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Original article

Dialkylaminoalkylindolonaphthyridines as potential antitumour agents: synthesis, cytotoxicity and DNA binding properties

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Abstract

The synthesis of new planar derivatives characterised by the presence of an indolonaphthyridine nucleus, carrying a dimethylaminoethyl or a dimethylaminopropyl side chain is reported. The antiproliferative activity of the new products was tested by means of an in vitro assay on human tumour cell lines (HL-60 and HeLa). A number of compounds (**1a**–**d**, **1h**) showed IC_{50} values comparable to that obtained with the well-known drug ellipticine on the HL-60 cell line. The interaction with DNA was also investigated. Linear flow dichroism measurements allowed us to understand the interaction geometry. The thermodynamic parameters of the binding process, i.e. intrinsic binding constant and exclusion parameter, were determined by fluorimetric titration.

Keywords: Indolonaphthyridine derivatives; Antiproliferative activity; DNA binding

1. Introduction

DNA replication constitutes the necessary precondition for cell division, and consequently the macromolecule has become one of the preferred targets for the development of new drugs intended to act as potential antitumour agents. Inside the wide field of drug-DNA interaction modes, the process of intercalation, first explicitly formulated by Lerman in 1961 [1], is shared by a variety of DNA-binding drugs, including several important antitumour agents [2,3].

As regards the mechanism of intercalation, the presence of a planar moiety is the chemical structural requirement that renders a drug molecule suitable for it to slip between the stacked base pairs of the double helix, i.e. for an intercalation mode of binding. It has been hypothesised that van der Waals contacts with the base pairs, as well as hydrophobic interactions and charge transfer forces, contribute to the binding energy. Moreover, specific contact between the drug and one of the helical grooves might take place and these interactions appear to be of great importance for the biological activity of intercalators. In this connection, it has been demonstrated in many cases that the insertion of a basic side chain into the planar chromophore of a DNA intercalator can induce an increase both in binding affinity and in solubility under physiological conditions [4,5].

In previous studies, our interest was devoted to the synthesis of new planar polycyclic derivatives, with the aim of obtaining drugs endowed with an antitumour activity. In detail, angular tetracyclic derivatives containing the purine or benzimidazole nucleus [6] and linear compounds characterised by the presence of a benzimidazoquinazoline system [7] were taken into account. Moreover, to improve the DNA binding properties and, consequently, the biological activity, in all the above-mentioned compounds the effect of the insertion of different dialkylamino-substituted side chains was evaluated. Spectroscopic studies revealed that these chromophores interact with the macromolecule mainly through an intercalative mode of binding. The type of side chain appears to have a significant influence on the

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cytotoxic activity, which, in some cases, was close to that of the well-known drug ellipticine.

As a result of our interest in polycondensed nitrogen heterocycles, in this paper we report the synthesis of eight new linear derivatives, **1a**–**h**, containing an indolo[3,2-*c*][1,8]naphthyridine nucleus carrying a dimethylaminoethyl or a dimethylaminopropyl side chain. Furthermore, in position 8 of the tetracyclic planar moiety, a hydrogen (**1a**, **1e**), a chlorine (**1b**, **1f**), a fluorine (**1c**, **1g**) atom, or a methoxy (**1d**, **1h**) group was linked. The ability of the new compounds to inhibit cell growth was evaluated by means of an in vitro assay, using two human tumour cell lines (HL-60 and HeLa). Spectroscopic techniques allowed us to investigate the formation of a complex between the new compounds and DNA and to establish their mode of interaction. The thermodynamic parameters for the binding process were calculated by fluorimetric titration.

2. Chemistry

The synthesis of a number of substituted 11*H*-indolo[3,2-*c*][1,8]naphthyridines, which represented a new heterocyclic ring system, has been previously reported by us [8].

Pursuing our investigation in this field, we now describe the preparation of new indolo[3,2-*c*][1,8]naphthyridines **1a**–**d** and **1e**–**h** functionalised with a dimethylaminoethyl or a dimethylaminopropyl side chain, respectively.

The synthetic pathway followed to prepare indolonaphthyridines **1a**–**h** is outlined in Fig. 1.

1-Dimethylaminoalkylnaphthyridinones (**7**, **8**) represented the key intermediates for the synthesis of the target compounds and were prepared under experimental conditions similar to those already reported for several *N*-alkylnaphthyridinones [9]. 7-Methyl-1,8-naphthyridin-4(1*H*)-one (**2**), which represented the starting reagent, was prepared following a described procedure [10]. Reaction of **2** with the appropriate dimethylaminoalkyl iodide hydroiodide in ethanol solution, in the presence of potassium hydroxide, gave N-substituted derivatives **3**, **4** in good yields. Dimethylaminoalkyliodide hydroiodides were prepared from the corresponding commercially available dimethylaminoalkylchloride hydrochlorides [11]. Several alkylation reactions of **2** performed using dimethylaminoalkylchloride hydrochlorides as starting materials led to poor yields; therefore, we decided to convert chlorides into iodides, which were then used as starting reagents.

Reduction of **3** or **4** with sodium borohydride in anhydrous ethanol solution afforded tetrahydro-4-hydroxy derivatives (**5**, **6**), which were converted into the corresponding 2,3-dihydronapthyridinones (**7**, **8**) in Oppenauer conditions. Analytical and spectral data of compounds **3**–**8** were consistent with the proposed structure and are reported in Table 1.

Compounds **7**, **8** were then treated with the appropriately substituted phenylhydrazine hydrochlorides in ethanol solution to give the corresponding phenylhydrazone dihydrochlorides **9a**–**h** (Table 2).

The title compounds **1a**–**h** were obtained by the Fischer indole cyclisation of **9a**–**h** in ethanol solution saturated with hydrogen chloride. The reaction proceeds through the initial formation of the 5,6-dihydroindolonaphthyridine derivatives, which proved not to be too stable, and are easily converted into the corresponding fully aromatic indolonaphthyridines by heating in an organic solvent. Therefore, the crude reaction mixture was refluxed in ethanol until complete conversion of the 5,6-dihydroindolonaphthyridines into the oxidised compounds (TLC), which, after purification by recrystallisation from the appropriate solvent, yielded the target compounds **1a**–**h**, whose analytical and spectral data are reported in Table 3.

Only from the cyclisation of phenylhydrazones **9a** and **9e** it was possible to isolate pure 5,6-dihydroderivatives **10a** and **10e**, which were characterised by analytical and spectral data (see Section 5).

An indication of the structure of indolonaphthyridine derivatives was obtained from an examination of the ^{1}H-NMR spectra of compounds **10a**, **e** and **1a**, **e**: for example, the spectrum of **10a** presented a singlet at $\delta = 5.01$ ppm, due to the 6-methylene protons of the 5,6-dihydroindolonaphthyridine system and a singlet at $\delta = 11.29$ ppm (exchangeable with D_2O) due to the 11 NH proton, while the ^{1}H-NMR spectrum of **1a** presented a singlet at $\delta = 9.29$ ppm (not exchangeable with D_2O), assigned to the aromatic 6-proton on the basis of literature data [8,12]. The ^{1}H-NMR spectra of **10a** and **10e** have to be recorded as quickly as possible, to avoid the partial conversion of the samples into the fully aromatic compounds. Moreover, in the MS spectra of compounds **10a** and **10e** the peaks corresponding to the value of the molecular weight were two units higher than those of compounds **1a** and **1e**.

For the biological assays, all the compounds **1a**–**h** were transformed into their dihydrochloride hydrates by treatment with hydrogen chloride-saturated ethanol solution (Table 4).

3. Results and discussion

3.1. Antiproliferative activity

The antiproliferative activity of compounds **1a**–**h** was evaluated by means of an in vitro assay performed on two human tumour cell lines, HL-60 and HeLa, and

was expressed as IC_{50} values (Table 5). The well-known drugs doxorubicin and ellipticine were used as reference compounds.

A comparison between the values obtained for the two cell lines reveals that HL-60, a human myeloid leukaemic cell line which lack a p53 protein [13], are more sensitive to the effect exerted by the new compounds with respect to HeLa, a human cervix adenocarcinoma cell line with low levels of p53 [14] and this behaviour is similar to that observed with doxorubicin. In this regard, it was already demonstrated that cells lacking p53 are more sensitive to a variety of chemotherapeutic agents damaging DNA [15].

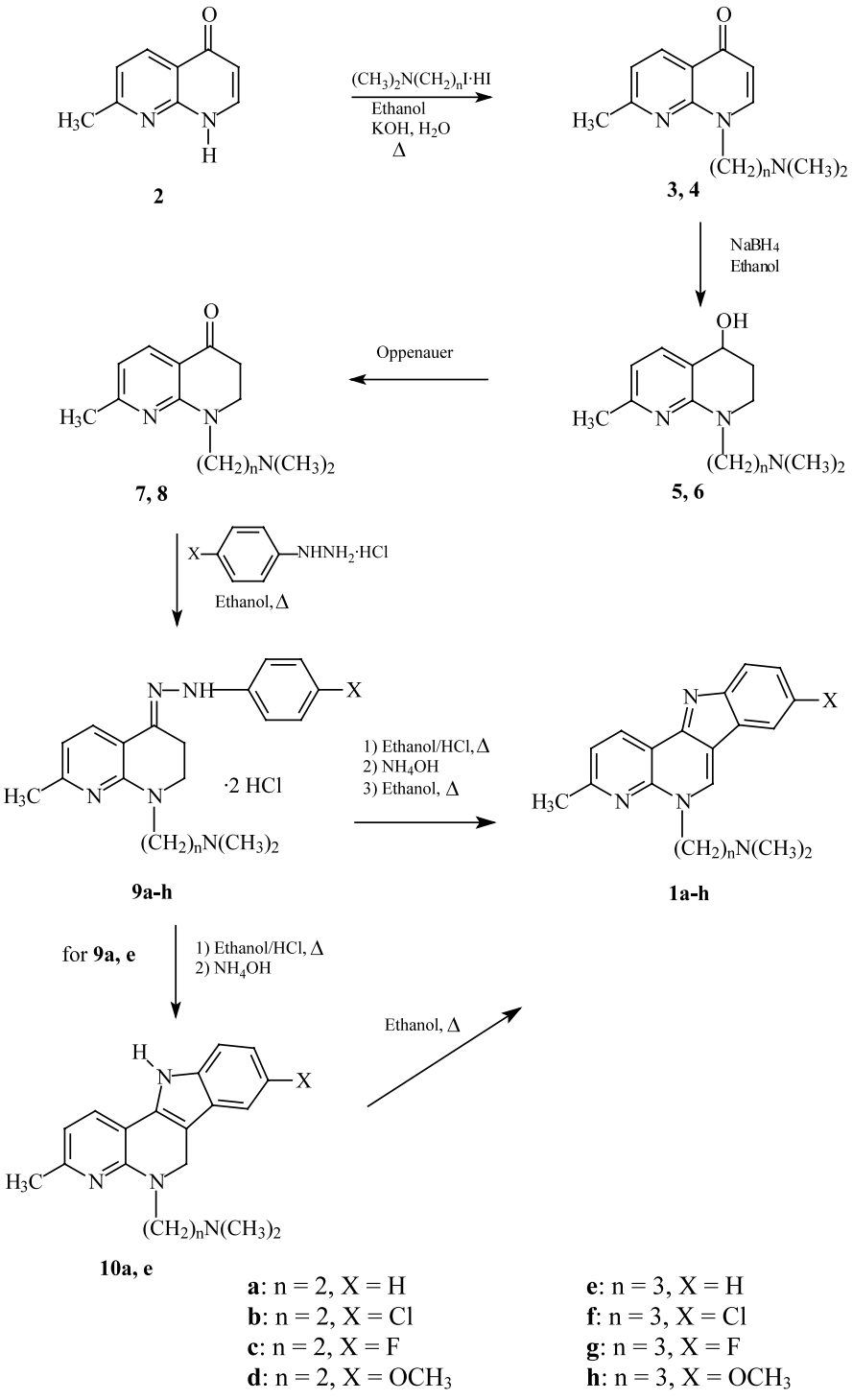
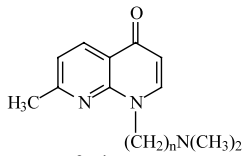
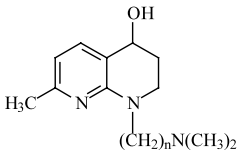


Fig. 1. Synthesis of indolonaphthyridine **1a**–**h**.

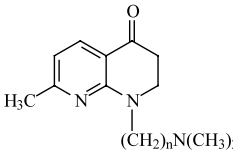
Table 1
Physical and spectral data of compounds **3**–**8**[a]



3, 4



5, 6



7, 8

N	*n*	Yield (%)	^{1}H-NMR (δ, ppm)[b]	MS *m/e* [M$^+$]	Formula[c]
3	2	41	2.32 (s, 6H, $N(CH_3)_2$); 2.65 (s, 3H, 7-CH_3); 2.70 (t, 2H, $CH_2CH_2N(CH_3)_2$); 4.46 (t, 2H, $CH_2CH_2N(CH_3)_2$); 6.28 (d, 1H, 6-H); 7.19 (d, 1H, 3-H); 7.68 (d, 1H, 2-H); 8.58 (d, 1H, 5-H)	231	$C_{13}H_{17}N_3O$
4	3	44	2.04 (m, 2H, $CH_2CH_2CH_2$); 2.24 (s, 8H, $N(CH_3)_2+CH_2CH_2CH_2N(CH_3)_2$); 2.63(s, 3H, 7-$CH_3$); 4.39 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 6.22 (d, 1H, 6-H); 7.13 (d, 1H, 3-H); 7.68 (d, 1H, 2-H); 8.53 (d, 1H, 5-H)[d]	245	$C_{14}H_{19}N_3O$
5	2	35	1.69 (m, 2H, 3-CH_2); 2.23 (s, 6H, $N(CH_3)_2$); 2.32 (s, 3H, 7-CH_3); 2.40–2.67 (m, 2H, $CH_2CH_2N(CH_3)_2$); 3.23–3.83 (m, 4H, $CH_2CH_2N(CH_3)_2+2$-CH_2); 4.58 (t, 1H, 4-H); 6.29 (d, 1H, 6-H); 7.16(d, 1H, 5-H)[d]	235	$C_{13}H_{21}N_3O$
6	3	43	1.60–2.00 (m, 4H, 3-$CH_2+CH_2CH_2CH_2$); 2.18 (s, 8H, $CH_2CH_2CH_2N(CH_3)_2$); 2.33 (s, 3H, 7-CH_3); 3.20–3.90 (m, 4H, 2-$CH_2+CH_2CH_2CH_2N(CH_3)_2$); 4.65 (t, 1H, 4-H); 6.29 (d, 1H, 6-H); 7.19 (d, 1H, 5-H)[d]	249	$C_{14}H_{23}N_3O$
7	2	46	2.30 (s, 6H, $N(CH_3)_2$); 2.38 (s, 3H, 7-CH_3); 2.43–2.73 (m, 4H, 3-$CH_2+CH_2CH_2N(CH_3)_2$); 3.48–3.89 (m, 4H, 2-$CH_2+CH_2CH_2N(CH_3)_2$); 6.44 (d, 1H, 6-H); 7.89 (d, 1H, 5-H)[d]	233	$C_{13}H_{19}N_3O$
8	3	76	1.60–2.00 (m, 4H, 3-$CH_2+CH_2CH_2CH_2$); 2.24 (s, 6H, $N(CH_3)_2$); 2.39 (s, 3H, 7-CH_3); 2.66 (t, 2H, $CH_2CH_2N(CH_3)_2$); 3.44–3.79 (m, 4H, 2-$CH_2+CH_2CH_2CH_2N(CH_3)_2$); 6.43 (d, 1H, 6-H); 7.90 (d, 1H, 5-H)[d]	247	$C_{14}H_{21}N_3O$

[a] All the compounds are oils with the exception of **4** (m.p. 67–68 °C; recrystallization solvent: petroleum ether 40–60 °C).
[b] Recorded in $CDCl_3$.
[c] Elemental analyses for C, H, N were within $\pm$ 0.4% of the calculated values.
[d] Recorded on a Varian CFT-20 NMR spectrometer, operating at 80 MHz.

Our previous studies indicated that even small chemical differences in the dialkylamino-substituted side chains inserted into the purinoquinazoline [6] or in the benzimidazoquinazoline [7] moieties could be decisive for the overall effect of a drug. Indeed, in both classes of derivatives, a noticeable difference in antiproliferative activity was found between the chromophores carrying a dimethylaminoethyl side chain with respect to the one where a dimethylaminopropyl side chain is present in the planar nucleus. In detail, the compounds having the dimethylaminoethyl side chain are able to exert a cytotoxic effect higher than those characterised by the presence of the dimethylaminopropyl one.

On the basis of these previous data, it appears to be interesting to make an initial comparison between the new indolonaphthyridine compounds **1a**–**d** with respect to **1e**–**h** dimethylaminoethyl- and dimethylaminopropyl-substituted, respectively. The data reported in Table 5 indicate that only the activity exerted by compounds **1a**–**c** and **1e**–**g** in HL-60 cells appears to depend on the length of the side chain. In this case the dimethylaminoethyl side chain seems to be responsible for a slight increase in cytotoxic ability with respect to the dimethylaminopropyl, as the IC_{50} values of **1a**–**c** range from one half to one third with respect to those of **1e**–**g**. The presence of the methoxy group in the tetracyclic system seems to abolish the effect attributable to the side chain: the same IC_{50} values were found both for **1d** and **1h**. It is noteworthy that in this cell line, the IC_{50} values of the new derivatives are comparable to that obtained with the well-known drug ellipticine, even if lower than that of the clinically used drug doxorubicin.

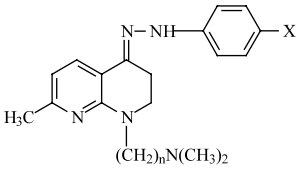
As regards HeLa cells, the IC_{50} values obtained do not allow us to affirm that a clear relationship exists between the antiproliferative effect and the type of side chain inserted into the planar nucleus.

Furthermore, in both HL-60 and HeLa, it seems that the presence of a substituent in position 8 of the indolonaphthyridine nucleus, whatever its electronic effect or steric hindrance, does not significantly influence the biological effect.

3.2. DNA binding studies

The study of the DNA binding properties of the new antiproliferative compounds arises from the fact that DNA is the central controlling element of cellular activities, and the compromising of any of its functions could have lethal consequences for the cell.

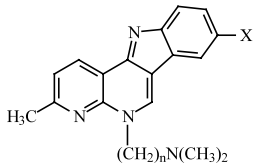
Table 2
Physical and spectral data of 1-dimethylaminoalkyl-7-methyl-2,3-dihydro-1,8-naphthyridin-4(1H)-ones phenylhydrazones dihydrochlorides **9a–h**



N	X	*n*	Yield (%)	M.p. (°C)	^{1}H-NMR (δ, ppm)	MS *m/e* [M$^+$]	Formula[a]
9a	H	2	89	149–151	2.52 (s, 3H, 7-CH_3); 2.86 (s, 6H, $N(CH_3)_2$); 3.20–3.65 (m, 4H, 3-CH_2+$CH_2CH_2N(CH_3)_2$); 3.80–4.60 (m, 4H, 2-CH_2+$CH_2CH_2N(CH_3)_2$); 6.66–7.22 (m, 6H, 6-H+ArH); 8.23 (d, 1H, 5-H); 10.60 (s, 1H, NH, exch. with D_2O)[b]	323	$C_{19}H_{25}N_5$·2 HCl
9b	Cl	2	35	173–175	2.13 (s, 3H, 7-CH_3); 2.71 (s, 6H, $N(CH_3)_2$); 2.99 (t, 2H, $CH_2CH_2N(CH_3)_2$); 3.05–3.14 (m, 4H, 2-CH_2+3-CH_2); 3.43 (t, 2H, $CH_2CH_2N(CH_3)_2$); 6.29 (d, 1H, 6-H); 6.66–6.93 (m, 4H, ArH); 7.77 (d, 1H, 5-H)[c]	357	$C_{19}H_{24}ClN_5$·2 HCl
9c	F	2	35	178–180	2.27 (s, 3H, 7-CH_3); 2.45 (t, 2H, $CH_2CH_2N(CH_3)_2$); 2.78 (s, 6H, $N(CH_3)_2$); 3.14 (t, 2H, 3-CH_2); 3.32 (t, 2H, 2-CH_2); 3.54 (t, 2H, $CH_2CH_2N(CH_3)_2$); 6.55 (d, 1H, 6-H); 6.81–6.85 (m, 4H, ArH); 8.00 (d, 1H, 5-H)[c]	341	$C_{19}H_{24}FN_5$·2 HCl
9d	OCH_3	2	31	138–140	2.26 (s, 3H, 7-CH_3); 2.47 (t, 2H, $CH_2CH_2N(CH_3)_2$); 2.78 (s, 6H, $N(CH_3)_2$); 3.16 (t, 2H, 3-CH_2); 3.31 (t, 2H, 2-CH_2); 3.52 (t, 2H, $CH_2CH_2N(CH_3)_2$); 3.65 (s, 3H, OCH_3); 6.58 (d, 1H, 6-H); 6.65–7.00 (m, 4H, ArH); 8.00 (d, 1H, 5-H)[c]	353	$C_{20}H_{27}N_5O$·2 HCl·H_2O
9e	H	3	37	141–144	1.82–2.30 (m, 2H, $CH_2CH_2CH_2$); 2.58 (s, 3H, 7-CH_3); 2.77 (s, 8H, $CH_2CH_2CH_2N(CH_3)_2$); 3.20 (t, 2H, 3-CH_2); 3.61 (t, 2H, 2-CH_2); 3.94 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 6.65–7.32 (m, 6H, 6-H+ArH); 8.28 (d, 1H, 5-H); 10.90 (s, 1H, NH, exch. with D_2O)[b]	337	$C_{20}H_{27}N_5$·2 HCl
9f	Cl	3	40	204–208 dec.	1.98–2.18 (m, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.60 (s, 3H, 7-CH_3); 2.75 (s, 6H, $N(CH_3)_2$); 2.88 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 3.15 (t, 2H, 3-CH_2); 3.61 (t, 2H, 2-CH_2); 3.95 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 6.75 (d, 1H, 6-H); 7.21–7.27 (m, 4H, ArH); 8.34 (d, 1H, 5-H); 10.90 (s, 1H, NH, exch. with D_2O)	371	$C_{20}H_{26}ClN_5$·2 HCl
9g	F	3	42	198–200	2.00–2.20 (m, 2H, $CH_2CH_2CH_2$); 2.57 (s, 3H, 7-CH_3); 2.75 (s, 6H, $N(CH_3)_2$); 2.84 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 3.14 (t, 2H, 3-CH_2); 3.59 (t, 2H, 2-CH_2); 3.90 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 6.73 (d, 1H, 6-H); 7.01–7.29 (m, 4H, ArH); 8.31 (d, 1H, 5-H); 10.90 (s, 1H, NH, exch. with D_2O)	355	$C_{20}H_{26}FN_5$·2 HCl
9h	OCH_3	3	39	148–150	1.70–1.98 (m, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.28 (s, 3H, 7-CH_3); 2.48 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.75 (s, 6H, $N(CH_3)_2$); 3.01 (t, 2H, 3-CH_2); 3.26 (t, 2H, 2-CH_2); 3.36 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 3.65 (s, 3H, OCH_3); 6.52 (d, 1H, 6-H); 6.69–6.82 (m, 4H, ArH); 8.04 (d, 1H, 5-H)[c]	367	$C_{21}H_{29}N_5O$·2 HCl·H_2O

[a] Elemental analyses for C, H, N were within ± 0.4% of the calculated values.
[b] Registered on a Varian CFT-20 NMR spectrometer operating at 80 MHz.
[c] Recorded in D_2O.

Table 3
Physical and spectral data of 3-methyl-5-dimethylaminoalkyl-5H-indolo[3,2-*c*][1,8]naphthyridines **1a–h**



N	X	*n*	Yield (%)	M.p. (°C) (recryst. solv)	^{1}H-NMR (δ, ppm)	MS *m/e* [M$^+$]	Formula[a]
1a	H	2	20	153–156 (ethyl acetate)	2.24 (s, 6H, $N(CH_3)_2$); 2.71(s, 3H, 3-CH_3); 2.78 (t, 2H, $CH_2CH_2N(CH_3)_2$); 4.83 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.20–8.95 (m, 5H, Ar-H+2-H); 8.91 (d, 1H, 1-H); 9.29 (s, 1H, 6-H)[b]	304	$C_{19}H_{20}N_4$
1b	Cl	2	24	172–175 (cyclohexane)	2.22 (s, 6H, $N(CH_3)_2$); 2.72 (s, 3H, 3-CH_3); 2.78 (t, 2H, $CH_2CH_2N(CH_3)_2$); 4.83 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.40 (dd, 1H, 9-H); 7.61 (d, 1H, 2-H); 7.75 (d, 1H, 10-H); 8.26 (d, 1H, 7-H); 8.93 (d, 1H, 1-H); 9.43 (s, 1H, 6-H)	338	$C_{19}H_{19}ClN_4$
1c	F	2	23	154–156 (cyclohexane)	2.22 (s, 6H, $N(CH_3)_2$); 2.72(s, 3H, 3-CH_3); 2.78 (t, 2H, $CH_2CH_2N(CH_3)_2$); 4.82 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.21–7.32 (m, 1H, 9-H); 7.60 (d, 1H, 2-H); 7.72–7.78 (m, 1H, 10-H); 7.91–7.97 (m, 1H, 7-H); 8.92 (d, 1H, 1-H); 9.39 (s, 1H, 6-H)	322	$C_{19}H_{19}FN_4$
1d	OCH_3	2	18	140–142 (ethyl acetate)	2.23 (s, 6H, $N(CH_3)_2$); 2.71 (s, 3H, 3-CH_3); 2.78 (t, 2H, $CH_2CH_2N(CH_3)_2$); 3.86 (s, 3H, OCH_3); 4.81 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.07 (dd, 1H, 9-H); 7.56 (d, 1H, 2-H); 7.66 (d, 1H, 10-H); 7.72 (d, 1H, 7-H); 8.88 (d, 1H, 1-H); 9.31 (s, 1H, 6-H)	334	$C_{20}H_{22}N_4O$
1e	H	3	21	190–195 (ethyl acetate)	2.22 (s, 8H, $CH_2CH_2CH_2N(CH_3)_2$); 2.47 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.76 (s, 3H, 3-CH_3); 4.85 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.29–7.82 (m, 4H, ArH); 8.18 (d, 1H, 2-H); 9.03 (d, 1H, 1-H); 9.62 (s, 1H, 6-H)[b]	318	$C_{20}H_{22}N_4 \cdot H_2O$
1f	Cl	3	31	146–147 (ethyl acetate)	1.98–2.12 (m, 2H, $CH_2CH_2CH_2$); 2.16 (s, 6H, $N(CH_3)_2$); 2.28 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.72 (s, 3H, 3-CH_3); 4.75 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.42 (dd, 1H, 9-H); 7.60 (d, 1H, 2-H); 7.75 (d, 1H, 10-H); 8.19 (d, 1H, 7-H); 8.92 (d, 1H, 1-H); 9.45 (s, 1H, 6-H).	352	$C_{20}H_{21}ClN_4 \cdot H_2O$
1g	F	3	26	96–98 (ethyl acetate)	2.01–2.08 (m, 2H, $CH_2CH_2CH_2$); 2.17 (s, 6H, $N(CH_3)_2$); 2.28 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.72 (s, 3H, 3-CH_3); 4.74 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.22–7.30 (m, 1H, 9-H); 7.59 (d, 1H, 2-H); 7.70–7.78 (m, 1H, 10-H); 7.89–7.95 (m, 1H, 7H); 8.91 (d, 1H, 1-H); 9.41 (s, 1H, 6-H).	336	$C_{20}H_{21}FN_4 \cdot H_2O$
1h	OCH_3	3	22	74–75 (ethyl acetate/cyclohexane)	2.01–2.08 (m, 2H, $CH_2CH_2CH_2$); 2.17 (s, 6H, $N(CH_3)_2$); 2.29 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 2.71 (s, 3H, 3-CH_3); 3.86 (s, 3H, OCH_3) 4.73 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.06 (dd, 1H, 9-H); 7.56 (d, 1H, 2-H); 7.66 (d, 1H, 10-H); 7.71 (d, 1H, 7-H); 8.88 (d, 1H, 1-H); 9.33 (s, 1H, 6-H).	348	$C_{21}H_{24}N_4O \cdot H_2O$

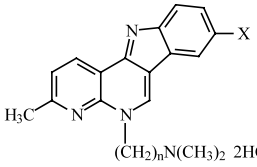
[a] Elemental analyses for C, H, N were within $\pm$ 0.4% of the calculated values.
[b] Recorded on a Varian CFT-20 NMR spectrometer operating at 80 MHz.

A first qualitative indication that an interaction takes place between the macromolecule and a possible ligand can be obtained by analysing the thermal stabilisation of the double helix, which can occur as a consequence of the formation of a molecular complex. The differences between the transition melting temperature of the DNA-drug complex and DNA alone (ΔT_m) have been calculated for the new derivatives. For all new compounds, it was possible to obtain ΔT_m values ranging from 6 to 8 °C thus indicating that a molecular complex takes place.

To look more in depth into the binding process that occurs between the macromolecule and **1a**–**h**, flow linear dichroism studies were performed. In Fig. 2 (middle panel), the flow linear dichroism spectra of **1d** for a [DNA]/[drug] = 12.5 is reported, as a representative spectrum. It can be seen from this figure that besides the strong negative signal at 260 nm, attributable to the presence of the macromolecule, also a dichroic signal is present at higher wavelengths (310–360 nm), where only the ligand molecule absorbs. Due to the fact that these small ligand molecules cannot themselves become oriented in the flow field, this signal has to be attributable to an induced orientation deriving from the formation of a molecular complex with DNA. A preliminary indication of the geometry of complexation can be derived from the sign of the signal. In this case the negative sign can be indicative of an orientation of the molecular plane of the ligand chromophore preferentially parallel to the plane of the DNA bases [6].

The calculation of the average orientation angle α_L, performed as indicated in the Section 5, provides specific information about the mode of binding of a ligand. In Fig. 2, the upper panel and the lower panel depict

Table 4
Physical and spectral data of 3-methyl-5-dimethylaminoalkyl-5H-indolo[3,2-*c*][1,8]naphthyridines dihydrochlorides **1a**–**h·2HCl**



N	X	*n*	Yield (%)	M.p. (°C)	^{1}H-NMR (δ, ppm)	Formula[a]
1a·2HCl	H	2	85	290 dec.	2.72 (s, 3H, 3-CH_3); 2.89 (s, 6H, $N(CH_3)_2$); 3.58 (t, 2H, $CH_2CH_2N(CH_3)_2$); 5.07 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.15–7.80 (m, 5H, Ar-H + 2-H); 8.42 (d, 1H, 1-H); 9.42 (s, 1H, 6-H)[b]	$C_{19}H_{20}N_4$·2 HCl·H_2O
1b·2HCl	Cl	2	90	278–280	2.74 (s, 3H, 3-CH_3); 2.96 (s, 6H, $N(CH_3)_2$); 3.66 (t, 2H, $CH_2CH_2N(CH_3)_2$); 5.15 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.03–7.66 (m, 3H, ArH); 7.68 (d, 1H, 2-H); 8.34 (d, 1H, 1-H); 9.39 (s, 1H, 6-H)[b]	$C_{19}H_{19}ClN_4$·2 HCl·H_2O
1c·2HCl	F	2	87	263–265 dec.	2.72 (s, 3H, 3-CH_3); 2.92 (s, 6H, $N(CH_3)_2$); 3.62 (t, 2H, $CH_2CH_2N(CH_3)_2$); 5.15 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.02–7.57 (m, 3H, ArH); 7.67 (d, 1H, 2-H); 8.44 (d, 1H, 1-H); 9.45 (s, 1H, 6-H)[b]	$C_{19}H_{19}FN_4$·2 HCl·H_2O
1d·2HCl	OCH_3	2	84	234–237 dec.	2.87 (s, 6H, $N(CH_3)_2$); 2.91 (s, 3H, 3-CH_3); 3.81 (t, 2H, $CH_2CH_2N(CH_3)_2$); 3.93 (s, 3H, OCH_3); 5.38 (t, 2H, $CH_2CH_2N(CH_3)_2$); 7.34 (d, 1H, 2-H); 7.80–7.99 (m, 3H, ArH); 8.06 (d, 1H, 1-H); 10.41 (s, 1H, 6-H)	$C_{20}H_{22}N_4O$·2 HCl·H_2O
1e·2HCl	H	3	88	285 dec.	2.26–2.66 (m, 2H, $CH_2CH_2CH_2$); 2.81 (s, 3H, 3-CH_3); 2.93 (s, 6H, $N(CH_3)_2$); 3.39 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 4.86 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.01–7.91 (m, 5H, ArH + 2-H); 8.35 (d, 1H, 1-H); 9.40 (s, 1H, 6-H)[b,c]	$C_{20}H_{22}N_4$·2 HCl·H_2O
1f·2HCl	Cl	3	90	253–255	2.25–2.35 (m, 2H, $CH_2CH_2CH_2$); 2.70 (s, 3H, CH_3); 2.79 (s, 6H, $N(CH_3)_2$); 3.22 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 4.83 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 6.99–7.49 (m, 3H, ArH); 7.61 (d, 1H, 2-H); 8.30 (d, 1H, 1-H); 9.25 (s, 1H, 6-H)[b]	$C_{20}H_{21}ClN_4$·2 HCl
1g·2HCl	F	3	85	290–292 dec.	2.45–2.55 (m, 2H, $CH_2CH_2CH_2$); 2.75 (s, 6H, $N(CH_3)_2$); 2.87 (s, 3H, 3-CH_3); 3.22 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 5.10 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.57–8.24 (m, 4H, ArH + 2-H); 9.35 (d, 1H, 1-H) ;10.52 (s, 1H, 6-H)	$C_{20}H_{21}FN_4$·2 HCl
1h·2HCl	OCH_3	3	86	265–270 dec.	2.38–2.58 (m, 2H, $CH_2CH_2CH_2$); 2.75 (s, 6H, $N(CH_3)_2$); 2.85 (s, 3H, 3-CH_3); 3.22 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 3.90 (s, 3H, OCH_3); 5.08 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 7.22–7.97 (m, 4H, ArH + 2-H); 9.19 (d, 1H, 1-H); 10.34 (s, 1H, 6-H)	$C_{21}H_{24}N_4O$·2 HCl·H_2O

[a] Elemental analyses for C, H, N were within ± 0.4% of the calculated values.
[b] Recorded in D_2O.
[c] Recorded on a Varian CFT-20 NMR spectrometer operating at 80 MHz.

Table 5
Cell growth inhibition in the presence of the test compounds against HL-60 and HeLa cells

Compound	IC_{50} (μM) [a]	
	HL-60	HeLa
1a	0.62 ± 0.21	2.9 ± 0.8
1b	0.50 ± 0.05	1.4 ± 0.5
1c	0.49 ± 0.03	1.5 ± 0.3
1d	0.50 ± 0.04	1.6 ± 0.2
1e	1.2 ± 0.06	3.2 ± 0.6
1f	0.89 ± 0.04	1.9 ± 0.2
1g	1.6 ± 0.3	1.8 ± 0.5
1h	0.55 ± 0.07	1.6 ± 0.1
Doxorubicin	0.0034 ± 0.0003	0.033 ± 0.005
Ellipticine	0.66 ± 0.02	0.29 ± 0.02

[a] Drug concentration required to inhibit the cell growth by 50% after 72 h of incubation. Data represent mean ± S.E. for three separate experiments.

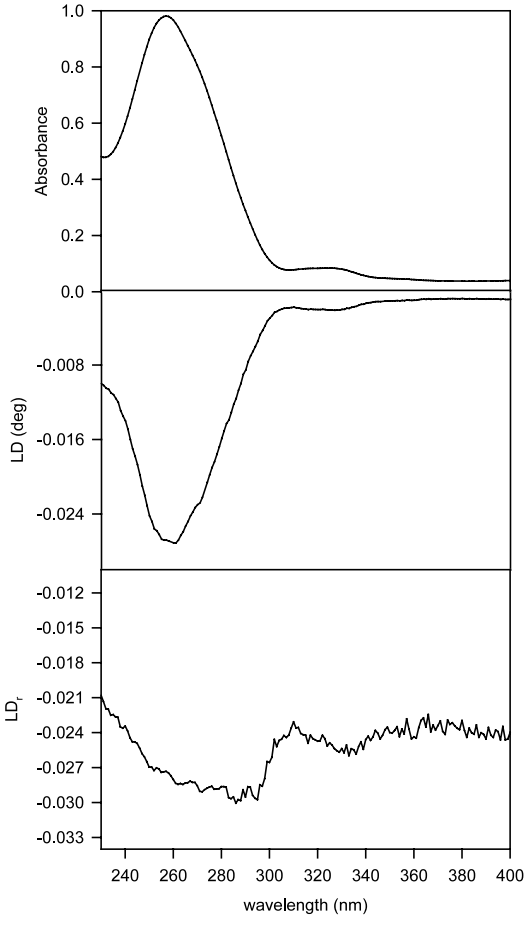


Fig. 2. Absorbance (upper panel), linear dichroism (LD, middle panel) and reduced linear dichroism (LD_r, lower panel) spectra of compound **1d** in the presence of [DNA] = 1.6×10^{-3} M for a [DNA]/[drug] = 12.5.

the light absorption and the reduced linear dichroism (LD_r) spectrum, respectively, for compound **1d**. For all the new derivatives **1a**–**h**, the α_L obtained ranges between 80 and 90°. The achievement of a large value of the average orientation angle is generally considered indicative of an intercalative mode of binding [16]. Consequently, it is reliable to assume that the planar aromatic moiety of **1a**–**h** adopts a perpendicular geometry with respect to the helix axis of the macromolecule.

The possibility of making a quantitative evaluation of the binding process through the determination of the intrinsic binding constant (K_i) and the exclusion parameter (n), is provided by fluorimetric titration. The presence of a detectable fluorescence signal in solutions containing both **1a**–**c** and **1e**–**g**, significantly quenched upon addition of DNA, allowed us to determine the above-mentioned parameters for all these compounds. On the contrary, this evaluation was not possible for **1d** and **1h** due to the absence of a suitable fluorescence emission. Fig. 3 presents as an example a fluorimetric titration (Fig. 3a) and the binding data represented as Scatchard plot (Fig. 3b), for compound **1e**. In Table 6, K_i and n values are reported for all the compounds considered. These parameters were obtained by fitting the experimental data with the McGhee and von Hippel equation [17]. It may be noted that in all cases the values obtained are very similar, thus indicating that these derivatives exert a similar affinity in the formation of a molecular complex with DNA.

4. Conclusions

The new indolonaphthyridine derivatives **1a**–**h** exert a significant antiproliferative effect on two human tumour cell lines, HL-60 and HeLa. The new compounds appear to be more active towards the first cell line taken into account and, in particular, it is noteworthy that for **1a**–**d** and **1h**, the IC_{50} values obtained are comparable to that evaluated for ellipticine, even if lower with respect to that of doxorubicin. Only in HL-60 a moderate different effect can be found between the derivatives carrying the dimethylaminoethyl or dimethylaminopropyl side chain: the former (**1a**–**c**) appear to be slightly more effective with respect to the latter (**1e**–**g**). Moreover, the insertion of substituents with different electronic and steric features in position 8 of the planar nucleus appears to be irrelevant.

As regards the antiproliferative effect evaluated on HeLa cells, it appears to be lower with respect to that obtained for HL-60, and no detectable differences could be attributed to the different side chains present in the test compounds.

The planar structure of the indolonaphthyridine moiety may suggest a possible intercalative interaction with DNA. Linear flow dichroism studies and the calculation of the average orientation angle α_L allowed us to

confirm this assumption. Indeed, for all the new compounds, a large value of α_L (80–90°) was found, which is known to be consistent with an intercalative mode of binding.

The values calculated for the binding thermodynamic parameters, i.e. the intrinsic binding constant and the exclusion parameter, indicate for all the new derivatives a similar affinity towards the macromolecule.

To make a comparison with the previously described related series of compounds [6,7], it has to be noted that these new indolonaphthyridines showed the highest affinity in the formation of a molecular complex with DNA, as their intrinsic binding constant (K_i) values were 2–3-fold higher than those of the described products. Unfortunately, this trend was not always paralleled by their citoxicity on HL-60 and HeLa cells, as they demonstrated to be more potent than the pyridopyrimidopurines and pyridopyrimidobenzimidazoles [6], as potent as the benzimidazoquinazolines [7], but less potent with respect to the purinoquinazolines [6], independently of the dialkylaminoalkyl chain type.

In conclusion, an effective interaction with DNA, together with a significant antiproliferative activity, has been demonstrated for this new nitrogen heterocyclic compounds. Therefore, this polycondensed structure may represent an interesting preliminary approach to the development of new intercalating molecules endowed with possible antitumour properties.

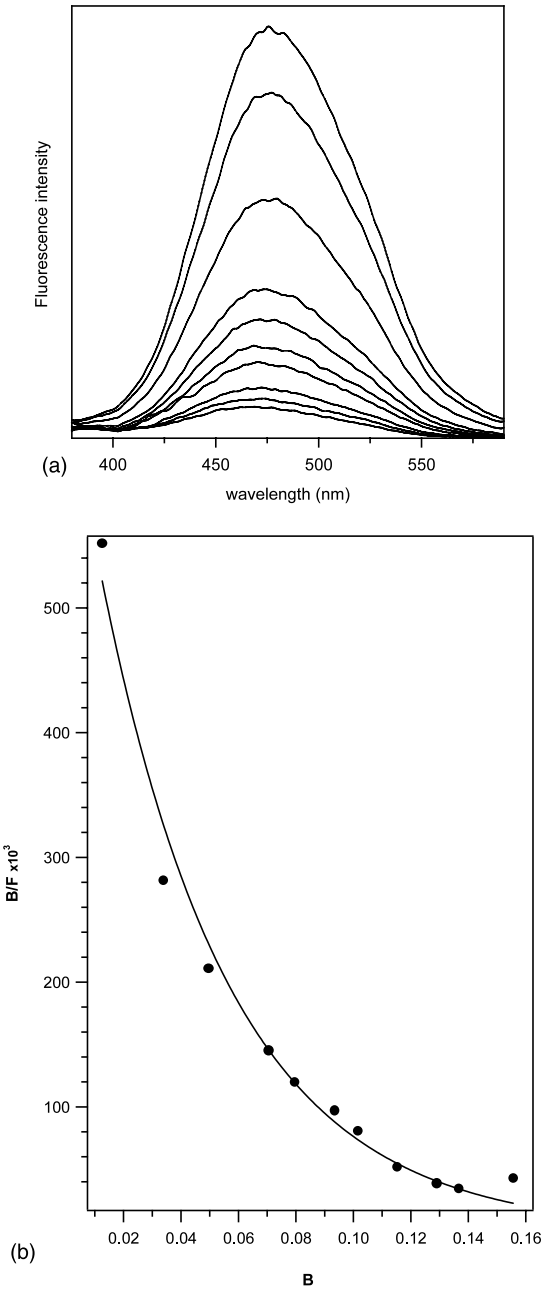


Fig. 3. (a) Fluorescence spectra of compound **1e** (5×10^{-6} M) in the absence and presence of DNA. (b) Scatchard plot for the interaction of compound **1e** with DNA. B is the ratio between bound drug and moles of DNA base pairs and F is the free drug concentration.

Table 6
Intrinsic binding constants (K_i) and exclusion parameters (n) of complexes between test compounds **1a–c** and **1e–f** and DNA

Compound	$K_i \times 10^{-5}$ [a]	n
1a	5.64	5
1b	5.74	5
1c	6.20	5
1e	6.01	6
1f	7.43	5
1g	5.04	5

[a] Intrinsic binding constant expressed as molar base pairs, mean ± 5% from at least four determinations.

5. Experimental protocols

5.1. Chemistry

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 in dimethyl-d_6 sulphoxide solution on a Varian GEMINI 200 spectrometer, unless otherwise reported. 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 sulphate was always used as the drying agent. Evaporations were performed in vacuo (rotary evaporator). Analytical TLC was carried out on Merck 0.2 mm precoated silica gel aluminium sheets (60 F-254), using chloroform–diethylamine (9:1) as the eluant.

Column chromatography was performed on aluminium oxide (grade I, 70–230 mesh) or silica gel 60 (230–400 mesh). Elemental analyses were performed by

our Analytical Laboratory and results agreed with theoretical values to within ± 0.4%.

*5.1.1. 1-Dimethylaminoalkyl-7-methyl-1,8-naphthyridin-4(1H)-ones (**3**, **4**)*

5.1.1.1. General procedure. The appropriate dimethylaminoalkyliodide hydroiodide (18.0 mmol) was added to an ethanolic solution (80 mL) of **2** (2.42 g, 15.0 mmol) and KOH (2.02 g, 36.0 mmol). The reaction mixture was refluxed under stirring until disappearance of the starting material (TLC) (4 days). After cooling, the solid precipitate (KI) was filtered off, the filtrate was evaporated to dryness and the residue was dissolved in water. The solution obtained was extracted with chloroform and the chloroform phase was dried and evaporated to give a residue, which was purified by column chromatography on neutral alumina using toluene–chloroform (9:1) as the eluant for **3**, or by recrystallisation from petroleum ether 40–60 °C for **4** (Table 1).

*5.1.2. 1-Dimethylaminoalkyl-4-hydroxy-7-methyl-1,2,3,4-tetrahydro-1,8-naphthyridines (**5**, **6**)*

5.1.2.1. General procedure. Sodium borohydride (4.60 g) was added, over a period of 10 min, to an ice-cooled, stirred solution of **3** or **4** (10.0 mmol) in absolute ethanol (100 mL). The mixture was stirred at room temperature until disappearance of the starting material (TLC) (4 days). After standing at room temperature for 48 h, the ethanol was removed and the residue was purified by column chromatography on silica gel, using chloroform–diethylamine (98:2) as the eluant, to yield pure **5** or **6** as colourless, viscous oils (Table 1).

*5.1.3. 1-Dimethylaminoalkyl-7-methyl-2,3-dihydro-1,8-naphthyridin-4(1H)-ones (**7**, **8**)*

5.1.3.1. General procedure. Aluminium isopropoxide (2.250 g, 11.0 mmol) and cyclohexanone (35 mL), both of them freshly distilled, were added to a solution of **5** or **6** (10.0 mmol) in anhydrous toluene (70 mL). The reaction mixture was refluxed until disappearance of the starting material (TLC) (3 h). After cooling, the suspension obtained was extracted with a 10% hydrochloric acid solution (4 × 25 mL). The aqueous acid solution was washed with diethyl ether and treated with an aqueous KOH solution, cooling at 0 °C, until precipitation and subsequent dissolution of the aluminium hydroxide. The basic solution was finally extracted with chloroform. The combined organic extracts were dried and evaporated to dryness; the residue was purified by column chromatography on neutral alumina, using toluene as the eluant, to yield pure **7** or **8** as yellow viscous oils (Table 1).

*5.1.4. 1-Dimethylaminoalkyl-7-methyl-2,3-dihydro-1,8-naphthyridin-4(1H)-ones phenylhydrazones dihydrochlorides (**9a–h**)*

5.1.4.1. General procedure. A mixture of **7** or **8** (1.6 mmol), methanol (2 mL) and the appropriate phenylhydrazine hydrochloride (1.8 mmol) was refluxed, until disappearance of the reagents (TLC) (1–2 h). After cooling at 0 °C, a yellow–orange solid formed by treatment with an ethanol solution saturated with hydrogen chloride (0.2 mL). The precipitate obtained was collected and recrystallised from methanol–diethyl ether, to yield the dihydrochlorides **9a**–**h** (Table 2).

*5.1.5. 3-Methyl-5-dimethylaminoalkyl-5H-indolo[3,2-c][1,8]naphthyridines (**1a–h**)*

5.1.5.1. General procedure. An ethanol solution saturated with hydrogen chloride (3 mL) was added to a solution of phenylhydrazone dihydrochloride **9a**–**h** (1.5 mmol) in a small amount of absolute ethanol; the mixture obtained was refluxed until disappearance of the starting material (TLC) (3–4 h).

After cooling, the yellow solid formed, composed of a mixture of ammonium chloride and variable quantities of 5,6-dihydroindolonaphthyridine and indolonaphthyridine, was collected and dissolved in water. The aqueous solution was basified with 30% NH_4OH solution, cooling at 0 °C, to give a solid, which was collected and dried over P_2O_5. The precipitate was dissolved in ethanol, and the solution obtained was refluxed until complete conversion into the fully aromatic compound **1a**–**h** (TLC) (3–24 h). After cooling, the ethanolic solution obtained was concentrated to dryness and the crude product was purified by recrystallisation from the appropriate solvent to yield pure **1a**–**h** (Table 3).

In the case of the reaction of **9a**, **e**, the intermediate 5,6-dihydroindolonaphthyridines **10a**, **e** were sufficiently pure to be characterised by analytical and spectral data (see below).

All the compounds **1a**–**h** were solubilised in absolute ethanol and treated with hydrogen chloride-saturated ethanol solution to give the corresponding dihydrochloride hydrates, which were purified by recrystallisation from methanol–ethyl ether (Table 4).

Compound **10a**. Yield 46%. Melting point (m.p.) 85–86 °C. ^{1}H-NMR (recorded on a Varian CFT-20 spectrometer, operating at 80 MHz): δ (ppm) = 2.23 (s, 9H, 3-CH_3 + $N(CH_3)_2$); 2.52 (t, 2H, $CH_2CH_2N(CH_3)_2$); 3.71 (t, 2H, $CH_2CH_2N(CH_3)_2$); 5.01 (s, 2H, 6-CH_2); 6.29–7.59 (m, 6H, ArH); 11.29 (s, 1H, NH, exchangeable with D_2O). MS (m/z): 306 [M^+].

Compound **10e**. Yield 44%. M.p. 70–71 °C. ^{1}H-NMR (recorded on a Varian CFT-20, spectrometer, operating at 80 MHz): δ (ppm) = 1.60–2.00 (m, 2H,

$CH_2CH_2CH_2$); 2.16 (s, 6H, $N(CH_3)_2$); 2.25 (m, 5H, 3-CH_3 + $CH_2CH_2CH_2N(CH_3)_2$); 3.59 (t, 2H, $CH_2CH_2CH_2N(CH_3)_2$); 4.97 (s, 2H, 6-CH_2); 6.29–7.54 (m, 6H, ArH); 11.26 (s, 1H, NH, exchangeable with D_2O). MS (*m*/*z*): 320 [M^+].

5.2. Biological activity

5.2.1. Cell cultures

HL-60 (human myeloid leukaemic cells) were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 15% heat-inactivated fetal calf serum (Seromed), and HeLa (human cervix adenocarcinoma cells) were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal calf serum (Seromed). 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 0.25 μg mL^{-1} amphotericin B (Sigma Chemical Co.) were added to the media. The cells were cultured at 37 °C in a moist atmosphere of 5% carbon dioxide in air.

5.2.2. Inhibition growth assay

HL-60 cells (3×10^4) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations of the test agents were added to the complete medium and incubated for a further 72 h.

HeLa (3×10^4) cells were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, the medium was replaced with an equal volume of fresh medium, and various concentrations of the test agents were added. The cells were then incubated in standard conditions for a further 72 h.

A trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as IC_{50} values, i.e. the concentrations of the test agent inducing 50% reduction in cell numbers compared with control cultures.

5.3. Interaction with DNA

5.3.1. Nucleic acid

Salmon testes DNA was purchased from Sigma Chemical Co. Aqueous solutions of nucleic acid containing Tris 10 mM, EDTA 1 mM (pH 7.0) and NaCl 0.01 M were used (ETN buffer).

5.3.2. Spectrophotometric determinations

UV absorption spectra were recorded at room temperature with a Perkin–Elmer model Lambda 5 spectrometer. DNA and test compound concentrations were determined by absorption measurements, using the following extinction coefficients: $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ at 260 nm for DNA, $\varepsilon = 11060\ M^{-1}\ cm^{-1}$ at 354 nm for **1a**, $\varepsilon = 10200\ M^{-1}\ cm^{-1}$ at 354 nm for **1b**, $\varepsilon = 7900\ M^{-1}\ cm^{-1}$ at 352 nm for **1c**, $\varepsilon = 16300\ M^{-1}\ cm^{-1}$ at 312 nm for **1d**, $\varepsilon = 11300\ M^{-1}\ cm^{-1}$ at 354 nm for **1e**; $\varepsilon = 10200\ M^{-1}\ cm^{-1}$ at 354 nm for **1f**, $\varepsilon = 9250\ M^{-1}\ cm^{-1}$ at 352 nm for **1g** and $\varepsilon = 18250\ M^{-1}\ cm^{-1}$ at 312 nm for **1h**.

5.3.3. Flow linear dichroism

Linear dichroism (LD) measurements were performed on a JASCO J500A circular dichroism spectropolarimeter converted for LD and equipped with an IBM PC and a JASCO J interface.

Linear dichroism is defined as:

$$LD_{(\lambda)} = A_{//(\lambda)} - A_{\perp(\lambda)}$$

where $A_{//}$ and $A_{\perp}$ correspond to the absorbances of the sample when polarised light is oriented parallel or perpendicular to the flow direction, respectively. The orientation is produced by a device designed by Wada and Kozawa [18] at a shear gradient of 500–700 rpm.

The reduced linear dichroism is defined as:

$$LD_r = LD_{(\lambda)}/A_{iso(\lambda)}$$

where $A_{iso(\lambda)}$ is the absorbance of the sample in the absence of flow. This quantity may be related to an orientation factor (S) and the angle between the active transition moment in the chromophore and the DNA helix axis, α [19,20]:

$$LD_r = 3/2(3\cos^2\alpha - 1)S$$

Assuming a value of $\alpha = 90°$ for the DNA base-pair chromophore with respect to a local helix axis, it is possible to evaluate α_L for a given ligand:

$$\alpha_L = \arccos[1/3 - (LD_r)_L/3(LD_r)_{DNA}]^{1/2}$$

where $(LD_r)_L$ is the reduced linear dichroism for the ligand, $(LD_r)_{DNA}$ is the reduced LD for DNA and α_L defines the ligand–DNA relative orientation. For the intercalated system, $(LD_r)_L \approx (LD_r)_{DNA}$ and $\alpha_L \cong 90°$.

A solution of salmon testes DNA (1.6×10^{-3}M) in ETN buffer was used. Spectra were recorded at 25 °C at 12.5, 25 and 50 [DNA]/[drug] ratios.

5.3.4. Fluorimetric determinations

Fluorescence spectra were recorded on a Perkin–Elmer LS50B luminescence spectrometer at $\lambda_{ex} = 354$ nm. The measurements were carried out at room temperature in ETN buffer.

Titration was performed by the addition of $5–12 \times 10^{-6}$ M test compound to samples containing different concentrations of DNA. The buffer background was subtracted from intensities for binding calculations. The amounts of free and bound ligand were determined as previously indicated [6].

Experimental binding data were plotted in accordance with the method of Scatchard [21] and analysed by using the approach of McGhee and von Hippel [17] to obtain the intrinsic binding constant (K_i).

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