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Chelating agents as potential antitumorals. 2-Quinolylhydrazones and bis-2-quinolylhydrazones. I

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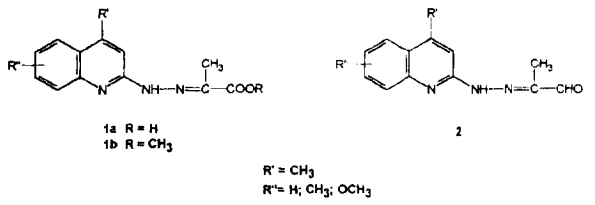
Summary — A number of pyruvic acid, methylpyruvate and pyruvic carboxaldehyde 2-quinolylhydrazones have been synthesized. Some bis-2-quinolylhydrazones were also obtained during the reaction with pyruvic carboxaldehyde. All these compounds have been evaluated for cytotoxicity and most display a significant *in vitro* activity against a variety of cell lines.

2-quinolylhydrazone / bis-2-quinolylhydrazone / pyruvic carboxaldehyde / chelating agent / cytotoxicity

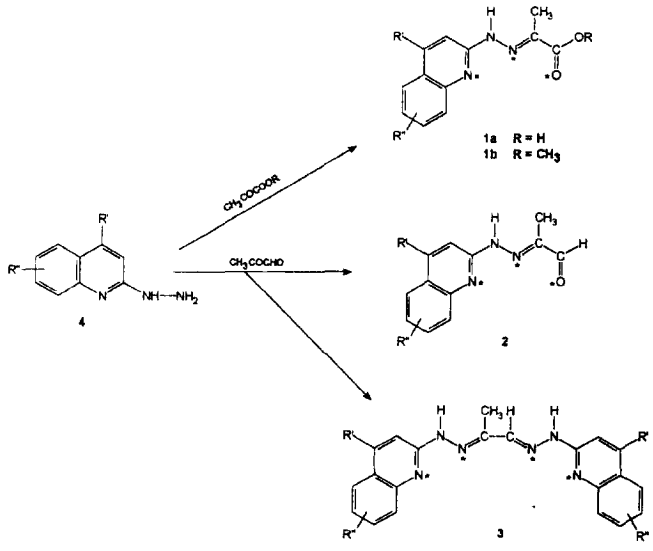
Introduction

In recent years there has been considerable interest in the development of chelating agents and their metal chelates as potential antineoplastic drugs. Several compounds containing an N*-N*-S* tridentate ligand system, *eg*, α-*N*-heterocyclic carboxaldehyde thiosemicarbazones (α-HCATs) and 2,2'-bipyridyl-6-carbothioamide derivatives [1–5], exhibit tumor-inhibitory actions, which are usually attributed to their ability to function as tridentate ligands. Our previous studies on some 2-quinolylhydrazones containing the N*-N*-N* tridentate ligand system showed that these compounds also possess some antitumoral activity [6]. As further development of this research, we have recently focused our attention on a new class of tridentate 2-quinolylhydrazones **1a**, **b** and **2**, containing the N*-N*-O* ligand system. The present paper describes the synthesis and the chemical characterization of the new 2-quinolylhydrazones **1a**, **b** and **2** and bis-2-quinolylhydrazones **3** as well as their cytotoxicity.



Chemistry

The 2-quinolylhydrazones **1a**, **b** and **2** were prepared (scheme 1) by condensing the corresponding 2-hydrazinoquinoline **4** with pyruvic acid, methylpyruvate or pyruvaldehyde in ethanolic or methanolic solution. From the reaction with pyruvaldehyde, a mixture of the quinolylhydrazones **2** and bis-quinolylhydrazones **3** was obtained (see *Experimental protocols*).



Scheme 1.

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The hydrazones **1a–b** and **2** separated as yellow crystals by cooling the reaction mixture. They may only behave as tridentate ligands *via* the N*-N*-O* system (starred atoms in scheme 1) if they adopt the *E* conformation, as reported for similar compounds [7–9].

The structures assigned to the synthesized compounds are in good agreement with elemental analyses and IR and ^1H NMR data (tables I and II). The IR spectra of compounds **1–3** had a weak band between 3350 and 3170 cm^{-1} due to the stretching vibration of the -NH group. In the ^1H NMR spectra of compounds **2** a singlet appears between 9.42 and 9.54 δ which is typical of the aldehyde proton; this structure is also confirmed by the mass spectra, in which the strongest peak clearly derives from the molecular ion by loss of the -CHO unit (*ie* mass spectrum of compound **2,6**, fig 1).

The ^1H NMR spectra of the bis-quinolylhydrazones **3** were recorded in DMSO-d_6, and showed two distinct proton chemical shifts attributable to two -NH; the azomethine proton resonates at 7.85–7.90 δ.

The synthesized compounds were examined *in vitro* for their cytotoxicity against P388 murine lymphocytic leukemia and HL-60 human promyelocytic leukemia; some selected compounds were also tested against three other human tumor cell lines. The resulting data, expressed as IC_{50} and IC_{90} values, are listed in tables III and IV.

Results and discussion

The results in table III show that several of the title compounds have an interesting antiproliferative activity. The most potent are the pyruvic aldehyde 2-quinolylhydrazones **2** and the pyruvaldehyde bis-2-quinolylhydrazones **3**, while the pyruvic and methyl pyruvate 2-quinolylhydrazones **1a** and **1b** were found not to be cytotoxic, with the exception of the 7-methoxy derivatives **1a,6** and **1b,6**. Compound **1a,6** showed inhibitor activity against the P388 and HL-60 leukemic cell lines, displaying IC_{50} values of 22.14 and 49.40, respectively, while **1b,6** only inhibited P388 with an IC_{50} value of 46.82 units.

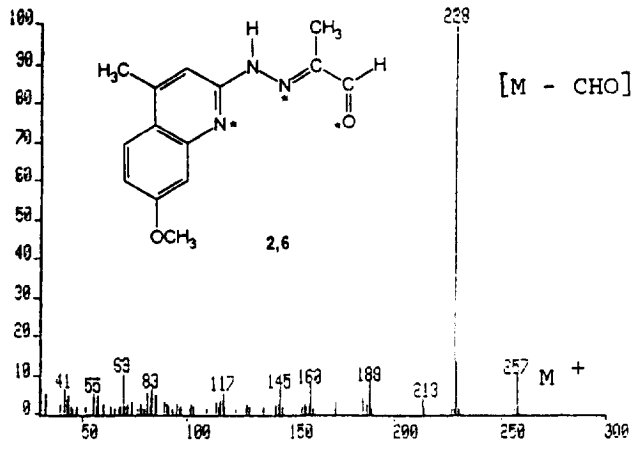


Fig 1. Mass spectrum of compound **2,6** recorded on a VG 70-250 S spectrometer.

Since the quinolylhydrazones **2,2**, **2,3**, **2,5**, **2,6** and the bis-quinolylhydrazones **3,2–5**, showed powerful activity against P388 and HL-60 leukemia, the *in vitro* antitumor screening was completed using other tumor cell lines (B16 murine melanoma, LLC murine lung carcinoma, LoVo human colon adenocarcinoma). These compounds were found to be active in each test system, indicating that they possess a broad spectrum of cytotoxic activity (table IV).

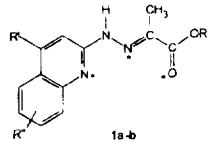
In conclusion, on the basis of the obtained data, it can be evidenced that the substitution at quinoline nucleus generally produces a favorable effect on the activity. In fact, the unsubstituted quinolylhydrazones are inactive (*ie* **1b,1**) or less active (*ie* **2,1** and **3,1**) when compared with the other substituted compounds. Compounds **2,1** and **3,1** showed activity against P388 leukemia only, with IC_{50} values of 36.57 and 34.77, respectively. Substitution at positions 4, 6 and 7 of the quinoline nucleus appear to be helpful in improving the cytotoxicity; substitution at position 8 is unfavorable. Derivative **2,4**, which has a methyl group in position 8 of the quinoline nucleus, is inactive and **3,4** is less active than the other compounds **3**. The most effective substituent is the methoxy group in 7, as also confirmed by **1a,6** and **1b,6**, which are the only quinolylhydrazones **1a** and **1b** with weak activity. Methyl and methoxy groups at position 6 have the same effect.

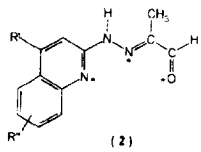
In solution compounds **3** could result in an N*-N*-N*-N* quadridentate ligand system. The higher cytotoxic activity of these compounds with respect to compounds **2** could be explained by the fact that **3** may have a higher affinity towards transition metals than **2**, which has an N*-N*-O* ligand system.

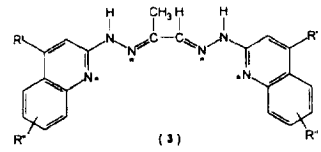
An important role is also played by the nature of carbonilic compound used. Of the synthesized quinolylhydrazones, the most active are those involving pyruvic aldehyde **2**.

The structures of the compounds presented here were developed in order to have chelating properties giving a good cytotoxicity. The inactivity of compound **2,4** and the increased activity of compound **3** with respect to compound **2** suggest that this goal has been reached. Several chelating compounds have the enzyme ribonucleotide reductase as the main intracellular target [10]. This enzyme is crucial in furnishing deoxyribonucleotides to growing cells. At present, the reported data do not allow us to ascertain

Table I. Physicochemical data of 2-quinolylhydrazones **1a**, **b** and **2** and bis 2-quinolylhydrazones **3**.







Compound	*R*	*R'*	*R"*	*Crystallization solvent*	*Mp (°C)*	*Molecular formula*	*Anal*[a]
1a,1	H	H	H	EtOH	212–215	$C_{12}H_{11}N_3O_2 \cdot H_2O$	C, H, N
1a,2	H	CH_3	H	$EtOH/H_2O$	232–234	$C_{13}H_{13}N_3O_2$	C, H, N
1a,3	H	CH_3	6-CH_3	$EtOH/H_2O$	240–242	$C_{14}H_{15}N_3O_2$	C, H, N
1a,4	H	CH_3	8-CH_3	abs EtOH	210–212	$C_{14}H_{15}N_3O_2$	C, H, N
1a,5	H	CH_3	6-OCH_3	MeCOOH	246–249	$C_{14}H_{15}N_3O_2$	C, H, N
1a,6	H	CH_3	7-OCH_3	MeCOOH	245 (dec)	$C_{14}H_{15}N_3O_3$	C, H, N
1b,1	CH_3	H	H	$EtOH/H_2O$	96–98	$C_{13}H_{13}N_3O_2$	C, H, N
1b,2	CH_3	CH_3	H	$EtOH/H_2O$	157–160	$C_{14}H_{15}N_3O_2$	C, H, N
1b,3	CH_3	CH_3	6-CH_3	$EtOH/H_2O$	176 (dec)	$C_{15}H_{17}N_3O_2 \cdot 1/4H_2O$	C, H, N
1b,4	CH_3	CH_3	8-CH_3	EtOH	172–175	$C_{15}H_{17}N_3O_2$	C, H, N
1b,5	CH_3	CH_3	6-OCH_3	$EtOH/H_2O$	171–173	$C_{15}H_{17}N_3O_3$	C, H, N
1b,6	CH_3	CH_3	7-OCH_3	$EtOH/H_2O$	127 (dec)	$C_{15}H_{17}N_3O_3 \cdot 1/2H_2O$	C, H, N
2,1		H	H	$EtOH/H_2O$	135 (dec)	$C_{12}H_{11}N_3O \cdot 1/4H_2O$	C, H, N
2,2		CH_3	H	$EtOH/H_2O$	127–130	$C_{13}H_{13}N_3O \cdot 1/4H_2O$	C, H, N
2,3		CH_3	6-CH_3	EtOH	137–139	$C_{14}H_{15}N_3O \cdot 1/4H_2O$	C, H, N
2,4		CH_3	8-CH_3	EtOH	128 (dec)	$C_{14}H_{15}N_3O$	C, H, N
2,5		CH_3	6-OCH_3	$EtOH/H_2O$	141–143	$C_{14}H_{15}N_3O_2 \cdot 1/4H_2O$	C, H, N
2,6		CH_3	7-OCH_3	$EtOH/H_2O$	108–111	$C_{14}H_{15}N_3O_2 \cdot 1/4H_2O$	C, H, N
3,1		H	H	EtOH	230–233	$C_{21}H_{18}N_6 \cdot 1/2H_2O$	C, H, N
3,2		CH_3	H	$EtOH/H_2O$	227–230	$C_{23}H_{22}N_6 \cdot 1/2H_2O$	C, H, N
3,3		CH_3	6-CH_3	DMF	254–257	$C_{25}H_{26}N_6 \cdot 1/2H_2O$	C, H, N
3,4		CH_3	8-CH_3	EtOH	220–223	$C_{25}H_{26}N_6 \cdot 1/2H_2O$	C, H, N
3,5		CH_3	6-OCH_3	DMF	289–291	$C_{25}H_{26}N_6 \cdot 1/4H_2O$	C, H, N

[a]Water of crystallization was determined by Karl–Fischer method.

whether our compounds inhibit ribonucleotide reductase by sequestering the iron (as Desferal does) or by destroying the tyrosyl free radical of the ribonucleotide reductases small subunit (as α-HCATs do). However, the structure of our compounds suggests that they could act in the same way as the α-HCAT family.

It is known that the complexes of transition metals may be more effective as antitumorals than free ligands, and it therefore seems advisable to continue our research by preparing transition metal complexes with known and newly synthesized ligands.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 240 C Elemental Analyzer and the data for C, H, N are within ±0.4% of the calculated values. IR spectra were determined in Nujol mull with a Perkin-Elmer 398 spectrophotometer. ^1H NMR spectra were recorded on Varian XL 200 FT and AC 200 Bruker spectrometers. The chemical shifts (δ) are relative to Me_4Si as internal standard; J in hertz; s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, mm = multiplets, td = triplet of doublets. TLC on silica-gel plates (Merck 60, F_{254}) and column flash chromatography on silica-gel 60 (Merck, 230-400 mesh) were used to control the purity of the products.

The 2-hydrazinoquinolines **4** were employed in the preparation of compounds **1a**, **b** and **2**, and were obtained from corresponding chloroquinolines and hydrazine hydrate [11]. The carbonilic compounds were purchased commercially and were purified by recrystallization or distillation.

Pyruvic acid 2-quinolylhydrazones 1a and methylpyruvate 2-quinolylhydrazones 1b

The compounds were prepared by refluxing equimolar amounts (0.01 mmol) of 2-quinolylhydrazines **4** and pyruvic acid (or methyl pyruvate) in methanol or ethanol for 1–2 h. From the

Table II. ^1H NMR in ppm and IR spectral data of synthesized compounds.

Compound	*^1H NMR (δ)*	*IR (cm^{-1})*
1a,1	(DMSO-d_6): 2.12 (s, 3H, CH_3-C=N); 7.35 (td, 1H, H-6), 7.58–7.83 (m, 4H, H-(3,4,5,7)); 8.22 (dd, 1H, H-8); 11.28 (br s, 1H, NH, D_2O exchangeable)	1660 (CO)
1a,2	(DMSO-d_6): 2.11 (s, 3H, CH_3-C=N); 2.61 (s, 3H, CH_3 quinoline); 7.37 (td, 1H, H-6); 7.56–7.69 (m, 3H, H-(3,5,7)); 7.90 (dd, 1H, H-8); 11.00 (br s, 1H, NH, D_2O exchangeable)	1665 (CO)
1a,3	(DMSO-d_6): 2.09 (s, 3H, CH_3-C=N); 2.44 and 2.59 (2s, 6H, CH_3 quinoline); 7.44 (dd, 1H, H-7, J_{7-8} = 8.5); 7.58 (d, 2H, H-(3,8), J_{8-7} = 8.5); 7.68 (unresolved d, 1H, H-5); 9.31 (br s, 1H, NH, D_2O exchangeable)	1670 (CO)
1a,4	(DMSO-d_6): 2.12 (s, 3H, CH_3-C=N); 2.56 and 2.63 (2s, 6H, CH_3 quinoline); 7.25 (t, 1H, H-6); 7.45–7.47 (m, 2H, H-(3,7)); 7.76 (dd, 1H, H-5); 12.60 (br s, 1H, NH, D_2O exchangeable)	1665 (CO)
1a,5	(DMF-d_7): 2.34 (s, 3H, CH_3-C=N); 2.73 (s, 3H, CH_3); 4.01 (s, 3H, OCH_3); 7.34–7.39 (m, 2H, H-(5,7)); 7.73 (d, 1H, H-8); 7.82 (br s, 1H, NH, D_2O exchangeable); 8.08 (s, 1H, H-3)	1670 (CO)
1a,6	(DMF-d_7): 2.34 (s, 3H, CH_3-C=N); 2.68 (s, 3H, CH_3); 3.99 (s, 3H, OCH_3); 7.09 (dd, 1H, H-6); 7.18 (d, 1H, H-8, J_{8-6} = 2.5); 7.60 (br s, 1H, NH, D_2O exchangeable); 7.83 (d, 1H, H-5, J_{5-6} = 9.0); 8.08 (s, 1H, H-3)	1690 (CO)
1b,1	($CDCl_3$): 2.18 (s, 3H, CH_3-C=N); 3.89 (s, 3H, $COOCH_3$); 7.40 (td, 1H, H-6); 7.65 (td, 1H, H-7); 7.71–7.77 (m, 3H, H-(3,4,5)); 8.11 (dd, 1H, H-8); 8.62 (br s, 1H, NH, D_2O exchangeable)	1710 (CO)
1b,2	($CDCl_3$): 2.17 (s, 3H, CH_3-C=N); 2.69 (s, 3H, CH_3 quinoline); 3.89 (s, 3H, $COOCH_3$); 7.38 (td, 1H, H-6); 7.60–7.76 (m, 3H, H-(3,5,7)); 7.89 (dd, 1H, H-8); 8.60 (br s, 1H, NH, D_2O exchangeable)	1745 (CO)
1b,3	($CDCl_3$): 2.16 (s, 3H, CH_3-C=N); 2.50 and 2.67 (2s, 6H, CH_3 quinoline); 3.89 (s, 3H, $COOCH_3$); 7.45 (dd, 1H, H-7); 7.55 (s, 1H, H-3); 7.64 (d, 2H, H-(5,8)); 8.50 (br s, 1H, NH, D_2O exchangeable)	1725 (CO)
1b,4	($CDCl_3$): 2.19 (s, 3H, CH_3-C=N); 2.67 and 2.69 (2s, 6H, CH_3 quinoline); 3.89 (s, 3H, $COOCH_3$); 7.27 (t, 1H, H-6); 7.47 (dd, 1H, H-7); 7.57 (s, 1H, H-3); 7.75 (d, 1H, H-5); 8.62 (br s, 1H, NH, D_2O exchangeable)	1710 (CO)
1b,5	($CDCl_3$): 2.16 (s, 3H, CH_3-C=N); 2.66 (s, 3H, CH_3); 3.89 (s, 3H, $COOCH_3$); 3.92 (s, 3H, OCH_3); 7.16 (d, 1H, H-5, J_{5-7} = 2 .6); 7.29 (dd, 1H, H-7, J_{7-5} = 2.6; J_{7-8} = 9.0); 7.57 (s, 1H, H-3); 7.66 (d, 1H, H-8, J_{8-7} = 9.0); 8.45 (br s, 1H, NH, D_2O exchangeable)	1740 (CO)
1b,6	($CDCl_3$): 2.17 (s, 3H, CH_3-C=N); 2.65 (s, 3H, CH_3); 3.89 (s, 3H, $COOCH_3$); 3.90 (s, 3H, OCH_3); 7.02 (dd, 1H, H-6); 7.08 (d, 1H, H-8); 7.42 (s, 1H, H-3); 7.76 (d, 1H, H-5, J_{5-6} = 8.9); 8.58 (br s, 1H, NH, D_2O exchangeable)	1715 (CO)
2,1	($CDCl_3$): 2.04 (s, 3H, CH_3-C=N); 7.35 and 7.66 (2td, 2H, H-(6,7)); 7.75–7.80 (m, 3H, H-(3,4,5)); 8.16 (dd, 1H, H-8); 8.90 (br s, 1H, NH, D_2O exchangeable); 9.53 (s, 1H, CHO)	1675 (CO)
2,2	($CDCl_3$): 2.03 (s, 3H, CH_3-C=N); 2.73 (s, 3H, CH_3 quinoline); 7.43 (td, 1H, H-6); 7.60–7.75 (m, 2H, H-(3,7)); 7.79 and 7.91 (2dd, 2H, H-(5,8)); 8.90 (br s, 1H, NH, D_2O exchangeable); 9.54 (s, 1H, CHO)	1670 (CO)
2,3	(DMSO-d_6): 1.99 (s, 3H, CH_3-C=N); 2.46 and 2.64 (2s, 6H, CH_3 quinoline); 7.37–7.44 (m, 4H, quinoline protons); 9.42 (s, 1H, CHO); 11.20 (br s, 1H, NH, D_2O exchangeable)	1690 (CO)
2,4	($CDCl_3$): 2.06 (s, 3H, CH_3-C=N); 2.68 and 2.71 (2s, 6H, CH_3 quinoline); 7.31 (t, 1H, H-6); 7.50 (dd, 1H, H-7); 7.62 (s, 1H, H-3); 7.80 (dd, 1H, H-5); 9.05 (br s, 1H, NH, D_2O exchangeable); 9.54 (s, 1H, CHO)	1665 (CO)
2,5	(DMSO-d_6): 1.98 (s, 3H, CH_3-C=N); 2.65 (s, 3H, CH_3); 3.88 (s, 3H, OCH_3); 7.27–7.35 (m, 2H, H-(5,7)); 7.58 (s, 1H, H-3); 7.68 (d, 1H, H-8, J_{8-7} = 8.9); 9.42 (s, 1H, CHO); 11.12 (br s, 1H, NH, D_2O exchangeable)	1680 (CO)

Table II. *(Continued.)*

Compound	*^1H NMR (δ)*	*IR (cm^{-1})*
2,6	(DMSO-d_6): 2.01(s, 3H, CH_3-C=N); 2.62 (s, 3H, CH_3); 3.88 (s, 3H, OCH_3); 7.04 (d, 1H, H-8, J_{8-6} = 2.5); 7.08–7.11 (m, 1H, H-6); 7.44 (s, 1H, H-3); 7.87 (d, 1H, H-5, J_{5-6} = 8.8); 9.43 (s, 1H, CHO); 11.14 (br s, 1H, NH, D_2O exchangeable)	1680 (CO)
3,1	(DMSO-d_6): 2.25 (s, 3H, CH_3-C=N); 7.29 (td, 2H, H-(6,6')); 7.52–7.80 (mm, 8H, H-(3,4,5,7,3',4',5',7')); 7.86 (s, 1H, CH=N); 8.17 (dd, 2H, H-(8,8')); 10.41 and 11.46 (2br s, 2H, NH, D_2O exchangeable)	
3,2	(DMSO-d_6): 2.26 (s, 3H, CH_3-C=N), 2.62 (s, 6H, $2CH_3$); 7.30–7.90 (m, 11H, quinoline protons and CH=N); 10.30 and 11.40 (2br s, 2H, NH, D_2O exchangeable)	
3,3	(DMSO-d_6): 2.25 (s, 3H, CH_3-C=N); 2.44 (s, 6H, $2CH_3$); 2.60 (s, 6H, $2CH_3$); 7.38–7.66 (mm, 8H, quinoline protons); 7.83 (s, 1H, CH=N); 10.23 and 11.31 (2br s, 2H, NH, D_2O exchangeable)	
3,4	(DMSO-d_6): 2.29 (s, 3H, CH_3-C=N); 2.58 and 2.62 (2s, 12H, CH_3 quinoline); 7.21 (td, 2H, H-(6,6')); 7.41–7.43 (m, 4H, H-(3,7,3',7')); 7.50 (dd, 2H, H-(5,5')); 7.91 (s, 1H, CH=N); 8.89 and 11.21 (2br s, 2H, NH, D_2O exchangeable)	
3,5	(DMSO-d_6): 2.31 (s, 3H, CH_3-C=N); 2.69 (s, 6H, $2CH_3$); 3.94 (s, 6H, $2OCH_3$); 7.29–7.69 (mm, 8H, quinoline protons); 7.88 (s, 1H, CH=N); 10.09 and 11.18 (2br s, 2H, NH, D_2O exchangeable)	

Table III. Concentrations (μM) inhibiting cell growth by 50% (IC_{50}) and 90% (IC_{90}) in leukemia cell lines.

Compound	*Quinolyl*	*P388*		*HL-60*	
		IC_{50}	*IC_{90}*	*IC_{50}*	*IC_{90}*
1a,2	4-CH_3-Quinolyl	> 50	> 50	> 50	> 50
1a,3	4,6-CH_3-Quinolyl	> 50	> 50	> 50	> 50
1a,5	4-CH_3-6-OCH_3-Quinolyl	> 50	> 50	> 50	> 50
1a,6	4-CH_3-7-OCH_3-Quinolyl	22.14	35.45	49.40	> 50
1b,1	Quinolyl	> 50	> 50	> 50	> 50
1b,2	4-CH_3-Quinolyl	> 50	> 50	> 50	> 50
1b,3	4,6-CH_3-Quinolyl	> 50	> 50	> 50	> 50
1b,5	4-CH_3-6-OCH_3-Quinolyl	> 50	> 50	> 50	> 50
1b,6	4-CH_3-7-OCH_3-Quinolyl	46.82	> 50	> 50	> 50
2,1	Quinolyl	36.57	> 50	> 50	> 50
2,2	4-CH_3-Quinolyl	18.68	33.40	10.18	15.76
2,3	4,6-CH_3-Quinolyl	12.38	18.09	7.76	10.12
2,4	4,8-CH_3-Quinolyl	> 50	> 50	> 50	> 50
2,5	4-CH_3-6-OCH_3-Quinolyl	17.59	26.20	10.52	16.11
2,6	4-CH_3-7-OCH_3-Quinolyl	8.51	11.52	3.64	8.54
3,1	Quinolyl	34.77	> 50	> 50	> 50
3,2	4-CH_3-Quinolyl	3.82	6.41	2.52	7.04
3,3	4,6-CH_3-Quinolyl	1.26	1.74	1.39	1.47
3,4	4,8-CH_3-Quinolyl	22.09	> 50	2.72	6.12
3,5	4-CH_3-6-OCH_3-Quinolyl	2.99	4.05	8.67	9.42

Table IV. Concentrations (μM) inhibiting cell growth by 50% (IC_{50}) and 90% (IC_{90}) in other tumor cell lines.

Compound	*Het*	*LoVo*		*B16*		*LLC*	
		IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
2,2	4-CH_3-Quinolyl	21.59	> 50	12.94	41.38	19.19	40.90
2,3	4,6-CH_3-Quinolyl	17.60	42.89	8.70	35.58	9.83	42.14
2,5	4-CH_3-6-OCH_3-Quinolyl	37.27	> 50	38.01	> 50	40.89	> 50
2,6	4-CH_3-7-OCH_3-Quinolyl	18.21	38.48	20.37	> 50	35.81	> 50
3,2	4-CH_3-Quinolyl	NO	NO	1.20	3.41	2.62	7.26
3,3	4,6-CH_3-Quinolyl	> 50	> 50	1.72	6.78	1.26	4.65
3,4	4,8-CH_3-Quinolyl	> 50	> 50	1.76	21.77	NO	NO
3,5	4-CH_3-6-OCH_3-Quinolyl	6.08	7.77	2.25	4.37	7.68	12.31

NO, not observed. The increasing of concentration did not cause a 50% and 90% inhibition (flat curve at any dose tested).

reaction mixture the quinolylhydrazones **1a** or **1b** precipitated by cooling and diluting with water. The products were filtered off and recrystallized from a suitable solvent (table I).

Pyruvic aldehyde 2-quinolylhydrazones 2 and bis-2-quinolylhydrazones 3

The reaction between 2-hydrazinoquinoline and pyruvaldehyde was carried out in the same way as for compounds **1a** and **1b**, and gave the expected 2-quinolylhydrazones **2** as well as the bis-2-quinolylhydrazones **3** (table I). A precipitate was obtained by cooling the reaction mixture and was identified as the bis-hydrazone **3** on the basis of analytical and spectral data (^1H NMR, IR). Dilution of the mother liquor with water afforded another crystalline solid which was either quinolylhydrazone **2** or, in some cases, a mixture of compounds **2** and **3**, which were separated by flash chromatography.

Pharmacology

Compound solution

Immediately before testing, compounds **1b,2**, **1b,6** and **2,6** were dissolved first in ethanol/polyethylene glycol 1500 (PEG) (3:1) (2 mg/ml) and then in water; **3,5** was dissolved first in dimethylformamide (DMF) (2.5 mg/ml) and then in water; the other compounds were dissolved first in dimethylsulfoxide (DMSO) (2.5 mg/ml) and then in water. The highest ethanol/PEG, DMSO and DMF concentrations used (2%, 0.5% and 0.1%, respectively) did not have any cytotoxic effect in our testing system.

In vitro antitumor evaluation

In order to determine cell growth inhibition, a partially modified tetrazolium-based colorimetric assay was used [12, 13]. The following cell lines were employed: P388 murine lymphocytic leukemia, B16 murine melanoma, LLC murine lung carcinoma, HL-60 human promyelocytic leukemia and LoVo human colon adenocarcinoma. Cell lines were maintained *in vitro* and with exponential growth. The LoVo cell line was maintained in Ham's F-12 medium containing antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml) supplemented with 10% fetal calf serum, 1% MEM Eagle vitamin mixture and 3 mM glutamine. The other cell lines were cultured in RPMI-1640 supplemented with antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml, gentamycin 50 μg/ml), 3 mM glutamine, 10 mM HEPES buffer and 15% (P388) heat-inactivated new-born calf serum or 10% (B16 cell line) or 15% (HL-60 cell line) heat-inactivated fetal calf serum.

Various concentrations of each drug were placed with tumor cell suspension (P388 and B16, 5 x 10^3 cells/well; LLC, 10^4 cells/well; LoVo, 2 x 10^4 cells/well; HL-60, 5 x 10^4 cells/well). Sixty-eight hours later, cell growth was determined by adding 50 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (2.5 mg/ml). It was reduced by mitochondrial dehydrogenase of viable cells in an insoluble blue formazan product during the 4 h contact at 37°C. After removal of supernatant, the formazan crystals were dissolved by adding 100 μl DMSO. The plates were read at 550 nm with a microELISA reader. At each dose level of compounds tested, cell growth inhibition was expressed as a percentage of the decrease of 550 nm absorbance in the treated cultures with respect to control cultures. Concentrations resulting in a 50% decrease of 550 nm absorbance was considered equal to that inhibiting 50% cell growth (IC_{50}) and was calculated as suggested by Chou [14]; the IC_{50} mean of at least three experiments was reported.

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