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Inhibition of *in vitro* transcription by 2-arylidene derivatives of thiazolo[3,2-α]benzimidazol-3(2H)-one

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Aim. *To evaluate a series of 2-substituted thiazolo[3,2-α]benzimidazolones as potential transcription inhibitors.* ***Methods***. *Compounds were tested in a model transcription system based on T7 RNA polymerase.* ***Results***. *The testing revealed a number of compounds able to inhibit transcription at micromolar concentrations. The most active inhibitor was dihydroxy derivative BT29 with* IC_{50} *= 1.6 μM.* ***Conclusions***. *Structure-functional dependence of the activity of tested compounds as transcription inhibitors was found. The key structural feature required for their high activity is a presence of hydroxy or dialkylamino group at p- or m-position of arylidene fragment.*

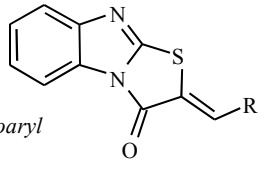
Keywords: thiazolo[3,2-α]benzimidazolones, transcription inhibitors, T7 RNA polymerase.

Introduction. Transcription is a key process required for cellular growth and replication. Specific inhibition of this process is a way to suppress viruses, bacteria and cancer cells. The search for novel transcription inhibitors remains one of the main directions of medicinal chemistry and drug design [1].

Molecular shape often determines the interactions of small molecules with biological targets, and shape complementarity is a critically important factor in the recognition. The notion that molecules with similar 3D shapes tend to have similar biological activity has been fully recognized and implemented in drug discovery [2].

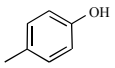
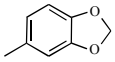
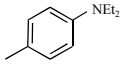
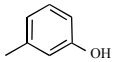
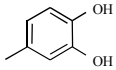
The literature search revealed that many transcription inhibitors were conjugated heteroaromatic compounds with S-like molecular shape. Molecular shape concept [2] allowed us to assume that 2-arylidene-substituted thiazolo[3,2-α]benzimidazoles, S-shaped molecules, could be inhibitors of RNA polymerases. These structures contain rigid bent tricyclic scaffold potentially able to interact with DNA thus affecting DNA-based enzymatic systems, with a relatively flexible arylidene fragment which can contain various substituents to ensure the efficient fitting to polymerase target, and a number of hydrogen bonding centers. So we designed a small library of 2-arylidene-[1,3]thiazolo[3,2-α]benzimidazol-3(2H)-ones (benzimidazothiazolones, BT) and studied their transcription inhibition activity. The *in vitro* screening was performed in a model transcription system based on bacteriophage T7 RNA polymerase (T7 RNAP).

BT



R – aryl, heteroaryl

Transcription inhibition activity of thiazolobenzimidazolone derivatives in T7 RNA polymerase assay

Compound	*R*	IC_{90}, μg/ml	IC_{50}, μg/ml	IC_{90}, M	IC_{50}, M
BT11		16.0	8.0	$5.4 \cdot 10^{-5}$	$2.7 \cdot 10^{-5}$
BT15		15.0	3.3	$4.6 \cdot 10^{-5}$	$1.0 \cdot 10^{-5}$
BT16		12.5	2.8	$4.4 \cdot 10^{-5}$	$9.8 \cdot 10^{-6}$
BT19		10.6	4.2	$3.0 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$
BT23		14.1	6.4	$4.8 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$
BT29		0.7	0.5	$2.3 \cdot 10^{-6}$	$1.6 \cdot 10^{-6}$

The transcription performed by this small single-subunit DNA-dependent RNA polymerase is fast and efficient and does not require ancillary transcription factors. Its mechanism of action has been thoroughly studied [3, 4], and the crystal structure is known [5] allowing the computer modeling of ligand binding to the transcription complex. Moreover, the structure of its active site is similar to that of other viral, bacterial and eukaryotic polymerases [5, 6]. T7 RNAP is thus a reliable *in vitro* model used for the studies of transcription [3–6] and mechanism of action of DNA-binding drugs [7–10]. This system was proposed in our previous papers for the screening of nucleic acids synthesis inhibitors [11–13].

Materials and Methods. T7 RNA polymerase and other components of the *in vitro* transcription reaction were purchased from «Fermentas» (Lithuania). 2-Arylidene-[1,3]thiazolo[3,2-α]benzimidazol-3(2H)-ones were prepared by three-step protocol based on methods [14, 15] (details will be published elsewhere).

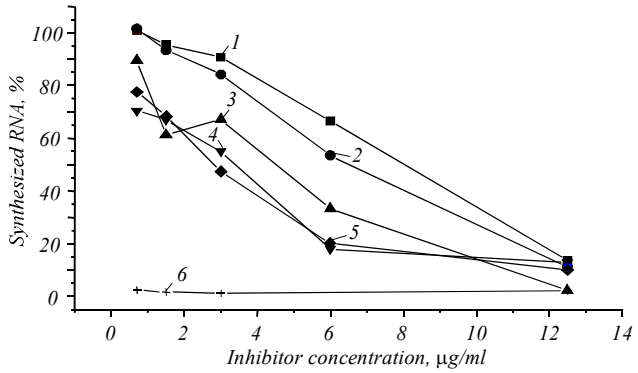
In vitro transcription assay. The screening was performed according to our protocol [11]. Reaction products were separated by electrophoresis in 1.2 % agarose gel. Gels stained with ethidium bromide were photographed with FujiFilm FinePix S5600 digital camera, and images were processed using TotallLab 1.10 software.

Inhibition activity was determined by comparing the amount of RNA produced in test reactions with that in a control (no inhibitor). IC_{50} and IC_{90} values (concentrations required for 50 and 90 % inhibition) were obtained from the concentration-activity plots. At least 3 independent experiments were performed for each compound. Standard deviations were below 10 %, except for BT23 where higher spread of data was observed (± 15 %).

Results and Discussion. Thiazolo[3,2-α]benzimidazoles have a wide range of biological activity [16]. Among 2-arylidene-[1,3]thiazolo[3,2-α]benzimidazol-3(2H)-ones were found antihelmintics [14], antibacterial agents [16], inhibitors of ubiquitin ligase [17] and other enzymes, and they may be used for the treatment of viral and inflammatory diseases, neurological disorders and cancer. In addition, some isosteric thiazolo[3,4-α]benzimidazoles inhibit enteroviruses [18] and tumors [19].

A series of 29 BT compounds containing aromatic and heteroaromatic arylidene fragments with halogen, hydroxy, alkoxy and dialkylamino substituents at various positions were synthesized. The testing was performed in an assay system involving T7 RNA polymerase. RNA was produced by T7 RNAP from linearized DNA template (*pTZ19R* plasmid containing T7 promoter and a 341 b. p. insert from which RNA transcript is synthesized).

All compounds were preliminary tested at the concentration of 25 μg/ml (70–80 μM, depending on the structure). *In vitro* transcription assay revealed the inhibition of transcription by most BT compounds. The results allowed us to evaluate the influence of arylidene substituent structure on biological activity. Six compounds (Table), that completely inhibited polymerase



The effect of inhibitor concentration on the efficiency of RNA synthesis *in vitro* (error bars are not shown (see the text)): *1* – BT11; *2* – BT23; *3* – BT19; *4* – BT15; *5* – BT16; *6* – BT29

at 25 μg/ml (inhibition above 99 %), were selected for further investigation.

Detailed study of the activity-concentration dependence of these compounds allowed determining their IC_{90} and IC_{50} values. A series of reactions with T7 RNAP was carried out with 2-fold inhibitor dilution at each next step. Five independent experiments were performed with all these BT compounds at each tested concentration. The activity plots were built by calculating the RNA product yield as a function of inhibitor concentration (Figure). IC_{90} and IC_{50} values were found from the plots by extrapolation. As can be seen from Figure, derivative BT29 demonstrated a very high activity and completely inhibited transcription at concentration below 1 μg/ml. An additional set of reactions at lower concentrations was performed to find that its IC_{50} is 1.6 μM. The other five compounds have IC_{50} in the range of 10–27 μM (Table).

Local deviation from linearity of the plot was observed for the compound BT19 that appeared as seeming enzyme stimulation at the concentration range of 2–3 μg/ml (Figure). A similar effect was reported for T7 RNA polymerase inhibition by pyrrolo[2,1-c][1, 4]benzodiazepines [8], although the authors were unable to provide any explanation.

We suppose that this deviation may indicate the presence of two binding sites for BT19 on the polymerase or its complex with DNA template, or two distinct binding modes. Mechanism of this effect will be the subject of the future study.

The analysis of experimental data revealed that the activity of BT compounds depended on the structure of arylidene fragment. The presence of halogen or multiple methoxy substituents in arylidene fragment significantly decreases the activity as compared to unsubstituted derivative (*R* = Ph, 94 % inhibition at 25 μg/ml)). The activity of compounds with heteroaryl moiety depends on its nature. Thiophen derivative almost totally inhibits RNA synthesis at 25 μg/ml, while 3- and 4-pyridyl derivatives are inactive; only 2-pyridyl derivative has noticeable activity (86 % inhibition at 25 μg/ml). Structure-activity relationship data suggest that the key feature of the most active compounds is the presence at *p*- or *m*-position of arylidene ring (but not at *o*-position) of either OH, alkoxy or dialkylamino group, i. e. functions able to form hydrogen and donor-acceptor bonds. The introduction of both *o*- and *n*-OH substituents into the structure of BT29 led to the dramatic increase of inhibitory activity.

Conclusions. A number of 2-aryliden-[1,3]thiazolo[3,2-α]benzimidazol-3(2H)-ones efficiently inhibit transcription in the T7 RNAP-based *in vitro* assay at micromolar concentrations. The dihydroxy derivative BT29 is the most active polymerase inhibitor with IC_{50} 1.6 μM. The structure of this compound will be further optimized using computer modeling to develop more efficient inhibitors. In our opinion, high activity of BT compounds *in vitro* allows to consider them potential antiviral drugs.

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Інгібування транскрипції *in vitro* 2-ариліденовими похідними тіазоло[3,2-α]бензімідазол-3(2H)-ону

Резюме

Мета. *Дослідити серію 2-заміщених тіазоло[3,2-α]бензімідазолонів як потенційних інгібіторів транскрипції.* ***Методи***. *Речовини тестували в модельній системі транскрипції на основі РНК-полімерази Т7.* ***Результати***. *Тестування виявило низку сполук, здатних інгібувати транскрипцію в мікромолярних концентраціях. Найактивнішим з-поміж них є дигідроксипохідне BT29 з* IC_{50} *= 1,6 μМ.* ***Висновки***. *Встановлено залежність активності вивчених речовин як інгібіторів транскрипції від їхнього структурно-функціонального стану. Ключовим фактором, що визначає їхню високу активність, є присутність гідрокси- чи діалкіламіногрупи в п- або м-положенні ариліденового фрагмента.*

Ключові слова: тіазоло[3,2-α]бензімідазолони, інгібітори транскрипції, РНК-полімераза Т7.

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Ингибирование транскрипции *in vitro* 2-арилиденовыми производными тиазоло[3,2-α]бензимидазол-3(2H)-она

Резюме

Цель. *Исследовать серию 2-замещенных тиазоло[3,2-α]бензимидазолонов как потенциальных ингибиторов транскрипции.* ***Методы***. *Вещества тестировали в модельной системе транскрипции на основе РНК-полимеразы Т7.* ***Результаты***. *Тестирование выявило ряд соединений, способных ингибировать транскрипцию в микромолярных концентрациях. Наиболее активным среди них является дигидрокси-производное BT29 с* $IC_{50} = 1,6$ *μM.* ***Выводы***. *Установлена зависимость активности изученных веществ как ингибиторов транскрипции от их структурно-функционального состояния. Ключевым фактором, определяющим их высокую активность, является присутствие гидрокси- или диалкиламиногруппы в п- или м-положении арилиденового фрагмента.*

Ключевые слова: тиазоло[3,2-α]бензимидазолоны, ингибиторы транскрипции, РНК-полимераза Т7.

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