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PROBING THE IMPORTANCE OF THE SECOND CHLOROETHYL ARM OF A BENZOIC ACID MUSTARD DERIVATIVE OF AN IMIDAZOLE-CONTAINING ANALOGUE OF DISTAMYCIN

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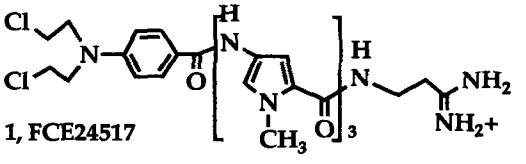
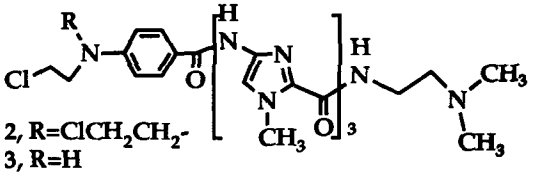
Abstract. The synthesis and biological evaluation of a half mustard derivative of an imidazole-containing analogue of distamycin **3** are described. Although binding efficiently to naked DNA the half mustard has a dramatically reduced cytotoxicity, and shows a distinctly different sequence specificity of alkylation from the parent mustard.

Nitrogen mustards are commonly used in the treatment of cancers[1], and they are known to react with guanine-N7 in the major groove to produce highly lethal interstrand crosslinks at 5'-GNC sites.[2] Recently, FCE24517, a benzoic acid mustard derivative of distamycin **1**, was developed.[3] It was shown to have significant activity against a number of solid cancers,[4] and is currently undergoing phase II clinical trials in Europe. In contrast to conventional N-mustards compound **1** was shown to bind in the minor groove of AT-rich sequences and alkylate at selective adenine-N3 sites with no evidence of guanine-N7 alkylation.[5] In our studies a highly cytotoxic imidazole-containing analogue **2** was synthesized which was selective for the minor groove of GC-rich sequences.[6] Although both compounds **1** and **2** have a bifunctional N-mustard moiety neither was shown to produce interstrand crosslinks with either isolated or cellular DNA at therapeutic doses,[5,7] suggesting cytotoxic monoalkylation may contribute to the biological activity. A half mustard analogue of **2** was therefore synthesized to determine the importance of the second chloroethyl arm for biological activity. In this communication we report the synthesis, cytotoxicity, and DNA binding properties of the half mustard **3**.



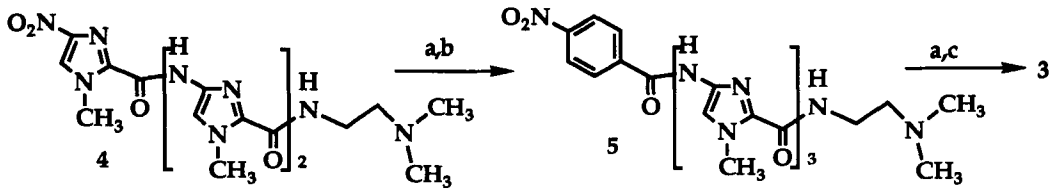
1, FCE24517



2, R=ClCH$_2$CH$_2$-
3, R=H

In the synthesis of half mustard **3**, given in scheme 1, the nitro group of compound **4**[6] was reduced by catalytic hydrogenation and the resulting amine was coupled with 4-nitrobenzoyl chloride to produce compound **5** in 44 percent yield (mp 170-173°C). The nitro group of

compound **5** was subsequently reduced by catalytic hydrogenation then condensed with chloroacetaldehyde and sodium cyanoborohydride in acidic methanol to produce the desired compound **3** in 16 percent yield (mp 235°C (dec)).[8]



Scheme 1. [a]H_2, 5% Pd-C, MeOH, r.t., 1 atm; [b]4-nitrobenzoyl chloride, triethylamine, dry THF, 0°C to r.t.; [c]$ClCH_2CHO$, $NaCNBH_3$, 1:1 6MHCl and MeOH, r.t.

The apparent DNA binding constants of compound **3** to various DNAs were determined using an ethidium displacement assay,[9] Table 1. Compound **3** binds more strongly to calf thymus and T4 DNAs than **2**, and has similar affinity for poly(dG-dC) and poly(dA-dT). Neither compounds has the AT sequence selectivity of distamycin which binds about 150 times more strongly to poly(dA-dT) than poly(dG-dC).[10] Binding to the minor groove of DNA is suggested because the major groove of T4 DNA is occluded by α-glycosylation of the 5-hydroxymethyl group of cytosine residues.[11] The binding of compound **3** to DNA was also investigated by measuring the melting temperature of its complexes with calf thymus DNA, poly(dA-dT) and poly(dG-dC). The experiments were performed with 0.46 mmoles of DNA b.p., and 0.16 mmoles of ligand in a 10 mM sodium phosphate buffer at pH 7.2. The ΔT_M values were 3.0±0.8, 1.1±0.5 and 3.0±0.4°C, respectively, thus showing a slight preference for poly(dG-dC).

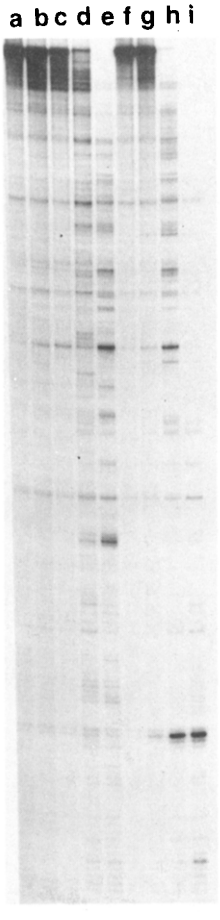
Table 1. Apparent binding constants, Kapp (x 10^5 M^{-1}).

Compound	Calf thymus DNA	T4 DNA	Poly(dG-dC)	Poly(dA-dT)
2[6]	5.9	1.7	5.0	5.5
3	22.7±1.0	38.9±1.5	9.0±0.8	5.5±1.0

The ability of compound **3** to covalently bind to calf thymus DNA was examined using a CD alkylation study.[3] A 140 μL solution of the DNA (0.02 mmoles b.p.) and **3** (0.08 mmoles) in 10 mM sodium phosphate, 0.25 mM EDTA at pH 7.2 was incubated at 37°C for 18 hours, and the CD spectrum showed a positive Cotton effect band at 336 nm (0.4 mdeg). Upon dilution of the solution by half with 1% SDS the ellipticity of the DNA induced ligand band was reduced to 0.15 mdeg thus suggesting that about 75% of **3** was irreversibly bound to the DNA. These results are in agreement with other reports which showed that half mustard containing compounds can alkylate DNA effectively.[12] The full mustard **2** was previously shown to alkylate DNA effectively under identical conditions.[6]

The cytotoxicity of compound **3** against the growth of chronic human myeloid leukemia K562 cells in culture was determined using the MTT assay,[13] producing an IC_{50} value >100 μM following one hour exposure for compound **3**, and 0.3 μM for **2** under identical conditions. The half mustard was therefore significantly less cytotoxic than the full mustard analogue **2** suggesting that the second chloroethyl group of **2** must play a role in enhancing the biological activity.

The covalent binding specificities of **2** and **3** were examined using a polymerase stop assay.[14] A photograph of a representative gel autoradiograph is given in Figure 1. Although showing similar overall reactivity with plasmid DNA the compounds gave distinctly different patterns of alkylation indicating that minor structural changes can produce marked alterations in sequence selectivity.



Removal of one arm of a benzoic acid mustard derivative of an imidazole-containing analogue of distamycin has produced a compound with dramatically reduced cytotoxicity over the parent mustard. Although DNA interstrand crosslinking is not thought to be important for the biological activity of agents of this type,[5,7] removal of one chloroethyl group may prevent the formation of other biologically relevant bifunctional lesions such as DNA intrastrand or DNA-protein crosslinks. Alternatively, the marked alteration in sequence selectivity may result in reduced monoalkylation at critical sequences. A detailed examination of the covalent sequence selectivities is currently in progress.

Figure 1. Autoradiograph of a 6% denaturing sequencing gel showing the blocks to Taq DNA polymerase on a fragment of pBR322 DNA by compounds **3** (lanes b-e) and **2** (lanes f-i). Lane a, control, b 0.1 μM **3**, c 1 μM **3**, d 10 μM **3**, e 50 μM **3**, f 0.1 μM **2**, g 1 μM **2**, h 10 μM **2**, i 50 μM **2**. Drug incubation time was 5 hours at 37°C.

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8. Data for compound **3**: mp 235°C (dec); tlc (25%MeOH:$CHCl_3$) Rf 0.44; IR ($CHCl_3$ cast) 3250, 2938, 1663, 1606, 1530, 1464, 1364, 1260, 1189, 1118, 1018 cm^{-1}; uv (water) 308 nm, $\varepsilon=1.87 \times 10^5$ $M^{-1}cm^{-1}$; ^{1}H-NMR ($CDCl_3$) 9.55 (s br, NH), 9.51 (s br, NH), 7.85 (d, 7.5 2H), 7.70 (t br, 4.2, NH), 7.59 (s, 1H), 7.45 (s, 1H), 7.42 (s, 1H), 6.66 (d, 7.5, 2H), 4.51 (t br, 5.7, NH), 4.06 (s, 3H), 4.05 (s, 3H), 4.03 (s, 3H), 3.73 (t, 5.7, 4H), 3.57 (q, 4.2, 2H) 3.47 (q, 5.7, 4H), 2.51 (t, 4.2, 2H), 2.27 (s, 6H); FAB-MS (NBA) (intensity) 639 (M+H, 5); HRMS-FAB (NBA) 639.2676 ($C_{28}H_{36}N_{12}O_4{}^{35}Cl$ requires 639.2671).
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