Synthesis of Novel 1,4-Dihydropyrido[3',2':5,6]thiopyrano[4,3-*c*]-pyrazoles and 5*H*-Pyrido[3',2':5,6]thiopyrano[4,3-*d*]pyrimidines as Potential Antiproliferative Agents

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The synthesis of new planar derivatives characterized by the presence of a pyridothiopyranopyrazole or pyridothiopyranopyrimidine nucleus, carrying a substituted aryl group, is reported. The novel 1,4-dihydropyrido[3',2':5,6]thiopyrano[4,3-*c*]pyrazole derivatives were obtained by condensation of 2,3-dihydro-3-hydroxymethylenethiopyrano[2,3-*b*]pyridin-4(4*H*)-ones with appropriate hydrazines. The preparation of 2-substituted pyrido[3',2':5,6]thiopyrano[4,3-*d*]pyrimidines was accomplished from the intermediate 2,3-dihydro-3-dimethylaminomethylenethiopyrano[2,3-*b*]pyridin-4(4*H*)-ones by reaction with the appropriate binucleophile amidines. The antiproliferative activity of some new products was tested by an *in vitro* assay on human tumour cell lines (HL-60 and HeLa), but none of them showed any significant effects in the tests performed. Accordingly, linear flow dichroism measurements indicated their inability to form a molecular complex with DNA.

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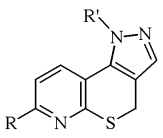
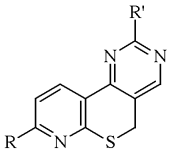
DNA replication constitutes the necessary condition for cell division, and consequently the macromolecule has become one of the preferred targets for the development of new drugs able to act as potential antitumor agents. Among the DNA binding compounds one of the most interesting groups is that of the intercalators, whose structure is characterized by the presence of a planar aromatic or heteroaromatic chromophore. These compounds interact with DNA thanks to the ability of their large, planar polycyclic moiety to form a molecular complex between the base pairs, by engaging hydrogen bonds, Van der Waals contacts, and hydrophobic interactions. The cellular events ascribable to the DNA-intercalative binding mode of the polycyclic compounds determine enzymatic blocking and reading errors during the replication process, leading to cell death. Moreover, it has been demonstrated that the presence of suitable side chains and/or basic nitrogens further strengthens the DNA binding, thus modulating the biological properties and, upon protonation, increasing solubility under physiological conditions [1-5].

In the light of the reported considerations, in previous studies our interest was devoted to the synthesis of new planar polycyclic derivatives with a potential antitumor activity [6-8], and, in recent papers, we described some tetracyclic derivatives containing the purine, benzimidazole or indole nucleus [9-11]. These compounds showed a cytotoxic activity, mainly through an intercalative mode of binding to DNA, which, in some cases, was close to that of the well-known drug ellipticine.

Pursuing our interest in polycondensed nitrogen heterocycles, in this paper we report the synthesis of some new pyrazole and pyrimido derivatives with the general formulas **I** and **II**, respectively, wherein the pyrazole and pyrimidine moieties are fused to a pyridothiopyrane nucleus, recently described [6,12].



I



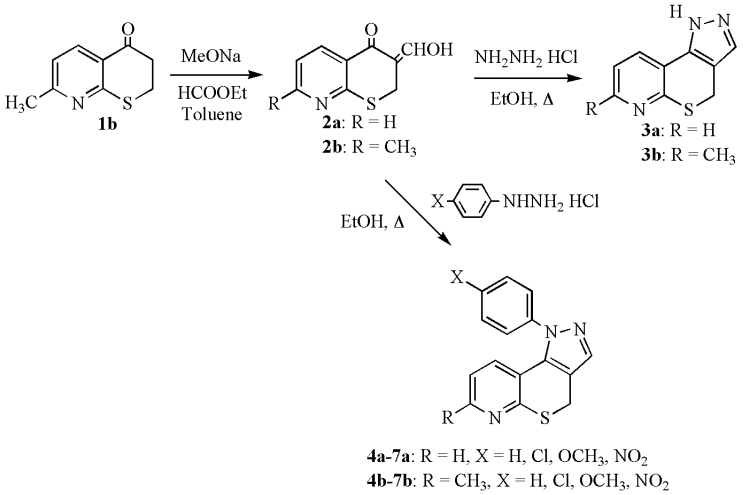
II

The ability of some of the new compounds to inhibit cell growth was evaluated by an *in vitro* assay, using two human tumor cell lines, HL-60 and HeLa. Furthermore, linear flow dichroism experiments were performed with the aim of investigating their capacity to form a molecular complex with DNA.

Results and Discussion.

The synthetic sequence utilized in the preparation of 1,4-dihydropyrido[3',2':5,6]thiopyrano[4,3-*c*]pyrazoles **3a-b** and of the 1-aryl substituted analogues **4a-b, 5a-b, 6a-b** and **7a-b** is illustrated in Scheme 1. The 2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-ones **1a** [12] and **1b** [6], which represented the starting materials for the preparation of the target compounds, have already been described, as well as the intermediate 2,3-dihydro-3-hydroxymethylenethiopyrano[2,3-*b*]pyridin-4(4*H*)-one **2a** [8]. The preparation of the corresponding 7-methyl derivative **2b** was easily accomplished from **1b**, by reaction with ethyl formate, adopting our previously reported procedure for the synthesis of **2a** (Scheme 1). The condensation of compounds **2a-b**, containing a methine active group, with hydrazine hydrochloride or with *p*-substituted phenylhydrazine hydrochlorides, in refluxing methanol, gave the desired pyrazoles **3a-b, 4a-b, 5a-b, 6a-b** and **7a-b**. Under these conditions, the cyclized compounds are directly obtained

Scheme 1



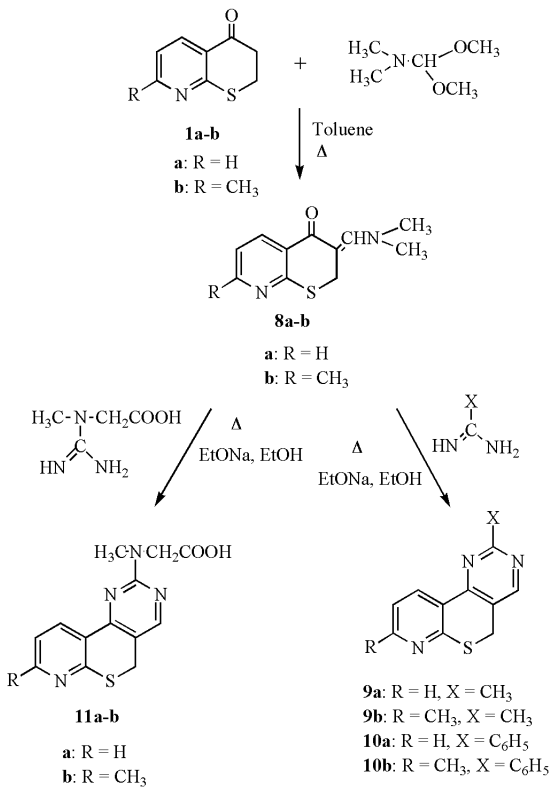
1b

2a: R = H
2b: R = CH_3

3a: R = H
3b: R = CH_3

4a-7a: R = H, X = H, Cl, OCH_3, NO_2
4b-7b: R = CH_3, X = H, Cl, OCH_3, NO_2

Scheme 2



1a-b
a: R = H
b: R = CH_3

8a-b
a: R = H
b: R = CH_3

11a-b
a: R = H
b: R = CH_3

9a: R = H, X = CH_3
9b: R = CH_3, X = CH_3
10a: R = H, X = C_6H_5
10b: R = CH_3, X = C_6H_5

as unique and pure derivatives (tlc analysis). Moreover, on the basis of the literature data [13-15], it is plausible to infer that the reaction of compounds **2a-b** with the hydrazines involves the more reactive methine group affording the intermediate 3-methylenehydrazono derivatives, which easily cyclize to the target products **3a-b, 4a-b, 5a-b, 6a-b** and **7a-b**, losing water intramolecularly. Analytical and spectral data of all compounds were in agreement with the proposed structures and are reported in Table I.

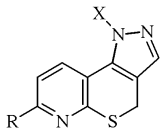
The synthetic route leading to the 2-substituted 5H-pyrido[3',2':5,6]thiopyrano[4,3-*d*]pyrimidines **9a-b** and **10a-b** takes advantage of the reactivity as 1,3-bielectrophiles of the intermediate 2,3-dihydro-3-dimethylaminomethylenethiopyrano[2,3-*b*]pyridin-4(4*H*)-ones **8a-b** in the reaction with the appropriate binucleophile amidines in a basic medium [16]. The 3-hydroxymethylene compounds **2a-b** failed to give directly the desired pyrimidine derivatives by reaction with substituted amidines under a variety of conditions. This lack of reactivity of **2a-b** was circumvented by converting the starting compounds **1a-b** into **8a-b**, with good yields, by reaction with an excess of dimethylformamide dimethylacetal (DMF-DMA) in refluxing toluene (Scheme 2).

Finally, compounds **11a-b**, bearing the *N*-methylglycine chain, were synthesized. It seemed reasonable that the presence of an ionizable side chain might result in the possibility of supporting the formation of a reinforced complex with DNA, ascribable to electrostatic interactions, contributing to the bonding energy. *N*-Methyl-*N*-(5*H*-pyrido[3',2';5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)glycines **11a-b** were obtained by reacting **8a-b** with creatine monohydrate, in an ethanolic refluxing solution, in the presence of sodium ethoxide (Scheme 2). The structures of compounds **9a-b, 10a-b** and **11a-b** were assigned on the basis of analytical and spectral data, which are reported inTable II.

The ability of **3a-7a** and **11a-b** to inhibit cellular growth was evaluated on two human tumor cell lines, HeLa and HL-60, in accordance with the experimental procedure previously described [11]. The test compounds, added at a concentration up to 20 μM, appeared to be ineffective on both the cell lines taken into consideration. The inability to give rise to any noticeable cytotoxic effect appeared to be

Table I

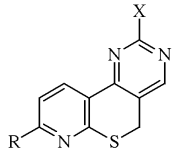
Physical and Spectral Data of Compounds **3a-b, 4a-b, 5a-b, 6a-b** and **7a-b**



Comp.	R	X	Yield (%)	M.p.°C (Recrystallization solvent)	^{1}H nmr (δ ppm)	Ms m/z	Molecular Formula	Analysis (%) Calcd./Found C	H	N
3a	H	H	58	185-189 (Ethylacetate)	4.19 (s, 2H, 4-CH_2); 7.14-7.21 (dd, 1H, ArH); 7.68 (s, 1H, ArH); 8.03 (d, 1H, ArH), 8.26 (dd, 1H, ArH); 13.05 (s, 1H, NH exch.).	189	$C_9H_7N_3S$	57.14 57.01	3.70 3.75	22.22 22.33
3b	CH_3	H	30	225-228 (Ethanol)	2.38 (s, 3H, 7-CH_3); 4.17 (s, 2H, 4-CH_2); 7.03 (s, 1H, ArH); 7.65 (s, 1H, ArH); 7.92 (d, 1H, ArH); 12.98 (s, 1H, NH exch.).	203	$C_{10}H_9N_3S$	59.11 58.81	4.43 4.55	20.69 20.48
4a	H	C_6H_5	35	160-162 (Ehanol)	4.18 (s, 2H, 4-CH_2); 6.92-6.97 (m, 2H, ArH); 7.36-7.41 (dd, 2H, ArH); 7.53-7.56 (dd, 3H, ArH); 7.69 (s, 1H, ArH); 8.21-8.24 (dd, 1H, ArH).	265	$C_{15}H_{11}N_3S$	67.92 67.67	4.15 4.27	15.84 15.51
4b	CH_3	C_6H_5	47	195-197 (Methanol)	2.34 (s, 3H, 7-CH_3); 4.14 (s, 2H, 4-CH_2); 6.81 (s, 2H, ArH); 7.33-7.38 (dd, 2H, ArH); 7.51-7.54 (m, 3H, ArH); 7.65 (s, 1H, ArH).	279	$C_{16}H_{13}N_3S$	68.82 68.61	4.66 4.91	15.05 15.04
5a	H	*p*-Cl-C_6H_4	35	158-160 (Ethanol)	4.17 (s, 2H, 4-CH_2); 7.03 (d, 2H, ArH); 7.43 (d, 2H, ArH); 7.60 (d, 2H, ArH); 7.72 (s, 1H, ArH); 8.25 (m, 1H, ArH).	299	$C_{15}H_{10}ClN_3S$	60.10 59.73	3.34 3.61	14.02 13.72
5b	CH_3	*p*-Cl-C_6H_4	18	194-195 (Methanol)	2.36 (s, 3H, 7-CH_3); 4.14 (s, 2H, 4-CH_2); 6.91 (s, 2H, ArH); 7.41 (d, 2H, ArH); 7.59 (d, 2H, ArH); 7.69 (s, 1H, ArH).	313	$C_{16}H_{12}ClN_3S$	61.24 61.06	3.83 4.00	13.40 13.72
6a	H	*p*-$OCH_3C_6H_4$	43	99-101 (Ethanol)	3.83 (s, 3H, OCH_3); 4.17 (s, 2H, 4-CH_2); 6.96 (dd, 1H, ArH); 6.98 (d, 1H, ArH); 7.08 (d, 2H, ArH); 7.31 (d, 2H, ArH); 7.64 (s, 1H, ArH); 8.20-8,23 (dd, 1H, ArH)	295	$C_{16}H_{13}N_3OS$	65.08 64.82	4.41 4.67	14.24 13.97
6b	CH_3	*p*-$OCH_3C_6H_4$	44	173-175 (Ethanol)	2.34 (s, 3H, 7-CH_3); 3.82 (s, 3H, OCH_3); 4.14 (s, 2H, 4-CH_2); 6.84 (s, 2H, ArH); 7.06 (d, 2H, ArH); 7.29 (d, 2H, ArH); 7.61 (s, 1H, ArH).	309	$C_{17}H_{15}N_3OS$	66.02 65.82	4.85 4.67	13.59 13.77
7a	H	*p*-$NO_2C_6H_4$	39	175-178 (Ethanol)	4.18 (s, 2H, 4-CH_2); 7.01-7.07 (d, 1H, ArH); 7.16 (d, 1H, ArH); 7.69 (d, 2H, ArH); 7.83 (s, 1H, ArH); 8.30 (d, 1H, ArH); 8.36 (d, 2H, ArH).	310	$C_{15}H_{10}N_4O_2S$	58.06 57.72	3.23 3.32	18.06 17.72
7b	CH_3	*p*-$NO_2C_6H_4$	57	242-245 (Ethanol)	2.39 (s, 3H, 7-CH_3); 4.14 (s, 2H, 4-CH_2); 6.89 (d, 1H, ArH); 7.05 (d, 1H, ArH); 7.66 (d, 2H, ArH); 7.79 (s, 1H, ArH); 8.38 (d, 2H, ArH).	324	$C_{16}H_{12}N_4O_2S$	59.26 58.89	3.70 4.01	17.28 17.14

Table II

Physical and Spectral Data of Compounds **9a-b**, **10a-b** and **11a-b**



Comp.	R	X	Yield (%)	M.p.°C (Recrystallization solvent)	^{1}H nmr (δ ppm)	Ms m/z	Molecular Formula	Analysis (%) Calcd./Found C	H	N
9a	H	CH_3	58	145-147 (Ehanol)	2.69 (s, 3H, 2-CH_3); 4.27 (s, 2H, 5-CH_2); 7.34-7.40 (dd, 1H, ArH); 8.50-8.53 (dd, 1H, ArH); 8.56-8.60 (dd, 1H, ArH); 8.71 (s, 1H, ArH).	215	$C_{11}H_9N_3S$	61.39 61.26	4.19 4.27	19.53 18.92
9b	CH_3	CH_3	82	133-135 (Ethanol)	2.49 (s, 3H, 8-CH_3); 2.65 (s, 3H, 2-CH_3); 4.24 (s, 2H, 5-CH_2); 7.22 (d, 2H, ArH); 8.46 (d, 2H, ArH); 8.66 (s, 1H, ArH).	229	$C_{12}H_{11}N_3S$	62.88 62.61	4.80 4.91	18.34 18.16
10a	H	C_6H_5	52	158-160 (Ethanol)	4.36 (s, 2H, 5-CH_2); 7.40-7.46 (d, 1H, ArH); 7.56 (m, 3H, ArH); 7.48-8.57 (m, 3H, ArH); 8.81-8.85 (dd, 1H, ArH), 8.92 (s, 1H, ArH).	277	$C_{16}H_{11}N_3S$	69.31 68.98	3.97 3.61	15.16 14.99
10b	CH_3	C_6H_5	55	190-193 (Ethanol)	2.50 (s, 3H, 8-CH_3); 4.33 (s, 2H, 5-CH_2); 7.28 (d, 1H, ArH); 7.53-7.57 (m, 3H, ArH); 8.46-8.51 (m, 2H, ArH); 8.70 (d, 1H, ArH); 8.87 (s, 1H, ArH).	291	$C_{17}H_{13}N_3S$	70.10 69.98	4.47 4.53	14.43 14.27
11a	H	CH_3NCH_2COOH	44	220-225 (Ethanol)	3.22 (s, 3H, N'CH_3); 4.13 (s, 2H, N'CH_2); 4.33 (s, 2H, 5-CH_2); 7.31-7.37 (m, 1H, ArH); 840 (s, 1H, ArH); 8.48 (d, 2H, ArH); 12.58 (bs, 1H, COOH, exch.).	288	$C_{13}H_{12}N_4O_2S$	54.16 53.95	4.16 4.25	19.44 19.27
11b	CH_3	CH_3NCH_2COOH	91	173-175 (Ethanol)	2.46 (s, 3H, 8-CH_3); 3.21 (s, 3H, N'CH_3); 4.10 (s, 2H, N'CH_2); 4.31 (s, 2H, 5-CH_2); 7.19 (d, 1H, ArH); 8.36 (s, 1H, ArH); 8.42 (d, 1H, ArH); 12.56 (bs, 1H, COOH, exch.).	302	$C_{14}H_{14}N_4O_2S$	55.63 55.27	4.64 4.67	18.54 18.77

in agreement with the results obtained from linear flow dichroism experiments, performed as reported in ref. [11]. In particular, the dichroic spectra of solutions of DNA in the presence of test compounds, revealed the absence of any detectable dichroic signal in the spectral region at wavelengths higher than 300 nm, where only the chromophore of the drug absorbs, thus demonstrating the inability of these polycyclic moieties to form a molecular complex with the macromolecule.

EXPERIMENTAL

Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were obtained on a PYE/UNICAM Model PU 9561 spectrophotometer as Nujol mulls. Nuclear magnetic resonance spectra were recorded on a Varian Gemini 200 spectrometer, in dimethyl-d_6 sulfoxide solution. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Magnesium sulfate was always used as the drying agent. Evaporations were made *in vacuo* (rotating evaporator). Analytical tlc were carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254). Elemental analyses were performed by our Analytical Laboratory.

7-Methyl-2,3-dihydro-3-hydroxymethylenethiopyrano[2,3-*b*]-pyridin-4(4*H*)-one (**2b**).

A solution of ethyl formate (0.65 ml, 8 mmoles) in anhydrous toluene (3 ml) was added dropwise to freshly prepared sodium methoxide (0.184 g of sodium, 8 mmoles, in 2 ml of absolute methanol) in the same solvent (3 ml). Then a solution of 7-methyl-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-one **1b** (0.660 g, 4 mmoles) in anhydrous toluene (8 ml) was added dropwise, with stirring, to the ice-cooled mixture, under a nitrogen atmosphere. Stirring was continued at room temperature for 24 hours to give the sodium salt of **2b**, which was collected and treated with water. The solution obtained was acidified with hydrochloric acid to give pure **2b** (0.564 g, 54.4 % yield). An analytical sample was obtained by recrystallization from petroleum ether 40-60 °C, m.p. 155-156 °C; ^{1}H-nmr (dimethyl-d_6 sulfoxide): δ 1.22 (s, 3H, C*H*$_3$); 3.91 (s, 2H, 2-C*H*$_2$); 7.12-7.39 (m, 2H, 3-C*H*OH, ArOH); 8.10 (t, 1H, ArH); ms: m/z = 207 (M^+).

Anal. Calcd. for $C_{10}H_9NO_2S$: C, 55.96; H, 3.63; N, 7.25; Found: C, 56.25; H, 3.69; N, 7.54.

1,4-Dihydro- **3a**, 1-(*p*-Substituted-phenyl)-1,4-dihydro- **4a-7a**, 7-Methyl-1,4-dihydro- **3b** and 7-Methyl-1-(*p*-substituted-phenyl)-1,4-dihydropyrido[3',2';5,6]thiopyrano[4,3-*c*]pyrazoles **4b-7b**.

General Procedure.

Hydrazine hydrochloride or the required phenylhydrazine hydrochloride (1.1 mmoles) was added to a solution of **2a** or **2b** (1 mmole) in 15 ml of methanol, and the reaction mixture was stirred at room temperature for 24 hours, and then refluxed for 15 hours. After cooling, the yellow solid, if present, was collected and the solution was evaporated under reduced pressure. The solid and the residue were washed with an aqueous potassium carbonate solution to give crude pyrazoles **3a-b, 4a-b, 5a-b, 6a-b** and **7a-b**, which were purified by recrystallization (Table I).

2,3-Dihydro-3-dimethylaminomethylenethiopyrano[2,3-*b*]-pyridin-4(4*H*)-one **8a** and 7-Methyl Derivative **8b**.

General Procedure.

An excess of dimethylformamide dimethylacetal (1 ml, 7.5 mmoles) was added to a stirred solution of the appropriate 2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-one **1a-b** (3.03 mmoles) in anhydrous toluene (5 ml) and the mixture was refluxed for 16 or 6 hours, respectively. After cooling, the solution obtained was evaporated under reduced pressure, giving a residue which was treated with ethyl ether, collected and purified by recrystallization from toluene-petroleum ether 60-80 °C.

Compound **8a** was obtained in 73.5% yield; m.p. 100-101 °C; ^{1}H-nmr (dimethyl-d_6 sulfoxide): δ 3.17 (s, 6H, N(C*H*$_3$)$_2$); 4.17 (s, 2H, C*H*$_2$S); 7.19-7.25 (m, 1H, ArH); 7.49 (s, 1H, =C*H*N); 8.12-8.17 (dd, 1H, ArH); 8.39-8.42 (dd, 1H, ArH); ms: m/z = 220 (M^+).

Anal. Calcd. for $C_{11}H_{12}N_2OS$: C, 60.00; H, 5.45; N, 12.72; Found: C, 59.63; H, 5.21; N, 12,51.

Compound **8b** was obtained in 76.1% yield; m.p. 140-141 °C dec.; ^{1}H-nmr (dimethyl-d_6 sulfoxide): δ 2.42 (s, 3H, C*H*$_3$): 3.15 (s, 6H, N(C*H*$_3$)$_2$); 4.15 (s, 2H, C*H*$_2$S); 7.07 (d, 1H, ArH); 7.45 (s, 1H, =C*H*N); 8.04 (d, 1H, ArH); 8.39-8.42 (dd, 1H, ArH); ms: m/z = 234 (M^+).

Anal. Calcd. for $C_{12}H_{14}N_2OS$: C, 61.54; H, 5.98; N, 11.97; Found: C, 61.42; H, 5.67; N, 12,13.

2-Substituted-5*H*-pyrido[3',2';5,6]thiopyrano[4,3-*d*]pyrimidines **9a**, **10a**, and 8-Methyl Derivatives **9b**, **10b**.

General Procedure.

The appropriate amidine hydrochloride (1.70 mmoles) was added, at room temperature, under nitrogen atmosphere, to a stirred solution of sodium ethoxide (2.55 mmoles of sodium in 8 ml of anhydrous ethanol). The resulting suspension was stirred at room temperature for 15 minutes, then the appropriate dimethylaminomethylene derivative **8a-b** (0.85 mmole) was added and the reaction mixture was refluxed for 6 hours. After cooling, the suspension was concentrated under reduced pressure. The residue obtained was washed with water and collected, to give crude pyrimidines **9a-b** and **10a-b**, which were purified by recrystallization from ethanol (Table II).

N-Methyl-*N*-(5*H*-pyrido[3',2';5,6]thiopyrano[4,3-*d*]pyrimidin-2-yl)glycine **11a** and 8-Methyl Derivative **11b**.

General Procedure.

Creatine monohydrate (0.210 g, 1.4 mmoles) was added, at room temperature, under nitrogen atmosphere, to a stirred solution of sodium ethoxide in ethanol (0.034 g, 1.50 mmoles of sodium in 3 ml of anhydrous ethanol). The resulting suspension was stirred at room temperature for 15 minutes, and then the appropriate dimethylaminomethylene derivative **8a-b** (0.95 mmole) was added and the reaction mixture was refluxed for 4 hours. After cooling, the suspension obtained was concentrated to *ca* half volume, poured into water (10 ml) and acidified (pH 3-4) with 1 *M* hydrochloric acid. The solid precipitate was collected, washed with water and purified by recrystallization (Table II).

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