Journal of Fluorine Chemistry 188 (2016) 191–195

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

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

Short communication

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

Alexey Yu. Aksinenko*, Tatyana V. Goreva, Tatyana A. Epishina, Sergey V. Trepalin, Vladimir B. Sokolov

Institute of Physiologically Active Compounds of Russian Academy of Sciences, Severnyi Pr. 1, Chernogolovka, Moscow Region, 142432, Russia

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 27 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
Fluorinated heterocycles
pyrimido[4,5-d]pyrimidines
Cyclocondensation

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.

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 (Fig. 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

* Corresponding author.
E-mail address: alaks@ipac.ac.ru (A.Y. Aksinenko).

http://dx.doi.org/10.1016/j.jfluchem.2016.06.019
0022-1139/

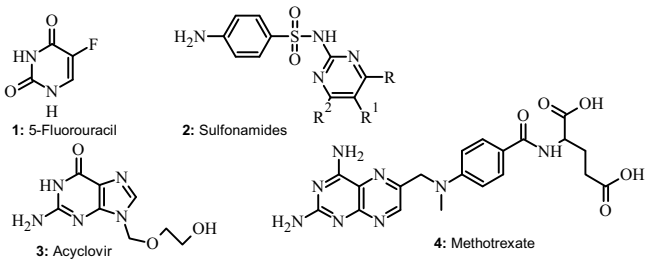


Fig. 1. Pyrimidine-containing drugs.

pyrimido[4,5-*d*]pyrimidines **4a-k** (Scheme 1). It should be mentioned that ^{19}F NMR spectrum of the reaction mixture containing uracil **1 g** and imine **2 g** that were kept in DMF for 0.5 h at room temperature in the absence of Et_3N showed two compounds – intermediate **3 j** and product **4j**. Broad singlets at −57.9 and −70.0 ppm could be assigned to the intermediate **3 j** 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 $\Delta_d = (\delta_{max} - \delta_{min})$ 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 **4 g**) 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

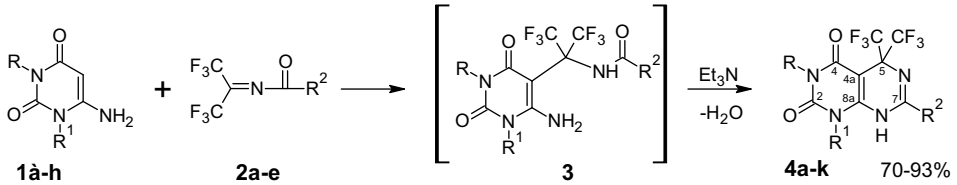
In the present work, a novel series of fluorinated pyrimido[4,5-d]pyrimidine-2,4-diones was prepared by the reaction of 6-aminouracils with *N*-acylimines of hexafluoroacetone. The obtained compounds were screened for their potential antimicrobial and antifungal activity. Most of the tested compounds 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 ^1H, ^{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



		R	R¹	R²			R	R¹	R²
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-$NO_2C_6H_4$					

Scheme 1. 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).