Synthesis and Evaluation of Tryprostatin B and Demethoxyfumitremorgin C Analogues

Haishan Wang,[†] Takeo Usui,[‡] Hiroyuki Osada,[‡] and A. Ganesan*[,†]

Institute of Molecular and Cell Biology, National University of Singapore, 30 Medical Drive, Singapore 117609, and The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan

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Tryprostatin B and demethoxyfumitremorgin C are fungal inhibitors of mammalian cell cycle progression at the G_2/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-carbolines 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.

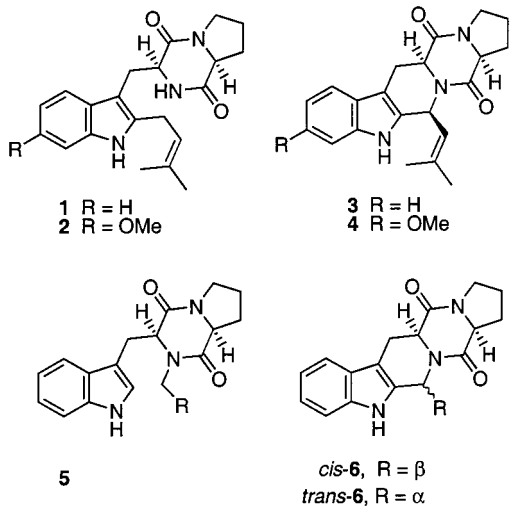
Introduction

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 G_1 phase, and microscopic examination of cell morphology identifies those that block G_2 cell cycle progression. With this screen, a family of prenylated Trp-Pro diketopiperazine alkaloids that cause cell cycle arrest at the G_2/M phase was isolated from fermentation broths of the fungus *Aspergillus fumigatus* BM 939, including[5] (Chart 1) tryprostatin B (**1**, MIC in the tsFT210 assay of 4.4 μM), tryprostatin A (**2**, MIC 16.4 μM), demethoxyfumitremorgin C (**3**, MIC 0.45 μM), and fumitremorgin C (**4**, MIC 4.1 μM).

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 adapted[8] 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 orthoformate[11]-promoted Schiff base (**7**) formation between L-tryptophan methyl ester and an aldehyde, followed by hydride reduction[12] to give secondary amine **8**. Coupling with the acid chloride[13] 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

* Address for correspondence: Department of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, United Kingdom. Tel: +44 2380 593897. Fax: +44 2380 596805. E-mail: ganesan@soton.ac.uk.
† National University of Singapore.
‡ RIKEN.

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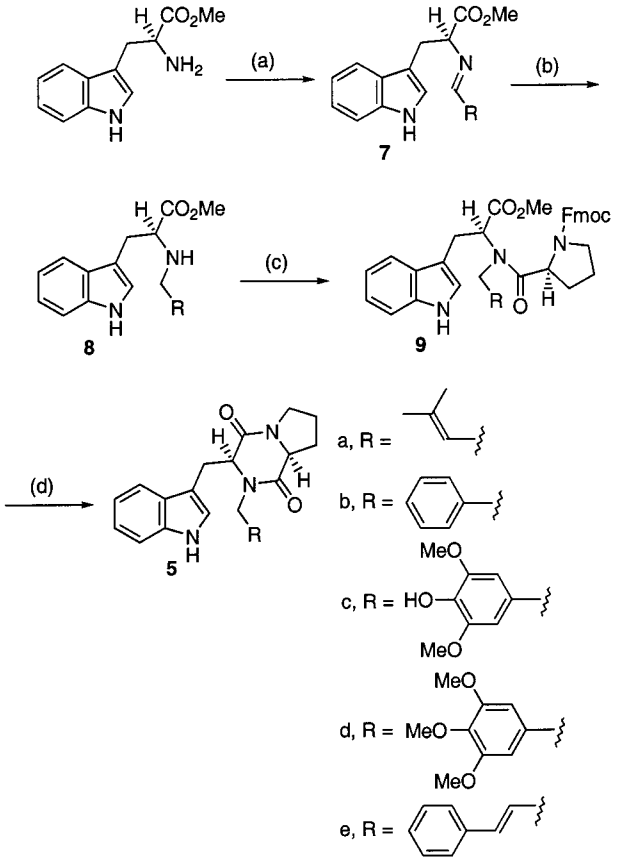
Scheme 1[a]

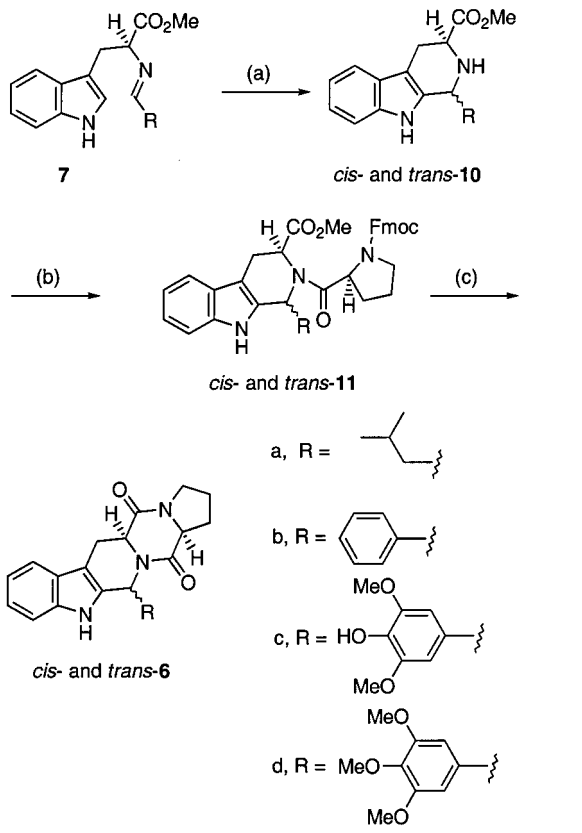


[a] Conditions: (a) R-CHO, $HC(OMe)_3$; (b) $NaBH(OAc)_3$; (c) Fmoc-L-ProCl, aq Na_2CO_3/CH_2Cl_2; (d) 20% piperidine/CH_2Cl_2.

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 pioneered[19] 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/CH_2Cl_2, 0 °C to rt; (b) Fmoc-L-ProCl, aq Na_2CO_3/CH_2Cl_2; (c) 20% piperidine/CH_2Cl_2.

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 methods[21] 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 $^1H-^{13}C$ 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 differences[21] 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. $\log_{10}$ IC_{50} (Molar) Averaged According to Cell Type in the NCI Panel

	panel/cell line[a]									mean averaged[b,c]	
compd	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
epi-**5a**	−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] GI_{50} = concentration that causes 50% growth inhibition; TGI = concentration for total growth inhibition, which signifies cytostatic effect; LC_{50} = concentration for killing 50% of cells, which signifies cytotoxic effect. [c] All the LC_{50} > −4.00 except for both **5d** and **5e** with LC_{50} = −4.01.

Table 2. $\log_{10}$ IC_{50} (Molar) Averaged According to Cell Type in the NCI Panel

	panel/cell line[a]									mean averaged[b]	
compd	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
12-*epi*-**3**	−4.16	−4.03	−4.02	−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.02	>−4.00
cis-**6a**	−4.44	−4.11	−4.17	−4.08	−4.06	−4.10	−4.13	−4.08	−4.20	−4.15	>−4.00
trans-**6a**	−4.11	−4.03	>−4.00	−4.07	>−4.00	>−4.00	−4.01	>−4.00	−4.06	−4.03	>−4.00
cis-**6b**	−4.86	−4.20	−4.38	>−4.00	−4.29	−4.24	−4.37	−4.07	−4.44	−4.32	>−4.00
trans-**6b**	−4.39	−4.28	−4.34	−4.21	−4.30	−4.25	−4.30	−4.20	−4.31	−4.29	>−4.00
cis-**6c**	−4.00	−4.39	−4.05	−4.48	−4.26	−4.40	−4.35	−4.18	−4.33	−4.27	>−4.03
trans-**6c**	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
cis-**6d**	−4.19	−4.15	−4.03	−4.02	−4.03	−4.14	−4.02	−4.04	−4.01	−4.06	>−4.00
trans-**6d**	−4.49	−4.16	−4.13	−4.13	−4.06	−4.11	−4.11	−4.14	−4.33	−4.18	−4.01
cis-**15c**	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
trans-**15c**	−4.42	−4.25	−4.33	−4.42	−4.34	−4.18	−4.26	−4.15	−4.31	−4.30	−4.01
cis-**15d**	−4.14	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.02	>−4.00	−4.03	−4.02	>−4.00
trans-**15d**	−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 LC_{50} > −4.00 except for *trans*-**15d** with LC_{50} = −4.04.

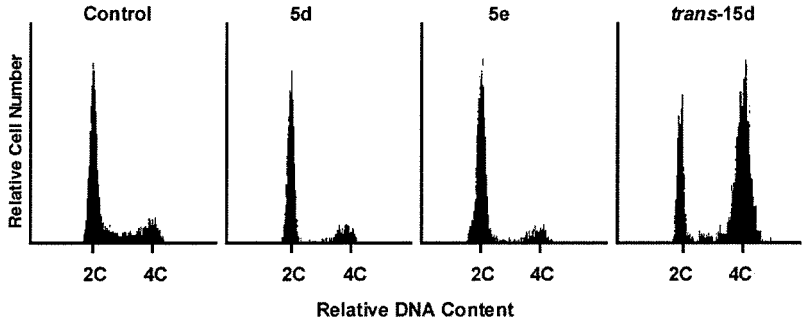


Figure 1. Cell cycle assay. Exponentially growing 3Y1 cells were treated with various compounds (**5d**, **5e**, and *trans*-**15d**) for 24 h, and the distribution of DNA content was determined by laser scanning cytometry.

Table 3. Summary of Bioassay Data for **5d**, **5e**, and *trans*-**15d**

		5d	**5e**	*trans*-**15d**
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:CH_2Cl_2 = 50:45:5).

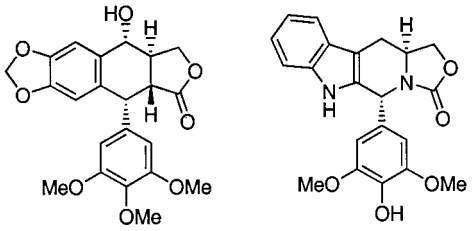
Analogues **5** and **6** were submitted for testing in the National Cancer Institute's in vitro panel of 60 tumor cell lines.[22] Cytotoxic activity (log GI_{50}) is summarized in Tables 1 and 2 according to cell type, together with mean panel inhibitory values. The data for demethoxyfumitremorgin C (**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

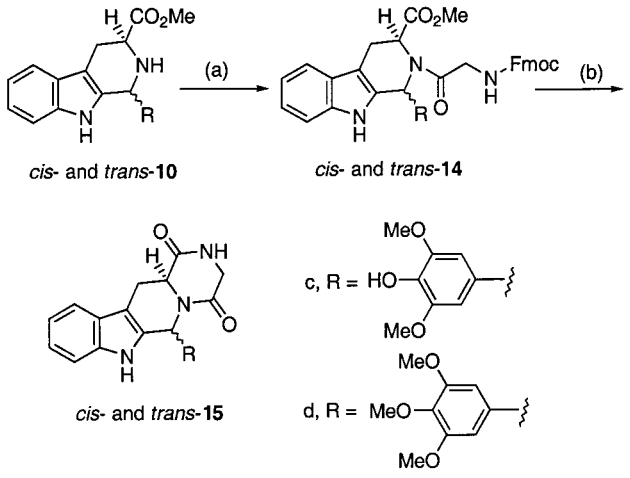
epimers were inactive. However, in the phenyl analogue, both *cis*-**6b** and *trans*-**6b** tetrahydro-β-carbolines were equally active, while *trans*-**6d** with the trimethoxyphenyl substituent was more active than *cis*-**6d**. We believed this shift in stereochemical preference might reflect a change 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 podophyllotoxin[23] (**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 Na_2CO_3/CH_2Cl_2; (b) 20% piperidine/CH_2Cl_2.

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 μM. 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 GI_{50} of 5.9 μM, is the most active of all the analogues prepared. It is relatively nontoxic, with a maximum tolerated dose of 400 mg/kg in mice,

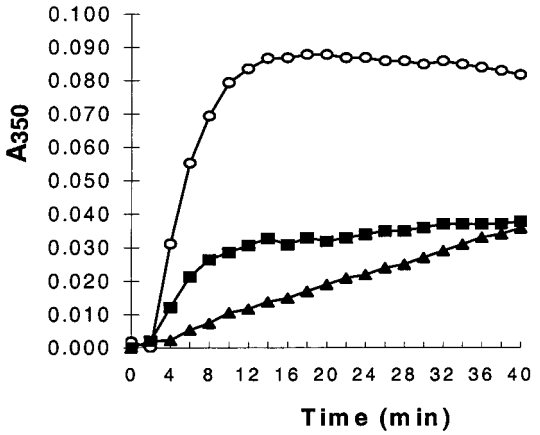


Figure 2. Time course of microtubule assembly in vitro. Microtubule proteins (2.0 mg/mL) were incubated with 1% DMSO containing 250 μM **1** (-■-), *trans*-**15d** (-▲-), or control (no compound, -○-).

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 CH_2Cl_2, which was distilled from CaH_2. TLC was carried out on precoated plates: analytical (Merck; Kieselgel 60 F_{254}), 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 ($CHCl_3$ solution or KBr pellet). ^1H and ^{13}C NMR were recorded at 300 or 400 and at 75 or 100 MHz, respectively, in $CDCl_3$ 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 = CH_2 and q = CH_3. The %ee was measured by using the chiral NMR chemical shift reagent europium tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorate] ((+)-Eu(tfc)$_3$). The conditions were optimized by gradual addition of Eu(tfc)$_3$ solution in $CDCl_3$ (20 or 6 mg/mL) into a NMR tube that contained a sample in $CDCl_3$ 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 C_{18} column (ODS Hypersil, 5 μm, 2.1 × 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 $MgSO_4$ or Na_2SO_4, 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 Na_2CO_3/CH_2Cl_2 and stirred until a clear solution was reached. The organic layer was separated and the aqueous phase extracted (CH_2Cl_2 ×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 CH_2Cl_2 (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 CH_2Cl_2 (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 Na_2CO_3 and extracted (CH_2Cl_2 ×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%): ^{1}H 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 C_2-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, OCH_3), 3.21 (dd, 1H, J = 13.9, 6.6 Hz, NCH_2), 3.15 (m, 2H, NCH_2), 3.11 (dd, 1H, J = 13.6, 6.6 Hz, NCH_2), 1.66 (d, 3H, J = 0.7 Hz, Me), 1.55 (d, 1H, J = 0.3 Hz, Me); ^{13}C 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 C_2-H and OCH_3 signals were split into two: δ 7.078 (*S*)/6.977 (*R*, indole C_2-H), 3.747 (*R*)/3.704 (*S*, OCH_3). The indole C_2-H signals were selected for calculation of %ee.

ent-**8a**: yellow oil prepared from *ent*-**7a**; ^{1}H and ^{13}C 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**; ^{1}H 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, C_2-H), 6.42 (s, 2H, ArH), 3.77 (d, 1H, J = 13.1 Hz), 3.74 (s, 6H, 2×OCH_3), 3.67 (dd, 1H, J = 7.4, 5.9 Hz, Trp-CHN), 3.66 (s, 3H, OCH_3), 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); ^{13}C 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 (OCH_3), 56.1 (OCH_3×2), 52.3 ($ArCH_2N$), 51.8 (Trp-CHN), 29.3 (Trp-CH_2); MS (ESI) m/z 385.2 ([M + H]$^+$).

8d: yellowish foam (82%) prepared from **7d**; ^{1}H 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, C_2-H), 6.43 (s, 2H, ArH), 3.80 (s, 3H, OCH_3), 3.79 (d, 1H, J = 12.6 Hz), 3.72 (s, 6H, 2×OCH_3), 3.67 (s, 3H, OCH_3), 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); ^{13} CNMR 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**; ^{1}H 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, C_2-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, OCH_3), 3.42 (ddd, 1H, J = 13.9, 6.2, 1.4 Hz, NCH_2), 3.30 (ddd, 1H, J = 14.0, 6.3, 1.3 Hz, NCH_2), 3.21 (dd, 1H, J = 14.4, 6.0 Hz, Trp-CH_2), 3.15 (dd, 1H, J = 14.3, 7.2 Hz, Trp-CH_2); ^{13}C 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 CH_2Cl_2 (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 Na_2CO_3 (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-carbolines *cis*- and *trans*-10a−d. Representative Example (*cis*- and *trans*-10d). Crude imine **7d** (1.01 g, 2.44 mmol) was dissolved in dry CH_2Cl_2 (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 Na_2CO_3 and extracted (CH_2Cl_2 ×3). After usual workup, the residue was purified by flash chromatography (ethyl acetate in CH_2Cl_2: 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**: ^{1}H 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, C_1-H), 3.88−3.84 (m, 1H, C_3-H), 3.74 (s, 6H, OMe×2), 3.72 (s, 3H, OMe), 3.67 (s, 3H, OMe), 3.03 (dd, 1H, J = 14.8, 2.8 Hz, C_4-H), 2.86 (ddd, 1H, J = 14.1, 11.2, 2.2 Hz, C_4-H), 2.79 (br s, 1H, N_2-H); ^{13}C 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 (OCH_3), 58.2 (C_1), 56.3 (C_3), 55.7 (OCH_3×2), 51.7 (OCH_3), 25.2 (C_4); NOE (DMSO-d_6) C_3-H (C_1-H, C_4-H); MS (ESI) m/z 397.2 ([M + H]$^+$). *trans*-**10d**: ^{1}H 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, C_1-H), 3.93 (m, 1H, C_3-H), 3.71 (s, 6H, OMe×2), 3.64 (s, 3H, OMe), 3.63 (s, 3H, OMe), 3.19 (br s, 1H, N_2-H), 3.09 (dd, 1H, J = 15.2, 4.7 Hz, C_4-H), 2.94 (ddd, 1H, J = 15.2, 6.2, 1.2 Hz, C_4-H); ^{13}C 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 (OCH_3), 55.7 (OCH_3×2), 54.1 (C_1), 52.2 (C_3), 51.5 (OCH_3), 24.3 (C_4); NOE (DMSO-d_6) no NOE between C_3-H and C_1-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 CH_2Cl_2−hexanes. Pure *trans*-**10b** was obtained as a white foam by flash chromatography (1% to 1.5% MeOH in CH_2Cl_2) of the mother liquor from recrystallization. The structures of *cis*-**10b** and *trans*-**10b** were confirmed by comparing their ^{1}H and ^{13}C NMR data with that reported[19] for (±)-*cis*-**10b** and (±)-*trans*-**10b**. Additional data: *cis*-**10b**: ^{1}H−^{13}C HMQC (DMSO-d_6) δ_H (δ_C) 5.21 (57.6, C_1), 3.88 (56.1, C_3); NOE (DMSO-d_6) C_1-H (C_3-H), C_3-H (C_1-H, C_4-H), N_2-H (C_1-H, C_3-H). *trans*-**10b**: ^{1}H−^{13}C HMQC (DMSO-d_6) δ_H (δ_C) 5.32 (53.9, C_1), 3.77 (51.6, C_3); NOE (DMSO-d_6) no NOE between C_3-H and C_1-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 CH_2Cl_2). *cis*-**10c**: ^{1}H 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, C_1-H), 3.86 (m, 1H, C_3-H), 3.72 (s, 9H, OMe×3), 3.02 (dd, 1H, J = 14.8, 2.7 Hz, C_4-H), 2.85 (ddd, 1H, J = 14.5, 11.2, 2.3 Hz, C_4-H), 2.68 (t, 1H, J = 5.6 Hz, N_2-H); ^{13}C 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 (C_1), 56.3 (C_3), 55.8 (OCH_3×2), 51.7 (OCH_3), 25.2 (C_4); NOE (DMSO-d_6) C_1-H (C_3-H, ArH), C_3-H (C_1-H), N_2-H (C_1-H); MS (ESI) m/z 383.2 ([M + H]$^+$). *trans*-**10c**: ^{1}H 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, C_1-H), 3.92 (t, 1H, J = 5.8 Hz, C_3-H), 3.69 (s, 6H, OMe×2), 3.63 (s, 3H, OMe), 3.10−3.05 (overlapped, 1H, N_2-H), 3.07 (dd, 1H, J = 14.7, 5.5 Hz, C_4-H), 2.93 (dd, 1H, J = 14.6, 6.2 Hz, C_4-H); ^{13}C 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 (OCH_3×2), 54.1 (C_1), 52.1 (C_3), 51.5 (OCH_3), 24.4 (C_4); 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*,8a*S*)-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*,8a*S*)-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 CH_2Cl_2 (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; $[\alpha]^{22}_D$ = +3.4 (*c* 0.46, MeOH); IR ν_{max} (KBr) 3228, 1667, 1633, 1332, 1305 cm^{-1}; ^{1}H 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); ^{13}C 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. ($C_{21}H_{25}N_3O_2$) 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 $[\alpha]^{22}_D$ = +3.2 (*c* 0.16, MeOH). *epi*-**5a**: white solid; $[\alpha]^{22}_D$ = −9.6 (*c* 0.38, MeOH); IR ν_{max} (KBr) 1651, 1457 cm^{-1}; ^{1}H 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); ^{13}C 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. ($C_{21}H_{25}N_3O_2$·2/5H_2O) C, H, N.

(3*S*,8a*S*)-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; $[\alpha]^{22}_D$ = +2.3 (*c* 0.10, MeOH); IR ν_{max} (KBr) 3265, 1667, 1657, 1632, 1473, 1452 cm^{-1}; ^{1}H 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); ^{13}C 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. ($C_{23}H_{23}N_3O_2$·2/3H_2O) C, H, N.

(3*S*,8a*S*)-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}; ^{1}H 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); ^{13}C 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. ($C_{25}H_{27}N_3O_5$·1/2H_2O) C, H, N.

(3*S*,8a*S*)-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; $[\alpha]^{22}_D$ = −6.3 (*c* 0.38, MeOH); IR ν_{max} (KBr) 3364, 1653, 1593, 1507, 1458, 1423 cm^{-1}; ^{1}H 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, OCH_3), 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); ^{13}C NMR δ 166.4, 164.4, 153.7, 137.9, 135.7, 131.0, 127.4 (s), 123.9 (indole C_2), 122.5, 119.8, 119.6, 111.0 (d), 108.5 (s), 105.7 (d, ArH), 60.9 (OCH_3), 59.5 (Trp-CHN), 59.3 (Pro-CHN), 56.3 (OCH_3×2), 46.3 (NCH_2), 44.5 (Pro-NCH_2), 28.3 (Pro-NCH**C**H_2), 26.8 (Trp-CH_2), 20.6 (Pro-NCH_2CH_2); MS (EI) m/z (relative intensity %) 463 (M$^+$, 8), 130 (100). Anal. ($C_{26}H_{29}N_3O_5$·1/2H_2O) C, H, N.

(3*S*,8a*S*)-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; $[\alpha]^{22}_D$ = −0.85 (*c*

0.46, MeOH); IR ν_{max} (KBr): 3261, 1661, 1637, 1461 cm^{-1}; ^{1}H 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=C*H*-), 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. ($C_{25}H_{25}N_3O_2$·1/4H_2O) C, H, N.

[5a*S*,12*S*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-(2-methylpropyl)-5*H*,14*H*-pyrrolo[1″,2″:4′,5′]pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-5,14-dione (*cis*-6a) and [5a*S*,12*R*,-14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-(2-methylpropyl)-5*H*,14*H*-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]

[5a*S*,12*S*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-phenyl-5*H*,14*H*-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; $[\alpha]^{22}_D$ = −5.0 (*c* 0.077, MeOH); IR ν_{max} (KBr) 3285, 1663, 1456, 1396 cm^{-1}; ^{1}H 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-NCH$_2$), 3.43 (dd, 1H, J = 15.8, 5.2 Hz, Trp-CH$_2$), 2.99 (dd, 1H, J = 15.7, 11.7 Hz, Trp-CH$_2$), 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. ($C_{23}H_{21}N_3O_2$·1/5H_2O) C, H, N.

[5a*S*,12*R*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-phenyl-5*H*,14*H*-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; $[\alpha]^{22}_D$ = −18.9 (*c* 0.016, MeOH); IR ν_{max} (KBr) 3345, 1657, 1627, 1424 cm^{-1}; ^{1}H 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-NCH$_2$), 3.44 (dd, 1H, J = 15.8, 4.9 Hz, Trp-CH$_2$), 3.34−3.26 (overlapped, 1H, Pro-NCH$_2$), 2.92 (dd, 1H, J = 15.3, 11.2 Hz, Trp-CH$_2$), 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. ($C_{23}H_{21}N_3O_2$·4H_2O) 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).

[5a*S*,12*S*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-(4-hydroxy-3,5-dimethoxyphenyl)-5*H*,14*H*-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}; ^{1}H 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×OCH$_3$), 3.53−3.45 (m, 2H), 3.40 (dd, 1H, J = 15.8, 5.7 Hz, Trp-CH$_2$), 3.01 (dd, 1H, J = 15.7, 11.7 Hz, Trp-CH$_2$), 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); ^{1}H−^{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. ($C_{25}H_{25}N_3O_5$·4/3H_2O) 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).

[5a*S*,12*R*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-(4-hydroxy-3,5-dimethoxyphenyl)-5*H*,14*H*-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}; ^{1}H 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×OCH$_3$), 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); ^{1}H−^{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. ($C_{25}H_{25}N_3O_5$·1/5H_2O) C, H, N.

[5a*S*,12*S*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-(3,4,5-trimethoxyphenyl)-5*H*,14*H*-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; $[\alpha]^{25}_D$ = −36.9 (*c* 0.20, MeOH); IR ν_{max} (KBr) 3312, 1656, 1593, 1507, 1456, 1418 cm^{-1}; ^{1}H 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×OCH$_3$), 3.56 (s, 3H, OCH$_3$), 3.51 (m, 2H, Pro-NCH$_2$), 3.45−3.40 (overlapped, 1H, Trp-CH$_2$), 3.02 (dd, 1H, J = 15.7, 11.7 Hz, Trp-CH$_2$), 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×OCH$_3$), 56.1 (ArCHN), 45.6, 28.6, 23.3, 22.0 (t); ^{1}H−^{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. ($C_{26}H_{27}N_3O_5$) C, H, N.

[5a*S*,12*R*,14a*S*]-1,2,3,5a,6,11,12,14a-Octahydro-12-(3,4,5-trimethoxyphenyl)-5*H*,14*H*-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; $[\alpha]^{25}_D$ = −238 (*c* 0.24, MeOH); IR ν_{max} (KBr) 3311, 1654, 1591, 1506, 1457, 1327 cm^{-1}; ^{1}H 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×2), 3.67 (overlapped, 1H, Pro-NCH$_2$), 3.63 (s, 3H, OMe), 3.48 (dd, 1H, J = 15.8, 4.9 Hz, Trp-CH$_2$), 3.30 (overlapped, 1H, Pro-NCH$_2$), 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; ^{1}H−^{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. ($C_{26}H_{27}N_3O_5$·H_2O) C, H, N.

[6*S*,12a*S*]-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}; ^{1}H 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×OCH_3), 3.43 (dd, 1H, J = 15.2, 5.1 Hz, Trp-CH_2), 2.97 (dd, 1H, J = 15.6, 11.8 Hz, Trp-CH_2); ^{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); ^{1}H−^{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. ($C_{22}H_{21}N_3O_5 \cdot 1/2H_2O$) C, H, N.

[6*R*,12a*S*]-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; ^{1}H 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×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); ^{1}H−^{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. ($C_{22}H_{21}N_3O_5 \cdot H_2O$) C, H, N.

[6*S*,12a*S*]-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; $[\alpha]^{30}_D$ = −50.4 (c 0.26, $CHCl_3$); IR ν_{max} (KBr) 3541, 3451, 3305, 1673, 1588, 1501, 1453, 1416 cm^{-1}; ^{1}H 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-CH_2), 4.05 (dd, 1H, J = 17.4, 3.6 Hz, Gly-CH_2), 3.75−3.69 (overlapped, 1H, Trp-CH_2), 3.75 (s, 3H, OMe), 3.72 (s, 6H, OMe×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); ^{1}H−^{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. ($C_{23}H_{23}N_3O_5 \cdot 3/4H_2O$) C, H, N.

[6*R*,12a*S*]-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; $[\alpha]^{30}_D$ = −251 (c 0.32, $CHCl_3$); IR ν_{max} (KBr) 3320, 1681, 1651, 1592, 1506, 1458, 1421 cm^{-1}; ^{1}H 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-CH_2), 4.11 (dd, 1H, J = 17.6, 2.5 Hz, Gly-CH_2), 3.83 (s, 3H, OMe), 3.72 (s, 6H, OMe×2), 3.56 (dd, 1H, J = 15.4, 4.0 Hz, Trp-CH_2), 3.05 (ddd, 1H, J = 15.4, 12.0, 1.3 Hz, Trp-CH_2); ^{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); ^{1}H−^{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. ($C_{23}H_{23}N_3O_5 \cdot 1/3H_2O$) 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% CO_2. 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 × 100) were counted.

In situ microtubule network disruption assay: For immunofluorescence observation, 3Y1 cells were plated on 24-mm × 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 $MgCl_2$, 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, *Pesudomonas aeruginosa* 9027, and *Candida albicans*1060. The quinolone antibiotic tosufloxacin 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 tosufloxacin gave 24 mm).

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References

(1) Waldman, T.; Zhang, Y.; Dillehay, L.; Yu, J.; Kinzler, K.; Vogelstein, B.; Williams, J. Cell-cycle Arrest Versus Cell Death in Cancer Therapy. *Nature Med*. **1998**, *3*, 1034−1036.

(2) Bunz, F.; Dutrriaux, A.; Lengauer, C.; Waldman, T.; Zhou, S.; Brown, J. P.; Sedivy, J. M.; Kinzler, K. W.; Vogelstein, B. Requirement for p53 and p21 to Sustain G2 Arrest after DNA Damage. *Science* **1998**, *282*, 1497−1501.

(3) Osada, H.; Cui, C.-B.; Onose, R.; Hanaoka, F. Screening of Cell Cycle Inhibitors from Microbial Metabolites by a Bioassay Using a Mouse cdc2 Mutant Cell Line, tsFT210. *Bioorg. Med. Chem*. **1997**, *5*, 193−203.

(4) Osada, H. Bioprobes for Investigating Mammalian Cell Cycle Control. *J. Antibiot*. **1998**, *51*, 973−982.

(5) (a) Cui, C.-B.; Kakeya, H.; Okada, G.; Onose, R.; Ubukata, M.; Takahashi, I.; Isono, K.; Osada, H. Tryprostatins A and B, Novel Mammalian Cell Cycle Inhibitors Produced by *Aspergillus Fumigatus*. *J. Antibiot*. **1995**, *48*, 1382−1384. (b) Cui, C.-B.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. Novel Mammalian Cell Cycle Inhibitors, Tryprostatins A, B and Other Diketopiperazines Produced by *Aspergillus Fumigatus* I. Taxonomy, Fermentation, Isolation and Biological Properties. *J. Antibiot*. **1996**, *49*, 527−533. (c) Cui, C.-B.; Kakeya, H.; Osada, H. Spirotryprostatin B, a Novel Mammalian Cell Cycle Inhibitor Produced by *Aspergillus fumigatus*. *J. Antibiot*. **1996**, *49*, 832−835. (d) Cui, C.-B.; Kakeya, H.; Osada, H. Novel Mammalian Cell Cycle Inhibitors, Spirotryprostatins A and B, Produced by *Aspergillus fumigatus*, Which Inhibit Mammalian Cell Cycle at G2/M Phase. *Tetrahedron* **1996**, *52*, 12651−12666. (e) Cui, C.-B.; Kakeya, H.; Osada, H. Novel Mammalian Cell Cycle Inhibitors, Cyclotryprostatins A−D, Produced by *Aspergillus fumigatus*, Which Inhibit Mammalian Cell Cycle at G2/M Phase. *Tetrahedron* **1997**, *53*, 59−72.

(6) Wang, H.; Ganesan, A. Concise Synthesis of the Cell Cycle Inhibitor Demethoxyfumitremorgin C. *Tetrahedron Lett*. **1997**, *38*, 4327−4328.

(7) For a review of earlier syntheses, see: Hino, T.; Nakagawa, M. Total Syntheses of Fumitremorgins and Verruculogens. *Heterocycles* **1997**, *46*, 673−704.

(8) Wang, H.; Ganesan, A. The *N*-Acyliminium Pictet−Spengler Condensation as a Multicomponent Combinatorial Reaction on Solid Phase and its Application to the Synthesis of Demethoxyfumitremorgin C Analogues. *Org. Lett*. **1999**, *1*, 1647−1649.

(9) Depew, K. M.; Danishefsky, S. J.; Rosen, N.; Sepp-Lorenzino, L. Total Synthesis of Tryprostatin B: Generation of a Nucleophilic Prenylating Species from a Prenylstannane. *J. Am. Chem. Soc*. **1996**, *118*, 12463−12464.

(10) (a) Gan, T.; Cook, J. M. Enantioselective Total Synthesis of Tryprostatin A. *Tetrahedron Lett*. **1997**, *38*, 1301−1304. (b) Gan, T.; Liu, R.; Yu, P.; Zhao, S.; Cook, J. M. Enantioselective Synthesis of Optically Active 6-Methoxytryptophan Derivatives and Total Synthesis of Tryprostatin A. *J. Org. Chem*. **1997**, *62*, 9298−9304. (c) Zhao, S.; Gan, T.; Yu, P.; Cook, J. M. Total Synthesis of Tryprostatin A and B as well as Their Enantiomers. *Tetrahedron Lett*. **1998**, *39*, 7009−7012.

(11) Look, G. C.; Murphy, M. M.; Campbell, D. A.; Gallop, M. A. Trimethylorthoformate: A Mild and Effective Dehydrating Reagent for Solution and Solid-Phase Imine Formation. *Tetrahedron Lett*. **1995**, *36*, 2937−2940.

(12) Szardenings, A. K.; Burkoth, T. S.; Look, G. C.; Campbell, D. A. A Reductive Alkylation Procedure Applicable to Both Solution- and Solid-Phase Syntheses of Secondary Amines. *J. Org. Chem*. **1996**, *61*, 6720−6722.

(13) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C. ((9-Fluorenylmethyl)oxy)-carbonyl (Fmoc) Amino Acid Chlorides. Synthesis, Characterization, and Application to the Rapid Synthesis of Short Peptide Segments. *J. Org. Chem*. **1986**, *51*, 3732−3734.

(14) For a discussion of racemization during reductive alkylation, see: Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. Solid-Phase Synthesis of 1,4-Benzodiazepine-2,5-Diones. Library Preparation and Demonstration of Synthesis Generality. *J. Org. Chem*. **1997**, *62*, 1240−1256.

(15) For recent reviews, see: (a) Bringmann, G.; Ewers, C. L. J.; Walter, R. Use of Carbonyl Derivatives for Heterocyclic Synthesis. In *Comprehensive Organic Synthesis, Vol. 6*; Winterfeldt, E., Ed.; Pergamon Press: Oxford, 1991; pp 736−740. (b) Cox, E. D.; Cook, J. M. The Pictet−Spengler Condensation: A New Direction for an Old Reaction. *Chem. Rev*. **1995**, *95*, 1797−1842.

(16) Massiot, G.; Mulamba, T. Synthesis of The Two Enantiomers of a Tetrahydro-β-carboline from L-(−)-Tryptophan. *J. Chem. Soc., Chem. Commun*. **1983**, *37*, 1147−1149.

(17) Nakagawa, K.; Fukushima, H.; Kawate, T.; Hongu, M.; Une, T.; Kodato, S.-I.; Taniguchi, M.; Hino, T. Synthetic Approaches to Fumitremorgins. III. Synthesis of Optically Active Pentacyclic Ring Systems, and Their Oxidation at Ring C. *Chem. Pharm. Bull*. **1989**, *37*, 23−32.

(18) Bailey, P. D.; Hollinshead, S. P.; McLay, N. R.; Morgan, K.; Palmer, S. J.; Prince, S. N.; Reynolds, C. D.; Wood, S. D. Diastereo- and Enantio-selectivity in The Pictet−Spengler Reaction. *J. Chem. Soc., Perkin Trans. 1* **1993**, 431−439.

(19) Soerens, D.; Sandrin, J.; Ungemach, F.; Mokry, P.; Wu, G. S.; Yamanaka, E.; Hutchins, L.; DiPierro, M.; Cook, J. M. Study of the Pictet−Spengler Reaction in Aprotic Media: Synthesis of the β-Galactosidase Inhibitor, Pyridindolol. *J. Org. Chem*. **1979**, *44*, 535−545.

(20) Madrigal, A.; Grande, M.; Avendaño, C. Stereocontrolled Synthesis of 3,6-Dimethyl-2,3,6,7,12,12a-hexahydropyrazino[1,2-*b*]-β-carboline-1,4-diones. *J. Org. Chem*. **1998**, *63*, 2724−2727.

(21) Ungemach, F.; Soerens, D.; Weber, R.; DiPierro, M.; Campos, O.; Mokry, P.; Cook, J. M.; Silverton, J. V. General Method for the Assignment of Stereochemistry of 1,3-Disubstituted 1,2,3,4-Tetrahydro-β-carbolines by Carbon-13 Spectroscopy. *J. Am. Chem. Soc*. **1980**, *102*, 6976−6984.

(22) Information on the NCI's screening program is available on the World Wide Web at http://dtp.nci.nih.gov.

(23) For a review, see: Damayanthi, Y.; Lown, J. W. Podophyllotoxins: Current Status and Recent Developments. *Curr. Med. Chem*. **1998**, *5*, 205−252.

(24) Leteurtre, F.; Madalengoitia, J.; Orr, A.; Cuzi, T. J.; Lehnert, E.; Macdonald, T.; Pommier, Y. Rational Design and Molecular Effects of a New Topoisomerase II Inhibitor, Azatoxin. *Cancer Res*. **1992**, *52*, 4478−4483.

(25) Madalengoitia, J. S.; Tepe, J. J.; Werbovetz, K. A.; Lehnert, E. K.; Macdonald, T. L. Structure−Activity Relationship for DNA Topoisomerase II−Induced DNA Cleavage by Azatoxin Analogues. *Biochem. Med. Chem*. **1997**, *5*, 1807−1815.

(26) (a) Usui, T.; Kondoh, M.; Cui, C.-B.; Mayumi, T.; Osada, H. Tryprostatin A, a Specific and Novel Inhibitor of Microtubule Assembly. *Biochem. J*. **1998**, *333*, 543−548. (b) Kondoh, M.; Usui, T.; Mayumi, T.; Osada, H. Effects of Tryprostatin Derivatives on Microtubule Assembly In Vitro and In Situ. *J. Antibiot*. **1998**, *5*, 801−804.

(27) (a) Muller, M. T.; Spitzner, J. R.; Didonato, J. A., Mehta, V. B.; Tsutsui, K.; Tsutsui, K. Single-Strand DNA Cleavages by Eukaryotic Topoisomerase. 2. *Biochemistry* **1988**, *27*, 8369−8379. (b) Spitzner, J. R.; Muller, M. T. A Consensus Sequence for Cleavage by Vertebrate DNA Topoisomerase II. *Nucleic Acid Res*. **1988**, *16*, 5533−5556.

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