Supplemental information

Colchicine Selective Interaction with Oncogene *RET* G-quadruplex Revealed by NMR

Fei Wang[a,b,§], Chunxi Wang[a,b,§], Yaping Liu[a,b], Wenxian Lan[a,b], Hao Han[c], Renxiao Wang[a,b], Shaohua Huang[c], Chunyang Cao[a,b,c,*]

Methods

G4-DNA samples preparation

Oligos G4-DNA strands, such as *RET* G4-DNA (5'-GGG GCG GGG CGG GGC GGG GT-3'), *c-myc* G4-DNA (5'-TGA GGG TGG GTA GGG TGG GTA A-3'), *c-kit* G4-DNA (5'-AGG GAG GGC GCT GGG AGG AGG G-3'), *Tel26wt* G4-DNA (5'-TTA GGG TTA GGG TTA GGG TTA GGG TT-3') and their variants, were commercially synthesized at an Ultra-PAGE grade from Sangon Biotech Co., Ltd., Shanghai, China. They were dialyzed three times against 100 mM K^+ solution (20 mM KH_2PO_4, 80 mM KCl, pH 6.8) and 10 mM K^+ solution (2 mM KH_2PO_4, 8 mM KCl, pH 6.8), successively, then were annealed at 98°C for 10 minutes, and cooled down to room temperature.

In silico high-throughput virtual screening

In 2010, Yun Tang, *et al*, reported that the two molecular docking programs GOLD and GLIDE reproduced the binding mode of small-molecule ligands to RNA pretty well (46) and could meet the basic requirements of virtual screening of binders to RNA targets. Based on their work, we optimized the parameters used by GOLD (version 5.1, released by CCDC) and GLIDE (version 5.7, released by the Schrödinger Inc.) for virtual screening of small-molecule ligands targeting G4-DNA. We randomly selected thirteen reported G4 complex structures with PDB codes 1NZM [1], 2A5R[2], 2L7V[3], 1L1H[4], 3CE5[5], 3EM2[6], 3EQW[6], 3ERU[6], 3ES0[6], 3ET8[6], 3EUM[6], 3NYP[7] and 3NZ7[7] from Protein Data Bank (https://www.rcsb.org/) as validation examples. Quality of each docking job was assessed by the RMSD value between the top-ranked docking pose and the native binding pose of the ligand observed in the X-ray crystal or NMR solution structures. The smaller the RMSD value is, the better quality the docking job is. The optimal parameters for running each docking software were selected accordingly.

Then, we operated virtual screening of an in-house repository of 3334 compounds targeting *RET* G4-DNA. The MOE software (version 2015, released by Chemical Computing Group) was used to generate the three-dimensional (3D) structural model of each compound from its chemical structure. For the screening job conducted with GOLD, size of the binding pocket was set to 20 Å. Center of the binding pocket was defined by the centroid of the four guanine residues on the

upper and lower planes of *RET* G4-DNA structure. A total of 10000 genetic generations were run for conformational search in each docking job. The GoldScore scoring function was applied to evaluate the interaction between the docked molecule and the target G4 structure. For the screening job conducted with GLIDE, the binding pocket was defined in the same way. The GlideScore-SP scoring function was applied to evaluate the interaction between the docked molecule and the target G4 structure. After the docking job of all compounds was finished, we extracted all of the top-ranked compounds by both software with the threshold of GoleScore > 45 and GlideScore < -6.0. We then visually inspected the binding mode of each extracted compound, eliminating those in unreasonable binding mode. Finally, a total of 87 compounds were selected out from the above virtual screening process as potential binders to *RET* G4-DNA.

One-dimensional ^{1}H-NMR titration experiments

To verify whether or not the 87 compounds from virtual screening interact with *RET* G4-DNA, we further performed NMR titration experiments by running one-dimensional (1D) ^{1}H NMR spectra at 20°C. All 87 chemicals were dissolved in isotope labeled d_6-DMSO (from Sigma-Aldrich) at about 100 mM concentration, working as stock solutions. To avoid chemical shift changes of *RET* G4-DNA resulted from d_6-DMSO addition, 5μL d_6-DMSO were added into 500 μL of 0.1 mM *RET* G4-DNA solution in the NMR buffer (20 mM KH_2PO_4, 80 mM KCl, pH 6.8, 10% D_2O), respectively, and then 1D ^{1}H-NMR spectra were conducted (**figure S1**). No chemical shift changes of imino protons of *RET* G4-DNA were observed. Thus, during NMR titration experiments of each compound into *RET* G4-DNA solution, the maximal volume 5μL of each compound in d_6-DMSO solution was thought as a final data point. Similarly, to test inter-G4s binding selectivity of colchicine, less than 5μL of colchicine in d_6-DMSO stocked solution was added into 500 μL of 0.1 mM *RET* G4-DNA, c-*kit* G4-DNA, c-*myc* G4-DNA and *Tel26wt* G4-DNA solution in NMR buffer at different mole ratios (G4-DNA *vs* colchicine), respectively, and then 1D ^{1}H-NMR spectra were acquired at 20°C.

To test interactions between *RET* G4-DNA and colchicine, *RET* G4-DNA was prepared in 10% D_2O buffer, containing 100 mM K^+ (2 mM KH_2PO_4, 8 mM KCl, pH 6.8), the 1D ^{1}H NMR spectra were acquired at 25°C using a set of ligand vs DNA molar ratios, as shown in **figures 1C and S8**. The binding affinity constant (K_D) was calculated by using the equation (1) [8], where $\Delta\delta_{obs}$ and $\Delta\delta_{max}$ represent the absolute values of observed chemical shift changes and of the maximal chemical shift changes, [L] and [P] are concentrations of the small molecule and the *RET* G4-DNA, n is the binding ratio between small molecule and *RET* G4-DNA, respectively.

$$\Delta\delta_{obs} = \frac{\Delta\delta_{max}(n[L]+n[P]+K_D-\sqrt{(n[L]+n[P]+K_D)^2-4n^2[L][P]}}{2n[P]} \quad (1)$$

Circular dichroism (CD) spectroscopy

Circular dichroism spectra of G4 with chemicals were recorded at 25°C on JASCO-815

spectropolarimeter using a 1-cm path length quartz cuvette with a reaction volume of 350 μL. DNA concentrations were 50 μM in a buffer containing 20 mM KH_2PO_4 , 80 mM KCl, pH 6.8. The chemicals were mixed with G4-DNA at a molar ratio 1:1. An average of three scans was taken, and the spectrum of the buffer was subtracted.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were carried out using an auto VP-CAP-DSC Microcalorimeter (Malvern Inc, USA). G4-DNA strands at 50μM were tested with or without the chemicals. Scans were performed at 1°C/min in the 20 - 110°C temperature range. A buffer-buffer scan was subtracted from the buffer-sample scans and linear-polynomial baselines were drawn for each scan. Baseline corrected thermograms were normalized with respect to the single strand molar concentration to obtain the corresponding molar heat capacity curves. Tm values were obtained as the temperatures corresponding to the maximum of each thermogram peak.

1D ^{1}H CPMG and STD experiments

Solvent-suppressed 1D ^{1}H CPMG (cpmgPr1d) experiments were employed to detect interactions between colchicine and G4-DNAs. All these NMR spectra were acquired at 20℃ on 600 MHz Bruker Avance III spectrometer equipped with a cryogenically cooled probe. Samples were dissolved in buffer (80 mM KCl, 20mM KH_2PO_4, pH 6.8, 1% d6-DMSO, 99% D2O), which contain 200 μM colchicine with different molar ratio of G4-DNAs, especially, the colchicine vs G4-DNAs molar ratio equals to 80:1, 40:1, 20:1 and 10:1, the final volume was 500 μL. The 90 pulse length was adjusted to about 11.07μs. A fixed total spin-spin relaxation delay 120 ms was applied to attenuate the broad NMR signals of slowly tumbling molecules with short T2 relaxation times and to retain signals of low-molecular weight compounds. 64 transients were collected into 64K data points with a spectral width of 7.8 kHz (13 ppm) and an acquisition time of 2.99 s.

STD data was acquired using 4 dummy scans and a relaxation delay of 3 s, followed by a 40 dB pulsed irradiation at frequency of 5.7ppm, the total acquisition time for STD spectrum was 21 min with 128 FIDs.

NMR data collection and analysis for structural determination

NMR experiments were performed on 600 MHz and 800 MHz Varian and Bruker spectrometers at 5°C, 10°C and 20°C, respectively. Most NMR resonances of bound *RET* G4-DNA could be assigned unambiguously based on the previous report of free *RET* G4-DNA structure [9], thus it's unnecessary for us to prepare site-specific low enrichment ^{15}N-labelled *RET* G4-DNA in complex with colchicine. We made NMR samples of unlabeled *RET* G4-DNA with colchicine at mole ratios of *RET vs* colchicine (1:1, 1:2, 1:3, 1:4, 1:8). 2D ^{1}H-^{1}H COSY, TOCSY (mixing time is 40 ms and 80 ms) and NOESY (mixing times are 50, 250 and 300 ms, respectively) spectra with a spectra width 16 ppm were acquired at 20°C on the samples in H_2O NMR buffer (20 mM KH_2PO_4, 80 mM KCl,

pH 6.8, 10% D_2O) and D_2O NMR buffer (20 mM KH_2PO_4, 80 mM KCl, pH 6.8, 100% D_2O). To assign imino proton signals, NOESY spectra were run at 5°C and 10°C, with a spectral width 20 ppm, mixing time 50 ms and 250 ms, respectively. The water signal in each NMR experiment was suppressed by watergate, jump-and-return or presat techniques. The acquisition data points were set to 2048 x (250-512) (complex points). All spectra were processed with the program NMRPipe [10]. The 45° or 60° shifted sine-squared functions were applied to two dimensional (2D) ^1H-^1H NOESY and TOCSY spectra. The fifth-order polynomial functions were employed for the baseline corrections. The final spectral sizes are 2048x 1024. The ^1H chemical shifts were referenced to 2, 2-dimethylsilapentane -5-sulfonic acid (DSS).

The signals of free colchicine were identified through ^1H-^1H COSY, TOCSY and NOESY spectra acquired on their samples dissolved in D_2O. These results were used as references in assigning the signals of colchicine in their bound states. Then, the NMR signals of the protons in colchicine bound to *RET* G4-DNA were assigned using 2D ^1H-^1H COSY, TOCSY and NOESY spectra acquired on the samples of *RET* G4-DNA in complexes with colchicine, respectively.

The imino proton assignments of bound *RET* G4-DNA were directly performed from our previous report of free *RET* G4-DNA structure [9]. NMR signals belonging to the protons in sugar ring and aromatic rings in the bases of bound *RET* G4-DNA were also obtained based on previous assignment of free *RET* G4-DNA, directly using 2D ^1H-^1H COSY, TOCSY and NOESY spectra acquired on the NMR samples of *RET* G4-DNA in complexes with colchicine, respectively. NMR peak assignments and NOE cross-peaks integrations were achieved using program Sparky (http://www.cgl.ucsf.edu/home/sparky/). The NOE peaks were integrated using the peak fitting function and volume integration in Sparky.

Solution structure determination

The distances between non-exchangeable protons were estimated based on the NOE cross-peak volumes at 50, 250 and 300 ms mixing times, and were divided into strong (1.8-2.9Å), medium (1.8-3.5Å) and weak (1.8-6.0Å) groups, respectively, using the proton H5-H6 distance (2.45Å) in cytosine base as a reference. Exchangeable proton restraints are based on NOESY data sets at two mixing times (50 ms and 250 ms) in H_2O buffer. Cross-peaks involving exchangeable protons were classified as strong (strong intensity at 50 ms), medium (weak intensity at 50 ms) and weak (observed only at 250 ms) NOEs. The G-tetrads within the *RET* G4-DNA were restrained with the distances corresponding to ideal hydrogen bond geometry. Each individual hydrogen bond was restrained using two distance restraints (heavy atom - heavy atom and heavy atom-proton, respectively). The aromatic rings of guanines in each G-tetrad were also restrained into one plane during calculation. 20 dihedral angle restraints were used to restrict the glycosidic torsion angle (χ) for the experimentally assigned *syn* configuration, *i.e*. G_1, G_7, G_{11} and G_{17} tetrad-guanines [60° ± 35°], as well as for the experimentally assigned *anti*-configuration bases, *i.e*. G_2, G_3, G_4, C_5, G_6, G_8, G_9, C_{10}, G_{12}, G_{13}, G_{14}, C_{15}, G_{16}, G_{18}, G_{19} and T_{20} [240° ± 70°].

To study how chemicals interact with *RET* G4-DNA, the topology parameters of colchicine were firstly generated from the PRODRG sever, a program for generating molecular topologies and unique molecular descriptors from the coordinates of small molecules (http://www.gromacs.org/Downloads/Related_Software/PRODRG), and then they were slightly modified so that they can be integrated into the program XPLOR-NIH[11]. The structural calculations of *RET* G4-DNA in complex with colchicine were carried out using a standard simulated annealing protocol implemented in the program XPLOR-2.47 (NIH version)[11]. A total of 364 or 314 NOE-derived distance restraints (**table S2**), of which 230 and 228 are from intra-residue NOEs, were incorporated into the NOE-restrained structure calculation, respectively. A total of ten iterations (50 structures in the initial ten iterations) were performed. 100 structures were computed in the last five iterations, 20 conformers with the lowest energy and minimal restrain violations are used to represent the best three-dimensional (3D) structures. In the ensemble of the simulated annealing 20 structures, there was no distance constraint violation larger than 0.3 Å and no torsion angle violation more than 5^{o}.

Cell culture, and reagents

Breast cancer cells were cultured in DMEM supplemented with 10% FBS, 2 mmol/L glutamine, and 100 mg/mL of penicillin/streptomycin (ThermoFisher Scientifi) at $37^{o}C$ in 5% CO_2. For experiments, colchicine (Sangon Biotech) was diluted to the indicated concentrations in cell culture media.

Cell growth assay

Cells were plated at a concentration of 5000 cells/well in a 96-well plate and incubated overnight, followed by the treatment with colchicine at increasing concentrations up to 96 hours. The cell growth was pictured by IncuCyte Zoom (Essen BioScience Inc.) and the confluence was calculated using the incuCyte software.

qRT-PCR

Total RNA was isolated from untreated and colchicine-treated cells using RNAeasy^{TM} Animal RNA Isolation Kit with Spin Column. Reverse transcription was performed as the manufacturer manual (Takara Bio Inc). Quantitative PCR (qRT-PCR) was done on ABI Q6 (Thermo-Fisher Scientific) to check the RET and ACTNB expression using ABI prime Master (Thermo-Fisher Scientific). The samples were normalized to the levels of b-actin (ACTNB). The primer sets are listed as follows: *RET* 1953-1971 forward, 5'-GCA GCA TTG TTG GGG GAC A-3', *RET* 2264-2245 reverse, 5'-CAC CGG AAG AGG AGT AGC TG-3', Actin 1359-1378 forward, 5'-CTG GAA GCC TGA AGG TGA CA-3', and Actin 1498-1476 reverse, 5'-AAGGGACTTCCT GTA ACA ACG CA-3', respectively.

Results

NMR signal assignment of colchicine binding to *RET* G4-DNA

Colchicine is an alkaloid obtained from the meadow saffron plant, *Colchicum autumnale* (Liliaceae) and other *Colchicum species*. As shown in the structure of colchicine in **table S1 and figure S7 or S9**, colchicine contains one phenyl group (ring C) and one seven-member aromatic ring (ring A) connected by seven-membered ring (ring B), which are conjugated, but, generally, are not in a plane. To correctly assign NMR signals of its protons of free colchicine, the ^{1}H atoms were termed based on the chemical component dictionary in ligand structure database in RCSB website (http://www.rcsb.org). About 30 mg colchicine was purchased at HPLC grade from Sangon Biotech Co., Ltd, and directly dissolved in NMR buffer containing 10% D_2O (the maximal concentration is about 45 mg/ml in H_2O, based on the product information provided by Sigma-Aldrich), and 2D ^{1}H-^{1}H COSY, TOCSY and NOESY spectra were then acquired in 600 MHz NMR machine. According to the previous report about the NMR signal assignment of free colchicine[12], the NMR signal belonging to 9-H (at 2.51 ppm and 2.39 ppm, **table S1**) and 10-H protons (at 2.23 ppm and 1.81 ppm) were easily identified as the starting points based on J-coupling correlation in COSY and TOCSY spectra. The assignment of 9-H was confirmed by NOE between 9-H and 7-H (at 6.51 ppm), while the assignment of 10-H was testified by J-coupling correlation among 11-H (4.63ppm),10-H, 11-NH (6.42 ppm), 11-H and 9-H. Then, NMR signals of other protons in colchicine were assigned based on NOE correlations (**figure S9**), and were summarized in **table S1**. These assignments of free colchicine were used as references to assign NMR signals of colchicine binding to *RET* G4-DNA.

Upon colchicine was mixed with *RET* G4-DNA at mole ratios of 1:1, 1:2, 1:3, 1:4 and 1:8 (*RET* vs colchicine) in NMR buffer, the NMR signals of colchicine were assigned using a process identical to that used in signal assignments of free colchicine, and basically through probing the differences in the ^{1}H-^{1}H NOESY spectra acquired on free *RET* G4-DNA and on the complex sample, respectively. Here, we used 1:1 NMR complex sample as an example to describe the signal assignment. Compared to the region (8.2 ppm - 9.2 ppm) of the ^{1}H-^{1}H spectrum of free *RET* G4-DNA (**figure S9B**), a unique, new strip of one amide proton 11-NH (at 8.63 ppm) was shown up in NOESY spectrum acquired on the complex (**figure S9A**), which indicates that 11-NH has NOEs with 9-H (*i.e.*, 9-H' and 9-H'', at 2.56 ppm and 2.25 ppm), 10-H (*i.e.*, 10-H' and 10-H'', at 1.84 ppm and 2.16 ppm), 11-H (at 4.26 ppm), 13-methyl group (at 1.91 ppm), 15-H (at 7.23 ppm), respectively. Similarly, compared to the region (6.4 ppm - 7.00 ppm) in the spectrum of free *RET* G4-DNA (**figure S9D**), in NOESY spectrum of the complex, newly shown up 7-H signal (at 6.71 ppm) of colchicine displays NOE correlations with 6-methyl group (*i.e.*, 6-CH_3, at 3.82 ppm), 9-H and 10-H (**figure S9C**), which confirmed the assignment of 9-H and 10-H in **figure S9A**. The assignments of 9-H and 10-H were confirmed by the J-coupling and NOE correlations between 9-H and 10-H protons were observed in TOCSY (**figure S9I**) and NOESY (**figures S9G and S9H**) spectra acquired on the complex sample. The assignment of 10-H was also testified by NOEs between 11-H and 10-H (**figure S9F**). Then, in the 6-CH_3 strip (at 3.82 ppm), 6-CH_3 displays NOEs with 4-CH_3 (3.77 ppm, close to diagonal peak), 9-H and 10-H (**figure S9E**), respectively. At same time, 4-CH_3 group

has NOE with 2-CH_3 (at 3.47 ppm), which further helps to assign NMR signal of 20-H (at 7.31 ppm). The 20-H assignment was confirmed by its NOE with 19-H (at 7.21 ppm) in 19-H strip (**figure S9F**), where 18-CH_3 (at 3.93 ppm) and 20-H (7.31 ppm) all have NOEs with 19-H, too. Taken together, based on NOE correlations and J-coupling correlation (**figure S9L**), we obtained all assignments of the chemical shifts of the protons in bound colchicine in NMR complex sample (**table S1**), which makes it possible for us to correctly assign intermolecular NOEs between colchicine and *RET* G4-DNA.

Intermolecular NOEs assignments between *RET* G4-DNA and colchicine

To determine the orientation of colchicine relative to *RET* G4-DNA, we assigned intermolecular NOEs between *RET* G4-DNA and colchicine by carefully comparing 2D ^{1}H-^{1}H NOESY spectra acquired on free *RET* G4-DNA and *RET* G4-DNA in complex with colchicine. As shown in **figure S10A**, firstly, in the strip of G16 imino proton (*i.e.*, G16 H1, at 11.25 ppm), four NOE correlations of G16 H1 with colchicine 9-H (in ring B, **figure S10F**), 6-CH_3 and 7-H were found, which were not shown in the same region of ^{1}H-^{1}H NOESY spectrum acquired on free *RET* G4-DNA. This observation indicates that the aromatic phenyl ring is positioned above G19, since G16 is close to G19. Secondary, in **figure S10B**, in strip of G9 imino proton (*i.e.*, G9 H1, at 10.82 ppm), three NOE cross-peaks between G9 H1 and colchicine 10-H (*i.e*., 10-H' and 10-H''), and between G9 H1 and colchicine 9-H were found, suggesting that the acetyl side of ring B is much closer to locate above G9 than the other atoms (such as 21-C and 22-C atoms, etc). Thirdly, in **figure S10C**, two NOE cross-peaks between colchicine 4-CH_3 (at 3.74 ppm) and base G13 H8, and between colchicine 18-CH_3 (at 3.79 ppm) and base G9 H8 were observed, further confirming the orientation of colchicine in the complex structure (**figure 3D**). Fourthly, in strips of colchicine 6-CH_3 (at 3.82 ppm) and 18-CH_3 (at 3.93 ppm)(**figure S10D**), two NOE cross-peaks between colchicine 6-CH_3 and base C15 H5'' (in sugar ring), and between colchicine 18-CH_3 and base G9 H2'' (in sugar ring) were also observed, implying that aromatic seven-membered ring (ring A) is above G9, and phenyl ring (ring C) is closely above base G19 (**figure 3D**). Finally, in **figure S10E**, a weak NOE correlation between colchicine 19-H and base G13 H8 was obtained, further suggesting whole molecule of colchicine is close to base G13 (**figure 3D**). All these intermolecular NOE assignments (**figure S10F**) were confirmed in two dimensions, and by one another, so that they can be used to generate distance constraints for structure determination.

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Supplemental figures and tables

Figure S1 NMR titration of d_6-DMSO into G4-DNA solutions. No chemical shift changes and no broadened line-width were observed, indicating less than 5 µL d_6-DMSO does not interact with (A) *RET* G4-DNA, (B) *Tel26wt* G4-DNA, (C) *c-kit* G4-DNA and (D) *c-myc* G4-DNA.

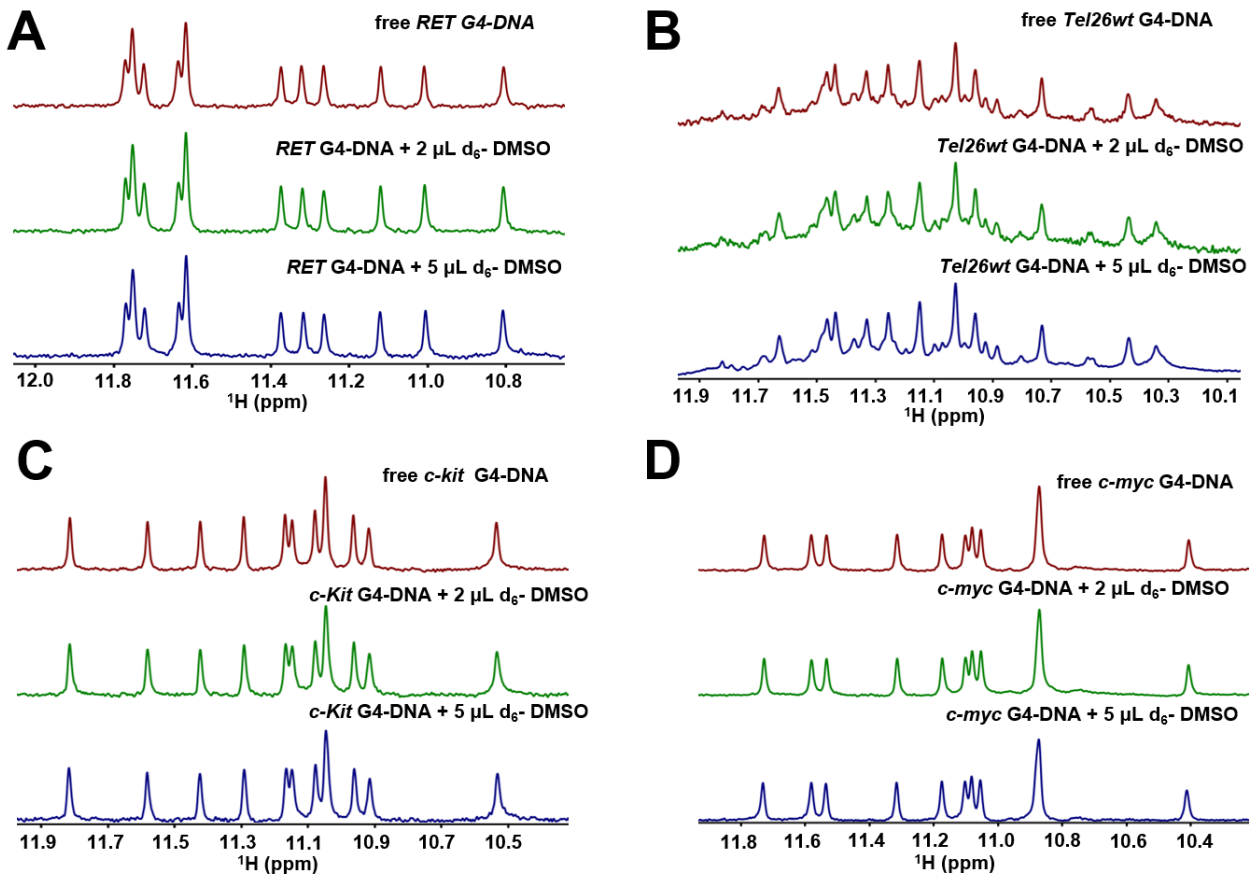

Figure S2 Chemical structures of 9 compounds which were screened out to interact with *RET* G4-DNA by NMR titration experiments. Among them, chemical compounds 26 is colchicine.

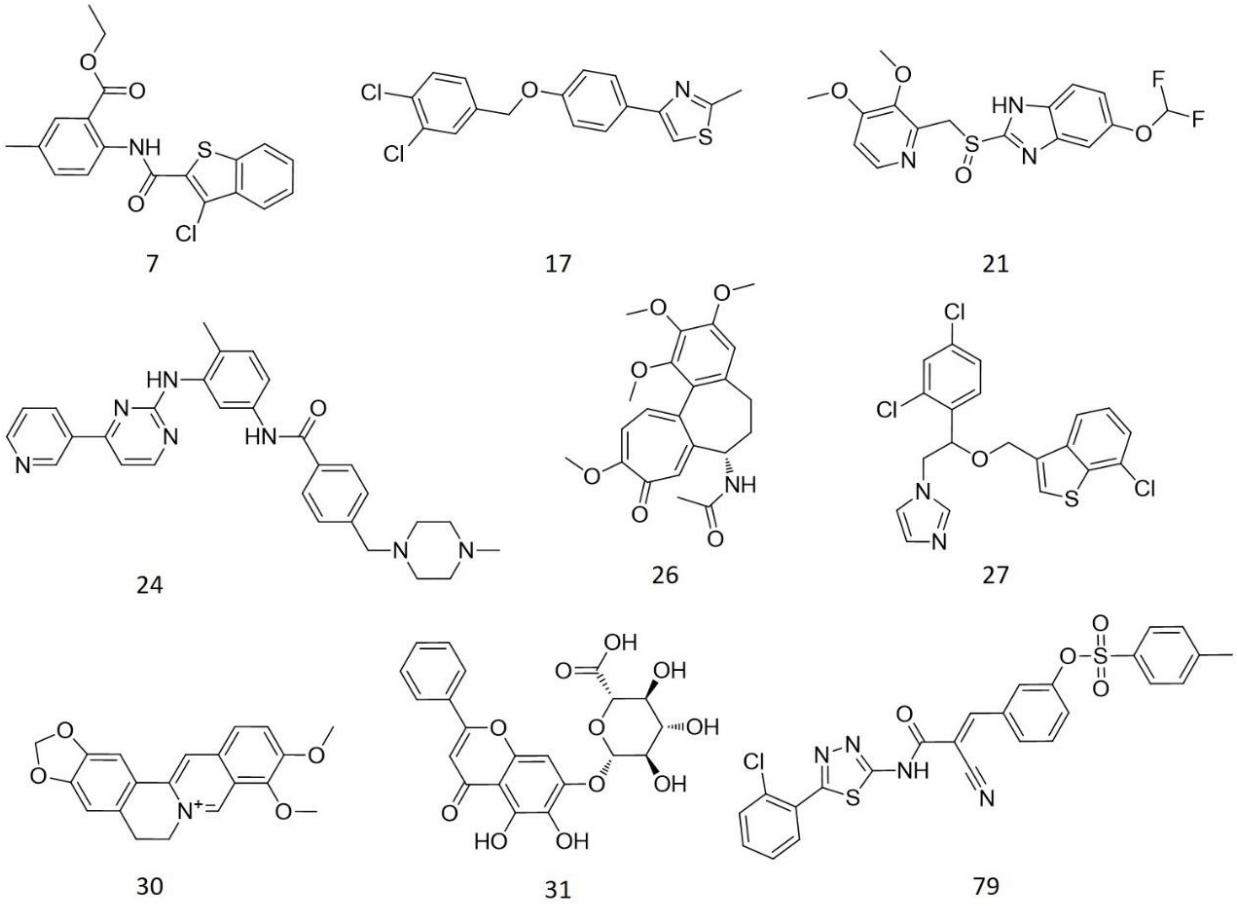

Figure S3 Structures of four G4-DNAs used in the manuscript. (A) *hybrid-2 wt Tel26* G4-DNA (pdb code 2JPZ). (B) *c-kit* G4-DNA (pdb code 2O3M). (C) *c-myc* G4-DNA (pdb code 1XAV). (D) *RET* G4-DNA (pdb code 2L88). In (A-D), the bases with *anti* and *syn* conformations were drawn in cyan and in pink, respectively.

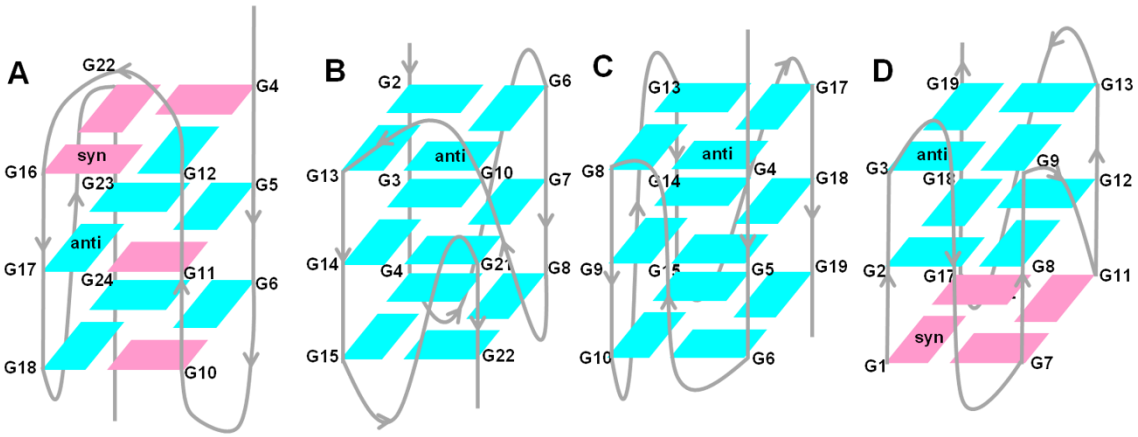

Figure S4 NMR titrations of nine compounds into *Tel26wt* G4-DNA solution at mole ratio 1:1. Only chemical 26 addition did not result in obvious changes in imino proton signals. The changed signals in each case were marked with red stars.

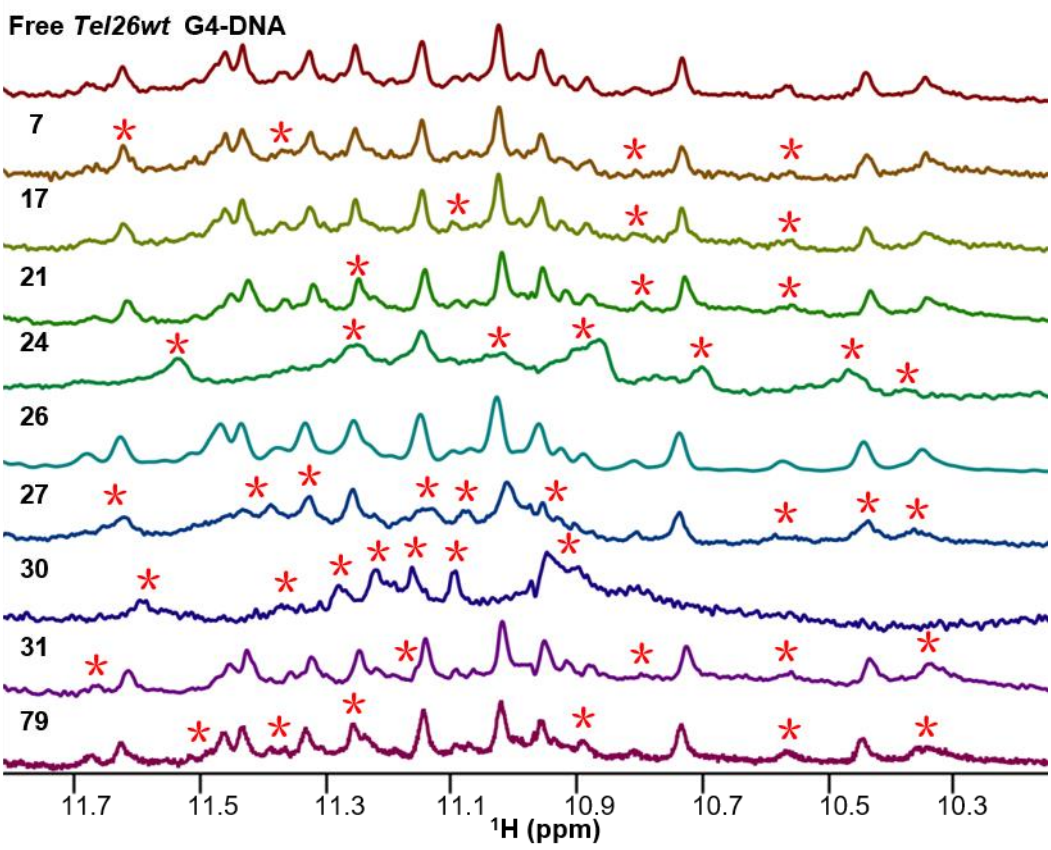

Figure S5 Inter-G4 selectivity of colchicine was determined based on the imino proton signal changes through NMR titration experiments at increased mole ratios (G4 vs colchicine), and effects on G4 folding by colchicine tested by CD spectra at mole ratios 1:1 (G4 vs colchicine). (A) *RET* G4-DNA. (B) *c-kit* G4-DNA. (C) *c-myc* G4-DNA. (D) *Tel26wt* G4-DNA.

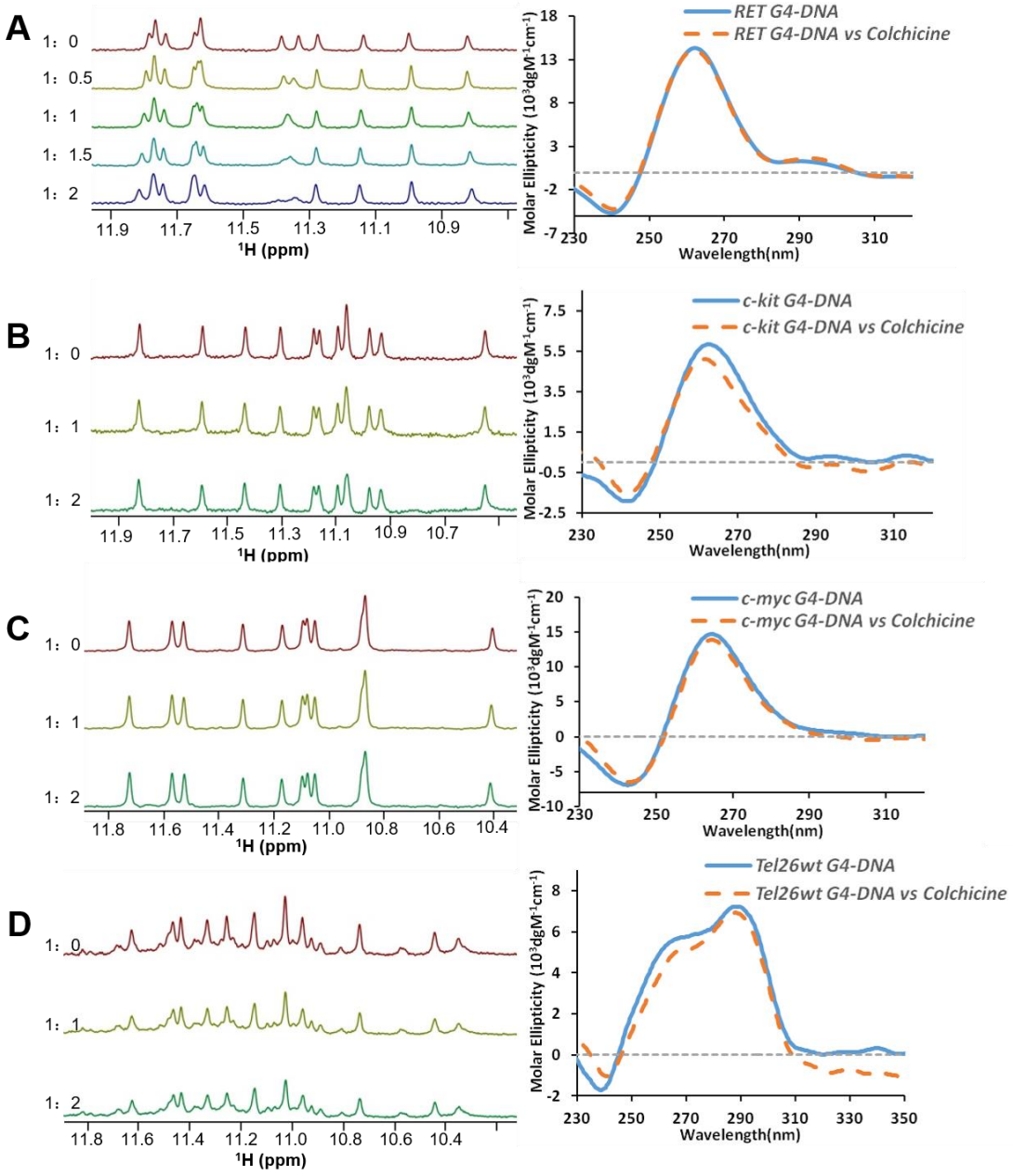

Figure S6 Colchicine selective interaction with *RET* G4-DNA confirmed by CPMG experiments. Stacked CPMG spectra of free colchicine (200 µM in 100mM K^+ buffer) was in blue lines and after being added with (A) 5 µM *RET* G4-DNA; (B) 5 µM c-*kit* G4-DNA; (C) 5 µM c-*myc* G4-DNA and (D) 5 µM *Tel 26wt* G4-DNA in red lines, respectively. 1%(v/v) d_6-DMSO was added as the reference, and the signals of the protons H7 and H4 of colchicine were showed in each top of spectrum, respectively.

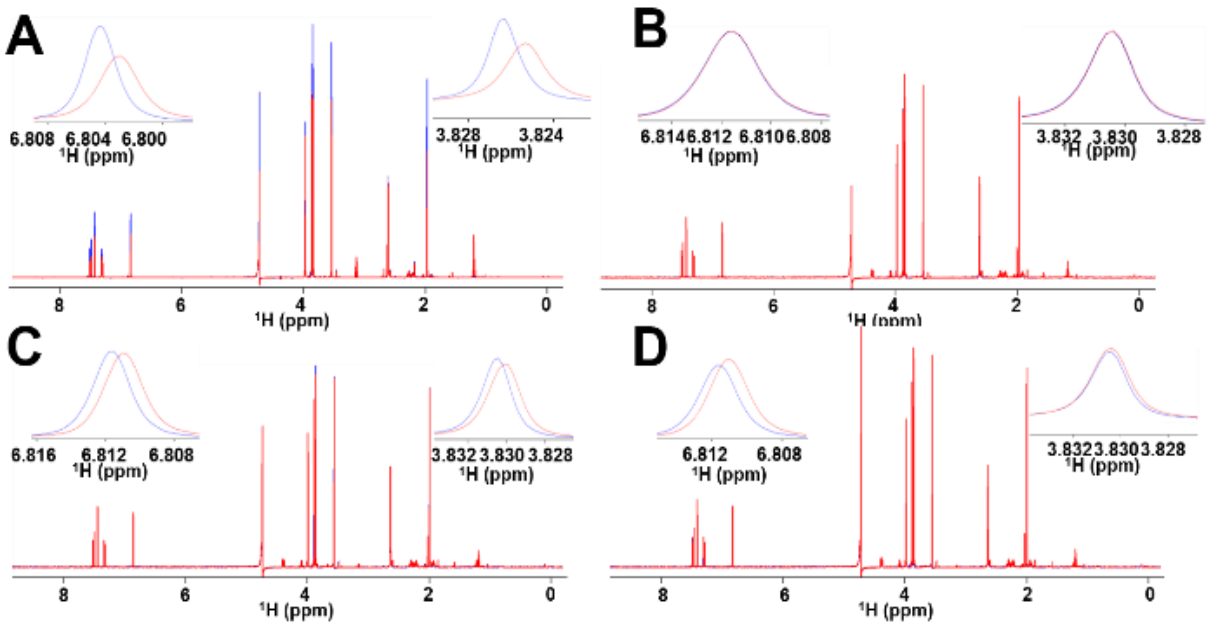

Figure S7 Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence spectra revealed that the interaction between *RET* G4-DNA and colchicine is dose-dependent. (A) Stacked CPMG spectra of colchicine (200 μM in 100mM K^+ buffer). After being added different molar ratio (*RET* G4-DNA vs colchicine) of *RET* G4-DNA is showed in blue (free), red (mole ratio is equal to 1:80), green (mole ratio is equal to 1:40), purple (mole ratio is equal to 1:20) and orange (mole ratio is equal to 1:10), respectively. (B)The CPMG spectra details of aromatic hydrogen H20, H15, H19 and H7 and aliphatic methyl group like H18, H6, H4, H2 and H13. The nomination of the carbon atoms in colchicine were inserted in (A).

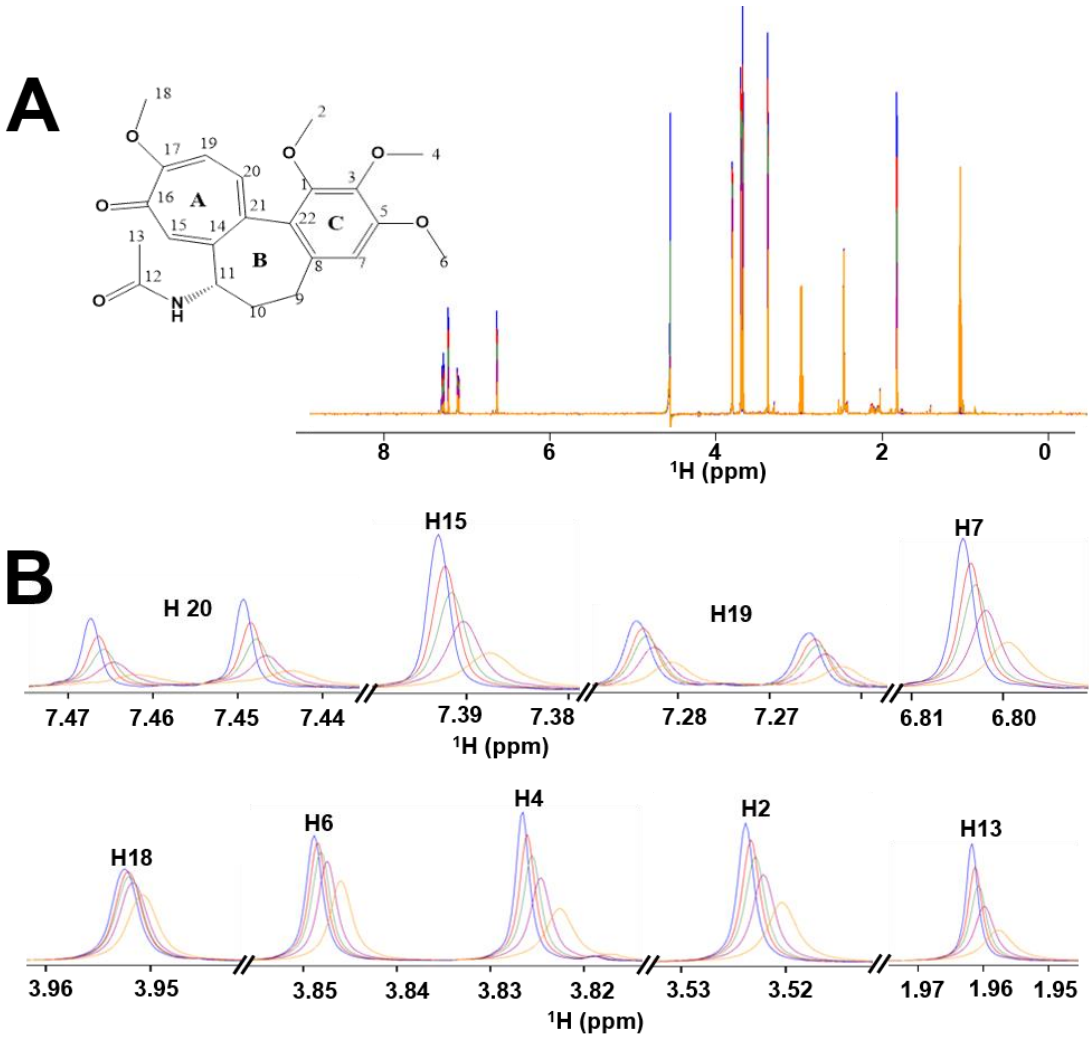

Figure S8 The imino proton region of 1D ^{1}H spectra of *RET* G4-DNA solution, titrated with colchicine at the mole ratios of 1:0, 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:5,1:6, 1:7, 1:8, 1:9 and 1:10 (*RET* G4-DNA vs colchicine), respectively.

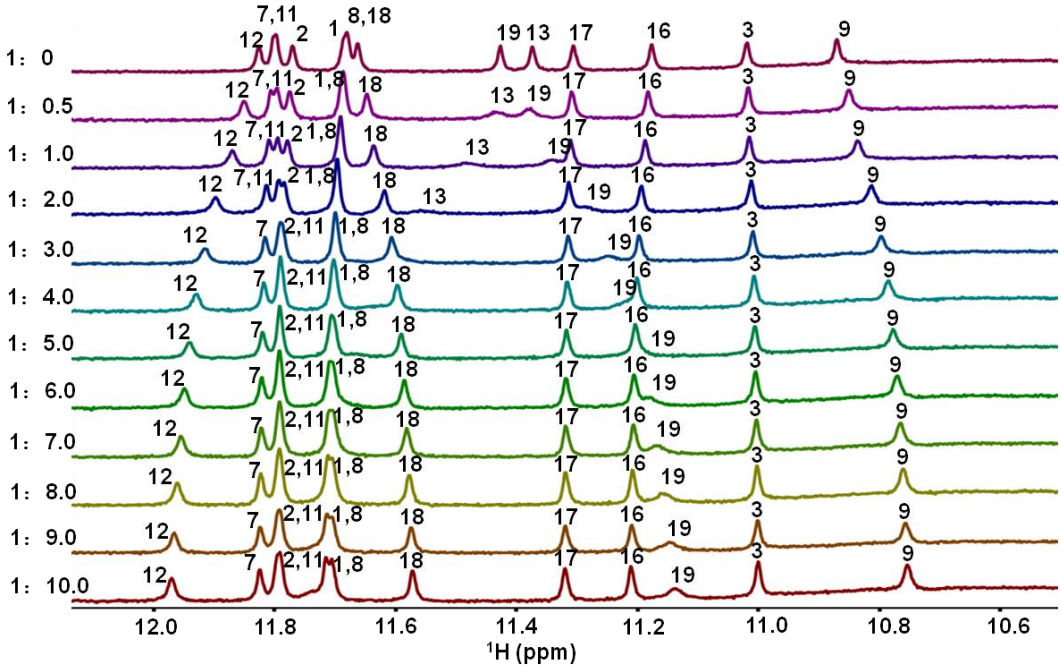

Figure S9 Assignment of NMR signals of colchicine protons in complex sample of colchicine with *RET* G4-DNA. **(A and B)** The region from 8.20 ppm to 9.20 ppm of the NOESY spectra acquired on (A) the complex and (B) free *RET* G4-DNA, respectively. The differences in (A) and (B) confirmed assignment of 11-NH and its NOEs with 9-H (*i.e.*, 9-H' and 9-H''),10-H (*i.e.*, 10-H' and 10-H''),11-H,13-CH_3 and 15-H. **(C and D)** The region from 6.40 ppm to 7.00 ppm of the NOESY spectra acquired on (C) the complex and (D) free *RET* G4-DNA, respectively. The differences in (C) and (D) confirmed assignment of 7-H and its NOEs with 10-H (*i.e.*, 10-H' and 10-H''), 9-H (*i.e.*, 9-H' and 9-H'') and 6-CH_3. **(E)** The region from 3.70 ppm to 4.30 ppm of NOESY acquired on the complex. This figure indicates 6-CH_3 has NOEs with aliphatic 4-CH_3 (close to diagonal peak, not labeled in the figure), 9-H (*i.e.*, 9-H' and 9-H'') and 10-H (*i.e.*, 10-H' and 10-H''), and 11-H has NOEs with 10-H. At the same time, in this region, 2-CH_3 displays NOEs with 4-CH_3 and 18-CH_3, respectively. **(F)** Observed NOEs between 19-H and 2-CH_3, between 19-H and 20-H, between 19-H and 18-CH_3, and between 20-H and 18-CH_3. **(G and H)** The region from 2.00 ppm to 3.70 ppm of the NOESY spectra acquired on (G) the complex and (H) free *RET* G4-DNA, respectively. The differences in (G) and (H) confirmed assignment of 9-H and 10-H and their NOE correlations. **(I)** The region from 2.00 ppm to 3.70 ppm of the TOCSY spectrum acquired on the complex. This figure confirms assignments of 9-H and 10-H by the J-coupling cross-peaks between 10-H and 9-H, labeled in red. **(L)** Chemical structure of colchicine, and all observed NOEs between protons in it were connected by blue lines.

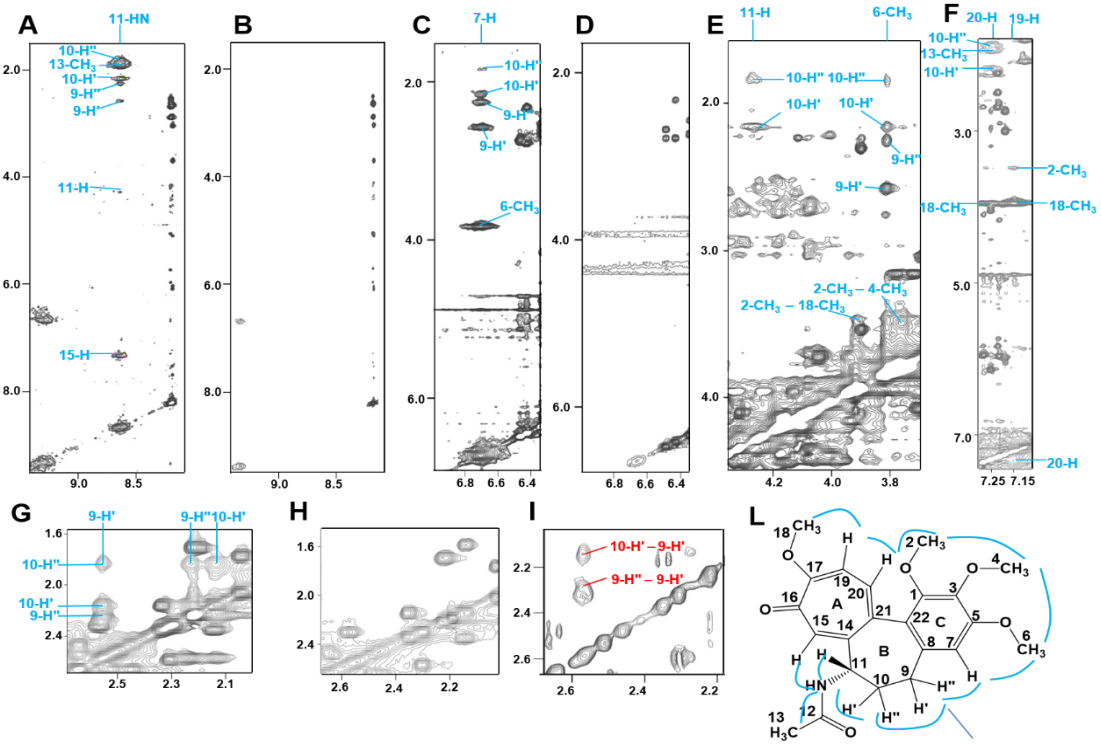

Figure S10 The assignment of intermolecular NOEs (labeled in orange) between colchicine (termed as LOC in all panels) and *RET* G4-DNA, using ^{1}H-^{1}H NOESY spectrum with a mixing time 250 ms. To distinguish them from intra-molecular NOEs, the intra-molecular NOEs of colchicine were labeled in blue. (**A**) Base G16 imino proton H1 displays intermolecular NOEs with 9-H (*i.e.*, 9-H' and 9-H''), 7-H and 6-CH_3 of colchicine. (**B**) G9 imino proton H1 demonstrates NOEs with colchicine 10-H (*i.e.*, 10-H' and 10-H'') and 9-H'. (**C**) Base G13 H8 has NOE with colchicine 4-CH_3, and G9 H8 displays NOE with 18-CH_3; (**D**) Colchicine 18-CH_3 has NOE with G9 H2' (in sugar ring), and colchicine 6-CH_3 displays NOE with C15 H5'' (in sugar ring). (**E**) G13 H8 has NOE correlation with colchicine 19-H. (**F**) The names of the protons and carbons in colchicine structure, and all intermolecular NOEs highlighted by orange lines between *RET* G4-DNA and colchicine. The G3-G9-G13-G19 tetrad was drawn by connecting these residues through grey lines. Residues G14, C15 and G16 and G4 were also displayed linked by palecyan lines and dashed arrows. The protons of bases G3, G9, G13, G14, C15 and G16, which have NOEs with colchicine, were also displayed beside these residues. In (A and D), the protons G16 H1 of *RET* G4-DNA and colchicine 18-CH_3, 6-CH_3 and 4-CH_3 were labeled on the top of their strips. The carbon atoms of colchicine were named based on the chemical component dictionary in ligand structure database in RCSB website (http://www.rcsb.org).

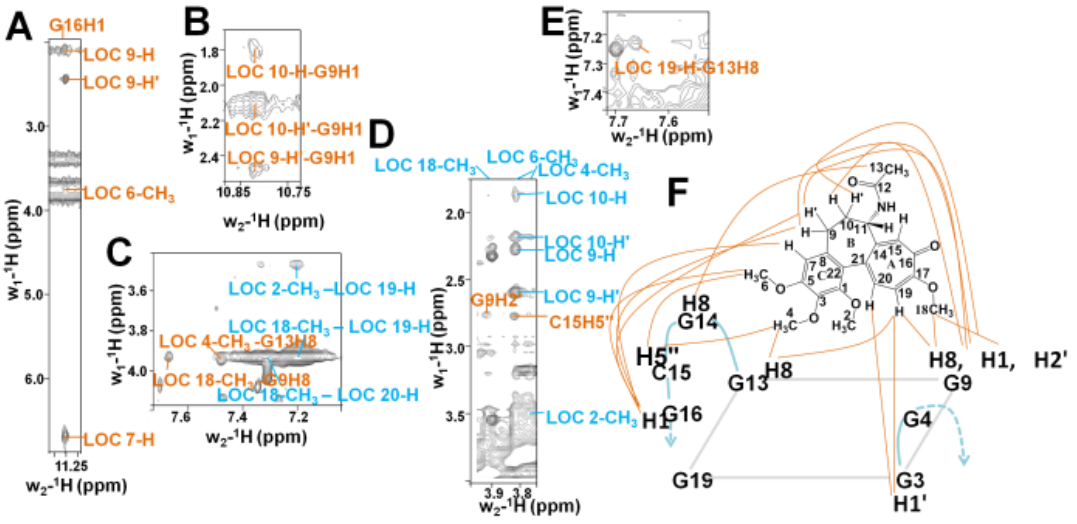

Figure S11 Residue G14 is important to *RET* G4-DNA stability. (**A**) G14 stacks with G13 in solution structure of free *RET* G4-DNA (pdb code 2L88). The bases with *anti* and *syn* conformations were drawn in cyan and in pink, respectively. (**B**) Hydrogen-bond (displayed in dashed lines) between the -NH2 group of G14 and carbonyl group of G19 (distance between them is 2.2Å) stabilizes the conformation of residue G14. (**C**) Replacement of G14 by I14 results in large chemical shift changes of imino protons of *RET* G4-DNA. (**D**) Melting temperature of *RET* G14I variant is smaller than that of the wild-type *RET* G4-DNA. (**E**) Intra-G14 NOEs (in purple) and inter-residue NOEs (in red) between G14 and C15 observed in ^{1}H-^{1}H NOESY spectrum acquired on free *RET* G4-DNA. (**F**) Intra-G14 and inter-residue NOEs between G14 and C15 disappear in the ^{1}H-^{1}H NOESY spectrum (mixing time 250 ms) acquired on *RET* G4-colchicine complex, indicating that G14 is flexible in structure of *RET* G4-colchicine complex. In (F), the intra-molecular NOEs of protons in colchicine (termed as LOC) were unanimously identified in blue. The names of protons in colchicine were shown in **Table S1**.

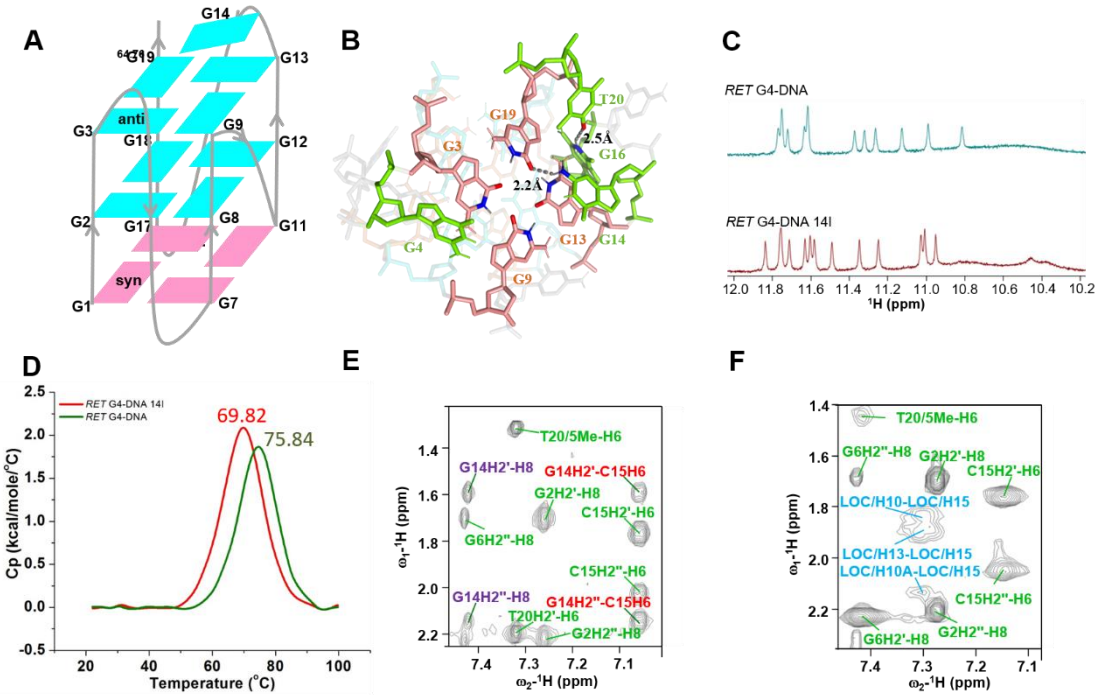

Figure S12 Chemical shift changes of the protons of *RET* G4-DNA upon being mixed with colchicine. The chemical shift changes (△δ, ppm) were calculated through the equation of $\triangle\delta = \delta_{bound} - \delta_{free}$, where δ_{bound} and δ_{free} are the chemical shift values of the protons of *RET* G4-DNA in bound state (with colchicine) and in free state, respectively. Different protons in each base of *RET* G4-DNA with varied chemical shift changes were displayed in different colors.

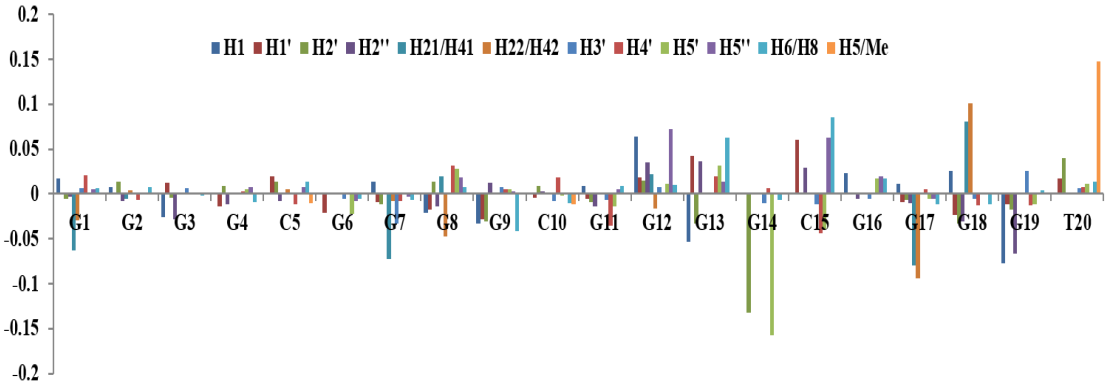

Figure S13 Analysis of stability of *RET* G4-DNA. (A) The stability of *RET* G4-DNA is dependent on the concentration of K^+. The melting temperatures were displayed on the top of the peaks. (B) The interaction of colchicine (*i.e.*, LOC) does not improve the stability of *RET* G4-DNA, even at higher mole ratios. In (B), the melting temperatures of *RET* G4-DNA were measured at different mole ratios of *RET* G4-DNA vs colchicine at H_2O.

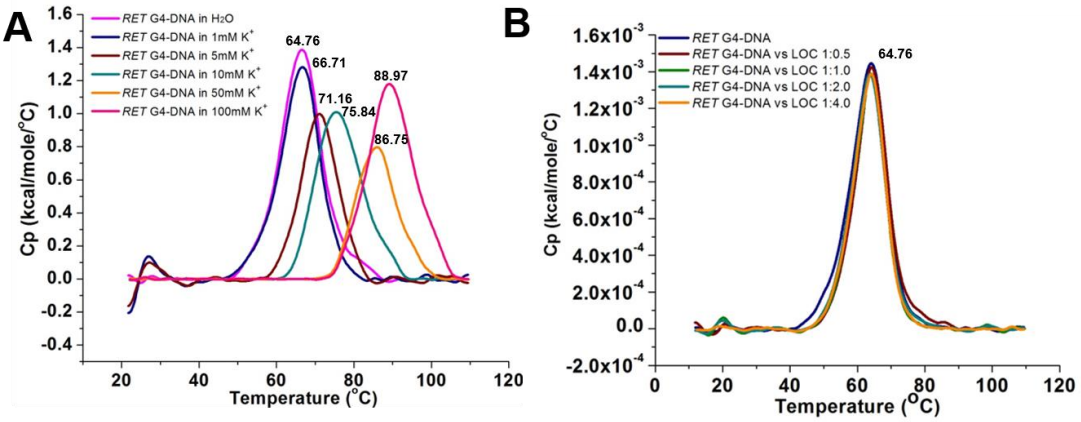

Table S1 Assignment of colchicine in the complex of *RET* G4-DNA with colchicine[ζ]

protons[&]	Reported assignments (free, in D_2O) (ppm) [$]	free colchicine (in this report, 10% D_2O, ppm)	*RET*-colchicine complex (in H2O, ppm, 1:1)	*RET*-colchicine complex (in H2O, ppm, 1:2)	*RET*-colchicine complex (in H2O, ppm, 1:3)	*RET*-colchicine complex (in H2O, ppm, 1:4)	*RET*-colchicine complex (in H2O, ppm, 1:8)
2-CH_3	3.42	3.63	3.47	3.50	3.50	3.51	3.51
4-CH_3	3.74	3.92	3.77	3.80	3.81	3.81	3.82
6-CH_3	3.76	3.88	3.82	3.83	3.84	3.85	3.85
7-H	6.64	6.51	6.71	6.74	6.74	6.74	6.72
9-H	2.40, 2.07	2.51,2.39	2.56, 2.25	2.54,2.32	2.54,2.32	2.51,2.32	2.51,2.29
10-H	1.77, 1.97	2.23,1.81	1.84, 2.16	1.86,2.18	1.86,2.13	1.85,2.13	1.85,2.13
11-H	4.23	4.63	4.26	4.29	4.29	4.28	4.28
11-NH	ND	6.42	8.63	8.64	8.65	8.66	8.66
13-CH_3	1.87	1.98	1.91	1.94	1.94	1.95	1.95
15-H	7.28	7.41	7.30	7.32	7.32	7.32	7.32
18-CH_3	3.79	3.98	3.93	3.93	3.93	3.92	3.90
19-H	7.07	6.82	7.21	7.22	7.20	7.20	7.18
20-H	7.27	7.30	7.31	7.34	7.34	7.35	7.35

[ζ] The chemical shift values presented in Table S1 do not correspond to a 1:1 complex but rather to averages from free and bound ligand.

[&] The protons were named based on the carbon termination in the structure below.

[$] Petty HR, Fernando M, Kindzelskii AL, Zarewych BN, Ksebati MB, Hryhorczuk LM, Mobashery S, Identification of colchicine in placental blood from patients using herbal medicines. *Chem Res Toxicol.* 2001 Sep;14(9):1254-8.

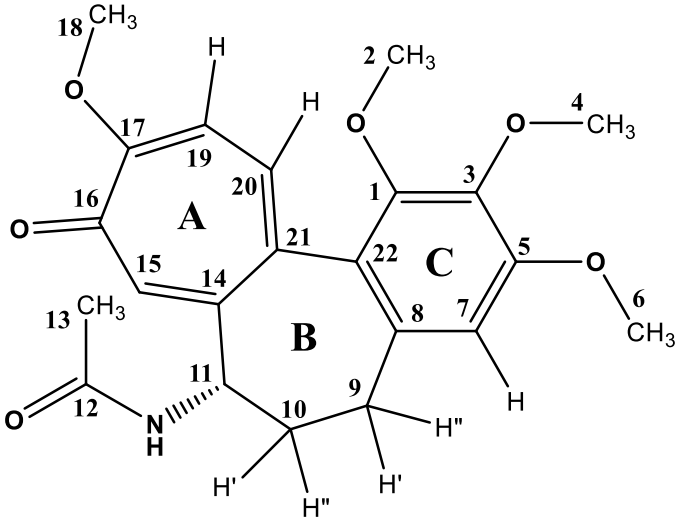

Table S2 Experimental restraints and structural statistics

	RET G4-colchicine[&]
NMR distance and dihedral constraints	
Distance restraints	
Total NOEs	366
Intra-residue	230
Inter-residue	97
Sequential (|i-j|=1)	66
Non-sequential (|i-j|>1)	31
Hydrogen bonds	52
Small molecule (intramolecular)	21
Intermolecular (small molecule/*RET*)	18
Total dihedral angle restraints	20
Structural statistics	
Violations (mean and SD)	
Distance constraints (Å)	0.048 ± 0.0048
Dihedral angle constraints(°)	0.051 ± 0.0061
Deviations from idealized geometry	
Bond lengths (Å)	0.0051 ± 0.00034
Bond angle (°)	1.15 ± 0.035
Impropers (°)	1.10 ± 0.020
Average pairwise r.m.s.d of all heavy atoms (Å)	
All residues	0.45 ± 0.08
All heavy atoms	0.63 ± 0.21

Note

[&] Here, RET G4-colchicine indicates that colchicine binds to RET G4-DNA at a mole ratio of 1:1.