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Research Article

The Reaction of Cyclopentanone with Cyanomethylene Reagents: Novel Synthesis of Pyrazole, Thiophene, and Pyridazine Derivatives

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The reaction of cyclopentanone with either malononitrile or ethyl cyanoacetate gave the corresponding condensated products. The latter underwent some heterocyclic reactions to give new pyrazole, thiophene, and pyridazine derivatives. The antitumor evaluation of the newly synthesized products against the three cancer cells, namely, breast adenocarcinoma (MCF-7), nonsmall cell lung cancer (NCI-H460), and CNS cancer (SF-268) showed that some of them have high inhibitory effect towards three cell lines which is higher than the standard.

1. Introduction

Heterocyclic compounds are worthy of attention for many reasons, chief among which are their biological activities, with many important drugs bearing thiazol, thiophene and pyridine derivatives. Therefore, organic chemists have been making extensive efforts to produce heterocyclic compounds by developing new and efficient synthetic transformations [1–10]. Many pyrazoles, thiophenes, and thiazoles were reported with a wide spectrum of biological activities which are known; they possess potent analgesic [11, 12], anticonvulsant, anti-inflammatory and antibacterial [13, 14], antipyretics [15], antitumor [16, 17], antiparasitic [18], antimicrobial [19], antihistaminic (H1) [20], antianxiety test in mice [21], antiarrhythmic [22], and serotonin antagonist [23]. In the present work, we studied the reaction of cyclopentanone with cyanomethylene reagents followed by heterocyclization of the products together with studying the antitumor evaluation of the newly synthesized products.

2. Experimental

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. IR spectra were recorded for KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were measured on a Varian EM-390 at 200 MHz in DMSO-d_6 as solvent using TMS as internal standard. The mass spectra were recorded with Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Analytical data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt. Antitumor evaluation for the newly synthesized products was performed by a research group at the National Research Center and the National Cancer Institute at Cairo University. Fetal bovine serum (FBS) and l-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (New Jersey, NJ, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin, and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, MO, USA). Stock solutions of all compounds were prepared in DMSO and kept at −20°C. Appropriate dilutions of the compounds were freshly prepared just prior to assays. Final concentrations of DMSO did not interfere with the cell growth. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), and NCI-H460 and SF-268 were kindly provided

Table 1: Physical and microanalysis of the newly synthesized compounds **3a–16b**.

Comp. no.	m.p.	Yield (%)	Solvent of crystallization	Molecular formulae	C%	H%	N%	S%
3a	148–150	78	Ethanol	$C_8H_8N_2$ 132.16	72.70	6.10	21.20	
3b	100–112	81	Ethanol	$C_{10}H_{13}NO_2$ 179.22	67.12	7.21	7.79	
5a	98–100	78	Ethanol	$C_{14}H_{12}N_4$ 236.27	71.02	5.22	12.59	
5b	60–62	86	Ethanol	$C_{14}H_{11}ClN_4$ 270.72	62.08	4.39	20.93	
5c	88–90	90	Ethanol	$C_{16}H_{17}N_3O_2$ 283.33	67.66	5.93	14.95	
5d	58–60	77	Ethanol	$C_{16}H_{16}ClN_3O_2$ 317.77	60.38	5.11	13.08	
7a	70–72	78	Ethanol	$C_{14}H_{18}N_6$ 270.33	62.36	6.83	31.11	
7b	80–82	82	Ethanol	$C_{20}H_{22}N_6$ 346.43	69.63	6.55	24.07	
7c	68–70	78	Ethanol	$C_{14}H_{17}N_5O$ 271.32	61.77	6.49	25.64	
7d	78–80	80	Ethanol	$C_{20}H_{21}N_5O$ 347.41	69.25	6.27	19.84	
9a	98–100	78	Ethanol	$C_{15}H_{12}N_2$ 220.27	81.67	5.33	12.57	
9b	40–42	70	Ethanol	$C_{17}H_{17}NO_2$ 267.32	76.51	6.58	5.33	
11a	98–100	82	Ethanol	$C_{17}H_{13}N_3$ 259.31	78.63	4.93	16.08	
11b	80–82	82	Ethanol	$C_{19}H_{18}N_2O_2$ 306.14	74.62	5.86	9.08	
11c	68–70	82	Ethanol	$C_{19}H_{18}N_2O_2$ 306.14	74.52	5.72	9.22	
11d	58–60	77	Ethanol	$C_{21}H_{23}NO_4$ 353.41	71.58	6.62	4.25	
13a	88–90	80	Ethanol	$C_8H_8N_2S$ 164.23	58.51	4.91	17.06	19.52
13b	70–72	72	Ethanol	$C_{10}H_{13}NO_2S$ 211.28	58.85	6.20	6.63	15.18
14a	120–122	86	Ethanol	$C_{14}H_{12}N_4S$ 268.34	62.74	4.75	20.67	12.28
14b	78–81	69	Ethanol	$C_{16}H_{17}N_3O_2S$ 315.39	61.31	5.67	13.15	10.06
15a	90–92	72	1,4-dioxane	$C_{16}H_{14}N_4OS$ 310.37	62.03	4.74	17.99	10.48
15b	68–70	83	1,4-dioxane	$C_{18}H_{19}N_3O_3S$ 357.43	60.58	5.52	11.84	9.32
16a	78–80	80	1,4-dioxane	$C_{14}H_{12}N_4$ 236.27	71.27	4.92	23.88	
16b	70–72	77	1,4-dioxane	$C_{14}H_{11}N_3O$ 237.26	70.64	4.83	17.93	

by the National Cancer Institute (NCI, Cairo, Egypt). The physical properties, yield %, solvent of crystallization, and microanalytical data of the synthesized products were indicated through Table 1. Spectral data were inserted through Table 2.

*2.1. 2-Cyclopentylidenemalononitrile **3a** and Ethyl 2-Cyano-2-cyclopentylideneacetate **3b***

General Procedure. To a dry solution of cyclopentanone **1** (0.84 g, 0.01 mol) either malononitrile **2a** (0.66 g, 0.01 mol) or

Table 2: Spectral data of the newly synthesized products.

Comp.	IR spectrum, ν, cm^{-1}	^{1}HNMR spectrum (200 MHz, DMSO), δ, ppm (J, Hz)	Mass spectrum (EI, 70 eV), m/z (Irel, %)
3a	2933 (CH_2); 2227, 2220 (2CN); 1634 (C=C)	1.34–2.44 (m, 8H, $4CH_2$)	132 (M^+, 15%)
3b	2936, 2890 (CH_3, CH_2); 2222 (CN); 1688 (C=O), 1634 (C=C)	1.13 (t, 3H, J = 7.01 Hz, CH_3), 1.36–2.40 (m, 8H, $4CH_2$), 4.22 (q, 2H, J = 7.01 Hz, CH_2)	179 (M^+, 22%)
5a	2936 (CH_2), 3058 (CH aromatic), 2228, 2220 (2CN); 1636 (C=C)	1.36–2.41 (m, 6H, $3CH_2$), 7.27–7.37 (m, 5H, C_6H_5), 8.77 (s, 1H, NH)	236 (M^+, 18%)
5b	3055 (CH aromatic), 2940 (CH_2), 2228, 2222 (2CN), 1630 (C=C)	1.32–2.39 (m, 6H, $3CH_2$), 7.29–7.39 (m, 4H, C_6H_4), 8.33 (s, 1H, NH)	270 (M^+, 77%).
5c	3058 (CH aromatic), 2946 (CH_2), 2220 (CN), 1632 (C=C)	1.13 (t, 3H, J = 7.22 Hz, CH_3), 1.30–2.38 (m, 6H, $3CH_2$), 4.25 (q, 2H, J = 7.22, CH_2), 7.26–7.36 (m, 5H, C_6H_5), 8.36 (s, 1H, NH)	283 (M^+, 40%)
5d	3060 (CH aromatic), 2923, 2891 (CH_3, CH_2), 2222 (CN), 1636 (C=C).	1.12 (t, 3H, J = 7.03 Hz, CH_3), 1.32–2.41 (m, 6H, $3CH_2$), 4.22 (q, 2H, J = 7.03 Hz, CH_2), 7.28–7.40 (m, 5H, C_6H_5), 8.42 (s, 1H, NH)	317 (M^+, 35%)
7a	3456–3435 (2 NH_2), 3058 (CH aromatic), 2942 (CH_2), 1667 (exocyclic C=N), 1638 (C=C)	1.32–2.36 (m, 6H, $3CH_2$), 2.81 (t, 1H, CH), 4.21, 4.85 (2s, 4H, $2NH_2$), 7.29–7.40 (m, 5H, C_6H_5), 8.38, 9.01 (2s, 2H, 2NH)	270 (M^+, 28%)
7b	3466–3465 (2 NH_2), 3052 (CH aromatic), 2938 (CH_2), 1668 (exocyclic C=N), 1632 (C=C).	1.33–2.38 (m, 6H, $3CH_2$), 2.80 (t, 1H, CH), 4.23, 4.86 (2s, 4H, $2NH_2$), 7.25–7.48 (m, 10H, $2C_6H_5$), 8.35, 8.88 (1s, 1H, 1NH).	346 (M^+, 21%)
7c	3566–3425 (OH, NH_2), 3053 (CH aromatic), 2934 (CH_2), 1666 (excyclic C=N), 1631 (C=C).	1.32–2.36 (m, 6H, $3CH_2$), 2.82 (t, 1H, CH), 4.21, 4.85 (2s, 4H, NH_2), 7.30–7.38 (m, 5H, C_6H_5), 8.33 (s, 1H, NH), 10.22 (OH)	271 (M^+, 21%).
7d	3520–3435 (OH, NH_2), 3050 (CH aromatic), 2931 (CH_2), 1670 (exocyclic C=N), 1634 (C=C).	1.33–2.38 (m, 6H, $3CH_2$), 2.80 (t, 1H, CH), 4.23, 4.86 (2s, 2H, NH_2), 7.32–7.37 (m, 10H, 2 C_6H_5), 8.32 (s, 1H, NH), 10.20 (OH)	347 (M^+, 18%).
9a	3058 (CH aromatic), 2920 (CH_2), 2227–2220 (2 CN), 1660 (C=C).	1.30–2.38 (m, 6H, $3CH_2$), 6.01 (s, 1H, C=CH), 7.28–7.37 (m, 5H, C_6H_5)	220 (M^+, 18%).
9b	3056 (CH aromatic), 2923 (CH_2), 2223 (CN), 1688 (C=O), 1663 (C=C).	1.12 (t, 3H, J = 7.05 Hz, CH_3), 1.32–2.34 (m, 6H, $3CH_2$), 4.22 (q, 2H, J = 7.05 Hz, CH_2), 6.03 (s, 1H, C=CH), 7.28–7.40 (m, 5H, C_6H_5)	267 (M^+, 15%).
11a	3466–3321 (NH_2), 3060 (CH aromatic), 2929 (CH_2), 2228, 2222 (2 CN), 1636 (C=C).	1.30–2.36 (m, 6H, $3CH_2$), 4.48 (s, 2H, NH_2), 7.26–7.38 (m, 5H, C_6H_5)	259 (M^+, 15%)
11b	3448–3318 (NH_2), 3058 (CH aromatic), 2933 (CH_2), 2220 (CN), 1690 (C=O), 1638 (C=C).	1.12 (t, 3H, J = 6.94 Hz, CH_3), 1.28–2.37(m, 6H, $3CH_2$), 4.22 (q, 2H, J = 6.94 Hz, CH_2), 4.48 (s, 2H, NH_2), 7.28–7.39 (m, 5H, C_6H_5)	306 (M^+, 22%)
11c	3453–3322 (NH_2), 3055 (CH aromatic), 2931 (CH_2), 2222 (CN), 1690 (C=O), 1638 (C=C)	1.12 (t, 3H, J = 6.94 Hz, CH_3), 1.26–2.37 (m, 6H, $3CH_2$), 4.24 (q, 2H, J = 6.94 Hz, CH_2), 4.49 (s, 2H, NH_2), 7.26–7.38 (m, 5H, C_6H_5)	306 (M^+, 22%)
11d	3460–3342 (NH_2), 3050 (CH aromatic), 2930 (CH_2), 1688, 1690 (2 C=O), 1636 (C=C).	1.13, 1.15 (2t, 6H, J = 6.66, 7.15 Hz, $2CH_3$), 1.28–2.39 (m, 6H, $3CH_2$), 4.21, 4.25 (q, 4H, J = 6.66, 7.15 Hz, $2CH_2$), 4.46 (s, 2H, NH_2), 7.29–7.39 (m, 5H, C_6H_5)	353 (M^+, 22%)
13a	3453–3321 (NH_2), 3056 (CH aromatic), 2940 (CH_2), 1633 (C=C).	1.22–2.38 (m, 6H, $3CH_2$), 4.43 (s, 2H, NH_2)	164 (M^+, 100%)
13b	3466–3348 (NH_2), 3055 (CH aromatic), 2936, 2884 (CH_3, CH_2), 1689 (C=O), 1638 (C=C).	1.12 (t, 3H, J = 7.22 Hz, CH_3), 1.25–2.36 (m, 6H, $3CH_2$), 4.22 (q, 2H, J = 7.22 Hz, CH_2), 4.46 (s, 2H, NH_2)	211 (M^+, 100%)
14a	3453–3321 (NH_2), 3056 (CH aromatic), 2940 (CH_2), 1633 (C=C). 29–7.36 (m, 5H, C6H5), 8.23 (s, 1H, NH).	1.22–2.38 (m, 4H, $4CH_2$), 4.43 (s, 2H, NH_2), 7.29–7.36 (m, 5H, C_6H_5), 8.23 (s, 1H, NH)	268 (M^+, 100%).
14b	3465–3342 (NH_2, NH), 3058 (CH aromatic), 2936, 2841 (CH_3, CH_2), 1694(C=O), 1663 (C=C).	1.12 (t, 3H, J = 7.44 Hz, CH_3), 1.24–2.36 (m, 4H, $2CH_2$), 4.22 (q, 2H, J = 7.44 Hz, CH_2), 4.52 (s, 2H, NH_2), 7.27–7.44 (m, 5H, C_6H_5), 8.63 (s, 1H, NH)	315 (M^+, 100%)

Table 2: Continued.

Comp.	IR spectrum, ν, cm^{-1}	^{1}HNMR spectrum (200 MHz, DMSO), δ, ppm (J, Hz)	Mass spectrum (EI, 70 eV), m/z (Irel, %)
15a	3453–3321 (NH_2), 3060 (CH aromatic), 2942, 2893 (CH_3, CH_2), 2220 (CN), 1685 (C=O), 1636 (C=C).	1.20–2.35 (m, 4H, $2CH_2$), 2.62 (s, 3H, CH_3), 7.28–7.40 (m, 5H, C_6H_5), 8.40, 8.63 (2s, 2H, 2NH)	310 (M^+, 60%)
15b	3466–3341 (NH_2), 3057 (CH aromatic), 2940, 2877 (CH_3, CH_2), 1684, 1689 (2C=O), 1639 (C=C).	1.14 (t, 3H, J = 7.42 Hz, CH_3), 1.20–2.37 (m, 4H, $2CH_2$), 2.68 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.42 Hz, CH_2), 7.28–7.39 (m, 5H, C_6H_5), 8.38, 8.52 (2s, 2H, 2NH)	357 (M^+, 38%)
16a	3477, 3320 (NH), 3054 (CH aromatic), 2936 (CH_2), 1663 (exocyclic C=N), 1638 (C=C).	1.22–2.39 (m, 6H, $3CH_2$), 7.32–7.43 (m, 5H, C_6H_5), 8.36 (s, 1H, NH)	236 (M^+, 100%).
16b	3050 (CH aromatic), 2940 (CH_2), 1687 (C=O), 1644 (C=C).	1.25–2.36 (m, 6H, $3CH_2$), 7.30–7.48 (m, 5H, C_6H_5)	237 (M^+, 100%).

ethyl cyanoacetate **2b** (1.13 g, 0.01 mol) was added followed by the addition of ammonium acetate (0.50 g). The reaction mixture, in each case, was heated in an oil bath at 120°C then left to cool. The solidified product was triturated with ethanol, and the formed solid product was collected by filtration to give compounds **3a**,**b**.

*2.2. Synthesis of 2-(2-Phenylhydrazono-cyclopentylidene)-malononitrile **5a,** 2-(2-(P-chlorophenyl)-hydrazono-cyclopentylidene)-malononitrile **5b,** Ethyl 2-Cyano-2-(2-phenylhydrazo)-cyclopentylideneacetate **5c,** Ethyl 2-Cyano-2-(2-(p-chlorophenylhydrazo)-cyclopentylideneacetate **5d**.* To a solution of either **3a** (1.32 g, 0.01 mol) or **3b** (1.79 g, 0.01 mol) in cold (0–5°C) ethanol (30 mL) containing sodium acetate (2.5 g) either of benzenediazonium chloride **4a**,**b** (0.01 mol or 4-chlorobenzenediazonium chloride (0.01 mol) (prepared by adding sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of the appropriate aniline or its derivative (0.01 mol) in concentrated hydrochloric acid (20 mL, 18 N) with continuous stirring) was added with stirring. The whole reaction mixture was kept at room temperature for 2 h, and the formed solid product was filtered off to yield **5a**–**d**.

*2.3. Synthesis of 3,5-Diamino-4-(2-phenylhydrazono-cyclopent-1-yl)pyrazol **7a,** 3-Amino-1-phenyl4-(2-phenylhydrazono-cyclopent-1-yl)pyrazol **7b,** 3-Amino-5-hydroxy-4-(2-phenylhydrazono-cyclopent-1-yl)pyrazol **7c** and 3-Amino-1-phenyl-5-hydroxy-4-(2-phenylhydrazono-cyclopent-1-yl) pyrazol **7d***

General Procedure. To a solution of either **5a** (2.36 g, 0.01 mol) or **5c** (2.83 g, 0.01 mol) in 1,4-dioxane (20 mL), either hydrazine hydrate **6a** (0.5 mL, 0.01 mol) or phenylhydrazine **6b** (1.08 g, 0.01 mol). The reaction mixture was heated under reflux for 1 h. The solid product, obtained upon cooling, was filtered off and dried to give compounds **7a**–**d**.

*2.4. Synthesis of 2-(Benzylidenecyclopentylidene)malononitrile **9a** and Ethyl 2-(2-Benzylidenecyclopentylidene)-2-cyano-acetate **9b***

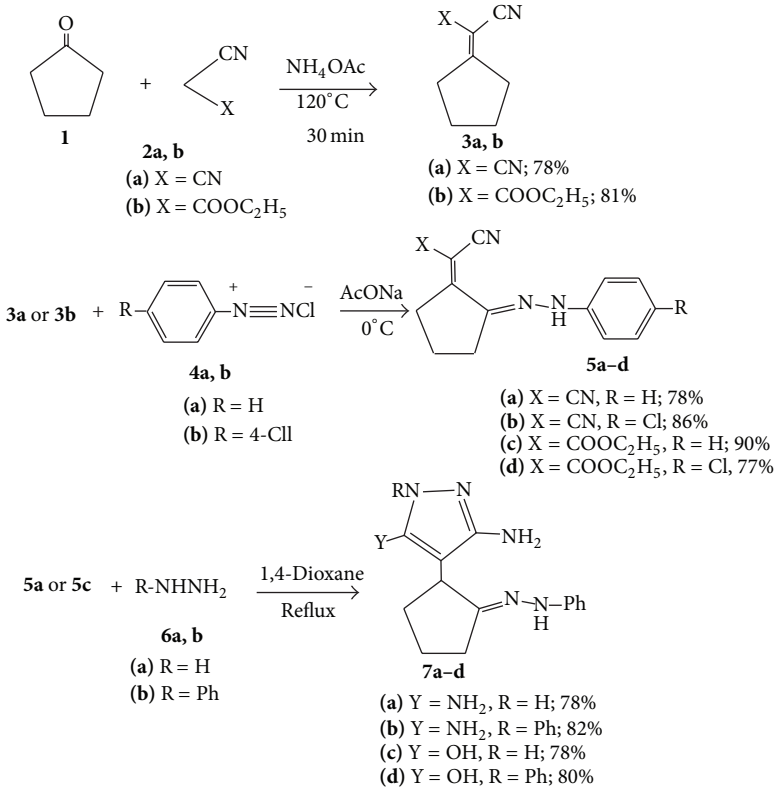
General Procedure. To a solution of either **3a** (1.32 g, 0.01 mol) or **3b** (1.79 g, 0.01 mol) in 1,4-dioxane (30 mL) containing piperidine (0.50 mL), benzaldehyde (1.06 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h. The solid product, obtained upon cooling, was filtered off to give compounds **9a**,**b**.

*2.5. Synthesis of 5-Amino-7-phenyl-2,3-dihydro-1H-indene-4,6-dicarbonitrile **11a,** Ethyl 6-Amino-7-cyano-4-phenyl-2,3-dihydro-1H-indene-5-carboxylate **11b,** Ethyl 5-Amino-6-cyano-7-phenyl-2,3-dihydro-1H-indene-4-carboxylate **11c** and Diethyl 5-Amino-7-phenyl-2,3-dihydro-1H-indene-4,6-dicarboxylate **11d***

General Procedure. To a solution of either **9a** (2.20 g, 0.0. mol) or **9b** (2.67 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL, 0.01 mol), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h. The solid product, obtained upon cooling, was collected by filtration to give compounds **11a**–**d**.

*2.6. Synthesis of 2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile **13a** and Ethyl 2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate **13b**.* To a solution of either **3a** (1.32 g, 0.01 mol) or **3b** (1.79 g, 0.01 mol) in ethanol (20 mL) containing triethylamine (0.50 mL, 0.01 mol) elemental sulphur (0.32 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 h. The separated solid was flittered off to afford **13a**,**b**.

*2.7. Synthesis of 2-Amino-4-(2-phenylhydrazono)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile **14a** and Ethyl*



Scheme 1

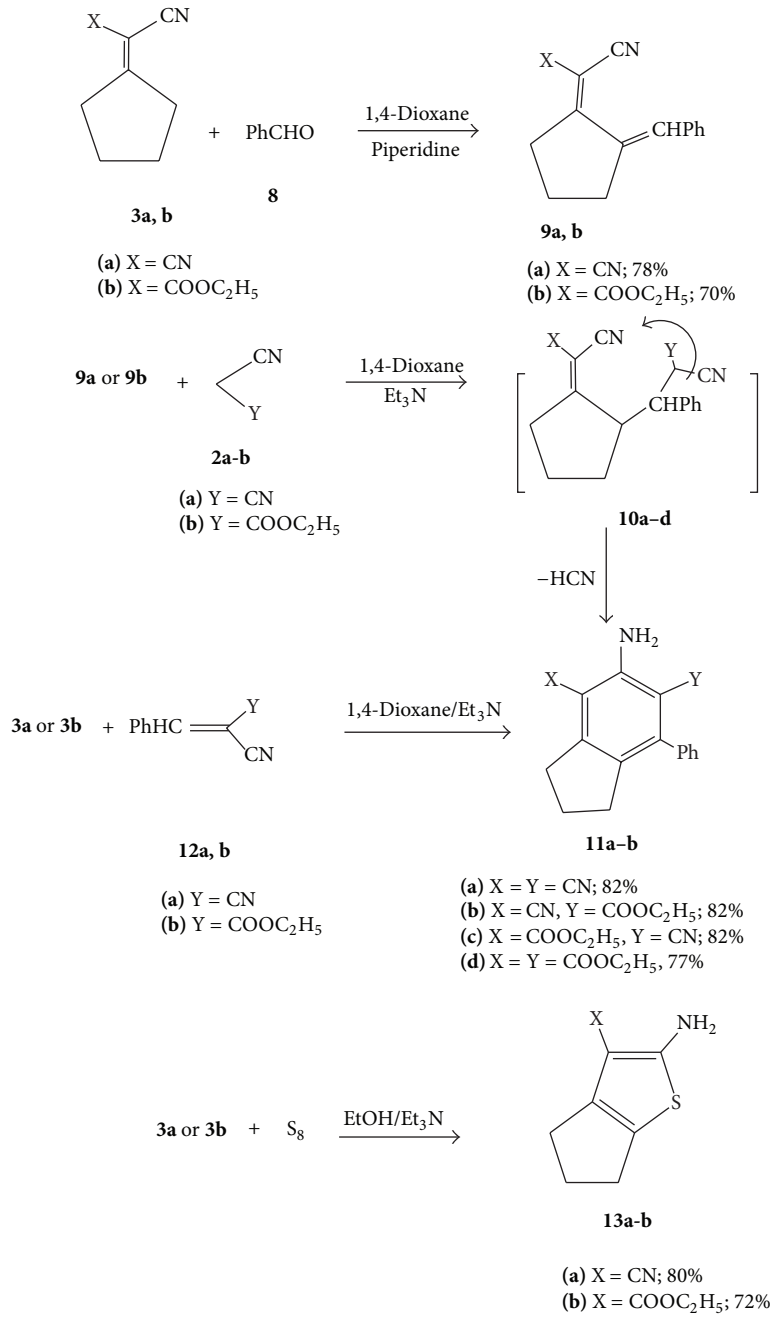
*2-Amino-4-(2-phenylhydrazono)-5,6-dihydro-4H-cyclopenta [b]thiophene-3-carboxylate **14b**.* To a solution of either **5a** (2.36 g, 0.01 mol) or **5b** (2.70 g, 0.01 mol) in 1,4-dioxane (20 mL) containing triethylamin (0.5 mL, 0.01 mol), elemental sulphur (0.32 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 1 h then left to cool. The separated solid was filtered off to afford **14a**,**b**.

*2.8. Synthesis of N-(3-Cyano-4-(2-phenylhydrazono)-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)acetamide **15a** and Ethyl 2-Acetamido-4-(2-phenylhydrazono)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate **15b**.* To a solution of either of compound **14a** (2.68 g, 0.01 mol) or **14b** (3.15 g, 0.01 mol) with acetic anhydride (1.02 g, 0.01 mol) in dimethyl formamide (20 mL) was heated under reflux for 2 h then left to cool. The solid product, so formed in each case, was collected by filtration. The reaction mixture was evaporated under vacuum and the remaining product was triturated with ethanol. The separated solid was filtered off to afford **15a**,**b**.

*2.9. Synthesis of 3-Amino-2-phenyl-3,5,6,7-tetrahydro-2H-4-carbonitrile **16a** and 3-Oxo-2-phenyl-3,5,6,7-tetrahydro-2H-cyclopenta[c]pyridazine-4-carbonitrile **16b**.* A solution of either **5a** (2.36 g, 0.01 mol) or **5b** (2.70 g, 0.01 mol) in 1,4-dioxane (20 mL) containing triethylamine (0.5 mL, 0.01 mol) was heated under reflux for 4 h, then the excess solvent was evaporated under vacuum. The remaining product, in each case, was triturated with diethyl ether, and the formed solid product was filtered off to give **16a**,**b**.

3. Results and Discussions

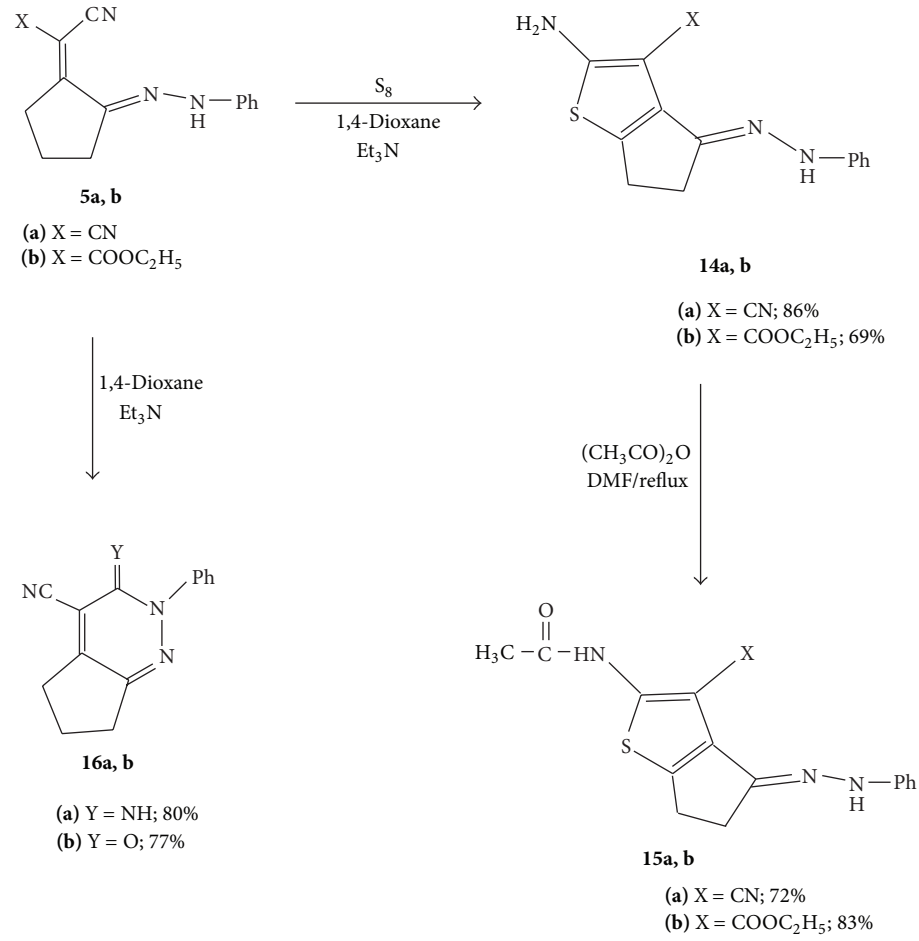
The reaction of cyclopentanone with either malononitrile or ethylcyanoacetate gave the Knoevenagel condensated products **3a** and **3b**, respectively. The structures of the latter products were based on analytical and spectral data. Thus, the ^{1}H NMR spectrum of **3a** showed a multiplet at δ 1.34–2.44 indicating the four CH_2 groups. Next, we studied the reaction of either **3a** or **3b** with either benzenediazonium chloride or p-chlorobenzenediazonium chloride. The reaction was carried out in ethanol 0–5°C and afforded the corresponding arylhydrazone derivatives **5a–d**, respectively. Either compound **5a** or **5c** reacts with either hydrazine hydrate or phenylhydrazine to give the corresponding pyrazole derivatives **7a–d**, respectively (Scheme 1). The analytical and spectral data of the latter products are in agreement with the proposed structures. Thus, the ^{1}H NMR spectrum of **7a** showed the presence of a multiplet at δ 1.32–2.36 corresponding to the three CH_2 groups, a triplet at δ 2.81 indicating the CH group, two singlets (D_2O exchangeable) at δ 4.21, 4.85 ppm for the two NH_2 groups, a multiplet at δ 7.29–7.40 ppm corresponding to one phenyl group, and two singlets (D_2O exchangeable) corresponding to the two NH groups. On the other hand, the reaction of either **3a** or **3b** with benzaldehyde afforded the benzylidene derivatives **9a** and **9b**, respectively.



Scheme 2

The reaction of either **(9a)** or **9b** with either malononitrile or ethyl cyanoacetate gave the benzocyclopentane derivatives **11a–d**, respectively. Formation of the latter products is explained in terms of the first addition of the cyanomethylene reagent to the ylidene moiety followed by the Micheal addition of the CH group to the nitrile and subsequent elimination of HCN. Structures of **11a–d** were confirmed on the basis of their analytical and spectral data, respectively. Thus, the ^{1}H NMR spectrum of **11a** showed a multiplet at δ 1.30–2.36 ppm, a singlet at δ 4.48 ppm for the NH_2 group, and a multiplet at δ 7.26–7.38 ppm indicating the C_6H_5 group. Further confirmations for the structures of such compounds were obtained through the reaction of either **3a** or **3b** with either α-cyanocinnamonitrile **12a** or ethyl α-cyanocinnamate **12b** to give the same products **11a–d**, respectively (identical finger print IR, mixed m.p.).

Next, we moved towards studying reactivity of either **3a** or **3b** towards Gewald's thiophene synthesis. Thus, the reaction of either **3a** or **3b** with elemental sulphur in 1,4-dioxane and the presence of a catalytic amount of triethylamine gave the cyclopenta[*b*]thiophene derivatives **13a** and **13b**, respectively (Scheme 2). The latter products were obtained previously by



Scheme 3

Mohareb and Al-Farouk [24] using another reaction route. Moreover, carrying the same reaction with the arylhydrazone derivatives **5a** and **5b** gave the thienocyclopentene derivatives **14a** and **14b**, respectively. Compounds **14a** and **14b** were reacted with acetic anhydride in dimethyl formamide to give the N-acetyl products **15a** and **15b**, respectively. Compounds **5a**,**b** underwent ready cyclization in 1,4-dioxane solution containing triethylamine to give the cyclopenta[*c*]pyridazine derivatives **16a** and **16b**, respectively (Scheme 3).

4. Antitumor Activity Tests

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

4.2. Cell Cultures. Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (nonsmall 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 μg/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.

4.3. Tumor Cell Growth Assay. The effects of **3a,b–15a,b** 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 [25]. 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 minimum concentration inhibition of 50% (IC_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere [26]. For our newly synthesized products, we selected the three cancer cell lines the breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460), and CNS cancer (SF-268) as our compounds are electron reach systems substituted with electronegative groups, and many reports from our previous work and others [25] used such cell lines together with the use of doxorubicin which was showed to be the best positive control against the three cell lines.

Materials, Methods and Reagents. Fetal bovine serum (FBS) and L-glutamine were obtained 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). Samples: stock solutions of compounds **(3a,b–15a,b)** were prepared in DMSO and kept at −20°C. Appropriate dilutions of the compounds were freshly prepared just prior to the assays. Final concentrations of DMSO did not interfere with the cell growth.

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 μg/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.

4.4. Structure Activity Relationship. Compounds **(3a,b–15a,b)** were evaluated for their capacities to inhibit the *in vitro* growth of breast adenocarcinoma (MCF-7).

4.4.1. Effect on the Growth of Human Tumor Cell Lines. The effect of selected compounds from the newly synthesized products **3a,b–16a,b** 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), after a continuous exposure of 48 h. The results are summarized in Table 3.

All the compounds were able to inhibit the growth of the human tumor cell lines in a dose-dependent manner (data not shown). The p-chlorophenylhydrazone derivative **15a**, the indene derivatives **11d**, and the pyridazin-6-one derivative **15b** showed the best results among the tested compounds, and such reactivity is higher than the standard doxorubicin. On the other hand, compounds **3b**, **7c**, **9a**, **9b**, **12a**, **12b**, **13a**, **14a**, **15a**, **16a**, and **16b** showed moderated growth inhibitory effect. Comparing the activities of **15a** and **15b**, it is observed that the chloro group in **15b** presents a stronger growth inhibitory effect than the amino substituent in **15a,** although the results in NCI-H460 cell line are comparable. It is clear from Table 3 that some compounds like **7a**, **7b**, and **11a** showed very low activity towards the three cancer cell lines.

Comparing the reactivities of compounds **11a–d**, it is obvious that compound **11d** with X = Y = COOEt showed the highest inhibitory effect among the four compounds. On the other hand, considering the cyclopentenopyridazine derivatives **15a** and **15b**, the presence of the electronegative C=N group is responsible for the higher cytotoxicity of **15b** over **15a**. It is clear from Table 3 that compounds **5b**, **11d**, **and 15b** showed the highest cytotoxicity among the

Table 3: Effect of compounds **3a,b–15a,b** on the growth of three human tumor cell lines.

Compound	GI_{50} (μmol L^{-1})		
	MCF-7	NCI-H460	SF-268
3a	10.2 ± 0.6	12.3 ± 1.2	20.3 ± 2.5
3b	20.3 ± 0.4	24.3 ± 0.8	30 ± 0.8
5a	30.6 ± 12.8	31.3 ± 6.4	44.2 ± 3.8
5b	0.01 ± 0.002	0.06 ± 0.002	0.04 ± 0.002
5c	35.4 ± 10.2	22.1 ± 0.8	6.7 ± 4.7
5d	0.8 ± 3.6	10.3 ± 0.9	16.7 ± 1.6
7a	60.7 ± 12.5	33.2 ± 10.6	66.0 ± 9.1
7b	50.1 ± 0.7	23.2 ± 4.8	10.4 ± 1.8
7c	22.0 ± 0.2	30.6 ± 1.4	32.6 ± 2.9
7d	32.0 ± 4.5	41.0 ± 2.4	22.5 ± 1.6
9a	22.0 ± 3.7	20.0 ± 4.4	30.5 ± 8.0
9b	6.4 ± 2.6	10.1 ± 2.8	4.2 ± 1.5
11a	70.9 ± 0.9	48.6 ± 4.8	50 ± 4.8
11b	30.4 ± 2.6	22.8 ± 2.8	56.2 ± 3.8
11c	23.9 ± 1.29	20.6 ± 4.8	22.8 ± 4.2
11d	0.04 ± 0.006	0.01 ± 0.008	0.02 ± 0.002
12a	22.0 ± 3.7	20.0 ± 4.4	30.5 ± 8.0
12b	6.4 ± 2.6	10.1 ± 2.8	4.2 ± 1.5
13a	16.6 ± 0.8	12.4 ± 2.6	14.8 ± 0.4
13b	33.4 ± 4.6	36.8 ± 1.8	34.2 ± 2.8
14a	20.9 ± 0.9	16.6 ± 4.8	18.8 ± 0.8
14b	30.4 ± 2.6	22.8 ± 2.8	56.2 ± 3.8
15a	20.9 ± 0.9	18.6 ± 4.8	16.8 ± 0.8
15b	0.01 ± 0.001	0.02 ± 0.006	0.02 ± 0.008
16a	30.4 ± 12.2	38.4 ± 8.4	22.4 ± 10.2
16b	24.4 ± 8.2	28.0 ± 6.1	20.4 ± 4.5
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.

newly synthesized products, and such activity is higher than that of the standard material doxorubicin.

5. Conclusions

In this work, we succeeded to synthesis a series of fused thiophene derivatives. The cytotoxicity of the newly synthesized products showed that compounds **5b**, **11d**, and **15b** are the most active compounds towards the three cancer cell lines.

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