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Design and synthesis of triazolopyridazines substituted with methylisoquinolinone as selective c-Met kinase inhibitors

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ABSTRACT

A series of triazolopyridazines substituted with methylisoquinolinone were designed and synthesized. Some of the triazolopyridazines strongly inhibited c-Met kinase and showed good anti-proliferative activity against a panel of c-Met-amplified gastric cancer cell lines (MKN-45, SNU-5 and Hs746T).

Receptor tyrosine kinases (RTKs) have been extensively studied as targets of anti-cancer drugs. Several small molecule inhibitors and a few antibodies against RTKs have been used successfully in the clinic for cancer treatment.[1,2] c-Met tyrosine kinase, a receptor for hepatocyte growth factor/scatter factor (HGF/SF), has recently attracted considerable interest as a therapeutic target for several types of cancers because it regulates cell survival, proliferation, migration and angiogenesis in both normal mammalian cell development and cancer metastasis.[3–5] Activating mutations or amplification of c-Met have frequently been found in various cancers including lung, gastric, renal, ovarian, prostate and liver cancers.[3,4] At the molecular level, binding of HGF to the extracellular domain of c-Met results in receptor dimerization and phosphorylation of multiple tyrosine residues. The activated c-Met binds and phosphorylates adaptor proteins including growth factor receptor-bound protein 2 (Grb2), Grb2-associated-binding protein 1 (Gab-1), Shc and c-Cbl, and subsequently induces the activation of a number of downstream signal transducers such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), signal transducers and activators of transcription (STAT), phosphoinositide-3-kinase (PI3K), Akt and focal adhesion kinase (FAK).[5]

A number of c-Met kinase inhibitors have already been reported and some of them have reached the clinical trial phase.[4] Among the known c-Met inhibitors, bicyclic triazole-based inhibitors showed

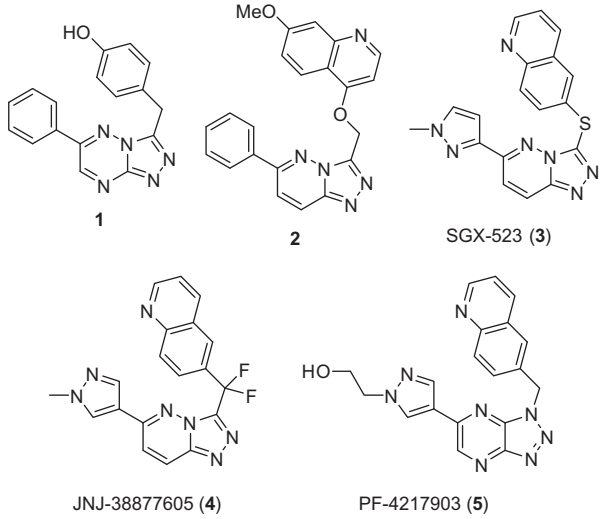

Figure 1. Structures of known bicyclic triazole-based inhibitors of c-Met kinase.

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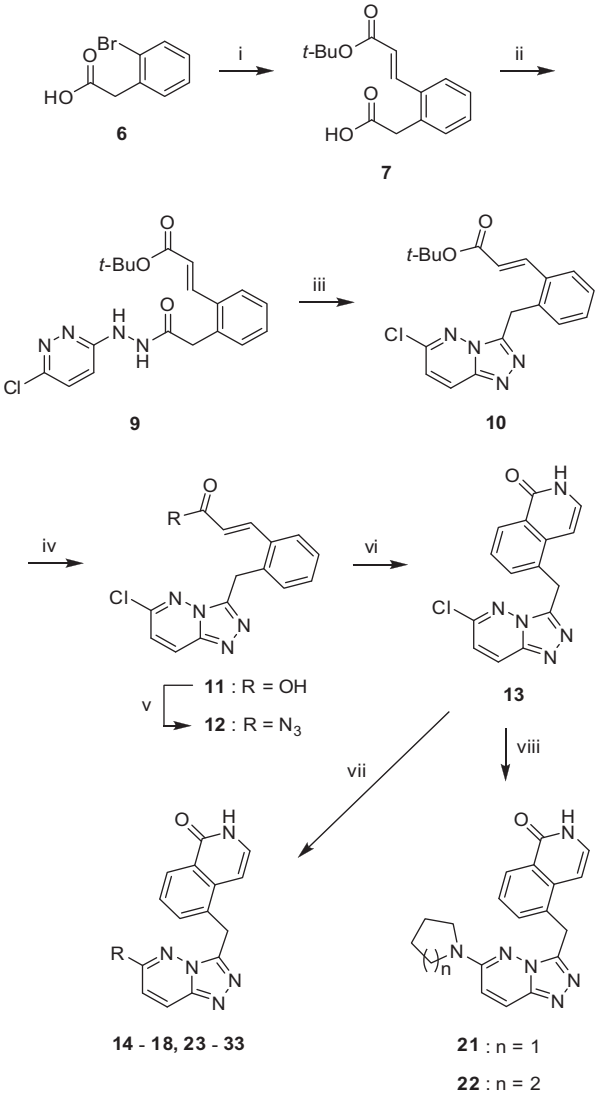
high c-Met inhibitory potency and excellent selectivity against other kinases (Fig. 1).[6] Some of the bicyclic triazole-based inhibitors have also been examined in clinical trials in which they exhibited good efficacy. However, further development of these was stopped or delayed due to toxicity[7] or unknown reasons. Thus, there is a need to discover new classes of bicyclic triazole-based inhibitors against c-Met for the treatment of cancer patients.

Previously, we have reported a series of aminopyridine based-inhibitors of c-Met kinase.[8] In an ongoing effort to discover novel c-Met inhibitors, we have designed and synthesized a new series of triazolopyridazine-based inhibitors against c-Met kinase.

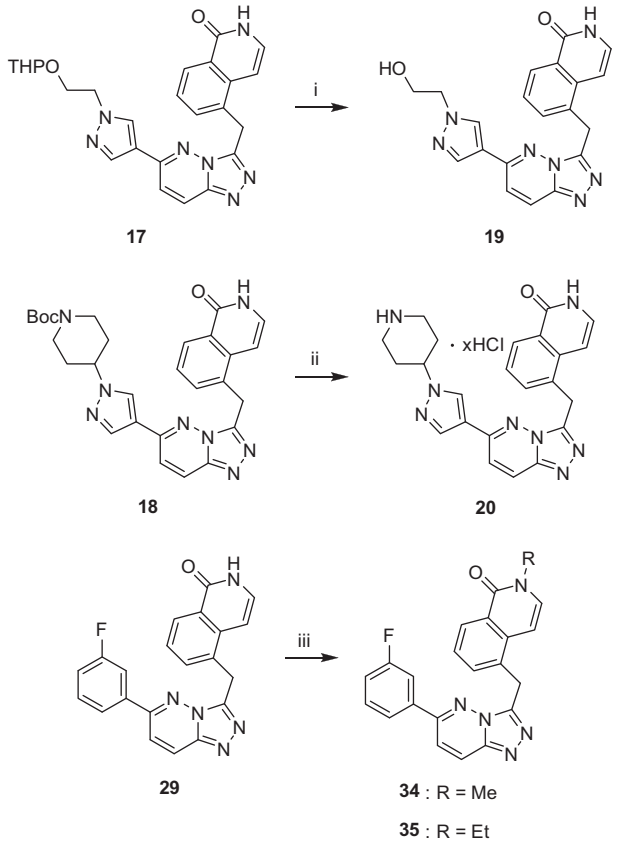
The synthesis of triazolopyridazines substituted with methylisoquinolinone commenced with the incorporation of an acrylate moiety into commercially available bromophenylacetic acid **6** by the Heck reaction as shown in Scheme 1.[9] Coupling of phenylacetic acid **7** with hydrazine **8** and subsequent treatment of the resulting hydrazide **9** with TsOH afforded triazolopyridazine **10**.[6a–c,e] The *t*-butyl ester group in triazolopyridazine **10** was hydrolyzed by treatment with trifluoroacetic acid and the resulting carboxylic acid **11** was transformed into acylazide **12**. Subjection of **12** to the Curtius rearrangement and concomitant cyclization yielded isoquinolinone **13**,[10] which was converted to disubstituted triazolopyridazines **14**–**18** and **23**–**33** by Suzuki coupling.[6a,b,11] Substitution of the chloride in the triazolopyridazine ring of **13** with pyrrolidine and piperidine furnished triazolopyridazines **21** and **22**, respectively.

The syntheses of **19**, **20**, **34** and **35** are depicted in Scheme 2. The respective removal of the THP group in **17** and the Boc group in **18** was effected to produce alcohol **19** and piperidine **20**, by treatment with hydrogen chloride solution and trifluoroacetic acid, respectively. N-Alkylation of isoquinolinone **29** with the corresponding alkyl iodides gave triazolopyridazines **34** and **35**.

To find a new class of triazole-based inhibitors that might target the ATP-binding site of c-Met, triazolopyridazines **14** and **15**[12] substituted with methylisoquinolinone were initially designed and synthesized (Table 1). Gratifyingly, both of these compounds showed excellent potency against c-Met. These results imply that the isoquinolinone ring works well as a hinge binding group, similar to the hydroxyphenyl and quinoline groups in the known triazole-based c-Met inhibitors **1–5**. Moreover, **14** and **15** revealed good selectivity in the kinase profiling assay against a panel of kinases consisting of structurally relevant kinases such as Ron, Flt3, TrkA and Aur A (IC_{50}s >10 μM). The potency and selectivity of **14** and **15** were similar to those of **1**–**5**. We sought to investigate the structure–activity relationships (SAR) in triazolopyridazines **14** and **15**, and the pyrazole moiety of **14** was modified first. Replacement of the methyl group in the pyrazole ring of **14** with a 2-hydroxyethyl group did not change the c-Met inhibitory

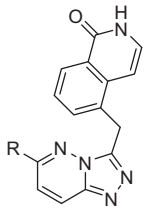


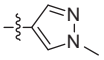
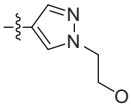
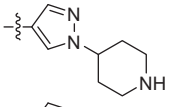
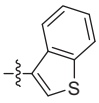
Scheme 1. Reagents and conditions: (i) *t*-butyl acrylate, (*o*-tolyl)$_3$P, *n*-Bu$_3$N, 1,4-dioxane, Pd(OAc)$_2$, reflux, 12 h, 94%; (ii) 3-chloro-6-hydrazinylpyridazine (**8**), EDCI, CH_2Cl_2, 0 °C, 2 h, 79%; (iii) *p*-TsOH, THF, 60 °C, 12 h, 81%; (iv) CF_3CO_2H, CH_2Cl_2, rt, 2 h, 95%; (v) Et_3N, $ClCO_2Et$, acetone, 0 °C 10 min then NaN_3, H_2O, 0 °C, 1 h, 83%; (vi) *n*-Bu$_3$N, Ph_2O, 140–210 °C, 50 min, 47%; (vii) RB(OH)$_2$ or RB(OR^1)$_2$, Cs_2CO_3 or Na_2CO_3, Pd(dppf)Cl$_2$ or Pd(Ph$_3$P)$_2$Cl$_2$, DME or 1,4-dioxane, H_2O, 80–110 °C, 2–12 h, 16–76%; (viii) pyrrolidine or piperidine, reflux, 2 h, 38–52%.



Scheme 2. Reagents and conditions: (i) 1 M aqueous HCl, CH_2Cl_2, rt, 2 h, 70%; (ii) CF_3CO_2H then 4 M HCl in dioxane, MeOH, rt, 4 h, 80%; (iii) NaH, DMF, MeI or EtI, 0 °C, 1 h, 25–30%.

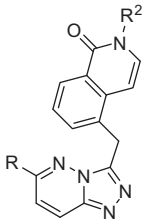
Table 1
Triazolopyridazines substituted with methylisoquinolinone

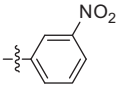
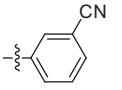
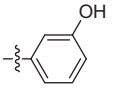
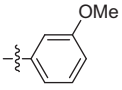


Compound	R	c-Met IC_{50}[a] (μM)
14		0.002
15		0.003
16		0.005
19		0.002
20		0.027
21		0.027
22		0.029
23		0.004
24		0.005
25		0.014

[a] For assay conditions, see Ref. 8.

Table 2
SAR of triazolopyridazine derivatives



Compound	R^1	R^2	c-Met IC_{50}[a] (μM)
26		H	0.072
27		H	0.004
28		H	0.007
29		H	0.003
30		H	0.004
31		H	0.003
32		H	0.003
33		H	0.005
34		Me	0.073
35		Et	0.072

[a] For assay conditions, see Ref. 8.

potency. However, attachment of a piperidinyl group to the pyrazole ring decreased c-Met inhibitory activity. The pyrazole group in compound **14** was also replaced with other heteroaryl or heterocycloalkyl groups. Of these, pyrrole **21** and piperidine **22** were not as active as pyrazole **14**, while thiophene **23** and pyridine **24** did show good potency against c-Met.

We then turned our attention to the modification of the phenyl ring of triazolopyridazine **15**, and synthesized a series of derivatives in which the phenyl group of **15** was replaced with a variety of substituted phenyl groups. Most of the compounds exhibited good c-Met inhibitory activity (Table 2). The potency in N-alkylated isoquinolinones **34** and **35** against c-Met had decreased, which suggests the free NH on the isoquinolinone ring might interact with a hinge residue of c-Met.

Modeling experiments suggested that triazolopyridazine **15** would dock strongly into the ATP-binding site of c-Met, as shown in Figure 2. This analysis utilized a crystal structure of c-Met complexed with **1** that was obtained from the Protein Data Bank (pdb entry: 3CCN).[6b] Calculations for this docking analysis were carried out using LibDock[13] interfaced with Discovery Studio 3.0 (Accelrys). The crystal structure of 3CCN was regenerated very well with a root mean square displacement (RMSD) of 0.7 Å. We set the H atom in the peptide backbone of M1160 as an interaction site with default parameters in LibDock. Triazolopyridazine **15** was bound very tightly by c-Met when the same docking parameters were used (DockScore, 137.0 for **15** vs 125.7 for **1**). These calculated values are in good agreement with the experimental results (c-Met IC_{50} 3 nM for **15** vs 6 nM for **1**). The strong interaction of **15** with c-Met is attributed both to H-bonding of the isoquinolinone moiety with hinge residues M1160 and P1158, and to π-stacking interactions between the triazolopyridazine moiety and Y1230. Hydrophobic interactions of the triazolopyridazine moiety with M1211 could also contribute to the tight binding. It is likely that the low potencies of **26** and **27** compared with those of **15** and **28–33** arise from disfavorable interactions between the ortho-substituents on the phenyl ring and backbone carbonyl groups of N1209 and R1208.

The anti-proliferative activity of the triazolopyridazines was evaluated by performing a cytotoxicity assay on a panel of c-Met-amplified gastric cancer cell lines (MKN-45, SNU-5 and Hs746T).[14,15] Most of the triazolopyridazines substituted with methylisoquinolinones were potent growth inhibitors for all the c-Met-amplified gastric cancer cell lines (Table 3). Several compounds showed double digit nanomolar anti-proliferative activity, but the proliferation of c-Met-independent N87 cells was hindered only slightly by treatment with the triazolopyridazines, even at

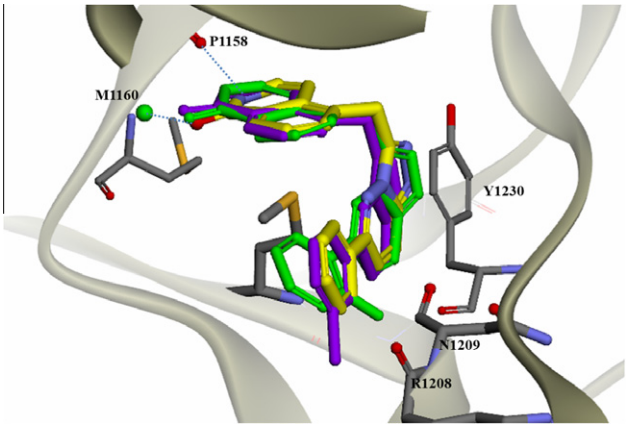


Figure 2. A proposed structure for the c-Met complex with triazolopyridazine **15** (yellow), **26** (green) and **28** (magenta). H-bonding interactions between the **15** and c-Met are shown in blue dotted lines.

Table 3
Anti-proliferative activity of triazolopyridazine derivatives against a panel of gastric cancer cell lines

Compound	GI_{50} (μM)[a]			
	MKN-45	SNU-5	Hs746T	N87
14	0.13	0.69	0.064	>10
15	0.038	0.059	0.071	>10
23	0.032	0.056	0.17	>10
24	0.42	0.45	0.40	>10
28	0.011	0.019	0.053	>10
29	0.042	0.052	0.13	>10
31	0.036	0.12	0.059	>10
32	0.47	1.1	0.60	>10
33	0.095	0.13	0.10	>10

[a] For assay conditions, see Ref. 15.

high concentration (10 μM). This difference represents a roughly 100-fold or more differential cytotoxicity toward c-Met-addicted gastric cancer cell lines.

A preliminary pharmacokinetic study of triazolopyridazine **15** showed a suitable elimination half life ($t_{1/2}$), slow absorption (T_{max}) and moderate bioavailability (F) (Table 4).

In summary, we have designed and synthesized a series of triazolopyridazines substituted with methylisoquinolinone, a moiety that strongly interacts with the hinge residues of c-Met, as selective c-Met kinase inhibitors. Some of the triazolopyridazines showed good anti-proliferative activity against a panel of c-Met-amplified gastric cancer cell lines, but the proliferation of c-Met independent cells was little affected by treatment with any of the triazolopyridazines. Further in vitro and in vivo pharmacological evaluation of these compounds is planned and the results will be reported in a due course.

Table 4
Preliminary pharmacokinetic profile for **15** in rats

Parameter	iv[a]	po[a]
C_{max} (μg/mL)		0.3
T_{max}		12.7
$t_{1/2}$	6.4	5.3
AUC (μg h/mL)	11.2	3.9
CL (L/kg h)	0.8	
V_{ss} (L/kg)	6.4	
F (%)		35

[a] Dose, 10 mg/kg (three rats were used).

Acknowledgments

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References and notes

1. Knight, Z. A.; Lin, H.; Shokat, K. M. *Nat. Rev. Cancer* **2010**, *10*, 130.
2. Jänne, P. A.; Gray, N.; Settleman, J. *Nat. Rev. Drug Disc.* **2009**, *8*, 709.
3. Christensen, J. G.; Burrows, J.; Salgia, R. *Cancer Lett.* **2005**, *225*, 1.
4. (a) Comoglio, P. M.; Giordano, S.; Trusolino, L. *Nat. Rev. Drug Disc.* **2008**, *7*, 504; (b) Sattler, M.; Hasina, R.; Reddy, M. M.; Gangadhar, T.; Salgia, R. *Ther. Adv. Med. Oncol.* **2011**, *3*, 171; (c) Underiner, T. L.; Herbertz, T.; Miknyoczki, S. J. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 7.
5. Jiang, W. G.; Martin, T. A.; Parr, C.; Davies, G.; Matsumoto, K.; Nakamura, T. *Crit. Rev. Oncol. Hematol.* **2005**, *53*, 35.
6. (a) Zhang, F.-J.; Vojkovsky, T.; Huang, P.; Liang, C.; Do, S. H.; Koenig, M.; Cui, J. WO2005010005, 2005.; (b) Albrecht, B. K.; Harmange, J.-C.; Bauer, D.; Berry, L.; Bode, C.; Boezio, A. A.; Chen, A.; Choquette, D.; Dussault, I.; Fridrich, C.; Hirai, S.; Hoffman, D.; Larrow, J. F.; Kaplan-Lefko, P.; Lin, J.; Lohman, J.; Long, A. M.; Moriguchi, J.; O'Connor, A.; Potashman, M. H.; Reese, M.; Rex, K.; Siegmund, A.; Shah, K.; Shimanovich, R.; Springer, S. K.; Teffera, Y.; Yang, Y.; Zhang, Y.; Bellon, S. F. *J. Med. Chem.* **2008**, *51*, 2879; (c) Boezio, A. A.; Berry, L.; Albrecht, B. K.; Bauer, D.; Bellon, S. F.; Bode, C.; Chen, A.; Choquette, D.; Dussault, I.; Hirai, S.; Kaplan-Lefko, P.; Larrow, J. F.; Lin, M.-H. J.; Lohman, J.; Potashman, M. H.; Rex, K.; Santostefano, M.; Shah, K.; Shimanovich, R.; Springer, S. K.; Teffera, Y.; Yang, Y.; Zhang, Y.; Harmange, J.-C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6307; (d) Buchanan, S. G.; Hendle, J.; Lee, P. S.; Smith, C. R.; Bounaud, P.-Y.; Jessen, K. A.; Tang, C. M.; Huser, N. H.; Felce, J. D.; Froning, K. J.; Peterman, M. C.; Aubol, B. E.; Gessert, S. F.; Sauder, J. M.; Schwinn, K. D.; Russell, M.; Rooney, I. A.; Adams, J.; Leon, B. C.; Do, T. H.; Blaney, J. M.; Sprengeler, P. A.; Thompson, D. A.; Smyth, L.; Pelletier, L. A.; Atwell, S.; Holme, K.; Wasserman, S. R.; Emtage, S.; Burley, S. K.; Reich, S. H. *Mol. Cancer Ther.* **2009**, *8*, 3181; (e) Lu, T.; Alexander, R.; Connors, R. W.; Cummings, M. D.; Galemmo, R.; Hufnagel, H. R.; Johnson, D. L.; Khalil, E.; Leonard, K. A.; Markotan, T. P.; Maroney, A. C.; Sechler, J. L.; Travins, J. M.; Tuman, R. W. WO2007075567, 2007.; (f) Timofeevski, S. L.; McTigue, M. A.; Ryan, K.; Cui, J.; Zou, H. Y.; Zhu, J. X.; Chau, F.; Alton, G.; Karlicek, S.; Christensen, J. G.; Murray, B. W. *Biochemistry* **2009**, *48*, 5339.
7. Diamond, S.; Boer, J.; Maduskuie, T. P., Jr.; Falahatpisheh, N.; Li, Y.; Yeleswaram, S. *Drug Metab. Dispos.* **2010**, *38*, 1277.
8. (a) Cho, S. Y.; Han, S.-Y.; Ha, J. D.; Ryu, J. W.; Lee, C. O.; Jung, H.; Kang, N. S.; Kim, H. R.; Koh, J. S.; Lee, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4223; Han, S.-Y.; Lee, C. O.; Ahn, S.-H.; Lee, M.-O.; Kang, S.-Y.; Cha, H.-J.; Cho, S. Y.; Ha, J. D.; Ryu, J. W.; Jung, H.; Kim, H. R.; Koh, J. S.; Lee, J. *Invest. New Drugs* in press. doi:10.1007/s10637-010-9584-2.
9. Kawata, S.; Ashizawa, S.; Hirama, M. *J. Am. Chem. Soc.* **1997**, *119*, 12012.
10. Briet, N.; Brookes, M. H.; Davenport, R. J.; Galvin, F. C. A.; Gilbert, P. J.; Mack, S. R.; Sabin, V. *Tetrahedron* **2002**, *58*, 5761.
11. Thomson, A. E.; Hughes, G.; Batsanov, A. S.; Bryce, M. R.; Parry, P. R.; Tarbit, B. *J. Org. Chem.* **2005**, *70*, 388.
12. *Characterization data for triazolopyridazine* **15**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (br s, 1H), 8.41 (d, J = 9.8 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 8.08–8.06 (m, 2H), 7.93 (d, J = 9.8 Hz, 1H), 7.76 (d, J = 6.8 Hz, 1H), 7.59–7.57 (m, 3H), 7.45 (t, J = 7.7 Hz, 1H), 7.22 (t, J = 6.2 Hz, 1H), 6.91 (d, J = 7.4 Hz, 1H), 4.86 (s, 2H) δ ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.9, 152.8, 148.1, 143.3, 136.8, 133.97, 133.93, 131.1, 131.0, 129.18, 129.15, 127.3, 126.7, 126.1, 125.9, 125.1, 119.7, 101.3, 27.4; HRMS (EI) m/z calcd for $C_{21}H_{15}N_5O$ (M^+) 353.1277, found 353.1282.
13. Diller, D. J.; Merz, M. L., III *Proteins: Structure, Function, and Genetics* **2001**, *43*, 113.
14. (a) Kuniyasu, H.; Yasui, W.; Kitadai, Y.; Yokozaki, H.; Ito, H.; Tahara, E. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 227; (b) Smolen, G. A.; Sordella, R.; Muir, B.; Mohapatra, G.; Barmettler, A.; Archibald, H.; Kim, W. J.; Okimoto, R. A.; Bell, D. W.; Sgroi, D. C.; Christensen, J. G.; Settleman, J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2316; (c) Asaoka, Y.; Tada, M.; Ikenoue, T.; Seto, M.; Imai, M.; Miyabayashi, K.; Yamamoto, K.; Yamamoto, S.; Kudo, Y.; Mohri, D.; Isomura, Y.; Ijichi, H.; Tateishi, K.; Kanai, F.; Ogawa, S.; Omata, M.; Koike, K. *Biochem. Biophys. Res. Commun.* **2010**, *394*, 1042; (d) Rege-Cambrin, G.; Scaravaglio, P.; Carozzi, F.; Ponzetto, C.; Comoglio, P. M.; Saglio, G. *Cancer Genet. Cytogenet.* **1992**, *64*, 170.
15. Cytotoxicity/MTS assay: MKN-45, SNU-5, Hs746T and N87 cells were purchased from American Type Culture Collection (ATCC) or Korean Cell Line Bank (KCLB). The cells were cultured in RPMI1640 or DMEM supplemented with 10% fetal bovine serum and gentamycin (50 μg/mL) at 37 °C and 5% CO_2. MKN-45, SNU-5, Hs746T and N87 cells were plated into 96-well plates (5000 or 10,000 cells/well) and treated with various concentrations of triazolopyridazines or DMSO for 72 h in triplicate. The anti-proliferative activity of the compounds was measured using CellTiter 96®AQueous NonRadioactive Cell Proliferation Assay (Promega).