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Synthesis of some new biologically-active coumarin derivatives

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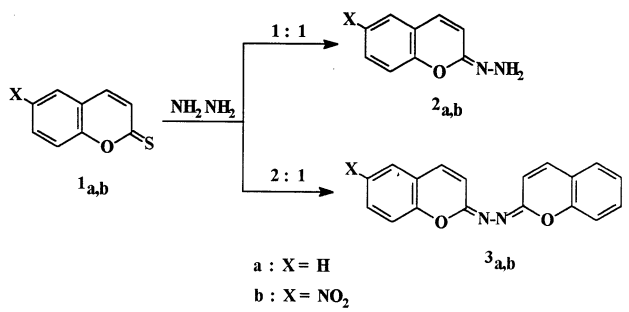
Abstract

Coumarin and 6-nitrocoumarin hydrazones (**2a,b**), respectively, were prepared via the reaction of 2-thiocoumarin (**1a,b**) derivatives with hydrazine hydrate. The hydrazones were used as key intermediates for the preparation of some benzopyrano-[2,3-c]pyrazoles (**21–24**) through the reaction of different acyl halides and subsequent cyclization in *N*,*N*-dimethylaniline. Benzopyrano[2,3-c]pyrazole-3-thione (**25a,b**) was prepared by the reaction of **1a** with CS_2 on which some alkylation, acylation and Mannich reactions were studied. Alternative procedures, other reactions and biological activity of some new compounds were given.

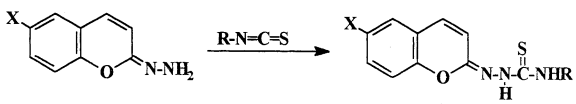
Keywords: Thiocoumarins; Coumarin hydrazones; Benzopyranopyrazoles; Biological activity

Several coumarin derivatives revealed pronounced medicinal value as antibacterial and antifungal agents [1–5]. Others displayed antitubercular activity [6] and some have insecticidal properties [7]. This prompted us to investigate the introduction of a new series of compounds containing coumarin moiety with different side chains or fused rings.

The reaction of thiocoumarins **1a,b** with hydrazine hydrate in equimolecular amounts gave the corresponding hydrazones **2a,b**, while the reaction of 2 mol of compounds **1a,b** with 1 mol of hydrazine hydrate afforded the coumarinazine derivatives **3a,b** in good yields.



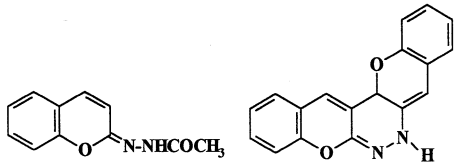
Treatment of compounds **2a,b** with phenyl (methyl) isothiocyanate afforded ω-phenyl(methyl)thiosemicarbazone derivatives **4a–d** (cf. Table 1).



2a,b 4a-d

4_a : X = H, R = $-C_6H_5$
4_b : X = NO_2, R = $-C_6H_5$
4_c : X = H, R = CH_3
4_d : X = NO_2, R = CH_3

Acetylation of compound **2a** with acetyl chloride in presence of triethylamine as a catalyst afforded ω-acetylcoumarin hydrazone **5a** in 70% yield and a byproduct in 30% yield which was identified as bis-[1]benzopyrano[2,3-c:2′,3′-e′]pyridazine **6a** in which two molecules of **2a** were condensed under the reaction conditions. The structure was confirmed by elemental, IR, NMR and MS analyses.



5a 6a

Chloroacetyl analogues **7a,b** were obtained by the reaction of **2a,b** with chloroacetyl chloride using triethyl-

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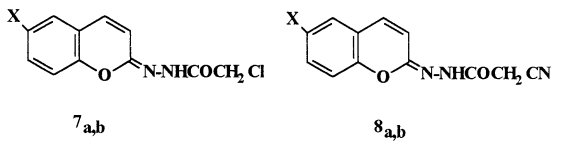
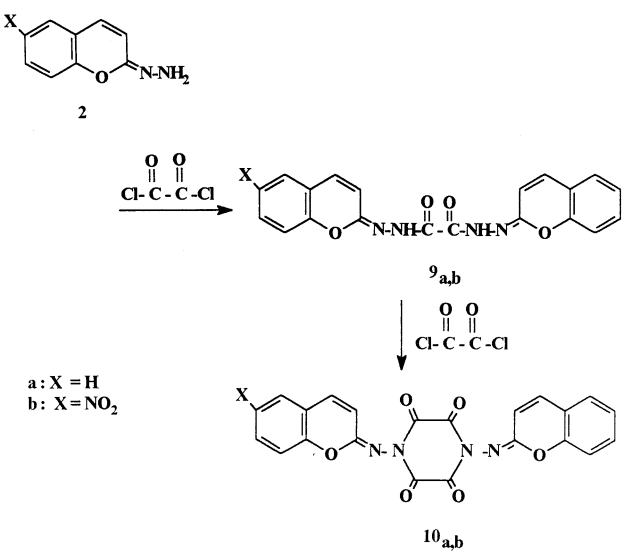
amine as a catalyst, while cyanoacetyl derivatives **8a,b** were prepared by the reaction of **2a,b** with ethyl cyanoacetate.



$^{7}_{a,b}$ $^{8}_{a,b}$

$_{a}$: X = H
$_{b}$: X = NO_2

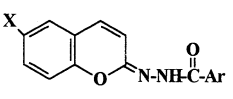
Also, compounds **2a,b** were allowed to react with oxalyl chloride in 2:1 molar ratio to give oxalyl bis-coumarin hydrazones **9a,b**, respectively. The MS of compound **8a** showed M^+ 374. Treatment of compounds **9a,b** again with oxalyl chloride (1:1) afforded piperazine-2,3,5,6-tetraone derivatives **10a,b**, respectively. The structure of products was confirmed using elemental and spectral analyses (cf. Table 1).



a: X = H
b: X = NO_2

$^{10}_{a,b}$

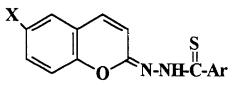
ω-Aroylhydrazones of coumarins could be prepared also by the reaction of thiocoumarins with acid hydrazides in presence of triethylamine as a catalyst. Representative examples **11a,b–15a,b** were prepared.



$^{11}_{a,b}$ - $^{15}_{a,b}$

11_a : X = H	Ar = C_6H_5	11_b : X NO_2	Ar = C_6H_5
12_a : X = H	Ar = isonicotyl-	12_b : X NO_2	Ar = isonicotyl-
13_a : X = H	Ar = nicotyl-	13_b : X NO_2	Ar = nicotyl-
14_a : X = H	Ar = 4-NH_2.C_6H_4	14_b : X NO_2	Ar = 4-NH_2.C_6H_4
15_a : X = H	Ar = 4-NO_2.C_6H_4	15_b : X NO_2	Ar = 4-NO_2.C_6H_4

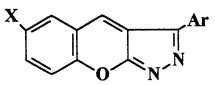
Many ω-aroylhydrazones were subjected to the reaction with phosphorus pentasulfide in dry pyridine to give thio analogues **16–20**.



16 - 20

16 : X = H	Ar = C_6H_5	17 : X = H	Ar = nicotyl-
18 : X = H	Ar = 4-NO_2.C_6H_4	19 : X = H	Ar = 4-$NH_2C_6H_4$
20 : X = NO_2	Ar = isonicotyl-		

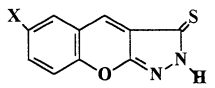
Benzopyranopyrazole compounds **21–24** were obtained by cyclization of the open chain aroyl or thioaroyl hydrazonocoumarins. The cyclization was achieved by refluxing in *N*,*N*-dimethylaniline [8].



21 - 24

21 : X = H	Ar = C_6H_5	22 : X = H	Ar = nicotyl-
23 : X = H	Ar = 4-$NO_2C_6H_4$	24 : X = NO_2	Ar = isonicotyl-

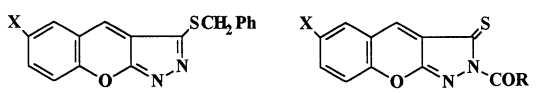
Benzopyranopyrazole thiones **25a,b** were prepared by the direct reaction of **2a,b** with carbon disulfide in alcoholic potassium hydroxide. M^+ of **25a** is 202 (77%).



$^{25}_{a,b}$

$_{a}$: X = H
$_{b}$: X = NO_2

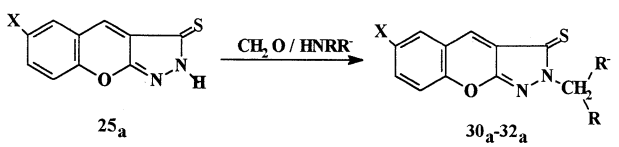
Reaction of compound **25a** with benzyl chloride afforded the corresponding sulfide derivative **26a**, while the reaction with aliphatic or aromatic acid chlorides yielded *N*-acylated products **27a–29a**. The elemental and spectral analyses of these compounds were in agreement with their structures (cf. Table 1).



$^{26}_{a}$ $^{27}_{a}$-$^{29}_{a}$

27_a : R = CH_3 28_a : R = C_6H_5 29_a : R = 4-NO_2 .C_6H_4

Application of Mannich reaction on compound **25a** gave the corresponding Mannich bases. Thus, when compound **25a** was reacted with formaldehyde and different primary or secondary amines in equimolecular ratio, the corresponding Mannich bases were obtained in fair yields.



$^{25}_{a}$ $^{30}_{a}$-$^{32}_{a}$

30a: NRR′ = 3-chloroaniline; **31a**: NRR′ = 4-acetylaniline; **32a**: NRR′ = morpholine.

Also the use of piperazine in 2:1 molar ratio leads to the bis-compound **33a**.

Table 1
Analytical and spectral data of the prepared compounds

Comp. no.	Reaction time (h)/temp. (°C)	M.p. (cryst. solvent)	Yield (%)	M_F (M_W)	Analytical data Calc. (Found) (%)			IR (Kbr) ν (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
					C	H	N		
2a	12/reflux	86 (benzene/pet. ether 40–60)	89	$C_9H_8N_2O$ (160.18)	67.49 (67.01)	5.03 (4.50)	17.49 (17.90)	3300, 3200 (NH_2), 1630 (C=C), 1600 (C=N)	7.50–6.20 (m, 6H, arom.+vinylic); 5.0 (s, 2H, NH_2)
2b	13/25	190 (dioxane)	90	$C_9H_7N_3O_3$ (205.17)	52.69 (52.20)	3.44 (3.84)	20.48 (20.88)	3450, 3350 (NH_2), 1640 (C=C), 1600 (C=N), 1510, 1340 (NO_2)	8.65–8.70 (m, 5H, arom.+vinylic); 6.30 (s, 2H, NH_2)
3a	12/25	80 (benzene)	73	$C_{18}H_{12}N_2O$ (288.31)	74.99 (75.40)	4.16 (4.57)	9.72 (9.79)	1610 (C=N)	7.30–6.40 (m, 12H, arom.+vinylic)
3b	12/20	233 (ethanol)	70	$C_{18}H_{10}N_4O_6$ (378.30)	57.15 (57.60)	2.66 (2.44)	14.81 (15.32)	1620 (C=N), 1500, 1340 (NO_2)	8.90–6.50 (m, 10H, arom.+vinylic)
4a	8/25	155 (ethanol)	90	$C_{16}H_{13}N_3SO$ (295.37)	65.06 (65.56)	4.44 (4.16)	14.23 (14.68)	3316, 3178 (2NH), 3056 (=CH), 1600 (C=N), 1200 (C=S)	9.30 (s, 1H, NH), 8.6–6.30 (m, 12H, arom.+vinylic, NH)
4b	7/25	245 (dioxane)	69	$C_{16}H_{12}N_4O_3S$ (340.36)	56.46 (56.01)	3.55 (3.44)	16.46 (16.07)	3546, 3417 (2NH), 3090 (=CH), 1607 (C=N), 1100 (C=S), 1525, 1400 (NO_2)	
4c	8/25	160 (acetone)	85	$C_{11}H_{11}N_3SO$ (233.29)	56.63 (56.15)	4.75 (4.23)	18.02 (17.60)	3321, 3229 (2NH), 3000 (=CH) 1605 (C=N), 1120 (C=S)	9.50 (s, 1H, NH), 8.50–6.80 (m, 7H, arom.+vinylic+NH), 1.20 (s, 3H, $-CH_3$)
4d	7/25	145 (chloroform)	75	$C_{11}H_{10}N_4O_3S$ (278.29)	47.48 (47.08)	3.62 (3.29)	20.13 (20.55)	3340, 3422 (NH), 3050 (=CH), 1600 (C=N), 1110 (C=S), 1524, 1334 (NO_2)	9.50 (s, 1H, NH); 8.50–6.50 (m, 6H, arom.+vinylic+NH), 1.00 (s, 3H, CH_3)
5a	4/25	120 (ethanol)	58	$C_{11}H_{10}N_2O_2$ (202.21)	65.34 (65.00)	4.98 (4.55)	13.85 (13.40)	3200 (NH), 3050 (CH), 1680 (C=O), 1600 (C=N)	9.10 (s, 1H, NH), 7.50–6.00 (m, 6H, arom.+vinylic), 2.20 (s, 3H, CH_3)
6a	4/25	210 (benzene)	40	$C_{18}H_{12}N_2O_2$ (288.33)	74.98 (74.50)	4.19 (3.70)	9.72 (10.10)	3350 (NH), 3050 (CH), 1640 (C=N)	7.90–6.60 (m, 11H, arom.+vinylic+NH), 3.55 (s, 1H, CH)
7a	4/20	204 (ethanol)	55	$C_{11}H_9N_2O_2Cl$ (236.66)	55.83 (56.01)	3.83 (3.88)	11.84 (11.60)	3300 (NH), 3000 (CH), 1670 (C=O), 1600 (C=N)	7.90–6.20 (m, 7H, arom.+vinylic+NH), 3.90 (s, 2H, CH_2)
7b	4/20	>300 (acetone)	60	$C_{11}H_8N_3O_2Cl$ (249.66)	52.92 (53.00)	3.23 (3.30)	16.83 (16.70)	3310 (NH), 3010 (CH), 1680 (C=O), 1610 (C=N), 1500, 1320 (NO_2)	9.60 (s, 1H, NH), 8.80–6.50 (m, 5H, arom.+vinylic), 4.30 (s, 2H, C–CH_2Cl)
8a	4/reflux	245 (ethanol)	75	$C_{12}H_9N_3O_2$ (227.23)	63.43 (63.37)	3.99 (3.89)	18.49 (18.59)	3400 (NH), 3050 (CH), 2150 (C≡N), 1650 (C=O), 1640 (C=N), 1600 (C=C)	11.30 (s, 1H, NH), 8.30–6.30 (m, 6H, arom.+vinylic), 3.40 (s, 2H, $-CH_2-CN$)
8b	4/reflux	295 (toluene)	70	$C_{12}H_8N_4O_4$ (272.22)	52.95 (52.75)	2.96 (2.90)	20.58 (20.98)	3300 (NH), 3100 (CH), 2040 (C≡N), 1680 (C=O), 1600 (C=N), 1660 (C=C)	8.65–6.50 (m, 6H, arom.+vinylic, NH), 4.20 (s, 2H, $-CH_2CN$)
9a	6/reflux	347 (chloroform)	80	$C_{20}H_{14}N_4O_4$ (374.36)	64.17 (64.25)	3.77 (3.67)	14.97 (15.16)	3400 (NH), 3050 (CH), 1690 (C=O), 1640 (C=N), 1600 (C=C)	9.80 (s, 2H, 2NH), 9.00–6.70 (m, 12H, arom.+vinylic)
9b	6/reflux	>350 (acetone)	82	$C_{20}H_{12}N_6O_8$ (464.35)	51.73 (51.80)	2.60 (2.70)	18.10 (18.50)	3400 (NH), 3100 (CH), 1690 (C=O), 1650 (C=N), 1610 (C=C), 1500, 1350 (NO_2)	
10a	3/reflux	>350 (acetic acid)	87	$C_{22}H_{12}N_4O_6$ (428.36)	61.69 (62.00)	2.82 (2.90)	13.08 (12.95)	3050 (C–H), 1770, 1720 (C=O), 1650, 1630 (C=N, C=C)	

(*Continued*)

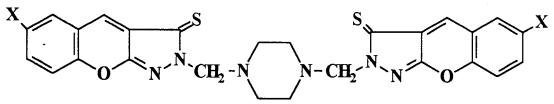
Table 1 (*Continued*)

10b	3/reflux	> 350 (dioxane)	90	$C_{22}H_{10}N_6O_{10}$ (518.36)	50.98 (51.05)	1.94 (2.05)	16.21 (16.10)	3100 (CH), 1700, 1680 (C=O), 1650, 1620 (C=N, C=C), 1510, 1350 (NO_2)	
11a	9/reflux	134 (benzene)	70	$C_{16}H_{12}N_2O_2$ (264.29)	72.71 (72.65)	4.58 (4.50)	10.60 (11.05)	3165 (NH), 3100 (CH), 1660 (C=O), 1630 (C=N)	9.50 (br, 1H, N–NH–C), 8.00–6.30 (m, 11H, arom.+vinylic)
11b	10/reflux	245 (ethanol)	71	$C_{16}H_{11}N_3O_4$ (309.28)	62.14 (62.28)	3.58 (3.62)	13.50 (13.45)	3200 (NH), 3000 (C–H), 1640 (C=O), 1600 (C=N), 1520, 1350 (NO_2)	10.60 (br, 1H, N–NH–C), 8.80–6.50 (m, 10H, arom.+vinylic)
12a	9/reflux	179 (chloroform)	60	$C_{15}H_{11}N_3O_2$ (265.27)	67.92 (67.80)	4.18 (4.15)	15.84 (16.08)	3160 (NH), 3000 (CH), 1660 (C=O), 1630 (C=N)	8.80 (br, 1H, N–NH–C), 8.10–6.30 (m, 10H, arom.+vinylic)
12b	10/reflux	305 (ethanol)	69	$C_{15}H_{10}N_4O_4$ (310.27)	58.07 (58.19)	3.25 (3.32)	17.49 (17.28)	3410 (NH), 3020 (C–H), 1650 (C=O), 1600 (C=N), 1510, 1340 (NO_2)	10.60 (br, 1H, N–NH–C), 8.80–6.50 (m, 10H, arom.+vinylic)
13a	9/reflux	164–165 (chloroform)	50	$C_{15}H_{11}N_3O_2$ (265.27)	67.92 (67.85)	4.18 (4.16)	15.84 (15.98)	3300 (NH), 3050 (CH), 1655 (C=O), 1635 (C=N)	9.70 (br, 1H, N–NH–C), 9.00–6.20 (m, 10H, arom.+vinylic)
13b	10/reflux	260 (benzene)	70	$C_{15}H_{10}N_4O_4$ (310.27)	58.07 (58.18)	3.25 (3.31)	17.49 (17.25)	3200 (NH), 3000 (C–H), 1680 (C=O), 1610 (C=N), 1520, 1350 (NO_2)	11.20 (br, 1H, N–NH–C), 9.00–6.50 (m, 9H, arom.+vinylic)
14a	9/reflux	232 (benzene)	62	$C_{16}H_{13}N_3O_2$ (279.30)	68.81 (68.72)	4.69 (4.59)	15.04 (15.24)	3500, 3400 (NH_2), 3250 (NH), 3050 (C–H), 1630 (C=O), 1600 (C=N)	10.50 (br, 1H, N–NH–C), 8.00–6.30 (m, 10H, arom.+vinylic), 5.20 (s, 2H, NH_2)
14b	10/reflux	240 (ethanol)	64	$C_{16}H_{12}N_4O_4$ (324.30)	59.26 (59.35)	3.73 (3.80)	17.28 (17.09)	3450, 3350, 3300 (NH_2, NH), 3100 (C–H), 1600 (C=N), 1520, 1340 (NO_2)	10.50 (br, 1H, N–NH–C), 8.40–6.30 (m, 9H, arom.+vinylic), 5.60 (s, 2H, NH_2)
15a	9/reflux	235 (dioxane)	59	$C_{16}H_{11}N_3O_4$ (309.28)	62.14 (62.10)	3.58 (3.49)	13.50 (13.75)	3440 (NH), 3120 (CH), 1630 (C=O), 1610 (C=N), 1520, 1345 (NO_2)	8.90 (br, 1H, N–NH–C), 8.25–6.30 (m, 10H, arom.+vinylic)
15b	10/reflux	310 (ethanol)	55	$C_{16}H_{10}N_4O_6$ (354.28)	54.24 (54.30)	2.85 (2.92)	15.81 (15.72)	3220 (NH), 3030 (C–H), 1660 (C=O), 1610 (C=N), 1510, 340 (NO_2)	9.35 (br, 1H, N–NH–C), 8.70–6.65 (m, 9H, arom.+vinylic)
16	7/reflux	200 (dioxane)	62	$C_{16}H_{12}N_2OS$ (280.35)	68.55 (58.45)	4.31 (4.25)	9.99 (10.05)	3160 (NH), 1100 (C=S)	
17	7/reflux	235 (ethanol)	58	$C_{15}H_{11}N_3OS$ (281.34)	64.04 (64.18)	3.94 (4.01)	14.94 (14.82)	3280 (NH), 1150 (C=S)	
18	7/reflux	170 (pyridine)	51	$C_{16}H_{11}N_3O_3S$ (285.35)	67.35 (67.26)	3.89 (3.82)	14.73 (14.89)	3250 (NH), 1170 (C=S)	
19	8/reflux	250 (ethanol)	70	$C_{16}H_{13}N_3OS$ (295.37)	65.06 (65.18)	4.44 (4.54)	14.23 (14.05)	3270 (NH), 1140 (C=S)	
20	7/reflux	150 (ethanol)	50	$C_{16}H_{10}N_4O_5S$ (370.35)	51.89 (51.80)	2.72 (2.61)	15.13 (15.26)	3300 (NH), 1180 (C=S)	
21	9/reflux	237 (ethanol)	70	$C_{16}H_{10}N_2O$ (246.27)	78.04 (78.14)	4.09 (4.15)	11.38 (11.19)	3056 (C–H), 1593 (C=N)	8.50–7.00 (m, 10H, arom.+vinylic)
22	9/reflux	220 (ethanol)	69	$C_{15}H_9N_3O$ (247.26)	72.87 (72.70)	3.67 (3.60)	16.99 (17.05)	3060 (C–H), 1600 (C=N)	8.90–6.80 (m, 9H, arom.+vinylic)
23	8/reflux	310 (ethanol)	52	$C_{16}H_9N_3O_3$ (291.27)	65.98 (65.87)	3.11 (3.05)	14.43 (14.51)	2900 (C–H), 1608 (C=N), 1516, 1340 (NO_2)	8.60–6.70 (m, 9H, arom.+vinylic)
24	7/reflux	217 (benzene)	67	$C_{15}H_8N_4O_3$ (292.26)	61.65 (61.55)	2.76 (2.70)	19.17 (19.28)	3063 (C–H), 1622 (C=N), 1528, 1340 (NO_2)	9.00–7.00 (m, 8H, arom.+vinylic)

(Continued)

Table 1 (*Continued*)

Comp. no.	Reaction time (h)/temp. (°C)	M.p. (cryst. solvent)	Yield (%)	M_F (M_W)	Analytical data Calc. (Found) (%)			IR (Kbr) ν (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
					C	H	N		
25a	20/reflux	274 (methanol)	70	$C_{10}H_6N_2OS$ (202.24)	59.39 (59.50)	2.99 (3.04)	13.85 (13.72)	3400 (NH), 3050 (CH), 1600 (C=N), 1180 (C=S)	14.20 (s, 1H, NH), 7.70, 6.70 (m, 5H, arom.+vinylic)
25b	19/reflux	280 (ethanol)	45	$C_{10}H_5N_3O_3S$ (247.23)	48.58 (48.70)	2.04 (2.12)	17.00 (16.85)	3450 (NH), 1600 (C=N), 1520, 1340 (NO_2), 1090 (C=S)	8.70–6.90 (m, 5H, arom.+vinylic, NH)
26a	1/reflux	185 (ethanol)	57	$C_{17}H_{12}N_2OS$ (292.36)	69.84 (69.90)	4.14 (4.20)	9.58 (9.41)	3000 (CH), 1600 (C=N)	8.70–6.90 (m, 10H, arom.+vinylic), 4.25 (s, 2H, $-CH_2$)
27a	12/25	190 (dioxane)	42	$C_{12}H_8N_2O_2S$ (244.27)	58.96 (58.84)	3.30 (3.25)	11.47 (11.62)	3084 (CH), 1750 (C=O), 1190 (C=S)	8.50–6.90 (m, 5H, arom.+vinylic), 2.50 (s, 3H, CH_3)
28a	12/20	125 (benzene)	56	$C_{17}H_{10}N_2O_2S$ (306.35)	66.65 (66.56)	3.29 (3.19)	9.14 (9.29)	3020 (CH), 1700 (C=O), 1050 (C=S)	8.60–6.80 (m, 10H, arom.+vinylic)
29a	12/25	246 (ethanol)	59	$C_{17}H_9N_3O_4S$ (351.34)	58.12 (58.06)	2.58 (2.52)	11.96 (12.09)	3100 (CH), 1700 (C=O), 1430, 1110 (NO_2, 1060 (C=S)	
30a	6/reflux	85 (benzene)	58	$C_{17}H_{12}N_3OSCl$ (341.82)	59.74 (59.87)	3.54 (3.60)	12.29 (12.14)	3300 (NH), 1600 (C=N), 1100 (C=S)	8.35–6.70 (m, 10H, arom.+vinylic+NH), 4.65 (s, 2H, N–CH_2–N)
31a	6/reflux	80 (ethanol)	70	$C_{19}H_{15}N_3O_2S$ (349.41)	65.31 (65.26)	4.33 (4.28)	12.03 (12.19)	3035 (NH), 2955 (CH), 1659 (C=O), 1595 (C=N), 1200 (C=S)	8.70–6.80 (m, 10H, arom.+vinylic+NH), 4.80 (s, 2H, N–CH_2–N), 2.50 (s, 3H, CH_3)
32a	5/reflux	75 (acetone)	51	$C_{15}H_{15}N_3O_2S$ (301.37)	59.78 (59.67)	5.02 (4.97)	13.94 (14.05)	2850 (CH), 1600 (C=N), 1120 (C=S)	8.00–6.90 (m, 5H, arom.+vinylic), 4.60 (s, 2H, N–CH_2–N), 4.00–3.60 (m, 4H, CH_2–O–CH_2), 3.10–2.50 (m, 4H, CH_2–N–CH_2)
33a	5/reflux	225 (ethanol)	73	$C_{26}H_{22}N_6O_2S_2$ (514.63)	60.68 (66.60)	4.31 (4.24)	16.33 (16.53)	2800 (CH), 1600 (C=N), 1150 (C=S)	8.00–6.60 (m, 10H, arom.+vinylic), 4.70 (s, 4H, 2 N–CH_2–N), 3.80–3.40 (m, 8H, CH_2 piperazine)



33a

1. Biological screening

Some representative examples of the new compounds were tested against brine shrimp (*Artemia sabina*) larvae to study their toxicity and tested against *Bacillus cereus* (Gram +ve bacteria) and *Escherichia coli* (Gram −ve bacteria). The results were compared with those for coumarin and thiocoumarin (cf. Table 2).

2. Experimental

2.1. Reaction of thiocoumarin with hydrazine hydrate: general procedure

To a solution of compound **1a** (0.02 mol) in ethanol (30 ml) or compound **1b** (0.02 mol) in dioxan (25 ml); hydrazine hydrate (0.02 mol) or (0.01 mol) was added in presence of two drops of triethylamine. The reaction mixture was refluxed (in case of compound **2**) or stirred at room temperature (r.t.) (in the case of compound **3**) until the H_2S ceased. The reaction mixture was concentrated. The product (**2** or **3**) was filtered and crystallized from the proper solvent (cf. Table 1).

2.2. Reaction of coumarin hydrazone (*2*) with isothiocyanates: general procedure

Phenyl or methyl isothiocyanate (0.01 mol) was stirred in ethanol (40 ml) or dioxan (30 ml) for 1 h and compound **2a** or **2b** (0.01 mol) was added to the solution. The reaction mixture was stirred for 7 h. The solid product (**4a–d**) was filtered and crystallized from the appropriate solvent (cf. Table 1).

2.2.1. Reaction of coumarin hydrazone (*2a*) with acetyl chloride

Acetyl chloride (0.01 mol) was added to a solution of compound **2a** (0.01 mol) in dry benzene (30 ml) in the presence of three drops of triethylamine. The reaction mixture was stirred for 4 h. The formed precipitate was filtered off and crystallized from ethanol to give ω-acetylcoumarin hydrazone (**5a**). The filtrate was concentrated and the precipitate was filtered off and crystallized from benzene to give compound **6a** (cf. Table 1).

MS: Compound **6a**: *m/e* (relative intensity)%: 290 (2.7), 288 (100.0), 118 (75.3), 89 (41.7), 63 (28.3).

2.2.2. Reaction of compound *2a* with chloroacetyl chloride

Chloroacetyl chloride (0.01 mol) was added to a solution of compound **2a** (0.01 mol) in dry benzene (30 ml) in presence of three drops of triethylamine. The reaction mixture was stirred for 4 h, then filtered. The precipitate was crystallized from ethanol to give compound **7a** (cf. Table 1).

MS: *m/e* (relative intensity)%: 236 (51.8), 238 (15.6), 219 (42.3), 159 (45.7), 102 (100.0).

2.3. Reaction of coumarin hydrazones (*2a,b*) with ethyl cyanoacetate: general procedure

Ethyl cyanoacetate (0.01 mol) was added to a solution of compound **2a** (0.01 mol) in ethanol (25 ml) or **2b** (0.01 mol) in dioxan (30 ml) in presence of two drops of acetic acid and piperidine. The reaction mix-

Table 2
Antifungal screening of some representative products[a]

Comp no.	1 (μg)			2 (μg)			3 (μg)		
	100	50	10	100	50	10	100	50	10
4a	+++	++	+	++	++	+	++	++	++
4b	+	-	-	++	++	++	++	++	+
7a	-	-	-	++	++	++	-	-	-
9a	-	-	-	++	++	++	-	-	-
10a	-	-	-	+++	++	++	++	++	++
11b	-	-	-	+	+	+	+	+	+
12a	-	-	-	++	++	++	++	+	+
14a	-	-	-	+	-	-	-	-	-
15a	+++	-	-	++	++	-	+	-	-
23	+	-	-	+	+	+	+	+	+
24	-	-	-	++	++	-	+	+	-
27	-	-	-	++	++	++	-	-	-
32a	-	-	-	-	-	-	-	-	-
33a	-	-	-	++	+	-	-	-	-

[a] (1) Brine shrimp test; (2) Gram +ve bacteria; (3) Gram −ve bacteria; (-) no effect; (+) weak; (++) moderate; (+++) strong.

ture was refluxed for 4 h. After cooling, the precipitate was filtered off and crystallized from a suitable solvent to afford compounds **8a,b**.

*2.4. Reaction of coumarin hydrazones (**2a,b**) with oxalyl chloride: general procedure*

Oxalyl chloride (0.01 mol) was added to a solution of compounds **2a,b** (0.02 mol) in dry benzene (30 ml). The reaction mixture was refluxed for 6 h under dry conditions. After cooling, the formed precipitate compounds **9a,b** was filtered and crystallized from the appropriate solvent (cf. Table 1).

*2.5. Synthesis of N,N′-di[2-imino-benzopyrane(6-nitro-benzopyrane)]piperazine-2,3,5,6-tetraone (**10a,b**)*

To a solution of compounds **9a,b** (0.005 mol) in dry benzene (30 ml), oxalyl chloride (0.005 mol) was added. The reaction mixture was refluxed and the crude product was crystallized from the appropriate solvent to give compounds **10a,b** (cf. Table 1).

2.6. Synthesis of ω-aroylcoumarin hydrazones

The appropriate acid hydrazide (benzoic, isonicotinic, nicotinic, *p*-aminobenzoic hydrazide or *p*-nitrobenzoic) (0.01 mol) was added to thiocoumarins (**1a,b**) (0.01 mol) in ethanol (30 ml) in presence of two drops of triethylamine. The reaction mixture was refluxed until the hydrogen sulfide ceased (~ 10 h). The reaction mixture was concentrated. After cooling, the precipitate compounds **11a,b–15a,b** were filtered off and crystallized from the appropriate solvent (cf. Table 1).

*2.7. Reaction of ω-aroylcoumarin hydrazones (**11–15**) with phosphorus pentasulfide: general procedure*

To a solution of the appropriate ω-aroylcoumarin hydrazone (0.005 mol) in dry pyridine (25 ml), phosphorus pentasulfide (0.005 mol) was added. The reaction mixture was refluxed for 7–10 h and filtered. The filtrate was cooled to r.t. The solid product was filtered and crystallized from the appropriate solvent to give the corresponding thio product compounds **16–20** (cf. Table 1).

*2.8. Synthesis of 3-aryl-[1]benzopyrano[2,3-c]pyrazole compounds **21–24**: general procedure*

The appropriate ω-aroylcounarin or thiocoumarin hydrazone (0.005 mol) was refluxed in *N*,*N*-dimethylaniline (20 ml) for 7–9 h. The reaction mixture was concentrated and cooled to r.t. The obtained product was filtered off and crystallized from the appropriate solvent to give compounds **21–24** (cf. Table 1).

MS: Compound **21**: *m*/*e* (relative intensity)%: 245 (58.6), 218 (16.4), 171 (30.73), 55 (100).

MS: Compound **23**: *m*/*e* (relative intensity)%: 290 (5.16), 254 (15.12), 238 (17.4), 139 (35.05), 20 (100).

*2.9. Synthesis of 2,3-dihydrobenzo- or (6-nitrobenzo)-pyrazo[2,3-c]-3-pyrazole-3-thione (**25a,b**): general procedure*

Alcoholic potassium hydroxide solution (0.01 mol in 7 ml ethanol/3 ml water) was added to a solution of coumarin hydrazone or 6-nitrocoumarin hydrazone (0.01 mol) in ethanol (50 ml), carbon disulfide (0.01 mol) was added to the mixture while stirring. After 1 h, the reaction mixture was refluxed until the hydrogen sulfide ceased (~ 20 h). The reaction mixture was concentrated, cooled to r.t. and poured on ice-water (100 ml) and filtered. The filtrate was acidified with concentrated hydrochloric acid. The precipitate obtained was filtered off and crystallized from the appropriate solvent to give compounds **25a,b** (cf. Table 1).

MS: Compound **25a**: *m*/*e* (relative intensity)%: 202 (76.6), 187 (12.9), 177 (3.88), 160 (100).

*2.10. Reaction of 3-benzylthio-2,3-dihydro[1]benzo-pyrano[2,3-c]pyrazole (**25a**) with benzyl chloride*

Compound **25a** (0.003 mol) was dissolved in sodium hydroxide solution (30 ml, 10%) and ethanolic solution of benzylchloride (0.003 mol) in 30 ml ethanol was added. The reaction mixture was refluxed for 1 h. After cooling, the precipitate was filtered off, washed with water and crystallized from ethanol to afford compound **26a** (cf. Table 1).

*2.11. Reaction of compound **25a** with acyl chlorides: general procedure*

A solution of acid chloride (acetyl chloride, benzoyl chloride or 4-nitrobenzoyl chloride), (0.01 mol) in 10 ml dry toluene was added to a solution of compound **25a** (0.01 mol) in toluene (30 ml) in presence of two drops of triethylamine and the reaction mixture was stirred overnight. The mixture was filtered and the solution was evaporated under reduced pressure. The residual solid was crystallized from the appropriate solvent to yield compounds **27a–29a** (cf. Table 1).

*2.12. Mannich reaction on 2,3-dihydrobenzopyrano-[2,3-c]pyrazole-3-thione (**25a**): general procedure*

Formaldehyde (1.5 ml; 40% solution) was added to a solution of compound **31a** (0.002 mol) in ethanol (20 ml). The reaction mixture was refluxed for 2 h. After

cooling, the appropriate primary amine (*m*-chloroaniline and *p*-aminoacetophenone) or secondary amine morpholine and piperazine) (0.002 mol) was added. The reaction mixture was refluxed for 4 h. The precipitate was filtered off and crystallized from the appropriate solvent to give compounds **30a–33a**.

2.13. Biological assays

For bioassays of coumarin and coumarin derivatives the following organisms were used as follows:

2.13.1. Brine shrimp test (animal organism)

(i) The immature stage (nauplii) or brine shrimp (*A. sabina* 1.) was used. Some 10–20 drops of brine shrimp eggs originating from Hadlow, Kent, UK, were hatched in filtered and autoclaved sea water (Red Sea) and kept at r.t. (22–24°C). Three days after the emergence of the first larvae, the hatched larvae were used as tested animals. (ii) 20, 50 and 100 μg of the appropriate compound (dissolved in methanol) were placed in test tubes, the methanol was evaporated and about 60–100 shrimp larvae in 1 ml sea water were transferred into the tubes. The tubes were kept at r.t. (22–24°C). Control tubes with methanol were also carried out. (iii) After 24 h, the mortality of larvae was determined with a stereoscopic microscope. (iv) All experiments were repeated three times.

2.13.2. Bacterial test

B. cereus, a Gram + ve bacteria, and *E. coli*, a Gram − ve bacteria, were used (kindly provided by Ali El-Sayed, Botany Department, Faculty of Science, South Valley University, Sohag, Egypt). Nutrient agar (NA) medium consisted of beef extract, 1 g; yeast extract, 2 g; peptone, 5 g; sodium chloride, 5 g; and agar, 15 g/l distilled H_2O. For soft agar, 2 g/l was used. The medium was adjusted to pH 7.2 (Spear and Sussmuch, 1987). A leg-phase bacteria suspension (0.05 ml in 3 ml soft agar) was poured onto the surface of the hard agar plate (NA) and the soft agar was left to solidify. A disc of filter paper (Whatman no. 1), 1 cm in diameter was saturated with a dose of 20, 50 or 100 μg of the appropriate compound (dissolved in methanol). After evaporation of methanol, the disc was placed in the center of the dish and incubated for 24 h at 37°C, after which time the diameter of the growth inhibition zone was measured. A control disc (methanol only) was also performed. The experiment was carried out twice for each compound.

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