Eur J Med Chem (1997) 32, 397–408

Thiazolyl and benzothiazolyl hydrazones derived from α-(*N*)-acetylpyridines and diazines: synthesis, antiproliferative activity and CoMFA studies

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(Received 5 June 1996; accepted 4 November 1996)

Summary — The synthesis of a series of thiazolyl and benzothiazolyl hydrazones derived from α-(*N*)-acylpyridines, -quinolines, -isoquinolines, -pyridazines, -pyrimidines, and -pyrazines is reported. The stereochemistry of these compounds was determined by NMR spectroscopic methods. The antiproliferative activity of the novel compounds was quantified in tissue culture (melanoma, breast carcinoma, colon adenocarcinoma, epitheloid cervix carcinoma, Burkitt's lymphoma, leukemia, and hydroxyurea sensitive and resistant myelogenous leukemia sublines). All compounds exhibited profound antiproliferative activity, in particular against Burkitt's lymphoma cells. Out of this series, compounds **6b**, **7b**, **7c**, **8c** and **8i** were found to be 13–900 times more potent than hydroxyurea and no cross-resistance to hydroxyurea was observed. A predictive 3D-QSAR model using the CoMFA approach was established.

antiproliferative agent / thiazolyl and benzothiazolyl hydrazone / α-(*N*)-acetylheteroarene derivative / hydroxyurea resistant tumor / CoMFA

Introduction

In 1956 Brockman et al showed that 2-formylpyridine thiosemicarbazone **1a** possesses moderate antileukemic activity in mice bearing leukemic cells [1]. Following this initial report, a large series of aliphatic, aromatic, and heteroaromatic carbaldehyde thiosemicarbazones (TSCs) was synthesized with the aim of studying structure–activity relationships (SAR) and also to obtain more efficacious antitumor agents. The chemistry and biological activities of a large number of such compounds have been reviewed [2, 3].

The α-(*N*)-heteroaromatic TSCs have been shown to interfere with the biosynthesis of DNA in mammalian cells and this has been attributed to the inhibition of the enzyme ribonucleotide reductase (RR) [4–6]. Mammalian RR is composed of two protein dimer subunits, designated as M1 and M2. Protein M1 contains binding sites for both nucleoside diphosphates and triphosphates. Protein M2 contains two moieties that are critical for the catalytic activity: a stable tyrosyl free radical and a nonheme iron center [7, 8]. Inhibition of RR by TSCs is due to the destruction of the M2 tyrosine-free radical [9].

Several of the TSCs mentioned above, especially those derived from 2-pyridine and 1-isoquinoline carbaldehydes, showed significant antitumor activity against a wide spectrum of transplanted murine neoplasms; their clinical utility, however, is limited due to extremely poor solubility in water [2]. Structural modifications have been carried out in order to overcome this problem by introduction of hydrophilic groups such as NH_2 or OH into the heterocyclic ring systems [10, 11]. Thus, these compounds can be formulated as water-soluble salts. From these studies, 5-hydroxypyridine-2-carbaldehyde TSC **2** has been chosen for clinical phase I evaluation. In this study, compound **2** showed extremely weak antileukemic activity due to its short biological half-life in man. Furthermore, high in vivo toxicity was observed [12, 13]. Considering these findings, Sartorelli and coworkers designed a new class of thiosemicarbazones in which the 2-pyridine or the 1-isoquinoline core bears a methyl group in *ortho* position to the hydrophilic substituent anticipating that this would provide steric protection against enzymatic acetylation [14].

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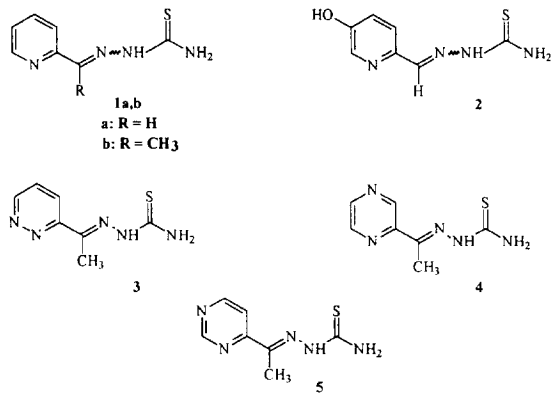


Recently, we have shown that replacement of the pyridine moiety in compounds of type **1a,b** by a diazine nucleus (in particular by the 1,2-diazine system) results in significantly improved water solubility. Moreover, in our test system compounds **3–5** turned out to be less toxic compared with **1a** and **1b** [15, 16]. It has been proposed that the in vivo toxicity of the α-(*N*)-heteroaromatic TSCs might be due to release of H_2S during the metabolism of such compounds. Hence, for example, replacement of the thiocarbamoyl substructure in compounds **1a,b** by a 2-pyridinyl moiety led to a drastic reduction in toxicity while the biological activity was retained [17].

To test this hypothesis further, we now synthesized compounds in which the thiocarbamoyl substructure of **1a,b** is formally replaced by a thiazole or a benzothiazole system. From this type of compounds release of H_2S during metabolism is unlikely to occur. Furthermore, a systematic structural modification in this series was carried out in order to gain insight into structure–activity relationships. The antiproliferative activity of these compounds was tested in a panel of human tumor cell-lines including hydroxyurea (HU) sensitive and resistant cells. Finally a 3D-QSAR study was performed using the CoMFA method in order to rationalize the biological activities observed.

Chemistry

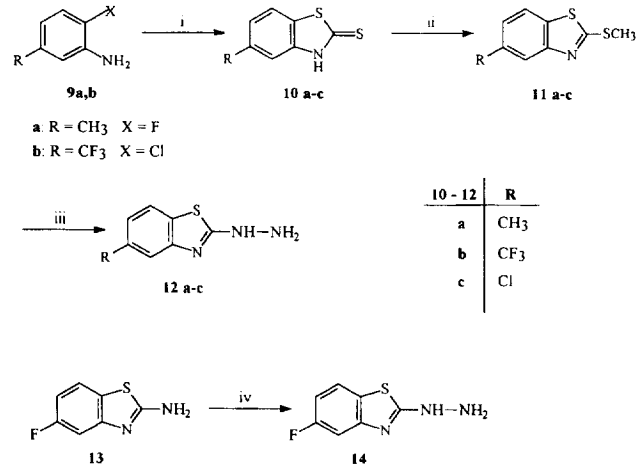
Synthesis

The synthesis of the 5-substituted 2-hydrazinobenzothiazoles **12a,b** depicted in scheme 1 was carried out in an analogous manner as described for the preparation of 5-chloro-2-hydrazinobenzothiazole (**12c**) [18]. Treatment of the aniline derivatives **9a,b** with carbon disulfide and sodium hydride in diethylene glycol monoethyl ether gave the 2-mercaptobenzothiazoles **10a,b** which were subsequently reacted with methyl iodide in 2 N sodium hydroxide solution to afford the 2-methylthiobenzothiazoles **11a,b** in high yields. The 2-hydrazinobenzothiazoles **12a,b** became accessible via displacement of the methylthio function in compounds **11a,b** by hydrazine (scheme 1). In analogy to the literature [19], 2-hydrazino-5-fluorobenzothiazole (**14**) was prepared in high yield by an exchange amination of 2-amino-5-fluorobenzothiazole [20] with a hydrazine hydrate/hydrazine hydrochloride mixture in ethylene glycol solution.

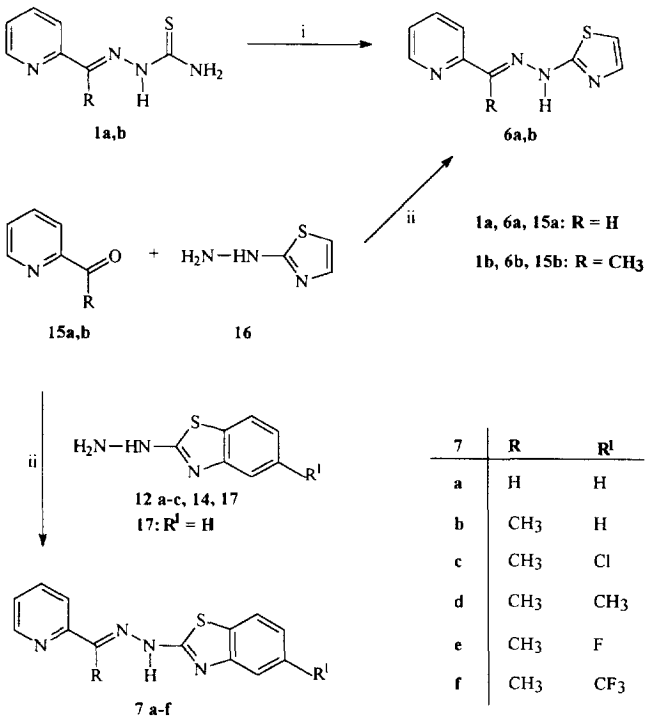
The preparation of the thiazolylhydrazones **6a,b** from the carbonyl compounds **15a,b** and 2-hydrazinothiazole (**16**) has been described previously [21]. The key reagent **16** in this synthesis was prepared via diazotization of 2-aminothiazole. Since compound **16** is obtained by this procedure in low yield only and is very unstable, we chose an alternative access to compounds **6a,b** namely reaction of **1a** or **1b** with chloroacetaldehyde and sodium acetate in DMF (scheme 2).

The synthesis of compounds **7c–f** was accomplished by reacting 2-acetylpyridine (**15b**) with the 2-hydrazinobenzothiazoles **12a–c** and **14** in methanol containing traces of glacial acetic acid (scheme 2). The synthesis of **7a,b** from **15a,b** and 2-hydrazinobenzothiazole (**17**) [22] has already been reported [23]. The additional novel compounds **8a–k** (scheme 3) were obtained in high yields by condensation of **17** with the ketones **18a–k** (table I).

The ketones **18a–k** are known compounds and were synthesized according to published procedures (see



Scheme 1. Synthesis of 2-hydrazinobenzothiazole: i) CS_2, NaH, diethylene glycol monoethylether; ii) MeI, NaOH; iii) $NH_2NH_2 \cdot H_2O$; iv) $NH_2NH_2 \cdot H_2O$, $NH_2NH_2 \cdot HCl$, ethylene glycol.



7	R	R^1
a	H	H
b	CH_3	H
c	CH_3	Cl
d	CH_3	CH_3
e	CH_3	F
f	CH_3	CF_3

Scheme 2. Synthesis of 2-thiazolyl and 2-benzothiazolyl hydrazones: i) $ClCH_2CHO$, NaAc, DMF; ii) MeOH, glacial acetic acid.

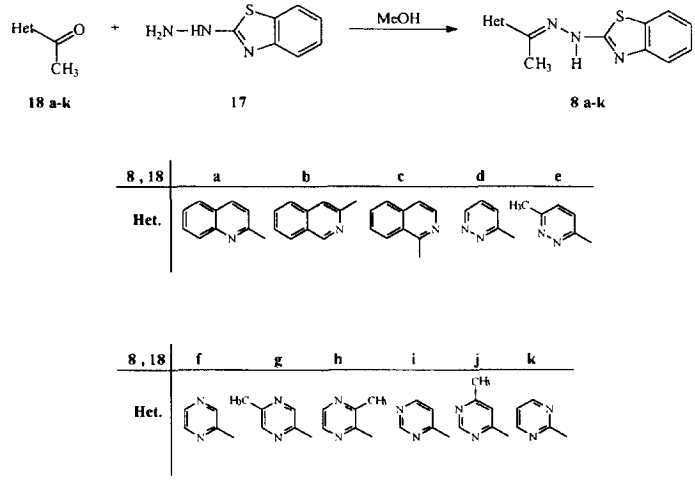
Experimental protocols) except for 1-acetylisoquinoline (**18c**). The reported synthesis of **18c** [24] was the direct adaptation of the reaction of 3-cyanoisoquinoline with MeMgI in diethyl ether/benzene mixture under reflux [25]. Applying this procedure, **18c** was obtained in 10% yield accompanied with much tarry material. We now found that simply by performing the Grignard reaction at –30 °C the yield of **18c** could be raised to 84%.

Spectroscopic investigations

The structures of all novel compounds were confirmed by elemental analyses, IR and NMR spectroscopy (table II). Compounds containing the -C=N- substructure (such as **7** and **8**) can exist in the *E*- or *Z*-form, or as mixtures of *E/Z*-isomers. According to the ^{1}H-NMR spectra all the hydrazones except compound **8c** turned out to be single isomers. Based on our previous findings with *N*-heteroaromatic TSCs, the most remarkable differences regarding the chemical shifts of the corresponding protons in the two isomeric forms are the resonance signals attributable to the NH protons: ie, δ (NH) = 14–15 ppm for the *Z*-form and δ (NH) = 9–12 ppm for the *E*-form [26, 27]. Considering these findings, the *E*-configuration was assigned to compounds **7a–f**, **8a,b**, and **8d–k** of which δ (NH) ≈ 11.78–12.43 ppm (table II). This conclusion is further supported by NOE difference experiments. Expectedly, irradiation of the NH resonance leads to a positive NOE on the acetyl-CH_3 protons. Compound **8c** was a mixture of *E*- and *Z*-isomers with the ratio 95:5 *(E/Z)*. Interestingly, **8c** in DMSO–d_6 solution is gradually transformed totally into the *Z*-configurated form with time.

Biological evaluation

To determine the in vitro cytotoxicity of the test compounds, exponentially growing human tumour



Scheme 3.

Table I. Yields and characteristics of benzothiazolyl hydrazones derived from acetyl *N*-(α)-heteroaromatics.

Compound	*Yield (%)*[a]	*Mp (°C)*	*Recrystallization solvent*[b]	*Analysis*
7c	68	208–210	96% EtOH	$C_{14}H_{11}ClN_4S$
7d	68	187–189	AcOET	$C_{15}H_{14}N_4S$
7e	98	174–176	AcOET	$C_{14}H_{11}FN_4S$
7f	79	183–185	MeOH	$C_{15}H_{11}F_3N_4S$
8a	76	135–137	2-PrOH	$C_{18}H_{14}N_4S$
8b	97	194–196	AcOET	$C_{18}H_{14}N_4S$
8c	82	192–194	2-PrOH	$C_{18}H_{14}N_4S$
8d	70	213–215	AcOET	$C_{13}H_{11}N_5S$
8e	98	282–284	MeOH/DIPE	$C_{14}H_{13}N_5S$
8f	93	218–220	96% EtOH	$C_{13}H_{11}N_5S$
8g	98	214–216	2-PrOH	$C_{14}H_{13}N_5S$
8h	70	190–193	EtOH	$C_{14}H_{13}N_5S$
8i	94	210–213	AcOET	$C_{13}H_{11}N_5S$
8j	88	188–191	AcOET	$C_{14}H_{13}N_5S$
8k	65	110–112	96 % EtOH	$C_{13}H_{11}N_5S$

[a]Isolated yield; [b]AcOET, ethyl acetate; DIPE, diisopropyl ether.

cells were exposed to a range of drug concentrations. Inhibition of cell proliferation of ZR-75-1, HeLa, HT-29 and MEXF-276 L was detected by the SRB-assay [28]. Dose–response curves for CCRF-CEM and Burkitt's lymphoma cells were detected by an MTT-assay [29]. The cytotoxicity assay of each compound was performed at least three times and two samples were taken within each experiment. The IC_{50} values were calculated by the 'Dose–effect analysis with microcomputers' software written by J Chou and TC Chou (Biosoft, Cambridge, UK). The results are reported in table III.

In addition, the in vitro cytotoxic activity of compounds **6b**, **7b**, **7c**, **7e**, **8c** and **8i** against hydroxyurea sensitive K562-WT (wild type) and resistant K562-DFMO^r human myelogenous leukemia cell-lines [30] was determined. The IC_{50} values are given in table IV.

Discussion

First we examined the effect of replacement of the thiocarbamoyl substructure of **1a/b** by a 2-thiazolyl (**6a/b**) or a 2-benzothiazolyl (**7a/b**) moiety. Compared to **1a**, the antiproliferative activity of **7a** is increased by a factor of two to nine dependent on the cell-line employed, whereas that of the thiazolyl congener **6a** is reduced by a factor of two to five. There is in general an enhancement of antiproliferative activity by a factor 80 to 200 if the thiocarbamoyl substructure of **1b** is replaced by a thiazole (**6b**) or a benzothiazole (**7b**) system. Not much difference is observed in the antiproliferative activities of compounds **1a** and **1b**. However, replacement of the formyl-H of **6a** by a CH_3 group (**6b**) results in a drastic enhancement of antiproliferative activity by a factor 20 to 300. The same phenomenon is observed for compounds **7a** and **7b** (table III). From this set of compounds, **6b** and **7b** displayed high cytotoxic activity against the tumour cell-lines tested (IC_{50} = 0.016–1.53 μM).

Although **7b** was not efficacious in an in vivo study using the murine lymphocytic leukemia P388 cells [23], we selected this compound for further modifications on the benzothiazole part of the molecule, because of the stability and ease of preparation of 2-hydrazinobenzothiazoles. The various substituents at the 5-position of the benzothiazolyl moiety were chosen to cover a range of lipophilic, electronic, and steric properties (ie, compounds **7c–f**). The inhibitory activities of the 5-fluoro analogue **7e** for the Burkitt's lymphoma (0.041 μM), HT-29 (0.52 μM), and MEXF-276 L (1.45 μM) and that of the 5-trifluoro analogue **7f** for the HT-29 (0.69 μM) and MEXF-276 L (1.90 μM) cell-lines were similar to that of **7b**. Introduction of a methyl group into the 5-position of the benzothiazole part of **7b** (compound **7d**) leads to a drastic reduction of cytotoxic activity by a factor of approximately 35 (table III). Comparing compounds 7**c–f**, the rank order of cytotoxic activity according to the nature of the substituent is as follows: $F \gg CF_3 \geq Cl > CH_3$.

In an attempt to obtain a more efficacious cytotoxic compound, the effects of replacement of the pyridine ring of compound **7b** by other heteroaromatic ring systems were studied. The cytotoxic activity of the 3-acetylisoquinoline and 1-acetylisoquinoline derived

Table II. Spectral data of compounds **7a–f** and **8a–k**[a].

Compound	*IR (KBr, cm^{-1})*	*^{1}H-NMR (DMSO-d_6, ppm)*
E-**7a**	3423, 3070, 2860, 1662 1607, 1576, 1560, 1147	7.12 (dt, *J* = 7 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.25–7.40 (m, 2H, Pyr-H5, Bth-H5), 7.48 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.75–7.94 (m, 3H, Pyr-H3, Pyr-H4 and Bth-H7), 8.14 (s, 1H, CH=N), 8.58 (ddd, *J* = 5 Hz, *J* = 2 Hz, *J* = 1 Hz, 1H, Pyr-H6), 12.43 (br s, 1H, NH)
E-**7b**	3449, 1609, 1560, 1553, 1465, 1151, 1142	2.42 (s, 3H, CH_3), 7.10 (dt, *J* = 7 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.22–7.44 (m, 3H, Pyr-H5, Bth-H4/5), 7.73 (br d, *J* = 7 Hz, 1H, Bth-H7), 7.84 (dt, *J* = 7 Hz, *J* = 2 Hz, 1H, Pyr-H4); 8.08 (br d, *J* = 7 Hz, 1H, Pyr-H3), 8.58 (ddd, *J* = 5 Hz, *J* = 2 Hz, *J* = 1 Hz, 1H, Pyr-H6), 11.91 (br s, 1H, NH)
E-**7c**	3435, 1620, 1602, 1564 1469, 1151, 1045	2.41 (s, 3H, CH_3), 7.13 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H, Bth-H6), 7.36 (ddd, *J* = 7 Hz, *J* = 5 Hz, *J* = 1 Hz, 1H, Pyr-H5), 7.46 (br d, 1H, Bth-H4), 7.77 (br d, *J* = 8 Hz, 1H, Bth-H7), 7.83 (dt, *J* = 7 Hz, *J* = 2 Hz, 1H, Pyr-H4), 8.04 (br d, *J* = 7 Hz, 1H, Pyr-H3), 8.58 (ddd, *J* = 5 Hz, *J* = 2 Hz, *J* = 1 Hz, 1H, Pyr-H6), 11.95 (br s, 1H, NH)
E-**7d**	3445, 2910, 1218, 1555, 1477, 1149, 1047	2.35 (s, 3H, Bth-CH_3), 2.42 (s, 3H, CH_3), 6.93 (br d, *J* = 8 Hz, 1H, Bth-H6), 7.20 (br s, 1H, Bth-H4), 7.37 (dt, *J* = 7 Hz, *J* = 5 Hz, *J* = 1 Hz, 1H, Pyr-H5), 7.58 (br d, *J* = 8 Hz, 1H, Bth-H7), 7.84 (dt, *J* = 7 Hz, *J* = 2 Hz, 1H, Pyr-H4), 8.08 (br d, *J* = 7 Hz, 1H, Pyr-H3), 8.59 (br d, *J* = 5 Hz, 1H, Pyr-H6), 11.78 (br s, 1H, NH)
E-**7e**	3431, 1620, 1557, 1476, 1143, 1049	2.43 (s, 3H, CH_3), 6.98 (dt, *J* = 9 Hz, *J* = 2 Hz, 1H, Bth-H6), 7.26 (br dd, *J* = 10 Hz, 1H, Bth-H4), 7.38 (ddd, *J* = 5 Hz, *J* = 3.8 Hz, *J* = 1 Hz, 1H, Pyr-H5), 7.78 (br dd, *J* = 9 Hz, *J* = 6 Hz, 1H, Bth-H7), 7.85 (dt, *J* = 7 Hz, *J* = 2 Hz, 1H, Pyr-H4), 8.07 (br d, *J* = 8 Hz, 1H, Pyr-H3), 8.59 (ddd, *J* = 5 Hz, *J* = 2 Hz, *J* = 1 Hz, 1H, Pyr-H6), 11.95 (br s, 1H, NH)
E-**7f**	3030, 2920, 2828, 1622, 1602, 1566, 1175, 1041	2.44 (s, 3H, CH_3), 7.36–7.45 (m, 2H, Bth-H6 and Pyr-H5), 7.70 (br s, 1H, Bth-H4), 7.86 (dt, *J* = 7 Hz, *J* = 2 Hz, 1H, Pyr-H4), 7.99–8.09 (m, 2H, Bth-H7 and Pyr-H3), 8.60 (ddd, *J* = 5 Hz, *J* = 2 Hz, *J* = 1 Hz, 1H, Pyr-H6), 11.93 (br s, 1H, NH)
E-**8a**	3449, 1597, 1553, 1501, 1447, 1121	2.55 (s, 3H, CH_3), 7.12 (dt, *J* = 7 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.30 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.42 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.54–8.05 (m, 5H, Bth-H7 and Quin-H5/6/7/8), 8.25 (ABd, *J* = 8.8 Hz, 1H, Quin-H4), 8.35 (ABd, *J* = 8.8 Hz, 1H, Quin-H3), 11.98 (br s, 1H, NH)
E-**8b**	3450, 1605, 1530, 1551, 1441, 1145	2.55 (s, 3H, CH_3), 7.11 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, 1H, Bth-H6), 7.30 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.40 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.63–7.83 (m, 3H, Bth-H7, 3-Iso-H6/7), 8.07 (br d, *J* = 8 Hz, 1H, 3-Iso-H5), 8.13 (br d, *J* = 8 Hz, 1H, 3-Iso-H8), 8.44 (s, 1H, 3-Iso-H4), 9.35 (s, 1H, 3-Iso-H1), 11.81 (br s, 1H, NH)
E-**8c**	3065, 2930, 1618, 1553, 1441, 1039	2.58 (s, 3H, CH_3), 7.10 (dddd, *J* = 1.4 Hz, *J* = 6.4 Hz, *J* = 7.2 Hz, 1H, Bth-H6), 7.30 (dt, *J* = 1.2 Hz, *J* = 6.6 Hz, *J* = 7.2 Hz, 1H, Bth-H5), 7.40 (br d, *J* = 7.4 Hz, 1H, 1-Iso-H8), 7.77–7.69 (m, 2H, Bth-H4/1-Iso-H), 7.86–7.79 (m, 2H, 1-Iso-H), 8.02 (br dd, *J* = 1.4 Hz, *J* = 7.2 Hz, Bth-H7), 8.55 (d, *J* = 5.4 Hz, 1H, 1-Iso-H4), 8.98 (br d, *J* = 7.4 Hz, 1H, 1-Iso-H), 11.68 (s, 1H, NH)
Z-**8c**		2.38 (s, 3H, CH_3), 7.04–6.94 (m, 2H, Bth-H6/1-Iso-H8), 7.17 (dt, *J* = 1.4 Hz, *J* = 7.6 Hz, 1H, Bth-H5), 7.58 (br d, 1H, 1-Iso-H), 7.86–7.64 (m, 3H, Bth-H4/1-Iso-H), 8.01 (br d, 1H, Bth-7), 8.54 (d, *J* = 5.8 Hz, 1-Iso-H4),11.51 (s, 1H, NH)
E-**8d**	3448, 3076, 1609, 1560, 1441, 1145	2.53 (s, 3H, CH_3), 7.10 (dt, *J* = 7 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.29 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.38 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.68–7.75 (m, 2H, Bth-H7 and Pydz-H5), 8.20 (dd, *J* = 8.8 Hz, *J* = 2 Hz, 1H, Pydz-H4), 9.19 (dd, *J* = 5 Hz, *J* = 2 Hz, 1H, Pydz-H6), 12.15 (br s, 1H, NH)
E-**8e**	3057, 2922, 1616, 1557, 1535, 1447, 1041	2.53 (s, 3H, CH_3), 2.65 (s, 3H, Pydz-CH_3), 7.11 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.30 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.39 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.58 (ABd, *J* = 9 Hz, 1H, Pydz-H5), 7.71 (br d, *J* = 8 Hz, 1H, Bth-H7), 8.11 (ABd, *J* = 9 Hz, 1H, Pydz-H4), 11.98 (br s, 1H, NH)
E-**8f**	3175, 3086, 2959, 1609, 1566,1516, 1443, 1128	2.39 (s, 3H, CH_3), 7.10 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.29 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.37 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.72 (br d, *J* = 8 Hz, 1H, Bth-H7), 8.56 (d, *J* = 2.6 Hz, 1H, Pyra-H6), 8.61 (dd, *J* = 2.6 Hz, *J* = 1.6 Hz, 1H, Pyra-H5), 9.24 (d, *J* = 1.6 Hz, 1H, Pyra-H3), 12.10 (br s, 1H, NH)
E-**8g**	3048, 2920, 1616, 1587, 1554, 1471, 1051	2.40 (s, 3H, CH_3), 2.52 (s, 3H, Pyra-CH_3), 7.11 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.30 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.39 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.74 (br d, *J* = 8 Hz, 1H, Bth-H7), 8.51 (d, *J* = 1.6 Hz, 1H, Pyra-H6), 9.13 (d, *J* = 1.6 Hz, 1H, Pyra-H3), 11.95 (br s, 1H, NH)
E-**8h**	1609, 1570, 1447, 1097	2.44 (s, 3H, CH_3), 2.86 (s, 3H, Pyra-CH_3), 7.11 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.30 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.39 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.74 (br d, *J* = 8Hz, 1H, Bth-H7), 8.47 (ABd, *J* = 2.4 Hz, 1H, Pyra-H6), 8.53 (ABd, *J* = 2.4 Hz, 1H, Pyr-H5), 11.87 (br s, 1H, NH)
E-**8i**	3073, 3028, 2941, 1605, 1553, 1468, 1155	2.39 (s, 3H, CH_3), 7.13 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.31 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.41 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.75 (br d, *J* = 8 Hz, 1H, Bth-H7), 8.01 (dd, *J* = 5 Hz, *J* = 1 Hz, 1H, Pymd-H5), 8.80 (d, *J* = 5 Hz, 1H, Pymd-H4), 9.19 (d, *J* = 1 Hz, 1H, Pymd-H2), 12.17 (br s, 1H, NH)
E-**8j**	3057, 2969, 2861, 2774, 1612, 1532, 1468, 1057	2.39 (s, 3H, CH_3), 2.53 (s, 3H, Pymd-CH_3), 7.14 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.32 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.42 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.77 (d, *J* = 8 Hz, 1H, Bth-H7), 7.87 (br s, 1H, Pymd-H5), 9.05 (d, *J* = 1 Hz, 1H, Pymd-H2), 12.10 (br s, 1H, NH)
E-**8k**	1613, 1562, 1543, 1470, 1437, 1051	2.46 (s, 3H, CH_3), 7.11 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H6), 7.30 (dt, *J* = 8 Hz, *J* = 1 Hz, 1H, Bth-H5), 7.44 (br d, *J* = 8 Hz, 1H, Bth-H4), 7.45 (t, *J* = 5 Hz, 1H, Pymd-H5), 7.76 (br d, *J* = 8 Hz, 1H, Bth-H7), 8.88 (d, *J* = 5 Hz, 2H, Pymd-H4/6), 11.87 (br s, 1H, NH)

[a]Bth: benzothiazole; Pyr: pyridine; Pydz: pyridazine; Pyra: pyrazine; Pymd: pyrimidine; Quin: quinoline; Iso: isoquinoline.

Table III. Concentrations (μM) inhibiting cell growth by 50% (IC_{50}) on human tumor cell lines.

Compound	*Inhibition of cell growth, IC_{50} (μM)*					
	CCRF-CEM	*Burkitt's*	*HeLa*	*ZR-75-1*	*HT-29*	*MEXF-276 L*
1a	1.29	2.38	3.03	> 10	9.85	6.56
1b	1.93	3.29	4.94	11.09	7.45	9.00
6a	5.71	5.26	8.33	ND	6.84	> 10
6b	0.27	0.016	0.061	ND	0.21	0.53
7a	0.15	0.31	1.40	1.88	5.31	5.13
7b	0.02	0.02	0.03	0.19	0.51	1.53
7c	0.34	0.16	0.26	ND	0.53	4.57
7d	0.75	0.35	0.50	ND	1.47	5.00
7e	0.18	0.041	0.15	ND	0.52	1.45
7f	0.30	0.15	0.41	ND	0.69	1.90
8a	> 10	> 10	> 10	> 10	> 10	> 10
8b	2.96	3.06	3.02	ND	5.33	6.23
8c	4.68	3.68	4.37	ND	5.74	> 10
8d	2.41	7.11	3.76	ND	6.96	8.35
8e	6.77	14.75	8.386	ND	8.26	> 10
8f	2.27	0.77	2.43	ND	3.50	6.13
8g	6.44	1.20	5.04	ND	9.50	> 10
8h	> 10	> 10	> 10	ND	> 10	> 10
8i	1.11	0.14	0.83	ND	9.50	3.01
8j	1.38	0.18	0.80	ND	2.51	3.93
8k	0.35	0.032	0.11	ND	0.52	1.02

ND: not determined.

Table IV. Concentration inhibiting cell growth by 50% of hydroxyurea sensitive (K562 WT) and resistant (K562 $DFMO^r$) human myelogenous leukemia K562 cells.

Compound	*Inhibition of cell growth, IC_{50} (μM)*		
	K562 WT	*K562 $DFMO^r$*	R_f[a]
Hydroxyurea	45.5	777.2	17.08
6b	0.051	0.072	1.41
7b	0.053	0.069	1.30
7c	0.355	0.566	1.59
7e	0.071	0.088	1.24
8c	3.469	4.515	1.30
8i	0.552	0.718	1.30

[a]The resistance factor R_f represents the ratio IC_{50} (K562 $DFMO^r$)/(K562 WT).

compounds **8b** and **8c** was found to be drastically reduced compared to **7b**. The 2-acetylquinoline analogue **8a** was totally devoid of cytotoxic activity. In this context it should be noted that, in the TSC series, the isoquinoline-1-carbaldehyde derivative has been found to be the most potent compound, whereas the activity of isoquinoline-3-carbaldehyde derivative is reduced and the quinoline-2-carbaldehyde congener is devoid of any carcinostatic activity [14]. Contrary to our expectations based on the findings reported in the literature [14], compound **8c** was less active than **8b**. During the course of the in vitro studies it was observed that the solutions of **8c** in DMSO must be freshly prepared, since even with cold stock solutions the activity is lost. According to ^{1}H-NMR investigations, this is due to transformation of *E*-configurated **8c** into its *Z*-isomer. Indeed, molecular modeling studies revealed that for *Z*-**8c** (a structure in which an intramolecular hydrogen bond can be formed easily), there exists an additional 'forbidden' volume area compared to the other compounds. These facts may perhaps explain the loss of activity for *Z*-configurated **8c**.

Finally, compounds obtained by formal replacement of a CH moiety of the pyridine ring of **7b** by a nitrogen atom to give the 1,2- or 1,4- or 1,3-diazine derivatives **8d**, **8f**, and **8i** were found to be less cytotoxic (IC_{50} = 0.77–9.50 μM). The cytotoxic activity of these compounds is further diminished by a factor of 5 to 100 upon introduction of a methyl substituent into the pyridazine or pyrazine ring systems (compounds **8e**, **8g**, and **8h**). However, in this series of compounds, a significant improvement of the antiproliferative activity (factor approximately 80) is observed for the pyrimidine derived compounds **8i**, **8j**, and **8k** (table III). Compared to the other cell-lines, the Burkitt's lymphoma cell-line was more sensitive to the inhibitory action of compounds **8f**, **8g**, and **8i–k** (IC_{50} = 0.032–1.20 μM).

Compounds **6b**, **7b**, **7c**, **8c**, and **8i** were 13 to 900 times more potent than hydroxyurea (HU) in the K562-WT cell-line. In accordance with our findings using the cell-lines discussed above, **6b**, **7b**, and **7e** turned out to be the most cytotoxic compounds (IC_{50} = 0.051–0.071 μM) also in the experiments employing the K562-WT cell-line. Moreover, unlike the clinically used antitumor agent HU, these compounds are also effective against the resistant cell-line K562-DFMO^r (IC_{50} = 0.069–4.515 μM), which overexpresses the ribonucleotide reductase M2 protein subunit. Whereas the K562-DFMO^r cell-line is 17 times more resistant to HU, it shows almost no cross-resistance to compounds **6b**, **7b**, **7c**, **7e**, **8c** and **8i** (table IV).

Statistical evaluation of biological data and CoMFA

A general problem in QSAR is that biological data are composed of different effects that are sometimes in competition, especially when originating from assays performed with intact cells. In our special case, we may assume that the inhibitory activity of the compounds tested depends at least on resorption, inhibition of ribonucleotide reductase, and an elimination mechanism. Moreover, these effects are markedly different for the cell-lines under investigation. A preliminary study concerning the finding of a possible dependence of antiproliferative activity of the compounds upon their lipophilicity revealed that no significant correlation of this parameter with growth inhibition exists for all the cell-lines. In order to detect intercorrelation between the sensitivity of the various cell-lines towards our compounds, we performed a principal component analysis of the activities. From the loadings plot (fig 1), it becomes evident that there exist three main types of cell-lines in our test system (the Burkitt's lymphoma, the HeLa and the HT-29 belonging to one type, CCRF-CEM and MEXF-276L forming two other types). Since the Burkitt's

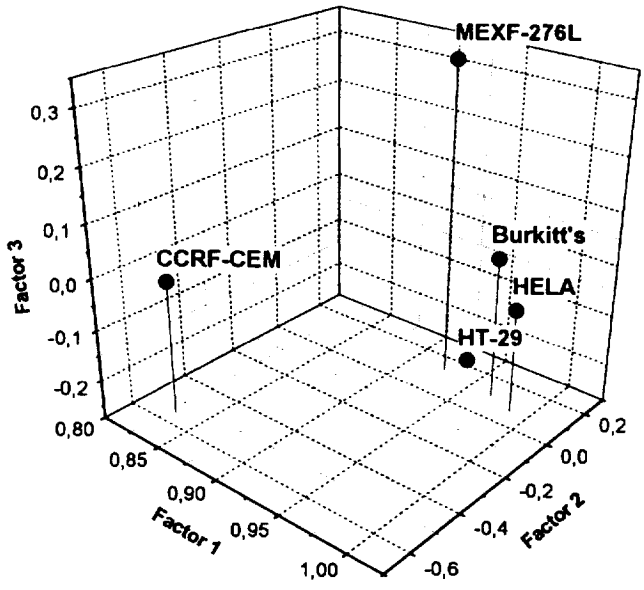


Fig 1. Loadings plot of principal component analysis.

lymphoma cell-line is known to belong to the most sensitive ones and the behaviour of this cell-line was comparable to at least two more cell-lines of our test systems, we focused our interest on establishing a 3D QSAR model for the inhibitory activity of our compounds only towards this tumor. The entire study was performed using the SYBYL molecular modeling software [31] and comparative molecular field analysis (CoMFA) [32] on a Silicon Graphics Indy workstation or on a Silicon Power Challenge XL parallel computer. Details are given in the *Experimental protocols*.

Two different alignments of the molecules structures were used in our study. All derivatives were superimposed in a way to give the most similar conformations. In the first case, the atoms of the *N*-heteroaromatic system and the hydrazone moiety were fitted utilizing a rigid body rotation and translation procedure. In the second alignment, the hydrazone and thiazolyl or benzothiazolyl atoms were superimposed (fig 2).

Whereas a less satisfactory 3D QSAR model could be generated when the first alignment was used (r^2_{cv} = 0.335), a predictive one was obtained with the second alignment using three components in the PLS analysis (r^2_{cv} = 0.447; r^2 = 0.904, s = 0.346, F = 40.630). The relative contribution of steric and electrostatic potential to the CoMFA regression equation for inhibitory activity was found to be 48% for steric

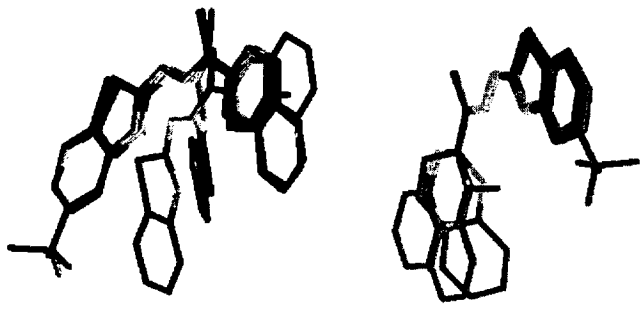


Fig 2. Alignment I and II as used for CoMFA study.

and 52% for electrostatic effects. The thus estimated pIC_{50} for compounds **6a,b**, **7a–f**, and **8a–k** as well as residual values are given in table V and the results are graphically represented in figures 3 and 4. In the CoMFA contour plots (a graphical representation of the model is shown in fig 5), regions where increased steric bulk is associated with enhanced activity are indicated in green, while regions where increased steric bulk is associated with diminished activity are indicated in yellow. Regions where increased positive charge is favorable for activity are indicated in red, while regions where increased negative charge is favorable for activity are indicated in blue. The inter-

Table V. Observed versus calculated pIC_{50} (M) values for compounds **6a,b**, **7a–f**, and **8a–k**.

Compound	*pIC_{50} (obsd)*	*pIC_{50} (calcd)*	*Residual*
6a	5.28	5.43	–0.15
6b	7.80	6.41	1.39
7a	6.51	6.59	–0.08
7b	7.70	7.21	–0.49
7c	6.80	6.98	–0.18
7d	6.46	6.50	–0.04
7e	7.39	7.41	–0.02
7f	6.82	6.78	0.04
8a	4.60	4.69	–0.09
8b	5.51	5.67	–0.16
8c	5.43	5.44	–0.01
8d	5.15	5.37	–0.22
8e	4.83	4.64	0.19
8f	6.11	6.26	–0.15
8g	5.92	5.89	0.03
8h	4.60	4.39	0.22
8i	6.85	6.75	0.11
8j	6.74	6.70	0.05
8k	7.49	7.65	–0.15

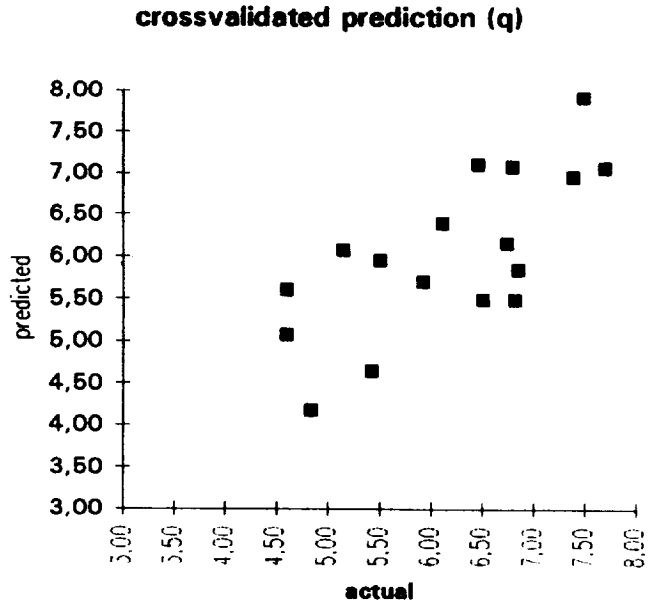


Fig 3. Results of cross-validated PLS analysis obtained from alignment II.

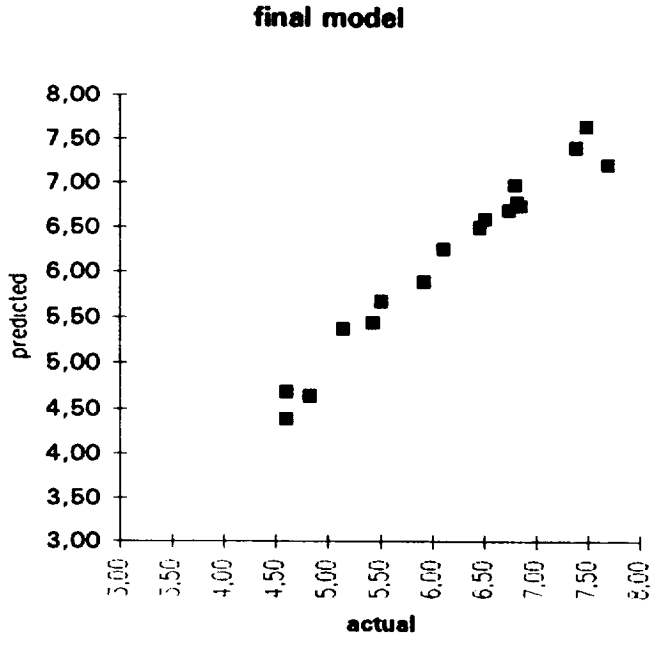


Fig 4. Results of final analysis obtained from alignment II.

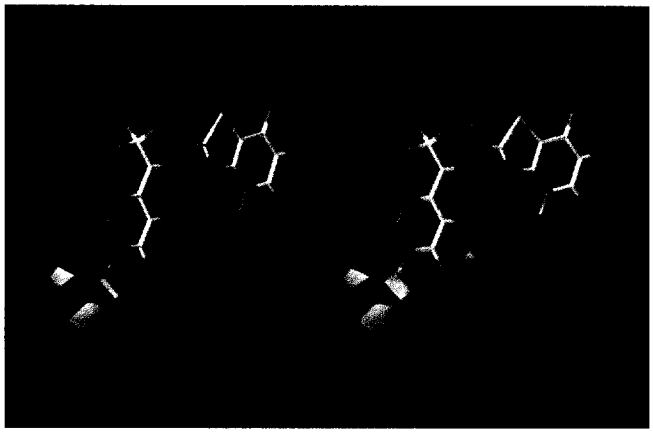


Fig 5. Stereoview of compound **7b** in the CoMFA steric and electrostatic contour plots (sdev* contribution) from the non-cross-validated PLS analysis based on alignment II.

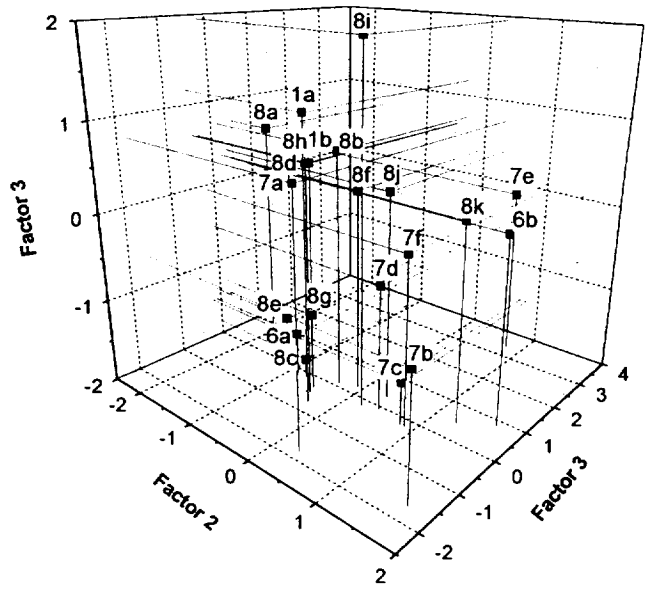


Fig 6. Scores plot of principal component analysis.

pretation of these contour plots is far from being evident, however, it should be possible to use the plots as guide for the development of more potent compounds. For instance, the effect of a methyl substitution in the diazine ring system becomes evident since this additional volume points into a region, where additional bulk is associated with diminished activity (compounds **8e**, **8g**, and **8h**). On the other hand, tolerance for steric bulk is indicated by the green contour regions on the 'thiazole' side of the molecules. In fact, the benzothiazolyl derivatives exhibit comparable activity. The effect of negative partial charge at the substituent in position 5 of the benzothiazolyl moiety can be deduced from the electrostatic contour map: a red region indicates negative charge favorable for activity, which is in good agreement with our findings (see ranking of compounds **7c–f**).

Additional help comes finally from interpretation of the scores plot resulting from principal component analysis of the biological data of all test compounds (fig 6), here compounds with unusual activities in certain cell assays are highlighted (**6b**, **7b**, **8i**).

Conclusion

We have shown in this study that replacement of the thiosemicarbazone (TSC) side chain of α-(*N*)-heteroaromatic TSCs by a 2-thiazolyl- or a 2-benzothiazolyl hydrazone (**6a,b**, **7a–f** and **8a–k**) generally leads to compounds with enhanced cytotoxic activity. Compared to the other cell-lines, the leukemia (CCRF-CEM) and the Burkitt's lymphoma cell lines were more sensitive to the inhibitory activity of the compounds described. Moreover, selected compounds (**6b**, **7b**, **7c**, **8c** and **8i**) were more potent than hydroxyurea (HU) and show no cross-resistance to the HU resistant cell-line K562-DFMO^r. In our opinion this could be a hint for the fact that the compounds described in this study either inhibit RR in a different fashion with respect to HU or that the cytotoxic activities are mediated through other cellular targets. Thus, this information together with the results of the theoretical methods presented might serve as a guide for the development of novel agents permitting to treat HU-resistant tumors.

Experimental protocols

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. The center of the solvent multiplet (DMSO-d_6) was used as internal standard, which was related to TMS with δ 2.49 ppm for ^{1}H and δ 39.50 ppm for ^{13}C. Assignments are based on chemical shift considerations, heteronuclear shift-

correlation (COLOC), as well as on NOE difference experiments. 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 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).

The following compounds utilized as starting materials were prepared according to the literature cited: 2-acetylquinoline **18a** [34], 3-acetylisoquinoline **18b** [35], 3-acetylpyridazine **18d** [15], 6-methyl-3-acetylpyridazine **18e** [16], 2-acetylpyrazine **18f** [36, 37], 5-methyl-2-acetylpyrazine **18g** [37], 4-acetylpyrimidine **18i** [16], 6-methyl-4-acetylpyrimidine **18j** [38] and 2-acetylpyrimidine **18k** [39, 40]. 3-Methyl-2-acetylpyrazine **18h** was purchased from Maybridge Co.

*General procedure for the preparation of compounds **6a,b***
To a suspension of thiosemicarbazoné **1a** (0.93 g, 5.15 mmol) or **1b** (1.00 g, 5.15 mmol) in DMF (5 mL) were added a 50% aqueous solution of chloroacetaldehyde (0.81 g, 5.15 mmol) and sodium acetate (0.42 g, 5.15 mmol). The mixture was heated at 70 °C for 6 h. After cooling to room temperature H_2O (20 mL) was added and cooled overnight. The precipitated product was filtered off and recrystallized.

*2-Formylpyridine 2-thiazolylhydrazone **6a**.* Yield: 0.58 g (55%); mp 184–187 °C (methanol), lit [21] mp 200 °C. ^{1}H-NMR δ: 6.92 (d, J = 3.7 Hz, 1H, thiazole-H), 7.27 (d, J = 3.7 Hz, 1H, thiazole-H), 7.30–7.39 (m, 1H, pyridine H-5), 7.80–7.88 (m, 2H, pyridine H-3/4), 8.04 (s, 1H, CH), 8.52–8.59 (m, 1H, pyridine H-6), 12.14 (s, 1H, NH).

*3-Acetylpyridine 2-thiazolylhydrazone **6b**.* Yield: 0.55 g (49%); mp 171–174 °C (methanol), lit [21] mp 175 °C. ^{1}H-NMR δ: 2.38 (s, 1H, CH_3), 6.87 (d, J = 3.7 Hz, 1H, thiazole-H), 7.27 (d, J = 3.7 Hz, 1H, thiazole-H), 7.29–7.37 (m, 1H, pyridine H-5), 7.81 (dt, J = 7.4 Hz, J = 8.1 Hz, J = 1.8 Hz, 1H, pyridine H-4), 8.02 (d, J = 8.1 Hz, 1H, pyridine H-3), 8.53 (dd, J = 4.8 Hz, J = 1.7 Hz, 1H, pyridine H-6), 11.28 (s, 1H, NH).

*General procedure for the preparation of compounds **10a,b***
Sodium hydride (3.07 g, 0.13 mol) was suspended in diethylene glycol monoethyl ether (80 mL) under a nitrogen atmosphere and the aniline **9a** (10.01 g, 0.08 mol) or **9b** (15.65 g, 0.08 mol) was added. After stirring for 30 min at room temperature, carbon disulfide (12.18 g, 0.16 mol) was added and the mixture heated at 140 °C for 5 to 6 h. After cooling the solution to room temperature, the product precipitating after addition of conc HCl (64 mL) was filtered off and recrystallized.

*5-Methyl-2-mercaptobenzothiazole **10a**.* Yield: 9.46 g (65%); mp 183–186 °C (EtOH/H_2O, light yellow crystals). IR 3099, 3068, 3045, 3034, 2989, 2939, 1604, 1512, 1454, 1406, 1255 cm^{-1}; ^{1}H-NMR δ: 2.37 (s, 3H, CH_3), 7.09–7.10 (m, 1H, H-6), 7.13 (br d, 1H, H-4), 7.54 (d, J = 8.8 Hz, 1H, H-7), 13.67 (s, 1H, NH); ^{13}C-NMR δ: 20.8 (CH_3), 112.5 (C-7), 121.3 (C-4), 125.3 (C-6), 126.2 (C-5), 137.0 (C-7a), 141.4 (C-3a), 189.9 (C=S); $C_8H_7NS_2$ (MW = 181.27) requires C53.01, H3.89, N7.73; found: C53,22, H3.93, N7.68.

*5-Trifluoromethyl-2-mercaptobenzothiazole **10b**.* Yield: 17.50 g (93%); mp 228–231 °C (DIPE, light yellow crystals); IR 3107, 3067, 3003, 2955, 2908, 1518, 1456, 1336, 1267, 1232, 1163cm^{-1}; ^{1}H-NMR δ: 7.50–7.51 (m, 1H, H-4), 7.63 (dd, J = 1.8 Hz, J = 8.0 Hz, 1H, H-6), 7.95 (d, J = 8 Hz, 1H, H-7), 14.02 (s, 1H, NH); ^{13}C-NMR δ: 108.6 (q, J = 4 Hz, C-4), 120.4 (q, J = 4 Hz, C-6), 122.9 (C-7), 123.9 (q, J = 271 Hz, CF_3), 127.6 (q, J = 32 Hz, C-5), 134.0 (C-7a), 141.4 (C-3a), 191.0 (C=S); $C_8H_7F_3NS_2$ (MW = 235.24) requires: C40.85, H1.71, N5.95; found: C41.07, H2.02, N5.93.

*General procedure for the preparation of compounds **11a,b***
The mercaptobenzothiazole **10a** (7.70 g, 42.50 mmol) or **10b** (10.00 g, 42.50 mmol) was dissolved in 1 N NaOH (55 mL) with stirring at room temperature. To this solution was added methyl iodide (9.05 g, 63.75 mmol) dropwise and the mixture was stirred for 3 h. The precipitate formed was filtered off, washed with water, dried, and recrystallized.

*5-Methyl-2-methylthiobenzothiazole **11a**.* Yield: 5.39 g (65%); mp 53–54 °C (EtOH/DIPE, colourless crystals); IR 3508–3468 (br), 2926, 1599 (w), 1550 (w), 1543 (w), 1437, 1412, 1311, 1153, 804, 685 cm^{-1}; ^{1}H-NMR δ: 2.42 (s, 3H, CH_3), 2.77 (s, 3H, SCH_3), 7.18 (br d, J = 8 Hz, J = 1 Hz, 1H, H-6), 7.65–7.67 (m, 1H, H-4), 7.86 (d, J = 8 Hz, 1H, H-7); ^{13}C-NMR δ: 15.4 (CH_3), 20.9 (SCH_3), 121.0 (C-6), 121.1 (C-7), 125.5 (C-4), 131.4 (C-5), 135.9 (C-7a), 153.2 (C-3a), 167.8 (C-2); $C_9H_9NS_2$ (MW = 195.30) requires: C55.35, H4.64, N7.17; found: C55.41, H4.68, N7.09.

*5-Trifluoromethyl-2-methylthiobenzothiazole **11b**.* Yield: 8.91 g (84%); mp 65–67 °C (DIPE, colorless crystals); IR 3097, 2930, 1608, 1466, 1442, 1421, 1167, 1141, 1113, 679, 669 cm^{-1}; ^{1}H-NMR δ: 2.82 (s, 3H, SCH_3), 7.67 (dd, J = 8 Hz, J = 2 Hz, H-6), 8.16 (m, 1H, H-4), 8.26 (br, d, J = 8 Hz, 1H, H-7); ^{13}C-NMR δ: 15.57 (SCH_3), 117.5 (q, J = 4 Hz, C-4), 120.1 (q, J = 4 Hz, C-6), 123.1 (d, J = 3.2 Hz, C-7), 124.2 (q, J = 270 Hz, CF_3), 127.2 (q, J = 31.8 Hz, C-5), 138.8 (d, J = 2 Hz, C-3a), 152.5 (C-7a), 171.4 (C-2); $C_9H_6F_3NS_2$ (MW = 249.27) requires: C43.37, H2.43, N5.62; found: C43.38, H2.37, N5.56.

*General procedure for the preparation of compounds **12a,b***
The methylthiobenzothiazole **11a** (3.91 g, 0.02 mol) or **11b** (4.99 g, 0.02 mol) was heated at 80 °C in 95% hydrazine hydrate (11.01 g, 0.22 mol) containing 10 drops of ethanol for 5 h (the reaction course was followed by TLC; eluent: ethyl acetate). The mixture was cooled to room temperature and then cooled overnight. The precipitate formed was filtered off, washed with water, and recrystallized.

*5-Methyl-2-hydrazinobenzothiazole **12a**.* Yield: 3.48 g (97%); mp 236–238 °C (1-propanol, colorless crystals); IR 3448, 3317, 3200, 3140, 3065, 2960, 1651, 1562, 1466, 1452, 1417 cm^{-1}. ^{1}H-NMR δ: 2.32 (s, 3H, CH_3), 4.98 (s, 2H, NH_2), 6.80 (m, 1H, H-6), 7.15 (m, 1H, H-4), 7.52 (d, J = 8 Hz, 1H, H-7), 8.93 (br s, 1H, NH); ^{13}C-NMR δ: 15.6 (CH_3), 118.3 (C-6), 120.4 (C-4), 121.3 (C-7), 127.2 (C-5), 134.5 (C-7a), 153.7 (C-3a), 174.0 (C-2); $C_8H_9N_3S_2$ (MW = 179.24) requires: C53.61, H5.06, N23.44; found: C53.97, H4.96, N23.57.

*5-Trifluoromethyl-2-hydrazinobenzothiale **12b**.* Yield: 3.28 g (70%); mp 123–125 °C (MeOH, colorless crystals); IR 3315, 3202, 3071, 2967, 1657, 1570, 1456, 1429, 1332, 1176, 1147 cm^{-1}; ^{1}H-NMR δ: 5.16 (s, 2H, NH_2), 7.27 (dd, 1H, J = 8 Hz, J = 3 Hz, H-6), 7.56 (m, 1H, H-4), 7.90 (d, 1H, J = 8 Hz, H-7), 9.36 (s, 1H, NH); ^{13}C-NMR δ: 113.7 (q, J = 4 Hz, C-4), 116.1 (q, J = 4 Hz, C-6), 121.98 (C-7), 124.6 (q, J = 270 Hz,

CF_3), 126.3 (q, J = 31 Hz, C-5), 134.8 (d, J = 2 Hz, C-3a), 153.7 (C-7a), 175.5 (C-2); $C_8H_6F_3N_3S_2$ (MW = 233.21) requires: C41.20, H2.59, N18.02; found: C41.35, H2.66, N18.02.

5-Fluoro-2-hydrazinobenzothiazole ***14***

The aminobenzothiazole **13** [19] (17.02 g, 0.10 mol) was suspended in ethylene glycol (100 mL) and 95% hydrazine hydrate (10.00 g, 0.20 mol) and hydrazine monohydrochloride (6.80 g, 0.10 mol) were added. The mixture was heated at 140 °C for 2 h under nitrogen. The solution was cooled to room temperature and water (100 mL) was added to precipitate the product. The crystals were filtered off, washed with water and dried. An analytically pure product was obtained by recrystallization from 2-propanol.

Yield: 18.11 g (98%); mp 220–222 °C; IR 3474, 3423, 3198, 3140, 3065, 2957, 1651, 1574, 1556, 1462, 1425, 1151, 958 cm^{-1}; ^{1}H-NMR δ: 5.07 (s, 2H, NH_2), 7.66 (dd, 1H, J = 8 Hz, $J_{(H7/F)}$ = 6 Hz, H-7), 7.10 (dd, 1H, J = 3 Hz, $J_{(H4/F)}$ = 11 Hz, H-4), 6.81 (dddd, 1H, J = 3 Hz, J = 8 Hz, $J_{(H6/F)}$ = 10 Hz, H-6); ^{13}C-NMR δ: 104.2 (d, J = 23.8 Hz, C-4), 107.3 (d, J = 23.8 Hz, C-6), 121.6 (d, J = 11 Hz, C-7), 125.9 (d, J = 2 Hz, C-7a), 154.8 (d, J = 12.4 Hz, C-3a), 161.1 (d, J = 235.8 Hz, C-5), 176.0 (C-2); $C_7H_6FN_3S_2$ (MW = 183.20) requires: C45.89, H3.30, N22.94; found: C46.30, H3.39, N23.04.

1-Acetylisoquinoline ***18c***

To a stirred solution of 1-cyanoisoquinoline (8.94 g, 0.058 mol) in a mixture of dry diethylether (100 mL) and dry THF (20 mL) was added slowly a solution of 3.0 M methylmagnesium iodide in diethyl ether (11.57 g, 0.696 mol) at –30 °C under argon. The mixture was allowed to warm to –15 °C and stirred for 1.5 h at this temperature. Then 2 N HCl (50 mL) was added and stirring was continued for another 15 min while keeping the temperature below 0 °C. The organic layer was separated, the aqueous phase was made alkaline with saturated $NaHCO_3$ solution and extracted several times with CH_2Cl_2. The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was successively purified by column chromatography (CH_2Cl_2/EtOAc 7:3) to give a pale-yellow oil which became a solid upon cooling.

Yield: 8.36 g (84%). ^{1}H-NMR δ: 2.77 (s, 3H, CH_3), 7.71–7.86 (m, 2H, H-6/7), 8.04–8.10 (m, 2H, H-5/8), 8.62 (d, J = 5.6 Hz, 1H, H-4), 8.75–8.90 (m, 1H, H-3); ^{13}C-NMR δ: 28.3 (CH_3), 124.6 (CH), 124.7 (C), 126.0 (CH), 127.2 (CH), 129.2 (CH), 130.5 (CH), 136.5 (C), 141.0 (C-3), 152.4 (C-1), 202.0 (C=O).

General procedure for the preparation of benzothiazolyl hydrazones ***7a–f*** *and* ***8a–k***

A mixture of 3.35 mmol of the appropriate carbonyl compound (**15a** or **15b**) and 3.35 mmol of the 2-hydrazinobenzothiazoles **12a–c**, **14** or **17** or an equimolar amount of **18a–k** and **17** in methanol (20 mL) containing 5 to 10 drops of glacial acetic acid was heated at 80 °C and the reaction followed by TLC (CH_2Cl_2/EtOAc 7:3). Reaction times varied between 6 to 10 h. The precipitates separating out after refrigeration overnight were filtered off and recrystallized.

Cell proliferation experiments

ZR-75-1 (breast carcinoma, ATCC CRL 1500), Burkitt's lymphoma (CA 46, ATCC CRL 1648), CCRF-CEM (acute lymphoblastic leukemia, ATCC CCL 119), HeLa (epitheloid cervix carcinoma, ATCC CCL 2) and HT-29 (colon adenocarcinoma, ATCC HTB 38) cells were obtained from the American Type Culture Collection, Rockville, MD. MEXF 276 L (melanoma) cells were kindly provided by HH Fiebig, Freiburg, Germany. K562-WT (wild type) and K562-DFMO^r (hydroxyurea resistant) cells [30] were kindly provided by O Heby, Lund, Sweden. HeLa, MEXF, ZR-75-1, Burkitt's lymphoma and CCRF-CEM were grown in RPMI 1640, HT-29 in McCoy's 5A medium 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 of ZR-75-1, HeLa, HT-29 and MEXF-276 L was detected by the SRB-assay. In this assay 3000–10 000 cells in 200 μL medium were seeded per well into 96-well plates. Dose–response curves for CCRF-CEM, Burkitt's lymphoma and K562 cells were detected by an MTT-assay from Boehringer Mannheim, Mannheim, Germany. Approximately 10 000 cells per 100 μL were seeded in 96-well plates. After an initial incubation of 4 h, various drug concentrations were added to the cells and exposed continuously for 72 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The drugs were dissolved in dimethylsulfoxide (DMSO). The concentration of DMSO was 0.5% and this was not toxic. Subsequently, the samples were processed and the absorption was detected by a microplate reader (Model 3550, Bio-Rad).

Molecular modeling

All molecules were built using structures from the Tripos standard fragment library. The potential energy of each structure was then refined by a molecular mechanics procedure (MAXIMIN) [41] and charges were calculated using the Gasteiger–Hückel [33] method. Conformational analysis was performed in a two-step procedure using the molecular dynamics and the random search methods, respectively (molecular dynamics: 100 ps equilibration at 1000 K or 800 K, respectively, and 100 ps at 300 K; conformers were recorded every 200 fs in the second period). The potential energies of the five conformers possessing the lowest energy of each dynamics simulation run were further minimized and then the conformer possessing the lowest energy was subjected to the random search procedure. The thus obtained minima structures were assumed to be located near the global minima structures. The comparative molecular field analysis was performed with default settings used throughout. The steric and electrostatic potentials were calculated using an sp^3 carbon probe with a 1+ charge. The grid used in the CoMFA study had a resolution of 2 Å and a size of 24 Å/18 Å/12 Å *(x/y/z)* resulting in 648 data points. The steric and electrostatic energy values were truncated to 30 kcal/mol. The electrostatic energy term was ignored at lattice intersections yielding maximal (30 kcal/mol) steric values. The linear expression of the CoMFA results was calculated with the partial least-squares analysis (PLS) algorithm, variable selection was done utilizing a minimum sigma of 2.0 for the initial analysis. Cross-validation was performed using the leave-one-out method to determine the optimal number of components in the PLS analysis. Statistical evaluation of the biological data was performed within the Sybyl QSAR module: 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).

Acknowledgments

Financial assistance was generously provided by the Austrian 'Fonds zur Förderung der Wissenschaftlichen Forschung', project No P 09879-MED. We thank W Mühlecker for recording the COLOC spectrum and A Schmidt for preparing compounds **8h** and **8k**.

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