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Reactions with Acetoacetanilide: Synthesis and Antibacterial Activity of some New Pyran, Pyrano[2,3-c]pyrazole and Pyrano[2,3-c]-pyridine Derivatives

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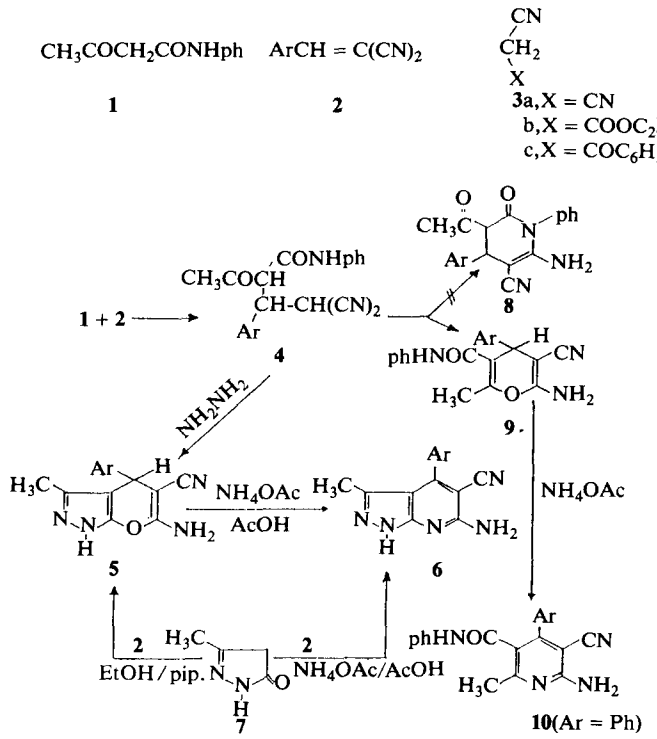
Abstract The reaction of acetoacetanilide (**1**) with the α-cyanocinnamonitrile derivatives **2** yielded the Michael adducts **4** which could be converted into the pyrano[2,3-c] pyrazole derivatives **5** via their reaction with hydrazine hydrate. Cyclisation of **4** afforded the cyanoaminopyrans **9** which could in turn be converted into the corresponding pyridine derivatives **10**. The pyranopyrazoles **9** reacted with different activated nitrile derivatives (**3**a-c) to give the pyrano[2,3-c]pyridine derivatives **13, 16** and **19** respectively. The biological activity of the synthesised heterocyclic derivatives was investigated and discussed.

Keywords acetoacetanilide, α-cyanocinnamonitrile, pyrano[2,3-c]pyrazole, pyrano[2,3-c] pyridine, antibacterial.

In the last few years we have been interested in the synthesis of heterocyclic derivatives of potential biological activity.[1-5] In conjuntion with our previous work, we report, here, on the synthesis of cetain pyranopyrazole, pyrazolopyridine and pyranopyridine derivatives required for a medicinal chemistry program. Recently[6] the reaction of **1** with **2** in boiling ethanol was reported to give 3-acetyl-6-amino-4-aryl-5-cyano-1-phenyl-1,2,3,4-tetrahydropyridin-2-ones. We now found that, when this reaction was carried out at room temperature, it yielded the acyclic Michael adducts **4** which could be cyclized to give the corresponding γ-pyran derivatives **9a,b**. The newly synthesised compounds bear latent functional substituents which make them interesting as candidates for biological activity studies, as well as for further chemical transformations.

It has been found that acetoacetanilide **1** reacted with the arylidene malononitriles **2** in ethanolic triethylamine at room temperature to yield adducts which were proved to be the Michael adducts **4a-e** based on the IR and ^{1}H-NMR spectra of the reaction products. Thus, the IR (cm^{-1}) spectrum of **4a** showed two peaks at 1700 and 1680 for two CO groups in addition to 2200 (CN) group. ^{1}H-NMR (δ ppm) of **4a** revealed signals at 2.2 (s, 3H, $\mathbf{CH_3}$CO); 2.8 (t, 1H); 4.4 (d, 1H) and 7.7-8.1 (m, 11H, ArH's and NH). Compounds **4a-e** reacted also with hydrazine hydrate in ethanol under reflux to give the 4-H pyrano[2,3-c]pyrazole derivatives **5a-e** respectively. The structure of **5** could be established via their synthesis from the reaction of **2** with 3-methyl-2-pyrazolin-5-one **7**.[7] The pyrano[2,3-c]pyrazoles **5a-e** could be converted into the corresponding pyrazolo[2,3-c]pyridines **6a-e** by reflux in acetic acid in the presence of ammonium acetate. Compounds **6** were also synthesised by refluxing **7** with **2** and ammonium acetate in acetic acid. The structure of **6** was confirmed by IR and ^{1}H-NMR spectra and elemental analyses. For example, the ^{1}H-NMR (δ ppm) spectrum of **6a** showed signals at 1.9 (s, 3H, $\mathbf{CH_3}$); 6.7 (s, 2H, NH_2) and 7.2-8.1 (m, 6H, ArH's and NH). The IR spectrum (cm^{-1}) of **6a** revealed the bands at 3400, 3320, 3200 (NH_2) and 2200 (CN).

Compounds **4a,b** were cyclized into **9a,b** and not **8a,b** by refluxing in a mixture of acetic acid-conc. hydrochloric acid (v/v, 4:1). Structure **8** was ruled out based on spectroscopic studies. Thus, the IR spectra of the reaction products showed only one carbonyl band at 1680 cm^{-1}, in addition to the peaks due to CN and NH_2 groups and their ^{1}H-NMR spectra revealed signals at (δ ppm) 1.9 (s, 3H, CH_3); 4.5 (s, 1H, pyran H-4); 6.8 (s, 2H, NH_2) and 7.2-8.3 (m 11H, ArH's and NH). Moreover, **9a** was refluxed with ammonium acetate in acetic acid



2,4-9 a,Ar = C_6H_5
b,Ar = C_6H_4-OCH_3-P
c,Ar = C_6H_4 CH_3-P
d,Ar = C_6H_4 Cl-P
e,Ar = C_6H_4 NO_2-P

Table I. Antibacterial Activities *in Vitro*

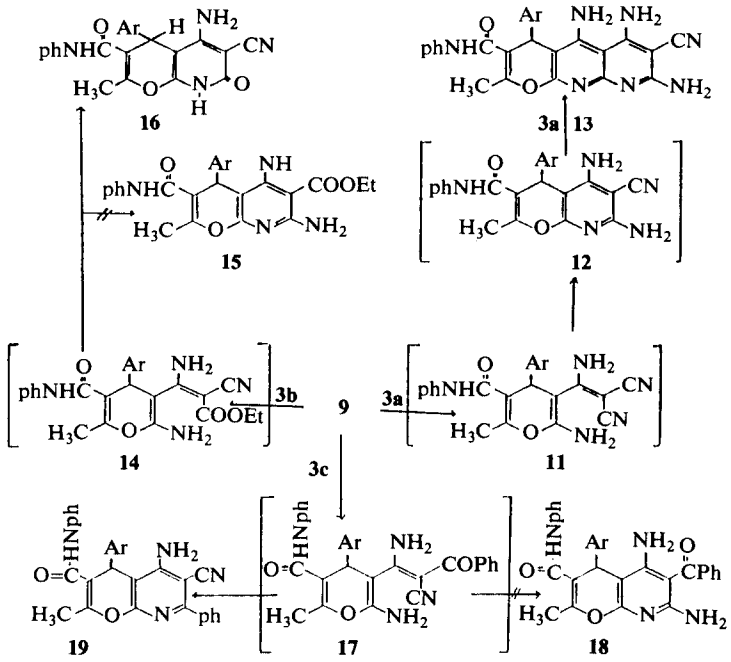
Organism	5a	5b	5c	5d	5e	6a	6b	6c	6d	6e	9a	9b	10	13a	13b	16a	16b	19a
Erwinia carotovora var. *carotovora*	+	2+	2+			+	4+	3+	+						+3			
Micrococcus luteus			+				+		+		+				+			
Clostridium acetobutylicum						4+			+						+			
Bacillus subtilis						2+			+			+			+			
Sarcinae sp.					4+													
Staphylococcus sp.	+	2+	3+	+	2+	2+	2+		+	+	+	+	+	2+	+	2+	+	+

inhibition zone around the disc: + = 1 mm 2+ = 2 mm
3+ = 3-4 mm 4+ = 4-5 mm

to yield the corresponding pyridine derivative **10**. The ^{1}H-NMR spectrum of **10** revealed the absence of signals for the proton in the region of 4-6 ppm and showed only signals at 1.9 (s, 3H, CH_3); 6.8 (s, 2H, NH_2) and 7.2-8.1 (m, 11H, ArH's and NH).

A solid mixture of each of **9a,b** and **3a** reacted at 160°C (bath temperature) to yield compounds **13a,b**. The structure of **13** was confirmed by elemental analyses and spectroscopic data studies (see experimental). **13a,b** were formed, most likely, via the intermediacy of **11a,b** which readily cyclized to the nonisolable **12a,b** which reacted with another molecule of **3a** to give the final isolable products **13a,b**.

In contrast to the behaviour of **3a**, ethyl cyanoacetate (**3b**) reacted with **9a,b** in refluxed ethanolic



13a,16a,19; Ar = C_6H_5
13b, 16b; Ar = $C_6H_4OCH_3$-p

triethylamine solution to yield the adducts **14a,b** which were readily cyclized to **16a,b** and not **15a,b** based on IR spectra, which revealed the presence of C≡N group. ^{1}H-NMR spectra of **16a,b** showed the absence of signals due to the ethoxycarbonyl group.

9a reacted with benzoylacetonitrile (**3c**) to yield the pyrano[2,3-b]pyridine derivative **19**. The reaction product was assumed to be formed via the formation of the intermediate **17** which readily lost one molecule of water to give **19** and not **18** based on the IR spectrum which revealed the presence of CN group at 2220 cm^{-1} in addition to the peaks at 1680 (CO anilide) and 3400, 3320, 3200 (NH_2) group. ^{1}H-NMR spectrum of **19** showed signals (δ ppm): 2.2 (s, 3H, CH_3); 4.8 (s, 1H, pyran H-4), 7.0-7.8 (m, 17H, ArH's and NH_2) and 8.3 (s, 1H, NH).

BIOLOGICAL RESULTS

The biological effects of these compounds were studied on some selected bacteria. The results reveal that compound **6b**, and **6c**, highly affected the bacteria *Erwinia carotovora* var. *carotovora* (isolated and identified by Saleh *et al.*[11]) while compounds **5c** and **5b** showed a moderate inhibiting action on this species. Most of the compounds showed a moderate bacteriocidal action on Staphylococcus sp.

Different effects on some species of bacteria are shown in Table I. On the other hand, these compounds were shown to have no effect on the following species of bacteria: *Aerobacter* sp., *Mycobacterium phlei, Micrococcus lutens, Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli, Salmonella typhimurum, Shigella flexneri, Pseudomonas putida, Bacillus licheniformis, Yersinia colitica* and *Proteus vulgaris*. All the above species of bacteria were kindly provided from the culture collection of Microbiological Research Center (MIRCEN). Cairo, Egypt.

EXPERIMENTAL

All melting points are uncorrected. The IR spectra were recorded (KBr) on a Pye-Unicam SP-1000 spectrophotometer. ^{1}H-NMR were obtained in $(CD_3)_2SO$ with a Varian 60 MHz spectrometer with $SiMe_4$ as internal standard. Microanalytical data were performed by the Microanalytical Centre, Cairo University. α-Cyanocinnamonitriles were prepared as previously described in the literature.[8-10]

Preparation of 4a-e

Equimolecular amounts of **1** (0.01 mol) and the

Table II. Characterisation data of the newly synthesised derivatives

Comp.	M.p. °C	Mol. formula	% Analysis		
			C %	H %	N %
4a	227-9	$C_{20}H_{17}N_3O_2$	72.49(72.30)	5.17(5.10)	12.68(12.80)
4b	220-1	$C_{21}H_{19}N_3O_3$	69.79(69.90)	5.29(5.20)	11.62(11.30)
4c	195	$C_{21}H_{19}N_3O_2$	73.02(73.10)	5.54(5.40)	11.26(11.80)
4d	210	$C_{20}H_{16}N_3O_2Cl$	65.66(65.70)	4.40(4.10)	11.48(11.60)
4e	175-6	$C_{20}H_{16}N_4O_3$	63.82(63.60)	4.28(4.10)	11.48(14.90)
5a	248[12]	$C_{14}H_{12}N_4O_2$	66.65(66.90)	4.79(4.60)	22.21(22.4)
5b	220[12]	$C_{15}H_{14}N_4O_2$	63.82(63.50)	4.99(4.80)	19.84(19.90)
5c	212	$C_{15}H_{14}N_4O$	67.65(67.40)	4.29(4.20)	21.03(20.90)
5d	220	$C_{14}H_{11}N_4OCl$	58.64(58.60)	3.86(4.10)	19.54(19.60)
5e	225[12]	$C_{14}H_{11}N_5O_3$	56.56(56.5)	4.44(4.70)	23.55(23.30)
6a	> 300	$C_{14}H_{11}N_5$	67.45(67.50)	4.44(4.60)	28.09(27.90)
6b	> 300	$C_{15}H_{13}N_5O$	64.50(64.60)	4.69(4.60)	25.07(25.40)
6c	> 300	$C_{15}H_{13}N_5$	68.42(68.30)	4.97(5.10)	26.59(26.30)
6d	> 300	$C_{14}H_{10}N_5Cl$	59.26(59.30)	3.55(3.70)	24.68(24.80)
6e	295	$C_{14}H_{10}N_6O_2$	57.14(56.90)	3.42(3.40)	28.55(28.30)
9a	195	$C_{20}H_{17}N_3O_2$	72.49(72.60)	5.17(5.00)	12.68(13.00)
9b	210	$C_{21}H_{19}N_3O_3$	69.79(71.00)	5.29(5.60)	11.62(11.30)
10	> 300	$C_{20}H_{16}N_4O$	73.15(73.20)	4.91(5.20)	17.06(16.80)
13a	230	$C_{26}H_{21}N_7O_2$	67.37(67.10)	4.56(4.90)	21.15(21.30)
13b	> 300	$C_{27}H_{23}N_7O_3$	65.70(65.80)	4.69(4.70)	19.86(19.70)
16a	> 310	$C_{23}H_{18}N_4O_3$	69.33(69.00)	4.55(4.40)	14.06(14.3)
16b	> 300	$C_{24}H_{20}N_4O_4$	67.28(67.30)	4.70(4.00)	1307(13.20)
19	> 300	$C_{29}H_{22}N_4O_2$	75.96(76.2)	4.83(5.20)	12.21(12.60)

appropriate **2a-e** (0.01 mol) in ethanol (20 m*l*) in the presence of catalytic amount of triethylamine (0.5 m*l*) were stirred for 2h at room temperature. The solid product was collected by filtration and crystallized from dimethylformamide to give **4a-e,** respectively (cf. Table II).

Synthesis of the pyran[2,3-c]pyrazoles 5a-e

A solution of each of **4a-e** and hydrazine hydrate (0.01 mol) in ethanol (25 m*l*) was heated under reflux for 2 h. The solid product so formed was collected and crystallized from DMF to give **5a-e,** respectively (cf. Table II and III).

Synthesis of the pyrazolo[3,4-b]pyridines 6a-e
Method A:

A solution of 0.01 mol of the appropriate **5a-e** and ammonium acetate (0.03 mol) in glacial acetic acid (30 m*l*) was refluxed for 2-3 h. The solid precipitated after cooling was collected and crystallized from acetic acid to give **6a-e,** respectively (cf. Table II and III).

Method B:

Equimolecular amounts of **7** (0.01 mol) and appropriate **2a-e** in glacial acetic acid (30 m*l*) containing ammonium acetate (4 g) were refluxed for 3 h. The solid product so formed was collected and crystallized from acetic acid to give **6a-e** respectively. The products obtained were identical in all respects with those obtained from method "A" (mp., mixed mp. and spectra).

Synthesis of the substituted pyrans 9a,b

A solution of each of **4a,b** (2 g) was heated under reflux in 20 m*l* acetic acid-hydrochloric acid (4:1) for 2 h. The reaction mixture was cooled and diluted with water. The solid so formed was collected and crystallized from diluted DMF to give **9a,b** respectively (cf. Table II).

Table III. IR and ^{1}H-NMR data

Comp.	IR [cm^{-1}]	^{1}H-NMR δ [ppm]
5a	3400, 3320, 3200 (NH_2); 2200(CN)	1.9 (s, 3H, CH_3); 4.5 (s, 1H, H-4); 6.8 (s, 2H, NH_2); 7.2-7.6 (m, 5H, ArH's); 10.1 (s, br, 1H, NH)
6a	3400, 3320, 3200 (NH_2, NH); 2220 (CN)	1.9 (s, 3H, CH_3); 6.8 (s, 2H, NH_2); 7.2-7.6 (m, 5H, ArH's); 10.0 (s, br, 1H, NH).
9a	3450 (NH); 2210 (CN); 1680 (CO).	1.9 (s, 3H, CH_3); 4.3 (s, 1H, H-4); 6.7 (s, 2H, NH_2); 7.2-8.4 (m, 11H, ArH's and NH).
10	3500 (NH); 2200 (CN); 1680 (CO).	1.9 (s, 3H, CH_3); 6.6 (s, 2H, NH_2); 7.2-7.5 (m, 11H, ArH's and NH).
13a	3500, 3450, 3320, 3200 (NH_2); 2200 (CN); 1680 (CO)	1.9 (s, 3H, CH_3); 4.3 (s, 1H, H-4); 6.8 (s, 2H, NH_2); 7.1-7.5 (m, 10H, ArH's); 8.2-8.4 (s, br, 5H, NH and two NH_2).
16a	3400, 3320, 3200 (NH and NH_2); 2200 (CN); 1690, 1670 (CO).	1.9 (s, 3H, CH_3); 4.3 (s, 1H, H-4); 6.8 (s, br, 2H, NH_2); 7.1-7.5 (m, 10H, ArH's); 8.5 (s, br, 2H, NH).
19	3400, 3320, 3200 (NH_2, NH); 2210 (CN); 1680 (CO)	1.8 (s, 3H, CH_3); 4.3 (s, 1H, H-4); 6.8 (s, br, 2H, NH_2); 7.1-8.5 (m, 16H, ArH's and NH).

Synthesis of 2-amino-3-cyano-6-methyl-4-phenyl-5-phenylcarbamoyl pyridine 10

A solution of **9a** (2 g) in glacial acetic acid (20 m*l*) containing ammonium acetate (4 g) was heated under reflux for 3 h. The reaction mixture was cooled and the solid formed was collected then crystallized from acetic acid to give the pyridine derivative **10** (cf. Table II and III).

Synthesis of 13a,b

A solid mixture of each of **9a,b** (0.01 mol) and **3a** was heated at 160 °C (bath T.) for 2 h in presence of a few drops of piperidine. The solid product, so formed, was crystallized from dimethylformamide as a brown powder and formulated as **13a,b** (cf. Table II).

Synthesis of the 4H-pyrano[2,3-b]pyridines 16a,b and 19

Equimolecular amounts (0.01 mol) of the appropriate **9a,b,** each of **3b** or **3c** in ethanol (20 m*l*) and catalytic amount of piperidine were heated under reflux for 4 h. The solid, so formed, was collected and crystallized from acetic acid to give **16a,b** and **19** respectively (cf. Tables II and III).

Methods used and test for antibacterial action

20 m*l* of nutrient agar medium were poured in each sterile petri dish (10 cm in diameter). Each plate was incubated by 2 m*l* of bacterial suspension containing 10^8 cells/m*l*. Discs containing 100 mg of different compounds were placed on the surface of the agar. The plates were incubated for 48 h. at 28 °C.

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