Electronic Supplementary Information

Metal binding properties of pyrimidinophanes and their acyclic counterparts

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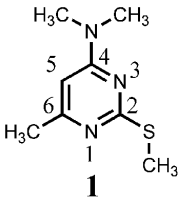
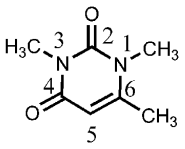
The structural formulae of the investigated compounds 1-8

Pyrimidine units

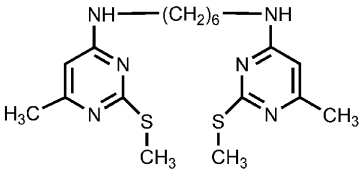


1



2

Podands



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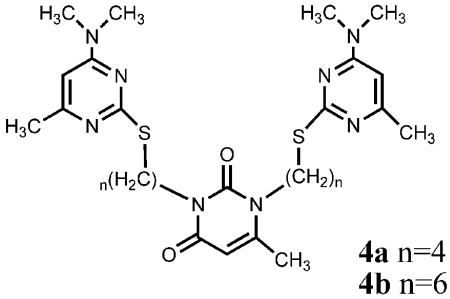
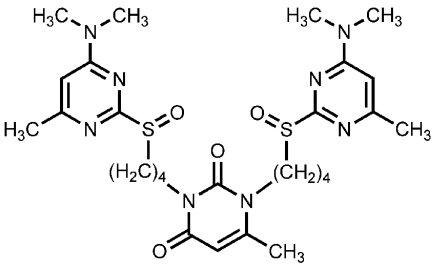


4a n=4
4b n=6



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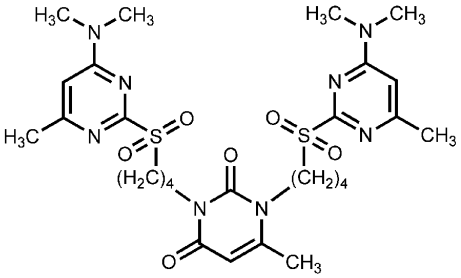
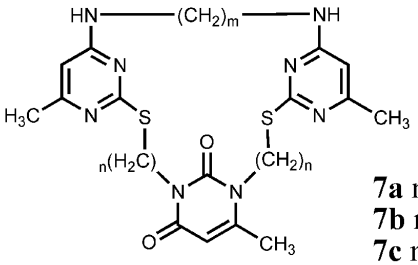
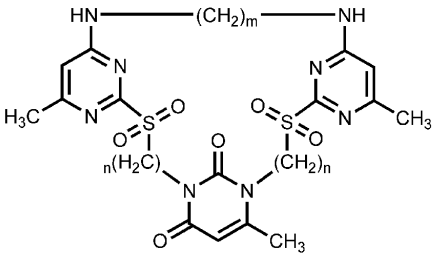


6

Pyrimidinophanes



7a m=3, n=6
7b m=5, n=6
7c m=7, n=6
7d m=4, n=4
7e m=6, n=4



8 m=6, n=4

Experimental extraction conditions

The $CHCl_3$ was saturated with H_2O before the use to prevent volume changes during the extraction. Aqueous metal picrate solutions (5 ml) buffered at 7.3 pH and the solutions of extractant (5 ml, 2.5×10^{-5} to 1×10^{-3} M) in $CHCl_3$ were magnetically stirred in a flask. The extraction equilibrium was reached after vigorous stirring for 1.5 h at 25°C. Then two phases were allowed to settle for 1 h and afterwards separated by centrifugation. The absorbances A_1 of aqueous phase after extraction and A_0 of aqueous phase before extraction were measured at λ_{max} = 355 nm (the wavelength of maximum absorption of the picrate ion). All data were obtained from three independent experiments. The aqueous metal picrate solutions ([metal salt] = 1×10^{-2} M; [picric acid] = 2.5×10^{-4} M) were prepared by stepwise addition of the 2.5×10^{-4} M aqueous picric acid solution to the calculated amounts of metal salts. The obtained solutions were stirred at pH 7.3 with using tris(hydroxymethyl)aminomethane-HNO_3 (0.05 M) as a buffer. The percent of extraction was calculated as a ratio $E\% = 100\times(A_0-A_1)/A_0 = \alpha\times100\%$. $E\%$ uncertainties are generally $\leq 2\%$.

To evaluate the stoichiometry of the complex formation with Ag^+ the Job's method was applied. Series of isomolar solutions containing various mole amounts of picric acid and ligand (their total concentration $2.5\cdot10^{-4}$ M) and constant amount of metal ions ($1\cdot10^{-2}$ M) were prepared. The obtained solutions were stirred in the buffer with pH 7.3 for 1 h, the water layer was separated and spectrophotometrically measured at λ_{max} = 355 nm. The maximum at the plot dependence of AgPic content in organic layer versus its initial mole fraction in water indicates the ratio of the stoichiometry coefficients of Ag^+ cation and ligand in the complex.

Experimental procedure of ^{1}H NMR spectroscopic titrations

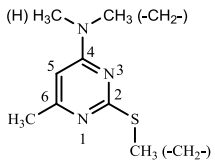
A Bruker AVANCE 600 spectrometer was used to measure the ^{1}H NMR shifts of the H protons of the receptors. NMR titrations were performed by adding aliquots of the mixture of the ligand (3mmol/l) and silver picrate AgPic·H_2O (12 mmol/l) o the solution of ligand (3 mmol/l) directly in the NMR tube. NMR ^{1}H spectra were recorded at every step of addition. The stability constants (logβ) were determined by the direct analysis of the molar ^{1}H NMR titration curves using the DynaFit software [S1].

S1. P.Kuzmic, *Anal. Biochem.*, 1996, **237**, 260.

^{1}H and ^{15}N NMR chemical shifts of 2-thiocytozine fragments in free ligands and Ag^+ complexes

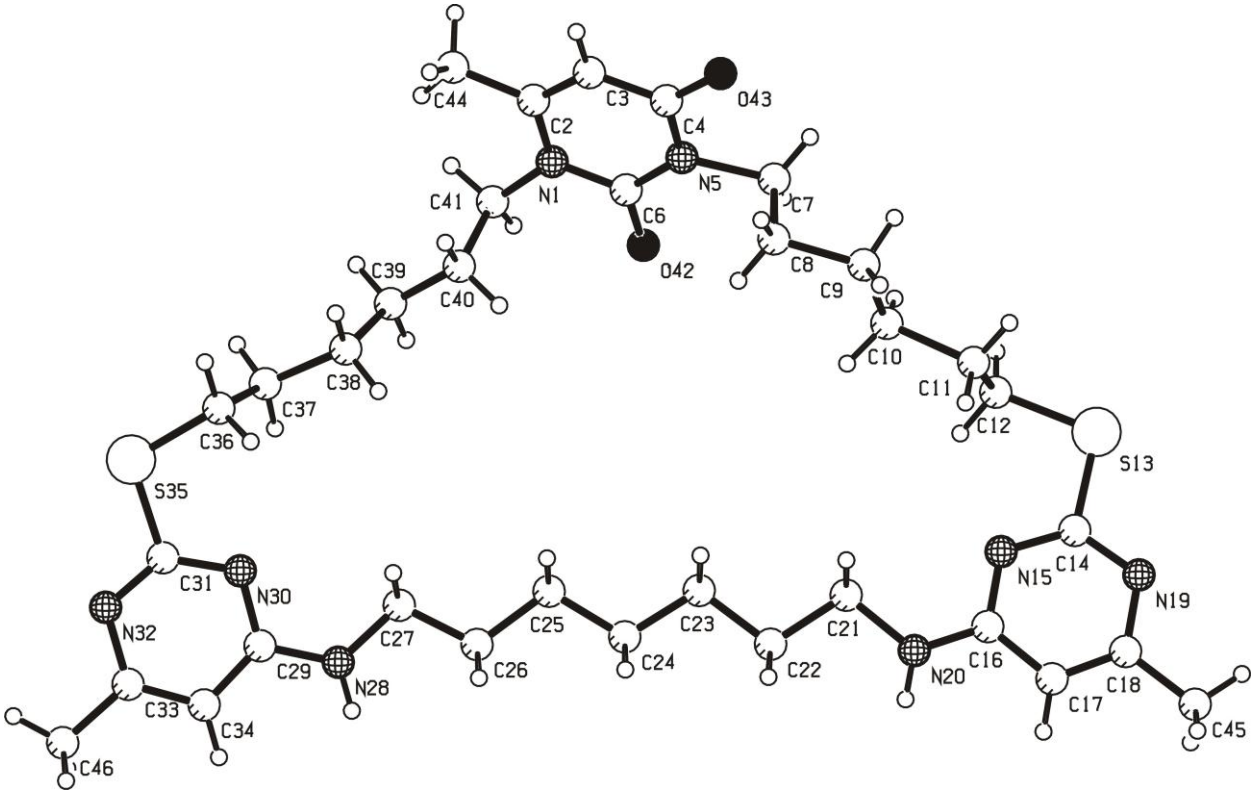
Table S1.

^{1}H and ^{15}N NMR chemical shifts (δ, ppm) of thiocytozine fragments in free ligands and Ag^+ complexes in $CDCl_3$/DMSO-d_6 = 1:1 (v/v) (CD_3OD/DMSO-d_6 = 1:1 (v/v)) at 303 K.

Compound 	Chemical shift, δ ppm							
	S-C<u>H</u>$_n$	Ar-<u>H</u>(5)	Ar-<u>CH</u>$_3$	N(4)-<u>CH</u>$_{n-}$	N(4)<u>H</u>	<u>N</u>(1)	<u>N</u>(3)	<u>N</u>(4)
1	2.39	6.02	2.17	3.02	-	244	(-)[a]	65
1+Ag^+	2.50	6.25	2.35	3.10	-	213	(-)[a]	(-)[a]
3	2.36	5.89	2.10	3.24	6.88	243	(-)[a]	86
3+Ag^+	2.43	6.05	2.23	3.34	7.71	210	(-)[a]	96
4b	2.99 (2.71)	6.03 (5.66)	2.17 1.83	3.02 2.71	-	243	228	65
4b+Ag^+	3.11 (2.83)	6.32 (5.92)	2.37 (2.03)	3.13 (2.78)	-	210	210	(-)[a]
7a	2.95	5.90	2.10	3.32	6.96	(-)[b]	(-)[b]	(-)[b]
7a+Ag^+	3.04	6.04	2.22	3.46	7.56	(-)[b]	(-)[b]	(-)[b]
7b	2.94	5.89	2.09	3.27	6.93	(-)[b]	(-)[b]	(-)[b]
7b+Ag^+	3.34	6.05	2.23	3.03	7.67	(-)[b]	(-)[b]	(-)[b]
7c	2.94	5.88	2.08	3.23	6.93	(-)[b]	(-)[b]	(-)[b]
7c+Ag^+	3.01	6.04	2.20	3.31	7.58	(-)[b]	(-)[b]	(-)[b]

[a] No signals for some nuclei were obtained
[b] No experimental data were obtained

Geometry of the pyrimidinophane 7c in the crystal and the numbering scheme



Determination of the minimal inhibitory concentrations of AgPic and its complexes with pyrimidinic ligands.

The in vitro antibacterial and antifungal activity of the synthesized compounds were investigated against *Pseudomonas aeruginosa* 9027, *Escherichia coli* F-50, *Staphylococcus aureus* 209p, *Bacillus subtilis* 6633 and yeast *Candida Albicans* 885-653. The antibacterial and antifungal assays were performed in nutrient broth (bacteria 3×10^5 cfu/mL) and Sabouraud dextrose broth (fungi $2\times10^{3\text{-}4}$ cfu/mL). Positive growth control and standard drug controls were also run simultaneously. The MICs were defined as the lowest concentrations that showed no growth and recorded by visual observation in every 24 h during 5 days for bacteria and after incubation during 14 days for fungi.

The bactericidal and fungicidal activity was determined as follows. Assay tubes were filled with 1 mL of test compound solution in nutrient agar. Concentrations of test compounds were varied from 12.5 to 10^4 μg/mL. Normal saline broth (bacteria 3×10^5 cfu/mL), 1 mL was added to the tubes and for 5 min, 10 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 4 h the inocula were prepared by transfering the broth onto petri plates containing meal-peptone agar. Petri plates were incubated at 37°C and minimum bactericidal concentration (MBC) recorded as the test compound dilution affecting total cell death. For fungicidal activity determination the tubes with the test compounds and fungi were incubated at 26°C. For 10 min, 20 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 6 h the inocula were prepared in Sabouraud dextrose broth and incubated at 26°C.

Results are presented in Table S2.

Table S2. *In vitro* antibacterial and antifungal activity AgPic and complexes of the salt with acyclic and macrocyclic pyrimidines[a]

Complexes	*Sa*	*Ba*	*Ec*	*Pa*	*Ca*
Minimal Bacteriostatic and Fungistatic Concentrations, μg/mL					
AgPic	3.9	31.3	7.8	15.6	31.3
Ag·L_{4b}·Pic	31.3	62.5	125	500	62.5
Ag·2L_1·Pic	31.3	62.5	125	500	62.5
Ag·L_3·Pic	15.6	62.5	125	500	250
Ag·L_{7a}·Pic	15.6	62.5	125	500	250
Minimal Bactericidal and Fungicidal Concentrations, μg/mL					
AgPic	>500	>500	>500	>500	>500
Ag·L_{4b}·Pic	>500	>500	>500	>500	>500
Ag·2L_1·Pic	>500	>500	>500	>500	>500
Ag·L_3·Pic	>500	>500	>500	>500	>500
Ag·L_{7a}·Pic	>500	>500	>500	>500	>500

[a] The tests were performed in duplicate and repeated twice; Pa, *Pseudomonas aeruginosa;* Ec, *Escherichia coli*; Sa, *Staphylococcus aureus;* Ba, *Bacillus subtilis*; Ca, *Candida Albicans.*

Minimal bacteriostatic concentrations (MBC) of the salt against bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* have values of 3.9, 31.3 and 7.8 μg/mL, respectively, minimal fungistatic concentrations (MFC)