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ORIGINAL PAPER

# 2-Methyl-3-chloro-9-hydroxy-4-oxo-4*H*-pyrido[1,2-a]pyrimidine Hydrochloride: Crystal Structure and Interaction with DNA

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**Abstract** The title compound, $C_9H_8ClN_2O_2^+ \cdot Cl^-$, is a hydrochloric acid salt of 2-methyl-3-chloro-9- hydroxy-4*H*-pyrido[1,2-a]pyrimidine-4-one, which is an important intermediate for the synthesis of biologically active heterocyclic compounds. The synthesized compound was characterized by $^1$H NMR and X-ray crystallography. The structure was solved in monoclinic, space group $P2_1/n$ with $a = 11.8295$ (12), $b = 6.2214$ (6), $c = 13.8133$ (15) Å, $\beta = 97.7860$ (10)°, $V = 1007.23$ (18) Å$^3$, $Z = 4$, and with $R_{int} = 0.077$. The cation of the title compound, as shown by the single-crystal structure determination, has two conjugated aromatic rings that are almost coplanar with a dihedral angle of 0.230°. The crystal packing is stabilized by intermolecular N–H···Cl, O–H···Cl hydrogen bonds, which link the molecules into centrosymmetric dimers, and by weak π–π stacking interactions (average distance 3.352 Å). The interaction of native calf thymus DNA (ctDNA) with the compound at physiological pH was monitored by UV–Vis spectroscopy and viscosimetric techniques. It was found that the compound might interact with ctDNA by a groove mode of binding via hydrogen bonds.

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**Keywords** Pyrido[1,2-a]pyrimidine · Crystal structure · DNA · Interaction

## Introduction

Aza-bridgehead fused heterocyclic compounds are attractive and such compounds have always been the main focus of interest in the search for new pharmaceutical candidates. Many pyrimidine and pyridopyrimidine derivatives show interesting pharmaceutical properties, such as: antiviral, antibacterial, anti-HIV, antiallergic, and antitumoral activities. For example, pyrimidine ring containing sulfonamides constitute one of the oldest groups of antibacterial agents. Bicyclic pyrimidines and their derivatives, especially those having substituents on the pyrimidine ring, are common drug intermediates and possess significant biological activities [1–9].

DNA is a major target for drugs and some toxic chemicals. Small molecules normally interact with DNA via noncovalent interaction modes, for instance, (i) intercalating between stacked base pairs, (ii) noncovalent groove binding, or (iii) electrostatic interaction with the negatively charged nucleic acid sugar–phosphate structure [10]. Research into the binding mechanism of some small molecules with DNA has been regarded as one of the key topics during the past few decades [11–14]. It is of interest to understand the action mechanism of some antitumor and antivirus drugs in order to design new and more efficient DNA targeted drugs to deal with genetic diseases.

Previously, we performed X-ray diffraction and spectroscopic studies of the pyrido[1,2-a] pyrimidine-4-one analogue [15]. Herein, we report the synthesis and crystal structural of the 2-methyl-3-chloro-9-hydroxy-4*H*-pyrido[1,2-a]pyrimidine-4-one hydrochloride (MCHPP). The

interaction of MCHPP with the native calf thymus DNA (ctDNA) using in vitro conditions was also investigated. We believe that this work will provide some useful information for the evaluation of the bioactivity of 2-methyl-3-chloro-9-hydroxy-4*H*-pyrido[1,2-a]pyrimidine-4-one.

## Experimental

### Chemicals and Reagents

The ctDNA was purchased from Sigma. The stock solution of ctDNA was prepared by dissolving ctDNA in 0.01 M of Tris buffer at pH = 7.2 (0.01 M of tris(hydroxymethyl)aminomethane [Tris] with NaCl concentration at 0.01 M)) and dialyzed exhaustively against the same buffer for 24 h. The ctDNA solution gave a UV 260/280 nm absorbance ratio of >1.8, indicating that DNA was sufficiently free of protein. The ctDNA concentration of the stock solution was determined by UV spectrophotometry in properly diluted samples, using a molar absorption coefficient ($\varepsilon$ = 6,600 $M^{-1}$ $cm^{-1}$) at 260 nm. The stock solution was stored at 4 °C and was used within 5 days. An individual stock solution for each compound containing $5 \times 10^{-5}$ mol $L^{-1}$ MCHPP, was prepared by dissolving an appropriate amount of the individual MCHPP in Tris buffer. A fresh working solution was prepared daily by diluting the stock solution with Tris buffer and used for different studies. Other used chemicals were of analytical reagent grade and were used as received. 2-Methyl-9-hydroxyl-pyrido[1,2-a]pyrimidine-4-one was synthesized as previously described [16].

### Apparatus and Methods

IR spectra were recorded in the range 400–4,000 $cm^{-1}$ on Perkin-Elmer Spectrum One FT-IR spectrometer using a KBr pellet. Elemental analyses (C, H and N) were performed on a Perkin Elmer 2400II CHN elemental analyzer. $^1$H NMR spectra were measured on a Bruker ARX300 spectrometer with DMSO as solvent. The UV–Vis spectra for MCHPP interactions with DNA were obtained using a Unico (UV 2100) spectrophotometer. Solutions of ctDNA and MCHPP were scanned using a 0.5-cm (1 mL) quartz cell. The spectra were recorded by progressive addition of pure MCHPP to the ctDNA solution. A SCHOT AVS450 viscometer in a constant water bath, set at 25 °C, was used to measure viscosity. Flow time was measured with a digital stopwatch and the mean values of three replicated measurements were used to evaluate the viscosity ($\eta$) of the samples. The data were reported as $(\eta/\eta_0)^{1/3}$ versus the [MCHPP]/[ctDNA] ratio in Tris buffers, where $\eta_0$ is the viscosity of the ctDNA solution alone.

### *Synthesis of 2-methyl-3-chloro-9-hydroxy-4H-pyrido[1,2-a]pyrimidine-4-one (**1**)*

A suspension of 0.88 g (0.005 mol) of the 2-methyl-9-hydroxy-4-pyrido[1,2-a]pyrimidine-4-one in 20 mL of 68 % hydrochloric acid was heated in a steam bath until complete dissolved, 6 mL of 20 % $H_2O_2$ was added, and the heating was continued for 1 h. After cooling, the precipitate was filtered off and recrystallized from a 7:1 ethanol–water mixture, 0.5 g of the title compound was obtained for a yield of 48 %. $^1$H NMR (DMSO-$d_6$, *ppm*): 14.46 (1H, phenolic), 8.43 (1H, *para* to nitrogen of pyridine), 7.20–6.06 (2H, aromatic), 1.79 (3H, methyl). Element analysis calcd (%) for $C_9H_7ClN_2O_2$: C 51.31, H 3.34, N 13.31; Found: C 61.13, H 3.59, N 13.72. IR (KBr, $cm^{-1}$):3530 (–OH stretching); 3057 (aromatic); 2984 (C–H stretching of methyl); 1735 (C=O stretching); 1605 (C=N stretching); 1510, 1471 (aromatic); 1278 (O–H deformation); 783 (C–Cl). MS (m/e): 209.6 [M+].

### *Synthesis of 2-methyl-3-chloro-9-hydroxy-4-pyrido[1,2-a]pyrimidin-4-one Hydrochloride (**2**)*

A weighed sample ($2 \times 10^{-3}$ mol) of compound **1** was dissolved in ethanol (10 mL), and then added a quantitative $ZnCl_2$. The pH of the solution was brought to 2 using HCl with a concentration of 1.0 mol/L. After 2 h stirring at room temperature, the solution was filtrated and then put in a peaceful environment. Several days later, the title salt, 2-methyl-3-chloro-9-hydroxy-4-pyrido[1,2-a]- pyrimidin-4-one hydrochloride (MCHPP), needle-shaped crystals precipitated. The isolated crystals were filtered off, washed with ethanol, and dried under vacuum.

### Crystallographic Data Collection and Refinement

Intensity data for the MCHPP hydrochloride was collected at 298 (2) K on a Siemens SMART CCD diffractometer with graphite monochromatic Mo $K_a$ radiation ($\lambda$ = 0.7107 Å). Data reductions and absorption corrections were performed with the SAINT [17] and SADABS software packages [18], respectively. The structures were solved by direct methods using SHELXS-97 [19, 20] and were refined by full matrix least-squares methods using SHELXL-97 [21]. Anisotropic displacement parameters were refined for all non-hydrogen atoms. All C-bound H-atoms were placed in idealized locations and were refined using a riding model, with C–H = 0.93 Å and $U_{iso}(H) = 1.2U_{eq}(C)$. The H-atoms attached to nitrogen were located in difference Fourier map and refined with the restraints N–H = 0.86 Å and

**Table 1** Crystal data and structure refinement information for MCHPP

| | |
|---|---|
| Molecular formula | $[C_9H_8ClN_2O_2]^+ \cdot Cl^-$ |
| Formula weight | 247.07 |
| T (K) | 298 (2) K |
| $\lambda$ (Å) | 0.71073 |
| Crystal system | Monoclinic |
| Space group | $P2_1/n$ |
| $a$ (Å) | 11.8295 (12) |
| $b$ (Å) | 6.2214 (6) |
| $c$ (Å) | 13.8133 (15) |
| $\beta$ (°) | 97.7860 (10) |
| $V$ (Å$^3$) | 1,007.23 (18) |
| $Z$ | 4 |
| $\mu$ (mm$^{-1}$) | 0.623 |
| $F$(0 0 0) | 504 |
| Total reflections | 4,647 |
| Unique reflections | 1,770 |
| Goodness-of-fit on $F^2$ | 1.064 |
| $R_{int}$ | 0.077 |
| $R_1$ [$I \geq 2\sigma(I)$] | 0.0458 |
| $wR_2$ (all data) | 0.1535 |
| Max/min electron density (e Å$^{-3}$) | 0.515/−0.551 |

$U_{iso} = 1.2U_{eq}(N)$. The crystallographic details and selected bond lengths (Å) and torsion angles (°) are provided in Tables 1 and 2. Images were created with the Diamond program [22].

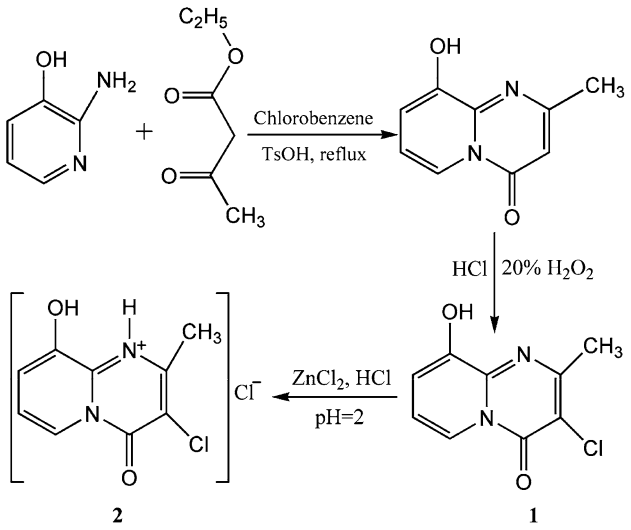
## Results and Discussion

### Syntheses of MCHPP and its Hydrochloride

The MCHPP was prepared according to Scheme 1. At the first step of the synthesis, 2-methyl-9- hydroxy-4*H*-pyrido[1,2-a]pyrimidine-4-one reacted with HCl in presence of the $H_2O_2$ under heated to provide the 2-methyl-3-chloro-

**Table 2** Selected bond lengths (Å) and torsion angles (°) for MCHPP

| Bond lengths | | | |
|---|---|---|---|
| Cl2–C2 | 1.725 (3) | N2–C8 | 1.383 (4) |
| N1–C4 | 1.352 (4) | N2–C3 | 1.469 (4) |
| N1–C1 | 1.358 (4) | O1–C3 | 1.211 (4) |
| N2–C4 | 1.353 (4) | O2–C5 | 1.339 (4) |
| Torsion angles | | | |
| C3–N2–C4–C5 | −179.5 (3) | C1–C2–C3–O1 | −176.1(4) |
| C8–N2–C4–N1 | −180.0 (3) | C9–C1–C2–Cl2 | −2.5 (5) |
| C3–N2–C4–N1 | −0.7 (5) | N2–C4–C5–O2 | 179.2 (3) |




**Scheme 1** The synthetic route of the compound (**1**) and its hydrochloride salt (**2**)

9-hydroxy-4*H*-pyrido[1,2-a]pyrimidine-4-one. The structure of the title compound was confirmed by mass spectrum, elemental analyses, and $^1$H NMR. The salt of the compound (**1**) was obtained by reacting with HCl in presence of the $ZnCl_2$. The structure of the salt was determined by single-crystal X-ray diffraction.

### Crystal Structure of MCHPP

Single-crystal X-ray analysis reveals that the title salt crystallizes in the monoclinic space group $P2_1/n$. The asymmetric unit of the crystal is illustrated in Fig. 1.

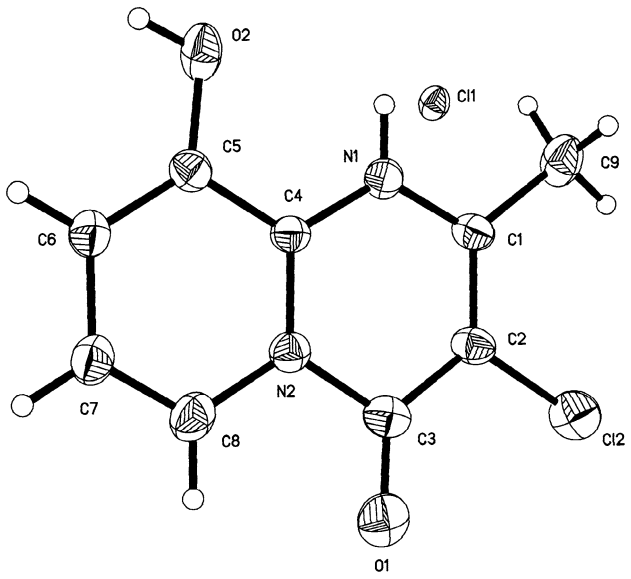



**Fig. 1** View of the molecule of MCHPP, showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 30 % probability level. H atoms are represented by *circles* of arbitrary size

Excepting methyl hydrogens, the cation of the salt is almost co-planar as evidenced by the two conjugated rings with a dihedral angle of only 0.230° and the C8–N2–C4–N1, Cl2–C2–C3–O1, and C9–C1–C2–C3 torsion angles of −180.0 (3), 3.5 (5), and 177.1 (4)°, respectively. The Cl2–C2 bond distance is 1.725(3) Å, which is similar to the found in the crystal structure of chlorophene analogue, i.e. 1.745(5) Å [23]. The C–N bond lengths of the pyrido[1,2-a]pyrimidine heterocyclic moiety are in the range of 1.352 (4)–1.469 (4) Å. The N2–C3 bond is longer than the remaining four C–N bonds within pyrido[1,2-a]pyrimidine heterocyclic moiety (Table 2). The elongation of the N2–C3 bond is presumed by the electron withdraw effect of the chlorine substituent that disturbs the delocalisation of the π electron of the double bond O1–C3 over these three atoms (O1, C3 and N2). The O1–C3 bond with a distance of 1.211 (4) Å is shorter than the O2–C5 bond (1.339 (4) Å), which is due to the double bond in O1–C3.

There are significant intermolecular hydrogen bond interactions in the crystal of title compound involving O–H···Cl (O2–Cl1 = 2.9635 Å) uncharged and anionic chlorine interactions and N–H···Cl (N1–Cl1 = 3.2055 Å) charge-assisted ones, which makes a discrete dimer (Fig. 2). The chloride anion is able to act as a proton acceptor, interacting with the partially charged -NH group. The results presented herein can form the basis for predicting hydrogen bonds between protonated multi-hetero-rings and chlorine anions. The dimers are extended into a ribbon via the face-to-face stacking interactions between conjugated rings of pyrido[1,2-a]pyrimidine heterocyclic moiety (average distance 3.352 Å) (Fig. 3). Furthermore, two neighbouring ribbons interact via the weak C3–O1···π interactions into two-dimension sheets. All hydrogen bond parameters are listed in Table 3.

## Study of the MCHPP Interaction with DNA

### Effect of the MCHPP on UV Spectra of ctDNA

The absorption band at around 260 nm of DNA arises because of the π–π* transition of DNA bases. Changes in absorbance and wavelength shifts of this characteristic band reflect the corresponding structural changes of the DNA, including alteration of stacking pattern, loss of the hydrogen bonds between complementary strands, covalent binding of DNA bases, intercalation between aromatic rings of molecules, etc. [24, 25]. Previous investigations have shown that hypochromism results from the contraction of DNA in the helix axis and from the changes in the conformation of DNA, whereas hyperchromism results from the disassociation, or melting, of the DNA double helix strands from each other [26]. The results obtained in Fig. 4 showed that with increasing amounts of MCHPP, a slight bathochromic shift (∼3 nm) of the band of DNA centered at the 263 nm was observed, with a significant

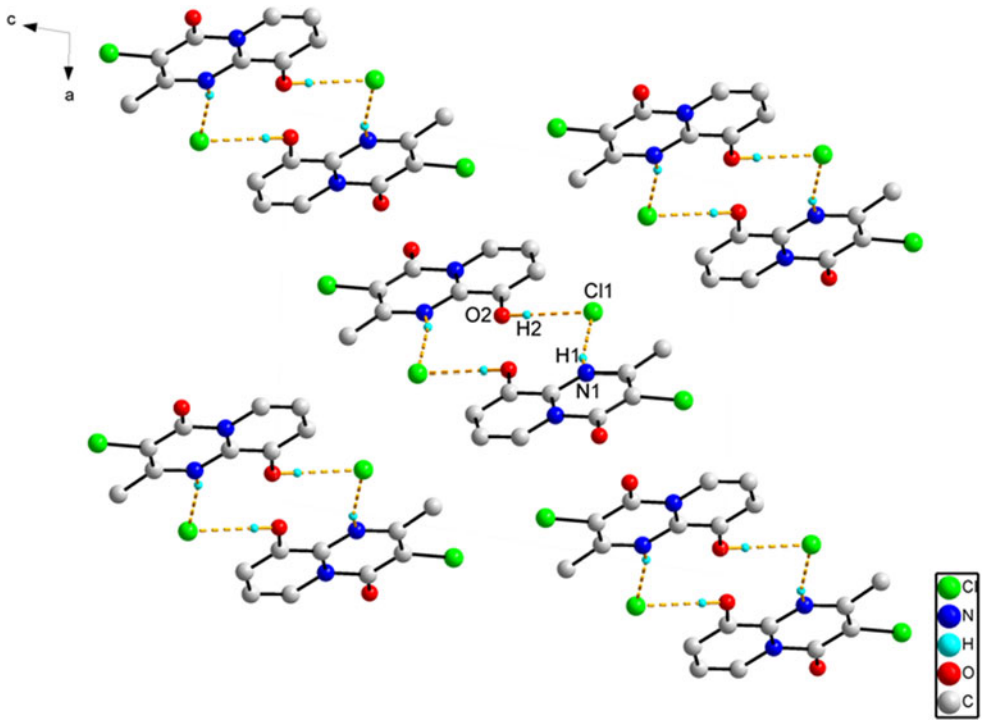



**Fig. 2** A view of the 1D ribbon in the compound along b-axis. All do not take part in H-bond (*dashed lines*) H atoms are omitted for clarity (Color figure online)

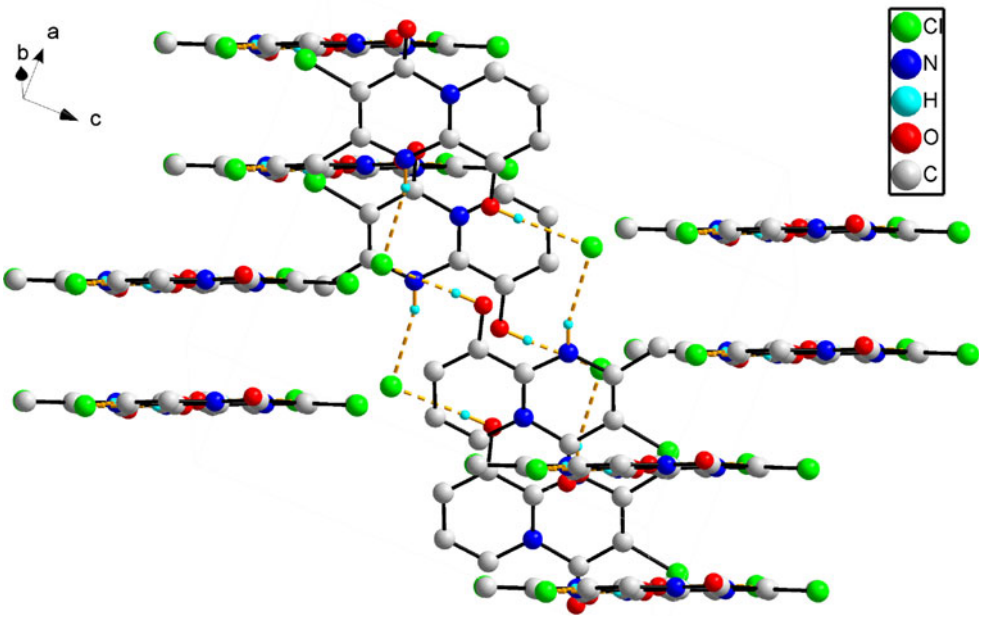



**Fig. 3** The π–π stacking interactions between the pyrido[1,2-a]pyrimidine rings of neighboring cations of the salt (Color figure online)

**Table 3** Hydrogen bonds for MCHPP [Å and °]

| D–H···A | *d*(D–H) | *d*(H···A) | *d*(D···A) | <DHA |
|---|---|---|---|---|
| N1-H1···Cl1[a] | 0.86 | 2.37 | 3.2055 | 164 |
| O2-H2···Cl1[b] | 0.82 | 2.14 | 2.9635 | 177 |

[a] x + 1/2, −y + 1/2, z−1/2

[b] −x + 3/2, y + 1/2, −z + 1/2

hypochromic effect. This indicated that the conformation of DNA double-helix structure was changed upon MCHPP addition, which suggests that there must be some interactions between MCHPP and the DNA. As only a slight bathochromic shift was observed in UV absorption band of DNA, the conformation changes of DNA structure is not via traditional intercalating mode but via a groove binding interaction with MCHPP [27].

## Viscosity Measurements

Optical and photophysical probes offer necessary, but not sufficient, clues to support a groove binding model. To further clarify the interaction between the MCHPP and ctDNA, viscosity measurements were carried out. Viscosity is a hydrodynamic measurements that is sensitive to length change and is considered the least ambiguous and the most important test for a binding model in the absence of crystallographic data [28, 29]. In classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to increased DNA

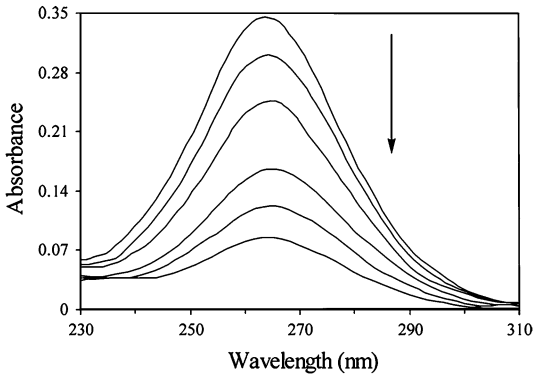



**Fig. 4** UV/Vis spectra of DNA ($5.0 \times 10^{-5}$ M) with increasing amounts of the MCHPP in Tris buffers (pH = 7.4) with $R_i$ = [MCHPP]/[ctDNA] 0.0, 0.2, 0.4, 0.6, 0.8, 1.0. The *arrow* shows the direction of the absorbance changes upon increasing MCHPP concentrations

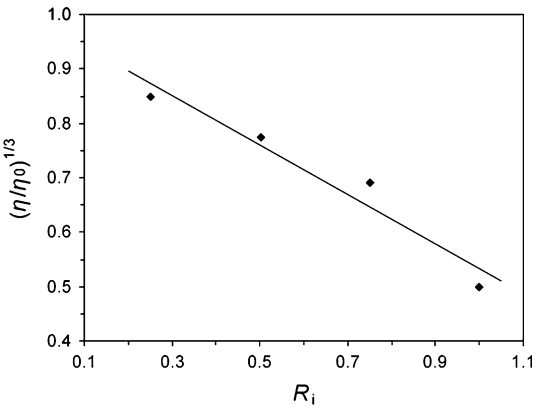



**Fig. 5** Effect of the compound on the viscosity of ctDNA ($5 \times 10^{-5}$ M) with different $R_i$ in Tris–HCl buffers (pH = 7.4) ($R_i$ = [compound]/[ctDNA] = 0.0, 0.25, 0.5, 0.75 and 1.0)

viscosity. In a partial nonclassical ligand intercalation or in groove binding the DNA helix will bend which will decrease its efficient length and thereby its viscosity. The effects of MCHPP on the viscosity of ctDNA are shown in Fig. 5. The specific viscosity of the DNA sample clearly decreases with the addition of the MCHPP. The relative specific viscosity of DNA exhibited a dependence on the concentration of MCHPP, which decreased with the value of [MCHPP]/[DNA]. The viscosity data suggests that the MCHPP interaction with the DNA leads to a contraction of the DNA helix and supports a groove mode of binding interaction between the MCHPP and the ctDNA.

## Conclusions

A distinct compound, MCHPP, has been prepared and characterized. We have performed a prime study on the title compound structure and its interaction with ctDNA. The interaction of the native ctDNA with the title compound in vitro was monitored by spectroscopic methods. The results from the fluorescence and viscosity studies suggested that title compound interacts with ctDNA through a groove mode of binding.

Despite the present results indicated that MCHPP might interact with native DNA, presumably in a groove mode, intensive investigation and mechanism studies need to be carried out for a deeper understanding.

## Supplementary Material

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallo- graphic Data center, CCDC No. 875504. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2, IEZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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