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Synthesis of New Thiazole-Pyridine Hybrids and Their Anticancer Activity

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Abstract—A series of new thiazole incorporated pyridine derivatives containing the phenoxyacetamide moiety as a linking bridge has been synthesized. The synthetic strategy involves condensation of 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)acetamide with cyanoacetic hydrazide followed by heterocyclization with acetylacetone, treatment of the produced acrylamides with malononitrile and substituted acetophenones, then heating the generated chalcones with mononitrile in acetic acid and ammonium acetate. In vitro anticancer activity of the newly synthesized thiazole-pyridine hybrids has been evaluated against prostate (PC3), liver (HepG2), laryngeal (Hep-2), and breast (MCF-7) cancer cell lines. One of thiazole-pyridine compounds **8c** demonstrates higher activity (IC_{50} 5.71 μM) against breast cancer than 5-fluorouracil used as a reference (IC_{50} 6.14 μM). Molecular docking procedure has provided valuable information on the binding sites of the synthesized compounds with rho-associated protein kinase 1 (ROCK-1).

Keywords: thiazole, 2-cyanoacetanilide, malononitrile, pyridone, cytotoxicity, molecular docking

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INTRODUCTION

Thiazole and its derivatives are used in medicine for treatment of hypertension [1], inflammation [2], schizophrenia [3], bacterial infections and HIV infections [4, 5]. Pyridine derivatives play an inmportant role in design of many pharmaceutically active compounds [6], and have been reported to possess various biological properties including anticancer activity [7–9].

In this respect, we have developed and synthesized a number of novel structural hybrids incorporating the thiazole and pyridine ring systems and evaluated their anticancer activity. Such combination of the structural blocks could help in understanding the influence of such hybridization on biological activity of the new compounds. Evaluation of anticancer properties of the constructed thiazole-pyridine hybrids was carried out against four cell lines, prostate cancer (PC3), liver carcinoma (HepG2), laryngeal carcinoma (Hep-2), and breast cancer (MCF-7).

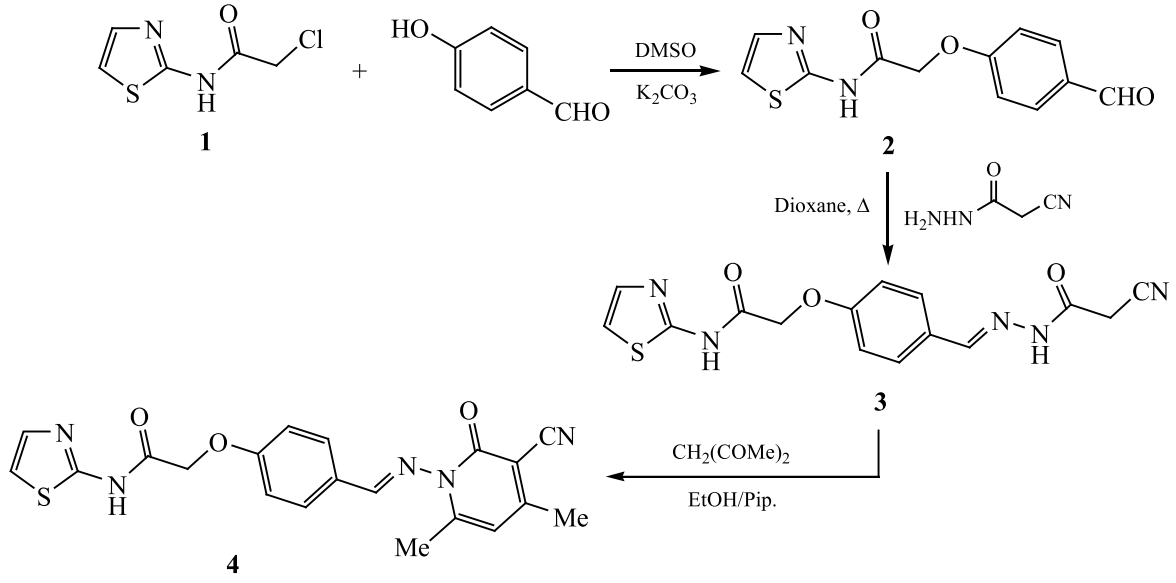
RESULTS AND DISCUSSION

The key precursor 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)acetamide (**2**) was synthesized by reacting 2-chloro-*N*-(thiazol-2-yl)acetamide (**1**) [10] with 4-hydroxybenzaldehyde in DMSO containing potassium carbonate via nucleophilic substitution of chlorine atom of 2-chloro-*N*-(thiazol-2-yl)acetamide (**1**) by oxygen nucleophilic center of hydroxybenzaldehyde (Scheme 1). The proposed structure of 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)acetamide (**2**) was supported by spectral data and elemental analysis. Reactivity of the formyl function in precursor **2** was studied with nucleophilic reagents. Condensation of compound **2** with 2-cyanoacetohydrazide gave the condensation product 2-(4-{[2-(2-cyanoacetyl)hydrazono]methyl}phenoxy)-*N*-(thiazol-2-yl)acetamide (**3**), heterocyclization with acetylacetone in the presence of piperidine furnished thiazolyl-pyridine hybrid **4** in 73% yield.

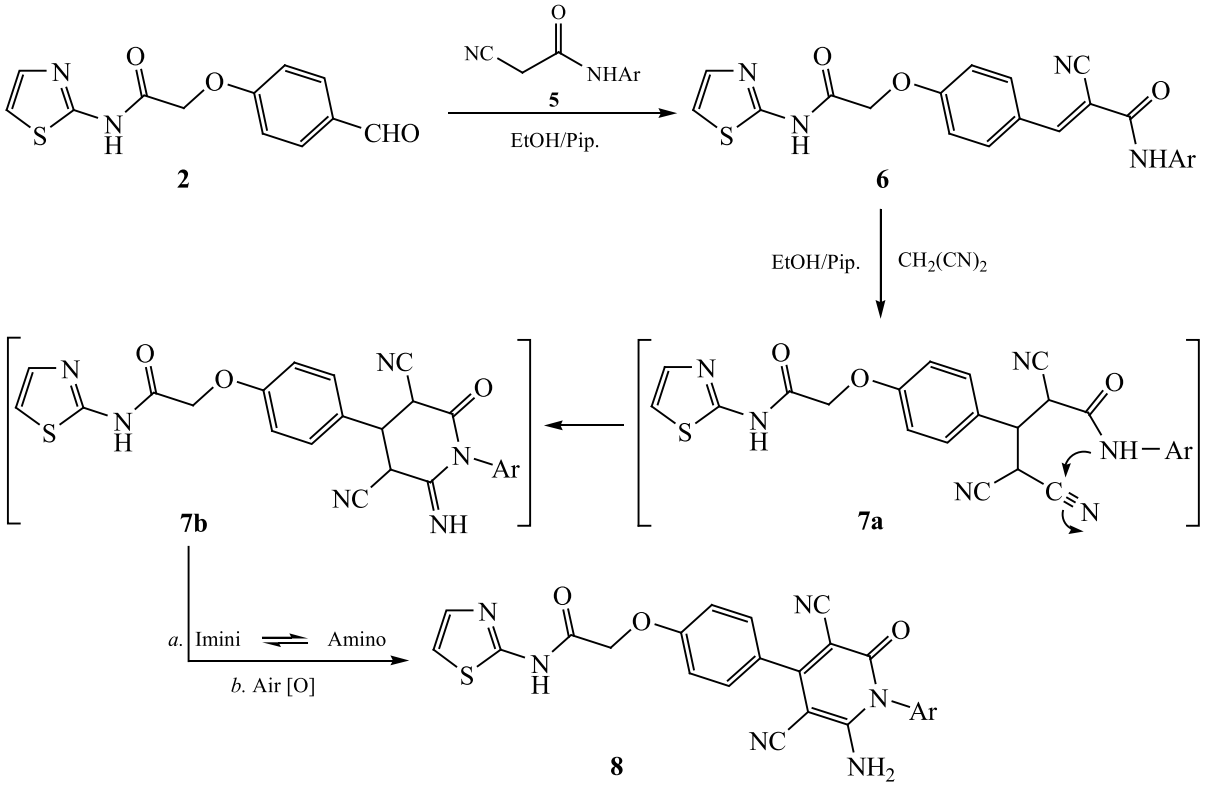
The formyl group of precursor **2** was subjected to the Knoevenagel condensation with substituted 2-cyanoacetanilides **5** [11, 12] which led to the corresponding *N*-aryl-2-cyano-3-{4-[2-oxo-2-(thiazolylamino)ethoxy]phenyl}acrylamide scaffolds **6a**–**6c** in high yields. The following refluxing of compounds **6a**–**6c** with malononitrile in ethanol with piperidine gave the corresponding 2-[4-(6-amino-1-arylpyridyl)phenoxy]-*N*-thiazolyl-acetamides **8a**–**8c** (yield 58–65%) (Scheme 2).

The Claisen–Schmidt reaction of the precursor **2** with *para*-substituted acetophenones in aqueous ethanolic solution of NaOH at room temperature gave the products identified as 2-[4-(3-aryl-3-oxo-prop-1-en-1-yl)phenoxy]-*N*-thiazolyl-acetamide derivatives **9a**–**9c**

Scheme 1. Synthesis of 2-(4-{[(3-cyano-2-oxopyridyl)imino]methyl}phenoxy)-*N*-(thiazol-2-yl)acetamide derivative **4**.



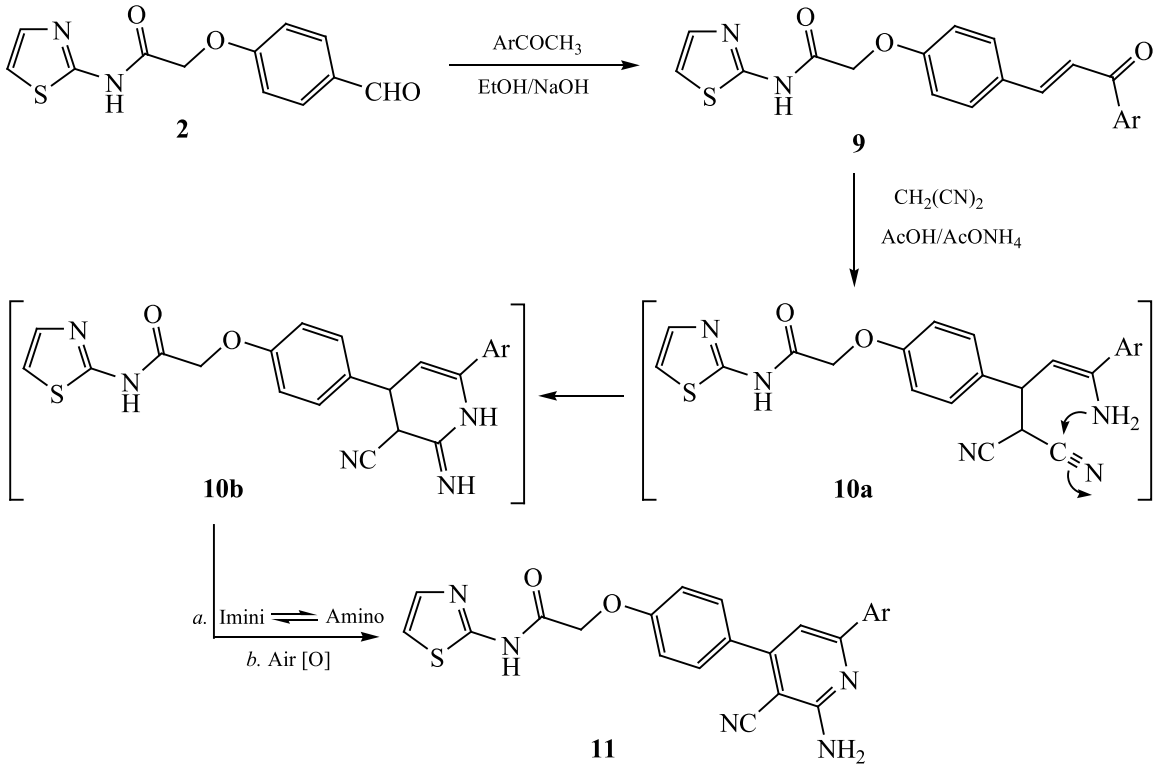
Scheme 2. Synthesis of 2-[4-(6-amino-1-aryl-pyridyl)-phenoxy]-*N*-thiazolyl-acetamides **8a**–**8c**.



Ar = 4-MeC_6H_4 (**a**), 4-$OMeC_6H_4$ (**b**), 4-ClC_6H_4 (**c**).

(Scheme 3). IR spectra of those demonstrated bands of the amide and α,β-unsaturated carbonyl groups. For example, ^{1}H NMR spectrum of **9b** demonstrated two singlets at 3.87 and 4.88 ppm attributed to the methoxy and methylene groups, respectively. Protons of the thiazole-C5 and -C4 were recorded as two doublets at 7.21 and 7.49 ppm. Two protons of the vinyl group resonated at 7.62 and 8.01 ppm (J = 15.60 Hz) and indicated the *E*-configuration of the synthesized chalcones **9a**–**9c**.

Scheme 3. Synthesis of 2-[4-(2-amino-6-aryl-3-cyano-pyridyl)-phenoxy]-*N*-(thiazolyl)-acetamide derivatives **11a**–**11c**.



Ar = 4-MeC_6H_4 (**a**), 4-$OMeC_6H_4$ (**b**), 4-ClC_6H_4 (**c**).

The α,β-unsaturated carbonyl (–CH=CH–C=O) fragment of chalcones **9a**–**9c** characterized by the presence of two electrophilic sites (carbonyl carbon and β-carbon atoms) could be involved in addition reactions via the carbonyl group (1,2-addition) or the β-carbon (1,4-addition) leading to the promising bioactive heterocyclic ring systems. Reaction of chalcones **9a**–**9c** with malononitrile carried out in acetic acid and ammonium acetate gave the corresponding 2-[4-(2-amino-6-aryl-3-cyanopyridyl)phenoxy]-*N*-(thiazolyl)-acetamide derivatives **11a**–**11c**. The plausible mechanism of the process included addition of malononitrile and ammonia to the α,β-unsaturated system and formation of the intermediate **10a**, intramolecular cyclization of which proceeded via nucleophilic addition of the amino function to the nitrile group leading to the imino-tetrahydropyridine intermediate **10b**. The latter species could undergo an imine-amino tautomeric transformation accompanied by autoxidation and formation of the pyridine moiety in compound **11**.

Anticancer activity. The synthesized thiazole-pyridine hybrids were tested for in vitro anticancer activity at the Bioassay-Cell Culture Laboratory at National Research Centre (Egypt) by the MTT assay [13] against prostate cancer (PC3), liver carcinoma (HepG2), laryngeal carcinoma (Hep-2), and breast cancer (MCF-7) cell lines [14] (Table 1) using 5-fluorouracil as a reference. All thiazole–pyridine hybrids demonstrated anticancer activity. The thiazole-pyridine hydrides **8a**–**8c** were determined to be the most potent against all tested cell lines. Thiazole-pyridine hybrids **11a**–**11c** were less active than compounds **8a**–**8c**. Thiazole-pyridine hybrid **4** was characterized by the lowest activity. Generally, the tested products demonstrated the most pronounced activity towards the particular cell line MCF-7.

According to the structure activity relationship (SAR) the thiazole-pyridine hybrid **8c**, in which the pyridone moiety was substituted by chlorine atom at the fourth position of aryl nucleus, demonstrated the highest activity against breast cancer. The next promising cytotoxic activity was exhibited by the corresponding hybrid **8b** that contained the methoxy group followed by the methyl substituted derivative **8a**. Thiazole-pyridine hybrids **11c** with 4-Cl substituent, **11b** (4-MeO substituent) and **11a** (4-Me substituent) displayed strong activity against breast cancer cell line. Generally, the order of pharmacological activity of the derivatives could be presented as follows: 4-Cl > 4-OCH_3 > 4-CH_3. Chlorine substituent on benzene

Table 1. In vitro cytotoxicity of the synthesized thiazole-pyridine hybrids (IC_{50}, μM)[a]

Compound	PC3	HepG2	Hep-2	MCF-7
4	38.98 ± 0.04	36.29 ± 0.03	34.28 ± 0.45	22.84 ± 0.27
8a	32.46 ± 2.15	20.34 ± 0.15	18.37 ± 0.13	11.03 ± 0.23
8b	30.57 ± 0.11	16.20 ± 0.03	13.52 ± 0.10	8.40 ± 0.16
8c	18.10 ± 0.01	13.18 ± 0.02	11.27 ± 0.05	5.71 ± 0.15
11a	37.48 ± 0.63	32.27 ± 0.44	27.13 ± 0.11	21.09 ± 0.27
11b	38.56 ± 0.81	29.32 ± 0.66	24.72 ± 0.73	17.41 ± 0.62
11c	24.82 ± 0.03	17.19 ± 0.07	18.23 ± 0.27	12.57 ± 0.49
5-Fu[b]	8.30 ± 0.23	7.19 ± 0.45	5.22 ± 0.82	6.14 ± 0.31

[a] Data are presented as the mean ± SD of three independent experiments.
[b] 5-Fluorouracil (5-Fu) is the reference drug for anticancer tests.

ring supported the anticancer activity (**8c** and **11c**) which was due to either its hydrophobicity or electronic effects (inductive and resonance) [15]. Chlorine substituent on aromatic ring is characterized by comparatively higher hydrophobicity constant than that of the other substituents (4-OCH_3 and 4-CH_3) [16].

Molecular docking. Molecular docking studies addressed binding modes of the synthesized thiazole-pyridine derivatives with rho-associated protein kinase 1 (ROCK-1) using MOE v10.2015.10 software. Crystal structure of the protein, PDB ID: 3TWJ was chosen from protein data bank and fluorouracil used as the reference ligand [17].

Thiazole-pyridine hybrid **4** could be involved in three types of interactions, one hydrogen bond between sulfur atom of thiazole ring and Glu 89 (4.00 Å), two π–π interactions of the phenyl ring with Glu 379 (3.83 Å) and pyridine ring with Thr 380 (3.84 Å), respectively (Fig. 1a). Thiazole-pyridine hybrid **8a** exhibited three intermolecular hydrogen bonds between sulfur atom of thiazole ring and Glu 89 (4.10 Å), the other H-bond was between oxygen atom of the amide group with Arg 147 (3.04 Å) and the third bond was revealed between nitrogen atom of the nitrile group and Ala 73 (3.02 Å) (Fig. 1b). The interactions were characterized by high binding energy score, S = –6.8482 kcal/mol.

Thiazole-pyridine hybrid **8b** participated in two intermolecular H-bonds between hydrogen atom of Asp 350 and sulfur atom of thiazole ring (3.40 Å) and the other one between oxygen atom of pyridone ring and Lys 100 (3.15 Å). The interactions were of moderate binding energy score, S = –6.0485 kcal/mol. Thiazole-pyridine hybrid **8c** demonstrated three intermolecular H-bonds. The first bond was between hydrogen atom of Asn 295 and chlorine atom (3.97 Å), the second one between oxygen atom of pyridone ring and His 293 (2.94 Å) and the third bond between nitrogen atom of the nitrile group and Arg 320 (3.58 Å). Also, there could be π-π interactions of thiazole ring with Tyr 254 (3.77 Å). All interactions were of binding energy score S = –6.7373 kcal/mol.

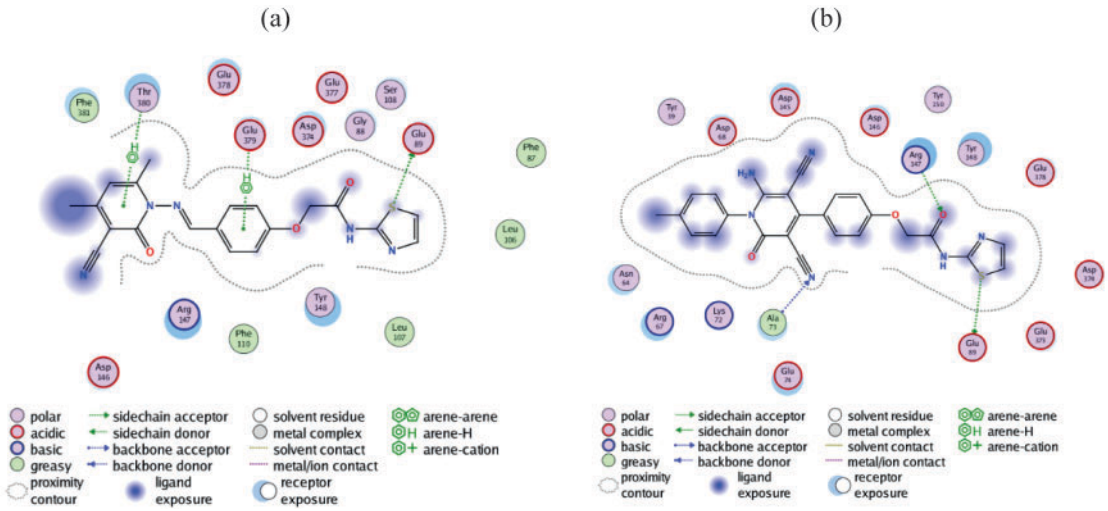


Fig. 1. (a) The interacting mode of thiazole-pyridine hybrid **4** and (b) thiazole-pyridine hybrid **8a** with Rho associated protein kinase 1.

Thiazole-pyridine hybrid **11a** revealed four intermolecular H-bonds including three bonds between hydrogen atom of Glu 378 and the methylene moiety, nitrogen atom of the amide moiety and sulfur atom of thiazole ring with various intermolecular distances 3.49, 3.12, 3.32 Å, respectively. The fourth H-bond was between sulfur atom of thiazole ring and Glu 377 (3.71 Å). Thiazole-pyridine hybrid **11b** revealed one intermolecular hydrogen bond between the amino group on pyridine ring and Glu 24 (3.04 Å). There were two π-π interactions between thiazole ring and Pro 393 (3.84 Å), and between phenyl ring and Trp 122 (3.86 Å). Thiazole-pyridine hybrid **11c** revealed three intermolecular hydrogen bonds, two bonds of sulfur atom in the fifth position of thiazole ring with both Leu 272 (3.87 Å) and Glu 172 (3.31 Å). The third bond could take place between the amino group on pyridine ring with Asp 302 (3.02 Å) characterized by binding energy score S = –6.5629 kcal/mol.

EXPERIMENTAL

Melting points were determined on a Gallenkamp electric device and were uncorrected. IR spectra (KBr discs) were recorded on a Thermo Scientific Nicolet iS10 FTIR spectrophotometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured on a JEOL spectrometer using DMSO-d_6 as a solvent. Mass spectra were measured on a Quadrupole GC-MS (DSQII) mass spectrometer at 70 eV. HCN analyses were carried out on a Perkin Elmer 2400 analyzer.

Synthesis of 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)acetamide (2). To a suspension of 2-chloro-*N*-(thiazol-2-yl)acetamide (**1**) (1.76 g, 0.01 mol) and 4-hydroxybenzaldehyde (1.22 g, 0.01 mol) in DMSO (30 mL), anhydrous sodium carbonate (1.38 g, 0.01 mol) was added. The reaction mixture was stirred at room temperature for 6 h and then diluted with 30 mL of cold water. The precipitate of product **2** was collected, dried and recrystallized from ethyl alcohol. Yield 66%, mp 132–133°C. IR spectrum, ν, cm^{-1}: 3302 (NH), 1702 (C=O), 1682 (C=O). ^{1}H NMR spectrum, δ, ppm: 4.82 s (2H, CH_2), 7.09 d (2H, J = 9.00 Hz, C_6H_4), 7.18 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.49 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.88 d (2H, J = 9.00 Hz, C_6H_4), 9.88 s (1H, CHO), 11.76 s (1H, NH). Found, %: C 54.82; H 3.87; N 10.77. $C_{12}H_{10}N_2O_3S$. Calculated, %: C 54.95; H 3.84; N 10.68. MS: *m/z*: 262 $[M]^+$.

Synthesis of 2-(4-{[2-(2-cyanoacetyl)hydrazono]-methyl}phenoxy)-*N*-(thiazol-2-yl)acetamide (3). A mixture of 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)-acetamide (**2**) (1.31 g, 0.005 mol) with cyanoacetic hydrazide (0.50 g, 0.005 mol) was dissolved in 20 mL dioxane and boiled for 4 h. The precipitate obtained upon cooling down was filtered off and recrystallized from ethyl alcohol. Yield 62%, mp 241–242°C. IR spectrum, ν, cm^{-1}: 3251, 3176 (NH), 2208 (C≡N), 1706 (C=O), 1684 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.86 s (2H, CH_2), 4.87 s (2H, CH_2), 7.12 d (2H, J = 9.00 Hz, C_6H_4), 7.22 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.51 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.76 d (2H, J = 9.00 Hz, C_6H_4), 8.37 s (1H, CH=N), 11.09 s (1H, NH), 11.88 s (1H, NH). Found, %: C 52.56; H 3.77; N 20.32. $C_{15}H_{13}N_5O_3S$. Calculated, %: C 52.47; H 3.82; N 20.40. MS: *m/z*: 343 $[M]^+$.

Synthesis of 2-(4-{[(3-cyano-4,6-dimethyl-2-oxo-pyrid-1(2*H*)-yl)imino]methyl}phenoxy)-*N*-(thiazol-2-yl)acetamide (4). To a solution of cyanoacetylhydrazone derivative **3** (0.68 g, 0.002 mol) in ethyl alcohol (30 mL) were added acetylacetone (0.20 mL, 0.002 mol) and piperidine (0.10 mL). The reaction mixture was refluxed for 4 h and then cooled down. The precipitated yellow solid was filtered off to give pyridyl-thiazole derivative **4**. Yield 73%, mp 288–290°C. IR spectrum, ν, cm^{-1}: 3231 (NH), 2215 (C≡N), 1704 (C=O), 1668 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.32 s (3H, CH_3), 2.41 s (3H, CH_3), 4.84 s (2H, CH_2), 6.21 s (1H, $C^5H_{pyridine}$), 7.09 d (2H, J = 8.50 Hz, C_6H_4), 7.18 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.49 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.82 d (2H, J = 8.50 Hz, C_6H_4), 8.46 s (1H, CH=N), 11.62 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 20.19, 21.24, 65.36, 108.42, 112.61, 115.24 (2C), 116.18, 118.36, 125.44, 129.31 (2C), 132.90, 135.82, 152.41, 155.26, 158.56, 160.58, 162.72, 168.63. Found, %: C 58.81; H 4.15; N 17.08. $C_{20}H_{17}N_5O_3S$. Calculated, %: C 58.96; H 4.21; N 17.19. MS: *m/z*: 407 $[M]^+$.

Synthesis of *N*-aryl-2-cyano-3-{4-[2-oxo-2-(thiazolylamino)ethoxy]phenyl}-acrylamides 6a–6c. To a suspension of 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)-acetamide (**2**) (1.31 g, 0.005 mol) in 30 mL of ethanol, the appropriate substituted 2-cyanoacetanilide derivative **5** (namely 2-cyano-*p*-methylacetanilide, 2-cyano-*p*-methoxyacetanilide or 2-cyano-*p*-chloroacetanilide) (0.005 mol) and 0.1 mL piperidine were added. The reaction mixture was refluxed for 2 h. The solid formed upon cooling down was filtered off and dried to give the corresponding 2-cyanoacrylamide derivatives **6a**–**6c**.

2-Cyano-3-{4-[2-oxo-2-(thiazol-2-ylamino)ethoxy]-phenyl}-*N*-(4-tolyl)acrylamide (6a). Yield 82%, mp 244–245°C. IR spectrum, ν, cm^{-1}: 3259 (NH), 3167 (NH), 2212 (C≡N), 1708 (C=O), 1665 (C=O). ^{1}H NMR

spectrum, δ, ppm: 2.37 s (3H, CH_3), 4.82 s (2H, CH_2), 7.01 d (2H, J = 8.00 Hz, C_6H_4), 7.18 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.31 d (2H, J = 7.50 Hz, C_6H_4), 7.47 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.61 d (2H, J = 7.50 Hz, C_6H_4), 7.92 d (2H, J = 8.00 Hz, C_6H_4), 8.11 s (1H, C=CH), 10.87 s (1H, NH), 11.71 s (1H, NH). Found, %: C 63.21; H 4.31; N 13.44. $C_{22}H_{18}N_4O_3S$. Calculated, %: C 63.14; H 4.34; N 13.39. MS: *m/z*: 418 $[M]^+$.

***N*-(4-Anisyl)-2-cyano-3-{4-[2-oxo-2-(thiazol-2-ylamino)ethoxy]phenyl}acrylamide (6b).** Yield 84%, mp 256-257°C. IR spectrum, ν, cm^{-1}: 3241 (NH), 3161 (NH), 2208 (C≡N), 1705 (C=O), 1662 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.87 s (3H, OCH_3), 4.84 s (2H, CH_2), 6.94 d (2H, J = 8.00 Hz, C_6H_4), 7.04 d (2H, J = 8.00 Hz, C_6H_4), 7.21 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.52 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.68 d (2H, J = 8.00 Hz, C_6H_4), 7.96 d (2H, J = 8.00 Hz, C_6H_4), 8.15 s (1H, C=CH), 11.04 s (1H, NH), 11.83 s (1H, NH). Found, %: C 60.91; H 4.20; N 12.97. $C_{22}H_{18}N_4O_4S$. Calculated, %: C 60.82; H 4.18; N 12.90. MS: *m/z*: 434 $[M]^+$.

***N*-(4-Chlorophenyl)-2-cyano-3-{4-[2-oxo-2-(thiazol-2-ylamino)ethoxy]phenyl}acrylamide (6c).** Yield 75%, mp 248–249°C. IR spectrum, ν, cm^{-1}: 3252 (NH), 3163 (NH), 2209 (C≡N), 1707 (C=O), 1671 (C=O). ^{1}H NMR spectrum, δ, ppm: 4.82 s (2H, CH_2), 7.02 d (2H, J = 8.00 Hz, C_6H_4), 7.17 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.38 d (2H, J = 8.00 Hz, C_6H_4), 7.55 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.78 d (2H, J = 8.00 Hz, C_6H_4), 7.98 d (2H, J = 8.00 Hz, C_6H_4), 8.12 s (1H, C=CH), 11.13 s (1H, NH), 11.87 s (1H, NH). Found, %: C 57.60; H 3.40; N 12.71. $C_{21}H_{15}ClN_4O_3S$. Calculated, %: C 57.47; H 3.45; N 12.77. MS: *m/z*: 438 $[M]^+$.

Synthesis of 2-[4-(6-amino-1-aryl-3,5-dicyano-2-oxo-1,2-dihydropyridyl)phenoxy]-*N*-(thiazolyl)-acetamides 8a–8c. A suspension of malononitrile (0.14 g, 0.002 mol) and the appropriate *N*-aryl-2-cyano-3-{4-[2-oxo-2-(thiazol-2-ylamino)ethoxy]phenyl}acrylamide derivative **6** (0.002 mol) in 15 mL of ethanol and 0.1 mL piperidine was refluxed for 4 h. The solid formed upon cooling to 25°C was recrystallized from EtOH and DMF mixture (5 : 1) to give the corresponding products **8a**–**8c**.

2-{4-[6-Amino-3,5-dicyano-2-oxo-1-(4-methylphenyl)-1,2-dihydropyrid-4-yl]phenoxy}-*N*-(thiazol-2-yl)acetamide (8a). Yield 61%, mp 281–283°C. IR spectrum, ν, cm^{-1}: 3327, 3257, 3181 (NH, NH_2), 2211 (C≡N), 1702 (C=O), 1656 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.34 s (3H, CH_3), 4.87 s (2H, CH_2), 6.64 s (2H, NH_2), 7.00 d (2H, J = 8.00 Hz, C_6H_4), 7.15 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.28–7.31 m (4H, C_6H_4), 7.46 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.60 d (2H, J = 8.00 Hz, C_6H_4), 12.06 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 20.87, 65.73, 76.61, 88.25, 113.11, 114.81 (2C), 115.04, 116.23, 123.56, 127.49 (2C), 128.57, 129.21 (2C), 130.08 (2C), 132.25, 137.63, 156.28, 157.44, 158.71, 160.06, 162.82, 169.07. Found, %: C 62.41; H 3.70; N 17.29. $C_{25}H_{18}N_6O_3S$. Calculated, %: C 62.23; H 3.76; N 17.42. MS: *m/z*: 482 $[M]^+$.

2-{4-[6-Amino-1-(4-methoxyphenyl)-3,5-dicyano-2-oxo-1,2-dihydropyrid-4-yl]phenoxy}-*N*-(thiazol-2-yl)acetamide (8b). Yield 65%, mp 308-309°C. IR spectrum, ν, cm^{-1}: 3307, 3216 (NH, NH_2), 2210 (C≡N), 1701 (C=O), 1658 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.84 s (3H, OCH_3), 4.87 s (2H, CH_2), 6.71 s (2H, NH_2), 6.92 d (2H, J = 8.00 Hz, C_6H_4), 7.02–7.21 m (5H, C_6H_4, $C^5H_{thiazole}$), 7.49 d (1H, J = 4.00 Hz, $C^4H_{thiazole}$), 7.61 d (2H, J = 8.00 Hz, C_6H_4), 11.91 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 55.38, 65.86, 77.88, 87.46, 112.79, 113.90 (2C), 114.67 (2C), 115.74, 116.38, 124.17, 129.84 (2C), 130.39 (2C), 132.78, 133.56, 156.41, 157.83, 158.65, 159.51, 161.83, 163.19, 168.94. Found, %: C 60.08; H 3.68; N 16.77. $C_{25}H_{18}N_6O_4S$. Calculated, %: C 60.23; H 3.64; N 16.86%. MS: *m/z*: 498 $[M]^+$.

2-{4-[6-Amino-1-(4-chlorophenyl)-3,5-dicyano-2-oxo-1,2-dihydropyrid-4-yl]-phenoxy}-*N*-(thiazol-2-yl)acetamide (8c). Yield 58%, mp 298–299°C. IR spectrum, ν, cm^{-1}: 3328, 3258, 3192 (NH, NH_2), 2214 (C≡N), 1705 (C=O), 1665 (C=O). ^{1}H NMR spectrum, δ, ppm: 4.85 s (2H, CH_2), 6.68 s (2H, NH_2), 6.98 d (2H, J = 8.00 Hz, C_6H_4), 7.15 d (1H, J = 4.00 Hz, $C^5H_{thiazole}$), 7.38–7.45 m (5H, C_6H_4, $C^4H_{thiazole}$), 7.62 d (2H, J = 8.00 Hz, C_6H_4), 11.96 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 65.91, 77.32, 88.06, 112.92, 114.47 (2C), 115.36, 115.94, 124.68, 128.78 (2C), 129.85 (2C), 130.28 (2C), 132.37, 133.61, 135.08, 155.73, 157.29, 158.84, 161.42, 164.21, 168.89. Found, %: C 57.46; H 3.08; N 16.82. $C_{24}H_{15}ClN_6O_3S$. Calculated, %: C 57.32; H 3.01; N 16.71. MS: *m/z*: 502 $[M]^+$.

Synthesis of 2-[4-(3-aryl-3-oxopropenyl)phenoxy]-*N*-(thiazolyl)acetamides 9a–9c. A mixture of 2-(4-formylphenoxy)-*N*-(thiazol-2-yl)acetamide (**2**) (1.31 g, 0.005 mol) with a substituted acetophenone (0.005 mol), 15% potassium hydroxide (10 mL) and 20 mL of methanol was stirred at 25–30°C for 6 h and then diluted with crushed ice (20 g). The corresponding solid product was filtered off and recrystallized from methanol.

2-{4-[3-Oxo-3-(4-methylphenyl)prop-1-en-1-yl]-phenoxy}-*N*-(thiazol-2-yl)acetamide (9a). Yield 56%, mp 173-174°C. IR spectrum, ν, cm^{-1}: 3214 (NH), 1694

(C=O), 1662 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.40 s (3H, CH_3), 4.91 s (2H, CH_2), 7.01 d (2H, *J* = 8.00 Hz, C_6H_4), 7.15 d (1H, *J* = 3.50 Hz, $C^5H_{thiazole}$), 7.34 d (2H, *J* = 7.50 Hz, C_6H_4), 7.47 d (1H, *J* = 3.50 Hz, $C^4H_{thiazole}$,), 7.58 d (1H, *J* = 15.50 Hz, COCH=C), 7.71 d (2H, *J* = 8.00 Hz, C_6H_4), 7.94 d (2H, *J* = 7.50 Hz, C_6H_4), 8.11 d (1H, *J* = 15.50 Hz, C=CH), 11.76 s (1H, NH). Found, %: C 66.48; H 4.86; N 7.49. $C_{21}H_{18}N_2O_3S$. Calculated, %: C 66.65; H 4.79; N 7.40%. MS: *m/z*: 378 $[M]^+$.

2-{4-[3-(4-Methoxyphenyl)-3-oxoprop-1-en-1-yl]-phenoxy}-*N*-(thiazol-2-yl)acetamide (9b). Yield 48%, mp 187–188°C. IR spectrum, ν, cm^{-1}: 3196 (NH), 1692 (C=O), 1664 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.87 s (3H, OCH_3), 4.88 s (2H, CH_2), 6.94 d (2H, *J* = 8.00 Hz, C_6H_4), 7.02 d (2H, *J* = 8.00 Hz, C_6H_4), 7.21 d (1H, *J* = 4.00 Hz, $C^5H_{thiazole}$), 7.49 d (1H, *J* = 4.00 Hz, $C^4H_{thiazole}$), 7.62 d (1H, *J* = 15.50 Hz, COCH=C), 7.77 d (2H, *J* = 8.00 Hz, C_6H_4), 8.01 d (1H, *J* = 15.50 Hz, C=CH), 8.18 d (2H, *J* = 7.50 Hz, C_6H_4), 11.48 s (1H, NH).). Found, %: C 63.84; H 4.55; N 7.02. $C_{21}H_{18}N_2O_4S$. Calculated, %: C 63.95; H 4.60; N 7.10. MS: *m/z*: 394 $[M]^+$.

2-{4-[3-(4-Chlorophenyl)-3-oxoprop-1-en-1-yl]-phenoxy}-*N*-(thiazol-2-yl)acetamide (9c). Yield 59%, mp 203–204°C. IR spectrum, ν, cm^{-1}: 3209 (NH), 1698 (C=O), 1658 (C=O). ^{1}H NMR spectrum, δ, ppm: 4.87 s (2H, CH_2), 6.95 d (2H, *J* = 8.00 Hz, C_6H_4,), 7.18 d (1H, *J* = 3.50 Hz, $C^5H_{thiazole}$,), 7.44–7.52 m (3H, C_6H_4, $C^4H_{thiazole}$), 7.62 d (1H, *J* = 15.50 Hz, COCH=C), 7.77 d (2H, *J* = 8.00 Hz, C_6H_4), 8.02 d (2H, *J* = 7.50 Hz, C_6H_4), 8.13 d (1H, *J* = 15.50 Hz, C=CH), 12.06 s (1H, NH). Found, %: C 60.44; H 3.87; N 7.15. $C_{20}H_{15}ClN_2O_3S$. Calculated, %: C 60.23; H 3.79; N 7.02. MS: *m/z*: 398 $[M]^+$.

Synthesis of 2-[4-(2-amino-3-cyano-6-arylpyrid-4-yl)phenoxy]-*N*-(thiazolyl)acetamides 11a–11c. A mixture of a compound **9a**–**9c** (0.002 mmol) with ammonium acetate (0.01 mol) in 20 mL of acetic acid and malononitrile (0.14 g, 0.002 mol) was refluxed for 6 h, then cooled down and diluted with 20 mL of cold water. The precipitate formed was filtered off and recrystallized from ethanol to give the corresponding pure product **11a**–**11c**.

2-{4-[2-Amino-3-cyano-6-(4-methylphenyl)pyrid-4-yl]phenoxy}-*N*-(thiazol-2-yl)acetamide (11a). Yield 68%, mp 261–262°C. IR spectrum, ν, cm^{-1}: 3327, 3274, 3157 (NH, NH_2), 2208 (C≡N), 1681 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.37 s (3H, CH_3), 4.93 s (2H, CH_2), 6.87 s (2H, NH_2), 7.07–7.18 m (5H, C_6H_4, $C^5H_{thiazole}$), 7.47 d (1H, *J* = 4.00 Hz, $C^4H_{thiazole}$), 7.73 d (2H, *J* = 8.00 Hz, C_6H_4), 7.91 s (1H, $C^5H_{pyridine}$), 8.04 d (2H, *J* = 7.50 Hz, C_6H_4), 11.81 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 20.96, 65.81, 86.77, 112.83, 114.26, 114.90 (2C), 115.67, 126.53 (2C), 129.45 (2C), 130.14 (2C), 131.17, 132.30, 135.22, 136.14, 156.88, 157.66, 159.31, 161.08, 163.21, 168.97. Found, %: C 65.38; H 4.41; N 15.98. $C_{24}H_{19}N_5O_2S$. Calculated, %: C 65.29; H 4.34; N 15.86. MS: *m/z*: 441 $[M]^+$.

2-{4-[2-Amino-6-(4-methoxyphenyl)-3-cyano-pyrid-4-yl]phenoxy}-*N*-(thiazol-2-yl)acetamide (11b). Brown solid, yield = 71%, mp 292–294°C. IR spectrum, ν, cm^{-1}: 3341, 3260, 3173 (NH, NH_2), 2210 (C≡N), 1677 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.87 s (3H, OCH_3), 4.92 s (2H, CH_2), 6.82 s (2H, NH_2), 7.02–7.10 m (4H, C_6H_4), 7.21 d (1H, *J* = 3.50 Hz, $C^5H_{thiazole}$), 7.46 d (1H, *J* = 3.50 Hz, $C^4H_{thiazole}$), 7.71 d (2H, *J* = 8.00 Hz, C_6H_4), 7.89 s (1H, $C^5H_{pyridine}$), 8.09 d (2H, *J* = 8.00 Hz, C_6H_4), 11.67 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 55.67, 66.04, 87.20, 112.41, 114.09, 113.92 (2C), 114.88 (2C), 115.73, 128.58 (2C), 129.47 (2C), 130.38, 131.15, 133.28, 156.34, 157.81, 159.02, 160.25, 161.79, 164.37, 169.11. Found, %: C 63.18; H 4.10; N 15.43. $C_{24}H_{19}N_5O_3S$. Calculated, %: C 63.01; H 4.19; N 15.31. MS: *m/z*: 457 $[M]^+$.

2-{4-[2-Amino-6-(4-chlorophenyl)-3-cyanopyrid-4-yl]phenoxy}-*N*-(thiazol-2-yl)acetamide (11c). Yield 65%, mp 275–277°C. IR spectrum, ν, cm^{-1}: 3335, 3270, 3168 (NH, NH_2), 2211 (C≡N), 1685 (C=O). ^{1}H NMR spectrum, δ, ppm: 4.91 s (2H, CH_2), 6.85 s (2H, NH_2), 7.08 d (2H, *J* = 8.00 Hz, C_6H_4), 7.19 d (1H, *J* = 3.50 Hz, $C^5H_{thiazole}$), 7.47 d (1H, *J* = 3.50 Hz, $C^4H_{thiazole}$), 7.62–7.72 m (4H, C_6H_4), 7.92 s (1H, $C^5H_{pyridine}$), 8.17 d (2H, *J* = 7.50 Hz, C_6H_4), 12.16 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 66.13, 85.94, 113.22, 114.41, 115.06 (2C), 115.72, 127.49 (2C), 129.02 (2C), 129.58 (2C), 131.17, 132.31, 134.89, 136.08, 157.11, 158.43, 159.67, 161.33, 163.17, 169.06. Found, %: C 59.62; H 3.42; N 15.05. $C_{23}H_{16}ClN_5O_2S$. Calculated, %: C 59.80; H 3.49; N 15.16. MS: *m/z*: 461 $[M]^+$.

Cytotoxicity assay. The MTT assay was applied to determine in vitro cytotoxicity of the new thiazole-pyridine compounds against prostate cancer (PC3), liver carcinoma (HepG2), laryngeal carcinoma (Hep-2), and breast cancer (MCF-7) cell lines. The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium (2×10^4 cells/mL) were plated in 96-well culture plates and incubated at 37°C in a 5% CO_2 incubator for 12 h. The tested sample (2 μL) was added to the cells (2×10^4) in 96-well plates and cultured at 37°C for 3 days. The cultured cells were mixed with

20 μL of MTT solution and incubated for 4 h at 37°C. The supernatant was carefully removed from each well and 100 μL of DMSO were added to each well to dissolve the formazan crystals that were formed by the cellular reduction of MTT. The absorbance of each well was measured by a microplate reader at 570 nm. The results were expressed as IC_{50} values.

Molecular docking. In docking procedure partial charges were added to the ligand atoms. Non-polar hydrogen atoms were conjoined, and rotatable bonds were illustrated. Docking was based on the ligand–protein configuration. All "fundamental hydrogen atoms, Kollman united atom type charges, and solvation parameters" were added before implementation of auto doc tools. Affinity (grid) maps by Å grid points and 0.375 Å spacing were generated via applying the Autogrid pattern. All parameters were applied in calculating Van der Waals data and electrostatic terms [18].

CONCLUSIONS

A new series of thiazole-pyridine hybrids containing different functional groups have been synthesized and their anticancer activity tested against a panel of four cancer cell lines. The thiazole-pyridine compounds exhibit potent cytotoxic activity and their order of potency can be presented in general as follows: **8a**–**8c** > **11a**–**11c** > **4**. The SAR study has revealed that the compounds **8a**–**8c** with the thiazole linked pyridone substituted with the amino, cyano and *N*-aryl groups demonstrate the highest anticancer activity against tested MCF-7, Hep-2, and HepG2 cell lines. Presence of chlorine atom on benzene ring of compounds **8c** and **11c** is decisive for their high breast anticancer activity. Molecular docking study has supported the same.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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