Solid-Phase Synthesis and Antitumor Evaluation of 2,4-Diamino-6-aryl-1,3,5-triazines

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2,4-Diamino-6-aryl-1,3,5-triazines were synthesized by using a solid-supported approach in which monoarylsubstituted triazines were captured directly from the crude reaction mixture by resin-bound amines. The effects of the synthesized compounds on inhibition activities against tumor cell lines (PC-3, K562, A549, and HO8910) were examined. Most of the obtained compounds demonstrated remarkable antiproliferative activities against K562, PC-3, and HO8910 cell lines. Particularly, compounds **8c** exhibited prominent inhibition activity with IC_{50} values of 1.01, 2.23, and 1.06 μM, respectively. The structure−activity relationships of 2,4-diamino-6-aryl-1,3,5-triazines are also discussed.

1. Introduction

The field of drug discovery has experienced significant advances with the introduction of combinatorial chemistry approaches.[1] Heterocyclic moieties commonly exist in biologically active natural products and medicinal agents. As a result, an increasing range and number of pharmaceutically useful heterocyclic compounds have been prepared using combinatorial solid-phase methodology.[2]

1,3,5-Triazines possess a broad range of biological properties, such as antimicrobial,[3] antitrypanosomal,[4] antiretroviral activities,[5] sorbitol dehydrogenase inhibition,[6] 5-HT_7 antagonism,[7] and estrogen receptor modulation.[8] Particularly noteworthy is their antitumor activity. Hexamethylmelamine (HMM) was discovered as a potent agent against breast, lung, and ovarian cancers. Unfortunately, it frequently caused severe toxicities including nausea, vomiting, abdominal cramps, anorexia, weight loss, and malaise.[9] Irsogladine, an antigastric ulcer agent, was shown to possess anticancer properties due to its antiangiogenic properties.[10] Additionally, some 1,3,5-triazine derivatives have proved to be promising new hits for further development of antitumor agents.[11] However, as far as aryl-1,3,5-triazines are concerned, to our knowledge, only few reports offer information concerning their biological properties. 4-(3-Methoxy-4-(oxazol-5-yl)phenylamino)-6-phenyl-1,3,5-triazin-2-ol was identified as an inosine monophosphate dehydrogenase II inhibitor with an IC_{50} of 18 nM[12] and *tert*-butyl 4-(4-phenyl-1,3,5-triazin-2-ylamino)phenylcarbamate was disclosed as a kinase inhibitor against IGFR, KDR-1, and Zap-1 with IC_{50} values in the range of 6.6−9.6 μM.[13] In continuation of our research program on anticancer agents and efforts directed toward the solid-phase synthesis of heterocyclic compounds,[14] herein, we wish to report the solid-phase synthesis of 2,4-diamino-6-aryl-1,3,5-triazines as well as their antitumor activity evaluation.

2. Results and Discussion

2.1. Solid Synthesis of 2,4-Diamino-6-aryl-1,3,5-triazines. The parallel synthesis of 2,4-diamino-6-aryl-1,3,5-triazines was carried out on the solid phase using the "tea-bag" methodology.[15] The reaction sequence is illustrated in Scheme 1.

Starting from 4-(4-formyl-3-methoxyphenoxy)butyryl AM resin **1**, in the presence of $NaBH_3CN$ in DMF, an amine **2** was attached to the resin by reductive amination. Reaction of cyanuric chloride **4** with 1.8 equiv of arylmagnesium bromide produced the mono- and diarylsubstituted crude reaction mixture **5**. Due to the different amination reactivity, the monoarylsubstituted triazine was selectively captured by the resin-bound amine **3** from the crude reaction mixture by nucleophilic substitution, resulting in a resin-bound triazine **6**. The chlorine of resin-bound triazine **6** was then displaced by heating in DMF with amines at 100 °C to obtain the resin-bound compound **7**. Cleavage of resin **7** was accomplished by treatment with 50% TFA in DCM, thus providing the liberated 2,4-diamino-6-aryl-1,3,5-triazines **8**. The products were characterized by electrospray LC-MS, ^{1}H NMR, and ^{13}C NMR. The results are summarized in Table 1.

From these results, the yields of products were dependent on the nature of the substituent R^2 of the amines **2**. Arylamines bearing electron-donating groups and aniline gave satisfactory results; however, arylamines with electron-withdrawing groups (4-chlorobenzenamine) gave modest yields. Besides, excellent yields of the products could also be obtained when alkylamines were used instead of arylamines.

2.2. Biological Evaluation. The synthesized 1,3,5-triazines were evaluated for their cytotoxic activity in vitro against

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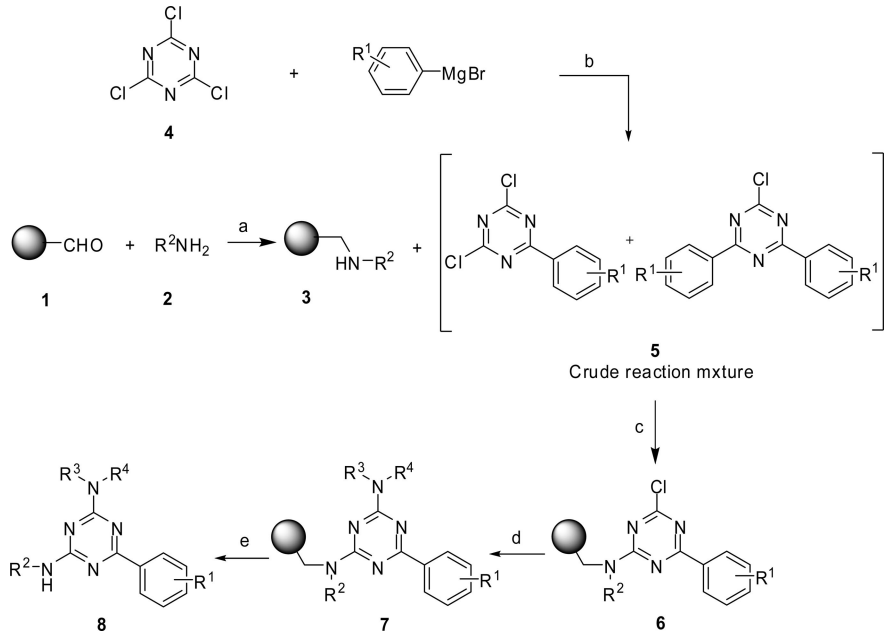
Scheme 1. Solid-Phase Synthesis of 2,4-Diamino-6-aryl-1,3,5-triazines **8**[a]



[a] Reagents and conditions: (a) $NaBH_3CN$, DMF/AcOH (99:1), rt, 24 h; (b) 1.8 equiv of ArMgBr, THF, 0 °C; (c) 0.1 equiv of **3**, 1.0 equiv of Et_3N, THF, rt, 24 h; (d) 10 equiv of R^3R_4NH, 10 equiv of Et_3N, DMF, 100 °C, 48 h; (e) TFA/DCM = 1:1, 1 h.

four human cancer cell lines (human lung carcinoma cell line A549, human leukemia cell line K562, human prostate cancer cell line PC-3, and human ovarian cancer cell line HO8910) using doxorubicin hydrochloride and IRESSA as the reference drugs. The results are summarized in Table 2.

As shown in Table 2, most of the 2,4-diamino-6-aryl-1,3,5-triazines exhibited inhibitions against the selected tumor cell lines, especially PC-3 and K562 cell lines. Preliminary structure−activity relationship of the 2,4-diamino-6-aryl-1,3,5-triazines was investigated. The data from compounds **8a**−**8j** in Table 2 summarizes the effect of different R^3R^4NH on their activity, with identical substitutions at R^1 and R^2 positions of these compounds. Generally, secondary amines with R^3R^4NH substitutions were beneficial for increasing the cytotoxicity of the 2,4-diamino-6-aryl-1,3,5-triazines. It was found that the introduction of piperidine (**8a**), piperazine (**8c**), or diethylamine (**8f**) at the R^3R^4NH position led to compounds with good activity against K562 and HO8910 cell lines. Particularly noteworthy is that the piperazine substitution yielded compound **8c** which was discovered as the most potent inhibitor against K562, PC-3, and HO8910 cell lines with IC_{50} values of 1.01, 2.23, and 1.06 μM, respectively. Additionally, the 1-methylpiperazine analogue **8d** showed a similar potency against K562 with an IC_{50} of 3.36 μM when compared to **8c**. However, benzyl amine (**8h**) and a heterocyclic derived piperazine (**8j**) substitution at the R^3R^4NH position caused the inhibitory potency against K562 and HO8910 cell lines to decrease significantly (IC_{50} > 50 μM). Compared with the data of **8k** (R^2 = phenyl), **8l** (R^2 = 4-chlorophenyl), **8m** (R^2 = benzyl), and **8n** (R^2 = *n*-butyl), various substitutions at the R^2 position have little effect on the compounds' cytotoxic activity against PC-3 and K562. The same is also observed in case of compounds **8p** (R^2 = 4-$CH_3C_6H_4$), **8r** (R^2 = C_6H_5), and **8u** (R^2 = 4-ClC_6H_4). With identical substitutions at R^2 and R^3R^4NH in compounds **8a** (R^1 = C_6H_5), **8p** (R^1 = 4-$CH_3OC_6H_4$), and **8w** (R^1 = 3-ClC_6H_4), phenyl (compound **8a**, IC_{50} = 3.49 μM) was found as the preferred substitutions at the R^1 position. Throughout our studies, we have found that inhibition activity against the selected tumor cell lines was primarily dependent on the R^3R^4NH substituent on the triazine scaffold.

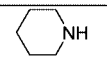
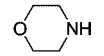
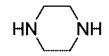
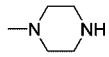
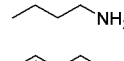
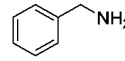
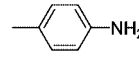
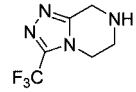
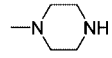
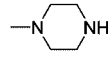
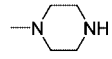
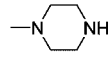
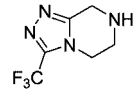
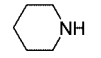
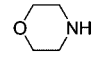
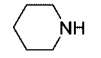
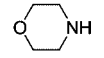
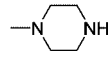
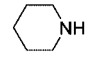
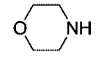
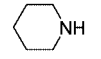
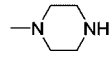
3. Conclusion

In summary, we have demonstrated an efficient approach for the parallel synthesis of 2,4-diamino-6-aryl-1,3,5-triazines on solid support by the resin capture of monoarylsubstituted triazines directly from the crude reaction mixture. In addition, the reaction conditions are readily amenable to the synthesis of individual and mixture-based combinatorial libraries. Meanwhile, the effects of the 2,4-diamino-6-aryl-1,3,5-triazines on inhibition activities against tumor cell lines (PC-3, K562, A549, and HO8910) were examined. Most of the obtained compounds showed potent antiproliferative activities against K562, PC-3, and HO8910 cell lines. The structure−activity relationships analysis indicated that the inhibition of tumor cells growth by the 2,4-diamino-6-aryl-1,3,5-triazines favored a piperazine moiety on the substituents. Further study on the mechanisms of these compounds in suppression of tumor cells growth is underway in our laboratory.

4. Experimental Section

4.1. Chemistry. DMF was treated with CaH_2 and THF was distilled from sodium and benzophenone before use. The other reagents were used directly without further purification. Melting points (mp) were obtained on a B-540 Büchi melting-point apparatus and are uncorrected. NMR spectra were recorded at 400 MHz (^{1}H NMR) or 100 MHz (^{13}C NMR) with DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard. *J* values are in Hertz. Chemical shifts are expressed in ppm downfield from internal TMS. The following abbreviations were used to designate

Table 1. Individual 2,4-Diamino-6-aryl-1,3,5-triazines

entry	product	R^1	R^2	R^3R^4NH	yield [a] (%)	MW(found)
1	8a	C_6H_5	4-$CH_3C_6H_4$		83	346.5 ($[M+H]^+$)
2	8b	C_6H_5	4-$CH_3C_6H_4$		81	348.6 ($[M+H]^+$)
3	8c	C_6H_5	4-$CH_3C_6H_4$		72	347.3 ($[M+H]^+$)
4	8d	C_6H_5	4-$CH_3C_6H_4$		83	361.4 ($[M+H]^+$)
5	8e	C_6H_5	4-$CH_3C_6H_4$		76	332.3 ($[M+H]^+$)
6	8f	C_6H_5	4-$CH_3C_6H_4$		78	334.5 ($[M+H]^+$)
7	8g	C_6H_5	4-$CH_3C_6H_4$		72	334.4 ($[M+H]^+$)
8	8h	C_6H_5	4-$CH_3C_6H_4$		74	368.4 ($[M+H]^+$)
9	8i	C_6H_5	4-$CH_3C_6H_4$		76	368.3 ($[M+H]^+$)
10	8j	C_6H_5	4-$CH_3C_6H_4$		65	453.3 ($[M+H]^+$)
11	8k	C_6H_5	C_6H_5		76	347.4 ($[M+H]^+$)
12	8l	C_6H_5	4-ClC_6H_4		52	381.3 ($[M+H]^+$)
13	8m	C_6H_5	$C_6H_5CH_2$		78	361.4 ($[M+H]^+$)
14	8n	C_6H_5	$CH_3CH_2CH_2CH_2$		73	327.4 ($[M+H]^+$)
15	8o	C_6H_5	$CH_3CH_2CH_2CH_2$		69	419.3 ($[M+H]^+$)
16	8p	4-$CH_3OC_6H_4$	4-$CH_3C_6H_4$		71	376.4 ($[M+H]^+$)
17	8q	4-$CH_3OC_6H_4$	4-$CH_3C_6H_4$		85	378.4 ($[M+H]^+$)
18	8r	4-$CH_3OC_6H_4$	C_6H_5		72	362.7 ($[M+H]^+$)
19	8s	4-$CH_3OC_6H_4$	C_6H_5		79	364.4 ($[M+H]^+$)
20	8t	4-$CH_3OC_6H_4$	C_6H_5		84	377.3 ($[M+H]^+$)
21	8u	4-$CH_3OC_6H_4$	4-ClC_6H_4		54	396.4 ($[M+H]^+$)
22	8v	4-$CH_3OC_6H_4$	4-ClC_6H_4		48	398.3 ($[M+H]^+$)
23	8w	3-ClC_6H_4	4-$CH_3C_6H_4$		81	380.4 ($[M+H]^+$)
24	8x	3-ClC_6H_4	C_6H_5		78	381.3 ($[M+H]^+$)

[a] Isolated yields calculated on the basis of the loading of the resin.

Table 2. Cytotoxicity of the 2,4-Diamino-6-aryl-1,3,5-triazines against Four Human Cancer Lines in Vitro

	cytotoxicity (IC_{50}, μM)[a]			
compd	PC-3	K562	A549	HO8910
doxorubicin	0.53 ± 0.26	0.15 ± 0.03	2.61 ± 0.37	0.2 ± 0.09
IRESSA	22.29 ± 5.75	7.74 ± 1.05	17.12 ± 1.18	2.25 ± 0.90
8a	38.10 ± 14.91	3.49 ± 2.15	>50	5.74 ± 1.49
8b	>50	23.66 ± 3.03	>50	>50
8c	2.23 ± 0.13	1.01 ± 0.19	>50	1.06 ± 0.66
8d	27.25 ± 14.21	3.36 ± 1.69	>50	>50
8e	>50	>50	>50	>50
8f	23.20 ± 1.17	5.64 ± 1.20	>50	2.96 ± 0.95
8g	22.26 ± 5.21	11.97 ± 2.76	>50	1.27 ± 0.98
8h	11.94 ± 2.26	>50	>50	>50
8i	41.77 ± 2.93	4.85 ± 4.06	46.61 ± 5.12	>50
8j	>50	>50	>50	>50
8k	12.06 ± 7.79	3.17 ± 4.24	14.06 ± 0.74	14.68 ± 3.26
8l	30.56 ± 12.67	2.76 ± 0.80	14.73 ± 5.57	12.28 ± 2.60
8m	19.56 ± 2.66	3.56 ± 0.70	22.80 ± 8.41	2.53 ± 1.64
8n	23.01 ± 3.80	6.23 ± 1.67	>50	4.87 ± 2.10
8o	>50	>50	>50	>50
8p	>50	20.75 ± 8.09	>50	30.46 ± 10.93
8q	21.62 ± 3.94	31.25 ± 5.12	>50	>50
8r	>50	29.25 ± 4.35	>50	8.67 ± 1.24
8s	>50	10.65 ± 4.59	>50	>50s
8t	>50	>50	>50	>50
8u	>50	>50	>50	>50
8v	>50	13.58 ± 3.22	>50	>50
8w	>50	>50	>50	>50
8x	24.86 ± 7.26	2.53 ± 0.62	19.59 ± 7.44	3.17 ± 2.30

[a] Each experiment was independently performed three times and expressed as means ± standard deviation (SD).

the multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. LC-MS (ESI) spectra were recorded on a Finnigan Mat LCQ mass spectrophotometer 214 nm using a Betasil C_{18} (3 μm, 100 Å, 3 × 50 mm) column. 4-(4-Formyl-3-methoxyphenoxy) butyryl AM resin (70−90 mesh; batch A31603, 1% DVB, substitution 0.88 mmol/g) was purchased from NovaBiochem.

4.1.1. General Procedure for the Synthesis of Crude Reaction Mixture 5. Oxide-free magnesium turnings (2.0 equiv, 130 mmol) were weighed out in a 100 mL three-necked round-bottom flask, to which a small crystal of iodine and 20 mL of anhydrous THF were added. Then substituted bromobenzene (1.8 equiv, 117 mmol) in 50 mL of THF were added dropwise to the flask maintaining a stead exotherm. When the addition was complete, the mixture was warmed to reflux for 2 h. After cooling, the Grignard solution was added dropwise to a mixture of cyanuric chloride **4** (12 g, 65 mmol) and THF (60 mL), while keeping the internal temperature of the mixture below 10 °C. Following addition, the mixture was stirred for 4 h at room temperature. The mixture was then diluted with toluene and washed with 10% aqueous HCl, water, and brine. The organic phase was dried over sodium sulfate, and the concentrated crude reaction mixture **5** was used without further purification in the next step.

4.1.2. General Procedure for the Synthesis of 2,4-Diamino-6-aryl-1,3,5-triazines 8. To the 4-(4-formyl-3-methoxyphenoxy)butyryl AM resin **1** (220 mg, 0.2 mmol sealed within a polypropylene mesh packet) was added an amine **2** (10 equiv, 0.1 mol/L), $NaBH_3CN$ (10 equiv, 0.1 mol/L) in anhydrous DMF/CH_3COOH (99:1). The mixture was shaken for 24 h at room temperature. The resin was then washed with DMF (3 times), methanol (3 times), and dichloromethane (3 times). The resulting resin-bound compound **3** was coupled with the crude reaction mixture **5** (10 equiv, 0.1 mol/L) in the presence of Et_3N (10 equiv, 0.1 mol/L) in anhydrous THF at room temperature for 24 h. The resin was washed with DMF (3 times), methanol (3 times), and dichloromethane (3 times). The final amination of resin-bound compound **6** was carried out using an amine (10 equiv, 0.1 mol/L) and Et_3N (10 equiv, 0.1 mol/L) in DMF at 100 °C for 48 h to afford resin-bound compound **7**. After being washed with DMF (3 times), methanol (3 times), and dichloromethane (3 times), the resin-bound compound **7** was treated with TFA/DCM = 1:1 at room temperature for 1 h and the solvent was removed under the reduced pressure. Following purification by flash column chromatography on silica gel, compounds **8** were characterized by electrospray LC-MS, ^{1}H NMR, and ^{13}C NMR.

4-Phenyl-6-(piperidin-1-yl)-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8a). White solid, mp 103−105 °C, LC-MS (ESI) *m*/*z* 346.5 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (1H, s), 8.35 (2H, d, J = 7.2 Hz), 7.65 (2H, d, J = 8.0 Hz), 7.49−7.55 (3H, m), 7.13 (2H, d, J = 8.0 Hz), 3.82−3.90 (4H, m), 2.26 (3H, s), 1.48−1.72 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 164.2, 137.2, 136.9, 131.4, 130.9, 128.8, 128.2, 127.8, 119.9, 43.7, 25.3, 24.2, 20.3.

4-Morpholino-6-phenyl-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8b). White solid, mp 228−229 °C, LC-MS (ESI) *m*/*z* 348.6 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (1H, s), 8.36 (2H, d, J = 6.8 Hz), 7.65 (2H, d, J = 8.0 Hz), 7.49−7.59 (3H, m), 7.14 (2H, d, J = 8.0 Hz), 3.85 (4H, t), 3.69 (4H, t), 2.27 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 165.5, 164.8, 137.1, 136.3, 131.6, 130.7, 128.6, 128.2, 127.9, 120.2, 65.9, 43.4, 20.3.

4-Phenyl-6-(piperazin-1-yl)-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8c). White solid, mp 225−227 °C, LC-MS (ESI) *m*/*z* 347.3 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (1H, s), 8.35 (2H, d, J = 7.2 Hz), 7.65 (2H, d, J = 8.0 Hz), 7.48−7.57 (3H, m), 7.12 (2H, d, J = 8.0 Hz), 3.86 (4H, t), 2.41 (4H, t), 2.26 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 164.4, 164.1, 137.2, 136.7, 131.6, 131.0, 128.8, 128.2, 127.9, 120.2, 51.8, 44.6, 20.3.

4-(4-Methylpiperazin-1-yl)-6-phenyl-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8d). White solid, mp 211−213 °C, LC-MS (ESI) *m*/*z* 361.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (1H, s), 8.35 (2H, d, J = 7.2 Hz), 7.64 (2H, d, J = 8.0 Hz), 7.49−7.57 (3H, m), 7.13 (2H, d, J = 8.0 Hz), 3.87 (4H, t), 2.39 (4H, t), 2.27 (3H, s), 2.23 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.7, 164.5, 164.2, 137.1, 136.7, 131.5, 131.1, 128.8, 128.2, 127.9, 120.1, 54.3, 45.7, 42.8, 20.3.

4-Phenyl-6-(pyrrolidin-1-yl)-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8e). White solid, mp 164−166 °C, LC-MS (ESI) *m*/*z* 332.3 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (1H, s), 8.34 (2H, d, J = 7.2 Hz), 7.72 (2H, d, J = 8.8 Hz), 7.47−7.55 (3H, m), 7.10 (2H, d, J = 8.8 Hz), 3.62 (4H, t), 2.24 (3H, s), 1.93 (4H, t). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 164.1, 137.3, 136.8, 131.3, 130.7, 128.8, 128.2, 127.6, 120.1, 42.4, 25.1, 20.3.

***N*,*N*-Diethyl-6-phenyl-*N*′-*p*-tolyl-1,3,5-triazine-2,4-diamine (8f).** White solid, mp 97−99 °C, LC-MS (ESI) *m*/*z* 334.5 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41(2H,

d, J = 8.0 Hz), 7.51(2H, d, J = 8.4 Hz), 7.42−7.47 (3H, m), 7.37 (1H, s), 7.09 (2H, d, J = 8.0 Hz), 3.70 (4H, q), 2.29 (3H, s), 1.25 (6H, t). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 163.6, 137.2, 136.8, 131.3, 130.9, 128.5, 128.1, 127.8, 119.9, 43.3, 20.4, 13.7.

***N*-Butyl-*N′*-*p*-tolyl-6-phenyl-1,3,5-triazine-2,4-diamine (8g).** White solid, mp 95−97 °C, LC-MS (ESI) m/z 334.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (1H, s), 8.30 (2H, d, J = 7.2 Hz), 7.72 (2H, d, J = 8.4 Hz), 7.65 (1H, s), 7.49−7.52 (3H, m), 7.10 (2H, d, J = 8.0 Hz), 3.34−3.44 (2H, m), 2.26 (3H, s), 1.54−1.61 (2H, m), 1.35−1.40 (2H, m), 0.92 (3H, t). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 165.8, 165.7, 137.4, 136.8, 131.2, 130.8, 128.8, 128.2, 127.8, 127.6, 30.9, 20.3, 19.6, 19.5, 13.7.

***N*^{2}-Benzyl-6-phenyl-*N*^{4}-*p*-tolyl-1,3,5-triazine-2,4-diamine (8h).** White solid, mp 91−93 °C, LC-MS (ESI) m/z 368.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (1H, s), 8.32 (2H, d, J = 6.4 Hz), 8.08 (1H, s), 7.72 (1H, d, J = 7.6 Hz), 7.05−7.55 (11H, m), 4.57 (2H, s), 2.25 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 164.3, 164.1, 143.2, 137.0, 136.7, 131.5, 131.0, 128.8, 128.5, 128.2, 127.8, 127.1, 126.3, 119.7, 44.7, 20.3.

6-Phenyl-*N*^{2},*N*^{4}-di-*p*-tolyl-1,3,5-triazine-2,4-diamine (8i). White solid, mp 128−131 °C, LC-MS (ESI) m/z 368.3 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (2H, s), 8.38 (2H, d, J = 6.8 Hz), 7.54−7.71 (7H, m), 7.15 (2H, d, J = 6.8 Hz), 2.29 (6H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 164.2, 136.8, 136.6, 131.6, 131.4, 128.8, 128.3, 127.9, 120.5, 120.4, 20.4.

4-Phenyl-*N*-*p*-tolyl-6-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1,3,5-triazin-2-amine (8j). White solid, mp 234−236 °C, LC-MS (ESI) m/z 453.3 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (1H, s), 8.40 (2H, d, J = 7.2 Hz), 7.65 (2H, d, J = 7.2 Hz), 7.52−7.59 (3H, m), 7.17 (2H, d, J = 8.0 Hz), 5.35 (2H, t), 4.43 (2H, t), 4.32 (2H, s), 2.29 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 164.2, 151.1, 142.7, 136.7, 136.2, 131.8, 131.6, 131.5, 128.9, 128.4, 128.0, 120.4, 119.8, 43.0, 42.9, 28.9, 20.4.

4-(4-Methylpiperazin-1-yl)-*N*,6-diphenyl-1,3,5-triazin-2-amine (8k). White solid, mp 161−163 °C, LC-MS (ESI) m/z 347.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (1H, s), 8.36 (2H, d, J = 6.8 Hz), 7.79 (2H, d, J = 8.0 Hz), 7.49−7.56 (3H, m), 7.33 (2H, t, J_1 = 7.6 Hz, J_2 = 8.0 Hz), 7.01 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.92 (4H, t), 2.40 (4H, t), 2.23 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 164.4, 164.2, 139.7, 136.6, 131.5, 128.4, 128.3, 127.9, 122.1, 119.9, 54.3, 45.7, 42.8.

***N*-(4-chlorophenyl)-4-(4-methylpiperazin-1-yl)-6-phenyl-1,3,5-triazin-2-amine (8l).** White solid, mp 186−188 °C, LC-MS (ESI) m/z 381.3 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (1H, s), 8.35 (2H, d, J = 8.0 Hz), 7.80 (2H, d, J = 8.0 Hz), 7.49−7.56 (3H, m), 7.37 (2H, d, J = 8.0 Hz), 3.92 (4H, t), 2.39 (4H, t), 2.22 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 164.4, 164.1, 138.7, 136.5, 131.6, 128.3, 128.2, 127.9, 125.7, 121.4, 54.3, 45.7, 42.8.

***N*-Benzyl-4-(4-methylpiperazin-1-yl)-6-phenyl-1,3,5-triazin-2-amine (8m).** White solid, mp 117−119 °C, LC-MS (ESI) m/z 361.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 7.87−8.06 (1H, m), 7.22−7.52 (8H, m), 4.51 (2H, d, J = 6.4 Hz), 3.81 (4H, t), 2.33 (4H, t), 2.19 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1 163.8, 150.4, 143.3, 131.6, 131.4, 128.8, 128.2, 127.9, 127.1, 126.3, 54.3, 45.7, 44.6, 42.8

***N*-Butyl-4-(4-methylpiperazin-1-yl)-6-phenyl-1,3,5-triazin-2-amine (8n).** White solid, mp 153−155 °C, LC-MS (ESI) m/z 327.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32−8.40 (m, 2H), 7.39−7.47 (m, 3H), 5.45 (s, 1H), 3.94 (t, 4H), 3.40−3.52 (m, 2H), 2.46 (t, 4H), 2.33 (s, 3H), 1.54−1.56 (m, 2H), 1.37−1.39 (m, 2H), 0.93 (t, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 163.4, 150.6, 131.5, 131.4, 128.2, 127.9, 54.3, 45.7, 42.8, 30.9, 19.6, 19.5, 13.6.

***N*-Butyl-4-phenyl-6-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1,3,5-triazin-2-amine (8o).** White solid, mp 172−173 °C, LC-MS (ESI) m/z 419.3 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (2H, d, J = 7.2 Hz), 8.34 (2H, d, J = 7.2 Hz), 7.69 (1H, t, J_1 = 5.6 Hz, J_2 = 5.6 Hz), 7.49−7.54 (3H, m), 5.24 (2H, t), 4.35 (2H, t), 4.28 (2H, s), 3.33−3.45 (2H, m), 1.52−1.57 (2H, m), 1.33−1.38 (2H, m), 0.90−0.94 (3H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.8, 165.7, 151.2, 136.5, 131.5, 131.4, 128.2, 127.9, 127.8, 119.8, 43.0, 42.9, 31.3, 30.9, 19.6, 19.5, 13.6.

4-(4-Methoxyphenyl)-6-(piperidin-1-yl)-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8p). White solid, mp 137−139 °C, LC-MS (ESI) m/z 376.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (1H, s), 8.30 (2H, d, J = 8.8 Hz), 7.65 (2H, d, J = 8.0 Hz), 7.12 (2H, d, J = 8.2 Hz), 7.04 (2H, d, J = 8.8 Hz), 3.83 (7H, s), 2.26 (3H, s), 1.56−1.65 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.3, 164.2, 161.9, 137.3, 130.8, 129.6, 129.2, 128.8, 119.9, 113.6, 55.3, 43.7, 25.4, 24.2, 20.3.

4-(4-Methoxyphenyl)-6-morpholino-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8q). White solid, mp 222−224 °C, LC-MS (ESI) m/z 378.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (1H, s), 8.31 (2H, d, J = 8.8 Hz), 7.64 (2H, d, J = 8.0 Hz), 7.12 (2H, d, J = 8.0 Hz), 7.05 (2H, d, J = 8.8 Hz), 3.83 (7H, s), 3.68 (4H, t), 2.26 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 164.6, 164.1, 162.1, 137.1, 130.9, 129.7, 128.9, 128.8, 120.1, 113.6, 65.9, 55.3, 43.3, 20.3.

4-(4-Methoxyphenyl)-*N*-phenyl-6-(piperidin-1-yl)-1,3,5-triazin-2-amine (8r). White solid, mp 148−151 °C, LC-MS (ESI) m/z 362.7 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (1H, s), 8.32 (2H, d, J = 8.8 Hz), 7.79 (2H, d, J = 8.0 Hz), 7.32 (2H, t, J_1 = 8.0 Hz, J_2 = 8.0 Hz), 7.05 (2H, d, J = 8.8 Hz), 6.99 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.83 (7H, s), 1.56−1.66 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 164.2, 164.1, 162.0, 139.9, 129.6, 129.1, 128.4, 121.9, 119.8, 113.6, 55.3, 43.7, 25.3, 24.2.

4-(4-Methoxyphenyl)-6-morpholino-*N*-phenyl-1,3,5-triazin-2-amine (8s). White solid, mp 197−199 °C, LC-MS (ESI) m/z 364.4 (M + H$^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.59 (1H, s), 8.33 (2H, d, J = 8.8 Hz), 7.78 (2H, d, J = 8.0 Hz), 7.32 (2H, t, J_1 = 8.0 Hz, J_2 = 8.0 Hz), 7.05 (2H, d, J = 8.8 Hz), 7.00 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.84 (7H, s), 3.69 (4H, t). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 164.6, 164.1, 162.1, 139.7, 129.7, 128.9, 128.4, 122.0, 119.9, 113.6, 65.9, 55.3, 43.4.

4-(4-Methoxyphenyl)-6-(4-methylpiperazin-1-yl)-*N*-phenyl-1,3,5-triazin-2-amine (8t). White solid, mp 225−228 °C, LC-MS (ESI) *m/z* 377.3 ($M + H^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (1H, s), 8.32 (2H, d, $J = 8.8$ Hz), 7.77 (2H, d, $J = 7.6$ Hz), 7.32 (2H, t, $J_1 = 8.0$ Hz, $J_2 = 8.0$ Hz), 7.05 (2H, d, $J = 8.8$ Hz), 6.99 (1H, t, $J_1 = 7.2$ Hz, $J_2 = 7.6$ Hz), 3.84 (7H, s), 2.39 (4H, t), 2.23 (3H, s).^{13}C NMR (100 MHz, DMSO-d_6) δ 164.4, 164.1, 162.1, 139.8, 129.7, 128.9, 128.4, 122.0, 119.8, 113.6, 55.3, 54.3, 45.7, 42.8.

***N*-(4-Chlorophenyl)-4-(4-methoxyphenyl)-6-(piperidin-1-yl)-1,3,5-triazin-2-amine (8u).** White solid, mp 182−185 °C, LC-MS (ESI) *m/z* 396.4 ($M + H^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (1H, s), 8.30 (2H, d, $J = 8.8$ Hz), 7.80 (2H, d, $J = 8.8$ Hz), 7.36 (2H, d, $J = 8.8$ Hz), 7.05 (2H, d, $J = 8.8$ Hz), 3.83−3.89 (7H, m), 1.56−1.66 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 164.1, 164.0, 162.1, 138.9, 129.7, 128.9, 128.3, 125.4, 121.2, 113.6, 55.3, 43.7, 25.3, 24.2.

***N*-(4-Chlorophenyl)-4-(4-methoxyphenyl)-6-morpholino-1,3,5-triazin-2-amine (8v).** White solid, mp 231−233 °C, LC-MS (ESI) *m/z* 398.3 ($M + H^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.74 (1H, s), 8.32 (2H, d, $J = 8.8$ Hz), 7.80 (2H, d, $J = 8.8$ Hz), 7.37 (2H, d, $J = 8.8$ Hz), 7.05 (2H, d, $J = 8.8$ Hz), 3.76−3.95 (7H, m), 3.69 (4H, t). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.5, 164.5, 164.1, 162.2, 138.8, 129.8, 128.7, 128.3, 125.6, 121.4, 113.6, 65.9, 55.3, 43.4.

4-(3-Chlorophenyl)-6-(piperidin-1-yl)-*N*-*p*-tolyl-1,3,5-triazin-2-amine (8w). White solid, mp 141−143 °C, LC-MS (ESI) *m/z* 380.4 ($M + H^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (1H, s), 8.27−8.31 (2H, m), 7.52−7.64 (4H, m), 7.12 (2H, d, $J = 8.0$ Hz), 3.80−3.89 (4H, m), 2.26 (3H, s), 1.48−1.64 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 164.2, 164.1, 139.1, 137.0, 133.2, 131.1, 130.2, 128.8, 127.4, 126.3, 120.0, 43.8, 25.3, 24.2, 20.3.

4-(3-Chlorophenyl)-6-(4-methylpiperazin-1-yl)-*N*-phenyl-1,3,5-triazin-2-amine (8x). White solid, mp 175−177 °C, LC-MS (ESI) *m/z* 381.3 ($M + H^+$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (1H, s), 8.31 (2H, t, $J_1 = 7.2$ Hz, $J_2 = 8.0$ Hz), 7.75 (2H, d, $J = 8.0$ Hz), 7.62−7.64 (1H, m), 7.55 (1H, t, $J_1 = 7.6$ Hz, $J_2 = 8.0$ Hz), 7.33 (2H, t, $J_1 = 7.6$ Hz, $J_2 = 8.0$ Hz), 7.01 (1H, t, $J_1 = 7.2$ Hz, $J_2 = 7.2$ Hz), 3.87 (4H, t), 2.40 (4H, t), 2.23 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 164.4, 164.1, 139.5, 138.8, 133.2, 131.3, 130.3, 128.5, 127.4, 126.4, 122.3, 120.0, 54.3, 45.7, 42.8.

4.2. Cytotoxic Assay. The tumor cell lines (A549, K562, PC-3, and HO8910) were obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and cultured in proper medium in a 5% CO_2 atmosphere at 37 °C. The cells were seeded to 96-well plates at a density of 5×10^3 cells/well. After 24 h, the cells were treated with 0.01, 0.1, 1, 10, and 100 μmol/L chemicals dissolved in DMSO (final concentration 0.1%), and the positive control cells were treated with doxorubicin hydrochloride and IRESSA, respectively. A 72 h period of time later, 20 μL of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) solution was added per well and the cells were cultured for another 4 h. Following this, the medium was discarded, and DMSO was added. The optical density (OD) of the resultant solution was measured at a wavelength of 570 nm with a microplate reader (EL × 800, Bio-Tek, Winooski, USA). The IC_{50} values were calculated with LOGIT method. Assays were performed in triplicate on three independent experiments.

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Supporting Information Available. Copies of LC−MS of **8a**, **8c**, **8j**, **8w** and ^{1}H and ^{13}C NMR spectra of **8a**, **8d**, **8i**, **8j**, **8l**, **8p**, **8r**, and **8w**. This material is available free of charge via the Internet at http://pubs.acs.org.

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