Article

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Synthesis and DNA Cleavage Activity of Piperazine Containing Guanidinoethyl and Hydroxyethyl Side Arms

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According to active groups synergetic catalysis principle, a novel phosphodiester receptor 1-(*N*-guanidinoethyl)-4-(*N*-hydroxyethyl)-piperazidine hydrochloride was firstly synthesized and the preliminary studies of its DNA cleavage activity. It is characterized and confirmed by methods as ^{1}H NMR and ^{13}C NMR. A "couple hardness with softness" piperazidine is designed to connect guanidinium group and hydroxyl group; The reserch was showed that the best cleaving conditions was 7.2 in pH; Cleaving DNA by the compound reaction was oxidation process that were proved through the free radicals quenches experiments; The compound can cleaved the pUC 19 DNA by phospholipid transferance was proved by BDNPP.

Keywords: Piperazidine; Phosphodiester bond; Guanidinium group; Spacer.

INTRODUCTION

Artificial nucleases including some small organic molecules and metal complexes have been widely investigated for their potential applications in the fields of molecular biological technology and drug development.[1-2] However, the pharmic use of metal complexes is hampered by concerns over the lability and toxicity due to free-radical generation during the redox processes of some transition metal,[3-5] such as Zn. Metal-free cleaving reagents which are identified safer for their hydrolytic pathway of cleaving the P-O bond of phosphodiester in nucleic acids and have shown clinical potential have been first put forward by Gobel and coworkers. Such small organic molecules as guanidinium derivatives,[6-8] cyclodextrin derivatives,[9-11] macrocyclic polyamines,[12-14] and dipeptides[15-17] have been used to cleave phosphodiester. Guanidinium receptors as nuclease mimics for cleavage of active phosphodiester, such as bis(*p*-nitrophenyl) phosphate (BNPP) and bis(2,4-dinitrophenyl) phosphate (BDNPP), have been reported by many research workers. DNA clevage activity of artificial receptors 1,4,7-triazacyclononane and tetrahydropyrimidin containing guanidinoethyl and hydroxyethyl side arms have been reported as phosphodiester receptor successfully.[18-19] In the cmpound, the guanidinium group serves to recognize, bind, and electrophilically activate the anionic phosphodiester through hydrogen bonding and electrostatic interaction. The hydroxyl group works as a nucleophilic groupin the transphosphorylation reaction, which is expected to be highly efficient because of the proximity effect.[20-22]

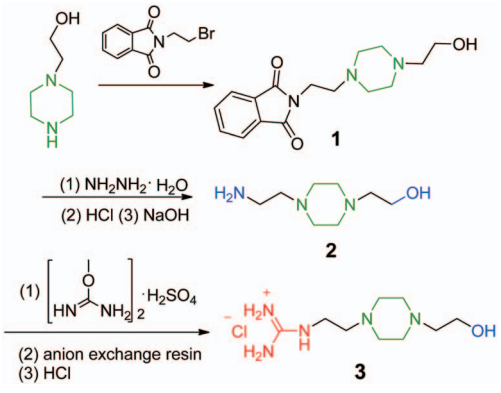
RESULTS AND DISCUSSION

Structural information

^{1}H NMR and ^{13}C NMR Spectra

Although the guanidine group is not a stronger electron with drawing group compared to the phthalimido group according to the ^{13}C NMR spectra and the alpha methyl protons in ^{1}H NMR. It gives a significant difference of 0.8-1.1 ppm between two compounds. The capacity of guanidine groups in the compound **3** to absorb electrons from methyl is more intensive than phthalimide groups in the compound **1**, which facilitates the reduction of the density of electron clouds surrounding the piperazine, abating the shielding that results the movement of electron clouds to the low-field.

Scheme I Synthetic routes of target compound **3**



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The chemical shift of the hydrogen in the same sites is relatively large under the existent of deuterium replaced water solvent and chlorine anions. So, the NMR chemical shift of methyl group of piperazinein compound **3** and compound **1** are downshift to around 3.2-3.5 ppm and 2.4-2.6 ppm due to spatial effect, respectively.

The hydrogen on hydroxyl and N-H of guanidine exhibit no chemical shift due to proton exchange effect when heavy water is used as a solvent for ^{1}H NMR nuclear magnetic resonance. (For NMR and ESI-MS data, see the Supporting Information.)

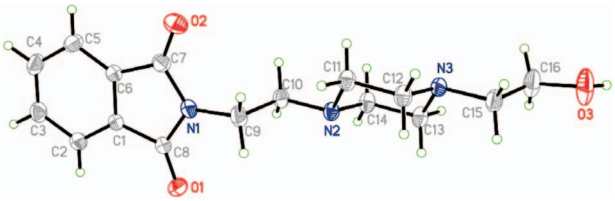


Fig. 1. Structure of the title compound **1**.

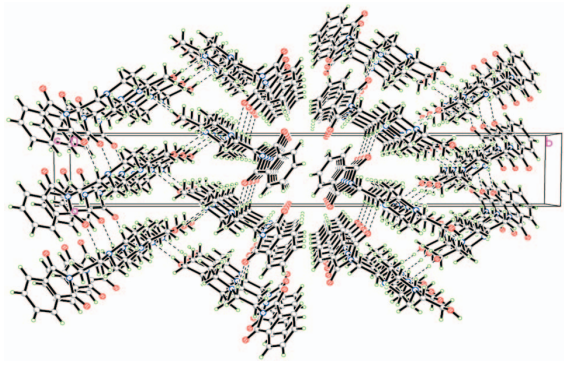


Fig. 2. Packing diagram of the title compound **1** of a unit cell.

In the molecule of the title compound **1**, the piperazine ring adopts a chair conformation, with its N-C bonds in pseudoequatorial orientations the phthalimide fragment is planar, structure shown in Fig. 1. In the crystal, the crystal packing is stabilized by O-H···N hydrogen bonding interactions and π–π interactions involving the benzene ring, see Fig. 2.

Agarose gel of pH dependence

(a) Agarose gel (1%) of pUC 19 DNA (0.05 mmol/L bp) incubated for 15 h at 37 °C with 0.042 mmol/L **4** in different pH buffer: Lanes 1-10, DNA control, pH 6.5, 6.75, 7.0, 7.25, 7.75, 8.0, 8.25, 8.5 and 9.0, respectively; (b) pH dependent profile for DNA cleavage promoted incubated for 24 h by 0.042 mmol/L **4** (■) in different pH buffer (50 mmol/L) at 37 °C.

Fig. 3a is the agarose gel of pH-dependence assays which indicates that the supercoiled DNA (form I) relaxes to form a nicked circular DNA (form II) in the presence of **3** and thelinear DNA (form III) forms when the pH is in the range of 6.5 to 9.0. The pH dependence data of **3** (Fig. 3b) presents a bell shaped profile which indicates that pH 7.2 is the optimal pH for DNA cleavage in the presence of **3**. A relatively low NaCl concentration (10 mM) was selected to control the ionic strength in all experiments.

Free radicals quenches experiments

(a) Histogram representing of pUC19 plasmid DNA (0.05 mM bp) cleaved by **3** (0.042 mM) in the presence of standard radical scavengers incubated for 24 h at 37 °C in pH 7.2 buffer (50 mM Tris–HCl). (b) Agarose gel of radical scavengers inhibition reactions. Lanes 1–6, DNA control, DNA + **3**, DNA + **3** + NaN_3 (10 mM), DNA + **3** + KI (10 mM), DNA + **3** + *t*-BuOH (1 mM), and DNA + **3** + DMSO (1 mM), respectively.

Fig. 4 shows the agarose gel of radical scavengers inhibition reactions which indicate that the oxidative species

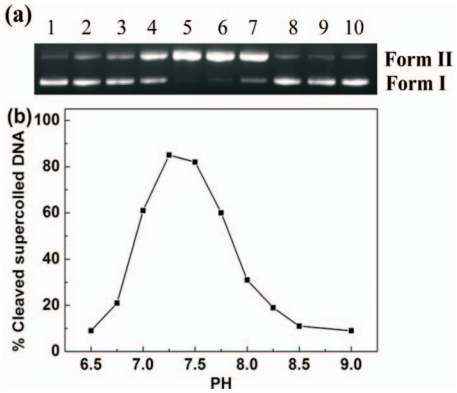


Fig. 3. The pH-dependence profile for DNA cleavage promoted by **4**.

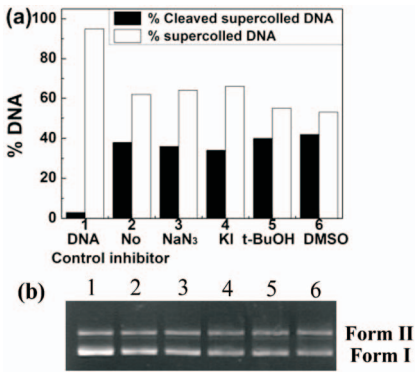


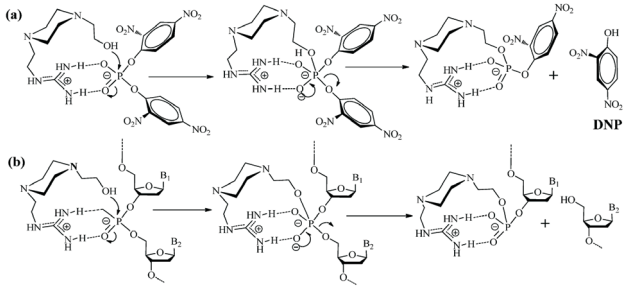
Fig. 4. The radical scavengers inhibition reactions for DNA cleaved by **4**.

effect was eliminated in the system. The reaction should be through a non redox process for DNA cleavage in the presence of **3**.

Reaction mechanism

To study the cleavage mechanism, BDNPP was used as the DNA mimics. BDNPP and **3** were dissolved in DMF/H_2O, and after 0.5 h equilibration time at room temperature, ESI-MS analysis was carried out. In ESI-MS spectrum, the peaks at *m*/*z* 428.9(-) and 182.95(-) show the signals of BDNPP (calcd $[BDNPP-H]^-$, 429.0) and 2,4-dinitrophenol (DNP) (calcd $[DNP-H]^-$, 183.0), respectively. The phosphodiester bond of BDNPP would be cleaved by **3** via a transphosphorylation pathway (Scheme IIa). Thus, similar to BDNPP, transphosphorylation is one of the possible mechanisms for the DNA cleavage promoted by **3**, which is schematically depicted in Scheme IIb.

Scheme II The mechanism for DNA cleavage promoted by **3**



EXPERIMENTAL

Materials and Apparatus. All reagents and chemicals were obtained from commercial sources and used without further purifications. *N*-(2-bromoethyl)phthalimide and *N*-(2-Hydroxyethyl)piperazine were purchased from Aladdin. pUC 19 plasmid DNA was purchased from TaKaRa Biotechnology (Dalian) Co. Ltd., and the purity was checked by agarose gel electrophoresis and their concentration was determined by UV spectroscopy using the extinction coefficient appropriate for double-stranded DNA (1.0 OD_{260} = 50 μg/mL^{-1}). Agarose was from Oxoid Limited of Basingstoke (UK). All solvents were purified by standard procedure.

^1H NMR and ^{13}C NMR data were recorded on a Brucker AM 500 spectrometer (Germany). Mass spectra were obtained on an electrospray mass spectrometer (LCQ, Finnigan) in positive mode. The pH value was confirmed by ORION868 pH meter with an Ag/AgCl electrode as the reference electrode in saturated KCl solution at room temperature. The gelelectrophoresis was conducted by DYY-5 electrophoresis apparatus.

Systhetic procedure and analysis. 1-(*N*-(2-Ethyl)phthalimido)-4-(*N*-hydroxyethyl)-piperazine (**1**): A stirred solution of compounds *N*-(2-hydroxyethyl)piperazine (0.36 g, 2.75 mmol), *N*-(2-bromoethyl)phthalimide (0.63 g, 2.50 mmol) and anhydrous potassium carbonate (0.90 g) in dry CH_3CN (15 mL) was heated at 80-85 °C for 10 h. The mixture was then allowed to cool to room temperature and filtered. Filter cake was washed with CH_3CN (3 × 5 mL) .The filtrate was concentrated under reduced pressure to give a flaxen solid. This solid was dissolved in deionized water (5 mL) and then a small amount of unreacted *N*-(2-bromoethyl)phthalimide was precipitated. The mixture was filtered and the filtrate was collected. After the water was removed under reduced pressure, a pale oil was obtained and purified by silica gel chromatographic column (petroleum ether/methanol, 2/1 then 1/1) to obtain compound **1** as a white needle solid (0.55 g, 1.80 mmol). Yield: 72.4%. m.p.: 133.2-133.7 °C. ^1H NMR (500 MHz/$CDCl_3$): δ 2.50-2.52 (m, 10H, 5 × NC**H**$_2$), 2.64 (t, *J* = 6.5 Hz, 2H, CONCH_2C**H**$_2$N), 3.59 (t, *J* = 5.3 Hz, 2H, C**H**$_2$OH), 3.82 (t, *J* = 6.5 Hz, 2H, CONC**H**$_2$), 7.71-7.73 (m, 2H, 2 × Ar-**H**), 7.84-7.86 (m, *2*H, 2 × Ar-**H**). ^{13}C NMR (125 MHz/$CDCl_3$): δ 35.3 (CONCH_2**C**H_2), 52.8 (N**C**H_2), 53.1 (N**C**H_2), 55.7 (N**C**H_2CH$_2$OH), 59.1 (HO**C**H_2), 123.2 (Ar-**C**), 132.2 (Ar-**C**), 133.86 (Ar-**C**), 168.35 (**C**O). ESI-MS: *m/z* $[M+H]^+$, calcd. 304.17, found 304.05.

1-(*N*-guanidinoethyl)-4-(*N*-hydroxyethyl)piperazine hydrochloride (**3**): Compound **2** (0.80 g, 4.62 mmol) was dissolved in water (5 mL) and then *O*-Methylisourea sulfate (0.54 g, 2.20 mmol) dissolved in sodium bicarbonate solution (10 mL, 5 M) was added. The mixture was stirred for 20 h at room temperature under an atmosphere of dry N_2 and then evaporated to remove the solvent. The crude product was dissolved in deionized water and chromatographed on a strong base anion-exchange resin column (eluted with deionized water). Subsequently, the eluent was evaporated under reduced pressure to remove water, and the residue was washed with ether (3 × 10 mL) to eliminate the unreacted reactant **2** and other organic impurities. The residue was then dissolved in deionized water (15 mL) and neutralized to pH 7.0 with 5% hydrochloric acid. Water was removed in vacuum to give compound **3** as strong hygroscopic brown solid (0.11 g, 0.37 mmol). Yield: 91.4%. ^1H NMR (500 MHz/D_2O): δ 3.27 (t, *J* = 6 Hz, 2H, NC**H**$_2$), 3.35 (t, 2H, *J* = 5 Hz, NHCH$_2$C**H**$_2$), 3.47 (br s, 4H, piperzaine-C**H**$_2$), 3.87 (t, *J* = 5.0 Hz, 2H, NHC**H**$_2$), 3.56 (t, *J* = 6.0 Hz, 2H, C**H**$_2$OH), 3.63 (brs, 4H, 2 × piperzaine-C**H**$_2$). ^{13}C NMR (1250 MHz/D_2O): δ 45.8 (**C**H_2OH), 49.6 (NHCH$_2$**C**H_2), 51.5 (piperzaine-**C**H_2), 55.0 (piperzaine-**C**H_2), 57.5 (N**C**H_2CH$_2$OH), 60.7 (NH**C**H_2), 157.12 (guanidine-**C**). ESI-MS: *m/z* $[M-Cl^-]^+$ calcd. 216.18, found 216.00.

Plasmid DNA cleavage. DNA cleavage experiments were

performed using 500 ng per reaction of pUC 19 derived plasmid of 2686 bp length. The DNA fragments after cleavage assays were separated and monitored by agarose gel electrophoresis. The supercoiled DNA in 50 mM Tris-HCl buffer containing 10 mM NaCl was treated with different compound concentrations followed by dilution with the buffer to a total volume of 15 μL. The sample was incubated at 37 °C. The loading buffer (30 mM EDTA, 0.05% (*w*/V) glycerol, 36% (V/V) bromophenol blue) 3 μL was added to end the reactions and the mixture was loaded on 1% agarose gel containing 1.0 μg/dm^3 EB. Electrophoresis was carried out at 80 V for 1.5 h in 0.5 M Tris-acetate EDTA (TAE) buffer. Bands were visualized by UV light and photographed. The proportion of DNA in the supercoiled, nicked, and linear forms after electrophoresis was estimated quantitatively from the intensities of the bands using TotalLab analysis software.

CONCLUSIONS

In this study, design and synthesis of a novel phosphodiester receptor **3** containing guanidinoethyl and hydroxyethyl side arms was achieved successfully. The reserch was showed that pH 7.2 is the optimal pH for DNA cleavage in the presence of **3**; Cleaving DNA by the compound reaction was oxidation process; The compound can cleave the pUC 19 DNA by phospholipid transferance. This substantial acceleration of cleavage reaction is due not only to the spatial proximity of the nucleophilic hydroxyl group but also the electrophilic activation for the phosphodiester of DNA by the binding guanidinium group.

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