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An efficient synthesis, characterization of some novel 6 -(R) phenyl-azo-coumarin -3- carboxy(3-chloro- 4-methoxy) anilide and 2- hydroxy- 5(R)-phenyl - azo - benzylidine (3- chloro - 4 -methoxy)aniline and their antibacterial activity

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ABSTRACT

An efficient synthesis of various substituted azo-coumarin & azo-Schiff base has been synthesized by the condensation of 3-chloro-4-methoxy phenyl malon anilic acid (1a) and different substituted phenyl benzene-azo-salicylaldehyde (5a-5l) with few drops of pyridine as a condensing agent. The constitution of the newly synthesized products has been established on the basis of their spectral studies viz: IR, ^{1}H NMR, Elemental analysis and physical properties as m.p, colour, yield%, molecular formula etc. and their antibacterial activity was carried out against *S.aureus* and *E.coli* micro-organisum.

Key words: Subs. Azo - Coumarins and Azo - Schiff's Bases, Pyridine, Condensation, Malonic Acid, Spectral Evaluation, Antibacterial Activity.

INTRODUCTION

Many of the synthetic, semi-synthetic, natural molecules containing the coumarin scaffold and included simple coumarin, azo-coumarin, pyrano-coumarin, iso-coumarin, benzo-coumarin have become an important class of heterocyclic compounds in drug research. Azo-coumarin have been reported to have multiple biological activities[1], some azo-coumarin and their derivatives have been reported to possess anti-coagulant[2], antibacterial[3], antifungal[4], antiallergic[5], antidiabe-tic[6], analgesic[7], activities. Azo-Schiff bases are also important class of heterocyclic compounds and had got wide applications in pharmacology and industrial fields[8]. Some substituted azo-Schiff bases and their derivatives have also been reported to possesses antiviral[9], anticancer[10], antibacterial[11], activities. In this laboratory also a number of substituted azo-coumarins and azo-Schiff bases and their derivatives have been prepared by various workers[12-15].

In continuation of our previous work[16-18] in the present study we report here the condensation reactions of 3-chloro-4-methoxy malon anilic acid (1a) with different substituted phenyl benzene-azo-salicylaldehydes (5a-5l) in the presence of pyridine as a condensing agent.

EXPERIMENTAL

Material and Methods

All the chemicals used were obtained from Sigma-Aldrich Company. All the recorded melting points were determined in open capillary tubes and are uncorrected. The purity of final products were checked by TLC using Silica-gel-coated Al-plates. IR (infrared spectrum) (Kbr-disc method cm^{-1}) were recorded on Perkin-Elmer Spectrum RX-1 FT-IR

spectrophotometer at ST. John's college, Agra. ^{1}H NMR spectra measured on Advance Bruker DRX-300 using solution in DMSO d_6. Chemical shifts are given in δ (ppm) and protons signals are indicated as: s = singlet, d = doublet, t = triplet, m = multiplet. Elemental analysis was performed on Elementor Vario EL III. All of the synthesized compounds gave satisfactory results. The physical and analytical properties of synthesized compounds are recorded in Table-1.

Synthesis of N - (3-chloro- 4-methoxy) phenyl malon anilic acid (1a), N : N^I - di - (3 - chloro - 4 methoxy) phenyl malonamide (2a), Ethyl-N-(3-chloro- 4 - methoxy) phenyl malonamate (3a), and N-(3-chloro- 4 -methoxy) phenyl malona-mic acid amide (4a)

To the substituted aniline (3 - chloro - 4 - methoxy; 0.025 mole), diethyl malonate (0.05 mo le) was added in the presence of catalyst (DMF), refluxed for about 45-60 minutes, cooling, filtered (1). The solid part was recrystallized by ethanol 99%, on analysis it was identified to be N:N^I di-(3-chloro - 4-methoxy) phenyl malonamide (2a). The filtrate (1) of main product was collected in a china dish and concentrated by heating it on boiling water-bath, the coloured mass was treated with 25 ml portion of petroleum ether (100-120^{O}C), recrystallized by petroleum ether several times, it was found to be ethyl N - (3 - chloro - 4 -methoxy) phenyl malonamate (3a). Ethyl N-(3-chloro- 4 - methoxy) phenyl malonamate (3a; 0.01 mole) was dissolved in 15 ml of ethanol in r.b.fla-sk was added 15 ml of liquor ammonia, the flask was tightly corcked vigorously shaken, cooling, filtered, purified by ethanol, it is identified to be N (3 - chloro - 4 - methoxy) phenyl malonamic acid amide (4a). In the filtrate (1) of main product add ethanol (20 ml) with a solution of Na_2CO_3 (20 ml), and then take the hydrolysis of reaction mixture for about 45 minutes, filtered, to the above filtrate add concentrated HCl drop-wise, thus the solid was separated, filtered,washed with distilled water, recrystallized and it was identified to be N (3-chloro- 4 -methoxy) phenyl malon anilic acid (1a).

Synthesis of 2-hydroxy-5 (R) phenyl benzene-azo benzaldehyde (5a-5l)

To substituted amine (0.025 mole) was diazotised with adding concentrated HCl (8 ml) in distilled water (6 ml) at 0°C in an ice-bath, then add solution of sodium nitrite (8 ml) to it with constant stirring, the solution of salicylaldehyde (0.025 mole) in 2N NaOH (20 ml) was added with stirring in to the above diazotised salt solution. The solid is separated out immediately, it was filtered, washed with cold water, recrystallized from absolute ethanol 99%.

Synthesis of 6(R)-phenyl azo-coumarin-3-carboxy (3- chloro - 4- methoxy) anilide (6a-6l) and 2-hydroxy-5(R) phenyl azo-banzylidine-(3-chloro - 4- methoxy) aniline (7a-7l)

A mixture of N (3-chloro-4-methoxy) phenyl malon anilic acid (0.001mole ; **1a**) and (0.001mole ;5a-5l) in equi molar quantity (1:1), with few drops of pyridine, the reaction mixture was heated for 4h in an oil-bath at 104-110^{O}C, the mixture was first melted and then soon set to a solid, cooling, then the product was digested with the saturated solution of Sodium bicarbonate, washed with water, the azo-Schiff base was removed by the extraction with hot ethanol (15 ml), the filtrate was concentrated and cooling, the obtained product was identified as substituted azo-Schiff base (7a - 7l), the residue was recrystallized from hot ethanol several times and identified to be substituted azo-coumarin (6a-6l).

Elemental Analysis for C, H, N of compound (6a) are as 53.90(53.87), 3.12(2.94), 8.25(8.19) and compound **(7a)** are as 54.16(54.01), 4.01(3.40), 9.77(9.45).

Antibacterial Activity

The newly synthesized compounds were screened for their antibacterial activity against S.aureus and E.coli bacterial strains by filter paper disc diffusion method[19-20] was followed by using special Hi-Media Sterile disc code SD-067. Streptomycin was used as a standard drug and compared with results, the compounds were screened at concentration of 25 µg/ml in DMF. The zone of inhibition produced by each compounds was measured in mm and the results of antibacterial activity are given in the Table-3.

RESULTS AND DISCUSSION

The infrared spectra of the newly synthesized compounds have been recorded in the

Table 1:Physical and Analytical data of Synthesized Compounds

S No.	Products codes	Molecular Formula	Molecular Weight	M.P 0°C	Yield %	Colour	%Analytical Analysis					
							C		H		N	
							cal.%	(found)	cal.%	(found)	cal.%	(found)
1.	1a	$C_{15}H_{10}N_1O_4Cl_1$	243.65	132	47.63	white	49.29	(49.32)	4.13	(4.14)	05.74	(05.79)
2.	2a	$C_{17}H_{16}N_2O_4Cl_2$	383.24	058	56.63	peach organza	53.28	(53.30)	4.20	(4.18)	07.31	(07.35)
3.	3a	$C_{12}H_{14}N_1O_4Cl_1$	271.70	068	48.75	mushroom	53.04	(53.06)	5.19	(5.17)	05.15	(05.18)
4.	4a	$C_{10}H_{11}N_2O_3Cl_1$	242.67	183	45.38	light dawn	49.49	(49.52)	4.56	(4.58)	11.54	(11.56)
5.	6a	$C_{23}H_{15}N_3O_4Cl_1Br_1$	512.77	157	57.19	light orange	53.87	(53.89)	2.94	(2.96)	08.19	(08.23)
6.	6b	$C_{23}H_{15}N_3O_4Cl_1F_1$	451.85	171	63.52	dirty yellow	61.13	(61.14)	3.34	(3.36)	09.30	(09.34)
7.	6c	$C_{24}H_{14}N_3O_4Cl_2F_3$	536.31	242	39.61	orange	53.74	(53.76)	2.63	(2.64)	07.83	(07.85)
8.	6d	$C_{23}H_{15}N_3O_4Cl_2$	468.31	155	77.62	light orange	58.98	(58.96)	3.22	(3.20)	08.97	(09.01)
9.	6e	$C_{24}H_{18}N_3O_4Cl_1$	447.88	217	54.54	dirty orange	64.36	(64.38)	4.05	(4.07)	09.38	(09.42)
10.	6f	$C_{25}H_{20}N_3O_5Cl_1$	477.91	178	47.08	orange	62.83	(62.85)	4.21	(4.19)	08.79	(08.83)
11.	6g	$C_{24}H_{18}N_3O_5Cl_1$	463.88	216	59.40	orange	62.14	(62.15)	3.91	(3.93)	09.05	(09.09)
12.	6h	$C_{23}H_{14}N_3O_4Cl_3$	502.76	234	70.12	thar desert	54.94	(54.96)	2.80	(2.78)	08.35	(08.31)
13.	6i	$C_{23}H_{14}N_3O_4Cl_3$	502.76	248	46.19	dirty orange	54.94	(54,95)	2.80	(2.82)	08.35	(08.38)
14.	6j	$C_{24}H_{17}N_3O_4Cl_2$	482.33	251	59.53	light volcano	59.76	(59.78)	3.55	(3.57)	08.71	(08.74)
15.	6k	$C_{24}H_{18}N_3O_4Cl_1$	447.88	195	45.86	orange	64.36	(64.33)	4.05	(4.07)	09.38	(09.35)
16.	6l	$C_{25}H_{20}N_3O_4Cl_1$	461.91	232	47.49	light sunrise	65.00	(64.98)	4.36	(4.38)	09.09	(09.03)
17.	7a	$C_{20}H_{15}N_3O_2Cl_1Br_1$	444.73	082	44.62	toffee orange	54.01	(54.03)	3.40	(3.42)	09.45	(09.48)
18.	7b	$C_{20}H_{15}N_3O_2Cl_1F_1$	383.82	119	58.60	light yellow	62.58	(62.60)	3.94	(3.92)	10.94	(10.97)
19.	7c	$C_{21}H_{14}N_3O_2Cl_3F_3$	468.28	098	47.29	brick red	53.86	(53.87)	3.01	(3.00)	08.97	(08.93)
20.	7d	$C_{20}H_{15}N_3O_2Cl_2$	400.27	137	42.17	mild orange	60.01	(60.02)	3.77	(3.79)	10.49	(10.52)
21.	7e	$C_{21}H_{18}N_3O_2Cl_1$	379.85	sticky	53.30	coffee brown	66.40	(66.41)	4.77	(4.79)	11.06	(11.09)
22.	7f	$C_{22}H_{20}N_3O_3Cl_1$	409.88	sticky	57.78	brown	64.46	(64.48)	4.91	(4.90)	10.25	(10.29)
23.	7g	$C_{21}H_{18}N_3O_3Cl_1$	395.85	069	56.60	brown	63.71	(63.69)	4.58	(4.56)	10.61	(10.65)
24.	7h	$C_{20}H_{14}N_3O_2Cl_3$	434.72	074	40.07	brown	55.25	(55.23)	3.24	(3.26)	09.66	(09.62)
25.	7i	$C_{20}H_{14}N_3O_2Cl_3$	434.72	078	49.53	light volcano	55.25	(55.26)	3.24	(3.25)	09.66	(09.61)
26.	7j	$C_{21}H_{17}N_3O_2Cl_2$	414.30	109	49.71	volcano	60.88	(60.90)	4.13	(4.11)	10.14	(10.17)
27.	7k	$C_{21}H_{18}N_3O_2Cl_1$	379.85	074	49.38	light volcano	66.40	(66.38)	4.77	(4.75)	11.06	(11.01)
28.	7l	$C_{22}H_{20}N_3O_2Cl_1$	393.88	069	52.20	rust	67.08	(67.07)	5.11	(5.10)	10.66	(10.62)

frequency region 4000-500 cm^{-1} and ^1H NMR spectral data are recorded in the Table 2.

The IR spectrum of compound (**1a**) shows -NH stretching vibrations at 3415.2 cm^{-1}, -CH_2 stretching at 1388.0 cm^{-1}, stretching vibration at 1723.6 cm^{-1} indicates the -COOH, presence of aromatic -C=C confirm by stretching at 1535.1 cm^{-1}, -CONH confirm by the stretching vibrations at 1655.4 cm^{-1}, linkage at 668.4 cm^{-1}. Above observations are lent support to the assigned structure of compound (**1a**).

IR spectrum of (2a) shows absorption at 3459.2 cm^{-1} indicates -NH stretching, absorption at 1388. 0 cm^{-1} (-CH_2 str.), while absorption at 1545.0 cm^{-1} (-C=C str.), absorption at 1650.3 cm^{-1} (-CONH str.), mono substitution at 668.3 cm^{-1}. Hence the assigned structure was in agreement of compound (2a).IR spectrum of (3a) shows absorption at 3412.9 cm^{-1} indicates (-NH str.), absorption at 1369.9 cm^{-1} (-CH_2 str.), while absorption at 1544.9 cm^{-1} show -C=C, absorption at 1657.4 cm^{-1} indicates the presence of -CONH, absorption at 1740.5 cm^{-1} indicates lactone -C=O, mono substitution at 688.8 cm^{-1}. By above observations the assigned structure was in agreement of the compound (3a).

The IR spectrum of compound (4a) shows -NH stretching vibrations at 3289.4 cm^{-1}, -CH_2 str. at 1370.4 cm^{-1}, stretching vibration at 1739.5 cm^{-1} indicates the -C=O, -C=C confirm by stretching at 1542.3 cm^{-1}, -CONH confirm by the stretching vibrations at 1653.3 cm^{-1}, C-Cl linkage at 693.6cm^{-1}. Above observations are lent support to the assigned structure of compound (4a).

The IR spectrum of compound (6a-6c & 6i) shows -NH str. in (3468.2-3413.6 cm^{-1}), -N=N str. in range 1477.5-1462.2 cm^{-1}, str. in range 17 86.4-1727.5 cm^{-1} indicates the -C=O, (C=C str.) in the range 1562.9-1546.6 cm^{-1},(-CONH str.) in the

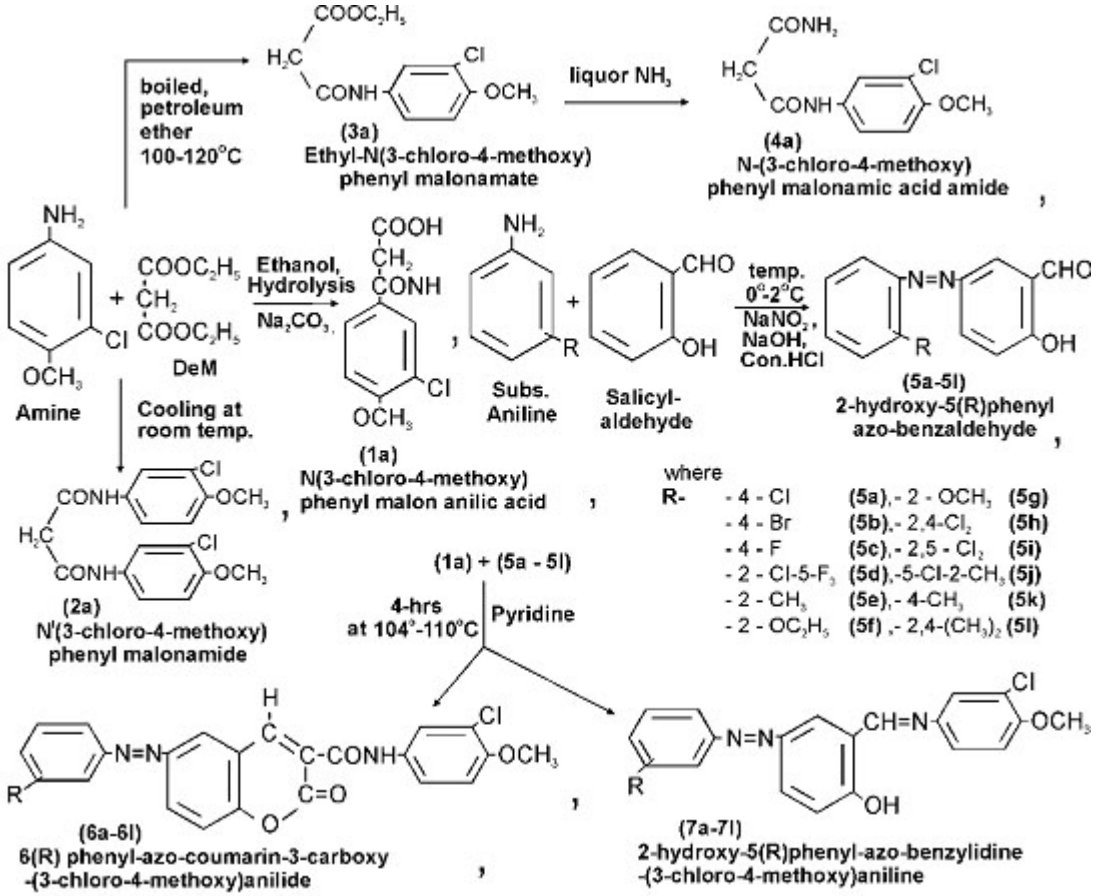

Table 2: Spectral data of the newly synthesized compounds

codes	IR absorption Bands in ν cm^{-1}									
	Ar C=C cm^{-1} stretching	HC=N cm^{-1} stretching	-OH cm^{-1} stretching	N=N cm^{-1} stretching	CONH cm^{-1} stretching	Lactone C=O cm^{-1}	-NH cm^{-1} stretching	$-CH_2$ cm^{-1} stretching	-COOH cm^{-1} stretching	mono substi.
1a	1535.1	-	-	-	1655.4	-	3415.2	1388.0	1723.6	668.4
2a	1545.8	-	-	-	1650.3	-	3459.2	1370.2	-	668.3
3a	1544.9	-	-	-	1657.4	1740.5	3412.9	1369.9	-	688.8
4a	1542.3	-	-	-	1653.3	1739.5	3289.4	1370.4	-	693.6
6a	1548.7	-	-	1477.5	1664.9	1764.1	3416.8	-	-	668.4
6b	1551.5	-	-	1465.9	1639.5	1786.4	3416.0	-	-	668.2
6c	1546.6	-	-	1467.2	1637.3	1785.9	3468.2	-	-	668.2
6i	1562.9	-	-	1462.2	1639.9	1727.5	3413.6	-	-	668.3
7a	1546.7	2361.6	3437.3	1467.1	-	-	-	-	-	668.3
7b	1548.4	2361.2	3468.0	1480.6	-	-	-	-	-	668.2
7c	1547.0	2361.1	3468.0	1484.0	-	-	-	-	-	668.3
7i	1546.6	2361.1	3436.1	1461.3	-	-	-	-	-	668.3

compounds codes	^{1}H-NMR Spectral data (in δ ppm)
1a	2.500(s,1H,COOH),3.318(s,2H,-CH_2),3.813(s,3H,Ar-H),7.379-7.416(m,6H,ring),10.160(s,1H, CONH)
6b	3.333(s,1H,Ar -CH),7.169(s,1H,-C=O),7.432(s,1H,-C=C),7.723(s,1H,-N=N),7.911-8.034(m,6H, -ring)
7b	1.227(s,3H,-C-CH_3),2.010(s,1H,-OH),2.500(s,1H,-CN=N),7.754(d,1H,-N=N),7.873-7.961(m,6H,-ring)

Table 3: Antibacterial data of synt. compounds

Codes	Zone of inhibition (in mm)	
	S.aureus **Gram +ve**	***E.coli*** **Gram -ve**
1a	+	+++
6a	+++	++
6b	+++	++
6c	++	+
6e	+	R
6f	R	+
6h	++	+++
6j	+++	+++
Streptomycin	++	+++

Key to symbols: Resistance=R; slighty active = + (inhibition zone 6-9 mm); moderately active = ++ (inhibition zone 9-12 mm);highly active = +++(inhibition zone >12).

range 1664.9-1639.3 cm^{-1}, str.of mono substitution in the range 668.4-668.2 cm^{-1}. Above observations are lent support to the assigned structure of compounds (6a-6c & 6i) and other compounds (6d-6h & 6j-6l).

The IR spectrum of compound (7a-7c & 7i) shows -OH str. In (3468.0-3436.1cm^{-1}), -N=N str. in (1484.0-1461.3 cm^{-1}), str. in (2361.6-2361.1 cm^{-1}) indicates the -HC=N, C=C confirm by str. in the range 1548.4-1546.6 cm^{-1}, str. vibrations in the range 668.3-668.2 cm^{-1} indicates the mono substitution. Above observations of these compounds are lent support to the assigned structure of compounds (7a-7c & 7i) and other compounds (7d-7h & 7j-7l).

The ^{1}H NMR spectra showed singlet at δ 2. 500(COOH), 3.813(Ar-H), 3.318(-CH_2), 10.160(CONH), confirming the structure of compound (1a), and the spectra of compound (6a) showed signal as singlet at ä 3.333(Ar-CH), 7.169(-C=O), 7.432(-C=C),7.723(-N=N), the above observations confirming the structure of compound (6a) and other compounds (6b-6l), in other compounds the spectra showed signal as singlet at δ 1.227(-C-CH_3), 2.010(-OH), 2.500(CH=N), 7.754(-N=N),7.873-7.961(ring), the above results confirm the structure of compound (**7b**) and other compounds (7a, 7c-7l).

The antibacterial activity results from Table-3 show that compound (6a,6b,6j) have moderate to highly active against S.aureus and compound (1a,6h,6j) have good activity against *E.coli.*

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