Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 24, pp. 3003-3006, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00526-9

RELATIONSHIP BETWEEN CYTOTOXICITY AND DNA-BINDING AFFINITY OF AMIDINE DERIVATIVES OF TETRAHYDROQUINO[4,3-*b*][1]BENZAZEPINES AND TETRAHYDROBENZO[*k*]NAPHTHYRIDINES.

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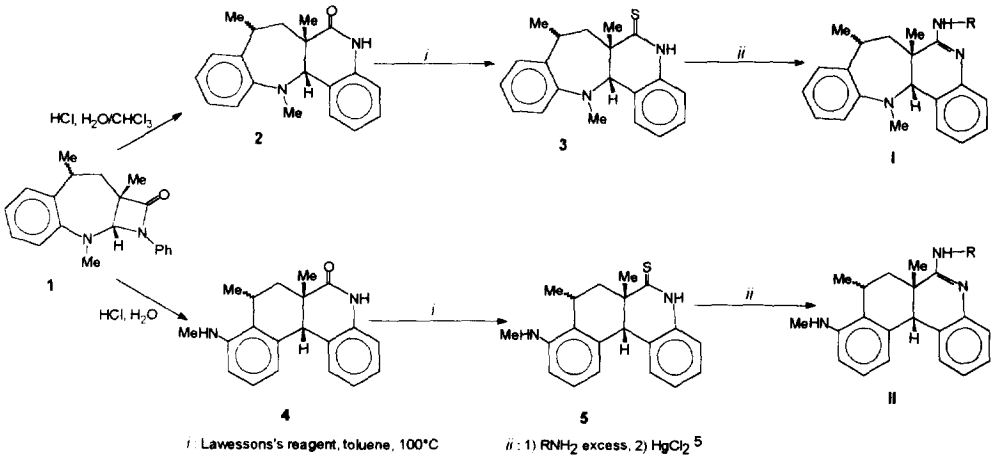
Abstract : Two sets of amidine derivatives of the non-planar tetracyclic systems : tetrahydroquino[4,3-*b*][1] benzazepine (**I**) and tetrahydrobenzo[*k*]naphthyridine (**II**), bearing three types of side chains (hydroxyl, amine and alkyl) have been synthesized. All the compounds were found to possess weak but significant DNA-binding affinity which correlated with *in vitro* cytotoxicity across two cell lines.

There is presently considerable interest in DNA-binding molecules as potential antitumour agents, DNA probes, or for possible use as therapeutic agents to treat genetic-based disorders [1a-d]. Compounds have been reported that can bind either in the major groove (e.g. the nitrogen mustards) or the minor groove (e.g. mitomycin), or that can intercalate between base pairs of duplex DNA (e.g. adriamycin)[1c]. Furthermore, compounds can bind either covalently or non-covalently to DNA [1a].

In the present work, two novel non-planar heterocyclic compounds derived from tetrahydroquino[4,3-*b*][1]benzazepine (**I**) and tetrahydrobenzo[*k*]naphthyridine (**II**) systems have been studied. To increase the possibility of DNA-interaction an amidine moiety (protonated at pH = 7.4) was included. The exocyclic nitrogen of these amidines was substituted by alkyl-, hydroxyalkyl- or aminoalkyl-bearing side-chains. This approach has been already used in the case of ellipticine and 5*H*- pyrido[4,3-*b*]benzo[*e*]- and- benzo[*g*]indoles derivatives[2].

The β-amino-β-lactam **1** was prepared from the 4,5-dihydro-1*H*-[1]benzazepine as previously described[3], and lactams **2** and **4** were synthesized according to Scheme 1. These were reacted with Lawesson's reagent[4] in toluene at 100°C to give thiolactams **3** and **5** which were purified by column chromatography. Facile conversion

into the corresponding amidine derivatives occurred after treatment with a large excess of the primary amines and one equivalent of $HgCl_2$ added in one portion[5]. The precipitate was removed by centrifugation, the excess amine evaporated *in vacuo*, and the resulting solid residue treated with CH_2Cl_2 and washed with a saturated solution of $Na_2S_2O_3$. The hydrochlorides were prepared from the free bases by treatment with HCl-EtOH. All compounds were produced as mixtures (60/40 ratio) of two diastereomers according to the geometry of the methyl substituents ; the more abundant being those where the two CH_3 groups are in a *trans* relationship.



Scheme 1

The eight amidine compounds shown in Table 1 were examined for DNA-binding affinity using thermal denaturation [7,8] and for *in vitro* cytotoxicity in both CH1 and SKOV-3 (ovarian carcinoma) cell lines. All of the compounds appear to have significant DNA-binding affinity and some of them have pronounced *in vitro* cytotoxicity across both of the cell lines examined. Furthermore, there appears to be a good correlation between DNA-binding affinity and cytotoxicity for both sets of analogs. The benzo[*k*]naphthyridine system (**II**) shows a greater overall DNA-binding affinity compared to its isomeric quino[4,3-*b*][1]benzazepine (**I**) counterpart which may be due to secondary interactions with DNA through the methylamino-substituent remote from the amidine residue. This extra CH_3-NH group in the benzo[*k*]naphthyridine (**II**) series is well-positioned to enhance DNA interaction if the molecules act as minor-groove binding agents. Introduction of the aminoalkyl side-chain favours enhanced interaction with $(CH_2)_4NH_2$ > $(CH_2)_3NH_2$ in each system. The hydroxyl group present in **Ib** and **IIb** similary enhances overall interaction relative to the unsubstituted hydrophobic propyl residue of the "parent" compounds, **Ia** and **IIa**. However, the enhancement is somewhat smaller than that observed for the protonatable amine function in compounds **Ic-d** and **IIc-d**. In summary, the data provide a rank order of binding for both series of :

$$R = (CH_2)_4NH_2 > (CH_2)_3NH_2 > (CH_2)_3OH > (CH_2)_2CH_3$$

with the benzo[*k*]naphthyridine compounds having a greater overall affinity for double-stranded DNA than the

quino[4,3-*b*][1]benzazepine isomers.

TABLE 1: Cytotoxicities[*] (IC_{50}, μM) and thermal denaturation[8] of complexes of compounds Ia-d and IIa-d with calf thymus DNA

Compound	R	CH1	SKOV-3	ΔT_m/°C
Ia, HCl	$CH_2CH_2CH_3$	10.5	1.4	0.17+/-0.09
Ib, HCl	$CH_2CH_2CH_2OH$	14	32.5	0.24+/-0.10
Ic, 2HCl	$CH_2CH_2CH_2NH_2$	0.5	3.3	0.46+/-0.13
Id,2HCl	$CH_2CH_2CH_2CH_2NH_2$	1.6	1.6	0.58+/-0.14
IIa, 2HCl	$CH_2CH_2CH_3$	9	12	0.29+/-0.10
IIb, 2HCl	$CH_2CH_2CH_2OH$	6	12.5	0.35+/-0.09
IIc, 3HCl	$CH_2CH_2CH_2NH_2$	2	3.1	0.88+/-0.12
IId, 3HCl	$CH_2CH_2CH_2CH_2NH_2$	3.5	2.25	1.14+/-0.13

The behaviour and 3-dimensional "enveloppe"shape of these molecules (as judged by preliminary molecular modelling studies) suggests that they may bind to DNA *via* the minor-groove rather than through an intercalative process, although non-specific interaction with the DNA backbone (i.e. cation-phosphate) is also feasible. However, the binding is significant and represents a moderate differential stabilization of the DNA duplex towards thermal denaturation. Studies are currently underway to try to elucidate the preferred binding mode of these compounds which represent a completely new class of DNA-binding ligands.

Acknowledgments Mrs M.Le Roch, Mr J-F Cupif, Mr P.Guenot, Mr S.Sinbandhit and Mr J-P.Ternisien are thanked for technical assistance, and Dr Lloyd Kelland (CRC, Cancer Therapeutics Centre, ICR,Sutton, UK) is thanked for providing the cytotoxicity data.This work was supported in part (TCJ, DET) by the Cancer Research Campain, UK.

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6.This reaction requires the addition of $HgCl_2$ after mixing the thioamide and amine (in large excess) components.

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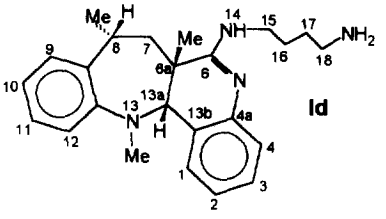
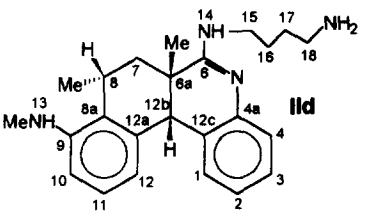
8. The compounds were studied in DNA thermal denaturation experiments using calf thymus (CT) DNA (type-I, highly polymerized).The CT-DNA had A_{260}/A_{280} = 1.9 and was satisfactorily free from protein; a molar extinction value at 260 nm of ε =6600$M^{-1}cm^{-1}$ was used. Aqueous solutions of DNA were prepared in Millipore-purified water buffered at pH 7.00±0.01 using 10mM sodium phosphate and 1mM EDTA; no added salt or support electrolyte was used. Working solutions containing 100µM of DNA alone and in the presence of 20µM of a compound were monitored at 260nm using a modified Shimadzu UV-2101 PC spectrophotometer fitted with a Shimadzu SPR-8 Peltier heating/cooling accessory. All compound-DNA solutions were incubated at 37°C for 18h prior to examination. Heating was applied at 1°C min^{-1} from 40°C until thermal denaturation of the DNA was complete, as judged from the increase in absorption. The optical absorbance versus temperature curves were sampled, normalized and analyzed.Thermal denaturation temperatures (T_m) were determined at a relative absorbance value of 0.50 and are reported as the mean ±s.e.m. of at least four determinations. The change in T_m (ΔT_m) following interaction of CT-DNA with an added compound was evaluated from :

$\Delta T_m = T_{m\ (DNA\text{-}Compound)} - T_{m\ (DNA)}$ and is reported (Table I) for a fixed [DNAp]: [compound] molar ratio of 5:1. This value was selected following trials to establish the saturation concentration of each compound, and from experience with other agents which bind either by intercalation or via the minor groove of duplex DNA. The ratio selected did not lead to saturation of DNA binding.

9. Analogs **Ia-d** and **IIa-d** were characterized by IR, mass spectroscopy and ^{1}H- and ^{13}C-NMR . Two typical examples from both series are given for the major stereoisomers of the free bases :





Id: IR (cm^{-1}): υ(NH+NH_2): 3352, broad; υC=N: 1582. ^{1}H NMR ($CDCl_3$, δ ppm, J Hz, *: coalescent): Ar (m: 7.3-6.6), CH_3-6a (s: 1.06*), H-7α (m: 2.23*), H-7β (m: 1.84*), H-8 (m: 3.49), CH_3-8 (d: 1.44), CH_3-13 (s: 2.53), H-13b (s: 4.03), CH_2-15 (m: 3.36), CH_2-16 (m: 1.62), CH_2-17 (m: 1.53), CH_2-18 (t: 2.74). ^{13}C NMR ($CDCl_3$): C-1, C-2, C-3, C-4, C-9, C-10, C-11, C-12 (118.0*; 120.3*; 121.6, 123.6, 126.4, 126.9*, 128.7, 129.7*), C-4a (145.6), C-12a (150.8), C-6 (165.1), C-6a (41.6), C-7 (41.8*), C-8 (30.8), CH_3-8 and CH_3-6a (23.3* and 23.9*), C-8a (134.8), CH_3-13 (37.0*; ^{1}JCH = 135), C-13a (65.8), C-13b (122.4*), CH_2-15 and CH_2-18 (41.3 and 40.9), CH_2-16 (26.4), CH_2-17 (30.7). MS: 376(6), 230(100), 213(5), 187(6), 147(16), 132(44), 117(10), 91(3). calc. $C_{24}H_{32}N_4$: 376.26268, found: 376.2615.

IId: IR: (cm^{-1}): υ(NH+NH_2+$NHCH_3$): 3348, broad; υC=N: 1584. ^{1}H NMR ($CDCl_3$): H-1 (d: 6.7), H-2 (t: 6.79), H-3 (t: 7.13), H-4 (d: 7.09), CH_3-6a (s: 1.10), H-7α (m: 1.76; JH7αH7β = 13.8; JH7αH8 = 8.9; JH7αH12b = 2.1), H-7β (m: 1.5, JH7βH8 = 7.3), H-8 (m: 2.69), CH_3-8 (d: 0.89), H-10 (d: 6.64), H-11 (t: 7.21), H-12 (d: 6.61), H-13 (s: 3.72), CH_3-13 (s: 2.90), CH_2-15 (m: 3.52), CH_2-16 (m: 1.70), CH_2-17 (dd: 1.57), CH_2-18 (t: 2.79). ^{13}C NMR ($CDCl_3$): C-1 (127.5), C-2 (122.1), C-3 (127.2), C-4 (122.8), C-4a (144.7), C-6 (164.9), C-6a (36.9), CH_3-6a (20.5), C-7 (37.1), C-8 (25.4), CH_3-8 (20.9), C-8a (129.4), C-9 (146.9), C-10 (108.8), C-11 (126.5), C-12 (121.1), C-12a (135.5), C-12b (47.7), C-12c (123.6), CH_3-13 (31.0, JCH = 135), CH_2-15 (41 ; JCH = 138), CH_2-16 (26.6), CH_2-17 (31.0), CH_2-18 (41.7 ; JCH = 134). (2D NMR-assisted attributions) .MS: 376 (38), 361(24), 346(17), 332(14), 318(21), 304(10) $C_{24}H_{32}N_4$ calc.376.2627, found 376.262.

(*Received in Belgium* 19 *May* 1995; *accepted* 9 *November* 1995)