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Synthesis and biological evaluation of substituted 1,2,3-benzotriazines and pyrido[3,2-*d*]-1,2,3-triazines as inhibitors of vascular endothelial growth factor receptor-2

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ABSTRACT

A novel series of substituted 1,2,3-benzotriazines and pyrido[3,2-*d*]-1,2,3-triazines were synthesized. The abilities of these compounds to inhibit the VEGFR-2 kinase activity and the proliferation of human microvascular endothelial cells (MVECs) were determined. 6-Methoxy-4-substituted-1,2,3-benzotriazines and 4-substituted-6-chloro-pyrido[3,2-*d*]-1,2,3-triazines have the abilities of inhibiting the VEGFR-2 kinase activity, but only the 4-substituted-6-chloro-pyrido[3,2-*d*]-1,2,3-triazines exhibit good growth inhibitory effects on MVECs. Compound 6-chloro-4-(3-trifluoromethylanilino)-pyrido[3,2-*d*][1,2,3]triazin (**11d**) is less half active than **PTK787** to inhibit the VEGFR-2 kinase activity, but is more active than **PTK787** to inhibit the growth of MVECs. The potential binding modes of **6d**, **11d**, and **CTZ12** in complex with their putative intracellular target, VEGFR-2, were predicted using *Surflex-Dock*.

1. Introduction

Vascular endothelial growth factor receptor-2 (VEGFR-2) is a receptor tyrosine kinase expressed on the endothelial cells, which belongs to the immunoglobulin subclass of the receptor tyrosine kinase superfamily and has seven Ig-like extracellular domains with a single transmembrane helix with an intracellular kinase region.[1,2] Following binding of vascular endothelial growth factor A (VEGFA), VEGFR-2 has the potential to form either a homodimer or a heterodimer complex with VEGFR-1, resulting in intracellular tyrosine phosphorylation.[3] The downstream effects of VEGFR-2 signaling which culminate in angiogenesis include a potent increase in vascular permeability and promotion of endothelial cell migration, proliferation, and survival.[4–9] Disruption of VEGF signaling pathway by either specific binding of circulating VEGF or inhibiting receptor tyrosine kinases with small molecules has been found to inhibit angiogenesis, tumor progression and dissemination.[10–13] The recombinant humanized anti-VEGF monoclonal antibody Avastin has been approved for the treatment of metastatic colorectal cancer.[14] The small molecule VEGFR-2 kinase inhibitor sunitinib (**SU11248**) was approved by the FDA for the treatment of advanced renal cell carcinoma.[15] A number of synthetic molecules have been developed as potent VEGFR-2 inhibitors for the treatment of various cancers and some are currently undergoing phase III clinical studies, including **PTK787** (vatalanib, ZK222584). **PTK787** inhibited VEGF-induced autophosphorylation of VEGFR-2 and endothelial cell proliferation.[16,17]

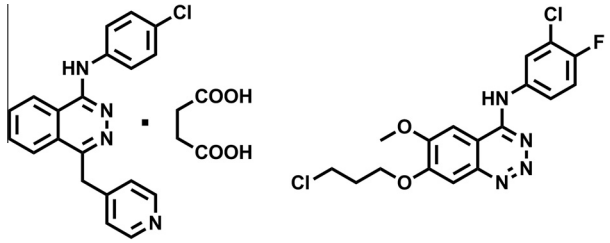

Figure 1. Structures of **PTK787** and **CTZ12**.

Previously we have synthesized a series of novel substituted 1,2,3-benzotriazines with a similar backbone structure and found that they could inhibit the proliferation of human microvascular endothelial cells (MVECs). The most effective compound 4-(3-chloro-4-fluoroanilino-)-7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine (**CTZ12**) was 4–10 fold more potent than **PTK787** in inhibiting the growth of MVECs (Fig. 1).[18] To improve the ability of

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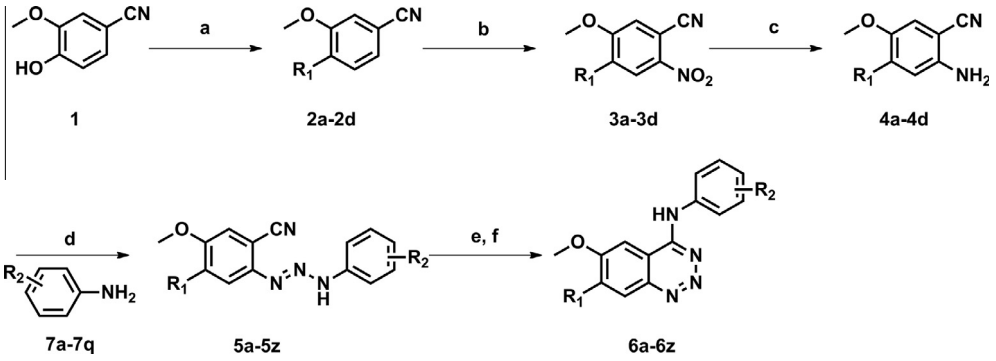


Scheme 1. Synthetic route to the target compounds **6a**–**6z**. Reagents and conditions: (a) alkyl halide, K_2CO_3, DMF, 37 °C, 6 h; (b) HNO_3, 30 °C, 2 h; (c) Pd/C, cyclohexene, EtOH, reflux, overnight; (d) $NaNO_2$/10 N HCl, 0 °C; substituted aniline, 4 °C, 2 h; (e) 70% EtOH, reflux, 1 h; (f) AcOH, reflux, 2 h.

CTZ12 to inhibit the proliferation of MVECs, a new series of substituted 1,2,3-benzotriazines as well as the pyrido[3,2-*d*]-1,2,3-triazine derivatives were synthesized. The anti-proliferative effects of these compounds on MVECs as well as their abilities of inhibiting VEGFR-2 kinase activity were tested and analyzed.

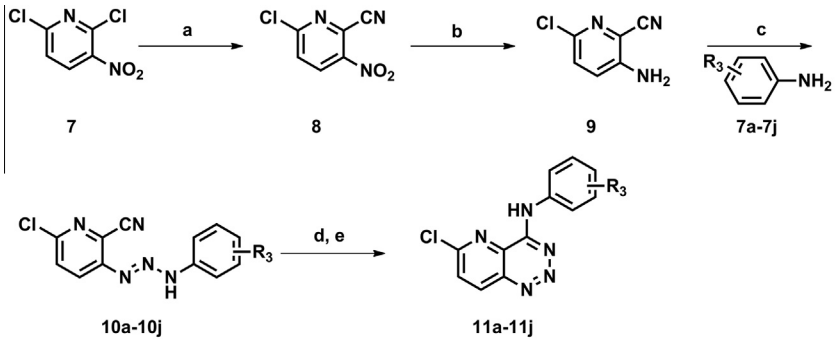
2. Chemistry

Compounds 7-substituted-6-methoxy-4-substituted-1,2,3-benzotriazines (**6a**–**6z**) were synthesized according to the synthetic route as described in Scheme 1. The intermediates 4-substituted-3-methoxybenzonitriles (**2a**–**2d**) were prepared by the reaction of different alkyl halides with 4-cyano-2-methoxyphenol (**1**) in the presence of potassium carbonate in DMF. The selectively nitration of compounds (**2a**–**2d**) in HNO_3 gave the nitro compounds (**3a**–**3d**) in good yields, following with the reduction reaction by Pd/C as catalyst to obtain the corresponding amines (**4a**–**4d**). Compounds (**4a**–**4z**) were diazotized and then coupled with substituted anilines at 0 °C, which were purified by recrystallization with ethyl acetate to afford triazenes (**5a**–**5z**). Compounds (**5a**–**5z**) were boiled in 70% ethanol, then directly rearranged to the final compounds (**6a**–**6z**) in refluxed acetic acid after evaporating the ethanol.

The preparation of target compounds 4-substituted-6-chloro-pyrido[3,2-*d*]-1,2,3-triazines (**11a**–**11j**) were described in Scheme 2. The intermediate 3-amino-6-chloropicolinonitrile (**9**) was synthesized by the reaction of 2,6-dichloro-3-nitropyridine with cuprous cyanide in *N*-methyl-pyrrolidone, followed by the reduction with sodium hyposulfite in dichloromethane/aqua solution using TBAB as a phase-transfer catalyst. Subsequently, the intermediate **9** was diazotized and then coupled with substituted anilines (**7a**–**7j**). After purification by recrystallization with ethyl acetate, compounds (**10a**–**10j**) were boiled in 70% ethanol, which were then directly rearranged to the final compounds (**11a**–**11j**) in refluxed acetic acid after evaporating the ethanol.

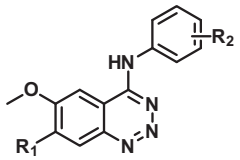
3. Biological evaluation and discussion

Although **CTZ12** was more effective than **PTK787** in inhibiting the proliferation of MVECs, it has minimal ability of inhibiting the VEGFR-2 kinase activity (Table 1). Replacement of 3-chloro-4-fluoro at the C4 anilino group (**CTZ12**) with 4-trifluoromethyl (**6y**) or 4-methyl (**6z**) did not improve the ability of inhibiting VEGFR-2 activity, but decreased the ability of inhibiting proliferation of MVECs. Replacement of 7-chloropropoxy at the C7 position of 1,2,3-benzotriazine with an ethoxy group (**6a**–**6i**), but not with a *n*-butoxy (**6j**–**6r**) or a pentlyoxy (**6s**–**6x**), improved the ability of inhibiting VEGFR-2 activity. However, all these compounds decreased the ability of inhibiting cell growth of MVECs. Also replacement of 3-chloro-4-fluoro at the C4 anilino group with a different substituted group (X) (**6a**–**6i**) did not significantly influence the ability of inhibiting VEGFR-2 activity. Replacing 7-(3-chloropropoxy)-6-methoxy-1,2,3-benzotriazine with 6-chloro-pyrido[3,2-*d*]-1,2,3-triazin evidently improved the ability of inhibiting VEGFR-2 activity (**11a**–**11j**) with similar or improved ability of inhibiting growth of MVECs comparing to **CTZ12** (Table 2). Although these 4-substituted-6-chloro-pyrido[3,2-*d*]-1,2,3-triazines showed less activities than **PTK787** to inhibit VEGFR-2 activity, they were more effective than **PTK787** to inhibit growth of MVECs. Based on these data we found that: (a) Introduction of a N atom at 5-position of 1,2,3-benzotriazines (6-chloro-pyrido[3,2-*d*]-1,2,3-triazine ring system) derivatives improved the ability of inhibiting VEGFR-2 activity (**11a**–**11j**) with similar or better ability of inhibiting cell growth of MVECs comparing to **CTZ12**; (b) 7-site alkoxyl group



Scheme 2. Synthetic route to the target compounds **11a**–**11j**. Reagents and conditions: (a) CuCN, NMP, DMF, 180 °C, 15 min; (b) $Na_2S_2O_4$, TBAB, CH_2Cl_2, H_2O, 30 °C, 4 h; (c) $NaNO_2$/10 N HCl, 0 °C; substituted aniline, 0 °C, 2 h; (d) 70% EtOH, reflux, 1 h; (e) AcOH, reflux, 2 h.

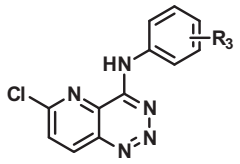
Table 1
The structures of target compounds **6a–6z** and their abilities of inhibiting VEGFR-2 activity and the proliferation of MVECs



Compd	R_1	R_2	VEGFR-2 inhibition [a] (%)	MVECs GI_{50} [b] (μM)	Compd	R_1	R_2	VEGFR-2 inhibition (%)	MVECs GI_{50} (μM)
6a	Ethoxy	3-F-4-Br	35.77 ± 0.56	73.30 ± 0.06	**6o**	*n*-Butoxy	2-F	12.03 ± 1.65	>80
6b	Ethoxy	3-CF_3	37.34 ± 1.43	>80	**6p**	*n*-Butoxy	3-Br	<10	16.80 ± 0.68
6c	Ethoxy	4-CF_3	24.66 ± 0.66	>80	**6q**	*n*-Butoxy	4-F	<10	>80
6d	Ethoxy	3-OCF_3	49.20 ± 2.46	23.28 ± 1.50	**6r**	*n*-Butoxy	4-CH_3	12.03 ± 0.62	>80
6e	Ethoxy	4-OCF_3	38.44 ± 1.48	>80	**6s**	Pentlyoxy	4-Cl	18.98 ± 2.51	25.34 ± 0.94
6f	Ethoxy	3-Cl	45.50 ± 0.19	59.99 ± 1.13	**6t**	Pentlyoxy	3-F-4-Br	19.78 ± 0.28	>80
6g	Ethoxy	3-CF_3-4-F	46.78 ± 0.22	31.65 ± 0.57	**6u**	Pentlyoxy	3,5-DiF	10.70 ± 1.17	27.93 ± 1.33
6h	Ethoxy	2-F	48.87 ± 1.15	>80	**6v**	Pentlyoxy	3-OCF_3	<10	42.64 ± 1.18
6i	Ethoxy	3-Br	44.10 ± 1.26	39.79 ± 0.79	**6w**	Pentlyoxy	4-OCF_3	11.22 ± 0.38	37.98 ± 1.14
6j	*n*-Butoxy	3-F-4-Br	<10	>80	**6x**	Pentlyoxy	4-F	11.50 ± 1.57	>80
6k	*n*-Butoxy	3,4-diCl	10.70 ± 2.49	15.50 ± 0.66	**6y**	Chloropropoxy	4-CF_3	<10	44.38 ± 1.37
6l	*n*-Butoxy	4-CF_3	<10	>80	**6z**	Chloropropoxy	4-CH_3	14.20 ± 1.99	17.08 ± 0.65
6m	*n*-Butoxy	4-OCF_3	10.96 ± 1.74	30.04 ± 0.47	**CTZ12**			<10	7.98 ± 0.35
6n	*n*-Butoxy	3-CF_3-4-F	12.57 ± 2.23	16.06 ± 1.48	**PTK787** [c]			100	38.15 ± 2.07

[a] VEGFR-2 inhibition: the inhibition rate of the compounds against VEGFR-2 kinase at the concentration of 10 μg/mL.
[b] GI_{50}: the concentration of the compounds (μM) producing 50% cell growth inhibition after 4 days of drug exposure, as determined by the MTT assay. Data shown are means ± SD of three independent experiments.
[c] Used as positive control.

Table 2
The structures of target compounds **11a–11j** and their abilities of inhibiting VEGFR-2 activity and the proliferation of MVECs



Compd	R_3	VEGFR-2 inhibition[a] (%)	MVECs GI_{50} [b] (μM)	Compd	R_3	VEGFR-2 inhibition (%)	MVECs GI_{50} (μM)
11a	4-Cl	29.83 ± 2.21	10.50 ± 0.55	**11g**	4-OCF_3	29.67 ± 2.23	11.49 ± 0.99
11b	3-F-4-Br	30.75 ± 1.16	9.56 ± 1.17	**11h**	3-CF_3-4-F	12.08 ± 2.99	10.78 ± 1.40
11c	3,5-DiCl	30.08 ± 0.99	7.93 ± 1.42	**11i**	2-F	31.56 ± 0.54	10.83 ± 0.14
11d	3-CF_3	40.00 ± 1.12	5.25 ± 0.09	**11j**	3-Br	30.80 ± 0.98	7.24 ± 0.09
11e	4-CF_3	17.24 ± 1.20	8.06 ± 0.10	**CTZ12**		<10	7.98 ± 0.35
11f	3-OCF_3	29.93 ± 2.21	4.28 ± 0.94	**PTK787** [c]		100	38.15 ± 2.07

[a] VEGFR-2 inhibition: the inhibition rate of the compounds against VEGFR-2 kinase at the concentration of 10 μg/mL.
[b] GI_{50}: the concentration of the compounds (μM) producing 50% cell growth inhibition after 4 days of drug exposure, as determined by the MTT assay. Data shown are means ± SD of three independent experiments.
[c] Used as positive control.

of the 1,2,3-benzotriazines derivatives is an important moiety to inhibit VEGFR-2 kinase activity. Replacement of 7-chloropropoxy at the C7 position of 1,2,3-benzotriazine with an ethoxy group (**6a–6i**), but not with a *n*-butoxy (**6j–6r**) or a pentlyoxy (**6s–6x**), improved the ability of inhibiting VEGFR-2 activity.

A docking analysis was carried out to compare the potential binding modes of some compounds with VEGFR-2. To illustrate the docking protocol, the native ligand 4-amino-furo[2,3-*d*]pyrimidine was removed from the Protein Data Bank structure (PDB entry **1YWN**)[19] and redocked into the ATP-binding site of VEGFR-2. The docked result was compared to the crystal structure of the bound ligand–protein complex and illustrated the post-docked ligand binds well to the cleft of the VEGFR-2. The RMSD value between the original ligand and post-docked ligand is 0.329. These docking results better reconstruct the crystal structure and the parameters are suitable for studying.

Using this model, **PTK787** was also docked into the ATP-binding site of VEGFR-2 kinase and the binding mode was almost consistent to the data reported previously.[20] The phthalazine bicycle makes hydrophobic contacts with residues Leu 1033, Gly 920, Cys917, and Leu 838, the aniline moiety occupies the hydrophobic pocket formed by residues Val 914, Val 912, Glu915, Val 897, Leu 887, Glu883, Lys 866, and Cys1043, and the pyridyl ring engages in a hydrophobic region containing the residues Gly920, Leu1033, Cys1043, and Asn1031 (Fig. 2).

Compounds **6d**, **11d** with medium inhibiting ability and **CTZ12** with minimal inhibiting ability on VEGFR-2 activity were selected for docking evaluation as representative examples. The predicted binding modes of **6d**, 11d, and **CTZ12** are shown in Figure 3. The anilino moiety of **6d**, **11d**, and **CTZ12** is located in the hydrophobic pocket formed by residues Lys 866, Val 897, Leu 887, Val 912, Val 914, Cys 1043, and Phe 1045, while the 1,2,3-benzotriazine and

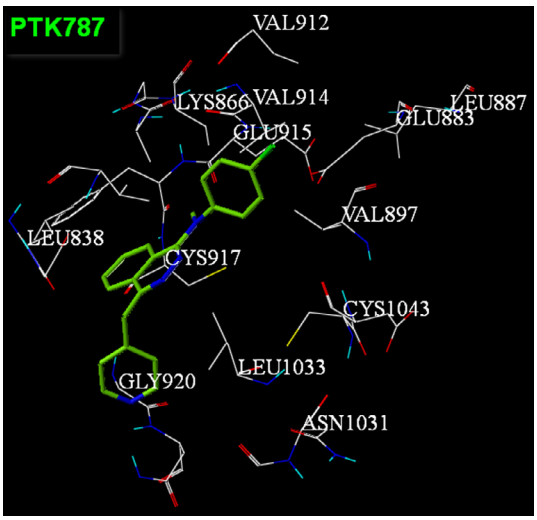


Figure 2. Binding model of compound **PTK787** in complex with VEGFR-2 kinase. **PTK787** is shown in stick model colored by green and the receptor is shown in lines model colored by atom type.

pyrido[3,2-*d*]-1,2,3-triazine bicycle make hydrophobic contacts with residues Leu 838, Gly 920, and Leu 1033. Unlike compounds **6d** and **CTZ12**, the anilino NH group of compound **11d** forms direct hydrogen bonds with the backbone of Cys 917. This may explain why compound **11d** shows more potent inhibitory activity against VEGFR-2 than **6d** and **CTZ12**. Glu 915 and Cys 917 are the residues of the hinge region in the ATP-binding site of VEGFR-2 which may be involved in hydrogen contracts with the adenine ring of ATP and maybe allow water-mediated hydrogen bonds with the anilino NH group of the inhibitor **PTK787**, **6d**, and **CTZ12**).[20] The reason that **CTZ12** is less effective to inhibit the VEGFR-2 activity than **6d** might be due to the steric hindrance of 3-chloropropoxy group, which is too large to enter into the hydrophobic region formed by the residues of Gly920, Leu1033, Cys1043, and Asn1031.

Based on the predicted binding modes in Figure 3, it seems that introducing a small substituted group at 7- or 8-position of 1,2,3-benzotriazines or pyrido[3,2-*d*]-1,2,3-triazines forming hydrogen bonds with CYS917, GLY920 would improve the activity. Also, in the cavity formed by VAL912, GLU883, and LEU887, adding hydrophobic groups with ability to form hydrogen bonds will elevate the activity.

4. Conclusion

A series of substituted 1,2,3-benzotriazines and pyrido[3,2-*d*]-1,2,3-triazines were synthesized and evaluated for their abilities to inhibit the VEGFR-2 kinase activity and the proliferation of MVECs. 6-methoxy-4-substituted-1,2,3-benzotriazines and 4-substituted-6-chloro-pyrido[3,2-*d*]-1,2,3-triazines have improved ability of inhibiting VEGFR-2 activity, but only 4-substituted-6-chloro-pyrido [3,2-*d*]-1,2,3-triazines exhibit good growth inhibitory effects on MVECs. 4-substituted-6-chloro-pyrido [3,2-*d*]-1,2,3-triazines are worthy of further studies.

5. Experimental protocols

5.1. Chemistry

The melting points were determined on an electrically heated X4 digital visual melting point apparatus and were uncorrected. Mass spectra (MS) were determined on either Finnigan MAT/USA spectrometer (LC–MS). ^{1}H NMR and ^{13}C NMR spectrum were

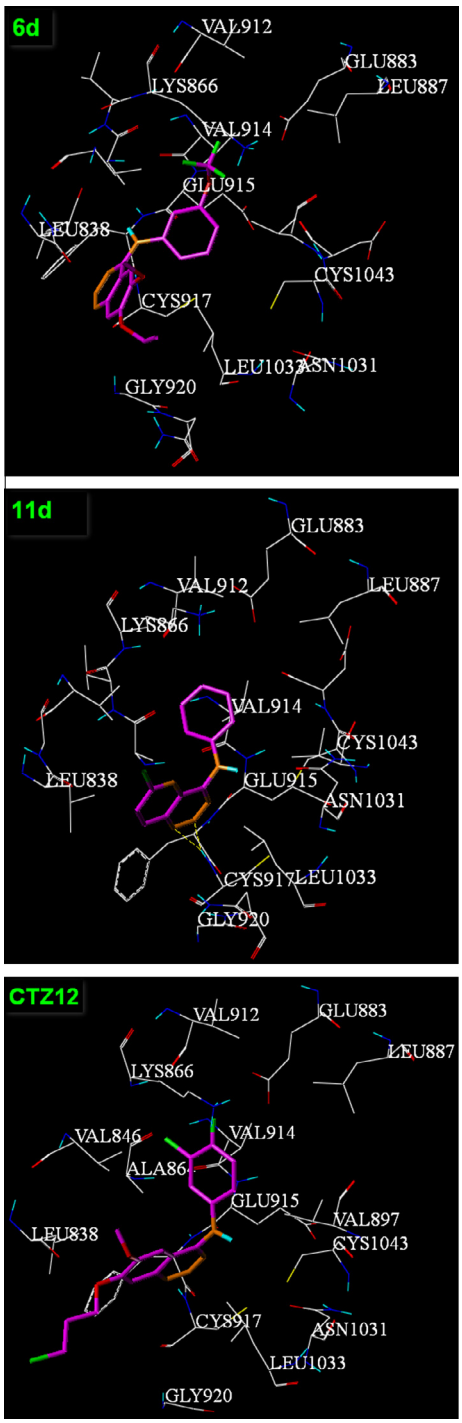


Figure 3. Binding models of compounds **6d**, **11d**, and **CTZ12** in complex with VEGFR-2 kinase. The hydrogen bonds are labeled as yellow broken lines. Compounds **6d**, **11d**, and **CTZ12** are shown in stick model colored by magenta and the receptor is shown in lines model colored by atom type.

recorded on a Bruker ARX-300 instrument with tetramethylsilane (TMS) as the internal standard. IR spectra (KBr disks) were recorded on a Bruker IFS55 instrument. Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). All chemicals were obtained from commercial suppliers and used without purification. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). 2-cyano-3-nitro-6-chloropyridine is synthesized in accordance with literature procedure.[21]

5.2. General procedure for the synthesis of 2-amino-5-methoxy-4-substituted benzonitriles (4a–4d)

A solution of 4-cyano-2-methoxyphenol (**1**) (6.70 mmol) in anhydrous DMF (4.00 mL) was stirred and cooled with an ice bath. K_2CO_3 (10.1 mmol) was added and the mixture was stirred at 20 °C for 1 h. The corresponding alkyl halide (8.13 mmol) was added dropwisely. The mixture was stirred at room temperature overnight, then heated at 37 °C for 6 h and finally poured into ice-water (100 mL). After stirring for 10 min, a precipitate was formed, then filtered off, washed with H_2O, and air-dried. Without any additional purification, the resulting 3-methoxy-4-substituted-benzonitriles (**2a**–**2d**) (9.10 mmol) in nitric acid (10 mL) was heated at 30 °C for 2 h, poured into ice-water (100 mL), filtered, and washed with H_2O to afford 5-methoxy-2-nitro-4-substituted-benzonitrile (**3a**–**3d**). Then a mixture of the intermediate (**3a**–**3d**) (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 dropwisely. 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 compounds (**4a**–**4d**) as yellow solids.

5.3. General procedure for the synthesis of final compounds 6a–6z

Compounds 2-amino-5-methoxy-4-substitutedbenzonitriles (**4a**–**4d**) (10.0 mmol) in 10 N hydrochloric acid (30.0 mL) were 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 the corresponding substituted anilines (10.0 mmol). The solution was kept overnight at 4 °C, filtered, and washed with water. The crude products were then purified by recrystallization with ethyl acetate to obtain intermediate (**5a**–**5z**). Compounds (**5a**–**5z**) were boiled in 70% ethanol (25.0 mL) for 1 h, and then 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. The crude products thus obtained were recrystallized from ethanol to give the final compounds 7-alkoxyl-6-methoxy-4-substituted-1,2,3-benzotriazines (**6a**–**6z**).

5.3.1. 7-Ethoxy-4-(3-fluoro-4-bromoanilino)-6-methoxy-1,2,3-benzotriazine (6a)

Yield: 68%. Mp: 238–240 °C. ESI-MS: *m*/*z*, 393.0 $[M+H]^+$. IR (KBr): 3326, 2918, 1682, 1623, 1509, 1432, 1289, 1243, 1106, 843 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.74 (s, 1H, NH), 8.13 (dd, 1H, J = 2.1 Hz, 11.7 Hz, arom), 7.89 (s, 1H, H-8), 7.72 (m, 2H, arom), 7.59 (s, 1H, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.04 (s, 3H, CH_3),1.44 (t, 3H, J = 6.9 Hz,CH_3). Anal. Calcd for $C_{16}H_{14}BrFN_4O_2$: C, 48.87; H, 3.59; N, 14.25; Found: C, 48.88; H, 3.61; N, 14.26.

5.3.2. 7-Ethoxy-6-methoxy-4-(3-trifluoromethylanilino)-1,2,3-benzotriazine (6b)

Yield: 70%. Mp: 228–230 °C. ESI-MS: *m*/*z*, 365.1 $[M+H]^+$. IR (KBr): 3344, 3132, 2936, 1702, 1619, 1572, 1509, 1447, 1332, 1290, 1243, 1163, 1112, 840, 797, 699.2 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.78 (s, 1H, NH), 8.30 (s, 1H, arom), 8.24 (d, 1H, arom), 7.92 (s, 1H, arom), 7.69 (t, 1H, arom), 7.60 (s, 1H, H-5), 7.52 (d, 1H, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.05 (s, 3H, CH_3), 1.45 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{17}H_{15}F_3N_4O_2$: C, 56.04; H, 4.15; N, 15.38; Found: C, 56.02; H, 4.13; N, 15.35.

5.3.3. 7-Ethoxy-6-methoxy-4-(4-trifluoromethylanilino)-1,2,3-benzotriazine (6c)

Yield: 71%. Mp: 242–244 °C. ESI-MS: *m*/*z*, 365.1 $[M+H]^+$. IR (KBr): 3439, 1614, 1508, 1427, 1326, 1103, 847 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.81 (s, 1H, NH), 8.16 (d, 2H, J = 8.4 Hz, arom), 7.94 (s, 1H, arom), 7.81 (d, 2H, J = 8.7 Hz, arom), 7.60 (s, 1H, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.05 (s, 3H, CH_3), 1.45 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{17}H_{15}F_3N_4O_2$: C, 56.04; H, 4.15; N, 15.38; Found: C, 56.07; H, 4.18; N, 15.41.

5.3.4. 7-Ethoxy-6-methoxy-4-(3-trifluoromethoxyanilino)-1,2,3-benzotriazine (6d)

Yield:70%. Mp: 207–209 °C. ESI-MS: *m*/*z*, 381.2 $[M+H]^+$. IR (KBr): 3426, 1616, 1510, 1444, 1423, 1255, 1166, 1105, 869 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.73 (s, 1H, NH), 8.04 (s, 1H, arom), 7.92 (m, 2H, arom), 7.58 (m, 2H, arom), 7.15 (d, 2H, J = 7.8 Hz, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.45 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{17}H_{15}F_3N_4O_3$: C, 53.69; H, 3.98; N, 14.73; Found: C, 53.65; H, 3.96; N, 14.69.

5.3.5. 7-Ethoxy-6-methoxy-4-(4-trifluoromethoxyanilino)-1,2,3-benzotriazine (6e)

Yield: 69%. Mp: 224–226 °C. ESI-MS: *m*/*z*, 381.2 $[M+H]^+$. IR (KBr): 3429, 1615, 1509, 1428, 1246, 1164, 1102, 1015, 851 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.70 (s, 1H, NH), 7.98 (d, 2H, J = 9 Hz, arom), 7.90 (s, 1H, arom), 7.57 (s, 1H, arom), 7.46 (d, 2H, J = 8.7 Hz, arom), 4.30 (q, 2H, J = 6.9 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.45 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{17}H_{15}F_3N_4O_3$: C, 53.69; H, 3.98; N, 14.73; Found: C, 53.72; H, 3.99; N, 14.76.

5.3.6. 4-(3-Chloroanilino)-7-ethoxy-6-methoxy-1,2,3-benzotriazine (6f)

Yield: 66%. Mp: 237–239 °C. ESI-MS: *m*/*z*, 331.1 $[M+H]^+$. IR (KBr): 3377, 3296, 2924, 1612, 1510, 1430, 1292, 1248, 1106, 1101, 839 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.65 (s, 1H, NH), 8.09 (s, 1H, arom), 7.90 (s, 1H, arom), 7.84 (d, 1H, J = 8.1 Hz, arom), 7.58 (s, 1H, arom), 7.48 (t, 1H, J = 8.1 Hz, arom), 7.22 (d, 1H, J = 8.1 Hz, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.03 (s, 3H, CH_3), 1.44 (t, 3H, J = 6.9 Hz, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 154.22, 153.27, 150.12, 141.65, 137.94, 126.57, 127.48, 123.97, 64.74, 56.73, 14.42. Anal. Calcd for $C_{16}H_{15}ClN_4O_2$: C, 58.10; H, 4.57; N, 16.94; Found: C, 58.14; H, 4.60; N, 16.96.

5.3.7. 7-Ethoxy-4-(4-fluoro-3-trifluoromethylanilino)-6-methoxy-1,2,3-benzotriazine (6g)

Yield: 70%. Mp: 216–218 °C. ESI-MS: *m*/*z*, 383.2 $[M+H]^+$. IR (KBr): 3451, 1615, 1508, 1433, 1326, 1326, 1291, 1236, 1131, 842 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.79 (s, 1H, NH), 8.28 (m, 2H, arom), 7.88 (s, 1H, arom), 7.61 (m, 2H, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.02 (s, 3H, CH_3), 1.45 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{17}H_{14}F_4N_4O_2$: C, 53.41; H, 3.69; N, 14.65; Found: C, 53.36; H, 3.65; N, 14.60.

5.3.8. 7-Ethoxy-4-(2-fluoroanilino)-6-methoxy-1,2,3-benzotriazine (6h)

Yield: 66%. Mp: 224–226 °C. ESI-MS: *m*/*z*, 315.2 $[M+H]^+$. IR (KBr): 3273, 2919, 2849, 1609, 1567, 1505, 1455, 1422, 1286, 1237, 1105, 763 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.67 (s, 1H, NH), 7.88 (s, 1H, arom), 7.59 (m, 2H, arom), 7.36 (m, 3H, arom), 4.31 (q, 2H, J = 6.9 Hz, CH_2), 4.02 (s, 3H, CH_3), 1.45 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{16}H_{15}FN_4O_2$: C, 61.14; H, 4.81; N, 17.83; Found: C, 61.11; H, 4.79; N, 17.81.

5.3.9. 4-(3-Bromoanilino)-7-ethoxy-6-methoxy-1,2,3-benzotriazine (6i)

Yield: 68%. Mp: 232–234 °C. ESI-MS: *m*/*z*, 375.0 $[M+H]^+$. IR (KBr): 3429, 2925, 1613, 1506, 1428, 1291, 1243, 1105, 859, 781.9 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.64 (s, 1H, NH), 8.21 (s, 1H, arom), 7.91 (d,1H, J = 7.8 Hz, arom), 7.89 (s, 1H, arom), 7.58 (s, 1H, arom), 7.39 (m, 2H, arom), 4.30 (q, 2H, J = 6.9 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.44 (t, 3H, J = 6.9 Hz, CH_3). Anal. Calcd for $C_{16}H_{15}BrN_4O_2$: C, 51.22; H, 4.03; N, 14.93; Found: C, 51.23; H, 4.06; N, 14.96.

5.3.10. 7-*n*-Butoxy-4-(3-fluoro-4-bromoanilino)-6-methoxy-1,2,3-benzotriazine (6j)

Yield: 64%.Mp: 227–229 °C. ESI-MS: *m*/*z*, 421.2 $[M+H]^+$. IR (KBr): 3423, 2959, 1614, 1511, 1429, 1289, 1240, 1104, 846 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.75 (s, 1H, NH), 8.13 (d, 1H, J = 11.6 Hz, arom), 7.90 (s, 1H, arom), 7.72 (m, 2H, arom), 7.61 (s, 1H, arom), 4.25 (t, 2H, J = 6.4 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{18}H_{18}BrFN_4O_2$: C, 51.32; H, 4.31; N, 13.30; Found: C, 51.30; H, 4.28; N, 13.29.

5.3.11. 7-*n*-Butoxy-4-(3,4-dichloroanilino)-6-methoxy-1,2,3-benzotriazine (6k)

Yield: 55%. Mp: 213–215 °C. ESI-MS: *m*/*z*, 393.2 $[M+H]^+$. IR (KBr): 3424, 2959, 1614, 1508, 1425, 1239, 1104, 854 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.71 (s, 1H, NH), 8.31 (d, 1H, J = 2.1 Hz, arom), 7.92 (d, 1H, J = 6.9 Hz, arom), 7.88 (s, 1H, arom), 7.70 (d, 1H, J = 8.7 Hz, arom), 7.61 (s, 1H, arom), 4.25 (t, 2H, CH_2), 4.04 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{18}H_{18}Cl_2N_4O_2$: C, 54.97; H, 4.61; N, 14.25; Found: C, 54.99; H, 4.64; N, 14.28.

5.3.12. 7-*n*-Butoxy-6-methoxy-4-(4-trifluoromethylanilino)-1,2,3-benzotriazine (6l)

Yield: 50%. Mp: 238–240 °C. ESI-MS: *m*/*z*, 393.2 $[M+H]^+$. IR (KBr): 3333, 3133, 2964, 2937, 1608, 1569, 1508, 1446, 1336, 1292, 1244, 1162, 1127, 800 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.77 (s, 1H, NH), 8.31 (s, 1H, arom), 8.25 (d, 1H, arom), 7.90 (s, 1H, arom), 7.69 (t, 1H, arom), 7.60 (s, 1H, arom), 7.51 (d, 1H, arom), 4.25 (t, 2H, J = 6.4 Hz, CH_2), 4.05 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.98 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ: 154.93, 153.91, 150.46, 143.25, 142.28, 126.29, 125.86, 124.06, 123.87, 123.65, 122.14, 107.22, 104.39, 100.37, 69.13, 57.18, 30.85, 19.20, 14.12. Anal. Calcd for $C_{19}H_{19}F_3N_4O_2$: C, 58.16; H, 4.88; N, 14.28; Found: C, 58.20; H, 4.92; N, 14.32.

5.3.13. 7-*n*-Butoxy-4-(4-trifluoromethoxyanilino)-6-methoxy-1,2,3-benzotriazine (6m)

Yield: 63%. Mp: 232–234 °C. ESI-MS: *m*/*z*, 409.2 $[M+H]^+$. IR (KBr): 3298, 2963, 1615, 1509, 1425, 1249, 1200, 1166, 1100, 855 cm^{-1}; ^1H-NMR (DMSO-d_6) δ: 9.69 (s, 1H, NH), 7.97 (d, 2H, J = 9 Hz, arom), 7.90 (s, 1H, H-8), 7.58 (s, 1H, arom), 7.46 (d, 2H, J = 8.7 Hz, arom), 4.25 (t, 2H, J = 6.4 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{19}H_{19}F_3N_4O_3$: C, 55.88; H, 4.69; N, 13.72; Found: C, 55.91; H, 4.72; N, 13.74.

5.3.14. 7-*n*-Butoxy-4-(4-fluoro-3-trifluoromethylanilino)-6-methoxy-1,2,3-benzotriazine (6n)

Yield: 62%. Mp: 233–235 °C. ESI-MS: *m*/*z*, 411.2 $[M+H]^+$. IR (KBr): 3360, 2962, 1612, 1508, 1432, 1290, 1234, 1137, 830 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.78 (s, 1H, NH), 8.29 (m, 2H, arom), 7.87 (s, 1H, arom), 7.63 (d, 1H, J = 9.6 Hz, arom), 7.61 (s, 1H, arom), 4.25 (t, 2H, CH_2), 4.04 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{19}H_{18}F_4N_4O_2$: C, 55.61; H, 4.42; N, 13.65; Found: C, 55.58; H, 4.40; N, 13.61.

5.3.15. 7-*n*-Butoxy-4-(2-fluoroanilino)-6-methoxy-1,2,3-benzotriazine (6o)

Yield: 61%. Mp: 225–227 °C. ESI-MS: *m*/*z*, 343.2 $[M+H]^+$. IR (KBr): 3357, 2958, 1611, 1503, 1453, 1112, 840, 758 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.66 (s, 1H, NH), 7.87 (s, 1H, arom), 7.59 (m, 2H, arom), 7.34 (m, 3H, arom), 4.24 (t, 2H, J = 6.4 Hz, CH_2), 4.01 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{18}H_{19}FN_4O_2$: C, 63.15; H, 5.59; N, 16.36; Found: C, 63.16; H, 5.61; N, 16.38.

5.3.16. 4-(4-Bromoanilino)-7-*n*-butoxy-6-methoxy-1,2,3-benzotriazine (6p)

Yield: 64%. Mp: 231–232 °C. ESI-MS: *m*/*z*, 403.7 $[M+H]^+$. IR (KBr): 3426, 2930, 1613, 1508, 1428, 1291, 1106, 855, 780 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.66 (s, 1H, NH), 8.22 (s, 1H, arom), 7.91 (m, 2H, H-8, arom), 7.59 (s, 1H, arom), 7.36 (m, 2H, arom), 4.25 (t, 2H, J = 6.4 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{18}H_{19}BrN_4O_2$: C, 53.61; H, 4.75; N, 13.89; Found: C, 53.54; H, 4.68; N, 13.82.

5.3.17. 7-*n*-Butoxy-4-(4-fluoroanilino)-6-methoxy-1,2,3-benzotriazine (6q)

Yield: 57%. Mp: 220–222 °C. ESI-MS: *m*/*z*, 343.1 $[M+H]^+$. IR (KBr): 3430, 2960, 1614, 1572, 1509, 1428, 1222, 1107, 1014, 830 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.59 (s, 1H, NH), 7.88 (s, 1H, arom), 7.83 (m, 2H, arom), 7.71 (s, 1H, arom), 7.29 (m, 2H, arom), 4.26 (t, 2H, J = 6.4 Hz, CH_2), 4.03 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{18}H_{19}FN_4O_2$: C, 63.15; H, 5.59; N, 16.36; Found: C, 63.20; H, 5.63; N, 16.41.

5.3.18. 7-*n*-Butoxy-4-(4-methylanilino)-6-methoxy-1,2,3-benzotriazine (6r)

Yield:56%. Mp: 214–215 °C. ESI-MS: *m*/*z*, 339.2 $[M+H]^+$. IR (KBr): 3335, 2958, 2927, 2872, 2851, 1613, 1569, 1512, 1428, 1286, 1236, 1102, 852 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.51 (s, 1H, NH), 7.90 (s, 1H, arom), 7.70 (d, 2H, J = 8.2 Hz, arom), 7.54 (s, 1H, arom), 7.25 (d, 2H, J = 8.2 Hz, arom), 4.24 (t, 2H, J = 6.4 Hz, CH_2), 4.02 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 0.97 (t, 3H, J = 7.3 Hz, CH_3). Anal. Calcd for $C_{19}H_{22}N_4O_2$: C, 67.44; H, 6.55; N, 16.56; Found: C, 67.42; H, 6.53; N, 16.55.

5.3.19. 4-(4-Chloroanilino)-6-methoxy-7-pentyloxy-1,2,3-benzotriazine (6s)

Yield: 72%. Mp: 212–214 °C. ESI-MS: *m*/*z*, 373.2 $[M+H]^+$. IR (KBr): 3378, 2954, 1612, 1507, 1425, 1245, 1101, 1014, 817 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.63 (s, 1H, NH), 7.91 (m, 3H, H-8, arom), 7.57 (s, 1H, arom), 7.50 (d, 2H, J = 8.4 Hz, arom), 4.24 (t, 2H, J = 6.4 Hz, CH_2), 4.03 (s, 3H, CH_3), 1.81 (m, 2H, CH_2), 1.40 (m, 4H, 2×CH_2), 0.92 (t, 3H, J = 7.0 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ: 154.31, 153.27, 150.30, 141.63, 138.88, 128.73, 128.73, 124.04, 122.69, 122.69, 106.81, 103.82, 100.15, 68.98, 56.79, 28.17, 27.78, 21.98, 14.04. Anal. Calcd for $C_{19}H_{21}ClN_4O_2$: C, 61.21; H, 5.68; N, 15.03; Found: C, 61.25; H, 5.71; N, 15.06.

5.3.20. 4-(4-Bromo-3-fluoroanilino)-6-methoxy-7-pentyloxy-1,2,3-benzotriazine (6t)

Yield: 75%. Mp: 220–222 °C. ESI-MS: *m*/*z*, 435.0 $[M+H]^+$. IR (KBr): 3265, 2956, 1613, 1510, 1429, 1288, 1239, 1177, 1104, 841, 819, 786 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.75 (s, 1H, NH), 8.16 (m, 1H, arom), 7.90 (s, 1H, arom), 7.73 (m, 2H, arom), 7.61 (s, 1H,

arom), 4.25 (t, 2H, CH_2), 4.04 (s, 3H, CH_3), 1.83 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 0.93 (t, 3H, CH_3). Anal. Calcd for $C_{19}H_{20}BrFN_4O_2$: C, 52.43; H, 4.63; N, 12.87; Found: C, 52.41; H, 4.60; N, 12.84.

5.3.21. 4-(3,5-Difluoroanilino)-6-methoxy-7-pentyloxy-1,2,3-benzotriazine (6u)

Yield: 68%. Mp: 192–194 °C. ESI-MS: *m*/*z*, 375.1 $[M+H]^+$. IR (KBr): 3345, 2936, 1620, 1512, 1449, 1291, 1235, 1114, 992, 846, 784 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.77 (s, 1H, NH), 7.89 (s, 1H, arom), 7.75 (d, 2H, *J* = 8.7 Hz, arom), 7.62 (s, 1H, arom), 7.00 (t, 1H, *J* = 9.3 Hz, arom), 4.24 (t, 2H, *J* = 6.4 Hz, CH_2), 4.05 (s, 3H, CH_3), 1.83 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 0.92 (t, 3H, *J* = 7.2 Hz, CH_3). Anal. Calcd for $C_{19}H_{20}F_2N_4O_2$: C, 60.95; H, 5.38; N, 14.97; Found: C, 60.99; H, 5.41; N, 15.02.

5.3.22. 6-Methoxy-7-pentyloxy-4-(3-trifluoromethoxyanilino)-1,2,3-benzotriazine (6v)

Yield: 73%. Mp: 181–183 °C. ESI-MS: *m*/*z*, 423.2 $[M+H]^+$. IR (KBr): 3421, 2931, 1613, 1508, 1446, 1258, 1216, 1163, 1105, 845, 784 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.72 (s, 1H, NH), 8.04 (s, 1H, arom), 7.92 (d, 1H, *J* = 8.9 Hz, arom), 7.91 (s, 1H, arom), 7.60 (s, 1H, arom), 7.56 (t, 2H, *J* = 8.6 Hz, arom), 7.14 (d, 1H, *J* = 8.7 Hz, arom), 4.24 (t, 2H, *J* = 6.3 Hz, CH_2), 4.05 (s, 3H, CH_3), 1.83 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 0.92 (t, 3H, *J* = 6.9 Hz, CH_3). Anal. Calcd for $C_{20}H_{21}F_3N_4O_3$: C, 56.87; H, 5.01; N, 13.26; Found: C, 56.92; H, 5.08; N, 13.31.

5.3.23. 6-Methoxy-7-pentyloxy-4-(4-trifluoromethoxyanilino)-1,2,3-benzotriazine (6w)

Yield: 70%. Mp: 230–232 °C. ESI-MS: *m*/*z*, 423.2 $[M+H]^+$. IR (KBr): 3426, 2959, 1615, 1510, 1428, 1247, 1200, 1165, 1102, 850, 786 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.69 (s, 1H, NH), 7.98 (d, 2H, *J* = 9.0 Hz, arom), 7.90 (s, 1H, arom), 7.58 (s, 1H, arom), 7.46 (d, 2H, *J* = 8.7 Hz, arom), 4.24 (t, 2H, *J* = 6.3 Hz, CH_2), 4.04 (s, 3H, CH_3), 1.83 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 0.92 (t, 3H, *J* = 6.9 Hz, CH_3). Anal. Calcd for $C_{20}H_{21}F_3N_4O_3$: C, 56.87; H, 5.01; N, 13.26; Found: C, 56.82; H, 4.97; N, 13.22.

5.3.24. 6-Methoxy-4-(4-fluoroanilino)-7-pentyloxy-1,2,3-benzotriazine (6x)

Yield: 64%. Mp: 213–215 °C. ESI-MS: *m*/*z*, 357.2 $[M+H]^+$. IR (KBr): 3369, 2953, 1616, 1509, 1428, 1283, 1222, 1158, 1107, 832, 786 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.60 (s, 1H, NH), 7.88 (s, 1H, arom), 7.82 (m, 2H, arom), 7.55 (s, 1H, arom), 7.29 (m, 2H, arom), 4.23 (t, 2H, *J* = 6.3 Hz, CH_2), 4.03 (s, 3H, CH_3), 1.83 (m, 2H, CH_2), 1.42 (m, 4H, 2×CH_2), 0.92 (t, 3H, *J* = 7.0 Hz, CH_3). Anal. Calcd for $C_{19}H_{21}FN_4O_2$: C, 64.03; H, 5.94; N, 15.72; Found: C, 64.06; H, 5.96; N, 15.77.

5.3.25. 7-(3-Chloropropoxy)-6-methoxy-4-(4-trifluoromethylanilino)-1,2,3-benzotriazine (6y)

Yield: 70%. Mp: 179–180 °C. ESI-MS: *m*/*z*, 413.1 $[M+H]^+$. IR (KBr): 3387, 2933, 1616, 1567, 1509, 1425, 1321, 1249, 1121, 848 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.83 (s, 1H, NH), 8.16 (d, 2H, *J* = 8.5 Hz, arom), 7.96 (s, 1H, arom), 7.82 (d, 2H, *J* = 8.6 Hz, arom), 7.66 (s, 1H, arom), 4.37 (t, 2H, *J* = 6.0 Hz, CH_2), 4.06 (s, 3H, CH_3), 3.84 (t, 2H, *J* = 6.4 Hz, CH_2), 2.29 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ: 154.50, 153.81, 150.45, 143.20, 142.16, 126.25, 125.85, 124.05, 123.90, 123.68, 122.16, 107.42, 104.60, 100.56, 66.25, 57.25, 42.23, 31.86. Anal. Calcd for $C_{18}H_{16}ClF_3N_4O_2$: C, 52.37; H, 3.91; N, 13.57; Found: C, 52.40; H, 3.98; N, 14.61.

5.3.26. 7-(3-Chloropropoxy)-6-methoxy-4-(4-methylanilino)-1,2,3-benzotriazine (6z)

Yield: 70.1%; Mp: 245–247 °C. ESI-MS: *m*/*z*, 359.1 $[M+H]^+$. IR (KBr): 3420, 2924, 1615, 1513, 1425, 1282, 1242, 859 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 9.55 (1H, s, NH), 7.92 (1H, s, arom), 7.69 (2H, d, *J* = 8.4 Hz, arom), 7.59 (1H, s, arom), 7.25 (2H, d, *J* = 8.4 Hz, arom), 4.37 (2H, t, *J* = 6.0 Hz, CH_2), 4.03 (3H, s, CH_3), 3.84 (2H, t, *J* = 6.3 Hz, CH_2), 2.34 (3H, s, CH_3), 2.29 (2H, m, CH_2). ^{13}C NMR (DMSO-d_6) δ: 154.23, 153.52, 150.62, 141.59, 136.43, 133.64, 129.48, 123.22, 107.28, 104.42, 100.86, 66.14, 57.22, 42.24, 31.84, 20.99. 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.

5.4. General procedure for the synthesis of final compounds 4-substituted-6-chloro-pyrido-[3,2-*d*]-1,2,3-triazine (11a–11j)

A mixture of 2,6-dichloro-3-nitropyridine (**7**) (26 mmol), cuprous cyanide (0.59 mml) and appropriate amount of *N*-methyl pyrrolidone was heated at 180 °C for 15 min, then cooled to 10 °C and poured into ice-water (200 mL) and stirred for 30 min. The solution was then filtered, washed with water and dried. The resulting 2-cyano-3-nitro-6-chloropyridine (**8**) was extracted with boiling toluene (50 mL) for 3 times, combined the organic phases and washed with water and saturated sodium chloride solution. Then the organic phase was dried, filtered, evaporated under reduced pressure to dryness, and purified by recrystallization with a mixture of petroleum ether/ethyl acetate (4/1). The reduction of the intermediate **8** (10 mmol) was reacted with sodium hyposulfite (80 mmol, and 40 mmol 2 h later) in dichloromethane/aqua (20 mL/20 mL) solution, and added TBAB (1 mmol) as a phase-transfer catalyst. The mixture was stirred at room temperature for 3 h, and then extracted with dichloromethane (40 mL) for 3 times. The combined organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to yield 2-cyano-6-chloro-3-pyridinylamine (**9**). Compound **9** was added into 3 mL of hydrochloric acid (10 mol/L), and the aqueous solution of sodium nitrite (0.072 g/1.0 mL) was added dropwise under stirring for 20 min in an ice-salt bath. The diazonium solution was reacted with the corresponding substituted anilines (10.0 mmol) and then neutralized with excess of sodium acetate trihydrate, stirred for 2 h at 0 °C. The solution was kept overnight, filtered, and washed with water to yield the crude products. The crude products were then purified by recrystallization with ethyl acetate to obtain intermediates 1-substituted-3-(2-cyano-6-chloro-3-pyridyl)triazene (**10a**–**10j**). The intermediates **10a**–**10j** were boiled in 70% ethanol (25.0 mL) for 1 h, and then 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. The crude products thus obtained were recrystallized from ethanol to give the final compounds 4-substituted-6-chloro-pyrido[3,2-*d*][1,2,3]triazines (**11a**–**11j**).

5.4.1. 6-Chloro-4-(4-chloroanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11a)

Yield: 43%. Mp: 207–209 °C. ESI-MS: *m*/*z*, 314.0 $[M+H]^+$. IR (KBr): 3272, 3145, 1609, 1558, 1505, 1140, 827 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.49 (s, 1H, NH), 8.68 (d, 1H, *J* = 8.7 Hz, H-8), 8.20 (d, 1H, *J* = 8.7 Hz, H-7), 8.07 (d, 2H, *J* = 8.7 Hz, arom-2′ H, 6′ H), 7.51 (d, 2H, *J* = 8.7 Hz, arom-3′ H, 5′ H). ^{13}C NMR (DMSO-d_6) δ: 154.24, 150.66, 139.14, 138.28, 136.91, 131.71, 131.71, 128.55, 128.55, 126.01, 124.50, 124.50. Anal. Calcd for $C_{12}H_7Cl_2N_5$: C, 49.34; H, 2.42; N, 23.97 Found: C, 49.33; H, 2.41; N, 23.95.

5.4.2. 4-(4-Bromo-3-fluoroanilino)-6-chloro-pyrido[3,2-*d*]-1,2,3-triazine (11b)

Yield: 45%. Mp: 200–202 °C. ESI-MS: *m*/*z*, 354.0 $[M+H]^+$. IR (KBr): 3441, 3290, 2920, 1606, 1505, 1447, 1136, 840, 642 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.63 (s, 1H, NH), 8.72 (d, 1H, *J* = 8.7 Hz, H-8),8.23 (m, 2H, H-7, arom), 7.96 (d, 1H, *J* = 8.7 Hz, arom), 7.77 (t, 1H, *J* = 8.4 Hz, arom). Anal. Calcd for $C_{12}H_6BrClFN_5$: C, 40.65; H, 1.71; N, 19.75, Found: C, 40.71; H, 1.78; N, 19.79.

5.4.3. 6-Chloro-4-(3,4-dichloroanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11c)

Yield: 48%. Mp: 190–192 °C. ESI-MS: *m*/*z*, 326.0 $[M+H]^+$. IR (KBr): 3357, 1600, 1556, 1505, 1450, 1145, 843 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.64 (s, 1H, NH), 8.74 (d, 1H, J = 8.7 Hz, H-8), 8.28 (s, 2H, arom-3′ H, 5′ H), 8.24 (d, 1H, J = 8.7 Hz, H-7), 7.43 (s, 1H, arom-4′ H). Calcd for $C_{12}H_6Cl_3N_5$: C, 44.13; H, 1.85; Cl, 32.57; N, 21.45, Found: C, 44.16; H, 1.86; N, 21.47.

5.4.4. 6-Chloro-4-(3-trifluoromethylanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11d)

Yield: 47%. Mp: 190–192 °C. ESI-MS: *m*/*z*, 326.0 $[M+H]^+$. IR (KBr): 3303, 1606, 1548, 1509, 1465, 1335, 1105, 843, 802 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.66 (s, 1H, NH), 8.72 (d, 1H, J = 8.7 Hz, H-8), 8.52 (s, 1H, arom-2′ H), 8.38 (d, 1H, J = 8.4 Hz, arom-4′ H), 8.25 (d, 1H, J = 8.7 Hz, H-7), 7.70 (t, 1H, J = 8.1 Hz, arom-5′ H), 7.56 (d, 1H, J = 7.8 Hz, arom-6′ H). Calcd for $C_{13}H_7ClF_3N_5$: C, 47.94; H, 2.17; N, 21.50 Found: C, 47.92; H, 2.14; N, 21.48.

5.4.5. 6-Chloro-4-(4-trifluoromethylanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11e)

Yield: 46%. Mp: 206–208 °C. ESI-MS: *m*/*z*, 326.0 $[M+H]^+$. IR (KBr): 3250, 3051, 1614, 1499, 1454, 1321, 1110, 1067, 846 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.66 (s, 1H, NH), 8.72 (d, 1H, J = 8.7 Hz, H-8), 8.32 (d, 2H, J = 8.4 Hz, arom-3′ H, 5′ H), 8.23 (d, 1H, J = 8.7 Hz, H-7), 7.82 (d, 2H, J = 8.4 Hz, arom-2′ H, 6′ H). ^{13}C NMR (DMSO-d_6) δ: 154.88, 151.12, 142.04, 139.57, 138.77, 132.21, 126.61 (q, J = 3.75 Hz), 126.29, 126.18 (q, J = 3.6 Hz), 124.80 (q, J = 270 Hz, –$\underline{C}F_3$), 124.76 (q, J = 31.8 Hz, –$\underline{C}CF_3$), 122.98, 120.86. Calcd for $C_{13}H_7ClF_3N_5$: C, 47.94; H, 2.17; N, 21.50 Found: C, 47.98; H, 2.20; N, 21.56.

5.4.6. 6-Chloro-4-(3-trifluoromethoxyanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11f)

Yield: 40%. Mp: 147–149 °C. ESI-MS: *m*/*z*, 342.0 $[M+H]^+$. IR (KBr): 3292, 1613, 1505, 1464, 1286, 1133, 882, 841 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.61 (s, 1H, NH), 8.71 (d, 1H, J = 8.7 Hz, H-8), 8.18 (m, 3H, H-7, arom-2′ H, 4′ H), 7.58 (t, 1H, J = 8.1 Hz, arom-5′ H), 7.19 (d, 1H, J = 8.1 Hz, arom-6′ H). ^{13}C NMR (DMSO-d_6) δ: 154.78, 151.12, 148.76, 140.08, 139.56, 138.70, 132.15, 130.64, 126.32, 121.47 (q, J = 255 Hz, –$\underline{C}F_3$), 118.07, 116.92, 115.26. Calcd for $C_{13}H_7ClF_3N_5O$: C, 45.70; H, 2.06; N, 20.50; Found: C, 45.74; H, 2.09; N, 20.56.

5.4.7. 6-Chloro-4-(4-trifluoromethoxyanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11g)

Yield: 45%. Mp: 165–167 °C. ESI-MS: *m*/*z*, 342.0 $[M+H]^+$. IR (KBr): 3281, 1611, 1508, 1266, 1157, 1031, 847 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.56 (s, 1H, NH), 8.69 (d, 1H, J = 8.7 Hz, H-8), 8.21 (d, 1H, J = 8.7 Hz, H-7), 8.13 (d, 2H, J = 9.0 Hz, arom-3′ H, 5′ H), 7.47 (d, 2H, J = 8.4 Hz, arom-2′ H, 6′ H). Calcd for $C_{13}H_7ClF_3N_5O$: C, 45.70; H, 2.06; N, 20.50; Found: C, 45.77; H, 2.12; N, 20.53.

5.4.8. 6-Chloro-4-(4-fluoro-3-trifluoromethylanilino)-pyrido[3,2-*d*]-1,2,3-triazine (11h)

Yield: 43%.Mp: 234–236 °C. ESI-MS: *m*/*z*, 344.0 $[M+H]^+$. IR (KBr): 3307, 2924, 1593, 1510, 1457, 1133, 837 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.70 (s, 1H, NH), 8.71 (d, 1H, J = 8.7 Hz, H-8), 8.51 (m, 1H, arom-6′ H), 8.42 (m, 1H, arom-2′ H), 8.22 (d, 1H, J = 8.7 Hz, H-7), 7.62 (t, 1H, J = 9.6 Hz, arom-5′ H). Calcd for $C_{13}H_6ClF_4N_5$: C, 45.43; H, 1.76; N, 20.38; Found: C, 45.41; H, 1.75; N, 20.36.

5.4.9. 6-Chloro-4-(2-fluoroanilino)-pyrido[3,2-*d*][3,2-*d*]-1,2,3-triazine (11i)

Yield: 41%. Mp: 195–197 °C. ESI-MS: *m*/*z*, 276.1 $[M+H]^+$. IR (KBr): 3356, 3065, 1620, 1601, 1514, 1465, 1139, 1010, 841, 760 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.36 (s, 1H, NH), 8.69 (d, 1H, J = 8.7 Hz, H-8), 8.21 (d, 1H, J = 8.7 Hz, H-7), 7.71(t, 1H, J = 8.4 Hz, arom-6′ H), 7.36 (m, 3H, arom-3′ H, 4′ H, 5′ H). Calcd for $C_{12}H_7ClFN_5$: C, 52.28; H, 2.56; N, 25.40 Found: C, 52.35; H, 2.58; N, 25.44.

5.4.10. 4-(4-Bromoanilino)-6-chloro-pyrido[3,2-*d*]-1,2,3-triazine (11j)

Yield: 49%. Mp: 205–207 °C. ESI-MS: *m*/*z*, 336.0 $[M+H]^+$. IR (KBr): 3345, 1605, 1505, 1477, 1144, 843, 777 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.70 (d, 1H, J = 8.7 Hz, H-8), 8.38 (s, 1H, arom-2′ H), 8.21 (d, 1H, J = 9.0 Hz, H-7), 8.05 (d, 1H, J = 6.3 Hz, arom-6′ H), 7.43 (m, 2H, arom-4′ H, 5′ H). Calcd for $C_{12}H_7BrClN_5$: C, 42.82; H, 2.10; N, 20.81; Found: C, 42.88; H, 2.17; N, 20.87.

5.5. VEGFR-2 kinase inhibition assay

VEGFR-2 Kinase Assay Kit, purchased from CST (Cell Signaling Technology) with a serial number of 7788, was used to evaluate the inhibitory effects of target compounds on the VEGFR-2 kinase activity. **PTK787** was used as the positive control for VEGFR-2 kinase, and 0.1% (v/v) DMSO was the negative control. The assay was performed in 96-well polystyrene round-bottomed plates pre-coated with 3 μM poly (Glu, Tyr)$_{4:1}$ as a substrate and with a final volume of 100 μL in each well. A solution of 40 μM ATP and 10 μg/mL of the selected compound were added in each well. The reaction was initiated by adding 8 ng/μL of VEGFR-2 kinase and incubated for 5 min at room temperature. After adding ATP solution and incubating for 30 min at room temperature, the reaction was stopped by adding 50 mM EDTA. Next, 100 μL of Phosphotyrosine mAb antibody was added. After 1 h of incubation at room temperature, the plate was washed three times with 200 μL PBS-T. Then 100 μL of anti-rat IgG (1:500 dilution) and anti-rabbit IgG (1:1000 dilution) diluted with PBS-T containing 1% BSA was added. The plate was reincubated at room temperature for 30 min, and washed five times as before. Finally, 100 μL of TMB was added and incubated at room temperature for 15 min. The reaction was terminated by the addition of 100 μL of 2 N H_2SO_4, and A_{450} was measured on a microplate plate reader (Bio-Rad™). The enzyme inhibition rate (%) was calculated using the equation:Inhibition rate (%) = [1−(A_{450}/A_{450} control)] × 100%

5.5.1. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The anti-proliferative activities of target compounds on MVECs were evaluated by the MTT assay with **PTK787** as the positive control. MVECs cells were cultured in DMEM with 4 mM L-glutamine and adjusted to contain 4.50 g/L glucose and 10% (v/v) heat-inactivated fetal bovine serum. Cells (2×10^3 cells/well in 100 ml medium) were incubated for 24 h, then various concentrations of each compound mixed in 100 ml medium were added to each well. The cells were cultured for another 4 days at 37 °C. MTT solution (50 μL of 2 mg/mL) was added per well and the cultures were 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. All of the compounds were tested three times. Growth inhibition is reported as compared to untreated cells (%) and GI_{50} concentration was calculated.

5.6. Molecular modeling

The crystal structure of the VEGFR-2 kinase in complex with a 4-amino-furo[2,3-*d*]pyrimidine inhibitor was downloaded from the Protein Data Bank (PDB entry **1YWN**).[19] All calculations and manipulations were performed using the *Surflex-Dock* in the Sybyl

X 2.0 software package. All water molecules in the experimental structure were removed and hydrogen atoms were added.

Compounds **PTK787**, **6d**,**11d**, and **CTZ12** were built using the Sybyl sketcher model and fully minimized with the Powell method (Tripos force field and Gasteiger–Huckel charges) to an energy gradient of 0.050 kcal/(mol Å). For the *Surflex-dock* calculation, *default parameters* were used except for max conformation per fragment was 50, max number of rotatable bonds per molecule was 200 and the maximum number of pulses per legend was 50. The docking mode was chosen on the basis of binding affinity rank. The selected docking results are based on the number of occurrences of the same conformation and the rationality of the conformation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.10.009.

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