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Synthesis and structure elucidation of 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione, 3,3-disubstituted and 2-substituted derivatives and evaluation of their inhibitory activity against inducible nitric oxide synthase*

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Dedicated to Prof. Dr. Peter H. Richter, Bad Saarow-Pieskow, Germany, on the occasion of his 75th birthday.

**N,N-Coupled heterobicycles from cyclic hydrazine derivatives. Part 15. Part 14: Schulz et al. (2013)*

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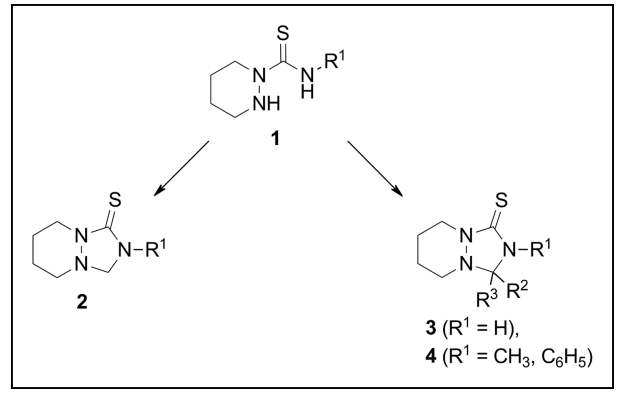
The 3-monosubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **3** (R^1, R^3 = H) were recently reported to possess inhibitory activity against inducible nitric oxide synthase in a cell based assay (Schulz et al. 2013). The 3,3-disubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **3** and **4** (R^2,$R^3 \neq$ H) were synthesized by cyclocondensation of the hexahydropyridazine-1-carbothioamides **1** with ketones. In order to access the 3,3-unsubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones, the unsubstituted parent system of these compounds, several synthetic routes were studied. By these methods the desired heterocyclic system **2a** as well as new *a*-anellated and *N*-substutited hexahydropyridazines were obtained. The biological evaluation of the title compounds confirmed the previously made finding that an aromatic moiety in position 3 of the substance is important for an inducible nitric oxide synthase (iNOS) inhibitory activity.

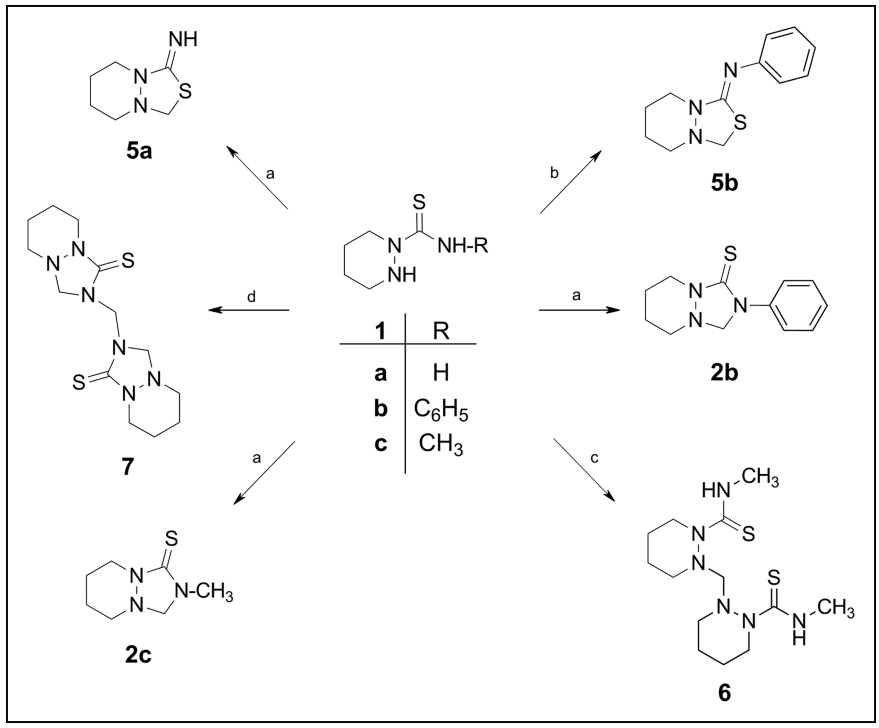
1. Introduction

Ever since the discovery of one of the smallest molecules in the human body, nitric oxide and its role to various diseases have been the focus of intense medicinal and pharmaceutical research. The inducible nitric oxide synthase (iNOS) is a mediator for many inflammatory processes, e.g. COPD or asthma. Although enzyme inhibition would appear to be an attractive approach to manage such diseases, a drug for this target is not yet available. Recently, we reported the synthesis of 3-monosubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones (compounds **3**, R^1, R^3 = H, Scheme 1) and their biological evaluation as inhibitors of inducible nitric oxide synthase in a cell based assay. In that work an overview of the current state of the relevant research is given (Schulz et al. 2013). A result of these investigations was that compounds with aryl substituents in position 3, and particularly those with cinnamyl radicals, were the most active. In continuation of these studies, further changes in the substitution pattern of the heterocyclic parent system (Scheme 1) on the inhibitory activity were investigated in an established biological test model. In this context the unsubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2–*a*]pyridazine-1-thione **2a** (R^1 = H, Scheme 1), representing the parent structure of compounds **3** and **4,** was of particular interest.

Moreover, in the present work the effects of the introduction of a second substituent in position 3, formally leading to the 3,3-disubstituted [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **3** (R^2, R^3 = alkyl, aryl), on the inhibitory activity was investigated. Additionally, for comparative chemical studies the syntheses of the 2-substituted [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **2** ($R^1 \neq$ H) and the **4,** starting from the *N*-substituted hexahydropyridazine-1-carbothioamides **1b** and **1c**, were investigated.



Scheme 1: General synthesis route for the 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **2** (R^1 = H, CH_3, C_6H_5, CO-C_6H_4-4-NO_2), **3** and **4** (for both of them R^2 = alkyl, aryl; R^3 = alkyl, aryl) starting from hexahydropyridazine-1-carbothioamides **1**.



Scheme 2: Results of cyclocondensation reactions of hexahydropyridazine-1-carbothioamides **1** with formaldehyde under different conditions.
(a) **5a** from **1a**, **2b** from **1b**, and **2c** from **1c**: CH_3OH, 30% aqueous HCHO solution, refluxing, 3 h
(b) **5b** from **1b**: 30% aqueous HCHO solution, room temperature, 2 days
(c) **6** from **1c**: method 1 - paraformaldehyde, molecular sieve, 140–150 °C, 15 min; method 2 - CH_3OH, 30% aqueous HCHO solution, refluxing, 6 xh, (d) **7** from **1a**: paraformaldehyde, molecular sieve, 140–150 °C, 25 min

2. Investigations, results and discussion

2.1. Synthesis

Analogously to the synthesis of the 3-monosubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2–*a*]pyridazine-1-thiones (Schulz et al. 2013), the title compounds **2**, **3** and **4** were obtained by reacting the hexahydropyridazine-1-carbothioamides **1** (Morgenstern et al. 2004) with carbonyl compounds (Scheme 1). In this context the results of the investigations to obtain compound **2a** ($R^1 = H$) are reported here as well.

2.1.1. 3-Unsubstituted compounds 2

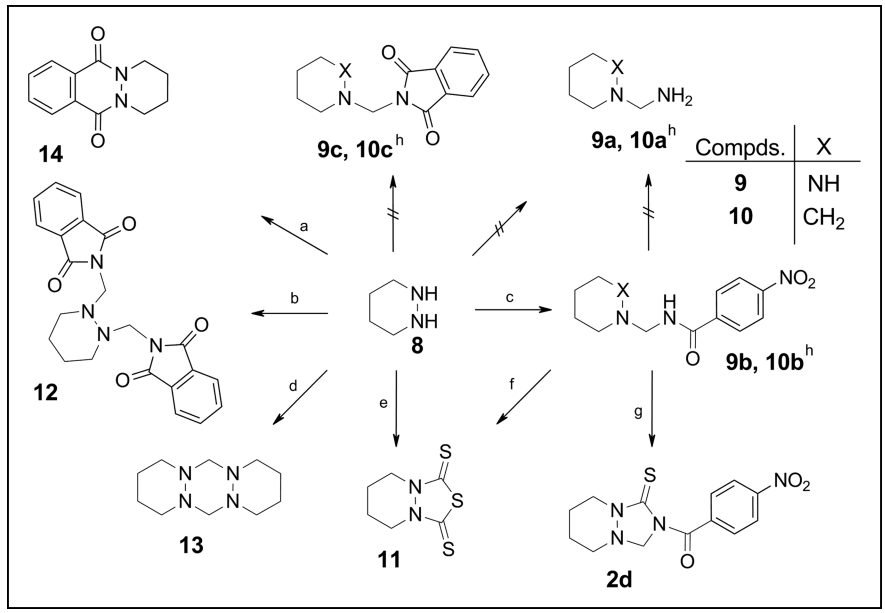
To synthesize **2a**, the hexahydropyridazine-1-carbothioamide **1a** ($R^1 = H$) (Morgenstern et al. 2004) was reacted with formaldehyde under different conditions. When heating **1a** in aqueous formaldehyde solution the reaction occurred rapidly and uniformly. However, the isomeric 1-imino[1,3,4]thiadiazolo[1,2–*c*]pyridazine **5a** (Scheme 2) was isolated exclusively.

The substituted carbothioamides **1b**,**c** (Morgenstern et al. 2004) actually yielded the desired [1,2,4]-triazolo[1,2–*a*]pyridazines **2b** and **2c** (Scheme 2) under the same conditions. However, the latter was obtained in considerably better yields. Applying milder conditions, **1b** reacted to the 1-phenylimino[1,3,4]thiadiazolo[1,2–*c*]pyridazine **5b** (isomer of **2b**). Heating the *N*-methylcarbothioamide **1c** in aqueous formaldehyde solution in the presence of methanol, compound **6** was formed from two molecules of the starting material. The suitability of paraformaldehyde as C1 reactant was alternatively tested. Unfortunately, the reaction of **1a** and paraformaldehyde in a melt did also not lead to **2a**. Although the ring closure gave the desired 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2–*a*]pyridazine system, the reaction proceeded most likely in a second step to **7**, where two molecules **2a** are linked by a methylene group (Scheme 2). The reaction of paraformaldehyde with **1c** also resulted in the formation of **6** in a slightly better yield as already observed utilizing aqueous formaldehyde. For **6** a methylene brigded structure was assigned.

An attempt to generate **2a** *via* cyclization of the piperidazin-1-ylmethane amine derivative **9a** (derived from **9b** by hydrolysis) or by elimination of the nitrobenzoyl residue from compound **2d** (Scheme 3) also failed. Although the piperidin-1-ylmethane amine **10a** has already been reported (Takeuchi et al. 1986, Takeuchi et al. 1988), the aza analogue **9a** is not yet known. The same applies for **10b** (Foldeak et al. 1962) and **9b**. In contrast to **9a**, compound **9b** could be directly synthesized from **8** hydrochloride. The hydrochloride of **9b** was obtained in analogy to **10b** by Mannich reaction utilizing 4-nitrobenzamide as acidic component. In this context, **10b** was prepared for comparison purposes. The ^1H and ^{13}C NMR data, until now unknown in literature, is reported here for the first time. However, all attempts to obtain **10a** and **9a** failed by targeted hydrolysis of both **10b** and **9b**. Instead, 4-nitrobenzamide was isolated in any case.

The attempt to cyclize **9b** with carbon disulphide by heating did not lead to **2d** but to [1,3,4]thiadiazolo [1,2-*c*]pyridazine-1,3-dithione **11,** the structure of which was confirmed also by an alternative synthesis starting from **8**. Under mild conditions, however, **9b** was transformed into the 2-(4-nitrobenzoyl)-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione **2d**. Although **2d** was somewhat stable, its conversion into the desired **2a** could not be realized.

Alternatively, it was examined whether **9c** can be synthesized and converted into **9a**. While **10c**, which is easily accessible by Mannich reaction, has long been known (Feldman and Wagner 1942), compound **9c** has not yet been reported. For comparative purposes, **10c** was synthesized and the ^1H and ^{13}C NMR spectra were recorded. However, all attempts to synthesize **9c** in the same manner failed, but gave instead



Scheme 3: Investigated synthetic routes to obtain suitable precursors for **2a**, the parent system of 2,3,5,6,7,8-hexahydro-1*H* [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **2**.
(a) **8** hydrochloride, potassium phthalimide, 30% aqueous HCHO, microwave irradiation (90 W, 130 °C, 3.3 bar), 5 min
(b) **8** hydrochloride, phthalimide, C_2H_5OH, 30% aqueous HCHO, $(C_2H_5)_3N$, refluxing, 5 h
(c) **8** hydrochloride 4-nitrobenzamide, paraformaldehyde, C_2H_5OH, refluxing, 5 h.
(d) **8** hydrochloride, potassium phthalimide, H_2O, 30% aqueous HCHO, refluxing, 6 h
(e) **8** hydrochloride, CH_3OH, $(C_2H_5)_3N$, CS_2, 80–90 °C, 30 min
(f) **9b** hydrochloride, C_2H_5OH, $(C_2H_5)_3N$, CS_2, 80–90 °C, 6 h
(g) **9b** hydrochloride, CH_3OH, $(C_2H_5)_3N$, CS_2, room temperature, 5 d
(h) **10a-c** are accessible starting from piperidine instead of **8** hydrochloride - **10a** (Takeuchi et al. 1986, Takeuchi et al. 1988) 10b (Foeldeák et al. 1962), and **10c** (Feldman, Wagner 1942)

1,2-bis(phthalimidomethyl)piperidazine **12**. When using potassium phthalimide instead of phthalimide the tetrazine **13** was obtained. The microwave-assisted reaction to obtain **9c** was also not successful; instead, the 2,3-tetramethylene phthalazine-1,4-dione **14** (Csampai et al. 1991; Rink and Grabowski 1956) was isolated.

The unsubstituted parent compound **2a** was finally obtained by the synthesis route presented in Scheme 4. Compound **16**, which was easily isolated from the reaction mixture, is rapidly formed by the heating of **1a** in formic acid. The formal didehydro analog is formed *via* the intermediate **15**, which was isolated and characterized. The formation of this intermediate is a suitable marker for optimizing the yield of compound **16** (Scheme 4). The hydrogenation of the 2,3-double bond of **16** went smoothly by modification of a procedure utilizing sodium borohydride under mild conditions (Irwin 1972). In this way the 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo [1,2-*a*]-pyridazine-1-thione **2a** was obtained in good yield.

2.1.2. 3,3-Disubstituted compounds *3* and *4*

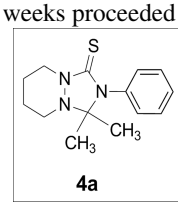
The reaction of hexahydropyridazine-1-carbothioamide **1a** with different ketones provided the 3,3-disubstituted title compounds **3a-k** (Scheme 1, Table) mostly in good yields. The reactants were heated without any solvent and under acid catalysis analogously as described for the 3-monosubstituted [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones (Schulz et al. 2013).

2.1.3. 3,3-Dialkyl derivatives *3a-c,g*, 3,3-pentamethylene derivative *3f*, and *4a*

The mentioned compounds **3** were usually synthesized by refluxing the carbothioamide **1a** (with the exception of **3c**, see below) with an excess of the respective ketone in the presence of small amounts of *p*-toluenesulfonic acid monohydrate. The time required for full conversion of **1a** was usually much longer (> 20 h) compared to various benzaldehydes and aromatic ketones, even when using a large excess of the carbonyl compound [acetone (**3a**), ethyl methyl ketone (**3b**), and diethyl ketone (**3 g**) (Table)]. In contrast, with only a small excess of cyclohexanone the 3,3-pentamethylene derivative **3f** was obtained within a short time and in good yield.

Another exception is the 3-cyclopropyl-3-methyl derivative **3c**, formation of which takes place only under special conditions. In this case, the use of aluminum chloride as a catalyst and the constant removal of the water that formed (through addition of molecular sieves) at room temperature led to an almost complete conversion of **1a**. However, the yield of **3c** was only sparingly because of its chemolability.

As already observed with the reaction of benzaldehyde with the *N*-phenyl derivative **1b** (Schulz et al. 2013), this substituted semicyclic compound reacts as easily as **1a** with acetone to the analogous 3,3-dimethyl derivative **4a**. Standing a solution of **1b** in acetone for several weeks proceeded to **4a** in a good yield.



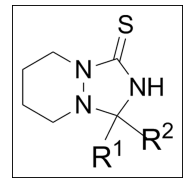
4a

Heating **1b** in acetone in the presence of aluminium chloride lead to a completion of the desired synthesis of **4a** within two hours.

2.1.4. 3-Aryl and 3,3-diaryl derivatives *3d,e,h-k*

The related alkylaryl or diaryl ketones were reacted with hexahydropyridazine-1-carbothioamide **1a** between 150 and 155 °C, as described for the aromatic aldehydes (Schulz et al. 2013). Depending on the reactivity of the ketones, the time until full conversion differs in comparison with the aldehydes [e.g. for benzaldehyde 75 min (Schulz et al. 2013)] in the expected order (Table).

Table: 3, 3-Disubstituted [1, 2, 4]triazolo[1,2-*a*]pyridazine-1-thiones 3a-k



3	R^1	R^2	Reaction time (Yield [%]) conventional[a,b]	Reaction time (Yield [%]) microwave-assisted[c]
a	CH_3	CH_3	33 h (81)	40 min (66)
b	CH_3	CH_2CH_3	44 h (66)	
c	CH_3	cyclopropyl	6 month (10)	
d	CH_3	C_6H_5	4 h (75)	30 min (49)
e	CH_3	4-CH_3O-C_6H_4	6 h (50)	
f	-$(CH_2)_5$-		45 min (60)	
g	C_2H_5	C_2H_5	20 h (76)	
h	C_6H_5	C_6H_5	13 h (75)	30 min (61)
i	C_6H_5	2-Cl-C_6H_4	15 h (38)	10 min (8)
j	C_6H_5	4-Cl-C_6H_4	21 h (52)	
k	C_6H_5	4-CH_3O-C_6H_4	14 h (40)	

[a] **3a,b,g**: refluxing **1a** in the appropriate ketone; **3d,e,h-k**: heating the educts at 150–155 °C
[b] **3c**: allowing to stand the educts with $AlCl_3$/molecular sieve at room temperature
[c] additional data are given in the experimental section

Although the yields after work-up are clearly lower (for **3i**: significantly), the microwave-assisted synthesis has the advantage that the reaction time are shortened in some cases, especially in the synthesis of **3a** (see Table for the preparation of the 3,3-disubstituted thiones **3**).

2.2. *Structural investigations*

The main focus of the structural studies was to verify the structures assigned to compounds **2**, **3**, and **4**. In this context, the exclusion of possible alternative monocyclic or bicyclic structures was of particular interest.

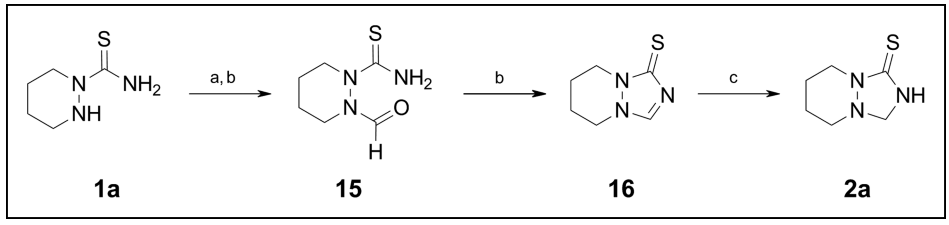
2.2.1. *Compounds **2a** and **5a***

The isomeric compounds **2a** (Scheme 4) and **5a** (Scheme 2) were obtained by different synthesis routes. In the IR spectrum of **2a,** the appearance of only one NH band at 3100 cm^{-1} is already a clear indication for the preservation of the bicyclic system in the process of the hydrogenation of **16**. The IR spectrum of **5a** compared to that of **2a** showed additional bands between 1500 cm^{-1} and 1600 cm^{-1}, indicating the simultaneous presence of a C = N- and NH-structure. The ^{1}H NMR spectrum of **2a** showed all characteristic signals as reported for the [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones (Schulz et al. 2013). Especially mentioned should be the signal splitting for the protons on C3, C5 and C8 indicating a magnetic nonequivalence due to different spatial positions (Fig. 1) and the singlet for NH in the hitherto observed normal range for this class.

Because of the poor solubility of compound **5a,** the hydrochloride had to be prepared for recording the ^{1}H and ^{13}C NMR spectra. A singlet at 5 ppm in the ^{1}H NMR spectrum of **5a** hydrochloride was an indication of the successful cyclization, as it can only be assigned to the protons at the C3 atom. In contrast to **2a**, no signal appears that would indicate a thioamide proton. In the ^{13}C NMR spectrum of **2a** and **5a** the expected six signals are found. But as for **2a**, comparable to the [1,2,4]triazolo [1,2-*a*]pyridazine-1-thiones (Schulz et al. 2013), the signal for C1 is found at about 178 ppm indicating the C = S carbon atom, the spectrum of **5a** showed a signal for C1 (C = N) at 169 ppm giving evidence for the existence of the thiadiazolo isomer.

2.2.2. *Compounds **2b** and **5b**, **2c***

In the IR spectra of the compounds **2b**, **5b** and **2c** (Scheme 2) no NH vibrational bands are present, indicating a successful cyclization reaction. Analogously to **2a**, in the ^{1}H NMR spectrum of **2b** the characteristics of the class (magnetic nonequivalence, see before) are found. Compared to the structurally isomeric **5b**, this signal for the C3 methylene group of **2b** appears at higher field at 4 ppm, caused by a lack of influence of the phenyl ring. In the ^{13}C NMR spectrum for both **2b** and **5b**, the expected signals for 10 carbon atoms are present. The signals for the methylene carbon atom C3 clearly differ for the compounds **2b** and **5b** (77.23 ppm *vs*. 58.11 ppm) due to the differences in electron attraction of the adjacent structures phenyl and sulfur, respectively. Additionally, the positions of the signal for the C1 atom in **2b** and



Scheme 4: Synthesis of 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione **2a** *via* the two isolable intermediates **15** and **16**.
(a) **15**: HCOOH, refluxing, 45 min
(b) **16** (directly from **1a**): HCOOH, refluxing, 1 h
(c) $NaBH_4$, propan-2-ol, room temperature,18 h

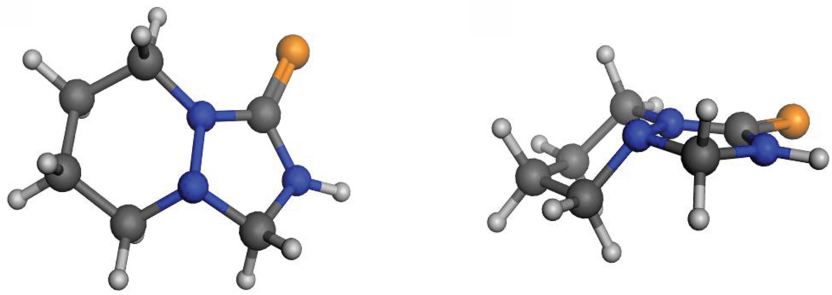


Fig. 1: Spatial representation of compound **2a** for visualization of the arrangement of the protons at the C3 atom.

5b differ as observed before (171.35 ppm vs. 160.73 ppm) for C = S and C = N, respectively. For the 2-methyl derivative **2c** (C1 at 178.87 ppm indicating C = S; Scheme 2), both alternative ring open and 1-methylimino-thiadiazolo[1,2-*a*]pyridazine structures can be excluded. Comparable to **2b,** the signal for the C3 atom is found at 72.68 ppm. In an alternative thiadiazolo [1,2-*a*]pyridazine structure it would be expected to appear at higher field (see above, **5b**).

2.2.3. *Compounds **2d** and **11***

The successful formation of the [1,2,4]thiadiazolo [1,2-*a*]pyridazine-1,3-dithione **11** from **9b** and carbon disulphide at higher temperature was already indicated by the IR and NMR spectra (lack of complete 4-nitrobenzoylaminomethyl partial structure, molecular symmetry) and could be verified by targeted synthesis using **8**-hydrochloride and carbon disulfide. In contrast, under mild conditions **2d** was formed instead. Thus, an additional signal at 198.03 ppm in comparison to compound **9b** appears in the ^{13}C NMR spectrum, supporting evidence for the insertion of the C = S fragment. Again, the signal for the protons at C3 appears split in the ^{1}H NMR spectrum, but compared to the previous **2**, the position of this comparable signal for **2d** at 5 ppm is shifted a little to lower field by the anisotropic effect of the adjacent carbonyl group at the C2 atom. The IR spectrum shows all the characteristic bands that are expected for **2d**. These findings finally confirmed the formation of compound **2d**. Unfortunately, in all spectra appear additional signals caused by an impurity formed from the starting compound **9b,** which is generated from **2b** due to its relatively low stability. Nevertheless, the HRMS confirmed the elemental composition calculated for **2d**.

2.2.4. *Compounds **3** and **4a***

The IR and the ^{13}C NMR spectra of all compounds **3** did not give any indications for the existence of a C = N partial structure of the conceivable alternatives [1,3,4]thiadiazolo[3,4-*a*]-pyridazin-1-imines of type **A** and a ring-open structures **B**, respectively (Fig. 2). In addition, none of the other analytical data yielded by NMR, IR, and MS gave evidences for these or other alternative structures. The same applies for **4a**.

Consistent with these findings, signals for the acidic proton at the N2 appear between 6 and 8 ppm in the ^{1}H NMR spectra and at about 176 ppm for the C = S carbon atom in the ^{13}C NMR spectra, which speaks for the formation of the **3**. The values are in accordance with those of **2a**, which was obtained in an alternative synthesis route (see above), and the related 3-monosubstituted [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones (Schulz et al. 2013), respectively. It is worth mentioning that the methyl substituents in position 3 of the derivatives **3a,c** appear in the spectra as very flat, broad peaks (barely visible) even after an exceptionally high rate of spectra accumulations, even though the corresponding methyl proton signals in the related ^{1}H NMR spectra are visible clearly and sharply. Interestingly, in the ^{1}H NMR of the 3-(2-chlorophenyl)-3-phenyl derivative **3i** occurred two broad singulets for the N(2)H. This finding could be explained with a different spatial alignment of the substituent in position 3. In particular different approximations of the 2-chlorophenyl moiety to the N(2)H should be possible. The behavior of the 3,3-disubstituted thiones **3a-k** in the EI mass spectrometry is comparable to those observed for the 3-monosubstituted compounds (Schulz et al. 2013). Unlike the other compounds with general structure **3**, the molecular ion peak for the 3-(2-chlorophenyl)-3-phenyl derivative **3i** is very weak, indicating its low stability. Ultimately, the chemical behavior of compounds **3** is also consistent with the assigned structure.

All recorded analytical data of **4a** including the absence of a NH signal (phenyl in position 2) in the ^{1}H NMR spectrum and NH bands in the IR spectrum, respectively, verify the expected ring closure. Interestingly, the carbon atoms of the two methyl groups in position 3, as observed with **3a,c** (see above), are also extremely difficult to detect. The same applies to for the carbon atom of the thioxo group.

2.3. *Biological results*

The biological tests were performed as described before (Schulz et al. 2013) using the rat insulinoma cell line RIN5F stimulated

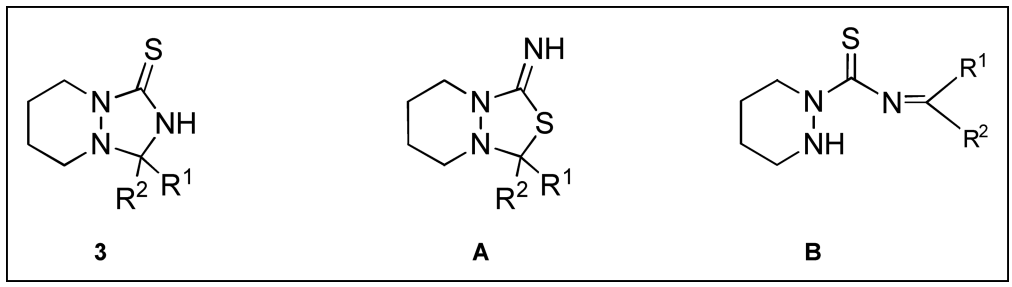


Fig. 2: Title compounds **3** and their possible alternative isomeric [1,3,4]thiadiazolo[3,4-*a*]pyridazin-1-imine (**A**) and open-ring substituted hexahydropyridazine-1-carbothioamide (**B**) structure.

with IL-1β and IFN-γ to induce inducible nitric oxide synthase (iNOS). The amount of produced NO was determined by measuring the oxidation product nitrite based on the Griess reaction (Griess 1879). Cytotoxicity was investigated by using a MTT based assay (Mosmann 1983). Due to the poor solubility of most of the substances only relatively low test concentrations (78 and 39 μM giving about 20 % resp. 10 % inhibition for the reference inhibitor AG) could be used.

To determine whether a change in the substitution pattern influences the potency as an iNOS inhibitor, several synthesized bicyclic thiones were exemplary evaluated. Thus, only substances with a modification of the substitution pattern in position 3 adjacent to an unsubstituted N2 atom were selected to ensure the comparability of the results with those of our previous investigations (Schulz et al. 2013).

As can be seen in Fig. 3A, the results show no activity for compound **2a,** the parent structure of the 3-substituted [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **3,** and the tested thiones with one or two aliphatic substituents, respectively. As the results of our previous investigations on monosubstituted thiones clearly indicated that a phenyl moiety is needed for iNOS inhibitory activity, it was assumed that this applies for disubstituted derivatives as well. The data obtained support this thesis. The inactivity of the 3,3-disubstituted methyl phenyl derivative **3d** might be explained by the fact that the aromatic structure is located in a very opposite direction to one of our hitherto best hits from our previous investigations. This was a 3-cinnamyl derivative ($IC_{50} = 9.81 \pm 2.21$ μM) that had an ethenylene group acting as spacer between the position 3 and the phenyl ring and determining the spatial orientation of the aromatic moiety (Fig. 4 B, structures aligned). This implies that for compounds **3** the optimal orientation of the aromatic ring is influenced by an adjacent aliphatic substituent at the same atom (C3). Considering the iNOS inhibitory potential only the 3,3-diaryl substituted thiones **3 h** (R^2, R^3 = phenyl) and **3k** (R^2 = phenyl, R^3 = 4-methoxyphenyl) were significantly more active than AG. Interestingly the 3-(2-chlorphenyl), 3-phenyl derivate **3i** is not active. It is possible that the chlorine atom sterically blocks interactions with the N(2)H the binding site or prevents interactions with the proton by forming intramolecular hydrogen bonds. The low-energy conformations of the three diphenyl substituted thiones **3 h,i,k** are quite similar (see Fig. 4 A, structures aligned). For the two candidates that were identified in the primary screening, an IC_{50} value had been determined which was for **3 h** at 65.88 ± 19.84 μM and for **3k** at 68.04 ± 38.23 μM (mean ± SD; n = 17). Compared to aminoguanidine with an IC_{50} value of 132.01 ± 14.96 μM (mean ± SD; n = 7) (Schulz et al. 2013) the tested substances are only slightly more active, especially in comparison to the activity of the best hit from previous investigations ($IC_{50} = 9.81 \pm 2.21$ μM). In addition, the determination of the IC_{50} gave relatively large fluxuations, as noted by the high SD, for the inhibitory concentrations were at the borderline solubility of the compounds.

The results obtained strengthen the hypothesis that an aromatic substituent in position 3 of the 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones, is essential for an iNOS inhibitory activity. It was repeatedly demonstrated that the orientation of the phenyl moiety plays an important role. However, a second substituent at this position is not advantageous. On the contrary, it is clearly detrimental to inhibitory potential.

Finally, like the reference substance aminoguanidine (AG) itself, also none of the tested compounds showed a cytotoxic effect (Fig. 3B).

In summary, it could be shown that the new class of 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones can be successfully extended to include the unsubstituted parent structure and a number of 3,3-disubstituted derivatives. While investigating different synthesis routes for compound **2a**, several other new substances possessing a hexahydropyridazine ring were obtained. The biological investigations strengthen the previously proposed hypothesis that an aromatic substitution at position 3 and its orientation are essential for iNOS inhibitory activity. The results also revealed that the insertion of a second substituent in position 3 is disadvantageous. For the further development of this substance class computational chemistry methods may be useful.

3. Experimental

3.1. General

The reported melting temperature ranges were determined on a Kofler-Boëtius apparatus type PHMK 81/3035 (VEB Wägetechnik Rapido) and are uncorrected. Elemental analyses were done with the 2400 CHN Elemental Analyzer (Perkin- Elmer). For the microwave-assisted syntheses the Discover LabMate with the IntelliVent™ Pressure Control System, circular, single-mode-self-tuning-system, frequency 2.45 GHz, and CEM's SynergyT software (CEM Kamp-Lintfort) was used. The spectra were recorded with the following instruments and conditions: IR spectra: FT-IR 1600 (Perkin-Elmer), transmission technique (KBr pressed disks) and for the compounds **2a-d, 5a, 6, 10c, 12-16** only IR 200 FT-IR (Thermo Electron Corporation Nicolet), ATR technique (diamond); ^{1}H, ^{1}H,^{1}H-COSY, DEPT-135**,** ^{13}C NMR spectra: AVANCE DPX 200 and for compounds **15** and **16** only the above named and additionally HSQC- and HMBC spectra: FT-NMR-Spektrometer Avance III™ 400 (both spectrometers from Bruker Analytische Messtechnik GmbH); temperature 25°C, solvents used are specified in the data of the related compound; internal standard tetramethylsilane; chemical shift δ in ppm. To verify the signal assignments 2D NMR techniques as HSQC, HMBC and ^{1}H,^{1}H-COSY were used. Mass spectra: EI, M 40 AMD (Intectra GmbH), electron impact, energy 70 eV, (with the exception of the molecular ion peaks normally only peaks > 10% are listed). High Resolution Mass Spectra: for the compounds **2a**, **2c**, **2b**, **2d**, **5a** hydrochloride, **5b**, **6**, **9b** hydrochloride **10b**, **10c**, **12**, **13**, **14, 15**: ESI, Shimadzu High Performance Liquid Chromatograph/Mass Spectrometer LCMS-IT-TOF with the system characterized as following: solvent delivery module LC-20AD Prominence, autosampler SIL-20AC HT Prominence, column oven CTO-20A Prominence, system controller CBM-20A Prominence, UV/VIS photodiode array detector SPD-M20A Prominence, evaporative light scattering detector ESD-LT II, spectrometric detector RF-10A XL, LCMS-IT-TOF workstation, software LCMS solution Version 3.41; column Chromolith® SpeedRod RP-18 endcapped, 50 mm; mobile phases (m. ph.) used for the compounds:

m. ph. IX: acetonitrile/water 1:1 (0.1% HCOOH in H_2O) (v/v), flow rate 0.2 mL·min^{-1} (compounds **2a**, **2c**, **2b**, **2d**, **5a** hydrochloride, **10c**, **13**, **14**)

m. ph. X: acetonitrile/water from 2:8 to 9:1 within 15 min (0.1% HCOOH in H_2O) (v/v), flow rate 0.2 mL·min^{-1} (compound **12**)

m. ph. XI: methanol/water 1:1 (0.1% HCOOH in H_2O) (v/v), flow rate: 0.4 mL·min^{-1} (compound **15**)

m. ph. XII: methanol/water 6:4 (0.1% HCOOH in H_2O) (v/v), flow rate: 0.4 mL·min^{-1} (compound **9b** hydrochloride, **10b**); flow rate: 0.2 mL·min^{-1} (compound **6**)

m. ph. XIII: methanol/water 8:2 (0.1% HCOOH in H_2O) (v/v), flow rate: 0.2 mL·min^{-1} (compound **5b**)

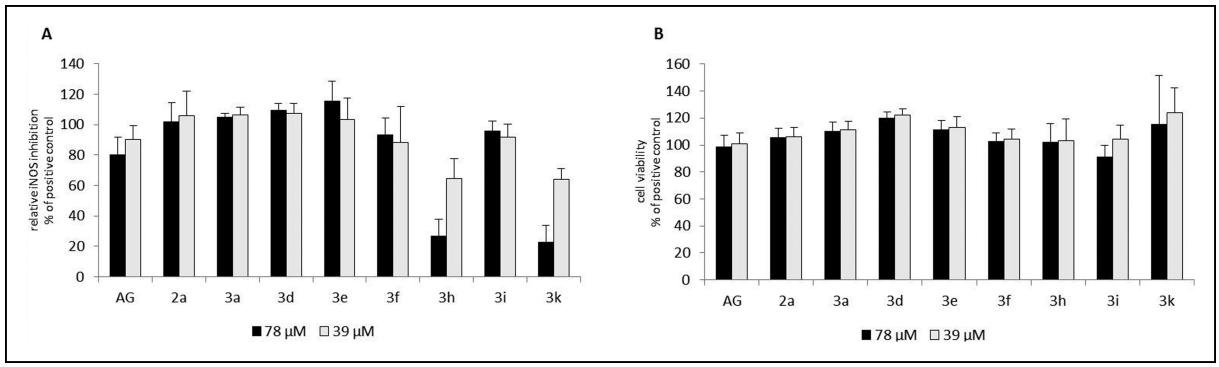


Fig. 3: Concentration-dependent inhibition of iNOS by aminoguanidine (AG), 3-unsubstituted thione **2a**, and 3,3-disubstituted thiones **3** (**A**). NO production was determined by using GRIESS reaction. Cell viability was tested on substance treated RINm5F cells using MTT assay (**B**). Both data sets are displayed as percent of positive control (RIN cells treated with IL and IFN but without substance). Aminoguanidine as the reference inhibitor was tested in every concentration as well. Mean ± SD; n = 6 (> 30 for AG, as control on every plate).

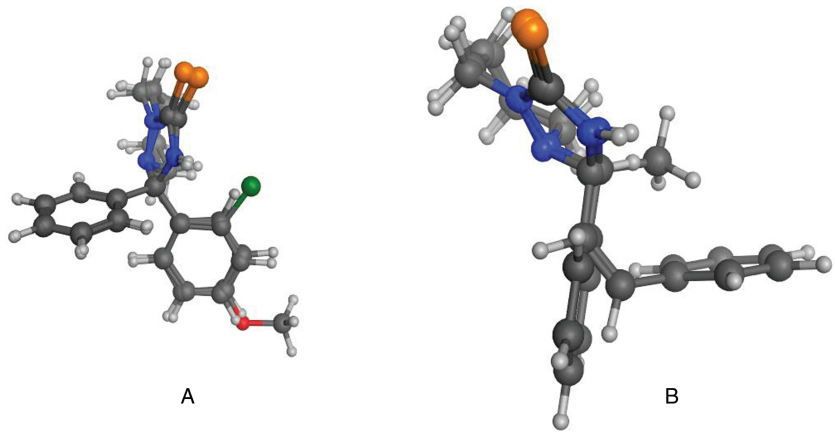


Fig. 4: (A) compound **3h**, **3i** and **3k** superposed; (B) compound **3d** superposed with one of the best hits from previous investigations, the 3-phenylethenyl derivative of the parent structure **2a**.

High Resolution Mass Spectra: for the compounds **3d, 11, 16**: ESI, Bruker Daltonics MicroOTOF-LC (ESI-TOF); external calibration (Agilent ESI TuneMix); column Zorbax RP-C18; 2.1 x 30 mm, 3.5 µm; mobile phase (m. ph.): m. ph. XVII: gradient propan-2-ol/water 20% Ba' 50% B.; flow rate: 0.3 mL/min.
For silica gel aluminum foil covered with silica gel 60 F_{254} (Merck) was utilized as stationary phase. The running distance for the front of the mobile phase was 6.5 cm. The following mixtures were used as mobile phases:

m. ph. I: *n*-hexane/ethyl acetate 1:1 (v/v)
m. ph. II: *n*-hexane/ethyl acetate 2:8 (v/v)
m. ph. III: dichloromethane/acetone 1:1 (v/v)
m. ph. IV: dichloromethane/acetone/triethylamine 10:10:1 (v/v/v)

Each mobile phase (m. ph.) used is specified in the data of the related compound. The substances were detected with UV radiation (λ = 254 nm) or Munier spray reagent (Munier 1953)_ENREF_1 and iodine azide reagent by AWE (Awe 1948). The HPLC investigations were done under the following conditions:
system: LaChrom (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 (λ = 220 nm or 240 nm); *stationary phase:* RP columns (given in the HPLC data of the compounds); the dead time t_0 was determined with uracil; each mobile phase (m. ph.) is given in the data for the related compound

m. ph. V: acetonitrile/0.02 M KH_2PO_4 (pH 2.85) 1:1 (v/v)
m. ph. VI: acetonitrile/0.02 M KH_2PO_4 (pH 2.85) 3:7 (v/v)
m. ph. VII: acetonitrile/water 1:1 (v/v)
m. ph. VIII: acetonitrile/0.02 M KH_2PO_4 (pH 2.85) 4:6 (v/v)

The values for the dead time t_0 and the retention times t_R are given in minutes.

3.2. Chemistry

3.2.1. Hexahydropyridazine-1-carbothioamides *1, 6* and *15*

Hexahydropyridazine-1-carbothioamides **1a-c**
Synthetic procedures and experimental data are already reported (Schulz et al. 2013).

N,N'-Dimethyl-2,2'-methylenedi(hexahydropyridazine-1-carbothioamide) **6**
Method 1: - from 1c and paraformaldehyde: A well prepared mixture of 0.7 mmol (0.112 g) *N*-methylhexahydropyridazine-1-carbothioamide **(1c)** and 0.8 mmol (0.024 g) paraformaldehyde was heated together with 0.2 g of dried molecular sieve in a metal bath at 140-150 °C until the reaction was terminated (TLC control, about 15 min). The cooled solid melt was dissolved in a little acetone and the solution was slowly concentrated at room temperature. The formed crystals were collected, washed twice with a little methanol and dried in vacuo. Yield 10%. Colorless crystals. M.r. 244-246 °C (acetone).
TLC (m. P. II): R_f = 0.42. HPLC (m. P. VI): RP-18, t_0 = 1.77; t_R = 4.98; k' = 1.81. IR (cm^{-1}): $\tilde{\nu}$ = 1185 (C = S); 1522 (NH); 2854 (CH_3); 2941 (aliph. H); 3325 (NH). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.30-1.84 (bm, 10H, heterocycl. CH_2); 2.73 (bt, 2H, heterocycl. CH_2); 2.93 (d, J = 4.6 Hz, 6H, 2 x -CH_3); 3.01 (bt, 1H, heterocycl. CH_2); 3.19 (bd, 1H, heterocycl. CH_2); 3.85

(d, J = 11.0 Hz, 1H, heterocycl. CH_2); 4.08 (d, J = 11.6 Hz, 1H, heterocycl. CH_2); 5.05 and 5.11 (bs, 2 x 1H, CH_2); 7.97 (q, J = 4.6 Hz, 2H, 2 x NH). ^{13}C NMR (DMSO-d_6, ppm): δ = 17.70 (2 x C5); 23.38 (2 x C4); 31.79 (2 x CH_3); 40.76 (2 x C3); 48.16 (2 x C6); 69.41 (CH_2); 181.11 (2 x C = S). MS (70 eV, 180 °C) m/z (%): 171.7 (54) [$M^{+\cdot}$ - .CH_2-(NC_4H_8N)-CS-NH-CH_3]; 97.9 (100) [$C_4H_8N_2$-CH_2^+]; 84.9 (23) [$C_4H_8N_2^+$]; 70.8 (12); 57.3 (13); 42.0 (14); 28.0 (50). HRMS [(ESI) m/z (%)]: Compound 6: calcd. [$C_{13}H_{26}N_6S_2$ + Na]$^+$: 353.1553; found: 353.1555 (35) (calcd. for [$C_{13}H_{26}N_6S_2$]$^+$: 330.1660; found: 330.1663) and compound 2c as a splitting product, calcd. [$C_7H_{13}N_3S$ + H]$^+$: 172.0903; found 172.0899 (100). Elemental analysis [$C_{13}H_{26}N_6S_2$ (330.5), %]: calcd. C, 47.24; H, 7.93; N, 25.43; S, 19.04; found C, 46.57; H, 8.00; N, 24.62; S, 18.64.

Method 2: - from 1c and aqueous formaldehyde solution: To a suspension of 1 mmol *N*-methylhexahydropyridazine-1-carbothioamide **(1c)**(0.159 g) in 1 mL methanol was added 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) and the reaction mixture was refluxed for 7 h (bath temperature 120 °C). After cooling overnight, the formed crystals were separated, washed with a little methanol and dried on a clay plate. Yield 3%. Colorless crystals. M.r. 226-229 °C (water).

2-Formyl-hexahydropyridazine-1-carbothioamide **(15)**
This compound is an intermediate in the synthesis of compound **16** (synthetic procedure see below).
A mixture of 5 mmol (0.726 g) hexahydropyridazine-1-carbothioamide **(1a)** and 2 mL 99% formic acid was refluxed for 45 min. Subsequently, the formed solution was concentrated in a warm air flow. The resulting crystals were recrystallized from ethanol whereby compound **16** was included in the residue. The mother liquor was concentrated again in a warm air flow and the formed solid was recrystallized from water. The yielded crystals were collected and washed with a little acetone. Yield 5%. Light beige crystals. M.r. 166-168 °C (water).
TLC (m. P. III): R_f = 0.70. IR (cm^{-1}): $\tilde{\nu}$ = 1184 (C = S); 1607 (NH_2); 1670 (C = O); 2852, 2930, 2958 (aliph. and formyl H); 3170; 3255, 3347 (NH_2). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.25 (s, 3H, CH_3); 1.76 (m, 3H; C(4)H_2 and C(5)H); 1.89 (m, 1H, C(5)H); 2.89 (dt, 1H, C(3)H); 3.10 (dt, 1H, C(6)H); 4.39 (dd, 1H, C(3)H); 5.48 (dd, 1H, C(6)H); 6.32 (bs, 2H, NH_2); 8.30 (s, 1H, COH). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.7 (C4); 23.0 (C5); 42.0 (C3); 51.6 (C6); 164.9 (C = O); 184.2 (C = S). HRMS [(ESI) m/z]: calcd. [$C_6H_{11}N_3OS$ + H]$^+$: 174.0696; found [M + H]$^+$: 174.0733.

*3.2.2. 5,6,7,8-Tetrahydro-1H-[1,2,4]triazolo [1,2-a]pyridazine-1-thione **16***

A mixture of 5 mmol (0.726 g) hexahydropyridazine-1-carbothioamide **(1a)** and 2 mL 99% formic acid (approximately 52.5 mmol) was refluxed for 1 h. After the excess of formic acid was removed the formed crystals were recrystallized from ethanol, filtered off, washed with ethanol and dried in vacuo. Yield 80%. Colorless crystals. M.r. 276–279 °C (ethanol).
TLC (m. P. III): R_f = 0.14. HPLC (m. P. VI): RP-select B, t_0 = 2.10; t_R = 2.37; k' = 0.13. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1178 (C = S); 1505 (C = N); 1849 (S = C-N = C); 2865, 2925, 2959 (CH_2); 3072 (=C-H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.95 (bm, 4H, C(6)H_2 and C(7)H_2); 3.93 (t, J = 6.0 Hz, 2H, C(5)H_2); 4.14 (t, J = 5.2 Hz, 2H, C(8)H_2); 8.48 (s, 1H, C(3)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 19.42 (C6); 20.03 (C7); 45.18 (C5); 46.65 (C8); 146.60 (C = N); 175.20 (C = S). MS (70 eV, 350 °C) m/z (%): 154.8 (100) [$M^{+\cdot}$]; 126.9 (35) [$M^+ - CH_2 = CH_2$]; 99.9 (33); 72.7 (15); 68.9 (17); 55.3 (14); 42.1 (16); 41.0 (41); 28.0 (48); 27 (10). Elemental analysis [$C_6H_9N_3S$ (155.2), %]: calcd. C, 46.43; H, 5.84; N, 27.07; S 20.65; found C, 47.25; H, 5.95; N, 27.55; S, 21.02.

*3.2.3. 2,3,5,6,7,8-Hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thiones **2a—d, 7***

2,3,5,6,7,8-Hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione **(2a)**
A mixture of 1 mmol (0.155 g) tetrahydro-1*H*-[1,2,4] triazolo[1,2-*a*]pyridazine-1-thione **(16)**, 3.8 mmol (0.144 g) $NaBH_4$, and 4.5 mL propan-2-ol was stirred at room temperature for 18 h. Subsequently, 7 mL water were added and the resulting solution was three times extracted each with 8 mL dichloromethane and the combined organic phases were dried over sodium sulphate. After filtration the organic phase was carefully concentrated, whereby crystals formed. These were sucked off and dried at room temperature in vacuo. Yield 91%. Colorless crystals. M.r. 85-93 °C (dichloromethane).
TLC (m. P. III): R_f = 0.76. HPLC (m. P. VII): RP-18, t_0 = 1.75; t_R = 2.37; k' = 0.35. IR (cm^{-1}): $\tilde{\nu}$ = 1174 (C = S); 1486 (NH); 2834, 2912, 2959 (–CH_2–); 3173 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.64 (bs, 2H, C(7)H_2); 1.80–1.84 (m, 2H, C(6)H_2); 2.82 (bs, C(5)H_2); 3.19 (bs, 1H, C(8)H); 4.20 (bs, 1H, C(3)H); 4.54 (bs, 2H, C(8)H u. C(3)H); 6.69 (bs, 1H, NH).^{13}C NMR ($CDCl_3$, ppm): δ = 23.18 (C7); 23.69 (C6); 45.21 (C8); 53.40 (C5); 67.03 (C3); 178.49 (C(1) = S). HRMS [(ESI) m/z]: calcd. for $C_6H_{11}N_3S$ + H^+: 158.0746; found [M + H^+]: 158.0707.

2-Phenyl-2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo [1,2-*a*]pyridazine-1-thione **(2b)**
A mixture of 1 mmol (0.221 g) *N*-phenylhexahydropyridazine-1-carbothioamide **(1b),** 1 mL methanol and 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) was refluxed for 6 h (bath temperature 120 °C). After cooling overnight, the formed crystals were separated and washed with a little methanol and dried on a clay plate. Yield 3%. Colorless crystals. M.r. 218-221 °C (methanol).
TLC (m. P. II): R_f = 0.86. HPLC (m. P. V): RP-18, t_0 = 1.75; t_R = 12.64; k' = 6.22. IR (cm^{-1}): $\tilde{\nu}$ = 1172 (C = S); 1588, 1686 (arom. ring). **^{1}**H NMR ($CDCl_3$, ppm): δ = 1.80 (bs, 4H, C(6)H_2 and C(7)H_2); 3.23 (t, J = 10.4, 2H, C(5)H_2); 4.06 (t, J = 10.6, 2H; C(8)H_2); 6.81-6.92 (t, J = 15.4 Hz, 3H, arom. H from C10, C12 and C14); 7.14 (t, J = 15.4 Hz, 2H, arom. H an C11and C13); 7.32-7.34 (d, 2H, C(3)H_2). **^{13}**C NMR ($CDCl_3$, ppm): δ = 22.52, 22.57 (C6 and C7); 45.41 (C8); 48.17 (C5); 77.23 (C3); 121.55 (2 arom. C); 122.44 (1 arom. C); 128.89 (2 arom. C); 134.03 (1 arom. C); 146.62 (C3); 171.35 (C(1) = S). HRMS [(ESI) m/z]: calcd. for $C_{12}H_{15}N_3S$ + H^+: 234.1059; found [M + H^+]: 234.1052.

2-Methyl-2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo [1,2-*a*]pyridazine-1-thione **(2c)**
A mixture of 1 mmol (0.159 g) *N*-methylhexahydropyridazine-1-carbothioamide **(1c)**, 1 mL water, and 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) was refluxed for 4 h (bath temperature 120 °C). After cooling overnight, the formed crystals were separated, washed with a little water and dried at room temperature in vacuo. Yield 71%. Colorless crystals. M.r. 32-36 °C (water).
TLC (m. P. II): R_f = 0.31. HPLC (m. P. VII): RP-18, t_0 = 1.75; t_R = 3.17; k' = 0.81. IR (cm^{-1}): $\tilde{\nu}$ = 1186 (C = S); 2853, 2924, 2950 (–CH_3, –CH_2–). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.62-1.67 (m, 2H, C(6)H_2); 1.77-1.84 (m, 2H; C(7)H_2); 2.76 (t, J = 10.2 Hz, 4H, C(5)H_2 and C(8)H_2); 3.11 (s, 3H, CH_3); 4.20-4.34 (bd, 2H, C(3)H_2). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.91 (C6); 23.78 (C7); 32.76 (CH_3); 45.98 (C8); 53.30 (C5); 72.68 (C3); 178.87 (C = S).

HRMS [(ESI) m/z]: calcd. for $C_7H_{13}N_3S + H^+$: 172.0903; found $[M + H^+]$: 172.0903.

2-(4-Nitrobenzoyl)-2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione **(2d)**
To a solution of 1 mmol (0.300 g) 4-nitro-*N*-(hexahydropyridazin-1-ylmethyl)benzamide hydrochloride **(9b)** HCl in 10 mL methanol were added 1 mmol (0.101 g) triethylamine and 45 mmol (3.426 g) carbon disulphide. The reaction mixture was allowed to stand for 5 d at room temperature. The formed crystals were separated, washed with methanol and dried on a clay plate. Yield 19%. Yellow crystals. M.r. 208-215 °C (methanol). TLC (m. P. IV): $R_f = 0.93$. HPLC (m. P. VII): RP-18, $t_0 = 1.75$; $t_R = 14.75$; k' = 7.43. IR (cm^{-1}): $\tilde{\nu} = 1190$ (C = S); 1544 ($-NO_2$); 1515, 1598 (arom. –C = C–); 1652 (C = O). ^1H NMR (DMSO-d_6, ppm): δ = 1.43-1.98 (m, 4H, C(6)H_2 and C(7)H_2); 3.13 (bt, 4H, C(5)H_2 and C(8)H_2); 4.76-4.96 (m, 2H, C(3)H_2); 8.05 (d, J = 8.8 Hz, 2H, arom. H); 8.30 (d, J = 8.8 Hz, 2H, arom. H). ^{13}C NMR (DMSO-d_6, ppm): δ = 18.30 (C6); 23.44 (C7); 44.56 (C8); 50.45 (C5); 55.68 (C3); 123.37 (2 arom. C); 128.94 (2 arom. C); 139.62 (1 arom. C); 149.08 (1 arom. C); 165.33 (C = O); 198.03 (C = S). HRMS [(ESI) m/z]: calcd. for $C_{13}H_{14}N_4O_3S + H^+$: 307.0859; found $[M + H^+]$: 307.0807.

2,2'-Methylene di(2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione) **(7)**
A mixture of a well prepared trituration of 0.7 mmol (0.102 g) hexahydropyridazine-1-carbothioamide **(1a)** with 0.8 mmol (0.024 g) paraformaldehyde and 0.2 g molecular sieve was heated at 140–150 °C until the reaction was completed (TLC monitoring, about 25 min). After cooling, the formed solid was dissolved in a little acetone and the solution was slowly concentrated. The formed crystals were sucked off, washed twice with a little methanol and dried in vacuo. Yield 15%. Colorless crystals. M.r. 154–157 °C (acetone).
TLC (m. P. II): $R_f = 0.74$. HPLC (m. P. VI): RP-select B, $t_0 = 2.10$; $t_R = 3{,}86$; k' = 0.84. IR (KBr, cm^{-1}): $\tilde{\nu} = 1188$ (C = S); 1480; 2842, 2851, 2941 (aliph. H); 3260 (ass. OH). ^1H NMR ($CDCl_3$, ppm): δ = 1.63 (bs, 4H, C(6)H_2 and C(6')H_2); 1.82 (m; 4H, C(7)H_2 and C(7')H_2); 2.74 (bs, 4H, C(5)H_2 and C(5')H_2); 3.19 (bs, 2H, je 1H of C(8)H_2 and C(8')H_2); 4.51 (bs, 6H, je 1H von C(8)H_2 and C(8')H_2 and je 2H of C(3)H_2 and C(3')H_2); 5.4 (s, 2H, ring-linking CH_2-group). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.01 (C7 and C7'); 23.85 (C6 and C6'); 45.88 (C5 and C5'); 53.70 (C8 and C8'); 55.58 (-CH_2-); 71.09 (C3 and C3'); 178.64 (2 x C = S). MS (70 eV, 295 °C) m/z (%): 325.7 (35) $[M^{+\cdot}]$; 169.8 (41) [2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thion-3-ylmethyl$^+$]; 155.8 (100) [2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thionyl$^+$]; 96.9 (46); 72.6 (15); 55.2 (17); 42.1 (28); 32.0 (11), 30.0 (12); 28.0 (59). Elemental analysis [$C_{13}H_{22}N_6S_2$ (326.5) · H_2O [344.5], %]: calcd. C, 45.33; H, 7.02; N, 24.40; S, 18.61; found C, 44.62; H, 6.70; N, 24.12; S, 19.22.

*3.2.4. 3,3-Disubstituted 2,3,5,6,7,8-hexahydro-1H-[1,2,4] triazolo[1,2–a]pyridazine-1–thiones **3** and **4a***

2,3,5,6,7,8-Hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **3a,b,g**
A mixture of 5 mmol (0.726 g) hexahydropyridazine-1-carbothioamide **(1a),** the given amount of the appropriate ketone, and 0.25 mmol (0.48 g) *p*-toluenesulfonic acid monohydrate was refluxed until the starting compound was completely reacted (TLC monitoring). Then the excess of the ketone was removed at room temperature. The formed crystals, were washed with a little ice-cold propan-2-ol, separated and dried in vacuo. In this manner the following compounds were obtained:

3,3-Dimethyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4] triazolo[1,2-a]pyridazine-1-thione **(3a)**
With 25 mL (approximately 340 mmol) acetone. Reaction time 33 h. Yield 81%. Light beige crystals. M.r. 156–157 °C (propan-2-ol).
TLC (m. P. I) $R_f = 0.32$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1172$ (C = S); 1460;1485; 1626 (NH); 2850, 2923, 2942, 2962, 2975 (aliph. C-H); 3158 (NH). ^1H NMR ($CDCl_3$, ppm): δ = 1.40 (s, 6H, C(3)(CH_3)$_2$); 1.64 (m, 2H, C(6)H_2); 1.82 (m, 2H, C(7)H_2); 2.76 (t, 2H, C(8)H_2); 3.78 (s, 2H, C(5)H_2); 6.1 (s, 1H, (N(2)H). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.15; 23.71 (C6 and C7); 23.94 (broad and flat signal, 2 x CH_3); 45.3 (C8); 47.5 (C5); 77.3 (C3); 176.4 (C = S). MS (70 eV, 115 °C) m/z (%): 185.1 (80) $[M^{+\cdot}]$; 170.0 (100) $[M^{+\cdot} - {}^{\cdot}CH_3]$; 115.5 (17); 100.0 (17); 85.0 (65) $[C_4H_9N_2^+]$; 70.9 (35); 56.3 (37); 55.3 (32); 41.1 (58); 30.0 (56); 28 (67). Elemental analysis [$C_8H_{15}N_3S$ (185.3), %]: calcd. C, 51.86; H, 8.16; N, 22.68; found C, 51.77; H, 8.44; N, 22.39.

3-Ethyl-3-methyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo [1,2-a]pyridazine-1-thione **(3b)**
With 10 mL (approximately 112 mmol) ethyl methyl ketone. Reaction time 44 h. Yield 66%. Colorless crystals. M.r. 103-104 °C (propan-2-ol).
TLC (m. P. I) $R_f = 0.53$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1180$ (C = S); 1419; 1449; 1482; 1635 (NH), 2846 (CH_3); 2914, 2956 (aliph. C-H); 3167 (NH). ^1H NMR ($CDCl_3$, ppm): δ = 0.95 (t, 3H, -$CH_2C\underline{H_3}$); 1.35 (s, 3H, CH_3); 1.65 (m, 4H, C(7)H_2 and -$C\underline{H_2}CH_3$); 1.80 (m, 2H, C(6)H_2) 2.73 (t, 2H, C(5)H_2); 3.45 (bs, 1H, C(8)H_2); 4.19 (bd, 1H, C(8)H_2); 6.5 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): δ = 8.26 (-$CH_2\underline{C}H_3$); 19.56 (CH_3); 23.24, 23.78 (C6 and C7); 31.06 (-$\underline{C}H_2CH_3$); 45.25 (C8); 47.52 (C5); 80.01 (C3); 175.88 (C = S). MS (70 eV, 180 °C) m/z (%): 199.1 (40) $[M^{+\cdot}]$; 184.0 (15) $[M^{+\cdot} - {}^{\cdot}CH_3]$; 170 (100) $[M^{+\cdot} - {}^{\cdot}C_2H_5]$; 115.5 (11); 85.0 (26) $[C_4H_9N_2^+]$; 70.9 (12); 69.9 (11); 56.3 (22); 55.3 (38); 42.1 (27); 41.1 (24); 32.0 (11); 30.0 (21); 29 (14); 28 (62); 27 (12). Elemental analysis [$C_9H_{17}N_3S$ (199.3), %]: calcd. C, 54.24; H, 8.60; N, 21.08; found C, 53.29; H, 8.71; N, 20.21.

3,3-Diethyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione **(3 g)**
With 10 mL (94 mmol) diethyl ketone. Reaction time 20 h. Yield 76%. Colorless crystals. M.r. 127-129 °C (propan-2-ol).
TLC (m. P. I) $R_f = 0.73$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1184$ (C = S); 1421; 1441; 1473; 1634 (NH); 2849, 2880, 2929, 2954, 2972 (aliph. C-H); 3175 (NH). ^1H NMR ($CDCl_3$, ppm): δ = 0.92 (t, 6H, (2 x -$CH_2C\underline{H_3}$); 1.69 (m, 8H C(6)H_2, C(7)H_2 and 2 x -$C\underline{H_2}CH_3$); 2.75 (t, 2H, C(5)H_2); 3.80 (bt, 2H, C(8)H_2); 6.47 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): δ = 8.11 (2 x $CH_2\underline{C}H_3$); 23.15, 23.71 (C6 and C7); 25.95 (2 x -$\underline{C}H_2CH_3$); 45.25 (C8); 47.24 (C5); 82.39 (C3); 175.67 (C = S). MS (70 eV, 150 °C) m/z (%): 213.0 (27) $[M^{+\cdot}]$; 185.1 (10); 184.0 (100) $[M^{+\cdot} - {}^{\cdot}C_2H_5]$; 85.0 (15) $[C_4H_9N_2^+]$; 83 (11); 56.3 (20); 55.3 (19); 41.1 (21); 30.0 (12); 29 (12); 28 (32). Elemental analysis [$C_{10}H_{19}N_3S$ (213.3), %]: calcd. C, 56.30; H, 8.98; N, 19.70; found C, 56.66; H, 8.69; N, 19.43.

3-Cyclopropyl-3-methyl-2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione (**3c**)
A mixture of 0.5 mmol (0.073 g) hexahydropyridazine-1-carbothioamide **(1a),** 1 mL (approximately 11 mmol) cyclopropyl methyl ketone, 0.05 mmol (0.007 g) anhydrous aluminium chloride, and 0.1 g fresh dehydrated molecular sieve was allowed to stand at room temperature. After the starting compound **1a** was completely reacted (about 6 month), the suspension was passed through a glass sinter frit and the resulting

clear filtrate was concentrated at room temperature. The remaining dark and viscous residue was digested several times with little boiling hexane. During cooling of the hexane phase crystals were formed which were separated, washed with hexane, and dried in vacuo. Yield 10%. Colorless crystals. M.r. 112-114 °C (hexane).

TLC (m. P. I) $R_f = 0.57$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1202$ (C = S); 1434; 1476; 1583; 1634 (NH); 2839, 2924, 2947, 2981; 3016 (aliph. H); 3177 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 0.29–0.33 (m, 1H, cyclopropyl); 0.52–0.55 (m, 3H cyclopropyl); 1.31 (s, 3H, CH_3); 1.60 -1.64 (m, 3H, 2H of C(6)H_2 and 1H of cyclopropyl);1.79–1.83 (m, 2H, C(7)H_2; 2.72–2.88 (m, 2H, C(5)H_2); 3.81 (bs, 2H, C(8)H_2); 5.98 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): δ = 1.51 (C2 and C3 cyclopropyl), 17.15 (very broad, less intensively, C1 cyclopropyl), 21.58 (very broad, less intensively, CH_3), 23.51 (C6), 24.02 (C7), 45.42 (C5), 48.45 (C8), 79.94 (C3), 176.06 (C = S). MS (70 eV, 115 °C) m/z (%): 211.0 (52) [$M^{+\cdot}$]; 196.0 (30) [$M^{+\cdot}$ – $^{\cdot}CH_3$]; 170.0 (39) [$M^{+\cdot}$ – $^{\cdot}C_3H_5$]; 85.0 (100) [$C_4H_9N_2^+$]; 70.9 (11); 68.9 (12); 67.9 (13); 56.3 (30); 55.3 (27); 42.1 (31); 41.1 (48); 39.1 (22); 42.1 (31); 32.0 (15); 30.1 (43); 29 (11); 28 (86); 27 (14). Elemental analysis [$C_{10}H_{17}N_3S$ (211.3), %]: calcd. C, 56.84; H, 8.11; N, 19.88; found C, 55.79; H, 8.91; N, 19.89.

3,3-Disubstituted 2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo [1, 2-*a*]pyridazine-1-thiones **3d-f,h-k**

Method 1: Preparation by conventional heating of 1a with ketones - general procedure: A well prepared mixture of 5 mmol (0.726 g) hexahydropyridazine-1-carbothioamide **1a**, 5.5 mmol of the appropriate ketone, and 0.25 mmol (0.048 g) p-toluenesulfonic acid monohydrate was heated at 150–155 °C until the starting compound **1a** was completely reacted (TLC monitoring). Subsequently, the cooled mass was dissolved in a little methanol, and the solution was concentrated at room temperature whereby crystals formed. The formed crystals were washed with little cold propan-2-ol, separated, and dried in vacuo.

In this manner the following compounds were obtained:

*3-Methyl-3-phenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4] triazolo[1,2-a]pyridazine-1-thione (**3d**)*

With acetophenone (0.661 g). Reaction time 4 h. Yield 75%. Colorless crystals. M.r. 144–146 °C (propan-2-ol).

TLC (m. P. I): $R_f = 0.64$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1204$ (C = S); 1477; 1493; 1582, 1627 (NH); 2952; 2970; 2987 (aliph. C-H); 3057 (ar-H); 3154 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.60–1.76 (m, 10H, C(6)H_2 and C(7)H_2 and C(3)–CH_3); 2.49 (s, C(8)H_2); 3.83 (s, C(5)H_2); 7.14 (s, 1H, NH), 7.32–7.54 (m, 5 arom. H). ^{13}C NMR ($CDCl_3$, ppm); δ = 23.17 (C7); 23.84 (C6); 24.21 (CH_3); 45.39 (C8); 48.68 (C5); 81.32 (C3); 126.07, 128.57, 128.54, 139.97 (all arom. C); 176.63 (C = S). MS (70 eV, 180 °C) m/z (%): 247.2 (39) [$M^{+\cdot}$]; 232.1 (21) [$M^{+\cdot}$ – $^{\cdot}CH_3$]; 161.9 (11); 102.8 (17); 91.0 (11); 85.1 (100) [$C_4H_9N_2^+$]; 77.4 (22); 56.4 (25); 41.1 (15); 30.0 (40); 28.0 (49). Elemental analysis [$C_{13}H_{17}N_3S$ (247.4), %]: calcd. C, 63.12; H, 6.93; N, 16.99; found C, 62.35; H, 6.38; N, 16.62.

*3-(4-Methoxyphenyl)-3-methyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3e**)*

With *p*-methoxyacetophenone (0.826 g). Reaction time 6 h. Yield 50%. Colorless crystals. M.r. 137–138 °C (propan-2-ol).

TLC (m. P. I): $R_f = 0.70$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1178$ (C = S); 1513; 1584; 1610 (broad peak basis, arom. ring and NH); 2850, 2930, 2953 (aliph. C-H); 3000 (arom. C-H); 3176 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.61 (q, 3H, C(6)H_2 and C(7)H); 1.64 (s, 3H, -CH_3); 1.71 (s, 1H, C(7)H); 2.40 (q, 2H, C(5)H_2); 3.72 (bs, 1H, C(8)H); 3.81 (s, 3H, -OCH_3); 3.99 (bs, 1H, C(8)H); 6.56 (s, 1H, NH); 6.90 (d, J = 12 Hz, 2H, arom. H); 7.41 (d, J = 12 Hz, 2H, arom. H). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.14 (C6); 23.81 (C7); 24.35 (-$\underline{C}H_3$) 45.49 (C8); 48.71 (C5); 55.30 (-O$\underline{C}H_3$); 80.99 (C3); 113.87 127.55, 159.84 (arom. C); 176.62 (C = S). MS (70 eV, 270 °C) m/z (%): 277.0 (41) [$M^{+\cdot}$]; 262.1 (18); 191.9 (77) [$M^{+\cdot}$ - $^{\cdot}C_4H_9N_2$]; 161.9 (11); 134.0 (13); 91.0 (17); 85.0 (100) [$C_4H_9N_2^+$]; 56.3 (20); 41.0 (13); 32.0 (13); 30.0 (32); 28.0 (66). Elemental analysis [$C_{14}H_{19}N_3OS$ (277.4), %]: calcd. C, 60.62; H, 6.90; N, 15.15; found C, 60.56; H, 6.85; N, 14.62.

*3,3-Pentamethylen-2,3,5,6,7,8-hexahydro-1H-[1,2,4] triazolo[1,2-a]pyridazine-1-thione (**3f**)*

With cyclohexanone (0.540 g). Reaction time 45 min. Yield 60%. Colorless crystals. M.r. 184–185 °C (propan-2-ol).

TLC (m.P. I): $R_f = 0.62$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1186$ (C = S); 1485; 1633 (NH); 2852, 2921 (aliph. C-H); 3193 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.30–1.84 (bm, 14H); 2.72 (t, 2H, CH_2 of the cyclohexane ring); 3.94 (bs, 2H, C(8)H_2), 7.26 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.80, 23.39, 23.84, 25.05, 32.73 (5 pentamethylene C); 23.38 (C7); 23.85 (C6); 45.07 (C8); 46.89 (C5); 79.21 (C3); 175.90 (C = S). MS (70 eV, 180 °C) m/z (%): 225.1 (48) [$M^{+\cdot}$]; 182.0 (86); 169.0 (18); 85.0 (44) [$C_4H_9N_2^+$]; 81.0 (18); 70.9 (16); 56.3 (15); 55.3 (22); 42.1 (13); 41.0 (35); 39.1 (10); 32.0 (20); 30.0 (23); 28.0 (100). Elemental analysis [$C_{11}H_{19}N_3S$ (225.4), %]: calcd. C, 58.63; H, 8.50; N, 18.65; found C, 57.95; H, 8.26; N, 18.38.

*3,3-Diphenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo [1,2-a]pyridazine-1-thione (**3h**)*

With benzophenone (1.002 g). Reaction time 13 h. The synthesis differed from the general method by washing the isolated crystals with a little methanol. Yield 75%. Colorless crystals. M.r. 252–255 °C (methanol).

TLC (m. P. I): $R_f = 0.84$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1166$ (C = S); 1452; 1472; 1634 (NH); 2853; 2922; 2944; 2960 (aliph. C-H); 3060 (arom. C-H); 3152 (NH). ^{1}H NMR (CDCl3, ppm): δ = 1.58–1.86 (m, 4H, C(6)H_2 and C(7)H_2); 2.25 (t, 2H, C(8)H_2); 3.98 (s, 2H, C(5)H_2); 7.28–7.41 (m, 10H, arom. H); 7.94 (s, 1H, NH). ^{13}C NMR (CDCl3, ppm): δ = 23.22; 24.08 (C6, C7); 45.12 (C8); 49.90 (C5); 85.78 (C3); 127.27, 128.33, 128.41, 139.81 (all arom. C); 175.96 (C = S). MS (70 eV, 250 °C) m/z (%): 309.5 (65) [$M^{+\cdot}$]; 232.4 (16) [$M^{+\cdot}$ – $^{\cdot}C_6H_5$]; 224.4 (28); 165.3 (37); 121.3 (12); 103.9 (15); 85.2 (100) [$C_4H_9N_2^+$]; 77.5 (16); 56.6 (14); 41.2 (12) 30.1 (27). Elemental analysis [$C_{18}H_{19}N_3S$ (309.4), %]: calcd. C, 69.87; H, 6.19; N, 13.58; found C, 69.56; H, 5.81; N, 13.53.

*3-(2-Chlorophenyl)-3-phenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3i**)*

With *o*-chlorobenzophenone (1.192 g). Reaction time 15 h. The synthesis differed from the general method by treating the melt with acetone and washing the formed crystals with a little acetone. Yield 38%. Colorless crystals. M.r. 244–246 °C (acetone).

TLC (m. P. I): $R_f = 0.89$. IR (KBr, cm^{-1}): $\tilde{\nu} = 753$ (ar–Cl); 1166 (C = S); 1443; 1460; 1632 (NH); 2840; 2926; 2937; 2956 (aliph. C-H); 3066 (arom. C-H); 3222 (NH). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.63–1.78 (m, 4H, C(6)H_2 and C(7)H_2); 1.94–2.01; 2.38–2.48; 3.33; 4.59 (m and m and t and d, 8H; C(8)H_2; C(5)H_2); 7.27–7.38 (m, 9H, arom. H); 8.13 and 8.17 (each bs, 0.5 H, NH). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.06 (C7); 24.14 (C6); 45.17 (C8); 49.83 (C5); 85.12 (C3); 126.55, 127.83, 128.39, 128.67, 128.97, 129.94, 131.45, 131.95 (all arom. C); 176.58 (C = S). MS (70 eV, 300 °C) m/z (%): 343.0 (3) [$M^{+\cdot}$]; 308.0 (21) [$M^{+\cdot}$ – $^{\cdot}Cl$]; 165.0 (11); 85.0 (45) [$C_4H_9N_2^+$]; 32.0 (23); 30.0 (12); 28.0 (100). Elemental analysis [$C_{18}H_{18}ClN_3S$ (343.9), %]: calcd. C, 62.87; H, 5.28; N, 12.22; found C, 60.54; H, 5.76; N, 12.43.

*3-(4-Chlorophenyl)-3-phenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3j**)*
With *p*-chlorobenzophenone (1.192 g). Reaction time 21 h. The synthesis differed from the general method by dissolving the melt in propan-2-ol and subsequent concentrating the solution. Thereby formed crystals, which were washed with a little acetone. Yield 52%. Colorless crystals. M.r. 252–254 °C (propan-2-ol/acetone).
TLC (m. P. I) $R_f = 0.82$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1167$ (C = S); 1450; 1472; 1593; 1632 (NH); 2853, 2944, 2962, (aliph. C-H); 3158 (broad band, arom. C-H and NH). ^{1}H NMR ($CDCl_3$, ppm): $\delta = 1.64$ (m, 2H, $C(6)H_2$) and 1.82 (m, 2H, $C(7)H_2$); 2.21 (m, 2H, $C(5)H_2$); 3.83 (bs, 1H, $C(8)H_2$); 4.07 (bs, 1H, $C(8)H_2$); 7.32 (m, 9H, arom. H); 8.17 (s, 1H, (NH). ^{13}C NMR ($CDCl_3$, ppm): $\delta = 23.18$ (C7); 24.03 (C6); 45.17 (C7); 49.90 (C8); 85.42 (C3); 127.34, 128.49, 128.55, 128.58, 128.75, 134.34, 138.84 (all arom. C); 175.93 (C = S). MS (70 eV, 250 °C) m/z (%): 342.8 (14) [$M^{+\cdot}$]; 258.0 (8); 164.9 (16); 85.0 (100) [$C_4H_9N_2^+$]; 28 (50). Elemental analysis [$C_{18}H_{18}ClN_3S$ (343.9), %]: calcd. C, 62.87; H, 5.28; N, 12.22; found C, 62.24; H, 5.05; N, 12.04.

3-(4-Methoxyphenyl)-3-phenyl-2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione (**3k**)
With p-methoxybenzophenone (1.167 g). Reaction time 14 h. Yield 40%. Colorless crystals. M.r. 218–220 °C (propan-2-ol).
TLC (m. P. I): $R_f = 0.78$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1176$ (C = S); 1509; 1583; 1609 (boad basis; arom. ring and NH); 2842, 2958 (aliph. C-H); 3007 (arom. C-H); 3165 (NH). ^{1}H NMR ($CDCl_3$, ppm): $\delta = 1.63$ (q, 2H, $C(6)H_2$); 1.77 (q, 2H, $C(7)H_2$); 2.22 (q, 2H, $C(5)H_2$); 3.78 (s, 3H, $-OCH_3$); 4.04 (bs, 2H, $C(8)H_2$); 6.82 (d, 2H, arom. H); 7.28 (d, 7H, arom. H); 7.76 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, ppm): $\delta = 23.24$ (C6); 24.09 (C7); 45.15 (C8); 49.86 (C5); 55.27 ($-O\underline{C}H_3$); 85.52 (C3); 113.66, 127.10, 128.28, 128.78, 131.28, 140.48, 159.64 (all arom. C); 175.81 (C = S). MS (70 eV, 225 °C) m/z (%): 339.0 (27) [$M^{+\cdot}$]; 254.0 (100); 85.0 (51) [$C_4H_9N_2^+$]; 56.3 (12); 41.0 (13); 32.0 (12); 30.1 (67) 28 (11); 26 (15). Elemental analysis [$C_{19}H_{21}N_3OS$ (339.5), %]: calcd. C, 67.23; H, 6.24; N, 12.38; found C, 66.81; H, 5.96; N, 12.13.

Method 2: Preparation by heating of 1a with ketones in a microwave reactor - general procedure: 1 mmol (0.145 g) hexahydropyridazine-1-carbothioamide **1a** and 1.1 mmol of the appropriate ketone were reacted in a microwave reactor under the described conditions (power, temperature, pressure). Subsequently, the mixture was cooled at 4 °C for 24 h. The product was suspended in a small amount of the solvent given below. The formed crystalline product was collected, washed carefully with the mentioned solvent and dried.
In this manner the following compounds were obtained:

*3,3-Dimethyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3a**)*
With acetone (0.063 g). 150 W, 125 °C, 10 bar. Reaction time 40 min. Acetone as solvent for working up. Yield 66%.

*3-Methyl-3-phenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3d**)*
With acetophenone (0.132 g). 300 W, 130 °C, 15 bar. Reaction time 30 min. Methanol as solvent for working up. Yield 49%.

*3,3-Diphenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3h**)*
With benzophenone (0.200 g). 300 W, 130 °C, 15 bar. Reaction time 30 min. Methanol as solvent for working up. Yield 61%.

*3-(2-Chlorophenyl)-3-phenyl-2,3,5,6,7,8-hexahydro-1H-[1,2,4]triazolo[1,2-a]pyridazine-1-thione (**3i**)*
With 2-chlorobenzophenone (0.238 g). 300 W, 130 °C, 15 bar. Reaction time 10 min. Ethyl acetate as solvent for working up. Yield 8%.

3,3-Dimethyl-2-phenyl-2,3,5,6,7,8-hexahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione (**4a**)
A mixture of 0.5 mmol (0.073 g) hexahydropyridazine-1-carbothioamide **1a,** 5 mL acetone, and 0.5 mmol (0.067 g) anhydrous aluminium chloride was refluxed for 2 h. Subsequently, the batch was carefully concentrated, and the residue was treated with 1 mL water, whereby crystals formed. The crystals were sucked off, washed twice with a little water and then once with a little cold propan-2-ol, and dried. Yield 64%. Colorless crystals. M.r. 138–140 °C (acetone).
TLC (m. P. I): $R_f = 0.70$. IR (KBr, cm^{-1}): $\tilde{\nu} = 1179$ (C = S); 1497, 1597 (arom. ring); 2846, 2933, 2948, 2965, 2980 (aliph. H); 3032, 3063 (arom. H). ^{1}H NMR ($CDCl_3$, ppm): $\delta = 1.35$ (s, 6H, 2 x CH_3); 1.68 (m, 2H, $C(6)H_2$); 1.88 (m, 2H, $C(7)H_2$); 2.82 (t, 2H, $C(5)H_2$); 4.03 (bs, 2H, $C(8)H_2$); 7.23–7.28 (m, 2H, arom. H); 7.38–7.50 (m, 3H, arom. H). ^{13}C NMR ($CDCl_3$, ppm): $\delta = 22.13$ (broad and flat signal, 2 x CH_3); 22.97 (C7); 23.94 (C6); 45.89 (C8); 47.59 (C5); 82.66 (C3); 128.37, 129.11, 130.31, 137.12 (all arom. C); 177.50 (C = S). MS (70 eV, 320 °C) m/z (%): 260.9 (56) [$M^{+\cdot}$]; 245.8 (100) [$M^{+\cdot}$ - $\cdot CH_3$]; 126.0 (18); 117.7 (10); 102.7 (15), 77.3 (19); 70.8 (21); 41.9 (11); 41.0 (12); 27.9 (38). Elemental analysis [$C_{14}H_{19}N_3S$ (261.4), %]: calcd. C, 64.33; H, 7.33; N, 16.08; found C, 63.58; H, 7.52; N, 15.88.

*3.2.5. 5,6,7,8-Tetrahydro-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-imines **5***

5,6,7,8-Tetrahydro-[1,3,4]thiadiazolo[3,4-*a*]pyridazin-1-imine (**5a**)
A mixture of 1 mmol (0.145 g) hexahydropyridazine-1-carbothioamide **(1a)**, 1 mL methanol, and 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) was refluxed (bath temperature 120 °C) for 3 h. After cooling, overnight the resulting viscous mass was triturated with some methanol and the formed crystals were separated. The crystals were washed with a little methanol and dried on a clay plate. Yield 23%. Colorless crystals. M.r. 209–213 °C (methanol).
TLC (m. P. II): $R_f = 0.73$. IR (cm^{-1}): $\tilde{\nu} = 1526$ (NH); 1563 (C = N); 2854, 2940 (CH_2); 3183 (NH). MS (70 eV, 255 °C): m/z (%): 157.1 [$M^{+\cdot}$] (24); 105.0 (14); 98 (19); 97.0 (30); 87.0 (21); 77.1 (13); 75.0 (12); 74.1 (28); 70.0 (16); 57.1 (21); 56.1 (12); 55.0 (25); 43.0 (34); 42.0 (30); 41.0 (30); 31.9 (25); 28.9 (19); 28.9 (15); 27.8 (100); 26.9 (14).

Hydrochloride of **5a**:
0.1 mmol (0.016 g) **5a** was dissolved in concentrated hydrochloric acid (some drops) and after dilution with methanol (about 2 mL) the solution was slowly concentrated at room temperature. The formed crystalline **5a** hydrochloride was filtered off, washed with a little methanol and dried at room temperature in vacuo. It was utilized for the following analytical investigations. Yield 85%. Yellow crystals. M.r. 205–230 °C (methanol).
HPLC (m. P. V): RP-Select B, $t_0 = 1.98$; $t_R = 2.55$; k' = 0.29. ^{1}H NMR (DMSO-d_6), ppm): $\delta = 1.57$ (bs, 2H, $C(7)H_2$); 1.79 (t, J = 10.4 Hz, 2H, $C(6)H_2$); 2.95 (t, J = 10.0 Hz, 4H, C(5(H_2 and $C(8)H_2$); 4.89 (s, 2H, $C(3)H_2$); 10.02 (bs, 1H, $-NH_2^+$); 10.47 (bs, 1H, $-NH_2^+$). ^{13}C NMR (DMSO-d_6), ppm): $\delta = 22.33$(C6); 22.66 (C7); 46.19 (C8); 51.45 (C5); 58.49 (C3); 169.20 (C = N). HRMS [(ESI) m/z]: calcd. for $C_6H_{11}N_3S$ (base) + H^+: 158.0746; found [M (base) + H^+]: 158.0783

N-Phenyl-5,6,7,8-tetrahydro-[1,3,4]thiadiazolo[3,4-*a*]pyridazin-1-imine (**5b**)

A mixture of 0.5 mmol (0.111 g) *N*-phenyl-hexahydropyridazine-1-carbothioamide **1b** and 0.070 g 30% aqueous formaldehyde solution (approximately 0.7 mmol formaldehyde) was allowed to stand at room temperature for 2 days in a sealed reaction vessel. Subsequently, 5 mL water were added and the formed crystals were separated, washed with some water, and dried in vacuo. Yield 75%. Colorless crystals. M.r. 59-62 °C (water).

TLC (m. P. I): $R_f = 0.70$. HPLC (m. P. VIII): RP-select B, $t_0 = 1.95$; $t_R = 7.61$; k' = 2.90. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1487, 1586 (arom. ring); 1612 (C = N); 2824, 2851, 2938, 2993 (aliph. H); 3028, 3053, 3076 (arom. H). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.61 (m) and 1.87 (m, 4H, C(6)H_2 and C(7)H_2); 2.72 (bs, 1H, C(5)H); 3.03 (bs, 1H, C(5)H); 3.34 (bd, 1H, C(8)H); 4.23 (bs, 1H, C(3)H_2) and 4.35 (bs, 1H, C(3)H_2); 4.73 (bs, 1H, C(8)H); 6.93–7.10 (m, 3H, arom. H); 7.26–7.39 (m, 2H, arom. H). ^{13}C NMR ($CDCl_3$, ppm): δ = 20.60 (C6); 24.02 (C7); 45.66 (C8); 50.35 (C5); 58.11 (C3); 121.52, 123.39, 128.93, 152.91 (6 arom. C) 160.73 (C = N). MS (70 eV, RT) m/z (%): 232.9 (23) [$M^{+\cdot}$]; 134.9 (3) [C_6H_5-N = C = S^+]; 98.0 (100) [$M^{+\cdot} - {}^{\cdot}C_6H_5$-N = C = S]; 96.9 (35); 77.3 (10) [$C_6H_5^+$]; 42.1 (21); 28 (13). HRMS [(ESI) m/z): calcd. for [$C_{12}H_{15}N_3S + H]^+$: 234.1059; found [M + H]$^+$: 234.1060

3.2.6. *4-Nitrobenzamides **9b** und **10b***

4-Nitro-*N*-(hexahydropyridazin-1-ylmethyl)benzamide hydrochloride (**9b** HCl)

A mixture of 5 mmol (0.981 g) **8** hydrochloride, 5 mmol (0.831 g) 4-nitrobenzamide, 5 mmol (0.150 g) paraformaldehyde and 10 mL ethanol was refluxed for 5 h. Subsequently, the reaction mixture was allowed to stand for 12 h at room temperature. The formed crystals were filtered off, washed with acetone, and recrystallized from water. The formed crystals were separated and washed once again with acetone. Yield 62%. Colorless crystals. M.r. 189–192 °C (water/acetone).

TLC (m. P. IV): $R_f = 0.50$. HPLC (m. P. V): RP-select B, $t_0 = 1.75$; $t_R = 2.82$; k' = 0.61.

IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1344, 1518 (NO_2); 1530 (NH); 1602; 1669 (C = O); 2448, 2533, 2558, 2715, 2795, 2854, 2933, 2953 (peaks of a broad absorption band, aliph. H); 3038, 3069 (arom. H); 3105, 3246 (NH). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.70 (bs, 4H, heterocycl. C(4)H_2 and C(5)H_2); 2.95 (bs, 2H; heterocycl. C(6)H_2); 3.17 (bs, 2H, heterocycl. C(3)H_2); 4.56 (d, $^3J_{HH}$ = 6.2 Hz, 2H, -N-C$\underline{H_2}$-NH-); 8.23 (d, $^3J_{HH}$ = 8.8 Hz, 2H, arom. C(2')H and C(6')H); 8.36 (d, $^3J_{HH}$ = 8.8 Hz, 2H, arom. C(3') and C(5')H); 9.65 (t, $^3J_{HH}$ = 6.2 Hz, 1H, amide NH); 10.28 (bs, 2H, heterocycl. NH_2^+). ^{13}C NMR (DMSO-d_6, ppm); δ = 19.49 (C4), 21.96 (C5), 48.69 (C6), 48.72 (C3) (all heterocycl. C); 59.17 (-N-$\underline{C}H_2$-NH-); 123.38 (C3', C5'), 129.29 (C2', C6'), 139.18 (C1'), 149.28 (C4'); 166.28 (C = O). MS (70 eV, 345 °C) m/z (%): 263.9 (5) [$M^{+\cdot}{}_{Base}$]; 165.9 (15) [$M^+ - CH_2C_4H_8N_2$]; 149.0 (28); 103.7 (11); 98.0 (21); 85.0 (22) [$C_4H_9N_2^+$]; 36.0 (15); 32.0 (22); 30.0 (13); 28 (100). HRMS [(ESI) m/z]: calcd. for [M for $C_{12}H_{16}N_4O_3$ (base) + H^+]: 265.1292; found [M (base) + H^+]: 265.1295. Elemental analysis [$C_{12}H_{17}ClN_4O_3$ (300.74), %]: calcd. C, 47.92; H, 5.70; N, 18.63; found C, 47.69; H, 5.74; N, 18.90.

4-Nitro-*N*-(piperidin-1yl-methyl)benzamide (**10b**)

Prepared by the procedure as described before (Foldeak et al. 1962). Yield 78%. Yellowish crystals. M.r. 122-128 °C (ethanol) ((Foldeak et al. 1962): 205 °C).

TLC (m. P. IV): $R_f = 0.12$. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1523 (NO_2); 1534 (NH); 1599; 1660 (C = O); 2703, 2796, 2850, 2914, 2930, 2969 (peaks of a broad absorption band, aliph. and arom. H,); 3168 (amide NH); 3286 (heterocycl. NH^+). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.06 (bs, 2H, heterocycl. C(4)H_2); 1.79 (m, 4H, heterocycl. C(3)H_2 and C(5)H_2); 2.96 (bs, 2H, heterocycl. C(2)H_2 and C(6)H_2) and 3.34 (bs, 2H, heterocycl. C(2)H_2 and C(6)H_2); 4.61 (bdd, $^3J_{H(N)H}$ = 6.4 Hz, 2H, -N-C$\underline{H_2}$-NH-); 8.22 (d, $^3J_{HH}$ = 9.0 Hz, 2H, arom. C(2')H and C(6')H); 8.38 (d, $^3J_{HH}$ = 9.0 Hz, 2H, arom. C(3') and C(5')H). 10.03 (t, $^3J_{H(N)H}$ = 6.4 Hz, 1H, amide NH); 10.23 (bs, 1H, heterocycl. NH^+). ^{13}C NMR (DMSO-d_6, ppm); δ = 21.08 (C4), 22.11 (C3 and C5), 49.98 (C2 and C6) (all heterocycl. C); 59.27 (N–$\underline{C}H_2$–NH-); 123.57, 129.34, 138.22, 149.60 (all arom. C); 166.24 (C = O). HRMS [(ESI) m/z]: calcd. for [M for $C_{12}H_{16}N_4O_3$ (base) + H^+]: 264.1336; found [M (base) + H^+]: 264.1343. Elemental analysis [$C_{13}H_{17}N_3O_3$ (263.3), %]: calcd. C, 59.30; H, 6.51; N, 15.96; found C, 58.07; H, 6.08; N, 16.16.

Hydrochloride of **10b**:

Colorless crystals. M.r. 191–201 °C (ethanol) ((Foldeak et al. 1962): 215 °C).

3.2.7. *N-Substituted phthalimide derivatives **10c** and **12***

2-(Piperidin-1-ylmethyl)-1,3-dihydroisoindoline-1,3-dione (**10c**)

Prepared by the described procedure (Feldman, Wagner 1942) however, in a small batch containing 1 mmol piperidine (0.085 g), 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) and 1 mmol (0.147 g) phthalimide and utilizing ethanol as a solvent. Yield 86%. Colorless crystals. M.r. 121–123 °C (ethanol) (Feldman, Wagner 1942): 119–119.5 °C (ethanol)).

TLC (m. P. IV): $R_f = 0.88$. HPLC (m. P. VII): RP-18, $t_0 = 1.75$; $t_R = 2.78$; k' = 0.59. IR (cm^{-1}): $\tilde{\nu}$ = 1463; 1611; 1702, 1769 (C = O); 2811, 2852 (aliph. H); 2928 (peak of an absorption band, arom. H). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.37 (m, 2H, piperidino C(4)H_2); 1.57 (m, 4H, piperidino C(3)H_2 and C(5)H_2); 2.61 (t, $^3J_{H(C3/5)H}$ = 10.4 Hz, 4H, piperidino C(2)H_2 and C(6)H_2); 4.63 (s, 2H, N-C$\underline{H_2}$-N); 7.74 (m, 2H, arom. H); 7.88 (m, 2H, arom. H). ^{13}C NMR ($CDCl_3$, ppm): δ = 23.79 (C4), 25.99 (C3 and C5), 51.95 (C2 and C6) (all piperidino C); 60.35 (N-$\underline{C}H_2$-N); 123.40 (arom. C4' and C7'); 132.05 (arom. C3a', C7a'); 134.06 (arom. C5' and C6'); 169.42 (2 x C = O). HRMS [(ESI) m/z]: calcd. for [$C_{14}H_{16}N_2O_2 + H]^+$: 245.1285; found: 245.1248 [M + H]$^+$.

2,2'-(Hexahydropyridazin-1,2-ylendimethylene)-di(1,3-dihydroisoindoline-1,3-dione) (**12**)

A mixture of 1 mmol (0.123 g) **8** hydrochloride, 1 mmol (0.147 g) phthalimide, 5 mL ethanol, 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) and 1 mmol (0.101 g) triethyl amine was refluxed (bath temperature 120 °C) for 5 h. After cooling and during standing overnight, crystals were formed, which were separated, washed with ethanol, and dried on a clay plate. Yield 5%. Pale yellow crystals. M.r. 194–196 °C (ethanol).

TLC (m. P. IV): $R_f = 0.93$. HPLC (m. P. VII): RP-18, $t_0 = 1.75$; $t_R = 7.19$; k' = 3.11. IR (cm^{-1}): $\tilde{\nu}$ = 1706, 1772 (C = O); 2858 (aliph. H)., 2940 (arom. H). ^{1}H NMR ($CDCl_3$, ppm): δ = 1.65 (t, J = 7.0 Hz, 4H, hexahydropyridazine C(4)H_2 and C(5)H_2); 3.16 (bs, 4H, hexahydropyridazine C(3)H_2 and C(6)H_2); 4.56 (s, 4H, 2 x N-C$\underline{H_2}$-N); 7.47–7.60 (m, 8H, all arom. H). ^{13}C NMR ($CDCl_3$, ppm): δ = 19.97 (hexahydropyridazine C4 and C5); 43.37 (hexahydropyridazine C3 and C6); 54.95 (2 x N-$\underline{C}H_2$-N); 123.00 (arom. C4', C7', C4", C7"); 132.04 (arom. C3a', C7a' C3a", C7a"); 133.48 (arom. C4', C5', C4", C5"); 167.55 (C = O, C1', C3', C1", C3"). MS (70 eV, 180 °C) m/z (%): 405.1 (8) [M + H^+]; 244.7 (51) [$C_{13}H_{14}N_3O_2^+$]; 160.4 (100) [$C_9H_6NO_2^+$]; 133.3 (13);

105.2 (10); 104.2 (17); 97.2 (69); 77.2 (24); 76.2 (17). HRMS [(ESI) m/z]: calcd. for $[C_{22}H_{20}N_4O_4 + H]^+$: 405.1557; found: 405.1549 $[M + H]^+$.

3.2.8. Anellated hexahydropyridazine derivatives **11,13**, and **14**

5,6,7,8-Tetrahydro[1,3,4]thiadiazolo[3,4-*a*]pyridazine-1,3-dithione (**11**)

Method 1 - from 8 hydrochloride and carbon disulphide: A mixture of 1 mmol (0.123 g) **8** hydrochloride, 5 mL methanol, 1 mmol (0.101 g) triethyl amine, and 15 mmol (1.142 g) carbon disulphide was heated (bath temperature 80–90 °C). Within 10 min fine needles crystallized. After the reaction was completed (TLC monitoring, about 30 min), the crystals were filtered off and washed with some ethanol. Yield 82%. Colorless needles. M.r. 188–190 °C (ethanol).
TLC (m. P. I): $R_f = 0.65$. HPLC (m. P. VII): RP-select B, $t_0 = 1.88$; $t_R = 6.94$; k' = 2.69. IR (KBr, cm^{-1}): $\tilde{\nu} = 1191$ (C = S, extraordinary intensively); 1403; 2858, 2929, 2952 (CH_2). ^1H NMR ($CDCl_3$, ppm): δ = 2.05 (m, 4H, $C(6)H_2$, $C(7)H_2$); 4.20 (m, 4H; $C(5)H_2$, $C(8)H_2$). ^{13}C NMR ($CDCl_3$, ppm): δ = 20.73 (C6, C7); 49.76 (C5, C8); 181.97 (2 x C = S). MS (70 eV, 60 °C) m/z (%): 204.1 (100) $[M^{+\cdot}]$; 84.1 (6) $[C_4H_8N_2^+]$; 72.0 (27); 41.1 (29); 32 (14), 29 (12); 27.9 (65). Elemental analysis $[C_6H_8N_2S_3$ (204.3), %]: calcd. C, 35.27; H, 3.95; N, 13.71; S, 47.07; found C, 35.96; H, 3.91; N, 13.59; S, 46.66.

Method 2 - as a product of the attempt of the cyclization of 9b hydrochloride with carbon disulphide: To a suspension of 0.33 mmol (0.099 g) **9b** hydrochloride in 5 mL ethanol 1 mmol (0.101 g) triethyl amine and 15 mmol (1.142 g) carbon disulphide were added. The mixture was heated (bath temperature 80–90 °C) for 6 h. Subsequently, the solution was concentrated and with the resulting product (about 50 mg) was done a column chromatographic separation (solid phase silica gel H Merck; mobile phase petroleum ether/ethyl acetate 5:1 (v/v), column: length 10 cm, diameter 1 cm). The first fraction (TLC monitoring) was evaporated to dryness, the resulting crystals were collected, washed with some acetone and dried in vacou. Yield 31%. Pale yellow crystals. M.r. 180–184 °C [petroleum ether/ethyl acetate 5:1 (v/v)].
TLC (m. P. I): $R_f = 0.65$. Mixed melting temperature with the substance yielded by method 1: 184–189 °C.

1,2,3,4,8,9,10,11-Octahydrodipyridazino [1,2-*a*:1',2'-*d*][1,2,4,5]tetrazine (**13**)

A mixture of 1 mmol (0.123 g) **8** hydrochloride, 1 mmol (0.185 g) potassium salt of phthalimide, 2 mL water, and 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) was refluxed for 6 h (bath temperature 120 °C). After cooling overnight, the mixture was allowed to concentrate at room temperature. The resulting solid was digested several times with anhydrous diethyl ether. The combined diethyl ether fractions were evaporated to dryness, and the resulting product was dried at room temperature. Yield 13% Yellowish crystals. M.r. 150–160 °C (diethyl ether, impurities by phthalimide and **8** hydrochloride). ((Schulz et al. 2013): 168 °C (ethanol/diethyl ether)).
TLC (m. P. IV): $R_f = 0.80$. HPLC (m. P. VII): RP-18, $t_0 = 1.75$; $t_R = 3.02$; k' = 0.53. IR (cm^{-1}): $\tilde{\nu} = 2696$, 2735, 2776, 2848, 2936 (aliph. H). ^1H NMR ($CDCl_3$, ppm): δ = 1.64 (bs, 8H, $C(2)H_2$, $C(3)H_2$, $C(9)H_2$ and $C(10)H_2$); 2.37 (bs, 4H, je 1H of $C(1)H_2$, $C(4)H_2$, $C(8)H_2$, $C(11)H_2$); 2.78 (d, 4H, J = 10.2 Hz, je 1H of $C(1)H_2$, $C(4)H_2$, $C(8)H_2$, $C(11)H_2$); 3.06 (d, J = 8.4 Hz, 2H, je 1H von $C(6)H_2$, $C(13)H_2$); 3.53 (d, J = 8.4 Hz, 2H, je 1H of $C(6)H_2$, $C(13)H_2$) [(Nelsen, Hintz 1972), the τ values given there are transformed into δ values here: 1.65 (m, 8H); 2.39 (m, 4H, –N–CH_2–C); 2.78 (d, J = 11 Hz, 4H, –N–CH_2–CH_2–); 3.05 (d, J = 9 Hz, 2H, –N–CH_2–N–); 3.55 (d, J = 9 Hz, 2H, –N–CH_2–N–]. ^{13}C NMR ($CDCl_3$, ppm): δ = 23.84 (C2, C3, C9, C10); 52.60 (C1, C4, C8, C11); 79.77 (C6 and C13). HRMS [(ESI) m/z): calcd. for $[C_{10}H_{20}N_4 + H]^+$: 197.1761; found $[M + H]^+$: 197.1698

1,2,3,4,6,11-Hexahydropyridazino[1,2-*b*]phthalazine-6,11-dione (**14**)

To a solution of 1 mmol (0.123 g) **8** hydrochloride and 1 mmol (0.185 g) potassium salt of phthalimide in 2 mL water 0.1 g 30% aqueous formaldehyde solution (approximately 1 mmol formaldehyde) was added and the mixture was irradiated for 5 min in a microwave reactor (90 W, 130 °C, 3.3 bar). After cooling, the resulting crystals were collected, washed with water, and dried in vacuo at room temperature.
Yield 9%. Yellowish crystals. M.r. 135-141 °C (water) [(Rink and Grabowski 1956):158-160 °C (water); (Clement 1960): two forms of crystals were isolated - swords 145.5-146.5 °C (water) and fine needles 156-156.5 °C (water)].
TLC (m. P. IV): $R_f = 0.86$. HPLC (m. P. VII): RP-18, $t_0 = 1.75$; $t_R = 3.01$; k' = 0.53. IR (cm^{-1}): $\tilde{\nu} = 1602$; 1633; 1664; 1710 (C = O); 2850 (aliph. H); 2949 (arom. H). ^1H NMR ($CDCl_3$, ppm): δ = 1.97 (m, 4H, $C(2)H_2$, $C(3)H_2$); 4.19 (t, $^3J = 1.4$ Hz, 4H, $C(1)H_2$, $C(4)H_2$); 7.28–7.85 (m, 2H, C(7)H, C(10)H); 8.26–8.35 (m, 2H, C(8)H, C(9)H). ^{13}C NMR ($CDCl_3$, ppm): δ = 22.14 (C2, C3); 44.32 (C1, C4); 127.70 (C7, C10), 128.85 (C6a, C10a); 133.39 (C8, C9); 158.51 (C = O, C6, C11). HRMS [(ESI) m/z]: calcd. for $[C_{12}H_{12}N_2O_2 + H]^+$: 217.0972; found $[M + H]^+$: 217.0964.

3.3. Biology

Material and methods are described in detail in a previous publication (Schulz et al. 2013). Here are the key parameters given.

3.3.1. Cell culture materials

All chemicals as well as interleukin-1β, fetal calf serum and trypane blue were from Sigma Aldrich. Cell culture plastics, PBS, trypsin/EDTA and interferon-γ were obtained from Biochrom AG, RPMI1640 and penicillin/streptomycin from Lonza.

3.3.2. Cell line

The insulin-producing rat insulinoma cell line RIN5F (ECACC catalogue No. 95090402) was used. The adherent cells were grown under standard conditions (95% humidity, 37 °C, 5% CO_2) in RPMI1640 supplemented with L-glutamine (2 mmol/L), 10% heat-inactivated FCS, penicillin (100 I.E.) and streptomycin (100 I.E.).

3.3.3. iNOS expression and inhibition

Tests were performed with 1×10^5 cells per well in 200 μL culture media in 96-well plates at standard culture conditions. Twenty-four hours after cells had been seeded out, iNOS was induced by adding 1 ng/mL IL-1β (human, recombinant) and 10 ng/mL IFN-γ (rat, recombinant). For the primary screening, test compounds were added to the cells at concentrations of 78 μM and 39 μM with 0.5% DMSO as solutizer. The reference inhibitor aminoguanidine was tested at the same concentrations. DMSO was added to the controls as well. Selected substances with considerably higher inhibition of iNOS than aminoguanidine were chosen for determination of IC_{50} that was calculated

from linear regression of logarithmized concentrations versus percentage of inhibition.

3.3.4. NO assay

Nitrite concentration, an indication for the degree of NO production, was determined from 50 μL cell-free supernatant (two samples from each cavity) using the Griess reaction (Green et al. 1982, Griess 1879). For this, each 50 μM of a 0.1% sulfanilamide solution in 5% phosphoric acid and an aqueous 0.01% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution were separately pipetted to the supernatant and incubated for 5 min in the dark. Afterwards, the absorption was measured in a plate reader at a wavelength of 550 nm. For calibration, a dilution series of nitrite (0 to 100 μM) was included on every plate.

3.3.5. MTT assay

The remaining cells in the test plates were used to perform the MTT assay as a test of cytotoxicity (Mosmann 1983). Medium was completely removed and replaced with 100 μL of fresh medium then 20 μL MTT in PBS (2.5 mg/mL) were added. After 4 h incubation at 37 °C medium was carefully removed and 100 μL DMSO were added. After dissolution of the formazan crystals the absorption was immediately measured at a wavelength of 570 nm.

3.3.6. Statistics

Normality was tested and if positive F- and t-test were performed. Otherwise Mann-Whitney-test was executed. Statistical significance was assumed for $p < 0.05$ or lower.

3.4. 3D structures

3D structure models of compound 2a was obtained with the program MOE 2011.10 ((2011) by using the MMFF94x force field.

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