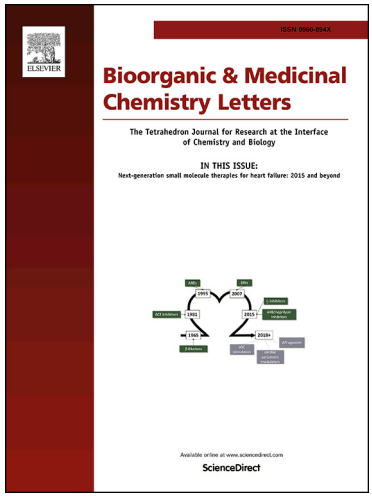
Accepted Manuscript



Design, synthesis, and biological evaluation of novel aminopyrimidinylisoindolines as AXL kinase inhibitors

Min Jung Choi, Eun Joo Roh, Wooyoung Hur, So Ha Lee, Taebo Sim, Chang-Hyun Oh, Sun-Hwa Lee, Jong Seung Kim, Kyung Ho Yoo

PII: S0960-894X(18)30803-5
DOI: https://doi.org/10.1016/j.bmcl.2018.10.013
Reference: BMCL 26072

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 24 August 2018
Revised Date: 1 October 2018
Accepted Date: 10 October 2018

Please cite this article as: Choi, M.J., Roh, E.J., Hur, W., Lee, S.H., Sim, T., Oh, C-H., Lee, S-H., Kim, J.S., Yoo, K.H., Design, synthesis, and biological evaluation of novel aminopyrimidinylisoindolines as AXL kinase inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.10.013

Design, synthesis, and biological evaluation of novel aminopyrimidinylisoindolines as AXL kinase inhibitors

Min Jung Choi[a,d], Eun Joo Roh[a], Wooyoung Hur[a], So Ha Lee[a], Taebo Sim[a], Chang-Hyun Oh[b], Sun-Hwa Lee[c], Jong Seung Kim[d], Kyung Ho Yoo[a,*]

[a] *Chemical Kinomics Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 02792, Republic of Korea*

[b] *Center for Biomaterials, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 02792, Republic of Korea*

[c] *Efficacy Assessment Support Department, New Drug Development Center, 80 Chembok-ro Dong-gu, Daegu 41061, Republic of Korea*

[d] *Department of Chemistry, Korea University, Seoul 02841, Republic of Korea*

* Corresponding author. Tel.: +82 2 958 5152; fax: +82 2 958 5549.

E-mail address: khyoo@kist.re.kr (K. H. Yoo).

ABSTRACT

A novel series of aminopyrimidinylisoindoline derivatives **1a-w** having an aminopyrimidine scaffold as a hinge region binding motif were designed and synthesized. Among them, six compounds showed potent inhibitory activities against AXL kinase with IC_{50} values of submicromolar range. Especially, compound **1u** possessing (4-acetylpiperazin-1-yl)phenyl moiety exhibited extremely excellent efficacy (IC_{50} = < 0.00050 μM). Their *in vitro* antiproliferative activities were tested over five cancer cell lines. Most compounds showed good antiproliferative activities against HeLa cell line. The kinase panel profiling of 50 different kinases and the selected inhibitory activities for the representative compound **1u** were carried out. The compound **1u** exhibited excellent inhibitory activities (IC_{50} = < 0.00050, 0.025, and 0.050 μM for AXL, MER, and TYRO3, respectively) against TAM family, together with potent antiproliferative activity against MV4-11 cell line (GI_{50} = 0.10 μM) related to acute myeloid leukemia (AML).

Keywords: Aminopyrimidinylisoindolines; Inhibitors; AXL kinase; TAM family; Enzyme inhibitory activity; Antiproliferative activity

AXL is a receptor tyrosine kinase (RTK) involved in the growth, differentiation, survival, and motility of many different cell types.[1] In recent years, AXL kinase has emerged as a key facilitator of immune escape and drug-resistance by eliminating intercellular antibodies and regulating the secretion, and release of cytokines. The AXL protein was latter classified as a RTK belonging to the TAM (TYRO3, AXL, and MER) subfamily.[2] The TAM RTKs are overexpressed in a large number of human cancers including myeloid leukemias, colon, liver and prostate cancer.[3,4] Elevated expression of one or more TAM receptors is often associated with cancer progression, resistance to targeted therapies, and metastasis.[5-7] Overexpression of AXL was shown to correlate with poorer prognosis, increased invasiveness, and xenograft growth of human cancers, further indicating that AXL has strong oncogenic potential.[8] AXL has been implicated as a cancer driver and correlated with poor survival in numerous aggressive tumors including glioblastoma multiforme,[9] breast[10] and lung[11] cancers, osteosarcoma,[12] and acute myeloid leukemia.[13] Therefore, AXL has recently been proposed as an potential oncology target due to its overexpression in several types of cancers and a number of small molecule inhibitors have been developed.[14-17]

Many kinase inhibitors utilize the pyrimidine scaffold as a hinge anchor with various substitutions at 2- and 4-positions.[18,19] In this work, a novel series of aminopyrimidinylisoindoline derivatives **1a-w** having an aminopyrimidine scaffold as a hinge region binding motif, based on the structural features of TP-0903,[15] TAE-684,[20] and Fedratinib,[21] were designed and synthesized (Fig. 1). Here isoindoline moiety having a rigid bicyclic structure to enhance efficacy was introduced at 4-position of pyrimidine nucleus.

Their *in vitro* enzyme inhibitory and antiproliferative activities were tested over AXL kinase and five cancer cell lines. Also the kinase panel profiling of 50 different kinases and the selected inhibitory activities for the representative compound **1u** are reported.

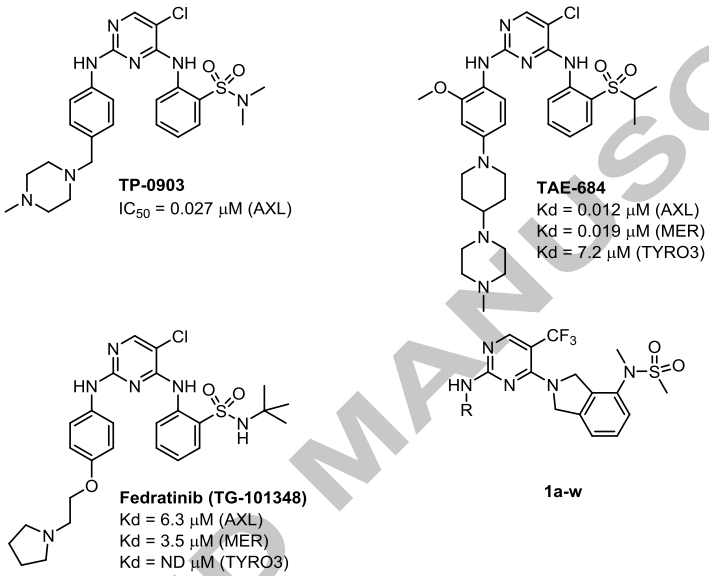
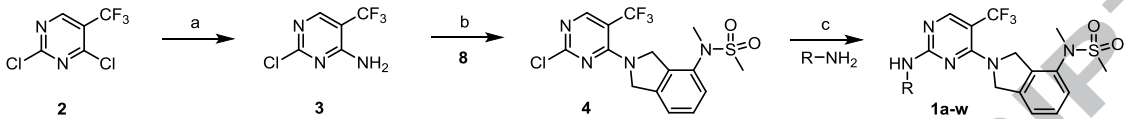


Fig. 1. Structures of aminopyrimidine derivatives as AXL and TAM inhibitors and the target compounds **1a-w**.

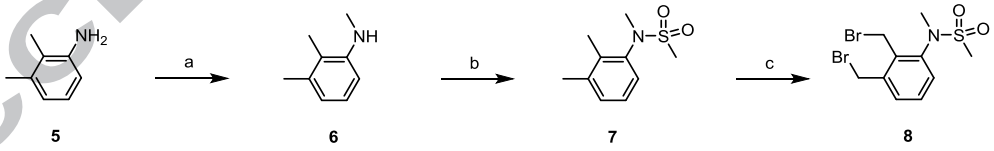
Aminopyrimidinylisoindolines **1a-w** were synthesized by the pathways illustrated in Scheme 1. Amination of 2,4-dichloro-5-trifluoromethylpyrimidine (**2**) as a starting material with 7*N* ammonia solution in methanol gave 2-chloro-5-(trifluoromethyl)pyrimidin-4-amine (**3**),[22] which was then cyclized with *N*-(2,3-bis(bromomethyl)phenyl)-*N*-methylmethanesulfonamide (**8**) in presence of sodium hydride to yield the key intermediate **4** in good yield. Nucleophilic substitution of the chloro group of compound **4** with the appropriate aryl amines in presence of *p*-toluenesulfonic acid led to the corresponding title

compounds **1a–w**.[23]



Scheme 1. Reagents and conditions: (a) 7*N* ammonia solution in MeOH, rt, 2.5 h, 38%; (b) NaH, THF, rt, 24 h, 84%; (c) *p*-TsOH (2*M* in dioxane), DMF, 90 ^{o}C, 24 h, 11-70%.

Synthesis of *N*-(2,3-bis(bromomethyl)phenyl)-*N*-methylmethanesulfonamide (**8**) was carried out by the sequence of reactions shown in Scheme 2. *N*-2,3-Trimethylaniline (**6**) was obtained by *N*-methylation of 2,3-dimethylaniline (**5**) with tetrabutylammonium bromide and dimethylsulfate in toluene followed by 15% aqueous sodium hydroxide solution. Treatment of **6** with methanesulfonyl chloride gave *N*-(2,3-dimethylphenyl)-*N*-methylmethanesulfonamide (**7**) in good yield, which was then brominated with *N*-bromosuccinimide in presence of AIBN under reflux condition to produce the desired compound **8**.[24]



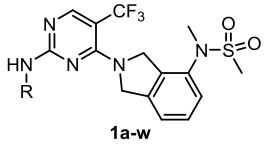
Scheme 2. Reagents and conditions: (a) tetrabutylammonium bromide, dimethylsulfuric acid, toluene, 15% aqueous NaOH solution, rt, 24 h, 51%; (b) methanesulfonyl chloride, pyridine, rt, 30 min, 98%; (c) N-bromosuccinimide, AIBN, CH_2Cl_2, reflux, 4 h, 73%.

The *in vitro* inhibitory activity of the target compounds was tested against AXL

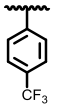
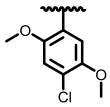
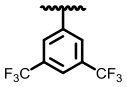
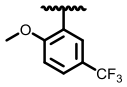
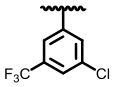
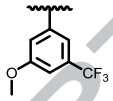
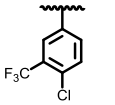
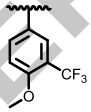
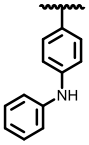
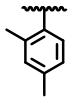
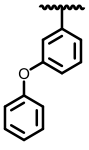
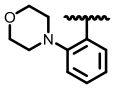
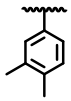
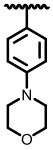
kinase, together with staurosporine as a reference standard because of its high potency.[25] The ability of aminopyrimidinylisoindolines **1a-w** to inhibit the activity of AXL kinase is summarized in Table 1. As shown in Table 1, compounds **1f**, **1j**, **1l**, **1r** showed good potencies (IC_{50} = 1.5 – 9.1 μM) and compounds **1a**, **1d**, **1v** exhibited potent inhibitory activities with IC_{50} values below submicromolar range (IC_{50} = 0.080 – 0.89 μM). Upon investigating the effect of substituents onto aryl nucleus, compounds with electron-donating group were generally found to be more potent than compounds having electron-withdrawing group. Compounds **1a**, **1d**, **1f**, **1j** with methoxy or methyl group showed superior potencies to other compounds having the different substituents, except for compound **1l**. This may be rationalized that the electronic effect of substituents at receptor site may affect the affinity and potency. Bicyclic compound **1w** exhibited excellent inhibitory activity with IC_{50} value of 0.0045 μM. Especially, compounds **1t** (IC_{50} = 0.00094 μM) and **1u** (IC_{50} = < 0.00050 μM) with longer chains at 2-position were remarkably more potent than other compounds having the chains of relatively short length. This may be attributed to that the longer chain, piperazinyl moiety, may geometrically permit appropriate fitting of the molecule at the ATP-binding site of the receptor kinase domain. In our series, compound **1u** possessing (4-acetylpiperazin-1-yl)phenyl moiety was selected as a representative compound. As compared with parent compound **1u**, compound **1v** having additional methoxy group on aryl nucleus displayed the result of much lowered efficacy. Interestingly, the comparison of compound **1t** with *p*–orientation and compound **1s** of *o*-orientation showed a very surprising difference (IC_{50} = 0.00094 and > 20 μM, respectively) in efficacy. Namely, compound **1s** did not possess any meaningful activity. It can be

concluded that the introduction of solubilizing group at the para position of the phenyl group greatly improved the potency and that steric and/or electronic properties of the substituents of the phenyl group play a very important role in the drug-receptor interaction.

Table 1. The *in vitro* enzyme inhibitory activity of aminopyrimidinylisoindolines **1a-w** against AXL kinase.



1a-w

Compd. No.	R	IC_{50} (μM)	Compd. No.	R	IC_{50} (μM)
1a		0.080	**1m**		> 20
1b		> 20	**1n**		> 20
1c		> 20	**1o**		> 30
1d		0.89	**1p**		> 20
1e		> 20	**1q**		> 20
1f		2.9	**1r**		2.6
1g		> 20	**1s**		> 20
1h		> 20	**1t**[26]		0.00094

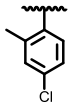
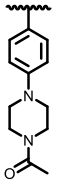
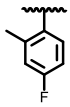
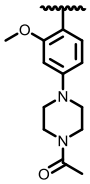
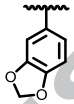
1i		> 20	**1u**[26]		< 0.00050
1j		9.1	**1v**		0.79
1k		> 20	**1w**		0.0045
1l		1.5	Staurosporine		0.0039

Table 2 shows the *in vitro* antiproliferative activity[27] of aminopyrimidinylisoindolines **1a-w** to inhibit the growth of five cancer cell lines. Most compounds showed superior antiproliferative activities against HeLa cell line to other cell lines. As previous, the long-chained compounds **1t-w** were found to be more potent than those of the shorter length against all the tested cell lines. Compound **1t** having 4-morpholinophenyl moiety showed good activities (GI_{50} = 0.79 - 6.8 μM) for all of these. In particular, the representative compound **1u** exhibited potent antiproliferative activities with GI_{50} values of submicromolar range (GI_{50} = 0.73 and 0.97 μM, respectively) against HeLa and OVCAR-3 cell lines.

Table 2. The *in vitro* antiproliferative activity of aminopyrimidinylisoindolines **1a-w** against five cancer cell lines.

Compd. No.	GI_{50} (μM)				
	A549	U87-MG	HeLa	OVCAR-3	Hep G2
1a	24.8	> 30	7.0	0.63	1.8
1b	7.3	6.2	16.5	> 30	25.6
1c	> 30	> 30	17.2	> 30	> 30
1d	> 30	> 30	5.1	> 30	22.1
1e	> 30	29.4	14.0	> 30	> 30
1f	20.7	> 30	10.6	> 30	19.1
1g	> 30	> 30	> 30	> 30	> 30
1h	> 30	> 30	> 30	> 30	> 30
1i	25.5	28.3	17.4	> 30	19.6
1j	> 30	> 30	6.5	> 30	16.8
1k	> 30	> 30	10.9	> 30	> 30
1l	> 30	> 30	5.3	> 30	> 30
1m	> 30	> 30	> 30	> 30	> 30
1n	> 30	16.8	9.2	> 30	10.3
1o	6.7	5.5	4.3	21.5	9.9
1p	15.2	> 30	11.1	>50	16.0
1q	6.2	29.8	7.3	>50	8.6
1r	19.0	19.5	4.3	24.9	23.0
1s	> 30	> 30	23.9	>50	> 30
1t	2.5	6.8	0.79	2.4	1.9
1u	1.3	22.1	0.73	0.97	1.4
1v	3.3	4.7	1.3	1.6	3.8
1w	12.1	> 30	6.3	> 30	9.8
PF-562271	2.1	3.7	1.7	1.8	1.2

Compound **1u** selected as a representative compound was tested at a single dose concentration of 10 μM over a panel of 50 oncogenic kinases at Reaction Biology Corporation (Malvern, PA) (Table 3). As shown in Table 3, good potencies more than 90% inhibition were observed against ten kinases at this concentration. Compound **1u** exhibited excellent enzymatic inhibitions above 95% against four kinases (Aurora A, FAK, PLK1, SYK). The inhibition exerted in twenty-four kinases was below 50%.

Table 3. Percentages of enzymatic inhibitions exerted by the representative compound **1u** over a panel of 50 kinases.

Kinase	% Inhibition[a,b]	Kinase	% Inhibition
ABL1	63.1	CK1 epsilon	-2.5
ACK1	91.1	DAPK1	42.2
AKT1	-3.6	DNA-PK	-4.0
ALK	71.3	EGFR	2.0
Aurora A	95.3	EPHA1	13.1
AXL	92.1	FAK/PTK2	96.4
BRAF	6.7	FGFR1	26.0
c-KIT	17.8	FGR	80.6
c-MET	17.9	FLT1/VEGFR1	49.0
c-SRC	88.2	FYN	62.2
CDK1/cyclin B	20.5	HIPK1	83.8
CHK1	37.2	IKKa/CHUK	14.1
JAK1	75.4	IR	89.8
JNK1	45.1	PKCa	19.3
KDR/VEGFR2	55.8	PLK1	95.2
LCK	84.1	RAF1	-14.6
LYN	69.1	RET	85.2
MEK1	3.6	ROCK1	6.9

MER	90.6	RON/MST1R	40.1
MST4	25.3	ROS/ROS1	92.2
MUSK	66.6	SYK	95.3
P38a/MAPK14	-34.8	TIE2/TEK	65.4
p70S6K/RPS6KB1	65.5	TRKA	92.2
PAK4	16.2	TYRO3/SKY	87.7
PIMI	9.8	YES/TES1	90.5

[a] Test compound was used in a single dose concentration of 10 μM.

[b] % Inhibition was calculated by subtracting % activity from 100.

The selected eight enzyme inhibitory and fourteen antiproliferative activities for the representative compound **1u** are summarized in Table 4. As shown in Table 4, compound **1u** exhibited excellent inhibitory activities with IC_{50} values of < 0.00050 μM (AXL), 0.025 μM (MER), and 0.050 μM (TYRO3) against TAM family. Four kinases such as Aurora A, FLT3, PLK1, and SYK showed 1-digit nanomolar IC_{50} values. Compound **1u** have shown good antiproliferative activities less than 1-digit micromolar range (GI_{50} = 0.10 – 5.2 μM) against all the tested cell lines, except U87-MG. It was found to possess potent activities with GI_{50} values of submicromolar range for seven cell lines. Among them, MV4-11 cell line related to acute myeloid leukemia (AML) displayed superior potency with GI_{50} value of 0.10 μM to other cell lines.

Table 4. Determination of the selected enzyme inhibitory and antiproliferative activities for the representative compound **1u**.

Kinase	IC_{50} (μM)	Cell line	GI_{50} (μM)
Aurora A	0.0030	U87-MG[a]	22.1
AXL[a]	< 0.00050	IMR-32	0.26
MER	0.025	MDA-MB-231	0.50
FAK	0.011	HCC-78	5.2
FLT3	0.0046	A549[a]	1.3
PLK1	0.0088	NOMO-1	3.8
SYK	0.0051	MV4-11	0.10
TYRO3	0.050	Hep G2[a]	1.4
		Panc-1	0.43
		OVCAR-3[a]	0.97
		HeLa[a]	0.73
		HCT 116	0.42
		A375	1.0
		HT-1080	1.8

[a] These data were used from Tables 1 and 2.

As a continuation of our ongoing anticancer development research project, a novel series of aminopyrimidinylisoindoline derivatives **1a-w** were designed and synthesized. The target compounds were evaluated for inhibitory activities against AXL kinase and antiproliferative activities against five cancer cell lines. Among them, compound **1u** possessing (4-acetylpiperazin-1-yl)phenyl moiety exhibited extremely excellent efficacy with IC_{50} value of < 0.00050 μM against AXL kinase. In our series, a representative compound **1u** exhibited the best combination of enzyme inhibitory activities (IC_{50} = < 0.00050, 0.025, and 0.050 μM for AXL, MER, and TYRO3, respectively) against TAM

family and antiproliferative activity (GI_{50} = 0.10 μM for MV4-11). It can be used as a promising lead for the development of potent AXL and TAM inhibitors.

Acknowledgments

This work was funded by the KIST Institutional Program (Grant No. 2E28010) from Korea Institute of Science and Technology, and by the Creative Fusion Research Program through the Creative Allied Project funded by the National Research Council of Science & Technology (CAP-17-01-KIST).

References and notes

1. Linger, R. M.; Keating, A. K.; Earp, H. S.; Graham, D. K. *Expert Opin. Ther. Targets* **2010**, *14*, 1073.

2. Baladi, T.; Abet, V.; Piguel, S. *Eur. J. Med. Chem*. **2015**, *105*, 220.

3. Graham, D. K; DeRyckere, D.; Davies, K. D.; Earp, H. S. *Nat. Rev. Cancer* **2014**, *14*, 769.

4. Linger, R. M. A.; Keating, A. K.; Earp, H. S.; Graham, D. K. *Adv. Cancer Res*. **2008**, *100*, 35.

5. Debruyne, D. N.; Bhatnagar, N.; Sharma, B. Luther, W.; Moore, N. F.; Cheung, N. K.; Gray, N. S.; George, R. E. *Oncogene* **2016**, *35*, 3681.

6. Giles, K. M.; Kalinowski, F. C.; Candy, P. A.; Epis, M. R.; Zhang, P. M.; Redfem, A. D.; Stuart, L. M.; Goodall, G. J.; Leedman, P. J. *Mol. Cancer Ther*. **2013**, *12*, 2541.

7. Akalu, Y. T.; Rothlin, C. V.; Ghosh, S. *Immunol. Rev*. **2017**, *276*, 165.

8. Paccez, J. D.; Vogelsang, M.; Parker, M. I.; Zerbini, L. F. *Int. J. Cancer* **2014**, *134*, 1024.

9. Hutterer, M.; Knyazev, P.; Abate, A.; Reschke, M.; Maier, H.; Stefanova, N.; Knyazeva, T.; Barbieri, V.; Reindl, M.; Muigg, A.; Kostron, H.; Stockhammer, G.; Ullrich, A. *Clin. Cancer Res*. **2008**, *14*, 130.

10. Wang, X.; Saso, H.; Iwamoto, T.; Xia, W.; Gong, Y.; Pusztai, L.; Woodward, W. A.; Reuben, J. M.; Warner, S. L.; Bearss, D. J.; Hortobagyi, G. N.; Hung, M. C.; Ueno, N. T. *Cancer Res*. **2013**, *73*, 6516.

11. Niederst, M. J.; Engelman, J. A. *Sci. Signaling* **2013**, *6*, re6.

12. Han, J.; Tian, R.; Yong, B.; Luo, C.; Tan, P.; Shen, J.; Peng, T. *Biochem. Biophys. Res. Commun.* **2013**, *435*, 493.

13. Ben-Batalla, I.; Schultze, A.; Wroblewski, M.; Erdmann, R.; Heuser, M.; Waizenegger, J. S.; Riecken, K.; Binder, M.; Schewe, D.; Sawall, S.; Witke, V.; Cubas-Cordova, M.; Janning, M.; Wellbrock, J.; Fehse, B.; Hagel, C.; Krauter, J.; Ganser, A.; Lorens, J. B.; Fiedler, W.; Carmeliet, P.; Pantel, K.; Bokemeyer, C.; Loges, S. *Blood* **2013**, *122*, 2443.

14. Myers, S. H.; Brunton, V. G.; Unciti-Broceta, A. *J. Med. Chem*. **2016**, *59*, 3592.

15. Mollard, A.; Warner, S. L.; Call, L. T.; Wade, M. L.; Bearss, J. J.; Verma, A.; Sharma, S.; Vankayalapati, H.; Bearss, D. J. *ACS Med. Chem. Lett*. **2011**, *2*, 907.

16. Corno, C.; Gatti, L.; Lanzi, C.; Zaffaroni, N.; Colombo, D.; Perego, P. *Curr. Med. Chem.* **2016**, *23*, 1496.

17. Shen, Y.; Chen, X.; He, J.; Lioa, D.; Zu, X. *Life Sci*. **2018**, *198*, 99.

18. Appari, D. R.: Chen, X.: Chilukuri, R.: Crew, P. A.: Dong, H.: Ferraro, C.: Foreman, K.: Gupta, C. R.: Li, A.H.: Sherman, D.: Stolz, M. K.: Volk, B.: Zahler, R. WO 2010141406.

19. Combs, A. P. WO 2012125629.

20. Galkin, A. V.; Melnick, J. S.; Kim, S.; Hood, T. L.; Li, N.; Li, L.; Xia, G.; Steensma. R.; Chopiuk, G.; Jiang, J.; Wan, Y.; Ding, P.; Liu, Y.; Sun, F.; Schultz, P. G.; Grat, N. S.; Warmuth, M. *Proc. Natl. Acad. Sci. U. S. A*. **2007**, *104*, 270.

21. Wernig, G.; Kharas, M. G.; Okabe, R.; Moore, S. A.; Leeman, D. S.; Cullen, D. E.; Gozo, M.; McDowell, E. P.; Levine, R. L.; Doukas, J.; Mak, C. C.; Noronha, G.; Martin, M.; Ko, Y. D.; Lee, B. H.; Soll, R. M.; Tefferi, A.; Hood, J. D.; Gilliland, D. G. *Cancer Cell* **2008**, *13*, 311.

22. Ren, P.; Liu, Y.; Li, L.; Chan, K.; Wilson, T. E.; Castro, A. C.; Evans, C. A.; Snyder, D. A. WO 2012064973.

23. Xu, Y.; Ding, S. WO 2010065721.

24. Rishton, G.; Catalano, S. M.; Look, G. C.; Gogoris, A. WO 2015116923.

25. *In vitro* kinase assay. The AXL assay is performed using the AXL Kinase Enzyme System (Promega, USA) and ADP-Glo™ Kinase Assay Kit (Promega, USA). Because the AXL reaction utilizes ATP and generates ADP, the kinase reaction is terminated simultaneously and the remaining ATP is depleted, when the ADP-Glo™ reagent is added. After the reaction, the kinase detection reagent is added, and then the ATP newly synthesized is converted to light, using the luciferase/luciferin reaction. First, the enzyme, substrate, ATP and inhibitor are diluted in kinase buffer. The mixture is added to the wells of 384 low volume plate: 1 µL of inhibitor or (5% DMSO), 2 µL of enzyme, and 2 µL of substrate/ATP mix. After incubated at room temperature for an hour, 5 µL of ADP-Glo™ reagent is added. Again, incubate at room temperature for 40 min, and then add 10 µL of kinase detection reagent. Lastly, incubate at room temperature for 30 min and record luminescence.

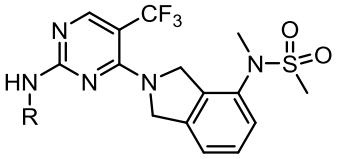
26. Selected data. Compound **1t**: Grey solid; mp: 247.5 – 249.5 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.37 (s, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.49 (q, J = 4.3 Hz, 1H), 7.46 (s, 1H), 7.44 (d, J = 1.5 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 5.16 (s, 2H), 5.03 (s, 2H), 3.75 (t, J = 4.7 Hz, 4H), 3.24 (s, 3H), 3.08 - 3.35 (m, 7H); HRMS (ESI, positive) calcd for $C_{25}H_{27}F_3N_6O_3SNa$ $[M+Na]^+$ 571.1715, found 571.1713. Compound **1u**: Pale brown solid; mp: 229.5 – 232.0 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.37 (s, 1H), 7.66 (d, J = 8.7 Hz, 2H), 7.50 (q, J = 4.3 Hz, 1H), 7.46 (s, 1H), 7.42 - 7.46 (m, 1H), 6.98 (d, J = 8.9 Hz, 2H), 5.16 (s, 2H), 5.03 (s, 2H), 3.53 - 3.63 (m, 4H), 3.24 (s, 3H), 3.11 (t, J = 4.9 Hz, 2H), 3.08 (s, 3H), 3.04 (t, J = 5.1 Hz, 2H), 2.05 (s, 3H); HRMS (ESI, positive) calcd for $C_{27}H_{30}F_3N_7O_3SNa$ $[M+Na]^+$ 612.1981, found 612.1990.

27. Cellular antiproliferation assay. Lung cancer cells (A549), glioblastoma cells (U87-MG), cervix adenocarcinoma cells (HeLa), ovarian carcinoma cells (OVCAR-3), and hepatocellular carcinoma cells (Hep G2) were obtained from ATCC (Manassas, VA).

Stock cultures were grown in 5 mL RPMI 1640 supplemented with 5% fetal bovine serum. Extracted cells in 96-well plates (100 µL cells/well) were exposed to different sample concentrations in DMSO/RPMI. And then, they are incubated at 37 ^{o}C with 5% CO_2 for 2 days. Afterwards, cells were fixed with 50% trichloroacetic acid and cell proliferation was estimated by spectrophotometric quantification (540 nm wavelength) of cellular protein content. The measurements were obtained three times (time zero, 48 hr post-incubation for compound free, and tested cells) and concentration-response curve for each cell line was plotted through an equation. For the concentration-response curve for each cell line, TGI (concentration that produces 100% of cell growth inhibition) value was determined.

Design, synthesis, and biological evaluation of novel aminopyrimidinylisoindolines as AXL kinase inhibitors

Min Jung Choi[a,d], Eun Joo Roh[a], Wooyoung Hur[a], So Ha Lee[a], Taebo Sim[a], Chang-Hyun Oh[b], Sun-Hwa Lee[c], Jong Seung Kim[d], Kyung Ho Yoo[a,*]



1a-w

1u: R = (4-acetylpiperazin-1-yl)phenyl

Enzymatic Assay

IC_{50} = < 0.00050 μM (AXL)

IC_{50} = 0.025 μM (MER)

IC_{50} = 0.050 μM (TYRO3)

Cell-based Assay

GI_{50} = 0.10 μM (MV4-11)

Highlights

- Six compounds showed potent inhibitory activities against AXL kinase
- Most compounds showed good antiproliferative activities against HeLa cell line
- **1u** exhibited extremely excellent efficacy (IC_{50} = < 0.00050 μM) against AXL kinase
- **1u** showed the best combination of enzyme inhibitory and antiproliferative activities