Supplementary Material

Synthesis and evaluation of benzochalcogenazole-benzimidazole derivatives as potential DNA-binding radioprotectors

Thomas Fellowes,a Colin E. Skene,a Roger F. Martin,a Pavel Lobachevsky,b,c Tze Cin Owyong,a,d Yuning Hong,d and Jonathan M. White*a

School of Chemistry and Bio-21 Institute, University of Melbourne, Australia
bMolecular Radiation Biology Laboratory, Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia
cAdvanced Analytical Technologies, Melbourne, Australia
dDepartment of Chemistry and Physics, La Trobe Institute for Molecular Science, La Trobe University, Australia

Email: whitejm@unimelb.edu.au

Table of Contents

NMR data Compound 6 ................................................................. S2
NMR data Compound 7 ................................................................. S3
NMR data Compound 10 ............................................................... S4
NMR data Compound 11 ............................................................... S5
NMR data Compound 11s ............................................................. S6
NMR data Compound 12-H2 ......................................................... S7
NMR data Compound 13 ............................................................... S8
NMR data Compound 16 ............................................................... S9
NMR data Compound 17 ............................................................... S10
NMR data Compound 18 ............................................................. S10
NMR data Compound 19 ............................................................. S11
Ligand-DNA binding analysis ...................................................... S12
Compound 6.
Compound 7.
Compound 10
Compound 11₀
Compound 11

Signals at 3.42 (q) and 1.05 (t) attributed to residual ethanol
Compound 12-H2
Compound 130

2.922 (s), 2.764 (s), 1.937 (s) attributed to N,N-dimethylacetamide impurity (0.3 eq)
Compound 16
Compound 17

Compound 18
Ligand-DNA binding Analysis

1 Theoretical approach

The purpose of the analysis described in the following section is to calculate the ligand-DNA binding parameters, namely the binding dissociation constant $K_d$ (measured in M – molar) and the frequency of binding sites $N$ (measured in 1/bp – per base pair of DNA), from the changes in the absorbance spectrum of the ligand upon binding to DNA.

1.1 General formalism

The basic equilibrium equation for binding of species $A$ to species $B$ is described by the equation:

$$K_d = \frac{[A][B]}{[AB]} \quad \text{S1}$$

where $[A]$ is the concentration of free species $A$, $[B]$ is the concentration of free species $B$, $[AB]$ is the concentration of $AB$ complexes and $K_d$ is the binding dissociation constant. Application of the equation S1 to the case of ligand-DNA binding produces the following equation:

$$K_d = \frac{(Nx - \alpha h)(1 - \alpha h)}{\alpha h} \quad \text{S2}$$

where $x$ is the DNA concentration, $h$ is the total ligand concentration, $\alpha$ is the fraction of the bound ligand and $N$ is the frequency of binding sites.

In the equation S2, $(Nx - \alpha h)$ is the concentration of the free (not occupied) binding sites (species $A$ in equation S1), $(1 - \alpha)h$ is the concentration of free ligand (species $B$ in equation S1), and $\alpha h$ is the concentration of complexes (equals to the concentration of bound ligand). Equation S2 is easily converted to the standard quadratic equation relative to unknown variable $\alpha$:

$$h\alpha^2 - (K_d + Nx + h)\alpha + Nx = 0. \quad \text{S3}$$

The positive solution of this equation relative to $\alpha$ produces the following expression:

$$\alpha = \frac{K_d + Nx + h - \sqrt{(K_d + Nx + h)^2 - 4Nxh}}{2h} \quad \text{S4}$$

Expression S4 can be regarded as a basis for non-linear regression analysis of the experimental data for the measured fraction of the bound ligand $\alpha$ assuming $K_d$ and $N$ as free parameters to be estimated as a result of the regression, and $x$ and $h$ as experimentally measurable variables.

1.2 Data Analysis Method

Experimental data for evaluation of DNA-ligand binding parameters consists of a range of absorbance spectra obtained following titration of the ligand solution with DNA. Two variables are under control: the DNA concentration (denoted as $x$) and the total ligand concentration (denoted as $h$). In a general case, both variables may change for each titration point. Assuming that there are $n$ titration points, we can denote the absorbance spectrum for each titration point $x_i$ and $h_i$ as $Y_i, (i = 0, 1, ..., n)$. We consider spectrum $Y_i$ as a vector (or an array) each element of which is the absorbance at a particular wavelength. The DNA concentration at the first titration point ($i = 0$) $x_0 = 0$, so $Y_0$ is the spectrum of free ligand. Unfortunately, it is very difficult to achieve conditions providing 100% binding of the ligand, so at the titration point with the highest DNA concentration $x_n$ the measured spectrum $Y_n$ is not the spectrum of bound ligand. Therefore we denote the spectrum of bound ligand which is usually unknown as $Y_b$. Any spectrum $Y$ of a ligand-DNA solution at particular values of $x$ and $h$ can be then represented as a linear combination of the $Y_0$ and $Y_b$ spectra by the following expression:

$$Y = (1 - \alpha)Y_0 + \alpha Y_b \quad \text{S5}$$

Since absorbance of the ligand is proportional to the ligand concentration $h$, we assume that in expression S5 all spectra are normalised to the same ligand concentration. When applied to the spectra $Y_i$, normalisation is
done to the concentration at the first point (measured spectrum multiplied by $h_0/h$). To compensate the contribution of DNA absorbance, we perform the titration using the dual beam mode and add DNA to both the reference and sample cuvette. Since the spectrum $Y_b$ in expression S5 is not available, direct calculation of $\beta$ from experimental data using this expression is not possible. However it is easy to demonstrate that any spectrum $Y$ can be represented by a linear combination of any two fixed spectra from the $Y_i$ range. An obvious choice of these two spectra is $Y_0$ and $Y_n$, since one of them is the spectrum of the first species (free ligand) and the second one has the largest contribution of the second species (bound ligand). Then:

$$ Y = \beta_0 Y_0 + \beta_1 Y_n, $$  

S6

where $\beta_0$ and $\beta_1$ are coefficients.

To establish the relationship between coefficients $\beta_0$ and $\beta_1$, we can substitute for $Y_n$ in expression S6 using the following expression:

$$ Y_n = (1 - \alpha_n) Y_0 + \alpha_n Y_b. $$  

S7

This substitution transforms expression S6 into:

$$ Y = (\beta_0 + \beta_1(1 - \alpha_n)) Y_0 + \beta_1 \alpha_n Y_b. $$  

S8

Comparison of expressions S5 and S8 allows deriving following expressions for $\beta_0$ and $\beta_1$:

$$ \beta_0 = 1 - \frac{\alpha}{\alpha_n}. $$  

$$ \beta_1 = \frac{\alpha}{\alpha_n}. $$  

S9

Considering that $\beta_1 = 1 - \beta_0$ (as follows from expressions S9), and provided that both spectra in expression S6 $Y_0$ and $Y_n$, are available from the titration experiment, we applied the non-linear regression analysis for each experimental spectrum $Y_i$ according to expression S6 using $Y_0$ and $Y_n$, as independent variables to obtain the best fit values of coefficient $\beta_1$ for each titration point ($\beta_{1i}$).

A substitution in expression S9 for $\alpha$ from expression S4 produces the following expression for $\beta_1$:

$$ \beta_1 = \frac{h_n \left(K_d + N x + h - \sqrt{(K_d + N x + h)^2 - 4 N x h}\right)}{h \left(K_d + N x_n + h_n - \sqrt{(K_d + N x_n + h_n)^2 - 4 N x_n h_n}\right)}. $$  

S10

We used expression S10 for the non-linear regression analysis of values $\beta_{1i}$ obtained at the previous step, considering $h$ and $x$ as independent variables and $K_d$ and $N$ as fitting parameters values of which are obtained as a result of this analysis. The spectrum of bound ligand $Y_b$ can be calculated from expression S7 as follows:

$$ Y_b = \frac{Y_n - (1 - \alpha_n) Y_0}{\alpha_n}. $$  

S11

2 Experimental
Figure S1. M2PB DNA binding analysis.

Left panel - experimental absorbance spectra of M2PB following titration with DLB16A DNA (symbols) and the results of regression analysis (lines). The range of DNA concentrations is from 0 to 730 μM bp. The range of ligand concentration is from 8.1 to 5.7 μM.

Right panel - non-linear regression analysis of fractions of bound ligand α. Values of DNA binding parameters are: $K_d = 3.92 \pm 0.30 \mu M$, $N = 0.0451 \pm 0.002 \, \text{1/bp}$. 