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Oligopyrrole carboxamides linked with a nucleobase as potential DNA minor groove binding ligands: synthesis, DNA binding and biological evaluation

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The synthesis of a series of new oligopyrrole carboxamides closely related to netropsin and distamycin A, linked with a nucleobase is reported. The new compounds possess similar structure elements as the known peptide nucleic acids which are interesting sequence reading DNA ligands. Cytotoxicity *in vitro*, the DNA binding characteristics and the inhibition of topoisomerase I were studied. Four of the compounds, **27**, **31**, **33** and **37** bind to DNA probably at AT sequences like netropsin or distamycin A in the minor groove. Surprisingly, no cytotoxicity and no inhibition of topoisomerase I was found.

1. Introduction

The need for new anticancer agents is pressing. Much efforts are currently directed toward the discovery of cytostatic agents targeting the cell-cycle pathway, angiogenesis, or cell differentiation, but conventional cytotoxic agents interfering with DNA metabolism remain actively searched as well (Anthoney and Twelves 2001). Promising anticancer agents that bind directly to DNA or inhibit DNA enzymes such as topoisomerase I or telomerase have been identified over the past few years (Demeunynck et al. 2003; Bailly 2000; Pindur and Lemster 1998). In this context creating DNA-binding ligands which additionally recognise specific sequences is a central goal in the development of DNA-targeted drugs (Bailly 1998; Pindur 2001; Bischoff and Hoffmann 2002; Pindur and Fischer 1996; Baird and Dervan 1996; Hurley 2002). Among these compounds there exists a structural class, which combines a sequence reading oligopyrrole carboxamide chain as a potential carrier linked with intercalating or alkylating groups or heterocyclic systems (Pindur 2001; Pindur and Fischer 1996; Hurley 2002; Bailly and Chaires 1998). Some of these ligands (so called combilexins) also interfere with DNA-dependent enzymes, as for example topoisomerases. The natural occurring antibiotics netropsin **1** or distamycin A **2** preferably binding to the DNA-AT sequence in the minor groove constituted the basic lead structure for these studies (Pindur and Fischer 1996; Hurley 2002).

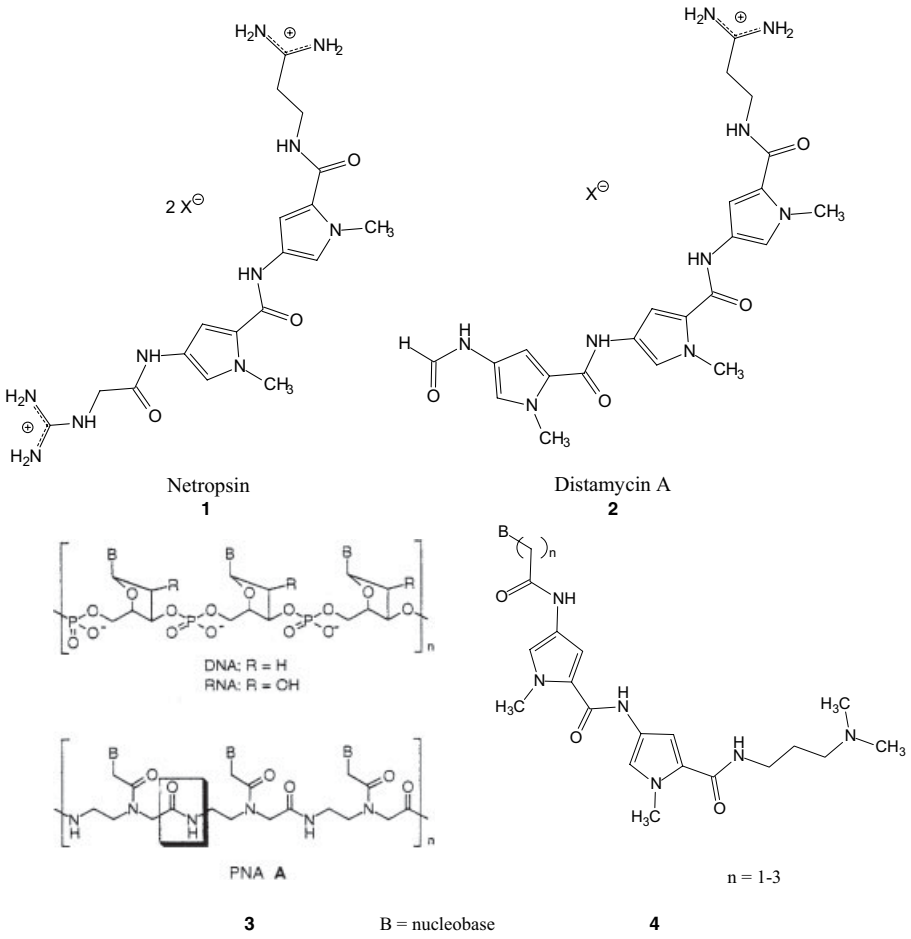
In the last years some interesting chemical and biological investigations were performed with the new synthetic molecules **3**, which possess a nucleobase linked with a peptide chain so called peptide nucleic acids (PNAs) binding the DNA double helix (Fox 2000; Ferrer et al. 2000; Gangamani et al. 1999; Nielsen and Haaima 1997; Diederichsen 1998; Nielsen 1997; Krotz et al. 1998; Nielsen 2001). These molecules seem to be promising candidates as sequence selective DNA triplex formers or strand invaders, which have a strong potential as therapeutic agents as antisense equivalent, diagnostic tools and probes in molecular biology as artificial transcription promoters (Ferrer et al. 2000). Mostly inspired by these results and in continuation of our studies of chemistry and biology of new DNA ligands possessing a propylamine oligopyrrole carboxamide group (Pindur and Fischer 1996; Hurley 2002; Lemster and Pindur 2002; Marotto et al. 2002; Hotzel et al. 2002, 2003), we report the first comprehensive synthesis, DNA binding and biology of nucleic acid base derived hybrid molecules **4**. This class of DNA ligands could probably undergo Hoogsteen base pairing in combination with minor groove binding and moreover should be able to inhibit DNA key enzymes. Based on the results of our previous work the linker length varied between C_2 and C_4 (Hotzel et al. 2002, 2003).

2. Investigations and results

2.1. Synthesis of the compounds

We formerly designed and synthesized a series of new pyrrole carboxamides with variations of the N- and C-terminus of distamycin A or netropsin. The highly basic amidine group at the C-terminal end of the natural compounds was replaced by an electronically equivalent propylamine function (Hotzel et al. 2002, 2003).

For the synthesis of nucleobase linked oligopyrrole carboxamides (see Table 1) a newly optimized strategy for the construction of a polyamidic group was used (Scheme 1). The sequence starts from *N*-methylpyrrole **5**, followed by transformation to trichloroacetyl pyrrole **6** and regioselective nitration with acetic-anhydride/HNO_3 to afford nitro derivative **7**. Nucleophilic displacement of the trichloromethyl group with the propylenediamine produces the monopyrrole

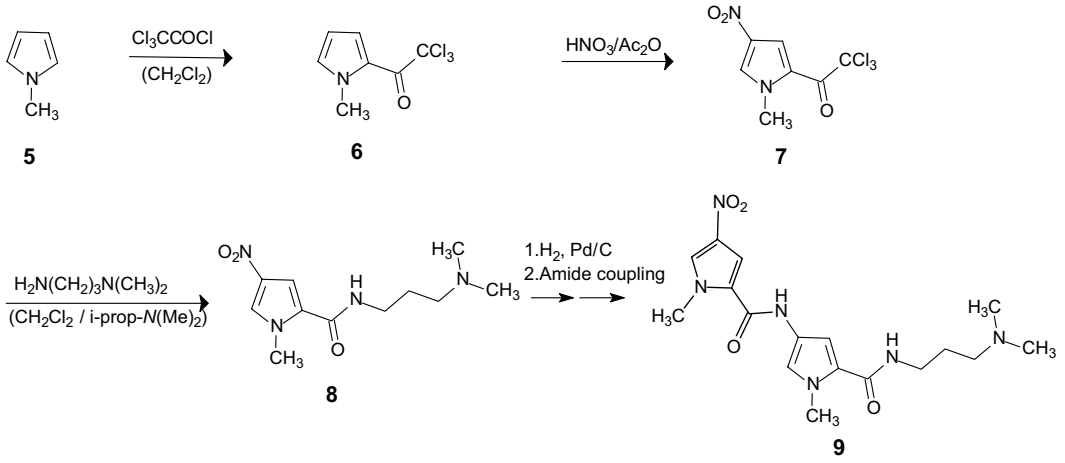


Netropsin **1**; Distamycin A **2**; **3** upper: DNA, RNA strand; lower: Peptide nucleic acid (PNA A-type) with the original N-(2-aminoethyl)glycine backbone (Gangamani et al. 1999); **4**: A nucleobase linked bispyrrole-carboxamide as a synthetic target for structural comparison, B = nucleobase

building block **8**, ready for coupling at the *N*-terminal end. For the construction of the appropriate *N*-terminal groups, for example adding an additional pyrrole carboxyl group and finally connecting the nucleobases, the nitro group was hydrogenated to a primary amine by Pd/C (10%) catalysis. Thus, compounds **8** (for the monopyrrole series) and the bispyrrole **9** (for the bispyrrole series, also trispyrroles are accessible by this method (Hotzel et al. 2003)) represent the decisive key compounds for the selective construction of the nucleobase linked target products (Scheme 1).

However, from the series of the nucleobases thymine, uracil and adenine three respective alkanoic acids (acetic acid, propionic acid, butanoic acid) were used for analysing the influence of the length of the alkyl chain at the *N*-

Scheme 1



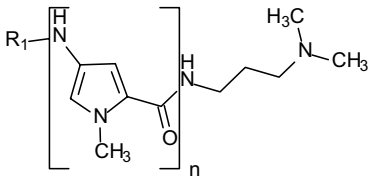
terminal end for DNA-binding. The carboxylic acids were coupled with the 4-aminopyrrole carboxamide building blocks subsequently generated from the nitro-pyrrole derivatives **8, 9**. The products **24**–**37** are listed in Table 1.

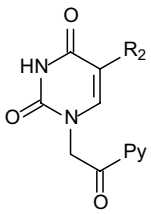
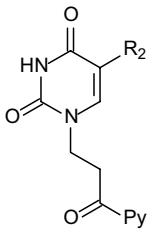
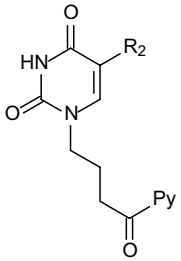
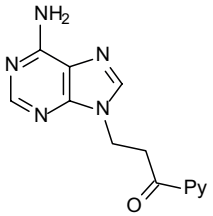
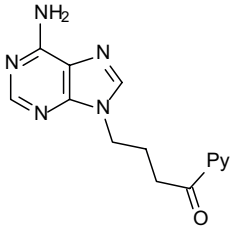
The thymine acetic acid **10** was commercially available. The compounds **11**–**15** were synthesized by selective routine alkylation procedures of thymine or uracil with bromo-carboxylic esters and subsequent alkaline hydrolysis to the free carboxylic acids (Hisatome et al. 1996). Especially in the case of the synthesis of the nucleobase butyric acids, the S_N-alkylation reaction was catalyzed by tetrabutylammonium chloride. In these syntheses also thymine and uracil diesters were formed beside the desired monosubstituted products **11**–**15** in a yield of 20–35%. Both could be separated and purified by column chromatography.

For a related direct alkylation reaction of the highly ambident nucleophilic adenine a complex product pattern is possible. The 9-adenyl-acetic acid, readily available by hydrolysis of the commercially available ethyl ester is highly insoluble in the most polar solvents appropriate for the amide coupling reactions. Thus, this compound could not be transformed anyway. In order to solve the problem of the insolubility of the 9-adenyl-acetic acid for the coupling reaction and to reduce the possibility of selfcondensation of the free 9-adenyl-acetic acid a lipophilic p-methoxy-benzoyl protecting group at the aminofunction was introduced (Scheme 3). However the introduction of this protecting group was difficult to control and starting from the ester **20** mostly the bisbenzoylated product **21** was formed. Compound **21** could be readily hydrolyzed to the carboxylic acid **22**. The carboxylic acid **22** was soluble enough for a coupling reaction with the 4-aminopyrrole building block to give rise to the monobenzoylated product **23**. Unfortunately complete deblocking of the p-methoxybenzoyl-group was not possible also with the reagent (chloromethylene)-dimethylammonium chloride without degradation of the molecule **23**.

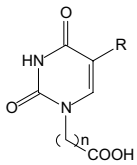
However, the direct alkylation reaction of adenine with 3-bromo-propionic and 4-bromo-butanoic acid proceeded with sufficient N-9-selectivity. Besides the main products **16** and **17** also the 3-alkylated mono-products could be isolated. The regiochemistry of these isomers was exemplarily analysed for compound **18** by application of combined ^1H and ^{13}C NMR-techniques including 2D-methods like HMBC and HMQC. For this analysis the key ^3J-couplings H-11 > C-4 and H-11 > C-8 are decisive.

Table 1: Nucleobase-linked pyrrole carboxamides

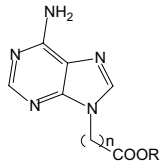


Compd.	R_1	R_2	n	Yield [%]
24		−H	1	47
25		$-CH_3$	1	49
26		−H	1	31
27			2	27
28		$-CH_3$	1	25
29			2	36
30		−H	1	35
31			2	33
32		$-CH_3$	1	45
33			2	37
34			1	30
35			2	19
36			1	37
37			2	22

Py = pyrrole carboxamide

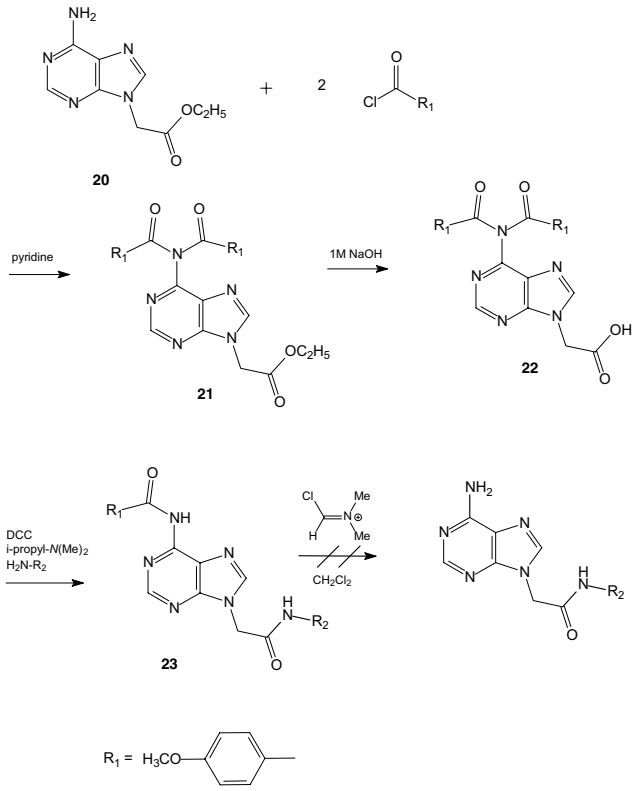


	R	n	Ethyl esters	
10	H	1	**12a**	s. exp. part
11	CH_3	1	**13a**	
12	H	2	**14a**	
13	CH_3	2	**15a**	
14	H	3		
15	CH_3	3		



	R	n	Ethyl esters	
16	H	2	**16a**	s. exp. part
17	H	3		
18	C_2H_5	3		
19	H	1		

Scheme 2



In general, for the last step to the hybrid molecules **24**–**37**, the amide coupling reaction (see e.g. Scheme 2, reaction from **22** to **23**) at the *N*-terminal end of a pyrrole unite is a crucial process and many synthetic efforts have been done in our group in the last years to optimize the procedure in order to synthesize new combilexines (Hotzel et al. 2002, 2003). A variety of coupling strategies from peptide chemistry (Jones 1999) were tested. However, the method with the best results comprises the reaction of the *N*-terminal 4-aminopyrrole unit with ethyl chloroformiate in DMF/THF and diethylisopropylamine. The correct reaction temperature and a high concentration of the reactants are very important factors (details of the procedure are given in the experimental part). For the amide coupling reaction procedure the thymine- and uracil-carboxylic acids were sufficiently soluble in DMF. The general solubility problem by the coupling reaction of **16** and **17** (equilibrium with betainic structures) in polar aprotic solvents, which must be used for amid coupling reactions in peptide chemistry, was solved by the addition of pyridinium chloride in dimethyl formamide (Hisatome et al. 1996). In this case ethyl chloroformiate as a carbonic acid group activator was used successfully.

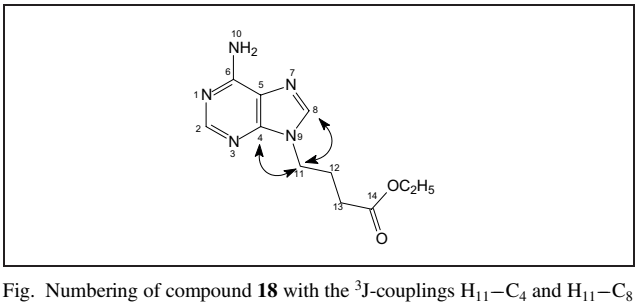


Fig. Numbering of compound **18** with the ^{3}J-couplings $H_{11}-C_4$ and $H_{11}-C_8$

Table 2: Results of the DNA-binding- and topoisomerase-inhibition-assays

Compd.	ΔT_m CT DNA r = 0.5	ΔT_m polydAdT r = 0.5	ΔT_m polydAdT r = 0.1	CD	UV	topo I inhibition	
						−BET	+BET
24	0	1.3	0	−	−	−	−
26	0	0	0	−	−	−	−
27	0	5.2	0	+	+(h, b)	−	−
28	0	0	0	−	−	−	−
30	0	0	0	−	−	−	−
31	1.3	6.5	2.8	+	+(h, b)	−	−
32	0	0	1.4	−	−	−	−
33	1.1	5.9	1.2	+	+(h, b)	−	−
37	1.3	6.8	2.8	+	+(h, b)	−	−

ΔT_m: variations of the melting temperatures (T_m drug-DNA complex – Tm DNA alone in °C) of the complex between CT-DNA (calf thymus DNA), polydAT and the compounds. The T_m were performed at several DNA/drug ratios (r). CD: Circular dichroism (+ correspond to bands that appear + upon addition of CT-DNA, – correspond to bands that appear − upon addition of CT-DNA). UV: UV/VIS spectroscopy. (b) bathochrome, (h) hypochrome. Topoisomerase I inhibition assays were analyzed in agarose gel in presence and absence of ethidiumbromide (BET) (for methods see [Bourdouxhe-Housiaux et al. 1996 Bailly 2001])

2.2. Cytotoxicity assay

All compounds were submitted at the NCI antitumor screening program (http://dtp.nci.nih.gov). In the pre-screening anticancer assay with the three tumor cell lines MCF7 (breast carcinoma), NCI-H460 (non small cell lung carcinoma), SF-268, (glioma) no significant inhibition of cell growth was performed, throughout. Probably, the high polarity (log P values about −1.2 and −2.1 were calculated) is mainly responsible for reduced cell membrane penetration.

2.3. DNA binding and topoisomerase I inhibition

DNA-binding and topoisomerase I inhibition assays were performed in an established biochemical laboratory (Bourdouxhe-Housiaux et al. 1996). The ΔT_m values obtained from the UV-melting curves with calf thymus DNA and polydAT for a DNA/drug ratio of 0.5 were significant for the bispyrrole derivatives **27** (5.2 °C), **31** (6.5 °C), **33** (5.9 °C) and **37** (6.8 °C) (Table 2). Moreover, the same compounds gave positive circular dichroism (CD) with calf thymus DNA and in the UV-spectra both bathochromic and hypochromic effects, supporting DNA-binding. Especially the positive sign of the CD curve proved the interaction with the minor groove, known for netropsin **1** and analogues. However, in the topoisomerase I assay all fourteen hybrid compounds did not inhibit the enzyme (Bourdouxhe-Housiaux et al. 1996; Bailly 2001).

3. Discussion

The new nucleobase linked mono- and bispyrrole carboxamides **24**–**37**, synthesized by a specially developed sequential coupling method, were evaluated as potential DNA ligands with different biophysical methods. Moreover, the topoisomerase I inhibition was tested. However, in the NCI screening no significant tumor cell cytotoxicity was found, probably due to low cell penetration in the assay. In the case of the bispyrrole uracil derivatives **27**, **31**, the thymine derivative **33** and the adenine-butanoyl derivative **37** the DNA melting curves with two different types of DNA (calf-thymus and polydAT) demonstrate weak binding to DNA, preferably to polydAT-strands like netropsin or distamycin A (Pindur 2001). Moreover, the positive sign of the CD curve (CT-DNA) shows AT minor groove binding of these

promising candidates. One reason for this surprisingly weak interaction could be the unsufficient linker length for dual binding in the minor and major groove of DNA. All fourteen compounds did not inhibit topoisomerase I.
In summary, the first biologically/biophysical results of the new nucleobase derivatives represent a useful basis for further investigations and structural optimization, first of all in the context of enhancement of cell penetration or increase of linker length to C_5 or C_6 to get more conformative flexibility for Hoogsteen-interaction.

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 experiments were recorded on a Bruker AC-300 apparatus (300 MHz). HMBC-Spectra were measured on a Bruker DRX 600 (600 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 **24**–**37** were chromatographically pure (tlc). Nonstoichiometric inclusion of solvent molecules (typical for oligopyrrole carboxamides) gave C,H,N-analysis with divergence of $>0.3\%$.

4.1.1. Ethyl 3-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl) propanoate (**13a**)

A suspension of 3.44 g (19 mmol) ethyl 3-bromopropanoate in 20 ml DMF was stirred. 1.59 g (12.6 mmol) of thymine and 5.75 g (41.6 mmol) of potassium carbonate were added. The mixture was stirred at 70 °C for 24 h. Potassium carbonate was removed by filtration. The DMF of the filtrate was removed under vacuum. The residue was chromatographed on silica gel ($CHCl_3$–MeOH (40 : 1)). White solid (1100 mg) (4.86 mmol) (39%), m.p. 71 °C [m.p. lit. 68–70 °C], ^1H NMR (DMSO-d_6): δ 1.15 (t, 3 H, $^3J = 7.1$ Hz, CH_3), 1.75 (s, 3 H, thymine-CH_3), 2.48 (t, 2 H, CH_2), 4.0 (2 t, 4 H, 2 CH_2), 7.31 (s, 1 H, thymine-H-6), 10.93 (s, 1 H, thymine-NH); EI-MS: m/z 226 [M^+], Anal. $C_{10}H_{14}N_2O_4$.

4.1.2. Ethyl 4-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoate (**15a**)

A suspension of 2 g thymine (15.9 mmol) in distilled DMF (160 ml) was treated with 0.4 g NaH (95%) (15.9 mmol) under nitrogen atmosphere, with stirring at room temperature. After 1 h 6.2 g ethyl 4-bromobutanoate (31.8 mmol) and tetrabutylammonium iodide were added. The mixture was stirred for 48 h at room temperature. Then the solvent was removed in vacuum and the residue was chromatographed on silica gel ($CHCl_3$–MeOH (20 : 1)). White solid (980 mg) (4.08 mmol) (26%), m.p. 134–135 °C [m.p. lit. 134–135 °C]; ^1H NMR (DMSO-d_6): δ 1.2 (t, 3 H, $^4J = 7.2$ Hz, CH_3), 1.75 (d, 3 H, thymine-CH_3), 1.8 (quint., 2 H, $^4J = 7.2$ Hz, CH_2), 2.3 (t, 2 H, $^4J = 7.4$ Hz, CH_2), 3.6 (t, 2 H, $^4J = 6.9$ Hz, CH_2), 4.0 (q, 2 H, $^4J = 7.1$ Hz, CH_2), 7.5 (d, 1 H, $^4J = 1.15$ Hz, thymine-H-6), 11.2 (s, 1 H, thymine-NH); EI-MS: m/z 240 [M^+], Anal. $C_{11}H_{16}N_2O_4$.

4.1.3. 2-(2,4-Dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)acetic acid (**10**)

Uracil (1.12 g, 10 mmol), 1.12 g potassium hydroxide (20 mmol) and 1.42 g chloro acetic acid (15 mmol) were solved in 100 ml water and stirred under reflux. After a few minutes the white precipitate was removed by the dropwise addition of a saturated solution of potassium hydroxide. After 24 h the mixture was cooled to room temperature and acidified with 1 M HCl. The water was removed under vacuum until a white precipitate was formed. It was filtered off and used without further purification.
White solid (1100 mg) (6.47 mmol) (65%), m.p. 285 °C [m.p. lit. 285 °C]; ^1H NMR (DMSO-d_6): δ 4.39 (s, 2 H, CH_2), 5.59 (d, 1 H, $^3J = 7.7$ Hz, uracil-H-5), 7.61 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-6), 11.36 (s, 1 H, COOH), EI-MS: m/z 170 [M^+], Anal. $C_6H_6N_2O_4$.

4.1.4. Ethyl 3-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)propanoate (**12a**)

Compound **12a** was synthesized analogous to **13a**. Uracil (1.41 g, 12.6 mmol), ethyl 3-bromopropanoate (3.44 g, 19 mmol), potassium carbonate (5.75 g, 41.6 mmol). White solid (900 mg, 4.24 mmol) (34%), m.p. 94 °C [m.p. lit. 68–70 °C], ^1H NMR (DMSO-d_6): δ 1.14 (t, 3 H, $^3J = 7.1$ Hz, CH_3), 2.51 (t, 2 H, CH_2), 3.99 (m, 4 H, 2 CH_2), 5.57 (d, 1 H, $^3J = 7.6$ Hz, uracil-H-5), 7.42 (d, 1 H, $^3J = 7.6$ Hz, uracil-H-6), 11.15 (s, 1 H, uracil-NH), EI-MS: m/z 212 [M^+], Anal. $C_9H_{12}N_2O_4$.

4.1.5. Ethyl 4-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoate (**14a**)

Compound **14a** was synthesized analogous to **15a** from uracil (1.78 g, 15.9 mmol), ethyl 4-bromobutanoate (6.2 g, 31.8 mmol), NaH (95%) (0.4 g, 15.9 mmol). White solid (800 mg, 3.54 mmol) (22%), m.p. 120–125 °C, ^1H NMR (DMSO-d_6): δ 1.15 (t, 3 H, $^4J = 7.1$ Hz, CH_3), 1.82 (quint., 2 H, $^4J = 7.1$ Hz, CH_2), 2.29 (t, 2 H, $^4J = 7.4$ Hz, CH_2), 3.66 (t, 2 H, $^4J = 6.9$ Hz, CH_2), 4.0 (q, 2 H, $^4J = 7.1$ Hz, CH_2), 5.51 (d, 1 H, $^4J = 7.9$ Hz, uracil-H-5), 7.59 (d, 1 H, $^4J = 7.6$ Hz, uracil-H-6), 11.2 (s, 1 H, uracil-NH), EI-MS: m/z 226 [M^+], Anal. $C_{10}H_{14}N_2O_4$.

4.1.6. Ethyl 3-(6-amino-9 H-9-purinyl)propanoate (**16a**)

Compound **16a** was synthesized analogous to **13a** from adenine (1.7 g, 12.6 mmol), ethyl 3-bromopropanoate (3.44 g, 19 mmol), and potassium carbonate (5.75 g, 41.6 mmol). Potassium carbonate was filtered off. The DMF of the filtrate was removed in vacuum. The product was recrystallized from the residue with 20 ml of a mixture of methanol and water (1 : 1). White solid (1800 mg, 7.66 mmol) (61%), m.p. 170 °C [m.p. lit. 170–171 °C], ^1H NMR (DMSO-d_6): δ 1.1 (t, 3 H, $^3J = 7.1$ Hz, CH_3), 2.93 (t, 2 H, $^3J = 6.6$ Hz, CH_2), 4.0 (q, 2 H, $^3J = 7.1$ Hz, CH_2), 4.38 (t, 2 H, $^3J = 6.7$ Hz, CH_2), 7.21 (s, 2 H, NH_2), 8.08 (s, 1 H, adenine-H-8), 8.13 (s, 1 H, adenine-H-2), EI-MS: m/z 235 [M^+], Anal. $C_{10}H_{13}N_5O_2$.

4.1.7. Ethyl 4-(6-amino-9 H-9-purinyl)butanoate (**18**)

Compound **18** was synthesized analogous to **15a** from adenine (2 g, 14.8 mmol), DMF (200 ml), ethyl 4-bromobutanoate (4.4 g, 22.57 mmol), NaH (95%) (0.4 g, 15.9 mmol). The residue was chromatographed on silica gel with MeOH–$CHCl_3$ (10 : 1). White solid (2600 mg, 10.44 mmol) (71%), m.p. 108–109 °C [m.p. lit. 108–109 °C], ^1H NMR (DMSO-d_6): δ 1.11 (t, 3 H, $^3J = 7.1$ Hz, CH_3), 2.05 (quint., 2 H, $^3J = 6.8$ Hz, CH_2), 2.27 (t, 2 H, $^3J = 7.4$ Hz, CH_2), 3.97 (q, 2 H, $^3J = 7.2$ Hz, CH_2), 4.16 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 7.22 (s, 2 H, NH_2), 8.11 (s, 1 H, adenine-H-8), 8.12 (s, 1 H, adenine-H-2); ^{13}C NMR (DMSO-d_6): δ 14.0 (p), 24.9 (s), 30.6 (s), 42.2 (s), 59.9 (s), 118.7 (q), 140.8 (t), 149.6 (q), 152.4 (t), 156.0 (q), 172.1 (q), EI-MS: m/z 249 [M^+], Anal. $C_{11}H_{15}N_5O_2$.

4.1.8. General procedure for the preparation of nucleobase-alkanoic acid by hydrolysis of the corresponding ester

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

4.1.8.1. 3-(5-Methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl) propionic acid (**13**)

White solid, quantitative reaction, m.p. >250 °C, ^1H NMR (DMSO-d_6): δ 1.75 (s, 3 H, thymine-CH_3), 2.42 (t, 2 H, $^3J = 7.6$ Hz, CH_2), 3.96 (t, 2 H, $^3J = 7.7$ Hz, CH_2), 7.29 (s, 1 H, thymine-H-6), 10.92 (s, 1 H, NH), 12.32 (s, 1 H, COOH), EI-MS: m/z 198 [M^+], Anal. $C_8H_{10}N_2O_4$.

4.1.8.2. 4-(5-Methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoic acid (**15**)

White solid, quantitative reaction, m.p. >250 °C, ^1H NMR (DMSO-d_6): δ 1.73 (s, 3 H, thymine-CH_3), 1.77 (quint., 2 H, $^3J = 7.2$ Hz, CH_2), 2.21 (t, 2 H, $^3J = 7.4$ Hz, CH_2), 3.62 (t, 2 H, $^3J = 7.0$ Hz, CH_2), 7.48 (s, 1 H, thymine-H-6), 11.19 (s, 1 H, thymine-NH), EI-MS: m/z 212 [M^+], Anal. $C_9H_{12}N_2O_4$.

4.1.8.3. 3-(2,4-Dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)propionic acid (**12**)

White solid, quantitative reaction, m.p. >250 °C, ^1H NMR (DMSO-d_6): δ 2.43 (t, 2 H, $^3J = 8.0$ Hz, CH_2), 3.93 (t, 2 H, $^3J = 7.7$ Hz, CH_2), 5.56 (d, 1 H, $^3J = 7.3$ Hz, uracil-H-5), 7.4 (d, 1 H, $^3J = 7.6$ Hz, uracil-H-6), 11.2 (s, 1 H, NH), EI-MS: m/z 184 [M^+], Anal. $C_7H_8N_2O_4$.

4.1.8.4. 4-(2,4-Dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoic acid (**14**)

White solid, quantitative reaction, m.p. >250 °C, ^1H NMR (DMSO-d_6): δ 1.78 (quint., 2 H, $^3J = 7.1$ Hz, CH_2), 2.23 (t, 2 H, $^3J = 7.3$ Hz, CH_2), 3.65 (t, 2 H, $^3J = 6.8$ Hz, CH_2), 5.55 (d, 1 H, $^3J = 7.1$ Hz, uracil-H-5), 7.6 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-6), 11.22 (s, 1 H, NH), EI-MS: m/z 198 [M^+], Anal. $C_8H_{10}N_2O_4$.

4.1.8.5. 3-(6-Amino-9 *H*-9-purinyl)propionic acid (**16**)

White solid, quantitative reaction, m.p. >250 °C, ^1H NMR (DMSO-d_6): δ 2.85 (t, 2 H, $^3J = 6.7$ Hz, CH_2), 4.32 (t, 2 H, $^3J = 6.8$ Hz, CH_2), 7.20 (s,

2 H, NH_2), 8.07 (s, 1 H, adenine-H-8), 8.12 (s, 1 H, adenine-H-2), EI-MS: m/z 207 [M^+], Anal. $C_8H_9N_5O_2$.

4.1.8.6. 3-(6-Amino-9 *H*-9-purinyl)butanoic acid (**17**)

White solid, quantitative reaction, m.p. 298 °C [>300 °C], ^{1}H NMR (DMSO-d_6): δ 2.01 (quint., 2 H, $^3J = 7.0$ Hz, CH_2), 2.2 (t, 2 H, $^3J = 7.2$ Hz, CH_2), 4.15 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 7.21 (s, 2 H, NH_2), 8.12 (s, 2 H, adenine-H-2 + -H-8), 12.1 (s, 1 H, COOH), EI-MS: m/z 221 [M^+], Anal. $C_9H_{11}N_5O_2$.

4.1.9. 2-{6-[Di(4-methoxybenzoyl)amino]-9 H-9-purinyl}acetic acid (**22**)

(Adenin-9-yl)acetic acid (442 mg) (2 mmol) was suspended in dried pyridine and stirred at 80 °C for 30 min. Then 1.7 g (10 mmol) of *p*-methoxybenzoylchloride were added and the mixture was stirred for 24 h at room temperature. The solvent was evaporated. This was repeated several times after treatment of the residue with toluol. The solvent was dissolved in 70 ml of CH_2Cl_2 and washed twice with 30 ml of aqueous citric acid solution (10%, m/V). The organic layer was dried with $MgSO_4$ and then the solvent was evaporated. The residue was dissolved in warm ethanol (60 °C, 40 ml), cooled down to room temperature and treated with 15 ml of a 1 M NaOH solution. After stirring for 3 h 3 ml of 1 M NaOH solution were added and the reaction was stopped by acidification with 1 M HCl solution to pH 5 after 4 additional hours. The product was filtered and dried. White solid (277 mg, 0.6 mmol) (30%), m.p.: 97 °C, ^{1}H NMR (DMSO-d_6): δ 3.8 (s, 3 H, CH_3), 3.84 (s, 3 H, CH_3), 5.11 (s, 2 H, CH_2), 7.03 (m, 4 H, *p*-methoxybenzoyl-H), 7.87 (pd, 2 H, $^3J = 8.8$ Hz, *p*-methoxybenzoyl-H), 8.04 (pd, 2 H, $^3J = 8.9$ Hz, *p*-methoxybenzoyl-H), 8.44 (s, 1 H, adenine-H-8), 8.7 (s, 1 H, adenine-H-2), 11.0 (s, 1 H, COOH); EI-MS: m/z 461 [M^+], Anal. $C_{23}H_{19}N_5O_6$.

4.1.10. N2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1 H-3-pyrrolyl]-4-{[(2-(6-[(4-methoxybenzoyl)amino)-9H-9-purinyl}acetyl)amino]-1-methyl-1 H-2-pyrrole-carboxamide (**23a**)

414 mg (1.1 mmol) of **9** were reduced to the primary amine with 200 mg Pd (10%/C) in 20 ml dried DMF under hydrogen atmosphere. After stirring for 12 h at room temperature the Pd/C was removed by filtration and the filtrate was cooled down to 0 °C. 327 mg (1 mmol) **22** dissolved in 40 ml of DMF and a few mg of DMAP were added. Then 248 mg (1.2 mmol) DCC dissolved in 10 ml CH_2Cl_2 were dropped to the mixture, which was stirred for 24 h at room temperature. The product could not be purified without decomposition; ^{1}H NMR (DMSO-d_6): δ 1.61 (quint., 2 H, $^3J = 6.9$ Hz, CH_2), 2.12 (s, 6 H, 2 CH_3), 2.25 (t, 2 H, $^3J = 6.7$ Hz, CH_2), 3.16 (q, 2 H, $^3J = 6.7$ Hz, CH_2), 3.71 (s, 3 H, CH_3), 3.77 (s, 3 H, CH_3), 3.78 (s, 3 H, CH_3), 5.15 (s, 2 H, CH_2), 6.24 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-3), 6.34 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-5), 6.77 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-3′), 6.8 (d, 1 H, $^4J = 1.3$ Hz, pyrrole-H-5′), 7.12 (m, 2 H, *p*-methoxybenzoyl-H), 8.09 (m, 2 H, p-methoxybenzoyl-H), 8.44 (s, 1 H, adenine-H-8), 8.69 (s, 1 H, adenine-H-2), 9.56 (s, 1 H, NH), 9.88 (s, 1 H, NH), 10.48 (s, 1 H, NH); FD-MS: m/z 656 [M^+], Anal. $C_{32}H_{37}N_{11}O_5$.

4.1.11. N2-[3-(Dimethylamino)propyl]-4-[(2-{6-[(4-methoxybenzoyl)amino]-9 H-9-purinyl}acetyl)amino}-1-methyl-1 H-2-pyrrole-carboxamide (**23b**)

The synthesis was performed in analogy to **23a**. Instead of 1.1 mmol of **9** 1.1 mmol (280 mg) of **8** were used. The product could not be purified without decomposition; ^{1}H NMR (DMSO-d_6): δ 1.63 (quint., 2 H, $^3J =$ 6.8 Hz, CH_2), 2.26 (s, 6 H, 2 CH_3), 2.39 (t, 2 H, $^3J = 6.6$ Hz, CH_2), 3.18 (q, 2 H, $^3J = 7.7$ Hz, CH_2), 3.76 (s, 3 H, CH_3), 3.85 (s, 3 H, CH_3), 5.13 (s, 2 H, CH_2), 6.71 (d, 1 H, pyrrole-H-3), 7.07 (d, 1 H, pyrrole-H-5), 7.07 (m, 2 H, *p*-methoxybenzoyl-H), 8.04 (m, 2 H, *p*-methoxybenzoyl-H), 8.1 (t, 1 H, NH), 8.43 (s, 1 H, adenine-H-8), 8.68 (s, 1 H, adenine-H-2), 10.43 (s, 1 H, NH); FD-MS: m/z 535 [M^+], Anal. $C_{26}H_{31}N_9O_4$.

4.1.12. General procedure for the synthesis of thymine-/uracil- pyrrole and bispyrrole carboxamides

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 pyrrole amine (prepared from the nitropyrrole analogous by hydrogenation in DMF over Pd on charcoal (10%) (Hotzel et al. 2002, 2003)) 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 (MeOH−NH_3 (25%, 97 : 3)).

4.1.12.1. *N*2-[3-(Dimethylamino)propyl]-1-methyl-4-{[2-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)acetyl]amino}-1*H*-2-pyrrole carboxamide (**24**)

Yellow solid (178 mg, 0.47 mmol) (47%), m.p. >250 °C; IR (KBr, cm^{-1}): 3300, 3160, 3040, 2940, 2810, 1660, 1580, 1520, 1460, 1430, 1400, 1350, 1220, 1150, 800, 760; ^{1}H NMR (DMSO-d_6): δ 1.65 (quint., 2 H, $^3J = 7.0$ Hz, CH_2), 2.3 (s, 6 H, 2 CH_3), 2.45 (t, 2 H, CH_2), 3.16 (q, 2 H, $^3J = 6.1$ Hz, CH_2), 3.76 (s, 3 H, pyrrole-CH_3), 4.46 (s, 2 H, CH_2) 5.57 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-5), 6.68 (d, 1 H, pyrrole-H-3), 7.07 (d, 1 H, $^4J = 1.1$ Hz, pyrrole-H-5), 7.58 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-6), 8.13 (t, 1 H, NH), 10.16 (s, 1 H, NH), 11.32 (s, 1 H, uracil-NH); ^{13}C NMR (DMSO-d_6): δ 26.65 (s), 36.33 (p), 36.82 (s), 44.58 (2 p), 50.0 (s), 58.1 (s), 100.77 (t), 104.3 (t), 117.91 (t), 121.4 (q), 121.52 (q), 123.42 (t), 147.1 (q), 151.4 (q), 161.4 (q), 164.16 (q); FD-MS: m/z 377 [M^+], Anal. $C_{17}H_{24}N_6O_4$.

4.1.12.2. *N*2-[3-(Dimethylamino)propyl]-1-methyl-4-{[2-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)acetyl]amino}-1*H*-2-pyrrole carboxamide (**25**)

Yellow solid (192.4 mg, 0.49 mmol) (49%), m.p. >250 °C; IR (KBr, cm^{-1}): 3303, 3157, 3040, 2945, 2812, 1660, 1577, 1524, 1463, 1435, 1406, 1346, 1223, 1144, 805, 760; ^{1}H NMR (DMSO-d_6): δ 1.6 (quint., 2 H, $^3J = 6.5$ Hz, CH_2), 1.75 (s, 3 H, thymine-CH_3), 2.2 (s, 6 H, 2 CH_3), 2.4 (t, 2 H, $^3J = 7.0$ Hz, CH_2), 3.2 (q, 2 H, $^3J = 6.7$ Hz, CH_2), 3.75 (s, 3 H, pyrrole-CH_3), 4.4 (s, 2 H, CH_2), 6.7 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-3), 7.1 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-5), 7.5 (s, 1 H, thymine-H), 8.1 (t, 1 H, $^3J = 6.7$ Hz, NH), 10.1 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 12.71 (p), 27.25 (s), 36.31 (p), 37.23 (s), 45.24 (2 p), 49.81 (s) , 56.85 (s), 103.6 (t), 108.23 (q), 117.83 (t), 121.52 (q), 123.51 (q), 142.79 (t), 151.41 (q), 161.34 (q), 164.3 (q), 164.78 (q). FD-MS: m/z 391 [M^+], Anal. $C_{18}H_{26}N_6O_4$.

4.1.12.3. *N*2-[3-(Dimethylamino)propyl]-1-methyl-4-{[3-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)propanoyl]amino}-1*H*-2-pyrrole carboxamide (**26**)

Yellow solid (121 mg, 0.31 mmol) (31%), m.p. 140−145 °C; IR (KBr, cm^{-1}): 3380, 3060, 2900, 2820, 2780, 1680, 1640, 1520, 1440, 1380, 1350, 1260, 1220, 1110, 780, 760, 610; ^{1}H NMR (DMSO-d_6): δ 1.6 (quint., 2 H, $^3J = 7.0$ Hz, CH_2), 2.18 (s, 6 H, 2 CH_3), 2.3 (t, 2 H, $^3J = 7.0$ Hz, CH_2), 2.47 (t, 2 H, CH_2), 3.14 (q, 2 H, $^3J = 6.7$ Hz, CH_2), 3.75 (s, 3 H, pyrrole-CH_3), 3.98 (t, 2 H, $^3J = 7.6$ Hz, CH_2), 5.57 (d, 1 H, $^4J = 7.7$ Hz, uracil-H-5), 6.61 (d, 1 H, $^4J = 1.5$ Hz, pyrrole-H-3), 7.07 (s, 1 H, pyrrole-H-5), 7.41 (d, 1 H, $^3J = 7.6$ Hz, uracil-H-6), 8.08 (t, 1 H, $^3J = 5.3$ Hz, NH), 9.85 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 27.16 (s), 33.78 (s), 36.24 (p), 36.6 (s), 37.16 (s), 45.16 (2 p), 57.14 (s), 100.09 (t), 103.62 (t), 117.85 (t), 122.04 (q), 123.25 (q), 140.91 (t), 151.56 (q), 161.43 (q), 163.3 (q), 167.15 (q); FD-MS: m/z 392 [M^+], Anal. $C_{18}H_{26}N_6O_4$.

4.1.12.4. *N*2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-3-pyrrolyl]-1-methyl-4-{[3-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)propanoyl]amino}-1*H*-2-pyrrole carboxamide (**27**)

Yellow solid (138 mg, 0.27 mmol) (27%), m.p. >250 °C, IR (KBr, cm^{-1}): 3380, 2900, 1680, 1620, 1550, 1500, 1420, 1370, 1080, 780, 750, 600; ^{1}H NMR (DMSO-d_6): δ 1.67 (quint., 2 H, $^3J = 7.3$ Hz, CH_2), 2.32 (s, 6 H, 2 CH_3), 2.5 (2 t, 4 H, 2 CH_2), 3.18 (q, 2 H, $^3J = 6.6$ Hz, CH_2), 3.78 (s, 3 H, pyrrole-CH_3), 3.81 (s, 3 H, pyrrole-CH_3), 4.01 (t, 2 H, $^3J = 7.6$ Hz, CH_2), 5.58 (d, 1 H, $^3J = 7.7$ Hz, uracil-H-5), 6.83 (2 d, 2 H, 2 pyrrole-H), 7.13 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-3′), 7.17 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-5′), 7.42 (d, 1 H, $^3J = 7.7$ Hz, uracil-H-6), 7.94 (s, 1 H, NH), 8.1 (t, 1 H, NH), 9.87 (s, 1 H, NH), 9.9 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 26.67 (s), 30.1 (s), 33.8 (s), 36.26 (p), 36.61 (p), 36.85 (s), 44.55 (2 p), 56.64 (s), 100.1 (t), 104.32 (t), 104.54 (t), 118.17 (t), 118.51 (t), 121.99 (q), 122.27 (q), 122.38 (q), 123.00 (q), 140.94 (t), 151.58 (q), 158.68 (q), 161.7 (q), 163.32 (q), 167.31 (q); FD-MS: m/z 513 [M^+], Anal. $C_{24}H_{32}N_8O_5$.

4.1.12.5. *N*2-[3-(Dimethylamino)propyl]-1-methyl-4-{[3-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)propanoyl]amino}-1*H*-2-pyrrole carboxamide (**28**)

Yellow solid (101 mg, 0.25 mmol) (25%), m.p. 130−140 °C; IR (KBr, cm^{-1}): 3230, 2900, 2680, 2420, 1680, 1610, 1500, 1420, 1360, 1260, 1180, 1100, 750, 600; ^{1}H NMR (DMSO-d_6): δ 1.62 (quint., 2 H, $^3J = 7$ Hz, CH_2), 1.76 (s, 3 H, thymine-CH_3), 2.2 (s, 6 H, 2 CH_3), 2.34 (t, 2 H, $^3J = 7.1$ Hz, CH_2), 2.46 (t, 2H, $^3J = 7.3$ Hz, CH_2), 3.17 (q, 2 H, $^3J = 5.6$ Hz, CH_2), 3.77 (s, 3 H, pyrrole-CH_3), 4.1 (t, 2 H, $^3J = 7.8$ Hz, CH_2), 6.64 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-3), 7.07 (d, 1 H, $^4J = 1.5$ Hz, pyrrole-H-5), 7.3 (s, 1 H, thymine-H), 8.11 (t, 1 H, NH), 9.84 (s, 1 H, NH), 10.9 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 12.76 (p), 26.32 (s), 33.79 (s), 36.28 (p), 36.63 (p), 36.84 (s), 44.17 (2 p), 56.34 (s), 103.83 (t), 107.47 (q), 117.98 (t), 122.09 (q), 123.12 (q), 136.69 (t), 151.44 (q), 161.59 (q), 164.00 (q), 167.26 (q); FD-MS: m/z 406 [M^+], Anal. $C_{19}H_{28}N_6O_4$.

4.1.12.6. *N*2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-3-pyrrolyl]-1-methyl-4-{[3-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)propanoyl]amino}-1*H*-2-pyrrole carboxamide (**29**)

Yellow solid (190 mg, 0.36 mmol) (36%), m.p. 180−190 °C; IR (KBr, cm^{-1}): 3380, 3250, 3100, 2820, 2660, 1640, 1620, 1520, 1480, 1420, 1370,

1260, 1150, 800, 620; ^{1}H NMR (DMSO-d_6): δ 1.61 (quint., 2 H, $^3J = 5.9$ Hz, CH_2), 1.77 (s, 3 H, thymine-CH_3), 2.2 (s, 6 H, 2 CH_3), 2.31 (t, 2 H, $^3J = 6.4$ Hz, CH_2), 3.16 (q, 2 H, CH_2), 3.78 (s, 3 H, pyrrole-CH_3), 3.81 (s, 3 H, pyrrole-CH_3), 4.03 (t, 2 H, $^3J = 6.4$ Hz, CH_2), 6.81 (d, 1 H, pyrrole-H-3), 6.84 (d, 1 H, pyrrole-H-5), 7.14 (d, 1 H, pyrrole-H-3′), 7.17 (d, 1 H, pyrrole-H-5′), 7.31 (s, 1 H, thymine-H), 8.08 (t, 1 H, NH), 9.86 (s, 1 H, NH), 9.9 (s, 1 H, NH), 10.9 (s, 1 H, thymine-NH); ^{13}C NMR (DMSO-d_6): δ 12.77 (p), 27.15 (s), 33.83 (s), 36.24 (p), 36.4 (p), 36.85 (s), 37.15 (s), 45.1 (2 p), 57.07 (s), 104.29 (t), 104.35 (t), 107.48 (q), 118.1 (t), 118.51 (t), 122.0 (q), 122.25 (q), 122.36 (q), 123.04 (q), 136.84 (t), 151.42 (q), 158.66 (q), 161.51 (q), 164.0 (q), 167.31 (q); FD-MS: m/z 528 [M$^+$], Anal. $C_{25}H_{34}N_8O_5$.

4.1.12.7. *N*2-[3-(Dimethylamino)propyl]-1-methyl-4-{[4-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoyl]amino}-1*H*-2-pyrrole carboxamide (**22**)

Yellow solid (142 mg, 0.35 mmol) (35%), m.p. >250 °C, IR (KBr, cm^{-1}): 3410, 1660, 1620, 1520, 1450, 1390, 1280, 1110, 800, 620; ^{1}H NMR (DMSO-d_6): δ 1.63 (quint., 2 H, $^3J = 7.2$ Hz, CH_2), 1.84 (quint., 2 H, $^3J = 7.2$ Hz, CH_2), 2.22 (t, 2 H, CH_2), 2.26 (s, 6 H, 2 CH_3), 2.4 (t, 2 H, $^3J = 7.0$ Hz, CH_2), 3.15 (q, 2 H, CH_2), 3.67 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 3.75 (s, 3 H, pyrrole-CH_3), 5.53 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-5), 6.62 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-3), 7.06 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-5), 7.61 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-6), 8.07 (t, 1 H, $^3J = 5.3$ Hz, NH), 9.78 (s, 1 H, NH), 11.23 (s, 1 H, uracil-NH); ^{13}C NMR (DMSO-d_6): δ 24.97 (s), 27.12 (s), 32.61 (s), 36.24 (p), 37.0 (s), 45.01 (2 p), 47.42 (s), 56.85 (s), 101.21 (t), 103.62 (t), 117.82 (t), 122.18 (q), 123.21 (q), 145.96 (t), 151.25 (q), 161.46 (q), 164.05 (q), 168.79 (q); FD-MS: m/z 405 [M$^+$], Anal. $C_{19}H_{28}N_6O_4$.

4.1.12.8. *N*2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-3-pyrrolyl]-1-methyl-4-{[4-(2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoyl]amino}-1*H*-2-pyrrole carboxamide (**31**)

Yellow solid (173 mg, 0.33 mmol) (33%), m.p. >250 °C, IR (KBr, cm^{-1}): 3420, 1660, 1560, 1530, 1450, 1420, 1380, 1340, 1250, 1090, 800, 770, 610; ^{1}H NMR (DMSO-d_6): δ 1.7 (quint., 2 H, $^3J = 7.1$ Hz, CH_2), 1.86 (quint., 2 H, $^3J = 7.2$ Hz, CH_2), 2.24 (t, 2 H, $^3J = 7.4$ Hz, CH_2), 2.39 (s, 6 H, 2 CH_3), 2.57 (t, 2 H, $^3J = 7.3$ Hz, CH_2), 3.18 (q, 2 H, $^3J = 6.4$ Hz, CH_2), 3.7 (t, 2 H, $^3J = 6.8$ Hz, CH_2), 3.78 (s, 3 H, pyrrole-CH_3), 3.8 (s, 3 H, pyrrole-CH_3), 5.54 (d, 1 H, $^3J = 7.8$ Hz, uracil-H-5), 6.83 (s, 2 H, 2 pyrrole-H), 7.13 (d, 1 H, $^4J = 1.4$ Hz, pyrrole-H-3′), 7.16 (d, 1 H, $^4J = 1.5$ Hz, pyrrole-H-5′), 7.63 (d, 1 H, $^3J = 7.9$ Hz, uracil-H-6), 8.11 (t, 1 H, $^3J = 5.3$ Hz, NH), 9.85 (s, 2 H, 2 NH), 11.24 (d, 1 H, $^4J = 0.6$ Hz, uracil-NH); ^{13}C NMR (DMSO-d_6): δ 25.01 (s), 26.32 (s), 32.64 (s), 36.28 (p), 36.38 (p), 36.64 (s), 44.14 (2 p), 47.44 (s), 56.31 (s), 101.22 (t), 104.25 (t), 104.53 (t), 118.22 (t), 118.45 (t), 122.24 (q), 122.38 (q), 123.01 (q), 123.09 (q), 145.98 (t), 151.27 (q), 158.69 (q), 161.68 (q), 164.09 (q), 168.87 (q); FD-MS m/z 528 [M$^+$], Anal. $C_{25}H_{34}N_8O_5$.

4.1.12.9. *N*2-[3-(Dimethylamino)propyl]-1-methyl-4-{[4-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoyl]amino}-1*H*-2-pyrrole carboxamide (**32**)

Yellow solid (188 mg, 0.45 mmol) (45%), m.p. 105 °C; IR (KBr, cm^{-1}): 3400, 2920, 1650, 1520, 1450, 1440, 1390, 1340, 1270, 1200, 1110, 720, 610; ^{1}H NMR (DMSO-d_6): δ 1.6 (quint., 2 H, $^3J = 7.4$ Hz, CH_2), 1.72 (s, 3 H, thymine-CH_3), 1.84 (quint., 2 H, $^3J = 7.2$ Hz, CH_2), 2.2 (t, 2 H, $^3J = 6.6$ Hz, CH_2), 2.3 (s, 6 H, 2 CH_3), 2.5 (t, 2 H, CH_2), 3.2 (q, 2 H, $^3J = 6.3$ Hz, CH_2), 3.65 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 3.75 (s, 3 H, pyrrole-CH_3), 6.63 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-3), 7.05 (d, 1 H, $^4J = 1.45$ Hz, pyrrole-H-5), 7.5 (d, 1 H, thymine-H), 8.05 (t, 1 H, $^3J = 5.5$ Hz, NH), 9.8 (s, 1 H, NH), 11.2 (s, 1 H, thymine-NH); ^{13}C NMR (DMSO-d_6): δ 12.26 (p), 24.99 (s), 26.77 (s), 32.71 (s), 36.24 (p), 36.92 (s), 44.7 (2 p), 47.18 (s), 55.21 (s), 103.65 (t), 108.78 (q), 117.83 (t), 122.22 (q), 123.19 (q), 141.67 (t), 151.21 (q), 161.5 (q), 164.62 (q), 168.8 (q); FD-MS: m/z 420 [M$^+$], Anal. $C_{20}H_{30}N_6O_4$.

4.1.12.10. *N*2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-3-pyrrolyl]-1-methyl-4-{[4-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydro-1-pyrimidinyl)butanoyl]amino}-1*H*-2-pyrrole carboxamide (**33**)

Yellow solid (200 mg, 0.37 mmol) (37%), m.p. 170–175 °C; IR (KBr, cm^{-1}): 3380, 2880, 1650, 1600, 1550, 1500, 1440, 1410, 1380, 1340, 1260, 1180, 1100, 1040, 700, 590; ^{1}H NMR (DMSO-d_6): δ 1.6 (quint., 2 H, $^3J = 7.1$ Hz, CH_2), 1.73 (s, 3 H, thymine-CH_3), 1.85 (quint., 2 H, $^3J = 7.2$ Hz, CH_2), 2.2 (s, 6 H, 2 CH_3), 2.2 (t, 2 H, CH_2), 2.3 (t, 2 H, $^3J = 6$ Hz, CH_2), 3.2 (q, 2 H, $^3J = 6.3$ Hz, CH_2), 3.65 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 3.78 (s, 3 H, pyrrole-CH_3), 3.8 (s, 3 H, pyrrole-CH_3), 6.8 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-3), 6.83 (d, 1 H, $^4J = 1.9$ Hz, pyrrole-H-5), 7.13 (d, 1 H, $^4J = 1.4$ Hz, pyrrole-H-3′), 7.16 (d, 1 H, $^4J = 1.6$ Hz, pyrrole-H-5′), 7.5 (s, 1 H, thymine-H), 8.1 (t, 1 H, $^3J = 5.5$ Hz, NH), 9.84 (d, 1 H, $^4J = 1.3$ Hz, NH), 11.2 (s, 1 H, thymine-NH); ^{13}C NMR (DMSO-d_6): δ 12.26 (p), 25.02 (s), 27.1 (s), 32.72 (s), 36.24 (p), 36.37 (p), 37.13 (s), 45.05 (2 p), 47.19 (s), 57.04 (s), 104.21 (t), 104.38 (t), 108.83 (q), 118.11 (t), 118.41 (t), 122.25 (q), 122.36 (q), 123.05 (q), 123.25 (q), 141.72 (t), 151.24 (q), 158.67 (q), 161.56 (q), 164.65 (q), 168.89 (q); FD-MS: m/z 542 [M$^+$], Anal. $C_{26}H_{36}N_8O_5$.

4.1.13. General procedure for the synthesis of adenine- pyrrole and bis-pyrrole carboxamides

Carboxylic acid (1.3 mmol) was suspended in 40 ml DMF and stirred fast at room temperature. Pyridinium chloride (789 mg, 6.89 mmol) was dissolved in 20 ml DMF, added to the suspension and the mixture was stirred for a few more minutes. Then the resulting 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. After that 1 mmol of the pyrrole amine (prepared from the nitropyrrole analogous by hydrogenation in DMF over Pd on charcoal (10%)) and 1 mmol (129 mg) *N,N*-diisopropylethylamine were added. The mixture was stirred at room temperature in the dark. After 24 h a large quantity of chloroform was added to the mixture, the organic layer was washed with 10% aqueous NaOH and saturated aqueous NaCl and dried over Na_2SO_4. The solvents were evaporated under reduced pressure and the residue was chromatographed on silica gel (MeOH−NH_3 (25%, 97 : 3)).

4.1.13.1. *N*2-[3-(Dimethylamino)propyl]-4-{[(3-(6-amino-9*H*-9-purinyl)propanoyl]amino}-1-methyl -1*H*-2-pyrrole carboxamide (**34**)

Yellow solid (125 mg, 0.30 mmol) (30%), m.p. 235 °C, IR (KBr, cm^{-1}): 3380, 2910, 2700, 2070, 1620, 1510, 1420, 1390, 1100, 790, 760, 710, 610; ^{1}H NMR (DMSO-d_6): δ 1.69 (quint., 2 H, $^3J = 7.0$ Hz, CH_2), 2.45 (s, 6 H, 2 CH_3), 2.65 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 2.84 (t, 2 H, $^3J = 6.5$ Hz, CH_2), 3.17 (q, 2 H, $^3J = 6.0$ Hz, CH_2), 3.75 (s, 3 H, pyrrole-CH_3), 4.39 (t, 2 H, $^3J = 6.5$ Hz, CH_2), 6.62 (d, 1 H, pyrrole-H-3), 7.06 (d, 1 H, $^4J = 1.1$ Hz, pyrrole-H-5), 7.19 (s, 2 H, NH_2), 8.0 (s, 1 H, adenine-H-8), 8.1 (t, 1 H, $^3J = 5.2$ Hz, NH), 8.14 (s, 1 H, adenine H-2), 9.88 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 26.19 (s), 35.48 (s), 36.28 (p), 36.54 (s), 40.1 (s), 44.01 (2 p), 56.20 (s), 103.73 (t), 117.94 (t), 119.02 (q), 121.87 (q), 123.16 (q), 141.26 (t), 149.72 (q), 152.68 (t), 156.23 (q), 161.56 (q), 166.90 (q); FD-MS: m/z 415 [M$^+$], Anal. $C_{19}H_{27}N_9O_2$.

4.1.13.2. *N*2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-3-pyrrolyl]-4-{[3-(6-amino-9H-9-purinyl)propanoyl]amino}-1-methyl-1*H*-2-pyrrole carboxamide (**35**)

Yellow solid (100 mg, 0.19 mmol) (19%), m.p.145 °C, IR (KBr, cm^{-1}): 3380, 3350, 2880, 2700, 2050, 1620, 1500, 1440, 1400, 1370, 1100, 790, 750, 600; ^{1}H NMR (DMSO-d_6): δ 1.68 (quint., 2 H, $^3J = 7.1$ Hz, CH_2), 2.38 (s, 6 H, 2 CH_3), 2.6 (t, 2 H, $^3J = 6.9$ Hz, CH_2), 2.86 (t, 2 H, $^3J = 6.4$ Hz, CH_2), 3.18 (q, 2 H, $^3J = 6.0$ Hz, CH_2), 3.78 (s, 3 H, pyrrole-CH_3), 3.8 (s, 3 H, pyrrole-CH_3), 4.4 (t, 2 H, $^3J = 6.3$ Hz, CH_2), 6.8 (d, 1 H, $^4J = 1.7$ Hz, pyrrole-H-3), 6.83 (d, 1 H, $^4J = 1.6$ Hz, pyrrole-H-5), 7.12 (d, 1 H, $^4J = 1.5$ Hz, pyrrole-H-3′), 7.15 (d, 1 H, $^4J = 1.4$ Hz, pyrrole-H-5′), 7.19 (s, 2 H, NH_2), 8.0 (s, 1 H, adenine-H-8), 8.1 (t, 1 H, $^3J = 3.0$ Hz, NH), 8.15 (s, 1 H, adenine-H-2), 9.84 (s, 1 H, NH), 9.93 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 26.18 (s), 35.44 (s), 36.56 (p), 36.62 (p), 36.84 (s), 40.1 (s), 44.1 (2 p), 57.1 (s), 104.01 (t), 104.45 (t), 117.8 (t), 118.01 (t), 118.56 (q), 121.89 (q), 122.47 (q), 123.06 (q), 123.15 (q), 141.2 (t), 149.81 (q), 152.74 (t), 156.23 (q), 159.7 (q), 161.3 (q), 166.91 (q); FD-MS m/z 537 [M$^+$], Anal. $C_{25}H_{33}N_{11}O_3$.

4.1.13.3. *N*2-[3-(Dimethylamino)propyl]-4-{[(4-(6-amino-9*H*-9-purinyl)butanoyl]amino}-1-methyl-1*H*-2-pyrrole carboxamide (**36**)

Yellow solid (160 mg, 0.37 mmol) (37%), m.p. 201 °C, IR (KBr, cm^{-1}): 3380, 2910, 2700, 2070, 1620, 1510, 1450, 1420, 1380, 1100, 785, 760, 710, 640, 610; ^{1}H NMR (DMSO-d_6); δ 1.67 (quint., 2 H, $^3J = 7.0$ Hz, CH_2), 2.09 (quint., 2 H, $^3J = 6.5$ Hz, CH_2), 2.2 (t, 2 H, $^3J = 7.4$ Hz, CH_2), 2.39 (s, 6 H, 2 CH_3), 2.57 (t, 2 H, $^3J = 6.8$ Hz, CH_2), 3.18 (q, 2 H, $^3J = 6.0$ Hz, CH_2), 3.75 (s, 3 H, pyrrole-CH_3), 4.16 (t, 2 H, $^3J = 6.4$ Hz, CH_2), 6.63 (s, 1 H, pyrrole-H-3), 7.07 (s, 1 H, pyrrole-H-5), 7.2 (s, 2 H, NH_2), 8.08 (t, 1 H, $^3J = 5.6$ Hz, NH), 8.13 (s, 2 H, adenine H-8+H-2), 9.79 (s, 1 H, NH); ^{13}C NMR (DMSO-d_6): δ 26.04 (s), 26.37 (s), 32.82 (s), 36.26 (p), 36.64 (s), 42.84 (s), 44.21 (2 p), 56.37 (s), 103.74 (t), 117.89 (t), 119.06 (q), 122.19 (q), 123.10 (q), 141.14 (t), 149.88 (q), 152.69 (t), 156.28 (q), 161.60 (q), 168.69 (q); FD-MS: m/z 429 [M$^+$], Anal. $C_{20}H_{29}N_9O_2$.

4.1.13.4. *N*2-[5-({[3-(Dimethylamino)propyl]amino}carbonyl)-1-methyl-1*H*-3-pyrrolyl]-4-{[4-(6-amino-9*H*-9-purinyl)butanoyl]amino}-1-methyl-1*H*-2-pyrrole carboxamide (**37**)

Yellow solid (120 mg, 0.22 mmol) (22%), m.p. >250 °C, IR (KBr, cm^{-1}): 3380, 2900, 1620, 1580, 1560, 1520, 1450, 1420, 1390, 1250, 1190, 1130, 1110, 1090, 1050, 790, 760, 640, 610; ^{1}H NMR (DMSO-d_6): δ 1.66 (quint., 2 H, $^3J = 7.0$ Hz, CH_2), 2.1 (quint., 2 H, $^3J = 6.9$ Hz, CH_2), 2.21 (t, 2 H, $^3J = 7.4$ Hz, CH_2), 2.32 (s, 6 H, 2 CH_3), 3.19 (t + q, 4 H, 2 CH_2), 3.78 (s, 3 H, pyrrole-CH_3), 3.8 (s, 3 H, pyrrole-CH_3), 4.17 (t, 2 H, $^3J = 6.6$ Hz, CH_2), 6.83 (s, 2 H, NH_2), 7.13 (d, 1 H, $^4J = 1.4$ Hz, pyrrole-H-3), 7.16 (d, 1 H, $^4J = 1.4$ Hz, pyrrole-H-5), 7.21 (s, 2 H, pyrrole-H-3′ + 5′), 8.09 (t, 1 H, $^3J = 5.9$ Hz, NH), 8.14 (s, 2 H, adenine H-8 + H-2), 9.85 (s, 2 H,

2 NH); ^{13}C NMR (DMSO-d_6): δ 26.05 (s), 26.7 (s), 32.84 (s), 36.26 (p), 36.39 (p), 36.84 (s), 42.86 (s), 44.57 (2 p), 56.65 (s), 104.22 (t), 104.45 (t), 118.16 (t), 118.43 (t), 119.08 (q), 122.22 (q), 122.38 (q), 123.03 (q), 123.15 (q), 141.13 (t), 149.9 (q), 152.7 (t), 156.29 (q), 158.67 (q), 161.62 (q), 168.76 (q); FD-MS: m/z 552 [M^+], Anal. $C_{26}H_{35}N_{11}O_3$.

4.2. DNA-binding methods and topoisomerase I inhibition

According to Bourdouxhe-Housiaux et al. (1996).

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References

Anthoney DA, Twelves C (2001) DNA: Still a target worth aimining at? – a review of new DNA-interactive agents. Am J Pharmacogenomics 1: 67–81.

Bailly C (1998), Advances in DNA Sequence Specific Agents 3: 97–115.

Bailly C, Chaires JB (1998) Sequence-specific DNA minor groove binders. Design and synthesis of netropsin and distamycin analogues. Bioconj Chem 9: 513–538.

Bailly C (2000) Topoisomerase I poisons and suppressors as anticancer drugs. Curr Med Chem 7: 39–58.

Bailly C (2001) Methods in Enzymology, Vol. 340, Academic Press.

Baird EE, Dervan PB (1996) Solid phase synthesis of polyamides containing imidazole and pyrrole amino acids. J Am Chem Soc 118: 6141–6146.

Bischoff G, Hoffmann S (2002) DNA-binding of drugs used in medicinal therapies. Curr Med Chem 9: 321–348.

Bourdouxhe-Housiaux C, Colson P, Houssier C, Waring MJ, Bailly C (1996) Interaction of a DNA-threading netropsin-amsacrine combilexin with DNA and chromatin. Biochemistry 35: 4251–4264.

Demeunynck M, Bailly C, Wilson WD (2003) Small Molecule DNA and RNA Binders, Wiley VCH, Weinheim, Germany.

Diederichsen U (1998) Oligomere mit intercalierenden Cytosin-Cytosin$^+$-Basenpaaren und Peptidrückgrat: Analoga des DNA-i-Motivs. Angew Chem 110: 2395–2397.

Ferrer E, Shevchenko A, Eritja R (2000) Synthesis and hybridization properties of DNA-PNA chimeras carrying 5-bromouracil and 5-methylcytosine. Bioorg Med Chem 8: 291–297.

Fox KR (2000) Targeting DNA with triplexes. Curr Med Chem 7: 17–37.

Gangamani BP, Kumar VA, Ganesh KN (1999) Chiral analogues of peptide nucleic acids: synthesis of 4-aminoprolyl nucleic acids and DNA complementation studies using UV/CD spectroscopy. Tetrahedron 55: 177–192.

Hisatome M, Maruyama N, Ikeda K, Furutera T, Ishikawa T, Yanakawa K (1996) Synthesis and some spectroscopic properties of porphyrin derivatives connected with nucleobases (adenine, thymine, guanine and cytosine) by alkanamide chains. Chem Pharm Bull 44: 1808–1811.

Homepage National Cancer Institute: http//dtp.nci.nih.gov

Hotzel C, Marotto A, Pindur U (2002) Design, synthesis, DNA-binding and cytotoxicity evaluation of new potential combilexines. Eur J Med Chem 37: 367–378.

Hotzel C, Marotto A, Pindur U (2003) New propylamine oligopyrrole carboxamides linked to a heterocyclic or anthraquinone system: synthesis, DNA binding, topoisomerase I inhibition and cytotoxicity. Eur J Med Chem 38: 189–197.

Hurley LH (2002) DNA and its associated processes as targets for cancer therapy. Nature com 2: 188–200.

Jones J (1999) Synthese von Aminosäuren und Peptiden, Wiley-VCH Verlag Weinheim.

Krotz AH, Larsen S, Burchardt O, Eriksson M, Nielsen PE (1998) A 'Retro-Inverso' PNA: structural implications for DNA and RNA binding. Bioorg Med Chem 6: 1983–1992.

Lemster T, Pindur U (2002) Design synthesis and biological/biophysical evaluation of new oligopyrrole carboxamides, biscarbazoles, oxocarbazoles and benzo[*a*]carbazoles: Antitumour and antioxidative compounds. Recent Res Devel Org Bioorg Chem 5: 99–115 and ref. therein.

Marotto A, Kim YS, Pindur U (2002) New Indolocarbazoles as proteinkinase-C-inhibitors and antitumour active compounds. Pharmazie 57: 124–127.

Nielsen PE (1997) Design of sequence-specific DNA-binding ligands. Chem Eur J 3: 505–508.

Nielsen PE, Haaima G (1997) Peptide nucleic acid (PNA). A DNA mimic with a pseudopeptide backbone. Chem Soc Rev: 73–78.

Nielsen PE (2001) Targeting double stranded DNA with peptide nucleic acid (PNA). Curr Med Chem 8: 545–550.

Pindur U, Fischer G (1996) DNA Complexing minor groove-binding ligands: perspectives in antitumour and antimicrobial drug design. Curr Med Chem 3: 379–406.

Pindur U, Lemster T (1998) Antitumour drug design: DNA-binding ligands, which inhibit the topoisomerase I. Pharmazie 53: 79–86.

Pindur U (2001) Recent Advances in biologically active DNA Groove Binding agents and DNA recognition. Curr Med. Chem, special issue 8: 475–551.