Azinyl and Diazinyl Hydrazones Derived from Aryl *N*-Heteroaryl Ketones: Synthesis and Antiproliferative Activity[†,‡]

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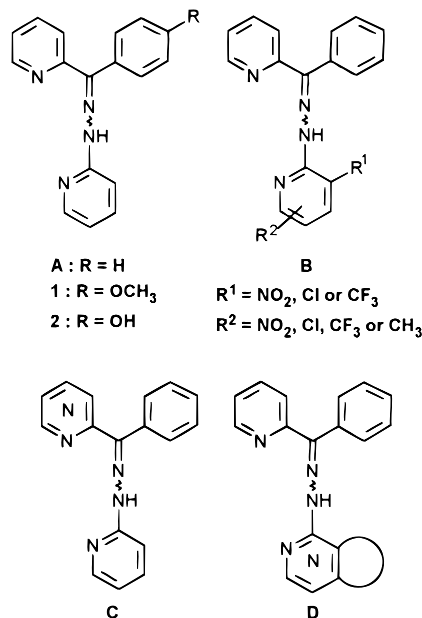
A series of *N*-heteroaryl hydrazones derived from aryl *N*-heteroaryl or bis-*N*-heteroaryl methanones was prepared in search for potential novel antitumor agents. The stereochemistry of these compounds was established by means of NMR spectroscopy. Antiproliferative activity was determined in a panel of human tumor cell lines (CCRF-CEM, Burkitt's lymphoma, HeLa, ZR-75-1, HT-29, and MEXF 276L) in vitro. Generally, the new compounds were found to be more potent (IC_{50} = 0.011−0.436 μM) than the ribonucleotide reductase inhibitor hydroxyurea (IC_{50} = 140 μM). Most of the compounds exhibited the highest activity against Burkitt's lymphoma with an IC_{50} of 0.011−0.035 μM. [^{14}C]Cytidine incorporation into DNA was quantitated for selected hydrazones (***Z*-A**, ***E*-1**, ***Z*-3**, ***Z*-4**, ***E*-5**, ***Z*-5**, ***E*-13**, ***E*-18**, ***Z*-19**, ***Z*-24**, and ***E*-26**) as a measure of the inhibition of ribonucleotide reductase in Burkitt's lymphoma cells. The *E*-configurated compounds were found to inhibit [^{14}C]cytidine incorporation to a greater extent (IC_{50} = 0.67−5.05 μM) than the *Z*-isomers (IC_{50} = 7.20 to >10 μM). Principal component analysis of the IC_{50} values obtained for inhibition of cell proliferation revealed that the cell lines tested can be grouped into three main families showing different sensitivities toward the compounds in our series [(i) CCRF-CEM, Burkitt's lymphoma, and Hela; (ii) HT-29; and (iii) MEXF 276 L].

Introduction

The production of deoxyribonucleotides is a tightly controlled cellular function. The enzyme responsible for this reaction is ribonucleotide reductase (RR).[1] The mammalian RR consists of two subunits referred to commonly as M1 and M2. Whereas the activity of RR is low in resting cells and high in rapidly growing normal cells, it is further increased in malignant transformed cells.[2] Thus, inhibitors of this enzyme are considered as potential antitumor agents.[3] Inhibitors directed at the M2 portion of the enzyme have been studied in detail, and they fall into two categories: metal ion chelators or radical scavengers.[4] Thus, compounds which can act as metal chelators and with the ability to undergo one-electron oxidation may combine two molecular features which may be important for RR inhibitory activity. One such compound previously described is 2-benzoylpyridine 2′-pyridylhydrazone (**A**, Chart 1) which inhibits the proliferation of the hormone dependent human mammary tumor cell line MDA-MB231 (IC_{50} = 0.097 μM) and the [^{3}H]thymidine incorporation into DNA (IC_{50} = 0.089 μM).[5] However, there are no reports on the stereochemistry of the sample employed in this study. In our test systems, ***Z*-A** turned out to be a potent cytotoxic agent with a broad spectrum of activity (IC_{50} = 0.022−0.380 μM, Table 1) against a panel of human tumor cell lines.

Chart 1



We now set out to prepare various analogues of compound **A** in which (a) the phenyl ring bears a OCH_3 or OH function (**1**,**2**), (b) electron-withdrawing or -donating groups are attached to the 2′-pyridylhydrazone moiety (**B**), (c) the 2-benzoylpyridine system is replaced formally by a benzoyldiazine partial structure (**C**), and (d) the 2′-pyridylhydrazone moiety is replaced by a diazinyl hydrazone or a benzoazinyl hydrazone substructure (**D**) (see Chart 1). Moreover, we investigated the antiproliferative activity of compounds of types **1**, **2**, **B**, **C**, and **D** in a panel of human tumor cell lines as well as the incorporation of cytidine into DNA

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Table 1. Antiproliferative Activity of 2-Benzoylpyridine 2′-Pyridylhydrazone and Related Compounds Against Various Human Tumor Cell Lines[a]

		cytoxicity IC_{50} (μM)				
compd	config	CCRF-CEM	Burkitt's	HeLa	HT-29	MEXF 276 L
***Z*-A**	*Z*	0.040	0.022	0.073	0.280	0.380
***E*-1**	*E*	0.081	0.011	0.048	0.154	0.293
***Z*-2**	*Z*	0.269	0.518	0.440	0.736	0.847
***Z*-3**	*Z*	0.162	0.014	0.054	0.160	0.264
***Z*-4**	*Z*	0.068	0.010	0.046	0.77	0.370
***E*-5**	*E*	0.286	0.032	0.113	0.101	0.619
***Z*-5**	*Z*	0.506	0.151	0.515	0.484	8.098
***E*/*Z*-6**	*E*/*Z*	3.210	3.245	3.541	2.957	4.370
***E*-7**	*E*	0.082	0.022	0.041	0.155	0.182
***Z*-7**	*Z*	0.110	0.023	0.060	0.053	0.669
***E*/*Z*-8**	*E*/*Z*	0.546	0.105	0.301	0.326	3.141
***Z*-9**	*Z*	0.140	0.134	0.228	0.342	1.910
***Z*-10**	*Z*	1.032	0.423	0.793	0.702	1.493
***Z*-11**	*Z*	1.676	0.591	1.246	0.705	2.183
***E*/*Z*-12**	*E*/*Z*	0.586	0.585	1.614	1.990	3.157
***E*-13**	*E*	1.161	0.283	1.209	1.585	2.423
***E*/*Z*-14**	*E*/*Z*	0.147	0.032	0.273	0.638	1.570
***E*/*Z*-15**	*E*/*Z*	0.212	0.035	0.142	0.668	1.203
***E*-16**	*E*	> 10	> 10	> 10	> 10	> 10
***E*/*Z*-17**	*E*/*Z*	0.145	0.103	0.331	0.855	1.844
***E*-18**	*E*	0.112	0.045	0.211	0.372	0.493
***Z*-19**	*Z*	1.507	0.347	0.739	3.078	3.217
***Z*-20**	*Z*	1.128	0.463	0.583	4.645	> 10
***Z*-21**	*Z*	> 10	12.361	> 10	> 10	> 10
***E*/*Z*-22**	*E*/*Z*	0.658	0.338	0.417	2.740	2.295
***E*-23**	*E*	1.093	1.424	1.011	1.006	2.563
***Z*-24**	*Z*	0.877	0.463	0.517	0.608	2.614
***E*/*Z*-25**	*E*/*Z*	0.312	0.257	0.314	1.264	1.820
***E*-26**	*E*	0.022	0.015	0.014	0.029	0.244
***E*/*Z*-27**	*E*/*Z*	0.068	0.154	0.084	0.301	0.774
***E*-28**	*E*	0.979	0.266	0.647	0.696	0.969

[a] Data represent the mean of at least three independent experiments in which duplicate determinations were taken within each experiment.

as a measure of RR inhibition with several selected compounds. Finally, we tried to find out whether it is possible to deduce a 3D-QSAR model in this series.

Chemistry

The hydrazones **1** and **2** were prepared by reaction of the substituted 2-benzoylpyridines **29b/c**[6] with 2-pyridylhydrazine (**30**). The synthesis of **3** from bis-2,2′-pyridylmethanone (**29d**) and the hydrazine **30** has been described previously.[7] Compounds **4**−**11** became accessible by reacting equimolar amounts of **29a** and the appropriately substituted 2-pyridylhydrazines **32a**−**h**. The 2-pyridylhydrazines **32b**,[8] **32c**,[9] **32d**,[10] and **32f**[2] were prepared according to the cited references. Reaction of 2-chloro-5-nitropyridine (**31a**) or 2-chloro-5-(trifluoromethyl)pyridine (**31e**) with hydrazine hydrate gave the 2-pyridylhydrazine derivatives **32a** and **32e** in 90% yield (Scheme 1).

Phenyl 2-pyrazinyl ketone (**33a**)[12] was prepared in a 57% yield from methyl 2-pyrazinecarboxylate[13] and phenyllithium at −70 °C. Reaction of pyrazine-2-carbonitrile with (*p*-methoxyphenyl)magnesium bromide at −30 °C afforded **33b**[12] in 54% yield. Treatment of methyl 4-pyrimidinecarboxylate[14] with phenyllithium in a mixture of THF/diethyl ether at −90 °C gave the ketone **33c**[15] in 73% yield. In a one-step reaction, pyrimidine-2-carbonitrile[16] was obtained in 49% yield from 2-chloropyrimidine and sodium cyanide. Thus, ketone **33d**[15] was obtained in 82% yield by reacting the pyrimidine-2-carbonitrile with phenylmagnesium chloride in diethyl ether at −5 to −10 °C. The novel hydrazones **12**−**15** then became accessible by reacting equimolar amounts of the ketones **33a**−**d** and 2-pyridylhydrazine (**30**) in methanol containing traces of acetic acid. The hydrazone **16** in which the 2-benzoylpyridine system is replaced by a 4-benzoylpyridine moiety became accessible from the ketone **33e** and hydrazine **30**.

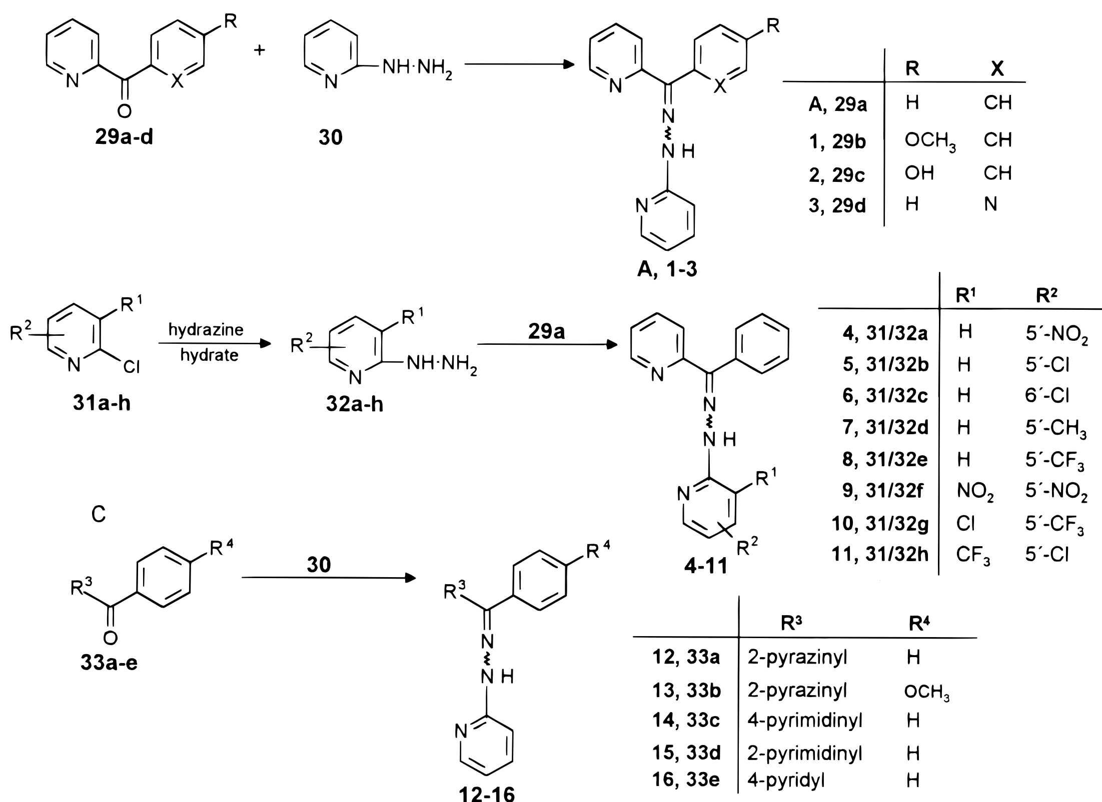
Condensation of the ketone **29a** with the hydrazines **34a**,[17] **34b**,[5] **34c**, **34d**[18] **34e**,[19] **34f**, **34g**,[20] **34h**,[21] **34i**,[22] **34j**,[5] and **34k** in methanol containing traces of glacial acetic acid afforded the hydrazones **17**, **18**, and **20**−**28** in high yields. The 4-pyrimidinylhydrazine which was obtained by treatment of **34b** with Pd/C and ammonium formate in methanolic solution was reacted without further purification with the ketone **29a** to give the hydrazone **19** in 45% yield (Scheme 2). Reaction of methyl acetoacetate with formamidine acetate in a sodium methoxide−methanol solution in analogy to the procedure reported by Butters[23a] for the preparation of 6-ethyl-4(3*H*)-pyrimidinone afforded 6-methyl-4(3*H*)-pyrimidinone[23b] in 72% yield. Thus, 4-chloro-6-methylpyrimidine then became available in 70% yield by chlorination of the methylpyrimidinone with phosphorus oxychloride. It was subsequently reacted with hydrazine hydrate (neat) at 40 °C to give **34c**[23c] in 75% yield.

Considering the fact that compounds of type **A**−**D** can exist as the *E*- or *Z*-isomer, or as *E*/*Z*-mixtures, a detailed examination of the stereochemistry at the −C=N− bond was performed. The configuration of the isolated compounds was determined by ^{1}H and ^{13}C NMR spectroscopy and homonuclear NOE-difference experiments in analogy to the reported findings related to thiosemicarbazones.[24] Thus, the *E*-configuration has to be assigned to compounds **1**, **13**, **16**, **18**, **23**, **26**, and **28** and the *Z*-configuration to compounds **A**, **2**−**4**, **9**−**11**, **19**−**21**, and **24**. The hydrazones **5**−**8**, **12**, **14**, **15**, **17**, **22**, **25**, and **27** were found to be mixtures of *E*/*Z*-isomers of various ratios. Due to problems of solubility, only the *E*/*Z*-isomeric mixtures of compounds **5** and **7** were separable by careful fractional crystallization from ethyl acetate.

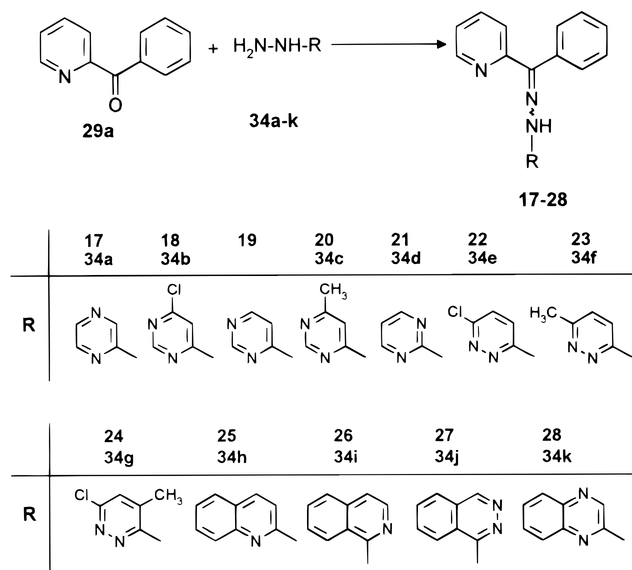
Results and Discussion

Inhibition of cell proliferation was determined in five human cell lines (see the Experimental Section). Since ***Z*-A** is more active against leukemia and Burkitt's lymphoma compared to colon and melanoma cells by a factor of 10 (Table 1), we studied the effect of subtle changes to ***Z*-A** on cytotoxic activity. Compounds bearing a *p*-methoxybenzylidene group (***E*-1**) or the bis-2,2′-pyridyl moiety (***Z*-3**) showed a marginal improvement in cytotoxic activity as compared to ***Z*-A**. The activity of the *p*-OH congener (compound ***Z*-2**) is reduced by a factor of 5−23, depending on the cell line. Further variations were made in the 2-pyridylhydrazone moiety of ***Z*-A** (i.e. compounds **4**−**11**). Comparing the effect of monosubstitution on cytotoxic activity, the substituents in the 5′-position (compounds ***Z*-4**, ***E*-5**, ***E*-7**, and ***E*/*Z*-8**) generally led to retention of activity with the rank order of $CH_3 \geq NO_2 > Cl > CF_3$. In contrast, a chloro substituent in the 6′-position (***E*/*Z*-6**) led to reduced activity by a factor of 100 (Burkitt's lymphoma, IC_{50} = 3.245 μM) compared to the 5′-chloro analogue ***E*-5** (IC_{50} = 0.032 μM) and ***Z*-A** (IC_{50} = 0.022 μM). Furthermore,

Scheme 1



Scheme 2



we studied the influence of stereochemistry on cytotoxic activity. Whereas ***E*-5** shows enhanced activity (IC_{50} = 0.032−0.619 μM) compared to ***Z*-5** (IC_{50} = 0.151−8.098 μM), there is only a small difference between the data obtained for ***E*-7** and ***Z*-7** for the cell lines tested except for MEXF 276L (***E*-7**, IC_{50} = 0.182 μM, and ***Z*-7**, IC_{50} = 0.669 μM). For the disubstituted compounds ***Z*-9−11**, cytotoxic activity is lowered compared to ***Z*-A** and the parent monosubstituted analogues ***Z*-4**, ***E*-5**, and ***E/Z*-8** (Table 1).

To gain further insight into structure−activity relationships, compounds characterized by replacement of the 2-benzoylpyridine substructure of ***Z*-A** by a 2-benzoylpyrazine (***E/Z*-12**), the *p*-OCH_3 derivative of **12** (i.e. ***E*-13**), 4-benzoylpyrimidine (***E/Z*-14**) or 2-benzoylpyrimidine (***E/Z*-15**) moieties (series **C**) were synthesized and evaluated for cytotoxic activity. The data given in Table 1 indicate a reduction in activity for all compounds as compared to ***Z*-A**. Furthermore, to study the relevance of the point of attachment of the benzylidene moiety to the pyridine ring for cytotoxic activity, the hydrazone **16** which is a derivative of 4-benzoylpyridine was synthesized. A total loss of activity was observed for ***E*-16** (IC_{50} >10 μM). This confirms that like observed for *N*-heteroaromatic thiosemicarbazones,[25] the point of attachment of the side chain should be α to the nitrogen of the *N*-heteroaromatic ring.

For the series **D** compounds, replacement of the 2′-pyridylhydrazone substructure of ***Z*-A** by either a 2′-pyrazinylhydrazone (compound ***E/Z*-17**) or a 4′-pyrimidinylhydrazone (compound ***Z*-19**) was found to lead to a reduction in activity by a factor of 8−37. For the compounds bearing a pyrimidinylhydrazone moiety (***E*-18** and ***Z*-19**−***Z*-21**), the 6′-Cl derivative (***E*-18**) turned out to be the most active. Interestingly, the shift of the hydrazine moiety from the 4-position of the pyrimidine ring (***Z*-19**, IC_{50} = > 0.35−3.22 μM) to the 2-position (***Z*-21**, IC_{50} = > 10 μM) results in a total loss of activity (Table 1). Also with compounds ***E/Z*-22**, ***E*-23**, and ***Z*-24**, the activity is lowered compared to ***Z*-A**, indicating that replacement of the 2′-pyridylhydrazone by a pyridazinylhydrazone and the various substituents it bears offers no advantage. Of the hydrazones (i.e. compounds **25**−**28**) derived from the benzoanulated azinyl- and diazinylhydrazines (**34h**−**k**), the 1-isoquinoline derivative ***E*-26** (IC_{50} = 0.015−0.244 μM) showed a significant improvement in cytotoxic activity as compared to ***Z*-A** (IC_{50} = 0.022−0.380 μM). In general, all the other compounds were less active compared to ***Z*-A** and ***E*-26**, with the loss of activity being greater with ***E/Z*-25**.

Table 2. Comparison of Inhibition of Cell Proliferation and [^{14}C]Cytidine Incorporation into DNA as a Measure of Ribonucleotide Reductase Activity of Several Selected Compounds

	IC$_{50}$ (μM)		
compd	cell proliferation[a]	[^{14}C]cytidine incorporation[b]	ratio of IC$_{50}$ values[c]
hydroxyurea	140	25.69	0.18
***Z*-A**	0.022	7.76	353
***E*-1**	0.011	0.67	60.9
***Z*-3**	0.014	0.57	40.7
***Z*-4**	0.010	7.20	720
***E*-5**	0.032	3.84	120
***Z*-5**	0.150	9.04	60.3
***E*-13**	0.283	3.31	11.7
***E*-18**	0.045	5.05	122
***Z*-19**	0.347	>10	28.8
***Z*-24**	0.463	8.44	18.2
***E*-26**	0.015	2.51	167

[a] IC$_{50}$ values taken from Table 1 (Burkitt's lymphoma). [b] Determined in Burkitt's lymphoma cells. The mean of at least two independent experiments which duplicate determinations were taken within each experiment. [c] The ratio IC$_{50}$ is IC$_{50}$ ([^{14}C]cytidine incorporation)/IC$_{50}$(cell proliferation).

[^{14}C]Cytidine Metabolism in Burkitt's Lymphoma Cells

Incorporation of [^{14}C]cytidine into DNA was measured in intact Burkitt's lymphoma cells in order to provide evidence that our novel compounds are capable of inhibiting RR activity in situ. Table 2 shows that all compounds inhibited cell proliferation (IC$_{50}$ = 0.010–0.463 μM) and [^{14}C]cytidine incorporation (IC$_{50}$ = 0.57 to >10 μM) to a larger extent than hydroxyurea (IC$_{50}$ = 140 μM and 25.69 μM, respectively). The *E*-configurated compounds **1**, **5**, **13**, **18**, and **26** inhibited [^{14}C]cytidine incorporation into DNA to a greater extent (IC$_{50}$ = 0.67–5.05 μM) than the *Z*-configurated compounds **A**, **4**, **5**, **19**, and **24** (IC$_{50}$ = 7.20 to >10 μM). Surprisingly, **3**, which can be considered to be in a pseudo-*Z*-form, turned out to be the most active compound, inhibiting the incorporation of [^{14}C]cytidine into DNA with an IC$_{50}$ of 0.57 μM. In general the IC$_{50}$ values for antiproliferative activity are lower than those of inhibition of [^{14}C]cytidine incorporation into DNA. Since the ratios of IC$_{50}$ values for [^{14}C]cytidine incorporation to the values for cell proliferation with our compounds is >1 but is less than one with hydroxyurea (Table 2), we conclude that the novel compounds do not exert their antiproliferative activity solely by RR inhibition. Investigations of other possible modes of action, however, showed that the cell cycle regulators cdc2 kinase, and cdc25 phosphatase are not affected by our compounds even at concentrations of >100 μM.[26]

Statistical Evaluation of Biological Data

Preliminary studies concerning the finding of QSARs revealed that no significant correlation exists between cell growth inhibition and structural properties of the compounds, using neither lipophilicity values nor 3D molecular interaction field descriptors calculated within the CoMFA approach.[27] Therefore we focused our interest in the detection of similarities between the sensitivity of the various cell lines toward our compounds. For determination of intercorrelation among biological data resulting from the assays done with the different cell lines, a principal component analysis (PCA) of the inhibitory activity of the compounds was

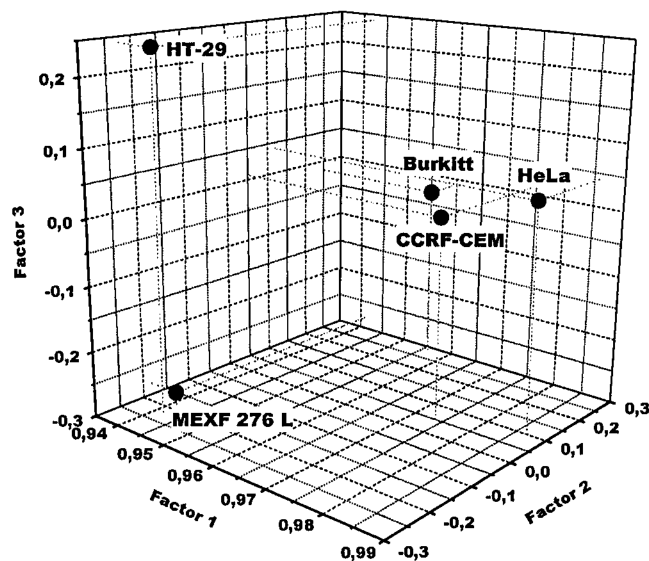


Figure 1. Loadings plot of principal component analysis.

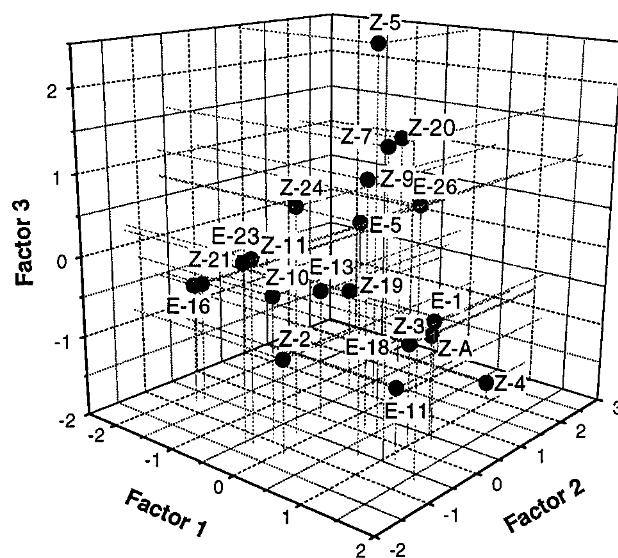


Figure 2. Scores plot of principal component analysis.

performed. The latent variables obtained after PCA of the data matrix as statistical scores are called principal properties (PPs) and represent in an appropriate way the multidimensional system by a few descriptors. Since PPs are orthogonal to each other, they are particularly suitable as design variables, permitting a few representative compounds to be selected out of a larger set. The results of our calculations are given in Figures 1 and 2. From the loadings plot (Figure 1) it becomes evident that there exist three main types of cell lines exhibiting comparable sensitivity toward the compounds in our test system (the Burkitt's lymphoma, CCRF-CEM, and HeLa cells belonging to one type, the HT-29 and MEXF-276L cells forming two other types). From the scores plot (Figure 2), compounds exhibiting unusual activities in certain cell assays can be detected, since they appear as outliers (***Z*-2**, ***Z*-4**, ***Z*-5**, ***E*-7**). These results may serve as a guide for further synthesis and efficient biological testing of selected compounds toward representatives of sensitive cell lines.

Conclusion

In general, it turned out that the various structural changes made to compound ***Z*-A** are well tolerated and

that several of the newly synthesized compounds are even more effective in inhibiting cell proliferation than ***Z*-A**. However, a substituent in the 6′-position of the 2′-pyridylhydrazone moiety (e.g. ***E/Z*-6**) and the replacement of the 4-pyrimidinyl group in compound ***Z*-19** by a 2-pyrimidinyl system (compound ***Z*-21**) leads to a substantial loss of activity. Most sensitive to the new compounds were Burkitt's lymphoma, CCRF-CEM, and HeLa; less sensitive were HT-29 and MEXF 276 L cells. The proliferation of Burkitt's lymphoma cells is inhibited in particular by ***E*-1, *Z*-3, *Z*-4, *E*-7**, ***Z*-7**, ***E/Z*-14**, ***E/Z*-15**, ***E*-18**, and ***E*-26** with IC_{50} in the range of 0.01−0.04 μM. A difference in cytotoxic activity is observed for *E*- and *Z*-isomers (e.g. with compounds **5** and **7**), and this is even more pronounced in the [^{14}C]cytidine incorporation assay where all of the *E*-configurated compounds are more active than the *Z*-isomers by a factor of 10−60. In conclusion, our investigations indicate that inhibition of ribonucleotide reductase seems not to be the sole mechanism of action of these compounds.

Experimental Section

Chemistry. Infrared spectra (IR) were recorded from KBr pellets on a Mattson Galaxy Series FTIR 3000 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded from DMSO-d_6 solutions on a Varian Gemini 200 (^{1}H, 199.98 MHz; ^{13}C, 50.29 MHz) spectrometer with δ 2.49 ppm for ^{1}H and δ 39.50 ppm for ^{13}C as internal standard. Melting points were determined on a Reichert Thermovar hot stage microscope and are uncorrected. Elemental analyses were performed at the "Institut für Physikalische Chemie", University of Vienna, Austria, and the data for C, H, N are within ±0.4% of the calculated values. Reactions were monitored by TLC using Polygram SIL G/UV_{254} (Macherey-Nagel) plastic backed plates (0.25 mm layer thickness) and visualized using UV lamp. Column chromatography was performed using Kieselgel 60 (0.040−0.063 mm).

2-Benzoylpyridine 4-Pyrimidinylhydrazone (*Z*-19). To a solution of **34b**[5] (1.72 g, 12 mmol) and ammonium formate (7.05 g, 112 mmol) in methanol (80 mL) was added 10% Pd/C (0.5 g). The mixture was heated at 50−60 °C for 15 h, cooled to room temperature, and filtered over Celite. After concentration of the methanolic solution to about 10 mL, 5 drops of concentrated H_2SO_4 and **33a** (1.00 g, 5.5 mmol) were added, and the mixture was refluxed for 4 h. After being cooled to room temperature, the mixture was evaporated to dryness, and the remaining solid was recrystallized from 95% ethanol to give 0.69 g (46%) **19** as colorless needles, mp 156−158 °C. Anal. ($C_{16}H_{13}N_5$) C, H, N.

Preparation of Hydrazines 32a and 32e. 2-Chloro-5-nitropyridine (**31a**) (5.50 g, 34.69 mmol) or 2-chloro-5-(trifluoromethyl)pyridine (**31e**) (18.15 g, 0.1 mol) was dissolved in ethanol (50 mL) with warming, and to this solution was added 6 equiv of 95% hydrazine hydrate.

5-Nitro-2-pyridylhydrazine (32a). The mixture was then stirred at room temperature overnight. The dark yellow precipitate formed was filtered off, washed with cold ethanol, and dried. Compound **32a** was obtained as fine yellow needles after recrystallization from 95% ethanol: yield 4.28 g (80%); mp 213-215 °C; ^{1}H NMR δ 8.87 (d, J = 3 Hz, 1H, H-6), 8.16 (dd, J = 3 Hz, J = 9 Hz, 2H, H-4/NH), 6.81−6.77 (m, 1H, H-3) 5.13−4.35 (br s, 2H, NH_2). Anal. ($C_5H_6N_4O_2$) C, H, N.

5-(Trifluoromethyl)-2-pyridylhydrazine (32e). The solution was refluxed for 5 h, the mixture was cooled overnight, and the product was filtered off, washed with cold water, and recrystallized from a water−ethanol mixture to give 14.17 g (80%) of **36e** as colorless needles. mp 65−67 °C; ^{1}H NMR δ 8.26 (s, 2H, H-6/NH), 7.68 (dd, J = 3 Hz, J = 9 Hz, 1H, H-4/NH), 6.81 (d, J = 9 Hz, 1H, H-3), 4.33 (s, 2H, NH_2); ^{13}C NMR δ 163.5 (C-2), 145.2 (q, J = 4 Hz, C-6), 133.6 (q, J = 3 Hz, C-4), 125.0 (q, J = 268 Hz, CF_3), 112.8 (q, J = 32 Hz, C-5), 105.4 (C-3). Anal. ($C_6H_6F_3N_3$) C, H, N.

Phenyl(2-pyrimidinyl)methanone (33d). (a) To a stirred solution of sodium cyanide (12.90 g, 263 mmol) in dry DMF (200 mL) was added 2-chloropyrimidine (20.00 g, 175 mmol) portionwise, and the mixture was heated at 60−70 °C for 24 h. After cooling, the mixture was diluted with water (650 mL), stirred for another 30 min, and extracted with CH_2Cl_2 (6 × 250 mL). The combined organic phases were washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The solid obtained after Kugelrohr distillation in vacuo was recrystallized from DIPE/PE to give 8.99 g (49%) of pyrimidine-2-carbonitrile: colorless crystals; mp 39−41 °C (lit.[16] mp 41−42 °C).

(b) To a stirred solution of pyrimidine-2-carbonitrile (5.00 g, 48 mmol) in dry diethyl ether (100 mL) at −5 to −10 °C was added dropwise a 25% solution of phenylmagnesium chloride in THF (6.54 g, 48 mmol). The mixture was allowed to warm to room temperature, and after 4 h of stirring, 6 N HCl solution (50 mL) was added, and stirring continued for 1 h. The ether layer was separated and extracted with 2 N HCl solution (3 × 50 mL). The combined acidic aqueous phases were adjusted to pH 7−8 with solid $NaHCO_3$ and then extracted several times with diethyl ether. The combined ether phases were washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The solid remaining was subjected to Kugelrohr distillation at 180 °C to give 7.15 g (82%) of compound **33d** as colorless crystals: mp 87−88 °C (lit.[15] mp 84−85 °C); ^{1}H NMR δ 8.94 (d, J = 5 Hz, 2H, pyrimidine H-4,6), 8.03 (dd, J = 1.6 Hz, J = 7 Hz, 2H, phenyl H-2,6), 7.66−7.57 (m, 1H, phenyl H-4), 7.52−7.44 (m, 3H, pyrimidine H-5 and phenyl H-3,5).

General Procedure for the Synthesis of Hydrazones 1−28. A mixture of equimolar amounts of ketone and hydrazine in methanol containing traces of glacial acetic acid was refluxed until TLC monitoring indicated no further conversion. Reaction times varied from 6 to 12 h. The resulting hydrazones separated when the solutions were cooled to 5 °C overnight. In the case of the more soluble hydrazones, the solutions were evaporated to dryness, and the residues obtained were dissolved in hot ethyl acetate or in an ethyl actate/diisopropyl ether mixture and chilled. Then, the crystals which separated were collected by filtration and recrystallized.

For compounds of Scheme 1, ***Z*-A**: 70% yield; mp 150 °C. Anal. ($C_{17}H_{11}N_4$) C, H, N. ***E*-1**: 80% yield; mp 143−145 °C (EtOH). Anal. ($C_{18}H_{15}N_4O$) C, H, N. ***Z*-2**: 76% yield; mp 240−242 °C (EA/DIPE). Anal. ($C_{17}H_{13}N_4O$) C, H, N. ***Z*-4**: 67% yield; mp 199−202 °C (EtOH). Anal. ($C_{17}H_{13}N_5O_2$) H, N; C: calcd, 63.93, found, 64.57. ***E*-5**: 35% yield; mp 134−136 °C (EtOH). Anal. ($C_{17}H_{13}ClN_4$) C, H, N. ***Z*-5**: 45% yield; mp 154−156 °C (EtOH). Anal. ($C_{17}H_{13}ClN_4$) C, H, N. ***E/Z*-6**: 75% yield; mp 166−169 °C (EA/DIPE). Anal. ($C_{17}H_{13}ClN_4$) C, H, N. ***E*-7**: 38% yield; mp 148−151 °C (DIPE). Anal. ($C_{18}H_{16}N_4$) C, H, N. ***Z*-7**: 55% yield; mp 110−113 °C (MeOH). Anal. ($C_{18}H_{16}N_4$) C, H, N. ***E/Z*-8**: 85% yield; mp 108−110 and 120−123 °C (EA). Anal. ($C_{18}H_{13}F_3N_4$) C, H, N. ***Z*-9**: 60% yield; mp 237−239 °C (EtOH/EA). Anal. ($C_{17}H_{12}N_6O_4$) C, H, N. ***Z*-10**: 70% yield; mp 167−169 °C (MeOH). Anal. ($C_{18}H_{12}ClF_3N_4$) C, H, N. ***Z*-11**: 65% yield; mp 142−144 °C (EA/DIPE). Anal. ($C_{18}H_{12}ClF_3N_4$) C, H, N. ***E/Z*-12**: 71% yield; mp 163−165 °C (EtOH) (lit.[7] mp 188 °C). ***E*-13**: 58% yield; mp 173−175 °C (EA/DIPE). Anal. ($C_{17}H_{15}N_5O$) C, H, N. ***E/Z*-14**: 77% yield; mp 152−155 °C (EtOH). Anal. ($C_{16}H_{13}N_5$) C, H, N. ***E/Z*-15**: 73% yield; mp 170−172 °C (EtOH). Anal. ($C_{16}H_{13}N_5$) C, H, N. ***E*-16**: 44% yield; mp 141−144 °C (PrOH/H_2O). Anal. ($C_{17}H_{11}N_4$) C, H, N.

For compounds of Scheme 2, ***E*-18**: 64% yield; mp 140−144 °C (EtOH/DIPE). Anal. ($C_{16}H_{12}ClN_5$) C, H, N. ***Z*-19**: 50% yield; mp 156−158 °C (EtOH). Anal. ($C_{16}H_{13}N_5$) C, H, N. ***Z*-20**: 78% yield; mp 97−100 °C (EA). Anal. ($C_{17}H_{15}N_5$) C, H, N; C: calcd, 70.56, found, 71.21. ***E/Z*-22**: 87% yield; mp 166−170 °C (EtOH/DIPE). Anal. ($C_{16}H_{12}ClN_5$) C, H, N. ***E*-23**: 45% yield; mp 209−212 °C (EtOH). Anal. ($C_{17}H_{15}ClN_5$) C, H, N. ***Z*-24**: 60% yield; mp 208−210 °C (THF). Anal. ($C_{17}H_{14}N_5$) C, H, N. ***E/Z*-25**: 77% yield; mp 136−139 and 179−183 °C (EtOH) (lit.[29] mp 193 °C). Anal. ($C_{20}H_{16}N_4$) C, H, N.

***E*-26**: 70% yield; mp 172−174 °C (toluene) (lit.[30] mp 172 °C). ***E/Z*-27**: 64% yield; mp 179−183 °C (MeOH/H_2O) (lit.[7] mp 177−179 °C). ***E*-28**: 55% yield; mp 218−220 °C (2-PrOH) (lit.[31] mp 222 °C). Recrystallization solvents: EtOH = ethanol, EA = ethyl acetate, DIPE = diisopropyl ether, MeOH = methanol, PrOH = propanol, 2-PrOH = 2-propanol, THF = tetrahydrofuran.

In Vitro Cytotoxicity Assay and [^{14}C]Cytidine Incorporation Studies. CCRF−CEM (acute lymphoblastic leukemia, ATCC CCL 119), Burkitt's lymphoma (CA 46, ATCC CRL 1648), HeLa (epitheloid cervix carcinoma, ATCC CCL2), and MEXF 276 L (melanoma) cells were grown in RPMI 1640, and HT-29 (colon adenocarcinoma, ATCC HTB 38) in McCoy's 5A medium. The media were supplemented with 10% fetal calf serum (except Burkitt's lymphoma with 15%), 2 mM glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. Inhibition of cell proliferation in HeLa, HT-29, and MEXF-276 L was detected by the SRB assay,[32] that of CCRF-CEM and Burkitt's lymphoma cells by the MTT assay[33] from Boehringer Mannheim, Mannheim, Germany.

[^{14}C]Cytidine incorporation into DNA was performed by a modification of the procedure previously described[34] in intact Burkitt's lymphoma cells. Three milliliters ($(1-2) \times 10^6$/mL) of exponentially growing cells were incubated at 37 °C in medium with various drug concentrations of each compound. After 90 min 0.7 μCi [^{14}C]cytidine (2−10 Ci/mmol, ICN Biomedicals, Meckenheim, Germany) was added to each sample for 45 min. Subsequently, the cells were washed twice in ice-cold phosphate-buffered saline, resuspended in 1 mL of ice-cold trichloroacetic acid, and transferred to Eppendorf tubes. The samples were then treated as described by Nocentini et al.[34]

Principal Component Analysis. Statistical evaluation of the biological data was performed within the Sybyl QSAR module:[35] factor analysis (NIPALS algorithm without rotation) yielded principal components (cumulative percentage of variance in the first three components: 86.7, 94.2, and 97.9 respectively).

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Supporting Information Available: ^{1}H NMR data for compounds **A**, **1**−**28** (5 pages). Ordering information is given on any current masterhead page.

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