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8-(Dichloromethylene)-3,4,7,8-tetrahydro-6*H*-imidazo[5,1-*c*][1,2,4,6]thiatriazin-6-one 2,2-dioxides: synthesis, structure and antiproliferative activity evaluation

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Abstract

Through cyclization of 4-(dichloromethylene)-5-(aminosulfonylimino)imidazolidin-2-ones by formaldehyde action series of 8-(dichloromethylene)-3,4,7,8-tetrahydro-6*H*-

imidazo[5,1-*c*][1,2,4,6]thiatriazin-6-one 2,2-dioxides were synthesized. Their structure parameters were discovered by NMR experiments and X-Ray analysis. The separate products (with allyl, carbethoxymethyl, 2-*N*-Boc-aminoethyl, 2-(*N*-Boc-*N*-isopropoxymethylamino)ethyl substituents in position 3) demonstrated high antiproliferative activity.

Keywords

4-(dichloromethylene)-5-(aminosulfonylimino)imidazolidin-2-one; cyclization; aminal; imidazo[5,1-*c*][1,2,4,6]thiatriazine; antiproliferative activity

Introduction

The value of 1,2,4,6-thiatriazine derivatives for medicinal chemistry is supported by a number of examples (Figure 1) [1–5]. Both the variety of the synthetic approaches to this heterocyclic system and the possibilities of its modification (altering substituents near the Carbon and Nitrogen atoms, changing the saturation of the cycle, or the oxidation state of the Sulfur atom, *etc*.) are a powerful tool for the design of molecules with required properties [6, 7].

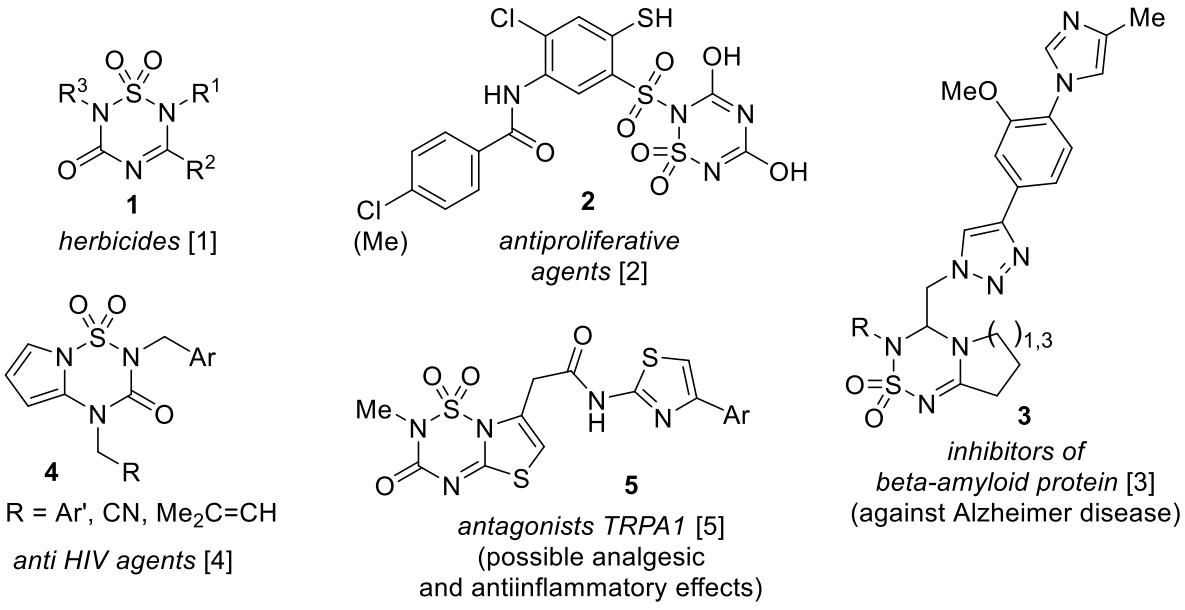
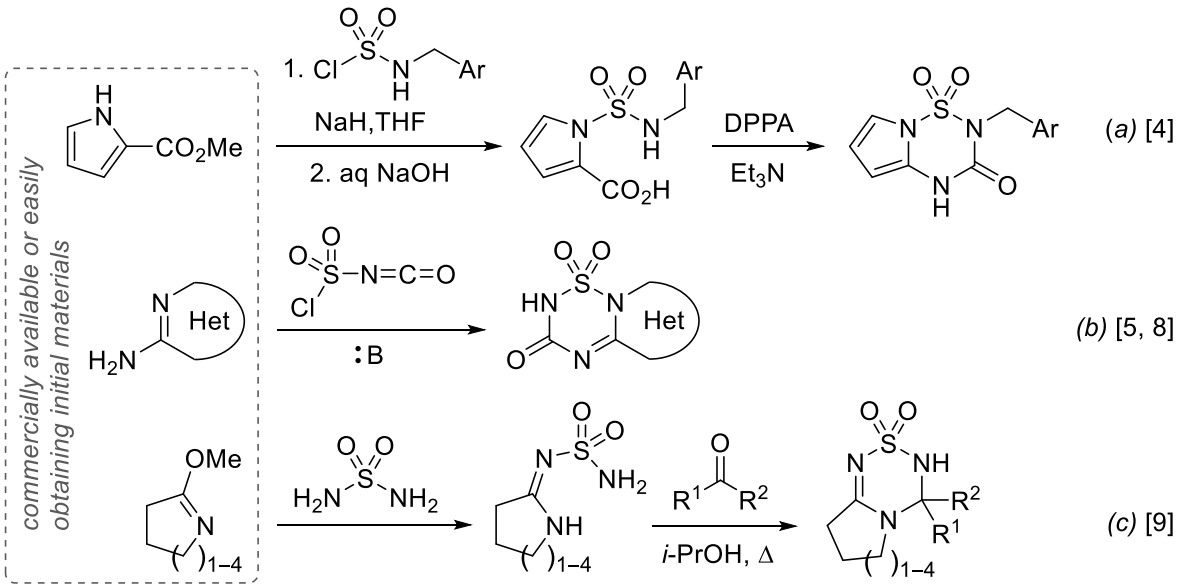


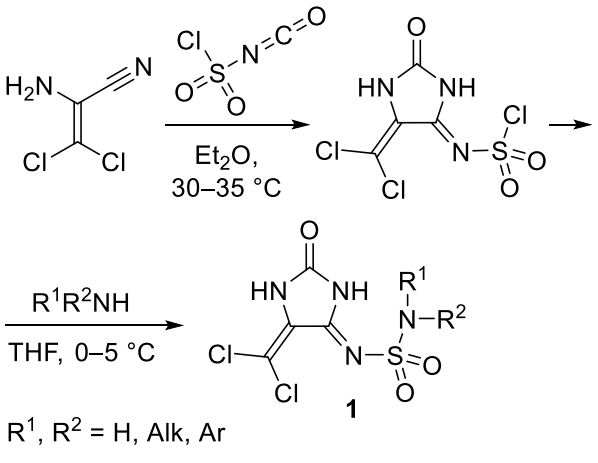
Figure 1: Bioactive 1,2,4,6-thiatriazines.

Literature data analysis easily discloses the fact that there is a significant fraction of heterocondensed derivatives among bioactive 1,2,4,6-thiatriazines (Figure 1). It is partly caused by the features of their preparation: some synthetically available heterocyclic derivatives have functional groups convenient for annulation of the 1,2,4,6-thiatriazine system (Scheme 1).



Scheme 1: The examples of [1,2,4,6]thiatriazine cycle annulation (literature data).

We paid attention to 5+1 cyclizations, which are carried out by the action of aldehydes or ketones on *N*-aminosulfonylamidines, such as the transformation in Scheme 1c, taking into account the fact that previously sulfonyl iminohydantoins of a general formula **1** with a similar fragment were synthesized by us (Scheme 2).



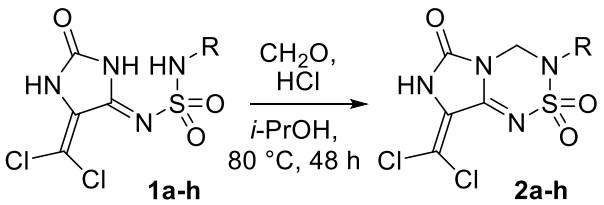
Scheme 2: 4-(Dichloromethylene)-5-(aminosulfonylimino)imidazolidin-2-ones synthesis (our previous works).

These compounds showed significant anticancer and antiviral activity [10–13]; and docking of individual representatives confirmed the important role which a polar SO_2-fragment plays in the enzyme binding center [14]. We may expect that the inclusion of a sulfamide group in the cycle and, therefore, the restriction of conformational conversions of this fragment will have an impact on bioactivity. In addition, the use of formaldehyde for such cyclization will cause minimal electronic impact on the system. In addition, such a small linker does not noticeably increase the molecular weight, which is important specifically in the field of medicinal chemistry.

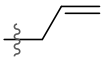
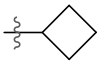
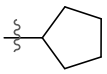
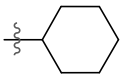
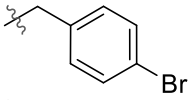
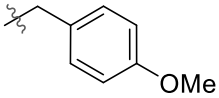
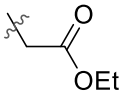
Results and Discussion

Synthesis

The interaction of sulfamides **1a-h** with formaldehyde solution (formalin) and addition of hydrochloric acid was carried out in a closed vial by prolonged boiling in *i*PrOH (Scheme 3). After using column chromatography for purification, the target products **2a-h** were obtained with yields, on average, about 50%. Such modest yields are explained by the requirement to stop the reaction before achieving complete conversion, since over time the reaction mixture destruction becomes too significant, and it decreases in the quantity and quality of the target product. At the same time, chromatographic purification of the product from residues of the starting compound is not difficult.



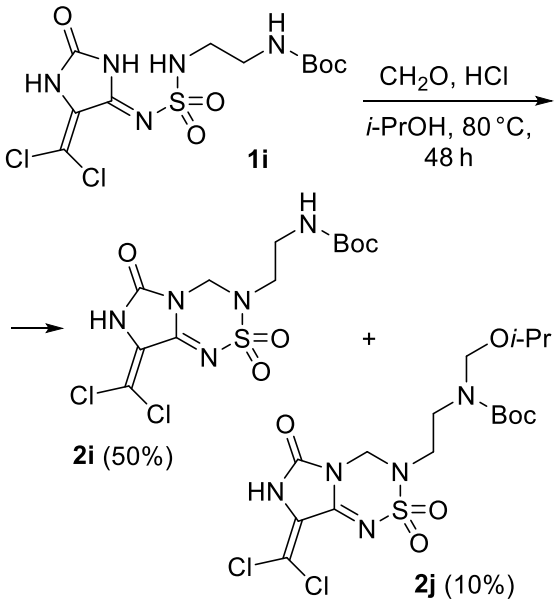
1a-h **2a-h**

	R	Product yield, %
a		64
b		31
c		37
d		41
e		53
f		64
g		53
h		53

Scheme 3: Cyclization of 4-(dichloromethylene)-5-(aminosulfonylimino)imidazolidin-2-ones into 8-(dichloromethylene)-3,4,7,8-tetrahydro-6*H*-imidazo[5,1-*c*][1,2,4,6]-thiatriazin-6-one 2,2-dioxides.

It is worth noting, that the *Z*-configuration of the C=N-SO_2 double bond in compounds **1**, shown in previous works [11], is certainly one of the favourable factors for this cyclization and the formation of a new heterocyclic system.

Interestingly, in cyclization conditions substrate **1i** with a Boc-protected ethane-1,2-diamine fragment, along with the target compound **2i**, also produces the isopropyl *N*,*O*-acetal **2j** (Scheme 4).



Scheme 4: The formation of by-product **2j** during substrate **1i** to **2i** cyclization.

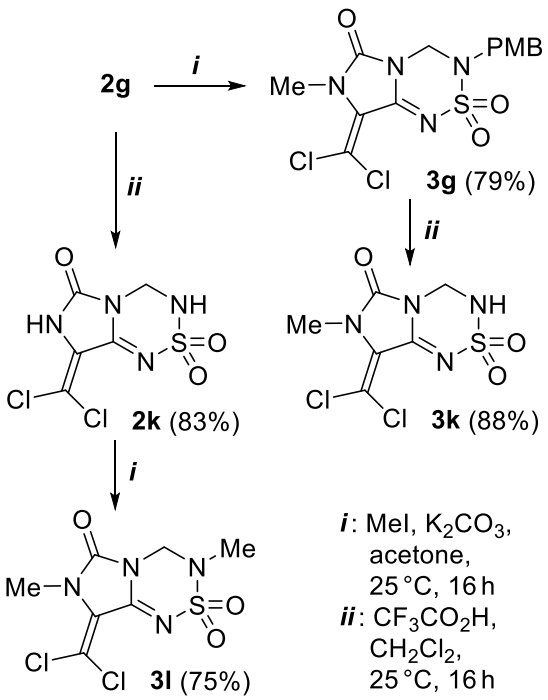
The initial molecule **1i** contains two NH groups with potential capacity for such a transformation, but a series of 2D NMR experiments confirmed that the *N*,*O*-acetal group is formed precisely with the participation of the side substituent's NH-group, and not with the heterocyclic NH: it can be confirmed, in particular, by numerous homo- and heteronuclear correlations between the atoms of the aliphatic residues (see Supporting Information File 1). Unexpectedly, in the ^{13}C NMR spectrum of *N*,*O*-acetal **2j** a doubling of some signals is observed. We consider it as a consequence of amide bond rotamerism [15], due to the existence of the equilibrium shown in the relevant section of Supporting Information File 1. The doubling of signals, rather than mere broadening, results from the fact that this process is relatively "slow" on the NMR timescale.

Using the PMB derivative **2g**, we performed some typical transformations to discover the stability of the newly formed heterocyclic core and its possibilities for further synthetic modifications. The stability issue was essential due to the possibility of the

3,4-dihydro-2*H*-1,2,4,6-thiatriazine 1,1-dioxide cycle existence in solution as an equilibrium mixture with its opened imino form, which was mentioned in Ref. [9].

The interaction with methyl iodide expectedly led to the methylation of the NH-group of the compound **2g** five-membered cycle and the formation of compound **3g** (Scheme 5).

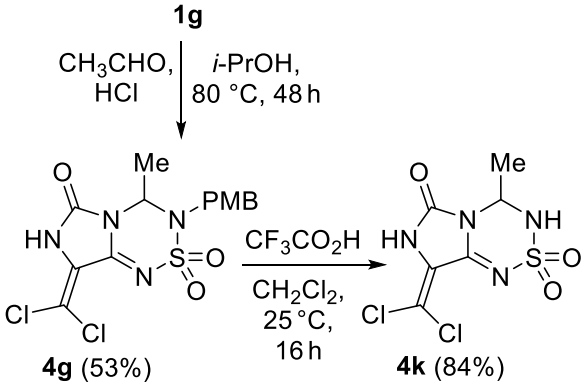
Treatment of compound **2g**, as well as its methyl derivative **3g**, with trifluoroacetic acid caused the desired removal of the PMB group and the formation of compounds **2k** and **3k**, respectively (Scheme 5). These reactions proceeded in sufficient yields, without the formation of any specific by-products, and no signs of the heterocyclic system instability were observed.



Scheme 5: Modifications of PMB derivative **2g**.

Both NH-fragments of compound **2k** appeared equally active in methylation reactions, which, due to interaction with an appropriate amount of methyl iodide, caused the formation of the dimethyl derivative **3l** (Scheme 5).

The obvious question of the possibility of cyclization of substrates **1** with other, than formaldehyde, carbonyl compounds has a positive answer at least for acetaldehyde: under similar conditions we obtained compound **4g** (Scheme 6). Unfortunately, under these conditions it was not possible to carry out the reaction with the less active benzaldehyde, since the reaction mixture undergoes severe destruction before appreciable conversion is achieved. The removal of the PMB group was also successfully realized for substance **4g** (Scheme 6).



Scheme 6: Substrate **1g** cyclization by acetaldehyde; further PMB-deprotection.

X-Ray analysis

X-Ray analysis of derivative **2k** allowed us to identify the structural features of this heterocyclic system (Figure 2).

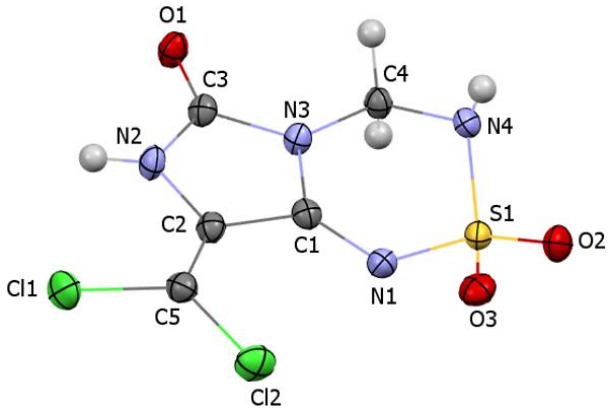


Figure 2: Molecular structure of derivative **2k** (X-ray data). Thermal ellipsoids of non-hydrogen atoms are shown at 50% probability level.

The partially saturated heterocycle of compound **2k** (Fig. 2) adopt a sofa conformation. The S1, N1, C1, N3 and C4 atoms lie within the plane with an accuracy of 0.012 Å, while the N4 atom deviates from this plane by 0.655(2) Å. The N4 atom has pyramidal configuration, the sum of bond angles centered at it is 340.9º.

In the crystal phase, molecules **2k** form the centrosymmetric dimers (Fig. 3) due to the intermolecular bonds N2–H…O1’ (symmetry operation is –x,1-y,1-z; H…O distance is 2.07(3) Å, N–H…O angle is 166(3)º). In turn, dimers are bound through the intermolecular hydrogen bonds N4–H…O3’ (symmetry operation is 1.5-x,0.5+y,0.5-z; H…O distance is 2.46(3) Å, N–H…O angle is 129(2)º).

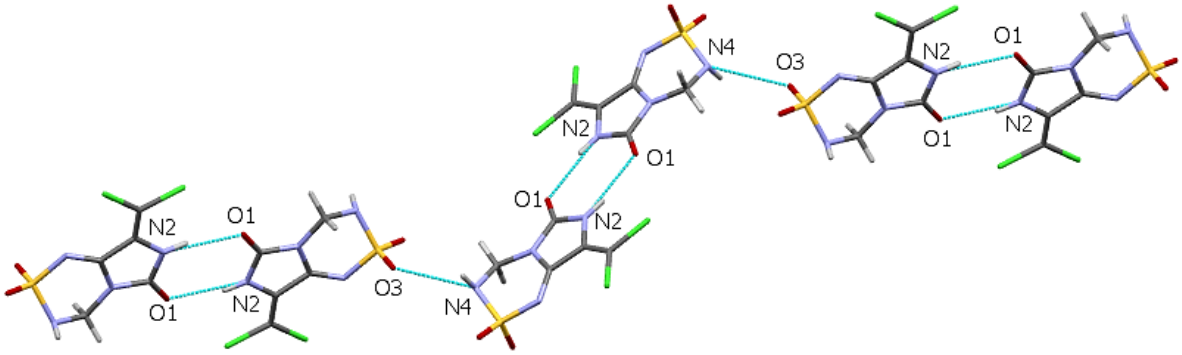


Figure 3: Packing of molecules **2k** in the crystal phase. Hydrogen bonds are shown in blue dashed lines.

For the details of experiment [16–18] see Supporting Information File 1.

Antiproliferative activity

In our previous investigations of the sulfamide derivatives of iminohydantoins effect on cancer cell growth, one of the best results was shown by the allylic derivative **1m** (see Scheme 2, in general formula **1** R^1 = Me, R^2 = allyl) [12]. Therefore, exploring the compound **2a** antiproliferative effect and comparing this data with the activity of the non-cyclic analogue **1m**, we were able to evaluate the effect of cyclization on anticancer activity.

The biological assay of compounds **1m, 2a, 2h-j** was carried out in cooperation with the National Cancer Institute (NCI, Bethesda, Maryland, USA) according to the International Program of the National Institutes of Health – DTP (Developmental Therapeutic Program, https://dtp.cancer.gov) of the National Cancer Institute (NCI, Bethesda, Maryland, USA); the details of the technique are also given in Ref. [19]. Complete data for all 60 cell lines is provided in Supporting Information File 1.

Single High Dose (10^{-5} M) assay of anticancer activity of compounds **2a**, **2h-j** showed that they also possess quite high activity; in particular, these derivatives caused fairly high percentage of lethality against certain lines of non-small cell lung cancer, as well as colon cancer, melanoma, ovarian, renal and breast cancer.

Comparison of the data of compounds **1m** and **2a** revealed that cyclization on average contributed to the antiproliferative activity increasing. For the majority of lines, the inhibitory effect of imidazothiatriazinone **2a** was stronger than iminohydantoin **1m** action (e.g., for lines SF-539 CNS cancer, SN12C renal cancer, MDA-MB-231/ATCC breast cancer). At the same time, a number of examples demonstrate a sharp change in the activity profile – from a very low inhibitory effect of substance **1m** to a high percentage of lethality caused by substance **2a** (e.g., EKVX non-small cell lung cancer, UO-31 renal cancer, BT-549 breast cancer).

On average, substances **2h,i** with, respectively, ester and NHBoc groups in the side substituent, showed anticancer activity that was slightly lower than the allylic derivative **2a**, but higher than the iminohydantoin **1m**. And the most active of the four studied imidazothiatriazinones was unexpectedly the *N*,*O*-acetal **2j**, which was lethal for 55 cancer cell lines. Without further targeted studies, it is impossible to say confidently which of the factors will play a key role here: a change in the geometric parameters of the molecule, its polarity, or the possibility of formaldehyde release under hydrolytic conditions. But the obtaining data are another verification of the significant prospects as anticancer agents for the sulfonyl iminohydantoins in general and their heterocondensed derivatives (in this case – imidazothiatriazinones) in particular.

The promising results of Single Dose tests of compounds **2a**, **2h-j** motivated a Five Doses study. In this experiment (for all data see Supporting Information File 1), the average anticancer activities of iminohydantoin **1m** and its cyclization product

imidazothiatriazinone **2a** were very close, the activity of derivatives **2h,i** was slightly lower, and compound **2j** was slightly higher. The last one even showed a GI_{50} of less than 10^{-6} M against several lines; these values are especially hopeful in cases where the TGI and LC_{50} were also low, namely, for the lines NCI-H522 non-small cell lung cancer, HCT-15 colon cancer, and MDA-MB-468 breast cancer.

As in Single Dose tests, there is a tendency for bigger imidazothiatriazinones actions on certain colon cancer, melanoma, breast cancer, renal cancer and non-small cell lung cancer lines. Interestingly, for many lines the GI_{50}, TGI and LC_{50} values are quite close, i.e. the concentration that is lethal for a culture of malignant cells is only 3–5 times higher than that causing 50% growth inhibition.

Conclusion

So, the action of formaldehyde on 4-(dichloromethylene)-5-(aminosulfonylimino)imidazolidin-2-ones were proved to be a practical method of 8-(dichloromethylene)-3,4,7,8-tetrahydro-6*H*-imidazo[5,1-*c*][1,2,4,6]thiatriazin-6-one 2,2-dioxides synthesis. This reaction can be provided for wide scope of aminosulfonyl moieties in iminohydantoin core, it was just limited by the activity of a cyclizing agent (using acetaldehyde instead of formaldehyde was successful, but benzaldehyde – not). The PMB-deprotection from the 3rd position and imidazolone fragment alkylation were confirmed to be productive techniques of further modifications of cyclization products. The significant antiproliferative activity of certain products demonstrated the possibilities of these heterocycles' practical usage.

Supporting Information

Supporting information (File 1) consists of four sections and contains, respectively: 1) descriptions of synthetic methods and characterization of compounds; 2) details of X-Ray analysis of compound **2k**; 3) copies of NMR spectra; 4) the details of primary *in vitro* assay of compounds **1m, 2a**, **2h-j** action in One Dose and Five Dose experiments against full NCI 60 cells panel.

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File Format: pdf

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Disclaimer

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