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Synthesis and antifungal activity of new hybrids thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4)triazole

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ABSTRACT

Synthesis and antifungal activity of hybrids of thiazolo[4,5-*d*]pyrimidines with (1H-1,2,4)triazoles are presented. The solubility and lipophilicity of compounds was assessed and it was discovered that compounds with piperazine linker exhibited significant antifungal activity against filamentous and yeast fungi.

The last few decades have seen a considerable increase in fungal diseases and emergence of resistance to the most popular antifungal drugs belonging to many chemical classes, which makes treatment of patients more difficult. Fungal infections are especially dangerous in patients with a weakened immune system destroyed by cancer, HIV, immunosuppressive drugs or other diseases.[1,2] The enormous amount of available data on the chemical characteristics and biological activity of (1*H*-1,2,4)triazole derivatives allows researchers to consider these substances to be one of the most promising classes of drug compounds.[3,4]

Several compounds bearing (1*H*-1,2,4)triazole core, display diverse bioactivities exhibit antibacterial, analeptic, anti-inflammatory, cardioprotective and other types of activity.[5] Common antifungal drugs – fluconazole, itraconazole, isavuconazole, efinaconazole, etc. – have a triazole fragment in the structure of their molecules. The mechanism of azole action is associated with inhibiting P450 cytochrome enzymes catalyzing lanosterol transformation into ergosterol, which leads to the destruction of the fungus cell membrane.[6]

Hybridization approach combining triazole and different heterocycle via a linker has been extensively used for discovery of new antifungal agents.[7–9] This principle has been used to obtain a lot of drugs of the triazole group, including ravuconazole and its commercial water-soluble analogue – isavuconazole – representing a triazole hybrid with thiazole.[10] Sulfur-containing heterocycles are often used by researchers as one of the components of the hybrid molecule based on triazole. An example would be the tetrahydro-thiazolo-pyridine hybrids with high antifungal activity and water solubility.[11,12] The effectiveness of the action of the hybrids with various thienopyrimidones was demonstrated on experimental models of systemic candida infections.[13] The high activity indicator was also achieved in the tests of various condensed pyrimidones with benzothiazoles.[14] In the present work, thiazolo[4,5-*d*]pyrimidine was used as a component of the hybrid molecule. The derivatives with this fragment potentially have biological relevance as analgesics, central nervous system depressants, and bronchodilators and are also recommended for prevention and treatment of atherosclerosis, diabetes and other diseases.[15,16]

Another important fragment incorporated into the molecular structure of the synthesized compounds is piperazine.[17,18] In combination with various heterocyclic fragments piperazine significantly improves the biological properties of the compounds, including their antimicrobial activity.[4,19] Obtained hybrid compounds consist of three components specified earlier: (1*H*-1,2,4)triazole, thiazolo-pyrimidine, and piperazine. We have also studied the effect of the structure of the linker between the heterocycles and substituents of various chemical nature on the *in vitro* antifungal activity of the synthesized substances. Besides, since lipophilicity is the key parameter responsible for the biological action of a drug,[20] one of the aims of the work was to experimentally determine the coefficient of distribution of the hybrid derivatives in the 1-octanol/buffer pH 7.4, as well as the equilibrium solubility of the

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compounds in the aqueous and organic phases used. The aim of the synthesis of the hybrids of thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4) triazole and of the comprehensive study of their pharmaceutically relevant physicochemical properties was to find the "structure–activity" correlation in the series of the heterocyclic substances obtained.

Triazole oxirane was synthesized by the known method from 2,4-difluoroacetophenone by treating it with trimethylsulfoxonium iodide in toluene.[21] The obtained amino derivative of triazole (2) is acylated with acetyl chloride in dichloromethane to form the acetylamine derivative of triazole (3) (Scheme 1).

The sulfur-containing fragment in the thiazolo[4,5-*d*]pyrimidines was prepared by the known method developed by Gewald[22] and described for one of the compounds with a fluorine substituent in the *para*-position of the phenyl core in position 3 of the thiazole core that was used.[23] The synthesis of the hybrids of thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4)triazole (Groups I, II) was carried out according to Scheme 2.

As the first step, we studied and tested the scheme of synthesizing thiazolo-pyrimidine hybrids with triazole through direct condensation of the dihydrothiazolopyrimidine derivatives with triazole oxirane prepared in advance. Among the possible variants of preparation of the target products in different solvents – acetonitrile, ethanol, and toluene with triethylamine and ammonium chloride additives – we selected synthesis with triazole oxirane in dimethyl formamide for 2–3 days with the formation of 6-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-(R^1,R^2-phenyl)-2-thioxo-thiazolo[4,5-*d*]pyrimidin-7-one that had not been described earlier. Three compounds with different substituents in the phenyl ring were obtained: R^1–CH_3, R^2–H (**Ia**), R^1–F, R^2–H (**Ib**) and R^1–H, R^2–OCH_3 (**Id**). Their molecular structures are shown in Scheme 2.

The reaction of the acetamide triazole chloro-derivative (prepared from triazole oxirane in the process of consecutive interaction with aqueous ammonia and then with chloroacetyl chloride in methylene chloride) with thiazolo pyrimidines led to the formation of hybrid derivatives with an amide linker (Group **II**). As a result, the target 6-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]-2-[3-(R^1,R^2-phenyl)-7-oxo-2-thioxo-thiazolo[4,5-*d*]pyrimidin-6-yl]acetamides (**IIa-d**) were obtained.

The synthesis of the thiazolo[4,5-*d*]pyrimidines hybrids with (1*H*-1,2,4)triazole (Groups **III-V**) was carried out according to Scheme 3.

A series of hybrid derivatives containing a certain linker between the heterocycles – triazole and pyrimidino[4,5-*d*]thiazol – was obtained by alkylation of the piperidine fragment imino group with haloalkyl (**8**). For that purpose we used commercial haloalkyl Boc-piperazines: *tert*-butyl 4-(2-bromoethyl)piperazin-1-carboxylate and *tert*-butyl 4-(3-bromopropyl)piperazine-1-carboxylate, as well as the product of Boc-piperazine condensation with racemic epichlorohydrin produced by the method described in patent literature.[24]

After removing the Boc-protection from compound the obtained products (**9**–**11**) by trifluoroacetic acid in methylene chloride, the resulting piperazinyl thiazolo[4,5-*d*]pyrimidine derivatives (**12**–**14**) were condensed by triazole oxirane in an alcohol with triethylamine and ammonium chloride to obtain target derivatives: 6-[3-[4-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]piperazin-1-yl]-2-hydroxy-propyl]-3-(R^1,R^2-phenyl)-2-thioxo-thiazolo[4,5-*d*]pyrimidin-7-one (**IIIa-d**), 6-[3-[4-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl]piperazin-1-yl]propyl]-3-(R^1,R^2-phenyl)-2-thioxo-thiazolo[4,5-*d*]pyrimidin-7-one (**IVa-c**) and 6-[2-[4-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propyl]piperazin-1-yl]ethyl]-3-(R^1,R^2-phenyl)-2-thioxo-thiazolo[4,5-*d*]pyrimidin-7-one (**Va, b**).

The detailed description of synthesis of 16 novel hybrids has been presented in Supplementary material. The purity of synthesized compounds has been determined by ^{1}H NMR and ^{13}C NMR measurements and all results were 95–98%. The graphical ^{1}H NMR and ^{13}C NMR spectra of compounds also have been provided in Supplementary material. The obtained hybrids thiazolo[4,5-*d*]pyrimidines with 1,2,4-triazole are amorphous, which was confirmed by PXRD method. As example, the typical PXRD pattern (amorphous halo) for compound **Ia** was shown in Fig. S1 (Supplementary material).

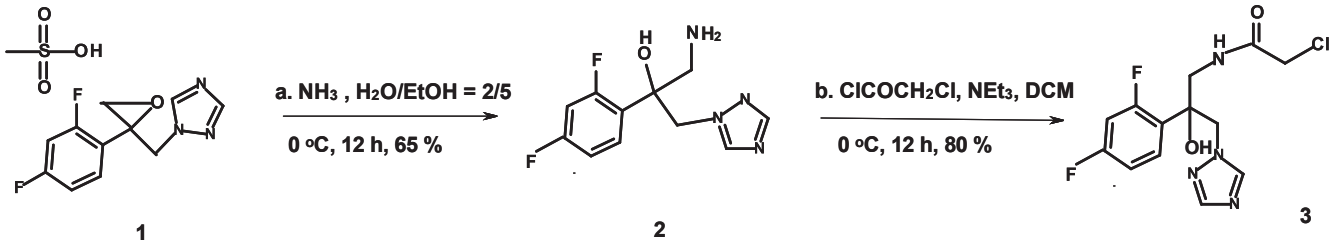
Sixteen novel hybrids of thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4) triazole have been synthesized in this work. Their structural formulas are shown in Fig. 1. It should be noted that in all compounds the methyl- (**a**), fluoro- (**b**) and chloro- (**c**) substituents are introduced in the para position of the phenyl ring, while the methoxy group (**d**) is the *ortho* substituent.

The bioactivity of the synthesized hybrids depends on their solubility in pharmaceutically relevant media and lipophilicity to a large extent. That is why we also experimentally measured the solubility of some derivatives under study in the buffer pH 7.4 simulating the intestinal fluid. Another important solvent used was 1-octanol, which simulates amphipathic lipids of biological membranes. Besides, in order to study the lipophilicity, we determined the distribution coefficients of the compounds in the 1-octanol/buffer pH 7.4. The results are summarized in Table 1. It is quite interesting to compare the data about the solubility and distribution of the synthesized hybrid derivatives containing a fluconazole fragment in the molecular structure with the characteristics of individual fluconazole that have been determined by us earlier[25] and are given in Table 1.

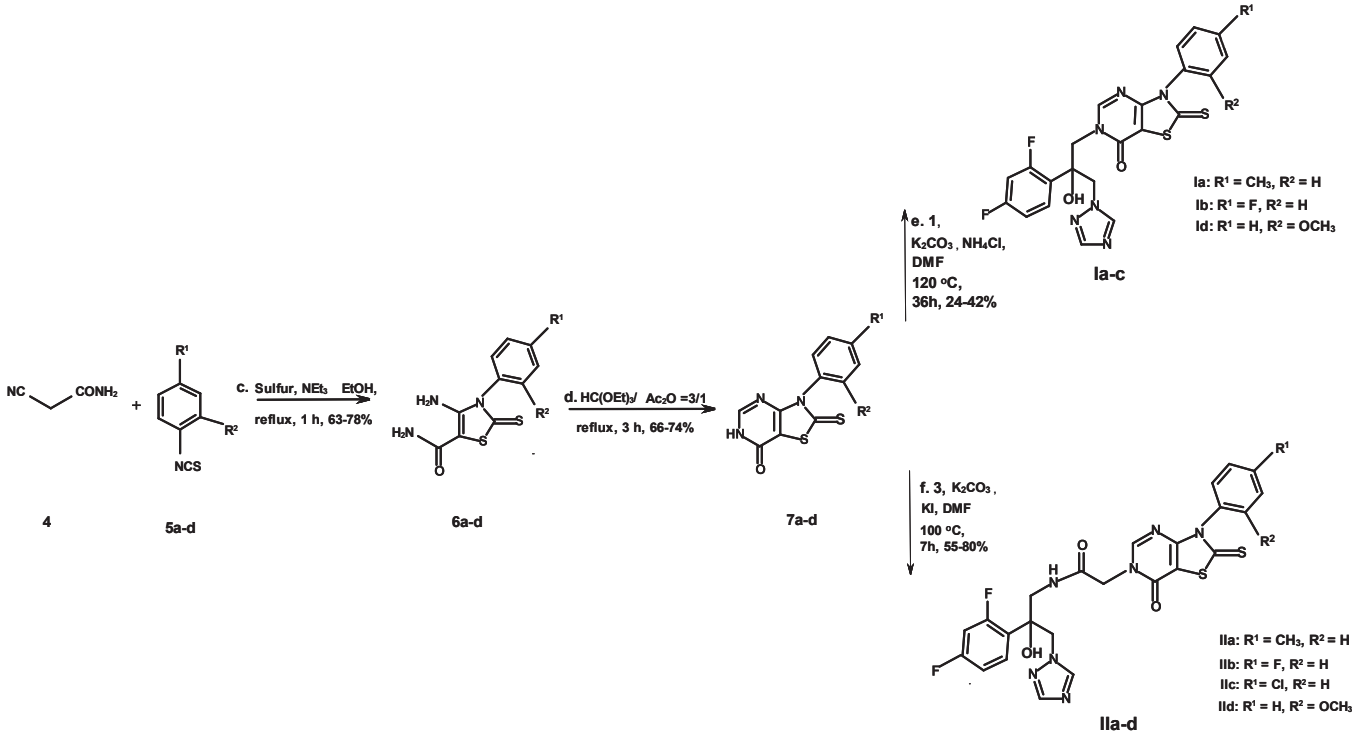
The molar solubility of the substances in the buffer pH 7.4 ($\log S_b$) changes by three orders of magnitude depending on the linker structure and the substituent introduced into the thiazolo-pyrimidine fragment. And in this case, all the compounds have much lower solubility in the pH 7.4 buffer than fluconazole.

An analysis of the effect of the chemical nature of the substituents in the phenyl ring of the compounds with an acetamide linker on the solubility in the aqueous medium ($\log S_b$) has shown that the derivatives can be arranged in the following order of increasing solubility: **IIb**(*p*-fluoro-) < **IIc**(*p*-chloro-) < **IIa**(*p*-methyl-) < **IId**(*o*-methoxy-). It has been established that all the synthesized substances with a methoxy-substituent have better solubility than the methyl-derivatives. According to the literature data,[26] the obtained result is explained by a stronger destructive effect of the compounds with substituents in the *ortho*-position of the phenyl ring on the solvent structure than that of the *para*-substituted derivatives.

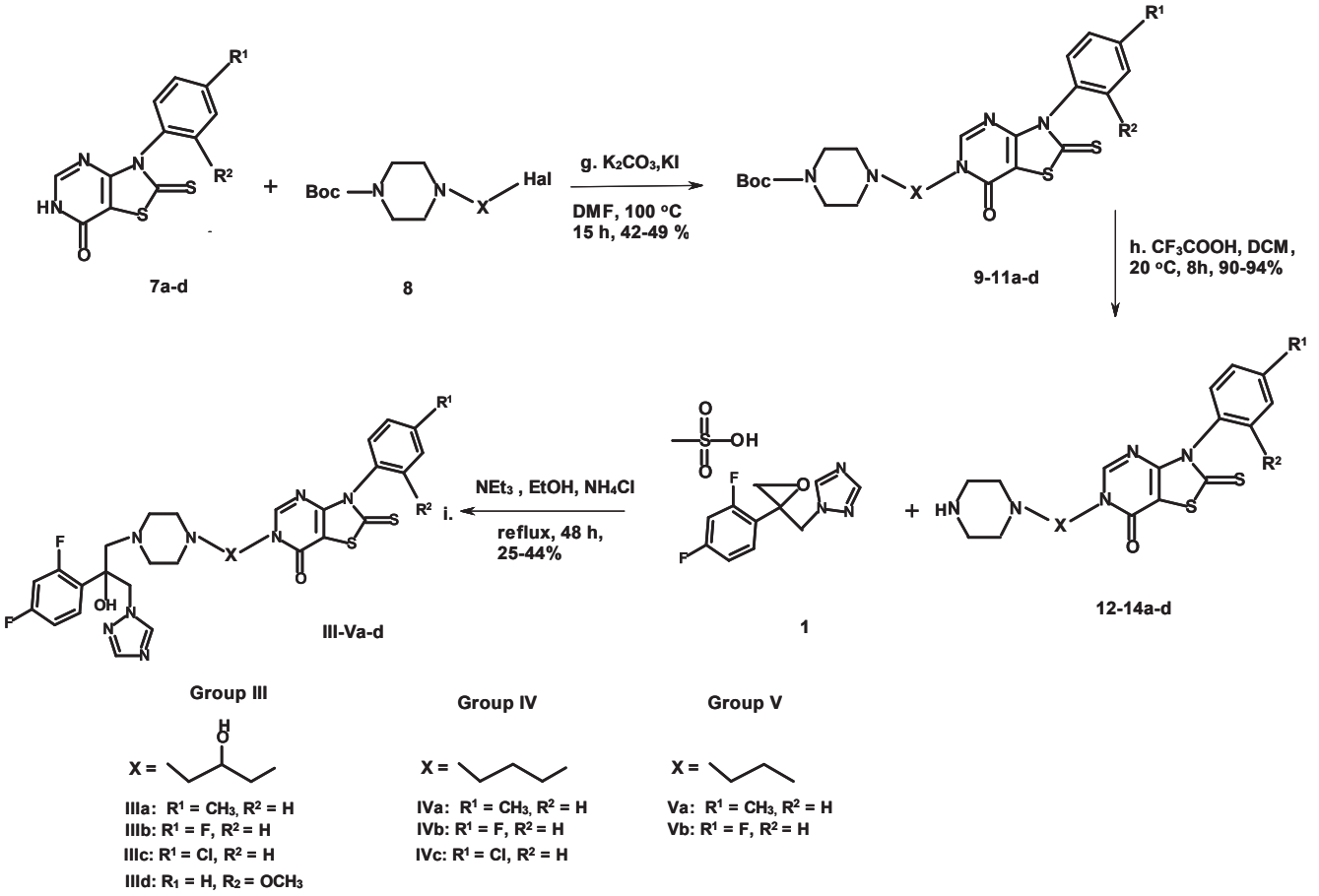
The results of the study of the linker structure of the compounds' solubility allowed us to make some conclusions about the interconnection between the molecular structure and solubility in the buffer pH 7.4.



Scheme 1. Synthesis of triazole derivatives (2, 3).



Scheme 2. Synthesis of the hybrids of thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4)triazole (compounds of Groups **I, II**).



Scheme 3. Synthesis of the hybrids of thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4)triazole (compounds of Groups **III-V**).

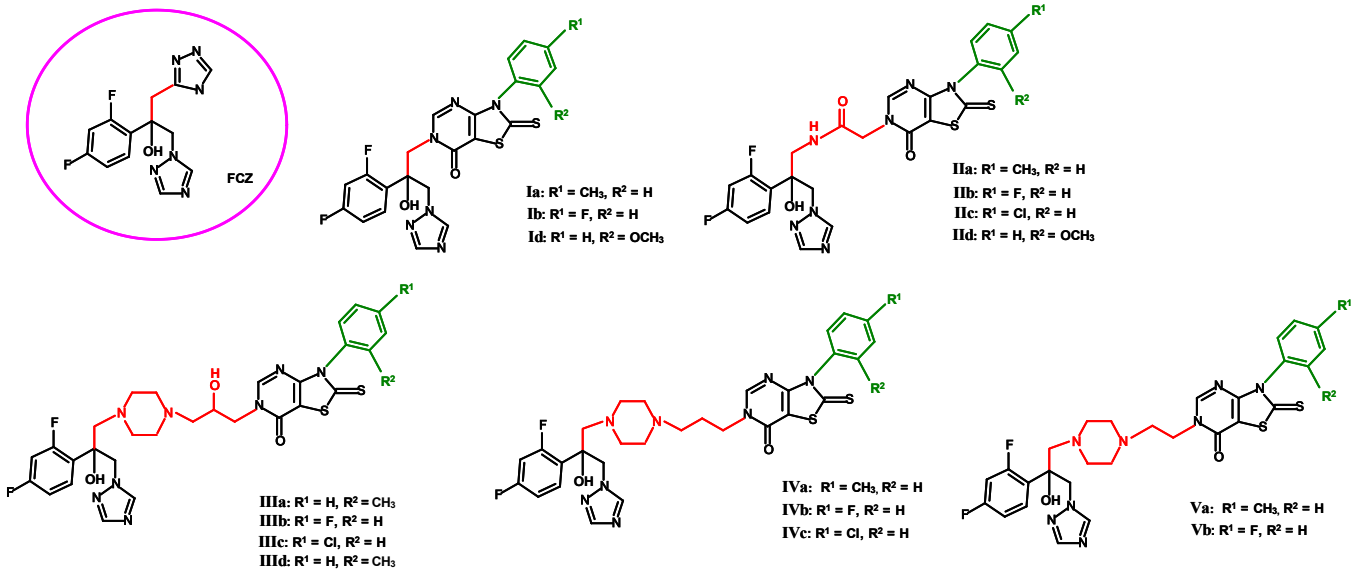


Fig. 1. Structural formulas of the synthesized compounds (Groups **I-V**).

Table 1
Solubility and lipophilicity of synthesized compounds.

No	[a]logS_b	[b]logS_o	[c]log$P_{o/b}$
1a	−3.85	−1.35	0.79
1b	−3.55	−1.50	0.47
1d	−3.59	−1.55	1.19
IIa	−3.75	−2.63	1.27
IIb	−4.12	−2.62	0.79
IIc	−3.81	−2.41	1.16
IId	−3.27	−1.88	0.80
IIId	−3.38	−1.41	2.91
IVa	−3.91	−0.74	4.10
IVb	−3.50	−2.25	2.40
IVc	−4.17	−1.91	1.65
Va	−4.40	−2.54	2.9
FCZ	−1.85	−1.56	0.44

[a] logS_b – solubility in buffer pH 7.4 (mol/L).
[b] logS_o – solubility in 1-octanol (mol/L).
[c] log$P_{o/b}$ – distribution coefficients in 1-octanol/ buffer pH 7.4 system.

The best solubility values among the methyl-substituted derivatives were found in compounds **I a** with a methylene bridge group and **IIa** with an acetamide fragment. The solubility is lower in compounds **IVa** and **Va** also with methyl- substituents and it is reduced by piperazine introduction. However, if the number of methylene units increases from two in compound **Va** to three in compound **IVa**, the solubility becomes higher. This may be the result of higher flexibility of the bridge group caused by the increase in the number of alkyl chain units. A study of the compounds with halogen atoms as substituents has shown that the solubility of chloro-derivative **IVc** with a linker consisting of piperazine and methylene groups, is lower than in chloro-substituted compound **IIc** with an acetamide linker. On the contrary, fluoro-derivative **IVb** dissolves better than compound with fluoro-group **IIb**.

The molar solubility of the synthesized substances in 1-octanol (logS_o) is 1–2 orders of magnitude higher than in the buffer pH 7.4 and ranges from −2.63 to −0.74. The solubility of compound **IVa** in 1-octanol is highest. The better solubility of the compounds in 1-octanol than in the buffer is explained by the strong intermolecular interaction between the solute and the solvent due to dipole–dipole forces and hydrogen bonds.

The log$P_{o/b}$ values of the synthesized derivatives are below 3 (with the only exception being compound **IVa**). For comparison, the log$P_{o/b}$ value of fluconazole is equal to 0.44.[27] It should be noted that the drug lipophilicity limit determined empirically is expressed by the inequality: $-0.5 < \log P_{o/b} < 5$.[28,29] The log$P_{o/b}$ values of the studied compounds range from 0.44 to 4.10, which indicates their optimal lipophilicity for pharmaceutics. The combination of several pharmacophore groups in the hybrid molecules has led to a considerable increase in the molecular weight of the substances up to over 500 g·mol^{-1}, which is a violation of Lipinski's rule of five.[29] It should be said that the molecular weight of the commercial antifungal drugs is often higher than the limit set by this rule. The number of hydrogen bond donors in the molecules of the derivatives does not exceed three, which corresponds to the values recommended for drug compounds. However, the number of hydrogen bond acceptors only for the compounds of the first group is less than ten, whereas the number for the other substances is higher than the limit set by the rule of five.

The serial dilution micromethod was used to determine the antifungal activity of the substances under consideration.[30] The antifungal activity was studied on the following cultures: *C. parapsilosis ATCC 22019, C. albicans ATCC 24433, C. albicans 8R, C. albicans CBS8837, C. albicans 604M, C. utilis 84, C. tropicalis 3019, C. glabrata 61 L, C. krusei 432M, Cryptococcus neoformans, A. niger 37a yeast fungi* and *filamentous fungi: M. canis B-200 and T. rubrum 2002*. Commercially available antifungal agent such as Fluconazole (Quimica Sintetica S.A, Spain, purity ≥ 0.99%) were used as positive control drugs for comparison. By combining triazole and thiazolo-pyrimidine fragments in a hybrid molecule we managed to obtain compounds with a wide spectrum of antifungal activity. The results of the biological tests showed that the change in the bridge group structure and introduction of substituents of various chemical nature into the phenyl ring had a great effect on the antifungal activity of the substances. All the synthesized compounds (except derivatives of II group) exhibited microbiological activity against the strains of the *C. parapsilosis* ATCC 22019 pathogenic fungi with the MIC values ranging from 0.06 to 8 μg/mL.

The introduction of a piperazine group into the hybrid molecule led to a considerable increase in the antifungal action, which was observed in all the compounds containing this group. The most active substances among those studied are methyl-(**IVa**), fluoro-(**IVb**), chloro- (**IVc**), methyl-(**Va**) and fluoro-(**Vb**), derivatives. The results of the study of the MIC values of these compounds are summarized in Table 2.

Besides, we defined the antifungal activity as excellent (0.06–2 μg/mL), good (4–8 μg/mL), moderate (16–32 μg/mL), or poor (≥32 μg/mL) based on the MIC values. As can be seen, the derivatives shown have a piperazine group in the linker structure and differ from each other in the

Table 2
The MIC results of the synthesized compounds.*

Test microorganisms	MIC (μg/mL)											
	FCZ		**IVa**		**IVb**		**IVc**		**Va**		**Vb**	
	24 h	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*	*24 h*	*48 h*
C. parapsilosis ATCC 22019	2	4	0.5	0.5	0.06	0.125	0.25	0.25	0.5	0.5	0.5	1
C. albicans ATCC 24433	4	4	16	32	8	8	16	32	8	16	8	8
C. albicans 8R	128	128	16	32	32	32	32	32	32	32	32	32
C. albicans CBS 8837	128	128	16	32	16	32	16	32	16	32	32	32
C. albicans 604M	64	64	32	32	32	32	32	32	16	32	32	32
C. utilis 84	2	2	4	4	1	1	2	2	2	4	32	32
C. tropicalis 3019	1	2	8	16	4	4	8	8	4	8	32	32
C. glabrata 61L	8	32	2	32	4	32	2	32	4	32	32	32
C. krusei 432M	32	64	16	32	8	16	16	32	16	32	32	32
Cryptococcus neoformans	16	16	64	64	16	16	32	32	32	32	32	32
A. niger 37a	64	64	32	32	16	16	32	32	32	32	32	32
M. canis B-200	32	32	32	32	4	4	32	32	32	32	32	32
T. rubrum 2002	64	128	32	32	32	32	16	16	32	32	32	32

* The averaged data of 3 repeated experiments are given.
Values of antifungal activity: ■ - excellent, ■ - good, ■ - moderate and ■ - poor.

number of carbon atoms in the alkyl chain connecting this group with the thiazolo-pyrimidine fragment. An analysis of the biotests has shown that the antifungal activity is affected by both the structure of the bridge group and the introduction of substituents into the aromatic ring (Fig. 2).

For compounds with methylene linker (Group **I**), we observed only a weak antifungal activity against the *C. parapsilosis* strain, and for compounds with substituents – fluorine in the *para* position (**Ib**) and methoxy – (**Id**) in the *ortho* position of the phenyl ring (MIC 8–16 μg / ml). The introduction of an acetamide linker (Group **II**), regardless of the substituents in the phenyl ring, completely deprived the molecule of microbiological activity, and only with the addition of a cyclic amine – piperazine (compounds of Groups **III–V**), we observed the appearance of high activity against various strains of the yeast-like *Candida fungus*, comparable to the activity commercial drug fluconazole.

In the presence of 3 methylene units (group **IV**), compounds with methyl-, fluoro- and chloro-substituents, respectively, exhibited maximum activity (MIC 0.06–0.25 μg/ml), which slightly decreases when the central hydrogen atom is replaced by hydroxyl (group **III**, MIC 2.0–8.0 μg/ml). A reduction in the number of methylene units to 2 (group **V**) also had some negative effect on the microbiological activity of the *C. parapsilosis* strain, remaining rather high (MIC 0.5–1.0 μg/ml).

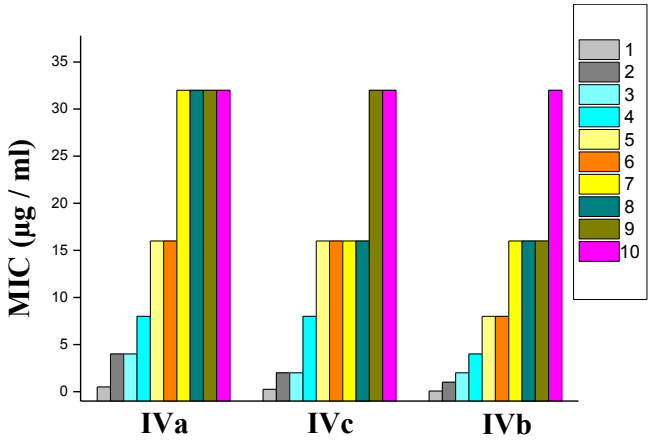


Fig. 2. Histogram MIC value (μg/mL) comparison of activity compounds **IVa-c** against different fungi: 1 – *C. parapsilosis ATCC 22019,* 2 – *C. utilis 84, 3 – C. glabrata 61L.* 4 – *C. tropicalis 3019,* 5 – *C. krusei 432M, 6 – C. albicans ATCC* 24433, *7 – C. albicans CBS 8837, 8 – Cryptococcus neoformans,* 9 – *A. Niger 37a,* 10 – *C. albicans 604M.*

The compounds can be arranged in the following order of increasing biological activity against most of the fungus strains studied: **IVa** (methyl-), **IVc** (chloro-) and **IVb** (fluoro-). Thus, by synthesizing hybrid compounds based on a triazole group, a thiazolo-pyrimidine fragment and a phenyl radical with halogen atoms, we were able to obtain new antifungal substances with a wide spectrum of biological activity, which can be used for further modifications.

To sum it up, a series of 16 novel derivatives of thiazolo[4,5-*d*]pyrimidines with (1*H*-1,2,4)-triazole have been designed and synthesized using the molecular hybridization approach. An analysis has been done made of the effect produced by the linker structure and chemical nature of the substituents introduced into the phenyl ring on the antifungal activity of the compounds. Several of the derivatives containing an alkylpiperazinyl linker exhibited strong *in vitro* antifungal activity, comparable to fluconazole, against a broad spectrum of cultures: *C. parapsilosis ATCC, 22019, C. albicans ATCC 24433, C. albicans 8R, C. albicans CBS 8837, C. albicans 604M, C. utilis 84, C. tropicalis 3019, C. glabrata 61L, Cryptococcus neoformans, and C. krusei 432M yeast cultures, A. niger 37a, and filamentous fungi: M. canis B-200 and T. rubrum 2002.*

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127944.

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