Synthesis of a Novel Purine-Containing Heterocyclic Ring System: 8,10-Dimethylindolo[2',3':5,6][1,2,4]triazino[4,3-*f*]purine-9,11(8*H*,10*H*,13*H*)-dione

Antonio Da Settimo, Giampaolo Primofiore, Federico Da Settimo*, Francesca Simorini, Anna Maria Marini, Concettina La Motta and Sabrina Taliani

Dipartimento di Scienze Farmaceutiche, Via Bonanno 6, I-56126 Pisa, Italy

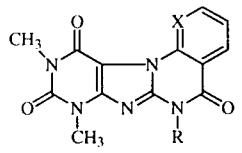
Received July 20, 1999

The preparation of a novel purine containing heterocyclic ring system, indolotriazinopurine, by the condensation of 8-hydrazinotheophylline with 5-substituted isatins *via* the intermediate hemiaminal and hydrazone derivatives, is described.

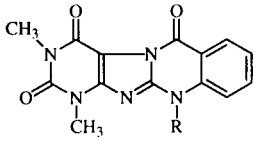
J. Heterocyclic Chem., **37**, 373 (2000).

DNA complexing agents are important anticancer drugs, as they can lead to cell death by inhibition of replicative enzymes and DNA repair systems or by interfering with topoisomerases. These compounds generally possess the following common structural features: an aromatic or heteroaromatic polycyclic ring system with a planar or pseudoplanar shape of the resulting molecule [1-4].

On the basis of these results and in connection with our program of preparing new polyheterocyclic compounds, which might exhibit an antiproliferative activity, in the last few years we have reported the synthesis of numerous molecules which contain the purine nucleus fused with several heterocyclic systems, such as purinoquinazoline **1a**, **2** [5], purinopyridopyrimidine **1b** [6], 2,4-benzodiazepinopurine **3** and 2,3,5-triazocinopurine **4** [7] derivatives.



1a, X=CH
1b, X=N
R=H, CH_3, $(CH_2)_nNR_2$



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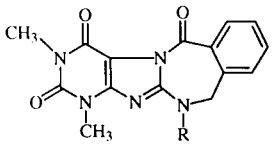

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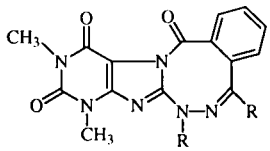

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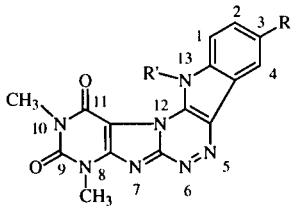
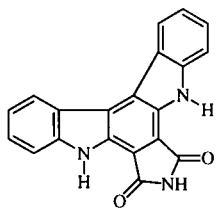
Some of these molecules, functionalized with an alkylamino-substituted side chain, exhibited an antiproliferative activity, as these compounds are able to form a complex with DNA and to inhibit topoisomerase II [6].

As a continuation of our study on new heteropolycyclic derivatives as potential antitumor agents, in particular those containing the purine nucleus, we now report the synthesis of indolo-1,2,4-triazinopurine derivatives **5** and **6**, possessing a new heterocyclic 6,5,6,5,6 ring system.

It is worth noting that the title compounds **5** have some structural features in common with indole[2,3-*a*]carbazole derivatives such as arcyriaflavin-A **7**, which is a potent inhibitor of protein kinase C (PKC) [8]. This enzyme has been implicated in the regulation of various cellular processes including growth, differentiation and tumor promotion. Indolocarbazole derivatives have also been found to exert a potent antitumor activity acting as human topoisomerase I (Topo I) poison [9,10].



5: R'=H
6: R'=CH_3
R=H, F, NO_2



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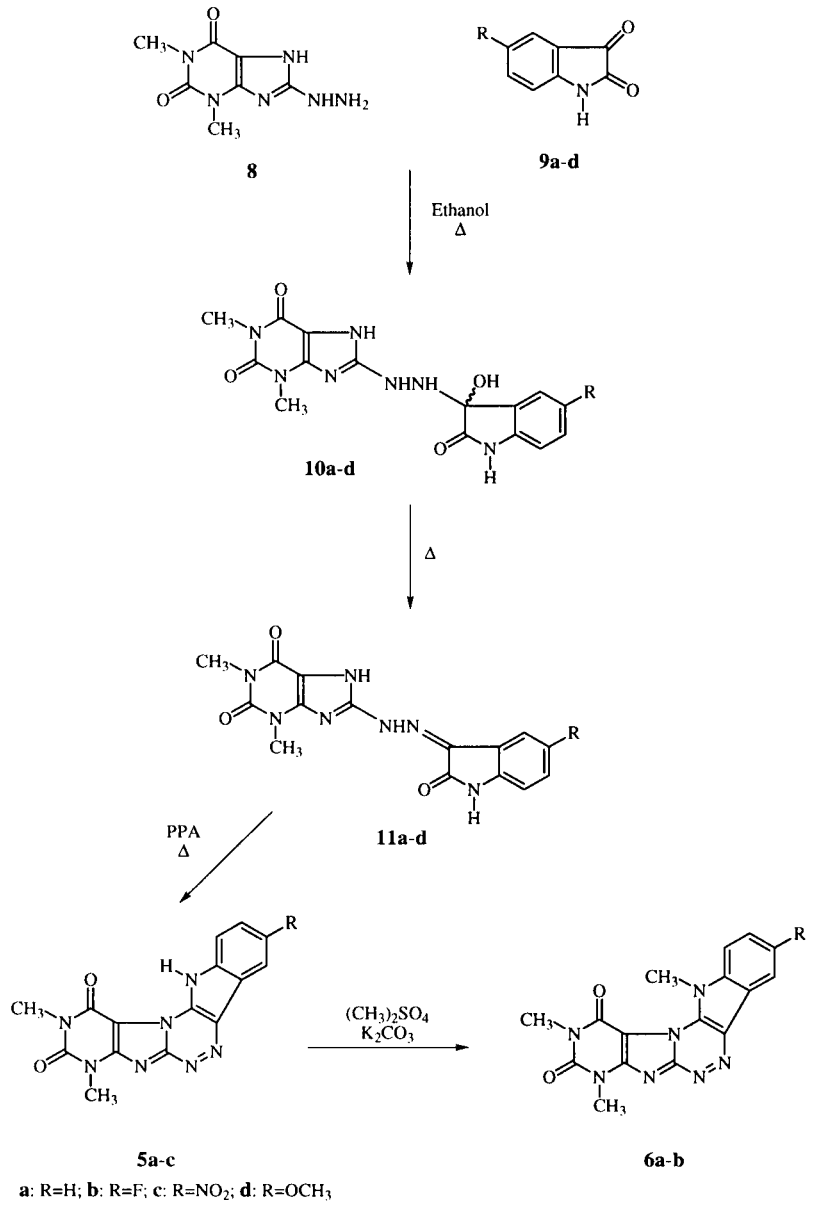
The target compounds **5** were synthesized as shown in Scheme 1.

The starting materials 8-hydrazinotheophylline **8** and 5-methoxyisatin **9d**, which were not commercially available, were easily prepared following described procedures [11,12].

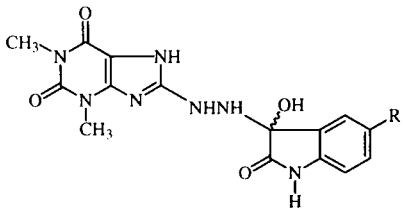
Scheme 1



The reaction of **8** with the appropriate isatin **9a-d** in ethanol at reflux for several hours (16-24 hours) gave, after cooling, yellow-orange crystals. The careful examination of analytical and spectral data made it possible to assign to these products the hemiaminal structure **10a-d** and not the expected hydrazone **11a-d**. The ir spectrum showed broad peaks between 3525 and 3450 cm^{-1} (with the exclusion of **10c**), relative to the OH stretching, and a strong C-O stretching vibration in the region from 1190 to 1060 cm^{-1}, but no absorption in the C=N region (Table 1). Compounds **10a-d**, when heated at 250 °C for 2 hours in a glass tube oven, remained unchanged, but they proved to be labile in solution. It was not possible to purify them by recrystallization from dimethylformamide because of their transformation to the corresponding hydrazones **11a-d**. The ease with which compounds **10a-d** lose a molecule of water was also confirmed by the ms spectrum, obtained with a direct injection probe, using an electron beam energy of 70 eV, in which it was not possible to detect the molecular peak (M^+), but only the peak relative to the molecular weight minus eighteen (M^+-H_2O). When compounds **10a,b,d** were refluxed in dimethylformamide for 1 hour, the hydrazones **11a,b,d** were obtained in high yields. A good yield of **11c** was obtained when **10c** was refluxed for 1 hour in ethanol saturated with hydrogen chloride.

Table 1

Physical Properties and Spectral Data of *N*-(Theophyllin-8-yl)-*N'*-(5-substituted 3-hydroxy-2-oxoindolin-3-yl)hydrazine Derivatives **10a-d**



N	R	Yield (%)	Mp (°C)	IR (cm^{-1})	^1H NMR [a] δ (ppm)	MS m/z	Molecular Formula	Analysis (%) Calcd./Found C	H	N
10a	H	95	>300	3450, 3150, 1690, 1640, 1600, 1180, 740	3.57 (s, 3H, 1-CH_3); 3.71 (s, 3H, 3-CH_3); 7.02-8.14 (m, 4H, ArH)	339 (M^+ -H_2O)	$C_{15}H_{15}N_7O_4$	50.42 50.78	4.23 3.95	27.44 27.45
10b	F	97	>300	3450, 3150, 1700, 1640, 1600, 1520, 1180, 750	3.55 (s, 3H, 1-CH_3); 3.68 (s, 3H, 3-CH_3); 7.02-7.98 (m, 3H, ArH)	357 (M^+ -H_2O)	$C_{15}H_{14}FN_7O_4$	48.00 47.78	3.76 3.90	26.12 26.44
10c	NO_2	96	>300	3150, 1680, 1640, 1620, 1520, 1060, 750	3.56 (s, 3H, 1-CH_3); 3.68 (s, 3H, 3-CH_3); 7.25 (d, 1H, 7'-H); 8.37 (dd, 1H, 6'-H); 8.71 (d, 1H, 4'-H)	384 (M^+ -H_2O)	$C_{15}H_{14}N_8O_6$	44.78 45.01	3.51 3.35	27.85 27.54
10d	OCH_3	99	>300	3525, 3475, 3150, 1710, 1650, 1630, 1190, 750	3.57 (s, 3H, 1-CH_3); 3.71 (s, 3H, 3-CH_3); 3.96 (s, 3H, O-CH_3); 7.07-7.80 (m, 3H, ArH)	369 (M^+ -H_2O)	$C_{16}H_{17}N_7O_5$	49.61 49.28	4.42 4.08	25.31 25.54

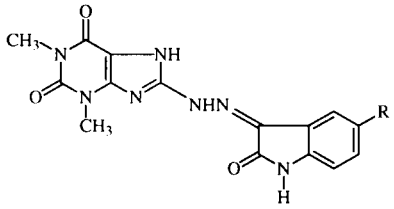
[a] Solvent: 20% trifluoroacetic acid-d in deuteriochloroform.

All compounds **11a-d** were very high-melting yellow-orange crystalline solids. The structures of **11a-d** were unequivocally confirmed by elemental analysis, ir, ^1H nmr and mass spectral data (Table 2). The ir spectrum showed C=N absorption bands in the region from 1560 to 1550 cm^{-1}, while the absorption in the OH region typical of the hemiaminal **10a-d** disappeared.

Hydrazones **11a-c** were easily cyclized to **5a-c** with a satisfactory yield by treatment with an excess of polyphosphoric acid (PPA), at 220 °C, for 15-20 minutes (Table 3).

Table 2

Physical Properties and Spectral Data of 5-Substituted 3-(Theophyllin-8-yl)hydrazonoindolin-2-one Derivatives **11a-d**

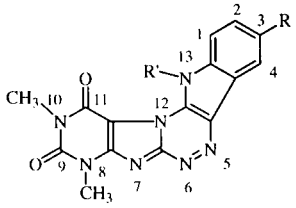


N	R	Yield (%)	Mp (°C)	IR (cm^{-1})	^1H NMR [a] δ (ppm)	MS m/z	Molecular Formula	Analysis (%) Calcd./Found C	H	N
11a	H	85	>300	3280, 1680, 1640, 1560, 1140, 760	3.56 (s, 3H, 1-CH_3); 3.70 (s, 3H, 3-CH_3); 7.01-7.97 (m, 4H, ArH)	339	$C_{15}H_{13}N_7O_3$	53.10 53.39	3.86 4.19	28.89 29.23
11b	F	80	>300	3260, 1700, 1650, 1560, 1160, 760,	3.56 (s, 3H, 1-CH_3); 3.70 (s, 3H, 3-CH_3); 7.03-7.71 (m, 3H, ArH)	357	$C_{15}H_{12}FN_7O_3$	50.42 50.19	3.39 3.04	27.44 27.64
11c	NO_2	77	>300	3180, 1700, 1680, 1650, 1550, 1160, 740	3.58 (s, 3H, 1-CH_3); 3.72 (s, 3H, 3-CH_3); 7.31 (d, 1H, 7'-H); 8.40 (dd, 1H, 6'-H); 8.75 (d, 1H, 4'-H)	ND [b]	$C_{15}H_{12}N_8O_5$	46.88 46.22	3.15 2.87	29.16 29.01
11d	OCH_3	90	>300	3260, 1700, 1685, 1645, 1550, 1140, 760	3.57 (s, 3H, 1-CH_3); 3.70 (s, 3H, 3-CH_3); 3.96 (s, 3H, O-CH_3); 7.05-7.54 (m, 3H, ArH)	369	$C_{16}H_{15}N_7O_4$	52.03 51.78	4.09 3.61	26.55 27.09

[a] Solvent: 20% trifluoroacetic acid-d in deuteriochloroform; [b] ND = not detectable.

Table 3

Physical Properties and Spectral Data of 3-Substituted 8,10-Dimethylindolo[2',3':5,6][1,2,4]triazino[4,3-*f*]purine-9,11(8*H*,10*H*,13*H*)-dione Derivatives **5a-c**, and 3-Substituted 8,10,13-Trimethylindolo[2',3':5,6][1,2,4]triazino[4,3-*f*]purine-9,11(8*H*,10*H*,13*H*)-dione Derivatives **6a-b**



N	R	R'	Yield (%)	Mp (°C)	IR (cm^{-1})	^1H NMR δ (ppm)	^{13}C NMR δ (ppm)	MS m/z	Molecular Formula	Analysis (%) Calcd./Found C	H	N
5a	H	H	45	>300	3230, 1660, 1590, 1550, 1240, 750	3.56 (s, 3H, 10-CH_3); 3.81 (s, 3H, 8-CH_3); 7.30-8.60 (m, 4H, ArH); 11.05 (s, 1H, NH)	155.8 (C-11); 153.3 (C-6a); 151.8 (C-9); 143.1 (C-7a); 139.4, 135.2, 133.3 (C-4b, C-12a, C-13a); 134.4 (C-2); 127.8 (C-3); 123.2 (C-4); 117.6 (C-4a); 116.0 (C-1); 100.5 (C-11a); 31.7 (10-CH_3); 29.5 (8-CH_3) [a]	321	$C_{15}H_{11}N_7O_2$	56.07 55.37	3.45 3.16	30.51 30.52
5b	F	H	54	>300	3150, 1700, 1660, 1580, 1540, 1240, 1160, 1050, 750	3.57 (s, 3H, 10-CH_3); 3.82 (s, 3H, 8-CH_3); 7.98-8.30 (m, 3H, ArH); 11.00 (s, 1H, NH)	161.7 (C-3, J = 250.0 Hz); 155.9 (C-11); 153.2 (C-6a); 151.7 (C-9); 143.1 (C-7a); 135.4 (J = 1.8 Hz); 133.5 (J = 1.8 Hz) (C-4b, C-13a); 134.7 (C-12a); 121.9 (C-2, J = 25.6 Hz); 119.1 (C-4a, J = 10.0 Hz); 117.7 (C-1, J = 9.2 Hz); 109.7 (C-4, J = 26.5 Hz); 100.7 (C-11a); 31.6 (10-CH_3); 29.4 (8-CH_3) [a]	339	$C_{15}H_{10}FN_7O_2$	53.10 52.75	2.97 2.99	28.90 28.87
5c	NO_2	H	18	>300	3220, 1710, 1650, 1590, 1160, 750	3.62 (s, 3H, 10-CH_3); 3.83 (s, 3H, 8-CH_3); 8.14-9.30 (m, 3H, ArH) [a]	156.2 (C-11); 153.4 (C-6a); 151.6 (C-9); 146.1 (C-3); 144.7 (C-7a); 141.8, 134.0, 133.6 (C-4b, C-12a, C-13a); 128.0 (C-2); 118.8 (C-4); 117.2 (C-4a, submerged by solvent signal); 116.5 (C-1); 100.4 (C-11a); 31.8 (10-CH_3); 29.6 (8-CH_3) [a]	366	$C_{15}H_{10}N_8O_4$	49.19 48.82	2.75 2.45	30.59 30.92
6a	H	CH_3	36	>300	1690, 1650, 1560, 1190, 750, 740	3.46 (s, 3H, 10-CH_3); 3.61 (s, 3H, 8-CH_3); 4.34 (s, 3H, 13-CH_3); 7.23-7.99 (m, 4H, ArH)	154.9 (C-11); 152.4 (C-6a); 151.5 (C-9); 149.9 (C-7a); 142.5, 140.5, 140.3 (C-4b, C-12a, C-13a); 131.6 (C-2); 124.2 (C-4a), 123.8 (C-4); 121.2, 121.0 (C-1, C-3); 101.5 (C-11a); 42.6 (13-CH_3); 30.3 (10-CH_3); 28.6 (8-CH_3) [b]	335	$C_{16}H_{13}N_7O_2$	57.31 57.21	3.90 3.73	29.24 29.51
6b	F	CH_3	32	>300	1700, 1660, 1580, 740	3.33 (s, 3H, 10-CH_3); 3.57 (s, 3H, 8-CH_3); 4.30 (s, 3H, 13-CH_3); 7.20-7.78 (m, 3H, ArH)	161.5 (C-3, J = 249.0 Hz); 155.6 (C-11); 152.3 (C-6a); 151.7 (C-9); 142.4 (C-7a); 135.5 (J = 1.8 Hz), 133.1 (J = 1.8 Hz) (C-4b, C-13a); 133.9 (C-12a); 121.9 (C-2, J = 25.6 Hz); 118.8 (C-4a, J = 10.0 Hz); 117.8 (C-1, J = 8.2 Hz); 109.5 (C-4, J = 27.5 Hz); 101.7 (C-11a); 45.8 (13-CH_3); 31.7 (10-CH_3); 29.4 (8-CH_3) [a]	353	$C_{16}H_{12}FN_7O_2$	54.39 54.29	3.42 3.09	27.75 28.07

[a] Solvent: 20% trifluoroacetic acid-d in deuteriochloroform; [b] Solvent: deuteriochloroform.

Surprisingly, several attempts to cyclize **11d** were unsuccessful. Neither treatment with polyphosphoric acid at 220 °C for 15-20 minutes, nor refluxing for 24 hours in ethanol saturated with hydrochloric acid or in glacial acetic acid gave the cyclization product.

The assignment of the structure of **5** was mainly based on the consideration that cyclization on the N(9) of theophylline is not likely, due to the steric hindrance of the 3-methyl, as reported by us for other similar cyclizations [5-7]. As ^{13}C nmr results are very useful for the characterization of complex polyheterocyclic systems, the ^{13}C nmr data of all the pentacyclic compounds **5** and **6** are reported in Table 3. The signals were ascribed taking into account the ^{13}C nmr literature data of systems such as theophylline, caffeine, indole and 2-aminobenzimidazole (data taken from the Integrated Spectral Data Base System for

Organic Compounds - SDBS), and the fluorine coupling in compounds **5b** and **6b** was a further confirmation of the proposed assignments.

By reaction with dimethyl sulfate in acetonitrile solution, in the presence of potassium carbonate, compounds **5a,b** afforded the methyl derivatives **6a,b** in good yields (Table 3). Taking into account that methylation at the N(7) was not possible due to the steric hindrance of the N(8)-methyl, the structure of compounds **6a,b** was inferred from ^1H and ^{13}C nmr spectral analysis. The ^1H nmr spectra (dimethyl-d_6 sulfoxide) of **6a** and **6b** showed a singlet at δ 4.34 ppm and δ 4.30 ppm, respectively, assigned to the methyl at position 13 and not 6, because of the downfield shift with respect to the singlet at δ 3.77 ppm of the N(13)-methyl in 1,3,13-trimethyl[2,3,5]benzotriazocino[5,3-*f*]purine-2,4,6(1*H*,3*H*)-trione **4** [7], and to the singlet at δ 3.96 ppm of the N(11)-methyl in 1,3,11-trimethylpurino[8,7-*b*]quinazoline-2,4,6(1*H*,3*H*,11*H*)-trione **2** [5]. This downfield shift should be due to the deshielding effect of the 11-carbonyl group facing the methyl bound at the N(13). Also the ^{13}C nmr signal at δ 42.6 ppm and δ 45.8 ppm relative to the N(13)-methyl of **6a** and **6b**, respectively, was downfield shifted with respect to the signal at δ 32.3 ppm of the N(13)-methyl in 1,3,13-trimethyl[2,3,5]benzotriazocino[5,3-*f*]purine-2,4,6(1*H*,3*H*)-trione **4** (unpublished results), thus confirming the proposed structure for compounds **6**.

EXPERIMENTAL

Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were obtained on a PYE/UNICAM mod. PU 9561 spectrophotometer in Nujol mulls. Nuclear magnetic resonance spectra were recorded on a Bruker AC200 spectrometer in dimethyl-d_6 sulfoxide solution, unless otherwise reported, using tetramethylsilane (TMS) as the internal standard. 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 performed *in vacuo* (rotating evaporator). Analytical tlc was carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254). Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ± 0.4 %.

N-(Theophyllin-8-yl)-*N'*-(5-substituted 3-hydroxy-2-oxoindolin-3-yl)hydrazine Derivatives **10a-d**.

General Procedure.

A suspension of the appropriate isatin (0.1 mmole) and of 8-hydrazinotheophylline (0.1 mmole) in 50 ml of absolute ethanol was refluxed for 16-24 hours, monitoring the reaction by tlc analysis. After cooling, the yellow-orange solid precipitate was collected and used in the following reaction without any purification (Table 1).

5-Substituted 3-(Theophyllin-8-yl)hydrazonoindolin-2-one Derivatives **11a-d**.

General Procedure.

The hydrazine derivative was refluxed in dimethylformamide for 1 hour (**10a,b,d**) or in ethanol saturated with hydrochloric acid for 3-4 hours (**10c**). After cooling, the yellow-orange solid precipitated was collected and recrystallized from dimethylformamide to give pure hydrazone derivatives **11a-d** (Table 2).

3-Substituted 8,10-Dimethylindolo[2',3':5,6][1,2,4]triazino[4,3-*f*]-purine-9,11(8*H*,10*H*,13*H*)-dione Derivatives **5a-c**.

General Procedure.

A mixture of the appropriate hydrazone derivative **11a-c** and an excess of polyphosphoric acid was heated in an oil bath at 220 °C for 20-30 minutes, monitoring the reaction by tlc analysis. The reaction mixture was allowed to cool at room temperature and then poured into ice. The solid precipitate was collected, washed with water, dried and recrystallized from dimethylformamide to give the pure derivatives **5a-c** (Table 3).

3-Substituted 8,10,13-Trimethylindolo[2',3':5,6][1,2,4]triazino[4,3-*f*]purine-9,11-(8*H*,10*H*, 13*H*)-dione Derivatives **6a-b**.

General Procedure.

A solution of dimethyl sulfate (3.70 mmoles) in acetone (3 ml) was added dropwise to an ice-cooled suspension of compound **5a,b** (0.44 mmole) and anhydrous potassium carbonate (0.46 mmole) in acetonitrile (10 ml). The reaction mixture was stirred at room-temperature for 3-4 days (tlc analysis). The precipitate formed was collected, washed with water and purified by recrystallization from dimethylformamide to yield the pure methyl derivatives **6a-b** (Table 3).

Acknowledgment.

This work was supported by grants from the Ministry of University and Scientific and Technological Research (MURST) (Research fund 60%).

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