Using Molecular Symmetry to Form New Drugs: Hydroxymethyl-Substituted 3,9-Diazatetraasteranes as the First Class of Symmetric MDR Modulators**

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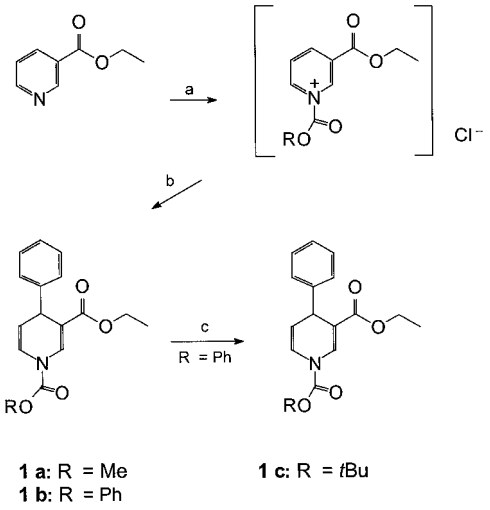
Integral proteins that function as ion channels, neural receptors, or transport proteins pose problems as target structures for the modern development of drugs, since the region that is recognized or bound by functionally modified pharmaceuticals is often not known or only insufficiently characterized.[1] Nonetheless, a design on the basis of structure–activity relationships requires exact knowledge of the spatial positioning of the amino acids in the bonding region.[1] Since the crystal structure analysis of integral membrane proteins is extraordinarily difficult, theories on how these proteins function and how they are affected by drugs are often based on models.[1,2] Examples of target structures that have so far only been described with models and that are currently of great interest for the battle against multidrug resistance (MDR) in the treatment of cancer include the transmembrane molecular pump P-glycoprotein (P-GP) and the multidrug resistance associated protein (MRP).[3,4] Owing to their position in the fluid mosaic membrane of the cell, integral proteins are difficult to crystallize. However, suitable crystals are needed to elucidate the structure of the bonding region for drugs that could modulate or reverse the multidrug resistance by blocking the molecular pump. As a result, even today the development of MDR modulators based on the target structure is not possible. Nevertheless, numerous drugs from various classes such as verapamil (VRP) or cyclosporin A have been characterized as MDR modulators in in vitro assays. These drugs inhibit the integral protein pumps and, when given together with substrates for the P-GP and MRP, reduce the expulsion of the latter from the tumor cells.[5] However, such drugs display a pharmacological action of their own, which is also shown by related compounds that have been further developed and structurally modified, and as a result cannot be used as MDR modulators in practice. The desired inhibitory effect requires concentrations in ranges where the native pharmacological action dominates or serious side effects appear.[6]

Here we present the only known class of compounds that are themselves symmetric modulators. Preliminary in vitro assays with MDR-resistant cancer cells show that concentrations below toxic levels are sufficient for a reversal of the MDR. These novel modulators belong to the group of hydroxymethyl-substituted 3,9-diazatetraasteranes and, in contrast to all other drugs known so far, display a symmetrical form. Owing to the rigid cage structure, the positioning of the functional groups for interaction with amino acids in the potential binding region is exactly defined. Since the compounds act as strong inhibitors, their rigid symmetrical structure could provide evidence for a possible, as yet unknown, symmetrical structure of the potential protein binding region.

The synthesis of the symmetric inhibitors **6a–c** started from the *N*-substituted 4-aryl-1,4-dihydropyridines **1a–c**. Compounds **1a** and **1b** were obtained by the reaction of nicotinic acid ethyl ester with the methyl and phenyl esters of chloroformic acid followed by regioselective arylation at the 4-position catalyzed by copper(I) iodide (Scheme 1).[7] The *N*-Boc derivative **1c** was prepared from **1b** with potassium *tert*-butoxide.



Scheme 1. Synthesis of the monomeric 4-aryl-1,4-dihydropyridines. a) CuI (5 mol%), ROCOCl (1 equiv), 10 min, −8°C, THF; b) PhMgCl (1 equiv), 30 min, RT, **1a** (75%), **1b** (83%); c) *t*BuOK (2 equiv), 30 min, −18°C, THF, **1c** (69%).

Interestingly, irradiation of solutions of **1a–c** with an Ultra-Vitalux lamp at $\lambda > 270$ nm resulted in excitation of the 1,4-dihydropyridine chromophore between 296 and 305 nm and formation of the 3,9-diazatetraasteranes **3a–c** as head-to-tail, centrosymmetric dimerization products. In addition, the corresponding novel *anti* dimers **4a–c** were also formed (Scheme 2).

Neither form of the dimerization products has yet been described as the direct reaction product for 5-unsubstituted 4-aryl-1,4-dihydropyridines. With the corresponding *N*-alkyl-substituted monomeric, 1,4-dihydropyridine derivative, tetra-

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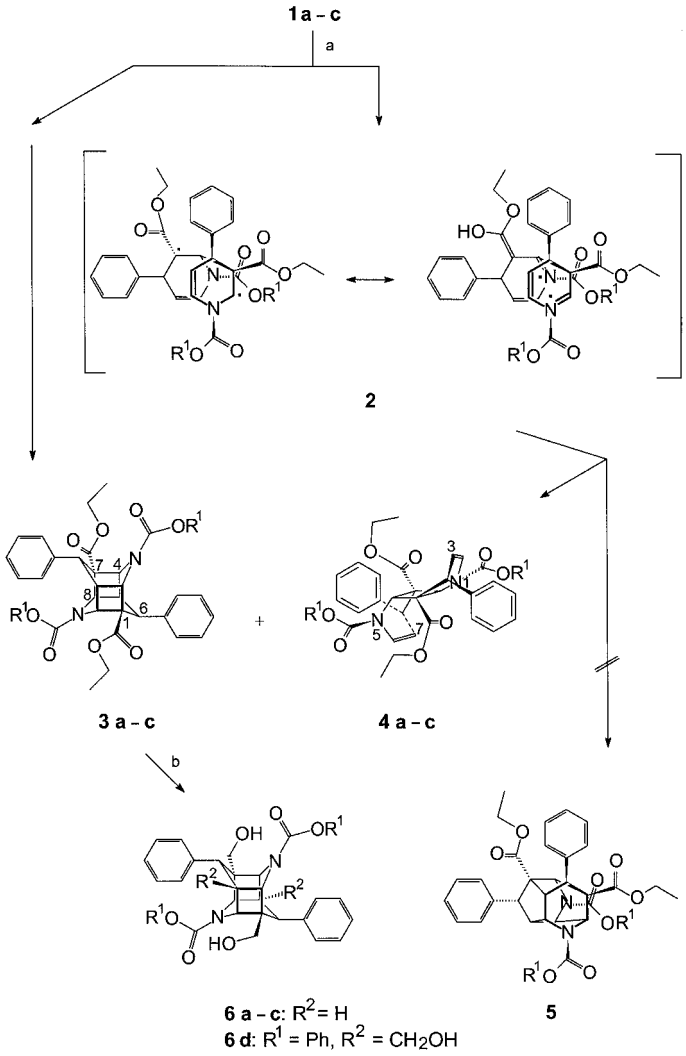

Scheme 2. Synthesis of the target structures **6a–c** starting from the monomeric 4-aryl-1,4-dihydropyridines. a) *hv*, $\lambda > 270$ nm, 4 weeks, 27 °C, THF, **3a** (44 %), **4a** (33 %), **3b** (46 %), **4b** (32 %), **3c** (41 %), **4c** (38 %); b) $LiAlH_4$ (1 equiv), 2 h, −8 °C, THF, **6a** (78 %), **6b** (75 %), **6c** (65 %).

kishomocubane **5** was formed.[7] We are currently investigating the causes of the unexpected outcome of this reaction. In the case of the *anti* dimers we propose a stabilizing conjugated effect of the *N*-oxycarbonyl group on a hypothetical intermediate biradical **2**, which then undergoes bond rotation to form the *anti* dimer. Subsequent selective reduction of the ester groups at the substituted cyclobutane ring of the dimers is accomplished with lithium aluminum hydride at low temperature and provides the target compounds **6a–c**.

The in vitro assay to determine the inhibition of the activity of the molecular pump made use of fluorescence measurements of untreated cells and cells that had been treated with P-GP modulators. The ratio of the uptake of a P-GP transporter substrate that shows specific binding and fluorescence, for example, rhodamine 123, by tumor cells and cells that have become resistant through MDR gene transfer was calculated as the ratio of P-GP inhibitory activity *R* (Tabelle 1).[8] This direct comparison of the fluorescence data ensures that the observed activities observed for the specific P-GP inhibitors is based on the MDR modulators.

Table 1. Effects of the hydroxymethyl-3,9-diazatetraasteranes **6a–d** on the MDR of tumor cells (mouse T-lymphoma).[a]

Compound	Activity ratio R[b,c] 3 μM	30 μM
6a	1.11	18.62
6b	28.10	30.87[d]
6c	6.81	7.42[d]
6d	0.97	5.40

[a] VRP control with 11 μM: 7.27. According to ref. [8] 11 μM is the relevant maximum concentration for MDR modulation. The compounds are active (A) when the ratio is $R > 1.1$, and very active (AA) for *R* values above 10.[8] [b] $R = \frac{\text{(MDR, treated/MDR, untreated control)}}{\text{(parent cell line, treated/parent cell line, untreated control)}}$. [c] Average value from two determinations. [d] Saturation concentration for P-GP inhibition.

In the case of the hydroxymethyl cage compounds **6a–c**, the *N*-methoxycarbonyl-substituted compound **6a** showed an activity similar to that of VRP; a linear relationship between the concentration and the inhibitory activity was determined. Structural characteristics required for sufficient activity of MDR modulators include the presence of two lipophilic moieties and a basic nitrogen atom, which was replaced for the first time by Ecker et al. by an ester group that functioned as a hydrogen bonding acceptor.[9,10] In our compounds the nitrogen atom, a hydrophilic structural element, is replaced by the hydroxymethyl group. The theory that sufficient lipophilicity is requirement for good MDR modulators was supported by the fact that compounds **6b** and **6c**, which contain more lipophilic groups at the N-substituents, showed increased activity at low concentrations.[11] It is interesting that an increase in the total number of potential hydrogen bond donor and acceptor functionalities from four (two hydroxymethyl and two carbamide ester groups) in **6b** to six (four hydroxymethyl and two carbamide ester groups) in **6d**[12] is accompanied by complete loss of activity at the same low concentration (3 μM).

Until now the presence of a large number of moieties that can participate in hydrogen bonding, for interaction with the amide groups that are in the proposed bonding site, was considered to be important for an optimal bonding affinity to the P-GP.[11,13,14] Initial studies on a series of 3,9-dialkyl-3,9-diazatetraasteranes[15] provide inhibitory activity ratios of 30.19 at 3 μM and 24.83 at 30 μM for a 3,9-dibenzyl derivative with four hydroxymethyl groups. This corresponds to the activity of compound **6b**, which contains two hydroxymethyl and two carbamide ester groups and therefore also presents a total of four hydrogen bonding sites. A 3,9-dimethyl-3,9-diazatetraasterane with four hydroxymethyl groups provides *R* values of 0.88 (3 μM) and 4.7 (30 μM) and thus does not display any noteworthy P-GP inhibitory activity until a concentration of 30 μM. This underlines the importance of a bulky lipophilic N-substituent for inhibitory activity, as shown by the comparison of the 3,9-dimethoxycarbonyl derivative **6a** with the 3,9-diphenoxycarbonyl derivative **6b**. A decrease in the number of hydroxymethyl groups from four to two leads to a decrease in the inhibitory activity ratio *R* to 16.42 (3 μM) and 10.69 for the 3,9-dibenzyl derivative (30 μM) and to 0.67 (3 μM) and 2.7 (30 μM) for the 3,9-dimethyl derivative. In the case of four hydroxymethyl groups, similar results are obtained as for the combination of two hydroxymethyl and

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two carbamide ester groups. In contrast, the presence of four hydroxymethyl and two carbamide ester groups, and therefore a total of six hydrogen bond donor and acceptor functionalities, is considered to be less favorable. Our results suggest a limit on the number of hydrogen bond donor and acceptor groups, at least for this first series of symmetric modulators, and therefore provide an important contribution to the discussion on the role of hydrogen bonding with respect to activity. A comparison of the concentrations required for sufficient inhibitory activity (< 3 μM) and the CC_{50} values for the cyctotoxicity of **6b** and **6c** (> 93 and 125 μM, respectively), obtained from MTT assays[16] for the determination of lactate dehydrogenase (LDH) activity in metabolically active normal cells (MT-4), makes clear that the novel MDR modulators evoke convincing inhibitory activities at concentrations below those causing cytotoxic effects.

In conclusion, hydroxymethyl-substituted 3,9-diazatetraasteranes represent an independent class of novel MDR modulators that show activity at concentrations below cytotoxic levels. A highly interesting characteristic of these compounds is the rigidly symmetric form, which, owing to the extraordinary effect on the target structure, could be a reflection of the composition of the molecular pump or the potential bonding region, whose structure is as yet unknown. The goal of further work is to examine this "symmetry hypothesis" through the synthesis and investigation of unsymmetrical compounds. In order to estimate the clinical potential experiments are planned on additional resistant cell lines, whose MDR is based on the expression of molecular pumps other than the P-GP.

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Fluorescence Spectroscopic Quantification of the Release of Cyclic Nucleotides from Photocleavable [Bis(carboxymethoxy)coumarin-4-yl]methyl Esters inside Cells**

Volker Hagen,* Stephan Frings, Jürgen Bendig, Dorothea Lorenz, Burkhard Wiesner, and U. Benjamin Kaupp

Caged compounds are an elegant means to produce rapid jumps in the concentration of chemical messenger molecules inside cells.[1] Caged compounds are photolabile inactive derivates of biologically active molecules. The biologically active substance is rapidly released by a photochemical reaction. Caged compounds allow the elucidation of complex intracellular processes and their resolution in time and space.

For many applications it would be useful to determine quantitatively the degree of photolysis of the caged compound inside cells. In a few cases this has been possible by a rather complicated calibration of the cellular reaction. Here we show that the determination of the amount of released cyclic nucleotides is feasible by fluorescence measurements, using the axial and equatorial diastereomers of [6,7-bis(carboxymethoxy)coumarin-4-yl]methyl (BCMCM) esters of cyclic adenosine-3′,5′-monophosphate (cAMP) **1** and cyclic guanosine-3′,5′-monophosphate (cGMP) **2**,[2] which are excellent phototriggers for cyclic nucleotides.

Upon irradiation with UV light in aqueous buffer solution and inside cells, **1** and **2** rapidly produce (within 2–5 ns) cAMP

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