

Synthesis and Evaluation of Tryprostatin B and Demethoxyfumitremorgin C Analogues

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Tryprostatin B and demethoxyfumitremorgin C are fungal inhibitors of mammalian cell cycle progression at the G₂/M transition. *N*-Alkyl derivatives of the L-Trp-L-Pro diketopiperazine were prepared as analogues of tryprostatin B, and two of these were more active than the natural product. A second series of *cis*- and *trans*-tetrahydro- β -carboline annulated to a diketopiperazine were prepared as analogues of demethoxyfumitremorgin C. The nature of the alkyl substituent, as well as its *cis* or *trans* relationship in the tetrahydro- β -carboline ring, was found to have a significant effect on cytotoxic activity. Small *cis*-alkyl substituents fall into the demethoxyfumitremorgin C family, whereas bulky benzyl *trans* compounds appear to act via a different mechanism of action.

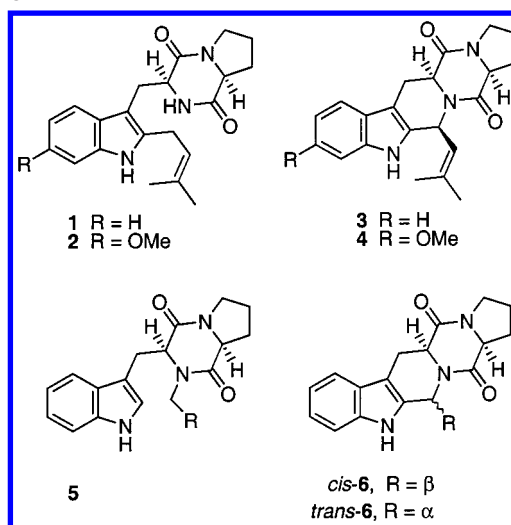
Introduction

The major chemotherapeutic agents in clinical use exert their primary action on a variety of targets, such as DNA (alkylation, intercalation, topoisomerase inhibition), purine or pyrimidine metabolism, or microtubule assembly. Nevertheless, the common consequence of these agents is cell cycle arrest induced by the tumor suppressor protein p53. Cell cycle progression is under tight regulation by a series of checkpoints, and recent evidence^{1,2} indicates that checkpoint control defects are a significant reason for the increased sensitivity of tumors over normal cells toward chemotherapy.

As our understanding of the molecular biology of cell cycle control increases, it has become possible to screen for compounds that specifically interfere with these processes. For example, the Osada group has developed^{3,4} a bioassay using a murine temperature-sensitive mutant cell line, tsFT210, defective in the *cdc2* gene. Test samples are incubated with cells synchronized in the G₁ phase, and microscopic examination of cell morphology identifies those that block G₂ cell cycle progression. With this screen, a family of prenylated Trp-Pro diketopiperazine alkaloids that cause cell cycle arrest at the G₂/M phase was isolated from fermentation broths of the fungus *Aspergillus fumigatus* BM 939, including⁵ (Chart 1) tryprostatin B (**1**, MIC in the tsFT210 assay of 4.4 μ M), tryprostatin A (**2**, MIC 16.4 μ M), demethoxyfumitremorgin C (**3**, MIC 0.45 μ M), and fumitremorgin C (**4**, MIC 4.1 μ M).

We have previously synthesized^{6,7} demethoxyfumitremorgin C, the most active of these diketopiperazines, by a concise three-step route featuring an acyliminium Pictet–Spengler condensation and also adapted⁸ this sequence to solid-phase conditions. Here, we report results of the first structure–activity study with these natural products, based on compounds of the general

Chart 1



structure **5** and **6** (Chart 1). Analogues **5** were intended as tryprostatin B mimics in which the alkyl substituent is moved from the indole ring to the diketopiperazine ring nitrogen. This was expected to result in compounds that are more readily accessible than the natural products, which require methodology^{9,10} for prenylation of tryptophan at C-2. The second series **6** were *cis*- and *trans*-tetrahydro- β -carboline analogues of demethoxyfumitremorgin C designed to probe the importance of the prenyl side chain as well as its stereochemistry.

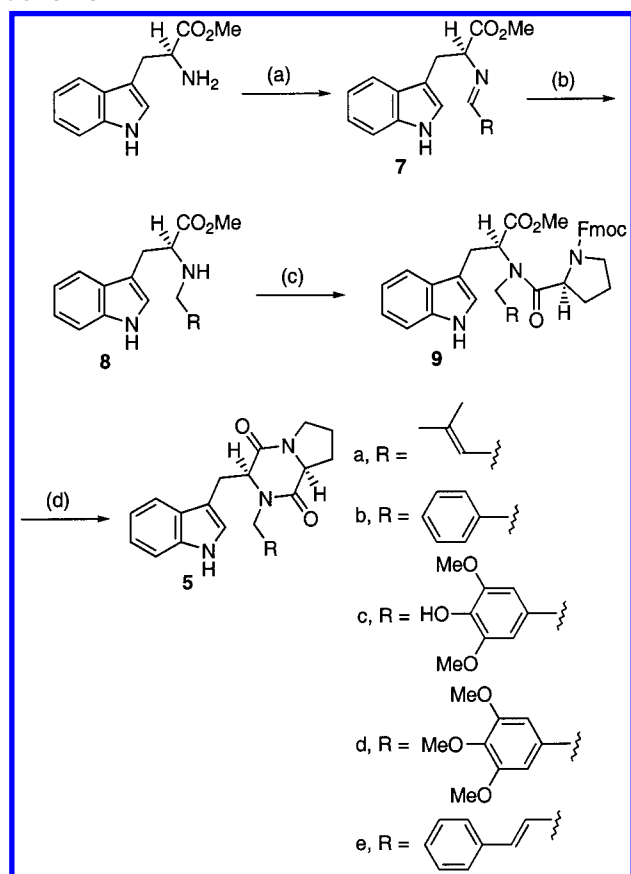
Results and Discussion

The preparation of **5** (Scheme 1) began with trimethyl orthoformate¹¹-promoted Schiff base (**7**) formation between L-tryptophan methyl ester and an aldehyde, followed by hydride reduction¹² to give secondary amine **8**. Coupling with the acid chloride¹³ of Fmoc-L-proline under two-phase Schotten–Baumann conditions furnished protected dipeptide **9**. Fmoc removal with concomitant cyclization completed the synthesis of diketopiperazine **5**. In the case of **7a**, this particularly unreactive α,β -unsaturated imine underwent significant

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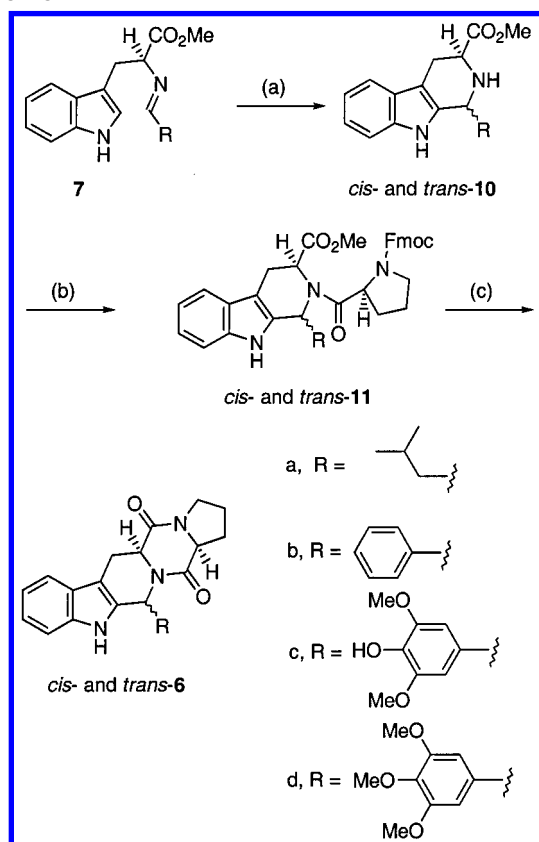
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Scheme 1^a

^a Conditions: (a) R-CHO, HC(OMe)₃; (b) NaBH(OAc)₃; (c) Fmoc-L-ProCl, aq Na₂CO₃/CH₂Cl₂; (d) 20% piperidine/CH₂Cl₂.

racemization,¹⁴ leading to *epi*-**5a** as a byproduct after the subsequent steps. Due to the additional chiral center from proline, this diastereomeric impurity was readily separable and its identity confirmed by independent synthesis according to Scheme 1 starting from D-tryptophan methyl ester. We also determined that **5a** was not significantly contaminated by its enantiomer (which would be formed if the acid chloride had epimerized as well). The enantiomer was not observed in NMR analysis with a lanthanide chiral shift reagent, control experiments having established that the detection limit of *ent*-**5a** (independently prepared from D-tryptophan and D-proline) was 1%. In the case of **7b–e**, a similar racemization of the imine was not observed, presumably due to their higher reactivity.

For the synthesis of analogues **6** (Scheme 2), imines **7** were subjected to Pictet–Spengler reaction.¹⁵ Since we desired access to both the *cis*- and *trans*-tetrahydro- β -carbolines, these reactions were carried out under acid-catalyzed nonequilibrating conditions (at or below room temperature), which are known^{16–18} to provide both isomers in high optical purity from tryptophan esters and amides, with the *cis* isomer predominating. Thus, for example, compound **10b** was obtained with a *cis*:*trans* ratio of 71:29 similar to that reported by Bailey et al.¹⁸ compared to the *trans*-selective high-temperature condensations pioneered¹⁹ by Cook. The *cis*- and *trans*-tetrahydro- β -carbolines **10a–d** were separated chromatographically, followed by two-phase Schotten–Baumann acylation with Fmoc-L-ProCl to afford dipeptides *cis*- and *trans*-**11a–d** in high yields. In the

Scheme 2^a

^a Conditions: (a) TFA/CH₂Cl₂, 0 °C to rt; (b) Fmoc-L-ProCl, aq Na₂CO₃/CH₂Cl₂; (c) 20% piperidine/CH₂Cl₂.

literature, there are reports^{17,20} of difficulties and lower yields in similar condensations using carbodiimide activating agents. Finally, Fmoc deprotection furnished the desired *cis*- and *trans*-tetrahydro- β -carbolines **6**.

The assignment of *cis*/*trans* stereochemistry in these tetrahydro- β -carbolines relied on the NMR methods²¹ well-established by Cook. Thus, both C-1 and C-3 of the *cis* isomer have larger δ_C values than the *trans*, due to the compression effect in the latter. Additionally, the methine proton at C-1 of the tetrahydro- β -carbolines is more shielded in the *cis* isomer resulting in a smaller δ_H value for the *cis* relative to the *trans*. The NMR signals for C-1 and C-3 were assigned by COSY and ¹H–¹³C HMQC (heteronuclear multiple quantum correlation spectroscopy) NMR experiments. For the known compounds **10a** and **10b**, spectra were also compared with that reported in the literature.

Although Cook's NMR method has been widely used to identify *cis*/*trans* isomers in simple 1,3- and 1,2,3-substituted tetrahydro- β -carbolines, we are unaware of examples in which N-2 and C-3 are tied up in a diketopiperazine ring as in **6**. For this reason, it was useful to independently check the assignment by the observation of a NOE between the C-1 and C-3 methine protons in the *cis* isomer. Provided the NOE is detectable, this method results in clear and unambiguous proof of stereochemistry. On a qualitative level, the *cis* and *trans* isomers also have characteristic differences²¹ in polarity. In the present series of tetrahydro- β -carbolines **10** and **11**, we observed that the *cis* isomer is less polar than the *trans*, as indicated by TLC *R_f* values (silica, ethyl acetate/hexanes developing solvent).

Table 1. log₁₀ IC₅₀ (Molar) Averaged According to Cell Type in the NCI Panel

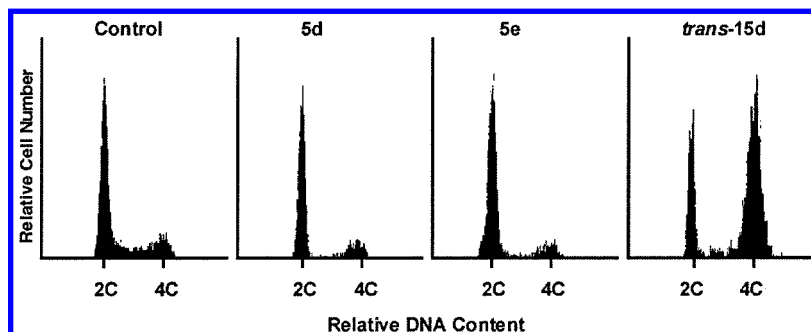
compd	panel/cell line ^a									mean averaged ^{b,c}	
	leukemia	NSL	colon	CNS	melanoma	ovarian	renal	prostate	breast	GI ₅₀	TGI
5a	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00
<i>epi-5a</i>	-4.21	-4.01	-4.02	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	-4.05	-4.07	-4.01
5b	-4.07	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	-4.01	>-4.00
5c	-4.19	-4.06	-4.06	>-4.00	-4.02	>-4.00	>-4.00	>-4.00	-4.02	-4.04	>-4.00
5d	-4.88	-4.37	-4.52	-4.56	-4.54	-4.39	-4.52	-4.24	-4.47	-4.51	-4.04
5e	-4.64	-4.49	-4.45	-4.43	-4.36	-4.35	-4.45	-4.40	-4.56	-4.50	-4.07

^a Abbreviations: NSL, non-small-cell lung cancer; CNS, CNS cancer. ^b GI₅₀ = concentration that causes 50% growth inhibition; TGI = concentration for total growth inhibition, which signifies cytostatic effect; LC₅₀ = concentration for killing 50% of cells, which signifies cytotoxic effect. ^c All the LC₅₀ > -4.00 except for both **5d** and **5e** with LC₅₀ = -4.01.

Table 2. log₁₀ IC₅₀ (Molar) Averaged According to Cell Type in the NCI Panel

compd	panel/cell line ^a									mean averaged ^b	
	leukemia	NSL	colon	CNS	melanoma	ovarian	renal	prostate	breast	GI ₅₀	TGI
3	-4.46	-4.28	-4.27	-4.36	-4.32	-4.15	-4.61	-4.30	-4.43	-4.36	-4.02
<i>12-epi-3</i>	-4.16	-4.03	-4.02	-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	-4.02	>-4.00
<i>cis-6a</i>	-4.44	-4.11	-4.17	-4.08	-4.06	-4.10	-4.13	-4.08	-4.20	-4.15	>-4.00
<i>trans-6a</i>	-4.11	-4.03	>-4.00	-4.07	>-4.00	>-4.00	-4.01	>-4.00	-4.06	-4.03	>-4.00
<i>cis-6b</i>	-4.86	-4.20	-4.38	>-4.00	-4.29	-4.24	-4.37	-4.07	-4.44	-4.32	>-4.00
<i>trans-6b</i>	-4.39	-4.28	-4.34	-4.21	-4.30	-4.25	-4.30	-4.20	-4.31	-4.29	>-4.00
<i>cis-6c</i>	-4.00	-4.39	-4.05	-4.48	-4.26	-4.40	-4.35	-4.18	-4.33	-4.27	>-4.03
<i>trans-6c</i>	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00
<i>cis-6d</i>	-4.19	-4.15	-4.03	-4.02	-4.03	-4.14	-4.02	-4.04	-4.01	-4.06	>-4.00
<i>trans-6d</i>	-4.49	-4.16	-4.13	-4.13	-4.06	-4.11	-4.11	-4.14	-4.33	-4.18	-4.01
<i>cis-15c</i>	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00
<i>trans-15c</i>	-4.42	-4.25	-4.33	-4.42	-4.34	-4.18	-4.26	-4.15	-4.31	-4.30	-4.01
<i>cis-15d</i>	-4.14	>-4.00	>-4.00	>-4.00	>-4.00	>-4.00	-4.02	>-4.00	-4.03	-4.02	>-4.00
<i>trans-15d</i>	-5.64	-5.12	-5.36	-5.43	-5.17	-5.23	-4.86	-5.26	-5.20	-5.23	-4.26

^a See footnote a of Table 1. ^b All the LC₅₀ > -4.00 except for *trans-15d* with LC₅₀ = -4.04.

**Figure 1.** Cell cycle assay. Exponentially growing 3Y1 cells were treated with various compounds (**5d**, **5e**, and *trans-15d*) for 24 h, and the distribution of DNA content was determined by laser scanning cytometry.**Table 3.** Summary of Bioassay Data for **5d**, **5e**, and *trans-15d*

		5d	5e	<i>trans-15d</i>
in vitro microtubule assembly	inhibition % at 250 μM	1.0 ± 0.0	2.9 ± 4.3	15.6 ± 3.5
disruption of in situ microtubule network 18 h	at 250 μM	no	no	disrupted
cell proliferation/cell cycle (control: at 24 h 185.8% cell number)	at 100 μM	153.0	130.9	96.4
	at 50 μM	145.7	177.0	95.9
	at 25 μM	not tested	not tested	105.1
	at 10 μM	not tested	not tested	153.9

Upon cyclization to diketopiperazines **6** (and **15**, vide infra), however, the *cis* isomers become more polar than the *trans* (TLC on silica, ethyl acetate or ethyl acetate: MeOH:CH₂Cl₂ = 50:45:5).

Analogues **5** and **6** were submitted for testing in the National Cancer Institute's in vitro panel of 60 tumor cell lines.²² Cytotoxic activity (log GI₅₀) is summarized in Tables 1 and 2 according to cell type, together with mean panel inhibitory values. The data for demethoxyfumitremorgin C (**3**) and its *trans* epimer,⁶ which were also tested, are included in Table 2 as a benchmark.

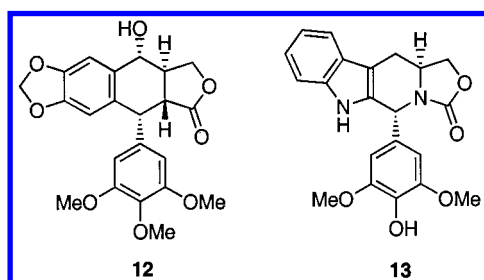
(a) Evaluation of *N*-Alkyldiketopiperazines **5.** Analogue **5a**, which most closely resembles tryprostatin

B, was inactive. Nevertheless, simple diketopiperazines without C-2 substitution on the indole ring can retain activity, as the trimethoxybenzyl (**5d**) and cinnamyl (**5e**) analogues were more potent than the natural products. Analogue **5d** did not show significant inhibition of cell cycle progression or effects on microtubules. Analogue **5e** was a weak inhibitor of cell cycle progression, but the growth arrest was cell cycle independent (Figure 1 and Table 3).

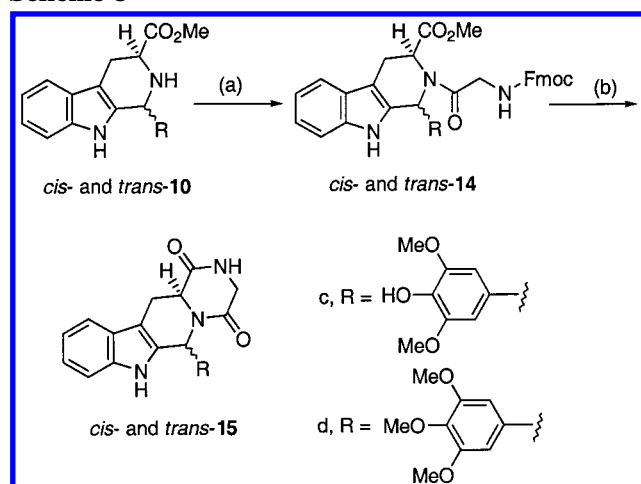
(b) Evaluation of Tetrahydro-β-carbolines **6.** With both demethoxyfumitremorgin C and its dihydro analogue (**6a**), the stereochemistry of the alkyl substituent was found to be significant. The unnatural *trans*

epimers were inactive. However, in the phenyl analogue, both *cis*-**6b** and *trans*-**6b** tetrahydro- β -carbolines were equally active, while *trans*-**6d** with the trimethoxyphenyl substituent was more active than *cis*-**6d**. We believed this shift in stereochemical preference might reflect a change in the mechanism of action. The compounds with a relatively small *cis* substituent fall into the demethoxyfumitremorgin C family, whereas those with a bulky *trans*-aryl substituent bear a resemblance to podophyllotoxin²³ (**12**; Chart 2) and particularly the synthetic analogue azatoxin^{24,25} (**13**).

Chart 2



Compared to azatoxin, our compounds contain an additional ring due to the proline unit. We considered that the similarity (and activity) would be improved by using glycine instead, and the corresponding tetrahydro- β -carbolines **15** were prepared in an analogous manner to **6** (Scheme 3). The *trans* epimers of both **15c** and **15d**

Scheme 3^a

^a Conditions: (a) Fmoc-GlyCl, aq Na₂CO₃/CH₂Cl₂; (b) 20% piperidine/CH₂Cl₂.

were found to be more active than the *cis*-tetrahydro- β -carbolines. *trans*-**15d** induces cell cycle arrest at the M phase (Figure 1) and disruption of the microtubule network at 250 μ M. In terms of inhibition of microtubule assembly, it is equally active or more active than tryprostatin B (time course shown in Figure 2). However, these compounds were inactive when tested for inhibition of topoisomerase I and II, which does not support our assumption that they are podophyllotoxin mimics. Meanwhile, it is unclear if *trans*-**15d** shares the same mechanism of action as the tryprostatins.²⁶ Further studies to identify the precise molecular target are planned. *trans*-**15d**, with a mean GI₅₀ of 5.9 μ M, is the most active of all the analogues prepared. It is relatively nontoxic, with a maximum tolerated dose of 400 mg/kg in mice,

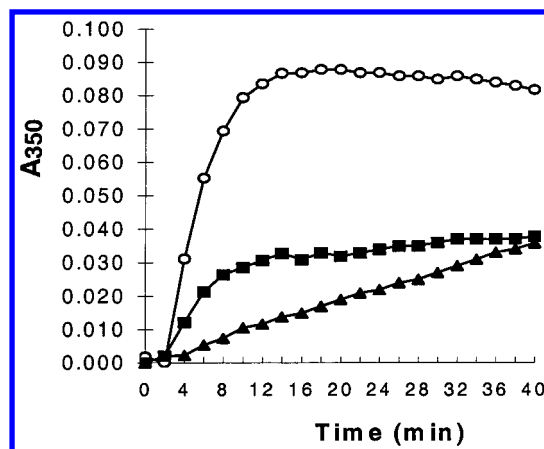


Figure 2. Time course of microtubule assembly in vitro. Microtubule proteins (2.0 mg/mL) were incubated with 1% DMSO containing 250 μ M **1** (■-), *trans*-**15d** (▲-), or control (no compound, -○-).

and currently undergoing evaluation in the National Cancer Institute's hollow fiber in vivo tumor model.

We have also checked our diketopiperazines for antimicrobial activity against a panel of bacterial and fungal strains. Both *trans*-**6d** and *trans*-**15d** showed some antibacterial activity against a sensitive strain of *Staphylococcus aureus* S1.

Summary

In this work, we have carried out the first structure–activity investigation into the cell cycle inhibitory effects of the fumitremorgins. The results show the nature and stereochemistry of the C-1 side chain in the tetrahydro- β -carboline ring to be important. The ability to access compounds with a different mechanism of action depending on *cis* or *trans* substitution highlights the versatility of the fumitremorgin skeleton, which combines features of tetrahydro- β -carbolines and diketopiperazines, as a template for drug discovery. Furthermore, the fact that unnatural analogues with comparable or higher biological activity than the fungal alkaloids were discovered illustrates the potential of combinatorially modifying a peptidomimetic scaffold used by Nature.

Experimental Section

All chemicals were obtained from commercial suppliers and used without further purification, except for CH₂Cl₂, which was distilled from CaH₂. TLC was carried out on precoated plates: analytical (Merck; Kieselgel 60 F₂₅₄), spots visualized with UV light and iodine vapor; preparative-scale (Aldrich; silica, 1 mm thick). Flash column chromatography was performed with silica (Merck; 230–400 mesh). Melting points were taken on a Büchi 535 melting point apparatus (capillary method) and are uncorrected. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Infrared spectra (IR) were recorded with a Perkin-Elmer 1600 series FTIR (CHCl₃ solution or KBr pellet). ¹H and ¹³C NMR were recorded at 300 or 400 and at 75 or 100 MHz, respectively, in CDCl₃ unless otherwise mentioned. Proton and carbon chemical shifts are expressed in ppm relative to internal tetramethylsilane. Multiplicity of carbons was assigned by DEPT experiments and is expressed as s = quaternary, d = CH, t = CH₂ and q = CH₃. The %ee was measured by using the chiral NMR chemical shift reagent europium tris[3-(trifluoromethylhydroxymethyl)-(+)-camphorate] ((+)-Eu(tfc)₃). The conditions were optimized by gradual addition of Eu(tfc)₃ solution in CDCl₃ (20 or 6 mg/mL) into a NMR tube that contained a sample in CDCl₃ and observing the splitting of the signals. Low-resolu-

tion mass spectra were determined using a Perkin-Elmer Sciex API 300 (electrospray, ESI, positive mode) or a VG Micromass 7035E instrument (EI). Microanalyses were performed on a Perkin-Elmer Elemental Analyzer 2400 CHN. Analytical HPLC was performed on a Hewlett-Packard 1050Ti series equipped with a diode array detector, using a C₁₈ column (ODS Hypersil, 5 μ m, 2.1 \times 200 mm, flow rate: 0.30 mL/min). Eluant solvent system A: acetonitrile/water (20:80 to 100:0) eluant linear gradient over 20 min, then 100% acetonitrile for additional 5 min, both solvents contain 0.1% TFA; solvent system B: methanol/water (40:60 to 100:0) eluant linear gradient over 20 min, then 100% methanol for additional 5 min. Compound purity was estimated from integrated peak areas of HPLC chromatographs generated at 220 nm. Usual workup means dried by MgSO₄ or Na₂SO₄, filtered, concentrated and dried under vacuum.

Imines 7a–d and ent-7a. Representative Example (7a). L-Tryptophan methyl ester hydrochloride salt was mixed with aqueous Na₂CO₃/CH₂Cl₂ and stirred until a clear solution was reached. The organic layer was separated and the aqueous phase extracted (CH₂Cl₂ \times 3). The combined organic layers gave the methyl ester as a white solid after usual workup in 98–99% yield. The free base (1.947 g, 8.92 mmol, 1 equiv) and 3-methyl-2-butenal (0.95 mL, 9.83 mmol, 1.10 equiv) were stirred in CH₂Cl₂ (30 mL) for 0.5 h, followed by addition of trimethyl orthoformate (4.0 mL, 36.5 mmol, 4.09 equiv). After stirring overnight (18.5 h), the solution was concentrated and dried under vacuum to remove all volatiles to afford crude imine **7a** as a yellowish solid (2.577 g, 102%) which was used without further purification. Imines *ent*-**7a** (starting from D-tryptophan methyl ester) and **7b–e** were prepared similarly from corresponding aldehydes.

Amines 8a–d and ent-8a. Representative Example (8a). Crude **7a** (0.307 g, 1.05 mmol) was dissolved in dry CH₂Cl₂ (10 mL), followed by addition of NaBH(OAc)₃ (0.440 g, 2.08 mmol) in two portions. After being stirred at room temperature for 44 h, the reaction mixture was basified with aqueous Na₂CO₃ and extracted (CH₂Cl₂ \times 3). After the usual workup, the crude product was purified by flash chromatography (ethyl acetate/hexanes: 33% to 70%) to give **8a** as a pale yellowish oil (0.121 g, 40%): ¹H NMR δ 8.33 (br s, 1H, NH), 7.59 (d, 1H, *J* = 7.9 Hz), 7.32 (d, 1H, *J* = 7.2 Hz), 7.17 (td, 1H, *J* = 7.5, 1.2 Hz), 7.10 (dd, 1H, *J* = 7.4, 1.0 Hz), 7.01 (d, 1H, *J* = 2.2 Hz, indole C₂-H), 5.15 (t-quintet, 1H, *J* = 7.0, 1.4 Hz), 3.66 (t, 1H, *J* = 6.7 Hz, Trp-CHN), 3.63 (s, 3H, OCH₃), 3.21 (dd, 1H, *J* = 13.9, 6.6 Hz, NCH₂), 3.15 (m, 2H, NCH₂), 3.11 (dd, 1H, *J* = 13.6, 6.6 Hz, NCH₂), 1.66 (d, 3H, *J* = 0.7 Hz, Me), 1.55 (d, 1H, *J* = 0.3 Hz, Me); ¹³C NMR δ 175.5, 136.3, 135.2, 127.4, 123.0, 122.2, 122.0, 119.4, 118.7, 111.2, 110.1, 61.4, 51.7, 45.6, 29.4, 25.7, 17.8; MS (ESI) *m/z* 287.2 ([M + H]⁺). The %ee of **8a** was 69% or *S/R* = 85/15. When 0.125 equiv of (+)-Eu(tfc)₃ was added, both the indole C₂-H and OCH₃ signals were split into two: δ 7.078 (*S*)/6.977 (*R*, indole C₂-H), 3.747 (*R*)/3.704 (*S*, OCH₃). The indole C₂-H signals were selected for calculation of %ee.

ent-**8a**: yellow oil prepared from *ent*-**7a**; ¹H and ¹³C NMR and MS data were identical to those of **8a**; %ee of *ent*-**8a** was 56% or *R/S* = 78/22 [0.10 equiv of (+)-Eu(tfc)₃].

8b: white solid quantitative yield from **7b**.

8c: white solid (95%) prepared from **7c**; ¹H NMR δ 8.19 (br s, 1H, NH), 7.58 (d, 1H, *J* = 7.9 Hz), 7.33 (dd, 1H, *J* = 8.1, 0.7 Hz), 7.17 (td, 1H, *J* = 7.0, 1.0 Hz), 7.09 (td, 1H, *J* = 6.7, 1.0 Hz), 7.02 (d, 1H, *J* = 2.0 Hz, C₂-H), 6.42 (s, 2H, ArH), 3.77 (d, 1H, *J* = 13.1 Hz), 3.74 (s, 6H, 2 \times OCH₃), 3.67 (dd, 1H, *J* = 7.4, 5.9 Hz, Trp-CHN), 3.66 (s, 3H, OCH₃), 3.57 (d, 1H, *J* = 13.1 Hz), 3.21 (dd, 1H, *J* = 14.4, 5.9 Hz), 3.13 (dd, 1H, *J* = 14.4, 7.4 Hz); ¹³C NMR δ 175.3, 146.9, 136.2, 133.5, 130.6, 127.4, 122.9 (d), 122.1 (d), 119.5 (d), 118.8 (d), 111.2 (s), 111.1 (d), 104.7 (d), 61.0 (OCH₃), 56.1 (OCH₃ \times 2), 52.3 (ArCH₂N), 51.8 (Trp-CHN), 29.3 (Trp-CH₂); MS (ESI) *m/z* 385.2 ([M + H]⁺).

8d: yellowish foam (82%) prepared from **7d**; ¹H NMR δ 8.16 (br s, 1H, NH), 7.60 (d, 1H, *J* = 7.9 Hz), 7.34 (d, 1H, *J* = 8.1 Hz), 7.18 (td, 1H, *J* = 7.2, 0.9 Hz), 7.10 (t, 1H, *J* = 7.1 Hz), 7.03 (d, 1H, *J* = 2.0 Hz, C₂-H), 6.43 (s, 2H, ArH), 3.80 (s, 3H,

OCH₃), 3.79 (d, 1H, *J* = 12.6 Hz), 3.72 (s, 6H, 2 \times OCH₃), 3.67 (s, 3H, OCH₃), 3.67 (overlapped, 1H, Trp-CH), 3.59 (d, 1H, *J* = 13.4 Hz), 3.22 (dd, 1H, *J* = 14.4, 5.8 Hz), 3.13 (dd, 1H, *J* = 14.4, 7.4 Hz); ¹³C NMR δ 175.4, 153.1, 136.7, 136.2, 135.5, 127.5, 122.9, 122.2, 119.5, 118.8, 111.4, 111.2, 104.7, 61.2, 60.8, 55.9, 52.3, 51.8, 29.4; MS (ESI) *m/z* 399.2 ([M + H]⁺). No split or enantiomeric signal was observed when 0.04 to 0.12 equiv of (+)-Eu(tfc)₃ was added.

8e: pale yellowish solid (30%) from **7e**; ¹H NMR δ 8.15 (br s, 1H, NH), 7.62 (d, 1H, *J* = 7.8 Hz), 7.34 (d, 1H, *J* = 7.3 Hz), 7.28–7.24 (m, 4H), 7.22–7.19 (m, 1H, PhH), 7.19 (td, 1H, *J* = 7.5, 1.0 Hz), 7.11 (ddd, 1H, *J* = 7.8, 7.0, 0.9 Hz), 7.04 (d, 1H, *J* = 2.2 Hz, C₂-H), 6.40 (br d, 1H, *J* = 15.9 Hz, PhCH=), 6.16 (dt, 1H, *J* = 15.9, 6.3 Hz, *trans*-PhCH=CH-), 3.72 (dd, 1H, *J* = 7.1, 6.1 Hz, Trp-CHN), 3.63 (s, 3H, OCH₃), 3.42 (ddd, 1H, *J* = 13.9, 6.2, 1.4 Hz, NCH₂), 3.30 (ddd, 1H, *J* = 14.0, 6.3, 1.3 Hz, NCH₂), 3.21 (dd, 1H, *J* = 14.4, 6.0 Hz, Trp-CH₂), 3.15 (dd, 1H, *J* = 14.3, 7.2 Hz, Trp-CH₂); ¹³C NMR δ 175.4, 137.0, 136.2, 131.6, 128.5, 127.7 (d), 127.4, 127.4, 126.3, 123.0, 122.2, 119.5, 118.8, 111.24, 111.16, 61.2, 51.9, 50.2, 29.4; MS (ESI) *m/z* 335.2 ([M + H]⁺). No split or enantiomeric signal was observed when 0.02 to 0.33 equiv of (+)-Eu(tfc)₃ was added.

Dipeptides 9a–d. Representative Example (9a). Secondary amine **8a** (69 mg, 0.24 mmol) was dissolved in CH₂Cl₂ (5 mL) and stirred at room temperature. Solid Fmoc-L-Pro-Cl (110 mg, 0.31 mmol) was added in one portion. After 2 min, aqueous Na₂CO₃ (1M, 3 mL) was added. After stirring for 3.5 h and usual workup, the crude acylation product was obtained as a mixture of **9a** and *epi*-**9a** (*R_f* = 0.46 and 0.60 respectively, silica, ethyl acetate/hexanes = 6:4). NMR spectra of these compounds were very complicated due to amide and carbamate bond rotamers.

9b: pale yellow solid (100%) prepared from **8b** with reaction time of 1 h.

9d: pale yellow solid prepared from **8d** with reaction time of 3.5 h.

9e: pale yellow solid prepared from **8e** with reaction time of 1.5 h.

Tetrahydro- β -carboline *cis*- and *trans*-10a–d. Representative Example (*cis*- and *trans*-10d). Crude imine **7d** (1.01 g, 2.44 mmol) was dissolved in dry CH₂Cl₂ (30 mL) and the resultant clear solution was cooled with an ice bath. Trifluoroacetic acid (0.39 mL, 3.90 mmol) was added dropwise. The ice bath was removed after 1.5 h and the solution kept stirring at room temperature for additional 6 h. The reaction mixture was then basified with aqueous Na₂CO₃ and extracted (CH₂Cl₂ \times 3). After usual workup, the residue was purified by flash chromatography (ethyl acetate in CH₂Cl₂: from 20% to 50%) to yield *cis*-**10d** as a white solid (0.654 g, 70%) and *trans*-**10d** as a white foam (0.183 g, 20%). *cis*-**10d**: ¹H NMR (DMSO-*d*₆) δ 10.31 (br s, 1H, indole NH), 7.43 (d, 1H, *J* = 7.5 Hz), 7.23 (d, 1H, *J* = 7.9 Hz), 6.99 (td, 1H, *J* = 8.1, 1.2 Hz), 6.95 (td, 1H, *J* = 7.5, 0.7 Hz), 6.70 (s, 2H, ArH), 5.15 (s, 1H, C₁-H), 3.88–3.84 (m, 1H, C₃-H), 3.74 (s, 6H, OMe \times 2), 3.72 (s, 3H, OMe), 3.67 (s, 3H, OMe), 3.03 (dd, 1H, *J* = 14.8, 2.8 Hz, C₄-H), 2.86 (ddd, 1H, *J* = 14.1, 11.2, 2.2 Hz, C₄-H), 2.79 (br s, 1H, N₂-H); ¹³C NMR (DMSO-*d*₆) δ 172.8, 152.7, 137.3, 136.8, 136.2, 135.4, 126.5, 120.6 (d), 118.3, 117.5, 111.1 (d), 106.6 (s), 105.5 (d), 59.8 (OCH₃), 58.2 (C₁), 56.3 (C₃), 55.7 (OCH₃ \times 2), 51.7 (OCH₃), 25.2 (C₄); NOE (DMSO-*d*₆) C₃-H (C₁-H, C₄-H); MS (ESI) *m/z* 397.2 ([M + H]⁺). *trans*-**10d**: ¹H NMR (DMSO-*d*₆) δ 10.53 (br s, 1H, indole NH), 7.44 (d, 1H, *J* = 7.7 Hz), 7.24 (d, 1H, *J* = 7.9 Hz), 7.02 (td, 1H, *J* = 7.1, 1.1 Hz), 6.96 (td, 1H, *J* = 7.4, 1.0 Hz), 6.64 (s, 2H, ArH), 5.30 (s, 1H, C₁-H), 3.93 (m, 1H, C₃-H), 3.71 (s, 6H, OMe \times 2), 3.64 (s, 3H, OMe), 3.63 (s, 3H, OMe), 3.19 (br s, 1H, N₂-H), 3.09 (dd, 1H, *J* = 15.2, 4.7 Hz, C₄-H), 2.94 (ddd, 1H, *J* = 15.2, 6.2, 1.2 Hz, C₄-H); ¹³C NMR (DMSO-*d*₆) δ 173.9, 152.6, 138.6, 136.6, 136.0, 134.3, 126.4, 120.7 (d), 118.2, 117.6, 111.0 (d), 106.0 (s), 105.3 (d), 59.8 (OCH₃), 55.7 (OCH₃ \times 2), 54.1 (C₁), 52.2 (C₃), 51.5 (OCH₃), 24.3 (C₄); NOE (DMSO-*d*₆) no NOE between C₃-H and C₁-H was observed; MS (ESI) *m/z* 397.2 ([M + H]⁺).

10a was prepared from imine **7a** (Scheme 2). The *cis* and *trans* isomers were separated by flash chromatography, and

their structures were confirmed by comparing their proton NMR data with those reported.¹⁷

10b, white solid (97%, *cis*:*trans* = 71:29), was prepared from **7b**. Pure *cis*-**10b** was isolated as a white solid from the mixture by recrystallization from CH₂Cl₂–hexanes. Pure *trans*-**10b** was obtained as a white foam by flash chromatography (1% to 1.5% MeOH in CH₂Cl₂) of the mother liquor from recrystallization. The structures of *cis*-**10b** and *trans*-**10b** were confirmed by comparing their ¹H and ¹³C NMR data with that reported¹⁹ for (±)-*cis*-**10b** and (±)-*trans*-**10b**. Additional data: *cis*-**10b**: ¹H–¹³C HMQC (DMSO-*d*₆) δ_H (δ_C) 5.21 (57.6, C₁), 3.88 (56.1, C₃); NOE (DMSO-*d*₆) C₁–H (C₃–H), C₃–H (C₁–H, C₄–H), N₂–H (C₁–H, C₃–H). *trans*-**10b**: ¹H–¹³C HMQC (DMSO-*d*₆) δ_H (δ_C) 5.32 (53.9, C₁), 3.77 (51.6, C₃); NOE (DMSO-*d*₆) no NOE between C₃–H and C₁–H was observed.

10c, white solid (quantitative yield, *cis*:*trans* = 1:1), was prepared from **7c**. Pure *cis*-**10c** (white solid, less polar) and *trans*-**10c** (white foam, more polar) were isolated by flash chromatography (first 60% ethyl acetate in hexanes then 50% ethyl acetate in CH₂Cl₂). *cis*-**10c**: ¹H NMR (DMSO-*d*₆) δ 10.26 (br s, 1H, indole NH), 7.42 (d, 1H, *J* = 7.6 Hz), 7.22 (d, 1H, *J* = 7.0 Hz), 7.00 (td, 1H, *J* = 7.1, 1.2 Hz), 6.95 (td, 1H, *J* = 7.2, 1.1 Hz), 6.63 (s, 2H, ArH), 5.10 (d like, 1H, *J* = 6.0 Hz, C₁–H), 3.86 (m, 1H, C₃–H), 3.72 (s, 9H, OMe×3), 3.02 (dd, 1H, *J* = 14.8, 2.7 Hz, C₄–H), 2.85 (ddd, 1H, *J* = 14.5, 11.2, 2.3 Hz, C₄–H), 2.68 (t, 1H, *J* = 5.6 Hz, N₂–H); ¹³C NMR (DMSO-*d*₆) δ 172.9, 147.7, 136.1, 135.8, 135.1, 131.5, 126.5, 120.5 (d), 118.2, 117.4, 111.1 (d), 106.5 (s), 105.7 (d), 58.1 (C₁), 56.3 (C₃), 55.8 (OCH₃×2), 51.7 (OCH₃), 25.2 (C₄); NOE (DMSO-*d*₆) C₁–H (C₃–H, ArH), C₃–H (C₁–H), N₂–H (C₁–H); MS (ESI) *m/z* 383.2 ([M + H]⁺). *trans*-**10c**: ¹H NMR (DMSO-*d*₆) δ 10.51 (br s, 1H, indole NH), 7.43 (d, 1H, *J* = 7.6 Hz), 7.24 (d, 1H, *J* = 8.0 Hz), 7.02 (td, 1H, *J* = 7.2, 0.8 Hz), 6.95 (t, 1H, *J* = 7.4 Hz), 6.57 (s, 2H, ArH), 5.25 (s, 1H, C₁–H), 3.92 (t, 1H, *J* = 5.8 Hz, C₃–H), 3.69 (s, 6H, OMe×2), 3.63 (s, 3H, OMe), 3.10–3.05 (overlapped, 1H, N₂–H), 3.07 (dd, 1H, *J* = 14.7, 5.5 Hz, C₄–H), 2.93 (dd, 1H, *J* = 14.6, 6.2 Hz, C₄–H); ¹³C NMR (DMSO-*d*₆) δ 173.9, 147.6, 136.0, 134.9, 134.7, 132.9, 126.4, 120.6 (d), 118.2, 117.5, 110.9 (d), 105.9 (s), 105.7 (d), 55.9 (OCH₃×2), 54.1 (C₁), 52.1 (C₃), 51.5 (OCH₃), 24.4 (C₄); MS (ESI) *m/z* 383.2 ([M + H]⁺).

N-Acyltetrahydro-β-carbolines 11a–d and 14c–d. *cis*- and *trans*-**11a–d** were prepared by acylation of *cis*- and *trans*-**10a–d** with Fmoc-L-Pro-Cl using similar conditions to those described for **9**. Similarly, *cis*- and *trans*-**14c–d** were prepared by acylation of *cis*- and *trans*-**10c–d** with Fmoc-Gly-Cl.

(3*S*,8*aS*)-Hexahydro-3-[(1*H*-indol-3-yl)methyl]-2-(3-methyl-2-butenyl)pyrrolo[1,2-*a*]pyrazine-1,4-dione (5a) and (3*R*,8*aS*)-Hexahydro-3-[(1*H*-indol-3-yl)methyl]-2-(3-methyl-2-butenyl)pyrrolo[1,2-*a*]pyrazine-1,4-dione (epi-5a). Crude **9a** (156 mg, 0.208 mmol) was dissolved in CH₂Cl₂ (2 mL), followed by addition of piperidine (0.5 mL). After 40 min, the solution was evaporated and dried under high vacuum to remove all volatiles. The residue was purified by preparative TLC (ethyl acetate:chloroform = 1:2) to give **5a** (56.9 mg, 78%) and the less polar *epi*-**5a** (9.1 mg, 13%). **5a**: white solid; [α]_D²⁵ = +3.4 (c 0.46, MeOH); IR ν_{max} (KBr) 3228, 1667, 1633, 1332, 1305 cm^{−1}; ¹H NMR δ 8.25 (br s, 1H), 7.65 (d, 1H, *J* = 7.7 Hz), 7.31 (d, 1H, *J* = 8.0 Hz), 7.17 (t, 1H, *J* = 7.3 Hz), 7.09 (t, 1H, *J* = 7.2 Hz), 6.92 (d, 1H, *J* = 2.3 Hz), 5.22 (m, 1H), 4.86 (dd, 1H, *J* = 14.5, 5.3 Hz), 4.35 (br s, 1H), 3.72–3.64 (m, 3H), 3.38 (m, 1H), 3.21 (dd, 1H, *J* = 14.7, 4.4 Hz), 2.91 (td, 1H, *J* = 11.0, 4.5 Hz), 1.79 (s, 6H, Me×2), 1.69 (m, 1H), 1.29 (m, 1H), 0.71 (m, 1H), −0.24 (quintet, 1H, *J* = 10.8 Hz); ¹³C NMR δ 166.0, 164.7, 138.6, 135.7, 127.4 (s), 124.1, 122.3, 119.7, 119.6, 117.8, 111.0 (d), 108.3 (s), 59.6, 59.3 (d), 44.4, 40.5, 28.2, 27.2 (t), 25.9 (q), 20.5 (t), 18.2 (q); MS (EI) *m/z* (relative intensity %) 351 (M⁺, 22), 130 (100). When 0.15 equiv of (+)-Eu(tfc)₃ was added to a 69/31 mixture of **5a** and *ent*-**5a** (prepared from *ent*-**8a** and Fmoc-D-Pro-Cl), both the methyl signals were split into two: δ 1.856 (*ent*-**5a**)/1.839 (**5a**), 1.743 (*ent*-**5a**)/1.731 (**5a**). No enantiomeric signal was observed when up to 0.24 equiv of (+)-Eu(tfc)₃ was added to the solution of **5a**. The detectable limit was 1% of *ent*-**5a** under the above condition. Anal. (C₂₁H₂₅N₃O₂) C, H, N.

epi-**5a**. Starting from *ent*-**8a**, *epi*-**5a** was prepared in 58% yield, as well as epimerized product **5a** in 16% yield with comparable [α]_D²⁵ = +3.2 (c 0.16, MeOH). *epi*-**5a**: white solid; [α]_D²⁵ = −9.6 (c 0.38, MeOH); IR ν_{max} (KBr) 1651, 1457 cm^{−1}; ¹H NMR δ 8.62 (br s, 1H), 7.58 (d, 1H, *J* = 7.7 Hz), 7.33 (d, 1H, *J* = 7.9 Hz), 7.16 (t, 1H, *J* = 7.3 Hz), 7.10 (t, 1H, *J* = 7.3 Hz), 6.93 (d, 1H, *J* = 1.9 Hz), 5.16 (t, 1H, *J* = 6.8 Hz), 4.58 (dd, 1H, *J* = 14.9, 5.5 Hz), 4.25 (t, 1H, *J* = 3.8 Hz), 3.63 (dd, 1H, *J* = 14.6, 8.7 Hz), 3.52 (dd, 1H, *J* = 14.9, 3.0 Hz), 3.43 (t, 1H, *J* = 10.3 Hz), 3.22 (dd, 1H, *J* = 14.8, 4.8 Hz), 3.00 (t, 1H, *J* = 9.9 Hz), 2.22 (dd, 1H, *J* = 10.7, 6.2 Hz), 1.97–1.87 (m, 2H), 1.75 (s, 3H, Me), 1.70 (s, 3H, Me), 1.69–1.52 (m, 1H), 1.25–1.11 (m, 1H); ¹³C NMR δ 167.6 (s), 165.7 (s), 138.0 (s), 136.2 (s), 127.3 (s), 124.1 (d), 122.3 (d), 119.7 (d), 118.9 (d), 118.4 (d), 111.3 (d), 109.1 (s), 61.8 (d), 57.9 (d), 44.8 (t), 41.3 (t), 29.2 (t), 27.9 (t), 25.9 (t), 21.6 (q), 17.81 (q); MS (EI) *m/z* (relative intensity %) 351 (M⁺, 20), 130 (100). Anal. (C₂₁H₂₅N₃O₂·2/5H₂O) C, H, N.

(3*S*,8*aS*)-Hexahydro-3-[(1*H*-indol-3-yl)methyl]-2-(phenylmethyl)pyrrolo[1,2-*a*]pyrazine-1,4-dione (5b): yield 96% from **9b**; white solid; mp 254–255 °C; [α]_D²⁵ = +2.3 (c 0.10, MeOH); IR ν_{max} (KBr) 3265, 1667, 1657, 1632, 1473, 1452 cm^{−1}; ¹H NMR δ 8.34 (br s, 1H), 7.61 (d, 1H, *J* = 8.0 Hz), 7.40–7.31 (m, 6H), 7.18 (td, 1H, *J* = 7.0, 1.1 Hz), 7.10 (ddd, 1H, *J* = 8.0, 7.1, 1.0 Hz), 6.95 (d, 1H, *J* = 2.4 Hz), 5.74 (d, 1H, *J* = 14.6 Hz), 4.20 (br s, 1H), 4.03 (d, 1H, *J* = 14.7 Hz), 3.78 (dd, 1H, *J* = 11.2, 5.8 Hz), 3.63 (dd, 1H, *J* = 14.9, 2.4 Hz), 3.42 (m, 1H), 3.34 (dd, 1H, *J* = 14.9, 4.7 Hz), 2.95 (td, 1H, *J* = 11.0, 4.7 Hz), 1.77 (m, 1H), 1.36 (m, 1H), 0.76 (m, 1H), −0.10 (m, 1H); ¹³C NMR δ 166.4, 164.4, 135.7, 135.3 (s), 129.0, 128.5, 128.2 (d), 127.4 (s), 123.9, 122.5, 119.7, 119.7, 111.0 (d), 108.5 (s), 59.5, 59.3 (d), 46.0, 44.5, 28.3, 26.6, 20.6 (t); MS (ESI) *m/z* calcd for M + H 374.2, found 374.2. Anal. (C₂₃H₂₃N₃O₂·2/3H₂O) C, H, N.

(3*S*,8*aS*)-Hexahydro-3-[(1*H*-indol-3-yl)methyl]-2-[(4-hydroxy-3,5-dimethoxyphenyl)methyl]pyrrolo[1,2-*a*]pyrazine-1,4-dione (5c): white solid, quantitative yield from crude **9c**; mp 204–206 °C dec; IR ν_{max} (KBr) 1651, 1517, 1459, 1336, 1321, 1218, 1115 cm^{−1}; ¹H NMR δ 8.35 (br s, 1H), 7.63 (d, 1H, *J* = 8.0 Hz), 7.33 (d, 1H, *J* = 7.9 Hz), 7.18 (td, 1H, *J* = 7.9, 1.2 Hz), 7.10 (td, 1H, *J* = 7.3, 1.2 Hz), 6.94 (d, 1H, *J* = 2.4 Hz), 6.56 (s, 2H), 5.65 (d, 1H, *J* = 14.7 Hz), 5.59 (s, 1H), 4.24 (m, 1H), 3.93 (d, 1H, *J* = 14.4 Hz), 3.89 (s, 6H), 3.76 (dd, 1H, *J* = 11.6, 5.5 Hz), 3.65 (dd, 1H, *J* = 14.7, 2.5 Hz), 3.40 (m, 1H), 3.36 (dd, 1H, *J* = 12.2, 3.7 Hz), 2.94 (td, 1H, *J* = 10.4, 4.3 Hz), 1.77 (m, 1H), 1.34 (m, 1H), 0.75 (m, 1H), −0.14 (m, 1H); ¹³C NMR δ 166.4, 164.5, 147.4, 135.7, 134.8, 127.4, 126.3 (s), 124.0, 122.5, 119.7, 119.6, 111.0 (d), 108.4 (s), 105.6 (d), 59.4, 59.3 (d), 56.5 (q), 46.3, 44.5, 28.2, 26.8, 20.6 (t); MS (ESI) *m/z* calcd for M + H 450.2, found 450.1. Anal. (C₂₅H₂₇N₃O₅·1/2H₂O) C, H, N.

(3*S*,8*aS*)-Hexahydro-3-[(1*H*-indol-3-yl)methyl]-2-[(3,4,5-trimethoxyphenyl)methyl]pyrrolo[1,2-*a*]pyrazine-1,4-dione (5d): yield 90% from **9d**; white solid; mp 139–141 °C; [α]_D²⁵ = −6.3 (c 0.38, MeOH); IR ν_{max} (KBr) 3364, 1653, 1593, 1507, 1458, 1423 cm^{−1}; ¹H NMR δ 8.23 (br s, 1H), 7.62 (d, 1H, *J* = 7.6 Hz), 7.34 (dd, 1H, *J* = 7.5, 0.9 Hz), 7.19 (td, 1H, *J* = 7.6, 1.2 Hz), 7.11 (td, 1H, *J* = 7.5, 1.0 Hz), 6.83 (d, 1H, *J* = 2.4 Hz), 6.53 (s, 2H), 5.67 (d, 1H, *J* = 14.4 Hz), 4.24 (br t, 1H, *J* = 3.3 Hz), 3.93 (d, 1H, *J* = 14.5 Hz), 3.86 (s, 6H, OMe×2), 3.85 (s, 3H, OCH₃), 3.77 (dd, 1H, *J* = 11.5, 6.1 Hz), 3.66 (dd, 1H, *J* = 14.8, 2.5 Hz), 3.44 (dt, 1H, *J* = 9.3, 5.4 Hz), 3.37 (dd, 1H, *J* = 14.8, 4.7 Hz), 2.96 (td, 1H, *J* = 11.1, 4.7 Hz), 1.78 (dddd, 1H, *J* = 13.0, 6.9, 5.2, 1.8 Hz), 0.85–0.70 (m, 1H), −0.11 (quintet-*d*, 1H, *J* = 10.8, 1.9 Hz); ¹³C NMR δ 166.4, 164.4, 153.7, 137.9, 135.7, 131.0, 127.4 (s), 123.9 (indole C₂), 122.5, 119.8, 119.6, 111.0 (d), 108.5 (s), 105.7 (d, ArH), 60.9 (OCH₃), 59.5 (Trp-CHN), 59.3 (Pro-CHN), 56.3 (OCH₃×2), 46.3 (NCH₂), 44.5 (Pro-NCH₂), 28.3 (Pro-NCH₂CH₂), 26.8 (Trp-CH₂), 20.6 (Pro-NCH₂CH₂); MS (EI) *m/z* (relative intensity %) 463 (M⁺, 8), 130 (100). Anal. (C₂₆H₂₉N₃O₅·1/2H₂O) C, H, N.

(3*S*,8*aS*)-Hexahydro-3-[(1*H*-indol-3-yl)methyl]-2-(3-phenyl-2-propenyl)pyrrolo[1,2-*a*]pyrazine-1,4-dione (5e): yield 99% from **9e**; white solid; mp 186–188 °C; [α]_D²⁵ = −0.85 (c

0.46, MeOH); IR ν_{\max} (KBr): 3261, 1661, 1637, 1461 cm^{-1} ; ^1H NMR δ 8.41 (br s, 1H), 7.65 (d, 1H, $J = 7.8$ Hz), 7.41–7.25 (m, 6H), 7.17 (t, 1H, $J = 7.2$ Hz), 7.09 (t, 1H, $J = 7.8$ Hz), 6.93 (d, 1H, $J = 2.2$ Hz), 6.69 (d, 1H, $J = 15.8$ Hz, PhCH=), 6.20 (ddd, 1H, $J = 15.8$, 8.5, 5.2 Hz, PhCH=CH-), 5.12 (dd, 1H, $J = 14.8$, 3.7 Hz), 4.44 (br s, 1H), 3.81–3.65 (m, 3H), 3.48–3.38 (m, 1H), 3.31 (dd, 1H, $J = 14.8$, 4.6 Hz), 2.95 (td, 1H, $J = 11.0$, 4.7 Hz), 1.79–1.65 (m, 1H), 1.32 (m, 1H), 0.74 (m, 1H), –0.16 (quintet, 1H, $J = 10.8$ Hz); ^{13}C NMR δ 166.2, 164.4, 136.0, 135.7 (s), 134.9, 128.7, 128.2, 127.4 (d), 126.5 (s), 123.9, 122.7, 122.5, 119.7, 119.7, 110.9 (d), 108.5 (s), 59.9, 59.3 (d), 45.1, 44.5, 28.3, 26.9, 20.6 (t); MS (ESI) m/z calcd for M + H 400.2, found 400.2. Anal. ($\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}_2 \cdot 1/4\text{H}_2\text{O}$) C, H, N.

[5aS,12S,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-(2-methylpropyl)-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (cis-6a) and [5aS,12R,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-(2-methylpropyl)-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (trans-6a). These two compounds were prepared in quantitative yields from *cis*-11a and *trans*-11a, respectively. Their NMR spectra were identical to those reported.¹⁷

[5aS,12S,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-phenyl-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (cis-6b): yield 91% from *cis*-11b; white solid; $[\alpha]_D^{25} = -5.0$ (c 0.077, MeOH); IR ν_{\max} (KBr) 3285, 1663, 1456, 1396 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 11.23 (br s, 1H, NH), 7.56 (d, 1H, $J = 7.7$ Hz), 7.32 (d, 1H, $J = 7.9$ Hz), 7.29–7.24 (m, 4H, PhH), 7.19–7.15 (m, 1H, PhH), 7.07 (td, 1H, $J = 7.0$, 1.2 Hz), 7.00 (t, 1H, $J = 7.4$ Hz), 6.32 (s, 1H, Ph-CHN), 4.55 (dd, 1H, $J = 11.6$, 5.0 Hz, Trp-CHN), 4.37 (t, 1H, $J = 7.8$ Hz, Pro-CHN), 3.54–3.45 (m, 2H, Pro-NCH₂), 3.43 (dd, 1H, $J = 15.8$, 5.2 Hz, Trp-CH₂), 2.99 (dd, 1H, $J = 15.7$, 11.7 Hz, Trp-CH₂), 2.22 (m, 1H), 1.95–1.84 (m, 3H); ^{13}C NMR (DMSO- d_6) δ 169.8, 165.3, 142.7, 135.9, 133.9 (s), 128.4, 126.8, 125.6 (d), 125.6 (s), 121.1, 118.8, 118.0, 111.3 (d), 104.4 (s), 58.3 (Pro-CHN), 56.2 (Trp-CHN), 54.8 (PhCHN), 44.8, 27.8, 22.5, 21.4 (t); NOE (DMSO- d_6) δ 6.32 (11.23, 7.29–7.24, 4.55), 4.55 (6.23); MS (ESI) m/z calcd for M + H 372.2, found 372.2. Anal. ($\text{C}_{23}\text{H}_{21}\text{N}_3\text{O}_2 \cdot 1/5\text{H}_2\text{O}$) C, H, N.

[5aS,12R,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-phenyl-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (trans-6b): yield 89% from *trans*-11b; white solid; mp >250 °C dec; $[\alpha]_D^{25} = -18.9$ (c 0.016, MeOH); IR ν_{\max} (KBr) 3345, 1657, 1627, 1424 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 10.97 (br s, 1H, NH), 7.52 (d, 1H, $J = 7.7$ Hz), 7.37–7.27 (m, 6H), 7.09 (td, 1H, $J = 7.6$, 1.1 Hz), 7.02 (td, 1H, $J = 7.4$, 0.9 Hz), 6.88 (s, 1H), 4.38 (dd, 1H, $J = 10.3$, 4.6 Hz, Trp-CHN), 4.20 (dd, 1H, $J = 9.0$, 6.2 Hz, Pro-CHN), 3.69 (dt, 1H, Pro-NCH₂), 3.44 (dd, 1H, $J = 15.8$, 4.9 Hz, Trp-CH₂), 3.34–3.26 (overlapped, 1H, Pro-NCH₂), 2.92 (dd, 1H, $J = 15.3$, 11.2 Hz, Trp-CH₂), 2.24 (m, 1H), 1.91–1.77 (m, 3H); ^{13}C NMR (DMSO- d_6) δ 165.0, 163.4, 139.7, 136.3, 130.5 (s), 128.5, 128.0, 127.8 (d), 125.8 (s), 121.4 (d), 118.7, 118.0, 111.2 (d), 106.5 (s), 58.5 (Pro-CHN), 53.0 (Trp-CHN), 51.6 (PhCHN), 44.5, 29.1, 26.9, 21.0 (t); MS (ESI) m/z calcd for M + H 372.2, found 372.1. Anal. ($\text{C}_{23}\text{H}_{21}\text{N}_3\text{O}_2 \cdot 4\text{H}_2\text{O}$) C, N; H: calcd, 6.59; found, 5.27. HPLC purity (retention time): solvent system A, 100.0% (13.29 min); solvent system B, 97.6% (15.86 min).

[5aS,12S,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-(4-hydroxy-3,5-dimethoxyphenyl)-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (cis-6c): yield 100% from crude *cis*-11c; white solid; mp 261 °C dec; IR ν_{\max} (KBr) 3256, 1671, 1656, 1516, 1460 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 11.28 (br s, 1H), 8.28 (s, 1H), 7.54 (d, 1H, $J = 7.7$ Hz), 7.35 (d, 1H, $J = 8.0$ Hz), 7.07 (td, 1H, $J = 7.1$, 1.0 Hz), 7.00 (td, 1H, $J = 7.2$, 0.6 Hz), 6.49 (s, 2H, ArH), 6.31 (s, 1H, Ar-CHN), 4.56 (dd, 1H, $J = 11.5$, 5.5 Hz, Trp-CHN), 4.39 (t, 1H, $J = 7.8$ Hz, Pro-CHN), 3.66 (s, 6H, 2 \times OCH₃), 3.53–3.45 (m, 2H), 3.40 (dd, 1H, $J = 15.8$, 5.7 Hz, Trp-CH₂), 3.01 (dd, 1H, $J = 15.7$, 11.7 Hz, Trp-CH₂), 2.21 (m, 1H), 2.01 (m, 1H), 1.87 (m, 2H); ^{13}C NMR (DMSO- d_6) δ 169.5 (s), 165.8 (s), 147.7 (s), 135.7, 134.3, 134.1, 132.5, 125.6 (s), 121.0 (d), 118.8 (d), 118.0 (d), 111.3 (s), 103.9 (s), 102.6 (d), 58.4, 55.8 (d), 55.8

(q), 53.9 (d), 44.9 (t), 27.7 (t), 22.9 (t), 20.9 (t); ^1H – ^{13}C HMQC (DMSO- d_6) δ_{H} (δ_{C}) 6.31 (53.9), 4.56 (55.9); MS (ESI) m/z calcd for M + H 448.2, found 448.0. Anal. ($\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}_5 \cdot 4/3\text{H}_2\text{O}$) C, N; H: calcd, 5.91; found, 5.17. HPLC purity (retention time): solvent system A, 100.0% (10.19 min); solvent system B, 98.0% (11.23 min).

[5aS,12R,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-(4-hydroxy-3,5-dimethoxyphenyl)-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (trans-6c): yield 100% from *trans*-11c; white solid; mp 270 °C; IR ν_{\max} (KBr) 3320, 1657, 1595, 1516, 1459 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 10.91 (br s, 1H), 8.49 (br s, 1H), 7.51 (d, 1H, $J = 7.8$ Hz), 7.30 (d, 1H, $J = 8.0$ Hz), 7.09 (t, 1H, $J = 7.5$ Hz), 7.01 (t, 1H, $J = 7.4$ Hz), 6.76 (s, 1H, Ar-CHN), 6.50 (s, 2H, ArH), 4.48 (dd, 1H, $J = 10.7$, 4.7 Hz), 4.20 (dd, 1H, $J = 10.4$, 6.0 Hz), 3.68 (overlapped, 1H), 3.66 (s, 6H, 2 \times OCH₃), 3.47 (dd, 1H, $J = 15.7$, 4.6 Hz), 3.28 (overlapped, 1H), 2.87 (dd, 1H, $J = 15.6$, 10.9 Hz), 2.23 (m, 1H), 1.90–1.80 (m, 3H); ^{13}C NMR (DMSO- d_6) δ 164.9, 163.6, 147.8, 136.4, 135.8, 130.9, 129.9, 125.8 (s), 121.4 (d), 118.6 (d), 118.0 (d), 111.2 (s), 106.5 (s), 105.7 (d), 58.5 (d), 56.0 (q), 53.1 (d), 52.0 (d), 44.5 (t), 29.2 (t), 27.1 (t), 21.1 (t); ^1H – ^{13}C HMQC (DMSO- d_6) δ_{H} (δ_{C}) 6.76 (52.3), 4.48 (53.3); MS (ESI) m/z calcd for M + H 448.2, found 448.0. Anal. ($\text{C}_{25}\text{H}_{25}\text{N}_3\text{O}_5 \cdot 1/5\text{H}_2\text{O}$) C, H, N.

[5aS,12S,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-(3,4,5-trimethoxyphenyl)-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (cis-6d): yield 94% from *cis*-11d; white solid; mp >270 °C; $[\alpha]_D^{25} = -36.9$ (c 0.20, MeOH); IR ν_{\max} (KBr) 3312, 1656, 1593, 1507, 1456, 1418 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 11.32 (br s, 1H, NH), 7.54 (d, 1H, $J = 7.8$ Hz), 7.36 (d, 1H, $J = 7.9$ Hz), 7.08 (t, 1H, $J = 7.5$ Hz), 7.00 (t, 1H, $J = 7.4$ Hz), 6.56 (s, 2H, ArH), 6.33 (s, 1H, Ar-CHN), 4.59 (dd, 1H, $J = 11.4$, 5.5 Hz, Trp-CHN), 4.41 (t, 1H, $J = 7.8$ Hz, Pro-CHN), 3.69 (s, 6H, 2 \times OCH₃), 3.56 (s, 3H, OCH₃), 3.51 (m, 2H, Pro-NCH₂), 3.45–3.40 (overlapped, 1H, Trp-CH₂), 3.02 (dd, 1H, $J = 15.7$, 11.7 Hz, Trp-CH₂), 2.22 (m, 1H), 2.02 (m, 1H), 1.92–1.84 (m, 2H); ^{13}C NMR δ 170.6, 166.4, 153.3, 137.6, 137.1, 136.7, 133.3, 126.0 (s), 122.1, 119.7, 118.4, 111.7 (d), 105.1 (s), 103.5 (d), 60.8 (q), 59.5 (Pro-CHN), 57.3 (Trp-CHN), 56.1 (2 \times OCH₃), 56.1 (ArCHN), 45.6, 28.6, 23.3, 22.0 (t); ^1H – ^{13}C HMQC (DMSO- d_6) δ_{H} (δ_{C}) 6.33 (54.0), 4.59 (55.9); NOE (DMSO- d_6) δ 6.33 (4.59, 4.41, 6.56), 4.59 (6.33, 4.41); MS (EI) m/z (relative intensity %) 461 (M^+ , 88), 278 (100). Anal. ($\text{C}_{26}\text{H}_{27}\text{N}_3\text{O}_5$) C, H, N.

[5aS,12R,14aS]-1,2,3,5a,6,11,12,14a-Octahydro-12-(3,4,5-trimethoxyphenyl)-5H,14H-pyrrolo[1'',2':4',5']pyrazino[1',2':1,6]pyrido[3,4-b]indole-5,14-dione (trans-6d): yield 89% from *trans*-11d; white solid; mp 158–160 °C; $[\alpha]_D^{25} = -238$ (c 0.24, MeOH); IR ν_{\max} (KBr) 3311, 1654, 1591, 1506, 1457, 1327 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 10.88 (br s, 1H), 7.52 (d, 1H, $J = 7.7$ Hz), 7.31 (d, 1H, $J = 8.0$ Hz), 7.09 (td, 1H, $J = 7.5$, 1.1 Hz), 7.01 (td, 1H, $J = 7.4$, 0.9 Hz), 6.74 (br s, 1H, Ar-CHN), 6.57 (br s, 2H, ArH), 4.56 (dd, 1H, $J = 10.6$, 4.7 Hz, Trp-CHN), 4.24 (dd, 1H, $J = 8.7$, 6.4 Hz, Pro-CHN), 3.70 (s, 6H, OMe \times 2), 3.67 (overlapped, 1H, Pro-NCH₂), 3.63 (s, 3H, OMe), 3.48 (dd, 1H, $J = 15.8$, 4.9 Hz, Trp-CH₂), 3.30 (overlapped, 1H, Pro-NCH₂), 2.89 (dd, 1H, $J = 15.8$, 10.7 Hz), 2.23 (m, 1H), 1.93–1.80 (m, 3H); ^{13}C NMR δ 164.6, 164.3, 153.2, 138.0, 136.7, 134.9, 129.7, 126.2, 122.5, 119.8, 118.5, 111.2, 108.6, 105.7, 60.7, 59.4, 56.1, 53.8, 52.6, 45.0, 30.2, 28.7, 21.5; ^1H – ^{13}C HMQC (DMSO- d_6) δ_{H} (δ_{C}) 6.74 (52.3), 4.56 (53.7); NOE (DMSO- d_6) no NOE between δ 6.74 and 4.56 was observed; MS (EI) m/z (relative intensity %) 461 (M^+ , 100). Anal. ($\text{C}_{26}\text{H}_{27}\text{N}_3\text{O}_5 \cdot \text{H}_2\text{O}$) C, H, N.

[6S,12aS]-2,3,6,7,12,12a-Hexahydro-6-(4-hydroxy-3,5-dimethoxyphenyl)pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione (cis-15c): yield 94% from crude *cis*-14c in two steps; white solid; mp 227–229 °C; IR ν_{\max} (KBr) 1668, 1616, 1517, 1462, 1427, 1322, 1217, 1113 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 11.17 (br s, 1H), 8.43 (d, 1H, $J = 4.2$ Hz, Gly-NH), 8.27 (s, 1H), 7.53 (d, 1H, $J = 7.6$ Hz), 7.33 (d, 1H, $J = 7.8$ Hz), 7.06 (t, 1H, $J = 7.3$ Hz), 6.99 (t, 1H, $J = 7.2$ Hz), 6.57 (s, 2H, ArH), 6.20 (s, 1H, Ar-CHN), 4.42 (dd, 1H, $J = 11.5$, 4.9 Hz, Trp-CHN), 4.06 (d, 1H, $J = 16.8$ Hz), 3.73 (dd, 1H, $J = 17.0$, 4.5 Hz), 3.69 (s,

6H, $2 \times \text{OCH}_3$), 3.43 (dd, 1H, $J = 15.2, 5.1$ Hz, Trp-CH₂), 2.97 (dd, 1H, $J = 15.6, 11.8$ Hz, Trp-CH₂); ^{13}C NMR (DMSO-*d*₆) δ 168.9, 167.6, 147.7, 135.8, 134.5, 134.1, 133.0, 125.6 (s), 121.0 (d), 118.7, 117.9 (d), 111.2 (s), 104.1 (s), 103.2 (d), 55.9 (q), 55.0 (d), 54.5 (d), 44.7 (t), 216 (t); ^1H - ^{13}C HMQC (DMSO-*d*₆) δ_{H} (δ_{C}) 6.20 (54.4), 4.42 (54.6); MS (ESI) m/z calcd for M + H 408.2, found 408.5. Anal. (C₂₂H₂₁N₃O₅·1/2H₂O) C, H, N.

[6R,12aS]-2,3,6,7,12,12a-Hexahydro-6-(4-hydroxy-3,5-dimethoxyphenyl)pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione (trans-15c): yield 100% from crude *trans*-14c; white solid; mp 260 °C dec; IR ν_{max} (KBr) 1680, 1651, 1613, 1520, 1461, 1327, 1116; ^1H NMR (DMSO-*d*₆) δ 11.09 (s, 1H), 8.58 (s, 1H), 8.30 (d, 1H, $J = 2.4$ Hz, Gly-NH), 7.52 (d, 1H, $J = 7.6$ Hz), 7.32 (d, 1H, $J = 8.0$ Hz), 7.11 (td, 1H, $J = 7.0, 1.0$ Hz), 7.02 (td, 1H, $J = 7.0, 0.9$ Hz), 6.82 (s, 1H, Ar-CHN), 6.44 (s, 2H, ArH), 4.13 (d, 1H, $J = 17.5$ Hz), 4.09 (dd, 1H, $J = 12.3, 4.2$ Hz, Trp-CHN), 3.90 (dd, 1H, $J = 17.6, 2.9$ Hz), 3.65 (s, 6H, OMe \times 2), 3.27 (dd, 1H, $J = 15.4, 4.2$ Hz), 3.00 (dd, 1H, $J = 14.4, 12.1$ Hz); ^{13}C NMR (DMSO-*d*₆) δ 166.4, 162.7, 147.9, 136.2, 136.0, 130.6, 129.0, 125.8 (s), 121.5 (d), 118.7 (d), 118.0 (d), 111.2 (s), 107.4 (s), 106.0 (d), 56.1 (q), 52.0 (d), 51.2 (d), 43.1 (t), 26.2 (t); ^1H - ^{13}C HMQC (DMSO-*d*₆) δ_{H} (δ_{C}) 6.82 (51.0), 4.09 (52.2); MS (ESI) m/z calcd for M + Na 430.1, found 430.0. Anal. (C₂₂H₂₁N₃O₅·H₂O) C, H, N.

[6S,12aS]-2,3,6,7,12,12a-Hexahydro-6-(3,4,5-trimethoxyphenyl)pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione (cis-15d): yield 95% from crude *cis*-14d; white solid; mp 256–258 °C; $[\alpha]_{\text{D}}^{25} = -50.4$ (c 0.26, CHCl₃); IR ν_{max} (KBr) 3541, 3451, 3305, 1673, 1588, 1501, 1453, 1416 cm⁻¹; ^1H NMR δ 8.30 (br s, 1H), 7.60 (dd, 1H, $J = 6.9, 1.4$ Hz), 7.29 (d, 1H, $J = 7.2$ Hz), 7.18 (td, 1H, $J = 7.1, 1.4$ Hz), 7.15 (td, 1H, $J = 7.0, 1.2$ Hz), 6.79 (br s, 1H, Gly-NH), 6.54 (s, 2H, ArH), 6.23 (s, 1H, Ar-CHN), 4.36 (dd, 1H, $J = 11.4, 4.5$ Hz, Trp-CHN), 4.11 (d, 1H, $J = 17.5$ Hz, Gly-CH₂), 4.05 (dd, 1H, $J = 17.4, 3.6$ Hz, Gly-CH₂), 3.75–3.69 (overlapped, 1H, Trp-CH₂), 3.75 (s, 3H, OMe), 3.72 (s, 6H, OMe \times 2), 3.25 (dd, 1H, $J = 15.8, 11.6$ Hz); ^{13}C NMR δ 169.4, 167.6, 153.2, 137.4, 137.2, 136.5, 132.9, 125.9 (s), 122.3, 120.0, 118.4, 111.3 (d), 105.6 (s), 103.8 (d), 60.7; 56.6, 56.0, 55.8, 45.3 (t), 23.1 (t); ^1H - ^{13}C HMQC δ_{H} (δ_{C}) 6.23 (56.9), 4.36 (56.0); NOE δ 6.23 (8.30, 6.54, 4.36, 4.11), 4.36 (6.32); MS (ESI) m/z calcd for M + Na 444.15, found 444.0. No split or enantiomeric signal was observed when 0.04–0.42 equiv of (+)-Eu(tfc)₃ was added. Anal. (C₂₃H₂₃N₃O₅·3/4H₂O) C, H, N.

[6R,12aS]-2,3,6,7,12,12a-Hexahydro-6-(3,4,5-trimethoxyphenyl)pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione (trans-15d): yield 100% from crude *trans*-14d; white solid; mp 182–184 °C; $[\alpha]_{\text{D}}^{25} = -251$ (c 0.32, CHCl₃); IR ν_{max} (KBr) 3320, 1681, 1651, 1592, 1506, 1458, 1421 cm⁻¹; ^1H NMR δ 8.36 (br s, 1H), 7.56 (d, 1H, $J = 7.8$ Hz), 7.36 (d, 1H, $J = 8.0$ Hz), 7.24 (td, 1H, $J = 8.2, 1.2$ Hz), 7.18 (td, 1H, $J = 7.5, 1.0$ Hz), 6.99 (s, 1H, Ar-CHN), 6.53 (d like, 1H, Gly-NH), 6.48 (s, 2H, ArH), 4.37 (dd, 1H, $J = 12.0, 4.1$ Hz, Trp-CHN), 4.19 (d, 1H, $J = 17.6$ Hz, Gly-CH₂), 4.11 (dd, 1H, $J = 17.6, 2.5$ Hz, Gly-CH₂), 3.83 (s, 3H, OMe), 3.72 (s, 6H, OMe \times 2), 3.56 (dd, 1H, $J = 15.4, 4.0$ Hz, Trp-CH₂), 3.05 (ddd, 1H, $J = 15.4, 12.0, 1.3$ Hz, Trp-CH₂); ^{13}C NMR δ 168.3, 161.8, 153.1, 137.6, 136.5, 134.2, 129.8, 126.1 (s), 122.5, 119.8, 118.3, 111.3 (d), 108.4 (s), 105.7 (d), 60.7; 56.0 (q), 52.7, 52.4 (d), 44.8, 27.4 (t); ^1H - ^{13}C HMQC δ_{H} (δ_{C}) 6.99 (52.3), 4.37 (52.6); MS (ESI) m/z calcd for M + H 422.2, found 421.8. The %ee = 96%. When 0.14 equiv of (+)-Eu(tfc)₃ was added, both the indole NH and ArH (2H) signals were split into two: δ 8.359 (100%, indole NH)/8.233 (1.4%), 6.694 (100%, 2H, ArH)/6.514 (2.5%). The averaged *ent-trans*-15d was 2%. Anal. (C₂₃H₂₃N₃O₅·1/3H₂O) C, H, N.

Bioassays. In vitro antitumor assay: See ref 22.

Cell proliferation and cell cycle assay: Rat normal fibroblast 3Y1 cells were grown in Dulbecco's modified MEM culture medium supplemented with 10% fetal calf serum under a humidified atmosphere containing 5% CO₂. Exponentially growing 3Y1 cells were treated with test compounds for 24 h. The distribution of DNA content was determined by laser scanning cytometry (Olympus LSC101, Tokyo, Japan) and relative cell numbers (cell number at 24 h per initial cell number at 0 h \times 100) were counted.

In situ microtubule network disruption assay: For immunofluorescence observation, 3Y1 cells were plated on 24-mm \times 24-mm glass coverslips at a low density and cultured for 1 day. After treatment with compounds for 18 h, the coverslips were washed with phosphate-buffered saline (PBS) and fixed with 3.7% formaldehyde in PBS for 5 min, then permeabilized for 5 min with PBS containing 0.2% (v/v) Triton X-100. After being washed with PBS-B [PBS containing 1% (v/v) bovine serum albumin and 0.02% sodium azide], the coverslips were overlaid with anti- α -tubulin antibody (N-356, Amersham Pharmacia Biotech) in PBS-B, then placed in a humidified container at 37 °C and incubated for 1 h. After being washed twice with PBS-B, the coverslips were overlaid with Alexa 488 goat anti-mouse IgG (H+L) conjugate (Molecular Probes, OR), incubated for 45 min, washed with PBS-B and mounted. The cytoskeletons were photographed using a cooled CCD camera (Olympus PROVIS AX70, Tokyo, Japan).

In vitro microtubule assembly assay: Calf brain microtubule protein was prepared by two cycles of assembly/disassembly and stored at –80 °C in Mes buffer [100 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes), 1 mM EGTA and 0.5 mM MgCl₂, pH 6.8]. Protein concentrations were determined by using the DC Protein Assay kit (BioRad, Hercules, CA).

Microtubule assembly was monitored by the turbidity assay.²⁶ In brief, microtubule protein (2.0 mg/mL in Mes buffer) was incubated at 37 °C and the change in absorbance at 350 nm was monitored with time. To examine the effect of compounds on polymerization, the microtubule or tubulin protein was preincubated with 1% dimethyl sulfoxide containing various concentrations of compound at 0 °C and polymerization was initiated with the addition of 1 mM GTP.

Topoisomerase I and II inhibition assay:²⁷ Topoisomerase I drug screening kit, topoisomerase II drug screening kit, human type I topoisomerase and human type II topoisomerase were purchased from TopoGEN, Inc. The assays were performed as described in their manuals, with compounds at a maximal concentration of 250 μM . Positive controls were camptothecin for topoisomerase I and etoposide for topoisomerase II.

Antimicrobial activity: Antibacterial and antifungal assays were carried out with the filter disk method. Test compounds were dissolved in methanol or DMSO (ca. 1 mg/mL). 20 μL of each solution was absorbed on filter paper disk. The air-dried disks (ca. 20 μg /disk) were placed on agar plates cultured with the following strains: *Staphylococcus aureus* S1, *Escherichia coli* 15153, *Pseudomonas aeruginosa* 9027, and *Candida albicans* 1060. The quinolone antibiotic tosoflaxacin was used as a positive control. *trans*-6d and *trans*-15d gave inhibition zones of diameter 8 mm and 6 mm, respectively, for *S. aureus* S1 (in comparison, 4 μg of tosoflaxacin gave 24 mm).

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