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Original article

Design, synthesis and biological evaluation of new oligopyrrole carboxamides linked with tricyclic DNA-intercalators as potential DNA ligands or topoisomerase inhibitors

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Abstract

In the context of the design and synthesis of minor groove binding and intercalating DNA ligands some new oligopyrrole carboxamides were synthesized. These hybrid molecules (combilexins) possess a variable and conformatively flexible spacer at the N-terminal end. As intercalating tricyclic systems acridone, acridine, anthraquinones and in a special case iminostilbene terminate the N-terminal end of the pyrrole chain. The cytotoxicity was examined by the NCI antitumor screening, furthermore, biophysical as well as biochemical studies were performed in order to get some information about the DNA binding properties and topoisomerase inhibition effect of this new series of molecules.

Keywords: DNA ligands; Oligopyrrole carboxamides; Hybrid molecules; DNA intercalation; DNA minor groove binding

1. Introduction

The interesting observation that the natural DNA ligand distamycin A **1** can bind side-by-side in the minor groove of DNA with selectivity for AT sequences [1] led to a pioneering research in several laboratories about gene targeted drugs [2,3] in order to create sequence-selective DNA minor groove binding molecules. The potential for the approach in the treatment of diseases such as cancer hardly needs elaboration. In this context and with a prospective view, specific artificial interference with gene expression through mediation of DNA site-specific transcriptional activation and repression [4,5] should have far-reaching implications concerning drug developments of compounds with polyamidic structure [6–8]. Meanwhile, there exist compounds, which combine structurally a sequence reading oligopyrrole carboxamide chain as potential carrier linked with intercalating, alkylating or other heterocyclic systems [6,8–11]. Some of these DNA ligands (so called combilexins [8]) possessing a dual binding mode also interfere with DNA-dependent enzymes as for example topoisomerases [12,13]. In connection with the development of new DNA ligands of the general type **3** (Scheme 1) some biophysical, biochemical and biological studies were evaluated by our group also including compounds linked

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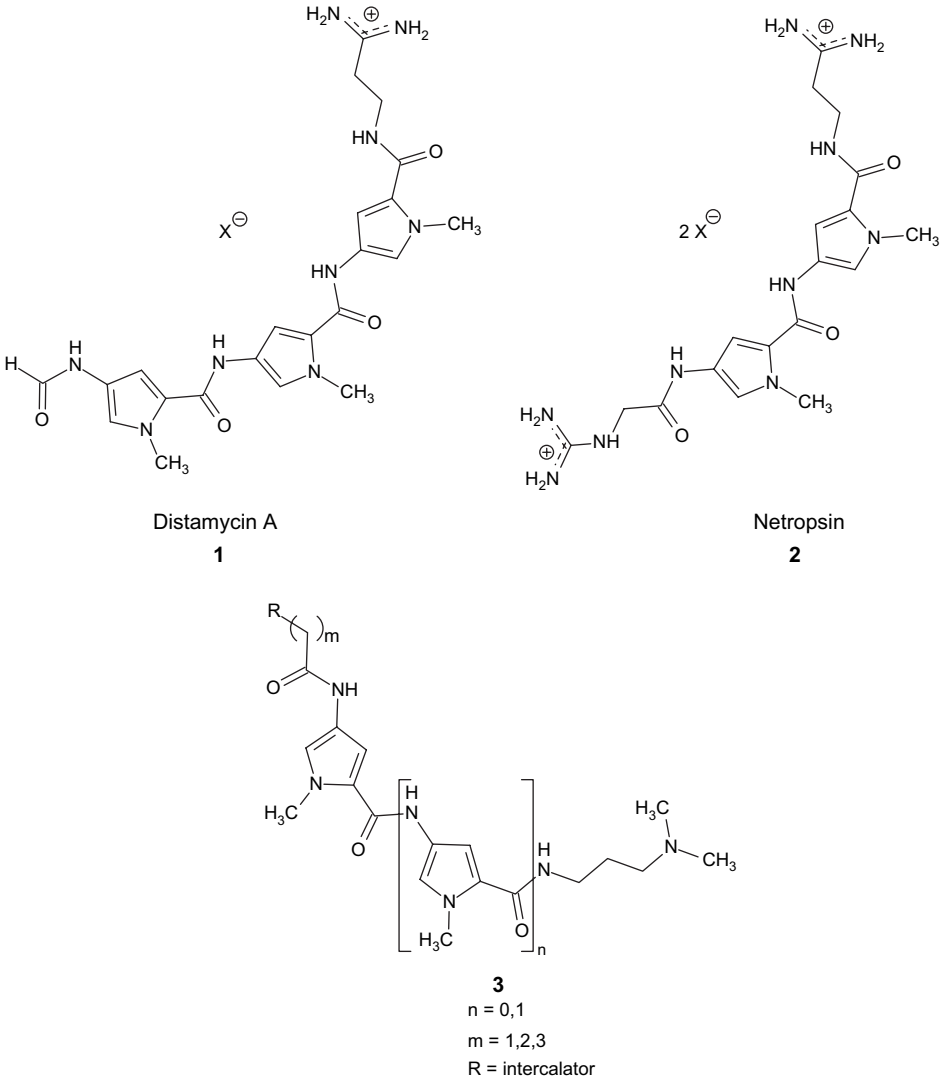


Scheme 1. Distamycin A **1**; netropsin **2**; general formula of combilexins **3**.

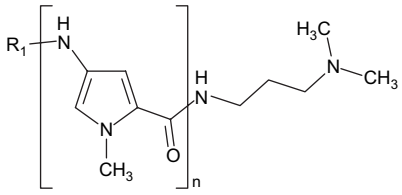
with nucleic bases [14,15]. Because of encouraging results, our research was focused on modifying the structure of the N-terminal end in **3** by using different intercalating tricyclic systems connected with the oligopyrrole carboxamide chain by a conformatively flexible tether. Moreover, as variable linkers at the N-terminal end, aliphatic acyl functions or amino substituted acyl functions were introduced. The modification of this class of potential anticancer drugs should further extend the knowledge of structure activity relationships. Therefore, hybrid molecules of type **3** were previously evaluated in the NCI antitumour screening [16] and concerning DNA binding experiments several biophysical and biochemical studies were performed [17–19].

2. Chemistry

In previous studies of our group [14,15,20] a variety of new combilexins of the oligopyrrole carboxamide series of type **3** (Scheme 1) with variations of the C- and N-terminal functions were presented. The biological and biochemical results demonstrated the superior DNA binding of those hybrid compounds which possess a C-terminal dimethylamino propyl function mimicking the highly basic amidine group of distamycin A **1** or netropsin **2** (Scheme 1) [14,20]. Thus, for new developments and in order to expand the preliminary structure activity relationships [14,15,20] further structural variations only were performed at the N-terminal end. In the present study about new combilexins of type **3** (Scheme 1) the intercalating chromophores acridone, (5-nitro-)naphthalimide, acridine and the photosensitizer anthraquinone were introduced. Moreover, to complete the ring-homology series starting with indole and carbazole in a former report [14,20] and now continued with acridine, the non-coplanar iminostilbene was introduced. However this heterocycle is better known as structural element in antidepressive and anticonvulsive drugs and was not mentioned in connection with cytotoxic or antitumor active compounds until now.

The access to the new combilexins of type **3** (see products **17–27**, **40–47**, Tables 1 and 2) started with the synthesis of the respective mono- or bis-pyrrole building blocks, which

Table 1
Intercalator-linked pyrrole carboxamides **17**–**27**, Py = pyrrole carboxamide



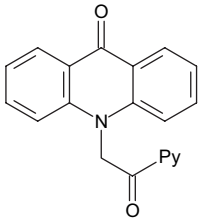
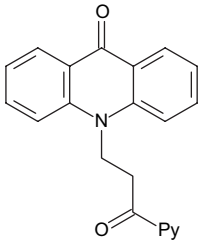
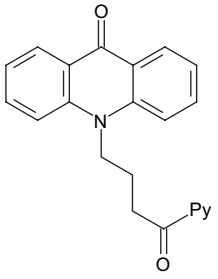
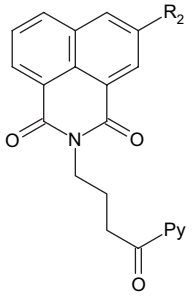
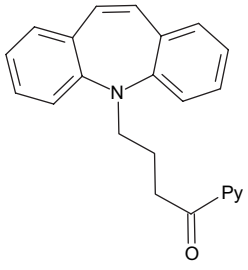
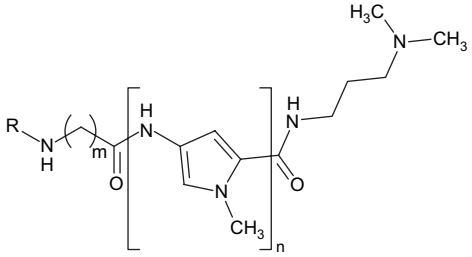
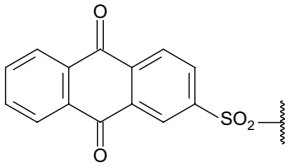
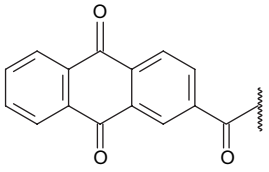
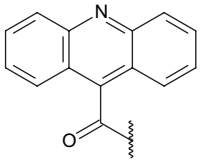
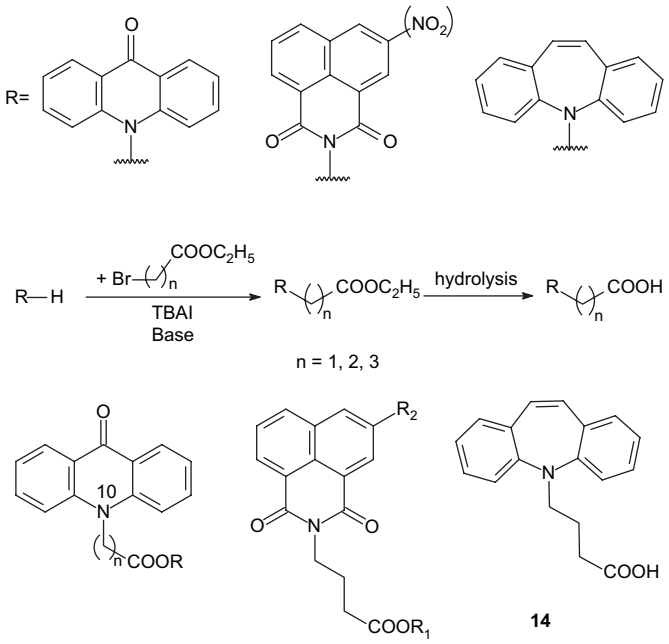
R_1	R_2	*n*	Yield [%]	Compound
		1	54	**17**
		2	51	**18**
		1	59	**19**
		2	49	**20**
		1	62	**21**
		2	48	**22**
	H	1	35	**23**
		2	31	**24**
	NO_2	1	35	**25**
		2	21	**26**
		2	20	**27**

Table 2
Intercalator-linked pyrrole carboxamides **40**–**47**



R	*m*	*n*	Yield [%]	Compound
	2	1	28	**40**
	3	1	17	**41**
	2	2	17	**42**
	3	2	13	**43**
	2	1	25	**44**
	3	1	14	**45**
	2	1	11	**46**
		2	10	**47**

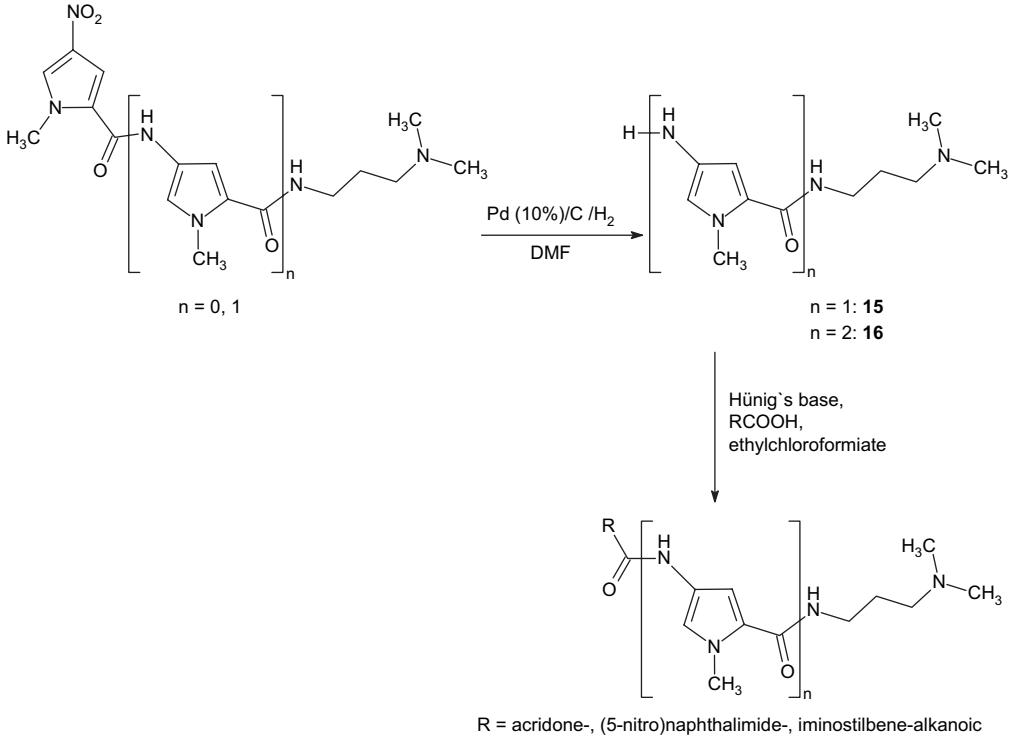
were C-terminated with a dimethylamino propyl group. The procedures to these synthetic building blocks were according to the proved strategy described in references [14,15,20]. The relatively weak NH-acidic heterocycles acridone, (5-nitro-) naphthalimide and iminostilbene were N-alkylated with ethyl-4-bromo-butanoate in the presence of a strong base (see compounds **8**, **10**, **12**, Scheme 2). In all cases as catalyst tetrabutylammonium iodide was used to increase the yield by an intermediary Finkelstein reaction. Additionally the esters of the propionic and acetic acids of the acridones **4** and **6** (Scheme 2) were synthesized to evaluate the influence of the linker-length at the N-terminal end of the combilexins of type **3** on DNA binding or antitumor cell cytotoxicity. The primarily produced acridone alkanoic esters **4**, **6**, and **8** (Scheme 2) and the iminostilbene butanoic ester – the last was accessible only in low yields – were hydrolysed to the carboxylic acids **5**, **7**, **9**, and **14** (Scheme 2) with 1 M NaOH. Due to the poor stability of the imide-function, for the (5-nitro-)naphthalimide alkanoic esters **10**, **12** (Scheme 2) LiOH or HCl (15%) were successfully used as hydrolysing reagents. In the following step the carboxylic acids **5**, **7**, **9**, **11**, **13**, and **14** (Scheme 2) were coupled with the amino substituted mono- or bis-pyrrole carboxamides **15** and **16** (Scheme 3), which were generated in situ by reduction of the nitro-derivatives with Pd/C (10%) in hydrogen atmosphere (Scheme 3). In all cases the coupling



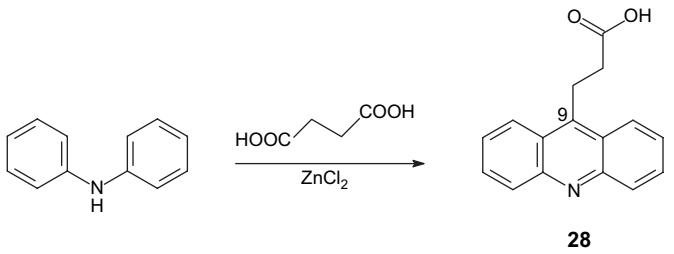
	R	n		R_1	R_2
4	C_2H_5	1	**10**	C_2H_5	H
5	H	1	**11**	H	H
6	C_2H_5	2	**12**	C_2H_5	NO_2
7	H	2	**13**	H	NO_2
8	C_2H_5	3			
9	H	3			

Scheme 2.

reactions were catalysed by ethyl chloroformate, which has been proved to be the most appropriate agent after having tested several other peptide-coupling reagents [15] (Scheme 3). Moreover the 9-acridine alkanoic acids were planned as coupling partners for the amino pyrrole carboxamide building block. The 9-acridine propionic acid **28** (Scheme 4) was synthesized from succinic acid and diphenylamine in 20% aqueous sulfuric acid with addition of $ZnCl_2$ as a catalyst (Scheme 4). However, the high insolubility of the product obtained (equilibrium with the betaine structure) prevented any successful coupling reaction with the amino pyrrole carboxamides. For this reason, alternative synthetic strategies had to be developed for the introduction of the appropriate acridine and anthraquinone residues. In order to provide further NH- and CO-functions in the N-terminal sphere (to increase DNA minor groove binding) the β-alanyl or γ-amino butanoyl group should be introduced as a linker in the acridine and anthraquinone including compounds. First of all the amino pyrrole carboxamides were coupled via the ethyl chloroformate method to BOC-protected β-alanine or γ-amino butyric acid (Scheme 5). Then the products **29–32** were deprotected with ethanolic HCl (Scheme 5). Finally, the resulting amines **33–36** (Scheme 5) were linked with the anthraquinone- or acridine-carbonyl- or sulfonyl chlorides **37–39** (Schemes 6 and 7), which were routinely produced by converting the corresponding acids with thionyl chloride (Schemes 6 and 7). The compounds **17–27** and **40–47** are listed in Tables 1 and 2.



Scheme 3.



28

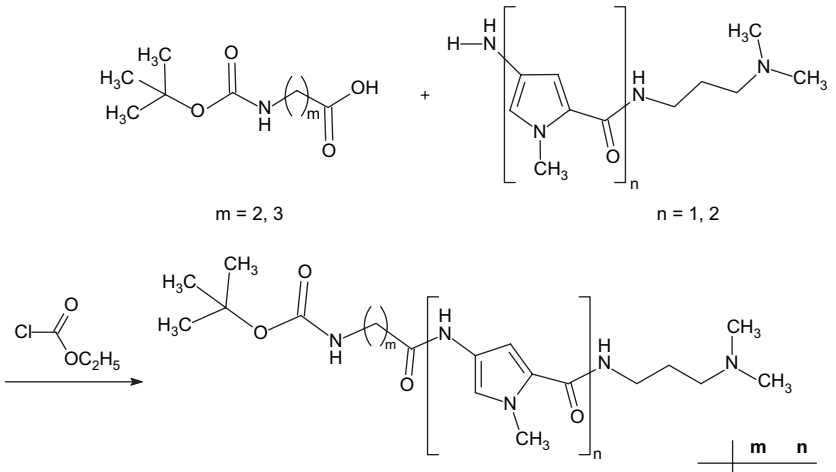
Scheme 4.

3. Biological and biophysical/biochemical methods

*3.1. Cytotoxicity assay of compounds **17–27** and **40–47***

This series of mono- and bis-pyrrole combilexins was selected by the US National Cancer Institute [22] for evaluation in the in vitro preclinical antitumor screening program. A first primary assay against three cell lines (breast, lung, CNS cancer cells) and for the most outstanding compound a screening program against 60 human tumor cell lines were used. In the primary screening cell proliferation was analysed with a 10^{-4} M concentration of the compounds in DMSO. The results are given in percent of cell growth compared to the untreated control cells. In the main screening the 60 human tumor cell lines derived from the nine cancer types, leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer were used. The compounds' dose response curves for each cell line were measured at a minimum of five concentrations at 10-fold dilutions in a protocol of 48 h continuous drugs exposure, and a sulfurhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth) and 50% cell death (LC_{50}, −50% growth) compared with the control was calculated. In general $\log_{10} GI_{50}$ values (the concentration of the drug resulting in inhibition of cell growth to 50% of control) were used for comparative discussion.

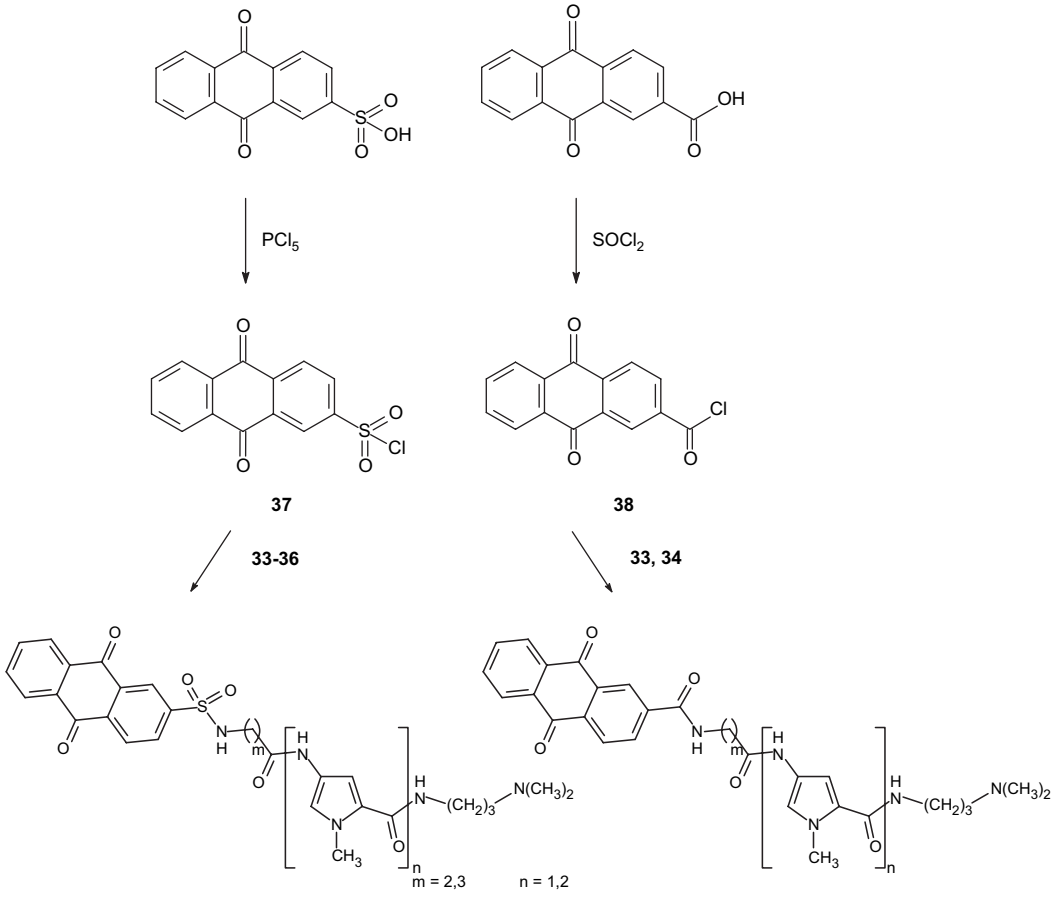
However, from the series of compounds **17–27** (Table 1) in the primary cell assay against breast cancer (MCF7), lung cancer (NCI-H460) and CNS cancer (SF-268) the iminostilbene derivative **27** was significantly active. All the three cell cultures showed 0% cell growth for **27**. In the further series



	m	n
29	2	1
30	3	1
31	2	2
32	3	2

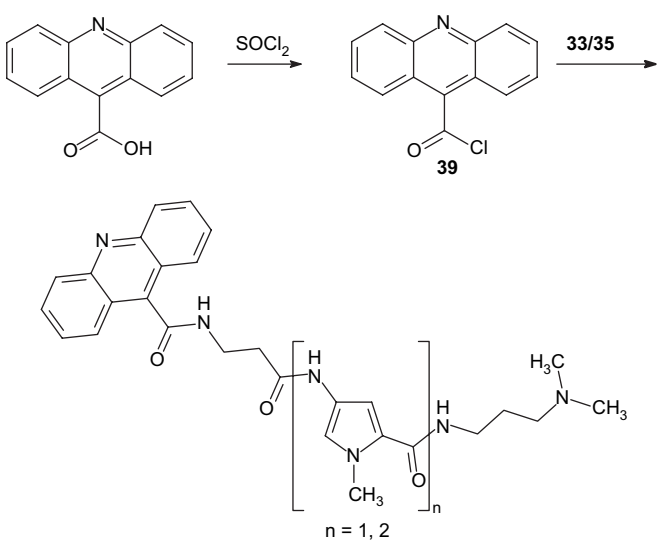
	m	n
33	2	1
34	3	1
35	2	2
36	3	2

Scheme 5.



Scheme 6.

of this compound selection cell growth inhibition of medium value was found for compounds **20** (52% growth in NCI-H460 cells) and for **22** (48% growth in MCF7 cells and 47% growth in NCI-H460 cells). NCI only continues the screening (60 panel assay) with compounds that induce a cell growth <32% in the primary assay. Thus, the iminostilbene derivative **27** passed this assay. According to the selectivity profile from NCI, the compound is sufficiently active but unselective. The mean graph midpoint value (MG_MID), which displays an averaged activity parameter over all cell lines is −5.23 (activity is defined as a $\log_{10} GI_{50}$ of <100 μM) (Table 4).



Scheme 7.

In the primary screening the compounds **40**, **42** and **44** (Table 3) showed good cell growth inhibition for some of the three cell lines. Thus, they also were selected to pass the 60 panel assay.

However, in the 60 panel assay (Table 4) compounds **40** and **42** showed good selectivity against melanoma and non-small cell lung cancer culture. Compound **27** presents the most potent cytotoxic effect against the breast cancer cell line MCF-7.

Table 3
Results of the primary NCI antitumor screening of **40**, **42** and **44** with three tumor cell lines

Compound	MCF7 (breast)	NCI-H460 (lung)	SF-268 (CNS)	Results
40	1	1	2	Active
42	27	84	98	Active
44	32	103	113	Active

Cell growth in %, concentration of drug 10^{-4} M in DMSO.

Table 4
Some results of the 60 panel NCI antitumor screening

Compound	From 60 cell lines	Cell line	GI_{50}[M]	MG_MID
27	MDA-MB-435	Breast cancer	5.75×10^{-5}	−5.23
40	UACC-257	Melanoma	5.44×10^{-5}	−4.01
42	NCI-H322 M	Non-small cell lung cancer	3.57×10^{-5}	−4.01
44		No selective inhibition	−4.13	

GI_{50}: molar concentration of compounds, which induces inhibition of cell proliferation of 50%. MG_MID: Mean graph midpoint, activity is defined as $\log_{10} G_{50}$ of <100 μM.

3.2. DNA binding and inhibition of topoisomerases I and II

3.2.1. UV–visible spectral absorbance

In order to address the DNA binding ability of this new series of combilexins, we first looked at the modification of the absorption spectra of the various compounds in the absence (solid line) and presence (dashed line) of calf thymus DNA (phosphate/drug ratio = 20). Some representative spectra are presented in Fig. 1. The acridone (**17**, **18**, **21**, **22**) and naphthalimide (**23**–**26**) derivatives presented here show either hypochromic (↓) or hypochromic and bathochromic (→) effects (Fig. 1) indicating that they all interact with the DNA helix. Comparison between compounds **17** and **18** or compounds **21** and **22** (Fig. 1) corresponding to mono- (compounds **17** and **21**) and bis-pyrroles containing derivatives (compounds **18** and **22**) of the acridone series reveals the same hypochromic effect but different bathochromic effects. At either 255 nm or 390–410 nm (acridone absorption band) the hypochromicity reaches 20–35%. The bis-pyrrole spectra differ from the mono-pyrrole derivative spectra by the presence of a medium peak at 296 nm that is shifted to higher wavelength in the presence of CT-DNA. By looking at the naphthalimide containing derivatives **23** and **24**, the bis-pyrrole corresponding peak cannot be clearly identified from the naphthalimide spectral band and only the hypochromic effect is seen. Moreover, the iminostilbene derivative **27** presents an important bathochromic effect at around 300 nm.

3.2.2. DNA-melting temperature

In order to confirm the DNA binding ability of those four compounds, we then looked at the stabilization of the DNA helix by the various combilexins using melting temperature studies. UV spectroscopic determination of the melting curve of DNA compared with the melting curve of a DNA ligand

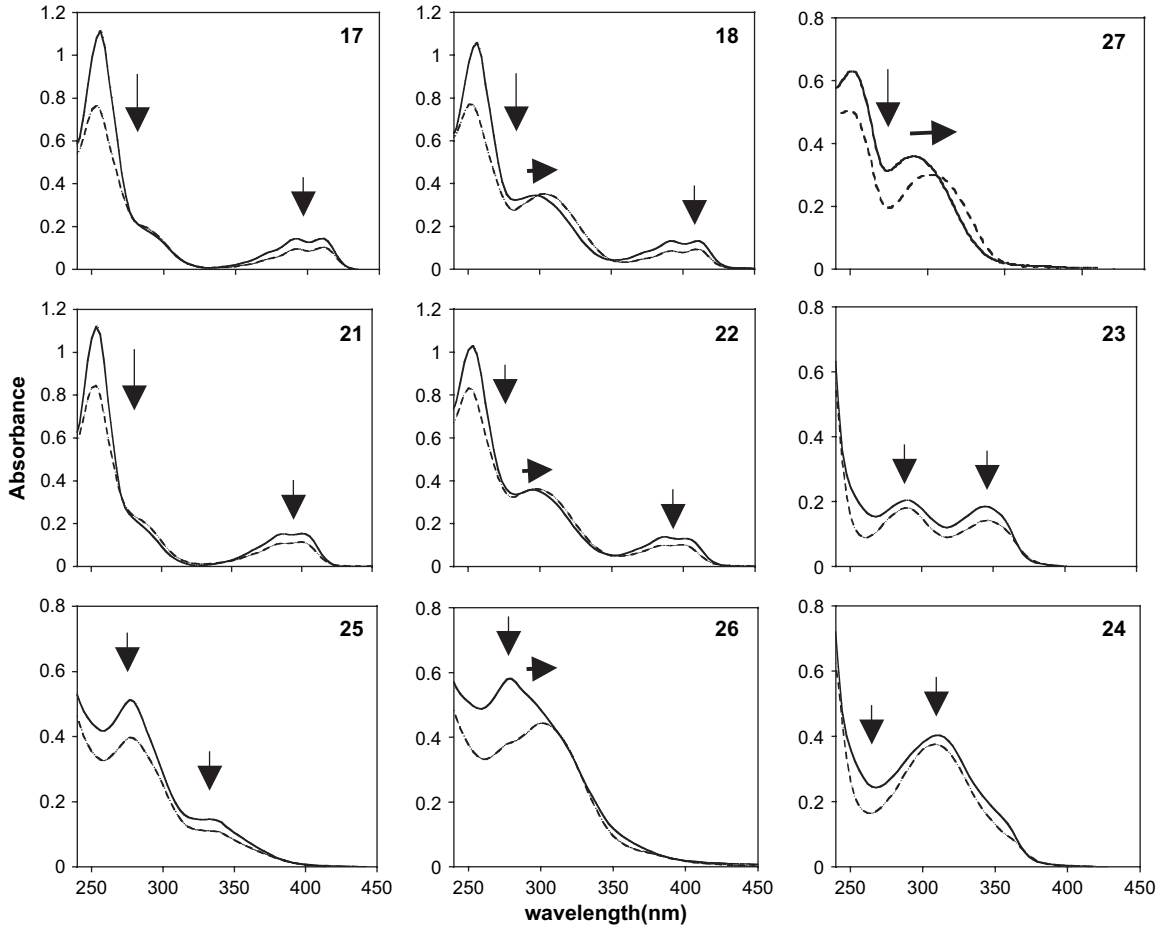


Fig. 1. UV–visible absorption spectra of acridone (**17**, **18**, **21**, **22**), naphthalimide (**23**–**26**) and iminostilbene derivatives (**27**) in the absence (solid lines) or presence (dashed lines) of calf thymus DNA (phosphate/drug ratio = 20). Arrows ↓ and → refer to hypochromic and bathochromic effects, respectively.

Table 5
Difference of DNA-melting temperature ΔT_m [°C] of calf thymus DNA and poly(dAdT) from complexation with various pyrrole carboxamides: r = molar ratio of ligand/DNA = 0.5

Compound	ΔT_m		Compound	ΔT_m	
	CT-DNA, $r = 0.5$	Poly(dAdT), $r = 0.5$		CT-DNA, $r = 0.5$	Poly(dAdT), $r = 0.5$
17	0	2.7	**41**	−1.3	6.8
18	2.7	18.5	**42**	3.9	17.4
19	2.6	11.8	**44**	3.9	13.1
21	2.7	12.0	**45**	1.3	10.5
22	9.5	26.6	**46**	1.1	5.9
23	1.7	7.0	**47**	5.7	18.2
24	5.7	20.4			
25	1.1	5.9			
26	5.7	18.2			
27	2.3	13.7			

complex gives a basic information about the binding potency of a ligand to DNA [23]. The ΔT_m values ($T_{m[CT\text{-}DNA+drug]} - T_{m[CT\text{-}DNAalone]}$) were determined with poly(dAdT)$_2$ and calf thymus DNA at DNA/drug ratio of 0.5. However, a ΔT_m value of more than 10 is a convenient sign for a good DNA binding of the ligand. Table 5 represents the ΔT_m data of a series of the new pyrrole carboxamides.

Derived from these data the compounds **18**, **22**, **24**, **26**, **42** and **47** reflect a significant affinity to the poly(dAdT)$_2$ −DNA sequence. Comparison between ΔT_m values obtained from mono-pyrrole (**17**, **21**, **23**, **25**, **41** and **46**) and bis-pyrrole carboxamides (**18**, **22**, **24**, **26**, **42** and **47**, respectively) clearly confirms the importance of the bis-pyrrole structure to increase the affinity for DNA and especially for AT-rich sequences. The presence of a NO_2 group on the naphthalimide moiety does not change the relative DNA binding affinity for CT-DNA or poly(dAdT)$_2$. Comparison of the bis-pyrrole derivatives bearing either an acridone (**22**), a naphthalimide

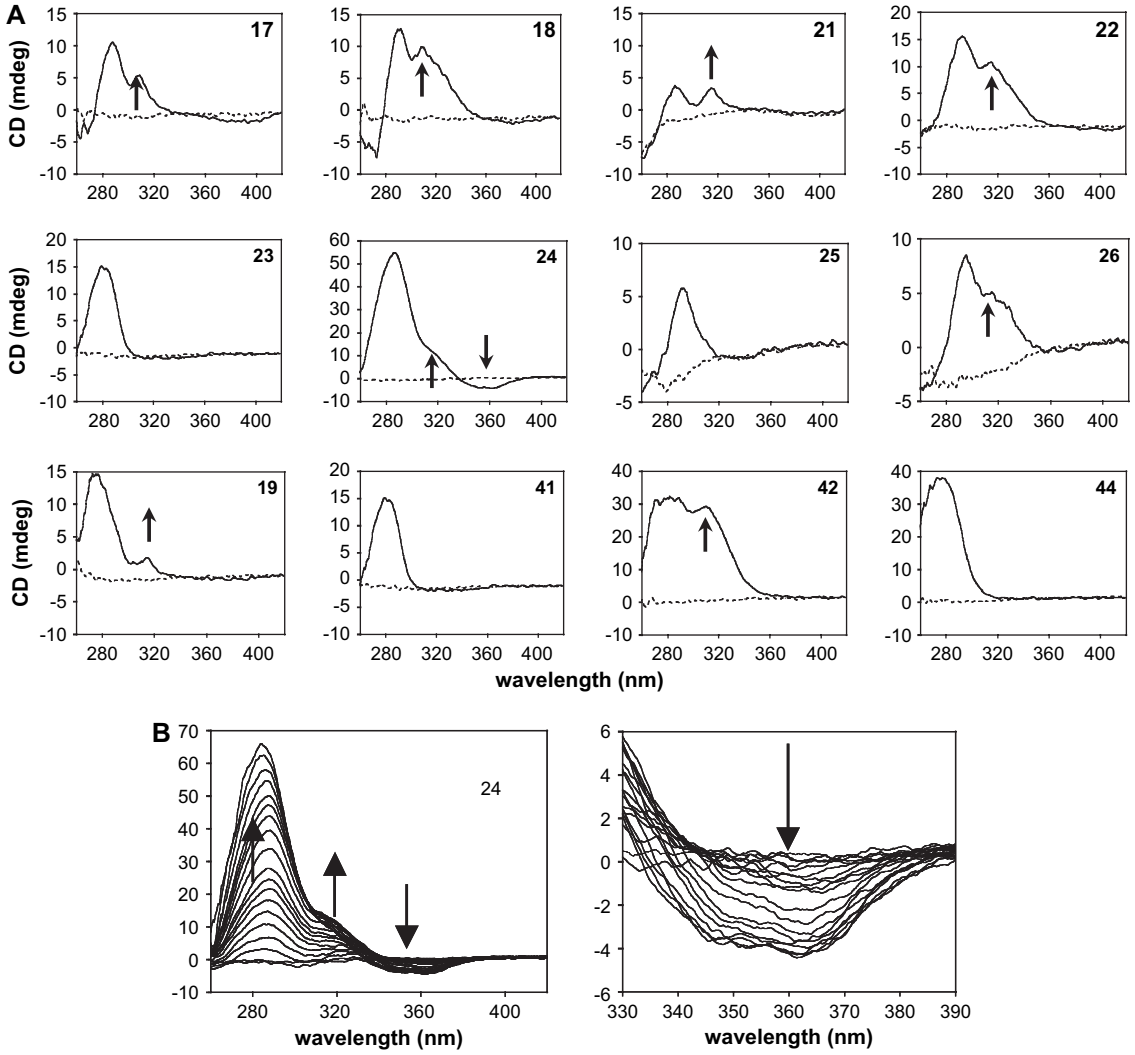


Fig. 2. Circular dichroism spectra of CT-DNA with the various combilexins. (A) CD spectra of 20 μM of the indicated compounds in the absence (dashed line) or presence (solid line) of calf thymus DNA at a phosphate/drug (P/D) ratio of 8. (B) CD titration of 50 μM of compound **24** with increasing concentrations of CT-DNA from 10 to 200 μM (P/D = 0.4–8). The right panel corresponds to the magnifying of the negative induced CD portion of the full CD graph presented on the left panel.

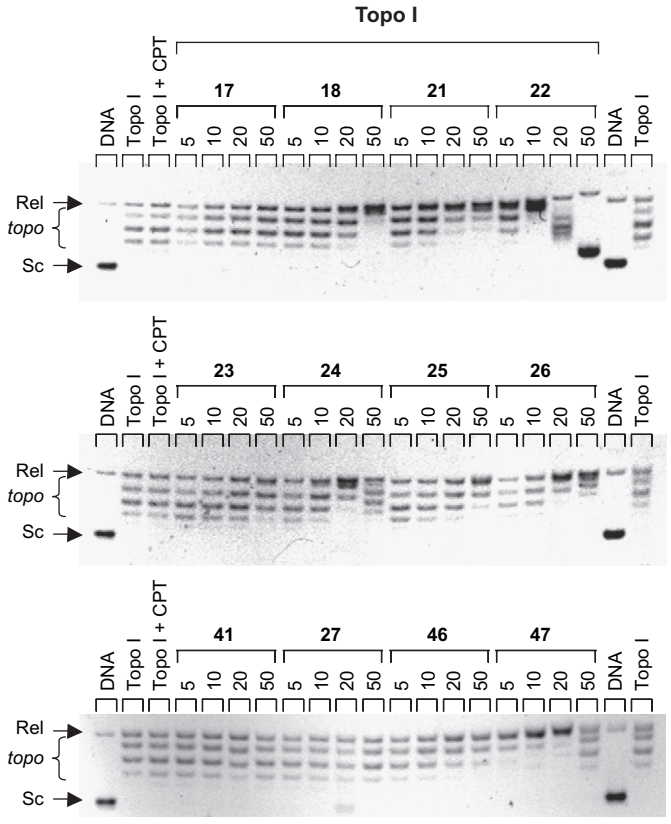


Fig. 3. Topoisomerase I-induced DNA relaxation. The effect of the various combilexins on the relaxation of supercoiled plasmid DNA by topoisomerase I was established using native supercoiled pLAZ DNA (130 ng; lane 0) incubated with topoisomerase I in the absence (lane Topo I) or presence of 5–50 μM of the various tested compounds. The various forms of plasmid DNA were separated by electrophoresis on a 1% agarose gel which was then stained with ethidium bromide. Rel: relaxed; Sc: supercoiled; *topo*: topoisomers.

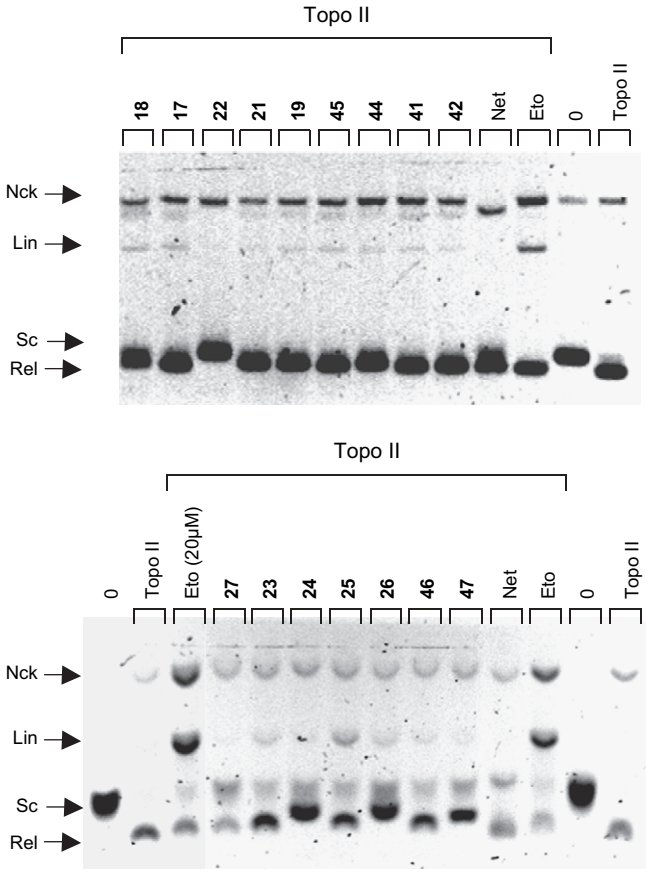


Fig. 4. Topoisomerase II Poisoning effects of various combilexins. Native supercoiled pLAZ plasmid DNA (130 ng, lane 0) was incubated with topoisomerase II (4 units) in the absence (lane Topo II) or presence of the 50 μM of the various combilexins. Etoposide (Eto) was used at 20 μM when indicated or otherwise at 50 μM. Reactions were stopped by treatment with SDS and proteinase K. DNA samples were separated by electrophoresis on an ethidium bromide containing agarose gel. The gels were photographed under UV light. Nck: nicked; Lin: linear; Rel: relaxed; Sc: supercoiled.

(**24**, **26**) or an iminostilbene ring (**27**) reveals that the acridone derivative is more potent to stabilise the DNA helix than the naphthalimide bearing compound and much more than the iminostilbene derivative. The length of the arm linking of the intercalating group to the pyrrole ring also affects the relative affinity for DNA. Indeed, the decrease of this arm from C-4 to C-2 reduces the ΔT_m value from 26 °C to 18 °C for the bis-pyrrole derivatives (compare compounds **22** and **18**) and from 12 °C to a very low temperature variation of 2 °C for the mono-pyrrole compounds **21** and **17**. This difference of DNA binding affinity suggested that the length of the arm is important for an easier conformative positioning of the intercalator and groove binder moieties in the DNA helix.

3.2.3. Circular dichroism measurements

These measurements with CT-DNA were used to define the binding mode – intercalation and/or groove binding – of the various hybrid compounds. Most of the compounds did not reveal intrinsic CD but the iminostilbene derivative **27** presents an intrinsic CD for up to 320 nm and so could not be analysed for its mode of binding to DNA using this approach to discriminate drug binding to the helix grooves or intercalation between adjacent base pairs. Compounds **21**, **25** and **26** show a small negative CD at the lower wavelengths (dashed lines) which complicates analysis of the mode of intercalation. The most common feature is a positive induced CD (ICD) in the absorption field of the oligopyrrole carboxamide ligand around 310–315 nm (Fig. 2) suggesting a groove binding of the combilexins through their pyrrole ring(s). By contrast, the naphthalimide pyrrole carboxamide compound **24** evidenced a clear negative induced CD at 350–365 nm additionally to the positive ICD at 310–315 nm. Incubation of compound **24** with CT-DNA at increasing DNA/drug ratio suggests the displacement of the compound from DNA groove binding to an intercalation binding mode between adjacent base pairs (Fig. 2B).

3.2.4. Topoisomerase I, II inhibition assay

In many cases effective DNA binders inhibit DNA-dependent enzymes as topoisomerase I and/or topoisomerase II [24].

The DNA relaxation assay induced by topoisomerase I [24] was performed, analysing the relaxed DNA. The electrophoretic test was performed on agarose gel in the presence and in the absence of ethidium bromide. However, incubation of

increasing concentration of the various combilexins with supercoiled DNA plasmid in the presence of topoisomerase I reveals that the bis-pyrrole compounds **18**, **22**, **24**, **26** and **47** efficiently intercalate with the coplanaric chromophore into the DNA helix as evidenced by the efficient formation of relaxed plasmid DNA using increasing concentrations of those four compounds (Fig. 3). A direct poisoning of the topoisomerase I (using gels pre-stained with ethidium bromide) was not detected (data not shown). These compounds cannot be considered as topoisomerase I poisons.

In an analogous assay the topoisomerase II poisoning effect of the combilexins was studied. Various compounds (50 μM each) were incubated with supercoiled plasmid DNA (Sc, see Fig. 4) in the presence of topoisomerase II enzyme. Separation of the samples on an ethidium bromide containing agarose gel reveals the blockade of the Topo II/DNA cleavable complex through linearized DNA (Lin) in the presence of some combilexins, but in a lesser extent than the reference drug etoposide (see Fig. 4). Therefore, compounds such as **17**, **18**, **25**, **44** and **45** can be qualified as mildly effective topoisomerase II poisons and some other as weak topoisomerase II poisoning agents.

3.2.5. DNase I footprinting experiments

DNase footprinting studies were used in order to clarify the sequence selectivity of a DNA ligand. These experiments were performed on a 117 bp radio-labelled DNA fragment to localize the binding sites of the compounds. The densitometric analyses were plotted by comparison with the effect of netropsin as control and reveal that bis-pyrrole derived compounds **24**, **26** and **47** share the same sequence selectivity as netropsin, whereas mono-pyrrole (**23**, **25** and **46**) derived molecules present only weak binding efficiency and selectivity (Fig. 5). This result exemplifies the importance of the tandem pyrrole rings on DNA binding and sequence selectivity. DNase I footprinting analysis also reveals that the iminostilbene derivative **27** interacts with DNA in a non-sequence specific manner (data not shown) suggesting that the iminostilbene ring interferes with the optimum binding of the two pyrrole rings in the minor groove of the DNA as does netropsin.

3.2.6. Discussion of the results and conclusion

The cell biological antitumor screening of the new pyrrole carboxamides chosen by the NCI commission shows significant cytotoxicities for the iminostilbene derivative **27** in the primary assays. First of all, measurements of the induced cell toxicity reveal that compounds **40**, **42** and **44** present interesting cell growth inhibitory effect (Table 3). Further tested in the NCI 60 cell line panel assay (Table 4), compounds **40** and **42** show good selectivity against melanoma and non-small cell lung cancer culture whereas the iminostilbene **27** derivative is particularly active against breast cancer cell lines. For those four compounds and various other derivatives, we performed DNA binding studies with the oligopyrrole carboxamides in order to address several structure activity relationships.

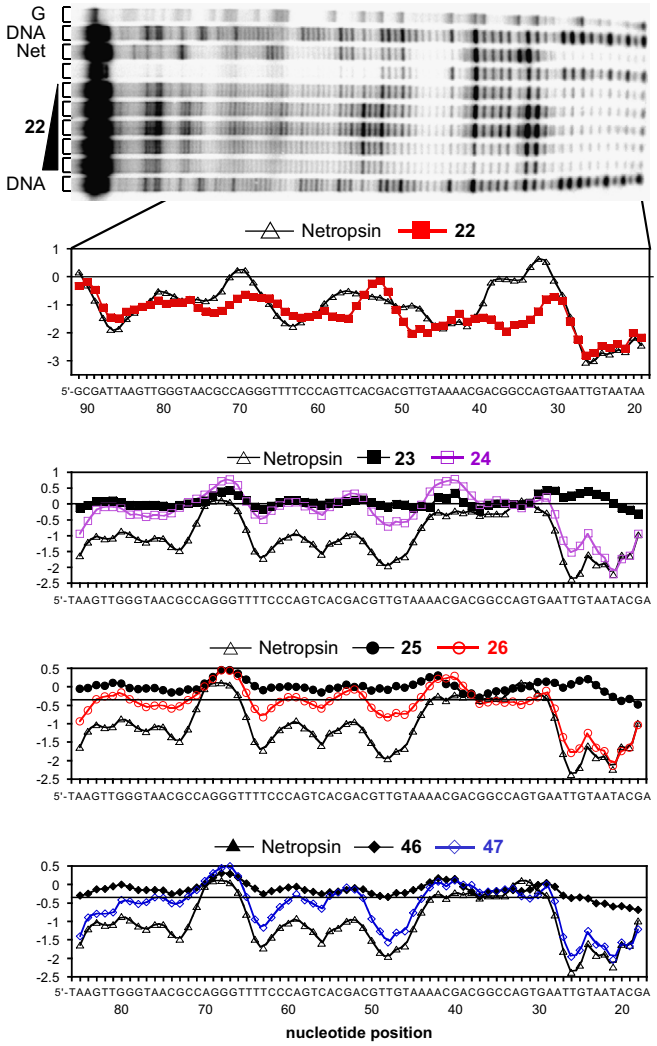


Fig. 5. DNase I footprinting experiments. Increasing amount of compound **22** (2, 5, 10, 20, 50 μM) was incubated with a 117 bp DNA fragment prior to be subjected to DNase I digestion. The resulting DNA fragments were separated on a denaturing 8% polyacrylamide gel. Net = netropsin; G = G-track.

The ΔT_m values of those four compounds **27**, **42**, **44**, and **45** range between 10.5 and 17.4 °C (medium to high DNA binding) highlighting their potent DNA binding activity.

ΔT_m values of this new series of combilexins bearing DNA intercalating and DNA minor groove structural elements confirm that the presence of two pyrrole rings instead of one increases the DNA affinity of the compounds. The C4-chain at the N-terminal end yields the most potent compounds. A maximum of four amide functions in the molecules is tolerated. However, the introduction of a further amide group in the molecules via a β-alanin or a γ-amino-butyric acid function at the N-terminal end did not lead to ligands with higher affinity or higher effects in the different spectral or biophysical investigations. In overall, among the various compounds, the acridone derivative **22** possesses the highest DNA binding affinity. On the basis of the UV and CD spectra, DNA ligand interactions are confirmed. Most of the bis-pyrrole containing combilexines showed a positive induced CD around 310–315 nm suggesting a groove binding through the pyrrole carboxamide moiety of the molecule.

Furthermore the DNA relaxation assay induced by topoisomerase I revealed that some of the bis-pyrrole compounds (**18**, **22**, **24**, **26** and **47**) act as potential intercalators by their coplanaric chromophore. Based on these preliminary results as mechanism of action sequence-selective minor groove binding combined with DNA intercalation can be supposed.

Although none of the compounds targeted topoisomerase I, some of them like the mono-pyrrole compounds bearing a naphthalimide or acridone moiety (compounds **19**, **23**, **25**), the tested compounds bearing an anthraquinone or acridine moiety (compounds **41**, **42**, **44**–**46**) or the acridone derivatives linked to mono- or bis-pyrrole carboxamide by a short spacer less than C-4 (compounds **17** and **18**) are able to block the topoisomerase II cleavable complex albeit with much weaker efficiency than the reference drug etoposide.

The overall preliminary results about the structure activity relationships were used in our group for the design and synthesis of new bioisosteric combilexins. In addition, concerning the ΔT_m and preliminary NCI results the iminostilbene oligopyrrole carboxamide **27** should be of further interest for structural modifications although it does not possess any Topo II poisoning effects or DNA intercalation potency.

4. Experimental

4.1. Chemistry

Melting points were measured with a Büchi 510 instrument and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1310 infrared-spectrometer using potassium bromide pellets (ν in cm^{-1}). ^1H NMR, ^{13}C NMR spectra including NOE were recorded on a Bruker AC-300 apparatus (300 MHz). The samples were dissolved in DMSO-d_6. The chemical shift values are reported in parts per million (ppm, δ units) and spin–spin coupling J were listed in Hz; 70 eV EI-mass spectra were obtained with a Mascom 311-A apparatus and FD mass spectra with a Finnigan MAT 7 instrument. Column chromatography was performed on silica gel (Merck, silica gel 60). The coupling products **17**–**27** and **40**–**47** were chromatographically pure (TLC). Non-stoichiometric inclusion of solvent molecules (typical for oligopyrrole carboxamides) gave C, H, N-analysis with divergence of >0.3%.

*4.1.1. Ethyl-2-(9-oxo-9,10-dihydro-10-acridinyl)acetate (**4**)*

NaH (0.15 g, 6.06 mmol) (97%) was suspended in 10 ml DMF. A suspension of 1.0 g (5.12 mmol) acridone in 20 ml DMF was added and the mixture was stirred for 0.5 h at room temperature. It was cooled to 0 °C, 1.28 g (7.66 mmol) ethyl-2-bromoacetate and 20 mg tetrabutylammonium iodide were added and it was stirred for further 24 h at room temperature. The reaction mixture was poured into 25 ml of cold water. The precipitate was filtered off, dried under vacuum and used without further purification. Yellow solid (1200 mg) (4.27 mmol) (83%), m.p. 172 °C; ^1H NMR (DMSO-d_6): δ 1.23 (t, 3H, $^3J = 7.2$ Hz, CH_3), 4.21 (q, 2H, $^3J = 7.2$ Hz, CH_2), 5.4 (s, 2H, CH_2), 7.35 (pt, 2H, $^3J = 7.4$ Hz, acridone-H), 7.65 (pd, 2H, $^3J = 8.6$ Hz, acridone-H-4 + 5), 7.8 (pt, 2H, $^3J = 7.1$ Hz, acridone-H), 8.35 (pd, 2H, $^3J = 7.9$ Hz, acridone-H-1 + 8); FD-MS: *m/z* 283 [M^+], $C_{17}H_{15}NO_3$.

*4.1.2. 2-(9-Oxo-9,10-dihydro-10-acridinyl)acetic acid (**5**)*

Compound **4**, 1.2 g, 4.27 mmol was dissolved in 200 ml of boiling ethanol; 4 ml of 2 M NaOH were added and the mixture was refluxed for 25 min. After 20 min a yellow solid precipitated. The suspension was cooled to −20 °C for 0.5 h and the precipitate was filtered off and washed with $CHCl_3$. The solid residue was dissolved in 20 ml H_2O and the solution acidified with 1 M HCl to pH 1. The precipitate was filtered off and dried. Yellow solid (500 mg) (1.97 mmol) (46%), m.p. > 250 °C; ^1H NMR (DMSO-d_6): δ 5.33 (s, 2H, CH_2), 7.35 (pt, 2H, $^3J = 7.5$ Hz, acridone-H), 7.66 (pd, 2H, $^3J = 8.7$ Hz, acridone-H-4 + 5), 7.81 (pt, 2H, $^3J = 8.3$ Hz, acridone-H), 8.34 (pd, 2H, $^3J = 7.9$ Hz, acridone-H-1 + 8); EI-MS: *m/z* 254 [M^+], $C_{15}H_{11}NO_3$.

*4.1.3. Ethyl-3-(9-oxo-9,10-dihydro-10-acridinyl)propanoate (**6**)*

Ethyl-3-bromopropanoate, 2.8 g, 15.46 mmol was dissolved in 20 ml DMF; 1.0 g (5.1 mmol) acridone and 2.35 g (17.03 mmol) potassium carbonate were added and the mixture was stirred for 24 h at 90 °C. The solvent was evaporated and the residue was chromatographed on silica gel (petrol ether/ethyl acetate (1.5:1)). Yellow solid (251 mg) (0.84 mmol) (17%), m.p. 97 °C; ^1H NMR (DMSO-d_6): δ 1.13 (t, 3H, $^3J = 7.1$ Hz, CH_3), 2.86 (t, 2H, $^3J = 7.5$ Hz, CH_2), 4.03 (q, 2H, $^3J = 7.2$ Hz, CH_2), 4.74 (t, 2H, $^3J = 7.5$ Hz, CH_2), 7.32 (m, 2H, acridone-H), 7.82 (m, 4H, acridone-H), 8.34 (m, 2H, acridone-H-1 + 8); EI-MS: *m/z* 296 [M^+], $C_{18}H_{17}NO_3$.

*4.1.4. Ethyl-4-(9-oxo-9,10-dihydro-10-acridinyl)butanoate (**8**)*

NaH, 0.15 g, 6.06 mmol (97%) was dissolved in 10 ml DMF. A suspension of 1.0 g (5.12 mmol) acridone in 20 ml DMF was added and the mixture was stirred for 0.5 h at room temperature; 3.0 g (15.38 mmol) ethyl-4-bromobutanoate and 20 mg tetrabutylammonium iodide were added and it was stirred for further 2 h at 80 °C. The solvent was evaporated and the residue was chromatographed on basic Al_2O_3 (petrol ether/ethyl acetate (2:1)). Yellow solid (270 mg) (0.87 mmol) (17%), m.p. 93 °C; ^1H NMR (DMSO-d_6): δ 1.21 (t, 3H, $^3J = 7.1$ Hz, CH_3), 2.05 (quint., 2H, $^3J = 7.6$ Hz, CH_2), 2.65 (t, 2H, $^3J = 6.6$ Hz, CH_2), 4.1 (q, 2H, $^3J = 7.1$ Hz, CH_2), 4.47 (t, 2H, $^3J = 8.2$ Hz, CH_2), 7.34 (m, 2H, acridone-H), 7.82 (m, 2H, acridone-H-4 + 5), 7.93 (m, 2H, acridone-H), 8.34 (m, 2H, acridone-H-1 + 8); FD-MS: *m/z* 309 [M^+], $C_{19}H_{19}NO_3$.

4.2. General procedure for the preparation of acridone propionic and butanoic acid by hydrolysis of the corresponding esters

The pure ester was dissolved in ethanol. The 2 molar excess of 2 M NaOH was added and the mixture stirred at room

temperature overnight. The progress of the reaction was controlled by TLC on silica gel. If necessary further 2 M NaOH was added. When the hydrolysis was completed the mixture was acidified by 1 M HCl to pH 1 and the precipitate was filtered off. The residue was dried under vacuum. The crude acid was used for the following amide coupling reaction without further purification.

*4.2.1. 3-(9-Oxo-9,10-dihydro-10-acridinyl)-propionic acid (**7**)*

Yellow solid, quantitative reaction, m.p. > 250 °C, ^{1}H NMR (DMSO-d_6): δ 2.69 (t, 2H, $^3J = 6.2$ Hz, CH_2), 4.7 (t, 2H, $^3J = 7.6$ Hz, CH_2), 7.31 (m, 2H, acridone-H), 7.83 (m, 4H, acridone-H), 8.32 (m, 2H, acridone-H-1 + 8); FD-MS: *m/z* 267 [M^+], $C_{16}H_{13}NO_3$.

*4.2.2. 4-(9-Oxo-9,10-dihydro-10-acridinyl)-butanoic acid (**9**)*

Yellow solid, quantitative reaction, m.p. > 250 °C, ^{1}H NMR (DMSO-d_6): δ 2.0 (quint., 2H, $^3J = 8.2$ Hz, CH_2), 2.57 (t, 2H, $^3J = 6.8$ Hz, CH_2), 4.47 (t, 2H, $^3J = 8.3$ Hz, CH_2), 7.33 (m, 2H, acridone-H), 7.83 (m, 2H, acridone-H-4 + 5), 7.94 (m, 2H, acridone-H), 8.34 (m, 2H, acridone-H-1 + 8); EI-MS: *m/z* 281 [M^+], $C_{17}H_{15}NO_3$.

*4.2.3. Ethyl-4-(1,3-dioxo-2,3-dihydro-1H-benzo[d,e]-isochinoline-2-yl)butanoate (**10**)*

NaH, 0.3 g, 12.12 mmol (97%) and 1.0 g (5.07 mmol) naphthalimide was suspended in 5 ml DMF and stirred for 20 min at 0 °C; 2.93 g (15.03 mmol) ethyl-4-bromobutanoate were added and the mixture was stirred at room temperature for further 24 h. The progress of the reaction was followed by TLC (silica gel; hexane/diethyl ether (1:1)). Fifty millilitres H_2O were added and the mixture was extracted three times with diethyl ether. The etherical phase was evaporated and 40 ml ethanol was added to the residue. The educt (ethyl-4-bromobutanoate) was soluble in ethanol while the product remained insoluble and could be filtered off. Further purification was not necessary. White solid (1000 mg) (3.21 mmol) (32%), m.p. 90 °C; ^{1}H NMR (DMSO-d_6): δ 1.1 (t, 3H, $^3J = 7.2$ Hz, CH_3), 1.9 (quint., 2H, $^3J = 7.2$ Hz, CH_2), 2.36 (t, 2H, $^3J = 7.4$ Hz, CH_2), 3.91 (q, 2H, $^3J = 7.1$ Hz, CH_2), 4.08 (t, 2H, $^3J = 6.8$ Hz, CH_2), 7.83 (pt, 2H, naphthalimide-H-5 + 8), 8.42 (m, 4H, naphthalimide-H-4 + 6 + 7 + 9); ^{13}C NMR (DMSO-d_6): δ 14.31 (p), 23.18 (s), 31.51 (s), one signal below the DMSO-peak, 60.04 (s), 122.39 (2q), 127.46 (2t), 127.69 (q), 130.93 (2t), 131.55 (q), 134.53 (2t), 163.84 (2q), 172.83 (q); FD-MS: *m/z* 312 [M^+], $C_{18}H_{17}NO_4$.

*4.2.4. 4-(1,3-Dioxo-2,3-dihydro-1H-benzo[d,e]-isochinoline-2-yl)butanoic acid (**11**)*

Seven hundred and eighty milligrams (2.51 mmol) of **10** were suspended in 21.5 ml HCl (15%) and refluxed for 6 h. The progress of the reaction was followed by TLC (silica gel; petrol ether/ethyl acetate (1:1)). The precipitate was filtered off and dried under vacuum. White solid, quantitative reaction, m.p. 185 °C; ^{1}H NMR (DMSO-d_6): δ 1.88 (quint., 2H, $^3J = 6.9$ Hz, CH_2), 2.3 (t, 2H, $^3J = 7.4$ Hz, CH_2), 4.07 (q, 2H, $^3J = 6.9$ Hz, CH_2), 7.84 (pt, 2H, naphthalimide-H-5 + 8), 8.44 (m, 4H, naphthalimide-H-4 + 6 + 7 + 9); FD-MS: *m/z* 284 [M^+], $C_{16}H_{13}NO_4$.

4.2.5. 5-Nitro-2,3-dihydro-1H-benzo[d,e]-isochinoline-1,3-dion(5-nitro-naphthalimide)

3-Nitro-naphthalic anhydride, 4.48 g, 18.42 mmol was suspended in 75 ml DMF: 80 ml NH_3 (26%) were added and the mixture was refluxed for 12 h at 200 °C. The progress of the reaction was monitored by TLC (silica gel; petrol ether/ethyl acetate (3:1)). The solid residue was filtered off and dried under vacuum. Pink solid (3570 mg) (14.74 mmol) (80%), m.p. > 250 °C, EI-MS: *m/z* 243 [M^+], $C_{12}H_6N_2O_4$.

*4.2.6. Ethyl-4-(5-nitro-1,3-dioxo-2,3-dihydro-1H-benzo-[d,e]isochinoline-2-yl)butanoate (**12**)*

5-Nitro-naphthalimide, 1.26 g, 5 mmol and 0.32 g (12.93 mmol) NaH (97%) was suspended in 20 ml DMF and the mixture was stirred for 30 min at room temperature: 3.0 g (15.38 mmol) ethyl-4-bromobutanoate were added and it was stirred for further 24 h. The progress of the reaction was followed by TLC (silica gel; petrol ether/ethyl acetate (3:1)). After complete turnover the solvent was evaporated and the residue suspended in 40 ml ethanol. The insoluble part was filtered off and dried under vacuum. The crude product could be used without further purification. Red-brown solid (1600 mg) (4.49 mmol) (90%), m.p. 157 °C (decomposition); ^{1}H NMR (DMSO-d_6): δ 1.11 (t, 3H, $^3J = 7.1$ Hz, CH_3), 1.91 (q, 2H, $^3J = 7.0$ Hz, CH_2), 2.38 (t, 2H, $^3J = 7.2$ Hz, CH_2), 3.95 (q, 2H, $^3J = 7.0$ Hz, CH_2), 4.07 (t, 2H, $^3J = 6.6$ Hz, CH_2), 8.01 (pt, 1H, naphthalimide-H-8), 8.62 (pd, 1H, naphthalimide-H-9), 8.72 (pd, 1H, naphthalimide-H-7), 8.87 (ps, 1H, naphthalimide-H-4), 9.42 (ps, 1H, naphthalimide-H-6); EI-MS: *m/z* 357 [M^+], $C_{18}H_{16}N_2O_6$.

*4.2.7. 4-(5-Nitro-1,3-dioxo-2,3-dihydro-1H-benzo[d,e]-isochinoline-2-yl)butanoic acid (**13**)*

One gram (2.81 mmol) of **12**, 28 ml THF, 14 ml H_2O and 142 mg (3.37 mmol) LiOH-monohydrate were stirred for 16 h at room temperature. After complete hydrolysis (TLC; silica gel; petrol ether/ethyl acetate (3:1)) the solution was acidified to pH 1 with 1 M HCl and the precipitate was filtered off. The crude product could be used without further purification. Ochre solid, quantitative reaction, m.p. 200 °C (decomposition); ^{1}H NMR (DMSO-d_6): δ 1.88 (q, 2H, $^3J = 6.9$ Hz, CH_2), 2.31 (t, 2H, $^3J = 7.3$ Hz, CH_2), 4.07 (t, 2H, $^3J = 6.7$ Hz, CH_2), 8.0 (pt, 1H, naphthalimide-H-8), 8.61 (pd, 1H, naphthalimide-H-9), 8.71 (pd, 1H, naphthalimide-H-7), 8.86 (ps, 1H, naphthalimide-H-4), 9.4 (ps, 1H, naphthalimide-H-6); EI-MS: *m/z* 328 [M^+], $C_{16}H_{12}N_2O_6$.

*4.2.8. 4-(5H-Dibenzo[b,f]azepine-5-yl)butanoic acid (**14**)*

NaH, 0.15 g, 6.06 mmol (97%) was suspended in 10 ml DMF. A suspension of 1.0 g (5.17 mmol) iminostilbene in 20 ml DMF was added under stirring at room temperature. After 0.5 h 3.0 g (15.38 mmol) ethyl-4-bromobutanoate and

20 mg tetrabutylammonium iodide were added and the mixture was stirred for further 48 h at 120 °C. The solvent was evaporated and the residue was chromatographed on silica gel (petrol ether/ethyl acetate (8:1)). The developed ethyl-4-(5*H*-dibenzo[*b*,*f*]azepine-5-yl)butanoate was used as a crude product for hydrolysis. For this 300 mg (0.98 mmol) were dissolved in 50 ml ethanol, 5 ml 2 M NaOH were added and the solution was stirred for 12 h at room temperature. After quantitative reaction (control by TLC (silica gel) petrol ether/ethyl acetate (8:1)) the solution was acidified to pH 1 with 1 M HCl and the resulting precipitate was filtered off and dried under vacuum. Green solid (200 mg) (0.72 mmol) (14%), m.p. >250 °C; ^{1}H NMR (DMSO-d_6): δ 1.63 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.24 (t, 2H, $^3J = 7.1$ Hz, CH_2), 3.68 (t, 2H, $^3J = 6.7$ Hz, CH_2), 6.69–7.35 (m, 10 H, iminostilbene-H); ^{13}C NMR (DMSO-d_6): δ 22.71 (s), 30.87 (s), 49.02 (s), 120.74 (2t), 123.55 (2t), 129.26 (4t), 132.29 (2t), 133.68 (2q), 150.73 (2q), 174.64 (q); FD-MS: *m/z* 280 [M^+], $C_{18}H_{17}NO_2$.

4.3. General procedure for the synthesis of pyrrole carboxamides with acridone alkanoic acids as building block

The carboxylic acid (1.3 mmol) was dissolved in 40 ml DMF. The solution was cooled to −20 °C, 1.2 mmol of ethyl chloroformiate were added and the mixture was stirred at this temperature for 20 min. Then 1 mmol of the mono- or bis-pyrrole amine (prepared from the nitropyrrole by hydrogenation in DMF over Pd on charcoal (10%) [15]) and 1 mmol (129 mg) *N*,*N*-diisopropylethylamine were added. The mixture was stirred at room temperature in the dark. After 24 h the solution was evaporated to dryness and the residue was chromatographed on silica gel (methanol–NH_3 (25%, 97:3)).

4.3.1. N-2-[3-(Dimethylamino)propyl]-1-methyl-4-{[2-(9-oxo-9,10-dihydro-10-acridinyl)acetyl] amino}-1H-2-pyrrole carboxamide (**17**)

Educt: 331 mg **5**, 254 mg **15**. Yellow solid (250 mg) (0.54 mmol) (54%), m.p. 206–208 °C; IR (KBr, cm^{-1}): 3350, 3200, 1570, 1470, 1440, 1380, 1350, 1270, 1220, 1160, 1090, 740, 660, 605; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, $^3J = 6.7$ Hz, CH_2), 2.4 (s, 6H, $2CH_3$), 2.6 (t, 2H, $^3J = 7.1$ Hz, CH_2), 3.17 (q, 2H, $^3J = 6.3$ Hz, CH_2), 3.76 (s, 3H, pyrrole-CH_3), 5.33 (s, 2H, CH_2), 6.76 (s, 1H, pyrrole-H-3), 7.09 (s, 1H, pyrrole-H-5), 7.35 (pt, 2H, $^3J = 7.5$ Hz, acridone-H), 7.65 (pd, 2H, $^3J = 8.8$ Hz, acridone-H-4 + 5), 7.79 (m, 2H, acridone-H), 8.11 (t, 1H, $^3J = 5.1$ Hz, NH), 8.35 (m, 2H, acridone-H-1 + 8), 10.43 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.29 (s), 36.32 (p), 36.58 (s), 44.13 (2p), 49.14 (s), 56.26 (s), 104.08 (t), 116.21 (2t), 118.12 (t), 121.56 (q), 121.79 (2t), 121.95 (2q), 123.36 (q), 126.88 (2t), 134.38 (2t), 142.91 (2q), 161.52 (q), 164.48 (q), 177.09 (q); FD-MS: *m/z* 460 [M^+], $C_{26}H_{29}N_5O_3$.

4.3.2. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-1-methyl-4-{[2-(9-oxo-9,10-dihydro-10-acridinyl)acetyl]amino}-1H-2-pyrrole carboxamide (**18**)

Educt: 331 mg **5**, 376 mg **16**. Yellow solid (297 mg) (0.51 mmol) (51%), m.p. 212 °C; IR (KBr, cm^{-1}): 3350, 3190, 1600, 1540, 1470, 1440, 1400, 1380, 1350, 1270, 1220, 1120, 1070, 910, 790, 740, 650, 600; ^{1}H NMR (DMSO-d_6): δ 1.67 (quint., 2H, $^3J = 6.9$ Hz, CH_2), 2.35 (s, 6H, $2CH_3$), 2.5 (t, 2H, $^3J = 7.1$ Hz, CH_2), 3.17 (q, 2H, $^3J = 6.4$ Hz, CH_2), 3.77 (s, 3H, pyrrole-CH_3), 3.8 (s, 3H, pyrrole-CH_3), 5.33 (s, 2H, CH_2), 6.82 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-3), 6.97 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H-5), 7.15 (s, 2H, 2 pyrrole-H), 7.35 (pt, 2H, $^3J = 7.4$ Hz, acridone-H), 7.65 (pd, 2H, $^3J = 8.8$ Hz, acridone-H-4 + 5), 7.79 (m, 2H, acridone-H), 8.08 (t, 1H, $^3J = 5.5$ Hz, NH), 8.35 (m, 2H, acridone-H-1 + 8), 9.86 (s, 1H, NH), 10.48 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.58 (s), 36.26 (p), 36.43 (p), 36.78 (s), 44.43 (2p), 49.2 (s), 56.56 (s), 104.43 (t), 104.56 (t), 116.21 (2t), 118.16 (t), 118.64 (t), 121.6 (q), 121.78 (2t), 121.99 (2q), 122.36 (q), 123.19 (q), 123.3 (q), 126.89 (2t), 134.38 (2t), 142.93 (2q), 158.58 (q), 161.65 (q), 164.54 (q), 177.11 (q); FD-MS: *m/z* 582 [M^+]; $C_{32}H_{35}N_7O_4$.

4.3.3. N-2-[3-(Dimethylamino)propyl]-1-methyl-4-{[3-(9-oxo-9,10-dihydro-10-acridinyl)propanoyl]amino}-1H-2-pyrrole carboxamide (**19**)

Three hundred and forty eight milligrams of **7**, 254 mg **15**. Yellow solid (280 mg) (0.59 mmol) (59%), m.p. > 250 °C; IR (KBr, cm^{-1}): 3400, 1610, 1590, 1570, 1480, 1440, 1390, 1360, 1280, 1170, 1100, 750, 670; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, $^3J = 7.2$ Hz, CH_2), 2.38 (s, 6H, $2CH_3$), 2.57 (t, 2H, $^3J = 6.4$ Hz, CH_2), 2.85 (t, 2H, $^3J = 7.2$ Hz, CH_2), 3.18 (q, 2H, $^3J = 6.3$ Hz, CH_2), 3.78 (s, 3H, pyrrole-CH_3), 4.78 (t, 2H, $^3J = 7.1$ Hz, CH_2), 6.67 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H-3), 7.15 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H-5), 7.35 (pt, 2H, $^3J = 7.3$ Hz, acridone-H), 7.86 (m, 4H, acridone-H), 8.1 (t, 1H, NH), 8.35 (m, 2H, acridone-H-1 + 8), 10.02 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.46 (s), 33.96 (s), 36.36 (p), 36.7 (s), 42.53 (s), 44.3 (2p), 56.43 (s), 103.72 (t), 116.03 (2t), 118.03 (t), 121.74 (2t), 121.98 (2q), 123.25 (2q), 127.12 (2t), 134.61 (2t), 141.76 (2q), 161.53 (q), 167.17 (q), 176.84 (q); FD-MS: *m/z* 474 [M^+]; $C_{27}H_{31}N_5O_3$.

4.3.4. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-1-methyl-4-{[3-(9-oxo-9,10-dihydro-10-acridinyl)propanoyl]amino}-1H-2-pyrrole-carboxamide (**20**)

Three hundred and forty eighty milligrams of **7**, 376 mg **16**. Yellow solid (290 mg) (0.49 mmol) (49%), m.p. 190–200 °C; IR (KBr, cm^{-1}): 3350, 1620, 1570, 1480, 1440, 1420, 1390, 1360, 1270, 1250, 1160, 1100, 750, 660, 610; ^{1}H NMR (DMSO-d_6): δ 1.69 (quint., 2H, $^3J = 7.1$ Hz, CH_2), 2.4 (s, 6H, $2CH_3$), 2.58 (t, 2H, $^3J = 6.9$ Hz, CH_2), 2.87 (t, 2H, $^3J = 7.0$ Hz, CH_2), 3.2 (q, 2H, $^3J = 6.3$ Hz, CH_2), 3.79 (s, 3H, pyrrole-CH_3), 3.83 (s, 3H, pyrrole-CH_3), 4.79 (t, 2H, $^3J = 6.9$ Hz, CH_2), 6.85 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H-3),

6.87 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-5), 7.17 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H-3′), 7.21 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H-5′), 7.35 (pt, 2H, $^3J = 7.0$ Hz, acridone-H), 7.86 (m, 4H, acridone-H), 8.1 (t, 1H, 5.7 Hz, NH), 8.35 (m, 2H, acridone-H-1 + 8), 9.87 (s, 1H, NH), 10.07 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.51 (s), 33.99 (s), 36.29 (p), 36.48 (p), 36.73 (s), 42.54 (s), 44.36 (2p), 56.49 (s), 104.23 (t), 104.5 (t), 116.04 (2t), 118.22 (t), 118.58 (t), 121.74 (2t), 121.98 (2q), 122.35 (2q), 123.15 (2q), 127.13 (2t), 134.62 (2t), 141.78 (2q), 158.63 (q), 161.67 (q), 167.24 (q), 176.84 (q); FD-MS: *m/z* 596 [M$^+$], $C_{33}H_{37}N_7O_4$.

4.3.5. N-2-[3-(Dimethylamino)propyl]-1-methyl-4-{[4-(9-oxo-9,10-dihydro-10-acridinyl)butanoyl] amino}-1H-2-pyrrole carboxamide (**21**)

Three hundred and sixty five milligrams of **9**, 254 mg **15**. Yellow solid (300 mg) (0.62 mmol) (62%), m.p. 140–145 °C; IR (KBr, cm^{-1}): 3200, 3000, 2880, 1600, 1570, 1500, 1460, 1440, 1420, 1370, 1350, 1260, 1240, 1160, 1120, 1080, 1020, 910, 730, 660, 590; ^{1}H NMR (DMSO-d_6): δ 1.67 (quint., 2H, $^3J = 6.9$ Hz, CH_2), 2.08 (quint., 2H, CH_2), 2.32 (s, 3H, $2CH_3$), 2.5 (t, 2H, CH_2), 2.58 (t, 2H, $^3J = 6.2$ Hz, CH_2), 3.19 (q, 2H, $^3J = 6.0$ Hz, CH_2), 3.79 (s, 3H, pyrrole-CH_3), 4.51 (t, 2H, $^3J = 8.0$ Hz, CH_2), 6.71 (s, 1H, pyrrole-H-3), 7.16 (s, 1H, pyrrole-H-5), 7.34 (pt, 2H, $^3J = 7.4$ Hz, acridone-H), 7.84 (pt, 2H, $^3J = 7.3$ Hz, acridone-H), 7.97 (pd, 2H, $^3J = 8.6$ Hz, acridone-H-4 + 5), 8.14 (t, 1H, NH), 8.35 (pd, 2H, $^3J = 7.9$ Hz, acridone-H-1 + 8), 9.92 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 22.58 (s), 26.67 (s), 32.01 (s), 36.3 (p), 37.02 (s), 44.58 (2p), 45.39 (s), 56.68 (s), 104.12 (t), 116.21 (2t), 117.95 (t), 121.61 (2t), 121.89 (2q), 122.17 (q), 123.29 (q), 127.06 (2t), 134.57 (2t), 141.86 (2q), 161.55 (q), 169.28 (q), 176.81 (q); FD-MS: *m/z* 488 [M$^+$], $C_{28}H_{33}N_5O_3$.

4.3.6. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-1-methyl-4-{[4-(9-oxo-9,10-dihydro-10-acridinyl)butanoyl]amino}-1H-2-pyrrole-carboxamide (**22**)

Three hundred and sixty five milligrams of **9**, 376 mg **16**. Yellow solid (290 mg) (0.48 mmol) (48%), m.p. > 250 °C; IR (KBr, cm^{-1}): 3360, 3220, 2900, 1610, 1570, 1480, 1440, 1420, 1380, 1360, 1340, 1280, 1250, 1190, 1160, 1080, 920, 800, 750, 660; ^{1}H NMR (DMSO-d_6): δ 1.67 (quint., 2H, CH_2), 2.09 (quint., 2H, CH_2), 2.37 (s, 6H, $2CH_3$), 2.59 (2 t, 4H, $2CH_2$), 3.2 (q, 2H, 6.3 Hz, CH_2), 3.8 (s, 3H, pyrrole-CH_3), 3.85 (s, 3H, pyrrole-CH_3), 4.53 (t, 2H, $^3J = 7.7$ Hz, CH_2), 6.86 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-3), 6.92 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-5), 7.18 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H-3′), 7.21 (d, 1H, pyrrole-H-5′), 7.34 (pt, 2H, $^3J = 7.4$ Hz, acridone-H), 7.84 (pt, 2H, $^3J = 7.0$ Hz, acridone-H), 7.99 (pd, 2H, $^3J = 8.8$ Hz, acridone-H-4 + 5), 8.1 (t, 1H, $^3J = 5.5$ Hz, NH), 8.36 (pd, 2H, $^3J = 8.2$ Hz, acridone-H-1 + 8), 9.88 (s, 1H, NH), 9.96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 22.59 (s), 26.58 (s), 32.03 (s), 36.28 (p), 36.44 (p), 36.78 (s), 44.41 (2p), 45.4 (s), 56.53 (s), 104.4 (t), 104.5 (t), 116.23 (2t), 118.21 (t), 118.54 (t), 121.62 (2t), 121.91 (2q), 122.22 (q), 122.4 (q), 123.13 (2q), 127.07 (2t), 134.59 (2t), 141.87 (2q), 158.69 (q), 161.65 (q), 169.36 (q), 176.81 (q); FD-MS: *m/z* 610 [M$^+$], $C_{34}H_{39}N_7O_4$.

4.4. General procedure for the preparation of pyrrole carboxamides with naphthalimide butanoic acid as building block

Synthesis: see Section 4.3.

4.4.1. N-2-[3-(Dimethylamino)propyl]4-{[4-(1,3-dioxo-2,3-dihydro-1H-benzo[d,e]isochinoline-2-yl)butanoyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**23**)

Educt: 370 mg **11**, 254 mg **15**. Yellow solid (170 mg) (0.35 mmol) (35%), m.p. 125 °C; IR (KBr, cm^{-1}): 3330, 2950, 1650, 1630, 1590, 1520, 1460, 1440, 1340, 1240, 1200, 1170, 1160, 1030, 970, 930, 850, 780, 670; ^{1}H NMR (DMSO-d_6): δ 1.63 (quint., 2H, $^3J = 6.8$ Hz, CH_2), 1.94 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.21 (s, 6H, $2CH_3$), 2.31 (2 t, 4H, $2CH_2$), 3.17 (q, 2H, $^3J = 6.0$ Hz, CH_2), 3.7 (s, 3H, pyrrole-CH_3), 4.08 (t, 2H, $^3J = 6.9$ Hz, CH_2), 6.56 (d, 1H, pyrrole-H-3), 6.94 (d, 1H, pyrrole-H-5), 7.82 (pt, 2H, naphthalimide-H-5 + 8), 8.04 (t, 1H, NH), 8.43 (m, 4H, naphthalimide-H-4 + 6 + 7 + 9), 9.73 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 24.15 (s), 27.02 (s), 33.66 (s), 36.18 (p), 37.11 (s), one signal below the DMSO-peak, 45.0 (2p), 57.01 (s), 103.48 (t), 117.65 (t), 122.2 (q), 122.4 (2q), 123.12 (q), 127.45 (2t), 127.72 (q), 131.0 (2t), 131.56 (q), 134.52 (2t), 161.44 (q), 163.81 (2q), 169.02 (q); FD-MS: *m/z* 490 [M$^+$], $C_{27}H_{31}N_5O_4$.

4.4.2. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-4-{[4-(1,3-dioxo-2,3-dihydro-1H-benzo[d,e]isochinoline-2-yl)butanoyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**24**)

Educt: 370 mg **11**, 376 mg **16**. Yellow solid (190 mg) (0.31 mmol) (31%), m.p. 165 °C; IR (KBr, cm^{-1}): 3300, 2940, 1650, 1590, 1530, 1430, 1400, 1385, 1235, 1140, 1100, 1045, 780; ^{1}H NMR (DMSO-d_6): δ 1.62 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 1.95 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.21 (s, 6H, $2CH_3$), 2.31 (2t, 4H, $2CH_2$), 3.17 (q, 2H, $^3J = 6.0$ Hz, CH_2), 3.75 (s, 3H, pyrrole-CH_3), 3.78 (s, 3H, pyrrole-CH_3), 4.11 (t, 2H, $^3J = 6.8$ Hz, CH_2), 6.78 (s, 1H, pyrrole-H-3), 6.81 (s, 1H, pyrrole-H-5), 6.99 (s, 1H, pyrrole-H-3′), 7.16 (s, 1H, pyrrole-H-5′), 7.84 (pt, 2H, $^3J = 7.6$ Hz, naphthalimide-H-5 + 8), 8.06 (t, 1H, NH), 8.45 (m, 4H, naphthalimide-H-4 + 6 + 7 + 9), 9.79 (s, 1H, NH), 9.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 24.17 (s), 27.14 (s), 33.68 (s), 36.24 (p), 36.32 (p), 37.14 (s), one signal below the DMSO-peak, 45.09 (2p), 57.05 (s), 104.13 (t), 104.35 (t), 118.09 (t), 118.3 (t), 122.27 (q), 122.37 (2q), 122.44 (q), 122.91 (q), 123.22 (q), 127.48 (2t), 127.75 (q), 131.02 (2t), 131.6 (q), 134.53 (2t), 158.64 (q), 161.54 (q), 163.84 (2q), 169.09 (q); FD-MS: *m/z* 613 [M$^+$], $C_{33}H_{37}N_7O_5$.

4.5. General procedure for the preparation of pyrrole carboxamides with 5-nitro-naphtalimide butanoic acid as building block

Synthesis: see Section 4.3.

4.5.1. N-2-[3-(Dimethylamino)propyl]-4-{[4-(5-nitro-1,3-dioxo-2,3-dihydro-1H-benzo[d,e]isochinoline-2-yl)-butanoyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**25**)

Four hundred and twenty seven milligrams of **13**, 254 mg **15**. Red-brown solid (185 mg) (0.35 mmol) (35%), m.p. 158 °C (decomposition); IR (FT, cm^{-1}): 3420, 2940, 1670, 1590, 1540, 1490, 1330, 1250, 1210, 1090, 1010, 970, 850, 810, 750; ^{1}H NMR (DMSO-d_6): δ 1.62 (quint., 2H, $^3J = 6.8$ Hz, CH_2), 1.98 (quint., 2H, $^3J = 6.3$ Hz, CH_2), 2.26 (s, 6H, $2CH_3$), 2.29 (t, 2H, CH_2), 2.4 (t, 2H, $^3J = 6.9$ Hz, CH_2), 3.14 (q, 2H, CH_2), 3.6 (s, 3H, pyrrole-CH_3), 4.1 (t, 2H, CH_2), 6.41 (d, 1H, pyrrole-H-3), 6.67 (d, 1H, pyrrole-H-5), 7.96 (m, 2H, naphthalimide-H-8 + NH), 8.58 (pd, 1H, naphthalimide-H-9), 8.67 (pd, 1H, naphthalimide-H-7), 8.84 (ps, 1H, naphthalimide-H-4), 9.35 (ps 1H, naphthalimide-H-6), 9.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 23.51 (s), 26.8 (s), 33.54 (s), 36.09 (p), 37.03 (s), one signal below the DMSO-peak, 44.81 (2p), 56.87 (s), 103.18 (t), 117.31 (t), 122.18 (q), 122.86 (2q), 122.99 (t), 124.21 (q), 129.31 (t), 129.62 (t), 129.79 (q), 131.01 (q), 134.08 (t), 136.3 (t), 145.85 (q), 161.31 (q), 162.67 (q), 163.11 (q), 168.9 (q); FD-MS: *m/z* 536 [M^+], $C_{27}H_{30}N_6O_6$.

4.5.2. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-4-{[4-(5-nitro-1,3-dioxo-2,3-dihydro-1H-benzo[d,e]isochinoline-2-yl)butanoyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**26**)

Four hundred and twenty seven milligrams of **13**, 376 mg **16**. Red-brown solid (80 mg) (0.21 mmol) (21%), m.p. 161–185 °C; IR (FT, cm^{-1}): 3350, 3100, 2960, 1700, 1660, 1600, 1540, 1460, 1430, 1400, 1340, 1330, 1245, 1210, 1140, 1110, 1060, 785, 760, 670; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, $^3J = 6.8$ Hz, CH_2), 2.0 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.34 (s + t, 8H, $2CH_3 + CH_2$), 3.19 (q + t, 4H, $2CH_2$), 3.68 (s, 3H, pyrrole-CH_3), 3.78 (s, 3H, pyrrole-CH_3), 4.18 (t, 2H, CH_2), 6.67 (s, 1H, pyrrole-H-3), 6.8 (s, 1H, pyrrole-H-5), 6.82 (s, 1H, pyrrole-H-3′), 7.15 (s, 1H, pyrrole-H-5′), 7.99 (pt, 1H, naphthalimide-H-8), 8.09 (t, 1H, NH), 8.62 (pd, 1H, naphthalimide-H-9), 8.69 (pd, 1H, naphthalimide-H-7), 8.89 (ps, 1H, naphthalimide-H-4), 9.39 (ps, 1H, naphthalimide-H-6), 9.74 (s, 1H, NH), 9.76 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 23.65 (s), 26.64 (s), 33.59 (s), 36.25 (2p), 36.88 (s), one signal below the DMSO-peak, 44.51 (2p), 56.61 (s), 103.94 (t), 104.48 (t), 118.03 (t), 118.18 (t), 122.25 (2q), 122.4 (2q), 122.97 (q), 123.08 (t), 124.36 (q), 129.36 (t), 129.72 (t), 129.92 (q), 131.09 (q), 134.14 (t), 136.37 (t), 145.99 (q), 158.57 (q), 161.63 (q), 162.75 (q), 163.2 (q), 169.04 (q); FD-MS: *m/z* 658 [M^+], $C_{33}H_{36}N_8O_7$.

4.5.3. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-4-{[4(5H-dibenzo[b,f]azepine-5-yl)butanoyl]amino}-1-methyl-1H-2-pyrrole carboxamide (**27**)

Synthesis: see Section 4.3. Educt: 182 mg (0.65 mmol) **14**, 188 mg (0.5 mmol) **16**, addition of 0.05 ml (0.6 mmol) ethyl chloroformiate. Green solid (60 mg) (0.1 mmol) (20%), m.p. > 250 °C; IR (KBr, cm^{-1}): 3270, 2940, 1640, 1580, 1520, 1460, 1430, 1400, 1250, 1200, 1140, 1040, 760; ^{1}H NMR (DMSO-d_6): δ 1.67 (2quint., 4H, $2CH_2$), 2.29 (s, 6H, $2CH_3$), 2.44 (t, 2H, CH_2), 3.18 (q/t, 4H, $2CH_2$), 3.72 (t, 2H, $^3J = 7.0$ Hz, CH_2), 3.77 (s, 6H, 2 pyrrole-CH_3), 6.75 (s, 2H, pyrrole-H-3 + 5), 6.81 (s, 2H, pyrrole-H-3′ + 5′), 6.93–7.35 (m, 10H, iminostilbene-H), 8.1 (t, 1H, NH), 9.73 (s, 1H, NH), 9.82 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 23.53 (s), 26.84 (s), 32.87 (s), 36.26 (p), 36.34 (p), 36.94 (s), 44.75 (2p), 49.5 (s), 56.8 (s), 104.23 (t), 104.4 (t), 118.12 (2t), 118.34 (2t), 120.72 (q), 122.28 (q), 122.97 (q), 123.18 (q), 123.54 (2t), 129.25 (4t), 132.33 (2t), 133.67 (2q), 150.82 (2q), 158.66 (q), 161.6 (q), 169.53 (q); FD-MS: *m/z* 608 [M^+], $C_{35}H_{41}N_7O_3$.

4.5.4. 3-(9-Acridinyl)propionic acid (**28**)

Diphenylamine, 1.7 g, 10 mmol , 3.55 g (30 mmol) succinic acid and 2 g $ZnCl_2$ were mixed and heated to 230 °C for 24 h; 50 ml H_2SO_4 (20%) were added and the mixture was refluxed for 4 h. After that the flask was cooled to room temperature and the black residue was filtered off. The filtrate was neutralised with NH_3 (25%) and then evaporated under vacuum. The product was isolated from the residue by column chromatography (silica gel; methanol/ethyl acetate (1:4)). Green solid (630 mg) (2.5 mmol) (25%), m.p. > 250 °C (lit. [21]: 300 °C); ^{1}H NMR (DMSO-d_6): δ 2.65 (t, 2H, $^3J = 8.0$ Hz, CH_2), 3.9 (t, 2H, $^3J = 7.85$ Hz, CH_2), 7.64 (pt, 2H, acridine-H-2 + 7), 7.84 (pt, 2H, acridine-H-3 + 6), 8.14 (pd, $^3J = 8.6$ Hz, 2H, acridine-H-4 + 5), 8.38 (pd, 2H, $^3J = 8.6$ Hz, acridine-H-1 + 8), 12.13 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6): δ 22.61 (s), 35.29 (s), 124.57 (t), 124.92 (t), 126.37 (2t), 130.16 (2t), 130.4 (2t), 142.3 (q), 145.45 (2q), 148.4 (2q), 173.74 (q); EI-MS: *m/z* 251 [M^+], $C_{16}H_{13}NO_2$.

4.5.5. 9,10-Dioxo-9,10-dihydro-2-anthracenesulfonyl chloride (**37**)

One gram (3.95 mmol) of sodium anthraquinone-2-sulfonate was mixed with 2.0 g (9.6 mmol) PCl_5. The mixture was heated for 30 min to 120 °C. After cooling down 20 ml toluene were added, the suspension was refluxed for 20 min, cooled to room temperature and the residue was filtered off. From the filtrate the product was gained by evaporation. Yellow solid (1100 mg) (3.59 mmol) (91%), m.p. 197 °C [lit. [14]: 197 °C]; IR (KBr, cm^{-1}): 3400, 3050, 2950, 1710, 1560, 1360, 1310, 1270, 1170, 1140, 1050, 950, 910, 840, 800, 780, 690, 650; ^{1}H NMR (DMSO-d_6): δ 7.89 (m, 2H, anthraquinone-H), 8.09 (pd, 1H, anthraquinone-H), 8.19 (m, 3H, anthraquinone-H), 8.38 (ps, 1H, anthraquinone-H); EI-MS: *m/z* 306 [M^+], $C_{14}H_7SO_4Cl$.

4.6. General procedure for the synthesis of BOC-protected GABA- and β-alanine functionalized mono- and bis-pyrrole carboxamides

Synthesis: see Section 4.3.

*4.6.1. tert-Butyl-N-(4-{[5-({[3-(dimethylamino)propyl]amino}-carbonyl)-1-methyl-1H-3-pyrrolyl] amino}-4-oxobutyl)-carbamate (**30**)*

Educts: 265 mg BOC-GABA, 254 mg **15**. Ochre solid (290 mg) (0.71 mmol) (71%), m.p. 125–130 °C; ^{1}H NMR (DMSO-d_6): δ 1.35 (s, 9H, $3CH_3$), 1.62 (2quint., 4H, $2CH_2$), 2.2 (t, 2H, CH_2), 2.27 (s, 6H, $2CH_3$), 2.37 (t, 2H, $^3J = 6.9$ Hz, CH_2), 2.91 (q, 2H, $^3J = 6.3$ Hz, CH_2), 3.16 (q, 2H, $^3J = 6.1$ Hz, CH_2), 3.76 (s, 3H, pyrrole-CH_3), 6.63 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-3), 6.84 (t, 1H, NH), 7.07 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H-5), 8.07 (t, 1H, $^3J = 5.3$ Hz, NH), 9.73 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.16 (s), 27.0 (s), 28.57 (3p), 33.39 (s), 36.22 (p), 37.07 (2s), 44.96 (2p), 56.97 (s), 77.76 (q), 103.6 (t), 117.74 (t), 122.26 (q), 123.22 (q), 155.9 (q), 161.47 (q), 169.37 (q); EI-MS: *m/z* 410 [M^+], $C_{20}H_{35}N_5O_4$.

*4.6.2. tert-Butyl-N-(4-{[5-({[3-(dimethylamino)propyl]amino}-carbonyl)-1-methyl-1H-3-pyrrolyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]amino}-4-oxobutyl)carbamate (**32**)*

Educts: 265 mg BOC-GABA, 376 mg **16**. Ochre solid (320 mg) (0.6 mmol) (60%), m.p. 160–170 °C; ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H, $3CH_3$), 1.63 (2quint., 4H, $2CH_2$), 2.21 (t, 2H, CH_2), 2.23 (s, 6H, $2CH_3$), 2.35 (t, 2H, $^3J = 7.0$ Hz, CH_2), 2.93 (q, 2H, $^3J = 6.4$ Hz, CH_2), 3.17 (q, 2H, $^3J = 6.4$ Hz, CH_2), 3.78 (s, 3H, pyrrole-CH_3), 3.8 (s, 3H, pyrrole-CH_3), 6.8 (s, 1H, pyrrole-H-3), 6.84 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-5), 7.13 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-3′), 7.16 (d, 1H, $^4J = 1.8$ Hz, pyrrole-H-5′), 8.07 (t, 1H, $^3J = 5.4$ Hz, NH), 9.78 (s, 1H, NH), 9.84 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.19 (s), 27.12 (s), 28.57 (3p), 31.0 (s), 33.41 (s), 36.23 (p), 36.36 (p), 37.13 (s), 45.06 (2p), 57.06 (s), 77.77 (q), 104.21 (t), 104.35 (t), 118.09 (t), 118.37 (t), 122.37 (2q), 123.03 (q), 123.25 (q), 155.9 (q), 158.68 (q), 161.55 (q), 169.46 (q); FD-MS: *m/z* 532 [M^+], $C_{26}H_{41}N_7O_5$.

*4.6.3. tert-Butyl-N-(3-{[5-({[3-(dimethylamino)propyl]amino}-carbonyl)-1-methyl-1H-3-pyrrolyl] amino}-3-oxopropyl)carbamate (**29**)*

Educts: 250 mg BOC-β-alanine, 254 mg **15**. Ochre solid (300 mg) (0.76 mmol) (76%), m.p. 140–145 °C; ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H, $3CH_3$), 1.59 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.16 (s, 6H, $2CH_3$), 2.27 (t, 2H, $^3J = 7.05$ Hz, CH_2), 2.36 (t, 2H, $^3J = 7.4$ Hz, CH_2), 3.16 (2q, 4H, $2CH_2$), 3.75 (s, 3H, pyrrole-CH_3), 6.61 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H-3), 6.81 (t, 1H, $^3J = 5.0$ Hz, NH), 7.08 (d, 1H, pyrrole-H-5), 8.06 (t, 1H, NH), 9.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 27.25 (s), 28.54 (3p), 36.22 (p), 36.93 (s), 37.15 (s), 37.29 (s), 45.29 (2p), 57.24 (s), 77.93 (q), 103.5 (t), 117.76 (t), 122.14 (q), 123.28 (q), 155.73 (q), 161.37 (q), 167.77 (q); FD-MS: *m/z* 397 [M^+], $C_{19}H_{33}N_5O_4$.

*4.6.4. tert-Butyl-N-(3-{[5-({[3-(dimethylamino)propyl]amino}-carbonyl)-1-methyl-1H-3-pyrrolyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]amino}-3-oxopropyl)-carbamate (**31**)*

Educts: 250 mg BOC-β-alanine, 376 mg **16**. Brown solid (350 mg) (0.68 mmol) (68%), m.p. 185–190 °C; ^{1}H NMR (DMSO-d_6): δ 1.36 (s, 9H, $3CH_3$), 1.61 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.19 (s, 6H, $2CH_3$), 2.3 (t, 2H, $^3J = 7.1$ Hz, CH_2), 2.38 (t, 2H, $^3J = 7.3$ Hz, CH_2), 3.17 (2q, 4H, $2CH_2$), 3.78 (s, 3H, pyrrole-CH_3), 3.8 (s, 3H, pyrrole-CH_3), 6.81 (s, 1H, pyrrole-H-3), 6.84 (s, 1H, pyrrole-H-5), 7.14 (s, 1H, pyrrole-H-3′), 7.16 (s, 1H, pyrrole-H-5′), 8.05 (2 t, 2H, 2NH), 9.85 (2 s, 2H, 2NH); ^{13}C NMR (DMSO-d_6): δ 27.22 (s), 28.55 (3p), 36.24 (2p), 36.39 (s), 37.08 (s), 37.2 (s), 45.2 (2p), 57.15 (s), 77.95 (q), 104.2 (t), 104.34 (t), 118.08 (t), 118.43 (t), 122.21 (q), 122.34 (q), 123.03 (q), 123.27 (q), 155.84 (q), 158.66 (q), 161.5 (q), 167.85 (q); FD-MS: *m/z* 518 [M^+], $C_{25}H_{39}N_7O_5$.

*4.7. General procedure for the deprotection of the amino-functions at compounds **29–32***

One millimole of the BOC-protected compound was dissolved in 60 ml ethanol. The 4.5 molar surplus of 2.3 M ethanolic HCl was added and the mixture was stirred at 60 °C. The progress of the reaction was followed by TLC (silica gel; methanol–NH_3 (97:3)) and if necessary further ethanolic HCl was added. After complete deprotection the mixture was cooled down to room temperature and 100 ml ethyl acetate were added. The white precipitate was filtered off and dried under vacuum.

Preparation of the 2.3 M ethanolic HCl: 170 ml ethanol were cooled in an ice bath; 41 ml (0.58 mmol) acetyl chloride were added dropwise under cooling and it was stirred at room temperature for 1.5 h. Finally ethanol was added to the volume of 250 ml.

*4.7.1. N-2-[3-(Dimethylamino)propyl]-4[(4-amino-butanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**34**)*

Educt: **30**. Brown solid, quantitative reaction, m.p. because of hygroscopicity not measurable; ^{1}H NMR (DMSO-d_6): δ 1.85 (2quint., 4H, $2CH_2$), 2.2 (t, 2H, CH_2), 2.35 (t, 2H, CH_2), 2.75 (s, 6H, $2CH_3$), 3.01 (quint., 2H, CH_2), 3.21 (q, 2H, CH_2), 3.78 (s, 3H, pyrrole-CH_3), 6.73 (d, 1H, $^4J = 1.6$ Hz, pyrrole-H-3), 7.11 (d, 1H, $^4J = 1.5$ Hz, pyrrole-H-5), 8.14 (2 t, 3H, NH + NH_2), 10.05 (s, 1H, NH); FD-MS: *m/z* 310 [M^+], $C_{15}H_{27}N_5O_2$.

*4.7.2. N-2-[5-({[3-(Dimethylamino)propyl]amino}-carbonyl)-1-methyl-1H-3-pyrrolyl]-4-[(4-amino-butanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**36**)*

Educt: **32**. Brown solid, quantitative reaction. Because of hygroscopicity no analytical data were obtained. Proof of the structure by analysis of the endproduct. $C_{21}H_{33}N_7O_3$.

*4.7.3. N-2-[3-(Dimethylamino)propyl]-4-[(3-aminopropanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**33**)*

Educt: **29**. Brown solid, quantitative reaction. Because of hygroscopicity no analytical data were obtained. Proof of the structure by analysis of the endproduct $C_{14}H_{25}N_5O_2$.

*4.7.4. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-4-[(3-aminopropanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**35**)*

Educt: **31**. Brown solid, quantitative reaction. Because of hygroscopicity no analytical data were obtained. Proof of the structure by analysis of the endproduct. $C_{20}H_{31}N_7O_3$.

*4.8. General procedure for the coupling of **37** with the amines **33–36***

Six hundred and fifteen milligrams (2 mmol) of **37** were dissolved in 30 ml DMF and a solution of 1.5 mmol amine in 30 ml DMF and 1 mmol (129 mg) *N*-ethyl-*N*,*N*-diisopropylamine were added. The mixture was stirred for 48 h at 100 °C. From time to time an additional small amount of **37** was added. Control of the progress of the reaction by TLC and purification of the product by column chromatography were done on silica gel (methanol–NH_3 (97:3)).

*4.8.1. N-2-[3-(Dimethylamino)propyl]-4-[(4-{[(9,10-dioxo-9,10-dihydro-2-anthracenyl)sulfonyl] amino}butanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**41**)*

Educt: 470 mg **34**. Brown solid (150 mg) (0.26 mmol) (17%), m.p. 210 °C (decomposition); IR (KBr, cm^{-1}): 3400, 1650, 1600, 1570, 1510, 1450, 1420, 1390, 1320, 1270, 1165, 1140, 1100, 950, 920, 700, 610; ^{1}H NMR (DMSO-d_6): δ 1.66 (2quint., 4H, $2CH_2$), 2.21 (t, 2H, $^3J = 6.8$ Hz, CH_2), 2.37 (s, 6H, $2CH_3$), 2.57 (q, 2H, $^3J = 6.5$ Hz, CH_2), 2.84 (t, 2H, CH_2), 3.17 (q, 2H, CH_2), 3.7 (s, 3H, pyrrole-CH_3), 6.58 (s, 1H, pyrrole-H-3), 6.98 (s, 1H, pyrrole-H-5), 7.95 (m, 2H, anthraquinone-H-6 + 7), 8.04 (t, 1H, NH), 8.15 (t, 1H, NH), 8.23 (m, 2H, anthraquinone-H-5 + 8), 8.36 (pd, 1H, anthraquinone-H-4), 8.39 (pd, 1H, anthraquinone-H-3), 8.51 (ps, 1H, anthraquinone-H-1), 9.69 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 25.44 (s), 26.46 (s), 32.52 (s), 36.21 (p), 36.73 (s), 42.45 (s), 44.34 (2p), 56.48 (s), 103.62 (t), 117.75 (t), 122.16 (q), 123.06 (q), 124.85 (t), 127.22 (2t), 128.54 (t), 131.86 (t), 133.32 (2q), 134.1 (q), 135.08 (2t), 135.63 (q), 145.77 (q), 161.5 (q), 168.88 (q), 181.95 (2q); FD-MS: *m/z* 580 [M^+], $C_{29}H_{33}N_5O_6S$.

*4.8.2. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-4-[(4-{[(9,10-dioxo-9,10-dihydro-2-anthracenyl)sulfonyl]amino}butanoyl)-amino]-1-methyl-1H-2-pyrrole carboxamide (**43**)*

Educt: 650 mg **36**. Brown solid (140 mg) (0.2 mmol) (13%), m.p. 230 °C (decomposition); IR (KBr, cm^{-1}): 3400, 1670, 1590, 1530, 1460, 1440, 1400, 1340, 1290, 1180, 1150, 1110, 990, 960, 930, 820, 780, 720, 660, 630; ^{1}H NMR (DMSO-d_6): δ 1.67 (2quint., 4H, $2CH_2$), 2.25 (t, 2H, CH_2), 2.35 (s, 6H, $2CH_3$), 2.71 (q, 2H, CH_2), 2.85 (t, 2H, CH_2), 3.18 (q, 2H, CH_2), 3.76 (s, 3H, pyrrole-CH_3), 3.78 (s, 3H, pyrrole-CH_3), 6.78 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H-3), 6.83 (d, 1H, $^4J = 1.3$ Hz, pyrrole-H-5), 7.04 (d, 1H, $^4J = 1.4$ Hz, pyrrole-H-3′), 7.15 (d, 1H, $^4J = 1.3$ Hz, pyrrole-H-5′), 7.93 (m, 2H, anthraquinone-H-6 + 7), 8.12 (t, 1H, NH), 8.22 (m, 2H, anthraquinone-H-5 + 8), 8.37 (pd, 1H, anthraquinone-H-4), 8.4 (pd, 1H, anthraquinone-H-3), 8.5 (ps, 1H, anthraquinone-H-1), 9.73 (s, 1H, NH), 9.8 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 25.4 (s), 26.54 (s), 32.78 (s), 36.21 (p), 36.36 (p), 36.75 (s), 42.9 (s), 44.44 (2p), 56.53 (s), 103.62 (t), 103.84 (t), 117.75 (t), 118.03 (t), 122.16 (q), 122.69 (q), 123.06 (q), 123.39 (q) 124.85 (t), 127.47 (2t), 128.57 (t), 132.03 (t), 133.33 (2q), 134.4 (q), 134.98 (2t), 135.73 (q), 145.87 (q), 158.6 (q), 161.72 (q), 169.07 (q), 182.00 (2q); FD-MS: *m/z* 702 [M^+], $C_{35}H_{39}N_7O_7S$.

*4.8.3. N-2-[3-(Dimethylamino)propyl]-4-[(3-{[(9,10-dioxo-9,10-dihydro-2-anthracenyl)sulfonyl]amino}-propanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**40**)*

Educt: 445 mg **33**. Brown solid (240 mg) (0.42 mmol) (28%), m.p. 150 °C (decomposition); IR (KBr, cm^{-1}): 3400, 1650, 1610, 1500, 1450, 1420, 1390, 1320, 1270, 1165, 1140, 1100, 950, 930, 700, 610; ^{1}H NMR (DMSO-d_6): δ 1.64 (quint., 2H, $^3J = 6.6$ Hz, CH_2), 2.29 (s, 6H, $2CH_3$), 2.37 (t, 2H, CH_2), 2.5 (t, 2H, CH_2), 3.13 (2q, 4H, $2CH_2$), 3.61 (s, 3H, pyrrole-CH_3), 6.48 (d, 1H, pyrrole-H-3), 6.9 (d, 1H, pyrrole-H-5), 7.94 (m, 2H, anthraquinone-H-6 + 7), 8.2 (m, 3H, anthraquinone-H-5 + 8 + NH), 8.25 (pd, 1H, anthraquinone-H-4), 8.33 (pd, 1H, anthraquinone-H-3), 8.5 (ps, 1H, anthraquinone-H-1), 9.73 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.64 (s), 36.1 (s), 36.17 (p), 36.79 (s), 36.91 (s), 44.61 (2p), 56.73 (s), 103.39 (t), 117.73 (t), 121.91 (q), 122.97 (q), 124.88 (t), 127.16 (2t), 128.39 (t), 131.82 (t), 133.24 (2q), 133.98 (q), 135.04 (2t), 135.94 (q), 145.94 (q), 161.24 (q), 166.86 (q), 181.84 (q), 181.91 (q); FD-MS: *m/z* 567 [M^+], $C_{28}H_{31}N_5O_6S$.

*4.8.4. N-2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]-4-[(3-{[(9,10-dioxo-9,10-dihydro-2-anthracenyl)sulfonyl]amino}propanoyl)-amino]-1-methyl-1H-2-pyrrole carboxamide (**42**)*

Educt: 630 mg **35**. Brown solid (180 mg) (0.26 mmol) (17%), m.p. 170 °C (decomposition); IR (KBr, cm^{-1}): 3400, 1670, 1590, 1530, 1460, 1440, 1400, 1350, 1290, 1180, 1150, 1100, 990, 960, 930, 820, 720, 660, 630; ^{1}H NMR (DMSO-d_6): δ 1.62 (quint., 2H, $^3J = 6.9$ Hz, CH_2), 2.19 (s, 6H, $2CH_3$), 2.31 (t, 2H, $^3J = 7.0$ Hz, CH_2), 2.4 (t, 2H, $^3J = 6.8$ Hz, CH_2), 3.15 (2q, 4H, $2CH_2$), 3.68 (s, 3H, pyrrole-CH_3), 3.78 (s, 3H, pyrrole-CH_3), 6.71 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-3), 6.78 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H-5), 6.97 (d, 1H, $^4J = 1.9$ Hz, pyrrole-H-3′), 7.14 (d, 1H, $^4J = 1.7$ Hz, pyrrole-H-5′), 7.92 (m, 2H, anthraquinone-H-6 + 7), 8.19 (t, 1H; NH),

8.21 (m, 2H, anthraquinone-H-5 + 8), 8.26 (pd, 1H, anthraquinone-H-4), 8.33 (pd, 1H, anthraquinone-H-3), 8.51 (pd, 1H, anthraquinone-H-1), 9.72 (s, 1H, NH), 9.8 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 27.22 (s), 36.12 (s), 36.24 (p), 36.33 (p), 37.06 (s), 37.18 (s), 45.16 (2p), 57.13 (s), 103.97 (t), 104.37 (t), 118.13 (t), 118.34 (t), 121.95 (q), 122.26 (q), 122.86 (q), 123.2 (q), 124.9 (t), 127.2 (2t), 128.4 (t), 131.83 (t), 133.24 (2q), 134.0 (q), 135.04 (2t), 135.52 (q), 145.92 (q), 158.45 (q), 161.55 (q), 167.95 (q), 181.9 (2q); FD-MS: *m/z* 689 [M^+], $C_{34}H_{37}N_7O_7S$.

*4.8.5. 9,10-Dioxo-9,10-dihydro-2-anthracenecarbonyl chloride (**38**)*

One gram (4 mmol) of anthraquinone carboxylic acid and 10 ml (140 mmol) thionyl chloride were refluxed for 6 h. The mixture was cooled to room temperature and the surplus thionyl chloride was evaporated. The residue was dried for 3 h under vacuum and could be used without further purification. Yellow solid (980 mg) (3.62 mmol) (91%), m.p. 141 °C; ^{1}H NMR (DMSO-d_6): δ 7.91 (m, 2H, anthraquinone-H-6 + 7), 8.2 (m, 2H, anthraquinone-H-5 + 8), 8.24 (pd, 1H, anthraquinone-H-4), 8.32 (pd, 1H, anthraquinone-H-3), 8.58 (pd, 1H, anthraquinone-H-1); $C_{15}H_7ClO_3$.

*4.9. General procedure for the coupling of **38** with the amines **33** and **34***

Synthesis: see Section 4.7. Two millimoles of **38** correspond to 542 mg.

*4.9.1. N-2-[3-(Dimethylamino)propyl]-4-[(4-{[(9,10-dioxo-9,10-dihydro-2-anthracenyl)carbonyl] amino}butanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**45**)*

Educt: 470 mg **34**. Brown solid (110 mg) (0.2 mmol) (14%), m.p. 210 °C (decomposition); IR (KBr, cm^{-1}): 3450, 2900, 1650, 1620, 1570, 1520, 1450, 1420, 1390, 1310, 1270, 1240, 1170, 1100, 920, 770, 700, 610; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, $^3J = 7.3$ Hz, CH_2), 1.86 (quint., 2H, $^3J = 7.0$ Hz, CH_2), 2.3 (t, 2H, CH_2), 2.38 (s, 6H, $2CH_3$), 2.55 (t, 2H, CH_2), 3.18 (q, 2H, CH_2), 3.36 (q, 2H, CH_2), 3.74 (s, 3H, pyrrole-CH_3), 6.63 (d, 1H, pyrrole-H-3), 7.1 (d, 1H, pyrrole-H-5), 7.94 (m, 2H, anthraquinone-H-6 + 7), 8.09 (t, 1H, NH), 8.22 (m, 2H, anthraquinone-H-5 + 8), 8.27 (pd, 1H, anthraquinone-H-4), 8.31 (pd, 1H, anthraquinone-H-3), 8.64 (pd, 1H, anthraquinone-H-1), 8.99 (t, 1H, NH), 9.8 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 25.44 (s), 26.38 (s), 32.09 (s), 33.1 (s), 36.25 (p), 36.72 (s), 44.28 (2p), 56.41 (s), 103.69 (t), 117.89 (t), 122.17 (q), 123.78 (q), 125.82 (t), 127.36 (2t), 127.97 (t), 133.14 (t), 133.38 (2q), 134.83 (q), 135.01 (2t), 135.73 (q), 139.69 (q), 161.57 (q), 164.95 (q), 169.44 (q), 182.48 (2q); FD-MS: *m/z* 545 [M^+], $C_{30}H_{33}N_5O_5$.

*4.9.2. N-2-[3-(Dimethylamino)propyl]-4-[(3-{[(9,10-dioxo-9,10-dihydro-2-anthracenyl)carbonyl]amino}-propanoyl)amino]-1-methyl-1H-2-pyrrole carboxamide (**44**)*

Educt: 445 mg **33**. Brown solid (195 mg) (0.37 mmol) (25%), m.p. 150 °C (decomposition); IR (FT, cm^{-1}): 3450, 2900, 1650, 1620, 1570, 1520, 1450, 1420, 1390, 1310, 1270, 1230, 1160, 1100, 920, 770, 700, 610; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, $^3J = 6.7$ Hz, CH_2), 2.36 (s, 6H, $2CH_3$), 2.54 (t, 2H, CH_2), 2.6 (t, 2H, CH_2), 3.19 (q, 2H, $^3J = 5.9$ Hz, CH_2), 3.59 (q, 2H, $^3J = 6.0$ Hz, CH_2), 3.78 (s, 3H, pyrrole-CH_3), 6.67 (d, 1H, pyrrole-H-3), 7.13 (d, 1H, pyrrole-H-5), 7.94 (m, 2H, anthraquinone-H-6 + 7), 8.11 (t, 1H, $^3J = 4.3$ Hz, NH), 8.21 (m, 2H, anthraquinone-H-5 + 8), 8.26 (pd, 1H, anthraquinone-H-4), 8.32 (pd, 1H, anthraquinone-H-3), 8.62 (pd, 1H, anthraquinone-H-1), 9.08 (t, 1H, $^3J = 5.0$ Hz, NH), 9.9 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.1 (s), 35.4 (s), 36.0 (p), 36.4 (2 s), 44.0 (2p), 56.1 (s), 103.4 (t), 117.6 (t), 121.9 (q), 122.9 (q), 125.5 (t), 126.8 (t), 126.9 (t), 127.1 (t), 132.8 (t), 133.0 (q), 133.1 (2q), 134.5 (q), 134.7 (2q), 139.3 (q), 161.2 (q), 164.7 (q), 167.5 (q), 182.1 (q), 182.2 (q); FD-MS: *m/z* 531 [M^+], $C_{29}H_{31}N_5O_5$.

*4.9.3. 9-Acridinecarbonyl chloride (**39**)*

Nine hundred milligrams (4 mmol) of 9-acridine carboxylic acid hydrate and 10 ml (140 mmol) thionyl chloride were refluxed for 6 h. The mixture was cooled to room temperature and the surplus thionyl chloride was evaporated. The residue was dried for 3 h under vacuum and could be used without further purification. Yellow solid (930 mg) (3.85 mmol) (96%), m.p. 225 °C; ^{1}H NMR (DMSO-d_6): δ 7.96 (pt, 2H, acridine-H), 8.27 (m, 4H, acridine-H), 8.6 (m, 2H, acridine-H); $C_{14}H_8ClNO$.

*4.10. General procedure for the coupling of **39** with the amines **33** and **35***

Synthesis: see Section 4.7. Two millimoles of **39** correspond to 485 mg.

*4.10.1. N-9-(3-{[5-({[3-(Dimethylamino)propyl]amino}-carbonyl)-1-methyl-1H-3-pyrrolyl]amino}-3-oxopropyl)-9-acridine carboxamide (**46**)*

Educt: 445 mg **33**. Ochre solid (80 mg) (0.16 mmol) (11%), m.p. 140 °C (decomposition); IR (FT, cm^{-1}): 3300, 3050, 2940, 1640, 1580, 1520, 1470, 1435, 1410, 1270, 1130, 1100, 1050, 750; ^{1}H NMR (DMSO-d_6): δ 1.63 (quint., 2H, $^3J = 6.7$ Hz, CH_2), 2.22 (s, 6H, $2CH_3$), 2.34 (t, 2H, $^3J = 6.7$ Hz, CH_2), 2.72 (t, 2H, $^3J = 6.3$ Hz, CH_2), 3.18 (2q, 4H, $2CH_2$), 3.79 (s, 3H, pyrrole-CH_3), 6.7 (s, 1H, pyrrole -H-3), 7.18 (s, 1H, pyrrole-H-5), 7.57 (pt, 2H, acridine-H-2 + 7), 7.85 (pt, 2H, acridine-H-3 + 6), 8.0 (pd, 2H, acridine-H-4 + 5), 8.14 (t, 1H, NH), 8.15 (pd, 2H, acridine-H-1 + 8), 9.12 (t, 1H, $^3J = 5.0$ Hz, NH), 10.03 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 27.04 (s), 35.68 (s), 36.31 (p), 36.43 (s), 37.18 (s), 45.01 (2p), 57.05 (s), 103.51 (t), 117.85 (t), 122.31 (q), 123.27 (q), 126.16 (2t), 126.89 (2t), 129.47

(2t), 130.9 (2t), 142.65 (q), 148.44 (4q), 161.49 (q), 166.37 (q), 167.67 (q); FD-MS: *m/z* 502 [M^+], $C_{28}H_{32}N_6O_3$.

*4.10.2. N-9-(3-{[5-({[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]amino}carbonyl)-1-methyl-1H-3-pyrrolyl]amino}-3-oxopropyl)-9-acridinecarboxamide (**47**)*

Educt: 630 mg **35**. Ochre solid (90 mg) (0.14 mmol) (10%), m.p. 165 °C (decomposition); IR (FT, cm^{-1}): 3290, 1640, 1580, 1530, 1460, 1430, 1400, 1260, 1140, 1090, 1060, 815, 760; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2H, $^3J = 6.4$ Hz, CH_2), 2.37 (s, 6H, $2CH_3$), 2.53 (t, 2H, CH_2), 2.74 (t, 2H, $^3J = 6.6$ Hz, CH_2), 3.21 (2q, 4H, $2CH_2$), 3.79 (s, 3H, pyrrole-CH_3), 3.84 (s, 3H, pyrrole-CH_3), 6.86 (s, 1H, pyrrole-H-3), 6.93 (s, 1H, pyrrole-H-5), 7.18 (s, 1H, pyrrole-H-3′), 7.24 (s, 1H, pyrrole-H-5′), 7.56 (pt, 2H, acridine-H-2 + 7), 7.85 (pt, 2H, acridine-H-3 + 6), 8.01 (pd, 2H, acridine-H-4 + 5), 8.1 (t, 1H, NH), 8.16 (pd, 2H, acridine-H-1 + 8), 9.14 (t, 1H, $^3J = 4.9$ Hz, NH), 9.91 (s, 1H, NH), 10.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 26.44 (s), 35.61 (s), 36.28 (p), 36.46 (p), 36.72 (s), 37.2 (s), 44.25 (2p), 56.41 (s), 104.19 (t), 104.55 (t), 118.25 (t), 118.49 (t), 122.07 (q), 122.38 (q), 123.04 (q), 123.12 (q), 126.14 (2t), 126.89 (2t), 129.48 (2t), 130.92 (2t), 142.64 (q), 148.46 (4q), 158.72 (q), 161.67 (q), 166.39 (q), 167.76 (q); FD-MS: *m/z* 624 [M^+], $C_{34}H_{38}N_8O_4$.

4.11. Absorption spectrometry spectra and melting temperature studies

CT-DNA and double stranded poly(dAdT)$_2$ oligonucleotide were purchased from Sigma. CT-DNA was dissolved in deionized water and deproteinized using sodium dodecyl sulfate followed by a dialysis against 1 mM sodium cacodylate (pH 7.0). The concentrations of both DNA were quantified by measuring their UV absorbance at 260 nm and calculated from their molar extinction coefficients of 6600 $M^{-1}\,cm^{-1}$. The various synthesized compounds were prepared at 10 mM concentration in DMSO and further dilutions were made freshly in the appropriate aqueous buffer.

For UV spectral absorption spectrometry, 20 μM of the various tested drugs were incubated in 1 ml of BPE buffer [6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.1] in the presence or absence of 20 μM of base pairs of CT-DNA. Each mixture was transferred in a quartz cuvette of 10 mm pathlength. The UV–visible spectrum was recorded from 240 nm to 450 nm using an Uvikon 943 spectrophotometer and is referenced against a cuvette containing DNA (without drug) at the same concentration as in the sample cuvette.

The variation of melting temperature was deduced from the melting temperature measurement of 20 μM of CT-DNA or poly(dAdT)$_2$ incubated alone (control T_m) or with increasing concentration of the various compounds (drug/base pair ratio of 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.5 or 1) in 1 ml of BPE buffer. The samples were transferred in quartz cells and the absorbance at 260 nm was measured using the Uvikon 943 spectrophotometer thermostated with a Neslab RTE111 cryostat. One measure of UV–visible absorption was performed at each min over a range of 20–100 °C with an increment of 1 °C/min. The T_m values were obtained from the midpoint of the hyperchromic transition. ΔT_m values $= T_{m(drug+DNA)} - T_{m(DNA\ alone)}$.

4.12. Circular dichroism

The various drugs (50 μM final concentration) were incubated in a quartz cell of 10 mm path length in 1 ml of sodium cacodylate (1 mM, pH 7.0) with or without (control) 200 μM (base pairs) of CT-DNA or increasing concentration of CT-DNA (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200 μM). The CD spectra were collected from 500 to 230 nm with a resolution of 0.1 nm using a J-810 Jasco spectropolarimeter at 20 °C controlled by a PTC-424S/L peltier type cell changer (Jasco).

4.13. Topoisomerase I-mediated DNA relaxation and topoisomerase I cleavage assay

Both experiments were performed as previously described in Bailly [21] with the following modifications. Graded concentrations of the tested compounds were incubated in the presence of supercoiled pLAZ plasmid DNA (130 ng) prior to the addition of 4 units of human topoisomerase I (Topogen) at 37 °C for 45 min in relaxation buffer (50 mM tris(hydroxymethyl)aminomethane (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA and ATP). The reactions were stopped by adding SDS to 0.25% and proteinase K to 250 μg/ml and incubating the samples at 50 °C for 30 min. DNA samples were completed with 3 μl of the electrophoresis dye mixture to be loaded on a 1% agarose gel and then separated by electrophoresis at room temperature for 2 h at 120 Vin. Gels for the relaxation studies were ran without ethidium bromide and then stained using a bath containing ethidium bromide whereas gels for the DNA cleavage assays contained ethidium bromide prior to the electrophoretic migration. Both gels were finally washed and photographed under UV light.

4.14. Topoisomerase II cleavage assay

Supercoiled pLAZ plasmid DNA (130 ng) was incubated with 50 μM of the various combilexins or etoposide (20 or 50 μM) as control prior to the addition of 4 units of human topoisomerase II (Topogen) at 37 °C for 45 min in the appropriate cleavage buffer. SDS (0.25%) and proteinase K (250 μg/ml) were then added to stop the reaction during 30 min at 50 °C. DNA samples were loaded on 1% agarose gels containing ethidium bromide for 2 h at 120 Vin TBE buffer. After migration, gels were washed and photographed under UV light.

4.15. DNase I footprinting

Experiments were performed essentially as previously described in Bailly and Waring [25]. Briefly, a 117 bp 3′-end-labelled DNA fragment was obtained from double

digestion of the pBS plasmid (Stratagene, La Jolla, CA) using *Eco*RI and *Pvu*II restriction enzymes for 1 h in their respective buffers followed by incubation with α-[^{32}P]-dATP (Amersham, Buckinghamshire, England) and AMV reverse transcriptase for 2 h. The 117 bp radio-labelled DNA fragment was then separated on a 6% polyacrylamide gel under native conditions in TBE buffer (89 mM Tris base, 89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3), cut off from the gel, crushed and dialysed overnight against 400 μl of Tris 10 mM pH 8.0, EDTA 1 mM, NaCl 100 mM. After filtration under a 0.45 μm filter, the purified DNA was ethanol precipitated. Appropriate concentrations of the various ligands were incubated with the 117 bp radio-labelled DNA fragment for 15 min at 37 °C to ensure equilibrium prior to digestion of the DNA by the addition of DNase I (0.01 unit/ml) in 20 mM NaCl, 2 mM $MgCl_2$, 2 mM $MnCl_2$, pH 7.3). After 3 min, the reaction was stopped by freeze drying and samples were lyophilized. The DNA samples were resuspended in 5 μl of denaturing loading buffer (80% formamide solution containing tracking dyes), heated at 90 °C for 4 min and chilled in ice for 4 min prior to be loaded on a classical 8% denaturing polyacrylamide gel containing 8 M urea for 90 min at 65 W in TBE buffer. The data were collected using a Molecular Dynamics 445SI PhosphorImager and analysed using the ImageQuant version 4.1 software. Each resolved band on the autoradiograph was assigned to a particular bond within the DNA fragment by comparison of its position relative to the guanines sequencing standard (G-track) classically obtained using DMS and piperidine treatment of the same DNA fragment.

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