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Drug to Genome to Drug: Discovery of New Antiplasmodial Compounds

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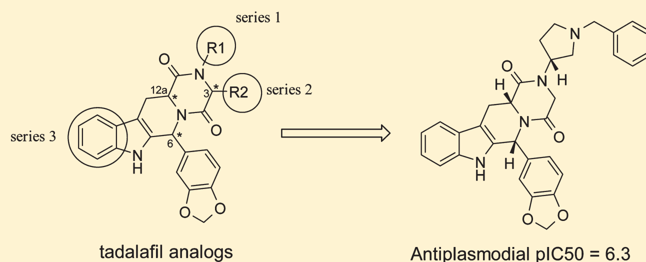
ABSTRACT: The dominant strategy for discovery of new antimalarial drugs relies on cell-free assays on specific biochemical pathways of *Plasmodium falciparum*. However, it appears that screening directly on the parasite is a more rewarding approach. The "drug to genome to drug" approach consists of testing a small set of structural analogues of a drug acting on human proteins that have plasmodial orthologues. Both man and plasmodium possess cyclic nucleotide phosphodiesterases (PDEs) that are key players of cell homeostasis. We synthesized and tested 40 analogues of tadalafil, a human PDE5 inhibitor, on *P. falciparum* in culture and obtained potent inhibitors of parasite growth. We discuss the structure−activity relationships, which support the hypothesis that our compounds kill the parasite via inhibition of plasmodial PDE activity. We also prove that antiplasmodial derivatives inhibit the hydrolysis of cyclic nucleotides of the parasite, validating the cAMP/cGMP pathways as therapeutic targets against *Plasmodium falciparum*.



INTRODUCTION

Difficulty of Antiplasmodial Drug Discovery. Malaria remains one of the most challenging diseases. Over three billion people live under the threat of malaria across the world and one million are killed each year, mostly children. *Plasmodium falciparum* accounts for the most severe and fatal form of the disease. Emergence of strains resistant to the usual drugs has made the situation worse,[1] and there is an urgent need for scientists to discover and develop new antimalarial drugs with a new mode of action. Finding biochemical pathways specific to the malaria parasite and identifying lead compounds against these pathways is the most employed strategy[2−4] to find new drug candidates.[4] The recent sequencing of the *P. falciparum* genome[5] could help in the identification of these metabolic pathways. However, the newly identified protein must also be functionally validated. In this long-term target-based strategy, the bottleneck is obtaining compounds active on the whole intracellular parasite. As a consequence, although target-based HTS currently dominates much of the early drug discovery process, whole parasite is a more valid and rewarding strategy.[6] This approach is supported by the World Health Organization, and academic laboratories have easy access to such platforms.[7] Even if this process has the advantage of identifying compounds able to enter erythrocyte and parasite to exert activity, functional testing on *P. falciparum* requires labor-intensive steps like parasite culture and is only just adapted in 384-well plates.[4,8]

We asked ourselves whether it could be possible to reconcile the "target-based" and whole parasite screening strategies and even combine their advantages. In other terms, is it possible to design a small library to specifically target a relevant class of parasitic proteins? The medium-throughput assays as proposed by WHO would then be sufficient to perform the primary screen. The selected target(s) should belong to a sufficiently documented class of proteins that are implicated in cellular homeostasis in such a way that modulators of these proteins could disrupt the parasite cycle. The rational selection of such target(s) can be based on a straightforward analysis of *P. falciparum* genome.

The sequencing of *P. falciparum* has not immediately been an easy gateway to new therapeutic targets.[5] However, it has become possible to compare the parasite genome to the human genome, in particular to the subset of human sequences coding for genuine drug targets. Figure 1 displays the overlapping maps of parasite and human proteomes'.

When it comes to safety issues, obvious antiparasitic targets are plasmodial proteins with no orthologue in humans (green zone in Figure 1). Research is often focused on this class to avoid selectivity issues during optimization of the drug candidate (green arrow). Indeed, in the case of a new protein class (red and blue zones), selectivity can be difficult to attain. However, the situation is radically different when the orthologous protein is

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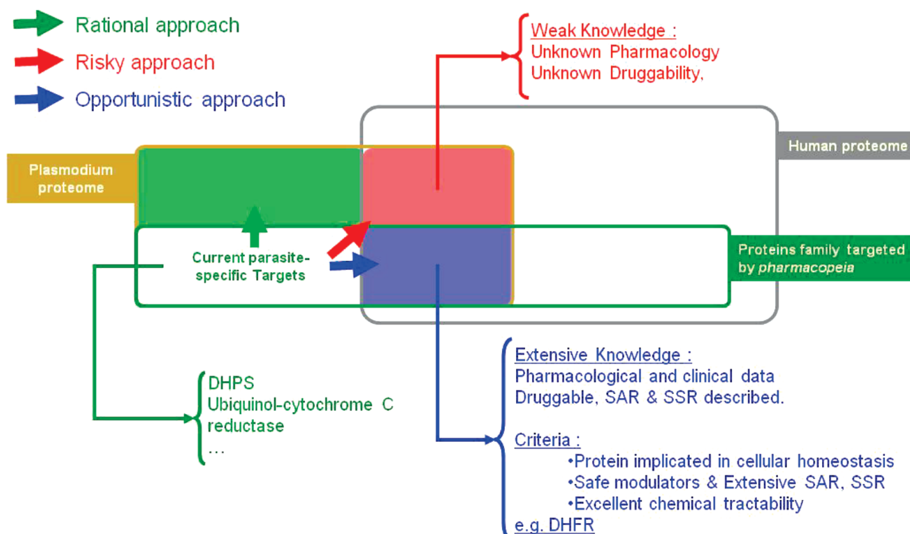


Figure 1. Schematic representation of human and plasmodial proteomes (respectively gray and brown rectangles) and current drug targets (in green rectangle). Rational, risky, and opportunistic approaches for the selection of plasmodial proteins as new potential drug targets (respectively green, red, and blue arrows pointing to green, red and blue shaded zones) are illustrated. The blue zone is explored by the drug-to-genome-to-drug approach.

the target of a marketed drug and a member of a well-documented protein family (blue zone). In this case, a wealth of data is available that helps in optimizing compounds, the selectivity issue can be anticipated and solved, and eventually the initial difficulty can be turned into an opportunity. In the drug-to-genome-to-drug approach described here, we propose to use drugs as entry points in the search for new antiparasitic targets in the plasmodial genome. This strategy has been referred to as "inverted silver bullet" by Wentzinger et al.[9] in opposition to Ehrlich's "magic bullet", which consists of targeting pathways that are essential for the parasite but absent in human. It can also be compared to the concept of selective optimization of side activities (SOSA) proposed by Wermuth in 2006[10] for the generation of new biological activities.[11] The strategy described in our paper can be compared to drug screening but differs from previously described works[16−17] by the fact that it does not rely on the systematic screening of a large collection of drugs. Rather it consists in systematic screening of analogues of one selected drug. Briefly, our strategy combines "black box" screening and "knowledgeable molecules" to discover rapidly active compounds with a good idea of their mode of action.

An Opportunistic Approach. The class 1 cyclic nucleotide-specific phosphodiesterases (PDEs) is a salient family to test this strategy. PDEs are enzymes that catalyze the hydrolysis of cGMP and cAMP and therefore are involved in cellular response to a large variety of stimuli.[15,16] They have been well studied as potential targets in various eukaryotic organisms.[17,18] In humans, 11 isoforms (PDE1−11) are expressed.[19] Some PDEs were recently characterized in protozoan parasites like *Trypanosoma*[18] and *Plasmodium*.[9,29] cAMP and cGMP play a pivotal role in the growth and differentiation of these parasites.[20] The genome of *P. falciparum* encodes four isoforms of PDEs. Yuasa et al.[21] have cloned PfPDEα and reported its enzymatic activity. Wentzinger and co-workers[9] in a recent article showed that the knockout of this enzyme is not lethal. However, Taylor et al.[20] showed that PfPDEδ knockout decreases cGMP hydrolysis and severely reduces gametogenesis. We then made the hypothesis that an inhibitor of *Plasmodium* phosphodiesterase activities could disrupt the malaria cycle.

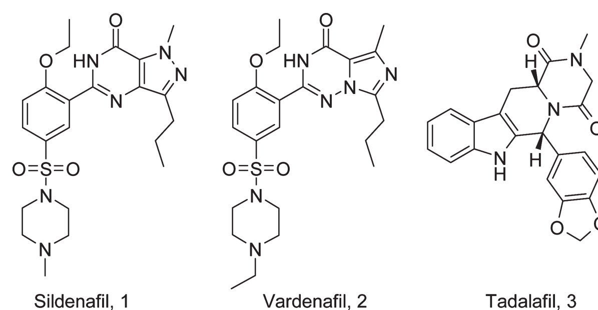


Figure 2. Three marketed inhibitors of human PDE5.

Design of Library: Choice of Scaffold. Among the large number of PDE inhibitors, the most studied in terms of activity and selectivity are human PDE5 inhibitors.[22−24] PDE5 has received considerable attention over the last 10 years, with three selective inhibitors now being on the market (sildenafil, vardenafil, and tadalafil[25]). The first two are purine analogues (Figure 2). Card and co-workers[26] related the lack of selectivity of sildenafil or vardenafil to the structural similarity between these compounds and enzyme substrates.

Tadalafil is the first non-purine PDE5 inhibitor on the market. It displays a novel tetrahydro-β-carboline ring fused to a diketopiperazine scaffold that accounts for to its original selectivity profile.[27] Despite a lack of selectivity over PDE11, the role of which is not yet well established,[28] tadalafil presents a better safety profile. Moreover, the tetrahydro-β-carboline moiety can be obtained in three easy steps.[25] This convenient route for the preparation of tadalafil analogues is well suited to the rapid synthesis of a small focused library.

Analogue Design. Catalytic sites of PfPDEs and hPDE5 display only 41−47% sequence homology. However, residues in contact with tadalafil in the active hPDE5 are highly conserved[26] (Figure 3). A glutamine residue (Q817 in PDE5), hydrogen-bonded to the indole ring of tadalafil, is conserved in all PDEs. In PDE5, F820 participates with V782 to form a hydrophobic clamp (Q1 pocket) recognizing the indole ring of

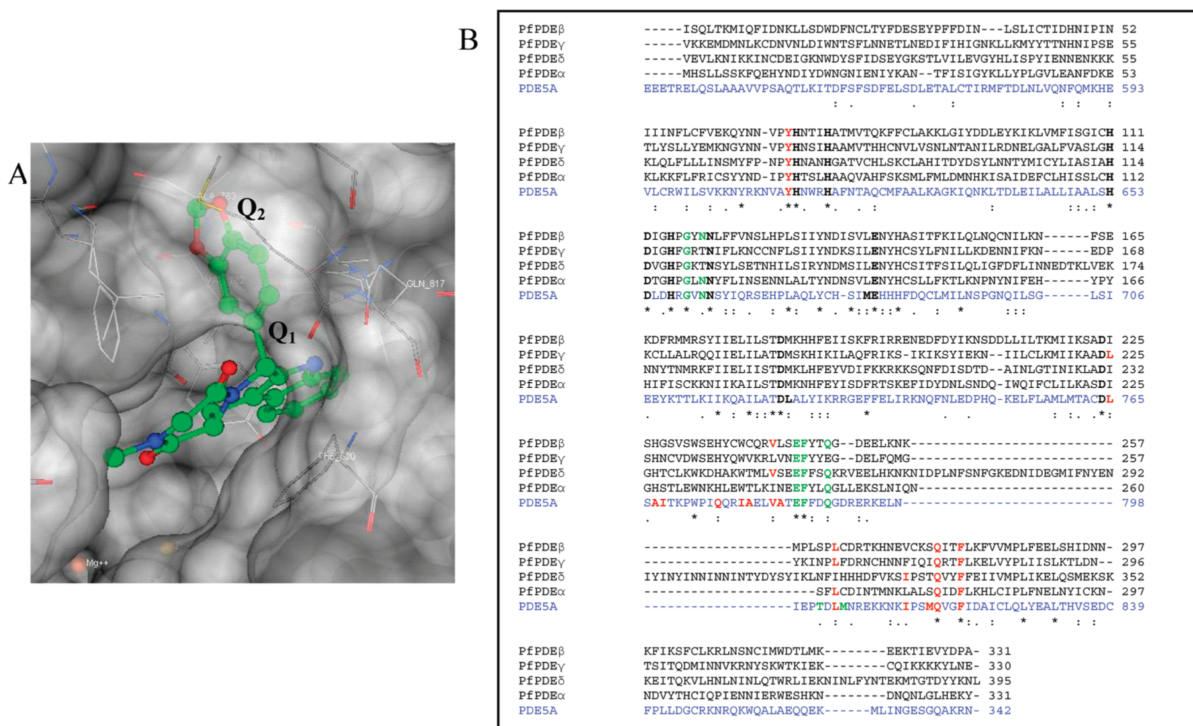


Figure 3. (A) Structure of human PDE5A cocrystallized with tadalafil. (B) ClustalW alignment of catalytic sites of human PDE5A and the four *Plasmodium* PDEs. Residues constituting the metal binding pocket are shown in boldface type; residues participating in the Q switch (Q1 and Q2 pockets) and the P clamp are shown in red; and residues exposed in the solvent-filled side pocket are shown in green.

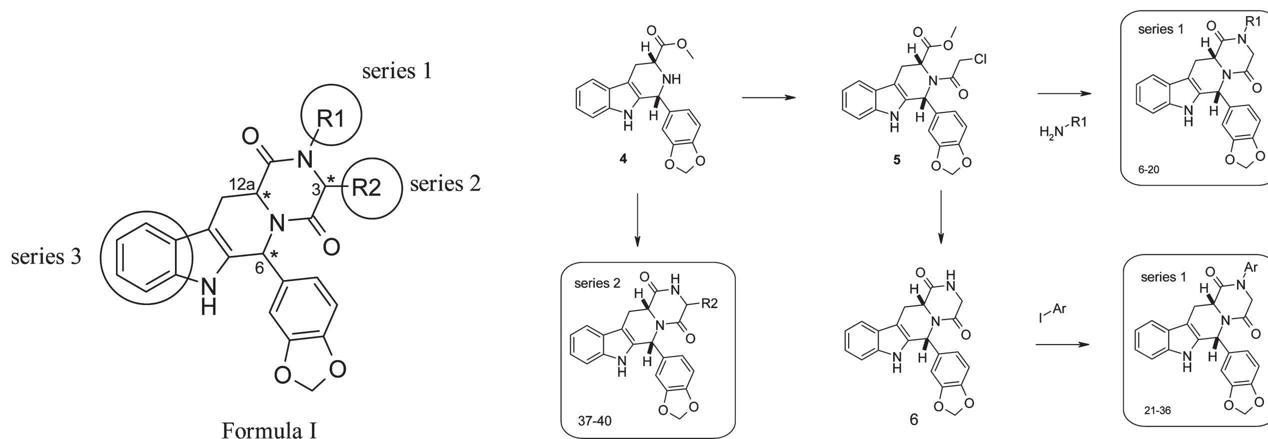


Figure 4. General formula of synthesized compounds.

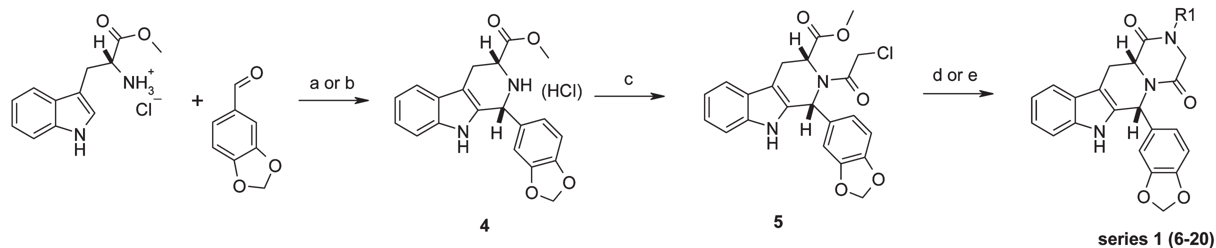
tadalafil. In plasmodial PDEs, F820 is fully conserved whereas V782 is conserved or replaced by homologous leucine (PfPDEγ) or isoleucine (PfPDEα). Interestingly, the methyl group of tadalafil points out toward an unexploited solvent pocket and could become a convenient point to anchor diversity in the library. The piperonyl group of tadalafil occupies the Q2 pocket, which is thought to be responsible for selectivity in the series.[26] The residues forming this pocket are variable within the parasitic PDEs family and are different from hPDE5. We believe that selectivity against hPDE5 could be obtained by modifying the piperonyl group once active compounds are obtained.

Using the structural information described above, we designed and synthesized three series of compounds closely related to tadalafil to establish preliminary structure–activity relationships (SAR) for *P. falciparum* growth inhibition. In this first step, the goal was to obtain active compounds, and then to work out the expected lack of selectivity against PDE5 by fine-tuning the interaction with the variable Q2 pocket.

The general formula I of the diketopiperazine derivatives is presented in Figure 4. Three points of pharmacomodulation were studied: (1) N-substitution of the 1,4-piperazinedione (series 1), (2) modifications at the C-3 position (series 2), and (3) introduction of diversity on the tryptophan moiety (series 3).

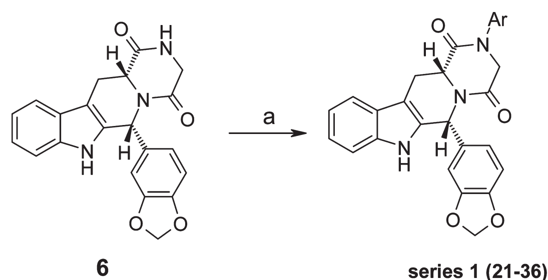
Series 1 derives from the tetrahydro-β-carboline (1*R*,3*R*) obtained by a stereoselective Pictet–Spengler reaction between D-tryptophan methyl ester and piperonal[29] (compound **4**). The secondary amine is then chloroacetylated (compound **5**). Cyclization in diketopiperazine occurs spontaneously in the presence

Scheme 1. General Synthetic Route for Compounds 6−20[a]



[a] Conditions: (a) CF_3COOH, CH_2Cl_2; diastereoisomers separation; (b) 2-PrOH, reflux; (c) chloroacetyl chloride, Et_3N, $CHCl_3$ 0 °C−RT; (d) R1-NH_2; MeOH, reflux or EtOH, microwave 100 W/5 min.

Scheme 2. General Synthetic Route for Derivatives 21−36[a]



[a] Conditions: (a) CuI, K_3PO_4, (±)-*trans*-1,2-diaminocyclohexane and iodoaryl derivatives in anhydrous dioxane (0.13 M), 14 °C for 14 days.

of the required primary amine (compounds **6**−**20**). Compounds **21**−**36** require the synthesis of the unsubstituted diketopiperazine (nortadalafil). The amide is then arylated under Buchwald conditions.[30] Series 2 is issued from the condensation of the tetrahydro-β-carboline and the required α-amino acid. Series 3 requires a Pictet−Spengler reaction between a correctly substituted tryptophan analogue and piperonal. The obtained tetrahydro-β-carbolines are then treated under the conditions described above for series 1.

■ RESULTS AND DISCUSSION

N-Substitution of the 1,4-Piperazinedione (Series 1). In these series, we kept two critical points of interaction with hPDE5 (the diketopiperazine and the piperonyl fragment), and designed a series of compounds where the methyl group is replaced by a larger substituent. To respect the stereochemical requirements established for PDE5 inhibition (6*R*,12a*R*), our starting material was preferably D-tryptophan methyl ester.

cis-Tetrahydro-β-carboline **4** was stereoselectively obtained by Pictet−Spengler reaction in refluxing 2-propanol, conditions inspired from the industrial synthesis of tadalafil (Scheme 1).[29] It is then acylated to generate the *cis*-chloroacetyl derivatives **5** in good yield. Ring closure in the presence of primary amines (R2-NH_2) can be performed in refluxing methanol to provide compounds of series 1 (**6**−**20**). This cyclization step could be improved by heating the chloroacetyl intermediate with the primary amine in ethanol via microwave for 5 min.

Aromatic substituents are introduced by a mild and efficient method, derived from the conditions described by Buchwald and co-workers[31] for the arylation of amides and lactams. Our aim was to close the diketopiperazine ring to form nortadalafil **6** in high yield and then introduce the aromatic substituent. We could thus tap from the large array of commercially available aryl or heteroaryl halides (compounds **21**−**36**). Our starting material nortadalafil **6** (in the 6*R*,12a*R* *cis* configuration) was easily prepared in three steps from D-tryptophan methyl ester, piperonal, chloroacetyl chloride, and ammonia[30] (Scheme 2).

Tadalafil and analogues were tested in vitro for inhibitory activity against *P. falciparum* (GHA chloroquine-sensitive strain) in culture and for cytotoxicity on MRC5 cells to confirm the specificity of action of the compounds. A compound is considered as "highly active" when the IC_{50} is lower than 1 μM and selective (index > 100).[32] Tables 1 and 2 present results of series 1.

Tadalafil itself (**1**) has no detectable activity against *P. falciparum*. The replacement of its methyl group by hydrogen (**6**) or an alkyl group (**7**) led to inactive compounds. However, substitution of the N2 position by a benzylaminopyrrolidine (**10**) resulted in potent antiplasmodial activity. Interestingly, the debenzylation of compounds **10** and **14** appeared very detrimental, leading to a loss in activity (**16**, **17**). Altogether these results were sufficient to draw a preliminary definition of the pharmacophore at the N2 position. An alicyclic protonable amine seems necessary but not sufficient. Indeed, hydrophobicity could be an important determinant of activity. Interestingly, the notable difference between the two epimers **10** and **11** argues in favor of a specific interaction between the target and the aryl group.

Introduction of any aromatic ring directly at the N2 position resulted in a loss of activity, in contrast with the results obtained for hPDE5.[30] Interestingly, antiplasmodial activity in this series could not be restored by introduction of a protonatable function (**21**−**25**) compared to derivatives **10** and **14**.

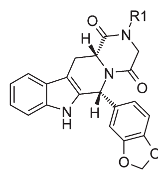
Most of the compounds are noncytotoxic, as IC_{50} values on MRC5 cells are above 64 μM, except for compounds **10**, **13**, **14**, **24**, and **25**. However, it is reassuring to observe no correlation between cytotoxicity on MRC5 and activity on parasite.

Modifications at the C-3 Position (Series 2). Further pharmacomodulations consisted of introducing diversity on the C-3 position of the tetrahydro-β-carbolines moiety. Tetrahydro-β-carbolines were reacted with N-protected aminoacid fluorides. Cyclisation occurred spontaneously upon deprotection of the amine to afford compounds **37**−**40** (Scheme 3, Table 3).

Substitution at the R2 position seems to be deleterious for activity, although compounds issued from glutamic acid and benzyl serine residues (respectively **37** and **40**) are described as human PDE5 inhibitors.[27] This series was not further explored.

Introduction of Diversity on the Tryptophan Moiety (Series 3). According to the radiocrystallographic data of PDE5, the core of tadalafil is clamped between two hydrophobic

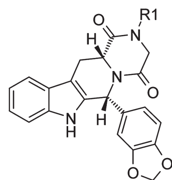
Table 1. In Vitro Antiplasmodial Activity and Cytotoxicity for Series 1 (6−20, 6*R*, 12a*R* Configuration)



Cpd	R1	Antiplasmodial activity IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
		PfGHA	MRC5
1	CH_3	>64.0	>64.0
6	H	>64.0	>64.0
7		>64.0	>64.0
8		22.0	>64.0
9		9.0	>64.0
10		0.5	10.0
11		10.0	>64.0
12		7.0	>64.0
13		8.0	8.0
14		1.0	7.0
15		4.0	62.0
16		12.0	>64.0
17		32.0	30.0
18	NH_2	>64.0	>64.0
19		16.0	>64.0
20		9.0	>64.0

residues, valine (V782) and phenylalanine (F820),[28] and the indole moiety is anchored deeply in the Q1 pocket. Only one hydrogen bond can be observed between the indole nitrogen and a glutamine residue (Q817 in PDE_5). This glutamine is very important for recognition of the substrates and selectivity between the cyclic nucleotides cAMP and cGMP. It is conserved in all PDEs across different species. In order to study the influence of modifications on the indole, we

Table 2. In Vitro Antiplasmodial Activity and Cytotoxicity of Series 1 (21−36, 6*R*, 12a*R* Configuration)



Cpd	R1	Antiplasmodial activity IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
		PfGHA	MRC5
21		19.0	>64.0
22		23.0	>64.0
23		>64.0	>64.0
24		7.0	8.0
25		10.0	8.0
26		>64.0	>64.0
27		>64.0	>64.0
28		>64.0	>64.0
29		>64.0	>64.0
30		>64.0	>64.0
31		>64.0	>64.0
32		>64.0	12.0
33		60.0	>64.0
34		22.0	>64.0
35		56.0	>64.0
36		>64.0	>64.0

synthesized series 3 of compounds substituted at the C-9, C-10, or N-7 position or possessing an azaindole moiety (Table 4). The synthesis of these derivatives is similar to the synthesis of series 1. Because of separation difficulties, compounds **41**, **44**, and **45** were isolated as mixture of *cis*-diastereisomers (6*R*,12a*R* and 6*S*,12a*S*).

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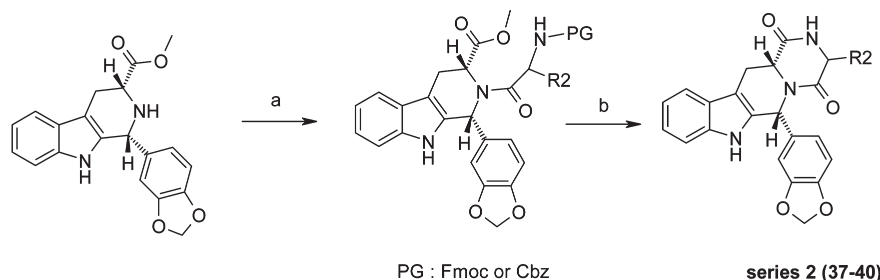
Scheme 3. General Synthetic Route to Compounds 37−40 (Series 2)[a]



[a] Conditions: (a) $FCOR_2NHProt$, pyridine, dioxane, darkness. (b) Deprotection: piperidine, 20% DMF (Fmoc) or H_2, $Pd(OH)_2/C$ (Cbz).

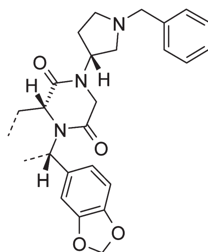
Table 3. Antiplasmodial in Vitro Activity and Cytotoxicity for Series 2

Cpd	R2	Antiplasmodial activity IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
		PfGHA	MRC5
37		60.0	>64.0
38		4.0	>64.0
39		25.0	35.0
40		>64.0	>64.0

Methylation of the indolic nitrogen led to a significant loss of activity, reminiscent of the SAR for PDE5. Substitution of the indole group in the 5-position with a bulky bromine atom (**41**) had a detrimental effect. This effect is less marked with the smaller fluorine atom (**44** and **45**). The azaindole group, with approximately the same hindrance as the indole group, seems to be better tolerated.

Overall, and although they present some differences, SARs on *Plasmodium* and on PDE5 display striking similarities. This observation suggests that active compounds described here are genuine inhibitors of the PDE activity of the parasite, leading to the disruption of its life cycle. We also verify that the stereochemical requirement for HsPDE5 inhibition[33] was also essential for antiplasmodial activity. The *trans*-epimer (6*S*,12a*R*) of the

Table 4. Antiplasmodial in Vitro Activity and Cytotoxicity for Series 3



Cpd	Indole moiety	Antiplasmodial activity IC_{50} (µM) PfGHA	Cytotoxicity IC_{50} (µM) MRC5
10	N H	0.50	10.00
41	Br, N H [a]	7.00	10.00
42	N CH_3	9.00	>64.00
43	N, N H	0.97	22.00
44	F, N H [a]	1.26	16.00
45	F, N H [a]	1.70	17.80

[a] Mixtures of two *cis*-diastereoisomers were tested (6*R*,12a*R* and 6*S*,12a*S*).

active compound **10** was tested (**46**) and it was confirmed that it is 100-fold less potent than compound **10** (Table 5). This difference in activity is also observed for hPDE5.

All SAR established by us on antiplasmodial activity (Figure 5) and by others on PDE5 suggest that our tetrahydro-β-carboline diketopiperazine are inhibitors of *Plasmodium* PDE activity. In order to validate this hypothesis, we evaluated compounds **10** and **14** as inhibitors of cyclic nucleotide (cXMP) hydrolysis in *P. falciparum*. cGMP and cAMP hydrolysis was measured in lysates in the presence or absence of inhibitors **10** and **14**. Dipyridamole (**47**) and zaprinast (**48**), two nonselective hPDE inhibitors, were also used (Table 6).[34]

In the vehicle sample, the cyclic phosphonucleotides are totally hydrolyzed by the lysate containing phosphodiesterase activity (0% inhibition). We confirmed that nonselective dipyridamole and zaprinast were able to inhibit the hydrolytic activity of the parasite lysate at 20 μM for both cAMP and cGMP. Despite this PDE inhibition, their activity on whole *Plasmodium* is weak, probably due to poor penetration within erythrocyte and parasite. Compounds **10** and **14** were able to inhibit 60% of the cGMP hydrolytic activity of the lysate, while we observed only weak inhibition of cAMP hydrolysis. These results suggest that our derivatives are inhibitors of at least one cGMP-hydrolyzing PfPDE, sufficient for antiparasitic activity. Activities on isolated enzymes should be confirmed as soon as functional recombinant PfPDEs can be isolated.

■ CONCLUSION

In this paper, we show that known drugs with no anti-infective activity can be used as an efficient source of inspiration for the design of antiplasmodial compounds. To maximize the chance of

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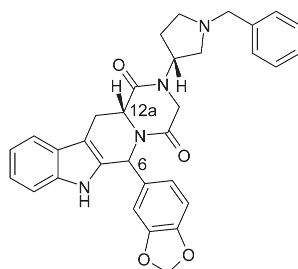
Table 5. Antiplasmodial in Vitro Activity, Cytotoxicity, and Configuration



		antiplasmodial activity IC_{50} (μM)	cytotoxicity IC_{50} (μM)
compd	configuration	PfGHA	MRC5
10	(6*R*,12a*R*)	0.5	10.0
46	(6*S*,12a*R*)	50.0	>64.0

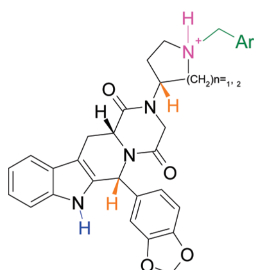


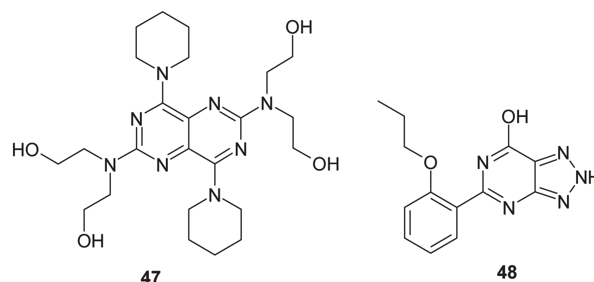
Figure 5. Schematic depiction of the pharmacophore: unsubstituted indole ring (blue), (*R*) configuration in positions 6 and 3′ (orange), tertiary amine included in a pyrrolidine or piperidine ring (pink) and substituted by a methylene aromatic ring (green).

success, we have deliberately chosen a drug that acts on a human enzyme having closely related orthologues in the parasite: the drug-to-genome-to-drug approach. Capitalizing on sequence homology between human and parasite proteins, we have rapidly obtained, with limited chemistry resources, highly potent antiplasmodial compounds. Consistent with the homology of sequence, the mode of action of our compounds is strongly supported by the striking similarities between SARs for hPDE5 and antiplasmodial activity, and we demonstrated that some compounds are able to significantly inhibit cGMP hydrolysis and weakly inhibit cAMP hydrolysis of the parasite, strongly suggesting that at least one PDE is inhibited. The partial inhibition of cGMP hydrolysis does not contradict the hypothesis that inhibiting a PDE can be lethal for the parasite. Indeed, the esterase activity that remains in the lysate treated with our compounds could be due to a noncritical PDE isoform that is not inhibited by our compounds. To fully validate this hypothesis and excludes other mechanisms of action, it will be necessary to test our compounds on isolated enzymes. These results shed light on the role of PDEs in parasite growth and deserve further work on selectivity to deliver eventually compounds with suitable therapeutic index and therapeutic utility. This work will be described in a following paper.

EXPERIMENTAL SECTION

Biology. The standard screening methodologies were adopted as described by Cos et al.[35]

Table 6. Antiplasmodial Activity of 10, 14, and Two Unspecific PDE Inhibitors



compd	antiplasmodial activity IC_{50} (μM)
10	0.5
14	1.0
47	64.0
48	35.0[a]

[a] IC_{50} value published by Yuasa et al.[21]

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. Aliquots (200 μL) of infected human red blood cells suspension (1% parasitemia, 2% hematocrit) were 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. Aliquots (100 μL) of Malstat reagent were transferred in 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 nitro blue tetrazolium/phenazine ethosulfate (NBT/PES) solution and 2 h of incubation in the dark, the absorbance was spectrophotometrically read at 655 nm (Bio-Rad 3550-UV microplate reader). Percentage growth inhibition was calculated compared to the negative blanks. IC_{50} values are calculated from duplicate determinations with relative difference below 25%.

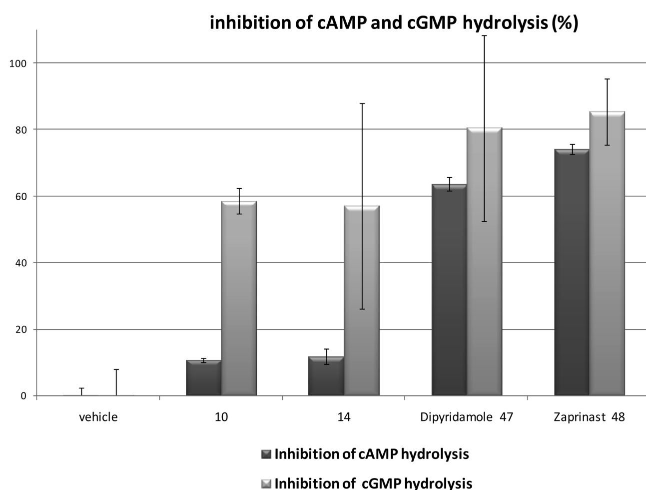


Figure 6. Inhibition of cXMP hydrolysis (100% corresponds to the signal measured in the absence of parasite lysate). Compounds were incubated at 20 μM for 6 h before measurement of cXMP concentrations.

Cytotoxicity Test upon MRC-5 Cells. MRC-5 SV2 cells, human fetal lung fibroblasts, were cultivated in minimum essential medium supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate, and 5% fetal calf serum at 37 °C and 5% CO_2. For the assay, 104 MRC-5 cells/well were seeded onto test plates containing the prediluted compounds and incubated at 37 °C and 5% CO_2 for 72 h. After 72 h of incubation, parasite growth was assessed fluorometrically by adding resazurin[8] for 24 h at 37 °C. Fluorescence was measured on a GENios Tecan fluorometer (excitation 530 nm, emission 590 nm). IC_{50} values are calculated from duplicate determinations with relative difference below 25%.

Inhibition of PDE Activity on Plasmodium Lysates. Fixed amounts of parasitic lysate (2 μL ~ 400 000 parasites) and cXMP (150 nM) were incubated 6 h at 37 °C in the presence of inhibitors at 20 μM (4% DMSO final). The assays were performed in black half area 96-well microplates (Corning 3694). Assay volume was 36.5 μL and the assay buffer contained 50 mM Tris-HCl, pH 7.4, 6 mM $MgCl_2$, 1 mM $MnCl_2$, and 4 mM dithiothreitol. At the end of the incubation, residual cXMP in the incubates were measured by use of a time-resolved fluorescence resonance energy transfer-based assay (HTRF technology) from Cisbio International. (quantification kits cGMP 62GMPPEB and cAMP 62AM6PEB). This method relies on competition between free cXMP and cXMP labeled with the dye d2 for binding to an anti-cXMP cryptate conjugate. Briefly, 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 read on a Victor[3]V (Wallac 1420 multilabel counter; Perkin-Elmer) and the 665/620 nm fluorescence ratio was calculated. Each inhibition assay was performed in duplicate and each well was read twice. Figure 6 reports the mean hydrolysis inhibition percentages with error bars.

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 by 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 LC-MS-MS triple-quadrupole system (Varian 1200ws). HPLC analyses were performed on a C18 TSK-GEL Super ODS 2 μm particle size column (50 × 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 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).

Protocol a: 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 sieve (4 Å). The solution was cooled to 0 °C, 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.

(1*R*,3*R*)-1-Benzo[1,3]dioxol-5-yl-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (4). Compound **4** was prepared according to general protocol a, starting from D-tryptophan methyl ester (3 g) and obtained as 950 mg of a white powder (23%). LC t_R = 3.7 min; MS (ESI+) m/z = 351 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.45 (d, J = 6.9 Hz, 1H), 7.39 (s, 1H), 7.16 (m, 1H), 7.10−7.01 (m, 2H), 6.81 (dd, J = 8.0 and 1.7 Hz, 1H), 6.75 (d, J = 1.7 Hz, 1H), 6.73 (d, J = 8.0 Hz, 1H), 5.87 (s, 2H), 5.09 (s, 1H), 3.88 (dd, J = 11.1 and 4.3 Hz, 1H), 3.75 (s, 3H), 3.14 (ddd, J = 15.0, 4.3, and 1.9 Hz, 1H), 2.91 (ddd, J = 15.0, 11.0, and 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 173.58, 148.58, 148.23, 136.56, 135.08, 127.52, 122.39, 120.04, 118.64, 111.36, 109.26, 109.20, 108.72, 101.64, 58.8, 57.27, 52.68, 26.07; mp = 160−161 °C.

(1*R*,3*R*)-1-Benzo[1,3]dioxol-5-yl-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester Tetrahydrochloride (4·HCl): Industrial Diastereoselective Synthesis. D-Tryptophan-OMe·HCl (1.0 g, 1 equiv) and piperonal (1.1 equiv) were suspended in 2-propanol (10 mL). The reaction mixture was refluxed under argon atmosphere for 22 h. The mixture was cooled in an ice bath to allow crystallization of the product. The residue was filtered and washed with cold 2-propanol to give **4**·HCl as a white powder (1.38 g, 90%). LC t_R = 3.8 min; MS (ESI+) m/z = 351 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 10.84 (s, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 6.88−7.14 (m, 5H), 6.10 (s, 2H), 5.85 (d, J = 1.2 Hz, 1H), 4.75 (s, 1H), 3.85 (s, 3H), 3.39 (s, 1H), 3.33 (d, J = 4.5 Hz, 1H), 3.29 (m, 1H), 3.25 (d, J = 1.8 Hz, 1H); mp = 220−222 °C.

Protocol b: Acylation. Intermediate tetrahydro-β-carboline **4** (1 equiv) was suspended in chloroform (0.2 M) with triethylamine. The reaction mixture was cooled at −10 °C, and 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 with brine, dried over $MgSO_4$, and evaporated to dryness.

(1*R*,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 (5). Compound **5** was prepared according to protocol b, starting from intermediate **4** (2.0 g) and obtained after precipitation in diethyl ether as a white powder (2 g, 83%). LC t_R = 6.2 min; MS (ESI+) m/z = 427 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.87 (s, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.15−7.32 (m, 3H), 6.84−6.90 (m, 2H), 6.65 (br s, 2H), 5.92 (s, 2H), 4.95 (br s, 1H), 4.20−4.40 (m, 2H), 3.70 (d, J = 16.2 Hz, 1H), 3.22 (m, 4H).

Protocol c: Cyclization under Classical Heating. Chloroacetyl intermediate (1 equiv) and amine (2 equiv) were 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.

Protocol d: Cyclization under Microwaves. Chloroacetyl intermediate was dissolved in ethanol (0.04 M) with 2.5 equiv of amine. The reaction mixture was heated by microwaves (100 W) for 2−15 min. The reaction mixture was totally evaporated and purified by chromatography.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6] pyrido[3,4-*b*]indole-1,4-dione (6). Compound **6** was prepared according to protocol c, starting from intermediate **5** (1 g) and ammonia (2 M in methanol) and obtained after precipitation in methanol as a white powder (535 mg, 60%). LC t_R = 4.6 min; MS (ESI+) m/z = 376 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 11.08 (s, 1H), 8.37 (s, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.30 (d, J = 7.9 Hz, 1H), 7.06 (td, J = 7.1 and 1.2 Hz, 1H), 6.99 (td, J = 7.0 and 1.0 Hz, 1H), 6.86 (d, J = 1.1 Hz, 1H), 6.77 (m, 2H), 6.17 (s, 1H), 5.92 (s, 1H), 4.40 (dd, J = 11.2 and 3.9 Hz, 1H), 4.04 (dd, J = 17.0 and 1.2 Hz, 1H), 3.72 (d, J = 16.9 Hz, 1H), 3.44 (dd, J = 15.8 and 4.8 Hz, 1H), 2.94 (dd, J = 15.6 and 11.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) 22.73, 45.15, 55.32, 55.64, 101.38, 105.01, 107.25, 108.49, 111.79, 118.54, 119.33, 119.48, 121.68, 126.21, 134.42, 136.56, 137.43, 146.54, 147.57, 168.20, 168.10.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-butyl-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 c, starting from intermediate **5** (100 mg) and butylamine (2 equiv) in ethanol and obtained after purification by flash chromatography (dichloromethane/methanol 99/1) as a white powder (45 mg, 44%). LC t_R = 6.0 min; MS (ESI+) m/z = 432 $[M + H]^+$; ^{1}H NMR (CD_2Cl_2) δ 8.11 (s, 1H), 7.57 (d, J = 6.5 Hz, 1H), 7.26 (d, J = 6.7 Hz, 1H), 7.12 (m, 2H), 6.82 (dd, J = 8.0 and 1.8 Hz, 1H), 6.73 (d, J = 1.7 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.13 (s, 1H), 5.85 (d, J = 1.3 Hz, 1H), 5.82 (d, J = 1.2 Hz, 1H), 4.26 (dd, J = 11.4 and 7.7 Hz, 1H), 4.05 (dd, J = 17.4 and 1.4 Hz, 1H), 3.87 (d, J = 17.4 Hz, 1H), 3.69 (dd, J = 16.0 and 4.6 Hz, 1H), 3.56 (m, 1H), 3.14 (ddd, J = 16.0, 11.5, and 1.3 Hz, 1H), 1.53 (m, 3H), 1.29 (s, J = 7.6 Hz, 2H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_2Cl_2) 14.57, 21.02, 25.18, 29.97, 46.99, 51.32, 57.22, 57.53, 102.37, 108.15, 109.02, 112.12, 119.57, 120.91, 121.31, 123.30, 125.44, 127.32, 134.13, 137.16, 137.60, 148.06, 148.90, 167.11, 168.27.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(2-morpholin-4-ylethyl)-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 c, starting from intermediate **5** (100 mg) and 2-(4-morpholino)ethylamine (2 equiv) in ethanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (45 mg, 39%). LC t_R = 4.0 min; MS (ESI+) m/z = 489 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 1H), 7.59 (m, 1H,), 7.15−7.27 (m, 3H), 6.84 (d, J = 7.8 Hz, 1H), 6.75 (s, 1H), 6.68 (d, J = 7.8 Hz, 1H), 6.17 (s, 1H), 5.85 (d, J = 5.3 Hz, 2H), 4.47 (m, 2H), 3.7−3.96 (m, 6H), 3.25 (m, 2H), 2.67 (m, 5H), 1.76 (m, 1H); ^{13}C NMR ($CDCl_3$) 23.40, 51.09, 53.63, 55.61, 56.12, 56.35, 66.77, 99.06, 101.19, 106.49, 107.44, 108.25, 111.25, 118.61, 120.14, 120.71, 122.55, 132.84, 135.34, 136.52, 147.16, 147.89, 149.49, 151.90, 167.34.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(3-imidazol-1-ylpropyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (9). Compound **9** was prepared according to protocol c, starting from intermediate **5** (100 mg) and 1-(3-aminopropyl)imidazole (2 equiv) in ethanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (32 mg, 28%). LC t_R = 4.0 min; MS (ESI+) m/z = 484 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 11.08 (s, 1H), 7.55 (d, J = 7.23 Hz, 1H), 7.32 (m, 2H), 7.02 (m, 3H), 6.86 (s, 1H), 6.78, (s, 2H), 6.16 (s, 1H), 5.92 (s, 2H), 4.45 (dd, J = 10.9 and 4.5 Hz, 1H), 4.23 (d, J = 17.0 Hz, 1H), 3.99 (m, 3H), 3.54 (m, 2H), 3.30 (m, 1H), 2.98 (dd, J = 15.6 and 11.7 Hz, 1H), 2.00 (qt, 2H); ^{13}C NMR (DMSO-d_6) δ 22.78, 27.93, 42.58, 44.02, 49.66, 55.09, 55.43, 100.93, 104.62, 106.87, 108.03, 111.35, 118.11, 118.90, 119.21, 119.71, 121.28, 125.74, 126.94, 133.88, 136.16, 136.88, 146.11, 147.09, 166.78, 167.09.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-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 (10). Compound **10** was prepared according to protocol c, starting from intermediate **5** (1.55 g) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (2 equiv) and obtained after purification by flash chromatography (dichloromethane/methanol 99/1) as a white powder (936 mg, 48%). LC t_R = 4.9 min; MS (ESI+) m/z = 535 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.60 (d, 1H, J = 6.6 Hz, 1H), 7.14−7.37 (m, 8H), 6.84 (dd, J = 8.1 and 1.5 Hz, 1H), 6.75 (d, J = 1.5 Hz, 1H), 6.68 (d, J = 8.1 Hz, 1H), 6.24 (s, 1H), 5.86 (d, J = 3.6 Hz, 2H), 5.12 (br s, 1H), 4.41 (br m, 1H), 4.22 (dd, J = 11.4 and 4.8 Hz, 1H), 3.99 (d, J = 17.7 Hz, 1H), 3.78 (br s, 2H), 3.70 (dd, J = 15.9 and 4.8 Hz, 1H), 3.22 (dd, J = 16.2 and 11.7 Hz, 1H), 1.79−3.09 (m, 6H); ^{13}C NMR ($CDCl_3$) 23.05, 29.66, 45.67, 51.53, 53.19, 55.98, 56.04, 56.64, 59.75, 101.02, 106.37, 107.40, 108.13, 111.11, 118.47, 119.99, 120.60, 122.38, 126.04, 127.02, 128.26, 128.39, 132.74, 135.09, 136.35, 138.68, 147.02, 147.75, 166.39, 168.21.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*S*)-1-benzylpyrrolidin-3-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (11). Compound **11** was prepared according to protocol c, starting from intermediate **5** (3.2 g) and (*S*)-(+)-1-benzyl-3-aminopyrrolidine (2 equiv) in methanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (2 g, 50%). LC t_R = 4.9 min; MS (ESI+) m/z = 535 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 11.1 (s, 1H), 7.54 (d, 1H, J = 7.6 Hz, 1H), 7.19−7.33 (m, 6H), 7.06 (t, J = 6.9 Hz, 1H), 6.99 (t, J = 7.6 Hz, 1H), 6.82 (s, 1H), 6.70−6.77 (m, 2H), 6.20 (s, 1H), 5.91 (s, 2H), 5.06 (br s, 1H), 4.39 (dd, J = 11.4 and 4.8 Hz, 1H), 4.20 (s, 2H), 3.34−3.59 (m, 3H), 2.85−3.01 (m, 2H), 2.56 (m, 1H), 2.43 (m, 1H), 2.15 (m, 2H), 1.77 (m, 1H); ^{13}CNMR (DMSO-d_6) 22.75, 27.83, 46.42, 51.53, 53.28, 55.11, 55.85, 58.21, 59.42, 101.40, 105.03, 107.14, 108.53, 111.83, 118.58, 119.43, 121.73, 126.20, 127.30, 128.67, 134.31, 136.57, 137.14, 139.31, 146.63, 147.64, 167.24, 168.20.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[2-(benzylmethylamino)ethyl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (12). Compound **12** was prepared according to protocol c, starting from intermediate **5** (100 mg), amine **12b** (2 equiv), and triethylamine (3 equiv) in ethanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a yellow powder (50 mg, 41%). LC t_R = 4.6 min; MS (ESI+) m/z = 523 $[M + H]^+$; ^{1}H NMR (MeOD) δ 7.54 (d, J = 7.2 Hz, 1H), 7.20−7.30 (m, 6H), 7.07 (m, 2H), 6.83 (m, 2H), 6.65 (d, J = 7.8 Hz, 1H), 6.24 (s, 1H), 5.81 (d, J = 5.4 Hz, 2H), 4.37 (dd, J = 11.4 and 3.6 Hz, 1H), 4.20 (dd, J = 17.1 and 1.5 Hz, 1H), 4.01 (d, J = 17.4 Hz, 1H), 3.47−3.69 (m, 5H), 3.12 (dd, J = 15.9 and 11.4 Hz, 1H), 2.62 (m, 2H), 2.23 (s, 3H); ^{13}C NMR (MeOD) 23.64, 42.06, 43.89, 51.11, 54.20, 56.12, 56.47, 62.54, 101.35, 107.15, 108.01, 111.10, 118.57, 119.92, 120.36, 122.31, 124.40, 124.53, 126.30, 127.02, 128.22, 128.94, 133.16, 136.20, 136.60, 147.90, 150.06, 166.19, 167.32.

[2-(Benzylmethylamino)ethyl]carbamic Acid *tert*-Butyl Ester (12a). *N*-Methylbenzylamine (114 μL, 1 equiv), 2-(Boc-amino)ethyl bromide (200 mg, 1 equiv), potassium iodide (74 mg, 0.5 equiv), and cesium carbonate (580 mg, 2 equiv) were dissolved in acetone and stirred at room temperature for 70 h. The reaction mixture was diluted with ethyl acetate, washed with water, dried over $MgSO_4$, and evaporated to dryness to give 160 mg of yellow oil (68%). LC t_R = 3.6 min; MS (ESI+) m/z = 265 $[M + H]^+$; ^{1}H NMR (CD_2Cl_2) δ 7.38−7.26 (m, 5H), 5.05 (m, 1H), 3.53 (s, 2H), 3.23 (q, J = 5.8 Hz, 2H), 2.51 (t, J = 6.0 Hz, 2H), 2.21 (s, 3H), 1.47 (s, 9H).

***N*^{1}-Benzyl-*N*^{1}-methylethane-1,2-diamine Dihydrochloride (12b).** Intermediate **12a** (160 mg, 600 μmol) was dissolved in dioxane (3 mL), 1.5 mL of HCl (4 N in dioxane) was added, and the

reaction mixture was stirred at room temperature for 20 h. Dioxane was removed by evaporation to give 125 mg of translucent oil (88%). LC t_R = 1.1 min; MS (ESI+) m/z = 165 [M + H]$^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[3-(benzylmethylamino)propyl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (13). Compound **13** was prepared according to protocol c, starting from intermediate **5** (100 mg), amine **13b** (2 equiv), and triethylamine (3 equiv) in ethanol (5 days) and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (30 mg, 24%). LC t_R = 4.7 min; MS (ESI+) m/z = 537 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.14 (s, 1H), 7.58 (dd, *J* = 6.2 and 1.9 Hz, 1H), 7.24 (m, 6H), 7.11 (m, 2H), 6.82 (dd, *J* = 8.0 and 1.8 Hz, 1H), 6.74 (d, *J* = 1.7 Hz, 1H), 6.66 (d, *J* = 7.9 Hz, 1H), 6.13 (s, 1H), 5.84 (d, *J* = 1.3 Hz, 1H), 5.81 (d, *J* = 1.3 Hz, 1H), 4.23 (dd, *J* = 10.7 and 3.9 Hz, 1H), 4.05 (dd, *J* = 17.4 and 1.4 Hz, 1H), 3.90 (d, *J* = 17.4 Hz, 1H), 3.67 (dd, *J* = 16.0 and 4.6 Hz, 1H), 3.58 (m, 1H), 3.45 (s, 2H), 3.34 (m, 1H), 3.13 (ddd, *J* = 16.0, 11.5, and 1.3 Hz, 1H), 2.38 (t, *J* = 6.9 Hz, 2H), 2.14 (s, 3H), 1.77 (m, 2H); ^{13}C NMR (CD_2Cl_2) 24.63, 25.83, 42.66, 45.71, 51.69, 55.39, 57.18, 57.47, 63.23, 97.29, 102.35, 107.46, 108.02, 109.02, 112.13, 119.55, 120.90, 121.35, 123.29, 128.02, 129.21, 130.09, 134.15, 137.15, 137.60, 138.25, 148.05, 148.91, 167.24, 168.28.

[3-(Benzylmethylamino)propyl]carbamic Acid *tert*-Butyl Ester (13a). *N*-Methylbenzylamine (108 μL, 1 equiv), 2-(Boc-amino)propyl bromide (200 mg, 1 equiv), potassium iodide (69 mg, 0.5 equiv), and cesium carbonate (547 mg, 2 equiv) were dissolved in acetone and stirred at room temperature for 70 h. The reaction mixture was diluted with ethyl acetate, washed 3 times with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and evaporated to dryness to give 210 mg of yellow oil (90%). LC t_R = 3.4 min; MS (ESI+) m/z = 279 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 7.34−7.25 (m, 5H), 5.62 (m, 1H), 3.51 (s, 2H), 3.18 (q, *J* = 6.1 Hz, 2H), 2.47 (t, *J* = 6.3 Hz, 2H), 2.18 (s, 3H), 1.69 (qt, *J* = 6.4 Hz, 2H), 1.47 (s, 9H).

***N*^{1}-Benzyl-*N*^{1}-methylpropane-1,3-diamine Dihydrochloride (13b).** Intermediate **13a** (210 mg) was dissolved in dioxane (3 mL), 1.5 mL of HCl (4 N in dioxane) was added, and the reaction mixture was stirred at room temperature for 20 h. Dioxane was removed by evaporation to give 190 mg of translucent oil (90%). LC t_R = 1.1 min; MS (ESI+) m/z = 179 [M + H]$^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(1-benzylpiperidin-4-yl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (14). Compound **14** was prepared according to protocol c, starting from intermediate **5** (100 mg) and 4-amino-1-benzylpiperidine (2 equiv) in ethanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (76 mg, 59%). LC t_R = 4.7 min; MS (ESI+) m/z = 549 [M + H]$^+$; ^{1}H NMR (MeOD) δ 7.54 (d, *J* = 7.2 Hz, 1H), 7.27−7.34 (m, 6H), 7.06 (m, 2H), 6.79 (dd, *J* = 8.1 and 1.8 Hz, 1H), 6.75 (d, *J* = 1.8 Hz, 1H), 6.67 (d, *J* = 8.1 Hz, 1H), 6.21 (s, 1H), 5.82 (d, *J* = 3.6 Hz, 2H), 4.37 (m, 2H), 4.06 (d, *J* = 17.1 Hz, 1H), 3.96 (d, *J* = 17.1 Hz, 1H), 3.63 (dd, *J* = 15.9 and 4.8 Hz, 1H), 3.53 (s, 2H), 3.13 (dd, *J* = 15.0 and 11.4 Hz, 1H), 2.97 (m, 2H), 2.09 (m, 2H), 1.68−1.88 (m, 3H), 1.54 (m, 1H); ^{13}C NMR (MeOD) 22.71, 27.54, 27.64, 45.10, 51.25, 52.17, 52.28, 56.03, 56.17, 62.25, 100.96, 104.73, 106.83, 107.51, 110.85, 117.52, 118.89, 119.77, 121.35, 125.96, 127.13, 127.96, 129.29, 133.22, 135.96, 136.89, 146.86, 147.70, 167.64, 168.14.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[1-(1-methyl-1*H*-imidazol-2-ylmethyl)piperidin-4-yl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione (15). Compound **15** was prepared according to protocol d, starting from intermediate **5** (100 mg) and 4-amino-1-benzylpiperidine **15b** (2 equiv) in ethanol and obtained after purification by chromatography (dichloromethane/methanol) as a white powder (32 mg, 25%). LC t_R = 4.25 min; MS (ESI+) m/z = 553 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 11.10 (s, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.08 (s, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.82−6.67 (m, 4H), 6.16 (s, 1H), 5.92 (s, 2H), 4.42 (dd, *J* = 11.3 and 4.2 Hz, 1H), 4.08 (d, *J* = 16.5 Hz, 1H), 3.89 (d, *J* = 16.9 Hz, 1H), 3.65 (s, 3H), 3.53−3.43 (m, 3H), 3.10−2.82 (m, 5H), 2.09 (t, *J* = 10.7 Hz, 2H), 1.73−1.44 (m, 3H). ^{13}C NMR (DMSO-d_6) 9.00, 22.90, 28.39, 33.16, 45.94, 51.00, 52.63, 53.54, 55.15, 55.92, 101.40, 104.98, 107.15, 108.53, 111.82, 118.57, 119.39, 121.71, 122.66, 126.00, 126.18, 134.31, 136.56, 137.15, 144.55, 146.59, 147.60, 167.28, 167.83.

[1-(1-Methyl-1*H*-imidazol-2-ylmethyl)piperidin-4-yl]carbamic Acid *tert*-Butyl Ester (15a). 2-(Chloromethyl)-1-methyl-1*H*-imidazole hydrochloride (800 mg, 1 equiv) and 4-(*N*-Boc-amino)piperidine (959 mg, 1 equiv) were dissolved in dichloromethane (48 mL), and DIEA (2.5 mL, 3 equiv) was added. The reaction mixture was stirred at room temperature for 48 h. Solvent was evaporated, and the residue was dissolved in dichloromethane, washed with saturated $NaHCO_3$ aqueous solution and water, dried over $MgSO_4$, and evaporated to dryness to give 1.25 g of a yellow powder (89%). LC t_R = 2.6 min; MS (ESI+) m/z = 295 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 7.05 (s, 1H), 6.73 (m, 2H), 3.61 (s, 3H), 3.45 (s, 2H), 3.18 (m, 1H), 2.68 (d, *J* = 11.8 Hz, 2H), 1.95 (t, *J* = 11.6 Hz, 2H), 1.64 (d, *J* = 10.7 Hz, 2H), 1.36 (s, 9H), 1.29 (m, 2H).

1-(1-Methyl-1*H*-imidazol-2-ylmethyl)piperidin-4-ylamine Trihydrochloridel (15b). Intermediate **15a** (940 mg) was dissolved in a mixture of methanol (16 mL) and 4 N HCl in dioxane (16 mL). The reaction mixture was stirred at 40 °C for 5 h. After evaporation, compound **15b** was obtained as a white powder (957 mg, 100%). LC t_R = 0.6 min; MS (ESI+) m/z = 195 [M + H]$^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(*R*)-pyrrolidin-3-yl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (16). Intermediate **10** (720 mg, 1 equiv) was dissolved in ethyl acetate (0.07 M), and $Pd(OH)_2$ on carbon (1 equiv w/w) was suspended. The reaction mixture was stirred at room temperature under H_2 atmosphere for 24−48 h. The reaction mixture was filtered on Celite, evaporated to dryness, and precipitated in diethyl ether to obtain compound **16** as a white powder (412 mg, 69%). LC t_R = 4.0 min; MS (ESI+) m/z = 445 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 11.12 (s, 1H), 8.31 (s, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.08−6.97 (m, 2H), 6.83−6.72 (m, 3H), 6.17 (s, 1H), 5.92 (s, 1H), 4.92 (m, 1H), 4.43 (dd, *J* = 11.1 and 4.8 Hz, 1H), 4.17 (d, *J* = 16.6 Hz, 1H), 4.00 (d, *J* = 16.8 Hz, 1H), 3.47 (dd, *J* = 15.8 and 4.9 Hz, 1H), 3.14−2.88 (m, 5H), 2.08−2.03 (m, 1H), 1.75−1.66 (m, 1H); ^{13}C NMR (DMSO-d_6) 22.95, 29.03, 45.60, 46.58, 47.41, 53.34, 55.29, 55.95, 101.41, 104.96, 107.22, 108.53, 111.83, 118.58, 119.36, 119.55, 121.74, 126.17, 134.30, 136.58, 137.16, 146.61, 147.60, 167.70, 167.86.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-piperidin-4-yl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (17). Intermediate **17** (50 mg) was dissolved in ethanol (3.75 mL), ammonium formate (5 equiv) was added, and Pd on carbon (0.2 equiv w/w) was suspended. The reaction mixture was stirred and refluxed for 30 min. The reaction mixture was filtered on Celite, evaporated to dryness, and precipitated in diethyl ether to give 30 mg of a white powder (77%). LC t_R = 4.1 min; MS (ESI+) m/z = 459 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 11.16 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 6.7 Hz, 1H), 7.07 (t, *J* = 7.1 Hz, 1H), 6.99 (t, *J* = 8.6 Hz, 1H), 6.83 (d, *J* = 1.6 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.73 (dd, *J* = 8.1 and 1.7 Hz, 1H), 6.19 (s, 1H), 5.92 (s, 2H), 4.45 (dd, *J* = 12.0 and 5.2 Hz, 1H), 4.12 (d, *J* = 15.9 Hz, 1H), 3.81 (d, *J* = 16.6 Hz, 1H), 3.48 (dd, *J* = 15.9 and 5.0 Hz, 1H), 3.26 (m, 2H), 3.16 (s, 1H), 2.96 (m, 3H), 1.90 (m, 2H), 1.72 (m, 1H), 1.62 (m, 1H); ^{13}C NMR (DMSO-d_6) 22.82, 25.92, 43.40, 43.47, 45.89, 48.73, 55.17, 55.91, 101.43, 104.88, 107.10, 108.55, 111.83, 118.60, 119.37, 121.75, 126.16, 134.25, 136.55, 137.09, 146.62, 147.63, 167.40, 167.63.

(6*R*,12a*R*)-2-Amino-6-benzo[1,3]dioxol-5-yl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (18). Compound **18** was prepared according to protocol d,

starting from intermediate **5** (100 mg, 1 equiv) and hydrazine hydrate (2.5 equiv) in ethanol and obtained after filtration as a white powder (55 mg, 56%). LC t_R = 4.51 min; MS (ESI+) m/z = 391 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 11.01 (s, 1H), 7.55 (d, J = 7.5 Hz, 1H), 7.30 (d, J = 7.5 Hz, 1H), 7.09−6.98 (m, 2H), 6.89−6.76 (m, 3H), 6.11 (s, 1H), 5.93 (s, 2H), 5.12 (s, 2H), 4.47−4.42 (m, 1H), 4.27 (d, J = 17.0 Hz, 1H), 3.97 (d, J = 17.0 Hz, 1H), 3.61−3.55 (m, 1H), 3.04−2.95 (m, 1H); ^{13}C NMR (DMSO-d_6) 23.93, 53.79, 55.87, 56.03, 101.36, 105.25, 107.39, 108.43, 111.77, 118.57, 119.34, 119.76, 121.71, 126.21, 134.47, 136.69, 137.57, 146.49, 147.48, 165.07, 166.74.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[2-(4-hydroxyphenyl) ethyl]-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (19). Compound **19** was prepared according to protocol c, starting from intermediate **5** (100 mg) and tyramine (2 equiv) in ethanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (73 mg, 62%). LC t_R = 5.5 min; MS (ESI+) m/z = 496 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 11.05 (s, 1H), 9.18 (s, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.30 (d, J = 7.6 Hz, 1H), 7.07−6.64 (m, 7H), 6.13 (s, 1H), 5.93 (s, 1H), 4.37 (d, J = 7.6 Hz, 1H), 4.09 (d, J = 17.4 Hz, 1H), 3.80 (d, J = 16.7 Hz, 1H), 3.59−3.46 (m, 3H), 2.91 (dd, J = 15.2 and 11.8 Hz, 1H), 2.7 (m, 2H); ^{13}C NMR (DMSO-d_6) 23.31, 32.28, 47.66, 50.67, 55.48, 55.89, 101.40, 105.09, 107.27, 108.49, 111.79, 115.66, 118.55, 119.39, 121.70, 126.21, 129.17, 130.07, 134.38, 136.61, 137.40, 146.55, 147.56, 156.19, 166.86, 167.50.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-morpholin-4-ylbenzyl)-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 d, starting from intermediate **5** (70 mg), 4-morpholinobenzylamine chlorhydrate (2 equiv), and triethylamine (4 equiv) in methanol and obtained after purification by flash chromatography (dichloromethane/methanol) as a white powder (63 mg, 57%). LC t_R = 5.9 min; MS (ESI+) m/z = 551 $[M + H]^+$; ^{1}H NMR (CD_2Cl_2) δ 8.02 (br s, 1H), 7.64 (dd, J = 6.6 and 2.7 Hz, 1H), 7.31 (m, 1H), 7.12−7.24 (m, 4H), 6.86−6.91 (m, 3H), 6.78 (d, J = 1.5 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.15 (s, 1H), 5.90 (dd, J = 8.1 and 1.5 Hz, 2H), 4.77 (d, J = 14.4 Hz, 1H), 4.35−4.44 (m, 2H), 3.77−3.98 (m, 7H), 3.26 (ddd, J = 16.2, 11.7, and 1.5 Hz, 1H), 3.15 (m, 4H); ^{13}C NMR (CD_2Cl_2) 24.91, 50.03, 50.17, 50.68, 57.39, 57.61, 67.88, 102.43, 107.59, 108.15, 109.08, 112.16, 116.68, 119.63, 121.01, 121.39, 123.39, 126.50, 127.50, 130.47, 131.10, 134.17, 137.21, 137.65, 152.75, 167.39, 168.21.

Protocol e for Nortadalafil Arylation. Intermediate **6** (1 equiv), CuI (2 equiv), K_3PO_4 (2 equiv), (±)-*trans*-1,2-diaminocyclohexane (4.4 equiv), and iodo derivatives (1 equiv) were dissolved in anhydrous dioxane (0.13 M) in inert vessels and stirred at 14 °C for 14 days, The reaction mixture was diluted with ethyl acetate and washed with NH_3/water (5/95) and water. The organic layer was dried over $MgSO_4$, evaporated to dryness, and purified by chromatography.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-morpholin-4-ylmethylphenyl)-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 e, starting from **6** (75 mg) and 4-(4-iodobenzyl)morpholine (60 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (43 mg, 40%). LC t_R = 4.3 min; MS (ESI+) m/z = 551 $[M + H]^+$; ^{1}H NMR (CD_2Cl_2) δ 8.12 (s, 1H), 7.65 (d, J = 7.1 Hz, 1H), 7.45 (d, J = 9.2 Hz, 2H), 7.32 (m, 3H), 7.17 (m, 2H), 6.93 (dd, J = 8.0 and 1.8 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.29 (s, 1H), 5.93 (d, J = 1.3 Hz, 1H), 5.90 (d, J = 1.3 Hz, 1H), 4.55 (m, 2H), 4.28 (d, J = 16.9 Hz, 1H), 3.79 (dd, J = 15.9 and 4.8 Hz, 1H), 3.71 (m, 4H), 3.55 (s, 2H), 3.33 (ddd, J = 15.9, 11.4, and 1.3 Hz, 1H), 2.48 (m, 4H); ^{13}C NMR (CD_2Cl_2) 23.42, 52.72, 56.29, 56.53, 62.55, 66.81, 101.35, 106.20, 107.34, 108.01, 111.16, 118.52, 119.91, 120.52, 122.30, 124.84, 126.21, 129.79, 133.09, 135.74, 136.56, 139.12, 147.12, 147.90, 166.50, 167.21.

4-[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]benzyl]piperazine-1-carboxylic Acid *tert*-Butyl Ester (22). Compound **22** was prepared according to protocol e, starting from **6** (75 mg) and *tert*-butyl-(3-iodobenzyl)tetrahydro-1(2*H*)-pyrazine carboxylate (80 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (30 mg, 23%). LC t_R = 5.25 min; MS (ESI+) m/z = 650 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 1H), 7.63 (d, J = 6.8 Hz, 1H), 7.36 (m, 5H), 7.18 (m, 2H), 6.90 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 1.6 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.32 (s, 1H), 5.90 (d, J = 5.2 Hz, 2H), 4.60 (d, J = 16.8 Hz, 1H), 4.49 (dd, J = 10.8 and 4.5 Hz, 1H), 4.34 (d, J = 16.8 Hz, 1H), 3.77 (dd, J = 16.0 and 4.9 Hz, 1H), 3.60 (m, 4H), 3.35 (dd, J = 15.9 and 11.4 Hz, 1H), 2.58 (m, 2H), 1.64 (m, 4H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) 23.29, 28.39, 43.14, 52.60, 52.90, 56.22, 56.56, 62.19, 79.92, 101.21, 106.36, 107.65, 108.29, 111.31, 118.60, 120.18, 120.96, 122.60, 126.12, 128.49, 129.43, 132.79, 134.93, 136.51, 140.09, 147.27, 147.93, 154.55, 166.55, 167.26.

4-[4-[(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]phenyl]piperazine-1-carboxylic Acid *tert*-Butyl Ester (23). Compound **23** was prepared according to protocol e, starting from **6** (75 mg) and *tert*-butyl-4-(4-iodophenyl)tetrahydro-1(2*H*)-pyrazine carboxylate (77 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (44 mg, 35%). LC t_R = 6.8 min; MS (ESI+) m/z = 636 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H), 7.64 (m, 1H), 7.16−7.32 (m, 8H), 6.90 (dd, J = 8 and 1.6 Hz, 1H), 6.80 (d, J = 1.5 Hz, 1H), 6.72 (d, J = 8 Hz, 1H), 6.3 (s, 1H), 5.90 (d, J = 6.0 Hz, 1H), 5.89 (d, J = 6.0 Hz, 1H), 4.50 (m, 2H), 4.28 (d, J = 17 Hz, 1H), 3.78 (m, 5H), 3.34 (dd, J = 16 and 11.6 Hz, 1H), 3.24 (m, 4H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$) 23.40, 28.42, 53.17, 56.33, 56.59, 80.22, 101.20, 106.46, 107.62, 108.28, 111.28, 117.56, 118.63, 120.16, 120.90, 122.58, 126.08, 132.80, 135.02, 136.52, 147.25, 147.93, 154.59, 166.51, 167.30.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-piperazin-1-ylphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione Hydrochloride (24). Intermediate **24** (15 mg) was dissolved in methanol (200 μL). HCl (4 N in dioxane, 10 μL) was added to the solution, and it was stirred at room temperature for 24 h. Solvents were removed by evaporation to give a white powder (14 mg, 100%). LC t_R = 4.4 min; MS (ESI+) m/z = 536 $[M + H]^+$; ^{1}H NMR (MeOD) δ 7.55 (d, J = 7.7 Hz, 1H), 7.32 (m, 3H), 7.10 (m, 4H), 7.09 (m, 2H), 6.88 (m, 2H), 6.73 (d, J = 7.8 Hz, 1H), 6.32 (s, 1H), 5.87 (s, 2H), 4.69 (d, J = 17.0 Hz, 1H), 4.62 (dd, J = 11.2 and 4.4 Hz, 1H), 4.16 (d, J = 16.8 Hz, 1H), 3.74 (m, 1H), 3.43 (m, 4H), 3.39 (m, 4H), 3.25 (m, 1H); ^{13}C NMR (MeOD) 22.89, 43.27, 46.32, 48.11, 52.87, 56.24, 56.44, 101.02, 104.69, 106.99, 107.59, 108.59, 110.90, 117.07, 117.57, 118.93, 119.95, 121.40, 125.97, 126.28, 126.78, 133.28, 133.40, 135.98, 136.91, 146.91, 147.75, 149.07, 167.75, 167.96.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(3-piperazin-1-ylmethylphenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6] pyrido[3,4-*b*]indole-1,4-dione Dihydrochloride (25). Intermediate **25** (15 mg) was dissolved in methanol (200 μL). HCl (4 N in dioxane, 10 μL) was added to the solution, and it was stirred at room temperature for 24 h. Solvents were removed by evaporation to give a white powder (14 mg, 100%). LC t_R = 4.1 min; MS (ESI+) m/z = 550 $[M + H]^+$; ^{1}H NMR (MeOD) δ 7.71 (s, 1H), 7.59 (m, 5H), 7.31 (d, J = 7.5 Hz, 1H), 7.09 (m, 2H), 6.88 (m, 2H), 6.73 (d, J = 7.8 Hz, 1H), 6.35 (s, 1H), 5.88 (m, 2H), 4.81 (d, J = 16.9 Hz, 1H), 4.68 (dd, J = 10.9 and 4.2 Hz, 1H), 4.49 (s, 2H), 4.31 (d, J = 16.6 Hz, 1H), 3.73 (dd, J = 20.6 and 16.1 Hz, 1H), 3.60 (m, 8H), 3.27 (dd, J = 16.2 and 12.1 Hz, 1H); ^{13}C NMR (MeOD) 22.78, 40.77, 52.36, 56.12, 56.41, 59.82, 101.03, 104.57, 107.06, 107.61, 110.93, 117.51, 118.96, 120.06, 121.45, 125.92, 126.76, 128.32, 129.51, 129.91, 130.02, 133.24, 135.89, 136.92, 140.88, 146.94, 147.75, 167.81, 167.95.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-phenyl-2,3,6,7,12,12a-hezxahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (26). Compound **26** was prepared according to protocol e, starting from **6** (50 mg) and iodobenzene (15 μL) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (50 mg, 83%). LC t_R = 5.91 min; MS (ESI+) m/z = 452 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.01 (s, 1H), 7.66 (d, *J* = 6.6 Hz, 1H), 7.46 (m, 2H), 7.35 (m, 4H), 7.18 (m, 2H), 6.94 (dd, *J* = 8.0 and 1.7 Hz, 1H), 6.85 (d, *J* = 1.7 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.29 (s, 1H), 5.92 (d, *J* = 7.8 Hz, 1H), 5.91 (d, *J* = 7.8 Hz, 1H), 4.56 (m, 2H), 4.30 (d, *J* = 17.0 Hz, 1H), 3.80 (dd, *J* = 15.9 and 4.7 Hz, 1H), 3.34 (dd, *J* = 16.0 and 11.3 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.49, 52.70, 56.50, 56.65, 101.36, 106.55, 107.32, 108.02, 111.10, 111.90, 118.57, 119.98, 120.62, 122.39, 125.10, 127.11, 129.14, 133.06, 135.75, 136.70, 139.04, 147.09, 147.74, 166.59, 167.76.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-thiophen-3-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 e, starting from **6** (50 mg) and 3-iodothiophene (14 μL) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (36 mg, 60%). LC t_R = 6.11 min; MS (ESI+) m/z = 458 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.16 (s, 1H), 7.65 (d, *J* = 6.8 Hz, 1H), 7.34 (m, 4H), 7.18 (m, 2H), 6.91 (dd, *J* = 8.0 and 1.7 Hz, 1H), 6.82 (d, *J* = 1.7 Hz, 1H), 6.73 (d, *J* = 8 Hz, 1H), 6.27 (s, 1H), 5.89 (d, *J* = 6.5 Hz, 1H), 5.88 (d, *J* = 6.5 Hz, 1H), 4.52 (m, 2H), 4.43 (d, *J* = 16.9 Hz, 1H), 3.82 (dd, *J* = 16.9 and 4.6 Hz, 1H), 3.34 (ddd, *J* = 15.9, 11.5, and 1.0 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.59, 29.69, 52.31, 56.34, 56.51, 101.36, 106.18, 107.31, 108.01, 111.18, 114.48, 118.53, 119.94, 120.51, 122.35, 122.89, 124.91, 126.20, 132.99, 135.61, 136.57, 138.24, 147.13, 147.89, 165.40, 166.75.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-methoxyphenyl)-2,3,6,7,12,12a-hexahydro-pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (28). Compound **28** was prepared according to protocol e, starting from **6** (50 mg) and 4-iodoanisole (31 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (30 mg, 47%). LC t_R = 5.93 min; MS (ESI+) m/z = 482 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.17 (s, 1H), 7.65 (d, *J* = 6.6 Hz, 1H), 7.34−7.13 (m, 5H), 6.98 (d, *J* = 6.8 Hz, 2H), 6.93 (dd, *J* = 8.1 and 1.7 Hz, 1H), 6.85 (d, *J* = 1.7 Hz, 1H), 6.75 (d, *J* = 8.1 Hz, 1H), 6.27 (s, 1H), 5.91 (d, *J* = 7.6 Hz, 1H), 5.90 (d, *J* = 7.6 Hz, 1H), 4.53 (m, 2H), 4.25 (d, *J* = 17.0 Hz, 1H), 3.84 (s, 3H), 3.78 (dd, *J* = 16.0 and 4.7 Hz, 1H), 3.33 (dd, *J* = 17.0 and 12.5 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.50, 55.48, 56.38, 56.52, 101.37, 106.22, 107.32, 108.01, 111.17, 113.69, 114.36, 118.54, 119.89, 120.49, 122.28, 126.21, 126.58, 132.97, 133.11, 135.82, 136.55, 147.10, 147.89, 158.54, 166.57, 167.28.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-butylphenyl)-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 e, starting from **6** (50 mg) and 4-*n*butylbenzene (35 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (32 mg, 48%). LC t_R = 7.49 min; MS (ESI+) m/z = 508 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.25 (s, 1H), 7.65 (d, *J* = 6.5 Hz, 1H), 7.33−7.13 (m, 7H), 6.92 (dd, *J* = 8 and 1.7 Hz, 1H), 6.85 (d, *J* = 1.7 Hz, 1H), 6.74 (d, *J* = 8 Hz, 1H), 6.29 (s, 1H), 5.90 (d, *J* = 7.0 Hz, 1H), 5.89 (d, *J* = 7.0 Hz, 1H), 4.53 (m, 2H), 4.27 (d, *J* = 17 Hz, 1H), 3.79 (dd, *J* = 16.0 and 4.7 Hz, 1H), 3.32 (dd, *J* = 15.9 and 11.3 Hz, 1H), 2.67 (t, *J* = 7.7 Hz, 2H), 1.65 (m, 2H), 1.40 (m, 2H), 0.97 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (CD_2Cl_2) 13.72, 22.37, 23.44, 29.70, 33.56, 35.13, 53.07, 56.29, 56.55, 101.36, 106.19, 107.32, 108.02, 111.20, 118.55, 119.89, 120.43, 122.26, 124.95, 125.28, 126.21, 126.43, 129.13, 129.43, 133.12, 135.78, 136.55, 137.76, 142.29, 147.09, 147.89, 166.51, 167.34.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(3-chlorophenyl)-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 e, starting from **6** (50 mg) and 4-iodonitrobenzene (16.5 μL) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (42 mg, 65%). LC t_R = 6.55 min; MS (ESI+) m/z = 486−488 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.34 (s, 1H), 7.65 (d, *J* = 6.5 Hz, 1H), 7.44−7.05 (m, 7H), 6.91 (dd, *J* = 7.9 and 1.7 Hz, 1H), 6.84 (d, *J* = 1.7 Hz, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 6.29 (s, 1H), 5.90 (d, *J* = 6.7 Hz, 1H), 5.89 (d, *J* = 6.7 Hz, 1H), 4.54 (m, 2H), 4.28 (d, *J* = 16.7 Hz, 1H), 3.78 (dd, *J* = 16.0 and 4.8 Hz, 1H), 3.34 (dd, *J* = 15.9 and 11.4 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.35, 52.62, 56.30, 56.47, 101.39, 106.10, 107.35, 108.04, 111.18, 118.54, 119.98, 120.58, 122.38, 123.05, 125.29, 126.17, 127.14, 130.19, 132.99, 134.43, 135.55, 136.54, 141.17, 147.18, 147.92, 166.55, 166.93.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-thiophen-2-yl-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 e, starting from **6** (50 mg) and 2-iodothiophene (15 μL) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (15 mg, 25%). LC t_R = 6.38 min; MS (ES+) m/z = 458 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.04 (s, 1H), 7.65 (d, *J* = 6.3 Hz, 1H), 7.33 (m, 1H), 7.18−7.08 (m, 4H), 6.98 (m, 1H), 6.90 (dd, *J* = 7.9 and 1.7 Hz, 1H), 6.80 (d, *J* = 1.7 Hz, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 6.24 (s, 1H), 5.90 (d, *J* = 7.3 Hz, 2H), 4.62 (m, 2H), 4.50 (d, *J* = 17.0 Hz, 1H), 3.87 (dd, *J* = 16.0 and 4.5 Hz, 1H), 3.33 (m, 1H); ^{13}C NMR (CD_2Cl_2) 23.92, 26.69, 51.19, 56.18, 56.75, 101.37, 106.11, 107.22, 108.02, 111.12, 112.76, 118.54, 120.00, 120.40, 120.57, 122.45, 126.18, 132.84, 135.57, 136.60, 140.44, 147.16, 147.91, 163.44, 165.51.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-trifluoromethylphenyl)-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 e, starting from **6** (50 mg) and 4-iodobenzotrifluoride (19 μL) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (21 mg, 30%). LC t_R = 6.90 min; MS (ESI+) m/z = 520 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.16 (s, 1H), 7.74 (d, *J* = 8.5 Hz, 2H), 7.65 (m, 1H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.34 (m, 1H), 7.18 (m, 2H), 6.92 (dd, *J* = 8.0 and 1.7 Hz, 1H), 6.84 (d, *J* = 1.7 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.31 (s, 1H), 5.90 (d, *J* = 7.0 Hz, 1H), 5.89 (d, *J* = 7.0 Hz, 1H), 4.63 (d, *J* = 16.6 Hz, 1H), 4.55 (dd, *J* = 11.4 and 4.6 Hz, 1H), 4.34 (d, *J* = 16.6 Hz, 1H), 3.80 (dd, *J* = 16.0 and 4.9 Hz, 1H), 3.37 (dd, *J* = 16 and 12.4 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.30, 52.36, 56.26, 56.50, 101.40, 106.04, 107.38, 108.03, 111.20, 118.54, 120.01, 120.64, 122.42, 124.01 (q, *J* = 288.75 Hz), 124.84, 126.16, 126.18 (q, *J* = 3.75 Hz), 128.42 (q, *J* = 32.8 Hz), 132.97, 135.44, 136.55, 143.14, 147.21, 147.92, 166.67, 166.90.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-(4-chlorophenyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (33). Compound **33** was prepared according to protocol e, starting from **6** (50 mg) and 1-chloro-4-iodobenzene (32 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (18 mg, 28%). LC t_R = 6.54 min; MS (ESI+) m/z = 486−488 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.16 (s, 1H), 7.65 (m, 1H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.34 (m, 3H), 7.17 (m, 2H), 6.91 (dd, *J* = 7.9 and 1.7 Hz, 1H), 6.83 (d, *J* = 1.7 Hz, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 6.27 (s, 1H), 5.90 (d, *J* = 7.4 Hz, 1H), 5.89 (d, *J* = 7.4 Hz, 1H), 4.50 (m, 2H), 4.27 (d, *J* = 16.7 Hz, 1H), 3.78 (dd, *J* = 16.0 and 5.0 Hz, 1H), 3.33 (dd, *J* = 14.9 and 12.4 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.37, 29.70, 52.67, 56.32, 56.47, 101.38, 107.34, 108.02, 111.17, 118.54, 119.96, 120.60, 122.37, 126.17, 126.32, 129.21, 132.42, 132.99, 135.57, 136.55, 138.64, 147.16, 147.90, 166.52, 167.02.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-pyridin-3-yl-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 e, starting from **6** (50 mg) and 3-iodopyridine (27 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (26 mg, 43%). LC t_R = 4.90 min; MS (ESI+) m/z = 453 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.66 (br s, 1H), 8.55 (br d, *J* = 3.2 Hz, 1H), 8.25 (s, 1H), 7.76 (m, 1H), 7.64 (m, 1H), 7.42 (m, 1H), 7.33 (m, 1H), 7.18

(m, 2H), 6.93 (dd, J = 8.0 and 1.7 Hz, 1H), 6.85 (d, J = 1.7 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.31 (s, 1H), 5.90 (d, J = 7.8 Hz, 1H), 5.89 (d, J = 7.8 Hz, 1H), 4.60 (m, 2H), 4.31 (d, J = 16.6 Hz, 1H), 3.80 (dd, J = 16.0 and 4.8 Hz, 1H), 3.36 (dd, J = 16.0 and 11.4 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.36, 52.36, 56.37, 56.41, 101.39, 106.04, 107.39, 108.03, 111.18, 118.53, 119.99, 120.69, 122.41, 123.69, 126.16, 132.33, 132.98, 135.50, 136.57, 136.73, 145.78, 147.20, 147.60, 147.92, 166.75.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-pyrimidin-5-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 e, starting from **6** (50 mg) and 5-bromopyrimidine (21 mg) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (10 mg, 17%). LC t_R = 5.05 min; MS (ESI+) m/z = 454 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 9.10 (s, 1H), 8.86 (s, 2H), 8.11 (s, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.19 (m, 2H), 6.93 (dd, J = 8.0 and 1.6 Hz, 1H), 6.84 (d, J = 1.6 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.32 (s, 1H), 5.91 (d, J = 6.2 Hz, 2H), 4.62 (m, 2H), 4.35 (d, J = 16.4 Hz, 1H), 3.81 (dd, J = 16.0 and 4.9 Hz, 1H), 3.39 (dd, J = 16.0 and 11.5 Hz, 1H); ^{13}C NMR (CD_2Cl_2) 23.31, 29.67, 31.91, 51.58, 56.31, 56.39, 101.40, 105.90, 107.44, 108.03, 111.17, 118.51, 120.05, 120.83, 122.50, 126.10, 132.85, 135.26, 136.57, 147.28, 147.95, 152.09, 166.18, 166.80.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-*m*-tolyl-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 e, starting from **6** (50 mg) and *m*-iodotoluene (16 μL) and obtained after purification by TLC (dichloromethane/methanol) as a white powder (32 mg, 52%). LC t_R = 6.34 min; MS (ESI+) m/z = 466 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 8.1 (s, 1H), 7.65 (d, J = 6.6 Hz, 1H), 7.33 (m, 2H), 7.18 (m, 5H), 6.93 (dd, J = 8.0 and 1.7 Hz, 1H), 6.85 (d, J = 1.7 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.29 (s, 1H), 5.92 (d, J = 7.8 Hz, 1H), 5.91 (d, J = 7.8 Hz, 1H), 4.55 (m, 2H), 4.30 (d, J = 17.0 Hz, 1H), 3.80 (dd, J = 15.9 and 4.7 Hz, 1H), 3.34 (dd, J = 16 and 11.3 Hz, 1H), 2.40 (S, 3H); ^{13}C NMR (CD_2Cl_2) 21.06, 23.44, 29.70, 53.07, 56.31, 56.56, 101.36, 106.21, 107.33, 108.03, 111.18, 118.54, 119.91, 120.46, 122.19, 122.28, 125.87, 126.23, 128.00, 128.96, 133.11, 135.78, 136.57, 139.44, 140.15, 147.12, 147.91, 166.52, 167.30

Protocol f: Coupling N-Z or N-Fmoc Amino Acid Fluoride. Intermediate **4** (1 equiv), acid fluoride (2 equiv), and pyridine (2 equiv) were dissolved in dioxane (0.1 M) and were stirred at room temperature in the dark. The reaction was monitored by LC-MS (samples were prepared in anhydrous methanol). Every time LC-MS showed it necessary, 1 equiv of acid fluoride and 1 equiv of pyridine were added, until the end of the conversion. The residue was dissolved in ethyl acetate, washed with an aqueous solution of $NaHCO_3$, dried over $MgSO_4$, and evaporated to dryness.

Protocol g: Deprotection of N-Z or N-Fmoc Derivatives. *Deprotection and Cyclization of N-Z Derivatives.* The crude product issued from coupling was dissolved in methanol (0.03 M), $Pd(OH)_2$ on carbon (1 equiv w/w) wet with ethyl acetate was added, and the reaction mixture was stirred at room temperature under H_2 atmosphere for 20 h. The reaction mixture was diluted with dichloromethane, filtered on Celite, evaporated to dryness, and purified by TLC (dichloromethane/methanol).

Deprotection and Cyclization of N-Fmoc Derivatives. The crude product issued from coupling was dissolved in piperidine, 20% in DMF (0.03 M), and the reaction mixture was stirred at room temperature for 20 h. DMF was removed by evaporation, and the residue was dissolved in ethyl acetate, washed with an aqueous solution of $NaHCO_3$, dried over $MgSO_4$, evaporated to dryness, and purified by TLC (dichloromethane/methanol).

Protocol h: Acid Fluoride Synthesis. To a solution of amino acid (1 equiv) and pyridine (1 equiv) in dichloromethane (0.4 M) was added cyanuric fluoride (4−8 equiv). The reaction mixture was flushed with argon and refluxed for 3 h. The reaction mixture was diluted with dichloromethane and washed with ice. The organic layer was dried over $MgSO_4$ and evaporated to dryness.

3-[(3*S*,6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-1,4-dioxo-1,2,3,4,6,7,12,12a-octahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indol-3-yl]propionic Acid *tert*-Butyl Ester (37). Compound **37** was prepared according protocol f and then protocol g, starting from **4** (100 mg), acid fluoride **37a** (9 equiv in total was added), and pyridine (9 equiv) and obtained after 26 days as a white powder (115 mg, 80%). LC t_R = 6.0 min; MS (ESI−) m/z = 502 [M − H]$^-$; ^{1}H NMR (MeOD) δ 7.53 (d, J = 6.6 Hz, 1H), 7.28 (d, J = 7.2 Hz, 1H), 7.06 (m, 2H), 6.82−6.86 (m, 2H), 6.69 (d, J = 8.1 Hz, 1H), 6.15 (s, 1H), 5.86 (m, 2H), 4.52 (dd, J = 11.4 and 4.5 Hz, 1H), 3.97 (dd, J = 7.2 and 7.2 Hz, 1H), 3.66 (dd, J = 15.6 and 4.5 Hz, 1H), 3.11 (dd, J = 14.7 and 11.4 Hz, 1H), 2.42 (t, J = 7.2 Hz, 2H), 2.09 (qd, J = 6.9 and 2.1 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (MeOD) 22.98, 26.95, 28.28, 30.53, 54.92, 55.18, 56.91, 80.51, 100.93, 104.44, 106.77, 107.54, 107.68, 108.45, 110.80, 117.52, 118.42, 118.84, 119.55, 120.64, 121.29, 125.97, 133.45, 136.49, 136.88, 146.73, 147.65, 169.17, 169.68, 172.22.

(*S*)-4-Benzyloxycarbonylamino-4-fluorocarbonylbutyric Acid *tert*-Butyl Ester [Z-L-Glu(OtBu)-F, 37a). Intermediate **37a** was prepared according to protocol h, starting from Z-L-Glu(OtBu)-OH (1 g, 2.96 mmol), pyridine (1 equiv), and cyanuric fluoride (6 equiv) and obtained as a white powder (1.2 g, 100%). LC-MS samples were prepared in anhydrous methanol. The corresponding methylic ester was analyzed. LC t_R = 6.3 min; MS (ESI+) m/z = 374 [methyl ester + H]$^+$.

(3*R*,6*R*,12a*R*)-3-(4-Aminobutyl)-6-benzo[1,3]dioxol-5-yl-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 protocol f and then protocol g, starting from intermediate **4** (70 mg), acid fluoride **38a** (5 equiv in total was added), and pyridine (5 equiv) and obtained after 25 days as a white powder (42 mg, 47%). LC t_R = 3.9 min; MS (ESI+) m/z = 447 [M + H]$^+$; ^{1}H NMR (MeOD) δ 7.52 (d, J = 6.9 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H) 7.05 (m, 2H), 6.81−6.87 (m, 2H), 6.70 (d, J = 8.1 Hz, 1H), 6.16 (s, 1H), 5.87 (d, J = 6.3 Hz, 2H), 4.40 (ddd, J = 11.7, 4.2, and 1.8 Hz, 1H), 4.15 (td, J = 4.2 and 1.5 Hz, 1H), 3.64 (dd, J = 15.9 and 4.5 Hz, 1H), 3.12 (dd, J = 14.7 and 11.7 Hz, 1H), 2.64 (m, 2H), 1.85 (m,2H), 1.45 (m, 4H); ^{13}C NMR (MeOD) 21.61, 27.49, 29.83, 35.04, 40.01, 52.01, 52.08, 55.00, 101.31, 107.12, 107.65, 108.42, 110.87, 117.57, 118.99, 121.82, 121.88, 126.01, 129.84, 132.56, 136.80, 148.00, 148.20, 165.38, 167.91.

[(*R*)-5-Benzyloxycarbonylamino-5-fluorocarbonylpentyl]carbamic Acid Benzyl Ester (Z-D-Lys(Z)-F, 38a). Intermediate **38a** was prepared according to protocol h, starting from Z-D-Lys(Z)-OH (500 mg, 1.2 mmol), pyridine (1 equiv), and cyanuric fluoride (8 equiv) and obtained as a colorless oil (500 mg, 100%). LC-MS samples were prepared in anhydrous methanol. The corresponding methylic ester was analyzed. LC t_R = 6.3 min; MS (ESI+) m/z = 429 [methyl ester + H]$^+$.

(3*S*,6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-3-(1*H*-indol-3-ylmethyl)-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido-[3,4-*b*]indole-1,4-dione (39). Compound **39** was prepared according protocol f and then protocol g, starting from intermediate **4** (100 mg), acid fluoride **39a** (3 equiv in total was added), and pyridine (3 equiv) and obtained after 5 days as a white powder (70 mg, 49%). LC t_R = 5.5 min; MS (ESI−) m/z = 503 [M − H]$^-$; ^{1}H NMR (MeOD) δ 7.59 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.5 Hz, 1H), 7.17 (d, J = 8.1 Hz, 2H), 6.91−7.07 (m, 5H), 6.79 (dd, J = 8.1 and 1.8 Hz, 1H), 6.76 (d, J = 1.8 Hz, 1H), 6.67 (d, J = 7.8 Hz, 1H), 5.84 (d, J = 3.3 Hz, 2H), 5.72 (s, 1H), 4.28 (t, J = 3.6 Hz, 1H), 3.56 (dd, J = 14.4 and 3.3 Hz, 1H), 3.20 (dd, J = 15.6 and 4.2 Hz, 1H), 3.12 (dd, J = 14.4 and 4.2 Hz, 1H), 2.75 (dd, J = 15.6 and 11.7 Hz, 1H), 2.44 (dd, J = 11.7 and 4.2 Hz, 1H); ^{13}C NMR (MeOD) 23.49, 30.38, 55.29, 56.59, 57.53, 100.86, 104.13, 106.64, 107.48, 107.86, 110.60, 110.95, 117.27, 118.35, 118.61, 118.76, 119.41, 121.08, 121.37, 124.72, 125.68, 126.83, 133.14, 136.56, 136.73, 136.84, 146.56, 147.54, 169.32, 170.41.

[(*S*)-1-Fluorocarbonyl-2-[(1*H*-indol-3-yl)ethyl]carbamic acid 9*H*-Fluoren-9-ylmethyl Ester (Fmoc-L-Trp-F, 39a). Intermediate **39a** was prepared according to protocol h, starting from Fmoc-L-TrpOH (1.264 g, 2.96 mmol), pyridine, (2 equiv) and cyanuric fluoride (4 equiv) and obtained after precipitation in petroleum ether as a white powder (1.2 g, 60%). LC-MS samples were prepared in anhydrous methanol. The corresponding methylic ester was analyzed. LC t_R = 7.1 min; MS (ESI+) m/z = 441 [methyl ester + H]$^+$.

(3*S*,6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-3-benzyloxymethyl-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (40). Compound **40** was prepared according protocol f and then protocol g, starting from intermediate **4** (100 mg), acid fluoride **40a** (7 equiv in total was added), and pyridine (8 equiv) and obtained after 11 days as a white powder (25 mg, 18%). LC t_R = 5.9 min; MS (ESI−) m/z = 496 [M − H]$^-$; ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H), 8.54 (d, *J* = 3.0 Hz, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.24 (m, 6H), 7.05 (td, *J* = 6.0 and 1.5 Hz, 1H), 6.98 (t, *J* = 6.9 Hz, 1H), 6.9 (d, *J* = 1.5 Hz, 1H), 6.83 (dd, *J* = 8.1 and 1.8 Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.09 (s, 1H), 5.92 (s, 2H), 4.48 (s, 2H), 4.33 (dd, *J* = 11.7 and 4.2 Hz, 1H), 4.05 (m, 1H), 3.85 (dd, *J* = 9.9 and 3.3 Hz, 1H), 3.58 (dd, *J* = 9.9 and 2.7 Hz, 1H), 3.52 (dd, *J* = 15.9 and 4.2 Hz, 1H), 2.93 (dd, *J* = 15.6 and 12.0 Hz, 1H); ^{13}C NMR (DMSO-d_6) 23.96, 56.10, 56.38, 56.52, 72.85, 73.01, 101.34, 105.29, 107.40, 108.45, 111.78, 118.53, 119.34, 119.66, 121.72, 126.18, 127.70, 128.01, 128.72, 134.45, 136.67, 137.74, 138.33, 146.46, 147.45, 168.67, 168.76.

[(*S*)-2-Benzyloxy-1-fluorocarbonylethyl]carbamic acid 9*H*-Fluoren-9-ylmethyl Ester (40a). Intermediate **40a** was prepared according to protocol h, starting from Fmoc-L-Ser(Bzl)OH (0.5 g, 1.2 mmol), pyridine (2 equiv), and cyanuric fluoride (4 equiv) and obtained after precipitation in petroleum ether as a white powder (360 mg, 71%). LC-MS samples were prepared in anhydrous methanol. The corresponding methylic ester was analyzed. LC t_R = 7.6 min; MS (ESI+) m/z = 432 [methyl ester + H]$^+$.

6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-benzylpyrrolidin-3-yl]-10-bromo-2,3,6,7,12.12a-hexahydropyrazino[1′,2′:1,6]pyrido[3.4-*b*]indole-1,4-dione (Mixture of 1*R*,6*R*,12a*R* and 1*R*,6*S*,12a*S* *cis*-Diastereoisomers) (41). Compound **41** was prepared according to protocol c, starting from intermediate **41b** (164 mg, 1 equiv), (*R*)-(−)-1-benzyl-3-aminopyrrolidine (112 μL, 2 equiv), and triethylamine (62 μL, 2 equiv) in methanol and obtained after purification by TLC (dichloromethane/methanol) as a white powder (94 mg, 47%). LC t_R = 5.69 min; MS (ESI+) m/z = 613−615 [M + H]$^+$; ^{1}H NMR (MeOD) δ 7.68−7.66 (m, 1H), 7.33−7.18 (m, 7H), 6.78−6.62 (m, 3H), 6.23 (s, 1H), 5.82 (d, *J* = 13.5 Hz, 2H), 5.10 (m, 1H), 4.40−4.30 (m, 2H), 4.12 (m, 1H), 3.71−3.51 (m, 3H), 3.10 (dd, *J* = 15.6 and 11.4 Hz, 1H), 2.94 (m, 1H), 2.75−2.58 (m, 1H), 2.48−2.41 (m, 1H), 2.24 (m, 2H), 1.85 (m, 1H); ^{13}C NMR (MeOD) 22.25, 22.34, 27.11, 29.09, 45.51, 45.95, 51.79, 51.98, 52.04, 52.60, 52.86, 55.67, 55.73, 55.78, 55.85, 57.23, 59.36, 59.45, 101.00, 104.43, 106.73, 107.57, 107.62, 107.70, 108.44, 112.00, 112.49, 119.65, 119.73, 120.26, 121.88, 124.07, 124.46, 126.79, 126.87, 127.68, 127.98, 128.02, 128.37, 128.46, 128.50, 128.88, 134.91, 135.42, 135.59, 138.26, 146.97, 147.75, 167.55, 168.67, 168.76.

1-Benzo[1,3]dioxol-5-yl-6-bromo-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Enantiomers) (41a). 5-Br-DL-tryptophan-OMe·HCl (500 mg, 1.5 mmol) was dissolved in $CHCl_3$ (40 mL) with 3 Å molecular sieves under argon. Piperonal (225 mg, 1.5 mmol) was added and the solution was cooled to 0 °C. TFA (345 μL, 4.5 mmol) in solution in $CHCl_3$ (5 mL) was added dropwise and the mixture was allowed to warm to room temperature and stirred overnight. After filtration and concentration, two pairs of enantiomers were separated by TLC (dichloromethane/methanol) to provide a yellow solid (140 mg, 22%). LC t_R = 4.64 min; MS (ESI+) m/z= 429−431 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 10.55 (s, 1H), 7.61 (d, *J* = 1.8 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.10 (dd, *J* = 8.4 and 1.8 Hz, 1H), 6.91 (d, *J* = 7.8 Hz, 1H), 6.87 (dd, *J* = 7.8 and 1.5 Hz, 1H), 6.82 (d, *J* = 1.2 Hz, 1H), 6.00 (d, *J* = 0.9 Hz, 2H), 5.13 (s, 1 H), 3.83 (dd, *J* = 11.1 and 4.2 Hz, 1H), 3.70 (s, 3H), 3.02 (m, 1H), 2.79 (m, 1H); mp = 132−140 °C.

1-Benzo[1,3]dioxol-5-yl-6-bromo-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Enantiomers) (41b). Intermediate **41b** was prepared according to protocol b, starting from intermediate **41a** (140 mg, 0.326 mmol, 1 equiv). A yellow solid was obtained (164 mg, 100%) and directly engaged in the cyclization step. LC t_R = 6.9 min; MS (ESI+) m/z = 504 [M + H]$^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-benzylpyrrolidin-3-yl]-7-methyl-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (42). Compound **42** was prepared according to protocol d, starting from intermediate **42b** (85 mg, 0.193 mmol, 1 equiv) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (73 μL, 2.2 equiv) in ethanol (2 mL), and obtained as a white powder (46 mg, 43%). LC t_R = 5.45 min; MS (ESI+) m/z = 549 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 7.63 (d, *J* = 4.7 Hz, 1H), 7.39 (d, *J* = 4.9 Hz, 1H), 7.36−7.21 (m, 5H), 7.15 (t, *J* = 4.7 Hz, 1H), 7.07 (t, *J* = 4.7 Hz, 1H), 6.82 (d, *J* = 0.9 Hz, 1H), 6.77 (d, *J* = 4.7 Hz, 1H), 6.74 (dd, *J* = 4.9 and 0.9 Hz, 1H), 3.37 (s, 1H), 5.95 (m, 2H), 5.00 (br s, 1H), 4.38 (dd, *J* = 6.9 and 2.9 Hz, 1H), 4.14 (s, 2H), 3.62−3.48 (m, 6H), 3.35 (s, 2H), 3.06 (dd, *J* = 9.2 and 7.1 Hz, 1H), 2.85 (m, 1H), 2.67 (dd, *J* = 6.2 and 1.6 Hz, 1H), 2.37 (dd, *J* = 6.2 and 4.7 Hz, 1H), 2.15 (m, 2H), 1.50 (m, 1H); ^{13}C NMR (DMSO-d_6) 22.90, 29.80, 30.07, 45.89, 51.38, 53.26, 54.21, 55.81, 55.89, 59.54, 101.51, 105.62, 108.49, 108.64, 110.16, 118.81, 119.63, 121.67, 121.89, 125.57, 127.31, 128.74, 135.26, 135.60, 137.42, 139.29, 146.87, 147.56, 167.13, 168.36.

(1*R*,3*R*)-1-Benzo[1,3]dioxol-5-yl-9-methyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (42a). 1-Methyl-D-tryptophan (2.3 mmol) and piperonal (1.1 equiv) were refluxed in 2-propanol for 24 h. The reaction mixture was diluted with ethyl acetate and washed with an aqueous solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude diastereoisomers mixture (477 mg) was dissolved in MeOH containing 3 equiv of thionyl chloride and stirred overnight at room temperature. Compound **42a** was purified by column chromatography: 155 mg (31%). LC t_R = 4.2 min; MS (ESI+) m/z = 365 [M + H]$^+$; ^{1}H NMR ($CDCl_3$) δ 7.56 (d, *J* = 7.8 Hz, 1H), 7.26 (m, 2H), 7.14 (m, 1H), 6.94 (dd, *J* = 8.0 and 1.7 Hz, 1H), 6.86 (d, *J* = 1.4 Hz, 1H), 6.79 (d, *J* = 7.9 Hz, 1H), 5.93 (m, 2H), 5.61 (br s, 1H), 4.07 (dd, *J* = 10.3 and 4.5 Hz, 1H), 3.76 (s, 3H), 3.36 (m, 1H), 3.26 (m, 1H), 3.24 (s, 3H).

(1*R*,3*R*)-1-Benzo[1,3]dioxol-5-yl-2-(2-chloroacetyl)-9-methyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic Acid Methyl Ester (42b). Intermediate **42b** was prepared according to protocol b, starting from intermediate **42a** (150 mg, 1 equiv) and obtained as a yellow powder (100 mg, 55%), which was directly engaged in the cyclization step. LC t_R = 5.2 min; MS (ESI+) m/z = 441 [M + H]$^+$.

(5a*R*,10*R*)-10-Benzo[1,3]dioxol-5-yl-7-[(*R*)-1-benzylpyrrolidin-3-yl]-5,5a,7,8,10,11-hexahydro-1,7,9a,11-tetraaza-benzo[*b*]fluorene-6,9-dione (43). Compound **43** was prepared according to protocol d, starting from intermediate **43c** (75 mg, 0.175 mmol, 1 equiv) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (66 μL, 2.2 equiv) in ethanol (1 mL). The diastereoisomer of interest was isolated by HPLC and obtained as a white powder (18 mg, 20%). LC t_R = 5.00 min; MS (ESI+) m/z = 536 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) 11.71 (s, 1H), 8.16 (d, *J* = 4.0 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.27 (m, 5H), 7.04 (dd, *J* = 7.7 and 4.7 Hz, 1H), 6.85−6.72 (m, 3H), 6.20 (s, 1H), 5.93 (s, 2H), 5.04 (br s, 1H), 4.42 (dd, *J* = 11.4 and 4.7 Hz, 1H), 4.17 (m, 2H), 3.63 (d, *J* = 13.0 Hz, 1H), 3.49 (m, 2H), 2.99−2.85 (m, 2H), 2.69 (d, *J* = 10.6 Hz, 1H), 2.38 (dd, *J* = 9.8 and 8.2 Hz, 1H), 2.26−2.15 (m, 2H), 1.51

(m, 1H); ^{13}C NMR (DMSO-d_6) 22.46, 27.82, 46.37, 51.52, 53.29, 54.69, 55.71, 58.27, 59.41, 101.44, 103.82, 107.03, 108.60, 115.92, 118.58, 119.34, 126.84, 127.31, 128.67, 134.91, 136.65, 139.31, 142.89, 146.72, 147.67, 149.03, 167.10, 168.17.

2-Amino-3-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)-propionic Acid Methyl Ester (43a). Intermediate **43a** was prepared starting from D,L-7-azatryptophan (975 mg) with 3 equiv of thionyl chloride in methanol (1.355 g, 97%). LC t_R = 1.85 min; MS (ESI+) m/z = 220 [M + H]$^+$

8-Benzo[1,3]dioxol-5-yl-6,7,8,9-tetrahydro-5*H*-dipyrido-[2,3-*b*;4′,3′-*d*]pyrrole-6-carboxylic Acid Methyl Ester (Mixture of 6*R*,8*R* and 6*S*,8*S* *cis*-Enantiomers) (43b). Intermediate **43a** (4.5 mmol) and piperonal (1 equiv) were suspended in pyridine (0.08 M) and heated to 75 °C for 48 h. Pyridine was removed by evaporation. The residue was dissolved in ethyl acetate and washed with an aqueous solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to dryness. Compound **43b** was purified by column chromatography to give 530 mg of a mixture of the two *cis*-enantiomers as a yellow powder (67%). LC t_R = 2.5 min; MS (ESI+) m/z = 352 [M + H]$^+$; ^{1}H NMR (CD_2Cl_2) δ 10.49 (s, 1H), 7.85 (dd, J = 7.7 and 1.3 Hz, 1H), 7.69 (d, J = 3.9 Hz, 1H), 6.99 (m, 2H), 6.86 (d, J = 7.9 Hz, 1H), 6.83 (d, J = 1.6 Hz, 1H), 5.97 (d, J = 1.2 Hz, 1H.). 5.93 (d, J = 1.3 Hz, 1H), 5.26 (m, 1H), 3.97 (dd, J = 11.1 and 4.2 Hz, 1H), 3.81 (s, 3H), 3.18 (ddd, J = 15.1, 4.2, and 1.8 Hz, 1H), 2.95 (ddd, J = 14.9, 11.1, and 2.5 Hz, 1H), 2.33 (m, 1H); mp = 246−249 °C.

8-Benzo[1,3]dioxol-5-yl-7-(2-chloroacetyl)-6,7,8,9-tetra-hydro-5*H*-dipyrido[2,3-*b*;4′,3′-*d*]pyrrole-6-carboxylic Acid Methyl Ester (Mixture of 6*R*,8*R* and 6*S*,8*S* *cis*-Enantiomers) (43c). Intermediate **43c** was prepared according to protocol b, starting from intermediate **43b** (351 mg, 1 equiv) and obtained as a yellow powder (392 g, 92%), which was directly engaged in the cyclization step. LC t_R = 4.25 min; MS (ESI+) m/z = 428 [M + H]$^+$.

Mixture of (6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-benzylpyrrolidin-3-yl]-9-fluoro-2,3,6,7,12,12a-hexahydro-pyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione and (6*S*,12a*S*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-benzylpyrrolidin-3-yl]-9-fluoro-2,3,6,7,12,12a-hexahydropyrazino[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (44). Compound **44** was prepared according to protocol d, starting from intermediate **44b** (100 mg, 1 equiv), (*R*)-(−)-1-benzyl-3-aminopyrrolidine (66 μL, 2 equiv) in ethanol (1 mL). Diastereoisomer mixture was isolated by flash chromatography (DCM/MeOH) and obtained as a white powder (62%). LC t_R = 5.00 min; MS (ESI+) m/z = 536 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 11.21 (s, 1H), 7.56 (dd, J = 5.2 and 3.3 Hz, 1H), 7.35−7.21 (m, 5H), 7.12 (dd, J = 6.0 and 1.4 Hz, 1H), 6.86 (m, 1H), 6.82 (dd, J = 2.7 and 1.0 Hz, 1H), 6.79 (dd, J = 7.1 and 4.8 Hz, 1H), 6.71 (dd, J = 4.8 and 0.8 Hz, 1H), 6.17 (d, J = 1.2 Hz, 1H), 5.93 (m, 2H), 5.04 (m, 1H), 4.40 (dd, J = 6.8 and 2.9 Hz, 1H), 4.17 (m, 2H), 3.56 (m, 2H), 3.45 (dt, J = 9.6 and 3.3 Hz, 1H), 2.95 (ddd, J = 9.0, 7.0, and 1.6 Hz, 1H), 2.89 (m, 1H), 2.70−2.57 (m, 1H), 2.50−2.37, (m, 1H), 2.16 (m, 2H), 1.80−1.52 (m, 1H); ^{13}C NMR (DMSO-d_6) 22.70, 27.79, 29.97, 45.92, 46.39, 51.44, 51.51, 53.29, 55.09, 55.64, 55.75, 56.37, 58.15, 59.39, 59.52, 97.94, 98.15, 101.43, 105.19, 107.14, 107.61, 107.80, 108.57, 119.45, 119.47, 119.60, 119.68, 123.08, 127.35, 127.41, 128.70, 128.75, 128.78, 134.87, 134.90, 136.39, 136.49, 137.04, 139.21, 139.30, 146.64, 147.63, 158.34, 160.21, 167.18, 168.18.

1-Benzo[1,3]dioxol-5-yl-7-fluoro-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Enantiomers) (44a). Intermediate **44a** was prepared according to protocol a, starting from 6-F-DL-tryptophan-OMe·HCl (1.4 g) and piperonal (940 mg) after stirring for 120 h and purification by column chromatography. Compound **44a** was obtained as a white powder (590 mg, 32%). LC t_R = 4.0 min; MS (ESI+) m/z = 369 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 7.42 (dd, J = 8.5 and 5.5 Hz, 1H), 6.97−6.77 (m, 5H), 6.00 (s, 2H), 5.11 (d, J = 5.4 Hz, 1 H), 3.84 (dt, J = 11.1 and 4.1 Hz, 1H), 3.70 (s, 3H), 3.00 (dd, J = 15.1 and 2.8 Hz, 1H), 2.85−2.69 (m, 2H).

1-Benzo[1,3]dioxol-5-yl-2-(2-chloroacetyl)-7-fluoro-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Enantiomers) (44b). Intermediate **44b** was prepared according to protocol b, starting from intermediate **44a** (378 mg) and obtained as a yellow powder (392 mg, 86%), which was directly engaged in the cyclization step. LC t_R= 6.4 min; MS (ESI+) m/z = 445 [M + H]$^+$.

(6*R*,12a*R*)-6-Benzo[1,3]dioxol-5-yl-2-[(*R*)-1-benzylpyrrolidin-3-yl]-10-fluoro-2,3,6,7,12,12a-hexahydropyrazino-[1′,2′:1,6]pyrido[3,4-*b*]indole-1,4-dione (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Diastereoisomers) (45). Compound **45** was prepared according to protocol d, starting from intermediate **45b** (102 mg, 0.23 mmol, 1 equiv), (*R*)-(−)-1-benzyl-3-aminopyrrolidine (2.2 equiv) in ethanol (2.3 mL). Compound **45** was isolated by HPLC and obtained as a white powder (50 mg, 33%). LC t_R = 5.164 min; MS (ESI+) m/z = 553 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 11.20 (s, 1H), 7.31 (m, 7H), 6.89 (td, J = 9.33 and 2.5 Hz, 1H), 8.00 (m, 1H), 6.76 (d, J = 7.6 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 6.18 (s, 1H), 5.92 (m, 2H), 5.03 (m, 1H), 4.38 (dd, J = 11.5 and 4.9 Hz, 1H), 4.17 (d, J = 11.1 Hz, 2H), 3.39−3.64 (m, 3H), 2.90 (m, 2H), 2.38−2.71 (m, 2H), 2.18 (m, 2H), 1.51−1.78 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 22.64, 27.78, 30.04, 45.92, 46.40, 51.43, 51.51, 53.29, 54.99, 55.68, 55.80, 59.39, 59.52, 101.44, 103.51, 103.82, 105.38, 105.44, 107.07, 108.59, 109.51, 109.85, 112.65, 112.78, 119.36, 126.35, 126.49, 127.39, 128.69, 128.74, 133.14, 136.35, 136.38, 136.88, 146.67, 147.66, 155.89, 158.96, 167.17, 168.19, 168.25.

1-Benzo[1,3]dioxol-5-yl-6-fluoro-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Enantiomers) (45a). Intermediate **45a** was prepared according to protocol a, starting from 5-F-DL-tryptophan-OMe·HCl (500 mg) and piperonal (276 mg) after stirring for 120 h and purification by column chromatography. Compound **45a** was obtained as a white powder (190 mg, 28%). LC t_R= 4.0 min; MS (ESI+) m/z = 369 [M + H]$^+$; ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 7.21−7.16 (m, 2H), 6.92−6.79 (m, 4H), 6.00 (s, 2H), 5.13 (s, 1H), 3.84 (dd, J = 11.0 and 4.0 Hz, 1H), 3.70 (s, 3H), 2.99 (ddd, J = 12.1, 4.0, and 1.5 Hz, 1H), 2.78 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 25.26, 51.77, 56.16, 57.47, 100.91, 102.37, 102.67, 107.18, 108.04, 108.34, 108.76, 111.90, 112.03, 121.99, 126.66, 126.79, 132.91, 135.70, 137.76, 146.80, 147.15, 155.20, 158.26, 172.81.

1-Benzo[1,3]dioxol-5-yl-2-(2-chloroacetyl)-6-fluoro-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (Mixture of 1*R*,3*R* and 1*S*,3*S* *cis*-Enantiomers) (45b). Intermediate **45b** was prepared according to protocol b, starting from intermediate **45a** (100 mg) and obtained as a yellow powder (119 mg, 100%), which was directly engaged in the cyclization step. LC t_R = 6.01 min; MS (ESI+) m/z = 445 [M + H]$^+$.

(6*S*,12a*R*)-6-Benzo[1,3]dioxol-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 (46). Compound **46** was prepared according to protocol c, starting from intermediate **46b** (160 mg) and (*R*)-(−)-1-benzyl-3-aminopyrrolidine (2 equiv) and obtained after purification by flash chromatography (dichloromethane/methanol 99/1) as a white powder (24 mg, 18%). LC t_R = 4.8 min; MS (ESI+) m/z = 535 [M + H]$^+$; ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1H), 7.52 (d, J = 7.6 Hz, 1H), 7.32−7.13 (m, 8H), 6.96 (s, 1H), 6.81 (s, 1H), 6.71 (s, 2H), 5.93 (s, 2H), 5.22 (br s, 1H), 4.33 (dd, J = 11.7 and 4.2 Hz, 1H), 4.29 (d, J = 17.6 Hz, 1H), 4.12 (d, J = 18.0 Hz, 1H), 3.57 (s, 2H), 3.50 (dd, J = 15.1 and 3.8 Hz, 1H), 2.93 (m, 2H), 2.73 (m, 1H), 2.47 (dd, J = 10.5 and 7.6 Hz, 1H), 2.21 (m, 2H), 1.75 (m, 1H).

(1*S*,3*R*)-1-Benzo[1,3]dioxol-5-yl-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic Acid Methyl Ester (46a). Intermediate

46a was prepared according to protocol a, starting from D-tryptophan methyl ester (3 g) and obtained as 870 mg of a white powder (21%). LC t_R = 3.9 min; MS (ESI+) m/z = 351 $[M + H]^+$; ^{1}H NMR (CDCl$_3$) δ 7.52 (s, 1H), 7.47 (d, J = 6.8 Hz, 1H), 7.17 (m, 1H), 7.11−7.02 (m, 2H), 6.67 (s, 3H), 5.85 (s, 2H), 5.25 (s, 1H), 3.90 (t, J = 6.2 Hz, 1H), 3.63 (s, 3H), 3.18 (dd, J = 15.5 and 5.5 Hz, 1H), 3.04 (dd, J = 15.3 and 6.6 Hz, 1H); ^{13}C NMR (CDCl$_3$) δ 174.4, 148.46, 147.90, 136.60, 136.15, 133.52, 127.34, 122.43, 122.25, 119.93, 118.68, 111.37, 109.18, 108.56, 101.59, 55.06, 53.88, 52.91, 52.58, 24.93; mp = 187−189 °C.

(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 (46b). Intermediate **46b** was prepared according to protocol b, starting from intermediate **46a** (996 mg, 1 equiv) and obtained as a yellow powder (1.06 g, 96%), which was directly engaged in the cyclization step. LC t_R = 6.3 min; MS (ESI+) m/z = 427 $[M + H]^+$.

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ABBREVIATIONS LIST

PDE, phosphodiesterase; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; HTS, high-throughput screening; HSQC, heteronuclear single quantum coherence spectroscopy; HMBC, heteronuclear multiple-bond correlation spectroscopy; ROESY, rotating Overhauser effect spectroscopy; COSY, correlation spectroscopy; DIEA, diisopropyl ethylamine; DCM, dichloromethane; TFA, trifluoroacetic acid; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; LC, liquid chromatography; t_R, retention time; MS, mass spectrometry; ESI, electrospray ionization; TLC, thick-layer chromatography; EtOAc, ethyl acetate; mp, melting point

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