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Optimization of 5-phenyl-3-pyridinecarbonitriles as PKCθ inhibitors

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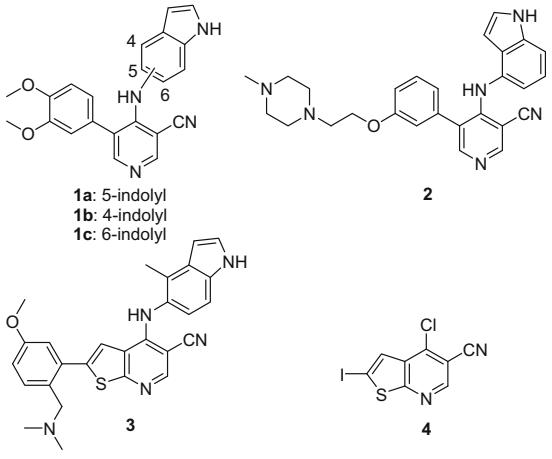
ABSTRACT

The key intermediate, 4-chloro-5-iodo-3-pyridinecarbonitrile, allowed for ready optimization of the PKCθ inhibitory activity of a series of 3-pyridinecarbonitriles. Analog **13b** with a 4-methylindol-5-ylamino group at C-4 and a 4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl group at C-5 had an IC_{50} value of 7.4 nM for the inhibition of PKCθ.

The protein kinase Cs (PKCs) are a family of serine threonine kinases that are subdivided into three classes based on their activation requirements.[1] The classical, or conventional, PKC isoforms, α, βIβII and γ, require the second messengers calcium and diacylglycerol (DAG). The novel isoforms, δ, ε, η, and θ, require DAG but not calcium. The atypical isoforms, ζ and λ, do not require either calcium or DAG. The PKC inhibitors currently in clinical trials are all structurally related to staurosporine. Midostaurin,[2] enzastaurin,[3] and ruboxistaurin,[4] target the classical isoforms, while sotrastaurin[5,6] targets both the classical and novel isoforms.

PKCθ, a novel isoform, is crucial for the activation and survival of T cells.[7–9] Proof-of-concept studies with mice where the PKCθ gene was deleted or 'knocked out' (KO) validated that the inhibition of this kinase could have therapeutic utility in diseases such as multiple sclerosis,[10,11] arthritis,[12] asthma,[13,14] inflammatory bowel disease,[15] and transplant rejection.[16,17] Surprisingly, there are only limited reports in the literature of small molecules designed to be selective inhibitors of PKCθ. Boehringer Ingelheim has disclosed a series of 2,4-diaminopyrimidine inhibitors,[18,19] and we previously reported that a thieno[2,3-*b*]pyridine-5-carbonitrile[20,21] and a 3-pyridinecarbonitrile[22,23] ring system can be used as templates for inhibitors of this kinase. Regarding the 3-pyridinecarbonitriles, the 4-indolyl isomer of **1a**, namely **1b**, was a more potent inhibitor of PKCθ, while the 6-indolylamino isomer, **1c**, was much less active.[23] Addition of a water solubilizing group to the *meta* position of the C-5 phenyl ring of **1b**, led to **2**, which inhibited PKCθ activity with an IC_{50} value of 18 nM.[23] PKCδ was used as the primary counter assay, and **2** inhibited this kinase with an IC_{50} value of 37 nM. Selectivity for PKCθ over PKCδ is thought to be desirable since PKCδ KO mice exhibit an increased proliferation of B cells making them susceptible to autoimmune disease.[24,25]

The SAR studies for the thieno[2,3-*b*]pyridine-5-carbonitriles, exemplified by **3**, were greatly facilitated by intermediate **4** which allowed for ready variation of both the 2 and 4 positions of the core.[20] While our initial route to the 3-pyridinecarbonitriles allowed for ready variation of the group at C-4, it required that the group at C-5 be established early in the synthesis. Therefore, a 3-pyridinecarbonitrile intermediate analogous to **4** was highly desired.



The route used to prepare this key intermediate is shown in Scheme 1. 3-Amino-2-butenenitrile was treated with aqueous hydrochloric acid to give acetoacetonitrile **5**. Reaction of **5** with

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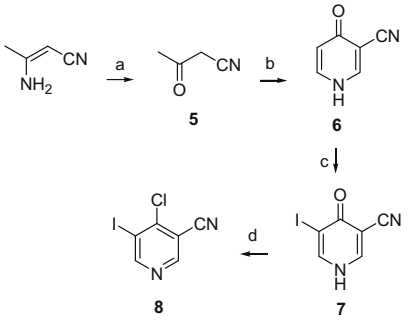
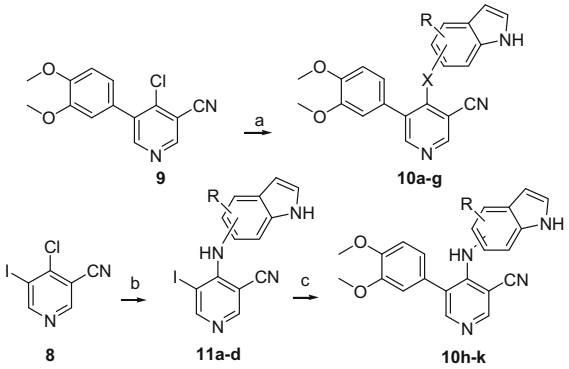


Scheme 1. Reagents: (a) aq HCl; (b) (1) *t*-butoxy bis(dimethylamino)methane, DMF–DMA, (2) NH_4OAc; (c) I_2, aq NaOH; (d) $POCl_3$.



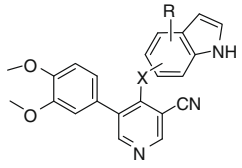
Scheme 2. Reagents: (a) for **10a**: 7-aminoindole, EtOH; for **10b**: 5-methylaminoindole, $EtOCH_2CH_2OH$; for **10c**: 5-hydroxyindole, K_2CO_3, CH_3CN; for **10d**: 5-indolylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$; for **10e**: 5-amino-4-methylindole, $EtOCH_2CH_2OH$; for **10f**: 5-amino-4-ethylindole, $EtOCH_2CH_2OH$; for **10g**: 5-amino-7-methylindole, $EtOCH_2CH_2OH$; (b) for **11a**: 5-amino-6-methylindole, EtOH, Et_3N; for **11b**: 5-amino-4-methoxyindole, EtOH, Et_3N; for **11c**: 5-amino-4-fluoroindole, EtOH, Et_3N; for **11d**: 5-amino-1,4-dimethylindole, EtOH; (c) 3,4-dimethoxyphenylboronic acid, $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$.

t-butoxy bis(dimethylamino)methane and the dimethylacetal of dimethylformamide followed by the addition of ammonium acetate provided the pyridone **6**. Iodination with iodine in aqueous sodium hydroxide gave **7**, with chlorination resulting in the desired intermediate **8**.

The analogs in Table 1 were prepared as shown in Scheme 2, starting from either **8**, or the earlier intermediate **9**. Using **1a** as the reference compound, the 7-aminoindolyl isomer **10a** was prepared from **9** and 7-aminoindole. This isomer was less potent in inhibiting PKCθ than **1a**, having a IC_{50} value of only 740 nM. Next the NH group at C-4 of **1a** was replaced with NMe, O and a bond, to provide **10b–d** all of which had reduced potency compared to **1a**. This SAR is consistent with what was seen in the thieno[2,3-*b*]pyridine-5-carbonitriles, where these changes also resulted in reduced inhibition of PKCθ activity.[20] Addition of a Me group at C-4 of the indole ring of **1a** resulted in increased activity with **10e** having an IC_{50} value of 6.3 nM for the inhibition of PKCθ. This increase was accompanied by enhanced selectivity over PKCδ (IC_{50} = 81 nM). This finding, and that the 4-Et analog, **10f**, had reduced activity, was also observed in the thieno[2,3-*b*]pyridine-5-carbonitrile series. Additional substituents on the indol-5-yl group were then studied.[26] While the 7-Me analog **10g** had an IC_{50} value of only 120 nM, increased potency was seen with the 6-Me analog **10h**. Increased selectivity over PKCδ was also observed, with **10h** having IC_{50} values of 10.5 and 280 nM for the inhibition of PKCθ and PKCδ, respectively. Good selectivity was also seen with the 4-OMe analog **10i** and good PKCθ potency was seen with the 4-F analog **10j**. The 1,4-diMe analog, **10k**, had greatly reduced activity illustrating the importance of the proton at N-1 of the indole.

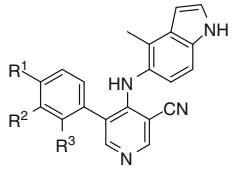
The initial optimization of the substituents on the C-5 phenyl ring was carried out with a 4-methylindol-5-yl group at C-4. As depicted in Scheme 3, **11e** was prepared by reaction of **8** with 5-amino-4-methylindole. Treatment of **11e** with phenylboronic acid under Suzuki conditions provided **12a**. The three isomers of methoxyphenylboronic acid were then coupled to **11e** to provide **12b–d**. As shown in Table 2, the unsubstituted phenyl analog **12a**, had reduced activity compared to the 3,4-dimethoxyphenyl analog **10e**. Walking the OMe group around the phenyl ring showed that the greatest inhibition of PKCθ activity was observed with the *para* OMe analog **12b**. The *para* position of the phenyl ring was therefore chosen as the site to add water solubilizing groups.

Table 1
PKCθ and PKCδ inhibitory activity of C-5 3,4-dimethoxyphenyl analogs with various groups at C-4



Ex	Indole isomer	X	R	PKCθ IC_{50} (nM)[27]	PKCδ IC_{50} (nM)[27]	θ/δ Ratio
1a	5	NH	H	70[22]	350[22]	5.0
1b	4	NH	H	13[23]	62[23]	4.8
1c	6	NH	H	2300[23]	NT[23,27]	NA[27]
10a	7	NH	H	740	NT	NA
10b	5	NMe	H	270	2100	7.8
10c	5	O	H	1700	NT	NA
10d	5	Bond	H	1700	NT	NA
10e	5	NH	4-Me	6.3	81	13
10f	5	NH	4-Et	55	980	18
10g	5	NH	7-Me	120	NT	NA
10h	5	NH	6-Me	10.5	280	27
10i	5	NH	4-OMe	10.6	350	33
10j	5	NH	4-F	5.3	69	13
10k	5	NH	1,4-diMe	260	1400	5.4

Table 2
PKCθ and PKCδ inhibitory activity of C-4 4-methylindol-5-ylamino analogs with various groups on the phenyl ring at C-5

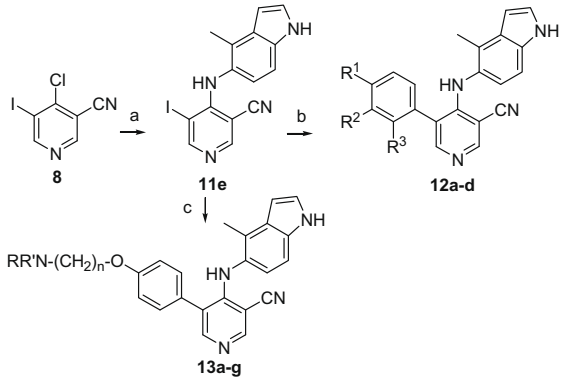


Ex	R^1	R^2	R^3	PKCθ IC_{50} (nM)[27]	PKCδ IC_{50} (nM)[27]	θ/δ Ratio
10e	OMe	OMe	H	6.3	81	13
12a	H	H	H	27	63	2.3
12b	OMe	H	H	5.6	85	15
12c	H	OMe	H	36	210	5.8
12d	H	H	OMe	82	340	4.1
13a	O–CH_2CH_2-morpholine	H	H	20	150	7.5
13b	O–CH_2CH_2–N-Me-piperazine	H	H	7.4	51	6.9
13c	O–CH_2CH_2-pyrrolidine	H	H	4.5	28	6.2
13d	O–CH_2CH_2–N–(CH_2CH_2OH)-piperazine	H	H	14	29	2.1
13e	O–CH_2CH_2–4-pyrrolidin-1-yl-piperidine	H	H	11	30	2.7
13f	O–CH_2CH_2–NH–$CH_2CH_2NMe_2$	H	H	5.9	18	3.1
13g	O–$CH_2CH_2CH_2$–N–Me-piperazine	H	H	19	110	5.8

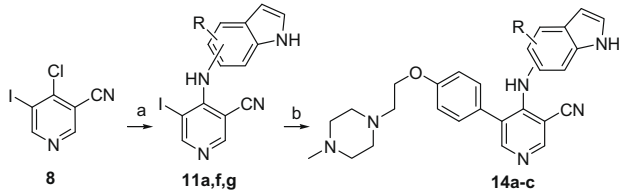
These analogs were prepared as shown in Scheme 3 where the corresponding boronic acids were generated in situ from either 1-bromo-4-(2-chloroethoxy)benzene or 1-bromo-4-(3-chloropropoxy)benzene. Palladium catalyzed coupling of these boronic acids with **11e** gave the intermediate alkyl chloro derivatives which were treated with amines in DME in the presence of NaI to provide the desired products **13a–g**. For analogs **13a–13f** with a 2-ethoxy linker, there was not a large variation in the IC_{50} values for the inhibition of PKCθ (4.5–20 nM). There was also not a large variation in selectivity for PKCθ over PKCδ with **13a–13c** being 6–7.5-fold selective and **13d–f** being 2–3-fold selective. Extension of the ethoxy linker of **13b** by a methylene group to give **13g**, decreased both the PKCθ and PKCδ activity 2-fold.

Keeping the group on the phenyl ring of **13b** constant, analogs were prepared varying the indole at C-4. Key intermediate **8** was reacted with 4-aminoindole and 5-aminoindole to provide **11f** and **11g** (Scheme 4). Conversion of 1-bromo-4-(2-chloroethoxy)benzene to the corresponding boronic acid, Pd catalyzed coupling with **11a**, **11f** and **11g** and displacement of the alkyl chloride with 1-methylpiperazine gave **14a–c**. As shown in Table 3, these three analogs were all weaker PKCθ inhibitors than **13b** and were also less selective over PKCδ. The decrease in selectivity observed with the 6-methylindol-5-yl analog **14c** was surprising since in the C-5 3,4-dimethoxyphenyl series **10h** showed 24-fold selectivity for PKCθ over PKCδ.

Analog **13b** was profiled against additional PKC family members. While **13b** only weakly inhibited PKCβ (IC_{50} = 22 μM) a classical isoform, more potent inhibition of PKCε and PKCη, two novel PKCs was observed, with **13b** having IC_{50} values of 54 and 450 nM, respectively. No inhibition of PKCζ, an atypical isoform, was observed (IC_{50} >100 μM). Additional kinase profiling of **13b** provided IC_{50} values of greater than 10 μM for Lyn, Lck, MK2, p38, IKK,

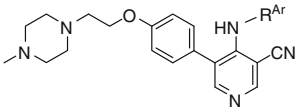


Scheme 3. Reagents: (a) 5-amino-4-methylindole, EtOH; (b) phenylboronic acids, $(Ph_3P)_4Pd$, DME, aq.$NaHCO_3$; (c) for **13a–f**: (1) 1-bromo-4-(2-chloroethoxy)benzene, *n*-BuLi, $B(OiPr)_3$, (2) $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, (3) RR′NH, NaI, DME; (c) for **13g**: (1) 1-bromo-4-(3-chloropropoxy)benzene, *n*-BuLi, $B(OiPr)_3$, (2) $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$; (3) 1-methylpiperazine, NaI, DME.



Scheme 4. Reagents: (a) for **11f**: 4-aminoindole, *n*-PrOH; for **11g**: 5-aminoindole, EtOH; (b) (1) 1-bromo-4-(2-chloroethoxy)benzene, *n*-BuLi, $B(OiPr)_3$, (2) $(Ph_3P)_4Pd$, DME, aq $NaHCO_3$, (3) 1-methylpiperazine, NaI, DME.

Table 3
PKCθ and PKCδ inhibitory activity of C-5 4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl analogs with various groups at C-4



Ex	R^{Ar}	PKCθ IC_{50} (nM)[27]	PKCδ IC_{50} (nM)[27]	θ/δ Ratio
13b	4-Me-5-indolyl	7.4	51	6.9
14a	4-Indolyl	14	41	2.9
14b	5-Indolyl	73	160	2.2
14c	6-Me-5-indolyl	33	130	3.9

PDGFR and ROCK1, demonstrating the selectivity of **13b** for inhibition of the novel PKCs.

The cellular activity of **13b** was evaluated in an assay using murine T cells stimulated with anti-CD3 and anti-CD28 to induce IL-2 expression.[27] With T cells from WT mice, **13b** blocked the production of IL-2 with an IC_{50} value of 160 nM, a 5-fold increase in activity compared to that of **2** (IC_{50} = 850 nM[23]). Reduced activity was seen in a corresponding assay with T cells isolated from PKCθ KO mice where **13b** had an IC_{50} value of greater than 15 μM. In pharmaceutical profiling assays, **13b** had a moderate permeability of 0.28×10^{-6} cm/s in a PAMPA format, with good solubility at pH 7.4 (67 μg/mL). However, in stability studies with liver microsomes from rats, mice, dogs, monkeys and humans, **13b** had half-lives of less than 15 min across the species.

Via the versatile 3-pyridinecarbonitrile intermediate **8**, we are continuing to develop the SAR of this series, with the goals of retaining potent activity against PKCθ and increasing both the selectivity against PKCδ and the metabolic stability. These efforts are focusing on variation of the ring at C-5 and the nature of the water solubilizing group.

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References and notes

1. Spitaler, M.; Cantrell, D. A. *Nat. Immunol.* **2004**, *5*, 785.
2. Fabbro, D.; Ruetz, S.; Bodis, S.; Pruschy, M.; Csermak, K.; Man, A.; Campochiaro, P.; Wood, J.; O'Reilly, T.; Meyer, T. *Anti-Cancer Drug Des.* **2000**, *15*, 17.
3. Sorbera, L. A.; Serradell, N.; Bolos, J.; Rosa, E. *Drugs Future* **2007**, *32*, 297.
4. Taulien, C. A.; Joy, S. V. *Drugs Today* **2006**, *42*, 577.
5. Wagner, J.; Albert, R.; Cooke, N. G.; Cottens, S.; Sedrani, R.; van Eis, M.; von Matt, P.; Ehrhardt, C.; Evenou, J.-P.; Zenke, G.; Brinkmann, V.; Beerli, C.; Brulisauer, A.; Weckbecker, G.; Bigaud, M.; Pally, C.; Wieczorek, G.; Burkhart, C.; Li, J.; Nusslein-Hildesheim, B.; Heusser, C.; Bruns, C.; Morris, R.; Rummel, G.; Stark, W.; Baier, G. *231st ACS National Meeting, Atlanta, GA* **2006**, MEDI-022.
6. Skvara, H.; Dawid, M.; Kleyn, E.; Wolff, B.; Meingassner, J. G.; Knight, H.; Dumortier, T.; Kopp, T.; Fallahi, N.; Stary, G.; Burkhart, C.; Grenet, O.; Wagner, J.; Hijazi, Y.; Morris, R. E.; McGeown, C.; Rordorf, C.; Griffiths, C. E. M.; Stingl, G.; Jung, T. *J. Clin. Invest.* **2008**, *118*, 3151.
7. Baier, G.; Telford, D.; Giampa, L.; Coggeshall, K. M.; Baier-Bitterlich, G.; Isakov, N.; Altman, A. *J. Biol. Chem.* **1993**, *268*, 4997.
8. Hayashi, K.; Altman, A. *Pharmacol. Res.* **2007**, *55*, 537.
9. Marsland, B. J.; Kopf, M. *Trends Immunol.* **2008**, *29*, 179.
10. Salek-Ardakani, S.; So, T.; Halteman, B. S.; Altman, A.; Croft, M. *J. Immunol.* **2005**, *175*, 7635.
11. Tan, S.-L.; Zhao, J.; Bi, C.; Chen, X. Y. C.; Hepburn, D. L.; Wang, J.; Sedgwick, J. D.; Chintalacharuvu, S. R.; Na, S. *J. Immunol.* **2006**, *176*, 2872.
12. Healy, A. M.; Izmailova, E.; Fitzgerald, M.; Walker, R.; Hattersley, M.; Silva, M.; Siebert, E.; Terkelsen, J.; Picarella, D.; Pickard, M. D.; LeClair, B.; Chandra, S.; Jaffee, B. *J. Immunol.* **2006**, *177*, 1886.

13. Marsland, B. J.; Soos, T. J.; Spaeth, G.; Littman, D. R.; Kopf, M. *J. Exp. Med.* **2004**, *200*, 181.
14. Salek-Ardakani, S.; So, T.; Halteman, B. S.; Altman, A.; Croft, M. *J. Immunol.* **2004**, *173*, 6440.
15. Nagahama, K.; Ogawa, A.; Shirane, K.; Shimomura, Y.; Sugimoto, K.; Mizoguchi, A. *Gastroenterology* **2008**, *134*, 459.
16. Manicassamy, S.; Yin, D.; Zhang, Z.; Molinero, L. L.; Alegre, M.-L.; Sun, Z. *J. Immunol.* **2008**, *181*, 513.
17. Wang, L.; Xiang, Z.; Ma, L.-L.; Chen, Z.; Gao, X.; Sun, Z.; Williams, P.; Chari, R. S.; Yin, D.-P. *Transplantation* **2009**, *87*, 507.
18. Cywin, C. L.; Dahmann, G.; Prokopowicz, A. S.; Young, E. R. R.; Magolda, R. L.; Cardozo, M. G.; Cogan, D. A.; DiSalvo, D.; Ginn, J. D.; Kashem, M. A.; Wolak, J. P.; Homon, C. A.; Farrell, T. M.; Grbic, H.; Hu, H.; Kaplita, P. V.; Liu, L. H.; Spero, D. M.; Jeanfavre, D. D.; O'Shea, K. M.; White, D. M.; Woska, J. R.; Brown, M. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 225.
19. Prokopowicz, A. S.; Cywin, C. L.; Dahmann, G.; Young, E. R. R.; Magolda, R. L.; Cardozo, M. G.; Cogan, D. A.; DiSalvo, D.; Ginn, J. D.; Kashem, M. A.; Wolak, J. P.; Homon, C. A.; Farrell, T. M.; Grbic, H.; Hu, H.; Kaplita, P. V.; Liu, L. H.; Spero, D. M.; Jeanfavre, D. D.; O'Shea, K. M.; White, D. M.; Woska, J. M.; Brown, M. L. *Abstracts of Papers, 234th ACS National Meeting, Boston, MA, United States, August 19–23*, **2007**, MEDI-368.
20. Boschelli, D. H.; Wu, B.; Barrios Sosa, A. C.; Chen, J.; Asselin, M.; Cole, D. C.; Lee, J.; Yang, X.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2850.
21. Wu, B.; Boschelli, D. H.; Lee, J.; Yang, X.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 766.
22. Cole, D. C.; Asselin, M.; Brennan, A.; Czerwinski, R.; Ellingboe, J. W.; Fitz, L.; Greco, R.; Huang, X.; Joseph-McCarthy, D.; Kelly, M. F.; Kirisits, M.; Lee, J.; Li, Y.; Morgan, P.; Stock, J. R.; Tsao, D. H. H.; Wissner, A.; Yang, X.; Chaudhary, D. *J. Med. Chem.* **2008**, *51*, 5958.
23. Dushin, R. G.; Nittoli, T.; Ingalls, C.; Boschelli, D. H.; Cole, D. C.; Wissner, A.; Lee, J.; Yang, X.; Morgan, P.; Brennan, A.; Chaudhary, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2461.
24. Miyamoto, A.; Nakayama, K.; Imaki, H.; Hirose, S.; Jiang, Y.; Abe, M.; Tsukiyama, T.; Nagahama, H.; Ohno, S.; Hatakeyama, S.; Nakayama, K. I. *Nature* **2002**, *416*, 865.
25. Mecklenbraeuker, I.; Saijo, K.; Zheng, N. Y.; Leitges, M.; Tarakhovsky, A. *Nature* **2002**, *416*, 860.
26. Synthetic routes to the indole starting materials can be found in US Patent Application 20070287708.
27. For assay protocols see Ref. 22 IC_{50} values represent the mean of at least two determinations. NT: Compound was not tested; NA: Selectivity ratio is not available.