Synthesis and Antiproliferative Evaluation of New Aryl Substituted Pyrido[3',2':5,6]thiopyrano[4,3-*c*]pyrazoles

G. Primofiore[†], A. M. Marini[†*], S. Salerno[†], F. Da Settimo[†], D. Bertini[†] and L. Dalla Via[§]

[†] Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy
[§]Dipartimento di Scienze Farmaceutiche, Università di Padova, via Marzolo 5, 35131 Padova, Italy

Received April 5, 2005

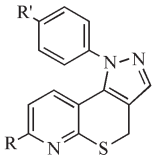
The preparation and the cytotoxic properties of new derivatives of the planar pyrido[3',2':5,6]thiopyrano-[4,3-*c*]pyrazole system, carrying an arylic side group in the 1 or 2 positions, are described. The novel substituted derivatives were obtained by reaction of suitable arylhydrazines with the appropriate key intermediate 3-hydroxymethylene-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-ones. Moreover the preparation was reported of the 2-carboxamidophenyl derivatives, which was accomplished from the previously obtained pyrido[3',2':5,6]thiopyrano[4,3-*c*]pyrazole nucleus, by reaction with phenylisocyanate. All the new compounds were evaluated for their antiproliferative ability, by an *in vitro* assay on human tumor cell lines (HL-60 and HeLa).

J. Heterocyclic Chem., **42**, 1357 (2005).

Introduction.

Many effective anticancer drugs in clinical use interact with DNA and some of them exhibit cytotoxic activity through DNA intercalation [1-3]. The intercalation process reflects the ability of a planar aromatic or heteroaromatic system to become inserted between adjacent base pairs of a DNA molecule; this intercalative reaction can affect many biological properties of DNA, including enzymatic blockade and reading errors during the replication and transcription process, thus leading to cell death [4-7]. Although the presence of a fused heterocyclic system plays an important role for the DNA-binding properties, the antiproliferative activity is often strongly dependent on the presence of suitable substituents or of a positive charge which could be provided both by an aminoalkyl side chain or by a protonable aromatic nitrogen of the chromophore [8].

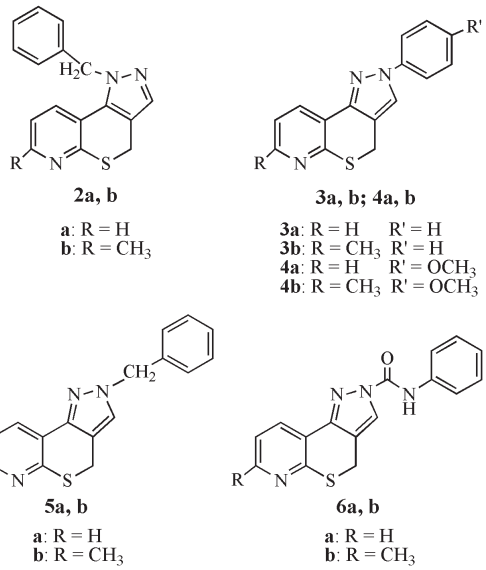
In the continual search of new classes of DNA-binding systems, our group has been interested for several years in the synthesis of new polycyclic derivatives, some of which, containing the purine, benzimidazole or indole nucleus, showed a cytotoxic activity, similar to that of Ellipticine, mainly attributable to an intercalative mode of binding to DNA [9-11]. As part of this screening research activity, we recently described a series of new 1-phenyl pyridothiopyrano[4,3-*c*]pyrazoles **1** (Figure 1) [12] which, evaluated for their antiproliferative activity on two human tumor cell lines, showed only moderate cytotoxic properties, with IC_{50} values higher than 20 μM.



1

R= H, CH_3; R'= H, Cl, OCH_3, NO_2

In an effort to enhance the cytotoxic activity of this class of compounds it seemed reasonable to further investigate how the position or the nature of the lipophilic moiety linked to the chromophore system was able to affect the capacity of intercalation with DNA base pairs. In this paper, we report the synthesis of the 1-benzyl derivatives **2** and of the 2-phenyl, 2-*p*-methoxyphenyl, 2-benzyl and 2-carboxamidophenyl derivatives **3**, **4**, **5** and **6**, respectively, in which the pendant phenyl ring was inserted at the 2-position of the heterocyclic scaffold both directly and with spacer groups of different length and grade of flexibility (Figure 2).



2a, b

a: R = H
b: R = CH_3

3a, b; 4a, b

3a: R = H R' = H
3b: R = CH_3 R' = H
4a: R = H R' = OCH_3
4b: R = CH_3 R' = OCH_3

5a, b

a: R = H
b: R = CH_3

6a, b

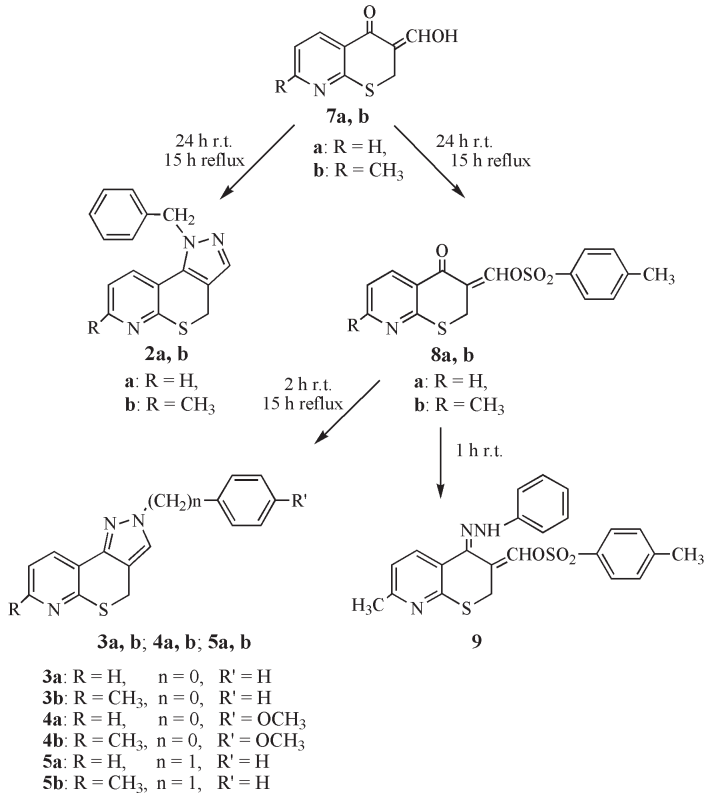
a: R = H
b: R = CH_3

The antiproliferative activity of the new compounds was evaluated *in vitro*, by means of a cell growth inhibition assay on two human tumor cell lines, human promyelocytic leukemic cells (HL-60) and human cervix adenocarcinoma cells (HeLa).

Results.

The synthetic procedure utilized in the preparation of the target compounds **2** and 2-aryl substituted analogues **3**, **4** and **5** is illustrated in Figure 3. The 3-hydroxymethylene-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-one **7a** and its 7-methyl derivative **7b**, which represent the starting key compounds, have been recently described by us [12,13].

The condensation of compounds **7** with benzylhydrazine hydrochloride, in refluxing methanol, gave the desired 1-benzyl pyrazoles **2** as unique and pure isomers, since the reaction of **7** with the hydrazines firstly involves the more reactive methine group adjacent to the C=O function [14-16]. Analytical and spectral data of compounds **2** confirm the proposed structures [12].



The synthetic sequence leading to the 2-aryl substituted 2,4-dihydropyrido[3',2':5,6] thiopyrano[4,3-*c*]pyrazoles **3**, **4** and **5** involved, as a first step, the conversion of the 3-hydroxymethylene compounds **7** into the intermediate *p*-toluensulphonates **8a** or **8b**, in which the reactive methine functionality has been protected [17]. The subsequent condensation of compounds **8** with the appropriate arylhydrazine hydrochloride, in anhydrous dimethylformamide at room temperature, involves only the carbonylic function in the 4-position, affording the intermediate arylhydrazones, which easily cyclize *in situ* by heating (100 °C) the reaction mixture. The removal of the protective tosyl group occurs under these conditions, thus affording the desired 2-substituted products **3**, **4** and **5**. All compounds were purified by flash chromatography and characterized by analytical and spectral data (Table 1). In particular the ^{1}H nmr spectra of compounds **3**, **4** and **5** showed, as the most discriminating feature, a singlet at ~ 8.3 ppm attributed to the proton in the 3-position of the pyrazole moiety, while the same proton of the analogous 1-aryl [12] and 1-benzyl (**2**) substituted compounds resonates at ~ 7.6 ppm. The proposed synthetic pathways have been confirmed by the isolation and characterization of the intermediate 4-phenylhydrazone derivative **9**, which was obtained by stirring **8b** with phenylhydrazine hydrochloride for 1 hour at room temperature. The ^{1}H nmr spectrum of **9** showed two singlet signals at δ 2.43 and δ 2.45 which were

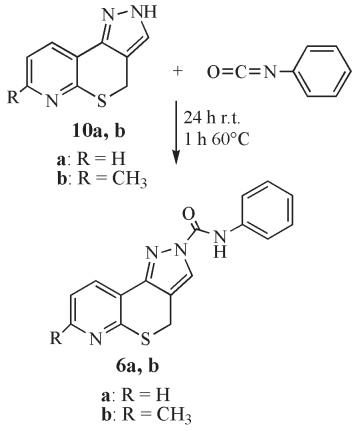
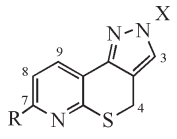


Table 1

Physical and Spectral Data of Compounds **3**, **4**, **5** and **6**



N	R	X	Yield (%)	m.p. °C	ir (cm^{-1})	^{1}H nmr (δ ppm)	Ms m/z	Molecular Formula	Analysis (%) Calcd./Found		
									C	H	N
3a	H	C_6H_5	43	157-160	1590, 1495, 1400, 965, 810, 750, 695	4.18 (s, 2H, *CH*$_2$S); 6.89-7.01 (m, 2H, 8-H, 4'Ph); 7.37-7.41 (m, 2H, 3',5'Ph); 7.51-7.56 (m, 3H,7-H, 2',6'Ph); 7.69 (s, 1H, 3-H); 8.23 (dd, 1H, $J_{9,8}$=6.8 Hz, $J_{9,7}$=1.9 Hz, 9-H).	265 (2, M$^+$), 214 (100)	$C_{15}H_{11}N_3S$	67.92 67.56	4.15 4.06	15.85 15.78
3b	CH_3	C_6H_5	35	177-179	1590, 1500, 1420, 960, 830, 765, 690	2.42 (s, 3H, 7-CH_3); 4.25 (s, 2H, *CH*$_2$S); 7.10 (d, 1H, J=7.8 Hz, 8-H); 7.33 (t, 1H, 4'Ph); 7.52 (t, 2H, 3',5'Ph); 7.86 (d, 2H, 2',6'Ph); 8.05 (d, 1H, J=7.8 Hz, 9-H); 8.44 (s, 1H, 3-H).	279 (27, M$^+$), 214 (100)	$C_{16}H_{13}N_3S$	68.82 68.56	4.52 4.66	14.80 15.05
4a	H	*p*-OCH_3-C_6H_4	28	79-82	1520, 1300, 1250, 1025, 830, 755, 720	3.80 (s, 3H, O*CH*$_3$); 4.27 (s, 2H, *CH*$_2$S); 7.08 (d, 2H, J=8.6 Hz, 2',6'Ph); 7.23 (dd, 1H, $J_{8,9}$=7.6 Hz, $J_{8,7}$=6.8 Hz, 8-H); 7.77 (d, 2H, J=8.6 Hz, 3',5' Ph); 8.14 (d, 1H, J=7.7 Hz, 9-H); 8.33 (d, 1H, J=6.8 Hz, 7-H); 8.34 (s, 1H, 3-H).	295 (5, M$^+$), 149 (100)	$C_{16}H_{13}N_3OS$	65.08 64.82	4.41 4.53	14.24 14.16
4b	CH_3	*p*-OCH_3-C_6H_4	27	103-106	1515, 1290, 1255, 1025, 830, 755, 660	2.42 (s, 3H, 7-CH_3); 3.80 (s, 3H, 4'-OCH_3); 4.24 (s, 2H, *CH*$_2$S); 7.07 (d, 2H, J=9 Hz, 2',6'Ph); 7.09 (d, 1H, J=7.8 Hz, 8-H); 7.76 (d, 2H, J=9 Hz, 3',5'Ph); 8.02 (d, 1H, J=7.8 Hz, 9-H); 8.31 (s, 1H, 3-H).	309 (3, M$^+$), 215 (100)	$C_{17}H_{15}N_3OS$	66.02 65.86	4.85 4.90	13.59 13.34
5a	H	$CH_2C_6H_5$	85	140-143	1485, 1400, 1095, 800, 750 710	4.12 (s, 2H, *CH*$_2$S); 5.65 (s, 2H, *CH*$_2$Ph); 7.03 (d, 2H, 2',6'Ph); 7.10-7.17 (dd, 1H, $J_{8,7}$ =4.7 Hz, $J_{8,9}$=7.9 Hz, 8-H); 7.24-7.35 (m, 3H, 3',4',5'Ph); 7.54 (s, 1H, 3-H); 7.80 (dd, 1H, $J_{9,7}$ =1.6 Hz, $J_{9,8}$=7.8 Hz, 9-H); 8.22 (dd, 1H, $J_{7,8}$ =4.6 Hz, $J_{7,9}$= 1.7 Hz, 7-H).	279 (67, M$^+$), 149 (100)	$C_{16}H_{13}N_3S$	68.82 68.96	4.66 4.78	15.05 14.89
5b	CH_3	$CH_2C_6H_5$	71	102-105	1575, 1500, 1130, 1100, 1050, 840, 750, 715	2.35 (s, 3H, 7-CH_3); 4.09 (s, 2H, *CH*$_2$S); 5.63 (s, 2H, *CH*$_2$Ph); 6.97-7.04 (m, 3H, 8-H, 2',6'Ph); 7.23-7.35 (m, 3H, 3',4',5'Ph); 7.51 (s, 1H, 3-H); 7.71 (d, 1H, 9-H).	293 (68, M$^+$), 149 (100)	$C_{17}H_{15}N_3S$	69.62 69.86	5.12 4.96	14.33 13.96
6a	H	$CONHC_6H_5$	33	118-121	3295, 1725, 1590, 1535, 1060, 880, 805, 765, 695	4.28 (s, 2H, *CH*$_2$S); 6.94 (t, 1H, 4'Ph); 7.13-7.28 (m,1H, 8-H); 7.29-7.50 (m, 2H, 3',5'Ph); 7.74 (d, 2H, 2',6'Ph); 8.31 (dd, 1H, $J_{9,7}$ =1.6 Hz, 9-H); 8.32 (s, 1H, 3-H); 8.41 (dd, 1H, $J_{7,9}$=1.6 Hz, 7-H); 10.34 (s, 1H, NH exch.).	308 (2, M$^+$), 149 (100)	$C_{16}H_{12}N_4OS$	62.34 62.00	3.80 4.03	18.18 17.86
6b	CH_3	$CONHC_6H_5$	30	158-161	3290, 1730, 1500, 1095, 890, 830, 755, 695	2.41 (s, 3H, 7-CH_3); 4.25 (s, 2H, *CH*$_2$S); 6.95 (t, 1H, 4'H); 7.27-7.36 (m, 2H, 3',5'Ph); 7.45 (d, 2H, 2',6'Ph); 7.74 (d, 1H, J=7.8 Hz, 8-H); 8.21 (d, 1H, J=7.9 Hz, 9-H); 8.33 (s, 1H, 3-H); 10.30 (s, 1H, NH exch.).	322 (2, M$^+$), 149 (100)	$C_{17}H_{14}N_4OS$	63.00 63.09	4.34 4.55	17.39 17.46

assigned to the methyl groups of the thiopyrane nucleus and of the tosyl moiety, respectively. Moreover, the presence of the nine-proton multiplet signals at δ 7.12-7.23 and δ 7.35-7.66, assigned to the aromatic protons either of the phenylhydrazine and of the tosyl group, was consistent with the structure of **9**.

The 2-carboxamidophenyl derivatives **6** were prepared from the pyridothiopyranopyrazoles **10** [12], by reaction with an excess of phenylisocianate (Figure 4). The assignment of the position of the carboxamido side chain was made on the basis of their proton nmr spectra, which showed important similarities to the above described compounds. The ^{1}H nmr spectra of **6** exhibited a singlet at ~ 8.3 ppm, which was assigned to the proton in the 3-position of the pyrazole ring, allowing us to infer that the carboxamidophenyl group is linked to the 2-position of the chromophore system (Table 1).

The antiproliferative activity of compounds **2-6** was evaluated by means of a cell growth inhibition assay on two human tumor cell lines, HL-60 and HeLa, in accordance with the experimental procedures previously described [11]. Moreover, the cytotoxic ability of the pyridothiopyranopyrazole nucleus **10** was also investigated in order to elucidate if the scaffold itself is biologically active or is a good carrier for other suitable molecular groups. The results, expressed as IC_{50} values and reported in Table 2, indicated that either 1-benzylderivatives **2** or those substituted in the 2-position (**3**, **4**, **5** and **6b**) do not exert any cytotoxic effect, while compound **6a** showed a detectable antiproliferative activity in both cell lines. As regard the pyrazole system, compound **10a** showed the ability to induce a cytotoxic effect, thus appearing an interesting chromophore system. It is also noteworthy that the insertion of a methyl group in the 7-position seemed to be detrimental for the biological activity.

Table 2

Antiproliferative activity of compounds **2**, **3**, **4**, **5**, **6** and **10**

Compound	Cellular lines IC_{50} (μM)	
	HeLa	HL-60
2a	>20	>20
2b	>20	>20
3a	>20	>20
3b	>20	>20
4a	>20	>20
4b	>20	>20
5a	>20	>20
5b	>20	>20
6a	6.6±0.3	13.5±0.5
6b	>20	>20
10a	8.8±0.2	9.0±0.5
10b	>20	>20

EXPERIMENTAL

Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra (ir) were obtained on a NICOLET/AVATAR, 360 FT spectrophotometer as Nujol mulls. Nuclear magnetic resonance (nmr) spectra were recorded on a Varian Gemini 200 spectrometer, in dimethyl-d_6 sulfoxide solution, using TMS as the internal standard. Mass spectra (ms) were obtained on a Finningan Polaris/GCQ Plus spectrometer using 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.

1-Benzyl-1,4-dihydropyrido[3',2':5,6]thiopyrano[4,3-*c*]pyrazole **2a** and 7-Methyl derivative **2b**.

General Procedure.

Benzylhydrazine hydrochloride (0.215 g, 1.10 mmoles) was added to a solution of 3-hydroxymethylene-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-one **7a** (0.193 g, 1.00 mmole) or 7-methyl-derivative **7b** (0.207 g, 1.00 mmole) in 15 mL of methanol, and the reaction mixture was stirred at room temperature for 24 hours, then refluxed for 15 hours. After cooling, the yellow precipitate, if present, was collected and the filtrate was evaporated under reduced pressure. The solid and the residue were treated with a saturated aqueous potassium carbonate solution to give crude pyrazoles **2**, which were purified by recrystallization from ethanol.

Compound **2a** was obtained as a yellow solid in 32% yield: m.p. 135-137 °C; ir: 1580, 1550, 1310, 1090, 990, 800, 750, 700 cm^{-1}; ^{1}H nmr: δ 4.12 (s, 2H, *CH*$_2$S), 5.65 (s, 2H, *CH*$_2$Ph), 7.03 (d, 2H, 2',6'-PhH), 7.10-7.17 (dd, 1H, $J_{8,7}$=4.7 Hz, $J_{8,9}$=8 Hz, 8-H), 7.24-7.36 (m, 3H, 3',4,'5'-PhH), 7.54 (s, 1H, 3-H), 7.80 (dd, 1H, $J_{9,8}$=8 Hz, $J_{9,7}$=1.6 Hz, 9-H), 8.23 (dd, 1H, $J_{7,8}$=4.7 Hz, $J_{7,9}$=1.6 Hz, 7-H); ms: m/z 279 (90, M$^+$), 149 (100).

Anal. Calcd. for $C_{16}H_{13}N_3S$: C, 68.82; H, 4.66; N, 15.05. Found: C, 68.69; H, 4.84; N, 14.84

Compound **2b** was obtained as an orange solid in 33% yield: m.p. 98-101 °C; ir: 1580, 1500, 1130, 845, 765, 710 cm^{-1}; ^{1}H nmr: δ 2.35 (s, 3H, *CH*$_3$), 4.09 (s, 2H, *CH*$_2$S), 5.63 (s, 2H, *CH*$_2$Ph), 6.97-7.05 (m, 3H, J=8 Hz, 8-H, 2', 6'-Ph), 7.27-7.32 (m, 3H, 3',4',5'-PhH), 7.52 (s, 1H, 3-H), 7.71 (d, 1H, J=8 Hz, 9-H); ms: m/z 293 (60, M$^+$), 149 (100).

Anal. Calcd. for $C_{16}H_{13}N_3S$: C, 69.0; H, 5.11; N, 14.33. Found: C, 68.86; H, 4.91; N, 13.96

3-[(*p*-Methylphenyl)sulhonyl]oxymethylen-2,3-dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-one **8a** and 7-Methyl Derivative **8b**.

General Procedure.

p-Toluensulphonylchloride (3.96 g, 20.8 mmoles) was added to a solution of **7a** (2.0 g, 10.4 mmoles) or **7b** (2.152 g, 10.4 mmoles) in 40 mL of anhydrous tetrahydrofurane in the presence of potassium carbonate (5.72 g, 41.6 mmoles). The reaction mixture, under nitrogen atmosphere, was stirred at room temperature for 24 hours then refluxed for 15 hours. After cooling, the suspension was concentrated under reduced pressure, and the residue obtained was treated with water and then extracted with chloroform. The organic layers were dried and evaporated to give

crude products 8 which were purified by flash chromatography on a silica gel column (60/0.040-0.063 mm) using petroleum ether 60-80°C/ethyl acetate 7/3 as the eluting system.

Compound **8a** was obtained as an orange solid in 39.4% yield: m.p. 60-63 °C; ir: 1670, 1600, 1200, 1130, 935, 840, 740, 675 cm^{-1}; ^1H nmr: δ 2.44 (s, 3H, 4'-CH_3), 4.02 (s, 2H, CH_2S), 7.32 (dd, 1H, $J_{6,7}$=4.6 Hz, $J_{6,5}$=7.9 Hz, 6-H), 7.49 (s, 1H, C*H*O), 7.55 (d, 2H, J=8.2 Hz, 3',5'-PhH), 7.97 (d, 2H, J=8.2 Hz, 2',6'-PhH), 8.24 (dd, 1H, $J_{5,6}$=7.9 Hz, $J_{5,7}$=1.6 Hz, 5-H), 8.56 (dd, 1H, $J_{7,6}$=4.6 Hz, $J_{7,5}$=1.6 Hz, 7-H); ms: m/z 347 (2, M^+), 149 (100).

Anal. Calcd. for $C_{16}H_{13}NO_4S_2$: C, 55.33; H, 3.74; N, 4.03. Found: C, 55.42; H, 3.55; N, 4.04

Compound **8b** was obtained as an orange solid in 57.7% yield: m.p. 135-137°C; ir: 1670, 1605, 1195, 1070, 940, 835, 760, 670 cm^{-1}; ^1H nmr: δ 2.43 (s, 3H, 4'-CH_3), 2.45 (s, 3H, 7-CH_3), 3.98 (s, 2H, CH_2S), 7.17 (d, 1H, J=8.0 Hz, 6-H), 7.45 (s, 1H, C*H*O), 7.55 (d, 2H, J=8.3 Hz, 3',5'-PhH), 7.97 (d, 2H, J=8.3 Hz, 2',6'-PhH), 8.13 (d, 1H, J=8.0 Hz, 5-H); ms: m/z 361 (1, M^+), 149 (100).

Anal. Calcd. for $C_{17}H_{15}NO_4S_2$: C, 56.51; H, 4.15; N, 3.88. Found: C, 56.09; H, 4.51; N, 3.87

2-(Phenyl)- **3a**, 2-(*p*-Methoxyphenyl)- **4a**, 2-(Benzyl)-2,4-dihydropyrido[3',2':5,6] thiopyrano[4,3-*c*]pyrazole **5a** and 7-Methyl derivatives **3b-5b**.

General Procedure.

The required arylhydrazine hydrochloride (2.4 mmoles) was added to a solution of **8a** (0.694 g, 2.00 mmoles) or **8b** (0.722 g, 2.00 mmoles) in 10 mL of anhydrous dimethylformamide. The reaction mixture, under nitrogen atmosphere, was stirred at room temperature for 2 hours, then refluxed at 100 °C for 15 hours. After cooling, the suspension was poured into a saturated aqueous potassium carbonate solution and extracted with chloroform. The organic layers were dried and evaporated under reduced pressure to give desired crude pyrazoles **3, 4** and **5**, which were purified by flash chromatography on a silica gel column (60/0.040-0.063 mm) using petroleum ether 60-80 °C/ethyl acetate 3/2 as the eluting system (Table 1).

7-Methyl-3-[(4-methylphenyl)sulphonyl]oxymethylen-2,3-Dihydrothiopyrano[2,3-*b*]pyridin-4(4*H*)-phenylhydrazone (**9**).

Phenylhydrazine hydrochloride (0.048 g, 0.332 mmole) was added to a solution of **8b** (0.100 g, 0.277 mmole) in 10 mL of anhydrous dimethylformamide. The reaction mixture was stirred at room temperature for 1 hour under nitrogen atmosphere then poured into a saturated aqueous potassium carbonate solution. The suspension obtained was filtered to give the desired crude phenylhydrazone **9** as an orange solid (84 % yield), which was analyzed without crystallization, due to its instability to warming up. m.p. 75-77 °C; ir: 3400, 1580, 1350, 1230, 1065, 940, 830, 760, 675 cm^{-1}; ^1H nmr: δ: 2.43 (s, 3H, 4'-CH_3), 2.44 (s, 3H, 7-CH_3), 3.99 (s, 2H, CH_2S), 6.82 (s, 1H, C*H*O), 7.12-7.23 (m, 3H, PhH), 7.35-7.66 (m, 6H, PhH), 7.96 (d, 1H, J=8.3 Hz, 6-H), 8.21 (d, 1H, J=8.4 Hz, 5-H); ms: m/z 451 (2, M^+), 162 (100), 225 (95).

Anal. Calcd. for $C_{23}H_{21}N_3O_3S_2$: C, 61.19; H, 4.66; N, 9.31. Found: C, 61.37; H, 4.55; N, 9.04

2-(*N*-Phenylcarboxamido)pyrido[2',3':5,6]thiopyrano[4,3-*c*]-pyrazole **6a** and 7-Methyl derivative **6b**.

General Procedure.

Phenylisocyanate (0.3 mL, 2.8 mmoles) was added dropwise to a stirred solution of compound **10a** (0.454 g, 2.4 mmoles) or **10b** (0.487 g, 2.4 mmoles) in 15 mL of anhydrous tetrahydrofurane, under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 24 hours adding during this time three additional amounts of 0.1 mL of phenylisocyanate, then the mixture was heated at 60 °C for 1 hour. After cooling, the suspension obtained was filtered and the pinkish solid was collected to give the crude products **6** which were purified by recrystallization from ethanol (Table 1).

Acknowledgments.

This work was supported by grants from MIUR (Research fund Cofin 2004).

REFERENCES AND NOTES

* To whom correspondence should be addressed. E-mail: *marini@farm.unipi.it*; Tel: +39-050-2219555; Fax: +39-050-2219605.

[1] E. Borowski, D. Shugar, in "Molecular Aspects of Chemotherapy", Pergamon Press, New York, (1991).

[2] S. Neidle, *Anti-Cancer Drug Des*., **12**, 433, (1997).

[3] S. Neidle, D. E. Thurston, In New Targets for Cancer Chemotherapy, D.J. Kerr, P. Workman, Eds.; CRC Press: Boca Raton, FL, (1994).

[4] L.P.G. Wakelin, M.J. Waring, "DNA intercalating agents" in Comprehensive Medicinal Chemistry, Pergamon Press, New York, (1990).

[5] B. C. Baguley, *Anti-cancer Drug Des*., **6**, 1 (1991).

[6] U. Pindur, G. Fisher, *Curr. Med. Chem*., **3**, 379 (1996), and references therein.

[7] M. F. Brana, M. Cacho, A. Gradillas, A. Ramos, *Curr. Pharmaceutical Des.*, **7**, 1745 (2001).

[8] S. D. G. Crow, C. Bailly, C. Garbay-Jaureguiberry, B. Proques, B. R. Shaw, M. J. Waring, *Biochemistry*, **42**, 8672, (2002).

[9] A. Da Settimo, A. M. Marini, G. Primofiore, F. Da Settimo, S. Salerno, G. Viola, L. Dalla Via, S. Marciani Magno, *Eur. J. Med. Chem*., **33**, 685 (1998).

[10] A. Da Settimo, A. M. Marini, G. Primofiore, F. Da Settimo, S. Salerno, L. Dalla Via, O. Gia, S. Marciani Magno, *Farmaco*, **56**, 159 (2001).

[11] L. Dalla Via, O. Gia, S. Marciani Magno, A. Da Settimo, G. Primofiore, F. Da Settimo, F. Simorini, A. M. Marini, *Eur. J. Med. Chem*., **37**, 475 (2002).

[12] G. Primofiore, A. M. Marini, F. Da Settimo, S. Salerno, D. Bertini, L. Dalla Via, S. Marciani Magno, *J. Heterocyclic Chem*., **40**, 783 (2003).

[13] A. Da Settimo, A. M. Marini, G. Primofiore, F. Da Settimo, S. Salerno, F. Simorini, G. Pardi, C. La Motta, D. Bertini, *J. Heterocyclic Chem*., **39**, 1001 (2002).

[14] A. Da Settimo, G. Primofiore, F. Da Settimo, F. Simorini, *Drug Design and Discovery*, **11**, 307 (1994).

[15] L. Mosti, G. Menozzi, P. Schenone, *J. Heterocyclic Chem.*, **21**, 361 (1984).

[16] G. Winters, A. Sala, B. Barone, E. Baldioli, *J. Med. Chem*., **28**, 934 (1985).

[17] I. K. Kostakis, P. Magiatis, N. Pouli, P. Marakos, A. Pierrè, *J. Med. Chem*., **45**, 2599 (2002).