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Design and synthesis of novel benzoxazole analogs as Aurora B kinase inhibitors

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ABSTRACT

A novel series of benzoxazole analogs was designed and synthesized, and their inhibitory activities against Aurora kinases were evaluated. Some of the tested compounds exhibited a promising activity with respect to the inhibition of Aurora B kinase. A structure-activity relationship study indicated that linker length, regiochemistry, and halogen substitution play important roles in kinase inhibitory potency. The binding modes between representative compounds and Aurora kinases were interpreted through a molecular docking study to explain the inhibitory activity and selectivity for Aurora A and B kinases. Compounds **13l** and **13q** also show an antiproliferative effect on the human tumor cell lines in a dose-dependent manner. The most potent **13q** demonstrated good efficacy in the prostate cancer PC-3 tumor xenograft model.

[1] Indicates equal contribution.

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Auroras, serine-threonine kinases, have emerged as key regulators in the control of the cell cycle and mitosis. Mammalian genomes contain three genes encoding Aurora kinases, denoted Aurora A, B, and C. Each Aurora kinase shows a different subcellular location. Aurora A localizes to the centrosome and spindle poles, and is mainly related to centrosome function and spindle assembly.[1] Aurora A may also be involved in cytokinesis, in accordance with its localization at the central spindle and midbody in the anaphase and telophase of mitosis.[2] Aurora B is a member of the chromosome passenger complex,[3] and localizes to centromeres during early mitosis, transfers to the midzone of the mitotic spindle and contractile ring during anaphase, and stays in the midbody during cytokinesis.[4,5] Aurora C is specifically expressed in the testis and plays many roles in meiosis. Aurora C has been reported to be a chromosomal passenger protein with maximal expression in the G2/M phase of the cell cycle, and can complement the functions of Aurora B. Aurora kinases are strongly associated with human cancer.[6] Accumulating evidence indicates that Aurora kinases are significantly overexpressed in a wide range of human cancer cell lines. Deregulation of Aurora kinase activity causes mitotic abnormalities, leading to genomic instability and ultimately tumorigenesis.[7,8] Considering their importance in mitosis and association with tumorigenesis, aurora kinases have been proposed as promising targets for cancer therapy.[7,8] A growing number of aurora kinase inhibitors are being developed, and many of these are in clinical trials. Recently, the pan-aurora kinase inhibitor Danusertib (PHA-739358)[9] and the selective Aurora B inhibitor Barasertib (AZD1152)[10] have been in clinical trials for the treatment of leukemia, myeloma, and other solid tumors (Fig. 1).[11,12]

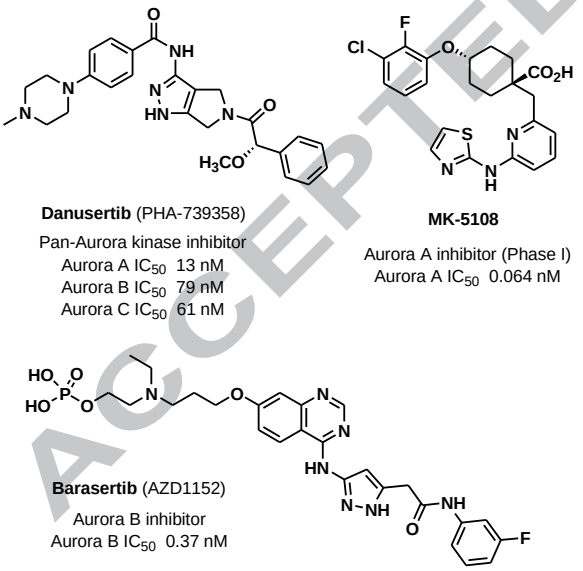


Figure 1. The structures of Aurora kinase inhibitors.

Typical kinase inhibitors are classified by their binding mode (type I - IV). Type I kinase inhibitors work on the active form of enzymes in a competitive manner with ATP. In contrast, type II inhibitors interact with the inactive conformation of enzymes.[13] In the case of type III kinase inhibitors, they bind in an allosteric pocket nearby active site, without any interaction with the ATP-binding pocket. Type IV kinase inhibitors placed to an allosteric site far from the ATP-binding pocket.[14] There are two remarkable differences between the active and inactive conformations of the kinases. Firstly, an Asp residue (Asp218 in Aurora B) interacting with the gamma phosphate is reoriented in the inactive conformation by the DFG motif taking the "out" position. The second important change is observed at a Glu residue (Glu125 in Aurora B), which is responsible for the construction of a salt bridge with β3 Lys (Lys106 in Aurora B), in the αC-helix.[15,16] These conformational changes generate an additional allosteric site that opens favorable interactions for selectivity.[17] In the present article, we report the discovery and identification of a novel series of 2-aminobenzoxazole analogs as type II kinase inhibitors. The representative structure of the targeted molecules is depicted in Figure 2. Similar to many other type II kinase inhibitors, our aminobenzoxazole analogs consist of three parts; a hinge binder, a solvent accessible group, and DFG and allosteric site-interacting groups.[13] Here, we examined their activities with respect to the inhibition of aurora kinases, cell growth, and tumor growth using an ADP-Glo™ kinase assay, a cell-based assay, and PC-3 tumor xenograft model, respectively. We found that compound **13q** potentially inhibited Aurora kinase B activity and suppressed cell proliferation in the human cancer cell lines *in vitro* and the growth of PC-3 xenograft tumors *in vivo*. These observations suggest that compound **13q** is worthy of further study directed towards the identification of more potent and selective Aurora B kinase inhibitors as anticancer agents.

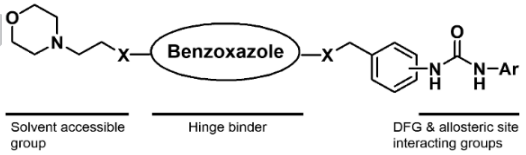
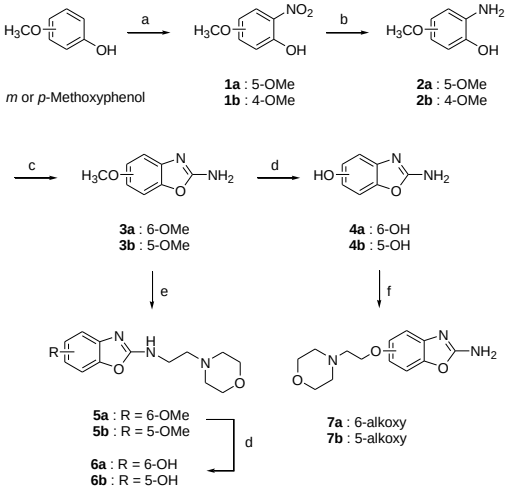


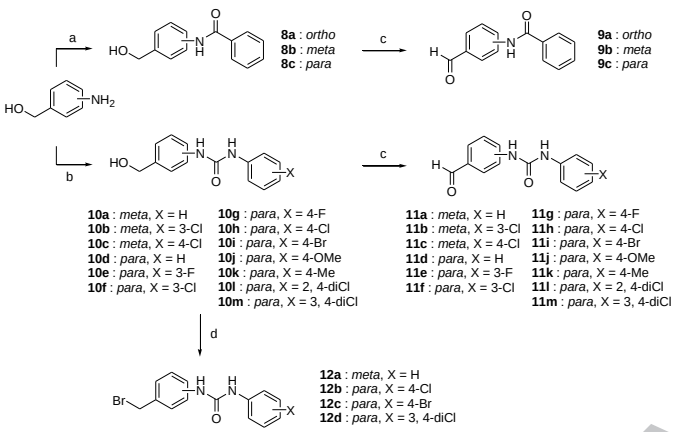
Figure 2. Representative structure of 2-aminobenzoxazole

The synthetic route for the aminobenzoxazole scaffold with a solvent accessible moiety is described in Scheme 1. Nitration of methoxyphenol and subsequent hydrogenation of the resulting nitro compound afforded compound **2**. Aminobenzoxazole **3** was formed by the reaction of compound **2** with BrCN in EtOH. The solvent accessible hydrophilic moiety, the morpholinylethyl group, was attached to the OH or NH_2 group of the aminobenzoxazle scaffold to yield compounds **6** and **7**.



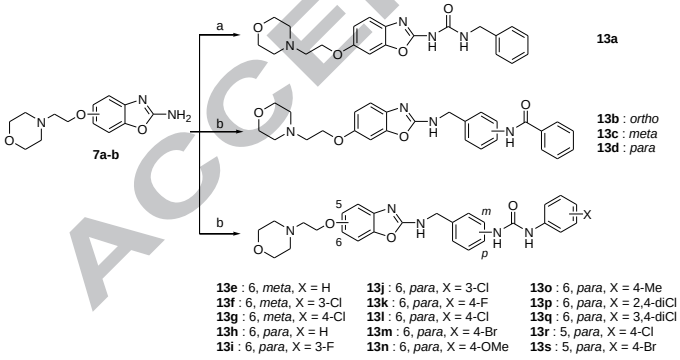
Scheme 1. Synthesis of the 2-aminobenzoxazole scaffold. Reagents and conditions: a) HNO_3, AcOH, 0 C, 30 min, 33%; b) H_2, Pd/C, MeOH, rt, 4 h, 75%; c) BrCN, EtOH, reflux, 2 h, 70%; d) 48% HBr, AcOH, reflux, 30 h, 75%; e) 4-(2-chloroethyl)morpholine HCl, Cs_2CO_3, DMF, 120 C, 3 h, 33%; f) 4-(2-chloroethyl)morpholine HCl, Cs_2CO_3, DMF, 80 C, 6 h, 40%.

We prepared two types of benzoxazole analogs. Two substituted groups of the aminobenzoxazole core were attached in an inverse fashion. In a series of type A analogs, the solvent accessible moiety, the morpholinyl alkoxy group, was attached to the benzene ring of benzoxazole, and additional H-bond donor/acceptor and hydrophobic moieties were attached to the nitrogen of aminobenzoxazole. Type B analogs were shaped in reverse (Fig. 2). Preparation of the hydrophobic moiety was started with aminobenzyl alcohol, which was treated with benzoyl chloride or substituted with phenyl isocyanate to form amide **8** or urea compound **10**, respectively. *N*-substituted aminobenzyl alcohol **8** and **10** were transformed to bromide **12** by treatment with PBr_3 or oxidized to respective aldehydes **9** and **11** by treatment with MnO_2 (Scheme 2).

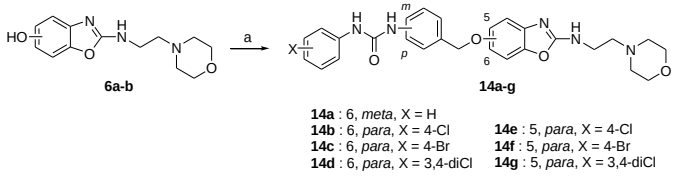


Scheme 2. Synthesis of hydrophobic substituents. Reagents and conditions: a) benzoyl chloride, NaOAc, THF, rt, 2 h, 80%; b) substituted phenyl isocyanate, THF, rt, 30 min, 80-90%; c) MnO_2, DMF, 70-80 C, 2-4 h, 60-90%; d) PBr_3, THF, 0 C, 30 min, 90%.

Type A compounds were prepared as depicted in Scheme 3. Addition of compounds **7** to isocyanate afforded urea **13a**, and reductive amination with aldehydes **9** or **11** gave *N*-benzyl substituted compounds **13b-s**. Type B compounds **14** were also prepared by *O*-alkylation of compounds **6** with various alkyl halides **12** (Scheme 4).



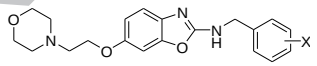
Scheme 3. Synthesis of type A compounds. Reagents and conditions: a) benzyl isocyanate, NaH (60%), DMF, 80 C, 2 h, 20%; b) compound **9a-c** or **11a-m**, Ti(*i*PrO)$_4$, THF, reflux, 3 h then $NaBH_3CN$, 40-50 C, 4 h - overnight, 10-30%.



Scheme 4. Synthesis of type B compounds. Reagents and conditions: a) compound **12a-d**, Cs_2CO_3, DMF or MeCN, rt, 2-5 h, 10-40%.

Enzymatic activity of the prepared compounds against Aurora A and B kinases was determined using the ADP-Glo assay (Promega, USA), which quantifies the ADP generation consumed from ATP during a kinase reaction. The inhibitory activity was represented as the relative percentage inhibition of the positive control staurosporine. Following assessment of all the prepared compounds for Aurora A and B enzymatic activity, selected compounds with sufficient activity against Aurora B were examined for their antiproliferative activity against the various human cancer cell lines. The results of the enzymatic assay are summarized in Tables 1 and 2. At the beginning of the study, we introduced an amide or urea group directly to the benzoxazole scaffold as depicted in **13a**, which was rarely active. Docking studies indicated that the lengthening of the molecule may be useful for activity. Introduction of a benzyl group as a linker between benzoxazole and the H-bond donor/acceptor moiety, amide or urea group, enhanced the activity depending on the position of substituents, as shown in Table 1.

Table 1
Aurora kinase inhibitory activities of aminobenzoxazole analogs

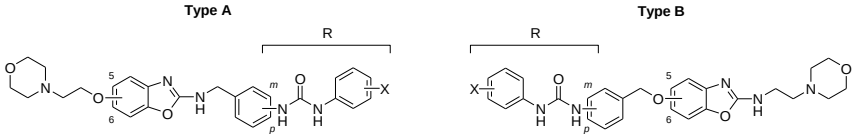


Cpd.	X		% inhibition[a]	
	Position	Substituent	AURA	AURB
13b	*o*	NHCOPh	ND[b]	NA[c]
13c	*m*	NHCOPh	ND	1
13d	*p*	NHCOPh	ND	21
13e	*m*	NHCONHPh	ND	30
13h	*p*	NHCONHPh	15	40

[a] Relative percent inhibitory activity against staurosporine at 10 μM. Determined IC_{50} value of staurosporine was 1.5 nM according to provided procedure of ADP-Glo assay kit by Promega. Compounds were treated at 10 μM. [b] ND = Not Determined. [c] NA = Not Active.

The attachment position of the H-bond donor/acceptor moiety (X) to the benzyl linker affected the activity. Better activity was seen when group X was substituted at the *para* position (compound **13d**) as compared with the *ortho* isomer **13b** and the *meta* isomer **13c**. A urea substituent group was more favorable than an amide as demonstrated when comparing **13c** vs **13e** and **13d** vs **13h**. Therefore, a further structure-activity relationship study was carried out with phenylurea analogs. As observed in Table 2, the *para*-substituted urea analogs (**13h**, **13j**, and **13l**) revealed a higher activity than the *meta*-substituted analogs (**13e-g**). We investigated the effect of a halogen substituent (X) on the terminal benzene ring. Introduction of a halogen atom generally increased activity. In the case of chlorine substitutions, the activities were in the order of; 2,4- or 3,4-diCl > 4-Cl > 3-Cl. The hydrophilic morpholine substituent was attached at the C6 or C5 position of the benzoxazole scaffold. 6-Alkoxy analogs were more potent than 5-alkoxy analogs when comparing **13l** and **13m** with **13r** and **13s**, respectively. With respect to type B analogs, most of the compounds revealed promising activities. However, the structure-activity relationships were more obscure than those of type A analogs. Compound **14b** showed the highest relative inhibitory activity but a lower IC_{50} value. The majority of the tested compounds showed selectivity for Aurora B kinase over Aurora A kinase. Considering the relative inhibitory activities, five representative compounds were chosen and their IC_{50} values were evaluated (Table 2). Dichloro-substituted compounds **13p** and **13q** were the most potent, with IC_{50} values of 0.7 and 0.6 μM, respectively.

Table 2
Aurora kinase inhibitory activities of benzoxazole compounds containing a phenylurea group



Cpd.	Type	Alkoxy position	R		% inhibition[a]		AURB IC_{50} (μM)
			Position	X	AURA	AURB	
13e	A	6	*m*	H	ND[b]	30	ND
13f	A	6	*m*	3-Cl	ND	NA[c]	ND
13g	A	6	*m*	4-Cl	8	45	ND
13h	A	6	*p*	H	15	40	ND
13i	A	6	*p*	3-F	7	63	ND
13j	A	6	*p*	3-Cl	13	58	ND
13k	A	6	*p*	4-F	NA	34	ND
13l	A	6	*p*	4-Cl	5	66	10.2
13m	A	6	*p*	4-Br	NA	69	ND
13n	A	6	*p*	4-OMe	10	76	ND
13o	A	6	*p*	4-Me	NA	65	ND
13p	A	6	*p*	2,4-diCl	8	86	0.7
13q	A	6	*p*	3,4-diCl	24	88	0.6
13r	A	5	*p*	4-Cl	8	42	ND
13s	A	5	*p*	4-Br	6	35	ND
14a	B	6	*m*	H	1	80	ND
14b	B	6	*p*	4-Cl	NA	94	50.4
14c	B	6	*p*	4-Br	NA	55	ND
14d	B	6	*p*	3,4-diCl	2	70	49.4
14e	B	5	*p*	4-Cl	4	37	ND
14f	B	5	*p*	4-Br	3	74	ND
14g	B	5	*p*	3,4-diCl	5	74	ND

[a] Relative percent inhibitory activity against staurosporine at 10 μM. Determined IC_{50} value of staurosporine was 1.5 nM according to provided procedure of ADP-Glo assay kit by Promega. Compounds were treated at 10 μM. [b] ND = Not Determined. [c] NA = Not Active.

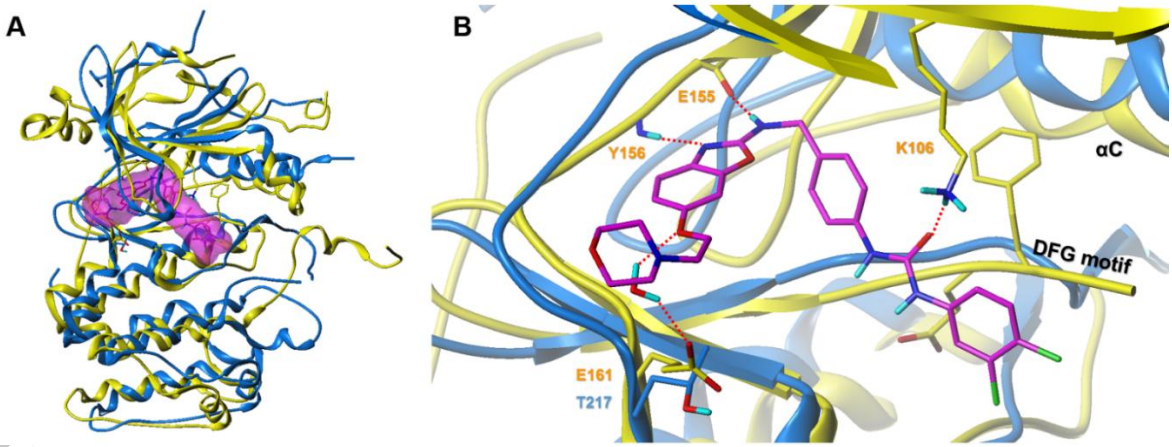


Figure 3. The predicted docking results of compound **13q** with Aurora A and Aurora B kinase. (A) Overlay structure of human Aurora A-TPX2 complex colored blue (PDB ID: 3E5A) and human Aurora B-INCENP complex colored yellow (PDB ID: 4AF3), with compound **13q** colored magenta. (B) Docked conformation of compound **13q** in the active site of Aurora B kinase colored yellow.

Compound **13q** was docked into the active site of Aurora B kinase using Tripos sybyl-X ver. 2.1.1. The crystal structure of human Aurora B kinase cocrystalized with its potent inhibitor VX-680 was used for the docking analysis (PDB ID: 4AF3).[16] The most likely binding conformations were selected based on the docking score and the binding mode. In addition, the crystal structure of human Aurora A kinase (PDB ID: 3E5A)[18] was aligned with Aurora B to provide a possible explanation for the selectivity of our compounds for Aurora B over Aurora A kinase (Fig. 3A). The binding mode of compound **13q** was similar to that of the reference compound VX-680 in the active site of Aurora B kinase. The nitrogen atom of the benzoxazole ring and the amino group of the aminobenzoxazole moiety interacted with Tyr156 and Glu155 at the hinge backbone, which is considered to be a key interaction in the maintenance of the activity against kinases. The terminal aromatic ring substituted with a halogen atom reached up to near the DFG motif. Additionally, the carbonyl group of the urea linker formed a H-bond interaction with Lys106 at the β3 strand. As colored in blue and yellow in Figure 3, respectively, Aurora A and B kinases have high sequence homology, however there is a critical difference at residue Glu161 in Aurora B (Thr217 in Aurora A), which can

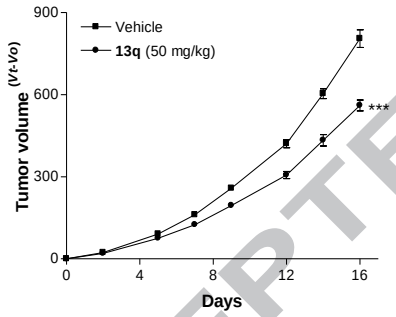
give different electrostatic and spatial states in the active site.[19,20] In the active site of Aurora B, the oxygen atom of the alkoxybenzoxazole moiety could make an additional H-bond with Glu161 through a water molecule (Fig. 3B). This water-mediated H-bond interaction could be the cause of selectivity.[20]

Table 3
Growth inhibitory effect of compound **13l** and **13q**

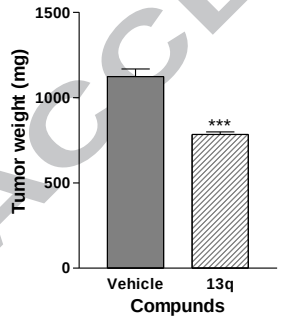
Cpd.	GI_{50} (μM)					
	ACHN	MDA-MB-231	PC-3	NUGC-3	HCT-15	NCI-H23
13l	2.2	7.6	6.2	5.5	4.8	4.0
13q	2.6	5.0	4.8	8.4	3.0	3.4

Cell proliferation assay was performed on various adenocarcinoma cell lines with the potent compounds **13l** and **13q**. GI_{50} values of compounds **13l** and **13q** ranged from 2.2 to 8.4 μM (Table 3). *In vivo* antitumor activity of compound **13q** was evaluated against prostate cancer PC-3 xenograft tumors in nude mice (Fig. 4). Compound **13q** was subsequently dosed orally at 50 mg/kg for 16 days (qdx16). *In vivo* efficacy was assessed as reduction of tumor growth in **13q**-treated versus vehicle-treated mice. In contrast to the vehicle-treated mice, tumor growth suppression was observed in all mice that treated **13q** without significant loss of body weight or visible toxicity. Taken together, we provide **13q** as a potential lead compound for the development of wide range of Aurora B kinase inhibitor with better activity and safety profile.

A. Growth curve



B. Tumor weight



C. Body weight

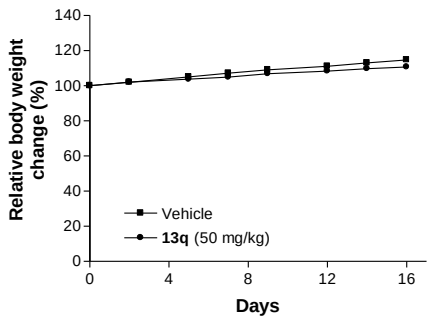


Figure 4. Antitumor activity of compound **13q** on prostate cancer PC-3 xenograft model. (A) Growth curve of the prostate cancer PC-3 xenograft in nude mice treated with compound **13q** (50 mg/kg) or vehicle (10% dimethylacetamide and 10% Tween 80 in water) once a day p.o. for 16 days. The tumor volumes in each group were measured every two or three days. (B) The weight of xenograft tumors from different groups that harvested on day 16 after the first administration of **13q** or vehicle. (C) Body weight change of mice during treatment with compound **13q** or vehicle. Student's t-test was used to determine the statistical significance: *** $p < 0.001$.

A novel series of aminobenzoxazole analogs were designed and synthesized as Aurora B kinase inhibitors and their structure activity relationship was investigated. Some of the tested compounds revealed promising inhibitory activities against Aurora B kinase. Among these compounds, dichloro-substituted compound **13q** was the most potent. A molecular docking study suggested the putative binding mode of the most potent compound **13q**, implying the cause of the Aurora B selectivity. Compound **13q** showed antitumor activity against the human adenocarcinoma cell lines and PC-3 tumor xenograft model *in vivo*. This work provides an attractive lead for further structure optimization in the discovery of more selective and potent Aurora B kinase inhibitors.

Acknowledgments

We dedicate this article to Professor Young-Ger Suh on the occasion of his retirement. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2011-0030074 and 2014R1A2A1A11052761).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at.

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Graphical Abstract

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