Modern Organic Chemistry Research, Vol. 2, No. 3, August 2017
https://dx.doi.org/10.22606/mocr.2017.23004

Synthesis and Anticancer Evaluation of Some New Heterocyclic Scaffolds Incorporating the Acetanilide Moiety

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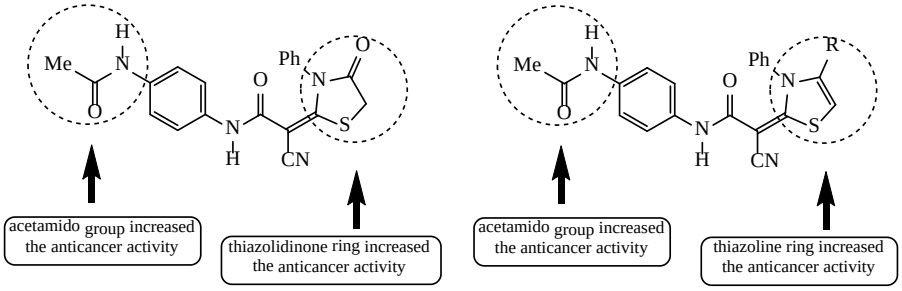
Abstract. New heterocyclic scaffolds containing acetanilide moiety were synthesized as anticancer agents. The precursor *N*-(4-acetamidophenyl)-2-cyanoacetamide (3) was coupled smoothly with (un)substituted phenyl diazonium chlorides producing the hydrazones 4. Various 2-pyridones 6 were picked up through the treatment of 3 with different arylidene malononitriles. The precursor underwent reaction with phenyl isothiocyanate in DMF/KOH followed by *in situ* addition of several α-halogenated reagents afforded the corresponding thiazole derivatives 9 and 10. Heating of thiocarbamoyl scaffold 11 with chloroacetone and phenacyl chloride resulted in the formation of 5-substitutedthiophene-3-carboxamides 12. Condensation of the synthesized ketene N,S-acetal 13 with NH_2NH_2 furnished the corresponding pyrazole-4-carboxamide 14. The newly synthesized scaffolds were characterized by considering their spectral analyses and they evaluated for their *in vitro* anticancer activities to show promising results.



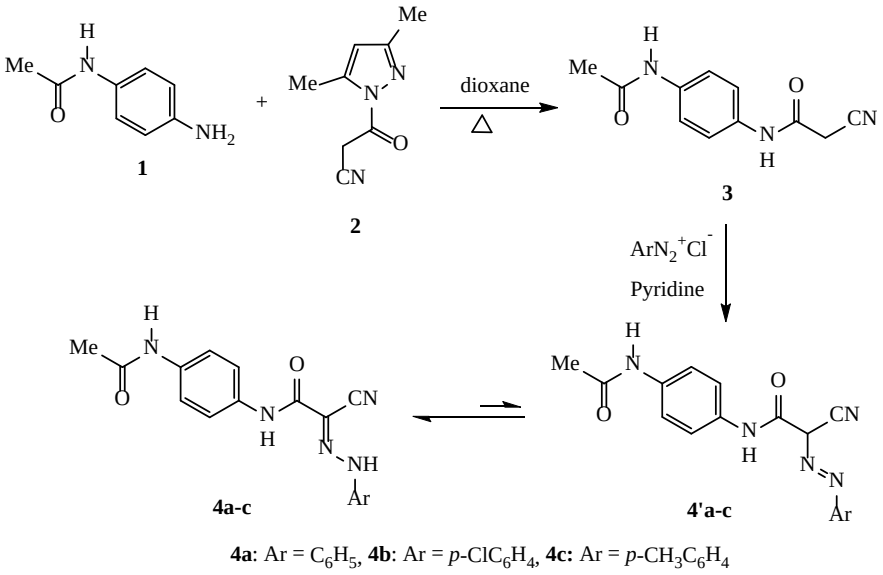
Keywords: 4-aminoacetanilide, hydrazones, 2-pyridones, thiazolidin-4-one, thiophene-3-carboxamides, anticancer activity.

1 Introduction

Cyanoacetamide scaffolds have great interest in various branches of medicine chemistry because of their multiplicity uses in the drug industry. The nucleus of cyanoacetamide is important pharmacophore in many biologically active compounds and found in many clinically used drugs. Their pharmaceutical activities include: insulin releasing [1], anti-inflammatory [2], antifungal [3], carbonic anhydrase inhibitory [4], antimicrobial [5,6], anti-cancer and anti-tumor properties [7]. Among heterocyclic compounds: pyrazoles, thiophenes and thiazoles are intriguing group of scaffolds which have across the board pharmacological characteristics, for example, analgesic, antipyretic and anti-androgenic activities [8-11]. Pyrazoles also possess antidepressant, anti-inflammatory and anti-rheumatic activities [12–14]. In recent years, several thiazole derivatives were found as anticonvulsant activities [15–18], antioxidant activities [19], potential neuro protective agents [20,21] and anti-tumor agents [22,23]. Also, some pyridones have antibacterial [24], antitumor [25] and other interesting biological activities [26,27]. In addition, many number of thiophene derivatives have known to give pharmacological effect [28-30]. Furthermore, the chromene nucleus is so important in the chemistry of biological active agents [31] which has been used as antithrombotic, anti-inflammatory, antibacterial, cardio protectors, enzymatic inhibitors, antitumor and antifungal [32-36]. In view of these findings, the present work focuses on the synthesis of new heterocyclic scaffolds containing the acetanilide moiety and evaluates their anti-breast cancer activity.

2 Results and Discussion

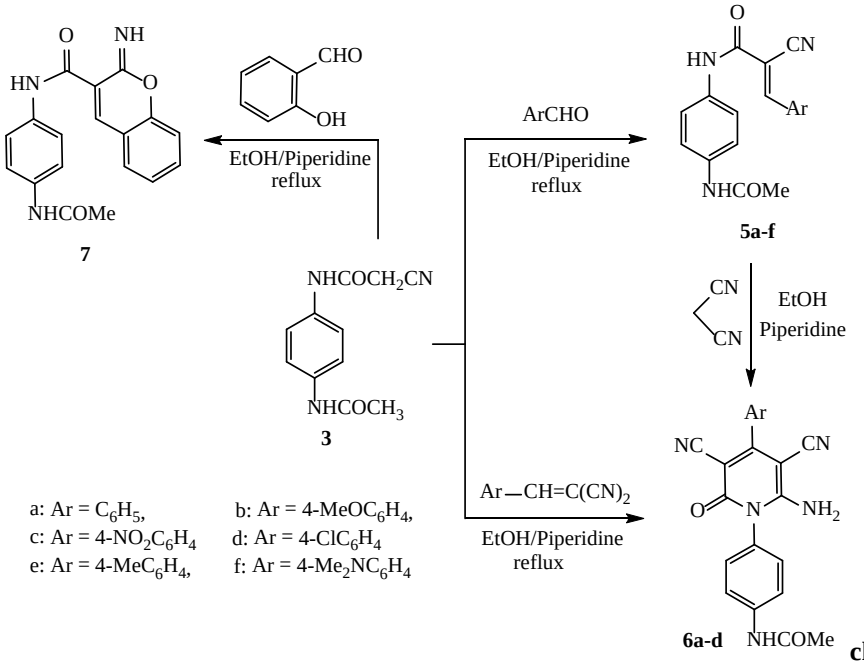
The important of aromatic amines activity is associated with the change probability of their - amino group to the cyanoacetamide group with furthermore function [23,24]. Cyanoacetylation of 4-aminoacetanilide (1) with *N*-cyanoacetyl-pyrazole reagent 2 furnished the key start *N*-(4-acetamidophenyl)-2-cyanoacetamide (3) (Scheme 1). Then, the activity of the methylene function of cyanoacetamide scaffold 3 towards reaction with diazonium chloride has been studied. Thus, cyanoacetamide scaffold 3 was diazocoupled with aryl diazonium chlorides, gotten from the suitable aromatic amines (aniline, 4-toluidine, and 4-chloroaniline) in pyridine to give the corresponding hydrazones 4a–c (Scheme 1). The proposed structures of the latter reaction products are obtained according to spectral and analytical data.



Scheme 1. Synthesis of N-(4-acetamidophenyl)-2-arylhydrazono-cyanoacetamides 4a-c.

In this manner, compound 3 was reacted with some aromatic aldehydes particularly benzaldehyde, 4-anisaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, 4-tolualdehyde, and 4-(dimethylamino)benzaldehyde to afford the conformity unsaturated nitrile scaffolds 5a–f (Scheme 2). The IR of 5a, as an example of the synthesized scaffolds, clearly demonstrated absorptions at 3450, 3288, 2222 and 1649 cm^{-1} to indicate the presence of NH, nitrile and carbonyl functional groups, respectively. ^1H NMR of the same scaffold demonstrated singlet for the protons of methyl function (2.03 ppm), multiplet for nine aromatic protons (7.54-7.74 ppm), singlet for olefinic proton (8.26 ppm) and two singlet signals for the protons of two NH functions (9.92 and 10.13 ppm). By heating in ethanol containing drops of piperidine as catalyst, pyridinone derivatives 6a–d were obtained by the reaction of malononitrile with the synthesized arylidenes 5a–d. The chemical structure of these pyridinone scaffolds was secured by their alternative synthesis via the reaction of cyanoacetamide derivative 3 with arylidene-malononitrile derivatives in hot ethanol containing drops of piperidine. The resulted compounds 6a–d were in perfect assent with the proposed structure according to elemental analyses and spectroscopic data.

In addition, cyclocondensation of cyanoacetanilide scaffold 3 with salicylaldehyde in ethanol containing drops of piperidine furnished *N*-(4-acetamidophenyl)-2-imino-2*H*-chromene-3-carboxamide (7). According to IR spectrum of 7, the absorptions at 3292, 3261 and 1683 cm^{-1} indicated the presence of two NH and C=O functions, respectively, while disappearance of any absorption band near 2200 cm^{-1} indicated the lack of nitrile function. The ^1H NMR spectrum of 7 revealed signals at 2.08 ppm (singlet for three protons, CH_3), 7.21-7.81 ppm (multiplet for eight aromatic protons), 8.31 ppm (singlet for the proton of chromene-C_4) and 9.28, 10.18 and 13.11 ppm for three protons of NH functions.

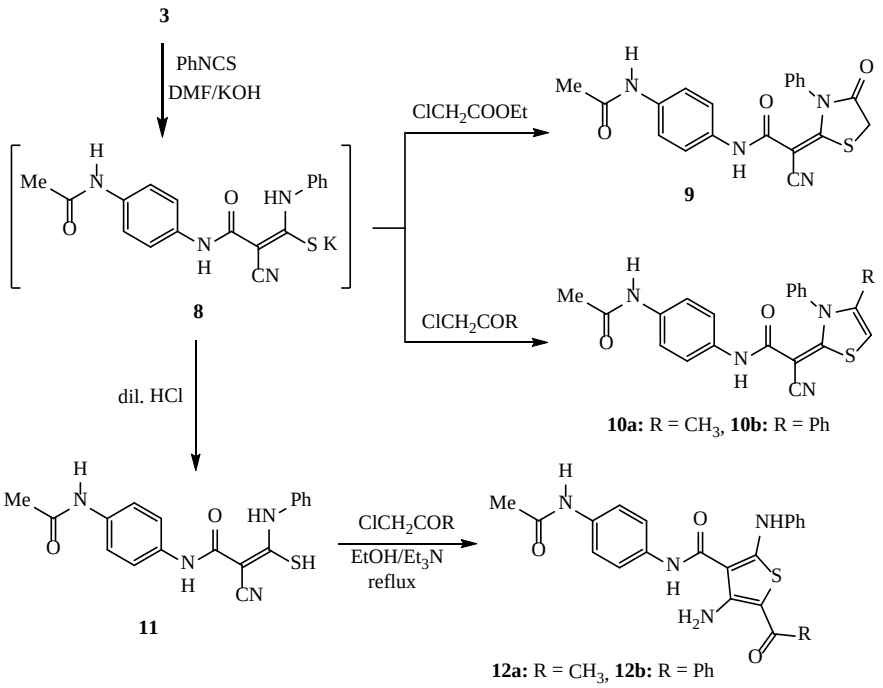


cheme 2. Synthesis of pyridones 6a–d and imino-2H-chromene derivative 7.

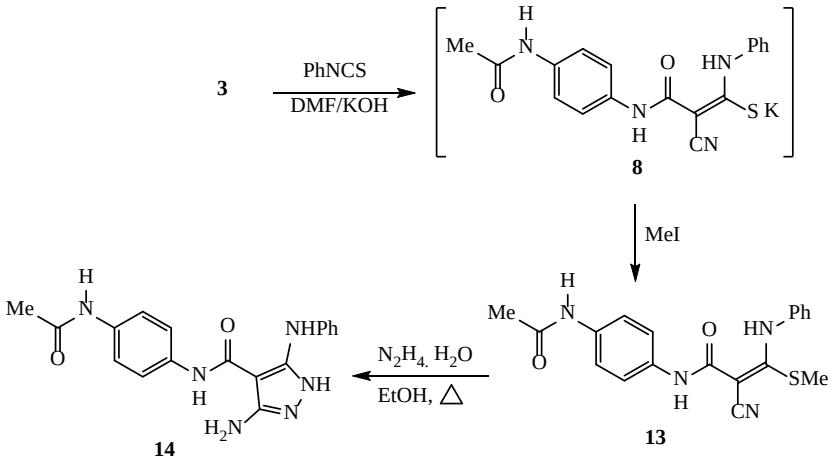
Treatment of cyanoacetamide scaffold 3 with phenyl isothiocyanate in dry DMF in the presence of an equimolar amount of KOH afforded the corresponding non-isolated sulphide salt 8, which underwent *in situ* stirring with ethyl chloroacetate, chloroacetone and phenacyl chloride furnished the corresponding thiazolidine-4-one, 4-methyl-3-phenylthiazoline and 3,4-diphenylthiazoline derivatives 9, 10a and 10b, respectively (Scheme 3). The absorption bands (3408, 3302, 2196 and 1743 cm^{-1}) in the IR of thiazolidine-4-one scaffold 9 clearly indicated the presence of NH, nitrile and cyclic carbonyl functions. The singlet signal for two protons at 3.99 ppm in the ^1H NMR spectrum of 9 was the best telltale for the cyclic methylene group. The structure of thiazoline scaffold 10a was secured by ^1H NMR spectrum, which characterized by the presence of singlet at 6.90 ppm for one olefinic proton (thiazole-C_5).

The synthesis of *N*-(4-acetamidophenyl)-3-mercapto-acrylamide scaffold 11 was achieved by treating non-isolated potassium salt 8 with dilute HCl. The IR spectrum of 11 clearly indicated the presence of NH (3457 and 3318 cm^{-1}), nitrile (2216 cm^{-1}) and carbonyl (broad 1665 cm^{-1}) functional groups. Refluxing of thiocarbamoyl scaffold 11 with chloroacetone and/or phenacyl chloride in ethanol and triethylamine resulted in the formation of *N*-(4-acetamidophenyl)-5-substitutedthiophene-3-carboxamides 12a and 12b, respectively. The IR spectra of 12a and 12b were characterized by the presence of NH and C=O absorption bands and the disappearance of nitrile absorption. In addition, ^1H NMR of 12a displayed four singlet signals at 2.01, 2.32, 11.55 and 11.88 ppm due to the protons of two methyl and two NH functions, respectively.

Furthermore, *in situ* alkylation of non-isolable salt 8 was achieved by addition of methyl iodide to furnish *N*-(4-acetamidophenyl)-2-cyano-3-(methylthio)-3-(phenylamino)acrylamide (13). The reaction of 13 with hydrazine hydrate was proceeded in boiling ethanol to afford *N*-(4-acetamidophenyl)-5-amino-3-(phenylamino)-1*H*-pyrazole-4-carboxamide (14) (Scheme 4). The IR spectrum indicated the lack of any absorption for the nitrile stretching and displayed absorption bands at 3445, 3261, 3176 and 1651 cm^{-1} due to NH_2, NH and carbonyl groups, respectively. The ^1H NMR spectrum displayed singlet at 6.05 ppm corresponding to NH_2 protons, doublet and multiplet signals in the region 6.25–7.55 ppm region due to aromatic protons, in addition to another three signals at 6.78, 8.59 and 8.78 ppm assignable to three NH protons.



Scheme 3. Synthesis of thiazole and thiophene derivatives 9, 10 and 12.



Scheme 4. Synthesis of pyrazole-4-carboxamide derivative 14.

3 *In Vitro* Anticancer Screening

In vitro cytotoxic action of the synthesized various heterocyclic scaffolds was assessed against human breast cancer cell line, MCF7. 5-Fluorouracil, which is a standout amongst 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 breast cancer cell line (MCF7) (Figure 1). The results of statistical analysis of drug in vitro anticancer revealed a significant rapprochement between the control and tested compounds 4c, 5f, 9, 10a and 10b which proved to be the most active members in our study. They showed very strong potency towards MCF7 cancer cell line.

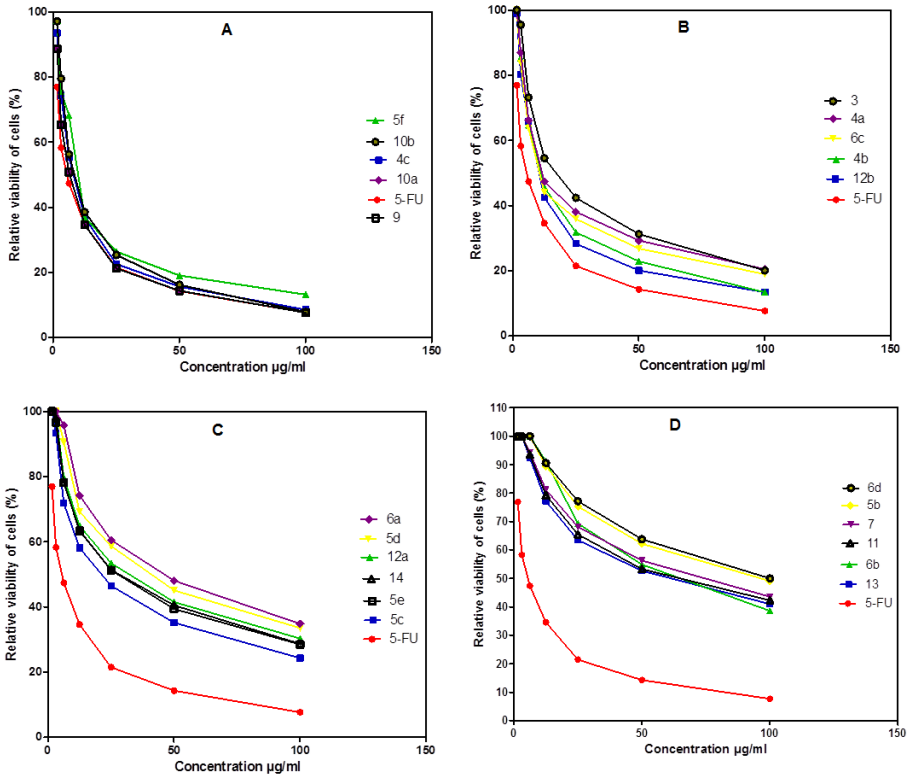


Figure 1. *In vitro* anticancer of relative viability of cells and drug concentration IC50 (µg/ml) compared with the reference drug 5-FU (5-fluorouracil): A- (very strong), B- (strong), C- (moderate) and D- (weak cytotoxic).

In vitro cytotoxic activities of the synthesized scaffolds were recognized in Table 1. IC50 values refer to the concentration required for 100% inhibition of cell viability. Analysis of the data in Table 1 indicated that compounds 3, 4a-c, 5f, 6c, 9, 10a,b and 12b exhibited the lowest IC50, which means that they are the most effective cytotoxic drugs. Accordingly compounds 4c, 5f, 9, 10a and 10b can be used as very potent cytotoxic drug for breast carcinoma cell, while compounds 5c, 5d, 6a, 6f and 12a have moderate IC50 which means that they are lower effective cytotoxic drug for breast carcinoma cell. On the other hand, the remaining compounds 5b, 6b, 6d, 7 and 13 are very weak cytotoxic drug compared with the reference drug, 5-fluorouracil.

Table 1. Cytotoxic activity of the synthesized scaffolds against human tumor cells.

Compound	In vitro Cytotoxicity IC50 (µg/ml)
	MCF-7
5-FU	5.5±0.21
3	19.8±1.86
4a	15.7±1.25
4b	12.8±1.10
4c	8.4±0.69
5a	>100
5b	86.8±4.82
5c	22.9±1.68
5d	40.8±2.94
5e	29.4±2.06
5f	10.4±0.88

6a	46.0±3.75
6b	61.4±4.31
6c	13.8±1.25
6d	91.3±5.69
7	67.3±4.82
9	5.3±0.28
10a	6.9±0.41
10b	9.3±0.98
11	65.5±4.64
12a	32.6±2.96
12b	11.4±1.03
13	57.2±3.87
14	30.4±1.92

IC50 (µg/ml): "1 – 10 (very strong); 11 – 20 (strong); 21 – 50 (moderate); 51 – 100 (weak); above 100 (non-cytotoxic); 5-FU = 5-fluorouracil"

The major findings gathered from the anticancer study of the unsaturated nitrile compounds 5a-f are highlighted as follow; the introduction of nitro and dimethylamino groups in compounds 5c and 5f provided higher anticancer activity than the other substituents (OMe, Cl, and Me). In addition, the newly synthesized thiazole scaffolds 9 and 10 exhibited similar or greater efficacy relative to the standard anticancer drug 5-fluorouracil. They could be important leads in continuing development against anticancer disease.

4 Experimental

4.1 General

All melting points (uncorrected) were measured on Gallenkamp electric melting point apparatus. Infrared spectra were determined on Mattson 5000 FTIR spectrometer (KBr discs). The ^{1}H NMR spectra were recorded on a Varian XL 300 MHz apparatus using DMSO-d_6 as a solvent. The mass spectra were acquired by EI mode at 70 eV with Kratos MS equipment. Elemental analyses (C, H and N) were determined on Perkin-Elmer 2400 analyzer.

4.2 Synthesis of N-(4-acetamidophenyl)-2-cyanoacetamide (3)

A suspension of 4-aminoacetanilide (1.50 g, 10 mmol) and *N*-cyanoacetyl-pyrazole reagent 2 (1.63 g, 10 mmol) was refluxed in dioxane (20 mL) for 4 h. On cooling to room temperature, the resulting precipitate was filtered, dried and recrystallized from EtOH/DMF mixture (5:1) to afford the cyanoacetamide scaffold 3.

Beige powder; yield (74%); m.p. 269-270°C. IR: 3289, 3172 (NH), 2256 (C≡N), 1662 (C=O). ^{1}H NMR (δ/ppm): 2.02 (s, 3H), 3.84 (s, 2H), 7.44 (d, 2H, J = 7.8 Hz), 7.51 (d, 2H, J = 7.8 Hz), 9.86 (s, 1H), 10.18 (s, 1H). Anal. Calcd. for $C_{11}H_{11}N_3O_2$ (217.23): C, 60.82; H, 5.10; N, 19.34%. Found: C, 60.73; H, 5.12; N, 19.22%.

4.3 Synthesis of N-(4-acetamidophenyl)-2-arylhydrazono-cyanoacetamides 4a-c

A suspension of 5 mmol aromatic amine (namely; aniline, 4-chloroaniline and 4-toluidine) in 1.5 mL conc. HCl was diazotized at 0-5°C with solution of $NaNO_2$ (0.35 g, 5 mmol in 10 mL water). The freshly prepared diazonium chloride solution was added drop by drop to a suspension of *N*-(4-acetamidophenyl)-2-cyanoacetamide (3) (1.08 g, 5 mmol) in pyridine (20 mL) at 0-5°C. When the addition is completed, the stirring was continued for 2 h and the solid that formed was filtered and recrystallized from EtOH/DMF mixture (3:1).

***N*-(4-Acetamidophenyl)-2-(phenylhydrazono)-2-cyanoacetamide (4a):**

Red powder; yield (82%); m.p. 245-246ºC. IR: 3306, 3241 (N–H), 2211 (C≡N), 1660 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 7.22–7.65 (m, 9H), 9.89 (s, 1H), 10.17 (s, 1H), 11.98 (br. s, 1H). Anal. Calcd. for $C_{17}H_{15}N_5O_2$ (321.34): C, 63.54; H, 4.71; N, 21.79%. Found: C, 63.41; H, 4.63; N, 21.87%.

***N*-(4-Acetamidophenyl)-2-(4-chlorophenylhydrazono)-cyanoacetamide (4b):**

Red powder; yield (70%); m.p. 210-211ºC. IR: 3351, 3221, 3188 (NH), 2208 (C≡N), 1684, 1665 (C=O). ^{1}H NMR (δ/ppm): 2.04 (s, 3H), 7.47 (d, 2H, J = 9 Hz), 7.56–7.62 (m, 4H), 7.92 (d, 2H, J = 9 Hz), 9.91 (s, 1H), 10.22 (s, 1H), 12.08 (s, 1H). Anal. Calcd. for $C_{17}H_{14}ClN_5O_2$ (355.78): C, 57.39; H, 3.97; N, 19.68%. Found: C, 57.30; H, 3.93; N, 19.57%.

***N*-(4-Acetamidophenyl)-2-(4-tolylhydrazono)-cyanoacetamide (4c)**

Yellow crystals; yield (80%); m.p. 255-256ºC. IR: 3350, 3308, 3223 (NH), 2205 (C≡N), 1681, 1664 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 2.29 (s, 3H), 7.18–7.62 (m, 8H), 9.81 (s, 1H), 9.89 (s, 1H), 11.78 (s, 1H). MS (m/z, %): 335 (M^+, 100.0), 293 (10.2), 217 (14.6), 175 (19.6), 150 (74.2), 134 (21.3), 121 (11.4), 107 (63.6), 91 (26.3), 77 (16.9), 65 (7.1). Anal. Calcd. for $C_{18}H_{17}N_5O_2$ (335.37): C, 64.47; H, 5.11; N, 20.88%. Found: C, 64.59; H, 5.04; N, 20.78%.

4.4 Synthesis of N-(4-acetamidophenyl)-3-aryl-2-cyano-acrylamides 5a–f

A suspension of cyanoacetamide scaffold 3 (1.08 g, 5 mmol) and the appropriate aromatic aldehyde (5 mmol) was heated under reflux for 3 h in EtOH (25 ml) containing five drops of piperidine as catalyst. The resulting products (on cooling) were separated by filtration and then recrystallized from the suitable solvent to obtain 5a–f.

***N*-(4-Acetamidophenyl)-2-cyano-3-phenylacrylamide (5a):**

Yellow powder; yield (92%); m.p. 245-246°C (EtOH). IR: 3450, 3288 (NH), 2222 (C≡N), 1649 (broad, C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 7.54-7.74 (m, 9H), 8.26 (s, 1H), 9.92 (s, 1H), 10.13 (s, 1H). Anal. Calcd. for $C_{18}H_{15}N_3O_2$ (305.334): C, 70.81; H, 4.95; N, 13.76%. Found: C, 70.94; H, 4.91; N, 13.83%.

***N*-(4-Acetamidophenyl)-2-cyano-3-(4-methoxyphenyl)acrylamide (5b):**

Pale Yellow crystal; yield (89%); m.p. 287-288°C (EtOH/DMF mixture). IR: 3311, 3287 (NH), 2224 (C≡N), 1675, 1654 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 3.87 (s, 3H), 7.17 (d, 2H, J = 9 Hz), 7.56 (s, 4H), 8.02 (d, 2H, J = 8.2 Hz), 8.18 (s, 1H), 9.93 (s, 1H), 10.21 (s, 1H). Anal. Calcd. for $C_{19}H_{17}N_3O_3$ (335.36): C, 68.05; H, 5.11; N, 12.53%. Found: C, 67.95; H, 5.01; N, 12.41%.

***N*-(4-Acetamidophenyl)-2-cyano-3-(4-nitrophenyl)acrylamide (5c):**

Red powder; yield (74%); m.p. 248-250°C (EtOH/DMF mixture). IR: 3415, 3358 (NH), 2221 (C≡N), 1676 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 7.39 (d, 2H, J = 9 Hz), 7.54-7.57 (m, 4H), 8.11 (d, 2H, J = 8.2 Hz), 8.33 (s, 1H), 9.92 (s, 1H), 10.30 (s, 1H). Anal. Calcd. for $C_{18}H_{14}N_4O_4$ (350.33): C, 61.71; H, 4.03; N, 15.99%. Found: C, 61.59; H, 4.07; N, 15.91%.

***N*-(4-Acetamidophenyl)-3-(4-chlorophenyl)-2-cyanoacrylamide (5d):**

Yellow powder; yield (80%); m.p. 288-290°C (EtOH). IR: 3326, 3269 (NH), 2220 (C≡N), 1673 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 7.41 (d, 2H, J = 9 Hz), 7.54-7.57 (m, 4H), 8.20 (d, 2H, J = 8.2 Hz), 8.35 (s, 1H), 9.95 (s, 1H), 10.34 (s, 1H). MS (m/z, %): 341 (M^+, 37.9), 339 (M^+, 100.0), 297 (94.2), 190 (10.3), 162 (8.5), 127 (12.6), 107 (94.7), 80 (50.8), 64 (20.3). Anal. Calcd. for $C_{18}H_{14}ClN_3O_2$ (339.78): C, 63.63; H, 4.15; N, 12.37%. Found: C, 63.54; H, 4.21; N, 12.44%.

***N*-(4-Acetamidophenyl)-2-cyano-3-(4-tolyl)acrylamide (5e):**

Yellow crystal, yield (89%); m.p. 257-258°C (EtOH). IR: 3324, 3282 (NH), 2220 (C≡N), 1677, 1656 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 2.40 (s, 3H), 7.40 (d, 2H, J = 9 Hz), 7.57 (s, 4H), 7.90 (d, 2H, J = 9 Hz), 8.20 (s, 1H), 9.91 (s, 1H), 10.26 (s, 1H). Anal. Calcd. for $C_{19}H_{17}N_3O_2$ (319.36): C, 71.46; H, 5.37; N, 13.16%. Found: C, 71.53; H, 5.30; N, 13.07%.

***N*-(4-Acetamidophenyl)-2-cyano-3-(4-dimethylaminophenyl)acrylamide (5f):**

Red powder; yield (84%); m.p. 280-281°C (EtOH). IR: 3373, 3353 (NH), 2200 (C≡N), 1687, 1664 (C=O). ^{1}H NMR (δ/ppm): 2.03 (s, 3H), 3.07 (s, 6H) 6.85 (d, 2H, J = 9 Hz), 7.54 (s, 4H), 7.90 (d, 2H, J = 9 Hz), 8.04 (s, 1H), 9.88 (s, 1H), 9.93 (s, 1H). Anal. Calcd. for $C_{20}H_{20}N_4O_2$ (348.41): C, 68.95; H, 5.79; N, 16.08%. Found: C, 68.82; H, 5.86; N, 16.19%.

4.5 Synthesis of 1-(4-acetamidophenyl)-6-amino-4-aryl-3,5-dicyano-2-oxopyridine derivatives 6a–d

Method A: To a mixture of arylidene derivatives 5a-d (5 mmol) and malononitrile (0.33 g, 5 mmol) in 20 mL of ethanol, five drops of piperidine was added and then heated under reflux for 3 h. On cooling to room temperature, the precipitated solid was filtered and recrystallized from EtOH/DMF mixture (3:1) to obtain the pyridine scaffolds 6a–d.

Method B: Equimolar weights of cyanoacetamide scaffold 3 (1.08 g, 5 mmol) and the appropriate (2-arylidenemalononitrile derivative (5 mmol) was refluxed for 3 h in 20 mL ethanol previously treated with drops of piperidine. The precipitate, which was found after cooling, was filtered and recrystallized from EtOH/DMF mixture (3:1).

1-(4-Acetamidophenyl)-6-amino-3,5-dicyano-2-oxo-4-phenylpyridine (6a):

Pale yellow powder; yield (70%); m.p. > 300°C. IR: 3451, 3373, 3191 (NH_2 and NH), 2211 (C≡N), 1684 (C=O). ^{1}H NMR (δ/ppm): 2.01 (s, 3H), 7.55–7.81 (m, 11H), 10.22 (s, 1H). Anal. Calcd. for $C_{21}H_{15}N_5O_2$ (369.38): C, 68.28; H, 4.09; N, 18.96%. Found: C, 68.16; H, 4.15; N, 18.88%.

1-(4-Acetamidophenyl)-6-amino-3,5-dicyano-4-(4-methoxyphenyl)-2-oxopyridine (6b):

Yellow crystal; yield (86%); m.p. > 300°C. IR: 3446, 3305, 3196 (NH_2 and NH), 2214 (C≡N), 1681, 1658 (C=O). ^{1}H NMR (δ/ppm): 2.02 (s, 3H), 3.86 (s, 3H), 7.11 (d, 2H, J = 9 Hz), 7.26 (d, 2H, J = 9 Hz), 7.51 (d, 2H, J = 9 Hz), 7.78 (d, 4H), 10.16 (s, 1H). Anal. Calcd. for $C_{22}H_{17}N_5O_3$ (399.41): C, 66.16; H, 4.29; N, 17.53%. Found: C, 66.08; H, 4.27; N, 17.47%.

1-(4-Acetamidophenyl)-6-amino-3,5-dicyano-4-(4-nitrophenyl)-2-oxopyridine (6c):

Brown powder; yield (54%); m.p. > 300°C. IR: 3447, 3292, 3174 (NH_2 and NH), 2257 (C≡N), 1666 (C=O). ^{1}H NMR (δ/ppm): 2.01 (s, 3H), 7.36 (d, 2H, J = 8.2 Hz), 7.45–7.62 (m, 6H), 8.10 (d, 2H, J = 9 Hz), 10.28 (s, 1H). Anal. Calcd. for $C_{21}H_{14}N_6O_4$ (414.38): C, 60.87; H, 3.41; N, 20.28%. Found: C, 60.63; H, 3.33; N, 20.16%.

1-(4-Acetamidophenyl)-6-amino-4-(4-chlorophenyl)-3,5-dicyano-2-oxopyridine (6d):

Pale yellow powder; yield (75%); m.p. > 300°C. IR: 3456, 3315, 3186 (NH_2 and NH), 2217 (C≡N), 1682, 1661 (C=O). ^{1}H NMR (δ/ppm): 2.02 (s, 3H), 7.39 (d, 2H, J = 8.2 Hz), 7.46-7.61 (m, 6H), 8.13 (d, 2H, J = 9 Hz), 10.31 (s, 1H). MS (m/z, %): 405 (M^+, 2.8), 403 (M^+, 8.0), 361 (14.4), 108 (7.6), 92 (5.6), 80 (17.4), 64 (100.0). Anal. Calcd. for $C_{21}H_{14}ClN_5O_2$ (403.83): C, 62.46; H, 3.49; N, 17.34%. Found: C, 62.61; H, 3.39; N, 17.22%.

4.6 Synthesis of N-(4-acetamidophenyl)-2-imino-2H-chromene-3-carboxamide (7)

A suspension of equivalent ratio of **3** (1.08 g, 5 mmol) and 2-hydroxybenzaldehyde (0.60 mL, 5 mmol) was heated under reflux for 3 h in ethanol (20 mL) and five drops of piperidine. The resulting product was filtered, dried and recrystallized from EtOH/DMF (4:1) to yield **7**.

Yellow crystals; yield (89%); m.p. 249-250°C. IR: 3292, 3261 (NH), 1683 (C=O). ^{1}H NMR (δ/ppm): 2.08 (s, 3H) 7.21–7.81 (m, 8H), 8.31 (s, 1H), 9.28 (s, 1H), 10.18 (s, 1H), 13.11 (s, 1H). MS (m/z, %): 321 (M^+, 100), 254 (34.0), 211 (27.4), 171 (88.2), 150 (74.2), 143 (65.1), 118 (32.3), 115 (33.3), 108 (87.6), 80 (34.2), 43 (18.6). Anal. Calcd. for $C_{18}H_{15}N_3O_3$ (321.34): C, 67.28; H, 4.71; N, 13.08%. Found: C, 67.11; H, 4.66; N, 13.17%.

4.7 Synthesis of N-(4-acetamidophenyl)-2-cyano-2-(3-phenylthiazolylidene)acetamides 9 and 10

A suspension of scaffold 3 (1.08 g, 5 mmol), phenyl isothiocyanate (0.60 mL, 5 mmol) and solid KOH (0.28 g) was stirred in 20 mL DMF at room temperature for 8 h. Stirring was continued for additional 4 h after *in situ* addition of the appropriate α-halogenated reagents (ethyl chloroacetate, chloroacetone and/or phenacyl chloride) to the reaction mixture. The precipitate, which obtained after dilution with ice-cold water, was filtered and recrystallized.

***N*-(4-Acetamidophenyl)-2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetamide (9)**

Pale Brown powder; yield (72%); m.p. 280-281°C (from EtOH/DMF mixture). IR: 3408, 3302 (NH), 2196 (C≡N), 1743, 1655 (C=O). ^{1}H NMR (δ/ppm): 2.01 (s, 3H), 3.99 (s, 2H), 7.40–7.55 (m, 9H), 9.28 (s, 1H), 9.84 (s, 1H). MS (m/z, %): 392 (M^+, 54.0), 243 (33.2), 215 (100.0), 150 (46.3), 132 (43.2), 108

(34.5), 77 (50.0). Anal. Calcd. for $C_{20}H_{16}N_4O_3S$ (392.43): C, 61.12; H, 4.11; N, 14.28%. Found: C, 61.24; H, 4.15; N, 14.15%.

***N*-(4-acetamidophenyl)-2-cyano-2-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)acetamide (10a):**

Pale Brown powder; yield (78%); m.p. 233-235°C (from EtOH/DMF mixture). IR: 3418, 3301 (NH), 2281 (C≡N), 1662 (C=O). ^{1}H NMR (δ/ppm): 1.84 (s, 3H), 2.01 (s, 3H), 6.90 (s, 1H), 7.30–7.60 (m, 9H), 8.75 (s, 1H), 9.80 (s, 1H). MS (m/z, %): 390 (M^+, 10.0), 217 (63.5), 175 (79.9), 150 (10.6), 134 (34.0), 107 (100.0), 80 (21.7), 77 (10.6). Anal. Calcd. for $C_{21}H_{18}N_4O_2S$ (390.46): C, 64.60; H, 4.65; N, 14.35%. Found: C, 64.41; H, 5.54; N, 14.20%.

***N*-(4-Acetamidophenyl)-2-cyano-2-(3,4-diphenylthiazol-2(3*H*)-ylidene)acetamide (10b):**

Brown powder, yield (70%); m.p. 264-265°C (from EtOH). IR: 3391, 3295 (NH), 2172 (C≡N), 1661 (C=O). ^{1}H NMR (δ/ppm): 2.07 (s, 3H), 7.19–7.57 (m, 14H), 7.88 (s, 1H), 9.81 (s, 1H), 9.86 (s, 1H). Anal. Calcd. for $C_{26}H_{20}N_4O_2S$ (452.53): C, 69.01; H, 4.45; N, 12.38%. Found: C, 68.13; H, 4.37; N, 12.26%.

4.8 Synthesis of N-(4-acetamidophenyl)-2-cyano-3-mercapto-3-(phenylamino)acrylamide (11)

A suspension of scaffold **3** (1.08 g, 5 mmol), phenyl isothiocyanate (0.60 mL, 5 mmol) and solid KOH (0.28 g) was stirred in 20 mL DMF. After stirring for 8 h, the mixture was decanted into ice-cold water and neutralized with dilute acid. The precipitate that obtained was separated by filtration and recrystallized from ethanol.

Orange powder; yield (75%), m.p. 139-140°C. IR: 3457, 3318 (NH), 2216 (C≡N), 1665 (C=O). ^{1}H NMR (δ/ppm): 2.07 (s, 3H), 7.46–7.71 (m, 10H), 9.85 (s, 1H), 10.17 (s, 1H). Anal. Calcd. for $C_{18}H_{16}N_4O_2S$ (352.41): C, 61.35; H, 4.58; N, 15.90%. Found: C, 61.51; H, 4.65; N, 15.81%.

4.9 Synthesis of N-(4-acetamidophenyl)-4-amino-2-(phenylamino)-5-substitutedthiophene-3-carboxamides 12

A suspension of thiocarbamoyl scaffold 11 (0.70 g, 2 mmol) and the appropriate chloroketone (2 mmol) was refluxed for 4 h in EtOH (20 mL) and five drops of Et_3N. The precipitate that formed on cooling to room temperature was separated by filtration and dried to furnish the target thiophene scaffolds.

***N*-(4-Acetamidophenyl)-5-acetyl-4-amino-2-(phenylamino)thiophene-3-carboxamide (12a):**

Brown powder; yield (65%), m.p. 188-190°C. IR: 3343, 3300 (NH_2 and NH), 1657 (C=O). ^{1}H NMR (δ/ppm): 2.01 (s, 3H), 2.32 (s, 3H), 7.04–7.52 (m, 10H), 9.88 (d, 2H), 11.55 (s, 1H), 11.88 (s, 1H). MS (m/z, %): 408 (M^+, 2.29), 392 (26.9), 241 (40.2), 232 (12.3), 217 (48.0), 187 (20.1), 175 (70.8), 150 (50.2), 134 (44.7), 107 (100.0), 80 (9.6), 77 (37.2), 51 (13.9). Anal. Calcd. for $C_{21}H_{20}N_4O_3S$ (408.48): C, 61.75; H, 4.94; N, 13.72%. Found: C, 61.54; H, 4.98; N, 13.61%.

***N*-(4-acetamidophenyl)-4-amino-5-benzoyl-2-(phenylamino)thiophene-3-carboxamide (12b)**

Pale brown powder; yield (80%), m.p. 275-276°C (from ethanol). IR: 3383, 3293, (NH_2 and NH), 1659 (C=O). ^{1}H NMR (δ/ppm): 2.07 (s, 3H), 7.29–7.87 (m, 15H,), 9.91 (d, 2H), 10.87 (s, 1H), 11.44 (s, 1H). MS (m/z, %): 470 (M^+, 3.4), 392 (57.6), 319 (12.0), 294 (18.0), 243 (34.8), 215 (100.0), 150 (50.0), 132 (38.0), 108 (28.5), 77 (57.1), 51 (8.9). Anal. Calcd. for $C_{26}H_{22}N_4O_3S$ (470.55): C, 66.37; H, 4.71; N, 11.91%. Found: C, 66.49; H, 4.77; N, 11.82%.

4.10 Synthesis of N-(4-acetamidophenyl)-2-cyano-3-(methylthio)-3-(phenylamino)-acrylamide (13)

A suspension of scaffold **3** (1.08 g, 5 mmol), phenyl isothiocyanate (0.60 mL, 5 mmol) and solid KOH (0.28 g) was stirred in 20 mL DMF. After stirring for 8 h, methyl iodide (0.35 mL, 5 mmol) was decanted to the reaction suspension and stirring was continued for additional 4 hr. The precipitate, which obtained after dilution with ice-cold water, was picked up filtration and recrystallized from ethanol.

Pale yellow powder, yield (80%), m.p. 207-208°C. IR: 3406, 3303, 3156 (NH), 2198 (C≡N), 1675 (C=O). ^{1}H NMR (δ/ppm): 2.01 (s, 3H), 2.23 (s, 3H), 7.24–7.49 (m, 9H), 9.45 (s, 1H), 9.84 (s, 1H), 11.76

(s, 1H). Anal. Calcd. for $C_{19}H_{18}N_4O_2S$ (366.44): C, 62.28; H, 4.95; N, 15.29%. Found: C, 62.17; H, 4.87; N, 15.12%.

4.11 Synthesis of *N*-(4-Acetamidophenyl)-5-amino-3-(phenylamino)-1*H*-pyrazole-4-carboxamide (14)

A mixture of 13 (0.73 g, 2 mmol) and hydrazine hydrate (0.20 ml, 5 mmol) was refluxed for 4 h in 20 ml EtOH. The precipitate that formed on cooling to room temperature was picked up by filtration and dried to furnish the target pyrazole scaffold.

Pale yellow powder, yield (60%), m.p. 184-185°C (EtOH). IR: 3445, 3261, 3176 (NH_2 and NH), 1651 (C=O). ^{1}H NMR (δ/ppm): 2.00 (s, 3H), 6.05 (s, 2H), 6.25 (s, 2H), 6.78 (t, 1H), 7.18–7.27 (m, 4H), 7.45 (d, 2H), 7.55 (d, 2H, J = 7.8 Hz), 8.59 (s, 1H), 8.78 (s, 1H). Anal. Calcd. for $C_{18}H_{18}N_6O_2$ (350.38): C, 61.70; H, 5.18; N, 23.99%. Found: C, 61.59; H, 5.25; N, 23.86%.

4.12 *In Vitro* Antitumor Activity

Mammary gland breast cancer (MCF-7) cell line was obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

MTT assay [37-39]: The cell lines were employed to reflect the inhibitory effects of compounds on cell growth using the MTT assay. This assay is depended on the change of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple formazan derivative containing mitochondrial succinate dehydrogenase in viable cells [37-39].

5 Conclusion

The present study has been focused on the synthesis and investigation the anticancer activity of new thiazole, pyridinone, thiophene, chromene and pyrazole derivatives containing biologically active acetanilide nucleus. Some of the newly synthesized compounds 4c, 5f, 9, 10a and 10b exhibited significant activity (very strong) compared to the control drug, 5-fluorouracil. Compounds 3, 4a 4b, 6c and 12b are strong cytotoxic drugs. Compounds 5c, 5d, 6a and 12a exhibited a moderate activity and compounds 5b, 6b, 6d, 7 and 13 showed a weak activity, while compound 5a is inactive cytotoxic drug.

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