

Synthesis and SAR of tetracyclic pyrroloquinolones as phosphodiesterase 5 inhibitors

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Abstract—The synthesis of the fused tetracyclic pyrroloquinolones **9a–i** in four steps is described. The PDE5 inhibitory activities of these compounds, their selectivities against PDE1, PDE2, PDE3, PDE4 and PDE6, the preclinical pharmacokinetic assessments and the in vivo efficacy in increasing intracavernosal pressure are presented and discussed.
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1. Introduction

Cyclic nucleotides, that is, cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) are important second messengers that control many physiological processes.¹ The levels of intracellular cyclic nucleotides are determined by the activities of cyclases that synthesize them and phosphodiesterases (PDEs) that degrade them. To date, 21 mammalian PDE genes have been cloned and are classified into 11 families (PDE1–PDE11) according to the sequence homology and their biochemical properties.^{1c} Phosphodiesterase type 5 (PDE5), a cGMP specific PDE, is abundant in smooth muscles, lung and platelets. In human corpus cavernosum, PDE5 is the major enzyme to hydrolyze cGMP to GMP. Upon sexual stimulation, release of nitric oxide from nonadrenergic, noncholinergic neurons activates soluble guanylyl cyclase, which cyclizes guanosine triphosphate (GTP) to generate cGMP. Increased cGMP levels eventually cause a decrease in intracellular calcium concentration. Lower calcium concentration leads to relaxation of smooth muscle in the corpus cavernosum, resulting in increased arterial blood flow to the penis and ultimately erection. PDE5 inhibition blocks cGMP degradation, facilitates cGMP accumulation, induces adequate relaxation of corpus cavernosal smooth muscle, and thus erection in patients with male erectile dysfunction (MED).

MED is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse. The incidence of MED^{2a} increases dramatically with age. The large patient population, estimated to be 30 million men in the United States alone,^{2b} combined with the success of Viagra® (sildenafil)³ have provided strong stimuli for the discovery and development of additional PDE5 inhibitors,⁶ such as recently launched Levitra® (vardenafil)⁵ and Cialis® (tadalafil).⁴ One important issue facing a new PDE5 inhibitor is its selectivity over other PDEs. Accumulation of clinical data suggested that some of the adverse side effects observed in sildenafil therapy, such as visual disturbances, might be the results of noticeable inhibition of sildenafil toward PDE6. So both potencies toward PDE5 and selectivities against other PDEs are important for the successful development of new PDE5 inhibitors. During the SAR studies on pyrroloquinolones in this group,⁸ we discovered that a wide variety of substituents are tolerated on the pyrrole nitrogen. These included acyl groups^{8j} and various heterocycles.^{8f} In addition, the β -carboline precursors such as **1a** were less potent than **1b**.^{8e} We decided to tie back the acyl group on the pyrrole nitrogen to the 3-position to generate conformationally constrained pyrroloquinolones such as **2b** (Fig. 1). We were also interested in the comparison of biological activities of these heterocyclic pyrroloquinolones with their β -carboline precursors such as **2a**.⁷ SAR study of pyrimidine pyrroloquinolones^{8e} showed that both potencies and PDE6/5 selectivities are very sensitive to the C-1 aromatic substituents. More importantly, pyrimidinyl pyrroloquinolones bearing methylenedioxyphenyl or dihydrobenzofuranyl groups are active and selective PDE5

Keywords: Pyrroloquinolone; β -Carboline; PDE5 inhibitor; MED (male erectile dysfunction).

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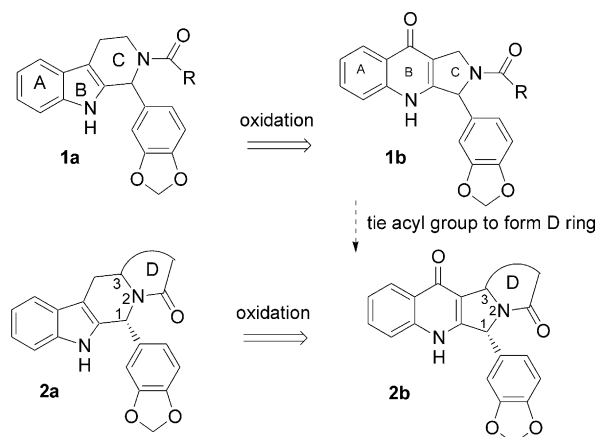


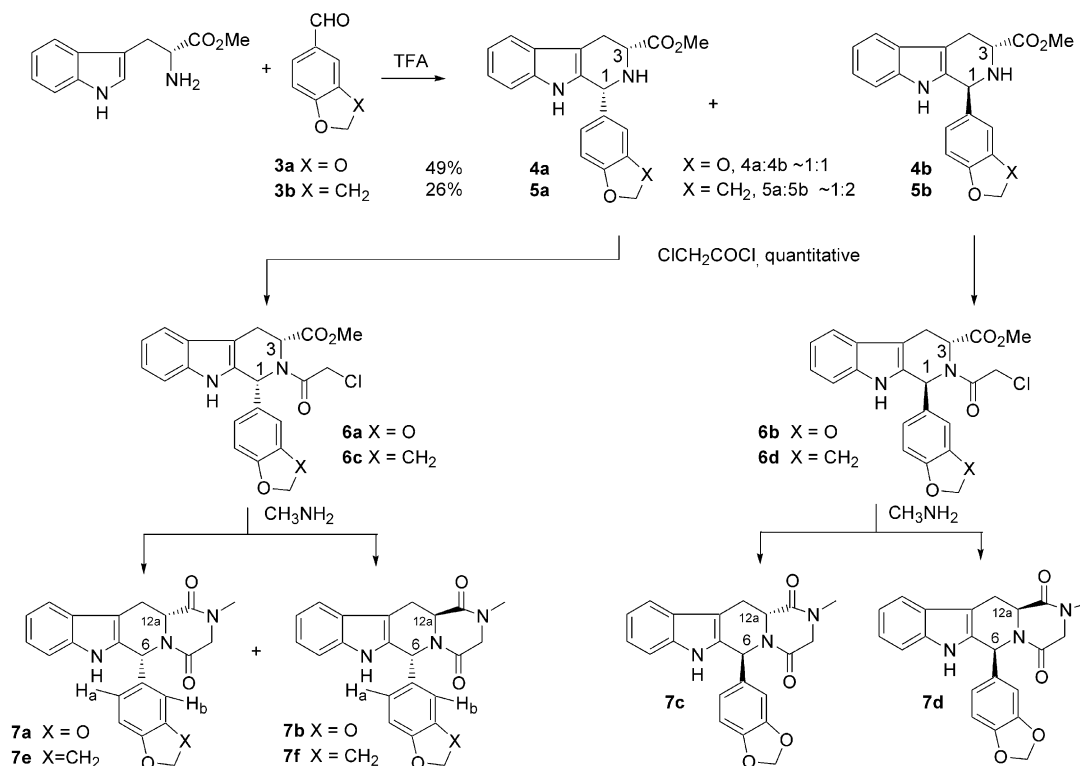
Figure 1. Derivatization of tri-cyclic pyrroloquinolones to tetra-cyclic pyrroloquinolones.

inhibitors.^{8c} We thus incorporated these two components in our initial synthetic plan.

2. Chemistry

The functional quinolone skeleton can be assembled by the application of the Winterfeldt oxidation reaction of β -carbolines, generated from the Pictet–Spengler reaction of D-tryptophan methyl ester with selected aldehydes. The implementation of our synthetic protocol to access these target pyrroloquinolone molecules is shown in Scheme 1. Thus, D-tryptophan methyl ester reacted with piperonal (**3a**) under Pictet–Spengler reaction condition (TFA/ $\text{CH}_2\text{Cl}_2/\text{MeOH}$) to furnish two diastere-

omers **4a** and **4b** in 25% and 24% yields, respectively.^{7g} The X-ray of **4b** was obtained to exclusively assign the absolute stereochemistry of **4a** and **4b**. Condensation of **4a** with chloroacetyl chloride provided acylated intermediate **6a** in almost quantitative yield. Subsequent cyclization of **6a** with *N*-methyl amine in methanol at 50 °C for 16 h provided diastereomers **7a** in 54% yield along with small amount of **7b** in 1.1% yield.^{7g} Compound **7a** is in full accordance with the literature data $\{[\alpha]_{\text{D}}^{20} + 71.4$ (c 1.00, CHCl_3); lit.^{7g} $[\alpha]_{\text{D}}^{20} + 71.2$ (c 1.00, CHCl_3)}. It should be noted that the amount of C-12a epimerized product **7b** generated from the cyclization reaction varied according to the length of reaction times. Thus, under the elongated reaction time, 48 h, compound **7c** was obtained from precursor **6b** with decreased yield of 21%, while C-12a epimerized product **7d** increased significantly to 18% yield. NOE studies were implemented on **7c/7d** for confirmation of the structural assignments. An NOE was observed between the two hydrogens on C-6 and C-12a for **7d**, while there is no NOE observed for the same pair of hydrogens in **7c**. When starting from 2,3-dihydro-benzofuran-5-carbaldehyde **3b**, compounds **5a** and **5b** were obtained in 17% and 9.0% yields, respectively. The stereochemistry of compound **5a** and **5b** were assigned by ^{13}C NMR spectroscopy according to the method reported by James Cook.^{10a} The signals for C-1 and C-3 in the *trans* diastereomer **5b** (55.9, 53.2 ppm) is more upfield than those of corresponding *cis* isomer **5a** (59.3, 57.8 ppm). Following the similar cyclization conditions (50 °C, 48 h) for compounds **7c** and **7d**, β -carbolines **7e** and **7f** were obtained from **6c** in 25% and 38% yields, respectively. An NOE was observed for **7f** between C-12a hydrogen and H_a and H_b on the 2,3-dihydro-benzofuran group,



Scheme 1. Synthesis of compound **7a–f**.

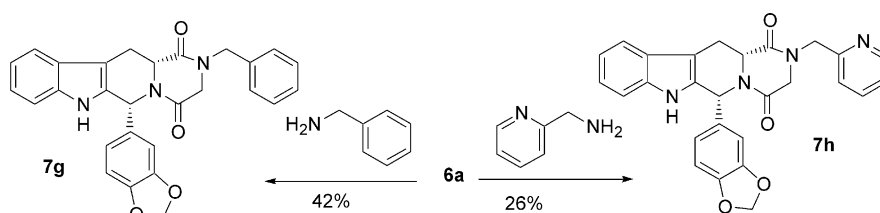
but not for **7e**. By carefully monitoring the reaction progress by HPLC-MS, we were able to stop the cyclization reaction right before significant C-12a epimerization had occurred. Thus, as shown in Scheme 2, when benzyl amine and 2-(aminomethyl)-pyridine was used in place of *N*-methyl amine in cyclization reaction, tetracyclic β -carboline **7g** and **7h** were obtained (50 °C, 24 h) as a single diastereomer in 42% and 26% yields, respectively.

In order to expand the substrate types, we turned our attention to 7-membered D ring. As shown in Scheme 3, we first obtained acylated compound **11a** from compound **5a** in 89% yield when treated with acryloyl chloride. Michael adduct **12a** was formed quickly in 90% yield after the addition of *N*-methyl amine to α,β -unsaturated ketone **11a**. However, compound **12a** was reluctant to cyclize after heated at 50 °C for 48 h. Longer reaction times or higher temperature only led to slow decomposition of starting material. This was presumably due to the 1,3-interaction of C-3-CO₂Me group and C-1 benzofuranyl group in *cis* compound **12a**, which forces both C-1 and C-3 substituents to *pseudo*-equatorial positions. Molecular modeling showed that it is difficult for -NHMe in the *N*-2 side chain to approach the C-3 carbonyl group when the C-3 substituent is *pseudo*-equatorial. On the contrary, the *trans* relationship of substituents on C-1 and C-3 in compound **12b** results in the most populated conformation of C-1 *pseudo*-equatorial and C-3 *pseudo*-axial since C-1 substituent is bigger than C-3. The *pseudo*-axial-CO₂Me of C-3 is more accessible when NHMe on *N*-2 side chain approaches in an *anti*-periplanar version, so that 7-membered D ring was formed to provide compound **7i** in 96% yield.

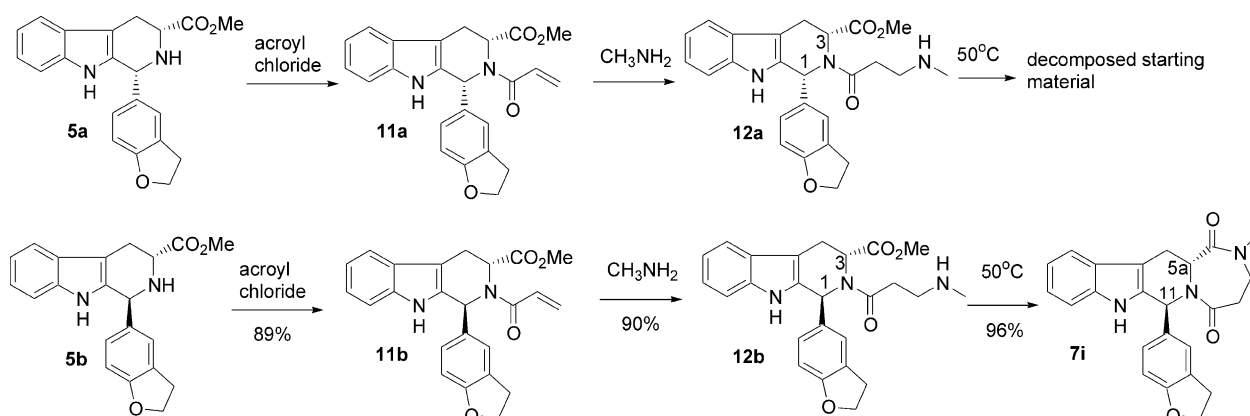
After the tetracyclic β -carboline precursors **7a–7i** were successfully obtained, they were subjected to Winterfeldt oxidation conditions.⁹ As shown in Scheme 4, when β -carboline **7a** was treated with KO^tBu/O₂, a very polar intermediate **8a** formed quickly with 32 mass units higher than compound **7a**. Attempts to isolate intermediate **8a** failed due to its high water solubility. We tentatively assigned intermediate **8a** as an oxidized product with concomitant D-ring hydrolysis when quenched with water. So, we stopped the reaction by neutralizing the excess amount of KO^tBu with HCl, and then subjected the reaction mixture to standard coupling condition (PyBrOP/DIEA). The desired pyrroloquinolone **9a** was isolated in 43% yield. This one-pot procedure has been applied to compounds **7b** through **7h** to provide compounds **9b–9h** in 8–63% yield.

Due to the less strain in a 7-membered ring than in a 6-membered ring, we observed the direct formation of pyrroloquinolone **9i** from **7i** with D-ring intact, under oxidation condition (KO^tBu/O₂). This was shown in Scheme 5. All of these reactions were carefully monitored by HPLC-MS, and no epimerization at C-3 was observed. Table 1 summarizes the experimental results for obtaining compounds **9a** through **9i**.

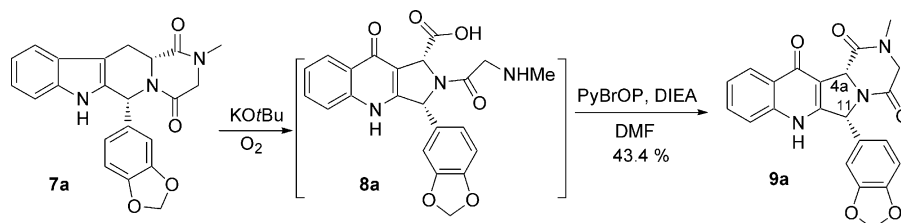
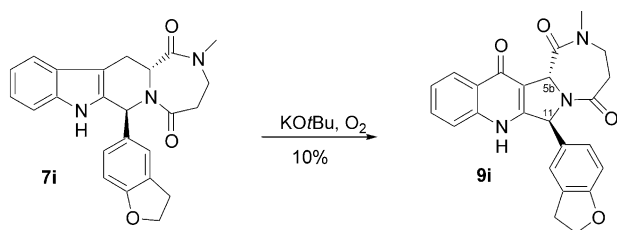
After considerable work had been carried out in this project, we discovered the potassium superoxide as an alternative reagent for Winterfeldt oxidation of β -carboline.^{8h} Compound **7a** was converted to compound **9a** in 38% yield upon treatment with KO₂/18-crown-6 (Scheme 6). This is in consistence with our findings that KO₂/18-crown-6 oxidation condition is complementary to Winterfeldt condition for base sensitive substrates.^{8h}



Scheme 2. Synthesis of compounds **7g** and **7h**.



Scheme 3. Synthesis of compound **7i**.

Scheme 4. Construction of compound **9a**.Scheme 5. Construction of compound **9i**.

3. Results and discussion

We have observed that all pyrimidinyl pyrroloquinolones in our previous studies are much more potent than their corresponding β -carboline precursors.⁸ Yet, for the tetracyclic compounds in this paper, the SAR was different (Table 2). Firstly, for compounds **7c/9c**, **7f/9f** and **7i/9i**, pyrroloquinolones are more potent than β -carbolines. For **7b/9b**, pyrroloquinolone has the same potency as that of β -carboline. For compounds **7a/9a**, **7d/9d**, **7e/9e**, **7g/9g** and **7h/9h**, pyrroloquinolones are less potent than β -carbolines. Secondly, this study demonstrated that, for pyrroloquinolones pairs **9a/9b**, **9d/9e** and **9e/9f**, *cis* isomers (C-4a and C-11) are more potent than *trans* isomers. When the substituent on the nitrogen in D-ring was changed from methyl to benzyl in **9a/9g**, potency decreased 9-fold. When benzyl group in **9g** was replaced by pyridinylmethyl in **9h**, potency slightly increased. Finally, 7-membered D-ring pyrroloquinolone **9i** was less potent than any 6-membered D-ring pyrroloquinolones **9a–h**. The potency of the best compound (**9e**) is approaching that of sildenafil. In addition, the tetracyclic analogues reported in this paper are generally less potent than pyrimidinyl pyrroloquinolones^{8e} and acyl pyrroloquinolones.^{8j}

One of the most important issues for the clinical utility of PDE5 inhibitors is that these inhibitors should demonstrate strong inhibitory potencies toward PDE5 while maintaining little or no inhibition toward other PDEs. The inhibitory activities of PDE1, PDE2, PDE3, PDE4 and PDE6 of all compounds in this series, together with sildenafil were tested. We controlled the substrate cGMP concentration relatively low (30 nM) to meet the condition of $[S] \ll K_m$, so that IC_{50} values approximate the K_i values. The values of K_i of PDE1–4 and PDE6 divided by K_i of PDE5 were calculated. All the data were shown in Table 3. Compounds **9d** and **9h** showed better PDE1/5 selectivity than sildenafil. Compounds **9a**, **e** and **f** had better PDE6/5 selectivity than sildenafil. Weaker inhibition of PDE6 will probably decrease the visual side effects observed in current Viagra[®] therapy. Compound **9e** showed better selectivities

than sildenafil against all tested PDEs though its potency was 4-fold less.

Preliminary pharmacokinetics studies have been conducted with selected analogues. In particular, compound **9a** had an oral bioavailability of 19% in male rats, compared with 11% for sildenafil in a parallel study.

Selected pyrroloquinolones from this series were studied in vivo in an anesthetized canine model for erectile dysfunction. In these studies, drugs were dosed via the intravenous route using sildenafil citrate as a positive control. Compound **9a** was found to be efficacious at 300 $\mu\text{g}/\text{kg}$ in the canine model (Fig. 2). Compound **9a** is 8-fold less potent than sildenafil with slightly higher oral bioavailability. Even at higher dosage (300 $\mu\text{g}/\text{kg}$), compound **9a** did not reach 50% of sildenafil's efficacy. This might be due to protein binding in the systemic circulation, leading to low concentration of unbound drug. This could also be explained by unfavorable local tissue distribution.

4. Conclusion

In summary, we have discovered a series of novel pyrroloquinolone analogues as potent and selective PDE5 inhibitors. Selected compounds are orally bioavailable in preliminary pharmacokinetics studies. Representative compound demonstrated in vivo efficacy in a canine model for erectile dysfunction.

5. Experimental

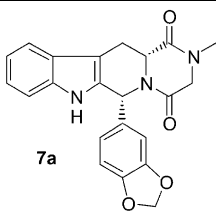
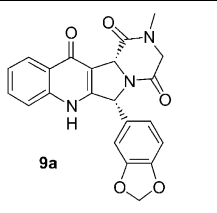
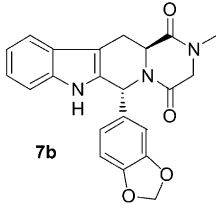
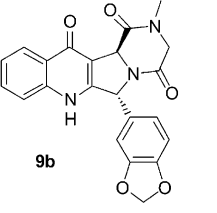
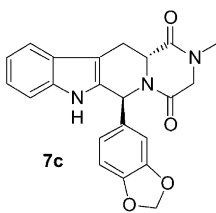
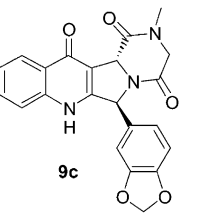
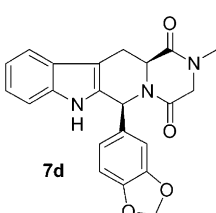
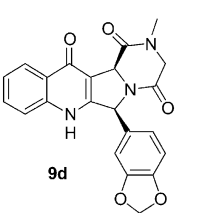
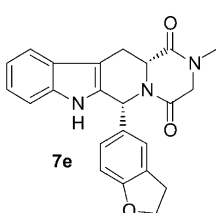
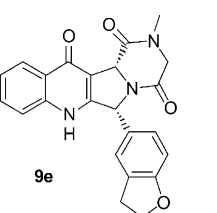
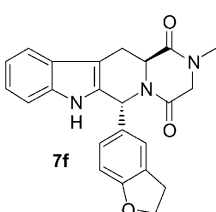
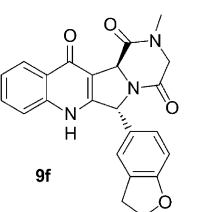
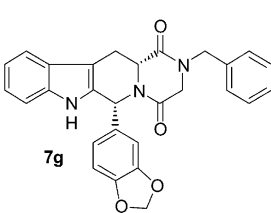
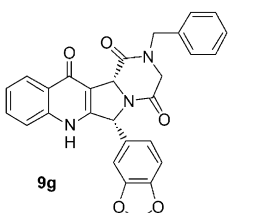
5.1. PDE Isolation

PDE1, 2, 3, 4, 5, and 6 were isolated from human heart, corpus cavernosum, platelet, skeletal muscle, corpus cavernosum, and retina respectively, as described in ref 8k.

5.2. PDE Assay and K_i Determination

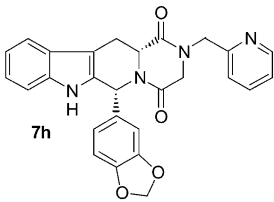
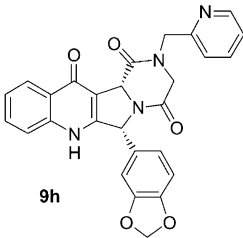
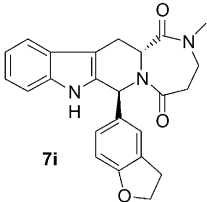
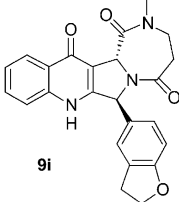
The PDE assays were carried out as described in reference 8k. Stock solutions of compounds were prepared in 100% DMSO, diluted in 100% DMSO to the appropriate concentrations, and added to the assay buffer to give a final concentration of 2% DMSO. The amount of enzyme used in each reaction was such that the hydrolysis of substrates did not exceed 15% so that the amount of product increased linearly with time. Duplicates were

Table 1. Transformation of compound **7** to compound **9^a**

Entry	Substrate	Product	Time ^c (h)	Yield (%)
1	 7a	 9a	4	43
2	 7b	 9b	3	50
3	 7c	 9c	6	63
4	 7d	 9d	5	20
5	 7e	 9e	8	30
6	 7f	 9f	5	8
7	 7g	 9g	3	35

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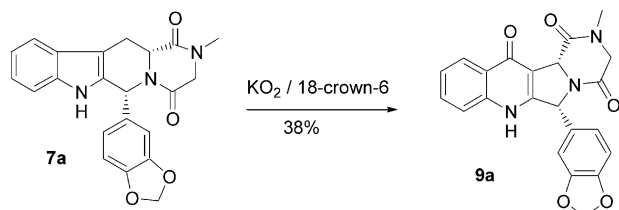
Table 1 (continued)

Entry	Substrate	Product	Time ^c (h)	Yield (%)
8			7	8
9 ^b			3	10

^a Reaction condition for entry 1–8: (1) KO^tBu/O₂, (2) HCl, (3) PyBrOP, DIEA.

^b Reaction condition for entry 9: KO^tBu/O₂.

^c Reaction time for the oxidation step.



Scheme 6. An alternative route to compound 9a.

Table 2. In vitro PDE5 inhibitory data for compounds 7 and 9

β-Carboline sildenafil	K _i (nM)	Pyrroloquinolone	K _i (nM)
	1.8		
7a	1.6	9a	14
7b	47	9b	48
7c	100	9c	80
7d	5.5	9d	17
7e	2.3	9e	7.1
7f	299	9f	69
7g	4.2	9g	123
7h	12	9h	63
7i	586	9i	148

run in each assay. IC₅₀ values were obtained from a nonlinear regression curve fitting program. At very low substrate concentrations ([S] < < K_m), IC₅₀ values approximate the K_i values.

5.3. In vivo efficacy study

All animals were handled in accordance with the NRC *Guide for Care and Use of Laboratory Animals* and the protocol was approved by Internal Animal Care and Usage Committee of Johnson & Johnson Pharmaceutical Research & Development, LLC. An anesthetized dog

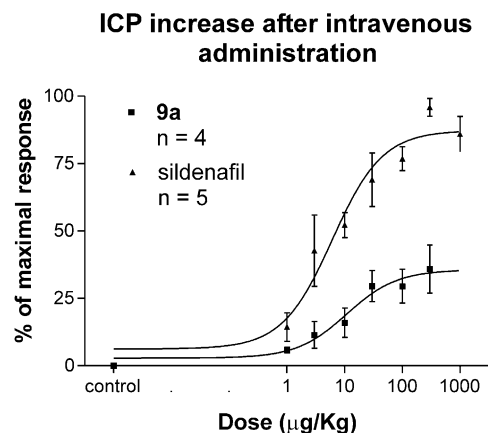
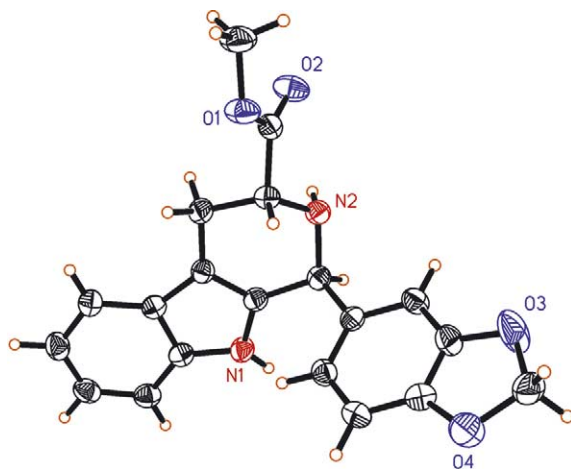
model was used and the animals were prepared as described in reference 8k. After period of stabilization, the control ICP response curves were generated at an appropriate current output setting (ICP increase to 20–30% of systolic pressure). When similar ICP increases were obtained from at least two control stimulations applied in 15-min interval, the baseline was established. Area under the curve was computed and designated as baseline. Ascending doses of compound were administered via bolus injection to the right femoral vein at 40 min interval. The effects of compound on ICP increase were evaluated 15 min after each dosing by electrical stimulation with the frequency setting the baseline. To determine the effect of the compound, the area under the curve (AUC) for ICP at each stimulation was computed and subtracted from the baseline AUC. At the end of each experiment, 300 μg/kg sildenafil was given intravenously. This dose was showed to induce a maximal response in our hands. For each animal, the highest ICP increase was designated as 100%.

5.4. Synthesis of compounds reported

5.4.1. General. NMR spectra were obtained at 400 MHz or 300 MHz on a Bruker AVANCE300 or AVANCE400 spectrometer. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Thin-layer chromatography was carried out using 2.5×7.5 cm silica gel 60 (250 μM layer) plates with UV detection. Magnesium sulfate was employed to dry organic extracts prior to concentration by rotary evaporation. Flash chromatography was performed using EM science silica gel 60 (230–400 mesh). Standard solvents from J. T. Baker were used as received. Anhydrous solvents from J. T. Baker or Aldrich and all other commercially available reagents were used without further purification. Melting points were taken using a Thomas-Hoover

Table 3. Selectivities of compound **9a–i** on PDE isoforms

Compd	K_i (nM)	PDE 1/5	PDE 2/5	PDE 3/5	PDE 4/5	PDE 6/5 (rod)	PDE 6/5 (cone)
sildenafil	1.80	170	6000	7110	2,610	5.4	9.6
9a	14	860	4500	7000	5,000	142	147
9b	48	430	2100	2100	2,100	12	22
9c	80	310	840	1250	940	37	35
9d	17	2160	5810	5810	3,150	39	64
9e	7.1	3660	11,410	12,440	9,820	385	554
9f	69	540	1450	1440	1,450	76	125
9g	123	810	70	810	70	15	43
9h	63	1580	1580	1580	530	41	42
9i	148	680	680	680	250	20	24

**Figure 2.** In vivo efficacy of **9a** in canine model.**Figure 3.** ORTEP picture of compound **4b**.

MelTemp apparatus without any correction. Micro-analysis was done by Quantitative Technologies Inc., Whitehouse, NJ. Mass spectra were obtained on a Hewlett-Packard 5989A quadrupole mass spectrometer. Silica gel (E. Merck, 230–400 mesh) was used for all flash chromatography. Thin-layer chromatography was performed on Analtech silica gel (250 μm). HPLC analysis was carried on Agilent 1100 Series LC/MSD. High resolution mass spectra were obtained on M-Scan's VG Analytical ZAB 2SE high field mass spectrometer.

5.4.2. (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 (4a**) and (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 (**4b**).** D-Tryptophan methyl ester hydrochloride (7.2 g, 28.3 mmol) was dissolved in methanol (74 mL) and dichloromethane (274 mL), followed by the addition of piperonal **3a** (4.7 g, 31.1 mmol) and TFA (5.0 mL). The reaction mixture was stirred at room temperature for 60 h. After cooled to 0 °C and carefully neutralized with saturated sodium bicarbonate aqueous solution, the organic layer was separated, dried and concentrated. The residue was purified by silica gel column (800 g) eluted with ethyl acetate/hexane (30/100) to provide *cis* isomer **4a** as a white solid (2.5 g, 25%). Continuous elution with ethyl acetate/hexane (40/60) yielded the *trans* isomer **4b** as a white solid (2.4 g, 24%). **4a**: ^1H NMR (CDCl_3) δ 3.11 (m, 1H), 3.23 (m, 1H), 3.69 (s, 3H), 3.98 (t, 1H, $J=6.1$ Hz), 5.31 (s, 1H), 6.78–7.62 (m, 7H). MS (m/z): 351 [$\text{M} + \text{H}$] $^+$. **4b**: ^1H NMR (CDCl_3) δ 2.95 (m, 1H), 3.20 (m, 1H), 3.81 (s, 3H), 3.92 (m, 1H), 5.18 (s, 1H), 5.89 (s, 2H), 6.78–7.52 (m, 7H); MS (m/z): 351 [$\text{M} + \text{H}$] $^+$. Anal. calcd for **4a** $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_4$: C 68.56, H 5.18, N 8.00; found, C 68.27, H 4.91, N 7.81. Recrystallization of **4b** from dichloromethane provided crystals suitable for X-ray analysis. X-ray ORTEP picture of **4b** was shown in Figure 3.

5.4.3. (1*R*,3*R*)-1-(2,3-Dihydro-benzofuran-5-yl)-2,3,4,9-tetrahydro-1*H*- β -carboline-3-carboxylic acid methyl ester (5a**) and (1*S*,3*R*)-1-(2,3-Dihydro-benzofuran-5-yl)-2,3,4,9-tetrahydro-1*H*- β -carboline-3-carboxylic acid methyl ester (**5b**).** Following the same procedure as **4a** and **4b**, starting from 2,3-dihydro-benzofuran-5-carbaldehyde **3b**, compound **5a** was obtained in 17% yield and compound **5b** was obtained in 9% yield. **5a**: ^1H NMR (CDCl_3) δ 2.90–3.28 (m, 4H), 3.78 (s, 3H), 3.91 (m, 1H), 4.51 (t, 2H, $J=8.2$ Hz), 5.12 (s, 1H), 6.71 (d, 1H, $J=6.7$ Hz), 7.12 (m, 5H), 7.52 (m, 1H); ^{13}C NMR (CD_3OD with two drops of $\text{DMSO}-d_6$) δ 26.4, 30.4, 52.6, 57.8, 59.3, 72.4, 108.7, 109.9, 112.1, 118.6, 119.8, 122.2, 126.5, 128.1, 128.9, 129.8, 134.1, 136.0, 138.0, 161.5, 174.4; MS (m/z): 349 [$\text{M} + \text{H}$] $^+$. **5b**: ^1H NMR (CDCl_3) δ 3.02–3.28 (m, 4H), 3.71 (s, 3H), 3.92 (m, 1H), 5.38 (s, 1H), 6.71 (d, 1H, $J=6.7$ Hz), 6.92–7.23 (m, 5H), 7.51 (m, 1H), 7.61 (s, 1H); ^{13}C NMR (CD_3OD with two drops of $\text{DMSO}-d_6$) δ 25.8, 30.6, 52.7, 53.2, 55.9, 72.5, 108.5, 110.1, 112.2, 118.9, 120.0, 122.5, 126.5, 128.2, 128.9, 129.7, 135.1, 135.6, 138.1, 161.3, 175.2; MS (m/z): 349 [$\text{M} + \text{H}$] $^+$.

5.4.4. (6*R*,12*aR*)-6-(Benzo[1,3]dioxol-5-yl)-2-methyl-2,3,6,7,12,12*a* hexahydropyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*a*) and (6*R*,12*aS*)-6-(Benzo[1,3]dioxol-5-yl)-2-methyl-2,3,6,7,12,12*a* hexahydropyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*b*). To a mixture of **4a** (1.01 g, 2.59 mmol) and sodium bicarbonate (0.30 g) in methylene chloride (30 mL) was added chloroacetyl chloride (0.54 mL, 6.78 mmol) in dichloromethane (5 mL) at 0 °C under nitrogen. After 1 h at 0 °C, the reaction mixture was poured into ice-cold saturated sodium bicarbonate aqueous solution (20 mL). The organic layer was separated and the aqueous layer was extracted with dichloromethane (2×50 mL). The combined organic layers were dried and concentrated to provide acylated compound **6a** as a yellow solid (1.12 g, 91%). The product was used for the next step without further purification.

To a solution of 1-benzo[1,3]dioxol-5-yl-2-(2-chloroacetyl)-2,3,4,9-tetrahydro-1*H*-*b*-carboline-3-carboxylic acid ethyl ester **6a** (1.12 g, 2.29 mmol) in methanol (100 mL) was added methylamine (2.0 M in methanol, 4 mL, 8.0 mmol) at room temperature under nitrogen and stirred for 1 h. The resulting mixture was heated at 50 °C for 16 h before cooling down to room temperature. After most of the solvent was removed under reduced pressure, the reaction mixture was then diluted with water (100 mL) and extracted with methylene chloride (1×100 mL, 2×30 mL). The combined organic layers were dried and concentrated. The residue was purified on silica gel column (50 g) eluted with dichloromethane/methanol (99:1) to provide **7a** (542 mg, 54%) as colorless crystals and **7b** (11.1 mg, 1.1%) as a white powder. **7a**: mp 303–305 °C; $[\alpha]_D^{20} + 71.4$ (*c* 1.00, CHCl₃); ¹H NMR (CDCl₃) δ 3.08 (s, 3H), 3.19 (m, 1H), 3.79 (m, 1H), 4.05 (AB quartet, 2H, *J* = 61.8, 18.5 Hz), 4.31 (m, 1H), 5.92 (d, 2H, *J* = 7.7 Hz), 6.18 (s, 1H), 6.70–7.82 (m, 7H); MS (*m/z*): 390 [M + H]⁺; HRMS calcd M⁺ for C₂₂H₁₉N₃O₄ 389.1376; found 389.1395. Anal. calcd for C₂₂H₁₉N₃O₄: C, 67.86; H, 4.92; N, 10.79; O, 16.43; found: C, 67.64; H, 4.89; N, 10.64. **7b**: mp 288–290 °C; ¹H NMR (CDCl₃) δ 2.91 (m, 1H), 3.02 (s, 3H), 3.51 (m, 1H), 4.07 (AB quartet, 2H, *J* = 58.0, 18.0 Hz), 4.35 (broad d, 1H, *J* = 7.6 Hz), 5.91 (s, 2H), 6.68–7.96 (m, 8H); MS (*m/z*): 390 [M + H]⁺; HRMS calcd M⁺ for C₂₂H₁₉N₃O₄ 389.1376; found 389.1386.

5.4.5. (6*S*,12*aR*)-6-(Benzo[1,3]dioxol-5-yl)-2-methyl-2,3,6,7,12,12*a* hexahydropyrazino[1',2',5:1,6]pyrido[3,4-*b*]indole-1,4-dione (7*c*) and (6*S*,12*aS*)-6-(Benzo[1,3]dioxol-5-yl)-2-methyl-2,3,6,7,12,12*a* hexahydropyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*d*). Following the same procedure as **7a** and **7b**, starting from **4b**, and in the reaction with methyl amine step, reaction was allowed to stir at 50 °C for 48 h. **7c** was obtained as a white solid in 21% yield; **7d** was obtained as an off-white solid in 18% yield. **7c**: $[\alpha]_D^{20} + 250$ (*c* 1.00, CHCl₃); mp 286–288 °C; ¹H NMR (CDCl₃) δ 2.95 (m, 1H), 3.02 (s, 3H), 3.56 (m, 1H), 4.10 (AB quartet, 2H, *J* = 55.6, 14.0 Hz), 4.39 (m, 1H), 5.96 (s, 2H), 6.72–7.88 (m, 8H); HRMS calcd M⁺ for C₂₂H₁₉N₃O₄ 389.1376, found 389.1367. **7d**: mp 302–304 °C decomposed; ¹H NMR (CDCl₃) δ 3.03 (s, 3H), 3.21 (m, 1H), 3.76 (m, 1H), 4.03 (AB

quartet, 2H, *J* = 60.2, 13.9 Hz), 4.31 (m, 1H), 5.91 (d, 2H, *J* = 7.6 Hz), 6.18 (s, 1H), 6.68–7.85 (m, 7H); MS (*m/z*): 390 [M + H]⁺; HRMS calcd M⁺ for C₂₂H₁₉N₃O₄ 389.1376, found 389.1387.

5.4.6. (6*R*,12*aR*)-6-(2,3-Dihydro-benzofuran-5-yl)-2-methyl-2,3,6,7,12,12*a*-hexahydro-pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*e*) and (6*R*,12*aS*)-6-(2,3-Dihydro-benzofuran-5-yl)-2-methyl-2,3,6,7,12,12*a*-hexahydro-pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*f*). Following the same procedure as **7a** and **7b**, starting from **5a**, in the reaction with methyl amine step, reaction was allowed to stir at 50 °C for 48 h. **7e** was obtained in 25% yield and **7f** was obtained in 38% yield. **7e**: ¹H NMR (CDCl₃) δ 2.96 (s, 3H), 3.12 (t, 2H, *J* = 8.6 Hz), 3.21 (m, 1H), 3.79 (m, 1H), 4.01 (AB quartet, 2H, *J* = 52.7, 17.0 Hz), 4.32 (m, 1H), 4.51 (t, 2H, *J* = 8.6 Hz), 6.17 (s, 1H), 6.65 (d, 1H, *J* = 5.7 Hz), 7.09–7.82 (m, 7H); MS (*m/z*): 388 [M + H]⁺; HRMS calcd M⁺ for C₂₃H₂₁N₃O₃ 387.1583, found 387.1590. **7f**: ¹H NMR (CDCl₃) δ 2.82 (m, 1H), 2.95 (s, 3H), 3.15 (t, 2H, *J* = 8.6 Hz), 3.52 (m, 1H), 4.08 (AB quartet, 2H, *J* = 45.6, 14.0 Hz), 4.38 (m, 1H), 4.52 (t, 2H, *J* = 8.6 Hz), 6.75–7.95 (m, 8H); MS (*m/z*): 388 [M + H]⁺; HRMS calcd M⁺ for C₂₃H₂₁N₃O₃ 387.1583, found 387.1590.387.1590.

5.4.7. (6*R*,12*aR*)-6-(Benzo[1,3]dioxol-5-yl)-2-benzyl-2,3,6,7,12,12*a*-hexahydro-pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*g*). Following the same procedure as **7a** using benzyl amine instead of methyl amine in the cyclization step (50 °C, 24 h) the product was obtained in 42% yield as a white solid. **7g**: mp 180–182 °C; ¹H NMR (CDCl₃) δ 3.28 (m, 1H), 3.82 (m, 1H), 3.91 (d, 2H, *J* = 4.6 Hz), 4.32 (m, 1H), 4.61 (AB quartet, 2H, *J* = 150.0, 13.8 Hz), 5.81 (d, 2H, *J* = 4.0 Hz), 6.15 (s, 1H), 6.60–8.24 (m, 12H); MS (*m/z*): 466 [M + H]⁺, 464 [M – H][–]; HRMS calcd M⁺ for C₂₈H₂₃N₃O₄ 465.1688; found 465.1686.

5.4.8. (6*R*,12*aR*)-6-(Benzo[1,3]dioxol-5-yl)-2-pyridin-4-yl-methyl-2,3,6,7,12,12*a*-hexahydro-pyrazino[1',2':1,6]pyrido[3,4-*b*]indole-1,4-dione (7*h*). Following the same procedure as **7a**, using *C*-pyridin-2-yl-methylamine instead of methyl amine in the cyclization step (50 °C, 24 h), the product was obtained as a yellow solid in 26% yield. **7h**: mp 238–240 °C; ¹H NMR (CDCl₃) δ 2.48 (m, 1H), 2.75 (m, 2H), 3.02 (s, 3H), 3.09 (t, 2H, *J* = 8.5 Hz), 3.42 (m, 1H), 4.73 (AB quartet, 2H, *J* = 129.0, 15.0 Hz), 6.60 (d, 1H, *J* = 7.6 Hz), 7.29–6.96 (m, 6H), 7.52 (d, 1H, *J* = 7.6 Hz), 8.98 (s, 1H); MS (*m/z*): 467 [M + H]⁺; 465 [M – H][–]; HRMS calcd (M + H)⁺ for C₂₇H₂₂N₄O₄ 467.1719; found 467.1721.

5.4.9. Preparation of compound 7*i*: (1*S*,3*R*)-2-Acryloyl-1-(2,3-dihydro-benzofuran-5-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline-3-carboxylic acid methyl ester (11*b*). To a solution of **5b** (1.80 g, 5.17 mmol) in dichloromethane (165 mL) was added acryloyl chloride (0.85 mL, 10.34 mmol) and sodium bicarbonate (0.504 g, 6.00 mmol). The reaction mixture was stirred at room temperature for 4 h before quenched with water (100 mL). The aqueous layer was further extracted with dichloromethane (100 mL). After washing with brine (2×150 mL), the

combined organic layers were dried and concentrated. The crude product was purified by silica gel column eluted with 10–50% ethyl acetate/hexane to provide the product as a white solid (1.84 g, 89%). **11b**: ^1H NMR (CDCl_3) δ 3.11 (m, 5H), 3.65 (m, 1H), 4.49 (t, 2H, $J=8.2$ Hz), 5.72 (d, 1H, $J=10.2$ Hz), 6.61 (m, 2H), 6.81 (m, 1H), 6.98 (m, 1H), 7.31–7.11 (m, 5H), 7.57 (d, 1H, $J=6.2$ Hz), 8.03 (s, 1H); MS (m/z): 403 $[\text{M}+\text{H}]^+$, 425 $[\text{M}+\text{Na}]^+$, 401 $[\text{M}-\text{H}]^-$.

5.4.10. (1*S*,3*R*)-1-(2,3-Dihydro-benzofuran-5-yl)-2-(3-methylamino-propionyl)-2,3,4,9-tetrahydro-1*H*- β -carboline-3-carboxylic acid methyl ester (12b**).** To a solution of **11b** (1.84 g, 4.58 mmol) in methanol (40 mL) was added methylamine (2.0 M in THF, 38.9 mL, 77.8 mmol). The reaction mixture was stirred at 50 °C for 2 h before concentrated to yield a yellow oil. The crude product was purified by silica gel column eluted with 5% methanol/dichloromethane to provide the product as a white solid (1.78 g, 90%). **12b**: ^1H NMR (CDCl_3) δ 2.38 (s, 3H), 2.51 (m, 1H), 2.75 (m, 2H), 3.05 (s, 3H), 3.10 (m, 2H), 3.48 (m, 2H), 4.51 (t, 2H, $J=6.8$ Hz), 4.71 (d, 1H, $J=4.0$ Hz), 6.61 (d, 1H, $J=7.6$ Hz), 6.91 (s, 2H), 7.09–7.29 (m, 4H), 7.52 (d, 1H, $J=4.0$ Hz), 9.0 (s, 1H); MS (m/z): 434 $[\text{M}+\text{H}]^+$, 432 $[\text{M}-\text{H}]^-$.

5.4.11. (1*S*,5*aR*)-11-(2,3-Dihydro-benzofuran-5-yl)-7-methyl-5,5*a*,8,9,11,12-hexahydro-7*H*-7,10*a*,12-triaza-cyclohepta[b]fluorene-6,10-dione (7i**).** A solution of **12b** (1.78 g, 4.13 mmol) in methanol (80 mL) was stirred at 50 °C for 48 h until the complete consumption of starting material monitored by HPLC. After concentration, the crude product was purified by silica gel column eluted with 5–10% methanol/dichloromethane to provide the product as a white solid (1.60 g, 96%). **7i**: mp 190–192 °C; ^1H NMR (CDCl_3) δ 2.89 (m, 2H), 3.08 (s, 3H), 8.01 (s, 1H), 3.15 (t, 2H, $J=6.2$ Hz), 3.22 (m, 1H), 3.48–3.38 (m, 3H), 3.75 (m, 1H), 4.61–4.48 (m, 5H), 6.65 (d, 1H, $J=6.0$ Hz), 7.31–6.92 (m, 6H), 7.48 (d, 1H, $J=5.2$ Hz); MS (m/z): 402 $[\text{M}+\text{H}]^+$, 424 $[\text{M}+\text{Na}]^+$, 825 $[\text{2M}+\text{Na}]^+$, 400 $[\text{M}-\text{H}]^-$; HRMS calcd ($\text{M}+\text{H})^+$ for $\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_3$ 402.1818, found 402.2047.

5.4.12. Procedure for converting **7a to **9a**.** (**4aR,11R**)-11-(Benzo[1,3]dioxol-5-yl)-3-methyl-2,3,4*a*,11-tetrahydro-10*H*-3,10,11*a*-triaza-benzo[b]fluorene-1,4,5-trione (**9a**). To a solution of compound **7a** (0.106 g, 0.272 mmol) in *N,N*-dimethylformamide (2.0 mL) was added KO^tBu (0.46 mL, 1.0 M in THF, 0.46 mmol). Dry air was bubbled through the reaction mixture under stirring for 4 h. Excess amount of KO^tBu was neutralized by HCl (0.23 mL, 2.0 M in ether, 0.46 mmol). To the reaction mixture, PyBrOP (0.14 g, 0.299 mmol, 1.1 equiv) and di-*iso*-propyl ethylamine (0.095 mL, 0.544 mmol) were then added. After 16 h at 25 °C, the reaction mixture was partitioned in ethyl acetate/water (30 mL/30 mL). The organic layer was washed with brine (3 $\times$ 30 mL), dried and concentrated. The crude product was purified by silica gel column eluted with 1% methanol/dichloromethane to provide the product as a yellow solid (16.2 mg, 44%). **9a**: mp 208–210 °C; ^1H NMR (CD_3OD) δ 3.12 (m, 2H), 3.20 (s, 3H), 3.52 (m, 2H), 4.68 (m, 1H), 5.88 (m, 2H), 6.74 (s, 1H), 6.84 (s, 1H), 6.94 (s, 1H),

6.98–7.16 (m, 2H), 7.25 (d, 1H, $J=10.0$ Hz), 7.48 (d, 1H, $J=10.0$ Hz); MS (m/z): 404 $[\text{M}+\text{H}]^+$, 426 $[\text{M}+\text{Na}]^+$, 829 $[\text{2M}+\text{Na}]^+$, 402 $[\text{M}-\text{H}]^-$; HRMS calcd ($\text{M}+\text{H})^+$ for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_5$ 404.1246; found 404.1245.

5.4.13. (4*aS*,11*R*)-11-(Benzo[1,3]dioxol-5-yl)-3-methyl-2,3,4*a*,11-tetrahydro-10*H*-3,10,11*a*-triaza-benzo[b]fluorene-1,4,5-trione (9b**).** Compound **9b** was prepared following the same procedure as compound **9a** in 50% yield. **9b**: mp 185–187 °C; ^1H NMR (CD_3OD) δ 3.02 (s, 3H), 3.33 (m, 1H), 3.53 (m, 1H), 4.73 (m, 1H), 5.92 (m, b, 2H), 6.76 (s, 1H), 6.87 (s, 1H), 6.94 (s, 1H), 7.12 (m, 2H), 7.29 (d, 1H, $J=8.7$ Hz), 7.52 (d, 1H, $J=8.7$ Hz), 7.98 (s, 1H). MS (m/z): 404 $[\text{M}+\text{H}]^+$, 426 $[\text{M}+\text{Na}]^+$, 829 $[\text{2M}+\text{Na}]^+$, 402 $[\text{M}-\text{H}]^-$; HRMS calcd ($\text{M}+\text{H})^+$ for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_5$ 404.1246, found 404.1236.

5.4.14. (4*aR*,11*S*)-11-(Benzo[1,3]dioxol-5-yl)-3-methyl-2,3,4*a*,11-tetrahydro-10*H*-3,10,11*a*-triaza-benzo[b]fluorene-1,4,5-trione (9c**).** Compound **9c** was prepared following the same procedure as compound **9a** in 63% yield. **9c**: mp 232–234 °C; ^1H NMR (CD_3OD) δ 3.01–3.27 (m, 4H), 3.22 (s, 3H), 3.58 (m, 1H), 4.75 (m, 1H), 5.92 (m, 2H), 6.74–7.19 (m, 5H), 7.34 (d, 1H, $J=10.3$ Hz), 7.52 (d, 1H, $J=10.3$ Hz); MS (m/z): 426 $[\text{M}+\text{Na}]^+$, 404 $[\text{M}+\text{H}]^+$, 829 $[\text{2M}+\text{Na}]^+$, 402 $[\text{M}-\text{H}]^-$; HRMS calcd ($\text{M}+\text{H})^+$ for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_5$ 404.1246, found 404.1261.

5.4.15. (4*aS*,11*S*)-11-(Benzo[1,3]dioxol-5-yl)-3-methyl-2,3,4*a*,11-tetrahydro-10*H*-3,10,11*a*-triaza-benzo[b]fluorene-1,4,5-trione (9d**).** Compound **9d** was prepared following the same procedure as compound **9a** in 20% yield. **9d**: mp 180–182 °C; ^1H NMR (CD_3OD) δ 3.03 (s, 3H), 3.54 (m, 1H), 4.03 (m, 1H), 4.24 (m, 1H), 4.74 (m, 1H), 5.88 (m, 2H), 6.69–7.21 (m, 5H), 7.32 (d, 1H, $J=10.8$ Hz), 7.51 (d, 1H, $J=10.8$ Hz); MS (m/z): 426 $[\text{M}+\text{Na}]^+$, 404 $[\text{M}+\text{H}]^+$, 829 $[\text{2M}+\text{Na}]^+$, 402 $[\text{M}-\text{H}]^-$; HRMS calcd ($\text{M}+\text{H})^+$ for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_5$ 404.1246, found 404.1236.

5.4.16. (4*aR*,11*R*)-11-(2,3-Dihydro-benzofuran-6-yl)-3-methyl-2,3,4*a*,11-tetrahydro-10*H*-3,10,11*a*-triaza-benzo[b]fluorene-1,4,5-trione (9e**).** Compound **9e** was prepared following the same procedure as compound **9a** in 30% yield. **9e**: ^1H NMR (CD_3OD) δ 3.05 (s, 3H), 3.15 (t, 2H, $J=9.3$ Hz), 3.54 (m, 1H), 4.05 (d, 1H, $J=18.0$ Hz), 4.28 (d, 1H, $J=18.0$ Hz), 4.57 (t, 2H, $J=9.3$ Hz), 4.72 (m, 1H), 6.65 (m, 1H), 6.92–7.32 (m, 5H), 7.51 (m, 1H), 7.98 (broad s, 1H; -NH); MS (m/z): 424 $[\text{M}+\text{Na}]^+$, 402 $[\text{M}+\text{H}]^+$, 825 $[\text{2M}+\text{Na}]^+$, 400 $[\text{M}-\text{H}]^-$; HRMS calcd ($\text{M}+\text{H})^+$ for $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_4$ 402.1454, found 402.1450.

5.4.17. (4*aS*,11*R*)-11-(2,3-Dihydro-benzofuran-6-yl)-3-methyl-2,3,4*a*,11-tetrahydro-10*H*-3,10,11*a*-triaza-benzo[b]fluorene-1,4,5-trione (9f**).** Compound **9f** was prepared following the same procedure as compound **9a** in 8% yield. **9f**: mp 190–192 °C; ^1H NMR (CD_3OD) δ 3.01–3.15 (m, 4H), 3.23 (s, 3H), 3.14 (t, 2H, $J=9.3$ Hz), 3.55 (m, 1H), 4.56 (t, 2H, $J=9.3$ Hz), 4.71 (m, 1H), 6.61 (m, 1H), 6.91–7.28 (m, 5H), 7.51 (m, 1H); MS (m/z): 424 $[\text{M}+\text{Na}]^+$, 402 $[\text{M}+\text{H}]^+$, 825 $[\text{2M}+\text{Na}]^+$, 400

$[M-H]^-$; HRMS calcd $(M+H)^+$ for $C_{23}H_{19}N_3O_4$ 402.1454, found 402.1472.

5.4.18. (4a*R*,11*R*)-11-(Benzo[1,3]dioxol-5-yl)-3-benzyl-2,3,4a,11-tetrahydro-10*H*-3,10,11a-triaza-benzo[*b*]fluorene-1,4,5-trione (9g). Compound **9g** was prepared following the same procedure as compound **9a** in 35% yield. **9g**: 1H NMR (CD_3OD) δ 3.03 (m, 1H), 3.54 (m, 2H), 3.95 (m, 1H), 4.12 (m, 1H), 4.78 (m, 1H), 5.88 (m, 2H), 6.69–7.51 (m, 12H); MS (m/z): 502 $[M+Na]^+$, 981 $[2M+Na]^+$, 478 $[M-H]^-$; HRMS calcd $(M+H)^+$ for $C_{28}H_{21}N_3O_5$ 480.1559, found 480.1558.

5.4.19. (4a*R*,11*R*)-11-(Benzo[1,3]dioxol-5-yl)-3-pyridin-2-ylmethyl-2,3,4a,11-tetrahydro-10*H*-3,10,11a-triaza-benzo[*b*]fluorene-1,4,5-trione (9h). Compound **9h** was prepared following the same procedure as compound **9a** in 8% yield. **9c**: 1H NMR (CD_3OD) δ 3.54 (m, 2H), 4.81 (m, 2H), 5.18 (m, 2H), 5.92 (s, 2H), 6.71–8.71 (m, 11H); MS (m/z): 481 $[M+H]^+$, 503 $[M+Na]^+$, 983 $[2M+Na]^+$, 479 $[M-H]^-$; HRMS calcd $(M+H)^+$ for $C_{27}H_{20}N_4O_5$ 481.1512, found 481.1554.

5.4.20. (5b*R*,11*S*)-11-(2,3-Dihydro-benzofuran-5-yl)-7-methyl-5,5a,8,9,11,12-hexahydro-7*H*-7,10a,12-triaza-cyclohepta[*b*]fluorene-6,10-dione (9i). To a solution of compound **7i** (0.095 g, 0.24 mmol) in *N,N*-dimethylformamide (2.5 mL) was added KO*t*Bu (0.47 mL, 1.0 M in THF, 0.47 mmol). The reaction mixture was stirred with dry air bubbling for 1 h. Then the reaction mixture was partitioned in ethyl acetate/water (30 mL/30 mL). The organic layer was washed with brine (3×15 mL), dried and concentrated. The crude product was purified on silica gel eluted with 1% methanol/methylene chloride to provide the product as a yellow solid **9i** (10.0 mg, 10%) with recovered starting material and dehydrogenated starting material (~10 mg, ~10%). **9i**: mp 193–195 °C; 1H NMR (CD_3OD) δ 2.68 (t, 2H, $J=8.2$ Hz), 3.21 (s, 3H), 3.23 (s, 1H), 3.39 (t, 2H, $J=8.2$ Hz), 3.85 (m, 2H), 4.68 (m, 2H), 6.98 (d, 1H, $J=9.6$ Hz), 7.38–7.86 (m, 6H); MS (m/z): 416 $[M+H]^+$, 414 $[M-H]^-$; HRMS calcd $(M+H)^+$ for $C_{23}H_{19}N_3O_5$ 416.1610, found 416.1612.

KO₂ oxidation of compound **7a** to compound **9a**

To a solution of β -carboline **7a** (38.9 mg, 0.10 mmol) and 18-crown-6 (26.4 mg, 0.10 mmol) in *N,N*-dimethylformamide (1.0 mL) was added KO₂ (35.5 mg, 0.50 mmol). The reaction mixture was allowed to stir at 25 °C for 6 h before quenched with a few drops of water. The crude mixture was then partitioned between ethyl acetate/brine (50 mL/50 mL). The organic layer was separated and washed with brine (3×50 mL). After dried and concentrated, the oily crude product was purified by silica gel column eluted with 1% methanol/methylene chloride to provide compound **9a** as a yellow solid (15.3 mg, 38%).

6. Supplementary material

Crystallographic data for the structure **4b** have been deposited with the Cambridge Crystallographic Data

Center as supplementary publication number CCDC 225080. Copies of the data can be obtained, free of charge, on application by e-mail to deposit@ccdc.cam.ac.uk.

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