Novel Symmetrical *trans*-Bis-Schiff Bases of *N*-Substituted-4-piperidones: Synthesis, Characterization, and Preliminary Antileukemia Activity Mensurations

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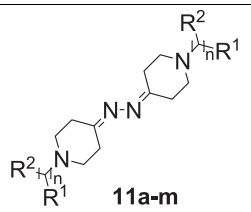

11a-m

11a: R_1= cyclohexyl; *n*=0; R_2= H
11b: R_1= C_6H_5; *n*=1; R_2= H
11c: R_1= 4-MeC_6H_4; *n*=1; R_2= H
11d: R_1= 4-MeOC_6H_4; *n*=1; R_2= H
11e: R_1= 3,4-methylenedioxyphenyl; *n*=1; R_2= H
11f: R_1= C_6H_5; *n*=1; R_2= CH_3
11g: R_1= C_6H_5; *n*=1; R_2= CH_3
11h: R_1= $C_6H_5CH_2$; *n*=1; R_2= H
11i: R_1= 3-pyridyl; *n*=1; R_2= H
11j: R_1= 6-chloro-3-pyridyl; *n*=1; R_2= H
11k: R_1= 2-tetrahydrofurfuryl; *n*=1; R_2= H
11l: R_1= 2-furfuryl; *n*=1; R_2= H
11m: R_1= 2-thienyl; *n*=1; R_2= H

A series of novel symmetrical *trans*-bis-Schiff bases (**11a-11m**) were designed and prepared as novel anticancer analogues, with the *trans*-configuration confirmed by X-ray diffraction. Preliminary inhibitory effects of these compounds on CML K562 cell growth were investigated, and the potential analogue **11e** showed an excellent anti-leukemia activity (IC_{50}=6.35 μg/mL), which is higher than that of the clinical drug 5-fluorouracil (IC_{50}=8.48 μg/mL). Complete assignments had been achieved for the title compounds by spectroscopic techniques, and their structure–activity relationships have been studied.

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INTRODUCTION

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by unregulated growth of myeloid leukemia cells in the bone marrow and accumulation of these cells in the blood, which is caused by clonal expansion of pluripotent hematopoietic stem cells retaining their differentiation potential [1]. It is usually a triphasic disease, having a chronic, an accelerated, and a blast phase [2–4], during which progressive resistance to therapy is acquired [5–10]. New evidence suggests that CML blasts arise from leukemic progenitors (rather than leukemic stem cells) that have restored self-renewal capacities [11,12]. Recent study also showed that CML cell line K562 was considerably more resistant to apoptosis than a number of other human hematopoietic cell lines. Therefore, the development of novel and effective anticancer drugs against CML is a high priority.

Schiff bases are an important class of organic compounds, some of which show significant biological activities [13–16]. Many studies have reported the biological activities of Schiff bases, including their anticancer [17–19], antibacterial [20–24], antifungal [23–25], and herbicidal activities [26–28]. In discovering biological schiff base molecules, a notable role is played by heterocyclic structures, which were reported to possess the cytotoxic [29], anticonvulsant [30], antiproliferative [31], anticancer, and antifungal activities [32]. Development of a new chemotherapeutic Schiff bases is now attracting the attention of medicinal chemists [33–36]. For example, Hwu et al. have reported that photolytic cleavage of the nitrogen–nitrogen single bond in benzaldehyde phenylhydrazones produced aminyl ($R_2N\cdot$) and iminyl ($R_2C{=}N\cdot$) radicals. This photochemical property was utilized in the development of hydrazones as photoinduced DNA cleaving agents [37]. Arylcarbaldehyd-4-(1-phenyl-3- methylpyrazolo-[3,4-b] pyridine) hydrazone derivatives were utilized as a new pharmacophoric tool for the development of more efficacious analgesics [38]. Moreover, due to the known therapeutic properties of piperidines and due to the presence of a keto function that facilitates the introduction of other substituents on the piperidine ring. Piperidine derivatives are useful as anti-osteoporotic raloxifene [39], vasodilator minoxidil [40,41], analgesic fentanyl **1** (Fig. 1) [42,43], anti-Pakinsonian

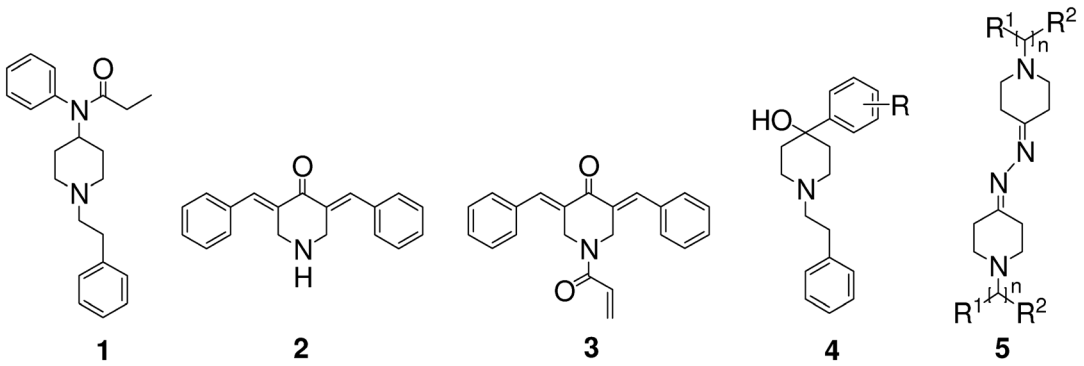


Figure 1. Structures of the compounds.

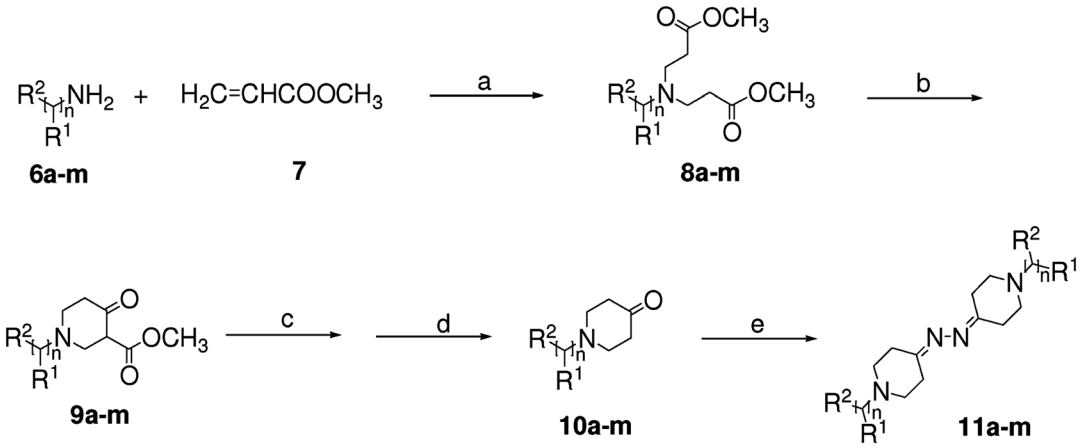
agent biperiden [44,45] and also as high antiproliferative activity which was shown by the parent molecule **2** and **3** (Fig. 1) towards leukemia and colon cancer cell lines [46,47].

On the basis of above observations and obeying the principle of active-factor-addition, the development of novel bis-Schiff bases of piperidones as potential anticancer agents is very attractive. Continuing our interest in nitrogen piperidine derivatives **4** (Fig. 1) endowed with anti-leukemia activity [48,49], we designed and synthesized a novel series of highly functionalized piperidone azines **5** (Fig. 1) and evaluated the effects of these compounds on CML K562 cell growth.

RESULTS AND DISCUSSION

Synthesis of compounds. A five-step synthetic strategy was adopted for the synthesis of symmetrical *trans*-*N*-substituted-4-piperidone azines **11a–m**. The general schematic representation describing the routes of syntheses is furnished in Scheme 1. *N*,*N*-Bis[2-(methoxycarbonyl) ethyl] substitutedamines **8a–m** was obtained by the Michael addition of substitutedamine, methyl acrylate, and acetic acid in 1:4:1 ratio, respectively. The *N*,*N*-bis [2-(methoxycarbonyl)ethyl] substitutedamines **8a–m** afforded *N*-substituted-4-piperidones **10a–m** upon reflux

Scheme 1. Synthesis of novel symmetrical *trans*-*N*-substituted-4-piperidone azines **11a–m**. Reagents and conditions: (a) acetic acid, absolute methanol, refluxing; (b) sodium/ absolute methanol, toluene, refluxing; (c) 25% HCl, refluxing; (d) 35% sodium hydroxide, r.t; (e) 85% hydrazine hydrate, acetic acid, absolute ethanol, refluxing.



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11e: R_1= 3,4-methylenedioxyphenyl; *n*=1; R_2= H
11f: R_1= C_6H_5; *n*=1; R_2= CH_3
11g: R_1= C_6H_5; *n*=1; R_2= CH_3
11h: R_1= $C_6H_5CH_2$; *n*=1; R_2= H
11i: R_1= 3-pyridyl; *n*=1; R_2= H
11j: R_1= 6-chloro-3-pyridyl; *n*=1; R_2= H
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11l: R_1= 2-furfuryl; *n*=1; R_2= H
11m: R_1= 2-thienyl; *n*=1; R_2= H

with sodium methoxide in the presence of absolute toluene and the followed mixture was extracted with 25% HCl, then directly refluxed. Azines **11a–m** was obtained by treatment of the *N*-substituted-4-piperidones **10a–m** with hydrazine hydrate in the presence of acetic acid in absolute ethanol in good yield 45–67.4%. For the formation of the azines, taking **11b** for example, according to Sayer mechanistic model [50], various pH value of buffers such as Tris 10 m*M*/EDTA 1 m*M* at pH 7.4 and acetic acid 100 m*M* at pH 4.6, other buffers were hydrochloric (HCl 200 m*M* + KCl 200 m*M*, pH 1–2), citratephosphate (citric acid 50 m*M* + Na_2HPO_4 100 m*M*, pH 2.6–7), acetic (acetic acid 100 m*M* + NaOH, pH 3.6–5), carbonate (K_2CO_3 50 m*M* + HCl, pH 8–9.6), and phosphate (Na_2HPO_4 50 m*M* + Na_3PO_4 50 m*M*, pH 10–12) under 70°C temperature were examined. Of these buffers, acetic acid at pH 4.6 was found to be the proficient condition for getting the excellent yields 67.4% of the azine **11b**. All the synthesized *N*-substituted-4-piperidone azines **11a–m** are freely soluble in absolute toluene but insoluble in polar aprotic solvent acetonitrile and partially soluble in polar protic solvents such as ethanol, methanol at room temperature. The structures of azine derivatives were determined by IR, ^{1}HNMR, mass spectroscopy and elementary analysis.

Single-crystal structural characterization of compound 11b by X-ray. The spatial structure of compound **11b** was determined by using X-ray diffraction analysis. The single crystals were grown from ethanol at room temperature. The molecular view of **11b** is shown in Figure 2. The X-ray analysis showed the two C N bonds are *trans* with respect to N N bond, which revealed unambiguous proof of the interesting point that since the azines contain two double bonds of imino group on **11b**, a free roatation of the imino double bond is restricted and formation of either geometrical *trans* or *cis* isomer is expected as ^{1}HNMR provide a symmetrical set of singals.

The molecule of **11b** is nonplanar, which consists of four fragments, namely two piperidine rings [N(1)/C(8)–C(13)] and [N(1)/C(13)–(17)], two benzene rings [C(1)–C(6)] and [C(19)–C(24)]. Because the two conjugated C N bonds, the piperidine rings do not show perfect chair conformation, the endocyclic torsion angles varying between 43.116 (178)° and 53.846(169) ° which deviated from the criterion region 56.5(4) to 56.7(4), respectively [51]. Moreover, for steric reasons and bulky rongsform trans conformation around the N(2)–N(3) bond, torsion angles C11–C10–N2–N3 [−179.36(16) °] and C14–C13–N3–N2 [176.76(16) °] close to 180°, which is different from the torsion angles C17–C13–N3–N2 [0.4(3)°] and C9–C10–N2–N3 [0.1(3)°] near to coplanarity. The axial C N bonds do not experience a repulsion with the axial nitrogen lone pair as provided by the opening of the following bond angles : N(1)C(12)C(11) [111.64(16)°], N(1)C(8)C(9) [112.90(17)°], N(4)C(15)C (114) [111.31(17)°] and N(4)C(16)C(17) [112.47(16)°]. In addition, the nitrogen lone pair could cause a slight change of C(8)–C(9), C(11)–C(12), C(13)–C(14) and C(13) –C(17) as provided by the comparison of bond distances (means 1.484(3) to 1.500(3) Å) with typical C C single bond distance 1.53 Å. Similarly, as the existence of chair conformation, the both conjugated C N bonds distance(1.271(3) Å) is shorter than the normal value of 1.34 Å. The C(7)–N(1) and C (18)–N(4) bond distances mean 1.472(3) and 1.458 (3) Å, however, are different from the C N bond distance 1.50 Å [52,53]. The crystal structures of **11b** are stabilized by C H…π bonds, without any intermolecular hydrogen bonds (Fig. 3). The representative single-crystal structural characterization of the compound **11b** should be valuable for further investigation.

Preliminary anti-leukemia activity. The growth inhibition activities of the title compounds and 5–fluorouracil (5–FU) against K562 cell were determined by the MTT assay according to the standard bioactivity test procedures of the Zooblast-molecular BiologyLaboratory of Shanghai Normal University of China [54]. The data obtained by MTT assay showed that compounds **11a–m** had inhibitory effects on the growth of K562 cells in dosage-dependent manners. As indicated in Figure 4, most of our designed compounds exhibited significant inhibition activities against K562 cells and had $>$ 80% inhibition at 100 μg/mL, which is higher than those of the anti-tumor drug of clinical practice 5-FU at the same concentration. Analogues **11e**, **11l,** and **11m** afforded the best in vitro activity,

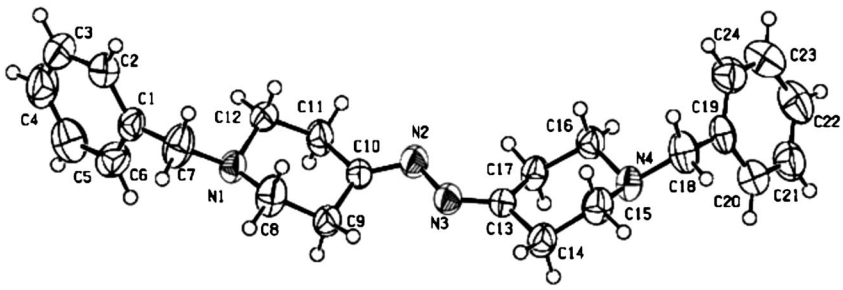


Figure 2. Molecular structure of compound **11b** (Number CCDC 739158).

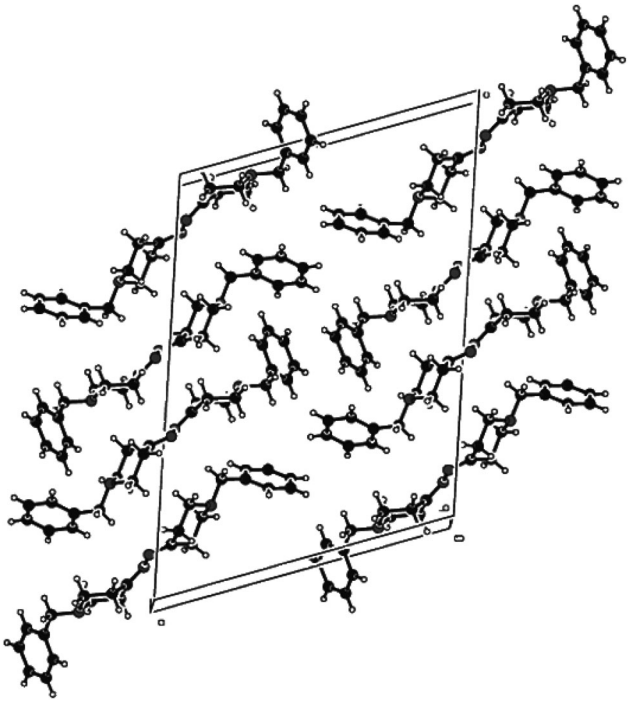


Figure 3. Packing diagram of compound **11b** viewed down the *b* axis.

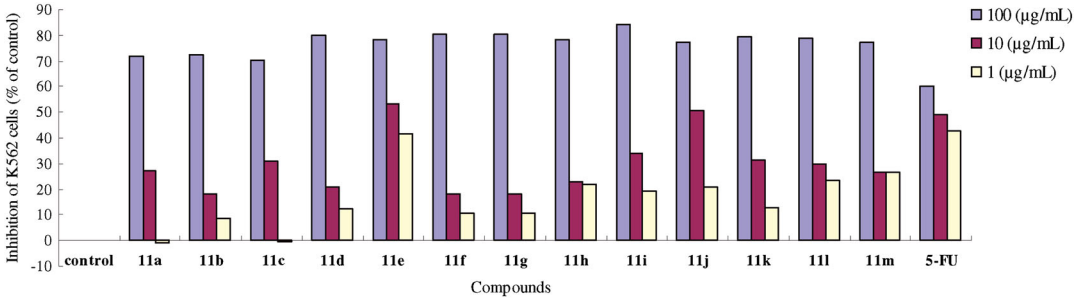


Figure 4. Inhibition of K562 cells treated with compounds **11a–m**. Cells were treated with the compounds at concentrations of 1, 10, and 100 μg/mL for 48 h. The cell viability was determined by MTT assay.

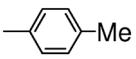
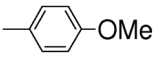
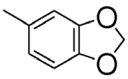
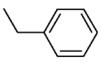
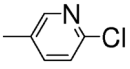
even at 1 μg/mL. Compounds **11a–m** exhibited a varying antileukemia IC_{50} values, ranging between 6.35 μg/mL and 29.17 μg/mL.

Structure–activity relationships. As indicated in Table 1, compound **11e** was the most potent compound in this series, having an IC_{50} value of 6.35 μg/mL in inhibiting the K562 cell growth, which is higher than that of the clinical drug 5-fluorouracil (IC_{50}=8.48 μg/mL). This compound was almost fourfold more potent than **11a**, **11b,** and **11c**, and the IC_{50} of other compounds varied drastically, depending upon the size, types, and characters of the 1-position substitution on piperidine rings. The introduction of 3-pyridylmethyl, 2-furanylmethyl, 6-chloro-3-pyridylmethyl, 2-thienylmethyl and 2-tetrahydrofuranyl methyl at the 1-position of piperidine rings in azines (**11i–m**) showed excellent inhibitory activities, $IC_{50} < 20$ μg/mL. Excepted for **11e**, the inhibition activities of all the corresponding analogues decreased in the order heterocyclic methyl (**11i–m**) > phenylethyl (**11h**) > phenylmethyl (**11b-d**) > cyclohexyl (**11a**). In addition, compounds **11g** and **11f**, with a methyl group at the methenyl demonstrated higher activities than **11b**. The observations herein corroborate our point of view that a 3,4-methylenedioxybenzyl or a heterocyclic methyl introduced at 1–position of piperidine rings may increase the inhibition activity of piperidone azine analogues.

In summary, this article has described a facile approach to prepare novel *N*- substitutedpiperidin-4-one azines derivatives (**11a–m**) via a series of Michael addition, Dieckmann condensation, hydrolysis decarhoxylation, and condensation

Table 1

Growth inhibitory properties IC_{50} (μg/mL) for the compounds **11a–m** and 5-FU at 48 h.

Compd	R^1	R^2	n	IC_{50}(μg/mL)
11a			0	29.17
11b		H	1	27.76
11c		H	1	26.70
11d		H	1	24.24
11e		H	1	6.35
11f[a]		CH_3	1	25.94
11g[b]		CH_3	1	23.78
11h		H	1	20.32
11i		H	1	12.37
11j		H	1	10.65
11k		H	1	18.83
11l		H	1	15.42
11m		H	1	17.11
5-FU				8.48

[a](±)-.
[b](*R*)-.

reactions. These compounds were characterized by IR, ^{1}HNMR, mass spectroscopy, and elementary analysis. The structural characterization of the compound **11b** was also studied by X-ray, which may be valuable for further investigation. Preliminary bioassay against the K562 cell showed all the tested compounds exhibited good anticancer activities at 100 μg/mL, and analogue **11e** was found to be the most effective molecule in inhibiting K562 cell growth, with its IC50 of 6.35 μg/mL, which is better than that of the anti-tumor drug of clinical practice (5-FU). Studies on the inhibition mechanisms of compound **11e** against CML cell line are ongoing and will be reported in due course. The structure–activity relationships described in this study will be highly useful for the development of new cancer inhibitors with high potency and selectivity.

EXPERIMENTAL

All the chemical reagents purchased were of analytical grade and used without further purification, except for the toluene, which was dried by refluxing in the presence of sodium and distilled prior to use. RPMI 1640 was obtained from Gibco BRL (Grand Island, NY, USA) and Bovine calf serum was supplied by Beijing DingGuo Biotechnology Co., China. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates Merck KGaA). ^{1}HNMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using $CDCl_3$ or DMSO as solvents and tetramethylsilane (TMS) as internal standard. Melting points were determined by an RK1 microscopic melting apparatus uncorrected. Elemental analysis was performed with a Perkin-Elmer 2400 instrument. IR spectra were obtained on a Nicolet 5DX FT-IR spectrophotometer in the region 4000-400 cm^{-1} using KBr discs. MS spectra were recorded on a Trace DSQ mass spectrograph. X-ray diffraction data were recorded on a Bruker Smart CCD diffractometer.

General procedure for the synthesis of 11a–m. To a stirred solution of derivatives **10a–m** [55] 1 m*M* and acetic acid 0.01 m*M* in 10 mL of ethanol, there was added a half amount of the appropriate hydrazine hydrate. The reaction mixture was maintained under reflux for 2–16 h, until TLC indicated the end of reaction. After this time, the reaction mixture stood over night and the solid formed was collected by filtration and washed with ethanol and recrystallized from ethanol to afford crystals. As a result of this process the compounds **11a–m** were prepared in yield of 45–67.4%.

***N*-Cyclohexyl-4-piperidone azine (11a).** Yellow solid, yield 57.3%, m.p. 116–118°C; IR (KBr, cm^{-1}) v: 1632 (C N), 1117 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 1.09-1.32 (m, 8H, cyclohexyl), 1.80 (t, *J* = 2.8Hz, 1H, cyclohexyl), 1.96 (d, *J* = 8.4Hz, 1H, cyclohexyl), 2.38 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.43 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.49 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.53 (t, *J* = 5.6Hz, 2H, CH_2, piperidine); MS (EI, 70 eV) *m/z* (%): 358.4 (M^+, 6.52), 179.3 (100), 83.3 (34.3). Anal. calcd for $C_{22}H_{38}N_4$: C 73.69, H 10.68, N 15.63%; found C 73.78, H 10.73, N 15.45%.

***N*-Benzyl-4-piperidone azine (11b).** Yellow solid, yield 67.4%, m.p. 134–135°C; IR (KBr, cm^{-1}) v: 1643 (C N), 1601, 1496, 1453 (C C), 1115 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.43 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.46 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.51 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.60 (t, *J* = 5.6Hz, 2H, CH_2, piperidine),3.56 (s, 2H, N-CH_2Ph), 7.25-7.36 (m, 5H, Ph-H); MS (EI, 70 eV) *m/z* (%): 374.5 (M^+, 8.24), 187.4 (100), 91.3 (41.70). Anal. calcd for $C_{24}H_{30}N_4$: C 76.97, H 8.07, N 14.96%; found C 76.84, H 8.12, N 14.88%.

***N*-(4-Methylbenzyl)-4-piperidone azine (11c).** Yellow solid, yield 61.2%, m.p. 147–149°C; IR (KBr, cm^{-1}) v: 1641 (C N), 1609, 1511, 1455 (C C), 1116 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.36 (s, 3H, CH_3), 2.41 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.56 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.59 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.63 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 3.55 (d, *J* = 2.2Hz, 2H, N-CH_2Ph), 7.15 (d, *J* = 8.0Hz, 2H, Ph-H), 7.24 (d, *J* = 8.0Hz, 2H, Ph-H); MS (EI, 70 eV) *m/z* (%): 402.3 (M^+, 7.84), 201.4 (100), 105.3 (41.23). Anal. calcd for $C_{26}H_{34}N_4$: C 77.57, H 8.51, N 13.92%; found C 77.61, H 8.37, N 13.83%.

***N*-(4-Methoxybenzyl)-4-piperidone azine (11d).** Yellow solid, yield 52.3%, m.p. 151–153°C; IR (KBr, cm^{-1}) v: 1646 (C N), 1611, 1510, 1452 (C C), 1112 (C N); ^{1}HNMR ([D_6]DMSO, 400 MHz) δ: 2.31 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.39 (d, 4H, *J* = 4.2Hz, CH_2, piperidine), 2.44 (d, *J* = 4.2Hz, 2H, CH_2, piperidine), 2.49 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 3.73(s, 3H, O-CH_3), 6.87 (d, *J* = 8.8Hz, 2H, Ph-H), 7.21 (d, *J* = 8.8Hz, 2H, Ph-H); MS (EI, 70 eV) *m/z* (%): 434.4 (M^+, 8.17), 217.4 (100), 121.3 (35.43). Anal. calcd for $C_{26}H_{34}N_4O_2$: C 71.86, H 7.89, N 12.89%; found C 71.75, H 7.98, N 12.71%.

***N*-(3,4-Methylenedioxybenzyl)-4-piperidone azine (11e).** Yellow solid, yield 50.8%, m.p. 171–173°C; IR (KBr, cm^{-1}) v: 1640 (C N), 1608, 1495, 1456(C C), 1120 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.47 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.52 (d, *J* = 4.8Hz, 2H, CH_2, piperidine), 2.57 (d, *J* = 4.8Hz, 2H, CH_2, piperidine), 2.60 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 3.46 (d, *J* = 4.8Hz, 2H, N-CH_2Ph), 5.95 (d, *J* = 5.2Hz, 2H, O-CH_2-O), 6.75(d, *J* = 4.4Hz, 2H, Ph-H), 6.88(d, *J* = 5.2Hz, 1H, Ph-H); MS (EI, 70 eV) *m/z* (%): 462.5 (M^+, 7.88), 231.4 (100), 135.3 (25.63). Anal. calcd for $C_{26}H_{30}N_4O_4$: C 67.51, H 6.54, N 12.11%; found C 67.27, H 6.65, N 12.28%.

***(±)-N*-(1-Phenylethyl)-4-piperidone azine (11f).** Yellow solid, yield 38.1%, m.p. 153–154°C; IR (KBr, cm^{-1}) v: 1643 (C N), 1610, 1496, 1451 (C C), 1115 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 1.27 (s, 3H, CH_3), 2.44 (t, *J* = 6.0 Hz, 2H, CH_2, piperidine), 2.54 (d, *J* = 4.8 Hz, 2H, CH_2, piperidine), 2.62 (d, *J* = 4.8 Hz, 2H, CH_2, piperidine), 2.77 (t, *J* = 5.6 Hz, 2H, CH_2, piperidine), 4.05 (s, 1H, Ph-CH), 7.29-7.36 (m, 5H, Ph-H); MS (EI, 70 eV) *m/z* (%): 401.5 (M^+, 8.16), 201.2 (100), 106.3 (41.32). Anal. calcd for $C_{26}H_{34}N_4$: C 71.86, H 7.89, N 12.89%; found C 71.98, H 7.81, N 12.77%.

***(R)-N*-(1-Phenylethyl)-4-piperidone azine (11g).** Yellow solid, yield 63%, m.p. 137–140°C; IR (KBr, cm^{-1}) v: 1635 (C N), 1611, 1498, 1457 (C C), 1109 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 1.40 (d, *J* = 2.8Hz, 3H, CH_3), 2.43 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.49 (d, *J* = 6.8Hz, 2H, CH_2, piperidine), 2.54 (d, *J* = 3.2Hz, 2H, CH_2, piperidine), 2.63 (t, *J* =5 .6Hz, 2H, CH_2, piperidine), 3.51-3.56 (dd, *J* = 6.4 Hz, 1H, Ph-CH), 7.24-7.36 (m, 5H, Ph-H); MS (EI, 70 eV) *m/z* (%): 402.3 (M^+, 11.93), 201.4 (100), 105.3 (39.87). Anal. calcd for $C_{26}H_{34}N_4$: C 71.86, H 7.89, N 12.89%; found C 71.83, H 7.75, N 12.96%; [Á] =+25.18° (C = 0.01 g/mL , $CHCl_3$)

***N*-Phenethyl-4-piperidone azine (11h).** Yellow solid, yield 58.3%, m.p. 103–105°C; IR (KBr, cm^{-1}) v: 1635 (C N), 1609, 1496, 1455 (C C), 1116 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.53 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.64-2.66 (m, 4H, CH_2, Ph-CH_2), 2.67-2.69 (m, 2H, CH_2, piperidine), 2.74 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.83-2.87 (m, 2H, N-CH_2), 7.20-7.33 (m, 5H, Ph-H); MS (EI, 70 eV) *m/z* (%): 402.5 (M^+, 8.73), 201.4 (100), 126.3 (38.62). Anal. calcd for $C_{26}H_{34}N_4$: C 77.57, H 8.51, N 13.92%; found C 77.66, H 8.43, N 13.77%.

***N*-(3-Pyridylmethyl)-4-piperidone azine (11i).** Yellow solid, yield 45%, m.p. 143–144°C; IR (KBr, cm^{-1}) v: 1627 (C N), 1607, 1501, 1457 (C C), 1115 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.52 (t, *J* = 5.6 Hz, 2H, CH_2, piperidine), 2.55 (d, *J* = 5.2Hz, 2H, CH_2, piperidine), 2.60 (d, *J* = 5.2Hz, 2H, CH_2, piperidine), 2.66 (t, *J* = 5.6 Hz, 2H, CH_2, piperidine), 4.63 (d, 2H, CH_2-Pyri), 7.12(d, *J* = 7.6 Hz, 1H, Pyri-H), 7.23-7.27(m, 1H, Pyri-H), 7.36(d, *J* = 8.0Hz, 1H, Pyri-H), 8.56-8.58 (dd, J_1 = J_2 = *2.4 Hz*, 1H, Pyri-H); MS (EI, 70 eV) *m/z* (%): 376.4 (M^+, 8.87), 188.3 (100), 92.4 (43.17). Anal. calcd for $C_{22}H_{28}N_6$: C 70.18, H 7.50, N 22.32%; found C 70.23, H 7.32, N 22.21%.

***N*-(6-Chloro-3-pyridylmethyl)-4-piperidone azine (11j).** Yellow solid, yield 49.2%, m.p. 156–157°C; IR (KBr, cm^{-1}) v: 1633 (C N), 1612, 1486, 1453(C C), 1112 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.46 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.52 (d, *J* = 4.8Hz, 2H, CH_2, piperidine), 2.59 (d, *J* = 4.8Hz, 2H, CH_2, piperidine), 2.77 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 4.64 (s, 2H, CH_2-Pyri), 7.37 (d, *J* = 8.0Hz, 1H, Pyri-H), 7.82-7.84 (dd, J_1 = 2.4 Hz, J_2 = 2.8, 1H, Pyri-H), 8.33 (d, *J* = 2.4 Hz, 1H, Pyri-H); MS (EI, 70 eV) *m/z* (%): 445.3 (M^+, 7.84), 222.4 (100), 126.3 (38.62). Anal. calcd for $C_{22}H_{26}Cl_2N_4$: C 59.33, H 5.88, N 18.87%; found C 59.42, H 5.71, N 18.89%.

***N*-(2-Tetrahydrofurfuryl)-4-piperidone azine (11k).** Yellow solid, yield 39.6%, m.p. 138–139°C; IR (KBr, cm^{-1}) v: 1634 (C N), 1607, 1499, 1452 (C C), 1108 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.03-2.16 (m, 2H, CH_2-THF), 2.44 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 2.48 (d, *J* = 4.0Hz, 2H, CH_2, piperidine), 2.56 (d, *J* = 4.4Hz, 2H, CH_2, piperidine), 2.63 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 3.87 (s, 2H, CH_2-THF); MS (EI, 70 eV) *m/z* (%): 362.5 (M^+, 6.57), 181.4 (100), 85.3 (28.97). Anal. calcd for $C_{20}H_{34}N_4O_2$: C 66.26, H 9.45, N 15.46%; found C 66.45, H 9.52, N 15.38%.

***N*-(2-Furfuryl)-4-piperidone azine (11l).** Yellow solid, yield 45.5%, m.p. 155–157°C; IR (KBr, cm^{-1}) v: 1627 (C N), 1615, 1500, 1461 (C C), 1114 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.51 (t, *J* = 5.6 Hz, 2H, CH_2, piperidine), 2.57 (d, *J* = 4.2 Hz, 2H, CH_2, piperidine), 2.61 (d, *J* = 4.2 Hz, 2H, CH_2, piperidine), 2.67 (t, *J* = 5.6Hz, 2H, CH_2, piperidine), 3.61 (s, 2H, CH_2-furan), 6.22 (d, *J* = 3.2 Hz, 1H, furan-H), 6.33-6.35 (m, 1H, furan-H), 7.40-7.41(dd, J_1 = J_2 = 0.8 Hz, 1H, furan-H); MS (EI, 70 eV) *m/z* (%): 354.4 (M^+, 9.37), 177.4 (100), 81.3 (34.18). Anal. calcd for $C_{20}H_{26}N_4O_2$: C 67.77, H 7.39, N 15.81%; found C 67.65, H 7.52, N 15.69%.

***N*-(2-Thienylmethyl)-4-piperidone azine (11m).** Yellow solid, yield 47.8%, m.p. 168–169°C; IR (KBr, cm^{-1}) v: 1636 (C N), 1611, 1493, 1449 (C C), 1117 (C N); ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 2.49 (t, *J* = 5.6 Hz, 2H, CH_2, piperidine), 2.58-2.61 (m, 4H, CH_2, piperidine), 2.68 (t, *J* = 5.6 Hz, 2H, CH_2, piperidine), 3.78 (d, *J* = 0.8 Hz, 2H, thio-CH_2), 6.92-6.98 (m, 2H, thio-H), 6.92-6.98(dd, J_1 = 1.6 Hz, J_2 = 1.2 Hz, 1H, thio-H); MS (EI, 70 eV) *m/z* (%): 386.3 (M^+, 12.13), 193.2 (100), 97.3 (28.84). Anal. calcd for $C_{20}H_{26}N_4S_2$: C 62.14, H 6.78, N 14.49%; found C 62.23, H 6.69, N 14.52%.

Cell culture. K562 cells were cultured in RPMI 1640 medium at 37 with 5% CO_2, and 95% air, supplemented with 10% (v/v) bovine calf serum and 80 U/mL gentamicin. The cells were seeded onto 96-well plates or other appropriate dishes containing the medium at the density of 6250/cm^2.

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REFERENCES AND NOTES

[1] Simona, F.; Riccardo, A.; Marilisa, B.; Margherita, G.; Chiara, C.; Isabelle, Z. C.; Michel, B.; Elise, C. K.; Giacomo, D. L. J Proteome Res 2007, 6, 4330.

[2] Faderl, S.; Talpaz, M.; Estrov, Z.; Kantarjian, H. M. Ann Intern Med 1999, 131, 207.

[3] Petzer, A. L.; Gunsiliusb, E. Arch Med Res 2003, 34, 496.

[4] Cotta, C. V.; Bueso-Ramos, C. E. Ann Diagn Pathol 2007, 11, 68.

[5] Druker, B. J.; Sawyers, C. L.; Kantarjian, H.; Resta, D. J.; Reese, S. F.; Ford, J. M.; Capdeville, R.; Talpaz, M. N. Engl J Med 2001, 344, 1038.

[6] Branford, S.; Rudzki, Z.; Walsh, S.; Grigg, A.; Arthur, C.; Taylor, K.; Herrmann, R.; Lynch, K. P.; Hughes, T. P. Blood 2002, 99, 3472.

[7] Sawyers, C. L.; Hochhaus, A.; Feldman, E.; Goldman, J. M.; Miller, C. B.; Ottmann, O. G.; Schiffer, C. A.; Talpaz, M.; Guilhot, F.; Deininger, M. W.; Fischer, T.; O'Brien, S. G.; Stone, R. M.; Gambacorti-Passerini, C. B.; Russell, N. H.; Reiffers, J. J.; Shea, T. C.; Chapuis, B.; Coutre, S.; Tura, S.; Morra, E.; Larson, R. A.; Saven, A.; Peschel, C.; Gratwohl, A.; Mandelli, F.; Ben-Am, M.; Gathmann, I.; Capdeville, R.; Paquette, R. L.; Druker, B. J. Blood 2002, 99, 3530.

[8] Shah, N.; Nicoll, J.; Nagar, B.; Gorre, M.; Paquette, R.; Kuriyan, J.; Sawyers, C. Cancer Cell 2002, 2, 117.

[9] Talpaz, M.; Silver, R. T.; Druker, B. J.; Goldman, J. M.; Gambacorti-Passerini, C.; Guilhot, F.; Schiffer, C. A.; Fischer, T.; Deininger, M. W.; Lennard, A. L.; Hochhaus, A.; Ottmann, O. G.; Gratwohl, A.; Baccarani, M.; Stone, R.; Tura, S.; Mahon, F. X.; Fernandes-Reese, S.; Gathmann, I.; Capdeville, R.; Kantarjian, H. M.; Sawyers, C. L. Blood 2002, 99, 1928.

[10] Randolph, T. R.. Clin Lab Sci 2005, 18, 49.

[11] Jamieson, C. H.; Ailles, L. E.; Dylla, S. J.; Muijtjens, M.; Jones, C.; Zehnder, J. L.; Gotlib, J.; Li, K.; Manz, M. G.; Keating, A.; Sawyers, C. L.; Weissman, I. L. N. Engl J Med 2004, 351, 657.

[12] Michor, F. Cell 2007, 25, 1114.

[13] Garnovskii, A. D.; Nivorozhkin, A. L.; Minkin, V. I. Coord Chem Rev 1993, 1, 126.

[14] Costamagna, J.; Vargas, J.; Latorre, R.; Alvarado, A.; Mena, G. Coord Chem Rev 1992, 67, 119.

[15] Walsh, C. T.; Orme-Johnson, W. H. Biochemistry 1987, 26, 4901.

[16] Witkop, B.; Ramachandran, L. K. Metabolism 1964, 13, 1016.

[17] Solomon, E. I.; Lowery, M. D. Science 1993, 259, 1575.

[18] Desai, S. B.; Desai, P. B.; Desai, K. R. Heterocycl Commun 2001, 7, 83.

[19] Pathak, P.; Jolly, V. S.; Sharma, K. P. Orient J Chem 2000, 16, 161.

[20] Gerdemann, C.; Eicken, C.; Krebs, B. Chem Res 2002, 35, 183.

[21] Kabeer, A. S.; Baseer, M. A.; Mote, N. A. Asian J Chem 2001, 13, 496.

[22] El-Masry, A. H.; Fahmy, H. H.; Abdelwahed, S. H. A. Molecules 2000, 5, 1429.

[23] More, P. G.; Bhalvankar, R. B.; Patter, S. C. J Ind Chem Soc 2001, 78, 474.

[24] Pandeya, S. N.; Sriram, D.; Nath, G.; Clereq, E. D. II Farmaco 1999, 54, 624.

[25] Singh, W. M.; Dash, B. C. Pesticides 1988, 22, 33.

[26] Samadhiya, S.; Halve, A. Orient J Chem 2001, 17, 119.

[27] Mallikarjun, S. Y.; Sangamesh, A. P. Transition Met Chem 1997, 22, 220.

[28] Yang, G. W.; Xia, X. P.; Tu, H.; Zhao, X. C. Chem Res Appl 1995, 7, 41.

[29] Tarafder, M. T.; Kasbollah, A.; Saravan, N.; Crouse, K. A.; Ali, A. M.; Tin, O. K. J Biochem Mol Biol Biophys 2002, 6, 85.

[30] Kucukguzel, I.; Kucukguzel, S. G.; Rollas, S.; Sanis, G. O.; Ozdemir, O.; Bayrak, I.; Altug, T.; Stables, J. II Farmaco 2004, 59, 839.

[31] Vicini, P.; Geronikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Kabras, C. A.; Colla, P. L. Bioorg Med Chem 2003, 11, 4785.

[32] Pignatello, R.; Panico, A.; Mazzane, P.; Pinizzotto, M. R.; Garozzo, A.; Fumeri, P. M. Eur J Med Chem 1974, 29, 781.

[33] Katia, B.; Simon, L.; Anne, R.; Gerard, C.; Francoise, D.; Bernard, M. Inorg Chem 1996, 35, 387.

[34] Drabent, K.; Bialoska, A.; Ciunik, Z. Inorg Chem Commun 2004, 7, 224.

[35] Klingele, M. H.; Brooker, S. Coord Chem Rev 2003, 241, 119.

[36] Arion, V. B.; Reisner, E.; Fremuth, M.; Jokupec, M. A.; Keppler, B. K.; Kukushkin, V. Y.; Pombeiro, A. J. L. Inorg Chem 2003, 42, 6024.

[37] Hwu, J. R.; Lin, C. C.; Chuang, S. H.; King, K. Y.; Suc, T.-R.; Tsay, S.-C. Bioorg Med Chem 2004, 12, 2509.

[38] Gaston, M. A.; Dias, L. R. S.; Freitas, A. C. C.; Miranda, A. L. P.; Barreiro, E. J. Pharm Acta Helv 1996, 71, 213.

[39] Heringa, M. Int J Clin Pharmacol Ther 2003, 41, 331.

[40] Traub, Y. M.; Redmond, D. P.; Rosenfeld, J. B. Israel J Med Sci 1975, 11, 991.

[41] Martín-Islán, A. P.; Martín-Ramos, D.; Sainz-Díaz, C. I. J Pharm Sci 2008, 97, 815.

[42] Bovill, J. G.; Sebel, P. S.; Stanley, T. H. Anesthesiology 1984, 61, 731.

[43] Stanley, T. S. Anaesth Intensive Care 1987, 15, 38.

[44] Brocks, D. R. J Pharm Pharm Sci 1999, 2, 39.

[45] Nishiyama, K.; Mizuno, T.; Sakuta, M. Adv Neurol 1993, 60, 479.

[46] Dimmock, J. R.; Arora, V. K.; Quail, J. W.; Pugazhenthi, U.; Allen, T. M.; Kao, G. Y.; De Clercq, E. J Pharm Sci 1994, 83, 1124.

[47] Dimmock, J. R.; Arora, V. K.; Wonko, S. L.; Hamon, N. W.; Quail, J. W.; Jia, Z.; Warrington, R. C.; Fang, W. D.; Lee, J. S. Drug Des Deliv 1990, 6, 183.

[48] Wang, H. F.; Xue, S. J.; Zhu, J.; Yang, D. R.; Jin, J.; Fang, Z. K. Chinese J Struct Chem 2009, 28, 742.

[49] Yang, D. R.; Xue, S. J.; Wang, H. F.; Zhu, J.; Jin, J.; Chen, J. J Mol Struc 2009, 929, 97.

[50] Sayer, J. M.; Pinsky, B.; Schonbrunn, A.; Washtien, W. J Am Chem Soc 1974, 96, 7998.

[51] Dega-Szafran, Z.; Jaskolski, M.; Szafran, M.; Dulewicz, E. J Mol Struct 2002, 615, 37.

[52] Dega-Szafran, Z.; Dulewicz, E.; Szafran, M.; Thaimattam, R.; Jaskolski, M. J Mol Struc 2007, 828, 19.

[53] Zhou, B. H.; Chen, Y. F.; Yin, G. D.; Wu, A. X. Chinese J Struct Chem 2006, 25, 127.

[54] Hansen, M. B.; Nielsen, S. E.; Berg, K. J. Immunol Methods 1989, 119, 203.

[55] Jiang, Z. L.; Wang, S. Y.; Duan, H. Chem World 2004, 9, 484.