Paullones, a Series of Cyclin-Dependent Kinase Inhibitors: Synthesis, Evaluation of CDK1/Cyclin B Inhibition, and in Vitro Antitumor Activity

Christiane Schultz,[‡] Andreas Link,[‡] Maryse Leost,[§] Daniel W. Zaharevitz,[†] Rick Gussio,[†] Edward A. Sausville,[†] Laurent Meijer,[§] and Conrad Kunick*[,‡]

Institut für Pharmazie, Abteilung für Pharmazeutische Chemie, Universität Hamburg, Bundesstrasse 45, D-20146 Hamburg, Germany, Centre National de la Recherche Scientifique, Station Biologique, BP 74, F-29682 Roscoff, France, and Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Rockville, Maryland 20852

Received February 2, 1999

The paullones represent a novel class of small molecule cyclin-dependent kinase (CDK) inhibitors. To investigate structure−activity relationships and to develop paullones with antitumor activity, derivatives of the lead structure kenpaullone (9-bromo-7,12-dihydroindolo-[3,2-*d*][1]benzazepin-6(5*H*)-one, **4a**) were synthesized. Paullones with different substituents in the 2-, 3-, 4-, 9-, and 11-positions were prepared by a Fischer indole reaction starting from 1*H*-[1]benzazepine-2,5(3*H*,4*H*)-diones **5**. Selective substitutions at either the lactam or the indole nitrogen atom were accomplished by treating kenpaullone with alkyl halides in the presence of sodium hydride/THF or potassium hydroxide/acetone, respectively. S-Methylation of the kenpaullone-derived thiolactam **18** yielded the methylthioimidate **19**, which gave the hydroxyamidine **20** upon reaction with hydroxylamine. The new paullones were tested both in a CDK1/cyclin B inhibition assay and in the in vitro antitumor cell line-screening program of the National Cancer Institute (NCI). With respect to the CDK1/cyclin B inhibition, electron-withdrawing substituents in the 9-position as well as a 2,3-dimethoxy substitution on the paullone basic scaffold turned out to be favorable. A 9-trifluoromethyl substituent was found to be equivalent to the 9-bromo substituent of kenpaullone. Replacement of the 9-bromo substituent of kenpaullone by a 9-cyano or 9-nitro group produced a substantial increase in enzyme-inhibiting potency. Substitutions in other positions or the replacement of the lactam moiety led to decreased CDK1 inhibition. Noteworthy in vitro antitumor activities (GI_{50} values between 1 and 10 μM) were found with the 9-bromo-2,3-dimethoxy-7,12-dihydroindolo[3,2-*d*]-[1]benzazepin-6(5*H*)-one (**4t**), its 9-trifluoromethyl analogue **4u**, the 12-Boc-substituted paullone **15**, and the methylthioimidate **19**, respectively. The 9-nitro-7,12-dihydroindolo[3,2-*d*][1]-benzazepin-6(5*H*)-one (**4j**, named alsterpaullone) showed a high CDK1/cyclin B inhibitory activity (IC_{50} = 0.035 μM) and exceeded the in vitro antitumor potency of the other paullones by 1 order of magnitude (log GI_{50} mean graph midpoint = −6.4 M).

Introduction

The cell division cycle is driven and regulated by a variety of complex processes. Among the manifold molecular entities that are involved in the surveillance of the cell cycle, the cyclin-dependent kinases (CDKs) play a central role. The CDKs are a group of serine threonine kinases, which control the transmission between successive stages of the cell cycle.[1] The activity of the CDKs is regulated by multiple mechanisms, including binding to cyclins, a diverse class of positive regulatory CDK-binding proteins. The oscillating concentration of the cyclins during the cell cycle is the basis for the stage-dependent activity of the CDKs. Binding to CDK inhibitory proteins (CKIs) results in deactivation of CDKs. In various human tumors, deregulations of CDK-related mechanisms have been found, e.g., overexpression of cyclins or deletion of genes encoding for CKIs.[2−5] Considering these observations, CDKs are attractive targets for the development of antitumor drugs.[6−9]

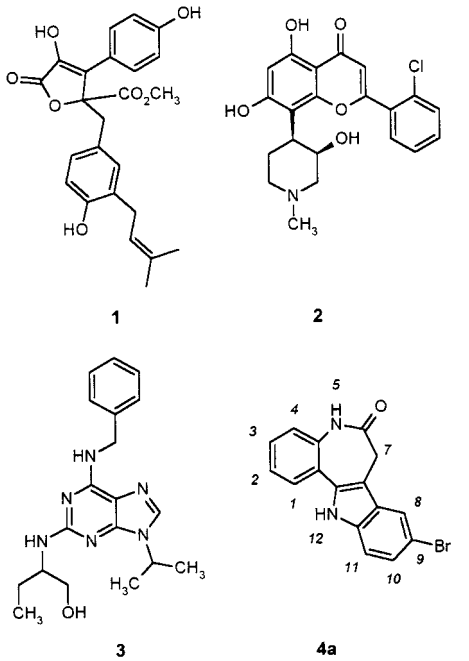
The number of chemical agents that act selectively as CDK inhibitors is limited; among them are a lactone (butyrolactone I (**1**)), flavonoids (e.g., flavopiridol (**2**)), and several purine derivatives (Chart 1). Butyrolactone I (**1**) has shown antiproliferative activity for colon and pancreatic carcinoma cell lines.[10,11] Flavopiridol (**2**) is the first CDK inhibitor that has entered clinical trials as an anticancer agent.[12,13] The class of purine-related derivatives comprises the majority of known CDK inhibitors, e.g., olomoucine,[14] roscovitine (**3**),[15,16] and the purvalanols.[17] X-ray structure determination of crystallized inhibitor−enzyme complexes revealed that the purine-derived compounds as well as flavopiridol bind to the ATP-binding pocket of the CDKs.[12,17−20]

When flavopiridol was used in a COMPARE search[21] performed at the National Cancer Institute (NCI) in a database of compounds tested in the NCI in vitro cancer cell line screening, the 9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**4a**, kenpaullone)[22] was identified as a potential CDK inhibitor.[23] Inhibition experiments revealed that **4a** indeed is a potent inhibitor of CDKs with selectivity for CDK1, CDK2, and CDK5. It was shown, that kenpaullone acts as an ATP competi-

[‡] Universität Hamburg.
[§] Centre National de la Recherche Scientifique.
[†] National Cancer Institute.

10.1021/jm9900570 CCC: $18.00
Published on Web 06/30/1999

Chart 1



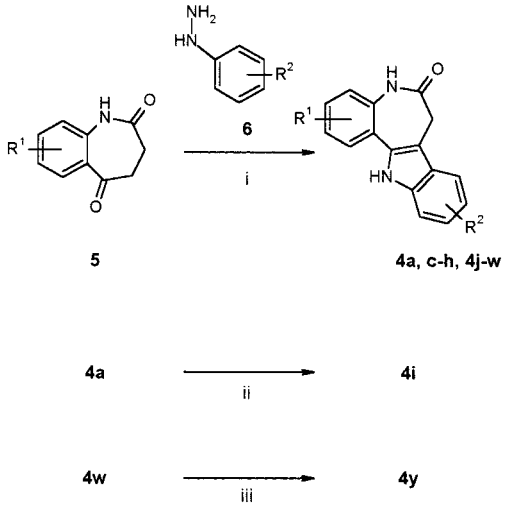
tive inhibitor of CDK1/cyclin B ($IC_{50} = 0.4\ \mu M$; apparent $K_i = 2.5\ \mu M$).[23] Although kenpaullone is equipotent to flavopiridol with respect to its inhibiting potency for CDK1/cyclin B, it exhibits only a modest antiproliferative activity in the in vitro cancer cell line screening (log GI_{50} MG_MID = −4.4 M), which is poor compared to the in vitro antitumor potency of flavopiridol (log GI_{50} MG_MID = −7.2 M).

A synthesis study was designed with a view to two main objectives. First, it was intended to search for paullone-related CDK-inhibitors with improved potency and antitumor activity. Second, information on structure−activity relationships (SAR) within the paullone series was needed for a further rational development. Special interest was devoted to the identification of the positions of the kenpaullone scaffold, which would tolerate the attachment of substituents, and to the question, which of these modifications would be detrimental for the CDK inhibitory activity.

Therefore, replacement of the 9-bromo substituent of kenpaullone by other moieties was undertaken to investigate the importance of this substitution position for CDK inhibitory activity. Additional substituents were introduced into the 2-, 3-, 4-, 10-, and 11-positions with or without keeping the 9-bromo substituent in place. The two nitrogen atoms of kenpaullone were substituted alternatively with alkyl chains, and Boc groups were introduced into the 5-, 7-, and 12-positions of kenpaullone to explore the ability of the target to accommodate bulky residues. To study the role of the secondary lactam partial structure, it was replaced by a thiolactam, a methylthioimidate, and a semicyclic hydroxyamidine group, respectively.

Results of molecular modeling experiments have been published, in which the energy-minimized structure of kenpaullone was docked to the ATP binding site of the CDK2 structure.[23] Using this model as a guide, structure-based considerations focused on the 9-position. It was postulated that substituting a hydrogen bond acceptor at this position could increase affinity by forming a

Scheme 1[a]



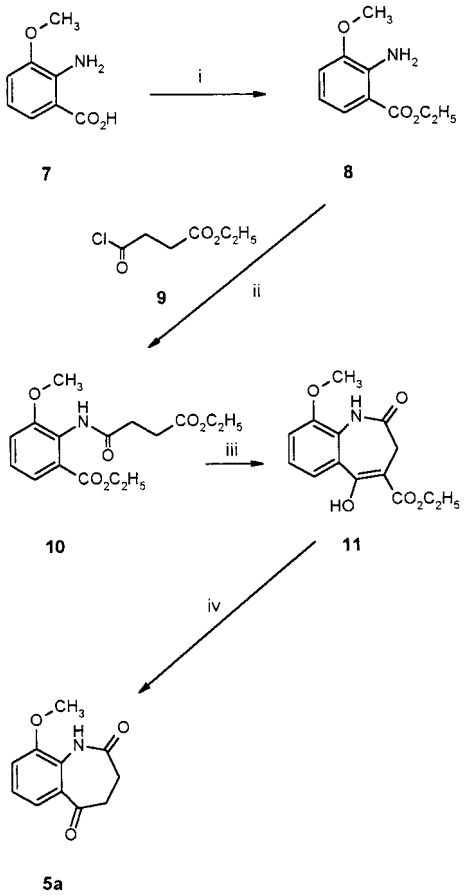
[a] Reagents and conditions: (i) 1. HOAc, 70 °C, 2. concd H_2SO_4, HOAc, 70 °C; (ii) CuCN, *N*-methyl-2-pyrrolidone, reflux; (iii) BBr_3, CH_2Cl_2, 20 °C.

hydrogen bond with a water molecule observed in the crystal structure of CDK2 with ATP bound.[24] It was further postulated that a substituent with the proper characteristics could enhance affinity by (a) increasing the strength of the hydrogen bonds to Leu-83 by increasing the resonant character of these bonds and (b) increasing the strength of the interactions with the hydrophobic side chains by rendering the ring systems relatively charge deficient. Ab initio quantum mechanical calculations, as well as molecular mechanics and hydropathic analysis, were used to investigate possible substituents. Results indicated that a 9-cyano substitution was likely to increase affinity. Details of the theoretical investigations will be reported elsewhere. Because of the remarkable improvement of the CDK1-inhibiting activity experienced with **4i**, the nitro analogue **4j** (named alsterpaullone) was prepared as an additional example of a paullone with similar electronic properties.

Chemistry

The preparation of the 7,12-dihydroindolo[3,2-*d*][1]-benzazepin-6(5*H*)-ones **4a**, **4c**, **4d**, **4k**, and **4p** by a Fischer indole synthesis has been reported previously.[22] Employing this method, the analogues **4c**−**h** and **4j**−**w** were prepared by reacting 1*H*-[1]benzazepine-2,5(3*H*,4*H*)-diones **5** with appropriate phenylhydrazines **6** in acetic acid. The intermediate phenylhydrazones were not isolated but were cyclized instantaneously by means of concentrated sulfuric acid (Scheme 1). For the preparation of the 1*H*-[1]benzazepine-2,5(3*H*,4*H*)-diones **5** several methods have been described.[25−32] For the synthetic sequences reported here, **5** were prepared by dealkoxycarbonylation of the corresponding 5-hydroxy-2-oxo-2,3-dihydro-1*H*-[1]benzazepine-4-carboxylic acid esters.[33] For example, the synthesis of the 9-methoxy-1*H*-[1]-benzazepine-2,5(3*H*,4*H*)-dione (**5a**), which was needed as a starting material to prepare the 4-methoxy paullone **4w**, is outlined in Scheme 2. The ethyl 2-amino-3-methoxybenzoate (**8**) was obtained by esterification of 2-amino-3-methoxybenzoic acid (**7**) with ethanol in the presence of gaseous hydrogen chloride. Acylation of **8**

Scheme 2[a]

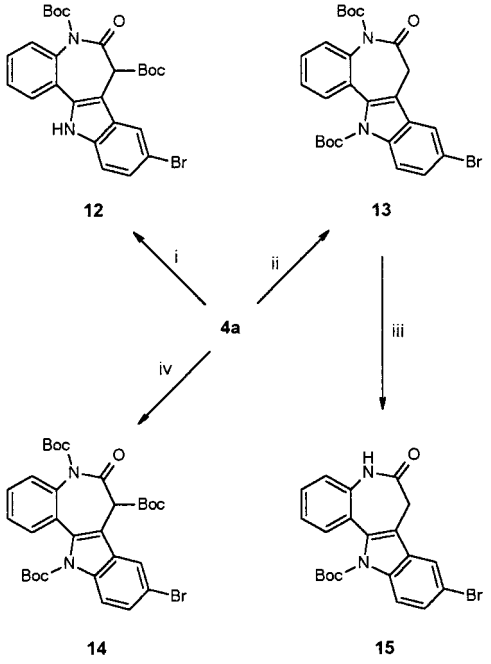


[a] Reagents and conditions: (i) HCl, EtOH, reflux; (ii) toluene, pyridine, <10 °C; (iii) KH, toluene, DMF, N_2, 80 °C; (iv) DMSO, H_2O, N_2, 150 °C.

with ethyl succinyl chloride (**9**) led to the diester **10**. A Dieckmann cyclization of (**10**) catalyzed by potassium hydride in a mixture of toluene and DMF furnished the 5-hydroxy-9-methoxy-2-oxo-2,3-dihydro-1*H*-[1]benzazepine-4-carboxylic acid ethyl ester (**11**). Heating **11** in wet DMSO yielded **5a**, which was reacted with 4-bromophenylhydrazine (**6a**) to give the 9-bromo-4-methoxy-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**4w**). The 4-hydroxy derivative **4y** of kenpaullone was obtained by treating the methyl ether **4w** with boron tribromide in dichloromethane. Employing a similar procedure, the hydroxy compound **4x** was prepared by ether cleavage from the corresponding methoxy precursor **4t**. The 9-cyano-substituted analogue **4i** was prepared by a Rosenmund−von Braun reaction upon heating kenpaullone (**4a**) with cuprous cyanide in *N*-methyl-2-pyrrolidone (Scheme 1).

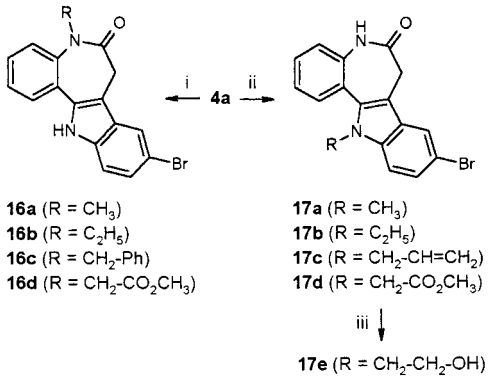
Employing varying reaction conditions and catalysts, the Boc group was introduced into three different positions of the kenpaullone molecule (Scheme 3). If kenpaullone was refluxed with 2 equiv di-*tert*-butyl dicarbonate in the presence of sodium hydride, the 5- and 7-positions were substituted to yield compound **12**. Carrying out the reaction under identical conditions with 3 equiv of di-*tert*-butyl dicarbonate led to the 3-fold substituted derivative **14**. The 5,12-disubstituted Boc derivative **13** was formed upon reacting **4a** with di-*tert*-butyl dicarbonate and 4-(dimethylamino)pyridine in dichloromethane. Treatment of **13** with 3 equiv of

Scheme 3[a]



[a] Reagents and conditions: (i) 2 equiv $(Boc)_2O$, 2 equiv NaH, THF, reflux; (ii) 3 equiv $(Boc)_2O$, DMAP, CH_2Cl_2, 20 °C; (iii) TFA, CH_2Cl_2, 20 °C; (iv) 3 equiv $(Boc)_2O$, 3 equiv NaH, THF, reflux.

Scheme 4[a]



[a] Reagents and conditions: (i) NaH, THF, alkyl halide, reflux; (ii) KOH, acetone, alkyl halide, 0−20 °C; (iii) $LiAlH_4$, THF, reflux.

trifluoroacetic acid at ambient temperature resulted in the selective cleavage of the Boc group in the 5-position, furnishing the 12-substituted kenpaullone derivative **15**.

A regioselective alkylation of the nitrogen atoms of kenpaullone was achieved using two different basic catalyst/solvent combinations (Scheme 4). On one hand, deprotonation of kenpaullone with sodium hydride in refluxing THF and the subsequent reaction with alkyl halides generated the tertiary lactams **16a**−**d**. On the other hand, reaction of kenpaullone with alkyl halides in the presence of potassium hydroxide in acetone led to substitution of the indole nitrogen atom yielding the 12-substituted derivatives **17a**−**d**.

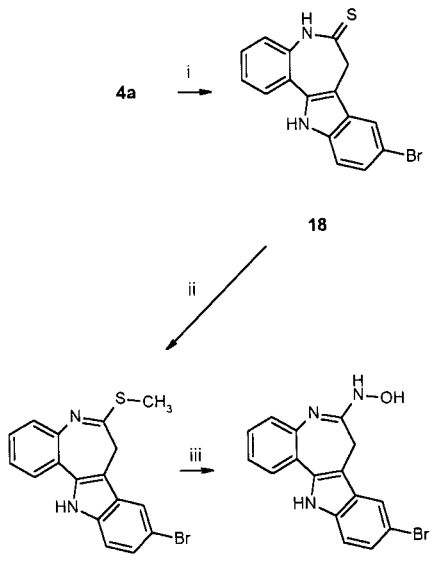
For the reduction of the ester moiety of **17d,** an excess of lithium alumino hydride was employed to yield the primary alcohol **17e** (Scheme 4). The lactam moiety remained unaffected under these conditions. A similar observation on the resistance of a lactam group for lithium alumino hydride reduction was reported for the related class of 5*H*-pyrido[3,2-*d*][1]benzazepin-6(7*H*)-ones.[34]

Table 1. CDK1/Cyclin B Inhibition and in Vitro Antitumor Activity of the Paullones **4a**−**y**

compd	R^1	R^2	IC_{50} CDK1/ cyclin B (μM)[a]	log GI_{50} HCT-116 (M)[b,c]	log GI_{50} MG_MID (M)[c,d]
2 (flavopiridol)			0.3	−7.6[e]	−7.2[e]
3 (roscovitine)[f]			0.65	−5.1/−5.1	−4.8/−4.7
4a (kenpaullone)	H	9-Br	0.4	−5.7/−5.7	−4.4/−4.3
4b	H	10-Br	1.3	−5.5	−4.8
4c	H	H	7	−4.8	−4.5
4d	H	9-Cl	0.6	−5.5	−4.1
4e	H	9-F	1.6	−5.1	−4.6
4f	H	9-OCH_3	0.9	−4.6	−4.6
4g	H	9-CH_3	2.0	>−4.0	−4.0
4h	H	9-CF_3	0.4	−5.4	−4.1
4i	H	9-CN	0.024	−4.7	−4.1
4j (alsterpaullone)	H	9-NO_2	0.035	−6.7/−7.4	−6.4/−6.5
4k	H	11-Cl	1.4	>−4.0	−4.0
4l	H	11-Br	1.3	NA[g]	−4.3
4m	H	11-Me	3.0	−4.8	−4.5
4n	H	11-Et	3.8	−4.1	−4.2
4o	H	8,10-di-Cl	2.5	>−4.0	−4.0
4p	2-Br	H	3.3	>−4.0	−4.0
4q	2-Br	9-Br	0.3	>−4.0	−4.0
4r	2-Br	9-CF_3	0.24	>−4.0	−4.0
4s	2,3-di-OCH_3	H	4.3	−4.6	−4.5
4t	2,3-di-OCH_3	9-Br	0.2	−5.8/−5.5	−5.2/−5.1
4u	2,3-di-OCH_3	9-CF_3	0.28	−5.7	−5.2/−5.3
4v	4-OCH_3	H	430	>−4.0	−4.1
4w	4-OCH_3	9-Br	250	−5.0	−4.6/−4.8
4x	2,3-di-OH	9-Br	3.0	>−4.0	−4.0
4y	4-OH	9-Br	40	−4.3	−4.4

[a] Tests were carried out in triplicate. [b] Colon cancer cell line. [c] Results of two test runs are separated by a slash. [d] MG_MID = mean graph midpoint. [e] Mean of six experiments. [f] Test results with racemic form. [g] NA, not available.

Scheme 5[a]



[a] Reagents and conditions: (i) P_2S_5, $NaHCO_3$, THF, N_2, reflux; (ii) NaH, THF, ICH_3, N_2, reflux; (iii) $H_2NOH{\cdot}HCl$, TEA, EtOH, 20 °C.

Treatment of kenpaullone with phosphorus pentasulfide led to the corresponding thiolactam **18,** as was reported previously for the parent compound **4c**.[35] S-Alkylation of **18** with iodomethane furnished the methylthioimidate **19**, which gave the hydroxyamidine **20** upon nucleophilic exchange of methanethiol for hydroxylamine (Scheme 5).

Test Results and Discussion

All new paullones were tested in a CDK1/cyclin B kinase inhibition assay described previously.[15] For this assay, CDK1/cyclin B was harvested from starfish oocytes and purified by affinity chromatography. CDK1/cyclin B activity was determined by measurement of the histone H1 phosphorylation catalyzed by the enzyme. Test results are given in Tables 1 and 2, with Table 1 including results for the paullone derivatives **4a**−**y** and the results with flavopiridol and roscovitine for comparison. Table 2 contains the results for paullones modified at the indole nitrogen atom or the lactam moiety (compounds **12**−**20**). The tests revealed that a substitution in the 9-position of the 7,12-dihydroindolo-[3,2-*d*][1]benzazepin-6(5*H*)-ones is favorable with respect to the CDK1/cyclin B inhibitory activity. Thus, the

Table 2. CDK1/Cyclin B Inhibition and in Vitro Antitumor Activity of the Kenpaullone Derivatives **12**−**20**

compd	IC_{50} CDK1/ cyclin B (μM)[a]	log GI_{50} HCT-116 (M)[b,c]	log GI_{50} MG_MID (M)[d,c]
12	80	>−4.0	−4.0
13	1000	NA[e]	−4.4
14	150	−5.1/−4.4	−4.4/−4.2
15	70	−5.6	−5.3
16a	20	−4.7	−4.5/−4.7
16b	470	>−4.0/−4.9	−4.6/−4.9
16c	35	−4.7	−4.5/−4.0
16d	6.4	−4.6/−4.8	−4.7/−4.5
17a	6.2	−5.1/−5.1	−4.5/−4.7
17b	23	−4.5	−4.3
17c	60	>−4.0	−4.2
17d	1.4	>−4.0	−4.2
17e	3.0	−4.8	−4.7
18	2.3	NA[e]	RO[f]
19	43	−5.7/−5.8	−5.5/−5.7
20	1.0	−5.2	−5.0

[a] Tests were carried out in triplicate. [b] Colon cancer cell line. [c] Results of two test runs are separated by a slash. [d] MG_MID, mean graph midpoint. [e] NA, not available. [f] RO, result omitted because of irregular shape of the dose−response curve.

9-bromo-substituted compounds **4a**, **4q**, **4t**, and **4w** showed higher activity compared to their unsubstituted counterparts **4c**, **4p**, **4s**, and **4v**, respectively. The shift of the bromo substituent from the 9-position in kenpaullone (**4a**) to the 10-position (**4b**), 11-position (**4l**), or 2-position (**4p**) resulted in decreased activity. The kinase selectivity has been investigated in detail with **4a** and its 10-bromo analogue **4b**, with results showing a considerable change in the inhibition pattern caused by the formal shift of the substituent.[23] Thus **4b**, which shows decreased inhibitory potency for CDK1, CDK2, and CDK5 (IC_{50} values > 1 μM, respectively), inhibits some protein kinase C isozymes in submicromolar concentrations. In contrast, kenpaullone (**4a**) does not inhibit protein kinase C isozymes in concentrations up to 100 μM.

Diminished CDK inhibitory activity was also found with analogues, in which the 9-bromo substituent of kenpaullone was replaced for moieties with +M or +I effects (**4f** and **4g**, respectively). Replacement of the 9-bromo substituent by a 9-trifluoromethyl group led to paullones with similar CDK1 inhibitory activity (e.g., pairs **4a**/**4h**, **4q**/**4r**, and **4t**/**4u**). The introduction of substituents into position 4 was clearly detrimental, for **4v**, **4w**, and **4y** were 2−3 orders of magnitude less active than kenpaullone. A comparison of the derivatives **4s**, **4t**, and **4u** with the analogues **4c**, **4a**, and **4h** shows that a substitution with two methoxy groups in the 2- and 3-positions is favorable regarding the CDK1/cyclin B inhibition. The replacement of the 9-bromo substituent of kenpaullone with hydrophilic, electron-withdrawing −M substituents resulted in improved CDK inhibitory potency. Thus, the 9-cyano paullone **4i** (IC_{50} = 0.024 μM) and the 9-nitro paullone **4j** (now named alsterpaullone, IC_{50} = 0.035 μM) exhibited a more than 10-fold higher potency compared to kenpaullone, flavopiridol, and roscovitine, respectively. However, **4i** and **4j** are less potent than the purvalanols (purvalanol A, IC_{50} = 0.004 μM; purvalanol B, IC_{50} = 0.006 μM[17]). None of the kenpaullone derivatives with substitution at either the lactam or the indole nitrogen atoms were superior to the lead structure with respect to the CDK1 inhibition (derivatives **12**−**17e**, Table 2). The introduction of bulky Boc groups into the kenpaullone scaffold resulted in dramatically decreased CDK1 inhibitory activity (analogues **12**−**15**). The thiolactam **18**, the thioimidate **19**, and the hydroxyamidine **20** also were inferior to the lead structure as CDK1 inhibitors.

Furthermore, the new paullones were tested in the NCIs in vitro anticancer drug discovery screen to evaluate their antiproliferative activity. The rationale,[36,37] applications,[21,38−41] and experimental procedures[42−44] of this cell line-based screening program have been described in detail. As a result of this screening, the antitumor activity of a test compound is reported for each out of 60 human tumor cell lines. For every cell line, the following parameters are calculated: log GI_{50} (GI_{50}, molar concentration inhibiting 50% net cell growth), log TGI (TGI, molar concentration for total inhibition of net cell growth), and log LC_{50} (LC_{50}, molar concentration leading to 50% net cell death). Averaged values, designated as mean graph midpoints (log MG_MID), are calculated for each of the three parameters by averaging the log parameters of all cell lines. For the calculation of the log MG_MID values, insensitive cell lines are included with the highest test concentration.

In Tables 1 and 2, the antiproliferative activities of the test compounds are given as log GI_{50} values both in terms of results on the colon cancer cell line HCT-116 and the MG_MID parameter. The HCT-116 results were selected, because this cell line was particularly sensitive to the growth inhibitory activity of kenpaullone (**4a**). A similar selectivity for HCT-116 cells was observed with several paullones (**4b**−**e**, **4h**−**j**, **4t**, **4u**, **4w**, see Table 1**)**. The results of the 9-trifluoromethyl paullones **4h**, **4r**, and **4u** closely resembled the results for the corresponding 9-bromo derivatives **4a**, **4q**, and **4t**. This observation is in accordance with the equivalence of the 9-bromo and 9-trifluoromethyl substituents with regard to CDK1 inhibition. Both 2,3-dimethoxy-substituted derivatives **4t** and **4u** exhibited interesting in vitro antitumor potency with GI_{50} values in the low-micromolar range. Alsterpaullone (**4j**) was the only compound from this study exhibiting GI_{50} activity in the submicromolar concentration range (log MG_MID GI_{50} = −6.40), representing a 100-fold improvement over kenpaullone. In contrast, the 9-cyano paullone **4i** was devoid of noteworthy in vitro antitumor activity, although it was more potent as CDK1 inhibitor compared to alsterpaullone. The reason for the marked difference between **4i** and **4j** with respect to antitumor activity is not yet clear. Interestingly, all members of the 2-bromo-substituted series of compounds (**4p**−**r**) are further examples exhibiting reasonable CDK1 inhibitory activity without showing in vitro antitumor potency. In contrast, the 12-Boc-substituted paullone **15** as well as the methylthioimidate **19**, which both are weak CDK1 inhibitors only, gave promising results in the antitumor cell line screening with GI_{50} concentrations between 1 and 10 μM. Apparently, the CDK1/cyclin B inhibition is not likely to be the crucial biological property determining the antiproliferative activity of the latter derivatives. Considering the structural similarity of the ATP binding site in different kinases, it may be speculated that the antitumor activity of the paullone derivatives **15** and **19** is due to the inhibition of other kinases involved in cell division. For a further rational development in the paullone series, inhibition studies on a broad array of kinases with representative derivatives would be helpful.

Conclusions

The present structure−activity relationship study revealed that electron-withdrawing substituents in the 9-position as well as a 2,3-dimethoxy substitution on the paullone basic scaffold are favorable with respect to the CDK1/cyclin B inhibitory activity. A 9-trifluoromethyl substituent was found to be equivalent to a 9-bromo substituent, whereas introduction of a 9-cyano or 9-nitro group produced a substantial increase in enzyme-inhibiting potency. Substitutions in the 4-, 5-, and 12-positions as well as replacement of the lactam structure for a thiolactam, a thioimidate, or a hydroxyamidine led to decreased CDK1 inhibition. Evaluation of the new paullones in a cancer cell line screening showed that the antiproliferative activity is not necessarily paralleling the CDK inhibitory potency. Hence, CDK1 inhibi-

tory paullones with a bromo substitution in the 2-position (**4p**−**r**) and the 9-cyano paullone **4i** were devoid of noteworthy in vitro antitumor activity. The 12-substituted paullone **15** and the methylthioimidate **19** exhibited reasonable antitumor activity, although both compounds turned out to be poor CDK1 inhibitors. The 9-nitro paullone (**4j**, alsterpaullone) exceeded the CDK1 inhibitory potency of both flavopiridol and roscovitine and exhibited a remarkable in vitro antitumor activity. Therefore, alsterpaullone was selected for preclinical development in a NCI program. Future studies will be directed to the design of new paullones incorporating a combination of substituents with beneficial effects. Furthermore, the biochemical mechanism underlying the antitumor activity of paullones with poor CDK inhibitory potency remains to be investigated.

Experimental Section

Synthetic Chemistry. Melting points were determined on an electric variable heater (Gallenkamp) and are not corrected. Elemental analyses were performed in the analytical department of the Institut für Pharmazie, Universität Hamburg. Results obtained were within ±0.4% unless indicated otherwise. Infrared spectra were recorded using KBr pellets on a Pye-Unicam SP 3-200 S, a Philips PU 9712, or a Perkin-Elmer 1660 FTIR spectrometer, respectively. Nuclear magnetic resonance spectra were recorded on a Bruker AMX 400 instrument, using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. NMR signals are reported in ppm on a δ scale. TLC analyses were carried out on fluorescent Polygram Sil G/UV_{254} silica gel plates. Spots were visualized under 254-nm UV illumination. The following compounds were prepared according to literature methods: 1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione,[33] 7-bromo-1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione,[33] and **4a**−**d**, **4k**, **4p**.[22]

Synthesis of 7,12-Dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-ones, General Procedure A. To a slurry of an appropriate 1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione (1 mmol) in glacial acetic acid (2 mL) was added a suspension of the appropriate corresponding phenylhydrazine (1.5 mmol) [or the appropriate substituted phenylhydrazine hydrochloride (1.5 mmol) and sodium acetate (123 mg, 1.5 mmol)] in glacial acetic acid (5 mL) dropwise with stirring. After stirring at 70 °C for 1 h the mixture was cooled to room temperature. Concentrated sulfuric acid (0.1 mL) was added, and stirring was continued for 1 h at 70 °C. After cooling to room temperature, the mixture was poured into a 5% aqueous sodium acetate solution (15 mL). A precipitate was formed, which was filtered off with suction and purified by crystallization from the given solvent.

Synthesis of Phenols by Cleavage of Methoxy Compounds, General Procedure B. Boron tribromide (1002 mg, 4 mmol) was added to a solution of the appropriate methoxy compound (1 mmol) in dichloromethane (10 mL). The mixture was stirred by means of a magnetic stirrer, and the reaction was monitored by thin-layer chromatography (silica gel, eluent acetone/toluene, 1:1). When the spot caused by the starting methoxy compound was no longer detectable, water (10 mL) was added and the mixture stirred for 1 h. A solid formed, which was filtered off with suction, washed with water, and crystallized for purification.

Synthesis of 12-Substituted 9-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-ones by Reaction with Alkyl Halides, General Procedure C. Powdered potassium hydroxide (56 mg, 1 mmol) was added with stirring and cooling by an ice bath to a solution of **4a** (327 mg, 1 mmol) in dry acetone (120 mL). After the mixture stirred for 1 h at 0 °C, the appropriate alkyl halide (10 mmol) was added and stirring continued for 3 days at room temperature. After addition of water (120 mL) a solid formed, which was filtered off and crystallized from ethanol/toluene.

4,5-Dimethoxy-2-[(4-methoxy-1,4-dioxobutyl)amino]-benzoic Acid Methyl Ester. A solution of methyl succinyl chloride (3764 mg, 25 mmol) (purchased from Aldrich) in toluene (5 mL) was added dropwise to a solution of 2-amino-4,5-dimethoxybenzoic acid methyl ester (purchased from Fluka) (4224 mg, 20 mmol) and pyridine (2.3 mL) in toluene (30 mL) with stirring and cooling. The resulting suspension was refluxed for 2 h. After the mixture cooled to room temperature, water (2 mL) was added. The mixture was then poured into a separating funnel, and a sufficient amount of dichloromethane was added to ensure a plain separation of the organic and aqueous layers. The organic layer was separated, washed successively with 10% hydrochloric acid (10 mL) and 5% aqueous sodium carbonate solution, dried over sodium sulfate, and evaporated. The residue was crystallized from ethanol to yield 87% colorless crystals: mp 115 °C; IR 3220 (NH), 1710, 1655 cm^{-1} (C═O); ^{1}H NMR (400 MHz) 2.63−2.68 (m, 4H, CH_2−CH_2), 3.61 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 7.39 (s, 1H), 8.13 (s, 1H), 10.81 (br s, 1H, NH). Anal. ($C_{15}H_{19}NO_7$) C, H, N.

7,8-Dimethoxy-5-hydroxy-2-oxo-2,3-dihydro-1*H*-[1]benzazepine-4-carboxylic Acid Methyl Ester. A solution of 4,5-dimethoxy-2-[(4-methoxy-1,4-dioxobutyl)amino]benzoic acid methyl ester (650 mg, 2 mmol) and *N*,*N*-dimethylformamide (1 mL) in toluene (12 mL) was added dropwise to a stirred suspension of powdered potassium hydride (400 mg, 10 mmol) in toluene (4 mL) with cooling and under nitrogen. [**Be cautious when handling potassium hydride! Carefully keep away water or moisture!**] After the hydrogen evolution had ceased, the mixture was stirred for 3 h at 80 °C under nitrogen. The mixture was cooled to room temperature, and successively acetic acid (0.6 mL) and water (6 mL) were added dropwise and with **caution** under nitrogen. The mixture was chilled in an ice bath and stirred for 15 min. The precipitate was filtered off with suction, washed with water and hexanes and crystallized from ethanol/toluene to yield 29% yellowish crystals: mp 256 °C dec; IR 3180 (NH), 1665, 1640 cm^{-1} (C═O); ^{1}H NMR (400 MHz) 2.91 (s, 2H, CH_2), 3.79 and 3.80 (2 s, 6 H, OCH_3, overlapping signals), 3.82 (s, 3H, OCH_3), 6.76 (s, 1H), 7.22 (s, 1H), 10.08 (s, 1H, NH), 12.54 (br s, 1H, OH). Anal. ($C_{14}H_{15}NO_6$) C, H, N.

7,8-Dimethoxy-1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione. A solution of 2,3-dihydro-7,8-dimethoxy-5-hydroxy-2-oxo-1*H*-[1]-benzazepine-4-carboxylic acid methyl ester (293 mg, 1 mmol) and water (0.5 mL) in dimethyl sulfoxide (10 mL) was stirred at 150 °C under nitrogen. Portions of water (0.5 mL) were added after 1 and 3 h of heating, respectively. After stirring for an overall 4 h at 150 °C, the mixture was allowed to cool to room temperature and then poured into water (20 mL). After standing for 12 h at 4 °C, crystals formed, which were filtered off and washed with water and hexanes. The material so obtained was analytically pure and used without further purification: yellowish crystals (90%), mp 234 °C (lit.[45] mp 183 °C dec); IR 3230 (NH), 1660, 1640 cm^{-1} (C═O); ^{1}H NMR (400 MHz) 2.60−2.85 (m, 4H, CH_2−CH_2), 3.76 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.79 (s, 1H), 7.32 (s, 1H), 9.86 (s, 1H, NH). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

9-Fluoro-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4e). Prepared according to general procedure A. Purification by column chromatography (6-cm column of silica gel 60A, 100−200 mesh, eluent dichloromethane) yielded 52% cream-colored powder: mp > 330 °C (shrinking starting at 180 °C); IR 3220 (NH), 1635 cm^{-1} (C═O); ^{1}H NMR (400 MHz) 3.50 (s, 2H, CH_2), 7.00 (d "t", 1H, 2.5/9.2/9.2 Hz), 7.23−7.31 (m, 2H), 7.35−7.44 (m, 2H), 7.48 (dd, 1H, 2.5/9.7 Hz), 7.73 (dd, 1H, 1.5/7.6 Hz), 10.08 (s, 1H, lactam-NH), 11.67 (s, 1H, indole-NH). Anal. ($C_{16}H_{11}FN_2O$); C, H, N.

9-Methoxy-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-(5*H*)-one (4f). Prepared according to general procedure A. Purification by column chromatography (6-cm column of silica gel 60A, 100−200 mesh, eluent dichloromethane) yielded 48% cream-colored powder: mp > 330 °C (shrinking starting at 290 °C); IR 3200 (NH), 1640 cm^{-1} (C═O); ^{1}H NMR (400 MHz) 3.49 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 6.81 (dd, 1H, 2.0/8.6 Hz), 7.17 (d, 1H, 2.5 Hz), 7.22−7.28 (m, 2H), 7.30−7.38 (m, 2H), 7.72

(dd, 1H, 7.6 Hz), 10.04 (s, 1H, lactam-NH), 11.38 (s, 1H, indole-NH). Anal. ($C_{17}H_{14}N_2O_2$) C, H, N.

9-Methyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4g). Prepared according to general procedure A to yield 59% cream-colored crystals: mp > 330 °C (ethanol); IR 3220 (NH), 1640 cm^{-1} (C═O); ^1H NMR (400 MHz) 2.41 (s, 3H, CH_3), 3.46 (s, 2H, CH_2), 7.00 (dd, 1H, 1.0/8.1 Hz), 7.22−7.38 (m, 4H), 7.43 (s, 1H), 7.73 (d, 1H, 6.9 Hz), 10.05 (s, 1H, lactam-NH), 11.42 (s, 1H, indole-NH). Anal. ($C_{17}H_{14}N_2O$) C, H, N.

9-(Trifluoromethyl)-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4h). Prepared according to general procedure A to yield 33% cream-colored crystals: mp > 330 °C (ethanol); IR 3200 (NH), 1650 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.61 (s, 2H, CH_2), 7.27−7.32 (m, 2H), 7.40−7.47 (m, 2H), 7.62 (d, 1H, 8.6 Hz), 7.78 (dd, 1H, 1.5/7.6 Hz), 8.13 (s, 1H), 10.15 (s, 1H, lactam-NH), 12.06 (s, 1H, indole-NH). Anal. ($C_{17}H_{11}FN_2O$) C, H, N.

9-Cyano-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4i). A mixture of **4a** (327 mg, 1 mmol) and copper(I) cyanide (180 mg, 2 mmol) in *N*-methyl-2-pyrrolidone (10 mL) was refluxed for 2 h. After the mixture cooled to room temperature, water (10 mL) was added. The precipitate was filtered off with suction and washed with water. The precipitate was then suspended in a mixture of water (10 mL) and 1,2-diaminoethane (25 mL). After stirring for 15 min, the solid was filtered off with suction, washed twice with 10% aqueous sodium cyanide solution, and crystallized twice from ethanol/toluene to yield 74% colorless crystals: mp > 330 °C; IR 3350, 3180 (NH), 2200 (CN), 1670 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.59 (s, 2H, CH_2), 7.27−7.32 (m, 2H), 7.43 (dt, 1H, 1.0/7.6 Hz), 7.51 (dd, 1H, 1.3/8.4 Hz), 7.59 (d, 1H, 8.1 Hz), 7.76 (dd, 1H, 1.0/7.6 Hz), 8.32 (s, 1H), 10.16 (s, 1H, lactam-NH), 12.19 (s, 1H, indole-NH). Anal. ($C_{17}H_{11}N_3O$) C, H, N.

9-Nitro-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (Alsterpaullone, 4j). A mixture of 1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione (175 mg, 1 mmol), 4-nitrophenylhydrazine hydrochloride (284 mg, 1.5 mmol), and sodium acetate (123 mg, 1.5 mmol) in glacial acetic acid (10 mL) was stirred at 70 °C. After 1 h sulfuric acid (0.1 mL) was added. While stirring was continued for 3 h at 70 °C, additional portions of concentrated sulfuric acid (0.1 mL, respectively) were added after each hour of stirring. The mixture was cooled to room temperature and poured into 5% sodium acetate solution (20 mL). A precipitate was formed, which was filtered off with suction, washed with water, and crystallized from ethanol/toluene to yield 33% yellow crystals: mp > 330 °C; IR 3380 (NH), 1660 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.65 (s, 2H, azepine-CH_2), 7.29−7.34 (m, 2H), 7.43−7.47 (m, 1H), 7.60 (d, 9.2 Hz, 1H), 7.77−7.79 (m, 1H), 8.08 (dd, 8.6/2.0 Hz, 1H), 8.74 (d, 2.0 Hz, 1H), 10.22 (s, 1H, NH), 12.39 (s, 1H, NH). Anal. ($C_{16}H_{11}N_3O_3$) C, H, N.

11-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-(5*H*)-one (4l). Prepared according to general procedure A yielding 58% yellow crystals: mp > 330 °C (ethanol); IR 3190 (NH), 1640 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.49 (s, 2H, CH_2), 7.04 ("t", 1H, 7.9 Hz), 7.22−7.31 (m, 2H), 7.40 (d, 2H, 7.6 Hz), 7.71 (d, 1H, 7.6 Hz), 7.91 (dd, 1H, 1.5/8.1 Hz), 10.10 (s, 1H, lactam-NH), 11.58 (s, 1H, indole-NH). Anal. ($C_{16}H_{11}BrN_2O$) H, N; C: calcd, 58.74; found 58.18. Br: calcd, 24.42; found, 23.70.

11-Methyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-(5*H*)-one (4m). Prepared according to general procedure A yielding 42% beige crystals: mp > 330 °C (ethanol); IR 3200 (NH), 1640 cm^{-1} (C═O); ^1H NMR (400 MHz) 2.54 (s, 3H, CH_3), 3.47 (s, 2H, azepine-CH_2), 6.95−7.01 (m, 2H), 7.24−7.30 (m, 2H), 7.37 (dt, 7.6/1.5 Hz), 7.47 (dd, 6.9/1.3 Hz, 1H), 7.86 (dd, 7.6/1.5 Hz, 1H), 10.07 (s, 1H, NH), 11.26 (s, 1H, NH). Anal. ($C_{17}H_{14}N_2O$) H, N; C: calcd, 77.84; found, 77.21.

11-Ethyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4n). Prepared according to general procedure A yielding 49% beige-yellow crystals: mp > 330 °C (ethyl acetate); IR 3250/3210 (NH), 1640 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.29 (t, 5.9 Hz, 3H, CH_3), 2.96 (q, 6.0 Hz, 2H, CH_2), 3.47 (s, 2H, azepine-CH_2), 6.99−7.03 (m, 2H), 7.25 (dd, 6.3/0.8 Hz, 1H), 7.28 (dt, 6.0/0.8 Hz, 1H), 7.37 (dt, 6.1/1.0 Hz, 1H), 7.47 (dd, 5.9/1.1 Hz, 1H), 7.86 (dd, 6.2/1.1 Hz, 1H), 10.05 (s, 1H, NH), 11.25 (s, 1H, NH). Anal. ($C_{18}H_{16}N_2O$) C, H, N.

8,10-Dichloro-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4o). Prepared according to general procedure A to yield 55% colorless crystals: mp > 330 °C (ethanol/toluene); IR 3280 (NH), 1650 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.80 (s, 2H, CH_2), 7.18 (d, 1H, 1.5 Hz), 7.27 (d, 1H, 6.6 Hz), 7.31 (t, 1H, 6.1 Hz), 7.42−7.44 (m, 1H), 7.45 (d, 1H, 1.2 Hz), 7.73−7.75 (m, 1H), 10.19 (s, 1H, lactam-NH), 12.17 (s, 1H, indole-NH). Anal. ($C_{16}H_{10}Cl_2N_2O$) C, H, Cl, N.

2,9-Dibromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4q). Prepared according to general procedure A to yield 41% brown crystals: mp > 330 °C (ethanol/toluene); IR 3200 (NH), 1640 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.55 (br s, 2H, CH_2), 7.20 (d, 1H, 8.6 Hz), 7.30 (dd, 1H, 1.5/8.6 Hz), 7.40 (d, 1H, 8.6 Hz), 7.56 (dd, 1H, 2.5/8.6 Hz), 7.92 (d, 1H, 2.6 Hz), 7.93 (d, 1H, 2.0 Hz), 10.20 (s, 1H, lactam-NH), 11.89 (s, 1H, indole-NH). Anal. ($C_{16}H_{10}Br_2N_2O$) C, H, Br, N.

2-Bromo-9-(trifluoromethyl)-7,12-dihydroindolo[3,2-*d*]-[1]benzazepin-6(5*H*)-one (4r). Prepared according to general procedure A, yielding 51% colorless crystals: mp > 330 °C (ethanol/toluene); IR 3300 (NH), 1635 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.64 (s, 2H, CH_2), 7.23 (d, 1H, 9.2 Hz), 7.48 (dd, 1H, 1.0/8.7 Hz), 7.58−7.63 (m, 2H), 7.95 (d, 1H, 2.0 Hz), 8.15 (s, 1H), 10.25 (s, 1H, lactam-NH), 12.15 (s, 1H, indole-NH). Anal. ($C_{17}H_{10}BrF_3N_2O$) C, H, Br, N.

2,3-Dimethoxy-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4s). Prepared according to general procedure A, yielding 73% cream-colored crystals: mp > 330 °C (ethanol/toluene); IR 3350, 3220 (NH), 1660 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.44 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.87 (s, 1H), 7.04−7.08 (m, 1H), 7.13−7.17 (m, 1H), 7.28 (s, 1H), 7.43 (d, 1H, 8.2 Hz), 7.62 (d, 1H, 7.6 Hz), 9.79 (s, 1H, lactam-NH), 11.46 (s, 1H, indole-NH). Anal. ($C_{18}H_{16}N_2O_3$) C, H, N.

9-Bromo-2,3-dimethoxy-7,12-dihydroindolo[3,2-*d*][1]-benzazepin-6(5*H*)-one (4t). Prepared according to general procedure A from 7,8-dimethoxy-1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione, yielding 55% red-brown crystals: mp > 330 °C (ethanol/toluene); IR 3340, 3210 (NH), 1660 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.45 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.87 (s, 1H), 7.23−7.27 (m, 2H), 7.39 (d, 1H, 8.6 Hz), 7.86 (d, 1H, 2.0 Hz), 9.83 (s, 1H, lactam-NH), 11.70 (s, 1H, indole-NH). Anal. ($C_{18}H_{15}BrN_2O_3$) C, H, Br, N.

2,3-Dimethoxy-9-(trifluoromethyl)-7,12-dihydroindolo-[3,2-*d*][1]benzazepin-6(5*H*)-one (4u). Prepared according to general procedure A yielding 38% pale-yellow crystals: mp > 330 °C (ethanol); IR 3240 (NH), 1635 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.55 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.90 (s, 1H), 7.31 (s, 1H), 7.43 (dd, 1H, 1.0/8.6 Hz), 7.61 (d, 1H, 8.6 Hz), 8.08 (s, 1H), 9.87 (s, 1H, lactam-NH), 11.96 (s, 1H, indole-NH). Anal. ($C_{19}H_{15}F_3N_2O_3$) C, H, N.

4-Methoxy-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6-(5*H*)-one (4v). Prepared according to general procedure A yielding 47% beige crystals: mp 285 °C (ethanol/toluene); IR 3380, 3250 (NH), 1640 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.48 (br s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 7.05−7.10 (m, 2H), 7.15−7.19 (m, 1H), 7.27 (t, 1H, 8.1 Hz), 7.35 (dd, 1H, 1.3/7.9 Hz), 7.44 (d, 1H, 7.6 Hz), 7.66 (d, 1H, 8.1 Hz), 8.77 (s, 1H, lactam-NH), 11.58 (s, 1H, indole-NH). Anal. ($C_{17}H_{14}N_2O_2$) C, H, N.

9-Bromo-4-methoxy-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4w). Prepared according to general procedure A, yielding 67% brownish crystals: mp > 330 °C (ethanol/toluene); IR 3300 (NH), 1650 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.49 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 7.12 (dd, 1H, 1.3/7.9 Hz), 7.27−7.35 (m, 3H), 7.40 (d, 1H, 8.6 Hz), 7.92 (d, 1H, 2.0 Hz), 8.84 (s, 1H, lactam-NH), 11.82 (s, 1H, indole-NH). Anal. ($C_{17}H_{13}BrN_2O_2$) C, H, Br, N.

9-Bromo-2,3-dihydroxy-7,12-dihydroindolo[3,2-*d*][1]-benzazepin-6(5*H*)-one (4x). Prepared according to general procedure B from **4t**, reaction time 1 h, yielding 30% colorless crystals: mp > 330 °C (ethanol/toluene); IR 3400 (OH), 3260 (NH), 1610 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.39 (s, 2H, CH_2), 6.68 (s, 1H), 7.06 (s, 1H), 7.20 (dd, 1H, 1.5/8.6 Hz), 7.33 (d,

1H, 8.7 Hz), 7.79 (d, 1H, 1.5 Hz), 9.03 (s, 1H, OH), 9.52 (s, 1H, OH), 9.69 (s, 1H, lactam-NH), 11.55 (s, 1H, indole-NH). Anal. ($C_{16}H_{11}BrN_2O_3$) C, H, N; Br: calcd, 22.25; found, 21.75.

9-Bromo-4-hydroxy-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (4y). Prepared according to general procedure B from **4w** (357 mg, 1 mmol), reaction time 24 h, yielding 35% colorless crystals: mp > 330 °C (ethanol); IR 3420, 3180 (NH), 1615 cm^{-1} (C═O); ^1H NMR (400 MHz) 3.49 (s, 2H, CH_2), 6.94 (dd, 1H, 1.3/7.9 Hz), 7.12 (t, 1H, 7.9 Hz), 7.20 (dd, 1H, 1.3/7.9 Hz), 7.26 (dd, 1H, 2.0/8.7 Hz), 7.39 (d, 1H, 8.7 Hz), 7.90 (d, 1H, 2.0 Hz), 8.57 (s, 1H, lactam-NH), 10.23 (br s, 1H, OH), 11.74 (s, 1H, indole-NH). Anal. ($C_{16}H_{11}BrN_2O_2$) C, H, Br, N.

2-Amino-3-methoxybenzoic Acid Ethyl Ester (8). 2-Amino-3-methoxybenzoic acid (167 mg, 1 mmol) (purchased from Aldrich) was dissolved in dry ethanol (7 mL). Gaseous hydrogen chloride was bubbled through the mixture, until 3 g of hydrogen chloride was absorbed (requires approximately 20 min). The mixture was refluxed for 5 h and subsequently evaporated to dryness. The residue was dissolved in water (20 mL), and the solution was neutralized by addition of 5% sodium acetate solution. The neutral solution was extracted five times with dichloromethane (each portion 10 mL). The combined organic layers were washed with 5% aqueous sodium carbonate solution (20 mL), dried by means of sodium sulfate, and evaporated to dryness to yield 81% colorless crystals, which were used without further purification: mp 40−41 °C, IR 3480, 3360 (NH), 1680 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.30 (t, 3H, 7.1 Hz, CH_2-CH_3), 3.82 (s, 3H, OCH_3), 4.26 (q, 2H, 7.1 Hz, CH_2-CH_3), 6.32 (br s, 2H, NH_2), 6.52 (t, 1H, 8.1 Hz), 6.98 (d, 1H, 7.6 Hz), 7.35 (d, 1H, 8.6 Hz). Anal. ($C_{10}H_{13}NO_3$) C, H, N.

2-[(4-Ethoxy-1,4-dioxobutyl)amino]-3-methoxybenzoic Acid Ethyl Ester (10). A solution of ethyl succinyl chloride (**9**) (2633 mg, 16 mmol) (purchased from Aldrich) in toluene (2 mL) was added dropwise to a stirred solution of 2-amino-3-methoxybenzoic acid ethyl ester (2340 mg, 12 mmol) and pyridine (1.4 mL) in toluene (5 mL) at 0−10 °C. After the resulting suspension stirred for 1.5 h below 10 °C, water (2 mL) was added. The resulting organic layer was separated using a separating funnel, washed with 10% hydrochloric acid (2 mL) and 5% aqueous sodium carbonate solution (2 mL), dried by means of sodium sulfate, and evaporated. After the oily residue was heated with ethanol (2 mL), a crystalline solid formed, which was crystallized from ethanol to yield 59% colorless crystals: mp 59−60 °C; IR 3250 (NH), 1710, 1690, 1650 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.18 (t, 3H, 7.1 Hz, CH_2-CH_3), 1.23 (t, 3H, 7.1 Hz, CH_2-CH_3), 2.50 (t, 2H, CH_2-CH_2, overlapping the DMSO signal), 2.59 (t, 2H, 6.6 Hz, CH_2-CH_2), 3.82 (s, 3H, OCH_3), 4.04 (q, 2H, 7.1 Hz, CH_2-CH_3), 4.14 (q, 2H, 7.1 Hz, CH_2-CH_3), 7.20−7.25 (m, 3H), 9.49 (s, 1H, NH). Anal. ($C_{16}H_{21}NO_6$) C, H, N.

5-Hydroxy-9-methoxy-2-oxo-2,3-dihydro-1*H*-[1]benzazepine-4-carboxylic Acid Ethyl Ester (11). **10** (323 mg, 1 mmol) was dissolved in a mixture of *N*,*N*-dimethylformamide (0.44 mL) and toluene (2 mL). The solution was added dropwise to a stirred suspension of powdered potassium hydride (0.2 g, 5 mmol) in toluene (2 mL) under nitrogen with cooling. [**Be cautious when handling potassium hydride! Carefully keep away water or moisture!**] After the hydrogen evolution had ceased, the mixture was stirred under nitrogen for 1.5 h at 80 °C. Subsequently, the mixture was neutralized by **cautious** addition of acetic acid (0.3 mL). Water (3 mL) was then added dropwise, and the organic layer was separated by means of a separating funnel. The organic layer was evaporated, and the residue was crystallized from ethanol, yielding 66% yellow crystals: mp 124 °C; IR 3160 (NH), 1650, 1625 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.31 (t, 3H, 7.1 Hz, CH_2-CH_3), 2.91 (s, 2H, azepine-CH_2), 3.87 (s, 3H, OCH_3), 4.30 (q, 2H, 7.1 Hz, CH_2-CH_3), 7.22−7.28 (m, 2H), 7.37 (dd, 1H, 2.5/6.6 Hz), 9.23 (s, 1H, NH), 12.53 (br s, 1H, OH). Anal. ($C_{14}H_{15}NO_5$) C, H, N.

9-Methoxy-1*H*-[1]benzazepine-2,5(3*H*,4*H*)-dione (5a). **11** (277 mg, 1 mmol) was dissolved in dimethyl sulfoxide (10 mL). Water (0.5 mL) was added, and the mixture was stirred under nitrogen at 150 °C. After 1 and 3 h of stirring, portions of water (0.5 mL, respectively) were added. After stirring for an overall 4 h at 150 °C, the mixture was allowed to cool to room temperature, and water (20 mL) was added. The mixture was extracted three times with dichloromethane (portions of 20 mL). The combined organic layers were then washed with water (20 mL), dried over sodium sulfate, and evaporated to yield 87% beige crystals: mp 99 °C; IR 3220 (NH), 1645 cm^{-1} (C═O); ^1H NMR (400 MHz) 2.65−2.94 (m, 4H, CH_2-CH_2), 3.87 (s, 3H, OCH_3), 7.19 (t, 1H, 8.1 Hz), 7.29 (dd, 1H, 1.5/8.1 Hz), 7.32 (dd, 1H, 1.5/7.6 Hz), 8.73 (s, 1H, NH). Anal. ($C_{11}H_{11}NO_3$) C, H, N.

9-Bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepine-5,7-dicarboxylic Acid Di-*tert*-butyl Ester (12). A solution of **4a** (327 mg, 1 mmol) in THF (40 mL) was refluxed with sodium hydride (48 mg, 2 mmol, 60% suspension in white oil) for 1 h. Di-*tert*-butyl dicarbonate (436 mg, 2 mmol) was added, and refluxing was continued for 12 h. After cooling to room temperature the mixture was poured on ice water (100 mL). A precipitate was formed, which consisted of a mixture of unreacted starting material, a side product, and the desired product. Repeated crystallization from ethanol afforded the title compound as colorless crystals in 25% yield: mp > 330 °C (dec starting at 285 °C); IR 3260 (NH), 1755, 1680 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.28 (br s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$), 6.82 (br s, 1H, azepine-CH), 7.32−7.37 (m, 2H), 7.44 (d, 1H, 8.6 Hz), 7.51−7.59 (m, 2H), 7.81 (dd, 1H, 1.5/7.6 Hz), 7.95 (d, 1H, 1.5 Hz), 12.15 (s, 1H, NH). Anal. ($C_{26}H_{27}BrN_2O_5$) C, H, Br, N.

9-Bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepine-5,12-dicarboxylic Acid Di-*tert*-butyl Ester (13). A solution of **4a** (327 mg, 1 mmol), (dimethylamino)pyridine (DMAP) (122 mg, 1 mmol), and di-*tert*-butyl dicarbonate (655 mg, 3 mmol) in dry dichloromethane (50 mL) was stirred at room temperature for 4 h. The mixture was then evaporated to dryness, and the residue was crystallized from ethanol to yield 85% colorless crystals: mp 196 °C; IR 1770, 1725, 1680 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.36 (s, 9H, $C(CH_3)_3$), 1.37 (s, 9H, $C(CH_3)_3$), 3.04 (d, 1H, 14.2 Hz, azepine-CH), 4.02 (d, 1H, 14.2 Hz, azepine-CH), 7.40−7.43 (m, 1H), 7.44−7.51 (m, 2H), 7.57 (dd, 1H, 2.0/9.1 Hz), 7.63 (dd, 1H, 1.8/7.4 Hz), 8.12−8.14 (m, 2H). Anal. ($C_{26}H_{27}BrN_2O_5$) C, H, Br, N.

9-Bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepine-5,7,12-tricarboxylic Acid Tri-*tert*-butyl Ester (14). A solution of **4a** (327 mg, 1 mmol) in THF (40 mL) was refluxed with sodium hydride (72 mg, 3 mmol, 60% suspension in white oil) for 1 h. Di-*tert*-butyl dicarbonate (655 mg, 3 mmol) was added, and refluxing was continued for 2 h. After the mixture cooled to room temperature, water (100 mL) was added. The mixture was extracted three times with dichloromethane (20 mL each portion). The combined organic layers were dried with sodium sulfate and evaporated to furnish a residue, which was crystallized from ethanol to yield 48% colorless crystals: mp 193 °C; IR 1760, 1715, 1660 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.30 (br s, 9H, $C(CH_3)_3$), 1.36 (s, 9H, $C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$), 6.87 (br s, 1H, azepine-CH), 7.35 (d, 1H, 8.1 Hz), 7.44−7.49 (m, 2H), 7.55−7.62 (m, 2H), 8.06−8.08 (m, 2H). Anal. ($C_{31}H_{35}BrN_2O_7$) C, H, Br, N.

9-Bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepine-12-carboxylic Acid *tert*-Butyl Ester (15). Trifluoroacetic acid (0.25 mL, 3.25 mmol) was added dropwise to a solution of **13** (527 mg, 1 mmol) in dry dichloromethane (20 mL). After the mixture stirred for 2 h at room temperature, water (40 mL) was added and the mixture was neutralized by addition of concentrated aqueous ammonia. The organic layer was separated by means of a separating funnel, subsequently dried with sodium sulfate, and evaporated to yield a solid, which upon crystallization from ethanol furnished 60% colorless crystals: mp > 330 °C; IR 3150 (NH), 1730, 1660 cm^{-1} (C═O); ^1H NMR (400 MHz) 1.37 (s, 9H, $C(CH_3)_3$), 3.18 (br s, 1H, azepine-CH, overlapping the H_2O signal), 3.66 (br s, 1H, azepine-CH, overlapping the H_2O signal), 7.23−7.27 (m, 2H), 7.38−7.43 (m, 1H), 7.49 (dd, 1H, 1.3/7.9 Hz), 7.54 (dd, 1H, 2.0/

9.2 Hz), 8.04 (d, 1H, 8.6 Hz), 8.08 (d, 1H, 1.5 Hz), 10.14 (s, 1H, NH). Anal. ($C_{21}H_{19}BrN_2O_3$) C, H, Br, N.

9-Bromo-5-methyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (16a). Sodium hydride (24 mg, 1 mmol, 60% suspension in white oil) was added to a solution of **4a** (327 mg, 1 mmol) in THF (25 mL). After the mixture refluxed for 45 min, iodomethane (71 mg, 0.5 mmol) was added and refluxing was continued. After 2 and 4 h portions of iodomethane (71 mg, 0.5 mmol, respectively) were added. After refluxing for an overall 8 h, the mixture was poured into ice water (50 mL). The precipitate which formed was filtered off with suction, washed with water, and crystallized from ethanol to yield 46% colorless crystals: mp 319 °C; IR 3250 (NH), 1630 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.25 (s, 3H, CH_3), 3.00−3.95 (br s, 2H, CH_2), 7.28 (dd, 1H, 2.0/8.7 Hz), 7.37−7.43 (m, 2H), 7.48−7.52 (m, 1H), 7.60 (dd, 1H, 1.0/8.1 Hz), 7.72 (dd, 1H, 1.3/7.9 Hz), 7.92 (d, 1H, 1.5 Hz), 11.90 (s, 1H, indole-NH). Anal. ($C_{17}H_{13}BrN_2O$) C, H, Br, N.

9-Bromo-5-ethyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (16b). Sodium hydride (40 mg of a 60% suspension in white oil, corresponding to 24 mg, 1 mmol, NaH) was added to a solution of **4a** (327 mg, 1 mmol) in THF (25 mL). After the mixture refluxed for 45 min, bromoethane (327 mg, 3 mmol) was added and refluxing was continued for 24 h. The mixture was then cooled to room temperature and poured into ice water (50 mL). A precipitate formed, which was filtered off with suction, washed with water, and crystallized twice from ethanol to yield 29% colorless crystals, mp 292 °C; IR 3260 (NH), 1630 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 0.89 (t, 6.9 Hz, 3H, CH_3), 2.97 (br. s, 1H, azepine-CH), 3.74 and 3.93 (2 br s, together 3H, CH_2 and azepine-CH, overlapping signals), 7.28 (dd, 8.6/1.5 Hz, 1H), 7.40−7.42 (m, 2H), 7.47−7.52 (m, 1H), 7.66 (d, 8.1 Hz, 1H), 7.73 (dd, 7.6/1.5 Hz, 1H), 7.91 (d, 1.5 Hz, 1H), 11.91 (s, 1H, NH). Anal. ($C_{18}H_{15}BrN_2O$) C, H, Br, N.

5-Benzyl-9-bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (16c). Sodium hydride (24 mg, 1 mmol, 60% suspension in white oil) was added to a solution of **4a** (327 mg, 1 mmol) in THF (25 mL). After the mixture refluxed for 45 min, a solution of benzyl bromide (257 mg, 1.5 mmol) in THF (5 mL) was added and refluxing was continued for 8 h. After the mixture cooled to room temperature, water (100 mL) was added and the mixture was extracted four times with portions of dichloromethane (20 mL each). The combined organic layers were dried with sodium sulfate and evaporated. The residue was crystallized from ethanol to furnish 57% yellowish crystals: mp 248 °C; IR 3300 (NH), 1640 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.20 (br s, 1H, azepine-CH, overlapping the H_2O signal), 4.01 (br s, 1H, azepine-CH), 5.08 (br s, 2H, benzyl-CH_2), 6.89−6.91 (m, 2H), 7.09−7.16 (m, 3H), 7.30 (dd, 1H, 1.5/8.7 Hz), 7.34 (d, 1H, 7.6 Hz), 7.39 (dd, 1H, 1.5/8.2 Hz), 7.43 (d, 1H, 8.6 Hz), 7.59 (d, 1H, 8.1 Hz), 7.67 (dd, 1H, 1.5/7.6 Hz), 7.96 (s, 1H), 11.93 (s, 1H, indole-NH). Anal. ($C_{23}H_{17}BrN_2O$) C, H, Br, N.

(9-Bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepin-5-yl)acetic Acid Methyl Ester (16d). A solution of **4a** (327 mg, 1 mmol) in THF (35 mL) was refluxed with sodium hydride (24 mg, 1 mmol, 60% suspension in white oil) for 1.5 h. Bromoacetic acid ethyl ester (153 mg, 1 mmol) was added, and refluxing was continued for 5 h. After the mixture cooled to room temperature, water (50 mL) was added. The mixture was extracted three times with dichloromethane (20 mL, respectively). The combined organic layers were dried with sodium sulfate and evaporated to furnish a residue, which was crystallized from ethanol to yield 46% colorless crystals: mp 240 °C; IR 3340 (NH), 1750, 1655 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.10 (br s, 1H, azepine-CH), 3.63 (s, 3H, $COOCH_3$), 3.94 (br s, 1H, azepine-CH), 4.43 (br s, 2H, CH_2COOCH_3), 7.29 (dd, 1H, 1.8/8.4 Hz), 7.40−7.43 (m, 2H), 7.46−7.52 (m, 2H), 7.72−7.74 (m, 1H), 7.93 (d, 1H, 1.5 Hz), 11.94 (s, 1H, NH). Anal. ($C_{19}H_{15}BrN_2O_3$) C, H, Br, N.

9-Bromo-12-methyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (17a). Prepared according to general procedure C employing iodomethane (1420 mg, 10 mmol) to furnish 28% yellowish crystals: mp 313 °C (ethanol/toluene); IR 3170 (NH), 1665 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.08−3.99 (very broad signal, 2H, CH_2, overlapping the H_2O signal) 3.84 (s, 3H, CH_3), 7.30−7.33 (m, 2H), 7.36 (dd, 1H, 1.6/6.9 Hz), 7.43−7.47 (m, 1H), 7.54 (d, 1H, 7.1 Hz), 7.74 (dd, 1H, 1.1/6.4 Hz), 7.95 (d, 1H, 1.5 Hz), 10.06 (s, 1H, NH). Anal. ($C_{17}H_{13}BrN_2O$) C, H, N.

9-Bromo-12-ethyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (17b). Prepared according to general procedure C employing iodoethane (1560 mg, 10 mmol) to furnish 24% colorless crystals: mp 298 °C (ethanol/toluene); IR 3180 (NH), 1660 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 1.24 (t, 3H, 5.7 Hz, CH_2-CH_3), 3.05 (br s, 1H, azepine-CH), 3.75 (br s, 1H, azepine-CH), 4.33 (q, 2H, 5.5 Hz, CH_2-CH_3), 7.32−7.36 (m, 3H), 7.44−7.47 (m, 1H), 7.58 (d, 1H, 7.0 Hz), 7.67−7.69 (m, 1H), 7.95 (d, 1H, 1.5 Hz), 10.03 (s, 1H, NH). Anal. ($C_{18}H_{15}BrN_2O$); C, H, N; Br: calcd, 22.49; found, 22.07.

9-Bromo-12-(2-propenyl)-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (17c). Prepared according to general procedure C employing allyl bromide (1210 mg, 10 mmol) to furnish 34% colorless crystals: mp 318 °C dec (ethanol/toluene); IR 3170 (NH), 1660 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.13 (br s, 1H, azepine-CH, overlapping the water signal), 3.78 (br s, 1H, azepine-CH), 4.80 (dd, 1H, 1.3/17.2 Hz), 4.89 (m, 2H, allyl-CH_2), 5.17 (dd, 1H, 1.3/10.5 Hz), 6.07 (ddt, 1H, 4.3/10.5/17.2 Hz), 7.28−7.32 (m, 2H), 7.35 (dd, 1H, 1.7/8.7 Hz), 7.42−7.46 (m, 2H), 7.62−7.64 (m, 1H), 7.98 (d, 1H, 1.6 Hz), 10.06 (s, 1H, NH). Anal. ($C_{19}H_{15}BrN_2O$) C, H, Br, N.

(9-Bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepin-12-yl)acetic Acid Methyl Ester (17d). Prepared according to general procedure C from bromoacetic acid ethyl ester (1530 mg, 10 mmol), yielding 27% colorless crystals from ethanol: mp 328 °C dec; IR 3220 (NH), 1745, 1665 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.20 (br s, 1H, azepine-CH), 3.79 (br s, 1H, azepine-CH), 3.66 (s, 3H, OCH_3), 5.16 (br s, 2H, CH_2-$COOCH_3$), 7.27−7.32 (m, 2H), 7.36 (dd, 1H, 1.8/8.9 Hz), 7.43−7.45 (m, 1H), 7.47−7.50 (m, 2H), 7.98 (d, 1H, 2.0 Hz), 10.08 (s, 1H, NH). Anal. ($C_{19}H_{15}BrN_2O_3$) C, H, Br, N.

9-Bromo-12-(2-hydroxyethyl)-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (17e). A solution of **17d** (399 mg, 1 mmol) in THF (80 mL) was added dropwise to a stirred suspension of lithium alumino hydride (19 mg, 0.5 mmol). After the addition was complete, the mixture was refluxed for 2 h. An additional portion of lithium alumino hydride was added, and refluxing was continued for 1 h. After the mixture cooled to room temperature, water was cautiously added until the hydrogen evolution was finished. A precipitate of aluminum hydroxide was redissolved by dropwise addition of 25% sulfuric acid. The solution was extracted twice with dichloromethane (20 mL, each portion). The combined organic layers were dried with sodium sulfate and evaporated. The residue was crystallized from ethanol to yield 48% colorless crystals: mp 267 °C; IR 3420, 3340 (OH), 3260 (NH), 1650 cm^{-1} (C=O); ^{1}H NMR (400 MHz) 3.05 (br s, 1H, azepine-CH), 3.35 (br s, 1H, azepine-CH, overlapping the H_2O signal), 3.68−3.75 (br m, 2H, CH_2-N), 4.32−4.35 (m, 2H, $O-CH_2$), 5.02 (t, 1H, 5.3 Hz, OH), 7.29−7.35 (m, 3H), 7.42−7.46 (m, 1H), 7.59 (d, 1H, 8.6 Hz), 7.94 (d, 1H, 2.0 Hz), 7.97 (d, 1H, 7.5 Hz), 10.01 (s, 1H, NH). Anal. ($C_{18}H_{15}BrN_2O_2$) C, H, Br, N.

9-Bromo-7,12-dihydroindolo[3,2-*d*][1]benzazepine-6-(5*H*)-thione (18). A solution of **4a** (327 mg, 1 mmol) in THF (30 mL) was stirred under nitrogen at 50 °C. Phosphorus pentasulfide (250 mg, 1.12 mmol) and sodium hydrogen carbonate (370 mg, 4.4 mmol) were added successively. After refluxing for 3 h under nitrogen, the mixture was allowed to cool to room temperature and then poured onto crushed ice (50 g). The mixture was then stirred until the ice was molten, and the precipitate which had formed was filtered off with suction, washed with water, and crystallized from ethanol/toluene yielding 67% pale-yellow crystals: mp > 330 °C; IR 3430, 3140 cm^{-1} (NH); ^{1}H NMR (400 MHz) 3.91 (s, 2H, CH_2), 7.30 (dd, 1H, 1.5/8.6 Hz), 7.39−7.45 (m, 4H), 7.79 (d, 1H, 7.1 Hz), 7.86 (d, 1H, 1.5 Hz), 11.92 (s, 1H, NH), 12.07(s, 1H, NH). Anal. ($C_{16}H_{11}BrN_2S$) C, H, Br, N, S.

9-Bromo-6-(methylthio)-7,12-dihydroindolo[3,2-*d*][1]-benzazepine (19). Sodium hydride (24 mg, 1 mmol, 60% suspension in white oil) was added to a solution of **18** (343 mg, 1 mmol) in THF (20 mL). After refluxing for 1 h with stirring under nitrogen, the mixture was cooled to room temperature and a solution of iodomethane (170 mg, 1.2 mmol) in THF (2 mL) was added. After further refluxing for 2 h, the mixture was cooled to room temperature and poured into ice water (150 mL). After stirring for 15 min, the precipitate was filtered off with suction, washed with water, and crystallized from ethanol to yield 44% colorless crystals: mp 199 °C; IR 3420 (NH), 1615 cm^{-1} (C═N); ^1H NMR (400 MHz) 2.35 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 7.26−7.32 (m, 2H), 7.36−7.43 (m, 3H), 7.80−7.82 (m, 1H), 7.97 (d, 1H, 1.5 Hz), 11.82 (s, 1H, NH). Anal. ($C_{17}H_{13}BrN_2S$) C, H, Br, N, S.

9-Bromo-6-(hydroxyamino)-7,12-dihydroindolo[3,2-*d*]-[1]benzazepine (20). Triethylamine (151 mg, 1.5 mmol) in ethanol (20 mL) was added to a solution of **19** (357 mg, 1 mmol) and hydroxylamine hydrochloride (104 mg, 1.5 mmol) in ethanol (40 mL). After the mixture stirred for 8 h at 20 °C, water (30 mL) was added. A precipitate formed, which was filtered off with suction and crystallized from ethanol to yield 83% colorless crystals: mp 233 °C dec; IR 3350 cm^{-1} (NH), 1635 cm^{-1} (C═N); ^1H NMR (400 MHz) 3.48 (s, 2H, azepine-CH_2), 7.11−7.14 (m, 1H, 7.22−7.30 (m, 2H), 7.35−7.39 (m, 2H), 7.61−7.63 (m, 1H), 7.81 (d, 1.5 Hz, 1H), 8.34 (s, 1H, OH), 9.52 (s, 1H, NH), 11.65 (s, 1H, NH). Anal. ($C_{16}H_{12}BrN_3O$) C, H, Br, N.

Biological Evaluation: p34^{cdc2}/Histone H1 Kinase Assay. P34^{cdc2}/cyclin B was purified from M phase oocytes of the starfish *Marthasterias glacialis* by affinity chromatography on p^{9CKhs1}−Sepharose beads,[15] from which it was eluted by free p^{9CKhs1}. The CDC2 assay mixture (final volume: 30 μL) contained 0.5−1 μL of purified enzyme, 5 μL of histone H1 (5 mg/mL), 5 μL of [γ-^{32}P]ATP (15 μM, 3000 Ci/mmol, 1 mCi/mL), and 3 μL of the inhibitor (0.1−1000 μM), all in reaction buffer C (60 mM β-glycerolphosphate, 15 mM *p*-nitrophenyl phosphate, 25 mM MOPS, pH 7.2, 5 mM EGTA, 15 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM sodium vanadate, 1 mM phenyl phosphate, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 10 μg/mL soybean trypsin inhibitor, 100 μM benzamidine). For determination of maximum phosphate incorporation, buffer C was used instead of inhibitor. Nonspecific binding was determined in the absence of histone H1 in the reaction mixture and subtracted from each volume. The assays were started by addition of radioactive ATP, and after 10-min incubation at 30 °C, 25-μL aliquots of the supernatant were spotted onto 2.5 × 3.0 pieces of Whatman P81 phosphocellulose paper. After 20 s, the filters were washed five times (for at least 5 min each time) in 0.1% phosphoric acid. The wet filters were transferred into 1 mL of ACS scintillation cocktail (Amersham), and after mixing, ^{32}P radioactivity was determined using a Packard Tri-Carb counter. Control analyses were also performed with appropriate dilutions of DMSO because the inhibitors were dissolved in DMSO as 100 mM stock solutions. However, the final DMSO concentration in the reaction mixture never exceeded 1%. The kinase activity is expressed as pmol of phosphate groups incorporated in histone H1 during a 10-min incubation or in percent of the maximal kinase activity. Dose−response curves were drawn for every compound tested and used for calculating IC_{50}s. All assays were carried out in triplicate.

Acknowledgment. We thank the fishermen of the Station Biologique de Roscoff for collecting the starfish. This research was supported by grants from the Association pour la Recherche sur le Cancer (ARC 9314) (to L.M.) and the Conseil Régional de Bretagne (to L.M.) and by a CNRS/NCI collaboration contract. Computer time and software were provided, in part, by the Advanced Biomedical Computing Center, Frederick, MD.

References

(1) Morgan, D. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 261−291.
(2) Draetta, G.; Pagano, M. Cell cycle control and cancer. *Annu. Rep. Med. Chem.* **1996**, *31*, 241−248.
(3) Hartwell, L. H.; Kastan, M. B. Cell cycle control and cancer. *Science* **1994**, *266*, 1821−1828.
(4) Hunter, T.; Pines, J. Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age. *Cell* **1994**, *79*, 573−582.
(5) Sherr, C. J. Cancer cell cycles. *Science* **1996**, *274*, 1672−1677.
(6) Meijer, L. Chemical inhibitors of cyclin-dependent kinases. In *Progress in Cell Cycle Research*; Meijer, L., Guidet, S., Tung, H. Y. L., Eds.; Plenum Press: New York, 1995; Vol. 1, pp 351−363.
(7) Meijer, L. Chemical inhibitors of cyclin-dependent kinases. *Trends Cell Biol.* **1996**, *6*, 393−397.
(8) Meijer, L.; Kim, S.-H. Chemical inhibitors of cyclin-dependent kinases. *Methods Enzymol.* **1997**, *283*, 113−128.
(9) Coleman, K. G.; Lyssikatos, J. P.; Ynag, B. V. Chemical inhibitors of cyclin-dependent kinases. *Annu. Rep. Med. Chem.* **1997**, *32*, 171−179.
(10) Yamamoto, H.; Monden, T.; Miyoshi, H.; Izawa, H.; Ikeda, K.; Tsujie, M.; Ohnishi, T.; Sekimoto, M.; Tomita, N.; Monden, M. Cdk2/cdc2 expression in colon carcinogenesis and effects of cdk2/cdc2 inhibitor in colon cancer cells. *Int. J. Oncol.* **1998**, *13*, 233−239.
(11) Wada, M.; Hosotani, R.; Lee, J.-U.; Doi, R.; Koshiba, T.; Fujimoto, K.; Miyamoto, Y.; Tsuji, S.; Nakajima, S.; Okuyama, A.; Imamura, M. An exogenous cdk inhibitor, butyrolactone-I, induces apoptosis with increased Bax/Bcl-2 ratio in p53-mutated pancreatic cancer cells. *Anticancer Res.* **1998**, *18*, 2559−2566.
(12) Sedlacek, H. H.; Czech, J.; Naik, R.; Kaur, G.; Worland, P.; Losiewicz, M.; Parker, B.; Carlson, B.; Smith, A.; Senderowicz, A.; Sausville, E. Flavopiridol (L86-8275, NSC-649890), a new kinase inhibitor for tumor therapy. *Int. J. Oncol.* **1996**, *9*, 1143−1168.
(13) Senderowicz, A. M.; Headlee, D.; Stinson, S. F.; Lush, R. M.; Kalil, N.; Villalba, L.; Hill, K.; Steinberg, S. M.; Figg, W. D.; Tompkins, A.; Arbuck, S. G.; Sausville, E. A. Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. *J. Clin. Oncol.* **1998**, *16*, 2986−2999.
(14) Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J.-Y.; Detivaud, L.; Leclerc, S.; Meijer, L. Inhibition of cyclin-dependent kinases by purine analogues. *Eur. J. Biochem.* **1994**, *224*, 771−786.
(15) Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P. J.; Blow, J. J.; Inagaki, N.; Inagaki, M.; Delcros, J.-G.; Moulinoux, J.-P. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Eur. J. Biochem.* **1997**, *243*, 527−536.
(16) Havlicek, L.; Hanus, J.; Vesely, J.; Leclerc, S.; Meijer, L.; Shaw, G.; Strnad, M. Cytokinin-derived cyclin-dependent kinase inhibitors: Synthesis and cdc2 inhibitory activity of olomoucine and related compounds. *J. Med. Chem.* **1997**, *40*, 408−412.
(17) Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; Leclerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G. Exploiting chemical libraries, structure, and genomics in the search of kinase inhibitors. *Science* **1998**, *281*, 533−538.
(18) De Azevedo, W. F.; Leclerc, S.; Meijer, L.; Havlicek, L.; Strnad, M.; Kim, S.-H. Inhibition of cyclin-dependent kinases by purine analogues. Crystal structure of human cdk2 complexed with roscovitine. *Eur. J. Biochem.* **1997**, *243*, 518−526.
(19) Schulze-Gahmen, U.; Brandsen, J.; Jones, H. D.; Morgan, D. O.; Meijer, L.; Vesely, J.; Kim, S.-H. Multiple modes of ligand recognition: crystal structures of cyclin-dependent protein kinase 2 in complex with ATP and two inhibitors, olomoucine and isopentenyladenine. *Proteins: Struct., Funct., Genet.* **1995**, *22*, 378−391.
(20) Kim, S.-H. Structure-based inhibitor design for CDK2, a cell cycle controlling protein kinase. *Pure Appl. Chem.* **1998**, *70*, 555−565.
(21) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of meangraph and COMPARE algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092.
(22) Kunick, C. Synthese von 7,12-Dihydro-indolo[3,2-d][1]benzazepin-6-(5*H*)-onen und 6, 11-Dihydro-thieno-[3′,2′:2,3]azepino[4,5-*b*]-indol-5(4*H*)-on. *Arch. Pharm. (Weinheim)* **1992**, *325*, 297−299.
(23) Zaharevitz, D. W.; Gussio, R.; Leost, M.; Senderowicz, A.; Lahusen, T.; Kunick, C.; Meijer, L.; Sausville, E. A. Discovery and initial characterization of the paullones, a novel class of small-molecule inhibitors of cyclin-dependent kinases. *Cancer Res.*, in press. A presentation of the molecular modeling results and methods included in this paper is accessible via the Internet from the following web site: Zaharevitz, D. W.; Gussio, R.; Leost,

M.; Senderowicz, A.; Lahusen, T.; Kunick, C.; Meijer, L.; Sausville, E. A. Model of kenpaullone (NSC-664704) fit in the ATP site of CDK2. URL: http://dtp.nci.nih.gov/Docs/Branches/Itb/tsddg/paullones/paullone.html.

(24) Schulze-Gahmen, U.; De Bondt, H. L.; Kim, S.-H. High-resolution crystal structures of human cyclin-dependent kinase 2 with and without ATP: bound waters and natural ligand as guides for inhibitor design. *J. Med. Chem.* **1996**, *39*, 4540−4546.

(25) Witte, J.; Boekelheide, V. Stereoselective syntheses of isoquinuclidones. II. *J. Org. Chem.* **1972**, *37*, 2849−2853.

(26) Rivett, D. E.; Stewart, F. H. C. Formation of substituted 1-benzazepine-2,5-diones. *Aust. J. Chem.* **1978**, *31*, 439−443.

(27) Rees, A. H. Azatropolones. *J. Chem. Soc.* **1959**, 3111−3116.

(28) Moriconi, E. J.; Maniscalco, I. A. π-Equivalent heterocyclic congeners of tropone. Azatropones. *J. Org. Chem.* **1972**, *37*, 208−215.

(29) Jones, G. Some basic and acidic derivatives of 2,5-dihydro-1*H*-1-benzazepine as potential therapeutic agents. *J. Chem. Soc.* **1967**, 1808−1813.

(30) James, D. M.; Rees, A. H. A chemical and pharmacological study of some compounds derived from 3,4-xylidine. *J. Med. Pharm. Chem.* **1962**, *5*, 1234−1239.

(31) Chen, W.-Y.; Gilman, N. W. Synthesis of 7-phenylpyrimido[5,4-*d*][1]benzazepin-2-ones. *J. Heterocycl. Chem.* **1983**, *20*, 663−666.

(32) Rossi, S.; Micheli, M.; Giannotti, M.; Salvatori, A.; Peruzzi, G. Benzocondensed heptaatomic heterocycles and their derivatives. VII. Synthesis of benzazepines and benzazepinones analogues of known anxiolytic drugs. *Gazz. Chim. Ital.* **1981**, *11*, 365−370.

(33) Kunick, C. Synthese [b]-kondensierter Azepindione durch Dealkoxycarbonylierung. *Arch. Pharm. (Weinheim)* **1991**, *324*, 579−581.

(34) Kunick, C.; Link, A.; Hropot, M. Synthesis and in vivo testing of a potential aquaretic agent. *Pharmazie* **1996**, *51*, 601−602.

(35) Kunick, C. Synthese und konformative Flexibilität von 4,9-Dihydroindolo[3,2-d][1,2,4]triazolo[4,3-a]benzazepinen. *Liebigs Ann. Chem.* **1993**, 1141−1143.

(36) Monks, A.; Scudiero, D. A.; Johnson, G. S.; Paull, K. D.; Sausville, E. A. The NCI anti-cancer drug screen: a smart screen to identify effectors of novel targets. *Anti-Cancer Drug Des.* **1997**, *12*, 533−541.

(37) Robert, J. Anticancer screening with in vitro models. *Drugs Future* **1997**, *22*, 739−746.

(38) Boyd, M. R.; Paull, K. D.; Rubinstein, L. R. Data display and analysis strategies for the NCI disease-oriented in vitro antitumor drug screen. In *Developments in Oncology. Twenty-Second Annual Cancer Symposium 1990, Detroit, Michigan*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic Publishers: Boston, 1992; pp 11−34.

(39) Bates, S. E.; Fojo, A. T.; Weinstein, J. N.; Myers, T. G.; Alvarez, M.; Paull, K. D.; Chabner, B. A. Molecular targets in the National Cancer Institute drug screen. *J. Cancer Res. Clin. Oncol.* **1995**, *121*, 495−500.

(40) Weinstein, J. N.; Myers, T. G.; O'Connor, P. M.; Friend, S. H.; Fornace, A. J., Jr.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sausville, E. A.; Zaharevitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. An information-intensive approach to the molecular pharmacology of cancer. *Science* **1997**, *275*, 343−349.

(41) Boyd, M. R.; Paull, K. D. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91−109.

(42) 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.

(43) 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 a protein assay against a diverse panel of human tumor cell lines. *J. Natl. Cancer Inst.* **1990**, *82*, 1113−1118.

(44) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, 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.

(45) Haq, M. A.; Rây, J. N.; Tuffail-Malkana, M. Quinoline Derivatives. Part III. β-2-Amino-4:5-dimethoxy-benzoylpropionic acid and its derivatives. *J. Chem. Soc.* **1934**, 1326−1328.

JM9900570