Functionalized Pyrrolidines Inhibit α-Mannosidase Activity and Growth of Human Glioblastoma and Melanoma Cells

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New substituted pyrrolidine-3,4-diol derivatives were prepared from D-(−)- and L-(+)-phenyl glycinol. The influence of the configuration and the substitution of the lateral side chain of these derivatives on the inhibition of 25 commercial glycosidases were determined. (2*R*,3*R*,4*S*)-2-({[(1*R*)-2-Hydroxy-1-phenylethyl]amino}methyl)pyrrolidine-3,4-diol ((+)-**7a**) was a potent and selective inhibitor of jack bean α-mannosidase (K_i = 135 nM). However, when evaluated on human tumor cells, **7a**, and the reference compound swainsonine, did not efficiently inhibit the growth of glioblastoma cells. Further derivatization of the hydroxyl group with lipophilic groups to increase bioavailability improved their growth inhibitory properties for human glioblastoma and melanoma cells. In particular, the 4-bromobenzoyl derivative **26** demonstrated high efficacy for human tumor cells whereas primary human fibroblasts were less sensitive to **26**. Therefore, functionalized pyrrolidines have the potential to inhibit the growth of tumor cells and display selectivity for tumor cells when compared to normal cells.

Introduction

Very few therapeutic options exist for the treatment of human glioblastoma and metastatic melanoma, in part because of their resistance to chemotherapeutic agents.[1] Therefore, new drugs have to be developed that are able to overcome resistance, and such novel approaches may be represented by agents targeting the glycosylation pathways of cancer cells. Aberrant glycosylation of glycoproteins and glycolipids was reported to be one of the molecular changes that accompany malignant transformations.[2] As both catabolic and processing glycosidases are involved in the transformation of normal cells to cancer cells and in tumor cell invasion and migration,[3] it has been proposed that the specific inhibition of α-mannosidases involved in the addition of N-linked carbohydrates to glycoproteins may provide a new anticancer strategy[4−6] able to overcome resistance to conventional chemotherapeutic agents. Clinical trials have demonstrated that swainsonine, a natural inhibitor of Golgi α-mannosidase II, which contains a 4-amino-4-deoxy-mannofuranoside moiety,[7] decreases the growth of solid tumors and hematological malignancies.[8] In particular, Kino and co-workers were the first to report that the subcutaneous administration of swainsonine completely inhibited the growth and the formation of lung metastases of a sarcoma.[9] Nevertheless, the toxicity observed for this alkaloid as well as the undesired coinhibition of lysosomal fucosidases resulted in the search for new, more selective α-mannosidases inhibitors. Some analogues of swainsonine such as **2** (Chart 1) as well as simpler derivatives have shown interesting inhibitory properties.[10−12] We previously reported that 3,4-dihydroxy-pyrrolidin-2-yl derivatives such as **3** and **4** are selective and competitive inhibitors of α-mannosidase from the jack bean.[13,14] Recently, we reported that 2-aminomethyl-5-(hydroxymethyl)pyrrolidine-3,4-diol derivatives of type **4** are competitive inhibitors of α-mannosidases with higher potency than the corresponding diamines with no substituent at the C(5) position of the pyrrolidine ring.[15] However, the potential of these molecules to inhibit tumor cell growth was not evaluted.

Here we report the synthesis and characterization of new derivatives of the diamine **3a** where a hydroxymethyl substituent was introduced on the lateral side chain of pyrrolidine and was able to inhibit purified glycosidases at low concentration. The effect of these derivatives on the proliferation of human glioblastoma and melanoma cells, two tumors associated with a high-proliferative and invasive potential, multiple resistance toward conventional chemotherapeutic agents, and poor prognosis, and on human fibroblasts as models for nontumoral cells was also determined. Drug resistance may result from the expression by tumor cells of enzyme systems able to reverse the cytostatic or cytotoxic effect of these agents or from the expression of efflux pumps able to extrude these agents out of tumor cells. Therefore novel strategies, which must be able to reverse tumor resistance to chemotherapeutic agents, have to be envisioned to treat these cancers. We have previously shown that molecules unrelated to conventional therapeutic agents, such as the drugs targeting the endothelin[16,17] and the renin−angiotensin[18] systems, or carbohydrate analogues,[19] may be of some interest. In this context, agents able to modify the glycosylation pathways of tumor cells may be particularly valuable.

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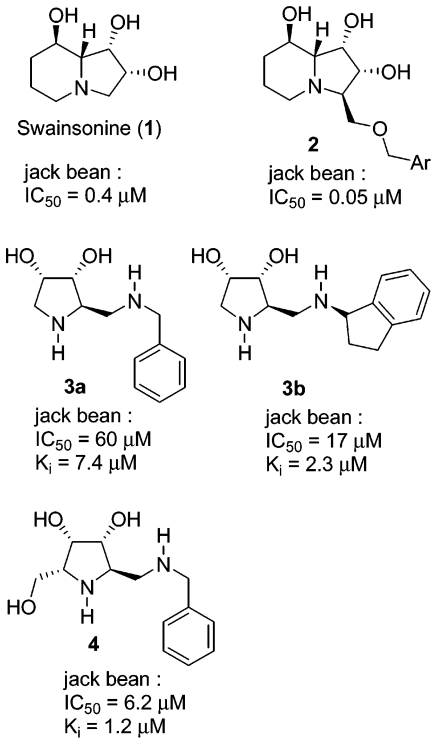
Chart 1. Swainsonine and Pyrrolidine Derivatives as Inhibitors of α-Mannosidases



Results and Discussion

Synthesis. The fully protected carbaldehyde **5**[20] was submitted to a reductive amination procedure in the presence of phenylglycinol derivatives and sodium triacetoxyborohydride for an in situ reduction of the soformed imines (Scheme 1). Acidic treatment of protected derivatives **6** afforded diamines **7a**−**7d** with a 40−74% yield (two steps). The previously reported alcohol **8**[21] was oxidized under Swern conditions and treated as above with D-(−)-α-phenylglycinol to provide diamine **10**, bearing two hydroxymethyl groups, with a 65% yield (three steps).

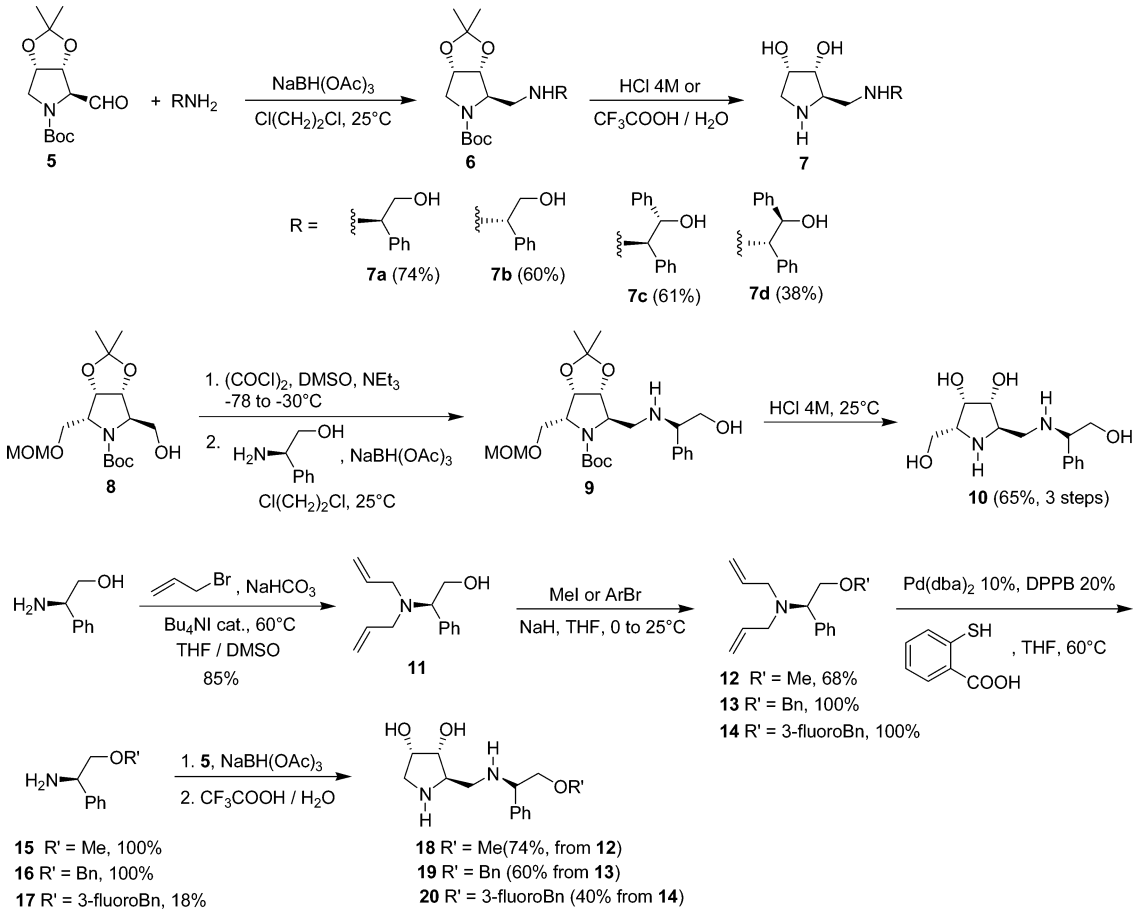
Functionalization of the primary alcohol of derivative **7a** was also performed in order to investigate the role of this hydroxyl moiety in enzyme recognition. D-(−)-α-phenylglycinol was transformed into diallylamine **11** with an 85% yield, allowing protection of the primary alcohol as simple ethers (benzyl and methyl ethers) with good to quantitative yields. Substituted benzyl ethers were also introduced. Palladium promoted cleavage of the allyl moieties in the presence of 2-mercaptobenzoic acid as an allyl scavenger,[21] followed by a reductive amination procedure with carbaldehyde **5**, led to diamines **18** and **19**, after quantitative deprotection in acidic medium. Deallylation of the 3-fluorobenzyl ether resulted in a poor yield of the corresponding amine **17**, which was then submitted to the same sequence of reductive amination/acidic deprotection to provide the functionalized pyrrolidine **20** in 40% yield.

Further derivatives containing lipophilic esters were prepared in order to determine whether modification of the hydroxyl group would improve efficacy (Scheme 2). Moreover, the introduction of lipophilic aromatic moieties should improve cell membrane penetration. D-(−)-α-Phenylglycinol was protected as a *tert*-butyl carbamate (**21**), allowing acylation of the primary alcohol with substituted aromatic acyl chlorides. After acidic treatment, the resulting amines were also engaged in a reductive amination procedure with pyrrolidine carbaldehyde **5** to provide compounds **23**−**26** after quantitative cleavage of the Boc and acetonide protecting groups. Intermediate **21** was also converted into the corresponding azido derivative which was used to introduce amide groups on the lateral side chain of the pyrrolidine such as the substituted benzamide **29**.

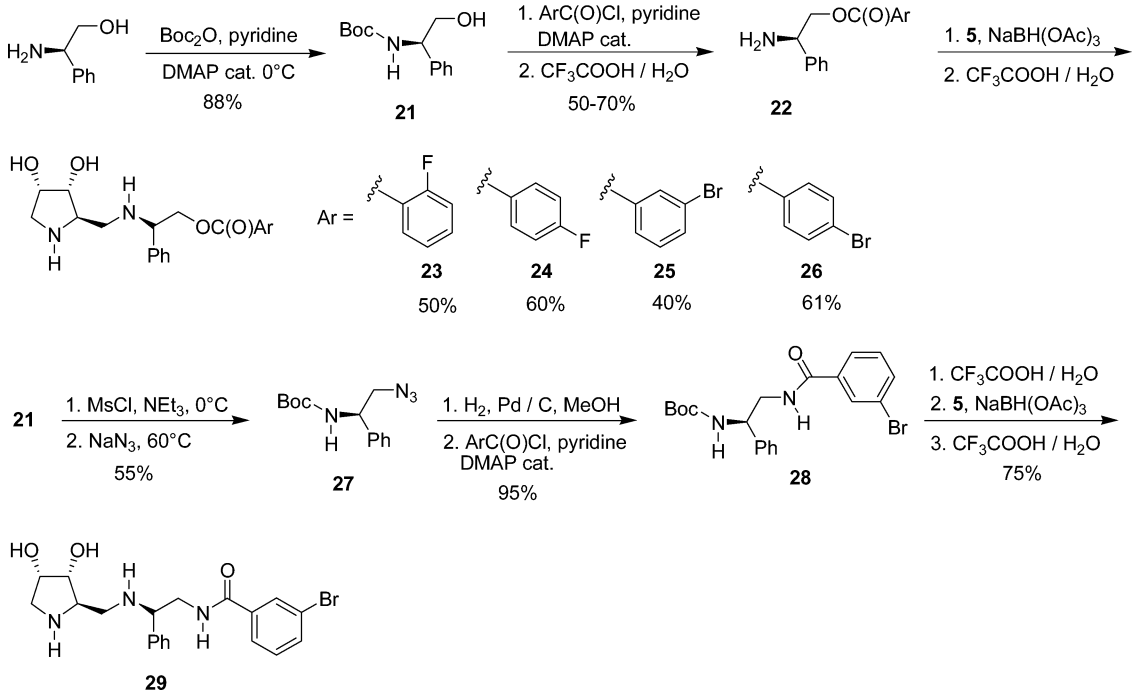
Inhibition of Purified Plant Glycosidases. The inhibitory potential of compounds **7a**−**7d**, **10**, **18**−**20**, **23**−**26**, and **29** toward 25 commercially available glycosidases[22] was determined and compared with the previously reported pyrrolidine derived inhibitors. The data are summarized in Table 1. The compounds (at 1 mM concentration) did not inhibit α-L-fucosidases (from bovine epididymis or human placenta), α- and β-galactosidases (from coffee beans, *Aspergillus niger*, *Aspergillus orizae*, *Escherichia coli,* or jack bean), α- and β-glucosidases (from yeast, rice, *Aspergillus niger*, rhizopus mold, almond, or *Caldocellum saccharolyticum*), β-mannosidases (from *Helix pomatia*), β-xylosidase (from *Aspergillus niger*), α-*N*-acetylgalactosaminidase, and β-*N*-acetylglucosaminidase (from chicken liver) (results not shown). However, α-mannosidases from jack bean and almond were inhibited by these derivatives with a high selectivity. In particular, the D-(−)-α-phenylglycinol-substituted derivative **7a** was a potent (K_i = 135 nM, IC_{50} = 700 nM), competitive (as determined on a Lineweaver−Burk plot) inhibitor of jack bean α-mannosidase, a reliable model enzyme for mammalian Golgi α-mannosidases II,[23] but was less potent for almond α-mannosidase. Introduction of an hydroxymethyl group on the lateral side chain of the pyrrolidine ring led to a considerable increase of the inhibitory potential in comparison with the nonsubstituted derivative **3a** (IC_{50} = 60 μM for **3a** vs IC_{50} = 700 nM for **7a**, factor of increase = 85). Its diastereoisomer **7b** was much less active (IC_{50} = 100 μM), demonstrating the influence of the stereochemistry of the lateral side chain of the pyrrolidine for optimal recognition by the enzyme. Introduction of a second aromatic group (**7c** and **7d**) resulted in a significant decrease of the inhibition of both α-mannosidases. The steric hindrance of these derivatives may be too important to allow them to enter the active site of the enzyme.

The free hydroxyl group on the lateral side chain seemed to also be a determinant for inhibition since the introduction of a methyl ether (**18**) led to a drastic loss of inhibitory activity (77% and 63% inhibition at 1 mM for α-mannosidases from the jack bean and almond, respectively). The benzyl-protected derivative **19** still presented an inhibition constant of 16 μM (IC_{50} = 58 μM) toward α-mannosidase from jack bean (80 times less active than **7a**). The ester and amide derivatives **23**−**26** and **29** were also less active than the parent unprotected compound **7a** with average IC_{50} values of 60 μM and a competitive type of inhibition. This can be explained by an increase of the steric hindrance as well as the loss of potential hydrogen bonding with the active site of the enzyme. Finally, introduction of a second hydroxymethyl group (compound **10**) improved the inhibitory potential on α-mannosidase from almond (IC_{50} = 3.0 μM, K_i = 600 nM) when compared with

Scheme 1. Synthesis of Phenylglycinol Derivatives



Scheme 2. Pyrrolidine Derivatives from Aromatic Phenylglycinol Esters and Amides



compound **7a**. Nevertheless, **10** was less active than **7a** on α-mannosidase from jack bean (K_i = 1.35 μM).

Evaluation of Functionalized Pyrrolidines in Human Glioblastoma and Melanoma Cells or Fibroblasts. First, the effects of derivatives **3a**, **3b**, **7a**−**7d,** and **10** were determined in human LN18 and LNZ308 glioblastoma cells using the MTT ((3,4,5-dimethylthiazol-yl)-2,5-diphenyl tetrazolium) assay, which determines the number of metabolically active cells present. Derivatives **3a**, **7b**−**7d**, and **10** did not display any activity (results not shown). Compound **3b** at 250 μM induced a 23% decrease in the number of metaboli-

Table 1. Inhibitory Activities of Pyrrolidine Derivatives toward α-Mannosidases from Jack Bean or Almond

inhibitor/ enzyme	α-mannosidase (jack bean)	α-mannosidase (almond)
7a	100%[a] (C)[b] IC_{50} = 700 nM, K_i = 135 nM	93% (C) IC_{50} = 46 μM, K_i = 9.5 μM
7b	92% IC_{50} = 100 μM	55%
7c	88% IC_{50} = 110 μM	55%
7d	84% IC_{50} = 128 μM	50%
10	98% (C) IC_{50} = 4.2 μM, K_i = 1.35 μM	96% (C) IC_{50} = 3.0 μM, K_i = 600 nM
18	77%	63%
19	92% (C) IC_{50} = 58 μM, K_i = 16 μM	75%
20	99% (C) IC_{50} = 30 μM, K_i = 7 μM	nd
23	83% (C) IC_{50} = 63 μM, K_i = 24 μM	nd
24	92% (C) IC_{50} = 60 μM, K_i = 19 μM	nd
25	79%	nd
26	92% IC_{50} = 55 μM	nd
29	95% IC_{50} = 89 μM	nd

[a] Percent inhibition at 1 mM and optimal pH. [b] Competitive inhibition.

Table 2. Time-course of Growth Inhibition of Glioblastoma Cells[a] by Functionalized Pyrrolidines

		LN18			LNZ 308		
	conc [μM]	24 h	48 h	72 h	24 h	48 h	72 h
7a	300	22	23	22	26	25	22
	200	19	26	12	8	19	13
	100	13	16	15	0	19	5
7b	300	7			28		
	200	17			4		
	100	0			0		
20	300	27	39	76	34	56	53
	200	19	14	41	14	29	27
	100	2	6	12	2	6	6
23	300	26	8	15	23	18	26
	200	17	10	14	18	15	17
	100	0	7	3	0	6	0
24	300	20	24	31	21	17	32
	200	22	16	14	16	18	24
	100	9	5	4	4	10	8
25	300	16	29	36	17	31	34
	200	10	25	24	5	23	23
	100	8	21	18	5	17	13
26	300	92	95	97	87	78	96
	200	67	48	68	60	33	56
	100	5	17	31	11	15	24
29	300	52	50	65	50	42	59
	200	30	46	61	20	40	52
	100	15	20	43	2	19	43

[a] Cells were exposed for 24, 48, or 72 h to 0, 100, 200, or 300 μM of the various synthetic derivatives, then the MTT assay was performed for the last 2 h of incubation. The percent of residual mitochondrial activity was calculated as the ratio of treated to control cells.

cally active cells. We observed some growth inhibitory activity of **7a** on LN18 and LNZ308 at high concentration (results not shown); however this compound was unstable under biological conditions, and we did not investigate it in more detail. As the hydrophilic character of these molecules may hinder their transport across a cell membrane, we prepared more lipophilic derivatives and evaluated them on the growth of human glioblastoma cells exposed for 24, 48, or 72 h to these molecules. These compounds demonstrated a time- and concentration-dependent inhibition of growth, with **26** being the most rapidly active molecule, producing almost complete inhibition within 24 h. These results are summarized in Table 2.

Swainsonine, an α-mannosidase inhibitor with promising antitumoral properties,[4,7–9] only inhibited by 20% glioblatoma cell growth at 250 μM and was not active at a lower concentration (Figure 1A). Compound **7a**, the most active inhibitor for plant α-mannosidases, was not active on human glioblastoma cells. These discrepancies could result from a poor cell membrane permeability and a low uptake of these compounds by glioblastoma cells. Among the lipophilic substituents of the lateral side chain of **7a**, the 4-bromobenzoyl ester **26** was the most efficient. The replacement of the bromo by a fluoro (**24**), or a bromo (**25**), or a fluoro (**23**), in position 3 of the aromatic ring resulted in the loss of antiproliferative properties. The use of an amide bond rather than an ester bond to link the bromobenzoyl moiety to the pyrrolidine backbone, resulting in **29**, decreased the efficacy. 4-Bromobenzoïc acid or ethyl 4-bromobenzoate, which may result from the hydrolysis of **26** by cell esterases, were without effect on glioblastoma and melanoma cell growth (results not shown) excluding an effect from the aromatic substituent. A dose−response evaluation (300−100 μM) of the antiproliferative effects of **26** and **29** (parts B and C of Figure 1) in both glioblastoma cells demonstrated the increased efficacy of **26** when compared to swainsonine or **29**. The IC_{50} values for **26** and **29** were determined to be 125 and 225 μM in LN 18 and LNZ 308 cells, respectively. These results suggest that cellular esterases, rather than peptidases, are able to release the more hydrophilic derivative **7a** with a free terminal alcohol, which was shown to be the most active and selective mannosidases inhibitor of this series of pyrrolidine derivatives.

We then evaluated whether **26** has the potential to diminish cell growth by inhibiting the synthesis of DNA and/or proteins. The evaluation of the incorporation of [^{3}H]-thymidine and [^{3}H]-Leu following 6 h of exposure of the cells to these molecules demonstrated that **26** inhibited thymidine incorporation (DNA synthesis) (Figure 2A) at slightly lower concentrations and to a higher extent than inhibiting leucine incorporation (protein synthesis) (Figure 2B). These results suggest that this molecule acts initially by inhibiting DNA synthesis (93% inhibition at 300 μM in LN18), then the rate of protein synthesis will decrease (82% at 300 μM in LN18), resulting in diminished cell survival.

Table 3 summarizes and compares the effects on cell growth (MTT assay) after 24 h of exposure to swainsonine, **7a** (the best inhibitor for plant α-mannosidases), and **26** in glioblastoma and melanoma, demonstrating the increased efficiency of **26**; however it has to be emphasized that relatively high concentrations (>100 μM) must be applied in order to have complete inhibition

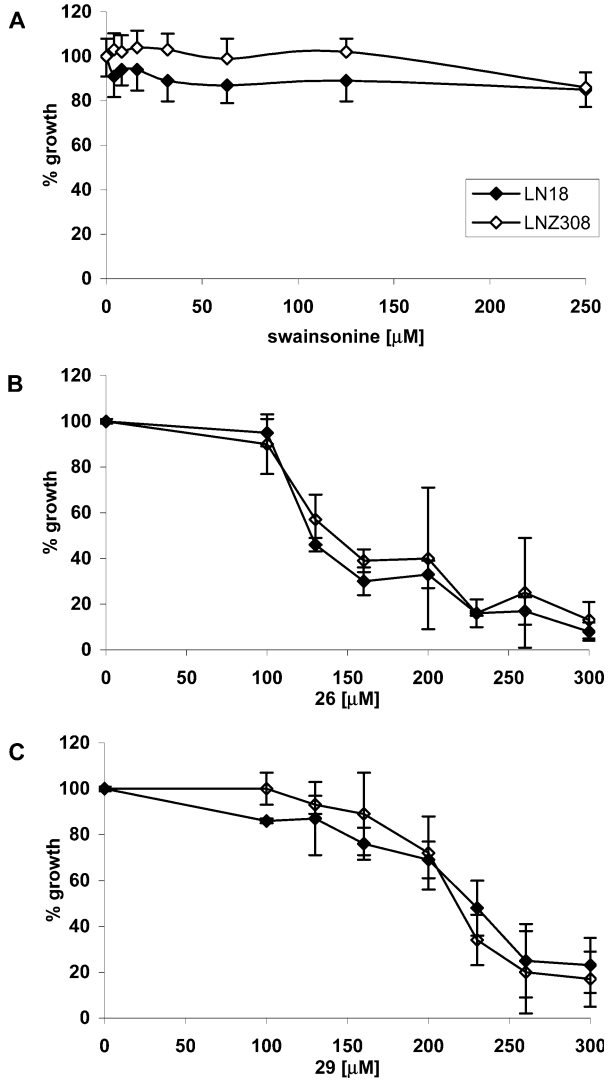


Figure 1. Growth inhibition of human glioblastoma cells by swainsonine, **26,** or **29**. Cells were exposed for 24 h to an increasing concentration of either swainsonine (A), **26** (B), or **29** (C), then the MTT assay was performed for the last 2 h and the percent of growth was calculated as the ratio of the MTT reduction of treated to untreated cells. Results are shown as means ± SD of triplicate wells of one representative experiment out of three.

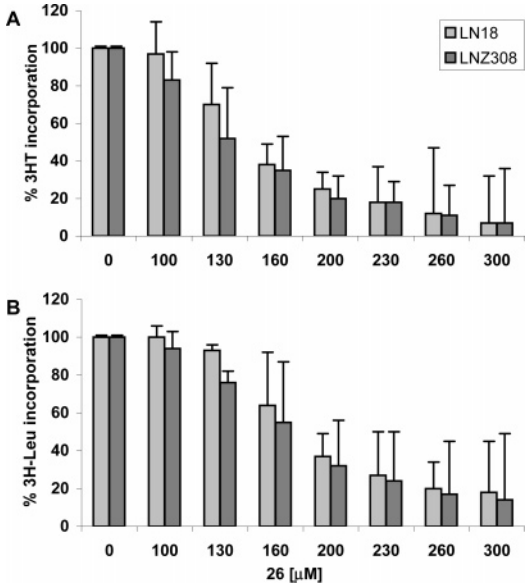


Figure 2. Inhibition of DNA and protein synthesis by **26** in human glioblastoma cells: light gray bars, LN18 and dark gray bars, LNZ308. Cells were exposed for 6 h to an increasing concentration of **26**. The incorporation of either (A) radioactive thymidine [^{3}HT] or (B) radioactive leucine [^{3}H]Leu was performed for the last 2 h. Results are shown as means ± SD of triplicate wells of one representative experiment out of three.

Table 3. Comparison of Dose Dependency of Growth Inhibition of Glioblastoma and Melanoma Cells[a] by Functionalized Pyrrolidines

		300 μM	200 μM	100 μM
swainsonine	LN18	13	18	7
	LNZ308	16	24	25
	Me275	15	22	14
	Me237	20	21	8
compound **7a**	LN18	22	19	13
	LNZ308	26	8	0
	Me275	14	16	15
	Me237	29	30	16
compound **26**	LN18	92	67	5
	LNZ308	87	60	11
	Me275	100	88	16
	Me237	100	98	54

[a] Cells were exposed for 24 h to either swainsonine, compound **7a**, or **26** at 0, 100, 200, or 300 μM, then the MTT assay was performed for the last 2 h of incubation. The percent of growth inhibition was calculated by comparing treated to untreated cells.

of cell growth, suggesting that the bioavailability of this series of molecules may be further optimized.

Therefore, **26** was the most promising derivative of this series in glioblastoma cells. Furthermore, we determined whether this compound was also able to inhibit DNA and protein synthesis and survival in human cancer cells originating from a tumor different from glioblastoma, the melanoma. Exposure of human Me237 and Me275 melanoma cells for 6 h to **26** resulted in a blockade of DNA synthesis (Figure 3A) and protein synthesis (Figure 3B), and after 24 h, the number of metabolically active, melanoma cells decreased (Figure 3C), as determined using the MTT assay.

Finally, we evaluated the sensitivity of human fibroblasts, as models for nontumoral cells to **26** (Figure 4). Fibroblasts were less sensitive to this compound than glioblastoma and melanoma cells for the inhibition of DNA synthesis after 6 h of exposure (Figure 4A) or survival after 24 h of exposure (Figure 4B) suggesting some cell selectivity of **26** between tumoral and nontumoral cells.

Conclusion

In conclusion, a series of functionalized pyrrolidine inhibitors of mannosidases have been prepared and evaluated. The phenylglycinol derivative **7a** was shown to be a potent, selective and competitive inhibitor of α-mannosidase from the jack bean (K_i = 135 nM). However, it did not inhibit the growth of human tumor cells. We postulated that its hydrophilic character prevented its internalization by cells. In support of this hypothesis, the more lipophilic derivative **26** inhibited the growth of human glioblastoma and melanoma cells more than the growth of human fibroblasts and more efficiently than swainsonine, a potential antitumoral agent. The presence of lipophilic substituents increased the efficacy making these derivatives able to inhibit DNA and protein synthesis and tumor cell survival.

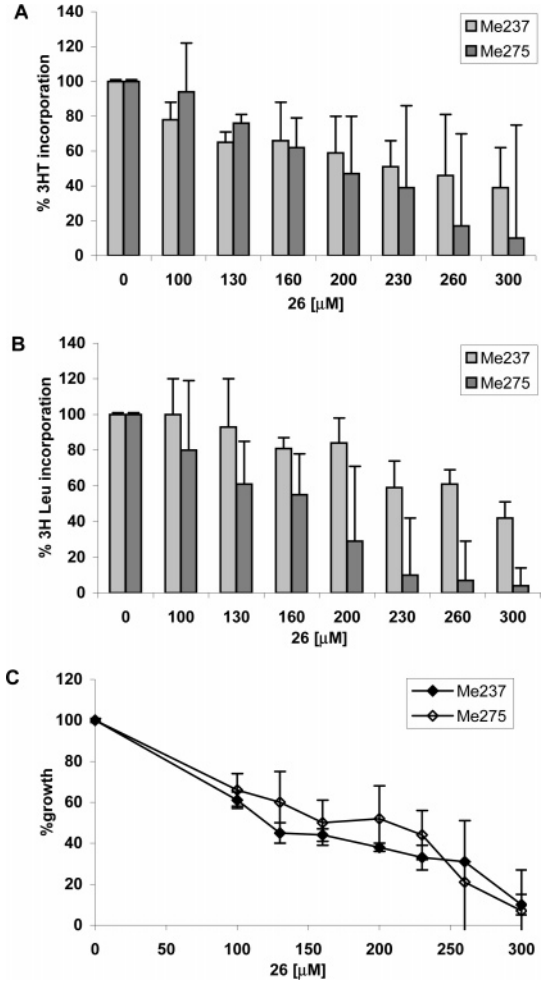


Figure 3. Inhibition of growth and DNA synthesis in human melanoma cells by **26**: light gray bars, Me237 and dark gray bars, Me275. Cells were exposed to an increasing concentration of **26**, either (A) for 6 h and the incorporation of radioactive thymidine (^{3}HT) was performed for the last 2 h or (B) for 24 h, and the MTT assay was performed for the last 2 h,and the percent of growth was calculated as the ratio of thymidine incorporation or MTT reduction of treated to untreated cells. Results are shown as means ± SD of triplicate wells of one representative experiment out of three.

Thus the exposure of tumor cells to ester derivatives of prodrugs analogue of **26** would result in two advantages: the hydrolysis of the ester would release molecules more active on mannosidases than the prodrugs, and the hydrophilic character of the active molecules following its intracellular hydrolysis by esterases would prevent its passive diffusion out of the cells. Such a strategy already proved to be efficient for ester derivatives of aminolevulinic acid in the context of photodynamic therapy protocols.[24]

Therefore, functionalized pyrrolidines represent promising lead agents able to control the progression of human glioblastoma and melanoma, to inhibit DNA and protein synthesis and tumor cell survival, and to display some selectivity for tumor cells compared to nontumoral cells.

Experimental Section

Chemistry. Commercial reagents (Fluka, Aldrich) were used without purification. Solvents were distilled prior to use: tetrahydrofuran (THF) from Na and benzophenone, MeOH from Mg and I_2, and CH_2Cl_2 from CaH_2. The light petroleum ether used refers to the fraction boiling at 40−60 °C. Solutions after reactions and extractions were evaporated in a rotatory evaporator under reduced pressure. Liquid/solid flash chromatography (FC): columns of silica gel (0.040−0.63 mm, Merck No. 9385 silica gel 60, 240−400 mesh). Thin-layer chromatography (TLC) for reaction monitoring: Merck silica gel 60F_{254} plates; detection by UV light; Pancaldi reagent ($(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O) or $KMnO_4$. IR spectra: Perkin-Elmer-1420 spectrometer. ^{1}H NMR spectra: Bruker-ARX-400 spectrometer (400 MHz); δ (H) in ppm relative to the solvent's residual ^{1}H signal ($CHCl_3$, δ (H) 7.27; CH_3OD, δ (H) 3.34) as internal reference; all ^{1}H assignments were confirmed by 2D-correlation spectroscopy-45 and 2D-nuclear Overhauser effect spectrometry spectra. ^{13}C NMR spectra: same instrument as above (101 MHz); δ (C) in ppm relative to solvent's C-signal ($CDCl_3$, δ(C) 77.0; CD_3OD, δ(C) 48.5) as internal reference; coupling constants J in hertz. MS: Nermag R-10-10C, chemical ionization (NH_3) mode *m/z* (amu) (% relative base peak (100%)). Electrospray mass spectra were obtained from the Swiss Institute of Technology Mass Spectral Facility. Elemental analyses: Ilse Beetz, D-96301 Kronach, Germany. Analytical high-performance liquid chromatography (HPLC) was performed on a Waters 600 apparatus, equipped with a Waters absorbance detector set at 214 nm and C_4, C_8, and C_{18} Grace Vydac columns. Elution was performed using the following gradient over 30 min, 100% (0.9% TFA in H_2O) to 100% (0.9% TFA in MeCN).

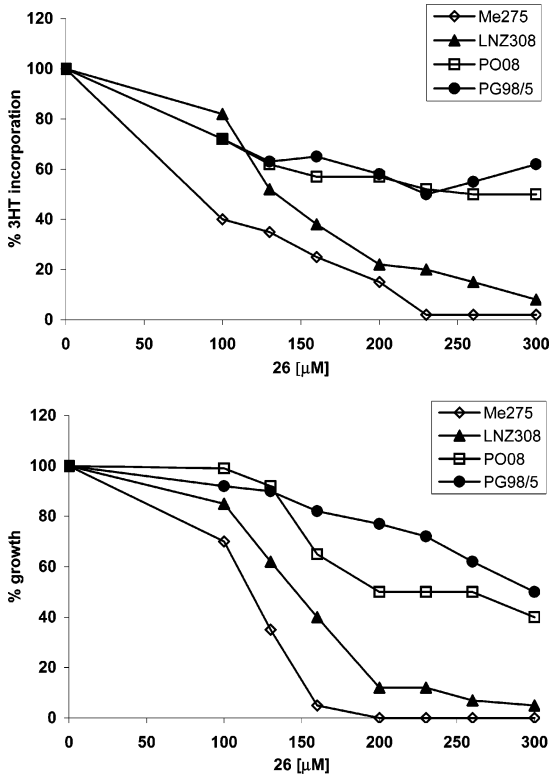


Figure 4. Comparison of the growth inhibition induced by **26** in human primary fibroblasts and human tumor cells. Human fibroblasts (PG98/5 and PO08) or tumor cells (LNZ308 glioblastoma and Me237 melanoma) were exposed to an increasing concentration of **26**, either (A) for 6 h and the incorporation of radioactive thymidine (^{3}HT) was performed for the last 2 h or (B) for 24 h, and the MTT assay was performed for the last 2 h, and the percent of growth was calculated as the ratio of thymidine incorporation or MTT reduction of treated to untreated cells. Results are shown as means ± SD of triplicate wells of one representative experiment out of three.

Procedure 1: General Method for the Reductive Aminations. $NaBH(OAc)_3$ (1.4 equiv) was added portionwise to a stirred solution of aldehyde RCHO (0.1−0.3 M) and primary

amine R′NH_2 (0.1−0.3 M) in 1,2-dichloroethane at 25 °C. After the complete disappearance of the reagents (TLC monitoring), the solution was poured into a saturated aqueous solution of $NaHCO_3$ (5 mL per mmol). The organic layer was collected, and the aqueous layer was extracted with EtOAc (10 mL per mmol, 3 times). The combined organic extracts were dried ($MgSO_4$). Solvent evaporation in vacuo and flash chromatography on silica gel gave pure diamines.

Procedure 2: General Method for the Acidic Cleavage of Boc and Acetonide Moieties. A 5% solution of protected diamines in 4:1 CF_3COOH/H_2O or in 4 M aqueous HCl was stirred at 25 °C for 1 h. After solvent evaporation in vacuo, the residue was purified by flash chromatography on silica gel or alumina.

***tert*-Butyl (3a*R*,4*R*,6a*S*)-4-({[(1*R*)-2-Hydroxy-1-phenylethyl]amino}methyl)-2,2-dimethyl tetrahydro-5*H*-[1,3]dioxolo-[4,5-c]pyrrole-5-carboxylate ((−)-6a).** Procedure 1: **5** (261 mg, 0.96 mmol), D-(−)-α-phenylglycinol (132 mg, 0.96 mmol), $NaBH(OAc)_3$ (285 mg, 1.34 mmol), $ClCH_2CH_2Cl$ (10 mL). FC (EtOAc): 281 mg (74%), 1.2:1 mixture of rotamers. ^{1}H NMR (400 MHz, MeOD) δ: 7.38−7.25 (m, 5H), 4.75−4.60 (m, 2H), 4.10 (dd, 1H), 4.03 (dd, 1H), 3.75 (m, 2H), 3.66−3.51 (m, 2H), 3.37 (m, 1H), 2.64−2.43 (m, 2H), 1.48, 1.40 (2s, 9H), 1.43 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ: 157.3, 154.6, 142.9, 130.4, 129.6, 129.4, 113.4, 85.4, 84.9, 82.2, 82.1, 81.6, 80.9, 68.9, 68.7, 67.5, 67.2, 66.2, 65.8, 53.9, 53.6, 50−49, 29.5, 28.1, 25.9. MS (CI−NH_3): 394 (16), 393 (M + H$^+$, 12), 362 (3), 338 (12), 305 (15), 262 (6), 199 (10), 142 (42), 118 (100), 91 (95), 77 (26). Anal. ($C_{21}H_{32}N_2O_5$) C 64.49, H 8.07.

(2*R*,3*R*,4*S*)-2-({[(1*R*)-2-Hydroxy-1-phenylethyl]amino}methyl)pyrrolidine-3,4-diol ((+)-7a). Procedure 2: **6a** (25 mg), 4 M HCl. FC (CH_3CN/NH_4OH 4:1): 16 mg (100%). ^{1}H NMR (400 MHz, MeOD) δ: 7.42−7.29 (m, 5H), 4.26 (ddd, 1H), 3.97 (dd, 1H), 3.83 (dd, 1H), 3.70 (dd, 1H), 3.64 (dd, 1H), 3.49−3.43 (d and m, 2H), 3.25 (dd, 1H), 2.93 (dd, 1H), 2.78 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 142.6, 130.6, 129.6, 129.4, 75.5, 71.9, 68.3, 67.5, 63.7, 51.5, 48.1). MS (CI−NH_3): 253 (M + H$^+$, 9), 221 (8), 150 (7), 120 (100), 91 (17). Electrospray: 253.31. Anal. ($C_{13}H_{20}N_2O_3$) C 61.76, H 7.95, N 11.08.

***tert*-Butyl (3a*R*,4*R*,6a*S*)-4-({[(1*S*)-2-Hydroxy-1-phenylethyl]amino}methyl)-2,2-dimethyl tetrahydro-5*H*-[1,3]dioxolo-[4,5-c]pyrrole-5-carboxylate ((+)-6b).** Procedure 1: **5** (97 mg, 0.36 mmol), L-(+)-α-phenylglycinol (49 mg, 0.36 mmol), $NaBH(OAc)_3$ (106 mg, 0.50 mmol), $ClCH_2CH_2Cl$ (3 mL). FC (EtOAc): 84 mg (60%), 1.7:1 mixture of rotamers.^{1}H NMR (400 MHz, MeOD) δ: 7.37−7.26 (m, 5H), 4.76 (m, 1H), 4.56 (d, 1H$_\alpha$), 4.49 (d, 1H$_\beta$), 4.14 (dd, 1H$_\beta$), 4.00 (dd, 1Hα), 3.76 (m, 2H), 3.68−3.52 (m, 2H), 3.36 (m, 1H), 2.66 (m, 1H), 2.46 (dd, 1H), 1.50 (s, 3H), 1.43, 1.41 (2s, 9H), 1.30 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ: 157.2, 142.8, 130.4, 129.7, 129.5, 113.4, 85.7, 85.3, 82.2, 82.1, 81.7, 80.9, 68.8, 67.4, 66.3, 65.7, 54.4, 53.6, 50−49, 29.5, 28.1, 25.8. MS (CI−NH_3): 394 (100), 393 (M + H$^+$, 49), 362 (55), 338 (16), 306 (35), 262 (13), 199 (16), 150 (72), 91 (48). Anal. ($C_{21}H_{32}N_2O_5$) C 64.33, H 8.33, N 7.01.

(2*R*,3*R*,4*S*)-2-({[(1*S*)-2-Hydroxy-1-phenylethyl]amino}methyl)pyrrolidine-3,4-diol ((+)-7b). Procedure 2: **6b** (30 mg), 4 M HCl. FC (CH_3CN/NH_4OH 4:1): 19 mg (100%). $[\alpha]_{589}^{25}$ = +42, $[\alpha]_{577}^{25}$ = +46, $[\alpha]_{546}^{25}$ = +50, $[\alpha]_{435}^{25}$ = +85, $[\alpha]_{405}^{25}$ = +99 (c = 0.55, H_2O). ^{1}H NMR (400 MHz, MeOD) δ: 7.42−7.31 (m, 5H), 4.24 (ddd, 1H), 3.97 (dd, 1H), 3.83 (dd, 1H), 3.70 (dd, 1H), 3.64 (dd, 1H), 3.57 (ddd, 1H), 3.43 (dd, 1H), 3.27 (dd, 1H), 2.91 (dd, 1H), 2.80 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 142.5, 130.6, 129.6, 129.5, 75.4, 72.0, 68.5, 66.9, 62.9, 51.8, 47.7. MS (CI−NH_3): 253 (M + H$^+$, 14), 221 (8), 235 (2), 195 (4), 138 (36), 116 (48), 98 (100), 84 (47). Anal. ($C_{13}H_{20}N_2O_3$) C 61.91, H 7.92, N 11.22.

***tert*-Butyl (3a*R*,4*R*,6a*S*)-4-({[(1*R*,2*S*)-2-Hydroxy-1,2-diphenylethyl]amino}methyl)-2,2-dimethyltetrahydro-5*H*-[1,3]dioxolo[4,5-c]pyrrole-5-carboxylate ((−)-6c).** Procedure 1: **5** (114 mg, 0.42 mmol), (1*S*,2*R*)-2-amino-1,2-diphenylethanol (90 mg, 0.42 mmol), $NaBH(OAc)_3$ (125 mg, 0.59 mmol), $ClCH_2CH_2Cl$ (4 mL). FC (EtOAc/light petroleum 1:1): 120 mg (61%), 1.2:1 mixture of rotamers. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41−7.14 (m, 10H), 4.77 (d, 1H), 4.56 (m, 1H), 4.47 (m, 1H), 4.08 (m, 1H$_\beta$), 3.94 (m, 1H$_\alpha$), 3.88 (d, 1H), 3.82 (d, 1H$_\alpha$), 3.73 (d, 1H$_\beta$), 3.28 (m, 1H), 2.51 (m, 2H), 1.45, 1.36, 1.32 (3s, 15 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.4, 154.2, 140.4, 139.2, 128.0, 127.9, 127.7, 126.7, 111.5, 83.8, 83.0, 79.7, 79.3, 78.6, 77.0, 69.1, 68.9, 63.8, 63.4, 52.1, 48.0, 47.4, 28.3, 26.9, 24.9. MS (CI−NH_3): 469 (M + H$^+$, 100), 413 (21), 361 (14), 305 (22), 261 (7), 186 (5), 142 (12). Anal. ($C_{27}H_{36}N_2O_5$) C 69.29, H 7.83, N 5.95.

(2*R*,3*R*,4*S*)-2-({[(1*R*,2*S*)-2-Hydroxy-1,2-diphenylethyl]amino}methyl)pyrrolidine-3,4-diol ((+)-7c). Procedure 2: **6c** (50 mg), 4:1 CF_3COOH/H_2O. FC (CH_3CN/NH_4OH 4:1): 35 mg (100%). ^{1}H NMR (400 MHz, MeOD) δ: 7.29−7.12 (m, 10H), 5.06 (d, 1H), 4.17 (m, 1H), 4.07 (d, 1H), 3.88 (dd, 1H), 3.52 (m, 1H), 3.35 (dd, 1H), 3.23 (dd, 1H), 2.98 (dd, 1H), 2.67 (m, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 143.5, 130.8, 130.0, 129.7, 129.3, 128.8, 77.8, 75.8, 71.8, 71.2, 62.5, 51.7, 48.0. MS (CI−NH_3): 329 (M + H$^+$, 57), 308 (2), 275 (3), 244 (3), 221 (100), 197 (9), 152 (6), 118 (35), 106 (66), 91 (40), 77 (49).

***tert*-Butyl (3a*R*,4*R*,6a*S*)-4-({[(1*S*,2*R*)-2-Hydroxy-1,2-diphenylethyl]amino}methyl)-2,2-dimethyltetrahydro-5*H*-[1,3]dioxolo[4,5-c]pyrrole-5-carboxylate ((−)-6d).** Procedure 1: **5** (104 mg, 0.38 mmol), (1*R*,2*S*)-2-amino-1,2-diphenylethanol (82 mg, 0.38 mmol), $NaBH(OAc)_3$ (114 mg, 0.54 mmol), $ClCH_2CH_2Cl$ (4 mL). FC (EtOAc/light petroleum 1:1): 69 mg (38%), 1.2:1 mixture of rotamers. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27−7.08 (m, 10H), 4.79 (d, 1H$_\alpha$), 4.75 (d, 1H$_\beta$), 4.63 (m, 1H), 4.39 (d, 1H$_\beta$), 4.34 (d, 1H$_\alpha$), 4.19 (dd,1H$_\beta$), 4.02 (d, 1H$_\alpha$), 3.97 (dd, 1H$_\beta$), 3.85−3.74 (m, 2H), 3.15 (m, 1H), 2.60−2.38 (m, 2H), 1.45, 1.42 (2s, 9H), 1.39, 1.28 (2s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 155.0, 154.5, 140.1, 139.2, 128.3, 128.2, 128.1, 127.8, 127.6, 126.9, 126.7, 111.5, 83.9, 83.1, 80.0, 79.9, 79.3, 78.7, 77.2, 69.1, 68.1, 63.9, 62.8, 52.1, 51.8, 47.8, 47.2, 28.3, 26.9, 25.0. MS (CI−NH_3): 469 (M + H$^+$, 100), 413 (26), 361 (10), 305 (20), 261 (5), 186 (2), 142 (10), 84 (12). Anal. ($C_{27}H_{36}N_2O_5$) C 69.30, H 7.69, N 5.94.

(2*R*,3*R*,4*S*)-2-({[(1*S*,2*R*)-2-Hydroxy-1,2-diphenylethyl]amino}methyl)pyrrolidine-3,4-diol ((+)-7d). Procedure 2: **6d** (52 mg), 4:1 CF_3COOH/H_2O. FC (CH_3CN/NH_4OH 4:1): 36 mg (100%). ^{1}H NMR (400 MHz, MeOD) δ: 7.30−7.14 (m, 10H), 4.95 (d, 1H), 4.20 (ddd, 1H), 3.96 (d, 1H), 3.88 (dd, 1H), 3.49 (ddd, 1H), 3.32 (dd, 1H), 3.22 (dd, 1H), 2.82 (dd, 1H), 2.76 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 143.8, 141.2, 130.6, 129.9, 129.7, 129.3, 129.0, 78.9, 75.4, 72.0, 70.5, 62.9, 51.7, 47.7. MS (CI−NH_3): 329 (M + H$^+$, 23), 221 (19), 197 (44), 180 (10), 133 (33), 105 (100), 91 (61), 77 (52).

***tert*-Butyl (3a*R*,4*R*,6*R*,6a*S*)-4-({[(1*R*)-2-Hydroxy-1-phenylethyl]amino}methyl)-6-[(methoxymethoxy)methyl]-2,2-dimethyltetrahydro-5*H*-[1,3]dioxolo[4,5-c]pyrrole-5-carboxylate ((−)-9).** Dimethylsulfoxide (DMSO) (35 μL, 0.50 mmol, 2.4 equiv) was added to a solution of oxalyl chloride (21 μL, 0.24 mmol, 1.15 equiv) in anhydrous dichloromethane (1.5 mL) and cooled to −78 °C. After 20 min, alcohol **8** (72 mg, 0.21 mmol, 1 equiv) in solution in anhydrous dichloromethane (2.5 mL) was added dropwise. After 20 min, triethylamine (144 μL, 1.04 mmol, 5 equiv) was added, and the mixture was warmed to −30 °C for 20 min. The mixture was poured into water (5 mL) and extracted with dichloromethane (5 mL, 3 times). The combined organic phases were washed with brine (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude aldehyde. Sodium triacetoxyborohydride (62 mg, 0.29 mmol, 1.4 equiv) was added portionwise to a stirred solution of the crude aldehyde (0.21 mmol, 1 equiv) and D-(−)-α-phenylglycinol (29 mg, 0.21 mmol, 1 equiv) in anhydrous dichloroethane (2 mL). After being stirred at room temperature overnight, the solution was poured into a saturated aqueous solution of $NaHCO_3$ (5 mL). The organic layer was collected, and the aqueous layer was extracted with CH_2Cl_2 (5 mL, 3 times). The combined organic extracts were dried ($MgSO_4$). Solvent evaporation in vacuo and flash chromatography on silica gel (EtOAc) afforded (−)-**9** (63 mg, 65%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.38−7.26 (m, 5H), 4.79 (dd, 1H), 4.67 (s, 2H), 4.64 (m, 1H), 4.39−3.99 (m, 2H), 3.89 (m, 1H), 3.81−3.69 (m, 4H), 3.55 (dd, 1H), 3.39 (s, 3H), 2.66 (m, 2H), 1.53, 1.39 (2s, 15 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 154.2, 140.2,

128.7, 127.8, 127.0, 111.6, 96.8, 82.0, 80.3, 79.5, 66.8, 64.9, 64.1, 61.0, 55.2, 47.8, 28.3, 26.2, 25.0. MS (CI−NH_3): 467 (M + H^+, 100), 437 (17), 379 (9), 335 (6), 150 (7). Anal. ($C_{24}H_{38}N_2O_7$) C 61.88, H 8.24, N 6.12.

(2*R*,3*S*,4*R*,5*R*)-2-(Hydroxymethyl)-5-({[(1*R*)-2-hydroxy-1-phenylethyl]amino}methyl)pyrrolidine-3,4-diol ((+)-10). Procedure 2: **9** (26 mg), 4 M HCl. FC (CH_3CN/NH_4OH 4:1): 16 mg (100%). ^{1}H NMR (400 MHz, D_2O) *δ:* 7.58−7.50 (m, 5H), 4.35 (dd, 1H), 4.20 (m, 1H), 4.16 (dd, 1H), 4.03 (dd, 1H), 3.95 (m, 3H), 3.79 (m, 1H), 3.65 (m, 1H), 3.14 (bd, 2H). ^{13}C NMR (101 MHz, D_2O) *δ*: 138.8, 131.7, 131.5, 130.5, 76.6, 72.7, 66.7, 66.2, 64.3, 61.4, 60.4, 48. MS (CI−NH_3): 283 (M + H^+, 12), 247 (2), 189 (3), 163 (15), 138 (100), 106 (54), 80 (9).

(2*R*)-2-(Diallylamino)-2-phenylethanol ((−)-11). $NaHCO_3$ (2.45 g, 29.2 mmol, 2 equiv) and allyl bromide (5.05 mL, 58.3 mmol, 4 equiv) were added to a solution of D-(−)-α-phenylglycinol (2 g, 14.6 mmol, 1 equiv) in THF/DMSO (40 mL/10 mL). A catalytic amount of tetrabutylammonium iodide (1.62 g, 4.38 mmol, 0.3 equiv) was added, and the reaction mixture was stirred at 60 °C for 3 h. Water (30 mL) was added after the reaction warmed to room temperature. The reaction mixture was extracted with CH_2Cl_2 (75 mL, 3 times), dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography (EtOAc/light petroleum 1:1) afforded (−)-**11** as a pale yellow oil (2.70 g, 85%). ^{1}H NMR (400 MHz, MeOD) *δ*: 7.39−7.30 (m, 3H, 7.22−7.20 (m, 2H), 5.82 (dddd, 2H), 5.19 (m, 4H), 4.01 (m, 2H), 3.63 (dd, 1H), 3.40 (m, 2H), 2.73 (dd, 2H). ^{13}C NMR (101 MHz, MeOD) *δ*: 136.4, 135.7, 129.0, 128.3, 127.8, 117.7, 63.4, 60.4, 52.4. MS (CI−NH_3): 218 (M + H^+, 100), 186 (32), 98 (4). Anal. ($C_{14}H_{19}NO$) C 77.51, H 8.93.

***N*,*N*-Diallyl-*N*-[(1*R*)-2-methoxy-1-phenylethyl]amine ((−)-12).** Diallylamine **11** (169 mg, 0.78 mmol, 1 equiv) was dissolved in anhydrous THF (3 mL), under argon atmosphere, and the temperature was cooled to 0 °C. Sodium hydride (41 mg, 0.93 mmol, 1.2 equiv, 55% in oil) was added and the reaction mixture was stirred at 0 °C for 10 min. Methyl iodide (86 μL, 0.93 mmol, 1.2 equiv) was added, and the mixture was allowed to warm to room temperature. After 2 h, the resulting mixture was poured into a saturated aqueous solution of $NaHCO_3$ (5 mL) and the aqueous layer was extracted with EtOAc (5 mL, 3 times). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography on silica gel (EtOAc/light petroleum 1:9) afforded (−)-**12** (122 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.37−7.26 (m, 5H), 5.91−5.82 (m, 2H), 5.16 (m, 4H), 4.04 (t, 1H), 3.81 (dd, 1H), 3.71 (dd, 1H), 3.35 (s, 3H, Me), 3.28 (dd, 2H), 2.99 (dd, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 139.3, 136.6, 128.5, 128.1, 127.1, 117.0, 73.8, 62.7, 58.9, 53.2. MS (CI−NH_3): 232 (M + H^+, 100), 186 (97), 135 (12), 117 (2), 91 (11). Anal. ($C_{15}H_{21}NO$) C 72.71, H 9.12, N 6.11.

***N*,*N*-Diallyl-*N*-[(1*R*)-2-(benzyloxy)-1-phenylethyl]-amine ((−)-13).** Diallylamine **11** (307 mg, 1.41 mmol, 1 equiv) was dissolved in anhydrous THF (5 mL), under argon atmosphere, and the temperature was cooled to 0 °C. Sodium hydride (74 mg, 1.70 mmol, 1.2 equiv, 55% in oil) was added, and the mixture was stirred at 0 °C for 10 min. Benzyl bromide (201 μL, 1.70 mmol, 1.2 equiv) was added, and the mixture was allowed to warm to room temperature for 12 h. The resulting mixture was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL), and the aqueous layer was extracted with EtOAc (15 mL, 3 times). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography on silica gel (EtOAc/light petroleum 1:9) afforded (−)-**13** (430 mg, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.44−7.25 (m, 10H), 5.95−5.85 (m, 2H), 5.24−5.15 (m, 4H), 4.55 (s, 2H), 4.13 (t, 1H), 3.92 (dd, 1H), 3.82 (dd, 1H), 3.31 (dd, 2H), 3.07 (dd, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 138.3, 136.4, 128.5, 128.2, 128.0, 127.5, 127.4, 127.0, 117.0, 73.0, 71.2, 63.0, 53.2. MS (CI−NH_3): 308 (M + H^+, 71), 186 (100), 144 (6), 91 (60).

(2*R*,3*R*,4*S*)-2-({[(1*R*)-2-Methoxy-1-phenylethyl]amino}-methyl)pyrrolidine-3,4-diol ((+)-18). A mixture of $Pd(dba)_2$ (4.11×10^{-5} mol) and DPPB (8.22×10^{-5} mol) in THF (0.5 mL) was stirred at room temperature, under argon atmosphere for 15 min. The so-formed catalyst and 2-mercaptobenzoid acid (2.2 equiv, 0.904 mmol) were added to the solution of **12** (95 mg, 0.411 mmol) in anhydrous THF (3 mL), and the reaction was stirred at 60 °C for 12 h. The mixture was poured into a 1 M HCl solution (10 mL) and extracted with EtOAc (10 mL, 2 times). The aqueous layer was treated with 1 M NaOH and extracted with EtOAc (10 mL, 3 times). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to afford **15** (62 mg, 100% crude). Procedure 1 was applied on **5** (111 mg, 0.41 mmol) and **15** (62 mg, 0.41 mmol) in the presence of $NaBH(OAc)_3$ (122 mg, 1.34 mmol) in $ClCH_2CH_2Cl$ (3 mL). FC (EtOAc/light petroleum 1:1): 123 mg (74%), 1.4:1 mixture of rotamers. This intermediate was treated according to procedure 2 in 4:1 CF_3COOH/H_2O. FC (CH_3CN/NH_4OH 4:1): (+)-**18**, 81 mg (100%).

Data for Intermediate *tert*-Butyl (3a*R*,4*R*,6a*S*)-4-({[(1*R*)-2-Methoxy-1-phenylethyl]amino} methyl)-2,2-dimethyltetrahydro-5*H*-[1,3]dioxolo[4,5-c]pyrrole-5-carboxylate. ^{1}H NMR (400 MHz, MeOD) *δ*: 7.39−7.26 (m, 5H), 4.69 (m, 1H), 4.60 (d, 1$H_α$), 4.55 (d, 1$H_β$), 4.09 (dd, 1$H_β$), 4.04 (dd, 1$H_α$), 3.88 (m, 1H), 3.76 (m, 1H), 3.50 (m, 2H), 3.38 (s, 3H), 3.33 (m, 1H), 2.60−2.40 (m, 2H), 1.48 (s, 3H), 1.41 (s, 9 H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, MeOD) *δ*: 157.4, 157.1, 142.5, 130.4, 129.6, 129.5, 113.4, 85.4, 84.9, 82.3, 82.1, 81.6, 80.9, 79.4, 79.2, 66.2, 65.7, 64.9, 64.5, 59.9 53.8, 53.3, 50−49, 29.5, 28.1, 25.9. MS (CI−NH_3): 407 (M + H^+, 100), 361 (10), 305 (5), 164 (9), 106 (3). Anal. ($C_{22}H_{34}N_2O_5$) C 64.95, H 8.50.

Data for (+)-18. ^{1}H NMR (400 MHz, MeOD) *δ:* 7.50−7.37 (m, 5H), 4.26 (m, 1H), 4.23 (ddd, 1H), 3.98 (dd, 1H), 3.68 (m, 2H), 3.63 (m, 1H), 3.50 (dd, 1H), 3.42 (s, 3H), 3.29 (dd, 1H), 3.20 (m, 1H), 3.08 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) *δ*: 138.4, 131.0, 130.8, 130.0, 76.7, 75.9, 71.4, 65.0, 61.3, 60.2, 52.0, 47.9. CI-MS (CI−NH_3): 267 (M + H^+, 100), 221 (34), 164 (22), 152 (9), 135 (35), 120 (39), 106 (19), 95 (10).

(2*R*,3*R*,4*S*)-2-({[(1*R*)-2-(Benzyloxy)-1-phenylethyl]-amino}methyl)pyrrolidine-3,4-diol ((−)-19). A mixture of $Pd(dba)_2$ (3.5×10^{-5} mol) and DPPB (7.0×10^{-5} mol) in THF (0.5 mL) was stirred at room temperature, under argon atmosphere for 15 min. The so-formed catalyst and 2-mercaptobenzoid acid (2.2 equiv, 0.7 mmol) were added to the solution of **13** (108 mg, 0.35 mmol) in anhydrous THF (3 mL), and the reaction was stirred at 60 °C for 12 h. The mixture was poured into a 1 M HCl solution (10 mL) and extracted with EtOAc (10 mL, 2 times). The aqueous layer was treated with 1 M NaOH and extracted with EtOAc (10 mL, 3 times). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to afford **16** (79 mg, 100% crude). Procedure 1 was applied on **5** (94 mg, 0.35 mmol) and **16** (79 mg, 0.35 mmol) in the presence of $NaBH(OAc)_3$ (103 mg, 0.49 mmol) in $ClCH_2CH_2Cl$ (2.5 mL). FC (EtOAc/light petroleum 1:1): 101 mg (60%), 1.1:1 mixture of rotamers. This intermediate was treated according to procedure 2 in 4:1 CF_3COOH/H_2O. FC (CH_3CN/NH_4OH 4:1): (−)-**19**, 72 mg (100%).

Data for Intermediate *tert*-Butyl (3a*R*,4*R*,6a*S*)-4-({[(1*R*)-2-(Benzyloxy)-1-phenylethyl] amino}methyl)-2,2-dimethyltetrahydro-5*H*-[1,3]dioxolo[4,5-c]pyrrole-5-carboxylate. ^{1}H NMR (400 MHz, MeOD) *δ*: 7.39−7.26 (m, 10H), 4.77−4.60 (m, 2H), 4.56 (s, 2H), 4.14−4.02 (m, 1H), 3.92 (m, 1H), 3.76 (d, 1$H_α$), 3.74 (d, 1$H_β$), 3.55 (m, 2H), 3.36 (m, 1H), 2.61−2.42 (m, 2H), 1.47, 1.38 (2s, 9H), 1.42 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, MeOD): 157.3, 157.1, 142.6, 130.4, 130.3, 129.8, 129.6, 129.5, 113.4, 85.4, 85.0, 82.3, 82.1, 81.6, 80.9, 77.0, 76.9, 75.0, 66.2, 65.8, 65.1, 64.6, 53.9, 53.4, 50−49, 29.5, 28.1, 25.9. MS (CI−NH_3): 484 (M + H^+, 100), 428 (3), 362 (62), 305 (18), 241 (6), 142 (14), 91 (30). Anal. ($C_{28}H_{38}N_2O_5$) C 67.55, H 7.66.

Data for (−)-19. ^{1}H NMR (400 MHz, MeOD) *δ*: 7.42−7.29 (m, 10H), 4.56 (s, 2 H), 4.21 (dd, 1 H), 3.96 (dd, 1H), 3.91 (dd, 1H), 3.63 (m, 2H), 3.40 (m, 2 H), 3.19 (dd, 1H), 2.88 (dd, 1H), 2.73 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) *δ*: 142.6, 140.2, 130.6, 130.3, 129.9, 129.7, 129.5, 76.7, 75.6, 75.2, 72.0, 65.3, 63.7, 51.5, 48.4. MS (CI−NH_3): 343 (M + H^+, 32), 306 (3), 229 (11), 195 (7), 159 (6), 120 (38), 106 (67), 91 (100).

Data for (2*S*)-2-({[(2*R*, 3*S*, 4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl}amino)-2-phenylethyl 2-Fluorobenzoate ((−)-23). ^{1}H NMR (400 MHz, MeOD) δ: 7.88 (m, 1H), 7.66−7.62 (m, 1H), 7.48−7.22 (m, 7H), 4.50 (m, 2H), 4.22 (ddd, 1H), 4.14 (t, 1H, ^3J = 6.1 Hz, H−C(1″)), 3.96 (dd, 1H), 3.48−3.38 (m, 2H), 3.23 (dd, 1H), 2.98 (dd, 1H), 2.75 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 167.1, 141.2, 136.3, 136.2, 133.1, 129.8, 129.1, 128.6, 125.4, 118.1, 117.9, 74.8, 71.2, 69.7, 63.3, 62.7, 50.8, 47.5. MALDI-TOF: 375 $(M + H)^+$.

Data for (2*S*)-2-({[(2*R*, 3*S*, 4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl}amino)-2-phenylethyl 4-Fluorobenzoate ((+)-24). ^{1}H NMR (400 MHz, MeOD) δ: 8.05 (m, 2H), 7.48−7.33 (m, 5H), 7.22 (m, 1H), 4.53 (dd, 1H), 4.45 (dd, 1H), 4.22 (m, 1H), 4.15 (m, 1H), 3.95 (dd, 1H), 3.48 (ddd, 1H), 3.41 (dd, 1H), 3.24 (dd, 1H), 2.99 (dd, 1H), 2.74 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 173.0, 141.3, 133.4, 130.0, 129.8, 129.4, 129.0, 128.6, 116.7, 74.8, 71.1, 69.5, 63.4, 62.7, 50.7, 47.6. MALDI-TOF: 375 $(M + H)^+$.

Data for (2*S*)-2-({[(2*R*, 3*S*, 4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl}amino)-2-phenylethyl 3-Bromobenzoate ((−)-25). ^{1}H NMR (400 MHz, MeOD) δ: 8.11 (s, 1H), 7.97 (d, 1H), 7.80 (dd, 1H), 7.49−7.41 (m, 5H), 7.35 (d, 1H), 4.55 (dd, 1H), 4.46 (dd, 1H), 4.21 (ddd, 1H), 4.14 (dd, 1H), 3.95 (dd, 1H), 3.48−3.42 (dd, 1H), 3.41−3.34 (m, 1H), 3.22 (dd, 1H), 2.98 (dd, 1H), 2.72 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 163.3 , 144.4, 141.3, 138.8, 137.2, 133.4, 131.5,129.9, 129.3, 129.1, 128.6, 74.9, 71.2, 69.7, 63.4, 62.7, 50.8, 47.7. MALDI-TOF: 437 $(M + 2)^+$, 435 $M^{+\bullet}$.

Data for (2*S*)-2-({[(2*R*, 3*S*, 4*S*)-3,4-Dihydroxypyrrolidin-2-yl]methyl}amino)-2-phenylethyl 4-Bromobenzoate ((−)-26). ^{1}H NMR (400 MHz, MeOD) δ: 7.90 (d, 2H), 7.68 (d, 2H), 7.48−7.40 (m, 4H), 7.28 (m, 1H), 4.54 (dd, 1H), 4.46 (dd, 1H), 4.22 (m, 1H), 4.14 (dd, 1H), 3.95 (dd, 1H), 3.46 (ddd, 1H), 3.41 (m, 1H, H−C(5)), 3.23 (dd, 1H), 2.99 (dd, 1H), 2.73 (dd, 1H). ^{13}C NMR (101 MHz, MeOD) δ: 170.0, 141.3, 133.0, 132.3, 130.3, 129.9, 129.2, 129.1, 128.6, 74.8, 71.1, 69.6, 63.4, 62.7, 50.7, 47.7. MALDI-TOF: 437 $(M + 2)^+$, 435 $M^{+\bullet}$.

Data for 3-Bromo-*N*-[(2*S*)-2-({[(2*R*,3*S*,4*R*)-3,4-dihydroxypyrrolidin-2-yl]methyl}amino)-2-phenylethyl]benzamide ((+)-29). ^{1}H NMR (400 MHz, MeOD): 7.93 (s, 1H), 7.72 (dd, 2H), 7.41−7.31 (m, 6H), 4.25 (m, 1H), 3.99 (m, 2H), 3.67 (dd, 1H), 3.59 (dd, 1H), 3.51−3.43 (m, 2H, 3.28 (dd, 1H), 2.96 (dd, 1H), 2.73 (dd, 1H). ^{13}C NMR (101 MHz, MeOD): 169.0, 142.4, 137.7, 135.6, 131.4, 129.8, 128.9, 128.4, 127.1, 123.5, 74.7, 71.1, 64.1, 62.7, 50.6, 47.4, 46.8. MALDI-TOF: 436 $[(M + 2)^+]$, 434 $(M^{+\bullet})$.

Biology. (A) Inhibition of Commercially Purified Plant Glycosidases. The experiments were performed essentially as previously described.[18] Briefly, 0.01−0.5 units/mL of enzyme (1 unit = 1 μmol of glycoside hydrolyzed/min), preincubated for 5 min at 20 °C with the inhibitor, and increasing concentration of aqueous solution of the appropriate *p*-nitrophenyl glycoside substrates buffered to the optimum pH of the enzyme were incubated for 20 min at 37 °C (45 °C for the amyloglucosidases). The reaction was stopped by the addition of a 2.5 volume of 0.2 M sodium borate buffer pH 9.8. The *p*-nitrophenolate formed was quantified at 410 nm, and IC_{50} values were calculated or double-reciprocal (Lineweaver−Burk) plots were used to determine the inhibition characteristics.

(B) Cell Cultures and Treatments. Human LN18 and LNZ308 glioblastoma cell lines[25] were routinely maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Basel, Switzerland) containing 4.5 g/glucoses, 5% fetal calf serum (FCS, Gibco), and antibiotics. Human Me237 and Me275 cell lines[26] were grown in RPMI medium (Gibco) containing 10% FCS and antibiotics. Human primary fibroblasts were prepared from surgical skin (PG 98/5) or lung (PO 08) biopsies using the explant technique and grown in DMEM medium containing 4.5 g/L glucose, 10% FCS, and antibiotics. For the experiments, cells were grown in 48 well plates (Costar, Corning, NY) for 24−72 h in the presence of FCS, and then exposed for the indicated time to increasing concentrations of pyrrolidine derivatives (from 0 to 300 μM, stock solutions in methanol/phosphate buffered saline (1:20)) in fresh complete medium. Then either metabolically active cells were quantified using the MTT assay (cf. below) or DNA synthesis and protein synthesis were evaluated using [^{3}H]-thymidine or [^{3}H]-leucine incorporation (cf. below). Experiments were performed in triplicate wells and were repeated at least three times. Means ± SD have been calculated.

(C) Evaluation of Cell Proliferation by the MTT Assay. MTT (Sigma, Buchs, Switzerland) was used to quantify the number of metabolically active cells. The cells were exposed to 0.2 mg/mL MTT in DMEM medium for 2 h following treatment with pyrrolidine, the supernatant was aspirated, and the precipitated formazan was dissolved in 0.1 N HCl in 2-propanol and quantified at 540 nm in a multiwell plate reader (iEMS Reader MF, Labsystems, Bioconcepts, Switzerland).

(D) Evaluation of DNA and Protein Synthesis. Thymidine and leucine incorporation were used to assess DNA and protein synthesis, respectively. [^{3}H]-Thymidine (Amersham-Pharmacia, Dübendorf, Switzerland, 400 nCi/well) or [^{3}H]-Leu (American Radiolabeled Chemicals, St. Louis MO, 400 nCi/puit) were added to treated or control cells for the last 2 h of incubation with the pyrrolidine derivatives. The cell layer was washed and precipitated with 10% trichloracetic acid, the precipitate was dissolved in 1% sodium dodecyl sulfate in 0.1 N NaOH; then 5 mL of scintillation cocktail (Optiphase, Wallac, Regensdorf, Switzerland) was added, and the radioactivity was counted in a β-counter (WinSpectral, Wallac).

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Supporting Information Available: Analytical and spectroscopic data for compounds (−)-**6a**, (+)-**7a**, (+)-**6b**, (+)-**7b**, (−)-**6c**, (+)-**7c**, (−)-**6d**, (+)-**7d**, (−)-**9**, (+)-**10**, (−)-**11**, (−)-**12**, (−)-**13**, (+)-**18**, (−)-**19**, (−)-**23**, (+)-**24**, (−)-**25**, (−)-**26**, and (+)-**29**. Elemental analyses or HPLC data for target compounds is also present. This material is available free of charge via the Internet at http://pubs.acs.org.

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