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Synthesis and First Biological Evaluation of 1-Aza-9-oxafluorenes as Novel Lead Structures for the Development of Small-Sized Cytostatics

Kristin Brachwitz and Andreas Hilgeroth*

Institute of Pharmaceutical Chemistry, Department of Pharmacy, Martin-Luther-University, Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle, Germany

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Abstract—A first series of novel 1-aza-9-oxafluorenes has been prepared from 3-carbonyl substituted 1,4-dihydropyridines and *p*-benzoquinone as small-sized cytostatics. Biological evaluation has been carried out in various cancer cell-lines. First structure–activity relationships proved the 4-phenyl substituent to be more favorable than the 4-methyl substituent. Cytostatic properties are discussed.

Among the diverse number of important cytostatic agents which have been introduced in clinical practice, the DNA-intercalating inhibitors of topoisomerase I or II are one of the most perspective classes of anticancer drugs.[1] One important feature for potential intercalating properties of such drug candidates is a planar aromatic system. Almost all developed intercalating agents have polycyclic structures consisting of more than three annelated rings. Indolo- or quinolo-annelated systems predominate within the promising candidates like intoplicine **1**, nitidine **2** (Fig. 1) or rebeccamycin-derived indolopyrazole compounds.[1]

One main problem of the polycyclic systems that contributed to clinical failure of cytostatically potent ellipticine **3** and derivatives was poor solubility of the substances so that absorption rates were insufficient for an oral therapy.[2] Among the series of highly potent tetracyclic anthracycline compounds, cardiotoxicity is one additional problem besides poor solubility.[1] Tricyclic anthracendione derivative mitoxanthrone shows better solubility and less cardiotoxicity.[3] Idarubicin shows satisfying oral bioavailability.[3] As was shown for the class of anthracycline compounds, one strategy within the development of novel cytostatic agents is an improvement of oral absorption rates due to a better solubility. The reported number of small-sized tricyclic cytostatic compounds like mitoxanthrone with promising better solubility is poor.

Low cytostatic activities of recently reported β-carboline compounds as azacarbazole derivatives with DNA-intercalating properties could be increased by an alkylamine side chain that showed additional binding affinities to the DNA-helix groove.[4] Among a series of α-carboline derivatives **4** (Fig. 1) only some candidates

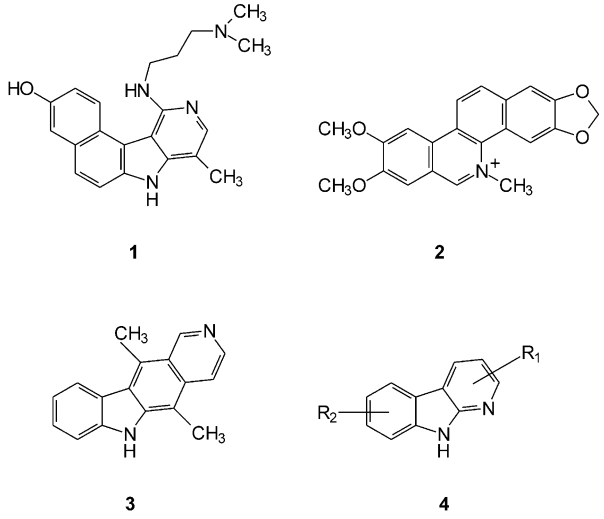

Figure 1.

*Corresponding author. Tel.: +49-345-55-25168; fax: +49-345-55-27026; e-mail: hilgeroth@pharmazie.uni-halle.de

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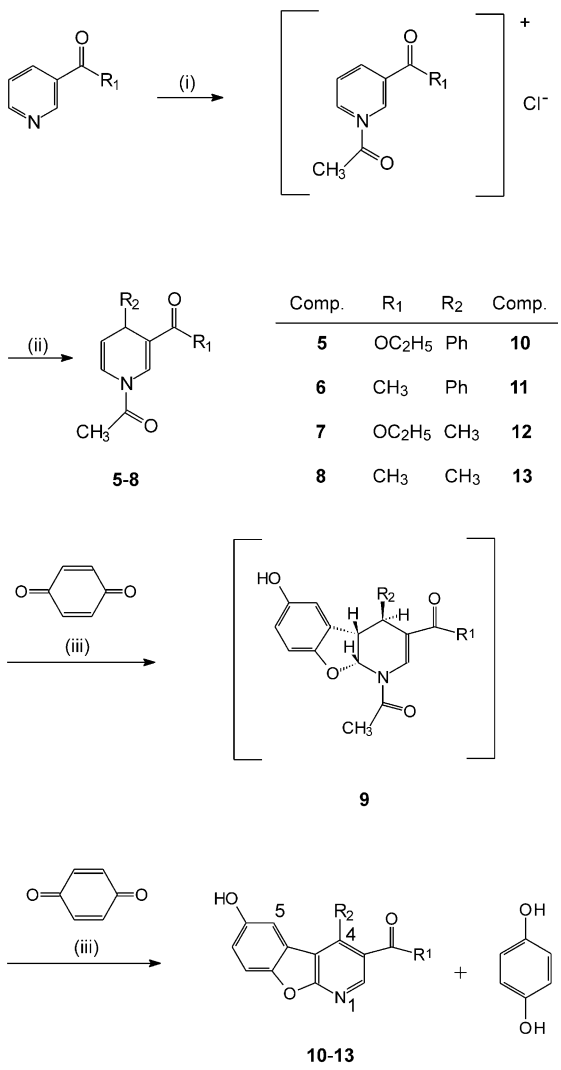
showed poor cytostatic activities.[5] Introduction of a 6-hydroxy function in the carboline scaffold was promising because 9-hydroxyellipticine showed improved cytostatic activity compared to ellipticine.[3] However, the 6-hydroxycarbolines were found inactive because of metabolic inactivation by dimerization via the indolo-nitrogen.[6]

We present the synthesis and cytostatic evaluation of a first series of novel 1-aza-9-oxafluorenes **10**–**13** as tricyclic aromatic compounds with higher activity as were reported for comparable tricyclic carboline systems. Compared to α-carbolines, we formally replaced the indolo-nitrogen by oxygen so that metabolic inactivation of the compounds via dimerization could be excluded. The compounds **10** and **11** showed cytostatic activity in the range of ellipticine derivatives. Supported by experimental data, the cytostatic action is discussed as non-intercalative.

Synthesis

The *N*-acetyl 1,4-dihydropyridine starting compounds **5**–**8** were prepared from ethyl nicotinate and 3-acetyl pyridine. First, acetylation was carried out with acetyl chloride at low temperature (−15 °C) in dried tetrahydrofurane. The intermediate *N*-acetyl pyridinium salts were regioselectively arylated or alkylated at the 4-position of pyridinium ring by Grignard reagents and catalytic amounts of copper(I) iodide (Scheme 1).[7]

The isolated 4-substituted 1,4-dihydropyridines **5**–**8** were then stirred with equimolar amounts of *p*-benzoquinone in dioxane/perchloric acid (5%) at room temperature. A regioselective formation of a primary cycloaddition product tetrahydro-1-aza-9-oxafluorene **9** took place. After the cycloaddition reaction was completed, the mixture was treated with an excess of *p*-benzoquinone leading to oxidized 1-aza-9-oxaflourenes **10**–**13** as aromatic compounds besides hydroquinone (Scheme 1). The structures of target compounds **10**–**13** were confirmed by IR and ^{1}H NMR spectroscopy, mass spectrometry and elemental analysis after their purification by recrystallization from ethanol or by silica gel column chromatography.



Comp.	R_1	R_2	Comp.
5	OC_2H_5	Ph	**10**
6	CH_3	Ph	**11**
7	OC_2H_5	CH_3	**12**
8	CH_3	CH_3	**13**

Scheme 1. Key: (i) CH_3COCl, THF, −15 °C; (ii) R_2MgCl, CuI, −15 °C; (iii) dioxane/$HClO_4$ (5%), rt.

Table 1. The GI_{50} values (μM) of compounds **10**–**13** to five cancer cell-lines[a]

Compd	SF-268	KM12	SNB-75	IGROVI	NCI/ADR-RES
10	29.7	5.1	16.5	2.5	4.7
11	27.7	40.8	2.6	5.0	19.6
12	69.9	70.3	26	86	41
13	>100	—	—	—	—

[a]SRB protein cytotoxicity assay, primary screening method performed at the National Cancer Institute, Bethesda, MD, USA, using a reported procedure.[9,10]

Cytostatic Activity

Cytotoxic evaluation was carried out in various cell-lines as shown in Table 1. Highest activities in the range of reported data for ellipticine of about $GI_{50} = 1$–10 μM in severe cell-lines (KB,[8] L1210[3]) were found for the 4-phenyl substituted compound **10** in KM12 (colon cancer), IGROVI (ovarian cancer) and NCI/ADR-RES (breast cancer) with $GI_{50} = 2.5$–5 μM and for **11** in SNB-75 (CNS cancer) and IGROVI with $GI_{50} = 2.6$–5 μM.

A significant decrease in cytostatic activity was found for the 4-methyl derivatives: compound **12** showed relatively moderate inhibitory activity as was reported for the alkyl chain substituted β-carboline derivatives.[4]

The 3-acetyl compound **13** was found almost inactive with $GI_{50} > 100$ μM tested in just one cell-line.

Discussion and Conclusion

Obviously the 4-phenyl substituent plays an important role in the cytostatic activity of the 1-aza-9-oxafluorenes as a novel class of potent small-sized cytostatics. The most decisive feature for intercalating property of potential cytostatics is the already mentioned planar aromatic system. The tricyclic 1-aza-9-oxafluorene scaffold,

however, fulfils this requirement. Cytostatic activities of the comparable tricyclic α-carboline systems in the millimolar range[11] are much lower than those found for the 4-phenyl-1-aza-9-oxafluorenes **10** and **11**. So we questioned whether the 4-phenyl ring contributes to planarity of the whole molecule. A coplanar 4-phenyl substituent may result in an enhanced intercalation potential of a quasi-tetracyclic aromatic system, thus giving an explanation for the increased activity of our compounds **10** and **11** compared to the reported tricyclic α-carboline derivatives. We analyzed the ^{1}H NMR spectra of all target compounds **10**–**13** and of the isolated tetrahydro-intermediates focusing on the chemical shifts of the 5-H, 7-H and 8-H: we found highfield-shifts of the 5-H in **10** to 6.46 ppm and in **11** to 6.28 ppm compared to the resonances of 5-H in both 4-phenyl tetrahydro-precursors **9** at 6.73 and 6.66 ppm. The resonances of 7-H and 8-H were found at higher ppm values in **10** with 6.96 and 7.47 ppm and in **11** with 6.97 and 7.48 ppm due to the enhanced aromatic systems after complete molecule oxidation. The 5-H signals of both 4-methyl derivatives **12** and **13** at 7.53 and 7.56 ppm were found with comparably high ppm-values, like 7-H and 8-H between 7.03 and 7.53 ppm.

The significant highfield-shift of 5-H in **10** and **11** of more than 1 ppm compared to the resonances of 5-H in the 4-methyl derivatives is caused by a shielding effect of the neighboring phenyl substituent that will consequently not lie within the plane of the aromatic system. Otherwise the resonances of both 5-protons would have been found at higher ppm values. These experimental results suggest that the novel cytostatics may not act as intercalators.

Finally, we presented a novel class of cytostatic agents. Among the classes of tricyclic compounds they are more potent than intercalative carboline systems. The 4-phenyl derivatives show cytostatic activity like tetracyclic ellipticine compounds. So 1-aza-9-oxafluorenes are promising lead structures in the group of small-sized cytostatics with potentially better bioavailability. In present studies, we investigate the mode of cytostatic action which may be novel within the group of tricyclic cytostatics.

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