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Synthesis and Anticancer Activity of new N-mustard Substituted Coumarin Derivatives

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ABSTRACT

A series of N-mustard Substituted- coumarin have been synthesized characterized and evaluated for their in vitro cytotoxicity and anticancer activity against PC-3 human cancer cell lines. The methodology involves nucleophilic substitution of readily accessible 3-Cyano-4-chloro coumarin with ethanol amine further on chlorination gives substituted 4-(bis(2-chloroethyl)amino)-3-nitro-2H-chromen-2-one derivatives. Among these compounds screened, three compounds (NT-2g, NT-2f and NT-2e) showed GI50 range from 80 to 95 µg/ml. All synthesized compounds were characterized by IR, NMR and mass spectral analysis and screened for Anticanceractivity using PC-3 cell line. All the compounds showed moderate to good anticancer activity.

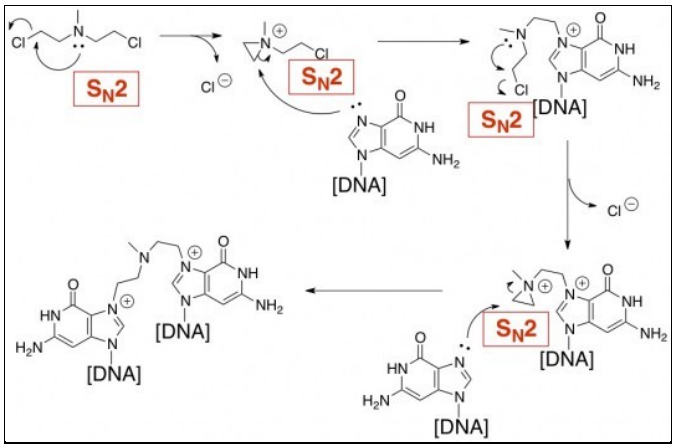
Keywords : 4-chloro,3-nitro coumarin; aniline mustard; anticancer activity

I. INTRODUCTION

The N-mustards were among the very earliest class of anticancer agents developed, and perhaps most extensively studied of the DNA alkylating agents[1]. The pronounce cytotoxicity of the N-mustard derivatives is attributed to their ability to induce inter strand cross-links between the two strands of DNA thereby inhibiting replication. The overall process of DNA alkylation by N-mustard is a two-step process (Figure 2). The nitrogen atom is able to displace a chloride ion intra-molecularly to form the highly elctrophilic aziridinium ion. Alkylation of DNA can then take place via nucleophilic attack on that intermediate by DNA[2]. For N-mustards, the regiospecificity of alkylation of DNA is largely governed by electronic and stearic properties of DNA. Therefore, they target DNA at the most electronegative sites, with mono adducts occurring primarily at the N-7 of guanines[3] and the inter strand cross-links between the N-7 positions of guanines in each strand at 5'-GNC sequences[4]. The present work will serve number of novel N-nitrogen mustard containing functionalized coumarin as potent antitumor agents using novel synthetic approaches. Coumarin and its hydroxyl derivatives have been prominently accepted as natural pharmaceuticals[5] worldwide, has revealed new biological activities with interesting therapeutic applications, besides their traditional employment as anticoagulants(anti-vitamin K activity)[6], antibiotics(novobiocin and analogues[7]) and anti AID[8]. Apart from this, they also possess anti-cancerous[9], antibacterial[10], neurotropic[11], immunosuppressive[12], anti-inflammatory[13], antiulcerous[14], antiPAF(anti platelet activating factor)[15] and antimutagenic[16] effects we chose it as basic nucleus.

The main significance of this work is it will provide libraries of highly functionalized heterocyclic molecules in search of stable nitrogen N-mustard derivatives as DNA alkylating agents. However, search is continuously to identify a more potent molecule as these molecules are developing resistance over an area. As we mentioned above, the significance and biological profile of this class of molecule so our continue efforts towards the synthesis of pharmacologically potential heterocyclic molecules. Seeking for novel antitumor drug with low side effect is a hot spot in the field of Medicinal chemistry. Based on our experience with various classes of heterocyclic compounds, our aim is to design some new biodynamic compounds. During the study of anti-cancer activity of benzopyraonobenothiazinones and anilinoacridines (Under the collaborative efforts), we have found these classes of compounds showed potent anti tumor activity

with stable nitrogen mustards. As we discussed, bifunctional alkylating agents particularly N- mustards have played an important role in anticancer drug development. Using this alkylating for chemotherapy have a number drawbacks including high chemical reactivity resulting in loss of drug's therapeutic efficacy by reacting with other cellular nucleophlies such as proteins and thiols producing many unwanted side-effects including bone marrow toxicity. To overcome these drawbacks, one of the strategies is to design DNA-directed alkylating agents by linking the alkylating pharmacophore with DNA-affinic molecules. Another approach to minimize these drawbacks is to prepare prodrug *via* introducing urea, carbamate or carboxamide linker. Thus, in present work, we will utilize coumarin as DNA affinic scaffolds (carrier) for N-mustard (alkylating pharmacophore) to stabilize its reactivity and to make more stable and targeted N-mustard as potent anti tumor agents.



II. METHODS AND MATERIAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of AR grade and were used without further purification. Melting points were taken in open capillary method and are uncorrected. IR spectra were recorded on FTIR-8400 spectrophotometer (Shimadzu, Kyoto, Japan), using DRS probe KBr pallet. 1H-NMR spectra of the synthesized compounds were recorded on a Bruker-Avance-II (400 MHz) DMSO-*d*6 solvent.

Chemical shifts are expressed in 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 the synthesized compounds **NT-2a to NT-2l** are shown in Table 1.

Synthesis of 4-hydroxy coumarin (int-1)

Various Substituted phenols (0.1 mole) and malonic acid were added to a mixture of phosphorus oxychloride (40 ml) and anhydrous zinc chloride (30 gms) which was preheated to get rid of any moisture. The reaction mixture was heated on a water bath at 70 ^{0}C for 8-10 hours. It was cooled and decomposed with ice and water to afford buff-yellow coloured solid. The solid was then filtered and washed thoroughly with water. It was then triturated with 10% sodium carbonate solution and filterd. The filtrate was slowly acidified with dilute HCl till the effervescence ceased. The product was filtered, dried and recrystallized with methanol.

General synthesis of various substituted 4-hydroxy 3-nitro coumarin (INT-02)

Various 4-hydroxy3-nitrocoumarins were prepared by heating the corresponding 4-hydroxycoumarins in with HNO_3 and acetic acid at 80-85°C for 1.5 hour. Mixture of 2 equivalent nitric acid and acetic acid was mixed with 4-hydroxy coumarin and acetic acid at 80-85^{0}C temperature. The reaction mass was heated at 80-85^{0}C for 1.5 hours. After completion of the reaction, reaction mass was cooled at room temperature and poured into crushed ice than filtered and washed with water to afford yellow colored solids of 4-hydroxy 3-nitro coumarins. These were characterized from their elemental analysis and spectroscopic data.

General synthesis of various substituted 4-chloro 3-nitro coumarin (INT-03)

Mixture of DMF and $POCl_3$ (2.5 equivalent) cooled at 0 °C for 20 min then it was stirred at room temperature for 15 min followed by dropwise addition of 4-hydroxy 3-nitrocoumarin dissolved in minimum quantity of DMF. Reaction mixture stirred at R.T. for 2 hour. After completion of reaction, it was poured into the ice and filtered. Solid mass is washed with water to afford yellowish 4-chloro 3-nitrocoumarin. These were characterized from their elemental analysis and spectroscopic data.

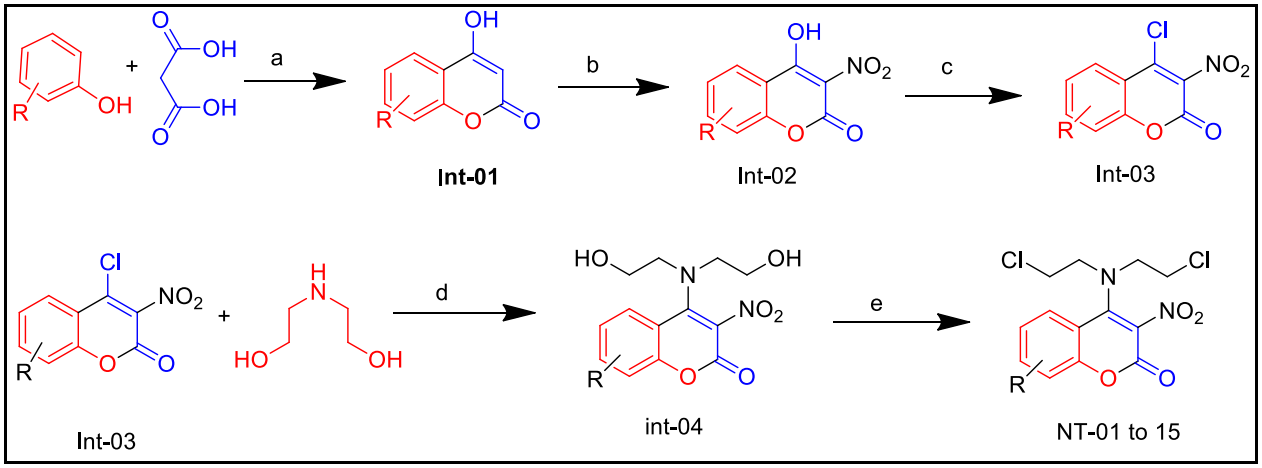
General synthesis of substituted 4-(bis(2-hydroxyethyl)amino)-3-nitro-2H-chromen-2-one (INT-04)

4-chloro-3-nitro coumarin (0.01 mol) was dissolved in 10 mL IPA and allowed to stir between 0-5°C followed by addition of diethanol amine(0.2 mol) was carefully added to the solution so that the temperature do not rise 10°C. Allow it to stir for 30 min and slowly rise to room temperature. After completion of the reaction, was poured into crushed ice, filtered and washed with water. Crystallization from chloroform gives 4-substituted4-(bis(2-hydroxyethyl)amino)-3-nitro-2H-chromen-2-one. Yield 61-88%

General synthesis of substituted 4-(bis(2-chloroethyl)amino)-3-nitro-2H-chromen-2-one derivatives (NT-2a-2l)

To a solution of 4-(bis(2-hydroxyethyl)amino)-3-nitro-2H-chromen-2-one and Dimethyl formamide at 0°C, thionyl chloride is added drop wise with continuous stirring. After addition of thionyl chloride reaction mixture is stirred at room temperature. After completion of reaction, reaction mixture is poured into crushed ice.

REACTION SCHEME



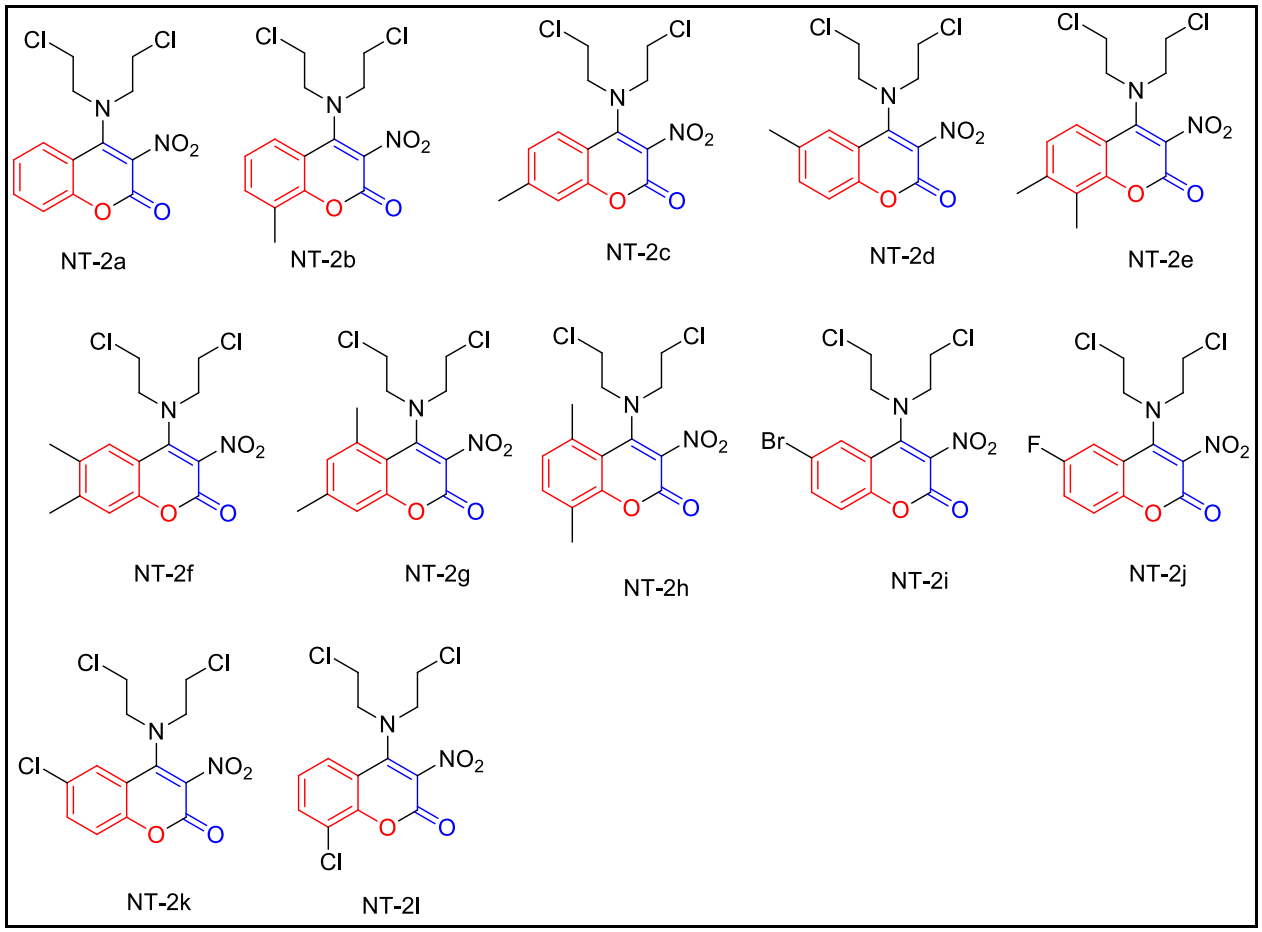
Scheme 1;(a)$POCl_3$,Anhy,$ZnCl_2$,80°C,5-6hr (b)HNO_3, gly.CH_3COOH, 0-80°C (c) DMF, $SOCl_2$ (d) IPA, excess ethanol amine(e)DMF,$SOCl_2$, 0°C to rt.

Table-1: Physical property of synthesized coumarin based N-nitrogen mustard

Code	Molecular Formula	R	Molecular Weight	Melting Point °C	Yield %
NT-2a	$C_{13}H_{12}Cl_2N_2O_4$	H	330	182-184	73
NT 2b	$C_{14}H_{14}Cl_2N_2O_4$	2-CH_3	344	184-186	68
NT-2c	$C_{14}H_{14}Cl_2N_2O_4$	3-CH_3	344	146-148	63
NT-2d	$C_{14}H_{14}Cl_2N_2O_4$	4-CH_3	344	206-208	66
NT-2e	$C_{15}H_{16}Cl_2N_2O_4$	2,3-diCH_3	358	172-174	69
NT-2f	$C_{15}H_{16}Cl_2N_2O_4$	3,4-diCH_3	358	176-178	78
NT-2g	$C_{15}H_{16}Cl_2N_2O_4$	3,5-diCH_3	358	192-194	67
NT-2h	$C_{15}H_{16}Cl_2N_2O_4$	2,5-diCH_3	358	188-190	55
NT-2i	$C_{13}H_{11}BrCl_2N_2O_4$	4-Br	407	194-196	64
NT-2j	$C_{13}H_{11}Cl_2FN_2O_4$	4-F	348	214-216	61

NT-2k	$C_{13}H_{11}Cl_3N_2O_4$	4-Cl	363	218-220	63
NT-2l	$C_{13}H_{11}Cl_3N_2O_4$	2-Cl	363	220-222	68

Scope of Substrate



III. RESULTS AND DISCUSSION

4. Spectral data of the synthesized compounds

4-(bis(2-chloroethyl)amino)-3-nitro-2H-chromen-2-one (NT-2a);Brown solid; R_f 0.41 (8:2 EA-hexane); mp 188-190°C; IR (KBr, cm^{-1}): 3278, 1680, 1608, 1558, 1516, 1481, 1375, 1317, 1242, 1062,840, 752, 690, 623 cm^{-1}; ^1H NMR: δ $_{PPM}$ 8.62 to 8.35 (m, 1H, Ar-H), 7.74(tri, 1H, Ar-H), 6.783(s, 2H, Ar-H), 3.749(s, 8H, (-CH_2CH_2-)$_2$). ^{13}C NMR (400 MHz, DMSO): 41.05, 52.09, 111.61, 114.50, 124.55, 125.14, 126.74, 134.05, 145.18, 151.30, 155.38. MS (*m/z*): 330 (M^+); Anal. Calcd for: $C_{13}H_{12}Cl_2N_2O_4$: C, 54.04; H, 3.89; Cl, 22.79; N, 9.00; Found: C, 54.12; H, 4.02; N, 9.9.

4-(bis(2-chloroethyl)amino)-8-methyl-3-nitro-2H-chromen-2-one (NT-2b): Brown solid; R_f 0.39 (8:2 MDC-hexane); mp 181-183°C; IR (KBr, cm^{-1}): 3298, 1681, 1605, 1515, 1495, 1314, 1202, 1102, 867, 786, 723, 645 cm^{-1}; MS (*m/z*): 324 (M^+); Anal. Calcd for: $C_{15}H_{14}Cl_2N_2O_2$: C, 55.40; H, 4.34; Cl, 21.80; N, 8.6; Found: C, 55.14; H, 4.21; N, 9.40.

4-(bis(2-chloroethyl)amino)-7-methyl-3-nitro-2H-chromen-2-one (NT-2c) Brown solid; R_f 0.45 (8:2 EA-hexane); mp 146-148°C; IR (KBr, cm^{-1}): 3294, 1680, 1604, 1514, 1313, 1203, 1103, 866, 821, 786, 723, 644 cm^{-1}; MS (*m/z*): 344 (M^+); Anal. Calcd for: $C_{14}H_{14}Cl_2N_2O_4$: C, 55.40; H, 4.34; Cl, 21.80; N, 8.6; Found: C, 55.14; H, 4.21; N, 9.40.

4-(bis(2-chloroethyl)amino)-6-methyl-3-nitro-2H-chromen-2-one (NT-2d): Yellowish solid; R_f 0.41 (8:2 EA-hexane); mp 202-204°C; IR (KBr,cm^{-1}): 3302, 1681, 1603, 1513, 1451, 1315, 1204, 1104, 1061, 867, 788, 723, 643 cm^{-1}; ^{1}H NMR: δ_{PPM} 8.28(s, 1H, Ar-H), 7.55(d, J=7.0 Hz, 1H, Ar-H), 7.32(d, J=7.01 Hz, 1H, Ar-H), 3.74(s, 8H, $(-CH_2CH_2-)_2$), 2.40(s, 3H, $-CH_3$). ^{13}C NMR (400 MHz, DMSO): 20.50, 41.05, 52.10, 111.60, 114.05, 124. 12, 125.01, 126.76, 133.99, 134.82, 145.14, 145.35,149.39, 155.50. MS (*m/z*): 344 (M^+); Anal. Calcd for $C_{14}H_{14}Cl_2N_2O_4$: C, 55.40; H, 4.34; Cl, 21.80; N, 8.6; Found: C, 55.14; H, 4.21; N, 9.40

4-(bis(2-chloroethyl)amino)-7,8-methyl-3-nitro-2H-chromen-2-one (NT-2e): Brown solid; R_f 0.43 (8:2 EA-hexane); mp 172-174°C; IR (KBr, cm^{-1}): 3307, 1678, 1602, 1537, 1485, 1336, 1205, 1103, 1062, 894, 775, 723, 646 cm^{-1}; ^{1}H NMR: δ_{PPM} 8.16(d, J=8.2Hz, 1H, Ar-H), 7.25(d, J=8.6Hz, 1H, Ar-H), 3.74(s, 8H, $(-CH_2CH_2-)_2$), 2.36(s, $-CH_3$), 2.26(s, $-CH_3$). ^{13}C NMR (400 MHz, DMSO): 11.45, 19.93, 41.06, 52.12, 111. 66, 111.96, 115.85, 121.29, 124.39, 125.02, 125.72, 127.05, 143.52, 145. 06, 146.08, 149.29, 155.39. MS (*m/z*): 358 (M^+); Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_4$: C, 56.65; H, 4.75; Cl, 20.90; N, 8.26; Found: C, 56.12; H, 4.54; N, 9.12.

4-(bis(2-chloroethyl)amino)-6,7-methyl-3-nitro-2H-chromen-2-one (NT-2f): Brown solid; R_f 0.42 (8:2 EA-hexane); mp 174-176°C; IR (KBr, cm^{-1}): 3305, 1724, 1678, 1514, 1427, 1311, 1203, 1101, 1058, 896, 819, 788, 723, 644 cm^{-1}; ^{1}H NMR: δ_{PPM} 8.165(s, 1H, Ar-H), 7.193(s, 1H, Ar-H), 2.322(s, 3H, $-CH_3$), 2.287(s, 3H, $-CH_3$), 3.745(s, 8H, $(-CH_2CH_2-)_2$). ^{13}C NMR (400 MHz, DMSO): 18.96, 19.57, 41.05, 52.10, 111.62, 116.01, 117.49, 124.25, 124.99, 126.87, 133.21, 144.14, 145.57, 149.59, 155.56. MS (*m/z*): 358 (M^+); Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_4$: C, 56.65; H, 4.75; Cl, 20.90; N, 8.26; Found: C, 56.17; H, 4.59; N, 9.19.

4-(bis(2-chloroethyl)amino)-5,7-dimethyl-3-nitro-2H-chromen-2-one (NT-2g): Brown solid; R_f 0.40 (8:2 EA-hexane); mp 194-196°C; IR (KBr, cm^{-1}): 3304, 1681, 1602, 1514, 1450, 1315, 1205, 1104, 1060, 868, 788, 723, 644 cm^{-1}; MS (*m/z)*: 358 (M^+); Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_4$: C, 56.65; H, 4.75; Cl, 20.90; N, 8.26; Found: C, 56.07; H, 4.69; N, 9.49.

4-(bis(2-chloroethyl)amino)-5,8-dimethyl-3-nitro-2H-chromen-2-one(NT-2h): Brown solid; R_f 0.42 (8:2 EA-hexane); mp 187-189°C; IR (KBr, cm^{-1}): 3305, 1724, 1678, 1536, 1483, 1337, 1205, 1103, 1062, 894, 775, 723, 646 cm^{-1}; MS (*m/z)*: 358 (M^+); Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_4$: C, 56.65; H, 4.75; Cl, 20.90; N, 8.26;Found: C, 56.02; H, 4.37; N, 9.53.

4-(bis(2-chloroethyl)amino)-6-bromo-3-nitro-2H-chromen-2-one (NT-2i): Brown solid; R_f 0.39 (8:2 EA-hexane); mp 190-192°C; IR (KBr, cm^{-1}) : 3306, 1723, 1604, 1536, 1483, 1335, 1206, 1104, 1062, 894, 776, 723, 646 cm^{-1}; MS (*m/z*): 407 (M^+); Anal. Calcd $C_{13}H_{11}BrCl_2N_2O_4$: C, 43.11; H, 2.84; Br, 20.49; Cl, 18.18; N, 7.18; Found: C, 45.34; H, 3.29; N, 8.18.

4-(bis(2-chloroethyl)amino)-6-fluoro-3-nitro-2H-chromen-2-one (NT-2j): Brown solid; R_f 0.39 (8:2 EA-hexane); mp 212-214°C; IR (KBr, cm^{-1}): 3303, 1723, 1604, 1514, 1427, 1312, 1203, 1102, 1058, 896, 819, 785, 723, 645 cm^{-1}; MS (*m/z*): 348 (M^+); Anal. Calcd $C_{13}H_{11}Cl_2FN_2O_4$: C, 51.09; H, 3.37; Cl, 21.54; F, 5.77; N, 8.51;Found: C, 50.10; H, 3.80; N, 6.20.

4-(bis(2-chloroethyl)amino)-6-chloro-3-nitro-2H-chromen-2-one (NT -2k):

Brown solid; R_f 0.41 (8:2 EA-hexane); mp 217-219°C; IR (KBr, cm^{-1}): 3303, 1722, 1676, 1517, 1429, 1314, 1205, 1104, 1058, 896, 820, 784, 723, 645 cm^{-1}; MS (*m/z*): 363 (M^+); Anal. Calcd $C_{13}H_{11}Cl_3N_2O_4$: C, 48.65; H, 3.21; Cl, 30.77; N, 8.11;Found: C, 49.27; H, 3.40; N, 9.11.

4-(bis(2-chloroethyl)amino)-8-chloro-3-nitro-2H-chromen-2-one (NT-2l):

Brown solid; R_f 0.41 (8:2 EA-hexane); mp 217-219°C; IR (KBr, cm^{-1}): 3303, 1722, 1676, 1517, 1429, 1314, 1205, 1104, 1058, 896, 820, 784, 723, 645 cm^{-1}; MS

(*m/z*): 363 (M^+); Anal. Calcd $C_{13}H_{11}Cl_3N_2O_4$: C, 48.65; H, 3.21; Cl, 30.77; N, 8.11;Found: C, 49.27; H, 3.40; N, 9.11.

Biological Activity

Principle : Anticancer sensitivity testing

The sulforhodamine B (SRB) assay is used for cell density determination, based on the measurement of cellular protein content. The method described here has been optimized for the toxicity screening of compounds to adherent cells in a 96-well format. After an incubation period, cell monolayers are fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min, after which the excess dye is removed by washing repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for OD determination at 510 nm using a microplate reader. The results are linear over a 20-fold range of cell numbers and the sensitivity is comparable to those of fluorometric methods. The method not only allows a large number of samples to be tested within a few days, but also requires only simple equipment and inexpensive reagents. The SRB assay is therefore an efficient and highly cost-effective method for screening.

	Drug concentrations (µg/ml) calculated from graph		
PC-3	**LC_{50}**	**TGI**	**GI_{50}**
NT-2a	>100	>100	>100
NT -2b	>100	>100	>100
NT -2c	>100	>100	>100
NT -2d	>100	>100	98.26102
NT -2e	>100	>100	97.2
NT -2f	>100	>100	87.2
NT -2g	>100	>100	90.4
ADR	61.2	19.1	<10

Cytotoxicity Data of synthesized compounds

Adriamycin is taken as standard for this experiment

	Human Prostate Cancer Cell Line PC3															
	% Control Growth															
	Drug Concentrations (µg/ml)															
Compound code	**Experiment 1**				**Experiment 2**				**Experiment 3**				**Average Values**			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
NT-2a	100.0	100.0	100.0	57.8	100.0	100.0	100.0	63.5	100.0	100.0	96.3	61.1	100.0	100.0	98.8	60.8
NT -2b	100.0	100.0	100.0	64.3	100.0	100.0	97.4	66.1	100.0	100.0	95.4	72.9	100.0	100.0	97.6	67.8
NT -2c	100.0	100.0	98.0	60.4	100.0	100.0	90.5	57.7	100.0	100.0	87.3	77.1	100.0	100.0	91.9	65.1
NT -2d	100.0	100.0	99.6	52.7	100.0	100.0	97.0	53.5	100.0	100.0	90.6	54.2	100.0	100.0	95.7	53.5
NT -2e	100.0	100.0	100.0	52.5	100.0	100.0	95.2	47.7	100.0	100.0	100.0	55.5	100.0	100.0	98.4	51.9
NT -2f	100.0	100.0	100.0	39.9	100.0	100.0	100.0	43.3	100.0	100.0	100.0	50.5	100.0	100.0	100.0	44.6
NT -2g	100.0	100.0	100.0	40.7	100.0	100.0	100.0	50.1	100.0	100.0	100.0	50.1	100.0	100.0	100.0	47.0
ADR	-30.8	-42.4	-44.9	-48.4	-31.3	-39.3	-45.5	-46.3	-42.9	-43.2	-46.7	-50.1	-35.0	-41.7	-45.7	-48.3

IV. CONCLUSION

We have serve number of novel nitrogen mustard containing functionalized coumarin as potent antitumor agents using novel synthetic approaches in high yield and purity. The reaction of diethanol amine and various 4-chloro 3-nitro coumarins was carried out by simply in IPA and excess diethanol amine as a base. Latter on chlorination of 4-chloro-3-nitro-2H-chromen-2-one result in 4-(bis(2-chloroethyl)amino)-3-nitro-2H-chromen-2-one derivatives. The formation of coumarin N-mustards by this method was first developed by us. All the synthesized compounds were evaluated for their anticancer activity. The investigation of anticancer screening data revealed that the compounds show moderate activity at higher concentration. All synthesized compounds were obtained in good to moderate yield. All synthesized compounds were characterized by IR, NMR and Mass spectrometry.

V. REFERENCES

[1]. Kramer, B. S., Gohagan, J. K. & Prorok, P. C. Cancer screening: theory and practice. (CRC Press, 1999).

[2]. Rink, S. M. et al. F THE CHEMICAL. 1993,115, 2551–2557

[3]. Kohn, K. W., Hartley, J. A. & Mattes, W. B. Mechanisms of DNA sequence selective alkylation of guanine-N7 positions by nitrogen mustards. Nucleic Acids Res. 1987,15, 10531–10549

[4]. Millard, J. T., Raucher, S. & Hopkins, P. B. Mechlorethamine Cross-Links Deoxy guanosine Residues. J. Am. Chem. Soc. 1990,2459–2460

[5]. Vogel Gilbert's ann. Phys.,1820,64,161.

[6]. (a).M.Stahmann, T. Wolff, K. Link, J. Am.Chem.Soc.,1943,65,2285.(b).E.Renk. W. Stoll, Prog.Drug.Res.,1968,14,226.(c).F.Kazmier,Mayo Clinic. Proc.,1974,49,918.(d).S.Moran, Crop. Protect.,2001,20,529.

[7]. J.Hinmann, H. Hoeksema, E. Carbon, W. Jackson, J. Am. Chem.Soc.,1956,78,1072.

[8]. A. Bourinbaiar, X. Tan, R. Nagomy, Acta Virol.,1993,37,241.

[9]. I.Kostava, Curr. Med. Chem.- Anti Cancer Agents, 2005,5,29.

[10]. P. Laurin, M. Klich, C. Dupis-Hamelin, P. Mauvais, P. Lassaigne, A. Bonnefoy, B.Musicki, Bioorg. Med.Chem. Lett.,1999,9,2079.

[11]. V. Savelev, N. Pryanishinikova, O. Artamonova, I. Fenida, V. Zagorevskii, Khimiko FarmasevticheskiiZhurnal, 1975,9(6),10,18.

[12]. A. Ruwet, C. Draguet, M.Renson, Bull.Soc.Chem.Belg.,1970,79,639.

[13]. V. Trapov, E. Perfanov, L. Smirnov, Khim.-Farm. Zh.,1996,30,20.

[14]. G. Raskob, P. Comp, G. Pineo, R. Hull, In Anticoagulant: Physiologic, Pathologic band Pharmacologic, D. Green, Ed.6, Eds.;CRC Press, Boca Raton,1994,231.

[15]. S. Pillai, S. Menon, L. Mitscher, C. Pillai, D. Shankel, J. Nat. Prod.,1999,62,1358.

[16]. W.Gao, W. Hou, M. Chang, Y. Cui, Y. Li, X. wang, L. Tang and S. Sun; Farming Zhuanli Shenqing Gongkai Shuomingshu, CN101220016,2008.