SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF COMPOUNDS CONTAINING BOTH THIOPYRAN (THIAPYRYLIUM) AND PYRROLIDONE CYCLES

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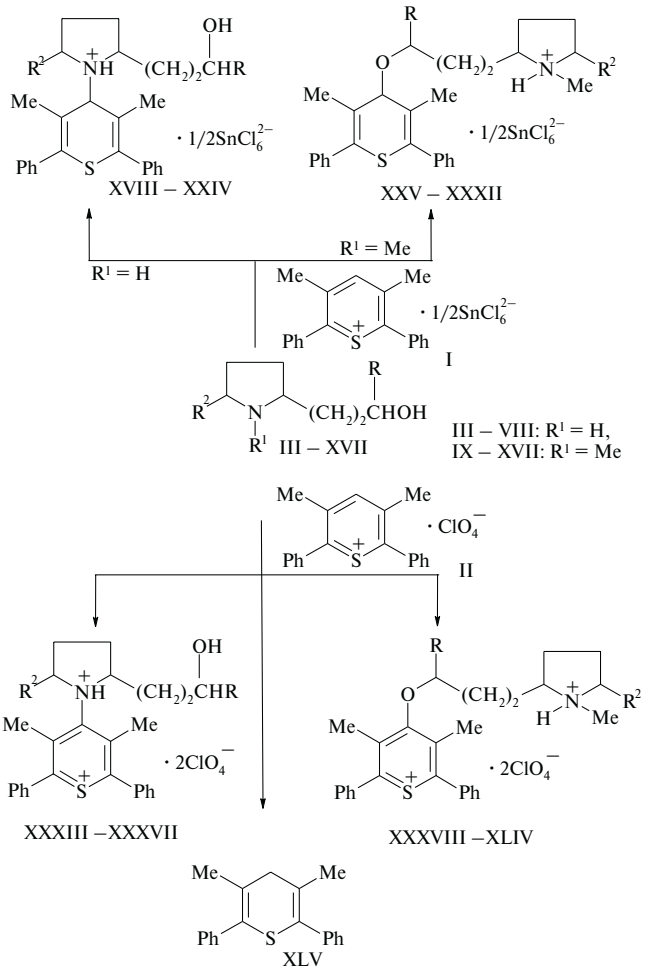
Previously we reported on the search for new cholinesterase inhibitors among hydroxyalkyl pyrrolidines [1]. In continuation of our systematic investigations devoted to the synthesis of biologically active compounds based on heterocyclic amines, we studied the interaction of hydroxyalkyl pyrrolidines and furfurylamines with thiapyrylium salts unsubstituted at the γ-position. This study was inspired by data reported previously on the antimicrobial activity of thiapyrylium salts [2] and acyl derivatives of the furan and pyrrolidine series [3]. In this context, the interaction of thiapyrylium salts with mono- and heterocyclic binucleophilic molecules seems to offer a promising pathway to the synthesis of compounds possessing antimicrobial properties.

Substrates for the modification were 3,5-dimethyl-2,6-diphenylthiapyrylium salts (I, II), in which the site for the preferential nucleophilic attack was determined by a substituent-free position 4 in the thiapyrylium cycle. The anion character was selected so as to obtain a salt that can be readily isolated from the reaction mixture in the form of stable nonhygroscopic crystals. Heterocyclic reagents III – VIII and XLVI, XLVII differed in the number and character of nucleophilic centers and, for hydroxyalkylpyrrolidines, in the character of substitution, geometry, and absolute configuration. The reactions of pyrrolidylalkanols with thiapyrylium salts were conducted in anhydrous dioxane at a temperature in the range from 10 to 60°C (Scheme 1).

It was found that the character of the nucleophilic attack and the reaction type were determined both by the reagent structure and by the nature of the substrate counterion. In the case of amino alcohols with tertiary amino groups, the nucleophilic attack is performed by a spatially open oxygen atom, whereby the tertiary nitrogen atom shielded by alkyl groups plays the role of an organic base. The NH-containing reagents unsubstituted with respect to nitrogen interact due to a stronger nucleophilic center. In this case, thiapyrylium hexachlorostannates always enter (irrespective of the nucleophile character) into the nucleophilic attachment reaction, which is probably related to a poor solubility of γ-thiopyrans XVIII – XXXII under the experimental conditions studied.

Scheme 1



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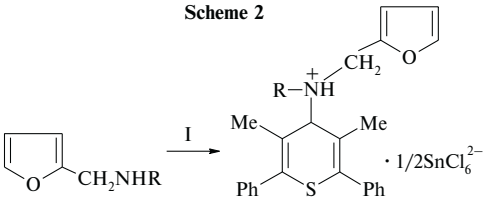
III, XVIII, XXXIII: R = R^2 = H; IV, XIX, XXXIV: R = H, R^2 = Me-*cis*; V, XX, XXXV: R = H, R^2 = Me-*trans*; VI, XXI: R = H, R^2 = *iso*-C_4H_9-*cis*; VII, XXII: R = H, R^2 = *iso*-C_4H_9-*trans*; VIII, XXIII, XXXVI: R = H, R^2 = Ph-*cis*; IX, XI, XXIV, XXVI, XXXVII, XXXIX: R = H, R^2 = Me-*cis*; X, XXV, XXXVIII: R = R^2 =H; XII, XXVII, XL: R = H, R^2 = Me-*cis*(+)2*S*,5*R*; XIII, XXVIII, XLI: R = H, R^2 = Me-*cis*(–)2*R*,5*S*; XIV, XXIX, XLII: R = Me, R^2 = Me-*cis*; XV, XXX, XLIII: R = H, R^2 = *iso*-C_4H_9-*cis*; XVI, XXXI: R = H, R^2 = *iso*-C_4H_9-*trans*; XVII, XXXII, XLIV: R = H, R^2 = Me-*cis*.

As a result, the latter compounds were isolated from the reaction mixture with a yield of up to 90%. Stabilized by the ClO_4^- anion, the soluble products of the nucleophilic attachment reaction are subject to a deeper conversion. As a result of the intermolecular hydride transfer these products are converted by the initial perchlorate into substituted thiapyrylium salts XXXIII – XLIV with a yield of 25 – 65%. The role of the hydride ion acceptor is apparently played by the initial thiapyrylium perchlorate I, which is confirmed by the formation of a side product – 3,5-dimethyl-2,6-diphenyl-4H-thiopyran (XLV), isolated at an amount corresponding to the reaction stoichiometry.

In order to study the possible dependence of antimicrobial properties on the nature of substituents in the γ-position of a sulfite heterocycle, we conducted reactions between hexachlorostannate I and furfurylamines XLVI and XLVII. As a result, we obtained furfurylammonium-substituted thiopyrans XLVIII and XLIX (Scheme 2).

Scheme 2



XLVI, XLVII XLVIII, XLIX

XLVI, XLVIII: R = H; XLVII, XLIX: R = Me.

The proposed structure of compounds XVIII – XLIV, XLVIII, and XLIX were confirmed by the data of elemental analyses and by the results of IR, UV, and ^{1}H NMR measurements. The IR spectra of compounds XVIII – XXXII, XLVIII, and XLIX display the characteristic absorption bands due to skeletal vibrations of γ-thiopyrans in the region of 1630 – 1680 cm^{-1}. The presence of thiapyrylium cations in compounds XXXIII – XLIV was confirmed by medium-intensity bands in the region of 1530 – 1595 cm^{-1}; ClO_4^- ions were manifested by intense absorption bands at 1060 – 1100 cm^{-1}.

The presence of an associated hydroxy group in compounds XVIII – XXIV and XXXIII – XLVII was confirmed by intense absorption in the region of 3200 – 3400 cm^{-1} (ν_{OH}). The spectra of thiopyrans XXV – XXXII have absorption bands in the regions of 1060 – 1085 and 1180 – 1200 cm^{-1} characteristic of the stretching vibrations of –O–C bonds. In addition, the spectra of compounds XVIII – XLIV, XLVIII, and XLIX display a group of bands in the region of 2250 – 2750 cm^{-1} attributed to ν_{NH^+} in the salts of alkylated amines.

The IR absorption bands due to the =C–O–C fragment are observed in the regions of 1270 – 1295 and 1000 – 1050 cm^{-1}. The presence of phenyl substituents and the aliphatic chain in compounds XVIII – XLIV, XLVIII, and XLIX is manifested by absorption bands in the regions of 3000 – 3100 cm^{-1} ($\nu_{=CH}$), 700 – 760 cm^{-1} ($\delta_{=CH}$), 2700 – 2940 cm^{-1} ($\nu^{as}_{CH_3CH_2}$, $\nu^{s}_{CH_3CH_2}$, ν_{NCH_3}), and 1385 – 1470 cm^{-1} ($\delta_{CH_3CH_2}$).

The UV absorption spectra of perchlorates XXXIII – XLII exhibit two bands peaked at 248 – 250 nm (log ε = 4.42 – 4.54) and 380 – 383 nm (log ε = 4.24 – 4.31). The band maxima in the spectra of hexachlorostannates XVIII – XXXII and XLIII, XLVIII, and XLIX occur in the regions of 245 – 255 nm (log ε = 4.00 – 4.15) and 370 – 390 nm (log ε = 3.80).

The ^{1}H NMR spectra were measured only for pyrrolidyl-containing thiapyrylium salts XXXIII – XLIV, which is explained by limited solubility of the γ-substituted thiopyrans XVIII – XXXII. The spectra of perchlorates XXXIII – XLIV contain a singlet with the chemical shift δ = 2.28 – 2.30 ppm corresponding to six protons of CH_3 groups and a multiplet at 7.20 – 7.40 ppm belonging to ten protons of the aromatic substituents. The resonance of protons in the N–CH_3 groups takes place at 2.60 – 2.70 ppm. A multiplet signal in the region of 3.10 – 3.81 ppm is attributed to protons of the aliphatic chain of a substituent in the γ-position of the thiapyrylium cycle.

It was established that all the substituted thiopyrans and thiapyrylium salts studied exhibit a pronounced selective effect with respect to *Staphylococcus aureus* 209p (Table 1). It must be pointed out that the antistaphylococcal activity of the products of nucleophilic reactions of thiapyrylium salts with both methylated hydroxypropylpyrrolidines and those unsubstituted at the nitrogen atom exceeded the activity of the initial thiapyrylium salts [2].

The antimicrobial and antifungal effect of γ-substituted thiopyrans increases on the passage from furfurylammonium-containing (XLVIII, XLIX) to pyrrolidinium-containing (XVIII, XIX, XXIII – XXX) compounds. The *cis*-isomer salts (XXI, XXX) are more active than the corresponding *trans* analogs (XXII, XXXI). The bacteriostatic effect was most pronounced in hydroxyalkylpyrrolidines containing an isobutyl radical in position 5 of the pyrrolidine cycle (XXI, XXX). The introduction of phenyl substituents in the same position of the heterocycle (XXIII, XXXII, XXXIV, XLIV) leads to a decrease in the antimicrobial activity.

Thiopyrans XVIII, XIX, and XXI and thiapyrylium salts XXXIII and XXXV containing free hydroxy groups exhibit, in addition to a high antistaphylococcal activity, a significant antifungal effect with a minimum inhibiting concentrations (MIC) ranging within 1.56 – 6.00 μg/ml. The presence of a

TABLE 1. The Antimicrobial Activity and Acute Toxicity of the Initial Salts I, II and the Functionally Substituted Thiopyrans and Thiapyrylium Salts XVIII – XLIX

Compound	MIC, μg/ml					LD_{50}, mg/kg
	St. aureus 209p	*E. coli* 675	*Pr. vulgaris* 38	*Ps. aeruginosa* 165	*C. albicans* 45	
XVIII	1.50	50.00	25.00	25.00	1.50	…
XIX	6.00	50.00	25.00	25.00	1.50	265
XX	12.00	100.00	50.00	50.00	12.00	…
XXI	1.50	50.00	25.00	25.00	0.78	…
XXII	12.00	100.00	50.00	50.00	12.00	…
XXIII	6.00	50.00	25.00	25.00	6.00	…
XXIV	6.00	50.00	25.00	25.00	25.00	…
XXV	3.00	50.00	25.00	25.00	25.00	…
XXVI	6.00	50.00	25.00	25.00	12.00	265
XXVII	1.50	50.00	25.00	25.00	6.00	221
XXVIII	3.00	50.00	25.00	25.00	6.00	332
XXIX	6.00	50.00	25.00	25.00	25.00	…
XXX	1.56	50.00	25.00	25.00	12.00	150
XXXI	12.00	100.00	50.00	50.00	12.00	…
XXXII	6.00	50.00	25.00	25.00	12.00	…
XXXIII	1.50	50.00	25.00	25.00	1.50	…
XXXIV	1.50	50.00	25.00	25.00	1.50	…
XXXV	12.00	100.00	50.00	50.00	12.00	200
XXXVI	6.00	50.00	25.00	25.00	6.00	…
XXXVII	3.00	50.00	25.00	25.00	25.00	…
XXXVIII	1.56	50.00	25.00	25.00	25.00	…
XXXIX	3.00	50.00	25.00	25.00	25.00	200
XL	1.50	50.00	25.00	25.00	6.00	180
XLI	1.50	50.00	25.00	25.00	6.00	290
XLII	3.00	50.00	25.00	25.00	25.00	160
XLIII	0.78	50.00	25.00	25.00	6.00	150
XLIV	6.00	50.00	25.00	25.00	25.00	155
XLVIII	12.00	100.00	50.00	50.00	12.00	…
XLIX	12.00	100.00	50.00	50.00	12.00	…
Erythromycin	0.01 – 20	…	…	…	…	…
Streptomycin	5.0 – 25	…	…	…	…	…
Levomycetin	1.0 – 10	…	…	…	…	…
Neomycin	0.5 – 200	…	…	…	…	…
Monomycin	0.8 – 10	…	…	…	…	…
Nistatin	–	…	…	…	3.1	…
Levorin	–	…	…	…	3.1	…
Amphotericin B	–	…	…	…	0.5 – 3.7	…
I	12.00	…	…	…	50.00	…
II	25.00	…	…	…	50.00	…

secondary carbon atom at oxygen in compounds XXIV, XXIX, XXXVII, and XLII leads to a two- to tenfold decrease in the activity with respect to *Candida albicans* 45. Compounds based on the enantiomers (XXVII, XXVIII, XL, XLI) were more active when compared to the corresponding racemic derivatives (XXVI, XXXIX).

A comparative analysis of the antimicrobial properties of the synthesized compounds and widely used reference drugs showed that the antistaphylococcal activity of the functionally substituted (γ-position) thiopyrans (XVIII, XXI, XXVII, XXX) and thiapyrylium salts (XXXIII, XXXIV, XXXVIII, XL, XLI, XLIII) is comparable with that of ampicillin, streptomycin, neomycin, erythromycin, and other well-known antibiotics [4]. The fungistatic activity of some ammonium-substituted thiopyrans (XVII, XIX, XXI) and thiapyrylium salts (XXXIII, XXXIV) exceeded that of nistatin, levorin, and amphotericin.

Thus, the antimicrobial activity of the functionally substituted thiopyrans and thiapyrylium salts, as well as the stereospecific biological action observed in the series of compounds studied, indicate that these substances are worthy of further investigation as potential drugs.

EXPERIMENTAL CHEMICAL PART

The IR absorption spectra were obtained using an UR-20 spectrophotometer (Germany) using samples suspended in Vaseline oil or hexachlorobutadiene. The UV spectra were recorded on a Specord M-40 spectrophotometer (Germany) using samples dissolved in dichloroethane at a concentration of $10^{-2} - 10^{-4}$ M. The ^{1}H NMR spectra were measured at 20 – 25°C on an 80-MHz Varian FT-8A spectrometer (USA) using $CDCl_3$ as the solvent and TMS as the internal standard.

The initial thiapyrylium salts I and II were obtained using a method described in [2]; 2-hydroxypropylpyrrolidines III – XVII and furfurylamines XLVI and XLVII were synthesized by the well-known methods as reported in [5, 6].

Bis-[*cis*-5-methyl-1-(3,5-dimethyl-2,6-diphenyl-4H-4-thiopyranyl)-2-(3-hydroxypropyl)pyrrolidinium] hexachlorostannate (XIX). To a suspension of 3.6 g (4 mmole) of bis-(3,5-dimethyl-2,6-diphenylthiapyrylium) hexachlorostannate (I) in 50 ml of anhydrous dioxane was gradually (over 1 h) added a solution of 1.26 g (8 mmole) of 3-(*cis*-5-methyl-2-pyrrolidyl)propan-1-ol (IV) in 10 ml of the same solvent and the reaction mixture was stirred for 30 min at 10 – 15°C. The precipitated colorless crystals were separated by filtration, washed with ether, and reprecipitated from DMF with ether to obtain 3.2 g (70%) of compound XIX.

Compounds XVIII, XX – XXIV, XLVIII, and XLIX were obtained similarly, proceeding from the corresponding pyrrolidylpropanols and furfurylamines.

Bis-[1-methyl-2-(3,5-dimethyl-2,6-diphenyl-4H-4-thiopyranylhydroxypropyl)pyrrolidinium] hexachlorostannate (XXV). To a suspension of 3 g (3.4 mmole) of hexachlorostannate I in 60 ml of anhydrous dioxane was added a solution of 0.96 g (6.8 mmole) of 3-(1-methyl-2-pyrrolidyl)-propan-1-ol (X) in 10 ml of the same solvent. The reaction mixture was stirred for 4 h at 40 – 50°C and allowed to stand at room temperature for 8 – 10 h. The precipitated crystals were separated by filtration and washed with ether to obtain 3.2 g (89%) of compound XXV.

A similar procedure was used to obtain compounds XXVI – XXXII.

3,5-Dimethyl-2,6-diphenyl-4-(*cis*-5-methyl-2-hydroxypropyl-1-pyrrolidinium)thiapyrylium diperchlorate (XXXIV). To a suspension of 3.2 g (8.6 mmole) of 3,5-dimethyl-2,6-diphenylthiapyrylium perchlorate (II) in 60 ml of anhydrous dioxane was added a solution of 0.6 g (4 mmole) of pyrrolidylpropanol IV in 10 ml of the same solvent and the reaction mixture was kept for 2 h at 10 – 15°C. Then 5 drops of 70% aqueous $HClO_4$ were added and the reaction mixture was stirred for 30 min. The precipitated oil was separated and triturated with ether. Finally, the product was recrystallized from petroleum ether to obtain 2.24 g (51%) of compound XXXIV.

A similar method was used to obtain perchlorates XXXIII and XXXV – XXXVII.

3,5-Dimethyl-2,6-diphenyl-4-[3-(1-methyl-2-pyrrolidinium)propyloxy]thiapyrylium diperchlorate (XXXVIII). To a suspension of 3 g (7.8 mmole) of perchlorate II in 20 ml of anhydrous dioxane was added with stirring a solution of 0.56 g (3.9 mmole) of pyrrolidylpropanol X in 10 ml of the same solvent and the reaction mixture was stirred for 4 h at 55 – 60°C. Then 8 drops of 70% aqueous $HClO_4$ were added and the reaction mixture was allowed to stand at room temperature until crystalline product ceased to precipitate. Then 100 ml of anhydrous ether were added and the precipitated crystals were separated by filtration and recrystallized from dichloroethane to obtain 2.8 g (61%) of compound XXXVII.

A similar method was used to obtain diperchlorates XXXIX – XLIV (Table 2).

TABLE 2. Yields and Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula
XVIII	70	250 – 253	$C_{52}H_{64}Cl_6N_2O_2S_2Sn$
XIX	70	179 – 180	$C_{54}H_{68}Cl_6N_2O_2S_2Sn$
XX	84	158 – 160	$C_{54}H_{68}Cl_6N_2O_2S_2Sn$
XXI	85	249 – 250	$C_{60}H_{80}Cl_6N_2O_2S_2Sn$
XXII	82	258 – 259	$C_{60}H_{80}Cl_6N_2O_2S_2Sn$
XXIII	48	214 – 216	$C_{64}H_{72}Cl_6N_2O_2S_2Sn$
XXIV	68	172 – 174	$C_{56}H_{72}Cl_6N_2O_2S_2Sn$
XXV	90	244 – 245	$C_{54}H_{68}Cl_6N_2O_2S_2Sn$
XXVI	86	216 – 219	$C_{56}H_{72}Cl_6N_2O_2S_2Sn$
XXVII	70	246 – 247	$C_{56}H_{72}Cl_6N_2O_2S_2Sn$
XXVIII	80	246 – 247	$C_{56}H_{72}Cl_6N_2O_2S_2Sn$
XXIX	65	139 – 140	$C_{58}H_{76}Cl_6N_2O_2S_2Sn$
XXX	79	247 – 250	$C_{62}H_{84}Cl_6N_2O_2S_2Sn$
XXXI	55	258 – 259	$C_{62}H_{84}Cl_6N_2O_2S_2Sn$
XXXII	55	245 – 247	$C_{66}H_{76}Cl_6N_2O_2S_2Sn$
XXXIII	40	143 – 144	$C_{26}H_{31}Cl_2NO_9S$
XXXIV	51	136 – 137	$C_{27}H_{33}Cl_2NO_9S$
XXXV	42	139 – 140	$C_{27}H_{33}Cl_2NO_9S$
XXXVI	40	140 – 142	$C_{32}H_{35}Cl_2NO_9S$
XXXVII	43	149 – 150	$C_{28}H_{35}Cl_2NO_9S$
XXXVIII	61	156 – 157	$C_{27}H_{33}Cl_2NO_9S$
XXXIX	25	138 – 139	$C_{28}H_{35}Cl_2NO_9S$
XL	58	134 – 135	$C_{28}H_{35}Cl_2NO_9S$
XLI	50	134 – 135	$C_{28}H_{35}Cl_2NO_9S$
XLII	41	122 – 123	$C_{29}H_{37}Cl_2NO_9S$
XLIII	65	158 – 159	$C_{31}H_{41}Cl_2NO_9S$
XLIV	37	139 – 141	$C_{33}H_{37}Cl_2NO_9S$
XLVIII	62	148 – 149	$C_{48}H_{48}Cl_6N_2O_2S_2Sn$
XLIX	58	150 – 151	$C_{50}H_{52}Cl_6N_2O_2S_2Sn$

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial activity of the synthesized compounds was studied by the standard method [7] of double serial dilutions in a beef-infusion broth (pH 7.2 – 7.4). The tests were performed with respect to the standard strains of *Staphylococcus aureus* 209p, *Escherichia coli* 675, *Proteus vulgaris* 38, *Pseudomonas aeruginosa* 165, and *Candida albicans* 45.

The acute toxicity (LD_{50}) upon intraperitoneal injection was studied in a group of white mongrel mice weighing 20 – 22 g by a conventional method [8].

REFERENCES

1. I. N. Klochkova, N. N. Semenova, A. A. Safonova, et al., *Khim.-Farm. Zh.*, **33**(12), 12 – 14 (1999).
2. V. G. Kharchenko, M. V. Noritsina, S. N. Chalaya, et al., *Khim.-Farm. Zh.*, **10**(1), 80 – 83 (1976).
3. I. N. Klochkova, L. K. Kulikova, M. K. Krasheninnikova, et al., *Khim.-Farm. Zh.*, **18**(8), 931 – 934 (1983).
4. S. M. Navashin and I. P. Fomina, *A Handbook on Antibiotics* [in Russian], Meditsina, Moscow (1974), pp. 99 – 238, 260 – 266.
5. M. V. Noritsina, I. N. Klochkova, and N. N. Sorokin, *Khim. Geterotsikl. Soedin.*, No. 12, 1648 – 1651 (1984).
6. I. N. Klochkova, A. V. Shlyakhtin, N. N. Semenova, et al., in: *The Chemistry and Technology of Furan Compounds. A Collection of Papers* [in Russian], Kuban State Technical University, Krasnodar (1997), Part 2, pp. 12 – 20.
7. G. N. Pershin (ed.), *Methods of Experimental Chemotherapy* [in Russian], Meditsina, Moscow (1971), pp. 100, 109 – 117.
8. I. V. Sanotskii, *Methods for Determining the Toxicity and Danger of Chemicals (Toxicometry)* [in Russian], Meditsina, Moscow (1970).