Current Topics

Drug Discovery: Recent Progress and the Future

Regular Article

Discovery of *N*-{2-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (ASP3026), a Potent and Selective Anaplastic Lymphoma Kinase (ALK) Inhibitor

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Anaplastic lymphoma kinase (ALK) is a validated therapeutic target for treating echinoderm microtubule-associated protein-like 4 (EML4)-ALK positive non-small cell lung cancer (NSCLC). We synthesized a series of 1,3,5-triazine derivatives and identified ASP3026 (14a) as a potent and selective ALK inhibitor. In mice xenografted with NCI-H2228 cells expressing EML4-ALK, once-daily oral administration of 14a demonstrated dose-dependent antitumor activity. Here, syntheses and structure–activity relationship (SAR) studies of 1,3,5-triazine derivatives are described.

Key words 1,3,5-triazine; non-small cell lung cancer; anaplastic lymphoma kinase; echinoderm microtubule-associated protein-like 4

The receptor tyrosine kinase anaplastic lymphoma kinase (ALK) was identified in anaplastic large-cell lymphoma (ALCL) as a fusion gene, comprising portions of the nucleophosmin (*NPM*) gene and the *ALK* gene that contains the kinase catalytic domain, which encodes the NPM-ALK fusion protein.[1] In 2007, the echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion gene was identified in a subset of non-small cell lung cancer (NSCLC) patients.[2,3] EML4-ALK oncogenic fusion kinase plays an essential role in the pathogenesis of NSCLC.[4] In addition, EML4-ALK has constitutive tyrosine kinase activity.[2,5] Furthermore, a number of ALK inhibitors have been reported to date,[6] with crizotinib having been approved by the U.S. Food and Drug Administration (FDA) in 2011[7] (Fig. 1). Given these previous findings, ALK is a validated therapeutic target for treating EML4-ALK-positive NSCLC.

Several compounds with inhibitory activity against ALK, such as NVP-TAE684[8,9] (Fig. 1), had been previously reported when we started our project to identify novel ALK inhibitors. In the course of exploring novel ALK inhibitors, 1,3,5-triazine derivatives were discovered. As a result of detailed structure–activity relationship (SAR) studies on every part of 1,3,5-triazine derivatives, **14a** (ASP3026[10]) was identified and selected as a clinical candidate. Here, we describe the syntheses and SAR studies of 1,3,5-triazine derivatives as novel ALK inhibitors. We also report the kinase selectivity and antitumor activity of **14a**.

Results and Discussion

Chemistry Compounds **5a**–**5e** were synthesized by reacting **3a**–**3c**, **3d**[11] and **3e**[12] with 4-piperidone monohydrate monohydrochloride, followed by reductive amination with 1-methylpiperazine and sodium triacetoxyborohydride ($NaBH(OAc)_3$) and hydrogenation with catalytic palladium on carbon (Chart 1). Compounds **8** and **10** were synthesized by introduction of the commercially available corresponding amines into **6** and **3a**, followed by hydrogenation of the nitro groups (Charts 2, 3, respectively).

The synthesis of compounds **14a**–**14l** is shown in Chart 4. These were prepared by reacting compounds **11a**–**11c**[13] with corresponding 1,3,5-triazine derivatives **12a**, **12b** and **12c**[14] to afford 4-chloro-1,3,5-triazine analogues **13a**–**13e**, followed by the introduction of corresponding aniline derivatives using methanesulfonic acid (MsOH) in ethanol (EtOH).

The synthesis of **20** is shown in Chart 5. Compound **16**

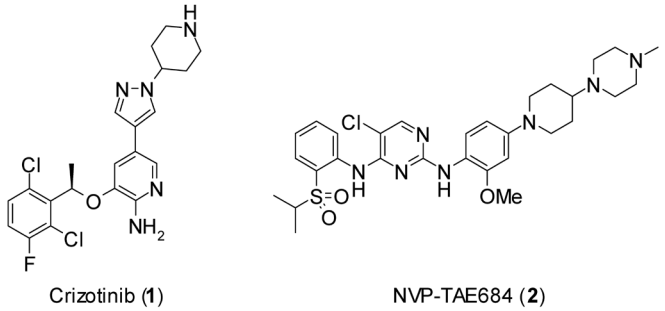

Fig. 1. Structures of Crizotinib and NVP-TAE684

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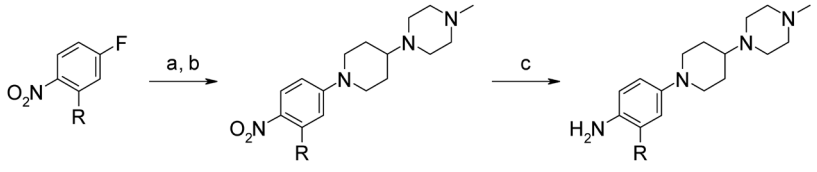
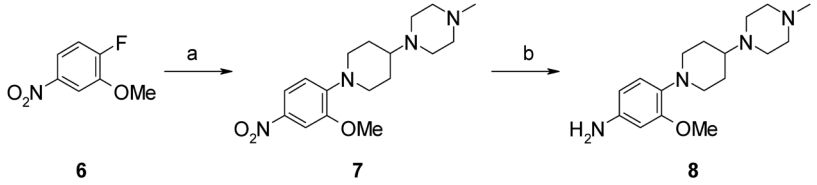


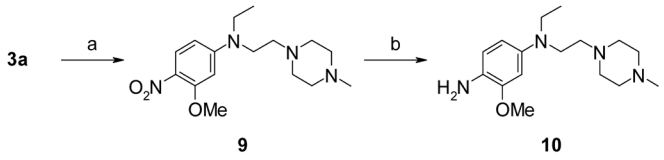
Reagents and conditions: (a) 4-piperidone monohydrate monohydrochloride, K_2CO_3, *N*,*N*-dimethylformamide (DMF), 70 or 80°C; (b) 1-methylpiperazine, $NaBH(OAc)_3$, dichloromethane (DCM) or 1,2-dichloroethane (DCE), rt; (c) H_2, 10% Pd/C, EtOH or EtOH/tetrahydrofuran (THF), rt.

Chart 1. Synthesis of Compounds **5a**–**5e**



Reagents and conditions: (a) 1-methyl-4-(piperidin-4-yl)piperazine, K_2CO_3, DMF, 80°C; (b) H_2, 10% Pd/C, EtOH/THF, rt.

Chart 2. Synthesis of Compound **8**



Reagents and conditions: (a) *N*-ethyl-2-(4-methylpiperazin-1-yl)ethanamine, K_2CO_3, DMF, 80°C; (b) H_2, 10% Pd/C, EtOH, rt.

Chart 3. Synthesis of Compound **10**

was synthesized by reacting commercially available **15** with 2-bromopropane using a sodium hydroxymethanesulfinate promoted one-pot reaction.[16] Oxidation of **16** with *m*-chloroperoxybenzoic acid (*m*CPBA), followed by reduction of the nitro group using iron powder in acetic acid (AcOH), provided compound **18**, which was converted to **20** in two steps by employing procedures similar to those described for **14a**–**14l**.

The synthesis of **25** is shown in Chart 6. Commercially available 1,4-dioxa-8-azaspiro[4.5]decane was reacted with **3a** to give **21**, which was then hydrogenated to give compound **22**. The introduction of **22** into **13a** successfully proceeded using *N*,*N*-diisopropylethylamine (DIPEA) in *N*-methylpyrrolidinone (NMP) under microwave conditions at 120°C to give **23**. After acidic hydrolysis of **23**, ketone **24** was subjected to reductive amination conditions with morpholine, then treated with 4 M HCl in ethyl acetate (EtOAc) to obtain **25** as a trihydrochloride salt.

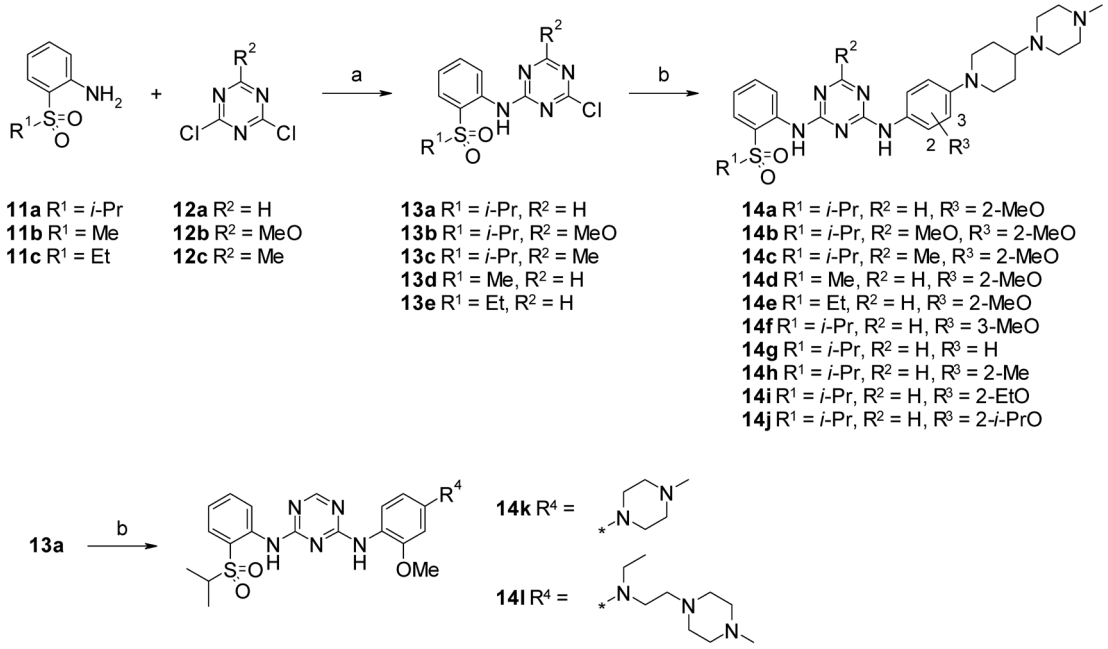
Compound **29** was synthesized by reacting **26**[17] with **13a** under basic conditions, followed by the removal of the *tert*-butoxycarbonyl group and reductive amination (Chart 7).

Biological Evaluation The synthesized compounds were evaluated *via* EML4-ALK enzyme and cell growth assays using Ba/F3 expressing EML4-ALK. The SARs of the synthesized compounds are summarized in Tables 1–4.

As shown in Table 1, **14a** inhibited EML4-ALK with an IC_{50} value of 17 nM.[18] Compounds **14b** and **14c**, with a methoxy group and a methyl group on the 1,3,5-triazine ring of **14a**, showed inhibitory activity against EML4-ALK with IC_{50} values of 73 and 360 nM, respectively. Our docking model of **14a** with wild type ALK indicates the formation of two hydrogen bonds between one of the nitrogen atoms of the 1,3,5-triazine ring and the NH hydrogen atom of Met1199, and between the hydrogen atom of the 1,3,5-triazine ring and the carbonyl oxygen atom of Glu1197 in the hinge region (Fig. 2). Therefore, the reduced inhibition of EML4-ALK by **14b** and **14c** compared to that of **14a** could be attributed to the attenuation of the interaction between these compounds and the hinge moiety in ALK.

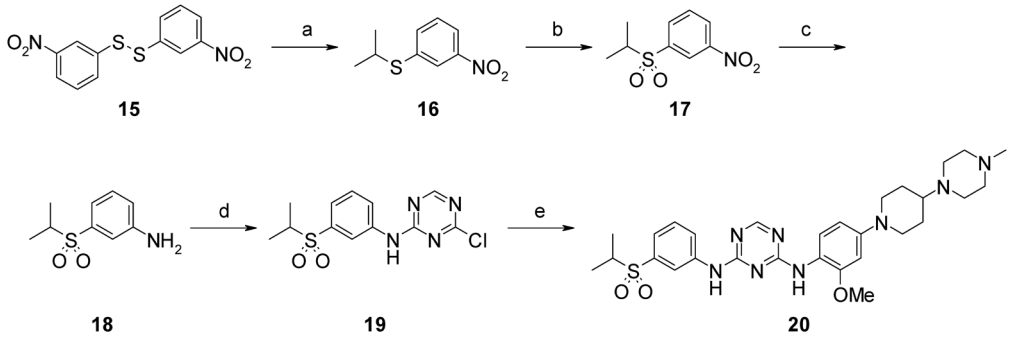
Table 2 shows SARs of the sulfonyl moiety of **14a**. Substitution of the 2-isopropylsulfonyl group of **14a** with a 3-isopropylsulfonyl group (**20**) led to a loss of inhibitory activity against EML4-ALK, possibly due to the steric hindrance between **20** and the amino acid residues in ALK, including Gly1125 and Ala1126, as shown in Fig. 3. The methylsulfonyl derivative **14d** exhibited reduced inhibition of EML4-ALK (IC_{50}=530 nM), whereas the ethylsulfonyl analogue **14e** maintained inhibitory activity against EML4-ALK (IC_{50}=21 nM). Our docking model suggests that the 2-isopropylsulfonyl moiety plays two important roles in the potent inhibitory activity of EML4-ALK (Fig. 3). First, the oxygen atom of the sulfone interacts with Lys1150 in ALK. Second, one of the two methyl groups in the isopropyl moiety extends into and forms a hydrophobic interaction with the hydrophobic pocket created by Leu1256 in ALK. Therefore, the decrease in inhibitory activity of **14d** could be attributed to the loss of the interaction between **14d** and the hydrophobic pocket. In contrast, **14e** retained its inhibitory activity, as the ethyl group in **14e** can interact with the hydrophobic pocket.

Substituent effects of the methoxy component of **14a** were then examined, with results shown in Table 3. Replacement of the 2-methoxy group (**14a**) with a 3-methoxy group (**14f**) resulted in a three-fold reduction in inhibition of EML4-ALK. An unsubstituted phenyl ring (**14g**) resulted in a 19-fold decrease in inhibitory activity against EML4-ALK relative to



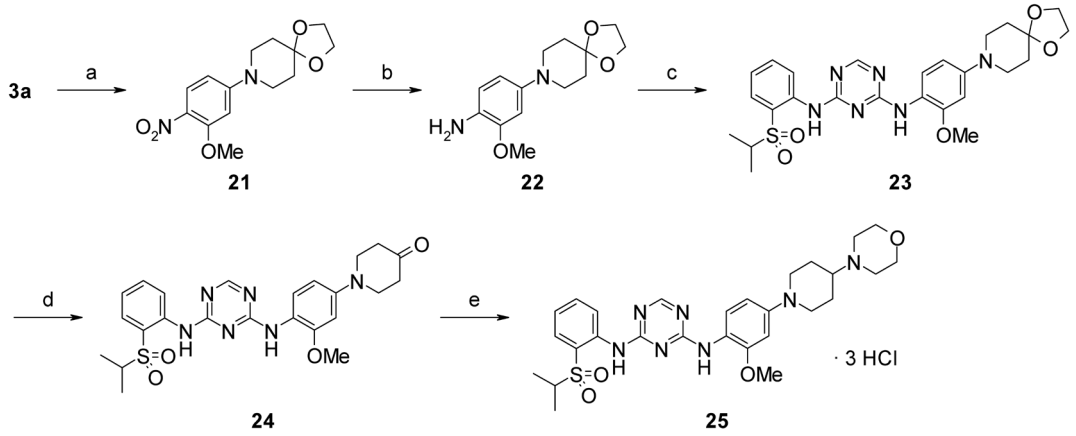
Reagents and conditions: (a) DIPEA, THF, rt or 70°C; (b) **5a**–**5e**, **8**, 2-methoxy-4-(4-methylpiperazin-1-yl)aniline,[15] or **10**, MsOH, EtOH, 80 or 100°C.

Chart 4. Synthesis of Compounds **14a**–**14l**



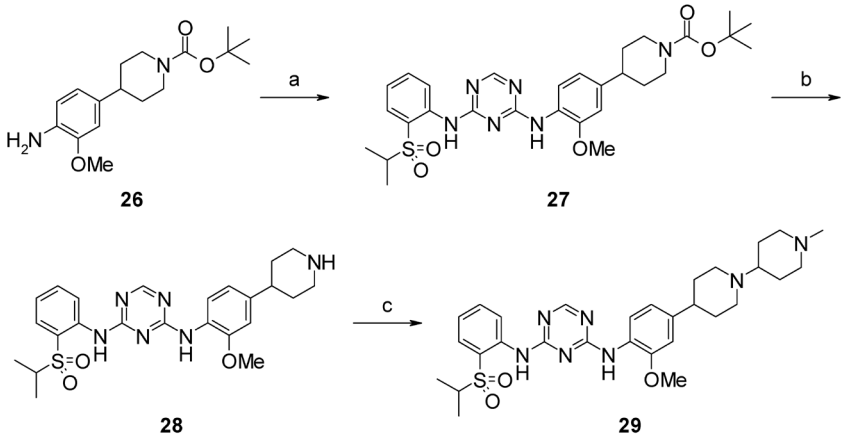
Reagents and conditions: (a) 2-bromopropane, sodium hydroxymethanesulfinate, K_2CO_3, DMF/H_2O, rt; (b) mCPBA, $CHCl_3$, rt to 50°C; (c) Fe, AcOH, 80°C; (d) **12a**, DIPEA, THF, 0°C; (e) **5a**, MsOH, EtOH, 100°C.

Chart 5. Synthesis of Compound **20**



Reagents and conditions: (a) 1,4-dioxa-8-azaspiro[4.5]decane, K_2CO_3, DMF, 70°C; (b) H_2, 10% Pd/C, EtOH/THF, rt; (c) **13a**, DIPEA, NMP, microwave, 120°C; (d) 4 M HCl aq, 1,4-dioxane, 80°C; (e) morpholine, $NaBH(OAc)_3$, DCM, rt, then 4 M HCl/EtOAc, THF, rt.

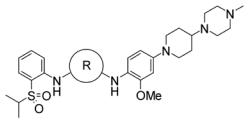
Chart 6. Synthesis of Compound **25**



Reagents and conditions: (a) **13a**, DIPEA, NMP, microwave, 120°C; (b) 4 M HCl/EtOAc, EtOAc/MeOH, rt; (c) 1-methylpiperidin-4-one, $NaBH(OAc)_3$, DCM, rt.

Chart 7. Synthesis of Compound **29**

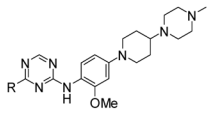
Table 1. Structure–Activity Relationships of Compounds **14a–14c**

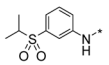


Compound	R	IC_{50} (nM)	
		EML4-ALK (enzyme)	Ba/F3 (cell)
14a		17	42
14b		73	304
14c		360	NT[a]

a: Not tested.; EML4-ALK enzyme IC_{50} value of compound **2** was 0.63 nM.[18]

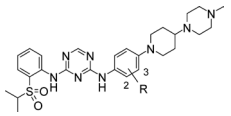
Table 2. Structure–Activity Relationships of Compounds **14a**, **14d**, **14e** and **20**



Compound	R	IC_{50} (nM)	
		EML4-ALK (enzyme)	Ba/F3 (cell)
14a		17	42
20		> 1000	NT[a]
14d		530	NT[a]
14e		21	146

a: Not tested.

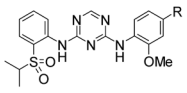
Table 3. Structure–Activity Relationships of Compounds **14a** and **14f–14j**

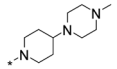
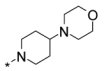
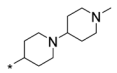
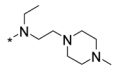


Compound	R	IC_{50} (nM)	
		EML4-ALK (enzyme)	Ba/F3 (cell)
14a	2-MeO	17	42
14f	3-MeO	56	107
14g	H	330	NT[a]
14h	2-Me	340	NT[a]
14i	2-EtO	93	197
14j	2-*i*-PrO	210	NT[a]

a: Not tested.

Table 4. Structure–Activity Relationships of Compounds **14a**, **14k**, **14l**, **25** and **29**



Compound	R	IC_{50} (nM)	
		EML4-ALK (enzyme)	Ba/F3 (cell)
14a		17	42
14k		29	67
25[a]		28	208
29		7.9	61
14l		81	ND[b]

a: 3HCl salt.; *b*: Not determined.

14a. Substitution of the methoxy group with a methyl group (**14h**) also reduced potency. These results indicate that the oxygen atom at the 2-position is important for the inhibitory activity of **14a**, possibly due to the formation of a dipole–dipole interaction between the methoxy group of **14a** and NH at the 2-position of the 1,3,5-triazine ring, and stabilization of the desirable conformation, as shown in Fig. 2. Replacing the methoxy group of **14a** with an ethoxy group (**14i**) or an isopropoxy group (**14j**) also resulted in a reduction of inhibitory activity (IC_{50}=93 and 210 nM, respectively). This finding is consistent with observations in our docking model suggesting that the space between the 2-methoxy group of **14a** and ALK is relatively narrow (Fig. 2).

Table 4 shows SARs of the amine moiety in **14a**, which projects into the solvent region located outside the ATP binding pocket and adjacent to Glu1210, as shown in Fig. 2. Compound **14k** retained inhibitory activity against EML4-ALK with an IC_{50} value of 29 nM.[18] Replacement of the piperazine ring of **14a** with a morphorine ring (**25**) also maintained inhibition of EML4-ALK. Compound **29** potently inhibited

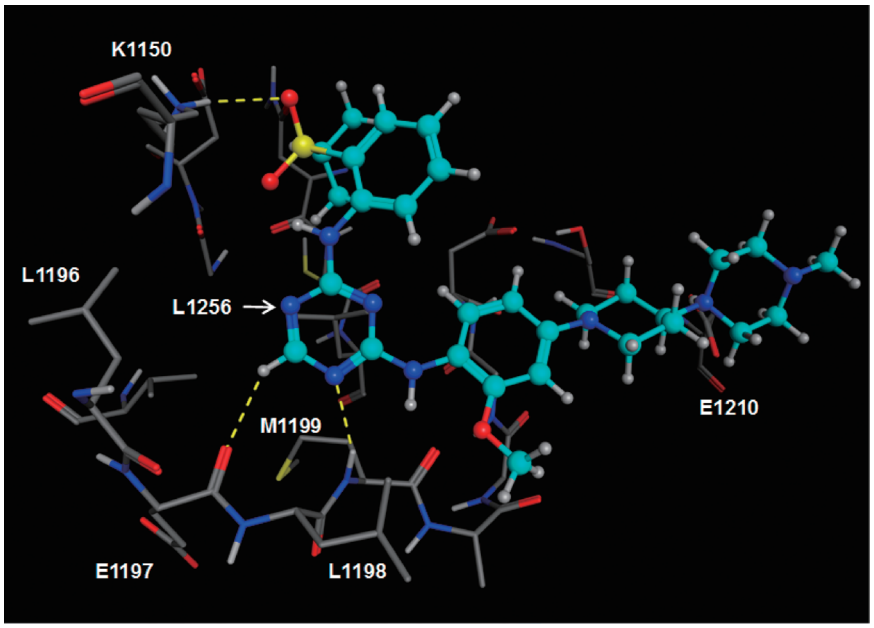


Fig. 2. The Docking Mode of **14a** with Wild Type ALK

14a and ALK are represented as a ball-and-stick and stick model, respectively. All of the atoms are colored according to element (white: hydrogen, cyan: carbon of **14a**, gray: carbon of ALK, blue: nitrogen, red: oxygen, yellow: sulfur). The yellow dotted lines indicate the hydrogen bonds formed between **14a** and ALK. For clarity, the nonpolar hydrogen atoms of the protein are omitted, and any atoms of ALK closer than the front of the gatekeeper residue are hidden.

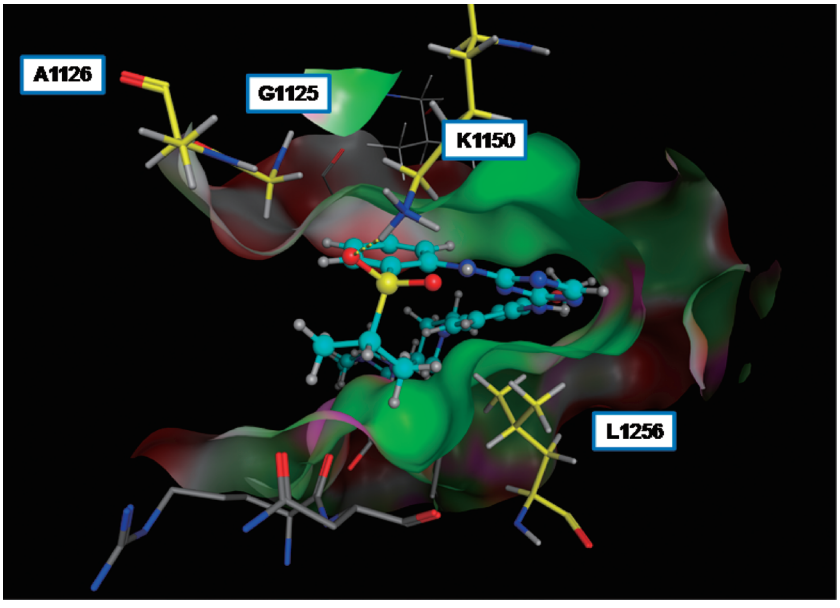


Fig. 3. The Docking Mode of **14a** with Wild Type ALK

14a and ALK are represented as a ball-and-stick and stick model, respectively. The protein surface is colored by the characteristics of the pocket (green: hydrophobic, magenta: polar, red: solvent exposed). The carbon atoms of A1126, G1125, K1150, and L1256 are highlighted in yellow. The other coloring and visualizing schemes are the same as in Fig. 2.

Table 5. Kinase Selectivity of **1** and **14a**[10)]

Kinase	Compound **1** IC_{50} (nM)	Selectivity[a]	Kinase	Compound **14a** IC_{50} (nM)	Selectivity[a]
ROS	0.95	0.63	ALK	3.5	—
ALK	1.5	—	ALK R1275Q	5.4	1.5
MET	1.5	1.0	ACK	5.8	1.7
ALK F1174L	1.8	1.2	NPM1-ALK	6.8	1.9
LTK	1.8	1.2	ROS	8.9	2.5
NPM1-ALK	2.0	1.3	ALK F1174L	10	2.9
ALK R1275Q	2.0	1.3	TNK1	27	7.7
TRKA	3.2	2.1	FMS	30	8.6
AXL	3.3	2.2	YES	32	9.1
MER	3.4	2.3	DDR1	35	10
TRKC	4.4	2.9			
TRKB	4.6	3.1			
RON	5.5	3.7			
MUSK	9.1	6.1			
EPHA1	11	7.3			
JAK2	11	7.3			
LCK	11	7.3			

An inhibitory assay for a panel of 86 tyrosine kinases, including ALK, was conducted. Kinases with IC_{50} values 10-fold or less, than that for ALK are shown (minor modification of ref. 10). *a*: The ratio of the IC_{50} value for each tyrosine kinase relative to ALK.

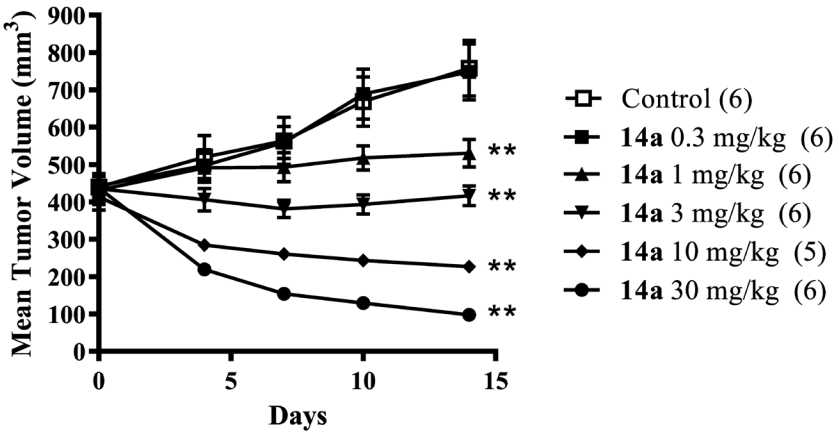


Fig. 4. Antitumor Activity of **14a**

Subcutaneously xenografted mice with NCI-H2228 cells were treated with once-daily oral administration of **14a** at the indicated doses for 14 d. Tumor volume was measured to assess antitumor activity. Each point represents the mean±S.E.M., and the number of animals used is shown in parentheses. The values obtained on day 14 were statistically analyzed and compared. **, $p<0.01$ compared with the value of the control group on day 14 (Dunnett's multiple comparison test).[10)]

EML4-ALK with an IC_{50} value of 7.9 nM, whereas compound **14l** resulted in a five-fold loss of inhibitory activity against EML4-ALK. In consideration of the inhibitory activity in cells, in addition to that in enzymes, compound **14a** exhibited the most promising inhibitory activity against EML4-ALK.

The inhibition exerted by **1** and **14a** on a panel of 86 tyrosine kinases, including ALK, was examined. Compound **14a** inhibited six kinases (ACK, ROS, TNK1, FMS, YES and DDR1) with IC_{50} values 10-fold or less, than that for ALK except ALK R1275Q, NPM1-ALK and ALK F1174L, while compound **1** inhibited 13 kinases (ROS, MET, LTK, TRKA, AXL, MER, TRKC, TRKB, RON, MUSK EPHA1, JAK2 and LCK) in the same range (Table 5).

The antitumor activity of **14a** was evaluated in mice xenografted with NCI-H2228, a human NSCLC tumor cell endogenously expressing EML4-ALK (Fig. 4). Compound **14a** inhibited the growth of NCI-H2228 cells with an IC_{50} value of 65 nM.[10)] Once-daily oral administration of **14a** demonstrated tumor growth inhibition at doses of 0.3 mg/kg (4% inhibition) and 1 mg/kg (69% inhibition), and tumor regression at doses of 3 mg/kg (4% regression), 10 mg/kg (45% regression), and 30 mg/kg (78% regression) in a dose-dependent manner. Body weight was not affected by **14a** at the doses used in this experiment.[10)]

Conclusion

We identified **14a** (ASP3026) as a potent and selective ALK inhibitor *via* SAR studies of 1,3,5-triazine derivatives. Once-daily oral administration of **14a** to mice bearing NCI-H2228 tumor xenografts demonstrated dose-dependent antitumor activity and induced tumor regression.

Experimental

Chemistry ^{1}H-NMR spectra were recorded on JEOL AL400 or Varian 400-MR, and chemical shifts were expressed in δ (ppm) values with trimethylsilane as an internal reference

(s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad peak). MS were recorded on Thermo Electron LCQ Advantage, Waters ultra performance liquid chromatography (UPLC)/ZQ, Waters UPLC/SQD LC/MS system, JEOL GC-mateII, Thermo Electron TRACE DSQ or Waters Micromass LCT Premier Mass Spectrometer. Elemental analyses were performed with Yanaco MT-6 (C, H, N) and DIONEX DX-500 (S, halogen) instruments, and results were within ±0.3% of theoretical values.

1-[1-(3-Methoxy-4-nitrophenyl)piperidin-4-yl]-4-methylpiperazine (4a) To a mixture of 4-fluoro-2-methoxy-1-nitrobenzene **3a** (3.00 g, 17.5 mmol) and K_2CO_3 (6.10 g, 44.1 mmol) in DMF (30 mL) was added 4-piperidone monohydrate monohydrochloride (3.20 g, 20.8 mmol). The reaction mixture was stirred at 70°C overnight. Water was added to the mixture, and the resulting slurry was extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was washed with Et_2O to give an ocher solid (3.85 g).

To this product were added 1,2-dichloroethane (40 mL) and 1-methylpiperazine (2 mL, 18.2 mmol). After the mixture was stirred for 30 min, sodium triacetoxyborohydride (3.90 g, 18.4 mmol) was added to the mixture, and the reaction mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ solution was added to the mixture, and the resulting slurry was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% aqueous NH_3=30 : 1 : 0.1 to 15 : 1 : 0.1). The resulting solid was washed with *n*-hexane to give **4a** (3.30 g, 56%) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.52–1.69 (2H, m), 1.90–2.03 (2H, m), 2.20–2.80 (9H, m), 2.31 (3H, s), 2.90–3.03 (2H, m), 3.89–4.01 (2H, m), 3.95 (3H, s), 6.31 (1H, d, J=2.0 Hz), 6.42 (1H, dd, J=2.0, 9.2 Hz), 7.96–8.03 (1H, m). Electrospray ionization (ESI)-MS m/z: 335 $[M+H]^+$.

1-Methyl-4-[1-(4-nitrophenyl)piperidin-4-yl]piperazine (4b) Compound **4b** was prepared with a yield of 14% from 1-fluoro-4-nitrobenzene **3b** using a procedure similar to that described for **4a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.52–1.73 (2H, m), 1.88–2.02 (2H, m), 2.18–2.76 (9H, m), 2.29 (3H, s), 2.89–3.05 (2H, m), 3.91–4.05 (2H, m), 6.75–6.85 (2H, m), 8.06–8.15 (2H, m). ESI-MS m/z: 305 $[M+H]^+$.

1-Methyl-4-[1-(3-methyl-4-nitrophenyl)piperidin-4-yl]-piperazine (4c) Compound **4c** was prepared with a yield of 20% from 4-fluoro-2-methyl-1-nitrobenzene **3c** using a procedure similar to that described for **4a**. ^{1}H-NMR (400 MHz, dimethyl sulfoxide (DMSO)-d_6) δ: 1.32–1.47 (2H, m), 1.75–1.88 (2H, m), 2.12 (3H, s), 2.15–2.63 (8H, m), 2.55 (3H, s), 2.85–3.00 (2H, m), 3.36–3.44 (1H, m), 3.95–4.11 (2H, m), 6.81–6.93 (2H, m), 7.93–8.02 (1H, m). ESI-MS m/z: 319 $[M+H]^+$.

1-[1-(3-Ethoxy-4-nitrophenyl)piperidin-4-yl]-4-methylpiperazine (4d) Compound **4d** was prepared with a yield of 55% from 2-ethoxy-4-fluoro-1-nitrobenzene[11] **3d** using a procedure similar to that described for **4a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.50 (3H, t, J=7.0 Hz), 1.53–1.67 (2H, m), 1.88–2.01 (2H, m), 2.22–2.75 (9H, m), 2.29 (3H, s), 2.86–3.00 (2H, m), 3.84–3.98 (2H, m), 4.14 (2H, q, J=6.9 Hz), 6.31 (1H, d, J=2.8 Hz), 6.41 (1H, dd, J=2.6, 9.4 Hz), 7.97 (1H, d, J=9.2 Hz). FAB-MS m/z: 349 $[M+H]^+$.

1-Methyl-4-{1-[4-nitro-3-(propan-2-yloxy)phenyl]piperidin-4-yl}piperazine (4e) Compound **4e** was prepared with a yield of 43% from 4-fluoro-1-nitro-2-(propan-2-yloxy)benzene[12] **3e** using a procedure similar to that described for **4a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.41 (6H, d, J=6.4 Hz), 1.52–1.68 (2H, m), 1.88–2.01 (2H, m), 2.19–2.76 (9H, m), 2.29 (3H, s), 2.87–2.99 (2H, m), 3.82–3.95 (2H, m), 4.53–4.67 (1H, m), 6.35 (1H, d, J=2.4 Hz), 6.42 (1H, dd, J=2.8, 9.6 Hz), 7.94 (1H, d, J=9.6 Hz). ESI-MS m/z: 363 $[M+H]^+$.

2-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]-aniline (5a) To a mixture of 1-[1-(3-methoxy-4-nitrophenyl)-piperidin-4-yl]-4-methylpiperazine **4a** (2.18 g, 6.52 mmol) in EtOH (50 mL) was added 10% palladium on carbon (wet, contains 53% water; 600 mg). The reaction mixture was stirred at room temperature for 8 h under 1 atm hydrogen atmosphere. The insoluble material was removed by filtration through Celite, and the filtrate was concentrated *in vacuo* to give **5a** (1.96 g, 99%) as a pale purple solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.62–1.80 (2H, m), 1.86–1.98 (2H, m), 2.22–2.84 (11H, m), 2.31 (3H, s), 3.23–3.74 (4H, m), 3.83 (3H, s), 6.42 (1H, dd, J=2.4, 8.0 Hz), 6.52 (1H, d, J=2.4 Hz), 6.63 (1H, d, J=8.4 Hz). Electron ionization (EI)-MS m/z: 304 $[M]^+$.

4-[4-(4-Methylpiperazin-1-yl)piperidin-1-yl]aniline (5b) Compound **5b** was prepared with a yield of 87% from **4b** using a procedure similar to that described for **5a**. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.39–1.56 (2H, m), 1.72–1.86 (2H, m), 2.07–2.61 (11H, m), 2.13 (3H, s), 3.25–3.46 (2H, m), 4.53 (2H, s), 6.43–6.51 (2H, m), 6.63–6.70 (2H, m). ESI-MS m/z: 275 $[M+H]^+$.

2-Methyl-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]-aniline (5c) Compound **5c** was prepared from **4c** using a procedure similar to that described for **5a**. ESI-MS m/z: 289 $[M+H]^+$.

2-Ethoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]-aniline (5d) Compound **5d** was prepared from **4d** using a procedure similar to that described for **5a**. ESI-MS m/z: 319 $[M+H]^+$.

4-[4-(4-Methylpiperazin-1-yl)piperidin-1-yl]-2-(propan-2-yloxy)aniline (5e) Compound **5e** was prepared from **4e** using a procedure similar to that described for **5a**. ESI-MS m/z: 333 $[M+H]^+$.

1-[1-(2-Methoxy-4-nitrophenyl)piperidin-4-yl]-4-methylpiperazine (7) To a solution of 1-fluoro-2-methoxy-4-nitrobenzene **6** (4.74 g, 27.7 mmol) in DMF (47 mL) were added 1-methyl-4-(piperidin-4-yl)piperazine (5.33 g, 29.1 mmol) and K_2CO_3 (4.59 g, 33.2 mmol). The reaction mixture was stirred at 80°C for 15 h. Water was added to this reaction mixture under cooling in an ice bath, and the resulting precipitate was filtered and washed with water to give **7** (8.79 g, 95%) as a yellow solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.44–1.60 (2H, m), 1.77–1.89 (2H, m), 2.06–2.61 (9H, m), 2.14 (3H, s), 2.65–2.80 (2H, m), 3.63–3.76 (2H, m), 3.90 (3H, s), 7.00 (1H, d, J=8.8 Hz), 7.67 (1H, d, J=2.4 Hz), 7.82 (1H, dd, J=2.4, 8.8 Hz). ESI-MS m/z: 335 $[M+H]^+$.

3-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]-aniline (8) Compound **8** was prepared with a yield of 94% from **7** using a procedure similar to that described for **5a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.72–1.94 (4H, m), 2.29 (3H, s), 2.32–2.77 (11H, m), 3.32–3.60 (4H, m), 3.81 (3H, s), 6.19–6.29 (2H, m), 6.77 (1H, d, J=8.4 Hz). ESI-MS m/z: 305 $[M+H]^+$.

***N*-Ethyl-3-methoxy-*N*-[2-(4-methylpiperazin-1-yl)ethyl]-4-nitroaniline (9)** A mixture of 4-fluoro-2-methoxy-1-nitroben-

zene **3a** (900 mg, 5.26 mmol), *N*-ethyl-2-(4-methylpiperazin-1-yl)-ethanamine (901 mg, 5.26 mmol) and K_2CO_3 (727 mg, 5.26 mmol) in DMF (10 mL) was stirred at 80°C for 6 h. The solvent was concentrated, then water was added to the residue. The slurry was extracted with EtOAc, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$ to $CHCl_3$/MeOH/28% aqueous NH_3=10/1/0.1) to give **9** (730 mg, 43%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.14 (3H, t, J=6.6 Hz), 2.14 (3H, s), 2.17–2.60 (10H, m), 3.42–3.59 (4H, m), 3.90 (3H, s), 6.19–6.24 (1H, m), 6.31–6.38 (1H, m), 7.86–7.93 (1H, m). ESI-MS m/z: 323 $[M+H]^+$.

N^4-Ethyl-2-methoxy-N^4-[2-(4-methylpiperazin-1-yl)ethyl]-benzene-1,4-diamine (10) Compound **10** was prepared from **9** using a procedure similar to that described for **5a** and directly used in the next reaction. ESI-MS m/z: 293 $[M+H]^+$.

4-Chloro-*N*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazin-2-amine (13a) To a mixture of 2,4-dichloro-1,3,5-triazine **12a** (460 mg, 3.07 mmol) and THF (5 mL) was added a mixture of 2-(propane-2-sulfonyl)aniline[13] **11a** (600 mg, 3.01 mmol) and DIPEA (0.58 mL, 3.33 mmol) in THF (10 mL). After the reaction mixture was stirred at room temperature for three days, water (60 mL) and saturated aqueous $NaHCO_3$ solution were added. The resulting slurry was extracted with EtOAc, and the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$) to give **13a** (340 mg, 36%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.32 (6H, d, J=7.2 Hz), 3.15–3.30 (1H, m), 7.30–7.38 (1H, m), 7.67–7.76 (1H, m), 7.92 (1H, dd, J=1.5, 7.8 Hz), 8.47–8.53 (1H, m), 8.61 (1H, s), 9.66–10.13 (1H, br). ESI-MS m/z: 313 $[M+H]^+$.

4-Chloro-6-methoxy-*N*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazin-2-amine (13b) To a mixture of 2,4-dichloro-6-methoxy-1,3,5-triazine **12b** (370 mg, 2.06 mmol) and THF (10 mL) was added a mixture of 2-(propane-2-sulfonyl)aniline[13] **11a** (400 mg, 2.01 mmol) and DIPEA (0.72 mL, 4.13 mmol) in THF (5 mL). After the reaction mixture was stirred at room temperature overnight and at 70°C for 7 h, water (60 mL) was added under ice-cooling. The resulting solid was purified by silica gel column chromatography ($CHCl_3$) and washed with *n*-hexane to give **13b** (200 mg, 29%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 3.13–3.28 (1H, m), 4.06 (3H, s), 7.19–7.38 (1H, m), 7.60–7.74 (1H, m), 7.90 (1H, dd, J=1.6, 7.6 Hz), 8.47 (1H, dd, J=1.0, 8.2 Hz), 9.69 (1H, s). ESI-MS m/z: 343 $[M+H]^+$.

4-Chloro-6-methyl-*N*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazin-2-amine (13c) Compound **13c** was prepared with a yield of 18% from 2-(propane-2-sulfonyl)aniline[13] **11a** and 2,4-dichloro-6-methyl-1,3,5-triazine[14] **12c** using a procedure similar to that described for **13a**. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.14 (6H, d, J=6.8 Hz), 2.40 (3H, s), 3.44–3.57 (1H, m), 7.47–7.60 (1H, m), 7.78–7.86 (1H, m), 7.91 (1H, dd, J=1.6, 8.0 Hz), 7.99–8.10 (1H, m), 10.00 (1H, s). FAB-MS m/z: 327 $[M+H]^+$.

4-Chloro-*N*-[2-(methanesulfonyl)phenyl]-1,3,5-triazin-2-amine (13d) To a mixture of 2-(methanesulfonyl)aniline monohydrochloride **11b** (600 mg, 2.89 mmol) and THF (10 mL) were added DIPEA (1.2 mL, 6.89 mmol) and 2,4-dichloro-1,3,5-triazine **12a** (880 mg, 5.87 mmol) under ice-cooling. After the reaction mixture was stirred at room temperature overnight, saturated aqueous $NaHCO_3$ solution and water were added. The resulting slurry was extracted with EtOAc, and the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=3 : 1 to 2 : 1) and washed with Et_2O to give **13d** (430 mg, 52%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.10 (3H, s), 7.31–7.41 (1H, m), 7.67–7.78 (1H, m), 7.97–8.07 (1H, m), 8.42–8.51 (1H, m), 8.63 (1H, s), 9.48–9.74 (1H, br). FAB-MS m/z: 285 $[M+H]^+$.

4-Chloro-*N*-[2-(ethanesulfonyl)phenyl]-1,3,5-triazin-2-amine (13e) Compound **13e** was prepared with a yield of 47% from 2-(ethanesulfonyl)aniline[13] **11c** and 2,4-dichloro-1,3,5-triazine **12a** using a procedure similar to that described for **13d**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.29 (3H, t, J=7.4 Hz), 3.16 (2H, q, J=7.5 Hz), 7.31–7.40 (1H, m), 7.67–7.78 (1H, m), 7.96 (1H, dd, J=1.6, 8.0 Hz), 8.45–8.52 (1H, m), 8.62 (1H, s), 9.61–9.90 (1H, br). ESI-MS m/z: 299 $[M+H]^+$.

***N*-{2-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14a, ASP3026)** A mixture of **5a** (210 mg, 0.690 mmol) and MsOH (0.13 mL, 2.00 mmol) in EtOH (3 mL) was stirred at room temperature for 15 min. To this mixture was added **13a** (170 mg, 0.543 mmol), and the reaction mixture was stirred at 100°C for 2 h. After the mixture was cooled to room temperature, water and saturated aqueous $NaHCO_3$ solution were added. The resulting slurry was extracted with $CHCl_3$, and the organic layer was dried over Na_2SO_4, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% aqueous NH_3=50 : 1 : 0.1 to 30 : 1 : 0.1) to give **14a** (180 mg, 57%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 1.62–1.79 (2H, m), 1.90–2.02 (2H, m), 2.23–2.81 (11H, m), 2.30 (3H, s), 3.18–3.32 (1H, m), 3.63–3.75 (2H, m), 3.88 (3H, s), 6.45–6.62 (2H, m), 7.14–7.29 (1H, m), 7.43–7.71 (2H, m), 7.88 (1H, dd, J=1.6, 8.0 Hz), 8.02–8.17 (1H, m), 8.27–8.61 (2H, m), 9.28 (1H, s). ESI-MS m/z: 581 $[M+H]^+$. High resolution (HR)-MS (ESI) m/z: 581.3022 $[M+H]^+$ (Calcd for $C_{29}H_{41}N_8O_3S$: 581.3022).

6-Methoxy-*N*-{2-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14b) Compound **14b** was prepared with a yield of 50% from **5a** and **13b** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.30 (6H, d, J=6.8 Hz), 1.50–1.79 (2H, m), 1.88–2.01 (2H, m), 2.25–2.81 (11H, m), 2.30 (3H, s), 3.19–3.32 (1H, m), 3.62–3.74 (2H, m), 3.89 (3H, s), 3.99 (3H, s), 6.44–6.60 (2H, m), 7.13–7.24 (1H, m), 7.47 (1H, s), 7.56–7.66 (1H, m), 7.87 (1H, dd, J=1.6, 8.0 Hz), 8.08–8.27 (1H, br), 8.47–8.62 (1H, m), 9.20 (1H, s). ESI-MS m/z: 611 $[M+H]^+$. HR-MS (ESI) m/z: 611.3140 $[M+H]^+$ (Calcd for $C_{30}H_{43}N_8O_4S$: 611.3128).

***N*-{2-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-6-methyl-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14c)** Compound **14c** was prepared with a yield of 19% from **5a** and **13c** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 1.50–1.79 (2H, m), 1.89–2.01 (2H, m), 2.22–2.79 (11H, m), 2.30 (3H, s), 2.41 (3H, s), 3.19–3.32 (1H, m), 3.61–3.74 (2H, m), 3.87 (3H, s), 6.45–6.58 (2H, m), 7.14–7.23 (1H, m), 7.35–7.55 (1H, m), 7.55–7.68 (1H, m), 7.87 (1H, dd, J=1.6, 8.0 Hz), 8.04–8.30 (1H, br), 8.53–8.67 (1H, m),

9.23 (1H, s). ESI-MS *m/z*: 595 $[M+H]^+$. HR-MS (ESI) *m/z*: 595.3171 $[M+H]^+$ (Calcd for $C_{30}H_{43}N_8O_3S$: 595.3179).

***N*-[2-(Methanesulfonyl)phenyl]-*N′*-{2-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-1,3,5-triazine-2,4-diamine (14d)** Compound **14d** was prepared with a yield of 31% from **5a** and **13d** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.49–1.79 (2H, m), 1.88–2.01 (2H, m), 2.30 (3H, s), 2.32–2.83 (11H, m), 3.09 (3H, s), 3.62–3.75 (2H, m), 3.88 (3H, s), 6.44–6.61 (2H, m), 7.18–7.29 (1H, m), 7.49–7.71 (2H, m), 7.97 (1H, dd, *J*=1.6, 8.0 Hz) 8.01–8.16 (1H, m), 8.28–8.56 (2H, m), 9.00 (1H, s). ESI-MS *m/z*: 553 $[M+H]^+$. HR-MS (ESI) *m/z*: 553.2701 $[M+H]^+$ (Calcd for $C_{27}H_{37}N_8O_3S$: 553.2709).

***N*-[2-(Ethanesulfonyl)phenyl]-*N′*-{2-methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-1,3,5-triazine-2,4-diamine (14e)** Compound **14e** was prepared with a yield of 18% from **5a** and **13e** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.27 (3H, t, *J*=7.4 Hz), 1.49–1.78 (2H, m), 1.89–2.01 (2H, m), 2.20–2.80 (11H, m), 2.30 (3H, s), 3.17 (2H, q, *J*=7.5 Hz), 3.64–3.75 (2H, m), 3.88 (3H, s), 6.46–6.60 (2H, m), 7.17–7.28 (1H, m), 7.43–7.70 (2H, m), 7.92 (1H, dd, *J*=1.4, 7.8 Hz), 8.03–8.15 (1H, m), 8.27–8.60 (2H, m), 9.14 (1H, s). ESI-MS *m/z*: 567 $[M+H]^+$. HR-MS (ESI) *m/z*: 567.2868 $[M+H]^+$ (Calcd for $C_{28}H_{39}N_8O_3S$: 567.2866).

***N*-{3-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14f)** Compound **14f** was prepared with a yield of 56% from **8** and **13a** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, *J*=6.8 Hz), 1.73–1.97 (4H, m), 2.30 (3H, s), 2.35–2.79 (11H, m), 3.17–3.32 (1H, m), 3.47–3.58 (2H, m), 3.87 (3H, s), 6.80–7.70 (6H, m), 7.88 (1H, dd, *J*=1.6, 8.0 Hz), 8.35–8.50 (1H, br), 8.53–8.64 (1H, m), 9.26–9.62 (1H, br). ESI-MS *m/z*: 581 $[M+H]^+$. HR-MS (ESI) *m/z*: 581.3020 $[M+H]^+$ (Calcd for $C_{29}H_{41}N_8O_3S$: 581.3022).

***N*-{4-[4-(4-Methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14g)** Compound **14g** was prepared with a yield of 38% from **5b** and **13a** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, *J*=6.8 Hz), 1.49–1.78 (2H, m), 1.90–2.01 (2H, m), 2.30 (3H, s), 2.24–2.86 (11H, m), 3.18–3.31 (1H, m), 3.66–3.79 (2H, m), 6.87–7.16 (3H, m), 7.17–7.24 (1H, m), 7.33–7.48 (2H, m), 7.49–7.71 (1H, br), 7.88 (1H, dd, *J*=1.6, 8.0 Hz), 8.29–8.47 (1H, br), 8.48–8.61 (1H, m), 9.24–9.43 (1H, br). ESI-MS *m/z*: 551 $[M+H]^+$. HR-MS (ESI) *m/z*: 551.2926 $[M+H]^+$ (Calcd for $C_{28}H_{39}N_8O_2S$: 551.2917).

***N*-{2-Methyl-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14h)** Compound **14h** was prepared from **5c**, which was obtained from **4c**, and **13a** using a procedure similar to that described for **14a** (13% in two steps from **4c**). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, *J*=6.8 Hz), 1.50–1.80 (2H, m), 1.88–2.02 (2H, m), 2.21–2.87 (11H, m), 2.25 (3H, s), 2.30 (3H, s), 3.15–3.33 (1H, m), 3.68–3.80 (2H, m), 6.48–7.75 (6H, m), 7.78–7.94 (1H, m), 8.30–8.70 (2H, m), 9.24–9.45 (1H, br). ESI-MS *m/z*: 565 $[M+H]^+$. HR-MS (ESI) *m/z*: 565.3077 $[M+H]^+$ (Calcd for $C_{29}H_{41}N_8O_2S$: 565.3073).

***N*-{2-Ethoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14i)** Compound **14i** was prepared from **5d**, which was obtained from **4d**, and **13a** using a procedure similar to that described for **14a** (11% in two steps from **4d**). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, *J*=6.8 Hz), 1.46 (3H, t, *J*=7.0 Hz), 1.53–1.79 (2H, m), 1.88–2.00 (2H, m), 2.24–2.79 (11H, m), 2.30 (3H, s), 3.18–3.32 (1H, m), 3.60–3.74 (2H, m), 4.10 (2H, q, *J*=7.1 Hz), 6.45–6.59 (2H, m), 7.16–7.29 (1H, m), 7.47–7.70 (2H, m), 7.89 (1H, dd, *J*=1.6, 8.0 Hz), 8.04–8.20 (1H, m), 8.27–8.48 (1H, br), 8.49–8.60 (1H, m), 9.22–9.34 (1H, br). ESI-MS *m/z*: 595 $[M+H]^+$. HR-MS (ESI) *m/z*: 595.3180 $[M+H]^+$ (Calcd for $C_{30}H_{43}N_8O_3S$: 595.3179).

***N*-{4-[4-(4-Methylpiperazin-1-yl)piperidin-1-yl]-2-(propan-2-yloxy)phenyl}-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14j)** Compound **14j** was prepared from **5e**, which was obtained from **4e**, and **13a** using a procedure similar to that described for **14a** (9% in two steps from **4e**). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, *J*=6.8 Hz), 1.38 (6H, d, *J*=6.0 Hz), 1.48–1.79 (2H, m), 1.86–2.02 (2H, m), 2.23–2.80 (11H, m), 2.31 (3H, s), 3.18–3.34 (1H, m), 3.59–3.74 (2H, m), 4.52–4.66 (1H, m), 6.44–6.60 (2H, m), 7.15–7.29 (1H, m), 7.50–7.71 (2H, m), 7.89 (1H, dd, *J*=1.4, 7.8 Hz), 8.05–8.24 (1H, m), 8.30–8.47 (1H, br), 8.49–8.60 (1H, m), 9.19–9.36 (1H, br). ESI-MS *m/z*: 609 $[M+H]^+$. HR-MS (ESI) *m/z*: 609.3335 $[M+H]^+$ (Calcd for $C_{31}H_{45}N_8O_3S$: 609.3335).

***N*-[2-Methoxy-4-(4-methylpiperazin-1-yl)phenyl]-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14k)** Compound **14k** was prepared with a yield of 39% from 2-methoxy-4-(4-methylpiperazin-1-yl)aniline[15] and **13a** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, *J*=6.8 Hz), 2.40 (3H, s), 2.54–2.73 (4H, m), 3.16–3.32 (5H, m), 3.89 (3H, s), 6.46–6.60 (2H, m), 7.16–7.29 (1H, m), 7.47–7.73 (2H, m), 7.88 (1H, dd, *J*=1.6, 8.0 Hz), 8.04–8.18 (1H, m), 8.26–8.65 (2H, m), 9.29 (1H, s). ESI-MS *m/z*: 498 $[M+H]^+$. HR-MS (ESI) *m/z*: 498.2279 $[M+H]^+$ (Calcd for $C_{24}H_{32}N_7O_3S$: 498.2287).

***N*-(4-{Ethyl[2-(4-methylpiperazin-1-yl)ethyl]amino}-2-methoxyphenyl)-*N′*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (14l)** Compound **14l** was prepared from **10**, which was obtained from **9**, and **13a** using a procedure similar to that described for **14a** (16% in two steps from **9**). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.18 (3H, t, *J*=7.0 Hz), 1.31 (6H, d, *J*=6.8 Hz), 2.30 (3H, s), 2.34–2.77 (10H, m), 3.18–3.31 (1H, m), 3.32–3.52 (4H, m), 3.87 (3H, s), 6.24–6.38 (2H, m), 7.10–7.24 (1H, m), 7.32–7.75 (2H, m), 7.82–8.10 (2H, m), 8.26–8.67 (2H, m), 9.19–9.33 (1H, br). ESI-MS *m/z*: 569 $[M+H]^+$. HR-MS (ESI) *m/z*: 569.3014 $[M+H]^+$ (Calcd for $C_{28}H_{41}N_8O_3S$: 569.3022).

1-Nitro-3-(propan-2-ylsulfanyl)benzene (16) A mixture of 1,1′-disulfanediylbis(3-nitrobenzene) **15** (3.00 g, 9.73 mmol) and K_2CO_3 (2.69 g, 19.5 mmol) in DMF (200 mL) was stirred at room temperature for 2 min. To this mixture were added 2-bromopropane (2.01 mL, 21.4 mmol), sodium hydroxymethanesulfinate (3.45 g, 29.2 mmol) and H_2O (3 mL), and the reaction mixture was stirred at room temperature for 2 h. Water was added to this mixture, and the resulting slurry was extracted with Et_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give **16** (2.95 g, 77%) as a yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.32–1.40 (6H, m), 3.45–3.61 (1H, m), 7.41–7.52 (1H, m), 7.62–7.70 (1H, m), 8.01–8.09 (1H, m), 8.17–8.24 (1H, m). EI-MS *m/z*: 197 $[M]^+$.

1-Nitro-3-(propane-2-sulfonyl)benzene (17) To a solution of 1-nitro-3-(propan-2-ylsulfanyl)benzene **16** (2.95 g, 15.0 mmol) in $CHCl_3$ (60 mL) was added mCPBA (contains *ca*. 25% water, 8.60 g, 37.4 mmol), and the reaction mixture was stirred at room temperature for 2 h, then at 50°C for 12 h. Saturated aqueous $NaHCO_3$ solution (100 mL) and 5% aqueous sodium sulfite solution (100 mL) were added to this mixture, and the resulting slurry was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give **17** (3.38 g, 99%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.19 (6H, d, J=6.8 Hz), 3.57–3.70 (1H, m), 7.92–8.04 (1H, m), 8.27–8.37 (1H, m), 8.50–8.56 (1H, m), 8.56–8.65 (1H, m). Chemical ionization (CI)-MS *m/z*: 230 $[M+H]^+$.

3-(Propane-2-sulfonyl)aniline (18) To a mixture of 1-nitro-3-(propane-2-sulfonyl)benzene **17** (3.38 g, 14.3 mmol) and AcOH (35 mL) was added iron powder (2.64 g, 47.2 mmol), and the reaction mixture was stirred at 80°C for 3 h. To this mixture was added EtOAc; the insoluble material was removed by filtration, and the filtrate was concentrated *in vacuo*. To the residue was added EtOAc, and the insoluble material was again removed by filtration. The organic layer was washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=100 : 0 to 50 : 1) to give **18** (1.79 g, 63%) as a pale yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.30 (6H, d, J=6.8 Hz), 3.10–3.26 (1H, m), 3.82–4.08 (2H, br), 6.86–6.96 (1H, m), 7.13–7.18 (1H, m), 7.19–7.25 (1H, m), 7.25–7.36 (1H, m). EI-MS *m/z*: 199 $[M]^+$.

4-Chloro-*N*-[3-(propane-2-sulfonyl)phenyl]-1,3,5-triazin-2-amine (19) Compound **19** was prepared with a yield of 92% from **12a** and **18** using a procedure similar to that described for **13d**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (6H, d, J=6.8 Hz), 3.18–3.33 (1H, m), 7.54–7.65 (1H, m), 7.66–7.74 (1H, m), 7.76–8.00 (2H, m), 8.01–8.34 (1H, br), 8.49–8.72 (1H, br). ESI-MS *m/z*: 313 $[M+H]^+$.

***N*-{2-Methoxy-4-[4-(4-methylpiperazin-1-yl)piperidin-1-yl]phenyl}-*N*′-[3-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (20)** Compound **20** was prepared with a yield of 36% from **5a** and **19** using a procedure similar to that described for **14a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.32 (6H, d, J=6.8 Hz), 1.51–1.78 (2H, m), 1.88–2.01 (2H, m), 2.30 (3H, s), 2.33–2.80 (11H, m), 3.12–3.29 (1H, m), 3.63–3.75 (2H, m), 3.87 (3H, s), 6.48–6.61 (2H, m), 7.16–7.36 (1H, m), 7.45–7.62 (2H, m), 7.85–8.25 (3H, m), 8.30–8.44 (1H, br). ESI-MS *m/z*: 581 $[M+H]^+$. HR-MS (ESI) *m/z*: 581.3033 $[M+H]^+$ (Calcd for $C_{29}H_{41}N_8O_3S$: 581.3022).

8-(3-Methoxy-4-nitrophenyl)-1,4-dioxa-8-azaspiro[4.5]-decane (21) To a mixture of 4-fluoro-2-methoxy-1-nitrobenzene **3a** (15 g, 87.7 mmol) and K_2CO_3 (30.0 g, 217 mmol) in DMF (150 mL) was added 1,4-dioxa-8-azaspiro[4.5]decane (15 g, 105 mmol), and the reaction mixture was stirred at 70°C overnight. The insoluble material was removed by filtration, and ice water was added to the filtrate. The resulting solid was filtered and washed with Et_2O to give **21** (23.8 g, 92%) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.78–1.85 (4H, m), 3.51–3.59 (4H, m), 3.95 (3H, s), 4.01 (4H, s), 6.33 (1H, d, J=2.8 Hz), 6.43 (1H, dd, J=2.4, 9.2 Hz), 8.00 (1H, d, J=9.2 Hz). ESI-MS *m/z*: 295 $[M+H]^+$.

4-(1,4-Dioxa-8-azaspiro[4.5]decan-8-yl)-2-methoxyaniline (22) Compound **22** was prepared with a yield of 82% from **21** using a procedure similar to that described for **5a**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.83–1.94 (4H, m), 3.10–3.22 (4H, m), 3.45–3.65 (2H, br), 3.83 (3H, s), 3.99 (4H, s), 6.45 (1H, dd, J=2.4, 8.4 Hz), 6.55 (1H, d, J=2.4 Hz), 6.63 (1H, d, J=8.4 Hz). ESI-MS *m/z*: 265 $[M+H]^+$.

***N*-[4-(1,4-Dioxa-8-azaspiro[4.5]decan-8-yl)-2-methoxyphenyl]-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (23)** A mixture of 4-chloro-*N*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazin-2-amine **13a** (1.00 g, 3.20 mmol), 4-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-2-methoxyaniline **22** (845 mg, 3.20 mmol) and DIPEA (0.56 mL, 3.20 mmol) in NMP (3.5 mL) was irradiated with microwaves at 120°C for 40 min. Water was added to this reaction mixture, and the resulting solid was filtered and dried to give **23** (1.62 g, 94%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 1.80–1.92 (4H, m), 3.18–3.35 (5H, m), 3.88 (3H, s), 4.01 (4H, s), 6.48–6.62 (2H, m), 7.17–7.31 (1H, m), 7.43–7.70 (2H, m), 7.82–7.95 (1H, m), 8.03–8.18 (1H, m), 8.28–8.64 (2H, m), 9.25–9.32 (1H, br). ESI-MS *m/z*: 541 $[M+H]^+$.

1-[3-Methoxy-4-({4-[2-(propane-2-sulfonyl)anilino]-1,3,5-triazin-2-yl}amino)phenyl]piperidin-4-one (24) A mixture of *N*-[4-(1,4-dioxa-8-azaspiro[4.5]decan-8-yl)-2-methoxyphenyl]-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine **23** (2.27 g, 4.20 mmol) and 4 M aqueous HCl solution (24 mL) in 1,4-dioxane (20 mL) was stirred at 80°C for 2 h. The reaction mixture was concentrated *in vacuo*, and saturated aqueous $NaHCO_3$ solution was added to the residue. The resulting slurry was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=100 : 0 to 20 : 1) to give **24** (1.49 g, 71%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.32 (6H, d, J=6.8 Hz), 2.51–2.68 (4H, m), 3.18–3.34 (1H, m), 3.52–3.68 (4H, m), 3.91 (3H, s), 6.51–6.68 (2H, m), 7.17–7.74 (3H, m), 7.84–7.95 (1H, m), 8.04–8.26 (1H, m), 8.30–8.68 (2H, m), 9.24–9.38 (1H, m). ESI-MS *m/z*: 497 $[M+H]^+$.

***N*-{2-Methoxy-4-[4-(morpholin-4-yl)piperidin-1-yl]-phenyl}-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine Trihydrochloride (25)** To a solution of 1-[3-methoxy-4-({4-[2-(propane-2-sulfonyl)anilino]-1,3,5-triazin-2-yl}amino)phenyl]piperidin-4-one **24** (200 mg, 0.403 mmol) in dichloromethane were added morpholine (0.14 mL, 1.61 mmol) and sodium triacetoxyborohydride (128 mg, 0.604 mmol), and the reaction mixture was stirred at room temperature for 2 h. Water and saturated aqueous $NaHCO_3$ solution were added to the mixture, and the resulting slurry was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH=100 : 0 to 10 : 1). The obtained product was dissolved in THF and treated with 4 M HCl in EtOAc, and then the mixture was concentrated *in vacuo*. The residue was treated with acetonitrile, EtOH and water to give **25** (133 mg, 49%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.15 (6H, d, J=6.8 Hz), 1.82–2.06 (2H, m), 2.18–2.31 (2H, m), 2.78–3.01 (2H, m), 3.01–3.21 (2H, m), 3.29–3.55 (4H, m), 3.80 (3H, s), 3.82–4.06 (6H, m), 6.57–6.99 (2H, m), 7.27–7.47 (2H, m), 7.55–7.91 (2H, m), 8.12–8.64 (2H, m), 9.25 (1H, s), 9.51 (1H, s), 11.08–11.35 (1H, br). ESI-MS *m/z*: 568 $[M+H]^+$. *Anal.* Calcd

for $C_{28}H_{37}N_7O_4S.2.8HCl.H_2O$: C, 48.89; H, 6.13; N, 14.25; S, 4.66; Cl, 14.43. Found: C, 48.62; H, 6.05; N, 14.14; S, 4.73; Cl, 14.43.

***tert*-Butyl 4-[3-Methoxy-4-({4-[2-(propane-2-sulfonyl)-anilino]-1,3,5-triazin-2-yl}amino)phenyl]piperidine-1-carboxylate (27)** A mixture of 4-chloro-*N*-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazin-2-amine **13a** (700 mg, 2.24 mmol), *tert*-butyl 4-(4-amino-3-methoxyphenyl)-piperidine-1-carboxylate[17] **26** (686 mg, 2.24 mmol) and DIPEA (0.47 mL, 2.69 mmol) in NMP (4 mL) was irradiated with microwaves at 120°C for 20 min. Water was added to this reaction mixture, and the resulting solid was filtered and dried *in vacuo*. This solid was dissolved in EtOAc, and the organic layer was dried over anhydrous $MgSO_4$, then concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=100 : 0 to 50 : 50) to give **27** (960 mg, 74%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 1.49 (9H, s), 1.53–1.71 (2H, m), 1.77–1.90 (2H, m), 2.58–2.70 (1H, m), 2.73–2.91 (2H, m), 3.18–3.32 (1H, m), 3.91 (3H, s), 4.16–4.37 (2H, m), 6.74–6.78 (1H, m), 6.80–6.86 (1H, m), 7.21–7.30 (1H, m), 7.53–7.79 (2H, m), 7.86–7.94 (1H, m), 8.26 (1H, d, J=8.4 Hz), 8.35–8.60 (2H, m), 9.33 (1H, s). ESI-MS *m/z*: 583 $[M+H]^+$.

***N*-[2-Methoxy-4-(piperidin-4-yl)phenyl]-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (28)** To a solution of *tert*-butyl 4-[3-methoxy-4-({4-[2-(propane-2-sulfonyl)anilino]-1,3,5-triazin-2-yl}amino)phenyl]piperidine-1-carboxylate **27** (980 mg, 1.68 mmol) in EtOAc (10 mL) and MeOH (10 mL) was added 4 M HCl in EtOAc (20 mL). After stirring at room temperature for 1 h, the mixture was concentrated *in vacuo*. Saturated aqueous $NaHCO_3$ solution was added to the residue, and the resulting slurry was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give **28** (840 mg, quantitative). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 1.68–2.30 (4H, m), 2.58–2.71 (1H, m), 2.74–2.90 (2H, m), 3.17–3.37 (3H, m), 3.91 (3H, s), 6.77–6.82 (1H, m), 6.82–6.90 (1H, m), 7.20–7.29 (1H, m), 7.49–7.83 (2H, m), 7.89 (1H, dd, J=1.6, 8.0 Hz), 8.25 (1H, d, J=8.0 Hz), 8.33–8.48 (1H, br), 8.48–8.62 (1H, m), 8.90–9.70 (1H, br). ESI-MS *m/z*: 483 $[M+H]^+$.

***N*-[2-Methoxy-4-(1′-methyl[1,4′-bipiperidin]-4-yl)-phenyl]-*N*′-[2-(propane-2-sulfonyl)phenyl]-1,3,5-triazine-2,4-diamine (29)** A mixture of *N*-[2-methoxy-4-(piperidin-4-yl)phenyl]-*N*′-[2-(propane-2-sulfonyl)-phenyl]-1,3,5-triazine-2,4-diamine **28** (300 mg, 0.622 mmol), 1-methylpiperidin-4-one (87 μL, 0.746 mmol) and sodium triacetoxyborohydride (158 mg, 0.746 mmol) in dichloromethane (11 mL) was stirred at room temperature overnight. Water and saturated aqueous $NaHCO_3$ solution were added to the reaction mixture, and the resulting slurry was extracted with $CHCl_3$. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/28% aqueous NH_3=100 : 0 : 0 to 10 : 1 : 0.1) to give **29** (140 mg, 39%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.31 (6H, d, J=6.8 Hz), 1.49–2.04 (10H, m), 2.22–2.40 (3H, m), 2.28 (3H, s), 2.42–2.55 (1H, m), 2.88–2.99 (2H, m), 3.00–3.12 (2H, m), 3.18–3.33 (1H, m), 3.89 (3H, s), 6.77–6.83 (1H, m), 6.83–6.92 (1H, m), 7.19–7.29 (1H, m), 7.53–7.82 (2H, m), 7.89 (1H, dd, J=1.6, 8.0 Hz), 8.22 (1H, d, J=8.4 Hz), 8.34–8.47 (1H, br), 8.47–8.60 (1H, m), 9.31 (1H, s). ESI-MS *m/z*: 580 $[M+H]^+$. HR-MS (ESI) *m/z*: 580.3077 $[M+H]^+$ (Calcd for $C_{30}H_{42}N_7O_3S$: 580.3069).

***In Vitro* Kinase Inhibitory Assay** EML4-ALK variant 1 protein was isolated from Ba/F3 cells transformed with EML4-ALK. Kinase activity was measured using HTRF® KinEASE™-TK (Cisbio Bioassays, Codolet, France). The final concentration of ATP for EML4-ALK variant 1 protein was 100 μM.

An inhibitory assay for a panel of 86 tyrosine kinases was conducted using ATP concentrations that were approximately equal to the Km value for each kinase using a TK-enzyme-linked immunosorbent assay (ELISA) or Off-chip Mobility Shift Assay (MSA) at Carna Biosciences, Inc. (Kobe, Japan). The percent inhibition of **1** and **14a** against kinase activity was first determined at concentrations of 100 and 1000 nM in a single experiment. IC_{50} values of **1** and **14a** were then determined in the presence of various concentrations of each compound in three individual experiments. The 86 tyrosine kinases were as follows: ABL, ACK, ALK, ALK F1174L, ALK R1275Q, ARG, AXL, BLK, BMX, BRK, BTK, CSK, CTK, DDR1, DDR2, EGFR, EGFR L858R, EGFR L861Q, EGFR T790M, EGFR T790M/L858R, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHB1, EPHB2, EPHB3, EPHB4, FAK, FER, FES, FGFR1, FGFR2, FGFR3, FGFR4, FGR, FLT1, FLT3, FLT4, FMS, FRK, FYN, HCK, HER2, HER4, IGF1R, INSR, IRR, ITK, JAK1, JAK2, JAK3, KDR, KIT, LCK, LTK, LYNa, LYNb, MER, MET, MUSK, NPM1-ALK, PDGFRα, PDGFRβ, PYK2, RET, RON, ROS, SRC, SRM, SYK, TEC, TIE2, TNK1, TRKA, TRKB, TRKC, TXK, TYK2, TYRO3, YES, and ZAP70.[10]

***In Vitro* Cell Growth Assay** Ba/F3 cells transformed with EML4-ALK variant 1 were seeded in 96-well plates at 2×10^3 cells per well and treated with various concentrations of compounds (**14a**, **14b** and **14k**) for 3 d, then cell viability was measured using the CellTiter-Glo™ Luminescent Cell Viability Assay (Promega Corporation, Madison, WI, U.S.A.) or seeded in 384-well plates at 2.5×10^2 or 5×10^2 cells per well, and treated with various concentrations of compounds (**14e**, **14f**, **14i**, **25** and **29**) for 2 d, then cell viability was measured with alamarBlue® Cell Viability Reagent (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.).

NCI-H2228 cells were seeded in 96-well spheroid plates (Sumitomo Bakelite Co., Ltd., Tokyo, Japan) at 2×10^3 cells per well and incubated overnight. Cells were then exposed to **14a** for 5 d. Cell viability was measured using the CellTiter-Glo™ Luminescent Cell Viability Assay (Promega Corporation).[10]

***In Vivo* NCI-H2228 Xenograft Model**[10] All experiments were performed in accordance with the regulation of the Animal Ethics Committee of Astellas Pharma Inc. NCI-H2228 cells were subcutaneously inoculated into the flank of male non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice (NOD.CB17-*Prkdc*^{scid}/J, Charles River Laboratories Japan, Inc., Kanagawa, Japan) at 5×10^6 cells/0.1 mL/mouse. **14a** was suspended in 0.5% methylcellulose solution and orally administered once daily. Tumor diameter was measured using a caliper, and tumor volume was determined by calculating the volume of an ellipsoid using the following formula: length$\times$width$^2\times$0.5.

Computational Modeling of ALK Inhibitors Docking simulation of **14a** with wild-type ALK was performed using

the docking software GLIDE (Schrödinger, LLC, New York, NY, U.S.A.). The coordinate of wild-type ALK with compound **2** (PDB ID: 2XB7[19]) was used as a template for the docking, and hydrogen atoms were added using the modeling software MOE with the function Protonate3D (Chemical Computing Group Inc., Montreal, Quebec, Canada). The docking mode with the highest docking score was employed.

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Conflict of Interest Kazuhiko Iikubo, Yutaka Kondoh, Itsuro Shimada and Takahiro Matsuya are inventors of the following patents: WO 2009/008371 (A1), US 8318702 (B2) and JP 5233996 (B2). Kazuhiko Iikubo, Itsuro Shimada, Takahiro Matsuya, Kenichi Mori, and Yoko Ueno are employees of Astellas Pharma Inc. as of the date of submitting this article. Minoru Okada has no conflict of interest.

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