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Synthesis, chemical behavior, structure elucidation and iNOS inhibitory activity of 1-substituted 3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazines[+]

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[+] *N,N-Coupled heterobicycles from cyclic hydrazine derivatives. Part 16. Part 15: Schulz et al. (2014)*

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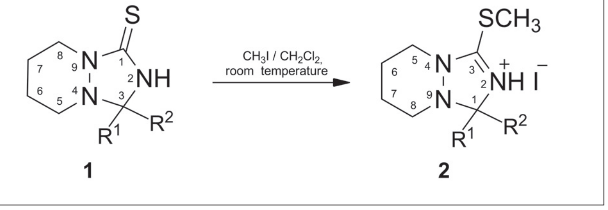
Novel slim and shapely sp^3-rich nitrogen containing heterocyclic ring systems are sought-after platforms for the expansion of molecular diversity in lead discovery. The present work describes the synthesis and characterization of a series of derivatives of hitherto unknown 3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazines **2**. This approach was guided by a computational study, aiming at an optimization of previously reported [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **1** known to inhibit the inducible nitric oxide synthase (iNOS). The title compounds are accessible by methylation of compounds **1** under mild conditions. The products were biologically evaluated by the same cell-based assay as applied for previous products of type **1** using RINm5F cells, which were stimulated to produce NO on the influence of proinflammatory cytokines IL-1β and IFN-γ. Compounds **2** did not display the anticipated improved iNOS inhibitory activity in the selected assay but contribute to SAR in the field. In addition, an unprecedented formation of side-products **3** via oxidation has been investigated. The novel scaffolds represent attractive starting points for the construction of diverse molecules which differ considerably from known compounds based on flat and lipophilic aromatic scaffolds.

1. Introduction

Inducible nitric oxide synthase (iNOS) inhibitors are potential drug candidates due to the critical role of an excessive production of nitric oxide in the pathophysiology of several human diseases. Recently we reported the synthesis of novel [1,2,4]triazolo[1,2-*a*]-pyridazine-1-thiones **1** (Scheme 1) varied in position 3 and their iNOS inhibitory activity in a cell-based assay. The 3,3-disubstituted (Schulz et al. 2014) and the 3-aryl derivatives (Schulz et al. 2013) were directly obtained from hexahydropyridazine-1-carbothioamide (Morgenstern et al. 2004) and the corresponding carbonyl compounds. In contrast, the synthesis of the 3-unsubstituted parent system required additional reaction steps and proceeded via intermediate products (Schulz et al. 2014). The bicyclic thiones **1** showed the desired iNOS inhibitory activity but were not potent enough in comparison with published lead compounds (Salerno et al. 2002). An overview of the status of structurally simple drug-like aromatic 1400W (Garvey et al. 1997) or more sophisticated slim and shapely sp^3-rich natural product-like iNOS inhibitors such as ONO-1714 (Naka et al. 2000) is given in our previous publication (Schulz et al. 2013).



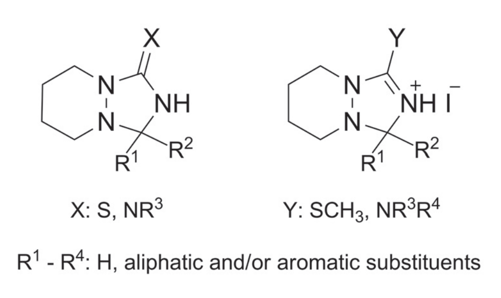
Scheme 1: Route for the synthesis of the title compounds **2** starting from the reported (Schulz et al. 2014; Schulz et al. 2013) thiones **1** (for reasons of clarity the numbering of the systems according to the rules of IUPAC is provided)

Since the active site is highly conserved among the different NOS isoenzymes, the design of potent and selective inhibitors remains a challenging problem. Thus, in accordance with the recent proposal by Tsukamoto (2013), we concentrated on underrepresented though nontrivial compounds, rather than synthesizing modular and sp^2-flat fragments through routine operations. We applied computer-aided methods to support the optimization of the [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **1** in continuation of our previous studies on this versatile sp^3-rich structure. The impact of a methyl group on the sulfur atom on the inhibitory activity against iNOS became the focus of our investigations. Here, we compare the *in silico* predictions to the *in vitro* results in the context of formerly obtained data on thiones **1** (Schulz et al. 2014, 2013). The synthesis of the hitherto unknown 3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazines **2** from thiones **1** was performed using methyl iodide (Scheme 1). Since the low solubility of compounds **1** hampered the biological screening in our previous investigations (Schulz et al. 2014, 2013), the synthesis of hydroiodides was envisioned to overcome current application problems.

2. Investigations, results and discussion

2.1. *Positioning* in silico *studies*

Based on our recent efforts towards potent and selective inhibitors of iNOS by the use of **1** (Schulz et al. 2014, 2013), we were interested in reasonable structure optimizations using computer-aided methods. Unfortunately, there was no suitable crystal structure of rat iNOS available, which would have been most favorable for a direct comparison of the upcoming *in silico* with our previous *in vitro* data. We therefore conducted an amino acid sequence alignment for human, rat and mouse iNOS (details available on request (Tafi et al. 2006; Garcin et al. 2008)). Thereby, we found the murine enzyme (1df1.pdb) of *mus musculus* most suitable for an *in silico* approach due to the overall protein consensus between rat and mouse isozymes of 94%. In the docking library, a variety of [1,2,4]triazolo[1,2-*a*]pyridazines was assembled differing in the substitution in positions 1 and 3 (Scheme 2). The aim was to identify further substance classes of aminoguanidine analogs as potential iNOS inhibitors as well as suitable functional groups for the differentiation within these new or known classes. Of course, the biologically hitherto investigated derivatives of our previous works (Schulz et al. 2013, 2014) were included as well to determine the quality of our model. As a result of the applied docking study we predicted the methylsulfanyl compounds **2** to be very promising test candidates. In general, the predicted activities correlated with the substitution patterns of our in vitro results on thiones **1**, indicating the suitability of our model. However, all thiones **1** were predicted inactive, which was attributed to the overall low two-digit micro molar activity found for these candidates in our previously reported *in vitro* studies. Nevertheless, since the 3-methylsulfanyl derivatives **2** consistently scored superior, we hypothesized that the synthesis of these compounds would contribute to SAR in the field and could lead to compounds with improved activity profiles.

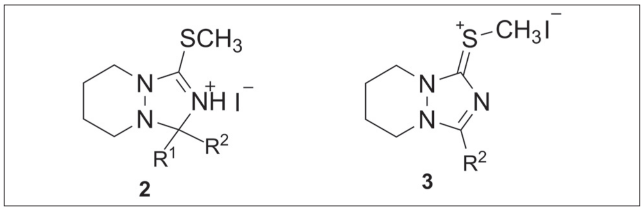


Scheme 2: Modifications in the positions 1 and 3 of the molecular basic structures of the investigated [1,2,4]triazolo[1,2-*a*]pyridazines to create the docking library

2.2. *Synthesis and chemical properties*

The 3-methylsulfanyl derivatives **2** (Table) were obtained by the reaction of methyl iodide and the corresponding thiones **1** at room temperature in dichloromethane in good yields (Scheme 1). Typically, 1.5-fold molar excess of methyl iodide was enough to drive the reaction to completion within six days, which in all cases was monitored by TLC control. However, the removal of the solvent by slow evaporation under atmospheric pressure at room temperature often resulted in non-crystalline semi-solids. During the long term crystallization of reaction products **2** structural changes occurred, as a result of the reactive potential of the methylsulfanyl substituent in position 3. This led to losses in yield or incidental isolation of side-products **3**, respectively (see below). Due to the contact with a protic solvent, e.g. propan-2-ol, the starting compound **1** was partly recovered by thioether cleavage, most notably for the derivatives **2l-n**, **2f** and **2h**. In the case of **2f** nearly one third of the starting material **1f** was recovered. Similar *S*-dealkylation reactions in a solvent (Verma 1963) even without further additional reactants at different thermal conditions were already reported by other authors, e. g. for *S*-alkylated isothiuronium salts (Saville 1961), salts of methylsulfanyl imidazoles (Lempert et al. 1970; Lempert and Zauer 1964; Lin et al. 1974), and triazoles (Begtrup 1971), respectively. The slow crystallization of **2** further decreased the product formation, even in complete absence of a solvent, due to the formation of **3**, which was according to our investigations caused by oxidation by means of atmospheric oxygen. This finding is unprecedented; a report of a similar oxidation reaction of heterocyclic methylsulfanyl compounds could not be found in the literature. Presumably, this reaction was additionally triggered by the formation of iodine from iodide under these conditions. Consequently, the reaction was performed in a sealed flask preventing contact with additional oxygen. However, the attempted application of an inert gas atmosphere did not lead to improved results. After completion of the synthesis, the reaction mixtures were concentrated under reduced pressure, which sometimes led to spontaneous crystallization, e.g. in the case of **2e**. Nevertheless, a semisolid was obtained in most of these experiments.

Table 1: Reaction products 2 and 3 of the corresponding [1,2,4]-triazolo[1,2-*a*]pyridazine-1-thiones 1 (1a,s-z (Schulz et al. 2014), 1b-r (Schulz et al. 2013))



Compd.	R^1	R^2	Reaction time [d] (yield [%])	Free base [a]
2a	H	H	2 (84)	
3a	-	H	3 (1) [b]	
2b	H	C_6H_5-CH_2	3 (85)	
3c	-	C_6H_5-CH=CH	7 (45)	
3d	-	2-NO_2-C_6H_4-CH=CH	7 (55)	
2e	H	C_6H_5	6 (86)	✓
3e	-	C_6H_5	A: 21 (28) B: 0,5 (17) [c]	
2f	H	2-CH_3-C_6H_4	5 (64)	
3g	-	3-CH_3-C_6H_4	3,5 (38)	
2h	H	4-CH_3-C_6H_4	2,5 (38)	
3h	-	4-CH_3-C_6H_4	5 (9)	
2i	H	2-Cl-C_6H_4	5 (68)	
2j	H	3-Cl-C_6H_4	5 (77)	
3j	-	3-Cl-C_6H_4	5 (45)	
2k	H	4-Cl-C_6H_4	1,5 (72)	
2l	H	2-NO_2-C_6H_4	7 (53)	✓
2m	H	3-NO_2-C_6H_4	5 (88)	✓
2n	H	4-NO_2-C_6H_4	6 (73)	✓
3n	-	4-NO_2-C_6H_4	37,5 (10)	
2o	H	2-OH-C_6H_4	5 (74)	✓
3o	-	2-OH-C_6H_4	7 (28)	
2p	H	3-OH-C_6H_4	5 (88)	✓
3p	-	3-OH-C_6H_4	7 (47)	
2q	H	4-OH-C_6H_4	5 (71)	✓
3q	-	4-OH-C_6H_4	4 (58)	
2qa	H	4-CHO_3-C_6H_4	2,5 (86)	✓
3qa	-	4-CH_3O-C_6H_4	2,5 (2) [b]	
2r	H	4-$N(CH_3)_2$-C_6H_4	1,5 (90)	✓
2s	CH_3	CH_3	5 (57)	
2t	$(CH_2)_5$		2 (39)	
2u	CH_3	C_6H_5	5 (72)	
2v	CH_3	4-CH_3O-C_6H_4	2,5 (78)	
2w	C_6H_5	C_6H_5	5 (45)	✓
2x	C_6H_5	4-CH_3O-C_6H_4	2,5 (86)	
2y	C_6H_5	2-Cl-C_6H_4	5 (35)	✓
2z	C_6H_5	4-Cl-C_6H_4	1 (95)	

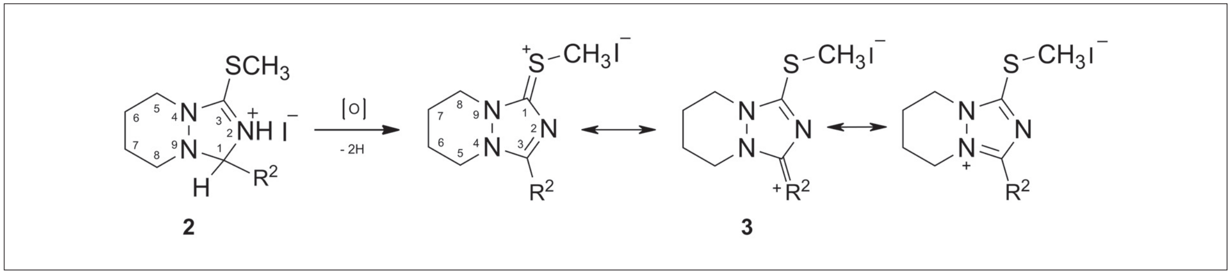
[a] Obtained from **2e**,**l**,**m**,**n**,**o**,**p**,**q**,**qa**,**r** by treatment of an aqueous solution of the respective compound with triethylamine and subsequent extraction with dichloromethane, and from **2w**,**y** by treatment of a solution of each compound in dichloromethane with ammonia, respectively (see Experimental)

[b] Yielded by extraction from mother liquor residue that was stored for 18 months

[c] Substance was obtained using two different methods (A and B, see Experimental)

To quickly purify the product and thus greatly reduce the risk of side-product formation by oxidation, the reaction mixture was treated with tetrahydrofuran, which supported the crystallization process.

The above described occurrence of oxidized methylsulfonium iodides **3**, e.g. **3c** and **3d**, during the alkylation of 3-monosubstituted thiones **1** to the expected and mostly yielded methylsulfanyl compounds **2**, required further investigations. A similar but much less pronounced tendency to form the corresponding oxidation products has already been observed in some 3-monosubstituted thiones **1** (Schulz et al. 2013). The formation of oxidation products **3** in the presence of atmospheric oxygen was observed when either one of the following factors or both applied, respectively: a slow formation of 3-methylsulfanyl derivative **2** from the thione **1** (**3c**,**d**,**o**-**q**) or an exceedingly slow product crystallization (**3g**, **j**). The middle of the resonance structures displayed in Scheme 3 illustrates, that the extension of the mesomeric system by incorporation of suitable 3-substituents (=$^+$R^2 means delocalization of the charge with participation of the substituent) such as (vinylogous) aryl substituents (additionally) favor the formation of the **3**. The far right one displayed resembles, because of possessing the positive charge at the bridging nitrogen atom, the only related methylsulfanyl 1,2,4-triazole system found in the literature, the 2,3-dimethyl-5-methylsulfanyl-1-(2,4,6-trichlorophenyl)-1*H*-1,2,4-triazolium hexachloro-antimonate synthesized by Wang et al. (1992). In summary, these observations explain the difficulties in isolating certain derivatives of type **2** and are the reason for failure to do so in those cases where the before illustrated problems favored the formation of the corresponding side-product of type **3**. The possibility that the thiones **1** were subject to oxidation first and then the methylation followed was also taken into account. But our hypothesis is that the methylation products **2** not the thiones **1** were oxidated, what was supported by both the methylation reaction of the 3-phenyl-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thione **1e** and the behavior of the formed **2e**. Within six days the starting material completely reacted to **2e** in very good yield, while storage for three weeks of an ethanolic solution of genuine compound **2e** resulted in the oxidation product **3e** (Table).



Scheme 3: Formation of methylsulfonium iodides **3** by oxidation of the methylsulfanyl derivatives **2** (for reasons of clarity the numbering of the systems according to the rules of IUPAC is provided)

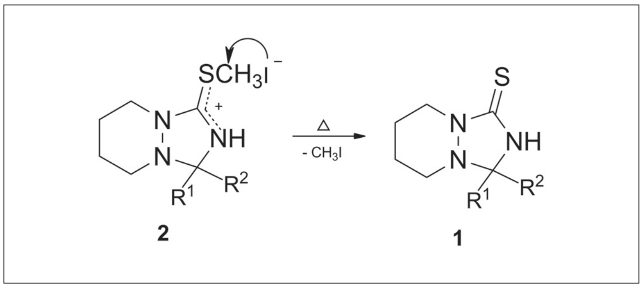
In addition the didehydro derivative **3e** was also obtained by targeted evaporation using an air flow over a large liquid surface of a solution of **2e** in propan-2-ol. However, crystalline solids of the methylsulfanyl derivatives **2** were stable over an extended period of time, when stored carefully sealed.

Despite all efforts to prevent side reactions of **2** in the course of the methylation reaction of the related thione **1** and in the subsequent work-up procedure, some products comprised considerable amounts of the oxidized analogs **3** apparent from ^{1}H NMR spectra. In order to obtain high quality analytical data, release of the free base from some products **2** (see Table) was carried out for further separation, which succeeded in most cases in good purity and high yield. Nevertheless, it was only possible to obtain NMR data as, except for **2w** and **2y**, all products were liquids, that analog to solutions of methylsulfanyl derivatives **2** (see below) were very susceptible to oxidation. Even short-term storage led to a considerable amount of byproduct. The NMR data had to be recorded within one hour after preparation and immediately after preparing the NMR sample. Therefore, no further structural analysis was taken into account. We also tried to determine the reason for the occasional occurrence of the oxidation products **3** in the reaction products of the releases. In some cases of prepared free bases without the detection of analog **3**, ^{1}H NMR data of the - supposed to be - impurified starting salts prior to the reaction surprisingly showed no impurity at all after repeated NMR analytics. This finding led to two conclusions. Firstly, liberation of the base is unfortunately no efficient way to separate **2** from **3**, as it was not able to completely eliminate the oxidation products **3** when they occur, and secondly, and even more importantly, a significant amount of **3** was found to be formed in the dissolved NMR samples of the methylsulfanyl derivatives **2** within only a few hours. Consequently, all further spectra were recorded immediately after sample preparation. However, even with this subsequent change in the analytical routine it was not possible to obtain NMR data free of impurification by the analog **3** for all methylsulfanyl derivatives **2**. For some substances (e.g. **2b**, **2o-qa**), the susceptibility to oxidation appeared to be just too high than that it could be prevented by the precautions taken during preparation.

Knowing that for the biological tests the methylsulfanyl derivatives **2** had to be sufficiently stable under the applied conditions, we investigated whether the 3-methylsulfanyl group is prone to react with nucleophiles. As a result, under mild conditions the compounds were found to be stable towards various *O*- and *N*-nucleophiles. Thus, **2e** did not react with piperidine at room temperature over a period of three weeks. The only newly formed product was the oxidation product **3e** (see above). Possibly the formation of the transition state necessary for the *S*-*N* exchange is sterically hindered (increasing the ring strain by transition of the carbon atom 3 of the [1,2,4]triazolo[1,2-*a*]pyridazine system from sp^2 to sp^3 hybridized state).

The observations of the reverse reactions leading to the formation of thiones **1** during the work-up of **2l**-**n**, **2f** and **2h**, e.g. the formation of **1e** during the GC-MS analysis of **2e** (140 °C), and the exceptionally broad melting ranges of the dried and TLC uniform 3-methylsulfanyl derivatives **2**, prompted a more detailed examination of their thermal behavior. This was performed utilizing a hot-stage microscope with 16-fold magnification. Particularly impressive was the observation of the transformation of the initially acicular crystals of compound **2w** upon heating (4 °C / min) beyond its softening point into compact crystals, which exhibited the same melting range as the thione **1w** (Schulz et al. 2014).

From our own earlier unpublished work on heating (150 °C) hexahydropyridazine-1-carbothioimidic acid methyl ester hydroiodide (Morgenstern et al. 2004), we already knew that *S*-demethylation into hexahydropyridazine-1-carbothioamide (partial structures of compounds **2** and **1**, respectively) occurs. TLC of the cooled melt using genuine **1w** as standard and the comparison of the IR spectra also confirmed that **2w** undergoes a uniform and complete thioether cleavage upon dry heating, yielding the analog thione **1w**. The series of compounds from **2a** to **2x** could be *S*-demethylated to the corresponding thiones **1** (Scheme 4). The 1-monoarylated compounds of series **2** and the 1-(4-methoxyphenyl)-1-phenyl

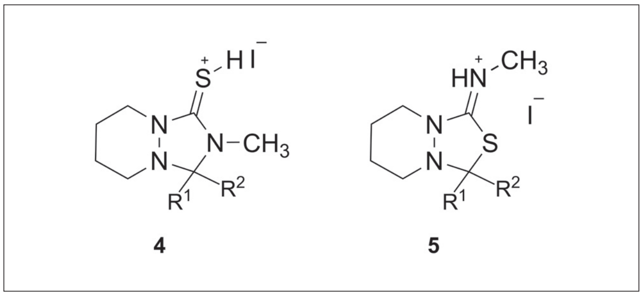


Scheme 4: Thioether cleavage of the methylsulfanyl derivatives **2** under formation of the thiones **1** by starting at an initial temperature of 70 °C, then increasing the temperature by 4 °C / min and, after melting, further heating to 30 °C above the melting temperature

compound **2x** were particularly easily converted. The alkylated compounds **2s** and **2t** reacted more slowly. Noteworthy is the thermal stability of the 1-(2-chlorophenyl)-1-phenyl derivative **2y**, for which the demethylation (in contrast to the 1-(4-chlorophenyl)-1-phenyl derivative **2z**) was almost not evident. To this day such thermal *S*-dealkylation reactions by simple dry heating of methylsulfanyl compounds have rarely been reported. For example, the hydrochlorides of some 3-methylsulfanyl-4-(2-pyridyl)-1,2,4-triazoles yield the related thiones (Bany and Santus 1980).

2.3. Structure elucidation

First evidence for the formation of the desired compounds **2** was given by the reaction behavior of the products obtained. In contrast to their behavior at room temperature towards *O*- and *N*-nucleophiles (see above), upon reaction with amines in boiling propan-1-ol, methanethiol was formed by all compounds **2**. This would not be expected in the case of formation of possible alternative reaction products such as the 2-methyl-[1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **4** or the related *N*-methyl-[1,3,4]thiadiazolo[3,4-*a*]pyridazin-1-imine isomers **5** (Scheme 5). The latter might also be conceivable as products if during the methylation of the compounds **1** (Schulz et al. 2013) an ring-opening and, subsequent, a ring-closure reaction with participation of the thioxo group would occure.



Scheme 5: Structure of possible alternative products **4** and **5** that have to be considered in the reaction of **1** with methyl iodide

Besides the chemical reactivity, spectroscopic data confirmed the structural assignment. The presence of a C=N stretching band in the IR spectra of compounds of type **2** (1500–1600 cm^{-1}), the lack of the NH stretching bands in the range of compounds of type **1**, and the absence of NH signals in the ^{1}H NMR spectra of the prepared free bases from several **2**, respectively, indicated a successful *S*-methylation. Further evidence was given by the position of the ^{13}C NMR signal of the methyl group between 17 ppm and 18 ppm and a signal for the S-C(3)=N partial structure between 145 ppm and 160 ppm. The respective C(1)=S signals were found at lower field for the thiones **1** with 170–180 ppm (Schulz et al. 2014, 2013). In the ^{1}H NMR spectra signals for the methylsulfanyl group were consistently detected between 3.00 ppm and 3.20 ppm. The detection of the two methyl groups at C(1) of **2s** in the ^{13}C NMR spectrum was challenging, due to the spectrum's indistinct, flat expression and the signal's close proximity to those of C(6) and C(7).This corresponds to findings for the related thione **1s** (Schulz et al. 2014). The location of methyl signals at lower field (23.99 ppm) allowed a good differentiation from the carbon atom of the methylsulfanyl group (17.87 ppm).

The $N^+(2)H$ signals were usually found around 11 ppm in the ^{1}H NMR spectra of the compounds, while the N(2)H signals of the corresponding thiones **1** appeared at significantly higher field, e.g. between 7.66 ppm (**2w**) and 6.20 ppm (**2b**) (Schulz et al. 2014, 2013).

A characteristic feature of the oxidation products **3** formed from the 1-monosubstituted methylsulfanyl derivatives **2** was a shift in the ^{1}H NMR spectra of the signal for the methyl group at the sulfur atom to lower field than for corresponding **2** derivatives. Additionally, the proton signal of C1 of the precursors **2** vanished, while the proton signals of C5 and C8 changed to two triplets or even multiplets at about 4 ppm to 5 ppm, suggesting a change in molecular geometry, related to partial planarization of the heterocycle based on sp^2 hybridization of the second carbon atom of the triazole moiety. In comparison, the proton signals of C5 and C8 of corresponding compounds **2** commonly appeared in close proximity of the methyl group, leading to four signals at about 3-4 ppm. While the signals for the tertiary C1 of compounds **2** were found in the ^{13}C NMR spectra at about 80 ppm, the corresponding signals for the sp^2 hybridized carbon atom C3 of compounds **3** occurred at about 155 ppm. Similar chemical shifts were reported for the appropriate sp^3 and sp^2 hybridized carbon atoms, respectively, of structurally related non-anellated 1,2,4-triazolium salts (Amer 1995). In accordance with the findings of the above discussed ^{1}H NMR spectra this indicated the absence of a tertiary sp^3-hybridized carbon atom and accounted for the presence of a C(3)=N(2) structure in the five-membered partial system. Due to the formation of a mesomeric system in **3** (Scheme 3) a separate C=S signal was not always detectable in the spectra. Characteristic IR bands of the spectra of compounds of series **3** in the region 1570–1635 cm^{-1} indicated the presence of a C=N bond in the molecules under investigation.

The EI mass spectrometric behavior of compound **3e** was distinct from **2e** and also supported the suggested structure of compounds **3**. While in the mass spectra of compounds **2** the molecular ion peak of the free bases can be detected, in the spectrum of compound **3e** an extremely small peak for the ion pair was found. Due to its poor volatility, all peaks generated from this substance are extremely small in magnitude.

Using the examples of **2e** and **3e** it becomes clear that the UV spectra of the compounds **2** and **3**, as expected, differ in shape, position and intensity of the absorption maxima (Fig. 1). Each of the spectra shows one absorption maximum, but the maximum of **3e** is bathochromically shifted compared with that of **2e** (**2e**: 224 nm; **3e**: 231 nm).

The absorption maximum of **3e** also shows a more intense absorption (approximately 11%) than that of compound **2e**. While the absorption curve of compound **2e** declines steeply from the maximum up to 240 nm, compound **3e** still shows an almost threefold absorption value compared at 250 nm. These facts support the suggested extension of the original chromophore of **2** in compounds **3**. Finally, the structure of **3d** was in addition unanimously determined by X-ray structural analysis. Compound **3d** crystallizes in the monoclinic space group $P2_1/c$ with four molecules per unit cell. The structure (Fig. 2 - to simplify the discussion a non-IUPAC numbering of the atoms of compound **3d** is given here, further information is available on request) is stabilized by inter- and intramolecular hydrogen bonds involving all three atoms of the nitro group (intra: O1-C9, N4-C9; inter: O2-C5), the imine nitrogen (intra: N3-C9) and the iodide ion (I1-C1); i.e. all hetero atoms except the two adjacent nitrogen atoms N1 and N2 of the heterocycle. All bonds of the 5-membered part of the N-heterocycle are in the range of 1.335 Å (N3-C2) to 1.368 Å (N1-N2) and the mean deviation from a perfect plane is only 0.014 Å indicating a considerable delocalization of the n-electrons, hence supporting the three mesomeric structures shown in Scheme 3. In addition

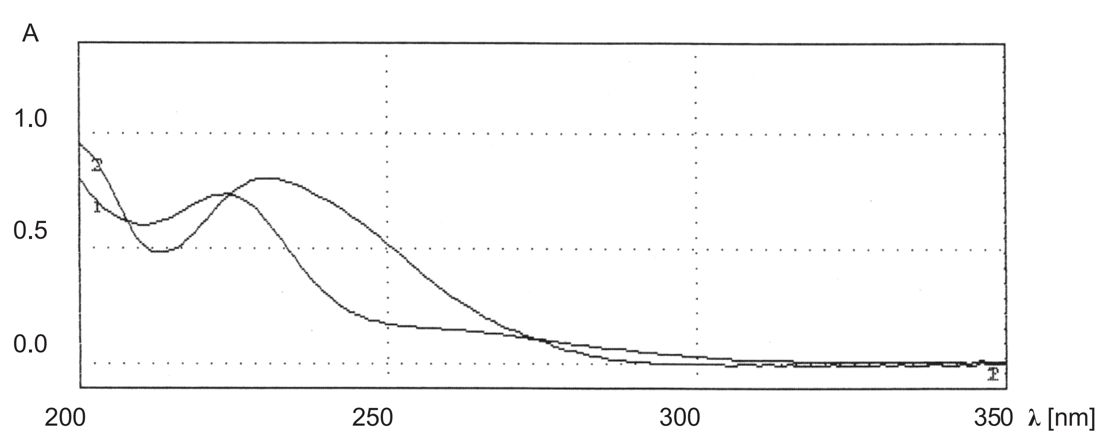


Fig. 1**:** UV spectra (solvent: water) of compound **2e** (curve 1, 3.23 · 10^{-5} mol · L^{-1}) and **3e** (curve 2, 3.28 · 10^{-5} mol · L^{-1})

there is nearly no torsion between the N-heterocyclic system and the phenyl ring of the substituent in position 3. This is most likely due to crystal packing effects as the bridge between the two rings comprises clearly localized single bonds (C7-C8 and C9-C10) and one localized double bond (C8-C9) being inconsistent with an extended aromatic system.

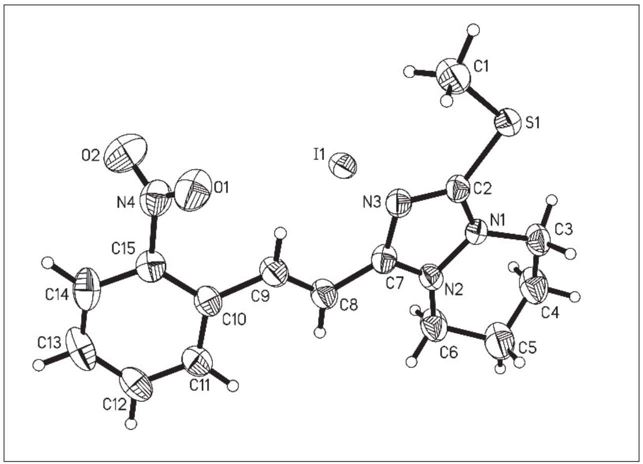


Fig. 2: The molecular structure of **3d**. Ellipsoids are drawn with 50% probability. Selected bond lengths [Å] and angles [°]: S1 – C1: 1.786(5); S1 – C2: 1.721(4); N1 – N2: 1.368(5); N1 – C2: 1.337(5); C2 – N3: 1.335(6); N3 – C7: 1.344(6); C7 – N2: 1.333(6); C1 – S1 – C2: 98.6(2).

The carbon sulfur bond lengths are 1.721 Å for the ring to sulfur bond (C2-S1) and 1.786 Å for the sulfur to methyl bond (C1-S1). These distances are similar to those of the only two related structures found in the Cambridge Crystallographic Database (1.737 and 1.784 Å and 1.723 and 1.831 Å respectively) (Berecz et al. 2002; Nakayama, Sanemitsu 1984). In both these structures the N_3C_2 heterocycle formally bears one double bond while the sulfide function doesn't, i.e. fewer n-electrons are present in these moieties than in compound **3d**. A structure resembling that of **3d** more closely could not be found emphasizing the novelty of all the compounds described above. With the shorter C-S distance clearly facing the heterocycle the sulfur carbon double bond appears to be more localized in its position than the n-system of the heterocyclic ring, although the S-C single bond is a bit shorter and the S=C bond a bit longer than expected for ideal single and double bonds respectively.

2.4. *Biological evaluation*

To evaluate the preliminary *in silico* predictions, the synthesized substances were biologically screened for inhibition of inducible nitric oxide synthase (iNOS), using the same cell-based assay as described earlier (Schulz et al. 2014, 2013). Rat insulinoma cell line RIN5F (Chick et al. 1977; Gazdar et al. 1980) was stimulated with proinflammatory cytokines IL-1β and IFN-γ to induce iNOS (Ankarcrona et al. 1994; Cetkovic-Cvrlje and Eizirik 1994) and thus NO production. The amount of produced NO was measured via detection of nitrite applying the Griess test (Green et al. 1982; Griess 1879) to the supernatant. Since the solubility of the methylsulfanyl derivatives **2** was in fact much better than that of the corresponding thiones **1** (Schulz et al. 2014, 2013), higher concentrations were reached in the biological screening (0.3125 mM and 0.156 mM). This is also evident from the inhibitory activity of the reference inhibitor aminoguanidine (Wolff and Lubeskie 1995) which increased inhibition from 50% to 90%. In Fig. 3 the residual NO production is displayed. The results provide a general overview of the inhibitory activity of this particular compound class. The products **3** were tested as well to compare the influence resulting from changes in the substitution at position 3. The substances' cytotoxicity was determined using the MTT assay (Mosmann 1983).

Regrettably, the *in vitro* data of the methylsulfanyl derivatives **2** did not correlate to the results of the *in silico* studies. As Fig. 3 shows, only **2x** reveals an inhibition comparable to aminoguanidine, but the reduction of NO production is most likely explained by its considerable cytotoxicity. Nevertheless, some additional conclusions can be drawn from the presented data. Substances **2k** and **2r**, both bearing a *para*-substituted phenyl moiety in position 1, show some activity. While the para substitution was already found to be advantageous for the thiones **1**, the 1-unsubstituted parent compound **2a** and the aliphatic 1,1-disubstituted derivative **2s** show no activity at all. Earlier similar results were observed for the thiones **1** as well (Schulz et al. 2014, 2013). Moreover, the oxidation products **3** exhibited only a marginal inhibitory influence, while possessing cytotoxic tendencies (**3d** and **3j**). Despite these findings, none of the tested compounds showed a strong cytotoxic effect.

The question remains what might account for the poor inhibition observed for the methylsulfanyl derivatives **2**. First, at physiological pH, the substances are supposedly predominantly protonated what would make it difficult for the molecule to enter the cell. Additionally, the oxidation sensitivity presumably plays a critical role, as for all monosubstituted compounds **2** a rapid oxidation to products **3** was observed. This was found to be even supported by an inevitable contact to the ambient atmosphere, since the cell culture plates needed to be well ventilated for providing a sufficient gas exchange.

2.5. *Conclusions*

Concluding can be summarized, that after computer-aided methods identified 3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazines **2** as reasonably well optimized relatives

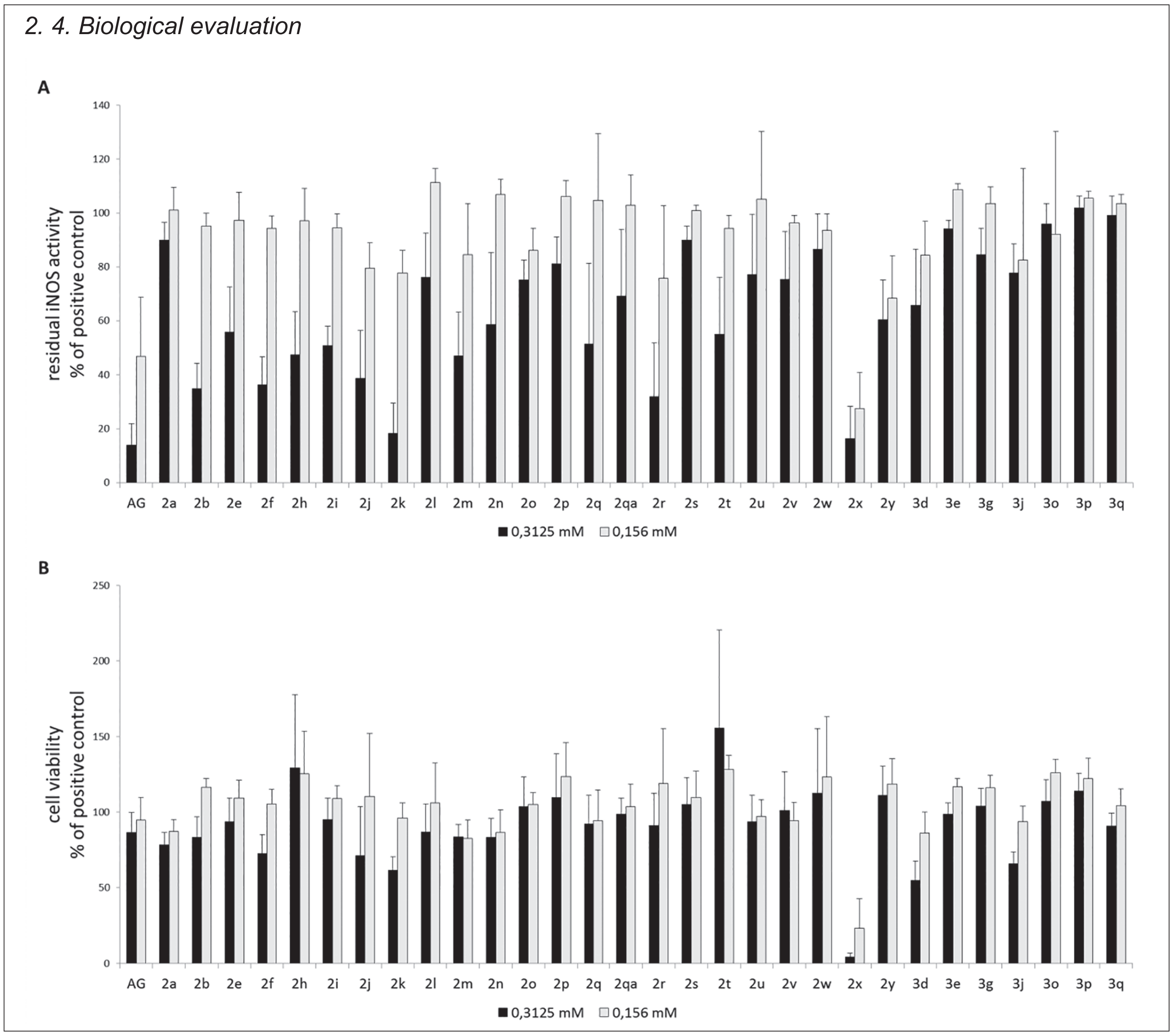


Fig. 3: Concentration-dependent inhibition of iNOS by aminoguanidine (AG), methylsulfanyl derivatives **2** and products **3** (**A**). NO production was determined using the Griess test. Cell viability was tested on substance treated RINm5F cells using MTT assay (**B**). Both data sets are displayed as percent of positive control (RIN cells treated with IL and IFN but without substance). Aminoguanidine (AG) was tested on each plate in the same concentrations as a reference inhibitor (n > 30). Values are means ± SD; n = 7–12.

of the previously introduced iNOS inhibitors [1,2,4]triazolo[1,2-*a*]pyridazine-1-thiones **1** (Schulz et al. 2014, 2013), numerous derivatives of this new compound class were successfully synthesized, characterized and biologically evaluated. The substances show rather distinct oxidation tendencies and because of that some of the oxidation products methyl-(5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazin-1-ylidene)sulfonium iodides **3** could be isolated and studied as well. Contrary to expectations, compounds **3** were shown to be less shapely than anticipated due to electronic effects and **2** did not show the inhibitory potency that was predicted by the *in silico* studies performed.

In this context, a new mechanism for the inactivation of the neuronal nitric oxide synthase which is one of the structurally closely related isoforms of the nitric oxid synthase (Raman et al. 1998; Fischmann et al. 1999; Crane et al. 1997), recently reported by Tang et al. (Tang et al. 2015) might be interesting. According to the studies of the authors the mechanism of inhibition includes both the oxidative *S*-demethylation of the investigated selective inhibitor (*S*)-2-amino-5-(2-methylsulfanyl)acetimidamido)pentanoic acid by the heme iron atom of the nNOS oxygenase domain and the binding of the resulting thiol to the same. Moreover, the before mentioned new nNOS inhibitor is also not selective to the neuronal isoform as it inhibited the iNOS in a comparable extent (Litzinger et al. 2006). While the synthesized and tested compounds **2** described in the present paper are as a rule susceptible to oxidation, they also differ significantly from the above mentioned in both the spatial distance of the sulfur atom of the amidine carbon atom by a direct link (*vs.* a methylene group as a spacer) and by the ring-shaped structure in the molecular flexibility. Therefore, the findings of Tang et al. (2015) might provide further approaches for subsequent studies.

3. Experimental

3.1. *In silico investigations*

The *in silico* screening was done using Molecular Operating Environment (MOE), version 2009.10 (2009). Missing parameters for the amber99 forcefield of cofactor heme were supplemented to the software (Shahrokh et al. 2012), while cofactor BH_4 was fixed due to missing parameters. Conformational analysis was performed with standard parameters, except for the following: method (stochastic), rejection limit (100), iteration limit (100), MM iteration limit (200), amid bond rotation (allowed), conformation limit (100). Docking was carried out using standard conditions and a 4.5 Å radius of both cofactors plus the following amino acids: H-Pocket, active site, Gln specificity pocket (see Tab. 3, Supporting Information (Garcin et al. 2008; Aparna et al. 2007; Tafi et al. 2006; Knowles, Moncada 1994)). The ligands were placed by the Triangle Matcher of MOE, scoring as well as rescoring were calculated with London dG.

3.2. *Chemistry - 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). The spectra were recorded with the following instruments and conditions: UV spectra: UVIKON 930 (Kontron), the molar absorption coefficients (ε) of the related absorption maxima of the compounds **2e** and **3e** were determined for each of them calculating the mean values of the values of three graded concentrations (highest concentrations yielded absorptions not higher than A = 1.20 for **2e** and A = 1.35 for **3e**); IR spectra: FT-IR 1600 (Perkin-Elmer), transmission technique (KBr pressed disks) and for the compounds **2a**, **2j**, **2o**, **2p**, **2q**, **2qa**, **3a**, **3h**, **3n** only IR 200 FT-IR (Thermo Electron Corporation Nicolet), ATR technique (diamond); ^{1}H, ^{1}H,^{1}H-COSY, DEPT-135, ^{13}C NMR and additionally HSQC- and HMBC spectra: FT NMR spectrometer AVANCE III™ 400, only for compounds **2z**, **3c**, **3g**, **3j**, **3o-q**: ^{1}H, ^{1}H,^{1}H-COSY, DEPT-135, ^{13}C NMR spectra: AVANCE DPX 200; (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 shifts δ in ppm. 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 compounds **2a**, **2e**, **2o-qa**, **2t**, **2v**, **2x**, **3a**, **3d**, **3h**, **3n**, **3qa**: 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-18e endcapped, 50 mm; mobile phase (m. ph. 3): methanol/water 6:4 (0.1% HCOOH in H_2O) (v/v), flow rate: 0.4 mL·min^{-1}.

For TLC 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.):

m. ph. 1: cyclohexane/ethyl acetate/triethylamine: 3:3:1 (V/V/V);
m. ph. 2: cyclohexane/ethyl acetate/triethylamine 6:3:1 (V/V/V);
m. ph. 3: hexane/ethyl acetate/triethylamine 5:5:1 (V/V/V);
m. ph. 4: water/conc. acetic acid/*n*-propanol 2:2:6 (V/V/V)

Each mobile phase (m. ph.) used is specified in the data of the related compound. The substances were detected by UV radiation (λ = 254 nm) or Munier spray reagent (Munier 1953) and iodine azide reagent by Awe (Awe 1948).

3.3. Synthesis of the compounds

3.3.1. 3-Methylsulfanyl-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]-pyridazine hydroiodides 2

To a solution of 2.5 mmol of the appropriate thione **1** (Schulz et al. 2014, 2013) in 25 mL dichloromethane 3.75 mmol methyl iodide were added. The sealed batch was allowed to stand at room temperature until the reaction was completed (TLC monitoring, reaction time is given below). In the following the solvent was removed without warming under reduced pressure. The resulting residue was allowed to solidify and subsequently re-crystallized from tetrahydrofuran (**2b**: dichloromethane; **2s**,**2z**: propan-2-ol). The formed crystals were collected by suction filtration, washed with tetrahydrofuran (**2b**,**2z**: dichloromethane; **2s**: propan-2-ol) and dried in vacuum. In this manner were obtained:

3-Methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine hydroiodide **(2a)**
Reaction time 2 d. Yield: 84%. Colorless crystals. M.r. 109–120 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.19. IR (cm^{-1}): $\tilde{\nu}$ = 1522 (C=N$^+$H); 2852, 2913, 2977 (aliph. H); 3050 (N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.61 (bs, 2H, C(6)H$_2$); 1.77 (quint, 2H, ^{3}J = 5.6 Hz, C(7)H$_2$); 2.76 (s, 3H, SCH$_3$); 2.85 (t, 2H, ^{3}J = 4.8 Hz, C(8)H$_2$); 3.66 (bs, 2H, C(5)H$_2$); 4.76 (s, 2H, C(1)H$_2$); 10.76 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.75 (SCH$_3$); 22.42 (C7); 23.39 (C6); 45.15 (C5); 53.17 (C8); 68.58 (C1); 165.34 (C(3)-S). HRMS [(ESI) m/z]: Calcd. for [$C_7H_{13}N_3S$ (base) + H]$^+$: 172.0903. Found [M$_{base}$ + H]$^+$: 172.0901.

3-Methylsulfanyl-1-phenylmethyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine hydroiodide **(2b)**
Reaction time 3 d. Yield: 85%. Colorless crystals. M.r. 135–140 °C (dichloromethane). TLC (m. ph. 3): R$_f$ = 0.76. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1492; 1542 (C=N$^+$H); 1600; 2848, 2913, 2942, 2972 (all aliph. H); 3092 (arom. H/N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.42–1.45 (m, 1H, C(6)H); 1.75 (m, 3H, C(6)H and C(7)H$_2$); 2.71 (s, 3H, SCH$_3$); 2.79 (m, 1H, C(8)H); 2.86–2.88 (m, 3H, C(8)H and CH$_2$); 3.26 (m, 1H, C(5)H); 3.66–3.70 (m, 1H, C(5)H); 5.27 (t, 1H, ^{3}J = 5,2 Hz, C(1)H); 7.29–7.34 (m, 5H, arom. H); 10.72 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.69 (SCH$_3$); 22.62 (C7); 23.81 (C6); 39.62 (CH$_2$ → superposed by solvent signal); 44.76 (C5); 54.08 (C8); 79.95 (C1); 126.83 (arom. C4); 128.04 and 129.76 (arom. C2, C3, C5, C6); 134.40 (arom. C1); 161.64 (C(3)-S). Elemental analysis [$C_{14}H_{20}IN_3S$ (389.3), %]: Calcd. C, 43.19; H, 5.18; N, 10.79. Found: C, 42.63; H, 4.88; N, 10.79.

3-Methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine hydroiodide **(2e)**
Reaction time 6 d. Yield: 86%. Beige crystals. M.r. 132–141 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.75. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1501; 1539 (C=N$^+$H); 1587; 1640; 2795, 2948, (aliph. H); 3022 (arom. H/N$^+$H). UV [H_2O, nm (lg ε)]: λ$_{max}$ = 224 (4,343), flat and elongated shoulder in the range of 250 (3.684) up to 310 (2.230). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.56–1.81 (m, 4H, C(7)H$_2$ and C(6)H$_2$); 2.77 (s, 3H, SCH$_3$); 2.89 (t, 1H, ^{3}J =11.2 Hz, C(8)H); 3.05 (d, 1H, ^{3}J = 10.0 Hz, C(8)H); 3.37–3.47 (m, 1H, C(5)H → superposed by HDO); 3.89 (d, 1H, ^{3}J = 13.2 Hz; C(5)H); 5.92 (s, 1H, C(1)H); 7.47–7.52 (m, 5H, arom. H); 11.13 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.46 (SCH$_3$); 22.48 (C7); 23.10 (C6); 45.58 (C5); 53.50 (C8); 81.51 (C1); 127.36 (arom. C2 and C6); 128.77 (arom. C3 and C5); 129.81 (arom. C4); 136.20 (arom. C1); 162.34 (C(3)-S). MS (70 eV, 25 °C): m/z (%) = 246.8 (26) (M$^{+\cdot}_{base}$); 184.9 (17); 169.8 (92); 147.2 (40); 141.7 (28); 127.8 (31); 126.7 (21); 84.9 (15) [$C_4H_9N_2$]$^+$; 55.2 (12); 41.0 (14); 31.9 (23); 27.9 (100). Elemental analysis [$C_{13}H_{18}IN_3S$ (375.3), %]: Calcd. C, 41.61; H, 4.83; N, 11.20. Found: C, 41.71; H, 4.56; N, 11.09.

1-(2-Methylphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2f)**
Reaction time 5 d. Yield: 64%. Colorless crystals. M.r. 105–112 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.83. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1487; 1531 (C=N$^+$H), 1606; 2826, 2925, 2952, (aliph. H); 3179 (arom. H/N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.60 (m, 1H, C(6)H); 1.76–1.80 (m, 3H, C(6)H and C(6)H$_2$); 2.40 (s, 3H, CH$_3$), 2.82 (m, 4H, C(8)H and SCH$_3$); 3.04 (d, 1H, ^{3}J = 10.4 Hz, C(8)H); 3.37 (m, 1H, C(5)H → superposed by HDO); 3.90 (d, 1H, ^{3}J = 13.2 Hz, C(5)H); 6.11 (s, 1H, C(1)H); 7.29–7.41 (m, 4H, 1st section arom. C(3)H and C(5)H, 2nd section arom. C(4)H, 3rd section arom. C(6)H); 11.07 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.57 (SCH$_3$); 18.79 (CH$_3$); 22.59 (C7); 23.28 (C6); 45.53 (C5); 53.44 (C8); 79.35 (C1); 126.24 (arom. C5); 126.97 (arom. C6); 129.56 (arom. C4); 131.01 (arom. C3); 133.35 (arom. C1); 136.96 (arom. C2); 162.95 (C(3)-S). Elemental analysis [$C_{14}H_{20}IN_3S$ (389.3), %]: Calcd. C, 43.19; H, 5.18; N, 10.79. Found: C, 43.38; H, 4.33; N, 9.96.

1-(4-Methylphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2h)**
Reaction time 2.5 d. Yield: 38%. Colorless crystals. M.r. 116–123 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.81. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1491; 1517 (C=N$^+$H); 1584; 1615; 2855, 2947, 2988 (aliph. CH); 3058 (arom. H/N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.56–1.80 (m, 4H, 1st and 2nd section C(7)H, 3rd section C(6)H$_2$); 2.34 (s, 3H, CH$_3$), 2.77 (s, 3H, SCH$_3$), 2.86 (t, 1H, ^{3}J = 10.8 Hz; C(8)H); 3.01 (d, 1H, ^{3}J = 10.8 Hz; C(8)H); 3.37–3.45 (m, 1H, C(5)H → superposed by HDO); 3.88 (d, 1H, ^{3}J = 12.0 Hz, C(5)H); 5.85 (s, 1H, C(1)H); 7.28 (d, 2H, ^{3}J = 7.6 Hz, arom. C(3)H and C(5)H); 7.41 (d, 2H, ^{3}J = 7.6 Hz, arom. C(2)H and C(6)H); 11.06 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.42 (SCH$_3$); 20.83 (CH$_3$); 22.45 (C7); 23.04 (C6); 45.57 (C5); 53.30 (C8); 81.51 (C1); 127.36 (arom. C2 and C6); 129.26 (arom. C3 and C5); 133.14 (arom. C1); 139.41 (arom. C4); 162.30 (C(3)-S). Elemental analysis [$C_{14}H_{20}IN_3S$ (389.3), %]: Calcd. C, 43.19; H, 5.18; N, 10.79. Found: C, 41.80; H, 4.95; N, 10.42.

1-(2-Chlorophenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2i)**
Reaction time 5 d. Yield: 68%. Colorless crystals. M.r. 122–128 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.91. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 788 (Cl); 1494; 1532 (C=N$^+$H); 1574; 1593; 2779, 2828, 2906 (aliph. H); 3017 (arom. H/N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.56–1.62 (m, 1H, C(6)H); 1.69–1.85 (m, 3H, C(6)H and C(7)H$_2$); 2.83 (s, 3H, SCH$_3$); 2.99 (t, 1H, ^{3}J = 10.8 Hz; C(8)H); 3.14 (d, 1H, ^{3}J = 10,8 Hz, C(8)H); 3.41–3.48 (m, 1H, C(5)H → superposed by HDO); 3.91 (dd, 1H, ^{3}J = 3.6 Hz, ^{3}J = 12.8 Hz, C(5)H); 6.24 (s, 1H, C(1)H; 7.48–7.50 (m, 2H, arom. C(3)H and C(5)H); 7.57–7.59 (m, 2H, arom. C(4)H and C(6)H); 11.16 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.66 (SCH$_3$); 22.68 (C7); 23.38 (C6); 45.52 (C5); 54.05 (C8); 78.81 (C1); 127.75 (arom. C5); 128.86 (arom. C6); 130.10 (arom. C4); 131.39 (arom. C3); 132.65 (arom. C2), 133.40 (arom. C1); 162.75 (C(3)-S). MS (70 eV, room temperature) m/z (%): 280.8 (8) [M$^{+\cdot}_{base}$]; 266.8 (25) [M$^{+\cdot}_{base}$ – ·CH$_3$]; 266.8 (68); 231.8 (13); 206.8 (14); 181.7 (28); 169.9 (52); 155.8 (36); 141.8 (56); 127.8 (11); 126.8 (29); 88.9 (17) [$C_4H_9N_2^+$]; 84.9 (100); 56.3 (21); 55.2 (15); 41.0 (17); 32.0 (15); 30.0 (30); 28 (78). Elemental analysis [$C_{13}H_{17}ClIN_3S$ (409.7), %]: Calcd. C, 38.11; H, 4.18; N, 10.26. Found: C, 38.40; H, 4.09; N, 10.52.

1-(3-Chlorophenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2j)**
Reaction time 5 d. Yield: 77%. Brownish-yellow crystals. M.r. 112–118 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.74. IR (cm^{-1}): $\tilde{\nu}$ = 1438 (CH); 1478, 1531 (C=N$^+$H); 1596 (C=C, arom.); 2777, 2826, 2910 (CH); 2997 (CH$_3$). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.56–1.59 (m, 1H, C(6)H); 1.73–1.82 (m, 3H, C(6)H and C(7)H$_2$); 2.78 (s, 3H, SCH$_3$); 2.91 (t, 1H, ^{3}J = 9.6 Hz; C(8)H); 3.09 (d, 1H, ^{3}J = 10.8 Hz; C(8)H); 3.39–3.49 (m, 1H, C(5)H → superposed by HDO); 3.89 (dd, 1H, ^{3}J = 3.2 Hz, ^{3}J = 13.2 Hz, C(5)H); 5.97 (s, 1H, C(1)H); 7.50–7.53 (m, 3H, arom. C(4)H, C(5)H and C(6)H); 7.65 (s, 1H, arom. C(1)H); 11.14 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.40 (SCH$_3$); 22.40 (C7); 23.02 (C6); 45.53 (C5); 53.57 (C8); 80.49 (C1); 125.88 (arom. C4); 127.12 (arom. C2); 129.58 (arom. C6); 130.64 (arom. C5); 133.33 (arom. C3); 138.84 (arom. C1); 162.30 (C(3)-S). HRMS [(ESI) m/z]: Calcd. for [$C_{13}H_{16}ClN_3S$ (base) + H]$^+$: 282.0826. Found [M$_{base}$ + H]$^+$: 282.0804.

1-(4-Chlorophenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2k)**
Reaction time 1.5 d. Yield: 72%. Brown crystals. M.r. 113–123 °C (tetrahydrofuran). TLC (m. ph. 3): R$_f$ = 0.76. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 604 (Cl); 1493; 1523 (C=N$^+$H); 1581; 1596; 1631; 2853, 2945 (aliph. H); 3027 (arom. H/N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.55–1.60 (m, 1H, C(6)H); 1.71–1.85 m, 3H, C(6)H and C(7)H$_2$); 2.78 (s, 3H, SCH$_3$); 2.90 (dt, 1H, ^{3}J = 2.0 Hz, ^{3}J = 11.2 Hz, C(8)H); 3.06 (d, 1H, ^{3}J = 10.8 Hz, C(8)H); 3.41–3.48 (m, 1H, C(5)H → superposed by HDO); 3.89 (dd, 1H, ^{3}J = 3.6 Hz; ^{3}J = 13.2 Hz, C(5)H); 5.97 (s, 1H, C(1)H); 7.53–7.58 (m, 4H, arom. H); 11.18 (bs, 1H, NH$^+$).^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.54 (SCH$_3$); 22.50 (C7); 23.14 (C6); 45.61 (C5); 53.59 (C8); 80.55 (C1); 128.78 and 129.21 (arom. C2, C3, C5 and C6); 134.38 (arom. C4); 135.42 (arom. C1); 162.43 (C(3)-S). Elemental analysis [$C_{13}H_{17}ClIN_3S$ (409.7), %]: Calcd. C, 38.11; H, 4.18; N, 10.26. Found: C, 38.29; H, 4.07; N, 9.82.

3-Methylsulfanyl-1-(2-nitrophenyl)-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2l)**
Reaction time 7 d. Yield: 53%. Yellow crystals. M.r. 142–145 °C (tetrahydrofuran) TLC (m. ph. 3): R$_f$ = 0.70. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1353 (NO$_2$), 1532 (C=N$^+$H); 1564 (NO$_2$), 1616; 2857, 2927, 2943 (aliph. H); 3000 (arom. H/N$^+$H). ^{1}H NMR (DMSO-*d*$_6$, ppm): δ = 1.53–1.56 (m, 1H, C(6)H); 1.76–1.83 (m, 3H, C(6)H and C(7)H$_2$), 2.82 (s, 3H, SCH$_3$); 2.98 (t, 1H, ^{3}J =10.4 Hz, C(8)H); 3.08 (d, 1H, ^{3}J = 9.2 Hz, C(8)H); 3.47 (t, 1H, ^{3}J = 12.0 Hz, C(5)H → superposed by HDO); 3.87 (d, 1H, ^{3}J = 11.6 Hz, C(5)H); 6.54 (s, 1H, C(1)H); 7.70–7.78 (m, 2H, arom. C(4)H and C(6)H); 7.84 (t, 1H, ^{3}J = 7.6 Hz, arom. C(5)H); 8.12 (d, 1H, ^{3}J = 7.6 Hz, arom. C(3)H); 11.28 (bs, 1H., NH$^+$). ^{13}C NMR (DMSO-*d*$_6$, ppm): δ = 14.84 (SCH$_3$); 22.84 (C7); 23.59 (C6); 45.53 (C5); 54.14 (C8); 77.70 (C1); 125.14 (arom. C3); 128.44 (arom. C6); 130.79 (arom. C4); 131.62 (arom. C1); 134.21 (arom. C5); 147.83 (arom. C2); 163.28 (C(3)-S). Elemental analysis [$C_{13}H_{17}IN_4O_2S$ (420.3), %]: Calcd. C, 37.15; H, 4.08; N, 13.33. Found: C, 38.08; H, 3.83; N, 12.24.

3-Methylsulfanyl-1-(3-nitropheny)l-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2m)**

Reaction time 5 d. Yield: 88%. Yellow crystals. M.r. 155–157 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.64. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1352 (NO_2), 1532 ($C{=}N^+H$); 1564 (NO_2), 1616; 2857, 2927, 2943 (aliph. H); 3000 (arom. H/N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.57–1.60 (m, 1H, C(7)H); 1.75–1.83 (m, 3H, C(6)H and C(7)H_2); 2.78 (s, 3H, SCH_3); 2.96 (t, 1H; ^3J = 10.8 Hz, C(8)H); 3.13 (d, 1H, ^3J = 10.4 Hz, C(8)H); 3.48 (t, 1H, ^3J = 13.2 Hz, C(5)H → superposed by HDO); 3.90 (dd, 1H, ^3J = 3.6 Hz, ^3J = 13.6 Hz, C(5)H); 6.17 (s, 1H, C(1)H); 7.79 (t, 1H, ^3J = 8.0 Hz, arom. C(5)H); 8.01 (d, 1H, ^3J = 7.6 Hz, arom. C(6)H); 8.31 (d, 1H, ^3J = 8.0 Hz, arom. C(4)H); 8.40 (t, 1H, ^3J = 2.0 Hz, arom. C(2)H); 11.26 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.59 (SCH_3); 22.56 (C7); 23.20 (C6); 45.68 (C5); 53.70 (C8); 80.25 (C1); 122.15 (arom. C2); 124.47 (arom. C4); 130.53 (arom. C5); 133.74 (arom. C6); 138.93 (arom. C3); 147.91 (arom. C1); 162.68 (C(3)-S). Elemental analysis [$C_{13}H_{17}IN_4O_2S$ (420.3), %]: Calcd. C, 37.15; H, 4.08; N, 13.33. Found: C, 38.48; H, 3.96; N, 13.56.

3-Methylsulfanyl-1-(4-nitrophenyl)-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2n)**

Reaction time 6 d. Yield: 73%. Pale yellow crystals. M.r. 135–142 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.62. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1351 (NO_2); 1443; 1525 ($C{=}N^+H/NO_2$); 1603; 2870, 2936, 2956 (aliph. H); 3106 (arom. H/N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56–1.59 (m, 1H, C(6)H); 1.75–1.86 (m, 3H, C(6)H and C(7)H_2); 2.79 (s, 3H, (SCH_3); 2.98 (t, 1H, ^3J = 10.4 Hz, C(8)H); 3.12 (d, 1H, ^3J = 10.4 Hz, C(8)H); 3.47 (t, 1H, ^3J = 12.0 Hz, C(5)H → superposed by HDO); 3.91 (dd, 1H, ^3J = 3.6 Hz, ^3J = 13.6 Hz, C(5)H); 6.17 (s, 1H, C(1)H); 7.81 (d, 2H, ^3J = 8.4 Hz, arom. C(2)H and C(6)H); 8.31 (d, 2H, ^3J = 8.4 Hz, arom. C(3)H and C(5)H); 11.22 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.64 (SCH_3); 22.62 (C7); 23.32 (C6); 45.65 (C5); 53.87 (C8); 80.18 (C1); 123.86 (arom. C3 and C5); 128.53 (arom. C2 and C6); 143.71 (arom. C1); 148.18 (arom. C4); 162.74 (C(3)-S). Elemental analysis [$C_{13}H_{17}IN_4O_2S$ (420.3), %]: Calcd. C, 37.15; H, 4.08; N, 13.33. Found: C, 37.04; H, 3.56; N, 12.92.

1-(2-Hydroxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2o)**

Reaction time 5 d. Yield: 74%. Brownish-yellow crystals. M.r. 91–98 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.62. IR (cm^{-1}): $\tilde{\nu}$ = 1436 (CH); 1495, 1559 ($C{=}N^+H$); 1594 (C=C, arom.); 2849, 2947 (CH); 3192 (CH_3); 3385 (NH^+); 3535 (OH). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56–1.59 (m, 1H, C(6)H); 1.72–1.84 (m, 3H, C(6)H and C(7)H_2); 2.77 (s, 3H, SCH_3); 2.90 (t, 1H, ^3J = 10.8 Hz, C(8)H); 3.06 (d, 1H, ^3J = 11.2 Hz, C(8)H); 3.38–3.42 (m, 1H, C(5)H → superposed by HDO); 3.92 (d, 1H, ^3J = 13.2 Hz, C(5)H); 6.06 (s, 1H, C(1)H); 6.87–6.92 (m, 2H, arom. C(3)H and C(5)H); 7.27 (dt, 1H, ^3J = 1.2 Hz, ^3J = 7.6 Hz, arom. C(4)H); 7.36 (dd, 1H, ^3J = 1.2 Hz, ^3J = 7.6 Hz, arom. C(6)H); 10.10 (s, 1H, OH); 10.92 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.35 (SCH_3); 22.53 (C7); 23.22 (C6); 45.29 (C5); 53.80 (C8); 77.13 (C1); 115.49 (arom. C3); 119.00 (arom. C5); 127.72 (arom. C6); 130.63 (arom. C4); 155.47 and 155.53 (arom. C1 and C2); 161.78 (C(3)-S). **HRMS** [(ESI) m/z]: Calcd. for [$C_{13}H_{17}N_3OS$ (base) + H]$^+$: 264.1165. Found [M_{base} + H]$^+$: 264.1156

1-(3-Hydroxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2p)**

Reaction time 5 d. Yield: 88%. Beige crystals. M.r. 145–150 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.21. IR (cm^{-1}): $\tilde{\nu}$ = 1454 (CH); 1480, 1535 ($C{=}N^+H$); 1589, 1615 (C=C, arom.); 2849, 2929, 2958 (CH); 3087 (CH_3); 3248 (OH). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56–1.67 (m, 1H, C(6)H); 1.72–1.85 (m, 3H, C(6)H and C(7)H_2); 2.77 (s, 3H, SCH_3); 2.89 (dd, 1H, ^3J = 2.4 Hz, ^3J = 11.2 Hz, C(8)H); 3.03–3.06 (m, 1H, C(8)H); 3.39 (m, 1H, C(5)H → superposed by HDO); 3.89 (dd, 1H, ^3J = 4.0 Hz, ^3J = 13.2 Hz, C(5)H); 5,83 (s, 1H, C(1)H); 6.83–6.94 (m, 3H, arom. C(2)H, C(4)H and C(6)H); 7.25–7.32 (m, 1H, arom. C(5)H); 9.68 (s, 1H, OH); 11.08 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.45 (SCH_3); 22.47 (C7); 23.10 (C6); 45.56 (C5); 53.48 (C8); 81.44 (C1); 113.95 (arom. C2); 116.70 (arom. C4); 117.82 (arom. C6); 129.89 (arom. C5); 137.46 (arom. C1); 157.64 (arom. C3); 162.26 (C(3)-S). HRMS [(ESI) m/z]: Calcd. for [$C_{13}H_{17}N_3OS$ (base) + H]$^+$: 264.1165. Found [M_{base} + H]$^+$: 264.1158.

1-(4-Hydroxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2q)**

Reaction time 5 d. Yield: 71%. Beige crystals. M.r. 144–154 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.19. IR (cm^{-1}): $\tilde{\nu}$ = 1444 (CH); 1499, 1547 ($C{=}N^+H$), 1594, 1612 (C=C, arom.); 2947, 2976 (CH); 3083 (CH_3); 3223 (OH). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.55–1.79 (m, 4H, C(7)H_2 and C(6)H_2); 2.75 (s, 3H, SCH_3); 2.78–2.83 (m, 1H, C(8)H); 2.99 (d, 1H, ^3J = 11.2 Hz, C(8)H); 3.38 (m, 1H, C(5)H → superposed by HDO); 3.88 (d, 1H, ^3J = 12.4 Hz, C(5)H); 5.74 (s, 1H, C(1)H); 6.84 (d, 2H, ^3J = 7.6 Hz, arom. C(3)H and C(5)H); 7.35 (d, 2H, ^3J = 7.6 Hz, arom. C(2)H and C(6)H); 9.78 (s, 1H, OH); 10.95 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.30 (SCH_3); 22.37 (C7); 22.90 (C6); 45.54 (C5); 52.98 (C8); 81.82 (C1); 115.39 (arom. C3 and C5); 125.88 (arom. C1); 129.11 (arom. C2 and C6); 158.83 (arom. C4); 162.14 (C(3)-S). HRMS [(ESI) m/z]: Calcd. for [$C_{13}H_{17}N_3OS$ (base) + H]$^+$:264.1165. Found [M_{base} + H]$^+$: 264.1155.

1-(4-Methoxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2qa)**

Reaction time 2.5 d. Yield: 86%. Beige crystals. M.r. 143–150 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.68. IR (cm^{-1}): $\tilde{\nu}$ = 1435 (CH); 1408, 1512 ($C{=}N^+H$); 1587, 1610 (C=C, arom.); 2852, 2941 (CH); 3051 (CH_3). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.59–1.81 (m, 4H, C(7)H_2 and C(6)H_2), 2.76 (s, 3H, SCH_3); 2.85 (m, 1H, C(8)H); 3.01 (d, 1H, ^3J = 11.2 Hz, C(8)H); 3.39 (m, 1H, C(5)H → superposed by HDO); 3.79 (s, 3H, OCH_3); 3.89 (m, 1H, C(5)H); 5.82 (s, 1H, C(1)H); 7.03 (dd, 2H, ^3J = 2.0 Hz, ^3J = 6.8 Hz, arom. C(3)H and C(5)H); 7.47 (dd, 2H, ^3J = 2.0 Hz, ^3J = 7.2 Hz, arom. C(2)H and C(6)H); 11.00 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.24 (SCH_3); 22.30 (C7); 22.84 (C6); 45.47 (C5); 53.00 (C8); 55.24 (OCH_3); 81.39 (C1); 114.01 (arom. C3 and C5); 127.68 (arom. C1); 128.92 (arom. C2 and C6); 160.35 (arom. C4); 162.13 (C(3)-S). HRMS [(ESI) m/z]: Calcd. for [$C_{14}H_{19}N_3OS$ (base) + H]$^+$: 278.1322. Found [M_{base} + H]$^+$: 278.1316.

1-(4-Dimethylaminophenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine hydroiodide **(2r)**

Reaction time 1.5 d. Yield: 90%. Beige crystals. M.r. 135–145 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.73. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1483; 1526 ($C{=}N^+H$), 1565, 1611; 2800, 2851, 2924, 2950, 2981 (aliph. H); 3043 (arom. H/N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.56–1.81 (m, 4H, C(7)H_2 and C(6)H_2); 2.75–2.83 (m + s, 4H, C(8)H and SCH_3); 2.93–2.98 (m + s, 7H, C(8)H and CH_3); 3.40 (m, 1H, C(5)H); 3.88 (bd, 1H, ^3J = 13.2 Hz, C(5)H); 5.70 (s, 1H, C(1)H); 6.78 (dd, 2H, ^3J = 1.2 Hz, ^3J = 8.4 Hz, arom. C(3)H and C(5)H); 7.33 (dd, 2H, ^3J = 1.6 Hz, ^3J = 8.4 Hz, arom. C(2)H and C(6)H); 10.93 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.24 (SCH_3); 22.34 and 22.82 (C6 and C7); 39.72 (CH_3 → superposed by solvent signal); 45.54 (C5); 52.73 (C8); 82.15 (C1); 111.96 (arom. C3 and C5); 128.61 (arom. C2 and C6); 151.39 (arom. C1 and C4); 162.14 (C(3)-S). Elemental analysis [$C_{15}H_{23}IN_4S$ (418.3), %]: Calcd. C, 43.07; H, 5.54; N, 13.39. Found: C, 41.21; H, 4.92; N, 12.96.

1,1-Dimethyl-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-a]pyridazine hydroiodide **(2s)**

Reaction time 5 d. Yield: 57%. Yellowish crystals. M.r. 109–114 °C (propan-2-ol). TLC (m. ph. 3): R_f = 0.66. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1496; 1524 ($C{=}N^+H$);1631; 2858, 2930, 2955, 2986 (aliph. H); 3096 (N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.44 (s, 6H, CH_3); 1.60 (bquint, 2H, ^3J = 5.6 Hz, C(6)H_2); 1.76 (bquint, 2H, ^3J = 4.8 Hz, C(7)H_2); 2.82 (t, 2H, ^3J = 5.2 Hz, C(8)H_2); 3.64 (t, 2H, ^3J = 5.6 Hz, C(5)H_2); 10.65 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.64 (SCH_3); 22.56 (C7); 23.06 (C6); 23.32 (CH_3); 45.75 (C5); 47.50 (C8); 81.90 (C1); 162.42 (C(3)-S). MS (70 eV, RT) m/z (%): 198.9 (6) [$M^{+\cdot}_{base}$]; 184.8 (23) [$M^{+\cdot}_{base}$ – $\cdot CH_3$]; 183.9 (100); 169.9 (13); 127.8 (11); 99.8 (15); 42.0 (11); 41.0 (18); 31.9 (18); 28.0 (90). Elemental analysis [$C_9H_{18}IN_3S$ (327.2), %]: Calcd. C, 33.04; H, 5.54; N, 12.84. Found: C, 33.16; H, 5.79; N, 13.04.

1,1-Pentamethylen-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2t)**

Reaction time 2 d. Yield: 39%. Colorless crystals. M.r. 74–185 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.80. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1522 ($C{=}N^+H$); 1603; 2855, 2931, 3084 (aliph. H): 3347 (N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.38–1.51 (m, 4H, C(7)H_2 and CH_2 of pentamethylene); 1.58–1.70 (m, 6H, C(6)H_2 and 2x CH_2 of pentamethylene); 1.79–1.83 (m, 4H, C(3′)H_2 and further CH_2 of pentamethylene); 2.77 (s, 3H, SCH_3); 2.80 (t, 2H, ^3J = 5.2 Hz, C(8)H_2); 3.70 (t, 2H, ^3J = 5.6 Hz, C(5)H_2); 10.52 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 15.17 (SCH3); 21.90 (C2′ und C4′ of pentamethylene); 22.81 (C3′ of pentamethylene); 23.81 and 24.16 (C6 and C7); 31.63 (C1′ and C5′ of pentamethylene); 45.77 (C5); 47.31 (C8); 83.45 (C1); 162.98 (C(3)-S). MS (70 eV, 300 °C) m/z (%): 239.2 (4) [$M^{+\cdot}_{base}$]; 225.2 (58);196.1 (39); 182.1 (100); 169.2 (21); 142.0 (89); 127.0 (54); 85.1 (52); 81.1 (34); 70.9 (19); 69 (13); 56.4 (18); 55.4 (38); 53.2 (14); 42.1 (23); 41.0 (63); 39.1 (22); 32.0 (17); 30.0 (29); 29.0 (22); 28 (95); 27 (21). Elemental analysis [$C_{12}H_{22}IN_3S$ (367.3), %]: Calcd. C, 39.24; H, 6.04; N, 11.44. Found: C, 38.08; H, 6.56; N, 11.16. HRMS [(ESI) m/z]: Calcd. for [$C_{12}H_{21}N_3S$ (base) + H]$^+$: 240.1529. Found [M_{base} + H]$^+$: 240.1522.

1-Methyl-3-methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]-pyridazine hydroiodide **(2u)**

Reaction time 5 d. Yield: 72%. Pale yellow crystals. M.r. 143–153 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.71. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1524 ($C{=}N^+H$); 1584; 1621; 2784, 2854, 2922, 2983 (aliph. H); 3027 (arom. H); 3100 (N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.53–1.59 (m, 1H, C(6)H); 1.68–1.72 (m, 1H, C(6)H); 1.78–1.84 (m + s, 5H, C(7)H_2) and CH_3); 2.74 (m, 1H, C(8)H); 2.83 (m + s, 4H, C(8)H and SCH_3); 3.55–3.58 (m, 1H, C(5)H); 3.74–3.77 (m, 1H, C(5)H); 7.39–7.47 (m, 3H, arom. C(3)H, C(4)H and C(5)H); 7.60–7.62 (m, 2H, C(2)H and C(6)H); 11.52 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.95 (SCH_3); 22.22 (CH_3); 22.73 (C7); 23.26 (C6); 45.93 (C5); 48.31 (C8); 84.84 (C1); 125.60 (arom. C2 and C6); 128.46 (arom. C3 and C5); 128.84 (arom. C4); 140.89 (arom. C1); 162.99 (C(3)-S). MS (70 eV, 300 °C) m/z (%): 261.0 (5) [$M^{+\cdot}_{base}$]; 246.0 (100); 183.9 (45); 161.9 (50); 127.8 (26); 102.7 (25); 77.3 (23) [$C_6H_5^+$]; 43.1 (17); 42.1 (11); 41.0 (14); 32.0 (15); 28.0 (76). Elemental analysis [$C_{14}H_{20}IN_3S$ (389.3), %]: Calcd. C, 43.19; H, 5.18; N, 10.79. Found: C, 43.36; H, 5.41; N, 10.89.

1-(4-Methoxyphenyl)-1-methyl-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine hydroiodide **(2v)**

Reaction time 2.5 d. Yield: 78%. Yellowish-brown crystals. M.r. 116–126 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.61. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1484; 1510 ($C{=}N^+H$); 1584; 1608; 2781, 2832, 2929, 2995 (aliph. H); 3035 (N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.52–1.58 (m, 1H, C(6)H); 1.65–1.67 (m, 1H, C(6)H); 1.76 (m + s, 5H, C(7)H_2 and CH_3); 2.66–2.68 (m, 1H, C(8)H); 2.73 (m, 1H, C(8)H); 2.80 (s, 3H, SCH_3); 3.56–3.60 (m, 1H, C(5)H); 3.71–3.78 (m + s, 3H, C(5)H and OCH_3); 7.00 (d, 2H, ^3J = 8.8 Hz, arom. C(3)H and C(5)H); 7.51 (d, 2H, ^3J = 8.8 Hz, arom. C(2)H and C(6)H); 11.25 (NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.64 (CH_3); 22.31 (SCH_3); 22.62 (C6); 23.04 (C7); 45.85 (C5); 48.08 (C8); 55.16 (OCH_3); 84.64 (C1); 113.64 (arom. C3 and C5); 127.21 (arom. C2 and C6); 131.88 (arom. C1); 159.48 (arom. C4); 162.63 (C(3)–S). HRMS [(ESI) m/z]: Calcd. for [$C_{15}H_{21}N_3OS$ (base) + H]$^+$: 292.1478. Found [M_{base} + H]$^+$: 292.1483. Elemental analysis [$C_{15}H_{22}IN_3OS$ (419.3), %]: Calcd. C, 42.97; H, 5.29; N, 10.02. Found: C, 43.45; H, 5.53; N, 9.93.

3-Methylsulfanyl-1,1-diphenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine hydroiodide (2w)

Reaction time 5 d. Yield: 45%. Colorless crystals. M.r. 180–190 °C (tetrahydrofuran; dec.; from about 240 °C formation of compact crystals which melt at 251–253 °C (thione 1w). TLC (m. ph. 3): R_f = 0.91. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1478; 1507 ($C{=}N^+H$); 1585; 1591; 2776, 2853, 2920 (aliph. H); 3050 (arom. H/N^+H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.62 (bs, 2H, C(6)H_2); 1.80 (bs, 2H, C(7)H_2); 2.24 (bs, 2H, C(8)H_2); 2.82 (bs, 3H, SCH_3); 3.80 (bs, 2H, C(5)H_2); 7.47 (m, 10H, arom. H); 11.84 (bs, 1H, NH^+). ^{13}C NMR (DMSO-d_6, ppm): δ = 15.09 (SCH_3); 22.92 (C7); 23.39 (C6); 46.10 (C5); 50.36 (C8); 89.53 (C1); 127.41; 128.47 and 129.26 (arom. C2, C2′, C3, C3′, C4, C4′, C5, C5′, C6 and C6′); 137.73 (arom. C1 u.C1′); 163.10 (C(3)-S). MS (70 eV, 250 °C) m/z (%): 323.0 (6) [$M^{+\cdot}_{base}$]; 309 (7); 246.0 (100); 223.8 (60); 166.0 (11); 164.9 (44); 127.8 (16); 121.0 (17); 103.7 (12); 85.0 (60) [$C_4H_9N_2^+$]; 77.3 (17) [$C_6H_5^+$]; 56.3 (12);

41.0 (16); 32.0 (12); 30.0 (18); 28.0 (67). Elemental analysis [$C_{19}H_{22}IN_3S$ (451.4),%]: Calcd. C, 50.56; H, 4.91; N, 9.31. Found: C, 49.97; H, 4.94; N, 9.09.

1-(4-Methoxyphenyl)-3-methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine hydroiodide **(2x)**
Reaction time 2.5 d. Yield: 86%. Yellow crystals. M.r. 157–167 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.88. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1481; 1511 (C=N$^+$H); 1582; 1607; 2848, 2932, 2958 (aliph. H); 3061 (arom. H/N$^+$H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.62–1.63 (m, 2H, C(6)H$_2$); 1.78–1.81 (m, 2H, C(7)H$_2$); 2.20–2.27 (m, 2H, C(8)H$_2$); 2.82 (s, 3H, SCH$_3$); 3.78 (bs, 5H, C(5)H$_2$) and OCH$_3$); 7.00 (d, 2H, ^3J = 9.2 Hz, arom. C(3)H and C(5)H, subst. phenyl); 7.35 (d, 2H, ^3J = 8.0 Hz, arom. C(2)H and C(6)H, subst. phenyl); 7.44–7.51 (m, 5H, arom. H, unsubst. phenyl); 11.74 (NH$^+$). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.95 (SCH$_3$); 22.84 (C6); 23.32 (C7); 45.97 (C5); 50.23 (C8); 55.19 (OCH$_3$); 89.16 (C1); 113.66 (arom. C3 and C5, subst. Ring); 127.12 (arom. C3, C4 u.C5, unsubst. phenyl); 127.92 (arom. C1, unsubst. phenyl); 128.33 (arom. C2 and C6, unsubst. phenyl); 128.97 (arom. C2 and C6, subst. phenyl); 159.65 (arom. C1 and C4, subst. phenyl); 162.79 (C(3)–S). Elemental analysis [$C_{20}H_{24}IN_3OS$ (481.4), %]: Calcd. C, 49.90; H, 5.03; N, 8.73. Found: C, 48.92; H, 5.12; N, 8.58. HRMS [(ESI) m/z]: Calcd. for [$C_{20}H_{23}N_3OS$ (base) + H]$^+$: 354.1635. Found [M_{base} + H]$^+$: 354.1632.

1-(2-Chlorophenyl)-3-methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine hydroiodide **(2y)**
Reaction time 5 d. Yield: 35%. Colorless crystals. M.r. 240–244 °C (tetrahydrofuran). TLC (m. ph. 3): R_f = 0.91. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 764 (Cl); 1477; 1516 (C=N$^+$H); 1571; 1586; 1611; 2852; 2920; 2942; 2978 (aliph. H); 3054 (arom. H/N $^+$H). ^{1}H NMR (DMSO-d_6, ppm): δ = 1.63 (bs, 2H, C(6)H$_2$); 1.81 (bs, 2H, C(7)H$_2$); 2.09 (bs, 1H, C(8)H); 2.38 (m, 1H, C(8)H); 2.77 (bs, 3H, SCH$_3$); 3.85 (bs, 2H, C(5)H$_2$); 7.40–7.54 (m, 8H, arom. C(3)H, unsubst. phenyl and other arom. H of both rings); 8.21–8.22 (m, 1H, arom. C(3)H, subst. phenyl); 11.75 (bs, 1H, NH$^+$). ^{13}C NMR (DMSO-d_6, ppm): δ = 15.42 (SCH$_3$); 23.09 and 23.54 (C6 and C7); 45.70 (C5); 49.77 (C8); 89.49 (C1); 126.88; 127.24; 128.10; 129.51 (arom. C3, subst. phenyl); 131.31; 131.86 (arom. C2, subst. phenyl); 135.49 (arom. C1 both phenyl rings); 162.18 (C(3)-S). → other signals belong to the remaining aromatic carbon atoms but are not to assign without doubt. Elemental analysis [$C_{19}H_{21}ClIN_3S$ (485.8),%]: Calcd. C, 46.98; H, 4.36; N, 8.65. Found: C, 46.36; H, 4.31; N, 8.39.

1-(4-Chlorophenyl)-3-methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine hydroiodide **(2z)**
Reaction time 1 d. Yield: 95%. Yellowish, hygroscopic crystals. M.r. 166 °C [dichloromethane; dec.; recrystallization beginning from 212 °C, remelting at 254–254.5 °C (thione 1z)]. TLC (m. ph. 2): R_f = 0.87. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1477; 1512 (C=N$^+$H); 1575; 1593; 2774; 2847; 2915; 3032 (N$^+$H). ^{1}H NMR (CDCl$_3$, ppm): δ = 1.73 (m, 2H, C(6)H$_2$ → superposed by HDO); 1.89–1.92 (m, 2H, C(7)H$_2$); 2.27–2.28 (m, 2H, C(8)H$_2$); 3.19 (bs, 3H, SCH$_3$); 3.80–3.81 (m, 2H, C(5)H$_2$); 7.35–7.40 and 7.68 (m and bs, 9H, arom. H); NH$^+$ not visible. ^{13}C NMR (CDCl$_3$, ppm): δ = 17.41 (SCH$_3$), 24.00 and 24.35 (C6 and C7); 46.46 (C5); 49.83 (C8); 92.32 (C1); 128.28, 128.57, 128.65, 128.95 and 129.60 (arom. C2, C2', C3, C3',C4', C5, C5', C6 and C6'); 132.49 (arom. C1 and C1'); 134.71 (arom. C(4)Cl); 162,92 (C(3)-S).
Elemental analysis [$C_{19}H_{21}ClIN_3S$ (485.8) · H_2O (503.8), %]: Calcd. C, 45.30; H, 4.60; N, 8.34. Found: C, 44.73; H, 4.33; N, 7.92.

3.3.2. Free bases from 3-methylsulfanyl-5,6,7,8-tetrahydro-1H-[1,2,4]-triazolo[1,2-a]pyridazine hydroiodides 2

The related hydroiodide **2** (60 to 80 mg) was dissolved in about 30 mL water and 1 mL triethylamine was added. The whole mixture was transferred into a separating funnel and shaken twice with equivalent amounts of dichloromethane. The organic phases were combined and dried with sodium sulfate. The solvent was removed using a rotary evaporator without heating. In the following, the residue was additionally dried under low pressure (about 10 mbar) for at least one hour to remove the triethylamine as far as possible. It is nevertheless visible in most of the NMR spectra.

Free base from **2e:** 3-Methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.42–1.49 (m, 1H, C(6)H); 1.54–1.65 (m, 3H, C(7)H$_2$ and C(6)H); 2.47–2.50 (m+s, 4H, C(8)H and SCH$_3$); 2.72–2.74 (m, 1H, C(8)H); 3.03 (t, 1H, ^3J = 11.2 Hz, C(5)H); 3.44 (d, 1H, ^3J = 12.0 Hz, C(5)H); 5.09 (s, 1H, C(1)H); 7.19–7.29 (m, 3H, arom. C(3)H, C(4)H and C(5)H); 7.38–7.40 (m, 2H, arom. C(2)H and C(6)H).

Free base from **2l:** 3-Methylsulfanyl-1-(2-nitrophenyl)-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyrid-azine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.34–1.39 (m, 1H, C(6)H); 1.50–1.53 (m, 1H, C(6)H); 1.70 (m, 2H, C(7)H$_2$); 2.53 (s, 4H, SCH$_3$); 2.74–2.78 (m, 2H, C(8)H$_2$); 3.17 (dt, 1H, ^3J = 1.2 Hz, ^3J = 14.0 Hz, C(5)H); 3.48 (dd, 1H, ^3J = 4.0 Hz, ^3J = 14.0 Hz, C(5)H); 5.99 (s, 1H, C(1)H); 7.33 (t, 1H, ^3J = 8.0 Hz, arom. C(6)H); 7.46 (t, 1H, ^3J = 8.0 Hz, arom. C(4)H); 7.58 (d, 1H, ^3J = 7.6 Hz, arom. C(5)H); 7.74 (d, 1H, ^3J = 8.0 Hz, arom. C(3)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 13.55 (SCH$_3$); 22.93 and 23.10 (C6 and C7); 43.91 (C5); 50.94 (C8); 83.56 (C1); 123.24 (arom. C3); 127.45 and 127.60 (arom. C6 and C4); 131.32 (arom. C5); 134.27 (arom. C1); 148.31 (arom. C2); 162.18 (C(3)-S).

Free base from **2m:** 3-Methylsulfanyl-1-(3-nitrophenyl)-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.41–1.51 (m, 1H, C(6)H); 1.60–1.69 (m, 3H, C(6)H and C(7)H$_2$); 2.48 (s, 3H, SCH$_3$); 2.63–2.68 (m, 1H, C(8)H); 2.72–2.74 (m, 1H, C(8)H); 3.11 (dt, 1H, ^3J = 2.4 Hz, ^3J = 12.4 Hz C(5)H); 3.50 (d, 1H, ^3J = 12.8 Hz, C(5)H); 5.22 (s, 1H, C(1)H); 7.42–7.47 (m, 1H, arom. C(5)H); 7.74 (d, 1H, ^3J = 7.6 Hz, arom. C(6)H); 8.09 (dd, 1H, ^3J = 0.8 Hz, ^3J = 8.4 Hz, C(4)H); 8.29 (d, 1H, ^3J = 1.6 Hz, arom. C(2)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 12.96 (SCH$_3$); 22.83 and 22.87 (C6 and C7); 44.86 (C5); 50.60 (C8); 87.89 (C1); 121.85 and 122.12 (arom. C2 and C4); 128.11 (arom. C5); 133.00 (arom. C6); 141.32 (arom. C3); 147.24 (arom. C1); 162.83 (C(3)-S).

Free base from **2n:** 3-Methylsulfanyl-1-(4-nitrophenyl)-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.50–1.57 (m, 1H, C(6)H); 1.66–1.76 (m, 3H, C(6)H and C(7)H$_2$); 2.55 (s, 3H, (SCH$_3$); 2.72–2.80 (m, 2H, C(8)H$_2$); 3.16 (dt, 1H, ^3J = 2.4 Hz, ^3J = 12.4 Hz, C(5)H); 3.57 (d, 1H, ^3J = 11.2 Hz, C(5)H); 5.30 (s, 1H, C(1)H); 7.66 (d, 2H, ^3J = 8.4 Hz, arom. C(2)H and C(6)H); 8.20 (d, 2H, ^3J = 8.4 Hz, arom. C(3)H and C(5)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 14.17 (SCH$_3$); 24.05 (C6 and C7); 46.00 (C5); 51.85 (C8); 89.08 (C1); 123.61 (arom. C3 and C5); 128.76 (arom. C2 and C6); 147.38 and 147.95 (arom. C1 and arom. C4); 163.87 (C(3)-S).

Free base from **2o:** 1-(2-Hydroxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.48–1.54 (m, 1H, C(6)H); 1.63–1.73 (m, 3H, C(6)H and C(7)H$_2$); 2.46 (s, 3H, SCH$_3$); 2.64 (bs, 1H, C(8)H); 2.89 (bs, 1H, C(8)H); 3.09 (bs, 1H, C(5)H); 3.53 (d, 1H, ^3J = 12.8 Hz, C(5)H); 5.29 (bs, 1H, C(1)H); 6.76–6.79 (m, 2H, arom. C(3)H and C(5)H); 7.11–7.20 (m, 2H, arom. C(4)H and C(6)H); signal for OH not detectable. ^{13}**C NMR** (CDCl$_3$, ppm): δ = 12.98 (SCH$_3$); 22.63 and 22.72 (C6 and C7); 28.68 (C5); 45.26 (C8); 88.34 (C1); 116.20 (arom. C3); 118.34 (arom. C5); 127.99 (arom. C6); 128.73 (arom. C4); quaternary C not detectable.

Free base from **2p:** 1-(3-Hydroxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.44–1.47 (m, 1H, C(6)H); 1.57–1.64 (m, 3H, C(6)H and C(7)H$_2$); 2.47–2.48 (m, 4H, C(8)H and SCH$_3$ → superposed by triethylamine signal); 2.73 (m, 1H, C(8)H); 3.07 (t, 1H, ^3J = 12.0 Hz, C(5)H); 3.47–3.50 (m, 1H, C(5)H); 5.06 (s, 1H, C(1)H); 6.55 (d, 3H, ^3J = 7.6 Hz, arom. C(4)H); 6.80 (d, 1H, ^3J = 7.6 Hz, arom. C(6)H); 6.86 (s, 1H, arom. C(2)H); 7.01 (dt, 1H, ^3J = 2.4 Hz, ^3J = 7.6 Hz, arom. C(5)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 12.79 (SCH$_3$); 22.79 and 22.92 (C6 and C7); 44.99 (C5); 50.50 (C8); 88.73 (C1); 114.31 (arom. C2); 115.10 (arom. C4); 117.90 (arom. C6); 128.18 (arom. C5); 139.00 (arom. C1); 156.03 (arom. C3); 162.72 (C(3)-S).

Free base from **2q:** 1-(4-Hydroxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.51–1.57 (m, 1H, C(6)H); 1.66–1.69 (m, 3H, C(6)H and C(7)H$_2$); 2.52–2.56 (m+s, 4H, SCH$_3$ and C(8)H); 2.78–2.84 (m, 1H, C(8)H); 3.10 (t, 1H, ^3J = 11.2 Hz, C(5)H); 3.55 (d, 1H, ^3J = 11.6 Hz, C(5)H) 5.07 (s, 1H, C(1)H); 6.53 (d, 2H, ^3J = 8.4 Hz, arom. C(3)H and C(5)H); 7.15 (d, 2H, ^3J = 8.4 Hz, arom. C(2)H and C(6)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 12.72 (SCH$_3$); 22.69 and 22.82 (C6 and C7); 45.08 (C5); 50.28 (C8); 88.64 (C1); 114.84 (arom. C3 and C5); 127.99 (arom. C2 and C6); 128.46 (arom. C1); 156.10 (arom. C4); 162.86 (C(3)-S).

Free base from **2qa**: 1-(4-Methoxyphenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.51–1.66 (m, 4H, C(6)H$_2$ and C(7)H$_2$), 2.51–2.58 (s+m, 4H, SCH$_3$ + C(8)H); 2.77 (d, 1H, ^3J = 10.0 Hz, C(8)H); 3.06 (t, 1H, ^3J = 11.6 Hz, C(5)H); 3.50 (d, 1H, ^3J = 10.8 Hz, C(5)H); 3.77 (s, 3H, OCH$_3$); 5.09 (s, 1H, C(1)H); 6.85 (dd, 2H, ^3J = 2.0 Hz, ^3J = 8.8 Hz, arom. C(3)H and C(5)H); 7.36 (dd, 2H, ^3J = 2.0 Hz, ^3J = 8.8 Hz, arom. C(2)H and C(6)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 13.93 (SCH$_3$); 24.05 and 24.07 (C6 and C7); 46.32 (C5); 55.45 (C8); 55.85 (OCH$_3$); 89.90 (C1); 113.82 (arom. C3 and C5); 115.68 (arom. C1); 129.26 (arom. C2 and C6); 159.81 (arom. C4); 163.48 (C(3)-S).

Free base from **2r**: 1-(4-Dimethylaminophenyl)-3-methylsulfanyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine
^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.48–1.51 (m, 1H, C(6)H); 1.54–1.64 (m, 1H, C(7)H$_2$ and C(6)H); 2.51 (m + s, 4H, C(8)H and SCH$_3$); 2.76–2.79 (m, 1H, C(8)H); 2.90 (s, 6H, CH$_3$); 3.05 (t, 1H, ^3J = 8.4 Hz, C(5)H); 3.48 (d, 1H, ^3J = 12.0 Hz, C(5)H); 5.05 (s, 1H, C(1)H); 6.69 (dd, 2H, ^3J = 1.6 Hz, ^3J = 8.4 Hz, arom. C(3)H and C(5)H); 7.30 (dd, 2H, ^3J = 1.6 Hz, ^3J = 8.4 Hz, arom. C(2)H and C(6)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 13.91 (SCH$_3$); 24.10 and 24.12 (C6 and C7); 40.88 (CH$_3$); 46.40 (C5); 51.14 (C8); 90.20 (C1); 112.59 (arom. C3 and C5); 128.91 (arom. C2 and C6); 150.96 (arom. C1 and C4); 163.29 (C(3)-S).

Free base from **2w**: 3-Methylsulfanyl-1,1-diphenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]-triazolo[1,2-*a*]pyridazine
In a mixture of 0.33 mmol **2w,** 2 mL ethanol, and 1 mL dichloromethane ammonia was bubbled for 3 min. The stoppered batch was allowed to stand at room temperature. Within 2 days colorless crystals were formed which were collected by suction filtration, washed with a little dichloromethane and dried in vacuum.
Yield: 12%. Colorless crystals. M.r. 160–165 °C (dichloromethane).
An equivalent approach using propylamine instead of ammonia yielded 83% of free base from **2w** when the mixture was allowed to stand at room temperature for two month.
TLC (m. ph. 3): R_f = 0.91. **IR** (KBr, cm^{-1}): $\tilde{\nu}$ = 1536 (C=N); 1584;1596; 1620; 2837, 2915, 2936, 2947, 2962 (aliph. H); 3024, 3057, 3082 (arom. H). ^{1}**H NMR** (CDCl$_3$, ppm): δ = 1.46 (bs, 2H, C(6)H$_2$); 1.68–1.72 (m, 2H, C(7)H$_2$); 2.12 (t, 2H, C(8)H$_2$); 2.62 (s, 3H, CH$_3$); 3.47 (bs, 2H, C(5)H$_2$); 7.19–7.51 (m, 6H; arom. C(3)H, C(5)H and C(4)H of the phenyl substituents); 7.51 (bs, 4H, arom. C(2)H and C(6)H). ^{13}**C NMR** (CDCl$_3$, ppm): δ = 14.94 (CH$_3$); 24.11 and 24.48 (C6 and C7); 45.99 (C5); 48.32 (C8); 93.83 (C1); 127.25, 127.81 and 128.11 (arom. C2, C3, C4, C5 and C6 of both phenyl rings); 161.25 (C(3)-S). Note: The aromatic C3 was not detectable under the applied conditions. Whether this was caused by a rapid relaxation or the related signal lied underneath one of the other signals is not sure, as, due to leak of differentiation of proton signals, an identification via 2D NMR spectra was not possible. **Elemental analysis** [$C_{19}H_{21}N_3S$ (323.5), %]: Calcd. C, 70.55; H, 6.54; N, 12.99. Found: C, 69.76; H, 6.48; N, 12.98.

Free base from **2y**: 1-(2-Chlorophenyl)-3-methylsulfanyl-1-phenyl-5,6,7,8-tetrahydro-1*H*-[1,2,4]triazolo[1,2-*a*]pyridazine

The free base from **2y** was obtained analogously as described above for the free base from **2w**.
Yield: 12%. Colorless crystals. M.r. 140–141 °C (dichloromethane).
TLC (m. ph. 2): R$_f$ = 0.85. **IR** (KBr, cm^{-1}): $\tilde{\nu}$ = 760 (Cl); 1531 (C=N); 1568, 1585; 1683; 2841, 2899, 2924, 2938, 2974, 2990 (aliph. H); 3025, 3054 (arom. H). **^{1}H NMR** (DMSO-d_6, ppm): δ = 1.41 (s, 2 H, C(7)H$_2$); 1.68 (t, 2 H, C(6)H$_2$); 2.00 (t, 2 H, C(5)H$_2$); 2.53 (s, 3 H, S–CH$_3$ → superposed by solvent signal); 3.50 (s, 2 H, C(8)H$_2$ → superposed by HDO); 7.20-7.45 (9 H, all arom. H). **^{13}C NMR** (DMSO-d_6, ppm): δ = 14.20 (S–CH$_3$); 23.39, 23.60 (C6 and.C7); 44.74 (C5); 47.11 (C8); 93.03 (C1); 126.21, 126.68, 127.21, 127.54, 129.20, 129.82, 130.69, 132.54, 139.30, 140.01 (all arom. C); 159.40 (C(3)-S). **Elemental analysis** [$C_{19}H_{20}ClN_3S$ (357.9), %]: Calcd. C, 63.76; H, 5.63; N, 11.74. Found: C, 61.91; H, 5.49; N, 11.41.

3.3.3. Methyl-(5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene)sulfonium iodides 3

To a solution of 1 mmol (for **3a**, **3d** 2.5 mmol) of the appropriate thione **1** (Schulz et al. 2013) in 25 mL (for **3a**, **3d** 20 mL) dichloromethane 1.5 mmol (for **3a**, **3d** 3.75 mmol) methyl iodide was added. The sealed batch was allowed to stand at room temperature until the reaction was completed (TLC monitoring, reaction time is given below). Subsequently the solvent was removed at room temperature and the formed crystals were collected by suction filtration, washed with diethyl ether (**3a**, **3d**: tetrahydrofuran) and dried under reduced pressure.

Methyl-(5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene)sulfonium iodide ***(3a)***
Reaction time 3 d. Yield: 1% (extracted from residue of mother liquor stored 18 months). Beige crystals. M.r. 110–120 °C (tetrahydrofuran). TLC (m. ph. 4): R$_f$ = 0.16. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1432, 1458 (-CH-); 1505, 1538 (-C=N-); 2848, 2917, 2971 (-CH-); 3038 (-CH$_3$). ^{1}H NMR (DMSO-d_6, ppm): δ = 2.04–2.09 (m, 4H, C(6)H$_2$ and C(7)H$_2$); 2.78 (s, 3H, SCH$_3$); 4.21 (t, 2H, ^3J = 5.2 Hz, C(8)H$_2$); 4.47 (t, 2H, ^3J = 5.2 Hz, C(5) H$_2$); 9.20 (s, 1H, C(3)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.07 (SCH$_3$); 18.22 and 18.52 (C6 and C7); 46.65 (C8); 48.15 (C5); 147.29 (C3); 159.23 (C(1)=S$^+$). HRMS [(ESI) m/z]: Calcd. for [$C_7H_{12}N_3S$]$^+$ (cation): 170.0746. Found [M$_{cation}$]$^+$: 170,0747.

Methyl-[3-(2-phenylethenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-yliden]sulfonium iodide ***(3c)***
Reaction time 7 d. R$_f$ of the intermediate (**2c**): 0.70 (m. ph. 1). Yield: 45%. Grey-pinkish crystals. M.r. 179–187 °C (dichloromethane). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1438; 1509, 1576; 1634; 2871, 2926 (aliph. H); 3026, 3054 (arom. H). ^{1}H NMR (CDCl$_3$, ppm): δ = 1.64 (s, 2 H, C(6)H$_2$); 2.37 (s, 2 H, C(7)H$_2$); 2.87 (d, 3 H, S-CH$_3$); 4.31 (s, 2 H, C(5)H$_2$); 4.86 (s, 2 H, C(8)H$_2$); 7.22 (d, 1 H, -C$\underline{\text{H}}$=CH-aryl); 7.42-7.45 (m, 3 H, arom. C(3)H/C(4)H/ C(5)H); 7.74-7.79 (m, 2 H, arom. C(2)H/C(6)H); 8.00 (d, 1 H, -CH=C$\underline{\text{H}}$-aryl). ^{13}C NMR (CDCl$_3$, ppm): δ = 14.72 (S-CH$_3$); 19.03 (C6); 19.07 (C7); 47.37 (C8); 48.47 (C5); 108.09 (C3); 128.96 (-$\underline{\text{C}}$H=CH-aryl); 129.05 (2C, arom. C2/ C6); 131.15 (arom. C4); 133.94 (2C, arom. C3/C5); 145.42 (-CH=$\underline{\text{C}}$H-aryl); 156.19 (arom. C1); 160.43 (C(1)=S$^+$). Elemental analysis [$C_{15}H_{18}IN_3S$ (399.3), %]: Calcd. C, 45.12; H, 4.54; N, 10.52. Found: C, 41.95; H, 3.85; N, 9.24.

Methyl-{3-[2-(2-nitrophenyl)ethenyl]-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]-pyridazin-1-ylidene}sulfonium iodide ***(3d)***
Reaction time 7 d. R$_f$ of the intermediate (**2d**): 0.48 (m. ph. 1). Yield: 55%. Yellow crystals. M.r. 188-195 °C (dichloromethane). TLC (m. ph. 4): R$_f$ = 0,15. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1355 (NO$_2$); 1432; 1508, 1530 (NO$_2$); 1570, 1630; 2930, 2964 (aliph. H); 3010 (arom. H). ^{1}H NMR (DMSO-d_6, ppm): δ = 2.10 (bs, 4H, C(6)H$_2$ and C(7) H$_2$); 2.84 (s, 3H, SCH$_3$); 4.23 (bs, 2H, C(5)H$_2$); 4.60 (bs, 2H, C(8)H$_2$); 7.49 (d, 1H, ^3J = 15.6 Hz, C(1′)H=C(2′)); 7.75 (t, 1H, ^3J = 7.0 Hz, arom. C(4)H); 7.90 (t, 1H, ^3J = 7.0 Hz, arom. C(5)H); 8.14 (d, 2H, ^3J = 6.8 Hz, arom. C(3)H and C(6)H); 8.25 (d, 1H, ^3J = 15.6 Hz, C(1′)=C(2′)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.11 (SCH$_3$); 18.17 and 18.36 (C6 and C7); 46.92 and 47.22 (C8 and C5); 113.78 (C(1′)); 124.95 (arom. C3); 129.40 (arom. C1); 129.44 (arom. C6); 131.31 (arom. C4); 133.98 (arom. C5); 137.48 (C(2′)); 148.24 (arom. C2); 153.84 (C3); 159.21 (C(1)=S$^+$). Elemental analysis [$C_{15}H_{17}IN_4O_2S$ (444.3), %]: Calcd. C, 40.55; H, 3.86; N, 12.61. Found: C, 40.99; H, 3.51; N, 12.13. HRMS [(ESI) m/z]: Calcd. for [$C_{15}H_{17}N_4O_2S$]$^+$ (cation): 317.1067. Found [M$_{cation}$]$^+$: 317.1079.

Methyl-(3-phenyl-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene) sulfonium iodide ***(3e)***
Method A:
A solution of 0.3 mmol **2e** in 2 mL ethanol was allowed to stand for 3 weeks. Subsequently the solvent was removed at room temperature. The formed crystals were collected by suction filtration, washed with a little propan-2-ol and dried under reduced pressure. Reaction time 21 d. Yield: 28%. Colorless crystals. M.r. 170–172 °C (propan-2-ol).
Method B:
The related compound **2e** was dissolved in propan-2-ol.The solvent was allowed to volatilize while contacting the ambient atmosphere using a surface as large as possible. Therefore the solution was transferred into a two-necked flask with a boiling capillary on one neck ending shortly above the liquid surface. Through a plug the system was connected to a membrane pump creating a light underpressure. Thus, an airflow passed over the liquid. Additionally the solution was stirred. If necessary, the procedure was repeated. Reaction time: to dryness about 0.5 d. Yield: 17%. M.r. 168–174 °C.
TLC (m. ph. 4): R$_f$ = 0.36. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1524; 1600 (C=N); 2874; 2933; 2958; 3000 (CH); 3042 (arom. H). UV [H$_2$O, nm (lg ε)]: λ_{max} = 231 (4.388). ^{1}H NMR (DMSO-d_6, ppm): δ = 2.06–2.13 (m, 4H, C(6)H$_2$ and C(7)H$_2$); 2.85 (s, 3H, CH$_3$); 4.30 (t, 2H, C(8)H$_2$); 4.55 (t, 2H, C(5)H$_2$); 7.67–7.77 (m, 3H, arom. C(3)H, C(4) H and C(5)H); 7.91 (d, 2H, arom. C(2)H and C(6)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.14 (CH$_3$); 18.07 and 18.64 (C6 and C7); 47.20 (C8); 49.27 (C5); 123.90 (arom. C1); 129.31 and 129.34 (arom. C2, C3, C5 and C6); 132.60 (arom. C4); 155.78 (C3); 159.01 (C(1)=S$^+$). MS (70 eV, 180 °C): m/z (%) = 372.7 (0.5) (M$^{+\cdot}$); 245.8 (2); 230.8 (1.3); 191.8 (0.4); 127.8 (0.6); 102.7 (1.9); 77.3 (0.8); 31.9 (2); 27.9 (100). Elemental analysis [$C_{13}H_{16}IN_3S$ (373.3), %]: Calcd. C, 41.83; H, 4.32; N, 11.26. Found: C, 41.52; H, 4.42; N, 11.49. HRMS [(ESI) m/z]: Calcd. for [$C_{13}H_{16}N_3S$]$^+$ (cation): 246.1059. Found [M$_{cation}$]$^+$: 246.1055.

Methyl-[3-(3-methylphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]sulfonium iodide ***(3g)***
To a solution of 0.5 mmol of the thione **1g** (Schulz et al. 2013) in 7 mL dichloromethane prepared under warming 0.75 mmol methyl iodide were added and the batch was allowed to stand at room temperature. After the reaction was completed (TLC control) the solvent was removed at room temperature and the resulting non-crystalline residue was layered with diethyl ether. The crystals formed within 5 weeks were collected by suction filtration, washed with diethyl ether and dried under reduced pressure. Reaction time 3.5 d. R$_f$ of the intermediate **2g**: 0.80 (m. ph. 1). Yield: 38%. Yellowish crystals. M.r. 175–182 °C (dichloromethane/diethyl ether).
IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1458; 1464; 1477; 1518, 1586, 1608; 2872, 2928, 2945, 2963, 2992 (aliph. H); 3060 (arom. H). ^{1}H NMR (DMSO-d_6, ppm): δ = 2.07-2.10 (m, 4 H, C(6)H$_2$ and C(7)H$_2$); 2.44 (s, 3 H, arom. CH$_3$); 2.85 (s, 3 H, S-CH$_3$); 4.29 (t, 2 H, C(8) H$_2$); 4.54 (t, 2 H, C(5)H$_2$); 7.55-7.58 (m, 2 H, arom. C(4)H and C(6)H); 7.68-7.72 (m, 2 H, arom. C(2)H and C(5)H).^{13}C NMR (DMSO-d_6, ppm): δ = 15.19 (S-CH$_3$); 19.15 (arom. CH$_3$); 19.71 (C7); 21.89 (C6); 48.25 (C8); 50.35 (C5); 124.91 (C3); 127.49 (arom. C6); 130.28 (arom. C4); 130.57 (arom. C5); 134.28 (arom. C2); 140.02 (arom. C3); 156.99 (arom. C1); 160.06 (C(1)=S$^+$). Elemental analysis [$C_{14}H_{18}IN_3S$ (387.3), %]: Calcd. C, 43.42; H, 4.68; N, 10.85. Found: C, 42.98; H, 3.94; N, 10.25.

Methyl-[3-(4-methylphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]sulfonium iodide ***(3h)***
To a solution of 1 mmol of the appropriate thione **1** (Schulz et al. 2013) in 25 mL dichloromethane 3.75 mmol methyl iodide was added. The batch was allowed to stand at room temperature until the reaction was completed (TLC monitoring, reaction time is given below). Subsequently the solvent was removed at room temperature and the formed crystals were collected by suction filtration, washed with tetrahydrofuran and dried under reduced pressure. Reaction time 5 d. Yield: 9%. Beige crystals. M.r. 174–178 °C (propan-2-ol).
TLC (m. ph. 4): R$_f$ = 0.24. IR (cm^{-1}): $\tilde{\nu}$ = 1429 (CH); 1470 (C=N); 1536, 1612 (C=C, arom.); 2929 (CH). ^{1}**H** NMR (CDCl$_3$, ppm): δ = 2.37–2.39 (m, 2H, C(7)H$_2$); 2.45–2.48 (m + s, 5H, C(6)H$_2$ and CH$_3$); 2.86 (s, 3H, SCH$_3$); 4.44 (t, 2H, ^3J = 5.6 Hz, C(8)H$_2$); 4.77 (t, 2H, ^3J = 5.6 Hz, C(5)H$_2$); 7.37 (d, 2H, ^3J = 8.0 Hz, arom. C(2)H and C(6)H); 7.82 (d, 2H, ^3J = 8.4 Hz, arom. C(3)H and C(5)H). ^{13}C NMR (CDCl$_3$, ppm): δ = 14.81 (SCH$_3$); 18.58 and 19.52 (C6 and C7); 21.73 (CH$_3$); 47.64 (C8); 49.75 (C5); 120.70 (arom. C1); 129.73 and 130.06 (arom. C2, C3, C5 and C6); 143.67 (arom. C4); 157.58 (C3); 160.43 (C(1)=S$^+$). HRMS [(ESI) m/z]: Calcd. for [$C_{14}H_{18}N_3S$]$^+$ (cation): 260.1216. Found [M$_{cation}$]$^+$: 260.1211.

[3-(3-Chlorophenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]-methylsulfonium iodide ***(3j)***
Compound **3j** was prepared as described for the sulfonium iodide **3g**, but using 10 mL dichloromethane and 1 mmol methyl iodide. Reaction time 5 d. Yield: 45%. Yellowish crystals. M.r. 163–170 °C (dichloromethane/diethyl ether).
TLC (m. ph. 1): R$_f$ = 0,02. IR (KBr, cm^{-1}): $\tilde{\nu}$ = 735 (Cl); 1439, 1458; 1516, 1566; 2876, 2933, 2970 (aliph. H); 3036, 3073 (arom. H). ^{1}H NMR (CDCl$_3$, ppm): δ = 2.40-2.51 (m, 4 H, C(6)H$_2$ and C(7)H$_2$); 2.89 (s, 3 H, S-CH$_3$); 4.46 (t, 2 H, C(8)H$_2$); 4.78 (t, 2 H, C(5)H$_2$); 7.54-7.61 (m, 2 H, arom. C(2)H and C(4)); 7.88-7.96 (m, 2 H, arom. C(5) H and C(6)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 15.22 (S-CH$_3$); 19.12 (C6); 19.59 (C7); 48.36 (C8); 50.34 (C5); 126.92 (C3); 129.14 (arom. C6); 129.88 (arom. C4); 132.41 (arom. C2); 133.57 (arom. C5); 135.01 (arom. C3); 155.61 (arom. C1); 160.29 (C(1)=S$^+$). Elemental analysis [$C_{13}H_{158}ClIN_3S$ (407.7), %]: Calcd. C, 38.30; H, 3.71; N, 10.31. Found: C, 39.09; H, 3.54; N, 10.05.

Methyl-[3-(4-nitrophenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]sulfonium iodide ***(3n)***
To a solution of 2.5 mmol of the appropriate thione **1** (Schulz et al. 2013) in 20 mL dichloromethane 3.75 mmol methyl iodide were added. The sealed batch was allowed to stand at room temperature until the reaction was completed (TLC monitoring, 37,5 d). Subsequently the solvent was removed at room temperture and the formed crystals were collected by suction filtration, washed with tetrahydrofuran and dried under reduced pressure. From the solid residue of the mother liquor the substance was yielded by washing with propan-2-ol. Yield: 10%. Beige crystals. M.r. 172–178 °C (propan-2-ol).
TLC (m. ph. 4): R$_f$ = 0.31. IR (cm^{-1}): $\tilde{\nu}$ = 1433, 1463 (-CH-); 1524 (-C=N-); 1601, 1633 (-C=C-); 2921 (-CH-). ^{1}H NMR (DMSO-d_6, ppm): δ = 2.08–2.14 (m, 4H, C(6) H$_2$ and C(7)H2); 2.86 (s, 3H, SCH$_3$); 4.34 (t, 2H, ^3J = 5.6 Hz, C(8)H$_2$); 4.57 (t, 2H, ^3J = 5.6 Hz, C(5)H$_2$); 8.18 (d, 2H, ^3J = 8.0 Hz, arom. C(2)H and C(6)H); 8.49 (d, 2H, ^3J = 8.0 Hz, arom. C(3)H and C(5)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.22 (SCH$_3$); 18.02 and 18.48 (C6 and C7); 47.39 (C8); 49.43 (C5); 124.30 (arom. C3 and C5); 129.65 (arom. C1); 131.08 (arom. C2 and C6); 149.59 (arom. C4); 154.18 (C3); 159.38 (C(1)=S$^+$). **HRMS** [(ESI) m/z]: Calcd. for [$C_{13}H_{15}N_4O_2S$]$^+$ (cation): 291.0910. Found [M$_{cation}$]$^+$: 291.0917.

[3-(Hydroxyphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]-methylsulfonium iodides ***(3o-q)***
To a solution of 0.5 mmol of the appropriate thione **1** (Schulz et al. 2013) in 5 mL dimethyl sulfoxide 1 mmol methyl iodide was added and the stoppered batch was allowed to stand at room temperature until the reaction was completed (TLC monitoring). Subsequently the solvent was removed by warming on a water bath under reduced pressure and the resulting non-crystalline product was either layered with diethyl ether (**3o**) or dissolved in dichloromethane followed by slow evaporation of the solvent at room temperature and crystallizing the residue from propan-2-ol (**3p,q**). The formed crystals were collected by suction filtration, washed with diethyl ether (**3o**) and a little cold propan-2-ol (**3p,q**), respectively, and dried in vacuum.

[3-(2-Hydroxyphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]-methylsulfonium iodide ***(3o)***
Reaction time 7 d. R_f of the intermediate **2o**: 0.74 (m. ph. 1). Yield: 28%. Yellow-brownish crystals. M.r. 185–195.5 °C (diethyl ether). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1488; 1525, 1591, 1605; 1666; 2922, 2977 (aliph. H); 3010 (arom. H); 3100 (arom. H/OH). ^1H NMR (DMSO-d_6, ppm): δ = 2.09-2.11 (m, 4 H, $C(6)H_2$ and $C(7)H_2$); 2.82 (s, 3 H, S-CH_3); 3.98-4.04 and 4.25-4.34 (m/m, 4 H, $C(5)H_2$ and $C(8)H_2$); 7.01-7.12 (m, 2 H, arom. C(3)H and C(5)H); 7.40-7.59 (m, 2 H, arom. C(4)H and C(6)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.65 (S-CH_3); 18.78 (C7); 18.97 (C6); 47.60 (C8); 48.94 (C5); 111.42 (C3); 117.03 (arom. C3); 120.18 (arom. C5); 132.07 (arom. C4); 134.58 (arom. C6); 155.75 (arom. C1); 156.04 (arom. C2); 159.60 ($C(1)=S^+$). Elemental analysis [$C_{13}H_{16}IN_3OS$ (389.3), %]: Calcd. C, 40.11; H, 4.14; N, 10.79. Found: C, 40.05; H, 3.66; N, 10.75.

[3-(3-Hydroxyphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]-methylsulfonium iodide ***(3p)***
Reaction time 7 d. R_f of the intermediate **2p**: 0.34 (m. ph. 1). Yield: 47%. Yellow-beige crystals. M.r. 186–192 °C (propan-2-ol). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1439; 1462; 1480; 1528; 1588; 1603; 2880, 2962 (aliph. H); 3148 (arom H/OH). ^1H NMR (DMSO-d_6, ppm): δ = 2.08 (s, 4 H, $C(6)H_2$ and $C(7)H_2$); 2.83 (s, 3 H, S-CH_3); 4.27 (s, 2 H, $C(8)H_2$); 4.50 (s, 2 H; $C(5)H_2$); 7.10 (d, 1 H, arom. C(6)H); 7.28 (d, 2 H, C(2)H and C(4)H); 7.47 (t, 1 H, arom. C(5)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.61 (S-CH_3); 18.52 (C7); 19.13 (C6); 47.65 (C8); 49.76 (C5); 116.31 (C3); 120.07 (arom. C2); 120.21 (arom. C4); 125.37 (arom. C6); 131.03 (arom. C5); 156.24 (arom. C1); 158.27 (arom. C3); 159.39 ($C(1)=S^+$). Elemental analysis [$C_{13}H_{16}IN_3OS$ (389.3), %]: Calcd. C, 40.11; H, 4.14; N, 10.79. Found: C, 38.17; H, 3.55; N, 9.95.

[3-(4-Hydroxyphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]-methylsulfonium iodide ***(3q)***
Reaction time 4 d. R_f of the intermediate **2q**: 0.29 (m. ph. 1). Yield: 58%. Yellow-orange crystals. M.r. 205–208 °C (propan-2-ol). IR (KBr, cm^{-1}): $\tilde{\nu}$ = 1440; 1455; 1474; 1589; 1607; 2952, 3048 (aliph. H); 3133 (arom. H/OH). ^1H NMR (DMSO-d_6, ppm): δ = 2.07 (s, 4 H, $C(6)H_2$ and $C(7)H_2$); 2.82 (s, 3 H, S-CH_3); 4.25 (m, 2 H, $C(8)H_2$); 4.51 (m, 2 H, $C(5)H_2$); 6.99-7.03 (m, 2 H, arom. C(3)H/C(5)H); 7.76-7.80 (m, 2 H, arom. C(2)H/C(6)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.62 (S-CH_3); 18.59 (C7); 19.30 (C6); 47.59 (C8); 49.81 (C5); 114.72 (C3); 116.65 (arom. C3/C5); 131.83 (arom. C2/C6); 156.53 (arom. C1); 159.22 (arom C4); 161.84 ($C(1)=S^+$). Elemental analysis [$C_{13}H_{16}IN_3OS$ (389.3), %]: Calcd. C, 40.11; H, 4.14; N, 10.79. Found: C, 39.31; H, 3.90; N, 10.29.

[3-(4-Methoxyphenyl)-5,6,7,8-tetrahydro-1H-[1,2,4]triazolo[1,2-a]pyridazin-1-ylidene]-methylsulfonium iodide ***(3qa)***
To a solution of 2.5 mmol of the appropriate thione **1** (Schulz et al. 2013) in 20 mL dichloromethane 3.75 mmol methyl iodide were added. The sealed batch was allowed to stand at room temperature until the reaction was completed (TLC monitoring; 2,5 d). Subsequently the solvent was removed at room temperature and the formed crystals were collected by suction filtration, washed with tetrahydrofuran and dried under reduced pressure.
Substance was yielded combining several solid residues of mother liquors of syntheses to the related **2** stored for 18 months and washing with tetrahydrofuran.
Yield: 20 mg (2%). Beige crystals. M.r. 186–190 °C (tetrahydrofuran). TLC (m. ph. 4): R_f = 0.21. IR (cm^{-1}): $\tilde{\nu}$ = 1436 (CH); 1465 (C=N); 1578, 1605 (C=C, arom.); 2955 (CH). ^1H NMR (DMSO-d_6, ppm): δ = 2.06–2.11 (m, 4H, $C(6)H_2$) and $C(7)H_2$); 2.84 (s, 3H, SCH_3); 3.89 (s, 3H, OCH_3); 4.27 (t, 2H, ^3J = 11.6 Hz, $C(8)H_2$); 4.53 (t, 2H, ^3J = 11.2 Hz, $C(5)H_2$); 7.22 (d, 2H, ^3J = 8.8 Hz, arom. C(3)H and C(5)H); 7.89 (d, 2H, ^3J = 8.8 Hz, arom. C(2)H and C(6)H). ^{13}C NMR (DMSO-d_6, ppm): δ = 14.10 (SCH_3); 18.06 and 18.73 (C6 and C7); 47.11 (C8); 49.26 (C5); 55.71 (OCH_3); 114.82 (arom. C3 and C5); 115.88 (arom. C1); 131.20 (arom. C2 and C6); 155.70 (C3); 158.79 ($C(1)=S^+$); 162.45 (arom. C4). HRMS [(ESI) m/z]: Calcd. for $[C_{14}H_{18}N_3OS]^+$ (cation): 276.1165. Found $[M_{cation}]^+$: 276.1176.

3.4. X-ray structural analysis

Diffraction data were collected at low temperature (-103.0 °C) on a STOE-IPDS 2T diffractometer with graphite-monochromated molybdenum K_α radiation, λ = 0.71073 Å. The structure was solved by direct methods (SHELXS-13) and refined by full-matrix least-squares techniques (SHELXL-13) (Sheldrick 2008). All non-hydrogen-atoms were refined with anisotropic displacement parameters. The hydrogen atoms were refined isotropically on calculated positions using a riding model with their U_{iso} values constrained to 1.5 U_{eq} of their pivot atoms for terminal sp^3 carbon atoms and 1.2 times for all other carbon atoms. DMSOCrystallographic data have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 1417441. Copies of the data can be obtained free of charge by contacting the CCDC via e-mail: deposit@ccdc.cam.ac.uk.
Structure and refinement data for compound **3d** are available on request.

3.5. Biological tests

Material and methods are described in detail in a previous publication (Schulz et al. 2013). The key parameters are given in the following.
Cell culture materials. All chemicals as well as interleukin-1β, fetal calf serum and trypane blue were purchased from Sigma Aldrich. Cell culture plastics, PBS, trypsin/EDTA and interferon-γ were obtained from Biochrom AG, RPMI1640 and penicillin/streptomycin from Lonza.
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.).
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 0.3125 mM and 0.156 mM with 0.5% dimethyl sulfoxide as solutizer. The reference inhibitor aminoguanidine was tested at the same concentrations. Dimethyl sulfoxide was added to the controls as well.
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.
MTT assay. The remaining cells in the test plates were used to perform the MTT assay as a test of cytotoxicity (Mosmann 1983). The 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 the medium was carefully removed and 100 µL dimethyl sulfoxide were added. After dissolution of the formazan crystals the absorption was immediately measured at a wavelength of 570 nm.
Statistics. Normality was tested and if positive F- and t-test were performed. Otherwise the Mann-Whitney-test was executed. Statistical significance was assumed for $p < 0.05$ or lower.

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