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SYNTHESIS OF NOVEL DIPYRIDO-1,4-THIAZINES[#]

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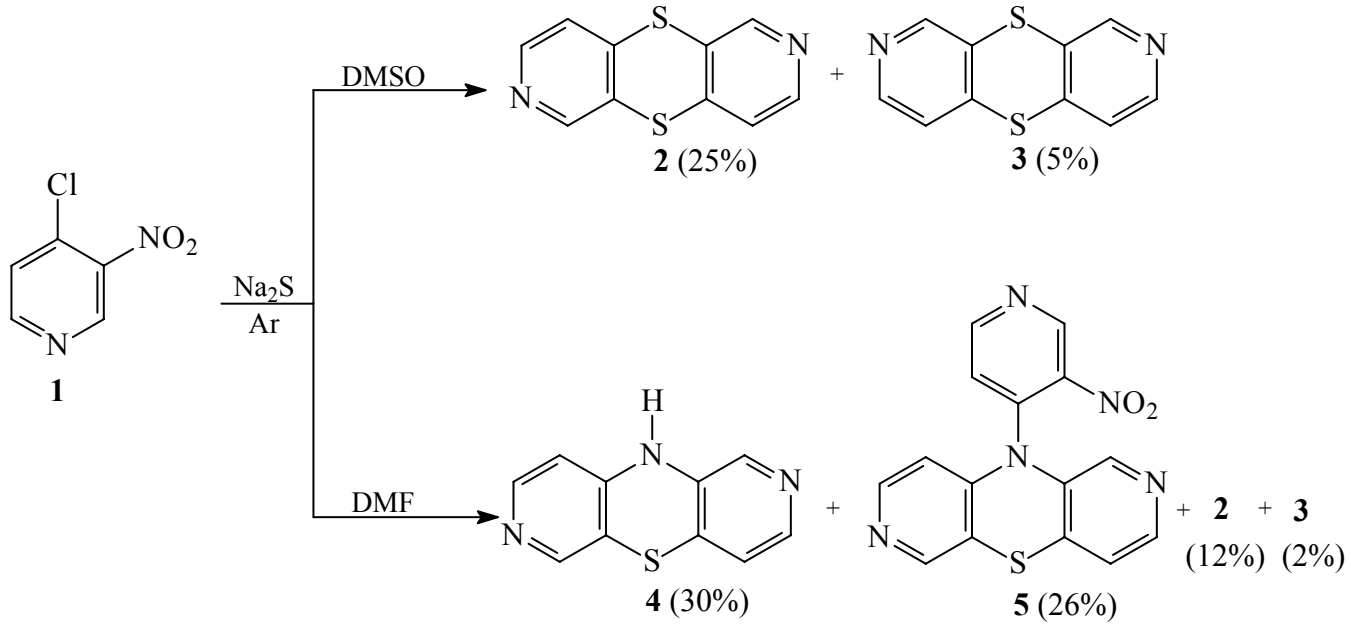
Abstract – An efficient synthesis of novel type of dipyrido-1,4-thiazine (**4**) was elaborated in the reactions of two pairs of 3,4-disubstituted pyridines in DMF. The reactions proceeded through the S→N type of the Smiles rearrangement of the resulting 4,4'-dipyridinyl sulfide. In the case of formation of 10-(3'-nitro-4'-pyridinyl)-2,7-diazaphenothiazine (**5**) double rearrangement was observed. 10*H*-2,7-diazaphenothiazine (**4**) was *N*-alkylated, *N*-arylated and *N*-heteroarylated to give 10-substituted (alkyl, arylalkyl, aryl, heteroaryl and dialkylaminoalkyl) derivatives (**5**) and (**17-29**). The NMR spectra were assigned with the help of the ^{1}H-^{1}H correlation (COSY) and NOE experiment of the methyl derivative (**17**). The crucial 10*H*-2,7-diazaphenothiazine (**4**) showed promising anticancer activity.

INTRODUCTION

Phenothiazines attract attention because of their wide chemical properties and very interesting biological activities (antipsychotic, antihistaminic, antitussive and antiemetic).[1] Recent reports have focused interests on anticancer, antiplasmid and antibacterial activities, reversal of multidrug resistance (MDR) and potential treatment in Alzheimer's and Creutzfeldt-Jakob diseases.[2-7] Some modifications of the phenothiazine structures were directed into azaphenothiazines, where one or two benzene rings were substituted with an azine ring, most often pyridine.[1] Out of ten theoretical dipyrido-1,4-thiazines only 4 types have been known, i. e. 1,6-, 1,9-, 3,6- and 3,7-diazaphenothiazines.[8-15] It is worth noting that 3,7-diazaphenothiazines were named incorrectly in the original papers[14,15] as 2,7-diazaphenothiazines (according to Beilstein's system). In our preliminary paper we found that reaction of 4-chloro-3-nitropyridine (**1**) reacted with sodium sulfide to give isomeric dipyrido-1,4-dithiins (**2**) and (**3**) and unknown dipyrido-1,4-thiazine (**4**) being 10*H*-2,7-diazaphenothiazine and its 3-nitropyridinyl derivative (**5**), depending on the reaction conditions (DMSO or DMF, respectively, Scheme 1).[16] Improved synthesis of both dipyrido-1,4-dithiins (**2**) and (**3**), their structure determination (based on the ^{1}H and ^{13}C NMR spectra and finally confirmed by X-ray analysis) and the 1,4-dithiin ring opening reactions were discussed

in our previous paper.[17] The formation of the thiazine ring was unexpected because of a reductive action of DMF and an unprecedented the Smiles rearrangement of the S→N type of the resulting 4,4'-dipyridinyl sulfide. *Ab initio* calculations of all dipyridinyl sulfides showed possibilities of the rearrangement only for the 2,2'-, 2,3'- and 2,4'-isomers.[18] In continuation of these studies we worked out an efficient synthesis of 10*H*-2,7-diazaphenothiazine (**4**) and its 10-substituted derivatives (**5**) and **(17-29)**, possessing alkyl, arylalkyl, aryl, heteroaryl and dialkylaminoalkyl substituents.



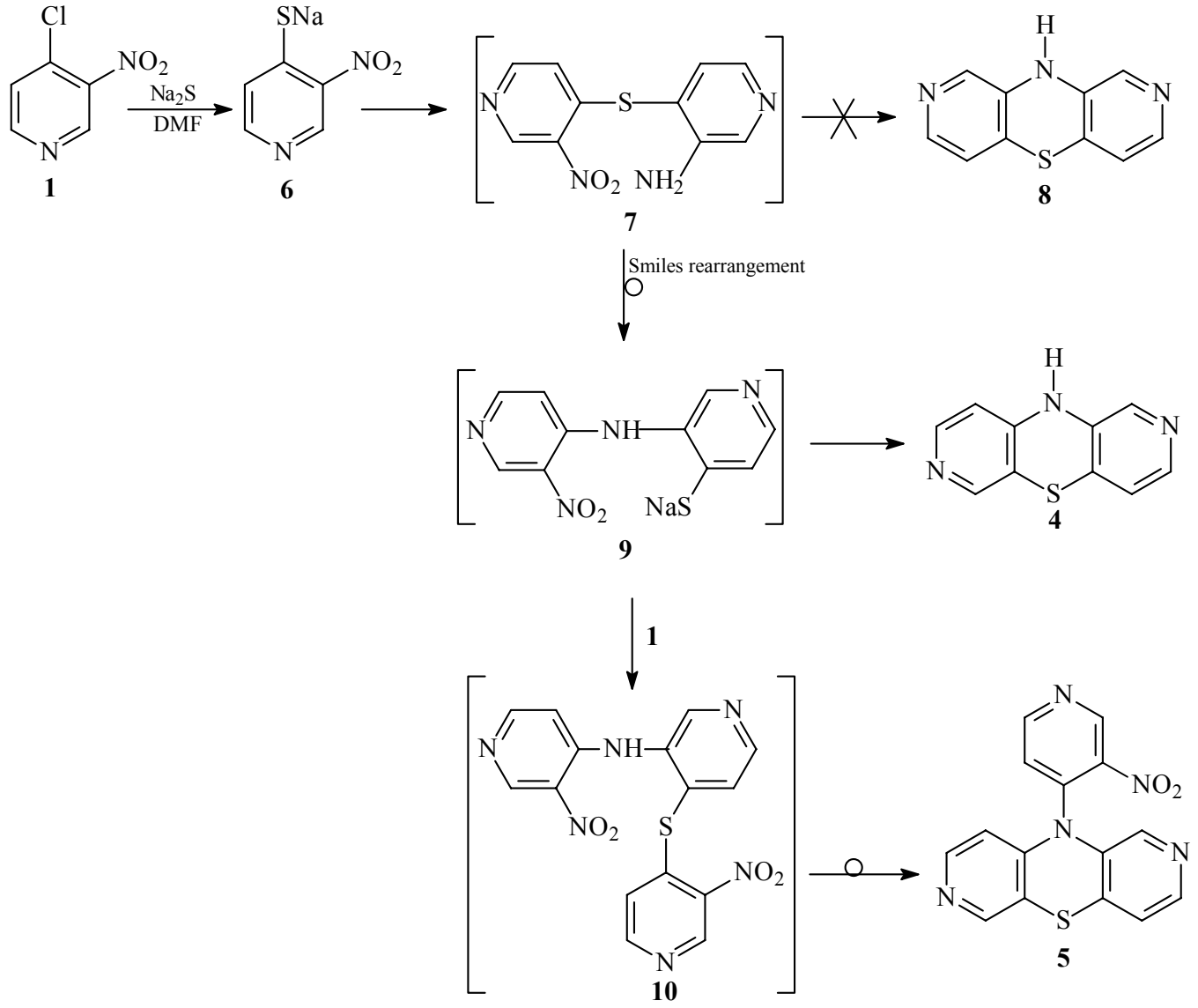
Scheme 1

RESULTS AND DISCUSSION

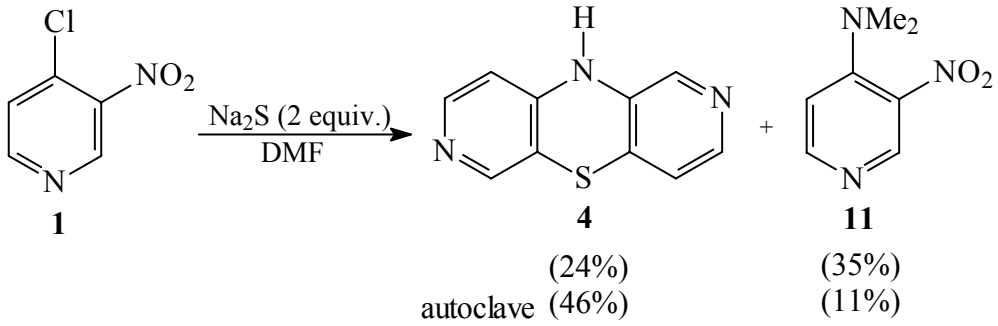
Synthesis

Taking into account a reductive action of DMF, a possible mechanism of formation of dipyridothiazines (**4**) and (**5**) is depicted on Scheme 2. 4-Chloro-3-nitropyridine (**1**) reacted with sodium sulfide giving first of all sodium 3-nitro-4-pyridinethiolate (**6**) which further reacted with substrate (**1**) under reductive action of DMF to form 3-nitro-3'-amino-4,4'-dipyridinyl sulfide (**7**). This sulfide did not undergo a cyclization to form symmetrical dipyrido-1,4-thiazine (**8**) (being 2,8-diazaphenothiazine) but underwent the Smiles rearrangement to dipyridinylamine (**9**) which cyclized to dipyridothiazine (**4**) or reacted with substrate (**1**) to give dipyridinylamine (**10**) and further dipyridothiazine (**5**), possessing three pyridine rings.

Since dipyridothiazines (**4**) and (**5**) were obtained in only 30% and 26% yields, we started to improve the reaction efficiency. To avoid the presence of both dithiins in the reaction mixture the amounts of sodium sulfide was lowered from 3 equivalents to 2. The reaction was more selective but still not efficient giving only two products, dipyridothiazine (**4**) (in 24% yield) and 4-dimethylamino-3-nitropyridine (**11**) (in 35% yield). A better yield of dipyridothiazine (**4**) (46%) was achieved when this reaction was carried in an autoclave at 170 ^{o}C (Scheme 3).



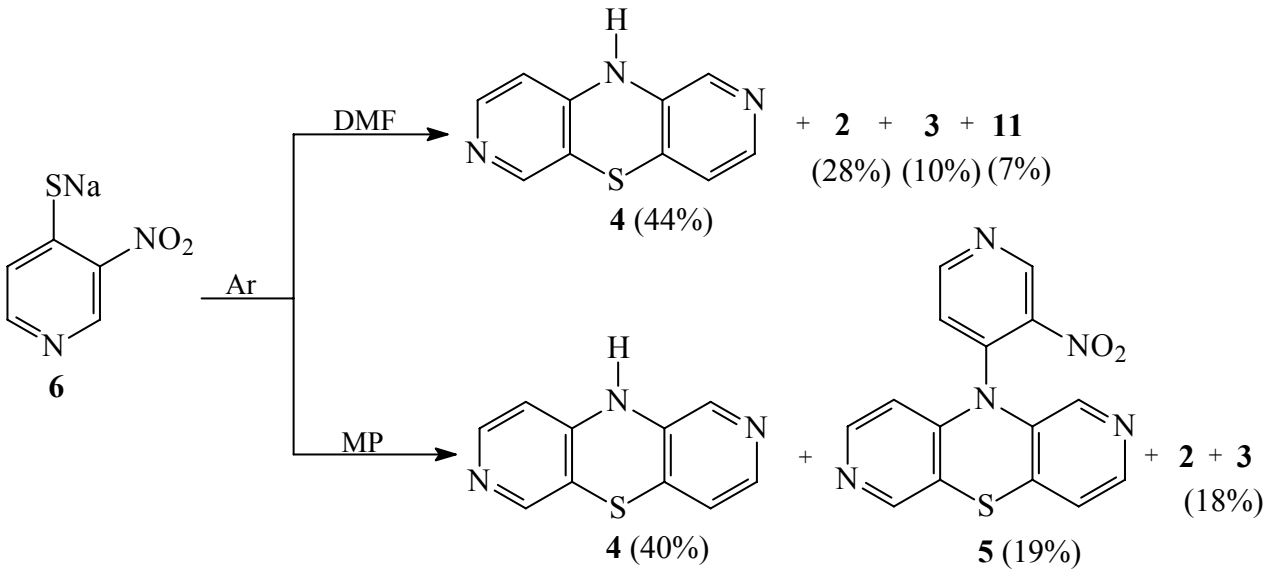
Scheme 2. A possible mechanism of the formation of dipyridothiazines (**4**) and (**5**).



Scheme 3

Next step was a check of the proposed mechanism using sodium 3-nitro-4-pyridinethiolate (**6**) (obtained from 4-mercapto-3-nitropyridine (**12**)). Heating this compound in boiling DMF led to dipyridothiazine (**4**) (in 44% yield) in the mixture of by-products: dithiins (**2**) and (**3**) and aminopyridine (**11**). Repeating this reaction at higher temperature, i. e. in boiling 1-methyl-2-pyrrolidinone (mP, 202 ^{o}C) did not bring better

results giving 2,7-diazaphenothiazines (**4**) and **5** together with dithiins (**2**) and (**3**) (Scheme 4). This reaction proves above all the formation of by-products (**2**) and (**3**) from compound (**6**) in the intermolecular process. It shows also that the formation of dipyridothiazine (**4**) is possible but in complicated process.

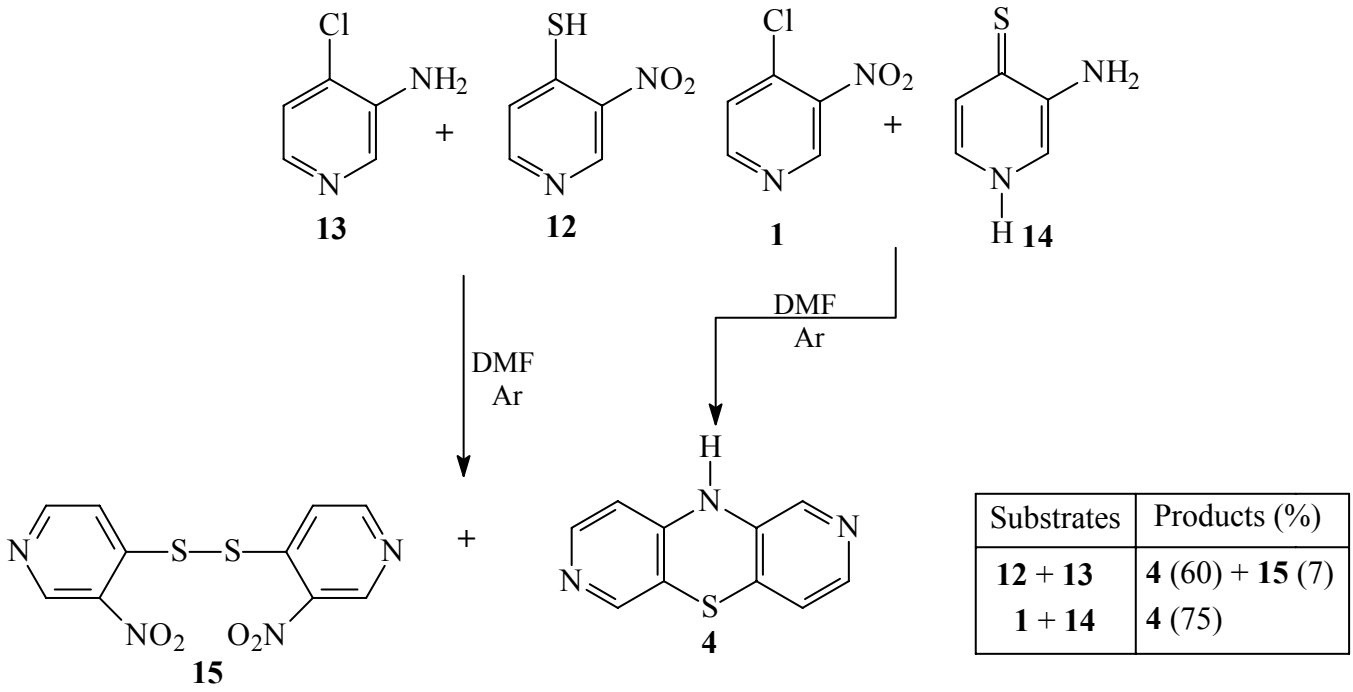


Scheme 4

Since the synthesis of 2,7-diazaphenothiazines (**4**) and (**5**) is considered to proceed through formation of sulfide (**7**), we decided to use pairs of the disubstituted pyridine substrates. Reaction of 4-mercapto-3-nitropyridine (**12**) with 3-amino-4-chloropyridine (**13**) (both obtained from substrate **1**) in DMF under argon (initially at room temperature for 24 hours to form sulfide (**7**) and next under reflux for 12 hours to ring closure) gave 2,7-diazaphenothiazine (**4**) in 60% yield and small amount of disulfide (**15**).

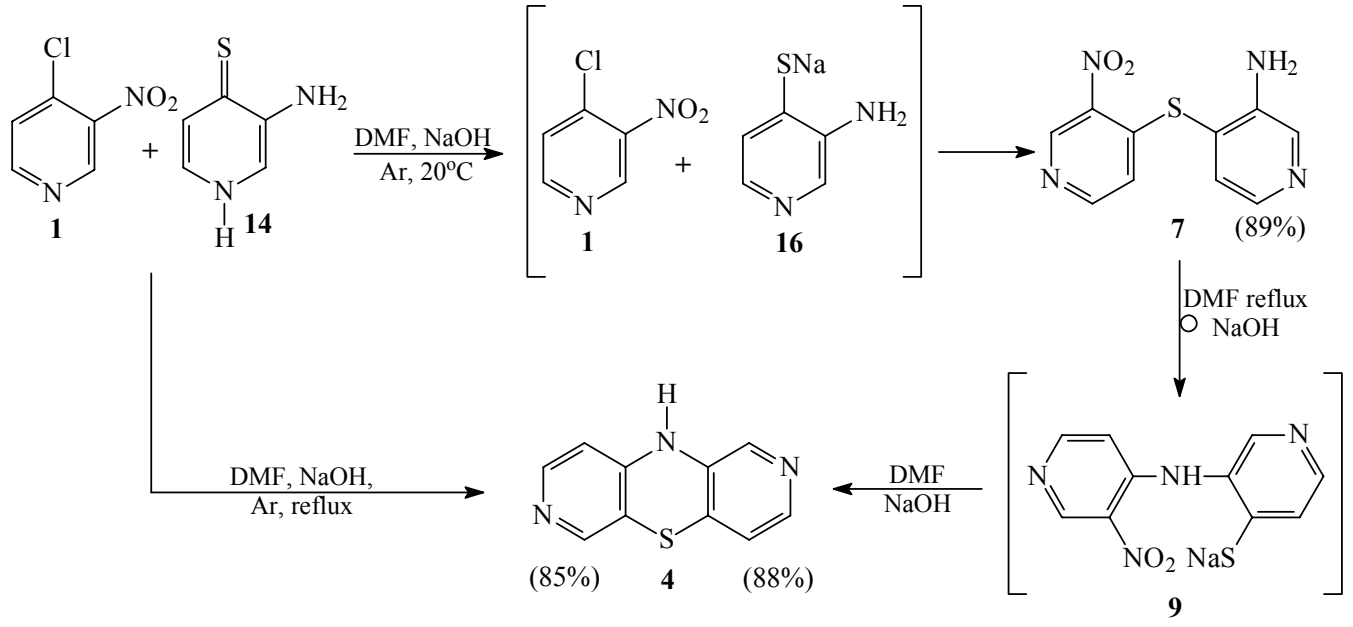
To avoid ready oxidation of compound (**12**) to disulfide (**15**), we checked another pair of substrates. Reaction of compound (**1**) with 3-amino-4(1*H*)-pyridinethione (**14**) in DMF under argon (at room temperature for 2 hours and under reflux for 12 hours) led to 2,7-diazaphenothiazine (**4**) in 75% yield (Scheme 5).

In order to overcome some inconvenience with long reaction time, we improved this reaction using sodium hydroxide which transform *in situ* 3-amino-4(*1*H)-pyridinethione (**14**) into more reactive sodium 3-amino-4-pyridinethiolate (**16**) and accelerate the rearrangement. The reaction proceeded 2 hours at room temperature and 4 hours under reflux giving the 2,7-diazaphenothiazine (**4**) in high yield (85%). When this reaction was carried only for 2 hours at room temperature sulfide (**7**) was also isolated in 89% yield. This sulfide heated in boiling DMF underwent the Smiles rearrangement followed the ring closure reaction to give compound (**4**) in 88% yield (Scheme 6).



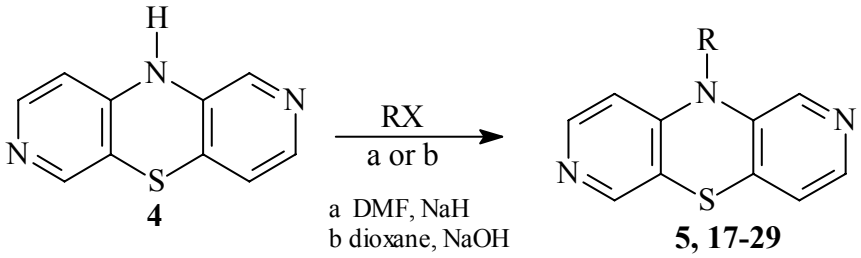
Substrates	Products (%)
12 + **13**	**4** (60) + **15** (7)
1 + **14**	**4** (75)

Scheme 5



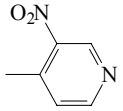
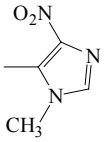
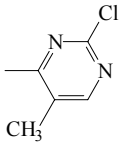
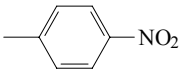
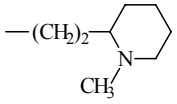
Scheme 6

Parent 10*H*-2,7-diazaphenothiazine (**4**) was transformed into various 10-substituted 2,7-diazaphenothiazines (**5**) and (**17-29**) in 58-88% yield in the reactions of *N*-alkylation and *N*-arylation with alkyl, arylalkyl, aryl and heteroaryl halides in DMF at room temperature in the presence of sodium hydride and with pharmacoactive hydrochlorides of dialkylaminoalkyl chlorides and phenacyl bromide in boiling dioxane in the presence of sodium hydroxide (Scheme 7, Table 1). *N*-Heteroarylation of 10*H*-2,7-diazaphenothiazine (**4**) with 4-chloro-3-nitropyridine (**1**) gave derivative (**5**) with higher yield than directly from the reaction of compound (**1**) with sodium sulfide.



Scheme 7

Table 1. 10-Substituted 2,7-diazaphenothiazines (**5**) and (**17-29**)

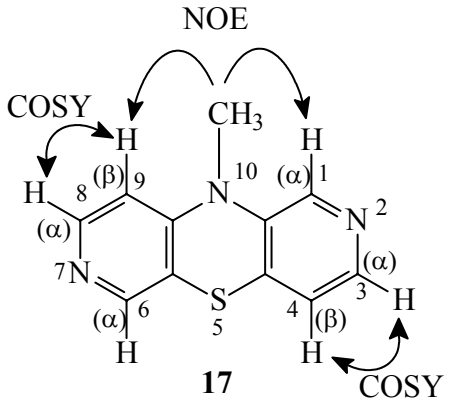
No	R	Yield (%)	No	R	Yield (%)
17	$-CH_3$	75	**5**		62
18	$-C_2H_5$	77	**24**		61
19	$-C_4H_9$	72	**25**		70
20	$-CH_2CH{=}CH_2$	69	**26**	$-(CH_2)_2N(C_2H_5)_2$	67
21	$-CH_2C_6H_5$	58	**27**	$-(CH_2)_3N(CH_3)_2$	70
22	$-CH_2COC_6H_5$	63	**28**	$-CH_2CHCH_2N(CH_3)_2$ CH_3	84
23		88	**29**		82

Physical and spectroscopic properties of 2,7-diazaphenothiazines

The synthetic routes to 10*H*-2,7-diazaphenothiazine (**4**) followed by *N*-alkylation and *N*-arylation reactions to substituted derivatives were observed by TLC analysis. All chromatograms of 2,7-diazaphenothiazines showed colour changing (from white to beige) during irradiation with UV lamp unlike to chromatograms of other compounds used and formed. Similar effect (yellow color for (**4**), (**17-22**), (**26-29**) or orange colour for (**5**), (**23-25**)) was observed when chromatograms of azaphenothiazines were sprayed with a phenothiazine detection mixture (sulfuric acid-water-ethanol 1:1:8).[19]

The ^{1}H NMR spectra of the obtained azaphenothiazines were recorded in deuteriochloroform and revealed two singlet signals and four doublet signals showing unsymmetrical structure of the

diazaphenothiazine system. Unquestionable assignment of these protons was based on homonuclear NOE experiment and homonuclear ^{1}H-^{1}H correlation (COSY) for 10-methyl derivative (**17**). Four signals at 8.05, 8.10, 8.15 and 8.29 ppm were considered as α–pyridinyl proton signals and two signals at 6.64 and 7.02 ppm as β–pyridinyl proton signals. Irradiation of the methyl protons at 3.38 ppm (Scheme 8) gave an enhancement of the singlet signal at 8.05 ppm by 3.27% (α–pyridinyl proton assigned as H-1) and doublet signal at 6.64 ppm by 3.08% (β–pyridinyl proton assigned as H-9). The second singlet signal at 8.10 ppm was assigned as H-6. The doublet signal at 8.29 ppm was assigned as H-8 because of COSY spectrum and coupling with H-9 (J = 5.6 Hz). The doublets at 8.15 ppm and 7.02 ppm with J = 4.9 Hz were considered as α– and β–pyridinyl proton signals and were assigned as protons H-3 and H-4, respectively, on the basis of COSY spectrum. Mass spectra (EI and FAB) of diazaphenothiazines (**4**), (**5**) and (**17-29**) revealed relatively high intensity of the molecular ions and some fragmentary ions. The X-ray study of nitropyridinyl derivative (**5**) confirmed the 2,7-diazaphenothiazine structure.[16]



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Scheme 8. NOE and COSY experiments in 10-methyl-2,7-diazaphenothiazine (**17**).

Table 2. Anticancer activity of 10*H*-2,7-diazaphenothiazine (**4**).

Anticancer activity	GI50 (x10^{-5}M)	TGI (x10^{-5}M)
Lung cancer HOP-62	1.43	5.74
Lung cancer HOP-92	0.85	7.66
Colon cancer COLO205	1.19	5.95
Colon cancer HCT-116	1.89	6.95
Renal cancer RXF393	1.56	4.23
Renal cancer A498	1.95	5.54
Leukemia Hl-60(TB)	2.05	6.85

GI50 – growth inhibition of 50%; TGI – total growth inhibition

All diazaphenothiazines (**4**), (**5**) and (**17-29**) show promising potential antipsychotic, antidepressant, antihistaminic, antiasthmatic, anticancer and sedative activity[20] and relatively low lipophilic character (logP = 1.58-3.07[21]) in comparison with neuroleptic phenothiazines (logP = 3.5-5.9[22]). Basic compound, 10*H*-2,7-diazaphenothiazine (**4**), was tested against 57 human cancer lines in National Cancer Institute in Bethesda showing promising activity against lung, colon and renal cancers, and leukemia.[23]

Conclusion

We report here an efficient synthesis of novel dipyrido-1,4-thiazine system. Reaction of 4-chloro-3-nitropyridine (**1**) with 3-amino-4(1*H*)-pyridinethione (**14**) in DMF in the presence of sodium hydroxide under argon proceeded through the Smiles rearrangement of the S→N type and led to 10*H*-2,7-diazaphenothiazine (**4**) in 85% yield. *N*-Alkylation and *N*-arylation of parent compound (**4**) gave fourteen 10-substituted 2,7-diazaphenothiazines (**5**) and (**17-29**) possessing alkyl, arylalkyl, dialkylaminoalkyl, aryl and heteroaryl substituents. Compound (**4**) shows significant anticancer activities.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Boetius melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Varian Unity-Inova-300 spectrometer at 300 MHz in deuteriochloroform with tetramethylsilane as the internal standard. The ^{1}H-^{1}H correlation and NOE experiment for compound (**17**) were recorded on a Bruker DRX spectrometer at 500 MHz. Electron impact (EI MS) and Fast Atom Bombardment (FAB MS, in glycerol) mass spectra were run on a Finnigan MAT 95 spectrometer at 70 eV. The thin layer chromatography were performed on silica gel 60 F_{254} (Merck 1.05735) with $CHCl_3$-EtOH (5:1 and 10:1 v/v) and on aluminum oxide 60 F_{254} neutral (type E) (Merck 1.05581) with $CHCl_3$-EtOH (10:1 v/v) as eluents.

4-Chloro-3-nitropyridine (1), 4-mercapto-3-nitropyridine (12), 3-amino-4-chloropyridine (13) and 3-amino-4(1*H*)-pyridinethione (14)

4-Chloro-3-nitropyridine (**1**) was obtained by nitration of 4(1*H*)-pyridinone with fuming nitric acid and 30% oleum to give 3-nitro-4(1*H*)-pyridinone followed by chlorination with phosphorus pentachloride and phosphorus oxychloride according to described procedures.[24,25] 3-Amino-4-chloropyridine (**13**) was obtained by reduction of 4-chloro-3-nitropyridine (**1**) with tin chloride according to described procedure.[26] 4-Mercapto-nitropyridine (**12**) was obtained from 4-chloro-3-nitropyridine (**1**) in the reaction with thiourea according to ref.[17] 3-Amino-4(1*H*)-pyridinethione (**14**) was obtained from 3-nitro-4(1*H*)-pyridinone *via* reduction followed by thionation with phosphorus pentasulfide and *via* reduction of 4-mercapto-3-nitropyridine (**12**) according to ref.[27]

Reaction of 4-chloro-3-nitropyridine (1) with sodium sulfide in DMF

A. With 3 equivalents of Na_2S

To a solution of 4-chloro-3-nitropyridine (**1**) (1.00 g, 6.3 mmol) in dry DMF (10 mL) under argon atmosphere anhydrous Na_2S (1.47 g, 18.9 mmol) was added. The reaction mixture was stirred at rt for 2 h and next was refluxed for 72 h. After cooling DMF was evaporated *in vaccuo* and the residue was extracted with $CHCl_3$ (3 x 10 mL). The extracts were washed with water, dried with anhydrous $CaCl_2$ and evaporated *in vaccuo*. The residue was purified by column chromatography (silica gel, $CHCl_3$-EtOH 10:1) to give:

a. 10*H*-2,7-Diazaphenothiazine (**4**) (0.190 g, 30%); mp 169-170 ^oC (EtOH).

^1H NMR ($CDCl_3$) δ: 6.39 (d, 1H, *J* = 5.0 Hz, H-9), 6.51 (broad s, 1H, NH), 6.82 (d, 1H, *J* = 5.0 Hz, H-4), 7.76 (s, 1H, H-1), 7.93 (s, 1H, H-6), 7.99 (d, 1H, *J* = 5.0 Hz, H-3), 8.06 (d, 1H, *J* = 5.0 Hz, H-8),. MS m/z: 201 (M^+, 100%), 174 (M-HCN, 23). Anal. Calcd for $C_{10}H_7N_3S$: C 59.68, H 3.51, N 20.88. Found: C 59.57, H 3.62, N 20.55.

b. 10-(3'-Nitro-4'pyridinyl)-2,7-diazaphenothiazine (**5**), **(**0.136 g, 26%); mp 231-232 °C (EtOH).

^1H NMR ($CDCl_3$) δ: 5.79 (d, 1H, *J* = 5.7 Hz, H-9), 6.93 (d, 1H, *J* = 5.1 Hz, H-4), 7.14 (s, 1H, H-1), 7.61 (d, 1H, *J* = 5.1 Hz, H_{arom}), 8.00 (d, 1H, *J* = 5.7 Hz, H-8), 8.06 (s+d, 2H, *J* = 5.1 Hz, H-3, H-6), 9.20 (d, 1H, *J* = 5.1 Hz, H_{arom}), 9.57 (s, 1H, H_{arom}). EI MS m/z: 323 (M^+, 100%), 277 (M-NO_2, 58), 200 (M-nitropyridinyl, 17.4). Anal. Calcd for $C_{15}H_9N_5O_2S$: C 55.72, H 2.81, N 21.66. Found: C 55.61, H 2.85, N 21.40.

c. Dipyrido-1,4-dithiin (**2**) (0.083 g, 12%); mp 174-175 ^oC (EtOH) (lit.,[17] mp 174-175 ^oC).

d. Dipyrido-1,4-dithiin (**3**) (0.014 g, 2%); mp 146-147 ^oC (EtOH) (lit.,[17] mp 146-147 ^oC).

B. With 2 equivalents of Na_2S

To a solution of 4-chloro-3-nitropyridine (**1**) (1.00 g, 6.3 mmol) in dry DMF (10 mL) under argon atmosphere anhydrous Na_2S (0.98 g, 12.6 mmol) was added. The reaction mixture was refluxed for 13 h. After cooling the reaction mixture was poured into water (30 mL) and extracted with $CHCl_3$ (3 x 10 mL). The extracts were worked up as described above and the residue was purified by column chromatography (aluminum oxide, $CHCl_3$-EtOH 10:1) to give:

a. 10*H*-2,7-Diazaphenothiazine (**4**) (0.15 g, 24%), mp 169-170 ^oC (EtOH).

b. 4-Dimethylamino-3-nitropyridine (**11**) (0.37 g, 35%), mp 48-49 °C (Et_2O) (lit.,[28] mp 48-49 °C).

C. 2 equivalents of Na_2S in autoclave at 170°C.

To a solution of 4-chloro-3-nitropyridine (**1**) (0.31 g, 1.96 mmol) in dry DMF (5 mL) under argon atmosphere in an autoclave anhydrous Na_2S (0.47 g, 6 mmol) was added. The mixture was heated at 170 ^oC for 6 h and worked up as described above to give:

a. 10*H*-2,7-Diazaphenothiazine (**4**) (0.09 g, 46%), mp 169-170 ^oC.

b. 4-Dimethylamino-3-nitropyridine (**11**) (0.05 g, 15%), mp 48-49 °C (Et_2O) (lit.,[28] mp 48-49 °C).

Cyclization of sodium 3-nitro-4-pirydinethiolate (6)

A. in DMF

To a solution of 4-mercapto-3-nitropyridine (**6**) (0.42 g, 2.69 mmol) in dry EtOH NaOMe (0.145 g, 2.69 mmol) was added. The solution was stirred for 5 min and then EtOH was evaporated to dryness *in vaccuo*. To the residue dry DMF (15 mL) was added and the solution was refluxed for 7 h under argon atmosphere. After cooling the reaction mixture was worked up as described above to give:

a. 10*H*-2,7-Diazaphenothiazine (**4**) (0.12 g, 44%),

b. 4-Dimethylamino-3-nitropyridine (**11**) (0.03 g, 7%),

c. Dipyrido-1,4-dithiin (**2**), (0.08 g, 28%),

d. Dipyrido-1,4-dithiin (**3**) (0.03 g, 10%).

B. in 1-methyl-2-pyrrolidinone (MP)

To a solution of 4-mercapto-3-nitropyridine (**12**) (0.156 g, 1 mmol) in dry EtOH NaOMe (0.054 g, 1 mmol) was added. The solution was stirred for 5 min and then EtOH was evaporated to dryness *in vaccuo*. To the residue dry DMF (5 mL) was added and the solution was refluxed for 2 h under argon atmosphere. After cooling the reaction mixture was worked up as described above to give:

a. 10*H*-2,7-Diazaphenothiazine (**4**) (0.040 g, 40%),

b. 10-(3'-Nitro-4'pyridinyl)-2,7-diazaphenothiazine (**5**) (0.020 g, 19%),

c. Dipyrido-1,4-dithiins (**2**) and (**3**) (0.020 g, 18%).

Reaction of 4-mercapto-3-nitropyridine (12) with 3-amino-4-chloropyridine (13)

To a solution of 3-amino-4-chloropyridine (**13**) (0.128 g, 1 mmol) in dry DMF (5 mL) 4-mercapto-3-nitropyridine (**12**) (0.156 g, 1 mmol) was added. The reaction mixture was stirred at rt under argon atmosphere for 24 h and next was refluxed for 24 h. After cooling the reaction mixture was worked up as described above to give:

a. 10*H*-2,7-Diazaphenothiazine (**4**) (0.12 g, 60%),

b. 3,3'-Dinitro-4,4'-dipyridinyl sulfide (**15**) **(**0.020 g, 7%), mp 234-235 °C (EtOH) (lit.,[25] mp 235 °C).

Reaction of 4-chloro-3-nitropyridine (1) with 3-amino-4(1*H*)-pyridinethione (14)

A. To a solution of 4-chloro-3-nitropyridine (**1**) (0.317 g, 2 mmol) in dry DMF (5 mL) 3-amino-4(1*H*)-pyridinethione (**14**) (0.252 g, 2 mmol) was added. The reaction mixture was stirred at rt under argon atmosphere for 2 h and next was refluxed for 24 h. After cooling the reaction mixture was worked up as described above to give 10*H*-2,7-diazaphenothiazine (**4**) (0.30 g, 75%).

B. In the presence of NaOH

To a solution of 3-amino-4(1*H*)-pyridinethione (**14**) (0.126 g, 1 mmol) in dry DMF (5 mL) NaOH (0.12 g, 3 mmol) and 4-chloro-3-nitropyridine (**1**) (0.158 g, 1 mmol) was added. The reaction mixture was stirred at rt under argon atmosphere for 2 h and next was refluxed for 4 h. After cooling the reaction mixture was worked up as described above to give 10*H*-2,7-diazaphenothiazine (**4**) (0.17 g, 85%).

Cyclization of 3-amino-3'-nitro-4,4`-dipyridinyl sulfide (7)

A. Synthesis of 3-amino-3'-nitro-4,4`-dipyridinyl sulfide (**7**)

When the above reaction was repeated at room temperature for 2 h only 3-amino-3'-nitro-4,4'-dipyridinyl sulfide (**7**) was isolated (0.22 g, 89%); mp 109-110 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 6.79 (d, *J* = 6.0 Hz, 1H, H_{arom}), 7.55 (d, *J* = 5.2 Hz, 1H, H_{arom}), 8.38 (d, *J* = 6.0 Hz, 1H, H_{arom}), 8.52 (d, *J* = 5.2 Hz, 1H, H_{arom}), 8.72 (s, 1H, H_{arom}), 9.35 (s, 1H, H_{arom}), 9.62 (broad s, 2H, NH_2). FAB MS m/z: 248 (M, 100). Anal. Calcd for $C_{10}H_8N_4O_2S$: C 48.38, H 3.25, N 22.57. Found: C 48.47, H 3.29, N 22.66.

B. Cyclization to 10*H*-2,7-diazaphenothiazine (**4**)

To a solution of 3-amino-3'-nitro-4,4'-dipyridinyl sulfide (**7**) (0.124 g, 0.5 mmol) in dry DMF (5 mL) NaOH (0.06 g, 1.5 mmol) was added. The reaction mixture was refluxed for 4 h under argon atmosphere. After cooling the reaction mixture was worked up as described above to give 10*H*-2,7-diazaphenothiazine (**4**) (0.088 g, 88%).

Synthesis of 10-substituted 2,7-diazaphenothiazines

A. *N*-Alkylation and *N*-arylation with alkyl and aryl halides – general procedure

To a solution of 10*H*-2,7-diazaphenothiazine (**4**) (0.100 g, 0.5 mmol) in dry DMF (5 mL) NaH (0.024 g, 1 mmol, 60% NaH in mineral oil was washed out with hexane) was added. The reaction mixture was stirred at rt for 1 h, alkyl halide (methyl iodide, ethyl iodide, butyl iodide, allyl bromide, benzyl chloride) or aryl halide (4-fluoronitrobenzen, 5-chloro-1-methyl-4-nitroimidazole, 4-chloro-3-nitro-pyridine (**1**), 2,4-dichloro-5-methylpyrimidine) (1.5 mmol) was added and the stirring was continued for 24 h. The reaction mixture was poured into water (15 mL) and extracted with $CHCl_3$ (3 x 5 mL). The extracts were worked up as described above and the residue was purified by column chromatography (aluminum oxide, $CHCl_3$) to give:

1. 10-Methyl-2,7-diazaphenothiazine (**17**), (0.080 g, 75%); mp 56-57 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 3.38 (s, 3H, CH_3), 6.64 (d, *J* = 5.6 Hz, 1H, H-9), 7.02 (d, *J* = 4.9 Hz, 1H, H-4), 8.05 (s, 1H, H-1), 8.10 (s, 1H, H-6), 8.15 (d, *J* = 4.9 Hz, H-3), 8.29 (d, *J* = 5.6 Hz, H-8). EI MS m/z: 215 (M^+, 100), 200 (M-CH_3, 75). Anal. Calcd for $C_{11}H_9N_3S$: C 61.37, H 4.21, N 19.52. Found: C 61.22, H 4.28, N 19.31.

2. 10-Ethyl-2,7-diazaphenothiazine (**18**), (0.088 g, 77%), an oil. ^{1}H NMR ($CDCl_3$) δ: 1.45 (t, *J* = 6.8 Hz, 3H, CH_3), 3.90 (q, *J* = 6.8 Hz, 2H, CH_2), 6.64 (d, *J* = 5.6 Hz, 1H, H-9), 6.94 (d, *J* = 5.2 Hz, 1H, H-4), 8.07 (m, 3H, H-1, H-3, H-6), 8.22 (d, *J* = 5.6 Hz, 1H, H-8). EI MS m/z: 229 (M^+, 94), 200 (M-C_2H_5, 100). Anal. Calcd for $C_{12}H_{11}N_3S$: C 62.86, H 4.84, N 18.33. Found: C 62.71, H 4.90, N 18.12.

3. 10-butyl-2,7-diazaphenothiazine (**19**), (0.092 g, 72%), an oil. ^{1}H NMR ($CDCl_3$) δ: 0.99 (t, *J* = 7.5 Hz, 3H, CH_3), 1.48 (m, 2H, CH_2), 1.80 (m, 2H, CH_2), 3.81 (t, *J* = 7.5 Hz, 2H, NCH_2), 6.63 (d, *J* = 5.7 Hz, 1H, H-9), 6.96 (d, *J* = 4.8 Hz, 1H, H-4), 8.08 (m, 3H, H-1, H-3, H-6), 8.24 (s, 1H, H-8), EI MS m/z: 257 (M^+, 93), 214 (M-C_3H_7, 100) 200 (M-C_4H_9, 35). Anal. Calcd for $C_{14}H_{15}N_3S$: C 65.34, H 5.87, N 16.33. Found: C 65.26, H 5.92, N 16.44.

4. 10-Allyl-2,7-diazaphenothiazine (**20**), (0.083 g, 69%); mp 118-119 °C (EtOH) ^{1}H NMR ($CDCl_3$) δ: 4.47 (s, 2H, NCH_2), 5.23 (d, *J* = 17.7 Hz, 1H, =CH_2), 5.42 (d, *J* = 10.8 Hz, 1H, =CH_2), 5.99 (m, 1H, =CH), 6.65 (d, *J* = 5.7 Hz, 1H, H-9), 6.95 (d, *J* = 4.5 Hz, 1H, H-4), 8.13 (m, 4H, H-1, H-3, H-6, H-8), EI MS m/z: 241 (M^+, 32), 200 (M-C_3H_5, 100). Anal. Calcd for $C_{13}H_{11}N_3S$: C 64.71 H 4.59, N 17.41. Found: C 64.50 H 4.62, N 17.19.

5. 10-Benzyl-2,7-diazaphenothiazine (**21**), (0.084 g, 58%); mp 45-46 °C (Et_2O). ^{1}H NMR ($CDCl_3$) δ: 5.06 (s, 2H, CH_2), 6.43 (d, *J* = 5.7 Hz, 1H, H-9), 6.96 (d, *J* = 4.8 Hz, 1H, H-4), 7.22-7.39 (m, 5H, C_6H_5), 7.83 (s, 1H, H_{arom}), 8.06 (m, 3H, $3H_{arom}$), EI MS m/z: 291 (M^+, 15), 200 (M-$CH_2C_6H_5$, 18), $C_6H_5CH_2^+$ (100). Anal. Calcd for $C_{17}H_{13}N_3S$: C 70.08, H 4.50, N 14.42. Found: C 69.92, H 4.58, N 14.20.

6. 10-(4'-Nitrophenyl)-2,7-diazaphenothiazine (**23**), (0.142 g, 88%); mp 237-238 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 5.92 (d, *J* = 7.6 Hz, 1H, H-9), 6.50 (d, *J* = 5.2 Hz, 1H, H-4), 6.57(s, 1H, $H_{arom.}$), 6.99 (d, *J* = 7.6 Hz, $1H_{arom.}$), 7.34 (d, *J* = 7.0 Hz, 2H, C_6H_2), 7.85 (d, *J* = 5.2 Hz, 1H, $H_{arom.}$), 7,90 (s, 1H, $H_{arom.}$), 8.35 (d, *J* = 7.0 Hz, 2H, C_6H_2). EI MS m/z: 322 (M^+, 84), 276 (M-NO_2, 100), 200(M-$C_6H_4NO_2$, 35). Anal. Calcd for $C_{16}H_{10}N_4O_2S$: C 59.62, H 3.13, N 17.38. Found: C 59.41, H 3.20, N 17.12.

7. 10-(3'-Nitro-4'-pyridinyl)-2,7-diazaphenothiazine (**5**), (0.100 g, 62%); mp 231-232 °C (EtOH).

8. 10-(1'-Methyl-4'-nitro-5'-imidazolyl)-2,7-diazaphenothiazine (**24**), (0.098 g, 61%); mp 129-130 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 3.70 (s, 3H, NCH_3), 5.89 (d, *J* = 7.6 Hz, 1H, $H_{arom.}$), 6.13 (d, *J* = 2.0 Hz, 1H, $H_{arom.}$), 6.51(s+d, *J* = 5.2 Hz, 2H, $2H_{arom.}$), 7.46 (s, 1H, $H_{arom.}$), 7.92 (d, *J* = 5.2 Hz, 1H, $H_{arom.}$), 7.94 (s, 1H, $H_{arom.}$). FAB MS m/z: 327 (M+H, 35) Anal. Calcd for $C_{14}H_{10}N_6O_2S$: C 51.53, H 3.09, N 25.75. Found: C 51.28, H 3.10, N 25.41.

9. 10-(2'-Chloro-5'-methyl-4'-pyrimidinyl)-2,7-diazaphenothiazine (**25**), (0.100 g, 70%); mp 205-206 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 2.34 (s, 3H, CH_3), 6.56 (d, *J* = 5.0 Hz, 1H, $H_{arom.}$), 7.4-8.3 (m, 6H, $6H_{arom.}$), 7.76 (s, 1H, $H_{arom.}$), 7.90 (d, *J* = 5.0 Hz, 1H, H-8), 8.37 (s, 1H, $H_{arom.}$). FAB MS m/z: 328 (M+H, 50). Anal. Calcd for $C_{15}H_{10}N_5ClS$: C 54.96, H 3.07, N 21.37. Found: C 54.82, H 3.10, N 21.13.

B. *N*-Alkylation with aminoalkyl chlorides and phenacyl bromide

To a solution of 10*H*-2,7-diazaphenothiazine (**4**) (0.100 g, 0.5 mmol) in dry dioxane (5 mL) NaOH (0.20 g, 5 mmol) and phenacyl bromide (0.30 g, 1.5 mmol) or hydrochlorides of dialkylaminoalkyl chloride (2-diethylaminoethyl, 3-dimethylaminopropyl, 3-dimethylamino-2-methylpropyl, 2-(1-methyl-2'-piperydinyl)ethyl, 1.5 mmol) was added. The reaction mixture was refluxed for 5 h. After cooling dioxane was evaporated *in vaccuo* and the residue was dissolved in $CHCl_3$ (10 mL). The extract was worked up as described above and the residue was purified by column chromatography (aluminum oxide, $CHCl_3$) to give:

1. 10-Phenacyl-2,7-diazaphenothiazine (**22**), (0.100 g, 63%); mp 59-60 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 5.24 (s, 2H, CH_2), 6.24 (d, *J* = 5.7 Hz, 1H, H-9), 7.00 (d, *J* = 4.8Hz, 1H, H-4), 7.73 (m, 2H, C_6H_2), 7.63 (m, 3H, C_6H_3), 8.10 (2s+d, 3H, H-1, H-3, H6), 8.15 (d, *J* = 5.4 Hz, H-8). EI MS m/z: 320 (M+H, 100). Anal. Calcd for $C_{18}H_{13}N_3OS$: C 67.69, H 4.10, N 13.16. Found: C 67.57, H 4.20, N 12.92.

2. 10-(2'-Diethylaminoethyl)-2,7-diazaphenothiazine (**26**), (0.100 g, 67%), an oil. ^{1}H NMR ($CDCl_3$) δ: 0.90 (t, *J* = 7.2 Hz, 6H, $2CH_3$), 2.02 (q, *J* = 7.2 Hz, 4H, $2CH_2$), 3.32 (t, *J* = 7.2 Hz, 2H, CH_2), 4.75 (t, *J* = 7.2 Hz, 2H, CH_2), 7.11 (d, *J* = 5.1 Hz, 1H, H_{arom}), 7.20 (d, *J* = 5.7 Hz, 1H, H_{arom}), 8.20 (m, 3H, $3H_{arom}$), 8.42 (d, *J* = 5.7 Hz, 1H, H_{arom}). FAB MS m/z: 301 (M+H, 40), 185 (2gly+H, 100). Anal. Calcd for $C_{16}H_{20}N_4S$: C 63.97, H 6.71, N 18.65. Found: C 63.80, H 6.82, N 18.38.

3. 10-(3'-Dimethylaminopropyl)-2,7-diazaphenothiazine (**27**), (0.100 g, 70%), an oil. ^{1}H NMR ($CDCl_3$) δ: 1.96 (m, 2H, CH_2), 2.26 (s, 6H, $2CH_3$), 2.44 (t, *J* = 6.8 Hz, 2H, NCH_2), 3.91 (t, *J* = 7.4 Hz, 2H, NCH_2), 6.72 (d, *J* = 5.6 Hz, 1H, H-9), 6.97 (d, *J* = 4.8 Hz, 1H, H-4), 8.05 (s, 1H, H-1), 8.10 (m, 2H, H-3, H-6), 8.23 (d, *J* = 5.6 Hz, 1H, H-8). FAB MS m/z: 287 (M+H, 100). Anal. Calcd for $C_{15}H_{18}N_4S$: C 62.91, H 6.33, N 19.56. Found: C 62.79, H 6.41, N 19.27.

4. 10-(3'-Dimethylamino-2'-methylpropyl)-2,7-diazaphenothiazine (**28**), (0.126 g, 84%); mp 120-121 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 0.95 (d, *J* = 6.8 Hz, 3H, CH_3), 2.35 (s, 6H, $2CH_3$), 2.2-2.4 (m, 3H, NCH_2, CH), 3.61 (m, 1H, NCH), 4.15 (m, 1H, NCH), 6.80 (d, *J* = 5.6 Hz, 1H, H-9), 7.05 (d, *J* = 4.8 Hz, 1H, H-4), 8.1-8.3 (m, 3H, H-1, H-6, H-3), 8.25 (d, *J* = 5.6 Hz, 1H, H-8). FAB MS m/z: 301 (M+H, 100), 202 (M+H-$CH_2CH(CH)CH_2N(CH_3)_2$, 15). Anal. Calcd for $C_{16}H_{20}N_4S$: C 63.97, H 6.71, N 18.65. Found: C 63.82, H 6.80, N 18.34.

5. 10-(1'-Methyl-2'-piperydinylethyl)-2,7-diazaphenothiazine (**29**), (0.134 g, 82%), an oil. ^{1}H NMR ($CDCl_3$) δ: 1.26-2,15 (m, 7H, $7H_{alif.}$), 2.28 (s, 3H, NCH_3), 2.86 (m, 1H, CH), 3.8-4.0 (m, 2H, NCH_2), 6.68 (d, *J* = 5.7 Hz, 1H, H-9), 6. 98 (d, *J* = 4.8 Hz, 1H, H-4), 8.09 (m, 3H, H-1, H-6, H-3), 8.24 (d, *J* = 5.7 Hz, 1-H, H-8). FAB MS m/z: 327 (M+H, 97), 109 ($CH_2CHC_5H_9N$, 100). Anal. Calcd for $C_{18}H_{22}N_4S$: C 66.23, H 6.79, N 17.16. Found: C 66.06, H 6.80, N 16.95.

REFERENCES

#Part XCV in the series of Azinyl Sulfides.

1. R. R. Gupta and M. Kumar, in Phenothiazines and 1,4-Benzothiazines – Chemical and Biological Aspects, ed. by R. R. Gupta, Elsevier, Amsterdam, 1988, pp. 1-161.
2. N. Motohashi, M. Kawase, T. Kurihara, A. Hever, S. Nagy, I. Ocsocvszki, M. Tanaka, and J. Molnar, *Anticancer Res*., 1996, **16**, 2525.
3. N. Motohashi, T. Kurihara, H. Sakagami, D. Szabo, K. Csuri, and J. Molnar, *Anticancer Res.,* 1999, **19**, 1859.
4. N. Motohashi, M. Kawase, S. Saito, and H. Sakagami, *Curr. Drug Targets*, 2000, **1**, 237.
5. L. Amaral, M. Vivieros, and J. E. Kristiansen, *Trop. Med. Int. Health*, 2001, **6**, 1016.
6. D. Ordway, M. Viveiros, C. Leandro, R. Bettencourt, J. Almeida, M. Martins, J. E. Kristiansen, J. Molnar, and L. Amaral, *Antimicrob. Agents Chemother*., 2003, **47**, 917.
7. N. Motohashi, M. Kawase, K. Satoh, and H. Sakagami, *Curr. Drug Targets*, 2006, **7**, 10557.
8. Y. Maki, *Yakugaku Zasshi,* 1957, **77**, 485.
9. T. Takahashi and Y. Maki, *Yakugaku Zasshi,* 1958, **78**, 417.
10. T. Takahashi and Y. Maki, *Chem. Pharm. Bull.,* 1958, **6**, 369.
11. O. R. Rodig, R. E. Collier, and R. K. Schlatzer, *J. Med. Chem*., 1966, **9**, 116.
12. S. Rath, US Patent 2 789 978 (1957).
13. C. Okafor, *J. Org Chem*., 1967, **32**, 2006.
14. E. Kopp, M. Strell, and R. Janson, German Patent DE 1 147 235 (1963).
15. E. Kopp and M. Strell, *Arch. Pharm*., 1962, **295**, 99.
16. B. Morak, K. Pluta, and K. Suwinska, *Heterocyclic Commun.,* 2002, **8**, 331.
17. B. Morak, K. Pluta, K. Suwińska, M. Grymel, C. Bernard, M. Schiltz, C. Kloc, and T. Siegrist, *Heterocycles*, 2005, **65**, 2619.
18. S. J. Dunne, L. A. Summers, and E. I. von Nagy-Felsobuki, *J. Heterocycl. Chem*., 1987, **24**, 851.
19. E. Stahl, Thin-layer chromatography, Springer-Verlag, Berlin, 1969, p. 508.
20. Prediction of Activity Spectra for Substance, http://ibmc.msk.ru/PASS/
21. B. Morak-Młodawska and K. Pluta, Unpublished data.
22. U. Franke, A. Munk, and M. Wiese, *J. Pharm. Sci.,* 1999, **88**, 89.
23. National Cancer Institute Developmental Therapeutics Program, In-Vitro Testing Results, Bethesda, USA.
24. J. B. Campbell, J. M. Greene, E. R. Lavagnino, D. N. Gardner, A. J. Pike, J. Snoddy, and E. C. Taylor, *J. Heterocycl. Chem*., 1986, **23**, 669.
25. S. Kruger and F. G. Mann, *J. Chem. Soc*., 1955, 2755.

26. H. W. Altland and G. A. Molander, *J. Heterocycl. Chem*., 1977, **14**, 129.
27. C. Okafor and U. C. Okaro, *Dyes & Pigments*, 1991, **16**, 149.
28. A. G. Burton, R. D. Frampton, C. D. Johnson, and A. R. Katritzky, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1940.