Triterpenoid Pyrazines and Benzopyrazines with Cytotoxic Activity

Milan Urban,[†,‡] Jan Sarek,*[,†] Miroslav Kvasnica,[†,‡] Iva Tislerova,[†] and Marian Hajduch[§]

Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43 Prague 2, Czech Republic, Institute of Organic Chemistry and Biochemistry Academy of Sciences of the Czech Republic, Flemingovo n. 2, 166 10, Prague 6, Czech Republic, and Laboratory of Experimental Medicine, Department of Pediatrics, Faculty of Medicine, Palacky University and Faculty Hospital in Olomouc, Puskinova 6, 775 20 Olomouc, Czech Republic

Received September 6, 2006

Twelve lupane, 18α-oleanane, and des-*E*-lupane derivatives (**1a**−**5b**) were either extracted from natural sources or synthesized from betulinic acid (**1a**) and betulin (**2**). Compounds **1b**, **1c**, **3b**, **3c**, **4b**, **4c**, **5a**, and **5b** were then used as starting materials for further synthesis of a series of pyrazines and benzopyrazines (**6a**−**18**); 20 of them are new (**6a**−**6e**, **7a**−**7d**, and **10a**−**18**). Activity of pyrazine **6a** against the T-lymphoblastic leukemia cell line CEM encouraged us to synthesize several new esters (**6b**−**6d**) to study structure−activity relationships with respect to substitution of the carboxyl group at position 28. The synthesized compounds were tested for cytotoxicity against a variety of cancer cell lines of different histogenetic origin, and the results were compared with cytotoxicity of the known starting compounds. Significant cytotoxic activity against A 549, K 562, and multidrug-resistant K 562-tax cell lines was found in pyrazines **6a**, **6d**, and **6e**.

Heterocyclic compounds occur widely in living organisms, and many possess a broad range of biological activities. Researchers have also demonstrated the interesting biological activities of many natural terpenoids.[1] However, terpenoids possessing a nitrogen-containing heterocycle condensed to an isoprenoid skeleton are not common. One example of a less common isoprenoid is cephalostatin, isolated[1] from the sea worm *Cephalodiscus gilchristie*. This bis-steroidal pyrazine is highly cytotoxic,[2] which sparked interest in the synthesis of similar structures.[3−5] To date no parallel studies have been reported in triterpene (especially lupane and oleanane) chemistry, a fact that motivated us to synthesize a group of novel pyrazines from betulinic acid (**1a**) and betulin (**2**). Acid **1a** and betulin (**2**) are pentacyclic triterpenes that occur in the bark of *Platanus hispanica* and *Betula pendula*, respectively, and many other plant sources.[6] Betulinic acid (**1a**) has anti-HIV[7,8] activity and cytotoxic[9,10] activity against human melanoma (MEL-2),[11] lung adenocarcinoma[12] (A 549), neuroblastoma,[13] medulloblastoma,[14] glioblastoma,[15] ovarian (OVCAR-3),[14] colon (HCT-15),[14] and glioma (XF498)[14] cell lines. It has also high *in vivo* activities against MEL-2[15] and other neuroectodermal tumors[15,16] including Ewings sarcoma.[16] Although betulin (**2**) itself does not seem to be cytotoxic, some derivatives were found to be active.[17,18]

Several heterocycles,[19,20] containing pyrazines,[21−23] have previously been synthesized from allobetulone **4b**, although no report concerning their cytotoxicity has been published. In this paper, we describe both the synthesis and structure−activity relationships of 20 new (**6a**−**6e**, **7a**−**7d**, **10a**, **10b**, **11a**, **11b**, and **12**−**18**) and 14 known (**1a**−**5b**, **6f**, **8**, and **9**) compounds. These include triterpenoid derivatives modified with pyrazine and benzopyrazine rings attached to the A-ring or E-ring of the triterpenes. Compounds **1b**, **3b**, and **5b** have previously shown interesting activities[18] and were selected as leading compounds for further modification. Allobetulon **4b** and diketones **4c** and **5a** were chosen as examples of inactive triterpenes, and their derivatives were useful for comparison of cytotoxicity with pyrazines prepared from active compounds (**1b**, **3b**, and **5b**).

Results and Discussion

3β-Hydroxylup-20(29)-en-28-oic acid (**1a**) was extracted from the bark of the plane tree, *Platanus hispanica*. Lup-20(29)-ene-3β,28-diol (**2**) was extracted from the outer layers of birch bark, *Betula pendula* (both collected in the Czech Republic). Betulonic acid (**1b**), dihydrobetulinic acid (**3a**), and dihydrobetulonic acid (**3b**) were synthesized[24] from betulinic acid (**1a**). Methyl betulonate (**1c**) and methyl dihydrobetulonate (**3c**) were prepared from free acids **1b** and **3b** by reaction with diazomethane. Allobetulone **4b** and diketones **5a** and **5b** were synthesized[18] from betulin (**2**). Diketone **4c** was synthesized from allobetulin (**4a**)[25] (Scheme 1).

The purpose of this work was to synthesize a series of pyrazine and benzopyrazine derivatives of triterpenes as summarized in Schemes 2 and 3. The reaction of ketone **4b** with ethylenediamine and sulfur in refluxing morpholine has been previously described.[22] We used an analogous procedure to prepare pyrazines **6a**, **6b**, **6e**, **6f**, **8**, and **17** from ketones **1b**, **1c**, **3b**, **3c**, **4b**, and **4c**, the only modification being in the amounts of reagents used. Reaction of ketones **1b**, **1c**, **3b**, **3c**, and **4b** with 1,2-phenylenediamine under the same conditions gave benzopyrazines **7a**−**7d** and **9**. We present a shorter alternative procedure for the synthesis of compound **9** than that previously described.[21]

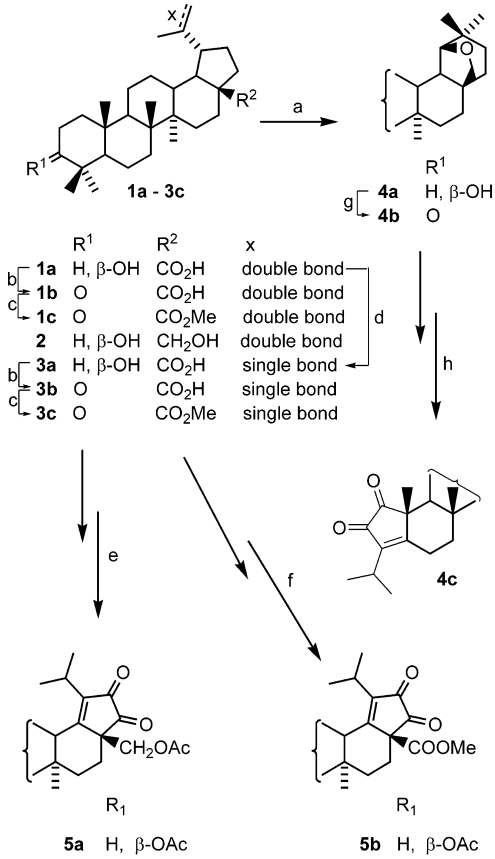
An analogous procedure for the preparation of pyrazines at the E-ring (Scheme 3), from diketones **5a** and **5b**, was not highly successful. Reaction of **5a** with ethylenediamine followed by acetylation gave pyrazine **12** (47%), along with multiple byproducts. In an analogous reaction, pyrazine **14** was obtained from **5b** as the major product (57%) instead of the desired compound **15**. Mixtures containing no benzopyrazine derivatives were obtained from reactions of **5a** and **5b** with 1,2-phenylenediamine. Since all of those side reactions could be caused by the basicity of morpholine, we optimized the reaction solvent. Compounds **13**, **15**, and **16** were obtained in yields of 50−70% in refluxing xylene. This approach led to fewer byproducts than the morpholine procedure and allowed easier purification. This methodology was then applied for the preparation of pyrazines at the A-ring. To our surprise, we found that using morpholine as a solvent is essential for the reaction of ketones in the A-ring with ethylenediamine, since no pyrazines were obtained in xylene. It appears that the basic solvent is important for an oxidative attack at the 2 position. The reaction of diketone **4c** with 1,2-phenylenediamine gave rise to compound **18** (63%).

Compound **6a** was treated with diazomethane, pivaloyloxymethyl chloride (PomCl), or acetoxymethyl bromide (AcmBr) to give esters **6b**−**6d**. We found previously,[26] in another group of triterpene acids, that Acm esters can be used as a prodrug and that cytotoxicity did not decrease significantly when compared with methyl and Pom

* To whom correspondence should be addressed. Tel: +420 221 951 332. Fax: +420 221 951 332. E-mail: jan.sarek@gmail.com.
† Charles University in Prague.
‡ Academy of Sciences.
§ Palacky University in Olomouc.

10.1021/np060436d CCC: $37.00
Published on Web 03/20/2007

Scheme 1. Preparation of Starting Material for Synthesis of Heterocycles



Key: (a) Montmorillonite, $CHCl_3$, reflux; (b) CrO_3, DMF, r.t.; (c) CH_2N_2, $CHCl_3$, Et_2O, r.t. (d) H_2, Pt, THF, EtOH, AcOH; (e) i: Ac_2O, pyridine, reflux, ii: HBr, toluene, AcOH, Ac_2O, reflux; iii: $Na_2Cr_2O_7.2H_2O$, toluene, AcOH, iv: SeO_2, dioxane, AcOH, reflux; (f) i: Ac_2O, pyridine, refl., ii: HBr, toluene, AcOH, Ac_2O, reflux; iii: $Na_2Cr_2O_7.2H_2O$, toluene, AcOH, iv: KOH, EtOH, toluene, v: RuO_2, $NaIO_4$, EtOAc, MeCN, H_2O, vi: CH_2N_2, $CHCl_3$; vii: SeO_2, dioxane, AcOH, reflux; (g) $Na_2Cr_2O_7.2H_2O$, toluene, AcOH; (h) i: PCl_5, toluene; ii: $Na_2Cr_2O_7.2H_2O$, toluene, AcOH; iii: SeO_2, dioxane, AcOH, reflux;

esters. Esters **6b**, **6f**, **7b**, and **7d** were reduced with LAH under standard conditions to give pyrazines **10a**−**11b**, which contain a 28-hydroxymethyl group (Scheme 2).

Cytotoxicity of the new pyrazines **6a**−**18** was then studied. Pyrazine **6a** showed interesting *in vitro* activity against the T-lymphoblastic leukemia CEM cell line (IC_{50} 14 μmol/L), which motivated us to synthesize a larger group of pyrazines and benzopyrazines for structure−activity relationship analysis. Among pyrazines **6a**−**18** (Table 1) we found that **6e** was the most active (IC_{50} 8 μmol/L) against the CEM cell line. Acm ester **6d** was the most active (IC_{50} 12 μmol/L) compound among esters **6b**−**6d**, with activity higher than that of free acid **6a**. Methyl ester **6b** and Pom ester **6c** were inactive. The cytotoxic activity of benzopyrazines (**7a**−**7d**, **9**, **11a**, **11b**, **13**, **16**, and **18**) was in the high micromolar ranges. All other changes of the terpene skeleton led to significant decrease of cytotoxicity. Pyrazines and benzopyrazines **8**, **9**, **17**, and **18** synthesized from inactive **4b** and diketone **4c** as well as compounds with a 28-hydroxymethyl group (**10a**−**11b**) were also inactive. Compounds with the heterocycle condensed to the E-ring (**12**−**16**) were less active than the starting diketones **5a** and **5b**.

The most active of these compounds against T-lymphoblastic leukemia CEM cells (**6a**, **6e**, and **6d**) along with several members of each structurally similar group of compounds were then tested on additional cancer cell lines of different histogenetic origin

Table 1. Cytotoxicity of Compounds **1a**−**18** to the CEM Cell Line

compound	IC_{50} (μmol/L[a]) CEM	compound	IC_{50} (μmol/L[a]) CEM
1a	30	**6f**	151
1b	20	**7a**	42
1c	250	**7b**	250
2	250	**7c**	124
3a	7	**7d**	250
3b	2	**8**	250
3c	173	**9**	250
4a	250	**10a**	101
4b	250	**10b**	74
4c	250	**11a**	178
5a	250	**11b**	35
5b	16	**12**	215
6a	14	**13**	106
6b	250	**14**	244
6c	232	**15**	250
6d	12	**16**	192
6e	8	**17**	250
		18	250

[a] The lowest concentration that kills 50% of tumor cells.

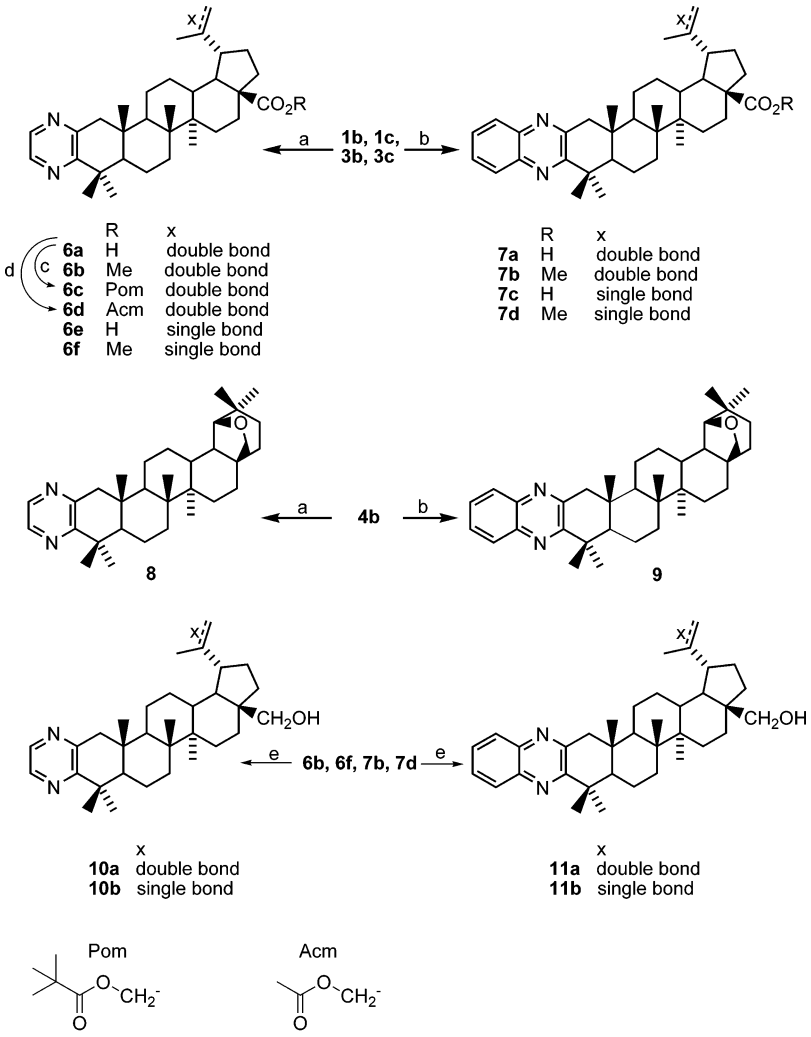
(Table 2). Of these tests the activities of compounds **6a**, **6d**, and **6e** against A 549, K 562, and the paclitaxel-resistant and Pgp-overexpressing[21a] line K 562-tax (IC_{50} 0.25−8 μmol/L) proved to be the most interesting. These compounds were preferentially cytotoxic against human carcinoma cell lines A 549 and HT-29 and less cytotoxic against prostate cancer PC-3 compared to otherwise highly sensitive CEM leukemia or neuroectodermal tumors (melanoma SK-MEL-2, glioblastoma U87-Mg). The high activity of **6a** and **6e** against the human myeloid leukemia line K 562 suggests that further studies should be performed using these compounds. These additional assays should pay particular attention to those bearing bcr-abl translocation. Compounds **6a**, **6d**, and **6e** were approximately 10 times less active against Pgp-positive multidrug-resistant cell line K 562-tax. This result suggests that further compounds having no affinity to multidrug resistance transporters should be synthesized. Nonetheless, the activity of **6e** was not affected by other multidrug resistance associated genes, represented by MRP protein in the case of the CEM-DNR-bulk cell line (Tables 1 and 2). No other derivative showed significant activity.

In conclusion, pyrazines and benzopyrazines **6a**−**18** were synthesized and their cytotoxic activity was compared with that of starting compounds **1a**−**5b**. The previously known[22] procedure for the preparation of pyrazines and benzopyrazines at the A-ring was optimized for the synthesis of corresponding heterocycles from 21,-22-diketones at the E-ring. Esters **6b**−**6d** and 28-hydroxymethyl derivatives **10a**−**11b** were prepared from pyrazines **6b** and **6f** and benzopyrazines **7b** and **7d**. Among all of the new derivatives, cytotoxicity of compounds **6a**, **6d**, and **6e** was found in a broad range of cancer cell lines having different histogenetic origins and drug sensitivity profiles. We confirmed[24,26] that methyl and Pom esters are less active than the corresponding free acids. The oleanane heterocycles **8** and **9** and 20(29)-lupene heterocycles **10a**−**11b** were inactive as well as compounds having pyrazine or benzopyrazine groups condensed to the five-membered ring (**12**−**18**).

Experimental Section

General Experimental Procedures. Melting points were determined using a Kofler block and are uncorrected. Optical rotations were measured using $CHCl_3$ solutions (unless otherwise stated) on an Autopol III (Rudolph Research, Flanders, NJ) polarimeter. NMR spectra were recorded on a Varian Unity INOVA 400 instrument (^{1}H NMR spectra at 399.95 MHz) using $CDCl_3$ solutions (unless otherwise stated), with $SiMe_4$ as an internal standard. EIMS were recorded on an INCOS 50 (Finigan MAT) spectrometer at 70 eV and an ion source temperature of 150 °C. The samples were introduced from a direct exposure probe

Scheme 2. Preparation of Pyrazines and Benzopyrazines at Six-Membered Rings



Key: (a) ethylenediamine, morpholine, S, reflux; (b) 1,2-phenylenediamine, morpholine, S, reflux; (c) PomCl, DBU, CH_2Cl_2, MeCN; (d) AcmBr, DBU, CH_2Cl_2, MeCN; (e) LAH, THF, refl.

at a heating rate of 10 mA/s. Column chromatography was performed using silica gel 60 (Merck 7734). The HPLC system consisted of a high-pressure pump (Gilson model 361), a Rheodyne injection valve, and a preparative column (25 × 250 mm) filled with silica gel (Biospher 7 μm), The differential refractometer detector (Laboratorni Pristroje, Praha, CZ) was connected with a PC (software Chromulan) and an automatic fraction collector (Gilson model 246). A mixture of EtOAc and hexane was used as the mobile phase, its composition specified in each experiment. TLC was carried out on Kieselgel 60 F_{254} plates (Merck) using toluene−Et_2O (5:1), detected by spraying with 10% aqueous H_2SO_4 and heating to 150−200 °C.

Workup refers to pouring the reaction mixture into H_2O, extraction of the product with organic solvent, washing the organic layer successively with H_2O, dilute aqueous HCl, H_2O, saturated aqueous $NaHCO_3$, and again H_2O, followed by drying over $MgSO_4$, filtration, and evaporation of the filtrate under reduced pressure. Analytical samples were dried over P_2O_5 under diminished pressure. AcmBr, PomCl, montmorillonite K10, DBU, morpholine, ethylenediamine, 1,2-phenylenediamine, THF, LAH, and selenium dioxide were purchased from Sigma-Aldrich Company.

Betulinic Acid (1a) and Betulonic Acid (1b). Those compounds were prepared according to literature procedures[24] and were identical with authentic samples.

Betulin (2). Bark of *Betula pendula* (500 g) was extracted with EtOH in a Soxhlet extractor, and the solvent was evaporated to give crude **2** as a light brown powder (50 g, 10% yield). Several crystallizations from EtOH gave a white crystalline powder, mp 256−258 °C; $[\alpha]^{25}_D$ +16 (*c* 0.37).[27]

Dihydrobetulinic Acid (3a) and Dihydrobetulonic Acid (3b). Compounds were prepared according to literature procedures.[28,29]

Allobetulin (4a). A solution of **2** (20 g, 45.2 mmol) in $CHCl_3$ (1 L) was vigorously stirred with montmorillonite K10 (50 g) under reflux for 2 days.[30,31] The solution was then filtered, $CHCl_3$ was evaporated, and the crude **4a** was crystallized from butanone to give colorless crystals (13.2 g, 66%): mp 264−266 °C; $[\alpha]^{25}_D$ +40 (*c* 0.35).[30,31]

Allobetulon 4b. Ac_2O (2 mL), AcONa (2 g), and $Na_2Cr_2O_7 \cdot 2H_2O$ (3 g, 10.1 mmol) were added to a solution of **4a** (10 g, 22.6 mmol) in a mixture of toluene (70 mL) and AcOH (30 mL). The mixture was worked up after 1 h. The crude product was chromatographed over silica gel (150 g) eluted with toluene, and crystallization from toluene−MeOH gave allobetulon **4b** (3.7 g, 37% yield) as a white powder: mp 228−230 °C; $[\alpha]^{25}_D$ +83 (*c* 0.35).[32]

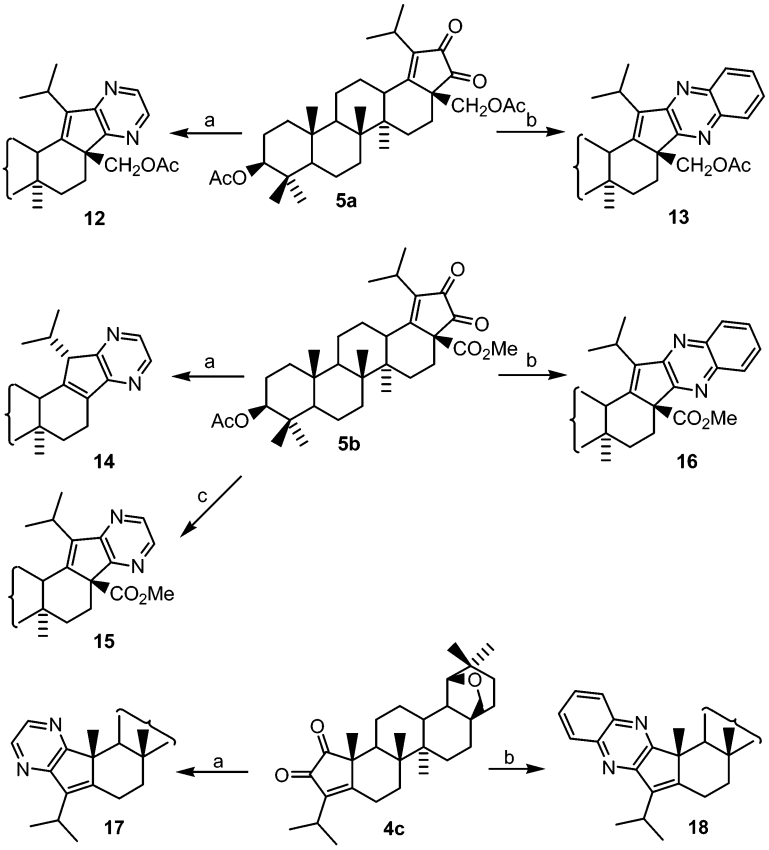
Diketone 4c. This compound was synthesized from **4a** according to literature procedures[25] and was identical with an authentic sample.

Diketones 5a and 5b. These compounds were obtained from **2** using four-step and seven-step reactions according to literature procedures[18] and were identical with authentic samples.

General Procedure for Preparation of Pyrazines at the A-Ring. Sulfur (1.5 g, 47 mmol) and ethylenediamine (1.5 mL, 25 mmol) were added to a solution of each ketone (**1b**, **1c**, **3b**, **3c**, **4b**, and **4c**; 5 mmol) in morpholine (20 mL). Each mixture was heated under reflux for 2−4 h and then worked up. Crude products were purified by chromatography on silica gel in a gradient from toluene to toluene−Et_2O (10:1) and then crystallized.

Pyrazine 6a. Pyrazine **6a** was prepared from **1b** (2.3 g, 5.1 mmol) by the general procedure. Crystallization from MeOH yielded **6a** (1 g, 41%): mp 240−243 °C; $[\alpha]^{25}_D$ +27 (*c* 0.30); IR ($CHCl_3$) ν_{max} 3521,

Scheme 3. Preparation of Pyrazines and Benzopyrazines at Five-Membered Rings



Key: (a) i: ethylenediamine, morpholine, S, reflux; ii: Ac_2O, pyridine (b) 1,2-phenylenediamine, xylene, S, reflux; (c) ethylenediamine, xylene, S, reflux.

1736, 1695, 1642 cm^{-1}; ^{1}H NMR δ 0.81, 1.02, 1.04, 1.27, 1.30 (15H, all s, 5 × CH_3), 1.72 (3H, s, H-30), 2.23−2.35 (2H), 2.46 (1H, d, *J*(H-1a, H-1b) = 16.6 Hz, H-1a), 3.04 (1H, td, *J*(H-19β, H-18α) = 10.5 Hz, *J*(H-19β, H-21α) = 10.5 Hz, *J*(H-19β, H-21β) = 5.2 Hz, H-19β), 3.05 (1H, d, *J*(H-1b, H-1a) = 16.6 Hz, H-1b), 4.65 (1H, m, H-29 *pro-E*), 4.77 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 8.29 (1H, d, *J* = 2.4 Hz), 8.42 (1H, dd, J_1 = 2.4 Hz, J_2 = 0.9, 2 × H-pyrazine); EIMS *m*/*z* 490 [M]$^+$ (39), 475 (100), 445 (19), 431 (16), 257 (30), 243 (28), 189 (33); *anal* C 78.25%, H 9.58%, N 5.58%; calcd for $C_{32}H_{46}N_2O_2$, C 78.31%, H 9.45%, N 5.71%.

Methyl Ester 6b. Methyl ester **6b** was prepared by the general procedure from crude **1c**, which was previously obtained from acid **1b** (4.5 g, 9.9 mmol). This yielded **6b** (4.2 g, 85%) as colorless crystals: mp 215−216 °C (MeOH); $[\alpha]^{25}_D$ +24 (*c* 0.38); IR ($CHCl_3$) ν_{max} 1642, 1720 cm^{-1}; ^{1}H NMR δ 0.81, 1.00, 1.02, 1.28, 1.30 (15H, all s, 5 × CH_3), 1.71 (3H, s, H-30), 2.23−2.33 (2H), 2.45 (1H, d, *J*(H-1a, H-1b) = 16.6 Hz, H-1a), 3.02 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19β, H-21β) = 4.3 Hz, H-19β), 3.04 (1H, d, *J*(H-1b, H-1a) = 16.5 Hz, H-1b), 3.68 (3H, s, O-CH_3), 4.64 (1H, m, H-29 *pro-E*), 4.77 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 8.27 (1H, d, *J* = 2.4 Hz), 8.41 (1H, dd, J_1 = 2.4 Hz, J_2 = 0.9 Hz, 2 × H-pyrazine); EIMS *m*/*z* 504 [M]$^+$ (67), 489 (100), 445 (23), 422 (10), 256 (47), 211 (9), 189 (21); *anal* C 78.52%, H 9.29%, N 5.61%; calcd for $C_{33}H_{48}N_2O_2$, C 78.53%, H 9.59%, N 5.55%.

Pom Ester 6c. PomCl (0.1 mL, 0.59 mmol) and DBU (0.1 mL, 0.60 mmol) were added to a solution of **6a** (150 mg, 0.31 mmol) in CH_2Cl_2 (3 mL) and MeCN (1 mL). The reaction was stirred at rt for 14 h and then worked up. The crude product was purified by HPLC in 15% EtOAc in hexane. Crystallization from MeOH gave colorless needles of **6c** (130 mg, 70%): mp 88−91 °C (MeOH); $[\alpha]^{25}_D$ +22 (*c* 0.32); IR ($CHCl_3$) ν_{max} 1748b, 1643 cm^{-1}; ^{1}H NMR δ 0.81, 1.01, 1.02, (9H, all s, 3 × CH_3), 1.23 (9H, s, 3 × CH_3-Pom), 1.28, 1.30 (6H, all s, 2 × CH_3), 1.71 (3H, m, H-30), 2.45 (1H, d, *J*(H-1a, H-1b) = 16.6 Hz, H-1a), 2.99 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.7 Hz, H-19β), 3.04 (1H, d, *J*(H-1b, H-1a) = 16.6 Hz, H-1b), 4.64 (1H, m, H-29 *pro-E*), 4.76 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.77 (1H, d, *J* = 5.5 Hz), 5.81 (1H, d, *J* = 5.5 Hz, CH_2-Pom), 8.27 (1H, d, *J* = 2.4 Hz), 8.41 (1H, dd, J_1 = 2.4 Hz, J_2 = 0.9 Hz, 2 × H-pyrazine); EIMS *m*/*z* 604 [M]$^+$ (88), 589 (76), 574 (14), 489 (33), 475 (22), 445 (100), 429 (23), 401 (43); *anal* C 75.39%, H 9.26%, N 4.51%; calcd for $C_{38}H_{56}N_2O_4$, C 75.46%, H 9.33%, N 4.63%.

Acm Ester 6d. AcmBr (0.1 mL, 0.78 mmol) and DBU (0.1 mL, 0.60 mmol) were added to a solution of **6a** (150 mg, 0.31 mmol) in CH_2Cl_2 (3 mL) and MeCN (1 mL). The reaction was stirred at rt for 14 h and then worked up. The crude product was purified by HPLC in 25% EtOAc in hexane and crystallized from MeOH−pentane to give needles of **6d** (100 mg, 58%): mp 63−66 °C (MeOH−pentane); $[\alpha]^{25}_D$ +21 (*c* 0.24); IR ($CHCl_3$) ν_{max} 1748b, 1643 cm^{-1}; ^{1}H NMR δ 0.81, 1.01, 1.02, 1.28, 1.30, (15H, all s, 5 × CH_3), 1.71 (3H, s, H-30) 2.11 (3H, s, CH_3-Acm), 2.22−2.33 (2H), 2.45 (1H, d, *J*(H-1a, H-1b) = 16.5 Hz, H-1a), 3.02 (1H, td, *J*(H-19β, H-18α) = 11.1 Hz, *J*(H-19β, H-21α) = 11.1 Hz, *J*(H-19β, H-21β) = 4.1 Hz, H-19β), 3.04 (1H, d, *J*(H-1b, H-1a) = 16.6 Hz, H-1b), 4.64 (1H, m, H-29 *pro-E*), 4.77 (1H, bd, *J* = 2.3 Hz, H-29 *pro-Z*), 5.73 (1H, d, *J* = 5.5 Hz), 5.82 (1H, d, *J* = 5.5 Hz, CH_2-Acm), 8.27 (1H, d, *J* = 2.4 Hz), 8.41 (1H, dd, J_1 = 2.5 Hz, J_2 = 0.8 Hz, 2 × H-pyrazine); EIMS *m*/*z* 562 [M]$^+$ (74), 547 (56), 502 (100), 489 (85), 475 (31), 445 (21), 429 (17), 401 (20); *anal* C 74.65%, H 9.07%, N 4.88%; calcd for $C_{35}H_{50}N_2O_4$, C 74.70%, H 8.95%, N 4.98%.

Pyrazine 6e. This compound was prepared from acid **3b** (0.5 g, 1.1 mmol) by the general procedure. Crystallization from $CHCl_3$−MeOH yielded **6e** (0.3 g, 55%) as white crystals: mp 267−269 °C; $[\alpha]^{25}_D$ −8 (*c* 0.25); IR ($CHCl_3$) ν_{max} 3518, 1734, 1693 cm^{-1}; ^{1}H NMR δ 0.78 (3H, d, *J* = 6.8 Hz), 0.82, 0.88 (3H, d, *J* = 7.2 Hz), 1.02 (6H, m), 1.27 (3H, s), 1.31 (3H, s, 7 × CH_3), 2.29 (3H, m), 2.49 (1H, d, *J*(H-1a, H-1b) = 16.8 Hz, H-1a), 3.10 (1H, d, *J*(H-1b, H-1a) = 16.6 Hz), 8.30 (1H, d, *J* = 2.4 Hz), 8.45 (1H, bd, *J* = 2.0 Hz, 2 × H-pyrazine); FABMS *m*/*z* 493 [M + H]$^+$, 477, 428, 303; *anal* C 78.00%, H 9.82%, N 5.69%; calcd for $C_{32}H_{48}N_2O_2$, C 77.70%, H 9.97%, N 5.75%

Table 2. Cytotoxic Activity of Selected Compounds on Additional Cell Lines

	IC_{50} (μmol/L[a])								
compound	A 549	HT 29	K 562	K 562-tax	PC-3	SK-MEL2	U87-Mg	B2-4	C-D-B
1b	15	17	6	17	15	26	159		
5a	103	250	250	250	208	170			
5b	13	7	6	9	14	14	74		5
6a	0.25	16	0.77	8	11	98			
6c	51	250	100	149	250	250			
6d	1	77	2	35	35	69			
6e	8	2	0.4	4	17		19	0.3	13
6f	220	164	201	91	227		231	94	235
7a	48	96	86	118	64	192			
7b	250	250	245	250	250		250	47	250
7c	122	123	113	117	103		148	99	198
13	101	229	70	234	233	231			

[a] The lowest concentration that kills 50% of tumor cells.

Methyl Ester 6f. Ester **6f** was prepared by the general procedure from **3c**, which was previously obtained by the reaction of acid **3b** (0.5 g, 1.1 mmol) with diazomethane. Crystallization from MeOH yielded **6h** (0.4 g, 74%) as colorless crystals: mp 121−123 °C (MeOH); $[\alpha]^{25}_D$ −7 (*c* 0.29) (lit.[23] mp 220 °C). Although the mp is different from the literature value, the ^{1}H NMR and MS are identical.

General Procedure for Preparation of Benzopyrazines at the A-Ring. Sulfur (6.0 g, 141 mmol) and 1,2-phenylenediamine (2.0 g, 18.5 mmol) were added to a solution of each ketone **1b**, **1c**, **3b**, **3c**, and **4b** (4.5 mmol) in morpholine (20 mL). The mixture was stirred under reflux for 2−4 h and then worked up. Crude products were chromatographed on silica gel (150 g) in a gradient from toluene to toluene−Et_2O (10:1) and then crystallized.

Benzopyrazine 7a. This compound was prepared from **1b** (2.0 g, 4.5 mmol) by the general procedure. Crystallization from $CHCl_3$−MeOH yielded **7a** (1.3 g, 55%) as yellow crystals: mp 305−307 °C; $[\alpha]^{25}_D$ +49 (*c* 0.42); IR ($CHCl_3$) ν_{max} 3513, 1736, 1694, 1641 cm^{-1}; ^{1}H NMR δ 0.85, 1.05, 1.06, 1.39, 1.42 (15H, all s, 5 × CH_3), 1.73 (3H, s, H-30), 1.97−2.09 (2H), 2.23−2.37 (2H), 2.60 (1H, d, *J*(H-1a, H-1b) = 17.6 Hz, H-1a), 3.04 (1H, td, *J*(H-19β, H-18α) = 11.7 Hz, *J*(H-19β, H-21α) = 11.7 Hz, *J*(H-19β, H-21β) = 4.7 Hz, H-19β), 3.34 (1H, d, *J*(H-1b, H-1a) = 16.2 Hz, H-1b), 4.66 (1H, m, H-29 *pro-E*), 4.78 (1H, bd, *J* = 2.0 Hz, H-29 *pro-Z*), 7.65 (2H, m), 7.99 (2H, m, 4 × H-benzopyrazine); EIMS *m*/*z* 540 [M]$^+$ (41), 525 (100), 496 (19), 481 (27), 427 (16), 413 (6), 368 (8), 307 (24), 293 (20), 237 (24), 223 (33),209 (31), 183 (16); *anal* C 80.05%, H 8.91%, N 5.18%; calcd for $C_{36}H_{48}N_2O_2$, C 79.96%, H 8.95%, N 5.18%.

Methyl Ester 7b. Methyl ester **7b** was prepared by the general procedure from methyl ester **1c**, which was previously obtained by the reaction of acid **1b** (2.0 g, 4.3 mmol) with diazomethane. Crystallization from MeOH yielded **7b** (1.3 g, 55%) as needles: mp 161−163 °C; $[\alpha]^{25}_D$ + 37 (*c* 0.34); IR ($CHCl_3$) ν_{max} 1720, 1644 cm^{-1}; ^{1}H NMR δ 0.84, 1.02, 1.04, 1.42, 1.43 (15H, all s, 5 × CH_3), 1.72 (3H, s, H-30), 1.84−1.98 (2H), 2.20−2.36 (2H), 2.60 (1H, d, *J*(H-1a, H-1b) = 16.5 Hz, H-1a), 3.02 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19β, H-21β) = 4.4 Hz, H-19β), 3.34 (1H, bd, *J*(H-1b, H-1a) = 16.0 Hz, H-1b), 3.68 (3H, s, OCH_3), 4.65 (1H, m, H-29 *pro-E*), 4.78 (1H, bd, *J* = 2.0 Hz, H-29 *pro-Z*), 7.66 (2H, m), 8.01 (2H, m, 4 × H-benzopyrazine); FABMS *m*/*z* 555 [M + H]$^+$, 539, 513; *anal* C 79.87%, H 9.21%, N 5.32%; calcd for $C_{37}H_{50}N_2O_2$, C 80.10%, H 9.08%, N 5.05%.

Benzopyrazine 7c. This compound was prepared from **3b** (1.0 g, 2.2 mmol) by the general procedure. Crystallization from MeOH yielded **7c** (0.7 g, 59%) as colorless crystals: mp 204−207 °C; $[\alpha]^{25}_D$ +11 (*c* 0.38); IR ($CHCl_3$) ν_{max} 3515, 1732, 1694 cm^{-1}; ^{1}H NMR δ 0.79 (3H, d, *J* = 6.4 Hz), 0.86 (3H, s), 0.88 (3H, d, *J* = 6.4 Hz), 1.03 (3H, s), 1.04 (3H, s), 1.41 (3H, s), 1.44 (3H, s, 7 × CH_3), 2.28 (3H), 2.65 (1H, d, *J*(H-1a, H-1b) = 16.4 Hz, H-1a), 3.46 (1H, bd, *J*(H-1b, H-1a) = 14.4 Hz, H-1b), 7.70 (2H, m), 8.06 (2H, m, 4 × H-benzopyrazine); FABMS *m*/*z* 543 [M + H]$^+$, 527, 497, 457; *anal* C 79.53%, H 9.34%, N 5.21%; calcd for $C_{36}H_{50}N_2O_2$, C 79.66%, H 9.28%, N 5.16%.

Methyl Ester 7d. This compound was prepared by the general procedure from crude **3c**, which was obtained by reaction of acid **3b** (1.1 g, 2.3 mmol) with diazomethane. This yielded **7d** (0.5 g, 38%) as colorless crystals: mp 153−155 °C (lyophilized from t-BuOH); $[\alpha]^{25}_D$ +11 (*c* 0.27); IR ($CHCl_3$) ν_{max} 1718 cm^{-1}; ^{1}H NMR δ (50 °C) 0.79 (3H, d, *J* = 6.8 Hz), 0.86 (3H, s), 0.89 (3H, d, *J* = 7.2 Hz), 1.02 (6H, s), 1.44 (3H, s), 1.45 (3H, s), 1.44 (3H, s, 7 × CH_3), 2.28 (3H), 2.66 (1H, d, *J*(H-1a, H-1b) = 16.8 Hz, H-1a), 3.51 (1H, bd, *J*(H-1b, H-1a) = 16.8 Hz, H-1b), 3.66 (3H, s, OCH_3), 7.70 (2H, m), 8.06 (1H, m), 8.13 (1H, m, 4 × H-benzopyrazine); FABMS *m*/*z* 557 [M + H]$^+$, 477, 471; *anal* C 79.64%, H 9.64%, N 4.97%; calcd for $C_{37}H_{52}N_2O_2$, C 79.81%, H 9.41%, N 5.03%.

Pyrazine 8. This compound was prepared from **4b** (5.0 g, 11.3 mmol) by the general procedure. Crystallization from $CHCl_3$−MeOH yielded **8** (4.1 g, 76%) as pale yellow crystals: mp 270−273 °C ; $[\alpha]^{25}_D$ +53 (*c* 0.25); IR ($CHCl_3$) ν_{max} 1452, 1403 cm^{-1}.[22]

Benzopyrazine 9. This compound was prepared from allobetulon **4b** (5 g, 11.3 mmol) by the general procedure. The crystallization from $CHCl_3$−MeOH yielded pyrazine **9** (4.4 g, 74%) as yellow crystals: mp 272 − 275 °C; $[\alpha]^{25}_D$ +62 (*c* 0.35); IR ($CHCl_3$) ν_{max} 1487, 1456 cm^{-1}.[21]

General Procedure for Reduction Methyl Esters. LAH (1.5 g, 39.5 mmol) was added to a solution of each ester (**6b**, **6f**, **7b**, **7d**; 1.0 mmol) in THF (20 mL), and the mixture was stirred for 30 min under reflux. Excess LAH was decomposed by addition of EtOAc (5 mL) and EtOH (5 mL), and the mixture was worked up. Crude hydroxyl derivatives were chromatographed and crystallized.

Pyrazine 10a. Pyrazine **10a** was prepared from ester **6b** (0.5 g, 1.0 mmol) by the general procedure. HPLC in a 20% solution of EtOAc in hexane followed by crystallization from MeOH yielded **10a** (210 mg, 44%) as colorless crystals: mp 247−249 °C (MeOH); $[\alpha]^{25}_D$ +29 (*c* 0.28); IR ($CHCl_3$) ν_{max} 3625, 1640 cm^{-1}; ^{1}H NMR δ 0.82, 1.04, 1.11, 1.30, 1.32 (15H, all s, 5 × CH_3), 1.71 (3H, s, H-30), 1.80−2.06 (3H), 2.36−2.46 (1H, H-19β), 2.48 (1H, d, *J*(H-1a, H-1b) = 16.6 Hz, H-1a), 3.11 (1H, d, *J*(H-1b, H-1a) = 16.6 Hz, H-1b), 3.36 (1H, d, *J*(H-28a, H-28b) = 10.8 Hz, H-28a), 3.82 (1H, d, *J*(H-28b, H-28a) = 10.8 Hz, H-28b), 4.62 (1H, m, H-29 *pro-E*), 4.78 (1H, bd, *J* = 2.0 Hz, H-29 *pro-Z*), 8.29 (1H, bd, *J* = 2.4 Hz), 8.48 (1H, bs, 2 × H-pyrazine); FABMS *m*/*z* 477 [M + H]$^+$, 461, 445; *anal* C 80.47%, H 9.95%, N 5.67%; calcd for $C_{32}H_{48}N_2O$, C 80.62%, H 10.15%, N 5.88%.

Pyrazine 10b. This compound was prepared from ester **6f** (0.5 g, 1.0 mmol) by the general procedure. Chromatography on silica gel (50 g) in toluene−Et_2O (10:1) and lyophilization from t-BuOH yielded **10b** (340 mg, 72%): mp 231−233 °C, $[\alpha]^{25}_D$ +7 (*c* 0.50); IR (KBr) ν_{max} 3626, 1672 cm^{-1}; ^{1}H NMR δ 0.79 (d, *J*(H-29, H-20) = 6.8 Hz), 0.83 (s), 0.87 (d, *J*(H-30, H-20) = 6.8 Hz), 1.02 (s), 1.11 (s), 1.29 (s), 1.31 (s) (21H, 7 × CH_3), 2.47 (1H, d, *J* (H-1a, H-1b) = 16.6 Hz, H-1a), 3.04 (1H, d, *J*(H-1b, H-1a) = 16.5 Hz, H-1b), 3.33 (1H, d, *J*(H-28a, H-28b) = 11.0 Hz, H-28a), 3.80 (1H, d, *J*(H-28b, H-28a) = 10.8 Hz, H-28b), 8.28 (1H, d, *J* = 2.8 Hz, H-pyrazine), 8.42 (1H, m, H-pyrazine); EIMS *m*/*z* 478 [M]$^+$ (24), 463 (23), 447 (100), 433 (41), 421 (19), 417 (10), 413 (82), 405 (74), 393 (51); *anal* C 80.07%, H 10.32%, N 5.74%; calcd for $C_{32}H_{50}N_2O$, C 80.28%, H 10.53%, N 5.85%.

Benzopyrazine 11a. This compound was prepared from ester **7b** (0.5 g, 0.9 mmol) by the general procedure. Chromatography on silica gel (50 g) in toluene−Et_2O (10:1) and lyophyllization from t-BuOH yielded **11a** (320 mg, 67%): mp 138−142 °C; $[\alpha]^{25}_D$ +42 (*c* 0.25); IR ($CHCl_3$) ν_{max} 3624, 1637 cm^{-1}; ^{1}H NMR δ 0.85, 1.05, 1.13, 1.43, 1.44 (15H, all s, 5 × CH_3), 1.72 (3H, s, H-30), 2.48 (1H, td, *J*(H-19β, H-18α) = 11.0 Hz, *J*(H-19β, H-21α) = 11.0 Hz, *J*(H-19β, H-21β) = 4.4 Hz, H-19β), 2.61 (1H, d, *J*(H-1a, H-1b) = 16.6 Hz, H-1a), 3.39 (1H, vbs, H-1b), 3.88 (1H, d, *J*(H-28a, H-28b) = 11.2 Hz, H-28a), 3.82 (1H, d, *J*(H-28b, H-28a) = 11.2 Hz, H-28b), 4.64 (1H, m, H-29 *pro-E*), 4.73 (1H, bd, *J* = 2.0 Hz, H-29 *pro-Z*), 7.68 (2H, m), 8.04 (2H, m, 4 ×

H-benzopyrazine); FABMS *m/z* 527 $[M + H]^+$, 511; *anal* C 82.46%, H 9.21%, N 5.59%; calcd for $C_{36}H_{50}N_2O$, C 82.08%, H 9.57%, N 5.32%.

Benzopyrazine 11b. This compound was prepared from ester **7d** (0.5 g, 0.9 mmol) by the general procedure. Chromatography on silica gel (50 g) in toluene−Et_2O (10:1) and crystallization yielded **11b** (250 mg, 53%): mp 170−174 °C; $[\alpha]^{25}_D$ +4 (*c* 0.23); IR ($CHCl_3$) ν_{max} 3626 cm^{-1}; ^1H NMR δ 0.80 (d, *J*(H-29, H-20) = 6.4 Hz), 0.86 (s), 0.88 (d, *J*(H30, H-20) = 6.4 Hz), 1.04 (s), 1.13 (s), 1.43 (s), 1.44 (s) (21H, 7 × CH_3), 2.61 (1H, d, *J* (H-1a, H-1b) = 16.3 Hz, H-1a), 3.35 (1H, d, *J*(H-1b, H-1a) = 16.3 Hz, H-1b), 3.32 (1H, d, *J*(H-28a, H-28b) = 10.8 Hz, H-28a), 3.81 (1H, d, *J*(H-28b, H-28a) = 10.8 Hz, H-28b), 7.66 (2H, m), 7.94−8.06 (2H, m, 4 × H-benzopyrazine); EIMS *m/z* 528 $[M]^+$ (42), 513 (37), 497 (100), 483 (48). C 82.03%, H 9.58%, N 5.61%; calcd for $C_{36}H_{52}N_2O$, C 81.77%, H 9.91%, N 5.30%.

Pyrazine 12. This compound was prepared from diketone **5a** (1.5 g, 2.7 mmol) by the general procedure. Before the purification, the crude product was reacetylated with Ac_2O in pyridine. This yielded pyrazine **12** (0.8 g, 47%): mp 242−246 °C (MeOH); $[\alpha]^{25}_D$ −94 (*c* 0.53); IR ($CHCl_3$) ν_{max} 1743, 1728, 1596, 1538 cm^{-1}; ^1H NMR δ 0.85, 0.86, 0.96, 1.23 (15H, all s, 5 × CH_3), 1.41 (3H, d, *J*(H-20, H-29) = 6.8 Hz, H-29), 1.44 (3H, d, *J*(H-20, H-30) = 6.8 Hz, H-30), 1.78 (3H, s, Ac), 2.06 (3H, s, Ac), 2.20 (1H), 2.98 (1H, dd, J_1 = 12.7 Hz, J_2 = 3.4 Hz, H-13), 3.56 (1H, septet, all *J* = 7.0 Hz, H-20), 4.37 (1H, d, *J* = 10.8 Hz, H-28a), 4.50 (1H, m, H-3), 4.79 (1H, d, *J* = 10.8 Hz, H-28b), 8.12 (1H, d, *J* = 2.9 Hz), 8.32 (1H, d, *J* = 2.9 Hz, 2 × H-pyrazine); EIMS *m/z* 576 $[M]^+$ (100), 561 (11), 516 (10), 503 (8), 473 (6), 443 (8), 312 (19), 299 (7), 253 (12), 183 (11); *anal* C 74.85%, H 9.07%, N 4.91%; calcd for $C_{36}H_{52}N_2O_4$, C 74.96%, H 9.09%, N 4.86%.

Benzopyrazine 13. 1,2-Phenylenediamine (2.0 g, 18.5 mmol) and sulfur (6.0 g, 141 mmol) were added to a solution of diketone **5a** (2.0 g, 3.6 mmol) in xylene (20 mL). The mixture was refluxed for 4 h and then worked up. The crude product was chromatographed over silica gel (50 g) eluted with toluene−Et_2O (15:1). Crystallization from EtOAc yielded benzopyrazine **13** (1.6 g, 71%) as colorless needles: mp 280−282 °C; $[\alpha]^{25}_D$ −59 (*c* 0.39); IR ($CHCl_3$) ν_{max} 1727, 1585, 1569 cm^{-1}; ^1H NMR δ 0.86, 0.89, 0.96, 1.24 (15H, all s, 5 × CH_3), 1.52 (3H, d, *J*(H-20, H-29) = 6.4 Hz, H-29), 1.54 (3H, d, *J*(H-20, H-30) = 6.4 Hz, H-30), 1.74 (3H, s, Ac), 2.06 (3H, s, Ac), 2.28 (1H), 3.07 (1H, dd, J_1 = 12.6 Hz, J_2 = 3.2 Hz, H-13), 3.62 (1H, septet, all *J* = 7.0 Hz, H-20), 4.42 (1H, d, *J* = 10.9 Hz, H-28a), 4.51 (1H, m, H-3), 5.00 (1H, d, *J* = 10.7 Hz, H-28b), 7.64 (2H, m), 8.03 (2H, m, 4 × H-benzopyrazine); EIMS *m/z* 626 $[M]^+$ (100), 611 (16), 583, (4), 567 (21), 553 (9), 523 (5), 493, (4), 363 (19), 349 (5); *anal* C 76.55%, H 8.53%, N 4.52%; calcd for $C_{40}H_{54}N_2O_4$, C 76.64%, H 8.68%, N 4.47%.

Pyrazine 14. This compound was prepared from diketone **5b** (1.5 g, 2.8 mmol) by the general procedure. Before purification, the crude product was reacetylated with Ac_2O in pyridine. Crystallization from MeOH yielded **14** (0.8 g, 57%): mp 224−226 °C; $[\alpha]^{25}_D$ +73 (*c* 0.42); IR ($CHCl_3$) ν_{max} 1724, 1620 cm^{-1}; ^1H NMR δ 0.34 (3H, d, *J*(H-20, H-29) = 7.2 Hz, H-29), 0.87, 0.93, 0.94, 1.08 (15H, all s, 5 × CH_3), 1.42 (3H, d, *J*(H-20, H-30) = 6.8 Hz, H-30), 2.06 (3H, s, Ac), 2.30−2.50 (2H), 2.56−2.73 (2H), 3.35 (1H, m, H-20), 4.49 (1H, m, H-3), 8.14 (1H, d, *J* = 3.0 Hz), 8.22 (1H, d, *J* = 3.1 Hz, 2 × H-pyrazine); EIMS *m/z* 504 $[M]^+$ (80), 489 (3), 461 (12), 444 (9), 429 (7), 419 (3), 401, (10), 309 (4), 174 (100); *anal* C 78.39%, H 9.51%, N 5.49%; calcd for $C_{33}H_{48}N_2O_2$, C 78.53%, H 9.59%, N 5.55%.

Pyrazine 15. Ethylenediamine (0.7 mL, 11.7 mmol) and sulfur (2.0 g, 47 mmol) were added to a solution of diketone **5b** (1.0 g, 1.9 mmol) in xylene (10 mL). The mixture was refluxed for 4 h and then worked up. The crude product was chromatographed over silica gel (50 g) eluted with toluene. Crystallization from EtOAc yielded **15** (0.7 g, 67%) as colorless crystals: mp 244−246 °C; $[\alpha]^{25}_D$ +136 (*c* 0.30); IR ($CHCl_3$) ν_{max} 1728, 1596, 1531, 1452 cm^{-1}; ^1H NMR δ 0.86, 0.88, 0.93, 1.09 (15H, all s, 5 × CH_3), 1.42 (3H, d, *J*(H-20, H-30) = 7.2 Hz, H-30), 1.48 (3H, d, *J*(H-20, H-29) = 6.8 Hz, H-29), 2.06 (3H, s, Ac), 2.12−2.23 (1H), 2.77 (1H, dd, J_1 = 12.8 Hz, J_2 = 3.1 Hz), 2.90 (1H, bd, *J* = 13.0 Hz), 3.56 (1H, septet, *J* = 7.0 Hz, H-20), 3.65 (3H, s, O-CH_3), 4.50 (1H, m, H-3), 8.12 (1H, d, *J* = 2.6 Hz), 8.37 (1H, d, *J* = 2.8 Hz, 2 × H-pyrazine); EIMS *m/z* 562 $[M]^+$ (100), 547 (7), 503 (8), 487 (3), 297 (11), 239 (18), 218 (11); *anal* C 74.79%, H 8.86%, N 4.95%; calcd for $C_{35}H_{50}N_2O_4$, C 74.70%, H 8.95%, N 4.98%.

Benzopyrazine 16. 1,2-Phenylenediamine (1.0 g, 9.3 mmol) and sulfur (3.0 g, 71 mmol) were added to a solution of **5b** (1.0 g, 1.9 mmol) in xylene (10 mL). The mixture was refluxed for 4 h and then worked up. Crystallization from $CHCl_3$−MeOH yielded **16** (0.6 g, 53%) as yellow crystals: mp 215−217 °C; $[\alpha]^{25}_D$ −115 (*c* 0.29); IR ($CHCl_3$) ν_{max} 1728, 1586, 1567 cm^{-1}; ^1H NMR δ 0.86, 0.92, 0.94, 1.11 (15H, all s, 5 × CH_3), 1.53 (3H, d, *J*(H-20, H-29) = 6.8 Hz, H-29), 1.58 (3H, d, *J*(H-20, H-30) = 6.8 Hz, H-30), 2.06 (3H, s, Ac), 2.16−2.24 (1H), 2.79 (1H, dd, J_1 = 12.8 Hz, J_2 = 3.2 Hz, H-13), 2.96 (1H, dm, *J* = 12.1 Hz), 3.62 (1H, septet, *J* = 6.7 Hz, H-20), 3.63 (3H, s, O-CH_3), 4.50 (1H, m, H-3), 7.58−7.69 (2H), 8.05 (2H, dm, *J* = 7.9 Hz, 4 × H-benzopyrazine); EIMS *m/z* 612 $[M]^+$ (100), 597 (8), 553 (12), 349 (19), 289 (17),259 (8); *anal* C 76.52%, H 8.43%, N 4.47%; calcd for $C_{39}H_{52}N_2O_4$, C 76.43%, H 8.55%, N 4.57%.

Pyrazine 17. This compound was prepared from diketone **4c** (1.0 g, 2.2 mmol) by the general procedure for a preparation of pyrazines at the A-ring. This yielded pyrazine **17** (0.61 g, 58% yield): mp 202−203 °C (MeOH); $[\alpha]^{25}_D$ −12 (*c* 0.37); IR ($CHCl_3$) ν_{max} 1619, 1535, 1032, 908 cm^{-1}; ^1H NMR δ 0.72, 0.76, 0.94, 1.23, 1.29 (15H, all s, 5 × CH_3), 1.38 (3H, d, *J*(H-23, H-4) = 6.8 Hz, H-23), 1.41 (3H, d, *J*(H-24, H-4) = 6.8 Hz, H-24), 2.40 (1H, td, J_1 = 13.3 Hz, J_2 = 4.1 Hz, H-6a), 2.51 (1H, m, H-11α), 2.81 (1H, dt, J_1 = 13.3 Hz, J_2 = 3.7 Hz, H-6b), 3.19 (1H, septet, all *J* = 6.9 Hz, H-4), 3.47 (1H, d, J(H-28 *pro-R*, H-28 *pro-S*) = 7.6 Hz, H-28 *pro-R*), 3.55 (1H, s, H-19α), 3.80 (1H, bd, *J*(H-28 *pro-S*, H-28 *pro-R*) = 7.6 Hz, H-28 *pro-S*), 8.07 (1H, d, J_1 = 2.9 Hz) , 8.27 (1H, d, J_1 = 2.9 Hz, 2 × H-pyrazine); EIMS *m/z* 474 $[M]^+$ (85), 459 (11), 432 (10), 401 (5), 28 (8), 255 (11), 211 (15), 188 (100); *anal* C 81.03%, H 9.72%, N 5.93%; calcd for $C_{36}H_{52}N_2O_4$, C 80.96%, H 9.77%, N 5.90%.

Benzopyrazine 18. 1,2-Phenylenediamine (0.3 g, 2.88 mmol) and sulfur (0.8 g, 25 mmol) were added to a solution of **4c** (0.3 g, 0.66 mmol) in xylene (6 mL). The mixture was refluxed for 4 h and then worked up. Crystallization from $CHCl_3$−MeOH yielded **18** (0.22 g, 63%) as white crystals: mp 197−199 °C; $[\alpha]^{25}_D$ −63 (*c* 0.425); IR ($CHCl_3$) ν_{max} 1619, 1568, 1032 cm^{-1}; ^1H NMR δ 0.72, 0.75, 0.94, 1.33, 1.34 (15H, all s, 5 × CH_3), 1.45 (3H, d, *J*(H-23, H-4) = 6.8 Hz, H-23), 1.49 (3H, d, *J*(H-24, H-4) = 6.8 Hz, H-24), 2.49 (1H, td, J_1 = 13.3 Hz, J_2 = 4.1 Hz, H-6a), 2.78 (1H, dq, J_1 = 12.4 Hz, J_2 = 5.8 Hz, H-11α), 2.89 (1H, dt, J_1 = 13.3 Hz, J_2 = 3.4 Hz, H-6b), 3.30 (1H, septet, all *J* = 6.9 Hz, H-4), 3.47 (1H, d, *J*(H-28 *pro-R*, H-28 *pro-S*) = 7.6 Hz, H-28 *pro-R*), 3.57 (1H, s, H-19α), 3.81 (1H, d, J(H-28 *pro-S*, H-28 *pro-R*) = 7.6 Hz, H-28 *pro-S*), 7.56−7.65 (2H), 8.00 (2H, d, *J* = 8.6 Hz, 4 × H-benzopyrazine); EIMS *m/z* 524 $[M]^+$ (80), 509 (66), 481 (73), 373 (7), 319 (8), 261 (24), 236 (96), 221 (100), 196 (91); *anal* C 76.52%, H 8.43%, N 4.47%; calcd for $C_{39}H_{52}N_2O_4$, C 82.44%, H 9.18%, N 5.36%; calcd for $C_{39}H_{52}N_2O_4$, C 82.39%, H 9.22%, N 5.34%.

Cell Lines. CEM, A 549, HT 29, K 562, PC-3, SK-Mel2, and U87-Mg cell lines were purchased from the American Tissue Culture Collection (ATTC). Paclitaxel/daunorubicin-resistant sublines of K 562/CEM cells were prepared and characterized in our laboratories. The human T-lymphoblastic leukemia cell line, CEM, was used for routine screening of compounds. To prove a common mechanism of action, selected compounds, which showed activity in the screening assay, were further tested in a panel of cell lines. These lines were of various histogenetic origin and drug resistance profiles. The cells were maintained in Nunc/Corning 80 cm^2 plastic tissue culture flasks and cultured in cell culture medium (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum, and $NaHCO_3$).

Cytotoxicity Assay. Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density (2500−30 000 cells/well based on cell growth characteristics). Cells were added by pipet (80 μL) into 96-well microtiter plates. Inoculates were allowed a preincubation period of 24 h at 37 °C and 5% CO_2 for stabilization. Four-fold dilutions, in 20 μL aliquots, of the intended test concentration were added at time zero to the microtiter plate wells. All compounds were dissolved in 10% DMSO and tested in quadruplicate. Incubation of the cells with the test compounds lasted for 72 h at 37 °C, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed using MTT. Aliquots (10 μL) of the MTT stock solution were pipetted into each well and incubated for a further 1−4 h. After this incubation period the formazan produced was dissolved by addition of 100 μL/well of 10% aqueous SDS (pH = 5.5), followed by a further incubation at 37 °C overnight. The optical density (OD) was measured at 540 nm with a Labsystem iEMS Reader MF. Tumor cell survival (TCS) was calculated using

the following equation: TCS = ($OD_{drug-exposed\ well}$/mean $OD_{control\ wells}$) × 100%. The TCS_{50} value, the drug concentration lethal to 50% of the tumor cells, was calculated from appropriate dose−response curves.

Acknowledgment. This study was supported in part by the Ministry of Education of the Czech Republic MSM 6198959216 and MSM 0021620857, which paid for instrumental equipment, and the Czech Science Foundation 203/03/D152, from which the chemicals and all other material support were paid. Biological testing was supported by the MPO FT-TA/027. All deuterosolvents for NMR spectra measurements were paid from Czech Science Foundation 203/05/P025 and from the Ministry of Education of the Czech Republic, project LC06070. We are grateful to Prof. J. Klinot for the interpretations of NMR spectra and to Dr. S. Hilgard for measurement of IR spectra. Special thanks to Mgr. B. Sperlichova for measurement of optical rotations.

References and Notes

(1) Dzubak, P.; Hajduch, M.; Vydra, D.; Hustova, A.; Kvasnica, M.; Biedermann, D.; Markova, L.; Urban, M.; Sarek, J. *Nat. Prod. Rep.* **2006**, *23*, 394−411.
(2) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006−2007.
(3) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828−6839.
(4) Kramer, A.; Ullmann, U.; Winterfeldt, E. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2865−2867.
(5) Morzycki, J. W.; Gryszkiewicz, A.; Lotowski, Z.; Szczepek, W. J. *Collect. Czech. Chem. Commun.* **1998**, *63*, 1589−1596.
(6) Fujioka, T.; Kashiwada, Y.; Kilkuskie, R. E.; Cosentino, L. M.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Chen, I. S.; Lee, K. H. *J. Nat. Prod.* **1994**, *57*, 243−247.
(7) Evers, M.; Poujade, C.; Soler, F.; Ribeill, Y.; James, C.; Lelicevre, Y.; Gueguen, J. C.; Reisdorf, D.; Morize, I.; Pauwels, R.; De Clercq, E.; Henin, Y.; Bousseau, A.; Mayaux, J. F.; Le Pecq, J. B.; Dereu, N. *J. Med. Chem.* **1996**, *39*, 1056−1068.
(8) Setzer, W. N.; Setzer, M. C.; Bates, R. B.; Jackes, B. R. *Planta Med.* **2000**, *66*, 176−177.
(9) Costantini, P.; Jacotot, E.; Decaudin, D.; Kroemer, G. *J. Natl. Cancer Inst.* **2000**, *92*, 1042−1053.
(10) Pisha, E.; Chai, H.; Lee, I. S.; Chagwedera, T. E.; Farnsworth, N. R.; Cordell, G. A.; Beecher, C. W. W.; Fong, H. H. S.; Kinghorn, A. D.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Hieken, T. J.; Gupta, T. K. D.; Pezzuto, J. M. *Nat. Med.* **1995**, *1*, 1046−1051.
(11) Jeong, H. J.; Chai, H. B.; Park, S. Y.; Kim, D. S. H. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1201−1204.
(12) Fulda, S.; Jeremias, I.; Pietsch, T.; Debatin, K. M. *Int. J. Cancer* **1999**, *82*, 435−441.
(13) Kim, Y. K.; Yoon, S. K.; Ryu, S. Y. *Planta Med.* **2000**, *66*, 485−486.
(14) Schmidt, M. L.; Kuzmanoff, K. L.; Ling-Indeck, L.; Pezzuto, J. M. *Eur. J. Cancer* **1997**, *33*, 2007−2010.
(15) Fulda, S.; Jeremias, I.; Pietsch, T.; Debatin, K. M. *Klin. Pediatr.* **1999**, *211*, 319−322.
(16) Ryu, S. Y.; Choi, S. U.; Lee, S. H.; Lee, Ch. O.; No, Z.; Ahn, J. W. *Arch. Pharm. Res.* **1994**, *17*, 375−377.
(17) Hajduch, M.; Sarek, J. PCT Intl. Patent Appl. Publ. WO 01/90046, CAN 136:6179, 2001.
(18) Sarek, J.; Klinot, J.; Brazinova, S.; Dzubak, P.; Klinotova, E.; Noskova, V.; Krecek, V.; Korinkova, J.; Thomson, J. O.; Janostakova, J.; Wang, S.; Parsons, S.; Fischer, P. M.; Zhelev, N. Z.; Hajduch, M. *J. Med. Chem.* **2003**, *46*, 5402−5415.
(19) You, Y.-J.; Kim, Y; Nam, N.-H.; Ahn, B.-Z. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3137−3140.
(20) Reddy, R. P.; Ravindranath, A.; Sudhakar, B.; Ramaiah, T. S.; Rao, M. V. *J. Ind. Chem. Soc.* **1988**, *65*, 688−690.
(21) Huneck, S. *Chem. Ber.* **1965**, *98*, 1837−1857.
(22) Noskova, V.; Dzubak, P.; Kuzmina, G.; Ludkova, A.; Stehlik, D.; Trojanec, R.; Janostakova, A.; Korinkova, G.; Mihal, V.; Hajduch, M. *Neoplasma* **2002**, *49*, 418−425.
(23) Sejbal, J.; Klinot, J.; Protiva, J.; Vystrcil, A. *Collect. Czech. Chem. Commun.* **1986**, *51*, 118−127.
(24) Pradhan, B. P.; Ghosh, P. *Ind. J. Chem., Sect. B* **1993**, *32*, 1068−1069.
(25) Urban, M.; Sarek, J.; Klinot, J.; Korinkova, G.; Hajduch, M. *J. Nat. Prod.* **2004**, *67*, 1100−1105.
(26) Kvasnica, M.; Tislerova, I.; Sarek, J.; Sejbal, J.; Cisarova, I. *Collect. Czech. Chem. Commun.* **2005**, *70*, 1447−1464.
(27) Urban, M.; Sarek, J; Tislerova, I; Dzubak, P.; Hajduch, M. *Bioorg. Med. Chem.* **2005**, *13*, 5527−5535.
(28) Hui, W.-H.; Li, M.-M. *J. Chem. Soc., Perkin Trans. 1* **1977**, *8*, 897−904.
(29) Otsuka, H.; Fujioka, S.; Komiya, T.; Goto, M.; Hirmatsu, Y.; Fujimora, H. *Chem. Pharm. Bull.* **1981**, *29*, 3099−3104.
(30) Hashimoto, F.; Kashiwada, Y.; Cosentino, L. M.; Chen, C. H.; Garrett, P. E.; Lee, K. H. *Bioorg. Med. Chem.* **1997**, *5*, 2133−2143.
(31) Li, T. S.; Wang, J. X.; Zheng, X. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3957−3965.
(32) Biedermann, D.; Sarek, J.; Klinot, J.; Hajduch, M.; Dzubak, P. *Synthesis* **2005**, *7*, 1157−1163.
(33) Huneck, S. *Tetrahedron Lett.* **1963**, *4*, 1977−1980.

NP060436D