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Short synthesis of piperizinohydroisoquinoline ring by selective Pictet–Spengler cyclization and evaluation of antitumor activity

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Abstract—A rapid access to the piperizinohydroisoquinoline motif, which has feature of the saframycins and related pentacyclic antitumor alkaloids is described. The key features of the synthetic strategy include (1) a controlled mono-Pictet–Spengler cyclization of the symmetrical 3,6-bis-[(2,5-dimethoxy-phenyl)methyl]piperizine-2,5-dione (**1**), with aldehydes to give **2**, under a critically controlled ratio of acetic acid and trifluoroacetic acid as solvent and (2) reduction of the activated amide to the hemiaminal, which then undergoes an unexpected dehydrogenation reaction to remove the steric hindrance for the second Pictet–Spengler cyclization to form the pentacyclic piperizinohydroisoquinoline **6**. The in vitro antitumor activity of these compounds was tested against five human cancer cell lines (A549 lung, HeLa cervical, SAS oral, SKHep1 hepatoma and PC-3 prostate carcinoma). The pentacyclic saframycin analogues **6**, **7** and **9** showed only weak activities. Interestingly, compound **6**, having a closer relation to cribrostatin IV, is selective towards oral cancer.

1. Introduction

The piperizinohydroisoquinoline alkaloids are a broad family of natural products and members of this family include the saframycins,[1] renieramycins,[2] safracins,[3] ecteinascidins[4] and cibrostatin IV[5] (Fig. 1). They possess potent antitumor, antibiotic and antimicrobial activity through the inhibition of RNA, DNA and protein synthesis.[4b,5,6] Ecteinascidin 743 (ET-743) is currently in phase II/III clinical trials for the treatment of ovarian, endometrial and breast cancer. The antiproliferative activity of ET-743 is greater than that of taxol, mitomycin C and cisplatin. The development of these compounds as antitumor drugs has been limited by their natural scarcity. Thus, none of these alkaloids or derivatives is currently on the market as therapeutics. Recently, it has been found that the biological acitivity of ET-743 is maintained in the simpler synthetic analogues such as phthalascidin.[7,8] The structural complexity of these piperizinohydroisoquinoline alkaloids has led to widespread synthetic attention, cumulating in numerous syntheses in both racemic[9–13] and enantiopure[14–18] form.

Most of the synthetic approaches have fundamental similarities in strategy by using Pictet–Spengler cyclization for the formation of both the AB and DE tetrahydroisoquinoline ring systems. The most common strategy is formation of the AB ring via Pictet–Spengler cyclization late in the synthesis. In one approach, C-2 symmetrical diketopiperazine was used as the key intermediate.[10,13] The reported methodology has the drawback of having to carry out multistep syntheses to differentiate between the two similar amide groups in diketopiperazine. Our retrosynthetic analysis is directed towards the piperizinohydroisoquinoline motif, which has a structural feature of antitumor alkaloids, saframycin A and related pentacyclic compounds as shown in Scheme 1 and makes use of two efficient Pictet–Spengler cyclizations. The key event was to take advantage of a mono-Pictet–Spengler cyclization on an unprotected 3,6-dibenzyl-piperazine-2,5-dione derivative to directly give the requisite intermediate, and this has not been reported. Thus, unlike most synthesis, we planned to install the ABC and E rings of the piperizinohydroisoquinoline alkaloids rapidly without the need of protection and deprotection. Construction of the bridged ring system will be realized from the selective reduction of amide to the hemiaminal followed by another Pictet–Spengler cyclization. Here, we report the results of our studies making use of this reinforced methodology.

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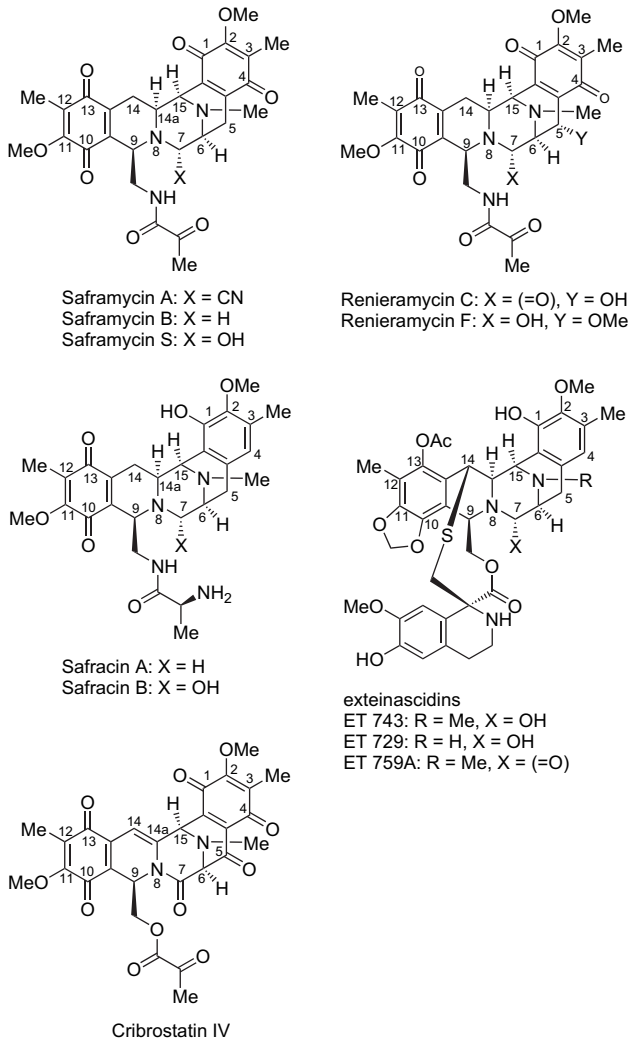
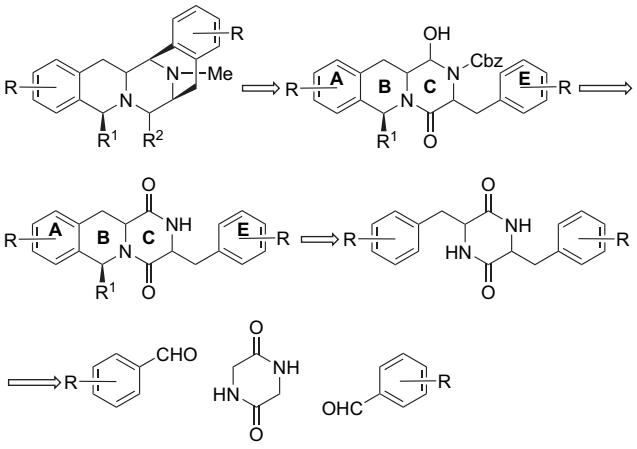


Figure 1. The structure of piperizinohydroisoquinoline alkaloids.



Scheme 1. Retrosynthesis of the piperizinohydroisoquinoline motif involving two efficient Pictet–Spengler cyclizations.

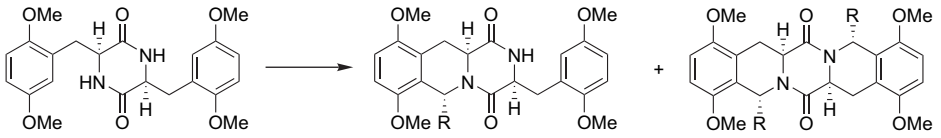
2. Results and discussion

3,6-Bis-[(2,5-dimethoxy-phenyl)methyl]piperizine-2,5-dione (**1**) was prepared in 75% yield by condensation of *N*,*N*′-diacetylpiperazine-2,5-dione with 2,5-dimethoxy-benzaldehyde in K_2CO_3/DMF, followed by hydrogenation of the benzylic double bonds with H_2/Pd/C in acetic acid.[19]

The first step in our conceived synthesis of the piperizinohydroisoquinoline motif requires a chemo-selectively controlled mono-Pictet–Spengler cyclization of compound (**1**) to furnish **2**. The stereoselectivity of the Pictet–Spengler cyclization leading to **2** has been reported by us[20] and Kubo.[10] We undertook to explore the modification of the standard procedure using acetic acid and trifluoroacetic acid for the Pictet–Spengler cyclization of **1** with aldehyde to furnish **3**.[19] A variety of conditions were employed; varying the equivalents of aldehydes, the ratios of CH_3COOH and CF_3COOH as solvent and the reaction temperature. The results are summarized in Table 1. Treatment of **1** with 4 equiv of acetaldehyde in CH_3COOH and CF_3COOH (5:1, v/v) at 80 °C gave a mixture of mono-cyclized product **2a** (52%) and di-cyclized product **3a** (20%). The lowering of the reaction temperature to 70 °C allowed nearly exclusive formation of **2a** in 50% yield, but 26% of starting material was isolated. In an attempt to drive the reaction to completion, 6 equiv of acetaldehyde was used (entry 4), which did succeed in consuming all the starting materials and the desired product **2a** was isolated in 75% yield along with **3a** in 24% yield. We have found that this reaction condition always give similar isolated yield. It was then observed that an increase in the amount of CF_3COOH favoured the formation of **3a** (Table 1, entry 6). The subtle reaction changes demonstrate the capricious reactivity of **1** during Pictet–Spengler cyclization. The Pictet–Spengler cyclization of **1** with propionaldehyde did not proceed at 70 °C in CH_3COOH and CF_3COOH (5:1, v/v). Reaction of compound **1** with 10 equiv of propionaldehyde at 70 °C under increasing amount of CF_3COOH in the solvent system (CH_3COOH/CF_3COOH=5:3, v/v) and a prolonged reaction time led to the formation of **2b** in 39% yield, together with **3b** in 9% yield and 34% recovery of starting material (Table 1, entry 9). Propionaldehyde (20 equiv) was necessary to drive the reaction to completion to give 61% yield of **2b**, together with **3b** in 18% yield. We have now successfully carried out a mono-Pictet–Spengler cyclization on **1**, and this new methodology should enable the rapid construction of piperizinohydroisoquinoline motif presence in saframycins and related pentacyclic antitumor alkaloids.

Transformation of **2a** into the desired piperizinohydroisoquinoline motif in saframycins is summarized in Scheme 2. The vicinal NH group in **2a** was acylated to give N–Cbz **4**, thereby enhancing the electrophilicity of this amide group. Selective reduction of this amide function of **4** to the corresponding hemiaminal **5** with lithium tri-*tert*-butoxy-aluminium hydride ($LiAl(O^tBu)_3H$)[21] in THF at 0 °C and subsequent cyclization of **5** after work-up (without purification) using formic acid at 60 °C gave the pentacyclic product formulated as the dehydro-derivate **6** in 75% overall yield. Analysis of the ^1H NMR of product **6** showed the absence of C-14 benyzlic methylene protons and C-14a methine proton at δ 2.28, 3.03 and 3.60, respectively,[10] and at the same time the appearance of an olefinic methine proton (CH) at δ 6.52. The spectroscopic data correlates well with the C-14 and C-14a dehydroderivatives of the saframycin motif reported.[13,18] We were able to obtain a crystal of **7**

Table 1. Mono-Pictet–Spengler cyclization reaction conditions



1 → **2a** R = Me, **2b** R = Et + **3a** R = Me, **3b** R = Et

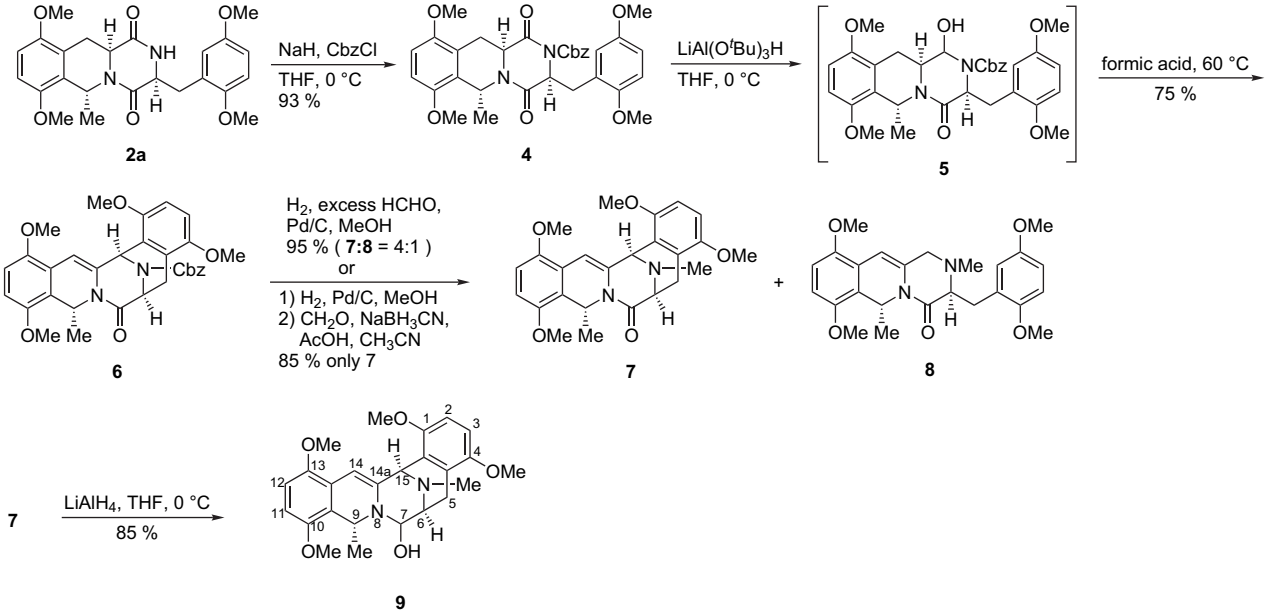
Entry	Aldehyde	v/v[a]	Time (h)	Temp (°C)	**2a** (%)	**3a** (%)	Recovery of **1**
1	4 equiv CH_3CHO	5/1	8–10	80	52	20	0
2	4 equiv CH_3CHO	5/1	8–10	70	50	Trace	26
3	5 equiv CH_3CHO	5/1	8–10	70	57	8	34
4	6 equiv CH_3CHO	5/1	8–10	70	75	24	0
5	10 equiv CH_3CHO	5/1	8–10	70	16	73	0
6	6 equiv CH_3CHO	5/3	4–6	70	7	80	0
7	10 equiv CH_3CHO	5/3	4–6	70	0	82	0
8	10 equiv CH_3CH_2CHO	5/1	14–16	70	Trace	0	99
9	10 equiv CH_3CH_2CHO	5/3	14–16	70	39	9	34
10	20 equiv CH_3CH_2CHO	5/3	14–16	70	61	18	0

[a] The volume ratio of CH_3COOH and CF_3COOH.

suitable for X-ray analysis[22] and this proved unequivocally its structure (Fig. 2). The dehydrogenation of **5** was unprecedented and the in situ cyclization of this intermediate has, to the best of our knowledge, never been reported. Fukuyama[9] states that the steric bulk around the C-14 stereocenter and the C-18 arylether group can prevent the cyclization of intermediate **5**. Furthermore, Liu[23] has also shown that desired cyclization without dehydrogenation can be accomplished in the absence of C-18 arylether group. Thus, dehydrogenation of the C14–C14a positions flattened the structure, whereby removing all the steric constrains for cyclization. We now provide an overall picture on those factors influencing cyclization to form the pentacyclic piperizinohydroisoquinoline ring via this route.

Cleavage of the *N*-benzyloxycarbonyl group in **6** by hydrogenation (5% Pd/C, H_2) followed by an in situ N-methylation gave the requisite **7** together with product **8** derived from the ring cleavage reaction by hydrogenolysis in 95% yield (ratio of **7**:**8**=4:1). Fortunately, we were able to convert **6** into **7** selectively by using a two step procedure in an overall 85% yield; first deprotection of the *N*-benzyloxycarbonyl under hydrogenation condition, followed by amine methylation with formaldehyde in the presence of $NaBH_3(CN)$.[24] Thus, the *N*-methylated product **7** activates the ring opening reaction by hydrogenolysis to give **8**. The amide group in **7** was finally converted to the hemiaminal **9** by control reduction with $LiAlH_4$ in THF.

We next attempt to provide some insight for the unexpected dehydrogenation during the second Pictet–Spengler cyclization of **4** to **6**. The dehydrogenated product **6** could be formed by two possible mechanisms. A salient feature of the mechanism was the need to remove the steric constrain



Scheme 2. Synthesis of the pentacyclic ring system of saframycin motif.

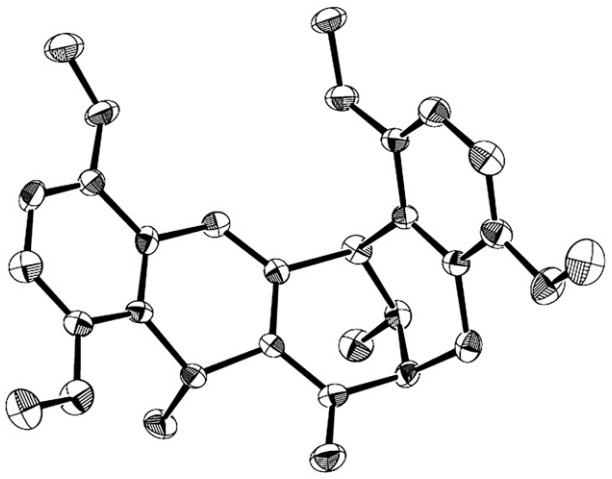
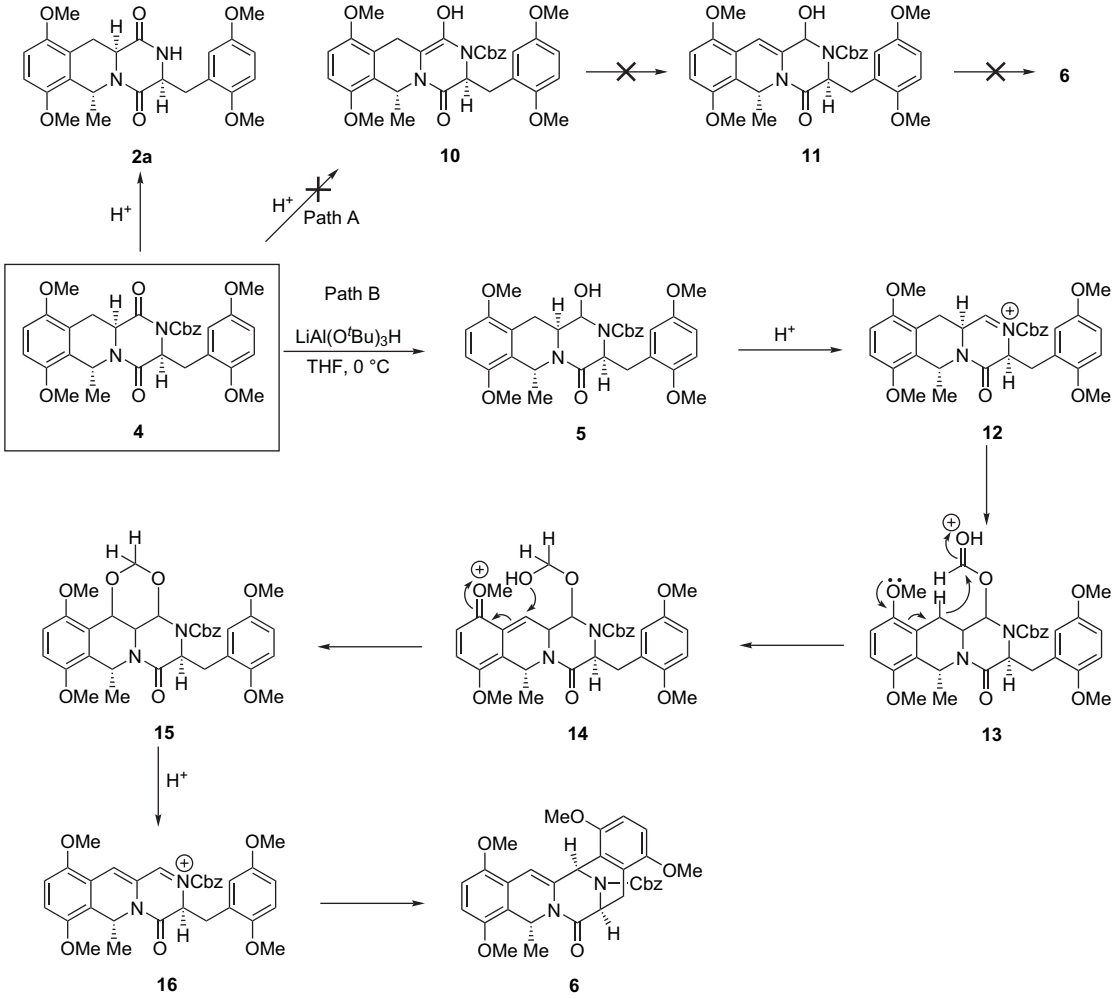


Figure 2. The X-ray spectrum of compound **7**.

around the C-14a stereocenter prior to Pictet–Spengler cyclization. The first mechanism could be the direct transformation of **4** to **6** promoted by formic acid (Path A). In this case, we assumed that the reduction of **4** to intermediate **5** did not take place. One could imagine protonation of the amide to form the iminium intermediate, which could deprotonate to the enamine intermediate **10** and further get transformed into intermediate **11**, which then cyclized to give **6** (Scheme 3). However, treatment of **4** in formic acid under the same reaction condition gave none of the anticipated product, **6**, and the sole product observed was from the N-deprotection, **2a**. A second mechanism was needed to rationalize the formation of **6** via the formation of the hemiaminal **5** (Path B). After selective reduction with lithium tri-*tert*-butoxyaluminium hydride ($LiAl(O^tBu)_3H$) in THF, **5** was isolated following preparative TLC purification. Particularly diagnostic ^{13}C NMR characteristic of **5** is the appearance of its tertiary hemiaminal carbon signal at δ 74.4, together with the disappearance of the amide carbon signal at δ 162.8. Treatment of the purified hemiaminal **5** with formic acid resulted in **6**. At this juncture, we pursued the opinion that the formation of *N*-acyliminium ion **12** cannot directly cyclize to **6** until the steric bulk around the C-14a stereocenter has been removed. We postulate that the formic acid attacks the *N*-acyliminium ion to give **13**. One could imagine an intramolecular hydride transfer from the benzylic position to the formate carbonyl moiety via a six-membered transition state through the aid of the electron rich methoxy group to form the oxonium intermediate **14**. This intermediate can then undergo an intramolecular addition with the hydroxyl group to form a cyclic acetal **15**. Through an extensive search for literature precedents, an example was found for the intermolecular abstraction of hydride from the benzylic position by *o*-chloranil



Scheme 3. Proposed mechanism for the formation of **6** from **4**.

Table 2. Antitumor activities of **6**, **7** and **9** (IC_{50} ($\mu g\ mL^{-1}$))

Compd	A549	HeLa	SAS	SKHep1	PC-3
6	ND	ND	25.8±2.2	ND	ND
7	ND	26.8±5.6	16.9±3.1	26.2±0.8	25.4±4.2
9	22.7±3.1	21.9±1.9	15.5±2.3	ND	ND

A549 is non-small cell lung cancer. HeLa is human cervical epithelioid carcinoma. SAS is oral squamous cell carcinoma. SKHep1 is hepatocellular carcinoma. PC-3 is human prostate carcinoma. ND expresses the value over 30 $\mu g\ mL^{-1}$.

followed by the formation of 1,3-benzodioxoles.[25] It was reasoned that deacetalation efficiently delivered an allylidine ammonium ion **16** having all the correct features for an intramolecular cyclization to give **6**.

The antitumor activity of **6**, **7** and **9** was determined using five human cancer cell lines and the results are shown in Table 2. Although these compounds are not very potent, a number of important points emerge from these data. It can be seen that compound **6** is very selective towards oral cancer, which warrants further design to improve the potency. Furthermore compounds **7** and **9** are also more toxic towards oral cancer cell line.

3. Conclusion

In summary, a strategy has been developed for the access of pentacyclic piperizinohydroisoquinoline motif that embodied a combination of the requisite functionalities present in the saframycin family of natural products. The simplicity and reproducibility of this new synthesis via selective mono-Pictet–Spengler cyclization of **1** are advantageous for the convenient development of new analogues as potential anticancer agents. The antitumor activity studies revealed the potential development of **6**, **7** and **9** as selective oral cancer drugs. We are currently investigating the potential application of this methodology to other analogues, as well as their biological activities.

4. Experimental section

4.1. General

All reactions were performed under an atmosphere of dry nitrogen. IR spectra were measured with a Hitachi I-2001 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 MHz and Varian Unity-INOVA-500 MHz spectrometer using $CDCl_3$ as solvent. Low-resolution mass and high-resolution mass spectra were measured with a Hitachi M-52-Instrument or Bruker APEX II mass spectrometer and elemental analyses were carried out on a HeraeusCHNO Rapid Elementary Analyzer. Melting points were uncorrected and were determined either using recrystallized samples or samples, which crystallized during concentration of the chromatography eluents.

4.1.1. Synthesis.

4.1.1.1. 7,10-Dimethoxy-3-(2,5-dimethoxybenzyl)-6-methyl-2,3,11,11a-tetrahydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (2a). To a solution of compound **1** (1.10 g, 2.66 mmol) in 10 mL acetic acid at 0 °C was added acetaldehyde (0.9 mL, 15.93 mmol, 6.0 equiv) quickly. The solution was stirred for 5 min, and then trifluoroacteic acid (2 mL) was added to the solution at 0 °C and stirred for an additional 5 min. The reaction mixture was warmed to 70 °C in an oil bath, and stirred overnight. After cooling to room temperature, the reaction mixture was extracted with CH_2Cl_2 and water. The organic layer was washed with satd $NaHCO_{3(aq)}$ and satd $NaCl_{(aq)}$, dried over $Na_2SO_{4(s)}$, then filtered and concentrated under vacuum. The crude product was purified by chromatography (SiO_2, hexane/ethyl acetate=1:1) to afford compound **2a** (874 mg, 75%) as a white solid. R_f=0.10 (SiO_2, 50% EtOAc in hexane). Mp: 201–202 °C. IR ($CHCl_3$): 1678, 1652 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 6.80 (d, 1H, J=9.0 Hz), 6.69–6.64 (m, 3H), 6.49 (d, 1H, J=3.0 Hz), 6.03 (br, 1H, –NH), 5.95 (q, 1H), 4.39 (br, 1H), 4.24 (dd, 1H, J=15.0, 4.5 Hz), 3.80 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 3.76 (s, 3H, $-OCH_3$), 3.37 (dd, 1H, J=15.0, 5.0 Hz), 3.28 (s, 3H, $-OCH_3$), 3.09 (dd, 1H, J=15.0, 5.0 Hz), 2.99 (dd, 1H, J=10.0, 5.5 Hz), 1.49 (dd, 1H, J=20.0, 12.5 Hz), 1.41 (d, 3H, J=6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.3 (C), 163.0 (C), 153.5 (C), 152.0 (C), 150.9 (C), 149.7 (C), 126.6 (C), 124.2 (C), 122.0 (C), 116.0 (CH), 114.2 (CH), 111.8 (CH), 107.9 (CH), 107.8 (CH), 56.2 (CH), 55.9 (CH_3), 55.5 (CH_3), 55.5 (CH_3), 55.1 (CH_3), 50.1 (CH), 44.5 (CH), 35.2 (CH_2), 28.2 (CH_2), 18.4 (CH_3). LRMS (FAB^+): 441 [$(M+H)^+$]. HRMS (ESI^+) calcd for $C_{24}H_{29}N_2O_6$ [$(M+H)^+$]: 441.2026; found $C_{24}H_{29}N_2O_6$ [$(M+H)^+$]: 441.2027. Anal. Calcd: C 65.44%, H 6.41%, N 6.36%; found: C 65.18%, H 6.42%, N 6.32%.

4.1.1.2. 2-Benzyloxycarbonyl-7,10-dimethoxy-3-(2,5-dimethoxybenzyl)-6-methyl-2,3,11,11a-tetrahydro-6*H*-pyrazino-[1,2-*b*]isoquinoline-1,4-dione (4). To a solution of sodium hydride (0.16 g, 6.82 mmol, 3.0 equiv) in 5 mL anhydrous THF at 0 °C was added compound **2a** (1.00 g, 2.27 mmol) dissolved in 15 mL anhydrous THF. The mixture was stirred for 30 min and then CbzCl (0.65 mL, 4.54 mmol, 2.0 equiv) was added dropwise slowly at 0 °C and stirred overnight. The reaction mixture was quenched with 10 mL satd $NaHCO_{3(aq)}$, filtered with Celite and concentrated under vacuum. The resulting mixture was dissolved in 100 mL CH_2Cl_2 and washed with water, satd $NaHCO_{3(aq)}$ and satd $NaCl_{(aq)}$, dried with $Na_2SO_{4(s)}$, then filtered and concentrated under vacuum. The crude product was purified by chromatography (SiO_2, hexane/ethyl acetate=3: 1) to afford compound **4** (1.21 g, 93%) as a white solid. R_f=0.12 (SiO_2, 25% EtOAc in hexane). Mp: 159–160 °C. ^1H NMR (500 MHz, $CDCl_3$): δ 7.34–7.51 (m, 5H), 6.61–6.70 (m, 4H), 6.35 (d, 1H, J=3.0 Hz), 5.95 (q, 1H), 5.39 (d, 1H, J=12.5 Hz), 5.30 (d, 1H, J=12.5 Hz), 5.14 (br, 1H), 4.33 (dd, 1H, J=12.0, 5.5 Hz), 3.79 (s, 3H, $-OCH_3$), 3.75 (s, 3H, $-OCH_3$), 3.69 (dd, 1H, J=14.3, 5.0 Hz), 3.50 (s, 3H, $-OCH_3$), 3.03–3.06 (m, 4H), 2.87 (dd, 1H, J=17.5, 5.5 Hz), 1.36 (d, 3H, J=6.5 Hz), 0.81 (dd, 1H, J=18.0, 12.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.6 (C), 162.8 (C), 153.2 (C), 152.2 (C), 151.9 (C), 150.9 (C), 149.5 (C), 134.9 (C), 128.6 (CH×2), 128.5 (CH), 128.4 (CH×2), 126.2 (C), 123.6 (C), 122.0 (C), 115.9 (CH), 115.3 (CH), 111.5 (CH), 107.8 (CH), 107.6 (CH), 68.9 (CH_2), 59.8 (CH), 55.5 (CH_3), 55.4 (CH_3), 55.4 (CH_3), 54.8 (CH_3), 51.5 (CH), 44.1 (CH), 33.0 (CH_2), 27.0

(CH_2), 18.4 (CH_3). LRMS (FAB^+): 574 [$(M+H)^+$]. HRMS (ESI^+) calcd for $C_{32}H_{35}N_2O_8$ [$(M+H)^+$]: 575.2393; found $C_{32}H_{35}N_2O_8$ [$(M+H)^+$]: 575.2396. Anal. Calcd: C 66.89%, H 5.96%, N 4.88%; found: C 66.72%, H 5.97%, N 4.63%.

4.1.1.3. Benzyl 1,4,10,13-tetramethoxy-9-methyl-7-oxo-(6*S,9*R**,15*S**)-5,6,9,15-tetrahydro-6,15-iminoisoquino-[3,2-*b*]-3-benzazocine-16-carboxylate (6).** To a solution of compound **4** (700 mg, 0.93 mmol) in 10 mL anhydrous THF at 0 °C was added dropwise $LiAl(O^tBu)_3H$ (1.1 M in THF, 1.7 mL, 1.86 mmol, 2.0 equiv) and stirred for 1 h. The reaction mixture was quenched with cool water, filtered with Celite and concentrated under vacuum. The resulting mixture was dissolved in 10 mL formic acid and warmed to 60 °C in an oil bath. The heat bath was removed until the solution became yellowish brown in colour and concentrated under vacuum. The resulting mixture was dissolved in CH_2Cl_2 and washed with satd $Na_2CO_{3(aq)}$ and satd $NaCl_{(aq)}$, dried with $MgSO_{4(s)}$, then filtered and concentrated under vacuum. The crude product was purified by chromatography (SiO_2, hexane/ethyl acetate=3:1) to afford compound **6** (507 mg, 75% yield) as a white solid. R_f=0.20 (SiO_2, 25% EtOAc in hexane). Mp: 203–204 °C. IR ($CHCl_3$): 1717, 1645 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.34 (s, 5H), 6.57–6.67 (m, 4H), 6.52 (br, 1H), 6.39 (br, 1H), 6.05 (q, 1H), 5.26 (br, 1H), 5.16 (br, 2H, $-OCH_2Bn$), 3.86 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 3.73 (s, 3H, $-OCH_3$), 3.72 (s, 3H, $-OCH_3$), 3.05 (br, 2H, $-CH_2-$), 1.24 (d, 3H, J=6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.2 (C), 153.9 (C), 151.1 (C), 149.7 (C), 149.0 (C), 148.2 (C), 136.0 (C), 133.4 (C), 128.8 (CH), 128.5 (CH), 128.0 (CH), 127.6 (CH), 124.6 (C), 122.6 (C), 121.6 (C), 120.6 (C), 110.0 (CH), 108.9 (CH), 108.7 (CH), 108.6 (CH), 108.1 (CH), 98.2 (CH), 67.7 (CH_2), 56.3 (CH_3), 55.6 (CH_3), 55.6 (CH_3), 55.4 (CH_3), 53.5 (CH), 47.7 (CH), 45.5 (CH), 29.7, 25.5 (CH_2), 20.0 (CH_3). LRMS (FAB^+): 557 [$(M+H)^+$]. HRMS (ESI^+) calcd for $C_{32}H_{33}N_2O_7$ [$(M+H)^+$]: 557.2288; found $C_{32}H_{33}N_2O_7$ [$(M+H)^+$]: 557.2286. Anal. Calcd: C 69.05%, H 5.79%, N 5.03%; found: C 68.92%, H 5.93%, N 5.05%.

4.1.1.4. 1,4,10,13-Tetramethoxy-9,16-dimethyl-(6*S,9*R**,15*S**)-5,6,9,15-tetrahydro-6,15-iminoisoquino-[3,2-*b*]-3-benzazocine-7-one (7).** A solution of compound **6** (500 mg, 0.90 mmol) and 5% Pd/C (50 mg) in 20 mL methanol was stirred under hydrogen (1 atm) for 2–3 h. The reaction was then filtered to remove the catalyst, and the filtrate was concentrated to give the crude product. The crude product was purified by chromatography (SiO_2, hexane/ethyl acetate=2:1) to afford amine (350 mg, 93%) as a white solid. R_f=0.27 (SiO_2, 50% EtOAc in hexane). ^1H NMR (500 MHz, $CDCl_3$): δ 6.56–6.66 (m, 4H), 6.42 (s, 1H), 6.09 (q, 1H), 5.38 (s, 1H), 4.27 (br, 1H), 3.85 (s, 3H, $-OCH_3$), 3.77 (s, 3H, $-OCH_3$), 3.73 (s, 3H, $-OCH_3$), 3.72 (s, 3H, $-OCH_3$), 3.03 (s, 1H), 3.02 (s, 1H), 1.98 (br, 1H, –NH), 1.32 (d, 3H, J=6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3 (C), 151.2 (C), 149.7 (C), 149.1 (C), 148.1 (C), 135.2 (C), 125.4 (C), 122.6 (C), 121.8 (C), 120.9 (C), 109.9 (CH), 108.7 (CH), 108.5 (CH), 108.4 (CH), 97.1 (CH), 56.3 (CH_3), 55.6 (CH_3), 55.6 (CH_3), 55.4 (CH_3), 53.3 (CH), 47.1 (CH), 45.0 (CH), 25.6 (CH_2), 19.9. LRMS (FAB^+): 423 [$(M+H)^+$]. HRMS (ESI^+) calcd for $C_{24}H_{27}N_2O_5$ [$(M+H)^+$]: 423.1920; found $C_{24}H_{27}N_2O_5$ [$(M+H)^+$]: 423.1919. To a solution of amine (350 mg, 0.83 mmol) and 37% formalin solution (2.43 mL, 29.94 mmol, 36.0 equiv) in acetonitrile and methanol (1:1, v/v, 20 mL) was added solid sodium cyanoborohydride (261 mg, 4.15 mmol, 5.0 equiv) and the mixture was stirred at room temperature for half an hour. Acetic acid (0.95 mL, 16.6 mmol, 20.0 equiv) was added dropwise and the resulting mixture was stirred at room temperature for another 1.5 h. The reaction was quenched with 10 mL satd $NaHCO_{3(aq)}$ and extracted with CH_2Cl_2 (25 mL×2). The organic layer was washed with satd $NaCl_{(aq)}$, dried with $MgSO_{4(s)}$, then filtered and concentrated under vacuum. The crude product was purified by chromatography (SiO_2, hexane/ethyl acetate=3:1) to afford compound **7** (329 mg, 91%) as a white solid. R_f=0.08 (SiO_2, 25% EtOAc in hexane). Mp: 213–214 °C. IR ($CHCl_3$): 1640 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 6.56–6.65 (m, 4H), 6.48 (s, 1H), 6.08 (q, 1H), 5.00 (s, 1H), 3.87 (d, 1H, J=5.0 Hz), 3.84 (s, 3H, $-OCH_3$), 3.78 (s, 3H, $-OCH_3$), 3.73 (s, 3H, $-OCH_3$), 3.72 (s, 3H, $-OCH_3$), 2.97–3.09 (m, 2H), 2.60 (s, 3H, $-NCH_3$), 1.36 (d, 3H, J=6.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.2 (C), 151.1 (C), 149.8 (C), 149.1 (C), 147.9 (C), 134.1 (C), 125.4 (C), 122.6 (C), 121.7 (C), 120.9 (C), 109.9 (CH), 108.7 (CH), 108.4 (CH), 108.4 (CH), 99.0 (CH), 60.1 (CH), 56.3 (CH_3), 55.6 (CH_3), 55.6 (CH_3), 55.4 (CH_3), 53.9 (CH), 45.3 (CH), 41.1 (CH_3), 25.8 (CH_2), 19.8 (CH_3). LRMS (FAB^+): 437 [$(M+H)^+$]. HRMS (ESI^+) calcd for $C_{25}H_{29}N_2O_5$ [$(M+H)^+$]: 437.2076; found $C_{25}H_{29}N_2O_5$ [$(M+H)^+$]: 437.2074. Anal. Calcd: C 68.47%, H 6.90%, N 6.39%; found: C 68.65%, H 6.84%, N 6.27%.

4.1.1.5. 7-Hydroxy-1,4,10,13-tetramethoxy-9,16-dimethyl-(6*S,9*R**,15*S**)-5,6,9,15-tetrahydro-6,15-iminoisoquino-[3,2-*b*]-3-benzazocine (9).** To a solution of compound **7** (250 mg, 0.57 mmol) with 20 mL anhydrous THF at 0 °C was added excess $LiAlH_{4(s)}$ slowly. The reaction mixture was stirred at room temperature for 1 day and quenched with cool water, then filtered with Celite and concentrated under vacuum. The resulting mixture was purified by thin layer chromatography (SiO_2, hexane/ethyl acetate=3:1) to afford compound **9** (213 mg, 85% yield) as an orange solid. R_f=0.44 (SiO_2, 100% EtOAc). Mp: 165–166 °C. IR ($CHCl_3$): 3450, 1477 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.61 (s, 1H), 6.70–6.64 (m, 4H), 5.25 (q, 1H), 4.83 (s, 1H), 3.88–3.85 (m, 2H), 3.82 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 3.77 (s, 3H, $-OCH_3$), 3.05 (dd, 1H, J=17.5, 6.5 Hz), 2.83 (d, 1H, J=17.5 Hz), 2.53 (s, 3H, $-NCH_3$), 1.23 (d, 3H J=6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.3 (C), 151.4 (C), 151.3 (C), 149.5 (C), 133.8 (C), 129.2 (C), 124.4 (C), 122.7 (C), 119.7 (C), 117.3 (CH), 109.4 (CH), 109.3 (CH), 108.4 (CH), 107.7 (CH), 107.6 (CH), 60.9 (CH), 55.5 (CH_3×2), 55.5 (CH_3), 55.4 (CH_3), 54.8 (CH), 52.8 (CH), 40.9 (CH_3), 25.3 (CH_2), 23.8 (CH_3). LRMS (ESI^+): 421 [$(M-OH)^+$] as a single peak. HRMS (ESI^+) calcd for $C_{25}H_{29}N_2O_4$ [$(M-OH)^+$]: 421.2127; found $C_{25}H_{29}N_2O_4$ [$(M-OH)^+$]: 421.2129.

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