Synthesis of Pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one Derivatives and Evaluation of their Interactions with DNA

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The synthesis of new derivatives of the planar tricyclic pyrimido[1,2-*a*]benzimidazole system featuring protonable side chains in the 3 and/or 10 positions is described. The reported literature procedures for the preparation of the intermediate 3-ethoxycarbonylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one **15**, starting from 2-aminobenzimidazole **18** and diethyl ethoxymethylenemalonate, were revised. The interaction with DNA, the intrinsic binding constants, and the antiproliferative activity of a number of compounds (**1-8**, **10**, **11**) were preliminarly investigated.

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Introduction.

The ability to intercalate into DNA constitutes a fundamental property for numerous antitumor compounds. Nowadays, a wide number of intercalating agents have an established place in clinical therapy for the treatment of cancer. Among them, the well-known drugs adriamycin and mitoxantrone represent important examples [1-4].

The molecular requirement for the intercalation process is the presence of a planar chromophore able to form a freely reversible complex between base pairs, whose driving force results from a combination of electrostatic, hydrogen-bonding, van der Waals and hydrophobic interactions [2,3]. Besides the undoubted role of the intercalating portion, the presence of appropriate groups or side chains on the planar moiety often appears to be decisive to increase the binding capacity to DNA of a molecule, and, in this connection, we may underline the role of protonable groups, which are believed to be essential for the binding of a drug to the negatively-charged nucleic acids.

In previous papers [5-7] we described some planar polycyclic derivatives characterized by different dialkylaminoalkyl side chains. The results obtained confirmed the importance of the side chains for the overall cellular effects of the intercalating nucleus. In particular, the structure-activity relationships showed that both the length and the steric hindrance of the side chains are decisive for cytotoxicity.

With the aim of studying new chemical structures with a planar chromophore bearing protonable groups, in this paper we describe the synthesis and the biological evaluation of pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one derivatives **1-14**, which feature various basic side chains in positions 3 and/or 10.

Linear flow dichroism experiments allowed us to study the interaction of compounds **1-8**, **10**, **11** with DNA and to establish their mode of binding. Furthermore, fluorimetric titration was performed to obtain the intrinsic binding constant values (K_i). The capacity of the new compounds to exert an antiproliferative activity was preliminary evaluated by means of an *in vitro* assay using two human tumor cell lines (HeLa and HL-60).

Chemistry.

The intermediates for the synthesis of compounds **1-6** are represented by the heterocycle **15** and its alkylated derivatives **16-17** (Figures 1 and 2).

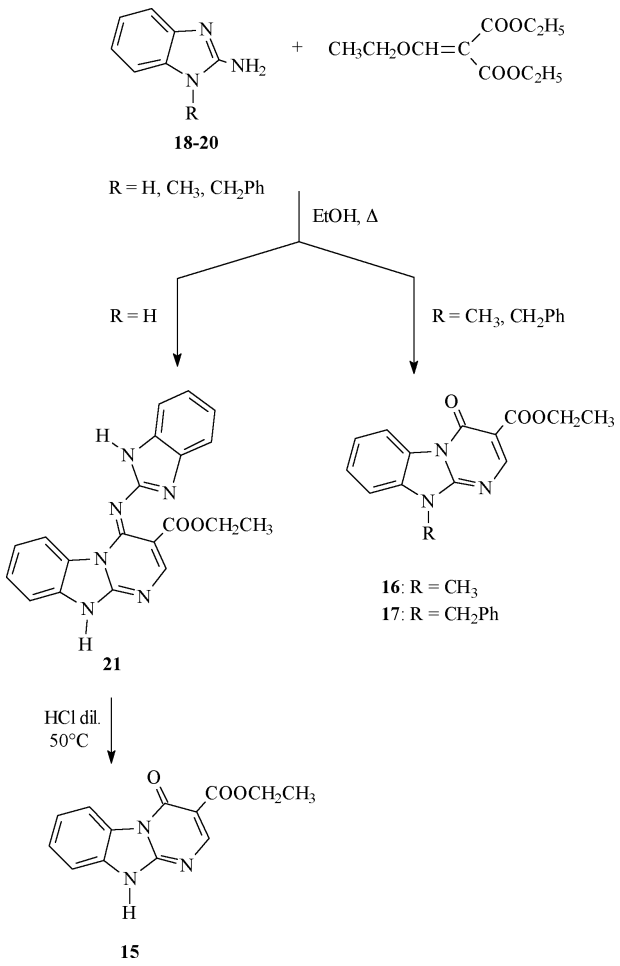


Figure 1

The pyrimidobenzimidazole derivative **15** was obtained by referring to an experimental procedure described in the literature [8], which consisted of the condensation of 2-aminobenzimidazole **18** with diethyl ethoxymethylenemalonate in refluxing ethanol (Figure 1). The authors report that from this reaction they directly obtain the 3-ethoxycarbonylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one **15**, which was characterized by analytical and spectral data [8].

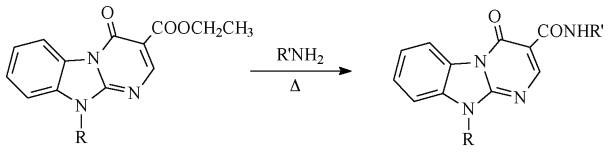
When we repeated this procedure, we obtained a white solid product which we identified as the imino derivative **21** (Figure 1 and experimental section). The structure of **21** was unequivocally confirmed by the analytical and spectral data. In particular, the ^{1}H nmr spectrum of **21** showed two multiplets, each integrating for two protons at δ 6.97-7.02 and 7.19-7.23 relative to the pendant benzimidazole nucleus, distinctly separated from the aromatic envelope of the pyrimidobenzimidazole system, which exhibited its signals between δ 7.25 and 8.65 (see experimental section).

We also obtained the same product **21** when the reaction was carried out following two other reported procedures: a mixture of 2-aminobenzimidazole **18** and diethyl ethoxymethylenemalonate was heated at 100° for 5 minutes, as described in reference [9], or at 110° for 30 minutes, as described in reference [10].

By acid hydrolysis with dilute hydrochloric acid at 50°, the imino derivative **21** gave compound **15** with a high yield (Figure 1). The ir and ^{1}H nmr data shown by our product **15** are in perfect agreement with those reported in the literature [8-10]. Probably the authors obtain **21** as their crude product, which in the recrystallization process undergoes a hydrolysis reaction to compound **15**. Actually, a pure sample of compound **15** was obtained when we recrystallized product **21** from acetic acid [9,10].

In the reaction of **19-20** [11] with diethyl ethoxymethylenemalonate in refluxing ethanol, the target esters **16** [12] and **17** [13] were directly obtained (Figure 1).

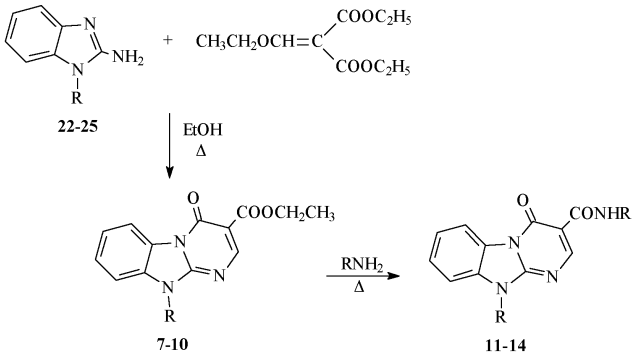
By heating at reflux in an excess of *N*,*N*-dimethylethylenediamine or *N*,*N*-diethylethylenediamine for 12 hours, the ethoxycarbonyl compounds **15-17** were converted to the corresponding amide derivatives **1**, **3**, **5**, and **2**, **4**, **6**, respectively (Figure 2).



R = H, CH_3, $CH_2C_6H_5$
R' = $(CH_2)_2N(CH_3)_2$, $(CH_2)_2N(CH_2CH_3)_2$

Figure 2

The synthetic procedure employed to prepare the pyrimidobenzimidazoles **7-10**, bearing the basic chain in position 10, involved condensation of the appropriate 1-aminoalkyl-2-aminobenzimidazole **22-25** [5,6] with diethyl ethoxymethylenemalonate by heating at reflux in a solution of ethanol (Figure 3). For the synthesis of derivatives **11-14**, which bear two basic chains in their structure, compounds **7-10** were heated at reflux in an excess of the appropriate diamine (Figure 3).



R = $(CH_2)_2N(CH_3)_2$, $(CH_2)_3N(CH_3)_2$, $(CH_2)_2N(CH_2CH_3)_2$, $(CH_2)_3N(CH_2CH_3)_2$

Figure 3

All products **1-14** were purified by recrystallization from ethanol and their structures were confirmed by ir, ms, ^{1}H nmr and elemental analyses (Tables 1 and 2).

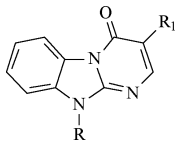
For the biological assays, compounds **1-8**, **10**, **11** were converted into their hydrochlorides by treatment with hydrogen chloride-saturated ethanol, for an easier solubilization in the test media.

Biological Results.

The ability of the new derivatives **1-8**, **10**, **11** to interact with DNA was investigated by means of both flow linear dichroism and fluorimetric titration measurements following the experimental procedures previously described [7]. For all the considered compounds, a dichroic signal at wavelengths higher than 260 nm (310-370 nm) appeared, thus indicating the occurrence of a molecular complex with the macromolecule [5]. To investigate the geometry of binding, the calculation of the average orientation angle α_L was performed [14,15] and the resulting values are shown in Table 3. The values of α_L (80°-83°) obtained for **3**, **4** and **11** are considered consistent with an intercalative mode of binding [16], whilst for the others derivatives the obtained values (64°-77°) indicate a divergence from coplanarity with DNA base plane, probably attributable to the occurrence of both intercalative and groove geometry of binding [6]. In addition, the new derivatives exert a significant fluorescence signal, which is quenched upon addition of DNA. This property allowed for the determination

Table 1

Physical Properties of Pyrimido[1,2-*a*]benzimidazole Derivatives **1-14**



Compd.	R	R_1	Yield (%)	Mp [a] (°C)	Formula	Elemental Analysis					
						Calcd. %			Found %		
						C	H	N	C	H	N
1	H	$CONH(CH_2)_2N(CH_3)_2$	37	145-146	$C_{15}H_{17}N_5O_2$	60.19	5.72	23.40	59.94	5.93	23.37
2	H	$CONH(CH_2)_2N(C_2H_5)_2$	35	60-63	$C_{17}H_{21}N_5O_2$	62.37	6.47	21.39	62.04	6.24	21.37
3	CH_3	$CONH(CH_2)_2N(CH_3)_2$	70	159-161	$C_{16}H_{19}N_5O_2$	61.33	6.11	22.35	61.09	6.35	22.13
4	CH_3	$CONH(CH_2)_2N(C_2H_5)_2$	41	115-117	$C_{18}H_{23}N_5O_2$	63.32	6.79	20.51	63.13	6.93	20.60
5	$CH_2C_6H_5$	$CONH(CH_2)_2N(CH_3)_2$	79	188-190	$C_{22}H_{23}N_5O_2$	67.85	5.95	17.98	67.68	6.13	17.92
6	$CH_2C_6H_5$	$CONH(CH_2)_2N(C_2H_5)_2$	68	135-137	$C_{24}H_{26}N_5O_2$	69.21	6.29	16.81	69.00	6.45	17.07
7	$(CH_2)_2N(CH_3)_2$	$COOC_2H_5$	87	150-152	$C_{17}H_{20}N_4O_3$	62.18	6.14	17.06	61.98	6.16	16.99
8	$(CH_2)_3N(CH_3)_2$	$COOC_2H_5$	90	138-140	$C_{18}H_{22}N_4O_3$	63.14	6.48	16.36	62.98	6.38	16.11
9	$(CH_2)_2N(C_2H_5)_2$	$COOC_2H_5$	64	133-135	$C_{19}H_{24}N_4O_3$	64.03	6.79	15.72	63.90	6.51	15.63
10	$(CH_2)_3N(C_2H_5)_2$	$COOC_2H_5$	82	105-107	$C_{20}H_{26}N_4O_3$	64.85	7.07	15.12	64.82	7.12	14.91
11	$(CH_2)_2N(CH_3)_2$	$CONH(CH_2)_2N(CH_3)_2$	53	168-170	$C_{19}H_{26}N_6O_2$	61.60	7.07	22.68	61.32	7.16	22.42
12	$(CH_2)_3N(CH_3)_2$	$CONH(CH_2)_3N(CH_3)_2$	42	110-112	$C_{21}H_{30}N_6O_2$	63.29	7.59	21.09	63.01	7.49	21.08
13	$(CH_2)_2N(C_2H_5)_2$	$CONH(CH_2)_2N(C_2H_5)_2$	32	73-75	$C_{23}H_{34}N_6O_2$	64.76	8.03	19.70	64.52	7.97	19.55
14	$(CH_2)_3N(C_2H_5)_2$	$CONH(CH_2)_3N(C_2H_5)_2$	37	106-108	$C_{25}H_{38}N_6O_2$	66.05	8.42	18.48	65.82	8.60	18.44

[a] Recrystallization solvent: ethanol.

of intrinsic binding constants whose values appeared in the range from $0.34x10^5$ to $0.60x10^5$ (M^{-1}). Cellular growth inhibition assays on two human tumor cell lines (HeLa e HL-60) [7] revealed that all the considered compounds are unable to exert a cytotoxic effect, despite their ability to form a molecular complex with DNA. On the basis of these results, it is possible to conclude that, despite an intrinsic capacity of compounds **1-8**, **10**, **11** to interact with DNA by forming a molecular complex, their low affinity for the macromolecule can be considered responsible for their inability to exert a detectable antiproliferative effect.

EXPERIMENTAL

Melting points were determined using a Reichert Köfler hot-stage apparatus and are uncorrected. Infrared spectra were recorded with a PYE/UNICAM Infracord Model PU 9516 spectrophotometer in Nujol mulls. Routine nuclear magnetic resonance spectra were recorded in DMSO-d_6 solution on a Varian Gemini 200 spectrometer operating at 200 MHz. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. Evaporation was performed *in vacuo* (rotary evaporator). Analytical tlc was carried out on Merck 0.2 mm precoated silica gel aluminum sheets (60 F-254). Elemental analyses were performed by our Analytical Laboratory and agreed with theoretical values to within ± 0.4%.

The following compounds were prepared in accordance with reported procedures: 1-methyl-2-aminobenzimidazole **19** [11]; 1-benzyl-2-aminobenzimidazole **20** [11]; 3-ethoxycarbonyl-10-methylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one **16** [12], using ethanol as the reaction solvent; 1-(2-dimethylaminoethyl)-2-aminobenzimidazole **22** [6]; 1-(3-dimethylaminopropyl)-2-aminobenzimidazole **23** [5]; 1-(2-diethylaminoethyl)-2-aminobenzimidazole **24** [5]; 1-(3-diethylaminopropyl)-2-amino benzimidazole **25** [5].

4-(Benzimidazol-2-ylimino)-3-ethoxycarbonyl-10*H*-pyrimido[1,2-*a*]benzimidazole (**21**).

A solution of 2-aminobenzimidazole **18** (5.00 g, 37.5 mmol) and diethyl ethoxymethylenemalonate (6.60 mL, 33.0 mmol) in 140 mL of ethanol was heated at reflux for 5 hours. After cooling, the separated white solid was collected, washed with ethanol and purified by recrystallization from DMF to give 6.38 g of the pure imino derivative **21** (52%), mp 293-295° (dec); ir: 3250, 3100, 1700, 1600, 1550, 1240, 780 cm^{-1}; ^1H nmr: δ 1.38 (t, 3H, CH_3, J = 7.1 Hz); 4.30 (q, 2H, CH_2, J = 7.1 Hz); 6.97-7.02 (m, 2H, 5'-, and 6'-H); 7.19-7.23 (m, 2H, 4'-, and 7'-H); 7.25-7.33 (m, 1H, 8-H); 7.38-7.47 (m, 1H, 7-H); 7.56 (dd, 1H, 9-H, J = 7.4, 0.5 Hz); 8.49 (dd, 1H, 6-H, J = 7.4, 0.7 Hz); 8.65 (s, 1H, 2-H); ms: m/z 257 (22), 212 (73), 133 (100).

Anal. Calcd. for $C_{20}H_{16}N_6O_2$: C, 64.51; H, 4.33; N, 22.57. Found: C, 64.31; H, 4.58; N, 22.33.

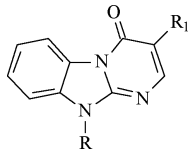
The same derivative **21** was also obtained following the synthetic procedure described in references [9] and [10]. Recrystallization of **21** from AcOH gave a pure sample of compound **15**.

3-Ethoxycarbonylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (**15**).

A suspension of **21** (3.91 g, 10.5 mmol) in 80 mL of dilute hydrochloric acid was heated at 50° for 5 hours. After cooling, the separated white solid was collected and purified by recrystallization

Table 2

Spectral Data of Pyrimido[1,2-*a*]benzimidazole Derivatives **1-14**



Compd.	R	R_1	^{1}H nmr (δ ppm)	ir nujol cm^{-1}	ms m/z (%)
1	H	$CONH(CH_2)_2N(CH_3)_2$	2.71 (s, 6H, $N(CH_3)_2$); 3.09 (t, 2H, $NHCH_2CH_2N$, J=5.7Hz); 3.58-3.64 (m, 2H, $NHCH_2CH_2N$); 7.14-7.23 (m,1H, 8-H); 7.32-7.40 (m, 1H, 7-H); 7.54 (d, 1H, 9-H, J=8.2Hz); 8.42 (d, 1H, 6-H, J=7.6Hz); 8.72 (s, 1H, 2-H); 9.33 (t, 1H, CONH, J=5.2Hz, deuterium oxide-exchangeable).	3250, 1650, 1600, 1290, 1220, 740.	255 (0.82); 212 (16); 58 (100).
2	H	$CONH(CH_2)_2N(C_2H_5)_2$	1.15 (t, 6H, $N(CH_2CH_3)_2$, J=7.1Hz); 2.98-3.07 (m, 6H, $NHCH_2CH_2N$, and $N(CH_2CH_3)_2$); 3.53-3.62 (m, 2H, $NHCH_2CH_2N$); 7.12-7.19 (m, 1H, 8-H); 7.29-7.36 (m, 1H, 7-H); 7.54 (d, 1H, 9-H, J=7.8Hz); 8.42 (d, 1H, 6-H, J=7.8Hz); 8.71 (s, 1H, 2-H); 9.41 (t, 1H, CONH, J=5.2Hz, deuterium oxide-exchangeable).	3250, 1640, 1590, 1290, 1040, 800.	255 (0.57); 212 (6.2); 86 (100)
3	CH_3	$CONH(CH_2)_2N(CH_3)_2$	2.24 (s, 6H, $N(CH_3)_2$); 2.45 (t, 2H, $NHCH_2CH_2N$, J=6.3Hz); 3.47 (q, 2H, $NHCH_2CH_2N$, J=5.9Hz); 3.90 (s, 3H, 10-CH_3); 7.48-7.56 (m, 1H, 8-H); 7.64-7.72 (m, 1H, 7-H); 7.85 (dd, 1H, 9-H, J=7.6,0.4Hz); 8.58 (d, 1H, 6-H, J=7.6Hz); 8.87 (s, 1H, 2-H); 9.05 (t, 1H, CONH, J=5.1Hz, deuterium oxide-exchangeable).	3300, 1660, 1560, 1240, 1140, 780.	269 (0.35); 226 (19); 58 (100).
4	CH_3	$CONH(CH_2)_2N(C_2H_5)_2$	1.00 (t, 6H, $N(CH_2CH_3)_2$, J=7.0Hz); 2.47-2.59 (m, 6H, $NHCH_2CH_2N$, and $N(CH_2CH_3)_2$); 3.33-3.43 (m, 2H, $NHCH_2CH_2N$); 3.85 (s, 3H, 10-CH_3); 7.44-7.52 (m, 1H, 8-H); 7.59-7.67 (m, 1H, 7-H); 7.80 (dd, 1H, 9-H, J=8.2,0.4Hz); 8.53 (d, 1H, 6-H, J=8.2Hz); 8.82 (s, 1H, 2-H); 9.03 (t, 1H, CONH, J=5.3Hz, deuterium oxide-exchangeable).	3250, 1640, 1580, 1240, 1140, 780.	269 (0.62); 226 (4.5); 86 (100).
5	$CH_2C_6H_5$	$CONH(CH_2)_2N(CH_3)_2$	2.20 (s, 6H, $N(CH_3)_2$); 2.42 (t, 2H, $NHCH_2CH_2N$, J=6.3Hz); 3.45 (q, 2H, $NHCH_2CH_2N$, J=6.0Hz); 5.65 (s, 2H, $CH_2C_6H_5$); 7.30-7.62 (m, 7H, Ar-H); 7.72 (dd, 1H, 9-H, J=7.8,0.4Hz); 8.59 (d, 1H, 6-H, J=8.0Hz); 8.87 (s, 1H, 2-H); 9.03 (t, 1H, CONH, J=5.3Hz, deuterium oxide-exchangeable).	3300, 1660, 1580, 1240, 1140, 780.	390 (0.12); 319 (6.9); 302 (9.9); 58 (100).
6	$CH_2C_6H_5$	$CONH(CH_2)_2N(C_2H_5)_2$	1.00 (t, 6H, $N(CH_2CH_3)_2$, J=7.0Hz); 2.48-2.58 (m, 6H, $NHCH_2CH_2N$, and $N(CH_2CH_3)_2$); 3.32-3.41 (m, 2H, $NHCH_2CH_2N$); 5.64 (s, 2H, $CH_2C_6H_5$); 7.27-7.61 (m, 7H, Ar-H); 7.71 (dd, 1H, 9-H, J=8.2,0.6Hz); 8.58 (d, 1H, 6-H, J=7.8Hz); 8.86 (s, 1H, 2-H); 9.06 (t, 1H, CONH, J=5.0Hz, deuterium oxide-exchangeable).	3250, 1640, 1520, 1300, 1140, 760.	319 (3.6); 302 (2.7); 86 (100).
7	$(CH_2)_2N(CH_3)_2$	$COOC_2H_5$	1.30 (t, 3H, OCH_2CH_3, J=7.2Hz); 2.18 (s, 6H, $N(CH_3)_2$); 2.71 (t, 2H, 10-CH_2CH_2N, J=6.4Hz); 4.25 (q, 2H, OCH_2CH_3, J=7.2Hz); 4.78 (t, 2H, 10-CH_2CH_2N, J=6.4Hz); 7.44-7.52 (m, 1H, 8-H); 7.57-7.66 (m, 1H, 7-H); 7.86 (d, 1H, 9-H, J=7.8Hz); 8.57 (dd, 1H, 6-H, J=8.2,0.8Hz), 8.73 (s, 1H, 2-H).	1720, 1660, 1520, 1260, 1120, 760.	283 (0.60); 225 (0.47); 71 (54); 58 (100).
8	$(CH_2)_3N(CH_3)_2$	$COOC_2H_5$	1.29 (t, 3H, OCH_2CH_3, J=7.1Hz); 1.93-2.03 (m, 2H, 10-$CH_2CH_2CH_2N$); 2.10 (s, 6H, $N(CH_3)_2$), 2.29 (t, 2H, 10-$(CH_2)_2CH_2N$, J=6.6Hz); 4.25 (q, 2H, OCH_2CH_3, J=7.1Hz); 4.44 (t, 2H, 10-$CH_2(CH_2)_2N$, J=6.8Hz); 7.44-7.52 (m, 1H, 8-H); 7.58-7.66 (m, 1H, 7-H); 7.85 (d, 1H, 9-H, J=8.2Hz); 8.57 (d, 1H, 6-H, J=8.2Hz); 8.72 (s, 1H, 2-H).	1720, 1660, 1520, 1260, 1120, 800.	297 (0.70); 225 (0.74); 85 (14); 58 (100).
9	$(CH_2)_2N(C_2H_5)_2$	$COOC_2H_5$	0.71 (t, 6H, $N(CH_2CH_3)_2$, J=7.1Hz); 1.30 (t, 3H, OCH_2CH_3, J=7.0Hz); 2.44 (q, 4H, $N(CH_2CH_3)_2$, J=7.0Hz); 2.81 (t, 2H, 10-CH_2CH_2N, J=6.1Hz); 4.25 (q, 2H, OCH_2CH_3, J=7.0Hz); 4.45 (t, 2H, 10-CH_2CH_2N, J=6.2Hz); 7.43-7.51 (m, 1H, 8-H); 7.58-7.66 (m, 1H, 7-H), 7.86 (d, 1H, 9-H, J=7.8Hz); 8.57 (d, 1H, 6-H, J=8.2Hz); 8.74 (s, 1H, 2-H).	1720, 1640, 1520, 1260, 1120, 800.	311 (0.51); 225 (0.68); 99 (88); 86 (100).
10	$(CH_2)_3N(C_2H_5)_2$	$COOC_2H_5$	0.83 (t, 6H, $N(CH_2CH_3)_2$, J=7.1Hz); 1.29 (t, 3H, OCH_2CH_3, J=7.1Hz); 1.90-1.97 (m, 2H, 10-$CH_2CH_2CH_2N$); 2.31-2.50 (m, 6H, $N(CH_2CH_3)_2$, and 10-$(CH_2)_2CH_2N$); 4.25 (q, 2H, OCH_2CH_3, J=7.1Hz); 4.41 (t, 2H, 10-$CH_2(CH_2)_2N$, J=6.9Hz); 7.43-7.51 (m, 1H, 8-H); 7.57-7.66 (m, 1H, 7-H); 7.84 (d, 1H, 9-H, J=8.2Hz); 8.56 (d, 1H, 6-H, J=7.8Hz); 8.72 (s, 1H, 2-H).	1720, 1640, 1520, 1260, 1120, 790.	370 (M^+, 5.8); 325 (6.3); 100 (14); 86 (100).
11	$(CH_2)_2N(CH_3)_2$	$CONH(CH_2)_2N(CH_3)_2$	2.22 (s, 6H, $NH(CH_2)_2N(CH_3)_2$); 2.24 (s, 6H, 10-$(CH_2)_2N(CH_3)_2$); 2.45 (t, 2H, $NHCH_2CH_2N(CH_3)_2$, J=6.5Hz); 2.76 (t, 2H, 10-$CH_2CH_2N(CH_3)_2$, J=6.4Hz); 3.47 (q, 2H, $NHCH_2CH_2N(CH_3)_2$, J=6.1Hz); 4.54 (t, 2H, 10-$CH_2CH_2N(CH_3)_2$, J=6.3Hz); 7.49-7.57 (m, 1H, 8-H); 7.64-7.72 (m, 1H, 7-H); 7.91 (d, 1H, 9-H, J=7.8Hz); 8.63 (d, 1H, 6-H, J=7.8Hz); 8.88 (s, 1H, 2-H); 9.05 (t, 1H, CONH, J=5.4Hz, deuterium oxide-exchangeable).	3250, 1660, 1570, 1260, 1160, 740.	300 (3.8); 283 (2.2); 71 (20); 58 (100).

Table 2 (continued)

Compd.	R	R_1	^{1}H nmr (δ ppm)	ir nujol cm^{-1}	ms m/z (%)
12	$(CH_2)_3N(CH_3)_2$	$CONH(CH_2)_3N(CH_3)_2$	1.62-1.70 (m, 2H, $NHCH_2CH_2CH_2N$); 1.88-1.98 (m, 2H, 10-$CH_2CH_2CH_2N$); 2.08 (s, 6H, NH-$(CH_2)_3N(CH_3)_2$); 2.16 (s, 6H, 10-$(CH_2)_3N(CH_3)_2$); 2.19-2.32 (m, 4H, $NH(CH_2)_2CH_2N$, and 10-$(CH_2)_2CH_2N$); 3.34-3.44 (m, 2H, $NHCH_2(CH_2)_2N$); 4.42 (t, 2H, 10-$CH_2(CH_2)_2N$, J=6.1Hz); 7.44-7.52 (m, 1H, 8-H); 7.60-7.68 (m, 1H, 7-H); 7.86 (d, 1H, 9-H, J=8.2Hz); 8.57 (d, 1H, 6-H, J=8.2Hz); 8.83 (s, 1H, 2-H); 8.99 (t, 1H, CONH, J=5.2Hz, deuterium oxide-exchangeable).	3300, 1680, 1540, 1300, 1170, 790.	342 (2.3); 325 (3.8); 86 (30); 58 (100).
13	$(CH_2)_2N(C_2H_5)_2$	$CONH(CH_2)_2N(C_2H_5)_2$	0.70 (t, 6H, 10-$(CH_2)_2N(CH_2CH_3)_2$, J=7.1Hz); 1.00 (t, 6H, $NH(CH_2)_2N(CH_2CH_3)_2$, J=7.1Hz); 2.38-2.58 (m, 10H, $NHCH_2CH_2N(CH_2CH_3)_2$, and 10-$(CH_2)_2N(CH_2CH_3)_2$); 2.80 (t, 2H, 10-CH_2CH_2N, J=6.0Hz); 3.36 (m, 2H, $NHCH_2CH_2N$); 4.46 (t, 2H, 10-CH_2CH_2N, J=6.2Hz); 7.46-7.52 (m, 1H, 8-H); 7.60-7.68 (m, 1H, 7-H); 7.86 (d, 1H, 9-H, J=7.8Hz); 8.57 (d, 1H, 6-H, J=8.2Hz); 8.84 (s, 1H, 2-H); 9.03 (t, 1H, CONH, J=5.6Hz, deuterium oxide-exchangeable).	3300, 1670, 1570, 1300, 1160, 800.	398 (1.2); 311 (2.0); 99 (53); 86 (100).
14	$(CH_2)_3N(C_2H_5)_2$	$CONH(CH_2)_3N(C_2H_5)_2$	0.79-0.96 (m, 12H, $NH(CH_2)_3N(CH_2CH_3)_2$, and 10-$(CH_2)_3N(CH_2CH_3)_2$); 1.60-1.73 (m, 2H, NH-$CH_2CH_2CH_2N$); 1.88-1.91 (m, 2H, 10-$CH_2CH_2CH_2N$); 2.09-2.50 (m, 12H, $NH(CH_2)_2CH_2N(CH_2CH_3)_2$, and 10-$(CH_2)_2CH_2N(CH_2CH_3)_2$); 3.29-3.39 (m, 2H, $NHCH_2(CH_2)_2N$); 4.42 (t, 2H, 10-$CH_2(CH_2)_2N$, J=6.0Hz); 7.44-7.52 (m, 1H, 8-H); 7.60-7.68 (m, 1H, 7-H); 7.85 (d, 1H, 9-H, J=8.4Hz); 8.57 (d, 1H, 6-H, J=8.2Hz); 8.83 (s, 1H, 2-H); 8.99 (t, 1H, CONH, J=5.4Hz, deuterium oxide- exchangeable).	3300, 1640, 1560, 1240, 1140, 780.	426 (0.68); 325 (1.6); 86 (26); 58 (100).

Table 3

Calculated Values of the Average Orientation Angle α_L for the Test Compounds **1-8**, **10**, **11**.[DNA]=1.6x10^{-3} *M*, [DNA]/[drug]=12.5

Compounds	α_L
1	68
2	68
3	80
4	82
5	64
6	65
7	76
8	73
10	77
11	83

from ethanol to give 2.56 g of pure **15** (95%), mp 292-293° (lit. ref. [8]: mp 292-295°).

10-Benzyl-3-ethoxycarbonylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (**17**).

A solution of 2-amino-1-benzylbenzimidazole **20** (8.36 g, 37.5 mmol) and diethyl ethoxymethylenemalonate (6.60 mL, 33.0 mmol) in 140 mL of ethanol was heated at reflux for 7 hours. After cooling, the separated white solid was collected, washed with ethanol and purified by recrystallization from ethanol to give 8.85 g of pure **17** (68%), mp 192-194°; ir: 1730, 1650, 1520, 1280, 1130, 700 cm^{-1}; ^{1}H nmr: δ 1.30 (t, 3H, CH_3, J = 7.1 Hz); 4.26 (q, 2H, CH_2CH_3, J = 7.0 Hz); 5.63 (s, 2H, CH_2Ph); 7.30-7.57 (m, 7H, ArH); 7.71 (d, 1H, 9-H, J = 7.2 Hz); 8.58 (d, 1H, 6-H, J = 7.0 Hz); 8.76 (s, 1H, 2-H); ms: m/z 347 (M^+, 4.7), 302 (3.7), 91 (100).

Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 68.99; H, 5.12; N, 12.04.

General Procedure for the Synthesis of 10-Substituted 3-[(*N*,*N*-Dialkylaminoethyl)aminocarbonyl]pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones (**1-6**).

A suspension of 2.0 mmol of the appropriate 3-ethoxycarbonyl pyrimido[1,2-*a*]benzimidazol-4-one **15-17** in an excess (44.0 mmol) of *N*,*N*-dimethylethylenediamine or *N*,*N*-diethylethylenediamine was heated at reflux for 6-12 hours (tlc analysis).

For derivatives **1-2**, the solution obtained was evaporated *in vacuo* to dryness. The oily residue was treated with acetone and then filtered to give crude compounds **1-2**, which were purified by recrystallization from ethanol (Tables 1 and 2).

For derivatives **3-6**, after cooling, the suspension obtained was filtered to give the desired crude products. The filtrate was concentrated and the separated precipitate was collected to yield an additional amount of crude products **3-6**. The quantities of derivatives **3-6** obtained from the initial insoluble precipitate or from the concentrated solution were variable, depending upon the solubility of the various derivatives. The crude products **3-6** were purified by recrystallization from ethanol (Tables 1 and 2).

General Procedure for the Synthesis of 3-Ethoxycarbonyl-10-(*N*,*N*-dialkylaminoalkyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones (**7-10**).

Diethyl ethoxymethylenemalonate 0.45 mL (2.2 mmol) was added to a solution of the appropriate 1-dialkylaminoalkyl-2-aminobenzimidazole **22-25** (2.5 mmol) in ethanol and the reac-

tion mixture was heated at reflux for 12 hours. After cooling, the solution obtained was concentrated to precipitate the desired crude products **7-10**, which were collected and purified by recrystallization from ethanol (Tables 1 and 2).

General Procedure for the Synthesis of 3-[(*N*,*N*-Dialkylaminoalkyl) aminocarbonyl]-10-(*N*,*N*-dialkylaminoalkyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones (**11-14**).

A solution of 1.22 mmol of compounds **7-10** in an excess (13.4 mmol) of the appropriate diamine was heated at reflux for 5-12 hours (tlc analysis). After cooling, the suspension obtained was filtered to give the crude compounds **11-14**, which were purified by recrystallization from ethanol (Tables 1 and 2).

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