Journal of Medicinal Chemistry Letter

J. Med. Chem. **2009,** *52,* 6193–6196 **6193**
DOI: 10.1021/jm901108b

Discovery of 3-(1*H*-Indol-3-yl)-4-[2-(4-methylpiperazin-1-yl)quinazolin-4-yl]-pyrrole-2,5-dione (AEB071), a Potent and Selective Inhibitor of Protein Kinase C Isotypes†

Jürgen Wagner,* Peter von Matt,* Richard Sedrani, Rainer Albert, Nigel Cooke, Claus Ehrhardt, Martin Geiser, Gabriele Rummel, Wilhelm Stark, Andre Strauss, Sandra W. Cowan-Jacob, Christian Beerli, Gisbert Weckbecker, Jean-Pierre Evenou, Gerhard Zenke, and Sylvain Cottens

Novartis Institutes for BioMedical Research, Basel CH-4002, Switzerland

Received July 27, 2009

Abstract: A series of novel maleimide-based inhibitors of protein kinase C (PKC) were designed, synthesized, and evaluated. AEB071 (**1**) was found to be a potent, selective inhibitor of classical and novel PKC isotypes. **1** is a highly efficient immunomodulator, acting via inhibition of early T cell activation. The binding mode of maleimides to PKCs, proposed by molecular modeling, was confirmed by X-ray analysis of **1** bound in the active site of PKCα.

Solid organ allotransplantation has become a common medical procedure with considerable impact on extending and improving the quality of life of patients with end stage renal, cardiac, hepatic, or pulmonary failure.[1] Currently known immunosuppressants can effectively modulate the immune system and induce acceptance of transplanted organs, but their use is limited by side effects. This challenge is addressed in clinical protocols by the use of drug combination treatments, which commonly consist of a calcineurin inhibitor (CNI,[a] cyclosporine A (CsA), or FK506) inhibiting T cell activation together with a T cell proliferation inhibitor (e.g., mycophenolic acid-based compounds or mTOR inhibitors) and steroids. However, as mechanism-based side effects limit the use of CNIs, a high medical need exists for safe and specific inhibitors of early T cell activation with a novel mechanism of action.

The protein kinase C (PKC) family of serine/threonine kinases consists of 10 isotypes that share sequence and structural homology and that are grouped into three categories based on their cofactor requirements.[2] Classical PKC isotypes α, β, and γ require Ca^{2+} and diacylglycerol (DAG) as cofactors. Novel isotypes δ, ε, η, and θ require DAG but are Ca^{2+} independent, and atypical isotypes ζ and ι/λ require neither Ca^{2+} nor DAG. Several isotypes of PKC play a central role in T cell signaling pathways which translate the engagement of the T cell receptor (TCR) and the coreceptor CD28 into amplification of IL-2 expression and T cell activation.[3] Thus, blockade of PKC is expected to inhibit T lymphocyte activation and to offer opportunities for novel T cell immunomodulators.

In this communication, we report on our project aimed at finding novel, low-molecular weight inhibitors of T cell activation acting via inhibition of classical and novel PKC isotypes. We describe the optimization of inhibitory potency and pharmacokinetic properties of a new class of maleimides. These efforts led to the identification of AEB071 (**1**, INN, sotrastaurin; for short, STN; Figure 1),[4] a representative of a new class of potent and selective PKC inhibitors based on a heteroarylindolylmaleimide scaffold. **1** is currently in phase II clinical trials for the inhibition of solid organ allograft rejection.

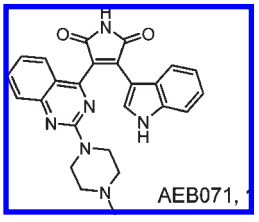


Figure 1. Structure of **1**.

At the outset of the project, typical maleimide-based PKC inhibitors contained two indole moieties at the C3 and C4 positions of the maleimide, with one of these indoles carrying a chain with a basic amine group (e.g., **2**,[5] Table 1).[6] Aiming to expand the structural diversity of the maleimide substituents, we tested the replacement of one of the two indoles with alternative (hetero)aromatic moieties. We first explored the possibility of replacing the unsubstituted indole (Table 1). The inhibitory potency on classical and novel PKC isotypes was determined by scintillation proximity assay (SPA) technology.[7] Results from these assays indicated that neither the 1-naphthyl (**3**),[8] 2-azaindol-3-yl (**4**),[9] nor the benzothiophen-3-yl (**5**)[8] moieties were suitable replacements of the indolyl, as these derivatives showed modest inhibitory activity on PKC (Table 1).

These disappointing results prompted us to refocus our efforts on the other indole and to design compounds in which the basic side chain was appended to a new (hetero)aromatic ring. This derivation strategy turned out to be successful, as it yielded novel, highly potent, and very selective PKC inhibitors (Table 2). The straightforward syntheses of the corresponding phenyl-, naphthyl-, and quinazolinyl-containing maleimide derivatives are described in Schemes 1−3 and are all based on the known base-mediated maleimide formation using a primary amide and a 2-oxoacetic acid ester derivative.[10]

The first compound (**9**) of this series, which contained a 3-(2-dimethylaminoethoxy)phenyl moiety, displayed promising nanomolar inhibitory activity in the biochemical assays on all PKC isoforms tested (Table 2). The docking of **9** into a homology model of PKCθ suggested that the active site could accommodate the extension of the phenyl ring to the

†The atomic coordinates of the X-ray crystal structure of **1** bound PKCα have been deposited with the Protein Data Bank: PDB code 3IW4.

*To whom correspondence should be addressed. For J.W.: phone, +41 61 69 62 385; fax, +41 61 69 62 455; e-mail, juergen.wagner@novartis.com. For P.v.M.: phone, +41 61 32 49 713; fax, +41 61 32 46 121; e-mail, peter.von_matt@novartis.com.

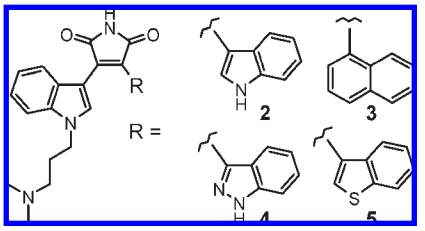
[a] Abbreviations: CNI, calcineurin inhibitor; CsA, cyclosporine A; DAG, diacylglycerol; GvH, rat graft-versus-host model; IL-2, interleukin 2; INN, international nonproprietary name; MLR, mixed lymphocyte reaction; pan-PKC inhibition, simultaneous inhibition of both classical and novel protein kinase C isotypes; PKC, protein kinase C; SPA, scintillation proximity assay; TCR, T cell receptor.

Published on Web 09/18/2009

corresponding naphthyl **10**, with a concomitant increase of lipophilic interactions.[11] In accord with the predictions of this molecular modeling study, derivative **10** afforded substantially improved pan-PKC inhibition (Table 2). **10** was also active in cellular assays, as it inhibited the T cell receptor (TCR)/CD28-mediated T cell activation with an IC_{50} of 69 nM.[11] However, **10** could not be progressed further because its exposure after oral dosing to rats was low, affording an oral bioavailability of only 1% (Table 3). Reasoning that the high basicity of the amine side chain (pK_a(**10**) = 8.4) might negatively impact the pharmacokinetic properties by lowering the levels of free base in the gastrointestinal tract, analogues with lowered basicity were tested. Thus, the replacement of the 2-dimethylaminoethoxy side chain by a piperazine moiety was studied. The *N*-methylpiperazine derivative **11** (pK_a = 7.5) was comparable in terms of inhibitory potency on PKC isoforms to the corresponding open chain phenyl derivative **9** (Table 2), indicating that this structural modification was tolerated. Furthermore, extending the phenyl ring to the naphthyl derivative **12** resulted in potent PKC inhibition and strong blockade of TCR/CD28-mediated T cell activation with an IC_{50} of 50 nM. Gratifyingly, the pharmacokinetic properties of **12** were significantly improved relative to **10** (Table 3). The exposure of **12** after oral dosing was more than 10-fold higher than that of **10**, which translated into an oral bioavailability of 12%. Further fine-tuning involved the replacement of the naphthyl of **12** with a quinazoline moiety. This modification led to AEB071 (**1**), a highly potent PKC inhibitor with IC_{50} in the single digit nanomolar range on all classical and novel PKC isotypes assayed.[12] **1** inhibited the TCR/CD28-mediated T cell activation with an IC_{50} of 54 nM. Most importantly, **1** (pK_a = 7.4) afforded good exposure after oral dosing and an oral bioavailability of 34%. The distal basic amine in the side chain of **1** is essential, since the corresponding morpholine derivative **13** displayed greatly reduced inhibitory activity. As expected, this weak inhibiton of PKC isoforms translated into substantially attenuated activity in the cellular assay.

The excellent fit of **1** to the active site of PKC isotypes was also revealed by an X-ray crystal structure of **1** bound to the catalytic domain of PKCα, which was obtained at 2.8 Å resolution (Figure 2). **1** binds in the ATP pocket through two hydrogen bonds with residues Glu418 and Val420 of the hinge region of the protein. A third hydrogen bond is formed

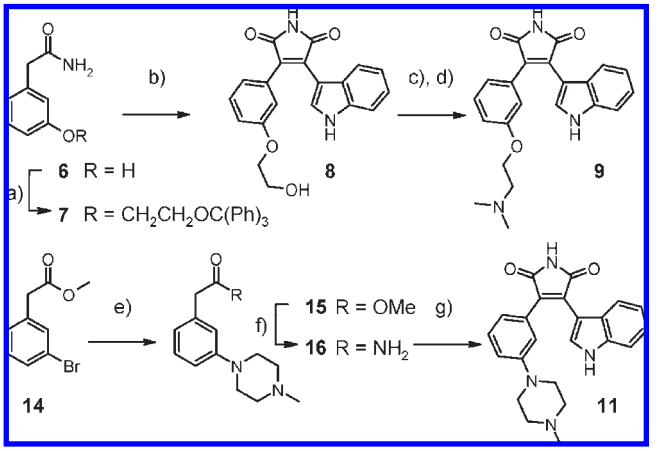
Table 1. Enzymatic Activity of Maleimide Derivatives **2**–**5**[a]



compd	PKCα	PKCβI	PKCδ	PKCε	PKCη	PKCθ
2	2.3 ± 0.7	1.6 ± 0.03	8.7 ± 1.5	6.6 ± 0.9	10 ± 2	6.7 ± 0.3
3	98 ± 2	60 ± 7	207 ± 5	> 315	349 ± 28	165 ± 50
4	> 315	> 315	> 315	> 315	> 315	> 315
5	73 ± 20	47 ± 5	183 ± 10	285 ± 29	293 ± 23	255 ± 18

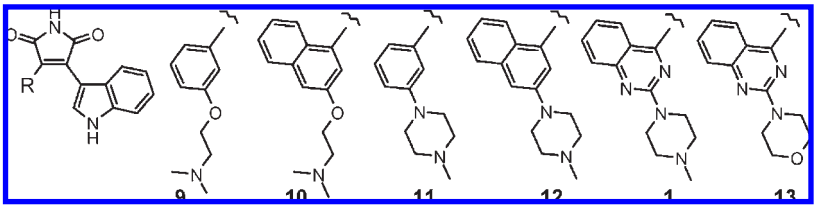
[a] IC_{50} values are reported in nM as the average ± SEM of ≥3 experiments.

Scheme 1[a]



[a] Reagents and conditions: (a) $TrOCH_2CH_2Br$, K_2CO_3, Bu_4NI, DMF, 60 °C, 75%; (b) (i) (1*H*-indol-3-yl)oxoacetic acid methyl ester, KO^tBu, THF, 0 °C to room temp, (ii) conc HCl (aq), 32%; (c) CH_3SO_2Cl, NEt_3, CH_2Cl_2, −20 °C, 57%; (d) $HNMe_2$ (33% in EtOH), THF, room temp, 64%; (e) 0.01 equiv of $Pd(OAc)_2$, 0.015 equiv of *rac*-BINAP, *N*-methylpiperazine, Cs_2CO_3, toluene, 100 °C, 27%; (f) NH_3, MeOH, 140 °C, 53%; (g) 1.2 equiv of (1*H*-indol-3-yl)oxoacetic acid methyl ester, 5.0 equiv of KO^tBu, DMF, 85 °C, 40%.

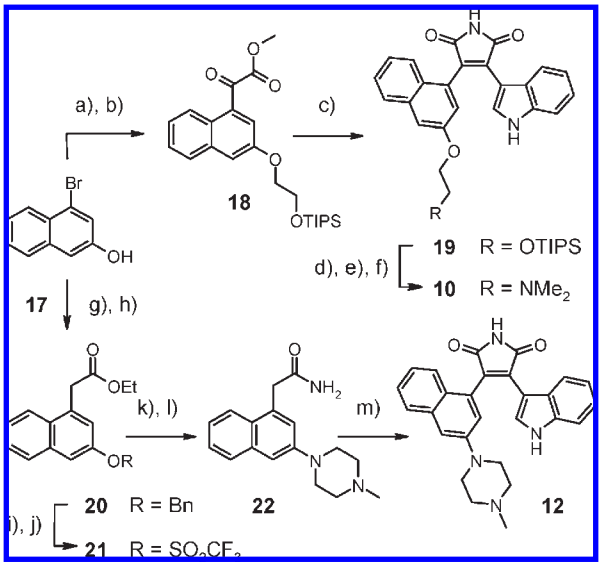
Table 2. Enzymatic and Cellular Activity of Maleimide Derivatives with Different Aromatic Moieties Bridging the Maleimide with the Basic Side Chain[a]



compd	PKCα	PKCβI	PKCδ	PKCε	PKCη	PKCθ	TCR/CD28
9	15 ± 2	58 ± 1	47 ± 6	29 ± 1	47 ± 11	52 ± 1	668 ± 54
10	2.2 ± 0.4	2.1 ± 0.1	4.4 ± 0.6	5.5 ± 0.7	11 ± 1	3.3 ± 0.3	69 ± 13
11	7.1 ± 2.0	36 ± 9	97 ± 25	59 ± 4	36 ± 2	76 ± 11	277 ± 45
12	0.9 ± 0.1	1.8 ± 0.2	7.3 ± 1.3	19 ± 6	25 ± 6	4.8 ± 0.6	50 ± 12
1	2.1 ± 0.2	2.0 ± 0.1	1.3 ± 0.1	6.2 ± 0.6	6.1 ± 0.4	1.0 ± 0.1	54 ± 8
13	1502 ± 556	1268 ± 450	794 ± 219	1825 ± 691	2545 ± 1006	1382 ± 486	> 3700

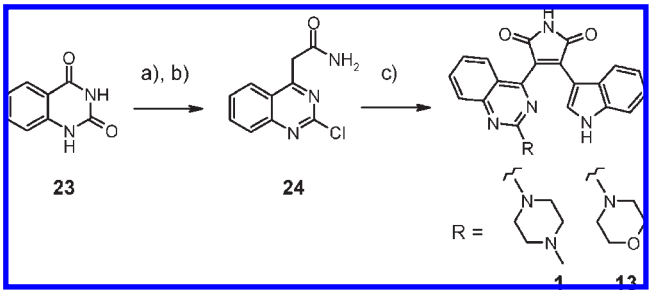
[a] IC_{50} values are reported in nM as the average ± SEM of ≥3 experiments.

Scheme 2[a]



[a] Reagents and conditions: (a) $TIPSOCH_2CH_2Br$, K_2CO_3, TBAI, DMF, 60 °C, 84%; (b) *n*-BuLi, oxalic acid dimethyl ester, THF, 0 °C, 63%; (c) 2-(1*H*-indol-3-yl)-acetamide, KO^{t}Bu, THF, 70 °C, 49%; (d) TBAF, THF, 0 °C, 95%; (e) $(CH_3SO_2)_2O$, pyridine, THF, 60 °C, 97%; (f) $HNMe_2$ (33% in EtOH), room temp, 70%; (g) NaH, BnBr, Bu_4NI, DMF, 93%; (h) tributylstannanylacetic acid ethyl ester, $ZnBr_2$, 0.2 equiv of $PdCl_2[P(o\text{-tolyl})_3]_2$, DMF, 80 °C, 42%; (i) Pd/C, H_2, MeOH, room temp, 95%; (j) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, room temp, 89%; (k) 0.05 equiv of $Pd_2(dba)_3$, 0.05 equiv of 2-(di-*tert*-butylphosphino)-biphenyl, 1-methylpiperazine, K_3PO_4, THF, 80 °C, 84%; (l) formamide, NaOMe, DMF, 105 °C, 92%; (m) (1*H*-indol-3-yl)oxoacetic acid methyl ester, KO^{t}Bu, THF, room temp, 66%.

Scheme 3[a]



[a] Reagents and conditions: (a) $POCl_3$, dimethylphenylamine, 150 °C, 81%; (b) (i) 3-oxobutyric acid ethyl ester, NaH, THF, 0 °C, then toluene, 140 °C; (ii) NH_4OH, room temp; (iii) EtOAc, 76 °C, 54%. For **1**: (c) (i) 1-methylpiperazine, 1-methyl-2-pyrrolidinone, 50 °C, 69%; (ii) (1*H*-indol-3-yl)oxoacetic acid methyl ester, KO^{t}Bu, THF, 0 °C to room temp, 47%. For **13**: (c) (i) morpholine, 1-methyl-2-pyrrolidinone, 50 °C, quant; (ii) (1*H*-indol-3-yl)oxoacetic acid methyl ester, KO^{t}Bu, THF, 0 °C to room temp, 25%.

between the distal nitrogen atom of the piperazine ring of **1** and the backbone carbonyl of Asp467 from the catalytic loop. van der Waals interactions make a strong contribution to the protein−ligand affinity. In particular, the hydrophobic contacts exist between the indolyl moiety of **1** and residues Leu345, Phe350, and Val353 from the glycine rich loop, where the tip of the loop bends down toward **1** and the phenylalanine side chain folds underneath it to form a complementary surface for the inhibitor.

1 is a highly selective inhibitor of PKC isotypes, since in a panel of selected kinases, the only enzyme that **1** inhibited with

Table 3. Pharmacokinetic Properties of Compounds **10**, **12**, and **1** in Rats[a]

	10	12	1
Intravenous Administration (iv), Dose = 5 mg/kg			
AUC_∞ (ng h mL^{-1})	2694	4320	1629
$t_{1/2E}$ (h)	20.6	4.5	3.2
Cl (mL min^{-1} kg^{-1})	30.9	19.3	51.2
V_{ss} (L/kg)	48.2	12.0	6.5
Oral Administration (po), Dose = 20 mg/kg			
T_{max} (h)	0.5	2.0	0.5
C_{max} (ng/mL)	16.2	242	622
AUC_∞ (ng h mL^{-1})	154	1992	2208
F (%)	1	12	34

[a] Data reported as an average of three animals (OFA rats for **10** and **1**; Lewis rats for **12**). Vehicle for iv: 75% saline (0.9%)−25% PEG400. Vehicle for po: 75% water−25% PEG400. AUC_∞: area under the curve, extrapolated to infinity.

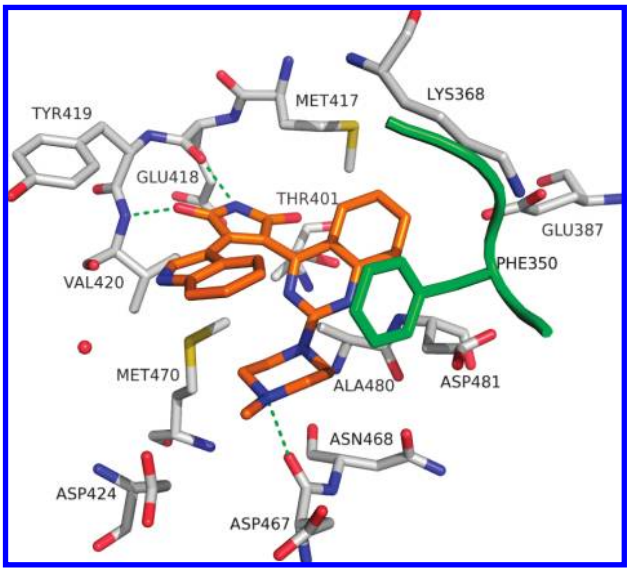


Figure 2. Detailed view of the X-ray crystal structure of **1** (carbons in orange) bound to the ATP-binding site of PKCα (white, with the glycin-rich loop in green). Hydrogen bonds formed between the ligand and the protein are represented by dashed green lines.

$IC_{50} < 1\,\mu M$ was GSK3β.[11] However, in a GSK3β-dependent cellular assay, **1** was active in the micromolar range only. **1** inhibited the mouse and human mixed lymphocyte reactions (MLR, in vitro T cell immune response assays),[11] with IC_{50} of 128 and 34 nM, respectively. For comparison, IC_{50} values of cyclosporine A in mouse and human MLR of 17 and 10 nM, respectively, were measured. **1** is not a general inhibitor of cell proliferation, as it affected the proliferation of mouse bone marrow cells only in the micromolar range ($IC_{50} = 3.7\,\mu M$).[11] Taken together, this cellular profile indicated that **1** exerted its immunomodulating effect via selective inhibition of early T cell activation. In vivo efficacy of **1** was demonstrated in the localized rat graft-versus-host model (GvH).[11] Furthermore, **1**, alone or in combination with adjunct immunosuppressive agents, prolonged rat heterotopic heart transplant survival and cynomolgus monkey renal allograft survival.[11]

In summary, **1** was found to be a potent and highly selective low molecular weight inhibitor of novel and classical PKC isotypes and was shown to be an effective inhibitor of early T-cell activation. **1** has demonstrated efficacy in a clinical

proof-of-concept study in psoriasis at well tolerated doses[13] and is currently in phase II clinical trials for the prevention of solid organ allograft rejection.

Supporting Information Available: Synthetic procedures and characterization data for **1** and **9**−**13**, docking study of **9** to a PKCθ homology model, kinase selectivity profile of **1**, and biological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Liu, E. H.; Siegel, R. M.; Harlan, D. M.; O'Shea, J. J. T Cell-Directed Therapies: Lessons Learned and Future Prospects. *Nat. Immunol*. **2007**, *8*, 25–30.

(2) Newton, A. C. Regulation of the ABC Kinases by Phosphorylation: Protein Kinase C as a Paradigm. *Biochem. J*. **2003**, *370*, 361–371.

(3) Marsland, B. J.; Kopf, M. T-Cell Fate and Function: PKC-θ and Beyond. *Trends Immunol*. **2008**, *29*, 179–185. Gruber, T.; Hermann-Kleiter, N.; Pfeifhofer-Obermair, C.; Lutz-Nicoladoni, C.; Thuille, N.; Letschka, T.; Barsig, J.; Baudler, M.; Li, J.; Metzler, B.; Nüssllein-Hildesheim, B.; Wagner, J.; Leitges, M.; Baier, G. PKCθ Cooperates with PKCα in Alloimmune Responses of T Cells in Vivo. *Mol. Immunol*. **2009**, *46*, 2071–2079.

(4) Albert, R.; Cooke, N. G.; Cottens, S.; Ehrhardt, C.; Evenou, J.-P.; Sedrani, R.; von Matt, P.; Wagner, J.; Zenke, G. Indolylmaleimide Derivatives as Protein Kinase C Inhibitors. PCT Int. Appl. WO 2002/038561 A1, **2002**.

(5) Barth, H.; Hartenstein, J.; Rudolph, C.; Schaechtele, C.; Betche, H. J.; Osswald, H.; Reck, R. Preparation of 3,4-Bis(indol-3-yl)-maleimides as Protein Kinase C Inhibitors. Eur. Pat. Appl. EP 397060, **1990**. **2** is also referred to as bisindolylmaleimide I (BIM I), Goe6850, or GF109203X.

(6) Hu, H. Recent Discovery and Development of Selective Protein Kinase C Inhibitors. *Drug Discovery Today* **1996**, *1*, 438–447. Pindur, U.; Kim, Y. S.; Mehrabani, F. Advances in Indolo[2,3-a]-carbazole Chemistry: Design and Synthesis of Protein Kinase C and Topoisomerase I Inhibitors. *Curr. Med. Chem*. **1999**, *6*, 29–69 and references cited therein.

(7) Evenou, J.-P.; Wagner, J.; Zenke, G.; Brinkmann, V.; Wagner, K.; Kovarik, J.; Welzenbach, K. A.; Weitz-Schmidt, G.; Guntermann, C.; Towbin, H.; Cottens, S.; Kaminski, S.; Letschka, T.; Lutz-Nicoladoni, C.; Gruber, T.; Hermann-Kleiter, N.; Thuille, N.; Baier, G. The Potent Protein Kinase C Selective Inhibitor AEB071 (Sotrastaurin) Represents a New Class of Immunosuppressive Agents Affecting Early T Cell Activation. *J. Pharmacol. Exp. Ther*. **2009**, *330*, 792–801.

(8) Zhang, H.-C.; Maryanoff, B. E.; McComsey, D. F.; White, K.; Ye, H.; Hecker, L.; Conway, B. R.; Demarest, K. Substituted Pyrrolines as Kinase Inhibitors. PCT Int. Appl. WO 2003/103663 A3, **2003**.

(9) Kozikowski, A. P.; Gaysina, I.; Petukhov, P.; Sridhar, J.; Tueckmantel, W. Inhibitors of Glycogen Synthase Kinase 3. PCT Int. Appl. WO 2007/008514, **2007**.

(10) Faul, M. M.; Winneroski, L. L.; Krumrich, C. A. A New, Efficient Method for the Synthesis of Bisindolylmaleimides. *J. Org. Chem*. **1998**, *63*, 6053–6058. Experimental details to the reactions shown in Schemes 1−3 are described in the Supporting Information.

(11) Details are described in the Supporting Information.

(12) **1** was only weakly active on the atypical PKCζ (IC_{50} = 5400 nM).

(13) 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. The PKC Inhibitor AEB071 May Be a Therapeutic Option for Psoriasis. *J. Clin. Invest*. **2008**, *118*, 3151–3159.