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Design and synthesis of 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles and pyrazolo[3,4-*b*]pyridines for Aurora-A kinase inhibitors

Jianyou Shi [a,b,†], Guobin Xu [a,†], Wei Zhu [a], Haoyu Ye [a], Shengyong Yang [a], Youfu Luo [a], Jing Han [a], Jincheng Yang [a,b], Rui Li [a,c,*], Yuquan Wei [a], Lijuan Chen [a,*]

[a] *State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medical School, Sichuan University, Keyuan Road 4, Gaopeng Street, Chengdu, Sichuan 610041, PR China*
[b] *Pharmacy College of West China of Sichuan University, Chengdu, Sichuan 610065, PR China*
[c] *State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China*

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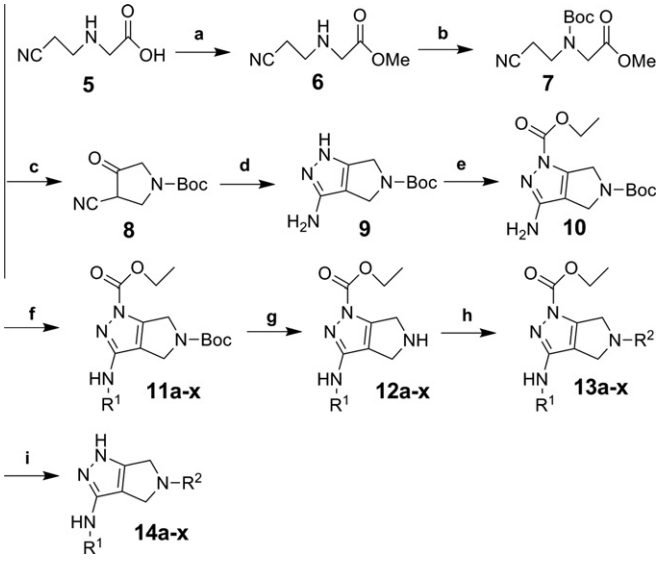
ABSTRACT

Two series of 3-aminopyrazole compounds including 24 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles and 16 pyrazolo[3,4-*b*]pyridines were synthesized and evaluated against HCT116, A549, and A2780 tumor cell lines. Among them, three compounds were found to have the ideal anti-proliferative activities in vitro. Docking experiments showed that the novel pyrazolo[3,4-*b*]pyridines share the similar interaction mode with Aurora-A kinase as PHA739358.

A large number of 3-aminopyrazole derivatives have been studied in the treatment of proliferative diseases,[1–9] such as cancer, inflammation, and arthritis. 3-Aminopyrazole is an important structure presented in a number of pharmaceutically compounds (Fig. 1). For example, 3-amino-1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles (**2**) have been discovered as a new class of CDK2 inhibitors and are able to efficiently inhibit CDK2-mediated tumor cell proliferation.[10] The skeleton of pyrazolo[3,4-*b*]pyridine (**3**) has been identified as potent inhibitors for glycogen synthase kinase-3 (GSK-3).[11] In addition, 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles (**1**), reported by Daniele Fancelli, can inhibit the activity of Aurora-A kinase.[1,12] 4′-(3-(Thiazol-5-ylamino)-1*H*-pyrazol-5-yl)biphenyl-2,4-diol (**4**), the Checkpoint 1 (G1) inhibitor, was proved as an attractive compound to fight cancer.[13]

The 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles and pyrazolo-[3,4-*b*]pyridines share the common nucleus as 3-aminopyrazole moiety, and the former have been discovered as effective Aurora kinase inhibitors. Recently, PHA739358 (**1**) has advanced into phase II clinical trials for the treatment of cancer. In order to find out more effective derivatives containing 3-aminopyrazole nucleus, a new series of derivatives were designed and synthesized. It was demonstrated that **14p**, **14s**, and **20i** have the ideal inhibi-



Scheme 1. Preparation of 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles **14a–x**. Reagents and conditions: (a) MeOH, H_2SO_4,7 h, reflux; (b) di-*tert*-butyl dicarbonate, DCM/aq $NaHCO_3$ (1:1), 24 h, 22 °C; (c) MeONa, toluene, 3 h, 80 °C, then 2 N HCl; (d) hydrazine hydrochloride, EtOH, 3 h, 60 °C, then aq $NaHCO_3$; (e) EtCOOCl, THF, 20 h, 0–5 °C; (f) RCOCl, DIEA, THF, 12 h, 22 °C; (g) TFA/DCM (1:1) 10 equiv, 1 h, 22 °C; (h) RCOCl, DIEA, THF, 6 h, 22 °C; (i) Et_3N 10% in MeOH, 3–6 h, 22 °C.

* Corresponding authors. Tel.: +86 28 85164063; fax: +86 28 85164060 (L.C.).
E-mail addresses: Lirui@scu.edu.cn (R. Li), lijuan17@hotmail.com (L. Chen).
† These authors contributed equally to this work.

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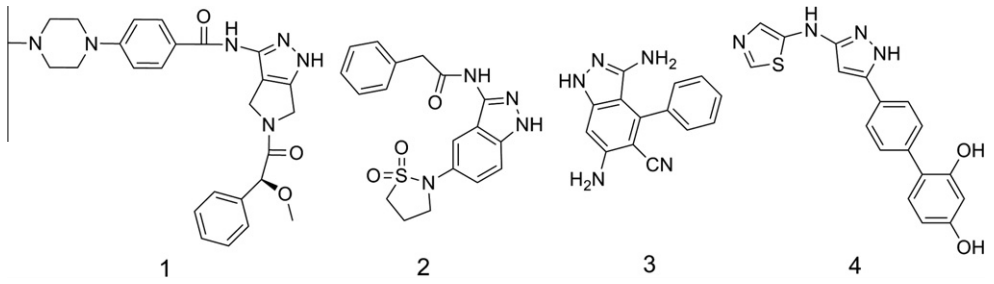
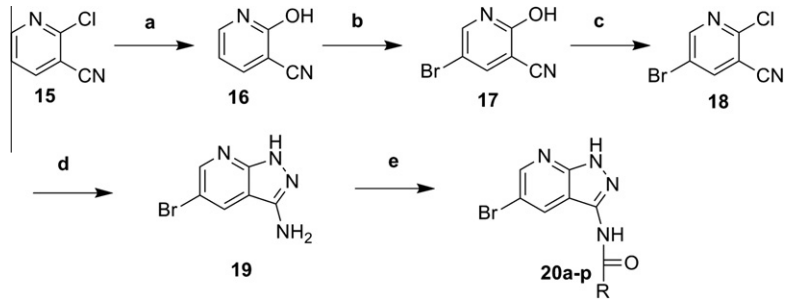


Figure 1. Aurora kinase inhibitor **1** (PHA739358) in clinical trials as anticancer treatment, CDK2 inhibitor **2**, GSK-3 inhibitor **3** and G1 inhibitor **4**.



Scheme 2. Preparation of pyrazolo[3,4-*b*]pyridines **20a**–**p**. Reagents and conditions: (a) AcOH, 125 °C (100%); (b) Br_2, AcOH (60%); (c) $POCl_3$, PCl_5, reflux (75%); (d) $N_2H_4{\cdot}H_2O$, EtOH, reflux (85%); (e) RCOCl, pyridine, rt (80%).

Table 1
Inhibition of cell proliferation by compounds **14a**–**x** and **1**

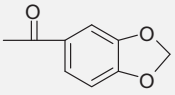
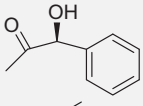
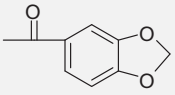
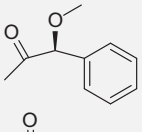
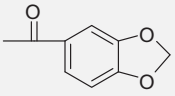
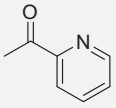
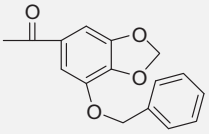
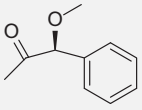
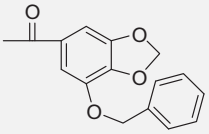
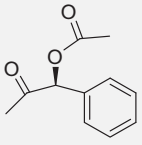
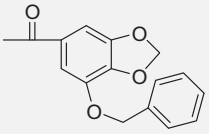
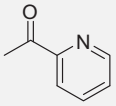
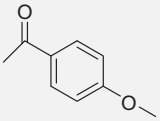
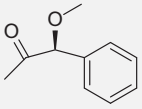
Compound	R^1	R^2	Cell line (IC_{50}, μM)		
			HCT116	A549	A2780
14a			135	84	>200
14b			>200	>200	>200
14c			>200	118	>200
14d			>200	>200	>200
14e			>200	>200	>200
14f			104	>200	>200
14g			207	72	>200

Table 1 (*continued*)

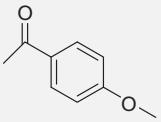
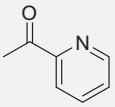
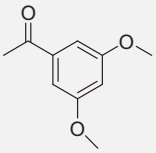
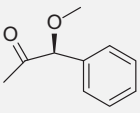
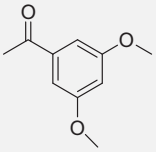
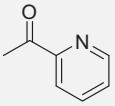
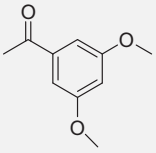
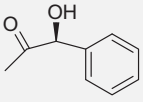
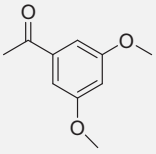
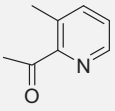
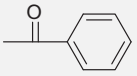
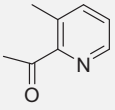
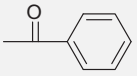
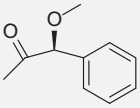
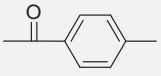
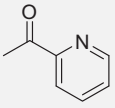
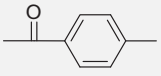
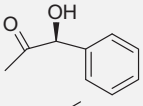
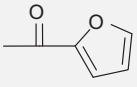
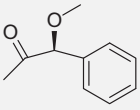
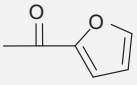
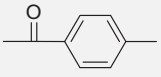
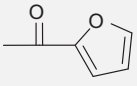
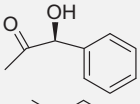
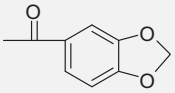
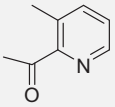
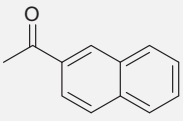
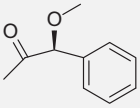
Compound	R^1	R^2	Cell line (IC_{50}, μM)		
			HCT116	A549	A2780
14h			>200	>200	>200
14i			103	99	>200
14j			236	>200	>200
14k			143	76	>200
14l			107	161	>200
14m			168	175	>200
14n			26	>200	>200
14o			>200	>200	>200
14p			13	42	82
14q			>200	>200	>200
14r			>200	>200	>200
14s			4	25	35
14t			>200	>200	>200
14u			38	16	23

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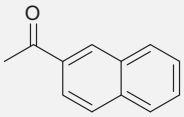
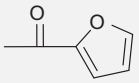
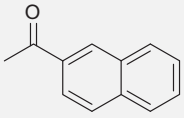
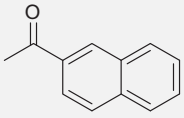
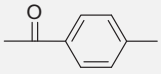
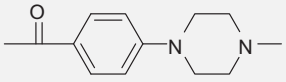
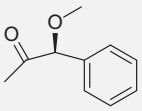
Table 1 (*continued*)

Compound	R^1	R^2	Cell line (IC_{50}, μM)		
			HCT116	A549	A2780
14v			45	34	54
14w			>200	>200	>200
14x			>200	>200	>200
1 (Cont)			13	43	11

Cont, positive control.

tion activity on HCT116, A549, and A2780 cell lines. Meanwhile, the interaction mode and SAR (structural and activity relationship) of this kind of compounds were also concluded.

The target compounds **14a–x** were synthesized according to the literatures with minor revision (Scheme 1).[1,2] Treatment of 2-(2-cyanoethylamino)acetic acid **5** with sulfuric acid and methanol afforded methyl ester **6**, which was protected with di-*tert*-butyl dicarbonate to give **7**. Reaction of **7** with sodium methoxide furnished 4-oxo-pyrrolidine-3-carbonitriles **8**, while cyclization of **8** to provide tetrahydropyrrolopyrazole **9** was accomplished by treatment with hydrazine in ethanol. To obtain **10**, ethyl chlorocarbonate was added to **9** in dry THF and DIEA under stirring at 0–5 °C. The reaction was kept at the same temperature for 2 h, allowed to reach room temperature, and stirred overnight. Acylation of the amino group of **10** yield **11a**. Then, the dihydropyrrole nitrogen of **11a** was unmasked with TFA to give the intermediate **12a**. Next, acyl chloride was added to **12a** to acquire **13a**. A solution of **13a** in MeOH and Et_3N was stirred at 30 °C for 3 h to obtain 14**a**. Compounds **14b–x** were obtained by using the same method. The compounds **20a–p** were synthesized using a modified literature method (Scheme 2).[11,12,14] 2-Chloronicotinonitrile **15** was transformed into 2-hydroxynicotinonitrile **16** by glacial acetic acid. Bromination of **16** by bromine in acetic acid afforded bromide **17**. Then, treatment of **17** with phosphorus oxychloride and phosphorus pentoxide at reflux afforded the corresponding chloropyridine **18**. Furthermore, **18** was treated with hydrazine hydrate in ethanol at reflux to yield the pyrazolopyridine **19**. Selective acylation of the C-3 amino group in pyridine would afford the corresponding amides **20a**–**p**.

With regard to 1,4,5,6-tetrahydropyrrolo[3,4-*c*]pyrazoles (Table 1), the structures of R^2 of **14a** and **14b** are similar with PHA739358, but their activities are lower than that of PHA739358 by one magnitude, illustrating that the piperonylic acid moiety is not a good substituent for R^1 as Aurora inhibitor. The activity of **14c** is also low. Moreover **14d–f**, with the benzyloxy group attached on the 3-position of piperonylic acid moiety, did not reach the ideal activity when varying different substituents of R^2. It is demonstrated that the R^1 group should not be too large, otherwise they cannot reach to the active site of Aurora-A successfully. When one or two methoxy groups attached to the phenyl group of R^1, the activities of compounds **14g–l** improved but still lower than PHA739358. When R^1 substituent was tolyl or benzyl, the activity of **14n** and **14p** improved greatly. However, **14m** and **14o** showed

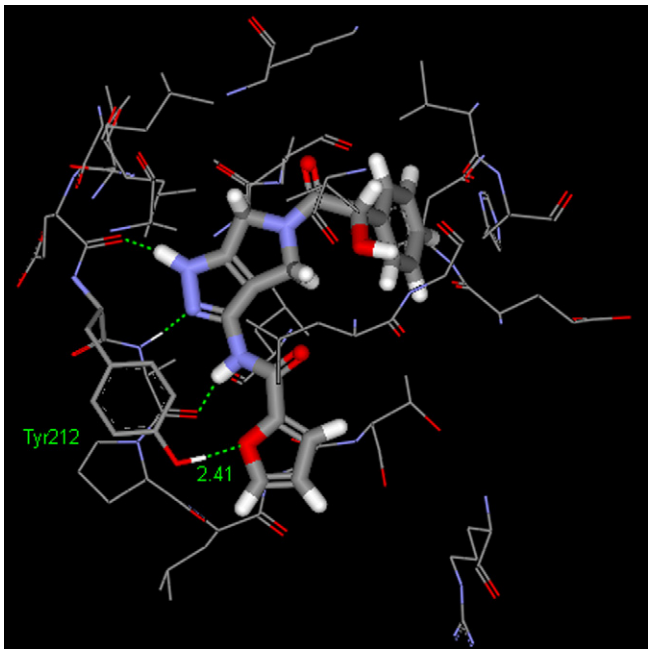


Figure 2. The binding mode of **14s** with Aurora-A kinase.

inactive, indicating that the methine of R^2 on the benzene ring played a key role in the Π–Π conjugation of benzene ring with HIS280. As for R^1, apart from benzyl derivatives, furan and naphthalene substituents were also tested, and it was found that **14s** and **14u** had the ideal activity. As for **14s**, the formation of extra hydrogen bonding between furan O atom and TYR212 (2.41 Å, obtained by docking experiment) (Fig. 2) may contribute to the binding energy. Moreover, **14v–x** bearing the same naphthalene group were synthesized and found that **14v** in which R^2 substituent is furan ring also showed good activity.

From the docking experiments by Autodock **4**,[15] it was found that the interaction of pyrazolo[3,4-*b*]pyridine derivatives with Aurora-A kinase is similar to that of PHA739358 (Fig. 3). The 3-aminopyrazole group would form three hydrogen bonds with GlU211 and AlA213 residues. So the pyrazolo[3,4-*b*]pyridine derivatives should bear

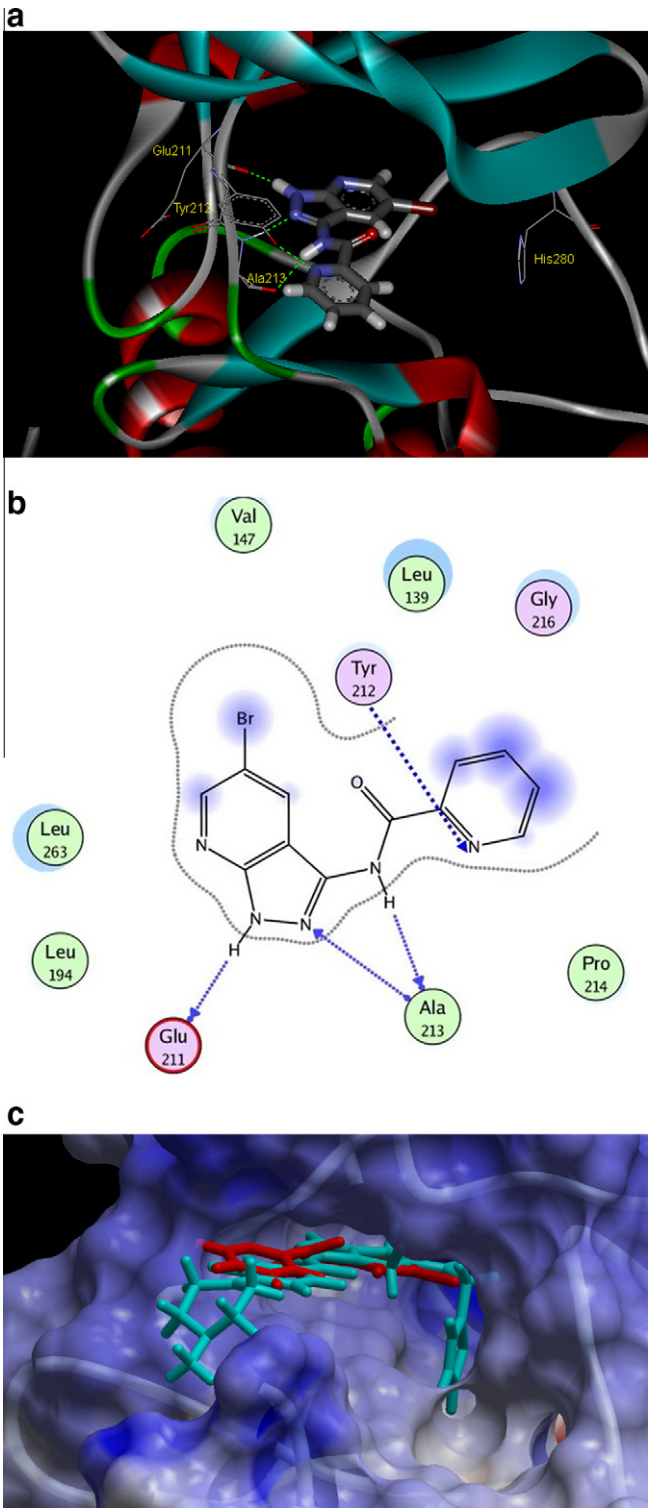
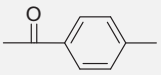
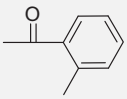
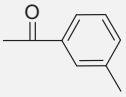
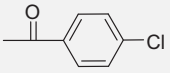
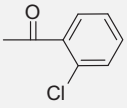
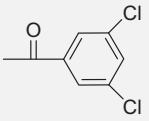
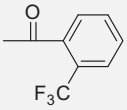
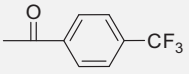
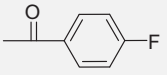
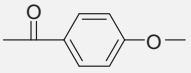
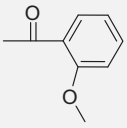
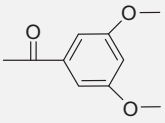
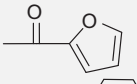
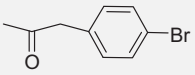
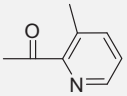
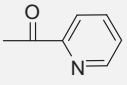


Figure 3. The binding mode of **20p** with ATP pocket of Aurora-A kinase obtained by molecular docking experiments (PDB code 2J4Z) (Fig. 3-1 and Fig. 3-2) and the molecular overlay of **20p** with PHA739358 (Fig. 3-3).

Table 2
Inhibition of cell proliferation by compounds **20a**–**p**

Compound	R	Cell line (IC_{50}, μM)		
		HCT116	A549	A2780
20a		39.82	>200	>200
20b		51.5	>200	>200
20c		25.49	>200	49.90
20d		34.69	51.4	51.56
20e		32.27	>200	46.69
20f		49.19	>200	>200
20g		40.29	75.1	>200
20h		21.88	>200	>200
20i		44.1	>200	50.03
20j		24.85	>200	>200
20k		15.63	>200	27.01
20l		19.31	53.7	37.86
20m		43.48	61.9	42.00
20n		31.7	43.10	22.82
20o		23.02	>200	>200
20p		14.29	45.25	46.45
1 (Cont)		13	43	11

Cont, positive control.

the similar anti-proliferative activity to PHA739358. Hence, a series of its derivatives were synthesized (Table 2). As for tolyl group, the activity of methyl in *meta* position (**20c**) is higher than *para* (**20a**) and *ortho* (**20b**) in HCT116 cell line. In terms of chlorine atom, the location and number have little effect on the activities (**20d**–**f**). As for the electron-withdrawing groups CF_3 and F, *para* position showed good activities. With regard to the electron-donating groups such as methoxy, *ortho* position **20k** is superior to *para* position **20j**, however **20k** was invalid on A549 cell line. Furthermore, compound **20l** having two methoxy groups substituted on the *meta* position

was synthesized and it was found that **20l** showed good inhibitory activities in three kinds of cell lines. In addition, furan, pyridine, and heterocyclic derivatives were also tested, and their activities were also well. Among them 2-substituted pyridyl derivative **20p** had the best activity, which might be ascribed to the formation of an extra hydrogen bond between the pyridine nitrogen and the phenol hydroxyl moiety of TYR212 (2.675 Å) (Fig. 3) besides the common three hydrogen bonds with the GlU211 and AlA213 residues reported by Fancelli et al.[1] and Talele and McLaughlin.[16]

In conclusion, a series of new compounds bearing pyrazolo[3,4-*b*]pyridine scaffold, represented as a novel class of compounds to inhibit the Aurora-A's activity, were synthesized and evaluated. They should interact with the Aurora-A kinases in the similar mode to PHA739358. The ongoing work aimed to explore the efficacy of compounds **14p**, **14s**, and **20p** in a range of in vivo models, will be the subjects of future reports.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.083.

References and notes

1. 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.
2. Fancelli, D.; Berta, D.; Bindi, S.; Cameron, A.; Cappella, P.; Carpinelli, P.; Catana, C.; Forte, B.; Giordano, P.; Giorgini, M. L.; Mantegani, S.; Marsiglio, A.; Meroni, M.; Moll, J.; Pittala, V.; Roletto, F.; Severino, D.; Soncini, C.; Storici, P.; Tonani, R.; Varasi, M.; Vulpetti, A.; Vianello, P. *J. Med. Chem.* **2005**, *48*, 3080.
3. Mortlock, A. A.; Foote, K. M.; Heron, N. M.; Jung, F. H.; Pasquet, G.; Lohmann, J.-J. M.; Warin, N.; Renaud, F.; Savi, C. D.; 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.
4. Soncini, C.; Carpinelli, P.; Gianellini, L.; Fancelli, D.; Vianello, P.; Rusconi, L.; Storici, P.; Zugnoni, P.; Pesenti, E.; Croci, V.; Ceruti, R.; Giorgini, M. L.; Cappella, P.; Ballinari, D.; Sola, F.; Varasi, M.; Bravo, R.; Moll, J. *Clin. Cancer Res.* **2006**, *12*, 4080.
5. Carpinelli, P.; Ceruti, R.; Giorgini, M. L.; Cappella, P.; Gianellini, L.; Croci, V.; Degrassi, A.; Texido, G.; Rocchetti, M.; Vianello, P.; Rusconi, L.; Storici, P.; Zugnoni, P.; Arrigoni, C.; Soncini, C.; Alli, C.; Patton, V.; Marsiglio, A.; Ballinari, D.; Pesenti, E.; Fancelli, D.; Moll, J. *Mol. Cancer Ther.* **2007**, *6*, 3158.
6. Pollard, J. R.; Mortimore, M. *J. Med. Chem.* **2009**, *52*, 2629.
7. Zhong, M.; Bui, M.; Shen, W.; Baskaran, S.; Allen, D. A.; Elling, R. A.; Flanagan, W. M.; Fung, A. D.; Hanan, E. J.; Harris, S. O.; Heumann, S. A.; Hoch, U.; Ivy, S. N.; Jacobs, J. W.; Lam, S.; Lee, H.; McDowell, R. S.; Oslob, J. D.; Purkey, H. E.; Romanowski, M. J.; Silverman, J. A.; Tangonan, B. T.; Taverna, P.; Yang, W.; Yoburn, J. C.; Yu, C. H.; Zimmerman, K. M.; O'Brien, T.; Lew, W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5158.
8. Chioua, M.; Samadi, A.; Soriano, E.; Lozach, O.; Meijer, L.; Marco-Contelles, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4566.
9. Salvati, M.E.; Kimbal, S. D. U.S. Patent 6,482,842, 2002.
10. Pevarello, P.; Fancelli, D.; Vulpetti, A.; Amici, R.; Villa, M.; Pittalàs, V.; _Vianello, P.; Cameron, A.; Ciomei, M.; Mercurio, C.; Bischoff, J. R.; Roletto, F.; Varasia, M.; Brascaa, M. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1084.
11. Witherington, J.; Bordas, V.; Garland, S. L.; Hickey, D. M. B.; Ife, R. J.; Liddle, J.; Saunders, M.; Smith, D. G.; Ward, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1577.
12. Coumar, M. S.; Wu, J. S.; Leou, J. S.; Tan, U. K.; Chang, C. Y.; Chang, T. Y.; Lin, W. H.; John, T. A.; Hsu, J. T.-A.; Chao, Y. S.; Wu, S. Y.; Hsieh, H. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1623.
13. Prudhomme, M. *Recent Patents Anti-Cancer Drug Discovery* **2006**, *1*, 55.
14. Lavecchia, G.; Berteina-Raboin, S.; Guillaumet, G. *Tetrahedron Lett.* **2004**, *45*, 6633.
15. Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. *J. Comput. Chem.* **2007**, *28*, 1145.
16. Talele, T. T.; McLaughlin, M. L. *J. Mol. Graphics Modell.* **2008**, *26*, 1213.