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Synthesis and anti-leukemia activity mensuration of 1-phenethyl-4-hydroxy-4-substituted piperidinium hydrochlorides: Structure of bis[1-phenethyl-4-hydroxy-4-(3-fluorophenyl) piperidinium hydrochloride] studied by X-ray and DFT methods

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ABSTRACT

Four unknown compounds have been synthesized based on the molecular motif of 1-phenethyl-4-hydroxy piperidinium hydrochloride with a variety of the substituted groups R on the same carbon atom bearing the hydroxy group, such as 3-fluorophenyl (**3a**), 4-methoxyphenyl (**3b**), 4-methylphenyl (**3c**) and cyclohexanyl (**3d**), and their molecular structures were characterized by ^{1}H NMR, MS and IR. To account for the stereo structure and provide more information on the effect of counter ions, hydrogen bonds on molecular conformation, the structure of [1-phenethyl-4-hydroxy-4-(3-fluorophenyl) piperidinium hydrochloride] [PHFPH·Cl] **3a** was determined by the single-crystal X-ray analysis and optimized by the B3LYP/6-31G (d, p) calculations. Two chloride anions, two PHFPH cations and a dichloromethane molecule formed a five-membered structure ($[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$) via the intermolecular hydrogen bonds. The core of the five-membered structure ($[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$) is formed by two PHFPH cations linked by N(1B)–H(2)···Cl(2) hydrogen bonds of lengths 3.033(4), which is engaged in two hydrogen bonds: N(1A)–H(1)···Cl(1) of 3.110(4) Å and O(1B)–H(2B)···Cl(1) of 3.099(4) Å. The anti-tumor activity tests indicated that these compounds could inhibit the growth of the K562 cells to some extent and have the potential bioactivity of anti-leukemia.

1. Introduction

Piperidine derivatives have important research value in the medical fields. They are used in the synthesis of analgesic, anti-psychotic, anti-tumor drugs and also as important intermediates in the organic synthesis [1]. Recently, we have found in our laboratory that piperidine derivatives have high inhibition to leukemia, as a part of a continuing effort to develop novel piperidine derivatives with potential anti-leukemia activity; we are currently involved in studies in which the 1-phenethyl-4-hydroxy piperidine system is being used as a conformationally restricted framework. In the framework system, we synthesized four new target compounds (**3a–3d**), which were characterized by ^{1}H NMR, MS and IR. The preliminary anti-tumor activity tests indicated that the investigated compounds inhibitory activities against K562 cells were higher than those of the anti-tumor drug of clinical practice 5-Fu (5-fluorouracil) in the same concentration of 100 μg/mL.

In order to confirm the structure and the steric configuration of the compounds, in this paper a crystal structure of 1-phenethyl-4-hydroxy-4-(3-fluorophenyl) piperidinium hydrochloride [PHFPH·Cl] is analyzed by X-ray diffraction. The crystal belongs to the monoclinic system space group $P2(1)/n$ with $a = 11.320(6)$, $b = 25.870(6)$, $c = 14.204(2)$ Å, $\beta = 104.447(19)$, $V = 4028(2)$ Å^3, $Z = 4$. The crystal structure suggests that there are two interesting contacts determining the conformation of the compound and also the packing of the molecule in the crystal cell. The piperidine ring adopts a chair conformation with the 3-fluorophenyl substituent in the equatorial and the OH group in the axial positions [2]. To get detailed information on the nature of **3a**, three isolated molecular conformers (**4a–6a**) were optimized by the B3LYP/6-31G (d, p) level of theory.

2. Experimental

2.1. Materials and physical measurements

All the starting chemical reagents and solvents were commercially available and used as received without further purification, except for tetrahydrofuran and toluene, which were dried by refluxing in the presence of sodium and were distilled prior to use. ^{1}H NMR spectrums (DMSO-d_6) were recorded on a Bruker AVANCE-

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400 MHz with TMS as an internal standard. The IR spectra were obtained from KBr discs in the range 4000–400 cm^{-1} on a Nicolet 5DXFT-IR spectrophotometer. Elemental analyses were performed with a Perkin-Elmer 2400 instrument and melting points were determined by an RK1 microscopic melting apparatus. The mass spectra were measured on a TRACE MS 2000 mass spectrometer.

2.2. Synthesis of 1-phenethyl-4-hydroxy-4-substituted piperidinium hydrochlorides, *3a–3d*

The synthesis of the title compounds **3a–3d** are presented in Scheme 1. Phenethylamine (**1**) was converted to the intermediate of 4-(2-phenethyl)-piperidone (**2**) according to the procedure given in the literature [3,4].

A solution of 2.732 g (0.016 mol) 3-bromofluorobene in 8 mL dry tetrahydrofuran was added dropwise, with stirring, to a stirred solution of 0.384 g magnesium and a little of iodine in 5 mL dry tetrahydrofuran, which was protected by N_2, with a speed of 3 mL/min under 60 °C. The mixture was heated under refluxing for 2 h, and added a solution of 1-phenethyl-4-piperidone (**2**) 4.06 g (0.02 mol) in dry tetrahydrofuran (10 mL) over a period of 20 min at 65 °C after the solid of Mg disappeared. It was cooled to 0–5 °C and 15 mL water was slowly added, concentrated hydrochloric acid was added dropwise with stirring to pH = 4. The mixture was extracted into CH_2Cl_2 (40 mL) and washed with concentrated salt solution (15 mL × 3). After drying with $MgSO_4$ (5 g) and removal of CH_2Cl_2, the residue was crystallized from acetone (30 mL) to get a white solid 4.187 g of the title compound **3a**.

The syntheses of **3b–3d** were carried out by the similar method. The analytical data for all the compounds **3a–3d** are summarized as follow:

3a: Yield 64.3%; m.p. 192–194 °C. $C_{19}H_{23}ClFNO$(335.8). Anal. Calc. for **3a**: C 67.90, H 6.87, N 4.17%; Found: C 67.93, H 6.84, N 4.19%. IR (KBr, cm^{-1}) *v*: 3318.37 (O–H,stretching), 2921.2 (C–H, stretching), 2586.1 (N^+–H, stretching), 1429.14 cm^{-1} (Ar, stretching). ^1H NMR (400 MHz in DMSO-d_6,) δ: 1.81–1.84 (m, 2H, CH_2, piperidine), 2.37–2.43 (m, 2H, CH_2, piperidine), 3.15–3.23 (m, 4H, N–CH_2, piperidine), 3.18–3.33 (m, 2H, ph–CH_2), 3.26–3.52 (m, 2H, N–CH_2), 5.64 (s, 1H, OH), 7.09–7.46 (m, 10H, Ar–H), 10.67 (s, 1H, N^+–H). MS *m/z* (%): 299.3 ([M–HCl]$^+$,5.37), 207.5 (100), 90.9 (30.67), 55.9 (54.01), 42.0 (84.72).

3b: Yield 60.1%; m.p. 190–193 °C. $C_{20}H_{26}ClNO_2$(347.9). Anal. Calc. for **3b**: C 68.99, H 7.47, N 4.02%; Found: C 69.02, H 7.48, N 4.04%. IR (KBr, cm^{-1}) *v*: 3317.39 (O–H,stretching), 2929.40 (C–H, stretching), 2561.33 (N^+–H, stretching), 1512.34 cm^{-1} (Ar, stretching). ^1H NMR (400 MHz in DMSO-d_6,) δ: 1.80–1.83 (m, 2H, CH_2, piperidine), 2.35–2.39 (m, 2H, CH_2, piperidine), 3.14–3.21 (m, 4H, N–CH_2, piperidine), 3.19–3.35 (m, 2H, ph–CH_2), 3.24–3.47 (m, 2H, N–CH_2), 3.73 (s, 3H, OCH_3) 5.33 (s, 1H, OH), 6.91–7.53 (m, 10H, Ar–H), 10.68 (s, 1H, N^+–H). MS *m/z* (%): 311.4 ([M–HCl]$^+$, 10.52), 220.3 (100), 90.7 (35.53), 55.7 (52.30), 41.8 (83.23).

3c: Yield 62.4%; m.p. 228–230 °C. $C_{20}H_{26}ClNO$(331.9): Anal. Calc. for **3c**: C 72.31, H 7.83, N 4.22%; Found: C 72.33, H 7.86, N 4.25%. IR (KBr, cm^{-1}) *v*: 3321.68 (O–H,stretching), 2921.23 (C–H, stretching), 2573.89 (N^+–H, stretching), 1489.3 cm^{-1} (Ar, stretching). ^1H NMR (400 MHz in DMSO-d_6,) δ: 1.78–1.81 (m, 2H, CH_2, piperidine), 2.26 (s, 3H, CH_3), 2.32–2.37 (m, 2H, CH_2, piperidine), 3.17–3.20 (m, 4H, N–CH_2, piperidine), 3.14–3.38 (m, 2H, ph–CH_2), 3.20–3.42 (m, 2H, N–CH_2), 5.41 (s, 1H, OH), 7.16–7.37 (m, 10H, Ar–H), 10.71 (s, 1H, N^+–H). MS *m/z* (%): 295.4 ([M–HCl]$^+$,9.41), 204.2 (100), 90.8 (31.42), 55.8 (53.32), 42.0 (82.65).

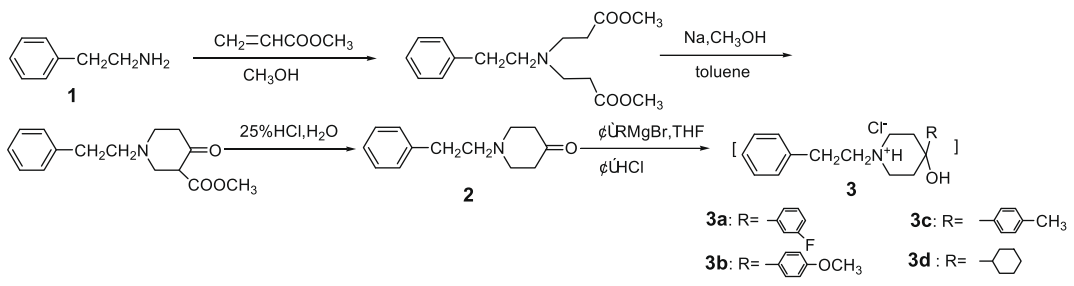
3d: Yield 51.3%; m.p. 203–205 °C. $C_{19}H_{30}ClNO$(323.8). Anal. Calc. for **3d**: C 70.41, H 9.26, N 4.32%; Found: C 70.44, H 9.27, N 4.35%. IR (KBr, cm^{-1}) *v*: 3348.72 (O–H, stretching), 2926.38(C–H, stretching), 2569.32(N^+–H, stretching), 1461 cm^{-1} (Ar, stretching). ^1H NMR (400 MHz in DMSO-d_6,) δ: 0.91–1.81 (m, 11H, 5CH_2, CH, cyclohexanyl), 1.86–1.90 (m, 2H, CH_2 piperidine), 2.46–2.57 (m, 2H, CH_2, piperidine), 3.02–3.37 (m, 4H, N–CH_2, piperidine), 3.21–3.36(m, 2H, ph–CH_2), 3.23–3.42 (m, 2H, N–CH_2), 5.31 (s, 1H, OH), 7.38–7.47 (m, 5H, Ar–H), 10.42 (s, 1H, N^+–H). MS *m/z* (%): 287.3 ([M–HCl]$^+$, 5.04), 196.3 (12.32), 91.1 (100), 60.1 (57.42), 42.2 (85.31).

2.3. X-ray crystallography

The colorless crystal of the title compound **3a** (grown from a mixed solution of petroleum dichloromethane) having approximate dimensions of 0.30 × 0.10 × 0.10 mm was mounted on a glass fiber in a random orientation. The data were collected by a Bruker Smart Apex CCD diffractometer with a graphite-monochromated Mo K_α radiation (λ = 0.71073 Å) by using a φ–ω scan mode in the range of $1.57 \leqslant \theta \leqslant 25.00°$ at 293(2) K. Empirical absorption correction was applied. A total of 16,531 reflections including 7065 unique ones (R_{int} = 0.0622) were measured. The structure was solved by direct methods and refined by full-matrix least-squares techniques on F^2 using the SHELXTL program package [5] on a legend Pentium (IV) computer. All the non-hydrogen atoms were refined anisotropically, and hydrogen atoms were located at their idealized positions. The final $R = 0.0687$, $wR = 0.1452$ ($w = 1/[\sigma^2(F_o^2) + (0.0284P)^2 + 3.5000P]$, where $P = (F_o^2 + 2F_c^2)/3$), $S = 1.096$, $(\Delta/\sigma)_{max} = 0.020$, $(\Delta\rho)_{max} = 0.447$ and $(\Delta\rho)_{min} = -0.495$ e/Å^3. The structural plots were drawn with SHELXTL-97 software package. Details of the data collection and refinement parameters are given in Table 1. Atomic coordinates and equivalent displacement parameters are list in Table 2. Other details of the structure have been deposited with the Cambridge Crystallographic Data Centre No. CCDC713410.

2.4. B3LYP calculation

The B3LYP [6,7] calculations with a 6-31G (d, p) [8] basis set were performed using the GAUSSION-O3 package [9].



Scheme 1. Structure and synthesis of 1-phenethyl-4-hydroxy-4-substituted piperidinium hydrochlorides.

Table 1
Crystal data and structure refinement for the 2:2:1 complex of 1-phenethyl-4-hydroxy-4-(3-fluorphenyl) piperidinium with hydrochloride and dichloromethane, $[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$.

Empirical formula	$C_{39}H_{48}Cl_4F_2N_2O_2$	$F(000)$	1592
Formula weight	756.59	Crystal size	$0.30 \times 0.10 \times 0.10\ mm^3$
Temperature	293(2) K	θ range for data collection	1.57 to 25.00°
Wavelength	0.71073 Å	Index ranges	$-13 \leqslant h \leqslant 13$, $-30 \leqslant k \leqslant 23$, $-16 \leqslant l \leqslant 16$
Crystal system	Monoclinic	Reflections collected	16531
Space group	$P2\ (1)/n$	Independent reflections	7065 [$R_{(int)} = 0.0622$]
Unit cell dimensions		Completeness to $\theta = 25.99°$	99.7%
a	11.320(6) Å	Absorption correction	Multi-scan
b	25.870(6) Å	Max. and min. transmission	0.9056 and 0.9670
c	14.204(2) Å	Refinement method	Full-matrix least-squares on F^2
α	90°	Data/restraints/parameters	7065/41/457
β	104.447(19)°	Goodness-of-fit on F^2	1.029
γ	90°	Final R indices [$I > 2$sigma(I)]	$R1 = 0.1152$, $wR2 = 0.1576$
Volume	4028(2) $Å^3$	R indices (all data)	$R1 = 0.0687$, $wR2 = 0.1452$
Z	4	Largest diff peak and hole	0.447 and −0.495 $eÅ^{-3}$
Density (calculated)	1.248 Mg/m^3		
Absorption coefficient	0.337 mm^{-1}		

Table 2
Atomic coordinates and equivalent isotropic displacements for the complex of $[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$.

Atom	x	y	z	U(eq)	Atom	x	y	z	U(eq)
F(1A)	−0.1071(3)	0.28347(14)	0.6027(3)	0.1471(14)	F(1B)	−0.1125(4)	0.27162(16)	0.1316(4)	0.192(2)
N(1A)	0.1814(3)	0.06498(12)	0.9650(2)	0.0553(8)	N(1B)	0.2151(3)	0.03767(12)	0.4306(3)	0.0588(9)
O(1A)	0.1664(3)	0.14754(11)	0.7696(3)	0.0641(8)	O(1B)	0.1483(3)	0.12037(11)	0.2314(3)	0.0649(8)
C(1A)	0.4857(5)	−0.0028(2)	1.2209(4)	0.1041(18)	C(1B)	0.3601(6)	−0.1085(2)	0.6131(4)	0.115(2)
C(2A)	0.5706(5)	−0.0386(3)	1.2659(4)	0.117(2)	C(2B)	0.4363(6)	−0.1504(3)	0.6432(5)	0.139(3)
C(3A)	0.5429(6)	−0.0885(3)	1.2649(5)	0.124(2)	C(3B)	0.4552(6)	−0.1842(2)	0.5756(6)	0.123(2)
C(4A)	0.4292(7)	−0.1027(2)	1.2174(6)	0.180(4)	C(4B)	0.4035(7)	−0.1767(3)	0.4800(6)	0.170(3)
C(5A)	0.3434(5)	0.0670(2)	1.1715(5)	0.144(3)	C(5B)	0.3299(6)	−0.1332(3)	0.4514(5)	0.155(3)
C(6A)	0.3720(4)	−0.01553(18)	1.1722(3)	0.0722(12)	C(6B)	0.3051(4)	−0.09948(17)	0.5180(4)	0.0779(13)
C(7A)	0.2828(5)	0.0236(2)	1.1215(3)	0.110(2)	C(7B)	0.2247(4)	−0.05379(17)	0.4871(4)	0.0915(15)
C(8A)	0.2713(4)	0.02513(15)	1.0143(3)	0.0654(11)	C(8B)	0.2918(4)	−0.00972(15)	0.4554(3)	0.0687(12)
C(9A)	0.2350(4)	0.11828(14)	0.9715(3)	0.0663(11)	C(9B)	0.2886(3)	0.08319(14)	0.4157(3)	0.0652(11)
C(10A)	0.1397(4)	0.15763(14)	0.9269(3)	0.0625(11)	C(10B)	0.2111(4)	0.13177(14)	0.3994(3)	0.0665(11)
C(11A)	0.0748(3)	0.14493(13)	0.8214(3)	0.0519(9)	C(11B)	0.0999(3)	0.12625(14)	0.3140(3)	0.0571(10)
C(12A)	0.0257(3)	0.08979(12)	0.8180(3)	0.0517(9)	C(12B)	0.0295(3)	0.07806(14)	0.3286(3)	0.0572(10)
C(13A)	0.1226(3)	0.05129(14)	0.8616(3)	0.0573(10)	C(13B)	0.1084(3)	0.03030(14)	0.3457(3)	0.0588(10)
C(14A)	−0.0264(3)	0.18321(14)	0.7833(3)	0.0560(10)	C(14B)	0.0171(4)	0.17350(16)	0.3043(4)	0.0697(12)
C(15A)	−0.0219(4)	0.21574(16)	0.7065(3)	0.0713(12)	C(15B)	−0.0112(4)	0.20180(17)	0.2202(4)	0.0839(15)
C(16A)	−0.1136(5)	0.25125(19)	0.6772(4)	0.0880(15)	C(16B)	−0.0859(5)	0.2440(2)	0.2162(6)	0.111(2)
C(17A)	−0.2078(5)	0.25708(19)	0.7190(4)	0.0909(15)	C(17B)	−0.1365(6)	0.2579(2)	0.2883(7)	0.124(2)
C(18A)	−0.2136(4)	0.22547(18)	0.7941(4)	0.0846(14)	C(18B)	−0.1093(6)	0.2303(3)	0.3710(6)	0.145(3)
C(19A)	−0.1228(4)	0.18863(16)	0.8258(3)	0.0700(12)	C(19B)	−0.0324(5)	0.1876(2)	0.3795(4)	0.1108(19)
C(20)	0.6995(6)	0.1460(3)	0.1020(8)	0.205(4)					
Cl(1)	0.95794(10)	0.07596(4)	0.05400(8)	0.0707(3)	Cl(2)	0.11064(10)	0.06608(5)	0.60063(8)	0.0810(4)
Cl(3)	0.6106(3)	0.09676(11)	0.0998(2)	0.2311(13)	Cl(4)	0.6421(4)	0.19297(15)	0.0142(3)	0.298(2)
H(1AA)	0.5081	0.0319	1.2242	0.125	H(1BA)	0.3461	−0.0856	0.6597	0.138
H(2AA)	0.6486	−0.0277	1.2977	0.140	H(2BA)	0.4738	−0.1552	0.7087	0.167
H(3AA)	0.6001	−0.1128	1.2958	0.149	H(3BA)	0.5044	−0.2130	0.5953	0.147
H(4AA)	0.4078	−0.1374	1.2155	0.180	H(4BA)	0.4166	−0.2001	0.4339	0.203
H(5AA)	0.2655	−0.0779	1.1397	0.172	H(5BA)	0.2972	−0.1271	0.3855	0.186
H(7AA)	0.3078	0.0575	1.1488	0.133	H(7BA)	0.1922	−0.0426	0.5409	0.110
H(7AB)	0.2036	0.0161	1.1330	0.133	H(7BB)	0.1565	−0.0636	0.4339	0.110
H(8AA)	0.3503	0.0327	1.0026	0.078	H(8BA)	0.3633	−0.0016	0.5070	0.082
H(8AB)	0.2456	−0.0086	0.9867	0.078	H(8BB)	0.3196	−0.0203	0.3990	0.082
H(9AA)	0.3000	0.1190	0.9381	0.080	H(9BA)	0.3217	0.0772	0.3599	0.078
H(9AB)	0.2699	0.1270	1.0392	0.080	H(9BB)	0.3563	0.0877	0.4724	0.078
H(10AA)	0.1781	0.1913	0.9292	0.075	H(10BA)	0.2602	0.1606	0.3875	0.080
H(10AB)	0.0798	0.1595	0.9651	0.075	H(10BB)	0.1844	0.1394	0.4578	0.080
H(12AA)	−0.0379	0.0885	0.8529	0.062	H(12BA)	−0.0051	0.0833	0.3839	0.069
H(12AB)	−0.0106	0.0804	0.7510	0.062	H(12BB)	−0.0373	0.0728	0.2716	0.069
H(13AA)	0.0868	0.0171	0.8589	0.069	H(13BA)	0.0605	0.0010	0.3576	0.071
H(13AB)	0.1838	0.0507	0.8245	0.069	H(13BB)	0.1369	0.0229	0.2881	0.071
H(15AA)	0.0416	0.2135	0.6759	0.086	H(15BA)	0.0192	0.1927	0.1674	0.101
H(17AA)	−0.2671	0.2821	0.6969	0.109	H(17BA)	−0.1892	0.2860	0.2812	0.149
H(18AA)	−0.2776	0.2284	0.8240	0.102	H(18BA)	−0.1419	0.2396	0.4225	0.174
H(19AA)	−0.1272	0.1670	0.8772	0.084	H(19BA)	−0.0143	0.1685	0.4368	0.133
H(20A)	0.7770	0.1338	0.0930	0.205	H(20B)	0.7150	0.1620	0.1657	0.205
H(1)	0.123(3)	0.0659(12)	0.994(2)	0.043(8)	H(2)	0.187(3)	0.0443(13)	0.480(2)	0.043(8)
H(1B)	0.149(4)	0.1324(16)	0.722(3)	0.063(16)	H(2B)	0.095(4)	0.1118(16)	0.193(3)	0.067(17)

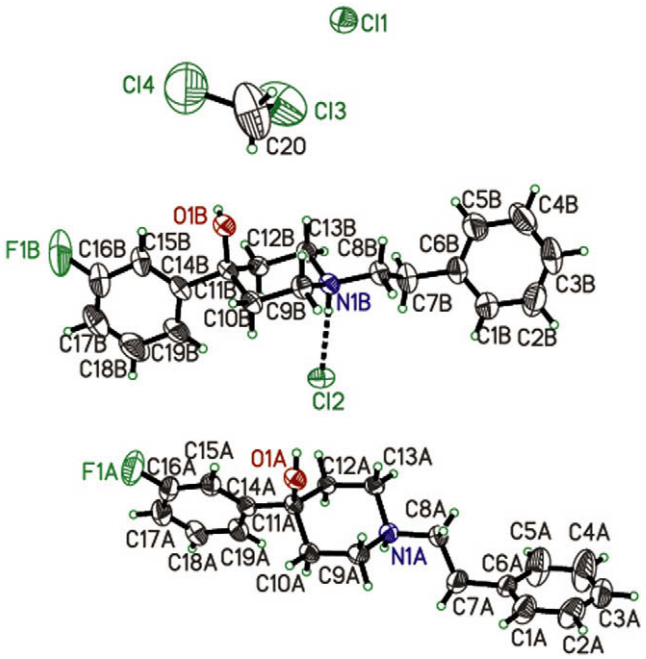


Fig. 1. Atom numbering scheme showing 50% probability displacement ellipsoids. Dashed lines show hydrogen bonds in the cluster of $[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$.

2.5. Anti-leukemia activity test

The anti-leukemia activity was evaluated according to the standard MTT experiments [10] of the Zooblast-molecular biology laboratory of Shanghai Normal University in China. About 10 mg of the title compounds were dissolved in 100 μL dimethylsulfoxide (DMSO) and then adjusted stepwise concentration of 1.0, 10.0, 100.0 μg/mL in each 96-well plates, the concentration of DMSO was below 1‰. One hundred thousands cells, 20 μL FBS, RPMI 1640 and diversified concentration of title compounds were seeded in each 100 μL well of a 96-well plates. Each group had eight wells. Setting up the comparison well of remedy color (5-FU, 5-fluorouracil) and incubate well (contain cells). After 44 h in a humidified atmosphere with 4% CO_2 at 33 °C, adding 10 μL MTT (5 mg/mL) into every well, then rejoined 100 μL 10% SDS to stop the reaction.

3. Result and discussion

3.1. Synthesis

The synthesis of title compounds were done in four steps (Scheme 1). The first step was Michael addition and the target products (**3a–3d**) were obtained via reaction of (**2**) with Grignard reagents.

Table 3
Selected bond lengths (Å) and bond angles (°) for the complex of $[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$.

Parameter	X-ray	B3LYP/6-31G (d, p)			Parameter	X-ray	B3LYP/6-31G (d, p)		
	3a	**4a**	**5a**	**6a**		**3a**	**4a**	**5a**	**6a**
Bond lengths									
F(1A)–C(16A)	1.363(5)	1.377	1.362	1.360	F(1B)–C(16B)	1.366(7)	1.379	1.359	1.362
N(1A)–C(8A)	1.494(5)	1.536	1.512	1.527	N(1B)–C(8B)	1.493(5)	1.491	1.515	1.529
N(1A)–C(13A)	1.496(5)	1.497	1.504	1.510	N(1B)–C(13B)	1.491(5)	1.492	1.507	1.514
N(1A)–C(9A)	1.500(5)	1.500	1.503	1.515	N(1B)–C(9B)	1.487(5)	1.444	1.493	1.502
O(1A)–C(11A)	1.415(4)	1.415	1.418	1.402	O(1B)–C(11B)	1.421(5)	1.411	1.431	1.418
C(6A)–C(7A)	1.484(6)	1.489	1.480	1.487	C(6B)–C(7B)	1.489(6)	1.485	1.488	1.491
C(7A)–C(8A)	1.496(6)	1.481	1.498	1.487	C(7B)–C(8B)	1.501(5)	1.486	1.511	1.497
C(9A)–C(10A)	1.503(5)	1.495	1.513	1.506	C(9B)–C(10B)	1.517(5)	1.502	1.519	1.510
C(10A)–C(11A)	1.532(5)	1.524	1.539	1.529	C(10B)–C(11B)	1.521(5)	1.518	1.516	1.523
C(11A)–C(14A)	1.509(5)	1.516	1.513	1.523	C(11B)–C(14B)	1.526(5)	1.519	1.521	1.530
C(11A)–C(12A)	1.527(5)	1.532	1.187	1.528	C(11B)–C(12B)	1.521(5)	1.526	1.530	1.532
C(12A)–C(13A)	1.497(5)	1.503	1.490	1.488	C(12B)–C(13B)	1.508(5)	1.505	1.513	1.518
C(20)–Cl(3)	1.619(7)				C(20)–Cl(4)	1.746(9)			
Bond angles									
C(8A)–N(1A)–C(13A)	112.4(3)	113.23	113.67	113.54	C(8B)–N(1B)–C(13B)	113.1(3)	113.64	113.34	113.42
C(8A)–N(1A)–C(9A)	112.8(3)	113.34	112.96	113.29	C(8B)–N(1B)–C(9B)	111.8(3)	112.31	112.43	112.52
C(13A)–N(1A)–C(9A)	110.6(3)	111.81	111.32	110.78	C(13B)–N(1B)–C(9B)	110.9(3)	111.52	111.24	111.64
C(1A)–C(6A)–C(7A)	122.1(5)	122.93	122.64	122.76	C(1B)–C(6B)–C(7B)	121.5(5)	121.93	121.45	121.65
C(5A)–C(6A)–C(7A)	121.9(5)	121.13	121.19	121.45	C(5B)–C(6B)–C(7B)	121.3(5)	120.92	120.87	121.47
C(6A)–C(7A)–C(8A)	112.8(4)	113.66	113.53	113.27	C(6B)–C(7B)–C(8B)	111.9(4)	112.52	111.48	111.87
N(1A)–C(8A)–C(7A)	111.8(3)	112.53	112.25	112.43	N(1B)–C(8B)–C(7B)	112.7(3)	113.37	112.84	112.91
N(1A)–C(9A)–C(10A)	111.1(3)	111.97	111.86	111.42	N(1B)–C(9B)–C(10B)	110.8(3)	111.38	111.14	111.42
C(9A)–C(10A)–C(11A)	112.7(3)	113.46	113.29	113.15	C(9B)–C(10B)–C(11B)	112.1(3)	113.44	112.56	112.52
O(1A)–C(11A)–C(14A)	111.8(3)	112.74	112.86	112.64	O(1B)–C(11B)–C(14B)	111.5(4)	112.25	111.87	111.96
O(1A)–C(11A)–C(12A)	109.7(3)	109.23	109.65	111.08	O(1B)–C(11B)–C(12B)	110.4(3)	109.81	110.35	109.75
C(14A)–C(11A)–C(12A)	111.3(3)	111.98	111.34	109.60	C(14B)–C(11B)–C(12B)	109.7(3)	110.23	109.79	110.38
O(1A)–C(11A)–C(10A)	105.3(3)	104.82	105.32	110.30	O(1B)–C(11B)–C(10B)	104.9(3)	104.19	104.79	111.39
C(14A)–C(11A)–C(10A)	110.2(3)	109.67	110.26	105.31	C(14B)–C(11B)–C(10B)	111.3(3)	111.80	111.34	104.79
C(12A)–C(11A)–C(10A)	108.2(3)	108.91	108.53	108.72	C(12B)–C(11B)–C(10B)	109.0(3)	109.45	109.52	109.27
C(13A)–C(12A)–C(11A)	112.7(3)	113.46	112.59	112.64	C(13B)–C(12B)–C(11B)	112.6(3)	113.29	112.87	112.14
N(1A)–C(13A)–C(12A)	110.5(3)	111.38	111.15	111.28	N(1B)–C(13B)–C(12B)	110.7(3)	111.24	110.54	110.34
C(19A)–C(14A)–C(11A)	121.5(4)	121.26	121.49	120.07	C(19B)–C(14B)–C(11B)	120.2(5)	120.91	120.27	121.41
C(15A)–C(14A)–C(11A)	120.4(4)	120.73	120.46	121.39	C(15B)–C(14B)–C(11B)	120.8(4)	120.18	120.78	120.04
C(17A)–C(16A)–F(1A)	118.3(5)	118.90	118.52	118.45	C(17B)–C(16B)–F(1B)	119.2(6)	118.72	118.83	118.89
F(1A)–C(16A)–C(15A)	117.6(5)	117.07	117.42	117.34	F(1B)–C(16B)–C(15B)	116.7(7)	117.27	117.19	117.15
Cl(3)–C(20)–Cl(4)	114.5(5)								

3.2. X-ray crystal structure discussion

The investigated compound crystallizes in monoclinic crystal system (space group *P*2 (1)/*n*) with two PHFPH cations, two chloride anion and a dichloromethane molecular in the asymmetric unit. The molecular structure and atomic numbering of bis[1-phenethyl-4-hydroxy-4-(3-fluorophenyl) piperidinium hydrochloride]-dichloromethane complex are shown in Fig. 1. Crystal data and structure refinement are presented in Table 1. The bond lengths, bond and torsion angles are listed in Table 3 and Table 4. The described compound is a salt of the cation–anion, where the cation is PHFPH and the anion is chloride ion. In the crystal structure, the benzene ring is connected through two carbon atoms via single bonds to the piperidinol derivative. The bond lengths for benzene and piperidine are typical for this type of compound [11,12]. The C(7A)–C(8A) bond length linking the benzyl ring with the piperidine ring is1.496(6) Å, which is slightly shorter than the sum of van der Waals radii of C–C(1.53 Å)

The torsion angle N(1A)C(8A)C(7A)C(6A) is −179.74(4)° indicating that, for steric reasons, bulky rongsform *trans* conformation around the C(7A)–C(8A) bond. The dihedral angles made by the central fragment of the piperidine rings (C(9A), C(10A), C(12A), C(13A) and C(9B), C(10B), C(12B), C(13B)) with two benzene rings are 24.03(2) and 62.04(2), respectively shows that the benzene ring(C(1B)–C(6B)) bonded to the piperidine ring through C(7B)–C(8B) bond is twisted away from the ring's symmetrical mirror [13], which maybe caused by a weak hydrogen bond (C–H···F^n = 2.71(5) Å, symmetry code *n*: 0.5 − *x*, −0.5 + *y*, 0.5 − *z*) interaction.

In a single molecular structure, the piperidine ring has a perfect chair conformation, the endocyclic torsion angles varying between 56.5(4) and 56.7(4), respectively [14]. The benzene ring (C(14A)–C(19A)) is rotated and coplanar with the oxygen atom O(1A) (the deviation of O(1A) atom from the plane formed by C(14A)–C(19A) is -0.117(4) Å). The axial oxygen O(1A) does not experience a repulsion with the axial nitrogen lone pair as provided by the opening of the following bond angles: N(1A)C(9A)C(10A) (111.09(3)°) and N(1A)C(13A)C(12A)(110.45(3)°), especially 105.3 (3)° for O(1A)C(11A)C(10A) [15]. In addition the nitrogen lone pair electrons could cause a slight change of C(9A)–C(10A) and C(13A)–C(12A), as provided by the comparison of bond distances (means 1.504(6) and 1.497(5) Å) with typical C–C single bond distance 1.53 Å.

The molecular arrangement in the crystal is clearly dominated by strong hydrogen bonds and contacts involving chlorine atoms. The N^+–H···Cl^- hydrogen bond is characterized by an N^+(1B)···Cl^-(2) distance of 3.033 Å and N^+(1B)–H(2)···Cl^-(2) angle of 176.1(3) °(Table 5, Fig. 1).The Cl^-(1) anion in the present structure is engaged in N^+(1A)–H(1)···Cl^-(1) (symmetry code *a*: −1 + *x*, *y*, 1 + *z*), O(1B)–H(2B)···Cl^-(1) (symmetry code *b*: −1 + *x*, *y*, *z*) hydrogen bonds (3.110(4) and 3.099(4) Å) and in an N^+(1A)···Cl^-(1) electrostatic interaction with neighboring piperidinium rings (Table 5, Fig. 2). As a consequence of these interatomic interactions, the crystal packing is governed by electrostatic interactions between the twosome cationic (N^+(1A)) and anionic (Cl^-(1)) and by van der Waals interactions (C(17B)–H(17BA)···O(1A)^c = 2.350 Å, symmetry code *c*: 1 − *x*, −*y*, 1−*z*). The dichloromethane solvent molecular is linked to Cl^-(1) anion though C(20)–H(20A)···Cl^-(1) hydrogen bond.

Table 4
Selected torsion angles for the complex of $[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$.

Parameter	X-ray	B3LYP/6-31G (d, p)		
	3a	**4a**	**5a**	**6a**
Torsion angles(°)				
C(2A)–C(1A)–C(6A)–C(7A)	−178.1(5)	−176.32	−177.85	−177.31
C(4A)–C(5A)–C(6A)–C(7A)	178.5(7)	177.12	178.29	178.56
C(1A)–C(6A)–C(7A)–C(8A)	100.4(6)	98.26	99.15	99.83
C(5A)–C(6A)–C(7A)–C(8A)	−79.1(7)	−76.27	−77.54	−75.32
C(13A)–N(1A)–C(8A)–C(7A)	−151.7(4)	−148.43	−149.35	−148.39
C(9A)–N(1A)–C(8A)–C(7A)	82.4(5)	85.46	86.33	87.33
C(6A)–C(7A)–C(8A)–N(1A)	−179.7(4)	−178.54	−178.26	−178.05
C(8A)–N(1A)–C(9A)–C(10A)	−176.3(3)	−173.48	−174.55	−172.49
C(13A)–N(1A)–C(9A)–C(10A)	−55.8(5)	−60.33	−59.34	−58.48
O(1A)–C(11A)–C(10A)–C(9A)	−63.9(4)	−68.5	−63.86	175.28
C(9A)–C(10A)–C(11A)–C(14A)	175.4(3)	173.61	174.69	172.90
C(9A)–C(10A)–C(11A)–C(12A)	53.4(4)	52.43	51.64	51.44
O(1A)–C(11A)–C(12A)–C(13A)	59.6(4)	60.2	59.70	−176.03
C(14A)–C(11A)–C(12A)–C(13A)	−176.1(3)	−174.37	−175.83	−174.39
C(10A)–C(11A)–C(12A)–C(13A)	−54.8(4)	−52.54	−53.42	−51.93
C(8A)–N(1A)–C(13A)–C(12A)	175.0(3)	176.66	177.32	178.48
C(9A)–N(1A)–C(13A)–C(12A)	−57.9(4)	−59.38	−58.48	−59.60
C(11A)–C(12A)–C(13A)–N(1A)	58.2(4)	56.53	57.34	55.08
O(1A)–C(11A)–C(14A)–C(19A)	−177.4(3)	−178.53	−86.96	−0.26
C(12A)–C(11A)–C(14A)–C(19A)	59.5(5)	63.43	62.65	63.06
C(10A)–C(11A)–C(14A)–C(19A)	−60.6(5)	−60.83	29.82	−120.05
O(1A)–C(11A)–C(14A)–C(15A)	−0.3(5)	−0.43	90.24	−179.75
C(12A)–C(11A)–C(14A)–C(15A)	−123.4(4)	−122.83	−32.77	−56.13
C(10A)–C(11A)–C(14A)–C(15A)	116.5(4)	114.66	114.21	115.04
C(11A)–C(14A)–C(15A)–C(16A)	−177.4(4)	−175.54	−179.21	−178.23
C(14A)–C(15A)–C(16A)–F(1A)	179.0(4)	178.48	178.83	178.07
F(1A)–C(16A)–C(17A)–C(18A)	−179.1(5)	−178.34	−178.72	−178.69
C(11A)–C(14A)–C(19A)–C(18A)	177.0(4)	175.38	176.98	177.35
C(2B)–C(1B)–C(6B)–C(7B)	−179.2(5)	−177.53	−178.02	−177.04
C(4B)–C(5B)–C(6B)–C(7B)	−178.8(6)	−177.38	−177.53	−177.01
C(1B)–C(6B)–C(7B)–C(8B)	96.7(6)	97.63	97.29	97.92
C(5B)–C(6B)–C(7B)–C(8B)	−81.0(7)	−78.39	−80.31	−78.21
C(13B)–N(1B)–C(8B)–C(7B)	−63.8(5)	−65.24	−64.39	−66.38
C(9B)–N(1B)–C(8B)–C(7B)	170.2(4)	172.32	171.59	173.03
C(6B)–C(7B)–C(8B)–N(1B)	−176.1(4)	−175.70	−175.89	−174.39
C(8B)–N(1B)–C(9B)–C(10B)	−175.0(3)	−173.42	−174.38	−173.49
C(13B)–N(1B)–C(9B)–C(10B)	57.8(4)	59.16	59.82	58.94
O(1B)–C(11B)–C(10B)–C(9B)	−64.5(4)	−65.8	−64.46	174.90
C(9B)–C(10B)–C(11B)–C(14B)	174.9(4)	173.15	173.63	172.58
C(9B)–C(10B)–C(11B)–C(12B)	53.7(4)	52.47	51.69	51.48
O(1B)–C(11B)–C(12B)–C(13B)	60.7(4)	60.7	60.76	−176.00
C(14B)–C(11B)–C(12B)–C(13B)	−176.0(3)	−174.64	−175.38	−173.79
C(10B)–C(11B)–C(12B)–C(13B)	−53.9(4)	−53.18	−54.15	−55.40
C(8B)–N(1B)–C(13B)–C(12B)	175.9(3)	176.52	176.16	176.93
C(9B)–N(1B)–C(13B)–C(12B)	−57.6(4)	−58.73	−57.43	−58.93
C(11B)–C(12B)–C(13B)–N(1B)	56.5(4)	55.38	55.69	54.04
O(1B)–C(11B)–C(14B)–C(19B)	−175.9(4)	−176.13	−90.32	−0.30
C(12B)–C(11B)–C(14B)–C(19B)	61.5(5)	62.43	62.72	63.66
C(10B)–C(11B)–C(14B)–C(19B)	−59.2(5)	−60.02	26.29	−120.91
O(1B)–C(11B)–C(14B)–C(15B)	5.5(5)	5.89	91.10	179.24
C(12B)–C(11B)–C(14B)–C(15B)	−117.0(4)	−116.52	−31.47	−58.53
C(10B)–C(11B)–C(14B)–C(15B)	122.3(4)	121.31	121.59	121.32
C(11B)–C(14B)–C(15B)–C(16B)	−179.8(4)	−179.18	−179.82	−179.87
C(14B)–C(15B)–C(16B)–F(1B)	179.8(5)	179.12	179.04	178.32
F(1B)–C(16B)–C(17B)–C(18B)	−180.0(6)	−179.31	−179.52	−178.55
C(11B)–C(14B)–C(19B)–C(18B)	−179.2(5)	−178.57	−177.43	−179.45

3.3. B3LYP/6-31G (d, p) calculations

Three conformers of bis[1-phenethyl-4-hydroxy-4-(3-fluorophenyl) piperidinium] hydrochloride, obtained by the B3LYP/6-31G (d, p) approach (**4a**–**6a**), were compared with the conformer found in the crystal structure **3a** in Fig. 3.Their geometrical data, energies are collected in Table 5. In conformer **4a** the oxygen atoms are almost coplanar with the plane of the 3-fluorophenyl substituents, while vertical in conformer **5a**. The 3-fluorophenyl substituents of two conformers are oriented in the equatorial positions, whereas the OH groups are axial. The main difference between conformers **4a** and **6a** lies in the orientation of hydroxyl groups and the 3-fluorophenyl substituents structural units, in conformer **4a** hydroxyl groups are in the axial and the 3-fluorophenyl substituents in the equatorial positions, while in conformer **6a** the arrangement of the substituents is the opposite, with the equatorial hydroxyl groups and the axial 3-fluorophenyl substituents. Bond lengths, bond and torsion angles are given in

Table 5
Experimental and calculated energies (a.u), relative energies (E_{rel}, kcal/mol) and hydrogen bonds (Å and °) for bis[1-phenethyl-4-hydroxy-4-(3-fluorphenyl) piperidinium] hydrochloride.

	X-ray	B3LYP/6-31G (d, p)		
	3a	**4a**	**5a**	**6a**
Energy (a.u)		−3814.587260	−3814.576321	−3814.567564
E_{rel}		0	7.46	12.31
N(1B)–H(2)···Cl(2)	3.033(4)	3.0612	3.0975	3.0845
N(1B)–H(2)	0.86(3)	0.871	1.005	1.004
H(2)···Cl(2)	2.18(3)	2.190	2.092	2.081
N(1B)–H(2)···Cl(2)	176 (3)	176.82	179.13	178.84
N(1A)–H(1)···Cl(1)[a]	3.110(4)			
N(1A)–H(1)	0.85(3)			
H(1)···Cl(1)	2.26(3)			
N(1A)–H(1)···Cl(1)	172(3)			
O(1B)–H(2B)···Cl(1)[b]	3.099(4)			
O(1B)–H(2B)	0.74(4)			
H(2B)···Cl(1)	2.37(4)			
O(1B)–H(2B)···Cl(1)	167 (5)			

Symmetry code: (a), −1 + *x*, *y*, 1 + *z*; (b), −1 + *x*, *y*, *z*.

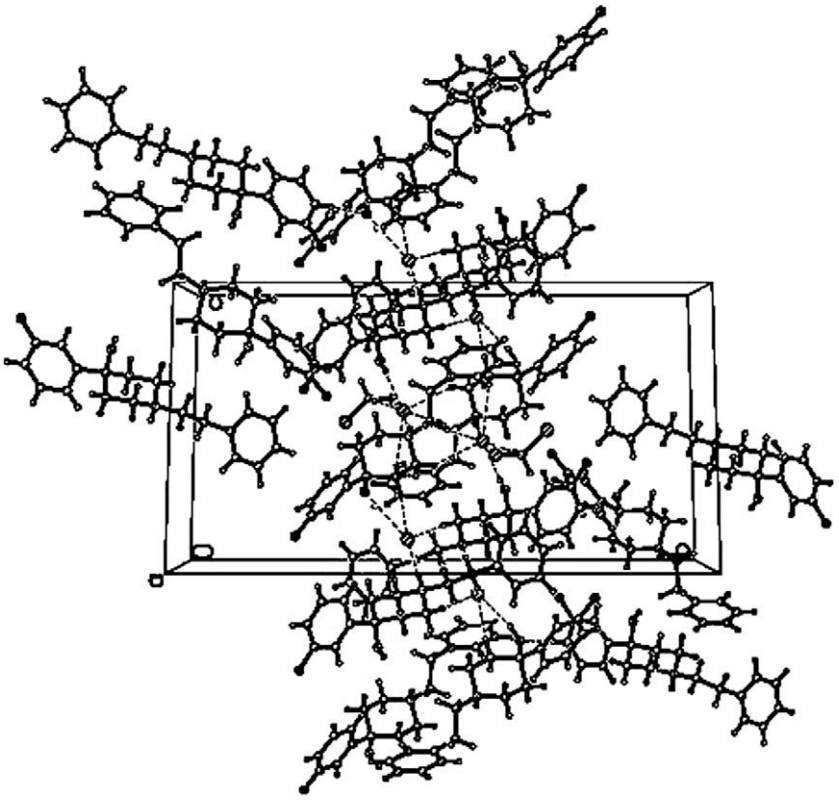


Fig. 2. Molecular arrangement of the cluster $[(PHFPH)_2Cl_2]\cdot CH_2Cl_2$ in the crystal.

Table 2 and Table 3. The differences between the conformers are observed in the torsion angles of O(1)–C(11)–C(14)–C(15) and O(1)–C(11)–C(14)–C(19) (Table 4) [16].

The isolated molecule of conformer **4a** is by 7.46 kcal/mol more stable than that of **5a**, which maybe caused by the steric exclusion and the weak C–H···F interaction. The conformer with 3-fluorophenyl substituents in the axial (**6a**) has higher energy than conformer **4a** (Table 5), which agrees well with the general rule that equatorial conformers of alkylcyclohexanes [17] and six-membered cycloamines [18] are more stable than the axial ones. In addition, the luck of the O(1A)–H(1B) ···Cl(2) interaction in **6a** is responsible for its high energy.

3.4. Spectroscopy

The IR, ^{1}H NMR and elemental analysis for the products are in good agreement with the title compounds **3a–3d**. The IR solid state spectra of the investigated compounds are shown in Fig. 4 [19]. In

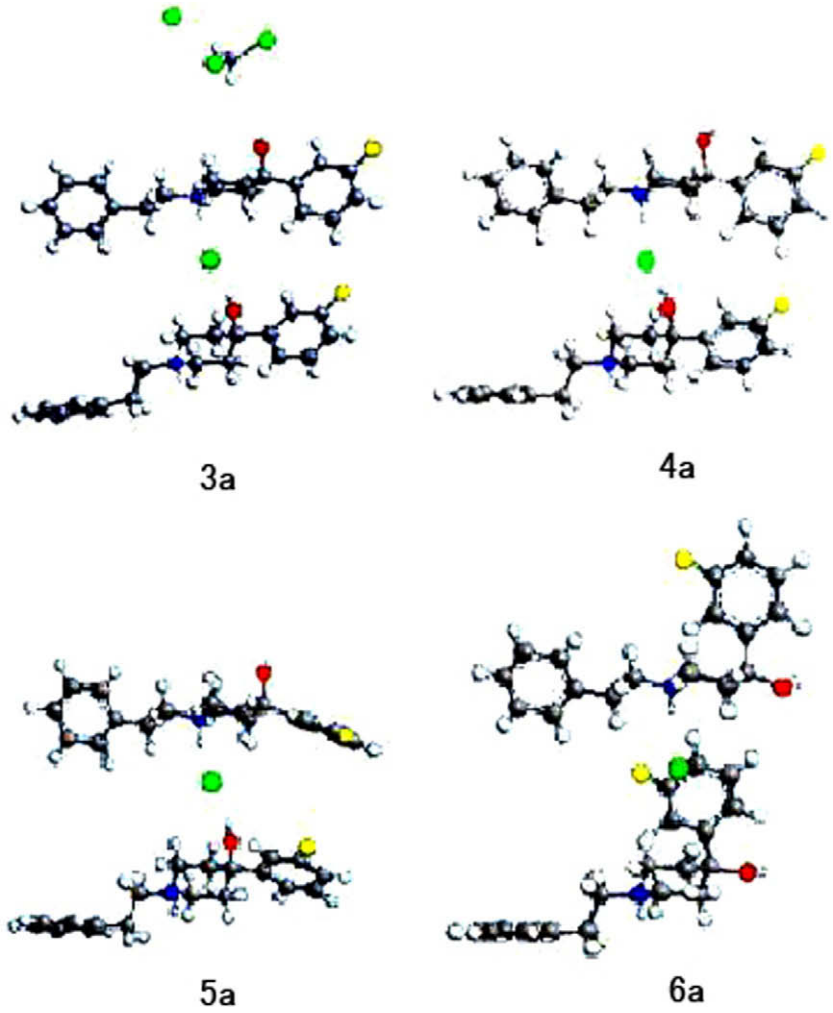


Fig. 3. comparison of the structure of $[(PFPH)_2Cl_2]\cdot CH_2Cl_2$ determined in the crystal (**3a**) and calculated for isolated molecules (**4a**–**6a**) by the B3LYP/6-31G (d, p) method.

the structure of complex **3a** the N^+–H···Cl^- and O(1B)–H(2B)···Cl^-(1) hydrogen bonds identified in the crystal structure are manifested in the IR spectrum by the overlapping *v*NH and *v*OH vibrations that give rise to a broad band in the 3350–2300 cm^{-1} region. There are five local maxima at 3318, 3267, 2921, 2686 and 2586 cm^{-1} on this broad band. The ^1H NMR (DMSO-d_6) spectra reveals that signal at about δ 10.67, δ 5.64 are attributed to the proton of N^+–H and O–H, respectively.

3.5. Anti-leukemia activity

The preliminary growth inhibition test results show that the compounds have good inhibitory activities against K562 cells (about 85.9–87.46%) in higher concentration (100 μg/mL). The inhibition percentage of title compounds to K562 cells is given in Table 6, from which we can find that the compounds inhibitory activities against K562 cells are higher than those of the anti-tumor drug of clinical practice 5-Fu (5-fluorouracil) in the same concentration of 100 μg/mL.

4. Conclusions

Four unknown compounds of the 1-phenethyl-4-hydroxy-4-substituted piperidinium hydrochlorides were synthesized and characterized. The X-ray data of **3a** shows that two 1-phenethyl-4-hydroxy-4-(3-fluorophenyl) piperidine [PHFP] with two Cl^- anions and a dichloromethane molecular form a five-membered structure linked by the N^+–H···Cl^- hydrogen bonds system of the lengths from 3.033(4) to 3.110(4) Å .The piperidine ring of the complex PHFP is protonated and adopts a perfect chair conformation with the 3-fluorophenyl substituent in the equatorial and the OH group in the axial position.

The structures of three conformers of $[(PHFPH)_2Cl]$ (**4a**–**6a**) were optimized by B3LYP/6-31G (d, p) calculations. The lowest energy is found for conformer **4a** with the OH group in the axial position and coplanar with 3-fluorophenyl substituents, as in the crystal structure **3a**. The steric effect and the lack of the O(1A)–H(1B)···Cl(2) interactions in **6a** are responsible for its high energy.

The IR spectrum shows a broad in the 3350–23,00 cm^{-1} region due to the overlapping *v*NH and *v*OH vibrations, and the ^1H NMR spectra reveals that signal at about δ 10.68 is attributed to the proton of N^+–H, which agree with the X-ray crystal structure .

The preliminary growth inhibition test results show that the compounds (**3a–3d**) have better inhibitory activities against K562 cells in higher concentration than 5-FU.

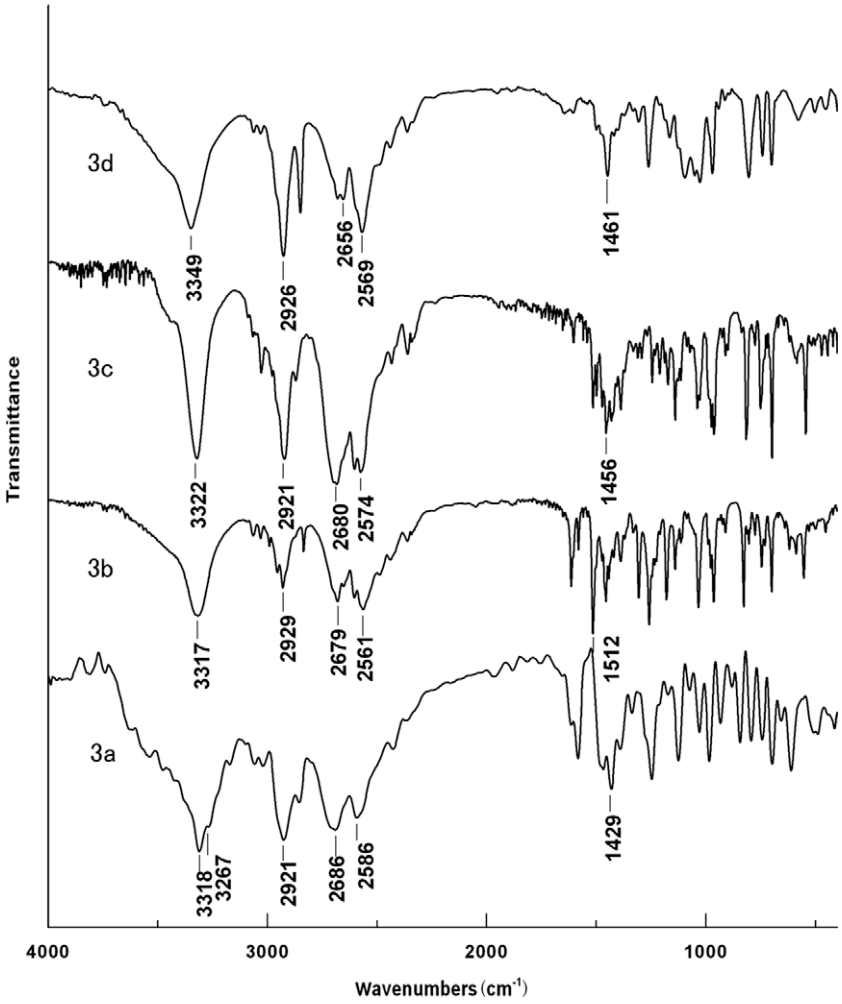


Fig. 4. Infrared spectra of compounds **3a**, **3b**, **3c**, **3d**.

Table 6
The growth inhibition percentage of the title compounds to K562 cells.

Compound	Inhibition rate (%)		
	100 μg/mL	10 μg/mL	1 μg/mL
3a	87.46	20.81	13.27
3b	87.19	20.25	11.71
3c	86.2	11.7	7.11
3d	85.9	25.31	14.64
5-FU	59.21	41.69	31.81

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