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Synthesis of a novel dimeric bis-benzimidazole with site-selective DNA-binding properties

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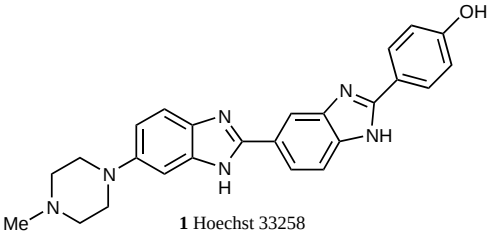
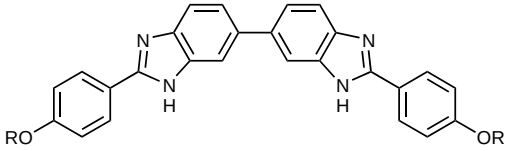
Abstract—We describe the synthesis of bis(3-{4-[2′-(4-methoxyphenyl)-3*H*,3′*H*-[5,5′]bibenzimidazolyl-2-yl]phenoxy}propyl)-methylamine **8**, which has high affinity for the oligonucleotide sequence $[A.T]_4$-[G.C]-$[A.T]_4$. © 2002 Published by Elsevier Science Ltd.

In cancer chemotherapy there is a move away from the traditional drugs, which damage DNA in a non-selective fashion, to new agents that interact non-covalently and modify the process of DNA replication. The possible parent of this new class of compounds is Hoechst 33258 **1**, which recognises A/T sequences in human DNA and was shown to inhibit binding of the so-called TATA-box binding protein to DNA, and also to be an effective inhibitor of mammalian DNA topoisomerase I.[1] It was shown to have activity against the mouse leukaemia L1210, and became the subject of several phase I clinical trials, but toxicity precluded further advanced trials.[2]



1 Hoechst 33258

Our redesign of this molecule began with a computer modelling study and the subsequent synthesis of a range of head-to-head bis-benzimidazoles[3] of general structure **2**. More recent biological evaluation has revealed that all of these compounds possess modest to good activities against a range of cultured human cancer cell lines with compound **2c** possessing the best overall potency—IC_{50} values down to 50 nM against lung, colon and breast tumours (Table 1).[4] There was also highly sequence-selective binding across four consecutive A/T base pairs of the dodecanucleotide sequence d(CGCGAATTCGCG), which is a model for longer oligonucleotide sequences in the A/T-rich region of the minor groove.



2a R = H, **2b** R = Me, **2c** R = $(CH_2)_3NMe_2$

With the aim of improving both the degree of binding and the biological potency, we embarked upon a synthesis of the dimer of compound **2c** and our synthetic route is shown in Scheme 1. In our original synthesis of compounds **2**, the condensation between an aryl aldehyde and 3,3′,4,4′-tetraaminobiphenyl was effected in

Table 1. IC_{50} values (μM) following a 96 h exposure to test compounds

Cell line	Compound **2b**	Compound **2c**
A2780 (ovarian)	13.5	0.235
A2780-Pt^R	5.1	0.115
CH1 (ovarian)	3.8	0.240
SKOV-3 (ovarian)	16.5	0.375
MCF-7 (breast)	0.174	0.051
RKO (colon)	0.645	0.158
H630 (colon)	0.232	0.167
OAW42 (ovary)	0.175	0.140
H838 (lung)	0.275	0.077

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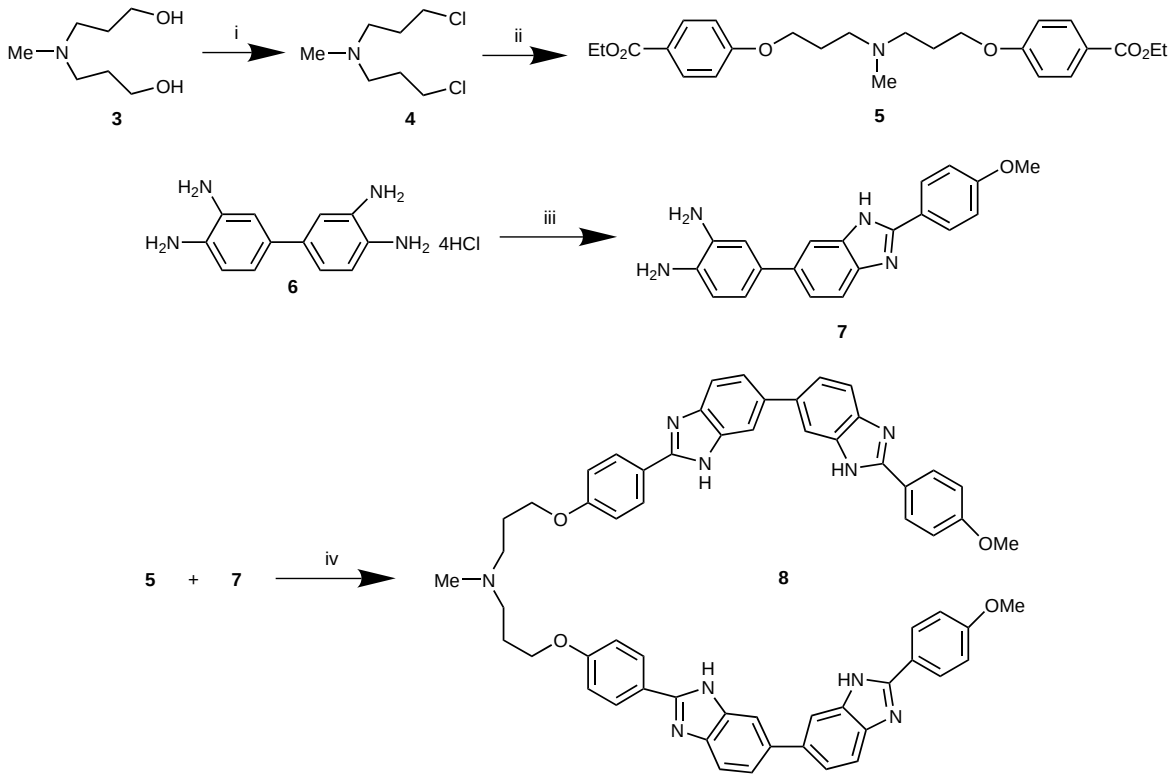

Scheme 1. *Reagents and conditions*: (i) $SOCl_2$, benzene, reflux, 4 h; (ii) $EtO_2CC_6H_4OH$, NaH, DMF, 80°C, 24 h; (iii) $MeOC_6H_4COOH$, 10% PPMA, 100°C, 2 h; (iv) 10% PPMA, 120°C, 6 h.

nitrobenzene at 150°C, with the nitrobenzene presumably acting as both solvent and oxidant. The yields of these reactions were never greater than about 30% and the non-availability of nitrobenzene in Northern Ireland necessitated a change of strategy. We now envisaged a condensation between diethyl 4,4′-[(methylimino)bis(propoxy)]dibenzoate **5** and 4-[2-(4-methoxyphenyl)-1*H*-benzimidazol-6-yl]benzene-1,2-diamine **7** to provide the dimeric bis-benzimidazole **8**.

The amine **5** was prepared (95% crude yield)[5] from the ethyl ester of 4-hydroxybenzoic acid (2 equiv.) and *N*,*N*-bis(3-chloropropyl)-*N*-methylamine **4**, which had been prepared by treatment of 3,3′-(methylimino)-dipropan-1-ol[6] **3** with thionyl chloride. The required benzimidazole **7** was prepared by a condensation[7] between 4-methoxybenzoic acid and 3,3′-diaminobenzidine tetrahydrochloride **6** using a solution of phosphorus pentoxide in methanesulphonic acid (PPMA)[8] (1:10 w/w) as the condensing agent. The yield of **7** was only modest (32%) but the reaction was more convenient (and certainly less hazardous) than our previous condensation methodology with nitrobenzene. Finally, compounds **5** and **7** were combined in the ratio of 1:2 and reacted with the same condensing agent (PPMA) at 120°C for 6 h to provide the desired dimeric bis-benzimidazole **8** (31% yield after recrystallisation from DMF–MeOH–H_2O).[9]

Preliminary biological evaluation of this compound against a range of cancer cells showed that it was about ten times less cytotoxic on average than the simpler benzimidazole **2c**.[4] However, DNA footprinting studies demonstrated that the compound had a high affinity (drug concentration for half-maximal footprinting was 0.15 μM) and selectivity for the oligonucleotide sequence $[A.T]_4$-[G.C]-$[A.T]_4$, which is not a common motif in human DNA. Further biological evaluation is underway and these results and all of the DNA binding studies will be described in due course.

Overall, we have devised an effective method of synthesising a new class of molecules with a high degree of sequence selectivity for A/T-rich regions of DNA (binding over a ten base pair sequence), and which do not exhibit generalised cytotoxicity. Additional studies will be required in order to fine-tune this selectivity and hopefully provide compounds with selective anti-tumour activities.

Acknowledgements

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5. Synthesis of diethyl 4,4′-[(methylimino)bis(propoxy)]-dibenzoate **5**: To a suspension of sodium hydride (0.80 g, 60% dispersion in mineral oil, 20 mmol) in dry DMF (10 cm^3) was added a solution of ethyl 4-hydroxybenzoate (3.32 g, 20 mmol) in dry DMF (8 cm^3) dropwise. After the evolution of hydrogen gas ceased, a solution of *N*,*N*-bis(3-chloropropyl)-*N*-methylamine **4** (1.84 g, 10 mmol) in dry DMF (2 cm^3) was added dropwise and the reaction mixture was stirred at 80°C for 24 h. It was then cooled in an ice bath, water was added slowly and the mixture was extracted with EtOAc. The combined organic extracts were washed with saturated Na_2CO_3 solution and brine successively, dried over $MgSO_4$, filtered and evaporated to yield compound **5** (4.20 g, 95%) as a viscous yellow oil used directly for the next step without further purification. IR (film)/cm^{-1} 2954, 1712, 1607, 1512, 1465, 1367, 1276, 1255, 1168, 1104, 1024 and 770; δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.96 (4H, d, $J=8.9$, ArH), 6.86 (4H, d, $J=8.9$, ArH), 4.34 (4H, q, $J=7.1$, $2\times CO_2CH_2CH_3$), 4.03 (4H, t, $J=6.3$, $2\times OCH_2$), 2.55 (4H, t, $J=7.0$, $2\times NCH_2$), 2.26 (s, 3H, NCH_3), 1.96 (4H, quintet, $J=6.6$, $2\times CH_2$) and 1.38 (6H, t, $J=7.1$, $2\times CO_2CH_2CH_3$); δ_C (75 MHz; $CDCl_3$; Me_4Si) 166.4, 162.7, 131.5, 122.8, 114.0, 66.1, 60.6, 53.9, 42.3, 27.0 and 14.4; *m*/*z* (EI) 443 (M^+, 1%), 250 (15), 121 (49), 97 (41), 85 (60), 69 (52), 57 (100) and 43 (98); HRMS (EI) calcd for $C_{25}H_{33}NO_6$ (M^+) 443.2308, found 443.2314.
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7. Synthesis of 4-[2-(4-methoxyphenyl)-1*H*-benzimidazol-6-yl]benzene-1,2-diamine **7**: 3,3′-Diaminobenzidine tetrahydrochloride dihydrate **6** (4.76 g, 12 mmol) was dissolved in PPMA (30 cm^3, 1:10 solution by weight of phosphorus pentoxide in methanesulphonic acid). When the bubbles of hydrogen chloride gas were eliminated, 4-methoxybenzoic acid (1.82 g, 12 mmol) was added to this solution and the mixture was stirred at 100°C for 2 h. The solution was cooled to room temperature, poured into ice water and neutralised with saturated Na_2CO_3 solution. The resulting solid was filtered under reduced pressure, washed with water and dried. It was then extracted with hot MeOH and the extract was evaporated to give the crude product, which was purified by flash column chromatography on silica gel eluting with DCM/MeOH (10:1) to give compound **7** (1.28 g, 32%) as a yellow solid; mp 219–220°C; IR (KBr)/cm^{-1} 3367, 2926, 1611, 1522, 1471, 1386, 1255, 1178, 1025, 809 and 732; δ_H (500 MHz; DMSO-d_6) 8.11 (2H, d, $J=8.9$, ArH), 7.57 (1H, s, ArH), 7.53 (1H, d, $J=8.2$, ArH), 7.32 (1H, dd, $J=8.2$ and 1.5, ArH), 7.11 (2H, d, $J=8.9$, ArH), 6.90 (1H, d, $J=2.0$, ArH), 6.75 (1H, dd, $J=7.9$ and 2.0, ArH), 6.60 (1H, d, $J=7.9$, ArH), 4.54 (4H, br s, $2\times NH_2$) and 3.85 (3H, s, OCH_3); *m*/*z* (EI) 330 (M^+, 8%), 195 (8), 184 (33), 168 (100), 153 (27), 141 (17) and 115 (23); HRMS (EI) calcd for $C_{20}H_{18}N_4O$ (M^+) 330.1481, found 330.1468.
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9. Synthesis of bis(3-{4-[2′-(4-methoxyphenyl)-3*H*,3′*H*-[5,5′]bibenzimidazolyl - 2 - yl]phenoxy}propyl)methylamine **8**: 4-[2-(4-Methoxyphenyl)-1*H*-benzimidazol-6-yl]benzene-1,2-diamine **7** (0.60 g, 1.8 mmol) was dissolved in PPMA (4.55 cm^3) and diethyl 4,4′-[(methylimino)bis(propoxy)]dibenzoate **5** (0.40 g, 0.9 mmol) was added to this solution. The reaction mixture was stirred at 120°C for 6 h, then the solution was cooled to room temperature, poured into ice water and neutralised with saturated Na_2CO_3 solution. The resulting solid was filtered under reduced pressure, washed with water and dried. It was recrystallised from DMF–MeOH–H_2O to give pure compound **8** (0.27 g, 31%) as a yellow solid; mp >300°C; IR (KBr)/cm^{-1} 3420, 2925, 1652, 1611, 1541, 1489, 1446, 1385, 1251, 1174, 1026, 834 and 803; δ_H (500 MHz; DMSO-d_6) 8.14 (8H, t, $J=8.6$, ArH), 7.95–7.40 (12H, m, ArH), 7.12 (4H, d, $J=8.9$, ArH), 7.09 (4H, d, $J=8.8$, ArH), 4.10 (4H, t, $J=6.0$, $2\times OCH_2$), 3.85 (6H, s, $2\times OCH_3$), 2.56 (4H, m, $2\times NCH_2$), 2.26 (3H, s, NCH_3) and 1.92 (4H, m, $2\times CH_2$); *m*/*z* (LSI) 976 ($M+H^+$, 100%), 544 (27) and 433 (56); HRMS (LSI) calcd for $C_{61}H_{54}N_9O_4$ ($M+H^+$) 976.4299, found 976.4333.