Novel Thiosemicarbazones Derived from Formyl- and Acyldiazines: Synthesis, Effects on Cell Proliferation, and Synergism with Antiviral Agents†,‡

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The synthesis of a series of novel thiosemicarbazones (TSC's) derived from various alkyl diazinyl (3-pyridazinyl, 4-pyrimidinyl, 2-pyrazinyl) ketones and 3-pyridazinecarbaldehyde and their evaluation against herpes simplex virus (HSV) and human immunodeficiency virus (HIV) as well as the determination of their cytotoxicity are described. In addition, the effects of combination of such TSC's with the well-known antiviral drugs acyclovir (ACV) and 3'-azido-3'-deoxythymidine (AZT) were studied. Under our experimental conditions, i.e. determination of virus-induced cytopathic effect upon infection of HUT78 cells with HSV-1 and upon infection of MT4 cells with HIV-1, no antiviral activity could be detected with any of the TSC's. However, pronounced effects on proliferation of these rapidly growing T4 lymphocyte cell lines were observed. Clear structure–activity relationships with regard to these cytotoxic effects could be established: compared to pyridine, pyrazine, or pyrimidine-derived TSC's most of the 3-pyridazinyl congeners investigated are less cytotoxic; introduction of a methyl group into C-6 of the pyridazine system or prolongation of the acyl moiety in these compounds has essentially no influence; all compounds bearing an *N,N*-dimethylamino or a cycloamino substituent are much more toxic than those with an NH_2 or NHR substituent; the nature of R in the latter type of compounds has only moderate influence. It has been reported that combination of TSC's with the antiviral agent acyclovir (ACV) results in potentiation of this well-known drug. We evaluated the potential of our series of novel TSC's in combination with ACV for inhibition of HSV-1-induced cytopathic effect in HUT78 cells and in combination with 3'-azido-3'-deoxythymidine (AZT) for inhibition of HIV-1-induced cytopathic effect in MT4 cells. Only four compounds out of this series, all characterized by an unsubstituted NH_2 group, exhibited moderate synergism with the above mentioned antiviral drugs. Our results do not support the previously expressed opinion that TSC's are selective antiviral agents. In our test systems no evidence for inhibition of virus-induced cytopathic effect was obtained. The TSC derivatives exhibited a broad range of cytotoxic effects, some at concentrations considerably below those reported to have antiviral efficacy. Several of our novel diazine-derived compounds proved advantageous over the previously described pyridine analogues with regard to cytotoxicity. Moderate synergism could be detected for relatively noncytotoxic TSC's with the antiviral drugs ACV (antiherpes) and AZT (anti-HIV).

Introduction

Thiosemicarbazone (TSC) derivatives have raised considerable interest in chemistry and biology due to their antibacterial,[2] antimalarial,[3] antineoplastic,[4] and antiviral[5,6] activities. Much effort has been devoted to structural modifications[7] and to the elucidation of structure—activity relationships with the objective to obtain more efficacious chemotherapeutic and antimicrobial agents. The antiviral, namely antiherpesvirus, activity of certain TSC derivatives has been shown to be based on inactivation of ribonucleotide reductases.[8] Herpesviruses encode distinct ribonucleotide reductases which are essential for viral replication.[9] The viral enzymes differ from the isofunctional cellular enzyme in their lack of being subject to allosteric regulation. It has been reported that the herpesvirus-encoded enzymes are more sensitive to TSC-mediated inactivation than the cellular homologue.[10] Furthermore, an inhibitory effect on plaque formation by various herpesviruses could be demonstrated in cell cultures. On the other hand, there was evidence for toxic effects on cells at drug concentrations similar to those required to inhibit virus. This result cast doubt on the selectivity of this type of drugs.[11] Inactivation of ribonucleotide reductases in general will lead to a reduction of intracellular pools of deoxynucleotides.[12] Thus, nucleotide analogues being antivirally effective by competing with the natural nucleotides may be enhanced in their efficacy in the presence of TSC's.

In a previous publication we described the evaluation of the cytotoxic and antiherpetic potential of various novel thiosemicarbazones[13] derived from pyridazinecarbaldehydes and alkyl pyridazinyl ketones. The result of these studies was that the 3-pyridazinyl derivatives were

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‡ This is the 63rd communication on pyridazines. For Pyridazines 62, see ref 1.

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(1) Pittenauer, E.; Allmaier, G.; Schmid, E.; Krenmayr, P.; Heinisch, G. Electron-Ionisation Mass Spectrometry of Phenylpyridazinylmethanols and Phenyl Pyridazinyl Ketones. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 421–424.

(2) Dobek, A. S.; Klayman, D. L.; Dickson, E. T.; Scovill, J. P.; Oster, C. N. Thiosemicarbazones of 2-Acetylpyridine, 2-Acetylquinoline, 1- and 3-Acetylisoquinoline and Related Compounds as Inhibitors of Clinically Significant Bacteria in vitro. *Arzneim.-Forsch./Drug Res.* **1983**, *33 (II)*, 1583–1591.

(3) Klayman, D. L.; Scovill, J. P.; Bartosevich, J. F.; Mason, C. J. 2-Acetylpyridine Thiosemicarbazones. 2. N^4,N^4-Disubstituted Derivatives as Potential Antimalarial Agents. *J. Med. Chem.* **1979**, *22*, 1367–1373.

(4) Klayman, D. L.; Scovill, J. P.; Mason, C. J.; Bartosevich, J. F.; Bruce, J.; Lin, A. J. 2-Acetylpyridine Thiosemicarbazones. Part 6. 2-Acetylpyridine and 2-Butyrylpyridine Thiosemicarbazones as Antileukemic Agents. *Arzneim.-Forsch./Drug Res.* **1983**, *33 (II)*, 909–912.

(5) Hamre, D.; Bernstein, J.; Donovick, R. Activity of p-Aminobenzaldehyde 3-Thiosemicarbazone on vaccina virus in the chick embryo and in the mouse. *Proc. Soc. Exp. Biol. Med.* **1950**, *73*, 275–278. Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. J. Antiviral Activity of 2-Acetylpyridine Thiosemicarbazones Against Herpes Simplex Virus. *Antimicrob. Agents Chemother.* **1981**, *19*, 682–685.

(6) Shipman, C., Jr.; Smith, S. H.; Drach, J. C.; Klayman, D. L. Thiosemicarbazones of 2-acetylpyridine, 2-acetylquinoline, 1-acetylisoquinoline, and related compounds as inhibitors of herpes simplex virus in vitro and in a cutaneous herpes guinea pig model. *Antiviral Res.* **1986**, *6*, 197–222.

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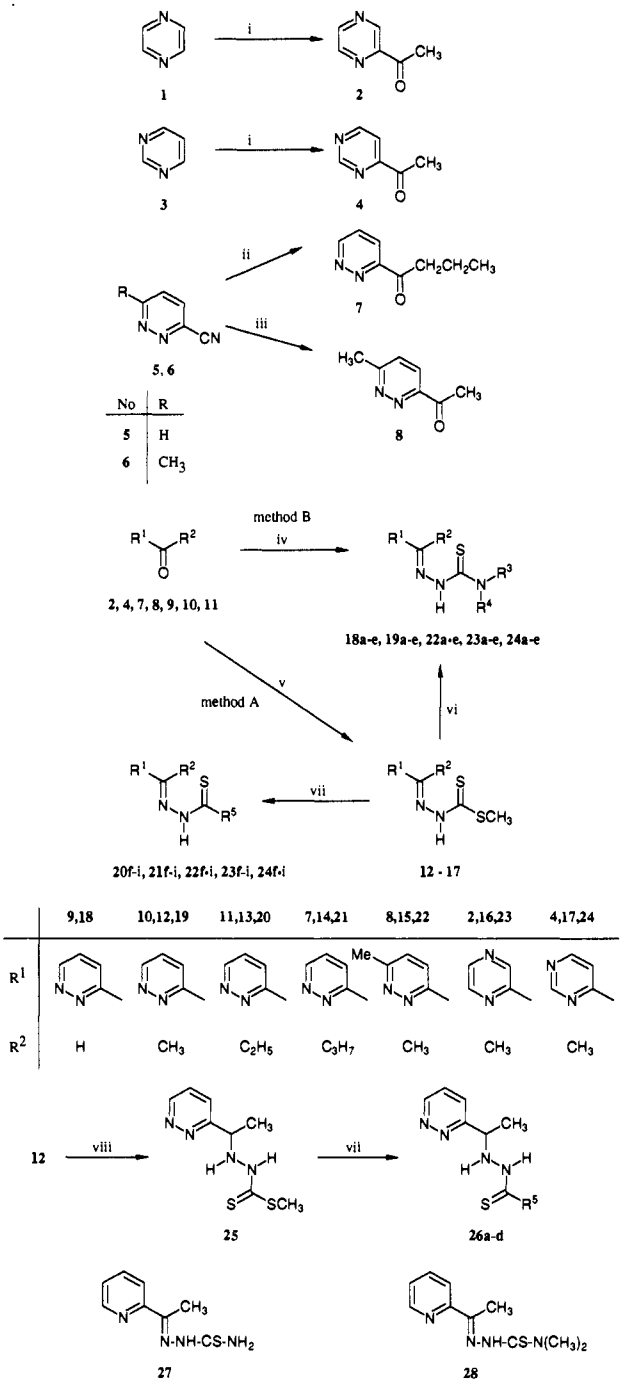
advantageous compared to the 2-pyridine congeners with regard to cytotoxicity and solubility in water.[13]

In continuation of these investigations we became interested in a series of so far unreported thiosemicarbazones derived from various alkyl diazinyl ketones (**2**, **4**, **7**, **8**, **10**, **11**) and 3-pyridazinecarbaldehyde (**9**). Here, we report the synthesis of these TSC's and the evaluation of their potential activity against herpes simplex virus (HSV) and human immunodeficiency virus (HIV) as well as the determination of their cytotoxicity. In addition, the effects of combination of such TSC's with the well-known antiviral drugs acyclovir (ACV) and 3′-azido-3′-deoxythymidine (AZT) were studied.

Synthesis

The starting 3-pyridazinecarbaldehyde (**9**) and the alkyl 3-pyridazinyl ketones **10** and **11** were prepared according to known procedures.[13,14] Methyl 2-pyrazinyl ketone (**2**)[15,16] was prepared in 50% yield by employing a method recently developed in our group.[17] It consists of radical substitution of the protonated parent heteroarene in a water/dichloromethane two-phase system and turned out to be advantageous to the procedures given in refs 15 and 16. In a similar manner, methyl 4-pyrimidinyl ketone (**4**)[18] could be prepared in 48% yield from protonated pyrimidine again using acetaldehyde as a source for acetyl radicals. The novel pyridazine-derived ketones **7** and **8** were obtained from 3-pyridazinecarbonitrile (**5**)[19] or 6-methyl-3-pyridazinecarbonitrile (**6**),[19] respectively, by means of Grignard-type reactions (Scheme I).

Scheme I



For the preparation of the target thiosemicarbazones **18–24** two approaches known from the literature[20–22] were employed. The products derived from 3-pyridazinecarb-

(7) Agrawal, K. C.; Sartorelli, A. C. The Chemistry and Biological Activity of α-(*N*)-Heterocyclic Carboxaldehyde Thiosemicarbazones. *Progr. Med. Chem.* **1978**, *15*, 321–356. Brockman, R. W.; Sidwell, R. W.; Arnett, G.; Shaddix, S. Heterocyclic Thiosemicarbazones: Correlation Between Structure, Inhibition of Ribonucleotide Reductase, and Inhibition of DNA Viruses (34528). *Proc. Soc. Exp. Biol. Med.* **1970**, *133*, 609–614. Agrawal, K. C.; Booth, B. A.; DeNuzzo, S. M.; Sartorelli, A. C. Potential Antitumor Agents. 14. 4-Substituted 2-Formylpyridine Thiosemicarbazones. *J. Med. Chem.* **1976**, *19*, 1209–1214. Agrawal, K. C.; Booth, B. A.; Sartorelli, A. C. Potential antitumor agents. 1. A series of 5-substituted 1-formylisoquinoline thiosemicarbazones. *J. Med. Chem.* **1968**, *11*, 700–703.

(8) Spector, T.; Jones, T. E. Herpes Simplex Type 1 Ribonucleotide Reductase. *J. Biol. Chem.* **1985**, *260*, 8694–8697. Turk, S. R.; Shipman, C., Jr.; Drach, J. C. Selective Inhibition of Herpes Simplex Virus Ribonucleoside Diphosphate Reductase by Derivatives of 2-Acetylpyridine Thiosemicarbazone. *Biochem. Pharmacol.* **1986**, *35*, 1539–1545.

(9) Averett, D. R.; Lubbers, C.; Elion, G. B.; Spector, T. Ribonucleotide Reductase Induced by Herpes Simplex Type 1 Virus. *J. Biol. Chem.* **1983**, *258*, 9831–9838. Averett, D. R.; Furman, P. A.; Spector, T. Ribonucleotide Reductase of Herpes Simplex Virus Type 2 Resembles That of Herpes Simplex Virus Type 1. *J. Virol.* **1984**, *52*, 981–983. Spector, T.; Stonehuerner, J. G.; Biron, K. K.; Averett, D. R. Ribonucleotide Reductase Induced by Varicella Zoster Virus. Characterisation, and Potentiation of Acyclovir by its Inhibition. *Biochem. Pharmacol.* **1987**, *36*, 4341–4346.

(10) Spector, T. Inhibition of Ribonucleotide Reductases Encoded by Herpes Simplex Viruses. *Pharm. Ther.* **1985**, *31*, 295–302.

(11) Field, H. J.; Reading, M. J. The inhibition of bovine herpesvirus-1 by methyl 2-pyridyl ketone thiosemicarbazone and its effects on bovine cells. *Antiviral Res.* **1987**, *7*, 245–256.

(12) Karlsson, A.; Harmenberg, J. Effects of Ribonucleotide Reductase Inhibition on Pyrimidine Deoxynucleotide Metabolism in Acyclovir-Treated Cells Infected with Herpes Simplex Virus Type 1. *Antimicrob. Agents Chemother.* **1988**, *32*, 1100–1102.

(13) Easmon, J.; Heinisch, G.; Holzer, W.; Rosenwirth, B. Synthesis and Antiviral Activity of Thiosemicarbazone Derivatives of Pyridazinecarbaldehydes and Alkyl Pyridazinyl Ketones. *Arzneim.-Forsch./Drug Res.* **1989**, *39* (*II*), 1196–1201.

(14) Heinisch, G.; Mayrhofer, A. Pyridazin-3-carbaldehyd: Synthese und Reaktionsstudien. *Monatsh. Chem.* **1977**, *108*, 213–224.

(15) Kushner, S.; Dalalian, H.; Sanjurjo, J. L.; Bach, F. L., Jr.; Safir, S. R.; Smith, V. K., Jr.; Williams, J. H. Experimental Chemotherapy of Tuberculosis. II. The Synthesis of Pyrazinamides and Related Compounds. *J. Am. Chem. Soc.* **1952**, *74*, 3617–3621.

aldehyde (compounds **18a–e**) as well as the alkyl diazinyl ketone derived compounds **19a,b**, **22a,b**, **23a,b**, and **24a,b** were obtained in satisfactory yields by employing method B consisting of reaction of the carbonyl compound with the appropriately N-4 substituted thiosemicarbazides. For the synthesis of the remaining target thiosemicarbazones the starting carbonyl compounds were reacted with methyl hydrazinecarbodithioate[22] to obtain compounds **12–17**. Displacement of the *S*-methyl group in the latter compounds by reaction with the appropriate primary or secondary amines then led to compounds **19c–e**, **20f–i**,[13] **21f–i**, **22c–i**, **23c–i**, and **24c–i** (method A). To obtain a series of selected thiosemicarbazides of type **26a–d**, the methyl dithioate **12** was reduced to **25** by sodium borohydride in ethanol solution in analogy to ref 23. Subsequent reaction of **25** with various cyclic amines afforded compounds **26a–d** in satisfactory overall yield. The pyridine-derived thiosemicarbazones **27** and **28**, required as comparison materials, were synthesized following reported procedures.[6,22]

On the basis of previous investigations of the configuration of a variety of structurally closely related thiosemicarbazones[24] (employing ^{1}H- and ^{13}C-NMR spectroscopy and homonuclear NOE-difference experiments), the stereochemistry of the novel thiosemicarbazones prepared could be unequivocally determined. In most cases only one isomer, namely the *E*-configurated compound was isolated, whereas compounds **21f**, **21i**, **21h**, **22f**, **22h**, **23h**, and **24h** were obtained as *E*/*Z*-mixtures with the *E*-isomer far predominating. A detailed presentation of these spectroscopic investigations will be published elsewhere.

For physical, analytical, and biological data of the thiosemicarbazones prepared, see Tables I and II; for the data of thiosemicarbazides **26a–d**, see Table III.

Results and Discussion

According to the literature,[5,6] 2-acetylpyridine thiosemicarbazone (**27**) and its *N*,*N*-dimethyl congener **28** exhibit activity against herpes simplex virus in vitro. The 50% inhibitory concentrations are in the range of 0.9–1.2 μg/mL (**27**) and 0.08–0.17 μg/mL (**28**) while inhibition of cellular DNA synthesis is observed at 2.0 (**27**) and 0.24 μg/mL (**28**); inhibition of cellular protein synthesis is only evident at more than 10-fold higher concentrations.

Under our experimental conditions, i.e. determination of virus-induced cytopathic effect upon infection of HUT78 cells with HSV-1 and upon infection of MT4 cells with HIV-1, no antiviral activity could be detected with any of the TSC's including compounds **27** and **28**. However, pronounced effects on proliferation of these rapidly growing T4 cell lines were observed at concentrations even lower than those reported for compounds **27** and **28** to have antiviral efficacy. The TSC derivatives varied considerably in their cytotoxic potential, and clear structure–activity relationships were evident (Tables I–III; the data shown are mean values of five determinations). It should be emphasized that completely parallel trends were observed with both cell lines used (HUT78 and MT4).

In our test systems derivative **27** inhibited cell proliferation at 0.35 (HUT78) and 0.049 μg/mL (MT4) while **28** was inhibitory at 0.00055 (HUT78) and 0.00011 μg/mL (MT4) (Table I).

Thus, in the pyridine-derived TSC **27** the replacement of the terminal NH_2 moiety by a *N*,*N*-dimethylamino group (compound **28**) results in a drastic enhancement of cytotoxicity (factor of ~100–1000). This result corroborates previous findings,[6] namely that replacement of its terminal NH_2 moiety by an *N*,*N*-dimethylamino group results in an increase in antiviral activity against HSV-1 and HSV-2, but a decrease in the in vitro therapeutic index as well as an increase in dermal toxicity during in vitro tests in guinea pigs.

In accordance with this observation, also in the series of novel diazine-derived TSC's **18**, **19**, **22**, **23**, and **24**, the corresponding dimethylamino derivatives (compounds **18e**, **19e**, **22e**, **23e**, **24e**) turned out to be the most cytotoxic ones compared to the related congeners with a primary or secondary amino function. The ED_{50} values of compounds **23e** and **24e**, in which an additional nitrogen atom is incorporated in position **4** or **5** of the *N*-heteroarene (pyrazine or pyrimidine derivatives), equal that of **28**. By contrast, replacement of C-6 of the pyridine ring in compound **28** by a nitrogen atom (3-pyridazinyl derivative **19e**) results in a decrease of cytotoxicity by a factor of 100–1000. A similarly low level of cytotoxicity is observed when an additional methyl group is attached to C-6 of the pyridazine system in **19e** (compound **22e**) and with the 3-pyridazinecarbaldehyde-derived TSC **18e**.

The evaluation of the inhibitory effect of compounds **23f–i** and **24f–i** on the proliferation of HUT78 and MT4 cells (Table II) revealed that switching from an *N*,*N*-dimethylamino function (as present in **23e** and **24e**) to various cycloamino functions does not influence the cytotoxicity significantly. The pyridazine-derived TSC's **22f–i**, which again displayed ED_{50} values similar to those of the *N*,*N*-dimethylamino congener **22e**, are considerably less cytotoxic than the before mentioned pyrazine or pyrimidine analogues. On the other hand, there is no significant difference in the cytotoxicity between the TSC's **22f–i** and the isomeric compounds **20f–i**, in which the methyl group attached to C-6 of the diazine nucleus is (formally) shifted to the side chain (3-propionylpyridazine derivatives). In addition, further homologation of the alkyl side chain as given in compounds **21f–i** again does not alter markedly the effect on cell proliferation nor does reduction of the C=N double bond as shown by the ED_{50} values of the thiosemicarbazides **26a–d** (Table III).

For the novel TSC's characterized by a free or monoalkylated terminal amino function (**18a–d**, **19a–d**, **22a–d**,

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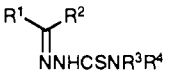
(17) Heinisch, G.; Lötsch, G. Marked Increase in the Regioselectivity of Radical Substitution: Minisci Reaction in a Two-Phase System (Entscheidende Steigerung der Regioselektivität bei radikalischen Substitutionen: Minisci-Reaktion im Zweiphasensystem). *Angew. Chem.* **1985**, *97*, 694–695; *Angew. Chem. Internat. Ed. Engl.* **1985**, *24*, 692–693.
(18) Robba, M. Contribution a L'Étude de Quelques Dérivés des Diazines. *Ann. Chim. (France)* **1960**, *5*, 351–414.
(19) Dostal, W.; Heinisch, G. Pyridazines XXXI. A Facile Synthesis of 3-pyridazinecarbonitriles vis 2-(4-Toluenesulfonyl)-2,3-dihydro-3-pyridazinecarbonitriles. *Heterocycles* **1986**, *24*, 793–797.
(20) McElhinney, R. S. Derivatives of Thiocarbamic Acid. Part I. Preparation of 4-Substituted Thiosemicarbazides. *J. Chem. Soc. (C)* **1966**, 950–955.
(21) Agrawal, K. C.; Lee, M. H.; Booth, B. A.; Moore, E. C.; Sartorelli, A. C. Potential Antitumor Agents. 11. Inhibitors of Alkaline Phosphatase, an Enzyme Involved in the Resistance of Neoplastic Cells to 6-Thiopurines. *J. Med. Chem.* **1974**, *17*, 934–938.
(22) Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. 2-Acetylpyridine Thiosemicarbazones. 1. A New Class of Potential Antimalarial Agents. *J. Med. Chem.* **1979**, *22*, 855–862.
(23) Klayman, D. L.; Scovill, J. P.; Bartosevic, J. F.; Bruce, J. 2-Acetylpyridine Thiosemicarbazones. 5. 1-[1-(2-Pyridyl)-ethyl]-3-thiosemicarbazides as Potential Antimalarial Agents. *J. Med. Chem.* **1983**, *26*, 35–39.
(24) Easmon, J.; Heinisch, G.; Holzer, W. Pyridazines 47. The Configuration of Novel Thiosemicarbazone Derivatives of Pyridazinecarbaldehydes and Alkyl Pyridazinyl Ketones. *Heterocycles* **1989**, *29*, 1399–1408.

Table I. Physical and Biological Data of Thiosemicarbazones **18a–e, 19a–e, 22a–e, 23a–e, 24a–e, 27, and 28**



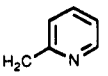
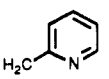
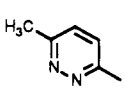
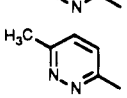
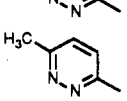
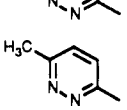
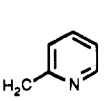
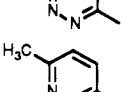
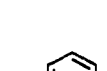
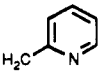
no.	R^1	R^2	R^3	R^4	mp, °C	formula	synth method	% yield	recryst solvent	inhibitory effect,[a] ED_{50}, μg/mL HUT 78	MT 4
18a		H	H	H	236–238	*b*	B	*b*	*b*	4.21[c]	2.18[d]
18b		H	H	CH_3	221–223 dec	$C_7H_9N_5S·0.25H_2O$	B	95	EtOH	4.52	2.42
18c		H	H	$CH_2CH{=}CH_2$	221–222	$C_9H_{11}N_5S$	B	74	EtOH	6.22	3.10
18d		H	H		207–209	$C_{12}H_{12}N_6S$	B	94	EtOH	5.62	3.90
18e		H	CH_3	CH_3	177–178	$C_8H_{11}N_5S$	B	60	EA[e]	0.11	0.11
19a		CH_3	H	H	198–199	$C_7H_9N_5S$	B	70	EtOH	0.99[c]	1.13[d]
19b		CH_3	H	CH_3	213–216 dec	$C_8H_{11}N_5S$	B	74	EtOH	1.86	1.62
19c		CH_3	H	$CH_2CH{=}CH_2$	155–158	$C_{10}H_{13}N_5S·0.3H_2O$	A	52	DMSO–H_2O	2.65	1.28
19d		CH_3	H		200–203	$C_{13}H_{14}N_6S$	A	43	EtOH	2.01	0.87
19e		CH_3	CH_3	CH_3	156–159	$C_9H_{13}N_5S·0.2MeOH$	A	68	MeOH	0.079	0.10
22a		CH_3	H	H	202–204 dec	$C_8H_{11}N_5S·0.5H_2O$	B	93	EtOH	1.58[c]	1.49[d]
22b		CH_3	H	CH_3	206–209	$C_9H_{13}N_5S$	B	78	EtOH	2.15	1.52
22c		CH_3	H	$CH_2CH{=}CH_2$	137–139	$C_{11}H_{15}N_5S·0.5H_2O$	A	81	EA[e]	2.81	1.47
22d		CH_3	H		213–214 dec	$C_{14}H_{16}N_6S$	A	76	EtOH	2.82	1.64
22e		CH_3	CH_3	CH_3	157–159	$C_{10}H_{15}N_5S$	A	70	MeOH	0.088	0.12
23a		CH_3	H	H	225–226 dec	*f*	B	95		1.39[c]	0.95
23b		CH_3	H	CH_3	231–233 dec	$C_8H_{11}N_5S$	B	69	EtOH	0.88	0.46
23c		CH_3	H	$CH_2CH{=}CH_2$	155–156 dec	$C_{10}H_{13}N_5S$	A	74	EtOH	0.036	0.014
23d		CH_3	H		200–203 dec	$C_{13}H_{14}N_6S$	A	82	EtOH	1.11	0.041
23e		CH_3	CH_3	CH_3	158–161 dec	$C_9H_{13}N_5S$	A	70	EtOH	0.00062	0.00045
24a		CH_3	H	H	211–213 dec	$C_7H_9N_5S$	B	84	MeOH	0.82	0.14
24b		CH_3	H	CH_3	202–205	$C_8H_{11}N_5S$	B	80	MeOH	0.022	0.020

Table I (Continued)

no.	R^1	R^2	R^3	R^4	mp, °C	formula	synth method	% yield	recryst solvent	inhibitory effect,[a] ED_{50}, μg/mL HUT 78	MT 4
24c		CH_3	H	$CH_2CH{=}CH_2$	105–107	$C_{10}H_{13}N_5S$	A	50	CHEX[g]	0.031	0.081
24d		CH_3	H		206–209	$C_{13}H_{14}N_6S$	A	61	EtOH	0.24	0.018
24e		CH_3	CH_3	CH_3	152–154	$C_9H_{13}N_5S$	A	71	EA[e]	0.00065	0.00049
27		CH_3	H	H	*h*	*h*	*h*	*h*	*h*	0.35	0.049
28		CH_3	CH_3	CH_3	*i*	*i*	*i*	*i*	*i*	0.00055	0.00011

[a] Inhibitory effect on the cell proliferation of HUT 78 and MT 4 cells. [b] Reference 30. [c] Synergism with ACV (compare Figure 1 and/or text). [d] Synergism with AZT (compare Figure 1 and/or text). [e] EA = ethyl acetate. [f] Reference 15. [g] CHEX = cyclohexane. [h] Reference 22. [i] Reference 3.

23a–d, **24a–d**) the following results were obtained: In general, all these compounds are significantly less cytotoxic than the corresponding compounds with an *N,N*-dimethylamino or cycloamino function. Also, the pyridazine derivatives (**18**, **19**, **22**) show a remarkably low level of toxicity, whereas most of the pyrazine and pyrimidine (**23**, **24**) congeners are more toxic. The inhibitory effect on cell proliferation of the 3-formylpyridazine-derived TSC's **18a–d** is only slightly enhanced when the formyl H is replaced by a methyl group (3-acetylpyridazine-derived compounds **19a–d**). No change is observed upon additional introduction of a methyl group into the 1,2-diazine system (compounds **22a–d**). It is of interest to note that, within the series investigated, the nature of the alkyl substituent attached to the terminal N-atom obviously has no strong influence on cytotoxicity, provided that this nitrogen atom still bears one hydrogen.

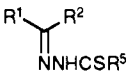
In summary, from these studies the following conclusions can be drawn with regard to inhibitory effects on cell proliferation: (a) compared to pyridine, pyrazine, or pyrimidine-derived TSC's, most of the 3-pyridazinyl congeners investigated are less cytotoxic, (b) introduction of a methyl group into C-6 of the pyridazine system or prolongation of the acyl moiety in these compounds has essentially no influence, (c) all compounds bearing an *N,N*-dimethylamino or a cycloamino substituent are much more toxic than those with an NH_2 or NHR substituent, and (d) the nature of R in the latter type of compounds has only moderate influence.

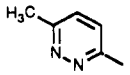
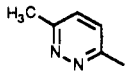
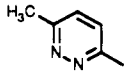
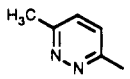
It has been reported that combination of 2-acetylpyridine 4-(2-morpholinoethyl)thiosemicarbazone (Burroughs Wellcome A723U) or 2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]thiocarbonohydrazone (Burroughs Wellcome A1110U) with the antiviral agent acyclovir (ACV) results in potentiation of this well-known drug.[25] We evaluated the potential of our series of novel compounds **18–24**, **26**, and the pyridine-derived congeners **27** and **28** in combination with ACV for inhibition of HSV-1-induced cytopathic effect in HUT78 cells and in combination with 3′-azido-3′-deoxythymidine (AZT) for inhibition of HIV-1-induced cytopathic effect in MT4 cells. Only four compounds out of this series (**18a**, **19a**, **22a**, and **23a**, compare Tables I–III), all characterized by an unsubstituted NH_2 group, exhibited reproducably moderate synergism with the above mentioned antiviral drugs. 3-Pyridazinecarbaldehyde thiosemicarbazone (**18a**) showed synergism with AZT with regard to inhibition of HIV and with ACV with regard to inhibition of HSV-1 (Figure 1). Replacement of the aldehyde H in **18a** by a methyl group (compound **19a**) and additional introduction of a methyl group into position 6 of the diazine system (compound **22a**) does not impair this activity. Surprisingly, the 2-acetylpyrazine-derived TSC **23a** showed synergism only with ACV (HSV-1 inhibition), but not with AZT. This may reflect some differences in pool sizes of dMTP's between the two cell lines used. With neither the pyrimidine-derived nor the pyridine-derived congeners (compounds **24a** and **27**) was potentiation of ACV or AZT observed. It should be emphasized that with the most cytotoxic compounds no synergism could be detected. One might have expected that the most cytotoxic derivatives, which presumably inhibit ribonucleotide reductase very effectively, but may, in addition, also have inhibitory effects at sites other than this enzyme, would reduce levels of cellular nucleotide pools and would thus enhance efficacy of the nucleotide analogues. However, it is known that virus replication requires cell metabolism, and therefore, only relatively intact cells support effective virus production. This delicate balance between the requirement for functioning cellular metabolism and presumed lowering of nucleotide pools by TSC's may explain why only relatively noncytotoxic compounds show enhancement of the antiviral effect of ACV and AZT.

In summary, our results do not support the previously expressed opinion[5,6,10] that certain TSC derivatives, e.g. **27** and **28**, are selective antiviral agents. In our test systems, i.e. virus replication in rapidly growing T4 lymphocyte cell lines, no evidence for inhibition of virus-induced cytopathic effect was obtained. The TSC derivatives exhibited a broad range of cytotoxic effects, some at concentrations considerably below those which were reported to be antiviral. Clear structure–activity relationships could be established. Several of our novel diazine-derived com-

(25) Spector, T.; Averett, D. R.; Nelson, D. J.; Lambe, C. U.; Morrison, R. W., Jr.; St. Clair, M. H.; Furman, P. A. Potentiation of antiherpetic activity of acyclovir by ribonucleotide reductase inhibition. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4254–4257. Spector, T.; Harrington, J. A.; Morrison, R. W., Jr.; Lambe, C. U.; Nelson, D. J.; Averett, D. R.; Biron, K.; Furman, P. A. 2-Acetylpyridine 5-[(dimethylamino)thiocarbonyl]thiocarbonohydrazone (A1110U), a potent inactivator of ribonucleotide reductases of herpes simplex and varicella-zoster virus and potentiator of acyclovir. *Proc. Natl. Acad. Sci. U. S.A.* **1989**, *86*, 1051–1055.

Table II. Physical and Biological Data of Thiosemicarbazones **20f–i, 21f–i, 22f–i, 23f–i, and 24f–i**

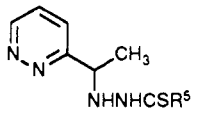


no.	R^1	R^2	R^5	mp, °C	formula	synth method	% yield	recryst solvent	inhibitory effect,[a] ED_{50}, μg/mL HUT 78	inhibitory effect,[a] ED_{50}, μg/mL MT 4
20f		CH_2CH_3		*b*	*b*	*b*	*b*	*b*	0.052	0.016
20g		CH_2CH_3		*b*	*b*	*b*	*b*	*b*	0.071	0.030
20h		CH_2CH_3		*b*	*b*	*b*	*b*	*b*	0.073	0.039
20i		CH_2CH_3		*b*	*b*	*b*	*b*	*b*	0.095	0.064
21f		$CH_2CH_2CH_3$		106–108	$C_{13}H_{19}N_5S$	A	67	MeOH	0.068	0.036
21g		$CH_2CH_2CH_3$		133–135	$C_{14}H_{21}N_5S$[c]	A	30	EA[d]	0.0023	0.0012
21h		$CH_2CH_2CH_3$		89–91	$C_{15}H_{23}N_5S$	A	86	MeOH	0.098	0.088
21i		$CH_2CH_2CH_3$		123–124	$C_{17}H_{25}N_5S$	A	66	EtOH	0.062	0.084
22f		CH_3		133–134 dec	$C_{12}H_{17}N_5S$	A	74	MeOH	0.073	0.036
22g		CH_3		169–171 dec	$C_{13}H_{19}N_5S$	A	91	MeOH	0.055	0.019
22h		CH_3		124–125 dec	$C_{14}H_{21}N_5S$	A	86	EA[d]	0.054	0.028
22i		CH_3		147–149 dec	$C_{16}H_{23}N_5S$	A	92	MeOH	0.053	0.055
23f		CH_3		177–178 dec	$C_{11}H_{15}N_5S$	A	85	MeOH	0.00049	0.00019
23g		CH_3		134–137 dec	$C_{12}H_{17}N_5S$	A	80	MeOH	0.00052	0.00055
23h		CH_3		126–127 dec	$C_{13}H_{19}N_5S$[e]	A	83	MeOH	0.0083	0.0059
23i		CH_3		135–138 dec	$C_{15}H_{21}N_5S$[e]	A	80	MeOH	0.0069	0.0054
24f		CH_3		178–180	$C_{11}H_{15}N_5S$	A	80	MeOH	0.00031	0.00026
24g		CH_3		122–125	$C_{12}H_{17}N_5S$	A	86	EA[d]	0.0037	0.0013
24h		CH_3		129–131	$C_{13}H_{19}N_5S$	A	73	EA[d]	0.0022	0.016
24i		CH_3		162–165	$C_{15}H_{21}N_5S$	A	78	EtOH	0.036	0.047

[a] Inhibitory effect on the proliferation of HUT 78 and MT 4 cells. [b] Reference 13. [c] No satisfactory elemental analysis obtained. High-resolution MS calcd for M^+ 291.1518, found 291.152 ± 0.005. [d] EA = ethyl acetate. [e] Compounds **23h** and **23i** are mentioned in ref 2, but no physical data are given.

pounds proved advantageous over the previously described pyridine analogues with regard to cytotoxicity. Moderate synergism could be detected for relatively noncytotoxic TSC's with the antiviral drug ACV (antiherpes) and AZT (anti-HIV). This enhancement of efficacy may be due to lowering of nucleotide pools by inhibition of ribonucleotide

Table III. Physical and Biological Data of Thiosemicarbazides **26a–d**



no.	R^5	mp, °C	formula	synth method	% yield	recryst solvent	inhibitory effect,[a] ED_{50}, μg/mL HUT 78	MT 4
26a		182–185	$C_{11}H_{17}N_5S$	A	70	MeOH	0.068	0.016
26b		164–167	$C_{12}H_{19}N_5S$	A	65	MeOH	0.074	0.033
26c		147–148	$C_{13}H_{21}N_5S$	A	52	MeOH	0.043	0.028
26d		151–153	$C_{15}H_{23}N_5S$	A	80	MeOH	0.038	0.034

[a] Inhibitory effect on the proliferation of HUT 78 and MT 4 cells.

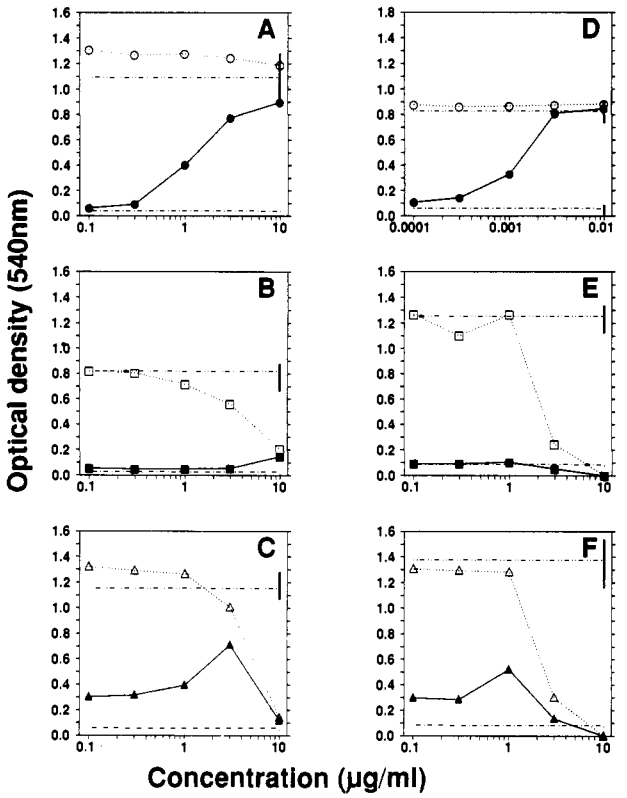


Figure 1. Viability of virus-infected and uninfected cell cultures grown in the presence or absence of various concentrations of test compounds as measured spectrophotometrically via in situ reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): —●—, —■—, —▲—, virus-infected cultures in the presence of test compounds; ··○··, ··□··, ··△··, uninfected cultures in the presence of test compounds; – - -, uninfected cultures without compound, average of five determinations; · · ·, virus-infected cultures without compound, average of five determinations; (1A) HUT78 cultures, with or without HSV-1, grown in the presence of various concentrations of ACV; (1B) HUT78 cultures, with or without HSV-1, grown in the presence of various concentrations of TSC 18a; (1C) HUT78 cultures, with or without HSV-1, grown in the presence of 1 μg/mL ACV plus various concentrations of TSC 18a; (1D) MT4 cultures, with or without HIV-1, grown in the presence of various concentrations of AZT; (1E) MT4 cultures, with or without HIV-1, grown in the presence of various concentrations of TSC 18a; (1F) MT4 cultures, with or without HIV-1, grown in the presence of 0.001 μg/mL AZT plus various concentrations of TSC 18a.

reductases; this hypothesis should be tested with biochemical methods.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot-stage microscope interfaced with a Mettler FP-2 digital thermometer and are uncorrected. Infrared spectra were run from KBr pellets on a Jasco IRA-1 spectrometer. Mass spectra (MS, electron-impact ionisation, 70 eV) were taken on a Varian MAT CH-7 mass spectrometer. NMR spectra were recorded from DMSO-d_6 solutions on a Bruker AC-80 (80.13 MHz for ^1H; 20.15 MHz for ^{13}C) spectrometer equipped with an Aspect 3000 computer and standard software. The center of the solvent multiplet (DMSO-d_6) was used as internal standard, which was related to tetramethylsilane with δ 2.49 ppm for ^1H and δ 39.50 ppm for ^{13}C. Microanalyses were obtained for C, H, N and are within ±0.4% of the theoretical values unless noted otherwise. Column chromatography was performed using Kieselgel 60 (70–230 mesh, Merck); medium-pressure liquid chromatography (MPLC) was carried out in Lobar glass columns filled with LiChroprep Si 60 (Merck) with detection at 280 nm. Gas liquid chromatography/mass spectrometry (GLC/MS) analyses were carried out on a Hewlett-Packard 5890A/5970B-GC/MSD instrument. Short-path distillations were performed with a Kugelrohr apparatus (Büchi GKR-50). Light petroleum refers to the fraction of bp 50–70 °C.

General Procedure for the Preparation of Compounds 2 and 4. At −5 to 0 °C, to a well stirred mixture of acetaldehyde (5.40 g, 123 mmol) and pyrazine (1, 1.60 g, 20 mmol) or pyrimidine (3, 1.60 g, 20 mmol) in 3.4 M sulfuric acid (10 mL) and dichloromethane (120 mL) were added simultaneously *tert*-butyl hydroperoxide (15 mL of an 80% solution in di-*tert*-butyl peroxide, 84 mmol) and iron(II) sulfate heptahydrate (33.40 g, 120 mmol) in water (100 mL) over a 20-min period. The resulting heterogeneous mixture was stirred for an additional hour and the temperature was kept below 5 °C. Then solid sodium iodide was added until the test with starch–iodide paper was negative. The phases were separated, and the aqueous phase was further extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residues thus obtained were treated as described below.

Methyl 2-Pyrazinyl Ketone (2). Column chromatography of the crude product (eluent, dichloromethane–ethyl acetate, 1:1) gave a cream solid which was 90% pure according to GLC/MS analysis. Further purification by MPLC (eluent, ethyl acetate–light petroleum, 1:1) gave 1.22 g (50%) of cream crystals, mp 75–77 °C (lit.[15] mp 76–78 °C).

Methyl 4-Pyrimidyl Ketone (4). The crude product was purified by column chromatography (eluent, dichloromethane–ethyl acetate, 1:1) followed by MPLC (eluent, ethyl acetate–light

petroleum, 2:1) to afford 1.17 g (48%) of colorless crystals, mp 68–69 °C (lit.[18] mp 68–69 °C).

General Procedure for the Preparation of Alkyl 3-Pyridazinyl Ketones 7 and 8. To a stirred solution of 3-pyridazinecarbonitrile[19] (10.51 g, 100 mmol) or 6-methyl-3-pyridazinecarbonitrile[19] (11.91 g, 100 mmol) in a mixture of dry diethyl ether (200 mL) and dry benzene (40 mL) was added a solution of the appropriate alkylmagnesium halide (120 mmol) in dry diethyl ether (120 mL) slowly at −15 °C under argon. After stirring for 1.5 h at this temperature, 2 N hydrochloric acid (60 mL) was added and stirring was continued for additional 15 min at 0 °C. The organic layer was separated; the aqueous phase was made alkaline with saturated sodium bicarbonate solution and was then exhaustively extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residues thus obtained were treated as described below.

***n*-Propyl 3-Pyridazinyl Ketone (7).** Compound **7** was prepared from 3-pyridazinecarbonitrile and *n*-propylmagnesium bromide. The crude product was purified by column chromatography (eluent, ethyl acetate–light petroleum, 1:1) followed by Kugelrohr distillation at 80 °C/0.03 mbar to give 4.51 g (30%) of a yellow oil. Further purification by MPLC (eluent, ethyl acetate–light petroleum, 1:1) afforded an analytically pure sample: IR (KBr) 3160, 2960, 1690 (C=O) cm^{-1}; MS *m/z* 150 (M^+, 18), 94 (22), 80 (100), 53 (41), 43 (56); ^1H NMR (DMSO-d_6) δ 0.94 (t, J = 7.2 Hz, 3 H, CH_3), 1.73 (m, J = 7.2 Hz, 2 H, CH_2), 3.27 (t, J = 7.2 Hz, 2 H, $COCH_2$), 7.79–7.97 (m, $J_{4,5}$ = 8.3 Hz, $J_{5,6}$ = 4.9 Hz, 1 H, pyridazine H-5), 8.03–8.17 (m, $J_{4,5}$ = 8.3 Hz, $J_{4,6}$ = 1.9 Hz, 1 H, pyridazine H-4), 9.38–9.46 (m, $J_{4,6}$ = 1.9 Hz, $J_{5,6}$ = 4.9 Hz, 1 H, pyridazine H-6); ^{13}C NMR (DMSO-d_6) δ 13.4 (CH_3), 16.6 (CH_2Me), 32.9 ($COCH_2$), 124.3 (pyridazine C-4), 127.9 (pyridazine C-5), 153.6 (pyridazine C-6), 155.4 (pyridazine C-3), 200.0 (C=O). Anal. ($C_8H_{10}N_2O$) C, H, N.

Methyl (6-Methyl-3-pyridazinyl) Ketone (8). From 6-methyl-3-pyridazinecarbonitrile and methylmagnesium iodide was obtained 12.12 g (89%) of crude **8** as light yellow crystals, mp 46–48 °C, which was employed in the following reaction steps without further purification. An analytically pure sample was obtained after MPLC (eluent, ethyl acetate–light petroleum, 1:1): IR (KBr) 3120, 1680 (C=O) cm^{-1}; MS *m/z* 136 (M^+, 38), 108 (20), 43 (100); ^1H NMR (DMSO-d_6) δ 2.73 (s, 3 H, $COCH_3$), 2.76 (s, 3 H, pyridazine 6-CH_3), 7.75 (AB system, A part, $J_{A,B}$ = 8.7 Hz, 1 H, pyridazine H-5), 8.01 (AB system, B part, $J_{A,B}$ = 8.7 Hz, 1 H, pyridazine H-4); ^{13}C NMR (DMSO-d_6) δ 21.8 (pyridazine 6-CH_3), 25.6 ($COCH_3$), 124.1 (pyridazine C-5), 127.8 (pyridazine C-4), 154.0 (pyridazine C-3), 162.6 (pyridazine C-6), 198.2 (C=O). Anal. ($C_7H_8N_2O$) C, H, N.

General Procedure for the Preparation of Methyl Hydrazinecarbodithioates 14–17. A stirred solution of 1 equiv of methyl hydrazinecarbodithioate[22] and 1 equiv of the appropriate alkyl diazinyl ketone in 2-propanol was heated at 65–70 °C for 1 h. The cooled solution was then refrigerated overnight; the crystals which had separated were filtered off, washed with cold 2-propanol, and dried. The products thus obtained were used without further purification in the following reaction step. Analytically pure samples were obtained by additional recrystallization from methanol.

Methyl 3-[1-(3-Pyridazinyl)butylidene]hydrazinecarbodithioate (14). Reaction of methyl hydrazinecarbodithioate (6.51 g, 53 mmol) with **7** (8.00 g, 53 mmol) in 2-propanol (100 mL) gave 11.11 g (82%) of **14** as cream needles: mp 131–133 °C dec; IR (KBr) 3190, 2960, 1600 cm^{-1}; MS *m/z* 254 (M^+, 81), 211 (100), 207 (89), 179 (77), 81 (78); ^1H NMR (DMSO-d_6) δ 0.96 (t, J = 7.6 Hz, 3 H, CCH_3), 1.56 (m, J = 7.6 Hz, 2 H, CH_2), 2.55 (s, 3 H, SCH_3), 3.21 (t, J = 7.6 Hz, 2 H, $COCH_2$), 7.69–7.86 (m, $J_{4,5}$ = 8.7 Hz, $J_{5,6}$ = 4.9 Hz, 1 H, pyridazine H-5), 8.15–8.29 (m, $J_{4,5}$ = 8.7 Hz, $J_{4,6}$ = 1.8 Hz, 1 H, pyridazine H-4), 9.23–9.31 (m, $J_{5,6}$ = 4.9 Hz, $J_{4,6}$ = 1.8 Hz, 1 H, pyridazine H-6), 12.94 (s, 1 H, exchangeable with D_2O, NH). Anal. ($C_{10}H_{14}N_4S_2$) C, H, N.

Methyl 3-[1-(6-Methyl-3-pyridazinyl)ethylidene]hydrazinecarbodithioate (15). Reaction of methyl hydrazinecarbodithioate (10.77 g, 88 mmol) and **8** (12.00 g, 88 mmol) in 2-propanol (200 mL) afforded 16.95 g (80%) of **15** as cream needles: mp 178–179 °C dec; IR (KBr) 3100, 2920, 1610 cm^{-1}; MS *m/z* 240 (M^+, 39), 165 (93), 165 (43), 94 (100), 91 (49), 42 (72), 39 (46); ^1H NMR (DMSO-d_6) δ 2.55 (s, 6 H, CH_3), 2.66 (s, 3 H, CH_3), 7.63 (AB system, A part, $J_{A,B}$ = 8.8 Hz, 1 H, pyridazine H-5), 8.10 (AB system, B part, $J_{A,B}$ = 8.8 Hz, 1 H, pyridazine H-4), 12.68 (s, 1 H, exchangeable with D_2O, NH). Anal. ($C_9H_{12}N_4S_2$), C, H, N.

Methyl 3-[1-(2-Pyrazinyl)ethylidene]hydrazinecarbodithioate (16). Reaction of methyl hydrazinecarbodithioate (8.00 g, 65 mmol) and **2** (8.00 g, 65 mmol) in 2-propanol (100 mL) afforded 13.04 g (88%) of **16** as yellow needles: mp 185–187 °C dec; IR (KBr) 3080, 2900, 1600 cm^{-1}; MS *m/z* 226 (M^+, 68), 179 (100), 147 (35), 91 (61), 80 (49), 79 (83), 52 (44); ^1H NMR (DMSO-d_6) δ 2.45 (s, 3 H, CH_3), 2.55 (s, 3 H, CH_3), 8.67 ("s", 2 H, pyrazine H-5,6), 9.25 ("s", 1 H, pyrazine H-3), 12.69 (s, 1 H, exchangeable with D_2O, NH). Anal. ($C_8H_{10}N_4S_2$) C, H, N.

Methyl 3-[1-(4-Pyrimidyl)ethylidene]hydrazinecarbodithioate (17). Reaction of methyl hydrazinecarbodithioate (8.00 g, 65 mmol) with **4** (8.00 g, 65 mmol) in 2-propanol (100 mL) gave 13.00 g (88%) of **17** as yellow crystals: mp 182–184 °C dec; IR (KBr) 3160, 2910, 1560 cm^{-1}; MS *m/z* 226 (M^+, 96), 179 (100), 151 (56), 147 (42), 91 (92), 80 (97), 79 (82), 52 (90), 42 (47); ^1H NMR (DMSO-d_6) δ 2.44 (s, 3 H, CH_3), 2.56 (s, 3 H, CH_3), 8.02 (dd, $J_{2,5}$ = 1.4 Hz, $J_{5,6}$ = 5.4 Hz, 1 H, pyrimidine H-5), 8.86 (d, $J_{5,6}$ = 5.4 Hz, 1 H, pyrimidine H-6), 9.25 (d, $J_{2,5}$ = 1.4 Hz, pyrimidine H-2), 12.74 (s, 1 H, exchangeable with D_2O, NH). Anal. ($C_8H_{10}N_4S_2$) C, H, N.

General Procedure for the Preparation of the Target Thiosemicarbazones 18–24. Method A. To 1 equiv of **12**,[13] **13**,[13] **14**, **15**, **16**, or **17** dissolved in warm methanol (30–40 mL) was added 1 equiv of the appropriate amine (except in a few cases where more than 1 equiv was used). The solution was heated under reflux until the evolution of methyl mercaptan almost ceased (methyl mercaptan was detected by the yellow color it imparts to moistened lead acetate paper placed at the mouth of the reflux condenser). Reaction times varied from 6 to 24 h. The resulting thiosemicarbazones frequently crystallized from the solution after cooling. To obtain the more soluble thiosemicarbazones, the reaction mixture was evaporated in vacuo, the residue was dissolved in hot ethyl acetate and chilled. The crystals which had separated were collected and recrystallized. For yields, recrystallization solvents and physical data see Tables I and II.

Method B. Equimolar quantities (10 mmol) of thiosemicarbazide or the appropriately N^4-substituted thiosemicarbazide [4-methylthiosemicarbazide,[20] 4,4-dimethylthiosemicarbazide,[20] 4-allylthiosemicarbazide,[26] 4-(2-pyridylmethyl)thiosemicarbazide; for the preparation, see below] and the carbonyl compound (aldehyde **9**,[14] ketones **2**, **4**, **8**, **10**[13]) in methanol (10–15 mL, containing 3 drops of glacial acetic acid) were refluxed for 3–5 h. The reaction mixture was cooled overnight, the separated crystals were collected by filtration and recrystallized. For yields, recrystallization solvents, and physical data see Table I.

4-(2-Pyridylmethyl)thiosemicarbazide (29). In analogy to ref 27, 2-picolylamine (1.73 g, 16 mmol) was added to a solution of 4-methyl-4-phenylthiosemicarbazide[28] (2.90 g, 16 mmol) in ethanol (6 mL) and the mixture was heated under reflux for 5 h. After rapid cooling, the ethanol was evaporated in vacuo. The residue was dissolved in water (50 mL) and exhaustively extracted with ethyl acetate. The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated in vacuo to a small volume. Light petroleum was added until the solution became cloudy. The crystals which separated upon cooling overnight were collected to afford 1.20 g (41%) of colorless crystals: mp 105–109 °C; MS *m/z* 182 (M^+, 34), 92 (100); ^1H NMR (DMSO-d_6) δ 4.55 (s, 2 H, exchangeable with D_2O, NH_2), 4.77 (d, J = 5.4 Hz, s after addition of D_2O, CH_2), 7.16–7.32 (m, 2 H, pyridine H-3,5), 7.64–7.85 (m, 1 H, pyridine H-4), 8.46 (s, 1 H, exchangeable with D_2O, NH),

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Methyl 3-[1-(3-Pyridazinyl)ethyl]hydrazinecarbodithioate (25). A stirred suspension of 12[13] (7.00 g, 31 mmol) in ethanol (100 mL) was treated portionwise with sodium borohydride (2.34 g, 62 mmol) over a period of 30 min. The solution was then stirred overnight at room temperature, diluted with water (100 mL), and cautiously neutralized with ~10 mL of glacial acetic acid. After evaporation of ethanol, the product separated from the remaining solution on cooling. Subsequent filtration and drying afforded 3.74 g (53%) of colorless needles, mp 159–160 °C. An analytically pure sample was obtained upon recrystallization from ethanol: IR (KBr) 3210, 3120, 3050, 2920, 2860, 1580 cm^{-1}; MS m/z 228 (M^+, 8), 181 (10), 122 (100), 108 (96), 107 (49); ^1H NMR (DMSO-d_6) δ 1.34 (d, J = 6.7 Hz, 3 H, CCH_3), 2.36 (s, 3 H, SCH_3), 4.51 (m, 1 H, q with J = 6.7 Hz after addition of D_2O, NCH), 6.18 (d, J = 4.0 Hz, 1 H, exchangeable with D_2O, CHN*H*), 7.58–7.74 (m, $J_{4,5}$ = 8.6 Hz, $J_{5,6}$ = 4.6 Hz, 1 H, pyridazine H-5), 7.77–7.91 (m, $J_{4,5}$ = 8.6 Hz, $J_{4,6}$ = 1.9 Hz, 1 H, pyridazine H-4), 9.08–9.17 (m, $J_{4,6}$ = 1.9 Hz, $J_{5,6}$ = 4.6 Hz, 1 H, pyridazine H-6), 10.90 (s, 1 H, exchangeable with D_2O, NHCS). Anal. ($C_8H_{12}N_4S_2$) C, H, N.

General Procedure for the Preparation of Thiosemicarbazides 26a–d. A mixture of compound 25 (1.50 g, 6.6 mmol) and 1 equiv of the appropriate cyclic amine in methanol (15 mL) was reacted and worked up as described for the preparation of thiosemicarbazones 18–24, method A. For yields, recrystallization solvents, and physical data, see Table III.

Biological Methods. Assays for Inhibition of Cell Proliferation and of Virus-Induced Cytopathic Effects. The assay procedure described by Pauwels et al.[29] for measuring inhibition of HIV was used with minor modifications. Briefly, the HTLV-I-transformed T4-cell line MT4, which was previously shown to be highly permissive for HIV-infection, was used as the target cell. Inhibition of HIV-1 (strain HTLV-IIIB) induced cytopathic effect was determined by measuring the viability of both HIV and mock-infected cells. Viability was assessed spectrophotometrically (at 540 nm) via in situ reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Virus-infected and uninfected cultures without compound were included as controls as were uninfected cells treated with compound. The cell concentration was chosen so that the number of cells/mL increased by a factor of 10 during the 4 days of incubation in the mock-infected cultures. Virus inoculum was adjusted to cause cell death in 90% of the target cells after 4 days of incubation. The virus was adsorbed to a 10-fold concentrated cell suspension at 37 °C for 1 h. Then, the infected cells were diluted 1:10 and added to microtiter plates containing the test compounds. Thus, compounds were added postadsorption.

The procedure was adopted for determination of herpes simplex virus type I (HSV-1) induced cytopathic effects in HUT78 cells, a T4-cell line derived from cutaneous T cell lymphoma. HSV-1 was found to cause death of these cells after 4 days of incubation. Inhibition of cell proliferation was measured as described above for MT4 cells by comparing cell numbers of drug treated and untreated uninfected control cells after 4 days by viability staining.

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4-Methyl-3-(arylsulfonyl)furoxans: A New Class of Potent Inhibitors of Platelet Aggregation

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A series of 4-methyl-3-(arylthio)furoxans were synthesized by oxidation of 1-(arylthio)-2-methylglyoxymes with dinitrogen tetroxide. Reduction with trimethyl phosphite of the furoxan derivatives afforded the corresponding furazans, while oxidation with an equimolar amount of 30% hydrogen peroxide in acetic acid or with an excess of 81% hydrogen peroxide in trifluoroacetic acid afforded the corresponding arylsulfinyl and arylsulfonyl analogues, respectively. All the furoxan and furazan derivatives showed activity as inhibitors of platelet aggregation. 4-Methyl-3-(arylsulfonyl)furoxans were the most potent derivatives of the series. 4-Methyl-3-(phenylsulfonyl)furoxan (**10a**), one of the most active derivatives, inhibits the AA-induced increase of cytosolic free Ca^{2+} and production of malondialdehyde. A primary action of the compound on cyclooxygenase is excluded, as a stable epoxymethano analogue of prostaglandin H_2 does not reverse the inhibitory effect of **10a**. This compound produces a significant increase in cGMP which is likely to cause inhibition at an early stage of the platelet activation pathway.

Several events are involved in the stimulus–response coupling in platelets. The major metabolic responses are phosphoinositide metabolism, increase of cytosolic calcium, protein phosphorylation, arachidonic acid release, and prostanoid synthesis.[1,2] These responses are not a linear progression of events, but probably there is a high degree of cooperativity and feed-back among them.[3] The cyclic nucleotides cAMP and cGMP play important regulatory roles in platelets. Increases in either cAMP and cGMP inhibit platelet activation through inhibition of agonist-induced Ca^{2+} elevation.[4]

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