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Drug-to-Genome-to-Drug, Step 2: Reversing Selectivity in a Series of Antiplasmodial Compounds

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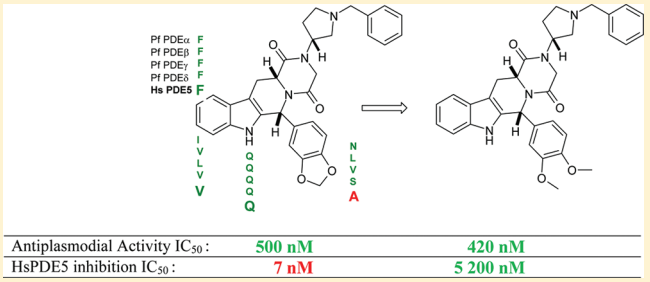
ABSTRACT: In a recent paper, we have described the discovery of antimalarial compounds derived from tadalafil, using a drug-to-genome-to-drug approach (*J. Med. Chem.* **2011**, *54* (9), pp 3222−3240). We have shown that these derivatives inhibit the phosphodiesterase activity of *Plasmodium falciparum* and the parasite growth in culture. In this paper, we describe the optimization of these compounds. A direct consequence of our approach based on gene orthology is the lack of selectivity of the compounds over the original activity on the human target. We demonstrate here that it is possible to take advantage of subtle differences in SAR between HsPDE5 inhibition and antiplasmodial activity to improve significantly the selectivity. In particular, the replacement of the piperonyl group in compound **2** by a dimethoxyphenyl group was the best way to optimize selectivity. This observation is consistent with the differences between human and plasmodial sequences in the Q2 pocket receiving this group.



INTRODUCTION

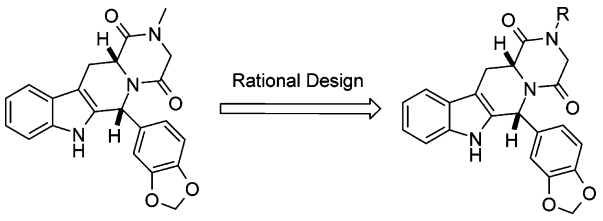
In a recent paper,[1] we described the drug-to-genome-to-drug approach for the discovery of new potent antiplasmodial compounds. This approach consists of screening compounds inspired from drugs acting on proteins that have orthologues in *Plasmodium falciparum*. We focused on PDE5 inhibitors inspired from tadalafil and showed that inhibition of *Plasmodium* phosphodiesterase activity by these compounds was linked to the disruption of the life cycle of the parasite in cell-based assay (Table 1).

Compound **2** and **3** are the most potent antiplasmodial compounds in our series. They were previously described by Maw et al. as potent human PDE5 inhibitor,[2] and we showed that they inhibit cGMP hydrolysis in a parasite lysate. In the first part of this article we report improved antiplasmodial compounds and structure−activity relationships (SARs). In the second part, we show that it is possible to find structural modifications that reverse the selectivity over human PDE5. The lead compound is further profiled to show that the original PDE selectivity of tadalafil is conserved while switching from HsPDE5 to antiplasmodial activity.

CHEMISTRY

Compound **2** was selected as a lead compound for variation of the side chain on the pyrrolidine ring in the first instance. The piperonyl moiety in the C6 position, the *R*-configuration of the pyrrolidine group, and the cis configuration (6*R*,12a*R*) were kept unchanged in this series. The benzyl group on the pyrrolidine of compound **2** was replaced by diverse aromatic or

Table 1. In Vitro Antiplasmodial Activity and Cytotoxicity



Cpd	R	Antiplasmodial activity ($IC_{50}\mu M$) PfGHA	Cytotoxicity ($CC_{50}\mu M$) MRC5
2		0.5	10.0
3		1.0	7.0

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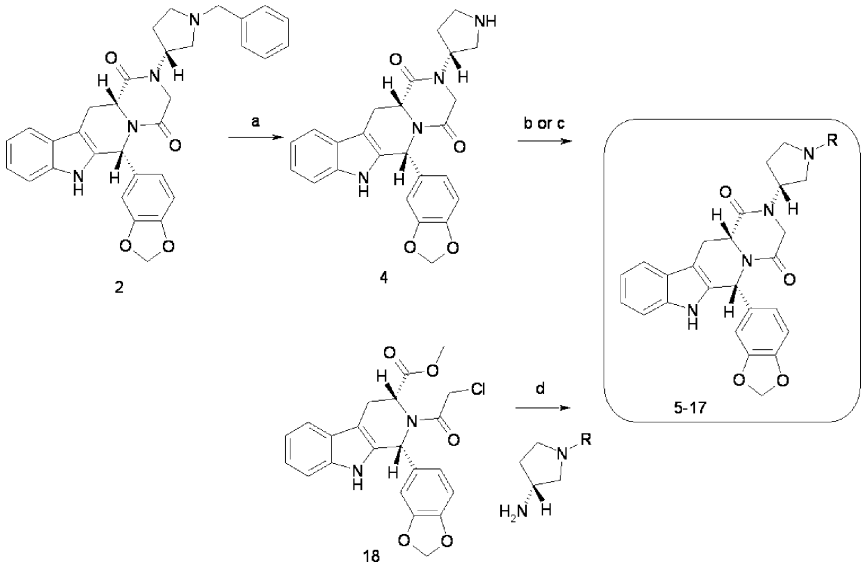
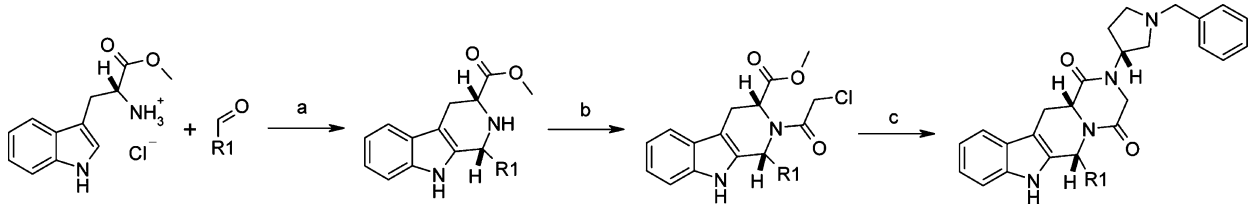
Scheme 1. General Synthetic Route[a]



[a]Conditions: (a) H_2, Pd/C, EtOH, reflux, 30 min; (b) aldehyde, (polystyrylmethyl)trimethylammonium cyanoborohydride resin, CH_2Cl_2/AcOH, 10/1, 12−24 h or (c) R-X, dioxanne, triethylamine, 70°C; (d) refluxed in methanol under basic conditions.

Scheme 2. General Synthetic Route of Lead 2 Analogues[a]



[a]Conditions: (a) CF_3COOH, CH_2Cl_2, diastereoisomer separation; (b) chloroacetyl chloride, Et_3N, $CHCl_3$ 0°C to room temp; (c) R1-NH_2, MeOH, reflux, or EtOH, microwave 100 W/5 min.

aliphatic derivatives. Compounds **4**−**16** were obtained following the synthetic route described in Scheme 1. The starting point of the synthesis was compound **2** itself. It was debenzylated in ethanol in the presence of hydrogen and Pd on charcoal to afford compound **4**, which was then alkylated using alkyl halides or reductive amination of aldehydes. Some derivatives were obtained by reaction of the corresponding N-substituted aminopyrrolidine with the chloroacetylated ester **18**. Intermediates **4** and **18** have been previously described by Beghyn et al.[1]

The synthesis of analogues where the piperonyl group is replaced by other substituents implies the use of the various aldehydes in the Pictet−Spengler reaction (Scheme 2). Recently, Dunn et al.[3] described the diastereoselective industrial synthesis of tadalafil in which the precipitation of the product in isopropanol is responsible for diastereoselectivity. Unfortunately, these diastereoselective conditions could not be applied to other substrates. Pictet−Spengler reactions were thus performed in dichloromethane in the presence of trifluoroacetic acid at room temperature, starting from D-tryptophan methyl ester hydrochloride (Scheme 2). Diastereoisomers cis (1*R*,3*R*) and trans (1*S*,3*R*) were separated by column chromatography.

RESULTS AND DISCUSSION

Structure−Activity Relationships. Compounds were tested in vitro for inhibitory activity against *P. falciparum* (GHA chloroquine-sensitive strain unless otherwise mentioned) in culture and for cytotoxicity on human fetal lung fibroblast (MRC5) to assess the specificity of action of the compounds (Table 2). A compound is considered as "highly active" when the IC_{50} is less than 1 μM and specific ratio is >100.[4]

The replacement of the benzyl group on the pyrrolidine by an alkyl chain led to a slightly decreased activity for **5** and **6**. The length of the chain between the aryl ring and the protonable nitrogen is important. Adding a methylene group is deleterious (**7**), while lengthening the chain by introducing an ether function restored activity (**8**). Compared to compound **2**, activity is retained with a piperonyl moiety but significantly reduced with other aryl groups (**10**−**12**) except for methylimidazole (**13**). Adding substituents at the para position of the benzyl group increased the cytotoxicity of compounds **10** and **11** (respectively methoxy and dimethylamino groups). Introduction of nonaromatic moieties such as a *N*,*N*-dimethylacetamide (**14**) led to a moderately active compound, whereas compound **15** bearing a *t*-Boc-aminoethyl substituent was very potent. A hydrophobic moiety (aromatic or aliphatic) such as the *tert*-butyl in compound **15** seems to be favorable for activity, whereas introduction of polar group appeared deleterious. The replacement of the aromatic ring by aliphatic cyclopentyl (**16**) or cyclohexyl (**17**) led to potent antiplasmodial compounds, but these compounds exhibit a significant cytotoxicity. The most promising compound (i.e., displaying

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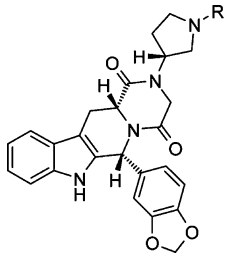
Table 2. In Vitro Antiplasmodial Activity



Cpd	R	antiplasmodial activity (IC_{50}µM)	cytotoxicity (CC_{50}µM)	ratio
2		0.50	10.00	20
5		12.00	8.00	<1
6		12.00	32.00	3
7		5.30	8.10	1
8		0.70	7.70	11
9		0.53	39.00	78
10		10.00	8.00	<1
11		7.00	2.00	<1
12		4.00	52.00	13
13		0.80	40.00	50
14		2.00	>64.00	>32
15		0.22	34.00	154
16		0.25	5.60	22
17		0.90	6.60	7

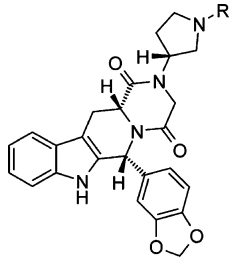
the best antiplasmodial activity and the best specificity ratio) is compound **15**.

Selectivity Issue. As a likely consequence of our approach, many of our compounds are also potent inhibitors of human PDE5. Activity measured by us confirm the trend published by Maw et al. (Table 3).[2] It is not surprising to note that **15**, one of the best antiplasmodial compounds in our series, is also a subnanomolar inhibitor of human PDE5.

We believed, however, that we could eventually overcome this selectivity issue. Indeed, a multiple alignment of the sequences of PDEs from human and *Plasmodium* reveals a few but possibly critical nonconservative differences in the residues of the Q2 pocket between the two species.[1] Therefore, the replacement of the piperonyl group of tadalafil known to bind to this pocket[5] could be a way to design compounds devoid of HsPDE5 inhibition while keeping antiplasmodial activity.

The choice of aldehydes for the Pictet reaction was based on SAR described for HsPDE5 inhibition in the tadalafil series. It has been demonstrated that the best interaction within the Q2 pocket of HsPDE5 is obtained with a piperonyl group or a *p*-methoxyphenyl group[6] and that a hindered group led to a loss of activity. It has also been shown that oxygen atoms in para- or meta-position are important for HsPDE5 inhibition. We selected several analogues of the piperonyl group, such as benzoxadiazole or benzothiadiazole group, fluorinated derivatives, and also vanilloyl, isovanilloyl, dimethoxyphenyl, and ethylenedioxyphenyl groups. Tetrazolo[1,5-*a*]pyridine-6-carbaldehyde **19** was prepared beforehand, reacting 6-chloropyridine-3-carbaldehyde with sodium azide in DMF (Scheme 3).[7] Intermediate **19a** was also included in the set of aldehydes.

Table 3. HsPDE5 Inhibition of Some Antiplasmodial Compounds[a]

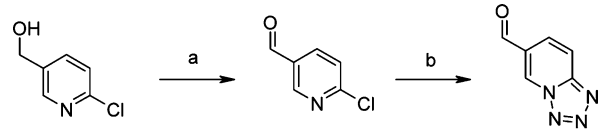


Cpd	R	HsPDE5 (IC_{50}nM)
2		5
12		*2
13		<1
14		*3
15		<1

[a]The asterisk (*) indicates data from Maw et al.[2]

In this series, we retained the benzylaminopyrrolidine side chain required at the N2 position for antiplasmodial activity. In vitro results for inhibitory activity against *P. falciparum* and

Scheme 3. Synthesis of 6-Chloropyridine-3-carbaldehyde and Tetrazolo[1,5-*a*]pyridine-6-carbaldehyde[a]



[a]Conditions: (a) MnO_2, $CHCl_3$, room temp, 12h; (b) NaN_3, EtOH, 60 °C, 24 h.

cytotoxicity on MRC5 cells of the series are presented in Table 4.

We confirmed that the benzothiadiazol group is a suitable bioisoster of the methylendioxyphenyl group (compound **21**), as Anzali et al. proposed in 1998,[8,9] better than the benzoxadiazole group (**20**). Moreover, it makes this compound original, slightly less toxic on MRC5, and expectedly more stable. At first, it seemed that the SAR at that position is identical for antiplasmodial activity and HsPDE5 inhibition.[10−12] Indeed, replacement of the piperonyl group by 6-chloropyridine and tetrazolo[1,5-*a*]pyridine (**22**, **23**) led to a loss of activity. Also, the presence of 2 oxygens at the meta and para positions (**24**, **25**, and **26**) is favorable except when these oxygens are substituted by electron withdrawing groups (**27**, **29**) or when the methylenedioxy group is replaced by a bulkier ethylenedioxy group (**28**). However, compound **24**, which is as potent as the lead compound, retained our attention because the dimethoxyphenyl group is deleterious for the inhibition of HsPDE5.[2] The most potent derivatives of the series were tested on HsPDE5 (Table 5). This experiment confirmed that introduction of dimethoxyphenyl group in place of piperonyl

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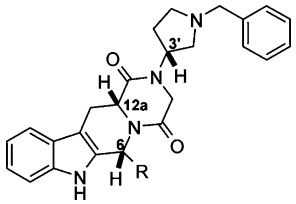
Table 4. In Vitro Antiplasmodial Activity and Cytotoxicity (Configuration 6*R*,12a*R*,3′*R*)



Cpd	R	antiplasmodial activity (IC_{50}μM)	Cytotoxicity (CC50μM)	ratio
2		0.50	10.00	20
20		6.00	>64.00	>10
21		0.60	30.00	50
22		11.00	14.00	1
23		7.00	21.00	3
24		0.42	32.00	76
25		0.61	22.00	36
26		0.52	23.00	44
27		11.00	8.00	1
28		5.00	>64.00	>13
29		11.00	>64.00	>6

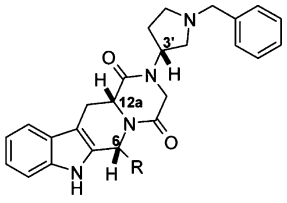
leads to a 1000-fold decrease on PDE5 inhibition. In all, these data also confirm that benzothiadiazol is a suitable bioisoster and that the Q2-pocket of human PDE5 does not tolerate a group larger than piperonyl. We thus synthesized a series of derivatives where the piperonyl group is replaced by a dimethoxyphenyl group in the C-6 position and compare the selectivity profile within the two series (Table 6).

We confirmed that the introduction of the dimethoxyphenyl group greatly reduces the activity on human PDE5 in all tested cases. We demonstrated that even slight differences in SAR can be exploited to remove the original undesired activity from the compound's profile. Noteworthy, the human to plasmodial cytotoxicity ratio is not correlated to the selectivity over PDE5. This suggests that another mechanism underlies the variable toxicity observed with our series on MRC5 cells. The phosphodiesterases inhibition profile has also been studied and confirms that the lead compound **24** does not inhibit significantly other phosphodiesterases (Table 7).

CONCLUSION

Compared to classical random screening on the whole parasite, the focused "drug-to-genome-to-drug" approach proved to be a work-efficient lead discovery process. It produced a novel and promising series of antimalarial compounds. One of the inherent weaknesses of the strategy is the issue of selectivity over HsPDE5, the original target of our series of compounds. A

Table 5. HsPDE5 Inhibition of the Most Potent Antiplasmodial Compounds



Cpd	R	HsPDE5 (IC_{50}nM)
2		5
21		28
24		5200
25		56
26		>1000

detailed analysis of structure−activity relationships published on tadalafil revealed that small modifications of the piperonyl moiety can be made that preserve antiplasmodial activity while almost abolishing the activity on HsPDE5. By use of this information, the selectivity index could be systematically inverted by replacing the piperonyl substituent by a 3,4-dimethoxyphenyl group. However, it needs to be further improved by increasing the antiplasmodial activity in the next round of optimization. Even if in vitro culture of the parasite in erythrocytes is known to be predictive of in vivo activity, we still need to formally prove the utility of our series in a rodent model of the disease. Characterization and optimization of ADME properties to achieve in vivo efficacy will be reported in due course.

EXPERIMENTAL SECTION

Biology. The standard screening methodologies were adopted as have been described by Cos et al.[13]

In Vitro *P. falciparum* Culture and Drug Assay. The chloroquine-susceptible *P. falciparum* GHA strain was used. Parasites were cultured in human erythrocytes A+ at 37 °C under a low oxygen atmosphere (3% O_2, 4% CO_2, and 93% N_2) in a modular incubation chamber. The culture medium was RPMI-1640, supplemented with 10% human serum. An amount of 200 μL of infected human red blood cells suspension (1% parasitemia, 2% hematocrit) was added to each well of the plates with test compounds and incubated for 72 h. After incubation, test plates were frozen at −20 °C. Parasite multiplication was measured by the Malstat method. An amount of 100 μL of Malstat reagent was transferred to a new plate and mixed with 20 μL of the hemolysed parasite suspension for 15 min at room temperature. After addition of 20 μL of NBT/PES solution and 2 h of incubation in the dark, the absorbance was spectrophotometrically read at 655 nm (Biorad 3550 UV microplate reader). Percentage growth inhibition was calculated compared to the negative blanks. IC_{50} values are calculated from the duplicate determinations with relative difference below 25%.

Cytotoxicity Test upon MRC-5 Cells. MRC-5 SV2 cells, human fetal lung fibroblast, were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate, and 5% FCS at 37 °C and 5% CO_2. For the assay, 104 MRC-5 cells/well were seeded onto the test plates containing the prediluted compounds and

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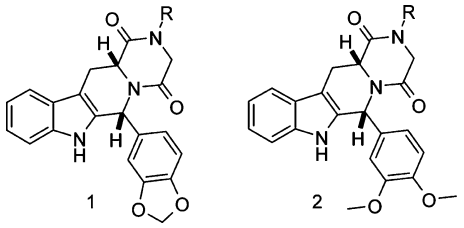
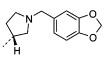
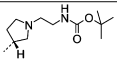
Table 6. Selectivity Profile of Dimethoxyphenyl Series over HsPDE5[a]



Compounds			Cell-based assays			Biochemical assays	
Cpd / Series		R	Antiplasmodial activity ($IC_{50}\mu M$)	Cytotoxicity ($CC_{50}\mu M$)	ratio	HsPDE5 IC_{50} (nM)	Selectivity index /PDE5
1 tadalafil	1		>64.00	>64.00	NC	5	NC
30	2		>64.00	>64.00	NC	>10000	NC
2	1		0.50	10.00	20	7	0.014
24	2		0.42	32.00	76	5200	12.4
9	1		0.53	39.00	73	7	0.013
31	2		*0.95	25.00	26	>10000	>10
13	1		0.80	40.00	50	2	0.003
32	2		*1.60	37.00	23	5600	3
15	1		0.22	34.00	154	4	0.018
33	2		*0.74	10.00	13	>10000	>13

[a]Antiplasmodial activities were tested on chloroquino-sensitive strains: PfGHA or PfK1. The asterisk (*) indicates that antiplasmodial activity was measured on the K1 strain.

Table 7. % Inhibition of Phosphodiesterases by Compound 24 at 10 μM of Control Values

PDE	% inhibition at 10 μM
PDE1B (h)	0
PDE2A (h)	0
PDE3A (h)	1
$PDE4A_{1A}$ (h)	1
PDE5 (h)	48
PDE6 (bovis)	18
PDE7A (h)	0
$PDE8A_1$ (h)	3
$PDE10A_1$ (h)	0
$PDE11A_4$ (h)	30

incubated at 37 °C and 5% CO_2 for 72 h. After 72 h of incubation, parasite growth was assessed fluorimetrically by adding resazurin[8] for 24 h at 37 °C. Fluorescence was measured using a GENios Tecan fluorimeter (excitation 530 nm, emission 590 nm). IC_{50} values are calculated from duplicate determinations with relative difference below 25%.

HsPDE5 Inhibition Assay: IC_{50} Measurement. Compounds were evaluated with a time-resolved fluorescence resonance energy transfer-based assay (HTRF technology) using the cGMP quantification kit (Cisbio International, No. 62GMPPEB) and bovine cGMP-specific PDE5 (Calbiochem, No. 524715). This quantitation method relies on the competition between free cGMP and a conjugate cGMP-fluorophore for the binding to a cGMP-specific antibody labeled with europium cryptate. Fixed amounts of bovine HSPDE5 (2 μL) and cGMP (150 nM) were incubated for 24 h at 37 °C in the presence of varying inhibitor concentrations. Theses assays were performed in black half-area 96-well microplates (Corning, No. 3694). Assay volume was 36 μL, and the assay buffer contained 50 mM Tris-HCl, pH 7.4, and 6 mM $MgCl_2$. At the end of the incubation, the detection reagents were added according to the manufacturer's protocol. After 1 h of incubation at room temperature in the dark, the HTRF signals were later read using a Victor3V (Wallac 1420 multilabel counter, Perkin-Elmer). Each inhibition assay was performed in duplicate and each well read twice. IC_{50} values were obtained using the curve fitting software XLfit4.2 (IDBS).

PDE Inhibition: % Inhibition. Compounds were evaluated on human recombinant (Sf9 cells) PDE1B, $PDE2A_1$, PDE3A, $PDE4A_{1A}$, PDE7A, $PDE8A_1$, $PDE10A_1$, and $PDE11A_4$ with HTRF technology after 30 min of incubation at room temperature using a cGMP or cAMP quantification kit.[14−19] Compounds were evaluated on human PDE5 isolated from platelets with scintillation counting of [^{3}H]5′GMP as described by Weishaar et al. in 1986, after 60 min of incubation at room temperature.[20] Compounds were evaluated on bovin PDE6 isolated from bovine retina with scintillation counting of [^{3}H]5′GMP as described by Ballard et al. in 1998, after 60 min of incubation at room temperature.[21] The results are expressed as a percent inhibition of control specific activity (100 − ((measured specific activity/control specific activity) × 100)) obtained in the presence of the test compound(s) and are the mean of duplicated experiments.

Chemistry. NMR spectra were recorded on a Bruker Avance 300 or Avance 500 spectrometer. Chemical shifts are in parts per million (ppm). The assignments were made using one-dimensional (1D) ^{1}H and ^{13}C spectra (classical or Jmod) and two-dimensional (2D) HSQC, HMBC, ROESY, and COSY spectra. Mass spectra were recorded with a LCMS−MS triple-quadrupole system (Varian 1200ws). HPLC analyses were performed using a C18 TSK-GEL Super ODS 2 μm particle size column (50 mm × 4.6 mm). HPLC gradient started from 100% H_2O/0.1% formic acid, reaching 20% H_2O/80% CH_3CN/0.08% formic acid within 10 min at a flow rate of 1 mL/min. All derivatives were isolated with purity higher than 95% (HPLC). Melting points

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were determined on a Büchi B-540 apparatus and were not corrected. All commercial reagents and solvents were used without further purification. Organic layers obtained after extraction of aqueous solutions were dried over $MgSO_4$ and filtered before evaporation in vacuo. Purification yields were not optimized. Thick layer chromatography was performed with silica gel 60 (Merck, 40-63 μm).

The syntheses of compounds **2**, **3**, and **18** are described in ref 1.

Protocol "a": Reductive Amination. The intermediate **12** (1 equiv) and the aldehyde (1.5 equiv) were dissolved in a mixture of dichloromethane/acetic acid, 10/1 (0.1 M). An amount of 2 equiv of (polystyrylmethyl)trimethylammonium cyanoborohydride resin was suspended, and the reaction mixture was stirred at room temperature for 12−24 h. The precipitate was dissolved with methanol. The resin was filtered, and the solution was evaporated to dryness. The reaction mixture was purified by chromatography (dichloromethane/methanol).

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-((*R*)-1-butylpyrrolidin-3-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-dione (5). **5** was prepared according to protocol "a", starting from intermediate **4** (45 mg) and butyraldehyde (1.5 equiv) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (20 mg, 39%). LC: t_R = 4.6 min. MS (ESI+): m/z = 501 $[M + H]^+$. ^{1}H NMR (CD_2Cl_2): δ 8.13 (s, 1H), 7.62 (dd, J = 6.9 Hz and J = 2.1 Hz, 1H), 7.33 (dd, J = 6.6 Hz and J = 1.8 Hz, 1H), 7.15 (m, 2H), 6.87 (dd, J = 8.1 Hz and J = 1.8 Hz, 1H), 7.78 (d, J = 1.5 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.21 (s, 1H), 5.90 (dd, J = 7.5 Hz and J = 1.2 Hz, 2H), 5.13 (m, 1H), 4.28−4.34 (m, 2H), 4.03 (dd, J = 17.4 Hz and J = 1.2 Hz, 1H), 3.70 (m, 1H), 3.21 (ddd, J = 16.2 Hz, J = 11.4 Hz and J = 1.2 Hz, 1H), 3.05 (m, 1H), 2.89 (dd, J = 10.8 Hz and J = 3.6 Hz, 1H), 2.48−2.62 (m, 3H), 2.25−2.39 (m, 2H), 1.77 (m, 1H), 1.47−1.54 (qt, J = 8.1 Hz, 2H), 1.38 (sxt, J = 7.8 Hz, 2H), 0.94 (t, J = 7.2 Hz, 3H). ^{13}C NMR (CD_2Cl_2): δ 21.68, 24.38, 30.20, 31.37, 47.12, 53.03, 56.42, 57.19, 58.28, 102.38, 103.09, 106.37, 107.44, 108.23, 109.05, 112.16, 119.56, 120.94, 121.42, 121.82, 123.33, 125.43, 125.51, 134.19, 137.08, 137.58, 153.73, 167.62, 169.06.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-(5-hydroxypentyl)pyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (6). **6** was prepared according to protocol "a" starting from intermediate **4** (45 mg) and 5-hydroxypentanal (1.2 equiv) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (32 mg, 60%). LC: t_R = 3.9 min. MS (ESI+): m/z = 531 $[M + H]^+$. ^{1}H NMR (MeOD): δ 7.53 (d, J = 6.9 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.07 (m, 2H), 6.79 (m, 2H), 6.69 (d, J = 8.4 Hz, 1H), 6.24 (s, 1H), 5.85 (s, 2H), 4.39 (dd, J = 11.4 Hz and J = 4.8 Hz, 1H), 4.2 (s, 2H), 3.65−3.55 (m, 3H), 3.14 (m, 3H), 2.82−2.57 (m, 5H), 2.30 (m, 1H), 1.85 (m, 1H), 1.59 (m, 5H), 1.45 (m, 3H). ^{13}C NMR (MeOD): δ 24.11, 28.07, 29.15, 32.77, 48.25, 49.25, 53.50, 54.15, 54.26, 56.10, 56.45, 56.92, 57.02, 62.19, 102.00, 105.50, 107.76, 108.43, 111.76, 117.80, 118.40, 119.80, 120.71, 122.26, 127.00, 136.50, 137.50, 168.88, 169.00.

Protocol "b": Cyclization under Classical Heating. Chloroacetyl intermediate (1 equiv) and amine (2 equiv) are refluxed in methanol or ethanol (0.075 M) for 12−24 h. Methanol was removed by evaporation. The residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, evaporated to dryness, and purified by chromatography.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-((*R*)-1-phenethylpyrrolidin-3-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (7). Compound **7** was prepared according to protocol "b" starting from intermediate **18** (100 mg), amine **7b** (1.1 equiv), and triethylamine (2 equiv) in methanol (22 h) and obtained after purification by HPLC as a white powder (54 mg, 46%). LC: t_R = 5.18 min. MS (ESI+): m/z = 549 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.12 (s, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.33−7.15 (m, 5H), 7.09−6.98 (m, 2H), 6.80 (m, 2H), 6.70 (dd, J = 1.7 Hz and J = 9 Hz, 2H), 6.18 (s, 1H), 5.92 (s, 2H), 5.03 (brs, 1H), 4.41 (brm, 1H), 4.10 (dd, J = 11.6 Hz and J = 4.6 Hz, 2H), 3.16 (m, 1H), 2.95 (m, 2H), 2.84 (brm, 1H), 2.75−2.60 (m, 3H), 2.38 (brm, 1H), 2.27−2.14 (m, 2H), 1.46 (brm, 1H), 1.23 (brm, 1H). ^{13}C NMR (DMSO-d_6): δ 22.65, 29.90, 34.86, 45.93, 51.47, 53.51, 55.05, 55.65, 56.43, 57.33, 101.46, 104.98, 107.09, 108.65, 111.84, 118.64, 119.36, 121.77, 126.31, 126.34, 128.69, 129.05, 134.25, 136. 54, 137.17, 140.84, 146.62, 147.64, 167.31, 168.30.

((*R*)-1-Phenethylpyrrolidin-3-yl)carbamic Acid *tert*-Butyl Ester (7a). (*R*)-(+)-3-(Boc-amino)pyrrolidine (200 mg) and Cs_2CO_3 (1.5 equiv) were suspended in anhydrous DMF (10 mL). An amount of 162 μL g of (2-bromoethyl)benzene (1.1 equiv) was slowly added, and the mixture was stirred at 100 °C for 20 h. The mixture was diluted with water and extracted with diethyl ether and evaporated to dryness. **39a** was obtained after flash chromatography (DCM/MeOH, 95/5) as a clear oil (110 mg, 35%). LC: t_R = 4.03 min. MS (ESI+): m/z = 291 $[M + H]^+$

((*R*)-1-Phenethylpyrrolidin-3-yl)carbamic Acid *tert*-Butyl Ester, Dihydrochloride (7b). **7a** (100 mg) was dissolved in dichloromethane. HCl gas was bubbled for a few minutes, and then the mixture was stirred for 2 h. Solvent was removed by evaporation to give 65 mg of oil (100%). LC: t_R = 1.65 min. MS (ESI+): m/z = 191 $[M + H]^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-(2-phenoxyethyl)-pyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (8). Compound **8** was prepared according to protocol "b" starting from intermediate **18** (100 mg), amine **8b** (1.1 equiv), and triethylamine (2 equiv) in methanol (22 h) and obtained after purification by HPLC as a white powder (54 mg, 46%). LC: t_R = 5.16 min. MS (ESI+): m/z = 565 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.12 (s, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.32−7.23 (m, 3H), 7.08−6.88 (m, 5H), 6.82−6.72 (m, 3H), 6.18 (s, 1H), 5.92 (s, 2H), 5.03 (brs, 1H), 4.41 (brm, 1H), 4.18 (s, 2H), 4.05 (t, 2H), 3.07−2.80 (m, 4H), 2.66 (brm, 1H), 2.22 (brm, 2H), 1.49 (brm, 1H), 1.25 (brm, 1H). ^{13}C NMR (DMSO-d_6): 22.70, 30.12, 49.05, 51.56, 53.83, 54.07, 55.03, 55.69, 56.71, 66.83, 101.50, 104.91, 107.13, 108.62, 111.74, 114.91, 118.57, 119.40, 120.96, 121.80, 126.17, 129.94, 134.25, 136.53, 137.19, 146.65, 147.61, 158.93, 167.31, 168.33.

[(*R*)-1-(2-Phenoxyethyl)pyrrolidin-3-yl]carbamic Acid *tert*-Butyl Ester (8a). (*R*)-(+)-3-(Boc-amino)pyrrolidine (500 mg) and Cs_2CO_3 (1.5 equiv) were suspended in anhydrous DMF (10 mL). An amount of 0.59 g of β-bromophenetole (1.1 equiv) was slowly added, and the mixture was stirred at 100 °C for 20 h. The mixture was diluted with water and extracted with diethyl ether and evaporated to dryness. **40a** was obtained after flash chromatography (DCM/MeOH 95/5) as a clear oil (380 mg, 46%). LC: t_R = 4.09 min. MS (ESI+): m/z = 307 $[M + H]^+$.

(*R*)-1-(2-Phenoxyethyl)pyrrolidin-3-ylamine, Dihydrochloride (8b). **8a** (100 mg) was dissolved in dichloromethane. HCl gas was bubbled for a few minutes, and then the mixture was stirred for 2 h. Solvent was removed by evaporation to give 67 mg of oil (100%). LC: t_R = 1.70 min. MS (ESI+): m/z = 207 $[M + H]^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-((*R*)-1-benzo[1,3]dioxol-5-ylmethylpyrrolidin-3-yl)-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (9). **9** was prepared according to protocol "a" starting from intermediate **4** (45 mg) and piperonal (1.2 equiv) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (18 mg, 31%). LC: t_R = 4.8 min. MS (ESI+): m/z = 579 $[M + H]^+$. ^{1}H NMR (MeOD): δ 5.53 (d, J = 6.9 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.06 (m, 2H), 6.90 (d, J = 1.2 Hz, 1H), 6.74−6.84 (m, 4H), 6.68 (m, 1H), 6.25 (s, 1H), 5.92 (s, 2H), 5.85 (s, 2H), 5.05 (m, 1H), 4.37 (dd, J = 11.7 Hz and J = 4.2 Hz, 1H), 4.31 (d, J = 16.8 Hz, 1H), 4.14 (d, J = 15.9 Hz, 1H), 3.69 (d, J = 12.6 Hz, 1H), 3.51−3.66 (m, 3H), 3.00−3.17 (m, 2H), 2.83 (dd, J = 10.8 Hz and J = 3.3 Hz, 1H), 2.58 (dd, J = 10.8 Hz and J = 7.8 Hz, 1H), 2.27−2.40 (m, 2H), 1.70−1.76 (m, 1H). ^{13}C NMR (MeOD): δ 22.41, 28.85, 45.99, 52.32, 52.82, 55.47, 55.86, 55.97, 59.00, 100.93, 100.99, 104.61, 106.80, 107.56, 108.80, 110.84, 117.50, 118.88, 119.70, 121.34, 121.88, 125.92, 133.17, 135.90, 136.83, 146.88, 147.04, 147.73, 147.86, 167.85, 168.70.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-(4-methoxybenzyl)pyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (10). **10** was prepared according to protocol "a" starting from intermediate **4** (50 mg) and para-anisaldehyde (2.0 equiv) and

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obtained after purification by TLC (dichloromethane/methanol) as a white powder (20 mg, 31%). LC: t_R = 5.0 min. MS (ESI+): m/z = 565 $[M + H]^+$. ^1H NMR (CD_2Cl_2): δ 8.06 (s, 1H), 7.55 (d, J = 6.8 Hz, 1H), 7.21−7.32 (m, 3H), 7.11 (m, 2H), 6.83 (m, 3H), 6.72 (d, J = 1.7 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.16 (s, 1H), 5.85 (d, J = 1.2 Hz, 1H), 5.83 (d, J = 1.2 Hz, 1H), 5.12 (m, 1H), 4.38 (d, J = 9.8 Hz, 1H), 4.21 (dd, J = 11.5 Hz and J = 4.3 Hz, 1H), 3.93 (d, J = 17.6 Hz, 1H), 3.74 (s, 3H), 3.57−3.66 (m, 2H), 3.44 (d, J = 12.7 Hz, 1H), 3.14 (dd, J = 16.0 Hz and J = 11.5 Hz, 1H), 2.94 (m, 1H), 2.70 (d, J = 9.7 Hz, 1H), 2.41 (m, 1H), 2.25 (m, 2H), 1.67 (m, 1H). ^{13}C NMR (CD_2Cl_2): δ 24.34, 30.56, 34.44, 46.94, 53.25, 54.85, 56.22, 57.12, 57.25, 57.30, 97.20, 102.38, 107.46, 108.23, 109.03, 112.14, 114.65, 119.55, 120.94, 121.40, 123.32, 127.50, 130.79, 132.25, 149.00, 149.50, 134.18, 137.07, 137.58, 154.00, 161.00, 167.50, 186.56.

(6R,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-(4-dimethylaminobenzyl)pyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (11). **11** was prepared according to protocol "a" starting from intermediate **4** (45 mg) and *p*-dimethylaminobenzaldehyde (1.5 equiv) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (16 mg, 27%). LC: t_R = 5.14 min. MS (ESI+): m/z = 578 $[M + H]^+$. ^1H NMR (MeOD): δ 7.52 (d, J = 6.9 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H), 7.20 (d, J = 8.7 Hz, 1H), 7.06 (m, 2H), 6.66−6.79 (m, 5H), 6.24 (s, 1H), 5.85 (s, 2H), 5.04 (brs, 1H), 4.34 (dd, J = 11.7 Hz and J = 5.4 Hz, 1H), 4.31 (d, J = 17.1 Hz, 1H), 4.09 (dd, J = 17.1 Hz and J = 1.2 Hz, 1H), 3.66 (d, J = 12.3 Hz, 1H), 3.58 (dd, J = 15.6 Hz and J = 4.8 Hz, 1H), 3.46 (d, J = 12.6 Hz, 1H), 3.12 (dd, J = 15.9 Hz and J = 11.7 Hz, 1H), 2.97 (m, 1H), 2.90 (s, 6H), 2.75 (dd, J = 10.8 Hz and J = 4.2 Hz, 1H), 2.54 (dd, J = 10.5 Hz and J = 7.8 Hz, 1H), 2.25−2.38 (m, 2H), 1.19−1.72 (m, 1H). ^{13}C NMR (MeOD): δ 24.50, 30.50, 41.50, 47.02, 52.77, 54.50, 57.12, 57.22, 60.05, 102.37, 107.48, 108.22, 109.03, 112.14, 113.41, 119.55, 120.93, 121.39, 123.31, 127.32, 130.47, 134.20, 137.11, 137.58, 148.94, 151.06, 167.46, 169.19.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-((*R*)-1-pyridin-2-ylmethylpyrrolidin-3-yl)-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (12). **12** was prepared according to protocol "a" starting from intermediate **4** (45 mg) and pyridine-2-carboxaldehyde (1.5 equiv) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (27 mg, 50%). LC: t_R =4.58 min. MS (ESI+): m/z = 536 $[M + H]^+$. ^1H NMR (MeOD): δ 8.53 (d, J = 4.8 Hz, 1H), 7.86 (td, J = 7.8 Hz and J = 1.8 Hz, 1H), 7.55 (m, 2H), 7.36 (dd, J = 6.6 Hz and J = 5.1 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.06 (m, 2H), 6.78−6.81 (m, 2H), 6.68 (d, J = 8.7 Hz, 1H), 6.25 (s, 1H), 5.85 (s, 2H), 5.01 (brs, 1H), 4.39 (dd, J = 11.7 Hz and J = 5.1 Hz, 1H), 4.3 (d, J = 17.1 Hz, 1H), 4.19 (d, J = 17.7 Hz, 1H), 3.92−4.03 (m, 2H), 3.61 (dd, J = 15.6 Hz and J = 4.8 Hz, 1H), 3.07−3.25 (m, 3H), 2.78−2.84 (m, 1H), 2.54−2.62 (m, 1H), 2.32−2.43 (m, 1H), 1.89−1.84 (m, 1H). ^{13}C NMR (MeOD): δ 24.37, 30.09, 44.55, 53.55, 54.14, 56.93, 57.21, 57.31, 102.38, 105.21, 107.31, 108.24, 109.04, 110.38, 112.91, 119.52, 120.92, 121.44, 123.31, 123.65, 124.33, 125.46, 127.30, 134.21, 137.02, 137.60, 137.83, 149.66, 150.14, 167.76, 168.76

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-(1-methyl-1*H*-imidazol-2-ylmethyl)pyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (13). **13** was prepared according to protocol "a" starting from intermediate **4** (45 mg) and 1-methyl-2-imidazolecarboxaldehyde (1.5 equiv) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (26 mg, 48%). LC: t_R =4.2 min. MS (ESI +): m/z = 539 $[M + H]^+$. ^1H NMR (CD_2Cl_2): δ 8.38 (s, 1H), 7.55 (d, J = 6.9 Hz, 1H), 7.25 (d, J = 7.5 Hz, 1H), 7.05−7.14 (m, 2H), 6.90 (d, J = 4.0 Hz, 2H), 6.79 (dd, J = 8.0 Hz and J = 1.7 Hz, 2H), 6.71 (d, J = 1.7 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 6.14 (s, 1H), 5.85 (d, J = 7.2 Hz, 1H), 5.82 (d, J = 1.2 Hz, 1H), 5.15 (brs, 1H), 4.18 (m, 2H), 3.86 (d, J = 17.1 Hz, 1H), 3.64 (m, 5H), 3.14 (dd, J = 15.6 Hz and J = 5.1 Hz, 1H), 2.83 (t, J = 7.76 Hz, 1H), 2.67−2.22 (m, 3H), 2.00 (s, 1H), 1.60 (m, 1H). ^{13}C NMR (CD_2Cl_2): δ 24.35, 30.59, 34.00, 46.67, 52.62, 52.90, 54.14, 57.14, 57.32, 57.60, 102.37, 107.34, 108.24, 109.02, 112.17, 119.54, 120.89, 121.39, 122.71, 123.28, 126.87, 127.28, 127.77, 134.17,137.09, 127.77, 146.50, 148.90, 167.42, 168.92.

2-[(*R*)-3-((6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-1,4-dioxo-3,4,6,7,12,12a-hexahydro-1*H*-pyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indol-2-yl)pyrrolidin-1-yl]-*N*,*N*-dimethylacetamide (14). The intermediate **4** (50 mg), *N*,*N*′-dimethylchloroacetamide (1 equiv), and triethylamine (1.1 equiv) were dissolved in dioxane (1 mL) and stirred at room temperature for 6 days. The reaction mixture was then heated to 70 °C for 15 h. The dioxane was removed by evaporation. The residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and evaporated to dryness, Purification by TLC (dichloromethane/methanol) gave 26 mg of a white powder (44%). LC: t_R = 4.0 min. MS (ESI+): m/z = 530 $[M + H]^+$. ^1H NMR (MeOD): δ 7.54 (d, J = 7.2 Hz, 1H), 7.29 (d, J = 7.2 Hz, 1H), 7.01−7.12 (m, 2H), 6.67−6.81 (m, 3H), 6.26 (s, 1H), 5.86 (s, 2H), 5.14 (m, 1H), 4.40 (dd, J = 11.4 Hz and J = 4.8 Hz, 1H), 4.32 (d, J = 17.1 Hz, 1H), 4.18 (d, J = 16.5 Hz, 1H), 3.61 (dd, J = 15.9 Hz and J = 5.1 Hz, 1H), 3.45 (d, J = 14.4 Hz, 1H), 3.30 (m, 1H), 2.98−3.18 (m, 3H), 3.12 (s, 3H), 2.94 (s, 3H), 2.55 (dd, J = 10.5 Hz and J = 7.8 Hz, 1H), 2.23−2.41 (m, 2H), 1.70 (m, 1H). ^{13}C NMR (MeOD): δ 24.35, 30.64, 36.19, 37.80, 46.80, 52.92, 54.36, 57.13, 57.24, 57.57, 58.30, 102.38, 103.86, 107.37, 108.25, 109.03, 112.19, 119.38, 120.88, 121.34, 123.25, 125.60, 126.52, 127.32, 134.29, 137.22, 137.62, 167.52, 169.17.

2-[(*R*)-3-((6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-1,4-dioxo-3,4,6,7,12,12a-hexahydro-1*H*-pyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indol-2-yl)pyrrolidin-1-yl]ethylcarbamic Acid *tert*-Butyl Ester (15). The intermediate **4** (50 mg), 2-(Boc-amino)ethyl bromide (25 mg, 1 equiv), and triethylamine (1.1 equiv) were dissolved in dioxane (1 mL) and stirred at room temperature. After 46 h, 0.5 equiv of 2-(Boc-amino)ethyl bromide, 0.5 equiv of TEA, and 0.5 mL of dioxane were added. After 96 h, the reaction mixture was heated to 70 °C for 20 h. The dioxane was removed by evaporation. The residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and evaporated to dryness, It was purified by TLC (dichloromethane/methanol) to give 29 mg of a white powder (44%). LC: t_R = 5.20 min. MS (ESI+): m/z = 604 $[M + H]^+$. ^1H NMR (MeOD): δ 7.53 (d, J = 7.2 Hz, 1H), 7.29 (d, J = 7.2 Hz, 1H), 7.07 (m, 2H), 6.68−6.80 (m, 3H), 6.27 (s, 1H), 5.86 (s, 2H), 5.10 (m, 1H), 4.41 (dd, J = 11.4 Hz and J = 4.5 Hz, 1H), 4.31 (d, J = 17.4 Hz, 1H), 4.18 (d, J = 17.1 Hz, 1H), 3.61 (dd, J = 15.6 Hz and J = 4.8 Hz, 1H), 2.87−3.23 (m, 5H), 2.50−2.63 (m, 3H), 2.23−2.32 (m, 2H), 1.65−1.71 (m, 1H), 1.44 (s, 9H). ^{13}C NMR (MeOD): 23.44, 28.37, 29.79, 39.50, 46.73, 53.22, 54.01, 55.72, 56.80, 56.90, 56.96, 63.02, 102.00, 105.66, 107.82, 108.56, 111.88, 118.54, 119.92, 120.69, 122.37, 126.95, 134.21, 137.05, 138.05, 147.75, 168.86, 169.70.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-((*R*)-1-cyclopentylmethylpyrrolidin-3-yl)-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (16). Compound **16** was prepared according to protocol "b" starting from intermediate **18** (100 mg), amine **16b** (2 equiv), and triethylamine (6 equiv) in methanol (32 h) and obtained after purification by HPLC as a white powder (15 mg, 12%). LC: t_R = 4.85 min. MS (ESI+): m/z = 527 $[M + H]^+$. ^1H NMR (CD_2Cl_2): δ 8.65 (s, 1H), 7.59 (dd, J = 7.0 Hz and J = 1.7 Hz, 1H), 7.32 (dd, J = 6.4 Hz an J = 1.86 Hz, 1H), 7.14 (m, 2H), 6.84 (dd, J = 8.0 Hz and J = 1.7 Hz, 1H), 6.78 (d, J = 1.7 Hz, 1H), 6.69 (d, J = 8.0 Hz, 1H), 6.25 (s, 1H), 5.88 (d, J = 1.3 Hz, 1H), 5.86 (d, J = 1.3 Hz, 1H), 5.10 (m, 1H), 4.36 (d, J = 17.1 Hz, 1H), 4.28 (dd, J = 11.7 Hz and J = 4.8 Hz, 1H), 4.03 (d, J = 17.5 Hz, 1H), 3.68 (dd, J = 15.9 Hz and J = 4.8 Hz, 1H), 3.19 (ddd, J = 15.9 Hz, J = 11.5 Hz and J = 0.9 Hz, 1H), 3.10 (dd, J = 8.7 Hz and J = 6.1 Hz, 1H), 2.95 (dd, J = 10.7 Hz and J = 3.2 Hz, 1H), 2.58 (dd, J = 11.1 Hz and J = 8.0 Hz, 1H), 2.46 (m, 2H), 2.31 (m, 2H), 2.07 (hept, J = 7.6 Hz, 1H), 1.8 (m, 3H), 1.6 (m, 4H), 1.25 (m, 2H). ^{13}C NMR (CD_2Cl_2): δ 23.27, 25.23, 25.27, 29.21, 31.24, 31.26, 38.45, 46.37, 52.24, 56.11, 56.22, 56.25, 61.18, 101.36, 106.20, 107.23, 107.02, 111.25, 118.48, 119.82, 120.26, 122.20, 126.26, 133.33, 136.02, 136.60, 147.05, 147.89, 166.71, 168.09.

((*R*)-1-Cyclopentylmethylpyrrolidin-3-yl)carbamic Acid *tert*-Butyl Ester (16a). (*R*)-(+)-3-(Boc-amino)pyrrolidine (300 mg), cyclopentanecarbaldehyde (1.1 equiv), and acetic acid (5 mol %) were dissolved in DCM (10 mL). $NaBH_3CN$ (1.1 equiv) was added, and the reaction mixture was stirred at room temperature over a weekend. The reaction mixture was diluted with EtOAc. The product was

extracted in aqueous HCl (pH 1), then basified to pH 9 to be extracted in dichloromethane. DCM was evaporated to dryness. **16a** was obtained as a clear oil (347 mg, 80%). LC: t_R = 4.09 min. MS (ESI +): m/z = 269 $[M + H]^+$.

(*R*)-1-Cyclopentylmethylpyrrolidin-3-ylamine, Trifluoroacetate Salt (16b). **16a** (434 mg) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (3 mL) was added, and then the mixture was stirred for 2 h. Solvent was removed by evaporation to give 642 mg of oil (95%). LC: t_R = 1.75 min. MS (ESI+): m/z = 169 $[M + H]^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-((*R*)-1-cyclohexylmethylpyrrolidin-3-yl)-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (17). Compound **17** was prepared according to protocol "b" starting from intermediate **18** (100 mg), amine **17b** (2 equiv), and triethylamine (6 equiv) in methanol (32 h) and obtained after purification by HPLC as a white powder (62 mg, 49%). LC: t_R = 5.03 min. MS (ESI+): m/z = 541 $[M + H]^+$. ^{1}H NMR (CD_2Cl_2): δ 8.81 (s, 1H), 7.59 (dd, J = 6.9 Hz and J = 1.6 Hz, 1H), 7.32 (dd, J = 6.5 Hz an J = 1.3 Hz, 1H), 7.14 (m, 2H), 6.84 (dd, J = 8.0 Hz and J = 1.7 Hz, 1H), 6.78 (d, J = 1.6 Hz, 1H), 6.69 (d, J = 8.0 Hz, 1H), 6.23 (s, 1H), 5.88 (d, J = 1.3 Hz, 1H), 5.86 (d, J = 1.3 Hz, 1H), 5.03 (m, 1H), 4.26 (m, 4H), 4.03 (d, J = 17.3 Hz, 1H), 3.68 (dd, J = 15.9 Hz and J = 4.8 Hz, 1H), 3.19 (m, 2H), 3.03 (dd, J = 10.8 Hz and J = 4.2 Hz, 1H), 2.78 (dd, J = 10.7 Hz and J = 8.1 Hz, 1H), 2.47 (m, 3H), 2.27 (m, 1H), 1.78 (m, 6H), 1.55 (m, 1H), 1.23 (m, 3H), 0.94 (m, 2H). ^{13}C NMR (CD_2Cl_2): δ 23.25, 25.89, 26.46, 28.59, 31.47, 31.50, 36.05, 46.88, 52.63, 55.82, 56.13, 56.20, 62.44, 101.30, 106.00, 107.16, 107.96, 111.22, 118.39, 119.73, 120.24, 122.12, 126.17, 133.28, 135.95, 136.56, 146.97, 147.81, 166.90, 167.74.

((*R*)-1-Cyclohexylmethylpyrrolidin-3-yl)carbamic Acid *tert*-Butyl Ester (17a). (*R*)-(+)-3-(Boc-amino)pyrrolidine (300 mg), cyclohexanecarbaldehyde (1.1 equiv), and acetic acid (5 mol %) were dissolved in DCM (10 mL). $NaBH_3CN$ (1.1 equiv) was added, and the reaction mixture was stirred at room temperture over a weekend. The reaction mixture was diluted with EtOAc. The product was extracted in aqueous HCl (pH 1), then basified to pH 9 to be extracted in dichloromethane. DCM was evaporated to dryness. **17a** was obtained as a clear oil (365 mg, 80%). LC: t_R = 4.20 min. MS (ESI +): m/z = 283$[M + H]^+$.

(*R*)-1-Cyclohexylmethylpyrrolidin-3-ylamine, Trifluoroacetate Salt (17b). **17a** (457 mg) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (3 mL) was added, and then the mixture was stirred for 2 h. Solvent was removed by evaporation to give 664 mg of oil (100%). LC: t_R = 1.75 min. MS (ESI+): m/z = 183 $[M + H]^+$.

Tetrazolo[1,5-*a*]pyridine-6-carbaldehyde (19). To a solution of **19a** (800 mg, 5.67 mmol) in ethanol (25 mL) was added NaN_3 (738 mg, 11.34 mmol). After the mixture was stirred at reflux overnight, the solvent was removed under reduced pressure. The product was purified by TLC (dichloromethane/methanol, 96:4) to provide **19** as a white solid (260 mg, 31%). LC: t_R = 2.58 min. MS (ESI+): m/z = 149 $[M + H]^+$. ^{1}H NMR (MeOD): δ 9.12 (d, J=1.2 Hz, 1 H), 8.07 (dd, J=9.3 Hz and J=0.9 Hz, 1H), 7.93 (dd, J=9.3 Hz and J=1.5 Hz, 1 H).

6-Chloropyridine-3-carbaldehyde (19a). To a solution of 2-chloro-5-hydroxymethylpyridine (1 g, 6.96 mmol) in chloroform (70 mL) was added manganese dioxide (1.8 g, 20.9 mmol). After being stirred at room temperature overnight, the reaction mixture was filtered on Celite and solvent evaporated under reduced pressure. **19a** was isolated as a white solid (870 mg, 88%). LC: t_R = 3.02 min. MS (ESI+): m/z = 142 $[M + H]^+$.

Protocol "c": Pictet–Spengler Reaction. D-Tryptophan-OMe·HCl or L-tryptophan-OMe·HCl (1 equiv) and piperonal (1 equiv) were dissolved in dry dichloromethane (0.05 M) containing activated molecular sieves (4 Å). The solution was cooled to 0 °C. An amount of 3 equiv of trifluoroacetic acid in dichloromethane was added dropwise, and the mixture was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was filtered and partially evaporated. The crude product was diluted with ethyl acetate and washed with aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The diastereoisomers were separated by column chromatography.

Protocol "d": Acylation. Intermediates tetrahydro-β-carboline 18 (1 equiv) was suspended in chloroform (0.2 M) with triethylamine. The reaction mixture was cooled at −10 °C, and an amount of 2.4 equiv of chloroacetyl chloride in chloroform (0.5 M) was added dropwise. The reaction mixture was stirred at −10 °C until completion and quenched with water. The organic layer was washed with an aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated to dryness.

(6*R*,12a*R*)-6-Benzo[1.2.5]oxadiazol-5-yl-2-((*R*)-1-benzylpyrrolidin-3-yl)-2.3.6.7.12.12a-hexahydropyrazino[1′.2′:1.6]-pyrido[3.4-*b*]indole-1.4-dione (20). Compound **20** was prepared according to protocol "b" starting from intermediate **20b** (150 mg, 0.35 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (123 μL, 2 equiv) in methanol and obtained after purification by TLC (dichloromethane/methanol, 95:5) as a yellow powder (115 mg, 50%). LC: t_R = 5.05 min. MS (ESI+): m/z = 533 $[M + H]^+$. ^{1}H NMR (MeOD): δ 7.96 (s, 1H), 7.70 (d, J = 9.30 Hz, 1H), 7.53 (d, J = 7.20 Hz, 1H), 7.44−7.30 (m, 6H), 7.26 (d, J = 7.50 Hz, 1H), 7.09 (td, J = 7.50 and 1.2 Hz, 1H), 7.02 (td, J = 7.20 and 1.2 Hz, 1H), 6.70 (s, 1H), 4.90 (m, 1H), 4.30 (dd, J = 11.7 and 4.5 Hz, 1H), 4.25 (d, J = 17.4 Hz, 1H), 4.16 (d, J = 17.4 Hz, 1H), 3.94 (d, J = 12.6 Hz, 1H), 3.75 (d, J = 12.6 Hz, 1H), 3.61 (dd, J = 15.9 and 4.5 Hz, 1H), 3.27 (m, 1H), 3.19 (dd, J = 15.9 and 11.4 Hz, 1H), 3.12−3.01 (m, 1H), 2.96−2.80 (m, 1H), 2.70−2.67 (m, 1H), 2.44−2.32 (m, 1H), 2.02−1.90 (m, 1H). ^{13}C NMR (MeOD): δ 23.50, 28.08, 53.36, 55.36, 56.11, 57.17, 58.69, 106.20, 110.98, 112.81, 116.27, 117.77, 119.15, 121.92, 125.89, 127.97, 128.45, 128.99, 130.56, 131.12, 132.66, 136.95, 145.98, 148.62, 149.16, 167.20, 168.26.

(1*R*,3*R*)-1-Benzo[1,2,5]oxadiazol-5-yl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester, CF_3COOH (20a). **20a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (1378 mg) and 2,1,3-benzoxadiazole-5-carbaldehyde (800 mg). The reaction mixture was stirred for 17 h. After filtration and concentration, the cis diastereoisomer was precipitated in EtOAc to provide product **20a** as a light yellow solid (750 mg, 40%). LC: t_R = 4.39 min. MS (ESI+): m/z = 349 $[M + H]^+$. ^{1}H NMR (MeOD): δ 8.29 (s, 1H), 8.08 (d, J = 9.3 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 9.3 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.20 (t, J = 6.6 Hz, 1H), 7.13 (t, J = 6.9 Hz, 1H), 6.21, (s, 1H), 4.85 (m, 1H), 3.98 (s, 3H), 3.66 (dd, J = 15.9 Hz and J = 4.5 Hz, 1H), 3.42 (m, 1H). Mp = 232−233 °C.

(1*R*,3*R*)-1-Benzo[1.2.5]oxadiazol-5-yl-2-(2-chloroacetyl)-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (20b). **20b** was prepared according to protocol "d" starting from intermediate **20a** (150 mg, 1 equiv) and obtained as a yellow powder (159 mg, 87%) which was directly engaged in the cyclization step. LC: t_R = 6.4 min. MS (ESI+): m/z = 423 $[M - H]^-$.

(6*R*,12a*R*)-6-Benzo[1.2.5]thiadiazol-5-yl-2-((*R*)-1-benzylpyrrolidin-3-yl)-2.3.6.7.12.12a-hexahydropyrazino[1′.2′:1.6]-pyrido[3.4-*b*]indole-1.4-dione (21). Compound **21** was prepared according to protocol "b" starting from intermediate **21b** (185 mg, 0.42 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (145 μL, 2 equiv) in methanol and obtained after purification by TLC (dichloromethane/methanol, 95:5) as a yellow powder (120 mg, 44%). LC: t_R = 5.03 min. MS (ESI+): m/z = 549 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.2 (s, 1H),) 8.01 (s, 1H), 7.97 (d, J = 9.30 Hz, 1H), 7.66 (dd, J = 9.0 and 1.5 Hz, 1H), 7.56 (d, J = 7.20 Hz, 1H), 7.38−7.35 (m, 5H), 7.28 (d, J = 7.80 Hz, 1H), 7.05 (td, J = 7.50 and 0.9 Hz, 1H), 7.02 (td, J = 7.80 and 1.2 Hz, 1H), 6.39 (s, 1H), 5.07 (m, 1H), 4.52 (dd, J = 11.4 and 4.2 Hz, 1H), 4.17 (s, 2H), 3.55 (dd, J = 15.9 and 4.5 Hz, 2H), 3.13 (dd, J = 15.3 and 11.7 Hz, 2H), 3.12−3.01 (m, 1H), 2.96−2.80 (m, 1H), 2.75 (m, 1H), 2.23 (m, 2H), 2.02−1.90 (m, 1H). ^{1}H NMR (DMSO-d_6): 23.56, 51.54, 55.99, 56.24, 105.82, 111.83, 111.89, 118.17, 118.76, 119.46, 120.76, 121.90, 122.04, 126.12, 126.16, 128.93, 129.44, 130.87, 132.68, 132.83, 136.72, 136.88, 145.06, 154.06, 154.53, 154.72.

(1*R*,3*R*)-1-Benzo[1,2,5]thiadiazol-5-yl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester, CF_3COOH (21a). **21a** was prepared according to protocol "c" starting from D-

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tryptophan-OMe·HCl (1241 mg) and 2,1,3-benzothiadiazole-5-carbaldehyde (800 mg). The reaction mixture was stirred overnight. After filtration and concentration, the cis isomer was precipitated in EtOAc to provide the product **21a** as a yellow solid (1180 mg, 66%). LC: t_R = 3.91 min. MS (ESI+): m/z = 365 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 10.85 (s, 1H), 8.31 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.78 (dd, J = 9.3 and 1.5 Hz, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 7.14−7.04 (m, 2H), 6.21 (s, 1H), 4.85 (m, 1H),) 3.86 (s, 3H), 3.42 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 162.70, 155.26, 137.47, 131.67, 126.12, 124.96, 122.85, 121.97, 119.95, 118.99, 112.22, 107.24, 96.23, 58.18, 56.01, 53.71. Mp = 237−238 °C.

(1*R*,3*R*)-1-Benzo[1.2.5]thiadiazol-5-yl-2-(2-chloroacetyl)-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (21b). **21b** was prepared according to protocol "d" starting from intermediate **21a** (200 mg, 1 equiv) and obtained as a yellow powder (205 mg, 84%) which was directly engaged in the cyclization step. LC: t_R = 6.2 min. MS (ESI+): m/z = 439 $[M - H]^-$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(6-chloropyridin-3-yl)-2.3.6.7.12.12a-hexahydropyrazino[1′.2′:1.6]pyrido[3.4-*b*]-indole-1.4-dione (22). Compound **22** was prepared according to protocol "b" starting from intermediate **22b** (0.44 mmol), triethylamine (123 μL, 2 equiv), and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (152 μL, 2 equiv) in methanol and obtained after purification by TLC (dichloromethane/methanol, 95:5) as a white powder (100 mg, 43%). LC: t_R = 4.69 min. MS (ESI+): m/z = 526 $[M + H]^+$. ^{1}H NMR (MeOD): δ 844 (d, J = 2.4 Hz, 1H), 7.63 (dd, J = 2.4 and 8.4 Hz, 1H), 7.55 (d, J = 7.20 Hz, 1H), 7.37−7.22 (m, 7H), 7.10 (td, J = 7.50 and 1.2 Hz, 1H), 7.04 (td, J = 7.20 and 1.2 Hz, 1H), 6.27 (s, 1H), 5.12 (m, 1H), 4.40 (dd, J = 11.4 and 4.8 Hz, 1H), 4.32 (d, J = 17.7 Hz, 1H), 4.12 (dd, J = 17.4 and 1.5 Hz, 1H), 3.70 (d, J = 12.9 Hz, 1H), 3.67 (dd, J = 15.9 and 4.5 Hz, 1H), 3.54 (d, J = 12.6 Hz, 1H), 3.15 (dd, J = 15.9 and 11.4 Hz, 1H), 2.94 (m, 1H), 2.75 (dd, J = 10.5 and 3.3 Hz, 1H), 2.48 (dd, J = 10.5 and 7.8 Hz, 1H), 2.34−2.20 (m, 2H), 1.74−1.65 (m, 1H). ^{1}H NMR (MeOD): δ 23.20, 28.66, 45.24, 52.01, 52.86, 54.02, 55.59, 56.00, 59.48, 105.70, 110.93, 117.78, 119.12, 120.02, 121.82, 124.17, 125.88, 126.86, 128.01, 128.47, 131.49, 137.16, 137.54, 137.97, 138.45, 148.62, 149.51, 167.01, 168.75.

(1*R*,3*R*)-1-(6-Chloropyridin-3-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (22a). **22a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (1.02 g) and 6-chloropyridine-3-carboxaldehyde (708 mg), after stirring for 67 h and purification by column chromatography. **22a** was obtained as a white powder (937 mg, 69%). LC: t_R = 3.8 min. MS (ESI+): m/z = 342 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 10.45 (s, 1H), 8.42 (d, J = 2.1 Hz, 1H), 7.72 (dd, J = 8.3 Hz and J = 2.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 7.0 Hz, 1H), 7.13 (m, 1H), 6.98 (m, 2H), 5.3 (d, J = 5.5 Hz, 1H), 3.89 (m, 1H), 3.71 (s, 3H), 3.21 (t, J = 5.5 Hz, 1H), 3.05 (dd, J = 14.9 Hz and J = 3.1 Hz, 1H), 2.85 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 150.74, 150.14, 140.74, 137.74, 137.04, 127.02, 125.15, 124.76, 121.63, 119.22, 118.39, 113.34, 111.80, 107.89, 56.71, 55.17, 52.46, 25.72. Mp = 190−192 °C.

(1*R*,3*R*)-2-(2-Chloroacetyl)-1-(6-chloropyridin-3-yl)-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (22b). **22b** was prepared according to protocol "d" starting from intermediate **22a** (681 mg, 1 equiv) and obtained as a white powder (667 mg, 80%) which was directly engaged in the cyclization step. LC: t_R = 6.0 min. MS (ESI+): m/z = 420 $[M + H]^+$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-tetrazolo[1.5-*a*]-pyridin-6-yl-2.3.6.7.12.12a-hexahydropyrazino[1′.2′:1.6]-pyrido[3.4-*b*]indole-1.4-dione (23). Compound **23** was prepared according to protocol "b" starting from intermediate **23b** (0.46 mmol), triethylamine (129 μL, 2 equiv), and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (159 μL, 2 equiv) in methanol and obtained after purification by TLC (dichloromethane/methanol, 95:5) as a white powder (80 mg, 33%). LC: t_R = 4.25 min. MS (ESI+): m/z = 533 $[M + H]^+$. ^{1}H NMR (MeOD): δ 9.19 (s, 1H), 7.90 (dd, J = 0.9 and 9.3 Hz, 1H), 7.63 (dd, J = 1.5 and 9.3 Hz, 1H), 7.58 (d, J = 6.9 Hz, 1H), 7.35−7.23 (m, 6H), 7.10 (td, J = 7.80 and 1.5 Hz, 1H), 7.05 (td, J = 7.50 and 1.2 Hz, 1H), 6.40 (s, 1H), 5.13 (m, 1H), 4.44 (dd, J = 11.4 and 4.8 Hz, 1H), 4.33 (d, J = 17.7 Hz, 1H), 4.13 (dd, J = 17.7 and 1.5 Hz, 1H), 3.73 (dd, J = 12.3 and 4.2 Hz, 1H), 3.68 (d, J = 12.3 Hz, 1H), 3.52 (d, J = 12.9 Hz, 1H), 3.26 (dd, J = 16.2 and 11.7 Hz, 1H), 2.92 (m, 1H), 2.76 (dd, J = 10.5 and 3.0 Hz, 1H), 2.48 (dd, J = 10.5 and 7.5 Hz, 1H), 2.33−2.21 (m, 2H), 1.79−1.74 (m, 1H). ^{13}C NMR (MeOD): δ 23.40, 28.48, 45.27, 52.06, 52.83, 54.18, 55.58, 56.06, 59.48, 106.53, 110.97, 114.63, 117.93, 119.22, 119.99, 122.06, 123.31, 124.64, 125.87, 126.85, 128.00, 128.46, 130.49, 132.33, 132.40, 137.31, 138.45, 147.81, 166.79, 169.10.

(1*R*,3*R*)-1-Tetrazolo[1,5-*a*]pyridin-6-yl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester, CF_3COOH (23a). **23a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (430 mg) and aldehyde **19** (250 mg). The reaction mixture was stirred overnight. After filtration and concentration, the cis isomer **23a** was precipitated in methanol to provide a white solid (160 mg, 27%). LC: t_R = 3.97 min. MS (ESI+): m/z = 349 $[M + H]^+$. ^{1}H NMR (MeOD): δ 10.47 (s, 1H), 9.42 (s, 1H), 8.12 (d, J = 9.3 Hz, 1H), 7.65 (dd, J = 9.3 Hz and J = 1.5 Hz, 1H), 7.48 (d, J = 6.9 Hz, 1H), 7.15 (dd, J = 6.9 Hz, 1H), 7.02 (td, J = 7.2 and J = 1.2 Hz, 1H), 6.96 (td, J = 7.2 and J = 1.5 Hz, 1H), 5.45 (s, 1H), 3.96 (dd, J = 11.1 Hz and J = 4.2 Hz, 1H), 3.73 (s, 3H), 3.10−3.07 (m, 1H), 2.94−2.85 (m, 1H). Mp = 115−119 °C.

(1*R*,3*R*)-2-(2-Chloroacetyl)-1-tetrazolo[1.5-*a*]pyridin-6-yl-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (23b). **23b** was prepared according to protocol "d" starting from intermediate **23a** (160 mg, 1 equiv) and obtained as an oil (194 mg, 100%) which was directly engaged in the cyclization step. LC: t_R = 5.73 min. MS (ESI+): m/z = 425 $[M + H]^+$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (24). Compound **24** was prepared according to protocol "b" starting from diastereoisomers mixture **24b** (100 mg, 0.226 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (87 mg, 0.50 mmol) in methanol and obtained after separation by TLC (dichloromethane/methanol, 95:5) as a yellow powder (29 mg, 23%). LC: t_R = 5.36 min. MS (ESI+): m/z = 551 $[M + H]^+$. ^{1}H NMR (DMSO-d_6) ppm: δ 11.22 (s, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.33−7.24 (m, 6H), 7.05 (t, J = 7.4 Hz, 1H), 6.98 (t, J = 7.1 Hz, 1H), 6.93−6.67 (m, 3H), 6.26 (s, 1H), 5.04 (brs, 1H), 4.42 (dd, J = 11.4 Hz and J = 5.0 Hz, 1H), 4.19 (s, 2H), 3.71−3.39 (m, 4H), 3.69 (s, 3H), 3.64 (s, 3H), 2.91 (m, 2H), 2.70 (d, J = 11.2 Hz, 1H), 2.41−2.14 (m, 2H), 1.51 (m, 1H). ^{13}C NMR (DMSO-d_6): 22.68, 30.32, 46.03, 51.43, 53.30, 54.50, 55.55, 55.82, 55.99, 59.53, 104.71, 110.28, 111.81, 112.39, 117.53, 118.53, 119.33, 121.63, 126.14, 127.39, 128.75, 134.43, 135.61, 136.39, 139.36, 148.18, 148.95, 167.46, 168.12.

Mixture of (1*R*,3*R*)-1-(3,4-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester and (1*S*,3*R*)-1-(3,4-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (24a). **24a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (637 mg, 2.5 mmol) and 3,4-dimethoxybenzaldehyde (415 mg, 2.5 mmol). The reaction mixture was stirred overnight. After filtration and concentration, a yellow solid was obtained (803 mg, 87%). LC: t_R = 3.96 and 4.08 min. MS (ESI+): m/z = 367 $[M + H]^+$, de (cis) = 22%. ^{1}H NMR (DMSO-d_6): δ 10.56(s, 1H), 10.26 (s, 0.65H), 7.43 (m, 1.65H), 7.20−7.24 (m, 1.65H), 6.91−7.04 (m, 6.6H), 6.85 (d, J = 8.3 Hz, 0.65H), 6.64 (dd, J = 8.2 Hz, J = 2 Hz, 1H), 5.26 (s, 1H), 5.15 (s, 0.65H), 3.79−3.89 (m, 2H), 3.75 (s, 1.95H) 3.71 (s, 9.9H), 3.62 (s, 3H), 3.03 (m, 2.3H), 2.85 (m,1.65H), 2.65 (m, 0.65H).

Mixture of (1*S*,3*R*)-2-(2-Chloroacetyl)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester and (1*R*,3*R*)-2-(2-Chloroacetyl)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (24b). **24b** was prepared according to protocol "d" starting from the diastereoisomers mixture **24a** (567 mg) and obtained as a brown powder (729 mg, 100%) which was directly engaged in the cyclization step. LC: t_R = 6.72 and 5.85 min. MS (ESI+): m/z = 443 $[M + H]^+$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(3-hydroxy-4-methoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (25). Compound **25** was prepared according to protocol "b" starting from diastereoisomers mixture **25b** (100 mg, 0.234 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (90

mg, 0.51 mmol) in methanol and obtained after separation by HPLC as a yellow powder (50 mg, 40%). LC: t_R = 4.60 min. MS (ESI+): m/z = 537 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): 11.12 (s, 1H), 8.21 (s, 1H), 7.53 (d, J = 7.1 Hz, 1H), 7.44−7.23 (m, 6H), 7.07 (t, J = 6.6 Hz, 1H), 6.99 (t, J = 7.2 Hz, 1H), 6.77 (d, J = 8.3 Hz, 1H), 6.62 (m, 2H), 6.16 (s, 1H), 5.04 (brs, 1H), 4.37 (dd, J = 11.34 Hz and J = 4.9 Hz, 1H), 4.15 (s, 2H), 3.71−3.39 (m, 7H), 2.92 (dd, J = 15.5 Hz and J = 11.3 Hz, 1H), 2.88 (m, 1H), 2.67 (d, J = 10.9 Hz, 1H), 2.37 (dd, J = 9.96 Hz and J = 8.01 Hz, 1H), 2.17 (m, 2H), 1.49 (brs, 1H). ^{13}C NMR (DMSO-d_6): 22.59, 30.13, 45.96, 51.38, 53.30, 54.62, 55.66, 56.11, 56.41, 59.54, 104.83, 111.78, 112.55, 113.98, 117.29, 118.51, 119.31, 121.59, 126.20, 127.38, 128.74, 134.74, 135.65, 136.46, 139.38, 146.74, 147.13, 167.38, 168.27.

Mixture of (1*R*,3*R*)-1-(3-Hydroxy-4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester and (1*S*,3*R*)-1-(3-Hydroxy-4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (25a). **25a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (500 mg, 1.9 mmol) and 3-methoxy-4-hydroxybenzaldehyde (299 mg, 1.9 mmol). The reaction mixture was stirred overnight. After filtration and concentration, a yellow solid was obtained (690 mg, 100%). LC: t_R = 3.67 and 3.82 min. MS (ESI+): m/z = 353 $[M + H]^+$, de (cis) = 0%.

Mixture of (1*R*,3*R*)-2-(2-Chloroacetyl)-1-(3-hydroxy-4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester and (1*S*,3*R*)-2-(2-Chloroacetyl)-1-(3-hydroxy-4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (25b). **25b** was prepared according to protocol "d" starting from the diastereoisomers mixture **25a** (662 mg). After a treatment with basic solution (H2O/MeOH, 1/2, pH 9) 737 mg of a brown powder was obtained (91%) which was directly engaged in the cyclization step. LC: t_R = 5.34 and 5.54 min. MS (ESI +): m/z = 429 $[M + H]^+$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(4-hydroxy-3-methoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (26). Compound **26** was prepared according to protocol "b" starting from diastereoisomers mixture **26b** (58 mg, 0.135 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (52 mg, 0.298 mmol) in ethanol and obtained after separation by HPLC as a yellow powder (22 mg, 30%). LC: t_R = 4.57 min. MS (ESI+): m/z = 537 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): 11.17 (s, 1H), 8.20 (s, 1H), 7.58−7.24 (m, 7H), 7.06 (t, J = 6.7 Hz 1H), 6.99 (t, J = 7.6 Hz, 1H), 6.87 (s, 1H), 6.61 (m, 2H), 6.24 (s, 1H), 5.04 (brs, 1H), 4.41 (dd, J = 11.4 Hz and J = 5.1 Hz, 1H), 4.18 (s, 2H), 3.70 (m, 3H), 3.66−3.38 (m, 6H), 2.94 (m, 2H), 2.69 (d, J = 10.6 Hz, 1H), 2.38 (dd, J = 9.96 Hz and J = 8.04 Hz, 1H), 2.19 (m, 2H), 1.49 (brs, 1H). ^{13}C NMR (DMSO-d_6): 22.33, 30.31, 46.06, 51.42, 53.30, 54.48, 55.56, 55.94, 56.42, 59.53, 104.66, 110.77, 111.80, 115.77, 118.10, 118.50, 119.30, 121.58, 126.18, 127.38, 128.74, 133.98, 134.67, 136.38, 139.38, 145.97, 147.72, 167.51, 168.12.

Mixture of (1*R*,3*R*)-1-(4-Hydroxy-3-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester and (1*S*,3*R*)-1-(4-Hydroxy-3-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (26a). **26a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (637 mg, 2.5 mmol) and vanilin (380 mg, 2.5 mmol). The reaction mixture was stirred overnight. After filtration and concentration, a yellow solid was obtained (914 mg, 100%). LC: t_R = 3.67 and 3.69 min. MS (ESI+): m/z = 353 $[M + H]^+$, de (cis) = 0%.

Mixture of (1*R*,3*R*)-2-(2-Chloroacetyl)-1-(4-hydroxy-3-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester and (1*S*,3*R*)-2-(2-Chloroacetyl)-1-(4-hydroxy-3-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (26b). **26b** was prepared according to protocol "d" starting from the diastereoisomers mixture **26a** (50 mg). After a treatment with basic solution (H_2O/MeOH, 1/2, pH 9) 58 mg of a brown powder was obtained (100%) which was directly engaged in the cyclization step. LC: t_R = 5.37 and 5.57 min. MS (ESI+): m/z = 429 $[M + H]^+$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(2,2-difluorobenzo[1,3]dioxol-5-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (27). Compound **27** was prepared according to protocol "b" starting from diastereoisomers mixture **27b** (100 mg) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (85 mg) in ethanol and obtained after flash chromatography (dichloromethane/methanol, 95:5) as a white powder (60 mg, 48%). LC: t_R = 5.4 min. MS (ESI+): m/z = 569 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.06 (s, 1H), 7.55 (d, J = 7.95 Hz, 1H), 7.34−7.24 (m, 9H), 7.13 (dd, J = 8.5 Hz and J = 1.7 Hz, 1H), 7.07 (t, J = 7.2 Hz, 1H), 7.00 (t, J = 7.2 Hz, 1H), 6.02 (s, 1H), 5.06 (brs, 1H), 4.42 (dd, J = 11.4 Hz and J = 4.7 Hz, 1H), 4.15 (s, 2H), 3.64−3.45 (m, 3H), 3.05 (dd, J = 14.9 Hz and J = 11.0 Hz, 1H), 2.88 (m, 1H), 2.70 (dd, J = 10.4 Hz and J = 4.1 Hz, 1H), 2.38 (t, J = 7.5 Hz, 1H), 2.19 (brs, 2H), 1.59 (brs, 1H). ^{13}C NMR (DMSO-d_6): δ 23.14, 29.69, 45.78, 51.47, 53.29, 55.72, 55.89, 56.36, 59.54, 105.42, 108.71, 110.38, 111.86, 118.72, 119.42, 121.92, 122.43, 126.16, 127.38, 128.73, 131.62, 133.50, 134.96, 136.73, 139.41, 140.48, 142.02, 143.04, 166.82, 168.35.

(1*R*,3*R*)-1-(2,2-Difluorobenzo[1,3]dioxol-5-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (27a). **27a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (1.03 g) and 2,2-difluoro-5-formylbenzodioxole (930 mg). The reaction mixture was stirred for 17 h. Compound **27a** was obtained after purification by column chromatography, as a white powder (862 mg, 56%). LC: t_R = 4.6 min. MS (ESI+): m/z = 387 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 10.45 (s, 1H), 7.45 (m, 2H), 7.36 (d, J = 1.5 Hz, 1H), 7.25 (dd, J = 1.5 Hz and J = 8.3 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (td, J = 1.5 Hz and J = 15 Hz, 1H), 6.95 (td, J = 1.1 Hz and J = 14.5 Hz, 1H), 5.42 (s, 1H), 4.09 (m, 1H), 3.75 (s, 3H), 3.10 (m, 1H), 2.93 (m, 1H). Mp = 124−126 °C.

(1*R*,3*R*)-2-(2-Chloroacetyl)-1-(2.2-difluorobenzo[1.3]dioxol-5-yl)-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (27b). **27b** was prepared according to protocol "d" starting from intermediate **27a** (662 mg, 1 equiv) and obtained as an orange powder (783 mg, 100%) which was directly engaged in the cyclization step. LC: t_R = 7.1 min. MS (ESI+): m/z = 383 $[M + H]^+$.

(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(2,3-dihydrobenzo[1,4]dioxin-6-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (28). Compound **28** was prepared according to protocol "b" starting from diastereoisomers mixture **28b** (100 mg) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (88 mg) in ethanol and obtained after separation by flash chromatography (dichloromethane/methanol, 95:5) as a yellow powder (63 mg, 50%). LC: t_R = 4.9 min. MS (ESI+): m/z = 549 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 8.11 (s, 1H), 7.60 (m, 1H), 7.35−7.12 (m, 8H), 6.82 (dd, J = 8.3 Hz and J = 2.1 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.20 (s, 1H), 5.18 (brs, 1H), 4.44 (d, J = 17.4 Hz, 1H), 4.22 (dd, J = 11.7 Hz and J = 4.5 Hz, 1H), 4.16 (s, 4H), 3.97 (d, J = 17.3 Hz, 1H), 3.69 (dd, J = 15.9 Hz and J = 4.7 Hz, 1H), 3.57 (m, 1H), 3.23 (dd, J = 15.9 Hz and J = 11.53 Hz, 1H), 3.04 (brs, 1H), 2.78 (brs, 1H), 2.48 (brs, 1H), 2.33 (m, 2H), 1.77 (brs, 1H). ^{13}C NMR ($CDCl_3$): δ 23.21, 29.57, 30.28, 51.92, 53.29, 55.91, 56.14, 57.47, 59.75, 64.23, 106.32, 111.25, 115.85, 117.39, 118.53, 120.02, 120.09, 122.38, 126,18, 127.37, 128.47, 128.68, 132.98, 134.62, 136.46, 138.41, 143.12, 143.44, 166.64, 168.06.

(1*R*,3*R*)-1-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (28a). **28a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (1.01 g) and 1,4-benzodioxan-6-carboxaldehyde (812 mg), after stirring for 43 h and purification by column chromatography. Compound **28a** was obtained as a white powder (319 mg, 22%). LC: t_R = 4.0 min. MS (ESI+): m/z = 365 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 10.33 (s, 1H), 7.42 (d, J = 7.3 Hz, 1H), 7.22 (d, J = 7.3 Hz, 1H), 6.96 (m, 2H), 6.82 (m, 3H), 5.10 (s, 1H), 4.22 (s, 4H), 3.83 (dd, J = 4.2 Hz and J = 11.1 Hz, 1H), 3.70 (s, 3H), 3.00 (dd, J = 2.8 Hz and J = 13.7 Hz, 1H), 2.81 (m, 1H). Mp = 133−135 °C.

(1*R*,3*R*)-2-(2-Chloroacetyl)-1-(2.3-dihydrobenzo[1.4]dioxin-6-yl)-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (28b). **28b** was prepared according to protocol "d" starting from intermediate **28a** (211 mg, 1 equiv) and obtained as an orange powder (203.9 mg, 80%) which was directly engaged in the cyclization step. LC: t_R = 6.3 min. MS (ESI+): m/z = 441 $[M + H]^+$.

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(6*R*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(4-difluoromethoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (29). Compound **29** was prepared according to protocol "b" starting from diastereoisomers mixture **29b** (100 mg) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (87 mg) in ethanol and obtained after separation by flash chromatography (dichloromethane/methanol, 95:5) as a yellow powder (99 mg, 79%). LC: t_R = 5.3 min. MS (ESI+): m/z = 557 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H), 7.62 (m, 1H), 7.30 (m, 10H), 6.99 (d, J = 11.6 Hz, 2H), 6.43 (t, J = 73.8 Hz, 1H), 6.26 (s, 1H), 5.23 (m, 1H), 4.50 (d, J = 17.7 Hz, 1H), 4.24 (dd, J = 11.5 Hz and J = 3.9 Hz, 1H), 3.97 (dd, J = 17.7 Hz and J = 1.2 Hz, 1H), 3.72 (m, 2H), 3.53 (d, J = 12.8 Hz, 1H), 3.24 (ddd, J = 16.0 Hz, J = 11.5 Hz and J = 1.2 Hz, 1H), 3.00 (t, J = 8.8 Hz, 1H), 2.73 (d, J = 12.0 Hz, 1H), 2.43−2.14 (m, 3H), 1.66 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 23.16, 29.83, 45.78, 51.68, 53.31, 55.67, 56.07, 56.39, 56.76, 59.85, 106.72, 111.29, 118.64, 119.67, 120.20, 122.63, 126.08, 127.18, 128.41, 128.53, 128.72, 129.20, 132.45, 136.51, 138.37, 138.75, 150.58, 166.46, 168.39.

(1*R*,3*R*)-1-(4-Difluoromethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (29a). **29a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (1.01 g) and 4-(difluoromethoxy)benzaldehyde (861 mg), after stirring for 72 h and purification by column chromatography. Compound **29a** was obtained as a white powder (845 mg, 57%). LC: t_R = 4.3 min. MS (ESI+): m/z = 373 $[M + H]^+$.

(1*R*,3*R*)-2-(2-Chloroacetyl)-1-(4-difluoromethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (29b). **29b** was prepared according to protocol "d" starting from intermediate **29a** (996 mg, 1 equiv) and obtained as an orange powder (711 mg, 94%) which was directly engaged in the cyclization step. LC: t_R = 6.5 min. MS (ESI+): m/z = 449 $[M + H]^+$.

(6*R*,12a*R*)-6-(3,4-Dimethoxyphenyl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (30). Compound **30** was prepared according to protocol "b" starting from **30a** (30 mg) and methylamine (25 μL, 3eq) in ethanol and obtained after purification by HPLC as a white powder (14 mg, 50%). LC: t_R = 4.85 min. MS (ESI+): m/z = 406 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.09 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.01 (m, 3H), 6.75 (m, 2H), 6.18 (s, 1H), 4.40 (dd, J = 11.7 Hz and J = 4.2 Hz, 1H), 4.20 (d, J = 17.1 Hz, 1H), 3.93 (d, J = 16.8 Hz, 1H), 3.72 (s, 3H), 3.64 (s, 3H), 3.52 (dd, J = 15.6 Hz and J = 4.5 Hz, 1H), 2.96 (dd J = 15.0 Hz and J = 12.0 Hz, 1H), 2.94 (s, 3H).

(1*R*,3*R*)-2-(2-Chloroacetyl)-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (30a). **30a** was prepared according to protocol "c" starting from intermediate **30b** (4.9 g, 1 equiv) and obtained as yellow powder (4.33 g, 82%) which was directly engaged in the cyclization step. LC: t_R = 6.09 min. MS (ESI+): m/z = 443 $[M + H]^+$.

(1*R*,3*R*)-1-(3,4-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (30b). D-Tryptophan-OMe·HCl (500 mg, 1 equiv) and dimethoxybenzaldehyde (2 equiv) were suspended in methanol (2.5 mL). The reaction mixture was refluxed overnight. The mixture was cooled in ice bath to allow crystallization of the product. The residue was filtered and washed with cold isopropanol to give **27** as a yellow powder (706 mg, 89%). LC: t_R = 3.85 min. MS (ESI+): m/z = 367 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 10.81 (s, 1H), 10.59 (brs, 1H), 10.24 (brs, 1H), 7.55 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 7.16 (d, J = 1.6 Hz, 1H), 7.14−7.02 (m, 4H), 5.85 (brs, 1H), 4.77 (brs, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.73 (s, 3H), 3.38 (dd, J = 15.6 Hz and J = 5.8 Hz, 1H), 3.31 (m, 1H).

(6*R*,12a*R*)-2-((*R*)-1-Benzo[1,3]dioxol-5-ylmethylpyrrolidin-3-yl)-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (31). Compound **31** was prepared according to protocol "b" starting from **30a** (100 mg), triethylamine (4.2 equiv), and **31a** (74 mg, 1.5eq) in methanol and obtained after purification by HPLC as a white powder (20 mg, 15%). LC: t_R = 5.25 min. MS (ESI+): m/z = 595 $[M + H]^+$. ^{1}H NMR (CD_2Cl_2): δ 8.09 (s, 1H), 7.62 (dd, J = 6.7 Hz and J = 2.01 Hz, 1H), 7.33 (m, 1H), 7.17 (dd, J = 4.9 Hz and J = 1.5 Hz, 1H), 7.15 (dd, J = 4.6 Hz and J = 1.4 Hz, 1H), 6.93−6.75 (m, 8H), 6.26 (s, 1H), 5.97 (s, 2H), 5.12 (brs, 1H), 4.36 (d, J = 17.6 Hz, 1H), 4.29 (dd, J = 11.5 Hz and J = 4.7 Hz, 1H), 4.02 (d, J = 17.4 Hz, 1H), 3.8 (d, J = 7.6 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.69 (dd, J = 15.8 Hz and J = 4.7 Hz, 2H), 3.21 (dd, J = 15.3 Hz and J = 11.5 Hz, 1H), 3.06 (m, 1H), 2.95 (m, 2H), 2.61 (m, 1H), 2.32(m, 3H), 1.81 (m, 2H). ^{13}C NMR (CD_2Cl_2): δ 21.60, 23.16, 26.04, 29.20, 52.90, 53.29, 55.76, 55.98, 56.08, 59.21, 101.19, 106.24, 107.87, 109.14, 110.48, 111.10, 111.34, 112.30, 118.44, 118.93, 119.85, 121.15, 122.20, 122.20, 122.43, 126.22, 133.3, 134.49, 136.45, 149.14, 166.72, 167.82.

(*R*)-1-Benzo[1,3]dioxol-5-ylmethylpyrrolidin-3-ylamine, Dihydrochloride (31a). (*R*)-(+)-3-(Boc-amino)pyrrolidine (200 mg) was dissolved in dichloromethane/acetic acid (90/10 3 mL). Then 196 mg of piperonal (1 equiv) and cyanoborohydride supported on polystyryl resin (2 equiv) were added to the solution and stirred overnight. Resin was then filtered and solvent removed by evaporation to give an oil. Then it was dissolved in dichloromethane. HCl gas was bubbled for a few minutes, and then the mixture was stirred for 2 h. Solvent was removed by evaporation to give **31a** (220 mg, 70%). LC: t_R = 0.87 min. MS (ESI+): m/z = 221 $[M + H]^+$.

(6*R*,12a*R*)-6-(3,4-Dimethoxyphenyl)-2-[(*R*)-1-(1-methyl-1*H*-imidazol-2-ylmethyl)pyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (32). Compound **32** was prepared according to protocol "b" starting from **30a** (100 mg), triethylamine (4.2 equiv), and **32a** (74 mg, 1.5 equiv) in methanol and obtained after purification by HPLC as a white powder (20 mg, 15%). LC: t_R = 4.57 min. MS (ESI+): m/z = 555 $[M + H]^+$. ^{1}H NMR (CD_2Cl_2): δ 8.88 (s, 1H), 7.59 (dd, J = 6.6 Hz and J = 1.7 Hz, 1H), 7.32 (d, J = 6.8 Hz, 1H), 7.13 (m, 2H), 6.91 (s, 2H), 6.87 (d, J = 1.6 Hz, 1H), 6.82 (d, J = 8.3 Hz and J = 1.6 Hz, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.26 (s, 1H), 5.20 (brs, 1H), 4.27 (m, 2H), 3.92 (d, J = 17.3 Hz, 1H), 3.79−3.64 (m, 12H), 3.20 (dd, J = 15.8 Hz and J = 11.7 Hz, 1H), 2.87 (m, 1H), 2.70 (d, J = 10.7 Hz, 1H), 2.51 (dd, J = 10.4 Hz and J = 7.7 Hz,1H), 2.26 (m, 2H), 1.65 (m, 1H). ^{13}C NMR (CD_2Cl_2): δ 23.09, 29.67, 32.87, 45.66, 50.85, 51.51, 55.71, 55.76, 55.88, 56.03, 56.25, 105.93, 110.38, 111.20, 111.33, 118.38, 118.73, 119.67, 121.63, 122.00, 125.99, 126.19, 133.43, 134.65, 136.53, 145.04, 148.56, 149.08, 166.59, 167.84.

(*R*)-1-(1-Methyl-1*H*-imidazol-2-ylmethyl)pyrrolidin-3-ylamine, Dihydrochloride (32a). (*R*)-(+)-3-(Boc-amino)pyrrolidine (200 mg) was dissolved in dichloromethane/acetic acid (90/10, 3 mL). Then 132 mg of 1-methyl-2-imidazole carboxaldehyde (1 equiv) and cyanoborohydride supported on polystyryl resin (2 equiv) were added to the solution and stirred overnight. Resin was then filtered and solvent removed by evaporation to give an oil. Then it was dissolved in cichloromethane. HCl gas was bubbled for a few minutes, and then the mixture was stirred for 2 h. Solvent was removed by evaporation to give **32a** (189 mg, 70%). LC: t_R = 0.67 min. MS (ESI +): m/z = 181 $[M + H]^+$.

(2-{(*R*)-3-[(6*R*,12a*R*)-6-(3,4-Dimethoxyphenyl)-1,4-dioxo-3,4,6,7,12,12a-hexahydro-1*H*-pyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indol-2-yl]pyrrolidin-1-yl}ethyl)carbamic Acid *tert*-Butyl Ester (33). The intermediate **33b** (70 mg), 2-(Boc-amino)ethyl bromide (27 mg, 2 equiv), and triethylamine (4 equiv) were dissolved in dioxane (1 mL) and stirred at 70 °C for 20 h, The dioxane was removed by evaporation. The residue was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and evaporated to dryness. It was purified by HPLC to give 23 mg of a white powder (31%). LC: t_R = 5.20 min. MS (ESI+): m/z = 604 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.17 (s, 1H), 7.52 (d, J = 7.1 Hz, 1H), 7.31 (d, J = 7.6 Hz, 1H), 7.08 (t, J = 7.7 Hz, 1H), 6.99 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 1.9 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 6.69 (dd, J = 8.2 Hz and J = 1.9 Hz, 1H), 6.24 (s, 1H), 5.01 (m, 1H), 4.46 (dd, J = 11.5 Hz and J = 5.3 Hz, 1H), 4.21 (d, J = 19.7 Hz, 1H), 4.10 (d, J = 16.7 Hz, 1H), 3.70 (s, 3H), 3.68 (m, 2H), 3.65 (s, 3H), 3.46−3.41 (m, 3H), 3.21 (m, 2H), 2.97 (dd, J = 15.6 Hz and J = 11.6 Hz, 2H), 2.26 (m, 2H), 1.39 (s, 9H), 1.37 (m, 1H).

(*R*)-3-[(6*R*,12a*R*)-6-(3,4-Dimethoxyphenyl)-1,4-dioxo-3,4,6,7,12,12a-hexahydro-1*H*-pyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indol-2-yl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (33a). Compound **33a** was prepared according to protocol "b" starting from **30a** (100 mg), triethylamine (2 equiv), and (*R*)-1-Boc-3-amino-

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pyrrolidine (42 μL, 1.1 equiv) in methanol and obtained as a white powder (119 mg, 94%). LC: t_R = 6.19 min. MS (ESI+): m/z = 561 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.15 (s, 1H), 7.54 (d, J = 7.4 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.08 (td, J = 5.3 Hz and J = 1.2 Hz, 1H), 6.99 (td, J = 7.0 Hz and J = 1.0 Hz, 1H), 6.95 (d, J = 1.9 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.71 (dd J = 8.3 Hz and J = 2.0 Hz, 1H), 6.22 (s, 1H), 4.94 (m, 1H), 4.45 (dd, J = 11.3 Hz and J = 5.0 Hz, 1H), 4.15 (d, J = 16.6 Hz, 1H), 3.90 (d, J = 17.1 Hz, 1H), 3.70 (m, 4H), 3.65 (s, 3H), 3.52−3.25 (m, 6H), 2.96 (dd, J = 15.7 Hz and J = 11.6 Hz, 1H), 1.40 (s, 9H).

(6*R*,12a*R*)-6-(3,4-Dimethoxyphenyl)-2-(*R*)-pyrrolidin-3-yl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]-indole-1,4-dione, Trifluoroacetate (33b). Compound **33a** (119 mg) was dissolved in dichloromethane (400 μL). TFA (100 μL) was slowly added. The reaction mixture was stirred at room temperature for 1 h. **21b** was precipitated in diethyl ether to give 74 mg of brown oil (60%). LC: t_R = 4.42 min. MS (ESI+): m/z = 461 $[M + H]^+$.

(6*S*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-methyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (34). Compound **34** was prepared according to protocol "b" starting from (1*S*,3*R*)-1-benzo[1,3]dioxol-5-yl-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid methyl ester[1] (100 mg) and methylamine (25 μL, 3 equiv) in ethanol and obtained after purification by HPLC as a white powder (75 mg, 82%). LC: t_R = 4.77 min. MS (ESI+): m/z = 390 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 7.95 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 6.97 (s, 1H), 6.81 (s, 1H), 6.71 (s, 2H), 5.93 (s, 2H), 4.35 (dd, J = 11.7 Hz and J = 4.2 Hz, 1H), 4.15 (d, J = 17.1 Hz, 1H), 3.99 (d, J = 16.8 Hz, 1H), 3.54 (dd, J = 15.6 Hz and J = 4.5 Hz, 1H), 2.99 (s, 3H), 2.96 (dd J = 15.0 Hz and J = 12.0 Hz, 1H).

(6*S*,12a*R*)-6-Benzo[1.2.5]thiadiazol-5-yl-2-((*R*)-1-benzylpyrrolidin-3-yl)-2.3.6.7.12.12a-hexahydropyrazino[1′.2′:1.6]-pyrido[3.4-*b*]indole-1.4-dione (35). Compound **35** was prepared according to protocol "b" starting from intermediate **35b** (190 mg, 0.43 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (148 μL, 2 equiv) in methanol and obtained after purification by TLC (dichloromethane/methanol, 95:5) as a yellow powder (147 g, 60%). LC: t_R = 5.05 min. MS (ESI+): m/z = 549 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.15 (s, 1H), 8.10 (d, J = 9.6 Hz, 1H), 7.58 (dd, J = 9.3 and 1.5 Hz, 1H), 7.64 (s, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.33−7.22 (m, 5H), 7.14 (td, J = 7.20 and 1.2 Hz, 1H), 7.08 (s, 1H), 7.05 (td, J = 7.50 and 0.9 Hz, 1H), 5.03−4.95 (m, 1H), 4.39 (d, J = 17.7 Hz, 1H), 4.15 (d, J = 17.4 Hz, 1H), 4.14 (dd, J = 12.3 and 4.5 Hz, 1H), 3.58 (d, J = 12.9 Hz, 1H), 3.51 (d, J = 12.9 Hz, 1H), 3.41 (dd, J = 15.6 and 4.2 Hz, 1H), 3.06 (dd, J = 15.0 and 12.3 Hz), 2.86−2.82 (m, 1H), 2.72 (dd, J = 10.2 and 2.7 Hz, 1H), 2.35 (dd, J = 10.2 and 7.8 Hz, 1H), 2.20−2.01 (m, 2H), 1.91−1.74 (m, 1H). ^{13}C NMR (DMSO-d_6): 26.90, 27.56, 45.32, 51.65, 53.35, 53.48, 56.93, 59.72, 67.70, 108.73, 112.05, 118.91, 119.66, 120.87, 122.40, 122.55, 124.65, 126.58, 127.58, 128.88, 129.00, 129.93, 130.07, 130.98, 136.96, 141.48, 154.57, 154.68, 164.17, 165.20.

(1*S*,3*R*)-1-Benzo[1,2,5]thiadiazol-5-yl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (35a). **35a** was prepared according to protocol "c" starting from D-tryptophan-OMe·HCl (1241 mg) and 2,1,3-benzothiadiazole-5-carbaldehyde (800 mg). The reaction mixture was stirred overnight. After filtration and concentration, the cis isomer was separated by precipitation in EtOAc and the residue was purified by column chromatography (dichloromethane/methanol) to provide the trans product **35a** as an orange solid (160 mg, 10%). LC: t_R = 4.22 min. MS (ESI+): m/z = 365 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 10.65 (s, 1H), 8.03 (d, J = 9.3 Hz, 1H), 7.80 (s, 1H), 7.70 (dd, J = 9.3 Hz and J = 1.8 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 7.06−6.95 (m, 2H), 5.57 (s, 1H), 3.89 (m, 1H), 3.62 (s, 3H), 3.07 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 154.54, 131.95, 124.46, 121.37, 120.11, 118.95, 118.29, 111.58, 96.17, 95.84, 54.28, 52.66, 52.14. Mp =156−160 °C.

(1*S*,3*R*)-1-Benzo[1.2.5]thiadiazol-5-yl-2-(2-chloroacetyl)-2.3.4.9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (35b). **35b** was prepared according to protocol "d" starting from intermediate **35a** (160 mg, 1 equiv) and obtained as a yellow oil (190 mg, 97%) which was directly engaged in the cyclization step. LC: t_R = 6.2 min. MS (ESI+): m/z = 441 $[M + H]^+$.

(6*S*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(3,4-dimethoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (36). Compound **36** was prepared according to protocol "b" starting from diastereoisomers mixture **24b** (100 mg, 0.226 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (87 mg, 0.50 mmol) in methanol and obtained after separation by TLC (dichloromethane/methanol, 95:5) as a yellow powder (29 mg, 23%). LC: t_R = 5.36 min. MS (ESI+): m/z = 551 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): δ 11.09 (s, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.47−7.22 (m, 6H), 7.10 (t, J = 6.6 Hz, 1H), 7.02 (t, J = 6.0 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 1.8 Hz, 1H), 6.83 (s, 1H), 6.58 (d, J = 9.9 Hz, 1H), 5.00 (brs, 1H), 4.13 (d, J = 16.9 Hz, 1H), 4.09 (m, 2H), 3.72 (s, 3H), 3.69 (s, 3H), 3.23 (dd, J = 10.9 Hz and J = 3.8 Hz, 1H), 2.99 (dd, J = 19.4 Hz and J = 8.8 Hz, 2H), 2.09 (m, 1H), 2.41−2.14 (m, 2H), 1.51 (m, 1H). ^{13}C NMR (DMSO-d_6): 26.40, 29.20, 45.30, 51.45, 52.83, 56.00, 56.03, 107.86, 111.76, 112.03, 112.41, 118.53, 119.31, 121.01, 122.08, 126.37, 128.86, 131.07, 131.71, 136.69, 149.33, 149.39, 163.1, 163.5.

(6*S*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(3-hydroxy-4-methoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (37). Compound **37** was prepared according to protocol "b" starting from diastereoisomers mixture **25b** (100 mg, 0.234 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (90 mg, 0.51 mmol) in methanol and obtained after separation by HPLC as a yellow powder (22 mg, 18%). LC: t_R = 4.68 min. MS (ESI+): m/z = 537 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): 11.11 (s, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.35−7.24 (m, 6H), 7.09 (t, J = 7.2 Hz, 1H), 7.01 (t, J = 7.6 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 6.76 (s, 1H), 6.64 (s, 1H), 6.55 (d, J = 8.2 Hz, 1H), 4.98 (brs, 1H), 4.33 (d, J = 17.5 Hz, 1H), 4.15−3.95 (m, 2H), 3.71−3.49 (m, 6H), 3.19 (dd, J = 15.3 Hz and J = 4.2 Hz, 1H), 2.99 (dd, J = 14.8 Hz and J = 12.2 Hz, 1H), 2.83 (m, 1H), 2.69 (d, J = 10.5 Hz, 1H), 2.39 (dd, J = 9.9 Hz and J = 7.9 Hz, 1H), 2.20−2.03 (m, 2H), 1.81 (m, 1H). ^{13}C NMR (DMSO-d_6): 26.68, 27.39, 45.15, 51.18, 51.47, 52.77, 53.27, 56.12, 57.03, 59.54, 107.70, 111.76, 112.80, 115.86, 118.49, 119.30, 119.51, 122.03, 126.41, 127.39, 128.73, 128.82, 131.30, 131.81, 136.64, 139.39, 147.09, 148.15, 163.24, 165.23.

(6*S*,12a*R*)-2-((*R*)-1-Benzylpyrrolidin-3-yl)-6-(4-hydroxy-3-methoxyphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]-pyrido[3,4-*b*]indole-1,4-dione (38). Compound **38** was prepared according to protocol "b" starting from diastereoisomers mixture **26b** (58 mg, 0.135 mmol) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (52 mg, 0.298 mmol) in ethanol and obtained after separation by HPLC as a yellow powder (15 mg, 20%). LC: t_R = 4.62 min. MS (ESI+): m/z = 537 $[M + H]^+$. ^{1}H NMR (DMSO-d_6): 11.07 (s, 1H), 7.50 (d, J = 7.7 Hz, 1H), 7.29 (m, 6H), 7.10 (t, J = 7.0 Hz 1H), 7.01 (t, J = 7.2 Hz, 1H), 6.87−6.71 (m, 3H), 6.49 (d, J = 7.1 Hz, 1H), 5.00 (brs, 1H), 4.33 (d, J = 17.5 Hz, 1H), 4.18−4.06 (m, 2H), 3.68 (m, 3H), 3.63−3.40 (m, 3H), 3.22 (dd, J = 15.2 Hz and J = 3.9 Hz, 1H), 2.99 (dd, J = 14.7 Hz and J = 12.2 Hz, 1H), 2.84 (m, 1H), 2.71 (d, J = 7.9 Hz, 1H), 2.41 (dd, J = 9.9 Hz and J = 8.0 Hz, 1H), 2.27−2.05 (m, 2H). ^{13}C NMR (DMSO-d_6): 26.76, 27.42, 45.15, 51.48, 52.80, 53.27, 56.17, 56.98, 59.54, 107.79, 111.75, 112.95, 115.66, 118.49, 119.26, 121.33, 122.00, 126.40, 127.35, 128.70, 128.80, 130.16, 131.32, 136.68, 139.40, 147.33, 148.18, 163.34, 165.27.

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■ ABBREVIATIONS USED

SAR, structure−activity relationship; PDE, phosphodiesterase; EtOH, ethanol; MeOH, methanol; AcOH, acetic acid; IC_{50}, concentration inhibiting 50%; CC_{50} cytotoxic concentration 50%; DMF, dimethylformamide; DCM, dichloromethane; TLC, thick layer chromatography; EtOAc, ethyl acetate; Boc, *tert*-butyloxycarbonyl; NC, not calculated; TFA, trifluoroacetic acid; DMSO, dimethylsulfoxide; LC, liquid chromatography; t_R, retention time; MS, mass spectrometry; ESI, electrospray ionization; Mp, melting point

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