Organic LETTERS

Letter

pubs.acs.org/OrgLett

Ru(II)-Catalyzed Site-Selective Hydroxylation of Flavone and Chromone Derivatives: The Importance of the 5-Hydroxyl Motif for the Inhibition of Aurora Kinases

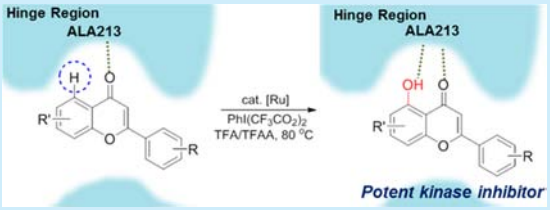
Kiho Kim,[†,‡,§] Hyeonjeong Choe,[†,‡,§] Yujeong Jeong,[†,‡] Jun Hee Lee,[‡] and Sungwoo Hong*[,†,‡]

[†]Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, 305-701, Korea

[‡]Center for Catalytic Hydrocarbon Functionalizations, Institute for Basic Science (IBS), Daejeon, 305-701, Korea

Supporting Information

ABSTRACT: An efficient protocol for Ru(II)-catalyzed direct C−H oxygenation of a broad range of flavone and chromone substrates was developed. This convenient and powerful synthetic tool allows for the rapid installation of the hydroxyl group into the flavone, chromone, and other related scaffolds and opens the way for analog synthesis of highly potent Aurora kinase inhibitors. The molecular docking simulations indicate that the formation of bidentate H-bonding patterns in the hinge regions between the 5-hydroxyflavonoids and Ala213 was the significant binding force, which is consistent with experimental and computational findings.



Chelation-assistance strategies have played key roles in the development of various kinds of transition-metal-catalyzed direct C−H oxidations, which enable facile construction of heteroatom-substituted arenes in a highly selective, efficient fashion.[1] Recently, the use of a weakly coordinating directing group (DG) has attracted much interest as a means to expand the substrate scope of the processes and to ultimately streamline late-stage drug modification.[2]

ortho-Acylphenols constitute a major class of naturally occurring biologically active compounds and are versatile intermediates in organic synthesis. Along these lines, the catalytic direct oxygenation of simple aromatic hydrocarbons through C−H bond activation has emerged as a powerful method for synthesizing various hydroxylated arenes.[3,4] The Ru(II)-catalyzed *ortho*-hydroxylations of aromatic esters and aromatic amides was developed by Rao and Ackermann, respectively.[5] Recently, Rao, Dong, and Kwong independently expanded the scope of weakly coordinating groups via Pd(II)-catalyzed ketone-directed arene hydroxylation reactions.[6] Moreover, Ackermann reported the monomeric Ru(II) complex-catalyzed C−H bond oxygenations of aromatic ketones.[7]

Despite these advances, there remain challenges to regio- and chemoselectivity in the direct catalytic hydroxylation of complex heterocyclic scaffolds that bear only weakly coordinating DGs. Our laboratory has been particularly interested in chemo- and/or regioselective C−H functionalization of medicinally important privileged structures, including chromones, flavones, and coumarins, by exploring the intrinsic characteristics of these scaffolds.[8] The site-selective hydroxylation of flavonoids at the C-5 position is particularly valuable because 5-hydroxyflavonoids are frequently associated with privileged biological moieties.[9] In particular, the naturally occurring flavonoid luteolin (**1b**) has recently been identified as a novel aurora kinase inhibitor by a high-throughput screening (Figure 1).[9c] Aurora kinases have been shown to be involved in a variety of human cancers.[10]

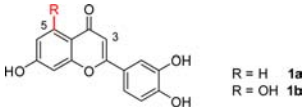


Figure 1. Naturally occurring flavonoid luteolin (**1b**).

Driven by our long-standing interest in developing potent aurora kinase inhibitors, we investigated the binding mode of luteolin (**1b**) in the ATP-binding site of aurora kinase (PDB code 1OL6)[11] to obtain structural insight into its underlying inhibitory activities. Our docking studies revealed that **1b** appears to be stabilized by bidentate H-bonding interactions with Ala213 in the hinge region, indicating that the 5-hydroxyl group confers significant binding forces that can stabilize luteolin in the active site (Figure 2).[12] On the basis of this analysis, we hypothesized that the overall activity of flavone derivatives could be improved by introducing a hydroxyl group into the 5 position of flavones to establish an additional H-bond. For this study, both 5-deoxy luteolin (**1a**) and luteolin (**1b**) were prepared to verify the important role of the 5-hydroxyl group. Notably, the inhibition of aurora A kinase activity by luteolin (**1b**: IC_{50} = 0.12 μM) was dramatically reduced when the C5-hydroxyl group was removed (5-deoxy luteolin **1a**: IC_{50} = 3.86 μM), probably due to the disruption of the key bidentate H-bonds with Ala213. To identify more potent aurora kinase inhibitors, we planned to develop a more general and efficient strategy to install the C5-hydroxyl group into the flavones. Herein, we report our investigation on

Received: April 18, 2015
Published: May 6, 2015

DOI: 10.1021/acs.orglett.5b01138
Org. Lett. 2015, 17, 2550−2553

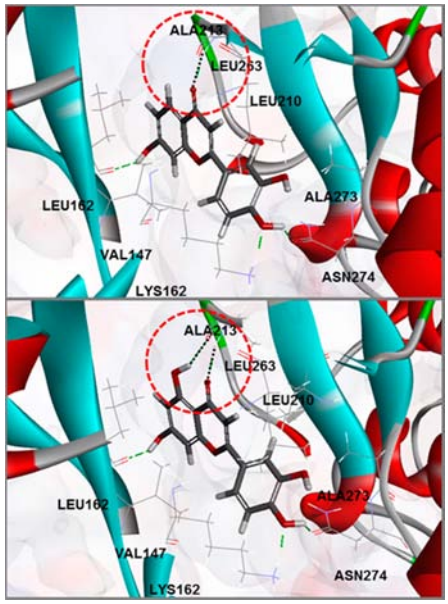


Figure 2. Predicted binding modes of 5-deoxy luteolin **1a** (up) and luteolin **1b** (down) in the ATP binding pocket of aurora kinase (PDB code 1OL6).

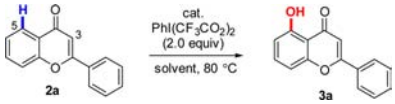
the site-selective hydroxylation of readily available flavones and related derivatives via Ru(II)-catalyzed C−H oxygenation and its application to late-stage modification of kinase inhibitors to enhance potency.

To this end, we explored the C-5 hydroxylation of flavone (**2a**) as a model substrate (Table 1). One of the challenges in this study might be the selective functionalization of the C5−H bond in the presence of a more nucleophilic C3 position. After much experimentation, we found that the desired 5-hydroxyflavone (**3a**) was formed after aqueous workup when **2a** was exposed to $[Ru(p\text{-cymene})Cl_2]_2$ (5 mol %) in TFA/TFAA (9:1, v/v) at 80 °C for 12 h, using $PhI(CF_3CO_2)_2$ as the oxidant (entry 3, 12%). Due to this preliminary result, intensive screening of the reaction media with the Ru(II) complex was conducted to optimize the reaction for C5 hydroxylation. Intriguingly, not only the amount of TFA but also the ratio of TFA/TFAA significantly affected the reaction efficiency. Fortunately, a marked increase in the conversion was observed when the ratio of TFA/TFAA was reversed (1:9) (entry 5, 34%). An extensive solvent system screening demonstrated that a mixture of TFA (3 equiv relative to **1**) and TFAA (1:65, v/v) displayed the best catalytic reactivity. The diminished product yields were obtained when the reaction was carried out with less TFA (<3 equiv). The use of other solvents, such as DCE and toluene, proved to be far less effective (see the Supporting Information (SI)). Other catalytic systems, such as $RuCl_3(H_2O)_n$, led to complete reactivity loss under the reaction conditions (see the SI). Notably, the use of a Ag^+ source in TFA/TFAA (1:65) increased the reaction yield, and the oxygenation of flavone **1** was efficiently achieved in 76% yield (entry 9). Also, careful monitoring of the reaction revealed that no formation of C-3 hydroxylated byproduct was observed,[13] highlighting the favorable coordination effect of the carbonyl group on the Ru catalyst.

The above results suggest that a monomeric $[Ru(p\text{-cymene})(CF_3CO_2)_2(H_2O)]$ (**4**)[14] is presumably generated as an active catalyst considering that $[Ru(O_2CMes)_2(p\text{-cymene})]$ (**5**) enabled C−H bond oxygenation.[5b,c,7,15] To confirm a working hypothesis, we prepared complex **4** (Table 2) and examined the hydroxylation. Indeed, complex **4** (5 mol %) catalyzed C−H bond oxygenation of flavone **2a** to afford an excellent yield of the desired product **3a** as a single regioisomer (entry 1, 87%). Again, the use of a TFA/TFAA cosolvent system was necessary to achieve high reaction efficiency. It was also found that $PhI(CF_3CO_2)_2$ was an optimal oxidant for the reaction efficiency, and switching the hypervalent iodine from $PhI(CF_3CO_2)_2$ to the less electrophilic $PhI(OAc)_2$ gave rise to a reduced yield of 67% (entry 1 vs 3). Inorganic oxidants, such as $K_2S_2O_8$, did not give any of the desired products. When using complex **5** as a catalyst, a comparable catalytic reactivity was observed (entry 5, 84%), demonstrating that **4** is a promising Ru complex for catalysis of oxidation.

Having established the optimal conditions, we explored the scope of the site-selective oxygenation with various heteroarenes, including flavones, and chromones (Scheme 1). In general, we observed that variation in the heteroarenes did not significantly affect the reaction efficiency. A variety of valuable functional groups including fluoro, chloro, trifluoromethyl, trifluorome-

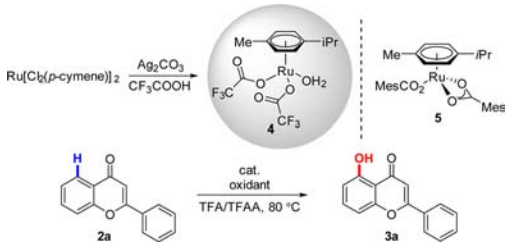
Table 1. Optimization of Reaction Conditions[a]



entry	cat. (5 mol %)	TFA/TFAA	additive (equiv)	yield[b] (%)
1	$Pd(OAc)_2$	9:1	–	0
2[c]	$Pd(OAc)_2$	DCE	–	6
3	$[RuCl_2(p\text{-cymene})]_2$	9:1	–	12
4	$[RuCl_2(p\text{-cymene})]_2$	0:1	–	31
5	$[RuCl_2(p\text{-cymene})]_2$	1:9	–	34
6	$[RuCl_2(p\text{-cymene})]_2$	1:65	–	63
7	$RuCl_3(H_2O)_n$	1:65	–	0
8	$RuCl_2(bpy)_3{\cdot}6H_2O$	1:65	–	0
9	$[RuCl_2(p\text{-cymene})]_2$	1:65	Ag_2CO_3 (1)	76
10	$[RuCl_2(p\text{-cymene})]_2$	1:65	AgTFA (1)	74

[a]Reaction conditions: Flavone (**1**, 0.10 mmol, 1.0 equiv), catalyst (mol % as indicated), $PhI(CF_3CO_2)_2$ (0.20 mmol, 2.0 equiv), TFA/TFAA, 80 °C for 12 h. [b]^{1}H NMR yield. [c]DCE was used as a solvent.

Table 2. Optimization of Reaction Conditions[a]

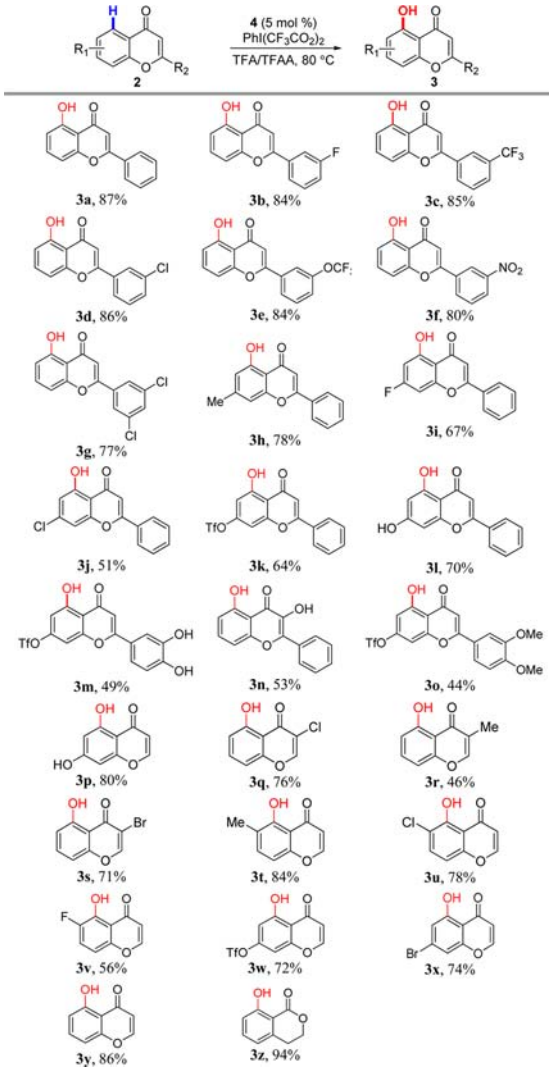


entry	cat. (5 mol %)	oxidant (2 equiv)	TFA/TFAA	yield (%)
1	**4**	$PhI(CF_3CO_2)_2$	1:65	87
2	**4**	$PhI(CF_3CO_2)_2$	1:45	78
3	**4**	$PhI(OAc)_2$	1:65	67
4	**4**	$K_2S_2O_8$	1:65	0
5	**5**	$PhI(CF_3CO_2)_2$	1:65	84

[a]Reaction conditions: Flavone (**1**, 0.15 mmol, 1.0 equiv), catalyst (mol % as indicated), $PhI(CF_3CO_2)_2$ (0.30 mmol, 2.0 equiv), TFA/TFAA, 80 °C for 12 h; isolated yield.

DOI: 10.1021/acs.orglett.5b01138
Org. Lett. 2015, 17, 2550−2553

Scheme 1. Substrate Scope Studies[a]

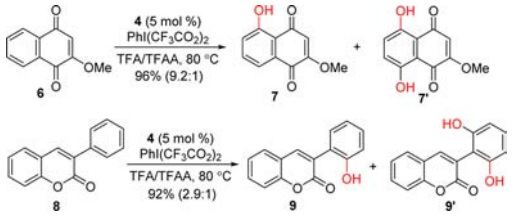


[a]Reaction conditions: Flavone (**1**, 0.15 mmol, 1.0 equiv), catalyst (5 mol %), $PhI(CF_3CO_2)_2$ (0.30 mmol, 2.0 equiv), TFA/TFAA, 80 °C and 8.5−20 h; isolated yield.

thoxy, nitro, methyl, trifluoromethanesulfonyl, and hydroxyl groups were well tolerated (**3a**−**3o**). Next, various chromone substrates (**3p**−**3y**) were subjected to the reaction. Chromones with various functional groups, such as fluoro, chloro, bromo, trifluoromethanesulfonyl, methyl, and hydroxyl groups, reacted well to afford the desired products in moderate to good yields under optimized conditions. Notably, synthetically useful intermediates **3k**, **3m**, **3o**, **3w**, and **3x** were produced with intact OTf or bromo functional groups which were used for further modification at the C7 position to afford kinase inhibitors.

Naphthoquinone was also suitable for this transformation. A coordination event between the Ru(II) and the more electron-rich carbonyl group on naphthoquinone **6** was preferred during the reaction, which resulted in **7** (83%) and the dihydroxylated product **7′** (9%). Given the frequency of the coumarin motif within biologically active molecules, coumarin **8** was subjected to the present reaction conditions to afford the mono- and dihydroxylated products **9** (71%) and **9′** (25%), respectively (Scheme 2). As flavones, chromones, naphthoquinones, and coumarins are common scaffolds in drugs and natural products,

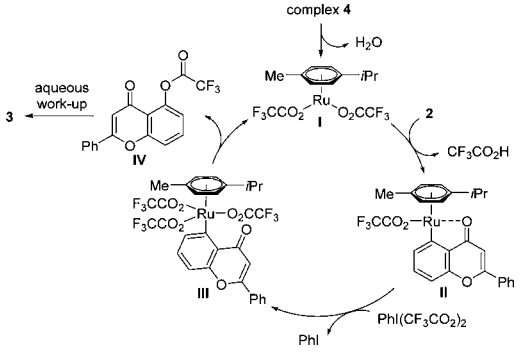
Scheme 2. Oxygenations with Naphthoquinone and Coumarin



this method can potentially open a facile route to rapidly accessing hydroxylated analogs.

On the basis of the experimental results above and previous reports,[15c] a possible mechanistic pathway for the C−H bond oxygenation is depicted in Scheme 3. The five-membered

Scheme 3. Proposed Mechanistic Pathway



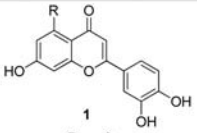
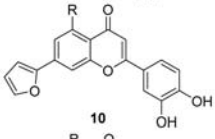
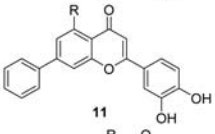
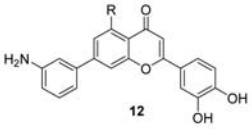
ruthenacycle **II** can be formed via chelate-directed C−H bond activation of the substrate. **II** can be oxidized by the hypervalent iodine to afford the Ru(IV)[15e,16] species **III**. Subsequent reductive elimination in **III** furnishes 5-(trifluoroacetyloxy)-flavone **IV** with the regeneration of the Ru(II) catalyst **I**. However, electrophilic cleavage of the Ar−Ru(II) bond in **III** by the hypervalent iodine cannot be ruled out at this stage. Finally, the trifluoroacetate **IV** readily converts into the corresponding product **3** during the aqueous workup.

The bioactivities of the synthesized 5-hydroxyflavone derivatives were then tested against aurora kinase, and the full IC_{50} values of the representative compounds were determined as illustrated in Table 3.[17] Intriguingly, the introduction of the hydroxyl group into the C5 position of the flavone scaffold yielded a dramatic increase in the inhibition of aurora kinase activity, suggesting that the bidentate H-bonding between the 5-hydroxyl motif and Ala213 is a critical factor in the inhibition of aurora kinase. The newly discovered aurora kinase inhibitors may serve as a good starting point for more potent inhibitors.

In summary, we developed an efficient protocol for Ru(II)-catalyzed direct C−H oxygenation of a variety of flavone and chromone substrates. We found that the monomeric [Ru(*p*-cymene)$(CF_3CO_2)_2(H_2O)$] (**4**) is an active catalyst for the C−H oxygenation. This powerful synthetic tool allows for the rapid and site-selective installation of the hydroxyl group into the flavone, chromone, and other related scaffolds for delivery of highly potent Aurora kinase inhibitors. The molecular docking simulation studies indicate that the formation of bidentate H-bonding patterns between the 5-hydroxyflavonoid and the backbone groups of Ala213 was the significant binding force that stabilized the inhibitors in the ATP-binding site, which

DOI: 10.1021/acs.orglett.5b01138
Org. Lett. 2015, 17, 2550−2553

Table 3. Chemical Structures and IC_{50} Values of Aurora A Inhibitors

compound[18]	aurora A IC_{50} (μM) R = H (**a**)	R = OH (**b**)
 **1**	3.86	0.12
 **10**	0.14	0.056
 **11**	0.84	0.066
 **12**	0.32	0.027
*staurosporin	0.0014	

should be considered in the design of potent aurora kinase inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedure and characterization of new compounds (^{1}H and ^{13}C NMR spectra). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01138.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: hongorg@kaist.ac.kr.

Author Contributions

§K.K. and H.C. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported financially by the Institute for Basic Science (IBS-R010-G1) and National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2010-0022179).

■ REFERENCES

(1) For selected recent reviews, see: (a) Ackermann, L. *Acc. Chem. Res.* **2014**, *47*, 281. (b) Engle, K. M.; Yu, J.-Q. *J. Org. Chem.* **2013**, *78*, 8927. (c) Mousseau, J. J.; Charrette, A. B. *Acc. Chem. Res.* **2013**, *46*, 412. (d) Arockiam, P. B.; Bruneau, C.; Dixneuf, P. H. *Chem. Rev.* **2012**, *112*, 5879. (e) Yu, D.-G.; Li, B.-J.; Shi, Z.-J. *Tetrahedron* **2012**, *68*, 5130. (f) Cho, S. H.; Kim, J. Y.; Kwak, J.; Chang, S. *Chem. Soc. Rev.* **2011**, *40*, 5068. (g) Wencel-Delord, J.; Dröge, T.; Liu, F.; Glorius, F. *Chem. Soc. Rev.* **2011**, *40*, 4740. (h) Ackerman, L. *Chem. Rev.* **2011**, *111*, 1315. (i) Yeung, C. S.; Dong, V. M. *Chem. Rev.* **2011**, *111*, 1215. (j) Sun, C.-L.; Li, B.-J.; Shi, Z.-J. *Chem. Rev.* **2011**, *111*, 1293. (k) Lyons, T. W.; Sanford, M. S. *Chem. Rev.* **2010**, *110*, 1147. (l) Colby, D. A.; Bergman, R. G.; Ellman, J. A. *Chem. Rev.* **2010**, *110*, 624. (m) Kulkarni, A. A.; Daugulis, O. *Synthesis* **2009**, 4087. (n) Park, Y. J.; Park, J.-W.; Jun, C.-H. *Acc. Chem. Res.* **2008**, *41*, 222.

(2) For reviews, see: (a) De Sarkar, S.; Liu, W.; Kozhushkov, S. I.; Ackermann, L. *Adv. Synth. Catal.* **2014**, *356*, 1461. (b) Mo, F.; Tabor, J. R.; Dong, G. *Chem. Lett.* **2014**, *43*, 264. (c) Engle, K. M.; Mei, T.-S.; Wasa, M.; Yu, J.-Q. *Acc. Chem. Res.* **2012**, *45*, 788.

(3) For the seminal reports on this area, see: (a) Zhang, Y.-H.; Yu, J.-Q. *J. Am. Chem. Soc.* **2009**, *131*, 14654. (b) Chen, X.; Hao, X.-S.; Goodhue, C. E.; Yu, J.-Q. *J. Am. Chem. Soc.* **2006**, *128*, 6790. (c) Desai, L. V.; Malik, H. A.; Sanford, M. S. *Org. Lett.* **2006**, *8*, 1141.

(4) Recent review on C−H oxygenation by Ru catalysis: Thirunavukkarasu, V. S.; Kozhushkov, S. I.; Ackermann, L. *Chem. Commun.* **2014**, *50*, 29.

(5) (a) Yang, Y.; Rao, Y. *Org. Lett.* **2012**, *14*, 2874. (b) Thirunavukkarasu, V. S.; Hubrich, J.; Ackermann, L. *Org. Lett.* **2012**, *14*, 4210. For the C−H oxygenation on aryl Weinreb amides, see: (c) Yang, F.; Ackermann, L. *Org. Lett.* **2013**, *15*, 718.

(6) (a) Shan, G.; Yang, X.; Ma, L.; Rao, Y. *Angew. Chem., Int. Ed.* **2012**, *51*, 13070. (b) Mo, F.; Trzepkowski, L. J.; Dong, G. *Angew. Chem., Int. Ed.* **2012**, *51*, 13075. (c) Choy, P. Y.; Kwong, F. Y. *Org. Lett.* **2013**, *15*, 270.

(7) Thirunavukkarasu, V. S.; Hubrich, J.; Ackermann, L. *Org. Lett.* **2012**, *14*, 6206.

(8) (a) Kang, D.; Hong, S. *Org. Lett.* **2015**, *17*, 1938. (b) Shin, Y.; Yoo, C.; Moon, Y.; Lee, Y.; Hong, S. *Chem.—Asian J.* **2015**, *10*, 878. (c) Kim, Y.; Moon, Y.; Kang, D.; Hong, S. *Org. Biomol. Chem.* **2014**, *12*, 3413. (d) Moon, Y.; Kim, Y.; Hong, H.; Hong, S. *Chem. Commun.* **2013**, *49*, 8323. (e) Kim, D.; Min, M.; Hong, S. *Chem. Commun.* **2013**, *49*, 4021. (f) Min, M.; Kim, Y.; Hong, S. *Chem. Commun.* **2013**, *49*, 196. (g) Moon, Y.; Kwon, D.; Hong, S. *Angew. Chem., Int. Ed.* **2012**, *124*, 11495. (h) Moon, Y.; Hong, S. *Chem. Commun.* **2012**, *48*, 7191. (i) Min, M.; Choe, H.; Hong, S. *Asian J. Org. Chem.* **2012**, *1*, 47. (j) Kim, D.; Hong, S. *Org. Lett.* **2011**, *13*, 4466.

(9) (a) Salmela, A.-L.; Pouwels, J.; Kukkonen-Macchi, A.; Waris, S.; Toivonen, P.; Jaakkola, K.; Mäki-Jouppila, J.; Kallio, L.; Kallio, M. K. *Exp. Cell Res.* **2012**, *318*, 578. (b) Xie, F.; Lang, Q.; Zhou, M.; Zhang, H.; Zhang, Z.; Zhang, Y.; Wan, B.; Huang, Q.; Yu, L. *Eur. J. Pharm. Sci.* **2012**, *46*, 388.

(10) For selected reviews, see: (a) Lens, S. M. A.; Voest, E. E.; Medema, R. H. *Nat. Rev. Cancer* **2010**, *10*, 825. (b) Keen, N.; Taylor, S. *Nat. Rev. Cancer* **2004**, *4*, 927.

(11) Bayliss, R.; Sardon, T.; Vernos, I.; Conti, E. *Mol. Cell* **2003**, *12*, 851.

(12) Discovery Studio software was used for docking simulation for Aurora binding modes.

(13) For the hypervalent iodine-mediated C-3 hydroxylation of chromones and flavones, see: Moriarty, R. M.; Prakash, O. *J. Heterocycl. Chem.* **1985**, *22*, 583.

(14) (a) Lo, V. K.-Y.; Guo, Z.; Choi, M. K.-W.; Yu, W.-Y.; Huang, J.-S.; Che, C.-M. *J. Am. Chem. Soc.* **2012**, *134*, 7588. (b) Tocher, D. A.; Gould, R. O.; Stephenson, T. A.; Bennett, M. A.; Ennett, J. P.; Matheson, T. W.; Sawyer, L.; Shah, V. K. *J. Chem. Soc., Dalton Trans.* **1983**, 1571.

(15) (a) Schinkel, M.; Wallbaum, J.; Kozhushkov, S. I.; Marek, I.; Ackermann, L. *Org. Lett.* **2013**, *15*, 4482. (b) Kumar, N. Y. P.; Jeyachandran, R.; Ackermann, L. *J. Org. Chem.* **2013**, *78*, 4145. (c) Diers, E.; Kumar, N. Y. P.; Mejuch, T.; Marek, I.; Ackermann, L. *Tetrahedron* **2013**, *69*, 4445. (d) Ackermann, L.; Pospech, J.; Potukuchi, H. K. *Org. Lett.* **2012**, *14*, 2146. (e) Ackermann, L.; Vincente, R.; Potukuchi, H. K.; Pirovano, V. *Org. Lett.* **2010**, *12*, 5032.

(16) (a) Shang, M.; Zeng, S.-H.; Sun, S.-Z.; Dai, H.-X.; Yu, J.-Q. *Org. Lett.* **2013**, *15*, 5286. (b) Ackermann, L.; Vincent, R.; Potukuchi, H. K.; Pirovano, V. *Org. Lett.* **2010**, *12*, 5032. (c) Flegeau, E. F.; Bruneau, C.; Dixneuf, P. H.; Jutand, A. J. *J. Am. Chem. Soc.* **2011**, *133*, 10161.

(17) IC_{50} determinations were performed at Reaction Biology Corp.

(18) Luteolin derivatives were synthesized via Suzuki cross-coupling from **3m** or **3y**.

DOI: 10.1021/acs.orglett.5b01138
Org. Lett. 2015, 17, 2550−2553