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Il Farmaco 55 (2000) 641–649

IL FARMACO

Synthesis and biological studies of some benzopyrano[2,3-*c*]pyrazole derivatives

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Received 8 February 2000; accepted 5 September 2000

Abstract

3-Chlorobenzopyrano[2,3-*c*]pyrazole (**2**) was prepared and reacted with sodium azide to give compound **3**, whereas its reaction with benzoyl hydrazide, ethyl glycinate, anthranilic acid or *o*-phenylenediamine afforded the products **4**–**7**, respectively. Compounds **8** and **9** were synthesized by the reaction of compound **2** with 2-mercaptobenzothiazole or piperidine. 3-Hydrazinobenzopyrano[2,3-*c*]pyrazole (**10**) was obtained and subjected to cyclization with different reagents such as CS_2, benzoic acid, acetyl acetone and diethyl malonate to give compounds **11**–**14**, respectively. Compound **10** was cyclized also with phenacyl cyanide, ylidenemalononitriles to afford the products **15**–**20**, respectively. On the other hand, compound **10** was cyclized with 3-[bis(methylthio)methylene]pentane-2,4-dione or 1,1-dicyano 2,2-dimethylthioethylene to give the corresponding compounds **22** and **23** which in turn were reacted with some compounds containing active methylene groups to afford the corresponding compounds **24**–**27**, respectively. The biological activities of some selected compounds were given.

Keywords: Benzopyrano[2,3-*c*]pyrazoles; Biological studies; Sodium azide

1. Introduction

Coumarin derivatives were found to possess useful applications in different areas of biological activity [1–5]. Some derivatives such as 3-pyridyl and 3-aminocoumarins had been reported to act as nervous system depressants [3] and antibacterial agents [4], whereas the others are reported as antibiotic novobiocine [5]. Accordingly, a new series of heterocyclic compounds containing benzopyran moiety were prepared in this work.

2. Results and discussion

2.1. Chemistry

3-oxo-2,3-Dihydrobenzopyrano[2,3-*c*]pyrazole[6,7] (**1**) was prepared by the reaction of 3-carbethoxybenzopyran-2-thione with hydrazine hydrate in good yield [6] (Scheme 1).

The first target compound, 3-chlorobenzopyrano[2,3-*c*]pyrazole (**2**) was prepared by the reaction of compound **1** with a mixture of phosphorus pentachloride and phosphorus oxychloride in good yield. The IR spectrum of compound **2** showed the absence of absorption bands corresponding to NH and C=O groups and the presence of absorption bands corresponding to C–Cl at 769 cm^{-1} and C=N at 1620 cm^{-1} (Table 1). Compound **2** was used as a key intermediate in this work. Its reaction with sodium azide [8] gave the tetrazole derivative **3** in good yield (Table 1). When compound **2** was allowed to react with benzoic acid hydrazide, it gave benzopyranodiazolotriazole derivative **4**. Also, compound **2** was reacted with another nucleophiles such as ethyl glycinate, anthranilic acid, *o*-phenylenediamine [9] in DMF to give imidazole derivatives **5**–**7**, respectively. The IR and ^1H NMR spectra of the products are in agreement with the proposed structures (Table 1). Finally, compound **2** was reacted with 2-mercaptobenzothiazole, piperidine and hydrazine hydrate [10] in DMF to produce the corresponding 3-(mercapto, amino and hydrazino) benzopyrano[2,3-*c*]pyrazoles (**8**–**10**), respectively. The structures of these products were established by elemental analysis, IR and ^1H NMR spectra (Table 1, Scheme 1).

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PII: S0014-827X(00)00090-2

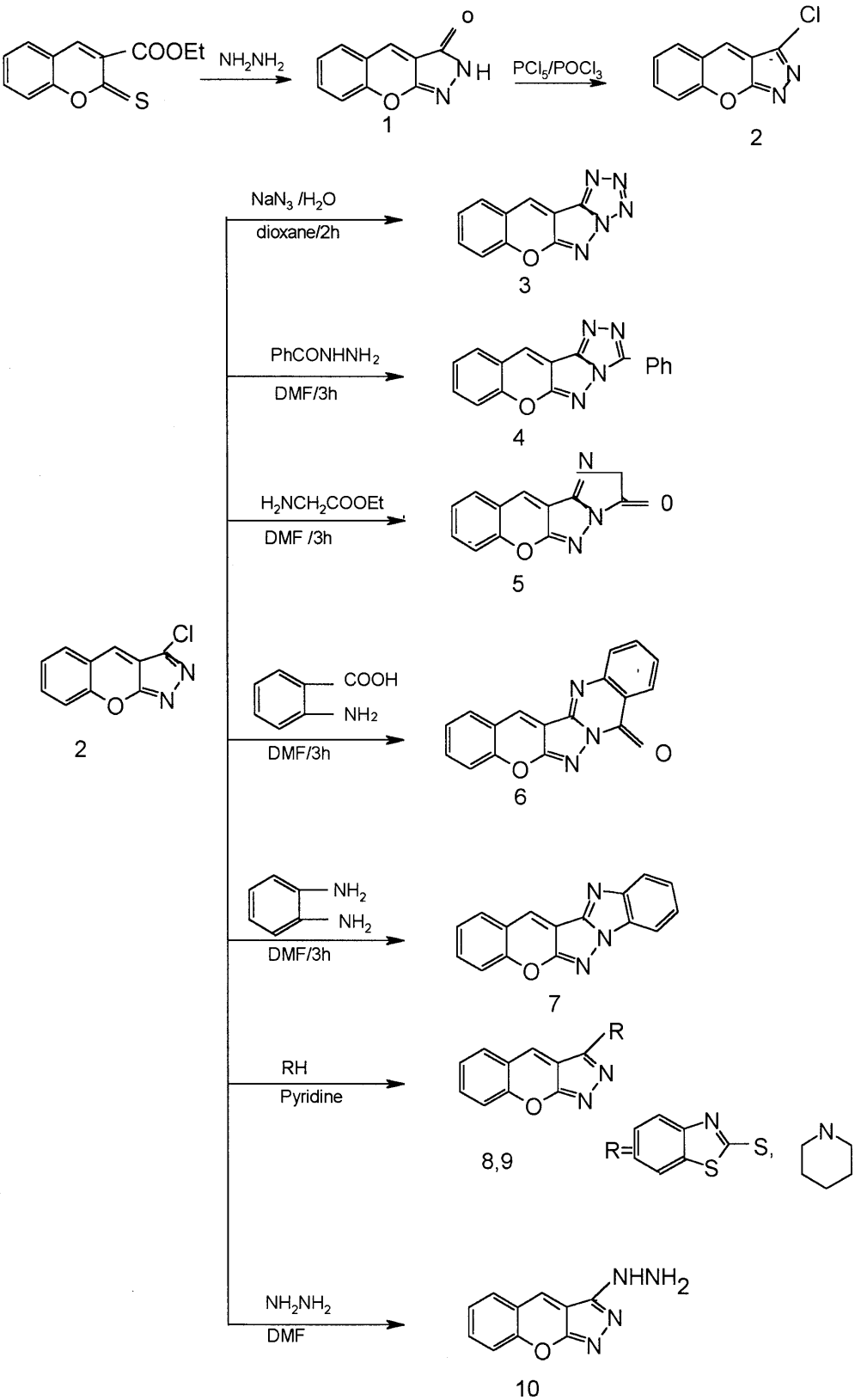
Table 1
Analytical and spectral data of the prepared compounds

Compound	M.p. (°C) (crystal solvent)	Yield (%)	M_F/M_w	Anal. data: (Calc. found) (%)			IR (KBr) ν (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
				C	H	N		
1	217–220 EtOH	80	$C_{10}H_6N_2O_2$ (186.170)	64.51 64.30	3.24 3.04	15.04 15.25	3200 (NH), 3127 (CH), 2928 (CH), 1670 (C=O), 1618 (C=N)	15.0 (s, 1H, NH), 8.6 (s, 1H, CH), 8.0-7.0 (m, 4H, arom.+vinylic)
2	175 Dioxane	78	$C_{10}H_5N_2OCl$ (204.615)	58.70 58.37	2.46 2.59	13.69 13.89	3140 (CH), 1620 (C=N), 769 (C=N)	8.5–7.0 (m, 5H, arom.+vinylic)
3	320 Dioxane	60	$C_{10}H_5N_5O$ (211.180)	56.87 56.39	2.38 2.69	33.16 32.78	3030 (CH), 2955 (CH), 1626 (C=N), 1575 (N=N)	8.6–7.0 (m, 5H, arom.+vinylic)
4	250 EtOH	73	$C_{17}H_{10}N_4O$ (286.291)	71.32 70. 96	3.52 3.71	19.57 19.88	3150 (CH), 2910 (CH), 1614 (C=N)	8.5–7.0 (m, 10H, arom.+vinylic)
5	280 EtOH	62	$C_{12}H_7N_3O_2$ (225.206)	63.99 63.60	3.13 3.23	18.65 18.82	3063 (CH), 2930 (CH), 1713 (C=O) 1618 (C=N)	8.4–7.0 (m, 5H, arom.+vinylic), 4.0 (s, 2H, CH_2)
6	210 Dioxane	76	$C_{17}H_9N_3O_2$ (287.277)	71.07 71.38	3.15 3.36	14.62 14.50	3030 (CH), 1660 (C=O), 1610 (C=N)	8.5–7.1 (m, 9H, arom.+vinylic)
7	170 Dioxane	81	$C_{16}H_9N_3O$ (259.266)	74.12 73.59	3.50 3.26	16.20 16.40	3060 (CH), 1600 (C=N)	8.5–7.0 (m, 9H, arom.+vinylic)
8	182 Dioxane	70	$C_{17}H_9N_3OS_2$ (335.405)	60.87 60.38	2.70 2.83	12.52 12.73	3050 (CH), 2957 (CH), 1610 (C=N)	8.8–6.9 (m, 9H, arom.+vinylic)
9	190 EtOH	82	$C_{15}H_{15}N_3O$ (253.303)	71.12 71.42	5.96 6.30	16.59 16.78	3100 (CH), 2850 (CH), 1600 (C=N)	8.6–7.0 (m, 5H, arom.+vinylic), 2.6–1.6 (m, 10H, cyclic CH_2)
10	320 EtOH	79	$C_{10}H_8N_4O$ (200.198)	59.99 59.50	4.02 3.82	27.98 27.85	3040 (CH), 3269, 3169 (NH_2), 3150 (NH), 2950 (CH), 1600 (C=N)	9.1 (s, 1H, NH), 8.5–7.0 (m, 5H, arom.+vinylic), 4.0–5.0 (br, 2H, NH_2)
11	170 Dioxane	79	$C_{11}H_6N_4OS$ (242.257)	54.53 54.04	2.49 2.26	23.12 23.31	3042 (CH), 3200 (NH), 2935 (CH), 1620 (C=N), 1200 (C=S)	9.0 (s, 1H, NH), 8.5–7.0 (m, 5H, arom.+vinylic)
12	200 Benzene	59	$C_{17}H_{10}N_4O$ (286.291)	71.32 71.52	3.52 3.75	19.57 19.74	3022 (CH), 2850 (CH), 1600 (C=N)	8.5–7.1 (m, 10H, arom.+vinylic)
13	130 EtOH	69	$C_{15}H_{12}N_4O$ (264.285)	68.17 68.36	4.57 4.76	21.19 21.41	3020 (CH), 2941 (CH), 1610 (C=N)	8.4–6.8 (m, 5H, arom.+vinylic), 3.5 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 1.3 (s, 3H, CH_3)
14	180 EtOH	59	$C_{13}H_8N_4O_3$ (268.231)	58.21 58.00	3.00 3.19	20.88 20.62	3020 (CH), 2937 (CH), 1703, 1620 (2C=O), 1590 (C=N)	9 (s, 1H, NH), 8.5–6.8 (m, 5H, arom.+vinylic), 3.5 (s, 2H, CH_2)
15	310 Chloroform	53	$C_{19}H_{13}N_5O$ (327.343)	69.71 70.11	4.00 3.57	21.39 21.18	3408 (NH_2), 3015 (CH), 2777 (CH), 1614 (C=N)	8.6–7.0 (m, 10H, arom.+vinylic), 3.9–3.0 (br, 2H, NH_2), 5.8 (s, 1H, CH)

Table 1 (*Continued*)

Compound	M.p. (°C) (crystal solvent)	Yield (%)	M_F/M_w	Anal. data: (Calc. found) (%)			IR (KBr) ν (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
				C	H	N		
16	212 Benzene	70	$C_{16}H_{13}N_5O_3$ (323.310)	59.44 59.77	4.05 4.36	21.66 21.35	3440–3340 (NH_2), 3020 (CH), 2900 (CH), 1700 (C=O)	9.0(s, 1H, CH), 8.5–7.0 (m, 5H, arom.+vinylic), 3.0–2.7 (br, 2H, NH_2), 2.0–1.3 (q, 2H, CH_2), 1.0–0.6 (t, 3H, CH_3)
17	310 Dioxane	68	$C_{20}H_{11}N_6ClO$ (386.800)	62.10 62.52	2.86 2.35	21.72 21.50	3422, 3322 (NH_2), 3011 (CH), 2777 (CH), 2210 (C=N), 1600 (C=N)	8.8–7.0 (m, 9H, arom.+vinylic), 4.6–3.3 (br, 2H, NH_2)
18	310 Dioxane	62	$C_{20}H_{10}N_5ClO_2$ (387.783)	61.94 62.46	2.59 2.76	18.06 18.17	3300 (OH), 3049 (CH), 2932 (CH), 2218 (CN), 1608 (C=N)	9.2 (s, 1H, OH), 8.3–7.0, (m, 9H, arom.+vinylic)
19	130 EtOH	71	$C_{20}H_{11}N_6ClO$ (386.800)	62.10 62.58	2.86 2.98	21.72 21.90	3426–3300 (NH_2), 3050 (CH), 2922 (CH), 2214 (C=N)	8.5–7.0 (m, 9H, arom.+vinylic), 3.2–3.5 (br, 2H, NH_2)
20	209 Dioxane	58	$C_{19}H_{20}N_6O_2$ (364.405)	62.62 62.31	5.53 5.73	23.06 23.18	3424–3300 (NH_2), 3190 (NH), 3060 (CH), 2920 (CH) 1620 (C=O)	9.0 (s, 1H, NH), 8.4–6.8 (m, 5H, arom.+vinylic), 4.4–4.1 (br, 2H, NH_2), 4.0–3.1 (m, 4H, $2CH_2$), 3.0–2.7 (m, 6H, $3CH_2$)
21	200 DMF	72	$C_{16}H_{17}N_5O$ (295.342)	65.06 65.37	5.70 5.59	23.71 23.83	3250 (NH), 3060 (CH), 2941 (CH)	8.9 (s, 2H, 2NH), 8.5–6.9 (m, 5H, arom.+vinylic), 4.3–3.2 (m, 4H, $2CH_2$), 3.0–2.5 (m, 6H, $3CH_2$)
22	168 EtOH	79	$C_{16}H_{12}N_4O_3$ (308.296)	62.33 62.03	3.92 3.70	18.17 18.01	3300 (NH), 3060 (CH), 2938 (CH), 1720 (2C=O), 1620 (C=N)	9.0 (S, 1H, NH),8.5–7.0 (m, 5H, arom.+vinylic), 2.2 (S, 6H, $2CH_3$)
23	174 Chloroform	82	$C_{14}H_6N_6O$ (274.238)	61.31 61.68	2.20 2.38	30.64 30.49	3020 (CH), 2934 (CH), 2197 (C≡N) 1608 (C=N)	8.5–7.0 (m, 5H, arom.+vinylic), 9.2 (s, 1H, NH)
24	250 Dioxane	77	$C_{20}H_{18}N_4O_2$ (346.388)	69.34 69.41	5.23 5.80	16.17 16.33	3400 (NH), 3080 (CH), 2943 (CH_2)	8.2–7.0 (m, 5H, arom.+vinylic), 6.8 (s, 1H, =CH), 4.6 (br, 1H, NH), 2.5 (s, 3H, CH_3), 2.1–1.0 (m, 8H, cyclic CH_2)
25	260 EtOH	52	$C_{24}H_{18}N_6O_2$ (422.444)	68.23 67.71	4.29 4.10	19.89 19.61	3280, 3200 (2NH), 1600 (C=N)	9.3 (br, 1H, NH), 8.5–6.9 (m, 10H, arom.+vinylic), 6.1 (br, 1H, =CH), 2.9 (s, 3H, CH_3 byrazole, 1.7 (s, 3H, CH_3)
26	255 Benzene	81	$C_{17}H_{11}N_9O_2$ (373.329)	54.69 54.20	2.97 2.89	33.76 33.56	3350 (NH), 3250, 3150 (NH_2), 3063 (CH), 2932 (CH), 2201 (CN), 1650 (C=O), 1600 (C=N)	8.3–7.0 (m, 6H, arom.+vinylic+NH), 5.0–3.3 (br, 4H, $2NH_2$), 1.3 (s, 1H, OH)
27	230 EtOH	69	$C_{20}H_{16}N_6O_4$ (404.384)	59.40 59.24	3.98 3.78	20.78 20.50	3410 (NH), 3200, 3100 (NH_2), 3050 (CH), 2980 (CH), 2203 (CN), 1710 (C=O)	8.5–6.9 (m, 5H, arom.+vinylic), 4.8–3.7 (br, 2H, NH_2), 3.3–2.6 (q, 2H, CH_2), 1.7 (s, 3H, CH_3), 1.0–0.6 (t, 3H, CH_3)

Because of the broad utility of heterocyclic hydrazines as intermediates for the synthesis of several condensed systems containing triazole and tetrazole nuclei [11,12], the second target compound, 3-hydrazinoben-



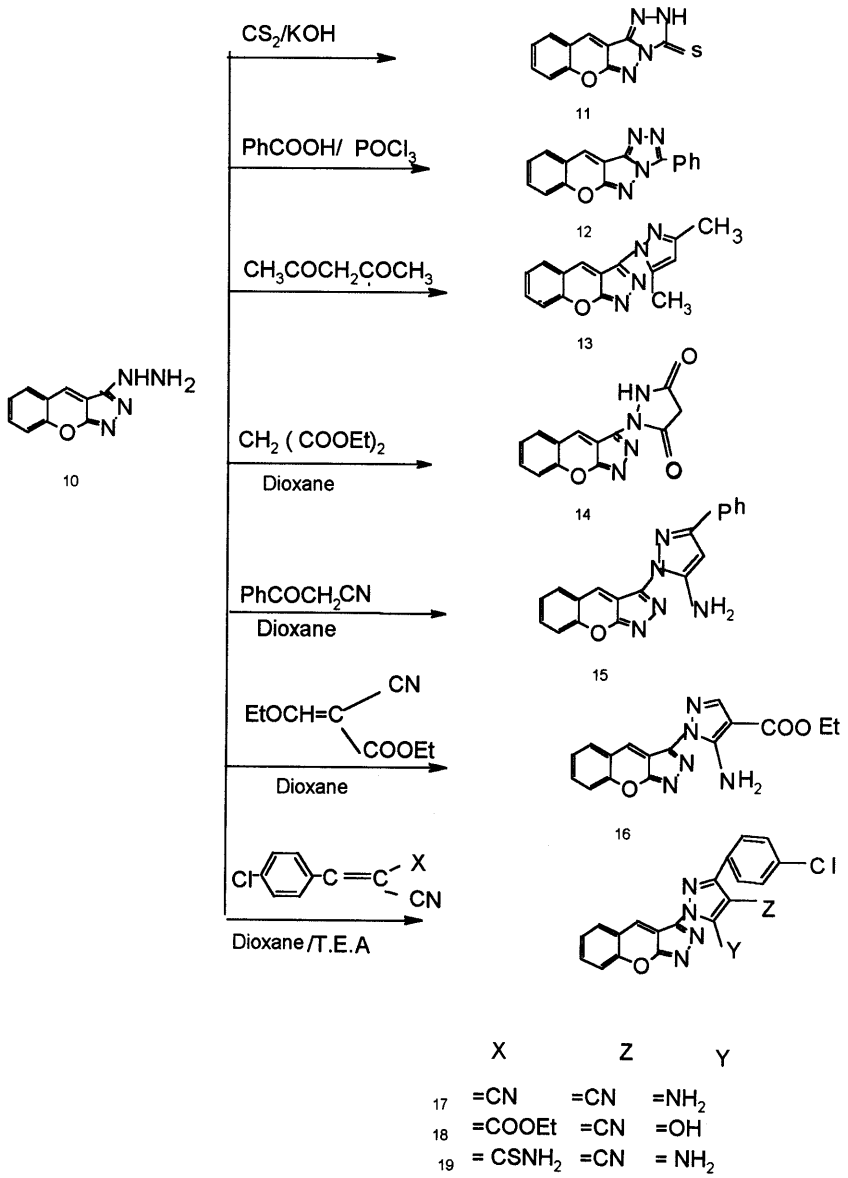
Scheme 1.

zopyrano[2,3-*c*]pyrazole (**10**) was used in preparing a new series of condensed heterotetracyclic compounds. The reaction of compound **10** with carbon disulfide [13] in the presence of alcoholic potassium hydroxide, as well as benzoic acid in the presence of phosphorus oxychloride, gave triazole derivatives **11** and **12**, respectively (Table 1, Scheme 2).

When compound **10** was allowed to react with compounds containing active methylene groups such as acetylacetone, diethyl malonate and phenacyl cyanide, it gave pyrazole and pyrazoledione derivatives **13**–**15**, respectively. The IR spectra of these compounds showed the absence of the NH and NH_2 absorption bands and the presence of new characteristic groups. The elemental and spectral analyses of these compounds were in agreement with their structures (Table 1, Scheme 2).

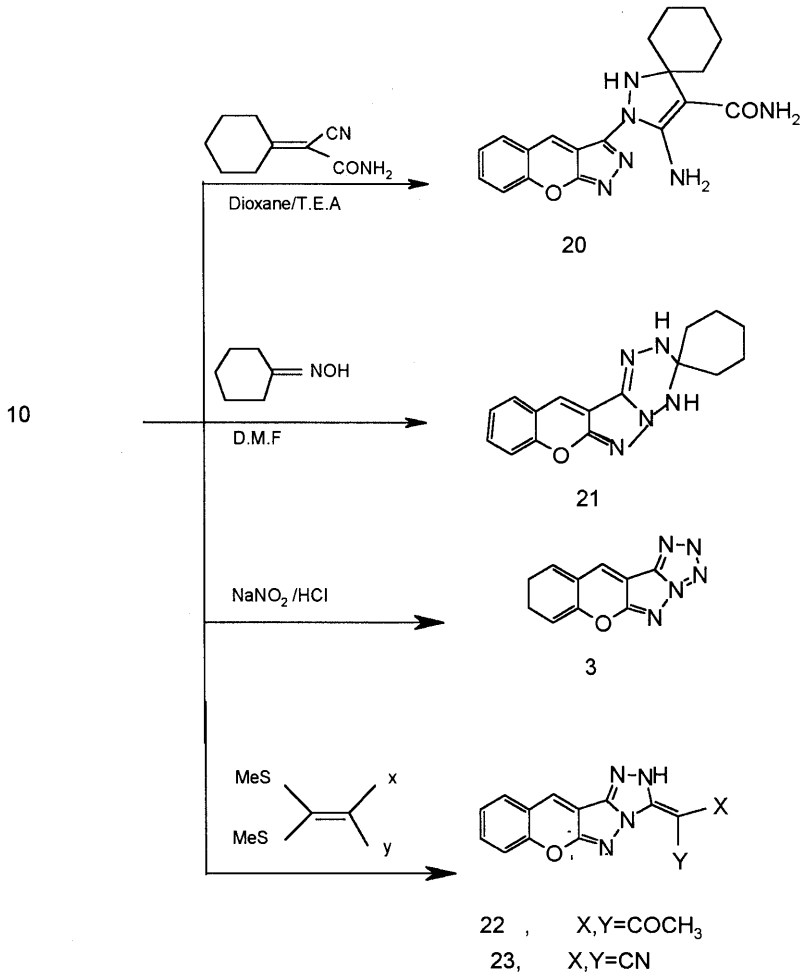
Compound **10** was reacted with ethylethoxymethylenecyanoacetate, *p*-chloro-phenylmethylenemalononitrile, *p*-chlorophenylmethylenecyanoacetate and *p*-chlorophenylmethylenecyanothioacetamide [14], to give the corresponding pyrazole derivatives **16**–**19**, respectively.

The mechanism of this reaction was suggested to proceed by nucleophilic addition of the NH_2 group to the activated double bond to give the intermediate Michael adduct which was cyclized by the addition reaction to C=N, C=O or C=S with the elimination of ethanol or H_2S molecules (**16**–**19**), respectively (Table 1, Scheme 2).



	X	Z	Y
17	=CN	=CN	$=NH_2$
18	=COOEt	=CN	=OH
19	$= CSNH_2$	=CN	$= NH_2$

Scheme 2.



Scheme 2. (*Continued*)

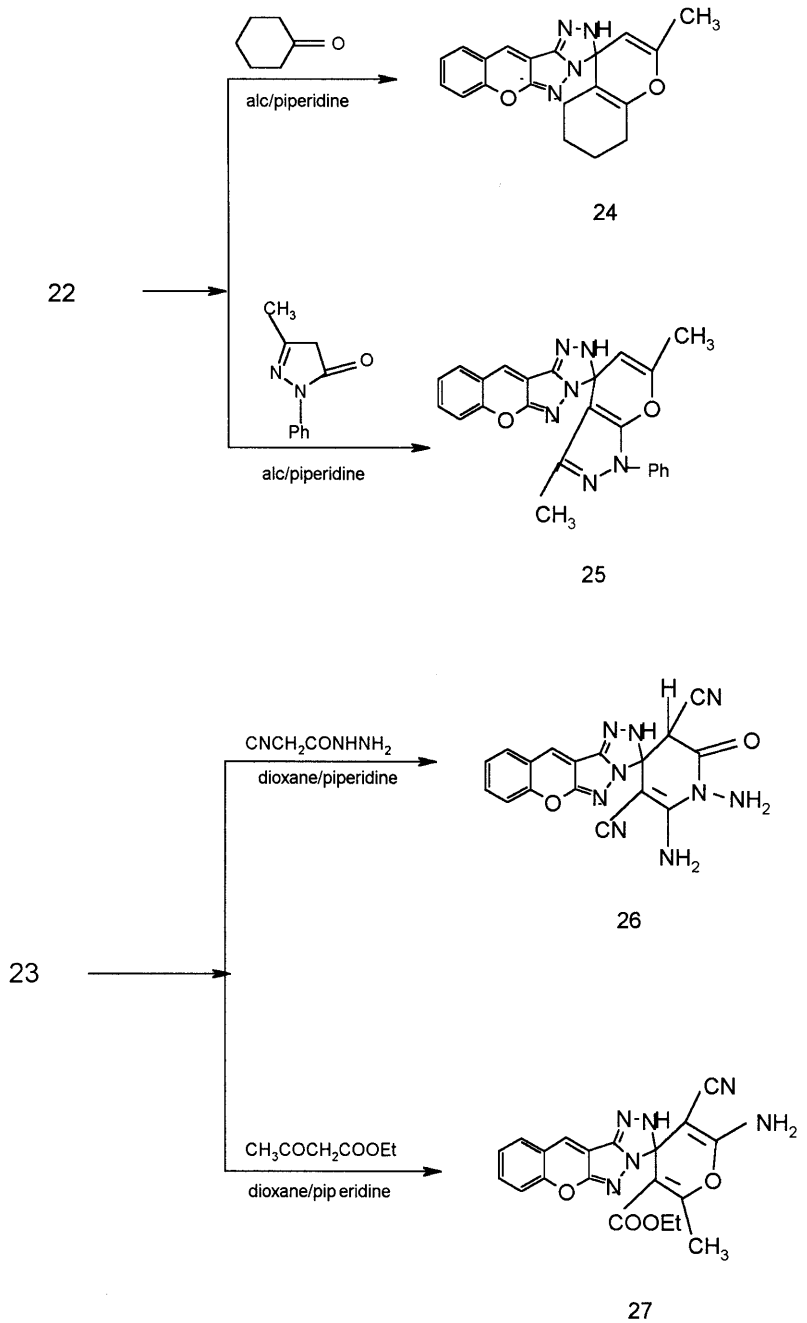
Treatment of compound **10** with cyclohexylidene-cyanoacetamide or with cyclohexylidene oxime gave the corresponding spiro compounds [15] **20** and **21**, respectively. The reaction pathway was assumed to follow nucleophilic addition of the NH_2 group at C=N or C=C and cyclization to give the desired spiro heterocycles. Compound **3** was also prepared by another method in which the hydrazino compound **10** was reacted with $NaNO_2$–HCl in an ice bath (Scheme 2).

Finally, compound **10** was reacted with 3-[bis-(methylthiomethylene)]pentan-2,4-dione [16] and 1,1-dicyano-2,2-dimethylthioethylene [16] to give compounds **22** and **23**, respectively. This reaction was assumed to go through nucleophilic attack on both NH_2 and NH groups to the ethylenic bond with the elimination of two molecules of methyl mercaptan. The structures were confirmed by elemental analysis (IR and ^1H NMR) spectral data (Table 1, Scheme 2).

Compound **22** was reacted with compounds containing active methylene groups such as cyclohexanone and 3-methyl-1-phenylpyrazol-5-one in refluxing ethanol containing piperidine as a catalyst to give the desired spiroheterocycles **24** and **25**, respectively. The reaction pathway was suggested to follow preliminary elimination of one acetyl group followed by nucleophilic addition of the formed carbanion to the ethylenic bond and cyclization (Table 1, Scheme 3). Finally, using the former mechanism, compound **23** was reacted with cyanoacetohydrazide and ethyl acetoacetate in refluxing dioxane containing piperidine as a catalyst, to afford compounds **26** and **27**, respectively (Table 1, Scheme 3).

2.2. Biological screening

When some of the new coumarin derivatives were tested for their toxicity on brine shrimp larvae and activity on four species of bacteria, the following results were obtained. Derivative **14** had good toxicity (100% dead larvae) and good activity against bacteria. Compound **10** proved to be of low toxicity against the larvae, with a high and/or moderate activity against



Scheme 3.

Gram + ve and Gram – ve bacteria. It is worth mentioning that the remaining derivatives (**11**, **12**, **13**, **15**, **16** and **17**) were non-toxic to experimental animals, with a variable degree of activity against bacteria. Also the activity against Gram + ve bacteria (*Bacillus cereus* and *staphylococcus albus*) were more than Gram – ve bacteria (*Pseudomonas aeruginosa* and *Escherichia Coli*) (Table 2).

3. Experimental

3.1. Chemistry

*3.1.1. Preparation of 3-chlorobenzopyrano[2,3-c]pyrazole (**2**)*

Phosphorus pentachloride (0.01 mol) was added to a solution of benzopyrano[2,3-*c*] pyrazole-3-one (0.01

mol) in phosphorus oxychloride (50 ml) and refluxed for 5 h. The reaction mixture was poured into ice-cooled water (100 ml). The precipitate was filtered off and crystallized from the appropriate solvent (Table 1).

*3.1.2. Synthesis of benzopyrano[2′,3′:3,4]pyrazolo[1,5-d]-[1,2,3,4]tetrazole **(3)** (Method A)*

Sodium azide solution (0.01 mol) in H_2O (3 ml) was added to a solution of compound **2** (0.01 mol) in dioxane (30 ml) and refluxed for 2 h. The solvent was concentrated and cooled; the precipitated solid was filtered off and washed with (3 ml) water. The solid product was crystallized from dioxane to afford compound **3** (Table 1).

*3.1.3. Synthesis of compound **(3)** (Method B)*

Sodium nitrite solution (0.01 mol) in H_2O (5ml) was added to solution of compound **10** (0.01 mol) in dilute HCl (10 ml) while stirring for 1 h in an ice bath. The formed precipitate was filtered off and crystallized from the appropriate solvent (Table 1).

3.1.4. General procedure for the reaction of 3-chlorobenzopyrano[2,3-c]pyrazole with different nucleophilic reagents

Benzoyl hydrazide, ethyl glycinate, anthranilic acid or *o*-phenylene diamine (0.01 mol) was added to a solution of compound **2** (0.01 mol) in DMF (20 ml). The reaction mixture was refluxed for 3 h, added to cold water containing a few drops of HCl. The precipitate was filtered off and crystallized from the suitable solvent to afford compounds **4**–**7** (Table 1).

*3.1.5. General procedure for the preparation of compounds **8** and **9***

2-Mercaptobenzothiazol or piperidine (0.01 mol) was added to a solution of compound **2** (0.01 mol) in pyridine (30 ml). The reaction mixture was refluxed for 2 h. After cooling the reaction mixture was poured into cold water containing drops of HCl. The precipitate was filtered off and crystallized from a suitable solvent (Table 1).

*3.1.6. Synthesis of 3-hydrazinobenzopyrano [2,3-c]pyrazole **(10)***

The solution of compound **2** (0.01 mol) in hydrazine hydrate (20 ml) was added and refluxed for 4 h. The reaction mixture was concentrated. The precipitate was filtered off and crystallized from ethanol (Table 1).

*3.1.7. Preparation of compound **11***

Alcoholic potassium hydroxide solution (0.01 mol) in ethanol (7 ml)–water (3 ml) was added to a mixture of 3-hydrazinobenzopyrano[2,3-*c*]pyrazole (0.01 mol), ethanol (50 ml) and carbon disulphide (0.01 mol) with stirring. The reaction mixture was refluxed until the hydrogen sulphide ceased ($\sim$ 20 h). The reaction mixture was concentrated, cooled to room temperature (r.t.), poured into an ice-water mixture (100 ml) and acidified with concentrated hydrochloric acid. The precipitate was filtered off and crystallized from dioxane to give compound **11** (Table 1).

3.1.8. Reaction of 3-hydrazinobenzopyrano-[2,3-c]pyrazole with benzoic acid

Benzoic acid (0.01 mol) was added to a solution of compound **10** (0.01 mol) in phosphorus oxychloride (20 ml). The reaction mixture was refluxed for 2 h, after cooling, the mixture was added to cold water (100 ml). The precipitated solid was filtered off and crystallized from benzene to give compound **12** (Table 1).

*3.1.9. General procedure for the preparation of compounds **13**–**20***

Acetylacetone, diethyl malonate, phenacyl cyanide or ethylethoxymethylenecyanoacetate, *p*-chlorophenylmethylenemalononitrile, ethyl-*p*-chlorophenylmethylenecyanoacetate, *p*-chlorophenylmethylenecyanothioacetamide and/or cyclohexylidenecyanoacetamide (0.01

Table 2
Biological activity of some coumarin derivatives against brine shrimp larvae Gram+ve and Gram−ve bacteria

Compound	Artemia salina [a]	*Bacillus cereus* (mm)	*Staphylocoecus albus* (mm)	*Pseudomonas* (mm)	*Escherichia coli* (mm)
2	D	2	2	-	-
3	D	2	4	3	3
11	D	4	3	2	2
14	D	1	2	1	2
19	A	4	4	3	2
21	D	2	3	2	2
24	C	2	4	3	3
25	D	2	2	2	2

[a] *Artemia salina* (brine shrimp) test. A, high toxicity, more than 100% dead larvae; B, moderate toxicity, 50–75% dead larvae; C, low toxicity, 25–50% dead larvae; D, non toxic, less than 25% dead larvae; *B. cereus* and *S. albus*, gram+ve bacteria; *P. aeruginosa* and *E. coli*, gram−ve bacteria.

mol) was added to a solution of compound **10** (0.01 mol) in dioxane (20 ml) and few drops of TEA. The reaction mixture was refluxed for 4 h, concentrated, cooled to r.t., and the precipitate was filtered off and crystallized from the appropriate solvent to give compounds **13**–**20** (Table 1).

*3.1.10. Reaction of compound **10** with cyclohexylidene oxime*

Cyclohexylidene oxime (0.01 mol) was added to a solution of compound **10** (0.01 mol) in DMF (20 ml). The reaction mixture was refluxed for 4 h, after cooling, the mixture was added to cold water containing few drops of HCl. The formed precipitate was filtered off and crystallized from DMF to give compound **21** (Table 1).

*3.1.11. General procedure for the preparation of compounds **22** and **23***

3-[Bis(methylthio)methylene]pentane-2,4-dione or 1,1-dicyano-2,2-dimethylthioethylene [16] (0.01 mol) was added to solution of compound **10** (0.01 mol) in 1-butanol (30 ml). The reaction mixture was refluxed for 48 h, and concentrated. The formed precipitate was filtered off and crystallized from the appropriate solvent (Table 1).

*3.1.12. General procedure for the reaction of compound **22** with active methylene compounds*

Cyclohexanone or 3-methyl-1-phenylpyrazol-5-one (0.01 mol) was added to a mixture of compound **22** (0.01 mol) in absolute ethanol (30 ml), and a few drops of piperidine. The reaction mixture was refluxed for 3 h. The procedure was performed as before to give compounds **24** and **25**, respectively (Table 1).

*3.1.13. General procedure for the preparation of compounds **26** and **27***

Cyanoacetohydrazide or ethylacetoacetate (0.01 mol) was added to a solution of compound **23** (0.01 mol) in dioxane (30 ml). Few drops of piperidine were added to the reaction mixture. The reaction mixture was refluxed for 3 h. The procedure was performed as before (Table 1).

3.2. Biological assay

For the bioassay of coumarin derivatives the following organisms were used:

1. Brine shrimp test. The larvae of brine shrimp (*Artemia Salina L.*) were used. The eggs were hatched on autoclaved seawater for 3 days at 22–24°C. A filter paper disc (1 cm) was saturated by acetone solution of 10 μg of the compound based on the method of Korpinen [17].
2. Bacterial test. Two Gram + ve (*B. cereus* and *S. albus*) and two Gram − ve (*P. aeruginosa* and *E. coli*) bacteria were used. The bacterial species were grown onto nutrient agar medium. A filter paper disc (1 cm) was saturated by acetone solution of (10 μg) of the compound. After the incubation period, the diameter of the inhibition zone was measured according Speer and Sussmuth [18].

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