Synthesis, Characterization, and Anticancer Activity (MCF-7) of Some Acetanilide-based Heterocycles

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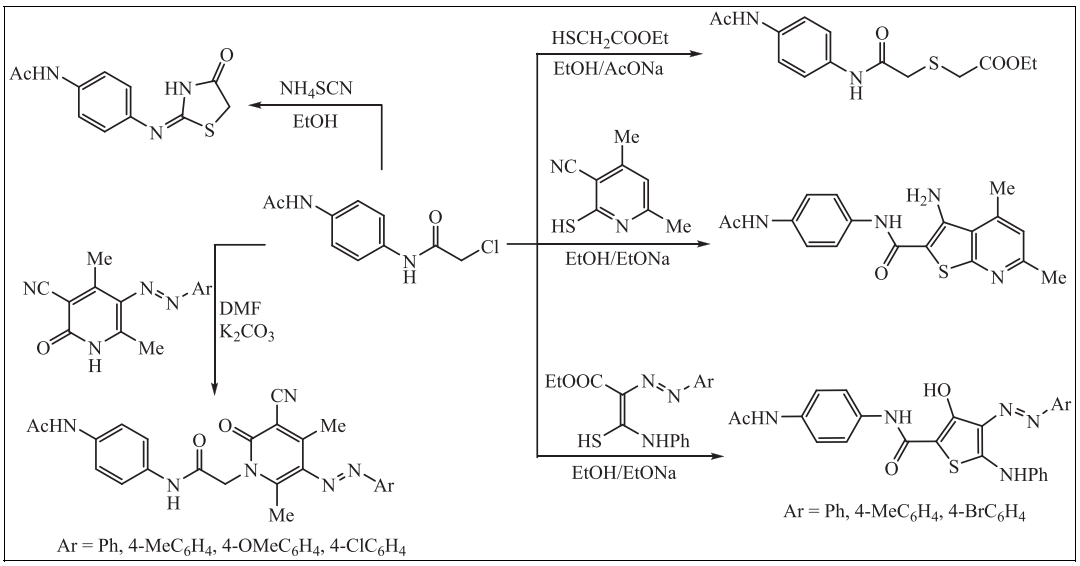

A series of some novel 4-(*N*-substituted amino)-acetanilide scaffolds was synthesized through the nucleophilic substitution reactions of the highly versatile *N*-(4-acetamidophenyl)-2-chloroacetamide (**3**) with various types of nucleophilic reagents such as benzothiazole-2-thiol, ethyl 2-mercaptoacetate, 4,6-dimethyl-2-mercapto-nicotinonitrile, various thiocarbamoyl derivatives, ammonium thiocyanate, 3-cyano-4,6-dimethyl-5-arylazopyridin-2-ones, and malononitrile. The synthesized 4-(*N*-substituted amino) acetanilide scaffolds were characterized by spectral analyses and assayed *in vitro* for breast anticancer activity. 2-(4-Acetamidophenylaminocarbonyl)-3-amino-thiophenes **11**, **13a**, and **13b** showed the highest cytotoxic activity.

INTRODUCTION

Since the discovery of acetanilide as analgesic under the name of Antifebrin [1], acetanilide derivatives have gained much attention in medicinal chemistry. Literature studies expose that acetanilide derivatives are an important structural backbone of biologically active molecules. They showed various activities such as antimicrobial [2,3], antiviral [4,5], anthelmintic [6], anti-inflammatory [7], cytotoxic [8,9], antifungal, and antibacterial [10,11]. Moreover, they possess anti-arthritic [12], analgesic [13], antileishmanial [14], antitumor [15], and insecticidal [16] properties.

Away from the famous activity of acetanilides, literature studies reveal a potent anticancer activity for those compounds. For example, AZD1152 [17] (Fig. 1) inhibited cell division and cell proliferation in Aurora kinase B overexpressed tumor cells. 2-[Benzimidazole-2-yl)sulfanyl]-*N*-[4-(2-methylthiazol-4-yl)phenyl]acetamide compounds [18] (Fig. 1) were found to have a potent effect against A549 and C6 tumor cell lines. Histone deacetylase inhibitors (HDACi) such as suberoylanilide hydroxamic acid [19,20] (Fig. 1) has shown potent cytotoxic effects against several tumor types with low toxicity towards normal cells.

Chloroacetamides are valuable synthetic intermediates as they allow the incorporation of unmined heteroatoms to afford a wide range of heterocycles. In this paper, we describe the synthesis and anticancer activity of many acetanilides incorporating thiophene, thiazole, pyridine, and/or pyrrole moieties through different linkages.

RESULTS AND DISCUSSION

Chemistry. The starting scaffold *N*-(4-acetamidophenyl)-2-chloroacetamide (**3**) has been prepared by the reported experimental conditions through treatment of

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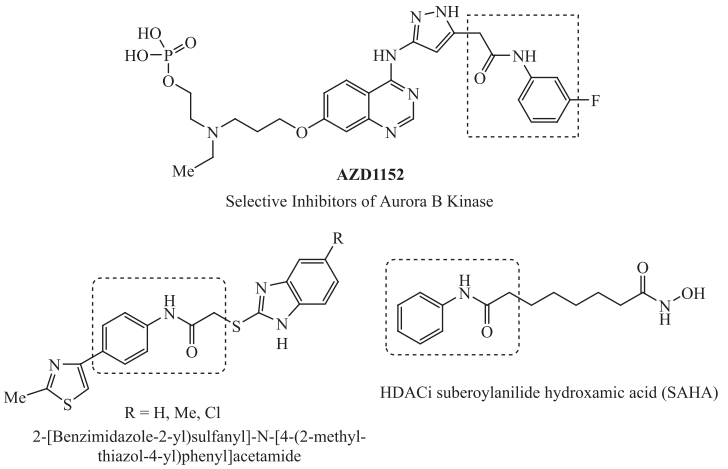


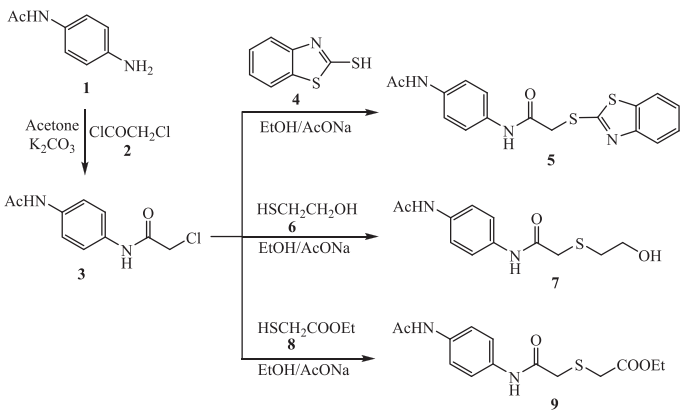
Figure 1. Selected representative examples for anticancer activity of acetanilide derivatives.

p-aminoacetanilide with chloroacetyl chloride in dry acetone in the presence of anhydrous potassium carbonate [21] (Scheme 1). The chemical behavior of 2-chloroacetamide derivative **3** was tested towards the reaction with several sulfur nucleophiles. Thus, heating of chloroacetamide derivative **3** with 2-mercaptobenzothiazole, 2-mercaptoethanol, and/or ethyl 2-mercaptoacetate in absolute ethanol and sodium acetate afforded the corresponding sulfide derivatives **5**, **7**, and **9**, respectively. The sulfide derivatives were isolated in 67 to 90% yields, and their structures were established based on spectral and elemental analyses. In the infrared spectrum of sulfide **5**, the absorption bands at 3286, 3150, and 1654 cm^{-1} clearly indicated the presence of NH and carbonyl functions. The ^1H NMR spectrum of **5** displayed the protons of methyl and methylene groups as two singlet signals at 2.02 and 4.37 ppm. The aromatic protons resonated as multiplet and doublet signals in the region 7.34–8.02 ppm. The protons of NH functions resonated as two singlet signals at 9.87 and 10.35 ppm.

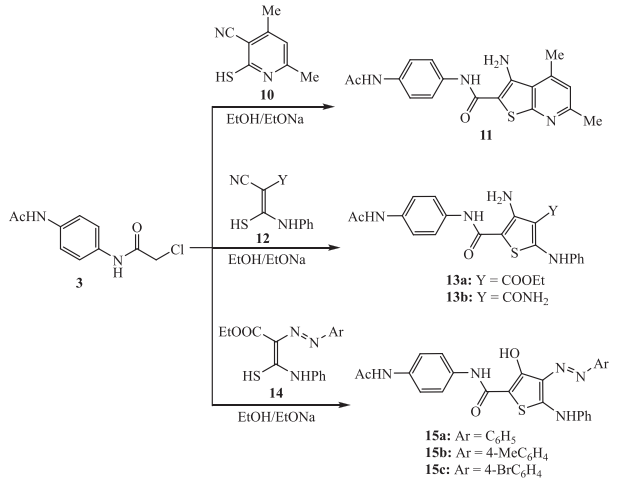
Refluxing equimolar amounts of **3** and 2-mercapto-4,6-dimethylnicotinonitrile (**10**) in ethanolic solution of sodium ethoxide furnished the corresponding 3-aminothieno[2,3-*b*] pyridine scaffold **11**, the reaction starts through nucleophilic substitution of the chlorine atom from chloroacetamide **3** followed by intramolecular cyclization at the nitrile function (Scheme 2). The IR spectrum of **11** indicated the absence of any absorption due to nitrile function and showed sharp bands of NH_2 group at 3490 and 3290 cm^{-1}. The ^1H NMR spectrum of **11** clearly secured the disappearance of any signal due to the methylene function and showed the recoupment singlet signal at 6.92 ppm for the protons of amino function.

Stirring of 2-chloroacetamide derivative **3** with thiocarbamoyl derivatives **12** that are derived from ethyl cyanoacetate and cyanoacetamide [22,23] in ethanolic solution of sodium ethoxide followed by refluxing at 60–70°C yielded the corresponding 2-(4-acetamidophenylaminocarbonyl)-3-aminothiophenes **13a**

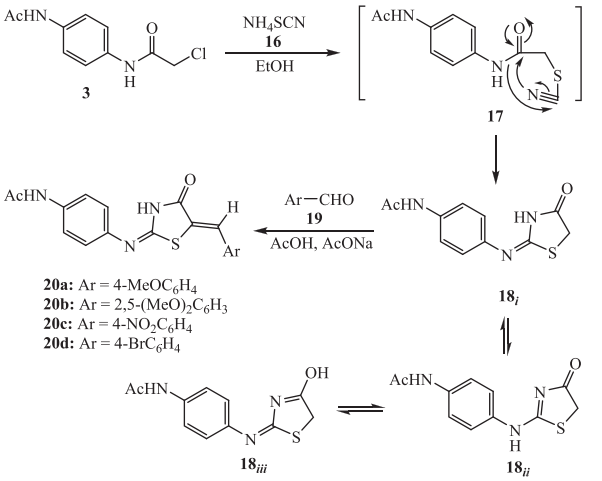
Scheme 1. Synthesis of sulfide derivatives **5**, **7**, and **9**.



Scheme 2. Synthesis of 2-(4-acetamidophenylaminocarbonyl)-3-amino (hydroxyl)-thiophenes **11**, **13**, and **15**.



Scheme 3. Synthesis of 2-(4-acetamidophenyl-imino)thiazolidin-4-ones **18** and **20**.



and **13b**. Elemental and spectral analyses were utilized to secure the chemical structure of compounds **13**, the IR absorption bands of **13b** at 3432, 3397, 3268, 3161, and 1661 referred to functional groups (NH, NH_2, and C=O), and the absence of nitrile function. In addition, the ^{1}H NMR signals of the same compound resonated as singlet at 2.01 ppm for three protons (CH_3), singlet at 4.98 for two protons (NH_2), multiplet in the region 6.93–7.52 ppm for the aromatic protons, three singlet at 8.80, 9.32, and 10.24 ppm for the protons of three NH groups, and singlet at 9.89 ppm for two protons (NH_2).

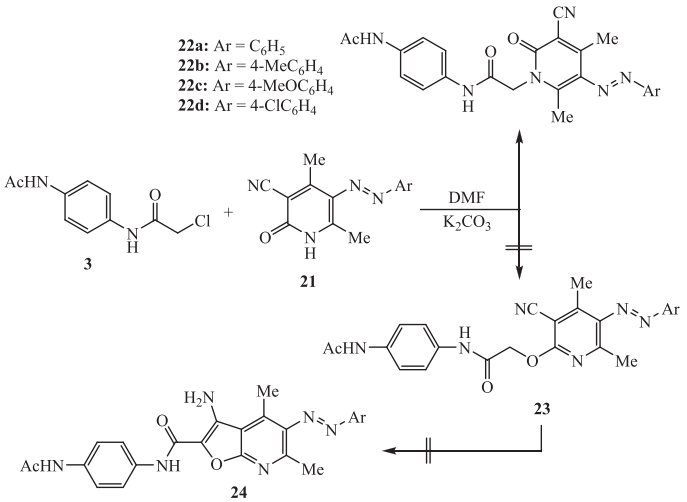
Treatment of 2-chloroacetamide derivative **3** with 2-(arylhydrazono)-2-ethoxycarbonyl-thioacetanilide derivatives **14** [24] in ethanolic sodium ethoxide yielded the corresponding 2-(4-acetamidophenylaminocarbonyl)-4-arylazo-3-hydroxythiophenes **15a–c**. The reaction starts *via* nucleophilic substitution of the chlorine atom from chloroacetamide **3** followed by intramolecular elimination of ethanol molecule to furnish the target 3-hydroxythiophene products. The chemical structures of **15a–c** were established based on their spectral and elemental analyses. The ^{1}H NMR spectrum of **15b** (as an example) displayed signals that agree with the designed structure, the two methyl substituents appeared at 2.03 and 2.35 ppm as two singlet signals. Aromatic protons were observed in the region 7.28 to 7.75 ppm as multiplet and doublet. The protons of NH and OH functions were observed as four singlet signals at 9.41, 9.90, 13.24, and 14.35 ppm.

Heterocyclization of 2-chloroacetamide **3** upon treatment with ammonium thiocyanate to generate the 2-(*p*-acetamidophenylimino)-thiazolidin-4-one (**18**) has been achieved by heating in ethyl alcohol under reflux for 4 h (Scheme 3). The formation of this lactam structure **18** proceeded as mentioned by Vicini *et al*. [25] through intramolecular cyclization of the thiocyanate intermediate **17** and the Dimroth-like rearrangements [26]. The amino–imino tautomerism of the 2-arylimino-thiazolidin-4-one **18** was established through the analysis of IR and ^{1}H NMR spectral data. The tautomeric structure $\mathbf{18}_{\boldsymbol{i}}$ finds support through the appearance of a lactam proton (-CONH-) in the ^{1}H NMR spectrum as singlet at lower field (11.12 ppm) rather than the imine proton of $\mathbf{18}_{\boldsymbol{ii}}$ which should appears at higher field (≈9.70 ppm). The IR absorption of the lactam NH group at 3109 cm^{-1}, together with a strong band at 1662 cm^{-1}, confirms the γ-lactam tautomeric form $\mathbf{18}_{\boldsymbol{i}}$ in the solid state. The possibility of the tautomeric structure $\mathbf{18}_{\boldsymbol{iii}}$ involving a hydroxylic group was diminished due to the absence of typical signals for OH group in IR and ^{1}H NMR spectra.

The methylene group in this thiazolidin-4-one derivative **18** proved to be reactive towards Knoevenagel condensation reaction with substituted benzaldehyde derivatives. The condensation proceeded readily by heating in glacial acetic acid and fused sodium acetate to furnish the corresponding 5-(substituted benzylidene)-thiazolidin-4-one derivatives **20a–d** with 70–85% yield. The structures of these targeted thiazolidine-4-ones were assigned based on spectroscopic and analytical data. The characteristic stretching absorptions for *Ac*N–H, N–H, and C=O bonds were observed at 3218–3214, 3162–3103, and 1676–1661 cm^{-1}, respectively. The ^{1}H NMR spectra of **20a–c** showed singlet for the proton of methine function (CH=C) deshielded to the range 7.72–7.77 ppm.

In an attempt to prepare the furo[2,3-*b*] pyridine derivatives **24** *via* intramolecular cyclization of the *O*-alkylated compounds **23**, heating of 2-chloroacetamide derivative **3** with 5-arylazo-3-cyano-4,6-dimethylpyridin-

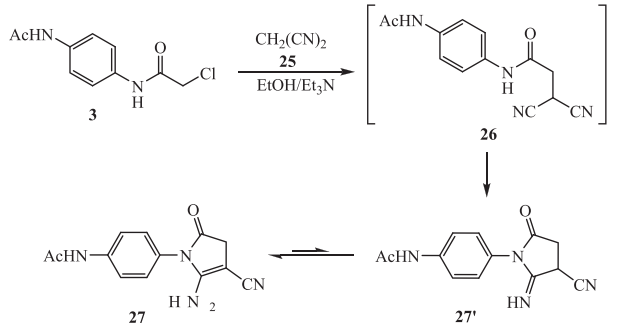
Scheme 4. Reaction of chloroacetamide **3** with 5-arylazo-3-cyano-4,6-dimethylpyridin-2-ones.



2-ones **21** in dimethylformamide containing potassium carbonate furnished the corresponding *N*-alkylated product, 2-(3-cyano-2-oxo-5-arylazopyridin-1-yl)-*N*-(*p*-acetamidophenyl)-acetamide derivatives **22a–d**, as a sole product (Scheme 4). The reaction failed to substitute the chlorine atom by the nucleophilic oxygen (*O*-alkylation), and the substitution proceeded by the more powerful nucleophilic nitrogen of the pyridine ring. Elemental and spectral analyses were utilized to secure the chemical structure of these synthesized pyridones **22a–d**, characteristic stretching infrared absorptions for N–H and C≡N bonds were observed at 3315–3275 and 2224–2223 cm^{-1}, respectively. The stretching absorption at about 1666–1661 cm^{-1} was recorded for C=O double bonds. In the ^1H NMR spectra, the methylene of acetamide moiety ($NHCOCH_2$) was observed as singlet at 5.01–5.09 ppm.

The reaction of 2-chloroacetamide derivative **3** with malononitrile as an example carbon nucleophile proceeded by heating in ethyl alcohol in the presence of base such as triethylamine (Scheme 5). The reaction product was designed and identified as the 1-acetamidophenyl-2-aminopyrrole scaffold **27** because of its correct elemental and spectral analyses. IR absorptions for the NH_2 and NH were observed at 3328, 3225, and 3185 cm^{-1}. The stretching absorptions at 2175, 1728, and 1647 cm^{-1} were recorded for C≡N and C=O bonds. The ^1H NMR spectrum clearly indicated the protons of methylene and amino functions as singlet signals at 3.36 and 6.78 ppm.

Scheme 5. Synthesis of 1-(*p*-acetamidophenyl)-2-amino-3-cyano-5-oxopyrrole **27**.



In vitro anticancer screening. *In vitro* cytotoxic action of the synthesized 4-(*N*-substituted amino)-acetanilide scaffolds was assessed against human breast cancer cell line, MCF7. Doxorubicin, which is a standout among the best anticancer agents, was utilized as the reference sedate as a part of this work. The relationship between relative viability of cells (%) and concentration (μg/mL) was plotted to acquire the survival curve of MCF7. The results of statistical analysis of drug *in vitro* anticancer revealed a significant rapprochement between the control and the tested 2-(4-acetamidophenylaminocarbonyl)-3-amino-thiophene compounds **11**, **13a**, and **13b** that proved to be the most active members in our study. They showed very strong potency towards MCF7.

In vitro cytotoxic activities of the synthesized scaffolds were recognized in (Table 1). IC_{50} values refer to the concentration required for 100% inhibition of cell viability. Analysis of the data in Table 1 indicated that compounds **11**, **13a**, and **13b** containing the thiophene moiety exhibited the lowest IC_{50}, which means that they are the most effective cytotoxic drugs. Accordingly, compounds **15a–c** and **27** can be used as very potent cytotoxic drugs for breast carcinoma cell, while compounds **3**, **20b**, and **22d** have moderate IC_{50}, which means that they are lower effective cytotoxic drugs for breast carcinoma cell. On the other hand, the remaining compounds **5**, **7**, **9**, **18**, **20a**, **20c**, **20d**, **22a**, and **22b** are

Table 1

Cytotoxic activity of the synthesized scaffolds against MCF-7 cell line.

Compound	*In vitro* cytotoxicity IC_{50} (μg/mL)
	MCF-7
DOX	4.17 ± 0.2
3	28.94 ± 1.9
5	81.65 ± 3.3
7	90.66 ± 4.3
9	67.04 ± 3.0
11	8.96 ± 0.7
13a	9.45 ± 0.9
13b	10.4 ± 0.88
15a	11.71 ± 1.0
15b	19.39 ± 1.6
15c	17.34 ± 1.4
18	52.70 ± 2.8
20a	88.97 ± 3.8
20b	46.83 ± 2.5
20c	72.43 ± 3.2
20d	94.26 ± 4.7
22a	59.84 ± 2.9
22b	84.72 ± 3.6
22c	<100
22d	38.92 ± 2.1
27	13.80 ± 1.2

IC_{50} (μg/mL): 1–10 (very strong); 11–20 (strong); 21–50 (moderate); 51–100 (weak); above 100 (non-cytotoxic). DOX, Doxorubicin.

very weak cytotoxic drugs compared with the reference drug, Doxorubicin.

Based on these preliminary screening results, aminothiophene compounds **11**, **13a**, and **13b** showed the highest cytotoxic activity in the tested cancer cell. In addition, the newly synthesized thiophene scaffolds **15a–c** exhibited good efficacy relative to the standard anticancer drug Doxorubicin. They could be important leads in continuing development against anticancer disease.

CONCLUSION

The present study has been focused on the synthesis of new thiophene, thiazolidine-4-one, pyridine, and pyrrole derivatives containing biologically active acetanilide nucleus and evaluation of their anticancer activity. Some of the newly synthesized compounds **11**, **13a**, and **13b** exhibited significant activity (very strong) compared with the control drug, Doxorubicin. Compounds **15a–c** and **27** are strong cytotoxic drugs while compounds **3**, **20b**, and **22d** exhibited a moderate activity.

EXPERIMENTAL

All melting points (uncorrected) were measured on Gallenkamp electric melting point apparatus. The infrared spectra were determined on a Thermo Scientific Nicolet iS10 FTIR spectrometer (Waltham, MA). The ^{1}H NMR spectra were recorded on a Varian XL 300 MHz apparatus (Palo Alto, CA) using DMSO-d_6 as a solvent. The mass spectra were recorded on a Quadrupole GC/MS Thermo Scientific Focus/DSQII (Waltham, MA) at 70 eV. Elemental analyses (C, H, and N) were determined on Perkin-Elmer 2400 analyzer (PerkinElmer Instruments, Shelton, CT). The NMR spectra of the products **15c**, **20d**, and **22d** are not provided due to their limited solubility in common NMR solvents.

Synthesis of *N*-(4-acetamidophenyl)-2-chloroacetamide (3). To a stirred suspension of 4-aminoacetanilide (10 mmol, 1.5 g) and anhydrous potassium carbonate (10 mmol, 1.38 g) in 40-mL acetone, 1.2 mL chloroacetyl chloride (15 mmol) has been added drop by drop while the stirring is continued for 2 h. The solid that obtained on dilution with cold water was filtered and recrystallized from ethyl alcohol.

White crystals, yield 72%, mp 248–250°C; lit. mp 240–243°C [21]; IR ($\bar{\nu}$/cm^{-1}): 3269, 3171 (NH), 1668 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.01 (s, 3H, CH_3), 3.47 (s, 2H, CH_2), 7.49 (s, 4H, Ar–H), 9.84 (s, 1H, NH), 10.04 (s, 1H, NH). *Anal*. Calcd. for $C_{10}H_{11}ClN_2O_2$ (226.05): C, 52.99; H, 4.89; N, 12.36%. Found: C, 52.91; H, 4.85; N, 12.38%.

General procedure for the synthesis of sulfide derivatives 5, 7, and 9. To a solution of chloroacetamide derivative **3** (1.13 g, 5 mmol) in 30-mL ethyl alcohol, 2-mercaptobenzothiazole, 2-mercaptoethanol, and/or ethyl 2-mercaptoacetate (5 mmol) in addition to 0.5 g sodium acetate was added. The mixture was heated under reflux for 3 h and then poured into ice water. The precipitate that formed was isolated by filtration and purified by recrystallization from the ethyl alcohol to furnish sulfide derivatives **5**, **7**, and/or **9**, respectively.

***N*-(4-Acetamidophenyl)-2-(benzothiazol-2-thio)acetamide (5).** White crystals, yield 90%, mp 208–210°C; IR ($\bar{\nu}$/cm^{-1}): 3286, 3150 (NH), 1654 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.02 (s, 3H, CH_3), 4.37 (s, 2H, CH_2), 7.34–7.44 (m, 2H, Ar–H), 7.50 (s, 4H, Ar–H), 7.83 (d, J = 8.1 Hz, 1H, Ar–H), 8.02 (d, J = 7.8 Hz, 1H, Ar–H), 9.87 (s, 1H, NH), 10.35 (s, 1H, NH). *Anal*. Calcd. for $C_{17}H_{15}N_3O_2S_2$ (357.06): C, 57.12; H, 4.23; N, 11.76%. Found: C, 57.26; H, 4.25; N, 11.84%.

***N*-(4-Acetamidophenyl)-2-((2-hydroxyethyl)thio)acetamide (7).** White powder, yield 70%, mp 143–145°C; IR ($\bar{\nu}$/cm^{-1}): 3259, 3169 (NH and OH), 1649 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.01 (s, 3H, CH_3), 2.70 (t, J = 6.75 Hz, 2H, CH_2), 3.28 (s, 2H, CH_2), 3.56 (t, J = 6.75 Hz, 2H, CH_2), 4.85 (s, 1H, OH), 7.48 (s, 4H, Ar–H), 9.86 (s, 1H, NH), 9.98 (s, 1H, NH). *Anal*. Calcd. for $C_{12}H_{16}N_2O_3S$ (268.09): C, 53.71; H, 6.01; N, 10.44%. Found: C, 53.81; H, 6.04; N, 10.36%.

***N*-(4-Acetamidophenyl)-2-(ethoxycarbonyl-methylthio) acetamide (9).** White powder, yield 67%, mp 148–150°C;

IR ($\bar{\nu}$/cm^{-1}): 3293, 3155 (NH), 1742, 1657 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 1.18 (t, J = 7.20 Hz, 3H, CH_3), 2.01 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.49 (s, 2H, CH_2), 4.08 (q, J = 7.20 Hz, 2H, CH_2), 7.48 (s, 4H, Ar–H), 9.84 (s, 1H, NH), 10.00 (s, 1H, NH). *Anal*. Calcd. for $C_{14}H_{18}N_2O_4S$ (310.10): C, 54.18; H, 5.85; N, 9.03%. Found: C, 54.07; H, 5.90; N, 9.08%.

General procedure for the synthesis of 2-(4-acetamidophenylaminocarbonyl)-3-amino (hydroxyl)-thiophenes 11, **13**, and **15**. *N*-(4-Acetamidophenyl)-2-chloroacetamide **3** (5 mmol, 1.13 g) was stirred for 10 min in sodium ethoxide solution (previously prepared by dissolving small granules of sodium, 0.23 g, in 30-mL absolute ethanol) and then the thiol derivatives (5 mmol) [namely, 2-mercapto-4,6-dimethylnicotinonitrile (**10**), thiocarbamoyl derivatives (**12**), or 2-(arylhydrazono)-2-ethoxycarbonyl-thioacetanilide derivatives (**14**)] were added. The reaction mixture was heated on a steam bath for 4 h and then allowed to pour into ice water. The solid that formed after neutralization by dilute HCl was filtered and recrystallized from EtOH/DMF mixture (4:1) to afford the thiophene products **11**, **13**, or **15**, respectively.

***N*-(4-Acetamidophenyl)-3-amino-4,6-dimethylthieno[2,3-b]pyridine-2-carboxamide (11).** Orange powder, yield 88%, mp 278–280°C; IR ($\bar{\nu}$/cm^{-1}): 3490, 3333, 3290, 3251 (NH and NH_2), 1684 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.02 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 6.92 (s, 2H, NH_2), 7.05 (s, 1H, pyridine-H_5), 7.50 (d, J = 9 Hz, 2H, Ar–H), 7.56 (d, J = 9 Hz, 2H, Ar–H), 9.33 (s, 1H, NH), 9.88 (s, 1H, NH); MS *m/z* (%): 355 (M^+ +1, 16.15), 354 (M^+, 71.93), 205 (82.62), 177 (19.46), 150 (100), 133 (26.56), 108 (40.74). *Anal*. Calcd. for $C_{18}H_{18}N_2O_3S$ (354.12): C, 61.00; H, 5.12; N, 15.81%. Found: C, 61.16; H, 5.06; N, 15.90%.

Ethyl 5-((4-acetamidophenyl)carbamoyl)-4-amino-2-(phenylamino)thiophene-3-carboxylate (13a). Green powder, yield 66%, mp 208–210°C; IR ($\bar{\nu}$/cm^{-1}): 3301, 3267, 3173 (NH and NH_2), 1668, 1653 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 1.21 (t, J = 7.20 Hz, 3H, CH_3), 2.04 (s, 3H, CH_3), 4.16 (q, J = 7.20 Hz, 2H, CH_2), 6.07 (s, 2H, NH_2), 7.14–7.72 (m, 9H, Ar–H), 9.84 (s, 1H, NH), 10.17 (s, 1H, NH), 11.53 (s, 1H, NH). MS *m/z* (%): 439 (M^+ +1, 38.50), 438 (M^+, 53.00), 428 (52.50), 408 (56.00), 398 (46.50), 390 (50.50), 368 (46.50), 342 (43.00), 323 (54.50), 314 (42.00), 288 (58.39), 215 (91.26), 187 (47.89), 143, (30.75), 77 (100.00). *Anal*. Calcd. for $C_{22}H_{22}N_4O_4S$ (438.14): C, 60.26; H, 5.06; N, 12.78%. Found: C, 60.12; H, 5.00; N, 12.70%.

N*$_2$*-(4-Acetamidophenyl)-3-amino-5-(phenylamino) thiophene-2,4-dicarboxamide (13b). Beige powder, yield 70%, mp 230–232°C; IR ($\bar{\nu}$/cm^{-1}): 3432, 3397, 3268, 3161 (NH and NH_2), 1661 (br, C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.01 (s, 3H, CH_3), 4.98 (s, 2H, NH_2), 6.93–7.52 (m, 9H, Ar–H), 8.80 (s, 1H, NH), 9.32 (s, 1H, NH), 9.89 (s, 2H, NH_2), 10.24 (s, 1H, NH). *Anal*. Calcd. for $C_{20}H_{19}N_5O_3S$ (409.12): C, 58.67; H, 4.68; N, 17.10%. Found: C, 58.78; H, 4.71; N, 17.16%.

2-(4-Acetamidophenylaminocarbonyl)-3-hydroxy-5-phenylamino-4-phenylazo-thiophene (15a). Reddish brown crystals, yield 58%, mp 224–225°C; IR ($\bar{\nu}$/cm^{-1}): 3364 (NH and OH), 1664, 1628 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.08 (s, 3H, CH_3), 7.32–7.89 (m, 14H, Ar–H), 9.46 (s, 1H, NH), 9.94 (s, 1H, NH), 13.32 (s, 1H, NH), 14.28 (s, 1H, OH). *Anal*. Calcd. for $C_{25}H_{21}N_5O_3S$ (471.14): C, 63.68; H, 4.49; N, 14.85%. Found: C, 63.82; H, 4.43; N, 14.95%.

2-(4-Acetamidophenylaminocarbonyl)-3-hydroxy-5-phenylamino-4-(4-tolylazo)-thiophene (15b). Reddish brown crystals, yield 80%, mp 235–237°C; IR ($\bar{\nu}$/cm^{-1}): 3364, 3297 (NH and OH), 1662, 1631 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.03 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.28–7.53 (m, 11H, Ar–H), 7.75 (d, J = 8.7 Hz, 2H, Ar–H), 9.41 (s, 1H, NH), 9.90 (s, 1H, NH), 13.24 (s, 1H, NH), 14.35 (s, 1H, OH); MS *m/z* (%): 485 (M^+, 56.40), 428 (45.44), 381 (73.22), 337 (51.68), 150 (48.81), 80 (100), 64 (97.98). *Anal*. Calcd. for $C_{26}H_{23}N_5O_3S$ (485.15): C, 64.31; H, 4.77; N, 14.42%. Found: C, 64.38; H, 4.79; N, 14.38%.

2-(4-Acetamidophenylaminocarbonyl)-4-(4-bromophenylazo)-3-hydroxy-5-phenyl amino-thiophene (15c). Brown powder, yield 76%, mp 290–292°C; IR ($\bar{\nu}$/cm^{-1}): 3298 (NH and OH), 1657 (br, C=O); MS *m/z* (%): 551 (M^+, Br-81, 9.94), 549 (M^+, Br-79, 6.08), 495 (32.6), 479 (61.26), 447 (100), 330 (28.7), 174 (45.04), 134 (25.8), 108 (29.8), 91 (16.85), 77 (56.10), 65 (43.70), 43 (49.4). *Anal*. Calcd. for $C_{25}H_{20}BrN_5O_3S$ (549.05): C, 54.55; H, 3.66; N, 12.72%. Found: C, 54.55; H, 3.61; N, 12.80%.

Synthesis of 2-(4-acetamidophenylimino)-thiazolidin-4-one (18). A suspension of chloroacetamide **3** (5 mmol, 1.13 g) and ammonium thiocyanate (10 mmol, 0.76 g) in 30-mL ethyl alcohol was heated under reflux for 4 h. The precipitate that obtained on cooling was picked up by filtration and then recrystallized by heating in ethyl alcohol.

Beige crystals, yield 70%, mp 280–282°C; IR ($\bar{\nu}$/cm^{-1}): 3248, 3109 (NH), 1662 (br, C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.08 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 7.56–7.59 (s, 4H, Ar–H), 9.95 (s, 1H, NH), 11.12 (s, 1H, NH). *Anal*. Calcd. for $C_{11}H_{11}N_3O_2S$ (249.06): C, 53.00; H, 4.45; N, 16.86%. Found: C, 53.12; H, 4.42; N, 16.79%.

General procedure for synthesis of 2-(4-acetamidophenylimino)-5-arylidene-thiazolidin-4-ones 20a–d. To a suspension of 2-(4-acetamidophenylimino) thiazolidin-4-one (**18**) (2 mmol, 0.5 g) and 0.5 g fused sodium acetate in 15-mL glacial acetic acid, the appropriate aromatic aldehyde derivative (2 mmol) was added. The reaction mixture was refluxed for 4 h and then allowed to cool to 25°C. The solid that formed, after dilution with cold water, was isolated by filtration. The resulting crude product was purified by recrystallization from EtOH/DMF mixture (1:1) to yield **20a–d**.

2-(4-Acetamidophenylimino)-5-(4-methoxybenzylidene)-thiazolidin-4-one (20a). Orange crystals, yield 80%, mp 294–295°C; IR ($\bar{\nu}$/cm^{-1}): 3218, 3103 (NH), 1661 (br, C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.06 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.14 (d, J = 8.00 Hz, 2H, Ar–H), 7.43–7.53 (m, 4H, Ar–H), 7.62 (d, J = 8.00 Hz, 2H, Ar–H), 7.72 (s, 1H, CH=C), 9.98 (s, 1H, NH), 12.30 (s, 1H, NH). *Anal*. Calcd. for $C_{19}H_{17}N_3O_3S$ (367.10): C, 62.11; H, 4.66; N, 11.44%. Found: C, 62.18; H, 4.61; N, 11.49%.

2-(4-Acetamidophenylimino)-5-(2,5-dimethoxybenzylidene)-thiazolidin-4-one (20b). Orange crystals, yield 75%, mp >300°C; IR ($\bar{\nu}$/cm^{-1}): 3217, 3107 (NH), 1662 (br, C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.05 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.84 (s, 1H, Ar–H), 6.98–7.06 (dd, 2H, Ar–H), 7.58–7.65 (m, 4H, Ar–H), 7.74 (s, 1H, CH=C), 9.99 (s, 1H, NH), 12.26 (s, 1H, NH). *Anal*. Calcd. for $C_{20}H_{19}N_3O_4S$ (397.11): C, 60.44; H, 4.82; N, 10.57%. Found: C, 60.33; H, 4.85; N, 10.49%.

(5Z)-2-(4-Acetamidophenylimino)-5-(4-nitrobenzylidene)-thiazolidin-4-one (20c). Orange crystals, yield 70%, mp >300°C; IR ($\bar{\nu}$/cm^{-1}): 3214, 3162 (NH), 1676 (br, C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.05 (s, 3H, CH_3), 7.63–7.75 (m, 4H, Ar–H), 7.77 (s, 1H, CH=C), 7.83 (d, J = 8.80 Hz, 2H, Ar–H), 8.34 (d, J = 8.40 Hz, 2H, Ar–H), 10.21 (s, 1H, NH), 12.40 (s, 1H, NH). *Anal*. Calcd. for $C_{18}H_{14}N_4O_4S$ (382.07): C, 56.54; H, 3.69; N, 14.65%. Found: C, 56.71; H, 3.63; N, 14.56%.

2-(4-Acetamidophenylimino)-5-(4-bromobenzylidene)-thiazolidin-4-one (20d). Brown powder, yield 65%, mp >300°C; IR ($\bar{\nu}$/cm^{-1}): 3218, 3106 (NH), 1662 (br, C=O); MS m/z (%): 417 (M^+, Br-81, 100), 415 (M^+, Br-79, 95.38), 375 (15.99). *Anal*. Calcd. for $C_{18}H_{14}BrN_3O_2S$ (415.00): C, 51.93; H, 3.39; N, 10.09%. Found: C, 51.77; H, 3.31; N, 10.21%.

General procedure for the synthesis of *N-(4-acetamidophenyl)-2-(3-cyano-4,6-dimethyl-2-oxo-5-(substituted phenylazo)pyridinyl)acetamides 22a–d.* A suspension of chloroacetamide derivative **3** (5 mmol, 1.13 g), 5-arylazo-3-cyano-4,6-dimethyl-pyridin-2-one (5 mmol), and 0.70 g of anhydrous potassium carbonate in 20-mL dimethylformamide was heated on water bath for 4 h. The reaction mixture was cooled and then poured onto ice water. The precipitate that formed was filtered off and recrystallized from dioxane to give *N*-substituted pyridine derivatives **22a**–**d**.

N-*(4-Acetamidophenyl)-2-(3-cyano-4,6-dimethyl-2-oxo-5-phenylazopyridin-1(2H)-yl)acetamide (22a).* Red crystals, yield 55%, mp 278–280°C; IR ($\bar{\nu}$/cm^{-1}): 3303 (NH), 2224 (C≡N), 1666 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.02 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 5.05 (s, 2H, CH_2), 7.26–7.64 (m, 9H, Ar–H), 9.90 (s, 1H, NH), 10.41 (s, 1H, NH). *Anal*. Calcd. for $C_{24}H_{22}N_6O_3$ (442.18): C, 65.15; H, 5.01; N, 18.99%. Found: C, 65.22; H, 5.06; N, 19.06%.

N-*(4-Acetamidophenyl)-2-(3-cyano-4,6-dimethyl-2-oxo-5-(4-tolylazo)pyridin-1(2H)-yl)acetamide (22b).* Orange powder, yield 67%, mp 285–286°C; IR ($\bar{\nu}$/cm^{-1}): 3275, 3171 (NH), 2224 (C≡N), 1664 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.03 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 5.01 (s, 2H, CH_2), 7.32 (d, J = 8.80 Hz, 2H, Ar–H), 7.41–7.47 (m, 4H, Ar–H), 7.67 (d, J = 8.80 Hz, 2H, Ar–H), 9.82 (s, 1H, NH), 10.39 (s, 1H, NH). *Anal*. Calcd. for $C_{25}H_{24}N_6O_3$ (456.19): C, 65.78; H, 5.30; N, 18.41%. Found: C, 65.66; H, 5.34; N, 18.32%.

N-*(4-Acetamidophenyl)-2-(3-cyano-4,6-dimethyl-2-oxo-5-(4-methoxyphenyl-azo)pyridin-1(2H)-yl)acetamide (22c).* Orange powder, yield 75%, mp 289–290°C; IR ($\bar{\nu}$/cm^{-1}): 3295 (NH), 2223 (C≡N), 1665 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.02 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 5.09 (s, 2H, CH_2), 7.11 (d, J = 8.80 Hz, 2H, Ar–H), 7.51–7.53 (m, 4H, Ar–H), 7.84 (d, J = 8.80 Hz, 2H, Ar–H), 9.89 (s, 1H, NH), 10.47 (s, 1H, NH). *Anal*. Calcd. for $C_{25}H_{24}N_6O_4$ (472.19): C, 63.55; H, 5.12; N, 17.79%. Found: C, 63.40; H, 5.06; N, 17.87%.

N-*(4-Acetamidophenyl)-2-(3-cyano-4,6-dimethyl-2-oxo-5-(4-chlorophenyl-azo)pyridin-1(2H)-yl)acetamide (22d).* Brown powder, yield 70%, mp 268–270°C; IR ($\bar{\nu}$/cm^{-1}): 3315 (NH), 2224 (C≡N), 1661 (C=O); MS m/z (%): 476 (M^+, 34.60), 416 (100), 415 (88.67), 374 (16.73), 353 (16.93), 329 (17.43), 327 (47.46). *Anal*. Calcd. for $C_{24}H_{21}ClN_6O_3$ (476.14): C, 60.44; H, 4.44; N, 17.62%. Found: C, 60.62; H, 4.37; N, 17.74%.

Synthesis of 2-amino-1-(4-acetamidophenyl)-3-cyano-5-oxo-4,5-dihydro-1H-pyrrole (27). A mixture of **3** (1.13 g, 5 mmol) and malononitrile (5 mmol) in EtOH (30 mL) containing 0.5 mL triethylamine was heated under reflux for 3 h. After cooling, the precipitate that formed was picked up by filtration and then recrystallized from the dioxane.

Gray powder, yield 78%, mp >300°C; IR ($\bar{\nu}$/cm^{-1}): 3328, 3225, 3185 (NH and NH_2), 2175 (C≡N), 1728, 1647 (C=O); ^{1}H NMR (DMSO-d_6): δ/ppm = 2.07 (s, 3H, CH_3), 3.36 (s, 2H, CH_2), 6.78 (s, 2H, NH_2), 7.17 (d, J = 8.70 Hz, 2H, Ar–H), 7.69 (d, J = 8.70 Hz, 2H, Ar–H), 10.13 (s, 1H, NH). *Anal*. Calcd. for $C_{13}H_{12}N_4O_2$ (256.10): C, 60.93; H, 4.72; N, 21.86%. Found: C, 60.81; H, 4.75; N, 21.78%.

In vitro anticancer activity. The evaluation of *in vitro* cytotoxicity effects of the synthesized aminoacetanilide scaffolds was carried out against mammary gland breast cancer (MCF-7) cell line. This cell line was obtained from ATCC *via* Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Cytotoxicity determinations are based the transformation of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in practical cells. The

method of this MTT test was performed as previously described in detail [27,30].

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