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Comparative binding study of steroidal adenine with flavin and uracil derivatives

Roopali Rai and Pramod S. Pandey*

Department of Chemistry, Indian Institute of Technology, Hauz Khas, New Delhi 110 016, India

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Abstract—A comparative binding study of a steroidal adenine derivative based on lithocholic acid with N^{10}-benzylisoalloxazine (flavin) and N^{1}-*iso*-propyluracil has been described.
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1. Introduction

Flavin adenine dinucleotide (FAD) is one of the primary cofactors in biological redox reactions. It has been shown earlier that flavin has the tendency of forming strong hydrogen-bonded complexes with adenine derivatives.[1–3] In recent years, there has been considerable interest on the study of interaction of RNA and flavin. RNA molecules that specifically bind riboflavin have been isolated by in vitro selection.[4–8] These flavin binding RNA motifs may provide a framework for generating new ribozymes that catalyze redox reactions. It has been found that uracil derivatives have the ability of releasing the flavin molecules from these RNA aptamers. Carboxy flavins have also been found to act as potent selective inhibitors of Taq DNA polymerase in a polymerase chain reaction.[7] Hence, it is of particular interest to understand the binding properties of flavin with adenine in the absence and in the presence of uracil derivatives. We were also interested to know whether flavin and uracil compete for the same binding site of the adenine molecule.

Thus, studies have been carried out to know the nature of the binding of steroidal adenine derivative with uracil and flavin derivatives. Scheme 1 outlines the synthesis of adenine derivative **2** based on lithocholic acid. For the synthesis of **2**, the methyl 3α-bromoacetyl lithocholate **1** was treated with an equivalent amount of adenine in dry DMF in the presence of anhydrous K_2CO_3 at room temperature for 24 h. ^{1}H NMR of receptor **2** showed singlets for H-8 and H-2 protons of adenine at 8.36 and 7.88 ppm, respectively. The NH_2 protons showed a broad singlet at 6.09 ppm. The 3β-methine signal appeared as a multiplet at 4.83 ppm while the methylene proton (–CH_2–N) appeared as a singlet at 4.94 ppm. The structure was further confirmed by ESI mass spectrum giving the molecular ion peak at 566.51 [M^++H].

The flavin derivative **5** was synthesized by a modified literature procedure[9] described in Scheme 2. For this, the 1-(*N*-benzyl)-2-aminobenzene **4** was first synthesized by treating *o*-phenylenediamine **3**, in excess (10 equiv), with benzyl bromide in methanol in the presence of anhydrous K_2CO_3 at room temperature for 12 h. ^{1}H NMR of **4** showed a broad singlet for the NH protons at 3.37 ppm. The two methylene and five phenyl protons of benzyl appeared at 4.26 ppm (singlet) and 7.37 ppm (multiplet), respectively. The protons of *o*-phenylenediamine ring appeared as multiplets at 6.68 ppm (three protons) and 6.81 ppm (one proton). Compound **4** was then treated with alloxan monohydrate and boric acid in acetic acid, which resulted in the formation of the required fluorescent flavin derivative **5** in 35–40% yield. The ^{1}H NMR spectrum of **5** in DMSO-d_6, depicted the NH proton signal at highly deshielded value, 11.47 ppm, due to the electron-withdrawing effect of two neighbouring carbonyl groups present. The methylene protons of *N*-benzyl group appeared at 5.90 ppm. A multiplet for the phenyl protons appeared in the range of 7.36–7.25 ppm. The four phenyl protons of isoalloxazine ring appeared at δ 8.16 (1H, doublet), 7.84–7.79 (1H, multiplet) and 7.68–7.57 (2H, multiplet). The ESI mass spectrum revealed the molecular ion at 305.40

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* Corresponding author. Fax: +91 11 26582037; e-mail: pramod@chemistry.iitd.ac.in

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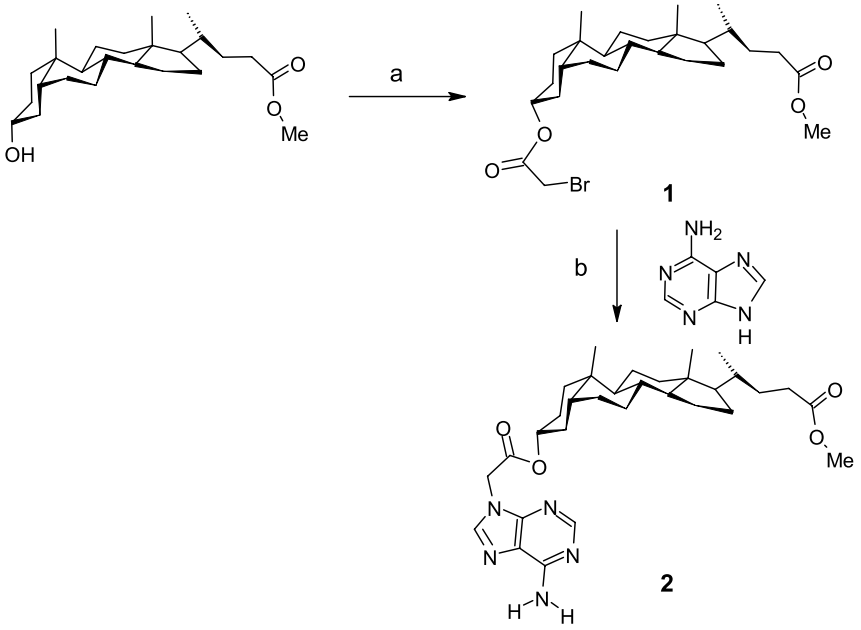
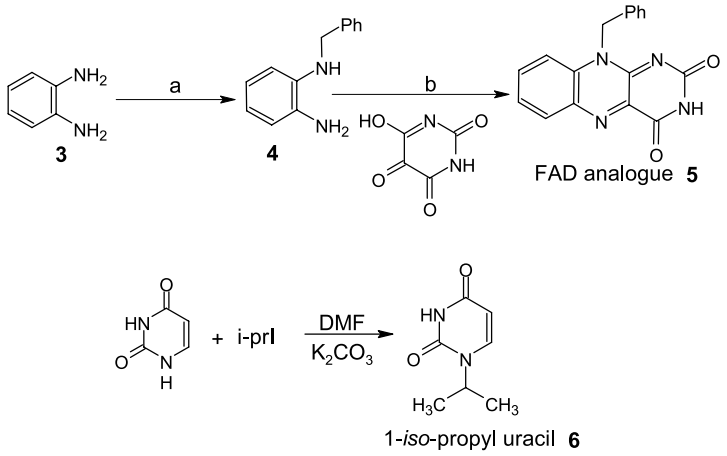


Scheme 1. Reagents and conditions: (a) $BrCH_2COBr$, anhydrous K_2CO_3, $CHCl_3$, 12 h, rt; (b) anhydrous K_2CO_3, DMF, 24 h, rt.



Scheme 2. Reagents and conditions: (a) $C_6H_5CH_2Br$, anhydrous K_2CO_3, CH_3OH, rt, 12 h. (b) H_3BO_3, CH_3COOH, 50 °C, 24 h.

$[M^+ + H]$. An *iso*-propyl derivative of uracil was synthesized by the treatment of uracil with isopropyl iodide (1 equiv) in DMF in the presence of K_2CO_3. The ^1H NMR spectrum of uracil derivative **6** revealed the imide N–H proton at 9.50 ppm. The C-6 and C-5 protons appeared as singlets at 7.29 and 5.76 ppm, respectively. The CH and CH_3 protons of *iso*-propyl unit appeared at 4.89 and 1.35 ppm, respectively. The binding of steroidal adenine derivative **2** was carried out separately with flavin **5** and uracil derivative **6**. The study was also carried out to know the competitive binding of adenine derivative with flavin and uracil derivatives.

2. Binding of adenine with flavin derivative 5

The binding study was carried out by using ^1H NMR titration method. To a 0.01 M solution of flavin derivative **5** in 2% CH_3OH in $CDCl_3$, various aliquots of 0.04 M solution of adenine derivative **2** were added and ^1H NMR spectra were recorded after each addition. The change in the chemical shift value of imide N–H proton of flavin derivative was observed. The chemical shift data were analyzed by WinEQNMR[10] software and the data fitted well for 1:1 complex giving a binding constant of $5.18 \times 10^2\ M^{-1}$. The analysis of the ^1H NMR spectra revealed that each addition of adenine derivative to the host molecule resulted in the upfield shift of only H-2 of adenine moiety. This suggested that Watson–Crick site of adenine is involved in the complexation (Fig. 1).

3. Binding of adenine with uracil derivative 6

The complexation of adenine derivative **2** (0.04 M) with *iso*-propyl uracil **6** (0.01 M) was studied in a similar manner as described earlier. The saturation data fitted

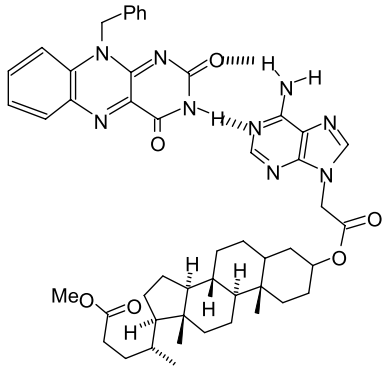


Figure 1. 1:1 complex of derivatives **2** and **5**.

well for 1:1 complex with the binding constant of $2.21 \times 10^3\ M^{-1}$. The proposed structure of the 1:1 complex is given in Figure 2.

4. Competitive binding of adenine with flavin and uracil derivatives

The comparative binding of the steroidal adenine with flavin and uracil derivatives was also carried out. To the mixture of flavin (0.005 M) and adenine derivative (0.01 M) in 2% CH_3OH in $CDCl_3$, different concentrations of uracil derivative were added and ^1H NMR was recorded after each addition. The ^1H NMR spectrum showed the changes in the chemical shift values of NH of all the three derivatives. It was observed that initially, addition of uracil showed slight effect on the chemical shifts of imide N–H of both flavin and uracil. However, as the addition of uracil was increased, the changes in the chemical shifts indicated the gradual replacement of the flavin derivative by uracil derivative. As the concentration of uracil reaches 0.088 M, the chemical shift value of imide proton of flavin approaches nearly the same value of imide proton of unbound flavin, thus showing that uracil replaces the flavin completely from the binding site of adenine (Table 1). This observation clearly indicates that both uracil and flavin are competing for the same binding site of the adenine, that is, the Watson–Crick site.

It has been observed that the gradual addition of uracil derivative to the complex of adenine–flavin initially decreases the chemical shit (upfield shift) of the –NH proton of the uracil derivative. However, when the concentration of uracil derivative becomes very high, there is a trend of increase in the chemical shift (downfield shift). This is quite understandable, because, as the concentration of uracil increases, the ratio of the concentration of the uncomplexed uracil to the complexed uracil also increases, which leads to the upfield shift of the average –NH proton signal. At the higher concentration of the uracil, the dimerization of uracil derivative may be responsible for the downfield shift of the –NH proton.

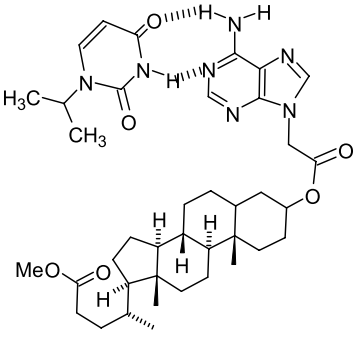


Figure 2. 1:1 complex of derivatives **2** and **6**.

Table 1. ^1H NMR titration data of adenine derivative **2**, flavin derivative **5** and uracil derivative **6**

Concn of uracil **6** added (M)	δ (N–H) Adenine **2**	δ (N–H) Flavin **5**	δ (N–H) Uracil **6**
0.000	6.190	10.390	—
0.008	6.307	10.319	9.825
0.016	6.395	10.213	9.758
0.024	6.458	10.119	9.710
0.032	6.517	10.070	9.690
0.040	6.554	10.021	9.665
0.048	6.581	9.984	9.646
0.056	6.631	9.973	9.660
0.064	6.668	9.967	9.670
0.072	6.697	9.956	9.673
0.080	6.723	9.954	9.685
0.088	6.761	9.936	9.690

In summary, we have carried out the comparative binding studies to establish the competitive binding of adenine towards flavin and uracil, which may be useful in future, for designing inhibitors based on flavin derivatives for DNA/RNA polymerases.

Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.bmcl.2005.03.097.

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