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Bioorganic & Medicinal Chemistry 12 (2004) 1071–1089
Bioorganic & Medicinal Chemistry

Synthesis, structural investigations and biological evaluation of novel hexahydropyridazine-1-carboximidamides, -carbothioamides and -carbothioimidic acid esters as inducible nitric oxide synthase inhibitors

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Received 22 July 2003; accepted 9 December 2003

Abstract—Local excess of nitric oxide (NO) has been implicated in β-cell damage, thus, a possible approach to the treatment of autoimmune IDDM is the selective inhibition of inducible nitric oxide synthase (iNOS). A series of variously substituted hexahydropyridazine-1-carbothioamides, -carbothioimidic acid esters and -carboximidamides was synthesized and dose-dependently evaluated as potential inhibitors of iNOS. The screening of the title compounds was performed with insulin-producing RIN-5AH cells and a combination of IL1-1β and IFN-γ as inducers of cellular NO production. The structure–activity analysis revealed that the variation of substituents in the position 1 of the hexahydropyridazine strongly influences the inhibitory activity to iNOS as well as being critical for RIN cell survival. Among the compounds tested, the hexahydropyridazine-1-carbothioamides showed particularly significant inhibitory effects. However, for an efficient iNOS inhibition substitution at the nitrogen of the 1-carbothioamide group is important. Thus, the introduction of aliphatic chains such as propyl or butyl and of cyclic moieties such as cyclohexyl, 3-methoxyphenyl, and 4-methoxyphenyl (IC_{50}: 0.5–2.1 mM), respectively, provided compounds with similar inhibitory activity to aminoguanidine (IC_{50}: 0.3 mM), a common standard substance used for the selective inhibition of iNOS. However, the 1-carboximidamides, which represent more structurally related semicyclic derivatives of aminoguanidine, caused only incomplete iNOS inhibition. The hexahydropyridazine-1-carbothioimidic acid esters caused dose- and substituent-dependent damage of RIN-5AH cells. The toxicity of the synthesized compounds increased markedly if aliphatic substituents at the exocyclic N atom(s) were replaced by variously substituted aromatic rings.

1. Introduction

The reactive metabolite nitric oxide (NO) is an intracellular messenger that mediates several physiological functions, including neurotransmission, vasodilatation, modulation of leukocyte adhesion, antimicrobial and antitumorial activities.[1–3] NO is synthesized by a family of three NO synthases (NOS). Whereas neuronal and endothelial NOS are constitutively expressed (cNOS), inducible NOS (iNOS) requires a stimulation by proinflammatory cytokines or bacterial lipopolysaccharides (LPS) and serves as defence enzyme against micoorganisms.[4] Besides these important functions, an overproduction of NO has been implicated in the pathogenesis of various inflammatory and autoimmune diseases.[5–8]

In insulin-dependent type-1 diabetes (IDDM) the autoimmune destruction of pancreatic β-cells is caused by an infiltration of pancreatic islets by mononuclear leucocytes. Autoreactive T-lymphocytes and a mixture of cytokines released from different mononuclear cells are responsible for β-cell damage.[9] The cytokine interleukin-1β (IL1β), alone or in combination with tumor necrosis factor-α (TNFα) or interferon-γ (IFNγ), induces functional impairment and damage of rodent and human islets in vitro.[10,11] One mechanism of IL1 β-induced β-cell damage is due to an activation of β-cell iNOS followed by an increased NO production.[12] NO

Keywords: Aminoguanidine; Hexahydropyridazine-1-carboximidamides; Inducible nitric oxide synthase; Rat insulinoma cells (RIN).
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doi:10.1016/j.bmc.2003.12.007

also affects the iron-containing respiratory chain enzyme aconitase.[13] The consequence is a decrease in ATP generation and subsequent cell death. The main producer of IL1β are macrophages and dendritic cells, infiltrating the pancreatic islets first before autoreactive T-lymphocytes appear.[14] Therefore, an inhibition of NO production may protect β-cells during an early phase of type-1 diabetes development.

However, for therapeutic use only selective inhibitors of iNOS are suitable because NO generated by cNOS is necessary for the maintenance of blood glucose concentration by regulation of both insulin and glucagon secretion.[15,16] A selective inhibitor of iNOS is aminoguanidine (hydrazine carboximidamide, AG). AG effectively reduces the cytokine-induced NO production of pancreatic islets and rat insulinoma cell line (RIN cells)[17,18] and reverses IL1β inhibited insulin release in vitro.[19] In transgeneic mice overexpressing iNOS in the pancreatic islets, it was reported that all animals developed diabetes within an age of 4 weeks. Treatment with AG prevented or delayed the onset of diabetes.[20] Administration of AG to young diabetes prone BB rats or NOD mice also delayed diabetes onset.[21,22] However, there are side effects that raise doubt about the use of AG as therapeutic drug. Thus, application of AG to wild type and iNOS knockout rats led to weight loss and rendered them more susceptible to infections.[23] Furthermore, rats injected intravenously with AG (1–50 mg/kg body weight) showed a decreased pancreatic blood flow.[24] In streptozotocin diabetic mice, administration of AG (50 mg/kg body weight) failed not only to prevent development of diabetes and insulitis but AG treated mice also showed an increased mortality compared to control mice treated with streptozotocin plus saline.[25] Finally, the selectivity of AG towards the iNOS isoform is only achieved at low inhibitor concentrations; higher AG doses inhibit all three NOS-isoforms, that is, inhibition of cNOS also takes place.[26,27]

The modification of the AG's structure might yield analogues with potent iNOS inhibitory activity but with less side effects. Moreover, it has been shown that the amidine partial structure is an effective iNOS inhibitory pharmacophore.[28,29] Open chained as well as cyclic amidines were identified as inhibitors of both cNOS and/or iNOS. In neuroinflammatory diseases pyridazine-based compounds were also used as inhibitors of iNOS.[30,31]

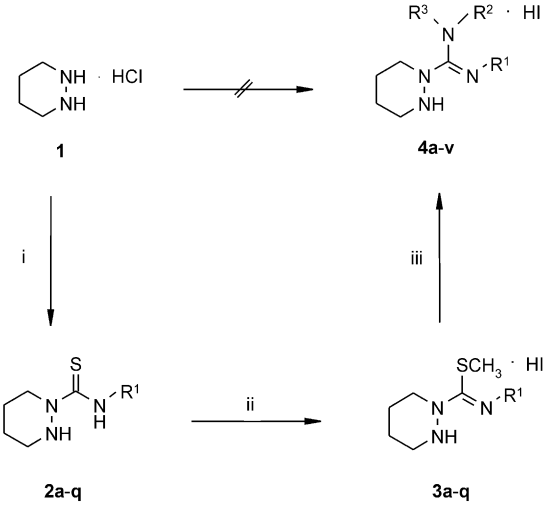
Here we report a series of hexahydropyridazine-1-carboximidamides representing novel semicyclic aminoguanidine derivatives with various stereochemical, electronical, and bonding properties. In relation to the known NOS inhibitory effects of *S*-alkyl isothioureas, for example, *S*-ethyl isothiourea (SEITU), the biological activities of the related isosters such as the hexahydropyridazine-1-carbothioimidic acid esters and the carbothioamides, respectively, were also of interest. All three types of hexahydropyridazine derivatives have been evaluated for concentration dependent inhibitory activity to cytokine-induced iNOS in insulin producing RIN-5AH cells as well as toxicity to these cells.

2. Results and discussion

2.1. Syntheses and structural investigations

2.1.1. Synthesis of hexahydropyridazine. In order to achieve the synthesis of the title compounds **4a–v** (Scheme 1, Table 2), it should be possible to start from hexahydropyridazine **1** (piperidazine; *N*,*N*′-tetramethylene hydrazine).[32] Hexahydropyridazine represents an important starting material for different herbicids and fungicids with [1,2,4]triazolo[1,2-a]-[33–36] or [1,3,4]thiadiazolo[3,4-a]pyridazine structure.[36–45] The classical preparation method published by Alder et al.[32] starts with the addition of buta-1,3-diene to azodicarboxylic acid dimethyl ester and has frequently been modified[40,46-49,54,55] or directly utilized by several authors.[50–53] Further patented[56] and modified[57,58] routes to **1** begin with the potassium salt of diisobutyric hydrazine,[59] with hydrazine-1,2-dicarboxylic acid esters,[60] with 1-aminopyrrolidine,[61] with butane-1,4-diamine,[62] and with 2,2-disubstituted hexahydropyrazolo[1,2-a]pyridazine-1,3-diones,[63] respectively. Up until now, only Marquis[64] reported the hydrogenation of pyridazine (with metallic sodium in ethanolic solution), but instead of the formation of **1** a ring cleavage occurs under formation of butan-1,4-diamine.

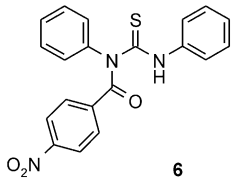
To achieve a more ready access to **1** in relation to the above mentioned methods, we again investigated the direct hydrogenation of commercially available pyridazine (hydrogen atmosphere, Adams catalyst, 5 bar; 25 °C) with the desired result. Because of the oxidation-sensitivity of the batches, immediately after hydrogenation the free hexahydropyridazine base was salified in etheric-ethanolic solution with dry hydrogen chloride gas to give the monohydrochloride[46] in good yields. To suppress the formation of mixtures with the dihydrochloride[32] a strict control of the hydrogen ion concentration is required.



Scheme 1. Reagents and conditions: (i) **2a**: KSCN, H_2O, Δ; **2b–q**: TEA, related R^1-N=C=S, CH_2Cl_2, room temperature; (ii) CH_3I, CH_2Cl_2, room temperature; (iii) NH_3 or related R^2R^3NH, methods A—E (see Experimental).

2.1.2. Syntheses of hexahydropyridazine-1-carbothioamides and hexahydropyridazine-1-carbothioimidic acid methyl esters. The subsequent preparation of the first members of the hitherto unknown title compounds **4** was planned in analogy to the pyrazolidine-1-carboximidamides. Their synthesis should be done as already reported[65] without isolation of intermediates, utilizing **1** and different *N*-aryl and *N*,*N*′-diaryl-*S*-methyl isothiouronium iodides, respectively, as reactants (Scheme 1).

With the *S*-methyl-*N*,*N*′-diphenyl isothiouronium iodide, which is frequently a reacting partner in the reaction with pyrazolidine,[65] considerable differences between the reactivities of **1** and the five membered homologue pyrazolidine, respectively, were observed. These differences in the reactivities of both heterocycles are evidently caused by their different stereochemical properties.[66,67] In addition, even with a varity of other substituted S-methylisothiouronium salts and by modifying the reaction conditions all attempts failed to transform the hexahydropyridazine **1**. For this reason, the synthesis of S-(4-nitrobenzoyl)-1,3-diphenyl **5** isothiourea from 1,3-diphenylthiourea and 4-nitrobenzoyl chloride and its reaction with **1** was attempted. However, instead of the desired **5**, the related, instable 1-acyl thiourea **6**[68] was isolated. Interestingly, in the reaction with **1** this compound acts as a donor of phenylisothiocyanate under formation of *N*-phenylcarbothioamide **2j** (Table 1) and 4-nitrobenzanilide.[73]



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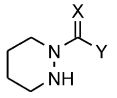
These findings lead us to attempt a new route (Scheme 1, steps i–iii) to the title compounds **4** via the hexahydropyridazine-1-carbothioamides **2a–q** (Table 1) and hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodides **3a–q** (Table 1), which was realized as reported in the past.[65] Whereas the **3** and the corresponding free bases are hitherto unknown, in various patents[34,37–42,44,45] several compounds with the general structure **2** are utilized as intermediates for heterocyclic crop protection chemicals. With a few exceptions,[34] these products represent polysubstituted and also halogenated *N*-phenyl derivatives. Various hexahydropyridazine-1-carbothioamides (ref 34: **2f**, **i**, **l**, **n**) also prepeared in our work, are explicitly named as starting products for [1,2,4]triazolo[1,2-a]pyridazines, but only very few of these have been directly characterized (ref 33: **2j**, **l**).

In principle, the compounds **2b–q** may be prepared from hexahydropyridazine (**1**) hydrochloride and related isothiocyanates (Scheme 1, step i) in the presence of a base in an aprotic solvent in good yields. They crystallize out very nicely and show, in contrast to pyrazolidine,[70] only a minor tendency to form 1,2-dicarbothioamide derivatives. The synthesis of **2a** proceeded in good yields by careful done evaporation of an aqueous solution of equimolar amounts of **1** hydrochloride and potassium thiocyanate.

Table 1. 1,2,3,4,5,6-Hexahydropyridazine-1-carbothioamides 2 (X=S, Y=NHR[1]) and 1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodides **3** (X=NH$^+$R^1 I$^-$, Y=SCH$_3$)



2,3	R[1]	k′[a]
a	H	0.36
b	CH_3	0.73
c	C_2H_5	1.08
d[b]	C_3H_7	1.55
e[b]	CH_2=CH–CH_2	1.34
f	$(CH_3)_2$CH	1.57
g[b]	C_4H_9	2.27
h	$C_6H_5CH_2CH_2$	2.74
i	Cyclohexyl	3.22
j	C_6H_5	2.05
k	3-CH_3–C_6H_4	2.76
l	4-CH_3–C_6H_4	2.37
m	2-CH_3O–C_6H_4	2.60
n	4-CH_3O–C_6H_4	1.64
o[c]	4-NO_2-C_6H_4	3.02
p	Naphth-1-yl	3.03
q	Naphth-2-yl	3.50

[a] HPLC capacity factor for compounds **2**, HPLC conditions: see Experimental.
[b] Non-crystalline mass with compounds **3**.
[c] Isolation of compound **3o** failed.

The combinatorial synthesis of **2b–q** was also attempted. Thus, appropriate conditions for the high performance liquid chromatography (HPLC) separation were developed (HPLC conditions see Experimental, k′ values are listed in Table 1). In summary, the combinatorial synthesis of compounds **2** was accompanied by undesired and not further characterized by-products; the rates of the **2**-formation were lower than 100% (exceptionally **2b**,**e**,**m**,**j**). These rates decrease at the same time when the reactivity of the isothiocyanate decreases; this point speaks for the enforced participation of the isothiocyanates selectively reacting in the formation of by-products.

Both with regard to the expected problems due to the different behavoir of the reactants and in order to an extensive characterization of the hitherto unknown hexahydropyridazine derivatives **3** and **4** the parallel synthesis approach was utilized in the preparation of these series of compounds.

The preparation of the hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodides **3a–q** proceeded under mild conditions by reacting iodomethane with the carbothioamides **2** (Scheme 1, step ii), where the *N*-alkyl substances took the longest reaction times. However, the preparation of compound **3o** from **2o** was not achieved even when heating with dimethyl sulfate. The isolation of the compounds **3** was complicated by poor tendency of most of these compounds to crystallize.

2.1.3. Synthesis of hexahydropyridazine-1-carboximid-amides. The synthesis of the compounds **4a–v** (Table 2) proceeded under mild conditions by allowing either ammonia or amines to react with the hydroiodides **3** (Scheme 1, step iii). Thus, **4a**, which embodies as the N^1,N^2-tetramethylene derivative of aminoguanidine the parent compound of the **4**, was synthesized by reacting **3a** with gaseous ammonia.

Because of the varying reactivity of the **3** and the amines utilized, monitoring of the reaction batches by TLC was done. As a result, the application of the weak nucleophilic aromatic amines was found to be unpractically, and for this reason the synthesis of N^1,N^2-diaryl substituted derivatives **4** is not reported here. However, the monoaryl substituted compounds **4i–k** and **4q–t** were obtained from the related *N*-aryl derivatives **3**. With the exception of the most of the aryl derivatives the isolated compounds **4** are mostly low melting substances that crystallized poorly (some are hygroscopic compounds; especially **4b**). In order to crystallize the title compounds, the products were dried and covered with diethylether for several days.

The investigations on the reaction conditions led to variations of the synthesis of compounds **4a–v** (methods A–E, see Experimental). If possible, methods A and B utilizing ammonia should be favoured because of the high volatility of the ammonia the working-up is simplified. The advantage of a non-aqueous reaction medium makes clear the transformation of **3h** to **4h** by reaction with ammonia (yield 87% versus 30%); the competing reactions consisting of the saponification of the applied **3** and the alkaline hydrolysis of the formed **4** are suppressed. The latter fact also becomes very clear in the preparation of the *N*-aryl derivatives **4i–k**.

Methylamine was used with an excess in a methanol-aqueous mixture; the undesired side reactions were attenuated because of the stronger nucleophility of the methylamine compared to ammonia. However, only moderate yields of the aromatic substituted derivatives **4r**,**s** were achieved.

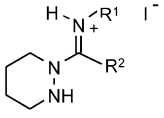
All attempts to synthesize some N^1,N^1,N^2-trisubstituted derivatives **4** in an analogous manner with dimethylamine failed. Most likely of this was because of stronger basicity in relation to methylamine (competing reactions, isolation of dimethylamine hydroiodide), and so these compounds are not reported here.

That the N^1,N^1-disubstituted and the N^1,N^1,N^2-trisubstituted compounds **4** can be prepared at all, was demonstrated with **4u**,**v**. These N^1,N^1-tetramethylene derivatives were obtained by reaction of **3a** and its *N*-methyl analogue **3b** with the secondary amine pyrrolidine, respectively. However, the successful preparation of further N^1,N^1-disubstituted and the N^1,N^1,N^2-trisubstituted derivatives **4** requires additional investigations with regard to sufficient reaction conditions, and this will be reported in a later paper.

2.1.4. Structural investigations. All the series of compounds **2**, **3**, and **4** described in the experimental part were analyzed by TLC and found to be chemically uniform. The data resulting from the elemental analysis were in accordance with the calculated values within the usual range of tolerances (±0.4% of the calculated values for C, H, N). Compared with hexahydropyridazine **1**, the *N*-substituted bases **2–4** are much less oxygen sensitive. There was no evidence in the NMR nor in the mass spectra for the formation of an unsaturated heterocyclic ring. The chemical shifts of the proton and carbon NMR signals for the hexahydropyridazine system of the synthesized **2–4** are in good agreement with the data given by other authors.[52,53] However, caused of the oxidation of iodide to iodine, **3** and **4** tended to form mixtures consisting of the hydroiodide and the related free base during long-term storage in the air.

The proton NMR spectra of the hexahydropyridazine-1-carbothioamides **2** show no evidence for the formation of 1,2-dicarbothioamides side products. Due to coupling with the $C(3)H_2$ structure of the heterocycle, the N(2)H signals appear as triplets (in the spectrum of **2i** as broad singulet). Moreover, the spectra did not provide any indication of the tautomeric carbothioimide structure. Thus, the proton NMR spectrum of **2a** shows the existence of two equivalent protons of the unsubstituted thiocarbamoyl group. In the case of the *N*-alkyl derivatives **2b,c–h** the signals from the *N*-alkyl substituents show splitting patterns, that are consistent with the assigned carbothioamide structure. The comparison of the carbon NMR spectra of the **2** with those of the corresponding **3** provides evidence of the favoured

Table 2. 1,2,3,4,5,6-Hexahydropyridazine-1-carboximid-amide hydroiodides **4**[a]



4	R^1	R^2
a	H	NH_2
b	CH_3	NH_2
c	C_2H_5	NH_2
d	C_3H_7	NH_2
e	CH_2–CH=CH_2	NH_2
f	H	$NHCH(CH_3)_2$
g	C_4H_9	NH_2
h	CH_2–$CH_2C_6H_5$	NH_2
i	C_6H_5	NH_2
j	2-CH_3O–C_6H_4	NH_2
k	4-CH_3O–C_6H_4	NH_2
l	CH_3	$NHCH_3$
m	CH_3	NHC_2H_5
n	CH_3	NHC_3H_7
o	CH_3	NHC_4H_9
p	CH_3	$NHCH_2CH_2C_6H_5$
q	C_6H_5	$NHCH_3$
r	4-CH_3–C_6H_4	$NHCH_3$
s	2-CH_3O–C_6H_4	$NHCH_3$
t	4-CH_3O–C_6H_4	$NHCH_3$
u	H	Pyrrolidino
v	CH_3	Pyrrolidino

[a] The given structures for the **4** are derived from the NMR spectra of the compounds (conditions: see Experimental).

existence of **2a–q** in the CS-NH form; the positions of the signal of the only sp^2 hybridized carbon atom of the compounds **2a–d**,**f**,**g**,**i** is clearly situated at lower field (about 180 ppm) than in the spectra of the analogous **3** (about 167 ppm). Similar results were obtained for the other isolated carbothioamides **2**.

The fragmentation behavior of the molecular ions of **2**, formed by electron impact in mass spectrometry, is mainly characterized by a retroaddition yielding the related isothiocyanates and hexahydropyridazine. This was especially the case with the *N*-alkyl derivatives **2**, which gave intensive peaks at *m*/*z* 86 ($M^{+\cdot}$–R–NCS). Furthermore, the mass spectra did not provide any indications of the elimination of $HS^{\cdot}$ or H_2S, which also speaks for the existance of the carbothioamide form.

The proton NMR spectra of the hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodides **3** gave no evidences for an alternative alkylation of the N(2) of the hexahydropyridazine ring. Normally, the signals of the N(2) partial structure appear in the range from 5.50 ppm (**3b**) to 6.23 ppm (**3m**), but in the case of the naphthyl derivatives **3p**,**q** the related signal was not observed. However, the carbon NMR spectra of all the compounds **3** argue for S-alkylation of the carbothioamide structure of the **2**. Assuming the *N*-methylation of the carbothioamide structure, the position of the signals of the C=S group related to the analogous **2** should not be shifted to such high fields. Moreover, the loss of methanethiol from the molecular ion of **3a** in the mass spectrometry and the evolution of this compound in the reaction of **3** with amines (subsequent transformation to the **4**, step iii, Scheme 1) prove the S-methylation of the carbothioamide structure.

With all the investigated carbothioimidic acid esters **3**, two types of exchangable protons exist in a 1:1 ratio (with **3a** 2:1). Because of the energetically favoured formation of mesomeric cations, the protonation of the carbothioimidic acid ester structure should occur at the nitrogen atom, and this was supported by the clear coupling with the α proton of the *N*-(cyclo)alkyl group in the spectra of **3f** and **3i**. With all the other compounds **3** the NH peaks for the $–C(SCH_3)=NH^{\oplus}$-structure appear as broad singulets between 8.29 ppm (**3a**) and 10.18 ppm (**3i**).

With the hexahydropyridazine-1-carboximidamide hydroiodides **4** the protonation site and the tautomerism involving the amidino group are of particular interest. The proton NMR spectra of the compounds **4a–v** clearly indicate that the amidino group is the site of protonation due to the formation of resonance-stabilized cations. No evidence was found that suggest protonation of the nitrogen atoms of the hexahydropyridazine ring. Thus, compounds **4a**,**u**,**v** possess two types of exchangeable protons [integrals ratio (amidino group protons : N(2)H atom): **4a**: 4:1; **4u**: 2:1; **4v**: 1:1]. The other compounds **4b–t** are characterized by three types of magnetically non-equivalent and exchangeable protons; these derivatives consequently have different, substitution dependent integrals ratios. Moreover, the proton resonance spectra allow an assignment as to which of the substituents are linked to each of the different hybridized nitrogen atoms N^1 (sp^3-hybridization) and N^2 (sp^2-hybridization) of the exocyclic amidino group. Assigning the farthest low field shifted signals of the exchangable protons to the positive charged nitrogen atom and simultaneously taking into account the integrals and the splitting patterns (coupling of the nitrogen linked protons with the α carbon protons of the alkyl substituents) of the signals of all the exchangable protons, the structures of the cations are clearly apparant (Table 2). In addition, no evidence was found in the proton spectra which might point to the simultaneously presence of further synionic structures.

The ^{13}C NMR peaks of the sp^2-hybridized carbon atoms of the amidino group appear between 154.32 ppm (**4f**) and 158.05 ppm (**4k**) for the N^2-unsubstituted carboximidamides, in a small range from 158.31 ppm (**4p**) to 158.92 ppm (**4l**) for the N^1,N^2-dialkylated compounds **4**, and again at a little higher field between 155.29 ppm (**4q**) and 157.92 (**4t**) for the N^2-aryl-N^1-methyl derivatives **4q–t**. In relation to those of the carbon atoms of the carbothioimidic acid ester structure of the **3** (see above), these are clearly shifted to higher field. In order to definitely assign the peaks of the carbon NMR spectra of the compounds **4n–o**,**r**,**t**, the corresponding DEPT 135 spectra were also analyzed.

The electron impact mass spectra of the hexahydropyridazine-1-carboximidamide hydroiodides **4** gave the free bases and are in accordance with the structures assigned. The intensities of the peaks of the molecular ions are greatest with N^1-unsubstituted **4a–k**, in which the molecular ions of the arylated derivatives **4i–k** are additionally stabilized by the aromatic system. The molecular ion peaks of the N^1,N^2-dialkylated **4** are characterized by intensities of only ca. 10% of the base peaks (normally at *m*/*z* 86). Again, compared with the N^1,N^2-dialkylated products, the N^2-aryl-N^1-methyl derivatives **4q–t** yield more intensive molecular ion peaks (17–41%). The most favoured initial fragmentation of the molecular ions of the bases **4** constists of a retroadditon, which obviously proceeds under formation of the neutral, related carbodiimides and the hexahydropyridazine cation radical (*m*/*z* 86). Exceptions to this were encountered with **4s** (resulting from the *ortho* located methoxy substituent) and the N^1,N^1-tetramethylene compounds **4u**,**v**. The latter, structurally related compounds, differ considerably in the fragmentation behavior of the molecular ions. However, both mass spectra of **4u**,**v** indicate the elimination of pyrrolidine from the molecular ion [**4u**: *m*/*z* 71 (75%); **4v**: *m*/*z* 30 (38%)]. In contrast, all the other mass spectra of the **4**-series did not give evidence for peaks arising form the amine component, which is generated from the carboximidamide structure.

2.2. Biological evaluation

The screening of the title compounds for iNOS inhibition was performed with insulin-producing RIN-5AH

cells (rat insulinoma cell line) and a combination of IL1-β and IFN-γ to induce NO production. Their usefulness was determined by the exclusion of compound-mediated cell death and by their inhibitory potency to iNOS. If there was a complete inhibition of NO production the efficiency of compounds was quantified by the IC_{50} value.

2.2.1. Evaluation of hexahydropyridazine-1-carbothioamides (HPC). The dose-dependent effects of the derivatives on cytokine-induced NO production in comparison with aminoguanidine (AG) are shown in Figure 1. AG caused a dose-dependent reduction of cytokine-induced NO-production. The inhibitory activity of AG on iNOS was characterized by an IC_{50} value of 0.3 mM.

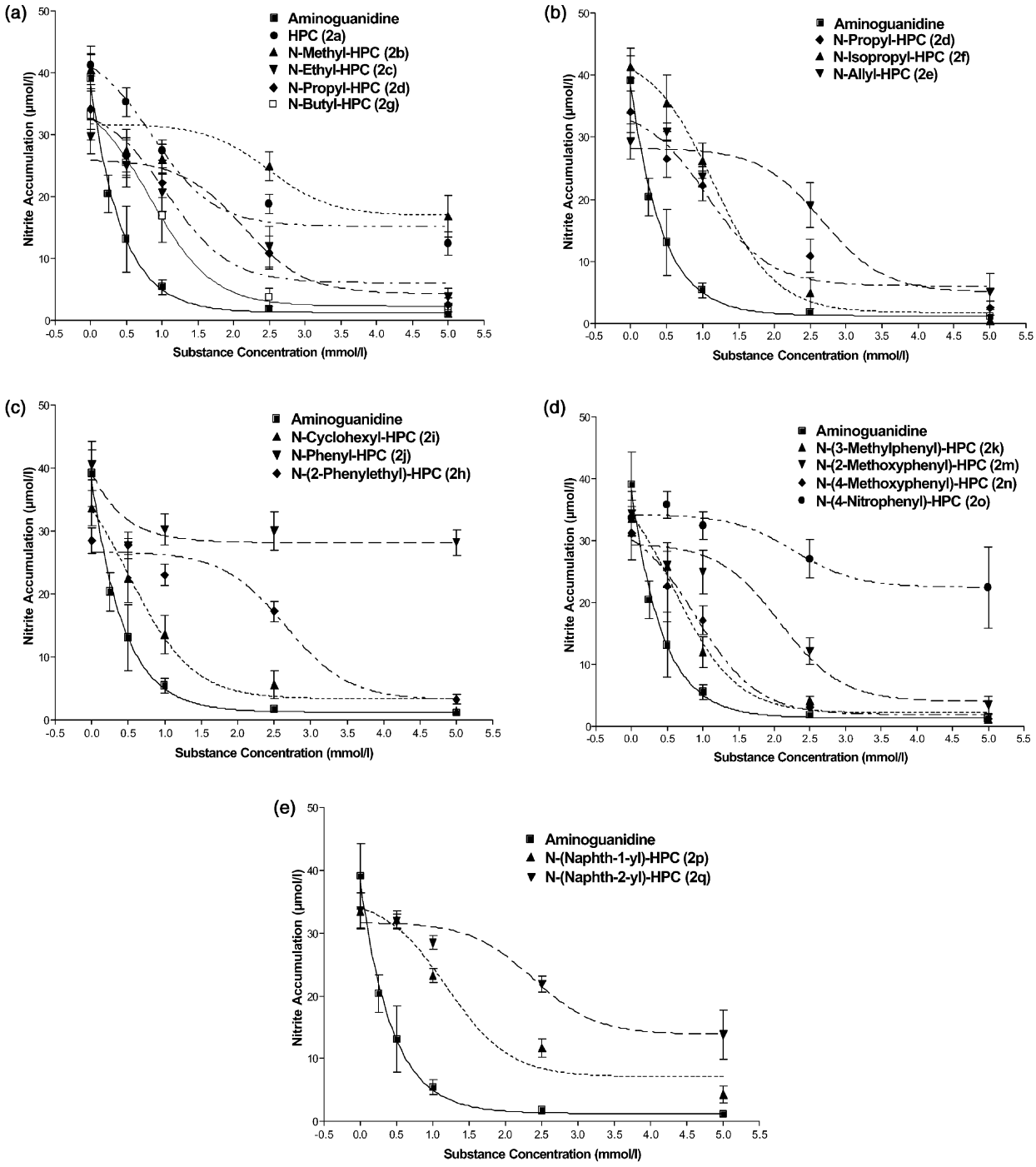


Figure 1. Inhibition of cytokine-induced iNOS by aminoguanidine and several hexahydropyridazine-1-carbothioamides (HPC) **2** depending on the concentration and the substitution of the carbothioamide group, respectively. Influence of HPC derivatives on cytokine-induced NO production: (a) and (b) exocyclic *N*-substitution with aliphatic chains, (c)–(e) exocyclic *N*-substitution with cyclic moieties. Curves were obtained by fitted nonlinear regression analysis using a sigmoidal dose response. Simultaneously to the determination of NO production using the Griess reaction the influence of inhibitors on RIN-5AH cell viability was tested to calculate a potential toxic effect of derivatives.

The hexahydropyridazines possessing a substituted 1-carbothioamide structure led to compounds with iNOS inhibitory properties, but compared to AG with reduced potency as expressed by the degree of inhibition and the enhanced IC_{50} values (Table 3). 5 out of 16 compounds (**2a**, **b**, **j**, **o**, **q**) showed only an incomplete inhibitory activity at the concentrations investigated. The *N*-substitution of the carbothioamide group by aliphatic residues brought about complete inhibition (Fig. 1a). The IC_{50} values decreased continuously from 2.1, 1.1 to 0.83 mM as a function of the chain length for the compounds *N*-ethyl, *N*-propyl, and *N*-butyl-HPC (**2c**,**d**,**g**). However, *N*-butyl-HPC **2g** showed also toxic side effects on RIN cells at a concentration of 5.0 mM. The exchange of the unsaturated allyl (**2e**, IC_{50}: 2.7 mM) for the (iso)propyl residue still increases further the inhibitory potency (**2d**, IC_{50}: 1.1 mM, **2f**, IC_{50}: 1.2 mM) (Fig. 1b).

The evaluation of such **2**-derivatives, which are characterized by a cyclic moiety, showed that cyclohexyl-HPC **2i** was a more effective compound (IC_{50}: 0.5 mM) than the aromatic phenyl-HPC **2j** and *N*-(2-phenylethyl)-HPC **2h** (non active and IC_{50}: 2.7 mM, respectively) (Fig. 1c). The insertion of a chain between the hexahydropyridazine ring and the phenyl ring not only increased the inhibitory potency but also enhanced the toxic behaviour (Fig. 1d). The inhibitory properties were improved depending on the substituent at the phenyl ring as follows: 4-nitro (**2o**) < 2-methoxy (**2m**) < 4-methoxy (**2n**) < 3-methyl (**2k**) (IC_{50}: 2.3, 2.1, 0.9 and 0.6 mM, respectively). According to these results the most effective compound in these series was **2k** with an IC_{50} of 0.6 mM and without any toxic side effects at the used concentrations. Toxic effects on RIN cells were detectable with *N*-(4-methoxyphenyl)-(**2n**) and *N*-cyclohexyl-HPC (**2i**) at 5.0 mM (Table 3).

Extension of the aromatic system (naphthyl derivatives) led to the compounds with both complete (**2p**, IC_{50}: 1.2 mM) and incomplete inhibitory activity (**2q**) (Fig. 1e). These results indicate that besides compounds containing the amidine function like aminoguanidine also some hexahydropyridazine derivatives **2** possessing a semicyclic thiosemicarbazide partial structure are able to inhibit iNOS, however, with a less activity (IC_{50} values of 0.6–2.7 mM).

2.2.2. Evaluation of hexahydropyridazine-1-carbothioimidic methyl esters. With one exception (**3b**) the *S*-methylation of the pyridazine-1-carbothioamide derivatives caused a dramatic increase in toxicity (Table 3). In general, the compounds bearing cyclic residues at the exocyclic N atom (**3i–q**) were more toxic than the aliphatic substituted derivatives (**3a–f**). Compound **3b** showed no toxicity at the used concentrations and was able to inhibit NO production completely with an IC_{50} value of 1.2 mM (data not shown). The results reveal that the exchange of the carbothioamide structure for the carbothioimidic acid methyl ester group is possibly crucial for the activities of compounds and therefore for the survival of RIN-5AH cells.

2.2.3. Evaluation of hexahydropyridazine-1-carboximidamides (HPCI). The degrees of inhibition of cytokine induced NO production by HPCI derivatives and their toxic dose to RIN-5AH cells are demonstrated in Table 3.

Table 3. Toxic effects of hexahydropyridazine-1-carbothioamides (compounds **2**),-carbothioimidic acid methyl ester hydroiodides (compounds **3**), and-carboximidamide hydroiodides (compounds **4**) on RIN-5AH cells and inhibition of inducible NO-synthase activity by **2** and **4**

2	TC (mM)[a]	Inhibition (%)[b]	IC_{50} (mM)	**3**	TC (mM)[a]	**4**	TC (mM)[a]	Inhibition (%)[b]
a	n.t.	69.8	n.d.	**a**	2.5	**a**	n.t.	61.7
b	n.t.	58.6	n.d.	**b**	n.t.	**b**	n.t.	61.2
c	n.t.	87.0	2.1	**c**	2.5	**c**	n.t.	32.0
d	n.t.	92.6	1.1	**d**	no data	**d**	n.t.	46.1
e	n.t.	82.6	2.7	**e**	no data	**e**	5.0	44.6
f	5.0	88.2	1.2	**f**	2.5	**f**	n.t.	43.9
g	5.0	88.8	0.8	**g**	no data	**g**	n.t.	78.4
h	n.t.	88.6	2.7	**h**	2.5	**h**	no data	no data
i	5.0	83.3	0.5	**i**	0.5	**i**	2.5	26.9
j	n.t.	30.3	n.d.	**j**	0.25	**j**	2.5	23.8
k	n.t.	96.8	0.6	**k**	0.25	**k**	2.5	22.9
l	no data	no data	no data	**l**	0.1	**l**	n.t.	32.2
m	n.t.	90.2	2.1	**m**	0.25	**m**	n.t.	33.6
n	5.0	90.5	0.9	**n**	0.1	**n**	n.t.	49.1
o	n.t.	33.3	n.d.	**o**	no data	**o**	no data	no data
p	n.t.	87.1	1.2	**p**	2.5	**p**	2.5	47.2
q	n.t.	59.0	n.d.	**q**	1.0	**q**	2.5	26.9
						r	2.5	31.9
						s	2.5	28.4
						t	2.5	23.5
						u	n.t.	35.0
						v	n.t.	24.8

[a] Toxic concentration (expressed in mM) of synthesized compounds to RIN-5AH cells measured by trypanblue exclusion test. At the given drug concentration all RIN cells were trypanblue positive; n.t. = not toxic up to a concentration of 5.0 mM.

[b] Degree of inhibition (%) of cytokine-induced NO production by a concentration of 5 mM or at the next lower concentration studied. IC_{50} values were only determined if the degree of iNOS inhibition was greater than 80%. All data based on the mean value of at least five to six independent experiments; n.d. = not determined. No data = compounds could not be dissolved completely.

Although the 1-substituent of the hexahydropyridazine ring represents an amidino group [–C(=N–R^1)NR^2R^3] and these compounds are structurally close to the lead AG, the inhibitory activities were dose-dependent but incomplete. The most active compounds were the unsubstituted parent compound (**4a**) of the HPCI **4** and the monosubstituted derivatives possessing the smallest (**4b**) and the largest (**4g**) aliphatic chain. Additional substitution, as found in the N^1,N^2-disubstituted (**4l–p**) and tetramethyl-HPCI derivatives (**4u**, **4v**), caused a further loss of inhibitory activity. In relation to the 1-carbothioamides **2**, the HPCI derivatives **4** probably may not fit efficiently into the active site of iNOS. Another reason may be that the **4** underlie a lag in cell uptake.

HPCI derivatives containing aromatic residues (**4i–k**, **p–t**) were toxic to RIN-5AH cells (Table 3). Therefore, the observed dose-dependent reduction of nitrite accumulation is due to the death of RIN cells and not to an inhibitory effect to iNOS.

3. Conclusions

A number of hexahydropyridazine-1-carbothioamides, -carboximidamides and -carbothioimidic acid methyl esters have been prepared and tested with regard to their in vitro inhibition of cytokine-induced NO production by RIN-5AH cells. Especially the substituent in the position 1 of the hexahydropyridazine derivatives was critical for their usefulness as inhibitor. Whereas most hexahydropyridazines with a 1-carbothioamide group (compounds **2**) caused a complete inhibition of NO production the compounds lost their activity completely after *S*-methylation forming hexahydropyridazine-1-carbothioimidic acid methyl ester (compounds **3**). The substitution by a carboximidamide group (compounds **4**) also resulted in a decrease of inhibitory activity which was characterized by an incomplete reduction of NO. This effect may be due to a structural mismatch between the active site of iNOS and the inhibitor or possibly to an ineffective uptake by the cells. Thus, only some few derivatives of hexahydropyridazine-1-carbothioamides particularly compound **2k** were identified as potential iNOS inhibitors. These compounds are taken up by the RIN cells and are sufficiently stable and efficient to exert their inhibitory activity in intact cells. Therefore, pyridazine-based derivatives are not only able to inhibit iNOS of neuronal cells[30,31] but also of pancreatic insulin-producing cells. However, before they should be used in vivo the compounds have to be tested with regard to their selectivity against iNOS and cNOS. First hints concerning the isoform selectivity are given by the spontaneous cNOS mediated NO production of RIN cells. A concentration of 5 mM aminoguanidine caused a decrease of spontaneous NO production and therefore a possible inhibition of cNOS whereas **2k** did not show this effect (data not shown). In case of a high iNOS specificity of **2k** this substance could be tested for their effect to isolated islets in vitro and could be valuable for the treatment of diabetic syndrome because low amounts of NO are still physiologically necessary for hormone release by pancreatic islet cells.

4. Experimental

4.1. Chemistry

4.1.1. General. The reported melting temperatures were determined on a Kofler-Boëtius apparatus type PHMK 78/1910 (VEB Analytik Dresden) and are uncorrected. Elemental analyses were done with the 2400 CHN Elemental Analyzer (Perkin Elmer) and were in accordance with the calculated values within the usual range (0.4%) of tolerances. The spectra were recorded with the followig instruments and conditions: IR spectra: FT-IR 1600 (Perkin–Elmer); ^{1}H and ^{13}C NMR spectra: AVANCE DPX 200 and ARX 300 (^{1}H NMR of **3p**,**q** and ^{13}C NMR of **4t** only) (both from Bruker Analytische Meßtechnik GmbH); room temperature, internal standard tetramethylsilane; mass spectra: M 40 AMD (Intectra GmbH), electron impact, energy 70 eV, (only peaks > 10% are listed). The gas chromatographic investigations were done with a FISON Instruments GC 8065 coupled with a mass spectrometric detector MD 800 (70 eV); column: Macherey-Nagel MN FS Hydrodex β-PM (25 m×0.25 mm); column prepressure: 10 psi (70 kP); column temperature 140 °C. The HPLC investigations were done with a LaChrom system (Merck Hitachi) consisting of pump L-7100, autosampler L-7200, column thermostat L-7350, solvent degasser L-7612, interface D-7000, and diode array detector L-7450 (further data are given below). For TLC aluminia foil covered with Kieselgel GF_{254} (Merck) was utilized as stationary phase, and as mobile phases the following mixtures were employed: *n*-hexane/acetic acid ethyl ester 1:1 (v/v) for the compounds **2**, and n-hexane/acetic acid ethyl ester/triethyl amine 6:3:1 (v/v/v) for the derivatives of **3** and **4**. The substances were detected with UV radiation (λ = 254 nm) or Munier spray reagent[71] (all the compounds) and iodine azide reagent by Awe[72] (**2** and **3**). All the members of the series of title compounds **2–4** characterized in the experimental part were obtained by parallel synthesis approach.

4.1.2. 1,2,3,4,5,6-Hexahydropyridazine hydrochloride (1·HCl). A mixture of 0.138 mol (11.04 g) pyridazine in 30 mL of absolute C_2H_5OH and 2.0 g Adams catalyst (PtO_2×x H_2O, 80% Pt) was shaken vigourously in a hydrogen atmosphere (initial pressure 5 bar) at 25 °C for 6 h. Subsequently, the catalyst was decanted off, the reaction vessel was rinsed with 20 mL of absolute C_2H_5OH, the remaining catalyst decanted again, and the liquor was filtered to became clear. After addition of 150 mL of $(C_2H_5)_2O$ under stirring and cooling with an ice-common salt-mixture dry HCl gas was passed through the solution until the neutral reaction was indicated by moistened test paper. It was stirred in the cooling bath for further 20 min. The precipitate was collected, washed with $(C_2H_5)_2O$ and dried. Yield 85% (crude product). The purification proceeded by recrystallization from C_2H_5OH. Colourless crystals, mp 167–168 °C (C_2H_5OH) (ref 46: 167–169 °C). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1402; 1445; 1503 (NH); 1593 ($+NH_2$); 1877, 2020; 2168; maxima of a broad band: 2423, 2471, 2560, 2623, 2737, 2808, 2890, 2954; 3217 (NH); 3428 ($^+NH_2$). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.67 (s, 4H, –C(4)H_2– and –C(5)H_2–); 2.99 (s, 4H, –C(3)H_2– and –C(6)H_2–); 3.42

(bs, 2H, NH); 10.26 (very broad and flat singulet, 1H, HCl). ^{13}C NMR (DMSO-d_6, ppm): δ = 21.30 (C4 and C5); 44. 66 (C3 and C6). MS (70 eV, 210 °C; free base): *m*/*z* (%): 86 (100) [M^{+}·]; 85 (20); 69 (8), 57 (88), 56 (30). Anal. [$C_4H_{11}ClN_2$ (122.6)] C, H, N.

4.1.3. 1,2,3,4,5,6-Hexahydropyridazine-1-carbothioamide (2a). 4 mmol (0.488 g) of **1**·HCl and 4 mmol (0.392 g) of KSCN were solved in 4 mL of H_2O and the solvent was evaporated carefully under gentle boiling. After cooling the residue was taken up in 1 mL of ice-cold H_2O. The crystals were collected by aspiration, washed with small amounts of both H_2O and C_2H_5OH and recrystallized from C_2H_5OH. Yield 54%. Colourless crystals, mp 175–176.5 °C (C_2H_5OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1514 (NH), 1577 (NH_2), 2849, 2930, 2944, 3155 (NH), 3250 ($NH_{2\ sym}$), 3398 ($NH_{2\ asym}$). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.66 (m, 2H, –C(4)H_2–); 1.80 (m, 2H, –C(5)H_2–); 2.95 (dt, 2H, –C(3)H_2–, $J_{\text{-C(3)H-NH}}$ = 7.3 Hz); 3.35 (t, 1H, NH, *J* = 7.3 Hz); 4.20 (bs, 2H, –C(6)H2–); 6.64 (bs, 2H, NH_2). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.83 (C4); 25.02 (C5); 47.55 (C3); 48.36 (C6); 181.11 (C=S). MS (70 eV, 30 °C): *m*/*z* (%): 145 (59) [M^{+}·], 86 (12) [M^{+}–HSCN], 71 (22), 61 (31) [H_2SCN^{+}], 58 (46), 57 (29), 43 (22), 41 (15), 32 (21), 28 (100). Anal. [$C_5H_{11}N_3S$ (145.2)] C, H, N.

4.1.4. 1,2,3,4,5,6-Hexahydropyridazine-1-carbothioamides 2b–q — general procedure. 5 mmol (0.613 g) of **1**·HCl were suspended in 5 mL of CH_2Cl_2, and after addition of 5 mmol of $(C_2H_5)_3N$ it was stirred at room temperature. When a clear solution had been formed, a solution of 5 mmol of the related isothiocyanate solved in 5 mL of CH_2Cl_2 was added dropwise whithin 10 min under contineously stirring at room temperature, and the stirring was continued for further 20 min. After evaporation of the solvent in vacuum the obtained product was washed with H_2O, recrystallized from 2-C_3H_7OH and dried in vacuum. In this manner were obtained.

4.1.4.1. *N*-Methyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2b). With CH_3–NCS. Yield 66%. Colourless crystals, mp 140–142 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1486; 1544 (NH); 2852, 2930 (–CH_3, –CH_2–); 3181 (NH); 3287 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.69 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.89 (dt, 2H, –C(3)H_2–); 3.12 (d, 3H, –CH_3); 3.18 (t, 1H, NH); 4.23 (bs, 2H, –C(6)H_2–); 7.83 (bs, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.86 (C4); 25.45 (C5); 31.53 (–CH_3); 47.31 (C3); 48.39 (C6); 181.23 (C=S). MS (70 eV, 140 °C): *m*/*z* (%): 159 (100) [M^{+}·], 87 (39), 86 (42) [M^{+}–CH_3-NCS] 76 (16), 75 (54), 71 (35), 70 (13), 58 (93), 57 (46), 32 (13), 30 (68), 28 (40). Anal. [$C_6H_{13}N_3S$ (159.3)] C, H, N.

4.1.4.2. *N*-Ethyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2c). With C_2H_5-NCS. Yield 64%. Colourless crystals, mp 109–110 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1485; 1546 (NH); 2858, 2938, 2968 (–CH_3, –CH_2–); 3178 (NH); 3288 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.21 (t, 3H, –CH_3); 1.69 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.90 (dt, 2H, –C(3)H_2–); 3.18 (t, 1H, NH); 3.62 (dq, 2H, NH–C<u>H</u>$_2$–); 4.21 (bs, 2H, –C(6)H_2–); 7.76 (bs, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 14.64 (–CH_2–<u>C</u>H_3); 23.84 (C4); 25.47 (C5); 39.64 (–<u>C</u>H_2–CH_3); 47.27 (C3); 48.26 (C6); 180.02 (C=S). Anal. [$C_7H_{15}N_3S$ (173.3)] C, H, N.

4.1.4.3. *N*-Propyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2d). With *n*-C_3H_7–NCS. Yield 65%. Colourless crystals, mp 72–75 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1486; 1547 (NH); 2869, 2926, 2960 (–CH_3, –CH_2–); 3196 (NH); 3299 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 0.95 (t, 3H, –CH_3); 1.62 (tq, 2H, –CH_2–); 1.71 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.89 (dt, 2H, –C(3)H_2–); 3.17 (t, 1H, NH); 3.56 (dt, 2H, NH–C<u>H</u>$_2$–); 4.22 (bs, 2H, –C(6)H_2–); 7.84 (bs, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 11.49 (CH_3); 22.60 (CH_2); 23.85 (C4); 25.60 (C5); 46.64 (CH_2); 47.34 (C3); 48.39 (C6); 180.54 (C=S). MS (70 eV, 110 °C): *m*/*z* (%): 187 (83) [M^{+}·], 87 (96), 86 (40) [M^{+}·–C_3H_7NCS], 71 (51), 61 (20), 59 (26), 58 (86), 57 (43), 43 (60), 42 (17), 41 (70), 39 (19), 30 (100), 28 (39), 27 (30). Anal. [$C_8H_{17}N_3S$ (187.3)] C, H, N.

4.1.4.4. *N*-Allyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2e). With CH_2=$CHCH_2$–NCS. Yield 74%. Colourless crystals, mp 105–109 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1483; 1537 (NH); 1638 (C=C); 2853, 2921, 2939 (–CH_2–); 3002 (=C–H); 3075 (=CH_2); 3180 (NH); 3305 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.72 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.91 (dt, 2H, –C(3)H_2–); 3.21 (t, 1H, NH); 4.30 (tt, 2H, allyl; bs: 2H, –C(6)H_2–); 5.17 (m, 2H, allyl); 5.91 (m, 1H, allyl); 7.90 (bs, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.84 (C4); 25.36 (C5); 47.26 (–<u>C</u>H_2–CH=CH_2); 47.32 (C3); 48.45 (C6); 116.15 (–CH_2–CH=<u>C</u>H_2); 134.38 (–CH_2–<u>C</u>H=CH_2); 180.22 (C=S). Anal. [$C_8H_{15}N_3S$ (185.3)] C, H, N.

4.1.4.5. *N*-Isopropyl-1,2,3,4,5,6-hexahydropyridazien-1-carbothioamide (2f). With i-C_3H_7-NCS. Yield 50%. Colourless crystals, mp 94–97 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1476; 1538 (NH); 2853, 2944, 2970 (–CH_3, –CH_2–, –CH); 3201 (NH); 3285 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.22 (d, 6H, 2-CH_3); 1.71 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.88 (dt, 2H, –C(3)H_2–); 3.13 (t, 1H, NH); 4.21 (bs, 2H, –C(6)H_2–); 5.49 (m, 1H, CH); 7.65 (bd, 1H, CS-N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.82 (2-CH_3); 23.90 (C4); 25.69 (C5); 46.32 (CH); 47.33 (C3); 48.32 (C6); 179.30 (C=S). Anal. [$C_8H_{17}N_3S$ (187.3)] C, H, N.

4.1.4.6. *N*-Butyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2g). With *n*-C_4H_9–NCS. Yield 70%. Colourless crystals, mp 75.5–78 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1492; 1552 (NH); 2858, 2928, 2855 (broad band, –CH_3, –CH_2–); 3181 (NH); 3296 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 0.94 (t, 3H, –CH_3); 1.37 (tq, 2H, –CH_2–); 1.57 (tt, 2H, –CH_2–); 1.71 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.89 (dt, 2H, –C(3)H_2–); 3.15 (t, 1H, NH); 3.59 (dt, 2H, NH–C<u>H</u>$_2$–); 4.28 (bs, 2H, –C(6)H_2–); 7.80 (bs, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 13.92 (CH_3); 20.25 (CH_2); 23.87 (C4); 25.62 (C5); 31.50 (CH_2); 44.72 (CH_2); 47.36 (C3); 48.40 (C6); 180.75 (C=S). Anal. [$C_9H_{19}N_3S$ (201.3)] C, H, N.

4.1.4.7. *N*-(2-Phenylethyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2h). With C_6H_5–C_2H_4–NCS.

Yield 88%. Colourless crystals, mp 115–119 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1474; 1494, 1542 (broad band, NH, arom. ring); 2856, 2936, 3181 (–CH_2–); 3022, 3058, 3083 (arom. =C–H); 3191 (NH); 3291 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.64 (m, 2H, –C(4)H_2–); 1.74 (m, 2H, –C(5)H_2–); 2.81 (dt, 2H, –C(3)H_2–); 2.92 (t, 2H, –C$\underline{H}_2$–C_6H_5); 3.10 (t, 1H, NH); 3.86 (dt, 2H, NH–C$\underline{H}_2$–); 4.21 (bs, 2H, –C(6)H_2–); 7.26 (m, 5H, phenyl); 7.85 (bs, 1H, CS–N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.85 (C4); 25.53 (C5); 35.52 (CH_2); 45.92 (CH_2); 47.25 (C3); 48.37 (C6); 126.38; 128.53 (2 C); 128.92 (2 C); 139.30; 180.33 (C=S). Anal. [$C_{13}H_{19}N_3S$ (249.4)] C, H, N.

4.1.4.8. *N*-Cyclohexyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2i). With C_6H_{11}–NCS. Yield 82%. Colourless crystals, mp 83–88 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1474; 1533 (NH); 1699; 2850, 2929 (broad band, –CH_2–, –CH); 3198 (NH); 3282 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.24 (m, 4H, cyclohexyl); 1.42 (m, 2H, cyclohexyl); 1.70 (m, 4H, –C(4)H_2– and –C(5)H_2–; 2H, cyclohexyl); 2.04 (m, 2H, cyclohexyl); 2.88 (bs, 2H, –C(3)H_2–); 3.14 (bs, 1H, NH); 4.19 (m, 2H, –C(6)H_2–; 1H, cyclohexyl); 7.75 (bd, 1H, CS-N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.83 (cyclohexyl; C4′); 24.92 (cyclohexyl; C3′, C5′); 25.62 (C4); 25.72 (C5); 33.07 (cyclohexyl; C2′, C6′); 47.26 (C3); 48.32 (C6); 53.00 (cyclohexyl; C1′); 179.02 (C=S). MS (70 eV, 120 °C): *m*/*z* (%): 227 (48) [$M^{+\cdot}$], 130 (12), 99 (26), 87 (100), 86 (26) [$M^{+\cdot}$–C_6H_{11}NCS], 84 (16), 71 (21), 58 (32), 57 (26), 56 (40), 44 (12), 42 (47), 39 (11). Anal. [$C_{11}H_{21}N_3S$ (227.4)] C, H, N.

4.1.4.9. *N*-Phenyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2j). With C_6H_5–NCS. Yield 83%. Colourless crystals, mp 133–135 °C (2-C_3H_7OH, ref 69: 133–134 °C). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1498; 1533 (broad band, NH, arom. ring); 1589, 1598 (arom. ring); 2854, 2938 (broad band, –CH_2–); 3182, 3215 (broad band, arom. =C–H, 2×NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.78 (m, 4H, –C(4)H_2– and –C(5)H_2–); 3.02 (dt, 2H, –C(3)H_2–); 3.40 (t, 1H, NH); 4.33 (bs, 2H, –C(6)H_2–); 7.18 (m, 1H, phenyl); 7.35 (m, 2H, phenyl); 7.56 (d, 2H, phenyl); 9.75 (s, 1H, CS–N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.78 (C4); 25.16 (C5); 47.53 (C3); 47.77 (C6); 124.57 (2 C); 125.32; 128.49 (2 C);139.06; 178.87 (C=S). MS (70 eV, 130 °C): *m*/*z* (%): 221 (90) [$M^{+\cdot}$], 136 (10) [C_6H_5-NCSH^+], 135 (12) [$M^{+\cdot}$–C_6H_5–NCS], 87 (100), 86 (22), 78 (46), 71 (21), 58 (45), 30 (46), 28 (52). Anal. [$C_{11}H_{15}N_3S$ (221.3)] C, H, N.

4.1.4.10. *N*-(3-Methylphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2k). With 3-CH_3–C_6H_4–NCS. Yield 88%. Colourless crystals, mp 93–98 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1484, 1526 (broad band, NH, arom. ring); 1608 (arom. ring); 2853, 2931, 2948, 2993 (broad band, –CH_3, –CH_2–); 3020 (arom. =C–H); 3183 (NH); 3236 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.78 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.35 (s, 3H, –CH_3); 3.00 (dt, 2H, –C(3)H_2–); 3.41 (t, 1H, NH); 4.32 (bs, 2H, –C(6)H_2–); 6.99 (d, 1H, m-subst. phenyl); 7.30 (m, 3H, m-subst. phenyl); 9.68 (s, 1H, CS–N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.00 (CH_3); 23.77 (C4); 25.31 (C5); 47.87 (C3); 48.17 (C6); 121.88; 125.37; 126.37; 128.33; 138.37; 138.93; 179.06 (C=S). MS (70 eV, 30 °C): *m*/*z* (%): 235 (19) [$M^{+\cdot}$], 149 (15) [C_7H_7-NCS$^+$], 92 (20), 87 (31), 86 (11), 58 (38), 57 (17), 32 (46), 30 (22), 28 (100). Anal. [$C_{12}H_{17}N_3S$ (235.4)] C, H, N.

4.1.4.11. *N*-(4-Methylphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2l). With 4-CH_3–C_6H_4–NCS. Yield 84%. Colourless crystals, mp 89–93 °C (2-C_3H_7OH, ref 46: 104–107 °C). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1481, 1527 (broad band, NH, arom. ring); 1583 (arom. ring); 2847, 2915, 2956, 2992 (broad band, –CH_3, –CH_2–); 3022 (arom. =C–H); 3209 (NH); 3264 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.77 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.33 (s, 3H, –CH_3); 3.01 (dt, 2H, –C(3)H_2–); 3.40 (t, 1H, NH); 4.31 (bs, 2H, –C(6)H_2–); 7.15 (d, 2H, p-subst. phenyl); 7.38 (d, 2H, p-subst. phenyl); 9.62 (s, 1H, CS–N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.10 (CH_3); 23.78 (C4); 25.34 (C5); 47.56 (C3); 47.98 (C6); 125.09 (2 C); 129.16 (2 C); 135.31; 136.45; 179.41 (C=S). Anal. [$C_{12}H_{17}N_3S$ (235.4)] C, H, N.

4.1.4.12. *N*-(2-Methoxyphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2m). With 2-CH_3O–C_6H_4–NCS. Yield 81%. Colourless crystals, mp 136–140 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1505, 1539 (broad band, NH, arom. ring); 1595 (arom. ring); 2835, 2852, 2936 (–CH_3, –CH_2–); 3002, 3064 (arom. =C–H); 3155 (NH); 3226 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.77 (m, 4H, –C(4)H_2– and –C(5)H_2–); 3.03 (m, 2H, –C(3)H_2–); 3.41 (t, 1H, NH);3.85 (s, 3H, –CH_3); 4.33 (bm, 2H, –C(6)H_2–); 7.00 (md, 3H, o-subst. phenyl); 8.55 (dd, 1H, o-subst. phenyl); 10.12 (bs, 1H, CS–N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.85 (C4); 25.30 (C5); 47.25 (C3); 47.46 (C6); 55.75 (CH_3); 110.28; 120.16; 123.55; 124.79; 128.52; 150.63; 178.06 (C=S). Anal. [$C_{12}H_{17}N_3OS$ (251.4)] C, H, N.

4.1.4.13. *N*-(4-Methoxyphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2n). With 4-CH_3O–C_6H_4–NCS. Yield 34%. Colourless crystals, mp 85–87 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1462, 1525 (broad band, NH, arom. ring); 1586, 1608 (arom. ring); 2834, 2855, 2938 (–CH_3; –CH_2–); 2999, 3047 (arom. =C–H); 3238 (NH); 3306 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.77 (m, 4H, –C(4)H_2– and –C(5)H_2–); 3.01 (m, 2H, –C(3)H_2–); 3.38 (t, 1H, NH);3.81 (s, 3H, –CH_3); 4.32 (bs, 2H, –C(6)H_2–); 6.89 (d, 2H, p-subst. phenyl); 7.37 (d, 2H, p-subst. phenyl); 9.53 (bs, 1H, CS–N$\underline{H}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.79 (C4); 25.28 (C5); 47.53 (C3); 48.06 (C6); 55.41 (CH_3); 113.88 (2 C); 126.94 (2 C); 132.36; 157.41; 177.72 (C=S). Anal. [$C_{12}H_{17}N_3OS$ (251.4)] C, H, N.

4.1.4.14. *N*-(4-Nitrophenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2o). With 4-NO_2–C_6H_4–NCS. Yield 97%. Yellow crystals, mp 155–158 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1328 (broad multipeak band, =C–NO_2), 1515, 1544 (broad band, NH, arom. ring); 1598 (arom. ring); 2924, 2947 (–CH_2–); 3063 (arom. =C–H); 3178 (broad band, 2×NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.80 (m, 4H, –C(4)H_2– and –C(5)H_2–); 3.05 (dt, 2H, –C(3)H_2–); 3.52 (t, 1H, NH); 4.35 (bs,

2H, –C(6)H_2–); 7.95 (dt, 2H, p-subst. phenyl); 8.21 (dt, 2H, p-subst. phenyl); 10.245 (s, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.72 (C4); 25.21 (C5); 47.65 (C3); 47.91 (C6); 121.99 (2 C); 122.48; 124.42 (2 C); 145.11; 178.01 (C=S). Anal. [$C_{11}H_{14}N_4O_2S$ (266.3)] C, H, N.

4.1.4.15. *N*-(Naphth-1-yl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2p). With $C_{10}H_7$–1-NCS. Yield 57%. Beige crystals, mp 119–122 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1478, 1525 (broad band, NH, arom. ring); 1594 (arom. ring); 2852, 2935 (–CH_2–); 3056 (arom. =C–H); 3179 (NH); 3227 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.78 (tt, 2H, –C(4)H_2–); 1.88 (tt, 2H, –C(5)H_2–); 3.13 (dt, 2H, –C(3)H_2–); 3.57 (t, 1H, NH); 4.38 (bs, 2H, –C(6)H_2–); 7.51 (m, 3H, naphth-1-yl); 7.83 (m, 4H, naphth-1-yl); 9.89 (s, 1H, CS–N<u>H</u>). Anal. [$C_{15}H_{17}N_3S$ (271.4)] C, H, N.

4.1.4.16. *N*-(Naphth-2-yl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2q). With $C_{10}H_7$–2-NCS. Yield 78%. Beige crystals, mp 142–148 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1507, 1525 (broad band, NH, arom. ring); 1578, 1598, 1631 (arom. ring); 2852, 2917, 2945 (–CH_2–); 3041 (arom. =C–H); 3267 (broad band, NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.74 (m, 2H, –C(4)H_2–); 1.84 (M, 2H, –C(5)H_2–); 3.08 (dt, 2H, –C(3)H_2–); 3.45 (t, 1H, NH); 4.34 (bs, 2H, –C(6)H_2–); 7.43 (m, 2H, naphth-2-yl); 7.67 (dd, 1H, naphth-2-yl); 7.79 (m, 3H, naphth-2-yl); 8.08 (d, 1H, naphth-2-yl); 9.91 (s, 1H, CS–N<u>H</u>). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.79 (C4); 25.33 (C5); 47.66 (C3); 47.90 (C6); 121.47; 124.32; 125.37; 126.16; 127.60; 127.69; 128.06; 131.36; 133.51; 136.59; 179.16 (C=S). MS (70 eV, 280 °C): *m*/*z* (%): 271 (23) [$M^{+\cdot}$], 186 (100) [$C_{10}H_7$-NCSH$^+$], 143 (13), 127 (51) [$C_{10}H_7^+$], 87 (44), 86 (15), 58 (24), 30 (27), 28 (12). Anal. [$C_{15}H_{17}N_3S$ (271.4)] C, H, N.

4.1.4.17. *N*-Phenyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioamide (2j) and 4-nitrobenzanilide from 1-(4-nitrobenzoyl)-1,3-diphenylthiourea (6). 1 mmol of **6** and subsequent 2 mmol of $(C_2H_5)_3N$ were added under stirring to a suspension of 1 mmol of **1**·HCl in 5 mL of CH_2Cl_2 at room temperature. After 10 min the stirring was ended, and the batch was stored at room temperature for 36 h. After evaporation to dryness with an air flow a solid residue was yielded, which was suspended in a small amount of water. The obtained product was pressed on an earthenplate and after drying it was recrystallized from C_2H_5OH. The residue yielding by the latter procedure was washed with a small amount of C_2H_5OH and dried: 4-nitrobenzanilide,[73] yield 37%, mp 218—219 °C. The crystals formed during the cooling down of the solution were sucked off, and recrystallized again form C_2H_5OH. **2j**, yield 63%, mp 133–135 °C.

4.1.5. Combinatorial synthesis of the 2 from 1-hydrochloride and isothiocyanates. Synthesis of the libraries. A solution of *Z* different isothiocyanates related to the respective derivative **2** (each 0.1 mmol in about 1 mL of CH_2Cl_2) in CH_2Cl_2 was added to a mixture of *Z*×0.1 mmol of **1** hydrochloride and *Z* 0.1 mmol of $(C_2H_5)_3N$, and the reaction occuring at room temperature was indicated by TLC. Group 1 (*Z* = 5): CH_3NCS (**2b**), C_2H_5NCS (**2c**), H_2C=CH-CH_2NCS (**2e**), $(CH_3)_2CH$-NCS, (**2f**), C_4H_9NCS (**2g**); group 2 (*Z* = 5): C_3H_7NCS (**2d**), $C_6H_{11}NCS$ (**2i**), C_6H_5NCS (**2j**), 2-CH_3O–C_6H_4NCS (**2m**),4-NO_2-C_6H_4NCS (**2o**); group 3 (*Z* = 2): 3-CH_3-C_6H_4NCS (**2k**), naphth-1-yl-NCS (**2p**). When the isothiocyanate was not anymore detectable (iodine azide reaction [72] on the TLC plate failed), the solvent was evaporated in vacuum, and the residue was solved in 10.00 mL of CH_3CN (ultrasonic bath).

For the HPLC measurements 1.00 mL of this solution was taken off and diluted with CH_3CN up to 10.00 mL. Injection volume:10 μL.

HPLC. Column: RP-select B (Merck); mobile phase acetonitrile/0.02 M KH_2PO_4 (pH 6.57) 1:1 (v/v). Investigation conditions: temperature 22 °C; flow 0.8 mL·min^{-1}, time of passage t_o determined with uracil. Detection: Near the absorption maximum utilizing the uv diode array detector L-7450 (Merck); group 1: 235 nm; group 2: 242 nm; group 3: 249 nm; **2a**,**h**,**n**: 246 nm; **2l**,**q**: 249 nm. Evaluation: The determination of each k′ value (Table 1) for the respective **2** prepared using the single synthesis mode was done in single runs with 0.01 mM concentrated solutions of these in CH_3CN. The chromatograms of the combinatorial libraries (groups 1 to 3) were evaluated by means of the k′ values and by comparison of the areas under the peaks with those in the chromatograms of the mixtures of the pure **2** (solved in CH_3CN, each of the compounds 0.01 mM).

4.1.6. 1,2,3,4,5,6-Hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodides 3 — general procedure. 2.5 mmol of the appropriate **2** were solved in 15 mL of CH_2Cl_2 and after addition of 3.75 mmol (0.535 g) of CH_3I the covered reaction vessel was stored at room temperature until the starting compound was completely reacted (24–48 h; reaction control utilizing TLC). The solvent was carefully evaporated in vacuum; the formed crystals were suspended in a very small amount of cold 2-C_3H_7OH, sucked off, washed with some 2-C_3H_7OH and dried in vacuum. Non-crystalline products isolated were frictionized, allowed to stand at −8 °C until the crystallization was completed and than they were worked up as described above. In this manner were obtained:

4.1.6.1. 1,2,3,4,5,6-Hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3a). From **2a**. Deviated from the given instruction the non-crystalline yielded product was dissolved in a small amount of 2-C_3H_7OH and crystallized by a subsequent careful addition of $(C_2H_5)_2O$. Yield 73%. Colourless crystals, mp 102–105 °C [2-C_3H_7OH/$(C_2H_5)_2O$]. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1478; 1592; 1632 (C=N); 2851; 2908; 2942; 2957; 2981; 3110; 3222; 3263; 3302. ^{1}H NMR ($CDCl_3$, ppm): δ = 1.88 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.94 (s, 3H, –SCH_3); 3.07 (bs, 2H, –C(3)H_2–); 4.29 (bs, 2H, –C(6)H_2–); 5.58 (t, 1H, NH); 8.29 (bs, 2H, =$NH_2^{\oplus}$). ^{13}C NMR ($CDCl_3$, ppm): δ = 17.15 (CH_3), 23.38 (C4); 23.96 (C5); 47.38 (C3); 49.36 (C6); 169.78 (<u>C</u>(SCH_3)=$NH_2^{\oplus}$).

MS (70 eV, 348 °C): *m*/*z* (%): 159 (56) [M^{+}_{base}], 145 (87) [$M^{+\cdot}{}_{base}$ — CH_2], 142 (95) [M^{+}_{base} — NH_3], 141 (59), 128 (96), 127 (100), 111 (15) [$M^{+\cdot}-CH_3SH$], 103 (25), 102 (39), 101 (23), 86 (61), 85 (77) [$C_4H_9N_2^{+}$, M^{+}_{base} — HN=C–SCH_3], 71 (76), 70 (37), 62 (32), 61 (71), 58 (88), 56 (77), 44 (32), 43 (62), 41 (60), 39 (25); 30 (96), 29 (31), 28 (85), 27 (31). Anal. [$C_6H_{14}IN_3S$ (287.2)] C, H, N.

4.1.6.2. *N*-Methyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3b). From **2b**. Yield 90%. Colourless crystals, mp 98.5–101 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1507; 1620 (C=N); 2852, 2938, 2997 (–CH_3, –CH_2–); 3144 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.86 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.69 (bs, 3H, –SCH_3); 3.11 (bs, 2H, –C(3)H_2–); 3.34 (d, 3H, =$NH^{\oplus}$-C$\underline{H}_3$); 4.29 (bs, 2H, –C(6)H_2–); 5.50 (bs, 1H, NH); 8.85 (bs, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 17.67 (–S$\underline{C}H_3$); 23.32 (C4); 24.61 (C5); 33.14 (=$NH^{\oplus}$–$\underline{C}H_3$); 47.47 (C3); 51.44 (C6); 167.65 ($\underline{C}(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_7H_{16}IN_3S$ (301.2)] C, H, N.

4.1.6.3. *N*-Ethyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3c). From **2c**. Yield 92%. Colourless crystals, mp 89.5–94 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1488; 1547 (NH); 1620 (C=N); 2862; 2938; 2968; 3152 (maximum of a broad band, NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.36 (t, 3H, –CH_3); 1.90 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.72 (bs, 3H, –SCH_3); 3.14 (m, 2H, –C(3)H_2–); 3.77 (dq, 2H, =$NH^{\oplus}$–C$\underline{H}_2$–); 4.30 (m, 2H, –C(6)H_2–); 5.67 (t, 1H, NH); 8.69 (bs, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 15.65 (–CH_2–$\underline{C}H_3$); 18.27 (–S–$\underline{C}H_3$); 23.29 (C4); 24.55 (C5); 41.68 (–$\underline{C}H_2$–CH_3); 47.43 (C3); 51.25 (C6); 166.95 ($\underline{C}(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_8H_{18}IN_3S$ (315.2)] C, H, N.

4.1.6.4. *N*-Isopropyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3f). From **2f**. Yield 84%. Colourless crystals, mp 101–103 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1480; 1536 (NH); 1604 (C=N); 2854; 2913; 2940; 3003; 3042; 3070; 3152 (NH); 3200 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.39 (d, 6H, 2-CH_3); 1.89 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.76 (s, 3H, –SCH_3); 3.09 (m, 2H, –C(3)H_2–); 4.32 (m, 3H, –C(6)H_2– and =$NH^{\oplus}$–$\underline{C}H$–); 5.87 (t, 1H, NH); 8.31 (bd, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 18.32 (–S–$\underline{C}H_3$); 23.21 (C4); 23.51 [–CH($\underline{C}H_3$)$_2$]; 24.32 (C5); 47.00 (C3); 49.76 (C6); 50.98 [–$\underline{C}H$(CH_3)$_2$]; 165.66 ($\underline{C}(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_9H_{20}IN_3S$ (329.2)] C, H, N.

4.1.6.5. *N*-(2-Phenylethyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3h). From **2h**. Yield 71%. Colourless crystals, mp 108–117 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1504 (NH); 1611 (C=N); 2854; 2933; 2992; 3090 and 3132 (maxima of a broad band, NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.85 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.42 (s, 3H, –SCH_3); 3.04 (m, 4H, –C(3)H_2– and –CH_2–); 3.98 (dt, 2H, =$NH^{\oplus}$–C$\underline{H}_2$–); 4.23 (m, 2H, –C(6)H_2–); 5.74 (t, 1H, NH); 7.28 (m, 5H, phenyl); 8.91 (bs, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 17.80 (–S$\underline{C}H_3$); 23.24 (C4); 24.60 (C5); 36.32 (–$\underline{C}H_2$–C_6H_5); 47.42 (C3); 47.75 (C6); 51.33 (=$NH^{\oplus}$–$\underline{C}H_2$–); 127.09 (phenyl; C4′); 128.84 (phenyl; C3′, C5′);′); 128.91 (phenyl; C2′, C6′);′); 136.97 (phenyl; C1′); 167.12 ($\underline{C}(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_{14}H_{22}IN_3S$ (391.3)] C, H, N.

4.1.6.6. *N*-Cyclohexyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3i). From **2i**. Yield 67%. Colourless crystals, mp 112–115 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1497; 1530 (NH); 1602 (C=N); 2858; 2934; 2974; 3070; 3111 (NH), 3140 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.44 (m, 4H, cyclohexyl); 1.66 (m, 2H, cyclohexyl); 1.90 (m, 8H, –C(4)H_2–, –C(5)H_2– and cyclohexyl); 2.75 (s, 3H, –SCH_3); 3.10 (m, 4H, –C(3)H_2– and –CH_2–); 3.94 (m, 1H, =$NH^{\oplus}$–C$\underline{H}$–); 4.29 (m, 2H, –C(6)H_2–); 5.81 (t, 1H, NH); 8.33 (d, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 18.66 (CH_3); 23.23 (cyclohexyl; C4′); 24.43 (C4); 24.61 (cyclohexyl; C3′, C5′); 24.76 (C5); 33.56 (cyclohexyl; C2′, C6′); 47.13 (C3); 51.06 (C6); 56.34 (cyclohexyl; C1′); 165.94 ($C(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_{12}H_{24}IN_3S$ (369.3)] C, H, N.

4.1.6.7. *N*-Phenyl-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3j). From **2j**. Yield 76%. Colourless crystals, mp 106.5–110 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1496; 1578; 1591 (C=N); 2940, 3060; 3105 (NH); 3153 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.92 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.22 (s, 3H, –SCH_3); 3.19 (m, 2H, –C(3)H_2–); 4.33 (m, 2H, –C(6)H_2–); 5.78 (bs, 1H, NH); 7.47 (m, 5H, phenyl); 10.18 (bs, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 16.80 (–S–$\underline{C}H_3$); 23.24 (C4); 24.18 (C5); 47.46 (C3); 51.32 (C6); 125.57 (arom C); 128.42 (arom. C); 129.78 (arom. C); 135.25 (arom. C); 167.78 ($\underline{C}(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_{12}H_{18}IN_3S$ (363.3)] C, H, N.

4.1.6.8. *N*-(3-Methylphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3k). From **2k**. Yield 78%. Colourless crystals, mp 107.5–111 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1498; 1585 (broad band); 2869; 2942; 3009; 3035; 3083; 3090; 3157. ^{1}H NMR ($CDCl_3$, ppm): δ = 1.92 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.26 (s, 3H, –SCH_3); 2.39 (s, 3H, –C_6H_4–C$\underline{H}_3$); 3.17 (m, 2H, –C(3)H_2–); 4.36 (m, 2H, –C(6)H_2–); 5.92 (bs, 1H, NH); 7.18 (bs, 1H, phenyl); 7.31 (m, 3H, phenyl); 10.08 (bs, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$, ppm): δ = 16.79 (–S–$\underline{C}H_3$); 21.32 (–C_6H_4–$\underline{C}H_3$); 23.28 (C4); 24.25 (C5); 47.45 (C3); 51.53 (C6); 122.27 (arom C); 125.75 (arom C); 129.19 (arom. C); 129.59 (arom C); 135.18 (arom. C); 140.12 (arom. C); 167.67 ($\underline{C}(SCH_3)$=$NH^{\oplus}$–). Anal. [$C_{13}H_{20}IN_3S$ (377.3)] C, H, N.

4.1.6.9. *N*-(4-Methylphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methl ester hydroiodide (3l). From **2l**. Yield 74%. Colourless crystals, mp 101–108 °C (CH_2Cl_2). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1510 (NH); 1679 (C=N); 2860; 2935; 3019; 3081; 3070; 3181 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.92 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.24 (s, 3H, –SCH_3); 2.38 (s, 3H, –C_6H_4–C$\underline{H}_3$); 3.18 (m, 2H, –C(3)H_2–); 4.33 (m, 2H, –C(6)H_2–); 5.92 (bs, 1H, NH); 7.23 (d, 2H, phenyl); 7.41 (d, 2H, phenyl); 10.02 (bs, 1H, =$NH^{\oplus}$–). ^{13}C NMR ($CDCl_3$,

ppm): δ = 16.77 (–S–CH$_3$); 21.16 (–C$_6$H$_4$–CH$_3$); 23.26 (C4); 24.23 (C5); 47.48 (C3); 51.38 (C6); 125.36 (2 arom. C); 130.34 (2 arom. C); 132.59 (arom. C); 138.64 (arom. C); 167.63 (C(SCH$_3$)=NH$^{\oplus}$–). Anal. [C$_{13}$H$_{20}$IN$_3$S (377.3)] C, H, N.

4.1.6.10. *N*-(2-Methoxyphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3m). From **2m**. Yield 75%. Colourless crystals, mp 118.5–123 °C (CH$_2$Cl$_2$). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1499; 1582 (C=N); 1600; 2923; 2952; 2975; 3083; 3219. ^{1}H NMR (CDCl$_3$, ppm): δ = 1.98 (m, 4H, –C(4)H$_2$– and –C(5)H$_2$–); 2.31 (s, 3H, –SCH$_3$); 3.20 (m, 2H, –C(3)H$_2$–); 3.91 (s, 3H, –C$_6$H$_4$–OCH$_3$); 4.43 (m, 2H, –C(6)H$_2$–); 6.23 (bs, 1H, NH); 7.02 (m, 2H, phenyl); 7.34 (m, 1H, phenyl); 7.56 (d, 1H, phenyl); 9.64 (bs, 1H, =NH$^{\oplus}$–). ^{13}C NMR (CDCl$_3$, ppm): δ = 16.92 (–S–CH$_3$); 22.97 (C4); 24.45 (C5); 47.45 (C3); 51.13 (C6); 56.00 (–O–CH$_3$); 111.73 (arom C); 121.16 (arom. C); 126.79 (arom. C); 130.11 (2 arom C); 153.33 (arom. C); 168.40 (C(SCH$_3$)=NH$^{\oplus}$–). Anal. [C$_{13}$H$_{20}$IN$_3$OS (393.3)] C, H, N.

4.1.6.11. *N*-(4-Methoxyphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide 3n. From **2n**. Yield 69%. Colourless crystals, mp 102–110 °C (CH$_2$Cl$_2$). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1513; 1597 (C=N, NH); 2940; 3055; 3125 (NH). ^{1}H NMR (CDCl$_3$, ppm): δ = 1.91 (m, 4H, –C(4)H$_2$– and –C(5)H$_2$–); 2.24 (s, 3H, –SCH$_3$); 3.17 (m, 2H, –C(3)H$_2$–); 3.38 (s, 3H, –C$_6$H$_4$–OCH$_3$); 4.33 (m, 2H, –C(6)H$_2$–); 5.86 (bs, 1H, NH); 6.94 (m, 2H, phenyl); 7.45 (m, 2H, phenyl); 9.97 (bs, 1H, =NH$^{\oplus}$–). ^{13}C NMR (CDCl$_3$, ppm): δ = 16.56 (–S–CH$_3$); 23.34 (C4); 24.15 (C5); 47.17 (C3); 51.54 (C6); 55.59 (–O–CH$_3$); 114.87 (4 arom C); 127.02 (2 arom. C); 159.49 (C(SCH$_3$)=NH$^{\oplus}$–). Anal. [C$_{13}$H$_{20}$IN$_3$OS (393.3)] C, H, N.

4.1.6.12. *N*-(Naphth-1-yl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3p). From **2p**. Yield 75%. Yellowish crystals, mp 89.5–95 °C (CH$_2$Cl$_2$). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1478; 1509; 1589 (very intensive; C=N and aromatic moiety); 2821; 2861; 2926; 2942; 2976; 3015; 3075; 3179. ^{1}H NMR (CDCl$_3$, ppm), spectrum of the free base: δ = 1.94 (m, 2H, –C(4)H$_2$–); 2.03 (m, 2H, –C(5)H$_2$–); 2.11 (s, 3H, –CH$_3$); 3.25 (t, 2H, –C(3)H$_2$–); N(2)H is not to detect; 4.47 (t, 2H, –C(6)H$_2$–); 7.49–7.73 (m, 4H, naphth-1-yl); 7.91 (m, 1H, naphth-1-yl); 7.93 (m, 1H, naphth-1-yl); 8.32 (d, 1H, naphth-1-yl). Anal. [C$_{16}$H$_{20}$IN$_3$S (413.3)] C, H, N.

4.1.6.13. *N*-(Naphth-2-yl)-1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (3q). From **2q**. Yield 60%. Yellowish crystals, mp 93.5–98 °C (CH$_2$Cl$_2$). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1476; 1496; 1511; 1589 (very intensive; C=N and aromatic moiety); 2857; 2942 (maximum of a multipeak band); 3032; 3090, 3110. ^{1}H NMR (CDCl$_3$, ppm), spectrum of the free base: δ = 1.87 (m, 2H, –C(4)H$_2$–); 1.98 (m, 2H, –C(5)H$_2$–); 2.23 (s, 3H, –CH$_3$); 3.19 (t, 2H, –C(3)H$_2$–); N(2)H is not to detect; 4.39 (t, 2H, –C(6)H$_2$–); 7.54–7.59 (m, 2H, naphth-1-yl); 7.64 (dd, 1H, naphth-1-yl); 7.84–7.92 (m, 3H, naphth-1-yl); 8.00 (d, 1H, naphth-1-yl). ^{13}C NMR (CDCl$_3$, ppm): δ = 16.81 (–S–CH$_3$); 23.28 (C4); 24.22 (C5); 47.52 (C3); 51.56 (C6); 123.08; 123.98; 127.23; 127.56; 127.87; 128.06; 129.90; 132.34; 132.63; 133.13; 168.21 (C(SCH$_3$)=NH$^{\oplus}$–). Anal. [C$_{16}$H$_{20}$IN$_3$S (413.3)] C, H, N.

4.1.7. 1,2,3,4,5,6-Hexahydropyridazine-1-carboximidamide hydroiodides 4a–v.

Method A — from different 3 and gaseous NH$_3$

Into a solution of 1 mmol of the related 1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide **3** dissolved in 4 mL of CH$_2$Cl$_2$ NH$_3$ was bubbled for 5 min and then the covered batch (pressure compensation!) was allowed to stand at room temperarature for 2 h. Thereafter (C$_2$H$_5$)$_2$O was carefully added until the solution became turbidly, and after allowing to stand for a longer time crystals precipitated which were aspirated off, washed with (C$_2$H$_5$)$_2$O/2-C$_3$H$_7$OH (3:1; V:V) and dried in vacuum. In this manner were obtained:

4.1.7.1. 1,2,3,4,5,6-Hexahydropyridazine-1-carboximidamide hydroiodide (4a). From **3a**. Yield 60%. Colourless crystals, mp 140–143 °C (CH$_2$Cl$_2$/(C$_2$H$_5$)$_2$O). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1598; 1640 (C=N); 2848; 2921; 2939; 3158; 3217; 3316; 3428. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56 (m, 4H, –C(4)H$_2$– and –C(5)H$_2$–); 2.79 (m, 2H, –C(3)H$_2$–); 3.52 (m, 2H, –C(6)H$_2$–); 5.17 (t, 1H, NH); 7.19 (s, 4H, –C(NH$_2$)=NH$_2^{\oplus}$). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.20 (C4); 23.38 (C5); 44.87 (C3); 46.39 (C6); 156.30 (C(NH$_2$)=NH$_2^{\oplus}$). MS (70 eV, 348 °C): *m*/*z* (%): 128 (100) [M$^{+\cdot}{}_{\text{base}}$, HI$^{+\cdot}$], 127 (55), 86 (30) [C$_4H_{10}N_2^{+\cdot}$, M$^{+\cdot}{}_{\text{base}}$ — HN=C=NH], 85 (13), 57 (38), 43 (27), 41 (10), 30 (27), 28 (37). Anal. [C$_5$H$_{13}$IN$_4$ (256.1)] C, H, N.

4.1.7.2. N^2-Methyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4b). From **3b**. Yield 58%. Colourless, markedly hygroscopic crystals, mp 41–47 °C (CH$_2$Cl$_2$/(C$_2$H$_5$)$_2$O). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1570; 1631 (C=N); 2856; 2940; 2939; 3212 and 3298 (maxima of a broad band). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.60 (m, 4H, –C(4)H$_2$– and –C(5)H$_2$–); 2.76 (d, 3H, CH$_3$); 2.79 (m, 2H, –C(3)H$_2$–); 3.53 (m, 2H, –C(6)H$_2$–); 5.13 (t, 1H, NH); 7.49 (s, 2H, NH$_2$); 7.70 (q, 1H, CH$_3$NH$^{\oplus}$=). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.19 (C4); 23.51 (C5); 28.20 (CH$_3$); 45.21 (C3); 46.22 (C6); 155.88 (C(NH$_2$)=NH$^{\oplus}$–). MS (70 eV, 120 °C): *m*/*z* (%): 142 (35) [M$^{+\cdot}{}_{\text{base}}$], 128 (66), 127 (31), 86 (90) [C$_4$H$_{10}$N$_2^{+\cdot}$, M$^{+\cdot}{}_{\text{base}}$ — HN=C=N–CH$_3$], 85 (19), 57 (100), 56 (15), 43 (16), 41 (13), 30 (34), 28 (26). Anal. [C$_6$H$_{15}$IN$_4$ (270.1)] C, H, N.

4.1.7.3. *N*^{2}-(2-Phenylethyl)-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4h). From **3h**. Yield 87%. Colourless crystals, mp 109.5–112 °C (CH$_2$Cl$_2$/(C$_2$H$_5$)$_2$O). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1497; 1566; 1642 (C=N); 2855; 2884; 2916; 2932; 2946; 2960; 3026; 3167, 3297; 3331. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.58 (bs, 4H, –C(4)H$_2$– and –C(5)H$_2$–); 2.76 (m, 2H, –C(3)H$_2$–); 2.80 (t, 2H, =NH$^{\oplus}$–CH$_2$–CH$_2$–C$_6$H$_5$); 3.41 (t, 2H, =NH$^{\oplus}$–CH$_2$–CH$_2$–C$_6$H$_5$); 3.52 (m, 2H, –C(6)H$_2$–); 5.16 (t, 1H, NH); 7.27 (m, 5H, arom. H); 7.56 (s, 2H, NH$_2$); 7.68 (t, 1H, =NH–CH$_2$–CH$_2$–C$_6$H$_5$). ^{13}C NMR

(DMSO-d_6, ppm): δ = 23.23 (C4); 23.48 (C5); 34.49 (–CH_2–$\underline{C}H_2$–C_6H_5); 42.31 (–$\underline{C}H_2$–CH_2–C_6H_5); 45.30 (C3); 46.21 (C6); 126.32; 128.27; 128.83; 138.32; 155.02 (–$\underline{C}(NH_2)$=$NH^{\oplus}$–CH_2–CH_2–C_6H_5). MS (70 eV, 348 °C): *m*/*z* (%):232 (30) [$M^{+\cdot}$ base], 204 (26), 197 (12), 195 (12), 193 (22), 189 (13), 183 (22), 182 (12), 181 (22), 172 (15), 157 (15), 155 (15), 146 (12), 141 (31), 139 (17), 128 (39), 127 (21), 120 (41), 119 (100) [C_6H_5–CH_2–CH=NH^{+}·], 105 (40), 104 (34), 103 (23), 102 (49), 101 (13), 92 (54), 91 (27), 86 (82) [$C_4H_{10}N_2^{+}$·,$M^{+\cdot}$ base — HN=C=N–CH_2–CH_2–C_6H_5], 85 (19), 79 (12), 78 (11), 77 (24), 76 (28), 75 (24), 66 (16), 58 (11), 57 (25), 56 (11), 52 (10), 51 (17), 50 (15), 43 (12), 41 (15), 39 (12), 30 (23), 28 (25). Anal. [$C_{13}H_{21}IN_4$ (360.2)] C, H, N.

4.1.7.4. N^2-(4-Methoxyphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4k)

From **3k**. Yield 65%. Colourless crystals, mp 138–141 °C (CH_2Cl_2/$(C_2H_5)_2O$). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1512; 1594; 1634 (C=N); 2845; 2938; 3105; 3196; 3280; 3426; 3449. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.65 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.88 (bs, 2H, –C(3)H_2–); 3.67 (bs, 2H, –C(6)H_2–); 3.78 (s, 3H,-OCH_3); 5.33 (t, 1H, NH); 7.01 (d, 2H, arom. H); 7.17 (d, 2H, arom. H); 7.40 (bs, 2H, NH_2); 9.36 (bs, 1H, =N$\underline{H}^{\oplus}$–C_6H_4–OCH_3). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.24 (C4); 23.41 (C5); 45.60 (C3); 46.41 (C6); 55.30 (–OCH_3); 114.61 (2 C), 127.72; 127.85; 155.06; 158.05 (–$\underline{C}(NH_2)$=$NH^{\oplus}$–). MS (70 eV, 348 °C): *m*/*z* (%): 234 (37) [$M^{+\cdot}$ base], 148 (25) [$M^{+\cdot}$ base — $C_4H_{10}N_2$], 147 (12), 133 (15), 128 (30), 127 (18), 123 (12), 92 (10), 86 (100) [$C_4H_{10}N_2^{+}$·, $M^{+\cdot}$ base — HN=C=N–C_6H_4–OCH_3], 85 (14), 77 (12), 57 (26), 30 (16), 28 (11). Anal. [$C_{12}H_{19}IN_4O$ (362.2)] C, H, N.

Method B — from different 3 and aqueous solution of NH_3

To a solution of 1.5 mmol of the related 1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide **3** in 5 mL of CH_3OH were added 2 mL of a 25 percentage aqueous solution of NH_3. Then the covered batch (pressure compensation!) was allowed to stand at room temperarature for 2 d. After the solvent was evaporated carefully, the dry product yielded was stored in a refrigerator to allow it to crystallize; the crystals were suspended in a small amount of 2-C_3H_7OH, then they were separated, recrystallized from 2-C_3H_7OH, sucked off, washed and dried in vacuum. In this manner were obtained:

4.1.7.5. N^2-Ethyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4c)

From **3c**. Yield 50%. Colourless crystals, mp 123–126 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1496; 1615 and 1641 (NH, C=N, maxima of an intensive band); 2922; 2946; 2979; 3175, 3204 and 3293 (maxima of a broad band). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.08 (t, 3H, –CH_2–$C\underline{H}_3$), 1.60 (m, 4H, –C(4)H_2– and –C(5)H_2–); 2.79 (m, 2H, –(3)H_2–); 3.20 (dq, 2H, –NH–$C\underline{H}_2$–CH_3); 3.54 (m, 2H, –C(6)H_2–); 5.15 (t, 1H, NH); 7.52 (s, 2H, NH_2); 7.68 (t, 1H, =N$\underline{H}^{\oplus}$–CH_2–CH_3). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.43 (–CH_2–$\underline{C}H_3$); 23.27 (C4); 23.55 (C5); 35.95 (–$\underline{C}H_2$–CH_3); 45.25 (C3); 46.27 (C6); 155.05 (–$\underline{C}(NH_2)$=$NH^{\oplus}$–CH_2–CH_3). MS (70 eV, 100 °C): *m*/*z* (%): 156 (30) [$M^{+\cdot}_{base}$], 128 (92), 127 (44), 86 (100) [$C_4H_{10}N_2^{+}$·, $M^{+\cdot}_{base}$ — HN=C=N–C_2H_5], 85 (19), 72 (12), 57 (54), 56 (12), 44 (23), 43 (47), 41 (13), 32 (20), 30 (33), 29 (14), 28 (100). Anal. [$C_7H_{17}IN_4$ (284.1)] C, H, N.

4.1.7.6. N^1-Isopropyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4f)

From **3f**. Yield 62%. Colourless crystals, mp 134–136.5 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1559; 1619; 1638 (C=N); 2856; 2928; 2940; 2974; 3182, 3193; 3297. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.16 (d, 6H, –CH($C\underline{H}_3$)$_2$); 1.60 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.79 (m, 2H, –C(3)H_2–); 3.54 (m, 2H, –C(6)H_2–); 3.77 (m, 1H, NH–C$\underline{H}$($C\underline{H}_3$)$_2$); 5.14 (t, 1H, NH); 7.28 (d, 1H, N$\underline{H}$–$CH(CH_3)_2$); 7.56 (s, 2H, =N$\underline{H}_2^{\oplus}$). ^{13}C NMR (DMSO-d_6, ppm): δ = 22.14 (–CH($\underline{C}H_3$)$_2$); 23.22 (C4); 23.51 (C5); 43.61 (–$\underline{C}$H(CH_3)$_2$); 45.23 (C3); 46.25 (C6); 154.32 (–$\underline{C}(NH_2^{\oplus})$–NH–$CH(CH_3)_2$). MS (70 eV, 348 °C): *m*/*z* (%): 170 (40) [$M^{+\cdot}_{base}$], 86 (100) [$C_4H_{10}N_2^{+}$·, $M^{+\cdot}_{base}$ — HN=C=N–$CH(CH_3)_2$], 85 (32), 57 (60), 56 (15), 44 (29), 43 (25), 41 (19), 32 (11), 30 (25), 28 (58). Anal. [$C_8H_{19}IN_4$ (298.2)] C, H, N.

4.1.7.7. N^2-(2-Phenylethyl)-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4h)

From **3h**. Yield 30%. For analytical data see 4.1.7.3.

4.1.7.8. N^2-Phenyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4i)

From **3i**. Yield 30%. Yellowish crystals, mp 153.5–156 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1497; 1520; 1604; 1634; 1642 (C=N); 2940; 3036; 3083, 3148; 3179; 3250; 3370. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.64 (m, 2H, –C(4)H_2–); 1.71 (m, 2H, –C(5)H_2–); 2.91 (m, 2H, –C(3)H_2–); 3.66 (m, 2H, –C(6)H_2–); 5.34 (t, 1H, NH); 7.24 (m, 2H, arom. H); 7.33 (m, 1H, arom. H); 7.46 (m, 2H, arom. H); 7.63 (bs, 2H, NH_2); 9.51 (s, 1H, =N$\underline{H}^{\oplus}$–C_6H_5). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.27 (C4); 23.42 (C5); 45.74 (C3); 46.49 (C6); 125.29 (broad signal, phenyl, C3′, C5′); 126.49 (phenyl, C4′); 129.43 (phenyl, C2′, C6′); 135.64 (phenyl, C1′); 154.64 (–$\underline{C}(NH_2)$=$NH^{\oplus}$–C_6H_5). MS (70 eV, 345 °C): *m*/*z* (%): 204 (56) [$M^{+\cdot}_{base}$], 128 (64), 127 (29), 119 (38) [H_2N–C=N–$C_6H_5^{+}$], 93 (14), 86 (100) [$C_4H_{10}N_2^{+}$·, $M^{+\cdot}_{base}$ — HN=C=N–C_6H_5], 85 (22), 71 (11), 77 (54), 58 (48), 56 (13), 51 (18), 43 (10), 41 (13), 30 (25), 28 (17). Anal. [$C_{11}H_{17}IN_4$ (332.2)] C, H, N.

4.1.7.9. N^2-(2-Methoxyphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4j)

From **3j**. Yield 35%. Yellowish crystals, mp 135–137 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1502; 1578; 1599; 1640 (C=N); 2916; 2884; 2946; 3172; 3305; 3437; 3538. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.65 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.87 (m, 2H, –C(3)H_2–); 3.65 (m, 2H, –C(6)H_2–); 3.81 (s, 3H, –OCH_3); 5.37 (t, 1H, NH); 7.01 (m, 1H, arom. H); 7.22 (m, 2H, arom. H); 7.35 (s, 2H, NH_2); 7.37 (m, 1H, arom. H); 9.11 (s, 1H, =N$\underline{H}^{\oplus}$–C_6H_4–OCH_3). ^{13}C NMR (+ DEPT 135) (DMSO-d_6, ppm): δ = 23.28 (C4); 23.43 (C5); 45.63 (C3); 46.40 (C6); 55.61 (–O–$\underline{C}H_3$); 112.50; 120.68; 123.15; 128.70; 129.16; 154.49; 155.01 ($\underline{C}(NH_2)$=$NH^{\oplus}$-). MS (70 eV, 40 °C): *m*/*z* (%): 234 (50) [$M^{+\cdot}_{base}$], 148 (14), 142 (11), 134 (27), 128 (65), 127 (35), 123 (13), 120 (13), 105 (10), 92 (15), 87

(14), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M_{base}^{+\cdot}$ — HN=C=N–C_6H_4–OCH_3], 85 (30), 77 (15), 65 (11), 58 (68), 57 (18), 56 (19), 43 (14), 41 (14), 30 (34), 28 (19). Anal. [$C_{12}H_{19}IN_4O$ (362.2)] C, H, N.

Method C — from 3a and different amines

To a solution of 1 mmol of **3a** in 4 mL of CH_2Cl_2 was added 1 mmol of the related amine, and the covered batch (pressure compensation!) was allowed to stand at room temperature for 5 d. Then the residue obtained by evaporation of the solvent was dried at 110 °C for 1 h. After cooling down to room temperature the non-crystalline product was covered with a small amount of $(C_2H_5)_2O$, and it was allowed to stand until the crystallization was completed. The formed crystals were suspended in $(C_2H_5)_2O$, collected by aspiration, washed with $(C_2H_5)_2O$/2-C_3H_7OH (3:1; V:V), and dried in vacuum. In this manner were obtained:

4.1.7.10. N^2-Propyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4d). With n-C_3H_7–NH_2. Yield 65%. Colourless crystals, mp 83–90 °C [$(C_2H_5)_2O$]. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1490; 1558; 1617 (C=N); 2869; 2916; 2955; 3159, 3190; 3310; 3325. ^1H NMR (DMSO-d_6, ppm): δ = 0.85 (t, 3H, –CH_2–CH_2–$C\underline{H}_3$), 1.50 (tq, 2H, –CH_2–$C\underline{H}_2$–CH_3); 1.60 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.79 (m, 2H, –C(3)H_2–); 3.13 (dt, 2H, –NH–$C\underline{H}_2$–CH_2–CH_3); 3.54 (m, 2H, –C(6)H_2–); 5.16 (t, 1H, NH); 7.52 (s, 2H, NH_2); 7.67 (t, 1H, =N$\underline{H}^{\oplus}$–CH_2–$C\underline{H}_2$–CH_3). ^{13}C NMR (DMSO-d_6, ppm): δ = 10.79 (–CH_2–CH_2–$\underline{C}H_3$); 21.86 (–CH_2–$\underline{C}H_2$–CH_3); 23.19 (C4); 23.52 (C5); 42.50 (–$\underline{C}H_2$–CH_2–CH_3); 45.24 (C3); 46.22 (C6); 155.12 (–$\underline{C}(NH_2)$=$NH^{\oplus}$–CH_2–CH_2–CH_3). MS (70 eV, 347 °C): *m*/*z* (%): 170 (26) [$M_{base}^{+\cdot}$], 128 (28), 127 (14), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M_{base}^{+\cdot}$ — HN=C=N–C_3H_7], 85 (25), 58 (12), 57 (49), 56 (13), 55 (12), 43 (50), 41 (21), 30 (22), 28 (29), 27 (11). Anal. [$C_8H_{19}IN_4$ (298.2)] C, H, N.

4.1.7.11. N^2-Allyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4e). With CH_2=CH–CH_2–NH_2. Yield 60%. Colourless crystals, mp 88–96 °C [$(C_2H_5)_2O$]. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1565; 1614; 1641 (C=N); 2854; 2919; 2945; 3153, 3211; 3298. ^1H NMR (DMSO-d_6, ppm): δ = 1.62 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.80 (m, 2H, –C(3)H_2–); 3.56 (m, 2H, –C(6)H_2–); 3.84 (m, 2H, =N$H^{\oplus}$–$C\underline{H}_2$–CH=CH_2); 5.12 (bs, 1H, NH); 5.20 (m, 2H, –CH_2–CH=$C\underline{H}_2$); 5.82 (m, 1H, –CH_2–$C\underline{H}$=CH_2); 7.52 (s, 2H, NH_2); 7.92 (t, 2H, =N$\underline{H}^{\oplus}$–CH_2–CH=CH_2). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.23 (C4); 23.45 (C5); 42.72 (–$\underline{C}H_2$–CH=CH_2); 45.33 (C3); 46.29 (C6); 115.53 (–CH_2–CH=$\underline{C}H_2$); 133.62 (–CH_2=$\underline{C}H$=CH_2); 155.17 (–$\underline{C}(NH_2)$=NH–CH_2–CH=CH_2). MS (70 eV, 348 °C): *m*/*z* (%): 168 (26) [$M_{base}^{+\cdot}$], 128 (80), 127 (40), 99 (20), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M_{base}^{+\cdot}$ — HN=C=N–CH_2–CH=CH_2], 85 (27), 84 (14), 83 (18), 71 (16), 57 (50), 56 (31), 55 (12), 43 (21), 41 (48), 39 (18), 30 (26), 28 (24). Anal. [$C_8H_{17}IN_4$ (296.2)] C, H, N.

4.1.7.12. N^2-Butyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4g). With n-C_4H_9–NH_2. Yield 80%. Colourless crystals, mp 100–105 °C [$(C_2H_5)_2O$]. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1560; 1619; 1639 (C=N); 2855; 2943; 3162, 3218; 3270; 3298; 3357. ^1H NMR (DMSO-d_6, ppm): δ = 0.88 (t, 3H, –$C\underline{H}_3$); 1.27 (tq, 2H, –CH_2–); 1.44 (tt, 2H, –CH_2–); 1.60 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.77 (m, 2H, –C(3)H_2–); 3.16 (dt, 2H, =N$H^{\oplus}$–$C\underline{H}_2$–); 3.54 (m, 2H, –C(6)H_2–); 5.15 (t, 1H, NH); 7.52 (s, 2H, NH_2); 7.64 (t, 2H, =N$\underline{H}^{\oplus}$–CH_2–). ^{13}C NMR (DMSO-d_6, ppm): δ = 13.55 (–CH_3); 19.15 (–CH_2–); 23.23 (C4); 23.53 (C5); 30.63 (–CH_2–); 40.75 (=N$H^{\oplus}$–$\underline{C}H_2$–); 45.26 (C3); 46.25 (C6); 155.13 (–$\underline{C}(NH_2)$=N$H^{\oplus}$–CH_2–CH_2–CH_2–CH_3). MS (70 eV, 348 °C): *m*/*z* (%): 184 (30) [$M^{+\cdot}{}_{base}$], 159 (17), 145 (61), 142 (94) [$M_{base}^{+\cdot}$ — H_2C=CH–CH_3], 141 (16), 139 (14), 128 (19), 127 (31), 98 (17), 97 (10), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M_{base}^{+\cdot}$ — HN=C=N–C_4H_9], 85 (20), 84 (10), 71 (11), 57 (50), 56 (19), 55 (12), 43 (35), 42 (12), 41 (24), 30 (34), 29 (16), 28 (33), 27 (13). Anal. [$C_9H_{21}IN_4$ (312.2)] C, H, N.

Method D—from different 3 with aqueous solution of CH_3NH_2

To a solution of 2 mmol of the related 1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide **3** in 5 mL of CH_3OH were added 2 mL of a 40percentage aqueous solution of CH_3NH_2 and the covered batch (pressure compensation!) was allowed to stand at room temperature for 1 d. The solvent was evaporated with slightly warming to dryness and the obtained residue was stored in the refrigerator until the crystallization started. The formed crystals were suspended in a small amount of 2-C_3H_7OH, then seperated, recrystallized from 2-C_3H_7OH, washed, and dried in vacuum. In this manner were obtained:

4.1.7.13. N^1,N^2-Dimethyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4l). From **3b**. Yield 45%. Colourless crystals, mp 118–119 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1495; 1615; 1625 (C=N); 2850; 2939; 2974; 3048; 3083; 3169; 3252. ^1H NMR (DMSO-d_6, ppm): δ = 1.57 (m, 2H, –C(4)H_2–); 1.67 (m, 2H, –C(5)H_2–); 2.83 [bd, 3H (CH_3) and 2H (–C(3)H_2–)]; 3.45 (m, 2H, –C(6)H_2–); 5.08 (t, 1H, NH); 7.48 (s, 2H, =N$\underline{H}^{\oplus}$–CH_3). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.48 and 23.57 (C4 and C5); 30.31 (–$\underline{C}H_3$); 46.51 (C3); 47.11 (C6); 158.92 ($\underline{C}$(NH–CH_3) = $NH^{\oplus}$–). MS (70 eV, 170 °C): *m*/*z* (%): 202 (45), 201 (65), 177 (24), 162 (13), 156 (11) [$M_{base}^{+\cdot}$], 128 (24), 127 (11), 124 (20), 122 (17), 105 (97), 86 (41) [$C_4H_{10}N_2^{+\cdot}$, $M_{base}^{+\cdot}$ — CH_3–N=C=N–CH_3], 78 (19), 77 (59), 72 (10), 57 (27), 51 (28), 47 (13), 43 (17), 41 (10), 32 (26), 30 (12), 28 (100). Anal. [$C_7H_{17}IN_4$ (284.1)] C, H, N.

4.1.7.14. N^1-Ethyl-N^2-methyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4m). From **3c**. Yield 55%. Colourless crystals, mp 98–100.5 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1605 (C=N); 2869; 2939; 3076, 3179 and 3248 (maxima of a broad band). ^1H NMR (DMSO-d_6, ppm): δ = 1.14 (t, 3H, –CH_2–$C\underline{H}_3$); 1.58 (m, 2H, –C(4)H_2–); 1.68 (m, 2H, –C(5)H_2–); 2.84 [bd, 3H (=N$H^{\oplus}$–$C\underline{H}_3$) and 2H (–C(3)H_2–)]; 3.24 (dq, 2H, –NH–$C\underline{H}_2$–CH_3); 3.47 (m, 2H, –C(6)H_2–); 5.12 (t, 1H, NH); 7.34 (t, 1H,

–NH–CH_2–CH_3); 7.53 (q, 1H, $CH_3NH^{\oplus}$=). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.92 (–CH_2–CH_3); 23.56 (C4 and C5); 30.50 (–CH_2–CH_3 and =$NH^{\oplus}$–CH_3); 46.57 (C3); 47.35 (C6); 158.33 (C(NH–CH_3)=$NH^{\oplus}$–). MS (70 eV, 300 °C): *m/z* (%): 170 (9) [$M^{+\cdot}_{base}$], 128 (44), 127 (19), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_2H_5], 85 (34), 78 (19), 57 (64), 30 (20), 28 (39). Anal. [$C_8H_{19}IN_4$ (298.2)] C, H, N.

4.1.7.15. N^2-Methyl-N^1-propyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4n). From **3d**, which resulted in form of a non-crystalline semisolid mass from the reaction of **2d** and CH_3I according the general procedure for the preparation of the 1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl esters **3**. Yield 85%. Colourless crystals, mp 94.5–96.5 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$? = 1498, 1609 (C=N), 2871, 2920, 2942, 2965, 3096. 3157, 3276. ^{1}H NMR (DMSO-d_6, ppm): δ = 0.87 (t, 3H, –CH_2–CH_2–CH_3), 1.55 [m, 2H (–CH_2–CH_2–CH_3) and 2H (–C(4)H_2–)]; 1.67 (m, 2H, –C(5)H_2–); 2.83 [bd, 3H (=$NH^{\oplus}$–CH_3) and 2H (–C(3)H_2–)]; 3.15 (dt, 2H, –NH–CH_2–CH_2-CH_3); 3.45 (m, 2H, –C(6)H_2–); 5.12 (t, 1H, NH); 7.33 (t, 1H, –NH–CH_2–CH_2–CH_3); 7.52 (q, 1H, =$NH^{\oplus}$–CH_3). ^{13}C NMR (+ DEPT 135) (DMSO-d_6, ppm): δ = 10.92 (–CH_2–CH_2–CH_3); 22.35 (–CH_2–CH_2–CH_3); 23.57 (C4, C5); 30.54 (=$NH^{\oplus}$–CH_3); 45.18 (–NH–CH_2–CH_2–CH_3); 46.57 (C3); 47.35 (C6); 158.48 (C(NH-CH_3)=$NH^{\oplus}$–). MS (70 eV, 350 °C): *m/z* (%): 184 (9) [$M^{+\cdot}_{base}$], 128 (42), 127 (23), 99 (12), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_3H_7], 85 (15), 70 (16), 57 (77), 56 (10), 43 (12), 41(16), 30 (25), 28 (19), 27 (10). Anal. [$C_9H_{21}IN_4$ (312.2)] C, H, N.

4.1.7.16. N^2-Methyl-N^1-(2-phenylethyl)-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4p). From **3h**. Yield 65%. Colourless crystals, mp 108–109 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1589; 1631 (C=N); 2852; 2918; 2935; 2950; 3001; 3168; 3260. ^{1}H NMR ($CDCl_3$, ppm): δ = 1.71 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.87 (bs, 2H, –C(3)H_2–); 2.93 (d, 3H, (=$NH^{\oplus}$–CH_3); 3.02 (t, 2H, –NH–CH_2–CH_2–C_6H_5); 3.69 (dt, 2H, –NH–CH_2–CH_2–C_6H_5); 3.79 (bs, 2H, –C(6)H_2–); 4.47 (m, 1H, NH); 6.77 (t, 1H, –NH–CH_2–CH_2–C_6H_5); 6.99 (q, 1H, =$NH^{\oplus}$–CH_3); 7.29 (m, 5H, arom. H). ^{13}C NMR (+ DEPT 135) (DMSO-d_6, ppm): δ = 23.48 (C4); 23.51 (C5); 30.28 (=$NH^{\oplus}$–CH_3); 34.96 (–CH_2–CH_2–C_6H_5); 44.81 (–CH_2–CH_2–C_6H_5); 46.54 (C3); 47.26 (C6); 126.34; 128.30 (2 C); 128.72 (2 C); 138.25; 158.31 (–C(NH–CH_3)=NH–). MS (70 eV, 370 °C): *m/z* (%): 246 (8) [$M^{+\cdot}_{base}$], 128 (35), 127 (19), 105 (31), 91 (13), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3-N=C=N–C_2H_4–C_6H_5], 85 (11), 70 (57), 57 (26), 30 (36), 28 (19). Anal. [$C_{14}H_{23}IN_4$ (374.3)] C, H, N.

4.1.7.17. N^1-Methyl-N^2-phenyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4q). From **3j**. Yield 55%. Yellowish crystals, mp 172–177 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1496; 1562; 1592; 1631 (C=N); 2854; 2938; 2979; 3038; 3155, 3196. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.60 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.69 (d, 3H, –CH_3); 2.92 (m, 2H, –C(3)H_2–); 3.40 (m, 2H, –C(6)H_2–); 5.37 (t, 1H, NH); 7.08 (m, 2H, arom. H); 7.18 (m, 1H, arom. H); 7.41 (m, 2H, arom. H); 8.39 (q, 1H, –NH–CH_3); 9.50 (s, 1H, =$NH^{\oplus}$–C_6H_5). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.39 (C4); 23.41 (C5); 30.24 (–NH–CH_3); 46.55 (C3); 47.94 (C6); 120.50 (phenyl, C3′, C5′); 124.22 (phenyl, C4′); 129.43 (phenyl, C2′, C6′); 138.08 (phenyl, C1′); 155.29 (–C(NHCH$_3$)=NH–C_6H_5). MS (70 eV, 350 °C): *m/z* (%): 218 (41) [$M^{+\cdot}_{base}$], 133 (69) [$M^{+\cdot}_{base}$ — $C_4H_9N_2$], 132 (14), 128 (57), 127 (31), 118 (31), 92 (10), 87 (10), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_6H_5], 85 (17), 77 (39), 58 (39), 51 (12), 41 (11), 30 (18), 28 (15). Anal. [$C_{12}H_{19}IN_4$ (346.2)] C, H, N.

4.1.7.18. N^1-Methyl-N^2-(4-methylphenyl)-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4r). From **3l**. Yield 30%. Colourless crystals, mp 177–178 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1512; 1562; 1624; 1637 (C=N); 2856; 2936; 3152; 3209; 3305. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.59 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.28 (s, 3H, –C_6H_4–CH_3); 2.66 (d, 3H, –NH–CH_3); 2.88 (bs, 2H, –C(3)H_2–); 3.35 (bs, 2H, –C(6)H_2–); 5.32 (t, 1H, NH); 6.97 (d, 2H, arom. H); 8.29 (q, 1H, –NH–CH_3); 9.38 (s, 1H, =$NH^{\oplus}$–C_6H_4–CH_3). ^{13}C NMR (+ DEPT 135) (DMSO-d_6, ppm): δ = 20.29 (Ar–CH_3); 23.40 (C4); 23.47 (C5); 30.19 (–NH–CH_3); 46.57 (C3); 47.88 (C6); 120.88 (2 C); 129.83 (2 C); 133.62; 135.40; 155.42 (–C(NHCH$_3$)=NH–). MS (70 eV, 270 °C): *m/z* (%): 232 (17) [$M^{+\cdot}$ base], 147 (26) [$M^{+\cdot}_{base}$ — $C_4H_9N_2$], 132 (11), 128 (26), 127 (12), 91 (14), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_6H_4–CH_3], 85 (10), 84 (10), 57 (22), 30 (13), 28 (18). Anal. [$C_{13}H_{21}IN_4$ (360.2)] C, H, N.

4.1.7.19. N^2-(2-Methoxyphenyl)-N^1-methyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4s). From **3m**. Yield 35%. Colourless crystals, mp 130–134 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1504; 1577, 1601; 1629; 2834; 2850; 2918; 2937; 3040; 3126; 3213. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.59 (d, 3H, –NH–CH_3); 2.83 (bs, 2H, –C(3)H_2–); 3.38 (bs, 2H, –C(6)H_2–); 3.84 (s, 3H, –OCH_3); 5.31 (t, 1H, NH); 6.97 (d, 2H, arom. H); 7.07 (d, 2H, arom. H); 8.17 (q, 1H, –NH–CH_3); 9.31 (s, 1H, =$NH^{\oplus}$–C_6H_4–OCH_3). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.17 (C4); 23.42 (C5); 29.79 (–NH–CH_3); 46.57 (C3); 47.59 (C6); 55.80 (–OCH_3); 112.13; 120.92; 124.87; 126.25 (tert. arom. C); 127.40; 152.20 (tert. arom. C); 156.46 (–C(NHCH$_3$)=$NH^{\oplus}$–). MS (70 eV, 350 °C): *m/z* (%): 248 (30) [$M^{+\cdot}_{base}$], 203 (49) [$M^{+\cdot}_{base}$ — CH_3–NH–CH_3], 202 (31), 163 (27) [$M^{+\cdot}_{base}$ — $C_4H_9N_2$], 162 (14), 148 (31), 147 (62), 146 (12), 142 (26), 134 (17), 133 (25), 128 (87), 127 (58), 112 (15), 120 (22), 119 (24), 105 (17), 100 (15), 92 (18), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_6H_4–OCH_3], 85 (16), 84 (10), 78 (16), 77 (14), 71 (20), 65 (12), 58 (28), 56 (12), 51 (12), 44 (11), 42 (17), 41 (14), 30 (27), 28 (22). Anal. [$C_{13}H_{21}IN_4O$ (376.2)] C, H, N.

4.1.7.20. N^2-(4-Methoxyphenyl)-N^1-methyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4t). From **3n**. Yield 55%. Colourless crystals, mp 129–131.5 °C (2-C_3H_7OH). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1514; 1574; 1612; 1643; 2862; 2941; 3012; 3044; 3167; 3262; 3321. ^{1}H

NMR (DMSO-d_6, ppm): δ = 1.60 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.64 (d, 3H, –NH–CH_3); 2.89 (bs, 2H, –C(3)H_2–); 3.42 (bs, 2H, –C(6)H_2–); 3.78 (s, 3H, –OCH_3); 5.30 (t, 1H, NH); 7.14 (m, 4H, arom. H); 8.16 (q, 1H, –NH–CH_3); 9.05 (s, 1H, =N$H^{\oplus}$–C_6H_4–OCH_3). ^{13}C NMR (+ DEPT 135; $CDCl_3$, ppm): δ = 23.90 (C4); 24.01 (C5); 31.34 (–NH–CH_3); 47.57 (C3); 49.34 (C6); 55.54 (–O–CH_3); 114.81 (2 C); 124.95 (2 C); 129.07; 156.33; 157.92 (–C(NHCH_3)=N$H^{\oplus}$–). MS (70 eV, 350 °C): *m*/*z* (%): 248 (21) [$M^{+\cdot}_{base}$], 163 (28) [$M^{+\cdot}_{base}$ — $C_4H_9N_2$], 162 (15), 147 (15), 146 (15), 133 (11), 128 (21), 127 (12), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_6H_4–OCH_3], 57 (20), 28 (42). Anal. [$C_{13}H_{21}IN_4O$ (376.2)] C, H, N.

Method E — from different 3 and different amines

To a solution of 1 mmol of the related 1,2,3,4,5,6-hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide **3** in 3 mL of CH_2Cl_2 was added 1 mmol of the appropriate amine and the covered batch (pressure compensation!) was allowed to stand at room temperature. After the reaction was completed, the solvent was removed with slightly warming to dryness and the residue obtained was stored in the refrigerator until the crystallization started. The formed crystals were pressed off on an earthenplate, then they were suspended in a small amount of $(C_2H_5)_2O$/2-C_3H_7OH (9:1; V/V), sucked off, washed with some $(C_2H_5)_2O$/2-C_3H_7OH (9:1; V/V), and dried in vacuum. In this manner were obtained:

4.1.7.21. N^2-Methyl-N^1-butyl-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4o). From **3b** and *n*-$C_4H_9NH_2$. Reaction time 1 day. Yield 85%. Colourless crystals, mp 47.5–53 °C. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1612, 1626 (maxima of an intensive band); 2857; 2873; 2933; 2952; 3107; 3170; 3285. ^{1}H NMR (DMSO-d_6, ppm): δ = 0.89 (t, 3H, –CH_3); 1.29 (tq, 2H, –CH_2–); 1.53 (tt, 2H, –CH_2–); 1.67 (bs, 4H, –C(4)H_2– and –C(5)H_2–); 2.83 [bd, 3H + 2H, =N$H^{\oplus}$–CH_3 (*J* = 4.2 Hz) + –C(3)H_2–]; 3.18 (dt, 2H, –NH–CH_2–); 3.46 (m, 2H, –C(6)H_2–); 5.09 (t, 1H, NH); 7.32 (t, 1H, NH–); 7.53 (q, 1H, =N$H^{\oplus}$–CH_3, *J* = 4.2 Hz). ^{13}C NMR (DEPT 135. DMSO-d_6, ppm): δ = 13.48 (–CH_3); 19.12 (–CH_2–); 23.55 and 23.53 (C4, C5); 30.47 (=N$H^{\oplus}$–CH_3); 31.04 (–CH_2–); 43.20 (–NH–CH_2–); 46.58 (C3); 47.35 (C6); 158.45 (C=N$H^{\oplus}$–). MS (70 eV, 200 °C): *m*/*z* (%): 198 (8) [$M^{+\cdot}_{base}$], 128 (26), 127 (12), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}_{base}$ — CH_3–N=C=N–C_4H_9], 85 (12), 57 (53), 41 (12), 30 (14), 29 (10), 28 (16). Anal. [$C_{10}H_{23}IN_4$ (326.2)] C, H, N.

4.1.7.22. N^1,N^1-Tetramethylene-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4u). From **3a** and pyrrolidine. Reaction time 3 days. Yield 40%. Colourless crystals, mp 90–94 °C. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1550; 1603; 1653 (C=N); 2852; 2870; 2906; 2947; 3168; 33308; 3364. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.55 (m, 2H, –C(4)H_2–); 1.69 (m, 2H, –C(5)H_2–); 1.85 [m, 4H, N(CH_2–CH_2–CH_2–CH_2)]; 2.82 (m, 2H, –C(3)H_2–); 3.43 [m, 2H (–C(6)H_2–) and 4H (N(CH_2–CH_2–CH_2–CH_2)]; 4.98 (t, 1H, NH); 7.57 (s, 2H, =N$H_2^{\oplus}$). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.60 (2 C, C4 and C5); 24.85 [2 C, N(CH_2–CH_2–CH_2–CH_2)]; 46.58 (C3); 47.40 (C6); 49.53 [2 C, N(CH_2–CH_2–CH_2–CH_2)]; 156.76 (C=$NH_2^{\oplus}$). MS (70 eV, 348 °C): *m*/*z* (%): 182 (44) [$M^{+\cdot}_{base}$], 128 (81), 127 (35), 112 (15), 97 (30), 86 (100) [$C_4H_{10}N_2^{+\cdot}$, $M^{+\cdot}$ — C_4H_8N–CN], 85 (27), 84 (10), 71 (75) [$C_4H_8N^{+\cdot}$, $M^{+\cdot}$ — $C_4H_9N_2$–CN], 57 (50), 56 (14), 55 (55), 43 (27), 42 (19), 41 (25), 30 (27), 28 (50), 27 (11). Anal. [$C_9H_{19}IN_4$ (310.2)] C, H, N.

4.1.7.23. N^2-Methyl-N^1,N^1-tetramethylene-1,2,3,4,5,6-hexahydropyridazine-1-carboximidamide hydroiodide (4v). From **3b** and pyrrolidine. Reaction time 5 days. Yield 50%. Colourless crystals, mp 165–171 °C. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1595 (C=N); 2852; 2929; 2970; 3056; 3151; 3226. ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56 (m, 2H, –C(4)H_2–); 1.72 (m, 2H, –C(5)H_2–); 1.86 [m, 4H, N(CH_2–CH_2–CH_2–CH_2)]; 2.83 [bd, 3H (=N$H^{\oplus}$–CH_3) and 2H (–C(3)H_2–)]; 3.45 [m, 2H (–C(6)H_2–) and 4H (N(CH_2–CH_2–CH_2–CH_2)]; 5.10 (t, 1H, NH); 7.38 (q, 1H, =N$H^{\oplus}$–CH_3). ^{13}C NMR (DMSO-d_6, ppm): δ = 23.56 (C4); 23.74 (C5); 24.74 [2 C, N(CH_2–CH_2–CH_2–CH_2)]; 31.13 (=N$H^{\oplus}$–CH_3); 46.39 (C3); 48.39 (C6); 48.96 [2 C, N(CH_2–CH_2–CH_2–CH_2)]; 157.42 (C=N$H^{\oplus}$–CH_3). MS (70 eV, 348 °C): *m*/*z* (%): 196 (10) [$M^{+\cdot}_{base}$], 128 (39), 127 (28), 111 (11), 110 (100) [$C_6H_{10}N_2^{+\cdot}$, $M^{+\cdot}$—$C_4H_{10}N_2$], 71 (13), 70 (38) [$C_4H_8N^+$], 57 (11), 55 (13), 42 (12), 41 (13), 30 (13), 28 (15). Anal. [$C_{10}H_{21}IN_4$ (324.2)] C, H, N.

4.1.8. 1-(4-Nitrobenzoyl)-1,3-diphenylthiourea (6). 0.01 mol of 1,3-diphenylthiourea was solved in 30 mL of CH_2Cl_2 with slightly warming, and it was added a warm solution of 0.01 mol of 4-nitrobenzoylchloride in 30 mL of CH_2Cl_2. The batch was allowed to stand at room temperature for 12 h, and an intensiv evolution of HCl occured. The solution was filtered and than at room temperature concentrated in vacuum. The obtained non-crystalline yellow coloured residue was grinded with n-hexane until the crystallization started and then the crystals were digested for four times with each 10 mL of boiling *n*-hexane. The product was sucked off, washed with *n*-hexane and dried on an earthenplate. Yield 81%. Bright yellow crystals. To prevent a quick decomposition the substance has to store proper enclosed and at a cool place, mp 123–129 °C (n-hexane; decomposition and recrystallisation are to observe between 130 and 135 °C, and beginning at about 195 °C sublimation takes place; the sublimate representing 4-nitrobenzanilide melts at 218–219 °C; reference:[73] 216–217 °C). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1490; 1523; 1592 (NH); 1669 (CO); maxima of a broad band: 3028, 3119, 3226, 3321. ^{1}H NMR ($CDCl_3$, ppm): δ = 7.24–8.32 (m, 14H, arom. H); 12.50 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6, ppm): δ = 123.18; 124.58; 125.16; 127.07; 128.21; 128.98; 129.03; 129.52; 130.59; 138.10; 140.49; 141.93; 147.86; 171.51 (C=S); 182.32 (C=O). Anal. [$C_{20}H_{15}N_3O_3S$ (377.4)] C, H, N.

4.2. Biology

4.2.1. Cell line. The rat insulinoma cell line RIN-5AH,[74] an adherent β-cell line, was grown in plastic culture

flasks (Greiner, Frickenhausen, Germany). Cells were maintained in RPMI-1640 medium (Biochrom, Berlin, Germany) containing 2 mmol/l L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 5% heat-inactivated fetal calf serum at 37 °C in a 5% CO_2 atmosphere. For the experiment cells were trypsinized, washed twice with Hanks medium and counted. Viability of cells was determined by trypan blue exclusion test.

4.2.2. Induction and inhibition of NO-production. Experiments were performed with 1×10^5 viable RIN-5AH cells/well in 200 μL RPMI medium (ICN, Ohio, USA) supplemented with 2 mmol/l L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 5% heat-inactivated fetal calf serum incubated in 96 well tissue culture plates (Greiner, Frickenhausen, Germany). Before addition of cytokines and hexahydropyridazine derivatives cells were allowed to form an adherent layer for 2 h at 37 °C and 5% CO_2. NOS was induced by treating cells with 1 ng/mL IL1β plus 100 U/mL IFNγ for 48 h at 37 °C in a 5% CO_2 atmosphere. Aminoguanidine and hexahydropyridazine derivatives were prepared as a 200 mM solution in culture medium or 50% alcohol. Substances were added to the cells in different concentrations ranging from 5.0 mM via 2.5 mM, 1.0 mM, 0.5 mM, 0.1 mM to 0.05 mM. After culture viability of RIN cells was determined by trypan blue exclusion test.

4.2.3. Measurement of NO. Accumulated NO was determined by measuring nitrite, a stable NO oxidation product.[75] The Griess reaction was performed in a two step incubation. Firstly, 50 μL of the cell free culture supernatents were incubated in duplicates with 50 μL 0.1% N-1-naphthylethylenediamine dihydrochloride solution at room temperature. After 10 min 1% sulfanilamide in 5% phosphoric acid was added to the wells and incubated for further 10 min. The resulting colour reaction was measured by spectrophotometry at 540 nm. The nitrite concentrations were calculated using the nitrite standard curve.

4.2.4. Data analysis. The inhibitory efficacy of tested hexahydropyridazine derivatives was calculated either by the degree of inhibition (a) and if there was an inhibition greater than 80% by the IC_{50} values (b). IC_{50} values were obtained by fitted nonlinear regression analysis using a sigmoidal dose response, where y is the determined value, Max is the maximal NO release, Min is the minimum value, ×is the dose of compound, and n is the slop.

(a) degree of inhibition (%) = 100−(Min×100/Max)

(b) $y = \mathrm{Min} + (\mathrm{Max}-\mathrm{Min})/1 + 10^{(\log IC_{50}-x)n}$

Acknowledgements

The authors wish to thank the Deutsche Forschungsgemeinschaft for the financial support of this work. They also thank Ulrich Käppler, Silke Graß, and Franz Studener for their assistance and Patrick Bednarski for his support.

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