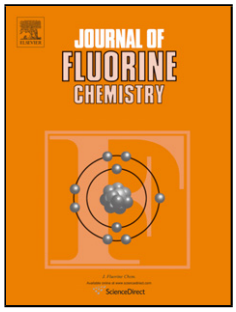

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PII: S0022-1139(16)30165-8
DOI: http://dx.doi.org/doi:10.1016/j.jfluchem.2016.06.019
Reference: FLUOR 8804

To appear in: *FLUOR*

Received date: 21-4-2016
Revised date: 27-6-2016
Accepted date: 28-6-2016

Please cite this article as: Alexey Yu.Aksinenko, Tatyana V.Goreva, Tatyana A.Epishina, Sergey V.Trepalin, Vladimir B.Sokolov, Synthesis of bis(trifluoromethyl)pyrimido[4,5-d]pyrimidine-2,4-diones and evaluation of their antibacterial and antifungal activities, Journal of Fluorine Chemistry http://dx.doi.org/10.1016/j.jfluchem.2016.06.019

Synthesis of bis(trifluoromethyl)pyrimido[4,5-*d*]pyrimidine-2,4-diones and evaluation of their antibacterial and antifungal activities

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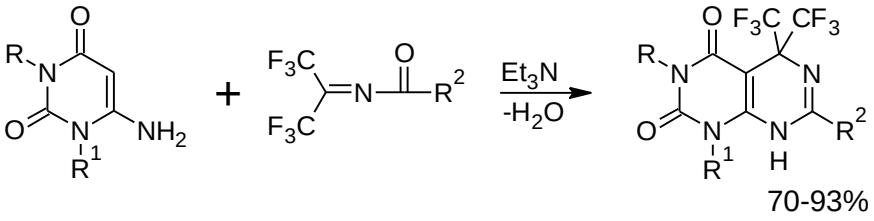
Graphical abstract



Highlights

- Fluorinated pyrimido[4,5-*d*]pyrimidine-2,4-diones have been prepared.
- Antibacterial and antifungal activities of synthesized compounds were evaluated.
- The obtained heterocycles are more active against the Gram-positive rather than the Gram-negative bacteria.

ABSTRACT

A series of 5,5-bis(trifluoromethyl)-5,6-dihydropyrimido[4,5-d]pyrimidine-2,4(1*H*,3*H*)-diones has been prepared by the cyclocondensation of *N*-acylimines of hexafluoroacetone and 6-aminouracils. The obtained compounds were screened for their activities against Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus anthracis*) and Gram-negative (*Escherichia coli*) bacteria, as well as fungus *Candida albicans.* A few of the title compounds showed promising antimicrobial activity.

Key words: fluorinated heterocycles, pyrimido[4,5-d]pyrimidines, cyclocondensation.

1. Introduction

Pyrimidines represent one of the important classes of heterocyclic compounds occurring widely in living organisms. Thus, pyrimidine fragment is present in uracil, thymine and cytosine that are three important constituents of nucleic acids [1]. The synthesis of new uracil-annelated heterocycles has received considerable attention in the field of drug discovery since many monocyclic uracils (for example, 5-fluorouracil **1** and sulfonamides **2**), as well as their fused derivatives (for examples, acyclovir **3**, methotrexate **4**) have found wide clinical applications (Figure 1).

Among them, the pyrimido[4,5-*d*]pyrimidines that have close resemblance with purine and pteridine systems, are of high interest as potential biologically active compounds. However, their synthesis and biological effects, mainly antimicrobial and antifungal, have been described only in a few works.

The pyrimido[4,5-*d*]pyrimidine bicycle was formed by the Biginelli reaction [2] from aldehydes, barbituric acid/thiobarbituric acid and urea/thiourea [3,4] or a variation of this reaction: a) from pyrimidine-5-carboxylate and thioureas[5], b) from 6-aminouracils, araldehydes and isothioureas [6], c) from pyrrole-2-carboxaldehyde, 2-aminobenzimidazole and 1,3-dimethylbarbituric acid [7], d) by a three-component reaction of 6-[(dimethylamino)-methylene]-1,3-dimethylaminouracil, terephthalaldehyde and amine derivatives [8], e) by the reaction between 2-pyrrolidones and 6-aminopyrimidines followed by the cyclocondensation with benzoyl chloride [9]. Some of the described compounds showed good antibacterial and antifungal activities.

One of the modern synthetic approaches used in drug discovery to increase pharmaceutical effectiveness, biological half-life, and bioabsorption consists in an incorporation of fluorine atom or fluoroalkyl group into a molecule of the potential drug candidate (for example, into an aromatic or heterocyclic system) or synthesis of new drug candidates from highly reactive fluorinated building blocks [10-17]. As of now, about 36% of approved drugs and 40% of drug candidates that are currently in phase II–III clinical trials contain at least one fluorine atom [18].

In our previous works, a new approach to the construction of trifluoromethylated pyrimido[4,5-*d*]pyrimidine ring systems by the cyclocondensation of *N*-acylimines of hexafluoroacetone or methyl trifluoropyruvate with 6-aminouracils/thiouracils was reported [19-22]. Thus, in continuation of our research in the field of bioactive heterocyclic compounds, a number of unexplored fluorinated pyrimido[4,5-*d*]pyrimidines were synthesized and their antibacterial and antifungal activities were evaluated.

2. Results and discussion.

2.1. Chemistry.

The synthesis of titled compounds was performed through the *C*-alkylation of 6-aminouracils **1a-h** with *N*-acylimines of hexafluoroacetone **2a-e**. The subsequent

cyclocondensation of the *in situ* generated intermediates **3** in the presence of Et_3N afforded pyrimido[4,5-*d*]pyrimidines **4a-k**. It should be mentioned that ^{19}F NMR spectrum of the reaction mixture containing uracil **1g** and imine **2g** that were kept in DMF for 0.5 h at room temperature in the absence of Et_3N showed two compounds – intermediate **3j** and product **4j**. Broad singlets at -57.9 and -70.0 ppm could be assigned to the intermediate **3j** that is a similar product of *C*-alkylation of 6-amino-1,3-dimethyluracil with benzoylimine of hexafluoroacetone [19], while a narrow singlet at -70.8 ppm corresponds to the product **4j**. Thus, it demonstrates that the cyclocondensation of the intermediates **3** to form target compounds **4** occurs easily. The structures of compounds **4a-k** were confirmed by ^1H and ^{19}F NMR, MS spectroscopies and elemental analysis.

In the ^{13}C NMR spectra, the signals of carbon atoms of pyrimido[4,5-*d*]pyrimidin-2,4-dione skeleton were assigned using "nmrshiftdb2" – a free NMR database [23]. The signals of carbon atoms of uracil ring are in the expected region of 149-155 ppm for C-2 and 159-161 ppm for C-4, 78-80 ppm for C-4a, and 150 ppm for C-8a, correspondingly. The signals of C-5 carbon atoms that are connected with two CF_3-groups appeared as septets at 64 - 66 ppm with the coupling constants $^2J_{CF}$ 30 - 32 Hz. Finally, the signals of C-7 carbon atoms of second pyrimidine ring are at 158 - 160 ppm. It should be noted that chemical shifts of C-4, C-4a, C-5, and, especially, C-8a, as well as CF_3 carbon atoms do not sufficiently depend on substituents at 1- and 7-positions, the deviations do not exceed ±1.0 ppm: the $\Delta_\delta = (\delta_{max} - \delta_{min})$ is 1.3 ppm for C-4, 1.7 ppm for C-4a, 1.9 ppm for C-5, 0.8 ppm for C-8a (see Table 1). The signals of carbon atoms of substituents at 1- and 7-positions are in accordance with their structures.

In the ^{19}F NMR spectra, the signals corresponding to CF_3 groups appeared as singlets at 4 - 6 ppm (**4a-g** and **4i-k**) in the range that is characteristic for this type of pyrimido[4,5-*d*]pyrimidines [19]. However, CF_3 groups in **4h** are nonequivalent and they appeared as two quartets at -70.3 and -71.1 ppm with the coupling constant of J_{FF} = 7 Hz. The signals of fluorine

atoms that are directly connected with a phenyl ring (compounds **4a**, **4e** and **4g**) appeared in the corresponding region.

2.2. Antimicrobial and antifungal activity.

The newly synthesized compounds **4a-k** were evaluated for their *in vitro* antibacterial activity by well diffusion method expressed by zone of inhibition (mm in diameter) against Gram-positive, namely, *Staphylococcus aureus (**SA**), Staphylococcus epidermidis (**SE**)* and *Bacillus anthracis(**BA**),*and Gram-negative - *Escherichia coli (**EC**)* bacterial strains. In addition, they were tested for their *in vitro* antifungal activity against *Candida albicans (**CA**)*. Streptocide and tetracycline were used as a reference to estimate the potency of the testing compounds under the same conditions. The results are presented in Table 2.

Most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria. Heterocycles **4a-c** derived from dimethyluracil **1a** are active against almost all tested microorganisms. Compounds **4f,i** exhibited similar activity against Gram-positive bacteria. All compounds, excluding **4a,c**, demonstrated low antifungal activity against *Candida albicans.*

3. Conclusion

In the present work, a novel series of fluorinated pyrimido[4,5-d]pyrimidine-2,4-diones was prepared by the reaction of 6-aminouracils with *N*-acylimines of hexafluoroacetone. The obtained compounds were screened for their potential antimicrobial and antifungal activity. Most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria. Synthesized compounds has low, except **4a,c** antifungal activity.

4. Experimental

4.1. General

4.1.1 Chemistry

The ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on Bruker DXP at 200, 50, and 188 MHz, respectively, in $CDCl_3$ and Me_2SO-d_6 using tetramethylsilane (TMS) as an internal standard and $CFCl_3$ as an external standard. Chemical shifts are reported in ppm units with the use of δ scale. Mass-spectra were recorded on a Finnigan 4021 spectrometer. Melting points were measured in open capillary tubes and are uncorrected.

4.1.2. Agar well diffusion method.

The assay was carried out by using 1 ml of inoculum ($1x10^9$ colony forming units) prepared from an overnight culture for given test microorganisms. 1 ml of the resultant spore/cell suspension was poured in the petri plate and the plates were poured with respective medium to seed each prepared plate. The medium was allowed to solidify. Using a sterilized cork-borer, wells of 5mm diameter were made in the solidified inoculated medium and the plate area uniformly. The wells were filled with 3 drop (0.1 mL) of compound solution. Plates were then incubated aerobically at 37± 2°C and the zone of inhibition if any was then measured in mm for the particular compound and specific organism after 24 hr. The efficiency of the tested compounds was compared to that of streptocide and tetracycline.

4.2. General procedure for preparation of 5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones ***4a-k.***

To a solution of 5 mmol of uracil **1** in 10 ml of DMF while stirring at 20ºC was added 5 mmol of imine **2**. The reaction mixture was stirred for 1 h at 20ºC, then 0.1 g Et_3N was added. The reaction mixture was heated at 80-90 ºC during 2 h and poured into 50 ml of H_2O, the precipitate formed was separated and crystallized from 50% EtOH.

4.2.1. 1,3- Dimethyl-7-(4-fluorophenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4a****.*

Yield 91%; mp 246-248 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 3.20 (s, 3H, CH_3), 3.50 (s, 3H, CH_3), 7.42 (t, 2H, CH_{Ar}, $^{3}J_{HH} = {}^{3}J_{HF}$= 8.9 Hz), 8.03 (dd, 2H, CH_{Ar}, $^{3}J_{HH}$ = 8.9 Hz, $^{4}J_{HF}$= 5.5 Hz), 10.10 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 27.8 (CH_3N), 29.9 (CH_3N), 65.9

(septet, $^{2}J_{CF}$ = 32 Hz, C-5), 78.7 (C-4a), 115.5 (d, $^{2}J_{CF}$ = 22 Hz, 3,5-C_{PhF}), 122.5 (q, $^{1}J_{CF}$ = 291 Hz, CF_3), 128.3 (d, $^{4}J_{CF}$ = 2 Hz, 1-C_{PhF}),131.8 (d, $^{3}J_{CF}$ = 9 Hz, 2,6-C_{PhF}), 150.8 (C-8a), 152.9 (C-2), 159.0 (C-7), 160.2 (C-4), 164.9 (d, $^{1}J_{CF}$ = 251 Hz, 4-C_{PhF}). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -105.1 (tt, 1F, $^{3}J_{FH}$ = 9.0 Hz, $^{4}J_{FH}$ 5.5 Hz), -70.8 (s, 6F). EI-MS (m/z): 424 $[M]^+$. Calc. for $C_{16}H_{11}F_7N_4O_2$: C, 45.29, H, 2.61, N, 13.21. Found: C, 45.48, H, 2.42, N, 13.27.

4.2.2. 1,3- Dimethyl-7-(4-cyanophenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4b***.

Yield 93%; mp 283-285 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 3.21 (s, 3H, CH_3), 3.50 (s, 3H, CH_3), 8.05 (s, 4H, CH_{Ar}), 10.38 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 27.9 (CH_3N), 30.0 (CH_3N), 66.0 (septet, $^{2}J_{CF}$ = 30 Hz, C-5), 79.1 (C-4a), 114.9 (4-C_{Ph}), 118.1 (C≡N), 122.6 (q, $^{1}J_{CF}$ = 290 Hz, CF_3), 129.6 (2,6-C_{Ph}), 132.3 (3,5-C_{Ph}), 136.0 (1-C_{Ph}), 150.7 (C-8a), 152.6 (C-2), 159.0 (C-7), 160.0 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -72.5 s. EI-MS (m/z): 431 $[M]^+$. Calc. for $C_{17}H_{11}F_6N_5O_2$: C, 47.34, H, 2.57, N, 16.24. Found: C, 47.52, H, 2.44, N, 13.42.

4.2.3. 1,3- Dimethyl-7-(3-nitrophenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4c***.

Yield 86%; mp 240-242 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 3.22 (s, 3H, CH_3), 3.52 (s, 3H, CH_3), 7.72 (t, 1H, $^{3}J_{HH}$ = 8.2 Hz),8.31 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 8.40 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 8.75 (s, 1H, CH_{Ar}), 10.08 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 27.9 (CH_3N), 30.0 (CH_3N), 65.8 (septet, $^{2}J_{CF}$ = 30 Hz, $\underline{C}CF_3$), 79.2 (C-4a), 122.6 (q, $^{1}J_{CF}$ = 291 Hz, CF_3), 123.5 (2-C_{PhNit}), 127.2 (4-C_{Ph}), 130.3 (5-C_{Ph}), 133.2 (1-C_{Ph}), 135.3 (6-C_{Ph}), 147.5 (3-C_{Ph}), 150.8 (C-8a), 152.7 (C-2), 159.0 (C-7), 159.4 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -70.4 s. EI-MS (m/z): 451 $[M]^+$. Calc. for $C_{16}H_{11}F_6N_5O_4$: C, 42.58, H, 2.46, N, 15.52. Found: C, 42.52, H, 2.40, N, 15.62.

4.2.4. 1- Phenyl-7-(3-nitrophenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4d***.

Yield 88%; mp 294-295 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 7.30 – 7.60 (m, 5H, CH_{Ar}), 7.73 (t, 1H, $^{3}J_{HH}$ = 8.2 Hz), 7.99 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 8.38 (s, 1H, CH_{Ar}), 8.41 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.9 Hz), 10.30 (s, 1H, NH), 11.64 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 64.4 (septet, $^{2}J_{CF}$ = 32 Hz, C-5), 80.4 (C-4a), 122.9 (q, $^{1}J_{CF}$ = 291 Hz, CF_3), 124.2 (2-C_{PhNit}), 127.8 (4-C_{PhNit}), 128.8 (4-C_{Ph}), 129.1 (3-C_{Ph}), 130.0 (2-C_{Ph}), 130.6 (5-C_{PhNit}), 133.3 (1-C_{PhNit}), 135,3 (6-C_{PhNit}), 136.2 (1-C_{Ph}), 147.9 (3-C_{PhNit}), 150.6 (C-8a), 155.1 (C-2), 159.0 (C-7), 160.5 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -70.7 s. EI-MS (m/z): 499 $[M]^+$. Calc. for $C_{20}H_{11}F_6N_5O_4$: C, 48.11, H, 2.22, N, 14.03. Found: C, 48.32, H, 2.04, N, 14.16.

4.2.5. 1- (4-Fluorophenyl)-7-(3-nitrophenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4e****.*

Yield 87%; mp 286-288 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 7.40 – 7.60 (m, 2H, CH_{Ar}), 7.76 (t, 1H, $^{3}J_{HH}$ = 8.2 Hz), 8.01 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.9 Hz), 8.39 (s, 1H, CH_{Ar}), 8.42 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 8.2 Hz), 10.31 (s, 1H, NH), 11.64 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 65.9 (septet, $^{2}J_{CF}$ = 30 Hz, C-5), 79.9 (br. s, C-4a), 115.3 (d, $^{2}J_{CF}$ = 22 Hz, 3,5-C_{PhF}), 122.5 (q, $^{1}J_{CF}$ = 290 Hz, CF_3), 123.6 (2-C_{PhNit}), 127.2 (4-C_{PhNit}), 130.1 (5-C_{PhNit}), 131.6 (d, $^{3}J_{CF}$ = 8 Hz, 2,6-C_{PhF}), 131.7 (1-C_{PhF}), 132.8 (1-C_{PhNit}), 134.7 (6-C_{PhNit}), 147.4 (3-C_{PhNit}), 150.1 (C-8a), 154.5 (C-2), 158.5 (C-7), 160.0 (C-4), 161.7 (d, $^{1}J_{CF}$ = 245 Hz, 4-C_{PhF}). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -112.7 (s, 1F), -70.7 (s, 6F). EI-MS (m/z): 517 $[M]^+$. Calc. for $C_{20}H_{10}F_7N_5O_4$: C, 46.43, H, 1.95, N, 13.54. Found: C, 46.33, H, 2.06, N, 13.38.

4.2.6. 1- (4-Methylphenyl)-7-(3-nitrophenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4f****.*

Yield 83%; mp 272-274 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 2.42 (s, 3H, CH_3), 7.28 (d, 2H, CH_{Ar}, *J* = 8.2 Hz), 7.76 (t, 1H, $^{3}J_{HH}$ = 7.6 Hz), 7.86 (d, 2H, CH_{Ar}, $^{3}J_{HH}$ = 8.2 Hz), 8.02 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 8.38 (s, 1H, CH_{Ar}), 8.41 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 9.82 (s, 1H, NH), 11.56 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 20.4 (CH_3), 65.6 (septet, $^{2}J_{CF}$ = 32 Hz, C-5), 79.8 (C-4a), 117.5 (2-C_{Tol}), 122.4 (q, $^{1}J_{CF}$ = 290 Hz, CF_3), 124.0 (2-C_{PhNit}), 127.4 (4-

C_{PhNit}), 128.9 (3-C_{Tol}), 130.4 (5-C_{PhNit}), 131, 2 (4-C_{Tol}), 132.9 (1-C_{PhNit}), 135,1 (6-C_{PhNit}), 137.8 (1-C_{Tol}), 147.5 (3-C_{PhNit}), 150.8 (C-8a), 152.4 (C-2), 158.7 (C-7), 160.1 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -70.6 s. EI-MS (m/z): 513 $[M]^+$. Calc. for $C_{21}H_{13}F_6N_5O_4$: C, 49.13, H, 2.55, N, 13.64. Found: C, 49.23, H, 2.48, N, 13.53.

4.2.7. 1- (4-Fluorophenyl)-7-(3-methylphenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4g***.

Yield 87%; mp 297-299 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 2.38 (s, 3H, CH_3), 7.05 – 7.32 (m, 6H, CH_{Ar}), 7.38 (d, 1H, CH_{Ar}, $^3J_{HH}$ = 7.6 Hz), 7.46 (s, 1H, CH_{Ar}), 9.40 (s, 1H, NH), 11.30 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 21.4 (CH_3), 66.3 (septet, $^2J_{CF}$ = 32 Hz, C-5), 80.0 (C-4a), 115.8 (d, $^2J_{CF}$ = 23 Hz, 3,5-C_{PhF}), 122.9 (q, $^1J_{CF}$ = 291 Hz, CF_3), 126.3 (6-C_{Tol}), 128.8 (5-C_{Tol}), 129.6 (2-C_{Tol}), 132.0 (4-C_{Tol}), 132.2 (d, $^3J_{CF}$ = 9 Hz, 2,6-C_{PhF}), 132.6 (d, $^4J_{CF}$ = 3 Hz, 1-C_{PhF}), 134.1 (1-C_{Tol}), 138.2 (3-C_{Tol}), 150.7 (C-8a), 155.5 (C-2), 160.6 (C-7), 161.1 (C-4), 162.1 (d, $^1J_{CF}$ = 245 Hz, 4-C_{PhF}). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -111.9 (tt, 1F, $^3J_{FH}$ = 9.2 Hz, $^4J_{FH}$ 5.7 Hz), -69.8 (s, 6F). EI-MS (m/z): 486 $[M]^+$. Calc. for $C_{21}H_{13}F_7N_4O_2$: C, 51.86, H, 2.69, N, 11.52. Found: C, 51.73, H, 2.78, N, 11.63.

4.2.8. 1- (2-Fluorophenyl)-7-(furan-2-yl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4h***.

Yield 78%; mp 261-264 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 6.71 (dd, 1H, CH_{Fur}, $^3J_{HH}$ = 3.4 Hz, $^3J_{HH}$ 1.5 Hz), 7.18 (d, 1H, CH_{Fur}, $^3J_{HH}$ = 3.7 Hz), 7.26 – 7.45 (m, 2H, CH_{Ar}), 7.46 – 7.61 (m, 2H, CH_{Ar}), 8.02 (br. s, 1H, CH_{Fur}), 9.88 (s, 1H, NH), 11.68 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 65.3 (septet, $^2J_{CF}$ = 32 Hz, C-5), 79.2 (C-4a), 113.1 (3-C_{Fur}), 122.2 (q, $^1J_{CF}$ = 290 Hz, CF_3), 122.3 (q, $^1J_{CF}$ = 290 Hz, CF_3), 115.7 (d, $^2J_{CF}$ = 19 Hz, 3-C_{PhF}), 119.0 (4-C_{Fur}), 123.2 (d, $^3J_{CF}$ = 13 Hz, 1-C_{PhF}), 124.5 (d, $^4J_{CF}$ = 3 Hz, 6-C_{PhF}), 130.8 (d, $^3J_{CF}$ = 8 Hz, 4-C_{PhF}), 131.7 (5-C_{PhF}), 144.5 (5-C_{Fur}), 148.6 (2-C_{Fur}), 149.4 (C-2), 150.7 (C-8a), 154.7 (C-7), 157.6 (d, $^1J_{CF}$ = 247 Hz, 2-C_{PhF}), 159.8 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -121.3 (m, 1F), -70.3

(q, 3F, $^4J_{FF}$ = 7.0 Hz), -71.15 (q, 3F, $^4J_{FF}$ = 7.0 Hz). EI-MS (m/z): 462 $[M]^+$. Calc. for $C_{18}H_9F_7N_4O_3$: C, 46.77, H, 1.96, N, 12.12. Found: C, 46.92, H, 2.07, N, 12.33.

4.2.10. 1- (4-Chlorophenyl)-7-(furan-2-yl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4i***.

Yield 81%; mp 274-276 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 6.68 (dd, 1H, CH_{Fur}, $^3J_{HH}$ = 3.4 Hz, $^3J_{HH}$ 1.7 Hz), 7.18 (d, 1H, CH_{Fur}, $^3J_{HH}$ = 3.2 Hz), 7.37 (d, 2H, CH_{Ar}, $^3J_{HH}$ = 8.5 Hz), 7.51 (d, 2H, CH_{Ar}, $^3J_{HH}$ = 8.5 Hz), 7.99 (d, 1H, CH_{Fur}, $^3J_{HH}$ = 1.7 Hz), 9.73 (s, 1H, NH), 11.51 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 65.2 (septet, $^2J_{CF}$ = 32 Hz, C-5), 79.3 (C-4a), 113.0 (3-C_{Fur}), 118.8 (4-C_{Fur}), 122.4 (q, $^1J_{CF}$ = 291 Hz, CF_3), 128.4 (2,6-C_{PhCl}), 131.5 (3,5-C_{PhCl}), 132.7 (1-C_{PhCl}), 134.7 (4-C_{PhCl}), 144.6 (5-C_{Fur}), 148.3 (2-C_{Fur}), 149.9 (C-2), 150.3 (C-8a), 154.8 (C-7), 159.8 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -72.4 s. EI-MS (m/z): 478 $[M]^+$. Calc. for $C_{18}H_9ClF_6N_4O_3$: C, 45.16, H, 1.89, N, 11.70. Found: C, 45.02, H, 1.77, N, 11.63.

4.2.11. 1-Benzyl-7-(thiophen-2-yl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4j***.

Yield 72%; mp 270-272 °C. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 5.24 (s, 2H, CH_2), 7.17 – 7.41 (m, 6H, CH_{Ar}), 8.03 (d, 1H, CH_{Ar}, $^3J_{HH}$ = 4.6 Hz), 8.47 (d, 1H, CH_{Ar}, $^3J_{HH}$ = 3.4 Hz), 9.78 (s, 1H, NH), 11.48 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 45.1 (CH_2), 66.2 (septet, $^2J_{CF}$ = 32 Hz, C-5), 79.6 (C-4a), 122.9 (q, $^1J_{CF}$ = 290 Hz, CF_3), 127.8 (4-C_{Ph}), 127.9 (2-C_{Ph}), 129.0 (3-C_{Ph}), 129.5 (3-C_{Th}), 133.6 (4-C_{Th}), 135.9 (2-C_{Th}), 136.3 (1-C_{Ph}), 138.2 (1-C_{Th}), 150.9 (C-8a), 154.6 (C-2), 155.4 (C-7), 160.0 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -72.4 s. EI-MS (m/z): 474 $[M]^+$. Calc. for $C_{19}H_{12}F_6N_4O_2S$: C, 48.11, H, 2.55, N, 11.81. Found: C, 48.03, H, 2.37, N, 11.68.

4.2.12. 1-[2-(3,4-Dimethoxyphenyl)ethyl]-7-(4-methylphenyl)-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione ***4k***.

Yield 69%; mp 223-225 ºC. ^{1}H NMR (200 MHz, Me_2SO-d_6, δ): 2.38 (s, 3H, CH_3), 2.83 (t, 2H, CH_2,$^3J_{HH}$ = 6.6 Hz), 3.66 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 4.06 (t, 2H, CH_2, $^3J_{HH}$ =

6.6Hz), 6.75 (dd, 1H, CH_{Ar}, ^{3}J = 7.9 Hz, $^{4}J_{HH}$ 1.2 Hz), 6.81 (d, 1H, CH_{Ar}, $^{4}J_{HH}$ = 1.2 Hz), 6.86 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.9 Hz), 7.30 (d, 2H, CH_{Ar}, $^{3}J_{HH}$ = 8.2 Hz), 7.82 (d, 2H, CH_{Ar}, $^{3}J_{HH}$ = 8.2 Hz), 9.83 (s, 1H, NH), 11.08 (s, 1H, NH). ^{13}C NMR (50 MHz, Me_2SO-d_6, δ): 39.2 (C$\underline{CH_2}$C), 42.2 (CH_2N), 55.5 (CH_3O), 55.7 (CH_3O), 65.8 (septet, $^{2}J_{CF}$ = 30 Hz, C-5), 80.1 (C-4a), 111.8 (2,5-C_{Ph}), 120.8 (6-C_{Ph}), 122.6 (q, $^{1}J_{CF}$ = 290 Hz, CF_3), 125.5 (2-C_{Tol}), 130.1 (3-C_{Tol}), 130.5 (1-C_{Tol}), 131.8 (1-C_{Ph}), 140.2 (4-C_{Tol}), 147.4 (4-C_{Ph}), 148.7 (3-C_{Ph}), 150.6 (C-2), 150.7 (C-8a), 158.8 (C-7), 159.9 (C-4). ^{19}F NMR (188.29 MHz, Me_2SO-d_6, δ): -71.6 s. EI-MS (m/z): 556 $[M]^+$. Calc. for $C_{25}H_{22}F_6N_4O_4$: C, 53.96, H, 3.98, N, 10.07. Found: C, 54.06, H, 3.77, N, 10.24.

*4.3. General procedure for preparation of imines **2b-d**.*

To mixture of 0.1 mol of benzamide in 50 mL of benzene, 0.2 mol of pyridine, 0.11 mol of hexafluoroacetone was bubbled at stirring at 20°C during 30 min. Then, 0.1 mol of SOCl2 was added, and the mixture was stirred during 2 h. The formed precipitate was filtered off. After the solvent removal, the residue was fractionated for **2b,d** or crystallized from hexane for **2c**.

*4.3.1. 4-Cyano-N-(1,1,1,3,3,3-hexafluoropropan-2-ylidene)benzamide **2b**.*

Yield 82%; bp 81-82 °C (1 Torr). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.86 (d, 2H, CH_{Ar}, $^{3}J_{HH}$ = 8.0 Hz), 8.05 (d, 2H, CH_{Ar}, $^{3}J_{HH}$ = 8.0 Hz). ^{19}F NMR (188.29 MHz, $CDCl_3$, δ): -68.3 s. Calc. for $C_{11}H_4F_6N_2O$: C, 44.91, H, 1.37, N, 9.52. Found: C, 44.76, H, 1.26, N, 9.44.

*4.3.2. N-(1,1,1,3,3,3-Hexafluoropropan-2-ylidene)-3-nitrobenzamide **2c**.*

Yield 71%; mp 83-84 °C. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.74 (t, 1H, $^{3}J_{HH}$ = 8.2 Hz), 8.32 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 8.38 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 8.65 (s, 1H, CH_{Ar}). ^{19}F NMR (188.29 MHz, $CDCl_3$, δ): -67.45 s. Calc. for $C_{10}H_4F_6N_2O_3$: C, 38.23, H, 1.28, N, 8.92. Found: C, 38.56, H, 1.46, N, 8.76.

*4.3.3. N-(1,1,1,3,3,3-Hexafluoropropan-2-ylidene)-3-methylbenzamide **2d**.*

Yield 80%; bp 101-102 °C (20 Torr). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.08 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 7.26 (t, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 7.38 (d, 1H, CH_{Ar}, $^{3}J_{HH}$ = 7.6 Hz), 7.46 (s, 1H,

CH_{Ar}). ^{19}F NMR (188.29 MHz, $CDCl_3$, δ): -69.53 s. Calc. for $C_{11}H_7F_6NO$: C, 46.66, H, 2.49, N, 4.95. Found: C, 46.52, H, 2.65, N, 4.73.

Acknowledgements

This publication was supported in the part by the Russian Foundation for Basic Research (project number 16-03-00696).

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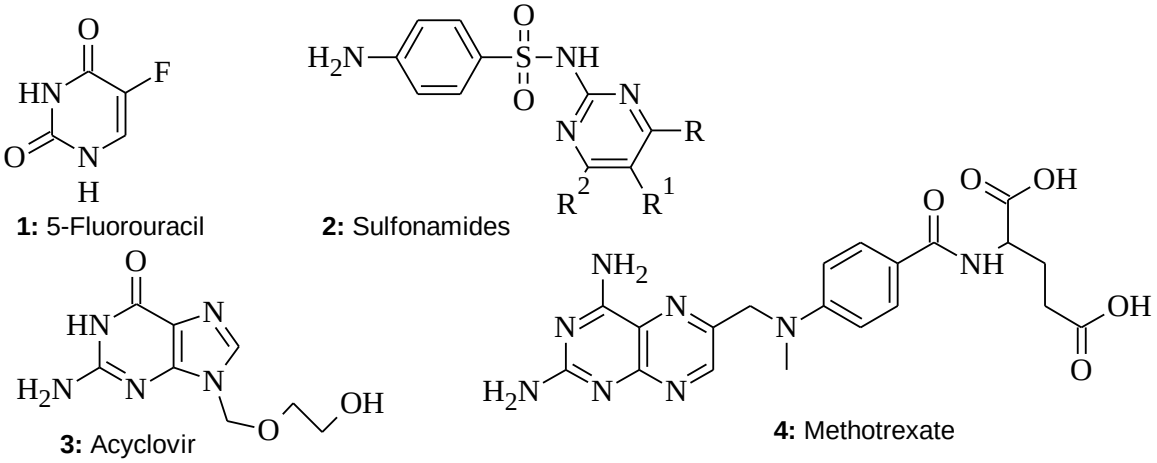
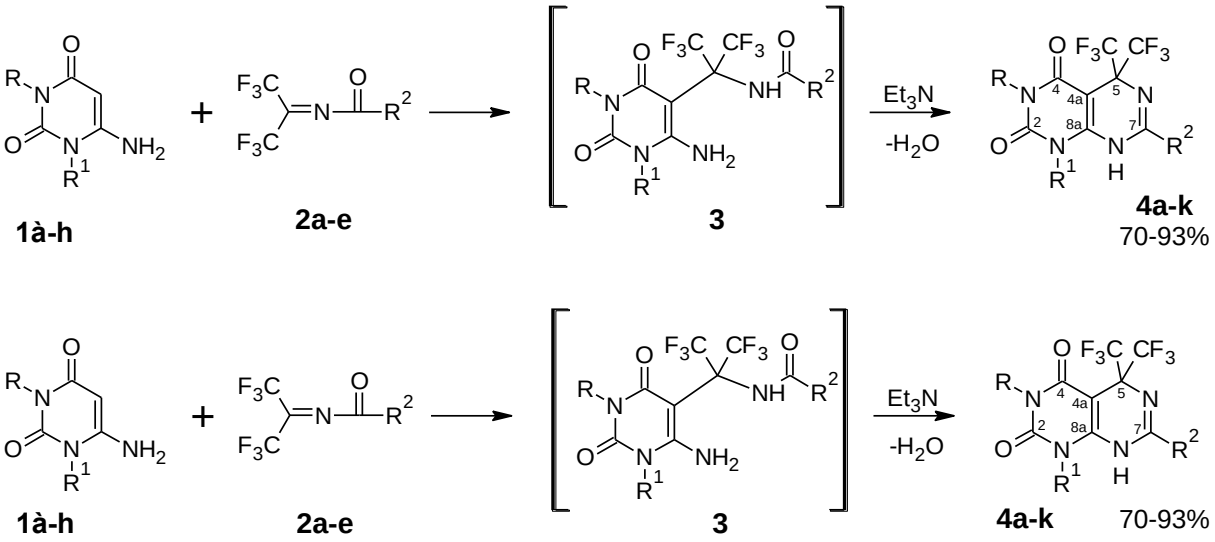


Figure 1. Pyrimidine-containing drugs.

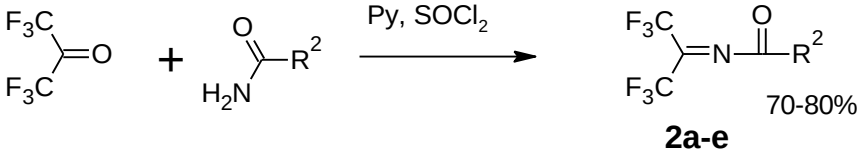
Scheme 1



		R	R^1	R^2			R	R^1	R^2
1a	**4a**	Me	Me	4-FC_6H_4		**4g**	H	4-FC_6H_4	3-MeC_6H_4
	4b	Me	Me	4-NCC_6H_4	**1e**	**4h**	H	2-FC_6H_4	2-Furyl
	4c	Me	Me	3-$NO_2C_6H_4$	**1f**	**4i**	H	4-ClC_6H_4	2-Furyl
1b	**4d**	H	Ph	3-$NO_2C_6H_4$	**1g**	**4j**	H	CH_2Ph	2-Thienyl
1c	**4e**	H	4-FC_6H_4	3-$NO_2C_6H_4$	**1h**	**4k**	H	3,4-$(MeO)_2C_6H_3CH_2CH_2$	4-MeC_6H_4
1d	**4f**	H	4-MeC_6H_4	3-$NO_2C_6H_4$					

Imines **2b-d** which were used in the synthesis of heterocycles **4** were synthesized for the first time according to the method described in [24] by a successive addition of hexafluoroacetone to a solution (or suspension) of equimolar amounts of benzamide, pyridine, and $SOCl_2$ in benzene at 20 °C; the reaction was completed within 1.5-2 h.

Scheme 2



2a-e

R^2 = 4-FC_6H_4 (**2a**), 4-CN-C_6H_4 (**2b**), 3-$NO_2C_6H_4$ (**2c**), 3-MeC_6H_4 (**2d**), 4-MeC_6H_4 (**2e**), 2-Furyl (**2f**), 2-Thienyl (**2g**)

Table 1.

Chemical shifts (δ) of carbon atoms of pyrimido[4,5-*d*]pyrimidin-2,4-dione skeleton for compounds **4a-k**

Comp.	C-2	C-4	C-4a	C-5	C-7	C-8a
4a	152.9	160.2	78.7	65.9	159.0	150.8
4b	152.6	160.0	79.1	66.0	159.0	150.7
4c	152.7	159.4	79.2	65.8	159.0	150.8
4d	155.1	160.5	80.4	64.4	159.0	150.6
4e	154.5	160.0	79.9	65.9	158.5	150.1
4f	152.4	160.1	79.8	65.6	158.7	150.8
4g	155.5	161.1	80.0	66.3	160.6	150.7
4h	149.4	159.8	79.2	65.3	154.7	150.7
4i	149.9	159.8	79.3	65.2	154.8	150.3
4j	154.6	160.0	79.6	66.2	155.4	150.9
4k	150.6	159.9	80.1	65.8	158.8	150.7
*Δ_δ	5.7	1.3	1.7	1.9	5.9	0.8

* $\Delta_\delta = (\delta_{max} - \delta_{min})$

Table 2.

Inhibition zones (in millimeters) of the synthesized compounds **4a-k**.

Compounds		Microorganisms and inhibition zone (mm)				
		Gram-positive			Gram-negative	Fungi
	%w	***SE***	***SA***	***BA***	***EC***	***CA***
4a	1.0	18	19	20	17	20
	0.1	21	22	33	17	25
	0.01	25	26	36	16	27
4b	1.0	14	17	22	11	14
	0.1	12	18	21	11	13
	0.01	11	18	20	0	12
4c	1.0	26	29	25	0	30
	0.1	23	24	21	0	25
	0.01	0	0	0	0	0
4d	1.0	13	16	15	0	12
	0.1	0	0	0	0	0
	0.01	0	0	0	0	0
4e	1.0	15	19	19	0	12
	0.1	11	14	12	0	11
	0.01	0	0	0	0	0
4f	1.0	15	21	20	0	13
	0.1	11	17	16	0	11
	0.01	0	14	14	0	0
4g	1.0	12	13	13	12	13
	0.1	0	0	12	11	12
	0.01	0	0	0	0	0
4h	1.0	11	12	17	0	0
	0.1	0	0	11	0	0
	0.01	0	0	0	0	0
4i	1.0	20	18	19	12	11
	0.1	11	12	12	0	0
	0.01	0	11	12	0	0
4j	1.0	11	11	13	0	13
	0.1	0	0	11	0	11
	0.01		0	0	0	0
4k	1.0	13	16	16	0	14
	0.1	0	0	13	0	12
	0.01	0	0	12	0	0
Streptocide	1.0	51	58	11	38	11
	0.1	35	29	0	30	0
	0.01	22	18	0	20	0
Tetracycline	10	25	38		32	
	1.0	23	36		29	
	0.1	16	30		23	

SA: *Staphylococcus aureus,* **SE**: *Staphylococcus epidermidis,* **BA**: *Bacillus anthracis,* **EC**: *Escherichia coli,* **CA**: *Candida albicans*