

Note

Synthesis, characterization and antimicrobial studies of some novel 3-arylazo-7-hydroxy-4-methylcoumarins

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A series of 3-arylazo-7-hydroxy-4-methylcoumarins have been synthesized in excellent yields (80-90%) and their structures established on the basis of IR, ¹H NMR, mass spectral data and elemental analyses. Their purity has been ascertained by chromatographic analysis using ethyl acetate-xylene (4:6, v/v) as irrigant. All the compounds have been tested for their antimicrobial activity against a number of microorganisms (*Agrobacterium tumefaciens*, *Azotobacter spp.*, *B. megatherium*, *B. subtilis* and *Rhodococcus rhodochorus*).

Coumarin is a simple oxygen containing heterocycle, present in melilot and in tonca beans. It is the odoriferous principle of wood ruff, which led to its wide spread use as a perfumery chemical¹ in industry. Coumarin derivatives have also been found applications as fluorescent dyes², CNS depressants³, antitumor agents⁴, HIV proliferator⁵ and as an intermediate for the synthesis of cardiac drug⁶ carbocromen. It is pertinent to mention here that potent antibiotics chartreusin⁷ and coumermycine⁸ are coumarin derivatives.

In view of greater demand for potent pharmacologically active agents, it was our zenith approach to develop a cheap and valuable facile synthesis of therapeutically significant 3-aryl substituted benzopyran-2-ones (coumarins) **33-48** (Scheme I). Initially, the synthetic procedure incorporates the coupling of a number of diazotized aniline derivatives (**1-16**) with a β-ketoester 1-ethoxy-1, 3-butanone in the presence of ethanol to afford 2-arylhydrazono-1-ethoxy-1, 3-butanones (**17-32**) as precursors, by the method developed in the laboratory⁹. The reaction course was found to be facilitated by enolisation owing to the presence of two carbonyl moieties on β-positions. However, the decrease in resonance energy due to the presence of

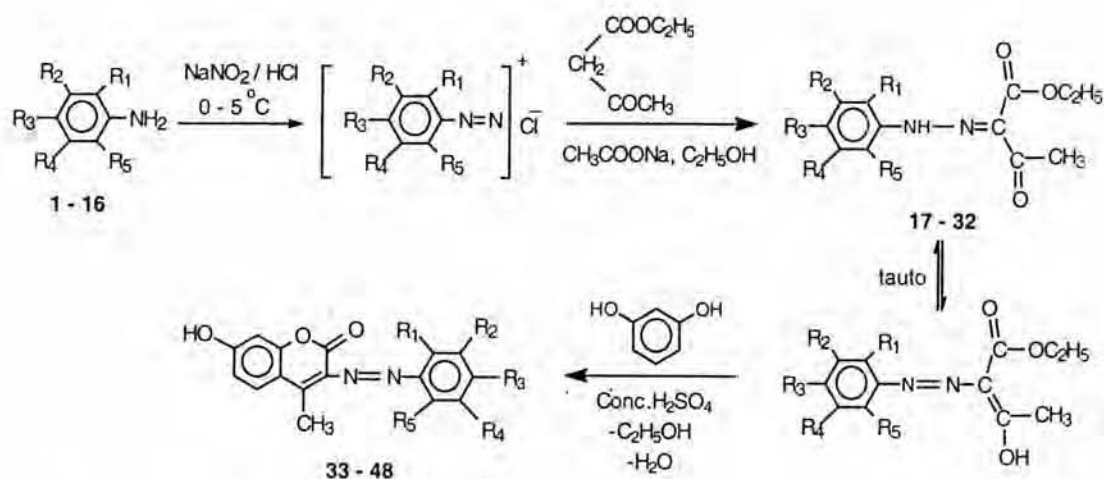
ester functionality, is circumvented by the presence of aromatic ring. Also, a careful temperature control is needed to ensure a good yield (82-96%) of the required coumarins **33-48** (Tables I and II). All the synthesized compounds were tested *in vitro* against a number of bacteria, and found to exhibit remarkable activity *cf.* Table III.

Antimicrobial screening. All the synthesized compounds were screened for their antimicrobial activity against the bacteria *A. tumefaciens*, *Azotobacter spp.*, *B. megatherium*, *B. subtilis* and *R. rhodochorus* (Table III) using standard antibiotic drug Sulphamethoxazole as control. The biological activity of these compounds have been evaluated by filter paper disc method¹⁰ at 500 ppm and 250 ppm concentrations using 5 cm size disc of filter paper.

The screening results indicates that compound **40** exhibits excellent antimicrobial activity against *A. tumefaciens* and *R. rhodochorus*, compound **41** shows good results on *B. subtilis* and *A. tumefaciens*. It is also observed that compounds having aryl group substituted by 2-NO₂(**42**), 3-NO₂(**43**), 2-CH₃-6-COOCH₃(**37**) and 4-CH₃-6-COOCH₃(**38**) associated with coumarin moiety also show good antimicrobial activity. Compounds **33**, **34**, **45**, **46**, **47** and **48** exhibit moderate activity. The remaining compounds **35**, **36**, **39** and **44** display weak activity against all microbes under investigation. The screening results also show that compound **40** owing to the presence of -Br group in phenyl ring, exhibit a higher zone of inhibition than that of exhibited by the standard drug against *A. tumefaciens*. It has been found that all the compounds are active at maximum concentration (500 ppm) but neutral at minimum concentration (250 ppm).

Experimental Section

General. Melting points were recorded on an electrically heated block and are uncorrected. UV spectra (λ_{max} in nm; log ε in parentheses) were recorded in 95% aldehyde free methanol on a Shimadzu UV 3101 PC spectrophotometer. IR spectra (ν_{max} in cm⁻¹) were recorded on a Perkin Elmer FT-IR Paragon 1000 spectrophotometer in KBr disc. ¹H NMR and ¹³C-NMR spectra were run in DMSO-*d*₆ on a Varian 270 MHz spectrophotometer using TMS as internal standard (chemical shifts in δ, ppm).



Compd	R ₁	R ₂	R ₃	R ₄	R ₅	Compd	R ₁	R ₂	R ₃	R ₄	R ₅
1	H	H	H	H	H	24	H	H	Br	H	H
2	COOH	H	H	H	H	25	NO ₂	H	NO ₂	H	Br
3	OH	H	H	H	H	26	NO ₂	H	H	H	H
4	H	H	OH	H	H	27	H	NO ₂	H	H	H
5	CH ₃	H	H	H	COOCH ₃	28	H	H	NO ₂	H	H
6	H	H	CH ₃	H	COOCH ₃	29	Cl	H	H	H	H
7	H	H	CH ₃	H	H	30	H	Cl	H	H	H
8	H	H	Br	H	H	31	H	H	Cl	H	H
9	NO ₂	H	NO ₂	H	Br	32	H	H	OCH ₃	H	H
10	NO ₂	H	H	H	H	33	H	H	H	H	H
11	H	NO ₂	H	H	H	34	COOH	H	H	H	H
12	H	H	NO ₂	H	H	35	OH	H	H	H	H
13	Cl	H	H	H	H	36	H	H	OH	H	H
14	H	Cl	H	H	H	37	CH ₃	H	H	H	COOH ₃
15	H	H	Cl	H	H	38	H	H	CH ₃	H	COOH ₃
16	H	H	OCH ₃	H	H	39	H	H	CH ₃	H	H
17	H	H	H	H	H	40	H	H	Br	H	H
18	COOH	H	H	H	H	41	NO ₂	H	NO ₂	H	Br
19	OH	H	H	H	H	42	NO ₂	H	H	H	H
20	H	H	OH	H	H	43	H	NO ₂	H	H	H
21	CH ₃	H	H	H	COOCH ₃	44	H	H	NO ₂	H	H
22	H	H	CH ₃	H	COOCH ₃	45	Cl	H	H	H	H
23	H	H	CH ₃	H	H	46	H	Cl	H	H	H
24	H	H	Br	H	H	47	H	H	Cl	H	H
25	NO ₂	H	NO ₂	H	Br	48	H	H	OCH ₃	H	H

Scheme I

Preparation of 2-arylhydrazone-1-ethoxy-1, 3-butanones 17-32. An aniline derivative (1-16) (0.01 mole) was dissolved in a mixture of 4.0 mL of concentrated hydrochloric acid and 4.0 mL of distilled water. The solution mixture was kept at freezing temperature. To this, an aqueous solution of sodium nitrite 2.0g (0.01 mole) in 5.0 mL of distilled water was added dropwise with continuous stirring keeping the temperature of the reaction vessel at 0-5 °C. Meanwhile in another beaker, 7.0 g (0.01 mole) of sodium acetate in a solution of 2.0 mL (0.01 mole) ethyl acetoacetate in 25.0 mL of ethyl alcohol was taken and cooled in an ice-bath. Now the diazotized solution was added to this solution dropwise with

thorough stirring. The reaction mixture was kept overnight, filtered under suction, washed thoroughly with cold water, dried and recrystallized from a mixture of dimethylformamide and ethyl alcohol (3:7, v/v) to give the corresponding butanone.

Preparation of 3-arylazo-7-hydroxy-4-methylcoumarins 33-48. In a one liter three-necked bolt head R.B. flask, fitted with a thermometer, mechanical stirrer and dropping funnel, 10.0 mL of conc. H₂SO₄ was taken and the flask placed in an ice-bath. After the temperature had fallen to 10 °C, 10.0g (0.091 mole) of resorcinol and 2-arylhydrazone-1-ethoxy-1, 3-butanone (0.01 mole) were added maintaining the temperature below 10 °C. The

Table I — Characterization data of 3-arylazo-7-hydroxy-4-methyl coumarins **33-48**.

Compd	mp °C	Yield (%)	Recrystallized from	Colour	Found (Calcd)(%)		
					C	H	N
33	113	88	Ethanol	Red	69.49 (69.84)	4.06 4.10	9.30 (9.58)
34	176	87	Diethylether	Cream	63.93 (64.23)	3.51 3.57	8.29 (8.33)
35	97	84	DMF	Green	65.83 (66.24)	4.01 3.89	9.01 (9.09)
36	98	85	DMF	Green	66.80 (66.24)	3.87 3.89	9.05 (9.09)
37	90	82	Acetone	Cream	65.20 (65.93)	4.12 4.39	7.21 (7.69)
38	110	83	Ethanol	Cream	65.39 (65.93)	4.22 4.39	7.54 (7.69)
39	86	89	Ethanol	Brown	70.19 (70.54)	4.50 4.57	9.10 (9.15)
40	104	93	Ethanol+DMF	Yellow	55.19 (55.08)	2.87 2.96	7.45 (7.54)
41	140	93	Ethanol+DMF	Brown	50.19 (50.38)	2.18 2.22	13.20 (13.80)
42	79	96	Ethanol	Brown	60.18 (60.55)	3.21 3.26	12.10 (12.43)
43	98	94	Ethanol	White	60.30 (60.55)	3.23 3.26	12.20 (12.42)
44	86	95	Ethanol	Green	60.23 (60.55)	3.22 3.26	12.20 (12.44)
45	124	95	DMF	Red	61.09 (62.45)	3.34 3.36	8.55 (8.57)
46	106	94	DMF	Brown	62.22 (62.44)	3.32 3.36	8.39 (8.57)
47	73	93	DMF	Yellow	62.13 (62.43)	3.32 3.36	8.52 (8.57)
48	118	89	DMF	Brown	66.81 (67.03)	4.22 4.34	8.61 (8.69)

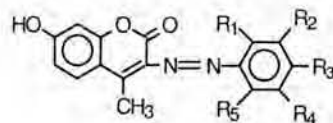
Table II — Spectral data of some 3-arylazo-7-hydroxy-4-methyl-coumarins.

Compd	UV[λ _{max} ; (log ε)]	NMR (δ ppm)		Compd	UV[λ _{max} ; (log ε)]	NMR (δ ppm)	
		¹ H	¹³ C			¹ H	¹³ C
34	218 (4.5), 282 (4.8), 440 (5.1).	1.4 (3H, s, CH ₃), 8.1 (1H, s, H-8), 6.92-7.3 (2H, m, J = 8.1Hz, H-5, H-6), 10.3 (1H, s, -COOH), 7.60 - 7.11 (2H, d, J = 8.8Hz, H-4', H-6'), 7.92 (1H, dd, H-5), 11.1 (1H, s, OH, D ₂ O ex.).	173 (COOH), 160 (C-2), 155 (C-2'), 132 (C-6'), 20.8 (CH ₃), 151 (C-7), 114 (C-6), 124.6 (C-5), 121 (C-8), 141 (C-3'), 128 (C-4').	42	228 (4.5), 308 (4.8), 415 (5.2).	-	157.6 (C-2), 138 (C-3), 114 (C-8), 148.8 (C-7), 118 (C-6), 21.1 (CH ₃), 121 (C-5), 159 (C-2'), 124 (C-5'), 127 (C-6'), 135 (C-3'), 131.6 (C-4').
41	225 (4.5), 268 (5.4), 335 (5.1).	1.3 (3H, s, CH ₃), 7.92 (1H, s, H-8), 7.0-7.61 (m, Ar-H), 11.9 (H, s, OH, D ₂ O ex.).	159.6 (C-2), 145.8 (C-3), 155.1 (C-1'), 20.1 (CH ₃), 116.5 (C-8), 114 (C-6), 128.8 (C-6'), 127.6 (C-3'), 129.8 (C-5').	47	239 (4.6), 358 (5.0).	1.1 (3H, s, CH ₃), 6.92 (2H, d, J = 8.8 Hz, H-3', H-5'), 7.47 (2H, d, J = 8.1 Hz, H-2', H-6'), 10.1 (1H, s, D ₂ O ex.).	117 (C-8), 21.8 (CH ₃), 155 (C-7), 115 (C-6), 127.8 (C-5), 161 (C-2), 155.7 (C-9), 133 (C-10), 143.1 (C-3 or C-1'), 128.3 (C-2', C-6'), 131.6 (C-4'), 129.1 (C-3', C-5').

reaction vessel was kept at room temperature for 18 hr. To this, approximately 50g of crushed ice was added with vigorous stirring. The precipitates, thus formed, were collected by filtration under suction and washed thrice with 5% sodium hydroxide solution (3-5mL). To the filtrate 2M H₂SO₄ (10 mL) was added slowly with vigorous stirring until the solution was just acidic to litmus, filtered at the pump, and

thoroughly washed with ice-cold water (4 × 10mL). Both the crops of crude coumarin were collected and recrystallized from a mixture of DMF and ethyl alcohol (3:7, v/v). Physical characteristics of all the synthesized products (**33-48**) are given in **Table I**.

Structures of all the compounds were established on the basis of their IR, UV and NMR spectral data reported in **Table II**.

Table III — Antibacterial activity of 3-arylaazo-7-hydroxy-4-methylcoumarins.

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Compd	Organism	Control drug α cm	Coumarin β cm	%inhibition* $\alpha-\beta/\alpha \times 100$ cm	Compd	Organism	Control drug α cm	Coumarin β cm	% inhibition* $\alpha-\beta/\alpha \times 100$ cm
33	<i>A. tumefaciens</i>	2.1	2.0	+4	42	<i>A. tumefaciens</i>	—	—	—
	<i>Azotobacter spp.</i>	2.0	2.3	-15		<i>Azotobacter spp.</i>	2.1	2.4	-14
	<i>B. subtilis</i>	1.8	1.8	-		<i>B. subtilis</i>	2.5	3.1	2.4
	<i>B. megatherium</i>	2.0	1.8	+10		<i>B. megatherium</i>	2.0	1.9	+5
	<i>R. rhodochorus</i>	2.0	2.3	-20		<i>R. rhodochorus</i>	2.0	2.3	-15
34	<i>A. tumefaciens</i>	2.5	3.1	-24	43	<i>A. tumefaciens</i>	1.9	2.5	-31
	<i>Azotobacter spp.</i>	2.1	2.4	-14		<i>Azotobacter spp.</i>	2.0	1.9	+5
	<i>B. subtilis</i>	2.9	3.0	-3.4		<i>B. subtilis</i>	—	—	—
	<i>B. megatherium</i>	2.1	2.5	-19		<i>B. megatherium</i>	3.0	3.6	-20
	<i>R. rhodochorus</i>	2.0	2.6	-30		<i>R. rhodochorus</i>	2.4	2.7	-12
37	<i>A. tumefaciens</i>	2.7	2.3	-17	45	<i>A. tumefaciens</i>	2.1	2.4	-14
	<i>Azotobacter spp.</i>	2.1	2.0	+4		<i>Azotobacter spp.</i>	2.0	2.3	-15
	<i>B. subtilis</i>	—	—	—		<i>B. subtilis</i>	2.0	2.2	-20
	<i>B. megatherium</i>	2.1	2.5	-19		<i>B. megatherium</i>	2.3	2.7	-17.3
	<i>R. rhodochorus</i>	2.1	3.1	-24		<i>R. rhodochorus</i>	2.1	2.5	-19
38	<i>A. tumefaciens</i>	1.8	2.5	-38	46	<i>A. tumefaciens</i>	1.8	1.9	-5.5
	<i>Azotobacter spp.</i>	2.0	1.9	+5		<i>Azotobacter spp.</i>	2.0	1.9	+5
	<i>B. subtilis</i>	2.4	2.4	-		<i>B. subtilis</i>	2.8	3.0	-7.1
	<i>B. megatherium</i>	2.9	3.6	-24		<i>B. megatherium</i>	2.1	2.0	+4
	<i>R. rhodochorus</i>	2.0	2.2	-20		<i>R. rhodochorus</i>	2.3	2.7	-17.3
40	<i>A. tumefaciens</i>	1.7	2.5	-47	47	<i>A. tumefaciens</i>	1.9	2.3	-21
	<i>Azotobacter spp.</i>	—	—	—		<i>Azotobacter spp.</i>	2.1	2.4	-14
	<i>B. subtilis</i>	—	—	—		<i>B. subtilis</i>	3.1	3.0	+3.2
	<i>B. megatherium</i>	2.3	2.7	-17		<i>B. megatherium</i>	2.0	2.6	-30
	<i>R. rhodochorus</i>	2.0	2.5	-25		<i>R. rhodochorus</i>	—	—	—
41	<i>A. tumefaciens</i>	2.2	3.0	-36	48	<i>A. tumefaciens</i>	2.0	2.2	-20
	<i>Azotobacter spp.</i>	2.0	2.3	-15		<i>Azotobacter spp.</i>	1.9	2.1	-10.5
	<i>B. subtilis</i>	2.9	3.6	-24		<i>B. subtilis</i>	2.1	2.0	+4
	<i>B. megatherium</i>	2.7	3.0	-11		<i>B. megatherium</i>	2.2	2.3	-4.5
	<i>R. rhodochorus</i>	2.0	1.8	+10		<i>R. rhodochorus</i>	1.8	2.0	-10.5

* At concentration of 500 ppm using 5 mm-size disc of filter paper.

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