Bioorg. Med. Chem. Lett. 30 (2020) 127653

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

New type of anti-influenza agents based on benzo[*d*][1,3]dithiol core

Tatyana M. Khomenko [a], Vladimir V. Zarubaev [b,*], Marina V. Kireeva [c], Alexandrina S. Volobueva [b], Alexander V. Slita [b], Sophia S. Borisevich [d], Dina V. Korchagina [a], Nina I. Komarova [a], Konstantin P. Volcho [a], Nariman F. Salakhutdinov [a]

[a] Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russia
[b] Department of Virology, Pasteur Institute of Epidemiology and Microbiology, 14 Mira St., 197101, St. Petersburg, Russia
[c] Saint Petersburg State University, 7/9 Universitetskaya nab.,199034, St. Petersburg, Russia
[d] Ufa Institute of Chemistry Ufa Federal Research Center RAS, pr. Oktyabrya, 71, 450054 Ufa, Russia

ARTICLE INFO

Keywords:
Heterocyclic compounds
Antiviral activity
Benzodithiols
H1N1 influenza virus
M2 channel
Hemagglutinin

ABSTRACT

We synthesized a series of amides with a benzo[*d*][1,3]dithiol core. The chemical library of compounds was tested for their cytotoxicity and inhibiting activity against influenza virus A/California/07/09 (H1N1)pdm09 in MDCK cells. For each compound, values of CC_{50}, IC_{50} and selectivity index (SI) were determined. Compounds of this structure type were for the first time found to exhibit anti-influenza activity. The structure of an amide substituent in the tested compounds was demonstrated to have a significant effect on their activity against the H1N1 influenza virus and cytotoxicity. Compound **4d** has a high selectivity index of about 30. **4d** was shown to be most potent at early stages of viral cycle. In direct fusogenic assay it demonstrated dose-dependent activity against fusogenic activity of hemagglutinin of influenza virus. Based on molecular docking and regression analysis data, viral hemagglutinin was suggested as possible target for these new antiviral agents.

Influenza viruses are major human pathogens that cause 3 to 5 million cases of severe illness worldwide.[1] The main means to fight the disease is vaccination, the effectiveness of which depends on the accuracy of prediction for the strain that will circulate in the upcoming epidemiological season and is far from 100% even in the case of a successful prediction. For example, the overall effectiveness of the 2017–2018 flu vaccine was 36%.[1] Therefore, chemotherapy is an important way to prevent and treat viral diseases. At present, few anti-influenza drugs are available: neuraminidase inhibitors (oseltamivir and zanamivir) and M2 channel blockers (amantadine and rimantadine)[2,3] as well as recently approved Baloxavir marboxil, selective inhibitor of influenza cap-dependent endonuclease.[4] However, a high variability of the influenza virus enables its resistance to drugs.[5,6] Therefore, the search for antiviral drugs of new structural types is an important task.[7–11]

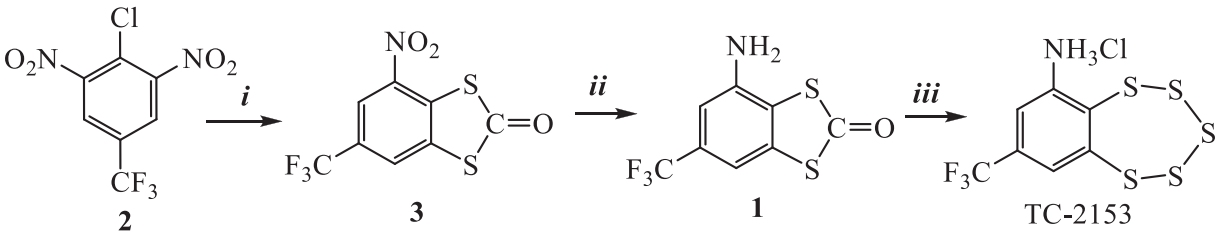
In this study, we screened compounds of various structural types for antiviral activity and found for the first time that 4-amino-6-(trifluoromethyl)benzo[*d*][1,3]dithiol-2-one **1** (Scheme 1) exhibited significant activity against the influenza virus, with IC_{50} in a lower micromolar range. This compound can be synthesized from 2-chloro-1,3-dinitro-5-(trifluoromethyl)benzene **2** in two stages and is a key intermediate in one of the methods for production of TC-2153; an inhibitor of the tyrosine phosphatase STEP.[12] TC-2153 exhibits antidepressant, anticonvulsant, and analgesic activities[13–16] and improves the condition of Alzheimer's disease and schizophrenia animal models.[12,17] However, the literature lacks data on the biological activity of compound **1**.

In this work, we synthesized a series of amides **4** and tested them for antiviral activity to elucidate the influence of a substituent at the amino group on the antiviral effect of compound **1**. For amine **1** modification nitrogen-containing heterocyclic compounds were chosen. Such substituents are often used in medicinal chemistry and can be found in many biologically active compounds. Thus, attachment of heterocycles such as piperidine and morpholine to borneol through ester bond allowed one to obtain compounds with high anti-flu activity.[11]

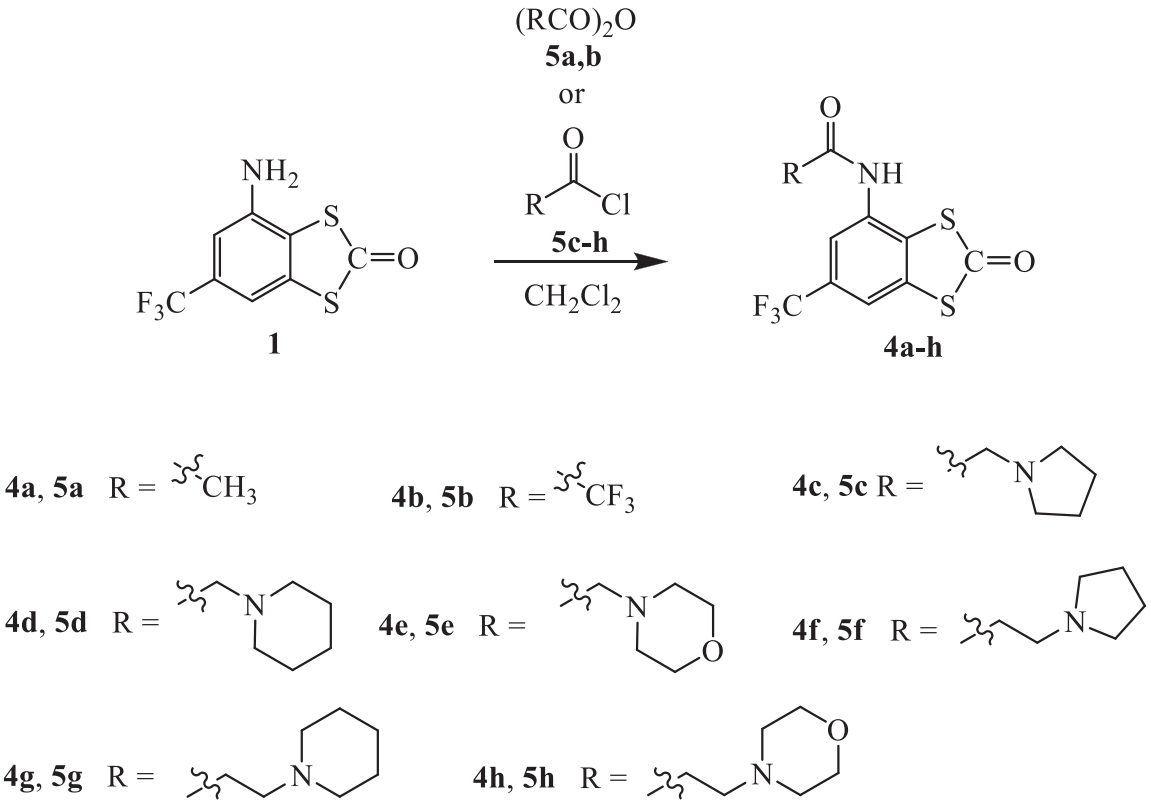
The interaction between compound **2** and sodium dimethyldithiocarbamate hydrate gave 4-nitro-6-(trifluoromethyl)benzo[*d*][1,3]dithiol-2-one **3** that was further reduced by the Zn/NH_4Cl system in accordance with a procedure[12] to form aminobenzodithiol **1** (Scheme 1). The resulting amine **1** reacted with a set of anhydrides **5a,b** or acid chlorides **5c-f** containing various substituents (Scheme 2).[18,19] Synthesis of the necessary chlorides **5c-f** was conducted in accordance with a

* Corresponding author.
E-mail address: zarubaev@gmail.com (V.V. Zarubaev).

https://doi.org/10.1016/j.bmcl.2020.127653
Received 10 September 2020; Received in revised form 22 October 2020; Accepted 24 October 2020
Available online 28 October 2020
0960-894X/



Scheme 1. Synthesis of compound **1**. (i) $NaSC(S)NMe_2$/DMSO, 44%; (ii) Zn/NH_4Cl, 84%; (iii) 1) $NaHS{\cdot}H_2O$, 24 h; HCl, 44%; 2) conc. HCl/Et_2O, 78%.



Scheme 2. Synthesis of compounds **4a-h**.

procedure.[20]

In general, the transformations proceeded smoothly, except the reactions of amine **1** with acid chlorides **5c,d**, where the starting amine **1** was incompletely converted under standard conditions. However, elongation of the linker by a CH_2-group upon transition to homologues **5f,g** significantly accelerated the reaction. Compound **4b** was produced with high purity; its yield was 84%. Compound **4a** was purified by recrystallization, which halved the yield. The yields of compounds **4c-h** after purification by chromatography on SiO_2 (eluent - hexane/benzene (1:1), ether, ethanol) amounted to 45–62%.

The synthesized compounds were tested for antiviral activity against the pandemic influenza virus A/California/07/09 (H1N1)pdm09 cultivated in cell culture using a technique described earlier.[21] Cytotoxicity of the compounds was evaluated in uninfected MDCK cells as described previously.[22] The obtained data were used to calculate the selectivity index for each derivative. Compounds with SI = 10 and higher were considered as active. The results are summarized in Table 1.

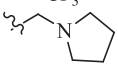
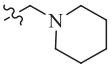
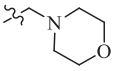
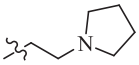
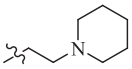
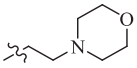
We found that 4-amino-6-(trifluoromethyl)benzo[*d*][1,3]dithiol-2-one **1** had high antiviral activity at low micromolar concentrations, which was combined with moderate cytotoxicity; the selectivity index was 11. Acetamide **4a** turned out to be both less active and more toxic, which led to a significant decrease in the selectivity index. On the contrary, trifluoroacetamide **4b** exhibited two orders of magnitude higher activity (in the submicromolar range) and toxicity than compound **1**, with the selectivity index being almost the same as that of **1**.

Compound **4c** comprising the amide substituent with a heterocyclic pyrrolidine moiety separated from the carbonyl group by a methylene spacer did not possess anti-flu activity. At the same time, compound **4d** with larger heterocycle exhibited high activity and low cytotoxicity, which increased the selectivity index to almost 30. The introduction of an oxygen atom into the heterocycle (compound **4e**) resulted in a drastic increase in cytotoxicity and, accordingly, a decrease in the selectivity index.

An increase in the length of a spacer between the acetamide group and the heterocycle, from methylene to ethylene, led to a change in the observed activities —compounds **4f** and **4h** with pyrrolidine and morpholine substituents had the highest activities with SI of 12 and 11, respectively, whereas compound **4g** with the piperidine substituent had both lower activity and higher cytotoxicity (Table 1). We suggest that the overall substituent size in this case plays a key role in the antiviral activity - bulky piperidine heterocycles provide activity with shorter spacers, while smaller pyrrolidine heterocycles are active with longer spacers (**4d** and **4g**; **4c** and **4f**).

Thus, we first found that compounds based on the benzo[*d*][1,3]

Table 1
Antiviral activity and cytotoxicity of compounds 4a-h against influenza virus A/California/07/09 (H1N1)pdm09 in MDCK cells.

Compound	R	CC_{50}[a]. μM	IC_{50}[b]. μM	SI[c]
1	–	212 ± 12	19 ± 3	11
4a		95 ± 7	38 ± 5	3
4b		2.2 ± 0.1	0.2 ± 0.1	12
4c		>828.7	191 ± 26	4
4d		532 ± 44	18 ± 3	29
4e		13 ± 1	5 ± 1	3
4f		75 ± 6	6 ± 1	12
4g		34 ± 2	>28.2	1
4h		104 ± 8	9 ± 2	11
Rimantadine		363 ± 20	41 ± 7	9
Ribavirin		>2130	44 ± 5	48

[a] CC_{50} is the median cytotoxic concentration, i.e. the concentration causing 50% cell death.
[b] IC_{50} is the 50% inhibiting concentration, i.e. the concentration causing 50% decrease of virus replication.
[c] SI is the selectivity index, which is the CC_{50}/IC_{50} ratio.

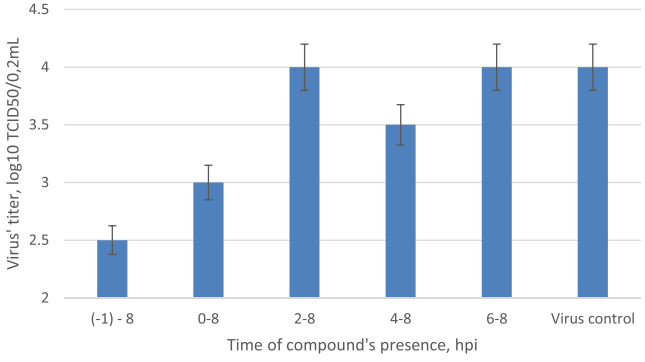


Fig. 1. Activity of **4d** against influenza virus A/California/07/09 (H1N1) pdm09 according to time-of-addition experiment. MDCK cells were infected with influenza virus, and **4d** was added at the indicated time points, where 0 corresponds to the moment when the cells were infected. The infectious activity of viral progeny was tested by further titration in the MDCK cells.

dithiol-2-one structure have high activity against the H1N1 influenza virus.

To study the mode of activity of **4d**, the compound having demonstrated the highest selectivity index, we performed time-of-addition experiments where the compound was added to the replicating virus at different time points corresponding to different stages of viral life cycle. As presented in Fig. 1, the compound was most effective in suppressing the viral replication when administrated at early stages of the viral cycle (0–2 h post infection). Therefore, target (or targets) for **4d** should be essential at early stages of viral cycle.

Two viral proteins are essential for viral cycle between 0 and 2 hpi: i) hemagglutinin (HA), the protein providing attachment of virions to cell surface and fusion of viral envelope with plasma membrane, and ii) M2 proton channel, the protein providing acidification of virion interior

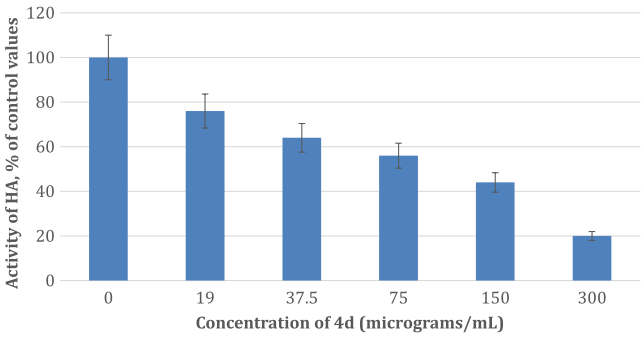


Fig. 2. Anti-membranotropic activity of **4d** against hemagglutinin of influenza virus A/California/07/09 (H1N1)pdm09. The compound was mixed with the virus, incubated at room temperature for 30 min, mixed with 0.75% chicken erythrocytes and incubated at +4 °C. After incubation with MES buffer (pH 5.0) and sedimentation of erythrocytes, optical density in the wells caused by free haemoglobin released after destruction of erythrocyte membranes, was measured at 405 nm. The HA-inhibiting activity of compound was calculated comparing to HA activity of influenza A virus without additives.

thus enabling dissociation of RNPs from envelope. To test whether **4d** is able to block activity of viral HA, we performed the direct assays for HA receptor-binding and fusogenic activity. No inhibiting effect of **4d** on receptor-binding activity of HA has been detected (data not shown). The results of fusogenic activity assay suggest that the compound demonstrates strong dose-dependent inhibiting activity against HA-driven membrane fusion process (Fig. 2).

Additionally, to identify potential molecular targets for the new antiviral compounds, we docked these compounds with several known targets for low molecular weight antiviral drugs and compared the estimated ligand binding energies to the measured IC_{50}. Active site of the hemagglutinin was considered as potential biological target. The crystal structures were downloaded from the Protein Data Bank.[23]

The following PDB code was explored: 3LZG corresponding[24] to hemagglutinin of virus strain A/California/04/2009 H1N1. Two monomers of the trimer in the case of HA were taken (the third one was excluded from analysis). All water molecules and other small molecules were removed. Hydrogen atoms were added to amino acid residues and optimized at pH values 5.0 ± 0.2 at which the crystallographic structures of proteins have been recorded. The geometrical parameters of the proteins and ligands were subjected to restrained minimizations using OPLS3 force field.[25]

The **4b**-binding site of HA was found near the fusion peptide: binding region of *tert*-butyl hydroquione (TBHQ), well-known HA inhibitor. The docking was performed under the following conditions: flexible ligand and protein, induced fit docking protocol, the grid-box size of 15 Å with the native ligand being located at the center (for more technical details see Supplementary Materials). All possible ionized states of the ligands were taken into account during the docking procedure. All calculations were carried out using Small-Molecule Drug Discovery Schrodinger Suite 2018-1.[26]

Both compounds, **4d** and **4f**, are located in the TBQH-binding site of HA near the fusion peptide (Fig. 3A). The molecules are bound by hydrogen bridges with tyrosine (Y94) and additionally **4b** forms a π-π stacking with tyrosine (Y94) and a hydrogen bond with lysine (K58). The values of docking score are from −6.1 to −9.1 units. In accordance with *in vitro* test results, **4b** compound (minimum value of IC_{50}) is characterized by high affinity for potential biological target: penultimate value to HA (the lowest value, −9.1 units, is for **4f**, see Supplementary Materials). The lead compound **4d** (highest SI) shows medium docking score −7.9 units.

To found the relationship between the values of biological tests and the results of theoretical calculations we created a regression model plotting the values of pIC_{50} against docking score (Fig. 3B). The docking

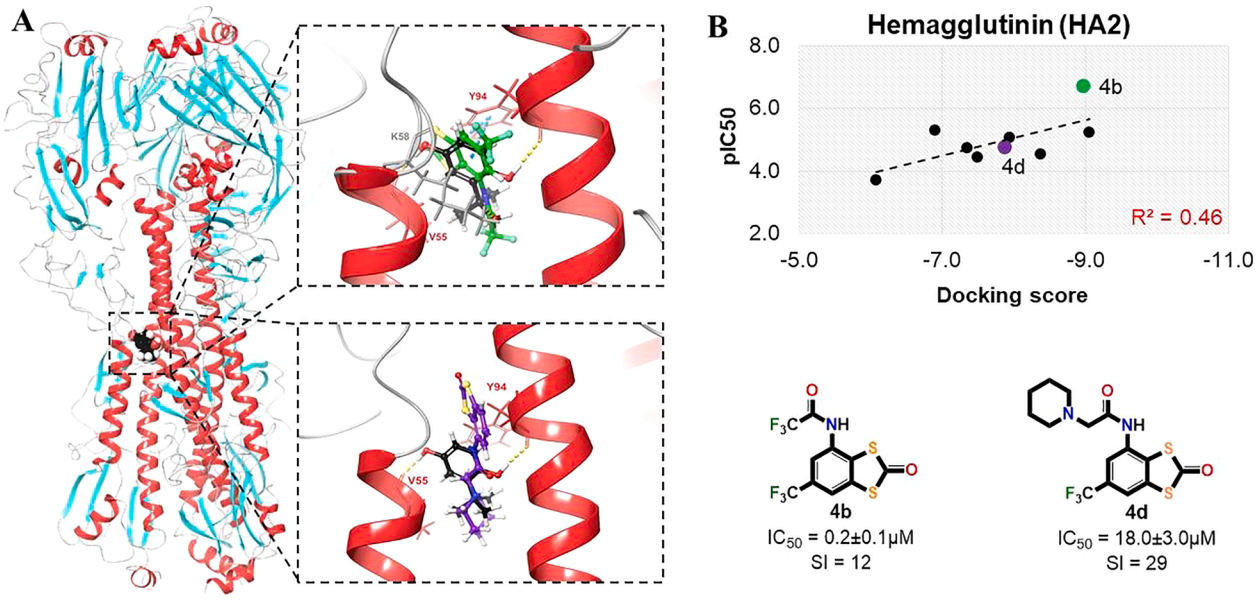


Fig. 3. A: location of lead compounds 4b (green) and 4d (violet) in the binding sites of hemagglutinin (PDB code: 3LZG): H-bridges are shown as yellow dotted lines, π-π stacking is blue; black molecule is *tert*-butylhydroquinone (TBQH); B: relationship between the values from biological tests and the results of theoretical calculations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

score values were considered as a parameter characterizing the affinity of the ligands for the proteins. The correlation coefficients characterizing the dependence of the pIC_{50} values on the ligands' affinity to the HA protein is equal to 0.46.

Based on the data of biological experiments and docking calculations, the antiviral activity of **1** and **4a-h** may be associated with inhibition of the hemagglutinin. Nevertheless, the possibility should not be ruled out that the target for these compounds is of cellular, not viral, origin, or they may demonstrate multitarget mode of activity. Further tests using a reference virus panel and specific target-oriented assays may be therefore important for deciphering the activity mechanism of benzo[*d*][1,3]dithiol-2-one-based compounds and subsequent improvement of their pharmacological properties.

In conclusion, we found a new type of antiviral compounds derived from benzo[*d*][1,3]dithiol-2-one, which exhibit high activity against the H1N1 influenza virus. We demonstrated the key role of the amide substituent structure on the antiviral activity and cytotoxicity of the studied compounds. It was demonstrated that **4d** was most effective when administered at early stages of viral cycle (0–2 h post infection) and able to suppress fusogenic activity of viral HA in dose-dependent manner. Based on the molecular docking and regression analysis data, we suggest that hemagglutinin might be the target for new antiviral agents. Probably, the compounds bind to the stem part of hemagglutinin (HA2), resulting in blocking the process of fusion of the viral and plasma membranes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Ministry of Science and Higher Education, Russian Federation for the financial support (grant N 075-15-2020-777). Authors also would like to acknowledge the Multi-Access Chemical Service Center SB RAS for spectral and analytical measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127653.

References

1 Halford B. Outsmarting influenza. C&EN, March, 2018, 42-47.
2 Fiore AE, Fry A, Shay D, Gubareva L, Bresee JS, Uyeki TM. Recommendations and Reports. 2011;601.
3 Tonelli M, Cichero E. Fight against H1N1 Influenza A virus: recent insights towards the development of druggable compounds. *Curr Med Chem*. 2016;23:1802.
4 Hayden FG, Sugaya N, Hirotsu N, et al. Baloxavir marboxil for uncomplicated influenza in adults and adolescents. *N Engl J Med*. 2018;379:913–923.
5 Govorkova EA, Baranovich T, Seiler P, et al. Antiviral resistance among highly pathogenic influenza A (H5N1) viruses isolated worldwide in 2002–2012 shows need for continued monitoring. *Antiviral Res*. 2015;117:10–19.
6 Dixit R, Khandaker G, Ilgoutz S, Rashid H, Booy R. Emergence of oseltamivir resistance: control and management of influenza before, during and after the pandemic. *Infect Disord Drug Targets*. 2015;13:34–45.
7 Wang Y, Hu Y, Xu S, et al. In vitro pharmacokinetic optimizations of AM2-S31N channel blockers led to the discovery of slow-binding inhibitors with potent antiviral activity against drug-resistant influenza A viruses. *J Med Chem*. 2018;61:1074–1085.
8 Ilyina IV, Zarubaev VV, Lavrentieva IN, et al. Highly potent activity of isopulegol-derived substituted octahydro-2h-chromen-4-ols against influenza A and B viruses. *Bioorg Med Chem Lett*. 2018;28:2061–2067.
9 Wang G, Wan J, Hu Y, et al. Synthesis and anti-influenza activity of pyridine, pyridazine, and pyrimidine C-nucleosides as favipiravir (T-705) analogues. *J Med Chem*. 2016;59:4611–4624.
10 Suslov E, Zarubaev VV, Slita AV, et al. Anti-influenza activity of diazaadamantanes combined with monoterpene moieties. *Bioorg Med Chem Lett*. 2017;27:4531–4535.
11 Sokolova AS, Yarovaya OI, Semenova MD, et al. Synthesis and in vitro study of novel borneol derivatives as potent inhibitors of the influenza A virus. *Med Chem Comm*. 2017;8:960–963.
12 Xu J, Chatterjee M, Baguley TD, et al. Inhibitor of the tyrosine phosphatase STEP reverses cognitive deficits in a mouse model of Alzheimer's disease. *PLoS Biol*. 2014;12, e1001923.
13 Khomenko TM, Tolstikova TG, Bolkunov AV, et al. 8-(Trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine: novel aminobenzopentathiepine having in vivo anticonvulsant and anxiolytic activities. *Lett Drug Des Discov*. 2009;6:464–467.
14 Tolstikova TG, Pavlova AV, Morozova EA, Khomenko TM, Volcho KP, Salakhutdinov NF. The analgesic activity of 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepine-6-amine and its hydrochloride. *Lett Drug Des Discov*. 2012;9:513–516.
15 Kulikova EA, Volcho KP, Salakhutdinov NF, Kulikov AV. Benzopentathiepine derivative, 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine hydrochloride (TC-2153), as a promising antidepressant of new generation. *Lett Drug Des Discov*. 2017;14:974–984.
16 Kulikova EA, Khotskin NV, Illarionova NB, et al. Inhibitor of striatal-enriched protein tyrosine phosphatase, 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine

hydrochloride (TC-2153), produces antidepressant-like effect and decreases functional activity and protein level of 5-HT2A receptor in the brain. *Neuroscience*. 2018;394:220–231.

17 Xu J, Kurup P, Baguley TD, et al. Inhibition of the tyrosine phosphatase STEP61 restores BDNF expression and reverses motor and cognitive deficits in phencyclidine-treated mice. *Cell Mol Life Sci*. 2016;73:1503–1514.

18 N-(2-Oxo-6-(trifluoromethyl)benzo[d][1,3]dithiol-4-yl)acetamide 4a. Ac2O (2.0 ml, 21.0 mmol) was added with stirring to the solution of compound 1 (0.206 g, 0.82 mmol) in CH2Cl2 (2.0 ml). The solution was stirred for 4 h at r.t. and allowed to stay overnight. Then ice was added, and the substance was extracted with CHCl3, washed with water, and dried over Na2SO4. The solvent was distilled off, the residue was recrystallized from ethanol to give amide 4a (0.102 g, 42%). 2,2,2-Trifluoro-N-(2-oxo-6-(trifluoromethyl)benzo[d][1,3]dithiol-4-yl)acetamide 4b. (CF3CO)2O (0.1 ml, 0.72 mmol) was added with stirring to the solution of compound 1 (0.072 g, 0.29 mmol) in CH2Cl2 (0.5 ml). The mixture was stirred for 2 h at r.t. The solvent was distilled off to give 4b. (0.085 g, 84%).

19 An appropriate chloroanhydride 5c-f (0.8 mmol) was added to a solution of compound 1 (0.4 mmol) in 2 ml CH2Cl2. The mixture was stirred at r.t. for the required period of time (control using HPLC). Then water and CH2Cl2 were added to the reaction mixture. The products were extracted by CH2Cl2, dried over Na2SO4. The solvent was distilled off, and the residue was purified on a SiO2 column.

20 Zakharenko A, Khomenko T, Zhukova S, et al. Synthesis and biological evaluation of novel tyrosyl-DNA phosphodiesterase I inhibitors with a benzopentathiepine moiety. *Bioorg Med Chem*. 2015;23:2044–2052.

21 Patrusheva OS, Zarubaev VV, Shtro AA, et al. Anti-influenza activity of monoterpene-derived substituted hexahydro-2*H*-chromenes. *Bioorg Med Chem*. 2016;24:5158–5161.

22 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55–63.

23 Berman HM, Westbrook J, Feng Z, et al. The protein data bank. *Nucleic Acids Res*. 2000;28:235–242.

24 Xu R, Ekiert DC, Krause JC, Hai R, Crowe Jr JE, Wilson IA. Structural basis of pre-existing immunity to the 2009 H1N1 pandemic influenza virus. *Science*. 2010;328:357–360.

25 Shivakumar D, Harder E, Damm W, Friesner RA, Sherman W. Improving the prediction of absolute solvation free energies using the next generation OPLS force field. *J Chem Theory Comput*. 2012;88:2553–2558.

26 Small-Molecule Drug Discovery Suite 2018-1, Schrödinger, LLC, New York, NY, 2018.