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Preparation and anti-HIV activities of retrojusticidin B analogs and azalignans

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Abstract—Ten lignans (**2**–**11**) and a series of azalignans including 1-aryl-pyrronaphthalenes **20**–**24** and 3-*N*-alkylaminomethyl-1-arylnaphthalenes **25**–**28**, structurally related to two HIV reverse transcriptase inhibitors, retrojusticidin B and phyllamyricin A, were prepared from phyllanthin (**1**) for evaluation of anti-HIV activities. Anti-HIV activity of these compounds on a R5 pseudotype virus, ConB/pNL43E-L+, in the U87-CD4-CCR5 cells has been measured. Compounds **5**, **22**, **23**, and **28** showed good anti-HIV activity with IC_{50} value of 0.25, 1.07, 0.01, 0.32 μg/mL, respectively.

1. Introduction

Reverse transcriptase (RT) is essential for the replication of human immunodeficiency virus (HIV), thus many attempts have been made to develop RT inhibitors for the treatment of AIDS.[1] Lignans, found in the roots, stems, bark, fruit, and seeds of many plant species, have been demonstrated to possess activities against cancers, viruses, and bio-oxidation.[2,3] Of these, lignans of aryltetrahydronaphthalene[4] type exhibit antiviral activity against HIV by inhibiting RT; those of the dibenzyl butanolide type inhibit both HIV protein production and reverse transcriptase.[3] In addition, two aryl naphthalene lignans, phyllamiricin B and retrojusticidin B isolated from *Phyllanthus myrtifolius* (Euphorbiaceae), possess strong inhibition on HIV-1 reverse transcriptase (HIV-1 RT) (IC_{50} 3.5 and 5.5 μM, respectively) and are relatively inactive toward human DNA polymerase-α (hDNAP) (IC_{50} 289 and 989 μM, respectively).[5–7] This potent and selective property and a computer-aided molecular modeling study of these two molecules suggested that they would be good lead compounds for the preparation of anti-HIV drugs.[7] Many synthetic entries to the arylnaphthalene type of lignan have been developed.[8–10] Of these, oxidative aryl–benzyl coupling reaction of the diarylbutane lignan, phyllanthin (**1**), using DDQ under ultrasonication, has been particularly useful and leads to an arylnaphthalene skeleton in one step.[11] Since our prior study had revealed **1** to be the most abundant constituent (6.63%) present in the alcoholic extract of *Phyllanthus urinaria*,[12] we utilized it as starting material to construct the arylnaphthalene skeleton. By using appropriate chemical reactions, a series of retrojusticidin B and phyllamiricin B related compounds and azalignans were prepared. The anti-HIV activity of the prepared compounds was evaluated. The following describe the outcome of this effort.

2. Results and discussions

Phyllanthin (**1**) was oxidized by DDQ under ultrasonic condition[11a] to give the aryl naphthaldehyde **2** (92%), $\delta_{C\text{-}9}$ 192.4 (d) and $\delta_{H\text{-}9}$ 10.38 (s) whose structure has been described previously in the literature.[11] Oxidation of the aldehyde **2** by sodium chlorite in the presence of sodium phosphate and 2-methyl-butene[13] produced the acid **3** (85%), $\delta_{C\text{-}9}$ 170.9 (s). *O*-Demethylation of the 9′-methoxy group by treating with trifluoroacetic acid at room temperature yielded the primary alcohol, which

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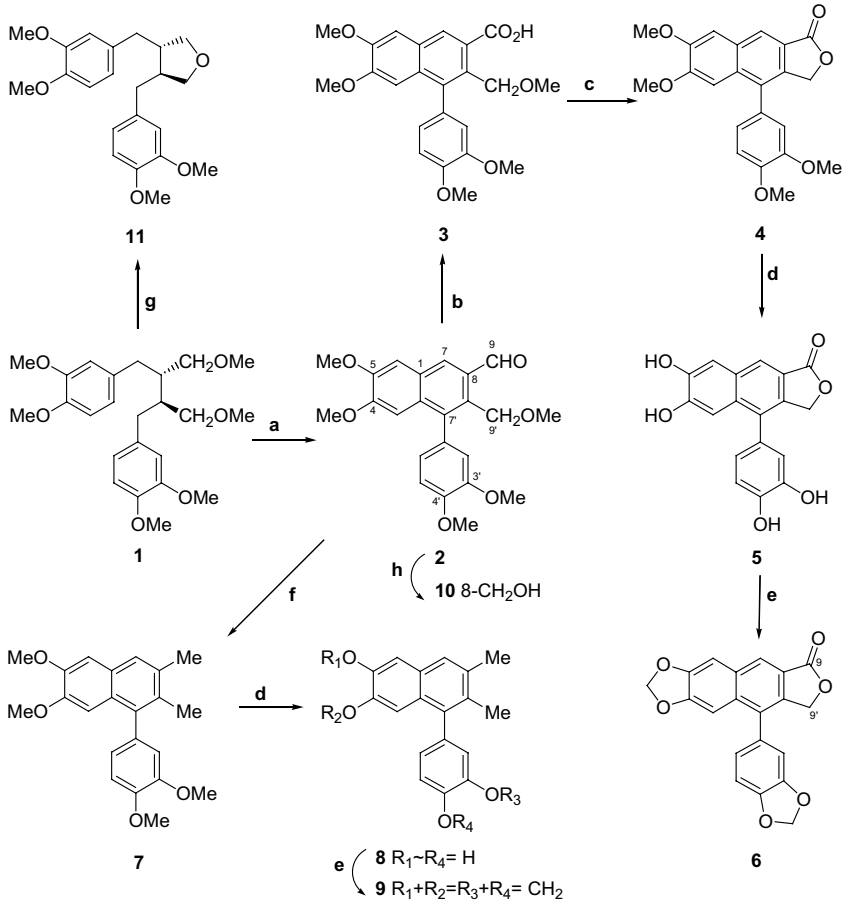
spontaneously lactonized to give the lactone **4** (98%),[14] $\delta_{H\text{-}9'}$ 5.26 and 5.17 (each doublet, $J = 15.0$ Hz), and $\delta_{C\text{-}9}$ 171.6 (s), $\delta_{C\text{-}9'}$ 69.6 (t) whose structure has been previously reported.[10,15,19] The lactone moiety of **4**, similar to that found in retrojusticidin B was thus accomplished in two steps. *O*-Perdemethylation of **4** with melted pyridinium hydrochloride (190 °C)[16] afforded the catechol **5** (79%),[17] which had been reported as an intermediate but none of its physical data had been listed. To compensate this, the physical data were measured and listed in the Experimental section. Treating **4** with the common *O*-demethylating agent BCl_3,[16] however, gave **5** in a poor yield. *O*,*O*-Methylenation of the catechol groups in **5** to give the known compound justicidin E (**6**),[18–20] δ (–OCH_2O–) 6.07 (2H, an AB system) and 6.06 (2H, an AB system), δ (–OCH_2O–) 101.8 (t) and 101.4 (t), was achieved by treating with dichloromethane in DMF (67%) (Scheme 1).

In order to confirm that the γ-lactone moiety on compound **4** was an essential pharmacophore, the corresponding compounds **7**–**9**, devoid of this moiety, were prepared (Scheme 1). Catalytic hydrogenation of **2** yielded the dimethyl naphthalene **7** (92%), which has been reported many times previously in the literature,[21] δ (2×aryl-CH_3) 2.43 (s) and 2.10 (s) and δ (2×aryl-CH_3) 20.9 (q) and 17.3 (q). *O*-Perdemethylation of **7** under similar conditions to those used for **4** yielded the catechol **8** (57%), whose ^{1}H NMR spectrum (methanol-d_4) revealed the absence of the four methoxy singlets. *O*,*O*-Methylenation of **8** by dichloromethane in DMF produced the known compound **9** (55%),[22] δ (2×–OCH_2O–) 6.02 and 5.93, and δ (2×–OCH_2O–) 101.0 (t) and 100.8 (t).

For comparison of biologic activity, another two compounds, **10** and **11**, were prepared (Scheme 1). Reduction of the aldehydic group in **2** with sodium borohydride produced the alcohol **10** (75% yield), $\delta_{H\text{-}9}$ 4.79 and 4.74, each doublet ($J = 12.2$ Hz); $\delta_{C\text{-}9}$ 70.7 (t). Treatment of **1** with $POCl_3$ in dry benzene and trifluoroacetic acid under reflux yielded the tetrahydrofuran **11** (45%),[11a] $\delta_{H\text{-}9/H\text{-}9'}$ 3.89 (2H, dd, $J = 8.6$, 6.7 Hz) and 3.51 (2H, dd, $J = 8.6$, 6.0 Hz); $\delta_{C\text{-}9/C\text{-}9'}$ 73.3 (t).

Bioassay of these prepared lignans **2**–**11** against HIV-1 RT was performed and it was found that 4,5,3′,4′-tetrahydroxy-2,7′-cycloligna-7,7′-dien-9,9′-olide (**5**) showed significant activity with an IC_{50} of 35 μM. When the catechol functions were protected, lower anti-HIV RT activity was observed [**4** (tetra-*O*-methylated), IC_{50}



Scheme 1. Preparation of compounds **2**–**11** from phyllanthin (**1**). Reagents and conditions: (a) DDQ, HOAc, ultrasonic wave, 50 °C, 40 min (92%); (b) 2-methyl-butene, *t*-BuOH, $NaClO_2$, NaH_2PO_4, rt, 18 h (85%); (c) TFA, MeCN, rt, 24 h (98%); (d) pyridinium HCl, N_2, 190 °C, 30 min (**5**: 79%; **8**: 57%); (e) CH_2Cl_2, DMF, 110 °C, 5 h (**6**: 67%; **9**: 55%); (f) H_2, 10% Pd–C, HOAc, rt, 18 h (92%); (g) $POCl_3$, C_6H_6, TFA, 0 °C, 6 h (45%); (h) $NaBH_4$, CH_2Cl_2, MeOH, rt, 10 h (75%).

226 μM and **6** (dimethylenated), IC_{50} 609 μM]. The remaining compounds in the series were almost inactive (IC_{50} >1000 μM). These data suggest that the 9,9′-γ-lactone in this series is essential for anti-HIV activity, consistent with conclusions from molecular modeling studies.[7] Since the γ-lactone moiety would be hydrolyzed easily in vivo and the catechol functionality is not stable, modification of these moieties is essential from the viewpoint of drug development. Hence a series of azalignans possessing a pyrrole moiety, corresponding to the γ-lactone, and a series of 9-*N*-alkylamino derivatives of **2**, were prepared (Scheme 2) in order to avoid the pharmacokinetic issue and to study the structure–activity relationship.

Treatment of **2** with dry HBr in CH_2Cl_2 yielded the 9′-bromo derivative **12** (89%), $\delta_{H\text{-}9'}$ 4.97 (1H, d) and 4.73 (1H, d), and $\delta_{H\text{-}7}$ 8.17 (s). Treatment of **2** with dry HBr and bromine in CH_2Cl_2 yielded the 6,9′-dibromolignan **13** (46%), $\delta_{H\text{-}7}$ 8.68 (s), and the 6′,9′-dibromolignan **14** (7.6%), $\delta_{H\text{-}2'}$ 6.92 (s) and $\delta_{H\text{-}5'}$ 7.28 (s), in addition to **12** (8.9%). Reaction of **12** with alkylamines, including methylamine, propylamine, isopropylamine, and isobutylamine, yielded *N*-alkyl 3a-alkylamino-5*H*-pyrro[4,3-*b*]-naphthalenes **15** (51%), **16** (41%), **17** (46%), and **18** (58%). The same reaction of **13** and methylamine yielded **19** (65%). These assigned structures were supported by the following common spectral evidences: IR absorption near 1649 cm^{-1} typical for C=N stretching, ^1H NMR signal for the 3a-N*H* near 10 ppm (br s) and ^{13}C NMR signal for a quaternary carbon near 160 ppm (C-3a). The 2D NMR spectra of **16** showed the vicinal coupling relationship of the protons in 3a-NH^{n}Pr (COSY-45), an NOE relationship between H-4 (δ 8.27) and 3a-NHCH_2– (δ 4.02) (NOESY) (Fig. 1), and the three-bond

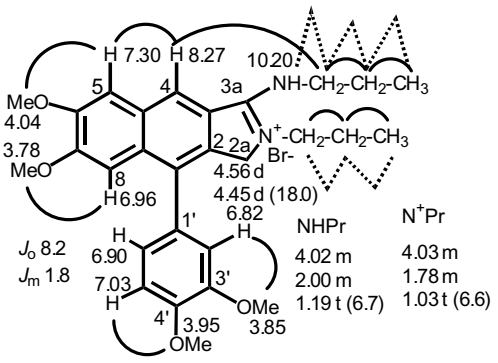
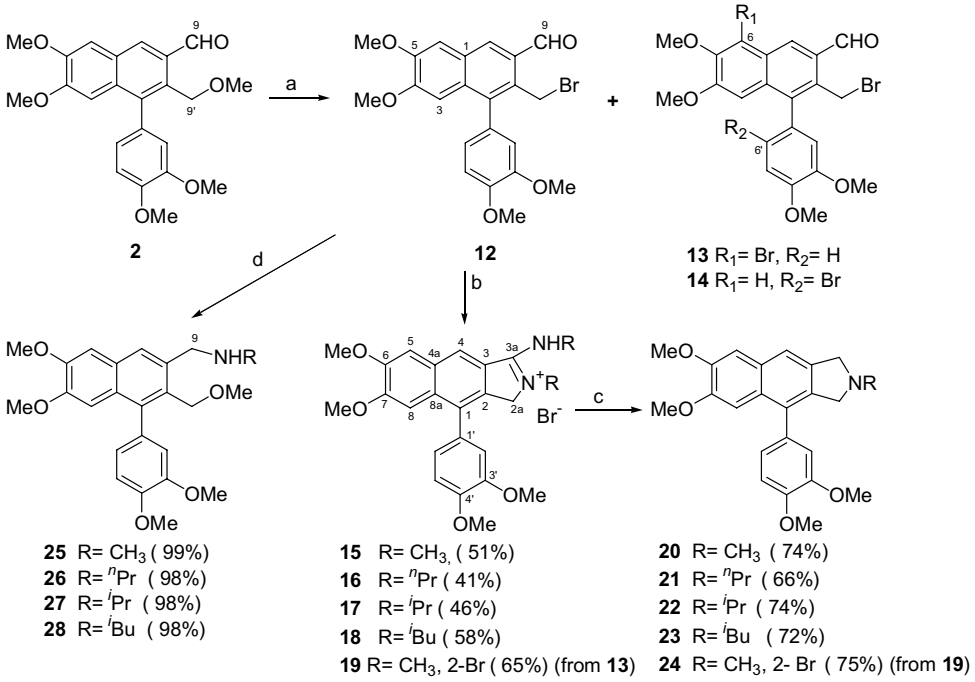


Figure 1. ^1H NMR data, NOESY (solid curves) and major COSY-45 (dash lines) of **16** ($CDCl_3$).

coupling between the C-3a singlet (δ 160.0) and the H-4 singlet (HMBC) (Fig. 2), confirming the assigned structure for **16**. With the assistance of these 2D NMR data, the complete assignment of ^1H and ^{13}C NMR data of **16** was made unambiguously and they are given in Figure 1 and listed in the Experimental section, respectively. The HR-EI-MS spectra of **15**–**19** gave the expected molecular ions as listed in the Experimental Section, and support the structural assignments.

The formation of these diamino adducts could be attributed to the mechanism shown in Scheme 3. The secondary amine formed by the reaction between the amine and the bromomethyl group, could react with the aldehyde to form an aminol that is air-oxidized to the lactam. Tautomerization of the lactam, followed by nucleophilic attack of alkylamine and subsequent dehydration would yield the products **15**–**19**.



Scheme 2. Preparation of azalignans **20**–**28** from the arylnaphthaldehyde **2**. Reagents and conditions: (a) HBr_g (**12**) or HBr_g/Br_2 (**12**–**14**); (b) RNH_2, CH_2Cl_2; (c) $NaBH_4$, MeOH; (d) RNH_2, $NaBH_4$, MeOH.

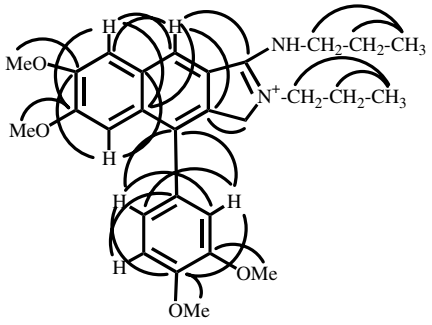


Figure 2. HMBC correlation in compound **16** ($CDCl_3$).

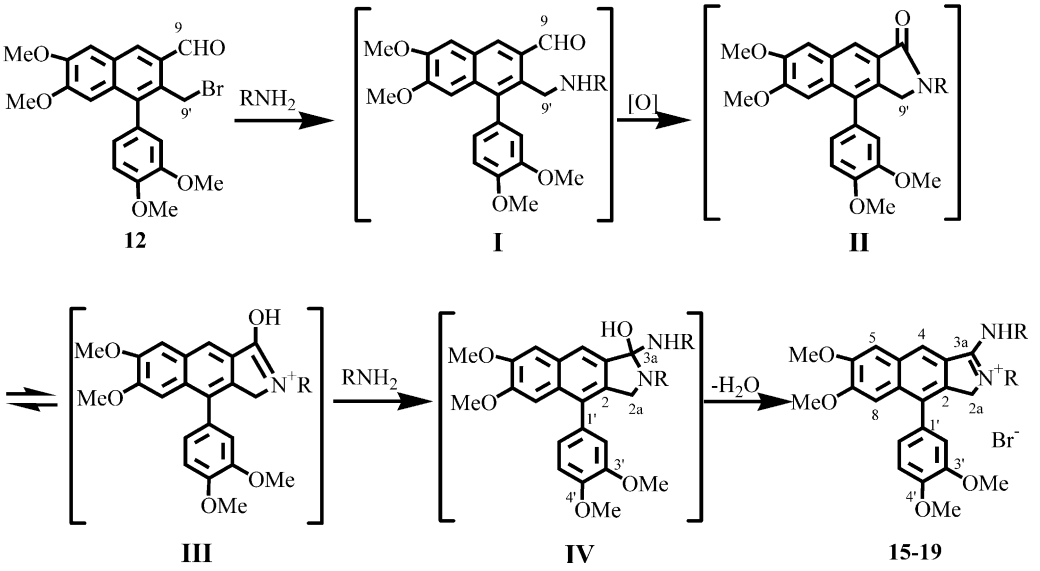
Sodium borohydride reduction of **15**–**19**, respectively, yielded *N*-alkyl-4-aryl-2,5-*H*-pyrro[4,3-*b*]naphthalenes **20** (74%), **21** (66%), **22** (74%), **23** (72%), and **24** (75%) (Scheme 2). The structures of compounds **21**–**24** were verified by ^{1}H NMR data, for instance in **20** $\delta_{H\text{-}3a}$ 3.84 (s) and $\delta_{H\text{-}2a}$ 4.05 (1H) and 4.08 (1H), an AB system, $J = 12.5$ Hz, and ^{13}C NMR data $\delta_{C\text{-}2a}$ 61.07 (t) and $\delta_{C\text{-}3a}$ 60.02 (t). Reductive *N*-alkylation of **2** with methylamine, propylamine, isopropylamine, and isobutylamine by sodium borohydride yielded 9-*N*-alkylaminolignans **25**–**28** (Scheme 2) quantitatively. Compounds **25**–**28** showed positive color reaction to the Dragendorff's reagent and their structures were verified by HR-EI-MS and ^{1}H NMR data, for instance in **25** $\delta_{H\text{-}9}$ 4.03 (s), $\delta_{H\text{-}9'}$ 4.29 (1H) and 4.34 (1H), an AB system, $J = 10.2$ Hz, and δ_{NHMe} 2.25 (s), and ^{13}C NMR data $\delta_{C\text{-}9}$ 58.3 (t), $\delta_{C\text{-}9'}$ 70.3 (t), and δ_{NHMe} 34.5 (q).

Compounds **2**–**11** and **20**–**28** were tested for anti-HIV activity on a R5 pseudotype virus, ConB/pNL43E-L+, in the U87-CD4-CCR5 cells. The IC_{50} and IC_{90} values were determined by using a virus infectivity assay and the CC_{50} and CC_{90} of these compounds were determined for comparison. The results (Table 1) indicate that compounds **5**, **22**, **23**, and **28** have good activity against HIV. It was noted that in the 9,9′-γ-lactone series (**4**–**6**), the dicatechol **5** is much more active than the tetramethoxy (**4**) and the dimethylenedioxy (**6**) derivatives, and the *N*-isobutyl azalignans **23** and **28** were the most active among the series of compounds **20**–**24**, **25**–**28**. Most strikingly the *N*-isobutyl-pyrro[4,3-*b*]naphthalene **23** possessed both best potency (IC_{50} 0.01 μg/mL) and therapeutic index ($CC_{50}/IC_{50} = 60{,}000$). It could serve as a lead for the development of anti-HIV drug.

3. Experimental

3.1. General experimental conditions

The physical data of the prepared compounds were obtained from the following instruments: Melting points: Fisher–Johns melting apparatus and not corrected; IR (KBr): JASCO FT/IR-410 spectrometer; UV (MeOH): Hitachi U-2000 spectrophotometer; NMR: Bruker Avance 400 MHz in deuterated solvent, using the residual solvent peak as internal standard ($CDCl_3$: δ_H 7.24 and δ_C 77.0; methanol-d_4: δ_H 3.30, δ_C 49.0; acetone-d_6: δ_H 2.04 and δ_C 29.8). Mass: Finnigan MAT 95S (EI,



Scheme 3. Proposed mechanism for the formation of compounds **15**–**19** from **12** reacting with amines.

Table 1. Anti-HIV activity (IC)[a] and cytotoxicity (CC) of tested compounds (μg/mL)[a]

Compound	IC_{50} (μg/mL)	CC_{50} (μg/mL)	CC_{50}/IC_{50}	IC_{90} (μg/mL)	CC_{90} (μg/mL)	CC_{90}/IC_{90}
5	0.25	74.8	299.2	17.6	134.5	7.6
22	1.07	63.5	59.3	15.43	114.3	7.4
23	0.01	60.0	6000	2.76	106.4	38.6
28	0.32	12.8	40.0	4.38	79.9	18.2

[a] AZT, the positive control, showed IC_{100} 0.2 μg/mL.

70 eV). Chromatographic system: 230–400 mesh silica gel for column; silica gel TLC: solvent A EtOAc–petroleum ether (30–70 °C) (6:4), solvent B EtOAc–petroleum ether (4:6).

3.2. 4,5,3′,4′,9′-Pentamethoxy-9-oxo-2,7′-cycloligna-7,7′-diene (2)

A mixture of phyllanthin (**1**, 2.9 g, 6.9 mmol), DDQ (4.92 g, 21.7 mmol), and acetic acid (glacial, 300 mL) was placed in an ultrasonic bath (37 ± 3 kHz) at 50 °C for 40 min and filtered. The filtrate was evaporated under vacuum and the residue (3.4 g) was recrystallized from hexane–EtOAc (9:1) to give **2** as colorless needles (2.6 g, 92%). The spectral and other properties were identical to those reported previously.[11]

3.3. 4,5,3′,4′,9′-Pentamethoxy-2,7′-cycloligna-7,7′-dien-9-oic acid (3)

To a mixture of **2** (400.1 mg, 1.0 mmol) and 2-methylbutene (0.78 mL) in *t*-BuOH (16 mL) was added aqueous solution (7.9 mL) of $NaClO_2$ (788.0 mg, 8.7 mmol) and NaH_2PO_4 (995.8 mg, 8.3 mmol). The resultant suspension was stirred overnight at rt and then concentrated. The residue was suspended in H_2O (20 mL) and the suspension was adjusted to pH 1 by adding concd H_2SO_4 dropwise, and was extracted with $CHCl_3$ (20 mL × 2). The $CHCl_3$ layers were concentrated to give a residue, which was passed through a silica gel column (230–400 mesh, 2 g, solvent A) to give **3** (354.0 mg, 85%): white amorphous solid; R_f 0.12 (solvent A); UV λ_{max} (log ε) 204 (4.50), 250 (4.67), 286 (3.97) nm; ^{1}H NMR ($CDCl_3$) δ 8.39 (1H, s, H-7), 7.21 (1H, s, H-6), 7.01 (1H, d, $J = 7.8$ Hz, H-5′), 6.86 (1H, br d, $J = 7.8$ Hz, H-6′), 6.84 (1H, br s, H-2′), 6.76 (1H, s, H-3), 4.52 (1H, d, $J = 10.6$ Hz, H-9′), 4.45 (1H, d, $J = 10.6$ Hz, H-9′), 4.00 (3H, s, 5-OMe), 3.99 (3H, s, 4′-OMe), 3.85 (3H, s, 3′-OMe), 3.72 (3H, s, 4-OMe), 3.33 (3H, s, 9′-OMe); ^{13}C NMR ($CDCl_3$) δ 170.9 (s, C-9), 151.2 (s, C-4), 150.2 (s, C-5), 148.7 (s, C-4′), 148.5 (s, C-3′), 140.0 (s, C-7′), 131.0 (s, C-8), 130.6 (d, C-7), 130.6 (s, C-1′), 128.3 (s, C-1), 128.3 (s, C-8′), 127.6 (s, C-2), 122.6 (d, C-6′), 113.4 (d, C-2′), 110.9 (d, C-5′), 107.1 (d, C-3), 105.9 (d, C-6), 70.2 (t, C-9′), 58.1 (q, 9′-OMe), 56.0 (q, 5-OMe), 56.0 (q, 3′-OMe), 55.7 (q, 4-OMe), 55.7 (q, 4′-OMe); EIMS m/z $[M]^+$ 412 (53), 380 (76), 379 (100), 349 (17), 335 (7), 305 (10), 181 (9); HREIMS m/z 412.1534 (calcd for $C_{23}H_{24}O_7$, 412.1517).

3.4. 4,5,3′,4′-Tetramethoxy-2,7′-cycloligna-7,7′-dien-9,9′-olide (4)

To a mixture of **3** (148.7 mg, 0.36 mmol) and MeCN (3 mL) was added TFA (130 μL) drop by drop. The reaction mixture was stirred at rt for 24 h, concentrated, and passed through a silica gel column (230–400 mesh, solvent A) to give **4** (135.0 mg, 98%) as white amorphous solid. The spectral and other properties were identical to those reported previously.[10,19]

3.5. 4,5,3′,4′-Tetrahydroxy-2,7′-cycloligna-7,7′-dien-9,9′-olide (5)

A mixture of **4** (10 mg, 0.03 mmol) and pyridinium HCl (300 mg, 2.6 mmol) was stirred under N_2 at 190 °C for 30 min and cooled. The mixture was partitioned between satd $NaHCO_3$ (10 mL) and EtOAc (10 mL × 2). The combined organic layers were washed with brine, dried by Na_2SO_4, and concentrated to give **5** (6.7 mg, 79%): white amorphous solid; R_f 0.40 [MeOH–$CHCl_3$ (2:8)]; UV λ_{max} (log ε) 204 (4.40), 223 (4.33), 257 (4.45), 322 (3.86) nm; ^{1}H NMR (acetone-d_6) δ 8.14 (1H, s, H-7), 7.48 (1H, s, H-6), 7.22 (1H, s, H-3), 7.00 (1H, d, $J = 8.0$ Hz, H-5′), 6.90 (1H, d, $J = 2.0$ Hz, H-2′), 6.76 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 5.23 (1H, d, $J = 8.1$ Hz, H-9′), 5.20 (1H, d, $J = 8.0$ Hz, H-9′); ^{13}C NMR (acetone-d_6) δ 171.9 (s, C-9), 150.0 (s, C-4), 147.9 (s, C-3′), 146.2 (s, C-5), 145.9 (s, C-4′), 137.6 (s, C-7′), 132.7 (s, C-8), 132.5 (s, C-1′), 130.8 (s, C-8′), 128.7 (s, C-1), 123.6 (d, C-7), 121.6 (d, C-6′), 121.4 (s, C-2), 117.1 (d, C-2′), 116.4 (d, C-5′), 111.9 (d, C-3), 108.5 (d, C-6), 69.9 (t, C-9′).

3.6. 4,5:3′,4′-Bis(methylenedioxy)-2,7′-cycloligna-7,7′-dien-9,9′-olide (6) (justicidin E)

A mixture of **5** (10 mg, 0.03 mmol), CH_2Cl_2 (0.2 mL, 3.12 mmol), DMF (0.2 mL), and K_2CO_3 (28 mg) was stirred in a sealed tube at 110 °C for 5 h, cooled, and concentrated. The residue was, partitioned between H_2O (5 mL) and $CHCl_3$ (5 mL × 3). The combined $CHCl_3$ layers were washed by brine, dried by Na_2SO_4, and concentrated to give **6** (7.2 mg, 67%) as white amorphous solid. The spectral and other properties were identical to those reported previously.[10,19]

3.7. 4,5,3′,4′-Tetramethoxy-2,7′-cycloligna-7,7′-diene (7)

A mixture of **2** (1.0 g, 2.52 mmol), EtOAc (200 mL), 10% Pd–C (697 mg), and acetic acid (20 mL) was stirred under H_2 at rt for 18 h, filtered through a layer of Celite pad, and concentrated. The residue was suspended in H_2O and the suspension was adjusted to pH 7 by adding NH_4OH_{aq} dropwise, and was extracted with $CHCl_3$ (30 mL). The $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and concentrated to give **7** (826.5 mg, 92%) as an off-white amorphous solid. The spectral and other properties were identical to those reported previously.[21]

3.8. 4,5,3′,4′-Tetrahydroxy-2,7′-cycloligna-7,7′-diene (8)

Under similar reaction conditions and workup procedure for the preparation of **5**, **8** (9.6 mg, 57%) was obtained from **7** (20 mg, 0.06 mmol) and pyridinium HCl (350 mg, 3.0 mmol). Compound **8**: white amorphous solid; mp 244–245 °C; R_f 0.24 (solvent A); ^{1}H NMR (methanol-d_4) δ 7.26 (1H, s, H-7), 6.92 (1H, s, H-6), 6.83 (1H, d, $J = 8.0$ Hz, H-5′), 6.61 (1H, s, H-3), 6.55 (1H, d, $J = 1.8$ Hz, H-2′), 6.43 (1H, dd, $J = 8.0$, 1.8 Hz, H-6′), 2.32 (3H, s, H-9), 2.01 (3H, s, H-9′); ^{13}C NMR

(methanol-d_4) δ 146.4 (s, C-4), 146.4 (s, C-5), 146.3 (s, C-4′), 145.1 (s, C-3′), 138.2 (s, C-7′), 134.2 (s, C-8), 133.4 (s, C-1′), 131.1 (s, C-8′), 129.3 (s, C-1), 129.3 (s, C-2), 126.1 (d, C-7), 122.7 (d, C-6′), 118.5 (d, C-2′), 116.3 (d, C-5′), 110.1 (d, C-3), 109.9 (d, C-6), 21.1 (q, C-9), 17.5 (q, C-9′); EIMS m/z $[M]^+$ 296 (13), 196 (8), 153 (14), 125 (16), 111 (32), 99 (28), 85 (62), 71 (100), 57 (68); HREIMS m/z $[M]^{+\cdot}$ 296.3317 (calcd for $C_{18}H_{16}O_4$, 296.1049).

3.9. 4,5:3′,4′-Bis(methylenedioxy)-2,7′-cycloligna-7,7′-diene (9)

Under similar reaction conditions and workup procedure for the preparation of **6**, **9** (1.9 mg, 55%) was obtained from **8** (3.2 mg, 0.01 mmol), CH_2Cl_2 (0.3 mL, 4.68 mmol), DMF (0.1 mL), and K_2CO_3 (16 mg). The spectral and other properties were identical to those reported previously.[22]

3.10. 4,5,3′,4′,9′-Pentamethoxy-9-hydroxy-2,7′-cycloligna-7,7′-diene (10)

To **2** (34.6 mg, 0.09 mmol) dissolved in CH_2Cl_2 (3 mL) and MeOH (2 mL) at rt was added $NaBH_4$ (60 mg, 0.63 mmol) portionwise and the mixture was stirred for 10 h. The mixture was concentrated to give a residue, which was partitioned between H_2O (60 mL) and $CHCl_3$ (60 mL). The organic layer was washed with brine, dried over anhyd Na_2SO_4, and concentrated to give **10** (26 mg, 75%): white amorphous solid; R_f 0.17 (solvent A); ^1H NMR ($CDCl_3$) δ 7.74 (1H, s, H-7), 7.13 (1H, s, H-6), 6.99 (1H, d, $J = 8.4$ Hz, H-5′), 6.86 (1H, br d, $J = 8.4$ Hz, H-6′), 6.85 (1H, br s, H-2′), 6.76 (1H, s, H-3), 4.79 (1H, d, $J = 12.2$ Hz, H-9a), 4.74 (1H, d, $J = 12.2$ Hz, H-9b), 4.43 (1H, d, $J = 10.4$ Hz, H-9′), 4.38 (1H, d, $J = 10.4$ Hz, H-9′), 3.99 (3H, s, 5-OMe), 3.98 (3H, s, 4′-OMe), 3.84 (3H, s, 3′-OMe), 3.70 (3H, s, 4-OMe), 3.29 (3H, s, 9′-OMe); ^{13}C NMR ($CDCl_3$) δ 149.8 (s, C-4), 149.5 (s, C-5), 148.6 (s, C-4′), 148.2 (s, C-3′), 139.6 (s, C-7′), 136.7 (s, C-8), 131.5 (s, C-1′), 129.9 (s, C-8′), 129.2 (s, C-1), 128.2 (s, C-2), 127.2 (d, C-7), 122.6 (d, C-6′), 113.5 (d, C-2′), 110.8 (d, C-5′), 106.3 (d, C-3), 106.0 (d, C-6), 70.7 (t, C-9), 65.0 (t, C-9′), 58.1 (q, 9′-OMe), 55.9 (q, 5-OMe), 55.9 (q, 3′-OMe), 55.9 (q, 4′-OMe), 55.7 (q, 4-OMe); EIMS m/z 398 (71, $[M]^+$), 367 (24), 366 (100), 335 (18), 305 (16), 275 (19); HREIMS m/z $[M]^{+\cdot}$ 398.1723 (calcd for $C_{23}H_{26}O_6$, 398.1729).

3.11. 3,4,3′,4′-Tetramethoxy-9,9′-epoxylignan (11)

To a mixture of **1** (20 mg, 0.05 mmol), dry benzene (0.5 mL), and trifluoroacetic anhydride (0.1 mL) was added $POCl_3$ (247 mg, 1.6 mmol) at 0 °C. The reaction solution was refluxed for 6 h and concentrated to give a residue, which was partitioned between 5% aqueous K_2CO_3 (10 mL), and EtOAc (10 mL). The organic layer was washed by brine, dried over anhyd Na_2SO_4, and concentrated to give **11** (8.0 mg, 45%). The spectral and other properties were identical to those reported previously.[11a]

3.12. Bromo-4,5,3′,4′-tetramethoxy-9-oxo-2,7′-cycloligna-7,7′-dienes (12–14: 12, 9′-Br; 13, 6-Br and 9′-Br; 14, 6′-Br and 9′-Br)

HBr gas, prepared by adding Br_2 to tetralin, was bubbled through a solution of **2** (500 mg) in anhyd CH_2Cl_2 (10 mL), via a $CaCl_2$ drying tube, for 30 min. The reaction mixture was capped and allowed to stand at room temperature for 3 h. Removal of the solvent yielded a residue, which was treated with H_2O (5 mL), acetone (10 mL), and $BaCO_3$ (500 mg), heated under reflux for 1 h. After cooling, the reaction mixture was extracted with $CHCl_3$ (10 mL × 3). The combined organic layers were dried over anhyd Na_2SO_4 and evaporated to give a residue (580 mg), which was crystallized from hexane–EtOAc (19:1) to furnish the light yellowish needles of **12** (501 mg, 89%).

During the process for preparation of **12** as described above a few drops of bromine was added after HBr bubbling and workup as that for **12** yielded a residue (125 mg) from **2** (100 mg, 0.28 mmol). This residue was chromatographed on silica gel (4 g) eluted with 10% EtOAc–hexane to give **12** (10 mg, 8.9%), (**13**) (61 mg, 46%), and **14** (10 mg, 7.6%).

Compound **12**: mp 174–75 °C; R_f 0.44 (solvent B); IR ν_{max}: 2958, 1689, 1617, 1506, 1255, 1026, 958 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.26 (1H, s, H-9), 8.17 (1H, s, H-7), 7.22 (1H, s, H-6), 7.00 (1H, d, $J = 8.4$ Hz, H-5′), 6.92 (1H, d, $J = 1.8$ Hz, H-2′), 6.86 (1H, dd, $J = 8.4$, 1.8 Hz, H-6′), 6.69 (1H, s, 1H, H-3), 4.97 (1H) and 4.73 (1H) (each d, $J = 9.2$ Hz, H-2a), 4.00 (3H, s, 5-OCH_3), 3.94 (3H, s, 3′-OCH_3), 3.85 (3H, s, 4′-OCH_3), 3.69 (3H, s, 4-OCH_3); ^{13}C NMR ($CDCl_3$) δ 192.1 (s, C-9), 152.1 (s, C-4), 150.5 (s, C-5), 148.8 (s, C-3′), 148.6 (s, C-4′), 140.2 (s, C-8′), 139.1 (s, C-7′), 135.4 (d, C-7) 130.6 (s, C-8), 129.6 (s, C-2), 129.2 (s, C-1), 128.2 (s, C-1′), 122.0 (d, C-6′), 112.9 (d, C-5′), 111.1 (d, C-2′), 107.5 (d, C-6), 106.8 (d, C-3), 56.1 (q, 5-OMe), 56.0 (q, 3′-OMe), 55.9 (q, 4′-OMe), 55.8 (q, 4-OMe), 29.4 (t, C-9′); EIMS m/z (rel. int.) 444 (8, M^+), 442 (90), 364 (80), 349 (49) 333 (19), 189 (38); HREIMS m/z $[M]^+$ 444.0575 (calcd for $C_{22}H_{21}O_5Br_{79}$, 444.0572), 446.0565 (calcd for $C_{22}H_{21}O_5Br_{81}$, 446.0553).

Compound **13**: R_f 0.46 (solvent B); ^1H NMR ($CDCl_3$) δ 10.33 (1H, s, H-9), 8.68 (1H, s, H-7), 7.00 (1H, d, $J = 8.2$ Hz, H-5′), 6.91 (1H, d, $J = 1.8$ Hz, H-2′), 6.86 (1H, dd, $J = 8.2$, 1.6 Hz, H-6′), 6.74 (1H, s, H-3), 5.00 (1H) and 4.74 (1H) (each d, $J = 9.2$ Hz, H-9′), 3.97 (3H, s, 5-O*C*H_3), 3.91 (3H, s, 3′-O*C*H_3), 3.87 (3H, s, 4′-O*C*H_3), 3.70 (3H, s, 4-OMe); ^{13}C NMR ($CDCl_3$) δ 192.1 (d, C-9), 154.1 (s, C-5), 148.9 (s, C-4), 148.8 (s, C-3′), 148.2 (s, C-4′), 140.5 (s, C-8′), 139.1 (s, C-7′), 135.4(d, C-7), 130.1 (s, C-8), 129.6 (s, C-2), 129.2 (s, C-1), 128.8 (s, C-1′), 122.1 (d, C-6′), 117.5 (d, C-6), 112.9 (d, C-5′), 111.1 (d, C-2′), 106.75 (d, C-3), 60.8 (q, 5-O*C*H_3), 56.0 (q, 3′-O*C*H_3), 55.9 (q, 4′-O*C*H_3), 55.8 (q, 4-O*C*H_3), 28.8 (t, C-9′); EIMS m/z (rel. int. %) 526 (2, $[M+4]^+$), 524 (4, $[M+2]^+$), 522 (2, $[M]^+$), 446 (81), 444 (100), 385 (9), 383 (8), 355 (10), 353 (9), 306 (34), 189 (12).

Compound **14**: R_f 0.45 (solvent B); ^{1}H NMR ($CDCl_3$) δ 10.32 (1H, s, H-9), 8.12 (1H, s, H-7), 7.28 (1H, s, H-5′), 7.22 (1H, s, H-6), 6.92 (1H, s, H-2′), 6.56 (1H, s, H-3), 5.35 (1H) and 4.31 (1H) (each d, $J = 9.2$ Hz, H-9′), 4.00 (3H, s, 5-OCH_3), 3.98 (3H, s, 3′-OCH_3), 3.87 (3H, s, 4′-OCH_3), 3.75 (3H, s, 4-OMe); EIMS m/z (rel. int. %) 526 (1, $[M+4]^+$), 524 (2, $[M+2]^+$), 522 (1, $[M]^+$), 446 (88), 444 (100), 364 (52), 306 (100), 189 (18).

3.13. 1-(3′,4′-Dimethoxyphenyl)-6,7-dimethoxy-[2-alkylamino-*N*-alkyl]-5*H*-pyrro[4,3-*b*]-naphthalenes (16: alkyl = ^{n}Pr; 17: alkyl = ^{i}Pr; 18: alkyl = ^{i}Bu; 19: 5-Br and alkyl = Me)

The mixture of **12** (459 mg, 1.03 mmol), CH_2Cl_2 (15 mL), and methylamine (197 μL) was stirring for 3 h and was evaporated to get a residue (660 mg). This residue was chromatographed on silica gel (15 g) eluted with 2% MeOH–$CHCl_3$ to give compound **15** (258 mg, 51%).

Using similar reaction conditions and workup to the preparation of **15**, compounds **16** (303 mg, 41%), **17** (336 mg, 46%), **18** (420 mg, 58%), and **19** (70 mg, 65%) were produced from **12** (600 mg, 1.35 mmol for **16** and **17**; 560 mg, 1.26 mmol for **18**), and **13** (100 mg, 0.19 mmol for **19**) by reacting with propylamine (279 μL, 3.37 mmol, 2.5 equiv for **16**), isopropylamine (270 μL, 3.37 mmol, 2.5 equiv for **17**), isobutylamine (237 μL, 3.15 mmol, 2.5 equiv for **18**), and methylamine (43 μL for **19**).

*Common ^{1}H NMR data for **15–18***: see Figure 1 for **16**.

*Common ^{13}C NMR data for **15–18***: δ ($CDCl_3$) 133.8–134.1 (s, C-2), 133.5–133.7 (s, C-1), 129.3–129.5 (s, C-4a), 124.3–124.6 (d, C-4), 123.4–123.6 (s, C-3), 107.8–108.0 (d, C-5), 150.1–150.6 (s, C-6), 152.5 (s, C-7), 103.9–104.0 (d, C-8), 130.7 (s, C-8a), 128.3–128.5 (s, C-1′), 111.9–120.0 (d, C-2′), 149.4–149.5 (s, C-3′), 149.2 (s, C-4′), 111.7–111.8 (d, C-5′), 121.4 (d, C-6′), 56.13–56.20 (q, 6-OMe), 55.92–56.0 (q, 7-OMe), 55.88–55.90 (q, 3′-OMe), 56.1 (q, 4′-OMe), ($\delta_{6\text{-OMe}} > \delta_{4'\text{-OMe}} > \delta_{7\text{-OMe}} > \delta_{3'\text{-OMe}}$).

Compound **15**: R_f 0.41 (8% MeOH–$CHCl_3$); IR ν_{max} 3417 (NH), 2964, 1649, 1619, 1506, 1257, 1119, 1026, 947 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 10.19 (1H, br s, 3a-NH*Me*), 8.45 (1H, s, H-4), 4.50 (1H) and 4.57 (1H) (each d, $J = 18.4$ Hz, H-2a), 3.64 (3H, s, N^+*Me*), 3.60 (3H, s, 3a-NH*Me*), and for the rest of the data see Figure 1; ^{13}C NMR ($CDCl_3$) δ 57.3 (t, C-2a), 161.2 (s, C-3a), 35.3 (q, s, N^+*Me*), 31.3 (q, 3a-NH*Me*), and for the rest of the data see above; FABMS m/z (rel. int. %) $[M+H]^+$ 407 (55), 307 (13), 289 (12), 154 (100), 136 (83); HREIMS m/z $[M]^+$ 406.1840 (calcd for $C_{24}H_{26}N_2O_4$, 406.1892), $[M-Me]^+$ 391.1643 (calcd for $C_{23}H_{23}N_2O_4$, 391.1657); EIMS m/z (rel. int. %) 406 (100, $[M]^+$), 405 (100, $[M-H]^+$), 389 (18), 149 (34).

Compound **16**: R_f 0.42 (8% MeOH–$CHCl_3$); IR (KBr) ν_{max}: 3416 (NH), 2964, 1649, 1619, 1506, 1257, 1119, 1026, 947 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Figure 1; ^{13}C NMR ($CDCl_3$) δ 57.1 (t, C-2a), 160.0 (s, C-3a), 45.4 (t, 3a-NH*C*$H_2C_2H_5$), 48.8 (t, N^+*C*$H_2C_2H_5$), 23.4 (t, 3a-NHCH$_2$*C*H_2CH$_3$), 21.0 (t, N^+CH_2*C*H_2CH_3), 11.3 (q, 3a-NHC$_2$H$_4$*C*H$_3$), 11.0 (q, $N^+C_2H_4$*C*H_3), and for the rest of the data see above; NOESY data, see Figure 1; HMBC data, see Figure 2; FABMS m/z (rel. int. %) $[M+H]^+$ 463 (100), 154 (24), 136 (22); HREIMS m/z $[M]^+$ 462.2500 (calcd for $C_{28}H_{34}N_2O_4$, 462.2518); EIMS m/z (rel. int. %) 462 (100, $[M]^+$), 433 (75), 420 (56), 378 (60).

Compound **17**: R_f 0.43 (8% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.98 (1H, br s, 3a-N*H*C$_3$H$_7$), 8.31 (1H, s, H-4), 4.44 (1H) and 4.36 (1H) (each d, $J = 18.4$ Hz, H-2a), 5.61 (1H, m, N^+C*H*Me$_2$), 4.84 (1H, m, 3a-NHC*H*Me$_2$), 1.35 (6H, d, $J = 6.4$ Hz, 3a-NHCH*Me*$_2$), 1.28 (6H, d, $J = 6.5$ Hz, N^+CH*Me*$_2$), and for the rest of the data see Figure 1; ^{13}C NMR ($CDCl_3$) δ 55.8 (t, C-2a), 158.7 (s, C-3a), 49.4 (d, N^+*C*HMe$_2$), 48.2 (d, 3a-NH*C*HMe$_2$), 23.3 (q) and 23.2 (q) (3a-NHCH*Me*$_2$), 20.4 (2C, q, N^+CH*Me*$_2$), and the rest data see above; FABMS m/z (rel. int. %) 495 (50, $[M+Na]^+$), 463 (100, $[M+H]^+$), 448 (16), 154 (60), 136 (55); HRMS m/z $[M]^+$ 462.2496 calcd for $C_{28}H_{34}N_2O_4$, 462.2518); EIMS m/z (rel. int. %) 462 (100, $[M]^+$), 447 (75), 432 (10), 419 (56), 405 (38).

Compound **18**: R_f 0.42 (7% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.38 (1H, br s, 3a-N*H*C$_3$H$_7$), 8.25 (1H, s, H-4), 4.53 (1H) and 4.45 (1H) (each d, $J = 18.4$ Hz, H-2a), 4.04 (2H, m, N^+C*H*$_2$CHMe$_2$), 3.86 (2H, m, 3a-NHC*H*$_2$CHMe$_2$), 2.36 (1H, m, 3a-NHCH$_2$C*H*Me$_2$), 2.21 (1H, m, N^+CH$_2$C*H*Me$_2$), 1.13 (6H, d, $J = 6.4$ Hz, 3a-NCH$_2$CH*Me*$_2$), 1.03 (6H, d, $J = 6.4$ Hz, N^+CH$_2$CH*Me*$_2$), and for the rest of the data see Figure 1; ^{13}C NMR ($CDCl_3$) δ 55.8 (t, C-2a), 160.4 (s, C-3a), 53.9 (t, N^+*C*H$_2$CHMe$_2$), 50.8 (t, 3a-NH*C*H$_2$CHMe$_2$), 29.1 (d, 3a-NHCH$_2$*C*HMe$_2$), 27.5 (d, N^+CH$_2$*C*HMe$_2$), 20.1 (2C, q, 3a-NCH$_2$CH*Me*$_2$), 19.6 (2C, q, N^+CH$_2$CH*Me*$_2$), and for the rest of the data see above; FABMS m/z (rel. int. %) 491 (62, $[M+H]^+$), 391 (18), 307 (22), 154 (100), 136 (65); HRMS m/z $[M]^+$ 490.2833 (calcd for $C_{30}H_{38}N_2O_4$, 490.2831); EIMS m/z (rel. int. %) 490 (80, $[M]^+$), 475 (8), 447 (100), 434 (2), 392 (22), 378 (95).

Compound **19**: semi-solid, R_f 0.42 (8% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.83 (1H, s, H-3a), 8.92 (1H, s, H-4), 7.04 (1H, d, $J = 8.2$ Hz, H-5′), 7.00 (1H, s, 8-H), 6.88 (1H, dd, $J = 1.8$ and 8.2 Hz, H-6′), 6.80 (1H, d, $J = 1.8$ Hz, H-2′), 4.60 (1H, d, $J = 18.8$ Hz, H-2a), 4.54 (1H, d, $J = 18.8$ Hz, H-2a), 3.98 (3H, s, 6-OCH$_3$), 3.97 (3H, s, 3′-OCH$_3$), 3.88 (3H, s, 4′-OCH$_3$), 3.79 (3H, s, 7-OCH$_3$), 3.71 (3H, s, N^+*Me*), 3.68 (3H, s, NH*Me*); ^{13}C NMR: δ_C 161.1 (s, C-3a), 155.3 (s, C-7), 149.6 (s, C-3′), 149.5 (s, C-4′), 148.5 (s, C-6), 135.6 (s, C-4a), 134.2 (s, C-2), 133.2 (s, C-1), 130.7 (s, C-8a), 128.9 (s, C-1′), 125.5 (d,C-4), 124.8 (s, C-3), 121.4 (d, C-6′), 118.3 (d, C-5), 111.9 (d, C-2′ and C-5′), 104.7 (d, C-8), 60.9 (q, 6-OMe), 56.2 (q, 3′-OMe), 56.0 (q, 4′- and 7-OMe), 35.2 (q, N^+*C*H$_3$), 31.4 (q, 3a-NH*C*H$_3$); EIMS m/z (rel. int. %) 483 (100, $[M-H]^+$), 469 (20), 456 (13), 262 (12).

3.14. 1-(3′,4′-Dimethoxyphenyl)-6,7-dimethoxy-*N*-alkyl-2″,5″*H*-pyrro[4,3-*b*]naphthalenes (20: *N*-Me; 21: *N*-^{n}Pr; 22: *N*-^{i}Pr; 23: *N*-^{i}Bu; 24: 5-Br and *N*-Me)

To the solution of **15** (81 mg, 0.17 mmol) in methanol (5 mL) was added sodium borohydride (31 mg,

0.83 mmol, 5 equiv) portionwise. After stirring 30 min at rt, the suspension was evaporated under vacuum and the residue was partitioned between H_2O (5 mL) and $CHCl_3$ (5 mL × 3). The combined organic layers were dried over anhyd Na_2SO_4 and evaporated to give a residue (105 mg), which was chromatographed on silica gel (3 g), eluted with 1% MeOH–$CHCl_3$, to give compound **20** (47 mg, 74%).

Under similar reaction conditions and workup to the preparation of **20**, compounds **21** (41 mg, 66%), **22** (46 mg, 74%), **23** (38 mg, 72%), and **24** (30 mg, 75%) were produced from **16** (82 mg, 0.15 mmol) for **21**, **17** (82 mg, 0.15 mmol) for **22**, **18** (72 mg, 0.13 mmol) for **23**, and **19** (50 mg, 0.09 mmol) for **24** by reduction with sodium borohydride (28 mg, 0.75 mmol, 5 equiv for **21** and **22**; 23 mg, 0.63mmol for **23**; 17 mg, 0.44 mmol for **24**).

*Common ^1H NMR data for **20–23***: δ ($CDCl_3$) 7.48 (1H, s, H-4), 7.09–7.10 (1H, s, H-5), 6.94–6.98 (1H, s, H-8), 6.86–6.88 (1H, d, $J = 1.9$ Hz, H-2′), 6.92–6.97 (1H, d, $J = 8.0$ Hz, H-5′), 6.88–6.90 (1H, dd, $J = 1.9$, 8.0 Hz, H-6′), ($\delta_{H\text{-}5'} > \delta_{H\text{-}6'} > \delta_{H\text{-}2'}$), 3.96–3.99 (3H, s, 6-OMe), 3.73–3.75 (3H, s, 7-OMe), 3.83–3.86 (3H, s, 3′-OMe), 3.94–3.96 (3H, s, 4′-OMe).

*Common ^{13}C NMR data for **20–23***: δ ($CDCl_3$) 148.8–149.4 (s, C-6), 148.8–149.2 (s, C-7), 148.7–148.9 (s, C-3′), 148.1–148.6 (s, C-4′), 131.8–132.3 (s, C-8a), 130.9–131.4 (s, C-3), 129.1–129.4 (s, C-4a), 127.2–127.5 (s, C-1′), 121.7–121.8 (d, C-6′), 118.5–118.9 (d, C-4), 112.7–112.8 (d, C-2′), 111.2–111.5 (d, C-5′), 106.5–106.6 (d, C-5), 104.8–104.9 (d, C-8), 55.9–56.0 (q, 6-OMe), 55.82–55.9 (q, 4′-OMe), 55.7–55.8 (q, 7-OMe), 55.6–55.7 (q, 3′-OMe), ($\delta_{6\text{-OMe}} > \delta_{4'\text{-OMe}} > \delta_{7\text{-OMe}} > \delta_{3'\text{-OMe}}$).

Compound **20**: R_f 0.41 (5% MeOH–$CHCl_3$); IR ν_{max}: 2959, 1620, 1509, 1254, 1119, 1026, 953 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.08 (1H) and 4.02 (1H) (each d, $J = 13.1$ Hz, H-2a), 3.81 (2H, s, H-3a), 2.57 (3H, s, N*Me*), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 131.9 (s, C-1), 136.9 (s, C-2), 61.1 (t, C-2a), 60.5 (t, C-3a), 42.4 (q, N*Me*), and for the rest of the data see above; HREIMS m/z $[M]^+$ 379.1742 (calcd for $C_{23}H_{25}NO_4$, 379.1785), $[M-2H]^+$ 377.1619 (calcd for $C_{23}H_{23}NO_4$, 377.1628); EIMS m/z (rel. int. %) 379 (52%, $[M]^+$), 378 (100), 363 (14), 242 (12), 189 (8), 149 (6).

Compound **21**: R_f 0.42 (5% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.24 (1H) and 4.17 (1H) (each d, $J = 12.8$ Hz, H-2a), 3.97 (2H, s, H-3a), 2.75 (2H, m, $NCH_2C_2H_5$), 1.64 (2H, m, $NCH_2CH_2CH_3$), 0.93 (3H, t, $J = 7.3$ Hz, $NCH_2CH_2CH_3$), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 133.9 (s, C-1), 134.7 (s, C-2), 59.0 (t, C-2a), 58.6 (t, C-3a), 58.2 (t, $NCH_2C_2H_5$), 21.1 (t, $NCH_2CH_2CH_3$), 11.7 (q, $NC_2H_4CH_3$), and for the rest of the data see above; HREIMS m/z $[M]^+$ 407.2055 (calcd for $C_{25}H_{29}NO_4$, 407.2096), $[M-2H]^+$ 405.1931 (calcd for $C_{25}H_{27}NO_4$, 407.1941); EIMS m/z (rel. int. %) 407 (58, $[M]^+$), 406 (100), 378 (68), 349 (36), 189 (13).

Compound **22**: R_f 0.43 (5% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.15 (1H) and 4.09 (1H) (each d, $J = 12.8$ Hz, H-2a), 3.86 (2H, s, H-3a), 2.75 (1H, m, $NCHMe_2$), 1.16 (6H, d, $J = 6.2$ Hz, $NCHMe_2$), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 135.3 (s, C-1), 136.2 (s, C-2), 57.2 (t, C-2a), 56.6 (t, C-3a), 54.9 (d, $NCHMe_2$), 21.5 (2C, q, $NCHMe_2$), and for the rest of the data see above; HRMS m/z $[M]^+$ 407.1809 (calcd for $C_{25}H_{29}NO_4$, 407.2096), $[M-2H]^+$ 405.1934 (calcd for $C_{25}H_{27}NO_4$, 405.1940); EIMS m/z (rel. int. %) 407 (50, M^+), 406 (100), 392 (64), 349 (24), 196 (10).

Compound **23**: R_f 0.42 (5% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.07 (1H) and 4.01 (1H) (each d, $J = 12.5$ Hz, H-2a), 3.79 (2H, s, H-3a), 2.48 (2H, d, $J = 7.3$ Hz, NCH_2CHMe_2), 1.79 (1H, m, NCH_2CHMe_2), 0.94 (6H, d, $J = 6.6$ Hz, NCH_2CHMe_2), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 135.8 (s, C-1), 136.8 (s, C-2), 59.5 (t, C-2a), 59.1 (t, C-3a), 65.0 (t, NCH_2CHMe_2), 27.3 (d, NCH_2CHMe_2), 20.9 (2C, q, NCH_2CHMe_2), and for the rest of the data see above; HRMS m/z $[M]^+$ 421.2209 (calcd for $C_{26}H_{31}NO_4$, 421.2253); EIMS m/z (rel. int. %) 421 (18, $[M]^+$), 419 (28), 378 (100), 349 (56), 318 (18), 189 (20).

Compound **24**: R_f 0.42 (5% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.99 (1H, s, H-4), 6.99 (1H, s, H-8), 6.97 (1H, d, $J = 8.0$ Hz, H-5′), 6.85 (1H, dd, $J = 8.0$, 1.8 Hz, H-6′), 6.82 (1H, d, $J = 1.8$ Hz, H-2′), 4.14 (1H) and 4.09 (1H) (each d, $J = 12.8$ Hz) (H-2a), 3.95 (3H, s, 6-OCH_3), 3.93 (3H, s, 3′-OCH_3), 3.84 (3H, s, 4′-OCH_3), 3.83 (2H, s, H-3a), 3.72 (3H, s, 7-OCH_3), 2.59 (3H, s, NCH_3); EIMS m/z (rel. int. %) 457 (100, $[M]^+$), 377 (75), 262 (10).

3.15. 4,5,3′,4′,9′-Pentamethoxy-9-alkylamino-2,7′-cycloligna-7,7′-dienes (25: alkyl = Me; 26: alkyl = ^{n}Pr; 27: alkyl = ^{i}Pr; 28: alkyl = ^{i}Bu)

The mixture of **2** (110 mg, 0.28 mmol), methanol (10 mL), and methylamine (64 μL, 0.56 mmol) was stirred for 10 min and then sodium borohydride (30 mg, 3 equiv) was added portionwise. The mixture was stirred for additional 30 min at rt and was evaporated to give a residue, which was partitioned between H_2O (10 mL) and $CHCl_3$ (10 mL × 3). The combined organic layers were dried over anhyd Na_2SO_4 and evaporated to give a residue (124 mg), which was chromatographed on silica gel (4 g), eluted with 1% MeOH–$CHCl_3$, to give compound **25** (113 mg, 99%).

Under similar reaction conditions and workup to the preparation of **25**, compounds **26** (109 mg, 98%), **27** (109 mg, 98%), and **28** (112 mg, 98%) were produced from **2** (each 100 mg, 0.25 mmol) and alkylamines (*n*-propylamine, 41 μL, 0.50 mmol, 2 equiv for **26**; isopropylamine, 43 μL, 2 equiv for **27**; isobutylamine, 50 μL, 0.5 mmol, 2 equiv for **28**) by reduction with sodium borohydride (each 28 mg, 3 equiv).

*Common ^1H NMR data for **25–28***: δ 6.73–6.75 (1H, s, H-3), 7.11–7.12 (1H, s, H-6), 7.72–7.74 (1H, s, H-7), 6.85–6.86 (1H, d, $J = 2.0$ Hz, H-2′), 6.97 (1H, d, $J = 8.0$ Hz, H-5′), 6.83–6.85 (1H, dd, $J = 2.0$, 8.0 Hz, H-6′), 4.33–

4.35 (1H) and 4.28–4.30 (1H) (each d, $J = 10.0 \sim 10.3$ Hz, H-9′), 3.68–3.69 (3H, s, 4-OMe), 3.95–3.97 (6H, s, 5-OMe and 4′-OMe), 3.83–3.84 (3H, s, 3′-OMe), 3.25–3.26 (3H, s, 9′-OMe).

*Common ^{13}C NMR data for **25**–**28***: δ 128.9–129.0 (s, C-1), 129.9–130.0 (s, C-2), 105.9–106.0 (d, C-3), 149.5–149.6 (s, C-4), 149.8 (s, C-5), 106.2 (d, C-6), 122.5 (d, C-7), 128.1–128.2 (s, C-8), 58.1–58.3 (t, C-9), 127.8–128.0 (s, C-1′), 113.5 (d, C-2′), 148.5 (s, C-3′), 148.1–148.2 (s, C-4′), 110.7 (d, C-5′), 122.5 (d, C-6′), 131.3–131.4 (s, C-7′), 139.9–140.0, C-8′), 70.2–70.3 (t, C-9′), 55.80–55.83 (q, 4-OMe), 55.86–55.90 (q, 5-OMe), 55.6 (q, 3′-OMe), 55.83–55.86 (q, 4′-OMe), ($\delta_{5\text{-OMe}} > \delta_{4'\text{-OMe}} > \delta_{4\text{-OMe}} > \delta_{3'\text{-OMe}}$).

Compound **25**: R_f 0.41 (4% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.03 (2H, s, H-9), 2.55 (3H, s, 9-NH*Me*), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 53.2 (q, 9′-OMe), 34.5 (q, 9-NH*Me*), and for the rest of the data see above; HREIMS m/z $[M]^+$ 411.1996 (calcd for $C_{24}H_{29}NO_5$, 411.2046); EIMS m/z (rel. int. %) 411 (2, $[M]^+$), 380 (100), 349 (28), 319 (16), 174 (10).

Compound **26**: R_f 0.42 (4% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.09 (2H, s, H-9), 2.74 (2H, t, $J = 7.2$ Hz, 9-NHCH_2C$_2$H$_5$), 1.61 (2H, m, 9-NHCH$_2$CH_2CH$_3$), 0.93 (3H, t, $J = 7.4$ Hz, 9-NHCH$_2$CH$_2$CH_3), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 51.7 (q, 9′-OMe), 50.4 (t, 9-NH*C*H$_2$C$_2$H$_5$), 22.0 (t, 9-NHCH$_2$-*C*H$_2$CH$_3$), 11.5 (q, 9-NHC$_2$H$_4$*C*H$_3$), and for the rest of the data see above; HREIMS m/z $[M-2H]^+$ 437.2196 (calcd for $C_{26}H_{31}NO_5$, 437.2202); EIMS m/z (rel. int. %) 439 (10, $[M]^+$), 407 (62), 380 (100), 349 (32), 174 (20), 160(19).

Compound **27**: R_f 0.43 (4% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.07 (2H, s, H-9), 3.02 (1H, m, 9-NHC*H*Me$_2$), 1.22 (6H, d, $J = 6.4$ Hz, 9-NHCH*Me*$_2$), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 49.1 (q, 9′-OMe), 48.6 (d, 9-NH*C*HMe$_2$), 21.6 (2C, q, 9-NHCH*Me*$_2$), and for the rest of the data see above; HRMS m/z $[M]^+$ 439.2309 (calcd for $C_{26}H_{33}NO_5$, 439.2359); EIMS m/z (rel. int. %) 439 (8, $[M]^+$), 421 (85), 407 (20), 380 (100), 349 (38), 319 (16), 175 (16).

Compound **28**: R_f 0.44 (4% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.10 (2H, s, H-9), 2.59 (2H, d, $J = 6.9$ Hz, 9-NHCH_2CHMe$_2$), 1.92 (1H, m, 9-NHCH$_2$C*H*Me$_2$), 0.92 (6H, d, $J = 6.4$ Hz, NCH$_2$CH*Me*$_2$), and for the rest of the data see above; ^{13}C NMR ($CDCl_3$) δ 70.2 (t, 9-NH*C*H$_2$CHMe$_2$), 52.1 (q, 9′-OMe), 27.6 (d, 9-NHCH$_2$*C*HMe$_2$), 20.5 (2C, q, 8-NHCH$_2$CH*Me*$_2$), and for the rest of the data see above; HRMS m/z $[M]^+$ 453.2446 (calcd for $C_{27}H_{35}NO_5$, 453.2515), 451.2317 (calcd for $C_{27}H_{33}NO_5$, 451.2359); EIMS m/z (rel. int. %) 453 (15, $[M]^+$), 421 (85), 380 (100), 349 (58), 175 (20).

3.16. Anti-HIV-1 RT activity assay

The method for bioactivity test against HIV-1 RT was based on Cheng's method and had been modified.[23] Compounds to be analyzed were prepared as 1 mM stock solution in 50% DMSO. HIV-1 RT was purchased from HT Biotechnology Ltd, Cambridge, UK. The chemicals and assay procedures were followed as described in an earlier paper.[5]

3.17. Virus infectivity assay

A one-round complementation assay was used to measure the anti-HIV activity of the compounds. They were tested using a R5 pseudotype virus, ConB/pNL43E-L+ in the U87-CD4-CCR5 cells. In addition, AZT, a FDA approved, prototype reverse transcriptase inhibitor, was also included in the analysis to provide a comparison of the anti-HIV activity between the tested compounds and a drug currently used in clinical therapy. Each compound was dissolved in 100% DMSO to a concentration of 10 mg/mL and diluted to 1 mg/mL as a working concentration. Different concentrations (0.02–20 μg/mL) were applied to the virus infectivity assay for the determination of the IC_{50} and IC_{90}, which were measured from the luciferase activity of the virus-infected cells. The CC_{50} and CC_{90} (cytotoxicity) were also determined.

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