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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF PHENOXAZINE DERIVATIVES

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ABSTRACT

Derivatives of phenoxazines namely N^{10}-pentylsubstituted-2-chloro phenoxazines, N^{10}-hexylsubstituted-2-chloro phenoxazines and N^{10}-hexylsubstituted phenoxazines were synthesized in an effort to find more specific and less toxic anti-cancer agents. The synthesized compounds were characterized by elemental analysis, UV, IR, ^{1}H, ^{13}C-NMR and mass spectral data. The anti-proliferative property of these compounds increased significantly by increasing the chain length to $(-CH_2)_5$ or $(-CH_2)_6$ from the corresponding $(-CH_2)_3$ or $(-CH_2)_4$ at N^{10}-position of the phenoxazine ring. The anti proliferative activity of various phenoxazine derivatives against rhabdomyosarcoma cell lines follows the order: N^{10}-hexyl > N^{10}-pentyl > N^{10}-butyl > N^{10}-propyl. Within the series, -Cl in C-2 position on the phenoxazine ring demonstrated a higher potency compared to phenoxazines with –H in C-2 position, suggesting that chlorine is playing a critical role on the growth inhibition.

KEYWORDS: Phenoxazine derivatives, rhabdomyosarcoma cell lines, antiproliferation, IC_{50} value.

INTRODUCTION

A number of phenoxazine derivatives have been shown to exert anticancer effects on a variety of cancer cells both *in vitro* and *in vivo*.[1-10] Some phenoxazine derivatives exerted strong anticancer activity against various cancer cells from human melanoma,[11] pancreatic cancer,[6] neuroblastoma,[12] glioma,[7] and multiple myeloma,[13] exerting apoptotic activity against these cancer cell lines.[6,7,11-13] Moreover, one derivative of phenoxazine inhibited human leukemia cells,[14] HTLV-1 negative T cell and lymphoblastoid cells.[15] Further, Shirato *et al.*,[12] investigated the anticancer effects of some phenoxazine derivatives on the human glioblastoma cell lines. There is an activation of Akt pathway in rhabdomyosarcoma cell lines which have tumerigenic potential. Thimmaiah *et al.*,[16-21] have reported the chemistry and biology of N^{10}- substituted phenoxazines.

They showed that phenoxazine derivatives inhibit Akt phosphorylation, induce apoptosis and exerts antiproliferative activity in rhabdomyosarcoma cells. Since the discovery of parent ring phenoxazine by Bernthsen,[22] researchers have continued to engage in its structural modifications to improve biological properties, reduce side effects and open new areas of applications. Several synthetic routes have been reported for the synthesis of phenoxazines, but the yields are poor and the methods are not generally applicable for the preparation of the wide variety of derivatives.[22,23] Although, the synthesis of 2-chlorophenoxazine was reported earlier,[24] the yield was very less. Thimmaiah *et al.*,[19] have synthesized phenoxazines with propyl and butyl group at N^{10}-position and H, -Cl or $-CF_3$ group at C-2 position of the phenoxazine ring. The potency of phenoxazine derivatives increased significantly when the chain length is increased from $(-CH_2)_3$ to $(-CH_2)_4$ at N^{10}-position of the phenoxazine ring due to increase in hydrophobicity and lipophilicity and those with acetyl bridge instead of alkyl exhibited no cell growth inhibition. Further it was shown that, within the series, –Cl at position-2 is more potent. Hence, in continuation we have synthesized phenoxazine derivatives by increasing the chain length to $(-CH_2)_5$ or $(-CH_2)_6$ and evaluated their effect on rhabdomyosarcoma cells.

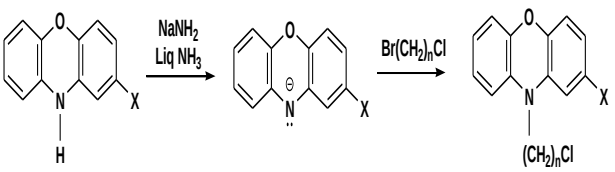
MATERIALS AND METHODS

Synthesis

The chemicals were obtained from standard commercial sources. Parent phenoxazine, secondary amines and

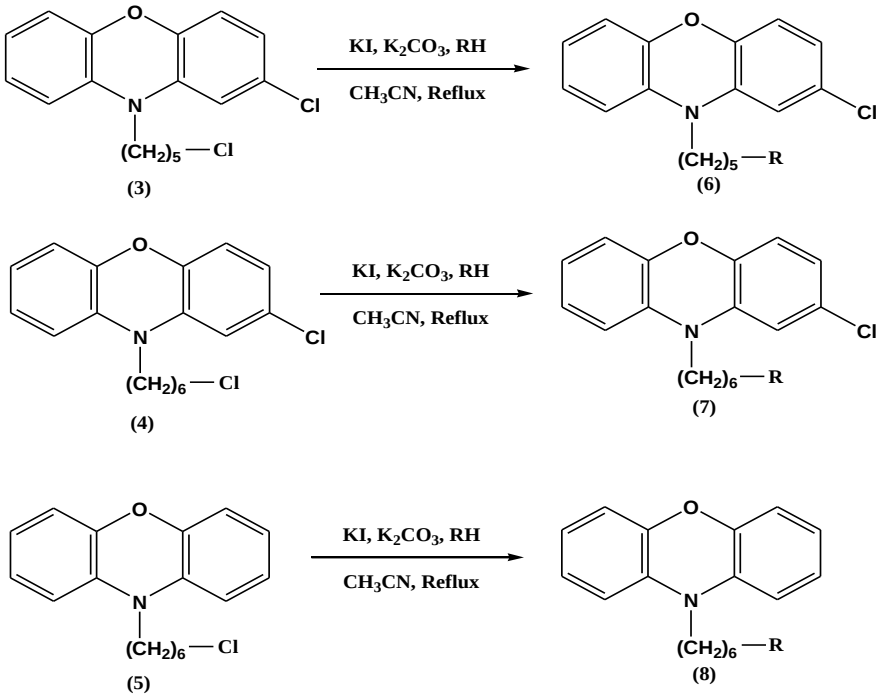
anhydrous solvents were purchased from Sigma-Aldrich and 2-chlorophenoxazine was prepared in pure form following the procedure published by G B Eregowda *et al*.[19] Progress of the Reactions and formation of the desired products were studied by TLC. For TLC, Analtech silica gel GF plates (20 x 20 cm, 250 µm, glass-backed), with petroleum ether-ethyl acetate and ethyl acetate-methanol as solvents were used. Silica gel was used in column chromatography. Melting points were recorded on an Electroral-9100 melting-point apparatus and are uncorrected. UV spectra were recorded in MeOH on a Schimatzu UV 1800 spectrophotometer. Infra-red spectra (IR) of the compounds were recorded as KBr pellets using Perkin Elmer FT-IR Spectrometer in the range 4000-400 cm^{-1}. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded in $CDCl_3$ solution/DMSO solution in a 5-mm tube on a Bruker drx 500 Fourier transform spectrometer (Agilant NMR or Varian NMR) with tetramethylsilane (TMS) as internal standard. The mass spectra of the compounds were obtained by both electron ionization (EI) and liquid secondary ionization (LSIMS) technique.

Alkylation of phenoxazine (1) or 2-chlorophenoxazine (2) was carried out according to the synthetic route outlined in **Scheme 1**. Under normal conditions, phenoxazine (1) or 2-chlorophenoxazine (2) resists to undergo *N*-alkylation with alkyl halides because of the weakly basic nature of the nitrogen atom of the phenoxazine nucleus. *N*-alkylation of these compounds was done by using stong base, sodamide in liquid ammonia. 2-chlorphenoxazine (2) was first converted in to an anionic species (2a) by treating with sodamide in liquid ammonia and then it was treated with 1-bromo-5-chloropentane or 1-bromo-6-chlorohexane to get N^{10}-(5'-chloropentyl)-2-chlorophenoxazine (3) and N^{10}-(6'-chlorohexyl)-2-chlorophenoxazine (4) respectively. Similarly, phenoxazine (1) was first converted in to an anionic species (1a) by treating with sodamide in liquid ammonia and then it was treated with 1-bromo-6-chlorohexane to get N^{10}-(6'-chlorohexyl) phenoxazine (5).



Scheme 1

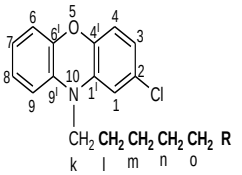
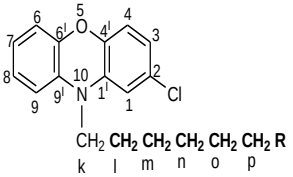
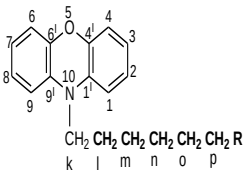
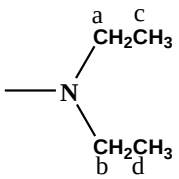
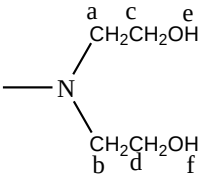
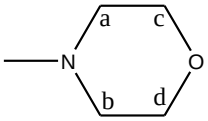
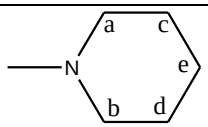
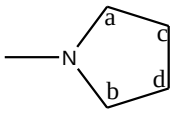
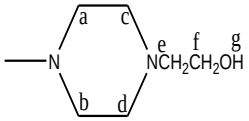
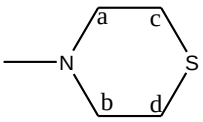
Compounds 3D-9D, 10D-16D and $10d_1$-$16d_1$ were prepared in good yield by the iodide catalyzed nucleophilic substitution of N^{10}-(5'-chloropentyl)-2-chlorophenoxazine(3), N^{10}-(6'-chlorohexyl)-2-chlorophenoxazine (4) and N^{10}-(6'-chlorohexyl)phenoxazine (5) respectively with various secondary amines (R-H :Diethylamine, Diethanolamine, morpholine, piperidine, pyrrolidine, β-hydroxyethylpiperazine, or thiomorpholine) **(Scheme 2)** by refluxing overnight with potassium carbonate in anhydrous acetonitrile.



R-H: Di ethylamine, Di ethaonolamine, Morpholine, Piperidine, Pyrrolidine, Hydroxyethylpierazine, Thiomorpholine

Scheme 2

Table 1: Structure of phenoxazine derivatives.

R	 Comp (6)	 Comp (7)	 Comp(8)
	3D	10D	$10d_1$
	4D	11D	$11d_1$
	5D	12D	12 d_1
	6D	13D	13 d_1
	7D	14D	14 d_1
	8D	15D	15 d_1
	9D	16D	16 d_1

N^{10}-[5'-Chloropentyl]-2-chlorophenoxazine (3)

6 g (0.074 mol) of 2-chlorophenoxazine (2) was added to 3.24 g (0.083 mol) of sodamide taken in 100 mL of liquid ammonia and the mixture was stirred for 45 min. 21 mL (0.159 mol) of 1-bromo-5-chloropentane was slowly added to this mixture with constant stirring. After 2 h, ammonia was allowed to evaporate and solid ice pieces were added carefully followed by cold water. When the reaction ceases, the product was extracted thrice with ether. The ether fraction was washed three times with water and dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel using (petroleum ether + chloroform) as elutant. A light yellow crystalline solid of pure compound (3) obtained was used for further synthesis.

Yield 68.7%; MS (m/z) 321 [$M^{+\cdot}$]; Anal. Cacld for $C_{17}H_{17}Cl_2NO$, C, 63.37; H, 5.32; N, 4.35. Found: C, 62.96; H, 5.31; N, 4.34; UV $\lambda_{max}(\varepsilon)$: 244 (13160), 329 (8520); IR (KBr) cm^{-1} : 2940, 1620, 1580, 1540, 1480, 1340, 1240, 1190, 1150, 1080, 930, 850, 790, 730; ^1H NMR (400 MHz, $CDCl_3$): δ 6.40-6.85 (m, 7H, H_1, H_3, H_4, H_6-H_9), δ 1.75-2.10 (m, 6H, H_l, H_m, H_n), 3.50-3.75 (m, 4H, H_k, H_o); ^{13}C NMR (100 MHz, $CDCl_3$): 111.12 (C_7), 114.42 (C_3), 115.25 (C_6), 115.87 (C_9), 119.83 (C_1), 121.22 (C_4), 123.68 (C_8), 128.16 (C_2), 131.68($C_{9'}$), 134.15 ($C_{1'}$), 143.45 ($C_{4'}$), 144.98 ($C_{6'}$), 22.30 (C_m), 24.43 (C_l), 30.60 (C_n), 43.30 (C_0), 44.50 (C_k).

N^{10}-[5'-(N-Diethylamino)pentyl]-2-chlorophenoxazine (3D).

2.9 g (0.01 mol) of compound (3) was dissolved in 150 mL of acetonitrile, 2.26 g (0.014 mol) of KI, and 2.68 g

(0.02 mol) of K_2CO_3 were added and refluxed for 45 min. 4.6 mL (0.04 mol) of N, N-diethylamine was added to the mixture and refluxed for 72h until a substantial amount of the product was formed as evidenced by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water three to four times, dried over anhydrous sodium sulfate and evaporated to give light yellow oil 3D (3.03 g, 94%). The obtained yellow oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure light green solid.

Yield: 94 %; mp 143 ^oC; MS: (m/z) 359.1 $[M+H]^+$; Anal: Calcd for $C_{21}H_{27}ClN_2O$: C, 70.28; H, 7.58; N, 7.81. Found: C, 70.26; H, 7.57; N, 7.80; UV $\lambda_{max}(\varepsilon)$: 204 (63000), 241 (98000), 329 (18400); IR (KBr) cm^{-1}: 3064, 2935, 2580, 2491, 1575, 1490, 1363, 1271, 1209, 1089, 1033, 921, 842, 780, 740; ^1H NMR (400 MHz, DMSO-d_6): δ 6.63-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.19 (t, 4H, H_c, H_d), δ 1.41-1.69 (m, 6H, H_l, H_m, H_n), 2.97 (m, 2H, H_o), δ 3.0-3.3 (q, 4H, H_a, H_b), 3.57 (t, 2H, H_k); ^{13}C NMR(100 MHz, $CDCl_3$): 111.32 (C_7), 111.73 (C_3), 115.57 (C_6), 116.11 (C_9), 120.19 (C_1), 121.57 (C_4), 123.93(C_8), 128.40 (C_2), 132.16 ($C_{9'}$), 134.34 ($C_{1'}$), 143.64 ($C_{4'}$), 144.65 ($C_{6'}$). 8.50 (C_c, C_d), 23.36 (C_m), 24.17 (C_l), 24.67 (C_n), 43.48 (C_k), 46.32 (C_a, C_b), 51.15 (C_o).

N^{10}-[5'-[N-Bis(hydroxyethyl)amino]pentyl]-2-chlorophenoxazine (4D).

2.45 g (0.008 mol) of compound (3) was dissolved in 150 mL of anhydrous acetonitrile, 2.13 g (0.13 mol) of KI, and 2g (0.015 mol) of K_2CO_3 were added and refluxed for 45 min. 6.3 mL (0.065 mol) of diethanolamine was added to the mixture and refluxed for 96h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 4D (2.45 g). The oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure white crystalline solid.

Yield: 82.58 %; mp 85 ^oC; MS: (m/z) 391 $[M+H]^+$; Anal: Calcd for $C_{21}H_{27}ClN_2O_3$: C, 64.52; H, 6.96; N, 7.17 Found: C, 64.51 H, 6.94; N, 7.15; UV $\lambda_{max}(\varepsilon)$: 203 (87000), 243 (78500), 328 (13800); IR (KBr) cm^{-1}: 3300, 2923, 1625, 1585, 1488, 1358, 1258,1129, 1081, 911, 827, 747; ^1H NMR (400 MHz $CDCl_3$): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.40-1.78 (m, 6H, H_l, H_m, H_n), 3.56 (t, 2H, H_k), 3.76(t, 4H, H_c, H_d), 3.15-3.28 (6H, m, H_a , H_b, H_o), 5.32 (s, 2H, H_e, H_f); ^{13}C NMR (100 MHz, $CDCl_3$): 111.37 (C_7), 111.78 (C_3), 115.54 (C_6), 116.10 (C_9), 120.17 (C_1), 121.54 (C_4), 123.97 (C_8), 128.39 (C_2), 132.17($C_{9'}$), 134.33 ($C_{1'}$), 143.60 ($C_{4'}$), 144.62 ($C_{6'}$). 23.39 (C_m), 23.85 (C_l), 24.57 (C_n), 43.54 (C_k), 54.85 (C_o), 56.13 (C_a , C_b), 56.88 (C_c , C_d).

N^{10}-[5'-(*N*-Morpholino)pentyl]-2-chlorophenoxazine (5D).

1.94 g (0.006 mol) of compound (3) was dissolved in 150 mL of anhydrous acetonitrile, 2.13 g (0.013 mol) of KI, and 2.0 g (0.015 mol) of K_2CO_3 were added and refluxed for 45 min. 8 mL of morpholine (0.09 mol) was added to the above mixture and refluxed for 25 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 5D (1.9 g). The oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 84.8 %; mp 85 ^oC; MS: (m/z) 373.1 $[M+H]^+$; Anal: Calcd for $C_{21}H_{25}ClN_2O_2$: C, 67.64; H, 6.76; N, 7.51. Found: C, 67.63; H, 6.75; N, 7.50; UV $\lambda_{max}(\varepsilon)$: 203 (40100), 241 (57200), 330 (10900); IR (KBr) cm^{-1}: 2948, 1625, 1487, 1359, 1272, 1110, 1081, 975, 919, 833, 742; ^1H NMR, (400 MHz, DMSO-d_6): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.40-1.78 (m, 6H, H_l, H_m, Hn), 2.99-3.40(m, 6H, H_a, H_b, H_o), 3.57 (t, 2H, H_k), 3.75-3.99 (m, 4H, H_c , H_d), ^{13}C NMR, 100 MHz, $CDCl_3$): 111.37 (C_7), 111.73 (C_3), 115.58 (C_6), 116.13 (C_9), 120.21 (C_1), 121.58 (C_4), 123.94 (C_8), 128.40 (C_2), 132.14 ($C_{9'}$), 134.32($C_{1'}$), 143.63($C_{4'}$), 144.65 ($C_{6'}$), 23.03 (C_m), 23.96 (C_l), 24.59 (C_n), 43.44 (C_k), 51.84 (C_a, C_b), 57.59 (C_o), 63.55 (C_c, C_d).

N^{10}-[5'-(*N*-Piperidino)pentyl]-2-chlorophenoxazine 6D

2.25 g (0.007 mol) of compound (3) was dissolved in 150 mL of acetonitrile, 2.13 g (0.013 mol) of KI and 1.91 g (0.014 mol) of K_2CO_3 were added and refluxed for 45 min. 6 mL of piperidine (0.061 mol) was added to the above mixture and refluxed for 24 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 6D (2.2 g). The oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 85 %; mp 185 ^oC; MS: (m/z) 371.1 $[M+H]^+$, Anal: Calcd for $C_{22}H_{27}ClN_2O$: C, 71.24; H, 7.34; N, 7.55. Found: C, 71.22; H, 7.33; N, 7.54; UV $\lambda_{max}(\varepsilon)$: 203 (25500), 241 (39700), 330 (7300); IR (KBr) cm^{-1}: 2937,

1625, 1485, 1357, 1269, 1128, 1200, 919, 827, 736; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.63-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.34-1.79 (m, 12H, H_l, H_m, H_n, H_c, H_d, H_e), 2.77-3.41 (m, 6H, H_a, H_b, H_o), 3.33-3.41 (m, 2H,), 3.57 (t, 2H), ^{13}C NMR,(100 MHz, $CDCl_3$): 111.35 (C_7), 111.74 (C_3), 115.54 (C_6), 116.09 (C_9), 120.15 (C_1), 121.54 (C_4), 123.94 (C_8), 128.39 (C_2), 132.15 ($C_{9'}$), 134.35 ($C_{1'}$), 143.62 ($C_{4'}$), 143.63 ($C_{6'}$), 22.11 (C_m), 22.49 (C_c, C_d), 23.39 (C_e), 24.12 (C_l), 24.59 (C_n), 43.50 (C_k), 53.15 (C_o), 57.12 (C_a , C_b).

N^{10}-[5'-(N-Pyrrolidino)pentyl]-2-chlorophenoxazine. 7D

2.21 g (0.007 mol) of compound (3) was dissolved in 150 mL of acetonitrile. 2.11 g (0.013 mol) of KI and 1.83 g (0.013 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 4 ml of pyrrolidine (0.049 mol) and refluxed for 25 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 7D (2.2 g). The oily product was purified by silica gel column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 89 %, mp 147 ^{o}C; MS: (m/z) 357.1 $[M+H]^+$; Anal: Calcd for $C_{21}H_{25}ClN_2O$: C, 70.67; H, 7.06; N, 7.85. Found: C, 70.62; H, 7.05; N, 7.83; UV λ_{max}(ε): 204 (42000), 241 (64100), 330 (12100); IR (KBr) cm^{-1}: 2939, 1625, 1566, 1487, 1361, 1272, 1199, 1132, 1093, 918, 831, 752; ^{1}H NMR (400 MHz, DMSO): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.42-1.58 (m, 4H, H_c, H_d), 1.70-1.97 (m, 6H, H_l, H_m, H_n), 2.89-3.09 (m, 4H, H_a, H_b), 3.45-3.48 (m, 2H, H_o), 3.54-3.58 (m, 2H, H_k); ^{13}C NMR (100 MHz, $CDCl_3$): 111.33 (C_7), 111.74 (C_3), 115.50 (C_6), 116.06 (C_9), 120.12 (C_1), 121.52 (C_4), 123.94 (C_8), 128.37 (C_2), 132.12 ($C_{9'}$), 134.31 ($C_{1'}$), 143.58 ($C_{4'}$), 144.58 ($C_{6'}$), 23.30 (C_m), 24.00 (C_c, C_d), 24.52 (C_l), 25.45 (C_n), 43.50 (C_k), 53.51 (C_o), 55.12 (C_a, C_b). 23.30 (C_m), 24.00 (C_c, C_d), 24.52 (C_l), 25.45 (C_n), 43.50 (C_k), 53.51 (C_o), 55.12 (C_a, C_b).

N^{10}-[5'-[N-(β-Hydroxyethyl)piperazino]pentyl]-2-chlorophenoxazine. 8D

3.22 g (0.01 mol) of compound (3) was dissolved in 150 mL of acetonitrile. 2.74 g of KI (0.02 mol) and 3.85 g (0.03 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 6.1 mL (0.05 mol) of N-(β-hydroxyethyl)piperazine and refluxed for 24 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 8D (3.56 g). The oily product was purified by silica gel column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 85 %; mp 243 ^{o}C; MS: (m/z) 416.1 $[M+H]^+$, Anal: Calcd for $C_{23}H_{30}ClN_3O_2$: C, 66.41; H, 7.27; N, 10.10 Found: C, 66.39; H, 7.26; N, 9.98; UV λ_{max}(ε): 203 (30700), 241 (44700), 330 (8300); IR (KBr) cm^{-1}: 3346, 2950, 1625, 1487, 1359, 1272, 1130, 1070, 923, 837, 738; ^{1}H NMR, (400 MHz , $CDCl_3$): δ 6.63-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.44-1.76 (m, 6H,), 3.57 (t, 2H,), 3.77(s, 1H$_g$,), 3.19-3.58(m, 14H); ^{13}C NMR(100 MHz, $CDCl_3$): 111.33 (C_7), 111.74 (C_3), 115.50 (C_6), 116.06 (C_9), 120.12 (C_1), 121.52 (C_4). 123.94 (C_8), 128.37(C_2), 132.12($C_{9'}$), 134.31($C_{1'}$), 143.58 ($C_{4'}$), 144.58 ($C_{6'}$), 25.10 (C_m), 27.30 (C_l), 28.10(C_n), 48.20(C_k), 53.00(C_a, C_b, C_c, C_d), 54.10 (C_o), 57.00 (C_e), 59.40 (C_f).

N^{10}-[5'-(N-Thiomorpholino)pentyl]-2-chlorophenoxazine 9D

1.22 g (0.004 mol) of compound (3) was dissolved in 150 mL of acetonitrile, 2.0 g (0.012 mol) of KI, and 2.09 g (0.015 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 3 mL (0.03 mol) of thiomorpholine and refluxed for 38 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 9D (1.42 g). The oily product was purified by silica gel column chromatography using ethyl acetate–methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 96 %, mp 98 ^{o}C, MS: (m/z) 389.3 $[M+H]^+$, Anal: Calcd for $C_{21}H_{27}ClN_2OS$: C, 64.85; H, 6.48; N, 7.20. Found: C, 64.80; H, 6.47; N, 7.17; UV λ_{max}(ε): 204 (23900), 241 (27900), 341 (5100); IR (KBr) cm^{-1}: 2921, 1587, 1488, 1360, 1273, 1130, 921, 849, 797, ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.44-1.74 (m, 6H, H_l, H_m, H_n), 3.56 (t, 2H, H_k), 2.99-3.40 (m, 6H, H_a, H_b, H_o), 3.75-3.99 (m, H_c, H_d ,4H); ^{13}C NMR(100 MHz, DMSO-d_6): 111.38 (C_7), 111.73 (C_3), 115.61 (C_6), 116.17 (C_9), 120.25 (C_1), 121.61 (C_4), 123.93 (C_8), 128.41 (C_2), 132.16 ($C_{9'}$), 134.33 ($C_{1'}$), 143.66 ($C_{4'}$), 144.69 ($C_{6'}$), 23.02 (C_m), 24.04 (C_l), 24.45 (C_n), 24.76 (C_c, C_d), 45.24 (C_k), 54.01 (C_o), 57.98 (C_a, C_b).

N^{10}-[6'-Chlorohexyl]-2-chlorophenoxazine (4)

3.24 g (0.083 mol) of sodamide was taken in 100 mL of liquid ammonia to which added 15.36 g (0.07 mol) of 2-chlorophenoxazine (2). After stirring for 45 min, 20 mL (0.134 mol) of 1-bromo-6-chlorohexane was added slowly with constant stirring. After 2 h, ammonia was allowed to evaporate and solid ice pieces were added carefully followed by cold water. It was extracted thrice with ether. The ether fraction was washed three times

with water dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on silica gel using Petroleum ether-chloroform as elutant. Compound (4) was obtained as a pure crystalline product (16.56 g).

Yield: 70%; MS: (m/z) 335.08 [$M^{+\cdot}$]; Anal: Calcd for $C_{18}H_{19}Cl_2NO$: C, 64.29; H, 5.70; N, 4.17. Found: C, 65.97; H, 5.68; N, 4.08; UV $\lambda_{max}(\varepsilon)$: 242 (47200), 330 (12900), IR (KBr) cm^{-1} : 2930, 1620, 1570, 1480, 1340, 1230, 1180, 1150 , 1080, 920. 840, 790, 730, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.4-6.85 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.75-2.10 (m, 6H, H_l, H_m, H_n, H_o), 3.50-3.75(m, 4H, H_k, H_p); ^{13}C NMR(100 MHz, $CDCl_3$): δ 111.12 (C_7), 114.42 (C_3), 115.25 (C_6), 115.87 (C_9), 119.83 (C_1), 121.22 (C_4), 123.68 (C_8), 128.16 (C_2), 131.68 ($C_{9'}$), 134.15 ($C_{1'}$), 143.45 ($C_{4'}$), 144.98 ($C_{6'}$), 22.30 (C_n), 24.43 (C_m), 27.6 (C_l), 33.30 (C_0), 44.50 (C_p), 48.2 (C_k).

N^{10}-[6'-(N-Diethylamino)hexyl]-2-chlorophenoxazine. 10D

2.85 g (0.009 mol) of compound (4) was dissolved in 140 mL of acetonitrile, 2.26 g (0.014 mol) of KI, and 2.68 g (0.02 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 6 mL (0.058 mol) of N,N-diethylamine and refluxed for 63 h until a substantial amount of the product was formed as evidenced by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 10D (2.6 g). The oily product was chromatographed on the silica gel with CH_3OH-$CHCl_3$ (3:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 74 %; mp: 85 ^{o}C; MS: (m/z) 373.1 $[M+H]^+$; Anal: Calcd for $C_{22}H_{29}ClN_2O$: C, 70.85; H, 7.84; N, 7.51 Found: C, 70.80; H, 7.83; N, 7.50; UV $\lambda_{max}(\varepsilon)$: 203 (67100), 241 (100300), 331 (8300); IR (KBr) cm^{-1} : 2944, 1625, 1585, 1486, 1359, 1271, 1204, 1129, 1091, 1042, 928, 831, 748; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.63-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.16-1.31 (m, 6H, H_c, H_d), 1.33-1.63 (m, 8H, H_l, H_m, H_n, H_o), 2.96-3.12 (m, 6H, H_a, H_b, H_p), 3.56 (t, 2H, H_k); ^{13}C NMR (100 MHz, $CDCl_3$): 111.32 (C_7), 111.68 (C_3), 115.42 (C_6), 115.96 (C_9), 119.98 (C_1), 121.39 (C_4), 123.85 (C_8), 128.33 (C_2), 132.22 ($C_{9'}$), 134.41 ($C_{1'}$), 143.59 ($C_{4'}$), 144.62 ($C_{6'}$). 8.55 (C_c, C_d), 23.28 (C_m), 24.80 (C_n), 26.18 (C_l), 26.62 (C_o), 43.72 (C_k), 46.51 (C_a, C_b), 51.18 (C_p).

N^{10}-[6'-[N-Bis(hydroxyethyl)amino]hexyl]-2-chlorophenoxazine 11D

3.1 g (0.009 mol) of compound (4) was dissolved in 280 mL of anhydrous acetonitrile, 1.7 g (0.01 mol) of KI, 2.71 g (0.02 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 4.12 mL (0.043 mol) of diethanolamine and refluxed for 47 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether .The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 11D (3.2 g). The oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 79 %; mp: 85 ^{o}C; MS: (m/z) 405.1 $[M+H]^+$; Anal: Calcd for $C_{22}H_{29}ClN_2O_3$: C, 65.25; H, 7.22; N, 6.92 Found: C, 62.21; H, 7.19; N, 6.91; UV $\lambda_{max}(\varepsilon)$: 203 (49800), 241 (77100), 340 (12800); IR (KBr) cm^{-1}: 3300, 2942, 1624, 1584, 1485, 1359, 1272, 1129, 1042, 929, 831, 732; ^{1}H NMR, (400 MHz, DMSO-d_6): δ 6.63-6.81 (m, 7H, H_1, H_3, H_4, H_6-H_9), δ 1.35-1.69 (m, 8H,), 3.55(t, 2H), 3.76(t, 4H), 3.15-3.28(6H, m,), δ 5.32 (s, 2H, H_e,H_f); ^{13}C NMR, (100 MHz, $CDCl_3$): 111.30 (C_7), 111.79 (C_3), 115.41 (C_6), 115.97 (C_9), 119.98 (C_1), 121.38 (C_4), 123.97 (C_8), 128.31 (C_2), 132.24 ($C_{9'}$), 137.63 ($C_{1'}$), 143.57 ($C_{4'}$), 144.56 ($C_{6'}$), 23.21 (C_m), 23.52 (C_n), 26.22 (C_l), 26.69 (C_o), 30.79 (C_k), 32.86 (C_p), 54.39 (C_a, C_b), 56.28 (C_c, C_d).

N^{10}-[6'-(N-Morpholino)hexyl]-2-chlorophenoxazine. 12D

2.1 g (0.006 mol) of compound (4) was dissolved in 300 mL of acetonitrile, 2.3 g (0.014 mol) of KI, and 2.35 g of K_2CO_3 (0.017 mol) were added and refluxed for 45 min. Then added 1.22 mL (0.014 mol) of morpholine and refluxed for 25 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 12D (2.3 g). The oily product was purified by column chromatography using ethyl acetate- methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 87 %: mp: 212 ^{o}C; MS: (m/z) 387.1 $[M+H]^+$; Anal: Calcd for $C_{22}H_{27}ClN_2O_2$: C, 68.29; H, 7.03; N, 7.24. Found: C, 68.02; H, 6.99; N, 7.17; UV $\lambda_{max}(\varepsilon)$: 203 (31300), 241 (44600), 331(8300); IR (KBr)cm^{-1} : 2938, 1584 , 1490, 1363, 1263, 1114, 975, 869, 734; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.40-1.78 (m, 8H, H_l, H_m, H_n, H_o), 2.99-3.40 (m, 6H, H_a, H_b, H_p), 3.57 (t, 2H, H_k), 3.75- 3.99 (m, 4H, H_c , H_d); ^{13}C NMR (100 MHz, $CDCl_3$): 112.01 (C_7), 112.85 (C_3), 115.60(C_6), 116.56 (C_9), 120.35 (C_1), 121.87 (C_4), 124.77 (C_8), 128.28 (C_2), 132.27 ($C_{9'}$), 134.75 ($C_{1'}$), 143.35 ($C_{4'}$), 144.21 ($C_{6'}$), 27.10 (C_m, C_n), 27.60 (C_l), 28.40 (C_o), 48.20 (C_k), 53.70 (C_a, C_b), 54.40 (C_p), 66.8 (C_c , C_d).

N^{10}-[6'-[N-Piperidino]hexyl]-2-chlorophenoxazine. 13D

2.3 g (0.007 mol) of compound (4) was dissolved in 270 mL of acetonitrile, 2.2 g (0.013 mol) of KI and 2.18 g (0.016 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 6.5 mL (0.07mol) of piperidine and refluxed for 24 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether .The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 13D (2.4 g). The oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 83 %; mp: 210 ^oC; MS: (m/z) 385.1 $[M+H]^+$, Anal: Calcd for $C_{23}H_{29}ClN_2O$: C, 71.76; H, 7.59; N, 7.28. Found: C, 71.47; H, 7.57; N, 7.12; UV $\lambda_{max}(\varepsilon)$: 203 (43300), 241 (61500), 331(11600); IR (KBr) cm^{-1} : 2942, 1625, 1585, 1488, 1359, 1271, 1200, 1131, 1066, 961, 929, 830, 754; ^1H NMR (400 MHz, DMSO-d_6): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.30-1.81 (m, 8H), 3.55 (t, 2H), 2.77-3.41 (m, 6H), 1.34-1.56 (m, 6H); ^{13}C NMR(100 MHz, $CDCl_3$): 111.30 (C_7), 111.69 (C_3), 115.41 (C_6), 115.95 (C_9), 119.97 (C_1), 121.38 (C_4), 123.88 (C_8), 128.32 (C_2), 132.21 ($C_{9'}$), 134.42 ($C_{1'}$), 143.58($C_{4'}$), 144.59 ($C_{6'}$). 25.9 (C_c, C_d, C_e), 27.1 (C_m, C_n), 27.6 (C_l), 28.4 (C_o), 48.2 (C_k), 54.4 (C_p), 54.6 (C_a , C_b).

N^{10}-[6'-(N-Pyrrolidino)hexyl]-2-chlorophenoxazine. 14D

2.3 g (0.007 mol) of compound 6 was dissolved in 300 mL of acetonitrile. 2.34 g (0.014 mol) of KI and 2 g (0.015 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 6.1 mL of pyrrolidine (0.073 mol) and refluxed for 25 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 14D (2.17 g). The oily product was purified by silica gel column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 77.5 %; mp: 180 ^oC; MS: (m/z) 371.1 $[M+H]^+$, Anal: Calcd for $C_{22}H_{27}ClN_2O$: C, 71.24; H, 7.34; N, 7.55. Found: C, 70.26; H, 7.17; N, 7.38; UV $\lambda_{max}(\varepsilon)$: 202 (71000), 240 (136300), 324(26600); IR (KBr) cm^{-1}: 2936, 1627, 1587, 1490, 1361, 1273, 1199, 1129, 1094, 1044, 907, 829, 793, 732; ^1H NMR(400 MHz, DMSO-d_6): δ 6.62-6.87 (m, 7H, H_1, H_3, H_4, H_6-H_9), 1.33-1.97 (m, 8H), 3.55 (t, 2H), 2.892-3.48 (m, 6H), 1.85–1.97 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 111.30 (C_7), 111.69 (C_3), 115.41 (C_6), 115.95 (C_9), 119.97 (C_1), 121.38 (C_4), 123.88 (C_8), 128.32 (C_2), 132.21 ($C_{9'}$), 134.42 ($C_{1'}$), 143.58 ($C_{4'}$), 144.59 ($C_{6'}$), 23.26 (C_c, C_d), 24.71 (C_m), 25.52 (C_n), 26.13 (C_l), 26.52 (C_o), 43.71 (C_k), 53.47 (C_a, C_b), 55.17 (C_p).

N^{10}-[6'-[N-(β-Hydroxyethyl)piperazino]hexyl]-2-chlorophenoxazine 15D

2 g (0.006 mol) of compound (4) was dissolved in 330 mL of acetonitrile. 2.12 g (0.013 mol) of KI and 2.85 g (0.02 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 5.65 mL (0.046 mol) of N-(β-hydroxyethyl)piperazine and refluxed for 48 h until a substantial amount of the product was formed and this is monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 15D (2.2 g). The oily product was purified by silica gel column chromatography using ethyl acetate –methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 79 %, mp: 243 ^oC; MS: (m/z) 430.1 $[M+H]^+$, Anal: Calcd for $C_{24}H_{32}ClN_3O_2$: C, 67.04; H, 7.50; N, 9.77. Found: C, 69.26; H, 7.47; N, 9.53, UV $\lambda_{max}(\varepsilon)$: 204 (70500), 241 (86300), 329(16700); IR (KBr) cm^{-1}: 3400, 2942, 1624, 1586, 1487, 1360, 1259, 1128, 1064, 929, 833, 754; ^1H NMR(400 MHz, DMSO-d_6): δ 6.62-6.88 (m, 7H, H_1, H_3, H_4, H_6-H_9), δ 1.40-1.71 (m, 8H), 3.56 (t, 2H)), 3.77 (s, 1H), 3.19- 3.58 (m, 14H); ^{13}C NMR, (100 MHz , $CDCl_3$): 111.30 (C_7), 111.69 (C_3), 115.41 (C_6), 115.95 (C_9), 119.97 (C_1), 121.38 (C_4), 123.88 (C_8), 128.32 (C_2), 132.21 ($C_{9'}$), 134.42 ($C_{1'}$), 143.58 ($C_{4'}$), 144.59 ($C_{6'}$), 27.1 (C_m, C_n), 27.6 (C_l), 28.4 (C_o), 48.2 (C_k), 53 (C_a, C_b, C_c ,C_d), 54.1 (C_p), 57 (C_e), 59.4 (C_f).

N^{10}-[6'-(N-Thiomorpholino)hexyl]-2-chlorophenoxazine 16D

1.6 g (0.005 mol) of compound (4) was dissolved in 200 mL of acetonitrile, 2 g (0.012 mol) of KI, and 2.13 g (0.015 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 4 mL (0.04 mol) of thiomorpholine and refluxed for 38 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether .The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 16D (1.82 g). The oily product was purified by silica gel column chromatography using ethyl acetate methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 95 %, mp: 170 ^oC; MS: (m/z) 403.1 $[M+H]^+$; Anal: Calcd for $C_{22}H_{27}ClN_2OS$: C, 65.57; H, 6.75; N, 6.95. Found: C, 64.93; H, 6.57; N, 6.80, UV $\lambda_{max}(\varepsilon)$: 203 (55800), 241 (70700), 340(11700); IR (KBr) cm^{-1}: 2921, 1587, 1488, 1360, 1273, 1130, 921, 849, 797, ^1H NMR(400 MHz, DMSO-d_6); δ 6.62-6.87 (m, 7H, H_1, H_3,

H_4, H_6-H_9), δ 1.41-1.76 (m, 8H), 3.56 (t, 2H), 2.99-3.40 (m, 6H), 3.75-3.99 (m, 4H); ^{13}C NMR, (100 MHz, $CDCl_3$) 111.46 (C_7), 112.31 (C_3), 115.03 (C_6), 115.99 (C_9), 119.73 (C_1), 121.29 (C_4), 124.23 (C_8), 127.73 (C_2), 131.71 ($C_{9'}$), 134.19 ($C_{1'}$), 142.80 ($C_{4'}$), 143.65 ($C_{6'}$). 22.46 (C_m), 23.27(C_n), 23.61(C_l , 23.85 (C_o), 44.4(C_c, C_d), 47.54 (C_k) , 52.57(C_p), 56.04 (C_a, C_b).

N^{10}-[6'-Chlorohexyl]phenoxazine (5)

3.53 g (0.09 mol) of sodamide was taken in 100 mL of liquid ammonia and cooled up to - 80 ^{o}C, to which added 16.66 g (0.09 mol) of phenoxazine 1 and the mixture was stirred for 45 min. 26.9 mL, (0.18 mol) of 1-bromo-6-chlorohexane was added drop wise to the above reaction mixture with constant stirring. The reaction was monitored by TLC. After the reaction was ceased (about 2 h), ammonia was allowed to evaporate. Solid ice pieces were added carefully to the reaction mixture followed by addition of cold water. The reaction mixture was extracted thrice with ether and the combined ether fraction was washed three times with water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel using petroleum ether-ethyl acetate (9:1) as elutant. The purified product was oily in nature which on storage at – 20 ^{o}C turned to white solid (5). (21 g).

Yield: 76 %; MS: (m/z) 301.12 [$M^{+\cdot}$], Anal: Calcd for $C_{18}H_{20}ClNO$: C, 71.63; H, 6.68; N, 4.64, Found: C, 69.23; H, 6.57; N, 4.57; UV λ_{max}(ε): 239(62147), 320(54235); IR (KBr) cm^{-1}: 3025, 2985, 1628, 1588, 1490, 1285, 1138, 920, 820; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), δ 1.75-1.85 (m, 8H, H_l, H_m, H_n, H_o), 3.60-3.90 (m, H_k, H_p); ^{13}C NMR (100 MHz, $CDCl_3$): 111.35 (C_3, C_7), 115.52 (C_4, C_6), 120.94 (C_1 , C_9), 123.78 (C_2 , C_8), 133.36 ($C_{1'}$, $C_{9'}$), 145.10 ($C_{4'}$, $C_{6'}$), 24.33 (C_n), 24.34 (C_m), 24.43 (C_l), 32.41 (C_o), 43.91 (C_p), 44.95 (C_k).

N^{10}-[6'-(N-Diethylamino)hexyl]phenoxazine 10d$_1$

2.5 g (0.008 mol) of compound (5) was dissolved in 120 mL of anhydrous acetonitrile. 2.03 g (0.012 mol) of KI and 2.15 g (0.016 mol) of K_2CO_3 was added and refluxed further for 45 min. Then added 20.6 mL (0.20 mol) of N,N-diethylamine and refluxed for 48 h until a substantial amount of the product as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The combined ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 10d$_1$ (2.22 g). The oily product was chromatographed on the silica gel with CH_3OH-$CHCl_3$ (1:3). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 71 %; mp 130 ^{o}C; MS: (m/z) 339.2 [M+H]$^+$; Anal: Calcd for $C_{22}H_{30}N_2O$: C, 78.06; H, 8.93; N, 8.28. Found: C, 77.26; H, 8.67; N, 8.25; UV λ_{max}(ε): 202(60400), 240(123000), 324(22600); IR(KBr)cm^{-1}: 2933, 1588, 1488, 1379, 1271, 1129, 1047, 926, 837, 751; ^{1}H NMR(400 MHz, $CDCl_3$): δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), 1.18-1.23 (m, 6H, H_c, H_d), 1.34-1.69 (m, 8H, H_l, H_m, H_n, H_o), 2.94-2.99 (m, 2H, H_p), 3.03-3.09 (m, 4H, H_a, H_b), 3.52-3.56 (t, 2H, H_k); ^{13}C NMR (100 MHz, $CDCl_3$); 112.40 (C_3, C_7), 115.44 (C_4, C_6), 121.15 (C_1 , C_9), 124.52 (C_2, C_8), 132.29 ($C_{1'}$, $C_{9'}$) , 144.50 ($C_{4'}$, $C_{6'}$), 23.14 (C_c, C_d), 24.52 (C_m, C_n), 25.98 (C_l), 26.39 (C_o), 43.18 (C_k), 46.25 (C_a , C_b), 50.67 (C_p).

N^{10}-[6'-[N-Bis(hydroxyethyl)amino]hexyl]phenoxazine 11d$_1$

To 2 g (0.007mol) of compound (5), 150 mL of acetonitrile was added and refluxed for 10 min. 2 g (0.012 mol) of KI was added and refluxed again for 45 min. To it 2 g (0.015mol) of K_2CO_3 was added and refluxed further for 15 min. Then added 12.65 mL (0.132mol) of diethanolamine and refluxed for 48 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 11d$_1$ (2.04 g). The oily product was purified by column chromatography using ethyl acetate-methanol (1:4). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt, which was dried, under high vacuum to get pure solid.

Yield: 82 %: mp 146 ^{o}C; MS: (m/z) 371.1 [M+H]$^+$; Anal: Calcd for $C_{22}H_{30}N_2O_3$: C, 71.32; H, 8.16; N, 7.56. Found: C, 70.06; H, 7.99; N, 7.40; UV λ_{max}(ε): 203(46400), 240(86000), 324(16200); IR (KBr) cm^{-1}: 3314, 2940, 1629, 1590, 1487, 1382, 1269, 1066, 894, 762, 731; ^{1}H NMR (400 MHz, $CDCl_3$), δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), 1.35-1.72 (m, 6H), 3.54 (t, 2H), 3.76 (t, 4H,), 3.15 -3.28 (6H, m), 5.32 (s, 2H, H_e, H_f); ^{13}C NMR (100 MHz ,$CDCl_3$): 111.34 (C_3,C_7), 115.43 (C_4,C_6), 120.80 (C_1 , C_9), 123.70 (C_2 ,C_8), 133.46 ($C_{1'}$, $C_{9'}$) , 145.08 ($C_{4'}$, $C_{6'}$), 25.06 (C_m, C_n), 27.19 (C_l), 27.26 (C_o), 44.01 (C_k), 54.74 (C_p), 56.08 (C_a , C_b), 59.4(C_c, C_d).

N^{10}-[6'-(N-Morpholino) hexyl]phenoxazine 12d$_1$

1.2 g (0.004 mol) of the compound (5), 215 mL of acetonitrile was added and refluxed for 10 min, 2.06 g (0.012 mol) of KI was added and refluxed for 45 min. To it 3.03 g (0.022 mol) of K_2CO_3 was added and refluxed for 15 min. Then added 10 mL (0.115 mol) of morpholine and refluxed for 23 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product 12d$_1$ (1.2 g). The oily product was purified by column chromatography using ethyl acetate -methanol (1:4). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 86 %; mp 146 ^{o}C; MS: (m/z) 353.1 $[M+H]^{+}$; Anal: Calcd for $C_{22}H_{28}N_2O_2$: C, 74.97; H, 8.01; N, 7.95. Found: C, 74.23; H, 7.92; N, 7.86; UV $\lambda_{max}(\varepsilon)$: 215(39900), 240(71100), 323(13700); IR (KBr) cm^{-1}: 2937, 1626, 1589, 1489, 1375, 1272, 1130, 1050, 962, 908, 867, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), δ 1.36-1.70 (m, 8H), 3.54 (t, 2H)), 2.99-3.40(m, 6H), 3.75-3.99 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 111.32 (C_3, C_7), 115.43 (C_4, C_6), 120.78 (C_1 ,C_9), 123.66 (C_2 , C_8), 133.47 ($C_{1'}$, $C_{9'}$) , 145.12 ($C_{4'}$, $C_{6'}$), 26.63 (C_m, C_n), 26.97 (C_l), 27.40 (C_o), 44.08 (C_k), 53.89 (C_a , C_b), 59.14 (C_p), 67.08 (C_c, C_d).

N^{10}-[6'-(N-Piperidino)hexyl]phenoxazines $13d_1$

2.01 g (0.007 mol) of the compound (5), was dissolved in 205 mL of acetonitrile, 1.97 g (0.012 mol) of KI was added and refluxed for 45 min. To it 1.96 g (0.014mol) of K_2CO_3 were added and refluxed for 15 min. Then added 6.5 m (0.066 mol) of piperidine and refluxed for 24 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulphate and evaporated to give an oily product $13d_1$ (1.6 g). The oily product was purified by column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 68 %; mp 140 ^{o}C; MS: (m/z) 351.2 $[M+H]^{+}$; Anal: Calcd for $C_{23}H_{30}N_2O$: C, 78.82; H, 8.63; N, 7.99. Found: C, 77.96; H, 8.57; N, 7.86; UV $\lambda_{max}(\varepsilon)$: 213(46100), 240(78700), 324(15500); IR (KBr) cm^{-1}: 2940, 1626, 1590, 1489, 1378, 1271, 1130, 1045, 950, 740; ^{1}H NMR(400 MHz, DMSO-d_6): δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), 1.31-1.80 (m, 8H), 3.54 (t, 2H), 2.77-3.41 (m, 6H), 1.34-1.56 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 111.33 (C_3, C_7), 115.39 (C_4, C_6), 120.74 (C_1 , C_9), 123.68 (C_2 , C_8), 133.48 ($C_{1'}$, $C_{9'}$) , 145.08 ($C_{4'}$, $C_{6'}$), 24.58 (C_c, C_d, C_e), 26.07 (C_m, C_n), 27.01 (C_l), 27.62 (C_o), 44.13 (C_k), 54.79 (C_p), 59.6 (C_a , C_b).

N^{10}-[6'-(N-Pyrrolidino)hexyl]phenoxazine $14d_1$

2.05 g (0.007 mol) of compound (5), was dissolved in 180 mL of acetonitrile. 2.04 g (0.012 mol) of KI and 2.84 g (0.021 mol) of K_2CO_3 were added and refluxed for 45 min. Then added 7 mL (0.085 mol) of pyrrolidine and refluxed for 11 h until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulphate and evaporated to give an oily product $14d_1$ (2.24 g). The oily product was purified by silica gel column chromatography using ethyl acetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 97 %; mp 118 ^{o}C; MS: (m/z) 337.1 $[M+H]^{+}$; Anal: Calcd for $C_{22}H_{28}N_2O$: C, 78.53; H, 8.39; N, 8.33. Found: C, 77.26; H, 8.27; N, 8.11; UV $\lambda_{max}(\varepsilon)$: 203(52200), 240(108400), 324(21000); IR (KBr) cm^{-1}: 2934, 1623, 1587, 1487, 1379, 1297, 1270, 1129, 1048, 925, 838, 752, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), 1.33-1.97 (m, 8H), 3.54 (t, 2H), 2.89-3.48 (m, 6H), 1.85-1.97 (m, 4H); ^{13}C NMR(100 MHz, $CDCl_3$); 111.35 (C_3, C_7), 115.39 (C_4, C_6), 120.74 (C_1 , C_9), 123.68 (C_2, C_8), 133.46 ($C_{1'}$, $C_{9'}$) , 145.08 ($C_{4'}$, $C_{6'}$), 23.46(C_c, C_d), 24.98 (C_m), 26.88 (C_n), 27.43 (C_l), 28.46 (C_o), 44.06 (C_k), 54.11 (C_p), 56.36 (C_a , C_b).

N^{10}-[6'-[N-(β-Hydroxyethyl)piperazino]hexyl]phenoxazine $15d_1$

2.07 g (0.007 mol) of compound (5), was dissolved in 140 mL of acetonitrile. 2.07 g of KI (0.013 mol) and 2.08 g (0.015 mol) of K_2CO_3 was added it and refluxed for 45 min. Then 11 mL (0.090 mol) of N-(β-hydroxy ethyl)piperazine was added and refluxed for 48 h until a substantial amount of the product was formed and this was monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulphate and evaporated to give an oily product $15d_1$ (2.22 g). The oily product was purified by silica gel column chromatography using ethylacetate-methanol (4:1). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 82 %; mp 222 ^{o}C; MS: (m/z) 396.2 $[M+H]^{+}$; Anal: Calcd for $C_{24}H_{33}N_3O_2$: C, 72.88; H, 8.41; N, 10.62, Found: C, 71.26; H, 8.27; N, 10.53; UV $\lambda_{max}(\varepsilon)$: 203(64900), 240(128500), 324(24700); IR (KBr) cm^{-1}: 3400, 2936, 1625, 1590, 1489, 1381, 1271, 1130, 1076, 965, 907, 752, 735; ^{1}H NMR (400 MHz, $CDCl_3$); δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), 1.40-1.71(m, 8H), 3.54 (t, 2H,) 3.77(s, 1H), 3.19-3.58 (m, 14H); ^{13}C NMR (100 MHz, $CDCl_3$); 111.32 (C_3, C_7), 115.42 (C_4, C_6), 120.77 (C_1, C_9), 123.67 (C_2, C_8), 133.47 ($C_{1'}$, $C_{9'}$) , 145.10 ($C_{4'}$, $C_{6'}$), 25.01(C_m), 26.98 (C_n), 27.48 (C_l), 44.10 (C_o), 52.94 (C_k), 53.44 (C_a, C_b, C_c, C_d), 57.79 (C_p), 58.72 (C_e), 59.25 (C_f).

N^{10}-[6'-(N-Thiomorpholino)hexyl]phenoxazine $16d_1$

1.51 g (0.005 mol) of compound (5), was dissolved in 165 mL of acetonitrile, 2.23 g (0.013 mol) of KI and 2.13 g (0.015 mol) of K_2CO_3 were added and refluxed for 18 min. Then added 18 mL (0.179 mol) of thiomorpholine and refluxed for 38 h, until a substantial amount of the product was formed as monitored by TLC. The contents were cooled, diluted with water and extracted with ether. The ether layer was washed with water thrice, dried over anhydrous sodium sulphate and evaporated to give an oily product $16d_1$ (1.63 g). An ether solution of the free base was treated with ethereal hydrochloride to give hydrochloride salt which was dried under high vacuum to get pure solid.

Yield: 88 %: mp 120 ^{o}C: MS: (m/z) 369.2 $[M+H]^{+}$; Anal: Calcd for $C_{22}H_{28}N_2OS$: C, 71.70; H, 7.66; N, 7.60. Found: C, 70.96; H, 7.57; N, 7.20; UV $\lambda_{max}(\varepsilon)$: 204(47200), 240(52800), 322(10800); IR (KBr) cm^{-1}: 2939, 1627, 1589, 1488, 1380, 1265, 1126, 1041, 950, 833, 732; ^{1}H NMR (400 MHz, DMSO-d_6); δ 6.62-6.85 (m, 8H, H_1-H_4, H_6-H_9), 1.41-1.74 (m, 8H), 3.56 (t, 2H), 2.99-3.40 (m, 6H), δ 3.75- 3.99 (m, 4H); ^{13}C NMR(100 MHz, $CDCl_3$): 111.31 (C_3, C_7), 115.42 (C_4, C_6), 120.77(C_1 , C_9), 123.66 (C_2, C_8), 133.45 ($C_{1'}$. $C_{9'}$), 145.07 ($C_{4'}$, $C_{6'}$), 26.52 (C_m), 27.55 (C_n), 28.06 (C_l), 31.02(C_o), 44.05(C_c, C_d), 47.92 (C_k), 55.12 (C_p), 59.38(C_a, C_b).

RESULTS AND DISCUSSION

The elemental analysis of all the compounds for carbon, hydrogen and nitrogen were obtained. The calculated values compared well with the experimentally found values.

The UV spectral data of 3D-9D, 10D-16D, and $10d_1$-$16d_1$ revealed that each compound exhibits three absorption bands (λ_{max}) at 201-207 nm, 238-244 nm, 323-345 nm and these bands assigned respectively to π→ π*, π→ π* and n→ π* transitions. It has been found that N^{10}-alkylation has only a slight influence upon the position and intensity of these bands and it merely produces small bathochromic shifts.

In the IR spectra bands in the range 3400-3260 cm^{-1} region in the spectra of compounds 4D, 8D, 11D, 15D, $11d_1$ and $15d_1$ may be assigned to the O-H stretching frequency. The strong bands in the spectra between 3042 and 2400 cm^{-1} may be assigned to the C-H stretching of the aromatic ring system. The bands observed at 1375-1250 cm^{-1} may be assigned to C-N aromatic stretching vibrations. The absorption bands in the range 1255-1160 cm^{-1} may be assigned to C-O diaryl stretching vibrations. The C-Cl stretching vibration was observed at 750-730 cm^{-1}.

The ^{1}H-NMR spectrum of 3D-16D and $10d_1$-$16d_1$ respectively, showed seven and eight aromatic protons (multiplet). The compounds 3D-16D showed the characteristic chemical shifts for the 2-chlorophenoxazine nucleus: ^{1}H-NMR δ 6.62-6.88 (m,7H, Ar-H, H_1, H_3-H_4, H_6-H_9). The compounds $10d_1$-$16d_1$ showed the characteristic chemical shifts for the phenoxazine nucleus: ^{1}H-NMR δ 6.62-6.85 (m, 8H). The multiplets at δ 1.75-2.10 ppm was assigned to -CH_2 protons (6H, H_l, H_m and H_n) and at δ 3.5-3.75 ppm was assigned to -CH_2 protons (4H, H_k and H_o) for pentyl series and at δ 1.75-2.10 ppm (m, 8H, H_l-H_o) and at δ 3.50-3.75 ppm (m, 4H, H_k and H_p) due to -CH_2 protons were assigned for hexyl series. A triplet at δ 3.57 ppm (t, 2H, H_k) is indicated by N-CH_2 due to deshielding by more electronegative N-atom and aromatic ring next to nitrogen.

In the ^{1}H-NMR spectrum of 3D, 10D and $10d_1$ the presence of a triplet at δ 1.19-1.31 ppm (6H, H_c and H_d) due to methyl protons and quartet at δ 3.0-3.3 ppm (4H, H_a and H_b) to methylene protons were observed. The signals due to H_a, H_b and H_o protons (in 3D) or H_p protons (in 10 D and $10d_1$) overlap with each other and appears as multiplet at δ 2.97 (2.96-3.12) ppm.

In the ^{1}H-NMR spectrum of 4D, 11D and $11d_1$ the –CH_2 groups next to the hydroxyl groups (H_c and H_d) were observed at δ 3.76(t, 4H, H_c, H_d), as a triplet and a singlet at δ 5.32 was assigned to the -OH protons (H_e and H_f) which disappeared on D_2O exchange. The N–CH_2 group nearer to the hydroxyl groups (H_a and H_b) and the –CH_2 group of the alkyl next to the nitrogen (H_o in 4D or H_p in 11 D and 11 d_1) appears as multiplet at δ 3.15-3.28 ppm.

In the ^{1}H-NMR spectrum of 5D, 12D and $12d_1$ the -CH_2 groups of the morpholino nucleus next to oxygen (H_c and H_d) were observed as a triplet at δ 3.75-3.99 ppm and the N-CH_2 of morpholino nucleus (H_a and H_b) and CH_2 attached to the nitrogen of the morpholine (H_o in 5D or H_p in 12D and $12d_1$) were observed as multiplet at δ 2.99-3.40 ppm.

In the ^{1}H-NMR spectrum of 6D, 13D and $13d_1$, the N-CH_2 of piperidine nucleus (H_a and H_b) and –CH_2 protons attached to the piperidine nitrogen (H_o in 6D / H_p in 13 D and 13 d_1) are observed as multiplet at δ 2.77-3.41 ppm. The three –CH_2 groups of piperidine (H_c, H_d and H_e) are observed as multiplet at δ 1.340-1.56 ppm.

In the ^{1}H-NMR spectrum of 7D, 14D and $14d_1$, the two -CH_2 groups of the pyrrolidino nucleus (H_c and H_d) are observed as multiplet at δ 1.42-1.58 ppm. The N-CH_2 of pyrrolidino nucleus (H_a and H_b) and the -CH_2 group attached to the nitrogen atom of the pyrrolidine (H_o in 7D / H_p in 14 D and 14 d_1) appears as mutiplet at δ 2.89-3.48 ppm.

In the ^{1}H-NMR spectrum of 8D, 15D and $15d_1$, the proton H_g from –OH group which exhibited a singlet at δ 3.77 ppm, disappeared on D_2O exchange. The presence of four –CH_2 groups of the piperazine ring (8H, H_a, H_b, H_c and H_d), and the two –CH_2 groups attached to the nitrogen atom of the piperazine ring (H_o (in 7D)or H_p (in 14 D and 14 d1) and H_e) and the –CH_2 group attached to the –OH group (H_f) were appeared as multiplet at δ 3.19-3.58 ppm.

In the ^{1}H-NMR spectrum of 9D, 16D and $16d_1$, the proton of N-CH_2 of thiomorpholino nucleus (H_a and H_b) and CH_2 protons attached to the nitrogen of the thiomorpholine (H_o in 7D or H_p in 14 D and 14 d_1) were observed at δ 2.97-3.41 ppm and the -CH_2 protons of thiomorpholino nucleus next to sulphur (H_c and H_d) were observed as multiplet at δ 3.74-3.99 ppm. A combination of the chemical shift, spin-spin coupling and the integration data permits the identification of the individual hydrogen at each side in the aromatic ring. The assignment of the protons is fully supported by the integration curves and all the derivatives showed the

characteristic chemical shifts for phenoxazine or 2-chlorophenoxazine.

The ^{13}C-NMR spectrum of 3D-16D exhibited twelve signals characteristic of 2-chlorophenoxazine nucleus representing twelve aromatic carbons: ^{13}C-NMR δ (^{1}H-decoupled) 111.12(C_7), 114.42(C_3), 115.25 (C_6), 115.87(C_9), 119.83(C_1), 121.22 (C_4), 123.68(C_8), 128.16(C_2), 131.68($C_{9'}$), 134.15($C_{1'}$), 143.45($C_{4'}$), 144.98($C_{6'}$). and $10d_1$-$16d_1$ showed six signals characteristic of phenoxazine nucleus representing 12 aromatic carbons: 112.40 (C_3, C_7), 115.44 (C_4, C_6), 121.15 (C_1 , C_9), 124.52 (C_2, C_8), 132.29 ($C_{1'}$, $C_{9'}$) , 144.50 ($C_{4'}$, $C_{6'}$). The resonances at δ 144 ppm and 143 ppm were assigned to the bridged head carbons ($C_{4'}$) and ($C_{6'}$) respectively and resonances at δ 131 ppm and 132 ppm were assigned to the bridged head carbons ($C_{1'}$) and ($C_{9'}$) respectively. The chemical shift at the lower field was assigned to the carbon adjacent to oxygen probably due to a larger deshielding effect of carbon resonances and higher electronegativity of oxygen. Similarly, the chemical shifts at δ 113.1 ppm and 113.9 ppm were assigned to the carbons *ortho* (C_1 and C_9) and δ 121 ppm and 122 ppm to the carbons *para* (C_3 and C_7) to nitrogen. The remaining resonances at δ 116.1 ppm and 114 ppm were assigned to carbons C_4 and C_6 and at δ 124.98 ppm and 124 ppm to carbons C_2 and C_8.The number of signals and their chemical shift values are in consistent with the structure of phenoxazine derivatives.

The mass spectra showed the intense molecular ion peak for each of these compounds. The molecular ion formed the base peak for all compounds. The molecular ions were observed in the form of protonated molecules $[M+H]^+$ for the phenoxazine derivatives because they contain secondary amines in the N^{10}-side chain. Mass spectral data showed that phenoxazine ring system remains stable, whereas the fragmentation reactions are observed due to the cleavage of bonds in the N^{10}-side chain portion of these compounds. In *toto*, elemental analysis and the spectral datas were consistent with the proposed structures of the phenoxazine derivatives.

Cell growth and cell viability assay

Rh1 cells were plated in a in a 6-well flat-bottom tissue culture plates (Falcon) in complete medium at a density of 6000 cells per well. After 24 h at 37 ^{o}C in a CO_2 incubator, medium was replaced with fresh medium containing 0.1 % DMSO or phenoxazines (3D-9D, 10D-16D or $10d_1$-$16d_1$) at concentrations ranging from 1.0 nM to 100 µM. The cells were further incubated for 6 days. Growth was assessed after lysing cells and counting nuclei. All measurements were made in triplicate.

Effects of phenoxazine derivatives on the viability of human rhabdomyosarcoma cells

The effects of various concentrations of phenoxazines (3D-16D and $10d_1$-$16d_1$) on the viability of human rhabdomyosarcoma cells (Rh1) after 6 days of treatment were investigated. To assess the effect on cell growth, cells grown in complete medium were exposed to graded concentrations (0.001-100 µM) of 3D-16D or $10d_1$-$16d_1$ for 6 days. Growth was assessed by lysing the cells and counting nuclei. The concentration response curves for 3D-9D in "Fig. 1", for 10D-16D in "Fig. 2", and for $10d_1$-$16d_1$ in "Fig. 3" are shown.

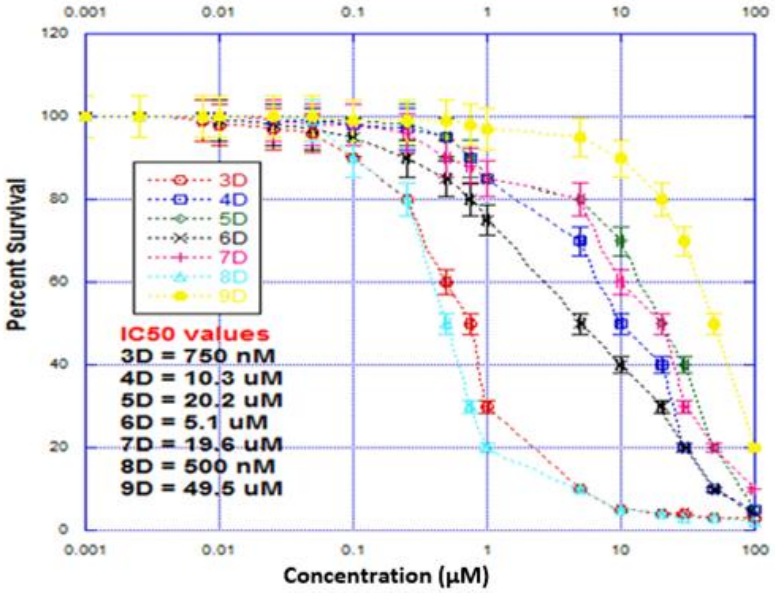


Fig 1: phenoxazine derivatives suppress cellular proliferation.

Rh1 cells were seeded in complete medium for overnight attachment. Next day, cells were exposed continuosly for 6 days to graded concentrations of 3D-9D (*A*), Growth was assessed by lysing the cells and counting the nuclei. Data represent the mean ±S.D. values of three separate experiments.

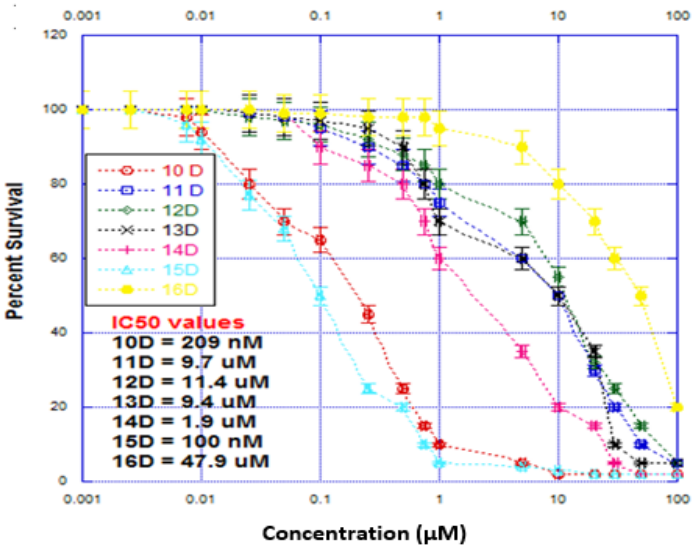


Fig 2: phenoxazines derivatives suppress cellular proliferation.

Rh1 cells were seeded in complete medium for overnight attachment. Next day, cells were exposed continuosly for 6 days to graded concentrations of 10D-16D. Growth was assessed by lysing the cells and counting the nuclei. Data represent the mean ±S.D. values of three separate experiments.

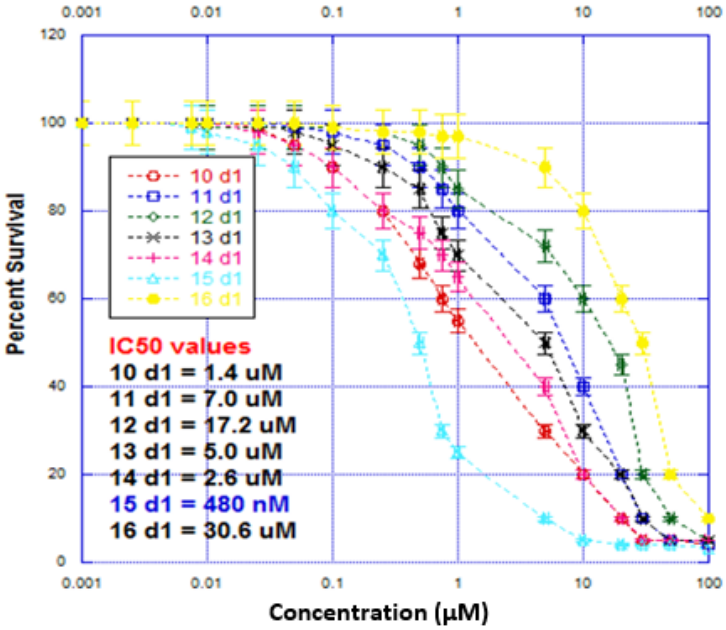


Fig 3: Phenoxazines derivatives supress cellular proliferation.

Rh1 cells were seeded in complete medium for overnight attachment. Next day, cells were exposed continuosly for 6 days to graded concentrations of $10d_1$-$16d_1$. Growth was assessed by lysing the cells and counting the nuclei. Data represent the mean ±S.D. values of three separate experiments.

Table 2: IC_{50} values of Phenoxazine derivatives for Rh1 cells.

3D-9D (Chloropentyl)		10D-16D (Chlorohexyl)		$10d_1$-$16d_1$ (Hexyl)	
Compound	**IC_{50} in µM**	**Compound**	**IC_{50} in µM**	**Compound**	**IC_{50} in µM**
3D	0.75	10D	0.209	$10d_1$	1.4
4D	10.3	11D	9.7	$11d_1$	7.0
5D	20.2	12D	11.4	$12d_1$	17.2
6D	5.1	13D	9.4	$13d_1$	5.0
7D	19.6	14D	1.9	$14d_1$	2.6
8D	0.5	15D	0.1	$15d_1$	0.48
9D	49.5	16D	47.9	$16d_1$	30.6

The IC_{50} values of the phenoxazine derivatives are summarized in **Table 2**. Phenoxazines inhibited the proliferation of cells in a dose-dependent manner. The substituent –Cl at position C-2 instead of hydrogen of the phenoxazine nucleus has a great bearing on anti-proliferative activity, as there is enhancement in the potency. The IC_{50} values within the series show the order of potency: -Cl > -H, other substituent's at position N^{10} being the same. Careful examination of IC_{50} values for N^{10}-chloropentyl (3D-9D) (0.5 µM– 49.5 µM) and N^{10}-chlorohexyl (10D-16D) (0.1 µM-47.9 µM) compounds against Rh1 cells revealed that anti-proliferative activity largely increased as the chain length increased from $(-CH_2)_5$ or $(-CH_2)_6$, suggesting that hydrophobic group is more effective.

Within the hexyl series, -Cl in C-2 position (10D-16D) demonstrated a higher potency compared to phenoxazines ($10d_1$-$16d_1$) with –H in C-2 position, suggesting that chlorine is playing a critical role on the growth inhibition. Comparison of the IC_{50} values within the series follow the order: 8D (0.5 µM) >3D (0.75 µM) >6D (5.1 µM) >4D (10.3 µM) >7D (19.6 µM) >5D (20.2 µM) >9D (49.5 µM) for 2-chloropentyl; 15D (0.1 µM) >10D (0.21 µM) >14D (1.9 µM) >13D (9.4 µM) >11D (9.7 µM) >12D (11.4 µM) >16D (47.9 µM) for 2-chlorohexyl; and $15d_1$ (0.48 µM) >$10d_1$ (1.4 µM) >$14d_1$ (2.6 µM) >$13d_1$ (5.0 µM) >$11d_1$ (7.0 µM) >$12d_1$ (17.2 µM) >$16d_1$ (30.6 µM) for hexyl phenoxazines with –H in C-2 position. A close look at the IC_{50} values revealed that the compound 15D demonstrated the greatest effect followed by 10D, $15d_1$ and so on. Of note was that thiomorpholino derivatives exhibited the least anti-proliferative effect in Rh1 cells. The structural features, within the series to cause a maximum anti-proliferative activity in Rh1 cells, include a hydrophobic phenoxazine ring nucleus with a –Cl substitution at position C-2 and a piperazinylamine with a *para*-β-hydroxyethyl group or N,N-diethylamino moiety joined by a six-carbon alkyl bridge to the phenoxazine nucleus at N^{10}-position. Thus, based on IC_{50} values, three most potent compounds 10D, 15D and $15d_1$ were selected for further evaluation. Further, cell viability experiments were done for 15D on Rh18 and Rh30 cell lines. The results reveal that both the cell lines were sensitive to this compound with an IC_{50} value of 0.5 µM and 0.6 µM, respectively (data not shown). Thus, growth inhibition appears to correlate well with the concentration of phenoxazines (10D, 15D or $15d_1$) that inhibit Akt in cells. In contrast, the remaining pentyl and hexyl compounds were at least 10-fold less inhibitory. In summary, both 10D and 15D exerted significant anti-proliferative effects in rhabdomyosarcoma cell lines, and the anti-proliferative effects of 10D and 15D in these cell lines were considerably stronger than those of other phenoxazine derivatives.

CONCLUSION

The experimental results provides evidence for the potent antiproliferative effects of phenoxazine derivatives. Compounds 10D, 15D shows significant antiproliferative activity *in vitro*, arising as good candidates for further studies.

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REFERENCES

1. Shirato K, Imaizumi K, Abe A, Tomoda A. Phenoxazine derivatives induce caspase-independent cell death in human glioblastoma cell lines, A-172 and U-251 MG. Oncology Reports, 2007; 17(1): 201-8.
2. Kurosaki NM, Ikegami K, Kurosaki K, Endo T, Aoyagi H, Hanami M, et al. Anticancer Effects of Phenoxazine Derivatives Revealed by Inhibition of Cell Growth and Viability, Disregulation of Cell Cycle, and Apoptosis Induction in HTLV-1–Positive Leukemia Cells. J pharmacol Sci., 2009; 110: 87-97.
3. Wesolowska O, Molnar J, Westman G, Samuelsson K, Kawase M, Ocsovszki I, Motohashi N, Michalak K. Benzo [a] phenoxazines: a new group of potent P-glycoprotein inhibitors. In vivo, 2006; 20(1): 109-13.
4. Ishihara M, Kawase M, Westman G, Samuelsson K, Motohashi N, Sakagami H. Quantitative structure-cytotoxicity relationship analysis of phenoxazine derivatives by semiempirical molecular-orbital method. Anticancer research, 2007; 27(6B): 4053-57.
5. Suzuki F, Hashimato K, Ishihara M, Westman G, Kawase M, Motohashi N, Sakagami H, Tumor-specificity and Type of Cell Death Induced by Phenoxazines. Anticancer Res, 2007; 27(6B): 4233-8.
6. Kato S, Shirato K, Imaizumi K, Toyota H, Mizuguchi J, Odawara M, Che XF, Tomoda A. Anticancer effects of phenoxazine derivatives combined with tumor necrosis factor-related apoptosis-inducing ligand on pancreatic cancer cell lines, KLM-1 and MIA-PaCa-2. Oncology Reports, 2006; 15(4): 843-48.
7. Kasuga T, Tabuchi T, Shirato K, Imaizumi K, Tomoda A. Caspase-independent cell death revealed in human gastric cancer cell lines, MKN45 and KATO III treated with phenoxazine derivatives. Oncology reports, 2007; 17(2): 409-15.
8. Tabuchi T, Che XF, Hiraishi K, Adachi M, Miyano K, Sumimoto H, Miyazawa K, Tomoda A. Selectively Induced Apoptosis in Human Neutrophils in the Presence of Oxidative Phenoxazines, 2-Amino-4,4*α*-dihydryo-4*α*-7*H*-phenoxazine-3-one and 2-Aminophenoxazine-3-one, Preceded by Decrease of Intracellular pH, Depolarization of the Mitochondria, and Inhibition of Superoxide Generation. J. Pharmacol. Sci., 2011; 117(3): 139-48.

9. Tomoda A, Miyazawa K, Tabuchi T. Prevention of Carcinogenesis and Development of Gastric and Colon Cancers by 2-Aminophenoxazine-3-one (Phx-3): Direct and Indirect Anti-Cancer Activity of Phx-3. International Journal of Molecular Sciences. 2013; 14(9): 17573-83.
10. Collins I, Caldwell J, Fonseca T, Donald A, Bavetsias V, Hunter LJ, Garrett MD, Rowlands MG, Aherne GW, Davies TG, Berdini V, Woodhead SJ, Davis D, Seavers LC, Wyatt PG, Workman P, McDonald E, Structure-based design of isoquinoline-5-sulfonamide inhibitors of protein kinase B. Bioorg Med Chem, 2006; 14(4): 1255-73.
11. Shimizu S, Suzuki M, Miyazawa K, Yokoyama T, Ohyashiki K, Miyazaki K, et al., Differentiation and apoptosis in human malignant melanoma G-361 cells induced by 2-aminophenoxazine-3-one. Oncol Rep., 2005; 14(1): 41-6.
12. Shirato K, Imaizumi K, Abe A, Tomoda A, Phenoxazine Derivatives 2-Amino-4,4α-dihydro-4α-phenoxazine-3-one and 2-Aminophenoxazine-3-one-Induced Apoptosis through a Caspase-Independent Mechanism in Human Neuroblastoma Cell Line NB-1 Cells, Biological and Pharmaceutical Bulletin, 2007, 30, 331-336.
13. Shirato K, Imaizumi K, Miyazawa K, Takasaki A, Mizuguchi J, Che XF, Akiyama S, Tomoda A. Apoptosis Induction Preceded by Mitochondrial Depolarization in Multiple Myeloma Cell Line U266 by 2-Aminophenoxazine-3-one. Biological and Pharmaceutical Bulletin, 2008: 31(1): 62-7.
14. Shimamoto T, Tomoda A, Ishida R, Ohyashiki K. Antitumor effects of a novel phenoxazine derivative on human leukemia cell lines in vitro and in vivo. Clin Cancer Res, 2001; 7(3): 704-708.
15. Koizumi J K, Akazawa M, Iwamoto T, et al. Antitumor activity of a phenoxazine compound, 2-amino-4,4α-dihydro-4α,7-dimethyl-3H-phenoxazine-3-one against human B cell and T cell lymphoblastoid cell lines: induction of mixed types of cell death, apoptosis, and necrosis. J Cancer Res Clin Oncol, 2002; 128: 363–68.
16. Thimmmaiah KN, Horton JK, Qian XD, Beck WT, Houghton JA, Houghton PJ. Structural determinants of phenoxazine type compounds required to modulate the accumulation of vinblastine and vincristine in multidrug-resistant cell lines. Cancer Commun, 1990; 2(7): 249-59.
17. Thimmaiah KN, Horton JK, Seshadri R, Israel M, Houghton JA, Harwood FC, Houghton PJ. Synthesis and chemical characterization of N-substituted phenoxazines directed toward reversing vinca alkaloid resistance in multidrug-resistant cancer cells. J Med Chem, 1992; 35: 3358-64.
18. Horton JK, Thimmaiah KN, Harwood FC, Kuttesch JF, Houghton PJ. Pharmacological characterization of N-substituted phenoxazines directed toward reversing Vinca alkaloid resistance in multidrug-resistant cancer cells. Mol Pharmacol, 1993; 44(3): 552-59.
19. Eregowda GB, Kalpana HN, Hegde R, Thimmaiah KN. Synthesis and analysis of structural features phenoxazine analogues needed to reverse vinblastine resistance in multidrug resistance (MDR) cancer cells. Indian J Chem, 2000; 39B(04): 243-59.
20. Eregowda GB, Krishnegowda G, Kalpana HN, Channu BC, Das C, Horton JK, Houghton PJ, Thimmaiah KN. Structural Requirements for Activity of Phenoxazines for Reversal of Drug Resistance in Cancer Cells. Asian Journal of Chemistry, 1999; 11(3), 878-905.
21. Thimmmaiah KN, Easton JB, Germain GS, Morton CL, Kamath S, Buolamwini JK, Houghton PJ, Identification of N10-substituted phenoxazines as potent and specific inhibitors of AKt signaling. J Biol Chem, 2005; 280(36): 31924-35.
22. Bernthsen A, *Ber Deut Chem Ges.*, 1887; 20: 942.
23. Fuhrer H, Scutter P, Weis CD. J Heterocyclic Chem, 1979; 16: 1121.
24. Olmsted MP, Craig PN, Lafferty JJ, Parloff AM. Zirkle CL. J Pharm Pharmocology, 1954; 6: 361.