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USES OF ACETOACETANILIDE FOR THE SYNTHESIS OF THIOPHENE DERIVATIVES WITH CYTOTOXIC ACTIVITIES

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ABSTRACT. The reaction of acetoacetanilide **1** with elemental sulfur and malononitrile **2** gave the thiophene derivative **3**. The latter was the key starting material for the synthesis of different thiophene and fused derivatives through its reaction with aryldiazonium chloride, benzaldehyde and acetic anhydride. Moreover, the reaction with phenylisothiocyanate produced fused derivative. The reaction of compound **3** with elemental sulfur produced dithiophene derivative. In addition, the reaction of compound **3** with ethylcyanoacetate produced N-cyanoacetamide derivative that was capable for further heterocyclizations through its reaction with different reagents. The cytotoxicity of the newly synthesized products was evaluated against the three cancer cell lines namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) where some compounds showed optimal cytotoxicity. The results showed that compounds **5a**, **5b**, **8**, **22**, and **23** exhibited optimal cytotoxic effect against cancer cell lines.

KEY WORDS: Thiophene, Thieno[2,3-*d*]pyrimidine, Coumarin, Cytotoxicity

INTRODUCTION

Thiophene derivatives represent a class of important and well-studied heterocycles [1]. The interest in this kind of heterocycles has spread from early dye chemistry [2] to modern drug design [3], biodiagnostics [4], electronic and optoelectronic devices [5], conductivity-based sensors [6], and self-assembled superstructures [7]. The general synthetic approaches to such kind of compounds either involve the functionalization at the positions a and b to the sulfur atom of the pre-constructed thiophene nucleus [8], or the construction of thiophene ring from appropriately substituted open chain precursors [9]. The latter becomes much attractive for its general applicability to achieve more complicated substitution patterns [10]. Gewald and co-workers developed the synthesis of 2-aminothiophenes from the multicomponent condensation of ketones or aldehydes, cyanoacetate and elemental sulfur [11]. Later on, there are several reviews and papers reported on the variations and improvements on the originally published Gewald's synthesis of polysubstituted thiophenes [12]. Recently, we were involved through comprehensive program involving the synthesis of thiophene [13, 14] derivatives together with their further reactions with chemical reagents to give heterocyclic and fused heterocyclic derivatives with antitumor activities. Moreover, we synthesized different thiophene derivatives that have been screened for antitumor activity against breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268); this is besides studying their cytotoxicity against the normal human cell line normal fibroblast cells WI 38 [15]. In this work we make extension for this program through the synthesis of thiophene derivatives using acetoacetanilide and maononitrile in the presence of elemental sulfur to produce potentially antitumor thiophene derivatives [16-23].

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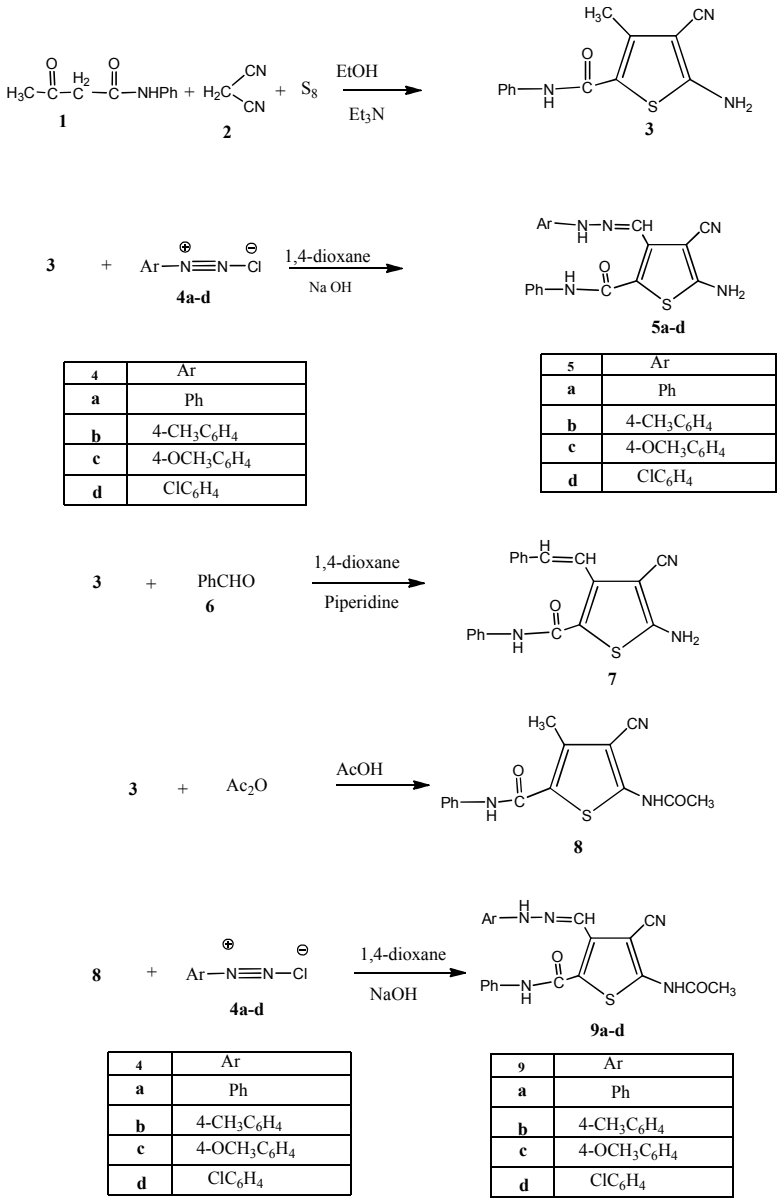
RESULTS AND DISCUSSION

In the present work, acetaacetanilide **1** reacted with elemental sulfur and malononitrile **2** in the presence of triethylamine to give the thiophene derivative **3**. The structure of compound **3** was based on analytical and spectral data. Thus, the ^{1}H NMR spectrum showed one singlet at δ 2.5 ppm corresponding to the presence of CH_3 group, one singlet at δ 3.33 ppm corresponding to the NH_2 group, two multiplets at δ 7.00-7.71 ppm corresponding to C_6H_5 group and a singlet at δ 9.54 ppm for the NH group. Moreover, the ^{13}C NMR data revealed the presence of signals at 16.9 (CH_3), 116.3 (CN), 119.0, 120.2, 123.8, 125.5, 134.2, 139.6, 143.2, 144.1 (C_6H_5, thiophene C), 163.9 (CO).

The 4-methyl group present in compound **3** showed interesting reactivity due to the effect of the electron withdrawal effect of the *o*-cyano group. Thus, compound **3** coupled with the aryldiazonium salts **4a-d** to give the aryl hydrazone derivatives **5a-d**, respectively. The analytical and spectral data of **5a-d** were the basis of their structure elucidation. On the other hand compound **3** reacted with benzaldehyde **6** to give the benzal derivative **7**. Compound **3** reacted with acetic acid/acetic anhydride (3:1) to give the 2-N-acetyl derivative **8**. The analytical and spectral data of the latter product were in agreement with its structure. Thus, ^{1}H NMR spectrum of **8** showed two singlet at δ 2.50 and 2.51 ppm corresponding to the presence of two CH_3 groups, one multiplets at δ 7.05-7.66 ppm corresponding to the C_6H_5 group and two singlets at δ 10.00, 11.96 ppm corresponding to the presence of two NH group. Moreover, ^{13}C NMR showed the presence of signals at 19.6, 20.3 ($2CH_3$), (C=C), 116.3 (CN), 123.2, 124.8, 125.3, 126.9, 128.9, 130.2, 132.7, 134.6, 142.2, 143.4 (C_6H_5, thiophene C), 164.3, 165.8 (2CO).

Compound **8** coupled with the aromatic diazonium salts **4a-d** to give the arylhydrazone derivatives **9a-d** (Scheme 1). Encouraged by the excellent results, we next investigated the reactivity of compound **3** towards phenylisothiocyanate **10**, where the reaction afforded the theino[*d*]pyrimidine derivative **12**. The reaction took place through the intermediate formation of the N-phenylthiourea derivative **11**. The structure of compound **12** was confirmed on the basis of analytical and spectral data. Thus, the ^{1}H NMR spectrum showed a singlet at δ 2.51 ppm for the CH_3 group, a multiplet at δ 7.30-7.66 ppm corresponding to the two C_6H_5 groups, a singlet at δ 7.71 ppm for SH group and two singlets at δ 9.54, 11.08 ppm corresponding to the presence of two NH group. Compound **3** reacted with elemental sulfur in the presence of triethylamine to give the thieno[*c*]thiophene derivative **14**. The reaction took place through the first reaction of the CH_3 group with elemental sulfur to give the thiol derivative **13** followed by the addition of the SH group to the CN group to give **14** (Scheme 2). On the other hand, the reaction of compound **3** with malononitrile (**15**) in 1,4-dioxane containing a catalytic amount of triethylamine gave the thieno[*b*]pyridine derivative **17**. Formation of the latter product took place through the intermediate formation of the acyclic intermediate **16**. The 2-amino group present in **3** showed interesting reactivity towards diazodization and coupling. Thus, compound **3** reacted with a cold solution of sodium nitrite in the presence of acetic acid/hydrochloric acid (3:1) to give the intermediate diazonium salt **18**. The latter reacted with acetyl acetone **19** to give the hydrazone derivative **20** (Scheme 3). The analytical and spectral data of the latter product were the basis of its structure elucidation. Thus the ^{1}H NMR spectrum showed three singlets at δ 2.32, 2.45, 2.62 ppm corresponding to the presence of three CH_3 groups, a multiplet at δ 7.27-7.62 ppm corresponding to the C_6H_5 group and two singlets at δ 7.72, 9.54 ppm for the two NH groups. In addition, the ^{13}C NMR spectrum showed the presence of signals at 19.8, 20.6, 21.3 (3 CH_3), 117.0 (CN), 120.4, 121.7, 122.9, 124.7, 125.9, 128.2, 130.1, 138.0 (C_6H_5, thiophene C), 163.2, 164.0, 165.2 (3CO), 173.2 (C=N).



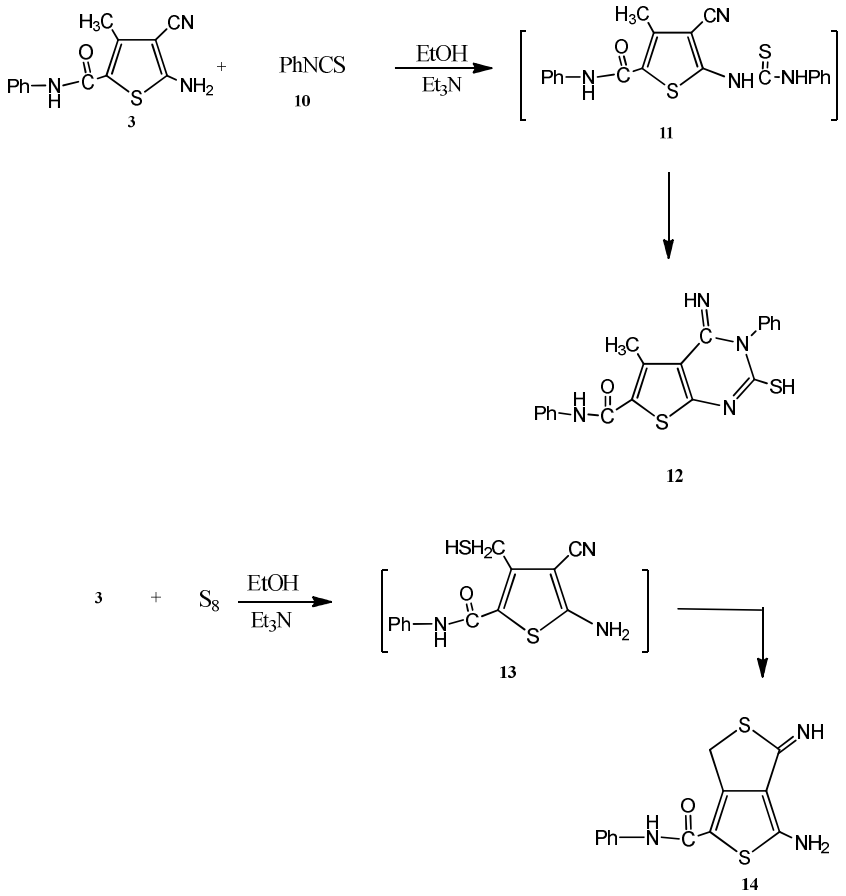
4	Ar
a	Ph
b	4-$CH_3C_6H_4$
c	4-$OCH_3C_6H_4$
d	ClC_6H_4

5	Ar
a	Ph
b	4-$CH_3C_6H_4$
c	4-$OCH_3C_6H_4$
d	ClC_6H_4

4	Ar
a	Ph
b	4-$CH_3C_6H_4$
c	4-$OCH_3C_6H_4$
d	ClC_6H_4

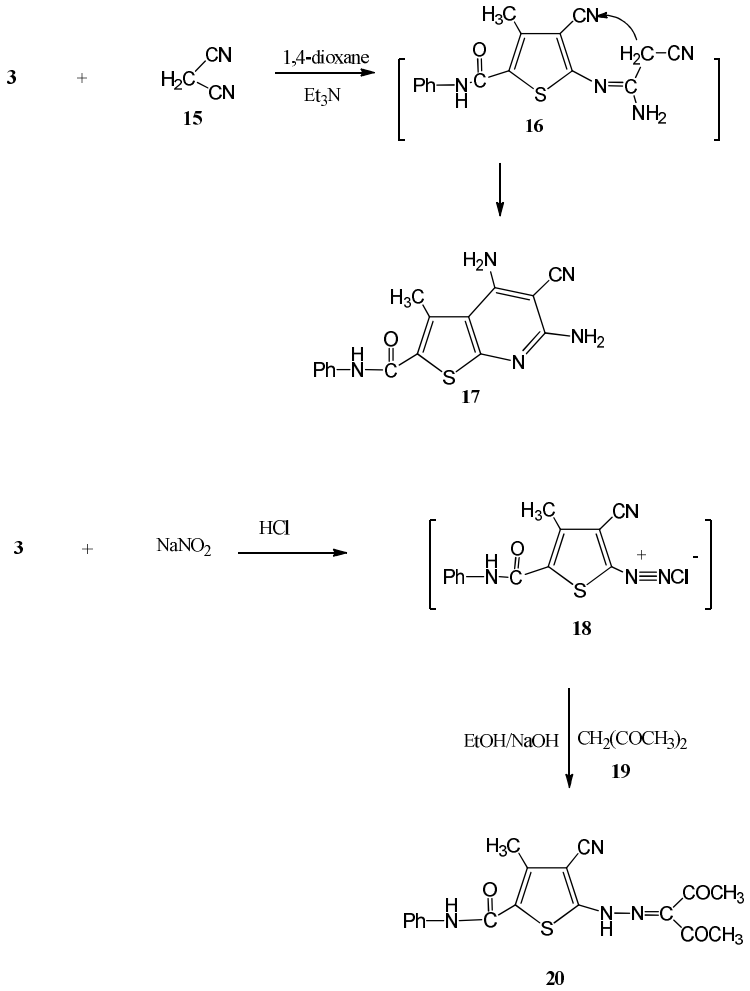
9	Ar
a	Ph
b	4-$CH_3C_6H_4$
c	4-$OCH_3C_6H_4$
d	ClC_6H_4

Scheme 1

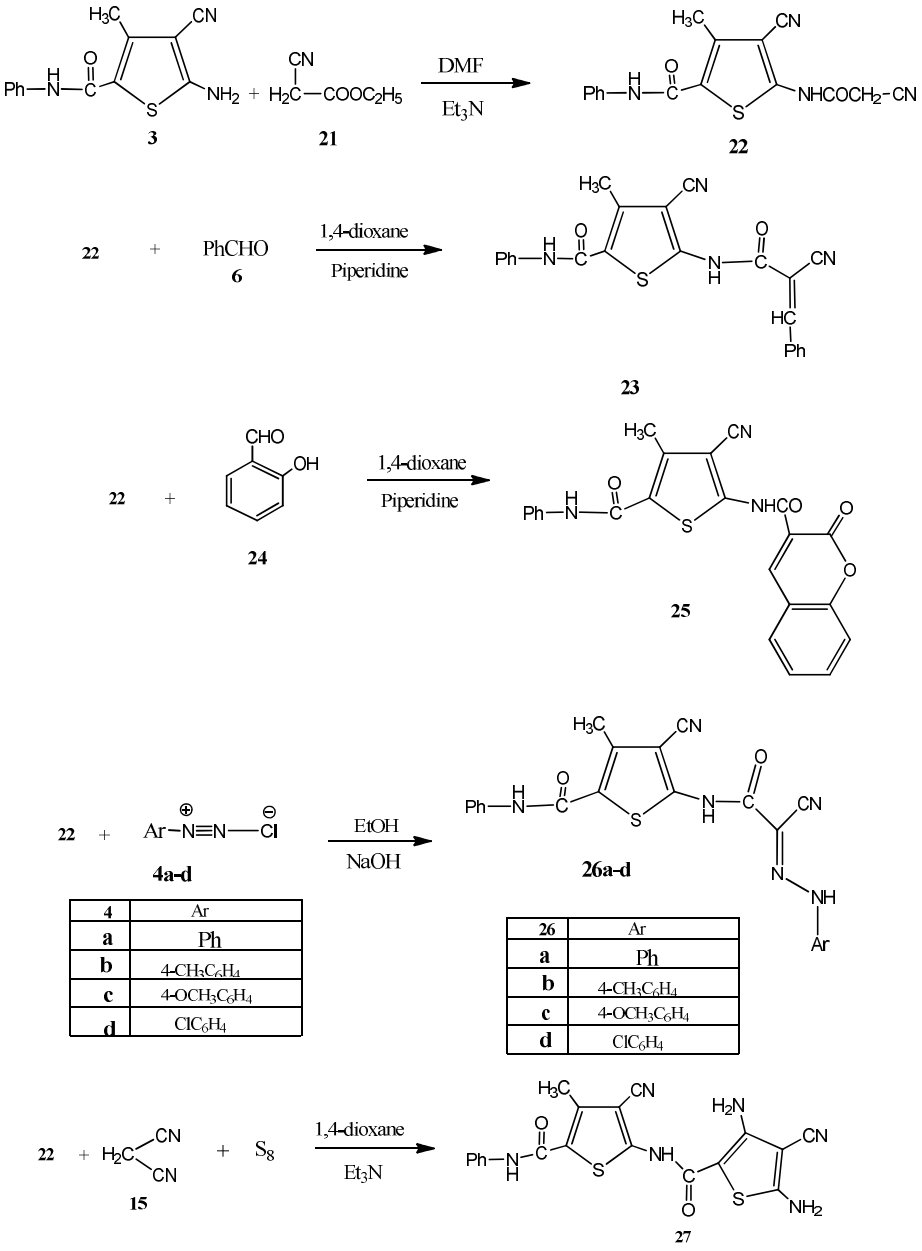


Scheme 2

Thus, compound **3** reacted with ethyl cyanoacetate **21** in refluxing dimethylformamide to give the amide derivative **22**. The analytical and spectral data of compound **22** are in agreement with its structure. The reaction of **22** with benzaldehyde **6** gave the benzylidene derivative **23**. Moreover, the reaction of **22** with salicyladehyde **24** gave the coumarin derivative **25**. The excellent yield of compound **22** encouraged us to study its reactivity towards aromatic diazonium salts in the aim of synthesizing new aryl hydrazine derivatives with different cytotoxic activities. Thus, the reaction of **22** with aromatic diazonium salts **4a-d** gave the arylhydrazone derivatives **26a-d**. The analytical and spectral data of the latter products were the basis of their structure elucidation. Finally the reaction of compound **22** with elemental sulfur and malononitrile **15** gave the thiophene derivative **27** (Scheme 4).


3
+
CN
H2C
CN
15
1,4-dioxane
Et3N
H3C
CN
H2C–CN
O
Ph–N
H
C
S
N=C
NH2
16
H2N
CN
H3C
NH2
O
Ph–N–C
H
S
N
17
3
+
NaNO2
HCl
H3C
CN
O
Ph–N–C
H
+
N≡NCl
-
S
18
EtOH/NaOH
CH2(COCH3)2
19
H3C
CN
O
Ph–N–C
H
S
N–N=C
H
COCH3
COCH3
20

Scheme 3



4	Ar
a	Ph
b	4-$CH_3C_6H_4$
c	4-$OCH_3C_6H_4$
d	ClC_6H_4

26	Ar
a	Ph
b	4-$CH_3C_6H_4$
c	4-$OCH_3C_6H_4$
d	ClC_6H_4

Scheme 4

Structure activity relationship

From Table 1 it is clear that the benzimidazole moiety was found to be crucial for the cytotoxic effect of the cyclic compounds **3** to **27**. Compounds **5a**, **5b**, **8**, **22**, and **23** exhibited optimal cytotoxic effect against cancer cell lines. On the other hand compound **9c**, **26d** and **27** showed very low activities toward the three cancer cell lines. Compounds **3**, **5c**, **5d**, **7**, **9d**, **14**, **20**, **25** and **26c** showed moderate activities. The thiophene derivative **3** showed low potency, however its reaction with difference aryl diazonium salts gave the arylhydrazone derivatives **5a-c**. It is obvious that compounds **5a** (R = H) and **5b** (R = CH_3) showed the highest cytotoxicity among the three compounds. The reaction of compound **3** with benzaldehyde produced compound **7** with low potency. The thiophene derivative **7** showed low potency although its acetylation gave compound **8** with high potency against the three cancer cell lines. For the thiophenes derivatives **9a-d**, it is clear that compound **9b** with 6- the 4-methylphenyl group showed the highest activity among the four compounds which is considered as a moderate activity. Compound **12** which was a thieno[*d*]pyrimidine derivative showed highest activity among the tested compounds. Similarly, compound **17** which is a thieno[*b*]pyridine derivative showed high potency towards the three cancer cell lines. Compounds **22**, **23**, **26a** and **26b** showed relatively high potency and such activity is attributed to the presence of the N-acyl moieties. From the activity of the compounds towards the three cancer cell lines it is obvious that the presence of N-rich and S-containing heterocyclic ring was the main effect through the high potency of the compound.

Table 1. Effect of the newly synthesized compounds on the growth of three human tumor cell lines GI_{50} (mmol L^{-1}).

Compound No.	MCF-7	NCI-H460	SF-268
3	22.6 ± 1.4	24.9 ± 2.8	13.8 ± 3.8
5a	0.4 ± 0.2	0.1 ± 0.02	0.9 ± 0.08
5b	0.01 ± 0.002	0.01 ± 0.004	0.04 ± 0.01
5c	30.6 ± 10.2	32.6 ± 8.6	24.4 ± 12.8
5d	18.1 ± 1.6	6.2 ± 2.8	8.6 ±2.6
7	28.6 ± 12.2	12.6 ± 8.6	52.4 ± 14.6
8	0.7 ± 0.50	0.2 ± 0.08	1.0 ± 0.02
9a	70.7 ± 18.5	40.2 ± 12.8	52.4 ± 8.6
9b	10.8 ± 2.6	4.5 ± 0.8	4.8 ± 1.8
9c	60.6 ± 16.9	38.9 ± 10.8	50.8 ± 8.6
9d	26.4 ± 2.2	34.1 ± 0.8	18.8 ± 4.8
12	0.01 ± 0.006	0.03 ± 0.001	0.02 ± 0.004
14	18.1 ± 0.6	16.3 ± 1.4	12.3 ±1.5
17	0.06 ± 0.004	0.03 ± 0.003	0.1 ± 0.02
20	32.8 ± 0.6	36.5 ± 0.8	30.7 ± 1.6
22	0.3 ± 0.1	0.2 ± 0.08	0.5 ± 0.01
23	0.9 ± 0.2	0.1 ± 0.02	0.3 ± 0.05
25	22.6 ± 2.6	24.3 ± 0.8	30.9 ± 3.8
26a	0.01 ± 0.003	0.02 ± 0.001	0.01 ± 0.001
26b	0.02 ± 0.008	0.03 ± 0.006	0.05 ± 0.00
26c	22.4 ± 2.2	32.6 ± 1.4	26.8 ± 6.4
26d	55.1 ± 2.7	23.2 ± 4.8	14.4 ± 2.6
27	36.0 ± 1.8	43.0 ± 0.8	30.5 ± 1.1
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

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

EXPERIMENTAL

Antitumor and normal cell line

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) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 mg/mL), at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x 10^5 cells/mL for MCF-7 and SF-268 and 0.75 x 10^4 cells/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.

Tumor cell growth assay. The effects of **3-27** 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. Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h 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., Powerwave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), 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.

Instruments

All melting points are uncorrected. IR spectra were recorded for KBr discs on a PyeUnicam SP-1000 spectrophotometer. ^{1}H NMR spectra were measured on a Varian EM-390–200 MHz in DMSO as solvent using TMS as internal standard and chemical shifts are expressed as δ. Analytical data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt.

*General procedure for the synthesis of 5-amino-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide **3***

To a solution of 3-oxo-N-phenylbutanamide (17.7 g, 0.1 mol) in absolute ethanol (50 mL) containing triethylamine (2.0 mL), malononitrile (6.6 g, 0.1 mol) and sulfur (3.2 g, 0.1 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The formed solid produce was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale yellow crystals. Yield 20.5 g (80%); m.p. 225 °C; anal. calcd for $C_{13}H_{11}N_3OS$ (257.06): C, 60.68; H, 4.31; N, 16.33; S, 12.46%; found: C, 60.76; H, 4.47; N,

16.60; S, 12.51%. IR (KBr) ν/cm^{-1} 3466-3320 (NH_2, NH), 3055 (CH aromatic), 2220 (CN), 1688 (CO), 1630 (C=C).^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 3.33 (s, 2H, NH_2), 7.00–7.71 (m, 5H, C_6H_5), 9.54 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 16.9 (CH_3), 116.3 (CN), 119.0, 120.2, 123.8, 125.5, 134.2, 139.6, 143.2, 144.1(C_6H_5, thiophene C), 163.9 (CO).

General procedure for the synthesis of the thiophenederivatives ***5a-d***

To a cold solution of **3** (2.57 g, 0.01 mol) in 1,4-dioxane (40 mL) containing sodium hydroxide (2.5 g) a cold solution of the respective diazonium salt [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of either aniline (0.94 g, 0.01 mol), *p*-toluidine (1.15 g, 0.01 mol), *p*-methoxy aniline (1.3 g, 0.01 mol) or *p*-chloroaniline (1.29 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added while stirring. The formed solid product, in each case, upon stirring at room temperature for 1 h was collected by filtration and dried. The obtained product was crystallized from ethanol to give brown crystals.

5-Amino-4-cyano-N-phenyl-3-((2-phenylhydrazono) methyl) thiophene-2-carboxamide ***(5a)***. Yield 2.7 g (75%); m.p. 135-140 °C; anal. calcd. for $C_{19}H_{15}N_5OS$ (361.42): C, 63.14; H, 4.18; N, 19.38; S, 8.87%; found: C, 63.06; H, 4.27; N, 19.44; S, 8.91%. IR (KBr) ν/cm^{-1} 3472-3328 (2NH, NH_2), 3055 (CH aromatic), 2220 (CN), 1687 (CO), 1656 (C=N), 1628 (C=C).^{1}H NMR (DMSO-d_6) δ 3.33 (s, 2H, NH_2), 6.39 (s, 1H, CH),6.40 (s,1H, NH), 7.05–7.71 (m,10H, $2C_6H_5$), 9.54 (s, 1H, NH).^{13}C NMR (DMSO-d_6) δ 116.6 (CN), 119.8, 121.6, 124.3, 125.8, 128.0, 129.6, 130.2, 134.5, 139.8, 143.8, 144.7 (two C_6H_5, thiophene C), 164.2 (CO), 172.1 (C=N).

5-Amino-4-cyano-N-phenyl-3-((2-(p-tolyl) hydrazono) methyl) thiophene-2-carboxamide ***(5b)***. Yield 2.62 g (70%); m.p. 140-150 °C; anal. calcd. for $C_{20}H_{17}N_5OS$ (375.54): C, 63.98; H, 4.56; N, 18.65; S, 8.54%; found: C, 63.79; H, 4.31; N, 18.72; S, 8.76%. IR (KBr) ν/cm^{-1} 3477-3318 (NH_2, 2NH), 3062 (CH aromatic), 2223 (CN), 1688 (CO), 1660 (C=N), 1628 (C=C).^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 3.30 (s, 2H, NH_2),7.05 (s,1H, CH), 7.12 (s,1H, NH), 7.32–7.71 (m, 9H, C_6H_4, C_6H_5), 9.54 (s, 1H, NH).^{13}C NMR (DMSO-d_6) δ 19.8 (CH_3), 116.6 (CN), 119.3, 122.5, 124.6, 125.2, 128.3, 129.8, 131.4, 132.9, 138.4, 142.7, 143.9 (C_6H_5, C_6H_4, thiophene C).

5-Amino-3-((2-(4-methoxyphenyl)hydrazono)methyl)-4-cyano-N-phenylthiophene-2-carboxamide ***(5c)***. Yield 3.24 g (83%); m.p. 120-125 °C; Anal. Calcd. for $C_{20}H_{17}N_5O_2S$ (391.45): C, 61.37; H, 4.38; N, 17.89;S, 8.19%; found: C, 61.43; H, 4.23; N, 18.02; S, 8.22%. IR (KBr) ν/cm^{-1} 3484-3322 (NH_2, 2NH), 3062 (CH aromatic), 2221 (CN), 1689 (CO), 1662 (C=N), 1625 (C=C).^{1}H NMR (DMSO-d_6) δ 3.74 (s, 2H, NH_2), 3.75 (s, 3H,CH_3),6.93 (s,1H,CH), 7.01 (s,1H, NH), 7.30–7.67 (m,9H, C_6H_5, C_6H_4), 9.50 (s, 1H, NH).^{13}C NMR (DMSO-d_6) δ 19.3 (CH_3), 116.3 (CN), 119.9, 123.2, 124.9, 125.1, 128.6, 129.1, 132.0, 132.9, 138.4, 142.7, 143.4 (C_6H_5, C_6H_4, thiophene C), 164.8 (CO), 171.2 (C=N).

5-Amino-3-((2-(4-chlorophenyl)hydrazono)methyl)-4-cyano-N-phenylthiophene-2-carboxamide ***(5d)***. Yield 3.36 g (85 %); m.p. 120 °C; anal. calcd. for $C_{19}H_{14}ClN_5OS$ (395.87): C, 57.65; H, 3.56; N, 17.69; S, 8.10%; found: C, 57.90; H, 3.47; N, 17.88; S, 8.28%. IR (KBr) ν/cm^{-1} 3489-3328 (NH_2, 2NH), 3051 (CH aromatic), 2220 (CN), 1687 (CO), 1665 (C=N), 1621 (C=C).^{1}H NMR (DMSO-d_6) δ 3.81 (s, 2H, NH_2), 6.90 (s, 1H, CH),7.02 (s,1H, NH), 7.30–7.67 (m, 9H, C_6H_4, C_6H_5), 9.52 (s, 1H, NH).^{13}C NMR (DMSO-d_6) δ 116.4 (CN), 119.9, 123.8, 124.9, 125.4, 128.1, 128.4, 132.3, 132.6, 134.6, 142.9, 143.5 (C_6H_5, C_6H_4, thiophene C), 164.1 (CO), 171.1 (C=N).

5-Amino-4-cyano-N-phenyl-3-styrylthiophene-2-carboxamide (7)

To a solution of **3** (2.57 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (1.0 mL), benzaldehyde (1.0 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 8 h then left to cool. The solid product formed upon evaporating the solution under vacuum followed by triturating the remaining product with ethanol, was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale brown crystals. Yield 2.76 g (80%); m.p. 180 °C; anal. calcd. for $C_{20}H_{15}N_3OS$ (345.42): C, 69.54; H, 4.38; N, 12.17; S, 9.28%; found: C, 69.69; H, 4.29; N, 12.28; S, 9.41%; IR (KBr) ν/cm^{-1} 3426–3365 (NH_2, NH), 3054 (CH, aromatic), 2219 (CN),1801 (C=O),1660 (C=N); ^{1}H NMR (DMSO-d_6) δ 3.31 (s, 2H, NH_2), 7.05–7.27 (2d, 2H, CH=CH), 7.30–7.59 (m,10H, $2C_6H_5$), 9.54 (s, 1H, NH),^{13}C NMR (DMSO-d_6) δ 90.6, 98.3 (C=C), 116.5 (CN), 120.4, 122.6, 124.6, 126.9, 128.3, 128.9, 132.1, 132.8, 134.6, 142.9, 143.9 (two C_6H_5, thiophene C), 164.13 (CO).

*5-Acetamido-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide (**8**)*

To a solution of **3** (2.57 g, 0.01 mol) in acetic acid (30 mL), acetic anhydride (1.02 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The solid product formed upon pouring onto ice/water containing a few drops of hydrochloric acid was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale yellow crystals. Yield 2.33 g (78%), m.p. 260 °C. anal. calcd. for $C_{15}H_{13}N_3O_2S$ (299.35): C, 60.18; H, 4.38; N, 14.04; S, 10.71%; found: C, 62.42; H, 4.36; N, 14.11; S, 10.84%. IR (KBr) ν/cm^{-1} 3446–3268 (NH_2, 2NH), 3051 (CH, aromatic), 2221 (CN), 1703 (C=O), 1643 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.50,2.51 (2s, 6H, $2CH_3$), 7.05–7.66 (m, 5H, C_6H_5), 10.00,11.96 (2s, 2H, 2NH).^{13}C NMR (DMSO-d_6) δ 19.6, 20.3 ($2CH_3$), (C=C), 116.3 (CN), 123.2, 124.8, 125.3, 126.9, 128.9, 130.2, 132.7, 134.6, 142.2, 143.4 (C_6H_5, thiophene C), 164.3, 165.8 (2CO).

*General procedure for the synthesis of thiophene-2-carboxamidederivatives **9a-d***

To a cold solution of **8** (2.99 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (2.5 g) a cold solution of the respective diazonium salt [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of either aniline (0.94 g, 0.01 mol), *p*-toluidine (1.15 g, 0.01 mol), *p*-methoxy aniline (1.3 g, 0.01 mol) or *p*-chloroaniline (1.29 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added while stirring. The formed solid product, in each case, upon stirring at room temperature for 1 h was collected by filtration and dried. The obtained product was crystallized from ethanol to give yellowish brown crystals for **9a** and **9b** and reddish yellow crystals for **9c** and **9d**.

*5-Acetamido-4-cyano-N-phenyl-3-((2-phenylhydrazono)methyl)thiophene-2-carboxamide (**9a**)*. Yield 2.82 g (70%); m.p. over 300 °C; anal. calcd. for $C_{21}H_{17}N_5O_2S$ (403.46): C, 62.52; H, 4.25; N, 17.36; S, 7.95%; found: C, 62.44; H, 4.11; N, 17.47; S, 8.05%. IR (KBr) ν/cm^{-1}: 3488-3328 (2NH), 3050 (CH aromatic), 2220 (CN), 1685, 1680 (2CO), 1660 (C=N), 1630 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, CH_3), 7.07 (CH=N), 7.08 (s, 1H, NH), 7.12–7.66 (m, 10H, $2C_6H_5$), 10.00, 11.96 (2s, 2H, 2NH).^{13}C NMR (DMSO-d_6) δ 19.8 (CH_3), 116.8 (CN), 119.4, 121.7, 122.0, 123.8, 124.9, 130.3, 133.8, 134.2, 138.5, 140.4, 144.2 (two C_6H_5, thiophene C), 163.8, 164.2 (2CO), 172.3 (C=N).

*5-Acetamido-4-cyano-N-phenyl-3-((2-(p-tolyl)hydrazono) methyl) thiophene-2-carboxamide (**9b**)*. Yield 3.33 g (80%); m.p. 290 °C; anal. calcd. for $C_{22}H_{19}N_5O_2S$ (417.48): C, 63.29; H, 4.59; N, 16.78; S, 7.68%; found: C, 62.88; H, 4.33; N, 16.92; S, 7.82%. IR (KBr) ν/cm^{-1} 3447–3267 (3NH), 3052 (CH, aromatic), 2970 (CH_3), 2223 (CN), 1704 (C=O),1643 (C=N),1529 (C=C); ^{1}H

NMR (DMSO-d_6) δ 2.25,2.50 (2s, 6H, $2CH_3$), 7.07 (s, 1H, NH), 7.09 (s,1H,CH=N), 7.30–7.60 (m, 9H, C_6H_5, C_6H_4) , 10.01,11.97 (2s, 2H, 2NH).^{13}C NMR (DMSO-d_6) δ 19.8, 21.6 ($2CH_3$), 116.4 (CN), 120.4, 121.8, 122.0, 124.1, 124.9, 130.6, 133.9, 134.3, 138.8, 140.1, 144.9 (C_6H_5, C_6H_4, thiophene C), 164.1, 164.8 (2CO), 172.1 (C=N).

*5-Acetamido-4-cyano-3-((2-(4-methoxyphenyl) hydrazono) methyl)-N-phenylthiophene-2-carboxamide (**9c**)*. Yield 3.76 g (87%); m.p. 280°C; anal. calcd. for $C_{22}H_{19}N_5O_3S$ (433.48): C, 60.96; H, 4.42; N, 16.16; S, 7.40%; found: C, 60.37; H, 4.36; N, 16.09; S, 7.66%. ^{1}H NMR (DMSO-d_6) δ 2.50,3.30 (2s, 6H, $2CH_3$), 6.93 (s, 1H, CH), 7.32–7.71 (m, 9H, C_6H_5, C_6H_4), 9.93, 9.94, 11.95 (3H, 3H, 3NH).^{13}C NMR (DMSO-d_6) δ 19.8, 22.4 ($2CH_3$), 116.8 (CN), 120.5, 121.1, 122.3, 124.4, 124.9, 130.6, 132.3, 133.6, 138.9, 140.3, 144.5 (C_6H_5, C_6H_4, thiophene C), 164.3, 164.9 (2CO), 172.3 (C=N).

*5-Acetamido-3-((2-(4-chlorophenyl)hydrazono)methyl)-4-cyano-N-phenylthiophene-2-carboxamide (**9d**)*. Yield 3.63 g (83%); m.p. 295°C; anal. calcd for $C_{21}H_{16}ClN_5O_2S$ (437.9): C, 57.60; H, 3.68; N, 15.99; S, 7.32%; found: C, 57.76; H, 3.45; N, 16.09; S, 7.46%. ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3), 7.07 (s, 1H, NH), 7.09 (s, 1H, CH=N), 7.30–7.60 (m, 9H, C_6H_5, C_6H_4), 10.01, 11.97 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 21.9 (CH_3), 116.6 (CN), 120.6, 121.4, 122.8, 124.2, 124.5, 130.8, 132.1, 133.6, 138.9, 140.2, 144.9 (C_6H_5, C_6H_4, thiophene C), 164.6, 164.8 (2CO), 172.2 (C=N).

*3,4-Dihydro-4-imino-2-mercapto-5-methyl-N,3-diphenylthieno[2,3-d]pyrimidine-6-carboxamide (**12**)*

To a solution of **3** (2.57 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL), phenyl isothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was heated underreflux for 2 h then left to cool. The solid product formed upon evaporating the solution under vacuum followed by triturating the remaining product with ethanol, was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale yellow crystals. Yield 2.70 g (75%); m.p. 120-135 °C; anal. calcd. for $C_{20}H_{16}N_4OS_2$ (392.5): C, 61.20; H, 4.11; N, 14.27; S, 16.34%; found: C, 61.31; H, 4.21; N, 14.38; S, 16.44%. IR (KBr) ν/cm^{-1} 3480-3320 (SH, 2NH), 3055 (CH aromatic), 1688 (CO) 1660 (exocyclic C=N), 1628 (C=C); ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H, CH_3), 7.30–7.66 (m, 10H, $2C_6H_5$), 7.71 (s, 1H, SH), 9.54, 11.08 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 19.6 (CH_3), 119.8, 120.3, 122.4, 123.7, 126.0, 128.9, 130.2, 131.2, 133.8, 138.9, 140.2, 142.6, 144.6, 146.8 (two C_6H_5, thiophene C), 164.2 (CO), 174.8 (C=N).

*3-Amino-4,6-dihydro-4-imino-N-phenylthieno[3,4-c]thiophene-1-carboxamide (**14**)*

To a solution of **3** (2.57 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (2.0 mL), element sulfur (3.2 g, 0. 1 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The formed solid product was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale yellow crystals. Yield 2.02 g (70%); m.p. 190 °C; anal. calcd. for $C_{13}H_{11}N_3OS_2$ (289.38): C, 53.96; H, 3.83; N, 14.52; S, 22.16%; found: C, 52.77; H, 3.67; N, 14.69; S, 22.09%. IR (KBr) ν/cm-^{1} 3364–3316 (NH_2, NH), 3057 (CH, aromatic), 1718 (C=O), 1624 (C=N); ^{1}H NMR (DMSO-d_6) δ 3.56 (s, 2H, NH_2), 7.05(s, 1H, thiophene CH_2), 7.27–7.60 (m, 5H, C_6H_5), 7.71, 9.54 (2H, 2H, 2NH).^{13}C NMR (DMSO-d_6) δ 64.6 (CH_2),119.4, 120.8, 122.6, 124.2, 126.5, 128.4, 131.2, 133.8, 138.9, 140.2, 142.6, 144.6 (C_6H_5, thiophene C), 164.8 (CO), 175.9 (C=N).

*4,6-Diamino-5-cyano-3-methyl-N-phenylthieno[2,3-b]pyridine-2-carboxamide (**17**)*

To a solution of **3** (2.57 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (1.5 mL), malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h. The solid product formed upon evaporating the solution under vacuum followed by triturating the remaining product with ethanol, was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale brown crystals. Yield 2.45 g (76%); m.p. 185 °C; anal. calcd. for $C_{16}H_{13}N_5OS$ (323.37): C, 59.43; H, 4.05; N, 21.66; S, 9.92%; found: C, 59.21; H, 3.89; N, 21.88; S, 10.05%. IR (KBr) ν/cm^{-1} 3424–3282 (2 NH_2, NH), 3056 (CH, aromatic), 2220 (CN), 1680 (C=O), 1629 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H, CH_3), 2.44, 3.56 (2s, 4H, NH_2),7.05–7.64 (m, 5H, C_6H_5), 9.54 (s, 1H, NH), ^{13}C NMR (DMSO-d_6) δ 22.3 (CH_3),116.9 (CN),119.4, 121.3, 122.6, 124.2, 126.8, 129.1, 130.5, 133.8, 138.3, 140.1, 143.8, 144.1 (C_6H_5, thiophene, pyridine C), 164.2 (CO), 172.2 (C=N).

*4-Cyano-5-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl)-3-methyl-N-phenylthiophene-2-carboxamide (**20**)*

To a cold solution of acetyl acetone (1.00 g, 0.01 mol) in ethanol (40 mL) containing sodium hydroxide (2.5 g) a cold solution of the respective diazonium salt [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of **3** (2.57 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added while stirring. The formed solid product, upon stirring at room temperature for 1 h was collected by filtration and dried. The obtained product was crystallized from ethanol to give black crystals. Yield 2.87 g (78%); m.p. 160-170 °C; anal. calcd. for $C_{18}H_{16}N_4O_3S$ (368.41): C, 58.68; H, 4.38; N, 15.21; S, 8.70%; found: C, 58.89; H, 4.25; N, 15.53; S, 8.88%. IR (KBr) ν/cm^{-1} 3422–3280 (2NH), 3053 (CH, aromatic), 2222 (CN), 1684 (C=O), 1622 (C=C); ^{1}H NMR (DMSO-d_6) δ 2.32, 2.45, 2.62 (3s, 9H, 3CH_3), 7.27–7.62 (m, 5H, C_6H_5) , 7.72, 9.54 (2s, 2H, 2NH), ^{13}C NMR (DMSO-d_6) δ 19.8, 20.6, 21.3 (3 CH_3), 117.0 (CN), 120.4, 121.7, 122.9, 124.7, 125.9, 128.2, 130.1, 138.0 (C_6H_5, thiophene C), 163.2, 164.0, 165.2 (3CO), 173.2 (C=N).

*4-Cyano-5-(2-cyanoacetamido)-3-methyl-N-phenylthiophene-2-carboxamide (**22**)*

To a solution of **3** (2.57 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.5 mL), ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h. The solid product formed upon pouring onto ice/water containing a few drops of hydrochloric acid was collected by filtration and dried. The obtained product was crystallized from ethanol to give brown crystals. Yield 2.59 g (80%); m.p. 195 °C; anal. calcd. for $C_{16}H_{12}N_4O_2S$ (324.36): C, 59.25; H, 3.73; N, 17.27; S, 9.89%; found: C, 59.86; H, 3.65; N, 17.06 ; S, 9.93. IR (KBr) ν/cm^{-1} 3477-3318 (2NH), 3045 (CH aromatic); ^{1}H NMR (DMSO-d_6) δ 2.54 (s, 3H, CH_3), 4.42 (s, 2H, CH_2). 7.09–7.60 (m, 5H, C_6H_5), 7.71, 9.54 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 20.6 (CH_3), 62.1 (CH_2), 116.8, 117.3 (2CN), 119.9, 120.6, 121.9, 123.1, 124.7, 125.5, 126.8, 130.2, 138.3, 140.2 (C_6H_5, thiophene C), 164.4, 164.5 (2CO).

*5-(2-Cyano-3-phenylacrylamido)-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide (**23**)*

To a solution of **22** (3.24 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (1.0 mL), benzaldehyde (1.0 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool. The solid product formed upon evaporating the solution under vacuum followed by triturating the remaining product with ethanol, was collected by filtration and dried. The obtained product was crystallized from 1,4-dioxane to give yellowish green crystals. Yield 3.37 g (82%); m.p. 175 °C; anal. calcd. for $C_{23}H_{16}N_4O_2S$ (412.46): C, 66.97; H, 3.91; N, 13.58;

S, 7.77%; found: C, 66.87; H, 3.69; N, 13.73; S, 8.01%. IR (KBr) ν/cm^{-1} 3424–3362 (2NH), 3059 (CH, aromatic), 2924 (CH_3), 1687, 1685 (2C=O), 1657 (C=C); ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, CH_3), 7.11 (s, 1H, CH=C), 7.39–7.68 (m, 10H, $2C_6H_5$), 8.88, 10.27 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 19.6 (CH_3), 87.3, 92.5 (CH=C), 116.4, 116.9 (2CN), 119.3, 120.8, 122.4, 123.6, 124.8, 125.9, 126.8, 130.1, 132.1, 136.2, 140.9, 142.6 (two C_6H_5, thiophene C), 164.6, 166.2 (2CO).

*N-(3-Cyano-4-methyl-5-(phenylcarbamoyl)thiophen-2-yl)-2-oxo-2H-chromene-3-carboxamide (**25**)*

To a solution of **22** (3.24 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (1.0 mL), salicyaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then left to cool. The solid product formed upon evaporating the solution under vacuum followed by triturating the remaining product with ethanol, was collected by filtration and dried. The obtained product was crystallized from ethanol to give orange yellow crystals. Yield 3.00 g (70%); m.p. 240-250 °C; anal. calcd. for $C_{23}H_{15}N_3O_4S$ (429.45): C, 64.33; H, 3.52; N, 9.78; S, 7.47%; found: C, 64.27; H, 3.34; N, 9.92; S, 7.64%. IR (KBr) ν/cm^{-1} 3479–3321 (2NH), 3053 (CH, aromatic), 2921 (CH_3), 2220 (CN), 1689-1685 (3C=O), 1653 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.53 (s, 3H, CH_3), 7.00 (s, H, coumarin H-4), 7.34–7.90 (m, 9H, C_6H_5, C_6H_4), 9.07, 10.24 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 18.3 (CH_3), 116.3 (CN), 96.8 (coumarin C-4), 120.5, 121.2, 121.9, 123.9, 125.2, 125.6, 129.1, 130.4, 132.7, 134.9, 139.2, 140.9, 141.8, 143.6, 152.0 (C_6H_5, coumarin, thiophene C), 164.1, 165.9, 166.3 (3CO).

*General procedure for the synthesis of 2-cyanoacetamido)-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide derivatives **26a-d***

To a cold solution of **22** (3.24 g, 0.01 mol) in 1,4-dioxane (40 mL) containing sodium hydroxide (2.5 g) a cold solution of the respective diazonium salt [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of either aniline (0.94 g, 0.01 mol), *p*-toluidine (1.15 g, 0.01 mol), *p*-methoxy aniline (1.3 g, 0.01 mol) or *p*-chloroaniline (1.29 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring]was added while stirring. The formed solid product, in each case, upon stirring at room temperature for 1 h was collected by filtration and dried. The obtained product was crystallized from ethanol to give brown crystals for **26a** and **26c** and black crystals for **26b** and **26d**.

*2-(2-Phenylhydrazono)-2-cyanoacetamido)-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide (**26a**)*. Yield 3.5 g (82%); m.p. 70-80 °C; anal. calcd. for $C_{22}H_{16}N_6O_2S$ (428.47): C, 61.67; H, 3.76; N, 19.61; S, 7.48. found: C, 61.21; H, 3.58; N, 19.88; S, 7.58%. IR (KBr) ν/cm^{-1} 3492–3329 (3NH), 3056 (CH, aromatic), 2923 (CH3), 2222, 2220 (2CN), 1689, 1687 (2C=O), 1660 (C=N), 1633 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3), 7.30 (s, 1H, NH), 7.40–7.66 (m, 10H, $2C_6H_5$), 8.40, 9.48 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 18.8 (CH3), 116.7, 117.2 (2CN), 121.0, 121.8, 122.3, 123.5, 124.2, 125.9, 129.1, 132.7, 132.9, 140.9, 141.8, 143.6 (two C6H5, thiophene C), 164.4, 165.2 (2CO), 172.0 (C=N).

*2-(2-p-Tolylhydrazono)-2-cyanoacetamido)-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide (**26b**)*. Yield 3.53 g (80%); m.p. 110-120 °C; anal. calcd. for $C_{23}H_{18}N_6O_2S$ (442.49): C, 62.43; H, 4.10; N, 18.99; S, 7.25%; found: C, 62.38; H, 3.91; N, 19.06; S, 7.41%. IR (KBr) ν/cm^{-1} 3488–3339 (3NH), 3053 (CH, aromatic), 2923 (CH_3), 2222, 2220 (2CN), 1693, 1689 (2C=O), 1661 (C=N), 1630 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.39, 2.40 (2s, 6H, $2CH_3$), 7.09 (s, 1H, NH), 7.37–7.71 (m, 9H, C_6H_5, C_6H_4), 8.80, 10.13 (2s, 2H, 2NH).^{13}C NMR (DMSO-d_6) δ

18.3, 19.6 ($2CH_3$), 116.2, 117.0 (2CN), 119.8, 122.0, 122.3, 123.8, 124.6, 126.4, 129.8, 132.2, 133.6, 140.5, 141.8, 143.8 (two C_6H_5, thiophene C), 164.2, 165.7 (2CO), 172.1 (C=N).

*2-(2-(4-Methoxyphenyl)hydrazono)-2-cyanoacetamido)-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide (**26c**)*. Yield 3.89 g (85%); m.p. 155°C; anal. calcd. for $C_{23}H_{18}N_6O_3S$ (458.49): C, 60.25; H, 3.96; N, 18.33; S, 6.99%; found: C, 59.89; H, 3.78; N, 18.09; S, 7.11%. IR (KBr) ν/cm^{-1} 3447–3267 (3NH), 3052 (CH, aromatic), 2970 (CH_3), 2223 (CN), 1704 (C=O), 1643 (C=N), 1529 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.49, 2.68 (2s, 6H, $2CH_3$), 7.09 (s, 1H, NH), 7.39–7.79 (m, 9H, C_6H_5, C_6H_4), 10.55, 11.11 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 18.6, 21.3 ($2CH_3$), 116.4, 117.3 (2CN), 120.9, 122.9, 122.3, 123.8, 124.8, 126.4, 129.3, 132.4, 133.8, 140.9, 141.3, 143.1 (two C_6H_5, thiophene C), 164.3, 165.9 (2CO), 172.0 (C=N).

*5-(2-(2-(4-Chlorophenyl)hydrazono)-2-cyanoacetamido)-4-cyano-3-methyl-N-phenylthiophene-2-carboxamide (**26d**)*. Yield 3.65 g (79%); m.p. 135°C; anal. calcd. for $C_{22}H_{15}ClN_6O_2S$ (462.91): C, 57.08; H, 3.27; N, 18.15; S, 6.93%; found: C, 56.82; H, 3.12; N, 17.99; S, 7.14%. IR (KBr) ν/cm^{-1} 3476–3320 (3NH), 3055 (CH, aromatic), 2924 (CH_3), 2224, 2220 (2CN), 1690, 1689 (2C=O), 1661 (C=N), 1624 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3H, CH_3), 7.06 (s, 1H, NH), 7.30–7.79 (m, 9H, C_6H_5, C_6H_4), 10.27, 11.10 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 18.9 (CH_3), 116.2, 117.1 (2CN), 120.8, 122.5, 122.8, 123.1, 124.8, 126.2, 129.3, 132.6, 133.8, 140.3, 142.8, 143.7 (two C_6H_5, thiophene C), 164.6, 165.4 (2CO), 172.1 (C=N).

*3,5-Diamino-4-cyano-N-(3-cyano-4-methyl-5-(phenylcarbamoyl)thiophen-2-yl)thiophene-2-carboxamide (**27**)*

To a solution of **22** (3.24 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (1.5 mL), malononitrile (0.66 g, 0.01 mol) was added followed by elemental sulfur (0.32 g, 0.01 mol). The reaction mixture was heated under reflux for 3 h. The solid product formed upon evaporating the solution under vacuum followed by triturating the remaining product with ethanol, was collected by filtration and dried. The obtained product was crystallized from ethanol to give pale brown crystals. Yield 3.16 g (75%); m.p. 185 °C; anal. calcd. for $C_{19}H_{14}N_6O_2S_2$ (422.48): C, 54.01; H, 3.34; N, 19.89; S, 15.18%; found: C, 53.72; H, 3.28; N, 20.11; S, 15.23. IR (KBr) ν/cm^{-1} 3491–3316 (3NH), 3056 (CH, aromatic), 2921 (CH_3), 2227, 2220 (2CN), 1692, 1686 (2C=O), 1660 (C=N), 1623 (C=C). ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 3.45–3.48 (2s, 4H, $2NH_2$). 7.03–7.60 (m, 5H, C_6H_5), 7.70, 9.54 (2s, 2H, 2NH). ^{13}C NMR (DMSO-d_6) δ 18.6 (CH_3), 116.1, 116.9 (2CN), 120.9, 122.8, 123.0, 124.3, 124.8, 126.8, 129.3, 132.9, 136.2, 140.7, 142.3, 143.1 (C_6H_5, two thiophene C), 164.3, 165.7 (2CO).

CONCLUSION

A series of thiophene derivatives were synthesized and evaluated against three cancer cell lines. The above results allow the conclusion that administration of the tested compounds to the cancer cell line showed promising anticancer activity. Compounds **5a**, **5b**, **8**, **22**, and **23** exhibited optimal cytotoxic effect against cancer cell lines.

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