Design and Synthesis of Novel 2,4-Diamino-5-pyrazol-4-yl Pyrimidine Derivatives as Selective Tyro3 Kinase Inhibitors

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The TAM (Tyro3, Axl, Mer) is a small family of kinase which was initially identified and cloned as a full-length Tyro3 by Crosier *et al*.[1] The TAM receptor tyrosine kinases and their cognate ligands GAS6 (growth arrest-specific 6) and Pros1 (protein S) play an important role in the processes of chronic inflammatory[2] and autoimmune diseases.[3] Knockouts of TAM receptors develop autoimmune diseases, including rheumatoid arthritis and lupus.[4] Moreover, the TAM family of receptor tyrosine kinase and Gas6 are aberrantly overexpressed in multiple hematological and epithelial malignancies in solid tumor.[5] Although, the role of Gas6/TAM receptor in solid tumors is not fully understood, Gas6/TAM expression was shown to be higher in tumor tissue, and the correlation to survival was not confirmed for different tumors.[6] Of the TAM receptor, Tyro3 is less studied and recently identified as Tyro3 expression decreases survival of malignant melanoma cell.[7] Expression level of Tyro3 has been evaluated in Gas6-mediated Akt-phosphorylation, and Tyro3 was specifically overexpressed in numerous melanoma cell lines.[7] Short-hairpin RNA-mediated knockdown of Tyro3 led to significant cell death via apoptotic mechanism regardless of BRAF mutation or NRAS mutation status in melanoma cell lines, which reflect the strong possibility for the efficient therapeutics in resistant or refractory melanoma patients.[7] Recently, it has been reported that Tyro3 is being proposed as a drug target for breast cancer, colorectal cancer, multiple myeloma and lung carcinoma.[8] It has been reported that Tyro3 was emerged as a potential therapeutic target in breast cancers especially for estrogen receptor positive/HER2 (human epidermal growth factor receptor 2)-non-amplified (luminal type) and estrogen negative/Her2 amplified (HER2 type cell) not triple negative cells by Tyro3 knockdown experiment.[9] Furthermore, Tyro3 was strongly upregulated in the tumor tissue of 42% of patients, and Tyro3 was deeply correlated with the key marker AFP (alpha-fetoprotein) and ALT (alanine aminotransferase) in hepatocellular carcinoma.[10]

Thus, the development of inhibitors against TAM receptors, particularly for Tyro3 selective inhibitor,[11] has been considered as a possible methodology to overcome the side effects[5,12] of TAM receptor inhibition and improve resistance from anticancer agents. To date, a number of Tyro3 inhibitors have been identified and being investigated in biological testing or in the early stage of preclinical trial. (Figure 1) Among the compounds, LDC1267 (Max-Planck-Gesellschaft, Munich, Germany) displayed strong potency against Axl, Tyro3, and Mer kinases. UNC569 (University of North Carolina, Chapel Hill, NC, USA) was observed in some degree of selectivity against Tyro3 over Axl, and Mer kinase. **1** and **2** (Pfizer, New York, NY, USA) were identified and developed as highly selective pyrimidine derivatives against Tyro3.[11]

In this report, we synthesized a series of novel *N*-cyclohexylpyrimidin-4-amine derivatives that were substituted with aniline moieties and evaluated structural-activity relationship (SAR) studies for TAM kinase inhibitory activity by me-too approach focused on the structures **2** (Figure 1). Some of the compounds displayed excellent selectivity toward Tyro3 *in vitro* enzyme assay. The general synthetic methods adopted in the preparation of *N*-cyclohexyl-5-pyrazol-4-yl-phenylpyrimidin-2,4-diamine derivatives are outlined in Scheme 1.[11]

Starting from a 5-bromo-2,4-dichloropyrimidine, 4-cyclohexylamino-5-bromo-2-chloropyrimidine (**3**) was obtained by

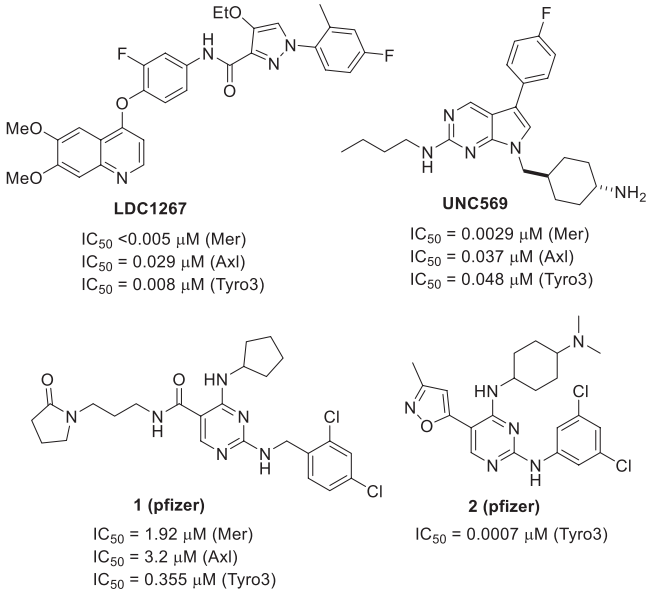
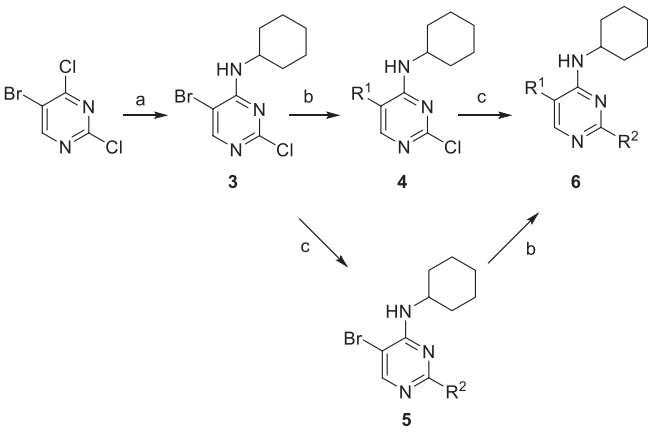


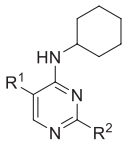
Figure 1. TAM inhibitors.[1]

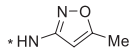
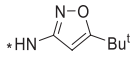
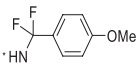
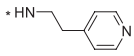
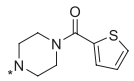


Scheme 1. Synthesis of N^4-cyclohexyl-5-aryl-N^2-arylpyrimidine-2,-4-diamine. Reagents and conditions: (a) cyclohexylamine, DIPEA, DMF, rt., 18 h, 65%; (b) $R^1B(OH)_2$, $Pd(dppf)_2Cl_2$, K_2CO_3, DMF, 80 °C, 98%; (c) R^2NH_2, Et_3N, CH_2Cl_2, rt., 18 h, 39%.

simple substitution of cyclohexylamine in the presence of diisopropylamine in DMF. Palladium catalyzed cross coupling of **3** with the appropriate arylboronic acid yielded 4-cyclohexylamino-5-aryl-2-chloropyrimidines (**4**).[11] Substitution of suitable arylamine to compound (**4**) afforded the final 2-arylamin-4-cyclohexylamino-5-arylpyrimidine derivatives **6**. Our initial structural-activity relationship studies in 2,4-diaminopyrimidine-5-aryl series demonstrated that highly selective Tyro3 compound can be derived from the modification of aryls at 4-position and aryl amine functional group at the 2-position. In an effort to secure excellent selectivity toward Tyro3 other than Axl or Mer kinases, we explored and optimized *in vitro* enzyme assay by structural modification of parent 2,4-diamine substituted pyrimidines. To understand the SAR of 2,4-disubstituted pyrimidines, all the compounds were tested *in vitro* enzyme assay ["Experimental procedure for enzyme assay against TAM receptors (Tyro3, Axl, Mer kinases)" in Appendix S1 (Supporting information)] toward Tyro3, Axl, and Mer kinases, respectively. The results were summarized in Table 1. Bromo compound (**5a**) displayed low degree of inhibitory activity in Axl. Introduction of 3,5-dichlorophenylamino at 2 position of pyrimidine, compound **5b**, micromolar range of inhibitory activity toward Tyro3 was observed. Interestingly, introduction of 4-methylphenylamino both at 2 and 4 positions, compound (**6a**), resulted in highly potent inhibitory activity toward Axl kinase. When we introduced methylpyrazole group at 5 position of pyrimidines instead of phenyl group, no inhibitory activity toward Tyro3 was observed with compound (**4a**)**,** which has chlorine at 2 position of pyrimidine. We decided to introduce aromatic groups at 2 position, such as 5-methylisoxazol-3-amine, that resulted in low degree of inhibitory activity (**6b**). In contrast, introduction of 5-(*tert*-butyl)isoxazol-3-amine at 2 position of pyrimidine (**6c**), slight increase of inhibitory activity against Axl and Mer was observed with no inhibitory activity toward Tyro3. Introduction of benzylamine group **(6d)** at 2 position resulted in increase of Tyro3 and Axl inhibitory activity with no activity in Mer kinase. On the contrary, introduction of polar substituent 2-(4-pyridyl)ethylamine group (**6f**) displayed results with the inhibitory activity in Axl and Tyro3 with no enzymatic activity in Mer kinase inhibition. In case of piperazin-1-yl(thiophen-2-yl)methanone (**6g**) at the same position of pyrimidine, selectivity and inhibitory activity were almost equivalent to that of compound **6d**. More potent inhibitory activity against Mer tyrosine kinase was observed with trifluoromethylbenzylamine group **(6e)** at 3 position of phenyl substituent with moderate activity in Tyro3 and Axl kinases. We next decided to introduce phenylamine instead of heterocyclic aromatic amine group. In this result, inhibitory activity and selectivity toward Tyro3 were dramatically increased with high degree of inhibitory activity against Tyro3 enzyme. Introduction of phenylamine **(6h)** secured selectivity toward Tyro3 with micromolar inhibitory activity except Axl and Mer enzymes. Enhancement of inhibitory activity was observed in the introduction of 3-chlorophenylamine **(6i)** with no inhibitory activity in Axl and low micromolar range of Mer kinase. Introduction of 3,5-dichlorophenylamino group at 2-position of pyrimidine (**6j**) displayed excellent selectivity over Axl and Mer kinases retaining strong inhibitory potency against Tyro3 kinase. Substitution of 3-chloro-4-methoxyphenylamino group (**6k**), 2-methylphenylamino group (**6l**), and 2-isopropylphenylamino group (**6m**) were detrimental to the inhibitory activity toward all of TAM kinases. Moderate inhibitory activity against Tyro3 and Mer kinases was observed in compound (**6n**) of 3-methylphenylamino group with no activity in Axl kinase. Introduction of 3-furyl (**6o**) and (**6p**) instead of pyrazol of compound was turned out to be little beneficial to inhibition of TAM kinases. In order to assess the binding mode of **6j** on Tyro3 kinase, we performed flexible docking study using Schrödinger Suite 2018–1 ("Molecular modelling study for 6j" in Appendix S1). Pyrimidin-2-amine scaffold of **6j** made hydrogen bonding with Met596 carbonyl and amide

Table 1. Results of TAM (Tyro3, Axl, and Mer) inhibition.



Entry	R^1	R^2	Tyro3 IC_{50} (μM)	Axl IC_{50} (μM)	Mer IC_{50} (μM)
4a		Cl	>10	8.5	>10
5a	Br	p-tolylamino	>10	2.8	>10
5b	Br	3,5-dichlorophenylamino	0.42	9.1	1.1
6a	p-tolyl	p-tolylamino	>10	0.0015	6.2
6b	a		>10	4.1	0.77
6c	a		>10	2.1	0.4
6d	a		1.1	0.5	>10
6e	a	3-trifluoromethylbenzylamino	0.61	0.52	0.085
6f	a		1.1	>10	0.52
6g	a		1.3	4.7	>10
6h	a	phenylamino	1.4	>10	>10
6i	a	3-chlorophenylamino	0.39	>10	2.4
6j	a	3,5-dichlorophenylamino	0.028	>10	12
6k	a	3-Cl-4-methoxyphenylamino	>10	>10	>10
6l	a	2-methylphenylamino	>10	6.3	1.2
6m	a	2-isopropylphenylamino	>10	>10	>10
6n	a	3-methylphenylamino	0.16	>10	0.11
6o		4-methylphenylamino	>10	>10	>10
6p	b	3,5-dichlorophenylamino	0.62	>10	>10

NH in the hinge region. Also pyrimidine ring formed hydrophobic interactions with Leu514, Ala538, Phe595, and Met652. The 1-methylpyrazole moiety of **6j** was positioned to the inside hydrophobic pocket including Val522, Ala571, Leu593, and Ala662, as well as 3,5-dichlorophenyl group was oriented toward solvent exposed area by forming hydrophobic interactions with Leu514. In addition, the orientation of cyclohexylamine sidechain was similar to that of 3-aminopropyl substituent of inhibitor bound to Tyro3 kinase. As shown in Figure S1 (Appendix S1), the proposed binding model of **6j** has similar binding mode to that of X-ray co-crystal structure in complexed with 2,4-diaminopyrimidine-5-carboxamide analog.

In summary, we synthesized a novel series of 2,4-disubstituted pyrimidine with aromatic amine and 5 position of pyrimidine with aryl group, such as pyrazole group and assayed TAM kinases, Tyro3, Axl, and Mer kinases *in vitro* enzyme assay. This effort resulted in the identification of analog **6j** as a potent Tyro3 kinase inhibitor with excellent selectivity over other TAM family kinase, such as Axl and Mer. Besides, compound **6a** displayed excellent inhibitory activity against Axl tyrosine kinase and selectivity over Tyro3 and Mer kinase. We found that it is possible to identify selective either Tyro 3 or Axl kinase by simple structural modification of 2,4,5-trisubstituted pyrimidines. Further studies on the development of potent and selective Tyro3 inhibitors and biological studies are being planned.

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Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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