Bioorganic & Medicinal Chemistry Letters 22 (2012) 3589–3593

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, biological evaluation, and molecular docking studies of N,1,3-triphenyl-1H-pyrazole-4-carboxamide derivatives as anticancer agents

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ARTICLE INFO

Article history:
Received 5 March 2012
Revised 30 March 2012
Accepted 13 April 2012
Available online 21 April 2012

Keywords:
N,1,3-triphenyl-1H-pyrazole-4-carboxamide
Aurora-A kinase
Structure–activity relationship
Molecular docking

ABSTRACT

A series of N,1,3-triphenyl-1H-pyrazole-4-carboxamide derivatives have been designed, synthesized and evaluated for their potential antiproliferation activity and Aurora-A kinase inhibitory activity. Among all the compounds, compound **10e** possessed the most potent biological activity against HCT116 and MCF-7 cell lines with IC_{50} values of 0.39 ± 0.06 μM and 0.46 ± 0.04 μM, respectively, which were comparable to the positive control. Compound **10e** also exhibited significant Aurora-A kinase inhibitory activity (IC_{50} = 0.16 ± 0.03 μM). Docking simulation was performed to position compound **10e** into the active site of Aurora-A kinase, in order to get the probable binding model for further study. The results of Western-blot assay demonstrated that compound **10e** possessed good Aurora-A kinase inhibitory activity against HCT116. Based on the preliminary results, it is deduced that compound **10e** with potent Aurora-A kinase inhibitory activity may be a potential anticancer agent.

Cancer is such a class of diseases that a group of cells display uncontrolled growth, invasion, and sometimes metastasis.[1,2] Strategies to block cell division by affecting the mitotic spindle have been a successful area of research for the advancement of cancer drugs for a long time.[3,4]

The Aurora kinases are a family of highly conserved serine/threonine protein kinases that play a key role in regulating many pivotal processes of mitosis and completion of cell division.[5–9] In mammals, three Aurora kinases are found: Aurora-A, Aurora-B and Aurora-C.[10] Aurora A and B, are well known for their distinct roles in regulating mitosis, but the role of Aurora C is still unclear. Aurora A is involved in centrosome maturation and separation, bipolar spindle assembly, and mitotic entry, while Aurora B is essential for accurate chromosome segregation and cytokinesis.[11] Overexpression of Aurora-A kinase contributes to genetic instability and tumorigenesis by disrupting the proper assembly of the mitotic checkpoint complex. Since its discovery, Aurora-A kinase has been identified as a colon-cancer-associated kinase that is overexpressed in a wide range of human tumors such as breast, colorectal, ovarian, as well as glioma.[12–14] Thus, targeted inhibition of Aurora-A kinase has become an attractive therapeutic strategy in cancer therapy, and more than 10 Aurora inhibitors have entered early clinical assessment.[15]

The scaffolds of the known inhibitors of Aurora-A kinase can be divided into four main groups labeled A–D, as shown in Figure 1: (A) contains a core of 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole; (B) contains a core of pyrrolo[2,3-b] pyrimidine; (C) contains a core of quinoline; and (D) contains a core of 2-anilino-diamino-pyrimidine.[16] Given the above information we have designed a series of new compounds by sharing a similar template with scaffold B and C, with the presumption that the pyrazole group is a common nucleus of Aurora-A kinase inhibitors which inhibits Aurora-A kinase by binding to its ATP side.[17]On the other hand, benzamide is an important pharmacophore of natural products and the synthetic precursors to various drugs. A variety of pharmacological activities of benzamide have been reported, including anticancer, anti-inflammatory and anti-fungal activities.[18,19] Given the previous reports, the phenyl group and carboxide group of benzamide template can also closely bind to the ATP side of Aurora-A kinase through the synergistic effect of hydrophobic interaction and π-cation interaction.[20] On that basis, a benzamide moiety was introduced into the pyrazole moiety to construct a *N*-phenyl-1H-pyrazole-4-carboxamide template with the reasoning that this template could react potently with the residues in Aurora-A kinase domain through the hydrophobic interactions. To sum up, in continuation to extend our research on antitumor compounds with Aurora-A kinase inhibitory activity, it is supposed to be worthful to synthesize a series of N,1,3-triphenyl-1H-pyrazole-4-carboxamide derivates as a novel class of Aurora-A kinase inhibitors combined with pyrazole and benzamide considering that they might exhibit synergistic effect in anticancer activities by the inhibition against Aurora-A kinase. Given the suggestions, we directed our research to evaluate their anticancer activities in HCT116 to further study the preliminary mechanism of their role in mitosis.

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http://dx.doi.org/10.1016/j.bmcl.2012.04.066

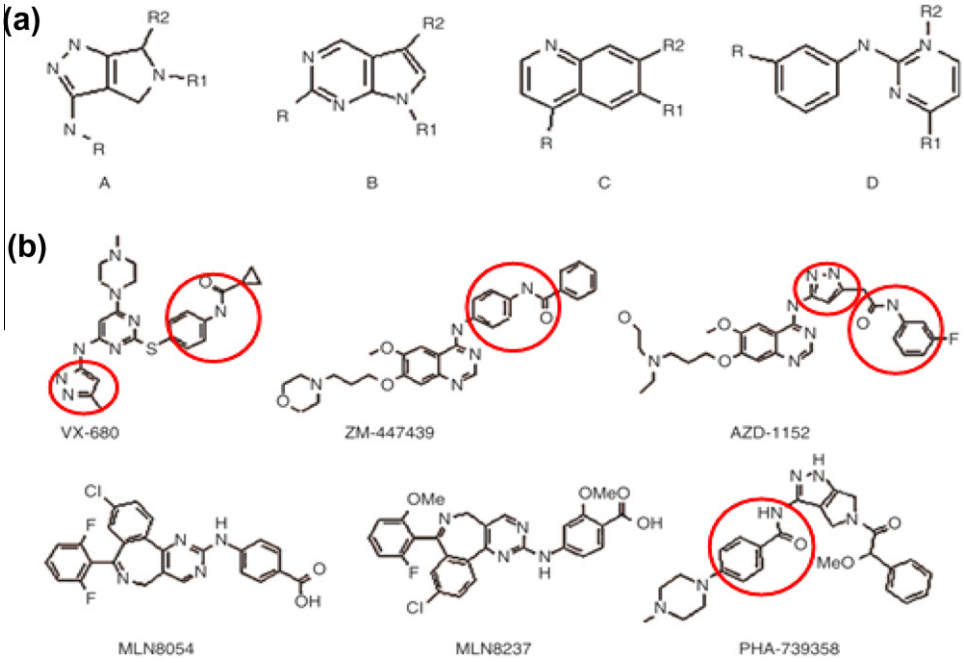
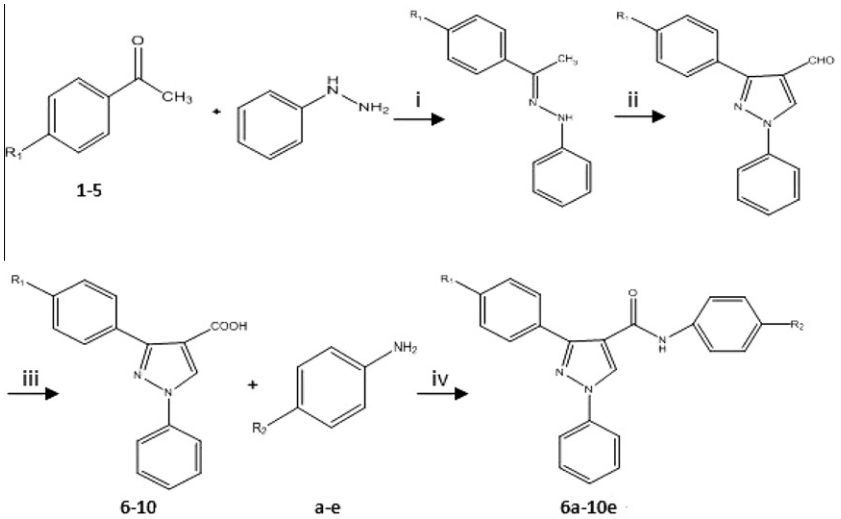


Figure 1. Four main types of scaffolds found in reported ATP competitive inhibitors of Aurora-A kinase and the potent Aurora-A kinase inhibitors under clinical study. **(a)** Four main types of scaffold found in reported ATP competitive inhibitors of Aurora-A kinase. (A) 1,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole scaffold; (B) pyrrolo[2,3-b] pyrimidine scaffold; (C) quinoline scaffold; (D) 2-anilino-diaminopyrimidine scaffold. Here, the groups R, R1 and R2 are variable. **(b)** Examples of potent Aurora-A kinase inhibitors current under clinical study.



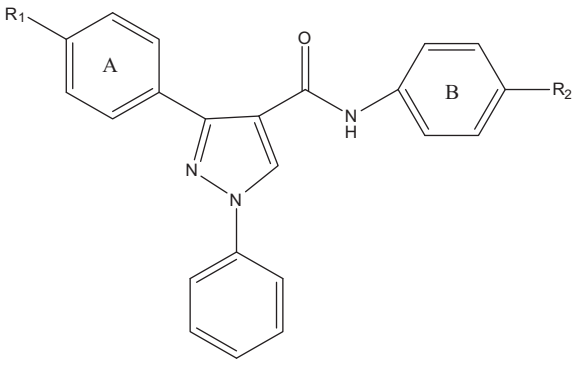
Scheme 1. General synthesis of N,1,3-triphenyl-1H-pyrazole-4-carboxamide derivatives (**6a–10e**). Reagents and conditions: (i) ethanol, 50–60 °C, 3 h; (ii) DMF, $POCl_3$, 50–60 °C, 5 h; (iii) $NaClO_2/NH_2SO_3$, 40–50 °C, 5 h; (iv) DMAP, K_2CO_3, dichloromethane, 50–60 °C, 12 h.

The synthetic route of the N,1,3-triphenyl-1H-pyrazole-4-carboxamide derivatives **6a–10e** is outlined in Scheme 1**.** As reported, the synthesis of N,1,3-triphenyl-1H-pyrazole-4-carboxamide begins with the interaction of substituted 1,3-diphenyl-1H-pyrazole-4-carboxylic and substituted anilines with the help of DMAP and K_2CO_3 in anhydrous methylene dichloride to give the desired compounds.[21] Among these compounds, **7e, 8e, 9b, 9e** and **10a–10e** are reported for the first time. All of the synthetic compounds (Table 1) gave satisfactory elementary analytical and spectroscopic data. ^{1}H NMR and ESI-MS spectra were consistent with the assigned structures.

To test the anticancer activities of the synthesized compounds, we evaluated antiproliferative activities of compounds **6a–10e** against HCT116 and MCF-7 cells. The results were summarized in Table 2. As illustrated in Table 2, the active analogs showed a remarkable potential antitumor activity. Particularly, it should be noticed that compound **10e** showed the most potent biological activity (IC_{50} = 0.39 ± 0.06 μM for HCT116 and IC_{50} = 0.46 ± 0.04 μM for MCF-7), comparable to the positive control VX-680 (IC_{50} = 0.30 ± 0.03 μM for HCT116 and IC_{50} = 0.38 ± 0.02 μM for MCF-7 cells respectively).

From the results listed in Table 2 we can conclude that the activity of the tested compounds may be correlated to structure variation and modifications. By investigating the variation in selectivity of the tested compounds over two cell lines, it was obviously revealed that different substitutes on the B-ring led to different antitumor activities, a comparison of the *para* substituents on the B-ring demonstrated that an electron-donating group have

Table 1
Structure of compounds **6a–10e**



Compound	R^1	R^2	Compound	R^1	R^2
6a	H	H	**8d**	OMe	OMe
6b	H	Cl	**8e**	OMe	OEt
6c	H	Br	**9a**	Cl	H
6d	H	OMe	**9b**	Cl	Cl
6e	H	OEt	**9c**	Cl	Br
7a	Me	H	**9d**	Cl	OMe
7b	Me	Cl	**9e**	Cl	OEt
7c	Me	Br	**10a**	NO_2	H
7d	Me	OMe	**10b**	NO_2	Cl
7e	Me	OEt	**10c**	NO_2	Br
8a	OMe	H	**10d**	NO_2	OMe
8b	OMe	Cl	**10e**	NO_2	OEt
8c	OMe	Br			

Table 2
Inhibition (IC_{50}) of HCT116 and MCF-7 cells proliferation and inhibition of Aurora-A by compounds **6a–10e**

Compound	IC_{50} ± SD (μM)		
	HCT116[a]	MCF-7[a]	Aurora-A[b]
6a	2.31 ± 0.14	2.34 ± 0.17	2.34 ± 0.36
6b	3.52 ± 0.33	3.86 ± 0.21	3.57 ± 0.35
6c	4.47 ± 0.45	5.75 ± 0.32	7.25 ± 0.81
6d	1.35 ± 0.07	1.71 ± 0.08	1.33 ± 0.17
6e	0.62 ± 0.03	0.88 ± 0.04	0.42 ± 0.12
7a	2.36 ± 0.14	2.45 ± 0.09	2.65 ± 0.29
7b	3.88 ± 0.16	4.01 ± 0.10	4.79 ± 0.83
7c	5.35 ± 0.27	6.08 ± 0.24	7.32 ± 0.69
7d	1.45 ± 0.18	1.62 ± 0.04	1.45 ± 0.05
7e	0.78 ± 0.04	1.03 ± 0.11	0.65 ± 0.09
8a	2.54 ± 0.20	2.53 ± 0.19	2.78 ± 0.14
8b	4.06 ± 0.38	4.35 ± 0.47	5.59 ± 0.37
8c	5.87 ± 0.42	8.21 ± 0.35	12.37 ± 0.85
8d	1.52 ± 0.09	1.77 ± 0.15	1.62 ± 0.11
8e	0.97 ± 0.10	1.10 ± 0.08	0.78 ± 0.13
9a	2.18 ± 0.15	2.34 ± 0.27	2.10 ± 0.13
9b	2.99 ± 0.32	3.75 ± 0.38	3.18 ± 0.17
9c	4.36 ± 0.42	5.31 ± 0.50	5.71 ± 0.52
9d	1.33 ± 0.09	1.55 ± 0.27	0.84 ± 0.10
9e	0.42 ± 0.08	0.56 ± 0.06	0.22 ± 0.08
10a	2.01 ± 0.11	2.26 ± 0.26	2.10 ± 0.15
10b	2.92 ± 0.26	3.24 ± 0.13	3.04 ± 0.48
10c	4.08 ± 0.31	5.30 ± 0.62	5.52 ± 0.55
10d	1.02 ± 0.12	1.21 ± 0.05	0.79 ± 0.07
10e	0.39 ± 0.06	0.46 ± 0.04	0.16 ± 0.03
VX-680	0.30 ± 0.03	0.38 ± 0.02	0.13 ± 0.01

[a] Inhibition of the growth of tumor cell lines.
[b] Inhibition of Aurora-A kinase.

improved antiproliferative activity, and the potency order is OEt>OMe, whereas Cl group substituent and Br group substituent had minor effects, which showed even lower activity compared with those without substituents on the B-ring. In the case of constant B ring substituents, change of substituents on the A-ring could also affect the activities of these compounds. Among the compounds, the compounds with NO_2 and Cl group as electron-withdrawing substituents on ring A are of better antitumor activity comparing to those with electron-donating substituents, quite different from ring B, the potency of *para*-substituents on the A-ring is ordered as: NO_2 > Cl > H > Me > MeO, followed that **8c** showed the lowest activity. Among all the compounds, **10e** with *para*-NO_2 group on the A-ring and *para*-OEt on the B-ring respectively, led to a noteworthy best activity.

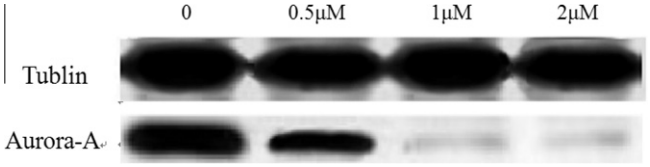


Figure 2. Compound **10e** was examined by Western blotting. Data are representative of three independent experiments.

To examine whether the compounds inhibit Aurora-A kinase, we screened compounds **6a–10e** against the Aurora-A kinase. The results were summarized in Table 2**.** Most of the tested compounds displayed potent Aurora-A kinase inhibitory. Among them, compounds **10e** showed the most potent inhibitory with IC_{50} of 0.16 ± 0.03 μM (the positive control VX-680 with an IC_{50} of 0.13 ± 0.01 μM for Aurora-A kinase). The results of Aurora-A kinase inhibitory activity of the tested compounds corresponded to the structure relationships (SAR) of their antitumor activities. This demonstrated that the potent antitumor activities of the synthetic compounds were probably correlated to their Aurora-A kinase inhibitory activities.

In an effort to study the preliminary mechanism of the compound with potent inhibitory activity, Western-blot experiment was performed to assay the effect of compound **10e**. The Western-blot results are summarized in Figure 2. The results indicated that compound **10e** showed excellent Aurora-A kinase inhibitory activity against HCT116.

To gain better understanding of the potency of the studied compounds and guide further SAR studies, we proceeded to examine the interaction of compound **10e** with Aurora A crystal structure (2BMC.pdb). The molecular docking was performed by inserting compound **10e** into ATP binding site of Aurora-A kinase. All docking runs were applied in LigandFit Dock protocol of Discovery Studio 3.1.

The binding modes of compound **10e** and Aurora-A kinase are depicted in Figure 3. All the amino acid residues which had interactions with Aurora-A kinase are exhibited in Figure 4. In the binding mode, compound **10e** is potently bound to the ATP binding site of Aurora-A kinase via hydrophobic interactions and binding is stabilized by two hydrogen bonds and one π-cation interaction. The oxygen atom of the OEt group formed one hydrogen bond with the amino hydrogen of Arg F:137 (bond length: 1.79 Å; bond angle: 153.9°) and the hydrogen atom of the amide bond formed another with the carboxylic oxygen of Leu F:139 (bond length: 2.28 Å; bond angle: 129.6°). The enzyme surface model was showed in Figure 3b, which revealed that the molecule was well embedded in the active pocket. This molecular docking result, along with the biological assay data, suggested that compound **10e** was a potential inhibitor of Aurora-A kinase.

In conclusion, a series of N,1,3-triphenyl-1H-pyrazole-4-carboxamide derivatives have been synthesized and evaluated for their antitumor activities. These compounds exhibited potent antiproliferative activities against HTC116, MCF-7 cells and Aurora-A kinase inhibitory activities. Among all of the compounds, **10e** showed the most potent inhibition activity which inhibited the growth of HTC116 and MCF-7 cell lines with IC_{50}

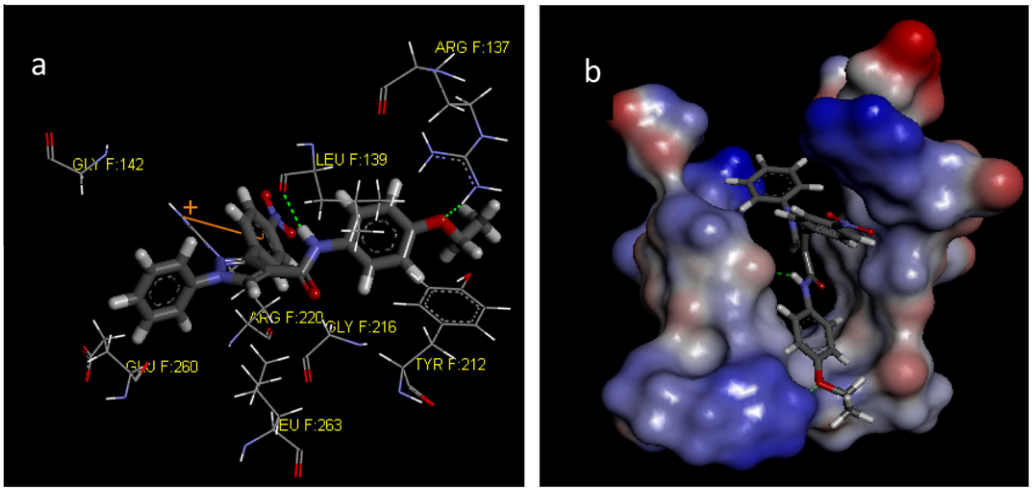


Figure 3. (**a**) Compound **10e** (colored by atom: carbons: gray; nitrogen: blue; oxygen: red) is bond into Aurora-A (entry 2BMC in the Protein Data Bank). The dotted lines show the hydrogen bond and the yellow line show the π-cation interaction interaction. Arg F:137 is the mean of Arg 137 in F chain. (**b**) The surface model structure of compound **10e** binding model with Aurora-A complex.

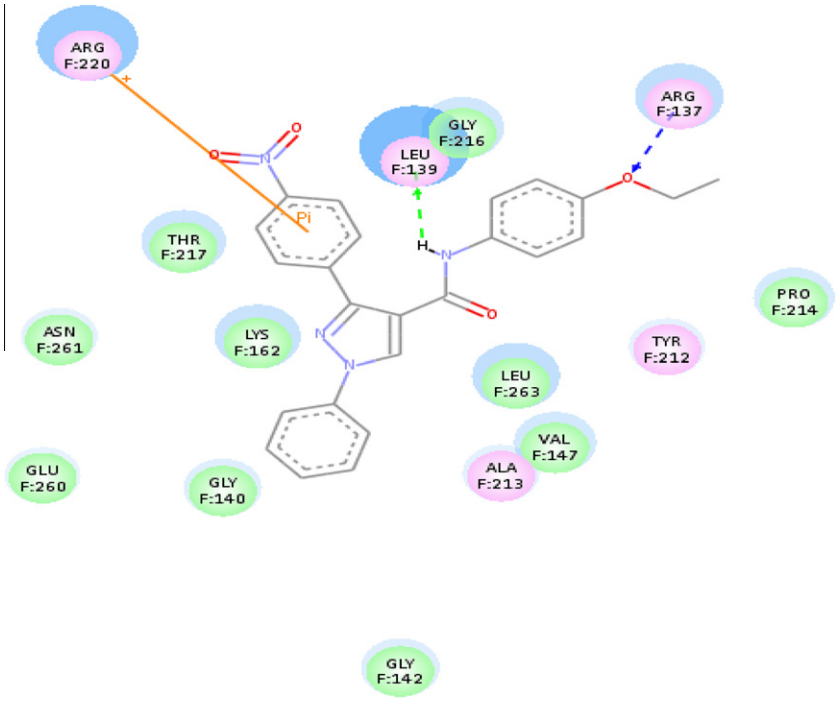


Figure 4. 2D Ligand interaction diagram of compound **10e** with Aurora-A using Discovery Studio program with the essential amino acid residues at the binding site are tagged in circles. The purple circles show the amino acids which participate in hydrogen bonding, electrostatic or polar interactions and the green circles show the amino acids which participate in the Van der Waals interactions.

values of 0.39 ± 0.06 μM and 0.46 ± 0.04 μM respectively and inhibited the Aurora-A kinase with IC_{50} of 0.16 ± 0.03 μM, which was comparable to the positive control VX680(IC_{50} of 0.13 ± 0.01 μM). Molecular docking was further performed to study the inhibitor- Aurora-A kinase interactions. After analysis of the binding model of compound **10e** with Aurora-A kinase, it was found that compound **10e** was potently bound to the Aurora-A kinase with two hydrogen bonds and a π-cation interaction. Western-blot results also showed compound **10e** was a potential antitumor agent. The information of this work might be helpful for the design and synthesis of a leading compound **10e** toward the development of new therapeutic agent to fight against cancer.

Acknowledgments

This work was supported by The Public Benefits Project sponsored by Ministry of Environmental Protection of PRC (201009023), Jiangsu Provincial Fund for Science and technology support projects (BE2011355), and Natural Science Foundation of Jiangsu Province (BK2009234).

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