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SYNTHESIS AND CYTOTOXICITY OF THIOPHENE, THIENO[2,3-C]PYRAZOLE, THIENO[2,3-D]PYRIMIDINE AND PYRIDINE DERIVATIVES DERIVED FROM 2-ACETYLFURAN

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

[1]*National Organization for Research and control of Biologicals, Giza, Egypt*
[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail, Kingdom of Saudi Arabia; National Organization for Drug Control & Research, Giza, Egypt*
[3]*National Organization for Drug Control & Research, Giza, Egypt*

(+ Corresponding author)

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ABSTRACT

This work deals with the synthesis and cytotoxicity of some heterocyclic compounds starting from 2-acetylfuran. The title compound was used for the synthesis of thiophene derivative **3** through its reaction with elemental sulfur and ethyl cyanoacetate. Compound **3** was used for the synthesis of thiophene, thieno[2,3-*c*]pyrazole, thieno[2,3-*d*]pyrimidine and pyridine derivatives through its reaction with different reagents. The newly synthesized compounds were screened through three cancer cell lines. Compound 13b showed the highest potency among the synthesized compounds.

Contribution/ Originality: This study contributes in the existing literature that thiophene derivatives can be produced from simple commercially available starting materials. The reactions of the produced thiophene derivative with different reagents gave both pyrazole and pyridine derivatives. The cytotoxicity of the produced products gave that some compounds with high potency.

1. INTRODUCTION

Thiophene and its derivatives constitute one of the major classes in heterocyclic chemistry. They have been shown to have interesting biological properties such as antiproliferative [1, 2] and anti-inflammatory [3]. Several synthetic routes for polyfunctional fused or pendant pyridine systems have been reported in the literature. They mainly involve intramolecular cyclization [4, 5] multi component intermolecular cyclization [6] metal assisted coupling [7] microwave assisted coupling [8, 9] or cycloaddition [10] azo electronic coupling [11] regioselective hetero Diels-Alder [12] and internal Mannich reaction [13]. They have been used in a wide variety of biological applications [14]. In this work we are demonstrating the synthesis of biologically active thiophene derivatives using 2-acetylfuran as the key starting reagent.

2. EXPERIMENTAL

2.1. General

All melting points were determined on an electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a FTIR plus 460 or Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR & ^{13}C NMR spectra were recorded with Varian Gemini-200 (200 MHz) instrument. Spectra were performed in DMSO-*d6* as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. MS (EI) spectra were recorded with Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Analytical data were obtained from the Micro-analytical Data Unit at Cairo University and were performed on Vario EL III Elemental analyzer.

2.2. Ethyl 2-amino-4-(furan-2-yl) thiophene-3-carboxylate (3)

To a solution of 2-acetylfuran (1.10 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL), ethyl cyanoacetate (1.13 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 h then left to cool. The solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration and crystallized to obtain pale yellow crystals from acetic acid, yield 55 % (1.30 g), m.p. = 105-107°C. Anal. Calculated for $C_{11}H_{11}NO_3S$ (237.27): C, 55.68; H, 4.67; N, 5.90; S, 13.51. Found: C, 55.77; H, 4.89; N, 5.61; S, 13.34. MS: m/e 237 (M^+, 22 %), IR, υ: 3470, 3321 (NH_2), 3058 (CH, aromatic), 1703 (CO), 1631 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz):δ=1.13 (t, 3H, J = 7.12 Hz, CH_3), 4.22 (q, 2H, J = 7.12 Hz, CH_2), 4.82 (s, 2H, D_2O exchangeable, NH_2), 6.04 (s, 1H, thiophene H-5), 7.31-7.38 (m, 3H, furan H). ^{13}C NMR (DMSO) δ : 16.7 (ester CH3), 53.8 (ester CH_2), 120.3, 121.8, 123.9, 128.9, 130.5, 133.9, 136.0, 139.4 (thiophene, furan C), 163.5 (CO).

2.3. Ethyl 2-acetamido-4-(furan-2-yl)thiophene-3-carboxylate (5)

A solution of compound **3** (2.37 g, 0.01 mol) in acetic acid (40 mL) containing acetic anhydride (10 mL) was heated under reflux for 3 h then poured onto ice/water. The formed solid product was collected by filtration and crystallized to obtain pale yellow crystals from 1,4-dioxane, yield 70 % (1.95 g), m.p. = 130-133 °C. Anal. Calculated for $C_{13}H_{13}NO_4S$ (279.31): C, 55.90; H, 4.69; N, 5.01; S, 11.48. Found: C, 55.68; H, 4.63; N, 4.98; S, 11.23. MS: m/e 279 (M^+, 20 %), IR, υ: 3466-3329 (NH), 3055 (CH, aromatic), 1707, 1693 (2CO), 1630 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 1.14 (t, 3H, J = 7.18 Hz, CH_3), 2.62 (s, 3H, CH_3), 4.23 (q, 2H, J = 7.18 Hz, CH_2), 6.04 (s, 1H, thiophene H-5), 7.29-7.37 (m, 3H, furan H), 8.30 (s, 1H, D_2O exchangeable, NH).

2.4. Ethyl 2-(benzylideneamino)-4-(furan-2-yl)thiophene-3-carboxylate (7)

To a solution of compound **3** (2.37 g, 0.01 mol) in 1,4-dioxane (40 mL), benzaldehyde (1.08 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized to obtain yellow crystals from 1,4-dioxane, yield 68 % (2.21g), m.p. = 187-189 °C. Anal. Calculated for $C_{18}H_{15}NO_3S$ (325.38): C, 66.44; H, 4.65; N, 4.30; S, 9.85. Found: C, 66.72; H, 4.80; N, 4.59; S, 10.02. MS: m/e 325 (M^+, 18 %), IR, υ: 3466-3329 (NH), 3056 (CH, aromatic), 1693 (CO), 1632 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 1.14 (t, 3H, J = 6.09 Hz, CH_3), 4.22 (q, 2H, J = 6.09 Hz, CH_2), 6.06 (s, 1H, thiophene H-5), 6.99 (s, 1H, CH=N), 7.30-7.43 (m, 8H, C_6H_5, furan H). ^{13}C NMR (DMSO) δ: 16.9 (ester CH_3), 53.4 (ester CH_2), 120.5, 121.2, 122.3, 124.6, 125.9, 128.4, 129.6, 130.8, 132.9, 134.2, 136.6, 139.1 (C_6H_5, thiophene, furan C), 163.8 (CO), 165.8 (C=N).

2.5. 2-Amino-4-(furan-2-yl)-N-phenylthiophene-3-carboxamide (9)

To a solution of compound **3** (2.37 g, 0.01 mol) in dimethylformamide (30 mL), aniline (0.93 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water containing few drops of

hydrochloric acid. The formed solid product was collected by filtration to obtain yellow crystals from 1,4-dioxane, yield 73 % (2.07 g), m.p. = 166-168 °C. Anal. Calculated for $C_{15}H_{12}N_2O_2S$ (284.33): C, 63.36; H, 4.25; N, 9.85; S, 11.28. Found: C, 63.51; H, 4.39; N, 9.54; S, 11.06. MS: m/e 284 (M^+, 16 %), IR, υ: 3493-3378 (NH_2, NH), 3050 (CH, aromatic), 1689 (CO), 1630 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 4.43 (s, 2H, D_2O exchangeable, NH_2), 6.05 (s,1H thiophene H-5), 7.26-7.38 (m, 8H,C_6H_5, furan H), 8.25 (s, 1H, D_2O exchangeable NH). ^{13}C NMR (DMSO) δ: 119.6, 120.2, 123.5, 124.2, 125.5, 127.6, 129.6, 131.3, 132.9, 134.5, 135.4, 139.3 (C_6H_5, thiophene, furan C), 163.6 (CO).

2.6. 4-(Furan-2-yl)-1H-thieno[2,3-*c*]pyrazol-3-ol (11)

To a solution of compound ***3*** (2.37 g, 0.01 mol) in 1,4-dioxane (40 mL), hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized to obtain orange crystals from ethanol, yield 70 % (1.44 g), m.p. = 225-228 °C. Anal. Calculated for $C_9H_6N_2O_2S$ (206.22): C, 52.42; H, 2.93; N, 13.58; S, 15.55. Found: C, 52.59; H, 3.06; N, 13.69; S, 15.62. MS: m/e 206 (M^+, 29 %), IR, υ: 3536-3440 (OH, NH), 3055 (CH, aromatic), 1631 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 6.03 (s, 1H, thiophene H-5), 7.30-7.37 (m, 3H, furan H), 8.28 (s,1H, D_2O exchangeable, NH),10.16 (s, 1H, D_2O exchangeable, OH).

2.7. General Procedure for the Synthesis of the Thiophene Derivatives (13a-c)

To a cold solution (0-5 °C) of compound ***3*** (2.37g, 0.01 mol) in ethanol (40 mL) containing sodium hydroxide (5 mL, 10 %) a solution of the aryl diazonium chloride [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol, in 10 mL water) to a cold solution of either aniline (0.93 g, 0.01 mol), 4-chloroaniline (1.28 g, 0.01 mol) or 4-methoxyaniline (1.24 g, 0.01 mol) dissolved in concentrated hydrochloric acid (8.0 mL)] was added with continuous stirring. The whole reaction mixture, in each case, was stirred at room temperature for 2h and the formed solid product was collected by filtration and crystallized from the suitable solvent.

2.7.1. Ethyl 2-amino-4-(furan-2-yl)-5-(phenyldiazenyl)thiophene-3-carboxylate (13a)

Orange crystals from ethanol, yield 84 % (2.86 g), m.p. = 189-192 °C. Anal. Calculated for $C_{17}H_{15}N_3O_3S$ (341.38): C, 59.81; H, 4.43; N, 12.31; S, 9.39. Found: C, 59.88; H, 4.64; N, 12.53; S, 9.47. MS: m/e 341 (M^+, 26 %), IR, υ: 3476, 3322 (NH_2), 3054 (CH, aromatic), 1692 (CO), 1630 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 1.13 (t, 3H, J = 6.91 Hz, CH_3), 4.22 (q, 2H, J = 6.91 Hz, CH_2), 4.76 (s, 2H, D_2O exchangeable, NH_2), 7.25-7.41 (m, 8H, C_6H_5, furan H).). ^{13}C NMR (DMSO) δ: 16.8 (ester CH_3), 53.5 (ester CH_2), 120.1, 120.6, 123.8, 124.2, 125.7, 128.8, 129.3, 131.3, 132.6, 133.2, 135.6, 139.4 (C_6H_5, thiophene, furan C), 164.1 (CO).

2.7.2. Ethyl 2-amino-5-((4-chlorophenyl)diazenyl)-4-(furan-2-yl)thiophene-3-carboxylate (13b)

Orange crystals from ethanol, yield 80 % (3.00g), m.p. = 231-233 °C. Anal. Calculated for $C_{17}H_{14}ClN_3O_3S$ (375.83): C, 54.33; H, 3.75; N, 11.18; S, 8.53. Found: C, 54.42; H, 3.80; N, 11.07; S, 8.73. MS: m/e 375 (M^+, 32 %), IR, υ: 3487, 3322 (NH_2), 3056 (CH, aromatic), 1693 (CO), 1631 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 1.15 (t, 3H, J = 7.17 Hz, CH_3), 4.23 (q, 2H, J = 7.17 Hz, CH_2), 4.73 (s, 2H, D_2O exchangeable, NH_2), 7.24-7.42 (m, 7H, C_6H_4, furan H).

2.7.3. Ethyl 2-amino-4-(furan-2-yl)-5-((4-methoxyphenyl)diazenyl)thiophene-3-carboxylate (13c)

Orange crystals from ethanol, yield 70 % (2.60 g), m.p. = 220-225 °C. Anal. Calculated for $C_{18}H_{17}N_3O_4S$ (371.41): C, 58.21; H, 4.61; N, 11.31; S, 8.63. Found: C, 58.44; H, 4.52; N, 11.08; S, 8.73. MS: m/e 371 (M^+, 22 %), IR, υ: 3485, 3429 (NH_2), 3056 (CH, aromatic), 1689 (CO), 1636 (C=C). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ= 1.13

(t, 3H, J = 7.15 Hz, CH_3), 3.68 (s, 3H, CH_3), 4.21 (q, 2H, J = 7.15 Hz, CH_2), 4.68 (s, 2H, D_2O exchangeable, NH_2), 7.29-7.41 (m, 7H, C_6H_4, furan H).

2.8. General Procedure for the Synthesis of the Thiophene Derivatives (15) and (17):

To a cold solution (0-5 °C) of compound ***3*** (2.37g, 0.01 mol) in acetic acid (30 mL) and hydrochloric acid (10 mL) a cold solution of sodium nitrite (0.70 g, 0.01 mol in water (5 mL) was added with continuous stirring. The formed intermediate diazonium salt was added to a cold solution of either ethyl cyanoacetate (1.13 g, 0.01mol) or acetylacetone (1.00 g, 0.01mol) in ethanol (50 mL) containing sodium hydroxide (5 mL, 10 %) with continuous stirring. The whole reaction mixture, in each case, was stirred at room temperature for 2h and the formed solid product was collected by filtration and crystallized from the suitable solvent.

2.8.1. Ethyl 2-(2-(1-cyano-2-ethoxy-2-oxoethylidene)hydrazinyl)-4-(furan-2-yl)thiophene-3-carboxylate (15)

Orange-red crystals from ethanol, yield 77 % (2.78 g), m.p. 231-234 °C. Anal. Calculated for $C_{16}H_{15}N_3O_5S$ (361.37): C, 53.18; H, 4.18; N, 11.63; S, 8.87. Found: C, 53.22; H, 3.92; N, 11.79; S, 8.92. MS: m/e 361 (M^+, 20 %), IR, υ: 3487- 3336 (NH), 3055 (CH, aromatic), 2222 (CN), 1690, 1685 (2CO), 1632 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ = 1.12, 1.15 (2t, 6H, J = 7.01, 7.33 Hz, $2CH_3$), 4.21, 4.27 (2q, 4H, J = 7.01, 7.33 Hz, $2CH_2$), 6.02 (s, 1H, thiophene H-5), 7.29-7.46 (m, 3H, furan H), 8.30 (s, 1H, D_2O exchangeable, NH).

2.8.2. Ethyl 2-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl)-4-(furan-2-yl)thiophene-3-carboxylate (17)

Orange crystals from acetic acid, yield 80 % (2.78 g), m.p. 190-193 °C. Anal. Calculated for $C_{16}H_{16}N_2O_5S$ (348.37): C, 55.16; H, 4.63; N, 8.04; S, 9.20. Found: C, 55.31; H, 4.51; N, 8.26; S, 8.94. MS: m/e 348 (M^+, 28%), IR, υ: 3489- 3338 (NH), 3055 (CH, aromatic), 1693-1686 (3CO), 1636 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.14 (t, 3H, J = 6.53 Hz, CH_3), 2.66, 2.83 (2s, 6H, $2CH_3$), 4.23 (q, 2H, J = 6.53 Hz, CH_2), 6.03 (s, 1H, thiophene H-5), 7.23-7.46 (m, 3H, furan H), 8.26 (s, 1H, D_2O exchangeable, NH).

2.9. Ethyl 4-(furan-2-yl)-2-(3-phenylthioureido)thiophene-3-carboxylate (19)

To a solution of compound ***3*** (2.37, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), phenylisothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized to obtain Orange-red crystals from ethanol, yield 74 % (2.75 g), m.p. = 188-191 °C. Anal. Calculated for $C_{18}H_{16}N_2O_3S_2$ (372.46): C, 58.04; H, 4.33; N, 7.52; S, 17.22. Found: C, 57.92; H, 4.25; N, 7.60; S, 17.39. MS: m/e 372 (M^+, 22 %), IR, υ: 3489- 3328 (2NH), 3055 (CH, aromatic), 1689 (CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.14 (t, 3H, J = 7.08 Hz, CH_3), 4.22 (q, 2H, J = 7.08 Hz, CH_2), 6.04 (s, 1H, thiophene H-5), 7.29-7.37 (m, 8H, C_6H_5, furan H), 8.27, 8.30 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C NMR (DMSO) δ: 16.6 (ester CH_3), 53.3 (ester CH_2), 119.3, 120.8, 123.5, 124.6, 126.9, 128.8, 129.8, 131.2, 132.6, 133.0, 135.3, 139.1 (C_6H_5, thiophene, furan C), 163.8 (CO), 180.3 (C=S).

2.10. General Procedure for the Synthesis of Thienopyrimidine Derivatives (23a,b)

To a solution of compound ***3*** (2.79, 0.01 mol) in acetic acid (40 mL) both of ethyl orthoformate (1.48, 0.01 mol) and either aniline (0.93 g, 0.01 mol) or 4-methylaniline (1.08 g, 0.01 mol) was added. The whole reaction mixture, in each case, was heated under reflux for 6 h then poured onto ice/water. The formed solid product was collected by filtration and crystallized from the suitable solvent.

2.10.1. 5-(Furan-2-yl)-3-phenylthieno[2,3-*d*]pyrimidin-4(3H)-one (23a)

Pale yellow crystals from acetic acid, yield 70 % (2.06 g), m.p. = 166-168 °C. Anal. Calculated for $C_{16}H_{10}N_2O_2S$ (294.33): C, 65.29; H, 3.42; N, 9.52; S, 10.89. Found: C, 65.40; H, 3.28; N, 9.63; S, 10.63. MS: m/e 294 (M^+, 18 %), IR, υ: 3063 (CH, aromatic), 1688 (CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 6.04 (s, 1H, thiophene H-5), 6.91 (s, 1H, pyrimidine CH), 7.25-7.38 (m, 8H, C_6H_5, furan H). ^{13}C NMR (DMSO) δ: 122.3, 124.2, 126.1, 127.4, 127.9, 128.2, 129.1, 131.7, 133.0, 133.2, 135.8, 137.2 (C_6H_5, thiophene, furan C), 164.8 (CO), 172.3 (C=N).

2.10.2. 5-(furan-2-yl)-3-(p-tolyl)thieno[2,3-*d*]pyrimidin-4(3H)-one (23b)

Yellow crystals from 1,4-dioxane, yield 68 % (2.09 g), m.p. = 205-208 °C. Anal. Calculated for $C_{17}H_{12}N_2O_2S$ (308.35): C, 66.22; H, 3.92; N, 9.08; S, 10.40. Found: C, 66.41; H, 3.82; N, 8.89; S, 10.29. MS: m/e 308 (M^+, 22 %), IR, υ: 3056 (CH, aromatic), 1691 (CO), 1631 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 2.87 (s, 3H,CH_3) , 6.03 (s, 1H, thiophene H-5), 6.82 (s, 1H, pyrimidine CH), 7.27-7.44 (m, 7H, C_6H_4, furan H).

2.11. General Procedure for the Synthesis of Thiophene Derivatives (25a, b)

To a solution of compound **3** (2.37 g, 0.01 mol) in dimethylformamide (40 mL) either ethyl cyanoacetate (1.13 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3h then poured onto ice/water. The formed solid product was collected by filtration and crystallized from the suitable solvent.

2.11.1. Ethyl 2-(2-cyanoacetamido)-4-(furan-2-yl)thiophene-3-carboxylate (25a)

Yellow crystals from 1,4-dioxane, yield 77 % (2.34 g), m.p. 210-213 °C. Anal. Calculated for $C_{14}H_{12}N_2O_4S$ (304.32): C, 55.25; H, 3.97; N, 9.21; S, 10.54. Found: C, 55.57; H, 3.84; N, 9.33; S, 10.39. MS: m/e 304 (M^+, 24 %), IR, υ: 3583-3358 (NH), 3056 (CH aromatic), 2220 (CN), 1690, 1684 (2CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.14 (t, 3H, J = 7.03 Hz, CH_3), 3.83 (s, 2H,CH_2), 4.23 (q, 2H, J = 7.03 Hz, CH_2), 6.03 (s, 1H, thiophene H-5), 7.26-7.39 (m, 3H, furan H), 8.30 (s, 1H, D_2O exchangeable,NH).

2.11.2. Ethyl 4-(furan-2-yl)-2-(3-oxobutanamido)thiophene-3-carboxylate (25b)

Yellow crystals from 1,4-dioxane, yield 70 % (2.25 g), m.p. 177-180 °C. Anal. Calculated for $C_{15}H_{15}NO_5S$ (321.35): C, 56.06; H, 4.70; N, 4.36; S, 9.98. Found: C, 56.22; H, 4.58; N, 4.52; S, 10.18. MS: m/e 321 (M^+, 28 %), IR, υ: 3484-3328 (NH), 3058 (CH, aromatic), 1692-1686 (3CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.13 (t, 3H, J = 7.33 Hz, CH_3), 2.87 (s, 3H, CH_3), 4.96 (s, 2H, CH_2), 4.23 (q, 2H, J = 7.33 Hz, CH_2), 6.04 (s, 1H, thiophene H-5), 7.27-7.39 (m, 3H, furan H), 8.32 (s, 1H, D_2O exchangeable, NH).

2.12. Ethyl 2-(2-cyano-2-(2-phenylhydrazono)acetamido)-4-(furan-2-yl)thiophene-3-carboxylate (26)

To a cold solution (0-5 °C) of compound **25a** (3.04 g, 0.01 mol) in ethanol (40 mL) containing sodium hydroxide (5 mL, 10 %), a solution of benzenediazonium chloride [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol, in 10 mL water) to a cold solution of aniline (0.93 g, 0.01 mol), dissolved in concentrated hydrochloric acid (8.0 mL)] was added with continuous stirring. The whole reaction mixture was stirred at room temperature for 2h. The formed solid product was collected by filtration and crystallized to obtain yellow crystals from 1,4-dioxane, yield 60 % (2.45 g), m.p. = 233-236 °C. Anal. Calculated for $C_{20}H_{16}N_4O_4S$ (408.43): C, 58.81; H, 3.95; N, 13.72; S, 7.85. Found: C, 58.64; H, 3.72; N, 13.55; S, 7.94. MS: m/e 408 (M^+, 26 %), IR, υ: 3449-3348 (2NH), 3053 (CH, aromatic), 2220 (CN), 1701, 1685 (2CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.14 (t, 3H, J = 7.30 Hz, CH_3), 4.23 (q, 2H, J = 7.30 Hz, CH_2), 6.03 (s, 1H, thiophene H-5), 7.24-7.42 (m, 8H, C_6H_5, furan H), 8.28, 8.36 (2s, 2H, D_2O exchangeable, 2NH).

2.13. Ethyl 2-(2-cyano-3-phenylacrylamido)-4-(furan-2-yl)thiophene-3-carboxylate (27)

To a solution of compound **25a** (3.04 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL), benzaldehyde (1.08 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized to obtain yellow crystals from ethanol, yield 78 % (3.06 g), m.p. = 244-247 °C. Anal. Calculated for $C_{21}H_{16}N_2O_4S$ (392.43): C, 64.27; H, 4.11; N, 7.14; S, 8.17. Found: C, 64.52; H, 4.06; N, 7.30; S, 8.25. MS: m/e 392 (M^+, 22 %), IR, υ: 3480-3332 (NH), 3056 (CH, aromatic), 2987, 2890 (CH_3, CH_2), 2220 (CN), 1710, 1685 (2CO), 1632 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.12 (t, 3H, J = 7.08 Hz, CH_3), 4.24 (q, 2H, J = 7.08 Hz, CH_2), 6.04 (s, 1H, thiophene H-5), 7.03 (s, 1H, CH=C), 7.24-7.39 (m, 8H, C_6H_5, furan H), 8.33 (s, 1H, D_2O exchangeable, NH).

2.14. Ethyl 4-(furan-2-yl)-2-(2-oxo-2H-chromene-3-carboxamido)thiophene-3-carboxylate (29)

To a solution of compound **25a** (3.04g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL), salicylaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized to obtain Yellow crystals from ethanol, yield 78 % (3.19 g), m.p. = 168-171 °C. Anal. Calculated for $C_{21}H_{15}NO_6S$ (409.41): C, 61.61; H, 3.69; N, 3.42; S, 7.83. Found: C, 61.83; H, 3.80; N, 3.51; S, 7.92. MS: m/e 409 (M^+, 23 %), IR, υ: 3480-3348 (NH), 3054 (CH, aromatic), 2988, 2890 (CH_3, CH_2), 1706-1685 (3 CO), 1634 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz); δ= 1.14 (t, 3H, J = 7.16 Hz, CH_3), 4.23 (q, 2H, J = 7.16 Hz, CH_2), 6.02 (s, 1H, thiophene H-5), 6.39 (s, 1H, coumarin H-4), 7.25-7.46 (m, 7H, C_6H_4, furan H), 8.30 (s, 1H, D_2O exchangeable, NH).

2.15. General Procedure for the Synthesis of Thiophene Derivatives (31a,b)

To a solution of compound **25a** (3.04 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), either ethyl cyanoacetate (1.13 g, 0.01 mol) or malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 4 h and the solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration and crystallized from the suitable solvent.

2.15.1. Ethyl2-(4-amino-3-cyano-6-hydroxy-2-oxopyridin-1(2H)-yl)-4-(furan-2-yl)thiophene-3 carboxylate (31a)

Yellow crystals from ethanol, yield 62 % (2.30 g), m.p. 155-158 °C. Anal. Calculated for $C_{17}H_{13}N_3O_5S$ (371.37): C, 54.98; H, 3.53; N, 11.31; S, 8.63. Found: C, 54.79; H, 3.62; N, 11.49; S, 8.80. MS: m/e 371 (M^+, 26 %), IR, υ: 3490-3370 (OH, NH_2), 3055 (CH, aromatic), 2982, 2893 (CH_3, CH_2), 2220 (CN), 1706, 1686 (2CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.13 (t, 3H, J = 6.05 Hz, CH_3), 4.23 (q, 2H, J = 6.05 Hz, CH_2), 4.82 (s, 2H, D_2O exchangeable, NH_2), 6.05 (s, 1H, thiophene H-5), 7.03 (s, 1H, pyridine H-5), 7.22-7.43 (m, 3H, furan H), 10.23 (s, 1H, D_2O exchangeable, OH).

2.15.2. Ethyl 2-(4,6-diamino-3-cyano-2-oxopyridin-1(2H)-yl)-4-(furan-2-yl)thiophene-3-carboxylate (31b)

Yellow crystals from ethanol, yield 59 % (2.18 g), m.p. = 130 °C. Anal. Calculated for $C_{17}H_{14}N_4O_4S$ (370.38): C, 55.13; H, 3.81; N, 15.13; S, 8.66. Found: C, 55.20; H, 3.58; N, 15.02; S, 8.72. MS: m/e 370 (M^+, 22 %), IR, υ: 3480-3338 ($2NH_2$), 3054 (CH, aromatic), 2989, 2883 (CH_3, CH_2), 2220 (CN), 1711, 1684 (2CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.13 (t, 3H, J = 7.31 Hz, CH_3), 4.24 (q, 2H, J = 7.31 Hz, CH_2), 4.48, 4.71 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.05 (s, 1H, thiophene H-5), 7.03 (s, 1H, pyridine H-5), 7.24-7.42 (m, 3H, furan H).

2.16. General Procedure for the Synthesis of the Thiophene Derivatives (32a, b)

To a solution of compound **25a** (3.04, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL), either of ethyl cyanoacetate (1.13g, 0.01 mol) or malononitrile (0.66 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 1 h then left to cool. The solid product formed upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration and crystallized from the suitable solvent.

2.16.1. Ethyl 2,4-diamino-5-((3-(ethoxycarbonyl)-4-(furan-2-yl)thiophen-2-yl)carbamoyl)thiophene-3-carboxylate (32a)

Yellow crystals from ethanol, yield 73 % (3.28 g), m.p. 220-223°C. Anal. Calculated for $C_{19}H_{19}N_3O_6S_2$ (449.50): C, 50.77; H, 4.26; N, 9.35; S, 14.27. Found: C, 50.82; H, 4.08; N, 9.44; S, 14.58. MS: m/e 449 (M^+, 38 %), IR, υ: 3489-3358 ($2NH_2$, NH), 3056 (CH, aromatic), 2993, 2887 (CH_3, CH_2), 1701-1685 (3CO), 1630 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.12, 1.14 (2t, 6H, J = 6.83, 7.29 Hz, $2CH_3$), 4.21, 4.26 (2q, 4H, J = 6.83, 7.29 Hz, $2CH_2$) 4.41, 4.59 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.04 (s, 1H, thiophene H-5), 7.23-7.39 (m, 3H, furan H), 8.23 (s, 1H, D_2O exchangeable, NH).

2.16.2. Ethyl 2-(3,5-diamino-4-cyanothiophene-2-carboxamido)-4-(furan-2-yl)thiophene-3-carboxylate (32b)

Yellow crystals from ethanol, yield 77 % (3.10 g), m.p. 190-192°C. Anal. Calculated for $C_{17}H_{14}N_4O_4S_2$ (402.45): C, 50.74; H, 3.51; N, 13.92; S, 15.93. Found: C, 50.92; H, 3.48; N, 14.09; S, 16.28. MS: m/e 402 (M^+, 22 %), IR, υ: 3493-3378 ($2NH_2$, NH), 3055 (CH, aromatic), 2983, 2890 (CH_3, CH_2), 2221 (CN), 1716, 1686 (2CO), 1633 (C=C). ^{1}H-NMR, (DMSO-d_6, 200 MHz): δ= 1.13 (t, 3H, J = 7.26 Hz, CH_3), 4.23 (q, 2H, J = 7.26 Hz, CH_2), 4.40, 4.58 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.03 (s, 1H, thiophene H-5), 7.28-7.39 (m, 3H, furan H), 8.32 (s, 1H, D_2O exchangeable, NH).

2.17. Antitumor Evaluations

2.17.1. Antitumor and Normal Cell Line Activity Tests

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

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), NCI-H460, SF-268 and normal fibroblast cells (WI 38) 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% CO2. Exponentially growing cells were obtained by plating 1.5x105cells/mL for MCF-7 and SF-268 and 0.75x104cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

2.17.2. Tumor Cell Growth Assay

The effects of compounds on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth, cells growing in 96-well plates were then

exposed for 48 hr to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Wincoski, USA). For each tested compound and cell line, a dose–response curve was obtained and the inhibitory concentration of 50% (IC50), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

2.17.3. Structure Activity Relationship

It is clear from Table 1 that some of the newly synthesized compounds showed high cytotoxicity towards the three cancer cell lines. The thiophene derivative **3** showed low potency. However, the acetyl derivative **5** showed high potency especially against MCF-7 and NCI-H460 cell lines. On the other hand, the Schiff's base **7** showed low potency but the anilide derivative **9** showed high cytotoxicity and this was attributed to the presence of the CONHPh moiety. Reaction of compound **3** with hydrazine hydrate gave the pyrazole derivative **11** which showed a moderate potency. But upon the reaction of compound **3** with any of the aromatic diazonium salts **12a-c** gave the arylazo derivatives **13a-c**. It is obvious from Table 1 that compound **13b** with the 4-chlorophenyl moiety showed high cytotoxicity. The reaction of the diazonium salt **14** with ethyl cyanoacetate gave the azo derivative **15** which showed a high potency towards the three cancer cell lines. Compounds **17**, **19**, **23a**, **23b** and **25a** showed low potency. It is of great value to notice that the thiophene derivative **25b** with the COOEt moiety showed high potency towards the three cancer cell lines. Compounds **26**, **27**, **31b** and **32a** showed low potency while compounds **29**, **31a** and **32b** showed moderate potency.

Table-1. Effect of compounds on the growth of the three human tumour cells

Compound	IC_{50} (μg L^{-1})			
	MCF-7	**NCI-H460**	**SF-268**	**WI 38**
3	64.29 ± 5.26	58.36 ± 822	48.40 ± 7.29	na
5	0.32 ± 0.06	0.86 ± 0.02	1.38 ± 0.69	68.11 ± 8.66
7	38.20 ± 1.36	32.69 ± 1.84	33.69 ± 3.50	28.81 ± 3.63
9	0.36 ± 0.20	0.83 ± 0.19	1.20 ± 0.66	70.45 ± 0.90
11	9.23 ± 1.69	12.61 ± 2.73	14.06 ± 2.69	na
13a	62.28 ± 6.53	60.51 ± 8.59	52.59 ± 6.80	na
13b	0.01 ± 0.008	0.02 ± 0.009	0.01 ± 0.003	na
13c	2.57 ± 1.26	13.50± 1.73	20.26 ± 5.73	na
15	0.06 ± 0.002	0.05 ± 0.008	1.53 ± 0.59	na
17	28.22 ± 2.73	38.8± 4.77	20.53 ± 4.62	na
19	30.27 ± 5.80	32.58 ± 11.41	29.36 ± 4.28	na
23a	16.84 ± 2.63	21.49 ± 3.69	30.37 ± 2.43	na
23b	38.22 ± 4. 18	39.03 ± 8.01	22.59 ± 4.01	na
25a	18.12 ± 2.36	12.74 ± 2.41	6.64 ± 1.34	77.32 ± 6.89
25b	3.80 ± 0.42	0.67 ± 0.31	1.39 ± 0.28	na
26	20.47 ± 2.64	26.8 ± 4.72	18.3 ± 2.63	na
27	33.61 ± 8.15	40.32 ± 12.43	30.40 ± 2.83	na
29	1.63 ± 0.88	0.68 ± 0.20	6.09 ± 1.87	na
31a	3.85 ± 1.39	18.62 ± 1.59	22.57 ± 2.51	na
31b	18.42 ± 3.52	18.42 ± 3.52	22.95 ± 0.46	72.77 ± 8.16
32a	12.48 ± 2.59	10.73 ± 3.61	18.09 ± 4.66	na
32b	6.08 ± 1.32	8.26 ± 2.44	8.39 ± 1.59	na
Doxorubicin	0.04 ± 0.008	0.09±0.008	0.09±0.007	> 100

Source: National Cancer Institute, Cairo, A. R. Egypt

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 hr and show means ± SEM of three-independent experiments performed in duplicate.

3. RESULTS AND DISCUSSION

In the present work we started with 2-acetylfuran (**1**) as the key starting material. Thus, the reaction of 2-acetylfuran (**1**) with elemental sulfur and ethyl cyanoacetate (**2**) gave the thiophene derivative **3**. The structure of compound **3** was based on analytical and spectral data. Thus, the ^{1}H-NMR spectrum showed the presence of a triplet at δ1.13 ppm for the ester CH_3 group, a quartet at δ 4.22 ppm for the ester CH_2 group, a singlet (D_2O exchangeable) at δ 4.82 ppm for the NH_2 group, a singlet at δ 6.04 ppm for the thiophene H-5 and a multiplet at δ 7.31-7.38 for the furan protons. Further confirmation for the structure of compound **3** was obtained through studying its reactivity towards some chemical reagents.

Thus, the reaction of compound **3** with acetic anhydride (**4**) and acetic acid gave the 2-acetamidothiophene derivative **5**. Moreover, the reaction of compound **3** with benzaldehyde (**6**) gave the Schiff's base **7**. The analytical and spectral data of compounds **5** and **7** were based on their respective analytical and spectral data (see experimental section). In addition, the reaction of compound **3** with aniline (**8**) gave the anilide derivative **9**, (Scheme1).

The reaction of compound **3** with hydrazine hydrate (**10**) gave the thieno[2,3-*c*]pyrazole derivative **11**. The latter product was formed through the first formation of the hydrazide derivative followed by ammonia elimination. The structure of compound **11** was based on the analytical and spectral data. Thus, the ^{1}H-NMR spectrum showed the presence of a singlet a δ 6.03 ppm indicating the presence of the thiophene H-5, a multiplet at δ 7.30-7.37 ppm for the furan protons and two singlets (D_2O exchangeable) at δ 8.28, 10.16 ppm for the NH and OH groups, respectively.

The C-5 of compound **3** reacted with any of benzenediazonium chloride (**12a**), 4-chlorobenzenediazonium chloride (**12b**) or 4-methoxybenzenediazonium chloride (**12c**) in ethanol containing sodium hydroxide solution to give the corresponding arylazo derivatives **13a-c**, respectively (Scheme 2).

On the other hand, the 2-amino group present in compound **3** is capable for diazotization. Thus, the cold solution (0-5 ºC) of compound **3** in acetic/hydrochloric acid reacted with sodium nitrite solution to give the intermediate 2-diazonium salt **14**. The latter coupled with either ethyl cyanoacetate (**2**) or acetylacetone (**16**) to afford the hydrazo derivatives **15** and **17**, respectively. On the other hand, the 2-amino group in compound **3** reacted with phenylisothiocyanate (**18**) to give the 2-N-phenylthiourea derivative **19** (Scheme 3).

Next we moved towards studying the reactivity of compound **3** towards the reaction with ethyl orthoformate followed by condensation reactions. Thus, compound **3** reacted with ethyl orthoformate (**20**) and either aniline (**21a**) or p-toluidine (**21b**) in acetic acid solution to give the thieno[2,3-*d*]pyridine derivatives **23a** and **23b**, respectively. The reaction took place through the intermediate formation of the 2-alkylatedaminothiophene derivative **22a, b**. The structure of compounds **23a** and **23b** was based on the analytical and spectral data. The reaction of compound **3** with either ethyl cyanoacetate (**2**) or ethyl acetoacetate (**24**) gave the 2-amido derivatives **25a** and **25b**, respectively. The analytical and spectral data of compounds **25a, b** was consistent with their respective structures (see experimental section). Compound **25a** reacted with benzenediazonium chloride in ethanolic sodium hydroxide solution to give the phenylhydrazo derivative **26** (Scheme 4).

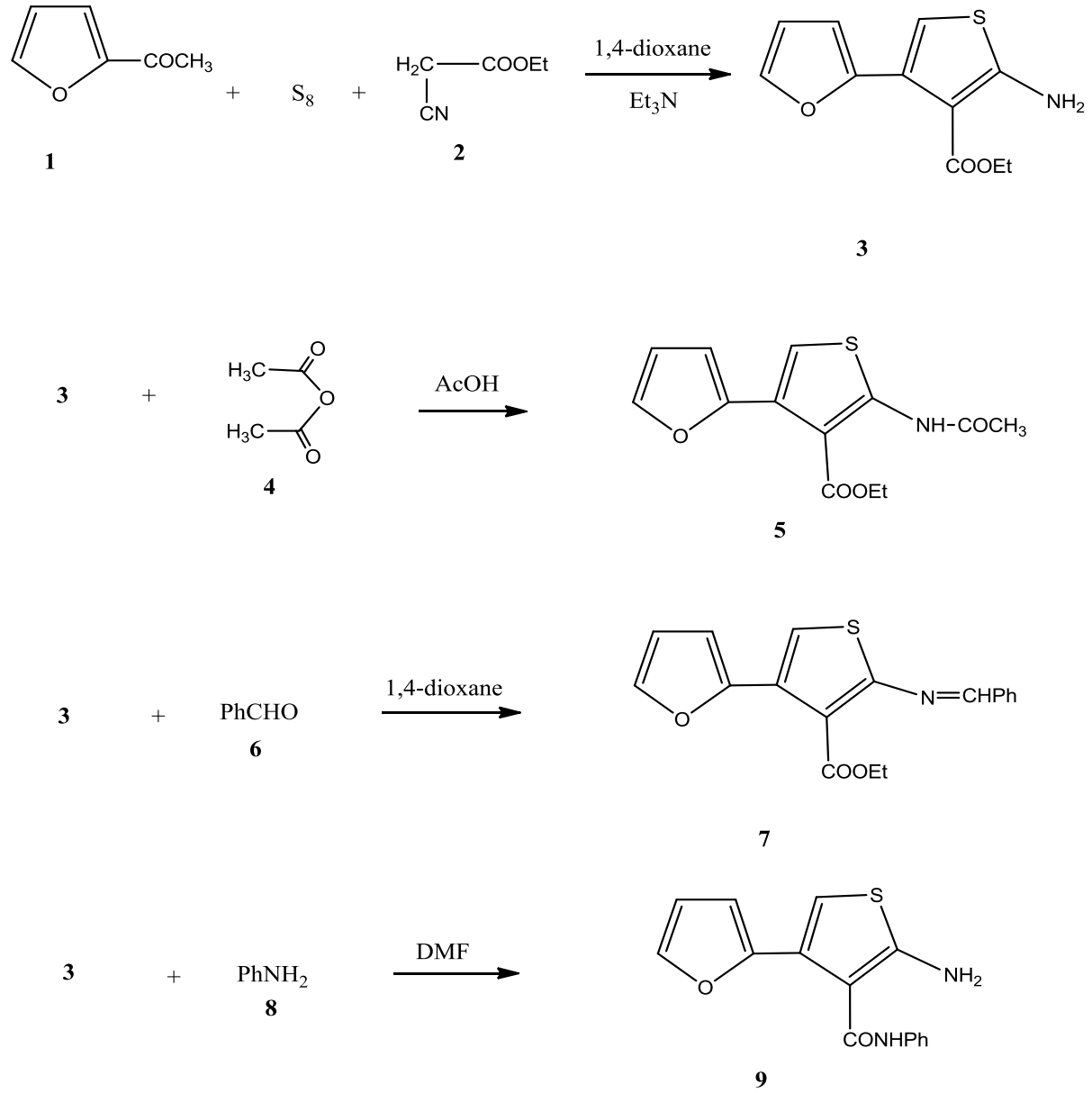
Furthermore, compound **25a** reacted with benzaldehyde (**6**) in piperidine solution to give the benzylidene derivative **27**. On the other hand, compound **25a** reacted with salicylaldehyde (**28**) to give the coumarin derivative **29**.

Our research program was directed towards the uses of compound **25a** towards the synthesis of pyridine derivatives with potential biological activities [15]. Thus, the reaction of compound **25a** with either ethyl cyanoacetate (**2**) or malononitrile (**30**) gave the pyridine derivatives **31a** and **31b**, respectively. Compound **25a**

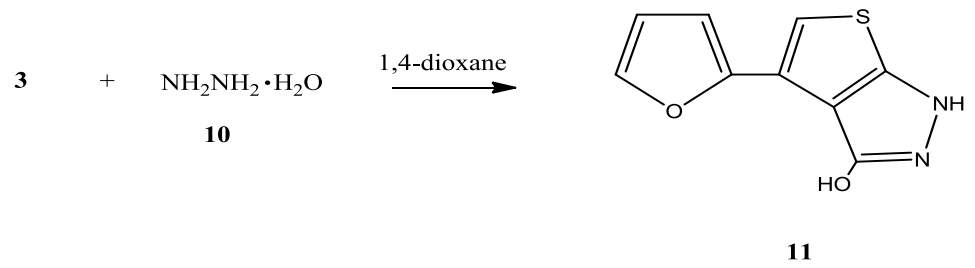
reacted with elemental sulfur and either of ethyl cyanoacetate (**2**) or malononitrile (**30**) to give the thiophene derivatives ***32a*** and ***32b***, respectively (scheme 5). The analytical and spectral data of the latter products are in agreement with their respective structures. Thus, the ^{1}H-NMR spectrum of compound ***32a*** (as an example) showed The presence of two triplets at δ 1.12, 1.14 ppm equivalent to the two ester CH_3 groups, two quartets at δ 4.21, 4.26 ppm for the two ester CH_2 groups, two singlets (D_2O exchangeable) at δ 4.41, 4.59 ppm indicating the two NH_2 groups, a singlet at δ 6.04 ppm equivalent to the thiophene H-5 proton, a multiplet at δ7.23-7.39 ppm for the furan protons and a singlet (D_2O exchangeable) at δ 8.23 ppm for the NH group.

4. CONCLUSION

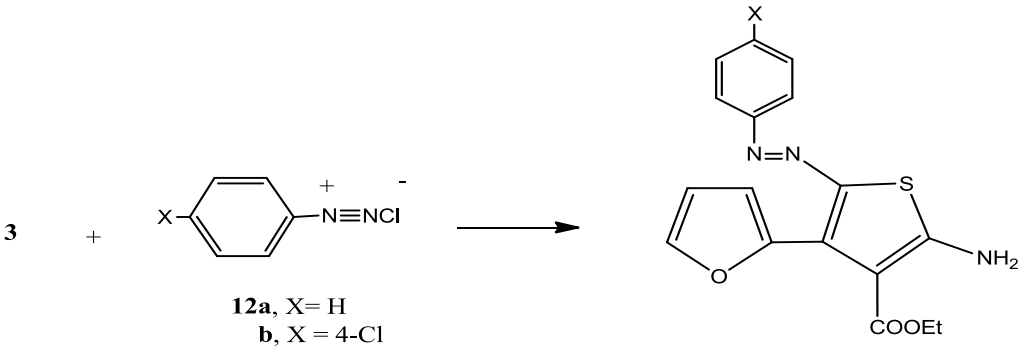
The work described here concerned with the synthesis of thiophene derivatives starting from 2-acetylfuran followed by studying of the chemical transformations through the thiophene ring system. The screening of the resulting compounds towards three cancer cell lines showed that compound **13b** gave the maximum inhibitory effect among the synthesized products.



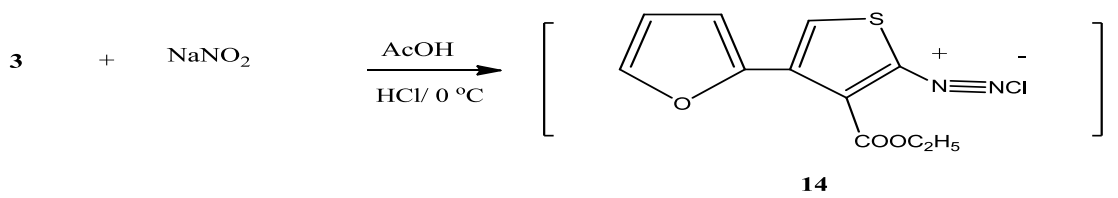
Scheme-1. Chemical Structure and Synthesis of compounds **3**,**5**,**7** and **9**

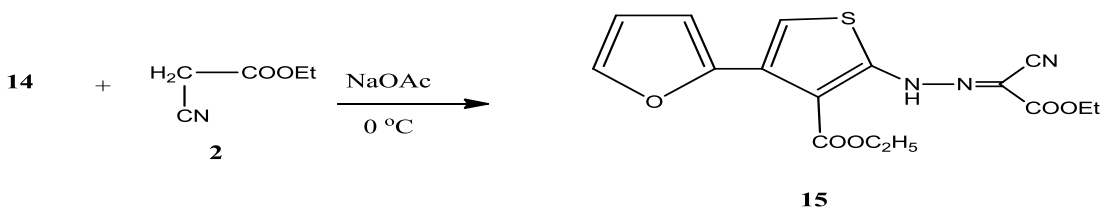


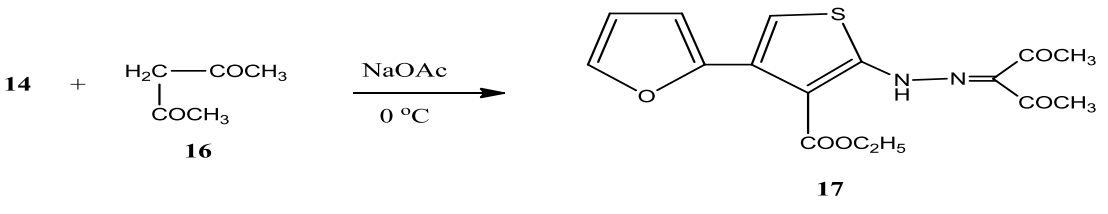
11

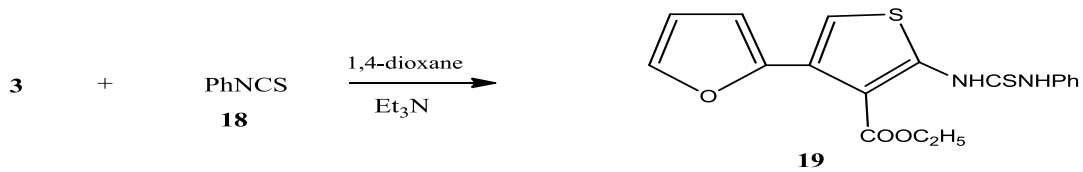


Scheme-2. Chemical Structure and Synthesis of compounds **11** and **13a-c**

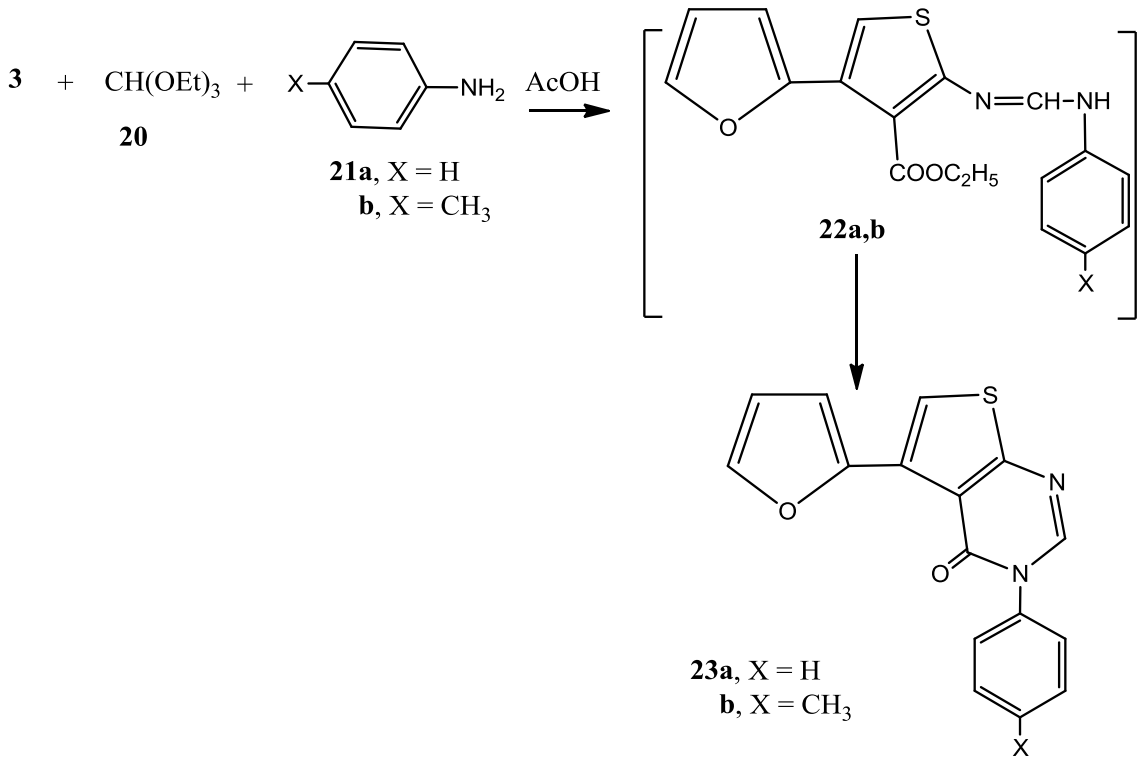


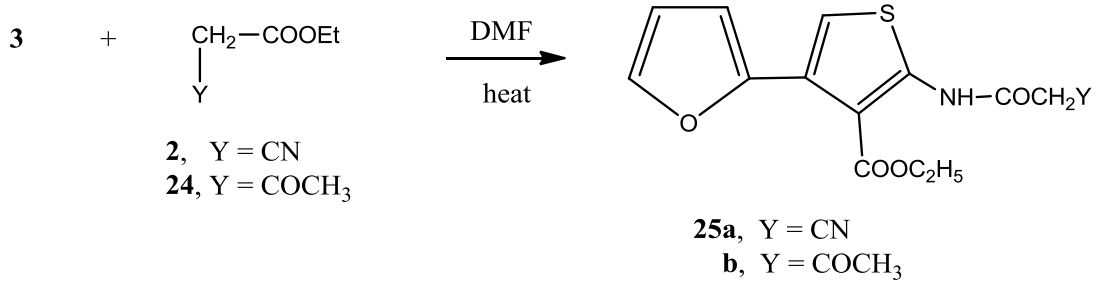


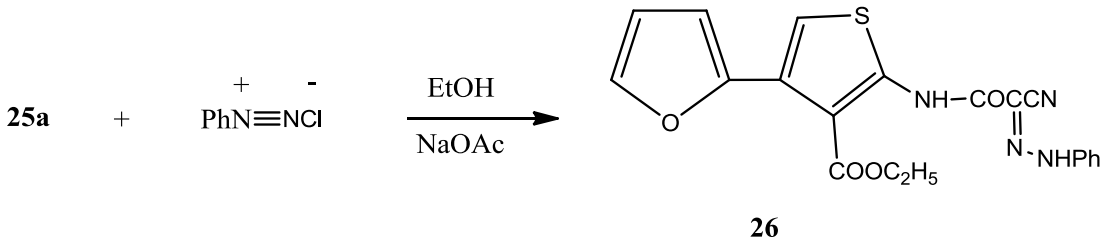




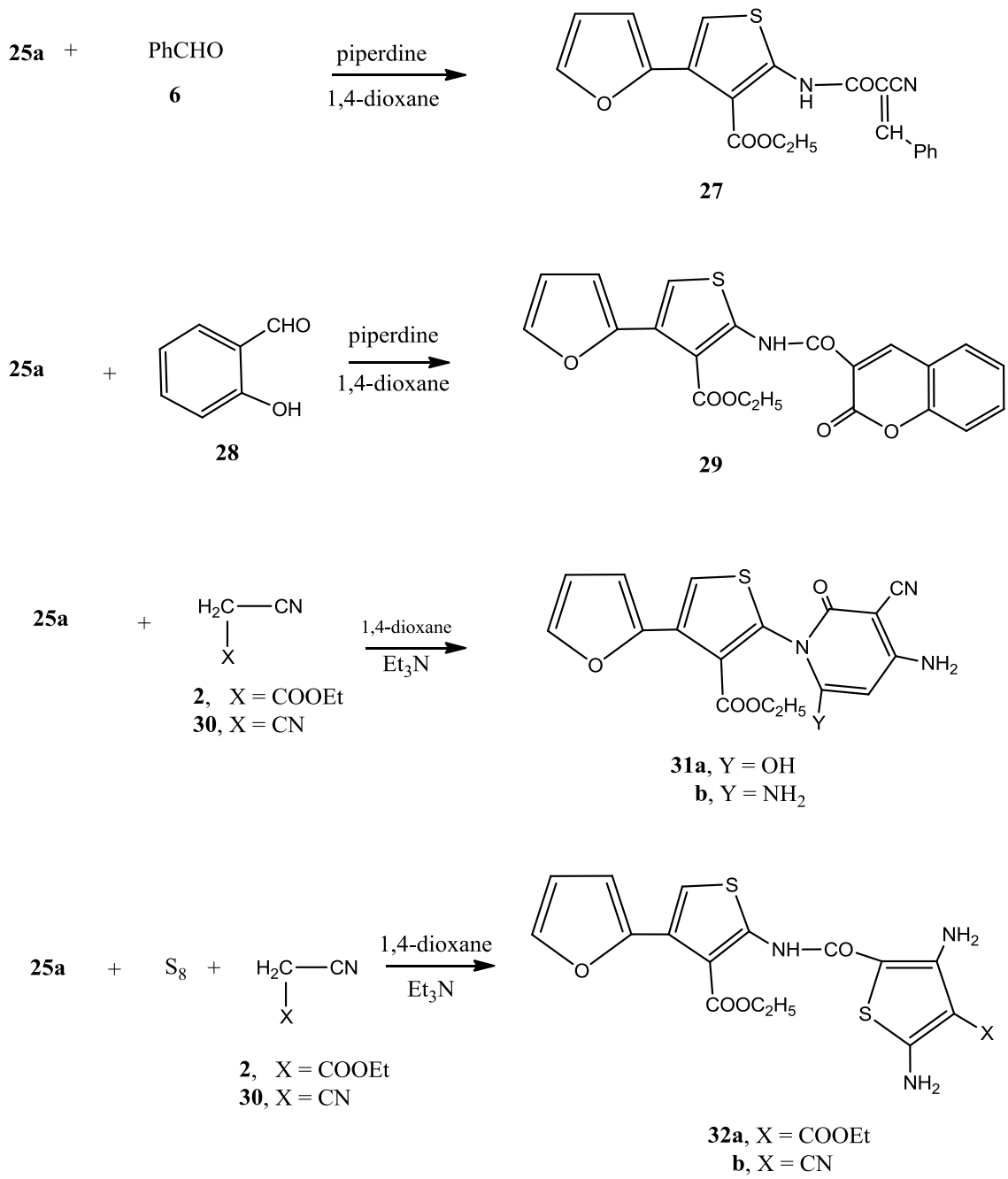
Scheme-3. Chemical Structure and Synthesis of **15**, **17** and **19**







Scheme-4. Chemical Structure and Synthesis of compounds **23a,b 25a,b** and **26**



Scheme-5. Chemical Structure and Synthesis of compounds**27**, **29**, **31a,b** and **32a,b**

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Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

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