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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and evaluation of aminobenzophenone derivatives containing nitrogen mustard moiety as potential central nervous system antitumor agent

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Abstract A series of novel substituted aminobenzophenone derivatives containing nitrogen mustard moiety **(5a–f)** were synthesized and characterized on the basis of their IR, ^{1}H NMR, ^{13}C NMR, CHN, and mass spectral data. All the compounds when evaluated for chemical 4-(4-nitrobenzyl) pyridine alkylating activity proved to be active alkylating agents. All the synthesized compounds were subjected to physicochemical parameters determination required for central nervous system (CNS) activity through computational, online software, and QikProp 3.2. The log *P* values and other in silico ADME physicochemical descriptors analyzed lay between the ranges those are required for good BBB penetration. The in vitro antiproliferative activity against human cancer cell lines viz. A 549 (lung), COLO 205 (colon), U 87 (glioblastoma), and IMR-32 (neuroblastoma) was investigated. Most of the test compounds showed potent antitumor activity, especially compound **(5f)** which displayed the highest activity against CNS cancer cell line comparable to that of chlorambucil and docetaxel. The preliminary structure–activity relationship (SAR) revealed that 5-chloroaminobenzophenone-mustard series (**5a–c**) exhibited better antitumor activity than 5-nitroaminobenzophenone-mustard series **(5d–f)**.

Design, synthesis and evaluation of aminobenzophenone derivatives containing nitrogen mustard moiety as potential central nervous system antitumor agent.

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Introduction

Cancer is considered as the most serious health problem all over the world. Of all the cancer, Brain cancers are the second leading cause of cancer-related deaths in males while the fifth leading cause of cancer-related deaths in women. It accounts for 85–90 % of all primary central nervous system (CNS) tumors. Approximately, 42 % of all brain and CNS tumors occurred in males and 58 % in females (Dolecek *et al*., 2012).

Nitrogen mustard classes of drug such as chlorambucil and mechlorethamine, bifunctional alkylators are still used extensively in the treatment of leukemia and solid tumors. The main demerits of mustard drug are their high reactivity so toxic to normal peripheral tissue and hydrophilicity which does not allow the molecule to cross blood–brain barrier (BBB) and, therefore, make these drugs not of much clinical significance to patients suffering from brain tumor (Francisco *et al.,* 2008). Various attempts have been made to overcome the limited access of nitrogen mustard into the brain such as synthesizing their lipophilic analogs (Genka *et al*. 1993) or by linking the active anticancer moiety to lipophilic carrier (Peng *et al*., 1975; Hirata and Driscoll, 1976; Bartzatt, 2004) and chemical drug delivery system (Bodor *et al*., 1989; El-Sherbeny *et al*., 2003). Moreover, recent study revealed that targeting N-mustard moiety by linking to various carriers having optimized physicochemical properties resulted in more potency and less peripheral cytotoxicity than the corresponding untargeted mustard (Mourelatos *et al*., 2012; Marvania *et al*., 2011;

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Scutaru *et al*., 2011; Kapuriya *et al*., 2011; Zheng *et al*., 2010; Reux *et al*., 2008; Fousteris *et al*., 2007). So, the aim of the present investigation is to improve N-mustard physicochemical properties by attaching it chemically to CNS active agent to obtain drug capable of penetrating the brain.

2-Aminobenzophenone derivatives are important compounds in organic chemistry because of their application in heterocyclic synthesis and medicines (Walsh, 1980). The 2-Aminobenzophenones have been used as starting material for the synthesis of various 1, 4-benzodiazepines having potent CNS therapeutic activity (Sternbach *et al*., 1962). It has also been used for the synthesis of peptidoaminobenzophenones, a novel class of ring open derivatives of 1, 4-benzodiazepines, evaluated for CNS activity (Hirai *et al*., 1980; Hirai *et al*., 1981). Recently, 2-aminobenzophenone derivatives were reported to exhibit antimitotic (Liou *et al*., 2002), p 38 MAP Kinase Inhibiting activity (Ottosen *et al*., 2003) and a novel class of bradykinin B1 receptor antagonists activity with good brain penetration (Su *et al*., 2008). 2-Aminobenzophenone derivatives were considered of our interest because they fulfill all the structure requirements as the benzodiazepine nucleus contains. According to SAR study, electron withdrawing substituents at ring A and B are important for the CNS activity of benzodiazepines as shown (Fig. 1).

Prompted by these claims, we assumed that incorporating these potent pharmacophores of benzodiazepine nucleus responsible for CNS action together with N-mustard moiety may result in strong anticancer agent that may pass BBB and effective against brain tumor.

In continuation of our efforts for the synthesis of CNS active therapeutic agent (Singh *et al*., 2011, 2012a, b, c, 2013), in the present work, new substituted 2-aminobenzophenones derivatives (**5a–f)** were synthesized, incorporating the N-mustard group and varying the substitution on the phenyl ring A at position 7 [Cl, NO_2] and the phenyl ring C at position 2 and 4 [Cl] to study the effect of the substitution at these positions on the antitumor activity against four cell lines.

Results and discussion

Chemistry

The target aminobenzophenone-mustard derivatives **(5a–f)** were synthesized as shown in Scheme 1. The starting compounds **(3a–f)** were prepared according to the previous reported methods (Sternbach *et al*., 1963). Treatment of **(3a–f)** with chloro/bromoacetylchloride in toluene under conventional and microwave irradiation, gave products **(4a–f).** Microwave method was found to give better yield in less time. For the representative compound **4a,** the characteristic IR spectra showed the disappearance of band of $–NH_2$ at 3,400 and 3,340 cm^{-1} and appearance of single band at 3,280 for secondary amide (–NHCO). The appearance of significant band at 1,646 cm^{-1} assigned to carbonyl of –NHCO further indicates the formation of **4a**. In ^{1}HNMR spectra, the appearance of ($COCH_2Cl$) protons as singlet at δ 4.0 ppm and (CONH) protons exchangeable to D_2O at δ 11.0 ppm as broad singlet confirms the formation of compound **4a** Table 1.

Target compounds **(5a–f)** were synthesized by two methods to optimize the yield and the purity. In method A, **(4a–f)** was treated with diethanolamine and then chlorination by phosphorous oxychloride to get desired compounds in 50–60 % yield. In order to increase the yield by reducing steps, **(4a–f)** were directly condensed with bis (2-chloroethyl)amine hydrochloride. But, the yield was even less (25–35 %) (Table 2). This is due to high reactivity of –Cl group and hence multiple side products may have

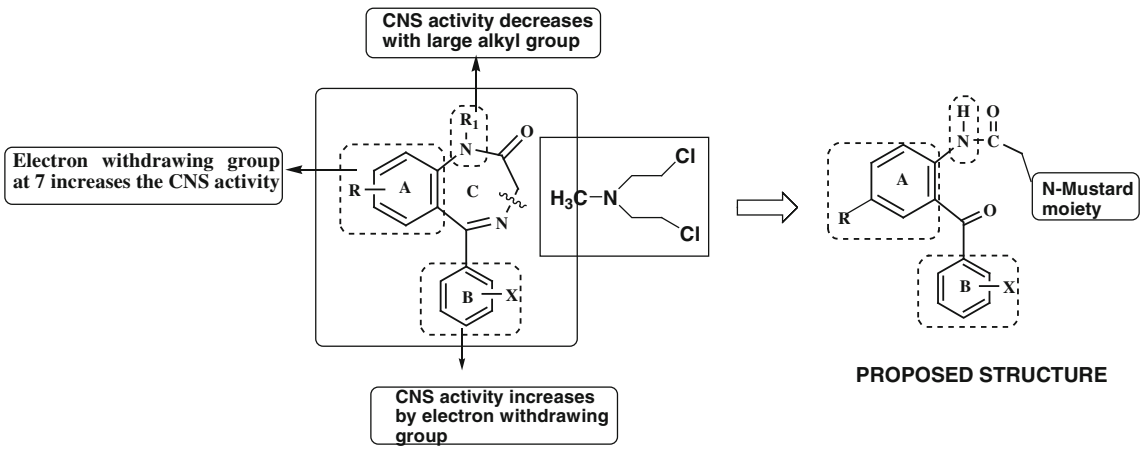
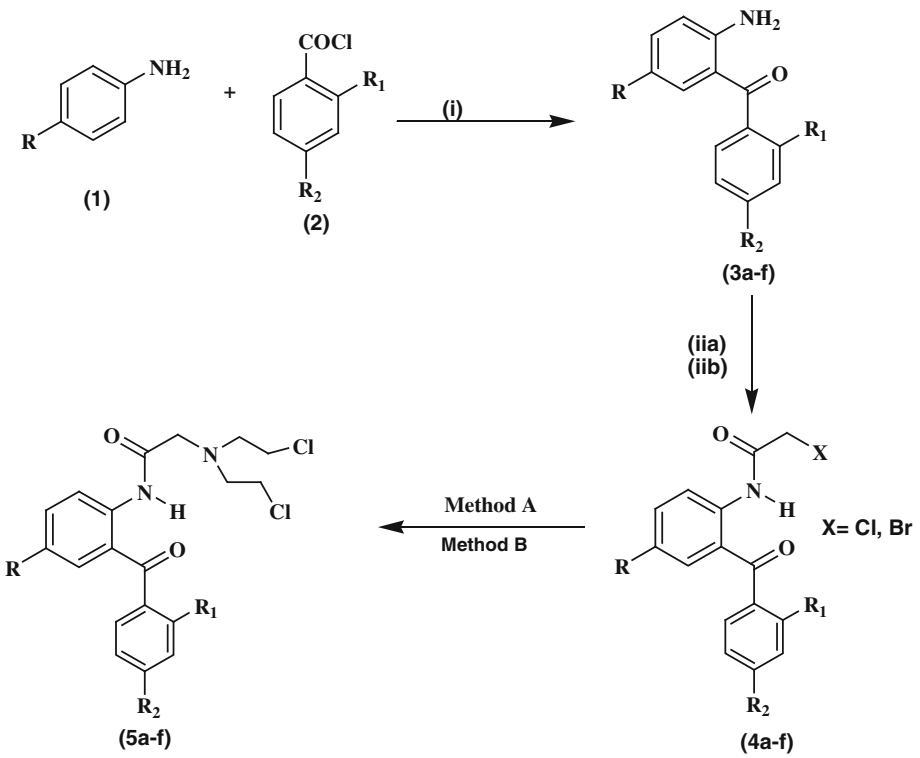


Fig. 1 Design of aminobenzophenone-mustard anticancer agents



Where

	R	R_1	R_2
5a	Cl	H	H
5b	Cl	Cl	H
5c	Cl	H	Cl
5d	NO_2	H	H
5e	NO_2	Cl	H
5f	NO_2	H	Cl

Scheme 1 Reagents and conditions (*i*) Zinc Chloride, (*iia*) Conventional, (*iib*) Microwave, $ClCH_2COCl$, $BrCH_2COBr$ (*iii*) **Method A**: Diethanolamine, DMF, NaI, $POCl_3$, **Method B**: Bis(2-chloroethylamine)hydrochloride, K_2CO_3/NaI, DMF

Table 1 Comparison of % yield and time taken by compounds **4(a–f)** from both methods

S. No.	Compounds	m.p. (°C)	Conventional		Microwave	
			Yield (%)	Time (h)	Yield (%)	Time (min)
1	4a	118–120	80	2.5	94	1.0
2	4b	132–134	67	3.0	88	2.5
3	4c	126–128	65	4.0	85	3
4	4d	154–156	66	3.5	85	2.0
5	4e	146–148	70	2.5	82	1.5
6	4f	156–160	63	3	78	2.5

Table 2 Comparison of % yield by methods A and B for the synthesis of compounds **(5a–f)**

S. No.	Compounds	m.p.	Method A % Yield	Method B %Yield
1.	5a	Oil	52	25
2.	5b	Oil	54	29
3.	5c	Oil	52	28
4.	5d	Oil	58	32
5.	5e	Oil	60	35
6.	5f	Oil	57	32

formed. For the series of aminobenzophenone-mustard derivatives **(5a–f),** the ^1H NMR spectra of these compounds shows the ethylene bridge yielding two triplets integrating for four protons each. One triplet appeared at δ 3.00–3.75 ppm which was assigned to $–CH_2$ group adjacent to nitrogen. The other triplet at δ 3.5–4.6 ppm was assigned to the $–CH_2–$ group adjacent to the –Cl group which is more deshielded due to the electronegativity effect of chlorine.

NBP alkylating activity assessment

The final compounds were evaluated by its alkylating activity using 4-(4-nitrobenzyl) pyridine (NBP) as an analytical reagent. It is hypothesized that there is correlation between the chemical alkylating activity and anti-tumor

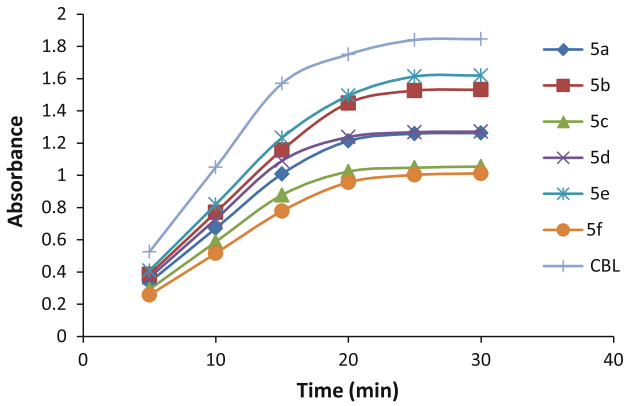


Fig. 2 Formation of the aminobenzophenone-mustard **(5a–f)**–NBP adduct in acetone/ethanol medium; variation in absorbance (λ_{max} 542–560 nm) with time; compared with chlorambucil

Table 3 The alkylating activity (NBP assay) of the synthesized compounds **(5a–f)**

Compounds	R	R_1	R_2	λ_{max}	Temp (°C)	K $min^{-1} \times 10^{-2}$	$t_{1/2}$ (min)
5a	Cl	H	H	542	65	5.21 ± 0.156	13.3
5b	Cl	Cl	H	545	65	6.72 ± 0.156	10.3
5c	Cl	H	Cl	545	65	7.78 ± 0.058	8.9
5d	NO_2	H	H	554	65	5.87 ± 0.175	11.8
5e	NO_2	Cl	H	558	65	7.29 ± 0.127	9.5
5f	NO_2	H	Cl	558	65	8025 ± 0.089	8.4
Chlorambucil				560	85	10.65 ± 0.122	6.5

activity (Gomez-Bombrelli *et al*., 2012). The reaction was determined at 65 °C in 50 % aqueous acetone using spectrophotometric quantitation. The 4-(4-nitrobenzyl) pyridine reacts with alkylating agents **(5a–f)** to form aminobenzophenone-mustard-NBP adduct which gives a purple color upon basification. The intensity of the produced color measured by UV spectrophotometer as absorbance is directly proportional to the degree of alkylation (Fig. 2). This method differentiates between the reactivities of the synthesized compounds **(5a–f)** under specified conditions using chlorambucil as positive control. All the compounds proved to be active alkylating agents (Table 3).

In silico physicochemical evaluation

Target compounds were designed to be CNS active; hence, the parameters were selected which affect BBB. Lipophilicity (logP), hydrophilicity (logS), and logBB were the principle descriptors to be identified as important for CNS penetration. Table 4 presents the partition coefficient values for synthesized derivative **(5a–f)** by computational, online software, and by QikProp. All the values are within required range indicating optimum lipophilicity. In addition, a compound possessing tertiary nitrogen (a feature of many CNS drugs) shows a higher degree of brain permeation (Pajouhesh and Lenz, 2005). All the compounds **(5a–f)** have tertiary nitrogen in their structure and hence indicate BBB permeability of the synthesized compounds.

Other physicochemical descriptors obtained by QikProp are presented in Table 5 support the clinical potential of these synthesized compounds. The physically significant descriptors of mechlorethamine, chlorambucil, and aminobenzophenone-mustard derivatives **(5a–f)** were analyzed for the CNS activity and drug-like properties. Polar Surface Area (PSA) has become an important molecular descriptor for drug absorption and blood–brain barrier penetration (Clark, 1999, Kelder *et al*., 1999). The values of PSA for all synthesized compounds **(5a–f)** are within the range that indicates good penetration of the blood–brain barrier.

QPPMDCK predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood-brain barrier (Wang *et al.,*2005). Higher the value of MDCK cell, higher the cell permeability. All the target compounds **(5a–f)** have QPPMDCK values within the range and hence good BBB permeation.

Table 4 Various partition coefficient parameters obtained by various tools important for CNS activity

S.No.	Compounds	miLogP^a < 5	CSLogP^b < 5	ClogP^c < 5	QP log$P$ o/w^d (−2.0–6.5)	QP logS^d (−6.5–0.5)	QP logBB^d (−3 to 1.2)
1.	5a	4.786	4.56	5.15	4.355	−3.823	0.522
2.	5b	5.416	4.84	5.66	4.637	−4.173	0.502
3.	5c	5.464	4.9	5.91	4.876	−4.690	0.658
3.	5d	4.067	3.841	4.35	3.253	−3.200	−0.663
4.	5e	4.697	4.181	4.83	3.400	−3.400	−0.643
5.	5f	4.745	4.481	5.09	3.580	−3.900	−0.494

[a] Calculated by method of Molinspiration
www.molinspiration.com/cgi-bin/properties

[b] Calculated by method of Chemsilico
www.chemsilico.com/cs_products/products.html

[c] Calculated by ChemDraw Ultra 8.0

[d] Calculated by QikProp

Table 5 In silico Pharmacokinetic parameters with their optimum range important for CNS activity and oral bioavailability obtained by QikProp tool

Descriptors	MCE[a]	CBL[b]	5a	5b	5c	5d	5e	5f
CNS (−2 to +2)	2	−1	2	2	2	−1	−1	−1
M.WT < 500	156.055	304.216	413.73	448.175	448.175	424.283	458.728	458.728
PSA (7.0–200)	5.6	55.2	59.28	59.0	59.26	103.85	105.8	107.2
Donar HB (0–6)	0	1	0	0	0	1	1	1
Accept HB (2.0–20)	2	3	5.5	5.5	5.5	6	6	6
QP logP o/w (−2.0–6.5)	1.967	4.671	4.355	4.637	4.876	3.253	3.40	3.580
Rot bond(0–15)	2	7	7	7	7	8	8	8
QP log S (−6.5–0.5)	−1.122	−5.347	−3.823	−4.173	−4.69	−3.20	−3.40	−3.90
QPPCaco < 25 poor > 500 good	2300.154	227.915	754.447	651.918	754.395	188.64	216.85	473.90
QP logBB (−3.0–1.2)	1.173	−0.583	0.522	0.502	0.658	−0.663	−0.643	−0.494
QPPMDCK < 25 poor > 500 good	8513.882	802.855	4233.643	5201.516	10000	188.6	216.8	474.0
Metablsm (1–8)	1	3	2	2	2	3	3	3
% Hu Ab > 80% high < 25% poor	100	96.495	100	100	100	81.2	83.2	96.95
Nand O < 2–15>	1	3	4	4	4	7	7	7
Violation of rule of five (max 4)	0	0	0	0	0	0	0	0
Violation of rule of three (max 3)	0	0	0	0	0	0	0	0

[a] Mechlorethamine

[b] Chlorambucil

In accordance with Lipkinski's rule of 5 (Lipinski *et al*., 1997), the molecular weights of compounds should have ≤500 Daltons, ≤5 hydrogen bond donors, ≤10 hydrogen bond acceptors, log P of ≤5 and rotatable bond ≤10. Compounds that satisfy these rules are considered drug-like. According to this rule, compounds with number of violations not more than 1 shows good bioavailability. All the synthesized compounds **(5a–f)** have no violation of rule of five. Compounds with H-bond donor less than 5 and H-bond acceptors less than 10 shows good BBB penetration (Osterberg and Norinder, 2000). All the synthesized compounds have H-bond donor values in the range of 0–1 which are less than 5 and H-bond acceptors value in the range of 5–6 which are less than 10. Compounds with molecular weight less than 500 Daltons shows significant passive lipid-mediated transport through BBB (Leeson and Davis, 2004). All the test compounds have molecular weight in the range of 413–458 which is <500. According to Jorgensen's rule of three (Jorgensen, 2009), there should be QPlogS >−5.7, QPPCaco >22 nm/s, # primary metabolites <7. In accordance with the data obtained from Table 5, all the compounds have no violation of Jorgensen's rule of three and hence potential to be CNS active.

In vitro anticancer screening

The newly synthesized compounds were evaluated for their in vitro cytotoxic activity against human cancer cell lines: A 549 (lung), COLO 205 (colon), U 87 (primary glioblastoma cells), and IMR-32 (neuroblastoma cell lines) using chlorambucil and

Table 6 The antiproliferative activity data of synthetic compounds **(5a–f)** assessed by the MTT reduction assay

Compounds		IC_{50} Values ± S.D.					
	R	R_1	R_2	A549	COLO 205	U87	IMR32
5a	Cl	H	H	37.093 ± 1.63	52.283 ± 2.09	29.013 ± 1.15	29.393 ± 2.03
5b	Cl	Cl	H	34.634 ± 1.92	32.573 ± 2.70	24.53 ± 1.89	30.816 ± 3.15
5c	Cl	H	Cl	37.336 ± 1.94	28.683 ± 2.02	27.134 ± 2.33	31.134 ± 2.96
5d	NO_2	H	H	64.806 ± 2.33	32.896 ± 2.87	29.464 ± 1.71	33.2 ± 2.27
5e	NO_2	Cl	H	64.806 ± 2.33	33.38 ± 2.19	43.744 ± 2.47	31.396 ± 1.50
5f	NO_2	H	Cl	36.673 ± 2.89	38.93 ± 1.46	23.267 ± 2.29	28.394 ± 1.67
STD DOCETAXEL				27.256 ± 1.75	30.706 ± 1.72	20.72 ± 1.19	23.71 ± 1.73
STD CHLORAMBUCIL				21.09 ± 1.72	26.67 ± 1.09	18.82 ± 1.09	20.09 ± 1.03

All experiments were carried out three times, independently. The data obtained were expressed in terms of mean, SD deviation values. Wherever appropriate, the data were also subjected to unpaired two tailed student's *t* test. A value of $p < 0.05$ was considered as significant

docetaxel as the reference drugs.The parameter used here is IC_{50}, which corresponds to the concentration required for 50 % inhibition of cell viability. The IC_{50} of the synthesized compounds compared to the reference drug are shown in Table 6. From the results in Table 6, it was found that most of the test compounds showed potent antitumor activity comparable to that of chlorambucil and docetaxel. In general, 5-chloroaminobenzophenone-mustard series (**5a–f**) exhibited better antitumor activity than 5-nitroaminobenzophenone-mustard series **(5d–f)**. Careful examination of the effect of –Cl group on ring B at *o* or *p* position showed that the presence of chlorine at these positions does not make much difference in activity of the compounds. Compound **5f** was the most active compound in this study against both CNS cancer cell lines, while, compound **5b** and 5**c** were the most active compounds against A549 and COLO 205 cell lines, respectively. It is also worthwhile mention that compounds **5a** and **5e** were not much active against A549 and COLO 205 cell lines, respectively.

Conclusion

In conclusion, a series of novel substituted 2-aminobenzophenone derivatives incorporating nitrogen mustard as cytotoxic moiety were synthesized and were subjected to physicochemical parameters determination for BBB penetration through online software. The in vitro NBP alkylation studies revealed that all compounds have active alkylating activity. Furthermore, a computational study was carried out for prediction of in silico physicochemical properties required for CNS activity and drug-likeliness. All pharmacokinetic descriptors were within the range set by Lipinski's rule of five and Jorgensen's rule of three and possessing favorable physicochemical properties for acting as CNS drugs, making them potentially promising agents for CNS tumor. The invitro antiproliferative activity of the newly synthesized compounds against human A 549, COLO 205, U 87, and IMR-32 was investigated. Most of the test compounds showed potent antitumor activity, especially compound **(5f)** which displayed the highest activity against CNS cancer cell line comparable to that of chlorambucil and docetaxel. Further studies are still needed to determine the invivo studies of the compounds as well as to explore the SAR of the other positions of the benzene ring.

Experimental

The melting points were determined on Veego-programmable melting point apparatus (microprocessor-based) and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained using Bruker Avance-II, 400 MHZ spectrometer. Infrared (IR) spectra were recorded on Perkin Elmer model 1600 FT-IR RX-I spectrometer as KBR disks. The ultraviolet spectra were recorded on Shimadzu, UV-1800 spectrophotometer. The TOF–MS ES^{+} spectra of the compounds were recorded on Waters micromass Q-TOF Mass spectrometer. Elemental analyses for C, H, and N were performed on Thermo-flash EA-1112 CHNS-O analyzer. The synthesis related to microwave irradiation were carried out in domestic LG little chef microwave oven. The Chromatography was carried out using Merck silica gel 60 (230–400 mesh). Anhydrous sodium sulfate was utilized as drying agents. A computational study of titled compounds was performed for prediction of ADME properties by QikProp 3.2 tool available in Schrödinger 9.0 version and Molinspiration online property calculation toolkit. Bis(2-chloroethylamine)hydrochloride was prepared by thionyl chloride-assisted chlorination of diethanolamine by reported procedure (Friedman and Seligman, 1954). The syntheses of substituted 2-aminobenzophenones **(3a–f)** were carried out by the literature method (Sternbach *et al*., 1962).

General method for the syntheses of substituted 2-(chloroacetamido)benzophenones (4a–f)

Conventional method

Compounds **(4a–f)** were prepared as per the published procedure (Sternbach *et al*., 1961, 1963) that involves treating substituted 2-aminobenzophenones **(3a–f)** (25 mmol) with chloroacetyl chloride/bromoacetyl bromide (50 mmol) in toluene (50 ml) and refluxing for 2 h to expel most of the formed hydrogen chloride. The solution was then cooled, washed with ice cold dilute aqueous ammonia solution, dried, filtered and concentrated in vacuo. The residue was recrystallized from alcohol to get pure substituted 2-(chloro/bromoacetamido)benzophenone **(4a–f)**.

Microwave irradiation method

A solution of substituted 2-aminobenzophenones (2.5 mmol) and chloroacetyl chloride/bromoacetyl bromide (5.0 mmol) in toluene (5 ml) was irradiated for 1–3 min in microwave oven (360 W). The solution was then cooled, washed with ice cold dilute aqueous ammonia solution, dried with anhydrous sodium sulfate, filtered and concentrated in vacuo. The recrystallization of the crude residue from alcohol afforded substituted 2-(chloro/bromoacetamido)benzophenones **(4a–f)**.

2-(chloroacetamido)-5-chlorobenzophenone (4a)

Anal

IR KBr (cm^{-1}) 3,280 (N–H), 3,059, 2,961 (C–H), 1,692 (C=O), 1,646 (NHC=O), 1,514 (C=C), 1,286 (C–N) and

752 (C–Cl); **^{1}H-NMR ($CDCl_3$)** δ (ppm): 4.19 (s, 2H, -COC*H*$_2$N), 7.50–7.70 (m, 8H, Ar*H*), δ 11.4 (brs, 1H, exchangeable, -N*H*COCH$_2$-); **^{13}C-NMR ($CDCl_3$)** δ (ppm): 43.3 (C–Cl), 122.4, 125.2, 128.4, 129.6, 130.0, 130.6, 132.8, 132.0, 139.2, 141.0 (Aromatic) 168.0 (NHC=O), 192.8 (C=O); Anal. calcd for $C_{15}H_{11}NO_2Cl_2$: C % 58.46, H % 3.60, N % 4.55; Found: C % 58.36, H % 3.72, N % 4.42.

2-(chloroacetamido)-5, 2′-dichlorobenzophenone (4b)

IR KBr (cm^{-1}) 3,284 (N–H), 3,065, 2,958 (C–H), 1,689 (C=O), 1,652 (NHC=O), 1,518 (C=C), 1,290 (C–N) and 757 (C–Cl); **^{1}H-NMR ($CDCl_3$)** δ (ppm): 3.8 (s, 2H, -COC*H*$_2$N), 7.40–7.60(m, 7H, Ar*H*), 11.3 (brs, 1H, exchangeable, -N*H*COCH$_2$-); **^{13}C-NMR ($CDCl_3$)** δ (ppm): 43.0 (C–Cl), 122.5, 125.7, 126.0, 128.8, 130.0, 131.2, 132.4, 133.8, 135.8, 136.6 (Aromatic), 164.0 (NHC=O), 198.8 (C=O); Anal. calcd for $C_{15}H_{10}NO_2Cl_3$: C % 52.59, H % 2.94, N % 4.09; Found: C % 52.36, H % 2.86, N % 4.16.

2-(chloroacetamido)-5,4′-dichlorobenzophenone (4c)

IR KBr (cm^{-1}) 3,283 (N–H), 3,065, 2,956 (C–H), 1,690 (C=O), 1,648(NHC=O), 1,514 (C = C), 1,288 (C–N) and 755 (C–Cl); **^{1}H-NMR ($CDCl_3$)** δ (ppm): 3.9 (s, 2H, -COC*H*$_2$N), 7.35–7.60 (m, 7H, Ar*H*), 11.2 (brs, 1H, exchangeable, -N*H*COCH$_2$–); **^{13}C-NMR ($CDCl_3$)** δ (ppm): 42.3 (C–Cl), 122.4, 125.9, 127.4, 129.3, 130.0, 131.2, 131.8, 133.6, 136.8, 138.0, 142.2 (Aromatic), 168.3 (NHC=O), 194.8 (C=O); Anal. calcd for $C_{15}H_{10}NO_2Cl_3$: C % 52.59, H % 2.94, N % 4.09; Found: C % 52.48, H % 2.88, N % 4.04.

2-(bromoacetamido)-5-nitrobenzophenone (4d)

IR KBr (cm^{-1}) 3,278 (N–H), 3,063, 2,968 (C–H), 1,692 (C=O), 1,655 (NHC=O), 1,518 (NO_2), 1,514 (C=C), 1,288 (C–N) and 624 (C–Br); **^{1}H-NMR ($CDCl_3$)** δ (ppm): 4.2 (s, 2H, –COC*H*$_2$N), 7.40–7.60(m, 8H, Ar*H*), 9.80 (brs, 1H, exchangeable, –N*H*COCH$_2$–); **^{13}C-NMR ($CDCl_3$)** δ (ppm): 30.6 (C–Br), 122.4, 125.4, 126.4, 127.8, 128.5, 130.6, 132.8, 140.0, 142.8, 148.0 (Aromatic), 162.0 (NHC=O), 195.8 (C=O); Anal. calcd for $C_{15}H_{11}N_2O_4Br$: C % 49.61, H % 3.05, N % 7.71; Found: C % 49.68, H % 3.15, N % 7.64.

2-(bromoacetamido)-2′-chloro-5-nitrobenzophenone (4e)

IR KBr (cm^{-1}) 3,282 (N–H), 3,074, 2,973 (C–H), 1,694 (C=O), 1,648 (NHC=O), 1,522 (NO_2), 1,522 (C=C), 1,270 (C–N) and 628 (C–Br); **^{1}H-NMR ($CDCl_3$)** δ (ppm): 4.0 (s, 2H, –COC*H*$_2$N), δ 7.17–7.55(m, 7H, Ar*H*), 9.67 (brs, 1H, exchangeable, –N*H*COCH$_2$–); **^{13}C-NMR ($CDCl_3$)** δ (ppm): 31.4 (C–Br), 122.5, 125.3, 125.8, 126.8, 127.2, 128.2, 131.4, 133.8, 136.8, 138.6, 144.3, 148.6 (Aromatic), 164.8 (NHC=O), 197.8 (C=O); Anal. calcd for $C_{15}H_{10}N_2O_4BrCl$: C % 45.31, H % 2.54, N % 7.05; Found: C % 45.38, H % 2.62, N % 7.12.

2-(bromoacetamido)-4′-chloro-5-nitrobenzophenone (4f)

IR KBr (cm^{-1}) 3,277 (N–H), 3,064, 2,970 (C–H), 1,692 (C=O), 1,650 (NHC=O), 1,520 (NO_2), 1,517 (C=C), 1,284 (C–N) and 630 (C–Br); **^{1}H-NMR ($CDCl_3$)** δ (ppm): 4.1 (s, 2H, –COC*H*$_2$N), 7.15–7.65 (m, 7H, Ar*H*), 11.3 (brs, 1H, exchangeable, –N*H*COCH$_2$–); **^{13}C-NMR ($CDCl_3$)** δ (ppm): 30.2 (C–Br), 122.5, 125.5, 126.0, 126.8, 128.8, 131.8, 136.8, 138.8, 144.6, 148.0 (Aromatic), 16.6 (NHC=O), 192.8 (C=O); Anal. calcd for $C_{15}H_{10}N_2O_4BrCl$: C % 45.31, H % 2.54, N % 7.05; Found: C % 45.36, H % 2.60, N % 7.16.

General method for the syntheses of 2-{N,N-bis(2′-chloroethyl)}aminoacetamido-benzophenone derivatives (5a–f)

Method A A mixture of substituted 2-(bromo/chloro acetamido) benzophenone derivatives **(4a–f)** (10 mmol), freshly distilled diethanolamine (30 mmol), anhydrous sodium iodide (30 mmol) in dry DMF was heated for 20 h at 120–130 °C on oil-bath. The reaction mixture was treated with saturated aqueous sodium bicarbonate solution. The solution was extracted with ethyl acetate, washed with brine and dried to get dihydroxy compound as oil which without further purification was treated with excess of phosphorous oxychloride (100 mmol) and heated under reflux for 2.5–3 h. The excess phosphorus oxychloride was evaporated to dryness in vacuo and added with stirring, to a mixture of ice/water. The mixture was extracted with ethyl acetate (3 × 50 ml) and washed with 10 % sodium bicarbonate solution. The combined organic layer was dried over anhydrous sodium sulfate, evaporated and passes through silica column using ethyl acetate to give desired final compounds **(5a–f)** as dark oil.

Method B A mixture of substituted 2-(bromo/chloro acetamido) benzophenone derivatives **(4a–f)** (10 mmol), bis(2-chloroethyl)amine hydrochloride (20 mmol), potassium carbonate (20 mmol), sodium iodide (10 mmol), and DMF was stirred for 48 h. The reaction mixture was poured into excess of water and extracted with ethyl acetate (3 × 50 ml). The recombined organic phases were dried on sodium sulfate and evaporated in vacuo, and residue

was chromatographed on silica gel using ethyl acetate to furnish compounds **(5a–f).**

2-{N,N-bis(2′-chloroethyl)}aminoacetamido-5-chlorobenzophenone (5a)

Yield = 65 %, brown oil. $\mathbf{R_f}$: 0.68 (chloroform: methanol: 9.5:0.5); **IR KBr ($\mathbf{cm^{-1}}$):** 3,336 (N–H), 3,094, 2,943 (C–H), 1,699 (C=O), 1,685 (NHC=O), 1,486 (C=C), 1,214, 1,120, 1,097 (C–N), 754 (C–Cl); **^{1}H-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 3.05 (t, 4H, J = 8.0 Hz, –N($CH_2CH_2Cl)_2$), 3.42 (s, 2H, –$COCH_2$N), 3.63 (t, 4H, J = 7.5 Hz, –N($CH_2CH_2Cl)_2$), 7.17–7.55(m, 8H, Ar*H*), 9.67 (br s, 1H, exchangeable, –N*H*$COCH_2$–); **^{13}C-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 42.8 (2xC-Cl), 56.4 (2xC-N), 58.0 (CH_2), 123.4, 125.7, 126.0, 128.4, 129.4, 130.4, 131.2, 132.8, 140.6, 142.8 (Aromatic), 174.0 (–NHC=O), 190.0 (C=O); **TOF MS ES$^+$m/z:** 437$(M + H + Na)^{+\cdot}$

2-{N,N-bis(2′-chloroethyl)}aminoacetamido-5,2′-dichlorobenzophenone (5b)

Yield = 68 %, brown oil. $\mathbf{R_f}$: 0.82 (chloroform: methanol: 9.8:0.2). **IR KBr ($\mathbf{cm^{-1}}$)**: 3,342 (N–H), 3,088, 2,940 (C–H), 1,694 (C=O), 1,680 (NHC=O), 1,476 (C=C), 1,219, 1,120, 1,086 (C–N), 756 (C–Cl); **^{1}H-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 3.20 (t, 4H, J = 7.6 Hz, –N($CH_2CH_2Cl)_2$), 3.70 (s, 2H, –$COCH_2$N), 3.90 (t, 4H, J = 7.8 Hz, –N($CH_2CH_2Cl)_2$), 7.20–7.70(m, 7H, Ar*H*), 12.0 (br s, 1H, exchangeable, –N*H*$COCH_2$–); **^{13}C–NMR ($\mathbf{CDCl_3}$)** δ (ppm): 42.5(2xC-Cl), 56.8 (2xC-N), 58.5 (CH_2), 123.2, 125.7, 126.8, 126.4, 127.9, 128.4, 129.4, 130.4, 131.2, 134.8, 140.8, 142.8 (Aromatic), 171.0 (–NHC=O), 197.0 (C=O); **TOF MS ES$^+$m/z:** 472$(M + H + Na)^{+\cdot}$

2-{N,N-bis(2′-chloroethyl)}aminoacetamido-5,4′-dichlorobenzophenone (5c)

Yield = 64 %, brown oil. $\mathbf{R_f}$: 0.91 (chloroform:methanol: 9.8:0.2). **IR KBr ($\mathbf{cm^{-1}}$):** 3,345 (N–H), 3,087, 2,950 (C–H), 1,696 (C=O), 1,683 (NHC=O), 1,470 (C=C), 1,216, 1,122, 1,090 (C–N), 754 (C–Cl); **^{1}H-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 3.02(t, 4H, J = 8.2 Hz, –N($CH_2CH_2Cl)_2$), 3.45 (s, 2H, –$COCH_2$N), 3.64 (t, 4H, J = 8.0 Hz, –N($CH_2CH_2Cl)_2$), 7.20–8.58 (m, 7H, Ar*H*), 11.27 (br s, 1H, exchangeable, –N*H*$COCH_2$–); **^{13}C-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 42.5 (2xC-Cl), 56.8 (2xC-N), 58.5 (CH_2), 123.2, 126.8, 128.4, 129.4, 130.4, 130.8, 131.2, 137.0, 138.0, 142.8 (Aromatic), 167.0 (–NHC=O), 194.0 (C=O); **TOF MS ES$^+$m/z:** 448$(M)^{+\cdot}$

2-{N,N-bis(2′-chloroethyl)}aminoacetamido-5-nitrobenzophenone (5d)

Yield = 54 %, red oil. $\mathbf{R_f}$: 0.71 (ethyl acetate). **IR KBr ($\mathbf{cm^{-1}}$):** 3,329 (N–H), 3,091, 2,952 (C–H), 1,689 (C=O), 1,671 (NHC=O), 1,524 (NO_2 assym stretch), 1,484 (C=C), 1,227, 1,123 (C–N), 759 (C–Cl); **^{1}H-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 3.75(t, 4H, J = 7.84 Hz, –N($CH_2CH_2Cl)_2$) 4.12 (s, 2H, –$COCH_2$N), 4.57 (t, 4H, J = 7.82 Hz, –N($CH_2CH_2Cl)_2$), 7.50–7.68 (m, 8H, Ar*H*), 11.26 (br s, 1H, exchangeable, –N*H*$COCH_2$–); **^{13}C-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 43.2 (2xC-Cl), 56.4 (2xC-N), 58.2 (CH_2), 122.4, 125.7, 126.8, 128.4, 130.5, 131.2, 132.8, 140.6, 142.8, 147.8 (Aromatic), 168.4 (–NHC=O), 195.0 (C=O); **TOF MS ES$^+$ m/z:** 447 $(M + Na)^{+\cdot}$

2-{N,N-bis(2′-chloroethyl)}aminoacetamido-2′-chloro-5-nitrobenzophenone (5e)

Yield = 52 %, red oil. $\mathbf{R_f}$: 0.52 (ethyl acetate). **IR KBr ($\mathbf{cm^{-1}}$):** 3,350 (N–H), 3,080, 2,967 (C–H), 1,691 (C=O), 1,672 (NHC=O), 1,526 (NO_2 assym stretch), 1,489 (C=C), 1,222, 1,124 (C–N), 758 (C–Cl); **^{1}H-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 3.75 (t, 4H, J = 8.0 Hz, –N($CH_2CH_2Cl)_2$), 4.11 (s, 2H, –$COCH_2$N), 4.55 (t, 4H, J = 8.1 Hz, –N($CH_2CH_2Cl)_2$), 7.20–7.70 (m, 7H, Ar*H*), 11.26 (br s, 1H, exchangeable, –N*H*$COCH_2$–); **^{13}C-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 42.3 (2xC-Cl), 56.4 (2xC-N), 58.3 (CH_2), 123.8, 125.7, 126.8, 127.2, 127.9, 128.4, 129.4, 130.4, 131.2, 134.8, 136.0, 152 (Aromatic), 168.0 (–NHC=O), 197.0 (C=O); **TOF MS ES$^+$ m/z:** 481 $(M + Na)^{+\cdot}$

2-{N,N-bis(2′-chloroethyl)}aminoacetamido-4′-chloro-5-nitrobenzophenone (5f)

Yield = 56 %, red oil. $\mathbf{R_f}$: 0.65 (ethyl acetate). **IR KBr ($\mathbf{cm^{-1}}$):** 3,354 (N–H), 3,016 (C–H), 1,685 (C=O), 1,677 (NHC=O), 1,521 (NO_2 assym stretch), 1,485 (C=C), 1,219, 1,124 (C–N), 754 (C–Cl); **^{1}H-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 3.75(t, 4H, J = 7.84 Hz, –N($CH_2CH_2Cl)_2$), 4.12 (s, 2H, –$COCH_2$N), 4.57 (t, 4H, J = 7.82 Hz, –N($CH_2CH_2Cl)_2$), 7.50–7.70 (m, 7H, Ar*H*), 11.38 (br s, 1H, exchangeable, -N*H*$COCH_2$-); **^{13}C-NMR ($\mathbf{CDCl_3}$)** δ (ppm): 42.4 (2xC-Cl), 55.6 (2xC-N), 57.5 (CH_2), 122.8, 125.0, 125.8, 128.6, 130.2, 131.2, 137.0, 138.0, 144, 148 (Aromatic), 167.0 (–NHC=O), 195.0 (C=O); **TOF MS ES$^+$ m/z:** 481 $(M + Na)^{+\cdot}$

NBP alkylation assay

All compounds were checked for their NBP alkylating activity by procedure mentioned before (Balazs *et al*., 1970). Chlorambucil (1.0 mg) was dissolved in 20.0 ml of 95 % EtOH. A 1.0 ml aliquot of this solution was placed in a 5.0 ml volumetric flask together with 1.0 ml of NBP solution (3.55 g/50 ml of acetone) and 1.0 ml of buffer solution (1.0 g of potassium hydrogen phthalate/100 ml of water). The flask was kept at 85 °C in a water bath for

30 min. The solution was cooled immediately for 2–3 min in an ice bath and 0.1 ml of a KOH solution (1.4 g/25 ml of 95 % EtOH) was added. The solution was diluted to 5.0 ml with 95 % EtOH and shaken. After 2 min, the absorbance from 540 to 560 nm was recorded. This procedure was used by varying the heating time to obtain the data tabulated in Table 3. The procedure was repeated thrice for compound **(5a–f).**

K values (i.e., rate constant) were calculated using the formula:

$K = (A_2 - A_1/T_2 - T_1)$ *Where A = absorbance at 542–560 nm and T = heating time.*

Since the K values are determined from initial rates in the presence of excess of NBP, they are proportional to (but not the same as) the pseudo-first-order rate constants for NBP alkylation.

Half life of compound was calculated using the formula:

$t_{1/2} = 0.693/K$

ADME prediction

ADME properties were calculated using Qikprop 2.5 tool of Schrodinger software program designed by Professor William L. Jorgensen. It evaluates the acceptability of analogs based on Lipinski's rule of five and Jorgensen's rule of three which are essential to insure drug-like pharmacokinetic profile while using rational drug design. All the analogs were neutralized before being used by Qikprop.

Antiproliferative assay in vitro

Cell culture

The following established in vitro human cancer cell lines were applied: A 549 (Human lung cancer cells), COLO 205 (Human colon cancer cells), U 87 (Human primary glioblastoma cells), and IMR-32 (Human neuroblastoma cell lines). All cell lines were obtained from NCCS Pune. All chemical and solvent were purchased from Himedia. Cell lines were maintained in desired media supplemented with 10 % inactivated fetal bovine serum, 100U/ml penicillin and 100ul/ml streptomycin incubated at 37 °C and 5 % CO_2 in humidifier incubator. After attaining 80 % confluence, cells were subcultured by trypsinization with 0.25 % trypsin solution under sterile conditions.

MTT assay

The cytotoxicity of synthesized compounds **(5a–f)** was determined by tetrazolium-based colorimetric assay in which mitochondrial activity is measured by splitting tetrazolium salts with mitochondrial dehydrogenases in viable cells only. For viability testing, 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) cell proliferation assay was carried out (Mosmann, 1983). The cells of all cell lines were plated out 24 h prior to testing in 96 well plates at a density of 3,000 cells/well in 100 µL of the medium. After overnight incubation, triplicate wells were treated with varying concentration of compounds ranging from (1–100 µg/ml) and incubated with standard chlorambucil and docetexal for 3 days. The cells were continuously exposed for a period of 72 h. After 3 days, medium was replaced with 2 µl of MTT (5 mg/ml), and cells were incubated for 3 h. The relative percentage of metabolically active cells compared with untreated controls and then determine on the basis of mitochondrial conversion of MTT to formazan crystals which were dissolved in dimethylsulfoxide (DMSO). Spectrophotometric absorbance of sample was determined by microplate reader (BIORAD) at 570–630 nm. Concentrations of sample showing 50 % reduction in cell viability (i.e., IC_{50}) were then calculated. An OD value of control cells (Unexposed cells) was taken as 100 % viability (0 % cytotoxicity).

$$\% \text{ inhibition} = \left[(\text{OD of control}) - (\text{OD of treated})/(\text{OD of control})\right] \times 100.$$

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