Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1623–1627
Bioorganic & Medicinal Chemistry Letters

Aurora kinase A inhibitors: Identification, SAR exploration and molecular modeling of 6,7-dihydro-4*H*-pyrazolo-[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione scaffold

Mohane Selvaraj Coumar,[a] Jian-Sung Wu,[a,†] Jiun-Shyang Leou,[a,†] Uan-Kang Tan,[b] Chung-Yu Chang,[a] Teng-Yuan Chang,[a] Wen-Hsing Lin,[a] John T.-A. Hsu,[a,c] Yu-Sheng Chao,[a] Su-Ying Wu[a,*] and Hsing-Pang Hsieh[a,*]

[a]*Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, No. 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, ROC*
[b]*Department of Chemical and Materials Engineering, Technology and Science Institute of Northern Taiwan, Taipei 112, Taiwan, ROC*
[c]*Department of Chemical Engineering, National Tsing Hua University, Hsinchu 300, Taiwan, ROC*

Received 23 October 2007; revised 12 January 2008; accepted 16 January 2008
Available online 19 January 2008

Abstract—Tricyclic 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione was identified as a novel scaffold for Aurora kinase A inhibition through virtual screening. SAR exploration coupled with molecular modeling of **8a** reveals the minimum pharmacophore requirements for Aurora kinase A inhibition.

Inhibition of Aurora kinase, a member of serine/threonine kinase involved in the regulation of cell division is emerging as a new molecular targeted cancer treatment option.[1–4] Three isoforms of Aurora kinase, A, B, and C are known, all of which have a conserved ATP binding site, but differ in amino acid length and sequence at the *N*-terminal domain.[3,5] Aurora A localizes on centrosomes from early S phase and is involved in centrosome maturation and separation, bi-polar spindle assembly, mitotic entry, and exit. Aurora B is a chromosome passenger protein, which localizes to the kinetochores from prophase to metaphase and to the central spindle and midbody during cytokinesis. It is essential for accurate chromosomal segregation, protein localization to the centromere and kinetochore, and for proper cytokinesis to take place. Aurora C co-localizes and complements the functions of Aurora B.[3,5]

More importantly, both Aurora A and B are overexpressed in many human cancers and are linked to chromosome instability, oncogenic transformation, tumor progression, and development of chemoresistance.[6,7] Inhibitors of Aurora kinase, regardless of their Aurora isoform specificity have shown to promote cancer cell death by induction of apoptosis and mitotic catastrophe. A handful of Aurora inhibitors are in various stages of development,[4] among them MK-0457/VX-680[8–10] (**1**), AZD1152[11,12] (**2**), and PHA-739358[13] (**3**) are in clinical development (Fig. 1). VX-680 is a potent inhibitor of all three Aurora kinases with K_i values of 0.6, 1.8, and 4.6 nM for A, B, and C isoforms, respectively.[9] It is undergoing clinical trials for solid tumors and hematological malignancies. AZD1152 is a quinazoline prodrug with selective Aurora kinase B/C inhibition profile. PHA-739358 is a non-selective agent like VX-680, with IC_{50} values of 13, 79, and 61 nM for Aurora A, B, and C, respectively.[13]

Based on the current success of Aurora kinase inhibitors in the development of kinase-based cancer therapy, we have initiated a virtual screening program for the identification of Aurora kinase inhibitors. Through virtual screening[14] of around 60,000 compound library,[15] we have identified a few potential hits with high priority scores. These compounds were purchased and assayed for Aurora kinase A inhibition. The most promising hit in terms of activity and novelty in structure,

Keywords: Aurora kinase inhibitors; Cancer; Molecular modeling.
* Corresponding authors. Tel.: +886 37 246166x35713; fax: +886 37586456 (S.-Y.W.); tel.: +886 37 246166x35708; fax: +886 37586456 (H.-P.H.); e-mail addresses: suying@nhri.org.tw; hphsieh@nhri.org.tw
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.068

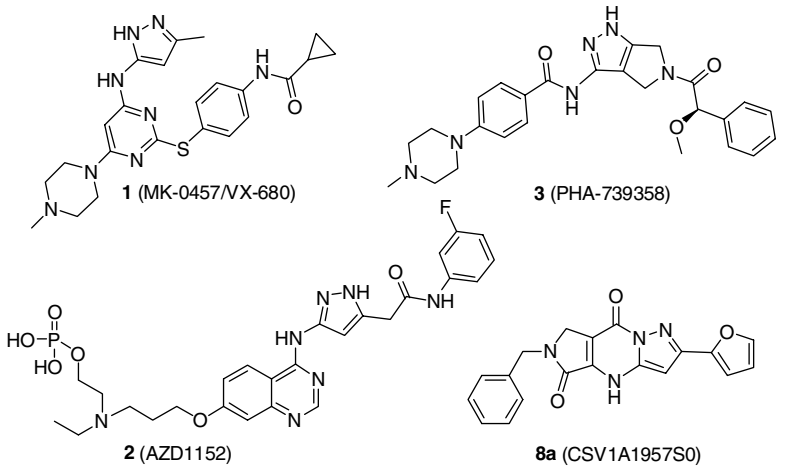


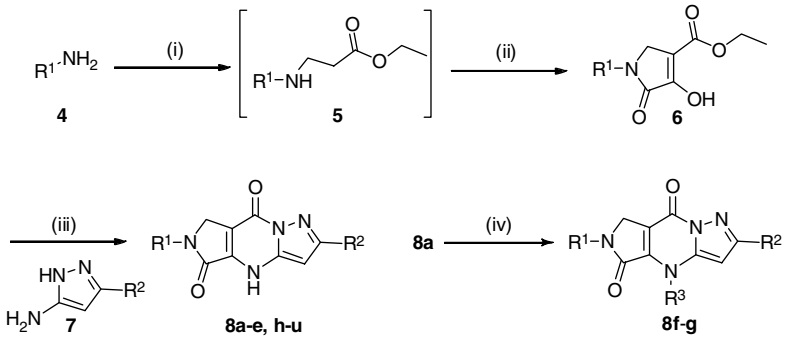
Figure 1. Aurora kinase inhibitors.

CSV1A1957S0 (**8a**) had a low micromolar Aurora A inhibition profiles with 90% inhibition at 50 μM concentration. Literature search showed that the 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione scaffold was reported as inhibitor of acyl CoA: cholesterol acetyl transferase,[16] but as such was unreported as inhibitor for any protein kinases. Considering this fact, the development of this new Aurora inhibitor could reveal interesting insight into the binding modes of **8a** to Aurora kinase A. This paper describes the SAR studies and molecular modeling of 6-benzyl-2-(furan-2-yl)-6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione **8a** for Aurora kinase A inhibition.

The general synthetic method[16] for the preparation of the fused tricyclic compounds is shown in Scheme 1. Commercially available amines **4** were alkylated with ethyl acrylate in absolute ethanol by stirring at RT to give the intermediate amines **5**. In the case of aniline (for **8h** compound), refluxing for 24 h is required for the completion of reaction. Intermediate amines **5** formed were cyclized in-situ to the pyrrolidinones **6** using diethyl oxalate and sodium ethoxide in refluxing ethanol. Condensation of pyrrolidinones **6** with various substituted pyrazoles **7** in refluxing glacial acetic acid gave the final tricyclic compounds **8a**–**e**, **h**–**u** in 20–25% overall yields after crystallizing from methanol–DMSO mixture. Compounds **8f**–**g** were synthesized from **8a** by alkylating with methyl iodide or 4-(2-chloroethyl)-morpholine hydrochloride using potassium carbonate. The structures of all the synthesized compounds were confirmed by ^{1}H NMR and LC–MS analysis.

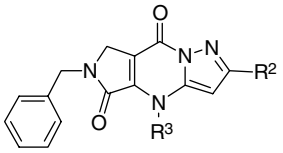
Aurora kinase A inhibition assay[17] was performed at two compound concentrations of 50 and 10 μM. For compounds with a good activity profile, IC_{50} was also determined. The original virtual screening hit **8a**, showed an IC_{50} of about 15 μM for Aurora A inhibition (Table 1). Initial attempts were focused on the replacement of furan ring (R^2 group), but either a thiophene (**8b**) or phenyl ring (**8c**) led to a decrease in activity. Complete removal of furanyl group (**8e**) or replacement with methyl group (**8d**) led to a dramatic loss of enzyme inhibition activity. This SAR trend clearly shows that presence of heterocyclic group (R^2 group) is essential for maintaining contact with the Aurora kinase. In the central ring NH group, substitution with either methyl (**8f**) or 4-ethyl morpoline group (**8g**) led to complete loss of kinase inhibition, suggesting the importance of free NH group for binding to Aurora kinase.

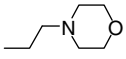
Next, we turned our attention to the pyrrolidinone ring substitution (R^1 group; Table 2). Decreasing the linker length that separates the phenyl ring from the tricyclic core by one-carbon (**8h**) or increasing the length to two- (**8i**) or three-carbon (**8j**) led to decreased activity.



Scheme 1. Reagents and conditions: (i) ethyl acrylate, ethanol, rt, 12 h; (ii) diethyl oxalate, sodium ethoxide, ethanol, reflux, 3 h; (iii) gl. acetic acid, reflux, 2 h; (iv) methyl iodide or 4-(2-chloroethyl)-morpholine hydrochloride, K_2CO_3, dichloromethane.

Table 1. Inhibition of Aurora kinase A by compounds **8a–g**



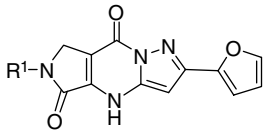
Compound	R^2	R^3	% inhibition of Aurora kinase A[a]	
			at 50 μM	at 10 μM
8a		H	90.2	35.6 (IC_{50} 15.1 μM)
8b		H	72.3	29.4
8c		H	47.2	25.9
8d	CH_3	H	20.8	1.8
8e	H	H	14.5	1.6
8f		CH_3	3.4	2.3
8g			1.1	0.0

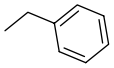
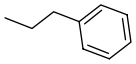
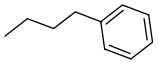
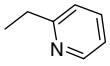
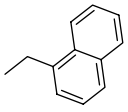
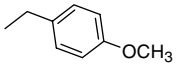
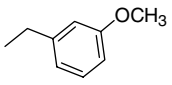
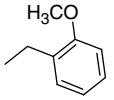
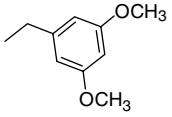
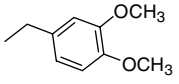
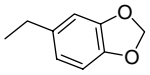
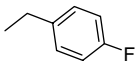
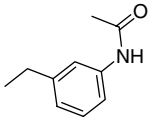
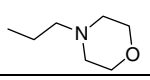
[a] Values are expressed as the mean of three independent determinations and are within ±15%.

Changing the phenyl ring to pyridyl (**8k**) or naphthyl ring (**8l**) system also has no positive effect on activity. As benzyl group was found optimal, the effects of substituents on the phenyl ring were investigated. Introduction of electron-donating substitution such as a methoxy group at three different positions (**8m–o**) of the phenyl ring led to the retention of activity. Of particular interest was the ortho substituted compound **8o**, which showed an enhanced activity with almost threefold improvements in IC_{50} of about 5 μM when compared to the unsubstituted lead compound **8a**. The meta substituted compound **8n** showed the lowest activity among the three compounds. When an additional methoxy substituent was introduced the activity level declined as in the case of **8p** and **8q**. Also, when a dimethoxy equivalent methylene dioxy group (**8r**) was introduced the activity level declined. On the other hand, introduction of electron-withdrawing substituent such as a fluoro substitution at para position (**8s**) led to a decreased activity; while acetamido group (**8t**) in the meta position retained the activity. In contrast to all of the above modifications, when the hydrophobic benzyl group of **8a** was replaced with a hydrophilic 4-ethyl morpholine group, the resulting compound **8u** completely lost the activity, showing that the binding region for this part is hydrophobic in nature and hydrophilic substitution may not be tolerated. Additionally, the two-carbon linker between the morpholine group and the tricyclic core could have contributed to the complete loss of activity, as one-carbon linker was found to be optimal (**8a** vs **8i**).

In order to understand the observed SAR trend and to study the possible binding modes of this class of compounds to Aurora kinase A, docking studies were car-

Table 2. Inhibition of Aurora kinase A by compounds **8h–u**



Compound	R^1	% inhibition of Aurora kinase A[a]		IC_{50} (μM)
		at 50 μM	at 10 μM	
8a		90.2	35.6	15.1
8h		73.7	7.6	—
8i		83.9	19.6	—
8j		84.4	16.4	—
8k		79.2	21.6	—
8l		79.9	30.4	—
8m		88.2	42.4	8.0
8n		90.0	29.3	15.2
8o		95.1	51.6	5.6
8p		75.3	10.1	—
8q		70.8	7.8	—
8r		75.2	12.3	—
8s		89.8	24.0	—
8t		87.8	35.8	18.3
8u		10.7	0.9	—

[a] Values are expressed as the mean of three independent determinations and are within ±15%.

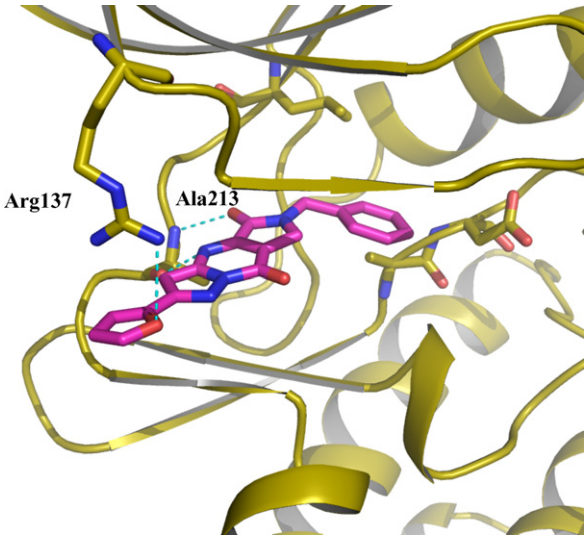


Figure 2. Docking model of **8a** in Aurora A protein.

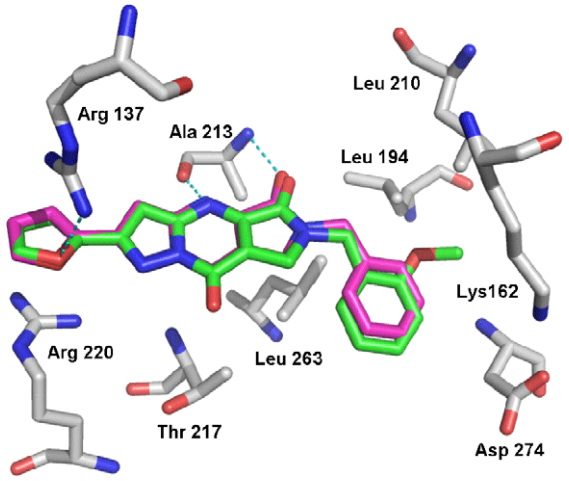


Figure 3. Superposition of the docking model of **8a** (magenta) with **8o** (green) in Aurora A protein.

ried out. Compound **8a** was first docked into the active site/ATP binding site of the Aurora kinase A by Gold 3.0 (CCDC Software Limited, Cambridge, UK) and then subjected to extensive docking by Glide 4.5 (Schrodinger, L.L.C., New York, USA) using the structure of Aurora A (PDB code: 1MQ4) as the template. The docking model (Fig. 2) showed the tricyclic ring fitted into the hinge region where the free NH group in the central ring formed the H-bond with the carbonyl group of Ala213 and the pyrrolidine ring carbonyl group 'O' atom formed the other H-bond with the NH on the main chain of Ala213. The important H-bond interactions of tricyclic ring with the hinge region could give the explanation for the loss of Aurora activity in **8f** and **8g**, where the central ring free NH group is substituted with methyl and 4-ethyl morpoline groups, respectively, which could interrupt in the hinge binding. Moreover, the tricyclic ring made hydrophobic interactions with the residues Leu139, Gly216, and Leu263. The furanyl ring formed a H-bond with Arg137 and had close contacts with Arg 220. In addition, there would be cation-π interactions between the furanyl aromatic ring and these two Arg residues.[18] The removal of furanyl ring, as seen in **8c**–**8e**, might loose these interactions with the protein thus resulting in the loss of activity. Finally, the phenyl ring of compound **8a** extended into the hydrophobic pocket next to the hinge region and formed hydrophobic interactions with Val147, Leu194, Leu210, Leu263, Ala273, and Asp274. Also, the docking pose of **8a** was compared with that of a known pyrimidine Aurora inhibitor (PDB code: 2NP8). It was found that both of them form two H-bonds with the hinge residue Ala213, and one H-bond with Arg137, while their phenyl groups orient differently in the hydrophobic region of the Aurora kinase.

To understand the improved potency of compound **8o** with an ortho methoxy substituent in the phenyl ring, docking was done following the same procedure as described for compound **8a**. As shown in Figure 3, the tricyclic ring and the furanyl ring of **8o** superimposed well with those of **8a**. The phenyl ring substituted with the methoxy group extended deeper into the hydrophobic pocket as compared with **8a** and the methoxy group had interactions with Lys162, Leu194, and Leu210. The protein–ligand intramolecular energy calculated by InsightII 2000.1 (Accelrys Inc., San Diego, USA) are −67.3 (kcal/mol) and −70.8 (kcal/mol) for compounds **8a** and **8o**, respectively, suggesting that the binding of **8o** with Aurora A is more energetically favorable and consequently leads to the improved potency of **8o**.

In conclusion, from virtual screening we have identified a novel tricyclic ring system showing micromolar Aurora kinase A inhibition. Since it is important and critical to develop Aurora kinase inhibitors possessing novel core structure, development of 6,7-dihydro-4*H*-pyrazolo[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione scaffold, which is unprecedented in the kinase domain is worthwhile and interesting. Molecular modeling of **8a** and **8o** reveals the tricyclic core made the important H-bonding interactions with the hinge region while the furanyl ring and the phenyl ring provided additional H-bond and hydrophobic interactions with the protein. Moreover, the modeling study is consistent with the SAR observed in this series of compounds and gives reasons for the improved potency of **8o**. Further skillful manipulation of the substituents around the central tricyclic core for lead optimization is intensively undergoing.

Acknowledgments

The study was financially supported by National Health Research Institutes and National Science Council (Grant Nos. NSC-95-2113-M-400-001-MY3 and NSC-95-2752-B-007), Taiwan, ROC.

Supplementary data

Superposition of the docking pose of **8a** and known pyrimidine Aurora inhibitor (PDB code: 2NP8); Syn-

thetic procedure and spectral data (^{1}H NMR and LC–MS) of **6a**, **h**–**u** and **8a**–**u**; Bioassay protocol for Aurora kinase inhibition available as supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.068.

References and notes

1. Glover, D. M.; Leibowitz, M. H.; McLean, D. A.; Parry, H. *Cell* **1995**, *81*, 95.
2. Gautschi, O.; Mack, P. C.; Davies, A. M.; Lara, P. N., Jr.; Gandara, D. R. *Clin. Lung Cancer* **2006**, *8*, 93.
3. Matthews, N.; Visintin, C.; Hartzoulakis, B.; Jarvis, A.; Selwood, D. L. *Expert Rev. Anticancer Ther.* **2006**, *6*, 109.
4. Naruganahalli, K. S.; Lakshmanan, M.; Dastidar, S. G.; Ray, A. *Curr. Opin. Investig. Drugs* **2006**, *7*, 1044.
5. Carmena, M.; Earnshaw, W. C. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 842.
6. Katayama, H.; Brinkley, W. R.; Sen, S. *Cancer Metastasis Rev.* **2003**, *22*, 451.
7. Fu, J.; Bian, M.; Jiang, Q.; Zhang, C. *Mol. Cancer Res.* **2007**, *5*, 1.
8. Cheetham, G. M.; Charlton, P. A.; Golec, J. M.; Pollard, J. R. *Cancer Lett.* **2007**, *251*, 323.
9. 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.; Miller, K. M. *Nat. Med.* **2004**, *10*, 262.
10. Young, M. A.; Shah, N. P.; Chao, L. H.; Seeliger, M.; Milanov, Z. V.; Biggs, W. H., 3rd; Treiber, D. K.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J.; Sawyers, C. L.; Kuriyan, J. *Cancer Res.* **2006**, *66*, 1007.
11. Mortlock, A. A.; Foote, K. M.; Heron, N. M.; Jung, F. H.; Pasquet, G.; Lohmann, J. J.; Warin, N.; Renaud, F.; De Savi, C.; Roberts, N. J.; Johnson, T.; Dousson, C. B.; Hill, G. B.; Perkins, D.; Hatter, G.; Wilkinson, R. W.; Wedge, S. R.; Heaton, S. P.; Odedra, R.; Keen, N. J.; Crafter, C.; Brown, E.; Thompson, K.; Brightwell, S.; Khatri, L.; Brady, M. C.; Kearney, S.; McKillop, D.; Rhead, S.; Parry, T.; Green, S. *J. Med. Chem.* **2007**, *50*, 2213.
12. Wilkinson, R. W.; Odedra, R.; Heaton, S. P.; Wedge, S. R.; Keen, N. J.; Crafter, C.; Foster, J. R.; Brady, M. C.; Bigley, A.; Brown, E.; Byth, K. F.; Barrass, N. C.; Mundt, K. E.; Foote, K. M.; Heron, N. M.; Jung, F. H.; Mortlock, A. A.; Boyle, F. T.; Green, S. *Clin. Cancer Res.* **2007**, *13*, 3682.
13. Fancelli, D.; Moll, J.; Varasi, M.; Bravo, R.; Artico, R.; Berta, D.; Bindi, S.; Cameron, A.; Candiani, I.; Cappella, P.; Carpinelli, P.; Croci, W.; Forte, B.; Giorgini, M. L.; Klapwijk, J.; Marsiglio, A.; Pesenti, E.; Rocchetti, M.; Roletto, F.; Severino, D.; Soncini, C.; Storici, P.; Tonani, R.; Zugnoni, P.; Vianello, P. *J. Med. Chem.* **2006**, *49*, 7247.
14. Details of virtual screening will be published in our next communication.
15. Commercially available compound database obtained from the Maybridge Chemical Company (Tintagel, Cornwall, UK).
16. Larsen, S. D.; Spilman, C. H.; Bell, F. P.; Dinh, D. M.; Martinborough, E.; Wilson, G. J. *J. Med. Chem.* **1991**, *34*, 1721.
17. Modified from Ref.: Koresawa, M.; Okabe, T. *Assay Drug Dev. Technol.* **2004**, *2*, 153.
18. Mecozzi, S.; West, A. P., Jr.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10566.