Synthesis of 1-(2-Naphthoyl) Benzotriazoles as Photoactivated DNA Cleaving Agents

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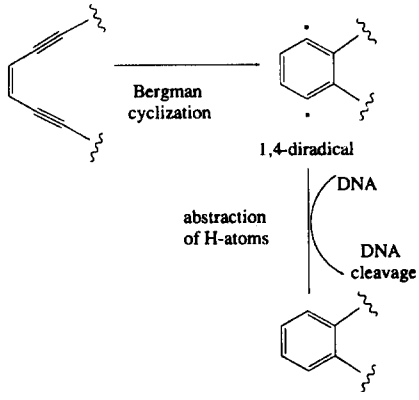
Currently, naturally occuring restriction enzymes (endonucleases) are utilized for DNA cleavage which is crucial to many molecular biological techniques such as gene isolation and production of recombinant DNA vectors (Watson *et al.*, 1983). But because of their relatively short DNA recognition sequences(4-8 base pairs), there have been widespread interest in the development of synthetic DNA-cleaving agents that are chemically stable and activatable by photoirradiation (Saito *et al.*, 1994). Such molecules would have great potentials in tumor photodynamic therapy (Dougherty, 1995).

Recent understanding of the activation mechanism of enediyne antibiotics such as Dynemicin (Wender, 1991, Danishefsky, 1995) and Neocarzinostatin (Goldberg, 1993) has stimulated the design of novel artificial DNA-cleaving molecules.

Via a Bergman cycloaromatisation, triggered by bioreduction or/and nucleophilic attack, phenylene or indenylene diradicals are generated and abstract hydrogen atoms from the ribose backbone of DNA, leaving eventually to single and double strand cleavage of DNA.



Scheme 1. Bergmann cyclization and DNA cleavage.

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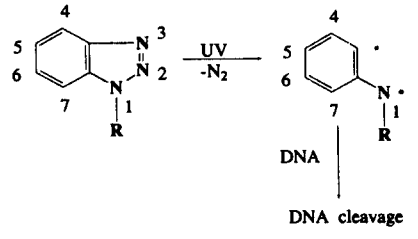
Since the early seventies, thermal and photochemical decomposition of 1,2,3-benzotriazole and its 1-substituted derivatives have been known to undergo loss of nitrogen upon UV irradiation to generate a 1,3-diradical intermediate (McCullagh, 1968). In this report, we describe our effort to examine the possibility of benzotriazole derivatives to cleave DNA as a part of our effort to develop new photoactivated DNA cleaving anticancer agents. We have thought this type of radicals also could be implicated in DNA cleavage.

So, we synthesized three benzotriazole analogs starting from benzotriazole or benzotriazole-5-carboxylic acid and tested their DNA cleaving ability. This trial is valuable because they can be synthesized in only a few steps from comparatively inexpensive starting materials.

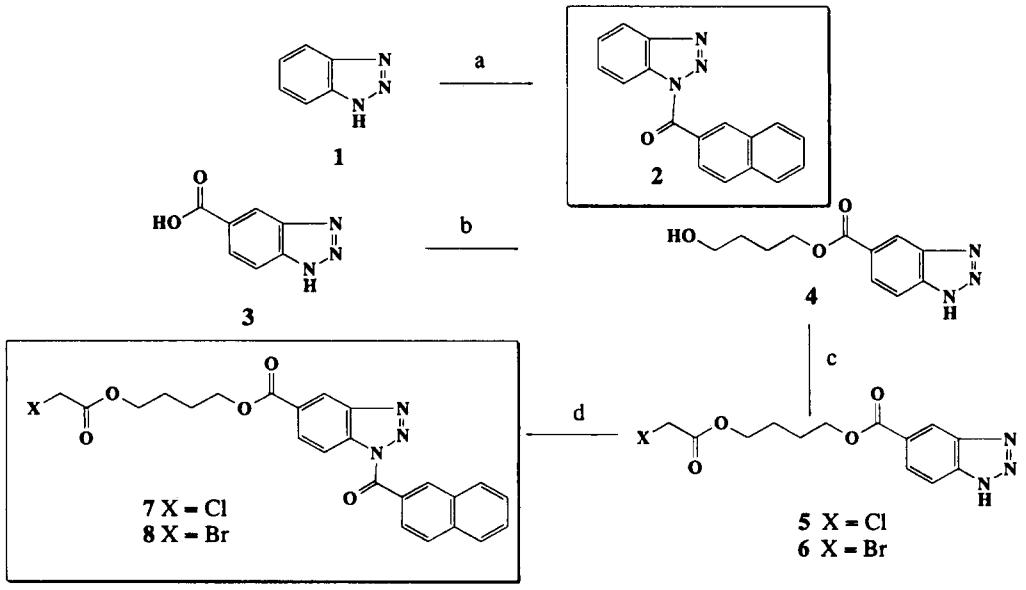
DNA absorbs light of less than 300 nm. So the functionalization of benzotriazole was necessary in order to extend bathochromically its UV absorption into the area less than 300 nm. 1-Alkenyl or 1-phenylbenzotriazole undergoes photocyclization upon UV-irradiation. However, the photolysis of 1-acylbenzotriazoles does not lead to photocyclization reaction. Naphthoyl group was the simplest one among the groups that satisfy our both requirements. So naphthoyl group was introduced to all of our benzotriazole analogs.

Benzotriazole-5-carboxylic acid (BTCA, **3**) also could be the material for the synthesis of DNA cleaving benzotriazole analogs. So α-chloro and α-bromoacetoxybutyl benzotriazole-5-carboxylate (**7, 8**) were synthesized from BTCA. Several methods including DCC coupling were considered for the esterification of BTCA with diol. Among them, Kadaba's BF_3 catalyzed esterification (Kadaba, 1972) was most successful. After haloacetylation of ω-hydroxyl group of hydroxyl ester (**4**), naphthoylation was performed to obtain **7, 8** in 25% and 20% yield respectively from BTCA (**3**).

The most convenient method to detect single and



Scheme 2. 1,3-Diradical generated from 1,2,3-benzotriazole.



Scheme 3. a. 2-naphthoyl chloride, K_2CO_3, acetone, reflux, overnight, 72% b. 1,4-butanediol, BF_3-etherate, 60°C, 16h, 80% c. chloroacetyl chloride, TEA, CH_2Cl_2, RT, 2h, 68%, (for **6**, bromoacetyl bromide, 60%) d. 2-naphthoyl chloride, TEA, CH_2Cl_2, RT, 2h, 63% (for **8**, 59%).

double strand cleavage of DNA was the conversion of supercoiled plasmid DNA (form I) to relaxed circular DNA (form II) which indicates single strand cutting of DNA and the conversion of supercoiled plasmid DNA (form I) directly to linear DNA (form III) which demonstrates coincident-site double strand cleavage. The supercoiled, circular and linear forms of DNA are readily separated by gel electrophoresis and easily visualized by means of UV transilluminator after staining with a fluorescent dye (ethidium bromide).

This procedure was applied to analyse the activity of the synthesized benzotriazole derivative.

Aliquots (20 ml) were prepared from the solution of DNA plamid ϕX174 RF in Tris buffer (pH=7.5, final concentration=30 μM/bp) and the solution of synthesized benzotriazoles in DMSO. These aliquots were placed in 0.5 ml plastic vials and were irradiated with pyrex-filtered-light from 450W Hanovia medium pressure mercury arc lamp for 30 min and analysis by electrophoresis on agarose gel showed, for lane 3, 4,5,6,8, the formation of a large amount of form II, consumption of form I. Analogs **7** and **8** showed better DNA cleaving ability than 1-(2-naphthoyl) benzotriazole(2). The availability as a DNA cleaving agent is presumably improved by introducing electrophilic group on alkyl chain of benzotriazole-5-carboxylate which allows nucleophilic site of DNA to attack electrophilic group before UV irradiation. In other words, introduction of electrophilic group on alkyl chain might have prevented them from flowing away without DNA cleaving. Bromoacetoxybutyl benzotriazole-5-carboxylate (**8**) showed better DNA cleaving ability comparing to **7**. This result was presumably attributed to better leaving ability of bromide than chloride. DNA cleavage was only slightly increased by incubating samples for 4 hours. That might be due to the reaction of DNA with a halide proceeded in a short time.

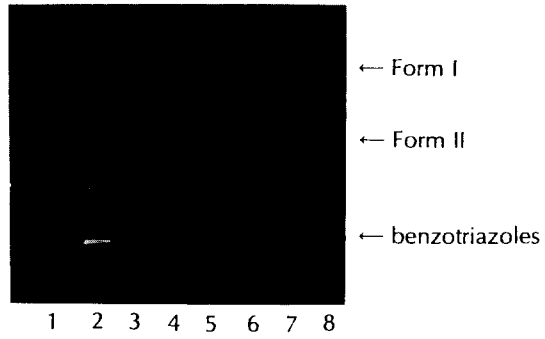


Fig. 1. DNA cleaving experiment with DNA plasmid ϕX174 RF in Tris buffer (pH=7.5, final concentration=30 μM/bp) and the solution of synthesized benzotriazoles in DMSO. Lane 1: DNA+UV, 2: DNA+**7** (150 μM), 3: DNA+**7** (75 μM)+UV, 4: DNA+**7** (150 μM)+UV, 5: DNA+**7** (75 μM)+UV, 6: DNA+**7** (150 μM)+UV, 7: DNA+**2** (75 μM)+UV, 8: DNA+**8** (75 μM)+UV. Lane 2, 5, 6, 7, 8 were incubated at room temperature for 4 hours at room termperature in the dark.

In conclusion, benzotriazole analogs prepared in this research showed strong possibility to be photochemically activated DNA cleaving agents. Electrophilic groups such as haloacetoxy groups on the alkyl chain of benzotriazole analogs promote the DNA cleaving ability.

ACKNOWLEDGEMENT

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Spectral data of **2**; ^{1}H NMR ($CDCl_3$): 8.88 (s, 1H), 8.46 (d, J=8.4, 1H), 8.24 (dd, J=8.4, 1.8, 1H), 8.21 (d, J=8.4, 1H), 8.05 (d, 6 Hz, 1H), 8.03 (d, J=8.1, 1H), 7.96 (d, J=8.4, 1H), 7.78-7.56 (m, 4H)

Spectral data of **4**; ^{1}H NMR (DMSO-d_6): 8.57 (s, 1H), 8.04 (d, J=8.7, 1H), 7.99 (d, J=8.7, 1H), 4.34 (t, J=6.3, 2H), 3.48 (t, J=6.3, 2H), 1.80 (m, 2H), 1.59 (m, 2H)

Spectral data of **5**; ^{1}H NMR ($CDCl_3$): 8.76 (s, 1H), 8.16 (d, J=8.7, 1H), 7.95 (d, J=8.7, 1H), 4.45 (t, J=6.0, 2H), 4.32 (t, J=6, 2H), 4.12 (s, 2H), 1.92 (m, 4H)

Spectral data of **7**; ^{1}H NMR ($CDCl_3$): 8.91 (s, 1H, 4), 8.90 (d, J=0.6, 1H, 11), 8.50 (dd, J=8.1, J=0.6, 1H, 6), 8.42 (dd, J=8.7, J=1.5, 1H, 7), 8.24 (dd, J=8.7, J=1.8, 1H, 3'), 8.06 (d, J=7.5, 1H, 8'), 8.05 (d, J=8.4, 1H, 4'), 7.97 (d, J=7.8), 7.70-7.61 (m, 2H, 6', 7'), 4.47 (t, J=6, 2H, 1"), 4.32 (t, J=6, 2H, 4"), 4.10 (s, 2H, $ClCH_2$-), 1.95-1.91 (m, 4H, 2", 3")