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Synthesis, Characterization, Industrial Application and Anticancer Activity of (E)-3-(((4-(Bis (2-Chloroethyl) Amino) Phenyl) Imino) Methyl)-4-Chloro-2H-Chromen-2-Onederivatives

Barasara Ashwin*¹, Bhatt Hardik¹ and Purohit Deepak²

¹School of Science, RK University, Kasturbadham, Rajkot (Gujrat -India)

²Shree Manibhai and Smt. Navalben Virani Science College,
Saurashtra University, Rajkot (Gujarat - India).

*Email: ashwinbarasara59@gmail.com

ABSTRACT

Some new (E)-3-(((4-(bis(2-chloroethyl)amino)phenyl)imino)methyl)-4-chloro-2H-chromen-2-one derivatives possessing coumarinas basic nucleus were synthesized. Characterizations of synthesised compounds were carried out by IR, NMR and mass spectral analysis. All synthesized compounds were tested for anticancer activity using colo-205 cell line against standard drug adriamycin.

KEYWORDS: Coumarin, Schiff base, Aniline mustard, Anticancer activity.

***Corresponding author**

Ashwin Barasara

School of Science, RK University,
Kasturbadham, Rajkot
(Gujrat -India)

Email: ashwinbarasara59@gmail.com

INTRODUCTION

Many biologically important Schiff bases have been reported in the literature possessing antimicrobial¹, antibacterial², antifungal³, anti-inflammatory⁴, anticonvulsant⁵, antitumour⁶ and anti-HIV⁷ activities. A complex of some transition metals with Schiff base shows analgesic and anti-inflammatory activities.⁸

MATERIAL AND METHODS

All compounds used in this study were of analytical grade and purchased from CDH Pvt. Ltd., Mumbai. Melting points of all compounds were taken in open capillary technique using Thieles tube. IR spectra of all compounds were taken on FTIR-8400 spectrophotometer apparatus using DRS probe and KBr pallet method. ¹H-NMR spectra of all the synthesized compounds were taken on a Bruker-Avance-II (400 MHz) using DMSO-*d*₆ as solvent. Chemical shifts are expressed in terms of δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Kyoto, Japan). Physical constants of all the synthesized compounds **ASW-1a** to **ASW-1l** are presented in Table 1. The method for the synthesis of aniline N-mustard is described in the experimental division.

General Procedure for the synthesis of 2,2'-((4-nitrophenyl)azanediyl)diethanol(Int a).

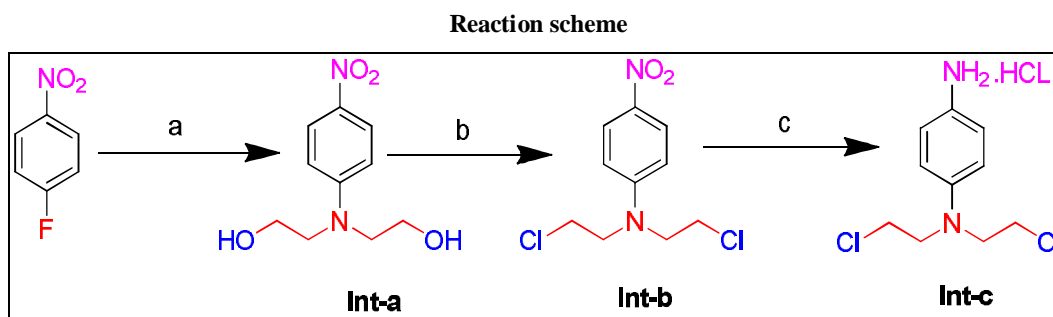
N,N-bisethanolamine(350 mmole) were added in RBF having 1-fluoro-4-nitrobenzene (300 mmole)and heatedfor 5-6 hr. at 80⁰C. Aftercompletion of the reaction,the reaction mixture was cooled to room temperature and poured in to the crushed ice. Separated solid was filtered and washed with water and dried to afford **int-a**. This compound was used in next step without further purification.

Procedure for the synthesis of N,N-bis(2-chloroethyl)-4-nitroaniline (Int-b).

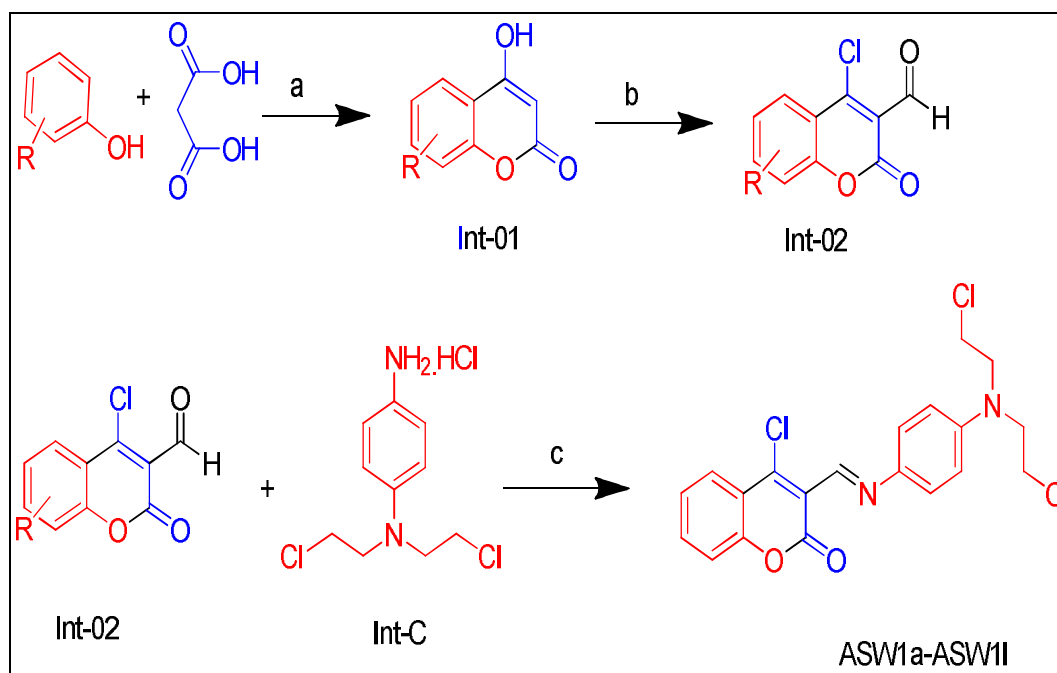
Thionyl chloride(250mmole) was added drop wise to a well stirred and cooled solution of the 2,2'-((4-nitrophenyl)azanediyl)diethanol (200 mmole) in 50ml DMF. After addition, theReaction mixturewas heated at 70-80⁰C for 3hr. After completion of the reaction, mixture was poured in to ice. Separated solid was filtered, washed it with water and dried to get **Int-b**.

Procedure for the synthesis of *N,N*-bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (Int-c).

The Tin metal (200mmole) was added in the mixture of **Int-b** (50 mmole) and 50 ml con.HCl at room temperature in the RBF. Then the reaction mixture was refluxed for 6 hr till all the tin metal dissolved and the clear solution obtained. After completion of the Reaction, mixture was filtered off and basified with the NaOH solution by keeping temperature below at 10⁰C. After neutralization, the solution was extracted with ethylacetate. After separated organic layer, combined organic layer was dried over sodium sulphate and acidified with ethylacetate HCl. Precipitated hydrochloride salt was filtered and washed with ethylacetate to get **Int-c**.



Scheme 1: (a) Diethanol amine, 80⁰C, 6hr, (b) SOCl₂, DMF, 70⁰C, 4hr (c) Sn, HCl, 90⁰C, Ethyl acetate



Scheme 2: (a) POCl₃, Anhy ZnCl₂, 80 °C, 5-6 hr (b) DMF, POCl₃, 0-60 °C (c) Gly.CH₃COOH, CH₃OH

General synthesis of Schiff base of 4-chloro-2-oxo-2H-chromene-3-carbaldehyde with aniline mustard (ASW1a-ASW1l)

At room temperature, aniline mustard was added in the solution of 4-chloro-2-oxo-2H-chromene-3-carbaldehyde (2gm, 0.0090mmole) and methanol (10 ml, 5v). After addition, reaction mixture was heated at reflux temperature for 30 min. After completion of reaction, mixture was cooled. The obtained solid was filtered, wash it with methanol and dried it in oven.

Table 1: Synthesized coumarin based aniline nitrogen mustard analogues.

Code	Molecular Formula	R	Molecular Weight	Melting Point °C	Yield %
Asw1a	C ₂₀ H ₁₇ Cl ₃ N ₂ O ₂	H	422	190	80
Asw1b	C ₂₁ H ₁₉ Cl ₃ N ₂ O ₂	2-CH ₃	436	183	71
Asw1c	C ₂₁ H ₁₉ Cl ₃ N ₂ O ₂	3-CH ₃	436	148	72
Asw1d	C ₂₁ H ₁₉ Cl ₃ N ₂ O ₂	4-CH ₃	436	204	63
Asw1e	C ₂₂ H ₂₁ Cl ₃ N ₂ O ₂	2,3-diCH ₃	451	174	69
Asw1f	C ₂₂ H ₂₁ Cl ₃ N ₂ O ₂	3,4-diCH ₃	451	176	76
Asw1g	C ₂₂ H ₂₁ Cl ₃ N ₂ O ₂	3,5-diCH ₃	451	196	65
Asw1h	C ₂₂ H ₂₁ Cl ₃ N ₂ O ₂	2,5-diCH ₃	451	189	58
Asw1i	C ₂₀ H ₁₆ BrCl ₃ N ₂ O ₂	4-Br	499	192	65
Asw1j	C ₂₀ H ₁₆ Cl ₃ FN ₂ O ₂	4-F	440	214	67
Asw1k	C ₂₀ H ₁₆ Cl ₄ N ₂ O ₂	4-Cl	456	219	64
Asw1l	C ₂₀ H ₁₆ Cl ₃ N ₃ O ₄	4-NO ₂	467	210	49

Synthesis of 4-hydroxy coumarin (int-1).

Anhydrous zinc chloride (30 gm) which was preheated to get rid of any moisture added to various substituted phenols (0.1 mole), malonic acid and phosphorus oxychloride (40 ml). After addition reaction, mixture was heated on a water bath at 700 °C for 8-10 hr. It was then cooled and poured into the crushed ice and water to get buff-yellow coloured solid. The solid was then filtered and washed with water. It was then triturated with 10 % sodium carbonate solution and filtered. The filtrate was slowly acidified with dilute HCl till the effervescence ceased. Finally, the product was filtered, dried and recrystallized with methanol.

Synthesis of 4-chloro-3-formyl coumarin (int-2).

The splution of POCl₃ (0.18 mole) were added to a stirred mixture of 4-hydroxy coumarin(0.06 mole) in anhydrous DMF (0.6 mole) at -10 °C to -5 °C. The reaction mixture was allowed to stirred additionally 1 hr at room temperature and after heated and stirred for 2 hr at 60 °C. After completion of the reaction, the mixture was poured in to crushed ice under vigorous and continue stirring. Separated pale yellow solid was collected by filtration and washed successively with Na₂CO₃ (5 %) and water, and then was air-dried. Recrystallization from acetone gave 87 % of 4-chloro-3-formyl coumarin (int-2) as a pale yellow powder with m.p. 115–120 °C.

RESULT AND DISSCUSION**Spectral data of the synthesized compounds**

(E)-3-(((4-(bis(2-chloroethyl)amino)phenyl)imino)methyl)-4-chloro-2H-chromen-2-one (ASW-1a); Brown solid; *R*_f0.43 (8:2 MDC-hexane); mp190 °C; **IR** (KBr, cm⁻¹): 3280, 1686, 1609, 1559, 1518, 1483, 1377, 1318, 1242, 1063,845, 753, 695, 625 cm⁻¹; **¹H NMR**: δ PPM 8.710 to 8.340 (m, 1H, Ar-H), 7.720(tri, 1H, Ar-H), 7.460(s, 2H, Ar-H), 7.086(s, 2H, Ar-H), 6.786(s, 2H, Ar-H), 6.673(s, H, -CH), 3.749(s, 8H, (-CH₂CH₂-)₂). **¹³C NMR**(400 MHz, DMSO): 41.07, 52.09, 111.66, 114.65, 116.36, 117.37, 124.66, 125.16, 127.75, 135.10, 145.16, 151.36, 155.34. **MS** (*m/z*): 422 (M⁺); Anal. Calcd for: C₂₀H₁₇Cl₃N₂O₂: C, 57.632; H, 4.38; Cl, 24.30; N, 6.40; Found: C, 54.13; H, 4.03; N, 9.9.

(E)-3-(((4-(bis(2-chloroethyl)amino)phenyl)imino)methyl)-4-chloro-6-methyl-2H-chromen-2-one (ASW-1d): Yellowish solid; *R*_f0.42 (8:2 MDC-hexane); mp204 °C; **IR** (KBr,cm⁻¹): 3306, 1685, 1605, 1516, 1455, 1316, 1206, 1105, 1066, 865, 789, 725, 645 cm⁻¹; **¹H NMR**: δ PPM 8.286(s, 1H, Ar-H), 7.557(d, J=7.0 Hz, 1H, Ar-H), 7.321(d, J=7.012 Hz, 1H, Ar-H), 7.063(d, J=6.4 Hz, 2H, Ar-H) 6.760(d, J=6.3 Hz, 2H, Ar-H), 6.651(s, H, -CH), 3.746(s, 8H, (-CH₂CH₂-)₂), 2.406(s, 3H, -CH₃). **¹³C NMR**(400 MHz, DMSO): 20.53, 41.16, 52.14, 111.65, 114.04, 116.23, 117.20, 124. 25, 125.08, 126.79, 134.10, 134.86, 145.16, 145.47,149.48, 155.53. **MS** (*m/z*): 436 (M⁺); Anal. Calcd for C₂₁H₁₉Cl₃N₂O₂: C, 57.64; H, 4.38; Cl, 24.31; N, 6.50; Found: C, 55.18; H, 4.21; N, 9.50

(E)-3-(((4-(bis(2-chloroethyl)amino)phenyl)imino)methyl)-4-chloro-7,8-dimethyl-2H chromen-2-one (ASW-1e): Brown solid; *R*_f0.44 (8:2 MDC-hexane); mp174 °C; **IR** (KBr, cm⁻¹): 3308, 1678, 1608, 1536, 1487, 1337, 1208, 1107, 1066, 897, 778, 727, 649 cm⁻¹; **¹H NMR**: δ PPM 8.162(d, J=8.2Hz, 1H, Ar-H), 7.262(d, J=8.6Hz, 1H, Ar-H), 7.063(d, J=8.3Hz, 2H, Ar-H), 6.730(d, J=8.3Hz, 2H, Ar-H), 6.674(s, H, -CH), 3.744(s, 8H, (-CH₂CH₂-) ₂), 2.361(s, -CH₃), 2.260(s, -CH₃). **¹³C**

NMR(400 MHz, DMSO): 11.46, 19.94, 41.08, 52.18, 111.76, 112.96, 115.88, 121.30, 124.37, 125.01, 125.82, 127.15, 143.53, 146.06, 146.10, 149.20, 155.30. **MS** (m/z): 451 (M^+); Anal. Calcd for $C_{22}H_{21}Cl_3N_2O_2$: C, 58.50; H, 4.68; Cl, 23.55; N, 6.21; Found: C, 56.13; H, 4.55; N, 9.13.

(E)-3-(((4-(bis(2-chloroethyl)amino)phenyl)imino)methyl)-4-chloro-6,7-dimethyl-2H-chromen-2-one (ASW-1f): Brown solid; R_f 0.42 (8:2 MDC-hexane); mp 176 °C; **IR** (KBr, cm^{-1}): 3311, 1725, 1675, 1515, 1425, 1315, 1206, 1107, 1055, 890, 825, 789, 726, 645 cm^{-1} ; **1H NMR**: δ PPM 8.166(s, 1H, Ar-H), 7.1974(s, 1H, Ar-H), 7.055(s, 2H, Ar-H), 6.734(s, 2H, Ar-H), 6.68(s, H, -CH), 2.321(s, 3H, -CH₃), 2.288(s, 3H, -CH₃), 3.746(s, 8H, (-CH₂CH₂)₂). **^{13}C NMR**(400 MHz, DMSO): 18.96, 19.58, 41.07, 52.11, 111.63, 116.02, 117.48, 124.24, 124.98, 126.97, 133.11, 144.24, 145.59, 149.55, 155.58. **MS** (m/z): 451 (M^+); Anal. Calcd for $C_{22}H_{21}Cl_3N_2O_2$: C, 58.50; H, 4.68; Cl, 23.55; N, 6.21; Found: C, 56.18; H, 4.58; N, 9.18.

Biological activity⁹⁻¹⁰

The biological activity has been performed as per standard protocols. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: $[Ti/C] \times 100 \%$.

Table 2 Anticancer activity

	Drug concentrations ($\mu g/ml$) calculated from graph		
Colo-205	LC50	TGI	GI50*
ASW-1a	NE	NE	48.0
ASW-1b	NE	65.7	<10
ASW-1c	NE	NE	NE
ASW-1d	NE	NE	>80
ASW-1e	NE	NE	NE
ASW-1f	NE	NE	<10
ADR	<10	<10	<10

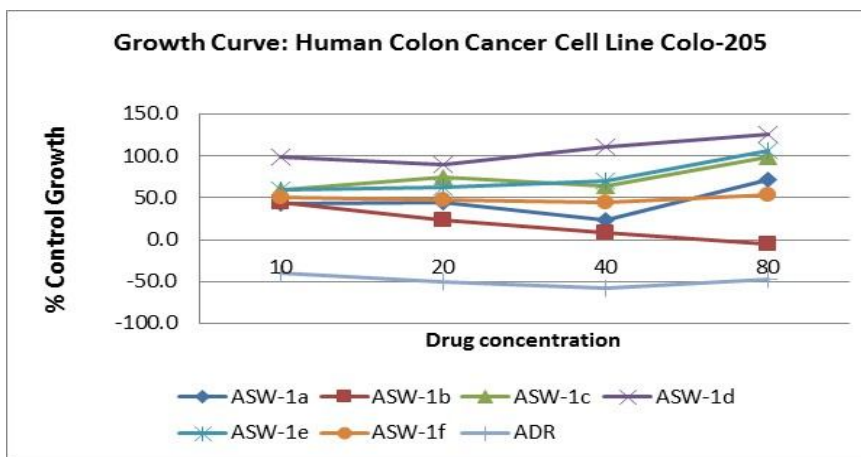


Figure 1 Anticancer activity

Table 2 Human Colon Cancer Cell Line Colo-205

Human Colon Cancer Cell Line Colo-205																
% Control Growth																
Drug Concentrations (µg/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
ASW-1a	53.0	51.3	19.2	35.2	20.6	41.8	26.8	81.5	56.1	40.7	26.3	97.9	43.2	44.6	24.1	71.5
ASW-1b	27.3	7.6	3.5	-21.1	26.5	10.8	-2.6	4.2	80.6	51.9	25.3	1.8	44.8	23.4	8.7	-5.0
ASW-1c	48.1	66.4	59.3	75.9	47.6	55.3	49.9	97.2	85.0	100.7	82.2	121.7	60.2	74.1	63.8	98.3
ASW-1d	110.6	122.8	122.4	139.6	88.0	57.8	107.7	123.4	95.3	88.9	99.9	111.9	98.0	89.8	110.0	124.9
ASW-1e	91.0	90.9	99.3	137.5	28.9	41.2	57.7	94.8	59.1	57.6	55.0	84.8	59.7	63.2	70.6	105.7
ASW-1f	80.5	82.3	83.6	87.9	26.6	20.7	24.4	31.1	44.8	39.4	26.4	43.7	50.7	47.5	44.8	54.2
ADR	-52.9	-45.1	-52.6	-54.8	-66.6	-60.6	-69.5	-57.9	-0.9	-43.3	-49.3	-29.6	-40.1	-49.7	-57.1	-47.5

CONCLUSION

We have recognised innovative and appropriate method for the synthesis of Schiff base of 4-chloro-2-oxo-2H-chromene-3-carbaldehyde with aniline mustard. All the produced compounds were acquired in good yield and pure by column chromatography using MDC as solvent system. Synthesized compounds were confirmed by IR, NMR and Mass spectrometry. Anticancer activities of all the synthesized compounds carried on colo-205 cell line using adriamycin as a standard drug. By the research of anticancer screening data shows that, the compounds ASW-1b and ASW-1f show very good activity compare to standard drug. Therefore, these compounds may be used as novel anticancer drugs after carrying out further evolution of cytotoxic research with advanced technology.

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