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Antineoplastic Agents. 551. Isolation and Structures of Bauhiniastatins 1−4 from *Bauhinia purpurea*[⊥]

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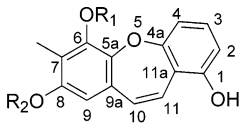
Bioassay-guided (P388 lymphocytic leukemia cell line) separation of extracts prepared from the leaves, stems, and pods of *Bauhinia purpurea*, and, in parallel, its roots, led to the isolation of four new dibenz[*b,f*]oxepins (**2a**, **3**−**5**) named bauhiniastatins 1−4, as well as the known and related pacharin (**1**) as cancer cell growth inhibitors. The occurrence of oxepin derivatives in nature is quite rare. Bauhiniastatins 1−4 were found to exhibit significant growth inhibition against a minipanel of human cancer cell lines, and bauhiniastatin 1 (**2a**) was also found to inhibit the P388 cancer cell line. Structures for these new cancer cell growth inhibitors were established by spectroscopic techniques that included HRMS and 2D NMR.

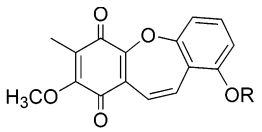
The relatively large *Bauhinia* genus (family Fabaceae) of trees and shrubs, some climbing, is distributed in a wide range of geographic locations in warm climates. Certain *Bauhinia* species have a long history of traditional medical applications. For example, in Africa, *B. reticulata* and *B. rufescens* have been employed against roundworm, conjunctivitis, anthrax, dysentery, blood-poisoning, leprosy, and lung diseases.[2a,b] Another species, *B. variegata*, has, additionally, found use in skin diseases and ulcerations in Africa, whereas in India, extracts of the bark of this species have been used for cancer.[3a] The history of the use of *Bauhinia* species for the treatment of cancer in India and Africa emphasizes the use of *B. tomentosa* L.[3a] and *B. purpurea* as described in a European compilation from the years 1831−1836, *B. purpurea* L.[3a] Despite the very encouraging traditional medical applications of some species of *Bauhinia*, especially *B. purpurea*, as cancer treatments, prior investigations of their anticancer constituents have not appeared in the literature.

In Taiwan, *Bauhinia purpurea* is a well-known ornamental tree, and careful chemical studies of the heartwood[3b] and of the bark[3c] led to the isolation of 27 components that included a chromone, carbohydrates, flavonoids, lipids, phenols, steroids, and triterpenes. Traditional use of *B. purpurea* in sub-Himalayan areas of India point to gastrointestinal activities with applications as a laxative (flowers),[4] as a carminative drug (roots), and for diarrhea (bark).[5a] A new flavone rhamnopyranoside has been isolated from this source,[5a] and there is earlier evidence for a chalcone glycoside constituent.[5b] Other chemical investigations of *B. purpurea* components have been concerned with flavonoids[6] such as the hypoglycemic and antioxidant kaempferol dirhamnoside from *B. forficata*, a traditional antidiabetic treatment in Brazil,[7] and earlier with hypoglycemic flavonoid-containing fractions from leaves of *B. purpurea* grown in Egypt.[8] Presently, *B. purpurea* seems best known for a lectin agglutinin that binds to dense cell surface glycoconjugates and may be of use in medical applications.[9]

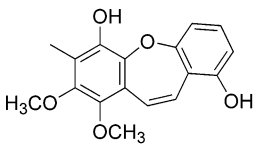
As part of the U.S. National Cancer Institute (NCI) worldwide exploratory survey of terrestrial plants for anticancer constituents, *B. purpurea* was collected in India (1963), and extracts from this plant gave evidence of activity against the NCI murine in vivo sarcoma 180 and Lewis lung carcinoma evaluation systems in the years following. By 1973−1976, fractions were isolated in NCI laboratories with a confirmed level of activity (34−60% life extension at 100 mg/kg) against the murine in vivo P388 lymphocytic leukemia. However, in these earlier studies, the biological results were erratic, and the antineoplastic constituents eluded isolation. Hence, in l979−1980, reinvestigation of *B. purpurea* was undertaken with extracts we prepared from a 36.3 kg re-collection (from India) of leaves, stems, and pods in parallel with a 5.5 kg sample of the roots, with use of the NCI P388 cell line (PS) for bioassay-guided separations. While the PS system represented a considerable improvement, we experienced at that time some inconsistencies that proved challenging. However, now with the advantage of being able to use improved purification and bioassay techniques compared to earlier, we have succeeded in isolating from *B. purpurea* four new, and very unusual, cancer cell growth inhibitors, which we have designated as bauhiniastatins 1−4 (**2a**−**5**).



1, R_1 = H, R_2 = CH_3
5, R_1 = CH_3, R_2 = H



2a, R = H
b, R = Ac



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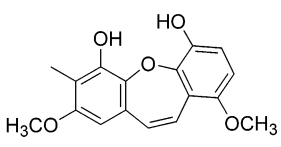

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Results and Discussion

A mixture of leaves, stems, and pods of *Bauhinia purpurea* L. was extracted with 95% EtOH, and the extract was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2-soluble extract was partitioned between CH_3OH-H_2O (4:1) and hexane, and then the aqueous phase was diluted to CH_3OH-H_2O (3:2) and extracted with CH_2Cl_2. The most encouraging PS activity (ED_{50} < 1 μg/

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

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Table 1. NMR Spectroscopic Data of Bauhiniastatin 1 (**2a**) in $CDCl_3$

position	δ_H [a]		*J*/Hz	$^1H-^1H$ COSY	NOE	δ_C		HMBC (C)[b]
1						157.1	(q)[c]	
2	6.85	dd	8.3, 0.9 (3, 4)	3	3	114.1	(t)	1, 4, 11a
3	7.22	t	8.3 (2, 4)	2, 4	2, 4	132.2	(t)	1, 4a, 11a
4	6.63	dd	8.3, 0.9 (3, 2)	3	3	112.8	(t)	1, 2, 4a, 11a
4a						153.8	(q)	
5a						150.6	(q)	
6						182.0	(q)	
7						128.4	(q)	
8						155.4	(q)	
9						182.5	(q)	
9a						128.6	(q)	
10	6.74	d	11.7 (11)	11	11	121.1	(t)	5a, 9, 11a
11	7.37	d	11.7 (10)	10	10	131.7	(t)	1, 4a, 9a, 11a
11a						118.5	(q)	
CH_3-7	1.98	s				8.8	(p)	6, 7, 8
OCH_3-8	3.99	s				61.1	(p)	8
OH-1	5.48	br s						

[a] ^1H chemical shift values (δ ppm from TMS) followed by multiplicity and then the coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position. [b] Long-range $^1H-^{13}C$ correlations from H to C observed in the HMBC experiment. [c] Letters, p, s, t, and q, in parentheses indicate, respectively, primary, secondary, tertiary, and quaternary carbons, assigned by DEPT.

mL) was located in the CH_2Cl_2 fraction. The latter was separated by bioassay (PS)-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography procedures as well as high-performance liquid chromatography (HPLC) to afford two new oxepins, bauhiniastatins 1 (**2a**) and 2 (**3**). Meanwhile, the EtOH extract of the roots of *B. purpurea* was partitioned successively between CH_3OH-H_2O, hexane, and CH_2Cl_2 using the same procedure as above. Back-extraction of the CH_2Cl_2-soluble fraction (ED_{50} 9.5 μg/mL) with CH_3OH-H_2O (1:1) gave a residue that was separated by the same fractionation procedure as above to give the known oxepin pacharin (**1**) and the four new analogues bauhiniastatins 1−4 (**2a**, **3**−**5**). The occurrence of oxepin derivatives appears to be extremely rare in nature. Pacharin (**1**) from the heartwood of *B. racemosa*[10a] and bauhinoxepins A and B from *B. saccocalyx*[10b] are the only previously reported natural dibenz[*b*,*f*]oxepin derivatives.

Bauhiniastatin 1 (**2a**) was isolated as colorless needles with the molecular formula $C_{16}H_{12}O_5$, as established by the molecular ion peak from high-resolution electron-impact mass spectrometry (HREIMS). The IR spectrum exhibited bands at 3345, 1664, 1657, 1607, and 1588 cm^{-1}, characteristic of a hydroxyl group, a conjugated ketone, and an aromatic ring. A close inspection of the ^1H and ^{13}C NMR spectra of oxepin **2a** (Table 1) by DEPT and ^1H and ^{13}C COSY experiments revealed the presence of the following functional groups: a vinyl methyl (CH_3-7), one methoxyl group (OCH_3-8), two ketones [δ 182.02 (C-6) and 182.51 (C-9)][11−13] in a cross-conjugated dienone system, one disubstituted and two tetrasubstituted double bonds (C-10, C-11, C-7, C-8, C-5a, and C-9a), a 1,2,3-trisubstituted benzene (C-1 to C-4a and C-11a), and one hydroxyl group, the presence of which was supported by the fact that acetylation of phenol **2a** gave monoacetate **2b**. The signals for one quaternary sp^2-carbon (C-8 and C-5a) of each of the two tetrasubstituted double bonds, as well as two quaternary sp^2-carbons (C-1 and C-4a) of the aromatic ring, appeared at low field (δ 150.62−157.14) in the ^{13}C NMR spectrum, implying that these four carbons are linked to an oxygen function. $^1H-^1H$ COSY analysis of oxepin **2a** led to two partial structural units, as shown by boldface lines in Figure 1, which were supported by HMBC correlations (Table 1). The connection of these two units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 1. HMBC correlations from the vinyl methyl protons to C-6 and C-8, from the methoxyl protons to C-8, and from one proton (H-10) of the ethylene to C-9 showed that the vinyl methyl, the methoxyl, and the two ketone groups are located at C-7, C-8, C-6, and C-9, respectively. That conclusion was supported further by an NOE between the acetyl proton signal and H-11 in acetate **2b**. HMBC correlations from one proton

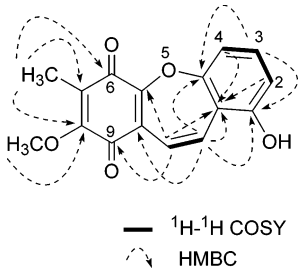

Figure 1. Selected $^1H-^1H$ COSY and HMBC correlations in bauhiniastatin 1 (**2a**).

(H-11) of the ethylene group to C-1, from the other proton (H-10) to C-5a, and from one proton triplet (H-3) in the ABC system to C-1 and C-4a in oxepin **2a** implied that the hydroxyl group is located at C-1 and that the ether linkage is between C-4a and C-5a. This evidence led to oxepin structure **2a** for bauhiniastatin 1.

Bauhiniastatin 2 (**3**) was assigned a molecular formula that contained one carbon and four protons more than that of oxepin **2a**, as deduced from HREIMS. The IR spectrum exhibited absorption bands for a hydroxyl group and an aromatic ring, but it did not show a band for a conjugated ketone. The general features of the ^1H and ^{13}C NMR spectra of oxepin **3** closely resembled those of **2a** except that the signals for additional hydroxyl [δ_H 5.86 (OH-6)] and methoxyl [δ_H 3.79, δ_C 61.35 (OCH_3-9)] groups appeared in the spectra of bauhiniastatin 2 (**3**), and the signals for two carbonyl carbons of the *p*-quinone in oxepin **2a** were replaced by those of two aromatic carbons [δ_C 142.23 (C-6) and δ_C 142.82 (C-9)] linked to the hydroxyl or the methoxyl function in oxepin **3** (Table 2). Observation of HMBC correlations from OH-6 to C-6, C-7, and C-5a, from OCH_3-9 to C-9, and from H-10 to C-9 implied that the hydroxyl and methoxyl groups newly observed in oxepin **3** are linked to C-6 and C-9, with the other HMBC correlations in bauhiniastatin 2 (**3**) being the same as those of oxepin **2a**. The above-mentioned evidence allowed assignment of structure **3** for bauhiniastatin 2.

Bauhiniastatin 3 (**4**) corresponded to the same molecular formula as oxepin **3**, as deduced from HREIMS. A close inspection of its IR and NMR spectra revealed the presence of two hydroxyl, one aromatic methyl, and two methoxyl groups and three aromatic protons, implying that oxepin **4** is the positional isomer of **3** (Table 3). Two of the three aromatic protons appeared as double doublets (J = 7.8 Hz) in an AB system at δ_H 6.52 (H-2) and 6.84 (H-3), while the other one occurred as a singlet at δ_H 6.23 (H-9), suggesting the presence of 1,2,3,4-tetrasubstituted and pentasubstituted benzene rings. HMBC correlations from the aromatic one-

Table 2. NMR Spectroscopic Data of Bauhiniastatin 2 (**3**) in $CDCl_3$

position	δ_H [a]		*J*/Hz	$^1H-^1H$ COSY	NOE	δ_C		HMBC (C)[b]
1						158.8	(q)[c]	
2	6.72	dd	8.0, 0.9 (3, 4)	3	3	113.3	(t)	1, 4, 11a
3	7.13	t	8.0 (2, 4)	2, 4	2, 4	129.8	(t)	1, 4a
4	6.61	dd	8.0, 0.9 (3, 2)	3	3	112.4	(t)	2, 4a, 11a
4a						153.6	(q)	
5a						140.1	(q)	
6						142.2	(q)	
7						119.6	(q)	
8						148.7	(q)	
9						142.8	(q)	
9a						121.8	(q)	
10	6.99	d	11.7 (11)	11	11	124.9	(t)	5a, 9, 11a
11	6.93	d	11.7 (10)	10	10	123.1	(t)	1, 4a, 9a
11a						118.6	(q)	
OH-1	5.19	br s						
OH-6	5.86	br s						5a, 6, 7
CH_3-7	2.19	s				9.2	(p)	6, 7, 8
OCH_3-8	3.79	s				60.6	(p)[d]	8
OCH_3-9	3.79	s				61.4	(p)[d]	9

[a−c] As in Table 1. [d] Interchangeable.

Table 3. NMR Spectroscopic Data of Bauhiniastatin 3 (**4**) in $CDCl_3$

position	δ_H [a]		*J*/Hz	$^1H-^1H$ COSY	NOE	δ_C		HMBC (C)[b]
1						150.9	(q)[c]	
2	6.52	d	8.7 (3)	3	OCH_3-1, -3	106.8	(t)	1, 4, 11a
3	6.84	d	8.7 (2)	2	2	115.7	(t)	4, 4a, 1
4						140.7	(q)	
4a						145.6	(q)	
5a						138.1	(q)	
6						146.2	(q)	
7						113.4	(q)	
8						154.9	(q)	
9	6.23	s			OCH_3-8, -10	101.1	(t)	7, 8, 5a, 10, 9a
9a						128.0	(q)	
10	6.76	d	11.7 (11)	11	9, 11	129.5	(t)	5a, 9, 9a, 11a
11	7.02	d	11.7 (10)	10	10	124.2	(t)	1, 4a, 9a, 11a
11a						120.9	(q)	
OCH_3 -1	3.78	s			2	56.2	(p)	1
OH-4	6.74[d]	br s						
OH-6	5.71[d]	br s						
CH_3-7	2.14	s				8.6	(p)	6, 7, 8
OCH_3-8	3.77	s			9	55.8	(p)	8

[a−c] As in Table 1. [d] Interchangeable.

proton singlet to C-7, C-8, C-5a, C-9a, and C-10 and from OCH_3-8 to C-8 showed that the aromatic proton singlet corresponds to H-9 and that one methoxyl group is located at C-8. This was supported by the observation of NOEs from H-9 to H-10 and OCH_3-8. HMBC correlations from one proton (H-2) in the AB system to C-11a and C-1 and from OCH_3-1 to C-1 implied that the other methoxyl group is located at C-1, which again was supported by a NOE correlation between H-2 and OCH_3-1. From the HMBC correlations (from CH_3-7 to C-7 and C-6 and from H-3 to C-4 and C-4a) it was deduced that the two signals (δ_C 140.73 and 146.17) for hydroxylated carbons correspond to C-4 and C-6. That evidence completed the assignment of structure **4** to bauhiniastatin 3.

For bauhiniastatin 4 (**5**), the molecular formula $C_{16}H_{14}O_4$ was established by a molecular ion peak in the HREIMS. The general features of its ^1H and ^{13}C NMR spectra (Table 4) closely resembled those of oxepins **3** and **4** except that one signal for the methoxyl groups in oxepins **3** and **4** was missing in this case (**5**). Three of the four aromatic protons constituted an ABC system, while the other one occurred as a singlet, implying the presence of 1,2,3-tetrasubstituted and pentasubstituted benzene rings. HMBC correlations from the aromatic proton singlet to C-7, C-8, C-5a, and C-10, from CH_3-7 to C-6 and C-8, and from OCH_3-6 to C-6 implied that the aromatic singlet proton corresponds to H-9, and the single hydroxyl and methoxyl groups are located at C-8 (δ_C 150.7) and C-6 (δ_C 150.9), respectively. Further support was realized by an NOE correlation between H-9 and H-10. The other hydroxyl group was found to be located at C-1 (δ_C 159.83) on the basis of HMBC correlation from H-11 to C-1. On the basis of this evidence, the oxepin structure **5** for bauhiniastatin 4 was established.

The previously known pacharin (**1**) was also isolated and has the same molecular formula as oxepin **5** as deduced from HREIMS. The general features of its ^1H and ^{13}C NMR spectra in $CDCl_3$ showed a striking resemblance to those of oxepin **5**, suggesting that it is a positional isomer of **5**. By deductions based on HMBC correlations (H-9 to C-7, C-8, and C-10, and OCH_3-8 to C-8) and NOE between H-9 and OCH_3-8, the methoxyl group in oxepin **1** could be located at C-8. Therefore, this compound was identified as pacharin (**1**), the structure of which has been established previously on the basis of spectroscopic and X-ray structure analyses.[10a] Furthermore, it was identified unequivocally by a comparison of the ^1H and ^{13}C NMR spectral data measured in DMSO-d_6 with published values.[10a] Assignments of the ^1H and ^{13}C NMR spectra of pacharin (**1**) using 2D NMR techniques ($^1H-^1H$ COSY, $^1H-^{13}C$ COSY, and HMBC) allowed revisions of the previously reported NMR spectral data of **1** in DMSO-d_6.[10a]

All of the isolated compounds (**1**−**5**) were evaluated for cancer cell growth inhibition against the P388 lymphocytic leukemia cell line. As shown in Table 5, bauhiniastatin 1 (**2a**) exhibited significant inhibition. Bauhiniastatins 1−3 (**2a**, **3**, **4**) and pacharin (**1**) were also evaluated against a minipanel of human cancer cell lines (Table 5), and each was found to exhibit significant inhibition against the cancer cell lines except for colon KM20L2 and, in the case of

Table 4. NMR Spectral Data of Bauhiniastatin 4 (**5**) in $CDCl_3$

position	δ_H [a]		J/Hz	$^1H-^1H$ COSY	NOE	δ_C		HMBC (C)[b]
1						159.8	(q)[c]	
2	6.89	dd	8.2, 0.9 (3, 4)	3	3	114.2	(t)	4
3	7.13	t	8.2 (2, 4)	2, 4	2, 4	129.9	(t)	
4	6.56	dd	8.2, 0.9 (3, 2)	3	3	111.7	(t)	2, 11a
4a						153.2	(q)	
5a						144.3	(q)	
6						150.9	(q)	
7						119.2	(q)	
8						150.7	(q)	
9	6.38	s			10	109.4	(t)	7, 8, 5a, 10
9a						129.7	(q)	
10	6.68	d	11.7 (11)	11	9, 11	129.4	(t)	5a, 9
11	6.93	d	11.7 (10)	10	10	123.7	(t)	1
11a						118.6	(q)	
OH-1	4.91[d]	br s						
OCH_3-6	3.99	s				61.6	(p)	6
CH_3-7	2.17	s				9.0	(p)	6, 7, 8
OH-8	4.56[d]	br s						

[a–c] As in Table 1. [d] Interchangeable.

Table 5. Cancer Cell Growth Inhibition Evaluation of Oxepins **1**−**5** against the P388 Lymphocytic Leukemia Cell Line and a Minipanel of Human Cancer Cell Lines[a]

		GI_{50} (μg/mL)					
cell type	cell line	1	2a	3	4	5	5-FU[b]
mouse leukemia	P388	15.5	0.65	23.20	15.70	61.5	0.073
human pancreas	BXPC-3	4.3	7.0	3.8	6.6		
breast	MCF-7	2.3	4.1	3.1	3.2		
CNS	SF268	3.1	2.4	3.2	5.8		
lung	NCI-H460	4.2	6.6	3.5	6.1		
colon	KM20L2	>10	>10	>10	>10		
prostate	DU-145	3.3	>10	3.8	5.8		

[a] DMSO was used as vehicle in the test of all compounds. [b] 5-FU was used as standard.

oxepin **2a**, except for prostate line DU-145. Evaluation of bauhiniastatin 4 (**5**) could not be carried out owing to lack of material and must await future availability. However, its very close isomeric relationship to pacharin (**1**) suggests that it will be found to show inhibition of human cancer cell growth and the current name will remain appropriate. Interestingly, the general structure of, for example, bauhiniastatin 3 (**4**) is reminiscent of the *cis*-stilbene geometry required for strong antiangiogensis activity in the combretastatin series of anticancer drugs from the *Combretum caffrum* tree, such as combretastatin A-1.[14]

Experimental Section

General Experimental Procedures. UV and IR spectra were recorded with a Shimadzu spectrophotometer and a Perkin-Elmer FT-IR spectrometer 1720X, respectively. NMR spectra were recorded at 27 °C on a Varian Unity INOVA-500 spectrometer with tetramethylsilane (TMS) as an internal reference. EIMS, including HRMS, was carried out with a Hitachi M-4000H mass spectrometer. Liquid chromatography over silica gel (mesh 230−400) was performed at ambient pressure. HPLC was performed with a Waters ALC-200 instrument equipped with a differential refractometer (R 401) and Shim-pack PREP-SIL or Shim-pack PREP-ODS (25 cm × 20 mm i.d.).

Plant Material. Specimens of the tree, *Bauhinea purpurea* L., were collected initially in India (1963). This exploratory sampling was conducted as part of the joint U.S. National Cancer Institute/U.S. Department of Agriculture research directed by Dr. Jonathan L. Hartwell of the NCI and Dr. Robert E. Perdue of the USDA. A voucher sample (No. BCS-2053) of *B. purpurea* is stored in the herbarium at the United States National Arboretum, 3501 New York Ave. NE, Washington, DC 20002-1958. A scale-up re-collection of *B. purpurea* leaves, stems, pods, and roots was made in 1979−80 and received in our Institute in 1980. The scale-up supply was accomplished under the direction of Drs. John D. Douros and Matthew I. Suffness of the NCI and Robert E. Purdue and James A. Duke of the USDA.

Extraction, Solvent Partition, and Isolation of Bauhiniastatins 1−4 (2a−5) and Pacharin (1). Part A. Leaves, Stems, and Pods. A mixture of leaves, stems, and pods of *B. purpurea*, finely ground (36.3 kg), was extracted with 95% EtOH, and the extract was partitioned between H_2O and CH_2Cl_2 to give a CH_2Cl_2 phase (402.9 g). A part of the CH_2Cl_2 extract (305.3 g) was partitioned between CH_3OH-H_2O (4:1) and hexane followed by dilution of the aqueous phase to CH_3OH-H_2O (3:2) and extraction with CH_2Cl_2. Removal of solvents gave the hexane (222 g, PS, ED_{50} 26 μg/mL), CH_2Cl_2 (54.6 g, PS, ED_{50} <1 μg/mL), and CH_3OH-H_2O (18.5 g, PS, ED_{50} 25 μg/mL) fractions. The CH_2Cl_2 fraction (54.6 g) was passed through a Sephadex LH-20 column, using CH_3OH as eluent. The fourth fraction (1.3 g) was chromatographed repeatedly on a silica gel column with a gradient of hexane−CH_2Cl_2−CH_3OH as eluent. The hexane−CH_2Cl_2 (1:1) eluate (47.0 mg) was separated by HPLC (SIL) using $CH_3OH-CH_2Cl_2$ (1:200) as eluent, affording bauhiniastatin 1 (**2a**, 12.5 mg) and bauhiniastatin 2 (**3**, 4.9 mg).

Part B. Roots. The roots of *B. purpurea*, finely ground (5.5 kg), were extracted with 95% EtOH. The EtOH extract (68.7 g) was partitioned between CH_3OH-H_2O (4:1) and hexane followed by CH_3OH-H_2O (3:2) and CH_2Cl_2 as above. Removal of solvents gave the hexane (4.23 g, PS, ED_{50} 27 μg/mL), CH_2Cl_2 (9.51 g, PS, ED_{50} 9.5 μg/mL), and CH_3OH-H_2O (15.52 g, PS, ED_{50} > 100 μg/mL) fractions. The 1:1 CH_3OH-H_2O-soluble fraction (5.6 g) of the CH_2Cl_2 fraction was passed through a Sephadex LH-20 column, with $CH_3OH-CH_2Cl_2$ (1:1) as eluent. The ninth (Fr. 1, 1.15 g) and eleventh fractions (Fr. 2, 141.2 mg) were chromatographed separately on a silica gel column with a gradient of hexane−CH_2Cl_2−CH_3OH as eluents. The hexane−CH_2Cl_2 (1:1) eluate (37.4 mg) from the column chromatography of Fr. 1 was separated further by HPLC (ODS) using CH_3OH-H_2O (8:2) as eluent to provide bauhiniastatin 2 (**3**, 3.5 mg), bauhiniastatin 1 (**2a**, 5.9 mg), and bauhiniastatin 3 (**4**, 3.7 mg). The hexane−CH_2Cl_2 (1:1), CH_2Cl_2, and CH_2Cl_2−CH_3OH (99:1) eluates from the silica gel column chromatogram of Fr. 2 gave pacharin (**1**, 36.8 mg), bauhiniastatin 2 (**3**, 14.8 mg), and Fr. 3 (18.2 mg), respectively. Fr. 3 was purified further by HPLC (ODS) using CH_3OH-H_2O (8:2) as eluent to yield bauhiniastatin 4 (**5**, 1.6 mg).

Pacharin (1): colorless needles (CH_2Cl_2); mp 211−213 °C; UV (CH_3OH) λ_{max} (log ϵ) 218 (4.29), 234 (4.14), 312 (4.02) nm; IR (KBr) ν_{max} 3410, 1612, 1585 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.12 (1H, t, J = 8.2 Hz, H-3), 6.92 (1H, d, J = 11.7 Hz, H-11), 6.73 (1H, dd, J = 8.2, 0.9 Hz, H-2), 6.72 (1H, d, J = 11.7 Hz, H-10), 6.59 (1H, dd, J = 8.2, 0.9 Hz, H-4), 6.19 (1H, s, H-9), 6.06 (1H, s, OH-6), 4.98 (1H, br s, OH-1), 3.78 (3H, s, OCH_3-8), 2.13 (3H, s, CH_3-7); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 158.96 (C-1), 154.97 (C-8), 153.50 (C-4a), 146.22 (C-6), 138.34 (C-5a), 130.01 (C-10), 129.81 (C-3), 127.05 (C-9a), 123.18 (C-11), 118.33 (C-11a), 113.97 (C-7), 113.31 (C-2), 112.16 (C-4), 101.13 (C-9), 55.78 (OCH_3-8), 8.61 (CH_3-7); ^1H NMR (DMSO-d_6, 500 MHz) δ 7.12 (1H, t, J = 8.2 Hz, H-3), 7.00 (1H, dd, J = 8.2, 0.9 Hz, H-4), 6.89 (1H, d, J = 11.7 Hz, H-10), 6.65 (1H, d, J = 11.7 Hz,

H-11), 6.64 (1H, dd, J = 8.2, 0.9 Hz, H-2), 6.29 (1H, s, H-9), 3.71 (3H, s, OCH_3-8), 2.02 (3H, s, CH_3-7); ^{13}C NMR (DMSO-d_6, 500 MHz) δ 158.86 (C-4a), 155.19 (C-1), 154.18 (C-8), 146.88 (C-6), 138.36 (C-5a), 129.50 (C-10), 128.72 (C-11), 127.90 (C-9a), 124.32 (C-3), 118.07 (C-11a), 113.43 (C-7), 112.26 (C-4), 111.56 (C-9), 100.55 (C-2), 55.49 (OCH_3-8), 8.55 (CH_3-7); EIMS m/z 270 $[M]^+$ (100); HREIMS m/z 270.0887 $[M]^+$ (calcd for $C_{16}H_{14}O_4$, 270.0891).

Bauhiniastatin 1 (2a): colorless needles (CH_2Cl_2); mp 200−202 °C; UV (CH_3OH) λ_{max} (log ϵ) 216 (4.14), 237 (3.95), 281 (4.12), 305 (3.75) nm; IR (KBr) ν_{max} 3345, 1664, 1657, 1607, 1588 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; EIMS m/z 284 $[M]^+$ (100); HREIMS m/z 284.0662 $[M]^+$ (calcd for $C_{16}H_{12}O_5$, 284.0641).

Bauhiniastatin 1 Acetate (2b). To a solution of oxepin **2a** (3.2 mg) in pyridine (1.0 mL) was added Ac_2O (1.0 mL), and the reaction mixture was left at room temperature overnight. The solution was concentrated to dryness under reduced pressure, and the residue was separated by HPLC (ODS) using CH_3OH-H_2O (9:1) as eluent to afford acetate **2b** (2.8 mg) as a colorless powder: IR (KBr) ν_{max} 1763, 1670, 1654, 1617, 1591 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.39 (1H, t, J = 8.3 Hz, H-3), 7.17 (1H, dd, J = 8.3, 0.9 Hz, H-2), 7.08 (1H, d, J = 11.7 Hz, H-11), 6.95 (1H, dd, J = 8.3, 0.9 Hz, H-4), 6.79 (1H, d, J = 11.7 Hz, H-10), 3.99 (3H, s, OCH_3-8), 2.34 (3H, s, $COCH_3$), 1.98 (3H, s, CH_3-7); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 182.17 (C-9), 181.72 (C-6), 168.73 ($COCH_3$), 156.60 (C-1), 155.37 (C-8), 151.25 (C-5a), 148.31 (C-4a), 131.67 (C-3), 130.29 (C-11), 128.44 (C-7), 127.89 (C-9a), 123.56 (C-11a), 123.05 (C-10), 120.09 (C-4), 119.63 (C-2), 61.10 (OCH_3-8), 20.80 ($COCH_3$), 8.79 (CH_3-7); EIMS m/z 326 $[M]^+$ (64).

Bauhiniastatin 2 (3): colorless powder; mp 180−182 °C; UV (CH_3OH) λ_{max} (log ϵ) 219 (4.29), 237 (4.11), 281 (3.76), 317 (4.01) nm; IR (KBr) ν_{max} 3382, 1609, 1581 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; EIMS m/z 300 $[M]^+$ (100); HREIMS m/z 300.0995 $[M]^+$ (calcd for $C_{17}H_{16}O_5$, 300.0997).

Bauhiniastatin 3 (4): colorless powder; mp 158−160 °C; UV (CH_3OH) λ_{max} (log ϵ) 218 (4.26), 237 (4.00), 305 (3.90), 328 (3.87) nm; IR (KBr) ν_{max} 3314, 1618, 1578 cm^{-1}; ^1H and ^{13}C NMR data, Table 3; EIMS m/z 300 $[M]^+$ (100); HREIMS m/z 300.0996 $[M]^+$ (calcd for $C_{17}H_{16}O_5$, 300.0997).

Bauhiniastatin 4 (5): colorless powder; mp 196−198 °C; UV (CH_3OH) λ_{max} (log ϵ) 210 (4.05), 228 (3.74), 298 (3.46) nm; IR (KBr) ν_{max} 3440, 1618, 1595 cm^{-1}; ^1H and ^{13}C NMR data, Table 4; EIMS m/z 270 $[M]^+$ (100); HREIMS m/z 270.0889 $[M]^+$ (calcd for $C_{16}H_{14}O_4$, 270.0891).

Cancer Cell Line Bioassay Methods. A summary of the inhibition of human cancer cell procedures has been presented in ref 15.

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References and Notes

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