, 68.86; H, 3.95; N, 12.68; S, 9.68. Found: C, 68.47; H, 4.16; N, 12.51; S, 9.38. MS: m/e 331 ($M^+$, 28%). IR, υ: 3462 - 3341 (NH), 3053 (CH, aromatic), 2220 (CN), 1682 (CO), 1635 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.09 (s, 1H, CH), 6.14 (s, 1H, thiazole H-5), 7.25-7.37 (m, 10H, $2C_6H_5$), 8.32 (s, 1H, $D_2O$ exchangeable, NH).

6) 3-(4-*chlorophenyl*)-2-*cyano*-*N*-(4-*phenylthiazol*-2-*yl*)*acrylamide* **(11b)**

Pale brown crystals from ethanol, yield 66% (2.41 g), m.p. 188˚C - 191˚C. *Anal.* Calculated for $C_{19}H_{12}ClN_3OS$ (365.84): C, 62.38; H, 3.31; N, 11.49; S, 8.76. Found: C, 62.19; H, 3.53; N, 11.60; S, 8.57. MS: m/e 365 ($M^+$, 40%). IR, υ: 3472 - 3329 (NH), 3056 (CH, aromatic), 2222 (CN), 1680 (CO), 1632 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.06 (s, 1H, CH), 6.12 (s, 1H, thiazole H-5), 7.23-7.42 (m, 9H, $C_6H_5$, $C_6H_4$), 8.34 (s, 1H, $D_2O$ exchangeable, NH).

7) 2-*cyano*-3-(4-*methoxyphenyl*)-*N*-(4-*phenylthiazol*-2-*yl*)*acrylamide* **(11c)**

Yellow crystals from ethanol, yield 78% (2.83 g), m.p. 166-169˚C. *Anal.* Calculated for $C_{20}H_{15}N_3O_2S$ (361.42): C, 66.46; H, 4.18; N, 11.63; S, 8.87. Found: C, 66.37; H, 3.86; N, 11.41; S, 8.72. MS: m/e 361 ($M^+$, 22%). IR, υ: 3463 - 3342 (NH), 3053 (CH, aromatic), 2220 (CN), 1682 (CO), 1631 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 3.68 (s, 3H, $OCH_3$), 6.08 (s, 1H, CH), 6.14 (s, 1H, thiazole H-5), 7.24-7.38 (m, 9H, $C_6H_5$, $C_6H_4$), 8.32 (s, 1H, $D_2O$ exchangeable, NH).

8) 2-*Oxo*-*N*-(4-*phenylthiazol*-2-*yl*)-2*H*-*chromene*-3-*carboxamide* **(13)**

To a solution of compound **9** (2.43 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL) salicylaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux (101˚C) for 2 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

Yellow crystals from ethanol, yield 62% (2.16 g), m.p. 122˚C - 124˚C. *Anal.* Calculated for $C_{19}H_{12}N_2O_3S$ (348.38): C, 65.51; H, 3.47; N, 8.04; S, 9.20. Found: C, 65.44; H, 3.59; N, 7.94; S, 9.38. MS: m/e 348 ($M^+$, 18%). IR, υ: 3439-3312 (NH), 3056 (CH, aromatic), 1690, 1682 (2CO), 1665 (C=N), 1628 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.13 (s, 1H, thiazole H-5), 6.29 (s, 1H, coumarin H-4), 7.21 - 7.43 (m, 9H, $C_6H_5$, $C_6H_4$), 8.30 (s, 1H, $D_2O$ exchangeable, NH).

9) *General procedure for the synthesis of the aryl hydrazone derivatives* **15a-c**

To a cold solution (0˚C - 5˚C) of compound **9** (2.43 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (2.50 g) any of the diazonium salts namely benzenediazonium chloride (**14a**) (0.01 mol) 4-chlorobenzene diazonium chloride (**14b**) (0.01 mol) or 4-methylbenzenediazonium chloride (**14c**) (0.01 mol) [prepared by the addition of sodium nitrite (0.70 g, 0.01 mol) to a cold solution (0-5 ˚C) of any of aniline (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methylaniline (1.14 g, 0.01 mol) in concentrated hydrochloric acid (16 mL)] was added. The whole reaction mixture, in each case, was stirred at room temperature for 2 h and the formed solid product was collected by filtration.

10) *2-Oxo-N'-phenyl-2-((4-phenylthiazol-2-yl)amino)acetohydrazonoyl cyanide* **(15a)**

Orange crystals from ethanol, yield 65% (2.24 g), m.p. 153˚C - 156˚C. *Anal.* Calculated for $C_{18}H_{13}N_5OS$ (347.39): C, 62.23; H, 3.77; N, 20.16; S, 9.23. Found: C, 62.41; H, 3.54; N, 20.08; S, 9.52. MS: m/e 347 ($M^+$, 36%). IR, υ: 3452-3316 (2NH), 3053 (CH, aromatic), 2220 (CN), 1686 (CO), 1660 (C=N), 1626 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.12 (s, 1H, thiazole H-5), 7.24-7.38 (m, 10H, $2C_6H_5$), 8.26, 8.30 (2s, 2H, $D_2O$ exchangeable, 2NH).

11) *N'-(4-Chlorophenyl)-2-oxo-2-((4-phenylthiazol-2-yl)amino)acetohydrazonoyl cyanide* **(15b)**

Orange crystals from ethanol, yield 74% (2.82 g), m.p. 177˚C - 179˚C. *Anal.* Calculated for $C_{18}H_{12}ClN_5OS$ (381.84): C, 56.62; H, 3.17; N, 18.34; S, 8.40. Found: C, 56.82; H, 3.38; N, 18.51; S, 8.29. MS: m/e 381 ($M^+$, 28%). IR, υ: 3458 - 3331 (2NH), 3056 (CH, aromatic), 2222 (CN), 1688 (CO), 1653 (C=N), 1628 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.18 (s, 1H, thiazole H-5), 7.22-7.41 (m, 9H, $C_6H_5$, $C_6H_4$), 8.28, 8.31 (2s, 2H, $D_2O$ exchangeable, 2NH).

12) *2-Oxo-2-((4-phenylthiazol-2-yl)amino)-N'-(p-tolyl)acetohydrazonoylcyanide* **(15c)**

Orange crystals from ethanol, yield 72% (2.59 g), m.p. 203˚C - 206˚C. *Anal.* Calculated for $C_{19}H_{15}N_5OS$ (361.42): C, 63.14; H, 4.18; N, 19.38; S, 8.87. Found: C, 62.97; H, 3.92; N, 19.26; S, 8.65. MS: m/e 361 ($M^+$, 38%). IR, υ: 3471 - 3369 (2NH), 3055 (CH, aromatic), 2220 (CN), 1687 (CO), 1656 (C=N), 1629 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 2.69 (s, 3H, $CH_3$), 6.17 (s, 1H, thiazole H-5), 7.24 - 7.45 (m, 9H, $C_6H_5$, $C_6H_4$), 8.26, 8.33 (2s, 2H, $D_2O$ exchangeable, 2NH).

13) *General procedure for the synthesis of the pyran derivatives* **17a-c**

To a solution of compound **9** (2.43 g, 0.01 mol) in ethanol (30 mL) containing triethylamine (0.50 mL) malononitrile (0.66 g, 0.01 mol) and any of benzalde-

hyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux (78˚C) for 3 h then left to cool and the formed solid product was collected by filtration.

14) 2-*Amino*-4-*phenyl*-6-(4-*phenylthiazol*-2-*yl)amino)*-4*H*-*pyran*-3,5-*dicarbonitrile* **(17a)**

Orange crystals from ethanol, yield 83% (3.29 g), m.p. 213˚C - 215˚C. *Anal.* Calculated for $C_{22}H_{15}N_5OS$ (397.45): C, 66.48; H, 3.80; N, 17.62; S, 8.07. Found: C, 66.73; H, 3.92; N, 17.94; S, 8.19. MS: m/e 397 ($M^+$, 26%). IR, υ: 3462 - 3338 (NH, $NH_2$), 3056 (CH, aromatic), 2221 (CN), 1662 (C=N), 1621 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 4.82 (s, 2H, $D_2O$ exchangeable, $NH_2$), 6.14 (s, 1H, thiazole H-5), 6.28 (s, 1H, $D_2O$ exchangeable, NH), 6.49 (s, 1H, pyran H-4), 7.28-7.42 (m, 10H, $2C_6H_5$).

15) 2-*Amino*-4-(4-*chlorophenyl*)-6-(4-*phenylthiazol*-2-*yl)amino)*-4*H*-*pyran*-3,5-*dicarbonitrile* **(17b)**

Orange crystals from ethanol, yield 76% (3.27 g), m.p. 183˚C - 185˚C. *Anal.* Calculated for $C_{22}H_{14}ClN_5OS$ (431.90): C, 61.18; H, 3.27; N, 16.22; S, 7.42. Found: C, 61.28; H, 3.37; N, 16.49; S, 7.80. MS: m/e 431 ($M^+$, 18%). IR, υ: 3462 - 3338 (NH, $NH_2$), 3056 (CH, aromatic), 2222 (CN), 1659 (C=N), 1626 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 4.80 (s, 2H, $D_2O$ exchangeable, $NH_2$), 6.17 (s, 1H, thiazole H-5), 6.26 (s, 1H, $D_2O$ exchangeable, NH), 6.49 (s, 1H, pyran H-4), 7.22 - 7.48 (m, 9H, $C_6H_5$, $C_6H_4$).

16) 2-*Amino*-6-(4-*phenylthiazol*-2-*yl)amino)*-4-(*p*-*tolyl*)-4*H*-*pyran*-3,5-*dicarbonitrile* **(17c)**

Orange crystals from ethanol, yield 68% (2.90 g), m.p. 97˚C - 99˚C. *Anal.* Calculated for $C_{23}H_{17}N_5O_2S$ (427.48): C, 64.62; H, 4.01; N, 16.38; S, 7.50. Found: C, 64.80; H, 4.26; N, 16.60; S, 7.44. MS: m/e 427 ($M^+$, 33%). IR, υ: 3462 - 3338 (NH, $NH_2$), 3055 (CH, aromatic), 2220 (CN), 1655 (C=N), 1628 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 3.77 (s, 3H, $CH_3$), 4.81 (s, 2H, $D_2O$ exchangeable, $NH_2$), 6.19 (s, 1H, thiazole H-5), 6.28 (s, 1H, D2O exchangeable, NH), 6.46 (s, 1H, pyran H-4), 7.24 - 7.45 (m, 9H, $C_6H_5$, $C_6H_4$).

17) *General procedure for the synthesis of the pyran derivatives* **18a-c**

To a solution of compound **9** (2.43 g, 0.01 mol) in ethanol (30 mL) containing triethylamine (0.50 mL) ethyl cyanoacetate (1.13 g, 0.01 mol) and any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux (78˚C) for 6 h then left to cool and the formed solid product was collected by filtration.

18) 2-*Hydroxy*-4-*phenyl*-6-(4-*phenylthiazol*-2-*yl)amino)*-4*H*-*pyran*-3,5-*dicarbonitrile* **(18a)**

Orange crystals from ethanol, yield 70% (2.79 g), m.p. 228˚C - 230˚C. *Anal.* Calculated for $C_{22}H_{14}N_4O_2S$ (398.44): C, 66.32; H, 3.54; N, 14.06; S, 8.05. Found: C, 66.88; H, 3.51; N, 14.14; S, 8.40. MS: m/e 398 ($M^+$, 19%). IR, υ: 3560 - 3328 (NH, OH), 3056 (CH, aromatic), 2223 (CN), 1660 (C=N), 1626 (C=C). $^1$H NMR

(DMSO-$d_6$, 200 MHz): $\delta$ = 6.16 (s, 1H, thiazole H-5), 6.46 (s, 1H, pyran H-4), 6.49 (s, 1H, $D_2O$ exchangeable, NH),7.29 - 7.36 (m, 10H, $2C_6H_5$), 10.20 (s, 1H, $D_2O$ exchangeable, OH).

19) 4-(4-*Chlorophenyl*)-2-*hydroxy*-6-(4-*phenylthiazol*-2-*yl)amino)*-4*H*-*pyran*-3,5-*dicarbonitrile* **(18b)**

Yellow crystals from ethanol, yield 80% (3.46 g), m.p. 220˚C - 223˚C. *Anal.* Calculated for $C_{22}H_{13}ClN_4O_2S$ (432.88): C, 61.04; H, 3.03; N, 12.94; S, 7.41. Found: C, 61.42; H, 3.02; N, 12.83; S, 7.83. MS: m/e 432 ($M^+$, 20%). IR, υ: 3560 - 3328 (NH, OH), 3054 (CH, aromatic), 2220 (CN), 1659 (C=N), 1624 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.13 (s, 1H, thiazole H-5), 6.47 (s, 1H, pyran H-4), 6.6 (s, 1H, $D_2O$ exchangeable, NH), 7.23 - 7.47 (m, 9H, $C_6H_5$, $C_6H_4$), 10.22 (s, 1H, $D_2O$ exchangeable, OH).

20) 2-*Hydroxy*-6-(4-*phenylthiazol*-2-*yl)amino)*-4-(*p*-*tolyl*)-4*H*-*pyran*-3,5-*dicarbonitrile* **(18c)**

Orange crystals from ethanol, yield 72% (3.08 g), m.p. 234˚C - 237˚C. *Anal.* Calculated for $C_{23}H_{16}N_4O_3S$ (428.46): C, 64.47; H, 3.76; N, 13.08; S, 7.48. Found: C, 64.43; H, 3.91; N; 13.22; S, 7.86. MS: m/e 428 ($M^+$, 20%). IR, υ: 3560 - 3328 (NH, OH), 3056 (CH, aromatic), 2223 (CN), 1652 (C=N), 1623 (C=C). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 3.79 (s, 3H, $CH_3$), 6.16 (s, 1H, thiazole H-5), 6.27 (s, 1H, $D_2O$ exchangeable NH), 6.48 (s, 1H, pyran H-4), 7. 28 - 7.41 (m, 9H, $C_6H_5$, $C_6H_4$), 10.18 (s, 1H, $D_2O$ exchan- geable, OH).

21) *General procedure for the synthesis of the pyrimidine derivatives* **19a-c**

To a solution of compound **9** (2.43 g, 0.01 mol) in ethanol (30 mL) containing triethylamine (0.50 mL) thiourea (0.76 g, 0.01 mol) and any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux (78˚C) for 6 h then left to cool and the formed solid product was collected by filtration.

22) 6-*Phenyl*-4-(4-*phenylthiazol*-2-*yl)amino)*-2-*thioxo*-1,2-*dihydropyrimidine*-5-*carbonitrile* **(19a)**

Orange crystals from ethanol, yield 66% (2.55 g), m.p. 162˚C - 165˚C. *Anal.* Calculated for $C_{20}H_{13}N_5S_2$ (387.48): C, 61.99; H, 3.38; N, 18.07; S, 16.55. Found: C, 62.32; H, 3.49; N; 18.33; S, 16.19. MS: m/e 387 ($M^+$, 25%). IR, υ: 3480 - 3337 (2NH), 3054 (CH, aromatic), 2220 (CN), 1663 (C=N), 1629 (C=C), 1205 (C=S). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.18 (s, 1H, thiazole H-5), 6.28 (s, 1H, $D_2O$ exchangeable, NH), 7.29 - 7.36 (m, 10H, $2C_6H_5$), 8.24 (s, 1H, $D_2O$ exchangeable, NH).

23) 6-(4-*Chlorophenyl*)-4-(4-*phenylthiazol*-2-*yl)amino)*-2-*thioxo*-1,2-*dihydropyrimidine*-5-*carbonitrile* **(19b)**

Yellow crystals from ethanol, yield 75% (3.15 g), m.p. 133˚C - 135˚C. *Anal.* Calculated for $C_{20}H_{12}ClN_5S_2$ (421.93): C, 56.93; H, 2.87; N, 16.60; S, 15.20. Found: C, 56.73; H, 2.99; N; 16.83; S, 15.69. MS: m/e 421 ($M^+$, 28%). IR, υ: 3487 - 3346 (2NH), 3056 (CH, aromatic), 2223 (CN), 1656 (C=N), 1628 (C=C), 1221 (C=S). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 6.14 (s, 1H, thiazole H-5), 6.6 (s, 1H,

$D_2O$ exchangeable, NH), 7.21 - 7.49 (m, 9H, $C_6H_5$, $C_6H_4$), 8.25 (s, 1H, $D_2O$ exchangeable, NH).

24) 6-(4-*Methoxyphenyl*)-4-(4-*phenylthiazol*-2-*yl)amino)*-2-*thioxo*-1,2-*dihydropyrimidine*-5-*carbonitrile* **(19c)**

Orange crystals from ethanol, yield 68% (2.83 g), m.p. 177˚C - 179˚C. *Anal.* Calculated for $C_{21}H_{15}N_5OS_2$ (417.51): C, 60.41; H, 3.62; N, 16.77; S, 15.36. Found: C, 60.52; H, 3.79; N; 16.55; S, 15.26. MS: m/e 417 ($M^+$, 15%). IR, υ: 3480 - 3329 (2NH), 3054 (CH, aromatic), 2221 (CN), 1658 (C=N), 1622 (C=C), 1205 (C=S). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 3.74 (s, 3H, $CH_3$), 6.16 (s, 1H, thiazole H-5), 6.36 (s, 1H, $D_2O$ exchangeable, NH), 7.23 - 7.46 (m, 9H, $C_6H_5$, $C_6H_4$), 8.23 (s, 1H, $D_2O$ exchangeable, NH).

25) 4-*Amino*-3-*phenyl*-*N*-(4-*phenylthiazol*-2-*yl)amino)*-2-*thioxo*-2,3-*dihydrothiazole*-5-*carboxamide* **(20)**

To a solution of compound **9** (2.43 g, 0.01 mol) in ethanol (30 mL) containing triethylamine (0.50 mL), elemental sulfur (0.32 g, 0.01 mol) and phenylisothiocyanate (1.35 g, 0.01 mol) were added. The reaction mixture, in each case, was heated under reflux (78˚C) for 6 h then left to cool then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Orange crystals from ethanol, yield 50% (2.05 g), m.p. 164˚C - 167˚C. *Anal.* Calculated for $C_{19}H_{14}N_4OS_3$ (410.54): C, 55.59; H, 3.44; N, 13.65; S, 23.43. Found: C, 55.73; H, 3.83; N; 13.83; S, 23.44. MS: m/e 410 ($M^+$, 35%). IR, υ: 3475 - 3342 (NH, $NH_2$), 3056 (CH, aromatic), 1688 (CO), 1655 (C=N), 1623 (C=C), 1230 (C=S). $^1$H NMR (DMSO-$d_6$, 200 MHz): $\delta$ = 4.34 (s, 2H, $D_2O$ exchangeable $NH_2$), 6.19 (s, 1H, thiazole H-5), 7. 23 - 7.46 (m, 10H, $2C_6H_5$), 8.39 (s, 1H, $D_2O$ exchangeable, NH).

## 4. Biological Activity

### 4.1. In Vitro Cytotoxic Assay

#### 4.1.1. Chemicals

Fetal bovine serum (FBS) and L-glutamine, were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

#### 4.1.2. Cell cultures

Were obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were 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 lg/mL), at 37˚C in a humidified atmosphere

containing 5% $CO_2$. Exponentially growing cells were obtained by plating $1.5 \times 10^5$ cells/mL for the seven human cancer cell lines including cells derived from $0.75 \times 10^4$ cells/mL 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 concentration (0.5%) of DMSO used in each assay.

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and a normal fibroblast cells (WI38). All of $IC_{50}$ values were listed in Table 1. Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested ($IC_{50}$ = 10 - 1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC50 > 10,000 nM). The reference compound used is the CHS-828 which is a pyridyl cyanoguanidine anti-tumor agent.

### 4.2. Structure Activity Relationship

It is clear from Table 1 that most of the tested compounds showed high cytotoxicity against the six cancer cell line. The thiazole derivative **3** showed moderate cytotoxicity against the six cancer cell lines. Acetylation of compound **3** to give the N-acetyl derivative **3** did not give a remarkable difference in activity compared with the original thiazole **3**. Reaction of compound **3** with phenylisothiocyanate to give the N-phenylthiourea derivative **7** showed also moderate potency. Similarly, the N-cyanoacetyl derivative **9** showed moderate potency against the six cancer cell lines. However, the reaction of compound **9** with any of the aromatic aldehydes gave the arylidene derivatives **11a-c**. It is obvious that compound **11b** (X = Cl) showed the highest potency among such series of compounds. On the other hand, **11c** (X = $OCH_3$) showed high potency against only NUGC cell lines and moderate potency against the other cell lines. The reaction of compound **9** with any of the diazonium salts **14a-c** gave the aryl hydrazone derivatives **15a-c**. Compound **15b** with the electronegative Cl group showed the highest cytotoxicity among the three compounds. The multicomponent reactions of compound **9** with any of the aromatic aldehydes **10a-c** and malononitrile gave the pyran derivatives **17a-c**. It is obvious from Table 1 that compounds **17b** and **17c** showed high potency against the six cancer cell lines. However compound **17a** showed high potency against NUGC, DLDI, HA22T, HEPG2and HONE1 cell lines and low potency against MCF cell lines. On the other hand, for the pyran derivatives **18a-c** it is clear from Table 1 that compound **18a** (X = H) showed high potency against DLDI, HEPG2 and MCF cell lines with $IC_{50}$'s 368, 224 and 310 nM and **18b** showed high potency against HONE1 cell line with $IC_{50}$ 666 nM. For the pyrimidine derivatives **19a-c**, compound **19c** with X = $OCH_3$ showed high potency against NUGC, DLDI, HA22,

**Table 1.** Cytotoxicity of novel derivatives against a variety of cancer cell lines [$IC_{50}$[b] (nM)].

| Compound | Cytotoxocity (IC50 in nM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | NUGC | DLDI | HA22T | HEPG2 | HONE1 | MCF | WI38 |
| 3 | 1220 | 2068 | 1028 | 1432 | 2019 | 1880 | na |
| 5 | 1329 | 3260 | 1120 | 1446 | 1240 | 1430 | na |
| 7 | 3228 | 1248 | 2077 | 1686 | 3120 | 1133 | na |
| 9 | 1487 | 3210 | 3240 | 2336 | 3382 | 2322 | 665 |
| 11a | 2760 | 1185 | 2688 | 3311 | 2276 | 2355 | na |
| 11b | 49 | 36 | 66 | 320 | 39 | 286 | na |
| 11c | 462 | 2102 | 1058 | 1260 | 1160 | 3320 | na |
| 13 | 2366 | 1089 | 260 | 82 | 71 | 559 | na |
| 15a | 1180 | 1500 | 1981 | 1371 | 1289 | 1133 | na |
| 15b | 182 | 1062 | 560 | 299 | 3254 | 210 | na |
| 15c | 1280 | 2872 | 1329 | 1258 | 2107 | 1169 | na |
| 17a | 270 | 348 | 163 | 331 | 178 | 2071 | na |
| 17b | 22 | 39 | 160 | 82 | 49 | 122 | na |
| 17c | 39 | 682 | 211 | 202 | 130 | 52 | na |
| 18a | 1750 | 368 | 1270 | 225 | 1140 | 310 | na |
| 18b | 2210 | 1011 | 1140 | 2134 | 666 | 1280 | na |
| 18c | 2130 | 1148 | 2013 | 2176 | 1670 | 1742 | na |
| 19a | 2080 | 2188 | 3840 | 2060 | 3160 | 2246 | na |
| 19b | 1026 | 2180 | 2138 | 2132 | 2470 | 2140 | na |
| 19c | 880 | 488 | 150 | 1040 | 1188 | 504 | na |
| 20 | 220 | 328 | 214 | 223 | 329 | 128 | na |
| CHS 828 | 25 | 2315 | 2067 | 1245 | 15 | 18 | na |

[a]NUGC, gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells.

and MCF cell lines. Finally, the thiazole derivative **20** showed high potency against the six cancer cell lines. Its high potency is attributed to the presence of high content of N and S together with the phenyl moiety through the molecule.

## 5. Conclusions

A series of new heterocyclic compounds with the thiazole nucleus were synthesized and characterized. Their cytotoxicity against six cancer cell lines was measured and the results showed that compounds **11b**, **11c**, **15b**, **17a**, **17b**, **17c**, **19c** and **20** were the most potent compounds among the synthesized compounds. The 2-amino-4-(4-chlorophenyl)-6-(4-phenylthiazol-2-yl)-4H-pyran-3, 5-dicarbonitrile (**17b**) showed the maximum cytotoxicity among the synthesized compounds towards the six cancer cell lines.

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