Total Solid Phase Syntheses of the Quinazoline Alkaloids: Verrucines A and B and Anacine

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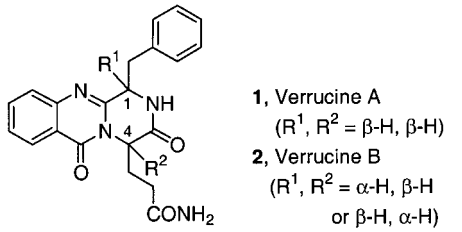
Received July 13, 2001

The first total syntheses of verrucines A and B and anacine (revised structure) were accomplished on Sasrin resin. This work confirmed the structure of verrucine A and unambiguously showed verrucine B to be a derivative of D-phenylalanine and L-glutamine. The study also proved that anacine and its epimer are quinazoline alkaloids, not benzodiazepines as originally proposed. 1-Hydroxyverrucine B, derived from air oxidization of verrucine B, was also isolated and characterized.

Fungi are capable of incorporating anthranilic acid and amino acids into a variety of fungal quinazoline metabolites.[1] Examples are glyantrypine from *Aspergillus clavatus*,[2] fumiquinazolines F and G from *Aspergillus fumigatus*,[3] and fiscalin B from *Neosartorya fischeri* and *Corynascus setosus*.[4] Recently Larsen et al. reported the isolation of verrucines A (**1**) and B (**2**) as a major and minor metabolite, respectively, from *Penicillium verrucosum*.[5] Verrucine A was derived from anthranilic acid, L-phenylalanine (Phe), and L-glutamine (Gln). However, the stereochemistry of its isomer, verrucine B, was not unambiguously assigned due to its epimerization. In the paper, the authors also pointed out that anacine, which was isolated from *Penicillium aurantiogriseum*, should have a quinazoline structure (**4**) rather than a benzodiazepine structure (**3**) as originally proposed by Mantle and co-workers.[6] The structure of anacine was revised according to the similarity of its UV and NMR spectra to those of verrucines A and B. To unambiguously confirm their structures as well as to establish their absolute configurations, we undertook the challenge of synthesizing verrucines A and B and anacine (revised structure).



3, anacine (originally proposed)

4, anacine (revised)

Results and Discussion

A general protocol for quinazoline alkaloid synthesis has been recently reported by Wang and Ganesan[7] and since utilized by several groups to synthesize other quinazoline alkaloids.[8] This protocol was adapted for our syntheses of verrucines A and B and anacine.

We foresaw that the steric hindrance of the 1,4-*syn* disubstituted bulky side chains in verrucine A and anacine could interfere with the cyclization step. Therefore, we envisioned that solid phase synthesis would be the best choice for the synthesis of these compounds for two reasons: (1) workup at each step would be easy, as excess reagents could be simply washed away; and (2) only the cyclized compound would be released from the resin. The primary amide in the glutamine side chain was protected in order to minimize dehydration of the amide to nitrile.[7b] Trityl (triphenylmethyl) was selected as a suitable protecting group for glutamine amide because of its ease of removal under acidic conditions.

Starting with Fmoc-L-Gln(Trt)-Sasrin-resin (**5**), the Fmoc was removed using piperidine, and the resulting amino resin was coupled with anthranilic acid to give peptide **6** (Scheme 1). The anthranilamide **6** was initially acylated with Fmoc-L-phenylalanine acid chloride,[9] added in two portions (5.1 and 4.7 equiv). Due to the highly acid labile property of Sasrin resin, the reaction solution was kept neutral to minimize the possible cleavage of the resin. The linear peptide **7a** was dehydrated to give benzoxazine[10] **8a**, which was further deprotected and transformed to the amidine intermediate[11] **9a**. Cyclization of **9a** in refluxing MeCN−$Cl(CH_2)_2Cl$ provided *N*-trityl verrucine A (**11a**) as the major product in 12% overall yield from **5**. A trace amount (<1%) of the *anti* epimer **10a** was also isolated. Refluxing the resin for an additional 13.5 h provided only minute amounts of **10a** (0.4%) and **11a** (1.5%). Apparently, the additional reaction time did not improve the yield, but increased the ratio of the epimerized product to the desired product. To improve the yield of **11a**, resin **6** was instead subjected to a double acylation process (2 × 5 equiv). Following the same cyclization protocol described above, **11a** was obtained in 17.0% final yield.

The 1,4-*syn* configuration of **11a** was studied and established by NOE. A NOE correlation was observed between one of the Phe methylene protons at 3.19 ppm and one of the Gln side chain methylene protons at 1.93 ppm, thus suggesting that the Phe and Gln side chains were on the same side, i.e., a 1,4-*syn* configuration. Similarly, a NOE correlation was observed between the methine of Phe of **10a** at 4.88 ppm and that of Gln methylene protons at 2.22 ppm, thus indicating that the Phe and Gln side chains were on the opposite side, i.e., a 1,4-*anti* configuration. The results were in accordance with the conformations calculated by MM2 (see Supporting Information).

Verrucine A was obtained in 84% yield by deprotecting **11a** with 40% TFA in CH_2Cl_2. The proton NMR of the crude

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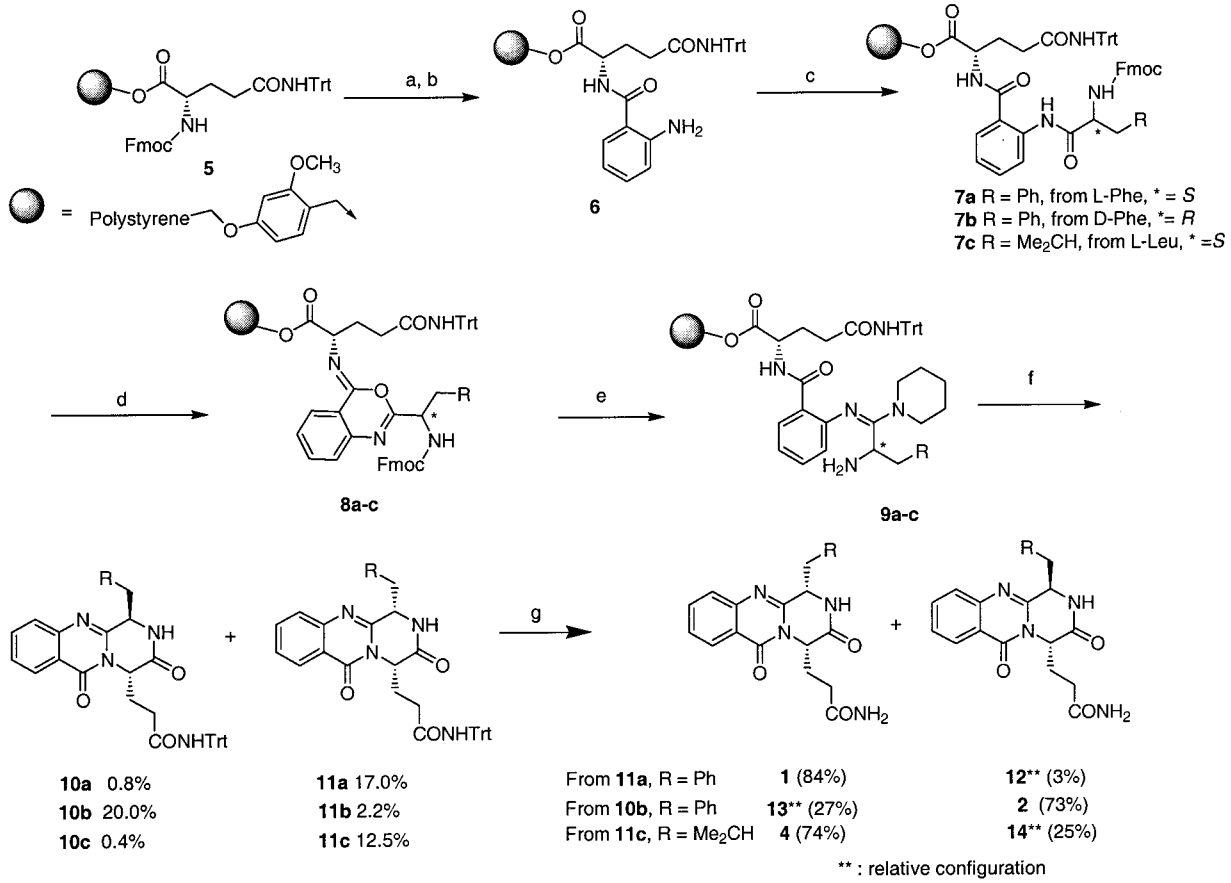
10.1021/np010342y CCC: $20.00 © 2001 American Chemical Society and American Society of Pharmacognosy
Published on Web 11/08/2001

Scheme 1. Total Solid Phase Syntheses of Quinazoline Alkaloids: Verrucines A and B and Anacine[a]



[a] Reagents and conditions: (a) 20% piperidine in DMF, 15 min × 2. (b) EDC (13.4 equiv), anthranilic acid (12.1 equiv), DMF (method A) or NMP (method B), rt 19 h. For **7a**−**11a**: (c) pyridine (7.9 equiv), Fmoc-L-Phe-Cl (5.1 equiv), CH_2Cl_2, rt 13 h, workup; repeat once in DMF. (d) Ph_3P (12.0 equiv), I_2 (11.1 equiv), Et(*i*-Pr)$_2$N (25.0 equiv), CH_2Cl_2, rt overnight (15 h). (e) 20% piperidine in CH_2Cl_2, rt 30 min. (f) MeCN/$(CH_2Cl)_2$ (1:1), reflux overnight. (g) TFA/Et_3SiH/CH_2Cl_2 (2:2:1), rt 15 min. Please refer to the Experimental Section for detailed conditions for **7b**−**11b** and **7c**−**11c**.

product showed that ca. 3% 1,4-*anti* epimer was also formed. The H-1 and H-4 signals of **1** were well separated in $CDCl_3$ solution. The NOE data reconfirmed the 1,4-*syn* configuration. Its NMR data in DMSO-d_6 were identical to those reported for verrucine A. The synthetic verrucine A was found to be optically purer than the one reported {$[\alpha]^{30}_D$ +57 ± 12° (*c* 0.25, EtOH), lit.[5] $[\alpha]^{22}_D$ +37° (*c* 0.1, EtOH)}.

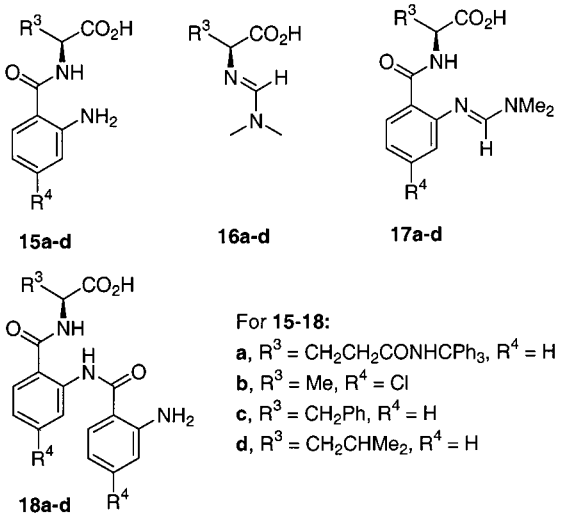
Total synthesis of verrucine B was achieved in a similar manner using Fmoc-D-Phe-Cl in the double acylation steps (Scheme 1). **10b** and **11b** were obtained in 20.0% and 2.2% yields, respectively. The 1,4-*syn* epimer (**11b**) had comparable optical data {$[\alpha]^{31}_D$ +63 ± 27° (*c* 0.12, $CHCl_3$)} to the previous major product (**11a**) {$[\alpha]^{31}_D$ +60 ± 4° (*c* 0.62, $CHCl_3$)}. The epimerization was observed to occur mainly at the C-1 position.

Deprotection of **10b** provided verrucine B (**2**, 73%) as well as the 1,4-*syn* epimer (**13**, 27%).[12] The NOE data of **2** showed that H-1 (4.96 ppm) and one of the Gln methylene protons at 2.27 ppm were on the same side. The optical rotation data of **2** showed that it had the same configuration but was optically purer {$[\alpha]^{29}_D$ +183° (*c* 0.19, EtOH), lit.[5] $[\alpha]^{22}_D$ +124° (*c* 0.08, EtOH)} than the natural one. Verrucine B was confirmed unambiguously as the derivative of D-phenylalanine and L-glutamine.

The synthesis of anacine started with Fmoc-L-Leu-Cl and was accomplished using the same synthetic route. 1,4-*syn* compound **11c** and 1,4-*anti* epimer **10c** were obtained in 12.5% and 0.4% yields, respectively. Deprotection of **11c** provided anacine (**4**, 74%) and the 1,4-*anti* epimer (**14**, 25%). The NMR data in DMSO-d_6 of **4** were identical to those reported for anacine. The optical rotation data of synthetic **4** {$[\alpha]^{27}_D$ +79 ± 13° (*c* 0.26, EtOH)} showed that it has the same absolute configuration as the natural one {$[\alpha]^{27}_D$ +81 ± 5° (*c* 0.60, EtOH)}.[13] The absolute configuration of **14** was not assigned in this work. However, identical proton NMR data were obtained for both compound **14** and the natural diastereoisomer of anacine. This confirmed the relative 1,4-*anti* configuration of the natural diastereoisomer of anacine.

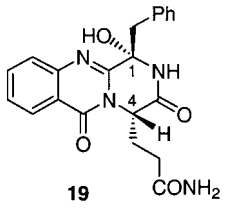
The overall yields for **11a**, **10b**, and **11c** were relatively low as compared to the previously reported less bulky pyrazino[2,1-*b*]quinazoline-3,6(1*H*,4*H*)-diones on Wang resins.[7c] The bulky Sasrin resin and the bulky trityl-protected Gln may have an important role in lowering the yields. To troubleshoot the problem, the Sasrin resins (**6**, **7a**, **7c**, **9a**, **9c**, post-refluxed **9a** and **9c**) were treated separately with 5% TFA in CH_2Cl_2 for 30 min at room temperature. Cleavage of resin **9a** provided the desired amidine **9a** and unreacted linear peptide (**7a**, Fmoc = H) in a ratio of 93:7 as estimated by MS (the MS peak intensities of the products were calibrated according to their respective concentrations). Similarly, cleavage of resin **9c** provided the desired amidine **9c** and unreacted linear peptide (**7c**, Fmoc = H) in a ratio of 89:11 (by MS). Cleavage of the post-MeCN-$(CH_2Cl)_2$ refluxed resin **9a** provided the linear peptide and some other unidentified products. This might account for the small amount of products obtained in the second cyclization. There was ca. 7% of amidine obtained from the cleavage of post-MeCN-$(CH_2Cl)_2$ refluxed resin **9c**. Cleavage of **6** and **7** gave **16a** and **17a** as the only byproducts. This clearly indicated that complete acylation

of **6** with amino acid chloride was achieved even though the yield of isolated Fmoc derivative was only 70% from **5**. The major byproduct, formamidine **16a**, was confirmed by MS and NMR. Apparently the Fmoc-deprotected **5** and **6** condensed readily with solvent DMF under dehydrative conditions to give formamidines **16a** and **17a**, respectively. The latter was obtained at about 14% of **16a** as estimated by MS. A trace amount (0−0.5% of **15a** as estimated by MS) of the dimer **18a** was also obtained. The ratio of **15a**:**16a**:**17a** was 78.4:19.1:2.6 as estimated by HPLC and ^{1}H NMR. The same study was carried out for **15b**−**d** on Wang resins (Ala, Phe, and Leu).[7c] All the cleaved (TFA-Et_3SiH-CH_2Cl_2, 50:5:45, rt, 1.5 h) products showed the respective peaks of **15b**−**d** through **18b**−**d** by MS, although the amount was less when compared to those obtained from cleaving **6**. The purities of **15b**, **15c**, and **15d** were estimated as 90%, 92%, and 88% (respectively) by ^{1}H NMR.



To avoid the formation of undesired **16** and **17**, the coupling reaction was carried out in 1-methyl-2-pyrrolidinone (NMP). The cleaved product **15a** was cleaner, containing only a trace amount of **18a** (2.4% as estimated by MS) as the only byproduct.

Compounds **2** and **4** in DMSO-d_6 and MeOH were found to degrade when they were kept at room temperature and exposed to air for several weeks. Purification of the decomposed mixture of **2** provided a polar major product, **19**. The proton NMR showed that H-1 has disappeared



while a new carbon appeared at 81.97 ppm in the ^{13}C NMR. With additional information from MS, **19** was assigned as an oxidized product of **2** at the C-1 position. The stereochemistry was established by NOE. Since natural products such as fumiquinazolines C and E contain oxygen at the C-1 positions,[3] it is therefore likely that an oxidation reaction will occur at the C-1 position of verrucine B.

In summary, (+)-verrucines A and B and anacine were synthesized in seven steps in overall yields of 14.3%, 14.5%, and 9.3%, respectively.[14] Although the yields obtained were relatively low, their purities were high,[7c] as only the desired compounds cyclize-released from the resins. Besides confirming the absolute configuration of verrucine A, verrucine B was shown unambiguously to be the derivative of D-phenylalanine and L-glutamine. Anacine and its natural diastereoisomer were confirmed as 1,4-*syn* and *anti* quinazolines (**4** and **14**), respectively, rather than benzodiazepines.

Experimental Section

General Experimental Procedures. Please see previous papers (ref 7) for details. All the 1D and 2D NMR experiments for ^{1}H (400.13 MHz), ^{13}C (100.61 MHz), and ^{15}N (40.55 MHz) nuclei were obtained on a Bruker AVANCE-400 digital NMR spectrometer. For solid phase, workup means filtered, washed with DMF (×5), 10% MeOH/CH_2Cl_2 (×4), MeOH (×4), 10% MeOH/CH_2Cl_2 (×4), and MeOH (×4), dried under vacuum. Cleavage means the Sasrin resin was shaken with TFA-Et_3-SiH-CH_2Cl_2 (5:5:90) at room temperature for 30 min, then filtered and washed with 10% MeOH/CH_2Cl_2 and MeOH, and the filtrate was evaporated to dryness.

***N*-(2-Aminobenzoyl)-L-Gln(Trt)-Sasrin Resin (6). Method A**. Fmoc-L-Gln(Trt)-Sasrin-resin (**5**, Feinchemikallen AG, loading 0.345 mmol/g, 1.919 g) was converted to resin **6** (1.738 g, new loading of 0.381 mmol/g) according to ref 7c.

The quality of resin **5** was checked by cleaving the deprotected resin **5**, and the cleaved L-Gln(Trt)-OH gave *m*/*z* 389.15 as the only peak. Resin **6** was cleaved, and the product was analyzed by MS, HPLC, and NMR. MS (ESI, positive mode): *m*/*z* (relative intensity) 508.2307 (M + H, 100%, **15a**, calcd for $C_{31}H_{29}N_3O_4$+H, 508.2236), 444.2352 (M + H, 41%, **16a**, calcd for $C_{27}H_{29}N_3O_3$+H, 444.2287), 563.2809 (M + H, 5%, **17a**, calcd for $C_{34}H_{34}N_4O_4$+H, 563.2658), 627.25 (M + H, 1%, **18a**, calcd for $C_{38}H_{34}N_4O_5$+H, 627.26); the formamidine **16a** is more sensitive than **15a**. Analytical HPLC (C_{18}, 2.1 × 200 mm, 5 μm, flow rate 0.3 mL/min, linear gradient: 30−100% MeCN/H_2O + 0.1% TFA in 40 min) showed **16a** (t_R 14.9 min) and **15a** (t_R 17.3 min) in a ratio of 20:80 (peak area at 220 nm). The crude product was also purified by preparative HPLC (C_{18}, 25 × 210 mm, 6 μm, flow rate 20 mL/min, linear gradient: 40−100% MeCN/H_2O + 0.04% TFA in 20 min) to afford **15a** and **16a**. The formamidine part was confirmed by 1D and 2D NMR (acetone-d_6). Two methyl groups at δ_H 2.85 (δ_C 37.1) and 3.46 (δ_C 44.2) were correlated with the quaternary carbon at 158.2 ppm by HMQC and HMBC. The purity of resin **6** was estimated as 78%.

Resin-bound **15b**−**d** were also cleaved with 50% TFA for 1.5 h. The formation of formamidines **16b**−**d** was also confirmed by MS and NMR. For example, the formamidine part of **16b** was identified at δ_H 8.04 (s, 1H, CH, δ_C 156.0, $^1J_{C,H}$ = 196 Hz), 3.11 (s, 3H, Me, δ_C 36.5), and 3.27 (s, 3H, Me, δ_C 43.8); all these protons were correlated with the carbon at 156.0 ppm by HMQC and HMBC (CD_3OD). Its purity was 90%, as estimated by ^{1}H NMR.

Method B. This method is similar to method A except NMP was used instead of DMF. The cleavage product showed only trace amounts of **18a** (2.4% by MS).

(1*S*,4*S*)-1,3,4,6-Tetrahydro-3,6-dioxo-1-(phenylmethyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-(*N*-trityl)propanamide (*N*-Trityl-verrucine A, 11a). Resin **6** (0.584 g, equal to 0.223 mmol of **5**, prepared by method A, purity 71%) was acylated with Fmoc-L-Phe-Cl (5.1 + 4.7 equiv), then dehydrated, deprotected, and cyclized according to ref 7c. The process provided **11a** (8.8 mg, 12.0% from **5**) and trace amounts of epimer **10a** (<1%). The resin was further reacted for 13.5 h to give additional **10a** (0.3 mg, 0.4%) and **11a** (1.1 mg, 1.5%) after purification.

Double Acylation. To a mixture of **6** (0.651 g, equal to 0.256 mmol of **5**, prepared by method A, purity 78%), CH_2Cl_2 (6.5 mL), and pyridine (2.03 mmol, 7.9 equiv to **5**) was added solid Fmoc-L-Phe-Cl (0.526 g, 1.30 mmol, 5.1 equiv). The reaction mixture was shaken at room temperature for 13 h and worked up. To the resin was added DMF (4 mL), pyridine (1.98 mmol, 7.7 equiv), and solid Fmoc-L-Phe-Cl (0.521 g, 1.28 mmol, 5.0 equiv). The mixture was shaken at room tempera-

ture overnight, followed by a workup to give resin **7a**. It was processed as above to give dibenzofulvene-piperidine adducts (69%), **10a** (R_f 0.57, 1.1 mg, 0.8% from **5**), and **11a** (R_f 0.31, 24.1 mg, 17.0% from **5**) after purification by preparative TLC (EtOAc/hexane, 2:1).

Compound 11a: white solid, mp 134 °C (dec); $[\alpha]^{28}_D$ +60 ± 4° (*c* 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.29 (dd, 1H, J = 8.0, 1.0, H-7), 7.80 (td, 1H, J = 7.8, 1.4, H-9), 7.70 (d, 1H, J = 8.1, H-10), 7.52 (t, 1H, J = 7.1, H-8), 7.30−7.21 (m, 20H, Ph + Ph_3C), 7.12 (s, 1H, Ph_3CNH), 6.18 (d, 1H, J = 3.7, H-2), 5.26 (dd, 1H, J = 9.9, 5.7, H-4), 4.71 (dt, 1H, J = 10.7, 3.8, H-1), 3.43 (dd, 1H, J = 13.4, 3.5) and 3.19 (dd, 1H, J = 13.4, 10.7, Phe-CH_2), 2.69 (dd, 1H, J = 15.2, 6.6) and 2.63 (dd, 1H, J = 15.2, 7.4, Gln-CH_2CONH), 2.33 (m, 1H) and 1.93 (m, 1H, Gln-CH_2CNH); ^{13}C NMR ($CDCl_3$) δ 170.12, 167.22, 160.53 (C-6), 149.77 (C-11a), 147.12 (C-10a), 144.73 (aromatic Cq of Ph_3C), 135.56 (Cq of Phe), 134.94 (C-9), 129.66 (CH × 2), 129.14 (CH × 2), 128.81 (CH × 6), 127.89 (CH × 6), 127.59 (d), 127.26 (d), 126.98 (d), 126.97 (d), 126.94 (CH × 3), 120.10 (s), 70.63 (Ph_3CN), 58.50 (C-1), 54.72 (C-4), 43.89 (Phe-CH_2), 34.15 (Gln-CH_2CONH), 29.50 (Gln-CH_2CHN); NOE δ_H 3.19 (3.43, 1.93), 1.93 (5.26, 3.19); HRMS (ESI, positive mode) *m/z* 619.2705 ([M + H]$^+$, calcd for $C_{40}H_{34}N_4O_3$+H, 619.2709).

(1*S*,4*S*)-1,3,4,6-Tetrahydro-3,6-dioxo-1-(phenylmethyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-propanamide (Verrucine A, 1). Compound **11a** (9.1 mg, 0.0147 mmol) was dissolved in CH_2Cl_2 (0.2 mL) and reacted with TFA (0.2 mL) and triethylsilane (0.1 mL) at room temperature for 15 min. The solution was evaporated, and the residue was purified by preparative TLC (EtOAc/MeOH/CH_2Cl_2, 50:5:45) to give recovered **11a** (1.2 mg, 13%), verrucine A (**1**) (4.6 mg, 84%), and a trace amount of *anti* epimer **12** (ca. 3%, estimated by proton NMR). Verrucine A (**1**): white solid; $[\alpha]^{30}_D$ +57 ± 12° (*c* 0.25, EtOH), lit.[5] $[\alpha]^{22}_D$ +37° (*c* 0.1, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.28 (dd, 1H, J = 8.0, 1.0, H-7), 7.81 (ddd, 1H, J = 8.3, 7.0, 1.4, H-9), 7.71 (d, 1H, J = 8.2, H-10), 7.53 (td, 1H, J = 7.6, 1.0, H-8), 7.39−7.28 (m, 5H, Ph), 6.40 (d, 1H, J = 3.6, H-2), 6.07 (br s, 1H) and 5.54 (br s, 1H, CONH_2), 5.20 (dd, 1H, J = 10.0, 5.4, H-4), 4.79 (dt, 1H, J = 10.5, 3.8, H-1), 3.51 (dd, 1H, J = 13.5, 3.7) and 3.27 (dd, 1H, J = 13.5, 10.4, Phe-CH_2), 2.64 (dt, 1H, J = 15.8, 7.4) and 2.61 (dt, 1H, J = 15.8, 6.8, Gln-CH_2-CONH$_2$), 2.31 (m, 1H) and 1.91 (m, 1H, Gln-CH_2CNH); ^{13}C NMR ($CDCl_3$) δ 173.61 (CONH$_2$), 167.25 (C-3), 160.73 (C-6), 149.71 (C-11a), 147.14 (C-10a), 135.58 (Cq of Phe), 135.02 (C-9), 129.68 (CH × 2), 129.20 (CH × 2), 127.69 (CH × 6), 127.33 (C-8), 127.02 (C-10 or C-7), 126.92 (C-7 or C-10), 120.03 (C-6a), 58.42 (C-1), 54.65 (C-4), 43.96 (Phe-CH_2), 32.26 (Gln-CH_2-CONH$_2$), 29.21 (Gln-CH_2CHN); NOESY and ROESY δ_H 3.27 with 1.91; HRMS (ESI, positive mode) *m/z* 377.1601 ([M + H]$^+$, calcd for $C_{21}H_{20}N_4O_3$+H, 377.1614); *m/z* 399.1418 ([M + Na]$^+$, calcd for $C_{21}H_{20}N_4O_3$Na, 399.1433). *anti* epimer **12**: HRMS (ESI, positive mode) *m/z* 377.1608 ([M + H]$^+$, calcd for $C_{21}H_{20}N_4O_3$+H, 377.1614); *m/z* 399.1417 ([M + Na]$^+$, calcd for $C_{21}H_{20}N_4O_3$Na, 399.1433).

(1*R*,4*S*)-1,3,4,6-Tetrahydro-3,6-dioxo-1-(phenylmethyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-(*N*-trityl)propanamide (*N*-Trityl-verrucine B, 10b). The procedure was similar to the one described for **11a** (double acylation), except that resin **6** (0.508 g, equal to 0.193 mmol of **5**, prepared by method A, purity 71%) was acylated twice with Fmoc-D-Leu-Cl (total 5.7 equiv × 2). The process provided dibenzofulvene-piperidine adducts (40.0 mg, 79%), **10b** [R_f 0.55, 23.9 mg (20.0%), and 1.1 mg (0.9%, 2nd batch)], and the *syn* epimer **11b** [R_f 0.27, 2.6 mg (2.2%) and 0.6 mg (0.5%, 2nd batch)] after purification by preparative TLC (MeOH/CH_2Cl_2/EtOAc/hexane, 2.5:47.5:25:25). *syn* epimer **11b**: $[\alpha]^{31}_D$ +63 ± 27° (*c* 0.12, $CHCl_3$); HRMS (ESI, positive mode) *m/z* 619.2743 ([M + H]$^+$, calcd for $C_{40}H_{34}N_4O_3$+H, 619.2709).

Compound 10b: white solid; $[\alpha]^{31}_D$ +149 ± 13° (*c* 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.28 (dd, 1H, J = 8.0, 1.0, H-7), 7.79 (td, 1H, J = 7.6, 1.4, H-9), 7.73 (d, 1H, J = 8.0, H-10), 7.50 (td, 1H, J = 7.5, 1.1, H-8), 7.39−7.31 (m, 3H, Ph), 7.29 (d like, 2H, Ph), 7.26−7.18 (m, 9H, Ph_3C), 7.17−7.14 (m, 6H, Ph_3C), 6.92 (s, 1H, Ph_3CNH), 5.82 (s, 1H, H-2), 5.43 (t, 1H, J = 8.0, H-4), 4.88 (dd, 1H, J = 10.3, 3.8, H-1), 4.06 (dd, 1H, J = 14.5, 3.7) and 2.95 (dd, 1H, J = 14.6, 10.4, Phe-CH_2), 2.59 (m, 1H) and 2.52 (m, 1H, Gln-CH_2CONH), 2.27 (m, 1H) and 2.22 (m, 1H, Gln-CH_2CNH); ^{13}C NMR ($CDCl_3$) δ 170.10, 167.95, 160.68 (C-6), 149.82 (C-11a), 146.82 (C-10a), 144.51 (aromatic Cq of Ph_3C), 135.28 (Cq of Phe), 134.85 (C-9), 129.46 (CH × 2), 129.32 (CH × 2), 128.67 (CH × 6), 127.89 (CH × 6), 127.81 (d), 127.63 (d), 127.54 (d), 126.99 (CH × 3), 126.95 (d), 120.35 (s), 70.61 (Ph_3CN), 55.44 (C-4), 53.56 (C-1), 37.55 (Phe-CH_2), 33.58 (Gln-CH_2CONH), 26.89 (Gln-CH_2CHN); NOESY and ROESY δ_H 4.88 with 2.22; ^{15}N NMR (obtained from ^{1}H−^{15}N HSQC and HMBC in $CDCl_3$) δ_N 112.0 (N-2, $^1J_{NH}$ = 90.5), 139.0 (CO**N**HTrt, $^1J_{NH}$ = 85.8), 165.9 (N-5) and 237.1 (N-11); HRMS (ESI, positive mode) *m/z* 619.2725 ([M + H]$^+$, calcd for $C_{40}H_{34}N_4O_3$+H, 619.2709); *m/z* 641.2526 ([M + Na]$^+$, calcd for $C_{40}H_{34}N_4O_3$Na, 641.2529).

(1*R*,4*S*)-(+)-1,3,4,6-Tetrahydro-3,6-dioxo-1-(phenylmethyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-propanamide (Verrucine B, 2). Compound **10b** (19.8 mg, 0.0320 mmol) was dissolved in CH_2Cl_2 (1 mL) and reacted with triethylsilane (0.2 mL) and TFA (0.8 mL) at room temperature for 30 min. The solution was evaporated, and the residue was purified by preparative TLC (EtOAc/MeOH/CH_2Cl_2, 50:5:45) to give verrucine B **2** (R_f 0.34, 8.7 mg, 73%) and the *syn* epimer **13** (R_f 0.29, 3.2 mg, 27%). Verrucine B **2**: white solid; $[\alpha]^{29}_D$ +183 ± 13° (*c* 0.19, EtOH), lit.[5] $[\alpha]^{22}_D$ +124° (*c* 0.08, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.28 (dd, 1H, J = 8.3, 1.0, H-7), 7.80 (td, 1H, J = 7.6, 1.5, H-9), 7.74 (d, 1H, J = 7.8, H-10), 7.53 (td, 1H, J = 7.5, 1.2, H-8), 7.41 (m, 2H, Ph), 7.37−7.32 (m, 3H, Ph), 6.47 (s, 1H, H-2), 5.78 (s, 1H) and 5.50 (s, 1H, CONH_2), 5.39 (t, 1H, J = 8.1, H-4), 4.96 (dd, 1H, J = 10.2, 3.6, H-1), 4.15 (dd, 1H, J = 14.6, 3.5) and 2.97 (dd, 1H, J = 14.6, 10.3, Phe-CH_2), 2.45 (t, 2H, J = 6.7, Gln-CH_2CONH$_2$), 2.31 (m, 1H) and 2.25 (m, 1H, Gln-CH_2CNH); ^{13}C NMR ($CDCl_3$) δ 173.71 (CONH$_2$), 168.25 (C-3), 160.81 (C-6), 149.99 (C-11a), 146.86 (C-10a), 135.72 (Cq of Phe), 134.86 (C-9), 129.53 (CH × 2), 129.37 (CH × 2), 127.68 (CH), 127.66 (C-10), 127.52 (C-8), 126.88 (C-7), 120.32 (C-6a), 55.39 (C-4), 53.98 (C-1), 37.57 (Phe-CH_2), 31.35 (Gln-CH_2CONH$_2$), 26.10 (Gln-CH_2CHN); NOESY and ROESY δ_H 4.96 with 2.27; HRMS (ESI, positive mode) *m/z* 377.1628 ([M + H]$^+$, calcd for $C_{21}H_{20}N_4O_3$+H, 377.1614); *m/z* 399.1418 ([M + Na]$^+$, calcd for $C_{21}H_{20}N_4O_3$Na, 399.1433); *syn* epimer **13**: MS (ESI, positive mode) *m/z* 377.159 ([M + H]$^+$) and 399.135 ([M + Na]$^+$).

(1*S*,4*S*)-1,3,4,6-Tetrahydro-3,6-dioxo-1-(2-methylpropyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-(*N*-trityl)propanamide (*N*-Trityl-anacine, revised structure, 11c). The procedure was similar to the one described for **11a** (double acylation), except that resin **6** (0.662 g, equal to 0.260 mmol of **5**, prepared by method A, purity 78%) was acylated twice with Fmoc-L-Leu-Cl (total 5.0 equiv × 2). The process provided dibenzofulvene-piperidine adducts (66%), **10c** (R_f 0.63, 0.5 mg, 0.4% from **5**), and **11c** (R_f 0.40, 16.7 mg, 12.5% from **5**) after purification by preparative TLC (EtOAc/hexane, 2:1). *anti* epimer **10c**: HRMS (ESI, positive mode) *m/z* 585.2892 ([M + H]$^+$, calcd for $C_{37}H_{36}N_4O_3$+H, 585.2866).

syn **11c**: white solid; mp 131 °C (dec); $[\alpha]^{29}_D$ +94 ± 7° (*c* 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.27 (dd, 1H, J = 8.0, 1.0, H-7), 7.77 (ddd, 1H, J = 8.3, 7.0, 1.4, H-9), 7.64 (dd, 1H, J = 8.2, 0.5, H-10), 7.49 (ddd, 1H, J = 8.0, 7.1, 1.0, H-8), 7.30−7.26 (m, 6H) and 7.26−7.20 (m, 9H, Ph_3C), 7.13 (s, 1H, Ph_3CNH), 6.91 (d, 1H, J = 4.2, H-2), 5.27 (dd, 1H, J = 9.6, 5.8, H-4), 4.52 (ddd, 1H, J = 9.6, 5.3, 2.7, H-1), 2.76 (ddd, 1H, J = 16.7, 7.1, 4.7) and 2.68 (dd, 1H, J = 15.5, 7.6, Gln-CH_2CONH), 2.38 (m, 1H) and 2.19 (m, 1H, Gln-CH_2CNH); 1.86−1.80 (m, 2H, Leu-CH_2), 1.77 (m, 1H, Leu-CHMe$_2$), 0.98 (d, 6H, J = 6.5, Leu-CHMe_2); ^{13}C NMR ($CDCl_3$) δ 170.19 (CONH$_2$), 168.00 (C-3), 160.73 (C-6), 150.79 (C-11a), 147.19 (C-10a), 144.71 (aromatic Cq of Ph_3C), 134.83 (C-9), 128.80 (CH × 6), 127.87 (CH × 6), 127.09 (C-8 or C-10), 127.02 (C10 or C-8), 126.93 (CH × 3), 126.85 (C-7), 120.00 (s), 70.58 (Ph_3CN), 54.98 (C-1), 54.77 (C-4), 46.87 (Leu-CH_2), 34.37 (Gln-CH_2CONH), 29.91 (Gln-CH_2-CHN), 24.65 (Leu-CHMe$_2$), 23.08 and 21.15 (Leu-Me$_2$); NOESY and ROESY δ_H 2.20 with 1.84; ^{15}N NMR (obtained from ^{1}H−^{15}N HSQC and HMBC in $CDCl_3$) δ_N 112.8 (N-2), 138.6 (CO*N*HTrt, $^1J_{NH}$ = 86), 166.1 (N-5) and 236.4 (N-11); HRMS

(ESI, positive mode) *m/z* 585.2861 ([M + H]$^+$, calcd for $C_{37}H_{36}N_4O_3$+H, 585.2866); *m/z* 607.2644 ([M + Na]$^+$, calcd for $C_{37}H_{36}N_4O_3Na$, 607.2685).

(1*S*,4*S*)-1,3,4,6-Tetrahydro-3,6-dioxo-1-(2-methylpropyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-propanamide (Anacine, revised structure, 4) and Epimer of Anacine (14). Compound **11c** (10.5 mg, 0.0180 mmol) was dissolved in CH_2Cl_2 (1 mL) and reacted with triethylsilane (0.2 mL) and TFA (0.8 mL) at room temperature for 30 min. The solution was evaporated and purified by preparative TLC (EtOAc/MeOH/CH_2Cl_2, 50:5:45) to give anacine **4** (R_f 0.29, 4.5 mg, 74%) and the *anti* epimer **14** (R_f 0.36, 1.5 mg, 25%). Anacine **4**: white solid; $[\alpha]^{27}_D$ +79 ± 13° (*c* 0.26, EtOH); ^{1}H NMR ($CDCl_3$) δ 8.26 (dd, 1H, *J* = 8.0, 1.2, H-7), 7.76 (td, 1H, *J* = 7.7, 1.3, H-9), 7.65 (d, 1H, *J* = 8.1, H-10), 7.49 (td, 1H, *J* = 7.6, 1.0, H-8), 7.22 (d, 1H, *J* = 4.0, H-2), 6.16 (s, 1H) and 5.74 (s, 1H, CON*H*$_2$), 5.23 (dd, 1H, *J* = 9.9, 5.5, H-4), 4.60 (m, 1H, H-1), 2.68 (t, 2H, *J* = 7.1, Gln-C*H*$_2$CONH$_2$), 2.40 (m, 1H) and 2.23 (m, 1H, Gln-C*H*$_2$CNH), 1.97 (overlapped m, 1H, Leu-C*H*Me$_2$), 1.94 (d, 2H, *J* = 5.4, Leu-CH$_2$), 1.08 (t, 3H, *J* = 6.5) and 1.06 (t, 3H, *J* = 6.5, Leu-CH*Me*$_2$); ^{13}C NMR ($CDCl_3$) δ 173.85 (s), 168.04 (s), 160.93 (s), 150.83 (s), 147.23 (s), 134.90 (C-9), 127.15 (C-8 or C-10), 127.07 (C-10 or C-8), 126.81 (C-7), 119.92 (s), 54.98 (C-1), 54.79 (C-4), 47.05 (Leu-*C*H$_2$), 32.35 (Gln-*C*H$_2$CONH$_2$), 29.45 (Gln-*C*H$_2$CHN), 24.73 (Leu-*C*HMe$_2$), 23.20 and 21.17 (Leu-*Me*$_2$); ROESY δ_H 2.68 with 1.07, 2.23 with 1.94; HRMS (ESI, positive mode) *m/z* 343.1777 ([M + H]$^+$, calcd for $C_{18}H_{22}N_4O_3$+H, 343.1770); *m/z* 365.1577 ([M + Na]$^+$, calcd for $C_{18}H_{22}N_4O_3Na$, 365.1590).

***anti* epimer 14**: ^{1}H NMR ($CDCl_3$) δ 8.26 (d, 1H, *J* = 7.9, H-7), 7.79 (t, 1H, *J* = 7.1, H-9), 7.70 (d, 1H, *J* = 8.0, H-10), 7.51 (t, 1H, *J* = 7.4, H-8), 6.13 (s, 1H, H-2), 5.78 (br s, 1H, one of CONH$_2$), 5.47 (t, 1H, *J* = 8.2, H-4), 5.40 (br s, 1H, one of CONH$_2$), 4.74 (dd, 1H, *J* = 9.4, 3.6, H-1), 2.61−2.49 (m, 3H, Gln-C*H*$_2$CONH$_2$ and one of Leu-C*H*$_2$), 2.36−2.30 (m, 2H, Gln-C*H*$_2$CNH), 1.87 (m, 1H, Leu-C*H*Me$_2$), 1.75 (ddd, 1H, *J* = 14.3, 9.5, 4.7, one of Leu-C*H*$_2$), 1.11 (d, 3H, *J* = 6.7) and 1.09 (d, 3H, *J* = 6.5, Leu-CH*Me*$_2$); ^{13}C NMR ($CDCl_3$) δ 173.38 (CONH$_2$), 168.54 (C-3), 160.97 (C-6), 150.65 (C-11a), 146.89 (C-10a), 134.75 (C-9), 127.70 (C-10), 127.41 (C-8), 126.82 (C-7), 120.22 (C-6a), 55.47 (C-4), 51.16 (C-1), 39.55 (Leu-CH$_2$), 31.48 (Gln-*C*H$_2$CONH$_2$), 25.98 (Gln-*C*H$_2$CHN), 24.69 (Leu-*C*HMe$_2$), 23.62 and 21.29 (Leu-CH*Me*$_2$); NOESY δ_H 4.74 with 2.34; HRMS (ESI, positive mode) *m/z* 343.1781 ([M + H]$^+$, calcd for $C_{18}H_{22}N_4O_3$+H, 343.1770); *m/z* 365.1604 ([M + Na]$^+$, calcd for $C_{18}H_{22}N_4O_3Na$, 365.1590).

(1*S*,4*S*)-1,3,4,6-Tetrahydro-3,6-dioxo-1-hydroxyl-1-(phenylmethyl)-2*H*-pyrazino[2,1-*b*]quinazoline-4-propanamide (1-Hydroxyverrucine B, 19). Oxidation of Verrucine B. The NMR sample of **2** in DMSO-d_6 was transferred into a flask. The verrucine B in DMSO-d_6 and MeOH solution was kept at room temperature for several weeks. After removal of the solvent under high vacuum, the oxidized mixture was purified by preparative TLC (EtOAc/MeOH/CH_2Cl_2, 50:5:45). Two polar compounds were isolated. The major one was assigned as 1-hydroxyverrucine B, while the minor one might be its isomer. Oxidized verrucine B (**19**): white solid; ^{1}H NMR ($CDCl_3$) δ 8.28 (d, 1H, *J* = 7.9, H-7), 7.96 (s, 1H, H-2), 7.85 (strong coupled dd, 1H, *J* = 8.0, 1.4, H-10), 7.82 (td, 1H, *J* = 8.3, 1.2, H-9), 7.54 (td, 1H, *J* = 7.5, 1.2, H-8), 7.45 (dd, 2H, *J* = 8.0, 0.8, Ph), 7.37−7.28 (m, 3H, Ph), 6.62 (br s, 1H, one of CON*H*$_2$), 6.47 (s, 1H, OH-1), 5.42 (s, 1H, one of CON*H*$_2$), 5.24 (dd 1H, *J* = 9.4, 7.7, H-4), 3.90 (d, 1H, *J* = 14.1) and 3.63 (d, 1H, *J* = 14.1, Phe-C*H*$_2$), 2.60 (m, 1H) and 2.49 (m, 1H, Gln-C*H*$_2$CONH$_2$), 2.58 (m, 1H) and 2.50 (m, 1H, Gln-C*H*$_2$CNH); ^{13}C NMR ($CDCl_3$) δ 176.06 (CONH$_2$), 169.28 (C-3), 160.76 (C-6), 150.00 (C-11a), 146.78 (C-10a), 134.82 (C-9), 134.39 (Cq of Phe), 131.70 (CH × 2), 128.55 (CH × 2), 128.08 (C-10), 127.67 (C-8), 127.34 (PhH), 126.71 (C-7), 120.45 (C-6a), 81.97 (C-1), 54.79 (C-4), 44.07 (Phe-CH$_2$), 30.39 (Gln-*C*H$_2$CONH$_2$), 26.37 (Gln-*C*H$_2$-CHN); NOESY δ_H 6.46 with 2.49; ^{1}H−^{13}C HMBC δ_H (δ_C) 7.96 (169.28, 150.00, 81.97, 54.79), 6.47 (81.97, 44.07); ^{1}H−^{15}N HSQC δ_H (δ_N) 7.96 (113.4), 6.62 and 5.42 (85.6); HRMS (ESI, positive mode) *m/z* 393.1545 ([M + H]$^+$, calcd for $C_{21}H_{20}N_4O_4$+H, 393.1563); *m/z* 415.1378 ([M + Na]$^+$, calcd for $C_{21}H_{20}N_4O_4Na$, 415.1382); *m/z* 375.1425 ([M + H − H_2O]$^+$, calcd for $C_{21}H_{19}N_4O_3$, 375.1457). Minor isomer: HRMS (ESI, positive mode) *m/z* 393.1559 ([M + H]$^+$, calcd for $C_{21}H_{20}N_4O_4$+H, 393.1563).

Acknowledgment. The authors thank Professor Peter G. Mantle (Imperial College of Science, Technology and Medicine, London SW7 2AY, UK) for providing an authentic sample of anacine. This work was supported by grants from the National Science and Technology Board of Singapore.

Supporting Information Available: ^{1}H and/or ^{13}C NMR spectra for **1**, **2**, **4**, **10b**, **11a**, **11c**, **14**, and **19** in $CDCl_3$. List of ^{1}H and ^{13}C NMR data for **1**, **2**, **4**, and **14** in DMSO-d_6. Conformations of **1**, **2**, **4**, **10b**, **11a**, **10c**, **11c**, and **14** calculated by MM2. This material is available free of charge via the Internet at http://pubs.acs.org.

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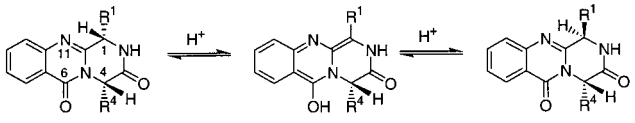
(12) Epimerization may be due to the strongly acidic condition. Numata et al. (ref 3) pointed out that the fumiquinazolines epimerized at both C-1 and C-4 positions under strongly basic conditions, but epimerized at C-1 only under strongly acidic conditions. C-6−benzene ring−N-11−C-11a−C-1 is a highly conjugated unsaturated ketone-like system. The interchangeable ketone and enol forms are the driving force for epimerization at the C-1 position.



(13) Optical rotation data of anacine and its natural diastereoisomer were not reported in the original paper (ref 6). The value reported here was obtained from the authentic sample provided by Professor Mantle.
(14) The authentic samples of verrucines A and B were not available.

NP010342Y