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Article

Design, Synthesis, and Antitumor Activities of Some Novel Substituted 1,2,3-Benzotriazines

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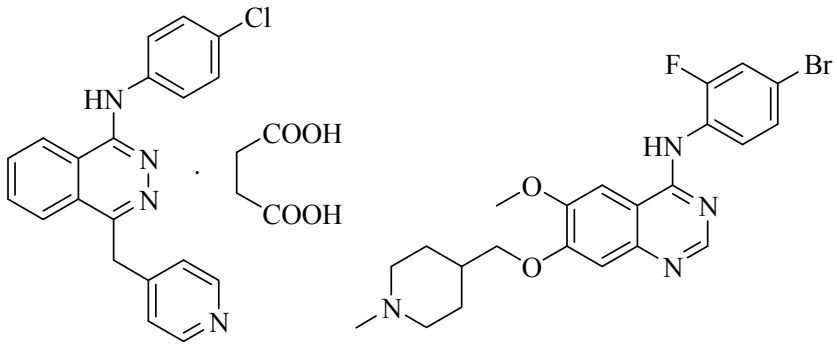
Abstract: A series of novel substituted 1,2,3-benzotriazines based on the structures of vatalanib succinate (PTK787) and vandetanib (ZD6474) were designed and synthesized. The antiproliferative effects of these compounds were tested on microvascular endothelial cells (MVECs) using the MTT assay. Introduction of a methoxy and a 3-chloropropoxy group into the 1,2,3-benzotriazines increased the antiproliferative effects. 4-(3-Chloro-4-fluoroanilino)-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**8m**) was the most effective compound. It was 4-10 fold more potent than PTK787 in inhibiting the growth of T47D breast cancer cells, DU145 and PC-3 prostate cancer cells, LL/2 murine Lewis lung cancer cells and B16F0 melanoma cells.

Keywords: 1,2,3-Benzotriazine, synthesis, antiproliferative activity, vatalanib succinate.

Introduction

Vascular endothelial growth factor (VEGF) plays an important role in both physiological and pathological angiogenesis by binding to its receptor, VEGFR [1]. The expression of VEGFR is increased in the majority of cancers and is associated with survival, migration and invasion of solid tumor cells [1]. Elevated VEGF levels have been found to increase resistance to chemotherapy [2, 3] and VEGF signaling has been used as a therapeutic target for the treatment of cancer. VEGFR inhibitors such as vatalanib succinate (PTK787/ZK222584) and vandetanib (ZD6474) have been used in clinical trials in several types of cancer [4-7]. Although both agents showed antitumor activities alone, combinations with chemotherapeutic agents seem to be required for reaching therapeutic effects [8-12]. To improve the antiproliferative abilities of PTK787 and ZD6474 (Figure 1), we have analyzed the structures of PTK787 and ZD6474. PTK787 contains 2,3-benzodizine and ZD6474 contains 1,3-benzodizine. We thought that the replacement of the benzodizine with 1,2,3-benzotriazine might have increased activities compared to PTK787. Thus, we designed and synthesized eighteen novel compounds (**8a-m**) with a basic 1,2,3-benzotriazine scaffold and the introduction of a methoxy group or a short alkoxy group. The antiproliferative effects of these compounds were determined in microvascular endothelial cells (MVECs) using MTT assay and the antiproliferative effects of compound **8m** were found to be the most profound in inhibiting growth of MVECs. Therefore, it was further investigated in T47D breast cancer cells, DU145 and PC-3 prostate cancer cells, LL/2 murine Lewis lung cancer cells and B16F0 melanoma cells.

Figure 1. Structures of vatalanib succinate (PTK787) and vandetanib (ZD6474).



PTK787 **ZD6474**

Results and Discussion

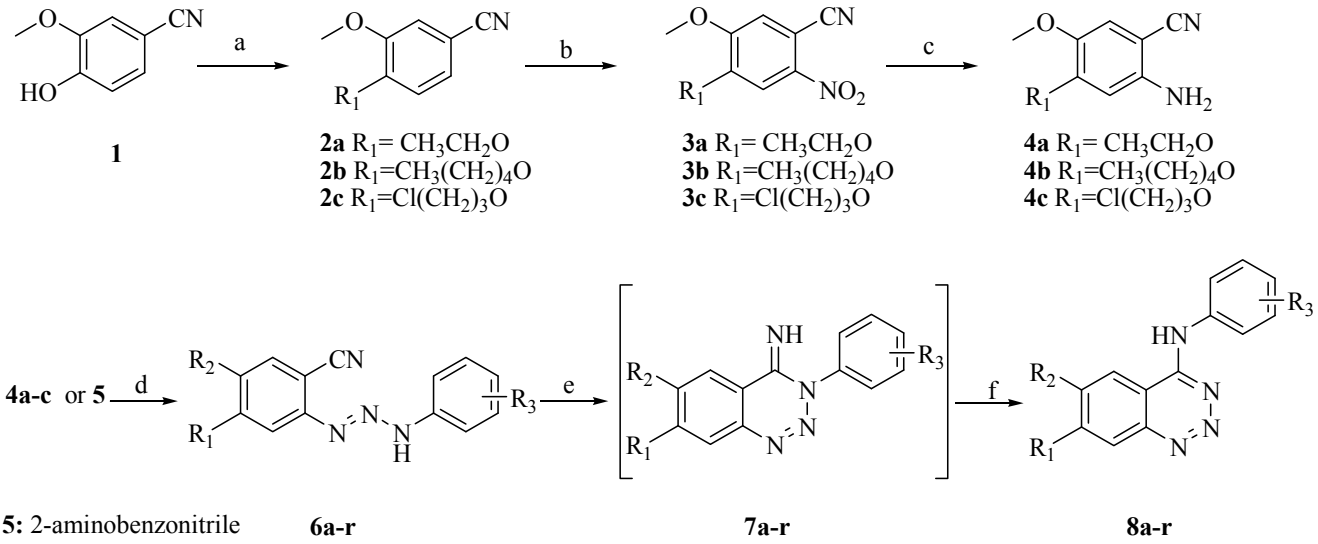
Design and synthesis of novel 1,2,3-benzotriazines

Recently, it was reported that 1,2,4-benzotriazines were inhibitors of Src and the binding model of 1,2,4-benzotriazines with Src showed the importance of the N1 and N2 atoms [13, 14]. We considered that VEGFR and Src might react with substrates in a similar fashion as both of them are tyrosine kinases. Both PTK787 and ZD6474 contain a substituted anilino group. ZD6474 contains a methoxy group at the C6 position and a short side chain at the C7 position. We thought that these substituents

are required for the antitumor activity. Thus, we decided using 1,2,3-benzotriazine to replace the phthalazine in PTK787 or the quinazoline in ZD6474 and to add a methoxy group at the C6 position and a short alkoxy group at the C7 position of 1,2,3-benzotriazine. A series of target compounds, **8a-r,** were obtained and their structures are listed in Table 1.

Compounds **8a-r** were prepared according to the synthetic route outlined in Scheme 1. 4-Cyano-2-methoxyphenol (**1**) reacted with various alkyl halides to afford intermediates **2a-c** in satisfactory yields. Compounds **2a-c** were selectively nitrated at 30 ^{o}C to give nitro compounds **3a-c** in 87-95% yields. The reduction of the nitro compounds **3a-c** to the corresponding amines **4a-c** was catalyzed by Pd/C [15]. Compounds **4a-c** were diazotized and then coupled with substituted anilines at 0 ^{o}C, followed by chromatographic purification to afford triazenes **6b-r**. Compound **6a** was prepared by the coupling of diazotized 2-aminobenzonitrile (**5**) and 4-chloroaniline. The cyclization of compounds **6a-r** in 70% ethanol formed intermediates **7a-r**, which then rearranged to compounds **8a-r** after refluxing in acetic acid [16]. As intermediates **7a-r** were unstable, they were used directly in the next step without further purification.

Scheme 1. Synthetic route to the target compounds.



Reagents and conditions: (a) alkyl halides, DMF, K_2CO_3, 37 ^{o}C; (b) HNO_3, 30 ^{o}C; (c) Pd/C, cyclohexene, ethanol, reflux; (d) $NaNO_2$/HCl, 0 ^{o}C; substituted aniline, 0 ^{o}C; (e) 70% ethanol, reflux; (f) AcOH, reflux.

Antiproliferative activities in MVECs

The antiproliferative effects of compounds **8a-r** in MVECs were determined (Table 1) and the structure-activity relationships were analyzed. The results revealed that compounds with a methoxy group (R_2) at the C6 position and an alkoxy group (R_1) at the C7 position (**8b-r**) had an increased activity compared to that of compound **8a** which did not have substitutions at the C6 and C7 positions. The compounds with a 3-chloropropoxy group (R_1) at the C7 position (**8h-r**) were more active than compounds with an ethoxy group (**8b-d**) or a pentyloxy group (**8e-g**).

By comparing the activities of compounds with a methoxy group at the C6 position and a 3-chloropropoxy group at the C7 position, but with a different substituted group (R_3) at the C4 anilino group (**8h-r**), it revealed that a substitution at the C4 anilino increased the antiproliferative activity in MVECs compared to compound **8r**, which did not have a substitution at the C4 anilino group. The electronic effect of a substituent on the anilino group did not influence the antiproliferative activities (comparing compounds **8k-o**). Compounds with two substituents at the C3' and C4' positions of the C4 anilino group (**8i**, **8m,** and **8p**) were more active than compounds with two substituents at the C3' and C5' positions (**8h**) or compounds with one substituent (**8j**, **8k**, **8l**, **8n**, **8o**, **8q**). Compound **8m** was the most potent one in inhibiting proliferation of MVECs with a GI_{50} value of 7.98 μM. The compounds with substituents at the anilino group (compounds **8h-q**) were more effective than PTK787 in inhibiting growth of MVECs after the introduction of a methoxy group at the C6 position and a 3-chloropropoxy group at the C7 position.

Table 1. The structures of target compounds and their antiproliferative effects in MVECs.

Compounds	R_1	R_2	R_3	GI_{50} (μM) [a]
8a	H	H	4-Cl	>80
8b	CH_3CH_2O	CH_3O	4-Cl	26.54±1.43
8c	CH_3CH_2O	CH_3O	4-CH_3	42.56±2.79
8d	CH_3CH_2O	CH_3O	3-Cl, 4-F	38.81±1.86
8e	$CH_3(CH_2)_4O$	CH_3O	4-Cl	25.35±1.02
8f	$CH_3(CH_2)_4O$	CH_3O	3-OCF_3	42.64±2.27
8g	$CH_3(CH_2)_4O$	CH_3O	4-OCF_3	37.98±1.98
8h	$Cl(CH_2)_3O$	CH_3O	3,5-di-Cl	28.84±1.40
8i	$Cl(CH_2)_3O$	CH_3O	3,4-di-Cl	11.02±0.49
8j	$Cl(CH_2)_3O$	CH_3O	4-Cl	18.59±1.31
8k	$Cl(CH_2)_3O$	CH_3O	4-CH_3	17.08±0.65
8l	$Cl(CH_2)_3O$	CH_3O	4-F	23.05±0.92
8m	$Cl(CH_2)_3O$	CH_3O	3-Cl, 4-F	7.98±0.35
8n	$Cl(CH_2)_3O$	CH_3O	3-OCF_3	24.86±0.86
8o	$Cl(CH_2)_3O$	CH_3O	4-OCF_3	23.19±1.12
8p	$Cl(CH_2)_3O$	CH_3O	3-CF_3, 4-F	15.22±0.51
8q	$Cl(CH_2)_3O$	CH_3O	3-CF_3	21.38±1.54
8r	$Cl(CH_2)_3O$	CH_3O	H	>80
vatalanib succinate (PTK787)				38.15±2.07

[a] GI_{50} is the concentration that inhibits 50% of cell growth. The cells were treated with various concentrations of the tested compounds for 4 days and cell growth inhibition was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data shown are means ± SD of three independent experiments.

The antiproliferative effects of compound **8m** were then tested in tumor cell lines. The antiproliferative effects of compound **8m** were determined in human T47 breast cancer cells, DU-145 and PC-3 prostate cancer cells, murine LL/2 Lewis lung cancer cells and B16F0 melanoma cells using MTT assay (Table 2). Compound **8m** was more effective than PTK787 to inhibit cell growth in all the tested cell lines.

Table 2. Antiproliferative effects of compound **8m** and PTK787 in several tumor cell lines.

Compounds	GI_{50} (μM) [a]				
	T47D	**DU-145**	**PC-3**	**LL/2**	**B16F0**
8m	5.04±0.32	4.96±0.43	6.91±0.30	3.79±0.19	5.19±0.22
PTK787	36.32±1.88	41.28±2.47	63.68±3.55	31.15±2.01	18.71±1.67

[a] Cells were treated with various concentrations of the tested compounds for 4 days and the cell growth inhibition was determined using the MTT assay. Data shown are means ± SD of three independent experiments.

Conclusions

In summary, our data indicate that 1,2,3-benzotriazines substituted with a methoxy group at the C6 position and a 3-chloropropoxy group at the C7 position exhibit antiproliferative activities. Most of these compounds are more effective than PTK787 in inhibiting proliferation of MVECs. Compound **8m** is the most potent and exhibits greater anti-tumor cell growth activity activities than PTK787. Since we did not stimulate the cell growth of MVECs and tumor cells with VEGF, the observed antiproliferative effects of these compounds, including PTK787, may be through a pathway independent of VEGFR inhibition. Because of the high similarity of 1,2,3-benzotriazine and 1,2,4-benzotriazine, the increased antiproliferative effects of the designed compounds in tumor cells may be due to the inhibition of Src, or other tyrosine kinases which is not inhibited by PTK787 [17]. The effects of our novel 1,2,3-benzotriazines on the activities of VEGFRs, Src and other kinases are under investigation.

Experimental

General

Reagents (analytical grade) were obtained from commercial suppliers and used without further purification unless otherwise noted. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker ARX-300 instrument with tetramethylsilane as the internal standard. IR spectra were recorded on a Bruker IR-27G spectrometer. MS were determined on either Finnigan MAT/USA spectrometer (LC-MS). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). The melting points were determined on an electrically heated X4 digital visual melting point apparatus and were uncorrected. The structures of the synthesized compounds were characterized

by melting point (mp), infrared (IR) spectra, nuclear magnetic resonance (NMR) spectra and mass spectral (MS) data.

General procedure for the synthesis of 4-substituted-3-methoxybenzonitriles **2a-c**

A solution of 4-cyano-2-methoxyphenol (**1**, 1.00 g, 6.70 mmol) in anhydrous DMF (4.00 mL) was stirred and cooled with a water bath. K_2CO_3 (1.39 g, 10.1 mmol) was added and the mixture was stirred at 20 ^oC for 1 h. The corresponding alkyl halide (8.13 mmol) was added dropwise, the mixture was stirred at room temperature overnight, then heated at 37 ^oC for 6 h and finally poured into a mixture of ice/H_2O (100 mL). After stirring for 10 min, a precipitate was formed. It was filtered off, washed with H_2O, and air-dried to yield the 4-substituted-3-methoxybenzonitriles **2a-c** as white solids.

4-Ethoxy-3-methoxybenzonitrile (**2a**): Yield: 91.8%; mp: 102-103 ^oC. ^{1}H-NMR (DMSO-d_6) δ: 7.36 (2H, m, H-2, H-6), 7.04 (1H, s, H-5), 4.11 (2H, q, J = 6.9 Hz, $CH_3C\underline{H}_2O$-), 3.81 (3H, s, -OCH_3), 1.35 (3H, t, J = 6.9 Hz, $C\underline{H}_3CH_2O$-); LC-MS: 178.1 $(M+H)^+$; Anal. Calcd. for $C_{10}H_{11}NO_2$: C 67.78, H 6.26, N 7.90; Found: C 67.79, H 6.24, N 7.91.

3-Methoxy-4-pentyloxybenzonitrile (**2b**): Yield: 89.0%; mp: 55-56 ^oC. ^{1}H-NMR (DMSO-d_6) δ: 7.37 (2H, m, H-2, H-6), 7.09 (1H, d, J = 8.1 Hz, H-5), 4.02 (2H, t, $CH_3(CH_2)_3C\underline{H}_2O$-), 3.79 (3H, s, -$OCH_3$), 1.71 (2H, m, $CH_3CH_2CH_2C\underline{H}_2CH_2O$-), 1.34 (4H, m, $CH_3C\underline{H}_2C\underline{H}_2CH_2CH_2O$-), 0.88 (3H, t, J = 6.9 Hz, $C\underline{H}_3CH_2CH_2CH_2CH_2O$-); LC-MS: 220.1 $(M+H)^+$; Anal. Calcd. for $C_{13}H_{17}NO_2$: C 71.21, H 7.81, N 6.39; Found: C 71.20, H 7.80, N 6.39.

4-(3-Chloropropoxy)-3-methoxybenzonitrile (**2c**): Yield: 87.2%. ^{1}H-NMR (DMSO-d_6) δ: 7.41 (2H, m, H-2, H-6), 7.15 (1H, d, J = 8.8 Hz, H-5), 4.16 (2H, t, J = 6.0 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 3.79 (5H, m, -$OCH_3$, $ClC\underline{H}_2CH_2CH_2O$-), 2.20 (2H, m, $ClCH_2C\underline{H}_2CH_2O$-); LC-MS: 226.1 $(M+H)^+$; Anal. Calcd. for $C_{11}H_{12}ClNO_2$: C 58.54, H 5.36, N 6.21; Found: C 58.52, H 5.37, N 6.20.

General procedure for the synthesis of 4-substituted-5-methoxy-2-nitrobenzonitriles, **3a-c**

A solution of 4-substituted-3-methoxybenzonitriles **2** (9.10 mmol) in nitric acid (10 mL) was heated to 30 ^oC for 2 h, poured into ice-water (100 mL), filtered, and washed with water to afford compounds **3** as light yellow solids.

4-Ethoxy-5-methoxy-2-nitrobenzonitrile (**3a**): Yield: 88.1%; mp: 198-199 ^oC. ^{1}H-NMR (DMSO-d_6) δ: 7.85 (1H, s, H-3), 7.70 (1H, s, H-6), 4.25 (2H, q, J = 6.9 Hz, $CH_3C\underline{H}_2O$-), 3.97 (3H, s, -OCH_3), 1.37 (3H, t, J = 6.9 Hz, $C\underline{H}_3CH_2O$-); LC-MS: 223.1 $(M+H)^+$; Anal. Calcd. for $C_{10}H_{10}N_2O_4$: C 54.05, H 4.54, N 12.61; Found: C 54.03, H 4.55, N 12.60.

5-Methoxy-2-nitro-4-pentyloxybenzonitrile (**3b**): Yield: 92.9%; mp: 136-137 ^oC. ^{1}H-NMR (DMSO-d_6) δ: 7.89 (1H, s, H-3), 7.80 (1H, s, H-6), 4.16 (2H, t, J = 6.3 Hz, $CH_3CH_2CH_2CH_2C\underline{H}_2O$-), 3.95 (3H, s, -$OCH_3$), 1.72 (2H, m, $CH_3CH_2CH_2C\underline{H}_2CH_2O$-), 1.35 (4H, m, $CH_3C\underline{H}_2C\underline{H}_2CH_2CH_2O$-), 0.89 (3H, t, J =

6.9 Hz, C<u>H</u>$_3$CH$_2$CH$_2$CH$_2$CH$_2$O-); LC-MS: 265.1 $(M+H)^+$; Anal. Calcd. for $C_{13}H_{16}N_2O_4$: C 59.08, H 6.10, N 10.60; Found: C 59.10, H 6.11, N 10.59.

4-(3-Chloropropoxy)-5-methoxy-2-nitrobenzonitrile (**3c**): Yield: 95.3%; mp: 133-134 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.91 (1H, s, H-3), 7.71 (1H, s, H-6), 4.32 (2H, t, J = 6.0 Hz, ClCH$_2$CH$_2$C<u>H</u>$_2$O-), 4.00 (3H, s, -OCH$_3$), 3.78 (2H, t, J = 6.3 Hz, ClC<u>H</u>$_2$CH$_2$CH$_2$O-), 2.23 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 271.0 $(M+H)^+$; Anal. Calcd. for $C_{11}H_{11}ClN_2O_4$: C 48.81, H 4.10, N 10.35; Found: C 48.82, H 4.11, N 10.34.

General procedure for the synthesis of 4-substituted-2-amino-5-methoxybenzonitriles, **4a-c**

A mixture of compound **3** (3.69 mmol) and Pd/C (10%, 7.00 g) in anhydrous ethanol (10.0 mL) was stirred and heated under reflux. Cyclohexene (2.30 mL, 22.6 mmol) was added dropwise. The mixture was refluxed overnight, then cooled to 40 °C, filtered, and washed with ethanol. The filtrate was concentrated to yield a solid. The crude product was suspended in ethanol, stirred at 40 °C for 30 min, cooled to room temperature, filtered to produce pure compound **4** as a yellow solid.

2-Amino-4-ethoxy-5-methoxybenzonitrile (**4a**): Yield: 49.1%; mp: 135-136 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.87 (1H, s, H-6), 6.39 (1H, s, H-3), 5.58 (2H, s, NH$_2$), 3.96 (2H, q, J = 6.9 Hz, CH$_3$C<u>H</u>$_2$O-), 3.65 (3H, s, -OCH$_3$), 1.33 (3H, t, J = 6.9 Hz, C<u>H</u>$_3$CH$_2$O-); LC-MS: 193.1 $(M+H)^+$; Anal. Calcd. for $C_{10}H_{12}N_2O_2$: C 62.49, H 6.29, N 14.57; Found: C 62.50, H 6.30, N 14.58.

2-Amino-5-methoxy-4-pentyloxybenzonitrile (**4b**): Yield: 50.1%; mp: 136-137 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.85 (1H, s, H-6), 6.38 (1H, s, H-3), 5.57 (2H, s, NH$_2$), 3.88 (2H, t, J = 6.3 Hz, CH$_3$CH$_2$CH$_2$CH$_2$C<u>H</u>$_2$O-), 3.63 (3H, s, -OCH$_3$), 1.70 (2H, m, CH$_3$CH$_2$CH$_2$C<u>H</u>$_2$CH$_2$O-), 1.34 (4H, m, CH$_3$C<u>H</u>$_2$C<u>H</u>$_2$CH$_2$CH$_2$O-), 0.88 (3H, t, J = 6.9 Hz, C<u>H</u>$_3$CH$_2$CH$_2$CH$_2$CH$_2$O-); LC-MS: 235.1 $(M+H)^+$; Anal. Calcd. for $C_{13}H_{18}N_2O_2$: C 66.64, H 7.74, N 11.96; Found: C 66.62, H 7.73, N 11.97.

2-Amino-4-(3-chloropropoxy)-5-methoxybenzonitrile (**4c**): Yield: 47.2%; mp: 118-119 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.89 (1H, s, H-6), 6.43 (1H, s, H-3), 5.62 (2H, s, NH$_2$), 4.03 (2H, t, J = 6.1 Hz, ClCH$_2$CH$_2$C<u>H</u>$_2$O-), 3.77 (2H, t, J = 6.4 Hz, ClC<u>H</u>$_2$CH$_2$CH$_2$O-), 3.65 (3H, s, -OCH$_3$), 2.18 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 241.1 $(M+H)^+$; Anal. Calcd. for $C_{11}H_{13}ClN_2O_2$: C 54.89, H 5.44, N 11.64; Found: C 54.88, H 5.45, N 11.63.

General procedure for the synthesis of 7-alkoxy-6-methoxy-4-substituted-1,2,3-benzotriazines **8a-r**

Compound **4** (or compound **5** when preparing **8a**, 10.0 mmol) in 10N-hydrochloric acid (30.0 mL) was cooled to 0 °C and diazotized with sodium nitrite (0.71 g) in water (10.0 mL). The diazonium solution was neutralized with excess of sodium acetate trihydrate and stirred for 2 h at 0 °C with a corresponding substituted aniline (10.0 mmol). The solution was kept overnight at 4 °C, filtered, and washed with water. The crude material was purified on a silica gel column with petroleum ether : ethyl acetate (v/v) = 10:1 to yield a yellow solid **6**. Compound **6** was boiled in 70% ethanol (25.0 mL) for 1

h, then the solution was evaporated under reduced pressure to dryness. Acetic acid (10.0 mL) was added and the solution was refluxed for 2 h, cooled, poured into water (100 mL), filtered, and dried to afford 7-alkoxyl-6-methoxy-4-substituted-1,2,3-benzotriazines **8**.

4-(4-chloroanilino)-1,2,3-benzotriazine (**8a**)*:* Yield: 68.9%; mp: 230 ^{o}C. IR (KBr): 3266, 3086, 1621, 1559, 1493, 1424, 1147, 766 cm^{-1}; ^{1}H-NMR (DMSO-d$_6$) δ: 10.02 (1H, s, NH), 8.62 (1H, d, J = 8.1 Hz), 8.23 (1H, d, J = 8.1 Hz), 8.13 (1H, t, J = 8.2 Hz), 8.05 (1H, m), 7.90 (2H, m), 7.52 (2H, m); LC-MS: 257.1 (M+H)$^{+}$; ^{13}C-NMR (DMSO-d$_6$) δ : 151.02, 143.53, 137.58, 134.73, 132.38, 128.66, 128.06, 127.47, 124.28, 121.87, 109.04; Anal. Calcd. for $C_{13}H_9ClN_4$: C 60.83, H 3.53, N 21.83; Found: C 60.79, H 3.51, N 21.85.

4-(4-Chloroanilino)-7-ethoxy-6-methoxy-1,2,3-benzotriazine (**8b**): Yield: 72.1%; mp: 230 ^{o}C. IR (KBr): 3296, 2928, 1612, 1560, 1494, 1423, 1291, 818 cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$) δ: 9.68 (1H, s, NH), 7.92 (3H, m, H-8, H-2', H-6'), 7.51 (3H, m, H-8, H-3', H-5'), 4.29 (2H, q, *J* = 6.6 Hz, $CH_3C\underline{H}_2O$-), 4.03 (3H, s, -OCH_3), 1.44 (3H, t, *J* = 6.9 Hz, $C\underline{H}_3CH_2O$-); LC-MS: 331.1 (M+H)$^{+}$; ^{13}C-NMR (DMSO-*d*$_6$) δ: 154.22 (C-7), 153.27 (C-6), 150.12 (C-10), 141.65 (C-9), 137.95 (C-1'), 128.57, 127.48, 123.95, 106.78 (C-5), 103.84 (C-4), 100.09 (C-8), 64.74 ($CH_3\underline{C}H_2O$-), 56.73 (-OCH_3), 14.42 ($\underline{C}H_3CH_2O$-); Anal. Calcd. for $C_{16}H_{15}ClN_4O_2$: C 58.10, H 4.57, N 16.94; Found: C 58.01, H 4.56, N 16.96.

7-Ethoxy-6-methoxy-4-(4-methylanilino)-1,2,3-benzotriazine (**8c**): Yield: 73.2%; mp: 249-251 ^{o}C. IR (KBr): 33381, 2980, 1614, 1512, 1426, 1283, 1244, 1103, 856, 816 cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$) δ: 9.50 (s, 1H, NH), 7.90 (s, 1H, H-8), 7.70 (d, 2H, *J* = 8.2 Hz, ArH-2'H, 6'H), 7.53 (s, 1H, H-5), 7.25 (d, 2H, *J* = 8.2 Hz, ArH-3'H, 5'H), 4.29 (q, 2H, *J* = 6.9 Hz, $CH_3C\underline{H}_2O$-), 4.02 (s, 3H, $C\underline{H}_3O$-), 2.34 (s, 3H, ArH-4'$C\underline{H}_3$), 1.44 (t, 3H, *J* = 6.8 Hz, $C\underline{H}_3CH_2O$-); LC-MS: 311.2 (M+H)$^{+}$; ^{13}C-NMR (DMSO-*d*$_6$) δ: 154.16, 153.19, 150.30, 141.62, 138.86, 128.72, 124.04, 122.70, 106.77, 103.82, 100.12, 64.73, 56.72, 20.89, 14.46; Anal. Calcd. for $C_{17}H_{18}N_4O_2$: C 65.79, H 5.85, N 18.05; Found: C 65.68, H 5.81, N 18.07.

4-(3-Chloro-4-fluoroanilino)-7-ethoxy-6-methoxy-1,2,3-benzotriazine (**8d**): Yield: 64.1%; mp: 248-250 ^{o}C. IR (KBr): 3288, 1609, 1554, 1500, 1417, 1292, 1237, 1222, 1106 cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$) δ: 9.66 (s, 1H, NH), 8.17 (m, 1H, ArH-2'H), 7.87 (s, 1H, H-8), 7.82 (m, 1H, ArH-5'H), 7.58 (s, 1H, H-5), 7.51 (d, 1H, *J* = 9 Hz, ArH-6'H), 4.31 (q, 2H, *J* = 6.9 Hz, $CH_3C\underline{H}_2O$-), 4.03 (s, 3H, $C\underline{H}_3O$-), 1.44 (t, 3H, *J* = 6.9 Hz, $C\underline{H}_3CH_2O$-); LC-MS: 349.2 (M+H)$^{+}$; ^{13}C-NMR (DMSO-*d*$_6$) δ: 154.43, 154.29, 153.62, 152.10, 150.35, 141.80, 136.65, 124.10, 123.00, 122.90, 119.55, 117.10, 116.80, 107.15, 104.13, 100.28, 64.69, 56.65, 14.46; Anal. Calcd. for $C_{16}H_{14}ClFN_4O_2$: C 55.10, H 4.05, N 16.06; Found: C 55.00, H 4.07, N 16.04.

4-(4-Chloroanilino)-6-methoxy-7-pentyloxy-1,2,3-benzotriazine (**8e**): Yield: 69.3%; mp: 213 ^{o}C. IR (KBr): 3378, 2954, 1612, 1562, 1507, 1426, 1245, 817 cm^{-1}; ^{1}H-NMR (DMSO-*d*$_6$) δ: 9.63 (1H, s, NH), 7.91 (3H, m, H-8, H-2', H-6'), 7.53 (3H, m, H-8, H-3', H-5'), 4.24 (2H, t, *J* = 6.0 Hz, $CH_3CH_2CH_2CH_2C\underline{H}_2O$-), 4.03 (3H, s, -$OCH_3$), 1.82 (2H, m, $CH_3CH_2CH_2C\underline{H}_2CH_2O$-), 1.40 (4H, m, $CH_3C\underline{H}_2C\underline{H}_2CH_2CH_2O$-), 0.92 (3H, t, *J* = 6.0 Hz, $C\underline{H}_3CH_2CH_2CH_2CH_2O$); LC-MS: 373.2 (M+H)$^{+}$;

^{13}C-NMR (DMSO-d_6) δ: 154.31 (C-7), 153.27 (C-6), 150.30 (C-10), 141.63 (C-9), 138.88 (C-1'), 128.73, 127.40, 123.91, 106.72 (C-5), 103.82 (C-4), 100.05 (C-8), 68.98 ($CH_3CH_2CH_2CH_2\underline{C}H_2O$-), 56.79 (-$OCH_3$), 28.17 ($CH_3CH_2CH_2\underline{C}H_2CH_2O$-), 27.78 ($CH_3CH_2\underline{C}H_2CH_2CH_2O$-), 21.98 ($CH_3\underline{C}H_2CH_2CH_2CH_2O$-), 14.04 ($\underline{C}H_3CH_2CH_2CH_2CH_2O$-); Anal. Calcd. for $C_{19}H_{21}ClN_4O_2$: C 61.21, H 5.68, N 15.03; Found: C 61.11, H 5.64, N 14.99.

6-Methoxy-7-pentyloxy-4-(3-trifluoromethoxyanilino)-1,2,3-benzotriazine (**8f**): Yield: 73.1%; mp: 181-183 ^{o}C. IR (KBr): 3421, 2931, 1613, 1508, 1446, 1258, 1216, 1163, 1105, 845, 784 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.72 (s, 1H, NH), 8.04 (s, 1H, ArH-2'H), 7.92 (d, 1H, J = 8.9 Hz, ArH-4'H), 7.91 (s, 1H, H-8), 7.60 (s, 1H, H-5), 7.56 (t, 2H, J = 8.6 Hz, ArH-5'H), 7.14 (d, 1H, J = 8.7 Hz, ArH-6'H), 4.24 (t, 2H, J = 6.3 Hz, $CH_3CH_2CH_2CH_2\underline{C}H_2O$-), 4.05 (s, 3H, $C\underline{H}_3O$-), 1.83 (m, 2H, $CH_3CH_2CH_2\underline{C}H_2CH_2O$-), 1.42 (m, 4H, $CH_3\underline{C}H_2\underline{C}H_2CH_2CH_2O$-), 0.92 (t, 3H, J = 6.9 Hz, $C\underline{H}_3CH_2CH_2CH_2CH_2O$-); LC-MS: 423.2 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.23, 153.52, 150.28, 148.76, 141.90, 141.06, 130.41, 124.25, 121.80, 118.10, 115.20, 114.04, 106.92, 104.11, 100.19, 68.98, 56.79, 28.17, 27.78, 21.98, 14.04; Anal. Calcd. for $C_{20}H_{21}F_3N_4O_3$: C 56.87, H 5.01, N 13.26; Found: C 56.81, H 5.00, N 13.23.

6-Methoxy-7-pentyloxy-4-(4-trifluoromethoxyanilino)-1,2,3-benzotriazine (**8g**): Yield: 70.2%; mp: 230-232 ^{o}C. IR (KBr): 3426, 2959, 1615, 1510, 1428, 1247, 1200, 1165, 1102, 850, 786 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.69 (s, 1H, NH), 7.98 (d, 2H, J = 9.0 Hz, ArH-3'H, 5'H), 7.90 (s, 1H, H-8), 7.58 (s, 1H, H-5), 7.46 (d, 2H, J = 8.7 Hz, ArH-2'H, 6'H), 4.24 (t, 2H, J = 6.3 Hz, $CH_3CH_2CH_2CH_2C\underline{H}_2O$-), 4.04 (s, 3H, $C\underline{H}_3O$-), 1.83 (m, 2H, $CH_3CH_2CH_2\underline{C}H_2CH_2O$-), 1.42 (m, 4H, $CH_3C\underline{H}_2C\underline{H}_2CH_2CH_2O$-), 0.92 (t, 3H, J = 6.9 Hz, $C\underline{H}_3CH_2CH_2CH_2CH_2O$-); LC-MS: 423.2 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.31, 153.27, 150.30, 144.05, 141.54, 137.91, 125.24, 123.93, 122.14, 121.45, 106.86, 103.83, 100.08, 68.97, 56.76, 28.17, 27.76, 21.97, 14.04; Anal. Calcd. for $C_{20}H_{21}F_3N_4O_3$: C 56.87, H 5.01, N 13.26; Found: C 56.88, H 5.03, N 13.27.

7-(3-Chloropropoxy)-4-(3,5-dichloroanilino)-6-methoxy-1,2,3-benzotriazine (**8h**): Yield: 72.9%; mp: 110-112 ^{o}C. IR (KBr): 3430, 2919, 1620, 1508, 1423, 1289, 849 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.74 (1H, s, NH), 8.10 (2H, d, J = 1.77 Hz, H-2', H-6'), 7.91 (1H, s, H-8), 7.68 (1H, s, H-5), 7.37 (1H, s, H-4'), 4.39 (2H, t, J = 6.15 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 4.06 (3H, s, -$OCH_3$), 3.84 (2H, t, J = 6.5 Hz, $ClC\underline{H}_2CH_2CH_2O$-), 2.30 (2H, m, $ClCH_2C\underline{H}_2CH_2O$-); LC-MS: 413.0 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.57 (C-7), 153.92 (C-6), 150.31 (C-10), 142.11 (C-9), 141.94, 134.28, 122.82, 120.06, 107.51 (C-5), 104.54 (C-4), 100.44 (C-8), 66.29 ($ClCH_2CH_2\underline{C}H_2O$-), 57.26 (-$OCH_3$), 42.19 ($Cl\underline{C}H_2CH_2CH_2O$-), 31.84 ($ClCH_2\underline{C}H_2CH_2O$-); Anal. Calcd. for $C_{17}H_{15}Cl_3N_4O_2$: C 49.36, H 3.65, N 13.54; Found: C 49.28, H 3.66, N 13.56.

7-(3-Chloropropoxy)-4-(3,4-dichlorophenyl)-6-methoxy-1,2,3-benzotriazine (**8i**): Yield: 72.4%; mp: 120-122 ^{o}C. IR (KBr): 3424, 1616, 1510, 1426, 1281, 848 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.74 (1H, s, NH), 8.31 (1H, d, J = 2.31 Hz, H-2'), 7.93 (2H, m, H-8, H-6'), 7.71 (1H, m, H-5'), 7.67 (1H, s, H-5), 4.38 (2H, t, J = 6.0 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 4.05 (3H, s, -$OCH_3$), 3.84 (2H, t, J = 6.4 Hz,

ClC<u>H</u>$_2$CH$_2$CH$_2$O-), 2.30 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 413.0 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.11 (C-7), 153.43 (C-6), 149.95 (C-10), 141.66 (C-9), 139.23, 130.84, 130.51, 125.02, 123.14, 121.90, 107.10 (C-5), 104.11 (C-4), 100.08 (C-8), 65.86 (ClCH$_2$CH$_2$<u>C</u>H$_2$O-), 56.85 (-OCH$_3$), 41.87 (Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 31.46 (ClCH$_2$<u>C</u>H$_2$CH$_2$O-); Anal. Calcd. for $C_{17}H_{15}Cl_3N_4O_2$: C 49.36, H 3.65, N 13.54; Found: C 49.31, H 3.66, N 13.56.

4-(4-Chloroanilino)-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**8j**): Yield: 67.9%; mp: 253 °C. IR (KBr): 3425, 2925, 1616, 1561, 1495, 1424, 1245, 839 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.66 (1H, s, NH), 7.91 (3H, m, H-8, H-2', H-6'), 7.62 (1H, s, H-5), 7.50 (2H, d, J = 8.8 Hz, H-3', H-5'), 4.37 (2H, t, J = 6.0 Hz, ClCH$_2$CH$_2$C<u>H</u>$_2$O-), 4.04 (3H, s, -OCH$_3$), 3.84 (2H, t, J = 6.4 Hz, Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 2.29 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 379.0 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 153.99 (C-7), 153.29 (C-6), 150.12 (C-10), 141.57 (C-9), 137.91 (C-1'), 128.59, 127.54, 123.99, 107.07 (C-5), 104.07 (C-4), 100.23 (C-8), 65.82 (ClCH$_2$CH$_2$<u>C</u>H$_2$O-), 56.82 (-OCH$_3$), 41.87 (Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 31.47 (ClCH$_2$<u>C</u>H$_2$CH$_2$O-); Anal. Calcd. for $C_{17}H_{16}Cl_2N_4O_2$: C 53.84, H 4.25, N 14.77; Found: C 53.79, H 4.24, N 14.71.

7-(3-Chloropropoxy)-6-methoxy-4-(4-methylanilino)-1,2,3-benzotriazine (**8k**): Yield: 70.1%; mp: 247 °C. IR (KBr): 3420, 2924, 1615, 1513, 1425, 1282, 1242, 859 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.55 (1H, s, NH), 7.92 (1H, s, H-8), 7.69 (2H, d, J = 8.4 Hz, H-2', H-6'), 7.59 (1H, s, H-5), 7.25 (2H, d, J = 8.4 Hz, H-3', H-5'), 4.37 (2H, t, J = 6.0 Hz, ClCH$_2$CH$_2$C<u>H</u>$_2$O-), 4.03 (3H, s, -OCH$_3$), 3.84 (2H, t, J = 6.3 Hz, Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 2.34 (3H, s, -CH$_3$), 2.29 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 359.1 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.23 (C-7), 153.51 (C-6), 150.62 (C-10), 141.59 (C-9), 136.43 (C-1'), 133.64, 129.48, 123.22, 107.28 (C-5), 104.42 (C-4), 100.86 (C-8), 66.14 (ClCH$_2$CH$_2$<u>C</u>H$_2$O-), 57.22 (-OCH$_3$), 42.24 (Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 31.84 (ClCH$_2$<u>C</u>H$_2$CH$_2$O-), 20.99(Ph-<u>C</u>H$_3$); Anal. Calcd. for $C_{18}H_{19}ClN_4O_2$: C 60.25, H 5.34, N 15.61; Found: C 60.28, H 5.33, N 15.56.

7-(3-Chloropropoxy)-4-(4-fluoroanilino)-6-methoxy-1,2,3-benzotriazine (**8l**): Yield: 71.2%; mp: 166 °C. IR (KBr): 3437, 1620, 1510, 1428, 1284, 842 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.63 (1H, s, NH), 7.91 (1H, s, H-8), 7.84 (2H, m, H-2', H-6'), 7.61 (1H, s, H-5), 7.29 (2H, t, J = 8.7 Hz, H-3', H-5'), 4.37 (2H, t, J = 6.0 Hz, ClCH$_2$CH$_2$C<u>H</u>$_2$O-), 4.03 (3H, s, -OCH$_3$), 3.84 (2H, t, J = 6.4 Hz, Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 2.29 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 363.1 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 160.67, 157.48, 154.28 (C-7), 153.56 (C-6), 150.64 (C-10), 141.79 (C-9), 135.46 (C-1'), 125.15, 125.04, 115.79, 115.50, 107.40 (C-5), 104.29 (C-4), 100.67 (C-8), 66.18 (ClCH$_2$CH$_2$<u>C</u>H$_2$O-), 57.17 (-OCH$_3$), 42.22 (Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 31.86 (ClCH$_2$<u>C</u>H$_2$CH$_2$O-); Anal. Calcd. for $C_{17}H_{16}ClFN_4O_2$: C 56.28, H 4.45, N 15.44; Found: C 56.20, H 4.43, N 15.56.

4-(3-Chloro-4-fluoroanilino)-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**8m**): Yield: 77.1%; mp: 138-139 °C. IR (KBr): 3314, 2923, 1616, 1571, 1511, 1427, 1285, 849 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.70 (1H, s, NH), 8.18 (1H, d, J = 4.4 Hz, H-2'), 7.90 (1H, s, H-8), 7.82 (1H, m, H-6'), 7.64 (1H, s, H-5), 7.51 (1H, t, J = 9.1 Hz, H-5'), 4.38 (2H, t, J = 5.9 Hz, ClCH$_2$CH$_2$C<u>H</u>$_2$O-), 4.04 (3H, s, -OCH$_3$), 3.84 (2H, t, J = 6.4 Hz, Cl<u>C</u>H$_2$CH$_2$CH$_2$O-), 2.29 (2H, m, ClCH$_2$C<u>H</u>$_2$CH$_2$O-); LC-MS: 397.0 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 155.47 (C-4'), 154.32 (C-7), 153.64 (C-6), 152.25 (C-4'), 150.32 (C-10),

141.81 (C-9), 136.57 (C-1'), 124.15 (C-2'), 122.93 (C-6'), 122.84 (C-6'), 119.47 (C-6'), 117.14 (C-5'), 116.85 (C-5'), 107.33 (C-5), 104.28 (C-4), 100.56 (C-8), 66.19 ($ClCH_2CH_2\underline{C}H_2O$-), 57.21 (-$OCH_3$), 42.18 ($Cl\underline{C}H_2CH_2CH_2O$-), 31.86 ($ClCH_2\underline{C}H_2CH_2O$-); Anal. Calcd. for $C_{17}H_{15}Cl_2FN_4O_2$: C 51.40, H 3.81, N 14.10; Found: C 51.41, H 3.85, N 14.08.

7-(3-Chloropropoxy)-6-methoxy-4-(3-trifluoromethoxyanilino)-1,2,3-benzotriazine (**8n**): Yield: 65.3%; mp: 154-155 °C. IR (KBr): 3427, 1615, 1572, 1511, 1448, 1256, 854 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.75 (1H, s, NH), 8.05 (1H, s, H-2'), 7.93 (2H, s, H-8, H-6'), 7.65 (1H, s, H-5), 7.59 (1H, t, *J* = 8.2 Hz, H-5'), 7.15 (1H, d, *J* = 7.9 Hz, H-4'), 4.38 (2H, t, *J* = 5.9 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 4.06 (3H, s, -$OCH_3$), 3.84 (2H, t, *J* = 6.4 Hz, $ClC\underline{H}_2CH_2CH_2O$-), 2.29 (2H, m, $ClCH_2C\underline{H}_2CH_2O$-); LC-MS: 429.1 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.34 (C-7), 153.65 (C-6), 150.40 (C-10), 148.82, 141.96 (C-9), 141.12 (C-1'), 130.54, 124.05, 122.30, 118.90, 115.91, 114.64, 107.32 (C-5), 104.41 (C-4),100.46 (C-8), 66.15 ($ClCH_2CH_2\underline{C}H_2O$-), 57.17 (-$OCH_3$), 42.17 ($Cl\underline{C}H_2CH_2CH_2O$-), 31.82 ($ClCH_2\underline{C}H_2CH_2O$-); Anal. Calcd. for $C_{18}H_{16}ClF_3N_4O_3$: C 50.42, H 3.76, N 13.07; Found: C 50.37, H 3.78, N 13.06.

7-(3-Chloropropoxy)-6-methoxy-4-(4-trifluoromethoxyanilino)-1,2,3-benzotriazine (**8o**): Yield: 63.0%; mp: 150 °C. IR (KBr): 3444, 1618, 1574, 1510, 1429, 1247, 852 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.68 (1H, s, NH), 7.93 (2H, d, *J* = 9.0 Hz, H-2', H-6'), 7.88 (1H, s, H-8), 7.59 (1H, s, H-5), 7.40 (2H, d, *J* = 8.7 Hz, H-3', H-5'), 4.33 (2H, t, *J* = 5.9 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 4.00 (3H, s, -$OCH_3$), 3.79 (2H, t, *J* = 6.6 Hz, $ClC\underline{H}_2CH_2CH_2O$-), 2.25 (2H, m, $ClCH_2C\underline{H}_2CH_2O$-); LC-MS: 429.1 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.41 (C-7), 153.70 (C-6), 150.54 (C-10), 144.48, 141.97 (C-9), 138.51 (C-1'), 125.64, 124.23, 122.34, 121.79, 107.46 (C-5), 104.43 (C-4), 100.68 (C-8), 66.23 ($ClCH_2CH_2\underline{C}H_2O$-), 57.22 (-$OCH_3$), 42.21 ($Cl\underline{C}H_2CH_2CH_2O$-), 31.85 ($ClCH_2\underline{C}H_2CH_2O$-); Anal. Calcd. for $C_{18}H_{16}ClF_3N_4O_3$: C 50.42, H 3.76, N 13.07; Found: C 50.39, H 3.75, N 13.04.

7-(3-Chloropropoxy)-4-(4-fluoro-3-trifluoromethylanilino)-6-methoxy-1,2,3-benzotriazine (**8p**): Yield: 71.1%; mp: 150 °C. IR (KBr): 3452, 2920, 1614, 1508, 1432, 1290, 1129 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.82 (1H, s, NH), 8.18 (2H, m, H-6', H-2'), 7.91 (1H, s, H-8), 7.65 (1H, s, H-5), 7.60 (1H, m, H-5'), 4.38 (2H, t, *J* = 6.0 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 4.05 (3H, s, -$OCH_3$), 3.84 (2H, t, *J* = 6.3 Hz, $ClC\underline{H}_2CH_2CH_2O$-), 2.30 (2H, m, $ClCH_2C\underline{H}_2CH_2O$-); LC-MS: 431.1 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 156.36, 154.04 (C-7), 153.35 (C-6), 150.01 (C-10), 141.53 (C-9), 135.74 (C-1'), 128.37, 128.26, 124.46, 120.85, 120.26, 117.61, 117.33, 107.05 (C-5), 103.94 (C-4), 100.10 (C-8), 65.83 ($ClCH_2CH_2\underline{C}H_2O$-), 56.80 (-$OCH_3$), 41.78 ($Cl\underline{C}H_2CH_2CH_2O$-), 31.44 ($ClCH_2\underline{C}H_2CH_2O$-); Anal. Calcd. for $C_{18}H_{15}ClF_4N_4O_2$: C 50.19, H 3.51, N 13.01; Found: C 50.17, H 3.53, N 13.00.

7-(3-Chloropropoxy)-6-methoxy-4-(3-trifluoromethylanilino)-1,2,3-benzotriazine (**8q**): Yield: 75.2%; mp: 180-181 °C. IR (KBr): 3438, 2925, 1623, 1576, 1510, 1448, 1244, 799 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 9.81 (1H, s, NH), 8.30 (1H, s, H-2'), 8.25 (1H, d, *J* = 8.4 Hz, H-6'), 7.94 (1H, s, H-8), 7.70 (1H, d, *J* = 7.8 Hz, H-5'), 7.66 (1H, s, H-5), 7.51 (1H, d, *J* = 8.1 Hz, H-4'), 4.38 (2H, t, *J* = 6.0 Hz, $ClCH_2CH_2C\underline{H}_2O$-), 4.06 (3H, s, -$OCH_3$), 3.84 (2H, t, *J* = 6.4 Hz, $ClC\underline{H}_2CH_2CH_2O$-), 2.29 (2H, m, $ClCH_2C\underline{H}_2CH_2O$-); LC-MS: 413.1 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 154.10 (C-7), 153.41 (C-6), 150.14 (C-10), 141.66 (C-9), 139.87 (C-1'), 129.88, 129.53, 129.32, 125.71, 119.93, 118.17, 107.09 (C-5), 104.11 (C-4), 100.14 (C-8), 65.85 ($ClCH_2CH_2\underline{C}H_2O$-), 56.83 (-$OCH_3$), 41.84

(Cl<u>C</u>$H_2CH_2CH_2O$), 31.45 ($ClCH_2$<u>C</u>H_2CH_2O-); Anal. Calcd. for $C_{18}H_{16}ClF_3N_4O_2$: C 52.37, H 3.91, N 13.57; Found: C 52.32, H 3.94, N 13.56.

4-Anilino-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**8r**): Yield: 70.1%; mp: 243 ^oC. IR (KBr): 3435, 2922, 1614, 1566, 1502, 1447, 1421, 1285, 1241, 1108, 850, 744 cm^{-1}; ^1H-NMR (DMSO-d_6) δ: 9.59 (1H, s, NH), 7.94 (1H, s, H-8), 7.84 (2H, d, *J* = 7.8 Hz, ArH-2'H, 6'H), 7.61 (1H, s, H-5), 7.45 (2H, t, *J* = 7.8 Hz, ArH-3'H, 5'H), 7.18 (1H, t, *J* = 7.5 Hz, ArH-4'H), 4.37 (2H, t, *J* = 6.0 Hz, $ClCH_2CH_2C$<u>H</u>$_2O$-), 4.04 (3H, s, $-OCH_3$), 3.84 (2H, t, *J* = 6.3 Hz, ClC<u>H</u>$_2CH_2CH_2O$-), 2.29 (2H, m, $ClCH_2C$<u>H</u>$_2CH_2O$-); LC-MS: 345.2 $(M+H)^+$; ^{13}C-NMR (DMSO-d_6) δ: 153.88 (C-7), 153.17 (C-6), 150.28 (C-10), 141.50 (C-9), 138.83 (C-1'), 128.68 (C-3', C-5'), 124.03 (C-4'), 122.88 (C-2', C-6'), 107.01 (C-5), 104.02 (C-4), 100.31 (C-8), 65.78 ($ClCH_2CH_2$<u>C</u>H_2O-), 56.81 ($-OCH_3$), 41.89 (Cl<u>C</u>$H_2CH_2CH_2O$-), 31.48 ($ClCH_2$<u>C</u>H_2CH_2O-); Anal. Calcd. for $C_{17}H_{17}ClN_4O_2$: C 59.22, H 4.97, N 16.25; Found: C 59.28, H 4.96, N 16.17.

Antiproliferative activity assay

Cell lines: The cell lines used were obtained from ATCC (Rockville, MD, USA). T47D human breast cancer cells, DU145 and PC-3 human prostate cancer cells were cultured in RPMI1640 medium (Gibco; New York, NY, USA) supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, 1 mM L-glutamine, 5 μg/mL insulin and 10% (v/v) heat-inactivated fetal bovine serum (FBS). MVECs, LL/2 murine lewis lung carcinoma cells and B16F0 melanoma cells were cultured in DMEM with 4 mM L-glutamine adjusted to contain 4.50 g/L glucose and 10% (v/v) heat-inactivated FBS.

Cell growth inhibition assay: The antiproliferative activities of these compounds were determined by the MTT assay. Cells (2×10^3 cells/well in 96-well plates) were incubated for 24 h, then various concentrations of each compound were added to each well and the cells were cultured for another 4 days at 37 ^oC. MTT solution (50 μL of 2 mg/mL) was added per well and the culture continued for an additional 4 h. The medium was removed by aspiration and the cells were dissolved in 200 μL DMSO. The absorbance at 570 nm was measured in the 96-well plate reader. Growth inhibition is reported as compared to untreated cells (%) and GI_{50} concentration was calculated [18].

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