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New Approaches for the Synthesis of Thiophene Derivatives with Anti-tumor Activities

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Abstract

The reaction of either cyclohexanone or cyclopentanone with cyanoacetylhydrazine and elemental sulfur gave the 2-aminocycloalkeno[*b*]thiophene derivatives **3a** and **3b**, respectively. The latter compounds reacted with either aromatic benzaldehydes or active methylene reagents to give the Schiff's bases **5a–d** and the pyrazole derivatives **7a–d** and **9a–d**, respectively. On the other hand, the reaction of 3-oxo-*N*-*p*-tolylbutanamide (**10**) with either of malononitrile or ethyl cyanoacetate gave the thiophene derivatives **13a** and **13b**, respectively. Compounds **13a**,**b** were subjected to a series of heterocyclization reactions to give heterocyclic derivatives. Their cytotoxicity against the three human tumor cells lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) together against the normal human cell line namely the normal fibroblast cells WI 38 were measured.

Keywords: Hydrazide-hydrazone, thiophene, pyrazole, arylhydrazone, antitumor activity.

1. Introduction

The pair cycloalkene/thiophene represents one of the most prominent examples of bioisosterism[1] (bioisosteres are isosteric[2] molecules that have similar or antagonistic properties in biological systems) and therefore the synthesis of thiophene analogues has attracted considerable attention especially in pharmaceutical research. The exploratory replacement of a benzene ring in successful drugs by a thiophene moiety has become a routine strategy in modern drug design and development.

The physiological effects of thiophene are similar to those of benzene (bioisostere), with frequently superior pharmacodynamic, pharmacokinetic, or toxicological properties. For example, thiophene replacement of the annulated benzene ring in derivatives of piroxicam, an anti-inflammatory agent used in arthritis patients, had no effect on activity.[3] Similarly, the thiophene analogue of amphetamine retains complete amphetamine-like activity.[4] Nevertheless, thiophene derivatives have shown numerous biological activities, such as nematocidal,[5] insecticidal,[6] antibacterial,[7] antifungal,[8] and antiviral activity.[9] Recently, substituted thiophenes have been shown to possess good anti-inflammatory activities in rats.[10] In view of reported biological activities of alkynyl substituted heterocycles[11–17] and our effort in the synthesis of thiophene derivatives[18–20] of potential pharmacological significance we became interested in the synthesis of substituted cycloalkeno thiophenes. Due to the known anticancer activities of thiophene derivatives[13] in this work we would like to report the synthesis of 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 beside studying their cytotoxicity against the normal human cell line namely the normal fibroblast cells WI 38.

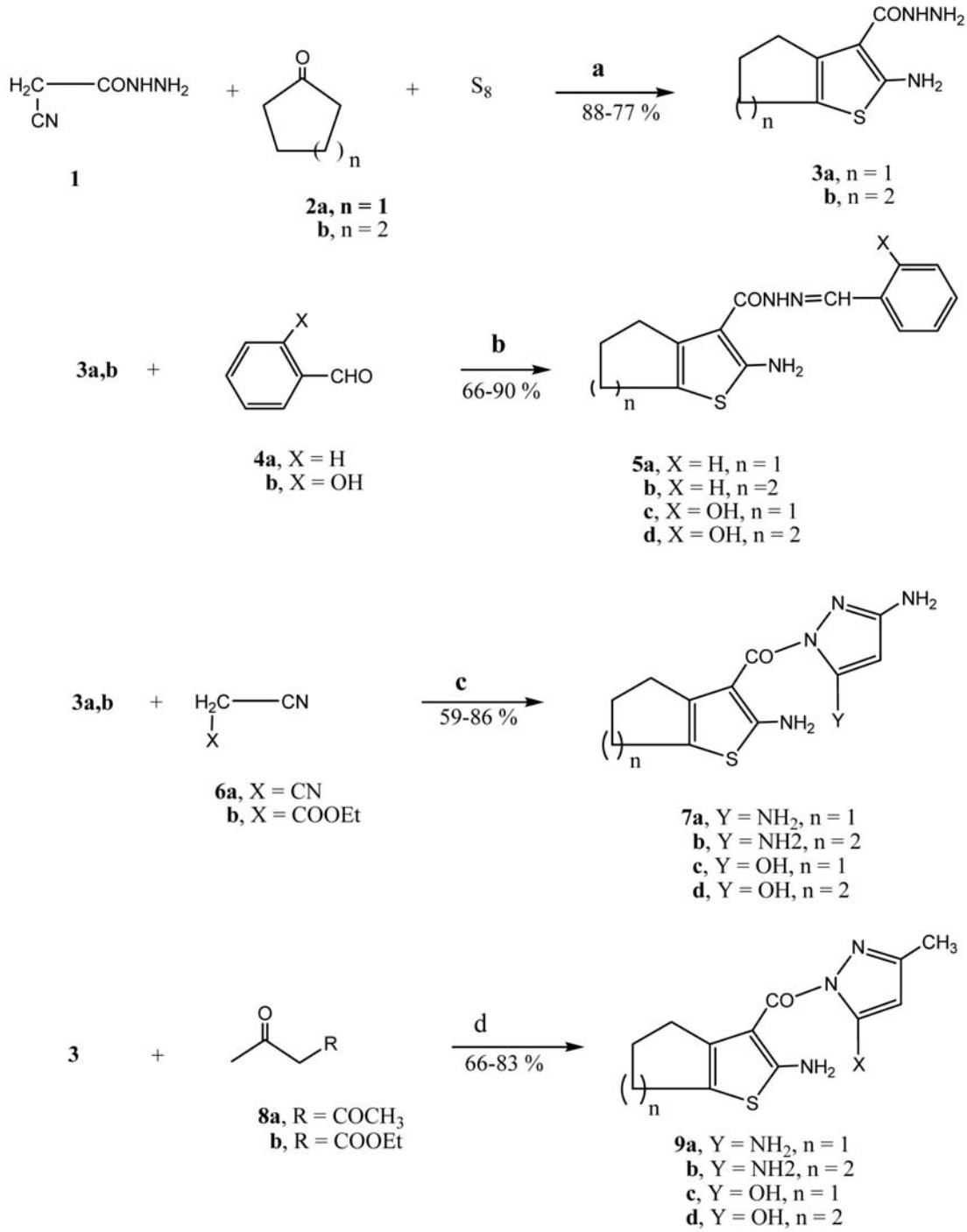
2. Results and Discussion

2. 1. Chemistry

In the present work, cyanoacetylhydrazine was reacted with either cyclohexanone (**2a**) or cyclopentanone (**2b**) and elemental sulfur in 1,4-dioxan and in the presence of triethylamine gave the cycloalkeno[*b*]thiophene derivatives **3a** and **3b**, respectively. Compound **3b** was pre-

viously reported.[21–25] The structure of compound **3a** was mainly elucidated by ^{1}H- and ^{13}C-NMR spectra. Thus, the ^{1}H-NMR spectrum of **3a** showed two multiplets at δ 1.77–1.79 and 2.20 ppm corresponding to the presence of three CH_2 groups, two multiplets at δ 4.20–4.22 ppm corresponding to the NH_2 group and a singlet at δ 9.01 (D_2O exchangeable) for the NH group. ^{13}C-NMR, δ: 22.0, 23.4, 30.9 (cyclophentene CH_2), 122.6, 136.3, 138.9, 144.5 (thiophene C), 166.8 (C=O).

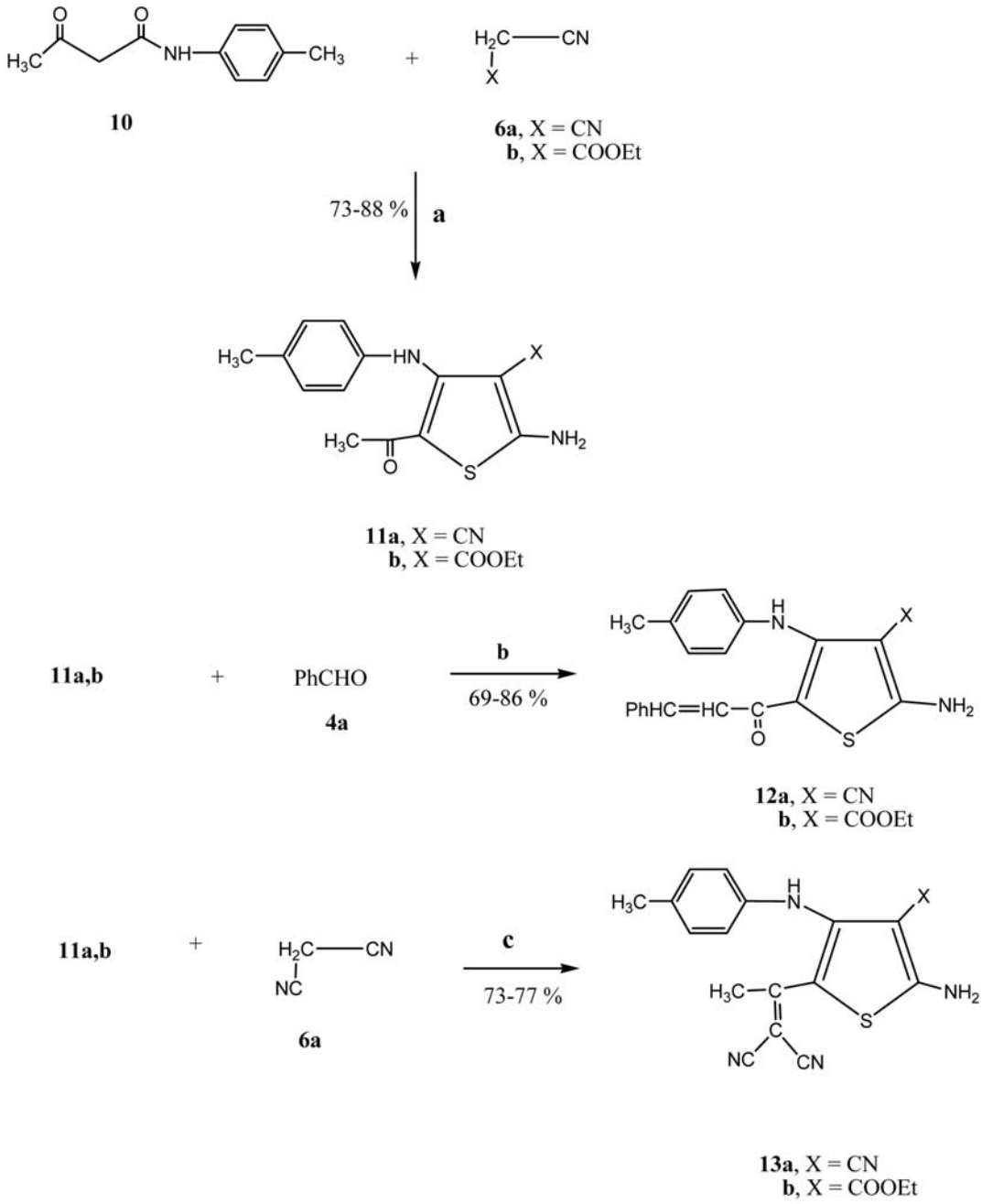
Compounds **3a**,**b** reacted with either benzaldehyde (**4a**) or salicylaldehyde (**4b**) to give the hydrazide-hydrazone derivatives **5a–d**, respectively. Compounds **5b**,**d** were identical to those reported by Jagtab et al.[21] The analytical and spectral data of compounds **5a**,**c** are consistent



Sheme 1. Reaction of 1 with elemental sulfur and the cycloketones **2a,b** and reaction of **3a,b** with aromatic aldehydes **4a,b** and synthesis of the pyrazole deravatives **7a,d** and **8a,d.** Reagents and conditions: (a) Et_3N, absolute ethanol, heat 3h; (b) 1,4-dioxan, heat 2 h; (c) 1,4-dioxan, Et_3N, heat 3 h; (d) 1,4-dioxan, Et_3N, heat 3h.

with their respective structures (see experimental section). On the other hand, the hydrazido moiety present in compounds **3a**,**b** showed high activity towards cyanomethylene reagents. Thus, their reaction with either malononitrile (**6a**) or ethyl cyanoacetate (**6b**) in 1,4-dioxan gave the pyrazole derivatives **7a–d**. Similarly the reaction of either **3a** or **3b** with either acetylacetone (**8a**) or ethyl acetoacetate (**8b**) gave the pyrazole derivatives **9a–d** (Scheme 1). The structures of compounds **7a–d** and **9a–d** were based on their respective ^{1}H- and ^{13}C-NMR data. Thus, the ^{1}H-NMR spectrum of **9a** (as an example) showed beside the expected signals, two singlets at δ 2.33, 2.82 ppm corresponding to the two CH_3 groups, a singlet at δ 4.23 ppm for NH_2 group and a singlet at δ 6.59 ppm for the pyrazole H-3 proton. Moreover, the ^{13}C-NMR spectrum showed δ: 16.1, 19.6 (2 CH_3), 21.5, 24.7, 26.8, 34.2 (cyclopentene CH_2), 123.8, 127.8, 134.2, 143.9, 145.2, 147.3, 152.8 (thiophene C, pyrazole C), 163.5 (C=O).

Next we moved towards the synthesis of another poly-functionally substituted thiophenes using 1,3-dicar-



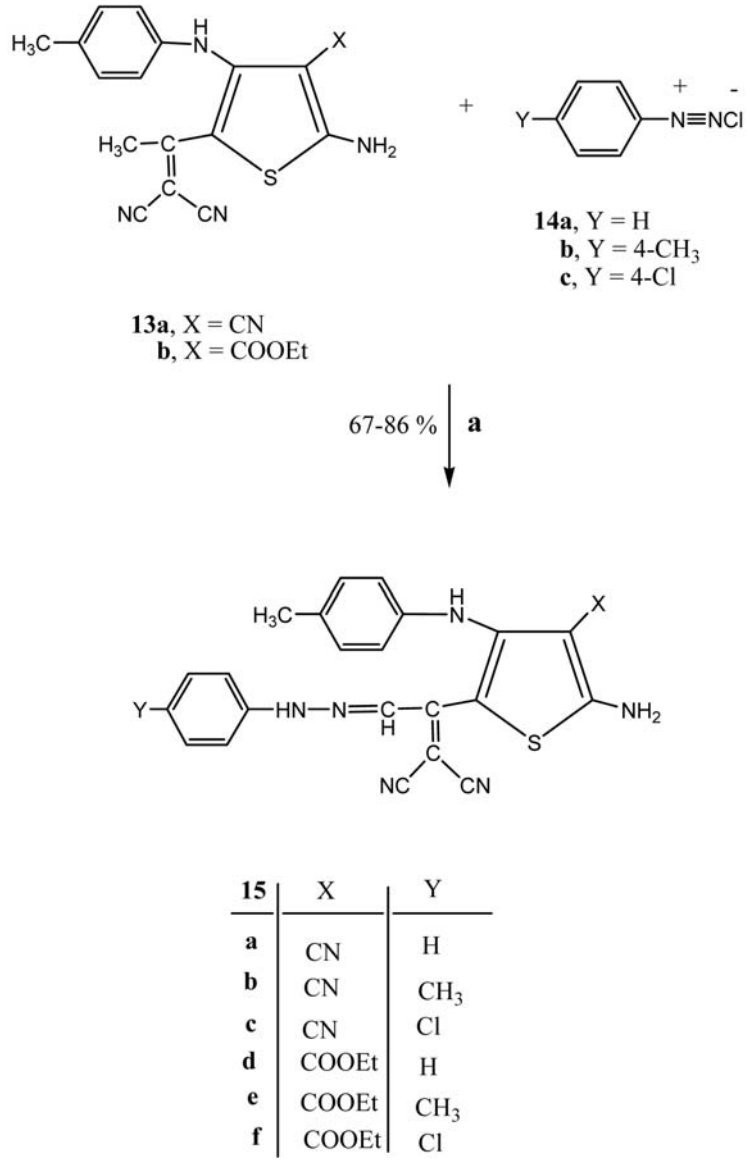
Sheme 2. Synthesis of the thiophene derivatives **11a,b** and their reaction with benzaldehyde and malononitrile. Reagents and conditions (a) S_8, EtOH, Et_3N, heat 1 h; (b) 1,4-dioxan, piperidine, heat 1 h; (c) 1,4-dioxan, Et_3N, heat 3 h.

bonyl compound and cyanomethylene reagents. Thus, the reaction of the 3-oxo-*N*-*p*-tolylbutanamide (**10**) with either malononitrile (**6b**) or ethyl cyanoacetate (**6b**) and elemental sulfur as an application of the well known Gewald's thiophene synthesis,[26] yielded the polyfunctionally substituted thiophene derivatives **11a**,**b**, respectively. The analytical and spectral data of the latter products are in agreement with the assigned structures (see experimental section).

The reactivity of **11a**,**b** towards some chemical reagents was studied in the aim of synthesizing thiophene derivatives with potential anti-tumor activity. Thus, the reaction of either **11a** or **11b** with benzaldehyde (**4a**) gave the benzal derivatives **12a**,**b**, respectively. Analogous to the known Knoevenagel condensation[27,28] we found that the reaction of either **11a** or **11b** with malononitrile (**6a**) gave the corresponding Knoevenagel condensated products **13a**,**b**, respectively (Scheme 2) .

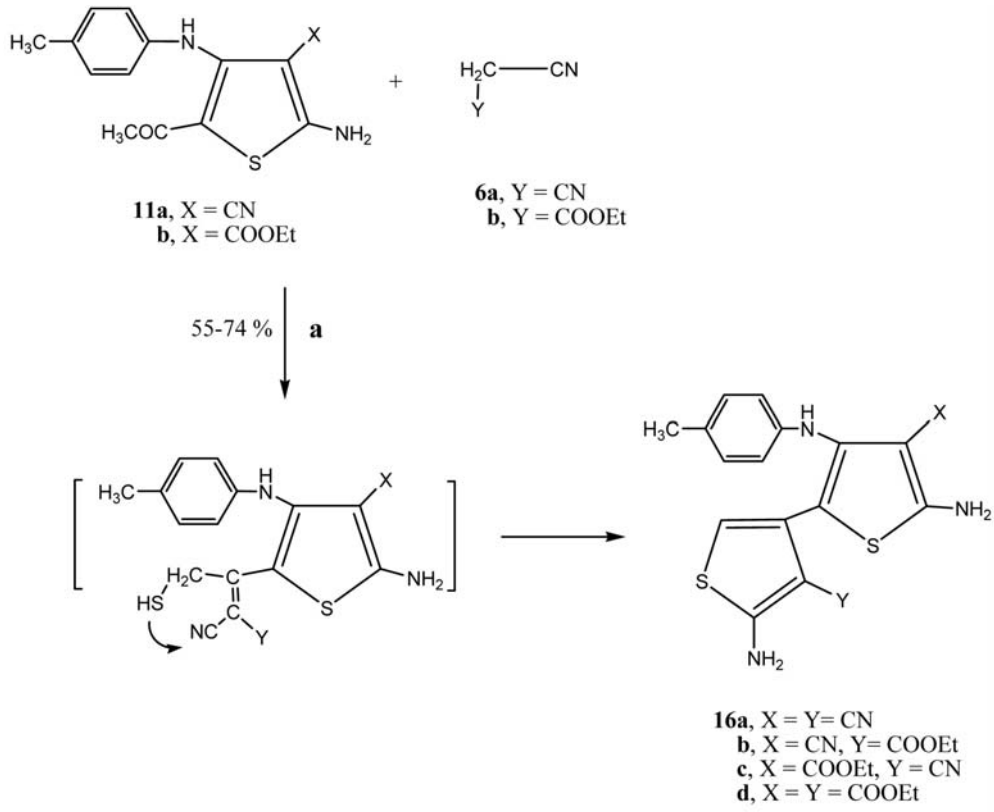
The excellent yields of compounds **13a**,**b** encouraged us to explore further reactions to study the coupling reactions of **13a**,**b** with aryl diazonium salts. Thus, with the diazonium salts namely, benzenediazonium chloride (**14a**), 4-methylbenzenediazonium chloride (**14b**) and 4-chlorobenzenediazonium chloride (**14c**) gave the aryl hydrazono derivatives **15a**–**f**, respectively (Scheme 3). The analytical and spectral data are in agreement with the assigned structures (see experimental section).

Encouraged by the excellent results, we next investigated the reactivity of the acetyl group present in **11a**,**b** using the well known Gewald's reaction.[29,30] Thus, the reaction of either **11a** or **11b** reacted with either malono-



15	X	Y
a	CN	H
b	CN	CH_3
c	CN	Cl
d	COOEt	H
e	COOEt	CH_3
f	COOEt	Cl

Sheme 3. Synthesis of the arylhydrazo derivatives **15a–f**. Reagents and conditions NaOH, EtOH, 0 °C.



Sheme 4. Synthesis of the thiophene derivatives **16a–d**. Reagents and conditions (a) 1,4 dioxan, Et_3N, S_8 heat 2 h.

nitrile (**6a**) or ethyl cyanoacetate (**6b**) and elemental sulfur in the presence of triethylamine gave the thio-3-yl thiophene derivatives **16a**–**d**, respectively (Scheme 4).

2. 2. Antitumor and Normal Cell Line Activity Tests

2. 2. 1. 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 a monolayer and are 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 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 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.

2. 2. 2. Tumor Cell Growth Assay

The effects of **3a**,**b**–**16a**–**d** 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 ž*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth (12). 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 mM. 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, Winooski, VT, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), correspon-

Table 1. Effect of compounds 3a,b–16a–d on the growth of three human tumor cell lines

Compound	GI_{50} (m mol L^{-1})			
	MCF-7	NCI-H460	SF-268	WI 38
3a	18.1 ± 1.6	6.2 ± 2.8	8.6 ±2.6	44.2 ± 10.2
3b	22.6 ± 2.6	24.3 ± 0.8	30.9 ± 3.8	60.2 ± 8.0
5a	70.6 ± 16.9	38.9 ± 10.8	52.8 ± 8.6	77.8 ± 16.8
5b	30.6 ± 10.2	32.6 ± 8.6	24.4 ± 12.8	33.4 ± 12.6
5c	6.4 ± 2.2	4.1 ± 0.8	8.8 ± 4.8	20.8 ± 8.2
5d	10.8 ± 2.6	4.5 ± 0.8	4.8 ± 1.8	28.0 ± 4.1
7a	70.7 ± 18.5	40.2 ± 12.8	52.4 ± 8.6	60.2 ±12.4
7b	55.1 ± 2.7	23.2 ± 4.8	14.4 ± 2.6	44.3 ±10.6
7c	20.4 ± 2.2	30.6 ± 1.4	20.8 ± 6.4	8.3 ± 3.8
7d	0.02 ± 0.008	0.03 ± 0.006	0.05 ± 0.001	> 100
9a	12.8 ± 2.6	22.0 ± 0.4	30.5 ± 6.0	14.8 ± 1.5
9b	10.8 ± 2.6	14.1 ± 0.6	22.3 ± 0.8	8.9 ± 4.2
9c	60.2 ± 2.4	43.6 ± 1.8	58.8 ± 0.8	66.2 ±8.2
9d	22.2 ± 0.8	22.3 ± 2.5	30.6 ± 0.8	8.7 ± 2.6
11a	12.8 ± 3.6	20.6 ± 3.4	24.2 ± 0.8	6.5 ± 1.4
11b	60.6 ± 10.4	40.8 ± 10.8	22.1 ± 2.8	12.2 ± 3.8
12a	0.02 ± 0.002	0.01 ± 0.002	0.06 ± 0.008	> 100
12b	40.6 ± 2.6	22.6 ± 2.6	35.2 ± 12.8	10.5 ± 5.1
13a	20.8 ± 6.8	18.2 ± 1.6	18.6 ± 4.8	> 100
13b	44.8 ± 8.6	38.3 ± 4.5	22.6 ± 5.5	14.2 ± 2.4
15a	32.4 ± 8.8	18.7 ± 6.2	30.4 ± 2.4	22.6 ± 9.2
15b	55.7 ± 8.1	26.9 ± 4.4	44.5 ± 6.9	64.0 ±12.4
15c	4.2 ± 1.8	2.8 ± 0.5	2.3 ± 0.8	2.4 ± 0.8
15d	22.4 ± 4.6	20.3 ± 2.8	18.8 ± 4.6	0.4 ± 0.01
15e	38.4 ± 6.2	24.2 ± 0.8	20.6 ± 2.6	26.6 ± 4.6
15f	68.2 ± 8.6	22.3 ± 2.6	50.5 ± 12.5	30.8 ± 4.2
16a	0.01 ± 0.002	0.03 ± 0.8	0.05 ± 0.01	> 100
16b	0.06 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.2 ± 0.6
16c	4.6 ± 1.8	2.3 ± 1.2	2.0 ± 0.4	> 100
16d	6.4 ± 0.8	4.3 ± 2.6	2.8 ± 0.6	0.2 ± 0.6
Doxorubicin	0.04 ± 0.008	0.09±0.008	0.09±0.007	> 100

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.

ding 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. 2. 3. Effect on the Growth of Human Tumor Cell Lines

The effect of compounds **3a**,**b**–**16a**–**d** was evaluated on the *in vitro* growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) together with the normal fibroblast cells (WI 38) after a continuous exposure for 48h. The results are summarized in Table 1. All of the tested compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependant manner. The results in Table 1 reveal that compounds **7d**, **12a**, **16a** and **16b** showed the highest inhibitory effect against all the three tumor cell lines«, such activity is higher than the refrence doxorubicin. The inhibitory effect of **7a**, **12a** and **16a** are very low against normal fibroblast cells (WI 38). While compounds **5d** and **16b** showed high inhibitory effects against non-small cell lung cancer (NCI-H460) and breast adenocarcinoma (MCF-7), respectively, which are less than the reference doxorubicin. Compounds **5b**, **5c**, **7a**, **7b**, **9c**, **11b**, **12a**, **13b**, **15a**, **15b**, **15e** and **15f** showed the lowest inhibitory effect. The rest of the compounds showed a moderate growth inhibitory effect. Comparing compound **5a–d**, it is obvious that the presence of the 2-OH group in compounds **5c** and **5d** is reposible for their reactivity over **5a** and **5b**. Similarly comparing of **12a** and **12b**, it is obvious that the introduction of the CN group in **12a** showed higher inhibitory effect towards the three cell lines than that of **12b**. On the other hand, comparing the inhibitory effect of compounds **15a–f**, one can say that compound **15c** with the X = CN and Y = Cl showed the highest inhibitory effect

among the six compounds but such reactivity is lower than that of the reference doxorubicin. Similarly, comparison of compounds **16a–d** showed that when X = Y = CN, like in **16a**, the maximum inhibitory effect among the four compounds was obtained. However, when X = CN and Y = COOEt as in the case of **16b**, the inhibitory effect was lowered but not by a large amount as the compound is still one of the most active compounds among all test compounds. On the other hand, introduction of the ester group, like in **16c**, decreases the reactivity and such observation was shifted towards lower reactivity in case of **16d** where X = Y = COOEt.

3. Experimental

3. 1. General

All melting points are uncorrected. IR spectra were recorded as KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H- and ^{13}C NMR spectra were measured on a Varian EM-390-200 MHz in CD_3SOCD_3 as the solvent using TMS as the internal standard and chemical shifts are expressed as δ. Analytical data were obtained from the Micro analytical Data Unit at Cairo University, Giza, Egypt. Physical and spectral data of compounds **3b**, **5b** and **5d** as reported in the literature.[21–25]

3. 1. 1. General Procedure for the Synthesis of Tetrahydobenzo[b]thiophene Derivatives 3a,b

To a solution of either cyclopentanone (8.50 g, 0.01 mol) or cyclohexanone (9.9 g, 0.1 mol) in abs. ethanol (50 mL) containing triethylamine (2.0 mL), cyanoacetylhydrazine (10.0 g, 0.1 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then left to cool. The formed solid product was collected by filtration and crystallized from the proper solvent.

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbohydrazide (3a). Yellow crystals from ethanol, yield 88% (17.42 g), m.p. 230–233 °C. *Anal*. Calculated for $C_8H_{11}N_3OS$ (197.26): C, 48.71; H, 5.62; N, 21.30; S, 16.26. Found: C, 49.01; H, 5.51; N; 21.17; S, 16.31. MS: *m/e* 197 (M^+, 20%), IR, ν: 3483–3205 (NH_2, NH), 3054 (CH, aromatic), 1699 (C=O). ^{1}H-NMR, δ: 1.77–1.79 (m, 4H, $2CH_2$), 2.20 (m, 2H, CH_2), 4.20–4.22 (2m, 4H, $2NH_2$), 9.01 (s, 1H, NH). ^{13}C-NMR, δ: 22.0, 23.4, 30.9, 122.6, 136.3, 138.9, 144.5, 166.8.

3. 1. 2. General Procedure for the Synthesis of the N-Arylidenocarbohydrazide Derivatives 5a–b

To a solution of either **3a** (1.97 g, 0.01 mol) or **3b** (2.11 g, 0.01 mol) in 1,4-dioxan (40 mL) either benzaldehyde (1.0 g, 0.01 mol) or salicylaldehyde (1.22 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h then poured onto ice/water. The formed solid product was collected by filtration and crystallized from the proper solvent.

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-(*N*-benzal)-carbohydrazide (5a). Yellow crystals from ethanol, yield 90% (2.56 g), m.p. 214–217 °C. *Anal*. Calculated for $C_{15}H_{15}N_3OS$ (285.26): C, 63.13; H, 5.30; N, 14.73; S, 11.24. Found: C, 63.01; H, 5.44; N; 14.82; S, 11.43. MS: *m/e* 285.36 (M^+, 5%), IR, ν: 3466–3223 (NH_2, NH), 3056 (CH, aromatic), 1689 (C=O). ^{1}H-NMR, δ: 1.71–1.74 (m, 4H, $2CH_2$), 2.19 (m, 2H, CH_2), 4.23 (m, 2H, NH_2), 6.78 (s, 1H, CH), 7.28–7.38 (m, 5H, C_6H_5), 9.30 (s, 1H, NH). ^{13}C-NMR, δ: 22.8, 25.6, 27.2, 33.8, 116.9, 117.4, 120.6, 122.6, 128.9, 134.3, 135.9, 145.1, 163.6, 169.8.

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-(*N*-benzal)-carbohydrazide (5c). Yellow crystals from ethanol, yield 66% (1.98 g), m.p. 120°C. *Anal*. Calculated for $C_{15}H_{15}N_3O_2S$ (301.36): C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 59.94; H, 5.31; N; 14.05; S, 10.48. MS: *m/e* 301 (M^+, 15%), IR, ν: 3475–3219 (NH_2, NH), 3053 (CH, aromatic), 1686 (C=O). ^{1}H-NMR, δ: 1.75–1.78 (m, 4H, $2CH_2$), 2.23 (m, 2H, CH_2), 4.26 (m, 2H, NH_2), 6.83 (s, 1H, CH), 7.29–7.36 (m, 5H, C_6H_5), 9.28 (s, 1H, NH), 10.51 (s, 1H, OH). ^{13}C-NMR, δ: 23.2, 25.5, 26.9, 34.1, 116.3, 118.0, 121.3, 122.8, 129.2, 133.8, 144.2, 148.6, 164.2, 168.4.

3. 1. 3. General Procedure for the Synthesis of the Pyrazole Derivatives 7a–d

To a solution of either **3a** (1.97 g, 0.01 mol) or **3b** (2.11 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.5 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h. The solid product, formed in each case, upon evaporation under vacuum and trituration with ethanol, was collected by filtration and crystallized from the proper solvent.

(3,5-Diamino-1*H*-pyrazol-1-yl)(2-amino-5,6-dihydro-4*H*-cycopenta[*b*]-thiophen-3-yl)methanone (7a). Orange crystals from ethanol, yield 82% (2.15 g), m.p. 188–191 °C. *Anal*. Calculated for $C_{11}H_{13}N_5OS$ (263.32): C, 50.17; H, 4.98; N, 26.60; S, 12.18. Found: C, 50.42; H, 5.24; N; 26.38; S, 12.41. MS: *m/e* 263 (M^+, 12%), IR, ν: 3472–3313 ($3NH_2$), 3053 (CH, aromatic), 1693 (C=O). ^{1}H-NMR, δ: 1.70–1.75 (m, 4H, $2CH_2$), 2.21 (m, 2H, CH_2), 4.21, 4.45, 5.42 (3s, 6H, $3NH_2$), 6.55 (s, 1H, pyrazole H-3). ^{13}C-NMR, δ: 21.2, 25.2, 28.0, 34.6, 125.0, 128.3, 136.3, 144.5, 146.8, 148.6, 154.2, 162.3.

(3,5-Diamino-1*H*-pyrazol-1-yl)(2-amino-4,5,6,7-tetrahydrobenzo[*b*]-thiophen-3-yl)methanone (7b). Yellow crystals from ethanol, yield 86% (2.38 g), m.p. 185–187 °C. *Anal*. Calculated for $C_{12}H_{15}N_5OS$ (277.35): C, 51.97; H, 5.45; N, 25.25; S, 11.56. Found: C, 51.75; H, 5.33; N; 25.06; S, 11.43. MS: *m/e* 277 (M^+, 8%), IR, ν: 3464–3236 (3 NH_2), 3059 (CH, aromatic), 1686 (C=O). ^{1}H-NMR, δ: 1.84–1.96 (m, 4H, $2CH_2$), 2.23–2.35 (m, 4H, $2CH_2$), 4.44, 4.48, 5.22 (3s, 6H, $3NH_2$), 6.59 (s, 1H, pyrazole H-3). ^{13}C-NMR, δ: 22.8, 24.8, 26.7, 32.6, 36.8, 124.7, 127.8, 134.0, 144.2, 147.3, 148.6, 153.8, 160.2

(2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophe-3-yl)(3-amino-5-hydroxy-1*H*-pyrazol-1-yl)methanone (7c). Orange crystals from ethanol, yield 77% (2.03 g), m.p. 145–146 °C. *Anal*. Calculated for $C_{11}H_{12}N_4O_2S$ (264.07): C, 49.99; H, 4.58; N, 21.20; S, 12.13. Found: C, 50.11; H, 4.66; N; 21. 32; S, 12.25. MS: *m/e* 264 (M^+, 10%), IR, ν: 3462–3333 (OH, $2NH_2$), 3056 (CH, aromatic), 1690 (C=O). ^{1}H-NMR, δ: 1.72–1.78 (m, 4H, $2CH_2$), 2.24 (m, 2H, CH_2), 4.30, 4.47 (2s, 4H, $2NH_2$), 6.58 (s, 1H, pyrazole H-3), 10.38 (s, 1H, OH). ^{13}C-NMR, δ: δ: 22.0, 25.3, 27.8, 34.3, 126.2, 126.8, 135.8, 138.9, 142.4, 146.6, 153.8, 164.0.

(2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(3-amino-5-hydroxy-1*H*-pyrazol-1-yl)methanone (7d). Orange crystals from ethanol, yield 59% (1.64 g), m.p. 142–144 °C. *Anal*. Calculated for $C_{12}H_{14}N_4O_2S$ (278.33): C, 51.78; H, 5.07; N, 20.13.; S, 11.52. Found: C, 51.52; H, 5.34; N; 20.08; S, 11.63. MS: *m/e* 278 (M^+, 32%), IR, ν: 3477–3232 (OH, 2 NH_2), 3052 (CH, aromatic), 1689 (C=O). ^{1}H-NMR, δ: 1.84–1.99 (m, 4H, $2CH_2$), 2.24–2.37 (m, 4H, $2CH_2$), 4.43, 4.49 (2s, 4H, $2NH_2$), 6.56 (s, 1H, pyrazole H-3), 10.11 (s, 1H, OH). ^{13}C-NMR, δ: 22.5, 24.5, 26.2, 32.6, 35.4, 124.0, 126.9, 132.8, 138.0, 145.3, 148.0, 154.3, 162.6.

3. 1. 4. General Procedure for the Synthesis the Pyrazole Derivatives 9a–d

To a solution of either **3a** (1.97 g, 0.01 mol) or **3b** (2.11 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.5 mL) either acetylacetone (1.0 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h. The solid product, formed in each case, upon evaporation under vacuum and trituration with ethanol, was collected by filtration and crystallized from the proper solvent.

(5-Amino-3-methyl-1*H*-pyrazol-1-yl)(2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophe-3-yl)methanone (9a). Orange crystals from ethanol, yield 79% (2.07 g), m.p. 135–138 °C. *Anal*. Calculated for $C_{13}H_{15}N_3OS$ (261.34): C, 59.74; H, 5.79; N; 16.08; S, 12.27. Found: C, C, 59.94; H, 5.58; N, 16.26; S, 12.29. MS: *m/e* 261 (M^+, 40%), IR, ν: 3466–3337 (2 NH_2), 3050 (CH, aromatic), 1690 (C=O). ^{1}H-NMR, δ: 1.71–1.78 (m, 4H, $2CH_2$), 2.23 (m, 2H, CH_2), 2.33, 2.82 (2s, 6H, $2CH_3$), 4.23 (s, 2H, NH_2), 6.59 (s, 1H, pyrazole H-3). ^{13}C-NMR, δ: 16.1, 19.6, 21.5, 24.7, 26.8, 34.2, 123.8, 127.8, 134.2, 143.9, 145.2, 147.3, 152.8, 163.5.

(5-Amino-3-methyl-1*H*-pyrazol-1-yl)(2-amino-4,5,6,7-tetrahydro-benzo[*b*]thiophen-3-yl)methanone (9b). Yellow crystals from ethanol, yield 66% (1.82 g), m.p. 133–135 °C. *Anal*. Calculated for $C_{14}H_{17}N_3OS$ (275.37): C, 61.06; H, 6.22; N, 15.26; S, 11.64. Found: C, 61.25; H, 6.73; N; 15.33; S, 16.52. MS: *m/e* 276 (M^+, 20%), IR, ν: 3458–3232 (2 NH_2), 3054 (CH, aromatic), 1689 (C=O). ^{1}H-NMR, δ: 1.86–1.99 (m, 4H, $2CH_2$), 2.21-2.56 (m, 4H, $2CH_2$), 2.49, 2.72 (2s, 6H, $2CH_3$), 4.43, 4.49 (2s, 4H, $2NH_2$), 6.63 (s, 1H, pyrazole H-3). ^{13}C-NMR, δ: 16.8, 19.9, 22.4, 25.1, 26.2, 30.6, 36.2, 124.9, 128.2, 134.4, 143.9, 146.6, 148.8, 154.2, 162.2.

(5-Hydroxy-3-methyl-1*H*-pyrazol-1-yl)(2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophe-3-yl)methanone (9c). Pale brown crystals from ethanol, yield 80% (2.10 g), m.p. 188–190 °C. *Anal*. Calculated for $C_{12}H_{13}N_3O_2S$ (263.32): C, 54.74; H, 4.98; N, 15.96; S, 12.18. Found: C, 54.93; H, 4.78; N; 16.09; S, 12.42. MS: *m/e* 263 (M^+, 22%), IR, ν: 3474–3382 (OH, NH_2), 3052 (CH, aromatic), 1688 (C=O). ^{1}H-NMR, δ: 1.73–1.80 (m, 4H, $2CH_2$), 2.26 (m, 2H, CH_2), 2.66 (s, 3H, CH_3), 4.38 (s, 2H, NH_2), 6.56 (s, 1H, pyrazole H-3), 10.42 (s, 1H, OH). ^{13}C-NMR, δ: δ: 22.6, 25.2, 28.2, 34.4, 125.8, 127.1, 134.5, 137.2, 143.4, 145.6, 158.2, 163.8.

(5-Hydroxy-3-methyl-1*H*-pyrazol-1-yl)(2-amino-4,5,6,7-tetrahydro-benzo[*b*]thiophen-3-yl)methanone (9d). Yellow crystals from ethanol, yield 83% (2.30 g), m.p. 110–112 °C. *Anal*. Calculated for $C_{13}H_{15}N_3O_2S$ (277.34): C, 56.30; H, 5.45; N, 15.15.; S, 11.56. Found: C, 56.45; H, 5.60; N; 15.28; S, 11.58. MS: *m/e* 277 (M^+, 14%), IR, ν: 3456–3241 (OH, NH_2), 3050 (CH, aromatic), 1685 (C=O). ^{1}H-NMR, δ: 1.83–1.95 (m, 4H, $2CH_2$), 2.22–2.38 (m, 4H, $2CH_2$), 2.69 (s, 3H, CH_3), 4.40 (s, 2H, NH_2), 6.58 (s, 1H, pyrazole H-3), 10.22 (s, 1H, OH). ^{13}C-NMR, δ: 22.9, 24.2, 26.5, 33.1, 35.4, 122.9, 124.2, 133.5, 137.8, 145.6, 147.3, 158.3, 163.5.

3. 1. 5. General Procedure for the Synthesis of the 2-Aminothiophene Derivatives 11a,b

To a solution of acetoacetanilide (1.78 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (1.5 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added followed by elemental sulfur (0.32 g, 0.01 mol). The whole reaction mixture was heated under reflux for 1 h then poured onto ice/water containing a few drops of hydrochloric acid. The solid product, for-

med in each case, was collected by filtration and crystallized using a suitable solvent.

4-(*p*-Tolylamino)-5-acetyl-2-aminothiophene-3-carbonitrile (11a). Orange crystals from ethanol, yield 73% (2.18 g), m.p. 221–223 °C. *Anal*. Calculated for $C_{14}H_{13}N_3OS$ (271.34): C, 61.97; H, 4.83; N, 15.49; S, 10.71. Found: C, 61.85; H, 4.60; N; 15.18; S, 10.59. MS: *m/e* 271 (M^+, 18%), IR, ν: 3458–3322 (NH_2, NH), 3054 (CH, aromatic), 2220 (CN), 1689 (C=O). ^{1}H-NMR, δ: 2.68, 2.93 (2s, 6H, $2CH_3$), 4.42 (s, 2H, NH_2), 7.28–7.33 (m, 4H, C_6H_4), 8.72 (s, 1H, NH). ^{13}C-NMR, δ: 24.8, 26.9, 118.5, 124.6, 126.0, 129.5, 132.5, 119.5, 135.8, 148.2, 155.7, 163.8.

Ethyl 4-(*p*-tolylamino)-5-acetyl-2-aminothiophene-3-carboxylate (11b). Orange crystals from ethanol, yield 88% (3.45 g), m.p. 172–175 °C. *Anal*. Calculated for $C_{16}H_{18}N_2O_3S$ (318.39): C, 60.36; H, 5.70; N, 8.80.; S, 10.07. Found: C, 60.78; H, 5.42; N; 8.93; S, 10.21. MS: *m/e* 318 (M^+, 20%), IR, ν: 3456–3320 (NH_2, NH), 3055 (CH, aromatic), 1693, 1687 (2 C=O). ^{1}H-NMR, δ: 1.16 (t, 3H, J = 7.22 Hz, CH_3), 2.68, 2.91 (2s, 6H, $2CH_3$), 4.25 (q, 2H, J = 7.22 Hz, CH_2), 4.46 (s, 2H, NH_2), 7.29–7.37 (m, 4H, C_6H_4), 8.68 (s, 1H, NH). ^{13}C-NMR, δ: 19.7, 24.2, 26.7, 44.2, 122.8, 124.7, 128.8, 134.2, 118.9, 136.3, 145.8, 154.8, 160.2, 163.8.

3. 1. 6. General Procedure for the Synthesis of Phenylacryloylthiophene Derivatives 12a,b

To a solution of either **11a** (2.99 g, 0.01 mol) or **11b** (3.46 g, 0.01 mol) in 1,4-dioxan (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 crystallized from a suitable solvent.

4-(*p*-Tolylamino)-2-amino-5-(3-phenylacryloyl)thiophene-3-carbontrile (12a). Orange crystals from ethanol, yield 86% (3.32 g), m.p. 232–235 °C. *Anal*. Calculated for $C_{21}H_{17}N_3OS$ (359.44): C, 70.17; H, 4.77; N, 11.69; S, 8.92. Found: C, 70.35; H, 4.68; N; 11.98; S, 8.99. MS: *m/e* 359 (M^+, 23%), IR, ν: 3468–3382 (NH_2, NH), 3055 (CH, aromatic), 2227 (CN), 1687 (C=O). ^{1}H-NMR, δ: 2.83 (s, 3H, CH_3), 4.46 (s, 2H, NH_2), 6.56, 6.99 (2d, 2H, CH=CH), 7.31–7.46 (m, 9H, C_6H_5, C_6H_4), 8.62 (s, 1H, NH). ^{13}C-NMR, δ: 26.4, 117.2, 118.1, 119.0, 120.3, 123.7, 129.5, 124.6, 130.0, 131.5, 132.5, 120.5, 135.8, 148.2, 155.7, 162.8.

Ethyl 4-(*p*-tolylamino)-2-amino-5-(3-phenylacryloyl) thiophene-3-carboxylate (12b). Orange crystals from acetic acid, yield 69% (2.99 g), m.p. 155–157 °C. *Anal*. Calculated for $C_{23}H_{22}N_2O_3S$ (406.14): C, 67.96; H, 5.46; N, 6.89; S, 7.89. Found: C, 67.66; H, 5.25; N; 6.61; S, 7.76. MS: *m/e* 406 (M^+, 30%), IR, ν: 3456–3342 (NH_2, NH), 3050 (CH, aromatic), 1689-1687 (2 C=O). ^{1}H-NMR, δ: 1.16 (t, 3H, J = 7.06 Hz, CH_3), 2.69 (s, 3H, CH_3), 4.25 (q, 2H, J = 7.06 Hz, CH_2), 4.64 (s, 2H, NH_2), 6.69, 6.86 (2d, 2H, CH=CH), 7.29–7.37 (m, 9H, C_6H_5, C_6H_4), 8.88 (s, 1H, NH). ^{13}C-NMR, δ: 22.2, 26.9, 46.0, 117.4, 118.9, 120.5, 126.4, 127.6, 136.1, 117.9, 138.0, 146.4, 154.2, 162.8, 163.2.

3. 1. 7. General Procedure for the Synthesis of the Dicyanoprop-1-en-2-yl thiophene Derivatives 13a,b

To a solution of either **11a** (2.99 g, 0.01 mol) or **11b** (3.46 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.0 mL), malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture, in each case, 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 crystallized from a suitable solvent.

4-(*p*-Tolylamino)-2-amino-5-(1,1-dicyanoprop-1-en-2-yl)thiophene-3-carbonitrile (13a). Orange crystals from ethanol, yield 73% (2.53 g), m.p. 152–154 °C. *Anal*. Calculated for $C_{17}H_{13}N_5S$ (319.38): C, 63.93; H, 4.10; N, 21.93; S, 10.04. Found: C, 63.57; H, 4.01; N; 21.78; S, 10.29. MS: *m/e* 319 (M^+, 38%), IR, ν: 3472–3342 (NH_2, NH), 3050 (CH, aromatic), 2228, 2220 (2 CN). ^{1}H-NMR, δ: 2.73, 3.02 (2s, 6H, $2CH_3$), 4.62 (s, 2H, NH_2), 7.32–7.45 (m, 9H, C_6H_5, C_6H_4), 8.76 (s, 1H, NH). ^{13}C-NMR, δ: 24.8, 26.4, 116.5, 117.2, 118.8, 117.8, 118.9, 123.7, 124.8, 130.5, 134.6, 122.6, 133.9, 146.8, 154.9.

Ethyl 4-(*p*-tolylamino)-2-amino-5-(1,1-dicyanoprop-1-en-2-yl)thiophene-3-carboxylate (13b). Yellow crystals from 1,4-dioxan, yield 77% (3.03 g), m.p. 133–135 °C. *Anal*. Calculated for $C_{19}H_{18}N_4O_2S$ (366.44): C, 62.28; H, 4.95; N, 15.29; S, 8.75. Found: C, 60.76; H, 4.51; N; 13.89; S, 8.62. MS: *m/e* 366 (M^+, 18%), IR, ν: 3642–3338 (NH_2, NH), 3053 (CH, aromatic), 1690 (C=O). ^{1}H-NMR, δ: 1.13 (t, 3H, J = 7.16 Hz, CH_3), 2.70, 3.01 (2s, 6H, $2CH_3$), 4.22 (q, 2H, J = 7.16 Hz, CH_2), 4.55 (s, 2H, NH_2), 7.30–7.35 (m, 5H, C_6H_4), 8.85 (s, 1H, NH). ^{13}C-NMR, δ: 22.0, 26.9, 29.1, 45.5, 116.4, 117.5, 117.4, 118.9, 120.8, 125.9, 126.8, 136.9, 118.3, 136.9, 145.5, 153.0, 164.6.

3. 1. 8. General Procedure for the Synthesis of the Arylhydrazone Derivatives 15a–f

To a cold solution of **13a** (3.74 g, 0.01 mol) or **13b** (3.94 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) 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 crystallized using a suitable solvent.

5-(3-(2-Phenylhydrazono)-1,1-dicyanoprop-1-en-2-yl)-4-(*p*-tolylamino) -2-aminothiophene-3-carbonitrile (15a). Brown crystals from ethanol, yield 80% (3.61 g), m.p. 182–184 °C. *Anal*. Calculated for $C_{23}H_{17}N_7S$ (423.49): C, 65.23; H, 4.05; N, 23.15; S, 7.57. Found: C, 65.66; H, 4.29; N; 23.48; S, 7.49. MS: *m/e* 423 (M^+, 12%), IR, ν: 3462–3348 (NH_2, 2NH), 3053 (CH, aromatic), 2226, 2222–2220 (3 CN). ^{1}H-NMR, δ: 3.02 (s, 3H, CH_3), 4.42 (s, 2H, NH_2), 6.62 (s, 1H, N=CH), 7.30–7.43 (m, 9H, C_6H_5, C_6H_4), 8.38, 8.56 (2s, 2H, 2NH). ^{13}C-NMR, δ: 22.6, 116.8, 117.6, 118.3, 117.4, 118.9, 120.3, 122.7, 123.0, 123.9, 124.2, 131.5, 136.3, 120.3, 126.8, 144.6, 152.5, 156.0, 158.3, 167.0.

5-(3-(2-*p*-Tolylhydrazono)-1,1-dicyanoprop-1-en-2-yl)-4-(*p*-tolylamino)-2-aminothiophene-3-carbonitrile (15b). Orange crystals from ethanol, yield 85% (3.95 g), m.p. 192–194 °C. *Anal*. Calculated for $C_{24}H_{19}N_7S$ (437.52): C, 65.88; H, 4.38; N, 22.41; S, 7.33. Found: C, 65.92; H, 4.29; N; 22.38; S, 7.28. MS: *m/e* 437 (M^+, 8%), IR, ν: 3460–3343 (NH_2, 2NH), 3056 (CH, aromatic), 2228, 2224–2220 (3 CN). ^{1}H-NMR, δ: 2.99, 3.01 (2s, 6H, $2CH_3$), 4.46 (s, 2H, NH_2), 6.59 (s, 1H, N=CH), 7.28–7.46 (m, 8H, 2 C_6H_4), 8.39, 8.45 (2s, 2H, 2NH). ^{13}C-NMR, δ: 24.3, 116.7, 117.4, 118.2, 119.4, 122.9, 123.4, 124.9, 134.4, 134.5, 143.9, 123.9, 125.6, 144.2, 154.8, 156.1, 159.3, 167.2.

2-(3-(2-*p*-Chlorohydrazono)-1,1-dicyanoprop-1-en-2-yl)-5-amino-4-(*p*-tolylamino)-2-aminothiophene-3-carbonitrile (15c). Orange crystals from ethanol, yield 67% (3.25 g), m.p. 222–225 °C. *Anal*. Calculated for $C_{23}H_{16}ClN_7S$ (457.94): C, 60.32; H, 3.52; N, 21.41; S, 7.00. Found: C, 60.36; H, 3.36; N; 21.31; S, 7.22. MS: *m/e* 485 (M^+, 44%), IR, ν: 3473–3339 (NH_2, 2NH), 3059 (CH, aromatic), 2224, 2224–2222 (3 CN). ^{1}H-NMR, δ: 3.01 (s, 3H, CH_3), 4.42 (s, 2H, NH_2), 6.62 (s, 1H, N=CH), 7.29–7.43 (m, 8H, 2 C_6H_4), 8.36, 8.29 (2s, 2H, 2NH). ^{13}C-NMR, δ: 116.3, 117.8, 118.4, 119.2, 122.4, 123.6, 123.9, 133.5, 134.7, 144.3, 124.2, 126.0, 144.1, 154.4, 156.2, 158.0, 167.0.

Ethyl 5-(3-(2-phenylhydrazono)-1,1-dicyano-1-en-2-yl)-4-(*p*-tolylamino)-2-aminothiophene-3-carboxylate (15d). Orange crystals from ethanol, yield 70% (3.49 g), m.p. > 300 °C. *Anal*. Calculated for $C_{25}H_{22}N_6O_2S$ (470.55): C, 63.81; H, 4.71; N, 17.86; S, 6.81. Found: C, 63.69; H, 4.54; N; 17.79; S, 6.68. MS: *m/e* 470 (M^+, 20%), IR, ν: 3658–3332 (NH_2, NH), 3056 (CH, aromatic), 1690 (C=O). ^{1}H-NMR, δ: 1.14 (t, 3H, *J* = 7.41 Hz, CH_3), 3.05 (s, 3H, CH_3), 4.24 (q, 2H, *J* = 7.41 Hz, CH_2), 4.63 (s, 2H, NH_2), 7.31–7.43 (m, 9H, C_6H_5, C_6H_4), 8.44, 8.65 (2s, 2H, 2NH). ^{13}C-NMR, δ: 16.8, 57.6, 116.8, 118.6, 119.9, 122.6, 122.9, 123.9, 133.3, 134.7, 144.0, 124.0, 126.4, 144.1, 154.8, 156.8, 158.4, 167.2.

Ethyl 5-(3-(2-*p*-tolylhydrazono)-1,1-dicyano-1-en-2-yl)-4-(*p*-tolylamino)-2-aminothiophene-3-carboxylate (15e). Reddish brown crystals from ethanol, yield 65% (3.33 g), m.p. 266–269 °C. *Anal*. Calculated for $C_{26}H_{24}N_6O_2S$ (484.57): C, 64.44; H, 4.99; N, 17.34; S, 6.62. Found: C, 64.33; H, 4.67; N; 17.39; S, 6.48. MS: *m/e* 484 (M^+, 6%), IR, ν: 3663–3338 (NH_2, NH), 3053 (CH, aromatic), 1688 (C=O). ^{1}H-NMR, δ: 1.13 (t, 3H, *J* = 7.22 Hz, CH_3), 2.94, 3.08 (2s, 6H, $2CH_3$), 4.25 (q, 2H, *J* = 7.22 Hz, CH_2), 4.77 (s, 2H, NH_2), 7.28–7.40 (m, 8H, $2C_6H_4$), 8.46, 8.63 (2s, 2H, 2NH). ^{13}C-NMR, δ: 16.5. 19.8, 58.2, 116.4, 118.2, 121.5, 122.8, 123.7, 133.3, 136.7, 144.2, 124.0, 126.4, 144.1, 154.8, 156.3, 158.6, 164.8, 167.0.

Ethyl 5-(3-(2-*p*-chlorophenylhydrazono)-1,1-dicyano-1-en-2-yl)-4-(*p*-tolylamino)-2-aminothiophene-3-carboxylate (15f). Reddish brown crystals from ethanol, yield 77% (4.10 g), m.p. 244–246 °C. *Anal*. Calculated for $C_{25}H_{21}ClN_6O_2S$ (504.99): C, 59.46; H, 4.19; N, 16.64; S, 6.35. Found: C, 59.53; H, 4.03; N; 16.44; S, 6.28. MS: *m/e* 533 (M^+, 35%), IR, ν: 3660–3336 (NH_2, NH), 3058 (CH, aromatic), 1688 (C=O). ^{1}H-NMR, δ: 1.15 (t, 3H, *J* = 7.22 Hz, CH_3), 2.96 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 7.22 Hz, CH_2), 4.68 (s, 2H, NH_2), 7.26–7.43 (m, 8H, $2C_6H_4$), 8.38, 8.53 (2s, 2H, 2NH). ^{13}C-NMR, δ: 16.9, 19.9, 58.8, 116.7, 118.4, 121.6, 122.6, 123.9, 134.2, 136.7, 144.8, 124.3, 126.8, 144.6, 154.2, 156.0, 158.8, 164.5, 167.3.

3. 1. 9. General Procedure for the Synthesis of the Thiophen-3-yl-thiophene Derivatives 16a–d

To a solution of either **11a** (2.99 g, 0.01 mol) or **11b** (3.46 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (1.0 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and elemental sulfur (0.32 g, 0.01 mol) were added. The reaction mixture, in each case, 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 from a suitable solvent.

4-(*p*-Tolylamino)-2-amino-5-(5-amino-4-cyanothiophen-3-yl)-thiophene-3-carbonitrile (16a). Pale yellow crystals from acetic acid, yield 74% (2.80 g), m.p. 199–202 °C. *Anal*. Calculated for $C_{17}H_{13}N_5S_2$ (351.45): C, 58.10; H, 3.73; N, 19.93; S, 18.25. Found: C, 58.29; H,

3.59; N; 19.91; S, 18.39. MS: *m/e* 351 (M^+, 12%), IR, ν: 34655–3332 (NH_2, 2NH), 3058 (CH, aromatic), 2229, 2220 (2 CN). ^{1}H-NMR, δ: 2.99 (s, 3H, CH_3), 4.95, 5.05 (2s, 4H, $2NH_2$), 6.70 (s, 1H, thiophene H-5), 7.28–7.39 (m, 4H, C_6H_4), 8.38, 8.22 (s, 1H, NH). ^{13}C-NMR, δ: 23.4, 116.4, 118.2, 120.3, 121.9, 123.4, 123.9, 124.3, 126.2, 133.8, 134.5, 144.8, 154.6.

Ethyl 4-(3-*p*-tolylamino)-5-amino-4-cyanothiophen-2-yl)-2-aminothiophene-3-carboxylate (16b). Colourless crystals from acetic acid, yield 66% (2.81 g), m.p. 158–162 °C. *Anal*. Calculated for $C_{20}H_{18}N_4O_3S_2$ (426.51): C, 56.32; H, 4.25; N, 13.14; S, 15.04. Found: C, 56.59; H, 4.39; N; 13.09; S, 14.87. MS: *m/e* 426 (M^+, 31%), IR, ν: 3478–3351 ($2NH_2$, NH), 3058 (CH, aromatic), 2222 (CN), 1689 (C=O). ^{1}H-NMR, δ: 1.25 (t, 3H, J = 5.44 Hz, CH_3), 2.85, (s, 3H, CH_3), 4.22 (q, 2H, J = 5.44 Hz, CH_2), 4.49, 5.20 (2s, 4H, $2NH_2$), 6.60 (s, 1H, thiophene H-5), 7.32–7.42 (m, 4H, C_6H_4), 8.26 (s, H, NH). ^{13}C-NMR, δ: 17.0, 18.9, 117.9, 120.8, 121.3, 123.8, 124.0, 124.6, 125.8, 133.2, 134.5, 144.9, 154.2, 163.2, 164.5.

Ethyl 4-(*p*-tolylamino)-2-amino-5-(5-amino-4-cyanothiophen-3-yl)thiophene-3-carboxylate (16c). Pale yellow crystals from acetic acid, yield 55% (2.34 g), m.p. 177–179 °C. *Anal*. Calculated for $C_{19}H_{18}N_4O_2S_2$ (398.50): C, 57.27; H, 4.55; N, 14.06; S, 16.09. Found: C, 57.46; H, 4.41; N; 14.36; S, 16.27. MS: *m/e* 398 (M^+, 26%), IR, ν: 3488–3352 ($2NH_2$, NH), 3056 (CH, aromatic), 2220 (CN), 1684 (C=O). ^{1}H-NMR, δ: 1.36 (t, 3H, J = 6.72 Hz, CH_3), 2.84, (s, 3H, CH_3), 4.26 (q, 2H, J = 6.72 Hz, CH_2), 4.46, 5.15 (2s, 4H, $2NH_2$), 6.62 (s, 1H, thiophene H-5), 7.30–7.40 (m, 4H, C_6H_4), 8.31 (s, H, NH). ^{13}C-NMR, δ: 17.3, 18.6, 118.6, 120.2, 121.5, 123.5, 124.2, 124.6, 124.9, 133.2, 134.7, 146.0, 156.8, 163.7.

Ethyl 5-(4-ethoxycarbonyl)-4-(*p*-tolylamino)-2-aminothiophen-3-yl)-2-amino-thiophene-3-carboxylate (16d). Orange crystals from ethanol, yield 64% (3.03 g), m.p. 188–190 °C. *Anal*. Calculated for $C_{21}H_{23}N_3O_4S_2$ (455.56): C, 56.61; H, 5.20; N, 9.43; S, 14.39. Found: C, 56.41; H, 5.54; N; 9.39; S, 14.58. MS: *m/e* 473 (M^+, 6%), IR, ν: 3675–3342 ($2NH_2$, NH), 3058 (CH, aromatic), 1692–1688 (2 C=O). ^{1}H-NMR, δ: 1.13, 1.16 (2t, 6H, J = 7.02, 6.53 Hz, $2CH_3$), 2.89 (s, 3H, CH_3), 4.23, 4.27 (2q, 4H, J = 7.02, 6.53 Hz, $2CH_2$), 4.48, 4.62 (2s, 4H, $2NH_2$), 7.26–7.40 (m, 4H, C_6H_4), 8.32 (s, 1H, NH). ^{13}C-NMR, δ: 17.0, 17.8, 18.4, 117.8, 120.4, 122.7, 123.8, 125.5, 125.6, 126.0, 133.7, 134.3, 145.6, 156.2, 163.3, 164.5.

4. Conclusions

The above results allow the conclusion that administration of the tested compounds to the cancer cell lines showed promising anticancer activity. The most potent compounds are **7d**, **12a**, **16a** and **16b** showing the highest inhibitory effects and displaying selectivities which are much higher than the reference doxorubicin.

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6. References

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Povzetek

Pri reakciji cikloheksanona oz. ciklopentanona s cianoacetilhidrazinom ter elementarnim žveplom sta nastala 2-aminocikloalkeno[*b*]tiofenska derivata **3a** oz. **3b**. Pri nadaljnji reakciji teh dveh spojin z aromatskimi benzaldehidi oz. reagenti, ki vsebujejo aktivno metilensko skupino, nastanejo Schiffove baze **5a**–**d** oz. pirazolski derivati **7a**–**d** in **9a**–**d**. Po drugi strani pa pri reakciji 3-okso-*N*-*p*-tolilbutanamida (**10**) z malononitrilom oz. etil cianoacetatom nastaneta tiofenska derivata **13a** oz. **13b**. Spojini **13a**,**b** sta v seriji heterociklizacijskih reakcij tvorili različne heterociklične derivate. Določena je bila tudi citotoksičnost pripravljenih spojin proti trem človeškim tumorskim celičnim linijam, t.j. adenokarcinomom dojke (MCF-7), pljučnemu raku (NCI-H460) and raku centralnega živčenga sistema (SF-268). Za primerjavo je bila še dodatno določena aktivnost proti normalnim človeškim fibroblastom WI 38.