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Synthesis, Characterization, Spectroscopic Properties of 2-(4-Dimethylaminophenyl)-5-fluoro-6-(morpholin-4-yl)-1H-benzimidazole and its Interaction with Calf Thymus DNA

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Abstract: 2-(4-Dimethylaminophenyl)-5-fluoro-6-(morpholin-4-yl)-1H-benzimidazole(**1**) has been synthesized and characterized by ^{1}H-NMR, MS and elemental analysis. UV-Vis spectra of the aqueous solutions at different pH values reveal that compound **1** can combine three protons. Its three protonation constants are determined by spectrophotometry and calculated by non-linear least squares. The results of steady-state fluorescence measurements indicate that a special interaction occurs between compound **1** and calf thymus DNA, of which the binding constant, K_b, is $(2.30 \pm 0.10)\times10^4$ L/mol. Compound **1** in the concentration range of 10^{-8} to 1.2×10^{-6} mol/L could be used for quantitative determination of DNA.

Keywords: 2-(4-Dimethylaminophenyl)-5-fluoro-6-(morpholin-4-yl)-1H-benzimidazole, calf thymus DNA.

Benzimidazole compounds have attracted a renewed interest recently owing to their potential applications in high-performance composite materials, electronic chemicals, photosensitive materials, and their special potentials in biological and/or medicinal application[1,2]. Typically, aromatic compounds with near planar structures and containing hydrogen-donor groups or groups, which are capable of being protonated, have special interactions with DNA *via* intercalation, hydrogen-bonding, and so on[3]. Meanwhile, such compounds are generally fluorescent and have strong molar absorbency in UV-Vis spectrum. Thus, UV-Vis and fluorescence measurements are effective tools for investigation of binding of these compounds to DNA[4].

A substituted benzimidazole, namely, 2-(4-dimethylaminophenyl)-5-fluoro-6-(morpholin-4-yl)-1H-benzimidazole (**1**) was synthesized and characterized by ^{1}H-NMR, MS and elemental analysis. Through UV-Vis and fluorescence measurements, the interaction between compound **1** and calf thymus DNA was investigated.

Compound **1** was synthesized through three steps as shown in **Scheme 1**. 4-Fluoro-5-morpholino-2-nitroaniline **3** (mp: 181-183°C) was obtained by condensation of 4, 5-difluoro-2-nitroaniline **2** and morpholine[5]. Instead of Pd-C/hydrazine[5], $TiCl_3$/

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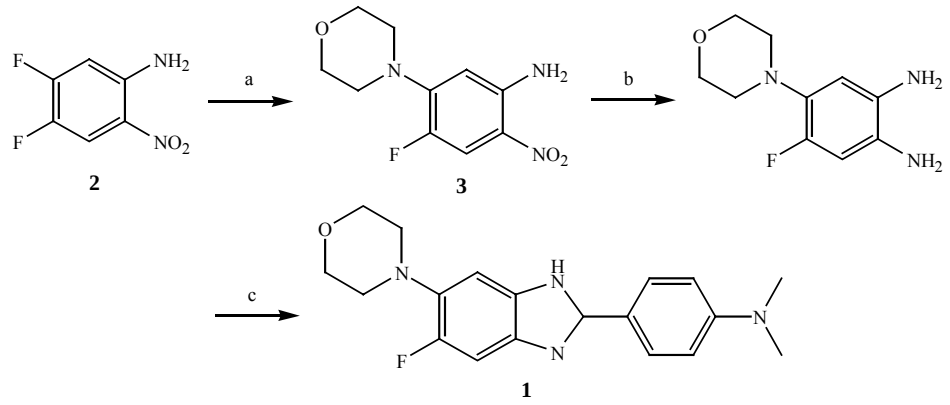
Scheme 1



a) morpholine, DMSO, 100°C, 6 h
b) $TiCl_3$, HCl, 40 min
c) 4-dimethylaminobenzaldehyde, $PhNO_2$, 145°C, 12 h

hydrochloric acid solution was used to reduce compound **3**. After neutralization with $NaHCO_3$, the resulted suspension was extracted into ethyl acetate. The organic layer was dried over magnesium sulphate and evaporated under vacuum to give 4-fluoro-5-morpholino-1,2-diaminobenzene **4**. Yield: 95% (ref.[5]: 80%). mp: 123-126°C. Under the nitrogen protection, the mixture of compound **4** and 4-dimethylamino- benzaldehyde in nitrobenzene was heated at 145°C for 12 h, cooled to room temperature and passed through a column of silica gel using petroleum ether:ethyl acetate (1:3 v:v) as eluent. A yellow product (compound **1**) was obtained after recrystallization from methanol. Yield: 27%. mp: 149-150°C. MS(EI) *m/z*: 340. ^{1}H NMR (400MHz, $CDCl_3$, δ ppm, J Hz): 2.963(t, 4H, J=4.5, -CH_2-), 2.994(s, 6H, -CH_3), 3.856(t, 4H, J=4.4, -CH_2-), 6.706(d, 2H, J=9.0, Ph-H), 7.028(d, 1H, J=7.6, Ph-H), 7.210(d, 1H, J=6.0, Ph-H), 7.970(d, 2H, J=7.1, Ph-H). Elemental Analysis Calcd. for $C_{19}H_{21}FN_4O{\cdot}0.5H_2O$: C65.32; H6.35; N16.03. Found: C65.77; H6.46; N15.86.

Effect of pH value of the solution on the UV-Vis spectrum of compound 1

UV-Vis spectra of solutions containing compound **1** (1.2×10^{-5} mol/L) in the pH from 1.0 to 10.0 have been measured from 260 to 440 nm. The results show that there are four absorption peaks at 300, 322, 339, 370 nm and three fairly clear isosbestic points at 314, 332, 350 nm, respectively, which mean that there are at least four species existing in the solution in the studied pH range. When the pH value is above 7, the neutral species is predominant. With the decrease of the pH value, the neutral one tends to convert to the univalent state, which is predominant in the pH range of 2.7~3.8. With the further decrease of pH value, the univalent species bind with another one or two protons, and lead to the formation of the bivalent and trivalent ions.

The univalent species of compound **1** may have three possible isomers since there are three different nitrogen atoms capable of binding with hydrogen cation. By

comparison of the relative energies of those possible structures calculated with *ab initio* molecular orbital theory, it can be deduced that the protonation sequence is as following: The tertiary nitrogen atom (=N–) of imidazole is the most favorite binding site to a proton; the second and third proton might combine with the nitrogen of morpholine and that of the dimethylamino group, respectively.

In this system, there might be four types of species for compound **1**, namely, the unprotonated base B, and three protonated species BH^+, BH_2^{2+} and BH_3^{3+}. $\bar{\varepsilon}(\lambda)$ denote the average molar absorbency of compound **1** in all chemical forms, and $\varepsilon_0(\lambda)$, $\varepsilon_1(\lambda)$, $\varepsilon_2(\lambda)$ and $\varepsilon_3(\lambda)$ denote the molar absorbency of species B, BH^+, BH_2^{2+}, and BH_3^{3+}, respectively, the average molar absorbency can be expressed as follows:

$$\bar{\varepsilon}(\lambda) = \frac{\varepsilon_0(\lambda) + \varepsilon_1(\lambda)\beta_1[H^+] + \varepsilon_2(\lambda)\beta_2[H^+]^2 + \varepsilon_3(\lambda)\beta_3[H^+]^3}{1 + \beta_1[H^+] + \beta_2[H^+]^2 + \beta_3[H^+]^3}$$

where β_1, β_2, and β_3 mean the three accumulative protonation constants of compound **1**. $\varepsilon_0(\lambda)$ can be easily obtained since only free base exists in solutions with pH higher than 7. By measuring the total absorbency A_j at wavelengths λ_j for different pH value, a set of ($\bar{\varepsilon}_k(\lambda_j)$, pH_k) data are collected. Based on these data, $\lg\beta_1$, $\lg\beta_2$, and $\lg\beta_3$ are calculated with a universal non-linear least squares fitting method and obtained to be 4.96±0.03, 5.72±0.07 and 7.95±0.10, respectively.

UV-Vis and fluorescent measurement of the interaction between compound 1 and calf thymus DNA

Aromatic compounds are characterized with a conjugated planar ring system. Their active hydrogen(s) usually have special interaction with DNA[3]. From the structure, one can find that compound **1** does not contain any active hydrogen. Thus, there might be no interaction between compound **1** and DNA. UV-Vis and fluorescent measurements confirmed the above inference. However, when the pH value of the solution is around 2.7~3.8, the compound **1** exists in its univalent form BH^+, which might combine with DNA. Thus, the interactions of compound **1** and calf thymus DNA were studied in phosphate and citric acid buffer at pH value of 3.40. UV-Vis spectra show that there are two isosbestic points at 327 and 376 nm. The absorption peak at 370 nm decreases with the increase of concentration of DNA, indicating the existence of interaction between compound **1** and calf thymus DNA. The relation between the concentration of DNA and the molar absorbency is as follows[6]:

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/(K_b(\varepsilon_b-\varepsilon_f))$$

where ε_a, ε_b and ε_f means the average molar absorbency of the solution (A_{obs}/[compound **1**]), the molar absorbency of the totally bonded compounds and that of the free compound **1**, respectively. K_b is the binding constant. Calf thymus DNA has no adsorption at this range. By plotting [DNA]/(ε_a-ε_f) against [DNA], K_b is found to be (2.30±0.10)×10^4 L/mol from the ratio of the slope to the intercept (data and figure not

shown).

The interaction of compound **1** and calf thymus DNA was further confirmed by fluorescence measurement. Differing from most molecules such as fluoroquinolones (FQs), the addition of DNA to the solution of compound **1** does not quench its fluorescence; on the contrary, the addition of DNA enhances the relative intensity of fluorescence[6]. When the concentration of compound **1** is in the range of 10^{-8} to 1.2×10^{-6} mol/L, the relative fluorescence intensity linearly increases. From the above observations one may expect that compound **1** at a proper concentration, namely, from 10^{-8} to 1.2×10^{-6} mol/L, could be used for quantitative determination of concentration of DNA.

Experimental

Calf thymus DNA (I_{260}/I_{280}=1.754) was purchased from Sino-American Biotechnology Company (China), the concentration of which was determined by absorption spectroscopy (ε_{260}=6600 $mol^{-1}Lcm^{-1}$)[7]. 2-Nitro-4,5-difluroaniline is purchased from Acros Company, purity>98%. $TiCl_3$, hydrochloric acid, phosphoric acid, NaOH, citric acid, *etc*, are all A.R. reagents. Triply distilled water is used in the absorption and fluorescence measurements. Absorption and fluorescence emission spectra are recorded with a Shimadzu UV-2401 and a HITACHI FL-4500 spectrophotometer, respectively.

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