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Synthesis, SAR studies, and pharmacological evaluation of 3-anilino-4-(3-indolyl) maleimides with conformationally restricted structure as orally bioavailable PKCβ-selective inhibitors

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Abstract—Conformationally restricted 3-anilino-4-(3-indolyl)maleimide derivatives were designed and synthesized aiming at discovery of novel protein kinase Cβ (PKCβ)-selective inhibitors possessing oral bioavailability. Among them, compounds having a fused five-membered ring at the indole 1,2-position inhibited PKCβ2 with IC_{50} of nM-order and showed good oral bioavailability. One of the most potent compounds was found to be PKCβ-selective over other 6 isozymes and exhibited ameliorative effects in a rat diabetic retinopathy model via oral route.

1. Introduction

Diabetes mellitus is one of the representative lifestyle-related diseases. The number of diabetic patients is over 150 million worldwide and continues to mount, especially in developed countries. Thanks to a variety of medicines including sulfonylureas, biguanide, thiazolidinediones, and α-glycosidase inhibitors, it has become possible to control patient's blood glucose level. Even with these medications, however, many patients are at risk for developing diabetic microvascular complications such as diabetic neuropathy, nephropathy, and retinopathy after suffering from long-term diabetes. Therefore, there is a need for agents which directly block signal transduction pathways leading to the onset of diabetic microvascular complications independent of blood glucose level control, thus improving the quality of life for the patient with late-stage diabetes mellitus. Recently, the signal transduction pathway has become clearer and protein kinase Cβ (PKCβ) activation was found to be implicated in the onset of diabetic microvascular complications.[1] A predominant factor of PKCβ activation is enhanced de novo synthesis of diacylglycerol (DAG) in hyperglycemia. Other factors associated with the activation of PKCβ include increased advanced glycated end products (AGE) and elevated oxidative stress in diabetic conditions.[2] Thus, activated PKCβ damages microvascular systems and causes a variety of symptoms of diabetic microvascular complications; hence PKCβ-selective inhibitors are expected to become therapeutic agents for diabetic microvascular complications.[3]

The discovery of staurosporine, a non-selective PKC inhibitor produced by *Streptomyces* sp., facilitated synthesis of numbers of its structural analogues possessing various inhibitory activities and selectivities.[4] Among them, ruboxistaurin[3a,5] exhibited selective PKCβ inhibitory activity and ameliorative effects in diabetic complications in clinical studies, demonstrating significant contribution of PKCβ to the onset of these diseases.[6] Ruboxistaurin selectively inhibits PKCβ1 and β2 with IC_{50} values of 4.7 and 5.9 nM, respectively, and IC_{50} for other PKC isoforms of 250 nM or greater.[3a] From the point of view of structural features, the hitherto synthesized staurosporine derivatives including ruboxistaurin were within a series having a pharmacophore of bisindolylmaleimide (Ro 31-6233 (**1**))[7] or a staurosporine-like carbazole substructure (Fig. 1). We

Keywords: PKCβ selective inhibitor; 3-Anilino-4-(3-indolyl)maleimide; Orally active compounds; Diabetic complications.
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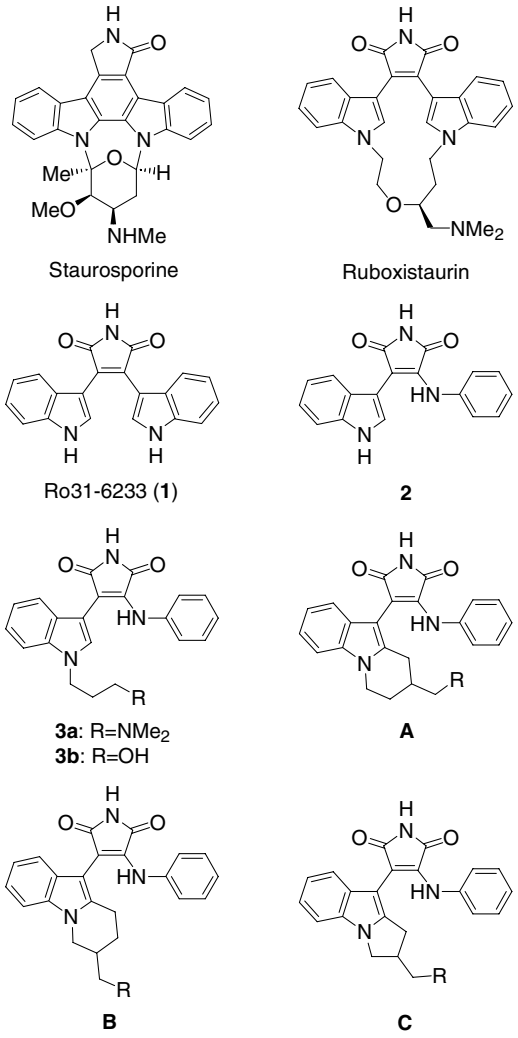


Figure 1. Structures of staurosporine and its derivatives.

reported a new structural class of PKCβ-selective inhibitors, which possess anilino-monoindolylmaleimides (**2**) as the pharmacophore.[8] The representative compound **3a** selectively inhibited PKCβ1 and β2 with IC_{50} values of 21 and 12 nM, respectively. Most recently, Zhang et al. have also reported a series of indolylindazolylmaleimides as an additional new structure class of PKCβ-selective inhibitors with inhibitory activity in the nM range.[9] We expected high in vivo efficacy for the previously reported derivatives of **3**, however they were ineffective in all in vivo models because of a lack of oral bioavailability. In this paper, we report the synthesis of further derivatives of compound **3**, which showed good oral bioavailability as well as curative effects in an animal model for diabetic complications. The most potent compound (***R***)-**23g** not only exhibited strong PKCβ inhibition in vitro (IC_{50} of 1.4 nM at PKCβ2), but also improved retinal vascular blood flow rate (an index of diabetic retinopathy) in the streptozotocin (STZ) induced diabetic rat model via oral administration.[3a]

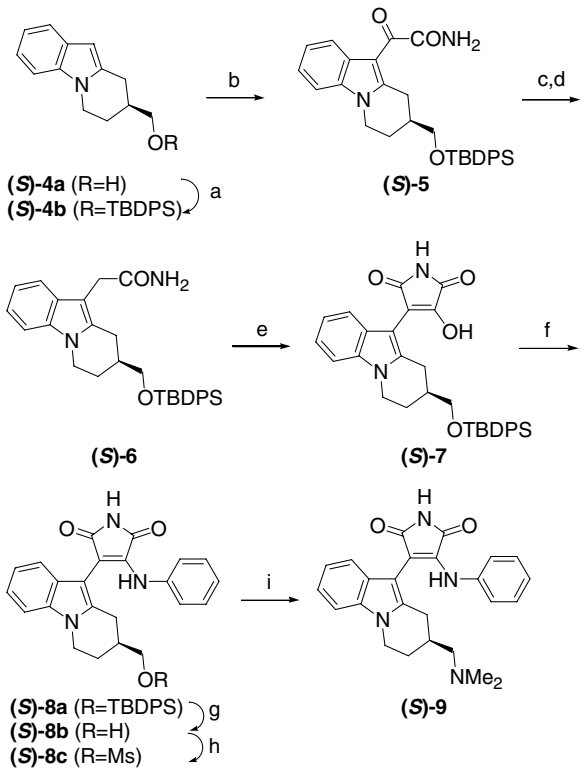
The SAR study was initially conducted based on PKCβ2 isozyme inhibitory activity since PKCβ1 and PKCβ2 had high degrees of homology, and previously synthesized derivatives of **3** inhibited both isozymes to approximately the same degree. The isozyme selectivity was monitored using selectivity over PKCα, which among the PKC family shares highest homology with PKCβ.[10] Based on these in vitro data we selected compound (***R***)-**23g**, and investigated a more detailed isozyme inhibitory profile as well as in vivo efficacy for this compound.

2. Design and synthesis

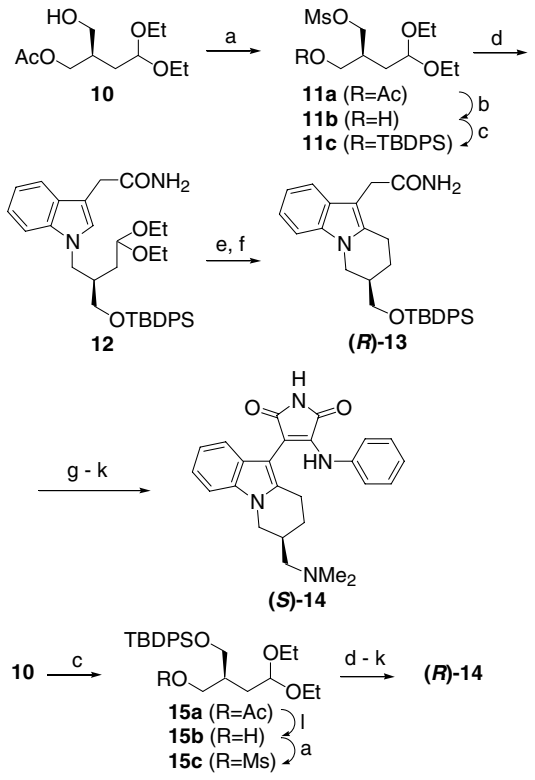
With the goal of obtaining more potent and orally available PKCβ-selective inhibitors starting from **3**, we designed cyclic derivatives including 8-substituted-6,7,8,9-tetrahydropyrido[1,2-*a*]indoles (series A), 7-substituted-6,7,8,9-tetrahydropyrido[1,2-*a*]indoles (series B), and 2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indoles (series C). In the previous SAR study of acyclic derivatives of **3**, we found a dramatic rise in potency when hydrophilic groups (R) were placed at the indole nitrogen of compound **2** via a three- to four-carbon linker so that the hydrophilic group could associate with the carboxylic acid residue of Asp427 and/or the main chain amide of Asp470 of PKCβ.[11] For instance, **3a** and **3b** were approximately 20 times and 10 times more potent than **2**, respectively. It was expected that, in these conformationally restricted cyclic systems, substituting a hydrophilic group would bring about elevated activity compared to the previous acyclic series due to a smaller change in entropy upon association with these amino acids in PKCβ. We also anticipated improvement in oral bioavailability in these cyclic derivatives based on a report that demonstrated that fewer rotatable bonds tended to have a positive effect on bioavailability in general.[12] These previous studies led us to develop a rational synthesis for these conformationally restricted cyclic compounds, with the intention of improving potency as well as oral bioavailability.

8-Substituted-6,7,8,9-tetrahydropyrido[1,2-*a*]indoles (series A, (***S***)-**8b** and (***S***)-**9**) were synthesized as shown in Scheme 1. Both enantiomers, (***S***)- and (***R***)-**4a**, were available in optically active forms using the method described in the literature.[13] After silyl protection of the hydroxyl group of (***S***)-**4a**, coupling reaction with oxalyl chloride followed by quenching with aqueous ammonia gave (***S***)-**5**,[14] which was then sequentially reduced with $NaBH_4$ and hydrosilane to provide (***S***)-**6**. Condensation of (***S***)-**6** with dimethyl oxalate in the presence of *t*-BuOK gave (***S***)-**7**.[15] The hydroxyl group of the maleimide of (***S***)-**7** was successfully replaced with aniline by heating with excess aniline in acetic acid to afford (***S***)-**8a**. Then the silyl protection was removed by TBAF to afford (***S***)-**8b**. The regenerated hydroxyl group was replaced with dimethylamine via the corresponding mesylate (***S***)-**8c** to give (***S***)-**9**. The antipode was similarly prepared from (***R***)-**4a**.

As shown in Scheme 2, the *S* and *R* enantiomers of 7-substituted-6,7,8,9-tetrahydropyrido[1,2-*a*]indoles (series B, **14**) were both synthesized from (*R*)-2-(2,2-diethoxyethyl)-1,3-propanediol monoacetate (**10**)[16]



Scheme 1. Reagents: (a) TBDPSCl, imidazole; (b) i. $(COCl)_2$, Et_3N, ii. aq NH_3; (c) $NaBH_4$; (d) TESH, TFA; (e) $(CO_2Me)_2$, *t*-BuOK; (f) $PhNH_2$, AcOH; (g) TBAF; (h) Ms_2O, pyridine; (i) Me_2NH.



Scheme 2. Reagents: (a) MsCl, Et_3N; (b) aq NaOH; (c) TBDPSCl, imidazole; (d) indole-3-acetamide, NaH; (e) TFA, H_2O; (f) H_2, 5% Pd–C; (g) $(CO_2Me)_2$, *t*-BuOK; (h) $PhNH_2$, AcOH; (i) TBAF; (j) Ms_2O, pyridine; (k) Me_2NH; (l) K_2CO_3, EtOH.

through **11c** and **15c**, respectively. **11c** was prepared by mesylation of the hydroxyl group of **10** followed by replacement of the acetyl group with the silyl-protective group, whereas **15c** was obtained by silyl-protection of the hydroxyl group of **10** prior to removal of the acetyl group, and subsequent mesylation. Alkylation of indole-3-acetamide with **11c** yielded **12** which was then converted into (***R***)-**13** via intramolecular Pictet-Spengler cyclization[17] and subsequent catalytic hydrogenation. The target compound (***S***)-**14** was achieved from (***R***)-**13** as in the preparation of (***S***)-**9** from (***S***)-**6** described in Scheme 1. The antipode ((***R***)-**14**) was similarly obtained from **15c**.

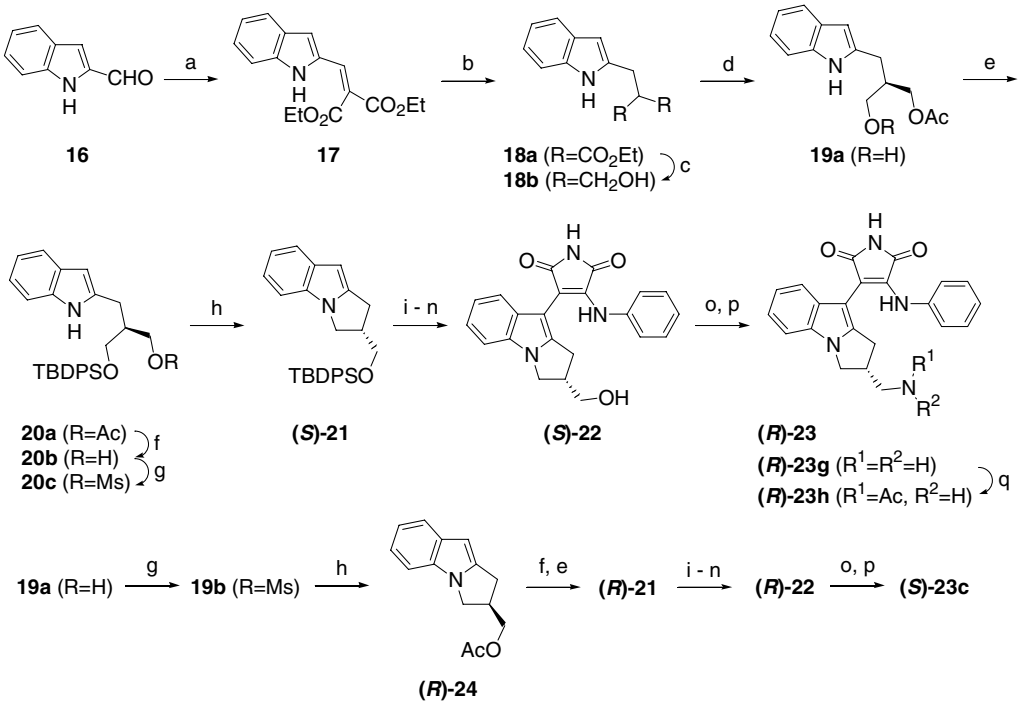
As shown in Scheme 3, *R* and *S* enantiomers of 2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indoles (series C, **23**) were both prepared from optically active monoacetate (**19a**) through (***S***)-**21** and (***R***)-**21**, respectively. Commercially available indole-2-carboxaldehyde (**16**) was transformed into diester (**17**) by Knoevenagel condensation reaction with diethylmalonate. Diol (**18b**) was obtained from **17** by 1,4-reduction with $NaBH_4$, followed by reduction of diethylesters with $LiAlH_4$. Lipase-catalyzed asymmetric acetylation[16,18] of **18b** was effective to obtain optically active **19a**, which was then cyclized into (***S***)-**21**[19] through conversion of the acetoxy group to a mesyl leaving group by sequential procedures containing silyl-protection of the hydroxyl group of **19a**, saponification of the acetoxy group, mesylation of the newly generated hydroxyl group and cyclization with sodium hydride. Meanwhile, (***R***)-**21** was prepared through (***R***)-**24**, which was obtained by cyclization of **19a** after conversion of the hydroxyl group into a mesyl leaving group. (***S***)-**21** and (***R***)-**21** were, respectively, transformed into (***R***)-**23** and (***S***)-**23** via (***S***)-**22** and (***R***)-**22** with the similar methods used in Scheme 1. The enantioselectivity of this enzymatic esterification was confirmed to be 90–95% by conversion of (***S***)-**21** into the corresponding MTPA ester.

The absolute chemistry of **23** resulted from the enzymatic differentiation of the two symmetric primary hydroxyl groups of **18b**. To investigate the enzymatic preference and determine the absolute configuration of **23**, (***S***)-**24** was synthesized using an alternative route as shown in Scheme 4. The racemic mixture of *cis*-hydroxy ester **26** obtained by Pd-catalyzed reduction of **25**[20] was chromatographically separated into two diastereomers of the Boc-L-alanine esters **27** and **28**. The absolute structure of the crystalline isomer **28** was successfully determined as depicted in Scheme 4 by X-ray crystallographic analysis.[21] Since (***S***)-**24** obtained from **28** showed $[\alpha]_D$ of +23.8°, the absolute stereochemistry of (***S***)-**23** derived from (***R***)-**24** having $[\alpha]_D$ of −20.4° was determined to be *S* and that of antipode (***R***)-**23** was assigned to *R*.

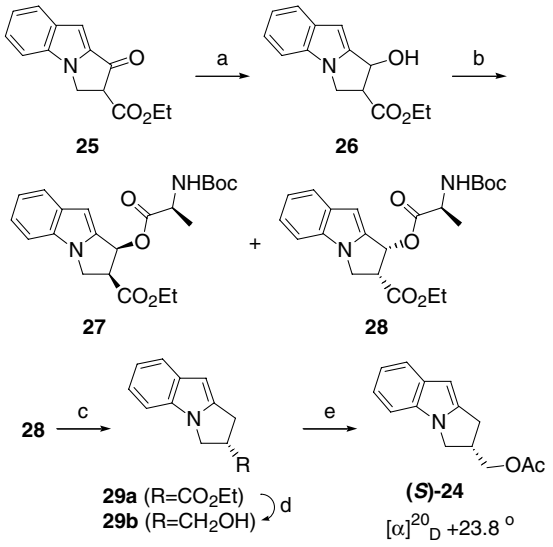
3. Results and discussion

3.1. In vitro SAR studies

It was expected that conformational restriction with cyclic structures might provide more potent inhibitors than



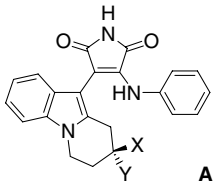
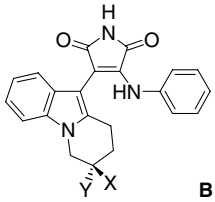
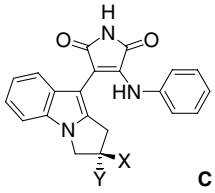
Scheme 3. Reagents: (a) $CH_2(CO_2Et)_2$, AcOH, piperidine; (b) $NaBH_4$; (c) $LiAlH_4$; (d) Lipase PS, vinyl acetate; (e) TBDPSCl, imidazole; (f) K_2CO_3, MeOH; (g) Ms_2O, pyridine; (h) NaH, NaI; (i) i. $(COCl)_2$, Et_3N, ii. aq NH_3; (j) $NaBH_4$; (k) TESH, TFA; (l) $(CO_2Me)_2$, *t*-BuOK; (m) $PhNH_2$, AcOH; (n) TBAF; (o) Ms_2O, pyridine or Tf_2O, 2,4,6-collidine; (p) R^1R^2NH; (q) Ac_2O.



Scheme 4. Reagents: (a) H_2, 5% Pd–C; (b) Boc-L-Ala, DCC, DMAP; (c) H_2 (2.7 atm), 10% Pd–C; (d) $LiAlH_4$; (e) Ac_2O, pyridine, DMAP.

acyclic inhibitor **3** by placing a hydrophilic group at a more favorable position to associate with Asp427 and/or Asp470 of PKCβ.[11] This idea was based upon our previous report, in which we advocated the significant effect of the linker length between the indole nitrogen and a hydrophilic group upon the inhibitory activity (in the case of **3**, the best linkers are $(CH_2)_3$ and $(CH_2)_4$).[8] Supporting this idea, in 8-substituted-6,7,8,9-tetrahydropyrido[1,2-*a*]indoles (series A), alcohol derivative (***S*)-8b** and dimethylamino derivative (***S*)-9** inhibited PKCβ2 five times more strongly than the corresponding acyclic analogue (**3b** and **3a**) showing IC_{50} values of 6 and 2 nM, respectively (Table 1). Moreover, these compounds were significantly more potent than their enantiomers ((***R*)-8b** and (***R*)-9**). We initially thought these facts implied that strict spatial arrangements were required in the recognition of the hydrophilic groups by Asp427 and/or Asp470 of the enzyme.[11] This enantiomeric preference, however, was not extended to other series including **14** (series B), or **22** and **23** (series C). In series B, (***S*)-14** and its enantiomer (***R*)-14** equally inhibited the enzyme showing IC_{50} values of 4 and 6 nM, respectively. In the case of series C, (***S*)-22** and (***R*)-23c** (enantiomers possessing hydrogen atom for X) exhibited IC_{50} values of 4 and 5 nM, whereas the IC_{50} of their enantiomers (***R*)-22** and (***S*)-23c** (enantiomers possessing hydrogen atom for Y) were 14 and 8 nM, respectively. The former set was scarcely more potent than the latter one showing opposite enantiomeric preference to that observed in series A. Although the cyclic derivatives showed significantly higher potency than their acyclic counterparts as expected (compare dimethylamino derivatives (***S*)-9**, (***S*)-14**, and (***R*)-23a** with **3a**, and hydroxyl derivatives (***S*)-8b** and (***S*)-22** with **3b**), the lack of obvious enantiomeric preference throughout these three series complicated attempts to gain insight into the Asp427 and Asp470 associable position. The increase in the inhibitory activity observed in the cyclic inhibitors may be attributed to their smaller number of rotatable bonds and the smaller change in entropy factors in associating with the enzyme compared to the acyclic inhibitors **3**. The existing rotatable bonds, including a C–C bond off the 5- and 6-membered

Table 1. PKCβ2 and PKCα inhibitory activity of series A, B, C and the acyclic compounds

Series	Compound	X	Y	IC_{50} (nM)	
				β2	α
 A	(*S*)-**8b**	CH_2OH	H	6	394
	(*S*)-**9**	CH_2NMe_2	H	2	96
	(*R*)-**8b**	H	CH_2OH	17	2131
	(*R*)-**9**	H	CH_2NMe_2	25	3057
 B	(*S*)-**14**	H	CH_2NMe_2	4	252
	(*R*)-**14**	CH_2NMe_2	H	6	445
 C	(*S*)-**22**	H	CH_2OH	4	458
	(*R*)-**23a**	H	CH_2NMe_2	4	148
	(*R*)-**23b**	H	CH_2NEt_2	32	1955
	(*R*)-**23c**	H	CH_2NMeEt	5	481
	(*R*)-**23d**	H	CH_2-1-pyrrolidinyl	8	542
	(*R*)-**23e**	H	CH_2NHMe	2.2	152
	(*R*)-**23f**	H	CH_2NHEt	2.4	212
	(*R*)-**23g**	H	CH_2NH_2	1.4	95
	(*R*)-**23h**	H	CH_2NHAc	7.9	903
	(*R*)-**22**	CH_2OH	H	14	1021
	(*S*)-**23c**	CH_2NMeEt	H	8	613
Acyclic	**3a**			12	460
	3b			30	1620

rings, could account for the relatively large tolerance for stereochemistry, ring size and substituent position throughout these ring systems. Molecular modeling studies implied that this flexibility allows the terminal hydrophilic groups to associate with the Asp427 and/or Asp470 in energetically acceptable conformations as seen in a staurosporine-PKA co-crystal, in which the lower NHMe group associates with Glu127 and Glu170 of PKA[22] and as also seen in the staurosporine-PKCθ co-crystal, in which the NHMe group binds to the main chain carbonyl of Asp508 of PKCθ.[23] The PKCβ2 selectivity of these cyclic compounds over PKCα was around 50- to 100-fold, and this value did not vary with the change in ring size, substituent position or *R*/*S* stereochemistry inversion.

To further increase the potency, derivatives having a variety of hydrophilic groups (substituents X or Y in Table 1) were investigated. We first synthesized and assessed several derivatives of series A and B. However, significant increase in activity was not observed in these series, and more importantly, poor oral bioavailability and unacceptable toxicity were found in series A and B, respectively (data not shown). Therefore, we shifted our focus to series C, and tested the effects of the hydrophilic group on potency and selectivity using series C as a platform. Replacement of the NMe_2 group of (***R***)-**23a** with larger substituents such as NEt_2 ((***R***)-**23b**), N(Me)Et ((***R***)-**23c**), and pyrrolidinyl ((***R***)-**23d**) resulted in reduced inhibitory activity, and the amide derivatives (***R***)-**23h** also showed lower activity than (***R***)-**23a**. In contrast, compounds having the smaller substituents showed the stronger inhibitory activity (see (***R***)-**23e**, (***R***)-**23f**, and (***R***)-**23g**). The size of the amino group did not influence the PKCβ2 selectivity over PKCα. The most potent compound was (***R***)-**23g**, which exhibited strong PKCβ2 inhibition (IC_{50} of 1.4 nM) and good selectivity over PKCα (67-fold). We selected this compound, in its developmental salt form as the mesylate (JTT-010), for more detailed investigation of the isozyme selectivity over other PKC isozymes (β1, γ, δ, ε, ζ, and μ). As shown in Table 2, JTT-010 selectively inhibited PKCβ1 and PKCβ2 with a selectivity profile similar to that of ruboxistaurin.[3a]

3.2. Ex vivo studies

Several potent compounds were selected for the rat ex vivo studies to observe oral absorption as well as duration of drug effect. After oral administration of the compounds to rats at a dose of 2 mg/kg (1 mg/kg for (***S***)-**9**), PKCβ2 inhibitory activity in plasma was measured at 2 and 6 h after dosing (Table 3). The inhibitory activity in animals receiving (***S***)-**9** was marginal (30%) at 2 h after administration and disappeared completely after 6 h, indicating poor oral bioavailability and short dura-

tion. In contrast, strong inhibition was detected at 2 and 6 h after administration of compounds in series B and C. Plasma from rats receiving (***S***)-**14**, (***R***)-**14**, and (***S***)-**22** all similarly inhibited the enzyme (64–79% inhibition at 2 h and 45–70% inhibition at 6 h) suggesting that oral bioavailability and duration of these three compounds were similar given their nearly equal in vitro IC_{50} values (~5 nM). Although (***R***)-**23d**, possessing a pyrrolidinyl group at the terminal of the molecule, was also efficiently absorbed, it appeared to be more rapidly cleared from plasma than (***S***)-**14**, (***R***)-**14**, and (***S***)-**22**, considering the lower inhibition (29%) after 6 h. Plasma from rats receiving (***R***)-**23a**, which has a dimethylamino group, strongly inhibited the enzyme and the effect lasted for more than 6 h. This strong and long-lasting inhibitory activity of (***R***)-**23a** in vivo was proven to be partially attributable to its more potent des-methyl metabolites (***R***)-**23e** and (***R***)-**23g** found in plasma of (***R***)-**23a** receiving animals. In response to this result, we conducted the ex vivo test using the most potent (***R***)-**23g**, and found that this compound almost completely inhibited the enzyme even after 6 h. Subsequent pharmacokinetic studies revealed that oral bioavailability and T1/2 of (***R***)-**23g** in the rat were 57% and 5.9 h, respectively (30 mg/kg, p.o. dosing). It could be speculated that decreasing the number of rotatable bonds was effective in increasing oral bioavailability in this series of PKCβ inhibitors.[12]

3.3. Ameliorative effect in STZ rat retinopathy model

PKCβ activation is known to mediate retinal vascular abnormalities including excessive vascular permeability and neovascularization, which can cause macular edemas and vascular occlusions, hallmarks of diabetic retinopathy. Ruboxistaurin, a selective PKCβ inhibitor, was reported to suppress these retinal symptoms in diabetic animal models,[24] demonstrating the involvement of PKCβ activity in diabetic retinopathy. Using the streptozotocin (STZ) induced diabetic rat retinopathy model, we investigated in vivo potency of our PKCβ-selective inhibitor (***R***)-**23g**, which was selected based on its in vitro and ex vivo potency (vide ante). In this animal model, mean circulation time (MCT) of fluorescein in retinal vascular segments was measured as an index of blood flow in retina.[25] From the day subsequent to STZ treatment, (***R***)-**23g** was orally given to the animals twice a day for two weeks and MCT was measured. As shown in Figure 2, STZ significantly prolonged MCT as seen in the vehicle group. This blood flow deceleration was, however, reversed in a dose-dependent manner by oral administration of (***R***)-**23g**. A 1 mg/kg dose given to the STZ rats significantly shortened the MCT to the levels comparable to the non-treated control group, demonstrating the in vivo potency of (***R***)-**23g**.

Table 3. PKCβ2 inhibitory activity of rat plasma after oral dosing

Series	Compound	Inhibitory activity (%) of rat plasma time after oral dosing[a]	
		2 h	6 h
A	(***S***)-**9**	30[b]	−9[b]
B	(***S***)-**14**	65	58
	(***R***)-**14**	79	70
C	(***S***)-**22**	64	45
	(***R***)-**23a**	84	82
	(***R***)-**23d**	82	29
	(***R***)-**23e**	92	91
	(***R***)-**23g**	97	93

[a] Dosing amount was 2 mg/kg otherwise noted.
[b] 1 mg/kg administration.

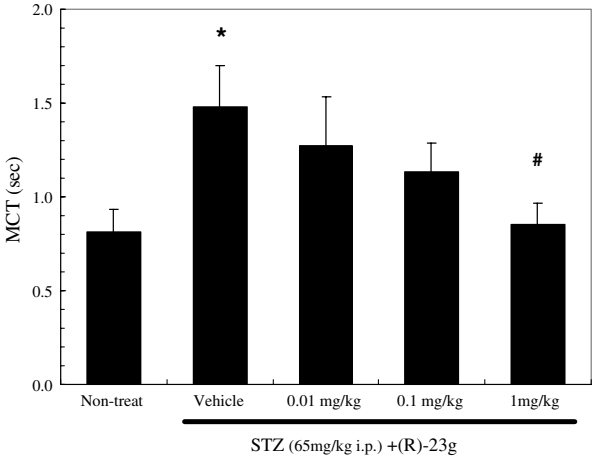


Figure 2. Effect of (***R***)-**23g** on retinal mean circulation time (MCT) in STZ diabetic rats. MCT was measured after 2 weeks treatment of (***R***)-**23g**. Values are expressed as means ± SE ($N = 9$–12). *significant difference from non-treated group by LSD test ($P < 0.05$). #Significant difference from STZ vehicle group by LSD test ($P < 0.05$).

4. Conclusion

Three new series of cyclic 3-anilino-4-(3-indolyl)maleimides, that is, series A, B, and C, were synthesized in optically active form in order to find PKCβ-selective inhibitors with good oral bioavailability starting from

Table 2. Comparison of PKCβ selectivity of (***R***)-**23g mesylate**, staurosporine, and ruboxistaurin

Compound	PKC isozyme IC_{50} (nM)							
	β1	β2	α	γ	δ	ε	ζ	μ
(*R*)-**23g** mesylate (JTT-010)	4.0	2.3	86	110	54	490	1700	>10000
Staurosporine	11	4.0	8.7	11	4.3	7.4	1700	24
Ruboxistaurin[a]	4.7	5.9	360	300	250	600	>100,000	NT

[a] These data were cited from reference 3a.

previously reported acyclic analogues **3** having no oral bioavailability. Among these series, compounds in series B and C exhibited good inhibitory activity as well as oral bioavailability in ex vivo studies. In addition to this, JTT-010 (mesylate of (***R***)-**23g**) in series C, which selectively inhibited PKCβ1 and β2 with IC_{50} values of 4.0 and 2.3 nM, respectively, remedied retinopathy in the STZ induced diabetic rat model at a dose of 1 mg/kg, oral administration. We have recently reported the ameliorative effects of JTT-010 also in diabetic neuropathy and nephropathy animal models.[26] It could be concluded that JTT-010 is a new structural class of orally bioavailable PKCβ-selective inhibitors showing in vivo curative effects in a variety of diabetic microvascular complication models.

5. Experimental

5.1. Chemistry

Melting points were determined using a Yanagimoto micro melting point apparatus or BÜCHI B-545 melting point instrument and were uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL JNM-A300W, Bruker AMX-300 or JEOL JNM-AL400 spectrometer in a solvent indicated. Chemical shifts (δ) are reported in parts per million relative to internal standard tetramethylsilane. Elemental analysis was performed with a Perkin-Elmer 2400 Series II CHNS/O analyzer. Mass spectra (FAB+) were recorded with a Finnigan TSQ 700 instrument and mass spectra (ESI+) were recorded with a ThemoQuest LCQ mass spectrometer. High-resolution mass spectra were obtained with a JEOL SX 102A spectrometer. $[\alpha]_D$ values were obtained at 20 or 25 °C with a Perkin-Elmer 241 polarimeter or Rudolph Research Analytical AUTOPOL V polarimeter.

5.1.1. (*S*)-8-(*tert*-Butyldiphenylsilyloxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indole ((*S*)-4b). To a solution of (***S***)-**4a**[13] (24.6 g, 122 mmol) and imidazole (20.0 g, 293 mmol) in DMF (300 mL), TBDPSCl (40.3 g, 146.6 mmol) was added at room temperature. The reaction mixture was stirred overnight and was partitioned between AcOEt and water. The organic layer was successively washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (100:1–50:1) to give (***S***)-**4b** (53.7 g, 100%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (9H, s), 1.84 (1H, ddd, J = 5.7, 13.0, 24.0 Hz), 2.03–2.18 (1H, m), 2.19–2.29 (1H, m), 2.68 (1H, dd, J = 11.0, 16.0 Hz), 3.10 (1H, dd, J = 3.7, 16.0 Hz), 3.62–3.76 (2H, m), 3.85 (1H, dt, J = 4.9, 11.7 Hz), 4.27 (1H, ddd, J = 2.9, 5.7, 11.7 Hz), 6.19 (1H, s), 7.02–7.18 (2H, m), 7.26 (1H, d, J = 6.7 Hz), 7.32–7.47 (6H, m), 7.52 (1H, d, J = 7.2 Hz), 7.61–7.71 (4H, m); MS (FAB) m/z 440 $(M+H)^+$.

5.1.2. 2-[(*S*)-8-(*tert*-Butyldiphenylsilyloxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]acetamide ((*S*)-6). To a stirring solution of (***S***)-**4b** (53.7 g, 122 mmol) and triethylamine (22.1 mL, 159 mmol) in CH_2Cl_2 (350 mL) was added oxalyl chloride (12.8 mL, 147 mmol) dropwise over 15 min at 0 °C. After stirring for 30 min at 0 °C, the reaction mixture was poured into ice-cooled 28% aqueous NH_3 (500 mL). The reaction mixture was allowed to warm to room temperature over 1 h and was partitioned between AcOEt and water. The organic layer was separated, successively washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give crude ketoamide (***S***)-**5** as a pale brown amorphous solid, which was used directly in the next step without any purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (9H, s), 1.81–1.92 (1H, m), 2.08–2.19 (1H, m), 2.28–2.37 (1H, m), 2.94 (1H, dd, J = 10.5, 18.9 Hz), 3.53 (1H, ddd, J = 1.2, 4.9, 18.7 Hz), 3.66 (1H, dd, J = 7.2, 10.4 Hz), 3.77 (1H, dd, J = 5.6, 10.4 Hz), 3.95 (1H, dt, J = 4.8, 11.2 Hz), 4.29 (1H, ddd, J = 2.8, 5.6, 12.4 Hz), 5.50 (1H, s), 6.68 (1H, s), 7.24–7.31 (3H, m), 7.35–7.46 (6H, m), 7.65–7.69 (4H, m), 8.18 (1H, dd, J = 5.1, 8.5 Hz); MS (FAB) m/z 511 $(M+H)^+$. To a stirring solution of all of resulting crude (***S***)-**5** in EtOH (1000 mL)–THF (250 mL) was added $NaBH_4$ (13.9 g, 367 mmol) portionwise at room temperature. After stirring for 2 h at room temperature, the reaction mixture was partitioned between AcOEt and a 2 M aqueous $KHSO_4$ solution. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give crude α-hydroxyacetoamide as a pale brown oil. To a solution of this compound in CH_2Cl_2 (800 mL) were successively added triethylsilane (39 mL, 244 mmol) and trifluoroacetic acid (68 mL, 883 mmol) at room temperature. After stirring for 2 h at room temperature, the reaction mixture was quenched by addition of a $NaHCO_3$ saturated aqueous solution at 0 °C. The layers were separated and the aqueous layer was extracted with $CHCl_3$. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (4:1–2:1) to give (***S***)-**6** (28.5 g, 47% from (***S***)-**4b**) as a pale brown solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (9H, s), 1.85 (1H, ddd, J = 5.6, 11.3, 24.5 Hz), 2.01-2.19 (1H, m), 2.20-2.32 (1H, m), 2.53 (1H, dd, J = 10.8, 16.2 Hz), 3.00 (1H, dd, J = 3.6, 16.2 Hz), 3.59 (1H, d, J = 23.1 Hz), 3.65 (1H, d, J = 23.1 Hz), 3.66-3.79 (2H, m), 3.88 (1H, dt, J = 4.9, 11.7 Hz), 4.29 (1H, ddd, J = 3.0, 5.9, 11.7 Hz), 5.31 (1H, brs), 5.58 (1H, brs), 7.09-7.21 (2H, m), 7.28 (1H, d, J = 7.5 Hz), 7.35-7.47 (6H, m), 7.50 (1H, d, J = 7.2 Hz), 7.66 (2H, d, J = 7.8 Hz), 7.67 (2H, d, J = 7.2 Hz); MS (FAB) m/z 497 $(M+H)^+$.

5.1.3. 3-[(*S*)-8-(*tert*-Butyldiphenylsilyloxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-4-hydroxy-1*H*-pyrrole-2,5-dione ((*S*)-7). To a stirring solution of (***S***)-**6** (24.5 g, 49.3 mmol) and dimethyl oxalate (6.40 g, 54.2 mmol) in THF (250 mL) was added *t*-BuOK (12.18 g, 108.4 mmol) in two equal portions, 15 min part at 0 °C. The reaction mixture was allowed to warm to room temperature over 1 h. To the resulting mixture were successively added a 0.5 M aqueous $KHSO_4$ solution and AcOEt, and the organic layer was separated. The organic layer was successively washed with water

and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. Thus obtained residue was chromatographed on silica gel eluting with hexane–AcOEt (4:1–2:1) to give (***S***)-**7** (24.4 g, 90%) as a brown amorphous solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (9H, s), 1.75–1.88 (1H, m), 2.03–2.15 (1H, m), 2.17–2.26 (1H, m), 2.63 (1H, dd, J = 11.1, 17.2 Hz), 3.00 (1H, dd, J = 3.3, 16.9 Hz), 3.71 (2H, d, J = 6.0 Hz), 3.88 (1H, dt, J = 4.9, 12.0 Hz), 4.29–4.38 (1H, m), 7.02 (1H, dt, J = 1.2, 8.4 Hz), 7.10 (1H, dt, J = 1.2, 7.2 Hz), 7.34–7.39 (2H, m), 7.40–7.49 (6H, m), 7.61–7.67 (4H, m), 10.49 (1H, s), 11.70 (1H, br s); MS (ESI) *m*/*z* 551 $(M+H)^+$.

5.1.4. 3-Anilino-4-[(*S*)-8-(hydroxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-1*H*-pyrrole-2,5-dione ((*S*)-8b). To a solution of (***S***)-**7** (24.4 g, 44.3 mmol) in AcOH (100 mL), aniline (20.2 mL) was added. The reaction mixture was stirred at 100 °C for 1 h, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (20:1–4:1) to give (***S***)-**8a** (23.0 g, 83%) as a red-orange amorphous solid. To a solution of all of resulting (***S***)-**8a** in THF (250 mL) was added a 1 M solution of tetrabutylammonium fluoride in THF (128.8 mL, 128.8 mmol) at room temperature. After stirring at room temperature for 2 h, a 0.5 M aqueous $KHSO_4$ solution was added to the resulting mixture, which was then extracted with AcOEt. The separated organic layer was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in vacuo. Thus obtained residue was chromatographed on silica gel eluting with hexane–AcOEt (1:1–1:3) to give (***S***)-**8b** (13.8 g, 97%) as a red-orange solid: mp 243–246 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92–1.12 (1H, m), 1.42–1.56 (1H, m), 1.67–1.80 (0.5H, m), 1.86–2.04 (1H, m), 2.06–2.19 (0.5H, m), 2.26–2.36 (0.5H, m), 2.53–2.64 (0.5H, m), 3.09–3.31 (2H, m), 3.43–3.54 (0.5H, m), 3.63–3.75 (0.5H, m), 4.07–4.23 (1H, m), 4.59 (1H, d, J = 6.0 Hz), 6.60 (2H, d, J = 8.6 Hz), 6.71 (3H, br s), 6.96 (1H, t, J = 7.2 Hz), 7.03 (1H, t, J = 7.5 Hz), 7.28 (2H, t, J = 6.8 Hz), 9.23 (0.5H, s), 9.26 (0.5H, s), 10.58 (1H, br s); MS (FAB) *m*/*z* 388 $(M+H)^+$; Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 70.88; H, 5.44; N, 10.73; HRMS (FAB–) calcd for $C_{23}H_{20}N_3O_3$ 386.1505. Found 386.1523; $[\alpha]_D^{25}$ −27.3° (*c* 0.26, MeOH).

5.1.5. 3-Anilino-4-[(*S*)-8-(methanesulfonyloxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-1*H*-pyrrole-2,5-dione ((*S*)-8c). To a solution of (***S***)-**8b** (13.8 g, 35.6 mmol) in THF (270 mL) were added pyridine (8.64 mL, 129 mmol) and methanesulfonic anhydride (12.4 g, 71.2 mmol) sequentially at room temperature. The reaction mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and quenched with a 1 M aqueous $KHSO_4$ solution. The organic layer was separated, sequentially washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Thus obtained residue was chromatographed on silica gel eluting with hexane–AcOEt (1:1–1:2) to give (***S***)-**8c** (12.4 g, 75%) as a red-orange solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.09–1.26 (1H, m), 1.54–1.72 (1H, m), 1.88–2.15 (1H, m), 2.22 (0.5H, dd, J = 11.2, 16.1 Hz), 2.33–2.43 (0.5H, m), 2.60–2.70 (0.5H, m), 3.19 (3H, s), 3.31–3.54 (1H, m), 3.69–3.80 (0.5H, m), 3.94–4.27 (3H, m), 6.57–6.64 (2H, m), 6.72 (3H, s), 6.98 (1H, t, J = 7.5 Hz), 7.06 (1H, t, J = 7.5 Hz), 7.30 (2H, t, J = 8.3 Hz), 9.28 (0.5H, s), 9.33 (0.5H, s), 10.64 (1H, s).

5.1.6. 3-Anilino-4-[(*S*)-8-[(dimethylamino)methyl]-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]-1*H*-pyrrole-2,5-dione ((*S*)-9). To a solution of (***S***)-**8c** (80 mg, 0.172 mmol) in THF (1.5 mL) was added 50% aqueous dimethylamine (0.774 mL, 8.60 mmol) at room temperature. The mixture was sealed in a stainless steel pressure tube and heated at 65 °C for 16 h. The reaction mixture was then allowed to cool to room temperature and was partitioned between AcOEt and water. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Thus obtained residue was purified by preparative thin-layer chromatography on silica gel (hexane–AcOEt (1:4) and then $CHCl_3$–MeOH (6:1)) to give (***S***)-**9** (51 mg, 72%) as an orange solid: mp 240–242 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88–1.01 (0.5H, m), 1.02–1.14 (0.5H, m), 1.36–1.54 (1H, m), 1.82–2.02 (4H, m), 2.07 (3H, s), 2.09 (3H, s), 2.21–2.31 (0.5H, m), 2.57–2.68 (0.5H, m), 3.41–3.52 (0.5H, m), 3.69–3.80 (0.5H, m), 4.02–4.11 (0.5H, m), 4.12–4.21 (0.5H, m), 6.54–6.63 (2H, m), 6.72 (3H, br s), 6.92–7.10 (2H, m), 7.25–7.36 (2H, m), 9.23 (0.5H, s), 9.29 (0.5H, s), 10.63 (1H, s); MS (FAB) *m*/*z* 415 $(M+H)^+$; Anal. Calcd for $C_{25}H_{26}N_4O_2 \cdot 0.25H_2O$: C, 71.66; H, 6.37; N, 13.37. Found: C, 71.69; H, 6.28; N, 13.39; HRMS (FAB–) calcd for $C_{25}H_{25}N_4O_2$ 413.1978. Found: 413.2019; $[\alpha]_D^{25}$ −36.4° (*c* 0.23, $CHCl_3$–MeOH 1:4).

(***R***)-**8b** and (***R***)-**9** were also obtained from (***R***)-**4a**[13] with the similar procedures used for the preparation of (***S***)-**8b** and (***S***)-**9**. All spectral data other than optical rotation ((***R***)-**8b**: $[\alpha]_D^{25}$ + 25.1° (*c* 0.21, MeOH) and (***R***)-**9**: $[\alpha]_D^{25}$ + 33.7° (*c* 0.20, $CHCl_3$–MeOH 1:4)) were consistent with those of (***S***)-**8b** and (***S***)-**9**.

5.1.7. (*S*)-2-(*tert*-Butyldiphenylsilyloxymethyl)-4,4-diethoxybutyl methanesulfonate (11c). To a stirring solution of **10**[16] (1.09 g, 4.67 mmol) and Et_3N (1.30 mL, 9.34 mmol) in THF (20 mL), methanesulfonyl chloride (0.398 mL, 5.14 mmol) was added at 0 °C. The reaction mixture was stirred for 25 min at 0 °C, and then was partitioned between AcOEt and $NaHCO_3$ saturated water. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give crude **11a** (1.53 g) as a pale yellow oil. To a solution of thus obtained crude **11a** (1.53 g) in 1,4-dioxane (22 mL)–H_2O (11 mL) was added 4 M NaOH (1.40 mL, 5.60 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then was partitioned between AcOEt and brine. The organic layer was separated, successively washed with $NaHCO_3$ saturated water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give crude **11b** (1.31 g) as a pale yellow oil. To a stirring solution of all of the crude **11b** and imidazole (636 mg, 9.34 mmol) in DMF (5 mL), TBDPSCl (1.42 g, 5.14 mmol) was added at

0 °C. The reaction mixture was allowed to warm to room temperature, stirred overnight, and then diluted with AcOEt. The mixture was washed with $NaHCO_3$ saturated water and brine successively, and concentrated in vacuo. The residue mixture was chromatographed on silica gel eluting with hexane–AcOEt (4:1) to give **11c** (2.11 g, 89% from **10**) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06 (9H, s), 1.16 (3Hx2, t, J = 7.2 Hz), 1.68 (2H, t, J = 6.0 Hz), 2.07–2.17 (1H, m), 2.93 (3H, s), 3.37–3.48 (2H, m), 3.53–3.65 (3H, m), 3.72 (1H, dd, J = 4.8, 10.4 Hz), 4.33 (1H, dd, J = 6.0, 9.6 Hz), 4.39 (1H, dd, J = 5.2, 9.6 Hz), 4.50 (1H, t, J = 6.0 Hz), 7.35–7.46 (6H, m), 7.62–7.67 (4H, m); $[\alpha]_D^{20}$ +1.25° (*c* 0.72, $CHCl_3$).

5.1.8. 2-{1-[(*R*)-2-(*tert*-Butyldiphenylsilyloxymethyl)-4,4-diethoxybutyl]-1*H*-indol-3-yl}acetamide (12). To a solution of indole-3-acetamide (1.08 g, 6.23 mmol) in DMF (10 mL) was added NaH (249 mg, 60% in mineral oil, 6.23 mmol) at 0 °C, and the reaction mixture was stirred for 15 min at 0 °C. To the resulting mixture, **11c** (2.11 g, 4.15 mmol) and NaI (62 mg, 0.415 mmol) were added. After stirring overnight at room temperature, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (1:1–1:3) to give **12** (1.42 g, 57%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (3H, t, J = 7.2 Hz), 1.11 (9H, s), 1.14 (3H, t, J = 7.2 Hz), 1.60–1.69 (1H, m), 1.72–1.82 (1H, m), 2.21–2.30 (1H, m), 3.27–3.38 (2H, m), 3.44–3.57 (4H, m), 3.64 (2H, s), 4.16 (1H, dd, J = 6.4, 14.4 Hz), 4.25 (1H, dd, J = 8.0, 14.4 Hz), 4.35 (1H, t, J = 5.6 Hz), 5.21 (1H, br s), 5.52 (1H, br s), 6.90 (1H, s), 7.13 (1H, t, J = 7.2 Hz), 7.21 (1H, t, J = 7.8 Hz), 7.30–7.37 (4H, m), 7.39–7.45 (3H, m), 7.53 (1H, d, J = 8.0 Hz), 7.57–7.63 (4H, m).

5.1.9. 2-[(*R*)-7-(*tert*-Butyldiphenylsilyloxymethyl)-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl]acetamide ((*R*)-13). To a solution of **12** (1.42 g, 2.36 mmol) in $CHCl_3$ (108 mL) was added a 50% aqueous trifluoroacetic acid solution (7.1 mL, 92.2 mmol) at room temperature. The reaction mixture was stirred vigorously for 30 min at room temperature. The reaction mixture was poured into $NaHCO_3$ saturated water and extracted with $CHCl_3$. The organic layer was separated, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Thus obtained residue was dissolved in EtOH (20 mL) and the solution was stirred for 3 h at room temperature under H_2 atmosphere in the presence of 50%-wet 5% palladium-carbon (100 mg). Then the insoluble catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (1:2–1:3) to give (***R***)-**13** (1.12 g, 95%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (9H, s), 1.58-1.69 (1H, m), 2.00–2.08 (1H, m), 2.28–2.40 (1H, m), 2.78 (1H, ddd, J = 6.0, 11.2, 16.8 Hz), 3.00 (1H, dt, J = 4.6, 17.2 Hz), 3.63 (2H, dd, J = 18.5, 24.5 Hz), 3.70–3.75 (2H, m), 3.81 (1H, dd, J = 5.4, 10.2 Hz), 4.30 (1H, dd, J = 5.6, 12.3 Hz), 5.20 (1H, br s), 5.57 (1H, br s), 7.15 (1H, dt, J = 1.3, 7.5 Hz), 7.20 (1H, dt, J = 1.4, 7.1 Hz), 7.27 (1H, d, J = 8.4 Hz), 7.32–7.48 (6H, m), 7.50 (1H, d, J = 7.8 Hz), 7.63–7.69 (4H, m); MS (ESI) *m*/*z* 497 $(M+H)^+$.

5.1.10. 3-Anilino-4-{(*S*)-7-[(dimethylamino)methyl]}-6,7,8,9-tetrahydropyrido[1,2-*a*]indol-10-yl}-1*H*-pyrrole-2,5-dione ((*S*)-14). (***S***)-**14** was obtained from (***R***)-**13** in 43% yield as an orange-red solid with similar procedures used for the preparation of (***S***)-**9** from (***S***)-**6**: mp 200–202 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.75 (1H, m), 1.24 (1H, m), 1.50–2.60 (5H, m), 2.12 (6H, s), 3.30 (1H, m), 4.06 (1H, m), 6.60 (br d, 2H, J = 6.6 Hz), 6.72 (3H, br s), 6.98 (1H, t, J = 7.3 Hz), 7.05 (1H, t, J = 7.3 Hz), 7.28 (1H, d, J = 7.3 Hz), 7.33 (1H, d, J = 7.3 Hz), 9.24 (1H, s), 10.61 (1H, br s); MS (FAB) *m*/*z* 415 $(M+H)^+$; Anal Calcd for $C_{25}H_{26}N_4O_2 \cdot 0.6H_2O$: C, 70.60; H, 6.45; N, 13.17. Found: C, 70.32; H, 6.37; N, 13.01; HRMS (FAB–) calcd for $C_{25}H_{26}N_4O_2$ 413.1978. Found: 413.2020; $[\alpha]_D^{25}$ +35.4° (*c* 0.28, MeOH).

5.1.11. (*S*)-2-(*tert*-Butyldiphenylsilyloxymethyl)-4,4-diethoxybutyl acetate (15a). To a stirring solution of **10**[16] (1.00 g, 4.27 mmol) and imidazole (581 mg, 8.54 mmol) in DMF (5 mL) was added TBDPSCl (1.29 g, 4.70 mmol) at 0 °C. The reaction mixture was stirred overnight and then poured into $NaHCO_3$ saturated water. The mixture was extracted with AcOEt and the organic layer was separated, washed with brine, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (9:1) to give **15a** (2.00 g, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05 (9H, s), 1.16 (6H, t, J = 7.2 Hz), 1.63 (1H, dt, J = 6.0, 14.4 Hz), 1.74 (1H, dt, J = 6.2, 14.4 Hz), 1.98 (3H,s), 1.99–2.07 (1H, m), 3.38–3.49 (2H, m), 3.55–3.65 (3H, m), 3.69 (1H, dd, J = 4.8, 10.4 Hz), 4.16 (1H, d, J = 6.0 Hz), 4.54 (1H, t, J = 5.8 Hz), 7.34–7.45 (6H, m), 7.62–7.66 (4H, m).

5.1.12. (*S*)-2-(*tert*-Butyldiphenylsilyloxymethyl)-4,4-diethoxybutan-1-ol (15b). To a solution of **15a** (2.00 g, 4.23 mmol) in EtOH (20 mL) was added potassium carbonate (643 mg, 4.65 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature and then poured into brine. The mixture was extracted with toluene, and the organic layer was separated, washed with brine, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (4:1) to give **15b** (1.79 g, 98%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06 (9H, s), 1.16 (3H, t, J = 7.2 Hz), 1.18 (3H, t, J = 7.2 Hz), 1.65 (2H, t, J = 5.8 Hz), 1.90–2.00 (1H, m), 2.77 (1H, t, J = 6.0 Hz), 3.39–3.49 (2H, m), 3.54–3.69 (3H, m), 3.70–3.77 (3H, m), 4.52 (1H, t, J = 5.6 Hz), 7.35–7.46 (6H, m), 7.63–7.69 (4H, m).

5.1.13. 3-Anilino-4-{(*R*)-7-[(dimethylamino)methyl]}-6,7,8,9-tetrahydropyridol[1,2-a]indol-10-yl]-1*H*-pyrrole-2,5-dione ((*R*)-14). To a solution of **15b** (1.78 g, 4.13 mmol) in THF (20 mL) were successively added Et_3N (1.15 mL, 8.26 mmol) and methanesulfonyl chloride (0.352 mL, 4.54 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and was partitioned between $NaHCO_3$ saturated water and AcOEt. The

organic layer was separated, washed with $NaHCO_3$ saturated water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give crude **15c** (2.14 g) as a pale yellow oil, which was converted to (***R***)-**14** showing $[\alpha]_D^{25}$ of −33.3° (*c* 0.23, MeOH) in 35% yield (nine steps from **15b**) with the same procedures used for the transformation of **11c** into (***S***)-**14**. All spectral data other than optical rotation were consistent with those of (***S***)-**14**.

5.1.14. Diethyl (1*H*-indol-2-ylmethylene)malonate (17). A mixture of **16** (10.0 g, 68.9 mmol), diethylmalonate (12.5 mL, 82.7 mmol), piperidine (0.684 mL, 6.89 mmol), and powdered 4 Å molecular sieves (10.0 g) in toluene (150 mL) was refluxed for 3 h. The reaction mixture was allowed to cool to room temperature, insoluble molecular sieves were removed by filtration, and then the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with hexane–AcOEt (4:1) followed by precipitation from hexane–*i*PrOH to give **17** (10.0 g, 51%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (3H, t, J = 7.1 Hz), 1.38 (3H, t, J = 7.1 Hz), 4.32 (2H, q, J = 7.2 Hz), 4.41 (2H, q, J = 7.2 Hz), 6.97 (1H, d, J = 1.2 Hz), 7.11 (1H, dt, J = 1.2, 8.1 Hz), 7.30 (1H, dt, J = 1.2, 8.4 Hz), 7.41 (1H, dd, J = 0.9, 8.4 Hz), 7.64 (1H, dd, J = 0.9, 8.1 Hz), 7.76 (1H, s), 10.49 (1H, br s); MS (ESI) *m*/*z* 288 $(M+H)^+$.

5.1.15. 2-(1*H*-Indol-2-ylmethyl)propane-1,3-diol (18b). To a suspension of $NaBH_4$ (1.32 g, 34.8 mmol) in THF (50 mL), a solution of **17** (10.0 g, 34.8 mol) in THF (50 mL) was added at 0 °C. After stirring for 3.5 h at 0 °C, brine, and a 1 M aqueous $KHSO_4$ solution were added to the reaction mixture. The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layers were washed with brine, and concentrated in vacuo to give crude **18a** (10.0 g) as a yellow-orange solid. A solution of resulting crude **18a** in THF (70 mL) was added to a suspension of $LiAlH_4$ (2.64 g, 69.6 mmol) in THF (80 mL) at 0 °C. After stirring for 2 h at 0 °C, crushed ice, brine and a 1 M aqueous $KHSO_4$ solution were successively added to the reaction mixture. The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layers were washed with brine and concentrated in vacuo to give crude **18b**. Thus obtained crude **18b** was azeotroped with vinyl acetate to afford AcOEt-free **18b** as a brown oil. No further purification was attempted on this compound, which was used directly in the next step. An analytical sample was purified by column chromatography on silica gel eluting with hexane–AcOEt (1:2–1:3) to afford pure **18b** as an off-white solid: mp 77–80 °C; ^{1}H NMR (300 MHz, $CDCl_3$); δ 2.06 (1H, m), 2.45 (2H, br s), 2.84 (2H, d, J = 7.3 Hz), 3.62–3.73 (2H, m), 3.76–3.86 (2H, m), 6.25 (1H, s), 7.01–7.17 (2H, m), 7.29 (1H, d, J = 7.9 Hz), 7.52 (1H, d, J = 7.5 Hz), 8.51 (1H, br s); MS (ESI) *m*/*z* 206 $(M+H)^+$.

5.1.16. (*R*)-3-Hydroxy-2-(1*H*-indol-2-ylmethyl)propyl acetate (19a). To a solution of thus obtained AcOEt-free **18b** in vinyl acetate (100 mL), Lipase PS[16,18] (348 mg) was added at room temperature. After stirring for 13 h at room temperature, the reaction mixture was passed through a Celite-filter. The filtrate was concentrated in vacuo to give crude **19a** (9.72 g) as a brown oil. No purification was attempted on this compound, which was used directly in the next step. An analytical sample was purified by column chromatography on silica gel eluting with hexane–AcOEt (5:1–1:1) to afford pure **19a** as a pale yellow solid: mp 66–69 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (3H, s), 2.15–2.27 (1H, m), 2.77–2.91 (2H, m), 3.55–3.69 (2H, m), 4.18 (2H, d, J = 5.9 Hz), 6.27 (1H, s), 7.01–7.16 (2H, m), 7.31 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 7.5 Hz), 8.43 (1H, br s); MS (ESI) *m*/*z* 248 $(M+H)^+$; $[\alpha]_D^{25}$ +8.33° (*c* 0.48, $CHCl_3$).

5.1.17. (*S*)-2-(*tert*-Butyldiphenylsilyloxymethyl)-3-(1*H*-indol-2-yl)-propan-1-ol (20b). To a solution of crude **19a** and imidazole (2.61 g, 38.3 mmol) in DMF (35 mL), TBDPSCl (8.98 mL, 38.3 mmol) was added at room temperature. The reaction mixture was stirred for 1.5 h and was partitioned between AcOEt and brine. The organic layer was separated, successively washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give crude **20a** (19.1 g) as a brown oil. Thus obtained **20a** was dissolved in MeOH (70 mL), and K_2CO_3 (4.81 g, 34.8 mmol) was added to the solution. After stirring for 40 min at room temperature, brine and a 1 M aqueous $KHSO_4$ solution were added to the reaction mixture at 0 °C, and the resulting mixture was extracted with AcOEt. The organic layer was separated, washed with brine and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (4:1) to give **20b** (11.6 g, 75% from **17**) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (9H, s), 2.03–2.11 (1H, m), 2.15 (1H, br s), 2.82 (1H, dd, J = 6.8, 14.8 Hz), 2.93 (1H, dd, J = 7.6, 14.8 Hz), 3.68 (1H, dd, J = 6.0, 11.2 Hz), 3.72–3.85 (3H, m), 6.18 (1H, s), 7.05 (1H, dt, J = 1.2, 7.6 Hz), 7.10 (1H, dt, J = 1.2, 8.0 Hz), 7.22 (1H, d, J = 8.0 Hz), 7.34–7.48 (6H, m), 7.50 (1H, d, J = 7.6 Hz), 7.62–7.70 (4H, m), 8.27 (1H, br s); MS (ESI) *m*/*z* 444 $(M+H)^+$; $[\alpha]_D^{20}$ +5.10° (*c* 1.11, $CHCl_3$).

5.1.18. (*S*)-2-(*tert*-Butyldiphenylsilyloxymethyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indole ((*S*)-21). To a stirring solution of **20b** (11.6 g, 26.1 mmol) in THF (200 mL) were successively added pyridine (6.30 mL, 78.3 mmol) and methanesulfonic anhydride (9.09 g, 52.2 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature over 2 h and then was partitioned between brine and AcOEt. The organic layer was washed with a 1 M aqueous $KHSO_4$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give crude **20c** as a yellow-orange amorphous solid. Thus obtained crude **20c** was dissolved in DMF (200 mL), and NaH (60% in mineral oil, 1.15 g, 6.23 mmol) and NaI (391 mg, 2.61 mmol) were added to the stirring solution at 0 °C. The reaction mixture was stirred for 40 min at 0 °C and for 12 h at room temperature. To the reaction mixture, a 1 M aqueous $KHSO_4$ solution was added and the product was extracted with AcOEt. The organic layer was separated, successively washed with water and brine, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–

AcOEt (19:1) to give (*S*)-**21** (8.94 g, 80%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06 (9H, s), 2.81 (1H, ddd, J = 0.8, 6.0, 16.8 Hz), 3.08 (1H, ddd, J = 0.8, 8.4, 16.8 Hz), 3.17–3.27 (1H, m), 3.73 (1H, dd, J = 7.6, 10.4 Hz), 3.79 (1H, dd, J = 6.4, 10.4 Hz), 3.95 (1H, dd, J = 5.6, 10.0 Hz), 4.17 (1H, dd, J = 8.0, 10.4 Hz), 6.12 (1H, s), 7.05 (1H, dt, J = 1.2, 7.6 Hz), 7.11 (1H, dt, J = 1.2, 7.2 Hz), 7.22 (1H, d, J = 8.0 Hz), 7.33–7.46 (6H, m), 7.53 (1H, d, J = 7.6 Hz), 7.62–7.66 (4H, m); MS (ESI) m/z 426 $(M+H)^+$; $[\alpha]_D^{20}$ +48.5° (c 0.84, $CHCl_3$). (*S*)-MTPA ester derived from (*S*)-**21**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.77 (1H, ddd, J = 1.1, 5.9, 16.1 Hz), 3.15 (1H, ddd, J = 1.1, 8.4, 16.1 Hz), 3.30–3.44 (1H, m), 3.53 (3H, s), 3.78 (1H, dd, J = 5.5, 10.3 Hz), 4.14 (1H, dd, J = 7.7, 10.6 Hz), 4.43 (2H, d, J = 6.6 Hz), 6.14 (1H, s), 7.01–7.19 (3H, m), 7.46–7.55 (3H, m), 7.66–7.77 (3H, m).

5.1.19. 3-Anilino-4-[(*S*)-2-(hydroxymethyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl]-1*H*-pyrrole-2,5-dione ((*S*)-22). (*S*)-**22** was obtained as an orange-red solid in 42% yield from (*S*)-**21** with the same procedures used for the preparation of (*S*)-**8b** from (*S*)-**4b**: mp 248–251 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.21 (1H, dd, J = 5.7, 16.5 Hz), 2.57 (1H, dd, J = 9.9, 16.5 Hz), 2.73 (1H, m), 3.09 (1H, m), 3.20–3.32 (1H, m), 3.73 (1H, dd, J = 5.5, 10.2 Hz), 3.99 (1H, dd, J = 7.7, 10.2 Hz), 4.75 (1H, t, J = 5.1 Hz), 6.62–6.73 (3H, m), 6.75–6.83 (2H, m), 6.88 (1H, t, J = 7.3 Hz), 6.98 (1H, t, J = 7.5 Hz), 7.20 (1H, d, J = 8.0 Hz), 7.30 (1H, d, J = 7.7 Hz), 9.15 (1H, s), 10.60 (1H, br s); MS (FAB) m/z 374 $(M+H)^+$; Anal Calcd for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.34; H, 5.07; N, 11.20; HRMS (FAB–) calcd for $C_{22}H_{18}N_3O_3$ 372.1348. Found: 372.1384; $[\alpha]_D^{25}$ −52.8° (c 0.22, MeOH).

5.1.20. 3-Anilino-4-{(*R*)-2-[(dimethylamino)methyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}-1*H*-pyrrole-2,5-dione ((*R*)-23a). (*R*)-**23a** was obtained as an orange-red solid in 57% yield from (*S*)-**22** with the similar procedures used for the preparation of (*S*)-**9** from (*S*)-**8b**: mp 203–206 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.93 (2H, d, J = 7.0 Hz), 2.08 (1H, m), 2.08 (6H, s), 2.58 (1H, dd, J = 8.4, 16.2 Hz), 2.73 (1H, m), 3.65 (1H, dd, J = 5.5, 10.3 Hz), 3.99 (1H, dd, J = 7.3, 10.3 Hz), 6.64–6.81 (5H, m), 6.91 (1H, dd, J = 7.0, 7.7 Hz), 7.00 (1H, dd, J = 7.0, 7.7 Hz), 7.22 (1H, d, J = 7.7 Hz), 7.35 (1H, d, J = 7.7 Hz), 9.17 (1H, s), 10.61 (1H, s); MS (ESI) m/z 401 $(M+H)^+$; Anal. Calcd for $C_{24}H_{24}N_4O_2$: C, 71.98; H, 6.04; N, 13.99. Found: C, 71.55; H, 6.01; N, 13.95; HRMS (FAB–) calcd for $C_{24}H_{23}N_4O_2$ 399.1821. Found: 399.1838; $[\alpha]_D^{25}$ −33.8° (c 0.20, MeOH).

5.1.21. 3-Anilino-4-{(*R*)-2-[(diethylamino)methyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}-1*H*-pyrrole-2,5-dione ((*R*)-23b). (*R*)-**23b** was similarly obtained as an orange-red solid in 49% yield: mp 167–169 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.90 (6H, t, J = 7.0 Hz), 1.90–2.15 (3H, m), 2.25–2.61 (5H, m), 2.70 (1H, m), 3.65 (1H, dd, J = 5.4, 10.3 Hz), 3.97 (1H, dd, J = 7.4, 10.3 Hz), 6.64–6.81 (5H, m), 6.91 (1H, dd, J = 7.0, 7.9 Hz), 7.00 (1H, dd, J = 7.0, 7.9 Hz), 7.22 (1H, d, J = 7.9 Hz), 7.35 (1H, d, J = 7.9 Hz), 9.20 (1H, s), 10.62 (1H, s); MS (ESI) m/z 429 $(M+H)^+$; Anal. Calcd for $C_{26}H_{28}N_4O_2{\cdot}0.4H_2O$: C, 71.67; H, 6.67; N, 12.86. Found: C, 71.72; H, 6.54; N, 12.93; HRMS (FAB–) calcd for $C_{26}H_{27}N_4O_2$ 427.2134. Found: 427.2139; $[\alpha]_D^{25}$ −50.4° (c 0.24, MeOH).

5.1.22. 3-Anilino-4-((*R*)-2-{[ethyl(methyl)amino]methyl}-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl)-1*H*-pyrrole-2,5-dione ((*R*)-23c). (*R*)-**23c** was similarly obtained as an orange-red solid in 49% yield: mp 184–185 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93 (3H, t, J = 7.0 Hz), 2.01 (3H, m), 2.09 (3H, s), 2.22 (2H, m), 2.58 (1H, dd, J = 8.4, 16.5 Hz), 2.73 (1H, m), 3.63 (1H, dd, J = 5.8, 10.3 Hz), 3.99 (1H, dd, J = 7.3, 10.3 Hz), 6.60–6.82 (5H, m), 6.91 (1H, t, J = 7.0 Hz), 7.00 (1H, t, J = 7.0 Hz), 7.22 (1H, d, J = 7.7 Hz), 7.35 (1H, d, J = 7.7 Hz), 9.17 (1H, s), 10.61 (1H, s); MS (FAB) m/z 415 $(M+H)^+$; Anal. Calcd for $C_{25}H_{26}N_4O_2$: C, 72.44; H, 6.32; N, 13.52. Found: C, 72.17; H, 6.07; N, 13.49; HRMS (FAB–) calcd for $C_{25}H_{25}N_4O_2$ 413.1978. Found: 413.1991; $[\alpha]_D^{25}$ −39.4° (c 0.31, MeOH).

5.1.23. 3-Anilino-4-[(*R*)-2-(pyrrolidin-1-ylmethyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl]-1*H*-pyrrole-2,5-dione ((*R*)-23d). (*R*)-**23d** was similarly obtained as an orange-red solid in 44% yield: mp 159–164 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.67 (4H, br s), 2.10 (3H, m), 2.37 (4H, m), 2.60 (1H, dd, J = 8.4, 16.1 Hz), 2.72 (1H, m), 3.67 (1H, dd, J = 5.5, 10.2 Hz), 4.01 (1H, dd, J = 7.3, 10.2 Hz), 6.64–6.82 (5H, m), 6.90 (1H, dd, J = 7.0, 8.1 Hz), 7.00 (1H, dd, J = 7.0, 8.1 Hz), 7.23 (1H, d, J = 8.1 Hz), 7.35 (1H, d, J = 8.1 Hz), 9.17 (1H, s), 10.61 (1H, s); MS (ESI) m/z 427 $(M+H)^+$; Anal. Calcd for $C_{26}H_{26}N_4O_2{\cdot}0.5H_2O$: C, 71.70; H, 6.25; N, 12.86. Found: C, 71.90; H, 6.53; N, 12.53; HRMS (FAB–) calcd for $C_{26}H_{25}N_4O_2$ 425.1978. Found: 425.2012; $[\alpha]_D^{25}$ −31.2° (c 0.23, MeOH).

5.1.24. 3-[(*R*)-2-(Aminomethyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl]-4-anilino-1*H*-pyrrole-2,5-dione ((*R*)-23g). (*R*)-**23g** was similarly obtained as an orange-red solid in 34%: mp 225–229 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.19 (1H, dd, J = 8.7, 19.5 Hz), 2.25–2.42 (2H, m), 2.51–2.65 (2H, m), 3.69 (1H, dd, J = 5.1, 10.5 Hz), 3.99 (1H, dd, J = 7.2, 10.2 Hz), 6.64–6.72 (3H, m), 6.74–6.81 (2H, m), 6.88 (1H, dt, J = 1.2, 7.5 Hz), 6.99 (1H, dt, J = 0.9, 7.5 Hz), 7.19 (1H, d, J = 8.1 Hz), 7.30 (1H, d, J = 7.5 Hz), 9.13 (1H, br s); MS (ESI) m/z 373 $(M+H)^+$; Anal. Calcd for $C_{22}H_{20}N_4O_2{\cdot}0.5EtOH$: C, 69.85; H, 5.86; N, 14.17. Found: C, 69.49; H, 5.47; N, 14.42; HRMS (FAB–) calcd for $C_{22}H_{19}N_4O_2$ 371.1508. Found: 371.1477; $[\alpha]_D^{25}$ −10.9° (c 0.26, MeOH).

5.1.25. 3-Anilino-4-{(*R*)-2-[(methylamino)methyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}-1*H*-pyrrole-2,5-dione ((*R*)-23e). To a stirring solution of (*S*)-**22** (100 mg, 0.268 mmol) and 2,4,6-collidine (0.106 mL, 0.802 mmol) in THF (1.0 mL) was added dropwise trifluoromethanesulfonic anhydride (0.135 mL, 0.802 mmol) at −78 °C. After stirring for 1.5 h at −78 °C, 40% methylamine in MeOH (0.820 mL, 8.04 mmol) was added to the reaction mixture at the same temperature. The reaction mix-

ture was allowed to warm to room temperature over 2 h. To the reaction mixture, $NaHCO_3$ saturated water was added and the product was extracted with AcOEt. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Thus obtained residue was purified by preparative thin-layer chromatography on silica gel ($CHCl_3$–MeOH–28% aqueous NH_3 150:20:2) to give (***R***)-**23e** (72 mg, 70%) as an orange solid: mp 218–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.08–2.34 (3H, br m), 2.23 (3H, s), 2.54–2.78 (2H, m), 3.67 (1H, dd, J = 5.3, 10.3 Hz), 3.99 (1H, dd, J = 7.5, 10.3 Hz), 6.62–6.83 (5H, m), 6.89 (1H, t, J = 7.0 Hz), 6.99 (1H, t, J = 7.0 Hz), 7.20 (1H, d, J = 8.1 Hz), 7.32 (1H, d, J = 8.0 Hz), 9.16 (1H, s); MS (FAB) *m*/*z* 387 $(M+H)^+$; Anal. Calcd for $C_{23}H_{22}N_4O_2$: C, 71.48; H, 5.74; N, 14.50. Found: C, 71.12; H, 5.74; N, 14.40; HRMS (FAB–) calcd for $C_{23}H_{21}N_4O_2$ 385.1665. Found: 385.1693; $[\alpha]_D^{25}$ −30.8° (*c* 0.28).

5.1.26. 3-Anilino-4-{(*R*)-2-[(ethylamino)methyl]-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-9-yl}-1*H*-pyrrole-2,5-dione ((*R*)-23f). (***R***)-**23f** was similarly obtained as an orange-red solid in 38% yield: mp 227–230 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (3H, t, J = 7.2 Hz), 2.08–2.28 (2H, m), 2.30–2.39 (2H, m), 2.40–2.50 (2H, m), 2.52–2.76 (2H, m), 3.68 (1H, dd, J = 5.4, 10.2 Hz), 4.00 (1H, dd, J = 7.2, 10.2 Hz), 6.61–6.82 (5H, m), 6.89 (1H, t, J = 7.5 Hz), 6.99 (1H, t, J = 7.4 Hz), 7.20 (1H, d, J = 7.5 Hz), 7.32 (1H, d, J = 7.8 Hz), 9.15 (1H, br s); MS (FAB) *m*/*z* 401 $(M+H)^+$; Anal. Calcd for $C_{24}H_{24}N_4O_2$: C, 71.98; H, 6.04; N, 13.99. Found: C, 71.55; H, 6.10; N, 13.73; HRMS (FAB–) calcd for $C_{24}H_{23}N_4O_2$ 399.1821. Found: 399.1807; $[\alpha]_D^{25}$ −21.5° (*c* 0.26, MeOH) for (***R***)-**23f**·HCl.

5.1.27. *N*-{[(*R*)-9-(4-Anilino-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-2-yl]methyl}-acetamide ((*R*)-23h). To a suspension of (***R***)-**23g** (100 mg, 0.269 mmol) in THF (1.0 mL) was added acetic anhydride (30.4 μL, 0.323 mmol) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was concentrated in vacuo. To the residue, EtOH (2 mL) was added and the resulting suspension was stirred at room temperature for 0.5 h. Deposited solid was collected by filtration to give (***R***)-**23h** (57 mg, 51%) as an orange-red solid: mp 153–155 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80 (3H, s), 2.23 (1H, dd, J = 5.7, 16.5 Hz), 2.55–2.79 (2H, m), 2.81–3.00 (2H, m), 3.64 (1H, dd, J = 5.4, 10.5 Hz), 4.00 (1H, dd, J = 7.2, 10.2 Hz), 6.65–6.72 (3H, m), 6.74–6.82 (2H, m), 6.87 (1H, dt, J = 1.2, 7.5 Hz), 6.98 (1H, dt, J = 1.2, 7.7 Hz), 7.18 (1H, d, J = 7.8 Hz), 7.28 (1H, d, J = 8.1 Hz), 7.97 (1H, t, J = 5.6 Hz), 9.19 (1H, s), 10.62 (1H, s); MS (ESI) **m/z** 415 $(M+H)^+$; Anal. Calcd for $C_{24}H_{22}N_4O_3 \cdot 0.75\,H_2O$: C, 67.35; H, 5.53; N, 13.09. Found: C, 67.59; H, 5.34; N, 13.21; HRMS (FAB–) calcd for $C_{24}H_{21}N_4O_3$ 413.1614. Found: 413.1641; $[\alpha]_D^{25}$ −29.1 (*c* 0.11, MeOH).

5.1.28. (*S*)-3-(1*H*-Indol-2-yl)-2-{[(methylsulfonyl)oxy]methyl}propyl acetate (19b). To a stirring solution of **19a** (150 mg, 0.607 mmol) in THF (6 mL) were successively added pyridine (0.098 mL, 1.21 mmol) and methanesulfonic anhydride (211 mg, 1.82 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was partitioned between brine and AcOEt, and the organic layer was separated, successively washed with 2 M HCl and brine, and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (2:1) to give **19b** (174 mg, 88%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (3H, s), 2.42–2.55 (1H, m), 2.90 (2H, d, J = 7.2 Hz), 3.02 (3H, s), 4.08–4.30 (4H, m), 6.30 (1H, s), 7.08 (1H, dt, J = 1.2, 8.4 Hz), 7.15 (1H, dt, J = 1.5, 7.5 Hz), 7.33 (1H, d, J = 8.1 Hz), 7.53 (1H, d, J = 7.5 Hz), 8.35 (1H, br s).

5.1.29. (*R*)-2,3-Dihydro-1*H*-pyrrolo[1,2-*a*]indol-2-ylmethyl acetate ((*R*)-24). To a stirring solution of **19b** (164 mg, 0.504 mmol) in DMF (5 mL) were successively added NaH (60% in mineral oil, 22.2 mg, 0.554 mmol) and NaI (8.3 mg, 0.0554 mmol) at 0 °C and the reaction mixture was stirred for 1 h at 0 °C. To the reaction mixture, a 1 M aqueous $KHSO_4$ solution was added and the mixture was extracted with AcOEt. The organic layer was separated, successively washed with water and brine and concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (20:1–10:1) to give (***R***)-**24** (98 mg, 85%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (3H, s), 2.83 (1H, dd, J = 6.4, 16.4 Hz), 3.19 (1H, ddd, J = 0.8, 8.4, 16.0 Hz), 3.28–3.40 (1H, m), 3.88 (1H, dd, J = 6.0, 10.4 Hz), 4.17 (1H, dd, J = 7.6, 11.6 Hz), 4.21 (1H, dd, J = 8.0, 10.4 Hz), 4.27 (1H, dd, J = 6.0, 11.2 Hz), 6.17 (1H, s), 7.07 (1H, t, J = 7.6 Hz), 7.13 (1H, t, J = 7.6 Hz), 7.22 (1H, d, J = 8.0 Hz), 7.55 (1H, d, J = 8.0 Hz); MS (ESI) *m*/*z* 230 $(M+H)^+$; $[\alpha]_D^{20}$ −20.4° (*c* 0.51, $CHCl_3$).

(***R***)-**22** and (***S***)-**23c** were obtained from (***R***)-**24** with the similar procedures used for the preparation of (***S***)-**22** and (***R***)-**23c**. All spectral data other than optical rotation ((***R***)-**22**: $[\alpha]_D^{25}$ +57.9 (*c* 0.15, MeOH) and (***S***)-**23c**: $[\alpha]_D^{25}$ +34.2 (*c* 0.12, MeOH)) were consistent with those of (***S***)-**22** and (***R***)-**23c**.

5.1.30. Ethyl (1*R,2*R**)-1-hydroxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indole-2-carboxylate (26).** To a solution of **25**[20] (30 g, 123 mmol) in THF (200 mL)-EtOH (100 mL) was added 50%-wet 5% palladium-carbon (6.0 g). The reaction mixture was stirred for 22.5 h at room temperature under hydrogen atmosphere. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel eluting with hexane–AcOEt (4:1–2:1) to give **26** (22.7 g, 75%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (3H, t, J = 7.2 Hz), 2.61 (1H, br s), 3.87 (1H, dt, J = 6.0, 8.8 Hz), 4.30 (2H, q, J = 7.2 Hz), 4.34 (1H, dd, J = 8.5, 10.3 Hz), 4.49 (1H, dd, J = 8.9, 10.5 Hz), 5.48 (1H, d, J = 6.0 Hz), 6.47 (1H, s), 7.10 (1H, dt, J = 0.9, 8.0 Hz), 7.20 (1H, dt, J = 0.9, 8.2 Hz), 7.29 (1H, d, J = 8.2 Hz), 7.62 (1H, d, J = 8.0 Hz); MS (ESI) *m*/*z* 246 $(M+H)^+$.

5.1.31. Ethyl (1*R*,2*R*)-1-{[(*S*)-*N*-(*tert*-butoxyoxycarbonyl)alanyl]oxy}-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indole-2-carboxylate (28). To a stirring solution of **26** (10.0 g, 40.8 mmol), Boc-L-alanine (8.50 g, 44.9 mmol), and DMAP (498 mg, 4.49 mmol) in CH_2Cl_2 (200 mL), DCC (9.26 g, 44.9 mmol) dissolved in CH_2Cl_2 (50 mL) was added at 0 °C. After stirring for 9 h at room temperature, the reaction mixture was filtered through a Celite pad. The filtrate was concentrated in vacuo, and resulting residue was chromatographed on silica gel eluting with hexane–AcOEt (10:1–6:1) to give **28** (3.70 g, 22%) as a colorless solid. Thus obtained **28** was recrystallized from a mixed solvent of hexane and AcOEt to give fine crystals that were used for X-ray crystallographic analysis:[21] ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (3H, d, J = 7.3 Hz), 1.33 (3H, t, J = 7.3 Hz), 1.41 (9H, s), 4.05 (1H, dd, J = 8.4, 15.0 Hz), 4.13–4.35 (3H, m), 4.38 (1H, dd, J = 8.1, 10.2 Hz), 4.57 (1H, t, J = 9.8 Hz), 4.95–5.10 (1H, m), 6.42 (1H, d, J = 6.3 Hz), 6.51 (1H, s), 7.11 (1H, t, J = 7.4 Hz), 7.23 (1H, t, J = 8.1 Hz), 7.31 (1H, d, J = 7.8 Hz), 7.61 (1H, d, J = 8.1 Hz).

5.1.32. (*S*)-2,3-Dihydro-1*H*-pyrrolo[1,2-*a*]indol-2-ylmethyl acetate ((*S*)-24). To a solution of **28** (3.65 g, 8.76 mmol) in THF (50 mL) was added 50%-wet 10% palladium-carbon (1.2 g). The reaction mixture was stirred for 6 h at room temperature under hydrogen atmosphere (2.7 kgf/cm^2). After removal of the catalyst by filtration, the filtrate was concentrated in vacuo, and the residue was chromatographed on silica gel eluting with hexane–AcOEt (10:1) to give **29a** (1.61 g, 80%) as a colorless solid. To a stirring suspension of lithium aluminum hydride (4.0 mg, 0.105 mmol) in THF (0.3 mL), a solution of **29a** (30 mg, 0.131 mmol) in THF (0.3 mL) was added at 0 °C. After stirring for 2 h at 0 °C, brine and a 1 M aqueous $KHSO_4$ solution were added to the reaction mixture. The resulting mixture was extracted with AcOEt and the organic layer was separated, washed with brine, and concentrated in vacuo to give crude **29b**. Thus obtained crude **29b** was dissolved in THF (0.5 mL), and to the solution were added pyridine (32 μL, 0.393 mmol), acetic anhydride (25 μL, 0.262 mmol), and DMAP (1.6 mg, 0.0131 mmol) at room temperature. After stirring for 6 h at room temperature, the reaction mixture was poured into brine and extracted with AcOEt. The organic layer was separated, successively washed with a 1 M aqueous $KHSO_4$ solution and brine, and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (hexane–AcOEt 4:1) to give (***S***)-**24** (22 mg, 73% from **29a**) as an off-white solid showing $[\alpha]_D^{20}$ of +23.8° (c 0.52, $CHCl_3$). All of spectral data other than optical rotation were consistent with those of (***R***)-**24**.

5.2. Enzymatic assay

PKC activity was measured with PKC-enzyme assay system (Amersham Biosciences).[26] The assay mixture (final volume 21 μL), containing 1.2 mM calcium acetate or 0.1 mM EGTA, 0.6 μg L-α-phosphatidyl-L-serine, 0.05 μg phorbol 12-myristate 13-acetate, 3 mM dithiothreitol, 0.1 mM ATP, 7 mM $MgCl_2$, 2.5 kBq [γ-^{32}P]ATP, 90 mM substrate peptide (RKRTLRRL-OH) or 2 μg PKC-ε substrate peptide (EMD Biosciences) or Syntide 2 (PLARTLSVAGLPGKK, Sigma–Aldrich), recombinant human PKC-α, -β1, -β2, -γ, -δ, -ε, -ζ or -μ (EMD Biosciences), and compound dissolved in DMSO (final concentration 4.8%), was incubated for 15 min at 37 °C. Reaction was stopped by addition of 300 mM H_3PO_4. The assay mixture was blotted onto phosphocellulose paper (Whatman), washed with 75 mM H_3PO_4, and evaluated by IP-autoradiography (Fujifilm). The IC_{50} value was calculated in a semi-logarithmic proportional manner from the two points enclosing 50% inhibition. All the assays were carried out in duplicate or triplicate and the average results are presented.

5.3. Ex vivo studies

Compounds were suspended in 0.5% methylcellulose and orally administered to 7-week-old male Sprague–Dawley rats (Charles River Japan) at a dose of 2 mg/kg. PKCβ2 inhibitory activity of plasma at 2 and 6 h after administration was evaluated as described above.

5.4. Retinal mean circulation time (MCT) in diabetic rats

Diabetes was induced with a single intraperitoneal injection of 65 mg/kg streptozotocin (STZ, Sigma–Aldrich) dissolved in 0.05 M citric acid buffer (pH 4.5) to 8-week-old male Sprague–Dawley rats. Animal protocols used in the study complied with our Laboratory Guidelines for Animal Experimentation. Distilled water or compound (***R***)-**23g** (0.01, 0.1, and 1 mg/kg) was administered twice per day for 14 days from the day following STZ injection. The day after the last administration, video fluorescein angiography was performed to evaluate retinal mean circulation time (MCT). Animals were anesthetized with sodium pentobarbital (20 mg/kg, i.p.) and 30 μL of 10% fluorescein was injected via catheter inserted in the jugular vein. Fluorescence circulation in retinal artery and vein was recorded by video angiogram and MCT was calculated.[25]

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References and notes

1. (a) Inoguchi, T.; Battan, R.; Handler, E.; Sportsman, J. R.; Heath, W.; King, G. L. *Proc. Natl. Acad. Sci. U.SA.* **1992**, *89*, 11059; (b) Inoguchi, T.; Xia, P.; Kunisaki, M.; Higashi, S.; Feener, E. P.; King, G. L. *Am. J. Physiol.* **1994**, *267*, E369.
2. Koya, D.; King, G. L. *Diabetes* **1998**, *47*, 859.
3. (a) Ishii, H.; Jirousek, M. R.; Koya, D.; Takagi, C.; Xia, P.; Clermont, A.; Bursell, S. E.; Kern, T. S.; Ballas, L. M.; Heath, W. F.; Stramm, L. E.; Feener, E. P.; King, G. L. *Science* **1996**, *272*, 728; (b) Nakamura, J.; Kato, K.;

Hamada, Y.; Nakayama, M.; Chaya, S.; Nakashima, E.; Naruse, K.; Kasuya, Y.; Mizubayashi, R.; Miwa, K.; Yasuda, Y.; Kamiya, H.; Ienaga, K.; Sakakibara, F.; Koh, N.; Hotta, N. *Diabetes* **1999**, *48*, 2090.

4. (a) Harris, W.; Wilkinson, S. E.; Nixon, J. S. *Exp. Opin. Ther. Patents* **1997**, *7*, 63; (b) Sridhar, J.; Pattabiraman, N. *Exp. Opin. Ther. Patents* **2005**, *15*, 1691; (c) Faul, M. M.; Gillig, J. R.; Jirousek, M. R.; Ballas, L. M.; Schotten, T.; Kahl, A.; Mohr, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1857; (d) Heath Jr, W. F.; Jirousek, M. R.; McDonald III, J. H.; Rito, C. J. U. S. Patent 5,624,949, 1997; (e) Zhang, H.-C.; White, K. B.; Ye, H.; McComsey, D. F.; Derian, C. K.; Addo, M. F.; Andrade-Gordon, P.; Eckardt, A. J.; Conway, B. R.; Westover, L.; Xu, J. Z.; Look, R.; Demarest, K. T.; Emanuel, S.; Maryanoff, B. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3049.
5. Jirousek, M. R.; Gillig, J. R.; Gonzalez, C. M.; Heath, W. F.; McDonald, J. H.; Neel, D. A.; Rito, C. J.; Singh, U.; Stramm, L. E.; Melikian-Badalian, A.; Baevsky, M.; Ballas, L. M.; Hall, S. E.; Winneroski, L. L.; Faul, M. M. *J. Med. Chem.* **1996**, *39*, 2664.
6. (a) The PKC-DRS Study Group. *Diabetes* **2005**, *54*, 2188; (b) Vinik, A. I.; Bril, V.; Kempler, P.; Litchy, W. J.; Tesfaye, S.; Price, K. L.; Bastyr, E. J., III *Clin. Ther.* **2005**, *27*, 1164; (c) Tuttle, K. R.; Bakris, G. L.; Toto, R. D.; McGill, J. B.; Hu, K.; Anderson, P. W. *Diabetes Care* **2005**, *28*, 2686; (d) Aiello, L. P.; Clermont, A.; Arora, V.; Davis, M. D.; Sheetz, M. J.; Bursell, S. E. *Invest. Ophthalmol. Vis. Sci.* **2006**, *47*, 86.
7. Davis, P. D.; Hill, C. H.; Lawton, G.; Nixon, J. S.; Wilkinson, S. E.; Hurst, S. A.; Keech, E.; Turner, S. E. *J. Med. Chem.* **1992**, *35*, 177.
8. Tanaka, M.; Sagawa, S.; Hoshi, J.; Shimoma, F.; Matsuda, I.; Sakoda, K.; Sasase, T.; Shindo, M.; Inaba, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5171.
9. Zhang, H.-C.; Derian, C. K.; McComesey, D. F.; White, K. B.; Ye, H.; Hecker, L. R.; Li, J.; Addo, M. F.; Croll, D.; Eckardt, A. J.; Smith, C. E.; Li, Q.; Cheung, W.-M.; Conway, B. R.; Emanuel, S.; Demarest, K. T.; Andrade-Gordon, P.; Damiano, B. P.; Maryanoff, B. E. *J. Med. Chem.* **2005**, *48*, 1725.
10. FASTA program was used against the SWISS-PROT database.
11. Kubo, K.; Ohno, S.; Suzuki, K. *FEBS. Lett.* **1987**, *223*, 138.
12. Veber, D. F.; Johnson, S. R.; Chen, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
13. Bit, R. A.; Davis, P. D.; Elliott, L. H.; Harris, W.; Hill, C. H.; Keech, E.; Kumar, H.; Lawton, G.; Maw, A.; Nixon, J. S.; Vesey, D. R.; Wardsworth, J.; Wilkinson, S. E. *J. Med. Chem.* **1993**, *36*, 21.
14. Speeter, N. E.; Anthony, W. C. *J. Am. Chem. Soc.* **1954**, *76*, 6208.
15. (a) Rooney, C. S.; Randall, W. C.; Streeter, K. B.; Ziegler, C.; Cragoe, E. J.; Schwam, H.; Michelson, S. R.; Williams, H. W. R.; Eichler, E.; Duggan, D. E.; Ulm, E. H.; Noll, R. M. *J. Med. Chem.* **1983**, *26*, 700; (b) Neel, D. A.; Jirousek, M. R.; McDonald, J. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 47.
16. Terao, Y.; Akamatsu, M.; Achiwa, K. *Chem. Pharm. Bull.* **1991**, *39*, 823.
17. Maarseveen, J. H.; van Mulders, S. J. E.; Aben, R. W. M.; Kruse, C. G.; Scheeren, H. W. *Tetrahedron* **1995**, *51*, 4841.
18. Takabe, K.; Hashimoto, H.; Sugimoto, H.; Nomoto, M.; Yoda, H. *Tetrahedron: Asymmetry* **2004**, *15*, 909, and references cited therein.
19. Beccalli, E. M.; Broggini, G.; Rosa, C. L.; Passarella, D.; Pilati, T.; Terraneo, A.; Zecchi, G. *J. Org. Chem.* **2000**, *65*, 8924.
20. Remers, W. A.; Weiss, M. J. *J. Med. Chem.* **1965**, *8*, 700.
21. Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 604892. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
22. Prade, L.; Engh, R. A.; Girod, A.; Kinzel, V.; Huber, R.; Bossemeyer, D. *Structure* **1997**, *5*, 1627.
23. Xu, Z.-B.; Chaudhary, D.; Olland, S.; Wolfrom, S.; Czerwinski, R.; Malakian, K.; Lin, L.; Stahl, M. L.; Joseph-McCarthy, D.; Benander, C.; Fitz, L.; Greco, R.; Somers, W. S.; Mosyak, L. *J. Biol. Chem.* **2004**, *279*, 50401.
24. (a) Aiello, L. P.; Bursell, S. E.; Clermont, A.; Duh, E.; Ishii, H.; Takagi, C.; Mori, F.; Ciulla, T. A.; Ways, K.; Jirousek, M.; Smith, L. E. H.; King, G. L. *Diabetes* **1997**, *46*, 1473; (b) Aiello, L. P. *Surv. Ophthalmol.* **2002**, *47*(Suppl. 2), S263.
25. Riva, C. E.; Feke, G. T.; Ben-Sira, I. *Am. J. Physiol.* **1978**, *234*, H315.
26. Sasase, T.; Yamada, H.; Sakoda, K.; Imagawa, N.; Abe, T.; Ito, M.; Sagawa, S.; Tanaka, M.; Matsushita, M. *Diabetes Obes. Metab.* **2005**, *7*, 586.