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3-Cyano-6-(5-methyl-3-pyrazoloamino)pyridines: Selective Aurora A kinase inhibitors

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ABSTRACT

A new class of Aurora A kinase inhibitor was created by transforming 4-(5-methyl-3-pyrazoloamino)pyrimidine moiety of VX-680 to 3-cyano-6-(5-methyl-3pyrazoloamino)pyridine. Compound **6** exhibited a potent Aurora A kinase inhibitory activity, excellent selectivity to Aurora B kinase and other 60 kinases, good cell permeability and good PK profile. Therefore compound **6** was effective in antitumor mice model at a dose of 30 mg/kg po qd without decrease of body weight.

The Aurora kinase is a family of highly conserved serine/threonine protein kinases[1] found to be involved in multiple mitotic events.[2] There are three homologs in human. Aurora A and Aurora B are ubiquitously present in various cells, whereas Aurora C is highly expressed only in testis.[3]

Aurora A or Aurora B kinase is one of attractive targets for cancer therapy. Some Aurora kinase inhibitors have proven effective on a wide range of tumor types in clinical trials.[4] These clinical data are very encouraging and promising for development of structurally different Aurora kinase inhibitors.

The most widely studied Aurora kinase inhibitor would be VX-680 (MK-0457) (Fig. 1) which inhibits Aurora A, B, and C kinases with K_i values of 0.6, 18, and 4.6 nM, respectively.[5] It also inhibits FLT-3 (fms-related tyrosine kinase 3), which is also attractive as antitumor target, with a K_i of 30 nM, but it shows good selectivity against other protein kinases.[5,6] One drawback of VX-680 is poor bioavailability. So it was administrated by iv infusion.[7]

VX-689 (MK-5108) is a successor of VX-680 by Vertex and Merck group (structure is unknown). It is administrated as po formulation. ENMD-2076 (Fig. 1) is developed as an orally active analogue of VX-680. IC_{50} values against Aurora A and Aurora B kinase inhibition are 14 and 290 nM, respectively, but this is a non-selective kinase inhibitor.[8]

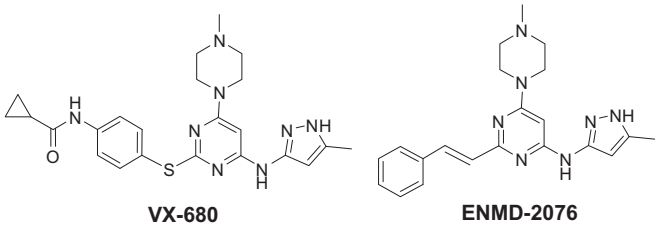
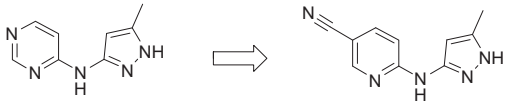


Figure 1. Structures of VX-680 and ENMD-2076.

We report herein design and synthesis of a new class of orally active Aurora A kinase inhibitor by transformation of core structure of VX-680.

We focused on a bioisoster structure of 4-(5-methyl-3-pyrazoloamino)pyrimidine moiety of VX-680. In our hypothesis, 5-methyl-3-pyrazoloamino moiety and near by one nitrogen atom of pyrimidine ring was essential to form multiple hydrogen-bonds with main-



Scheme 1. Transformation of core structure of VX-680.

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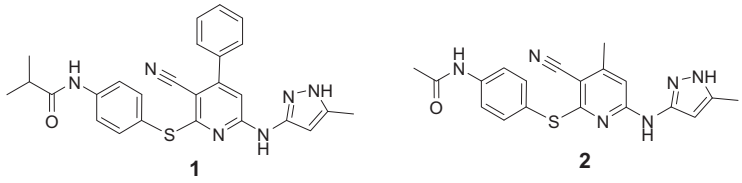


Figure 2. Structures of lead compounds.

chain amide residues of E211 and A213 at the hinge region of the kinase.[9,10] So we designed 3-cyano-6-(5-methyl-3-pyrazoloamino)pyridine as a new core structure in the point of electronically similar property of six-membered aromatic ring (Scheme 1).

As first specific compound, we designed compound 1 (Fig. 2); the substituent at 4-position of pyridine ring was phenyl group, not 4-methyl-1-piperazinyl group as seen in VX-680 or ENMD-2076, because of easiness of synthesis.[11]

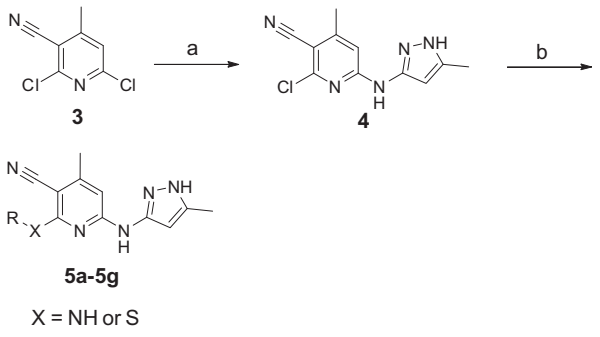
A K_i value of compound **1** against Aurora A kinase inhibition was 310 nM.[12] It was about 150 times less potent than VX-680 whose K_i value was 2.0 nM by our assay system, but we thought that 310 nM was potent enough as a new lead compound. One of problems of compound **1** was a high *C* log *P* value (6.03).

Further transformation of compound **1** encouraged us. Namely, replacement of 4-phenyl moiety of pyridine ring to methyl group improved inhibitory activity. For example, a K_i value of compound **2** (Fig. 2) was 5.3 nM. This is great advancement because the inhibitory activity was markedly improved, the starting compound 3-cyano-2,6-dichloro-4-methylpyridine **3** was commercially available, synthesis of derivatives is very easy, and *C* log *P* value became 3.94 which was much preferable as a lead compound to 6.03 of compound **1**.

Thus, we synthesized various derivatives of compound **2**. Firstly, reaction of compound **3** with 3-amino-5-methylpyrazole in the presence of diisopropylethylamine in DMSO afforded an intermediate **4** in 60% yield by substitution of the chlorine atom at 6-position of pyridine ring. Secondly, substitution reaction of the chlorine atom of compound **4** with nucleophiles such as alkylamines or alkylthiols in DMSO gave designed compounds **5a–5g** (Scheme 2).

We introduced various types of substituted alkyl moiety as R–X–, and typical results are summarized in Table 1. As a result of transformation of first step (**5a**–**5e**), hydroxyalkyl groups, especially 6-hydroxyhexyllthio (**5b**) or 6-hydroxyhexylamino (**5e**) group gave good results in the points of inhibitory activity against Aurora A kinase, cell permeability, and physiochemical properties. However, pharmacokinetic (PK) profile of these two compounds was very poor after po administration in rats.

We presumed that one of its reasons was rapid metabolism of the primary alcohol moiety. So we transformed the primary alcohol moiety to tertiary alcohol (**5f** and **5g**) to prevent conjugation of glucuronic acid or oxidation to carboxylic acid followed by β-oxidation. Indeed, their PK profiles were markedly improved. For example, C_{max} and AUC values of compound **5f** were 4000 times and 5700 times as high as those of compound **5b** at 30 mg/kg po in rats.



Scheme 2. Reagents and conditions: (a) 3-amino-5-methylpyrazole, diisopropylethylamine, DMSO, 100 °C, 60%; (b) R-XH, $NaHCO_3$, DMSO, 100 °C.

Table 1
Inhibitory activity against Aurora A kinase

Compound	R–X–	K_i[a] (nM)
5a	$CH_3(CH_2)_5$–*S*–	3.8
5b	$HO(CH_2)_6$–*S*–	8.5
5c	$H_2N(CH_2)_6$–*S*–	28
5d	$HO(CH_2)_2$–NH–	20
5e	$HO(CH_2)_6$–NH–	4.5
5f	$HO(CH_3)_2C(CH_2)_5$–*S*–	9.7
5g	$HO(CH_3)_2C(CH_2)_5$–NH–	2.3

[a] Values are means of three experiments.

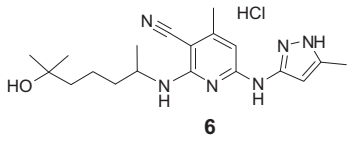


Figure 3. Structure of a representative compound.

An optimized compound in this alkyl series was compound **6**[13] (Fig. 3) which inhibited Aurora A kinase with a K_i value of 1.2 nM,[14] whereas a K_i value against Aurora B kinase inhibition was 101 nM. This compound exhibited good kinase selectivity. We examined inhibitory activity against typical 68 kinases, and IC_{50} values against 60 kinases were over 1000 nM.[15] Compound **6** also exhibited good cell permeability. It inhibited proliferation of HCT116 cells with an IC_{50} value of 115 nM.

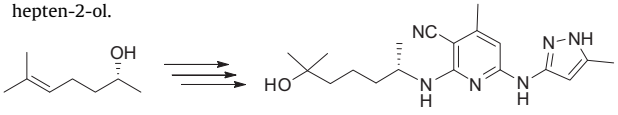
Compound **6** showed good PK profile; C_{max} value was 4930 nM (T_{max} = 1.2 h) and serum concentration after 24 h was 52 nM ($T_{1/2}$ = 3.3 h) at 30 mg/kg po in rats. So we tested antitumor activity in HCT-116 subcutaneous Xenograft model in mice. Compound **6** was administrated at 30 mg/kg po qd. Growth of tumor was inhibited by 59% after 14 days;[16] body weight was comparable to that of control mice at this dose during all period administrated.

In summary, we have created a new class of Aurora A kinase inhibitor by transforming the 4-(5-methyl-3-pyrazoloamino)-pyrimidine moiety of VX-680 to 3-cyano-6-(5-methyl-3pyrazoloamino)pyridine. Various substituted alkylthio or alkylamino group were introduced in its 2-position of pyridine ring. The optimized compound in this alkyl series (compound **6**) exhibited a potent Aurora A kinase inhibitory activity, excellent selectivity to Aurora B kinase and other 60 kinases, good cell permeability and good PK profile. Therefore compound **6** was effective in antitumor mice model at a dose of 30 mg/kg po qd without decrease of body weight.

References and notes

1. Glover, D. M.; Leibowitz, M. H.; McLean, D. A.; Parry, H. *Cell* **1995**, *81*, 95.
2. Fu, J.; Bian, M.; Jiang, Q.; Zhang, C. *Mol. Cancer Res.* **2007**, *5*, 1.

3. Lin, Y.; Su, L.; Yu, C. R.; Wong, F.; Yeh, H.; Chen, S.; Wu, J.; Lin, W.; Shiue, Y.; Liu, H.; Hsu, S.; Lai, J.; Huang, C. F. *Gene Expr.* **2006**, *13*, 15.
4. Pollard, J. R.; Mortimore, M. *J. Med. Chem.* **2009**, *52*, 2629.
5. Harrington, E. A.; Bebbington, D.; Moore, J.; Rasmussen, R. K.; Ajose-Adeogun, A. O.; Nakayama, T.; Graham, J. A.; Demur, C.; Hercend, T.; Diu-Hercend, A.; Su, M.; Golec, J. M. C.; Miller, K. M. *Nat. Med.* **2004**, *10*, 262.
6. Carter, T. A.; Wodicka, L. M.; Shah, N. P.; Velasco, A. M.; Fabian, M. A.; Treiber, D. K.; Milanov, Z. V.; Atteridge, C. E.; Biggs, W. H., III; Edeen, P. T.; Floyd, M.; Ford, J. M.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Mehta, S. A.; Patel, H. K.; Pao, W.; Sawyers, C. L.; Varmus, H.; Zarrinkar, P. P.; Lockhart, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11011.
7. Wang, Y.; Serradell, N. *Drugs Future* **2007**, *32*, 144.
8. Bray, M. R. In Proceedings of the 99th AACR Annual Meeting, San Diego, CA, Apr 12–16, 2008; American Association for Cancer Research: Philadelphia, PA, 2008.
9. There was no report regarding X-ray co-complex structure of VX-680 bound to Aurora A kinase when our project started.
10. Cheetham, G. M. T.; Charlton, P. A.; Golec, J. M. C.; Pollard, J. R. *Cancer Lett.* **2007**, *251*, 323.
11. All new compounds were fully characterized.
12. Recombinant Aurora A kinase was prepared using gene from Hela cells (ATCC CCL-2). Inhibition of phosphotransferases activity was measured by using peptide substrate kemptide and [γ-32P]ATP in a usual manner for measurement of kinase activity inhibition. A K_m value of kemptide was 28 μM.
13. Mp 177–179 °C; IR 3246, 2966, 2930, 2199, 1637, 1582, 1538, 1451, 1361, 1204, 763, 730 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.04 (s, 6H), 1.20 (d, J = 6.5 Hz, 3H), 1.30–1.50 (m, 5H), 1.64 (m, 1H), 2.24 (s, 3H), 2.28 (s, 3H), 4.13 (m, 1H), 6.20 (s, 1H), 6.28 (s, 1H), 6.76 (s, 1H), 10.54 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 10.736, 20.203, 20.731, 29.107, 29.235, 36.377, 43.369, 47.119, 68.610, 79.933, 94.817, 98.711, 117.227, 141,635, 145.995, 153.232, 153.845, 157.527. Anal. Calcd for $C_{19}H_{28}N_6O$·HCl: C, 58.08; H, 7.44; N, 21.39. Found: C, 57.79; H, 7.47; N, 21.21.
14. Racemate **6** was separated using chiral preparative HPLC (CHIRALPAK AD-H, Daicel Chemical Industries). K_i values of eutomer and distomer against aurora A kinase inhibition were 0.59 and 66 nM, respectively. The eutomer inhibited proliferation of HCT116 cells with an IC_{50} value of 52 nM. Intrinsic clearance (CL_{int}) in human hepatic microsomes of the racemate, the eutomer, and the distomer were 0.07, 0.05, and 0.11 mL/min/mg, respectively. Absolute configuration of the eutomer was (*S*)-form which was determined by chemical synthesis via an alternative route starting from (*R*)-6-methyl-5-hepten-2-ol.



eutomer

15. The less selective kinases were SRC-family; K_i values against yes, cSRC, and Fyn inhibition were 24, 26, and 28 nM, respectively.
16. Growth inhibitions by VX-680 (30 and 100 mg/kg ip qd) after 14 days were 60% and 68%, respectively.