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Synthesis and evaluation of novel substituted 5-hydroxycoumarin and pyranocoumarin derivatives exhibiting significant antiproliferative activity against breast cancer cell lines

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ABSTRACT

A library of novel 5-hydroxycoumarin and pyranocoumarin derivatives was constructed via silica sulfuric acid-catalyzed pechmann reaction and Pd(0)-catalyzed suzuki coupling in tandem, and their antiproliferative activities against breast cancer cells MCF-7 and MDA-MB-231 were evaluated. The results showed that compounds such as **6b**, **6d**, **6h**, and **6k** possess significant antiproliferative activity against MCF-7 cell line with the IC_{50} values of 7.2, 5.3, 3.3, and 6.5 μM, respectively.

Many molecules based on the coumarin ring system have been synthesized including hydroxycoumarins, pyranocoumarins, and coumarin sulfamates, which have been found to be useful in anticoagulant activity,[1] antipsoriasis activity,[2] inhibitory activity on viral proteases,[3] antibacterial/antitumoral activity,[4] antioxidant activity,[5] antiproliferative activity,[6] estrogen-like effects,[7] or central nervous system modulating activities.[8] Among the diverse biological activities of these coumarins, the most intriguing bioactivity is the notable effect of some of the coumarins against breast cancer. As breast cancer is one of the most commonly diagnosed cancer, comprising 23% of all the female cancers and the second leading cause of cancer deaths in women worldwide today,[9] there is a strong need to identify potential new drugs for breast cancer treatment and prevention.

As we know, most substituted hydroxycoumarins and pyranocoumarins are common moieties found in a variety of natural products, and are used as versatile intermediates in organic and natural product syntheses.[10] It was reported that 7-hydroxycoumarin exhibited growth-inhibitory cytostatic activity in human cancer cell lines[11] and coumarin sulfamate 667 Coumate (Fig. 1), set to enter clinical trials for the treatment of hormone-dependent breast cancer in postmenopausal women.[12] The further studies showed

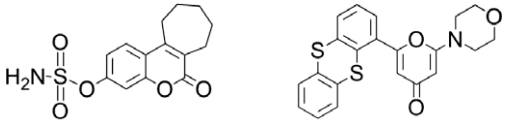

Figure 1. The structure of 667 COUMATE and KU-55933.

that coumarin's pharmacological and biochemical properties depended upon the pattern of substitutions, including the therapeutic applications, and can beneficially affect toxicity.[13] In this respect, herein, we expect to incorporate pyranocoumarin ring system as scaffold of the target molecules, together with the aryl sulfamate ester moiety. As it was reported that some anticancer agents have the core structure of benzo[*b*]thiophen[14] and thianthren, such as compounds KU-55933 (Fig. 1) and KU-5805,[15] we also expect to incorporate benzo[*b*]thiophen, thianthren, and 4-oxo-4-(phenylamino)butanoic acid instead of sulfamate ester moiety to investigate their primary biological activity against MCF-7 (estrogen receptor-positive) and MDA-MB-231 (estrogen receptor-negative) breast cancer cell lines.

For the synthesis of substituted 5-hydroxycoumarin and pyranocoumarin derivatives, we used commercially available 3,5-dimethoxyaniline (DMA) as the starting material, the successive

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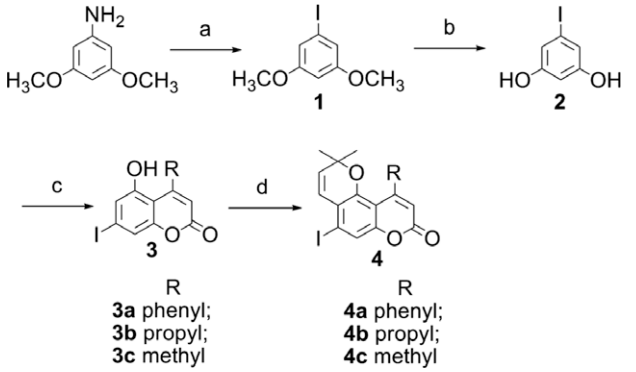


Scheme 1. Reagents and conditions: (a) H_2SO_4, $NaNO_2$, KI, 75%; (b) HI (aq), 95%; (c) β-ketoester, SSA, CH_2Cl_2, 30 °C, 89–94%; (d) 3-methylbut-2-enal, $PhB(OH)_2$, CH_3CH_2COOH, toluene, 140 °C, 79–82%.

Table 1
Reaction conditions for the coupling of **3a** with benzo[*b*]thiophen-2-yl-boronic acid[a]

Catalyst	Base	Solvent	Time (h)	Yield[b] % (**5a**)
$Pd(OAc)_2$	K_2CO_3	DMF	24	0
$Pd(OAc)_2$	Cs_2CO_3	DMF	24	0
$Pd(OAc)_2$	CsF	DMF	24	0
$Pd(OAc)_2$	CsF	Dioxane	24	0
$Pd(PPh3)_4$	K_2CO_3	DMF	24	Trace
$Pd(PPh3)_4$	Cs_2CO_3	DMF	24	Trace
$Pd(PPh3)_4$	CsF	DMF	28	9
$Pd(PPh3)_4$	CsF	Dioxane	28	11
$(dppf)PdCl_2$	K_2CO_3	DMF	18	12
$(dppf)PdCl_2$	Cs_2CO_3	DMF	18	25
$(dppf)PdCl_2$	CsF	DMF	16	58
$(dppf)PdCl_2$	CsF	Dioxane	16	81
$(dppf)PdCl_2$	KF	Dioxane	16	61

[a] All reactions were carried out at 80 °C by using Pd catalyst (3 mol %), compound **3a** (1 equiv), base (6 equiv), and benzo[*b*]thiophen-2-yl-boronic acid (2 equiv).
[b] Isolated yields.

diazotization and iodonation of DMA were achieved via Sandmeyer reaction in the presence of KI to afford 1-iodo-3,5-dimethoxybenzene (1), and then demethylation of iodine-substituted methoxy ether (1) smoothly to yield substituted 5-iodobenzene-1,3-diol (2) with 95% of yield (Scheme 1).

Silica sulfuric acid (SSA) is an inexpensive and non toxic catalyst, which can be easily prepared,[16] recycled, and re-used. Using of SSA as catalyst to synthesize coumarins was ever reported,[17] but some drawbacks existed in the corresponding procedure, such as high reaction temperature which resulted in more by-products and incomplete conversion of starting material. In our experiments, compound **2** and β-ketoester were dissolved in CH_2Cl_2 in the presence of SSA and heated to 30 °C in 4–6 h to give desired iodo-substituted 5-hydroxycoumarins (3) via Pechmann condensation. This condensation proceeded smoothly and resulted in the complete conversion of the β-ketoester with good to excellent yield (89–94%) (Scheme 1, compound **3**). The target compounds were obtained after filtration and washing the SSA with ethanol, respectively, and the remaining catalyst could be re-used again.

The ^{1}H NMR data for the phenolic OH signal of **3** were observed in an extremely low field (about 11 ppm), its structure and stereochemistry were further confirmed by the X-ray diffraction analysis (**3b**) (Fig. 2).[18] Finally, the 5-hydroxycoumarin (**3**) was converted to chromene **4** via annulation with 3-methylbut-2-enal (Scheme 1, compound **4**), and also we got the corresponding single crystal of **4b** to confirm its structure (Fig. 2).

Suzuki coupling is a considerable potential for application to the construction of our target molecules since a wide range of substituents on the aryl moiety are tolerated in this reaction,[19] so we hope to form the C–C bond via Suzuki coupling. However, we found that the yield was very low under the literature's condition.[20] In addition, proper selection of solvent and base is also essential, as the process is highly dependent on the conditions used for the couplings, and as little was known concerning the Pd(0) coupling reaction of 7-iodo-5-hydroxy-coumarin bearing an electron-donating hydroxyl group at C-5 with boronic acid derivatives. With this in mind, screening of reaction conditions for the coupling of **3a** with benzo[*b*]thiophen-2-yl-boronic acid to give compound **5a** was investigated, by using $Pd(OAc)_2$, $(dppf)PdCl_2$, or $Pd(PPh_3)_4$ as catalyst, and K_2CO_3, Cs_2CO_3, KF, or CsF as base in the reaction. The corresponding results are summarized in Table 1.

In the course of our experiments, optimum yields (82%) were obtained at 80 °C using degassed dioxane as solvent, CsF as base (6 equiv), and $(dppf)PdCl_2$ (3 mol %) as the catalyst within 16 h. Moreover, target compounds can be easily distinguished and purified due to its intensively fluorogenic speciality. With the optimal reaction conditions in hand, we attempted to explore the scope of substrates by employing a range of thianthren-1-ylboronic acid, 4-(4-boronophenylamino)-4-oxo-butanoic acid, and sulfonamide boronic acid. In general, the cross-coupling of **3b**, **3c**, **3d,** and **4** with benzo[*b*]thiophen-2-yl-boronic acid, thianthren-1-ylboronic acid, 4-(4-boronophenylamino)-4-oxo-butanoic acid, and 4-(methylsulfonamido)phenylboronic acid proceeded well affording the corresponding compounds **5b–d**, **6a–b**, **6d–f**, **6h–i,** and **6k** (Scheme 2).

Interestingly, using the same procedure for the synthesis of **6c**, **6g,** and **6j**, the yields were relatively low (average yields 45%), but when we used 4 equiv of 4-(4-boronophenyl-amino)-4-oxo-butanoic acid, it prolonged the corresponding reaction time and changed the base CsF to KF, and much improvement in yield (58–63%) was obtained.

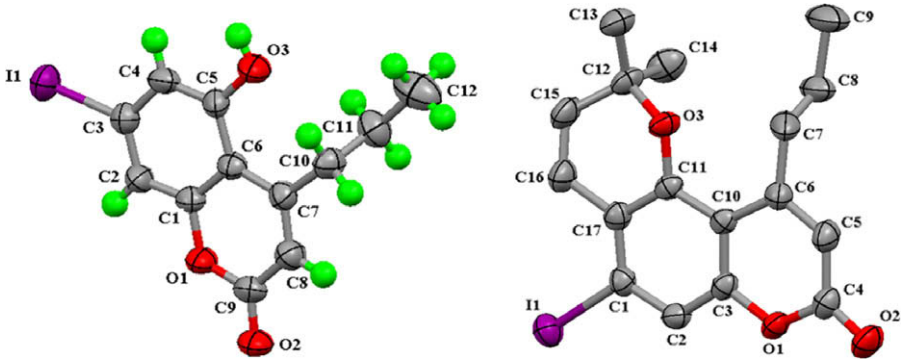
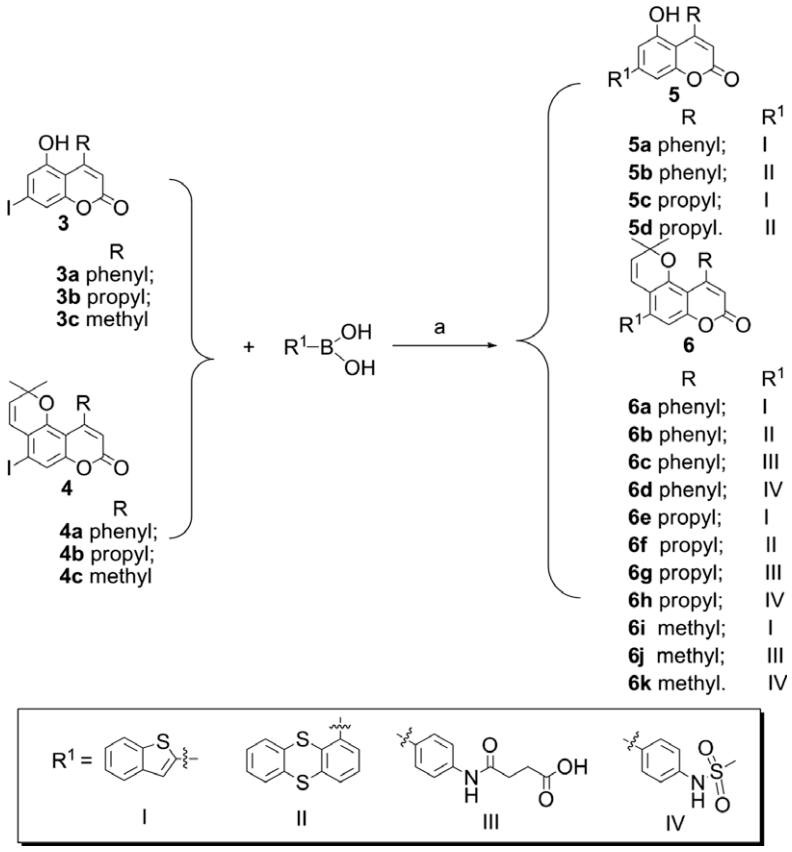


Figure 2. X-ray structure of **3b** and **4b**.



Scheme 2. Reagents and conditions: (a) (dppf)$PdCl_2$, CsF, or KF , dioxane, 80 °C, 58–83%.

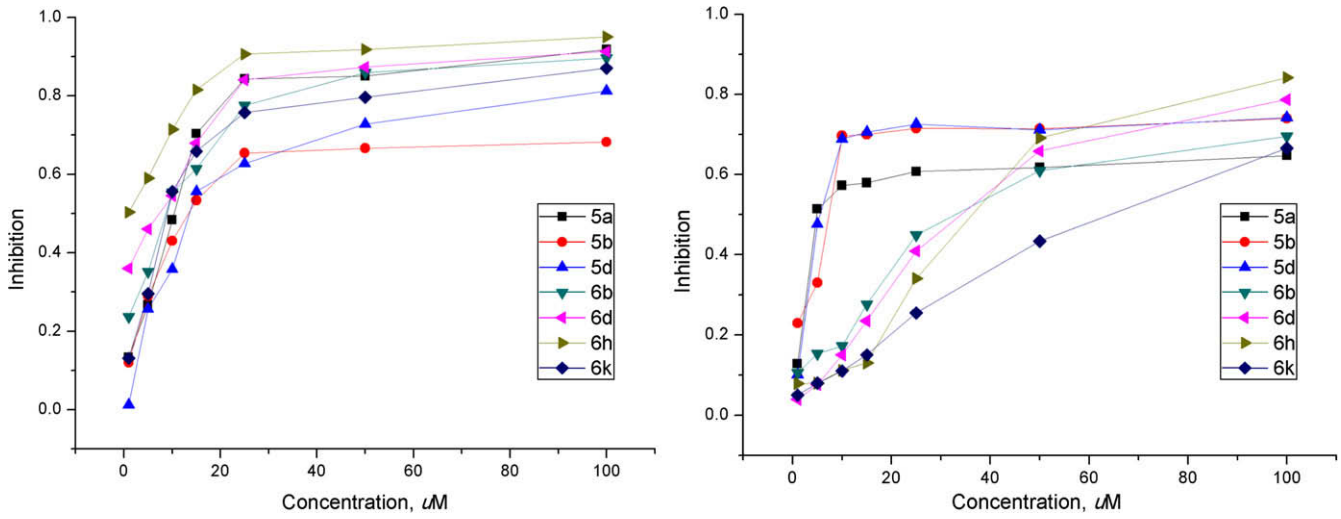


Figure 3. Inhibition of selected compound against breast cancer cell lines MCF-7 (left) and MDA-MB-231 (right) at different concentrations.

Upon completion of their syntheses, compounds **3a–c**, **4a–c**, **5a–d,** and **6a–k** were evaluated for antiproliferative activity in breast cancer cell lines MCF-7 and MDA-MB-231.[21] As shown in Figure 3, except compounds **6c**, **6g,** and **6j**, the others such as **5a–b**, **5d**, **6b**, **6d**, **6h,** and **6k** exhibited antiproliferative activity with good concentration-dependence against MCF-7 cell line, meanwhile, these compounds also exhibited antiproliferative activity with good concentration-dependence against MDA-MB-231 cell line.

The IC_{50} values obtained for these compounds are summarized in Table 2, showing that most of these compounds possess antiproliferative activity ranging from moderate to strong.

Generally, the antiproliferative activities of compounds **5a–d**, **6a–b**, **6d–e**, **6h–i**, and **6k** were better than those of **3a–c** and **4a–c**, suggesting that the incorporation of benzo[*b*]thiophen, thianthren, and sulfamate moieties to the basic structure of hydroxycoumarin and pyranocoumarin could increase antiproliferative activity in breast cancer cell lines MCF-7 and MDA-MB-231. Interestingly, compounds **3a–c**, **4a–c**, **6a**, and **6e** exhibited moderate antiproliferative activity in MCF-7 cell line but produced no activity in MDA- MB-231 cell line. To our surprise, the compounds **6c**, **6g**, and **6j** that bear the same carboxylic acid moiety at pyranocoumarin ring system produced no activity. It is remarkable that

Table 2
Antiproliferative activity of compounds **3a–c**, **4a–c**, **5a–d,** and **6a–k** reported in μM ($n = 3$)[a]

Compounds	MCF-7 (IC_{50})	MDA-MB-231 (IC_{50})
3a	25.0 ± 1.3	na
3b	26.8 ± 1.6	na
3c	18.3 ± 1.0	na
4a	54.3 ± 2.1	na
4b	13.7 ± 0.1	na
4c	30.7 ± 0.4	na
5a	7.6 ± 1.3	10.6 ± 0.8
5b	14.2 ± 2.4	9.3 ± 0.4
5c	22.3 ± 1.5	50.0 ± 1.2
5d	14.4 ± 0.9	7.9 ± 1.0
6a	77.7 ± 0.5	na
6b	7.2 ± 0.7	33.6 ± 1.4
6c	na[b]	na
6d	5.3 ± 1.1	28.9 ± 1.3
6e	22.8 ± 1.5	na
6f	>100	na
6g	na	na
6h	3.3 ± 0.2	27.2 ± 0.1
6i	25.4 ± 1.2	>100
6j	na	na
6k	6.5 ± 0.4	61.2 ± 0.5

[a] Each experiment was independently performed three times and expressed as 'means ± SD'.
[b] Not active.

compounds **5a**, **6b**, **6d**, **6h**, and **6k** exhibit stronger antiproliferative activity than other compounds in MCF-7 cell line, of which **6d**, **6h**, and **6k** were proved to be the most active in MCF-7 cell line with IC_{50} values of 5.3, 3.3, and 6.5 μM, respectively, accounting for bearing the same phenylmethanesulfonamide moiety at their pyranocoumarin ring system. Owing to bearing the benzo[*b*]thiophen or thianthren moiety at hydroxycoumarin system, compounds **5a**, **5b**, and **5d** exhibited strong antiproliferative activity in MDA-MB-231 cell line with IC_{50} values of 10.6, 9.3, and 7.9 μM, respectively.

In conclusion, we have developed a simple, efficient, and mild method for the synthesis of novel hydroxycoumarin and pyranocoumarin derivatives containing sulfamate, benzo[*b*]thiophen, thianthren, and 4-oxo-4-(phenylamino)butanoic acid moieties. The results of antiproliferative assay showed that most of these novel hydroxycoumarin and pyranocoumarin derivatives exhibited moderate to strong antiproliferative activity, suggesting that the sulfamate, benzo[*b*]thiophen, and thianthren moieties on the 5-hydroxycoumarin and pyranocoumarin ring system were effective for antiproliferative activity. Compounds **5a–d**, **6b**, **6d**, **6h**, and **6k** showed significant antiproliferative activity in breast cancer cell lines MCF-7 and MDA-MB-231, and thus could serve as new leads for further development of antibreast cancer agent. The mechanism of action of those compounds on the growth and metabolism of human breast cancer cell lines will be reported in due course.

Acknowledgment

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Supplementary data

Synthetic procedures and characterization for all new compounds, crystal structure data, and anti-proliferative assay are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.098.

References and notes

1. Bravic, G.; Gaultier, J.; Hauw, C. *C. R. Acad. Sci. Paris* **1968**, *267*, 1790.
2. McEvoy, M. T.; Stern, R. S. *Pharmacol. Ther.* **1987**, *34*, 75.
3. Nettleton, D. E. *Drugs Future* **1996**, *34*, 1257.
4. Hoeksema, H.; Caron, E. L.; Hinman, J. W. *J. Am. Chem. Soc.* **1956**, *78*, 2019.
5. Refouvelet, B.; Guyon, C.; Jacquot, Y.; Girard, C.; Fein, H.; Bévalot, F.; Robert, J.-F.; Heyd, B.; Mantion, G.; Richert, L.; Xicluna, A. *Eur. J. Med. Chem.* **2004**, *39*, 931.
6. Taniguchi, M.; Xiao, Y. Q.; Liu, X. H.; Yabu, A.; Hada, Y.; Guo, L. Q.; Yamazoe, Y.; Baba, K. *Chem. Pharm. Bull.* **1999**, *47*, 713.
7. (a) Livingston, A. L.; Bickoff, E. M.; Lundin, R. E.; Jurd, L. *Tetrahedron* **1964**, *20*, 1963; (b) Jacquot, Y.; Rojaz, C.; Refouvelet, B.; Robert, J.-F.; Leclercq, G.; Xicluna, A. *Mini-Rev. Med. Chem.* **2003**, *3*, 387; (c) Roelens, F.; Huvaere, K.; Dhooge, W.; van Cleemput, M.; Comhaire, F.; De Keukeleire, D. *Eur. J. Med. Chem.* **2005**, *40*, 1042.
8. Noeldner, M.; Hauer, H.; Chatterjee, S. S. *Drugs Future* **1996**, *21*, 779.
9. (a) Rosen, S. T.. In *Cancer Chemoprevention*; Bergan, R. C., Ed.; Academic Publisher: New York, 2001; Vol. 137, (b) Labrie, F.; Labrie, C.; Belanger, A.; Simard, J.; Gauthier, S.; Luu-The, V.; Mérand, Y.; Giguere, V.; Candas, B.; Luo, S.; Martel, C.; Singh, S. M.; Fournier, M.; Coquet, A.; Richard, V.; Charbonneau, R.; Charpenet, G.; Tremblay, A.; Tremblay, G.; Cusan, L.; Veilleux, R. *J. Steroid Biochem. Mol. Biol.* **1999**, *69*, 51.
10. (a) Lacy, A.; O'Kennedy, R. *Curr. Pharm. Des.* **2004**, *10*, 3797; (b) Musa, M. A.; Cooperwood, J. S.; Khan, M. O. F. *Curr. Med. Chem.* **2008**, *15*, 2664.
11. (a) Stanchev, S.; Momekov, G.; Jensen, F.; Manolov, I. *Eur. J. Med. Chem.* **2008**, *43*, 694; (b) Thornes, R. D.; Daly, L.; Lynch, G.; Breslin, B.; Browne, H.; Browne, H. Y.; Corrigan, T.; Daly, P.; Edwards, G.; Gaffney, E.; Henley, J.; Healy, F.; Keane, F.; Lennon, F.; McMurray, N.; O'Loughlin, S.; Shine, M.; Tanner, A. *J. Cancer Res. Clin. Oncol.* **1994**, *120*, S32.
12. Stanway, S. J.; Purohit, A.; Woo, L. W.; Sufi, S.; Vigushin, D.; Ward, R.; Wilson, R. H.; Stanczyk, F. Z.; Dobbs, N.; Kulinskaya, E.; Elliott, M.; Potter, B. V.; Reed, M. J.; Coombes, R. C. *Clin. Cancer Res.* **2006**, *12*, 1585.
13. (a) Murray, R. d. *Fortschr. Chem. Org. Naturst.* **2002**, *83*, 1; (b) Houlr, J. R. S.; Paya, M. *Gen. Pharmacol.* **1996**, *27*, 713; (c) Carotti, A.; Carrieri, A.; Chimichi, S.; Boccalini, M.; Cosimelli, B.; Gnerre, C.; Carrupt, P. A.; Testa, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3551.
14. Alonso, M. M.; Encío1, I.; Martínez-Merino, V.; Gil, M.; Migliaccio, M. *Br. J. Cancer* **2001**, *85*, 1400.
15. Hickson, I.; Yan, Z.; Richardson, C. J.; Green, S. J.; Martin, N. M. B.; Orr, A. I.; Reaper, P. M.; Jackson, S. P.; Curtin, N. J.; Smith, G. C. M. *Cancer Res.* **2004**, *64*, 9152.
16. Zolfigol, M. A. *Tetrahedron* **2001**, *57*, 9509.
17. Dabiri, M.; Salehi, P.; Zolfigol, M. A.; Baghbanzadeh, M. *Heterocycles* **2007**, *71*, 677.
18. The crystal structure of **3b** and **4b** has been registered at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 702721 and 702722, for details of the X-ray structure determination see the Supplementarydata.
19. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
20. (a) Hirano, T.; Hiromoto, K.; Kagechika, H. *Org. Lett.* **2007**, *9*, 1315; (b) Rozhkov, R. V.; Davisson, V. J.; Bergstroma, D. E. *Adv. Synth. Catal.* **2008**, *350*, 71.
21. (a) Chen, S.; Cho, M.; Karlsberg, K.; Zhou, D. J.; Yuan, Y. C. *J. Biol. Chem.* **2004**, *279*, 48071; (b) Donnelly, A. C.; Mays, J. R.; Burlison, J. A.; Nelson, J. T.; Vielhauer, G.; Holzbeierlein, J.; Blagg, B. S. J. *J. Org. Chem.* **2008**, *73*, 8901.