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Synthesis and evaluation of 9-O-substituted berberine derivatives containing aza-aromatic terminal group as highly selective telomeric G-quadruplex stabilizing ligands

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ABSTRACT

A series of new 9-O-substituted berberine derivatives (**4a**–**j**) as telomeric quadruplex ligands was synthesized and evaluated. The results from biophysical and biochemical assay indicated that introducing of positive charged aza-aromatic terminal group into the side chain of 9-position of berberine significantly improved the binding ability with G-quadruplex, and exhibited the inhibitory effect on the hybridization and on telomerase activity. These derivatives showed excellent selectivity for telomeric G-quadruplex DNA over duplex.

Telomerase can add telomere repeats onto chromosome ends and plays key role in the regulation of length of telomeric DNA. The activity of this enzyme is low in the somatic cells of normal tissues while dramatically raises in most of human tumors (more than 85%), which has made it as a highly selective target for anti-tumor drug design.[1–3]

A valid strategy for telomerase inhibition is to stabilize the higher-order quadruplex structures formed by telomeric G-rich sequence d[$(TTAGGG)_n$], which as a primer during the elongation catalyzed by telomerase.[4,5]

A few of small molecule ligands have been identified to stabilize G-quadruplex structures and inhibit telomerase activity in vitro,[6–9] and several classes of these ligands have been pronounced effects on cancer cell lines, such as growth inhibition and induction of cell apoptosis and senescence.[10,11]

Berberine, an alkaloid isolated from Chinese herbs, was initially used as anti-microbial agent.[12,13] Recently, berberine and some of its derivatives have been reported as G-quadruplex stabilizing ligands.[14–18]

Our previous studies on the interactions between G-quadruplex DNA and berberine or its derivatives, such as **zh-4d** (Fig. 1), showed that berberine with N^+-containing aromatic moiety appeared suitable for π–π stacking interactions with a G-quartet. Introduction of a side chain with terminal amino group in the 9-position of the berberine could lead to significant increase stabilization effect on telomeric G-quadruplex DNA.[15,17] It could be attributed to the terminal basic amino group in the side chain strengthen the electrostatic interaction with the phosphate backbone of G-quadruplex DNA. And it was suggested that terminal group of side chain should contribute much more for the interactions of the derivatives with G-quadruplex DNA.

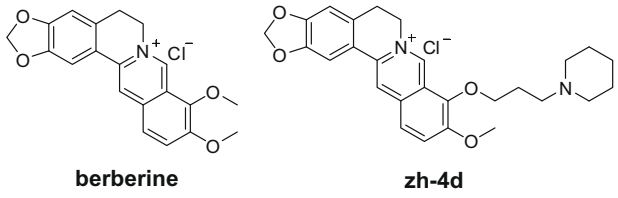

Figure 1. Structures of the berberine, 9-O-substitued derivative **zh-4d**.[15]

In present studies, in order to further improve the binding affinity and selectivity with G-quadruplex DNA, we designed a new series of 9-O-substituted berberine derivatives based on our previous studies. We introduced a positive charged terminal aza-aromatic group to increase the electrostatic interaction with the phosphate backbone and π–π stacking interaction with the bases of the loops. Molecular docking studies of the designed derivative **4f** with telomeric G-quadruplex structure were performed by using AUTODOCK 4.0 program[19] (Fig. 2). The results indicated that the terminal

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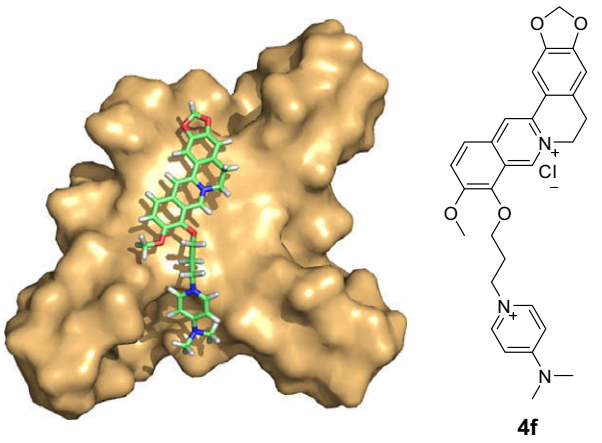


Figure 2. The interaction between **4f** and G-quadruplex created by PyMOL.[5] (view onto the plane of the 3′ surface of human G-quadruplex complex with **4f**).

aza-aromatic group N^+-containing in the side chain fitted well with grooves of G-quadruplex, and the derivative has much higher binding affinity with G-quadruplex compared to berberine and its derivative **zh-4d**, the free energies of binding were −8.66, −7.49, and −5.69 kcal/mol for **4f**, **zh-4d**, and berberine, respectively.

The synthetic pathway for 9-O-substituted berberine derivatives **4a–j** was shown in Scheme 1. The key intermediates, 9-bromoalkyl berberine **3a–c** were prepared according to the reported procedure.[15,20] The final products **4a–j** were prepared by nucleophilico-aromatic displacement of **3a–c** with pyridine analogs and exchanged with anion in 47–70% yield.

The interaction of compounds with telomeric DNA was identified by Circular Dichroism (CD) spectroscopy (see Supplementary data). Upon addition of compound **4f** to HTG21 in buffer containing KCl, the CD spectra changed with a disappearance of the small positive band at 270 nm, indicating the possible destruction of the parallel structure of the mixed G-quadruplex conformations, while the positive band at 290 nm increased and the negative band around 260 nm appeared, suggesting the formation of an antiparallel structure according to the previous studies.[21,22] The CD spectra of HTG21 titrated with **4f** in the absence of metal ion was found that the positive peak at 295 nm increased significantly, and a negative peak at 265 nm emerged, which indicated that **4f** had the ability to promote the formation of antiparallel G-quadruplex structure. Other derivatives exhibited the similar results to that of **4f**.

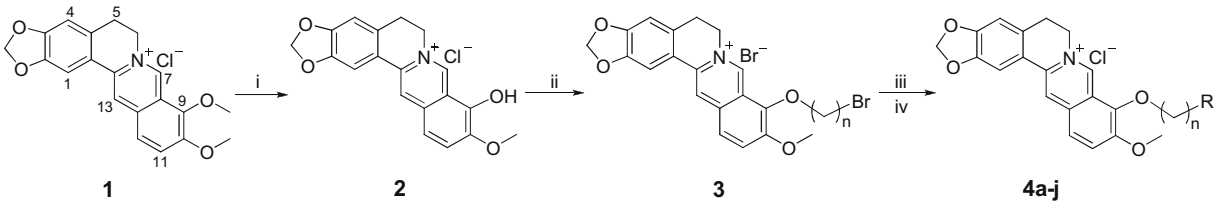
The stabilization ability of the compounds to DNA was evaluated using a FRET melting assay.[23] Two different oligomers were used here, including human telomeric G4 F21T and F10T which could self-associate a hairpin structure. To identify the competitive affinity of the compounds on G-quadruplex DNA to duplex DNA, a system containing a duplex oligomer ds26 was also applied.[24,25]

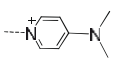
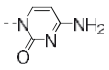
The ΔT_m values of the F21T G-quadruplex treated with the berberine and its derivatives listed in Table 1. As shown in Table 1, the derivative **4f** had the most distinct binding affinity with G-quadruplex (ΔT_m = 25 °C), and the ΔT_m value of **4a–4e** and **4i** are closely around 20 °C, while derivatives **4g** and **4j** showed lower ΔT_m value of 10–13 °C. The data showed derivatives **4a–j** possessed much stronger stabilizing ability on the telomeric G-quadruplex than berberine which inducing the ΔT_m of 4 °C. Especially, some compounds, such as **4b**, **4d**, and **4f** even excelled **zh-4d** that was the best ligand of the series in our previous work.[15] The results demonstrated that introducing of the positive charged aza-aromatic group made more contribution to the interaction with G- quadruplex structure than those neutral terminal groups (**4g**, **4j**, **zh-4d**). The electronic-donoring effect of the terminal group, such as the derivatives **4d** with methyl substituent and **4f** with *N*, *N*-dimethylamino substituent could help the binding ability. In addition, the length of the side chain played a slight favorable role in this interaction according to the data from **4a**, **4b**, and **4c**. Surprisingly, the

Table 1
G-Quadruplex FRET and binding constants (K_b, M^{-1})

Compd	ΔT_m[a] (°C)	ΔT_m[b] (°C)	K_b[c] × 10^{-6} (M^{-1})
4a	18	0.5	7.3
4b	21	0.5	8.1
4c	20	0	8.1
4d	21	0.5	7.3
4e	20	0	7.8
4f	25	0	12
4g	10	0	6.2
4h	17	0	8.9
4i	20	0	7.5
4j	13	0	6.5
zh-4d[d]	20	1	7.5
ber	4	1	2.0

Data by berberine derivatives with G-quadruplex.
[a] ΔT_m values of 0.2 μM F21T incubated in the presence of KCl 60 mM, compound 2.0 μM.
[b] ΔT_m values of 0.2 μM F10T incubated in the presence of KCl 60 mM, compound 2.0 μM.
[a,b] $\Delta T_m = T_m$ (DNA + ligand) − T_m (DNA). T_m (F21T) = 58 °C, T_m (F10T) = 60 °C.
[c] Binding constants (K_b, M^{-1}) by Fluorescence titration.
[d] **zh-4d** was reported as the best ligand during that series in our previous study.[15]



	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
n	3	5	6	3	3	3	3	3	3	3
R										

Scheme 1. Reagents and conditions: (i) 190 °C, 20–30 mmHg, 15 min; (ii) $Br(CH_2)_nBr$, CH_3CN, 60 °C, 3 h; (iii) pyridine analogs, DMSO, 90 °C; (iv) Dowex anion exchange resin.

stability of derivative **4i** with more bulky end group was equivalent to those with smaller groups (**4d**, **4e**), which indicated the quinolinium group also could be easily accommodated in quadruplex grooves or loops.

Moreover, the derivatives had a weak effect on the thermal stability of the duplex DNA (Table 1, ΔT_m <1 °C), implied that the derivatives were not the typical duplex DNA ligands. In the competition experiments (Fig. 3), the derivatives showed good selectivity for G-quadruplex versus 50 times excess of duplex DNA, which indicated that these derivatives could selectively bind to the quadruplex over duplex. Both results of the high binding affinity and good selectivity verified our original design that the more multiple interactions, such as π–π stacking interaction with G-quartet, electrostatic interactions with the phosphate backbone of grooves, and π–π stacking interactions and hydrophobic forces of terminal group with the nucleotide of loops (adenine or thymine) would improve the binding affinity and selectivity.

The binding affinities of berberine derivatives to HTG21 were also investigated by fluorescence titration assay. The DNA binding constant K_b obtained by Scatchard analysis was presented in Table 1.[18] All derivatives had higher DNA binding affinities compared with berberine. It was found derivative **4f** had the highest affinities, which was correlated with the data from FRET-melting assay. Derivatives **4g** and **4j** with neutral terminal group had higher binding affinity than berberine, but weaker than those with positive charged terminal group (**4a**–**f**, **4h**, **4i**). This suggested that the introduction of the side chain could remarkably increased binding affinity, and the positive charge on the end of side chain should play an important role in the interaction with G-quadruplex.

The induction of biologically relevant G-quadruplex formation in the HTG21 by berberine derivatives was investigated by PCR stop assay. After the PCR procedure and gel separation, the concentrations for inhibition of amplification by 50% (IC_{50}) were calculated and listed in Table 2. Derivatives with positive charged terminal group (**4a–f**, **4h**, **4i**) showed inhibitory effect on the hybridization of HTG21 at lower concentration than those with neutral ones. The data were also correlated to ΔT_m values in Table 1, which indicated the stability of human telomeric G-quadruplex induced by berberine derivatives was an important factor for inhibiting the gene amplification.

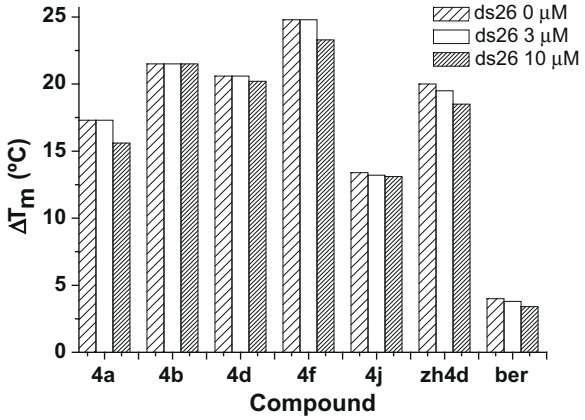


Figure 3. Competitive FRET results for derivatives and berberine without and with excess of duplex DNA competitor (ds26), the concentrations of ds26 were 0, 3, 10 μM.

Table 2
PCR-stop assay data and TRAP assay data

Compd	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	zh-4d
IC_{50} [a] (μM)	8	7	4	6	6	4	12	8	8	14	7
$^{Tel}IC_{50}$ [b] (μM)	17	15	15	14	16	14	28	16	15	22	16

[a] IC_{50}, concentration of compound required to achieve 50% inhibition of PCR.
[b] $^{Tel}IC_{50}$, the inhibitory concentrations by half values.

Telomerase inhibition was identified using telomerase repeat amplification protocol (TRAP) assay. The derivatives at certain concentrations were added into the telomerase reaction mixture containing extract from cracked MCF-7 breast carcinoma cell lines and the inhibitory concentrations by half ($^{Tel}IC_{50}$) values of these compounds were listed in Table 2. It was found the inhibitory effects on telomerase activity of derivatives were significantly enhanced compared with berberine ($^{Tel}IC_{50}$ = 70 μM), which were in line with the current thinking that a stronger ligand of the G-quadruplex was also a good inhibitor of telomerase activity.

In summary, a series of 9-O-substituted berberine derivatives containing aza-aromatic terminal group (**4a–j**) was designed and semi-synthesized. The interaction of derivatives with the human telomeric G-quadruplex DNA had been intensively evaluated by CD spectroscopy, fluorescence spectroscopy, molecular modeling, FRET-melting assay, PCR stop assay and TRAP assay. The CD results indicated the derivatives were capable of inducing the formation of antiparallel G-quadruplex and the results from fluorescence method, classical and competitive FRET melting assays, clearly showed that derivatives with positive charged aza-aromatic terminal group had high binding affinity and superior selectivity because of possible multiple interactions with G-quartet, grooves and loops of G-quadruplex DNA. The results of structure–activity relationships (SAR) study indicated that the central N^+-containing chromophore ring and the terminal positive charged aza-aromatic substituent might be important for G-quadruplex binding activity and selectivity, while the bond length of linker played slight role once the units were more than three.

Acknowledgments

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Supplementary data

Supplementary data (synthetic procedure, structural analysis data, activities assay methods, and molecular modeling method) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.030.

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