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The reaction of β-amino-α,γ-dicyanocrotononitrile with acetophenone: Synthesis of pyridine, pyridazine and thiophene derivatives with antimicrobial activities

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Condensation of β-amino-α,γ-dicyanocrotononitrile (**1**) with acetophenone gave 2-amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**2**). The latter product was used in a series of heterocyclization reactions with different reagents such as diazonium salts, hydrazines, hydroxylamines and elemental sulfur to give pyridazine, pyrazole, isoxazole and thiophene derivatives, respectively. On the other hand, it gave pyridine derivatives with aromatic aldehydes folowed by reaction with cyanomethylene reagents. The *MIC* values for the newly synthesized product were measured against *E. coli, B. cereus, B. subtilis* and *C. albicans.*

Keywords: pyridine, isoxazole, pyridazine, thiophene, antimicrobial activity

The continuing interest in the chemistry of β-amino-α,γ-dicyanocrotononitrile (**1**) (1-6) forms a part of the systematic efforts to obtain pyridines, pyrimidines, pyridazines, thiophenes, thiazoles and their analogs. The importance of such compounds is due to their diverse pharmaceutical activities in neurological disorders (7), as receptor antagonists (8, 9), tubulin inhibitors (10), kinase inhibitors (11) and for anticancer activity (12). Moreover, annulated nitrogen heterocycles, bearing pyridine and benzene, constitute a class of biologically active compounds that are potent anti-inflammatory agents (13), anti-bacterial agents (14), inhibitors of gastric acid secretion (15) and calcium channel blockers (16). Such biological and pharmaceutical importance of the different classes of heterocyclic derivatives prompted us to study the chemical reactivity of 2-amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile **2** towards some chemical reagents with the aim of forming biologically active heterocyclic derivatives.

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EXPERIMENTAL

Synthetic methods, analytical and spectral data

All melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. Elemental analyses were performed on a Yanaco CHN Corder elemental analyzer (Japan). IR spectra (ν, cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer (Shimadzu, Japan). ^1H NMR spectra were obtained on a Jeol 300 MHz (Japan) spectrometer in DMSO-d_6 as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75 eV) Ms equipment (Germany).

Synthetic pathways are presented in Schemes 1–3 and physicochemical and spectral data of the synthesized compounds are given in Tables I and II.

Table I. Physico-chemical data for synthesized compounds

Compd. No.	Yield (%)	M.p. (°C)	Mol. formula (M_r)	Found/calcd. (%)			
				C	H	N	S
2	91	75–77	$C_{14}H_{10}N_4$	71.78	4.30	23.92	–
			234.26	71.73	4.29	24.00	
3a	82	80–82	$C_{20}H_{14}N_6$	70.99	4.17	24.84	–
			338.37	70.72	4.21	24.82	
3b	81	120–122	$C_{20}H_{13}ClN_6$	64.43	3.51	22.54	–
			372.81	64.36	3.81	22.61	
3c	77	135–137	$C_{23}H_{17}N_7S$	65.23	4.05	23.23	7.57
			423.49	65.16	4.04	23.22	7.56
3d	81	207–209	$C_{25}H_{22}N_6O_2S$	63.81	4.71	17.86	6.81
			470.55	63.76	4.7	17.92	6.82
4a	80	222–225	$C_{20}H_{14}N_6$	70.99	4.17	24.84	–
			338.37	70.91	4.16	24.91	
4b	79	224–225	$C_{20}H_{13}ClN_6$	64.43	3.51	22.54	–
			372.81	64.34	3.84	22.69	
4c	61	189–192	$C_{23}H_{17}N_7S$	65.23	4.05	23.23	7.57
			423.49	65.16	4.04	23.22	7.56
4d	60	221–223	$C_{25}H_{22}N_6O_2S$	63.81	4.71	17.86	6.81
			470.55	63.56	4.87	17.62	6.80
5a	60	180–183	$C_{21}H_{14}N_4$	78.24	4.38	17.38	
			322.36	78.52	4.62	17.08	
5b	66	222–225	$C_{21}H_{14}N_4O$	74.54	4.17	16.56	
			338.36	74.56	3.85	16.38	
7a	81	75–77	$C_{23}H_{15}N_5$	76.44	4.18	19.38	–
			361.40	76.18	4.37	19.44	

7b	73	110–112	$C_{25}H_{20}N_4O_2$ 408.45	73.51 73.49	4.94 4.76	13.72 13.61	–
8a	81	166–169	$C_{23}H_{15}N_5$ 361.40	76.44 76.37	4.18 4.18	19.38 19.44	–
8b	87	160–164	$C_{25}H_{20}N_4O_2$ 408.45	73.51 73.47	4.94 4.93	13.72 13.76	–
9	86	125–126	$C_{14}H_{10}N_4S$ 266.32	63.14 63.10	3.78 3.78	21.01 21.30	12.21 12.30
10	64	175–177	$C_{14}H_{14}N_6S$ 298.37	56.36 56.28	4.73 4.72	28.17 28.25	10.75 10.45
11	81	160–162	$C_{20}H_{18}N_6S$ 374.46	65.15 65.14	4.85 5.22	22.44 22.66	8.56 8.86
13	68	240–242	$C_{14}H_{10}N_4O$ 250.26	67.19 67.12	4.03 3.82	22.39 22.78	–
14	71	80–82	$C_{14}H_{14}N_6$ 266.30	63.14 63.05	5.30 5.29	31.56 31.65	–
15	52	110–112	$C_{20}H_{18}N_6$ 342.40	70.16 70.08	5.30 5.59	24.54 24.62	–
16a	76	105–107	$C_{17}H_{12}N_6$ 300.32	67.99 67.90	4.03 4.02	27.98 27.62	–
16b	78	145–147	$C_{19}H_{17}N_5O_2$ 347.37	65.69 65.63	4.93 4.92	20.16 20.23	–

*2-Amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**2**)*

Equimolecular amounts of β-amino-α,γ-dicyanocrotononitrile (**1**) (1.32 g, 0.01 mol) and acetophenone (1.20 g, 0.01 mol) were heated in an oil bath at 140 °C for 1 h in the presence of anhydrous ammonium acetate (1.0 g). After cooling, the reaction mixture was heated in ethanol, then poured onto an ice/water mixture and the formed solid product **2** was collected by filtration and crystallized from ethanol.

*5-(2-Phenylhydrazono)-2-amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**3a**) and 5-(2-*p*-chlorophenylhydrazono)-2-amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**3b**). General procedure*

To a cold solution (0 °C) of compound **2** (2.34 g, 0.01 mol) in ethanol (50 mL) containing anhydrous sodium acetate (0.82 g, 0.01 mol), either benzenediazonium chloride (0.95 g, 0.01 mol) or 4-chlorobenzene-diazonium chloride (1.27 g, 0.01 mol) was added under continuous stirring. The reaction mixture was stirred at room temperature for 2 h and the formed solid product, in each case, was collected by filtration, dried and crystallized from acetic acid.

Table II. Spectral data of newly synthesized products

Compd. No.	IR (ν, cm^{-1})	^1H NMR (δ, ppm) (DMSO-d_6)	MS (M^+)
2	3475, 3360 (NH_2), 3055 (CH aromatic), 2974 (CH_3), 2227, 2224, 2220 (3CN), 1642 (C=C), 3475, 3360	2.93 (s, 3H, CH_3), 4.32 (s, 2H, NH_2), 7.32–7.38 (m, 5H, C_6H_5)	234
3a	3460–3325 (NH_2, NH), 3050 (CH aromatic), 2227, 2224, 2220 (3CN), 1655 (C=N), 1640 (C=C)	4.33 (s, 2H, NH_2), 6.78 (s, 1H, CH=N), 7.32–7.36 (m, 10H, $2C_6H_5$), 8.48 (s, 1H, NH)	338
3b	3444–3330 (NH_2, NH), 3060 (CH aromatic), 2226–2220 (3CN), 1655 (C=N), 1641 (C=C)	4.33 (s, 2H, NH_2), 6.54 (s, 1H, CH=N), 7.32–7.39 (m, 9H, C_6H_5, C_6H_4), 8.38 (s, 1H, NH)	372
3c	3460–3380 (NH_2, NH), 3055 (CH aromatic), 2890 (CH_2), 2228–2218 (4 CN), 1660 (C=N), 1645 (C=C)	2.25–2.27 (m, 4H, $2CH_2$), 2.34–2.38 (m, 4H, $2CH_2$) 4.32 (s, 2H, NH_2), 6.46 (s, 1H, CH=N), 7.31–7.34 (m, 5H, C_6H_5), 8.52 (s, 1H, NH)	423
3d	3455–3340 (NH_2, NH), 3060 (CH aromatic), 2985, 2819 (CH_3, CH_2), 2225–2219 (3CN), 1690 (C=O), 1660 (C=N), 1640 (C=C)	1.16 (t, 3H, CH_3), 2.24–2.26 (m, 4H, $2CH_2$), 2.34–2.38 (m, 4H, $2CH_2$), 4.23 (q, 2H, CH_2), 4.33 (s, 2H, NH_2), 6.68 (s, 1H, CH=N), 7.33–7.36 (m, 5H, C_6H_5), 8.72 (s, 1H, NH)	470
4a	3460–3345 (NH_2, NH), 3058 (CH aromatic), 2974, 2880 (CH_3, CH_2), 2225, 2220 (2CN), 1670 (exocyclic C=N), 1643 (C=C)	4.32 (s, 2H, NH_2), 6.89 (s, 1H, pyridazine H-3), 7.32–7.38 (m, 10H, $2C_6H_5$), 8.56 (s, br, 1H, NH)	338
4b	3460–3315 (NH_2, NH), 3060 (CH aromatic) 2223, 2220 (2CN), 1670 (exocylcic C=N), 1642 (C=C)	4.34 (s, 2H, NH_2), 6.88 (s, 1H, pyridazine H-3) 7.33–7.38 (m, 9H, C_6H_5, C_6H_4), 8.80 (s, 1H, NH)	372
4c	3448–3325 (NH_2, NH), 3055 (CH aromatic), 2890 (CH_2), 2225, 2222–2218 (3CN), 1665 (exocyclic C=N), 1644 (C=C)	2.24–2.27 (m, 4H, $2CH_2$), 2.33–2.36 (m, 4H, $2CH_2$), 4.32 (s, 2H, NH_2), 6.90 (s, 1H, pyridazine H-3), 7.28–7.33 (m, 5H, C_6H_5), 8.87 (s, 1H, NH)	423
4d	3460–3338 (NH_2, NH), 3062 (CH aromatic), 2978, 2880 (CH_3, CH_2), 2225, 2220 (2CN), 1682 (C=O), 1670 (exocyclic C=N), 1646 (C=C)	1.16 (t, 3H, J = 6.57 Hz, CH_3), 2.23–2.26 (m, 4H, $2CH_2$), 2.32–2.37 (m, 4H, $2CH_2$), 4.24 (q, 2H, J = 6.57 Hz, CH_2), 4.33 (s, 2H, NH_2), 6.90 (s, 1H, pyridazine H-3), 7.30–7.34 (m, 5H, C_6H_5), 8.87 (s, br, 1H, NH)	470
5a	3463, 3370 (NH_2), 3065 (CH aromatic), 2225, 2222, 2218 (3CN), 1645 (C=C)	4.33 (s, 2H, NH_2), 6.64, 6.78 (2d, 2H, CH=CH), 7.30–7.37 (m, 10H, $2C_6H_5$)	322

5b	3560–3365 (OH, NH_2), 3062 (CH aromatic), 2225, 2222, 2220 (3CN), 1646 (C=C)	4.31 (s, 2H, NH_2), 6.60, 6.73 (2d, 2H, CH=CH), 7.32–7.39 (m, 9H, C_6H_5, C_6H_4), 10.20 (s, 1H, OH)	338
7a	3455–3336 ($2NH_2$), 3050 (CH aromatic), 2227, 2223, 2220 (3CN), 1650 (C=C)	4.32, 5.68 (2s, 4H, $2NH_2$), 7.28–7.37 (m, 11H, $2C_6H_5$, C_6H)	361
7b	3470–3336 ($2NH_2$), 3057 (CH aromatic), 2890, 2877 (CH_3, CH_2), 2229, 2225 (2CN), 1644 (C=C).	1.16 (t, 3H, *J* = 7.02 Hz, CH_3), 4.19 (q, 2H, *J* = 7.02 Hz, CH_2), 4.32, 5.47 (2s, 4H, $2NH_2$), 7.28–7.37 (m, 11H, $2C_6H_5$, C_6H)	408
8a	3462–3325 ($2NH_2$), 3058 (CH aromatic), 2225, 2221 (2CN), 1645 (C=C)	5.85, 6.21 (2s, 4H, $2NH_2$), 7.29–7.36 (m, 11H, $2C_6H_5$, C_6H)	361
8b	3466–3380 ($2NH_2$), 3055 (CH aromatic), 2974, 2880 (CH_3, CH_2), 2225 (CN), 1687 (C=O), 1665 (C=N), 1645 (C=C)	1.16 (t, 3H, *J* = 5.97 Hz, CH_3), 4.25 (q, 2H, *J* = 5.97 Hz, CH_2), 5.32, 6.09 (2s, 4H, $2NH_2$), 7.28–7.33 (m, 11H, $2C_6H_5$, C_6H)	408
9	3480–3340 ($2NH_2$), 3060 (CH aromatic), 2225, 2220 (2CN), 1643 (C=C)	4.33, 5.46 (2s, 4H, $2NH_2$), 6.89 (s, 1H, thiophene H-5), 7.29–7.34 (m, 5H, C_6H_5)	266
10	3485–3362 ($4NH_2$), 3054 (CH aromatic), 1672 (C=N), 1648 (C=C)	4.48, 5.21–5.36 (4s, 8H, $4NH_2$), 6.63 (s, 1H, thiophene H-2), 7.33–7.39 (m, 5H, C_6H_5)	298
11	3460–3348 ($4NH_2$), 3060 (CH aromatic), 1662 (C=N), 1643 (C=C)	5.34, 5.20–5.68 (2s, 8H, $4NH_2$), 6.43 (1s, 1H, thiophene H-3), 7.32–7.38 (m, 10H, $2C_6H_5$)	376
13	3458–3370 (NH_2), 3060 (CH aromatic), 2985 (CH_3), 2225, 2223 (2CN), 1673 (C=N), 1648 (C=C)	2.91 (s, 3H, CH_3), 4.88 (s, 2H, NH_2), 7.30–7.38 (m, 10H, $2C_6H_5$)	250
14	3463–3348 ($3NH_2$), 3058 (CH aromatic), 2974 (CH_3), 1660 (C=C), 1638 (C=C).	3.23 (s, 3H, CH_3), 4.58, 5.23, 5.81 (3s, 6H, $3NH_2$), 7.31–7.38 (m, 5H, C_6H_5).	266
15	3455–3320 ($2NH_2$), 3062 (CH aromatic), 2984 (CH_3), 1668 (exocyclic C=N), 1656 (C=C)	3.02 (s, 3H, CH_3), 5.42, 5.64 (2s, 4H, $2NH_2$), 7.27–7.34 (m, 10H, $2C_6H_5$), 8.73 (s, 1H, NH)	342
16a	3480–3325 ($3NH_2$), 3057 (CH aromatic), 2225, 2222 (2CN), 1667 (C=C), 1648 (C=C)	4.84, 5.23, 5.46 (3s, 6H, $3NH_2$), 7.31–7.39 (m, 6H, C_6H_5, C_6H)	300
16b	3465–3342 ($3NH_2$), 3048 (CH aromatic), 2987, 2892 (CH_3, CH_2), 2220 (CN), 1685 (C=O), 1660 (C=N), 1642 (C=C)	1.16 (t, 3H, CH_3), 4.24 (q, 2H, CH_2), 5.32, 5.48–5.51 (3s, 6H, $3NH_2$), 7.29–7.34 (m, 6H, C_6H_5, C_6H)	347

*5-(3′-Cyano-4′,5′,6′,7′-tetrahydrobenzo[b]thieno-2-hydrazono)-2-amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**3c**) and 5-(ethyl 4′,5′,6′,7′-tetrahydrobenzo[b]thiopheno-3-carboxylato-2-hydrazono)-2-amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**3d**). General procedure*

A solution of 2-diazo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (2.25 g, 0.01 mol) or ethyl 2-diazo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (1.78 g, 0.01 mol), in glacial acetic acid (30 mL), was added to a cold solution (0 °C) of compound **2** (2.34 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (0.82 g, 0.01 mol) under continuous stirring. The reaction mixture was stirred at room temperature for 2 h and the formed solid product was collected by filtration, dried and crystallized from acetic acid.

*2-(Amino(2,3-dihydro-3-imino-2,5-diphenylpyridazin-4-yl)methylene)-malononitrile (**4a**), 2-(amino(2,3-dihydro-3-imino-2-(4′-chlorophenyl)-5-phenylpyridazin-4-yl)methylene) malononitrile (**4b**), 2-(amino(2,3-dihydro-3-imino-2-(3-cyano-4′,5′,6′,7′-tetrahydrobenzo[b]thieno-2-yl)-5-phenyl-pyridazin-4-yl)methylene)malononitrile (**4c**), and 2-(amino-(2,3-dihydro-3-imino-2-(ethyl 4′,5′,6′,7′-tetrahydrobenzo[b]thiopheno-3-carboxylato-2--yl)-5-phenylpyridazin-4-yl)methylene)malononitrile (**4d**). General procedure*

A suspension of 0.01 mol **3a-d** (**a**: 3.38 g), (**b**: 3.72 g) (**c**: 4.22 g) and (**d**: 4.69 g) in 0.46 g sodium ethoxide solution [prepared by dissolving sodium metal (0.02 mol) in 40 mL absolute ethanol] was heated in a boiling water bath for 3 h, then left to cool. The solid product formed upon pouring onto ice/water containing hydrochloric acid (pH ~ 6) was collected by filtration, dried and crystallized from dioxane (**4a**, **4c**), DMF (**4b**) and ethanol (**4d**).

*2-Amino-4,6-diphenyl-1,1,3-tricyanohexa-1,3,5-triene (**5a**)*

To a solution of compound **2** (2.34 g, 0.01 mol) in 1,4-dioxane (50 mL) containing piperidine (0.5 mL), benzaldehyde (1.08 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h, then poured onto ice/water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from ethanol.

*2-Amino-4-phenyl-1,1,3-tricyano-6-(2′-hydroxyphenyl)hexa-1,3,5-triene (**5b**)*

To a solution of compound **2** (2.34 g, 0.01 mol) in ethanol (50 mL) containing piperidine (0.5 mL), salicylaldehyde (1.18 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h, cooled at room temperature and poured onto ice/water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from dioxane.

*α-Cyano-β-amino-β-(2-amino-3-cyano-4,6-diphenylbenzen-1-yl)acrylonitrile (**7a**) and α--cyano-β-amino-β-(2-amino-3-ethoxycarbonyl-4,6-diphenylbenzen-1-yl)acrylonitrile (**7b**). General procedure*

To a solution of compound **5a** (3.22 g, 0.01 mol) in ethanol (50 mL) containing triethylamine (0.5 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 2 h, then poured onto ice/water containing a few drops of hydrochloric acid. The formed solid product, in each case, was collected by filtration, dried and crystallized from ethanol.

*2,4-Diamino-5,7-diphenylquinoline-3,8-dicarbonitrile (**8a**) and ethyl 2,4-diamino-5,7-diphenylquinoline-3-nitrilo-8-carboxylate (**8b**)*

Method (A). – A suspension of either **7a** (3.61 g, 0.01 mol) or **7b** (4.08 g, 0.01 mol) in sodium ethoxide [prepared by dissolving sodium metal (0.23 g, 0.01 mol) in absolute ethanol (30 mL)] was heated in a boiling water bath for 3 h, then poured onto ice/water containing a few drops of hydrochloric acid (till pH 6). The formed solid product was collected by filtration and crystallized from 1,4-dioxane.

Method (B). – To a suspension of compound **5a** (3.22 g, 0.01 mol) in sodium ethoxide (0.01 mol), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated in a boiling water bath for 3 h, then poured onto ice/water containing a few drops of hydrochloric acid (till pH 6). The formed solid product was collected by filtration.

Method (C). – To a solution of compound **2** (2.34 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (0.5 mL), either α-cyanocinnamonitrile (1.54 g, 0.01 mol) or α-ethoxycarbonyl cinnamonitrile (2.01 mol, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h, then poured onto ice/water and the formed solid product was collected by filtration.

*2-Amino-3-(β-amino-α-cyanoacrylonitrilo-3-yl)-4-phenylthiophene (**9**)*

To a solution of compound **2** (2.34 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (0.5 mL), elemental sulfur (0.32 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h. It was allowed to cool then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The reaction mixture was left overnight to settle and the formed solid product was collected by filtration, dried and crystallized from acetic acid.

*2-Amino-3-aminocarbonyl-(3′,5′-diaminopyrazol-4′-ylideno)-4-phenylthiophene (**10**) and 2-amino-4-phenyl-3-(aminocarbonyl-(3′amino-5′-imino-1′-phenylpyrazol-4′-ylideno)-thiophene (**11**). General procedure*

To a solution of compound **9** (2.66 g, 0.01 mol) in ethanol (50 mL), either hydrazine hydrate (0.5 g, 0.01 mol) or phenylhydrazine (1.18 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h, then poured onto an ice/water

mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration, dried and crystallized from acetic acid (**10**) and 1,4-dioxane (**11**).

*2-[3-Amino-4-(1-phenylethylideno)isoxazol-5-(4*H*)-ylideno]malononitrile (**13**)*

To a solution of compound **2** (2.34 g, 0.01 mol) in 1,4-dioxane (50 mL) containing sodium acetate (0.82 g, 0.01 mol), hydroxylamine hydrochloride (0.69 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1.5 h, during which time a non-isolable intermediate **12** formed and the latter afforded the final product **13**. After cooling the reaction mixture was poured onto ice/water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from ethanol.

*5-(1-Phenylethylideno)-5*H*-pyrazolo[3,4-b]pyridine-3,4,6-triamine (**14**)*

To a solution of compound **2** (2.34 g, 0.01 mol) in ethanol (50 mL), hydrazine hydrate (0.5 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h and the formed solid product, upon being poured into ice/water containing a few drops of hydrochloric acid, was collected by filtration, dried and crystallized from 1,4-dioxane.

*3-Amino-4-(1′-amino-2′-cyano-3′-phenyl-2′-buten-1′-ylideno)-5-imino-1-phenylpyrazole (**15**)*

To a solution of compound **2** (2.34 g, 0.01 mol) in ethanol (50 mL), phenylhydrazine (1.18 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h and then poured onto ice/water containing a few drops of hydrochloric acid. The formed solid product was collected by filtration, dried and crystallized from DMF.

*2,4,7-Triamino-5-phenylquinoline-3,8-dicarbonitrile (**16a**) and ethyl 2,4,7-triamino-3--cyano-5-phenylquinoline-8-carboxylate (**16b**). General procedure*

To a solution of compound **2** (2.34 g, 0.01 mol) in ethanol (50 mL) containing triethylamine (0.5 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 1.5 h and then poured onto ice-water containing a few drops of hydrochloric acid. The solid product formed, in each case, was collected by filtration, dried and crystallized from ethanol.

In vitro *antimicrobial and antifungal activity*

Evaluation of the antibacterial activity, using one Gram-negative (*Escherichia coli ECT* 101) and two Gram-positive bacteria (*Bacillus subtilis* CECT 498 and *Bacillus cereus* CECT 148), and the antifungal activity, using *Candida albicans* 1394 as a representative fungal species was made for the compounds. The minimal inhibitory concentration (*MIC*, in $\mu g\ mL^{-1}$) was determined using an adapted agar streak dilution method based on radial diffusion (17, 18). Under the same conditions, solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. Diameters of the inhibition zones corresponding to the *MICs* are presented in Table III.

Table III. Antimicrobial and antifungal activities of synthesized compounds

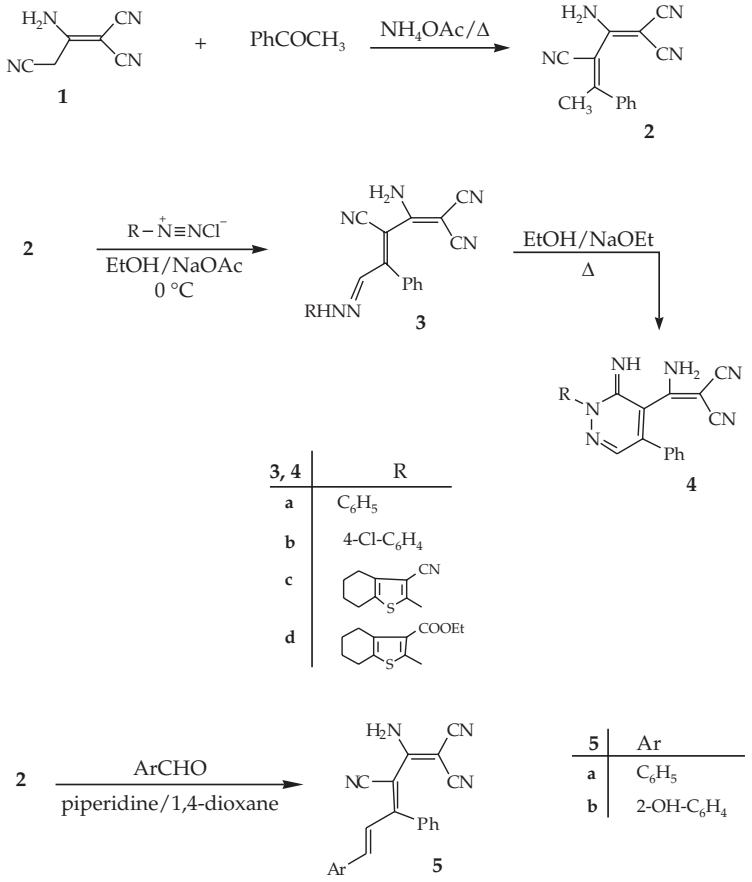
Compd. No.	*MIC* (μg mL^{-1}) (zone of inhibition, mm)			
	E. *coli*	*B. cereus*	*B. subtilis*	*C. albicans*
2	14.8 (6)	12.01 (4)	6.25 (5)	20 (11)
3a	16.50 (6)	20 (8)	6.25 (4)	50 (11)
3b	14.80 (6)	8.05 (9)	3.13 (10)	0.61 (6)
3c	18.21 (5)	20.15 (4)	26.16 (9)	16 (5)
3d	14.84 (6)	12.32 (3)	16.32 (8)	14.40 (4)
4a	NA	18.32 (5)	6.22 (2)	0.40 (10)
4b	NA	12.30 (4)	4.22 (6)	12.55 (12)
4c	NA	12.34 (7)	6.13 (4)	0.40 (5)
4d	NA	6.05 (6)	12.42 (2)	4.55 (10)
5a	NA	4.25 (15)	18 (8)	30 (6)
5b	10.33 (6)	0.01 (3)	0.48 (4)	25.60 (6)
7a	NA	6.66 (3)	20.33 (8)	10.20 (5)
7b	NA	13.39 (2)	12.33 (6)	10.66 (4)
8a	10.46 (4)	8.66 (6)	25.33 (5)	12.22 (8)
8b	NA	7.39 (4)	4.33 (5)	12.77 (5)
9	NA	0.08 (2)	2.22 (5)	6.44 (8)
10	NA	7.03 (8)	0.68 (2)	20.50 (5)
11	NA	0.08 (2)	2.22 (5)	6.44 (8)
13	NA	4.25 (3)	6.23 (8)	6.44 (6)
14	NA	6.22 (5)	12.89 (4)	18.42 (9)
15	NA	7.39 (4)	4.33 (5)	12.77 (5)
16a	8.8 (6)	1.03 (8)	0.68 (2)	100 (5)
16b	NA	25 (8)	23 (6)	26 (3)
Ampicillin	6.25 (8)	3.13 (8)	12.50 (10)	–
Cycloheximide	–	–	–	12.50 (10)

Solvent used: DMSO; solutions of 1.0 mg mL^{-1} of each compound.
NA – not active.

For the *in vitro* antimicrobial activity, microorganism suspensions were prepared to contain approximately 10^8 cfu mL^{-1} and the plates were inoculated. A stock solution of the synthesized compound (1.0 mg mL^{-1}) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3 mm, diameter 4 mm) made in the center of the Petri dish (nutrient agar for bacteria and Sabouraud *vs.* dextrose agar medium for fungi). The plates were incubated at 37 °C (for bacteria) and at 30 °C (for fungi) for 24 h in duplicate. A positive control using only inoculation and a negative control using only DMSO in the cavity were carried out.

RESULTS AND DISCUSSION

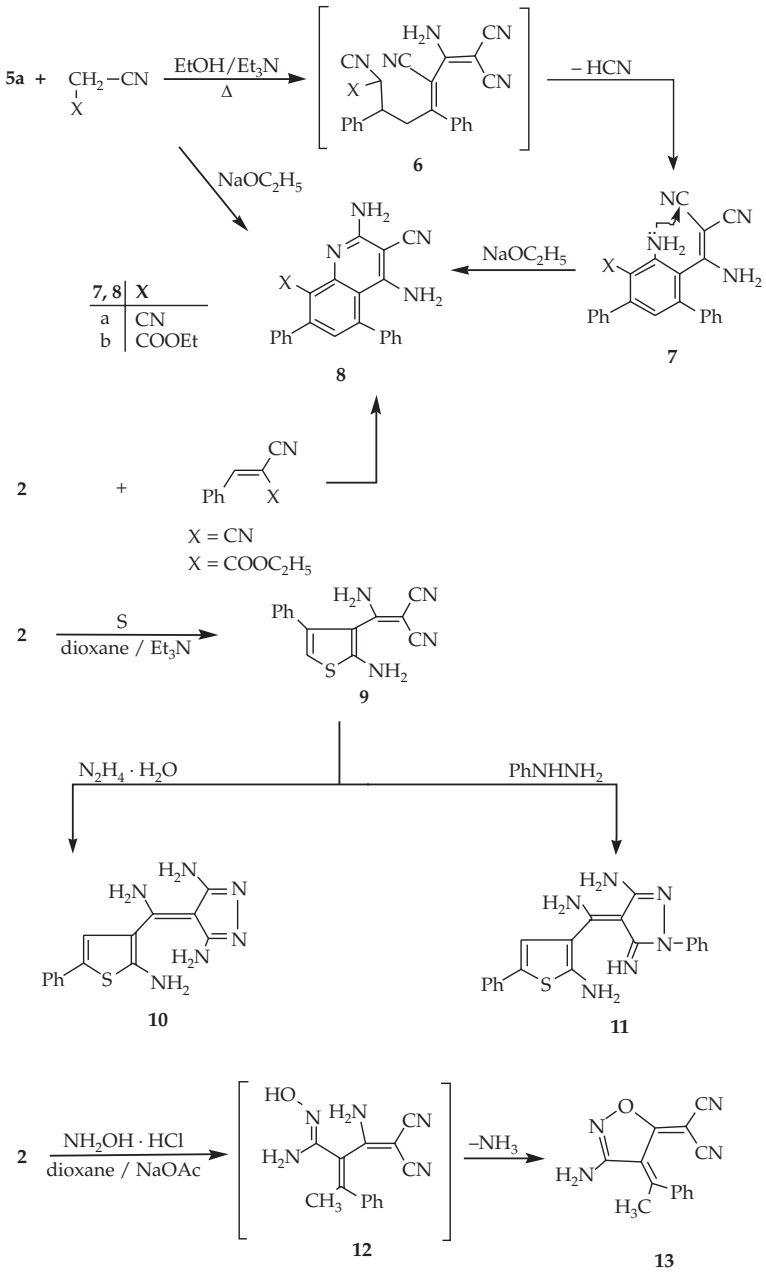
The starting material β-amino-α,γ-dicyanocrotononitrile (**1**) was prepared as previously described (19). Compound **1** reacted with acetophenone at 140 °C in the presence of anhydrous ammonium acetate to give a single product with the molecular formula $C_{14}H_{10}N_4$. 2-Amino-4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile **2** was considered for the reaction product on the basis of ^{1}H NMR of the reaction product which showed the presence of a singlet at δ 2.93 ppm due to the presence of a methyl group, a singlet at δ 4.32 ppm (D_2O-exchangeable) due to the existence of an NH_2 group and a multiplet at δ 7.32–7.38 ppm due to the existence of a phenyl group. The mass spectral fragmentations are in agreement with the structure of compound **2**. Further confirmation for the structure of compound **2** was made by through studying its reactivity towards various chemical reagents. Thus, coupling of compound **2** with aryl and heterocyclic diazonium salts was studied. Coupling of **2** with either benzenediazonium chloride or 4-chlorobenzenediazonium chloride gave the arylhydrazono derivatives **3a** and **3b**, respectively. Similarly, compound **2** was coupled with either 2-diazo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile or ethyl 2-diazo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate to give the hydrazo derivatives **3c** and **3d**, respectively. Formation of compounds **3a-d** was based on simple coupling of diazonium salts with a methyl group activated by α,β-unsaturated nitrile. Compounds **3a-d** underwent cyclization when heated under reflux in an ethanolic/NaOEt solution to give the pyridazine derivatives **4a-d**, respectively. Formation of the latter products is based on the addition of the hydrazo NH group to the cyano group. Reaction of compound **2** with aromatic aldehydes was studied with the aim of forming arylidene derivatives capable of reacting with cyanomethylene reagents forming polyfunctionally substituted pyridine or benzene derivatives of potential biological activities. Thus, condensation of compound **2** with benzaldehyde in 1,4-dioxane solution containing a catalytic amount of piperidine gave the 2-amino-4,6-diphenyl-1,1,3--tricyanohexa-1,3,5-triene (**5a**). In a similar manner, compound **2** reacted with salicylaldehyde to give 2-amino-4-phenyl-1,1,3-tricyano-6-(2′-hydroxyphenyl)hexa-1,3,5-triene **5b**. Compounds **5a,b** were formed on the basis of the first formation of alcohol followed by dehydration (Scheme 1). Compound **5a** reacted with either malononitrile or ethyl cyanoacetate in absolute ethanol containing a catalytic amount of triethylamine to give the substituted benzene derivatives **7a,b**, respectively. Formation of **7a,b** is explained in terms of intermediate formation of **6a,b** followed by hydrogen cyanide liberation. Compounds **7a,b** underwent ready cyclization when heated in sodium ethoxide solution to give the quinoline derivatives **8a,b** *via* the Michael addition of the NH_2 group to CN group. Moreover, the same quinoline derivatives **8a,b** were obtained by reacting **5a** with either malononitrile or ethylcyanoacetate in sodium ethoxide solution. Quinoline derivatives **8a,b** were chosen for the reaction products based on ^{1}H NMR of the reaction products, which showed for **8a** two downfield (D_2O exchangeable) singlets at δ 5.85, 6.21 ppm for two NH_2 groups, a multiplet at δ 7.29–7.36 ppm for two C_6H_5 and one benzene CH protons. Thus, the chemical shift of the enaminonitrile amino group, which appears at δ 4.32 ppm for compounds **7a,b**, was downfield to δ 5.85 ppm in structures **8a,b**. Addition of the NH_2 group to the cyano group in compounds **7a,b** leads to the formation of cyclized products **8a,b**. Further confirmations for structures **8a,b** were obtained in terms of their mass spectra (Table II). Compounds **8a,b** were also synthesized using another reaction



Scheme 1

route; thus, the reaction of compound **2** with cinnamonitrile derivatives like α-cyanocinnamonitrile and α-ethoxycarbonyl cinnamonitrile gave the same products **8a** and **8b**, respectively (m.p., mixed m.p. and fingerprint IR).

Reaction of compound **2** towards elemental sulfur to form thiophene derivatives applying Gewald's synthesis (20, 21) was undertaken. Thus, compound **2** reacted with elemental sulfur in the presence of triethylamine to give 2-amino-3-(β-amino-α-cyanoacrylonitrilo-3-yl)-4-phenylthiophene **9**, the structure of which was based on analytical and spectral data. The ^{1}H NMR showed the presence of two singlets (D_2O exchangeable) at δ 4.33 and 5.46 ppm due to the presence of two NH_2 groups, a singlet at δ 6.89 ppm due to the thiophene H-5 proton and a multiplet at δ 7.29–7.34 due to aromatic protons. Formation of the thiophene derivative **9** is explained in terms of the first addition of S to CH_3 of compound **2** to form an intermediate SH followed by addition of the latter to the CN group. Reaction of **9** with hydrazine hydrate gave the pyrazole derivative **10**.

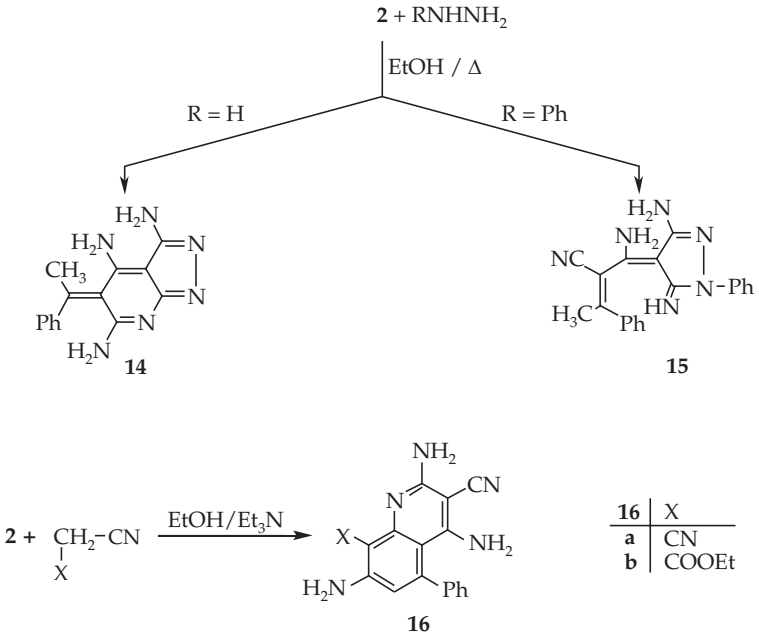

5a +
CH2–CN
X
EtOH/Et3N
Δ
6
– HCN
NaOC2H5
7
NaOC2H5
8
7, 8 | X
a | CN
b | COOEt
2 +
X = CN
X = COOC2H5
2
S
dioxane / Et3N
9
N2H4 · H2O
PhNHNH2
10
11
2
NH2OH · HCl
dioxane / NaOAc
12
–NH3
13

Scheme 2

However, the reaction of **10** with phenylhydrazine gave the thieno[2,3:2,3] pyrido-[5,6:4,5]pyrazole derivative **11**. Formation of compounds **10** and **11** is explained in terms of the addition of hydrazine to the dicyano group present in compound **9**.

Compound **2** reacted with hydroxylamine hydrochloride to give 2-(3-amino-4-(1-phenylethylideno)isoxazol-5-(4H)-ylideno)malononitrile (**13**), the structure of which is based on spectral and analytical data. Formation of **13** took place through the intermediacy of **12** followed by ammonia elimination (Scheme 2). Reaction of **2** with either hydrazine hydrate or phenylhydrazine was studied. Thus, in case of the reaction with hydrazine hydrate, 5-(1-phenylethylideno)-5*H*-pyrazolo[3,4-b]pyridine-3,4,6-triamine (**14**) is formed. However, the reaction of compound **2** with phenylhydrazine gave 3-amino-4-(1′-amino-2′-cyano-3′-phenyl-2′-buten-1′-ylideno)-5-imino-1-phenylpyrazole (**15**). Addition of hydrazine to the dicyano group in compound **2** to pyrazoles **14** and **15**. The analytical and spectral data are consistent with the proposed structure. Compound **2** reacted with either malononitrile or ethyl cyanoacetate to give the benzo[*b*]pyridine derivatives **16a** and **16b**, respectively. The tools used to confirm the structures of **16a,b** are the same as those used before to confirm **8a,b** (see experimental section). Formation of compounds **16a,b** is explained by the first addition of the CH_3 group of compound **2** to CN of malononitrile followed by the second addition of CH_2 of malononitrile moiety to one of the CN-groups (Scheme 3).

From the analysis in Table III it is possible to establish some SARs. The only active compounds against *E. coli* at the concentrations tested are compound **2**, **3a**, **3b**, **3c**, **3d**, **8a**



Scheme 3

and **16a** (average *MIC* 12.5 μg mL^{-1}), substituted thiophene, polyfunctionally substituted benzene and the benzopyridine moieties responsible for the activity. However, the benzo[*b*]pyridine derivative **16a** showed the highest activity. 2-Amino-4-phenyl-1,1,3-tricyano-6-(2′-hydroxyphenyl)hexa-1,3,5-triene (**5b**) (conjugated enaminonitrile derivative) showed the highest activity against *B. cereus* and *B. subtilis*. Compound **4a** (polyfunctionally substituted pyridazine derivative) showed the highest activity against *C. albicans*. Comparing the SAR of compounds **3a,c,d** (substituted hydrazone derivatives), one can notice that when the R group is phenyl as in the case of 5-(2-phenylhydrazono)-2-amino--4-phenylpenta-1,3-diene-1,1,3-tricarbonitrile (**3a**), the lowest activity against *E. coli* and *C. albicans* is observed. However, the presence of *p*-chlorophenyl substituted phenyl hydrazone in **3b** increases the activity against *C. albicans*. The presence of the 3-cyano-tetrahydrobenzo[b]thiophene substituent as in compound **3c** decreases the activity against *E. coli*. However, substitution of the 3-cyano group by the ethyl ester group, as in the case of **3d**, increases the activity against *E. coli*. Considering the pyridazine derivatives **4a-d**, one can notice that when the substituent is 3-ethoxycarbonyl-tetrahydrobenzo[b]thiophene as in the case of **4d**, the highest activity towards *B. cereus* is obtained. However, compound **4c** with 3-cyano-tetrahydrobenzo[b]thiophene showed the highest activity against *C. albicans*. Compounds **5b** (with the hydroxyphenyl group), thiophene derivatives **9**, **11** and benzo[b]pyridine **16a** are highly active against *B. cereus*. In addition compounds **5b** and **16a** are active against *B. subtilis* and compounds **3b**, **4a** and **4c** are highly active against *C. albicans*.

Comparing the reactivity of newly synthesized products towards *E. coli* relative to ampicillin, all synthesized products are less active except for compound **16a**, which showed comparable reactivity. However, comparing the reactivity towards *B. cereus*, compounds **5b**, **9**, **11** and **16a** showed higher activity than ampicillin. On the other hand, all compounds except **3c**, **3d**, **5a**, **7a**, **8a** and **16b** showed higher reactivity against *B. subtilis* than ampicillin. Concerning the reactivity against *C. albicans* compared to the reference cycloheximide, one can notice that all compounds showed higher activity, with the exception of **2**, **3a**, **3c**, **3d**, **5a,b**, **10**, **14** and **16a,b**.

CONCLUSIONS

The structure activity relationship suggested that heterocyclization reactions of the newly synthesized penten-1,3-diene-1,1,3-tricarbonitrile derivative (**2**) into pyrazole, isoxazole, thiophene, pyridine and pyridazine derivatives resulted in products some of which show high antimicrobial activity. These findings encourage us to explore new molecules by introducing potent moieties, such as heterocyclic and fused ring systems described in this work, into other enaminonitriles. Our prediction is that these compounds with new ring systems may show even better antimicrobial activity.

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S A Ž E T A K

Reakcija β-amino-α,γ-dicianokrotononitrila s acetofenonom: Sinteza derivata piridina, piridazina i tiofena s antimikrobnim djelovanjem

RAFAT M. MOHAREB, KARAM A. El-SHARKAWY i SHERIF M. SHERIF

Kondenzacijom β-amino-α,γ-dicijanokrotononitrila **1** s acetofenonom dobiven je 2--amino-4-fenilpenta-1,3-dien-1,1,3-trikarbonitril (**2**) koji je upotrebljen u reakcijama heterociklizacije s različitim reagensima poput diazonijevih soli, hidrazina, hidroksilamina i elementarnog sumpora pri čemu su nastali derivati piridazina, pirazola, izoksazola, odnosno tiofena. Spoj **2** je u reakciji s aromatskim aldehidima te naknadno sa cijanometilenima dao derivate piridina. Određene su *MIC* vrijednosti za novosintetizirane spojeve protiv *E. coli*, *B. cereus*, *B. subtilis* i *C. albicans*.

Ključne riječi: piridin, izoksazol, piridazin, tiofen, antimikrobno djelovanje

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