ChemPubSoc Europe

DOI: 10.1002/chem.201600308

CHEMISTRY
A European Journal
Full Paper

Library Synthesis

One-Step Synthetic Access to Isosteric and Potent Anticancer Nitrogen Heterocycles with the Benzo[*c*]phenanthridine Scaffold

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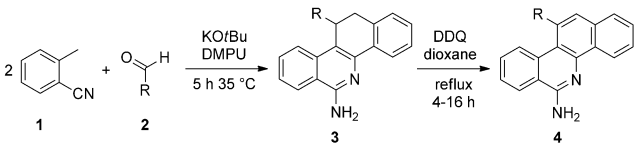
Abstract: A versatile one-step two-component cyclization to build new tetracyclic nitrogen heterocycles is described. *Ortho*-methylhetarenecarbonitrile components were condensed with aldehydes to access a large library of differently substituted ring systems. The heterocyclic core can be easily modified by variation of the position of the endocyclic nitrogen atom in the *o*-methylhetarenecarbonitrile substrate. The manner of the nucleophilic attack that leads to the condensation can be triggered by different electron-density distribution in the molecule induced by the position of the nitrogen atom. Taking this into account, there is an electronic preference that leads to either pyridophenanthrolines or the corresponding pyridoazacarbazoles as the main products. We demonstrate the high antitumor potential of some of our synthesized heterocycles, which is strongly dependent on the substitution pattern introduced through the aldehyde component. The position and number of endocyclic nitrogen atoms play an important role regarding cytotoxicity of the studied compounds.

Introduction

With 8.2 million deaths worldwide caused by a cancer burden in 2012, malignant tumor diseases are one of the leading causes of mortality. The number of individuals suffering from cancer is predicted to rise by about 70% in the next 20 years, thus therapy of malignancies becomes more and more important, in addition to prevention.[1] Research in the field of new antitumor agents is gaining a growing relevance in medicinal chemistry. Widely studied classes of compounds include substances that possess a benzo[*c*]phenanthridine core, which is known for its broad pharmacological activity. Besides antimicrobial and antiviral properties, antitumor activity has also been described for compounds with this structural element.[2] Naturally occurring compounds with the benzo[*c*]phenanthridine skeleton, including fagaronine (**5**) and nitidine (**6**), extracted from plants of the Rutaceae family, are among the most-commonly isolated and characterized alkaloids with respect to their antitumor activity. These two natural alkaloids are especially known for their anticancer activity based on inhibition of enzymes from the topoisomerase family and their DNA intercalation abilities.[3–7] The fact that 74% of all antitumor drugs approved between 1981 and 2002 were natural products or natural product-derived/inspired[8] in combination with their broad spectrum of pharmacological activity[9] reveals the importance of synthetic access to benzo[*c*]phenanthridine derivatives.

In 2005 we reported a surprisingly simple one-step synthetic procedure that involved condensation of *o*-tolunitrile (**1**; 2 equiv) with an aldehyde component **2** to give the complex 11-substituted 6-amino-11,12-dihydrobenzo[*c*]phenanthridine heterocyclic structure **3**. The corresponding fully aromatized structures **4** were accessible by subsequent oxidation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (Scheme 1).[10]



Scheme 1. One-step synthesis of 11-substituted 6-amino-11,12-dihydrobenzo[*c*]phenanthridines **3** with subsequent dehydrogenation to the fully conjugated systems **4**.[10] DMPU = 1,3-dimethyl-3,4,5,6-tetrahydropyrimidine-2-(1*H*)-one.

Investigations concerning the antitumor activity of the synthesized derivatives **3** and **4** at the U.S. National Cancer Institute (NCI) revealed promising results: the activities of **3** and **4** partially surpassed the cytotoxic activity of **5**.[10,11] Due to the poor water solubility and high lipophilicity (log*P* > 5) of this

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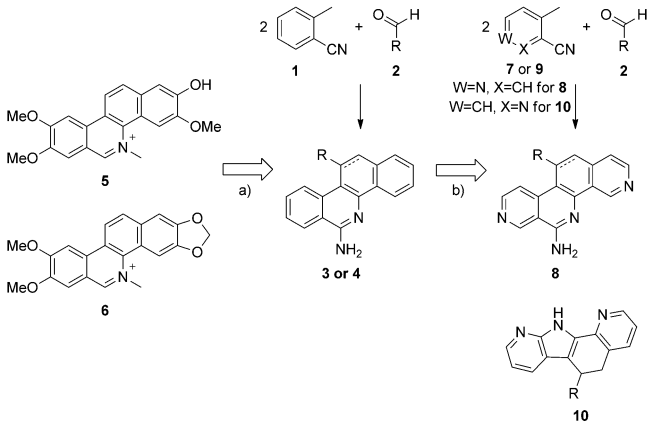
Supporting information for this article (including full experimental details and characterization) is available on the WWW under http://dx.doi.org/10.1002/chem.201600308.

class of compounds application as potential pharmaceutical product was very unlikely. To overcome these disadvantages aza-analogues of the benzo[*c*]phenanthridine class were synthesized by substitution of *o*-tolunitrile with heterofunctionalized 4-methyl-3-cyanopyridine (**7**) in the established condensation reaction to give 11-substituted 6-amino-pyrido[3,4-*c*][1,9]phenanthrolines (**8**). Dehydrogenation provided the fully aromatic ring systems. A remarkable decrease in lipophilicity relative to the corresponding benzo[*c*]phenanthridine derivatives **3** and **4** and an improvement in solubility was also demonstrated.[12] The cytotoxic effect of this newly derived substance class could be maintained through the introduction of two additional endocyclic nitrogen atoms.[12] Recently, we demonstrated that the directional selectivity of the ring-closing reaction that leads to four-membered heterocyclic systems can be triggered by the position of the endocyclic nitrogen atom in the *o*-methylhetarenecarbonitrile substrate. A new class of nitrogen heterocycle, 6-substituted 5,6-dihydro-11*H*-pyrido[3,2-*i*]-1-azacarbazoles (**10**), were accessible from 3-methyl-2-cyanopyridine (**9**) after optimization of the reaction conditions. Again, preliminary investigations concerning the cytotoxic activity of pyridoazacarbazoles **10** showed very promising results.[13] A schematic of the natural-product-inspired development of heterocyclic structures based on the benzo[*c*]phenanthridine ring system is shown in Scheme 2.



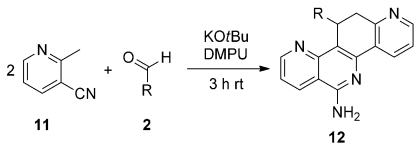
Scheme 2. a) Natural-product-inspired development of a one-step synthesis of benzo[*c*]phenanthridine derivatives **3** and **4**.[10] b) Access to two different nitrogen heterocycles, pyrido[3,4-*c*][1,9]phenanthrolines **8** and pyrido[3,2-*i*]-1-azacarbazoles **10**, by the established synthetic route.[12,13]

Results and Discussion

Chemistry

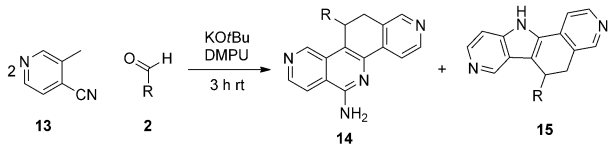
A common way to optimize the absorption, distribution, metabolism, excretion, and toxicity (ADMET) features of a compound whilst maintaining biological activity is "scaffold hopping". One possibility to realize the change required in the scaffold is isosteric replacement of certain atoms in the structural core.[14] This strategy has been successful for the development of new topoisomerase I (Top I) inhibitors as potential anticancer agents by optimization of the binding to the Top I–DNA covalent complex, as well as for marketed drugs, for example, identification of Vardenafil by scaffold hopping from Sildenafil.[15–17] One of the most-popular isosteric pairs, especially in compounds that possess a heterocyclic scaffold, are CH and N.[15,16,18] This replacement is often linked to improved solubility and reduced lipophilicity, which we have already shown for the pyrido[3,4-*c*][1,9]phenanthrolines **8**.[12,19] Additionally, pyridine and pyrimidine rings are common motifs in the structures of approved drugs.[19] The advantages regarding drug design and the possibility to trigger the direction of the nucleophilic attack and access new unknown heterocyclic classes with interesting chemical properties encouraged us to investigate the synthetic potential of our straightforward synthetic procedure.

A hitherto unreported heterocyclic class, 11-substituted 11,12-dihydropyrido[3,2-*c*][1,7]phenanthrolines (**12**) could be obtained from 2-methylnicotinonitrile (**11**) by using our one-step cyclization method (Scheme 3).



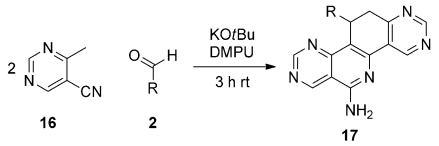
Scheme 3. Isosteric replacement of **1** by **11** leads to the unknown heterocyclic class of pyrido[3,2-*c*][1,7]phenanthrolines **12**.

When 3-methyl-4-cyanopyridine (**13**) was used as the *o*-methylhetarenecarbonitrile substrate a coincident synthesis of two new heterocyclic structures, 11-substituted 11,12-dihydropyrido[4,3-*c*][1,8]phenanthrolines (**14**) and 6-substituted 5,6-dihydro-11*H*-pyrido[3,4-*i*]-3-azacarbazoles (**15**), was realized (Scheme 4). The two compound classes could be separated easily by column chromatographic purification. Though separation could be realized, complete purification of byproducts **15** was not successful. Nevertheless, detailed NMR spectroscopic investigations proved the structures without any doubt.



Scheme 4. Cyclization of **13** with aldehydes **2** leads to two new heterocyclic systems, pyrido[4,3-*c*][1,8]phenanthrolines **14** and 11*H*-pyrido[3,4-*i*]-3-azacarbazoles **15**.

To investigate the applicability of our one-step synthetic procedure with pyrimidine moieties, 4-methyl-5-cyanopyrimidine (**16**) was condensed with different aldehydes **2**. Again, a hitherto unreported substance class with two additional nitrogen atoms in the scaffold could be obtained, namely 11-substituted 1-aza-11,12-dihydropyrimido[5,4-*c*][1,9]phenanthrolines (**17**; Scheme 5).



Scheme 5. The introduction of two additional nitrogen atoms into the scaffold was possible by utilizing substrate **16** in a straightforward cyclization reaction to afford 1-aza-11,12-dihydropyrimido[5,4-*c*][1,9]phenanthrolines **17**.

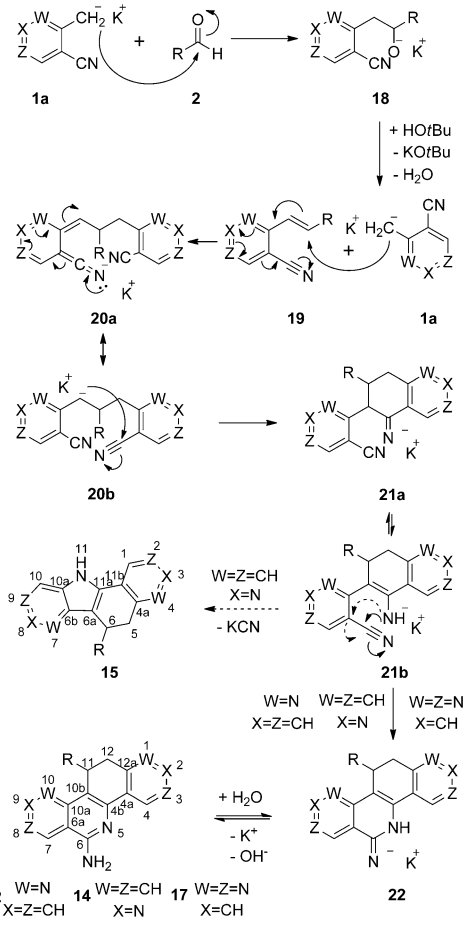
Mechanism of formation

The formation of either pyridoazacarbazoles **15**, pyridophenanthrolines **12** and **14**, or azapyrimidophenanthrolines **17** is strongly dependent on the *o*-methylhetarenecarbonitrile substrate. Two alternate nucleophilic attacks lead to the different heterocyclic classes, therefore the formation of each can be triggered by the position of the endocyclic nitrogen atom in the *o*-methylhetarenecarbonitrile substrate (Scheme 6).

Under the strongly basic reaction conditions deprotonation of the methyl group of the *o*-methylhetarenecarbonitrile component results in carbanion **1a**. Nucleophilic attack at the carbonyl group of aldehyde **2** forms alcoholate **18**, which is subsequently converted into the corresponding aza-analogous stilbene **19**. Next, a vinologous Michael reaction that comprises addition of a second molecule of carbanion **1a** to the stilbene double bond gives gives intermediates **20** which are represented by the mesomeric structures **20a** and **20b**. A 6-*exo-dig* intramolecular ring-closing reaction gives iminonitrile **21a**, which tautomerizes to provide enaminonitrile **21b**. Enaminonitrile **21b** is the key structure and directs the preference for either a subsequent 6-*exo-dig* reaction to afford pyridophenanthrolines **12** and **14** and azapyrimidophenanthroline **17** or a 5-*exo-trig* cyclization reaction to form pyridoazacarbazole **15**. According to Baldwin[20] both ring-closing reactions are kinetically favored. Therefore, the formation of distinct heterocyclic systems is determined solely by the charge distribution in the *o*-methylhetarenecarbonitrile substrate, which can be altered by different positions of the endocyclic nitrogen atom(s). Regarding the pyrido[3,2-*c*][1,7]phenanthrolines **12** and 1-aza-pyrimido[5,4-*c*][1,9]phenanthrolines **17** only the 6-*exo-dig* cyclization is observed due to negative polarization of the aromatic carbon atom next to the nitrile group. The 6-*exo-dig* ring closure is observed for pyrido[3,4-*c*][1,8]phenanthrolines **14**, the partial positive charge also favors the concurring 5-*exo-trig* cyclization, forming concomitantly the 11*H*-pyrido[3,4-*i*]-3-azacarbazoles **15** under elimination of cyanide. In contrast, we recently reported a postulated reaction mechanism for the formation of pyrido[3,2-*i*]-1-azacarbazoles **10**. The 5-*exo-trig* cyclization was strongly favored in this case. The corresponding 6-*exo-dig* cyclization leading to the pyridophenanthroline skeleton was only observed in very small amounts.[13] In a simplistic mechanistic picture, the influence of the *ortho*-nitrogen atom in enhancing the electrophilic character of the endocyclic carbon atom by polarization is much stronger for the pyrido[3,2-*i*]-1-azacarbazoles **10**. This explains the isolation of both **14** and **15** when the nitrogen atom is in a *para* position.

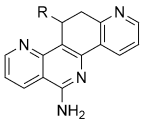
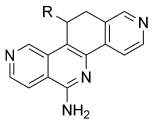
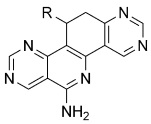
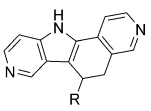
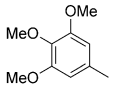
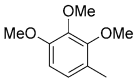
To reinforce the dependency of the manner of the nucleophilic attack on the position of the endocyclic nitrogen, Hückel analysis of the Hückel molecular orbital (HMO) electron densities for compounds **21b** was accomplished by using the MarvinSketch 6.0.6 programme. Indeed, for the pyrido[3,2-*c*][1,7]phenanthrolines **12** and 1-azapyrimido[5,4-*c*][1,9]phenanthrolines **17** comparably high charge densities (1.07 and 1.08) were calculated for the carbon atom next to the nitrile group, which confirmed the low electrophilic character of this atom. For heterocyclic classes **14** and **15** the charge density of the carbon atom next to the nitrile group is remarkably lower (1.04), which enhances its electrophilic character and is therefore responsible for the coincident formation of pyridophenanthrolines **14** and pyridoazacarbazoles **15**. In contrast, azacarbazole **10** was obtained almost exclusively when methylpyridine **9** was used.[13] This can be verified by the charge density of the carbon atom next to the nitrile group (1.02), which is the lowest in the considered series. The negative charge calculated for the nitrile carbon atom in **21b** is always 0.82 and is independent of the nitrogen atom position in this simple model.

To investigate the influence of the number of nitrogen atoms and their different positions in the diverse heterocyclic scaffolds, but also to examine the impact of the substitution



Scheme 6. Postulated mechanism for the formation of pyrido[3,2-*c*][1,7]phenanthrolines **12**, pyrido[3,4-*c*][1,8]phenanthrolines **14**, 1-azapyrimido[5,4-*c*][1,9]phenanthrolines **17**, or pyrido[3,4-*i*]-3-azacarbazoles **15**.

Table 1. Yields of 11-substituted pyridophenanthrolines **12** and **14**, azapyrimidophenanthrolines **17**, and 6-substituted pyridoazacarbazoles **15** synthesized by a one-pot two-step cyclization route ($\log D_{7.4}$ values are given in parentheses).

Compound	R	 **12**	 **14**	 **17**	 **15**
a		37% (2.91)	21% (3.06)	23% (2.75)	10%
b		66% (3.49)	19% (3.48)	31% (3.28)	16% (3.85)
c		25% (2.42)	18% (2.41)	20% (2.23)	–[a]
d		50% (3.41)	19% (3.41)	17% (3.32)	14% (3.77)
e		66% (3.95)	22% (3.89)	28% (3.90)	22% (4.12)
f		60% (2.44)	33% (2.69)	19% (2.23)	13% (2.96)
g		43% (2.89)	31% (3.12)	39% (2.95)	11% (3.27)
h		–[b]	–[b]	17% (2.71)	–[b]
i		–[b]	–[b]	14% (2.94)	–[b]
j		–[b]	–[b]	14% (3.48)	–[b]

[a] Could not be obtained. [b] Not synthesized.

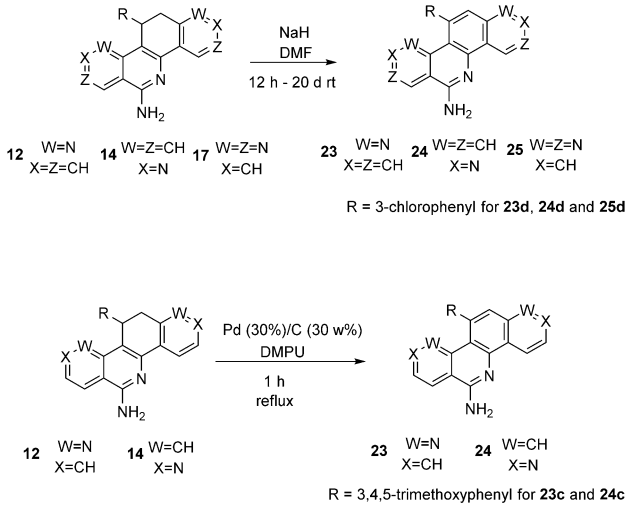
pattern at the C11 carbon atom, on the antitumor activity of these new compounds, we synthesized a library of about 30 heterocycles (Table 1).

No general trend concerning the nitrogen atom position was observed when the yields of the products obtained from *o*-methylhetarenecarbonitrile substrates with differing numbers and positions of nitrogen atoms in the one-step cyclization reaction with various aldehydes **2** were compared. Higher water solubility, strongly dependent on the C11 substituent, limited the product yields by impeding isolation after the hydrolysis. Therefore, compounds that possessed higher lipophilicity, for example compounds with a 3,4-dichlorophenyl residue (**12e**, **14e**, **17e**, **15e**) were isolated in higher yields.

To investigate the influence of the different number and position of the nitrogen atoms on lipophilicity, $\log D_{7.4}$ values were determined by HPLC analysis (Table 1). The C11- and C6 substituents had more influence on lipophility than the heterocyclic core. The lipophilicities of the new aza-analogues **12**, **14**, **17**, and **15** ($\log D_{7.4} = 2.23–4.12$) are similar to the lipophilicities of pyrido[3,4-*c*][1,9]phenanthrolines **8**. Therefore, the isosteric nitrogen heterocycles described herein showed a remarkable decrease in lipophilicity relative to the benzo[*c*]phenanthridines **4**.[12]

The potential of our versatile synthetic procedure is not limited to the broad range of substituents introduced via aldehyde **2** and the possibility to exchange the heterocyclic core, it also provides a way to further functionalize key structural elements.

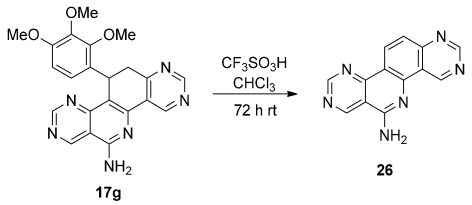
To increase the planarity of the scaffold, the C11HR–C12H_2 moiety was dehydrogenated, a procedure that has already been shown to enhance the antitumor activity of aminopyrido[3,4-*c*][1,9]phenanthrolines **8**.[12] For this substance class we developed two dehydrogenation methods depending on the C11 substituent. In the presence of a halophenyl substituent dehydrogenation was carried out with sodium hydride by using an altered method described by Capra and Le Gall,[21] whereas derivatives that bore no halogen atom on the C11 substituent were dehydrogenated by treatment with palladium on activated charcoal by utilizing a modified method described by Meier et al.[12] After optimization of the reaction conditions



Scheme 7. Dehydrogenation of the 11,12-position increases the planarity of the heterocyclic scaffolds of **23 d**, **24 d**, **25 d**, **23 c**, and **24 c**, obtained from three distinct cores **12**, **14**, and **17**.

the two dehydrogenation methods were applied to the new heterocyclic systems (Scheme 7).

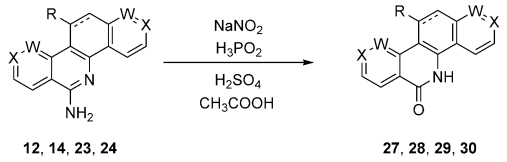
The 11-unsubstituted 1-aza-11,12-dihydropyrimido[5,4-*c*][1,9]phenanthroline **26**, the completely conjugated analogue of series **25**, was accessed in moderate yield by *ipso* protonation and subsequent elimination of the C11 substituent of **17 g** by using a method described by Li et al. (Scheme 8).[22] We have already showed the versatility of this concept in our synthesis of the completely conjugated system of the 11*H*-[3,2-*i*]-1-azacarbazoles **10**.[13]



Scheme 8. Completely conjugated ring system **26** was accessible by elimination of the C11 substituent.

To investigate the influence of the C6 substituent on the cytotoxic effect of the newly derived substance classes **12** and **14** we transformed the 6-amino substituent into a 6-oxo substituent, which generated a lactam functional group. Consequently, a hydrogen-bond acceptor replaced a hydrogen-bond donor. We used a diazotization method that was reported by Kornblum[23] and modified by Meier et al.[24] (Scheme 9). The corresponding 6-oxo-functionalized structures were accessible in yields between 22 and 83% and were strongly dependent on the heterocyclic core and the C11 substituent.

In compounds **28 c**, **28 d**, and **30 d** the C6 carbonyl moiety could be further linked via the oxygen atom. To reach a maximum scaffold planarity to improve possible DNA intercalation

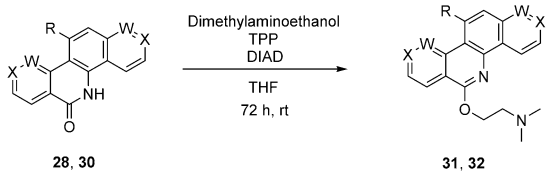


Scheme 9. Synthetic access to the 6-oxo structures of two different heterocyclic systems with various C11 substituents. **12**, **23**, **27**, **28**: W = N, X = CH, **14**, **24**, **29**, **30**: W = CH, X = N, **12** and **14**: 11,12-dihydro, **23** and **24**: 11,12-dehydro, **27** and **29**: 11, 12-dihydro, **28** and **30**: 11,12-dehydro, **12 c**, **14 c**, **23 c**: R = 3,4,5-trimethoxyphenyl, **14 g**: R = 2,3,4-trimethoxyphenyl, **23 d**, **24 d**: R = 3-chlorophenyl, **27 c**, **28 c**, **29 c**: R = 3,4,5-trimethoxyphenyl, **29 g**: R = 2,3,4-trimethoxyphenyl, **28 d**, **30 d**: R = 3-chlorophenyl.

only the 11,12-dehydrogenated derivatives were functionalized further.

Enhancement of the cytotoxicity by introduction of an aminoalkyl sidechain has been reported for non-camptothecin four-membered heterocycles.[25–27] The phenanthroline Topovale (ARC-111) showed promising anticancer activity in human tumor xenografts and its monomethyl sidechain analogue Genz-644282 is currently being investigated in phase I clinical trials.[28,29] Structurally related indenoisoquinolines LMP400 (indotecan) and LMP776 (indimitecan) are presently undergoing phase I clinical trials. The presence of an aminoalkyl sidechain in all the above-mentioned drug candidates combined with the first successful potentiation of antitumor activity through sidechain connection allowed us to apply this promising concept to our new heterocyclic classes.[28,29]

To realize a structural approach to Topovale (ARC-111) a dimethylaminoethyl sidechain was linked to the lactam oxygen atom of **28 c**, **28 d**, and **30 d** by a Mitsunobu reaction sequence reported by Kiselev et al. (Scheme 10).[16] The C6-functionalized derivatives **31** and **32** were obtained in yields between 34 and 95% depending on the heterocyclic structure and the C11 substituent.



Scheme 10. Synthesis of the 6-dimethylaminoethoxy derivatives **31 c**, **31 d**, and **32 d**. TPP = triphenylphosphane, DIAD = diisopropyl azodicarboxylate. **28**, **31**: W = N, X = CH, **30**, **32**: W = CH, X = N, **28 c**: R = 3,4,5-trimethoxyphenyl, **28 d**, **30 d**: R = 3-chlorophenyl, **31 c**: R = 3,4,5-trimethoxyphenyl, **31 d**, **32 d**: R = 3-chlorophenyl.

Antitumor activity

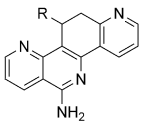
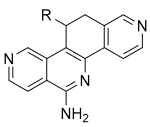
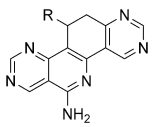
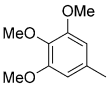
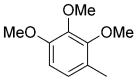
To investigate the antitumor activity of the new heterocyclic classes and to determine structure–activity relationships regarding the position of the nitrogen atom and the C11- and C6-substitution patterns, the cytotoxic effect on 60 human tumor cell lines was determined at the U.S. NCI. The antitumor

activity of each compound was evaluated in the NCI 60 in vitro cell line screening assay that comprises 60 cell lines derived from 9 different solid human tumor types.[30] In preliminary one-dose testing all compounds were screened at a concentration of 10 μM, which provided a mean-growth percent averaged over all 60 cell lines. Promising compounds (a mean-growth value typically below 60%) were selected for testing at five different concentrations. For each tested cell line a dose–response curve was obtained, which enabled calculation of the GI_{50} value, as well as other parameters. The GI_{50} value represents the concentration required to result in 50% growth inhibition for each cell line, and can be averaged over the whole cell line panel to gain a highly comparable quantity for the tested compounds, namely, the mean-graph midpoint (MGM) GI_{50}. In situations in which the GI_{50} value is greater than 10^{-4} M (maximum testing concentration) or less than 10^{-8} M (minimum testing concentration), the GI_{50} values are recorded and replaced as 10^{-4} M and 10^{-8} M.[31,32]

Regarding the 11,12-dihydro derivatives of the heterocyclic classes **12**, **14**, and **17** a strong dependency of the antitumor activity on the C11 substituent is clear (Table 2). For all three heterocyclic classes a bromo- or chloro-phenyl moiety at C11 (**12b**, **12d**, **14b**, **14d**, **17b**, and **17d**) is required for promising antitumor activity. Structural elements possessing a high-electron-density substituent (e.g., methoxyphenyl compound **17h**) proved to be less potent. A strong influence of the number of endocyclic nitrogen atoms and their positions on cytotoxicity is also clear from the data in Table 2. The 11,12-dihydropyrido[3,2-*c*][1,7]phenanthrolines **12** and the slightly less potent 1-aza-11,12-dihydropyrimido[5,4-*c*][1,9]phenanthrolines **17** possess MGM GI_{50} values in the range of the natural products **5** and **6** (9.48 μM and 1.75 μM, respectively).[33] However, the 11,12-dihydropyrido[4,3-*c*][1,8]phenanthrolines **14** surpassed these MGM GI_{50} values by one order of magnitude. For the 1-aza-11,12-dihydropyrimido[5,4-*c*][1,9]phenanthrolines **17** the introduction of two additional nitrogen atoms seems to reduce antitumor potency. Preliminary investigations concerning the antitumor activity of the 5,6-dihydro-11*H*-pyrido[3,4-*i*]-3-azacarbazoles **15** were less promising because none of the tested derivatives showed sufficient cell-growth inhibition in the NCI one-dose assay to enable selection for five-dose testing.

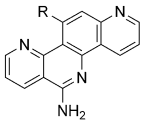
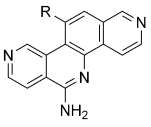
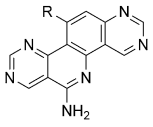
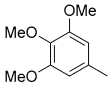
For the completely conjugated derivatives a converse dependency of the antitumor activity on the C11 substituent was observed for a few derivatives compared with their 11,12-dihydro analogues (Table 3). A 3,4,5-trimethoxyphenyl residue revealed the highest cytotoxic potential (**23c**) with an MGM GI_{50} value of 0.58 μM. Consequently the 11,12-dehydrogenation transformation resulted in a high in-

Table 2. NCI MGM GI_{50} values of 11-substituted 11,12-dihydropyridophenanthrolines **12** and **14** and 11,12-dihydroazapyrimidophenanthrolines **17**.[33]

Compound	R	 **12**	 **14**	 **17**
a		73% at 10 μM[a]	n.t.[b]	96% at 10 μM[a]
b		1.18 μM	0.51 μM	4.90 μM
c		95% at 10 μM[a]	84% at 10 μM[a]	101% at 10 μM[a]
d		2.34 μM	0.47 μM	4.68 μM
e		3.72 μM	0.66 μM	95% at 10 μM[a]
f		n.t.[c]	n.t.[c]	102% at 10 μM[a]
g		99% at 10 μM[a]	99% at 10 μM[a]	70% at 10 μM[a]
h		–[d]	–[d]	88% at 10 μM[a]
i		–[d]	–[d]	n.t.[c]
j		–[d]	–[d]	4.90 μM

[a] Not selected for five-dose testing due to insufficient inhibition of cell growth in preliminary one-dose testing at 10 μM. [b] Not tested. [c] Not selected for testing. [d] Not synthesized.

Table 3. NCI MGM GI_{50} values of 11-substituted pyridophenanthrolines **23** and **24** and azapyrimidophenanthrolines **25**.[33] (log$D_{7.4}$ values are given in parentheses).

Compound	R	 **23**	 **24**	 **25**
c		0.58 μM (3.16)	41% at 10 μM[a] (3.59)	–[b]
d		60% at 10 μM[b] (4.19)	36% at 10 μM[a] (4.33)	84% at 10 μM[a] (2.63)
26	-H	–[b]	–[b]	96% at 10 μM[a] (2.38)

[a] Not selected for five-dose testing due to insufficient inhibition of cell growth in preliminary one-dose testing at 10 μM. [b] Not synthesized.

crease of antitumor activity for this compound. In contrast 11,12-dehydro derivatives that bore a 3-chlorophenyl substituent (**23 d**, **24 d**, and **25 d**) were less potent than their 11,12-dihydro analogues (**12 d**, **14 d**, and **17 d**). In the case of the 1-aza-11,12-dihydropyrimido[5,4-*c*][1,9]phenanthrolines **17** the influence of a completely unsubstituted and fully aromatic system **26** on the cytotoxic potency was investigated. The growth-inhibitory effects of this compound were not efficient enough to be tested at five concentrations. These findings demonstrate the necessity of a bulky C11 substituent for antitumor properties to be displayed.

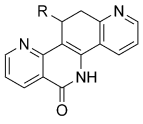
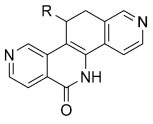
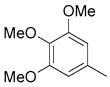
To investigate the influence of the nature of the C6 substituent on antitumor activity derivatives **27 c** and **29 c** were examined in the NCI 60 human tumor cell line screening assay (Table 4). The results are contrary for the two heterocyclic classes **27** and **29**, which differ only in the position of their two endocyclic nitrogen atoms. In the case of 6-oxo-pyridophenanthroline **27 c** a remarkable increase in antitumor potency compared with its 6-amino analogue **12 c** is produced by replacement of a hydrogen-bond donor by a hydrogen-bond acceptor; this was not the case for 6-oxo-pyridophenanthroline **29 c**. These findings demonstrate a predominant influence of the heterocyclic core on cytotoxicity in the case of 6-oxo functionalization, which is in contrast to the 6-amino derivatives of the tetracyclic ring systems, for which the impact of the C11 substituent seemed to dominate (Tables 2 and 3).

A slight decrease of antitumor activity was observed for 11,12-dehydro derivative **28 c** relative to the 11,12-dihydro analogue **27 c** (Table 4 and 5). In the case of 11-chlorophenyl-substituted derivative **30 d** higher activity was observed relative to its 6-amino analogue **24 d**, but **30 d** is still two orders of magnitude less potent than the corresponding 6-amino-11,12-dihydro derivative **14 d**. For the isosteric lactam derivative **28 d** a slight decrease in antitumor activity was observed relative to the 6-amino analogue **23 d**, and the latter was less potent than the related 6-amino-11,12-dihydro derivative **12 d**.

A significant improvement of cytotoxicity by introduction of an aminoalkyl sidechain was not observed (Table 6). The cytotoxicity of the new heterocyclic structures depends on the distinct positions of the nitrogen atoms in combination with the C11 substituent, rather than on a single substitution pattern such as the aminoalkyl sidechain. Nevertheless derivatives **31 c** and **31 d** showed promising antitumor activity with assumed higher water solubililty as a result of the basic sidechain.

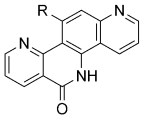
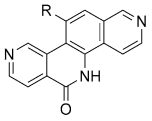
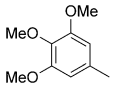
The antitumor activity of the new aza-analogues could be maintained by isosteric replacement. A similar, and partially even better, cytotoxic activity was observed for the new heterocyclic series relative to the benzo[*c*]phenanthrindines **4** and pyrido[3,4-*c*][1,9]phenanthrolines **8**.[10–12]

Table 4. NCI MGM GI_{50} values of 11-(3,4,5-trimethoxyphenyl)-substituted 11,12-dihydropyridophenanthrolin-6-ones **27 c** and **29 c**.[33] ($\log D_{7.4}$ values are given in parentheses).

Compound	R	 **27**	 **29**
c		0.71 μM (2.33)	98% at 10 μM[a] (2.05)

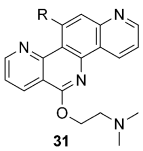
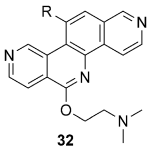
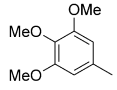
[a] Not selected for five-dose testing due to insufficient inhibition of cell growth in preliminary one-dose testing at 10 μM.

Table 5. NCI MGM GI_{50} values of 11-substituted 6-oxo-11,12-dehydrogenated pyridophenanthrolines **28** and **30**.[33] ($\log D_{7.4}$ values are given in parentheses).

Compound	R	 **28**	 **30**
c		1.12 μM (2.30)	–[a]
d		86% at 10 μM[b] (4.20)	39.8 μM (3.82)

[a] Not synthesized. [b] Not selected for five-dose testing due to insufficient inhibition of cell growth in preliminary one-dose testing at 10 μM.

Table 6. NCI MGM GI_{50} MGM values of 11-substituted 6-dimethylaminoethoxy-11,12-dehydrogenated pyridophenanthrolines **31** and **32**.[33]

Compound	R	 **31**	 **32**
c		0.53 μM (3.70)	–[a]
d		1.48 μM (4.62)	26% at 10 μM[b] (4.47)

[a] Not synthesized. [b] Not selected for five-dose testing due to insufficient inhibition of cell growth in preliminary one-dose testing at 10 μM.

Conclusion

The high applicability of a facile one-step synthesis to build tetracyclic nitrogen heterocycles was demonstrated. With this versatile synthetic tool it was possible to synthesize large libraries of differently substituted heterocyclic systems. The high variability of the substitution pattern, defined by the aldehyde component **2**, in addition to the ability to vary the position and number of endocyclic nitrogen atoms, specified by the *o*-methylhetarenecarbonitrile substrate, emphasize the practicability of our synthetic route. The direction of the nucleophilic attack of the cyclization reaction can now be easily predicted and triggered by the electronic distribution in the *o*-methylhetarenecarbonitrile substrate, which is determined by the position of the endocyclic nitrogen atom(s). This results in either pyridoazacarbazoles or pyridophenanthrolines and azapyrimidophenanthrolines, which can be further functionalized. Very promising antitumor activity was determined in the NCI 60 human tumor cell line screening assay for three of the heterocyclic series: some of the derivatives were more active than the naturally occurring derivatives **5** and **6**. The anticancer activity was strongly dependent on the C11 substituent, but also varied with the number of endocyclic nitrogen atoms and their position in the ring. We further demonstrated a distinct decrease in lipophilicity of the aza-analogues relative to the isosteric class of the benzo[*c*]phenanthridines.

The mechanism leading to the anticancer activity of these new heterocyclic classes and possible differences in the mechanism of action depending on the heterocyclic scaffold is the subject of future studies.

Acknowledgements

We thank Martin Clemen (Institut für Physikalische Chemie der Universität Kiel) for performing the straightforward HRMS measurements and Melissa Zietz for outstanding technical support. Antitumoral activity testing was performed by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI (http://dtp.cancer.gov). We thank the NCI for its excellent screening service.

Keywords: antitumor agents · fused-ring systems · medicinal chemistry · nitrogen heterocycles · synthetic methods

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Received: January 22, 2016
Published online on May 6, 2016