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Original article

New class of potent antitumor acylhydrazone derivatives containing furan

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ABSTRACT

A pair of chemical isomeric structures of *N*-acylhydrazone compounds **I** and **II** were designed and synthesized. The reaction was carried out with high diastereoselectivity to obtain one configurational isomer in excellent yields. The exact configuration and conformation of **IIa** and **IIe** were confirmed by the X-ray single crystal diffraction. The antitumor bioassay revealed that some compounds exhibited excellent activity against the selected cancer cell lines. In particular, **IIf** ($IC_{50} = 16.4$ μM) was better than doxorubicin ($IC_{50} = 53.3$ μM) against human promyelocytic leukemic cells (HL-60). Their toxicities were predicted *in silico*. The results showed that compounds **II** were safe and eligible to be development candidates. **IIf** showed great promise as a novel lead compound for further anticancer discovery.

1. Introduction

Recently metal ion chelation therapy [1–9] has attracted much attention. Many *in vitro* studies as well as clinical trials have revealed that some tumors, particularly leukemia and neuroblastoma are sensitive to metal ion chelation therapy. Tumor cells are more susceptible than normal cells to ion chelators as shown with the clinically used chelator desferrioxamine (DFO) [10–15] (Fig. 1). However, DFO suffers some disadvantages, such as high cost, a short plasma half-life, limited membrane permeability and poor absorption from the gut, which has led to poor antitumor activity [16]. In order to overcome these issues, it is necessary to develop new compounds with more favorable characteristics.

As ligands with metal ions, hydrazone and acylhydrazone moieties possess a wide range of excellent coordination properties [17–20]. Some compounds, such as **1** [21], **2** [22], **3** [23] and **4** [6] (Fig. 2), exhibited a variety of valuable biological effects [24–26], especially as multidentate ligands to prevent proliferation of cancer cells.

Furan rings are electron-rich systems amenable to being good ligands with metal ions [27–29]. It has been reported that derivatives of furan substituted at the 2- and 5-positions are frequently found in nature. These furan derivatives show broad-spectrum pharmacological properties [30–32]. In our previous work, many compounds containing this moiety were synthesized, in which the bridge linkage (X) was modified with ureide, acylhydrazine, and carbamic acid ester to obtain **5** [33], **6** [34], and **7** [35] (Scheme 1) respectively. Bioassays indicated that the compounds with different bridge linkages (X) showed different activities. For example, **5** had insecticidal activity, **6** showed antitumor activity and **7** possessed fungicidal activity. In the present work, structure optimization was carried out by converting the bridge linkage to acylhydrazone (Scheme 1). For the purpose of discovering novel lead compounds with antitumor bioactivity, a pair of chemical isomeric structures of *N*-acylhydrazone compounds **I** and **II** were designed and synthesized by the route shown in Scheme 2. Their anticancer activity was evaluated and their toxicity was predicted by *in silico* methods to determine whether or not the title compounds were safe and eligible to be lead compounds.

2. Results and discussion

2.1. Synthesis

The synthetic route of compounds **I** and **II** is shown in Scheme 2. Compounds of type **I** were obtained from starting materials of substituted anilines and benzoic acid. The key intermediates **9** were prepared from substituted aniline with furfural by the Meerwein

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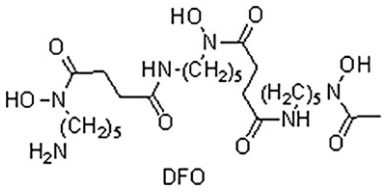


Fig. 1. Chemical structure of the iron chelator desferrioxamine (DFO).

arylation reaction. All type **I** compounds were finally synthesized by the condensation of substituted phenyl hydrazine **8** with 5-substituted phenyl-2-furfural **9** using acetic acid as the catalyst. In general, the condensation yields of the products were more than 90% as detected by HPLC (Table 1). By the same procedure as that of **I**, compounds of type **II** were obtained in higher yields of more than 95%. HPLC analysis (Table 1, Fig. 3 and Fig. 4) of the target compounds indicated that the condensation reaction was highly diastereoselective with only one isomer in excellent yields.

The structures of all the compounds were characterized by ^{1}H NMR, IR, UV and elemental analyses. In the IR spectra, the compounds showed absorption bands around 3400 cm^{-1} originating from the N—H stretching vibration. The bands between 1690 and 1630 cm^{-1} could be assigned to the C=N stretching vibration. The strong bands around 1650 cm^{-1} were carbonyl vibrations of the secondary amide. Absorption bands around 1610, 1520 and 1480 cm^{-1} were attributed to the frame vibration of the phenyl and furan ring.

In the ^{1}H NMR spectrum, one sharp peak from 11.50 to 12.20 ppm was due to the presence of the proton in secondary amide NH. Another sharp peak from 8.30 to 8.95 ppm was due to the proton in the imine N=CH in the Schiff base. Mostly, the protons on phenyl rings were split into multiple peaks from 7.10 to 8.30 ppm and the protons on the furan ring were split into a doublet from 6.92 to 7.40 ppm. The splits of most compounds were normal, except for the compounds with fluorine substitution, because of the coupling and splitting between fluorine and hydrogen. The fluorine atom splits a hydrogen proton into a doublet, which complicated the signal. For example, one of the protons on the furan ring was split into a triplet when the fluorine atom existed nearby.

Karabatsos *et al.* [36–38] reported that the presence of the singlet signals related to the imine hydrogen was differently attributed to the respective (*E*) and (*Z*)-diastereomers. The imine-attached hydrogen signal of the (*E*)-diastereomer is downfielded by 0.2–0.3 ppm from the corresponding hydrogen atom signal in the (*Z*)-diastereomer. The ^{1}H NMR spectra of the title compound indicated that only one isomer was obtained. However, its configuration could not be determined according to the present ^{1}H NMR spectra since the different substituted groups in the benzene ring lead to different effects on the shift of the imine hydrogen, which caused different results from that of Karabatsos. Thus, an alternative method was necessary to unambiguously characterize the geometry of the isomer and assure the relative configuration of the imine double bond of acylhydrazone derivatives.

2.2. Crystallography

The single crystals of the target compounds **IIa** and **IIe** were cultured for the X-ray diffractions to define the configuration. Their crystal data are presented in Table 5. Their perspective views with the atomic labeling system are shown in Figs. 5 and 6. Both **IIa** and **IIe** are the *E*-isomers.

The conformation between the double bond in the furan ring and the conjoint carbonyl group in **IIa** and **IIe** was different. **IIa** was in the *s-cis* conformation and **IIe** was in the *s-trans* conformation. In Fig. 7 the intermolecular hydrogen bonds between the target compound **IIa** gave the *s-cis* conformation and *via* hydrogen bonds led to infinite extending in the *a*-axis in the crystal lattice. Fig. 7 suggested that **IIa** possessed a dimer structure mode.

Fig. 8 shows the formation of a hydrogen bond between the carbonyl group and the solvent methanol resulting in different stabilization of the *s-trans* isomer. Two molecules of **IIe** were linked by methanol *via* hydrogen bonds, which led to infinite extending in the *a*-axis in the crystal lattice. The existence of the hydrogen bonds can stabilize the pack of the crystal to form the single crystal. If the methanol was replaced with other solvents (lower polarity and lack of active hydrogen as the hydrogen bond donor, such as ethyl acetate or ethyl ether), the single crystal could not be obtained.

2.3. Bioassay

Compounds **Id**, **If**, **IIa**, **IId**, and **IIf** exhibited better antitumor activity than the others in the preliminary test (Table 2). The IC_{50} values of these five compounds are shown in Table 3, in which the activity of **IIf** ($IC_{50} = 16.4$ μM) was better than that of the doxorubicin ($IC_{50} = 53.3$ μM) to human promyelocytic leukemic cells (HL-60). **IIf** exhibited good activity against the solid human gastric carcinoma cells (BGC-823) and human hepatocellular carcinoma cells (Bel-7402). Its activity against BGC-823 ($IC_{50} = 9.6$ μM) and Bel-7402 ($IC_{50} = 8.3$ μM) was close to that of doxorubicin ($IC_{50} = 4.8$ and 5.0 μM). **Id** also had good activity against BGC-823 ($IC_{50} = 9.5$ μM). However, the activity of these five compounds against human nasopharyngeal carcinoma cells (KB) was poor.

The preliminary structure–activity relationship analysis indicated that compounds in which the R^1 was 2-hydroxyl (**Id**, **If**, **IId**, and **IIf**) or 2-methoxyl (**II e**) group, showed better activity than the others. The oxygen atom on the *ortho*-position might play an important role on the chelation of ion combined with the nitrogen atoms of imine and carbonyl amide to form bidentate or tridentate ligands. The oxygen atom should make the coordination mode more favorable for bioactivity.

2.4. Prediction of toxicity

The discovery of novel chemical agents with good biological activity together with reduced mammalian toxicity is a difficult process and it is essential that an indication of the toxicity of selected compounds is established as rapidly as possible. Predictive *in silico* methods have been shown to be a useful tool for the generation of this information at a relatively low cost.

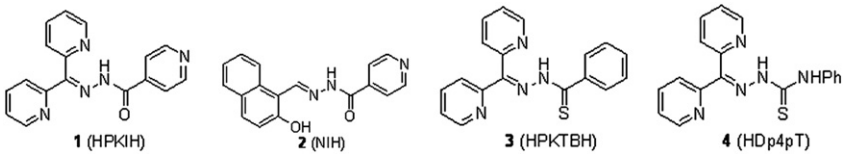
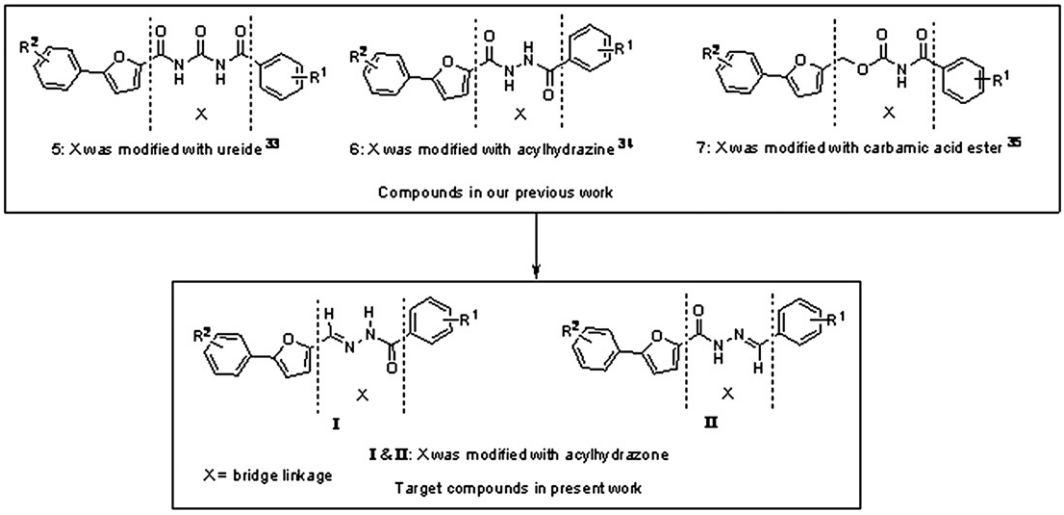


Fig. 2. Chemical structures of the iron chelators containing hydrazone moieties: di-2-pyridylketone isonicotinoyl hydrazone (HPKIH), 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (NIH), di-2-pyridylketone thiobenzoyl hydrazone (HPKTBH) and 4-phenyl-1-(di(pyridin-2-yl)methylene) thiosemicarbazide (HDp4pT).

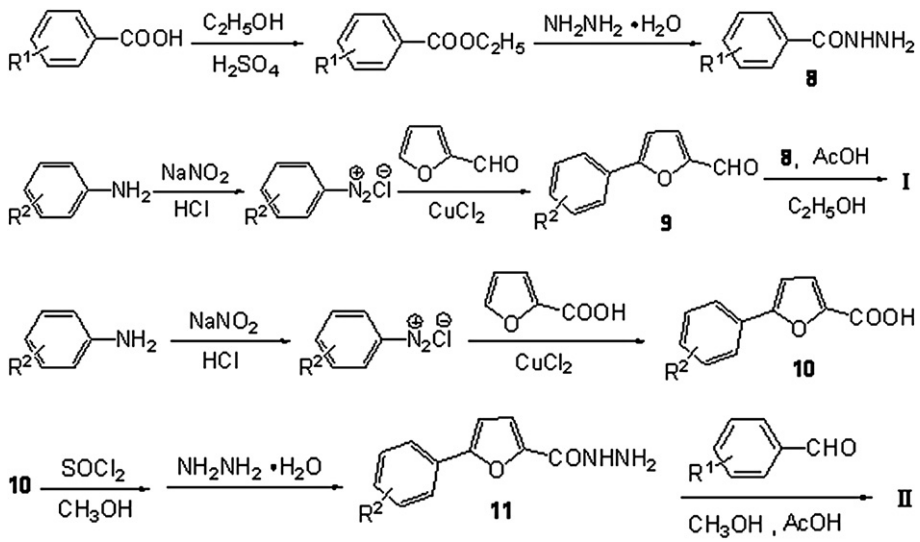


Scheme 1. Design strategy for compounds **I** and **II**.

All the predicted values of title compounds are listed in Table 4. For the carcinogenic toxicity, when the predicted value is more than 70%, and the value of CIP (Carcinogenic Impossibility) is more than 0.65, meanwhile its CIP value is greater than CP (Carcinogenic Possibility), the compound is considered as non-carcinogenic. Otherwise, they were considered to be carcinogenic. For the mutagenic toxicity, if the predictability value was more than 70% and its MIP (Mutagenic Impossibility) value was greater than the MP (Mutagenic Possibility), the compound was considered as non-mutagenic. Otherwise, they were considered to be mutagenic. Based on the results in Table 4, the carcinogenic toxicity data of compounds **II** were from 93% to 95% and their CIP values were from 0.849 to 0.951, much higher than their CP values of 0.001. The mutagenic toxicity data of compounds **II** were from 97% to 98% and their MIP values were from 0.630 to 0.950, much higher than the MP values of 0.01. Thus, compounds **II** were considered to be low carcinogenic toxicity and mutagenic toxicity. The results indicated that **IIf** was safe and could be regarded as a candidate for further study. Unfortunately, according to the judgement standard, the CIP values of compounds **I** were less than 0.65, suggesting potential carcinogenic toxicity.

3. Conclusion

In summary, a pair of isomeric structures were designed and synthesized. A single configurational isomer of each of the target compounds was obtained through a highly diastereoselective reaction. In general, the yields of the target compounds were more than 95%. The exact structures of **IIa** and **IIe** were confirmed by the X-ray single crystal diffraction. Both of them had the *E*-configuration about the C=N bond. The conformation about the single bond of the furan arene-carbonyl system in **IIa** was *s-cis* and in **IIe** was *s-trans*. The antitumor activity showed that some of the compounds exhibited excellent activity against the selected cancer cell lines compared to the positive control doxorubicin. The activity of **IIf** against human promyelocytic leukemic cells (HL-60) was better than that of doxorubicin. The predictive toxicity results showed that **IIf** was safe and eligible to be a lead compound for further development.



Scheme 2. The synthetic routes of compounds **I** and **II**.

Table 1
Yield and retention time analyses of **I** and **II** by HPLC.

Compd.	R^1	R^2	Yield/%	R_f[a] (min)
Ia	2-OCH_3	2.4-2F	92.02	7.629
Ib	4-Br	2-Cl	98.58	3.468
Ic	4-Cl	2-F	99.75	3.146
Id	2-OH	4-F	96.66	2.859
Ie	H	4-Cl	96.04	3.024
If	2-OH	2.4-2F	99.68	3.068
Ig	3-CH_3	2-NO_2	98.87	2.754
Ih	4-Br	3-F	81.80	3.127
IIa	2-OCH_3	2.4-2F	99.33	3.272
IIb	4-Br	2-Cl	95.99	3.678
IIc	4-Cl	2-F	98.09	3.415
IId	2-OH	4-F	98.50	7.051
IIe	H	4-Cl	98.21	3.225
IIf	2-OH	2.4-2F	99.71	9.374
IIg	3-CH_3	2-NO_2	98.73	2.86
IIh	2-Cl	4-F	98.87	3.304
IIi	4-OH	4-F	98.80	2.668
IIj	3-Br	2-F	98.71	3.433

[a] Retention factor was determined by HPLC by using a Rexchrom 5 lm RP-18 column (125 × 4.6 mm) and a mixture of methanol–water (9:1 v/v) as eluent at flow rate of 0.8 mL/min.

4. Materials and methods

4.1. Instruments

All the melting points were determined with a Cole-Parmer melting point apparatus while the thermometer was uncorrected. IR spectra were recorded on a NEXUS-470 FTIR(Nicolet) spectrometer with KBr pellets. ^{1}H NMR spectra were recorded with a Bruker DPX300 instrument, while tetramethylsilane was used as the internal standards. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F254), and spots were visualized with ultraviolet (UV) light. Elemental analysis, which was performed at the laboratories of the Institute of Chemistry, Chinese Academy of Sciences, was carried out with a Flash EA 1112 elemantal analyzer.

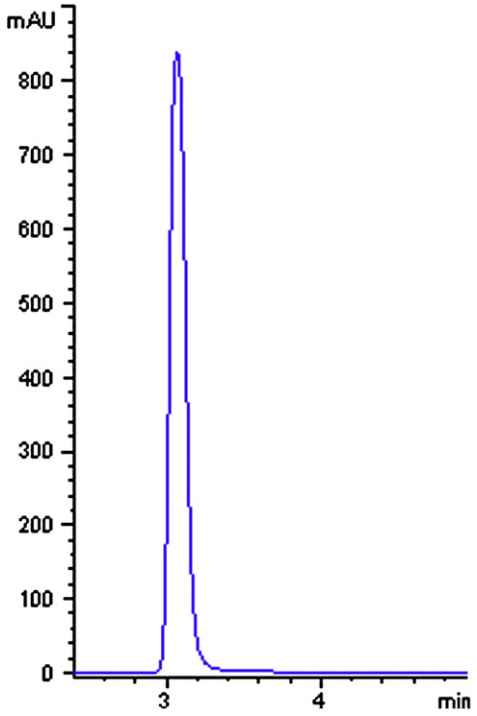


Fig. 3. HPLC chromatogram of the target compound **If**. Only one isomer was found at the retention time of 3.068 min.

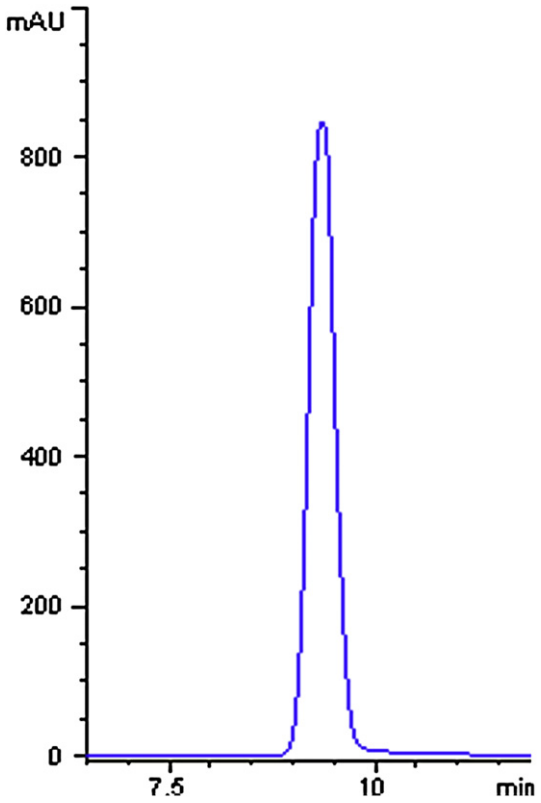


Fig. 4. HPLC chromatogram of the target compound **IIf**. Only one isomer was found at the retention time of 9.374 min.

4.2. Synthetic procedures

4.2.1. General synthetic procedure for the key intermediates

Intermediates **8** and **11** were synthesized according to the literature [34,39]. Intermediates **9** and **10** were synthesized from substituted aniline by Meerwein arylation reaction using the reported procedure [34,35,40].

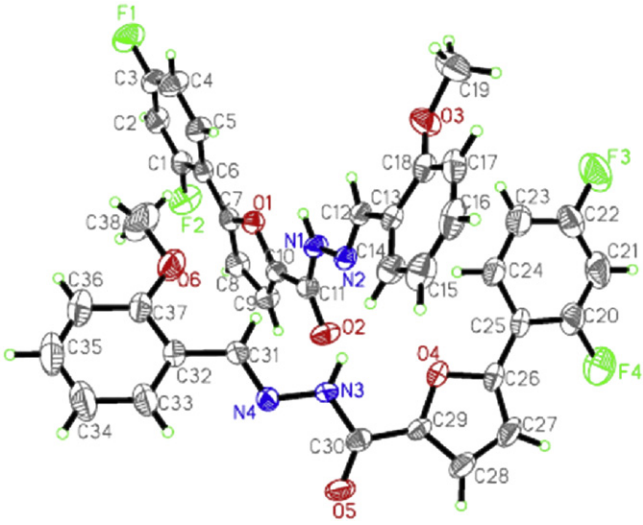


Fig. 5. Molecular structures of **IIa**. Displacement ellipsoids are drawn at the 30% probability level.

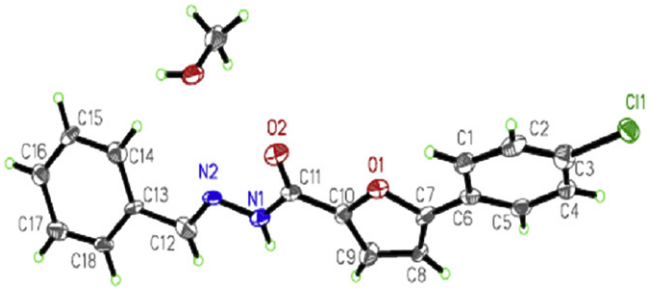


Fig. 6. Molecular structure of **IIe**. Displacement ellipsoids are drawn at the 30% probability level.

*4.2.2. General synthetic procedure for the target compounds **I** and **II***

A mixture of aldehyde **9** (0.05 mol) and acylhydrazine **8** (0.05 mol) reacted in ethanol under reflux for 5 h. The reaction was catalyzed by drops of acetic acid. After cooling, the solvent was removed under reduced pressure, and the solid was recrystallized from ethanol to obtain the target compounds **I**. Using the same method, compound **II** was obtained by the reaction of substituted aldehyde (0.05 mol) with 5-substituted phenyl-2-furoyl hydrazide **10** (0.05 mol).

All the synthesized compounds were solid. Their structures were confirmed by ^{1}H NMR, IR, UV and elemental analysis.

*4.2.2.1. (E)-5-(2′,4′-difluorophenyl)furan-2-carbaldehyde-2-methoxybenzoyl hydrazone (**Ia**).* Light yellow powdery crystals: m.p. 171–172 °C. IR (KBr) ν_{max}: 3248.7, 2935.8, 1661.5, 1605.7, 1550.0, 1485.1, 1297.5, 1269.8, 1251.7, 1141.2, 1103.6, 1024.8, 966.8, 845.1, 797.7, 751.8, 605.3 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.89 (s, 3H, OCH_3), 6.97–7.00 (m, 1H, FuH), 7.05–7.10 (m, 2H, FuH + ArH), 7.17–7.19 (m, 1H, ArH), 7.26–7.27 (m, 1H, ArH-Fu), 7.46–7.55 (m, 2H, ArH + ArH-Fu), 7.62 (dd, J = 7.56, 1.74 Hz, 1H, ArH), 7.90–7.93 (m, 1H, ArH-Fu), 8.31 (s, 1H, CH=N), 11.54 (s, 1H, NH). MS/ESI: *m/e* (%) 395.0 $[M+K]^+$ (2.5), 379.0 $[M+Na]^+$ (19.0), 357.1 $[M+H]^+$ (100). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 349.5 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{19}H_{14}F_2N_2O_3$: C, 64.04; H, 3.96; N, 7.86. Found: C, 63.92; H, 3.89; N, 7.92.

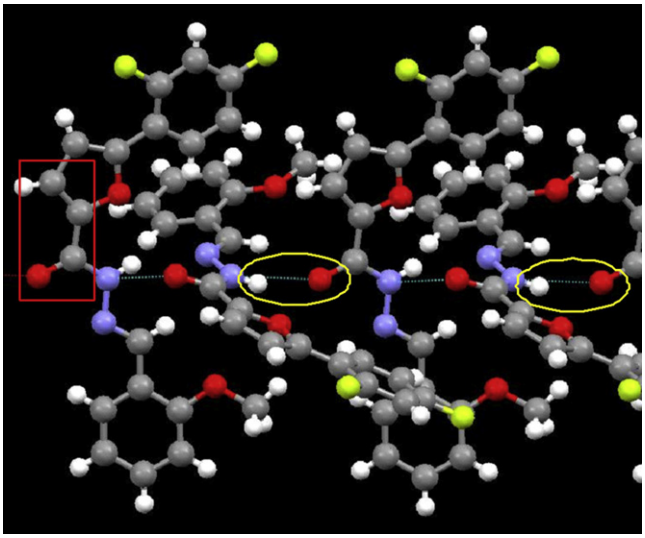


Fig. 7. IIa: H-bond with each other to form the *s-cis* conformation.

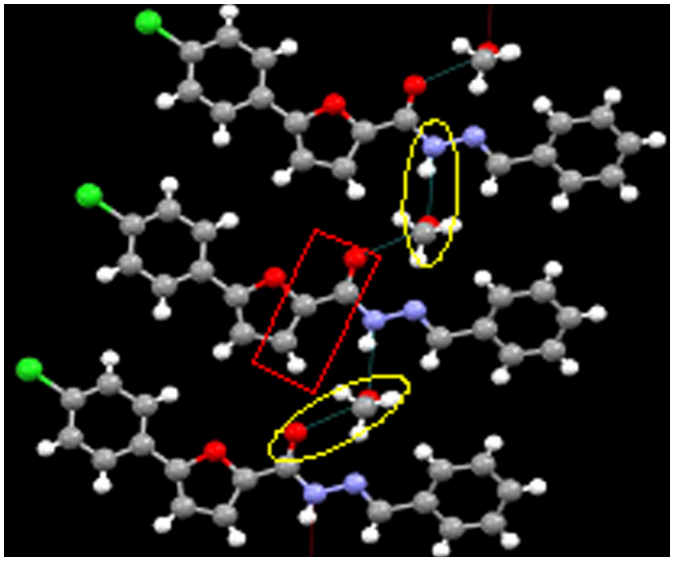


Fig. 8. IIe: H-bond with the methanol to form the *s-trans* conformation.

*4.2.2.2. (E)-5-(2′-chlorophenyl)furan-2-carbaldehyde-4-bromobenzoyl hydrazone (**Ib**).* Light yellow needle crystals: m.p. 200–201 °C. IR (KBr) ν_{max}: 3243.8, 3085.4, 3054.7, 1654.8, 1621.8, 1593.6, 1551.0, 1481.7, 1466.2, 1298.7, 1148.7, 1071.5, 1024.9, 900.7, 835.8, 798.1, 760.2, 648.9 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.14 (d, J = 3.60 Hz, 1H, FuH), 7.29 (d, J = 3.60 Hz, 1H, FuH), 7.41 (td, J = 1.68, 7.64 Hz, 1H, ArH-Fu), 7.48–7.53 (m, 1H, ArH-Fu), 7.60 (dd, J = 1.22, 7.89 Hz, 1H, ArH-Fu), 7.76 (d, J = 8.49 Hz, 2H, ArH), 7.86–7.93 (m, 3H, ArH-Fu + 2ArH), 8.41 (s, 1H, CH=N), 11.96 (s, 1H, NH). MS/ESI: *m/e* (%) 427.1 $[M+Na]^+$ (20.7) 405.2 $[M+H]^+$ (100). UV (nm) λ_{max}: 200.3 ($\pi \rightarrow \pi^*$), 240.2 ($n \rightarrow \sigma^*$), 350.7 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}BrClN_2O_2$: C, 53.56; H, 3.00; N, 6.94. Found: C, 53.94; H, 3.14; N, 7.04.

*4.2.2.3. (E)-5-(2′-fluorophenyl)furan-2-carbaldehyde-4-chlorobenzoyl hydrazone (**Ic**).* Yellow powdery crystals: m.p. 179–180 °C. IR (KBr) ν_{max}: 3300.2, 3036.0, 1649.0, 1597.7, 1554.4, 1484.9, 1292.1, 1273.4, 1220.5, 1147.0, 1030.2, 898.8, 805.6, 756.1, 661.8 cm^{-1}.

Table 2
The antitumor activity of the target compounds **I** and **II**.[a]

Compd.	Inhibition rate (%)			
	HL-60	BGC-823	Bel-7402	KB
Ia	7.77	35.87	1.69	10.07
Ib	–	–	–	–
Ic	6.13	31.54	2.96	4.62
Id	−2.08	71.53	81.64	84.59
Ie	15.05	19.44	−7.17	3.20
If	−9.50	68.43	63.42	61.81
Ig	6.25	36.09	5.67	5.90
Ih	25.91	46.74	28.19	25.96
IIa	27.41	66.76	53.06	59.54
IIb	–	–	–	–
IIc	6.49	32.56	16.88	11.26
IId	13.09	74.04	39.99	53.91
IIe	25.76	33.71	2.28	16.99
IIf	43.07	79.08	91.77	75.25
IIg	9.54	31.37	6.66	10.30
IIh	−3.22	32.03	4.81	19.26
IIi	1.07	46.30	10.64	11.03
IIj	27.65	43.61	12.54	26.46

– Means not detected.
[a] Mean values based on three independent experiments.

Table 3
The IC_{50} values of antitumor activity of the target compounds **I** and **II**.

Method	Cell line	Human tumor	IC50 (μM)					
			Id	**If**	**IIa**	**IId**	**IIf**	Doxorubicin
MTT	HL-60	Leukemia	61.6	125.2	>40	63.1	16.4	53.3
SRB	BGC-823	Gastric cancer	9.5	46.6	81.6	180.4	9.6	4.8
SRB	Bel-7402	Hepatoma	15.2	19.3	24.6	50.3	8.3	5.0
SRB	KB	Nasopharyngeal cancer	39.0	55.3	>40	>40	>40	5.8

^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.03 (t, $J = 3.51$ Hz, 1H, ArH-Fu), 7.14 (d, $J = 3.57$ Hz, 1H, FuH), 7.34–7.45 (m, 3H, 2ArH-Fu + FuH), 7.62–7.65 (m, 2H, ArH), 7.86–7.96 (m, 3H, ArH-Fu + 2ArH), 8.42 (s, 1H, CH═N),11.97 (s, 1H, NH). MS/ESI: *m*/*e* (%) 381.0 $[M + K]^+$ (17.7), 365.2 $[M + Na]^+$ (100), 343.3 $[M + H]^+$ (28.3). UV (nm) λ_{max}: 197.9 ($\pi \rightarrow \pi^*$), 233.2 ($n \rightarrow \sigma^*$), 349.5 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}ClFN_2O_2$: C, 63.08; H, 3.53; N, 8.17. Found: C, 63.04; H, 3.58; N, 8.15.

*4.2.2.4. (E)-5-(4′-fluorophenyl)furan-2-carbaldehyde-2-hydroxybenzoyl hydrazone (**Id**).* Light yellow powdery crystals: m.p. 243–244 °C. IR (KBr) ν_{max}: 3243.8, 1626.4, 1545.9, 1520.3, 1493.7, 1456.6, 1373.9, 1235.8, 1144.3, 1025.4, 834.4, 783.1, 752.4, 617.9 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.94–7.00 (m, 2H, ArH), 7.10 (d, $J = 3.60$ Hz, 1H, FuH), 7.16 (d, $J = 3.60$ Hz, 1H, FuH), 7.31–7.37 (m, 2H, ArH-Fu), 7.42–7.48 (m, 1H, ArH), 7.84–7.88 (m, 3H, ArH + 2ArH-Fu), 8.39 (s, 1H, CH═N), 11.85 (br s, 2H, NH + OH). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 355.5 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{13}FN_2O_3$: C, 66.66; H, 4.04; N, 8.64. Found: C, 66.53; H, 4.13; N, 8.91.

*4.2.2.5. (E)-5-(4′-chlorophenyl)furan-2-carbaldehyde benzoyl hydrazone (**Ie**).* White powdery crystals: m.p. 214–215 °C. IR (KBr) ν_{max}: 3224.4, 3194.8, 3032.8, 1651.0, 1616.5, 1555.0, 1473.5, 1279.9, 1146.6, 1090.5, 1026.1, 975.6, 829.4, 789.5, 689.6, 501.7 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.08 (d, $J = 3.48$ Hz, 1H, FuH), 7.21 (d, $J = 3.54$ Hz, 1H, FuH), 7.52–7.64 (m, 5H, 3ArH + 2ArH-Fu), 7.81–7.84 (m, 2H, ArH), 7.91–7.93 (m, 2H, ArH-Fu), 8.40 (s, 1H, CH═N), 11.88 (s, 1H, NH). MS/ESI: *m*/*e* (%) 363.0 $[M + K]^+$ (7.8), 347.1 $[M + Na]^+$ (100), 325.2 $[M + H]^+$ (63.0). UV (nm) λ_{max}: 199 ($\pi \rightarrow \pi^*$), 232.0 ($n \rightarrow \sigma^*$), 356.7 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{13}ClN_2O_2$: C, 66.57; H, 4.03; N, 8.63. Found: C, 66.51; H, 4.15; N, 8.40.

Table 4
The predictive toxicity of the target compounds **I** and **II**.

Compd.	Carcinogenic toxicity			Mutagenic toxicity			Clog *P*	Mark
	Pred. (%)	CP	CIP	Pred. (%)	MP	MIP		
Ia	97	0.001	0.386	98	0.01	0.800	3.70	Caution[a]
Ib	–	–	–	–	–	–	–	
Ic	97	0.001	0.257	98	0.01	0.570	4.17	Caution[a]
Id	99	0.001	0.390	99	0.01	0.410	4.10	Caution[a]
Ie	99	0.001	0.133	99	0.01	0.300	4.08	Caution[a]
If	97	0.001	0.561	98	0.01	0.760	4.15	Caution[a]
Ig	96	0.004	0.023	98	0.12	0.010	3.64	Caution[a]
Ih	99	0.001	0.106	99	0.01	0.210	4.59	Caution[a]
IIa	94	0.001	0.870	97	0.01	0.950	3.92	
IIb	93	0.001	0.873	97	0.01	0.750	5.25	
IIc	93	0.001	0.911	97	0.01	0.850	4.57	
IId	95	0.001	0.891	98	0.01	0.720	4.32	
IIe	95	0.001	0.849	98	0.01	0.630	4.48	
IIf	94	0.001	0.951	97	0.01	0.940	4.37	
IIg	–	–	–	–	–	–	–	
IIh	94	0.001	0.947	98	0.01	0.830	4.18	
IIi	95	0.001	0.748	98	0.01	0.670	3.45	
IIj	93	0.001	0.899	97	0.01	0.890	4.77	

– means not detected.
[a] the compound considered toxic.

*4.2.2.6. (E)-5-(2′4,-difluorophenyl)furan-2-carbaldehyde-2-hydroxybenzoyl hydrazone (**If**).* Light yellow powdery crystals: m.p. 248–249 °C. IR (KBr) ν_{max}: 3235.5, 3073.0, 2928.7, 1638.5, 1606.7, 1548.9, 1519.4, 1489.7, 1452.7, 1373.2, 1301.6, 1270.1, 1221.9, 1145.7, 1103.8, 1027.2, 969.6, 908.6, 850.7, 783.8, 753.0, 621.9 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.98–7.05 (m, 3H, 2ArH + FuH), 7.13 (d, $J = 3.54$ Hz, 1H, FuH), 7.27 (td, $J = 8.45$, 2.34 Hz, 1H, ArH-Fu), 7.41–7.48 (m, 2H, ArH-Fu + ArH), 7.86–7.93 (m, 2H, ArH-Fu + ArH), 8.42 (s, 1H, CH═N), 11.80 (s, 1H, OH), 11.87 (s, 1H, NH). MS/ESI: *m*/*e* (%) 381.0 $[M + K]^+$ (4.8), 365.0 $[M + Na]^+$ (100), 343.2 $[M + H]^+$ (20.4). UV (nm) λ_{max}: 200.3 ($\pi \rightarrow \pi^*$), 353.1 ($n \rightarrow \pi^*$). Anal. Calcd.

Table 5
Crystal structure data for compounds **IIa** and **IIe**.

	IIa	**IIe**
Empirical formula	$C_{19}H_{14}F_2N_2O_3$	$C_{38}H_{34}Cl_2N_4O_6$
Formula weight	356.32	713.59
T	113(2) K	113(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Triclinic	Triclinic
Space group	*P*1	*P*1
Unit cell dimensions	$a = 8.767(4)$ Å, $\alpha = 105.334(9)^\circ$ $b = 13.404(7)$ Å, $\beta = 99.257(9)^\circ$ $c = 16.815(8)$ Å, $\gamma = 107.126(10)^\circ$	$a = 6.4301(13)$ Å, $\alpha = 103.54(3)^\circ$ $b = 9.1796(18)$ Å, $\beta = 91.42(3)^\circ$ $c = 15.822(3)$ Å, $\gamma = 110.24(3)^\circ$
Volume	1759.1(15) Å^3	846.1(3) Å^3
Z	4	1
D_x	1.345 mg/m^3	1.400 mg/m^3
Absorption coefficient	0.106 mm^{-1}	0.247 mm^{-1}
F (0 0 0)	736	372
Crystal dimensions	0.30 × 0.20 × 0.10 mm	0.24 × 0.18 × 0.04 mm
θ range for data collection	1.30–25.02	2.45–25.02°
Limiting indices	$-10 \le h \le 9$, $-15 \le k \le 13$, $-16 \le l \le 20$	$-7 \le h \le 6$, $-8 \le k \le 10$, $-18 \le l \le 18$
Reflection collected/unique	9215/6179 [*R*(int) = 0.0390]	4405/2820 [*R*(int) = 0.0935]
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.9895 and 0.9688	0.9902 and 0.9432
Data/restraints/parameters	6179/0/471	2820/0/229
Goodness-of-fit on F^2	1.007	3.331
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0529$, $wR_2 = 0.0988$	$R_1 = 0.2656$, $wR_2 = 0.6557$
$2\theta_{max}$	50.04° with Mo *K*α	50.04° with Mo *K*α
$(\Delta\rho)_{max}$	0.190 e Å^{-3}	1.820 e Å^{-3}
$(\Delta\rho)_{min}$	−0.174 e Å^{-3}	−1.308 e Å^{-3}
Program system	SHELXS-97, SHELXL-97	SHELXS-97, SHELXL-97
Structure determination	Direct method	Direct method
Refinement	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
CCDC No.	761107	761106

(%) for $C_{18}H_{12}F_2N_2O_3$: C, 63.16; H, 3.53; N, 8.18. Found: C, 63.04; H, 3.71; N, 7.85.

*4.2.2.7. (E)-5-(2′-nitrophenyl)furan-2-carbaldehyde-3-methylbenzoyl hydrazone (**Ig**).* Yellow powdery crystals: m.p. 160–161 °C. IR (KBr) ν_{max}: 3192.8, 3015.1, 1644.0, 1605.6, 1559.2, 1527.0, 1346.8, 1306.5, 1287.9, 1220.1, 1036.5, 850.3, 785.9, 744.2, 703.9 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 7.06 (d, $J = 3.63$ Hz, 1H, FuH), 7.11–7.12 (m, 1H, ArH), 7.41–7.45 (m, 2H, ArH + FuH), 7.62–7.82 (m, 4H, 2ArH-Fu + 2ArH), 7.90–7.99 (m, 2H, ArH-Fu), 8.38 (s, 1H, CH=N), 11.86 (s, 1H, NH). MS/ESI: *m/e* (%) 388.0 $[M + K]^+$ (2.3), 372.1 $[M + Na]^+$ (100), 350.2 $[M + H]^+$ (33.8). UV (nm) λ_{max}: 197.9 ($\pi \rightarrow \pi^*$), 332.8 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{19}H_{15}N_3O_4$: C, 65.32; H, 4.33; N, 12.03. Found: C, 65.15; H, 4.27; N, 12.14.

4.2.2.8. (E)-5-(3′-fluorophenyl)furan-2-carbaldehyde-4-bromobenzoyl hydrazone (Ih). Yellow powdery crystals: m.p. 181–182 °C. IR (KBr) ν_{max}: 3444.4, 3209.4, 3062.8, 1658.7, 1598.6, 1571.5, 1479.7, 1313.4, 1270.5, 1220.0, 1173.8, 1070.2, 1039.8, 982.5, 955.4, 898.1, 814.9, 781.6, 754.8, 703.1, 680.0 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.11–7.28 (m, 3H, 2FuH + ArH-Fu), 7.48–7.70 (m, 3H, ArH-Fu), 7.73–7.75 (m, 2H, ArH), 7.86–7.89 (m, 2H, ArH), 8.40 (s, 1H, CH=N), 11.96 (s, 1H, NH). MS/ESI: *m/e* (%) 410.9 $[M + Na]^+$ (43.4), 389.1 $[M + H]^+$ (100). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 234.4 ($n \rightarrow \sigma^*$), 353.1 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}BrFN_2O_2$: C, 55.83; H, 3.12; N, 7.23. Found: C, 55.65; H, 3.38; N, 7.20.

*4.2.2.9. (E)-2-methoxybenzaldehyde-5-(2,4-difluorophenyl)-2-furoyl hydrazone (**IIa**).* Light yellow powdery crystals: m.p. 197–198 °C. IR (KBr) ν_{max}: 3196.1, 3034.2, 2939.9, 2837.9, 1655.0, 1602.4, 1550.9, 1487.4, 1464.4, 1432.4, 1358.1, 1274.3, 1251.9, 1155.8, 1106.6, 1068.8, 1028.6, 969.3, 886.5, 844.9, 806.2, 753.3 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.90 (s, 3H, OCH_3), 6.98–7.08 (m, 2H, FuH + ArH), 7.12–7.15 (m, 1H, ArH), 7.33 (td, $J = 8.38$, 1.79 Hz, 1H, ArH-Fu), 7.42–7.52 (m, 3H, ArH-Fu + ArH + FuH), 7.90 (d, $J = 6.81$ Hz, 1H, ArH), 8.25–8.27 (m, 1H, ArH-Fu), 8.88 (s, 1H, CH=N), 11.90 (s, 1H, NH). MS/ESI: *m/e* (%) 379.0 $[M + Na]^+$ (6.5), 357.1 $[M + H]^+$ (100). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 337.6 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{19}H_{14}F_2N_2O_3$: C, 64.04; H, 3.96; N, 7.86. Found: C, 64.13; H, 4.06; N, 7.80.

*4.2.2.10. (E)-4-bromobenzaldehyde-5-(2-chlorophenyl)-2-furoyl hydrazone (**IIb**).* Light yellow powdery crystals: m.p. 189–190 °C. IR (KBr) ν_{max}: 3433.8, 3263.1, 3076.5, 1665.3, 1591.9, 1549.0, 1484.7, 1465.6, 1356.2, 1299.8, 1249.7, 1169.6, 1069.0, 1027.7, 839.7, 809.3, 757.7 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.33 (d, $J = 3.69$ Hz, 1H, FuH), 7.42–7.56 (m, 3H, FuH + 2ArH-Fu), 7.61–7.74 (m, 5H, 4ArH + ArH-Fu), 8.15–8.17 (m, 1H, ArH-Fu), 8.50 (s, 1H, CH=N), 11.94 (s, 1H, NH). MS/ESI: *m/e* (%) 405.1 $[M + H]^+$ (100). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 329.2 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}BrClN_2O_2$: C, 53.56; H, 3.00; N, 6.94. Found: C, 53.34; H, 3.19; N, 7.02.

*4.2.2.11. (E)-4-chlorobenzaldehyde-5-(2-fluorophenyl)-2-furoyl hydrazone (**IIc**).* White powdery crystals: m.p. 209–210 °C. IR (KBr) ν_{max}: 3234.3, 3057.6, 3035.5, 1672.7, 1647.7, 1598.4, 1552.8, 1485.9, 1357.5, 1295.5, 1222.5, 1154.4, 1088.7, 1069.9, 1023.2, 835.0, 801.6, 754.5,585.6 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.05 (t, $J = 3.48$ Hz, 1H, ArH-Fu), 7.36–7.56 (m, 6H, 2FuH + 2ArH-Fu + 2ArH), 7.77–7.80 (m, 2H, ArH), 8.15–8.20 (m, 1H, ArH-Fu), 8.52 (s, 1H, CH=N), 11.96 (s, 1H, NH). MS/ESI: *m/e* (%) 381.0 $[M + K]^+$ (12.6), 365.2 $[M + Na]^+$ (100), 343.4 $[M + H]^+$ (23.8). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 328.0 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}ClFN_2O_2$: C, 63.08; H, 3.53; N, 8.17. Found: C, 63.22; H, 3.53; N, 8.51.

*4.2.2.12. (E)-2-hydroxybenzaldehyde-5-(4-fluorophenyl)-2-furoyl hydrazone (**IId**).* Yellow powdery crystals: m.p. 113–114 °C. IR (KBr) ν_{max}: 3404.6, 3027.4, 2883.7, 1650.6, 1622.9, 1604.4, 1586.6, 1486.0, 1375.2, 1315.8, 1274.3, 1234.3, 1160.5, 1035.1, 981.4, 959.9, 837.7, 810.3, 752.0, 601.9, 513.9 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 6.91–6.96 (m, 2H, ArH), 7.19 (d, $J = 3.66$ Hz, 1H, FuH), 7.29–7.42 (m, 4H, 2ArH + ArH-Fu + FuH), 7.59–7.61 (m, 1H, ArH), 8.01–8.05 (m, 2H, ArH-Fu), 8.73 (s, 1H, CH=N), 11.10 (s, 1H, OH), 12.06 (s, 1H, NH). MS/ESI: *m/e* (%) 363.0 $[M + K]^+$ (5.8), 347.1 $[M + Na]^+$ (100), 325.3 $[M + H]^+$ (47.5). UV (nm) λ_{max}: 214.4 ($\pi \rightarrow \pi^*$), 279.3 ($n \rightarrow \sigma^*$), 341.1 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{13}FN_2O_3$: C, 66.66; H, 4.04; N, 8.64. Found: C, 66.51; H, 4.16; N, 8.70.

*4.2.2.13. (E)-benzaldehyde 5-(4-chlorophenyl)-2-furoyl hydrazone (**IIe**).* Light yellow needle crystals: m.p. 188–189 °C. IR (KBr) ν_{max}: 3430.8, 3057.4, 2946.2, 1649.3, 1524.2, 1475.0, 1410.5, 1346.2, 1279.4, 1092.1, 1017.7, 949.5, 833.4, 754.9, 691.5, 506.0 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.26 (d, $J = 3.63$ Hz, 1H, FuH), 7.41–7.61 (m, 6H, FuH + 2ArH-Fu + 3ArH), 7.74–7.77 (m, 2H, ArH), 7.99–8.01 (m, 2H, ArH-Fu), 8.53 (s, 1H, CH=N), 11.86 (s, 1H, NH). MS/ESI: *m/e* (%) 363.2 $[M + K]^+$ (51.8), 347.3 $[M + Na]^+$ (91.4), 325.4 $[M + H]^+$ (100). UV (nm) λ_{max}: 197.9 ($\pi \rightarrow \pi^*$), 331.6 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{13}ClN_2O_2$: C, 66.57; H, 4.03; N, 8.63. Found: C, 66.44; H, 4.00; N, 8.61.

*4.2.2.14. (E)-2-hydroxybenzaldehyde-5-(2,4-difluorophenyl)-2-furoyl hydrazone (**IIf**).* Yellow powdery crystals: m.p. 165–166 °C. IR (KBr) ν_{max}: 3411.1, 3023.6, 2875.0, 1643.2, 1579.4, 1484.4, 1431.5, 1376.2, 1316.3, 1274.1, 1038.9, 969.8, 849.4, 810.2, 751.8, 602.9 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 6.92–7.03 (m, 3H, 2ArH + FuH), 7.29–7.37 (m, 2H, ArH-Fu + ArH), 7.44–7.53 (m, 2H, ArH-Fu + FuH), 7.60–7.62 (m, 1H, ArH), 8.18–8.26 (m, 1H, ArH-Fu), 8.74 (s, 1H, CH=N), 11.07 (s, 1H, OH), 12.11 (s, 1H, NH). MS/ESI: *m/e* (%) 381.0 $[M + K]^+$ (5.9), 365.1 $[M + Na]^+$ (100), 343.4 $[M + H]^+$ (20.6). UV (nm) λ_{max}: 199.1 ($\pi \rightarrow \pi^*$), 213.2 ($n \rightarrow \sigma^*$), 340.0 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}F_2N_2O_3$: C, 63.16; H, 3.53; N, 8.18. Found: C, 62.99; H, 3.78; N, 8.14.

*4.2.2.15. (E)-3-methylbenzaldehyde-5-(2-nitrophenyl)-2-furoyl hydrazone (**IIg**).* Light brown powdery crystals: m.p. 152–153 °C. IR (KBr) ν_{max}: 3252.0, 3091.7, 2915.8, 2858.0, 1659.6, 1554.4, 1534.1, 1461.8, 1358.5, 1301.1, 1183.3, 1163.9, 1066.2, 981.1, 850.5, 830.6, 793.8, 749.8, 696.3, 595.8 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 6.99 (br s, 1H, FuH), 7.26–7.28 (m, 1H, ArH), 7.34–7.39 (m, 1H, ArH), 7.45 (br s, 1H, FuH), 7.51–7.57(m, 2H, ArH), 7.68–7.74 (m, 1H, ArH-Fu), 7.82–7.87 (m, 1H, ArH-Fu), 7.99–8.05 (m, 2H, ArH-Fu), 8.42 (s, 1H, CH=N), 11.85 (s, 1H, NH). MS/ESI: *m/e* (%) 388.0 $[M + K]^+$ (10.9), 372.1 $[M + Na]^+$ (100), 350.2 $[M + H]^+$ (52.0). UV (nm) λ_{max}: 200.3 ($\pi \rightarrow \pi^*$), 313.7 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{19}H_{15}N_3O_4$: C, 65.32; H, 4.33; N, 12.03. Found: C, 65.07; H, 4.35; N, 12.13.

*4.2.2.16. (E)-2-chlorobenzaldehyde-5-(4-fluorophenyl)-2-furoyl hydrazone (**IIh**).* Yellow powdery crystals: m.p. 182–183 °C. IR (KBr) ν_{max}: 3204.0, 3025.0, 1646.1, 1595.1, 1547.2, 1484.9, 1366.6, 1297.4, 1276.8, 1234.3, 1155.9, 1047.9, 1022.2, 882.7, 836.2, 797.6, 753.6, 613.7 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.19 (d, $J = 3.63$ Hz, 1H, FuH), 7.35–7.57 (m, 6H, FuH + 2ArH-Fu + ArH), 8.04–8.07 (m, 3H, ArH + 2ArH-Fu), 8.92 (s, 1H, CH=N), 12.08 (s, 1H, NH). MS/ESI: *m/e* (%) 380.9 $[M + K]^+$ (14.4), 365.1 $[M + Na]^+$ (100). UV (nm) λ_{max}: 200.3 ($\pi \rightarrow \pi^*$), 313.7 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}ClFN_2O_2$: C, 63.08; H, 3.53; N, 8.17. Found: C, 63.39; H, 3.65; N, 7.93.

*4.2.2.17. (E)-4-hydroxybenzaldehyde-5-(4-fluorophenyl)-2-furoyl hydrazone (**IIi**).* Yellow powdery crystals: m.p. 246–247 °C. IR (KBr) ν_{max}: 3332.4, 3236.7, 3062.5, 1643.4, 1602.6, 1514.0, 1485.1, 1441.8, 1378.0, 1280.0, 1236.5, 1160.4, 1018.3, 836.6, 789.9, 527.2 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 6.85–6.88 (m, 2H, ArH), 7.16 (d,

J = 3.60 Hz, 1H, FuH), 7.32–7.40 (m, 3H, 2ArH-Fu + FuH), 7.57–7.60 (m, 2H, ArH), 8.00–8.04 (m, 1H, ArH-Fu), 8.41 (s, 1H, CH=N), 9.98 (s, 1H, OH), 11.63 (s, 1H, NH). MS/ESI: *m/e* (%) 347.0 $[M + Na]^+$ (100), 325.1 $[M + H]^+$ (78.9). UV (nm) λ_{max}: 197.9 ($\pi \rightarrow \pi^*$), 336.4 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{13}FN_2O_3$: C, 66.66; H, 4.04; N, 8.64. Found: C, 66.59; H, 4.16; N, 8.61.

*4.2.2.18. (E)-3-bromobenzaldehyde-5-(2-fluorophenyl)-2-furoyl hydrazone (**IIj**).* Yellow powdery crystals: m.p. 144–145 °C. IR (KBr) ν_{max}: 3169.9, 3059.2, 2961.2, 1650.7, 1564.6, 1520.9, 1482.4, 1447.0, 1392.6, 1344.5, 1268.0, 1218.1, 1174.5, 1032.3, 941.8, 881.0, 814.4, 757.9, 733.8, 678.5, 587.1 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 7.06 (t, J = 3.39 Hz, 1H, ArH-Fu), 7.37–7.51 (m, 5H, 2FuH + 2ArH-Fu + ArH), 7.64–7.67 (m, 1H, ArH), 7.75–7.78 (m, 1H, ArH), 7.93–7.97 (m, 1H, ArH), 8.15–8.20 (m, 1H, ArH-Fu), 8.49 (s, 1H, CH=N), 12.03 (s, 1H, NH). MS/ESI: *m/e* (%) 411.0 $[M + Na]^+$ (78.6), 389.1 $[M + H]^+$ (100). UV (nm) λ_{max}: 197.9 ($\pi \rightarrow \pi^*$), 329.2 ($n \rightarrow \pi^*$). Anal. Calcd. (%) for $C_{18}H_{12}BrFN_2O_2$: C, 55.83; H, 3.12; N, 7.23. Found: C, 55.49; H, 3.26; N, 7.18.

4.3. Crystallography

Compounds **IIa** and **IIe** were recrystallized from methanol to give colorless crystals suitable for X-ray single crystal diffraction. Cell constants at 173(2) K were determined by a least-square fit to the setting parameters. Cell constants at 113 K were determined by a least-square fit to the setting parameters of independent reflections measured on a Bruker SMART [41] 1000 CCD area-detector diffractometer with a graphite-monochromated Mo Kα radiation (λ = 0.071073 nm) and operating in the phi and scan modes. The structure was solved by the direct method with SHELXS-97 [42,43] and refined by the full-matrix least-squares method on F2 data using SHELXL-97 [43,44]. The empirical absorption corrections were applied to all intensity data. H atom of N–H was initially located in a different Fourier map and was refined with the restraint *U*iso(H) = 1.2 *U*eq(N). Other H atoms were positioned geometrically and refined using a riding model, with d(C…H) = 0.093–0.097 nm and *U*iso(H) = 1.2 *U*eq(C) or 1.5 *U*eq(C-methyl). The crystal data in CIF format have been deposited at the Cambridge Crystallographic Data Centre with deposition numbers CCDC 761106 and 761107.

4.4. Antitumor activity

All the title compounds were dissolved in DMSO and screened for preliminary anticancer activity against four different cell lines: a human promyelocytic leukemic cell line (HL-60), a human hepatocellular carcinoma cell line (Bel-7402), a human gastric carcinoma cell line (BGC-823), and a human nasopharyngeal carcinoma cell line (KB) at a concentration of 10 μM (Table 2). The commercial drug doxorubicin was used as positive control in the bioassay. Three replicates were performed. The IC_{50} values of some active target compounds were evaluated using logit analysis (Table 3) [45]. IC_{50} results were analyzed using the statistical data processing system (DPS, 10.15, Zhejiang, China).

The four types of cell line were grown and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in humidified incubators in an atmosphere of 5% CO_2.

All the experiments were performed on exponentially growing cancer cells. Numbers of viable cancer cells were determined by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazoliumbromide] [46] and SRB [47] assays. The cancer cells ($1–2.5 \times 10^4$ cells mL^{-1}) were inoculated in 96-well culture plates (180 μL/well). After 24 h, 20 μL of culture medium containing compounds of various concentrations were added to the wells and, the cells were incubated for 48 h (Table 3). 20 μL of RPMI-1640 medium was added to the control cells. HL-60 cells were assayed by MTT, and the Bel-7402, BGC-823 and KB cells were assayed by SRB. The absorbance of each well was measured using a microculture plate reader at 570 nm (MTT) and 540 nm (SRB). The inhibition rate was calculated according to the following formula:

$$\text{Inhibition rate} = (\text{ODcontrol} - \text{ODtreated})/\text{ODcontrol} \times 100\%$$

4.5. Prediction of toxicity

The database (Prediction System of Carcinogenic Toxicity Version1.0, Prediction System for Mutagenic Toxicity Version1.0 and CISOC-LOGP Version1.0) [48] of SIOC, CAS (Shanghai Institute of Organic Chemistry, Chinese Academy of Science) was used to predict the carcinogenic toxicity, mutagenic toxicity and the Log *P* value of the target compounds. The prediction consists of following steps: (1) reading a structure file, (2) generating all structural descriptors, and (3) calculating corresponding data.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.09.007.

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