The Discovery of Tadalafil: A Novel and Highly Selective PDE5 Inhibitor. 1: 5,6,11,11a-Tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione Analogues

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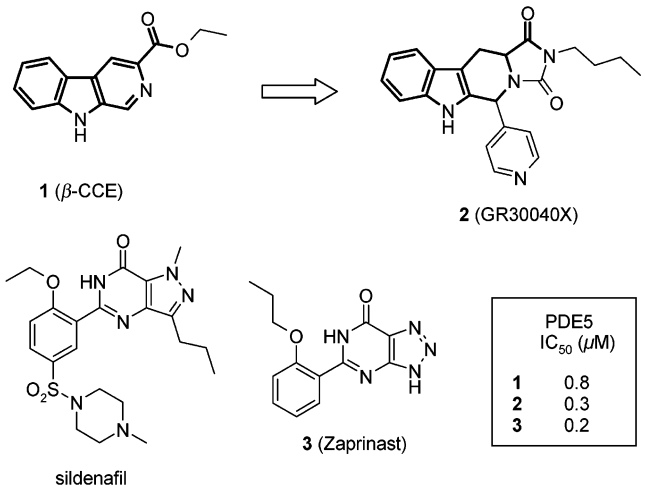
Starting from ethyl β-carboline-3-carboxylate (β-CCE), **1**, a modest inhibitor of type 5 phosphodiesterase (PDE5), a series of functionalized tetrahydro-β-carboline derivatives has been identified as a novel chemical class of potent and selective PDE5 inhibitors. Optimization of the side chain on the hydantoin ring of initial lead compound **2** and of the aromatic ring on position 5 led to the identification of compound **6e**, a highly potent and selective PDE5 inhibitor, with greater selectivity for PDE5 vs PDE1−4 than sildenafil. Compound **6e** demonstrated a long-lasting and significant blood pressure lowering effect after iv administration in the spontaneously hypertensive rat model but showed only moderate oral in vivo efficacy.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cyclic nucleotides cGMP and cAMP into their respective 5′ nucleoside monophosphates. To date, the phosphodiesterase superfamily has been classified into 11 different isoforms (PDE1−11) according to their primary protein and cDNA sequences, their specificities toward hydrolysis of cyclic AMP or cyclic GMP, their mechanisms of regulation, and their sensitivities to various pharmacological agents.[1−4] Phosphodiesterase type 5, (PDE5), one member of the superfamily of cyclic nucleotide hydrolyzing enzymes that specifically cleaves cyclic guanosine monophosphate (cGMP), is distributed throughout vascular smooth muscle tissue and to a lesser extent in the lung, kidney, and platelets. Inhibition of PDE5 as a therapeutic target has received considerable attention over the years, particularly for the treatment of cardiovascular diseases, e.g., angina, hypertension, and congestive heart failure.[5,6] More recently, it was demonstrated that PDE5 plays a critical role in the mechanism of penile erection and that PDE5 inhibition would be of therapeutic utility in treating male erectile dysfunction (MED).[7] After these discoveries, sildenafil (Viagra) was approved for the treatment of MED.[8,9]

The primary objective of our research effort in the early 1990s was to identify chemically novel, selective (PDE5 vs other PDEs), and orally active PDE5 inhibitors for the treatment of hypertension and congestive heart failure. β-Carbolines had been previously found to increase basal level of cGMP in rat cerebellum[10] and to induce a concentration-dependent relaxation of rat aortic rings precontracted with KCl.[11] Furthermore, β-carbolines were also reported to inhibit crude rat aortic cyclic nucleotide phosphodiesterase activity.[11] In fact, in our hands, ethyl β-carboline-3-carboxylate (β-CCE), compound **1**, displayed modest inhibitory activity toward PDE5 (IC_{50} = 0.8 μM). The β-carboline scaffold was then used as a basis for substructure searching in our internal database to find novel type 5 phosphodiesterase inhibitors (Chart 1). The hydantoin derivative **2** was identified as a promising PDE5 inhibitor, with a selectivity comparable to zaprinast **3**, the reference PDE5 inhibitor at that time (see Table 5).

Chart 1



In the present paper, we describe the discovery and optimization of a novel series of highly potent and selective inhibitors of PDE5 via a program of medicinal chemistry based upon hydantoin **2**.

Chemistry

The general synthesis of hydantoin compounds **6** is illustrated in Scheme 1. Racemic tryptophan methyl or ethyl ester and appropriate aldehydes **4** were subjected to a modified Pictet−Spengler reaction. Since we initially desired access to both the *cis*- and *trans*-isomers **5**, the reaction was carried out under nonstereospecific conditions. The *cis*- and *trans*-1,3-disubstituted tetrahydro-β-carbolines **5**, which can be separated by flash chromatography or crystallization, reacted with appropriate, commercially available isocyanates to afford

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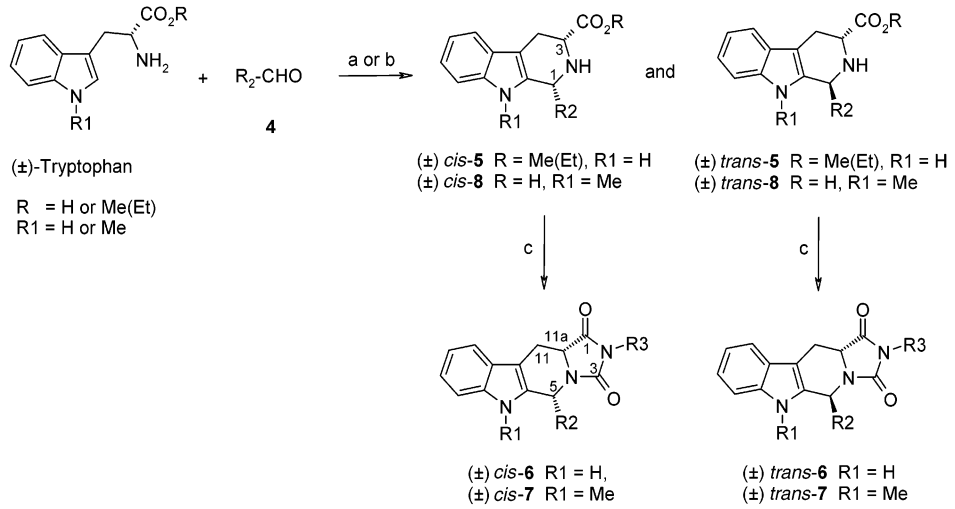
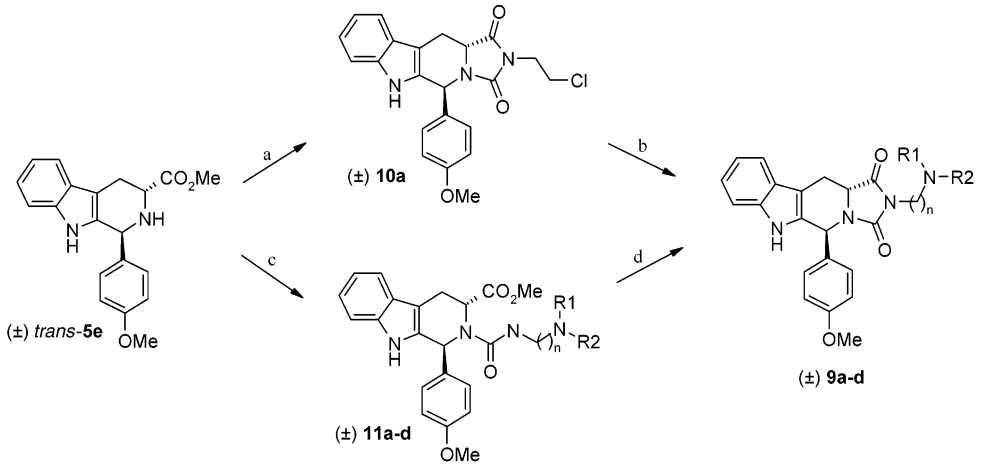
Scheme 1[a]



[a] Conditions: (a) R = Me(Et), CF_3CO_2H, CH_2Cl_2; (b) R = H, dil. H_2SO_4; (c) R_3–N═C═O, 2-butanone, reflux.

Scheme 2[a]



[a] Conditions: (a) Cl–$(CH_2)_2$–NCO, 2-butanone, reflux; (b) $Me_2NH{\cdot}HCl$, K_2CO_3, DMF; (c) R1R2N$(CH_2)_n NH_2$, CDI, THF; (d) THF, reflux.

the desired *cis*- and *trans*-hydantoins **6**, respectively. The *N*-alkyl indole derivatives **7** were prepared using a similar synthetic pathway as described in Scheme 1 but starting from racemic *N*-methyl tryptophan.[12]

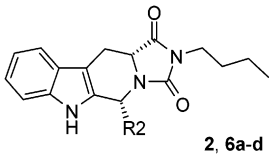
The assignment of *cis*/*trans*-stereochemistry for tetrahydro-β-carboline **5** was based on a detailed study of the ^{1}H and ^{13}C NMR spectroscopy and by comparison with the literature data, well-established by Cook.[13,14] Thus, the signals for C-1 and C-3 in the *trans*-isomer appeared at higher field in the carbon spectrum than the analogous carbons of the corresponding *cis*-isomer, probably due to the 1,3-interactions present in the *trans*-isomer. Moreover, the NMR signals for the proton at C-1 is more shielded in the *cis*-isomer compared to the *trans*-isomer. A correlation exists between R_f value on TLC and the stereochemistry of the 1,3-disubstituted tetrahydro-β-carbolines as described in the literature.[14] The *cis*-isomer **5** is systematically less polar than *trans*-**5**, as indicated by the order of elution during the purification on silica gel (toluene/ethyl acetate or dichloromethane/methanol as eluent). However, in the hydantoin series **6**, the polarity is reversed, the *cis*-isomer becomes more polar than the *trans*-isomer.

For the synthesis of analogues bearing a polar chain on the hydantoin ring **9a**–**d**, two different synthetic pathways were explored as illustrated in Scheme 2. The *trans*-1,3-disubstituted tetrahydro-β-carboline **5e** reacted with 2-chloroethyl isocyanate to give the corresponding hydantoin intermediate **10a** as the sole product. Subsequent attempts to substitute the halide **10a** with various secondary amines using different experimental conditions either failed or led to the desired compound **9a** in a very low yield. To improve general access to polar compounds, a second approach was used. Primary amines were treated with carbonyl diimidazole in THF and the resulting imidazolide intermediates were reacted with *trans*-1,3-disubstituted tetrahydro-β-carboline **5e** to give the intermediate ureas **11a**–**d** which underwent intramolecular cyclization in refluxing THF to produce the hydantoins **9a**–**d** in good yields.

Results and Discussion

Compounds were evaluated for inhibitory activity against bovine PDE5. Each compound was evaluated in two steps. The first step was the determination of the percentage of inhibition at 10 μM performed in triplicate. For compounds displaying a percentage of inhibition greater than 50% at 10 μM, the IC_{50} was determined from a concentration–response curve using concentrations of 1 nM, 10 nM, 100 nM, 1 μM, and 10

Table 1. PDE5 Inhibitory Potency of C-5 Aromatic or Heterocyclic Derivatives



2, **6a-d**

compd	R2	PDE5 IC_{50} (μM)[a]
2	4-pyridinyl	0.30
cis-**6a**	3-pyridinyl	0.09
cis-**6b**	3-thienyl	0.03
cis-**6c**	3-furanyl	0.10
cis-**6d**	phenyl	0.06

[a] IC_{50} values were reproducible to ±25%.

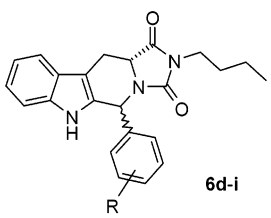
μM, each tested in duplicate. IC_{50} values were reproducible to ±25%. Selectivity toward other PDEs was evaluated using similar assays. Rat PDE5 was not available at the time, but it was known that there is 94.3% homology between rat and bovine PDE5.[15] We therefore made the assumption that our PDE5 inhibitors would exhibit similar potency against the rat enzyme as against bovine. Selected compounds were then tested in a cellular assay using atrial natriuretic factor treated rat aortic smooth muscle cells (RSMC). This assay is designed to reveal the ability of the tested compound to increase intracellular cGMP concentration in cells. In this assay the EC_{50} values were determined as the concentration giving half-maximal increase of cGMP observed with saturating compound concentration. Finally, spontaneously hypertensive rat model (SHR) was used to measure blood pressure lowering ability of the best compounds.

Modification at the C-5 Position. In an attempt to improve the potency and selectivity of the hydantoin lead **2**, we explored first the possibility of replacing the pendant pyridine ring of **2** with aromatic or other heteroaromatic systems (Table 1). Substitution with a 3-pyridinyl **6a** or with a five-membered heterocycle such as a thienyl **6b** or a furanyl ring **6c** showed improvement in potency compared to the initial hit **2**. More interestingly, the introduction of a phenyl ring **6d** led to very encouraging in vitro results.

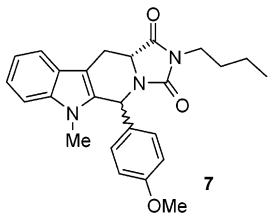
SAR on the Phenyl Ring in C-5 Position. Having shown that a phenyl ring could advantageously replace the pyridinyl moiety in compound **2**, a study of the structure−activity relationships around the phenyl group of compound **6d** was carried out (Table 2). Substitution at the 4-position on the phenyl ring with a methoxy group **6e** gave an increase in potency of PDE5 inhibition. Analogues with a chlorine atom in the 3- (**6f**) or the 4-position (**6g**) gave no improvement of in vitro activity compared with compound **6d**. Introduction of an electron-withdrawing substituent in the 4-position of the phenyl ring, such as cyano, had a deleterious effect on potency (Table 2, **6d** vs **6h)**. Introduction of a methoxy group in the 2-position resulted in loss of activity (**6i**), possibly suggesting that the presence of a 2-substituent prevents the optimal orientation of the phenyl ring.

Comparison of the activities of *cis*- and *trans*-compounds showed that there was no clear diastereospecificity for PDE5 inhibition in this series (Table 2, *cis*-**6e**, **-6f** vs *trans*-**6e**, **-6f**).

Table 2. PDE5 Inhibition and Cellular Activity of Substituted Phenyl Analogues



6d-i



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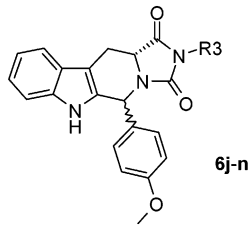
compd	R	PDE5 IC_{50} (μM)[a]	RSMC EC_{50} (μM)
cis-**6d**	H	0.060	5
trans-**6d**	H	0.020	2
cis-**6e**	4-OMe	0.008	0.7
trans-**6e**	4-OMe	0.005	1
cis-**6f**	3-Cl	0.050	4
trans-**6f**	3-Cl	0.050	>10
cis-**6g**	4-Cl	0.050	>10
trans-**6g**	4-Cl	0.020	1.5
cis-**6h**	4-CN	0.9	nd[b]
trans-**6h**	4-CN	0.3	nd[b]
trans-**6i**	2-OMe	1	nd[b]
cis-**7**	4-OMe	>10	nd[b]
trans-**7**	4-OMe	2	nd[b]

[a] IC_{50} values were reproducible to ±25%. [b] Not determined.

Table 3. PDE5 Inhibition and Cellular Activity of N-Substituted Hydantoins



6j-n

compd	R3	PDE5 IC_{50} (μM)[a]	RSMC EC_{50} (μM)
cis-**6e**	butyl	0.008	0.7
trans-**6e**	butyl	0.005	1
trans-**6j**	hydrogen	0.020	3
cis-**6k**	methyl	0.010	1
cis-**6l**	ethyl	0.010	0.4
trans-**6l**	ethyl	0.007	1.5
cis-**6m**	benzyl	0.004	0.15
trans-**6m**	benzyl	0.018	>10
cis-**6n**	c.hexyl	0.007	0.3
trans-**6n**	c.hexyl	0.003	0.1

[a] IC_{50} values were reproducible to ±25%.

Alkylation of the indole nitrogen atom led to a drastic fall in PDE5 inhibitory potency (cf. **6e** vs *cis*- or *trans*-**7**).

N-Substitution of the Hydantoin Ring. Given the increased potency of compounds bearing a *p*-methoxy substituent on the phenyl ring, this substitution was retained while we modified the chain on the hydantoin ring. The presence or the nature of the alkyl side-chain group on the hydantoin ring does not seem to significantly influence the potency (Table 3, **6e** vs **6j**−**l**). Moreover, the introduction of a benzyl group **6m** or a cycloalkyl group such as cyclohexyl ring **6n** gave compounds equipotent with butyl hydantoin **6e** (Table 3). The PDE5 enzyme appears to be tolerant of a wide

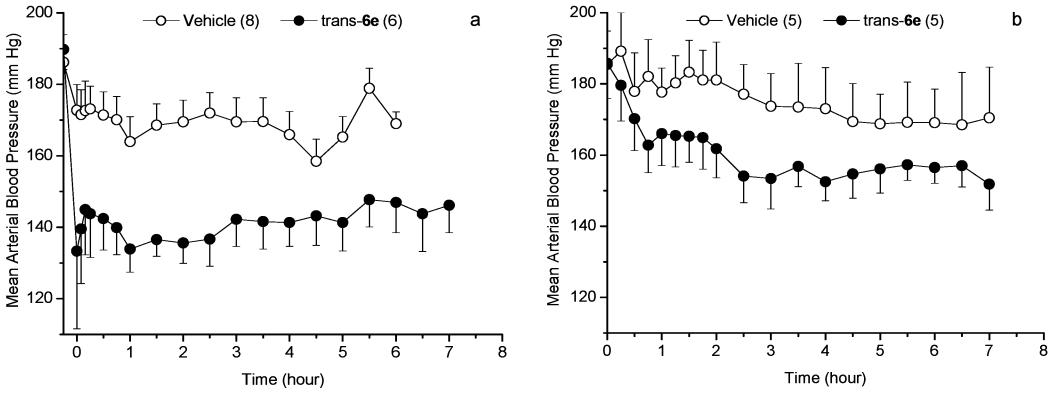
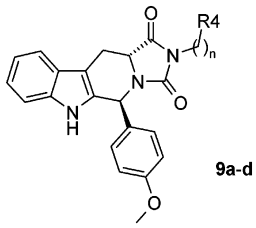


Figure 1. Effect of *trans*-**6e** on Blood Pressure after iv (a) and po (b) Administration in SHR Model. **1a**: Time course of the changes in mean arterial blood pressure produced by *trans*-**6e** (●) or its vehicule (○) after iv administration (10 mg/kg) to spontaneously hypertensive rats (SHR). **1b**: Time course of the changes in mean arterial blood pressure produced by *trans*-**6e** (●) or its vehicule (○) after po administration (30 mg/kg) to spontaneously hypertensive rats (SHR).

Table 4. PDE5 Inhibition and Cellular Activity of Hydantoins Substituted with Basic Chains



compd	n	R4	PDE5 IC_{50} (μM)[a]	RSMC EC_{50} (μM)
trans-**9a**	2	NMe_2	0.030	3.5
trans-**9b**	1	3-pyridinyl	0.020	5.5
trans-**9c**	2	2-pyridinyl	0.006	1.5
trans-**9d**	2	pyrrolidinyl	0.100	10

[a] IC_{50} values were reproducible to ±25%.

range of substituents on the hydantoin nitrogen. However, in the RSMC test, the benzyl *cis*-**6m** and the cyclohexyl **6n** compounds showed a significant increase in potency. Surprisingly, the *trans*-benzyl analogue *trans*-**6m** was completely inactive at concentrations up to 10 μM, despite an only 5-fold decrease in PDE5 inhibition.

Incorporation of basic groups into the hydantoin side chain **9a**−**d** led to somewhat decreased PDE5 inhibitory potency in both enzymatic and cellular assays (Table 4).

Phosphodiesterase Selectivity. To further evaluate the interest of this new class of PDE5 inhibitors, representative compounds were tested against other PDE isoforms. As shown in Table 5, all compounds tested in this series have high selectivity vs PDE1−PDE4 and much greater selectivity than sildenafil on PDE1, PDE3, and PDE4.

Acute Effects of Compound *trans*-6e on Blood Pressure. On the basis of the PDE5 inhibitory potency, isoform selectivity, and cellular potency (RSMC test), several compounds were evaluated in the conscious spontaneously hypertensive rat model (SHR) for antihypertensive activity. Compound *trans*-**6e** was representative of this chemical series. A significant (−25 mmHg) and long-lasting blood pressure lowering effect (>7 h) was observed following iv administration (10 mg/kg, Figure 1a). In contrast, only a modest effect on blood pressure was observed after oral dosing (30 mg/kg, Figure 1b), probably reflecting poor oral absorption of *trans*-**6e**.

Table 5. PDE5 Activity and Selectivity of Hydantoins on PDE Isoforms

compd	IC_{50} (μM)[c] PDE5[a]	IC_{50} (μM) PDE1[a]	PDE2[b]	PDE3[a]	PDE4[b]
lead-**2**	0.3	2	nd[d]	>10	>10
Zaprinast-**3**	0.2	2.6	>10	>10	>10
trans-**6e**	0.005	>10	>10	>10	>10
cis-**6e**	0.008	>10	>10	>10	>10
cis-**6m**	0.004	>10	>10	>10	>10
cis-**6n**	0.007	>10	>10	>10	>10
sildenafil	0.006	1.1	>10	9.2	7.8

[a] Bovine aorta PDE. [b] Human recombinant PDE. [c] IC_{50} values were reproducible to ±25%. [d] Not determined.

Conclusion

In this paper, we described the discovery of 5,6,11,-11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione analogues as a novel class of highly potent and selective PDE5 inhibitors. The best inhibitors in this series demonstrated high selectivity for PDE5 vs PDE1−4 when compared to sildenafil. However, a representative compound showed only moderate oral efficacy in conscious spontaneously hypertensive rats (SHR), probably due to limited oral bioavailability. Nevertheless, this new series of hydantoin compounds represents an attractive starting point for the design and synthesis of selective and potent PDE5 inhibitors with more desirable pharmacokinetic and pharmacological profiles (see Part 2).

Experimental Section

All starting materials were commercially available and used without further purification. All reactions were carried out with the use of standard techniques under an inert atmosphere (Ar or N_2). Organic extracts were routinely dried over anhydrous Na_2SO_4. Solvent removal refers to rotary evaporation under reduced pressure at 30−40 °C. The analytical thin-layer chromatography (TLC) was carried out on E. Merck 60-F_{254} precoated silica gel plates and components were usually visualized using UV light, iodine vapor, or Dragendorff preparation. Flash column chromatography was performed on silica gel 60 (E. Merck, 230−400 mesh). Melting points were determined on a hit-stage Kofler apparatus and are uncorrected. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were recorded at ambient temperature on a Bruker

Avance 300 DPX spectrometer using tetramethylsilane as internal standard, and proton chemical shifts are expressed in ppm in the indicated solvent. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quadruplet, dd = double doublet, m = multiplet. The elemental analysis were performed by Wolff Laboratories and are within ±0.4% of the theoretical value, unless stated otherwise.

Methyl 1-(4-Methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5e). Representative example: Racemic tryptophan methyl ester (10.76 g, 49.35 mmol, 1 equiv) and 4-methoxybenzaldehyde (7.39 g, 54 mmol, 1.1 equiv) were dissolved in CH_2Cl_2 (70 mL) and cooled to 0 °C with an ice bath. To this solution was added dropwise TFA (7.6 mL), and the mixture was stirred at room temperature for 4 days. The reaction mixture was then basified with aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3×). The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel eluting with toluene/AcOEt, 70/30, to give first the *cis*-isomer **5e** as a white solid (4.15 g, 25%), mp 132 °C, followed by the *trans*-isomer **5e** as a white solid (6.09 g, 37%), mp 202 °C. *cis*-**5e**: ^{1}H NMR ($CDCl_3$) δ 7.58−7.37 (m, 2H), 7.32−7.02 (m, 5H), 6.84 (d, 2H, J = 8.3 Hz), 5.14 (s, 1H, C_1–H), 3.91 (dd, 1H, J = 11.3, 4.3 Hz, C_3–H), 3.76 (br s, 6H), 3.16 (dd, 1H, J = 15.2, 4.3 Hz, C_4–H), 2.96 (dd, 1H, J = 15.2, 11.2 Hz, C_4–H), 2.20 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 173.8, 160.5, 136.8, 135.5, 133.2, 130.5, 127.8, 122.6, 120.3, 118.9, 114.9, 111.6, 109.5, 58.7 (C_1), 57.6 (C_3), 56, 52.9, 26.3; ^{1}H-^{13}C HMQC ($CDCl_3$) δ_H (δ_C) 5.14 (58.7, C_1), 3.91 (57.6, C_3); *trans*-**5e**: ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.61 (d, 1H, J = 7 Hz), 7.44−7.16 (m, 5H), 6.91 (d, 2H, J = 8.7 Hz), 5.41 (s, 1H, C_1–H), 4.14−4.0 (m, 1H, C_3–H), 3.84 (s, 3H), 3.77 (s, 3H), 3.32 (dd, 1H, J = 15.4, 5.4 Hz, C_4–H), 3.15 (dd, 1H, J = 15.5, 6.8 Hz, C_4–H), 2.53 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 174.7, 160.1, 136.8, 134.5, 134.1, 130.3, 127.6, 122.6, 120.2, 118.9, 114.7, 111.6, 108.9, 56, 54.9 (C_1), 53.2 (C_3), 52.8, 25.2; ^{1}H-^{13}C HMQC ($CDCl_3$) δ_H (δ_C) 5.41 (54.9, C_1), 4.14 (53.2, C_3).

The following compounds were prepared using a similar procedure with appropriate aldehydes.

Ethyl 1-(3-Pyridinyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5a). The title compounds were prepared from racemic tryptophan ethyl ester and 3-pyridinecarboxaldehyde. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 95/5, to give first *cis*-**5a** as a white solid (0.5 g, 13%), mp 230−232 °C, followed by *trans*-**5a** as a white solid (0.3 g, 7.9%), mp 210−214 °C.

Ethyl 1-(3-Thienyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5b). The title compounds were prepared from racemic tryptophan ethyl ester and 3-thiophenecarboxaldehyde. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 70/30, to give first *cis*-**5b** as a white solid (2.3 g, 51%), mp 130 °C, followed by *trans*-**5b** as a white solid (0.45 g, 10%), mp 182−184 °C.

Methyl 1-(3-Furanyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate as a Mixture of Isomers (*cis*- and *trans*-5c). The title compound was prepared from racemic tryptophan methyl ester and 3-furancarboxaldehyde. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 98/2, to give the mixture of *cis*- and *trans*-**5c** (3 g, 51%), mp 130 °C.

Ethyl 1-(Phenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5d). The title compounds were prepared from racemic tryptophan ethyl ester and benzaldehyde. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give first *cis*-**5d** as a white solid (0.9 g, 34%), mp 153−155 °C, followed by *trans*-**5d** as a white solid (0.55 g, 20%), mp 180−182 °C.

Methyl 1-(3-Chlorophenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate as a Mixture of Isomers (*cis*- and *trans*-5f). The title compound was prepared from racemic tryptophan methyl ester and 3-chlorobenzaldehyde. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give the mixture of *cis*- and *trans*-**5f** (4.4 g, 79%), mp 150−160 °C.

Methyl 1-(4-Chlorophenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5g). The title compounds were prepared from racemic tryptophan methyl ester and 4-chlorobenzaldehyde. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 90/10, to give first *cis*-**5g** as a white solid (3.6 g, 54%), mp 208−209 °C, followed by *trans*-**5g** as a white solid (0.56 g, 9%), mp 108−109 °C.

Ethyl 1-(4-Cyanophenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5h). The title compounds were prepared from racemic tryptophan ethyl ester and 4-cyanobenzaldehyde. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 80/20, to give first *cis*-**5h** as a white solid (3.47 g, 46%), mp 200 °C, followed by *trans*-**5h** as a white solid (1.54 g, 20.5%), mp 156 °C.

Methyl 1-(2-Methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylate (*cis*- and *trans*-5i). The title compounds were prepared from racemic tryptophan methyl ester and 2-methoxybenzaldehyde. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give first *cis*-**5i** as a white solid (7.89 g, 40%), mp 195 °C, followed by *trans*-**5i** as a white solid (2.01 g, 10%), mp 180 °C.

1-(4-Methoxyphenyl)-9-methyl-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid as a Mixture of Isomers (*cis*- and *trans*-8). 4-Methoxybenzaldehyde (0.68 g, 5 mmol, 1.1 equiv) was added to a stirred solution of *N*-methyl tryptophan (1 g, 4.58 mmol) in water (13 mL). A 1 N solution of H_2SO_4 (4.6 mL) was added, and the mixture was heated at 50 °C for 16 h. The mixture was then cooled at room temperature, and the precipitated product was collected by filtration, washed with water, and dried to give the mixture of *cis*- and *trans*-**8** as a beige solid (1.14 g, 74%), mp >230 °C.

5-(4-Methoxyphenyl)-2-(phenylmethyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6m). Representative example: To a stirred solution of tetrahydro-*β*-carboline *trans*-**5e** (369 mg, 1.1 mmol, 1 equiv) in methylethyl ketone (10 mL) was added benzyl isocyanate (135 μL, 1.1 mmol, 1 equiv), and the mixture was stirred at reflux for 16 h under a nitrogen atmosphere. The solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography eluting with cyclohexane/AcOEt, 80/20, to give *trans*-**6m** as a white solid (300 mg, 62.5%) after crystallization from 2-propanol, mp 208−212 °C; ^{1}H NMR ($CDCl_3$) δ 7.84 (s, 1H), 7.53 (d, 1H, J = 7 Hz), 7.43−7.10 (m, 10H), 6.83 (d, 2H, J = 8.7 Hz), 6.25 (s, 1H, C_5−H), 4.70 (d, 1H, J = 14.6 Hz), 4.60 (d, 1H, J = 14.6 Hz), 4.29 (dd, 1H, J = 11, 5.6 Hz, C_{11a}-H), 3.76 (s, 3H), 3.46 (dd, 1H, J = 15.3, 5.5 Hz), 2.82 (dd, 1H, J = 15.3, 11.1 Hz); ^{13}C NMR ($CDCl_3$) δ 173.1, 160.6, 155.2, 137.2, 136.7, 131.9, 131.4, 130.2, 129.4, 129.3, 128.6, 126.8, 123.5, 120.8, 119.1, 115.1, 111.9, 108.7, 56, 53.9 (C_{11a}), 52.2 (C_5), 43, 24.1; ^{1}H−^{13}C HMQC ($CDCl_3$) δ_H (δ_C) 6.25 (52.2, C_5), 4.29 (53.9, C_{11a}); Anal. ($C_{27}H_{23}N_3O_3$) C, H, N.

The following compounds were prepared using a similar procedure starting from appropriate tetrahydro-*β*-carbolines and isocyanates.

5-(4-Methoxyphenyl)-2-(phenylmethyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6m). The title compound was prepared from tetrahydro-*β*-carboline *cis*-**5e** and benzyl isocyanate. The crude residue was purified by flash column chromatography eluting with cyclohexane/AcOEt, 80/20, to give *cis*-**6m** as a pale yellow solid (630 mg, 46.6%) after crystallization from EtOH, mp 240−243 °C; ^{1}H NMR ($CDCl_3$) δ 7.62−7.53 (m, 2H), 7.41−7.11 (m, 10H), 6.84 (d, 2H, J = 8.6 Hz), 5.73 (s, 1H, C_5−H), 4.68 (d, 1H, J = 14.5 Hz), 4.55 (d, 1H, J = 14.5 Hz), 4.34 (dd, 1H, J = 11.5, 4.5 Hz, C_{11a}-H), 3.76 (s, 3H), 3.49 (dd, 1H, J = 14.9, 4.7 Hz), 3.05 (dd, 1H, J = 14.9, 11.3 Hz); ^{13}C NMR ($CDCl_3$) δ 172.1,

160.3, 155.2, 137.4, 136.8, 134.4, 131.1, 129.8, 129.5, 129.3, 128.6, 126.9, 123.4, 120.8, 119.1, 114.9, 111.9, 107.6, 58.8 (C_{11a}), 56.9 (C_5), 55.8, 42.9, 23.1; $^1H-^{13}C$ HMQC ($CDCl_3$) δ_H (δ_C) 5.73 (56.9, C_5), 4.34 (58.8, C_{11a}); Anal. ($C_{27}H_{23}N_3O_3$) C, H, N.

2-Butyl-5-(3-pyridinyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6a). The title compound was prepared from tetrahydro-β-carboline *cis*-**5a** and butyl isocyanate. The crude residue was purified by crystallization from 2-propanol to give *cis*-**6a** as a white solid (350 mg, 78%), mp 257−263 °C; ^1H NMR ($CDCl_3$) δ 10.27 (s, 1H), 8.44 (d, 1H, J = 3.8 Hz), 8.38 (s, 1H), 7.60 (d, 1H, J = 6.6 Hz), 7.48 (d, 1H, J = 8.1 Hz), 7.32 (d, 1H, J = 6.4 Hz), 7.25−7.10 (m, 3H), 5.85 (s, 1H), 4.39 (dd, 1H, J = 11.5, 4.5 Hz), 3.60−3.37 (m, 3H), 3.07 (dd, 1H, J = 14.8, 11.3 Hz), 1.61−1.49 (m, 2H), 1.35−1.21 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz); Anal. ($C_{22}H_{22}N_4O_2$) C, H, N.

2-Butyl-5-(3-thienyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6b). The title compound was prepared from tetrahydro-β-carboline *cis*-**5b** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 80/20, to give *cis*-**6b** as a white solid (250 mg, 22%) after crystallization from 2-propanol, mp 219−221 °C; ^1H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.55 (d, 1H, J = 8.3 Hz), 7.38 (s, 1H), 7.28−7.10 (m, 4H), 6.86 (d, 1H, J = 6.2 Hz), 5.97 (s, 1H), 4.34 (dd, 1H, J = 11.3, 4.6 Hz), 3.56−3.40 (m, 3H), 3.05 (dd, 1H, J = 14.8, 11.3 Hz), 1.63−1.50 (m, 2H), 1.37−1.24 (m, 2H), 0.90 (t, 3H, J = 7.2 Hz); Anal. ($C_{21}H_{21}N_3O_2S$) C, H, N.

2-Butyl-5-(3-furanyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6c). The title compound was prepared from tetrahydro-β-carboline *cis*- and *trans*-**5c** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 80/20, to give *cis*-**6c** as a white solid (160 mg, 13%) after crystallization from toluene, mp 155−160 °C; ^1H NMR ($CDCl_3$) δ 7.69 (s, 1H), 7.61 (s, 1H), 7.56 (d, 1H, J = 8.5 Hz), 7.37 (s, 1H), 7.26−7.13 (m, 3H), 6.18 (s, 1H), 5.88 (s, 1H), 4.30 (dd, 1H, J = 11.5, 4.7 Hz), 3.55−3.43 (m, 3H), 2.96 (dd, 1H, J = 15.1, 11.5 Hz), 1.65−1.50 (m, 2H), 1.40−1.25 (m, 2H), 0.91 (t, 3H, J = 7.4 Hz); Anal. ($C_{21}H_{21}N_3O_3$) C, H, N.

2-Butyl-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6d). The title compound was prepared from tetrahydro-β-carboline *cis*-**5d** and butyl isocyanate. The crude residue was purified by crystallization from toluene to give *cis*-**6d** as a white solid (150 mg, 33%), mp 225−227 °C; ^1H NMR ($CDCl_3$) δ 7.50 (s, 1H), 7.48 (s, 1H), 7.34−7.01 (m, 8H), 5.72 (s, 1H), 4.30 (dd, 1H, J = 11.3, 4.5 Hz), 3.50−3.28 (m, 3H), 2.97 (dd, 1H, J = 14.1, 11.5 Hz), 1.55−1.41 (m, 2H), 1.28−1.13 (m, 2H), 0.82 (t, 3H, J = 7.1 Hz); Anal. ($C_{23}H_{23}N_3O_2$) C, H, N.

2-Butyl-5-phenyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6d). The title compound was prepared from tetrahydro-β-carboline *trans*-**5d** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with cyclohexane/AcOEt, 80/20, to give *trans*-**6d** as a white solid (130 mg, 20%) after crystallization from 2-propanol, mp 240−243 °C; ^1H NMR ($CDCl_3$) δ 7.73 (s, 1H), 7.47 (d, 1H, J = 7.6 Hz), 7.30−7.06 (m, 8H), 6.22 (s, 1H), 4.20 (dd, 1H, J = 11.2, 5.7 Hz), 3.50−3.35 (m, 3H), 2.80 (dd, 1H, J = 15.3, 11.5 Hz), 1.56−1.43 (m, 2H), 1.30−1.14 (m, 2H), 0.83 (t, 3H, J = 7.3 Hz); Anal. ($C_{23}H_{23}N_3O_2$) C, H, N.

2-Butyl-5-(4-methoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6e). The title compound was prepared from tetrahydro-β-carboline *cis*-**5e** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give *cis*-**6e** as a white solid (280 mg, 63%) after crystallization from MeOH, mp 220−225 °C; ^1H NMR ($CDCl_3$) δ 7.71−7.58 (m, 2H), 7.30−7.17 (m, 5H), 6.91 (d, 2H, J = 8.6 Hz), 5.82 (s, 1H, C_5−H), 4.40 (dd, 1H, J = 11.3, 4.5 Hz, C_{11a}-H), 3.82 (s, 3H), 3.61−3.42 (m, 3H), 3.08 (dd, 1H, J = 14.9, 11.3 Hz), 1.70−1.55 (m, 2H), 1.42−1.27 (m, 2H), 0.94 (t, 3H, J = 7.4 Hz); Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

2-Butyl-5-(4-methoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6e). The title compound was prepared from tetrahydro-β-carboline *trans*-**5e** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give *trans*-**6e** as a white solid (520 mg, 49%) after crystallization from EtOH/H_2O, mp 173−174 °C; ^1H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.55 (d, 1H, J = 7 Hz), 7.35−7.10 (m, 5H), 6.85 (d, 2H, J = 8.7 Hz), 6.25 (s, 1H, C_5−H), 4.30 (dd, 1H, J = 11, 5.6 Hz, C_{11a}-H), 3.75 (s, 3H), 3.60−3.40 (m, 3H), 2.85 (dd, 1H, J = 15.3, 11.1 Hz), 1.70−1.55 (m, 2H), 1.45−1.25 (m, 2H), 0.95 (t, 3H, J = 7.4 Hz); Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

2-Butyl-5-(3-chlorophenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6f and *trans*-6f). The title compounds were prepared from tetrahydro-β-carboline *cis*- and *trans*-**5f** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 90/10, to give first *trans*-**6f** as a white solid (500 mg, 28%) after crystallization from EtOH, mp 207−209 °C; ^1H NMR ($CDCl_3$) δ 7.82 (s, 1H), 7.52 (d, 1H, J = 7.3 Hz), 7.27−7.11 (m, 7H), 6.22 (s, 1H), 4.24 (dd, 1H, J = 11, 5.5 Hz), 3.50−3.41 (m, 3H), 2.82 (dd, 1H, J = 15.5, 11.1 Hz), 1.63−1.48 (m, 2H), 1.37−1.19 (m, 2H), 0.87 (t, 3H, J = 7.4 Hz); Anal. ($C_{23}H_{22}ClN_3O_2$) C, H, N; followed by cis-**6f** as a white solid (500 mg, 28%) after crystallization from Et_2O/cyclohexane, mp 215−217 °C; ^1H NMR ($CDCl_3$) δ 7.60 (s, 1H), 7.55 (d, 1H, J = 8.7 Hz), 7.27−7.12 (m, 7H), 5.74 (s, 1H), 4.34 (dd, 1H, J = 11.3, 4.5 Hz), 3.54−3.36 (m, 3H), 3.03 (dd, 1H, J = 15.1, 11.5 Hz), 1.62−1.50 (m, 2H), 1.34−1.21 (m, 2H), 0.88 (t, 3H, J = 7.3 Hz); Anal. ($C_{23}H_{22}ClN_3O_2$) C, H, N.

2-Butyl-5-(4-chlorophenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6g). The title compound was prepared from tetrahydro-β-carboline *cis*-**5g** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 85/15, to give *cis*-**6g** as a pale yellow solid (340 mg, 57%) after crystallization from MeOH, mp 252 °C; ^1H NMR ($CDCl_3$) δ 7.58 (s, 1H), 7.56 (s, 1H), 7.36−7.14 (m, 7H), 5.79 (s, 1H), 4.36 (dd, 1H, J = 11.3, 4.6 Hz), 3.59−3.38 (m, 3H), 3.04 (dd, 1H, J = 14.9, 11.3 Hz), 1.64−1.51 (m, 2H), 1.38−1.23 (m, 2H), 0.90 (t, 3H, J = 7.4 Hz); Anal. ($C_{23}H_{22}ClN_3O_2$) C, H, N.

2-Butyl-5-(4-chlorophenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6g). The title compound was prepared from tetrahydro-β-carboline *trans*-**5g** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 85/15, to give *trans*-**6g** as a pale yellow solid (260 mg, 62%) after crystallization from MeOH, mp 174 °C; ^1H NMR ($CDCl_3$) δ 7.75 (s, 1H), 7.52 (d, 1H, J = 7.3 Hz), 7.35−7.12 (m, 7H), 6.25 (s, 1H), 4.22 (dd, 1H, J = 11.1, 5.6 Hz), 3.56−3.39 (m, 3H), 2.84 (dd, 1H, J = 15.3, 11 Hz), 1.63−1.49 (m, 2H), 1.36−1.21 (m, 2H), 0.88 (t, 3H, J = 7.4 Hz); Anal. ($C_{23}H_{22}ClN_3O_2$) C, H, N.

2-Butyl-5-(4-cyanophenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6h). The title compound was prepared from tetrahydro-β-carboline *cis*-**5h** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 80/20, to give *cis*-**6h** as a white solid (530 mg, 92%) after crystallization from 2-propanol, mp 260 °C; ^1H NMR ($CDCl_3$) δ 7.71−7.53 (m, 4H), 7.42 (d, 2H, J = 7.9 Hz), 7.32−7.11 (m, 3H), 5.86 (s, 1H), 4.38 (dd, 1H, J = 11.3, 4.6 Hz), 3.61−3.38 (m, 3H), 3.06 (dd, 1H, J = 14.8, 11.7 Hz), 1.65−1.49 (m, 2H), 1.36−1.19 (m, 2H), 0.90 (t, 3H, J = 7.5 Hz); Anal. ($C_{24}H_{22}N_4O_2$) C, H, N.

2-Butyl-5-(4-cyanophenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6h). The title compound was prepared from tetrahydro-β-carboline *trans*-**5h** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give *trans*-**6h** as a white solid (400 mg, 72.7%) after crystallization from Et_2O/cyclohexane, mp 158 °C; ^1H NMR ($CDCl_3$) δ 7.87 (s, 1H), 7.63 (d, 2H, J = 8.1 Hz), 7.56

(d, 1H, J = 7.5 Hz), 7.48 (d, 2H, J = 8.3 Hz), 7.35−7.14 (m, 3H), 6.36 (s, 1H), 4.24 (dd, 1H, J = 11.1, 5.7 Hz), 3.57−3.44 (m, 3H), 2.90 (dd, 1H, J = 15.5, 11.2 Hz), 1.67−1.53 (m, 2H), 1.39−1.26 (m, 2H), 0.92 (t, 3H, J = 7.4 Hz); Anal. ($C_{24}H_{22}N_4O_2$) C, H, N.

2-Butyl-5-(2-methoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6i). The title compound was prepared from tetrahydro-β-carboline *trans*-**5i** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with toluene/AcOEt, 70/30, to give *trans*-**6i** as a pale yellow solid (480 mg, 80%) after crystallization from 2-propanol, mp 181 °C; ^{1}H NMR ($CDCl_3$) δ 8.39 (s, 1H), 7.49 (d, 1H, J = 7.4 Hz), 7.33−6.92 (m, 7H), 6.60 (s, 1H), 4.59 (dd, 1H, J = 11, 5.5 Hz), 3.98 (s, 3H), 3.62−3.43 (m, 3H), 2.84 (dd, 1H, J = 15.1, 11 Hz), 1.71−1.59 (m, 2H), 1.41−1.29 (m, 2H), 0.93 (t, 3H, J = 7.4 Hz); Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

2-Butyl-5-(4-methoxyphenyl)-9-methyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-7 and *trans*-7). The title compounds were prepared from tetrahydro-β-carboline carboxylic acid *cis*- and *trans*-**8** and butyl isocyanate. The crude residue was purified by flash column chromatography eluting with cyclohexane/AcOEt, 80/20, to give first *trans*-**7** as a white solid (420 mg, 33.8%) after crystallization from diisopropyl ether, mp 149−151 °C; ^{1}H NMR ($CDCl_3$) δ 7.57 (d, 1H, J = 7.7 Hz), 7.30−7.15 (m, 5H), 6.85 (d, 2H, J = 8.8 Hz), 6.36 (s, 1H), 4.21 (dd, 1H, J = 11.2, 5.9 Hz), 3.78 (s, 3H), 3.57−3.44 (m, 3H), 3.34 (s, 3H), 2.90 (dd, 1H, J = 15.3, 11.2 Hz), 1.64−1.55 (m, 2H), 1.38−1.25 (m, 2H), 0.91 (t, 3H, J = 7.2 Hz); Anal. ($C_{25}H_{27}N_3O_3$) C, H, N; followed by *cis*-**7** as a white solid (250 mg, 20.1%) after crystallization from 2-propanol, mp 249 °C; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 1H, J = 7.5 Hz), 7.28−7.10 (m, 5H), 6.81 (d, 2H, J = 8.7 Hz), 5.90 (s, 1H), 4.30 (dd, 1H, J = 11.4, 4.3 Hz), 3.77 (s, 3H), 3.58−3.36 (m, 3H), 3.32 (s, 3H), 3.05 (dd, 1H, J = 14.7, 11.5 Hz), 1.61−1.48 (m, 2H), 1.35−1.22 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz); Anal. ($C_{25}H_{27}N_3O_3$) C, H, N.

5-(4-Methoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo-[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-6j). The title compound was prepared from tetrahydro-β-carboline *trans*-**5e** and potassium cyanate as previously described.[16] The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 96/4, to give *trans*-**6j** as a pale yellow solid (80 mg, 4%) after crystallization from MeOH, mp 300−310 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 7.56 (d, 1H, J = 7.4 Hz), 7.34−7.14 (m, 6H), 6.86 (d, 2H, J = 8.6 Hz), 6.26 (s, 1H), 4.37 (dd, 1H, J = 11, 5.5 Hz), 3.79 (s, 3H), 3.48 (dd, 1H, J = 15.2, 5.6 Hz), 2.98 (dd, 1H, J = 15.3, 11.2 Hz); Anal. ($C_{20}H_{17}N_3O_3$) C, H, N.

5-(4-Methoxyphenyl)-2-methyl-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6k). The title compound was prepared from tetrahydro-β-carboline *cis*-**5e** and methyl isocyanate. The crude residue was purified by crystallization from EtOH to give *cis*-**6k** as a white solid (220 mg, 39.3%), mp 233−240 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1H), 7.60 (d, 1H, J = 7.4 Hz), 7.30−7.17 (m, 5H), 6.90 (d, 2H, J = 8.7 Hz), 5.82 (s, 1H), 4.41 (dd, 1H, J = 11.3, 4.5 Hz), 3.82 (s, 3H), 3.55 (dd, 1H, J = 15.1, 4.7 Hz), 3.09 (dd, 1H, J = 15, 11.5 Hz), 3.02 (s, 3H); Anal. ($C_{21}H_{19}N_3O_3$) C, H, N.

2-Ethyl-5-(4-methoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6l and *trans*-6l). The title compounds were prepared from tetrahydro-β-carboline *cis*- and *trans*-**5e** and ethyl isocyanate. The crude residue was purified by flash column chromatography eluting with cyclohexane/AcOEt, 70/30, to give first *trans*-**6l** as a white solid (270 mg, 12.1%) after crystallization from 2-propanol, mp 245−248 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1H), 7.61 (d, 1H, J = 7.4 Hz), 7.36−7.18 (m, 5H), 6.90 (d, 2H, J = 8.6 Hz), 6.31 (s, 1H), 4.33 (dd, 1H, J = 11.1, 5.6 Hz), 3.83 (s, 3H), 3.69−3.49 (m, 3H), 2.93 (dd, 1H, J = 15.3, 11 Hz), 1.27 (t, 3H, J = 7.2 Hz); Anal. ($C_{22}H_{21}N_3O_3$) C, H, N; followed by *cis*-**6l** as a white solid (1320 mg, 59.1%) after crystallization from EtOH, mp 210−220 °C; ^{1}H NMR ($CDCl_3$) δ 7.65−7.54 (m, 2H), 7.30−7.10 (m, 5H), 6.85 (d, 2H, J = 8.7 Hz), 5.75 (s, 1H), 4.36 (dd, 1H, J = 11.3, 4.5 Hz), 3.78 (s, 3H), 3.60−3.41 (m, 3H), 3.05 (dd, 1H, J = 15, 11.5 Hz), 1.21 (t, 3H, J = 7.3 Hz); Anal. ($C_{22}H_{21}N_3O_3$) C, H, N.

2-Cyclohexyl-5-(4-methoxyphenyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*cis*-6n and *trans*-6n). The title compounds were prepared from tetrahydro-β-carboline *cis*- and *trans*-**5e** and cyclohexyl isocyanate. The crude residue was purified by flash column chromatography eluting with cyclohexane/AcOEt, 70/30, to give first *trans*-**6n** as a white solid (350 mg, 18.3%) after crystallization from 2-propanol, mp 265−269 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1H), 7.78 (d, 1H, J = 7.4 Hz), 7.56−7.36 (m, 5H), 7.08 (d, 2H, J = 8.7 Hz), 6.46 (s, 1H), 4.44 (dd, 1H, J = 10.9, 5.4 Hz), 4.18−4.09 (m, 1H), 3.99 (s, 3H), 3.70 (dd, 1H, J = 15.2, 5.4 Hz), 3.07 (dd, 1H, J = 15.3, 11.1 Hz), 2.5−1.4 (m, 10H); Anal. ($C_{26}H_{27}N_3O_3$) C, H, N; followed by *cis*-**6n** as a white solid (650 mg, 34.1%) after crystallization from EtOH, mp 250−260 °C; ^{1}H NMR ($CDCl_3$) δ 7.65−7.54 (m, 2H), 7.35−7.17 (m, 5H), 6.91 (d, 2H, J = 8.7 Hz), 5.80 (s, 1H), 4.31 (dd, 1H, J = 11.3, 4.5 Hz), 3.97−3.86 (m, 1H), 3.83 (s, 3H), 3.52 (dd, 1H, J = 15, 4.5 Hz), 3.07 (dd, 1H, J = 14.9, 11.3 Hz), 2.27−1.19 (m, 10H); Anal. ($C_{26}H_{27}N_3O_3$) C, H, N.

5-(4-Methoxyphenyl)-2-(3-pyridinylmethyl)-5,6,11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3-(2*H*)-dione (*trans*-9b). Representative example: To a solution of carbonyl diimidazole (0.28 g, 1.72 mmol, 1.16 equiv) in dry THF (5 mL) was added dropwise (3-pyridinylmethyl)amine (0.18 g, 1.66 mmol, 1.13 equiv), and the reaction mixture was stirred at room temperature. After 30 min, a solution of tetrahydro-β-carboline *trans*-**5e** (0.5 g, 1.49 mmol, 1 equiv) in THF (10 mL) was added, and the mixture was stirred at reflux under a nitrogen atmosphere overnight. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give *trans*-**9b** as a white solid (470 mg, 72.2%) after crystallization from EtOH, mp 160−165 °C; ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 1H), 8.50 (d, 1H, J = 4.9 Hz), 8.02 (s, 1H), 7.75 (d, 1H, J = 8 Hz), 7.54 (d, 1H, J = 7.1 Hz), 7.31−7.10 (m, 6H), 6.84 (d, 2H, J = 8.7 Hz), 6.25 (s, 1H), 4.72 (d, 1H, J = 14.7 Hz), 4.63 (d, 1H, J = 14.7 Hz), 4.33 (dd, 1H, J = 11.1, 5.4 Hz), 3.76 (s, 3H), 3.49 (dd, 1H, J = 15.3, 5.5 Hz), 2.84 (dd, 1H, J = 15.3, 11.1 Hz); Anal. ($C_{26}H_{22}N_4O_3$) C, H, N.

The following compounds were prepared using a similar procedure with appropriate primary amines.

2-[2-(Dimethylamino)ethyl]-5-(4-methoxyphenyl)-5,6,-11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-9a). The title compound was prepared from tetrahydro-β-carboline *trans*-**5e** and *N*,*N*-dimethyl-1,2-ethanediamine. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 92/8, to give *trans*-**9a** as a white solid (400 mg, 64.5%) after crystallization from MeOH, mp 145 °C; ^{1}H NMR ($CDCl_3$) δ 10.09 (s, 1H), 7.42−7.16 (m, 6H), 6.86 (d, 2H, J = 8.7 Hz), 6.59 (s, 1H), 4.14 (dd, 1H, J = 11, 6.2 Hz), 3.84 (s, 3H), 3.80−3.70 (m, 2H), 2.99−2.82 (m, 2H), 2.61−2.50 (m, 1H), 2.14 (s, 6H), 1.76−1.61 (m, 1H); Anal. ($C_{24}H_{26}N_4O_3$) C, H, N.

5-(4-Methoxyphenyl)-2-[2-(2-pyridinyl)ethyl]-5,6,11,-11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione (*trans*-9c). The title compound was prepared from tetrahydro-β-carboline *trans*-**5e** and [2-(2-pyridinyl)ethyl]-amine. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 99/1, to give *trans*-**9c** as a white solid (350 mg, 52.2%) after crystallization from EtOH/H_2O, mp 140−143 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, 1H, J = 4.2 Hz), 7.90 (s, 1H), 7.60−7.47 (m, 2H), 7.30−7.06 (m, 7H), 6.84 (d, 2H, J = 8.8 Hz), 6.23 (s, 1H), 4.24 (dd, 1H, J = 10.9, 5.5 Hz), 3.97−3.86 (m, 2H), 3.78 (s, 3H), 3.44 (dd, 1H, J = 15.3, 5.5 Hz), 3.11 (t, 2H, J = 6.9 Hz), 2.82 (dd, 1H, J = 15.3, 10.9 Hz); Anal. ($C_{27}H_{24}N_4O_3$) C, H, N.

5-(4-Methoxyphenyl)-2-[2-(1-pyrrolidinyl)ethyl]-5,6,-11,11a-tetrahydro-1*H*-imidazo[1′,5′:1,6]pyrido[3,4-*b*]indole-

1,3(2*H*)-dione (*trans*-9d). The title compound was prepared from tetrahydro-β-carboline *trans*-**5e** and [2-(1-pyrrolidinyl)-ethyl]amine. The crude residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH, 95/5, to give *trans*-**9d** as a white solid (340 mg, 64.4%) after crystallization from EtOH/H_2O, mp 126−130 °C; ^{1}H NMR ($CDCl_3$) δ 9.91 (s, 1H), 7.43−7.09 (m, 6H), 6.86 (d, 2H, J = 8.7 Hz), 6.51 (s, 1H), 4.12 (dd, 1H, J = 10.9, 6 Hz), 3.81 (s, 3H), 3.74 (t, 2H, J = 5.8 Hz), 3.12−2.88 (m, 2H), 2.79−2.54 (m, 5H), 1.60−1.29 (m, 5H); Anal. ($C_{26}H_{28}N_4O_3$) C, H, N.

Phosphodiesterase Preparations. PDE1, -3, and -5 were purified from bovine aorta as previously described in the literature.[17] Bovine PDE6 (ROS−PDE) was supplied by Dr. N. Virmaux (Inserm U338, Strasbourg, France). Human recombinant PDE2 and -4 were provided from Drs. Vince Florio and Tim Martins (ICOS Corporation, Bothell, WA).

Phosphodiesterase assays. The PDE assay[17,18] was based on the use of multiscreen plates (Millipore) and a vacuum manifold (Millipore). In such plates, both the reaction and the subsequent separation between substrates and products can be achieved. The assay (100 μL) contained 50 mM Tris pH 7.5, 5 mM Mg acetate, 1 mM EGTA, and 250 μg/mL snake venom nucleotidase. Fifty nanomolar [8-^{3}H]-cGMP (15 Ci/mmol; Amersham) or [8-^{3}H]-cAMP (25 Ci/mmol; Amersham) was added. Reactions were started by the addition of 25 μL of the diluted enzyme preparation. The assays were incubated for 30 min at 30 °C. Microcolumns were prepared by aliquoting 300 μL per well of QAE Sephadex previously swollen for 2 h in water (12 mL/g). At the end of the incubation, the total volume of the assays was loaded on microcolumn plate by filtration. The elution of free radioactivity was obtained by 200 μL of water from which 50 μL aliquots were analyzed by scintillation counting.

In this PDE assay, the substrate concentration never exceeded 30% of the K_m of the enzyme tested. Under such conditions, the IC_{50} obtained for any given compound closely corresponded to the K_i for such compound. In addition, all enzymes studies were performed under conditions of initial velocity (maximal substrate hydrolysis of 10−15%). Stock solutions of PDE inhibitors were prepared in dimethyl sulfoxide and the final solvent concentration in each assay was 2% (v/v).

Determination of cGMP Accumulation in RSMC. Rat aortic smooth muscle cells (RSMC) were prepared according to Chamley.[19] Cells were cultured in Dubelcco's modified Eagle medium (GIBCO) containing 10% fetal calf serum, 1% glutamine and 1% penicilin−streptomycin at 37 °C in a 95% air−5% CO_2 humidified atmosphere.

Cells were seeded in 24-well culture dishes at a density of (2−5) × 10^4 cells/well. Experiments were performed after 3−5 days in culture when cells reached confluence. Media were aspired and replaced with 0.5 mL of PBS containing the PDE inhibitor. After 30 min at 37 °C, particular guanylate cyclase was stimulated by addition of atrial natriuretic factor (0.1 μM) for 10 min at 37 °C. At the end of the incubation, the medium was removed and stored at −20 °C for extracellular cyclic nucleotides determinations. Intracellular cyclic nucleotides were extracted by two ethanolic (65%) washes at 4 °C for 5 min. The ethanolic extracts were pooled, evaporated to dryness using a speed-Vac system and stored at −20 °C. cGMP was measured by scintillation proximity immunoassay (Amersham). In all cases, any given treatment with effectors was performed in triplicate wells. Stock solutions of PDE inhibitors were made in dimethyl sulfoxide. In the assays, the final concentration of dimethyl sulfoxide never exceeded 0.1% (v/v).

Effect on Blood Pressure of Conscious SHR. The experiments were performed in spontaneously hypertensive rats (SHR, Charles River, France) weighting 340−380 g. The day before experiment, the left carotid artery was catheterized under pentobarbital anesthesia. On the day of experiment, the catheter was connected to pressure transducers for blood pressure measurement. After an equilibration period of ca. 30 min, the compound was dissolved in a mixture of 5% (v/v) DMF in olive oil for p.o. administration and in a mixture of DMF/tetraglycol/glucose (30/25/45%) for i.v. administration. Arterial blood pressure was monitored continuously over 7 h.

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