Evaluation of the First Cytostatically Active 1-Aza-9-oxafluorenes as Novel Selective CDK1 Inhibitors with P-Glycoprotein Modulating Properties

Kristin Brachwitz, Burkhardt Voigt, Laurent Meijer,[§] Olivier Lozach,[§] Christoph Schächtele,[†] Josef Molnár,[‡] and Andreas Hilgeroth*

Institute of Pharmaceutical Chemistry, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Strasse 4, 06120 Halle, Germany

Received November 6, 2002

The first series of synthetic 1-aza-9-oxafluorenes with cytostatic activities in the micromolar range was evaluated as cyclin-dependent kinase (CDK1) inhibitors. Activity was found to be selective in comparison to the inhibition of other kinases within the CDK family. Compounds were shown to inhibit the membrane-efflux pump P-glycoprotein responsible for multidrug resistance in cancer cells. First structure−activity relationships are discussed.

Introduction

Inhibition of cyclin-dependent kinases (CDK) as regulating enzymes within the cell cycle resulted in antiproliferative effects and made them an interesting target for the development of novel small-sized cytostatics for combined cytostatic therapies.[1−3] From the various subtypes of CDKs, the most important explored targets in cancer therapy have been CDK1, -2, and -4.[4] All these enzymes were inhibited by nonselective flavopiridol, which is presently undergoeing phase II clinical trials.[5] The present CDK inhibitors are either nonselective or show inhibition profiles toward various CDK subtypes such as CDK1, -2, and -5 and CDK4 and -6.[4] Despite intense efforts, no specific CDK inhibitor has been discovered so far.[4]

We report a novel class of small-sized cytostatic agents designed as planar aromatic DNA intercalators with cytostatic activities in the range of that for ellipticine. Without showing DNA-intercalating properties, they are demonstrated to act as selective CDK1 inhibitors. In addition, they inhibit the membrane-efflux pump P-glycoprotein (P-gp), which is responsible for multidrug resistance (MDR) in cancer treatment against most cytostatic agents.[6] With respect to an application in combined therapies including other cytostatics, this additional ability strengthens the importance of the new class of bifunctional cytostatics.

Chemistry

The 1-aza-9-oxafluorene target structures **5a**−**d** have been synthesized by oxidations of *N*-acyltetrahydro-1-aza-9-oxafluorenes **4a**−**d**, which have been reported as exclusive reaction products from 3-acyl-substituted 1,4-dihydropyridines and *p*-benzoquinone under acid catalysis with isolated yields of about 70%.[7] When lead tetraacetate is used as the oxidizing agent, the reaction proceeds within a few hours without any other oxidized fragmentation products being observed. In the case of the 3-acyl-1,4-dihydropyridine starting compounds, the total yields of about 50% for the target structures **5a**−**d** were unsatisfying. Therefore, the one-pot reaction of 3-acyl-1,4-dihydropyridines **6a**−**d** and *p*-benzoquinone without isolation of the tetrahydro intermediate products, **4a**−**d**, with an excess of the oxidant *p*-benzoquinone was investigated as an alternative route. Although some fragmentation to the pyridine oxidation products **7a**−**d** was observed, the final yields of the target compounds of about 70% of the crude reaction products, which were recrystallized from alcohol/water, was the superior method. Acetylation of compound **5a** to form **5e** has been carried out in acetic anhydride using catalytic pyridine as a basic auxiliary.

Results and Discussion

The cytostatic activities of the 1-aza-9-oxaflourenes **5a**−**e** have been evaluated at the National Cancer Institute (NCI) following the protocol of a sulforhodamine B protein cytotoxicity assay in an in vitro antitumor screen.[8−10] The 4-phenyl derivatives **5a** and **5b** show moderate cytostatic activities within the performed screen of the 60 cell lines with a mean graph midpoint (MG_MID) of −4.5, as has been demonstrated for cytostatically active kenpaullone (Table 1).[11] Some selectivity was found in the ovarian cancer cell line IGROVI with values of −5.6 μM for **5a** and −5.3 μM for **5b**.

With the replacement of the 4-phenyl substituent by a methyl group, the cytostatic activity significantly decreased with reduced cytostatic activities of compound **5c** and practically no activity for **5d** with GI_{50} values greater than 100 μM. Acetylation of the 6-hydroxy function of compound **5a** also led to a loss of cytostatic activity in **5e**. Thus, the 6-hydroxy and the 4-phenyl functions turned out as essential functional substituents for cytostatic activity.

DNA-intercalation properties of the cytostatically active compounds **5a**−**c** have been investigated in a described ethidium bromide displacement assay using ellipticine as reference compound.[12] When a drug displaces intercalating ethidium bromide from its fluores-

* To whom correspondence should be addressed. Phone: 49-345-55-25168. Fax: 49-345-55-27026. E-mail: hilgeroth@pharmazie.uni-halle.de.

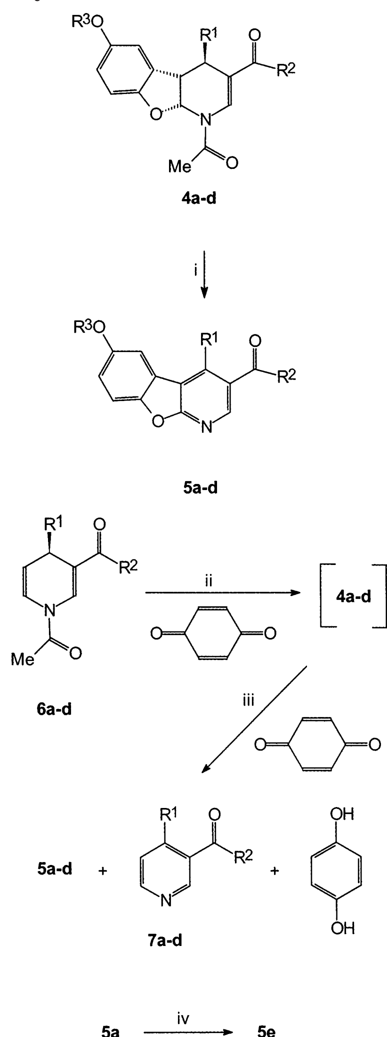
§ Present address: CNRS, Cell Cycle Group, Station Biologique, BP 74, Roscoff 29682 Cedex, France.

† Present address: ProQinase GmbH, Breisacher Strasse 117, 79106 Freiburg, Germany.

‡ Present address: Faculty of Medicine, Department of Medical Microbiology, University of Szeged, Dom ter 10, 6720 Szeged, Hungary.

10.1021/jm021090g CCC: $25.00 © 2003 American Chemical Society
Published on Web 02/01/2003

Scheme 1. Synthesis of 1-Aza-9-oxafluorenes **5a**−**e**[a]



[a] Reagents and conditions: (i) $PbAc_4$, THF, room temp, 6 h; (ii) dioxane, perchloric acid (5%), room temp, 12 h; (iii) dioxane, perchloric acid (5%), room temp, 120 h; (iv) acetic anhydride, pyridine, room temp, 12 h.

cent complex with DNA, the fluorescence intensity is reduced. All the investigated compounds **5a**−**c** showed a very small decrease in fluorescence up to 40 μM, which is comparable to that due to the solvent. Therefore, a DNA-intercalating mode of cytostatic action for the 1-aza-9-oxafluorenes was unlikely.

A COMPARE analysis was carried out with derivative **5a** using the correlation coefficient method (PCC).[13,14] This method uses log GI_{50} values of all 60 cell lines of the screen of **5a** and compares activity data of **5a** to those of the NCI-COMPARE database compounds[14] starting with one cell line. All database compounds with no activtiy in the first cell line are excluded for further comparison, finally leading to pairs of correlation coefficients between the investigated compound and the database substances of highest similarity of activity in all cell lines and correlation coefficients of $\leq$1. The similar activity profile then may give hints to a similar mode of cytostatic action of both substances.

The highest correlation coefficients have been calculated for a 3,5-diarylidene-4-piperidone (0.65), an indolocarbazole glycoside of the rebeccamycin class (0.64) and a 9-acridinylmethyl derivative (0.64). While the mode of cytostatic action of the 4-piperidone compound was not further investigated, the rebeccamycin-derived compounds are known to act as intercalating inhibitors of topoisomerase and as protein kinase inhibiting agents.[15] The compounds from the acridinyl series are proven DNA intercalators.[16] However, because we demonstrated that our 1-aza-9-oxafluorenes are unlikely to act as intercalators, we investigated their ability to inhibit protein kinases. One important class of protein kinases are the cyclin-dependent ones with CDK1, -2, and -4 involved in the cell proliferation process of cancer with similar significance.[4] We selected cyclin-B-dependent CDK1 from the series and evaluated the inhibition properties of our target structures **5a**−**e**.

While the 4-methyl derivatives **5c**, **5d**, and the acetylated compound **5e** were found to be inactive at 10 μM, the 4-phenyl substituent in **5a** and **5b** resulted in comparably high inhibitory activity up to 66% for **5b** at 10 μM with a calculated IC_{50} of 4.2 μM.

Thus, the 1-aza-9-oxafluorene compounds of highest cytostatic activities were identified as CDK1/B inhibitors. Because selectivity of protein kinase inhibition is an important objective in CDK-inhibitor design of today,[4] we also checked the inhibitory properties against CDK5/p25 also belonging to the CDK family. CDK5 itself plays an important role in the progression of Alzheimer disease.[17] Compared to flavopiridol and kenpallone, which are nonselective CDK1 inhibitors, we found a certain selectivity for our 4-phenyl compounds, which show merely some inhibitory activity of CDK5 at 10 μM and no activity of CDK2/E or CDK4/D1 with $IC_{50} > 100$ μM. Thus, they have been evaluated as the first selective CDK1 inhibitors for cancer therapy.

MDR mediated by multidrug efflux pumps is a major problem in cancer treatment. Thus, it is important to ascertain whether new potential CDK inhibitors are able to modulate the MDR against the broad variety of anticancer drugs as efflux-pump substrates especially with respect to suggested combined therapies for CDK inhibitors with other cytostatics for which MDR has been stated.[6] We investigated cytostatically active 1-aza-9-oxafluorenes **5a**−**c** as inhibitors of P-gp which is the most important efflux pump in multidrug-resistant cancer cells. Concentration-dependent MDR reversal was determined in a mouse T-cell lymphoma parental cell line and the multidrug resistant subline which was transfected with the human MDR-1 gene, thus ensuring that observed activity of compounds exclusively results from MDR efflux-pump inhibition.[18] In the present study, fluorescence uptake of the commonly used MDR marker rhodamine 123 as a specific binding substrate of P-gp was measured.[18] The given ratios of activity (R) in Table 2 have been calculated following eq 1.

$$R = \frac{\text{MDR treated/MDR untreated control}}{\text{parental treated/parental untreated control}} \qquad (1)$$

Compounds were found to be active (A) if the ratio was greater than 1.1 and very active (AA) for ratios greater than 10.[18]

Direct comparison of rhodamine fluorescence data in MDR-treated and parental-treated cells shows that the observed effects for the rhodamine dye result from specific P-gp inhibition by the MDR reversers, whereas any effects different from P-gp binding do not influence the activity ratios.

Table 1. Enzyme Inhibition and in Vitro Antitumor Activity of the 1-Aza-9-oxafluorenes

compd	R^1	R^2	R^3	IC_{50} [a] [μM] CDK1/B	CDK5	% inhibition at 10 μM[a] CDK5	log GI_{50} [a] IGROVI	MG_MID [M]
flavopiridol				0.2[b]	0.17[b]	NA[c]	NA[c]	−7.2[b]
kenpaullone				0.4[b]	0.85[b]	NA[c]	NA[c]	−4.3[b]
5a	Ph	OEt	H	28.8	nd[c]	6	−5.6	−4.5
5b	Ph	Me	H	4.2	nd[c]	15	−5.3	−4.5
5c	Me	OEt	H	na[c]	nd[c]	3	−4.1	−4.1
5d	Me	Me	H	na[c]	nd[c]	10	>−4	>−4
5e	Ph	OEt	Ac	na[c]		na[c]	>−4	>−4

[a] Mean of two determinations. [b] From literature.[11,17] [c] NA, not available. nd, not determined. na, not active.

Table 2. P-gp Inhibitory Activity Ratios (*R*) of Cytostatically Active 1-Aza-9-oxafluorenes **5a**−**c**

compd	inhibitory activity ratios[a] (*R*) 7 μM	70 μM
5a	9.50	9.14[b]
5b	1.26	6.74
5c	1.18	4.77
verapamil control at 11 μM[c]	7.27	

[a] Mean of two determinations. [b] Saturation concentration for P-gp inhibition. [c] Maximum concentration for relevant P-gp modulation.[18]

All compounds were found to be active at the low concentration of 7 μM, with the 4-phenyl compound **5a** as the best one showing higher activity than the standard reference compound verapamil at the given concentration relevant for effective MDR reversal in clinical investigations.[18] Within this first series of active compounds, the combination of the 4-phenyl and the 3-ethoxycarbonyl substituents in **5a** is most favorable for inhibitory activities. From the limited knowledge of common structure−activity relationships (SAR) of MDR reversers, two separated aromatic centers and a basic nitrogen are derived as essential structural features found within reported reversers.[18] Besides these structural elements, hydrogen bond acceptor groups are discussed as favoring inhibitory activities by increasing binding possibilities of the modulators to amino acids of P-gp binding regions.[18] Both 4-phenyl compounds **5a** and **5b** of highest activity have those two separated aromatic centers, while **5c** with the lowest activity has just one. A nitrogen atom as well as two hydrogen bond acceptor groups of the 3-carbonyl and 9-hydroxy functions are found in all the active compounds. However, the difference in observed activity between **5a** and **5b** has to be investigated in further studies for more insight into the SAR of this new class of MDR reversers.

Conclusion

From the first series of 1-aza-9-oxafluorenes, the 4-phenyl-substituted compounds showed cytostatic activities in the range of those of ellipticine compounds in the lower micromolar range.[16] Compared to planar aromatic ellipticine, they showed no DNA-intercalating properties but were shown to act as selective inhibitors of CDK1. With demonstrated P-gp inhibitory activities, they are a potential class of novel cytostatics with exclusive bifunctionality of CDK1 inhibition as well as P-gp modulation.

Experimental Section

General. Commercial reagents were used without further purification. ^{1}H NMR (500 MHz) spectra were recorded using tetramethylsilane as an internal standard. TLC was performed on E. Merck 5554 silica gel plates. Mass spectra were measured with an AMD 402 mass spectrometer. Elemental analysis was performed using a Leco CHNS-932 apparatus.

The synthesis of starting compounds **4a**−**d** and **6a**−**d** were recently reported in ref 7.

Representative Procedure for the Oxidation of Compounds 4a−d to 1-Aza-9-oxafluorenes 5a−d: Ethyl 6-Hydroxy4-phenylbenzo[4.5]furo[2.3-*b*]pyridine-3-carboxylate (5a). **4a** (0.04 g, 0.1 mmol) was dissolved in dry THF (40 mL) and treated with a 10-fold excess of lead tetraacetate (0.44 g, 1 mmol) under stirring. After 6 h, the solution was poured into ice/water and extracted with chloroform (30 mL) three times. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness under vaccuum, leaving a brownish solid that was stirred in ether. The resulting crude reaction product was recrystallized from alcohol/water, yielding 0.022 g (82%) of pure **5a** as a brownish powder: mp 209−213 °C; ^{1}H NMR ($CDCl_3$) δ 9.02 (s, 1 H), 7.54−7.52 (m, 3 H), 7.47 (d, J = 8.8 Hz, 1 H), 7.38−7.34 (m, 2 H), 6.96 (dd J = 8.8/2.6 Hz, 1 H), 6.28 (d, J = 2.6 Hz, 1 H), 4.62 (s, 1 H), 4.12 (q, J = 7.2 Hz, 2 H), 1.03 (t, J = 7.2 Hz, 3 H); MS (EI) *m*/*z* 333. Anal. ($C_{20}H_{15}NO_4$) C, H, N.

3-Acetyl-6-hydroxy-4-phenylbenzo[4.5]furo[2.3-*b*]pyridine (5b). Yield 0.025 (84%); mp 242−246 °C; ^{1}H NMR ($CDCl_3$) δ 8.74 (s, 1 H), 7.59−7.57 (m, 3 H), 7.48 (d, J = 9.0 Hz, 1 H), 7.44−7.42 (m, 2 H), 6.76 (dd, J = 9.0/2.5 Hz, 1 H), 6.46 (d, J = 2.5 Hz, 1 H), 4.85 (s, 1 H), 2.10 (s, 3 H); MS (EI) *m*/*z* 303 (M^+). Anal. ($C_{19}H_{13}NO_3$) C, H, N.

Ethyl 6-Hydroxy-4-methylbenzo[4.5]furo[2.3-*b*]pyridine-3-carboxylate (5c). Yield 0.021 g (78%); mp 250−255 °C; ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1 H), 7.53 (d, J = 2.4 Hz, 1 H), 7.50 (d, J = 9.0 Hz, 1 H), 7.03 (dd, J = 9.0/2.4 Hz, 1 H), 5.14 (s, 1 H), 4.40 (q, J = 7.0 Hz, 2 H), 2.69 (s, 3 H), 1.56 (t, J = 7.0 Hz, 3 H); MS (EI) *m*/*z* 271 (M^+). Anal. ($C_{15}H_{13}NO_4$) C, H, N.

3-Acetyl-6-hydroxy-4-methylbenzo[4.5]furo[2.3-*b*]pyridine (5d). Yield 0.019 g (80%); mp 273−296 °C; ^{1}H NMR ($CDCl_3$) δ 8.83 (s, 1 H), 7.56 (d, J = 2.5 Hz, 1 H), 7.53 (d, J = 9.0 Hz, 1 H), 7.06 (dd, J = 9.0/2.5 Hz, 1 H), 4.94 (s, 1 H), 3.00 (s, 3 H), 2.72 (s, 3 H); MS (EI) *m*/*z* 241 (M^+). Anal. ($C_{14}H_{11}NO_3$) C, H, N.

Representative Procedure for the One-Pot Formation of Compounds 5a−d and Pyridine Oxidation Side Products 7a−d: Ethyl 6-Hydroxy-4-phenylbenzo[4.5]furo[2.3-*b*]pyridine-3-carboxylate (5a) and Ethyl 4-Phenylnicotinate (7a). Compound **6a** (0.5 g, 1.8 mmol) and *p*-benzoquinone (0.23 g, 2.2 mmol) were dissolved in 100 mL of dried dioxane containing 5% of perchloric acid. The mixture was stirred overnight. Then additional portions of *p*-benzoquinone, each 0.08 g (2.2 mmol), were added until product formation from the detectable tetrahydro intermediate was complete as followed by TLC. Then the solution was poured into ice/water. After extraction with chloroform (3 × 50 mL), the organic layer was dried over sodium sulfate. Filtration was followed by evaporation to dryness under vacuum. The resulting oil was separated by preparative TLC using a mixture of chloroform/ethyl acetate/methanol (85:15:2). The resulting product zones of target compound **5a** and pyridine oxidation product **7a** were separated and washed with acetone. After evaporation of the organic layer to dryness, the resulting powder of **5a** was recrystallized from methanol, yielding 0.44 g (72%): mp 208−209 °C. The resulting oil of **7a** was 0.08 g (20%); the melting

point of the picrate was 125−126 °C (from ethanol) (ref 7, mp 126−129 °C).

3-Acetyl-6-hydroxy-4-phenylbenzo[4.5]furo[2.3-*b*]pyridine (5b). Yield 0.40 (72%); mp 240−244 °C.

Ethyl 6-Hydroxy-4-methylbenzo[4.5]furo[2.3-*b*]pyridine-3-carboxylate (5c). Yield 0.34 g (69%); mp 249−253 °C.

3-Acetyl-6-hydroxy-4-methylbenzo[4.5]furo[2.3-*b*]pyridine (5d). Yield 0.31 g (71%); mp 289−294 °C.

3-Acetyl-4-phenylpyridine (7b). Yield 0.06 g (18%); mp (picrate) 142−144 °C (ref 7, mp 145−148 °C).

Ethyl 4-Methylpyridine (7c). Yield 0.05 g (19%); mp (picrate) 126−131 °C (ref 7, mp 128−134 °C).

3-Acetyl-4-methylpyridine (7b). Yield 0.05 g (20%); mp (picrate) 136−138 °C (ref 7, mp 137−140 °C).

Ethyl 6-Acetoxy-4-phenylbenzo[4.5]furo[2,3-*b*]pyridine-3-carboxylate (5e). An amount of 0.04 g (0.1 mmol) of compound **5a** was stirred overnight in 50 mL of dried acetic anhydride containing 5 drops of dried pyridine base. Then the mixture was evaporated to dryness in vaccuum, leaving a colored solid that was stirred in ether, filtered off, and recrystallized leading to 0.03 g (80%) of acetylation product **5e**: mp 313−319 °C; ^{1}H NMR ($CDCl_3$) δ 9.04 (s, 1 H), 7.60 (d, J = 9.0 Hz, 1 H), 7.55−7.36 (m, 5 H), 7.17 (dd, J = 9.0/2.4 Hz, 1 H), 6.60 (d, J = 2.5 Hz, 1 H), 4.13 (q, J = 7.0 Hz, 2 H), 2.23 (s, 3 H), 1.03 (t, J = 7.0 Hz, 3 H); MS (EI) m/z 375 (M^+). Anal. ($C_{22}H_{17}NO_5$) C, H, N.

Enzyme Inhibition Assays.[11,17] Kinase activities were assayed at 30 °C at a final ATP concentration of 15 μM. Blank values were subtracted and activities were calculated as picomoles of phosphate incorporated for a 10 min incubation. CDK1/cyclin B was extracted from M phase starfish (*Marthasterias glacialis*) oocytes and purified as described. Kinase activity was assayed in a homogenization buffer containing 60 mM β-glycerophosphate, 15 mM *p*-nitrophenyl phosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, and 1 mM phenyl phosphate with 1 mg histone H1/mL in the presence of 15 μM [γ-^{32}P]ATP (3000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After incubation, 25 μL aliquots of the supernatant where spotted on phosphocellulose filter paper, which was washed five times with 10 mL of phosphoric acid per liter of water. Then scintillation counting was carried out. CDK5/p25 was reconstituted by mixing equal amounts of recombinant mammalian CDK5 and p25 as glutathione-*S*-transferase (GST) fusion proteins and purified as described. Its activity was assayed in the same homogenization buffer as used for CDK1/cyclin B inhibition.

Fluorescence Uptake Assay. Cells from the L5178Y mouse T-cell lymphoma parental cell line and from its multidrug-resistant subline L5178YvMDR were adjusted to a concentration of 2×10^6/mL in serum-free McCoys 5A medium from which 0.5 mL was incubated at room temperature with the test compounds. Then rhodamine 123 with 5.2 μM as the final concentration was added and incubation continued for 20 min at 37 °C. After it was washed twice with PBS, the fluorescence of 1×10^4 cells was measured by flow cytometry.

Acknowledgment. The work was financially supported within the framework of the COST B16 Action of the EU.

References

(1) Fischer, P. M.; Lane, D. P. Inhibitors of cyclin-dependent kinases as anti-cancer therapeutics. *Curr. Med. Chem.* **2000**, *7*, 1213−1245.

(2) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. Cyclin-dependent kinase inhibitors: useful targets in cell cycle regulation. *J. Med. Chem.* **2000**, *43*, 1−18.

(3) Kelland, L. R. Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinical: current status. *Expert Opin. Invest. Drugs* **2000**, *9*, 2903−2911.

(4) Knockaert, M.; Greengard, P.; Meijer, L. Pharmacological inhibitors of cyclin-dependent kinases. *TRENDS Pharmacol. Sci.* **2002**, *23*, 417−426.

(5) Webster, K. R. Therapeutic Potential of Targeting the Cell Cycle. *Chem. Res. Toxicol.* **2000**, *13*, 940−943.

(6) Krishna, R.; Mayer, L. D. Multidrug resistance (MDR) in cancer mechanism, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *Eur. J. Pharm. Sciences* **2000**, *11*, 265−283.

(7) Hilgeroth, A.; Brachwitz, K.; Baumeister, U. Regioselective Formation of Novel Functionalized 1-Aza-9-oxafluorenes. *Heterocycles* **2001**, *55*, 661−669.

(8) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer drug-screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

(9) Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. Comparison of in vitro anticancer drug-screening data generated with a tetrazolium assay versus protein assay against a diverse panel of human tumor cell lines. *J. Natl. Cancer Inst.* **1990**, *82*, 1113−1118.

(10) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langeley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.

(11) Schultz, C.; Link, A.; Leost, M.; Zaharevitz, D. W.; Gussio, R.; Sausville, E. A.; Meijer, L.; Kunick, C. Paullones, a Series of Cyclin-Dependent Kinase Inhibitors: Synthesis, Evaluation of CDK1/Cyclin B Inhibition, and in Vitro Antitumor Activity. *J. Med. Chem.* **1999**, *42*, 2909−2919.

(12) Marotto, A.; Kim, Y.-S.; Schulze, E.; Pindur, U. New indolocarbazoles as antitumor active compounds: evaluation of the target by experimental and theoretical studies. *Pharmazie* **2002**, *57*, 194−197.

(13) Developmental Therapeutics Program NCI/NIH. COMPARE Methodology. http://dtp.nci.gov./docs/compare_methodology.html (accessed 2001).

(14) Developmental Therapeutics Program NCI/NIH. Compare−User Defined COMPARE applet explaination and instructions. http://dtp.nci.nih.gov/docs/applets/java_compare/uc_explain.html (accessed 2001).

(15) Pindur, U.; Kim, Y.-S.; Mehrabani, F. Advances in Indolo[2,3-*a*]carbazole Chemistry: Design and Synthesis of Protein Kinase C and Topoisomerase I Inhibitors. *Curr. Med. Chem.* **1999**, *6*, 29−69.

(16) Insaf, S. S.; Danks, M. K.; Witiak, D. T. A Structure−Function Analysis of DNA Topoisomerase II Inhibitors. *Curr. Med. Chem.* **1996**, *3*, 437−466.

(17) Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J. A.; Snyder, G. L.; Grenngardt, P.; Biernat, J.; Wu, Y.-Z.; Mandelkow, E.-M.; Eisenbrand, G.; Meijer, L. Indirubins Inhibit Glycogen Synthase Kinase-3β and CDK5/P25, Two Protein Kinases Involved in Abnormal Tau Phosphorylation in Alzheimer's Disease. *J. Biol. Chem.* **2001**, *276*, 251−260.

(18) Hilgeroth, A.; Molnár, J.; De Clercq, E. Mit molekularer Symmetrie zu neuen Wirkstoffen: Hydroxymethyl-substituierte 3,9-Diazatetraasterane als erste eigenständige Klasse symmetrischer MDR-Modulatoren. *Angew. Chem.* **2002**, *114*, 3772−3775. Hilgeroth, A.; Molnár, J.; De Clercq, E. Using Molecular Symmetry to Form New Drugs: Hydroxymethyl-Substituted 3,9-Diazatetraasteranes as the First Class of Symmetric MDR Modulators. *Angew. Chem., Int. Ed.* **2002**, *41*, 3623−3625.

JM021090G