

## Research Article

# Effect of Ionic Strength on Porphyrin Drugs Interaction with Quadruplex DNA Formed by the Promoter Region of C-myc and Bcl2 Oncogenes

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C-myc and Bcl2 are well characterized oncogenes that are capable of forming G-quadruplex structures. Promoter regions of C-myc and Bcl2 forming G-quadruplex structures are chemically synthesized and G-quadruplex structure is formed in presence of 100 mM potassium ion. Three different porphyrin drugs, namely TMPyP2, TMPyP3, and TMPyP4 are allowed to interact with quadruplex DNA complex and the site and nature of interaction are studied. Drug interactions with quadruplex DNA were carried out in different potassium ionic strengths using fluorescence spectroscopy. It is found that fluorescence hypochromicity decreases with an increase in ionic strength in the case of TMPyP4, TMPyP3, and TMPyP2. Fluorescence titration studies and Job plots indicate that four molecules of TMPyP4, two molecules of TMPyP3 and TMPyP2 are interacting with one molecule of quadruplex DNA.

## 1. Introduction

Bcl2 (B-cell/lymphoma-2) is a proto-oncogene which encodes for 25 KD protein which has a peculiar function of blocking programmed cell death without affecting proliferation [1]. There are about 25 genes in the Bcl2 family. The Bcl2 gene is known to be responsible for a number of cancers like melanoma, breast, prostate, lung carcinoma, schizophrenia, and autoimmunity [2]. An increase in Bcl2 levels in the cell is reported to lead to poor prognosis [3]. Hence control of the production of Bcl2 in a cell has become a significant area of research. Like Bcl2, C-myc is also an oncogene which has a G-quadruplex forming sequence in its promoter region. The protein product of C-myc proto-oncogene controls a variety of genes that together enhance proliferation of cell [4, 5]. C-myc is overexpressed in many human cancers cells like leukemia, lung, breast, pancreatic, cervical, etc, [6–8]. The role of G-quadruplexes in cancer therapeutics is increasing. Work is under way in many laboratories elsewhere to identify

a suitable synthetic compound or macromolecule, which can stabilize the quadruplex complex in vivo [9]. Many synthetic chemicals were tested for their interaction with quadruplex DNA. Few among them are anthraquinones [10], acridines [11], perylenes [12], and triazines [13, 14]. Porphyrins are chemical compounds, until that can interact with quadruplex DNA structure and stabilize them [15, 16]. Today, there has been controversy in the nature and site of porphyrin binding to quadruplex DNA structure. Kimura et al. [17] have studied the interaction between G-quadruplex-TMPyP4 using 2-aminopurine substituted human telomeric DNA sequence. Han et al. [18] have demonstrated that TMPyP4 is a suitable telomerase inhibitor because of its low toxicity.

Different TMPyP4 binding modes to DNA are reported in the literature, like intercalation between the G-C base pairs [19, 20], binding to major groove [21] and minor groove [22, 23], and binding to the DNA surface [24]. Haq et al. [25] have studied the binding of cationic porphyrin

to G-quadruplex DNA using ITC, UV-Vis spectroscopy and have shown that the stoichiometry of porphyrin binding to G-quadruplex DNA depends on the number of intervals between successive G-tetrad planes. Freyer et al. [26] have recently reported that the binding of TMPyP4 to c-myc NHE III promoter is in the ratio of 4 : 1, using microcalorimetric and spectroscopic techniques.

## 2. Materials and Methods

**2.1. Oligonucleotide Synthesis.** For this study, two different quadruplex forming oncogenic sequences, corresponding to Bcl2 and C-myc promoter regions were synthesized and their interactions with three different porphyrin drugs were studied. The sequences of oligonucleotides used in the present study are shown below. The structure of G-quadruplex DNA and porphyrin drugs is shown in Figure 1. All the structures shown in Figure 1 are drawn using ACD/ChemSketch (Version 12):

27Bcl2 - CGG GCG CGG GAG GAA GGG GGC GGG  
AGC

C-myc - TGG GGA GGG TGG GGA GGG TGG GGA  
AGG

The 27Bcl2 and C-myc oligonucleotide stock solutions were prepared by dissolving each oligonucleotide into 100 mM KBPES buffer (0.01 M  $\text{KH}_2\text{PO}_4$ , 0.01 M  $\text{K}_2\text{HPO}_4$ , 0.001 M EDTA, 0.02–0.6 M KCl, KOH added drop wise to pH 7.0). DNA samples were dialyzed (1000 molecular weight cutoff membrane) against two changes of buffer (1L, 24 hours each) at 4°C. Concentration of all the oligonucleotides was measured using UV-Vis spectrophotometry with molar extinction coefficients determined by using the nearest neighbor calculation for single stranded DNA [27] and the absorbance of thermally denatured constructs extrapolated back to 25°C and/or a total phosphate analysis technique [28]. The extinction coefficient determined for two G-quadruplexes by these techniques was  $\epsilon_{254} = 207840 \text{ M}^{-1}\text{cm}^{-1}$  (27Bcl2) and  $\epsilon_{260} = 215330 \text{ M}^{-1}\text{cm}^{-1}$  (c-myc), respectively.

The cationic porphyrins (TMPyP4, TMPyP3, and TMPyP2) were purchased from Frontier Scientific (Logan, Utah, USA). All the porphyrin drugs were used without further purification. The porphyrin solutions were prepared by dissolution of each drug into a measured volume of final dialysate from the appropriate oligonucleotide solution preparation as the buffer. The concentration of each porphyrin drug was calculated using the molar extinction coefficient  $\epsilon_{424} = 2.26 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  [29],  $\epsilon_{417} = 2.50 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  [30],  $\epsilon_{414} = 1.82 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  [31] for TMPyP4, TMPyP3, and TMPyP2, respectively.

**2.2. CD Experimental Studies.** Circular dichroism experiments were performed using a JASCO 815 CD spectropolarimeter (Jasco, Tokyo, Japan). The CD spectra were recorded from 200 to 500 nm for characterizing the G-quadruplex DNA and for studying induced CD by porphyrins. For each CD experiment,  $5 \times 10^{-6} \text{ M}$  G-quadruplex

DNA was used. CD spectra were recorded using  $10 \times 10^{-6} \text{ M}$  (1 : 2) and  $20 \times 10^{-6} \text{ M}$  (1 : 4) TMPyP4. CD titrations were done in 100 mM KBPES buffer (pH 7.0) at 25°C. TMPyP4 was added using a calibrated micropipette. Each CD spectrum is recorded three times, and the average of 3 scans is considered.

**2.3. Fluorescence Experiments.** Fluorescence emission spectra were measured at 25°C using a FluoroMax-3 spectrofluorimeter (Horiba Jobin Yvon, Edison, NJ) using a 1 cm path length quartz cuvette. G-quadruplex DNA samples were measured in 2 mL of 100 mM KBPES buffer (pH 7.0). Through out the fluorescence experiment, concentration of G-quadruplex DNA is kept constant (200 nM) and titrated with increasing concentrations of cationic porphyrin (0 to 1200 nM). Fluorescence spectra were recorded after each addition of porphyrin to the fluorescent cuvette. G-quadruplex DNA and porphyrin drug mixture volume were kept constant throughout the fluorescence titration (2 mL). After each titration, the quartz cuvette was thoroughly washed with distilled water and dilute nitric acid (approximately 0.1 N, nitric acid) to remove traces of porphyrin sticking to the walls of quartz cuvette. TMPyP4, TMPyP3, and TMPyP2 were excited at 433 nm, 417 nm, and 424 nm, respectively and emission spectra for each titration were collected from 600 to 800 nm. Each spectrum was recorded three times and the average of three scans was taken. The difference in fluorescence emission maxima before and after the addition of G-quadruplex DNA was plotted against concentration of porphyrin to obtain the porphyrin-G-quadruplex binding curve. In the fluorescence binding curves (Figure 4), the points of intersection of two extrapolated lines (initial and final), at saturation levels, will indicate the stoichiometry of G-quadruplex DNA and porphyrin drugs.

To investigate the effect of ionic strength on fluorescence hypochromicity (% FH), four different KBPES buffer solutions (pH 7.0) with increasing concentration of K(I) ion were prepared. The KBPES (pH 7.0) buffer solutions used in the present study contained 30 mM, 100 mM, 200 mM, and 600 mM of potassium ion.

**2.4. UV-Vis Spectroscopic Titrations.** UV-Vis spectroscopic titrations were performed using an Agilent 8453 diode array (Agilent, Santa Clara, CA) at 25°C using a 1 cm path length quartz cuvette. Stock solutions of  $10 \mu\text{M}$  porphyrin and  $10 \mu\text{M}$  of G-quadruplex DNA were prepared in 100 mM KBPES buffer (pH 7.0). Two series of solutions were used for the experiments. First two mL of dilute G-quadruplex DNA ( $10 \mu\text{M}$ ) were placed in a 1 cm path length quartz cuvette, and twenty five  $\times 50 \mu\text{L}$  injections of porphyrin solution ( $10 \mu\text{M}$ ) were added manually. In the second part, two mL of dilute cationic porphyrin solution ( $10 \mu\text{M}$ ) were used and twenty five  $\times 50 \mu\text{L}$  injections of G-quadruplex DNA solution were added. Absorption spectra were collected from 350 nm to 500 nm. The quartz cells were thoroughly cleaned with distilled water and 0.1 N nitric acid to remove traces of porphyrin that were deposited on the walls of quartz cell. The

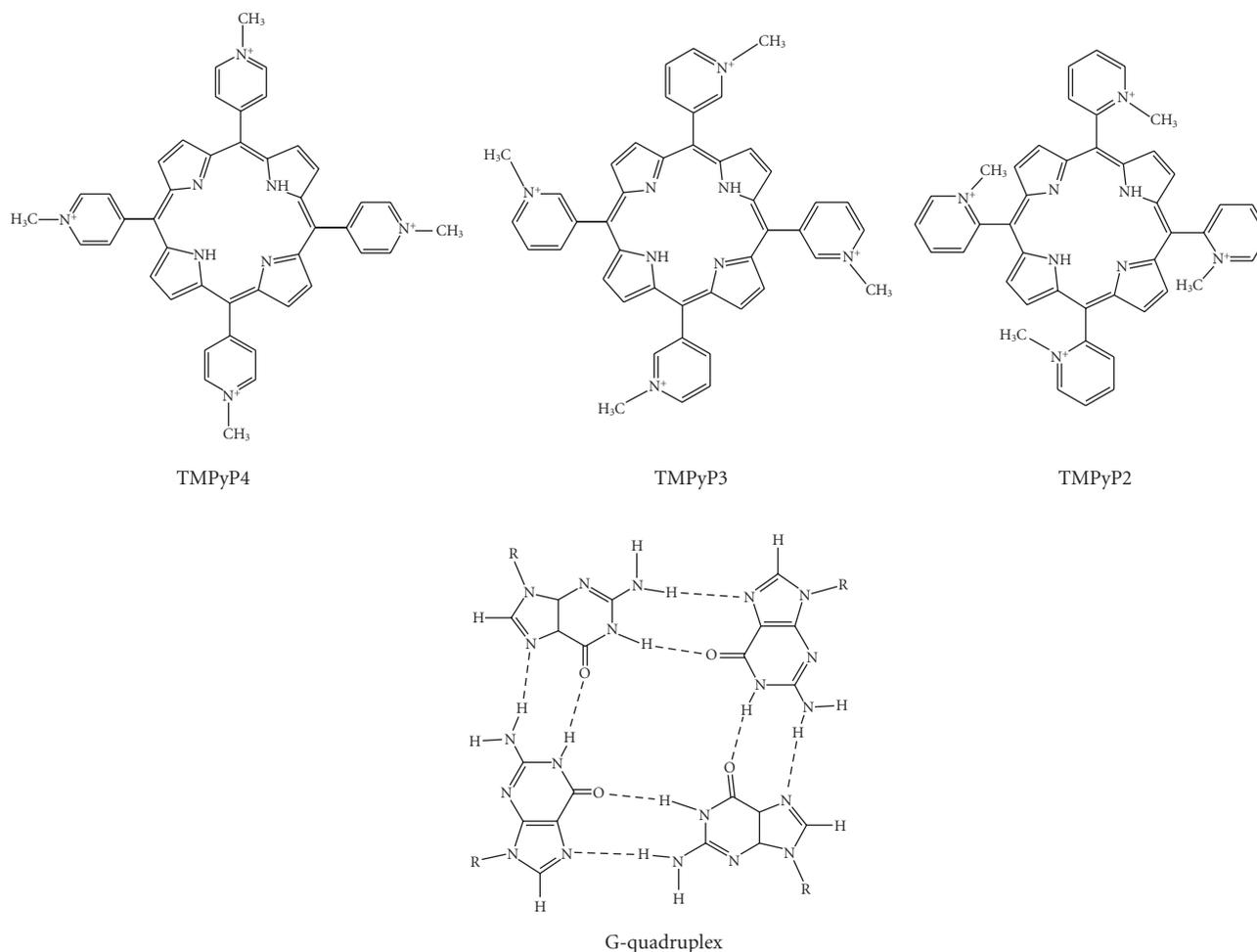


FIGURE 1: The structures of G-quadruplex DNA and TMPyP2, TMPyP3, and TMPyP4.

difference in the absorption maxima of the solet band was plotted versus the porphyrin mole fraction to generate a Job plot [32–34].

UV-Vis absorption titrations were done by adding G-quadruplex DNA stock solution (200–350 nM) in 100 mM KBPES buffer (pH 7.0) to the quartz cuvette containing approximately  $1\ \mu\text{M}$  porphyrin solution prepared in the same buffer. G-quadruplex DNA and porphyrin samples were freshly prepared before performing the experiment. UV spectra were collected from 200 nm to 500 nm. The titrations were carried out until the porphyrin solet band remains at a fixed wavelength upon five successive additions of G-quadruplex DNA.

### 3. Results and Discussion

**3.1. CD Studies.** The CD spectrum (Figure 2(a)) shows a prominent positive peak at 265 nm and a small hump at 295 nm, indicating that the 27Bcl2 is forming antiparallel quadruplex structure in solution. On addition of TMPyP4 (at 1 : 2 and 1 : 4 DNA : TMPyP4 ratio) a weak induced CD band (ICD) is seen around 435 nm, indicating that TMPyP4

is efficiently interacting with quadruplex DNA. Whereas Figure 2(b) demonstrates comparatively weaker interaction of TMPyP4 with C-myc quadruplex DNA. Furthermore, the CD spectrum shows a positive peak at 265 nm. This indicates that it is forming parallel quadruplex DNA structure in solution. When ( $10 \times 10^{-6}$  M) and ( $20 \times 10^{-6}$  M) concentrations of TMPyP4 is added to 27Bcl2 quadruplex DNA ( $5 \times 10^{-6}$  M), a negative cotton effect is observed. Small or no cotton effect is seen, when double and quadruple concentrations of drug are added to  $5 \times 10^{-6}$  M, C-myc DNA. A negative cotton effect in the case of 27Bcl2 is observed due to the difference in the conformation of Bcl2 quadruplex DNA. Occurrence of induced CD at 435 nm and negative cotton effect at 265 nm indicates that Bcl2 quadruplex complex has a marginally better interaction efficiency with TMPyP4.

**3.2. UV-Titration Studies.** UV-Vis spectroscopy is an excellent technique for finding the extent of drug interaction with DNA. In all the drug-DNA interactions, the solet band has shown a red shift. The solet band has shifted from 423 nm to 441 nm (18 nm shift) in case of TMPyP4 and the isobestic point is prominent at 436 nm (UV-Vis spectra are similar for

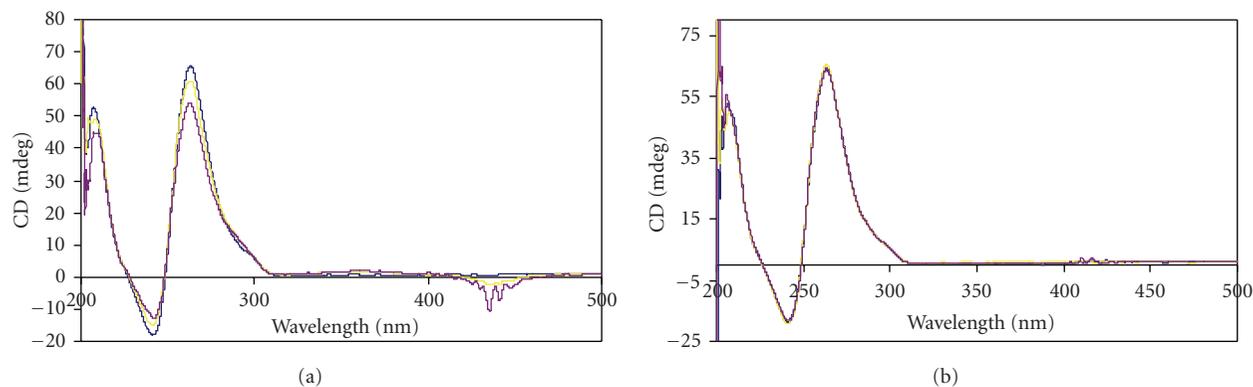


FIGURE 2: (a) CD spectra for the 27Bcl2 mid promoter sequence, wavelength in nm is plotted on  $x$ -axis, and Ellipticity,  $\theta$ , is plotted on the  $y$ -axis in mdeg. The  $5 \times 10^{-6}$  M quadruplex DNA is considered for each CD experiment. CD spectra are recorded at 1 : 2 and 1 : 4 quadruplex DNA: TMPyP4 stoichiometry. Each spectrum is recorded thrice, and the average of the three runs is considered. (b) CD spectra for the C-myc sequence, wavelength in nm is plotted on  $x$ -axis and Ellipticity,  $\theta$ , is plotted on the  $y$ -axis in mdeg. The  $5 \times 10^{-6}$  M quadruplex DNA is considered for each CD experiment. CD spectra are recorded at 1 : 2 and 1 : 4 quadruplex DNA: TMPyP4 stoichiometry. Each spectrum is recorded thrice, and the average of the three runs is considered.

TABLE 1: UV-Vis absorption titration parameters for TMPyP4, TMPyP3, and TMPyP2 with four different Bcl2 and C-myc G-quadruplex DNA. Soret band hypochromicity is calculated using the formula: % Hypochromicity =  $[(\epsilon_{\text{free}} - \epsilon_{\text{bound}})/\epsilon_{\text{free}}] \times 100$ .  $\epsilon_{\text{free}}$  and  $\epsilon_{\text{bound}}$  are the extinction coefficient of free and bound porphyrin, respectively.

S. No.	Name of quadruplex DNA and cationic porphyrin	Soret band shift (nm)	Isobestic point (nm)	Percentage hypochromicity
1.	27Bcl2-TMPyP4	423–441	435.5	59.7%
2.	27Bcl2-TMPyP3	417–431	426.5	41.5%
3.	27Bcl2-TMPyP2	No shift	422.0	23.0%
4.	C-myc-TMPyP4	423–439	432.2	52.3%
5.	C-myc-TMPyP3	417–429	425.3	39.5%
6.	C-myc-TMPyP2	No shift	421.7	14.7%

both 27Bcl2 and C-myc G-quadruplex sequences). In case of TMPyP3, the soret band has shifted from 417 nm to 431 nm (13-14 nm shift) and isobestic point is observed at around 426 nm. In the case of TMPyP2 the soret band does not show a shift from 414 nm and an isobestic point is observed at around 422 nm. Percentage of hypochromicity for each G-quadruplex DNA is calculated by following the procedure described by Keating and Szalai [32]. The details of UV-Vis spectral studies are shown in Table 1. Results demonstrate that both quadruplex DNA's can interact with TMPyP3 and TMPyP4 drugs. But among them, TMPyP4 interaction with C-myc and 27Bcl2 quadruplex DNA is more efficient than TMPyP3. TMPyP2 has very little or no interaction. Similar observations have been made by Anantha et al. [35]. They showed that TMPyP4 exhibits both intercalation and external binding. From UV spectral analysis, it is evident that the isobestic point when TMPyP4 interacts with 27Bcl2 quadruplex DNA shows slight deviation at the end (isobestic point is not a tight single point). This indicates that interaction involves multiple steps. Where as with C-myc quadruplex DNA, the isobestic point does

not show any deviation (a tight point is observed). Similar observations were made while observing the drug-DNA interaction, fluorimetrically.

**3.3. Job Plot.** Job plots make it possible to find out the stoichiometry interactions between drugs and quadruplex DNA. From Figure 3, it is evident that four molecules of TMPyP4 are interacting with each molecule of C-myc and 27Bcl2 quadruplex DNA. Similar observations have been made by Freyer et al. [26] only two molecules of TMPyP2 and TMPyP3 are interacting with each quadruplex DNA complex. The variation in the number of drug molecules interacting with quadruplex DNA is probably due to the placement and rotation of methyl groups placed on the porphyrin molecule. In the case of TMPyP4, the N-CH<sub>3</sub> group is in the para position and it has about 360° degree of rotation along the axis. Hence the molecules can attain perfect planar structure when it is interacting with quadruplex DNA. Due to this reason, it can intercalate as well as bind externally to quadruplex DNA molecule. In the case

TABLE 2: Data obtained from Job plot of Bcl2 and C-myc G-quadruplex DNA with TMPyP4, TMPyP3, and TMPyP2 porphyrin drugs.

S. No.	Name of gquadruplex DNA and porphyrin	Value of mole fraction of porphyrin matching with point of intersection	Stoichiometry between gquadruplex DNA porphyrin
1.	27Bcl2-TMPyP4	0.78	1 : 3.54
2.	27Bcl2-TMPyP3	0.70	1 : 2.33
3.	27Bcl2-TMPyP2	0.58	1 : 1.38
4.	C-myc-TMPyP4	0.76	1 : 3.17
5.	C-myc-TMPyP3	0.70	1 : 2.33
6.	C-myc-TMPyP2	0.58	1 : 1.38

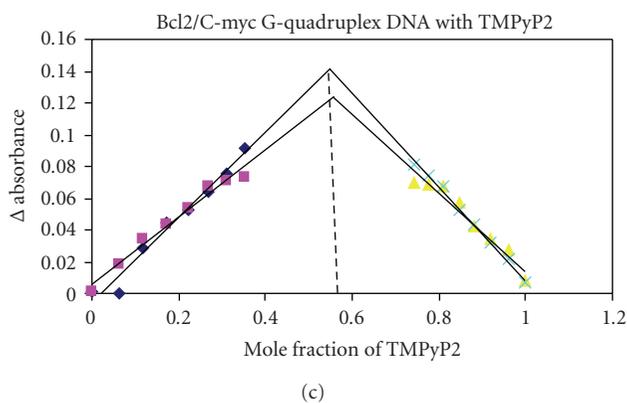
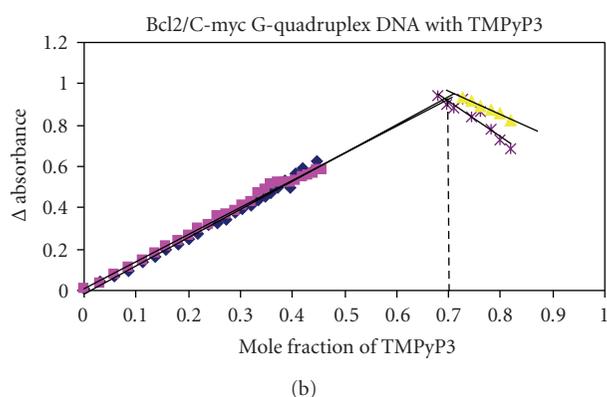
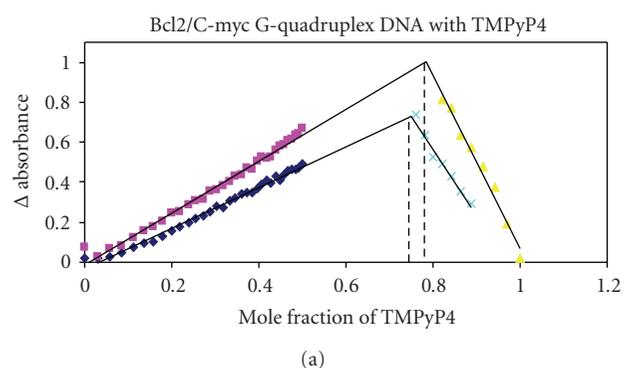


FIGURE 3: Job plots for the titration of the 27Bcl2 mid promoter and C-myc quadruplex DNA on interaction with TMPyP2, TMPyP3, and TMPyP4 drugs. Point of intersection of two lines is indicated by a dashed line.

of TMPyP3 the N-CH<sub>3</sub> group is in meta position and it can have a restricted rotation on its axis. Therefore, this molecule cannot attain perfect coplanarity, like TMPyP4. Hence it cannot intercalate completely in between two G-quartets but prefers to bind externally. With TMPyP2, the N-CH<sub>3</sub> groups are in ortho position. The N-CH<sub>3</sub> groups have much more restricted rotation on its axis, in case of TMPyP2. Hence, the molecule is incapable of attaining perfect planar structure, exhibits intercalation with quadruplex DNA and shows only external binding. Probably due to this reason, TMPyP4 can intercalate and bind externally, and the drug:quadruplex DNA stoichiometry is 4 : 1. Whereas, TMPyP2 and TMPyP3 prefers to bind externally and the stoichiometry is 2 : 1. Details were shown in Table 2.

**3.4. Fluorescence Hypochromicity.** In order to study the effect of buffer cationic strength on drug binding to quadruplex DNA, fluorescence titration was carried out in different KBPS buffers with variation in potassium ion concentrations (30 mM, 100 mM, 200 mM, and 600 mM). Results emphasize that fluorescence hypochromicity decreases with increase of buffer salt concentration. It demonstrates that as the positive ion cloud around quadruplex complex increases, it occupies the available binding sites on quadruplex complex surface and reduces the possibility for drug molecules to bind exterior of quadruplex DNA. A decrease in fluorescence intensity of the drug may also be due to quenching by the surrounding water molecules. But if the drug molecule shows an intercalative mode of binding, quenching of fluorescence intensity is comparatively less. Because drug molecules are sandwiched between two G-quartets, the distance between guanine bases in quadruplex and the drug molecules becomes less. As the drug molecules come close to guanines in quadruplex, energy transfer between them takes place, which results in an increase of fluorescence. An increase in fluorescence intensity by energy transfer between DNA bases and the drug molecules, on intercalation, was reported earlier [36, 37]. The extent of fluorescence attenuation is not the same with all the three porphyrin drugs. It is more with TMPyP2, moderate with TMPyP3 and less with TMPyP4. These results indicate that TMPyP4 can show both intercalative as well as end binding to quadruplex complex while TMPyP3, and TMPyP2 prefer to bind externally. From Tables 3(a) and 3(b) it is evident that percentage of

TABLE 3

(a) Effect of ionic strength on the percentage of fluorescence hypochromicity while 27Bcl2 interacts with porphyrin drugs.

S. No.	Ionic strength of KBPES buffer (pH7.0)	Stoichiometry DNA : drug	Percentage of fluorescence hypochromicity
1.		TMPyP4 with 27BCL2	
1.a	30 mM	1 : 4	34.2%
1.b	100 mM	1 : 4	28.5%
1.c	200 mM	1 : 4	20.3%
1.d	600 mM	1 : 4	6.5%
2.		TMPyP3 with 27BCL2	
2.a	30 mM	1 : 2 1 : 3	59.8% 51.5%
2.b	100 mM	1 : 2 1 : 3	52.5% 49.5%
2.c	200 mM	1 : 2 1 : 3	33.6% 16.6%
2.d	600 mM	1 : 2 1 : 3	8.6% 4.9%
3.		TMPyP2 with 27BCL2	
3.a	30 mM	1 : 2 1 : 3	37.4% 40.2%
3.b	100 mM	1 : 2 1 : 3	11.9% 12.3%
3.c	200 mM	1 : 2 1 : 3	6.5% 4.4%
3.d	600 mM	1 : 2 1 : 3	0.7% 0.3%

(b) Effect of ionic strength on the percentage of fluorescence hypochromicity while C-myc interacts with porphyrin drugs.

S. No.	Ionic strength of KBPES buffer (pH7.0)	Stoichiometry DNA : drug	Percentage of fluorescence hypochromicity
1.		TMPyP4 with C-myc	
1.a	30 mM	1 : 4	39.2%
1.b	100 mM	1 : 4	32.6%
1.c	200 mM	1 : 4	29.3%
1.d	600 mM	1 : 4	5.5%
2.		TMPyP3 with C-myc	
2.a	30 mM	1 : 2 1 : 3	52.8% 49.5%
2.b	100 mM	1 : 2 1 : 3	47.5% 42.2%
2.c	200 mM	1 : 2 1 : 3	29.6% 12.6%
2.d	600 mM	1 : 2 1 : 3	6.6% 3.9%
3.		TMPyP2 with C-myc	
3.a	30 mM	1 : 2 1 : 3	42.4% 37.2%

(b) Continued.

S. No.	Ionic strength of KBPES buffer (pH7.0)	Stoichiometry DNA : drug	Percentage of fluorescence hypochromicity
3.b	100 mM	1 : 2	12.6%
		1 : 3	7.2%
3.c	200 mM	1 : 2	7.8%
		1 : 3	5.4%
3.d	600 mM	1 : 2	0.6%
		1 : 3	0.2%

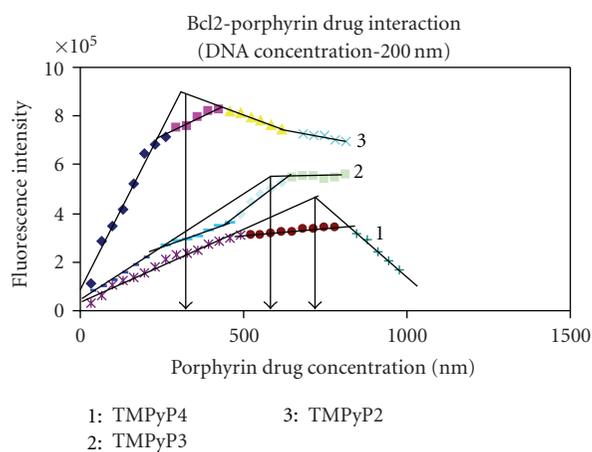
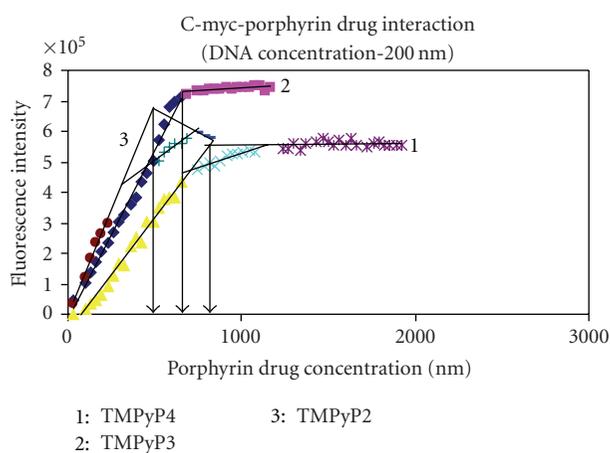


FIGURE 4: Fluorescence titration curves. 200 nM quadruplex DNA is titrated with 0–1200 nm concentration of three different porphyrin drugs. Stoichiometry is considered at saturation levels. Hence the slope of initial and end points are considered. 1: TMPyP4, 2: TMPyP3, and 3: TMPyP2.

fluorescence hypochromicity is comparatively higher (from lower to higher ionic strength), in the case of TMPyP3 and TMPyP2. This indicates that drug molecules are quenched by the surrounding water molecules. But, in the case of TMPyP4, fluorescence attenuation is gradual. This demonstrates that

though there is quenching of TMPyP4 fluorescence by surrounding water molecules, there is some increase in the fluorescence, due to energy transfer between TMPyP4 and guanines in the quadruplex on drug intercalation. Due to this, TMPyP4 could exhibit fluorescence even at 600 mM salt concentration. Whereas in case of TMPyP2 and TMPyP3 fluorescence intensity is almost quenched by the surrounding water molecules, as they are binding externally. The results obtained with 27Bcl2 as well as C-myc quadruplex are shown in Tables 3(a) and 3(b), respectively.

**3.5. Fluorescence Titration.** In this study, 200 nM of G-quadruplex DNA was considered for interaction with three different porphyrin drugs in the range of 0–1200 nM. From Figure 4 it is evident that the binding curve has multiple inflection points. 27Bcl2 shows a comparatively higher number of inflection points, indicating that the interaction occurs in multiple steps. Since it is intended to know the stoichiometry at saturation levels, only slopes of initial and final titrations are considered. The titration demonstrates that with one molecule of G-quadruplex DNA, four molecules of TMPyP4 and two molecules of TMPyP2 and TMPyP3 are interacting. It is also clear that the saturation point is occurring relatively at lower concentration in case of TMPyP2 (as the slope of binding curve is steep), moderate in TMPyP3, and at higher concentration in TMPyP4. The reason for difference in the nature of interaction of three porphyrins with quadruplex DNA is probably due to the orientation of N-CH<sub>3</sub> groups on the drug molecule and the nature of binding.

## 4. Conclusions

From the present study, it can be concluded that cationic porphyrins show different modes of binding to G-quadruplex DNA. The nature of binding depends upon the position of the bulky N<sup>+</sup>-CH<sub>3</sub> groups on porphyrin. Experiments with CD, fluorescence, and UV-Vis spectroscopic techniques indicate that TMPyP4 can bind externally as well as intercalate between the two G-tetrads. Two porphyrins, TMPyP3 and TMPyP2, prefer external binding to G-quadruplex DNA. Job plots and fluorescence binding curves indicate that four molecules of TMPyP4 and two molecules of TMPyP2 and TMPyP3 interact with 27Bcl2/C-myc G-quadruplex DNA. As the ionic strength of the buffer increases, fluorescence

hypochromicity decreases. The extent of reduction in fluorescence hypochromicity is high in TMPyP2 and TMPyP3, and moderate in the case of TMPyP4. The variation in fluorescence hypochromicity is probably due to the difference in the mode of drug interaction with quadruplex DNA.

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