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# Novel synthesis of pyran, thiophene, and pyridine derivatives incorporating thiazole ring and their antitumor evaluation

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**Abstract**

This study aims to design and synthesize a number of novel pyran, thiophene, and pyridine derivatives incorporating thiazole ring and evaluate their antitumor inhibition (μM) as significant anticancer agents. The reactivity of compound **1** [2-(4-oxo-4,5-dihydrothiazol-2-yl)acetonitrile] towards different chemical reagents was described. Furthermore, the reactivity of all the newly synthesized products was evaluated. The most active compounds towards all the three tumor cancer cell lines used such as MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer), and normal fibroblasts human cell line (WI-38) were compounds **6d**, **8**, and **10b**, which compared with the antiproliferative effects of the reference control doxorubicin. Also, some of the novel compounds indicate higher inhibition than doxorubicin against some of the cancer cell lines used such as **6c** (especially towards MCF-7) and **2b**, **6b** (especially towards SF-268).

**KEYWORDS**

antitumor, pyran, pyridine, thiazole, thiophene

## 1 | INTRODUCTION

Thiazole ring has been identified as a central structural element of a number of biological active natural products and of pharmacological active substances. In nature, thiazoles play an important role where thiazolium ring present in vitamin B1. Many pendant and fused thiazole systems exhibit various applications in different fields, such as in medicinal chemistry as antibacterial[1,2], antifungal[3,4], anti-inflammatory[5,6], anticonvulsant[7,8], antiviral[9,10], antioxidants[11,12], skin whitening agent (KHG22394)[13,14], and anticancer.[15–17] In addition, thiazoles are applied in agriculture as agrochemicals[18–20]. Also, used in biologically active natural products[21], in materials science as sensors[22], molecular switches[23], and in the cosmetic industry as sunscreens[24]. Due to the important role of thiazole derivatives in biological systems, which in turn encouraged us to synthesize some new pyran, thiophene, and pyridine derivatives, the thiazole ring has been incorporated.

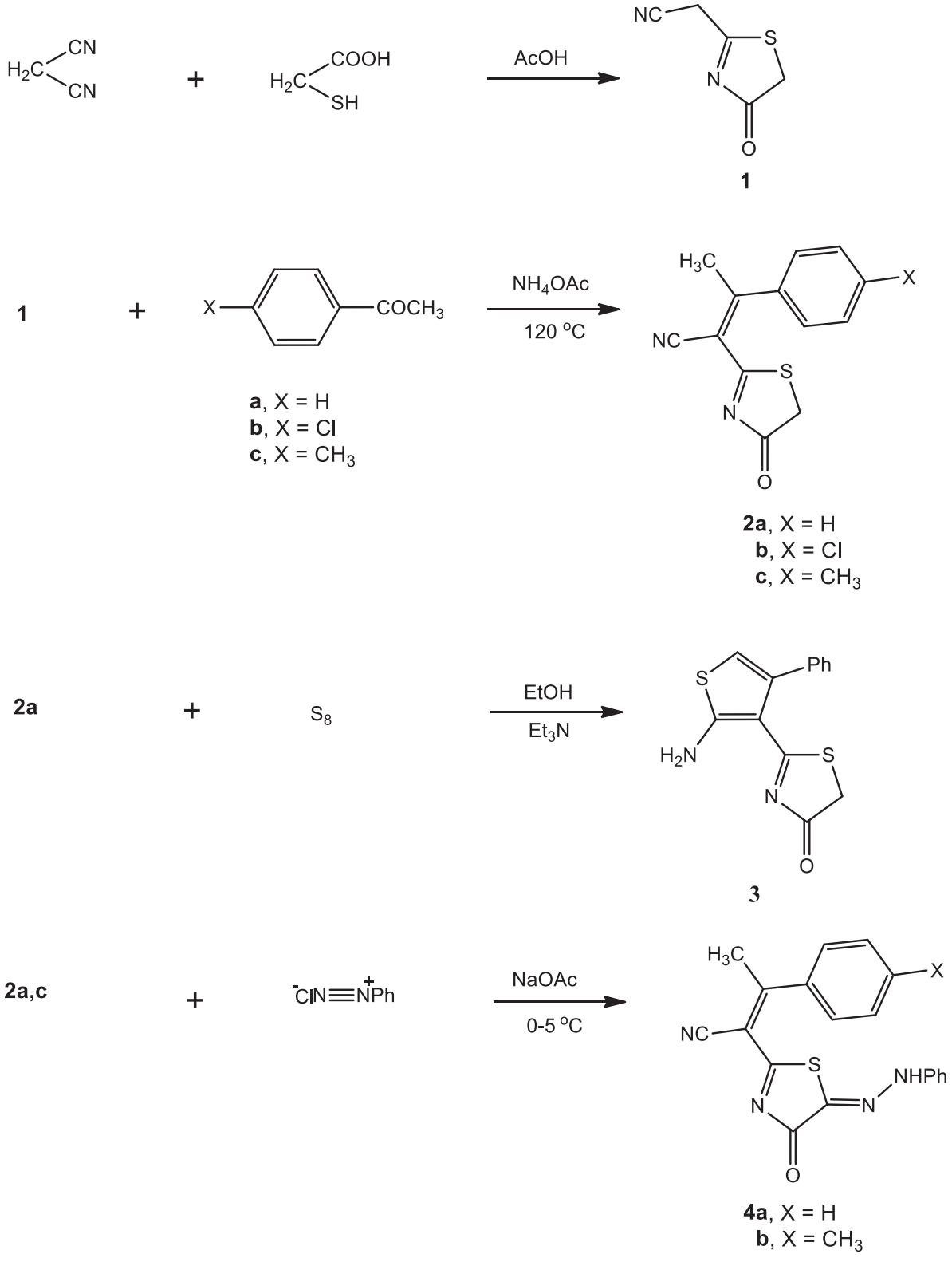
## 2 | RESULTS AND DISCUSSION

### 2.1 | Chemistry

In the current work, we are explaining the synthesis of thiazole derivatives together with their antitumor evaluation, in proceedings to our concern in synthesize of bioactive heterocyclic compounds[25–33]. Thus, compound (**1**)

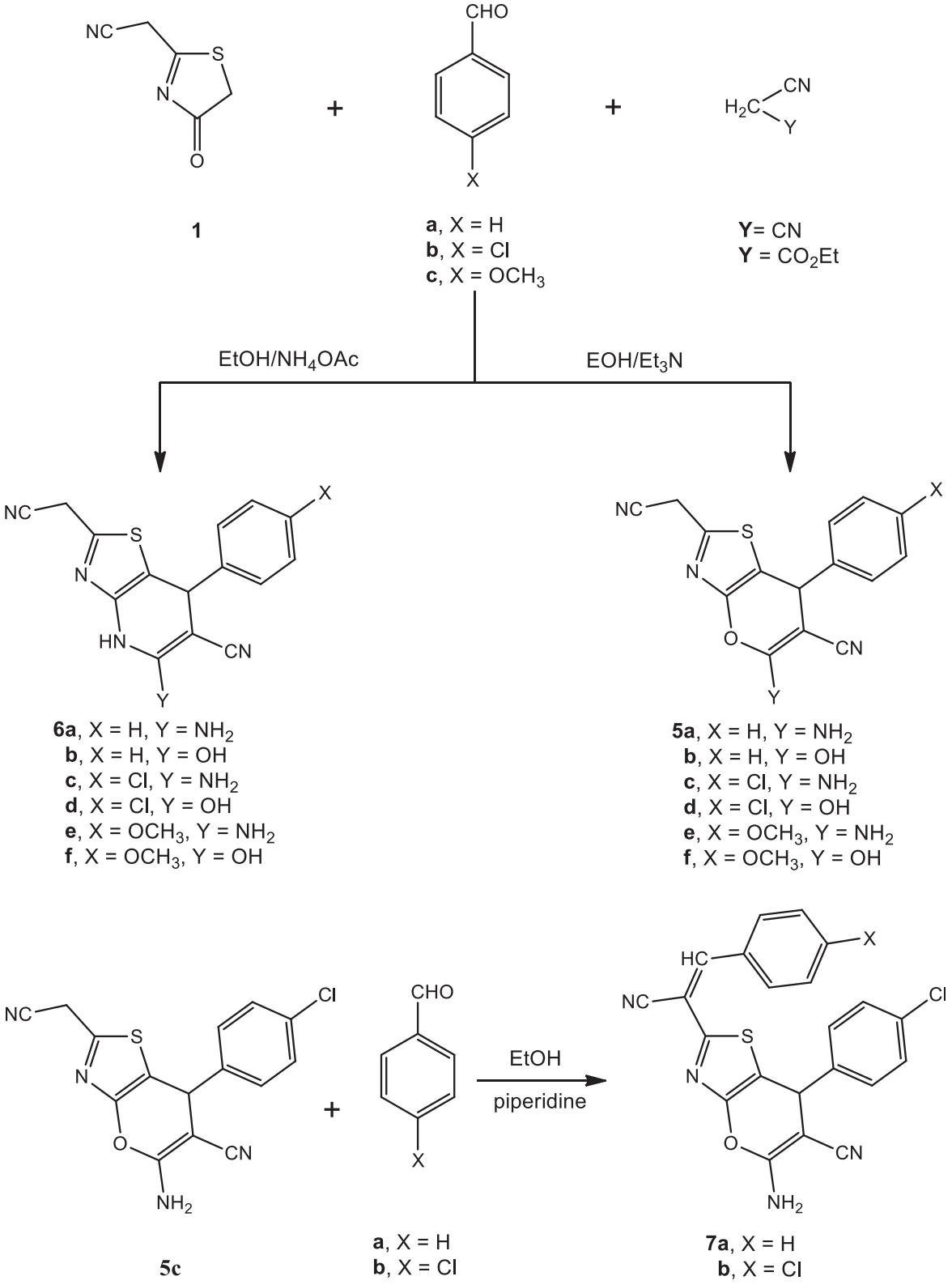
2-(4-oxo-4,5-dihydrothiazol-2-yl)acetonitrile, which was obtained as reported in literature[34] by the reaction of malononitrile with thioglycolloic acid in acetic acid solution, reacted with any of acetphenone, 4-chloroacetophenone, or 4-methylacetophenone in an oil bath at 120°C to afford the respective compounds **2a-c** through the Knoevenagel reaction (Scheme 1). The structures of the latter compounds were confirmed on the basis of their respective spectral and analytical data. Thus, the $^1$H-NMR spectrum of compound **2a** revealed a singlet at




**SCHEME 1** Synthesis of the thiazole derivatives **1**; **2a-c**; **3**; and **4a,b**

$\delta$ 2.50 ppm for $CH_3$ group, a singlet at $\delta$ 4.97 ppm for $CH_2$ group, and a multiplet at $\delta$ 7.33-7.46 ppm for the existence of the phenyl protons. Compound **2a** reacted with elemental sulfur in ethanol and triethylamine to afford the thiophene derivative **3** (Scheme 1) in an equivalent way like the reported Gewald thiophene synthesis[35].

The reaction of either compounds **2a** or **2c** with benzenediazonium chloride gave the phenylhydrazone




**SCHEME 2** Synthesis of the pyran **5a-f**, pyridine **6a-f**, and the benzylidene derivatives **7a,b**

derivatives **4a** and **4b**, respectively. The spectral and analytical data of compounds **4a** and **4b** are in concord with their structures (Scheme 1). Thus, the $^1$H-NMR spectrum of **4a** revealed a singlet at $\delta$ 2.50 ppm for $CH_3$ group, multiplet for two phenyl groups at $\delta$ 6.91-7.98 ppm, and a singlet at $\delta$ 8.55 for the presence of NH moiety. Also, compounds **4a,b** indicated a molecular formula $C_{19}H_{14}N_4OS$ (*m/z* 346 [$M^+$]) and $C_{20}H_{16}N_4OS$ (*m/z* 360 [$M^+$]) in their respective mass spectral data, which confirmed its structures.

Compound **1** underwent multi-component reactions with any of benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde and either malononitrile or ethyl cyanoacetate in ethanol containing triethylamine to afford the pyran derivatives **5a-f**, respectively (Scheme 2). The spectral and analytical data are consistent with their respective structures. The $^1$H-NMR spectrum of compound **5a** showed a singlet at $\delta$ 4.59 ppm corresponding to the $CH_2$ group, a singlet at $\delta$ 7.34 ppm equivalent to the pyran C4, a multiplet at $\delta$ 7.40-7.66 ppm indicating the phenyl protons, and a singlet at $\delta$ 7.84 ppm for the $NH_2$ group. On the other hand, compound **5c** showed a singlet at $\delta$ 4.66 ppm for $CH_2$ group, a singlet for pyran C-4 at $\delta$ 7.47 ppm, a multiplet at $\delta$ 7.53-7.69 ppm for $C_6H_4$ protons, and a $D_2O$ exchangeable singlet signal at $\delta$ 7.84 ppm corresponding to the $NH_2$ group. Besides, compound **5e** showed a singlet at $\delta$ 1.90 ppm for $CH_3$ group, a singlet at $\delta$ 4.52 ppm for $CH_2$ moiety, a singlet at $\delta$ 7.49 ppm for pyran C-4, a multiplet at $\delta$ 6.94-7.14 and 7.30-7.61 ppm for $C_6H_4$ group, and a singlet at $\delta$ 7.79 ppm indicating the presence of the $NH_2$ group.

The multi-component reaction of compound **1** with either benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde and ethyl cyanoacetate gave the pyran derivatives **5b**, **5d**, and **5f**, respectively. The structures of the previous compounds were confirmed according to their spectral data.

The multi-component reactions of compound **1** with any of the aromatic aldehydes, namely, benzaldehyde, 4-chlorobenzaldehyde, or 4-methoxybenzaldehyde and either malonnitrile or ethyl cyanocetate in ethanol containing ammonium acetate gave the pyridine derivatives **6a-f**, respectively (Scheme 2). In the mass spectra of **6a**, **6b**, **6d**, and **6f** revealed the existing [$M^+$] ions at $m/z = 293$, $m/z = 294$, $m/z = 329$, and $m/z = 324$ confirmed their respective molecular weights.

Compound **5c** reacted with either benzaldehyde or 4-chlorobenzaldehyde to afford the pyran derivatives **7a** and **7b**, respectively (Scheme 2). The mass spectra of compounds **7a** and **7b** revealed molecular ion peaks at [$M^+$] = 417 and [$M^+$] = 451 corresponding to their respective molecular formulae $C_{22}H_{13}N_4OSCl$ and $C_{22}H_{12}N_4OSCl_2$.

On the other hand, compound **1** reacted with salicylaldehyde and ethyl cyanoacetate in ethanol and triethylamine to give the chromeno[4′,3′:4,5]pyrano[2,3-*d*]thiazole derivative **8** (Scheme 3). Formation of compound **8** took place via the loss ethanol molecule through the intermediate of the pyran derivatives **A**.

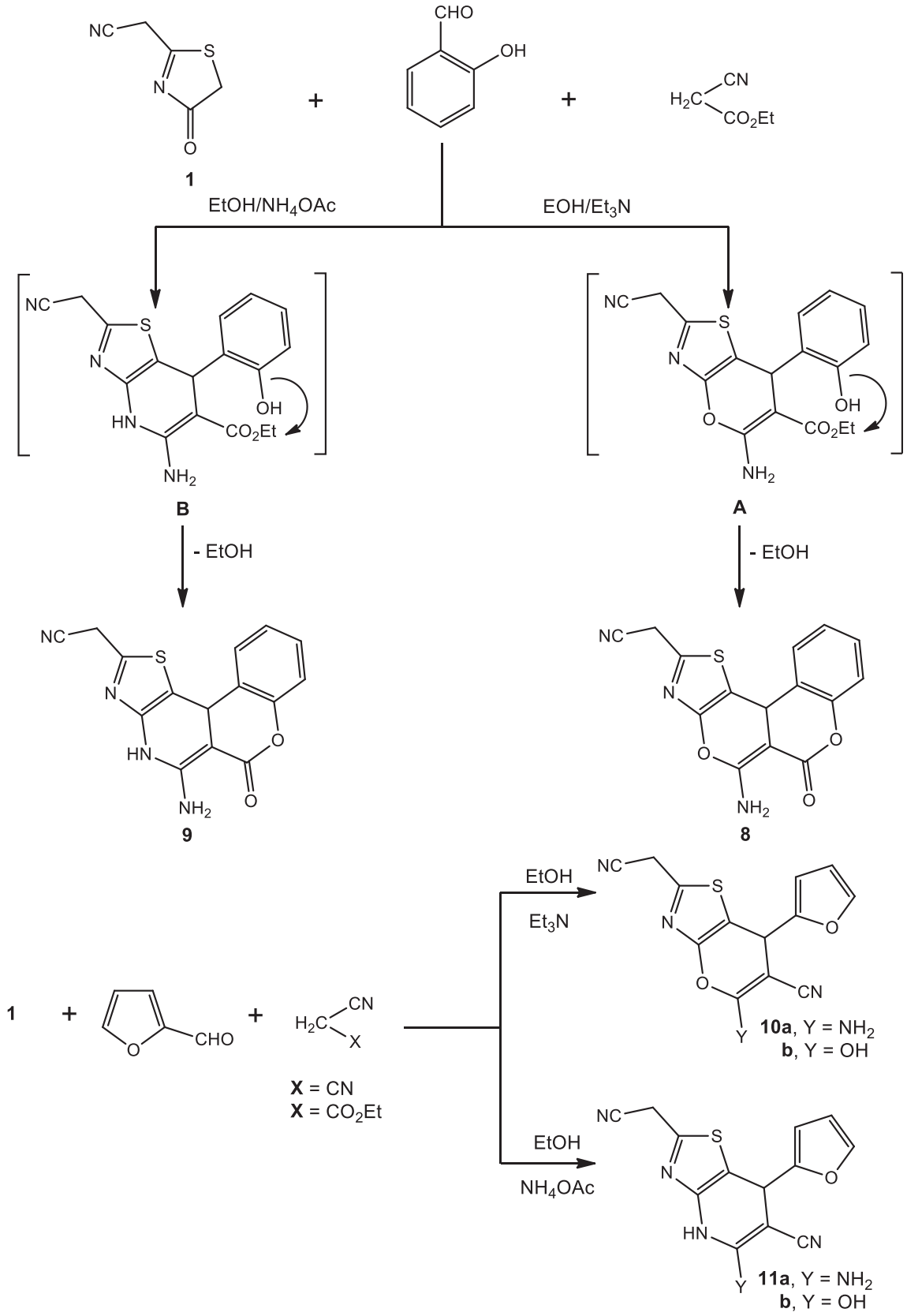
On the other hand, the reaction of compound **1** with salicylaldehyde and ethyl cyanoacetate in ethanol-containing ammonium acetate afforded the chromeno[4,3-*d*]thiazolo[4,5-*b*]pyridine derivative **9** (Scheme 3) through the intermediate formation of **B**. In addition, the reaction of compound **1** with furfural and either malononitrile or ethyl cyanoacetate in ethanol containing triethylamine gave the pyrano[2,3-*d*]thiazole derivatives **10a** and **10b**, respectively (Scheme 3). Finally, compound **1** reacted with furfural and either malononitrile or ethyl cyanoacetate in ethanol containing ammonium acetate gave the thiazolo[4,5-*b*]pyridine derivatives **11a** and **11b**, respectively (Scheme 3).

## 2.2 | In vitro cytotoxic effect on the synthesized compounds

The three cancer cell lines were used in the cytotoxicity evaluation and the results indicated that some of the prepared compounds revealed promising results (Table 1). The resulting values were compared with the antiproliferative effects of the reference control doxorubicin.[36] The tested compounds were dissolved in dimethyl sulfoxide (DMSO) at 1 mg/mL immediately before use and diluted just before addition to the cell culture. Most of the prepared compounds revealed substantial growth inhibitory effects at the concentrations tested towards the human tumor cells.

## 2.3 | Structure activity relationship

From Table 1, it is clear that compounds **6c** (especially towards MCF-7), **2b, 6b** (especially towards SF-268), **6d**, **8**, and **10b** exhibited optimal cytotoxic effect against the used cancer cell lines, with $IC_{50}$'s in the (μM) range. Considering the thiazole derivatives **2a-c**, it is obvious that compound **2b** exhibits the highest cytotoxicity among the three compounds, and this is attributed to the presence of the electronegative Cl group. In addition, this compound showed no inhibition towards the normal cell line WI38. The thiophenylthiazole derivative **3** and the thiazole derivatives **4a,b** showed low cytotoxicity towards the three cancer cell lines. Considering the pyrano[2,3-*d*]thiazole **5a-f**, it is clear such series of compounds were of low inhibitions toward the six cancer cell lines although compound **5f** (X═$OCH_3$, Y═OH) exhibited moderate cytotoxicity toward MCF-7 and NCI-460 cell lines with $CI_{50}$'s




**SCHEME 3** Synthesis of pyran **8**; pyridine **9**; pyrano[2,3-*d*]thiazole-6-carbonitrile **10a,b** and dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile derivatives **11a,b**

**TABLE 1** Antitumor effect of the newly prepared compounds in $GI_{50}$ (μM) on the growth of three human tumor cell lines and normal human cell line

| Compound | $GI_{50}$, μM[a] | | | |
|---|---|---|---|---|
| | MCF-7 | NCI-H460 | SF-268 | WI-38 |
| **2a** | 36.00 ± 4.29 | 23.85 ± 2.13 | 24.55 ± 4.60 | >100 |
| **2b** | 0.33 ± 10.84 | 0.21 ± 8.33 | 0.06 ± 0.008 | >100 |
| **2c** | 65.63 ± 10.42 | 62.01 ± 8.56 | 59.49 ± 6.39 | >100 |
| **3** | 10.36 ± 2.19 | 12.36 ± 2.29 | 19.44 ± 5.19 | 70.55 ± 12.2 |
| **4a** | 20.04 ± 10.01 | 18.07 ± 2.13 | 10.34 ± 4.33 | >100 |
| **4b** | 12.30 ± 2.43 | 10.60 ± 1.26 | 8.83 ± 2.36 | >100 |
| **5a** | 33.62 ± 2.8 | 60.49 ± 13.59 | 59.29 ± 4.65 | >100 |
| **5b** | 72.21 ± 12.88 | 69.31 ± 12.36 | 48.60 ± 5.37 | >100 |
| **5c** | 36.20 ± 2.4 | 48.60 ± 2.8 | 78.80 ± 8.5 | 80.20 ± 4.6 |
| **5d** | 33.72 ± 3.54 | 34.50 ± 2.38 | 30.50 ± 8.0 | >100 |
| **5e** | 26.20 ± 2.4 | 28.60 ± 2.8 | 26.80 ± 8.5 | 30.20 ± 2.6 |
| **5f** | 3.88 ± 0.18 | 2.05 ± 0.01 | 0.44 ± 4.01 | >100 |
| **6a** | 33.58 ± 4.09 | 30.60 ± 10.22 | 41.29 ± 12.18 | >100 |
| **6b** | 0.66 ± 0.08 | 0.52 ± 0.08 | 0.09 ± 0.001 | >100 |
| **6c** | 0.04 ± 0.001 | 0.42 ± 0.01 | 0.63 ± 0.13 | >100 |
| **6d** | 0.06 ± 0.006 | 0.06 ± 0.006 | 0.02 ± 0.008 | >100 |
| **6f** | 1.61 ± 0.05 | 4.36 ± 0.98 | 2.55 ± 0.39 | >100 |
| **7a** | 12.60 ± 2.01 | 18.60 ± 6.06 | 30.40 ± 2.36 | 30.6 ± 10.2 |
| **7b** | 0.10 ± 0.04 | 0.80 ± 0.08 | 0.10 ± 0.08 | >100 |
| **8** | 0.01 ± 0.001 | 0.02 ± 0.004 | 0.06 ± 0.002 | >100 |
| **9** | 70.22 ± 6.12 | 65.00 ± 4.7 | 55.39 ± 6.8 | >100 |
| **10a** | 58.00 ± 7.29 | 41.73 ± 4.50 | 38.41 ± 1.29 | 33.60 ± 6.21 |
| **10b** | 0.06 ± 0.002 | 0.04 ± 0.003 | 0.01 ± 0.002 | >100 |
| **11a** | 22.80 ± 8.30 | 22.80 ± 4.32 | 22.80 ± 6.23 | 44.80 ± 6.0 |
| **11b** | 12.60 ± 2.01 | 18.60 ± 6.06 | 30.40 ± 2.36 | 30.60 ± 10.2 |
| Doxorubicin | 0.0428 ± 0.0082 | 0.0940 ± 0.0087 | 0.0940 ± 0.0070 | > 100 |

*Note*: Doxorubicin was used as positive control.

[a]Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h; data are expressed as means ± SEM of three independent experiments performed in duplicates.

3.88 and 2.05μM, respectively, but it showed high inhibition towards SF-268 cell line with $GI_{50}$ 0.44μM. On the other hand, for the pyrido[2,3-*d*]thiazole **6a-f** interestingly compounds **6b** (X═H, Y═OH), **6c** (X═Cl, Y═$NH_2$), and **6d** (X═Cl, Y═OH) exhibited high inhibitions towards the six cancer cell lines. However, compound **6f** (X═$OCH_3$, Y═OH) showed moderate inhibitions. Considering the pyrano[2,3-*d*]thiazole **7a,b** where compound **7b** (X═Cl) showed higher inhibitions than **7a** (X═H). It is clear from Table 1 that the chromeno[4′,3′:4,5]pyrano[2,3-*d*]thiazole derivative **8** showed the highest cytotoxicity toward the three cancer cell lines. For the pyrano[2,3-*d*]thiazole derivatives **10a,b**, it is clear that compound **10b** (Y═OH) showed higher cytotoxities than **10a** (Y═$NH_2$) although

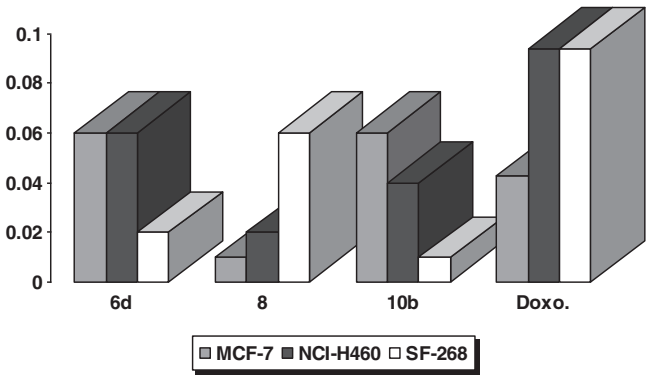



**FIGURE 1** The reactivity of the most active compounds towards the three cancer cell lines

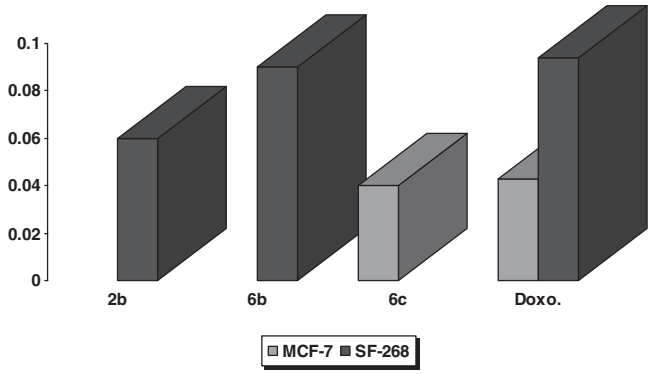



**FIGURE 2** The most active compounds towards the MCF-7 or SF-268 cancer cell lines

the pyrido[2,3-*d*]thiazole derivatives **11a,b** exhibited low inhibitions toward the three cancer cell lines.

From our study, it is clear that the presence of the electronegative OH, Cl, and $OCH_3$ hydrophobic groups in the thiazole derivatives might play a very important role in enhancing the cytotoxic effect. The antitumor activity of the newly synthesized compounds towards all the three cancer cell lines or towards some of them is indicated through Figures 1 and 2.

## 3 | CONCLUSIONS

Our study describes a simple protocol for preparing 26 newly synthesized thiazole derivatives. All data are in accordance with their proposed structures. Moreover, the new compounds were estimated for their antitumor activities on three human cancer cell lines and normal human cell line where most of the tested compounds were found to be promising as anticancer agents. The results indicated that compounds **6d**, **8**, and **10b** are the most active compounds towards the three tumor cell lines used such as MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer) tested, and normal fibroblasts human cell line (WI-38) compared with the standard reference doxorubicin. Also, some of the newly synthesized compounds showed optimal cytotoxic effect towards the two cell lines, MCF-7 (breast adenocarcinoma) such as compound **6c** and SF-268 (CNS cancer) for compounds **2b** and **6b**.

## 4 | EXPERIMENTAL

### 4.1 | General

All melting points were uncorrected and measured by an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube. IR spectra (KBr discs) were determined on a FTIR plus 460IR spectrophotometer (Shimadzu, Japan). $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Mercury-300BB (300 and 75 MHz, respectively), at Cairo University, in DMSO-$d_6$ as solvent, using TMS [$Si(CH_3)_4$] as internal standard and chemical shifts are expressed as $\delta$ ppm. Mass spectra were measured using Hewlett Packard 5988 (USA) A GC/MS system and GCMS-QP 1000 Ex Shimadzu (Japan) using EI (electron impact method). Elemental analyses were carried out on Vario EL III Elemental CHNS analyzer (Japan).

#### 4.1.1 | Synthesis of 2-(4-oxo-4,5-dihydrothiazol-2-yl)acetonitrile (1)

Compound **1** was synthesized according to the method reported earlier.[34]

#### 4.1.2 | General procedure for the synthesis of (Z)-2-(4-oxo-4,5-dihydrothiazol-2-yl)-3-phenylbut-2-enenitrile derivatives (2a-c)

To equimolar amount of **1** (1.40 g, 0.01 mol) containing a traces amount of ammonium acetate, either acetophenone (1.20 g, 0.01 mol), *p*-chloro acetophenone (1.54 g, 0.01 mol) or *p*-methyl acetophenone (1.34 g, 0.01 mol) was fused in an oil bath at 120°C under reflux for about 30 minutes and then boiled in absolute ethanol (20 mL) for few minutes. The solid products formed upon pouring onto ice/water mixture were crystallized from absolute ethanol.

#### 4.1.3 | 2-(4-Oxo-4,5-dihydrothiazol-2-yl)-3-phenylbut-2-enenitrile (2a)

Dark brown crystals from ethanol, yield (1.98 g, 82%), mp 235-238°C. IR (KBr, $cm^{-1}$): 3057 (CH aromatic), 2971 ($CH_2$, $CH_3$), 2200 (CN), 1692 (C═O) 1602, 1449 (C═C), 1563 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.50 (s, 3H, $CH_3$), 4.97 (s, 2H, $CH_2$), 7.33-7.46 (m, 5H, $C_6H_5$). MS (EI): *m/z* (%) 243 [$M^+$+1] (39.42), 242 [$M^+$] (45.26), 77 $[C_6H_5]^+$ (29.93), 54 (100.00). Elemental analysis, calculated for $C_{13}H_{10}N_2OS$ (242.30): C 64.44; H 4.16; N 11.56; S 13.23. Found: C 64.72; H 3.99; N 11.83; S 13.53.

#### 4.1.4 | 3-(4-Chlorophenyl)-2-(4-oxo-4,5-dihdrothiazol-2-yl)but-2-enenitrile (2b)

Dark brown crystals from ethanol, yield (2.10 g, 76%), mp: 127-130°C. IR (KBr, $cm^{-1}$): 3100 (CH aromatic),

2965-2926 ($CH_2$, $CH_3$), 2202 (CN), 1630 (C═O), 1580, 1485 (C═C) 1530 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.50 (s, 3H, $CH_3$) 4.86 (s, 2H, $CH_2$), 7.46 (d, $J$ = 8.7 Hz, 2H, Ar—H), 7.54 (d, $J$ = 8.7 Hz, 2H, Ar—H). MS (EI): $m/z$ (%) 279 [$M^+$+2] (5.29), 278 [$M^+$+1] (23.63), 277 [$M^+$] 15.97, 276 [$M^+$−1] (59.57), 275 [$M^+$−2] (5.84), 144 (100.00), 76 [$C_6H_4$] (13.66). Elemental analysis, calculated for $C_{13}H_9N_2OSCl$ (276.74): C, 56.42; H, 3.28; N, 10.12; S, 11.59. Found: C, 56.72; H, 3.18; N, 10.33; S, 11.93.

### 4.1.5 | 2-(4-Oxo-4,5-dihydrothiazol-2-yl)-3-(*p*-tolyl)-but-2-enenitrile (2c)

Brown crystals from ethanol, yield (1.69 g, 66%), mp: 177-180°C. IR (KBr, cm$^{-1}$): 3100 (CH aramatic) 2969-2922 ($CH_2$, $CH_3$), 2200 (CN), 1690 (C═O), 1603, 1450 (C═C), 1509 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 1.23 (s, 3H, $CH_3$), 2.50 (s, 3H, $CH_3$), 4.93 (s, 2H, $CH_2$), 7.16-7.18 (d, $J$ = 6.0 Hz, 2H, Ar—H), 7.84-7.86 (d, $J$ = 6.0 Hz, 2H, Ar—H). MS (EI): $m/z$ (%) 258 [$M^+$+2] (3.17), 257 [$M^+$+1) (10.46), 256 [$M^+$] (41.20), 80 (100.00), 76 [$C_6H_4$]$^+$ (3.65). Elemental analysis, calculated for $C_{14}H_{12}N_2OS$ (256.32): C, 65.60; H, 4.72; N, 10.93; S, 12.51. Found: C, 65.98; H, 4.39; N, 11.29; S, 12.41.

### 4.1.6 | Synthesis of 2-(2-amino-4-phenylthiophen-3-yl)thiazole-4(5*H*)-one (3)

To a solution of compound **2a** (2.42 g, 0.01 mol) in absolute ethanol (25 mL), in triethylamine (0.50 mL), an elemental sulfur (0.32 g, 0.01 mol) was added. Under reflux system, the reaction was heated for 3 hours. The obtained solid product was poured onto acidified ice/water mixture, collected by filtration and crystallized from absolute ethanol.

Dark brown crystals from ethanol, yield (2.69 g, 98%), mp: 207-210°C. IR (KBr, cm$^{-1}$): 3430, 3200 ($NH_2$), 3092 (CH aromatic), 2925 ($CH_2$), 1690 (C═O), 1630, 1442 (C═C). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.98 (s, 2H, $CH_2$), 6.70 (s, 1H, thiophene C5), 7.00 (s, 2H, $NH_2$), 7.34-7.50 (m, 5H, $C_6H_5$). MS (EI): $m/z$ (%) 275 [$M^+$+1] (2.87), 274 [$M^+$] (9.96), 273 [$M^+$−1] (4.67), 272 [$M^+$−2] (0.81), 148 (100.00). Elemental analysis, calculated for $C_{13}H_{10}N_2OS_2$ (274.36): C, 56.91; H, 3.67; N, 10.21; S, 23.37. Found: C, 57.08; H, 4.02; N, 10.29; S, 23.75.

### 4.1.7 | General procedure for the synthesis of phenyl hydrozone derivatives (4a,b)

To a cold solution (0-5°C) of **2a** (2.42 g, 0.01 mol) or **2c** (2.56 g, 0.01 mol) in sodium acetate (1.00 g), an equimolar amount of diazotized aniline (0.93 mL, 0.01 mol) was gradually added while stirring. The formed solid products upon cooling in an ice bath were collected by filtration, washed with water, and crystallized from absolute ethanol.

### 4.1.8 | 2-(4-Oxo-5-(2-phenylhydrazone)-4,5-dihydrothiazol-2-yl)-3-phenylbut-2-enenitrile (4a)

Brown crystals from ethanol, yield (3.39 g, 98%), mp: 147-150°C. IR (KBr, cm$^{-1}$): 3429 (NH), 3057 (CH aromatic), 2200 (CN), 1700 (C═O), 1602, 1488 (C═C), 1560 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 2.50 (s, 3H, $CH_3$), 6.91 (m, 1H, Ar—H), 7.53 (m, 1H, Ar—H), 7.56 (d, $J$ = 7.5 Hz, 2H, Ar—H), 7.64 (d, $J$ = 7.5 Hz, 2H, Ar—H), 7.69 (d, $J$ = 6.9 Hz, 2H, Ar—H), 7.98 (d, $J$ = 6.9 Hz, 2H, Ar—H), 8.55 (s, 1H, NH). MS (EI): $m/z$ (%) 347 [$M^+$+1] (23.08), 346 [$M^+$] (49.57), 345 [$M^+$−1] (4.27), 78 (100.00), 77 [$C_6H_5$]$^+$ (25.64). Elemental analysis, calculated for $C_{19}H_{14}N_4OS$ (346.41): C, 65.88; H, 4.07; N, 16.17; S, 9.26. Found: C, 65.88; H, 4.19; N, 16.55; S, 9.42.

### 4.1.9 | 2-(4-Oxo-5-(2-phenylhydrozono)-4,5-dihydrothiazol-2-yl)-3-(*p*-tolyl)but-2-enenitrite (4b)

Pale brown crystals from ethanol, yield (3.57 g, 99%), mp: 182-185°C. IR (KBr, cm$^{-1}$): 3424 (NH), 2920 (2$CH_3$), 2202 (CN), 1650 (C═O), 1601, 1453 (C═C). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 1.23 (s, 3H, $CH_3$), 2.35 (s, 3H, $CH_3$), 7.31-7.89 (m, 9H, $C_6H_4$, $C_6H_5$), 8.15 (s, 1H, NH). MS (EI): $m/z$ (%) 361 [$M^+$+1] (63.22), 360 [$M^+$] (66.67), 103 (100.00), 77 [$C_6H_5$]$^+$ (25.29). Elemental analysis, calculated for $C_{20}H_{16}N_4OS$ (360.43): C, 66.65; H, 4.47; N, 15.54; S, 8.90. Found: C, 67.01; H, 4.77; N, 15.93; S, 9.20.

### 4.1.10 | General procedure for the synthesis of 2-(cyanomethyl)-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitile derivatives (5a-f)

To a solution of compound **1** (1.40 g, 0.01 mol) in absolute ethanol (25 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and either benzaldehyde (1.06 g, 0.01 mol), *p*-chlorobenzaldehyde (1.40 g, 0.01 mol) or *p*-methoxybenzaldehyde (1.36 g, 0.01 mol) in triethylamine (0.50 mL) were added. The reaction mixture was heated under reflux for 30 minutes in case of malononitrile and 2 hours in case of ethyl cyanoacetate,

and the solid products being formed upon pouring onto acidified ice/water mixture was collected by filtration and crystallized from absolute ethanol.

### 4.1.11 | 5-Amino-2-(cyanomethyl)-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitile (5a)

Yellow crystals from ethanol, yield (1.76 g, 60%), mp: 252-255°C. IR (KBr, $cm^{-1}$): 3380, 3211 ($NH_2$), 3058-3026 (CH aromatic), 2926 ($CH_2$), 2250, 2201 (2 CN), 1617, 1489 (C═C), 1557 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.59 (s, 2H, $CH_2$), 7.34 (s, 1H, pyran C4), 7.40-7.66 (m, 5H, $C_6H_5$), 7.84 (s, 2H, $NH_2$). MS (EI): *m/z* (%) 296 [$M^+$+2] (0.22), 295 [$M^+$+1] (0.39), 294 [$M^+$] (1.42), 293 [$M^+$−1] (0.52), 292 [$M^+$−2] (0.33), 77 $[C_6H_5]^+$ (24.52), 134 (100.00). Elemental analysis, calculated for $C_{15}H_{10}N_4OS$ (294.33): C, 61.21; H, 3.42; N, 19.04; S, 10.89. Found: C, 61.49; H, 3.72; N, 18.88; S, 11.26.

### 4.1.12 | 2-(Cyanomethyl)-5-hydroxy-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (5b)

Yellow crystals from ethanol, yield (2.80 g, 95%), mp: 117-120°C. IR (KBr, $cm^{-1}$): 3406 (OH), 3063 (CH aromatic), 2979 ($CH_2$), 2260, 2205 (2 CN), 1603, 1448 (C═C) 1510 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.60 (s, 2H, $CH_2$), 6.93 (s, 1H, pyran C4), 7.26-7.28 (m, 1H, Ar—H), 7.37 (d, $J$ = 7.5 Hz, 2H, Ar—H), 7.56 (d, $J$ = 7.5 Hz, 2H, Ar—H), 8.61 (s, 1H, OH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 13.7, 38.7, 58.9, 116.1, 118.9, 126.5, 128.6, 128.6, 129.2, 129.2, 132.8, 144.1, 148.4, 169.8, 170.1. MS (EI): *m/z* (%) 297 [$M^+$+2) (5.72), 296 [$M^+$+1) (13.87), 295 [$M^+$] (4.91), 294 [$M^+$−1] (3.85), 293 [$M^+$−2] (2.94), 77 $[C_6H_5]^+$ (100.00). Elemental analysis, calculated for $C_{15}H_9N_3O_2S$ (295.32): C, 61.01; H, 3.07; N, 14.23; S, 10.86. Found: C, 60.99; H, 3.37; N, 13.89; S, 10.56.

### 4.1.13 | 5-Amino-7-(4-chlorophenyl)-2-(cyanomethyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbanitile (5c)

Yellow crystals from ethanol, yield (2.46 g, 75%), mp: 277-280°C. IR (KBr, $cm^{-1}$): 3378, 3208 ($NH_2$), 3024 (CH aromatic), 2924 ($CH_2$), 2257, 2203 (2 CN), 1614, 1485 (C═C), 1558 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.66 (s, 2H, $CH_2$), 7.47 (s, 1H, pyran C4), 7.53-7.69 (m, 4H, $C_6H_4$), 7.84 (s, 2H, $NH_2$). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 38.8, 39.6, 63.5, 115.6, 118.4, 120.5, 120.5, 130.0, 130.0, 131.7, 132.8, 140.6, 147.9, 165.0, 169.6. MS (EI): *m/z* (%) 330 [$M^+$+1] (21.69), 329 [$M^+$] (20.88), 328 [$M^+$−1] (29.32), 327 [$M^+$−2] (27.31), 76 $[C_6H_4]^+$ (17.27), 69 (100.00). Elemental analysis, calculated for $C_{15}H_9N_4OSCl$ (328.78): C, 54.80; H, 2.76; N, 17.04; S, 9.75. Found: C, 54.50; H, 3.09; N, 16.77; S, 10.13.

### 4.1.14 | 7-(4-Chlorophenyl)-2-(cyanomethyl)-5-hydroxy-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (5d)

Canary yellow crystals from ethanol, yield (1.98 g, 60%), mp: 82-85°C. IR (KBr, $cm^{-1}$): 3410 (OH), 3050 (CH aromatic), 2981 ($CH_2$), 2260, 2205 (2 CN), 1634, 1483 (C═C), 1520 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.93 (s, 2H, $CH_2$), 6.84 (s, 1H pyrano C4), 7.22 (d, $J$ = 8.7 Hz, 2H, Ar—H), 7.31 (d, $J$ = 8.7 Hz, 2H, Ar—H), 8.60 (s, 1H, OH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 13.9, 38.9, 59.2, 117.9, 119.6, 120.8, 120.8, 129.4, 129.4, 130.9, 131.7, 143.1, 148.4, 170.8, 174.5. MS (EI): *m/z* (%) 332 [$M^+$+2] (1.55), 331 [$M^+$+1] (2.71), 330 [$M^+$] (1.64), 329 [$M^+$−1] (8.21), 328 [$M^+$−2] (5.02), 264 (100.00). Elemental analysis, calculated for $C_{15}H_8N_3O_2SCl$ (329.76): C, 54.63; H, 2.45; N, 12.74; S, 9.72. Found: C, 54.33; H, 2.33; N, 13.13; S, 10.09.

### 4.1.15 | 5-Amino-2-(cyanomethyl)-7-(4-methoxyphenyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonirile (5e)

Orange crystal from ethanol, yield (2.27 g, 70%), mp: 257-260°C. IR (KBr, $cm^{-1}$): 3389, 3200, ($NH_2$), 3005 (CH aromatic), 2930-2840 ($CH_2$, $CH_3$), 2260, 2202 (2 CN) 1593, 1447 (C═C), 1560 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 1.90 (s, 3H, $CH_3$), 4.52 (s, 2H, $CH_2$), 6.94 (d, $J$ = 8.7 Hz, 1H, Ar—H), 7.14 (d, $J$ = 8.7 Hz, 1H, Ar—H), 7.30 (d, 1H, Ar—H), 7.49 (s, 1H, pyran C4), 7.61 (d, $J$ = 8.7 Hz, 1H, Ar—H), 7.79 (s, 2H, $NH_2$). MS (EI): *m/z* (%) 325 [$M^+$+1] (0.97), 324 [$M^+$] (0.81), 323 [$M^+$−1] (2.18), 76 $[C_6H_4]^+$ (16.25), 243 (100.00). Elemental analysis, calculated for $C_{16}H_{12}N_4O_2S$ (324.36): C, 59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 59.55; H, 4.02; N, 16.90; S, 10.04.

### 4.1.16 | 2-(Cyanomethyl)-5-hydroxy-7-(4-methoxyphenyl)-7H-pyrano[2,3-*d*]thiazole-6-carbonitrile (5f)

Yellow crystals from ethanol, yield (1.95 g, 60%), mp: 247-250°C. IR (KBr, $cm^{-1}$): 3380 (OH), 3060 (CH aromatic), 2969-2841 ($CH_2$, $CH_3$), 2258, 2206 (2 CN), 1637, 1450 (C═C), 1506 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 3.45 (s, 3H, $CH_3$), 4.52 (s, 2H, $CH_2$), 6.85 (d, $J$ = 8.7 Hz, 1H, Ar—H),

7.12-7.15 (d, 1H, Ar—H), 7.22 (d, $J$ = 8.7 Hz, 1H, Ar—H), 7.61 (d, $J$ = 8.7 Hz, 1H, Ar—H), 7.79 (s, 1H, pyran C4), 8.59 (s, 1H, OH). MS (EI): *m/z* (%) 326 [$M^+$+1]+ (65.00), 325 [$M^+$] (76.00), 324 [$M^+$−1] (63.00), 231 (100.00). Elemental analysis, calculated for $C_{16}H_{11}N_3O_3S$ (325.34): C, 59.07; H, 3.41; N, 12.92; S, 9.86. Found: C, 58.79; H, 3.71; N, 12.59; S, 10.03.

### 4.1.17 | General procedure for the synthesis of 2-(cyanomethyl)-7-phenyl-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile derivatives (6a-f)

To a solution of compound **1** (1.40 g, 0.01 mol) in absolute ethanol (25 mL) containing ammonium acetate, each of either benzaldehyde (1.06 g, 0.01 mol), *p*-chlorobenzaldehyde (1.40 g, 0.01 mol), or *p*-methoxybenzldehyde (1.36 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 30 minutes and then poured onto ice/water containing a few drops of hydrochloric acid. The formed solid product, in each case, was filtered of and crystallized from absolute ethanol.

### 4.1.18 | 5-Amino-2-(cyanomethyl)-7-phenyl-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile (6a)

Pale green crystals from ethanol, yield (2.82 g, 96%), mp: 127-130°C. IR (KBr, $cm^{-1}$): 3378-3209 (NH, $NH_2$), 3060-3027 (CH aromatic), 2900 ($CH_2$), 2250, 2195 (2 CN), 1618, 1493 (C═C), 1558 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.61 (s, 2H, $CH_2$), 7.21-7.23 (m, 1H, Ar—H), 7.29 (d, $J$ = 9 Hz, 2H, Ar—H), 7.52 (d, $J$ = 9 Hz, 2H, Ar—H), 7.61 (s, 1H, pyridine C4), 7.86 (s, 2H, $NH_2$), 8.02 (s, 1H, NH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 24.3, 33.3, 63.6, 115.9, 118.7, 127.0, 128.6, 128.6, 129.3, 129.3, 134.6, 142.8, 147.8, 165.3, 168.9. MS (EI): *m/z* (%) 295 [$M^+$+2] (3.23), 294 [$M^+$+1] (15.21), 293 [$M^+$] (6.09), 77 $[C_6H_5]^+$ (18.56), 91 (100.00). Elemental analysis, calculated for $C_{15}H_{11}N_5S$ (293.35): C, 61.42; H, 3.78; N, 23.87; S, 10.93. Found: C, 61.03; H, 4.02; N, 23.57; S, 11.29.

### 4.1.19 | 2-(Cyanomethyl)-5-hydroxy-7-phenyl-4,7-dihydro-thiazolo[4,5-*b*]pyridine-6-carbonitrile (6b)

Brown crystal from ethanol, yield (1.62 g, 55%), mp: 137-140°C. IR (KBr, $cm^{-1}$): 3406 (NH, OH), 3056-3025 (CH aromatic), 2980-2931 ($CH_2$), 2258, 2260, 2203 (2 CN), 1634, 1491 (C═C), 1507 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.04 (s, 2H, $CH_2$), 7.39-7.42 (m, 1H, Ar—H), 7.51 (d, $J$ = 7.8 Hz, 2H, Ar—H), 7.59 (d, $J$ = 7.8 Hz, 2H, Ar—H), 7.66 (s, 1H, pyridine C4), 7.85 (s, 1H, NH), 8.60 (s, 1H, OH). MS (EI): *m/z* (%) 296 [$M^+$+1] (0.63), 295 [$M^+$+] (0.64), 294 [$M^+$] (0.76), 293 [$M^+$−1] (0.20), 77 $[C_6H_5]^+$ (7.71), 59 (100.00). Elemental analysis, calculated for $C_{15}H_{10}N_4OS$ (294.33): C, 61.21; H, 3.42; N, 19.04; S, 10.89. Found: C, 61.51; H, 3.70; N, 18.73; S, 11.19.

### 4.1.20 | 5-Amino-7-(4-chlorophenyl)-2-(cyanomethyl)-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile (6c)

Pale green crystals from ethanol, yield (2.82 g, 86%), mp: 167-170°C. IR (KBr, $cm^{-1}$): 3425-3212 (NH, $NH_2$), 3060 (CH aromatic), 2900 ($CH_2$), 2250, 2209 (2 CN), 1618, 1491 (C═C), 1554 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.67 (s, 2H, $CH_2$), 7.40 (d, $J$ = 6.9 Hz, 2H, Ar—H), 7.63 (d, $J$ = 6.9 Hz, 2H, Ar—H), 7.69 (s, 1H, pyrano C4), 7.71 (s, 2H, $NH_2$), 7.86 (s, 1H, NH). MS (EI): *m/z* (%) 327 [$M^+$−1] (0.27), 326 [$M^+$−2] (0.63), 76 $[C_6H_4]^+$ (8.62), 145 (100.00). Elemental analysis, calculated for $C_{15}H_{10}N_5SCl$ (327.79): C, 54.96; H, 3.07; N, 21.37; S, 9.78. Found: C, 55.26; H, 3.42; N, 21.01; S, 10.12.

### 4.1.21 | 7-(4-Chlorophenyl)-2-(cyanomethyl)-5-hydroxy-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile (6d)

Yellow crystals from ethanol, yield (1.84 g, 56%), mp: 237-240°C. IR (KBr, $cm^{-1}$): 3378-3250 (NH, OH), 3050 (CH aromatic), 2980-2929 ($CH_2$), 2260, 2210 (2 CN) 1639, 1495 (C═C), 1594 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.61 (s, 2H, $CH_2$), 7.37 (s, 1H, pyridine C4), 7.61 (d, $J$ = 9 Hz, 2H, Ar—H), 7.66 (d, $J$ = 9 Hz, 2H, Ar—H), 7.83 (s, 1H, NH), 8.61 (s, 1H, OH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 13.8, 38.8, 59.1, 116.0, 119.7, 128.2, 128.2, 130.1, 130.1, 131.7, 135.1, 143.2, 148.5, 165.5, 167.8. MS (EI): *m/z* (%) 331 [$M^+$+1] (0.28), 330 [$M^+$+1] (0.49), 329 [$M^+$] (0.23), 328 [$M^+$−1] (0.11), 327 [$M^+$−1] (0.18), 76 $[C_6H_4]^+$ (1.56), 59 (100.00). Elemental analysis, calculated for $C_{15}H_9N_4OSCl$ (328.78): C, 54.80; H, 2.76; N, 17.04; S, 9.75. Found: C, 54.50; H, 3.06; N, 16.70; S, 10.05.

### 4.1.22 | 5-Amino-2-(cyanomethyl) 7-(4-methoxyphenyl)-4,7-dihydrothiazolo [4,5-*b*]pyridine-6-carbonitrile (6e)

Canary yellow crystals from ethanol, yield (2.81 g, 87%), mp: 262-265°C. IR (KBr, $cm^{-1}$): 3500-3399 (NH, $NH_2$), 3100 (CH aromatic), 2929 ($CH_2$, $CH_3$), 2200 (CN), 1562,

1416 (C═C), 1563 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 3.84 (s, 3H, $CH_3$), 4.52 (s, 2H, $CH_2$), 6.95 (d, $J$ = 8.7 Hz, 2H, Ar—H), 7.33 (s, 1H, pyridine C4), 7.47 (s, 2H, $NH_2$), 7.61 (d, $J$ = 8.7 Hz, 2H, Ar—H), 7.80 (s, 1H, NH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 27.0, 38.7, 55.5, 65.0, 114.1, 114.1, 115.9, 118.6, 125.3, 130.1, 130.1, 142.0, 147.7, 158.9, 161.1, 165.0. Elemental analysis, calculated for $C_{16}H_{13}N_5OS$ (323.37): C, 59.43; H, 4.05; N, 21.66; S, 9.92. Found: C, 59.10; H, 3.77; N, 21.44; S, 10.21.

### 4.1.23 | 2-(Cyanomethyl)-5-hydroxy-7-(4-methoxyphenyl)-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile (6f)

Yellow crystals from ethanol, yield (2.11 g, 65%), mp: 247-250°C. IR (KBr, cm$^{-1}$): 3379-3200 (NH, OH), 3065 (CH aromatic), 2970-2840 ($CH_2$, $CH_3$), 2260, 2206 (2 CN), 1636, 1453 (C═C), 1506 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 3.82 (s, 3H, $CH_3$), 4.52 (s, 2H, $CH_2$), 7.15 (s, 1H, pyridine C4), 6.85 (d, $J$ = 6.9 Hz, 1H, Ar—H), 7.13 (d, $J$ = 6.9 Hz, 1H, Ar—H), 7.22 (d, 1H, Ar—H), 7.60 (d, $J$ = 6.9 Hz, 1H, Ar—H), 7.79 (s, 1H, NH), 8.59 (s, 1H, OH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 13.7, 38.7, 55.0, 58.9, 114.9, 114.9, 115.4, 116.3, 128.8, 131.6, 131.6, 143.0, 148.3, 158.2, 167.9, 168.7. MS (EI): $m/z$ (%) 326 [$M^+$+2] (0.14), 325 [$M^+$+1] (0.45), 324 [$M^+$] (0.11), 323 [$M^+$−1] (0.12), 322 [$M^+$−2] (0.10), 76 $[C_6H_4]^+$ (0.70), 59 (100.00). Elemental analysis, calculated for $C_{16}H_{12}N_4O_2S$ (324.36): C, 59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 59.55; H, 4.11; N, 16.97; S, 10.23.

### 4.1.24 | General procedure for the synthesis of 5-Amino-7-(4-chlorophenyl)-2-(1-cyano-2-phenylvinyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile derivatives (7a,b)

To a solution of compound **5c** (3.28 g, 0.01 mol) in absolute ethanol (25 mL) in piperidine (0.5 mL), either benzaldehyde (1.06 g, 0.01 mol), or *p*-chlorobenzaldhyde (1.40 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 hour then poured onto ice/water mixture containing a few drops of hydrochloric acid. The formed solid products was filtered off and crystallized from absolute ethanol.

### 4.1.25 | 5-Amino-7-(4-chlorophenyl)-2-(1-cyano-2-phenylvinyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (7a)

Orange crystals from ethanol, yield (3.29 g, 79%), mp: 147-150°C. IR (KBr, cm$^{-1}$): 3438, 3300 ($NH_2$), 3100 (CH aromatic), 2942-2859 (CH), 2189, 2220 (2 CN), 1622, 1438 (C═C), 1549 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.45 (s, 1H, CH), 6.45 (m, $J$ = 6.6 Hz, 1H, Ar—H), 7.16 (s, 1H, pyran C4), 7.27 (d, $J$ = 8.4 Hz, 2H, Ar—H), 7.57 (d, $J$ = 8.4 Hz, 2H, Ar—H), 7.69 (d, $J$ = 6.6 Hz, 2H, Ar—H), 7.78 (s, 2H, $NH_2$), 7.92 (d, $J$ = 6.6 Hz, 2H, Ar—H),. $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 38.6, 57.0, 107.0, 115.3, 118.9, 120.7, 120.7, 127.3, 128.7, 128.7, 129.9, 129.9, 131.8, 131.8, 132.6, 132.8, 135.0, 143.2, 148.1, 158.6, 168.6, 168.7. MS (EI): $m/z$ (%) 419 [$M^+$+2] (1.53), 418 [$M^+$+1] (1.20), 417 [$M^+$] (1.71), 416 [$M^+$−1] (2.34), 415 [$M^+$−2] (1.91), 76 $[C_6H_4]^+$ (9.22), 77 $[C_6H_5]^+$ (19.59), 84 (100.000). Elemental analysis, calculated for $C_{22}H_{13}N_4OSCl$ (416.88): C, 63.38; H, 3.14; N, 13.44; S, 7.69. Found: C, 63.01; H, 3.50; N, 13.74; S, 8.06.

### 4.1.26 | 5-Amino-7-(4-chlorophenyl)-2-(2-(4-chlorophenyl)-1-cyanovinyl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (7b)

Yellow crystals from ethanol, yield (3.29 g, 73%), mp: 277-280°C. IR (KBr, cm$^{-1}$), 3424, 3380 ($NH_2$), 3100 (CH aromatic), 2922 (CH), 2292, 2207 (2 CN), 1618, 1488 (C═C), 1560 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.67 (s, 1H, CH), 7.48 (s, 1H, pyran C4), 7.63-7.71 (m, 8H, $2C_6H_4$), 7.86 (s, 2H, $NH_2$). MS (EI): $m/z$ (%) 453 [$M^+$+2] (3.02), 452 [$M^+$+1] (9.49), 451 [$M^+$] (6.77), 450 [$M^+$−1] (13.86), 449 [$M^+$−2] (4.81), 76 $[C_6H_4]^+$ (1.73), 339 (100.00). Elemental analysis, calculated for $C_{22}H_{12}N_4OSCl_2$ (451.33): C, 58.55; H, 2.68; N, 12.41; S, 7.10. Found: C, 58.73; H, 3.01; N, 12.70; S, 7.40.

### 4.1.27 | Synthesis of 2-(5-hydroxy-6-oxo-6,11*b*-dihydro-chromeno[4′,3′:4,5]pyrano[2,3-*d*]thiazol-2-yl)acetonitrile (8)

Compound (**1**) (1.40 g, 0.01 mol) in absolute ethanol (25 mL) and ethyl cyanoacetate (1.13 g, 0.01 mol) was added to salicyaldehyde (1.22 g, 0.01 mol) in triethylamine (0.5 mL). By heating the reaction mixture under reflux for 2 hours, the solid products will be formed. Collect the products by filtration after pouring it onto acidified ice/water mixture and crystallized from absolute ethanol.

Pale brown crystals from ethanol, yield (3.09 g, 99%), mp: 127-130°C. IR (KBr, cm$^{-1}$): 3410, 3316 ($NH_2$), 2977 ($CH_2$), 2204 (CN), 1672 (C═O), 1607, 1452 (C═C), 1521 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.20 (s, 2H, $CH_2$), 7.01 (s, 1H, pyran C4), 7.26-7.40 (m, 4H, $C_6H_4$), 7.73 (s, 2H, $NH_2$). MS (EI): $m/z$ (%) 312 [$M^+$+1] (0.95), 311 [$M^+$] (1.48), 310 [$M^+$−1] (1.88), 76 $[C_6H_4]^+$ (5.95), 128 (100.00). Elemental analysis, calculated for $C_{15}H_9N_3O_3S$ (311.32): C,

57.87; H, 2.91; N, 13.50; S, 10.30. Found: C, 57.59; H, 2.95; N, 13.88; S, 10.15.

### 4.1.28 | Synthesis of 2-(5-hydroxy-6-oxo-6,11*b*-dihydro-4*H*-chromeno[4,3-*d*]thiazolo[4,5-*b*]pyridin-2-yl)acetonitrile (9)

To a solution of compound **1** (1.40 g, 0.01 mol) in absolute ethanol (30 mL) containing ammonium acetate (0.50 g), each of ethyl cyanoacetate (1.13 g, 0.01 mol) and salicyaldehyde (1.22 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 30 minutes and then poured onto ice/water mixture. The formed solid product was collected by filtration and dried, then crystallized from absolute ethanol.

Orange crystals from ethanol, yield (3.07 g, 99%), mp: 222-225°C. IR (KBr, $cm^{-1}$): 3423, 3300 ($NH_2$, NH), 2973 ($CH_2$), 2205 (CN), 1642 (C═O), 1605, 1451 (C═C), 1540 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.01 (s, 2H, $CH_2$), 6.67 (s, 1H, pyridine C4), 7.10-7.30 (m, 4H, $C_6H_4$), 7.50 (s, 2H, $NH_2$), 7.92 (s, 1H, NH). MS (EI): *m/z* (%) 311 [$M^+$+1] (0.87), 310 [$M^+$] (1.50), 67 (100.00), 76 [$C_6H_4$]$^+$ (14.85). Elemental analysis, calculated for $C_{15}H_{10}N_4O_2S$ (310.33): C, 58.05; H, 3.25; N, 18.05; S, 10.33. Found: C, 57.73; H, 3.50; N, 18.22; S, 10.16.

### 4.1.29 | General procedure for the synthesis of 2-(cyanomethyl)-7-(furan-2-yl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile derivatives (10a,b)

To a solution of compound **1** (1.40 g, 0.01 mol) in absolute ethanol (25 mL) containing triethylamine (0.50 mL), each of either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and furfural (0.96 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 20 minutes in case of malononitrile and 3 hours in case of ethyl cyanoacetate, then poured onto acidified ice/water mixture, and then collected the formed products after filtration and crystallized from absolute ethanol.

### 4.1.30 | 5-Amino-2-(cyanomethyl)-7-(furan-2-yl)-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (10a)

Brown crystals from ethanol, yield (1.70 g, 60%), mp: 245-248°C. IR (KBr, $cm^{-1}$): 3388, 3326 ($NH_2$), 3121-3023 (CH aromatic), 2975 ($CH_2$), 2220, 2195 (2 CN), 1652, 1465 (C═C), 1539 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.84 (s, 2H, $CH_2$), 6.42-6.49; 6.78-6.85; 7.18-7.19 (m, 3H, furan C), 7.42 (s, 1H, pyran C4), 8.16 (s, 2H, $NH_2$). MS (EI): *m/z* (%) 285 [$M^+$+1] (0.30), 59 (100.00). Elemental analysis, Calculated for $C_{13}H_8N_4O_2S$ (284.29): C, 54.92; H, 2.84; N, 19.71; S, 11.28. Found: C, 54.59; H, 2.89; N, 19.39; S, 11.58.

### 4.1.31 | 2-(Cyanomethyl)-7-(furan-2-yl)-5-hydroxy-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (10b)

Brown crystals from ethanol, yield (1.62 g, 57%), mp: 227-230°C. IR (KBr, $cm^{-1}$): 3407 (OH), 3125-3064 (CH aromatic), 2979-2928 ($CH_2$), 2260, 2207 (2 CN), 1636, 1446 (C═C), 1503 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.80 (s, 2H, $CH_2$), 6.21-6.39; 6.74-6.79; 7.04-7.19 (m, 3H, furan C), 7.48 (s, 1H pyran C4), 8.59 (s, 1H, OH). $^{13}$C-NMR (75 MHz, DMSO-$d_6$) $\delta$: 13.9, 34.1, 59.1, 106.1, 110.6, 116.1, 117.4, 142.1, 146.9, 148.9, 155.2, 167.8, 168.5. MS (EI): *m/z* (%) 287 [$M^+$+2] (0.19), 286 [$M^+$+1] (0.62), 285 [$M^+$] (0.94), 284 [$M^+$−1] (1.97), 283 [$M^+$−2] (9.92), 255 (100.00). Elemental analysis, calculated for $C_{13}H_7N_3O_3S$ (285.28): C, 54.73; H, 2.47; N, 14.73; S, 11.24. Found: C, 54.59; H, 2.77; N, 15.05; S, 11.54.

### 4.1.32 | General procedure for the synthesis of 2-(cyanomethyl)-7-(furan-2-yl)-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile derivatives (11a,b)

To a solution of compound **1** (1.40 g, 0.01 mol) in absolute ethanol (20 mL) containing ammonium acetate (0.50 g), each of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and furfural (0.96 g, 0.01 mol) were added. The reaction mixture was heated under reflex for 1 hour then poured onto ice/water mixture. The obtained solid products, in each case, were collected by filtration and crystallized from absolute ethanol.

### 4.1.33 | 5-Amino-2-(cyanomethyl)-7-(furan-2-yl)-4,7-dihydrothiazolo[4,5-*b*]pyridine-6-carbonitrile (11a)

Dark red crystals from ethanol, yield (1.70 g, 60%), mp: 197-200°C. IR (KBr, $cm^{-1}$): 3403-3219 (NH, $NH_2$), 3041 (CH aromatic), 2973-2922 ($CH_2$), 2260, 2204 (2 CN), 1652, 1463 (C═C), 1540 (C═N). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.84 (s, 2H, $CH_2$), 6.41-6.48; 6.78-6.84; 7.18-7.19 (m, 3H, furan C), 7.41 (s, 1H pyridine C4), 7.58 (s, 2H, $NH_2$), 8.16 (s, 1H, NH). $^{13}$C-NMR (75 MHz,

DMSO-$d_6$) $\delta$: 31.7, 38.6, 60.8, 107.9, 110.6, 115.5, 116.2, 143.4, 143.6, 148.3, 152.6, 168.4, 168.7. MS (EI): *m/z* (%) 285 [$M^+$+2] (0.88), 284 [$M^+$+1] (1.32), 283 [$M^+$] (5.45), 282 [$M^+$−1] (4.53), 281 [$M^+$−2] (2.21), 124 (100.00). Elemental analysis, calculated for $C_{13}H_9N_5OS$ (283.31): C, 55.11; H, 3.20; N, 24.72; S, 11.32. Found: C, 54.81; H, 3.52; N, 24.37; S, 10.99.

#### 4.1.34 | 2-(Cyanomethyl)-7-(furan-2-yl)-5-hydroxy-4,7-dihydrothiazolo[4,5-*b*] pyridine-6-carbonitrile (11b)

Orange crystals from ethanol, yield (1.82 g, 64%), mp: 207-210°C. IR (KBr, $cm^{-1}$): 3409-3200 (OH, NH), 3100 (CH aromatic), 2978, 2928 ($CH_2$), 2206, 2195 (2CN), 1608, 1455 (C═C). $^1$H-NMR (300 MHz, DMSO-$d_6$) $\delta$: 4.82 (s, 2H, $CH_2$), 6.41-6.47; 6.76-6.83; 7.15-7.16 (m, 3H, furan C), 7.53 (s, 1H pyridine C4), 8.13 (s, 1H, NH), 8.30 (s, 1H, OH). Elemental analysis, calculated for $C_{13}H_8N_4O_2S$ (284.29): C, 54.92; H, 2.84; N, 19.71; S, 11.28. Found: C, 54.60; H, 3.10; N, 20.01; S, 11.50.

## 5 | ANTITUMOR ACTIVITY EVALUATION

### 5.1 | Materials and methods

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

### 5.2 | Samples

Stock solutions of compounds **2a-c** to **11a,b** were prepared in DMSO and kept at −20°C. Just before the assays, suitable dilutions of the tested compounds were freshly prepared. The concentrations of DMSO did not overlap with the cell growth.

### 5.3 | 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 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 µg/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 MCF-7 and SF-268 and $0.75 \times 10^4$ cells/mL for NCI-H460, followed by 24 hours 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.

## CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest.

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